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Review

# Physiologic functions of PP2A: Lessons from genetically modified mice☆

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ABSTRACT

Protein Phosphatase 2A (PP2A) encompasses a large family of Ser/Thr phosphatases, consisting of a catalytic C subunit and a structural A subunit that are, in most cases, further bound to a regulatory B-type subunit. The B-type subunits determine function and regulation of PP2A trimers, but despite their importance in PP2A biology, their roles in controlling dephosphorylation of a given substrate in a given cell or tissue remain poorly defined, particularly in the context of a complete organism. Besides twenty PP2A subunit encoding genes, some of which are tissue-specifically expressed, five additional genes encode major regulators of active PP2A trimer assembly, and at least seven genes encode cellular PP2A inhibitors, further adding to the complexity of the mammalian PP2A system. In this review, we summarize current knowledge on physiologic functions of PP2A in germ cell maturation, embryonic development, metabolic regulation, tumor suppression, and homeostasis of adult brain, heart, liver, immune system, lung, intestine, kidney, skin, bone and eye, all retrieved from *in vivo* studies using PP2A transgenic, knockout or knockin mice. Data from 63 mouse models, generated between 1998 and now, reveal the essentiality of PP2A *in vivo*, and shed light on tissue-specific functions of particular PP2A subunits on the one hand, and functional redundancies on the other hand. In future, it remains of utmost importance to further characterize the existing models, as well as to generate novel models, with the aim of deepening our insights in PP2A (patho)physiology and, particularly, in the therapeutic potential of PP2A targeting in human disease.

## 1. Introduction

Reversible protein phosphorylation is a major important mechanism for signal transduction. In physiologic conditions, phospho-regulation is strictly controlled and balanced through a network of tightly regulated protein kinases and protein phosphatases. The bulk of protein phosphorylation occurs on Ser and Thr residues. Together with protein phosphatases of type 1 (PP1), type 2A protein phosphatases (PP2A) make up > 90% of the Ser/Thr phosphatase activity in most cell types. Thus, it is not surprising that dysregulation of PP2A has been strongly associated with several disease states, including Alzheimer's disease, intellectual disability (ID), autoimmune disease, diabetes and diverse cancers [1–5].

Structurally, PP2A phosphatases are rather complex enzymes [6,7] (Fig. 1). A substantial amount of PP2A (around 30%) resides in the cell as a dimer [8], consisting of a catalytic C subunit and a structural A subunit. In mammals, both subunits are each encoded by two distinct genes, leading to an α and β protein isoform. Despite Cα and Cβ sharing 97% amino acid identity, the *Ppp2ca* (Cα) knockout (KO) mouse is embryonically lethal [9], implying a lack of redundancy between these isoforms. Likewise, *Ppp2r1a* (Aα) KO mice are neither viable, despite 86% of sequence identity between Aα and Aβ proteins [10]. The PP2A AC core dimers further associate with a range of regulatory B subunits, which are subdivided in B/PR55/B55, B′/PR61/B56, B″/PR72 and B‴/STRN families (Fig. 1). Given that they bind the core dimer in a mutually exclusive way, these regulatory subunits are known determinants of the substrate specificity, regulation and subcellular localization of the different PP2A holoenzymes, and, as such, largely determine the physiologic functions of the trimeric PP2A complexes [11,12]. In addition, several other, specific cellular regulators and inhibitors of PP2A have been identified (Fig. 1), each contributing to PP2A physiology in a unique, although usually still largely unexplored, manner [13–18].

In mice, PP2A expression was found in all of thirty adult or embryonic tissues examined by RNA-seq through the Mouse ENCODE

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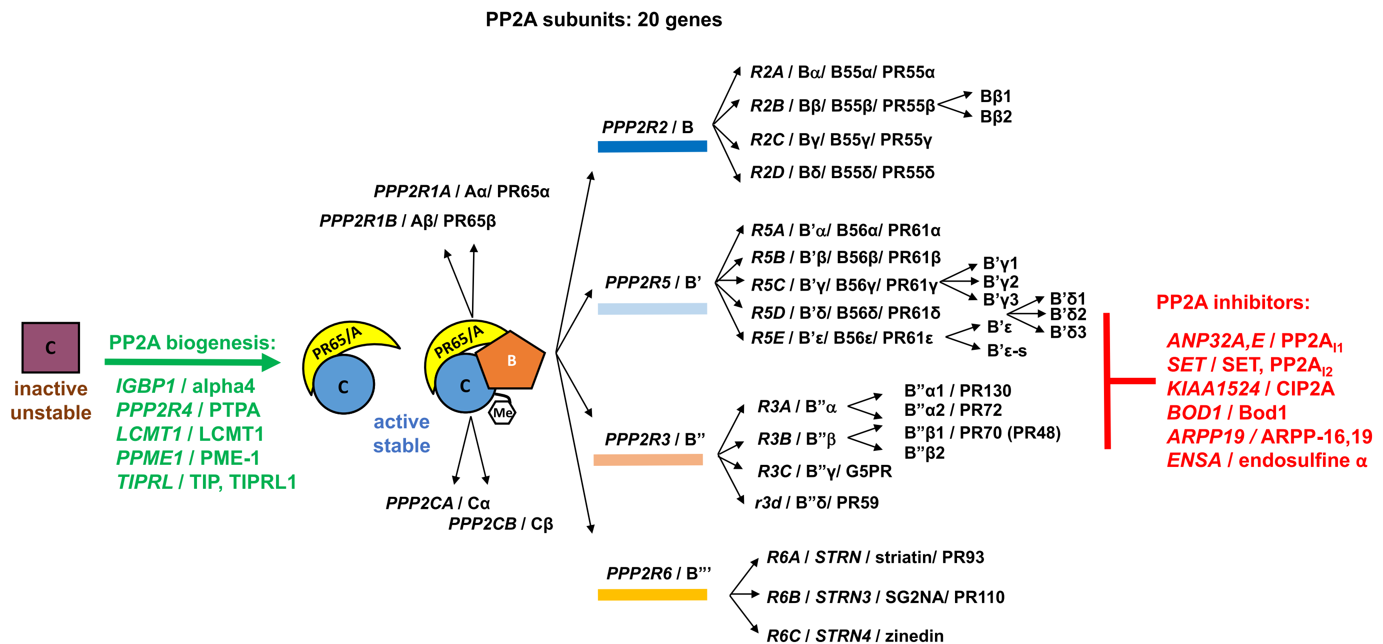


**Fig. 1.** Overview of mammalian PP2A subunit genes, as well as of the genes encoding PP2A biogenesis regulators (in green) and cellular inhibitors (in red). In mice, 2 genes encode PP2A catalytic C subunits, 2 genes encode scaffolding A subunits, and 15 genes encode the different B-type subunits (*PPP2R3B* is human-specific; *Ppp2r3d* is mouse-specific [22]). The C subunit is originally synthesized as an inactive, unstable protein that undergoes a number of stabilizing interactions, conformational changes and post-translational modifications (collectively denoted as 'biogenesis') before its incorporation into biologically active PP2A AC dimers or ABC trimers [14]. *IGBP1* encodes the α4 subunit (also called: Immunoglobulin Binding Protein 1), which stabilizes the C subunit as a latent form [16,25]. PTPA (PP2A Phosphatase Activator), encoded by *PPP2R4*, promotes folding of PP2A C into an active conformation, with ATP/$Mg^{2+}$ as a necessary co-factor [17]. *LCMT1* encodes Leucine Carboxyl Methyl Transferase 1, the enzyme catalyzing methylation (Me) of the carboxyterminus of the activated C subunit. This modification selectively promotes B-type subunit binding, and thereby, assembly of active PP2A trimers [13]. *PPME1* encodes the PP2A Methyl Esterase 1, which is the functional antagonist of LCMT-1 and demethylates the PP2A C carboxyterminus. In addition, PME-1 binds to and stabilizes an inactive PP2A AC pool in the cell, which can be reactivated by PTPA [14]. TIPRL1 (Two A Inhibitory Protein 1 or TOR signaling Pathway Regulator 1) is the isoform encoded by *TIPRL* that selectively binds the unmethylated C subunit and makes unusual wobble contacts with the A subunit, thereby also stabilizing inactive PP2A AC dimers [15]. The 'real' PP2A cellular inhibitors (denoted in red), all have the ability to proactively inhibit active PP2A holoenzymes [18] and are currently represented by seven genes: *ANP32A* and *ANP32E* (Acidic Nuclear Phosphoprotein 32, member A, or E; ANP32A also called: PP2A Inhibitor 1, $PP2A_{I1}$), *SET* (Suvar/Enhancer of zeste/Trithorax; also called: PP2A Inhibitor 2, $PP2A_{I2}$), *CIP2A* (Cancerous Inhibitor of PP2A), *BOD1* (Bi-orientation of chromosomes in cell division 1), *ARPP19* (cAMP-Regulated Phosphoprotein 19, encoding the splice variants ARPP-16 and ARPP-19) and *ENSA* (endosulfine α). These inhibitors either directly bind to the PP2A catalytic subunit or target very specific PP2A holoenzymes, thereby preventing dephosphorylation of a large variety of PP2A substrates [18].

Initiative, overall showing the highest expression in brain, and the lowest in adult liver (Fig. 2) [19]. While the large majority of B-type subunits appear ubiquitously expressed, some of them are restricted to specific tissues, and therefore, likely mediate tissue-specific PP2A functions (Fig. 2). This is probably best illustrated for B55γ (nearly exclusively expressed in brain) and B″α (highly expressed in heart), in accordance with previously published work of others [20–22]. In addition, some of the ubiquitously expressed B-type subunits show remarkably enhanced expression in certain tissues (*e.g.* the very high B56β expression in the adrenal gland compared to all other tissues examined), also suggestive for a more important role in those organs. Most PP2A biogenesis regulators and cellular PP2A inhibitors are ubiquitously expressed as well, except for CIP2A, which apart from thymus and testis, appears only reasonably well expressed in embryonic tissues (Fig. 2). Finally, some PP2A genes appear developmentally regulated, best illustrated for B55β and B55γ, the expression of which increases during embryonic brain development, while expression of the PP2A inhibitors SET, ANP32A, ANP32E and CIP2A, conversely, decreases during brain development, and also during liver development (Fig. 2). Thus, insights into the tissue distribution and developmental expression of the various PP2A genes may provide important clues as to their physiologic functions.

Mouse models have proven useful tools for research on physiologic functions of proteins, but also on disease and underlying pathology. Their implementation in PP2A research, in particular, has already lead to some interesting insights. Here, we review the data from 63 genetically modified mouse models (in short: strains), generated over the last 20 years, targeting specific PP2A subunit or PP2A regulator/inhibitor encoding genes. For the PP2A subunit genes, these models can be subdivided into complete or inducible, total-body *versus* tissue-specific knockouts (KO) of catalytic, structural or regulatory subunits; models with transgenic overexpression of wild-type (WT) or mutant catalytic, structural or regulatory subunits; and models with knockin (KI) of mutant subunits in the endogenous gene loci (Tables 1–3). For the genes encoding PP2A regulators/inhibitors, merely KO and some transgenic models (overexpression of WT) have been described so far (Table 4). Although, clearly, each of these models has contributed to a better understanding of many of the pleiotropic PP2A functions in mammalian cells and tissues, the interpretation of the precise PP2A dysfunction underlying a given, observed phenotype is not always as straightforward as it might seem at first sight. Indeed, because total PP2A levels are subject to tight regulation, both at transcriptional and post-transcriptional levels, overexpression or knockout of a given subunit may 'titrate' other endogenous subunits, and hence, the actual cause of the phenotype may not be the overexpression or lack of the holoenzyme containing the transgenic subunit, but rather, the lower or higher concentration of holoenzymes containing other subunits. Particularly in PP2A regulatory B subunit strains, this possibility of 'competitive adaptation' is important to keep in mind, although in some models, authors have attempted to shed light on potential (additional) changes in PP2A biochemistry or (compensatory) changes in subunit expression.

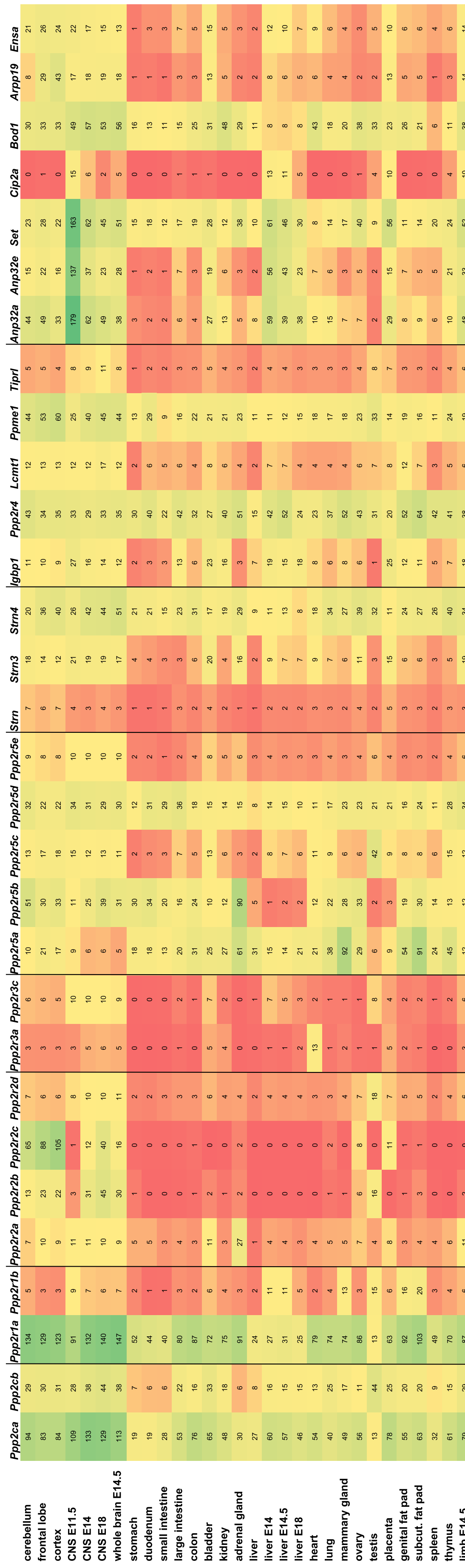


**Fig. 2.** Expression pattern of 'PP2A' genes in different mouse tissues as determined by RNA-seq on at least two biological replicates by the Mouse ENCODE consortium initiative [19]. The Mouse ENCODE dataset was queried through the NCBI Gene database (www.ncbi.nlm.nih.gov/gene), exported to Excel, and displayed as a heat map, with green indicating highest expression, and red indicating lowest expression. The numbers displayed denote 'the number of reads per kilobase per million reads placed' (RPKM). Unfortunately, no expression data were found for *Ppp2r3d* in this dataset. Overall, the data are consistent with previous reports on the expression of PP2A subunit genes in mouse tissues using other techniques, such as *e.g.* Northern blotting and *in situ* hybridisation [20–22].

**Table 1**
Overview of PP2A genetically modified strains, with manipulation of genes encoding Cα or Cβ subunits.

| Gene<br>Protein | Genetic manipulation and background | Affected tissue (promoter) | Main phenotype(s) | Ref. |
|---|---|---|---|---|
| *Ppp2ca*<br>Cα | > 0.5 kb of gene promoter, exon 1 and 0.3 kb of intron 1 replaced by Neo-cassette; C57BL/6 background | Total body, constitutive KO | Lethal; degenerated, smaller embryos at E6.5; no gastrulation or mesoderm formation; Wnt signaling defect | [9,34] |
| *Ppp2ca*<br>Cα | Exons 3–5: floxed; C57BL/6 background | Conditional KO in total body (*Ddx4*-Cre, germ-cell-specific) | Lethal; degenerated, smaller embryos at E6.5; no mesoderm formation | [35] |
| *Ppp2ca*<br>Cα | Exons 3–5: floxed; background: N/A | Conditional KO in oocytes at primary follicular stages (*Zp3*-Cre) | Normal female fertility | [28] |
| *Ppp2ca*<br>Cα | Exon 2: floxed; C57BL/6J background | Conditional KO in primordial germ cells at E12.5 (*Ddx4*-Cre); *Ppp2ca*$^{flox/-}$ mice were analyzed | Male infertility; meiosis defect suspected | [27] |
| *Ppp2ca*<br>Cα | Exons 3–5: floxed; Mixed 129/B6 background | Conditional KO in early hematopoietic and endothelial cells (*Tie2*-Cre) | -Embryonic lethal at E12.5; defective hematopoiesis in fetal livers; increased apoptosis of erythroid cells; STAT5 signaling defect<br>-No embryonic vasculature defects at E12.5 | [38] |
| *Ppp2ca*<br>Cα | Exons 3–5: floxed; Background: N/A | Conditional KO in macrophages (*LysM*-Cre) | Elevated host antiviral response; increased type I Interferon and NF-κB signaling | [84] |
| *Ppp2ca*<br>Cα | Exons 3–5: floxed; C57BL/6 background | Conditional KO in liver (*Alb*-Cre, hepatocyte-specific) | -Increased serum lipid levels; less hepatic lipid deposition<br>-Improved glucose tolerance; increased insulin signaling; suppressed gluconeogenesis; improved glycogen storage | [71] |
| *Ppp2ca*<br>Cα | Exons 3–5: floxed; Mixed 129/B6 background | Conditional KO in liver (*Alb*-Cre, hepatocyte-specific); 8–10-Week-old mice were *i.p* injected with $CCl_4$ for 5 weeks (3 × /week) | Protection against chronic liver injury; impaired TGF-β1/Smad2,3 signaling | [95] |
| *Ppp2ca*<br>Cα | 'Floxed', no further details; Background: N/A | Conditional KO, specifically in the CNS (*nestin*-Cre) | At P7: microcephaly, cortical degeneration; impaired learning/memory; increased pro-apoptotic Hippo and p73 signaling in NPCs | [47] |
| *Ppp2ca*<br>Cα | Exons 3–5: floxed; C57BL/6J background | Conditional KO in cardiomyocytes (*αMHC*-Cre, expression at P6.5) | Cardiac hypertrophy; altered cardiomyocyte metabolism; increased PLB phosphorylation | [62] |
| *Ppp2ca*<br>Cα | Exon 2: floxed; C57BL/6J background | Conditional KO in cardiomyocytes (*αMHC*-MerCreMer, tamoxifen-induced at 3 months) | Cardiac hypertrophy, fibrosis; AKT, GSK-3β and β-catenin phosphorylation affected | [64] |
| *Ppp2ca*<br>Cα | Exon 2: floxed; C57BL/6J background | Conditional KO in epidermis/ectoderm (*Krt14*-Cre) | Smaller animals; 15% die at 6 weeks; abnormal hair follicle morphogenesis; basal cell hyperproliferation; AKT phosphorylation defect | [89] |
| *Ppp2ca*<br>Cα | Expression of N-terminally HA-tagged Cα L199P mutant; C57BL/6 background | Transgene; neuron-specific expression (*Thy1.2* promoter) in Purkinje cells of the cerebellum and layers I–V of the cortex (high) and the hippocampus (low) | Increased Tau phosphorylation (S422, AT8) and aggregation; increased MEK/ERK/Elk-1 and JNK/c-jun signaling | [51,53] |
| *Ppp2ca*<br>Cα | Expression of N-terminally FLAG-tagged mouse Cα L199P mutant; BDF1 background | Transgene; osteoblast lineage-specific expression (*Col1α1* promoter) | Larger and heavier mice; increased bone mineral density and thickness; increased adipogenesis in bone marrow | [96] |
| *Ppp2ca*<br>Cα | Expression of N-terminally HA-tagged Cα L309A mutant; C57BL/6 background | Transgene; moderate expression in the Harderian gland (*mThy1.2* promoter), and high neuron-specific expression in hippocampus, cortex (III-VI), thalamus and cerebellum | -Delayed development and hypoplasia of the Harderian gland; enophthalmos (slit-eyes); Wnt signaling defect<br>-Increased Tau phosphorylation in forebrain; no neurodegeneration; when crossed with P301L Tau transgene: Tau phosphorylation aggravated, tangles | [49,50,91] |
| *Ppp2ca*<br>Cα | Expression of WT Cα coding region, including 69 bp of 5′UTR and 326 bp of 3′UTR; CD1 background | Transgene; cardiac-specific expression (*α-myosin heavy chain* promoter) | Cardiac hypertrophy; decelerated inactivation of L-type $Ca^{2+}$ channels; decreased phosphorylation of PLB, TnI and eEF-2 | [61] |
| *Ppp2ca*<br>Cα | Expression of WT Cα coding region, including 69 bp of 5′UTR and 326 bp of 3′UTR; CD1 background | Transgene; cardiac-specific expression (*α-myosin heavy chain* promoter); adult mice (7–11 m) were subjected to chronic myocardial infarction (MI) (*via* LAD ligation) and analyzed 28 days later | -Dilated cardiomyopathy, fibrosis<br>-Increased left ventricle infarct size: worse post-MI remodeling<br>-In basal conditions: dysregulated SERCA, CAMKIIα, RyR2, AKT, GSK-3β and β-catenin phosphorylation; upon MI: restoration of normal AKT, GSK-3β and β-catenin phosphorylation | [63] |
| *Ppp2ca*<br>Cα | Expression of WT Cα coding sequence; C57BL/6J background | Transgene; T cell-specific expression (human *CD2* promoter) | Increased susceptibility to glomerulonephritis (inflammation); increased IL-17 production by $CD4^{+}$ T cells; epigenetic upregulation of *Il17a* and *Il17f*; increased promoter binding of IRF4 | [85,86] |

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**Table 1** (*continued*)

| Gene<br>Protein | Genetic manipulation and background | Affected tissue (promoter) | Main phenotype(s) | Ref. |
|---|---|---|---|---|
| *Ppp2cb*<br>Cβ | Exon 3: floxed;<br>C57BL/6J background | Conditional KO in total body (EIIa-Cre) | Viable; no overt phenotypes | [35] |
| *Ppp2cb*<br>Cβ | Exon 3: floxed;<br>C57BL/6J background | Conditional KO in oocytes at primary follicular stages (*Zp3*-Cre) | Normal female fertility | [28] |
| *Ppp2ca*,<br>*Ppp2cb*<br>Cα, Cβ | *Ppp2ca*: exons 3–5 floxed;<br>*Ppp2cb*: exon 3 floxed;<br>C57BL/6J background | Double conditional KO in oocytes at primary follicular stages (*Zp3*-Cre) | Female infertility; oocytes: no meiosis-I; decreased dephosphorylation of Aurora Kinase B/C substrates | [28] |

The majority of PP2A strains represent models with genetic manipulation of the C subunits (Table 1, 19 models) or A subunits (Table 2, 10 models). Evidently, as all active PP2A holoenzymes contain at least one C and one A subunit, the observed phenotypes in these models are likely attributable to dysfunctions of more than one PP2A holoenzyme. Despite their inferred importance in PP2A biology and the fact that they are represented by no less than 15 different mouse genes, relatively few PP2A strains have manipulated a single regulatory B-type subunit (Table 3, 14 models). In part, this may be explained by the low targeting frequency in Embryonic Stem (ES) cells at the time; in part, by the concern that no overt phenotypes might be seen, due to compensatory mechanisms or functional redundancies. Nevertheless, it is clear that, upon careful examination of these mice, clear phenotypes could be observed in all cases, underscoring the physiologic need for the existence of the plethora of regulatory B-subunits in mammals. The latter also further warrants generating additional KO mice for those regulatory subunits whose genes have not been targeted until now, in order to obtain a more comprehensive image of the physiologic functions of specific PP2A trimers. Finally, the strains of endogenous PP2A biogenesis regulators (mostly KO models) often present with lethal or very severe phenotypes, while the strains of endogenous PP2A inhibitors (also mostly KO models), in contrast, often present with very subtle or no phenotypes at all (Table 4, 20 models). The former may again reflect the essentiality of PP2A *in vivo*, while the latter might suggest the feasibility of pharmacologic targeting of these inhibitors in diseases in which their upregulation is part of the pathogenic mechanism. Some caution in interpreting data for the PP2A biogenesis regulator strains is however warranted, as several of these proteins, besides PP2A, likely also affect the functions of the PP2A-like phosphatases PP4 and PP6 [23–26], and hence, their knockout or overexpression may not solely affect PP2A.

In the following sections, we will discuss in more detail the physiologic functions of PP2A during (embryonic) development and in adult mice, as they can be deduced from the 43 hitherto generated PP2A subunit strains and the 20 PP2A regulatory/inhibitory strains. The data reveal essential PP2A functions in virtually any tissue examined, and shed light on a complex pattern of tissue-specific functions of specific PP2A subunits on the one hand, and functional redundancies on the other hand.

## 2. PP2A function in germ cell production

Several mouse models have pinpointed an important role for PP2A already at the earliest stages of the murine life cycle.

Conditional KO of *Ppp2ca* in primordial germ cells at E12.5, resulted in male infertility [27]. Testes were smaller, normal tubules containing spermatogonia and primary and secondary spermatocytes were absent, germ cells were disorganized, and elongated spermatozoa were lacking – all suggestive for a spermatogenesis and meiosis defect. On the other hand, conditional KO of either *Ppp2ca* or *Ppp2cb* in oocytes at primary follicular stages resulted in normal female fertility [28]; only upon double *Ppp2ca* × *Ppp2cb* KO, female fertility was completely compromised [28]. While in these mice, ovary morphology and ovulation appeared normal, defective oocytes were observed, characterized by a lack of the first meiotic division, chromosome congression, alignment and segregation defects, and formation of an abnormal acentrosomal spindle in meiosis-I. These defects correlated with increased phosphorylation of the Aurora kinase substrates, hKNL1 (Ser24) and Histone H3 (Ser10), and could be partially rescued by the pharmacologic Aurora kinase B/C inhibitor, hesperadin. Thus, these data imply redundant roles of Cα and Cβ in oocyte formation at meiosis-I, but a non-redundant role of Cα in meiotic processes guiding spermatogenesis.

The redundant role of specific PP2A subunits in preserving female fertility and oocyte generation was further corroborated in mice with conditional KO of *Ppp2r1a* in oocytes of primordial follicles [29]. These mice suffered from severe female subfertility, without effects on ovarian

**Table 2**
Overview of PP2A genetically modified strains affecting the structural A subunit encoding genes.

| Gene Protein | Genetic manipulation and background | Affected tissue (promoter) | Main phenotypes | Ref. |
|---|---|---|---|---|
| *Ppp2r1a* Aα | Exons 5–6: floxed; FVB/NCrl background | Total-body KO (prion-Cre deleted mouse) | Embryonic lethal between E5 and E10.5 | [10] |
| *Ppp2r1a* Aα | Transcription stop cassette in intron 2 (*Ppp2r1a*$^{tm2a(KOMP)Mbp}$ allele); Background: N/A | Total-body KO, constitutive | Embryonic lethal; at E7.5: similar dysregulated gene expression pattern as in $t^{w18}/t^{w18}$ embryos | [36] |
| *Ppp2r1a* Aα | $t^{w18}$ allele (large chr 17 deletion); mixed 129/SvEv and C57BL/6 background | Total-body KO, constitutive | At E6.5: impaired Nodal and Wnt signaling; E7.5: no mesoderm; E8-E9: defective EMT; E10: embryonic death; complete rescue by re-expression of *Ppp2r1a* | [36] |
| *Ppp2r1a* Aα | Exons 5–6: floxed; FVB/NCrl background | Total body conditional KO in adult mice (tamoxifen-inducible CreER mouse) | All homozygous $^{Δ5–6/Δ5–6}$mice started to lose weight after the first tamoxifen injection, and had died 6 days later; hunched backs, walking difficulties | [10] |
| *Ppp2r1a* Aα | Exons 5–6: floxed; Mixed FVB/NJ and 129S4/SvJae and C57BL/6J background | Conditional KO in oocytes of primordial follicles (*Gdf9*-Cre, expressed from day P3 onwards) | Severe female subfertility; oocytes: abnormal meiosis-II; eggs with risk of aneuploidy; after fertilization: impaired embryonic development | [29] |
| *Ppp2r1a* Aα | Exons 5–6: floxed; C57BL/6J background | Conditional KO in $T_{reg}$ cells (*Foxp3*-YFP-Cre) | Severe, progressive, multi-organ autoimmunity; lymphoproliferative disorder; increased proliferation and glucose metabolism in $T_{reg}$ cells; upregulated mTORC1 signaling; phenotypic rescue by rapamycin | [81] |
| *Ppp2r1a* Aα | Exons 5–6: floxed; C57BL/6J background | Conditional KO in B cell lineage at pro-B cell stage (*Mb1*-Cre); or, tamoxifen-induced ablation in isolated bone marrow pre-B or splenic B cells, *in vitro* transduced with Cre-$ER^{T2}$ | -Impaired B-cell development; acute cell death upon Aα KD in a B-cell lineage-specific manner; increased PI3K-AKT-mTOR-S6K signaling; increased glycolysis, decreased expression of enzymes driving pentose phosphate pathway;<br>-Reduced leukemia burden and increased ROS in sublethally irradiated NSG mice injected with *BCR-ABL1* or *NRAS*$^{G12D}$ expressing Aα KD B-cells | [75] |
| *Ppp2r1a* Aα | Expression of Aα E64D mutant in *Ppp2r1a* locus; FVB/NCrl background; FVB background for lung carcinogenesis experiments | Constitutive KI; lung carcinogenesis induced by a single *i.p.* injection of benzopyrene in 5-week-old mice; or, by crossing with a Latent Allele of *Kras*$^{G12D}$, expressed in all tissues | -$^{E64D/E64D}$, $^{+/E64D}$ and $^{Δ5–6/E64D}$mice: viable and fertile<br>-Increased benzopyrene-induced lung carcinogenesis in $^{E64D/+}$, $^{Δ5–6/+}$ and $^{Δ5–6/E64D}$mice; rescued by dominant-negative *TP53* allele<br>-*Kras*$^{G12D}$ expressing $^{E64D/+}$, $^{Δ5–6/+}$ and $^{Δ5–6/E64D}$mice show decreased median survival than *Kras*$^{G12D}$ expressing $^{+/+}$mice | [10,108] |
| *Ppp2r1a* Aα | Expression of Aα E64G mutant in *Ppp2r1a* locus; FVB/NCrl background | Constitutive KI | Homozygous and heterozygous mice, and $^{Δ5–6/E64G}$mice: viable and fertile | [10] |
| *Ppp2r1a* Aα | Expression of Aα E64G or E64D mutant in *Ppp2r1a* locus; C57BL/6 background | Constitutive KI | Normal retinal morphology and function; slightly increased PKCα and mTOR phosphorylation | [94] |
| *Ppp2r1a* Aα | Expression of C-terminally EE-tagged human Aα cDNA, lacking HEAT-repeat 5 coding sequences (AαΔ5); C57BL/6 | Transgene; muscle-specific expression in heart, skeletal muscle and smooth muscle (chicken *β-actin* promoter and CMV enhancer) | -Dilated cardiomyopathy from P1 onwards; reduced septum thickness<br>-Starting at 3–4 months, 25% of animals showed extremely enlarged hearts in the 7–12 month age group, and these mice died earlier than WT | [65] |

**Table 3**
Overview of PP2A genetically modified strains, with manipulation of genes encoding diverse regulatory B subunits.

| Gene<br>Protein | Genetic manipulation and background | Affected tissue (promoter) | Main phenotypes | Ref. |
|---|---|---|---|---|
| *Ppp2r2a*<br>B55α | ENU-induced mutagenesis: creation of new splice acceptor site in *Ppp2r2a* intron 3, resulting in addition of 8 amino acids to sequence encoded by exons 1–3;<br>C3H/HeH background | Total body, constitutive KO, hypomorphic allele | -In heterozygous mice: modest insulin resistance, no diabetes<br>-When crossed with mice heterozygous for a null allele of the insulin receptor (IR): overt diabetes and insulin resistance; normal basal, but decreased insulin-induced AKT signaling | [74] |
| *Ppp2r3*<br>G5PR, B″γ | Exons 1–2: floxed;<br>C57BL/6 background | Conditional KO in pro-B cells and BCR-positive B cells (*CD19*-Cre) | Impaired B-cell development; increased BCR activation-induced apoptosis (*via* JNK and Bim activation); normal BCR activation-induced proliferation and signaling | [76] |
| *Ppp2r3c*<br>G5PR, B″γ | Exons 1–2: floxed;<br>C57BL/6 background | Conditional KO in T-cells (*Lck*-Cre) | Impaired T-cell development; normal proliferation and differentiation potential, but increased susceptibility of DP T-cells to apoptosis (*via* caspase-3, JNK and FasL) | [79] |
| *Ppp2r3c*<br>G5PR, B″γ | Expression of WT *G5pr* cDNA;<br>C57BL/6 background | Transgene; lymphoid cell selective expression (*Lck* proximal promoter, *immunoglobulin H-chain* intronic enhancer) | -No phenotypes in non-immunized young mice; upon immunization, more germinal center B-cells, specifically non-antigen-specific B cells; decreased antibody production<br>-Upon aging, more peritoneal B-1a cells in non-immunized mice due to prevention of BCR activation-induced apoptosis (decreased JNK and caspase-3 activity)<br>-In aged females, persistent B-1a levels result in autoimmunity | [80] |
| *Ppp2r5a*<br>B56α | Gene trap (gt) insertion in *Ppp2r5a* intron 1;<br>C57BL6 background | Total body, constitutive KO | Decreased heart rate, conduction defects and increased sensitivity to parasympathetic stimulation<br>-In isolated B56α$^{+/-}$ myocytes: reduced $Ca^{2+}$ waves and spark frequency upon sympathetic stimulation; reduced RyR2 phosphorylation | [68] |
| *Ppp2r5a*<br>B56α | Gene trap (gt) insertion in *Ppp2r5a* intron 1 (TIGM clone);<br>C57BL/6N background;<br>or, FVB background (for carcinogenesis analyses) | Total body, constitutive KO, hypomorphic gt allele | Spontaneous skin lesions, hyperproliferating epidermis and hair follicles; increased expression of phospho-S62-c-Myc; increased DMBA/TPA-induced skin carcinogenesis; more skin and bone marrow stem cells; increased immune cell infiltration in diverse tissues, suggestive for increased extramedullary hematopoiesis | [90] |
| *Ppp2r5a*<br>B56α | Expression of B56α cDNA;<br>DBA/C3H background | Transgene; cardiac-specific expression (mouse *α-myosin heavy chain* promoter) | Increased basal contractility, but impaired contractile response to β-adrenergic stimulation; increased myofilament $Ca^{2+}$ sensitivity in cardiomyocytes; decreased basal phosphorylation of several myofilament proteins; decreased β-adrenergic-induced phosphorylation of PLB | [70] |
| *Ppp2r5a*<br>B56α | Expression of B56α cDNA;<br>C57BL6 background | AAV9-mediated transduction *via* injection in the myocardium (with IRES mCherry reporter) | Higher resting heart rate, increased peak heart rates in response to exercise or β-adrenergic stimulation, blunted heart response to parasympathetic stimulus; increased RyR2 phosphorylation | [68] |
| *Ppp2r5c*<br>B56γ | Gene trap (gt) insertion in *Ppp2r5c* intron 2 (SIGTR clone XP0444);<br>C57BL/6 background | Total body, constitutive KO | -Increased neonatal death (P1–P2); born mice are less active and smaller; surviving mice become obese with more white adipose tissue; muscle strength and coordination defects<br>-Heart phenotypes at E16: no ventricular septum in 50% of cases; less ventricular tissue due to increased apoptosis | [67] |
| *Ppp2r5c*<br>B56γ | Tail vein injection of AAV-miRNA, targeting all *Ppp2r5c* isoforms; miRNA expression under control of a hepatocyte-specific promoter;<br>C57BL/6J background | Hepatocyte-specific knockdown | -Normal body weight and liver function; increased insulin sensitivity and improved glucose tolerance: increased hepatic glucose uptake; normal gluconeogenesis; increased triglyceride levels and *de novo* lipogenesis in liver; increased AMP kinase activity and altered activities of HIF1α and SREBP-1<br>-In $^{db/db}$mice: B56γ KD improves their hyperglycemia, but worsened their dyslipidemia | [73] |
| *Ppp2r5c*<br>B56γ | Expression of B56γ cDNA;<br>C57BL/6 background | Transgene; lung-specific expression (*Surfactant protein C* promoter) | Neonatal death; at E18: smaller lungs with no peripheral airway formation and no distal differentiation; no β-catenin expression | [37] |
| *Ppp2r5d*<br>B56δ | Gene trap (gt) insertion in *Ppp2r5d* intron 2 (Baygenomics clone RRK451);<br>Mixed 129/OlaHsd and C57BL/6J background (F4) | Total body, constitutive KO, hypomorphic allele | -Homozygous $^{gt/gt}$mice: lethal<br>-Heterozygous $^{+/gt}$mice: increased acoustic startle response due to decreased prepulse inhibition; normal locomotor activity; increased GSK-3β phosphorylation | [57] |

(*continued on next page*)

**Table 3** (*continued*)

| Gene<br>Protein | Genetic manipulation and background | Affected tissue (promoter) | Main phenotypes | Ref. |
|---|---|---|---|---|
| *Ppp2r5d*<br>B56δ | 146 bp of exon 3, exons 4–14 and 45 bp of exon 15 replaced by Neo-cassette;<br>mixed 129/OlaHsd - C57BL/6 genetic background | Total body, constitutive KO | -Homozygous $^{-/-}$mice: viable<br>-Tauopathy in the hindbrain; no tangles or neurodegeneration; increased GSK-3β and decreased CDK5 activity (loss of p35); tau-related motor coordination, but tau-unrelated sensorimotoric defects; normal learning and memory<br>-Spontaneous development of hematologic malignancies and HCC; in HCCs: increased oncogenicity of c-Myc; tumor predisposing mechanism may involve increased GSK-3β phosphorylation | [54,110] |
| *Ppp2r6a*<br>STRN | $Strn^{tm1a}$ $(KOMP)^{Wtsi}$ allele;<br>C57BL/6N background | Total body, constitutive KO | -Homozygous $^{-/-}$mice: lethal<br>-In $^{+/-}$mice: increased salt sensitivity of blood pressure; on liberal salt intake: increased renal expression of mineralocorticoid receptor and MR target genes; reduced phospho-AKT/total AKT level; increased aortic contraction and reduced aortic relaxation responses | [97,98] |

follicle growth or survival, or ovulation. Again, a meiotic maturation defect was observed, with in this case, a normal meiosis-I, but abnormal elongated spindle formation during meiosis-II, precocious sister chromatid separation due to loss of centromeric cohesion, and increased germinal vesicle breakdown. The latter correlated with increased Cyclin B/CDK1 activity, as witnessed by increased Cyclin B1 expression and increased CDK1 phosphorylation (Thr161). Eggs showed increased risk of aneuploidy, but could still be fertilized. Fertilization was however followed by impaired embryonic development. At E0.5, efficient pronuclear formation was defective, and at E4.0, the blastocyst stage was rarely reached and signs of apoptosis were seen. Together with the data from conditional C subunit KO mice, these data might infer redundant roles for Aα and Aβ in meiosis-I, while Aα would have a non-redundant role in meiosis-II of oocyte formation.

Interestingly, a spermatogenesis defect was also one of only two phenotypes that could be found in a constitutive KO mouse model for CIP2A (Cancerous Inhibitor of PP2A) [30]. CIP2A is an oncoprotein, presumably specifically targeting PP2A-B56 complexes [31] and inhibiting PP2A-mediated dephosphorylation of a very limited set of substrates, including *e.g.* c-Myc [32]. The gene-trapped *Kiaa1524* allele appeared hypomorphic, with CIP2A mRNA expression decreased for more than 90%, but not entirely eliminated, in all tissues examined. Testes were characterized by a smaller and lighter epididymis. Sperm counts were reduced, with a smaller number of PLZF-positive spermatogonial progenitor cells (SPCs). In seminiferous tubuli cells, reduced expression was found of self-renewal markers *Plzf*, *Oct4* and *Nanog*, indicative for reduced proliferation of SPCs [30].

## 3. PP2A in embryonic development

In general, Cα (*Ppp2ca*) is expressed to a 10-fold higher extent than Cβ (*Ppp2cb*), mainly due to the more active promotor region of *Ppp2ca* in most tissues [33] (Fig. 2). Constitutive, homozygous KO of *Ppp2ca* in mice resulted in embryonic lethality, with premature death and degeneration starting at embryonic day E6.5. At this stage, gastrulation could not be initiated, resulting in absence of differentiated mesoderm and accompanying Wnt target gene markers, Goosecoid and Brachyury in $C\alpha^{-/-}$ embryos [9]. In accordance with a potential defect in Wnt signaling and cell-cell adhesion, decreased expression and increased cytoplasmic localization of E-cadherin and β-catenin were observed [34]. The authors suggest a role for Cα in the stabilization of the E-cadherin/β-catenin complex at the cell membrane. In contrast, Cβ does not display this strict β-catenin co-localization, possibly explaining its inability to compensate for this Cα function [34]. Very similar observations were made in a conditional *Ppp2ca* KO mouse model, in which again smaller, degenerated embryos were observed at E6.5, with no mesoderm formation and no Brachyury expression [35]. On the other hand, *Ppp2cb* conditional KO mice showed no obvious embryonic or adult phenotype, behaved normally and were fertile [35]. In light of the higher abundance of Cα, the total amount of PP2A catalytic subunit was unaltered in $C\beta^{-/-}$ heart tissue as compared to WT mice, implying that Cα could in this case potentially compensate for loss of Cβ [35].

In line with the observations from Cα KO mice, constitutive KO of *Ppp2r1a* in two different models resulted in embryonic lethality between E5 and E10.5 [10,36]. This embryonic phenotype was further studied in detail in mice harboring the $t^{w18}$ allele, characterized by a 4.3 Mb deletion on chromosome 17, encompassing 74 genes including *Ppp2r1a* [36]. In line with the disorganized Wnt signaling in Cα KO mice, impaired Wnt signaling during development in this embryonically lethal $t^{w18}$ deletion model could indeed be entirely attributed to the loss of *Ppp2r1a*, as re-expression of the full *Ppp2r1a* gene, including sequences 3 kb upstream and 20 kb downstream of the transcription initiation site, resulted in a complete rescue of the observed embryonic lethality at E10.5 and E18.5. At E7.5, $t^{w18}/t^{w18}$ mice had functional endoderm, while neuroectoderm patterning was compromised and mesoderm was lacking. Further underscoring gastrulation

**Table 4**
Overview of genetically modified strains, manipulating genes encoding endogenous PP2A regulators or inhibitors.

| Gene<br>Protein | Genetic manipulation and background | Affected tissue (promoter) | Main phenotypes | Ref. |
|---|---|---|---|---|
| *Ppp2r4*<br>PTPA | Confirmed single gene trap (gt) insertion in *Ppp2r4* intron 1 (Baygenomics clone XH627);<br>Mixed 29P2/OlaHsd and C57BL/6N background | Total body, constitutive KO, hypomorphic gt allele | -Embryonic lethality suggested; decreased overall survival in $^{gt/+}$mice<br>-Decreased activity of a selective set of PP2A trimers (B56-type, not B55)<br>-Spontaneous cancer development in $^{gt/+}$ and $^{gt/gt}$mice > 12 months: mainly hematologic malignancies, less: HCC.<br>-Accelerated DMBA/TPA-induced skin carcinogenesis in $^{gt/+}$mice<br>-Sporadic activation of Wnt, c-Myc and Hedgehog signaling in $^{gt/gt}$tumors | [43] |
| *Lcmt1*<br>LCMT-1 | Gene trap (gt) insertion in *Lcmt1* intron 1 (German Gene Trap consortium clone);<br>C57BL/6 background | Total body, constitutive KO | -Embryonic lethality between E14.5 and E16.5 (5% die before E12.5)<br>-At E12.5: reduced embryo and liver weight; fetal liver hematopoiesis defect (all lineages affected); misshapen eyes; indentation of the back of the head<br>-In $^{-/-}$E14.5 MEFs: decreased methylation of PP2A, PP4 and PP6; selective defects in PP2A-B55 and PP4-PPP4R1 complex formation | [26,39,41] |
| *Lcmt1*<br>LCMT-1 | Confirmed single gene trap (gt) insertion in *Lcmt1* intron 1 (Baygenomics clone CSC099);<br>C57BL/6 background | Total body, constitutive KO, but hypomorphic gt allele | -Embryonic lethality suggested<br>-In surviving $^{gt/gt}$mice: decreased glucose tolerance and increased glucose-stimulated insulin secretion, indicative for insulin resistance | [42] |
| *Lcmt1*<br>LCMT-1 | Expression of *tetO*-driven 3xFLAG-tagged LCMT-1;<br>C57BL/6J × 129SVEV/Tac F1 background | Transgene; inducible expression in neurons, using *CaMKIIα*-tTA (FLAG expression throughout the forebrain) | -FLAG-LCMT-1 overexpression: 150%<br>-No effects on tau phosphorylation; decreased APP phosphorylation<br>-Protection against Aβ-induced cognitive impairments | [55] |
| *Ppme1*<br>PME-1 | 1.8 kb of *Ppme1* gene encompassing exon 7 (encoding amino acids 134–185, including catalytic S156 residue) was replaced by Neo-cassette;<br>129SvJ-C57BL/6 background | Total body, constitutive KO | Normal embryonic development, but perinatal death at P1; neonatal mice showed breathing and suckling difficulties | [56] |
| *Ppme1*<br>PME-1 | Expression of *tetO*-driven 3× FLAG-tagged PME-1;<br>C57BL/6J × 129SVEV/Tac F1 background | Transgene; inducible expression in neurons, using *CaMKIIα*-tTA (FLAG expression throughout the forebrain) | -FLAG-PME-1 overexpression: 350%<br>-Increased tau and APP phosphorylation<br>-Higher sensitivity to Aβ-induced cognitive impairments | [55] |
| *Igbp1*<br>α4 | Exons 1–2: floxed<br>(*Igbp1*: X-linked gene; all experiments done with male mice);<br>Background: N/A | Conditional KO in B-cells (*CD19*-Cre) | -In spleen, B-cell maturation defect<br>-In bone marrow, less pre-B cells<br>-In thymus, normal T-cell number<br>-Decreased proliferation of activated B-cells; reduced p70 S6K activation<br>-Reduced response to immunization, particularly with T-cell dependent antigens, suggesting impaired B-cell differentiation | [87] |
| *Igbp1*<br>α4 | Exons 1–2: floxed<br>(*Igbp1*: X-linked gene; all experiments done with male mice);<br>Background: N/A | Conditional KO in T-cells (*Lck*-Cre) | -Impaired early T-cell development<br>-Decreased proliferation capacity, but no increased apoptosis of thymocytes<br>-impaired CD3 signaling in thymocytes | [88] |
| *Igbp1*<br>α4 | Exons 3–5: floxed<br>(*Igbp1*: X-linked gene) | Conditional KO in developing T cells (*Lck*-Cre); or in MEFs (retroviral expression of Cre); or in differentiated adipocytes (=PPARγ-induced MEFs) (adenoviral expression of Cre); or in liver (adenoviral Cre, injected in tail vein); or in MEFs stably transfected with Bcl-xL (retroviral expression of MIGR1-GFP-Cre) | -Impaired T-cell development<br>-In $^{-/-}$MEFs: apoptosis (c-jun, p53 and caspase-3 activation, PARP cleavage); (partially) rescued by expression of papilloma E6 protein, or Bcl-xL<br>-In $^{-/-}$MEFs: increased basal phosphorylation of several established PP2A substrates; decreased reversal of stress-induced phosphorylations<br>-In $^{-/-}$adipocytes: apoptosis (c-jun, p53, caspase-3 activation)<br>-In liver: all mice died 6 days after adeno-Cre injection; livers showed signs of apoptosis and p53 and c-Jun activation<br>-In Bcl-$x_L$-expressing MEFs: slower cell spreading; decreased cell adhesion; reduced cell migration; reduced Rac1 activation; phenotypes rescued by active Rac1 V12 mutant | [25,44,45] |
| *Igbp1*<br>α4 | Expression of FLAG-tagged α4;<br>Background: N/A | Transgene, T-cell specific expression (*CD2* promoter/enhancer) | No effects on T-cell viability, proliferation or development; enhanced T-cell migration in transwell assay; increased levels of activated Rac1 | [45] |

(*continued on next page*)

**Table 4** (*continued*)

| Gene<br>Protein | Genetic manipulation and background | Affected tissue (promoter) | Main phenotypes | Ref. |
|---|---|---|---|---|
| *Igbp1*<br>α4 | Exons 1–2: floxed;<br>C57BL/6 background | Conditional KO in neurons (*α4-b*-Cre) | Normal brain development; no sensorimotor or motivational deficiencies; impaired learning and memory; increased activity of CaMKIIα in hippocampus | [48] |
| *Igbp1*<br>α4 | Exons 1–2: floxed;<br>Background: N/A | Conditional KO in the intestinal epithelium (IE) (*Villin*-Cre) | Shorter and thinner gastrointestinal tract; abnormal architecture of the small intestinal mucosal epithelium at the levels of crypts, villi and Paneth cells; impaired epithelial barrier function, causally linked to increased IKKα-mediated phosphorylation and degradation of the RNA binding protein HuR | [99] |
| *Kiaa1524*<br>CIP2A | Gene trap (gt) insertion in *Kiaa1514* intron 1 (clone CD0252 from IGTC);<br>C57BL6/J background | Total body, constitutive KO, but hypomorphic gt allele | -No histological abnormalities or phenotypes in 42 different tissues examined (German Mouse Clinic)<br>-In testis: reduced proliferation of SPCs and impaired spermatogenesis<br>-Impaired adaptive immune response, due to a T-cell proliferation defect | [30,83] |
| *Arpp19*<br>ARPP-16/19 | Not specified | Total body, constitutive KO | Embryonic lethal (unpublished) | [60] |
| *Arpp19*<br>ARPP-16/19 | Gene: floxed (no further details given);<br>Mixed C57BL/6/129 background | Conditional KO in neurons (*CaMKIIα*-Cre) | Increased motivation responses; normal baseline locomotor activity; decreased locomotor response to acute cocaine exposure; decreased basal phosphorylation of DARPP-32 and AKT; loss of forskolin-induced increase in ERK1 phosphorylation in striatum | [60] |
| *ANP32a*<br>LANP, $I1_{PP2A}$ | Exons 2–3: floxed;<br>C57BL/6J background | Constitutive, total body KO (targeted ES cells electroporated with Cre, before chimera generation) | Homozygous $^{-/-}$mice are viable and fertile and lack any discernable phenotype in any of the major organ systems, including brain | [112] |
| *ANP32e*<br>Cpd-1, $I1_{PP2A}$ | Gene trap insertion in intron 3 of the *Anp32e* gene (Baygenomics clone RRK001);<br>Mixed 129P2/OlaHsd × C57BL/6 background | Constitutive, total body KO | Homozygous $^{-/-}$mice are viable and fertile and lack any discernable phenotypes, except for a subtle neurological clasping phenotype | [113] |
| *ANP32e*<br>Cpd-1, $I1_{PP2A}$ | Exons 2–5 replaced by *pgk*-neo cassette;<br>C57BL/6 background | Constitutive, total body KO | Homozygous $^{-/-}$mice are viable and fertile and lack any discernable phenotypes, including in brain, in MEFs and in thymocytes | [114,115] |
| *ANP32a/e*<br>LANP, Cpd-1 | Double *Anp32a* × *Anp32e* KO mice (*cfr.* above) | Constitutive, total body, double KO | No discernable phenotypes | [113,114] |

defects, there was no notochord formation, overgrowth of the primitive streak and bulging of the cells into the amniotic cavity at E8-E9 due to lack of ability to undergo epithelial to mesenchymal transition (EMT). By E10, all embryos had died. Upon analysis at E6.5, the resulting disorganized embryos showed reduced expression of several Wnt and Nodal genes. Upregulation of genes involved in cell adhesion was accompanied by a persistent E-cadherin expression, preventing the switch to N-cadherin expression necessary for EMT and mesoderm differentiation [36]. Apart from its role in embryogenesis, Aα remains important perinatally and in adult mice, since tamoxifen-inducible loss of Aα in a Δ5–6/Δ5–6 KO mouse model, lacking *Ppp2r1a* exons 5 and 6, resulted in immediate weight loss and subsequent death, six days after induction. The mice also displayed hunched backs and walking difficulties [10].

Further support for a role of PP2A in Wnt signaling regulation during embryonic development came from transgenic mice with specific overexpression of the WT B56γ subunit in the epithelium of the developing lung [37]. The mice died neonatally. In E18 embryos, smaller lungs were found, lacking distal lung differentiation and peripheral airway formation, while proximal airway and blood vessel development were normal. The underdeveloped lungs were completely devoid of β-catenin expression, suggesting an important restricting role of PP2A-B56γ complexes in Wnt signaling in lung tissue. This view is in line with the drop in high B56γ levels, observed in this tissue at rat embryonic stage E20 under physiological circumstances [37].

Another function of PP2A during embryonic development was revealed in a conditional KO model of Cα in early hematopoietic and endothelial cells [38]. Homozygously targeted embryos looked pale and died at E12.5 due to defects in hematopoiesis, despite an incomplete KO and 35% remaining PP2A activity in fetal livers. At E12.5 and E14.5, total cellularity of fetal liver was dramatically reduced, with a pronounced 90% reduction in erythrocytes, while other hematopoietic lineages appeared in normal numbers. Specifically, primitive erythropoiesis was normal, but definitive erythropoiesis was impaired, as witnessed by an increased number of pro-erythroblasts, a decreased number of erythroid colony- and blast-forming units and reduced expression of $\beta^{maj/min}$*-globin*. In addition, increased apoptosis of committed erythroid cells was observed, correlating with decreased STAT5 phosphorylation (Tyr694) and decreased Bcl-$X_L$ expression in basal and EPO-stimulated cells [38]. Interestingly, a rather similar phenotype was observed in *Lcmt1* KO mice, lacking any detectable expression of the PP2A methyltransferase LCMT-1, which is predicted to majorly affect PP2A holoenzyme formation with B55 subunits [26,39–41]. At E12.5, > 95% decreased PP2A-C methylation was seen, accompanied by 30% reduction in total A and C subunit levels and a 92% reduction in PP2A-B55α/δ formation (due to 80% reduction in B55α/δ expression, and 40% reduction in C binding to B55). The large majority of LCMT-1 KO embryos died between E14.5 and E16.5; 5% died before E12.5 [41]. This is more or less in line with findings from another gene-trapped *Lcmt1* KO model, where many resorbing embryos were observed, already at E9.5 [42]. At E12.5, embryos showed a reduced weight, mainly attributable to a 50% reduction in liver weight. Again, a fetal liver hematopoiesis defect became apparent, with increased apoptosis of liver hematopoietic cells, and a dramatic reduction in the erythroid, and less pronounced, the myeloid lineages of fetal liver cells. Isolated liver cells retained normal proliferation and mitotic indices, but showed reduced colony-forming capacity, indicative for reduced presence of hematopoietic stem and progenitor cells. The latter was further confirmed in independent transplantation experiments [41]. Thus, the similarities between fetal liver phenotypes in both the conditional Cα KO and constitutive LCMT-1 KO mouse models [38,41], seem to suggest that PP2A-C is likely the main *in vivo* substrate of LCMT-1, and that the hematopoiesis defects seen in both models are most probably attributable to a defect in PP2A-B55α/δ functionality. In addition, as in the *Lcmt1* KO mice, no gastrulation defects were observed, it can be postulated that PP2A-B55α/δ trimers either do not play a role in mesoderm formation, or play a redundant role in this process.

The importance of PP2A in embryonic development was further demonstrated in several KO models affecting the function of other PP2A biogenesis regulators. For instance, homozygously targeted *Ppp2r4* KO mice (hypomorphic allele), with severely affected expression of the PP2A activator PTPA (Phosphatase Two A Phosphatase Activator) (on average only 20% of WT levels were left in most tissues), were born at less than Mendelian ratios, suggestive for embryonic lethality [43]. In the surviving PTPA KO mice, overall PP2A-C methylation and activity were significantly reduced (50%). Interestingly, however, a selective decrease in PP2A-B56γ/ε activity was found, while PP2A-B55 activity was unaffected. This infers a role for PP2A-B56γ/ε trimers in embryonic development. Likewise, knockout of *Igbp1*, encoding the PP2A biogenesis regulator alpha4 (α4), resulted in embryonic lethality [44]. Alpha4$^{-/-}$ MEFs showed increased rates of apoptosis and a 70% reduced activity of immunoprecipitated PP2A-C, decreased levels of A and C subunits, and decreased levels of AC dimers. These PP2A defects were accompanied by an overall increase in basal phosphorylation of several known PP2A substrates (*e.g.* transcription (co-)factors p53, RelA and FoxO1; kinases ATM, S6K and AMPK; and Histone H2AX) and a decreased ability of the cells to reverse stress-induced phosphorylations [25]. These *in vitro* biochemical data were further underscored in isolated adipocytes with induced α4 KO [44], as well as *in vivo*, upon conditional KO of α4 in liver, where, again, decreased levels of PP2A A and C subunits were observed, decreased PP2A-C methylation, increased phosphorylation of H2AX and induced stress response signals [25]. Moreover, all targeted mice died 6 days after induction of hepatic α4 KO, with livers showing signs of severe apoptosis and increased phosphorylation of p53 and c-jun transcription factors [44]. Notably, apoptosis of α4 KO MEFS could be rescued by overexpression of Bcl-$X_L$ [44], thereby revealing a cell migration defect, characterized by slower cell spreading, a decreased number of mature focal adhesions and reduced activity of the small GTPase Rac1 [45]. These cytoskeletal defects could be rescued by overexpression of a constitutively active Rac1 V12 mutant. Overall, these data further underscore the major role of α4 in preventing apoptosis and maintaining proper PP2A function and activity *in vivo*.

Another protein that has been shown to post-translationally modify PP2A-C, is nitric oxide synthase interacting protein (NOSIP), a ubiquitin E3 ligase [46]. Loss of NOSIP caused perinatal lethality and holoprosencephaly, a severe impairment in craniofacial development that is often associated with defective Hedgehog signaling. NOSIP interacts with the C, Aα and B55α subunits, and *in vivo* loss of NOSIP lead to an increase in PP2A activity, specifically in palatal and facial tissue. In NOSIP$^{-/-}$ MEFs, this was associated with loss of PP2A monoubiquitination [46]. The data from this model might therefore suggest that gain-of-function of PP2A could also result in severe effects on embryonic development.

## 4. PP2A functions in brain and neuropathologies

Conditional loss of *Ppp2ca* in the nervous system resulted in severe microcephaly and cortical atrophy at P7, characterized by loss of cortical neurons and decreased thickness of cortical layers II-V [47]. No remaining Cα protein expression was found in brain extracts of these mice. The animals performed poorly in a Morris water maze test, indicating learning and memory defects. Additional research in neuronal progenitor cells (NPCs) identified an upregulation of Hippo signaling, witnessed by an increased phosphorylation of MST1/2 (Ser180/183), LATS1 (Ser909) and YAP (Ser127), and inhibited nuclear translocation of YAP. Furthermore, p53-like protein p73 phosphorylation (Tyr99) was enhanced, which could additionally contribute to the observed increase in apoptotic signaling and diminished proliferation of the Cα$^{-/-}$ NPCs [47]. In contrast, conditional KO of α4 in neurons did not result in any gross abnormalities in brain development, despite clearly decreased overall PP2A activity in hippocampal extracts [48]. This

seems remarkable given that KO of α4 in several other cell and tissue contexts consistently resulted in major apoptosis [25,44]. Nevertheless, the brain-specific α4 KO mice did show impaired learning and memory (measured through spatial learning and shuttle box avoidance tests), but no sensorimotor or motivational deficiencies. The presumed dysfunction of the hippocampus correlated with increased activity of CaMKIIα [48].

A large number of brain-specific PP2A strains were specifically generated to address the presumed role of PP2A in dephosphorylation of the microtubule-associated protein tau. Aggregation of hyperphosphorylated tau is one of the pathological hallmarks of Alzheimer's disease (AD), a neurodegenerative disorder also characterized by the deposition of amyloid β (Aβ) plaques. Despite intensive research, the pathology of AD remains largely elusive. *In vitro* research identified PP2A-B55α/γ as important tau phosphatases [1], which was sustained by several *in vivo* PP2A transgenic models. *In vivo* neuron-specific expression of a Cα mutant, harboring the L309A substitution in the C-terminal tail that does not affect PP2A-C methylation but specifically prevents B55 subunit binding [40], resulted in increased tau phosphorylation (Ser202/Thr205) and impaired dephosphorylation of vimentin, but no neurodegeneration [49]. Interestingly, crossbreeding of these mice with P301L mutant tau transgenic pR5 mice intensified the tau pathology, further increasing tau Ser422 phosphorylation and neurofibrillary tangle (NFT) formation in hippocampus and amygdala [50]. Neuron-specific expression of the dominant negative L199P Cα mutant also induced tau hyperphosphorylation at the physiological AT8 (Ser202/205) and pathological $pS^{422}$ (Ser422) epitopes, accompanied by the formation of ubiquitin-containing tau aggregates in the somatodendritic neuronal compartments [51]. This Cα mutant is catalytically inactive, while showing normal A subunit binding, and thus presumably interferes with PP2A function through titration of B subunits [52]. Although an effect of overall reduced PP2A activity (34%) on direct tau dephosphorylation could not be excluded, a detailed analysis of the Purkinje cells also revealed increased phosphorylation of MEK/ERK/Elk and JNK/c-jun [53], implying that PP2A might also indirectly affect tau phosphorylation through regulation of the activities of tau kinases, ERK and JNK. This general idea was further sustained in a mouse model with constitutive loss of B56δ (*Ppp2r5d*), a PP2A regulatory subunit that is highly expressed throughout the brain [21] (Fig. 2). In this model, tauopathy was observed in the brainstem and the dorsal horn of the spinal cord, characterized by an altered tau conformation (MC-1 positive) and progressively increased tau phosphorylation (AT8, AT180), without tau filament or NFT formation and with no neurodegeneration [54]. The B56δ KO mice showed normal learning and memory, but had motor coordination defects that were more than additively affected in B56δ KO mice in which tau was additionally overexpressed. On the other hand, the mice exhibited a delayed latency on a hot plate that was unaffected by tau overexpression. Mechanistically, a nearly complete absence of the CDK5 activator p35 and decreased CDK5 activity were found in brainstem lysates, correlating with decreased GSK-3β inactivating Ser9 phosphorylation. Thus, the data from this mouse model were not only suggestive for indirect regulation of tau kinases CDK5 and GSK-3β by PP2A-B56δ, but also implied an additional role for PP2A-B56δ in regulation of sensorimotoric functions, independently of the observed tauopathy. Moreover, as the spatial distribution of the tauopathy did not correlate with the spatial expression pattern of B56δ or tau in WT brain, functional redundancies of PP2A-B56δ with other PP2A trimers or even other Ser/Thr phosphatases were suspected in the brain areas of the KO mice that were devoid of the tauopathy [54].

Genetic manipulation of PP2A-C methylation also affected tau pathology in mice. Decreased PP2A-C methylation, as observed in an overexpression model of the PP2A methylesterase PME-1 in the entire forebrain, resulted in increased phosphorylation of tau (Ser396/Ser404, Ser202/Thr205, Ser262) and of amyloid precursor protein (APP) at Thr668 in the hippocampus [55]. This was accompanied by an added impairment in several cognitive/behavioral tests and long-term potentiation (LTP) in the presence of pathological Aβ concentrations. No impact on motor performance, sensory perception, motivation or LTP was seen in the absence of Aβ, or in the presence of very low Aβ concentrations. Conversely, LCMT-1 overexpression in the forebrain protected against these Aβ-induced impairments, despite any detectable changes in PP2A-C methylation [55]. In these transgenic mice, no effects on tau phosphorylation were observed, while APP phosphorylation (Thr668) was decreased. Unfortunately, the effects of losses of LCMT-1 or PME-1 on tau or APP phosphorylation could not be addressed, given that constitutive KO of *Lcmt1* was embryonically lethal, and constitutive KO of *Ppme1* resulted in perinatal lethality, with all mice dying at P1 due to lack of ability to start normal breathing or suckling [39,56]. As PP2A-C methylation has the strongest impact on PP2A-B55 holoenzyme assembly [13], the PME-1 and LCMT-1 transgenic models provide nevertheless further evidence for a role of these PP2A trimers in tau dephosphorylation, and additionally, in the regulation of APP phosphorylation.

Another link between PP2A-B56δ and regulation of GSK-3β activity in brain was provided in mice, heterozygous for a hypomorphic *Ppp2r5d* allele, and showing a ~50% reduction of B56δ protein levels in total brain lysates [57]. These animals showed a weakened prepulse inhibition (PPI), an event in which, under normal circumstances, a startle reactivity is attenuated when a low-intensity stimulus is presented shortly before a high-intensity acoustic stimulus [58,59]. The increased acoustic startle response in the B56δ KO mice correlated with GSK-3β hyperphosphorylation (Ser9), and decreased phosphorylation of the presumed GSK-3β substrate, KCNQ2, an M-type potassium channel protein [57].

Finally, loss of inhibition of PP2A-B55α and PP2A-B56δ (and potentially other PP2A trimers) in a neuron-specific KO mouse of the PP2A inhibitor ARPP-16 significantly altered dopamine signaling in the striatum [60]. These mice showed increased motivation to respond to a food reinforcer, and a decreased locomotor response to acute cocaine exposure, while exhibiting normal baseline locomotor activity. The former phenotypes were associated with a decreased basal phosphorylation of DARPP-32 (Thr57) and AKT kinase (Thr308) in striatal slices, and a loss of forskolin-induced increase in phosphorylation of ERK1 (Thr202). As in both basal and PKA-stimulated conditions, no phosphorylation changes could be detected in established PP1 and PP2B substrates in these slices, these data provide *in vivo* evidence for the specificity of ARPP-16 as a PP2A inhibitory protein in the striatum [60]. Notably, the constitutive, total body KO of ARPP16/19 was embryonically lethal, suggestive for a major role of these PP2A inhibitors during embryogenesis as well [60].

## 5. PP2A in cardiac function

In view of PP2A's relatively high abundance in heart tissue and the muscle-specific expression of a number of PP2A regulatory subunits [22] (Fig. 2), it is not surprising that the role of PP2A in cardiac function has been well investigated. To prevent cardiac dysfunction, PP2A activity apparently needs to be strictly regulated, since both cardiomyocyte-specific overexpression as well as tamoxifen-induced, cardiomyocyte-specific deletion of *Ppp2ca* at P6.5 resulted in mice with cardiac hypertrophy and increased expression of hypertrophic markers ANP and BNP, ventricular fibrosis, and impaired heart contractility [61–63]. In the KO, an 80% reduction in Cα expression was observed at P11.5 [62], while in the transgene, total PP2A activity was 1.7-fold increased [61]. Likewise, when KO was induced in a cardiomyocyte-specific way in 3-month-old adult mice, cardiac hypertrophy, fibrosis and impaired cardiac pump function were observed, again associated with increased expression of ANP, BNP, α- and β-MHC [64]. Although in these mice Cα levels were decreased by only 50% and total PP2A activity by only 25%, a small but significant compensatory upregulation of B55α and B56ε mRNA expression was observed, while expression of

all other B subunits was unaffected. At the signaling level, a significant disruption of the AKT/GSK-3β/β-catenin pathway was seen in this model, characterized by decreased overall β-catenin levels and enhanced β-catenin phosphorylation (Ser552), and decreased phosphorylation of AKT (Thr308 and Ser473) and GSK-3β (Ser9) [64]. Conversely, increased AKT and GSK-3β phosphorylation, and increased β-catenin degradation were seen in the Cα transgenic mice [63]. In addition, the Cα KO mice showed increased phosphorylation of phospholamban (PLB) (Ser16) in the heart [62], while in the Cα transgenes, PLB phosphorylation (Ser16, Ser17) was found decreased [61]. These findings were further corroborated upon experimental induction of chronic myocardial infarction in 7-to-11-month-old Cα transgenic mice [63]. Upon analysis of the hearts one month later, an increased left ventricle infarct size was observed, suggestive for impaired *post*-myocardial infarction remodeling capacity. This was however not associated with a decreased survival, and unexpectedly, even resulted in improved survival in the subacute phase after infarction - potentially, because upon myocardial infarction, restoration of normal AKT and GSK-3β phosphorylation, and β-catenin levels could be noted [63].

Muscle-specific expression of a dominant negative Aα subunit lacking HEAT-repeat 5 (AΔ5), which mediates important interactions with all B-type subunits, also resulted in dilated cardiomyopathy from P1 onwards. This phenotype was characterized by an enlarged heart, impaired left ventricle function, reduced septum thickness, reduced thickness of the left ventricular posterior wall, and increased expression of β-MHC transcripts [65]. At 3–4 months, 25% of these mice died within a few weeks after showing extremely enlarged hearts and increased respiratory rates [65]. The AΔ5 mutant is incapable of binding any B subunit class [66] and would therefore behave as a dominant-negative mutant towards several different PP2A trimers. It is therefore interesting to note that some of the heart phenotypes seen in AΔ5 transgenic mice, were also found in mice with a total-body, constitutive KO of *Ppp2r5c*, encoding the B56γ subunits. These mice showed increased neonatal death (P1–P2), were less active and smaller in mass than their WT littermates. In their hearts, significantly thinner ventricular walls and less ventricular tissue were seen, with total lack of a ventricular septum observed in half of the B56γ KO fetuses at E16.5. Surviving adult mice also showed significantly thinner septums. This phenotype was attributed to increased cardiomyocytic apoptosis [67].

In contrast, the complete, constitutive KO of B56α (*Ppp2r5a*), remarkably, increased cardiac PP2A activity (measured on pNPP as a substrate and using the selective pharmacological PP2A inhibitor fostriecin as a specificity control), both in heterozygous and homozygous mice, leading to a completely distinct phenotype [68]. These mice suffered from a reduced intrinsic heart rate, conduction defects, and increased sensitivity to parasympathetic stimulation, as evident from their relative resistance to catecholamine and exercise-induced arrhythmias. In isolated B56α$^{-/-}$ cardiomyocytes, reduced $Ca^{2+}$ waves and spark frequency in the presence of sympathetic stimulation (β-adrenergic stimulus) were observed. Further in-depth analysis revealed a diminished phosphorylation of the ryanodine receptor ($RyR_2$) at Ser2808 and Ser2814, correlating with reduced $Ca^{2+}$ sensitivity of $RyR_2$ [68]. Conversely, in a model with increased cardiac B56α expression through injection of an adenoviral B56α expression vector in the myocardium, a higher resting heart rate was found, alongside an increased peak heart rate in response to exercise or β-adrenergic stimulation and a blunted response to parasympathetic stimulation. This was associated with an increase in $RyR_2$ phosphorylation (Ser2808, Ser2814) [68]. Remarkably, although increased B56α expression was confirmed, in the presence of unaltered A and C subunit expression levels, this correlated with a 2-fold decreased fostriecin-inhibitable pNPP phosphatase activity in heart, prompting the authors to suggest that B56α would actually act as an inhibitor of PP2A activity in the heart. $RyR_2$ Ser2808 hypophosphorylation was also seen in the *Ppp2ca* cardiac overexpression model, co-occurring with reductions of CaMKIIα and sarcoplasmic reticulum $Ca^{2+}$-ATPase (SERCA) protein expression [63]. While $RyR_2$ augments $Ca^{2+}$ flux from the SR into the cytosol during contraction, SERCA is important for restoring resting [$Ca^{2+}$] [69]. In addition, the observed decline in PLB phosphorylation, possibly subsequent to reduced CaMKIIα expression or increased PP2A activity, could reinforce its inhibition of SERCA, further exacerbating the phenotype [63].

Finally, in a cardiac-specific transgenic B56α overexpression model, increased basal contractility and myofilament $Ca^{2+}$ sensitivity were observed, while an attenuated contractile response was seen upon β-adrenergic stimulation [70]. The latter correlated with a localization shift of B56α from the myofilaments to the cytosol. Further, a decreased basal phosphorylation was seen for several myofilament proteins, including cardiac troponin inhibitor (Ser23/24), myosin-binding protein C (Ser282), myosin light chain-2 (Ser18) and troponin T (Ser208), as well as a decreased isoproterenol-induced phosphorylation of PLB (Ser16). In this model, the observed 2-fold overexpression of B56α correlated with a 2-fold higher expression level of the PP2A C subunit and a 2-fold higher, okadaic acid-inhibitable, phosphorylase-*a* activity in the cytoplasmic and myofilament fraction.

Taken together, although *in vivo* PP2A dysregulation results in a variety of cardiac phenotypes, a common impairment of the $Ca^{2+}$ response pathway might serve as the underlying cause of the heart failure phenotypes observed, and the B56α subunit is likely a major modulator of cardiac contractility and function. In addition, PP2A-B56γ complexes would rather be involved in proper heart development.

## 6. PP2A in carbohydrate and lipid metabolism

In the early (at P6.5) conditional KO model of Cα in heart, evidence was obtained for decreased glucose transport and glycolysis, increased aerobic glucose metabolism, and increased fatty acid transport and metabolism [62], suggesting a role for PP2A in the regulation of carbohydrate and lipid metabolism in cardiomyocytes. Metabolic homeostasis is crucial for normal functioning, and dysregulation can result in several pathologies such as diabetes type 2, obesity and atherosclerosis. Besides (cardiac) muscle and pancreas, a main organ player in this process is the liver. Liver-specific deletion of *Ppp2ca* resulted in improved glucose tolerance and enhanced insulin sensitivity and signaling in liver, but not in muscle [71]. The mice showed normal body weights, normal liver-body weight ratios and normal liver histology. Increased insulin signaling was exemplified by increased phosphorylation of AKT (Ser473, Thr308), GSK-3α/β (Ser21/Ser9), FoxO1 (Ser264) and GS (Ser641). The improved glucose tolerance could be prevented by prior treatment with wortmannin, a PI3K inhibitor. In addition, hepatic gluconeogenesis was suppressed, as witnessed by decreased expression of the FoxO1-regulated genes phosphoenolpyruvate carboxykinase (*Pepck*) and glucose-6-phosphatase (*G6P*) [72]. The livers also showed improved glycogen storage and decreased lipid deposition. The latter correlated with increased levels of triglycerides (TGD), cholesterol (CHOL), and high- and low-density lipoproteins (HDL and LDL) in serum, both when mice were given normal chow or high-fat diets [71].

At least two different PP2A trimeric complexes seem to play a role in mediating these metabolic phenotypes.

First, it was observed that the surviving mice in the constitutive total-body KO model of *Ppp2r5c* (encoding the B56γ subunits) became obese, exhibiting 30% increased body weights and increased amounts of white adipose tissue [67]. More insights into this phenotype were obtained in a hepatocyte-specific knockdown (KD) model of B56γ, achieved through tail vein injection of an adenovirus, expressing a *Ppp2r5c*-targeting miRNA under the control of a hepatocyte-specific promoter [73]. These mice showed a significant reduction in B56γ mRNA (85%) and protein (50%) levels in the liver. Although no effects on body weight could be detected, an increased insulin sensitivity and glucose tolerance phenotype was observed, very similar to the liver-specific Cα KO mice. However, in this case, this phenotype was not caused by any effects on gluconeogenesis or insulin-induced AKT or

GSK-3β phosphorylation, but rather by increased glucose uptake by the hepatocytes. In addition, and in contrast to the liver-specific Cα KO mice, increased triglyceride levels were found in the B56γ KD livers, suggestive for increased *de novo* lipogenesis. Together with an enhanced liver mass, elevated VLDL secretion in blood and decreased glycogen breakdown upon fasting, these phenotypes infer a more detrimental effect on lipid metabolism than on carbohydrate metabolism. This view was confirmed in diabetic db/db mice, where liver-specific *Ppp2r5c* knockdown worsened their dyslipidemia, while their glucose metabolism was considerably improved. Consistently, in livers of type 2 diabetic patients, *PPP2R5C* expression was significantly increased, and visceral obesity correlated with *PPP2R5C* transcription in non-diabetic patients [73]. Mechanistically, BIO-ID and PLA assays revealed a role for PP2A-B56γ in the direct control of AMP-activated protein Kinase (AMPK) activity (Thr172 phosphorylation was enhanced in the B56γ KD livers) and in the suppression of Hypoxia Inducible Factor 1α (HIF1α) transcriptional activity. Further, an indirect role for PP2A-B56γ was inferred in suppressing Sterol Regulatory Element Binding Protein 1 (SREBP-1)-regulated transcription [73].

A completely different role in glucose metabolism was established for PP2A-B55α trimers. Through use of a random chemical-induced mutagenesis approach, a mouse model was created, harboring a new splice acceptor site in *Ppp2r2a* intron 3, resulting in the generation of a premature translation stop [74]. Concomitantly, in heterozygous mice, a 35–45% reduction in B55α transcripts and a 22–43% reduction in B55α protein expression was seen in white adipose tissue, liver and skeletal muscle. These mice showed modest insulin resistance, but no overt diabetes. In contrast, in a digenic model, in which these *Ppp2r2a*$^{+/-}$ mice were crossed with mice heterozygous for a null allele of the insulin receptor, a severely aggravated and overt diabetic phenotype was observed, characterized by progressive hyperglycemia, hyperinsulinemia, glycosuria, impaired glucose tolerance and insulin resistance. This was accompanied by decreased total AKT levels and increased basal AKT phosphorylation (Thr308, Ser473), but decreased insulin-stimulated AKT, GSK-3β and p70 S6K phosphorylation. Additionally, elevated FoxO1 expression, resulting in increased *Pepck* and *G6P* mRNA levels, were observed both in *Ppp2r2a in vitro* knockdown and in the digenic *in vivo* model [74]. Interestingly, and consistent with the inferred essential role of LCMT-1 in PP2A-B55 holoenzyme assembly, homozygous mice for a hypomorphic gene-trapped *Lcmt1* allele, also showed an insulin resistance phenotype, with decreased glucose tolerance and increased glucose-stimulated insulin secretion [42]. In this model, decreased LCMT-1 expression was confirmed in cardiac and skeletal muscle (95% decrease), liver (60% decrease), brain (50% decrease) and kidney (40% decrease), and was accompanied by decreased PP2A-C methylation in these tissues [42].

Overall, these data revealed an undeniable role of PP2A in the regulation of carbohydrate and lipid metabolism homeostasis, which is however highly dependent on the particular holoenzyme involved, as distinct and even opposite phenotypes are seen upon deficiency of different regulatory B subunits. Therefore, the further elucidation of these holoenzyme-specific functions will be needed before PP2A can be rationally exploited as a therapeutic target in human metabolic diseases, such as type 2 diabetes.

## 7. PP2A functions in the immune system

Besides its crucial role in the development of the innate immune system, PP2A also plays a major role in the adaptive immune response and in preventing autoimmunity. This is perhaps best sustained by several PP2A strains manipulating the Aα subunit, and further supported by some models of manipulated Cα, and α4. Moreover, the B″γ subunit (also called G5PR, or G5-domain binding Phosphatase Regulator) seems to be one of the important mediators in some of these processes.

Conditional KO of Aα in the B-cell lineage (at pro-B cell stage) did not affect normal pro-B and early pre-B cell numbers, but severely diminished cell numbers once the pre-B-Cell Receptor (BCR)-dependent stages were reached, resulting in barely detectable mature B-cell numbers [75]. The mechanism behind this phenomenon was further studied in isolated bone marrow pre-B or splenic B-cells, transduced with Cre-ER$^{T2}$, in which ablation of Aα expression was induced by addition of tamoxifen. Induced loss of Aα apparently decreased PP2A-C expression as well, and resulted in acute cell death in a B-cell lineage-specific manner. The increased cell death correlated with moderate increases in ERK and AKT phosphorylation, increased activation of mTOR-S6 kinase signaling, inactivation of FoxO1 and -3a, and decreased expression of antioxidant, FoxO target genes (*e.g. Cat*). Overexpression of catalase rescued cell death, inferring the involvement of Reactive Oxygen Species (ROS) in the cell death mechanism. Alongside an amplified mTORC1 pathway, the Aα KO cells showed increased glycolysis in the absence of any changes in mitochondrial respiration. Instead, a decrease in expression of enzymes driving the pentose phosphate pathway was observed that was attributed to an increased phosphorylation of Pfkfb2 (Ser483), which promotes its 6-phosphofructo-2-kinase activity over its fructose 2,6-bisphosphatase activity. This change in enzyme activity/specificity, driving the pentose phosphate pathway, was causally linked to the phenotype, as overexpression of TIGAR, a *TP53*-induced glycolysis regulatory phosphatase that harbors a fructose 2,6 bisphosphatase activity, could rescue the Aα-depletion-induced cell death. Interestingly, increased cell death upon Aα deficiency was also demonstrated in several cell models of B-cell lineage-specific cancers *in vitro* and *in vivo*, but not in cancer models of the myeloid lineage - encouraging the authors to propose PP2A
inhibition (or inhibition of the induced antioxidant pentose phosphate pathway) as a novel therapeutic strategy to specifically target B-cell malignancies [75].

Likewise, the reduced number of mature B-cells in spleen, lymph nodes and peripheral blood that was seen in a B-cell specific KO of B″γ/G5PR (*Ppp2r3c*), was attributed to impaired B-cell survival and increased BCR-activation-induced apoptosis, rather than to a proliferation defect [76]. G5PR is a regulatory subunit of the B″ family known to interact with both PP2A and PP5 [77]. Upon analysis, cells showed prolonged Bim phosphorylation after BCR cross-linking, accompanied by sustained JNK activation and enhanced mitochondrial membrane depolarization. These results imply the existence of a G5PR-dependent resistance mechanism to BCR-activation-induced apoptosis in mature B-cells [76], which fits well with the observation that G5PR expression is upregulated in mature B-cells upon their activation [78]. Notably, G5PR appears to play a rather similar role in thymocytes. Thymocytes mature from double negative ($CD4^-CD8^-$) into double positive ($CD4^+CD8^+$), and eventually, single positive $CD4^+$ or $CD8^+$ T-cells. T-cell-specific loss of G5PR lead to thymic atrophy and 10-fold reduced thymocyte numbers, due to enhanced sensitivity of double positive (DP) thymocytes to apoptosis, while their proliferation and differentiation potential remained unaffected [79]. However, in contrast to the mechanism in G5PR-deficient B-cells [76], apoptosis seemed to be caspase-3 dependent and Bim-independent. Together with a prolonged JNK activation and increased FasL expression, this explained the considerable reduction in DP thymocytes and the nearly complete loss of single positive T-cells in the thymus [79]. The data from the B- and T-cell specific G5PR KO models were further corroborated in a transgenic model with selective overexpression of G5PR in lymphoid cells [76,80], in which a 4.5-times overexpression of G5PR was confirmed in isolated splenocytes. Young mice showed no apparent phenotypes in splenic B-cells under non-immunized conditions. However, upon immunization with nitrophenyl-conjugated chicken γ-globulin, more germinal center B-cells were noted, with an increase in non-antigen-specific B-cells and decreased numbers of antigen-specific B-cells, and decreased antibody production. Upon aging (> 40 weeks), non-immunized mice showed increased numbers of peritoneal B-1a cells

that were not caused by increased proliferation of B-1a or B-2 cells, but attributed to prevention of BCR-activation-induced apoptosis. This was associated with severely decreased JNK signaling and suppressed caspase-3 activation. More remarkably, in aged female but not male transgenic mice, these persistent B-1a cells resulted in development of autoimmunity and autoantibody production [80].

A severe, progressive, multi-organ autoimmunity and lymphoproliferative disorder was also observed in regulatory T-cell ($T_{reg}$)-specific KO mice of the Aα subunit [81]. By 10–14 weeks, these mice showed dermatitis, scaly tails and ears, eyelid crusting, skin rash, ulcerations, extensive inflammatory and lymphocytic infiltrates in multiple organs, and enlarged secondary lymphoid organs. This was accompanied by an increased number of activated $CD4^+$ and $CD8^+$ T-cells, and increased production of several cytokines in these cells [81]. $T_{regs}$ suppress autoimmunity through active targeting of autoreactive T-cells that are erroneously present in the periphery [82]. This is quite comparable to BCR-activation-induced apoptosis in (pre)mature B-cells, which is essentially an inherent mechanism to prevent self-recognition within the B-cell population. Aα-deficient $T_{reg}$ cells showed increased proliferation and an altered metabolic profile, characterized by higher glycolytic and oxidative-phosphorylation rates. Biochemically, this was associated with upregulated mTORC1 activity and increased S6 phosphorylation, while AKT activity was unaltered. Addition of the mTORC1 inhibitor rapamycin normalized the $T_{reg}$ phenotypes [81].

Likewise, reduction of CIP2A levels resulted in a differential expression of genes associated with regulation of autoimmune diseases, although these mice showed normal immune system development and did not display any immunological defects under baseline conditions [83]. However, when challenged with *Listeria monocytogenes*, an impaired adaptive immune response was provoked, with reduced numbers of $CD4^+$ T-cells, $CD8^+$ effector T-cells and IFNγ-producing $CD8^+$ T-cells, indicative for reduced proliferation of CIP2A-deficient T-cells. In WT T-cells, CIP2A expression was induced after anti-CD3/anti-CD28 stimulation, further implying a role of CIP2A in T-cell activation, particularly of effector $CD8^+$ T-cells [83]. Conversely, conditional KO of Cα specifically in macrophages, resulted in an elevated host antiviral response, as shown by an increased protection of these mice from lethal infection with the vesicular stomatitis virus (VSV) [84]. This was associated with enhanced NF-κB signaling in peripheral blood mononuclear cells (PBMCs) and increased type-I interferon signaling in peritoneal macrophages, when stimulated with LPS or upon Sendai virus infection, as shown by increased interferon regulatory factor 3 (IRF3) phosphorylation (Ser396), and increased expression of *Ifnb1*, *Isg15* and *Rantes*. These data are thus consistent with a direct role for PP2A in IRF3 dephosphorylation, a process that appears to be mediated by the RACK1 adaptor protein [84]. Together, the data from the CIP2A KO and macrophage-specific Cα KO mice seem to justify the careful conclusion that PP2A and CIP2A-regulated PP2A complexes might negatively affect the adaptive immune response. This view seems to be further underscored in transgenic mice with WT Cα overexpression, specifically in T-cells [85]. A 30% increase in Cα mRNA and protein expression was achieved. These mice showed increased susceptibility to immune-mediated glomerulonephritis, in the absence of other immune defects. An increased number of neutrophils in the blood sustained the increased inflammation characterizing this model. $CD4^+$ T-cells showed a deviated cytokine production profile, with increased levels of IL-17 (*Il17a* and *Il17b*). Indicative for a causal role, neutralization of IL-17 rescued the glomerulonephritis development [85]. Additional microarray analysis in naïve and activated $CD4^+$ T-cells from this model, revealed 124 upregulated and only 6 downregulated genes, with the upregulated genes primarily encoding cytokines and chemokines, including IL-17 [86]. Mechanistically, increased Histone H3 acetylation at *Il17a* and *Il17b* loci was detected, alongside increased recruitment of IRF4, implying a role for PP2A in epigenetic regulation in T-cells.

A role for PP2A in innate immunity is further underscored in several strains targeting *Igbp1*, encoding the PP2A biogenesis regulator α4.

Impaired B-cell differentiation and a reduced proliferation of activated B-cells were phenotypes observed in a B-cell-specific KO mouse of α4 [87]. In bone marrow, a normal number of pro-B cells and a reduced number of pre-B cells was noted, while in spleen, the number of mature $B220^+$ B-cells was significantly decreased. Upon activation by antigens, LPS or anti-CD40 antibodies, B-cells significantly proliferated less, showing an impaired G1/S transition, reduced p70 S6 kinase activation and reduced rapamycin sensitivity. The mice also showed reduced B-cell responses to immunization with T-cell independent, and particularly with T-cell-dependent antigens, as witnessed by a significant reduction in IgM and IgG production, isotype switching, V region somatic hypermutation and germinal center formation.

Impaired early T-cell development, associated with smaller and disorganized thymi, was the main phenotype in T-cell-specific KO mice of α4 [88]. T-cell development was arrested at the CD4/CD8 double-negative 3 stage. Thymocytes showed a decreased cytokine- and anti-CD3-induced proliferation capacity but no increased apoptosis, and impaired CD3 signaling, exemplified by a lack of anti-CD3-induced IL-2R expression, while anti-CD3-induced CD28 expression was normal. The impairment in T-cell development in the absence of α4 was also confirmed in another conditional KO model, where in the thymus more immature thymocytes were observed and no mature T-cells could be detected in peripheral blood [44]. Conversely, transgenic overexpression of α4 in thymus did not result in any effects on T-cell viability, proliferation or development. However, the α4 overexpressing T-cells exhibited enhanced migration in a transwell assay, correlating with increased levels of activated Rac1 [45].

Together, these data indicate the variety of essential PP2A functions in the homeostasis of the immune system, and might thus provide further clues for PP2A targeting in (auto)immune disease.

## 8. PP2A functions in the skin

Epidermal-specific loss of Cα resulted in smaller animals with aberrant hair morphogenesis and disruption of the hair follicle regeneration cycle [89]. Mice displayed significant hair loss, had a thicker epidermis with more fat cells, prominent melanin deposition in the paws and at the base of the claws and the tail, and excessive keratinization of the tail. Some animals had severe stool obstruction, and 15% of mice died at 4–6 weeks of age [89]. Abnormal hair follicle morphogenesis occurred from the first post-natal hair cycle onwards, and was characterized by failure of hair cycling and a differentiation defect of matrix cells into the inner root sheath and hair shaft. Upon histologic examination of the epidermis, a hyperproliferation of the basal cells was noted, as witnessed by increased expression of Ki67. Remarkably, this was associated with decreased phosphorylation of AKT (Thr308 and Ser473) [89].

The predominant phenotype in a constitutive, total-body KO mouse model of *Ppp2r5a*, encoding the B56α subunit, also manifested in the skin [90]. In these mice, B56α expression was significantly decreased but not entirely null, with 0.01–0.2% of control levels remaining in all tissues tested. No additional effects on mRNA expression of all other PP2A subunits were apparent, and no effects on overall PP2A activity, as measured on PP2A-C IPs, could be found. B56α KO mice spontaneously developed skin lesions, characterized by hyperproliferation of the epidermis, hair follicles and sebaceous glands. This was associated with increased expression of phosphorylated c-Myc (Ser62) and CDK4 (a Myc target gene). Notably, an increased number of skin (and bone marrow) stem cells was found, suggestive for a role of PP2A-B56α in suppressing stemness. Accordingly, the mice appeared more susceptible to carcinogen-induced skin carcinogenesis as well (see also Section 11) [90].

## 9. PP2A functions in the eye

Moderate transgenic expression of the dominant negative L309A Cα

mutant in the Harderian gland caused a delayed development and hypoplasia of this gland, accompanied by a slit-eye phenotype (enophthalmos) in a significant number of mice [91]. The Harderian or lacrimal gland is a tubular alveolar tissue located behind the eye, that serves to lubricate the eye and the nictitating membrane in mammals [92]. Retinal function in the transgenic animals remained unaffected [91]. A Wnt signaling defect was suspected, since the Harderian gland showed reduced E-cadherin and β-catenin levels and their expression was spatially shifted from the membrane to the cytosol. In addition, an increase in GSK-3β phosphorylation (Ser9) was observed [91].

Additionally, PP2A appeared to be developmentally regulated in the retina, with main expression in the photoreceptor inner segments. However, in an *in vivo* knockin model of the cancer-associated E64G Aα mutant, defective in B56α and B56δ (and potentially other B56 isoforms) binding [10,93], there was no impact on retina structure and function. Nevertheless, an enhanced phosphorylation of PP2A substrates PKCα and mTOR was observed, an effect that could also be induced under light-adapted conditions [94]. These data might rather imply a role of other, non-B56-type PP2A holoenzyme complexes in retinal development and function, although this certainly needs to be further established.

## 10. Additional functions of PP2A in liver, bone, kidney and intestine

In the **liver**, hepatocyte-specific KO of *Ppp2ca* expression did not only result in a metabolic phenotype (see Section 6), but also appeared protective against chronic liver injury, as induced upon repeated *i.p.* injections with $CCl_4$ [95]. Although severely decreased, Cα expression was not completely absent in this model, resulting in a 30% reduction in PP2A activity. Compared with WT mice, 50% less fibrotic lesions were found in Cα KO livers, accompanied by decreased collagen deposition, as well as reduced serum levels of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), two marker genes whose expression is indicative for decreased liver function. Histologically, injured Cα KO livers showed less activated stellate cells, and clearly reduced hepatocyte proliferation, which partially correlated with a slight increase in number of apoptotic hepatocytes. The decrease in fibrosis could possibly be attributed to a decline in serum levels of TGF-β, normally activated in response to injury. In line with decreased TGF-β signaling, phosphorylated Smad2 and Smad3, downstream mediators of TGF-β, were almost completely absent in the injured KO livers [95].

In **bone**, osteoblast-specific overexpression of the dominant-negative L199P Cα mutant, caused a 50% overall decrease in PP2A activity, associated with larger and heavier mice [96]. In the animals, bone mineral density and thickness were significantly increased, while cartilage thickness was not affected. In addition, in the bone marrow, increased adipogenesis was observed, suspected to be caused by a paracrine mechanism.

A potential role for PP2A in the **kidneys** and the regulation of blood pressure homeostasis was revealed in heterozygous mice for the PP2A B‴ subunit STRN [97,98]. Homozygous $Strn^{-/-}$ mice are likely not viable [97]. In the heterozygous mice, decreased STRN expression by ~50% was confirmed in heart, kidney, adrenal gland and aorta. Compared with WT mice, STRN-deficient mice showed an enhanced salt sensitivity of their blood pressure (BP), characterized by a higher increase in BP upon liberal salt intake, and a higher decrease in BP upon salt restriction. On liberal salt intake, an increased expression of the mineralocorticoid receptor (MR) and several MR target genes (*e.g.* the epithelial sodium channel ENaC and the SGK1 kinase) was observed [97]. Additionally, the BP effects were in part due to changes in vascular contraction/relaxation, as an increased aortic contraction was observed upon phenylephrine or KCl addition, and a reduced aortic relaxation upon acetylcholine addition. In both cases, endothelial NO and cGMP dampened the vascular sensitivity effects [98].

Finally, a role for PP2A in intestinal development and the regulation of intestinal epithelial barrier function was inferred from mice with a conditional KO of α4 in the **intestinal epithelium** [99]. Alpha4 mRNA and protein levels in the intestinal mucosa were undetectable, and this correlated with 40% decreased levels of PP2A-C, consistent with several other α4 KO models. The α4 KO mice were smaller, weighed less, and contained a shorter and thinner gastrointestinal tract. The mucosal epithelium of the small intestine was abnormally structured, showing inhibited mucosal maturation, villous shrinkages, crypt hyperplasia and displacement of the Paneth cells from the bases to the tops of the crypts or the villous areas. Overall, proliferation of crypt cells was enhanced, crypt-to-villus migration rates were decreased, and apoptosis in the mucosal epithelium was increased. An impaired epithelial barrier function was also apparent, characterized by increased gut permeability and decreased expression of several epithelial junction proteins (*e.g.* claudin-1/3, ZO-1 and E-cadherin). Mechanistically, an increased phospho-IKKα/total IKKα ratio was detected, resulting in increased IKKα-mediated phosphorylation and degradation of the RNA-binding protein HuR. The latter was causally involved, since forced overexpression of HuR could rescue the observed epithelial barrier function defects [99].

## 11. PP2A as a tumor suppressor, preventing cancer development

Although the tumor suppressive role of specific PP2A complexes had already been inferred for a relatively long time, from experiments with the tumor promotor and pharmacologic PP2A inhibitor okadaic acid [100], from *in vitro* cell transformation assays in a large variety of epithelial cells [101], and from a plethora of clinical data supporting PP2A dysfunction in many different cancer types [5,102,103], convincing *in vivo* data to sustain this function had been lacking for some time.

The first *in vivo* indications to sustain the tumor suppressive functions of PP2A arose from PP2A strains, in which cancer formation was either induced by carcinogens, or by co-expression of an established oncogene. These initial models were largely based on the discovery of mono-allelic mutations in the Aα encoding gene, *PPP2R1A*, in human cancers [104–106]. For instance, the Aα E64G and E64D mutants, respectively discovered in breast and lung carcinoma [104], were found to be defective in B56α and B56δ binding *in vivo* [10], and B56γ binding *in vitro* [93]. The Aα Δ5 variant, found in human breast carcinoma [104] and lacking HEAT-repeat 5, no longer bound any B subunit [66]. Ruediger et al. generated several KI models with these cancer-associated Aα variants [10]. E64D/E64D, E64D/+, E64G/E64G, E64G/+, Δ5–6/E64G, Δ5–6/E64D and Δ5–6/+ mice all turned out to be viable and fertile, with no discernable phenotypes. However, in E64D/+ and Δ5–6/+ mice, as well as in the double mutated Δ5–6/E64D mice, a 50% increase in benzopyrene-induced lung carcinogenesis incidence was observed, when compared with WT mice. This increase could be rescued upon co-expression of a dominant-negative *TP53* allele, prompting the authors to suggest that the protective effect of PP2A is dependent on the presence of p53 [10]. In subsequent research, these three Aα KI models were crossed with transgenic mice expressing the mutant $Kras^{G12D}$ allele in all tissues [107], and this resulted in a decreased median survival, when compared with $Kras^{G12D}$ expressing WT mice [108]. Unfortunately, it was not documented whether this difference in survival was anyhow related to an altered occurrence of $Kras^{G12D}$-induced malignancies.

Increased carcinogen-induced tumor formation was further underscored in B56α KO mice [90]. In this model, accelerated skin papilloma formation was observed upon DMBA/TPA treatment of the skin, although endpoint levels were similar to those in WT mice. No differences were seen in progression of the papillomas into squamous cell carcinomas, indicating that loss of B56α increases the initiation of carcinogenesis, without affecting progression. This phenotype is likely connectable to the induced stemness potential of skin cells in these mice, which on its turn could be related to increased expression of phosphorylated c-Myc (Ser62). The inability of the B56α-depleted skin

cells to counteract c-Myc activity in the presence of an oncogenic event, might therefore promote tumor initiation [90]. Notably, accelerated occurrence of DMBA/TPA-induced skin papillomas was also observed in heterozygous PTPA KO mice, in which activity of PP2A-B56γ/ε (and presumably other PP2A-B56 isoforms) was selectively decreased [43].

Only very recently, it was shown that PP2A ablation could also increase susceptibility of mice to spontaneous cancer development [109]. A very prominent spontaneous cancer phenotype was indeed observed in the total-body, constitutive KO model of the B56δ subunit [110] and in both homozygous and heterozygous mice of a hypomorphic *Ptpa* allele [43]. Loss of B56δ resulted in elevated levels of hematologic malignancies, mainly non-Hodgkin small B-cell lymphomas, in aging mice. More surprisingly, a high incidence of hepatocellular carcinomas (HCCs) was observed as well, in up to 60% of mice in the oldest age category, while HCC did not occur at all in aging WT mice. Of these HCCs, 70% arose in a normal liver context, with no signs of inflammation, fibrosis or necrosis. Unbiased transcriptome data revealed a commonly upregulated c-Myc activity in the HCCs, further corroborated by increased c-Myc phosphorylation (Ser62) and enhanced CIP2A expression (a Myc target gene) in all HCCs examined. A negative feedback loop between B56δ and c-Myc was reported before, involving PP2A-B56δ-mediated dephosphorylation and activation of GSK-3β, which on its turn promoted c-Myc degradation [111]. Interestingly, elevated Ser9 phosphorylation of GSK-3β was indeed seen in both non-cancerous and cancerous B56δ KO liver samples, implying a role for dysregulated GSK-3β activity in the tumor predisposing mechanism [110]. The spontaneous tumor phenotype observed in PTPA-deficient mice upon aging, was somewhat less penetrant, and mainly characterized by the development of hematologic malignancies, and less commonly, of HCC. In this case, no common signaling defects could be revealed, although sporadic activation of Wnt signaling (increased β-catenin expression), Hedgehog signaling (increased Gli1 expression) and c-Myc activation (Ser62 phosphorylation) were present in some of the tumor tissues analyzed [43].

Together, the data from the above PP2A cancer strains seem to specifically highlight the tumor suppressive role of the PP2A-B56-type of complexes *in vivo*, particularly of the B56α and B56δ isoforms, which seem to be able to prevent tumorigenesis in a context- and tissue-dependent manner. Whether other trimeric PP2A complexes might also contribute, perhaps in other tissues, awaits further research, as does the role of cellular PP2A inhibitors, such as CIP2A and SET. Transgenic models in which these oncoproteins are overexpressed in specific cells or tissues would indeed be highly instructive to further underscore their inferred role in cancer initiation and/or progression.

## 12. Conclusions and future perspectives

Protein Phosphatase 2A is clearly a far-reaching cellular regulator, operating in a variety of signaling mechanisms in virtually any tissue analyzed. From many of the PP2A strains described above, it became clear that PP2A phosphatases are essential enzymes, not only for maintaining homeostasis of the particular targeted tissue, but also for organismal survival as a whole. In other words, global PP2A inhibition is detrimental and underscores the importance of PP2A as a major homeostatic factor. Some of these PP2A functions are quite well-defined and mechanistically underbuilt *in vivo*, such as *e.g.* the general modulation of Wnt, Nodal (TGF-β) and, likely, Hedgehog signaling during (embryonic) development, which is mediated by different PP2A trimers, harboring B subunits from different families. In fully developed mice, different PP2A complexes regulate tissue homeostasis/function mainly through their ability to control or counteract the activities of diverse kinases and signaling pathways, the most important ones perhaps being the PI3K/AKT/GSK-3β, mTOR/S6K and several MAP kinase pathways, impacting on for instance, glucose and lipid metabolism (in general, but also in different cell types), tumor development, and direct or indirect regulation of tau dephosphorylation in brain. In addition, PP2A is involved in β-adrenergic and $Ca^{2+}$-mediated signaling in brain and heart, and in pro- as well as anti-apoptotic and proliferative pathways in a variety of tissues, but perhaps best illustrated in immune and germ cells.

Nevertheless, a substantial part of its physiologic functions remains unclear. This is mostly due to the highly complex structure and regulation of PP2A, stemming from the presence of no less than 19 different PP2A subunit encoding genes in mice, which have not all been studied *in vivo* to date. This is particularly true for the genes encoding the different regulatory B subunits, despite their inferred importance in determining the physiologic functions of PP2A. In the additional light of the expected therapeutic potential of targeting specific PP2A trimers for the treatment of many different human diseases, there is definitely a need to fill this current knowledge gap. New gene targeting technologies, such as gene editing using CRISPR/Cas9, may certainly hold the potential to move this field forward, and aid in the generation of additional, and improved PP2A B subunit mouse models. Moreover, it became evident from several of the PP2A subunit strains, that specific PP2A subunits can be functionally redundant in specific contexts, further complicating the interpretation of certain phenotypes (or lack of phenotypes), but certainly, also further warranting to join forces and crossbreed existing models to provide additional insights into this issue.

Finally, PP2A is subject of an extended network of inhibitory and activating mechanisms, making the unraveling of its main functions even more challenging. Even if these PP2A regulators might, in the end, turn out not to be entirely PP2A-specific, important insights were derived from the strains in which these regulators were manipulated, for instance, by highlighting particular PP2A functions in brain, the immune system and tumor development, but also by underscoring the therapeutic potential of indirect PP2A targeting, through these inhibitors or activators, for the improved treatment of certain diseases.

In conclusion, the 63 PP2A-related mouse models discussed here, have certainly represented a significant aid in elucidating PP2A's *in vivo* mechanisms, its intense modulation and its impact on (patho)physiology. In the future, the additional characterization of the existing models, as well as the generation of yet other models, will only deepen these insights, and thereby provide some of the missing clues for the full clinical development of PP2A as a promising therapeutic target in human disease.

## Transparency document

The Transparency document associated with this article can be found, in online version.

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## References

[1] J.M. Sontag, E. Sontag, Protein phosphatase 2A dysfunction in Alzheimer's disease, Front. Mol. Neurosci. 7 (2014) 16.

[2] G. Houge, D. Haesen, L.E. Vissers, S. Mehta, M.J. Parker, M. Wright, J. Vogt, S. McKee, J.L. Tolmie, N. Cordeiro, T. Kleefstra, M.H. Willemsen, M.R. Reijnders, S. Berland, E. Hayman, E. Lahat, E.H. Brilstra, K.L. van Gassen, E. Zonneveld-Huijssoon, C.I. de Bie, A. Hoischen, E.E. Eichler, R. Holdhus, V.M. Steen, S.O. Doskeland, M.E. Hurles, D.R. FitzPatrick, V. Janssens, B56delta-related protein phosphatase 2A dysfunction identified in patients with intellectual disability, J. Clin. Invest. 125 (2015) 3051–3062.

[3] J.C. Crispin, C.M. Hedrich, G.C. Tsokos, Gene-function studies in systemic lupus erythematosus, Nat. Rev. Rheumatol. 9 (2013) 476–484.

[4] A. Kowluru, A. Matti, Hyperactivation of protein phosphatase 2A in models of glucolipotoxicity and diabetes: potential mechanisms and functional consequences, Biochem. Pharmacol. 84 (2012) 591–597.

[5] P.P. Ruvolo, The broken “Off” switch in cancer signaling: PP2A as a regulator of tumorigenesis, drug resistance, and immune surveillance, BBA Clin. 6 (2016) 87–99.
[6] C. Lambrecht, D. Haesen, W. Sents, E. Ivanova, V. Janssens, Structure, regulation, and pharmacological modulation of PP2A phosphatases, Methods Mol. Biol. 1053 (2013) 283–305.
[7] D. Haesen, W. Sents, K. Lemaire, Y. Hoorne, V. Janssens, The basic biology of PP2A in hematologic cells and malignancies, Front. Oncol. 4 (2014) 347.
[8] E. Kremmer, K. Ohst, J. Kiefer, N. Brewis, G. Walter, Separation of PP2A core enzyme and holoenzyme with monoclonal antibodies against the regulatory A subunit: abundant expression of both forms in cells, Mol. Cell. Biol. 17 (1997) 1692–1701.
[9] J. Gotz, A. Probst, E. Ehler, B. Hemmings, W. Kues, Delayed embryonic lethality in mice lacking protein phosphatase 2A catalytic subunit Calpha, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 12370–12375.
[10] R. Ruediger, J. Ruiz, G. Walter, Human cancer-associated mutations in the Aalpha subunit of protein phosphatase 2A increase lung cancer incidence in Aalpha knock-in and knockout mice, Mol. Cell. Biol. 31 (2011) 3832–3844.
[11] M.J. Cundell, L.H. Hutter, R. Nunes Bastos, E. Poser, J. Holder, S. Mohammed, B. Novak, F.A. Barr, A PP2A-B55 recognition signal controls substrate dephosphorylation kinetics during mitotic exit, J. Cell Biol. 214 (2016) 539–554.
[12] E.P.T. Hertz, T. Kruse, N.E. Davey, B. Lopez-Mendez, J.O. Sigurethsson, G. Montoya, J.V. Olsen, J. Nilsson, A conserved motif provides binding specificity to the PP2A-B56 phosphatase, Mol. Cell 63 (2016) 686–695.
[13] V. Janssens, S. Longin, J. Goris, PP2A holoenzyme assembly: in cauda venenum (the sting is in the tail), Trends Biochem. Sci. 33 (2008) 113–121.
[14] W. Sents, E. Ivanova, C. Lambrecht, D. Haesen, V. Janssens, The biogenesis of active protein phosphatase 2A holoenzymes: a tightly regulated process creating phosphatase specificity, FEBS J. 280 (2013) 644–661.
[15] C.G. Wu, A. Zheng, L. Jiang, M. Rowse, V. Stanevich, H. Chen, Y. Li, K.A. Satyshur, B. Johnson, T.J. Gu, Z. Liu, Y. Xing, Methylation-regulated decommissioning of multimeric PP2A complexes, Nat. Commun. 8 (2017) 2272.
[16] L. Jiang, V. Stanevich, K.A. Satyshur, M. Kong, G.R. Watkins, B.E. Wadzinski, R. Sengupta, Y. Xing, Structural basis of protein phosphatase 2A stable latency, Nat. Commun. 4 (2013) 1699.
[17] F. Guo, V. Stanevich, N. Wlodarchak, R. Sengupta, L. Jiang, K.A. Satyshur, Y. Xing, Structural basis of PP2A activation by PTPA, an ATP-dependent activation chaperone, Cell Res. 24 (2014) 190–203.
[18] D. Haesen, W. Sents, E. Ivanova, C. Lambrecht, V. Janssens, Cellular inhibitors of protein phosphatase PP2A in cancer, Biomed. Res. 23 (2012) 197–211.
[19] J.A. Stamatoyannopoulos, M. Snyder, R. Hardison, B. Ren, T. Gingeras, D.M. Gilbert, M. Groudine, M. Bender, R. Kaul, T. Canfield, E. Giste, A. Johnson, M. Zhang, G. Balasundaram, R. Byron, V. Roach, P.J. Sabo, R. Sandstrom, A.S. Stehling, R.E. Thurman, S.M. Weissman, P. Cayting, M. Hariharan, J. Lian, Y. Cheng, S.G. Landt, Z. Ma, B.J. Wold, J. Dekker, G.E. Crawford, C.A. Keller, W. Wu, C. Morrissey, S.A. Kumar, T. Mishra, D. Jain, M. Byrska-Bishop, D. Blankenberg, B.R. Lajoie, G. Jain, A. Sanyal, K.B. Chen, O. Denas, J. Taylor, G.A. Blobel, M.J. Weiss, M. Pimkin, W. Deng, G.K. Marinov, B.A. Williams, K.I. Fisher-Aylor, G. Desalvo, A. Kiralusha, D. Trout, H. Amrhein, A. Mortazavi, L. Edsall, D. McCleary, S. Kuan, Y. Shen, F. Yue, Z. Ye, C.A. Davis, C. Zaleski, S. Jha, C. Xue, A. Dobin, W. Lin, M. Fastuca, H. Wang, R. Guigo, S. Djebali, J. Lagarde, T. Ryba, T. Sasaki, V.S. Malladi, M.S. Cline, V.M. Kirkup, K. Learned, K.R. Rosenbloom, W.J. Kent, E.A. Feingold, P.J. Good, M. Pazin, R.F. Lowdon, L.B. Adams, An encyclopedia of mouse DNA elements (Mouse ENCODE), Genome Biol. 13 (2012) 418.
[20] K. Schmidt, S. Kins, A. Schild, R.M. Nitsch, B.A. Hemmings, J. Gotz, Diversity, developmental regulation and distribution of murine PR55/B subunits of protein phosphatase 2A, Eur. J. Neurosci. 16 (2002) 2039–2048.
[21] E. Martens, I. Stevens, V. Janssens, J. Vermeesch, J. Gotz, J. Goris, C. Van Hoof, Genomic organisation, chromosomal localisation tissue distribution and developmental regulation of the PR61/B′ regulatory subunits of protein phosphatase 2A in mice, J. Mol. Biol. 336 (2004) 971–986.
[22] K. Zwaenepoel, J.V. Louis, J. Goris, V. Janssens, Diversity in genomic organisation, developmental regulation and distribution of the murine PR72/B″ subunits of protein phosphatase 2A, BMC Genomics 9 (2008) 393.
[23] C. Van Hoof, E. Martens, S. Longin, J. Jordens, I. Stevens, V. Janssens, J. Goris, Specific interactions of PP2A and PP2A-like phosphatases with the yeast PTPA homologues, Ypa1 and Ypa2, Biochem. J. 386 (2005) 93–102.
[24] J.L. McConnell, R.J. Gomez, L.R. McCorvey, B.K. Law, B.E. Wadzinski, Identification of a PP2A-interacting protein that functions as a negative regulator of phosphatase activity in the ATM/ATR signaling pathway, Oncogene 26 (2007) 6021–6030.
[25] M. Kong, D. Ditsworth, T. Lindsten, C.B. Thompson, Alpha4 is an essential regulator of PP2A phosphatase activity, Mol. Cell 36 (2009) 51–60.
[26] J. Hwang, J.A. Lee, D.C. Pallas, Leucine carboxyl methyltransferase 1 (LCMT-1) methylates protein phosphatase 4 (PP4) and protein phosphatase 6 (PP6) and differentially regulates the stable formation of different PP4 holoenzymes, J. Biol. Chem. 291 (2016) 21008–21019.
[27] X. Pan, X. Chen, X. Tong, C. Tang, J. Li, Ppp2ca knockout in mice spermatogenesis, Reproduction 149 (2015) 385–391.
[28] A. Tang, P. Shi, A. Song, D. Zou, Y. Zhou, P. Gu, Z. Huang, Q. Wang, Z. Lin, X. Gao, PP2A regulates kinetochore-microtubule attachment during meiosis I in oocyte, Cell Cycle 15 (2016) 1450–1461.
[29] M.W. Hu, Z.B. Wang, Z.Z. Jiang, S.T. Qi, L. Huang, Q.X. Liang, H. Schatten, Q.Y. Sun, Scaffold subunit Aalpha of PP2A is essential for female meiosis and fertility in mice, Biol. Reprod. 91 (2014) 19.
[30] S. Ventela, C. Come, J.A. Makela, R.M. Hobbs, L. Mannermaa, M. Kallajoki, E.K. Chan, P.P. Pandolfi, J. Toppari, J. Westermarck, CIP2A promotes proliferation of spermatogonial progenitor cells and spermatogenesis in mice, PLoS One 7 (2012) e33209.
[31] J. Wang, J. Okkeri, K. Pavic, Z. Wang, O. Kauko, T. Halonen, G. Sarek, P.M. Ojala, Z. Rao, W. Xu, J. Westermarck, Oncoprotein CIP2A is stabilized via interaction with tumor suppressor PP2A/B56, EMBO Rep. 18 (2017) 437–450.
[32] A. Khanna, J.E. Pimanda, J. Westermarck, Cancerous inhibitor of protein phosphatase 2A, an emerging human oncoprotein and a potential cancer therapy target, Cancer Res. 73 (2013) 6548–6553.
[33] Y. Khew-Goodall, B.A. Hemmings, Tissue-specific expression of mRNAs encoding alpha- and beta-catalytic subunits of protein phosphatase 2A, FEBS Lett. 238 (1988) 265–268.
[34] J. Gotz, A. Probst, C. Mistl, R.M. Nitsch, E. Ehler, Distinct role of protein phosphatase 2A subunit Calpha in the regulation of E-cadherin and beta-catenin during development, Mech. Dev. 93 (2000) 83–93.
[35] P. Gu, X. Qi, Y. Zhou, Y. Wang, X. Gao, Generation of Ppp2Ca and Ppp2Cb conditional null alleles in mouse, Genesis 50 (2012) 429–436.
[36] L. Lange, M. Marks, J. Liu, L. Wittler, H. Bauer, S. Piehl, G. Blass, B. Timmermann, B.G. Herrmann, Patterning and gastrulation defects caused by the t(w18) lethal are due to loss of Ppp2r1a, Biol. Open 6 (2017) 752–764.
[37] A.D. Everett, C. Kamibayashi, D.L. Brautigan, Transgenic expression of protein phosphatase 2A regulatory subunit B56gamma disrupts distal lung differentiation, Am. J. Phys. Lung Cell. Mol. Phys. 282 (2002) L1266–L1271.
[38] W. Chen, P. Gu, X. Jiang, H.B. Ruan, C. Li, X. Gao, Protein phosphatase 2A catalytic subunit alpha (PP2Acalpha) maintains survival of committed erythroid cells in fetal liver erythropoiesis through the STAT5 pathway, Am. J. Pathol. 178 (2011) 2333–2343.
[39] J.A. Lee, D.C. Pallas, Leucine carboxyl methyltransferase-1 is necessary for normal progression through mitosis in mammalian cells, J. Biol. Chem. 282 (2007) 30974–30984.
[40] S. Longin, K. Zwaenepoel, J.V. Louis, S. Dilworth, J. Goris, V. Janssens, Selection of protein phosphatase 2A regulatory subunits is mediated by the C terminus of the catalytic subunit, J. Biol. Chem. 282 (2007) 26971–26980.
[41] J.A. Lee, Z. Wang, D. Sambo, K.D. Bunting, D.C. Pallas, Global loss of leucine carboxyl methyltransferase-1 causes severe defects in fetal liver hematopoiesis, J. Biol. Chem. 293 (25) (2018) 9636–9650.
[42] K.B. MacKay, Y. Tu, S.G. Young, S.G. Clarke, Circumventing embryonic lethality with Lcmt1 deficiency: generation of hypomorphic Lcmt1 mice with reduced protein phosphatase 2A methyltransferase expression and defects in insulin signaling, PLoS One 8 (2013) e65967.
[43] W. Sents, B. Meeusen, P. Kalev, E. Radaelli, X. Sagaert, E. Miermans, D. Haesen, C. Lambrecht, M. Dewerchin, P. Carmeliet, J. Westermarck, A. Sablina, V. Janssens, PP2A inactivation mediated by PPP2R4 haploinsufficiency promotes cancer development, Cancer Res. 77 (2017) 6825–6837.
[44] M. Kong, C.J. Fox, J. Mu, L. Solt, A. Xu, R.M. Cinalli, M.J. Birnbaum, T. Lindsten, C.B. Thompson, The PP2A-associated protein alpha4 is an essential inhibitor of apoptosis, Science 306 (2004) 695–698.
[45] M. Kong, T.V. Bui, D. Ditsworth, J.J. Gruber, D. Goncharov, V.P. Krymskaya, T. Lindsten, C.B. Thompson, The PP2A-associated protein alpha4 plays a critical role in the regulation of cell spreading and migration, J. Biol. Chem. 282 (2007) 29712–29720.
[46] M. Hoffmeister, C. Prelle, P. Kuchler, I. Kovacevic, M. Moser, W. Muller-Esterl, S. Oess, The ubiquitin E3 ligase NOSIP modulates protein phosphatase 2A activity in craniofacial development, PLoS One 9 (2014) e116150.
[47] B. Liu, L.H. Sun, Y.F. Huang, L.J. Guo, L.S. Luo, Protein phosphatase 2ACalpha gene knock-out results in cortical atrophy through activating hippo cascade in neuronal progenitor cells, Int. J. Biochem. Cell Biol. 95 (2018) 53–62.
[48] T. Yamashita, S. Inui, K. Maeda, D.R. Hua, K. Takagi, K. Fukunaga, N. Sakaguchi, Regulation of CaMKII by alpha4/PP2Ac contributes to learning and memory, Brain Res. 1082 (2006) 1–10.
[49] A. Schild, L.M. Ittner, J. Gotz, Altered phosphorylation of cytoskeletal proteins in mutant protein phosphatase 2A transgenic mice, Biochem. Biophys. Res. Commun. 343 (2006) 1171–1178.
[50] N. Deters, L.M. Ittner, J. Gotz, Substrate-specific reduction of PP2A activity exaggerates tau pathology, Biochem. Biophys. Res. Commun. 379 (2009) 400–405.
[51] S. Kins, A. Crameri, D.R. Evans, B.A. Hemmings, R.M. Nitsch, J. Gotz, Reduced protein phosphatase 2A activity induces hyperphosphorylation and altered compartmentalization of tau in transgenic mice, J. Biol. Chem. 276 (2001) 38193–38200.
[52] D.R.H. Evans, T. Myles, J. Hofsteenge, B.A. Hemmings, Functional expression of human PP2Ac in yeast permits the identification of novel C-terminal and dominant-negative mutant forms, J. Biol. Chem. 274 (1999) 24038–24046.
[53] S. Kins, P. Kurosinski, R.M. Nitsch, J. Gotz, Activation of the ERK and JNK signaling pathways caused by neuron-specific inhibition of PP2A in transgenic mice, Am. J. Pathol. 163 (2003) 833–843.
[54] J.V. Louis, E. Martens, P. Borghgraef, C. Lambrecht, W. Sents, S. Longin, K. Zwaenepoel, R. Pijnenborg, I. Landrieu, G. Lippens, B. Ledermann, J. Gotz, F. Van Leuven, J. Goris, V. Janssens, Mice lacking phosphatase PP2A subunit PR61/B′delta (Ppp2r5d) develop spatially restricted tauopathy by deregulation of CDK5 and GSK3beta, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 6957–6962.
[55] R.E. Nicholls, J.M. Sontag, H. Zhang, A. Staniszewski, S. Yan, C.Y. Kim, M. Yim, C.M. Woodruff, E. Arning, B. Wasek, D. Yin, T. Bottiglieri, E. Sontag, E.R. Kandel, O. Arancio, PP2A methylation controls sensitivity and resistance to beta-amyloid-induced cognitive and electrophysiological impairments, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 3347–3352.

[56] S. Ortega-Gutierrez, D. Leung, S. Ficarro, E.C. Peters, B.F. Cravatt, Targeted disruption of the PME-1 gene causes loss of demethylated PP2A and perinatal lethality in mice, PLoS One 3 (2008) e2486.
[57] D. Kapfhamer, K.H. Berger, F.W. Hopf, T. Seif, V. Kharazia, A. Bonci, U. Heberlein, Protein phosphatase 2a and glycogen synthase kinase 3 signaling modulate prepulse inhibition of the acoustic startle response by altering cortical M-type potassium channel activity, J. Neurosci. 30 (2010) 8830–8840.
[58] H.S. Hoffman, J.L. Searle, Acoustic variables in the modification of startle reaction in the rat, J. Comp. Physiol. Psychol. 60 (1965) 53–58.
[59] J.R. Ison, G.R. Hammond, Modification of the startle reflex in the rat by changes in the auditory and visual environments, J. Comp. Physiol. Psychol. 75 (1971) 435–452.
[60] E.C. Andrade, V. Musante, A. Horiuchi, H. Matsuzaki, A.H. Brody, T. Wu, P. Greengard, J.R. Taylor, A.C. Nairn, ARPP-16 is a striatal-enriched inhibitor of protein phosphatase 2A regulated by microtubule-associated serine/threonine kinase 3 (Mast 3 kinase), J. Neurosci. 37 (2017) 2709–2722.
[61] U. Gergs, P. Boknik, I. Buchwalow, L. Fabritz, M. Matus, I. Justus, G. Hanske, W. Schmitz, J. Neumann, Overexpression of the catalytic subunit of protein phosphatase 2A impairs cardiac function, J. Biol. Chem. 279 (2004) 40827–40834.
[62] D. Dong, L. Li, P. Gu, T. Jin, M. Wen, C. Yuan, X. Gao, C. Liu, Z. Zhang, Profiling metabolic remodeling in PP2Acalpha deficiency and chronic pressure overload mouse hearts, FEBS Lett. 589 (2015) 3631–3639.
[63] M. Hoehn, Y. Zhang, J. Xu, U. Gergs, P. Boknik, K. Werdan, J. Neumann, H. Ebelt, Overexpression of protein phosphatase 2A in a murine model of chronic myocardial infarction leads to increased adverse remodeling but restores the regulation of beta-catenin by glycogen synthase kinase 3beta, Int. J. Cardiol. 183 (2015) 39–46.
[64] L. Li, C. Fang, D. Xu, Y. Xu, H. Fu, J. Li, Cardiomyocyte specific deletion of PP2A causes cardiac hypertrophy, Am. J. Transl. Res. 8 (2016) 1769–1779.
[65] N. Brewis, K. Ohst, K. Fields, A. Rapacciuolo, D. Chou, C. Bloor, W. Dillmann, H. Rockman, G. Walter, Dilated cardiomyopathy in transgenic mice expressing a mutant A subunit of protein phosphatase 2A, Am. J. Physiol. Heart Circ. Physiol. 279 (2000) H1307–H1318.
[66] R. Ruediger, N. Brewis, K. Ohst, G. Walter, Increasing the ratio of PP2A core enzyme to holoenzyme inhibits Tat-stimulated HIV-1 transcription and virus production, Virology 238 (1997) 432–443.
[67] P. Varadkar, D. Despres, M. Kraman, J. Lozier, A. Phadke, K. Nagaraju, B. McCright, The protein phosphatase 2A B56gamma regulatory subunit is required for heart development, Dev. Dyn. 243 (2014) 778–790.
[68] S.C. Little, J. Curran, M.A. Makara, C.F. Kline, H.T. Ho, Z. Xu, X. Wu, I. Polina, H. Musa, A.M. Meadows, C.A. Carnes, B.J. Biesiadecki, J.P. Davis, N. Weisleder, S. Gyorke, X.H. Wehrens, T.J. Hund, P.J. Mohler, Protein phosphatase 2A regulatory subunit B56alpha limits phosphatase activity in the heart, Sci. Signal. 8 (2015) ra72.
[69] Z. Yuchi, L. Kimlicka, F.V. Petegem, Structural insights into disease mutations of the ryanodine receptor, in: M. Puiu (Ed.), Genetic Disorders, InTech, 2013(pp. Ch. 05, Place Published).
[70] U. Kirchhefer, C. Brekle, J. Eskandar, G. Isensee, D. Kucerova, F.U. Muller, F. Pinet, J.S. Schulte, M.D. Seidl, P. Boknik, Cardiac function is regulated by B56alpha-mediated targeting of protein phosphatase 2A (PP2A) to contractile relevant substrates, J. Biol. Chem. 289 (2014) 33862–33873.
[71] L. Xian, S. Hou, Z. Huang, A. Tang, P. Shi, Q. Wang, A. Song, S. Jiang, Z. Lin, S. Guo, X. Gao, Liver-specific deletion of Ppp2calpha enhances glucose metabolism and insulin sensitivity, Aging (Albany NY) 7 (2015) 223–232.
[72] P. Puigserver, J. Rhee, J. Donovan, C.J. Walkey, J.C. Yoon, F. Oriente, Y. Kitamura, J. Altomonte, H. Dong, D. Accili, B.M. Spiegelman, Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction, Nature 423 (2003) 550–555.
[73] Y.S. Cheng, O. Seibert, N. Kloting, A. Dietrich, K. Strassburger, S. Fernandez-Veledo, J.J. Vendrell, A. Zorzano, M. Bluher, S. Herzig, M. Berriel Diaz, A.A. Teleman, PPP2R5C couples hepatic glucose and lipid homeostasis, PLoS Genet. 11 (2015) e1005561.
[74] M. Goldsworthy, Y. Bai, C.M. Li, H. Ge, E. Lamas, H. Hilton, C.T. Esapa, D. Baker, W. Baron, T. Juan, M.M. Veniant, D.J. Lloyd, R.D. Cox, Haploinsufficiency of the insulin receptor in the presence of a splice-site mutation in Ppp2r2a results in a novel digenic mouse model of type 2 diabetes, Diabetes 65 (2016) 1434–1446.
[75] G. Xiao, L.N. Chan, L. Klemm, D. Braas, Z. Chen, H. Geng, Q.C. Zhang, A. Aghajanirefah, K.N. Cosgun, T. Sadras, J. Lee, T. Mirzapoiazova, R. Salgia, T. Ernst, A. Hochhaus, H. Jumaa, X. Jiang, D.M. Weinstock, T.G. Graeber, M. Muschen, B-cell-specific diversion of glucose carbon utilization reveals a unique vulnerability in B cell malignancies, Cell 173 (2018) 470–484.
[76] Y. Xing, H. Igarashi, X. Wang, N. Sakaguchi, Protein phosphatase subunit G5PR is needed for inhibition of B cell receptor-induced apoptosis, J. Exp. Med. 202 (2005) 707–719.
[77] Y. Kono, K. Maeda, K. Kuwahara, H. Yamamoto, E. Miyamoto, K. Yonezawa, K. Takagi, N. Sakaguchi, MCM3-binding GANP DNA-primase is associated with a novel phosphatase component G5PR, Genes Cells 7 (2002) 821–834.
[78] F.M. Huq Ronny, H. Igarashi, N. Sakaguchi, BCR-crosslinking induces a transcription of protein phosphatase component G5PR that is required for mature B-cell survival, Biochem. Biophys. Res. Commun. 340 (2006) 338–346.
[79] Y. Xing, X. Wang, H. Igarashi, H. Kawamoto, N. Sakaguchi, Protein phosphatase subunit G5PR that regulates the JNK-mediated apoptosis signal is essential for the survival of CD4 and CD8 double-positive thymocytes, Mol. Immunol. 45 (2008) 2028–2037.
[80] M. Kitabatake, T. Toda, K. Kuwahara, H. Igarashi, M. Ohtsuji, H. Tsurui, S. Hirose, N. Sakaguchi, Transgenic overexpression of G5PR that is normally augmented in centrocytes impairs the enrichment of high-affinity antigen-specific B cells, increases peritoneal B-1a cells, and induces autoimmunity in aged female mice, J. Immunol. 189 (2012) 1193–1201.
[81] S.A. Apostolidis, N. Rodriguez-Rodriguez, A. Suarez-Fueyo, N. Dioufa, E. Ozcan, J.C. Crispin, M.G. Tsokos, G.C. Tsokos, Phosphatase PP2A is requisite for the function of regulatory T cells, Nat. Immunol. 17 (2016) 556–564.
[82] T.K. Starr, S.C. Jameson, K.A. Hogquist, Positive and negative selection of T cells, Annu. Rev. Immunol. 21 (2003) 139–176.
[83] C. Come, A. Cvrljevic, M.M. Khan, I. Treise, T. Adler, J.A. Aguilar-Pimentel, B. Au-Yeung, E. Sittig, T.D. Laajala, Y. Chen, S. Oeder, J. Calzada-Wack, M. Horsch, T. Aittokallio, D.H. Busch, M.W. Ollert, F. Neff, J. Beckers, V. Gailus-Durner, H. Fuchs, M. Hrabe de Angelis, Z. Chen, R. Lahesmaa, J. Westermarck, CIP2A promotes T-cell activation and immune response to *Listeria monocytogenes* infection, PLoS One 11 (2016) e0152996.
[84] L. Long, Y. Deng, F. Yao, D. Guan, Y. Feng, H. Jiang, X. Li, P. Hu, X. Lu, H. Wang, J. Li, X. Gao, D. Xie, Recruitment of phosphatase PP2A by RACK1 adaptor protein deactivates transcription factor IRF3 and limits type I interferon signaling, Immunity 40 (2014) 515–529.
[85] J.C. Crispin, S.A. Apostolidis, F. Rosetti, M. Keszei, N. Wang, C. Terhorst, T.N. Mayadas, G.C. Tsokos, Cutting edge: protein phosphatase 2A confers susceptibility to autoimmune disease through an IL-17-dependent mechanism, J. Immunol. 188 (2012) 3567–3571.
[86] S.A. Apostolidis, T. Rauen, C.M. Hedrich, G.C. Tsokos, J.C. Crispin, Protein phosphatase 2A enables expression of interleukin 17 (IL-17) through chromatin remodeling, J. Biol. Chem. 288 (2013) 26775–26784.
[87] S. Inui, K. Maeda, D.R. Hua, T. Yamashita, H. Yamamoto, E. Miyamoto, S. Aizawa, N. Sakaguchi, BCR signal through alpha 4 is involved in S6 kinase activation and required for B cell maturation including isotype switching and V region somatic hypermutation, Int. Immunol. 14 (2002) 177–187.
[88] D.R. Hua, S. Inui, T. Yamashita, K. Maeda, K. Takagi, J. Takeda, N. Sakaguchi, T cell-specific gene targeting reveals that alpha4 is required for early T cell development, Eur. J. Immunol. 33 (2003) 1899–1906.
[89] C. Fang, L. Li, J. Li, Conditional knockout in mice reveals the critical roles of Ppp2ca in epidermis development, Int. J. Mol. Sci. 17 (2016) 756.
[90] M. Janghorban, E.M. Langer, X. Wang, D. Zachman, C.J. Daniel, J. Hooper, W.H. Fleming, A. Agarwal, R.C. Sears, The tumor suppressor phosphatase PP2A-B56alpha regulates stemness and promotes the initiation of malignancies in a novel murine model, PLoS One 12 (2017) e0188910.
[91] A. Schild, S. Isenmann, N. Tanimoto, F. Tonagel, M.W. Seeliger, L.M. Ittner, A. Kretz, E. Ogris, J. Gotz, Impaired development of the Harderian gland in mutant protein phosphatase 2A transgenic mice, Mech. Dev. 123 (2006) 362–371.
[92] G.R. Buzzell, The Harderian gland: perspectives, Microsc. Res. Tech. 34 (1996) 2–5.
[93] W. Chen, J.D. Arroyo, J.C. Timmons, R. Possemato, W.C. Hahn, Cancer-associated PP2A Aalpha subunits induce functional haploinsufficiency and tumorigenicity, Cancer Res. 65 (2005) 8183–8192.
[94] A. Rajala, Y. Wang, S.F. Abcouwer, T.W. Gardner, R.V.S. Rajala, Developmental and light regulation of tumor suppressor protein PP2A in the retina, Oncotarget 9 (2018) 1505–1523.
[95] N. Lu, Y. Liu, A. Tang, L. Chen, D. Miao, X. Yuan, Hepatocyte-specific ablation of PP2A catalytic subunit alpha attenuates liver fibrosis progression via TGF-beta1/Smad signaling, Biomed. Res. Int. 2015 (2015) 794862.
[96] K. Yoshida, J. Teramachi, K. Uchibe, M. Ikegame, L. Qiu, D. Yang, H. Okamura, Reduction of protein phosphatase 2A Calpha promotes in vivo bone formation and adipocyte differentiation, Mol. Cell. Endocrinol. 470 (2018) 251–258.
[97] A.E. Garza, C.M. Rariy, B. Sun, J. Williams, J. Lasky-Su, R. Baudrand, T. Yao, B. Moize, W.M. Hafiz, J.R. Romero, G.K. Adler, C. Ferri, P.N. Hopkins, L.H. Pojoga, G.H. Williams, Variants in striatin gene are associated with salt-sensitive blood pressure in mice and humans, Hypertension 65 (2015) 211–217.
[98] A.E. Garza, L.H. Pojoga, B. Moize, W.M. Hafiz, L.A. Opsasnick, W.T. Siddiqui, M. Horenstein, G.K. Adler, G.H. Williams, R.A. Khalil, Critical role of striatin in blood pressure and vascular responses to dietary sodium intake, Hypertension 66 (2015) 674–680.
[99] H.K. Chung, S.R. Wang, L. Xiao, N. Rathor, D.J. Turner, P. Yang, M. Gorospe, J.N. Rao, J.Y. Wang, alpha4 coordinates small intestinal epithelium homeostasis by regulating stability of HuR, Mol. Cell. Biol. 38 (2018) (in the press).
[100] V. Janssens, J. Goris, C. Van Hoof, PP2A: the expected tumor suppressor, Curr. Opin. Genet. Dev. 15 (2005) 34–41.
[101] W.C. Hahn, R.A. Weinberg, Rules for making human tumor cells, N. Engl. J. Med. 347 (2002) 1593–1603.
[102] J. Sangodkar, C.C. Farrington, K. McClinch, M.D. Galsky, D.B. Kastrinsky, G. Narla, All roads lead to PP2A: exploiting the therapeutic potential of this phosphatase, FEBS J. 283 (2016) 1004–1024.
[103] O. Kauko, J. Westermarck, Non-genomic mechanisms of protein phosphatase 2A (PP2A) regulation in cancer, Int. J. Biochem. Cell Biol. 96 (2018) 157–164.
[104] G.A. Calin, M.G. di Iasio, E. Caprini, I. Vorechovsky, P.G. Natali, G. Sozzi, C.M. Croce, G. Barbanti-Brodano, G. Russo, M. Negrini, Low frequency of alterations of the alpha (PPP2R1A) and beta (PPP2R1B) isoforms of the subunit A of the serine-threonine phosphatase 2A in human neoplasms, Oncogene 19 (2000) 1191–1195.
[105] D. Haesen, L. Abbasi Asbagh, R. Derua, A. Hubert, S. Schrauwen, Y. Hoorne, F. Amant, E. Waelkens, A. Sablina, V. Janssens, Recurrent PPP2R1A mutations in uterine cancer act through a dominant-negative mechanism to promote malignant cell growth, Cancer Res. 76 (2016) 5719–5731.
[106] M. Shih Ie, T.L. Wang, Mutation of PPP2R1A: a new clue in unveiling the

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pathogenesis of uterine serous carcinoma, J. Pathol. 224 (2011) 1–4.
[107] L. Johnson, K. Mercer, D. Greenbaum, R.T. Bronson, D. Crowley, D.A. Tuveson, T. Jacks, Somatic activation of the K-ras oncogene causes early onset lung cancer in mice, Nature 410 (2001) 1111–1116.
[108] G. Walter, R. Ruediger, Mouse model for probing tumor suppressor activity of protein phosphatase 2A in diverse signaling pathways, Cell Cycle 11 (2012) 451–459.
[109] B. Meeusen, V. Janssens, In vivo pieces of the PP2A onco-puzzle fallen into place, Oncoscience 4 (2017) 154–155.
[110] C. Lambrecht, L. Libbrecht, X. Sagaert, P. Pauwels, Y. Hoorne, J. Crowther, J.V. Louis, W. Sents, A. Sablina, V. Janssens, Loss of protein phosphatase 2A regulatory subunit B56delta promotes spontaneous tumorigenesis in vivo, Oncogene 37 (2018) 544–552.
[111] L. Liu, R.N. Eisenman, Regulation of c-Myc protein abundance by a protein phosphatase 2A-glycogen synthase kinase 3beta-negative feedback pathway, Genes Cancer 3 (2012) 23–36.
[112] P. Opal, J.J. Garcia, A.E. McCall, B. Xu, E.J. Weeber, J.D. Sweatt, H.T. Orr, H.Y. Zoghbi, Generation and characterization of LANP/pp32 null mice, Mol. Cell. Biol. 24 (2004) 3140–3149.
[113] R.K. Kular, R.G. Gogliotti, P. Opal, Cpd-1 null mice display a subtle neurological phenotype, PLoS One 5 (2010) e12649.
[114] P.T. Reilly, S. Afzal, A. Wakeham, J. Haight, A. You-Ten, K. Zaugg, J. Dembowy, A. Young, T.W. Mak, Generation and characterization of the Anp32e-deficient mouse, PLoS One 5 (2010) e13597.
[115] P. Wong, V.I. Leo, M. Low, T.W. Mak, X. Zhang, P.T. Reilly, Targeted ANP32E mutant mice do not demonstrate obvious movement defects, PLoS One 8 (2013) e63815.