

The ryanodine receptor is a novel target for Bcl-2

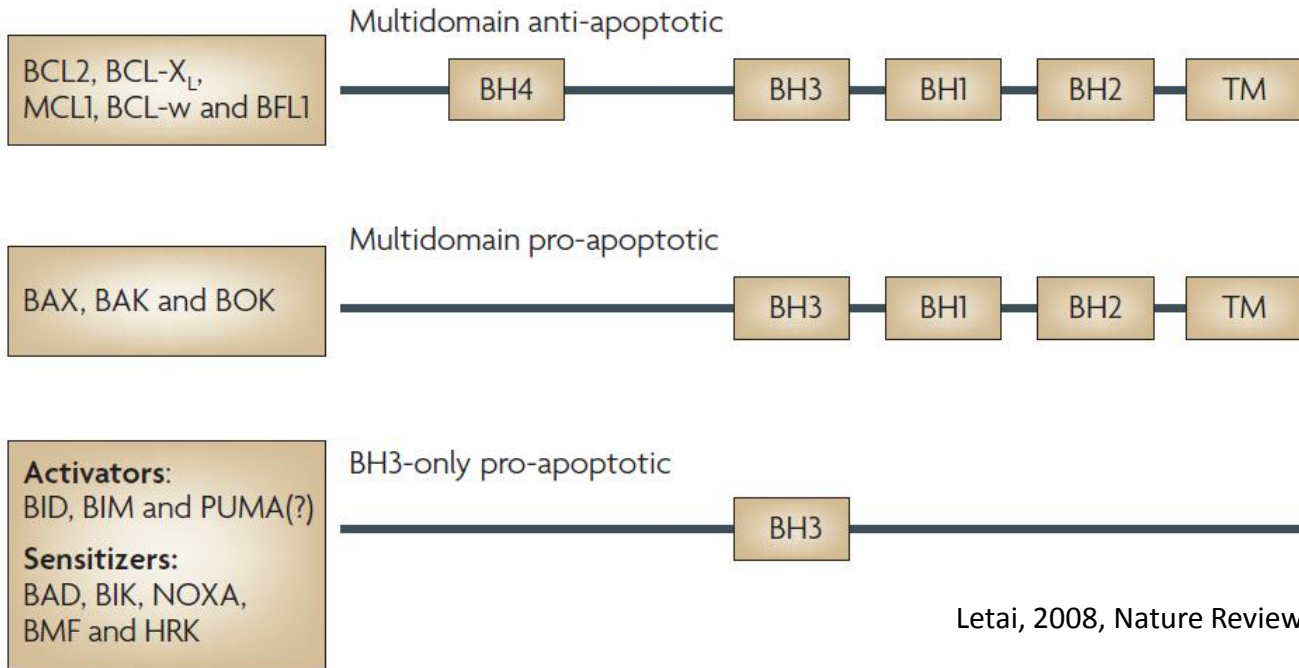
Tim Vervliet
26/10/2012

Promoter: Geert Bultynck
Co-promoter: Jan Parys
Laboratory for Molecular and Cellular Signaling

IP₃Rs ↔ RyRs

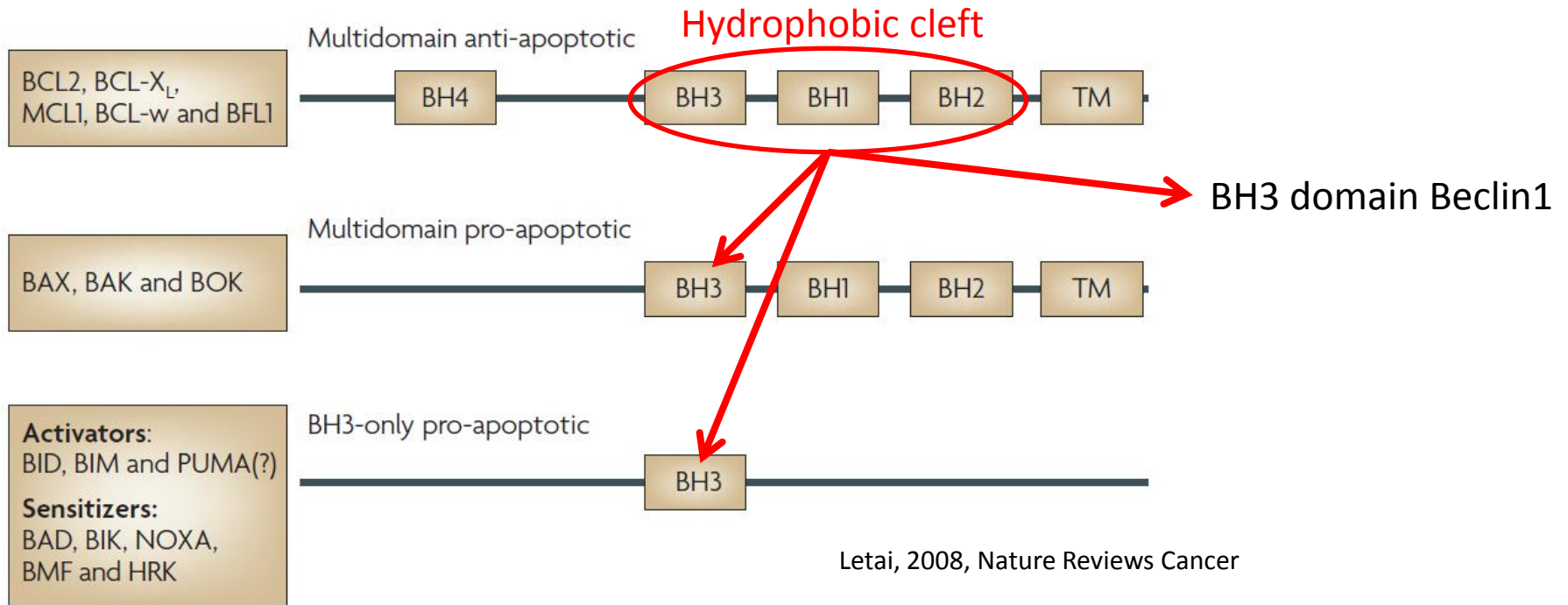
	IP ₃ R	RyR
Molecular weight	±300kDa	±500kDa
Number of isoforms	3	3
Expression	All cells	High expression in specific cell types: skeletal muscle, heart, brain...
Functions	Cell survival, differentiation, proliferation, autophagy, cell death...	Muscle contraction Neuronal signaling: LTP, LTD...

The Bcl-2-protein family

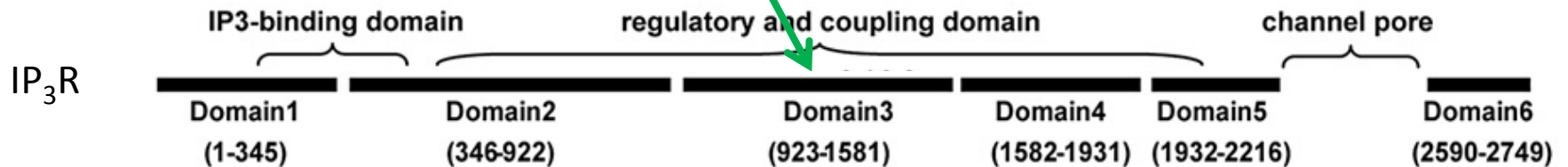
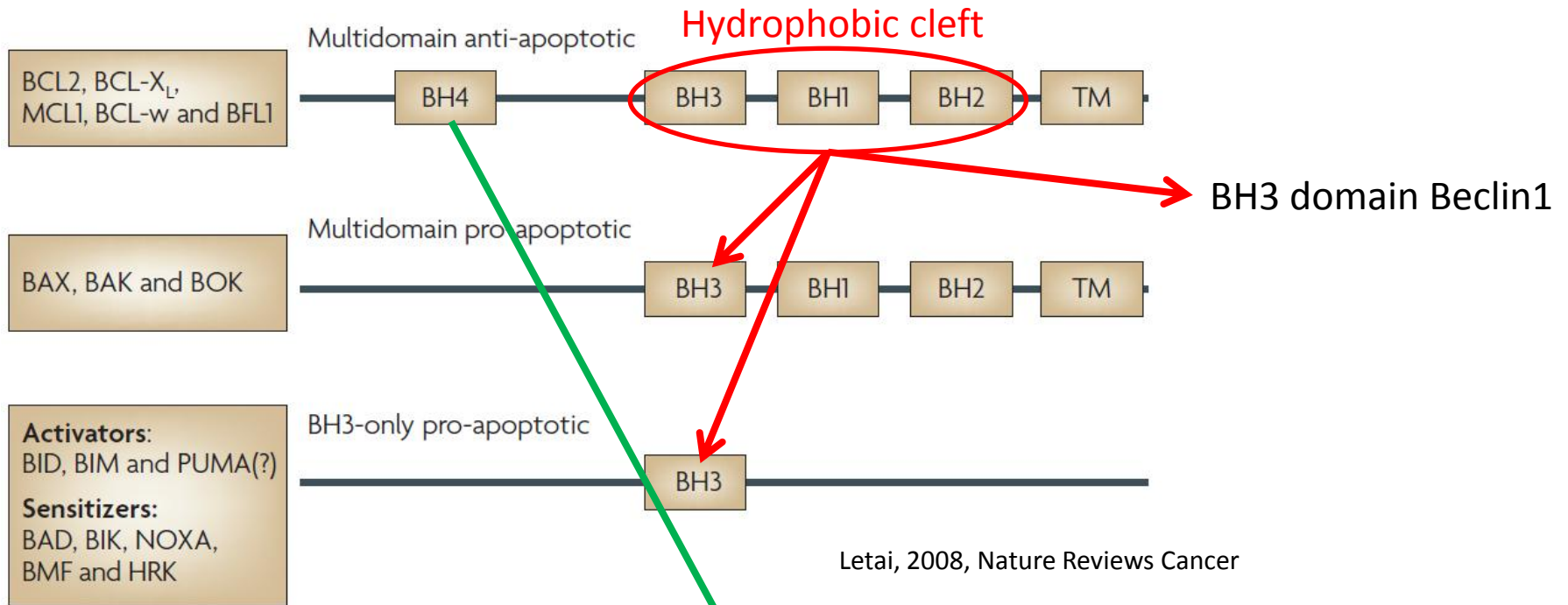


Letai, 2008, Nature Reviews Cancer

The Bcl-2-protein family



The Bcl-2-protein family



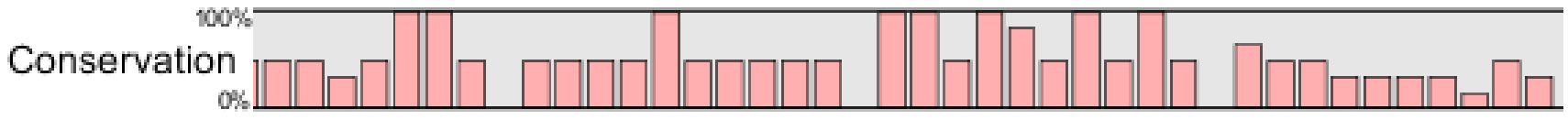
Why study the Bcl-2/RyR interaction?

Bcl-2-binding site IP₃R

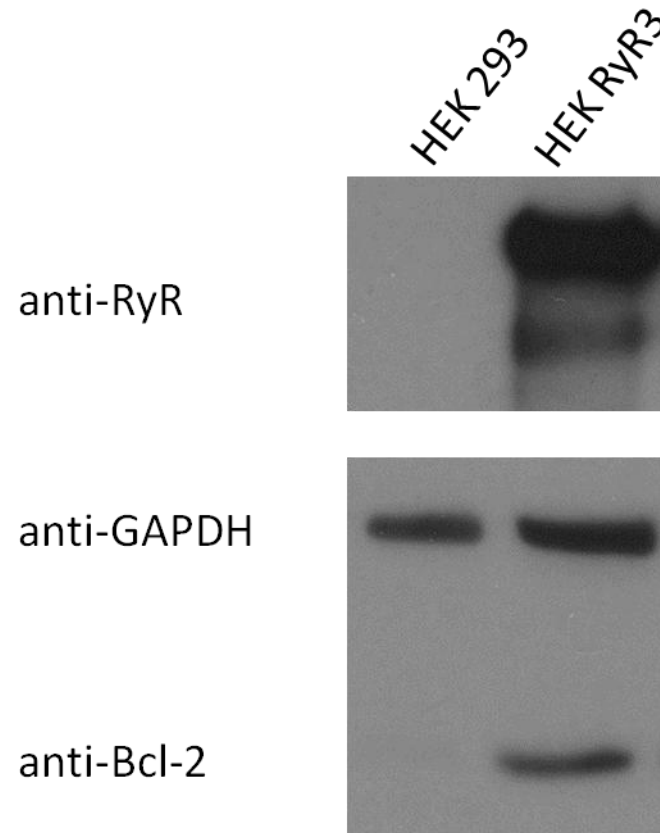


IP3R1	- - -	E G K N	V Y T E	I K C	- -	N	S L L P	L D D	I V R	V V T	- - - - -
IP3R2	- - -	E G K N	V Y T E	I K C	- -	N	S L L P	L D D	I V R	V V T	- - - - -
IP3R3	- - -	E G K N	V Y T E	I K C	- -	T	S L L P	L E D	V V S	V V T	- - - - -
RyR 1	L	I Q A G K G	E A L R	I R A	I L R		S L V P	L E D	L V G	I I S L	P L Q I P T
RyR 2	L	I H A G K G	E A I R	I R S	I L R		S L I P	L G D	L V G	V I S I	A F Q M P T
RyR 3	L	I Q T G K G	E A I R	I R S	I L R		S L V P	T E D	L V G	I I S I	P L K L P S

Consensus | **L I - E G K X** **X X T X I X C I L R** **S L L P L E D L V G** **V X X - - - - P -**



Upregulation of Bcl-2 in HEK RyR3 cells

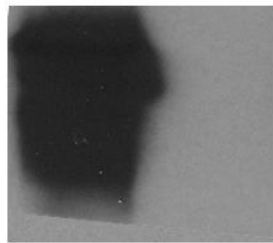


Full-size interaction

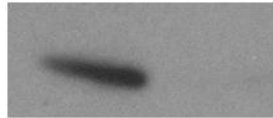
HEK RyR3 cells

RyR antibody	+	-
IgG	-	+

anti-RyR



anti-Flag



3xFlag

Bcl-2

Full-size interaction

HEK RyR3 cells

RyR antibody

+ -

IgG

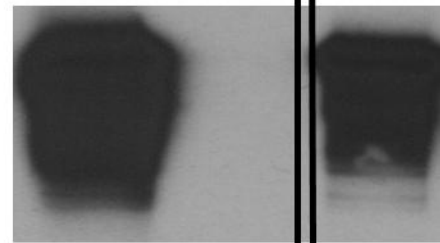
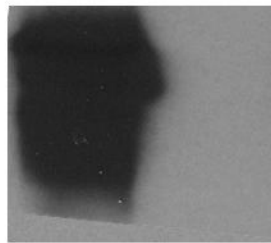
- +

+ -

+ +

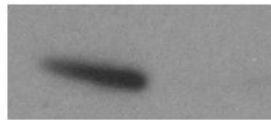
Input

anti-RyR



anti-RyR

anti-Flag



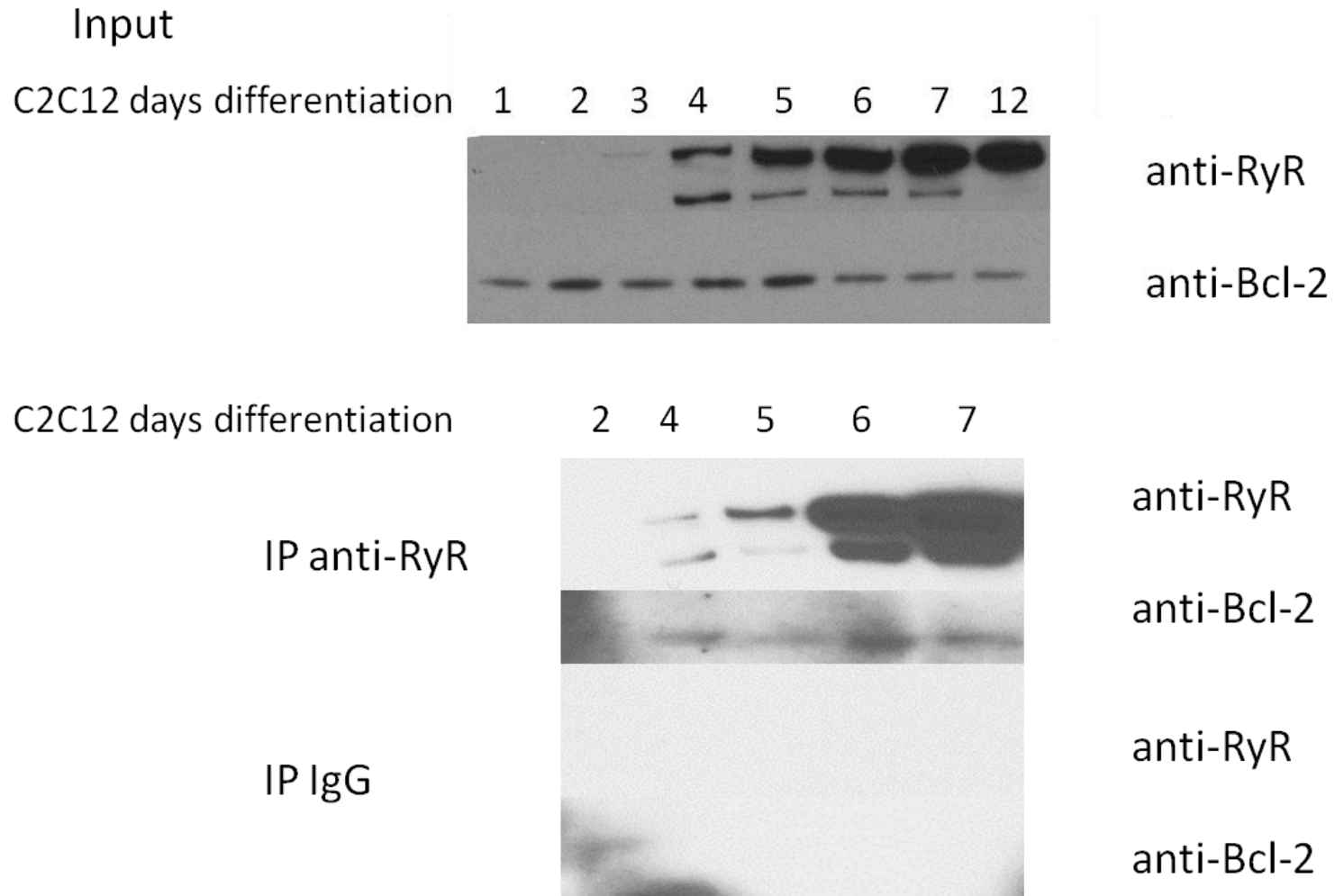
anti-Bcl-2

3xFlag

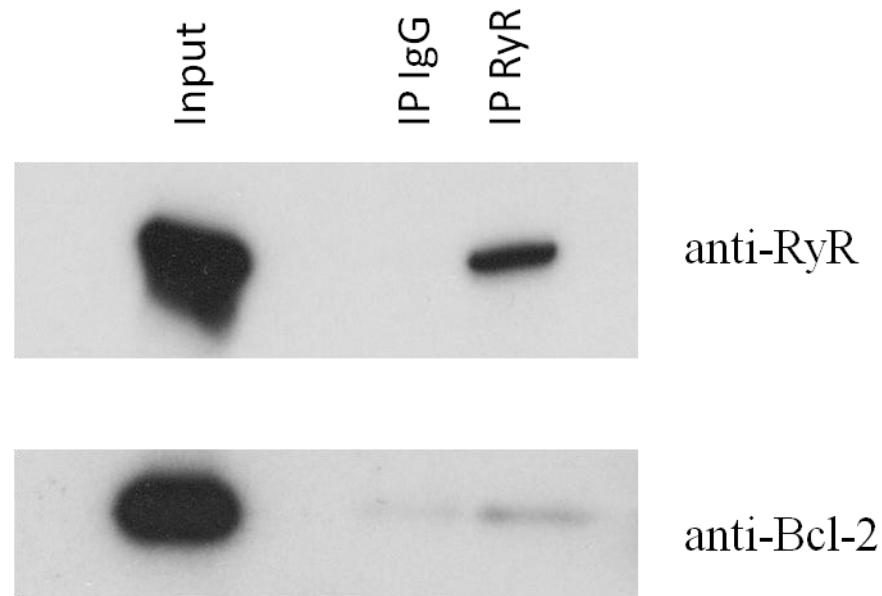
Bcl-2

Endogenous
Bcl-2

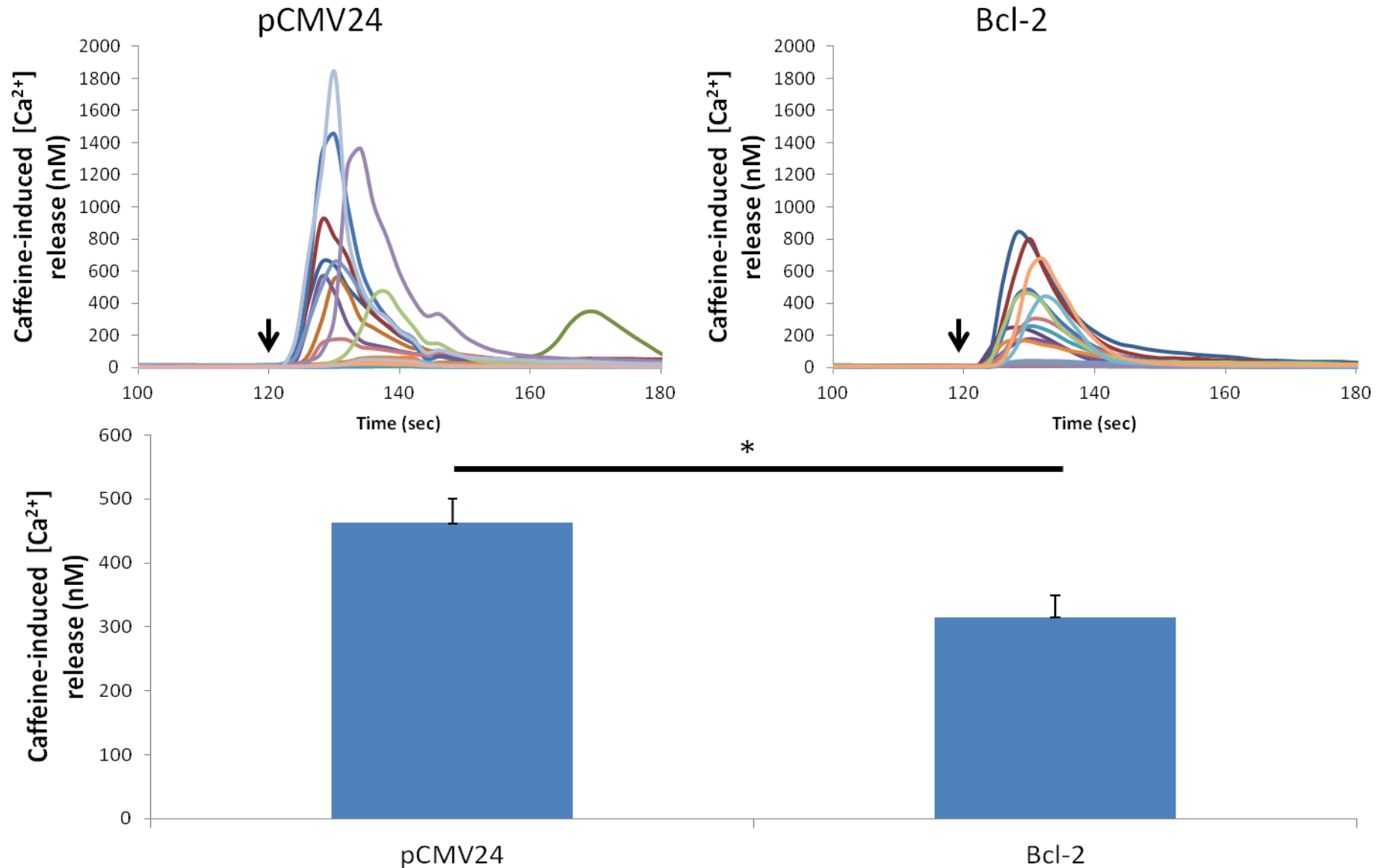
Bcl-2 interacts with the RyR during differentiation of C2C12 cells



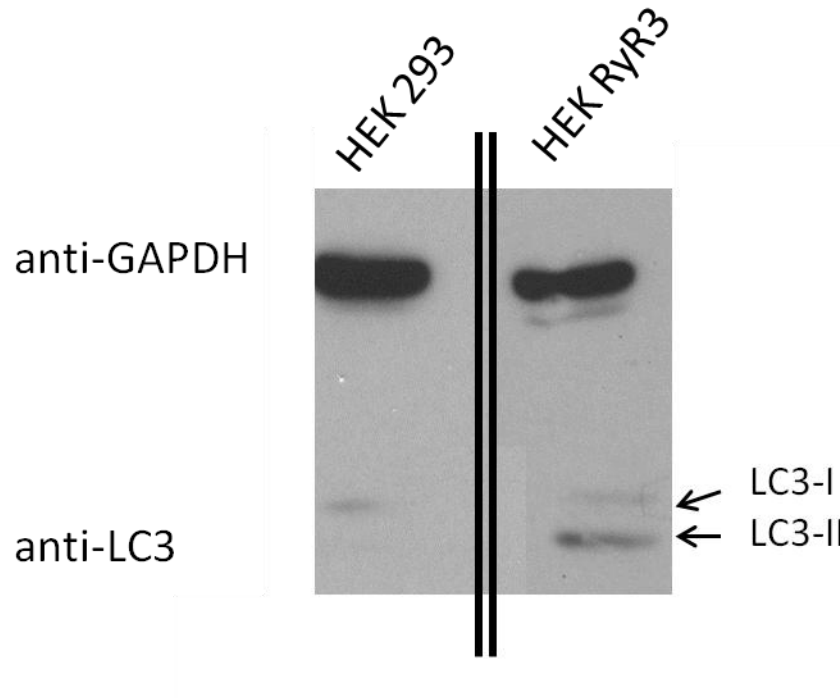
Bcl-2 interacts with RyR in hippocampal rat-brain lysates



Bcl-2 inhibits caffeine-induced Ca^{2+} release

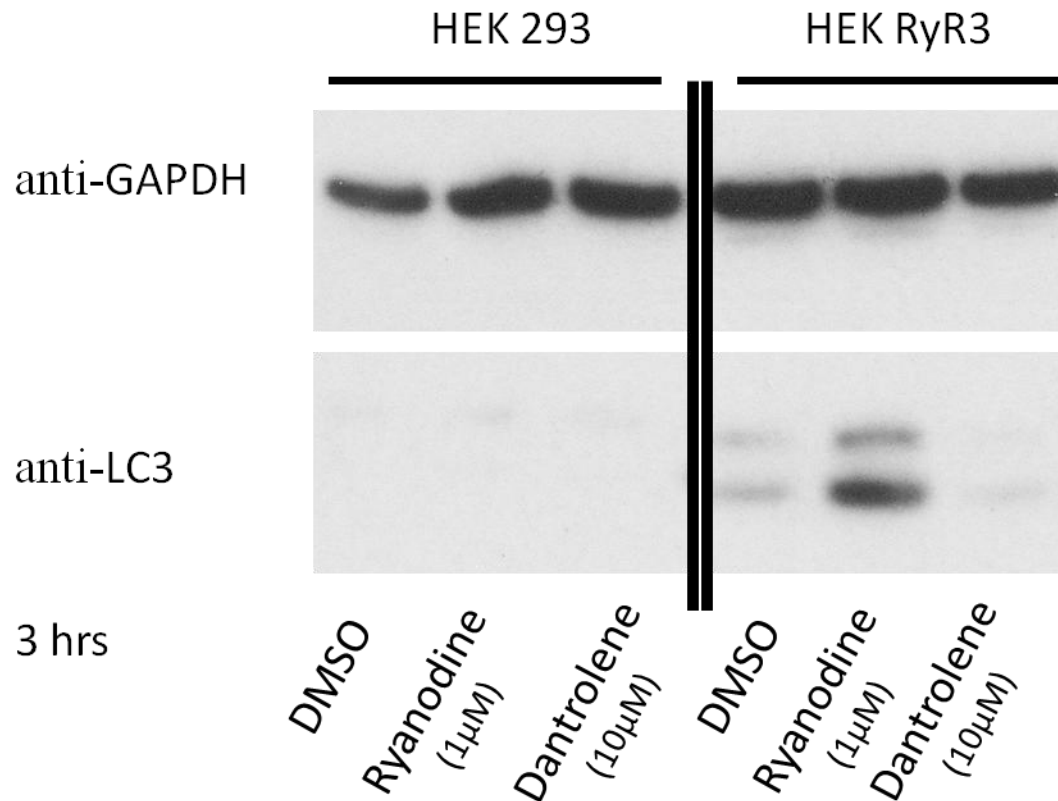


Cell biological role in cell fate: Autophagy?



- HEK RyR3 cells show increased basal levels of LC3-II

Modulating RyR3 activity influences autophagy in HEK RyR3 cells



- RyR3 activity influences LC3-II formation in HEK RyR3 cells

Conclusions

- Bcl-2 interacts with the RyR
- Interaction of Bcl-2 with RyR3 inhibits caffeine-induced Ca^{2+} release
- Role in autophagy?

Acknowledgements

Laboratory for Molecular
and Cellular Signaling:

Giovanni Monaco
Santeri Kiviluoto
Kirsten Welkenhuyzen
Tomas Luyten

Humbert De Smedt
Ludwig Missiaen
Jan Parys
Geert Bultynck

Physiology group UGENT:

Elke Decrock

Luc Leybaert

Laboratory of Intracellular
Ion Channels Bratislava:

Zuzana Tomaskova

Karol Ondrias

Donders Institute for Brain,
Cognition and Behaviour Nijmegen:

Nael Nadif Kasri

Molecular Medicine Section
university of Sienna:

Vincenzo Sorrentino