The potential roles of the Transient Receptor Potential Vanilloid 2 (TRPV2) in post myocardial infarction immune reactions

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Disclosures

None

Fold- change of known Ca^{2+} channel genes genes (MI vs SHAM) Affymerix GC GeneChip array of post MI (5 days) vs Sham- cardiac tissue

Gene	Fold Change (MI vs SHAM)	p-value
SLC8A.1 (NCX.1)	-1.02	0.95
CACNA1C (L-type)	-1.1	0.02
SERCA2	-1.12	0.72
RyR2	-1.52	0.42
PLN (phospholamban)	-1.6	0.085
CASQ2 (Calsequestrin)	-1.51	0.35

Fold change of variance TRP genes (MI vs SHAM) Affymerix GC GeneChip array of post MI (5 days) vs Sham- cardiac tissue

Gene	Fold Change (MI vs SHAM)	p-value
TRPV1	+1.06	0.17
TRPV2	+1.54	0.006
TRPV4	-1.1	0.11
TRPV5	+1.06	0.2
TRPV6	-1.02	0.7
TRPM1	-1.3	0.6
TRPM2	+1.21	0.2
TRPM7	+1.21	0.3

Real-time validation analysis:

Fold change (MI vs Sham): Rats- 4.5; Mice- 10.0

Entin-Meer M et.al ,J Clinic Experiment Cardiol (2012)

TRP relatively non-selective cation channels

33 channels composed of 6 membrane-spanning helices with intracellular N- and C termini:

TRPC (canonical) – associated with Focal segmental glomerulosclerosis

TRPA (ankyrin) - Stress (mechanical) receptor

TRPM (melastatin) – associated with hypomagnesemia with secondary hypocalcemia

TRPP (polycystin) – associated with polycystic kidney disease

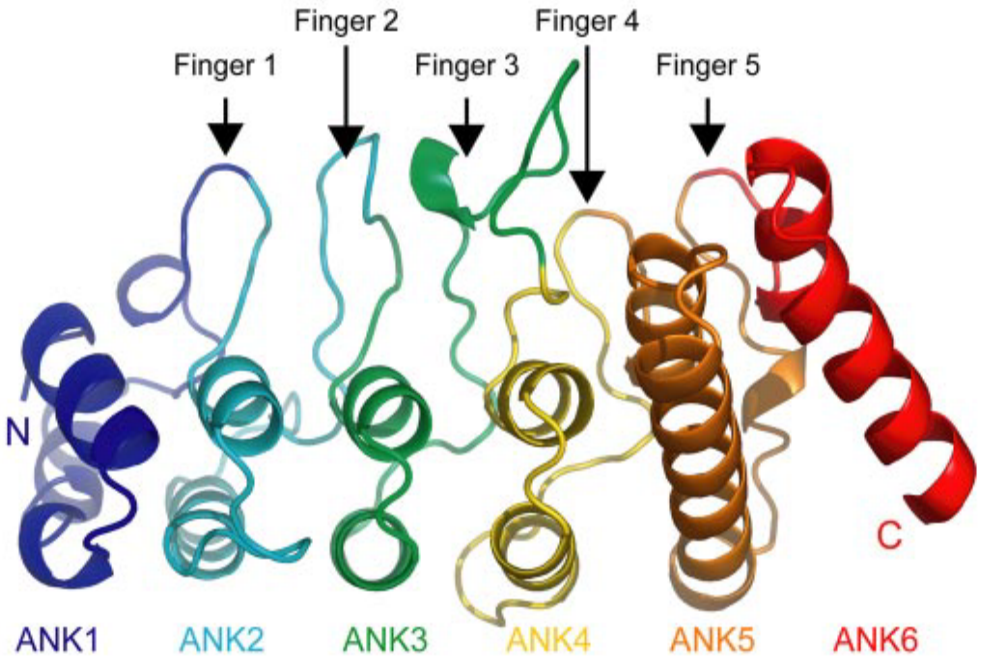
TRPML (mucolipin) - associated with mucolipidosis

TRPN (NOMPC) – no mechanoreceptor potential C - not found in mammals

TRPV (vanilloid)- mediates pain/hot sensations

The TRPV Family

-All TRPV subfamily are trans-membrane proteins contain an intracellular N-terminal ankyrin repeat domain (ARD), a prevalent protein interaction motif.



TRPV1 and TRPV2

From a cardiovascular point of view- TRP channels are important in controlling:

- **Vascular function including endothelial permeability**
- **Responses to oxidative stress**
- **Myogenic tone**

TRPV-2

-A weak non- voltage gated quite Ca^{2+}-selective cation channel

-Regulated by Insulin-Like Growth Factors (IGF) -

Activated by high temperatures (>52^{o}C) and by specific pharmacologic agonists such as: -2-Aminoethoxydiphenyl borate (2-APB), Inhibited by Ruthenium red.

- Expressed in CNS, brain, spleen, liver, lung and slightly expressed in the heart, aortic smooth muscle cells.

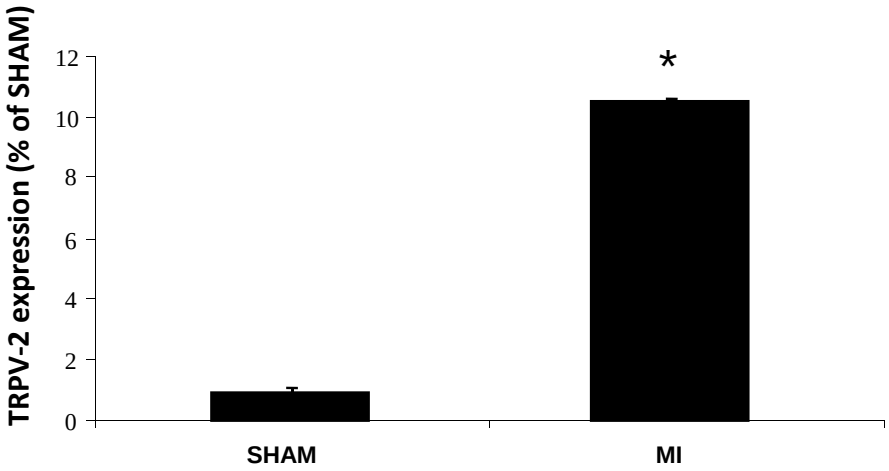
-TRPV2 is expressed in phagocyte populations and has a pivotal role in macrophage particle binding and phagocytosis (Caterina , Nature Immunology, 2010).

-May be involved in the induction of apoptotic cell death (Yamada T, Urology, 2010)

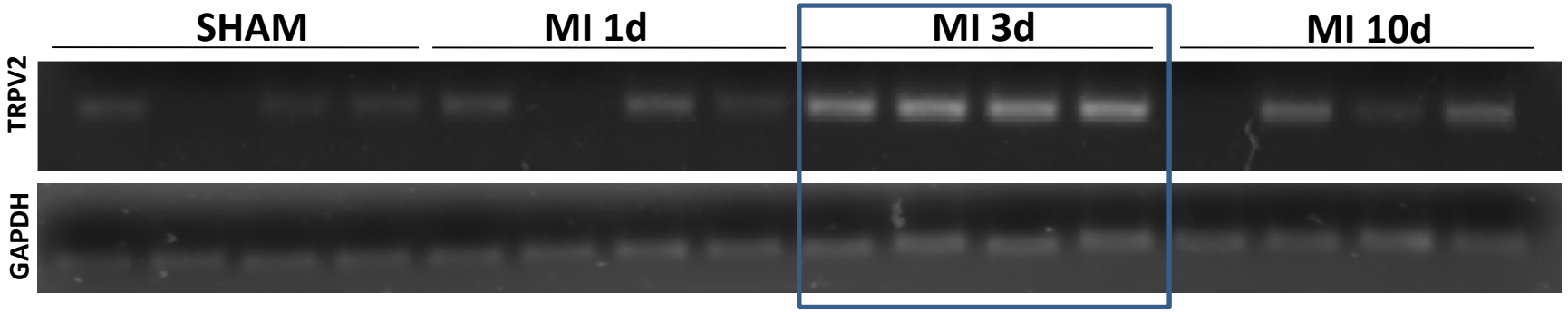
-**Nevertheless, the role of TRPV2 in cardiac function and myocardial ischemia is still unknown**

TRPV2 expression upon acute MI (In vivo model)

mRNA levels of TRPV2 are significantly upregulated in the cardiac tissue within 3-5 days post acute MI

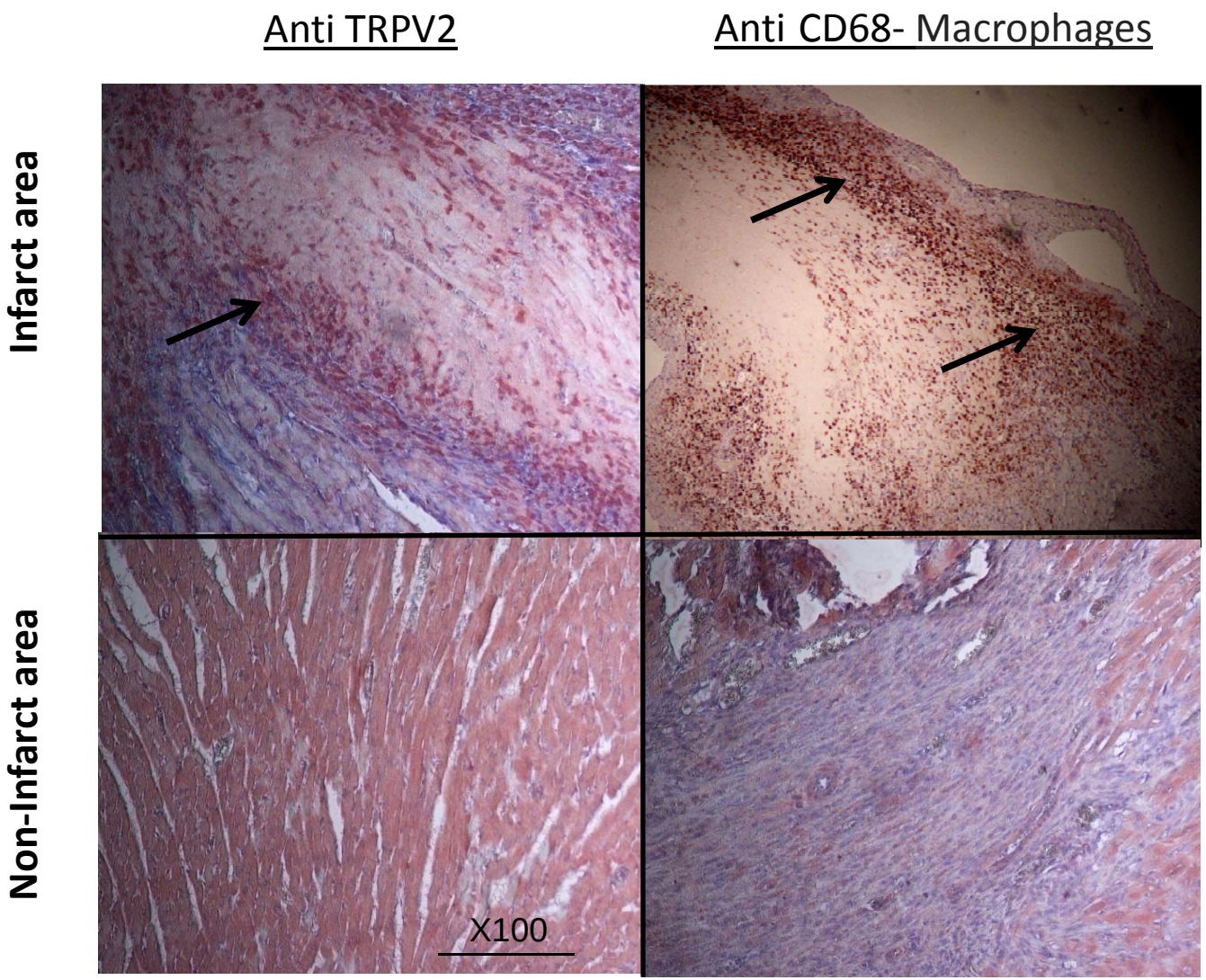


Agarose gel electrophoresis of TRPV2 fragment



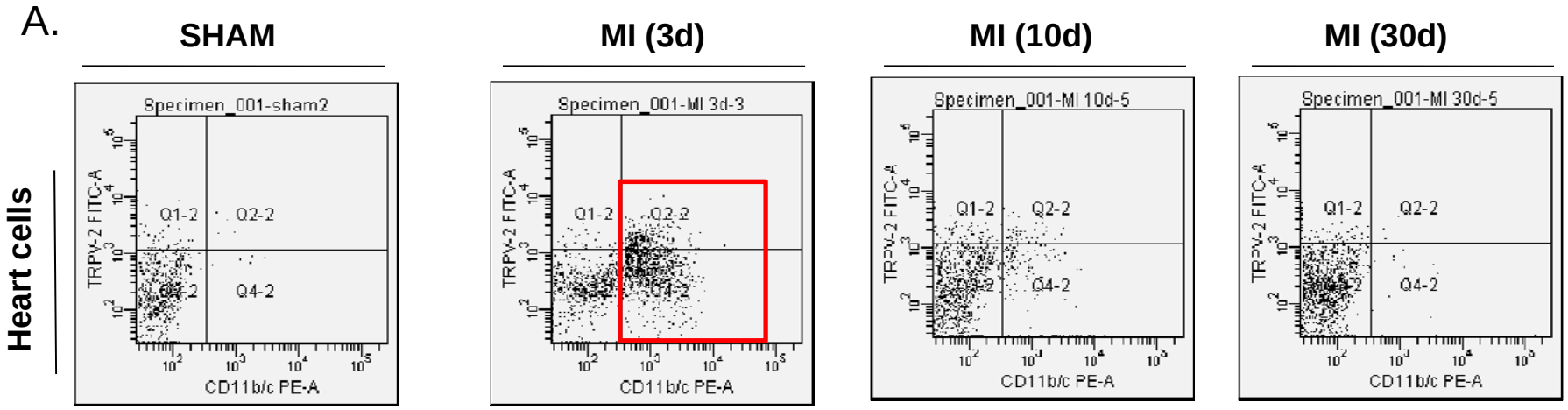
→ PCR analysis demonstrated a clear upregulation of TRPV2 expression in the LV three days post MI

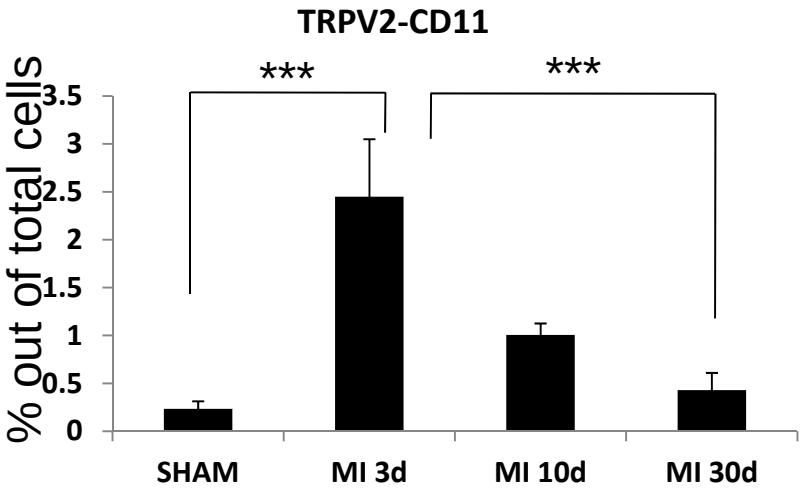
Peri-infarct infiltrating macrophages harbor TRPV2 expression 3 days post acute MI

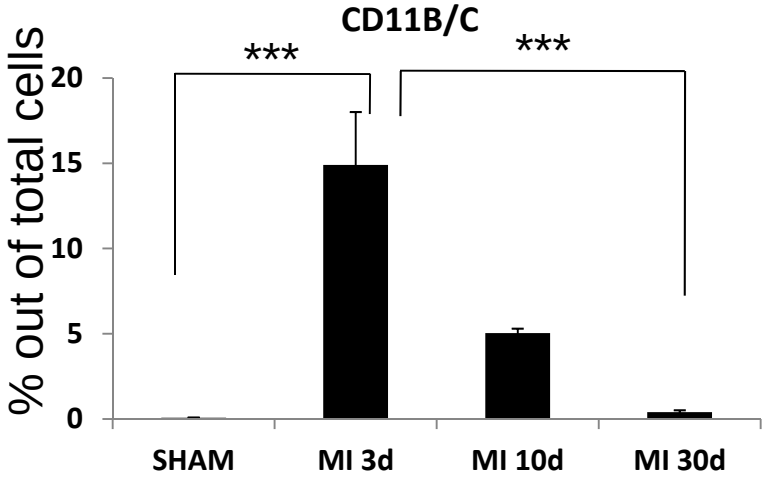


TRPV2 is upregulated in immune cells that migrated to the infracted cardiac tissue of rats

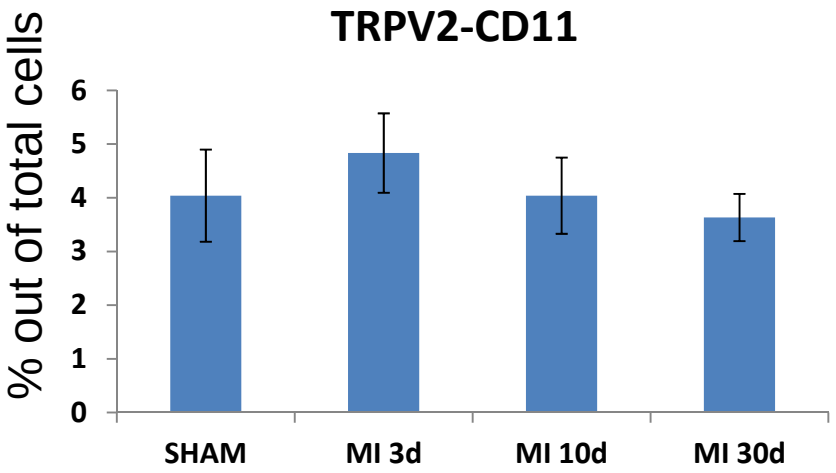
FACS analysis:

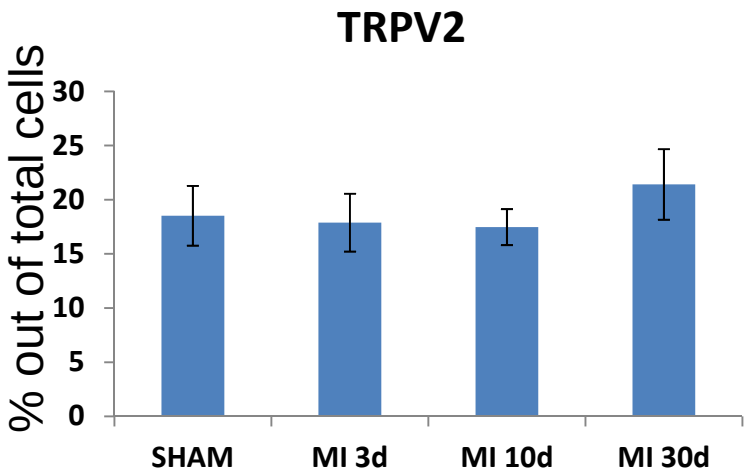


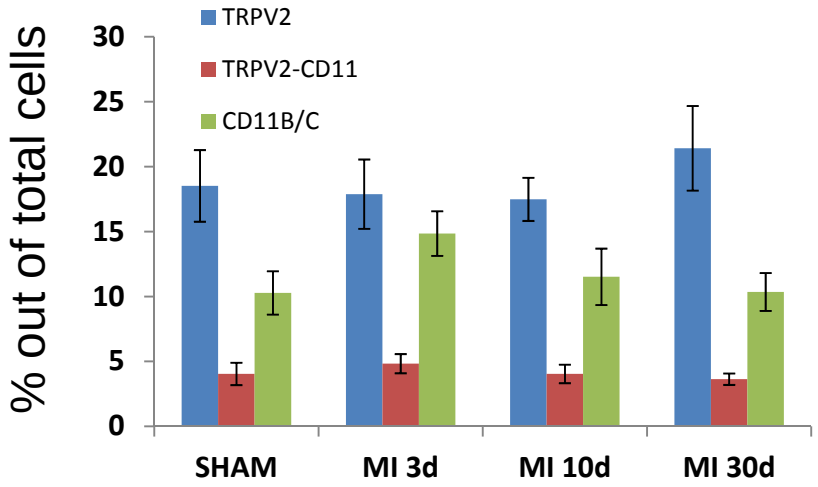


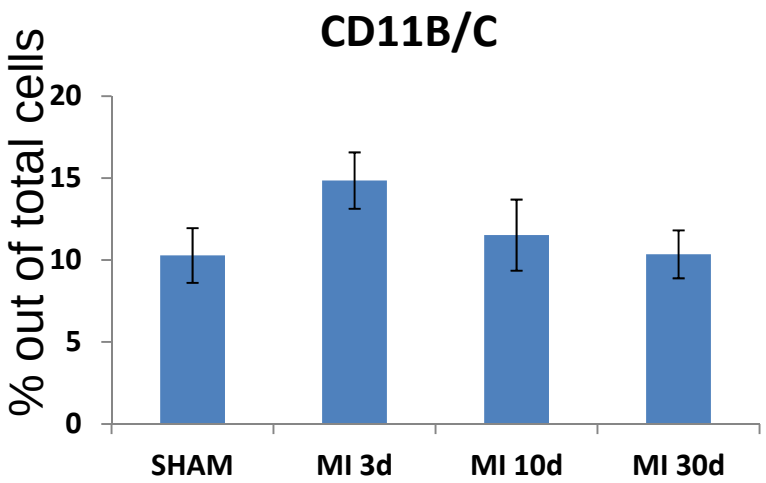


Facs Analysis TRPV2-FITC + CD11B/C-PE-Blood Cells





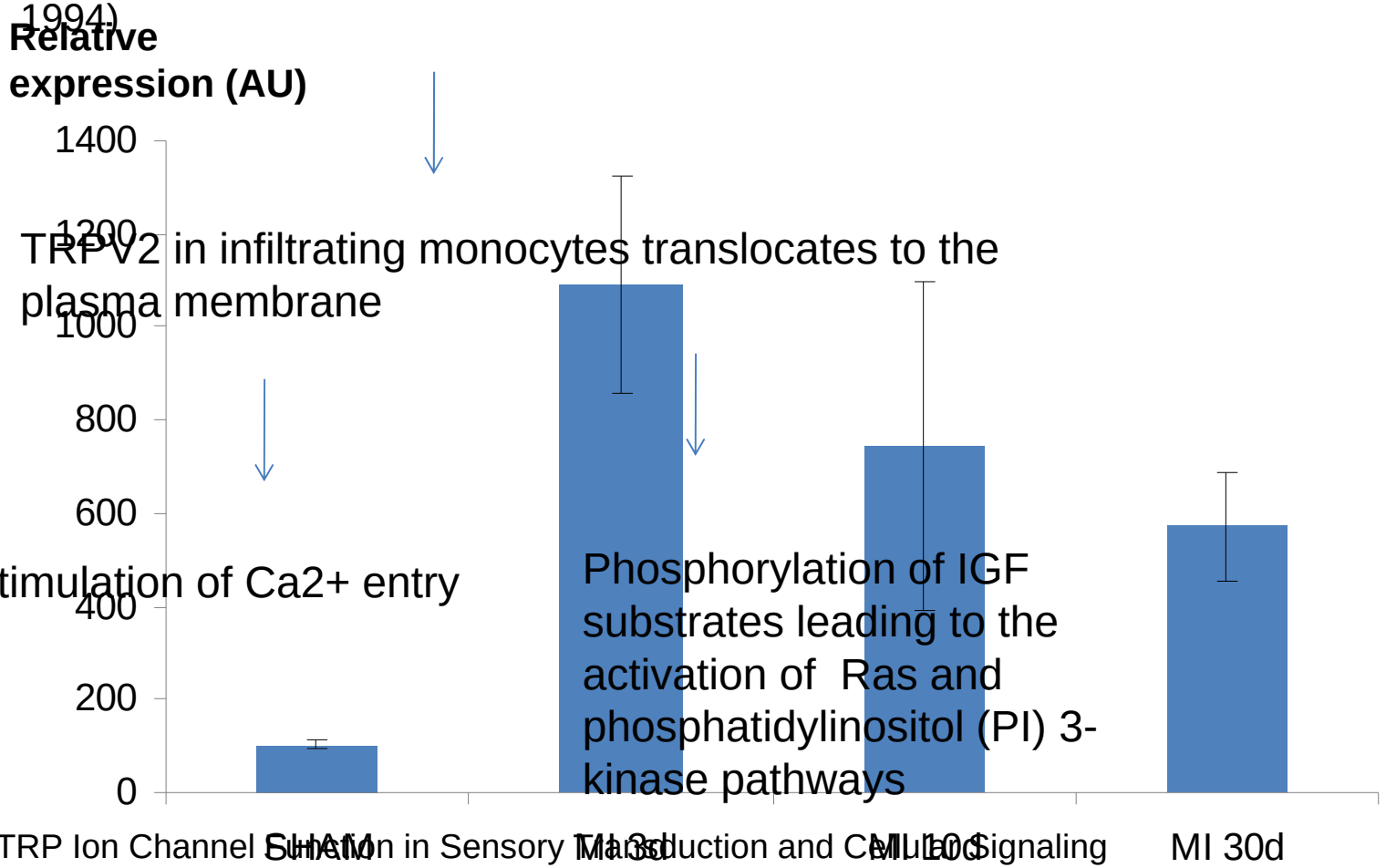




Hypothesis

Insulin growth factor (IGF) is upregulated in the LV concomitant with TRPV2 overexpression

IGF1 is overexpressed in the healthy cardiomyocytes after acute MI (Reiss, Exp Cell Res, 1994)



TRP V2 in infiltrating monocytes translocates to the plasma membrane

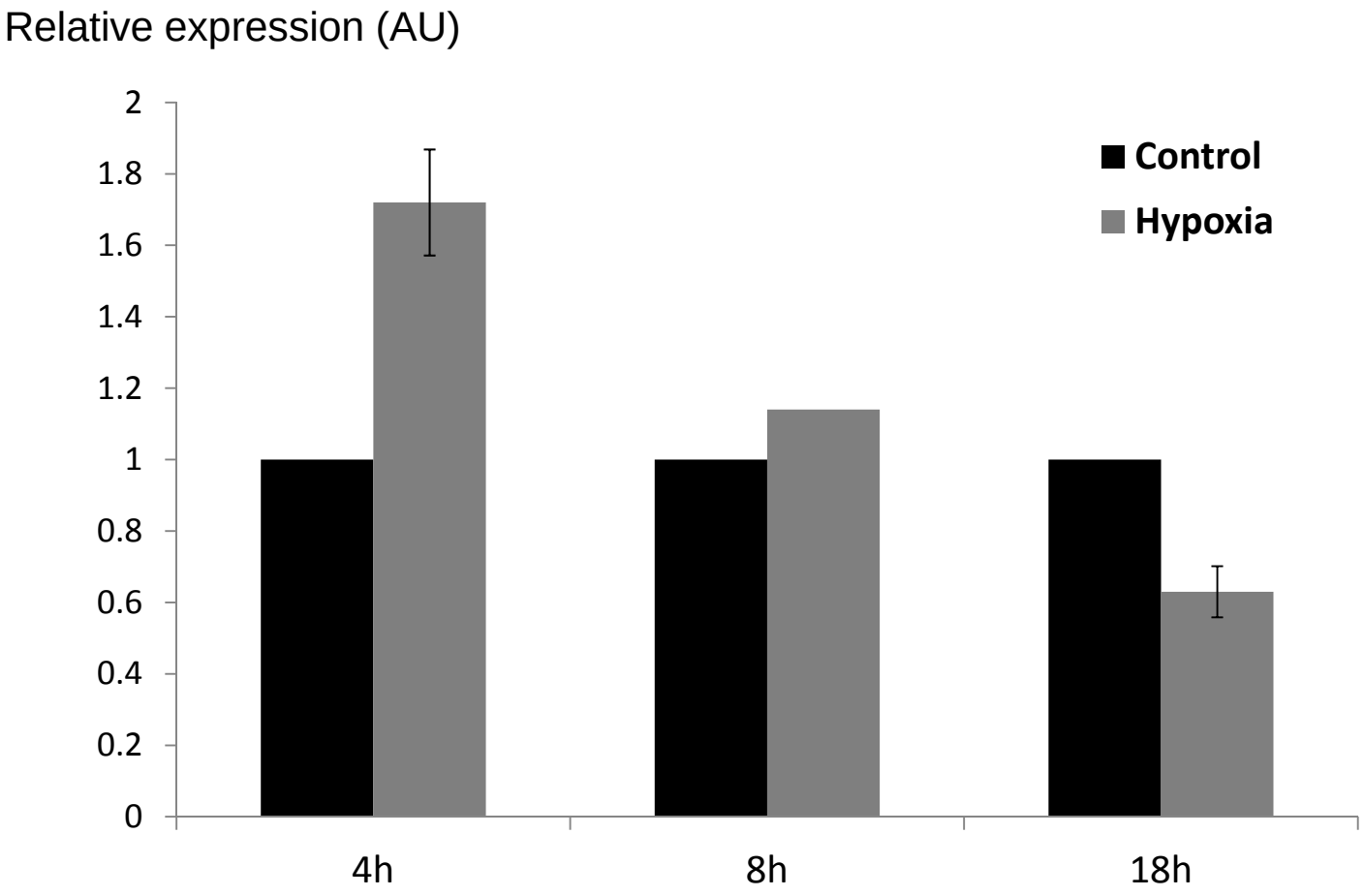
Stimulation of Ca2+ entry

Phosphorylation of IGF substrates leading to the activation of Ras and phosphatidylinositol (PI) 3-kinase pathways

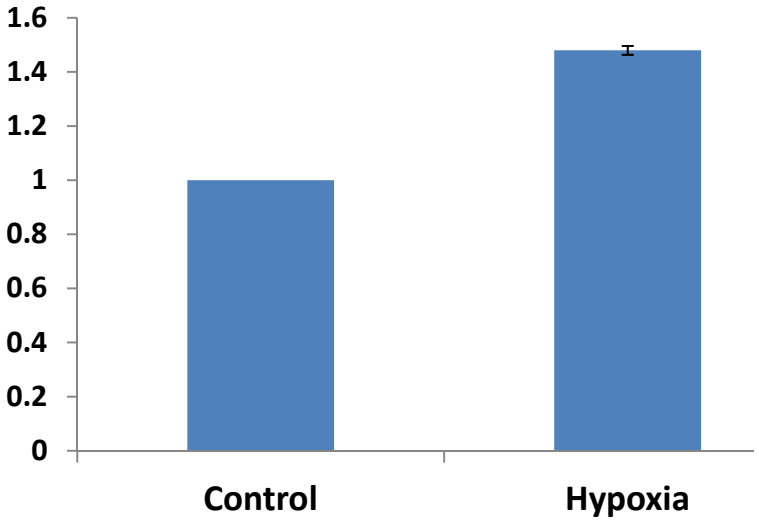
TRP Ion Channel Function in Sensory Transduction and Cellular Signaling Cascades. Liedtke WB, Heller S, editors. Boca Raton (FL): CRC Press; 2007.

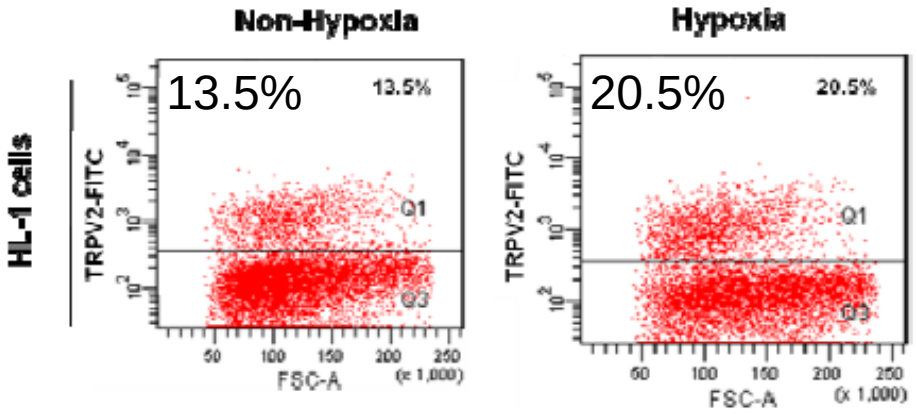
TRPV2 expression in cardiomyocytes exposed to hypoxic conditions (In vitro model)

Real Time PCR-HL-1-TRPV2

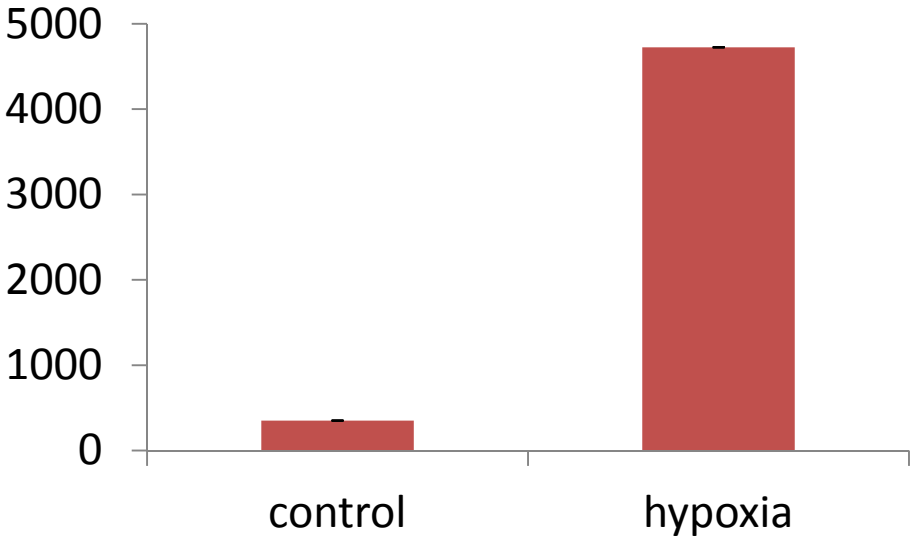


FACS Analysis – 18h-TRPV2 FITC

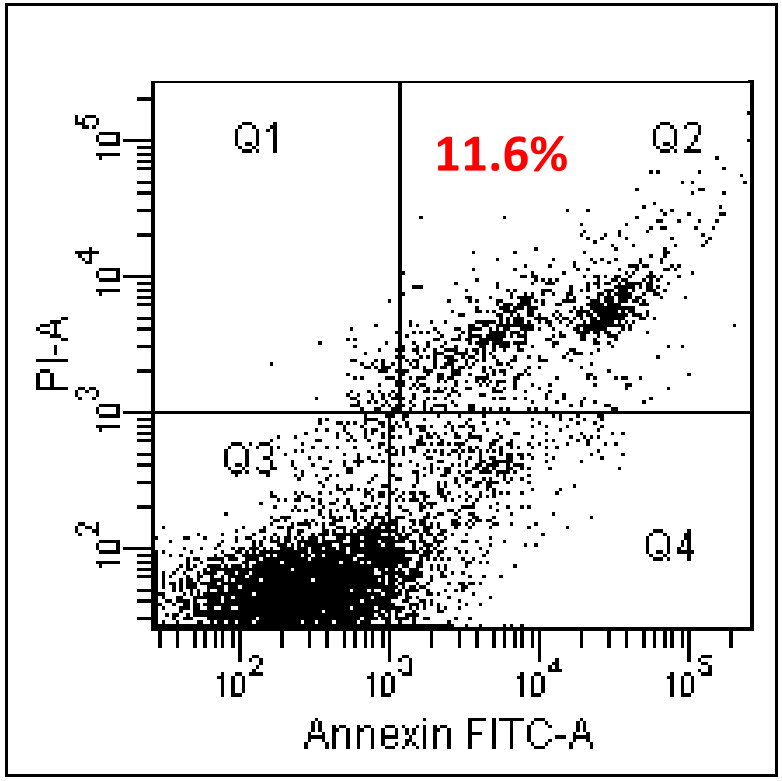




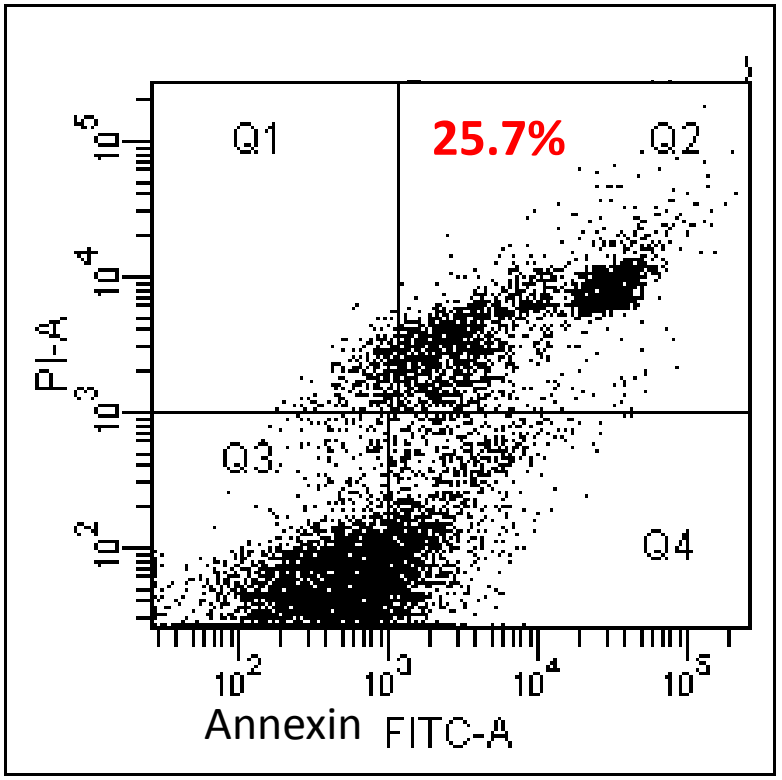
Number of dead cells in the media



HL-1 Normal



HL-1 Hypoxia



Upregulated expression of TRPV2 is associated with apoptosis of HL-1 cells

Summary

- TRPV2 is highly expressed in the peri-infarct infiltrating monocytes (M1/M2) 3 -10 days post MI

  TRPV2 may play a pivotal role in phagocytic processes (Caterina , Nature Immunology, 2010).

- TRPV2 is upregulated in murine cardiomyocyte cell line shortly after exposure to hypoxic conditions.
- This upregulation is associated with increased cell death, including apoptosis (TRPV2 siRNA).
  Up to 24 h post MI

Cardiomyocyte cell death → **Removal of the dead cardiomyocytes**

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Future Plans:

- Define the sub-type of the TRPV2 expressing monocytes in post MI reactions: TRPV2/ CD68/ CD80- M1 (pro-inflammatory)
  TRPV2/ CD68/ CD163- M2 (anti-inflammatory)

- Assess whether the migratory capacity of monocyte cell lines towards the suprnatant of HL-1 cells undergoing hypoxia is TRPV2-dependent

- MI model in TRPV2 KO mice in order to assess whether TRPV2 expression is beneficial or detrimental for post MI recovery

- Assess the TRPV2 levels in circulating lymphocytes in post MI patients compared to normal coronaries

- Can siRNA to TRPV2 attenuate cardiomyocyte cell death upon hypoxia?