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Phosphorylation of Vasodilator-Stimulated Phosphoprotein Prevents Platelet-Neutrophil Complex Formation and Dampens Myocardial Ischemia-Reperfusion Injury

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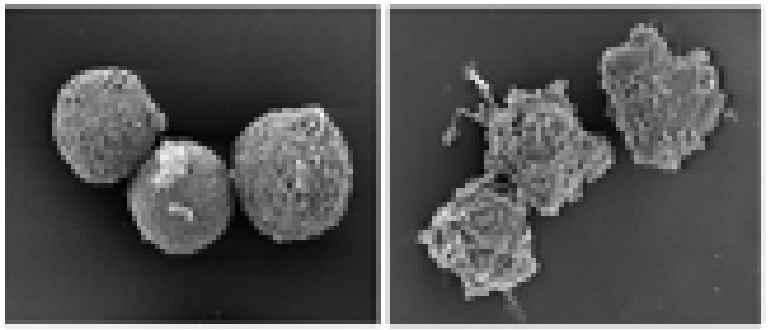
PNC and AMI

- PNC (platelet-neutrophil complexes) are directly associated with I/R injury → inflammatory tissue damage
- PNC: neut: CD11b/CD18
  platelets: GPIIb/IIIa
  } fibrinogen

→ Changes in cytosceletal conformation pivotal
VASP mediated

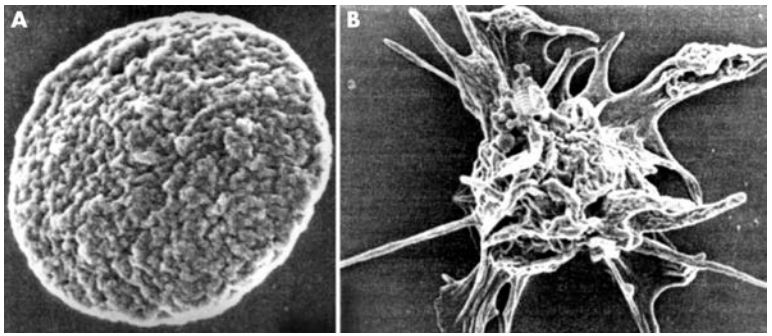
VASP (vasoactive-stimulated phosphoprotein)

neutrophils



Pharmacol Rev. 2010 Dec;62(4):726-59.

platelets



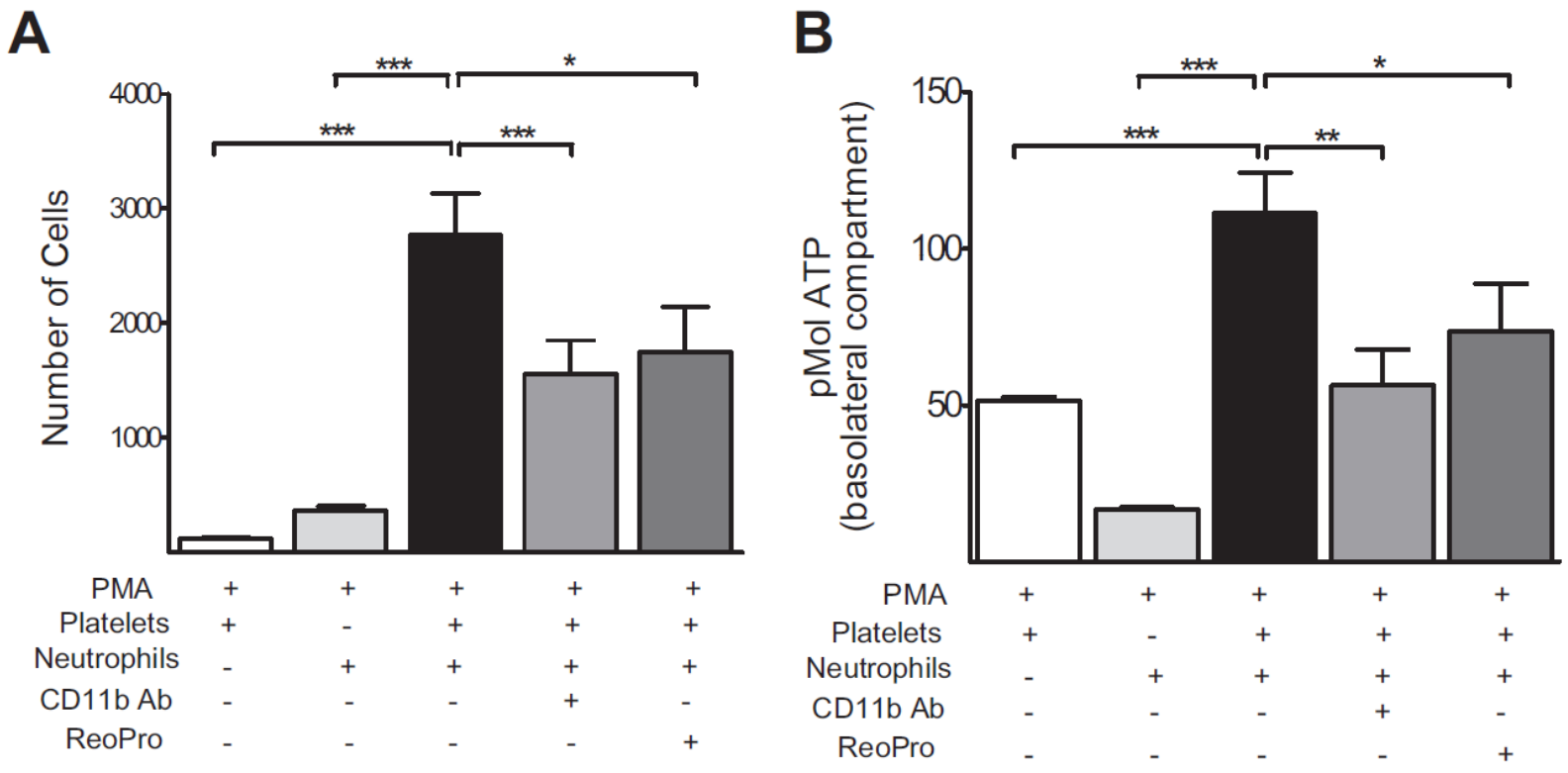
Heart. 2003 Oct;89(10):1273-8.

VASP

- Phosphorylated form: inhibition of cytosceletal reorganisation in neutrophils and platelets
- Ser^{157}: cAMP
- Ser^{239}: cGMP

Vasodilator-Stimulated Phosphoprotein Phosphorylation Affects Neutrophil Facilitated Transendothelial Platelet Movement

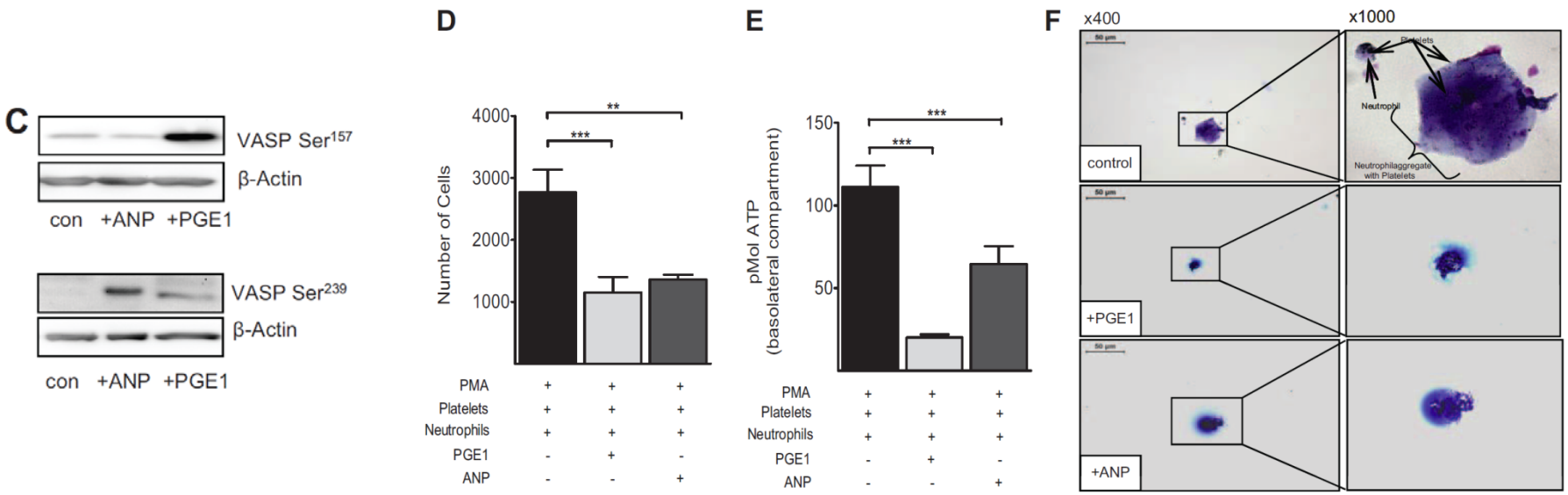
PNC formation augments transmigration of Neu and Platelets
(confirmation of previously published data)



PMA: phorbol-12-myristate-13-acetate

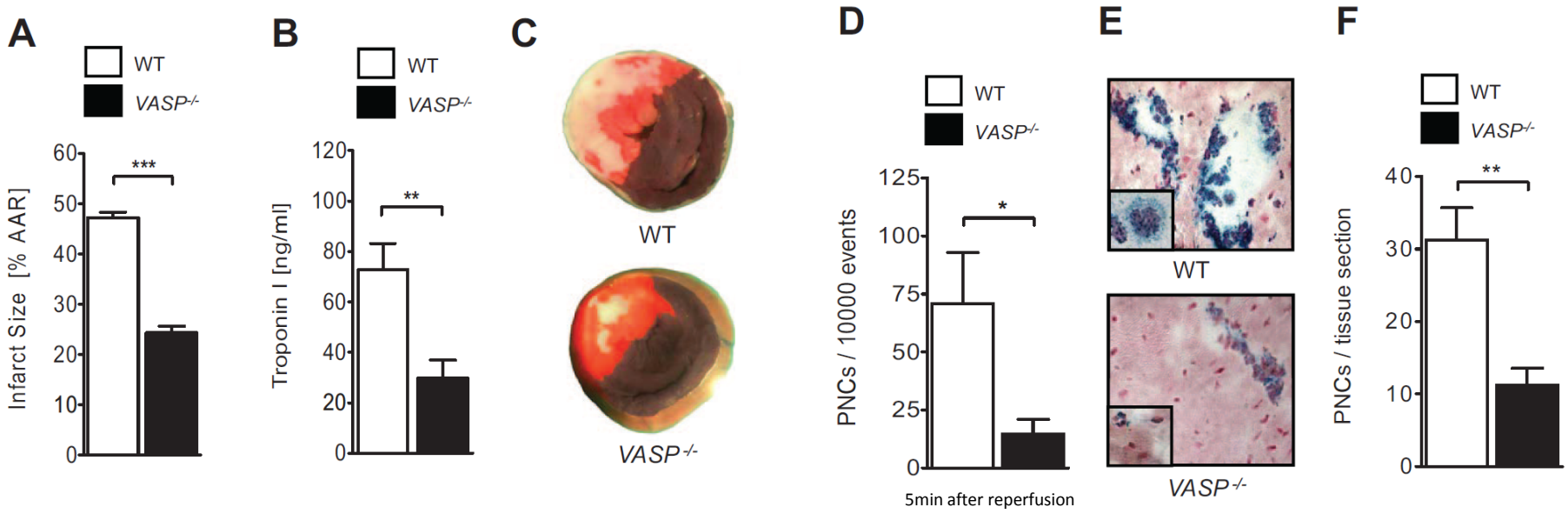
Vasodilator-Stimulated Phosphoprotein Phosphorylation Affects Neutrophil Facilitated Transendothelial Platelet Movement

Is VASP responsible for formation of PNC and increased transmigration?



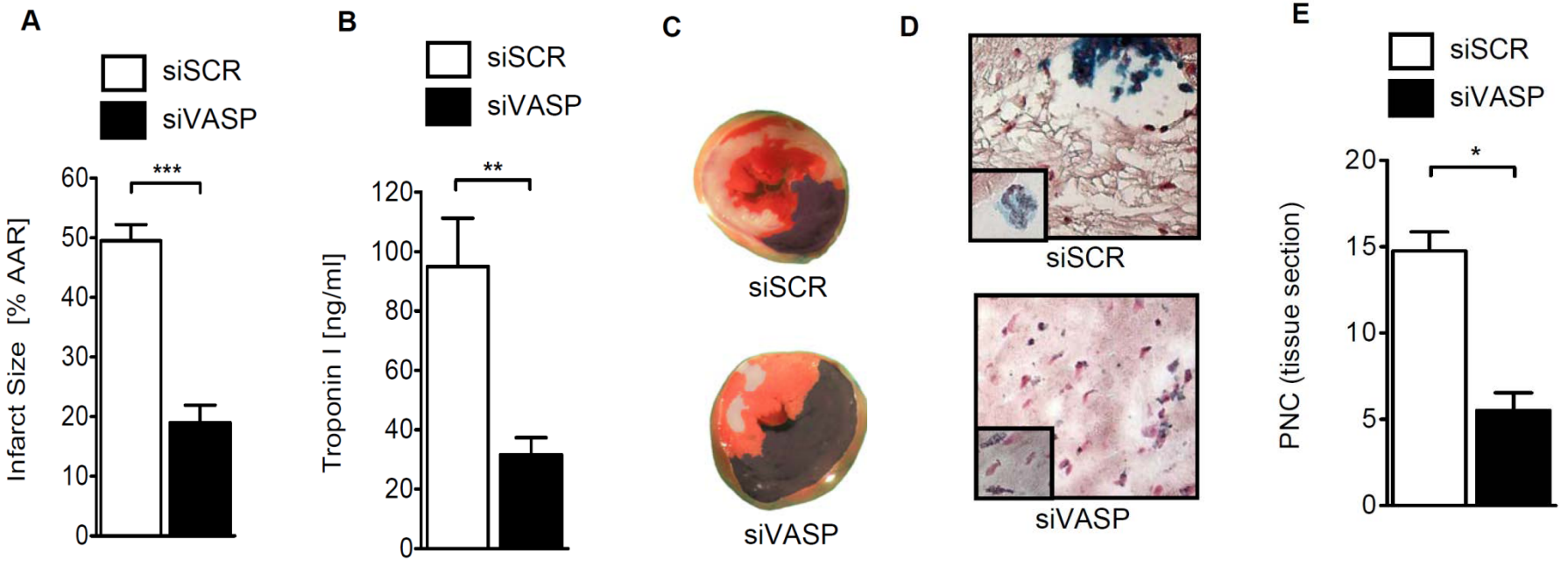
ANP: atrial natriuretic peptide
PGE1: prostaglandin E1

Vasodilator-Stimulated Phosphoprotein$^{-/-}$ Mice Demonstrate Reduced Platelet-Neutrophil Complexes Formation and Attenuated Myocardial IR Injury

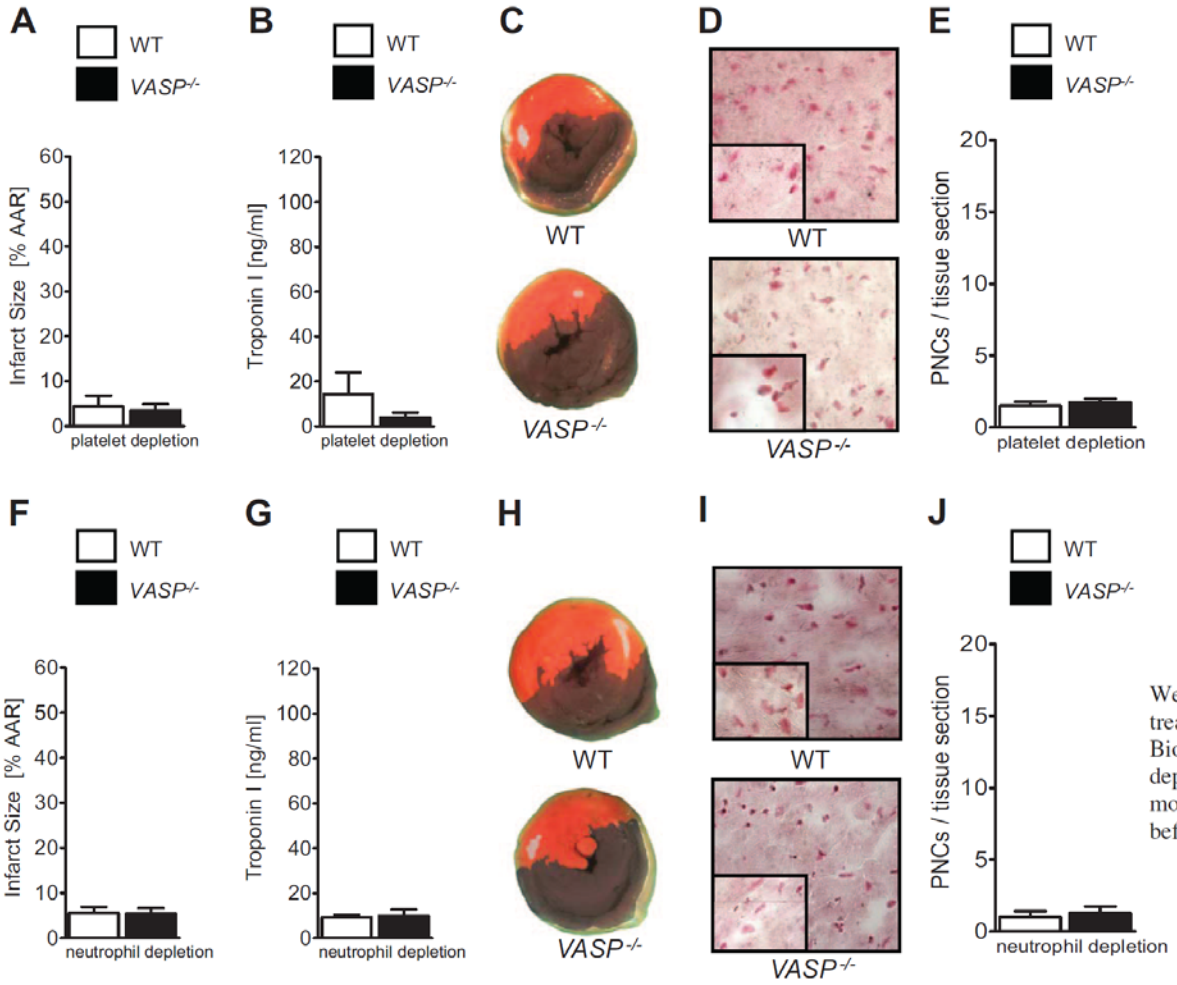


After left parasternal thoracotomy, the left coronary artery was visually identified and an 8.0 nylon suture (Prolene, Ethicon, Norderstedt, Germany) was placed around the vessel. 60 minutes of myocardial ischemia followed by 3 hours of reperfusion. VASP–deficient mice demonstrated

Vasodilator-Stimulated Phosphoprotein$^{-/-}$ Mice Demonstrate Reduced Platelet-Neutrophil Complexes Formation and Attenuated Myocardial IR Injury

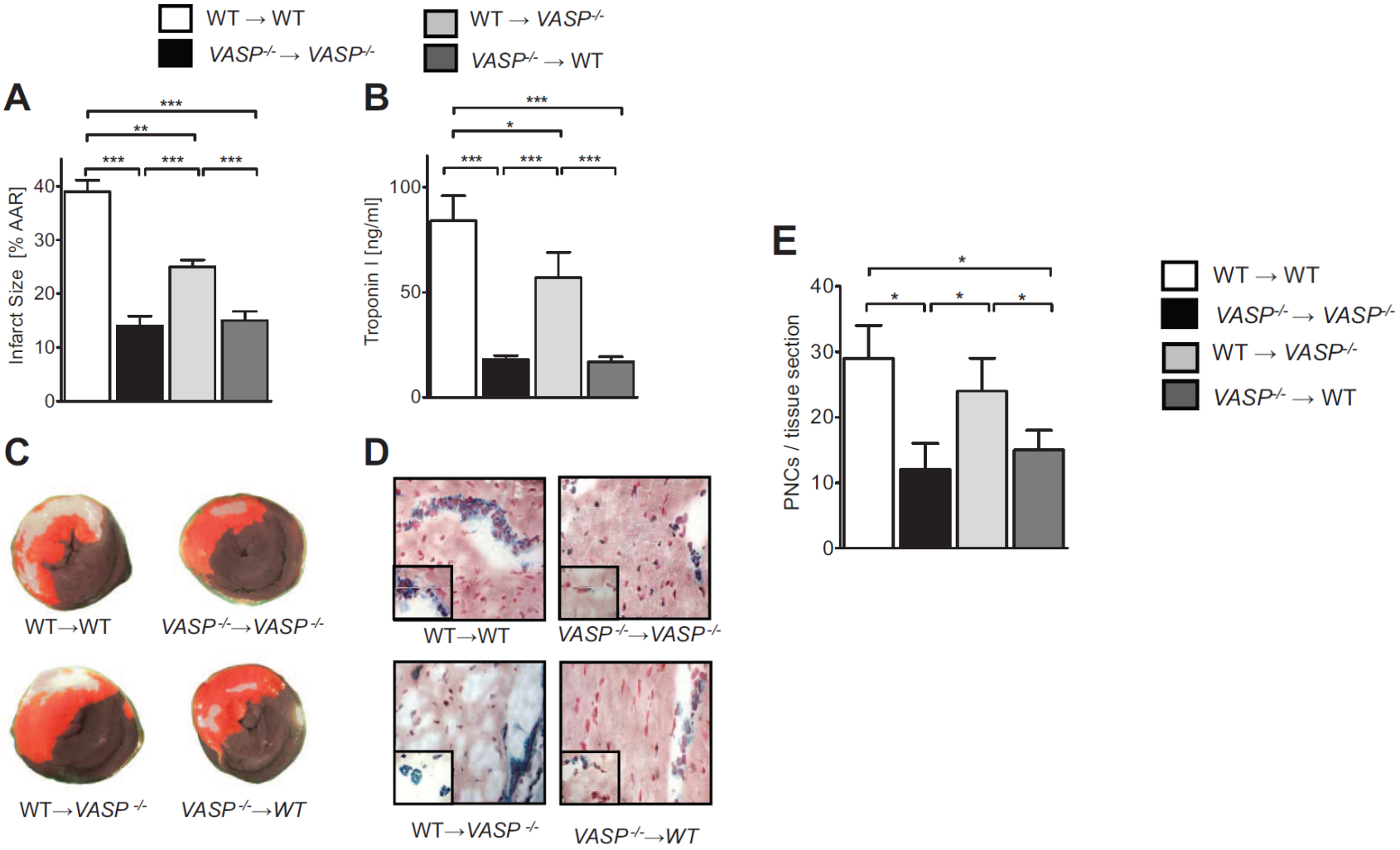


Platelet Depletion, Neutrophil Depletion, and Crossover Injection Identify the Importance of Platelet-Neutrophil Complexes for Myocardial Ischemia-Reperfusion Injury

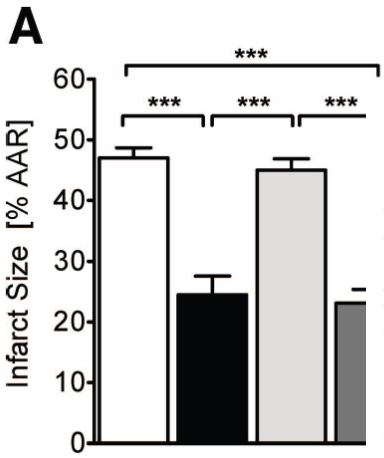


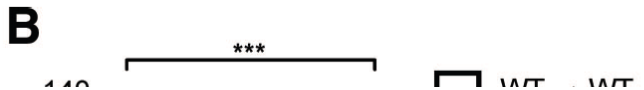
We achieved neutrophil depletion using neutrophil-specific antibody treatment (RB6-8C5, 150 μg per mouse, intraperitoneally; BD Bioscience) 24 hours before the experiments. We achieved platelet depletion using an antibody-to-mouse thrombocyte serum (50 μl per mouse, intravenously; Accurate Chemical and Scientific) 2 hours before the experiments.

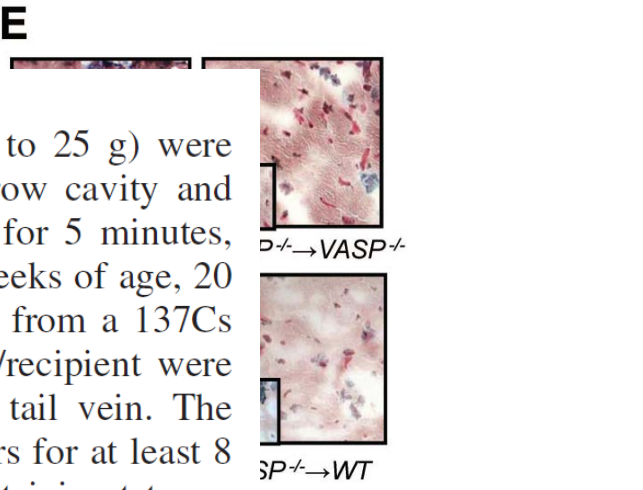
Platelet Depletion, Neutrophil Depletion, and Crossover Injection Identify the Importance of Platelet-Neutrophil Complexes for Myocardial Ischemia-Reperfusion Injury



Platelet-Neutrophil Complexes Formation Is Dependent on Hematopoietic Vasodilator-Stimulated Phosphoprotein Expression

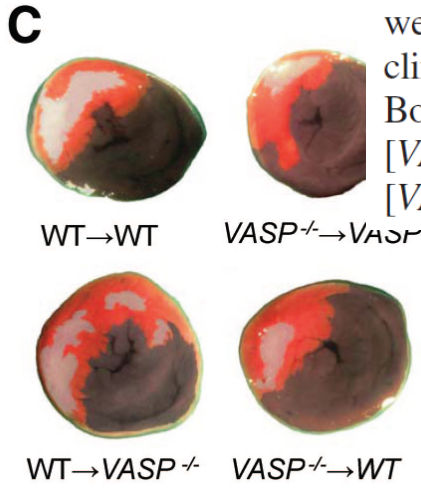


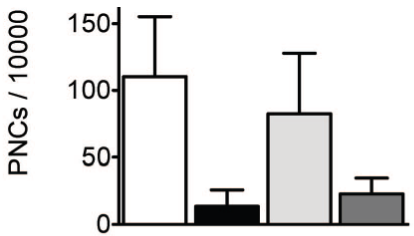


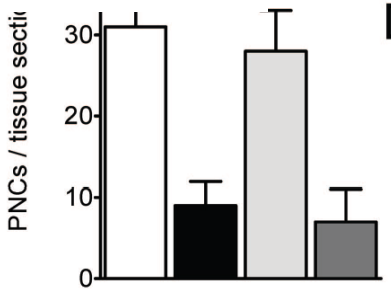


Generation of Bone Marrow Chimeras

In short, male donor mice (8 to 10 weeks old, 20 to 25 g) were euthanized, marrow harvested by flushing the marrow cavity and bone marrow cells were then centrifuged at 400 g for 5 minutes, resuspended and counted. Recipient mice (8 to 10 weeks of age, 20 to 25 g) were irradiated with a total dose of 12 Gy from a 137Cs source. Immediately after irradiation, 10^7 BM cells/recipient were injected in 150 μl 0.9% sodium chloride into the tail vein. The resulting chimeric mice were housed in microisolators for at least 8 weeks before experimentation and fed with water containing tetracycline (100 mg/L) in the first 2 weeks after bone marrow transplantation. Bone marrow cells were transplanted to generate (1) [WT→WT], (2) [$VASP^{-/-}$→$VASP^{-/-}$] mice as controls, (3) [WT→$VASP^{-/-}$], and (4) [$VASP^{-/-}$→WT] chimeric mice.

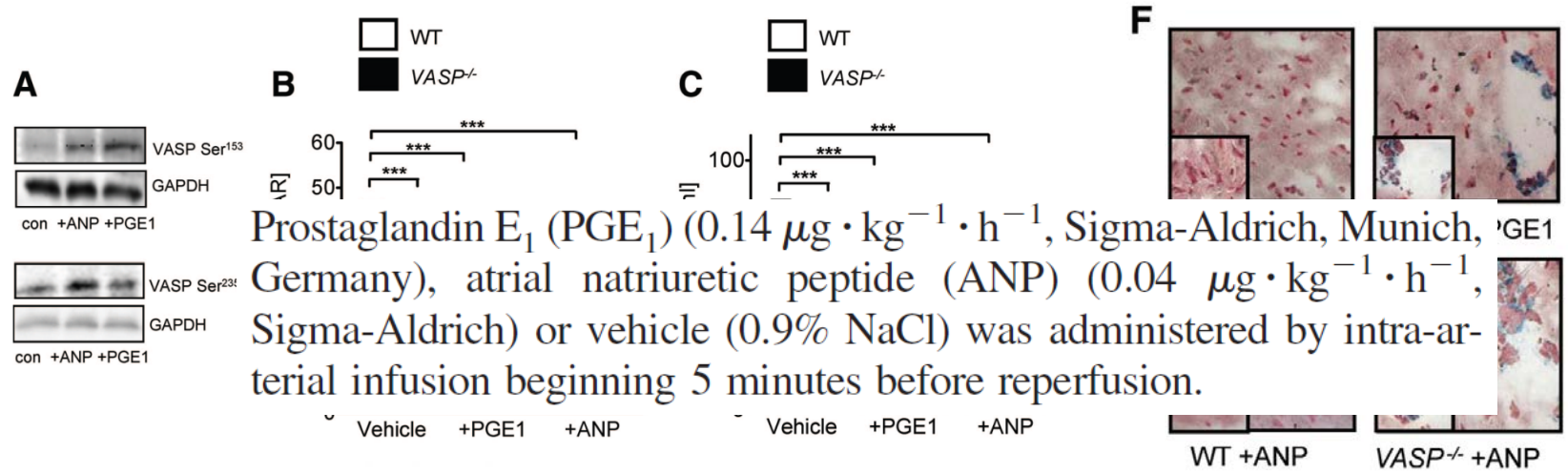


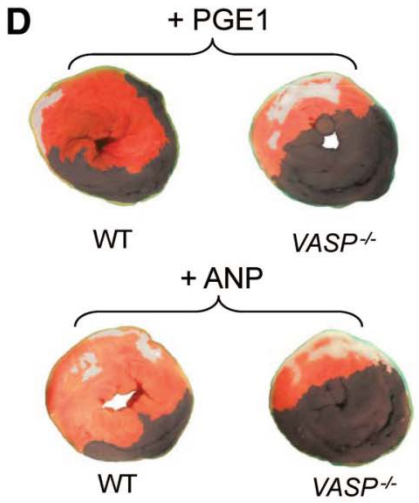


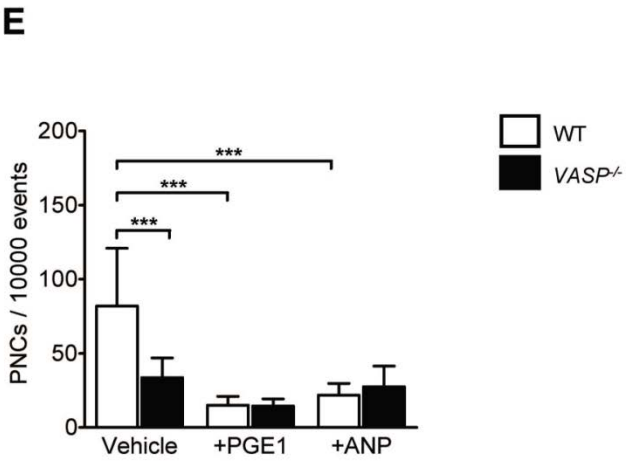


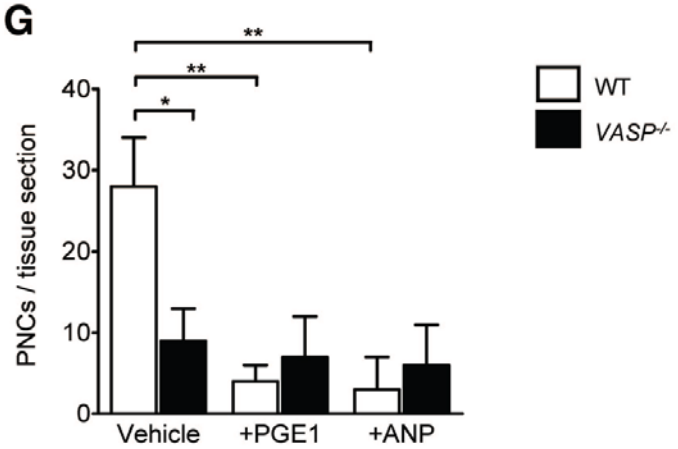
Vasodilator-Stimulated Phosphoprotein Phosphorylation Reduces In Vivo Platelet-Neutrophil Complexes Formation and Myocardial IR Injury

Prostaglandin E_1 (PGE_1) (0.14 $\mu g \cdot kg^{-1} \cdot h^{-1}$, Sigma-Aldrich, Munich, Germany), atrial natriuretic peptide (ANP) (0.04 $\mu g \cdot kg^{-1} \cdot h^{-1}$, Sigma-Aldrich) or vehicle (0.9% NaCl) was administered by intra-arterial infusion beginning 5 minutes before reperfusion.









Conclusions

- VASP is responsible for PNC mediated I/R injury
- Phosphorylation of VASP results in smaller infarct size