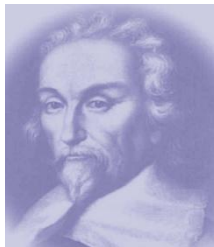


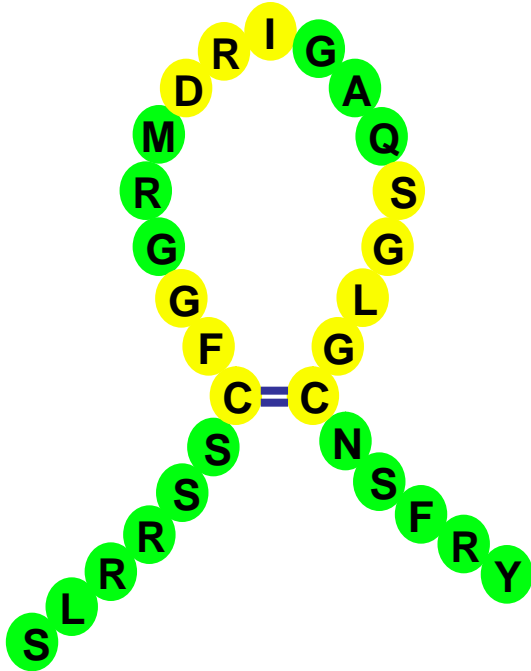
C-type natriuretic peptide signalling and cardiovascular disease

Adrian Hobbs

Professor of Cardiovascular Pharmacology
William Harvey Research Institute
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Queen Mary University of London
Charterhouse Square
London EC1M 6BQ

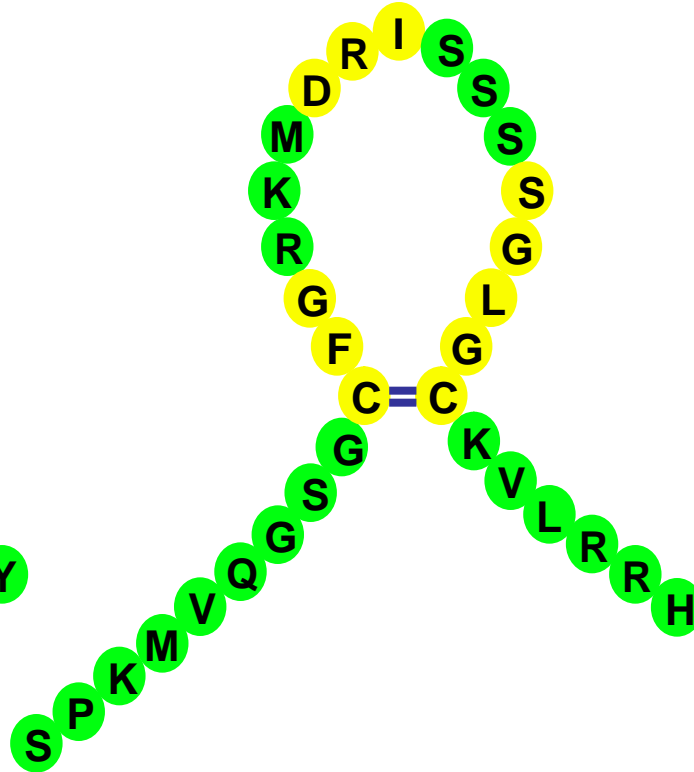


Natriuretic peptide family



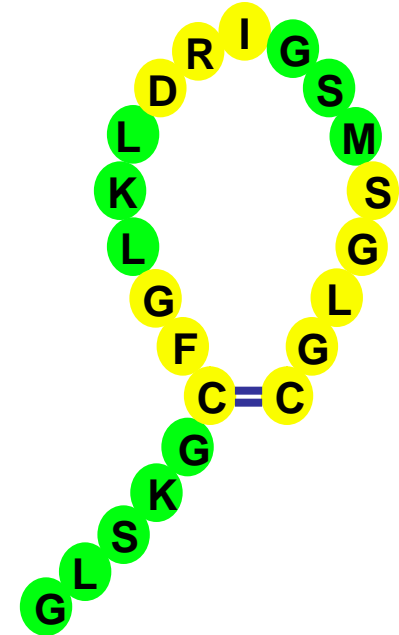
ANP

28aa peptide



BNP

32aa peptide



CNP

22aa peptide



Biological actions of natriuretic peptides

ENDOCRINE

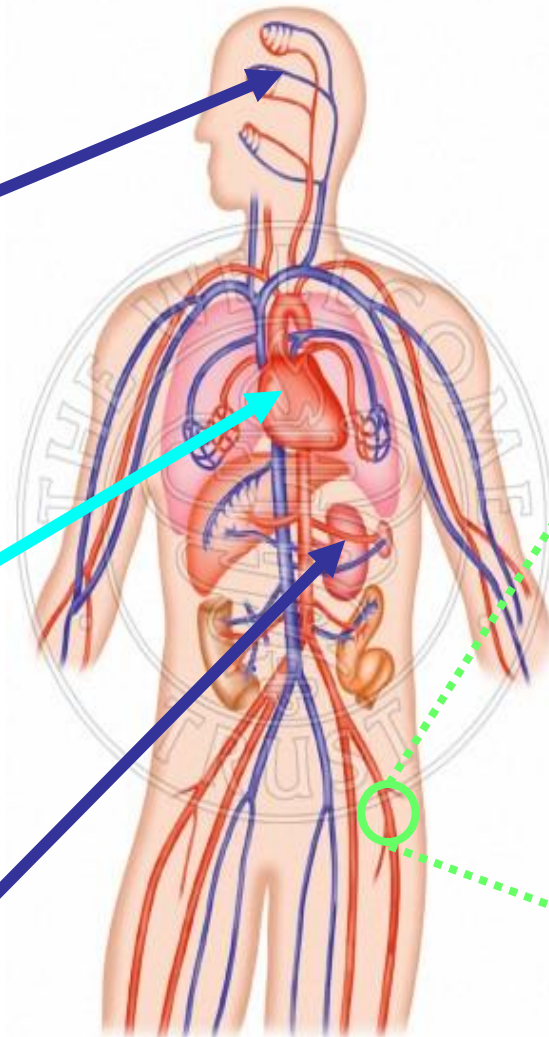
↓ Thirst
↓ Vasopressin
↓ Sympathetic



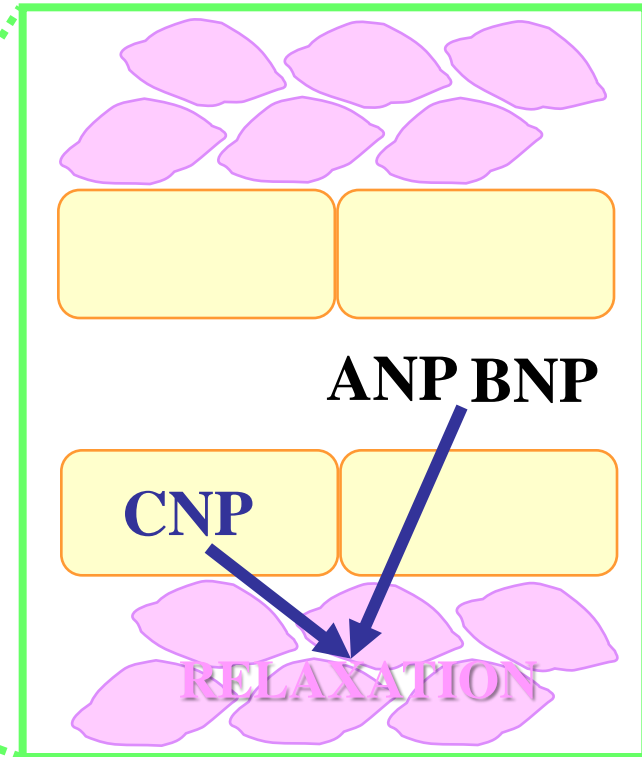
ANP (atria)
BNP (ventricles)



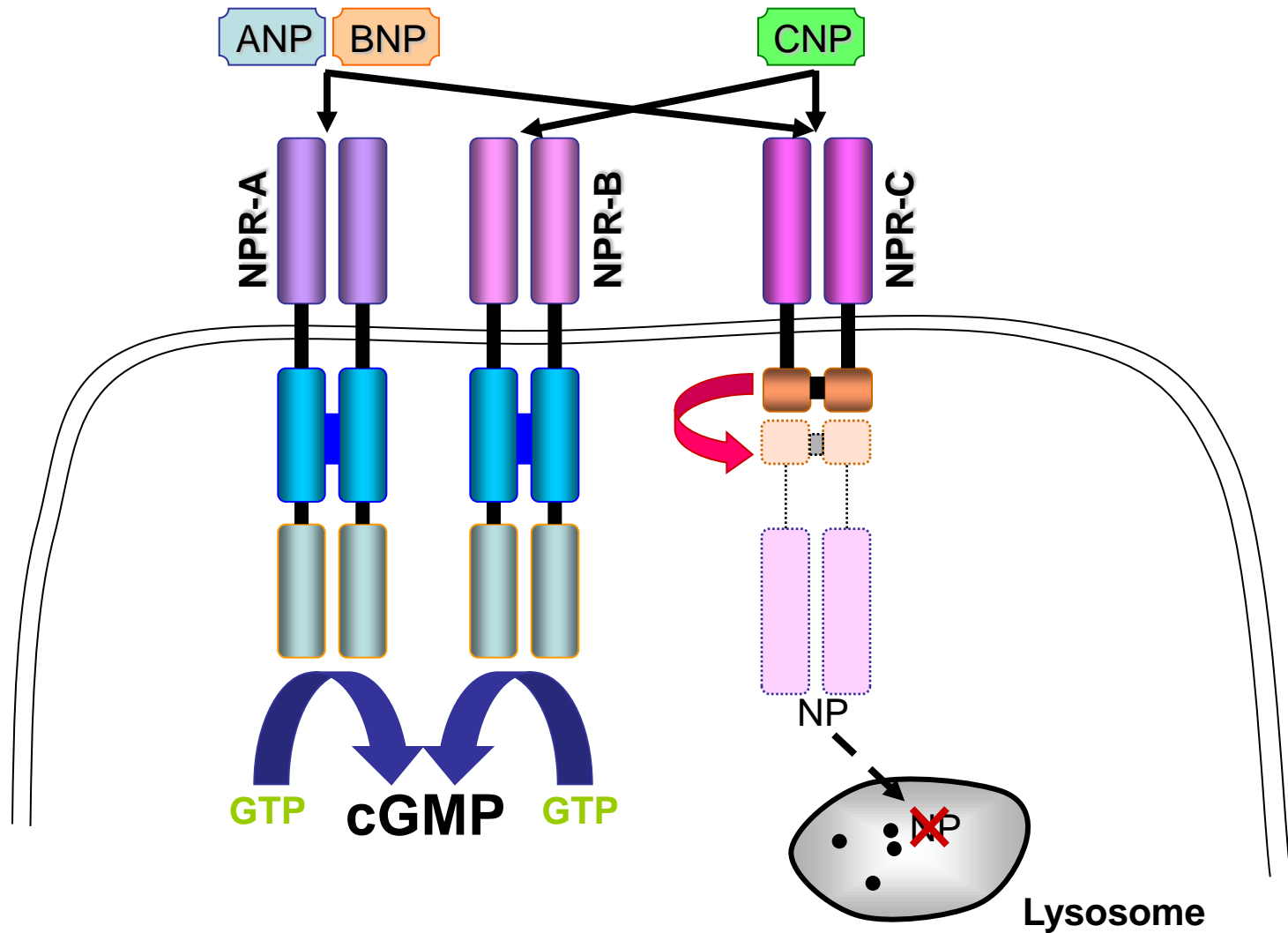
↑ Na⁺/H₂O loss
↓ Aldosterone



PARACRINE



Natriuretic peptide receptor (NPR) subtypes

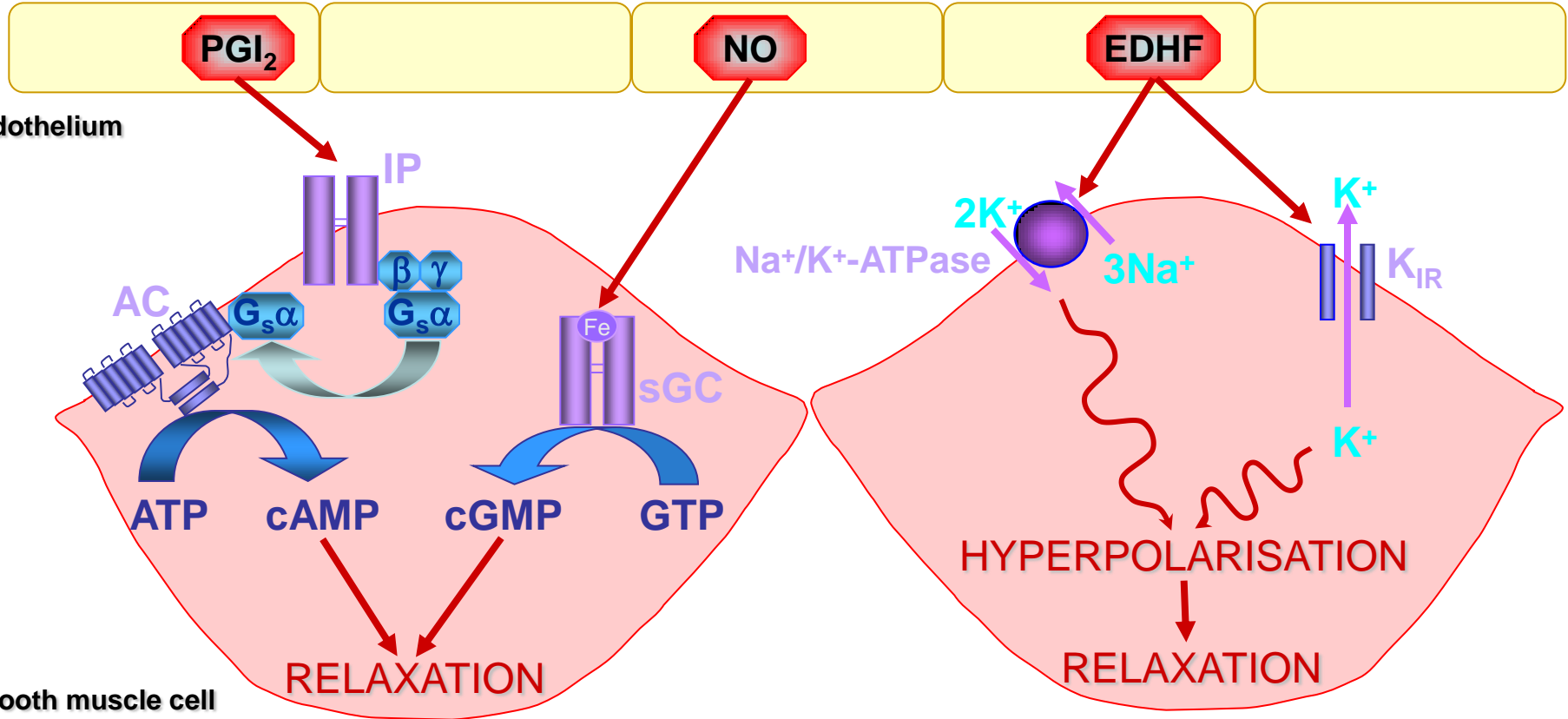


Endothelium-dependent dilatation

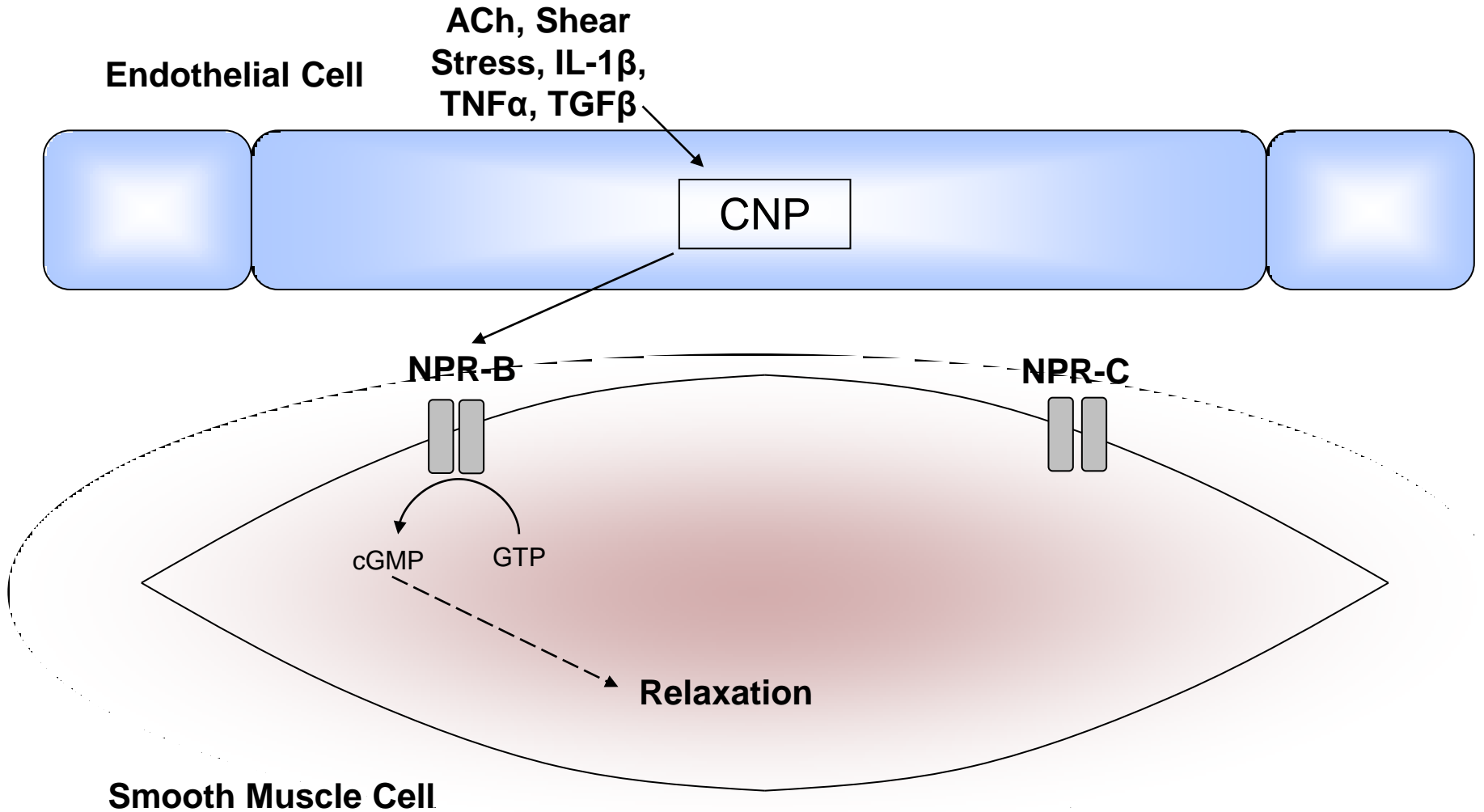
Blood vessel lumen

Endothelium

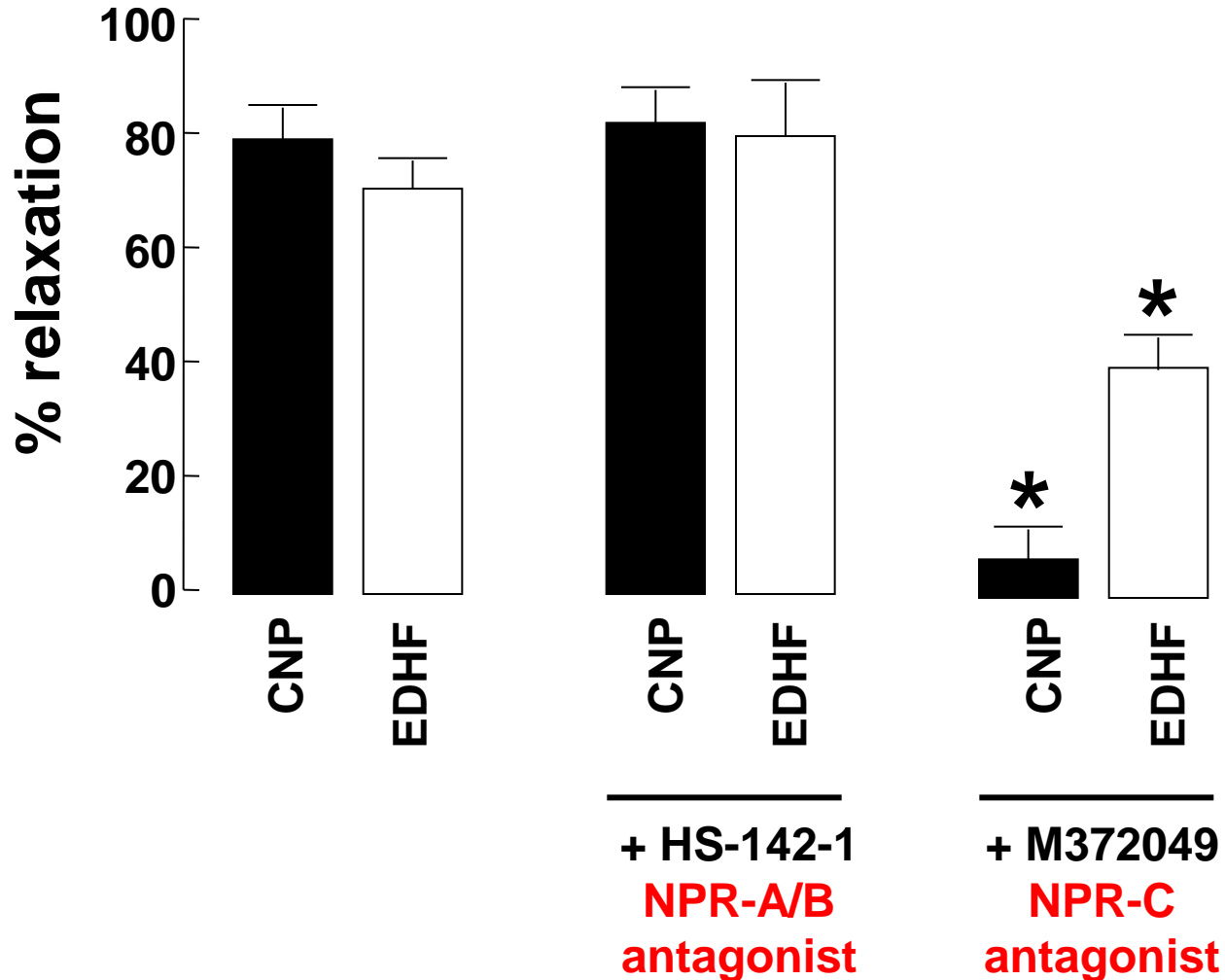
Smooth muscle cell



Vascular pharmacology of CNP

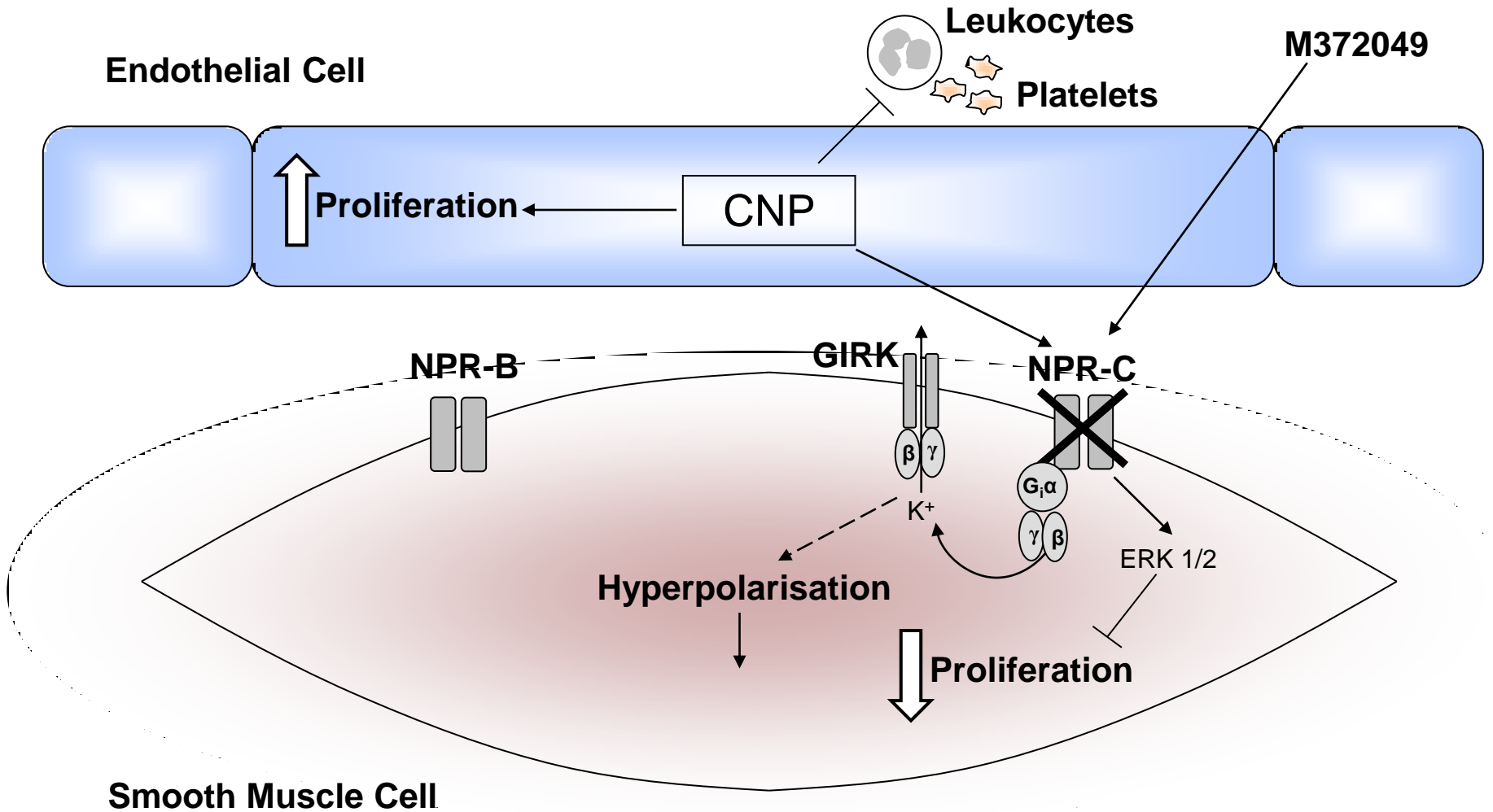


CNP and EDHF activate NPR-C!

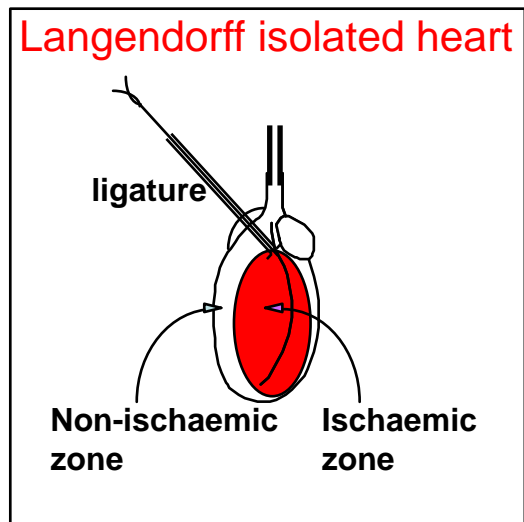
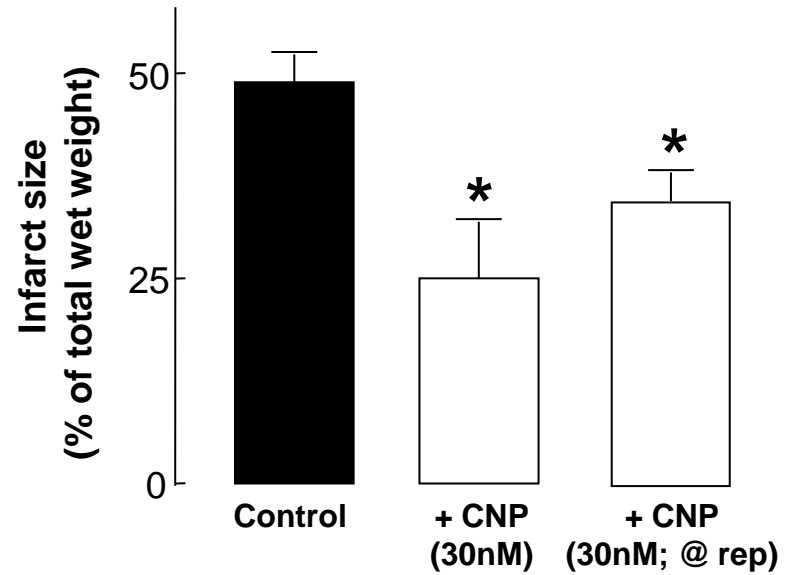
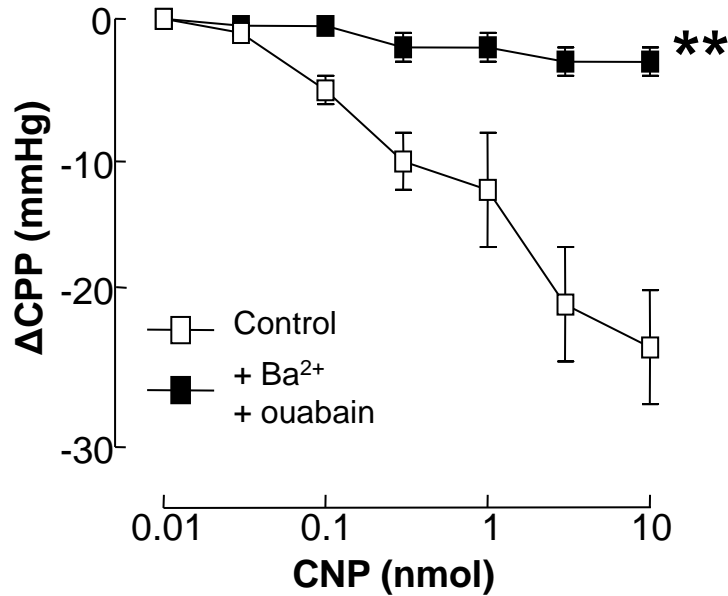


Rat isolated mesenteric arteries

Vascular pharmacology of CNP



A cytoprotective role for CNP in ischaemia-reperfusion (I/R) injury

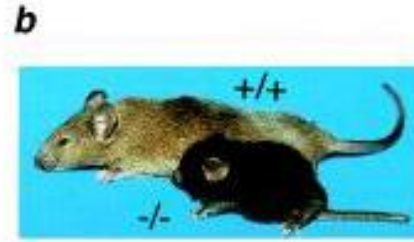
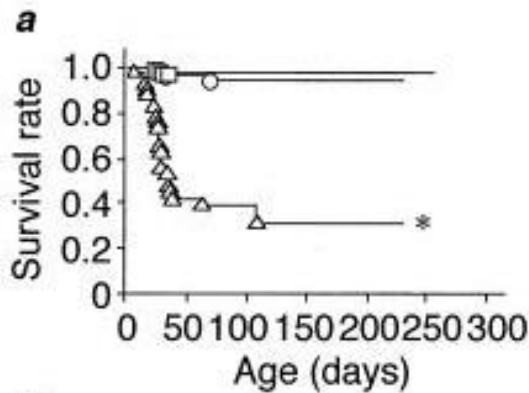
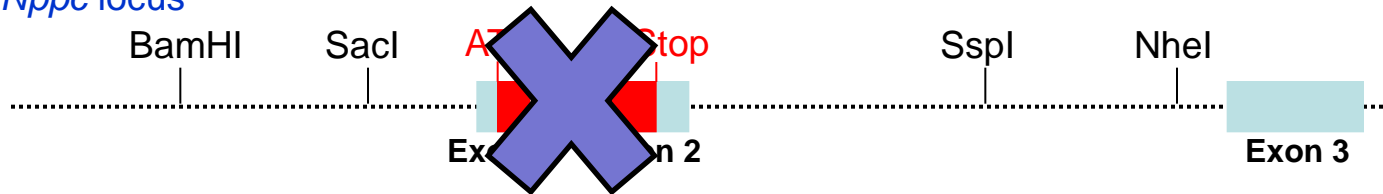


A physiological role for CNP?

- ① Interesting pharmacodynamic profile!
- ② Presence of CNP in the endothelium suggests it has tissue localisation & functional remit to preserve vascular homeostasis
 - Acts as an EDHF in mesenteric and coronary arteries
 - Prevents the activation of leukocytes & platelets
 - Maintains integrity of blood vessel wall (regulates EC and VSMC proliferation)
- ③ Paucity of selective pharmacological interventions targeting natriuretic peptide signalling
 - Difficult to define a physiological function

Development of an endothelium-specific CNP knockout

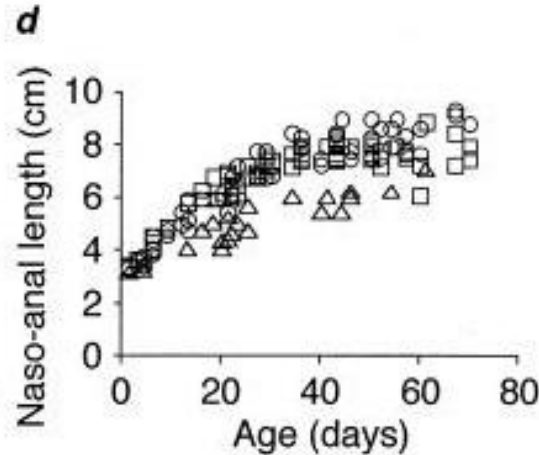
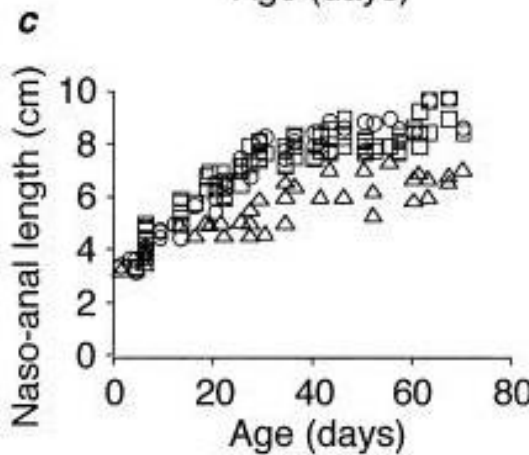
Endogenous *Nppc* locus



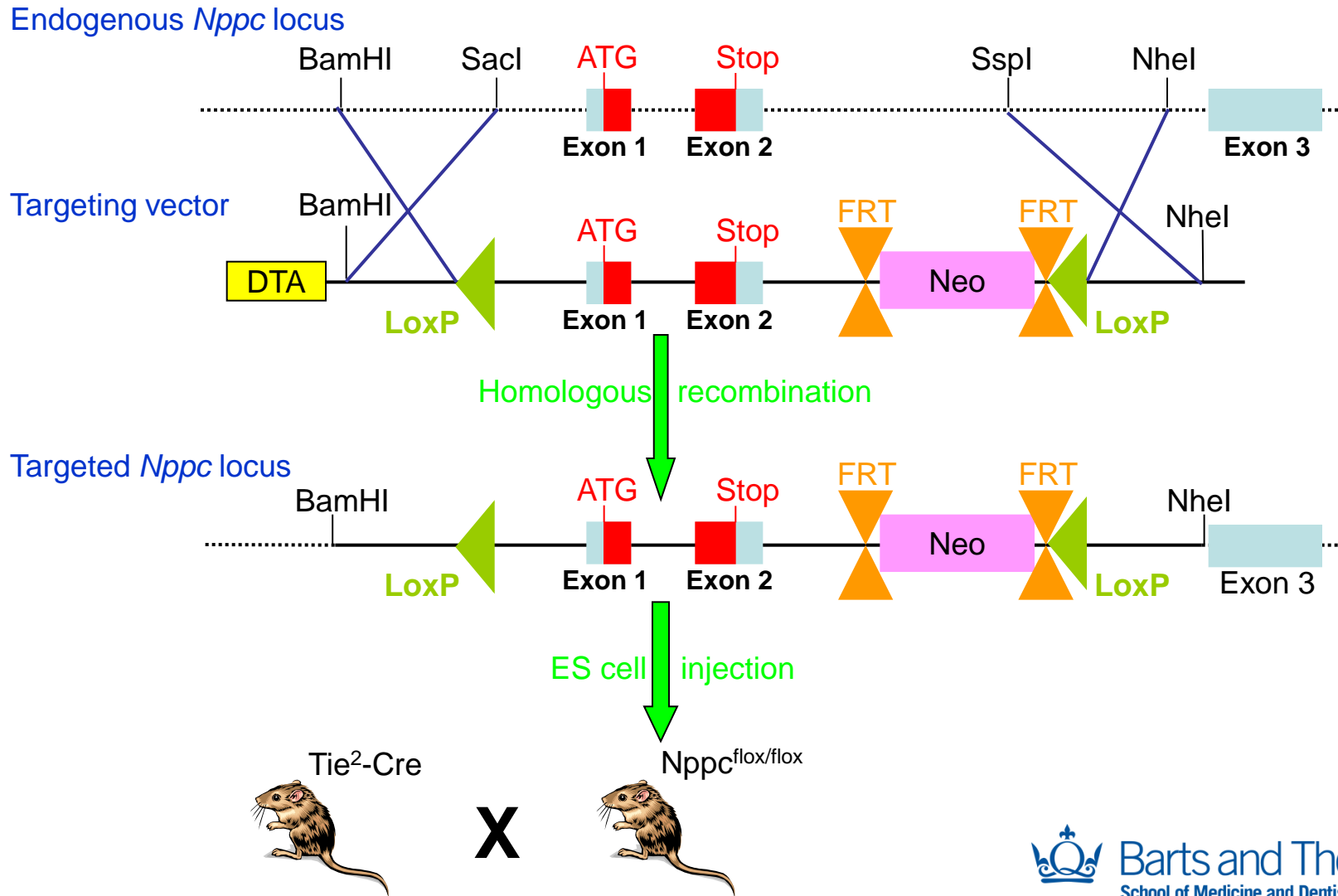
Dwarfism and early death in mice lacking C-type natriuretic peptide

Hideki Chusho^{1*}, Naohisa Tamura^{1*}, Yoshihiro Ogawa^{1*}, Akihiro Yasoda^{1*}, Michio Suda^{1*}, Takashi Miyazawa^{1*}, Kenji Nakamura², Kazuki Nakao², Tatsuya Kurihara², Yasato Komatsu³, Hiroshi Itoh⁴, Kiyoshi Tanaka⁴, Yoshihiko Saito⁴, Motoya Katsuki⁵, and Kazuwa Nakao⁴

4016 4021 | PNAS | March 27, 2001 | vol. 98 | no. 7



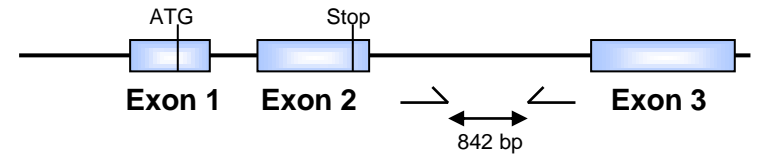
Development of an endothelium-specific CNP knockout



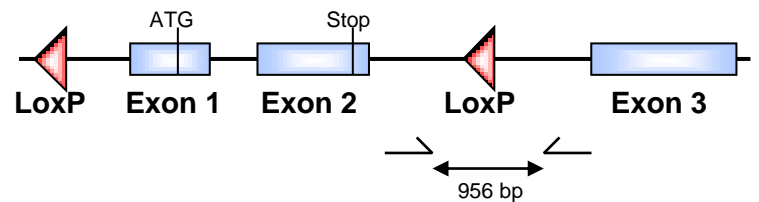
Development of an endothelium-specific CNP knockout



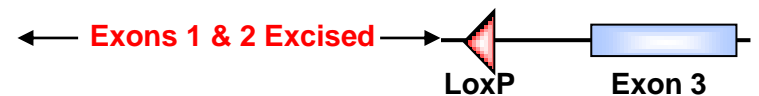
Endogenous Mouse CNP Locus



LoxP (Floxed) Mouse

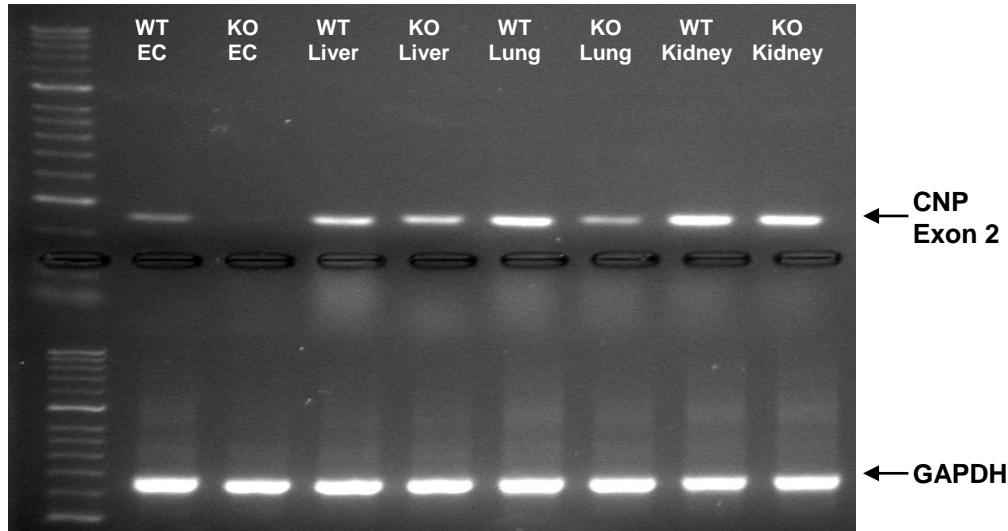


ecCNP KO Mouse

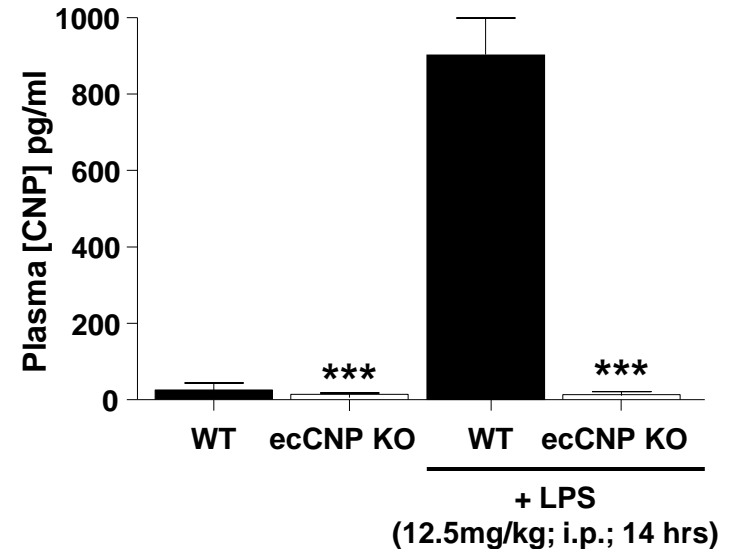


Development of an endothelium-specific CNP knockout

CNP mRNA expression

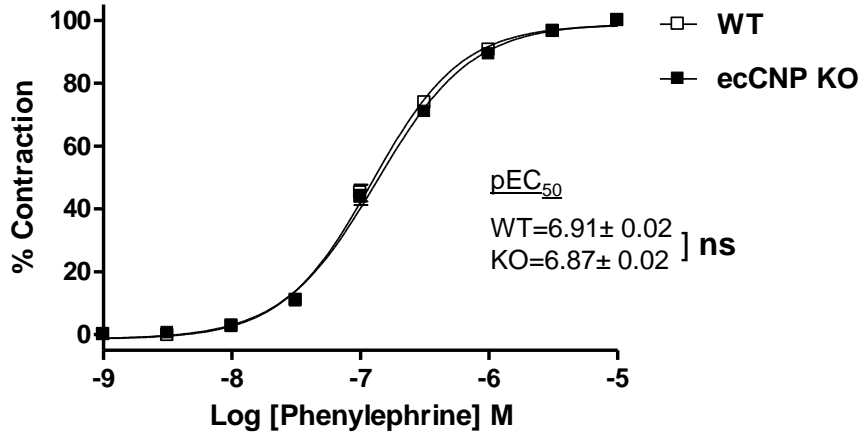


Plasma [CNP]

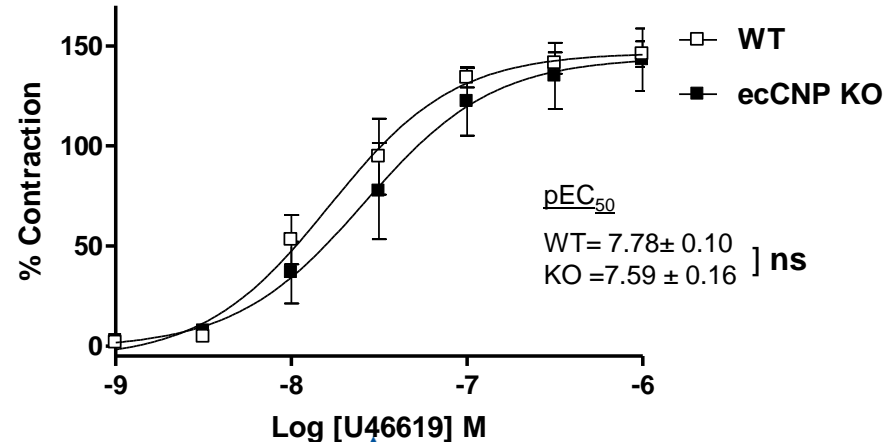
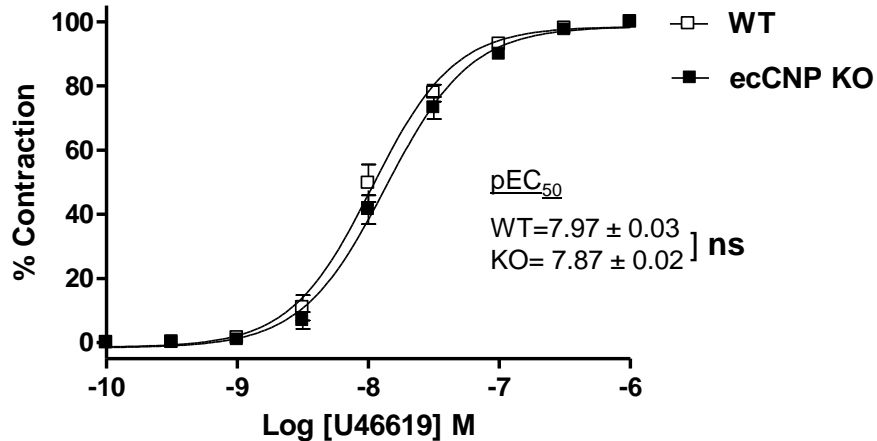
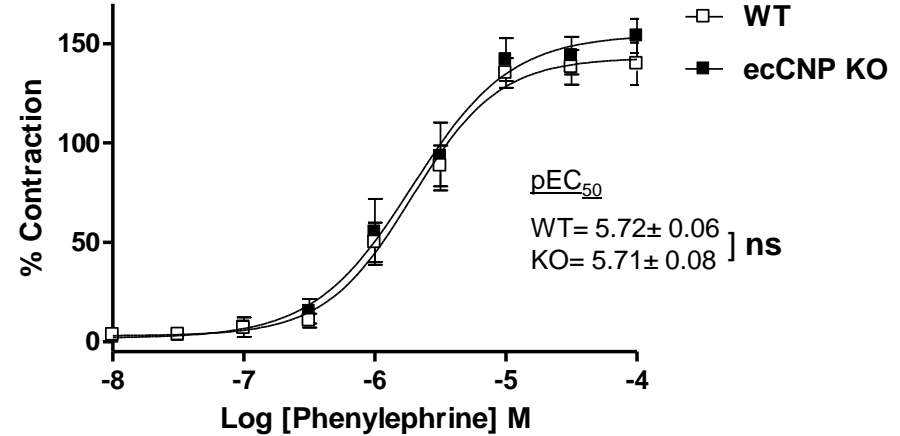


Vasoconstrictor responses are unaltered in ecCNP KO vessels

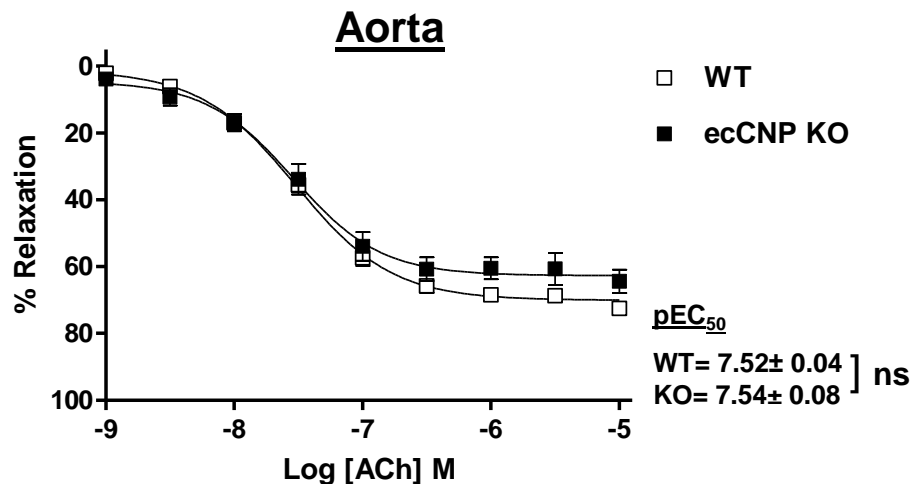
Aorta



Mesentery

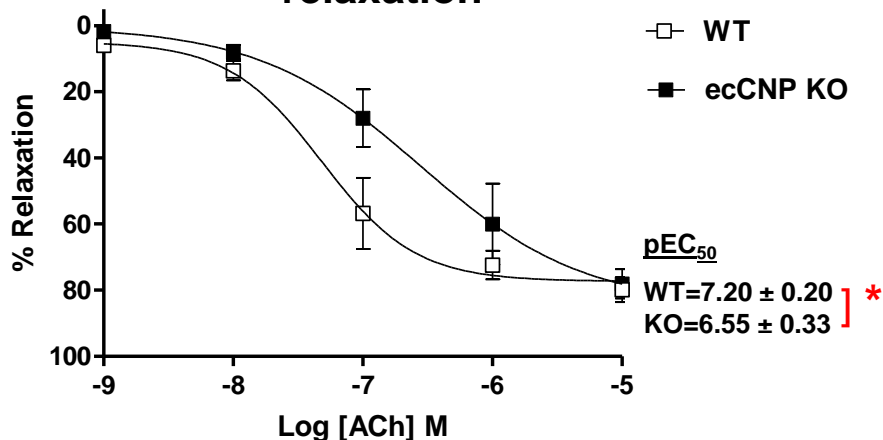


Endothelium-dependent relaxation is impaired in resistance arteries of ecCNP KO mice

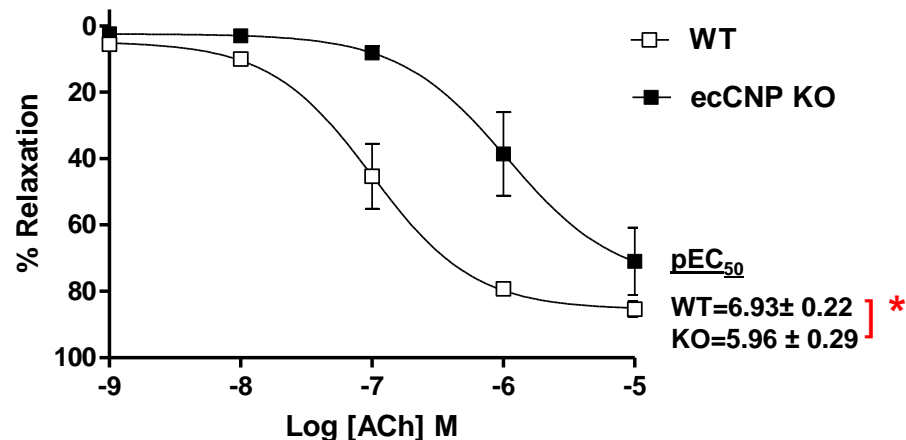


Mesentery

NO, PGI₂ & EDHF-dependent relaxation

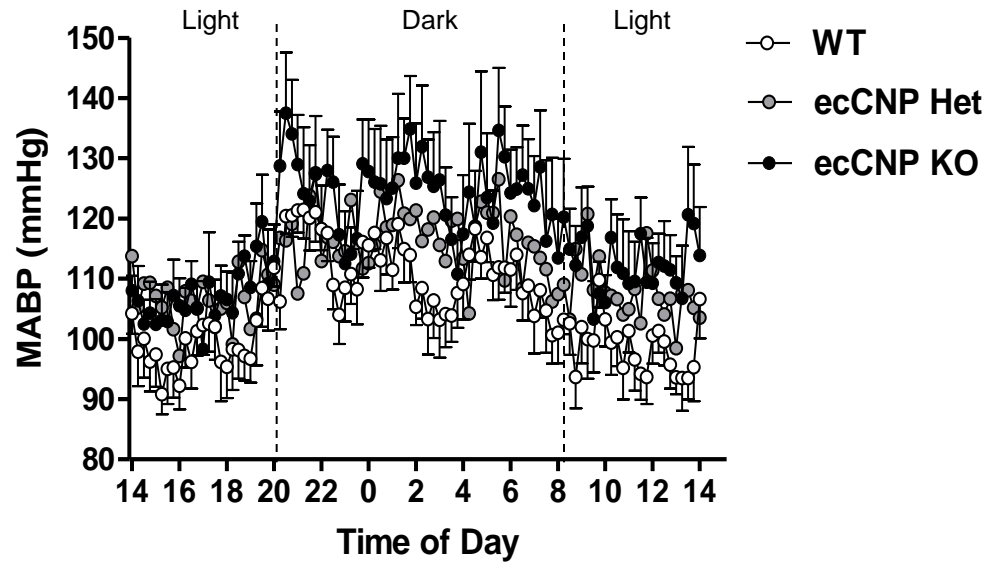


EDHF-dependent relaxation

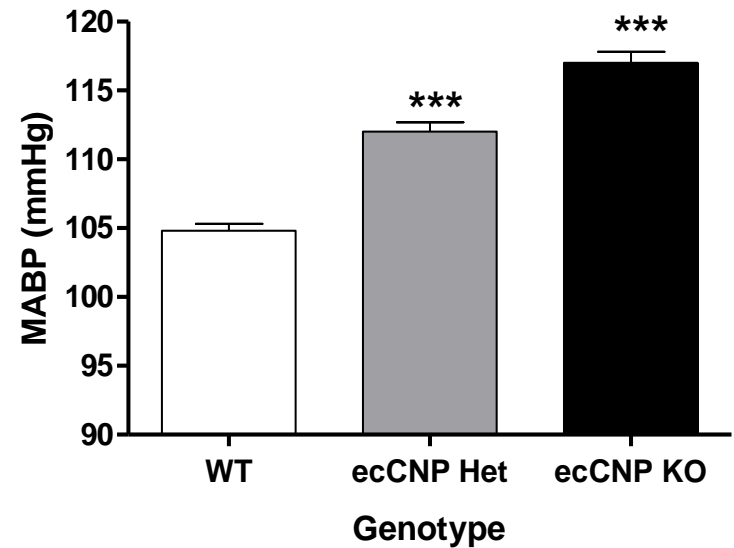


Blood pressure is elevated in ecCNP KO mice

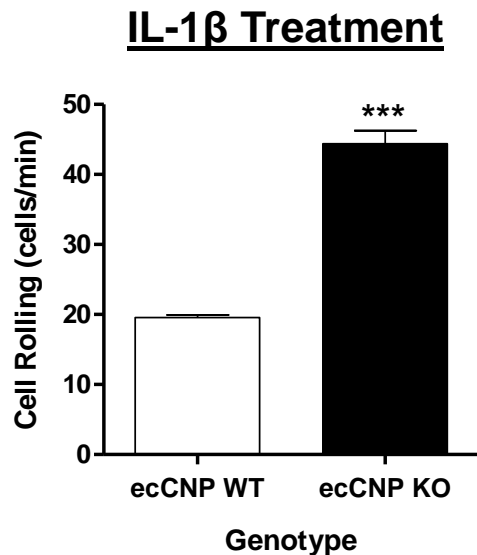
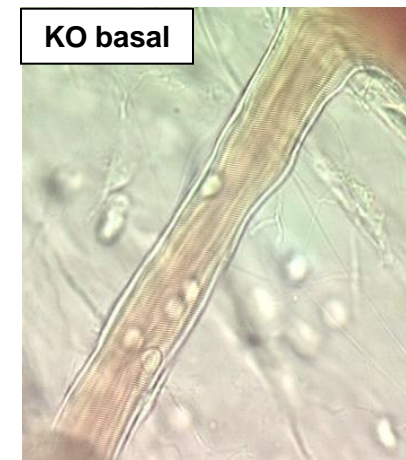
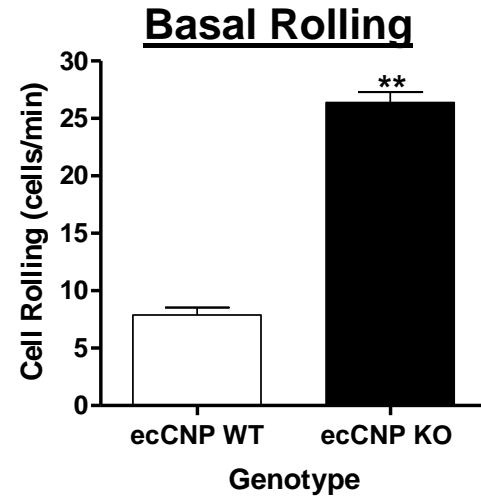
Circadian Rhythm



MABP (24hr Mean)

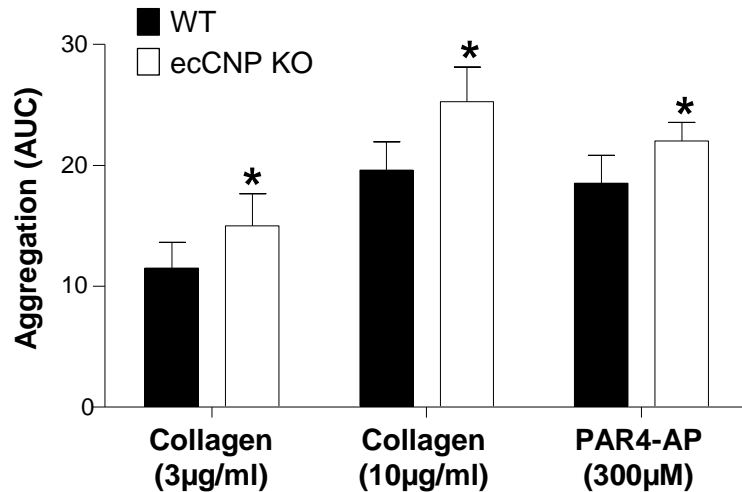


Loss of endothelial CNP results in increased leukocyte rolling

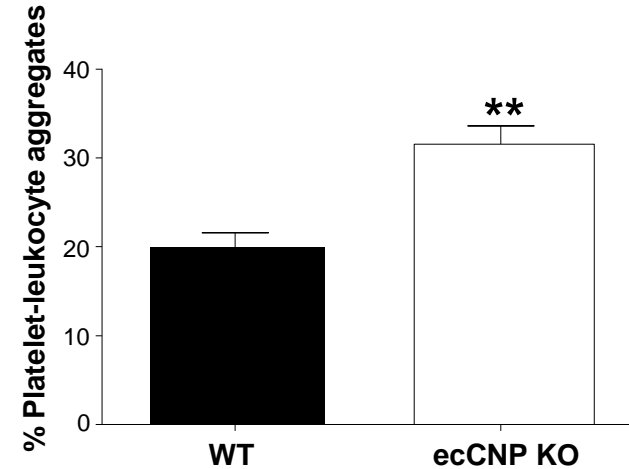


Loss of endothelial CNP: platelet function?

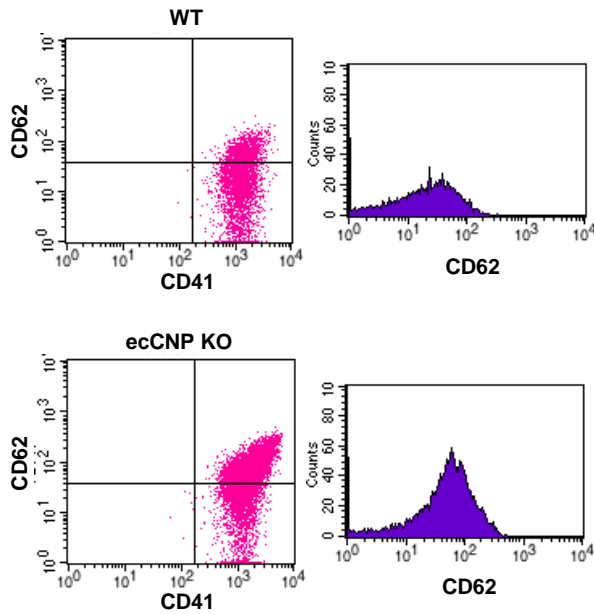
Impedance aggregometry



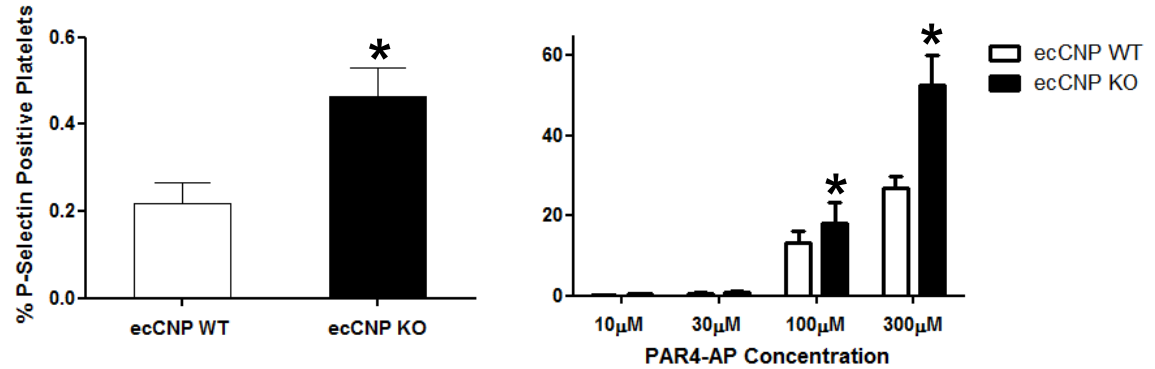
Platelet-leukocyte aggregates



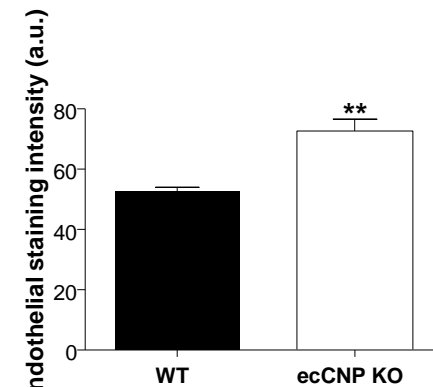
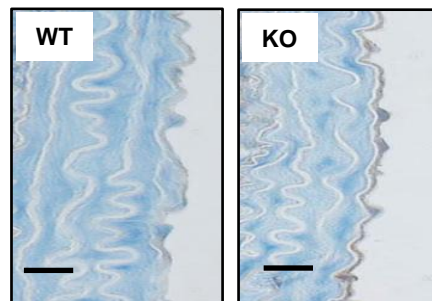
Increased platelet & endothelial P-selectin expression in ecCNP KO mice



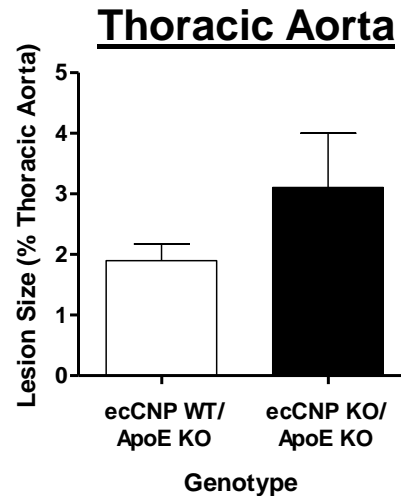
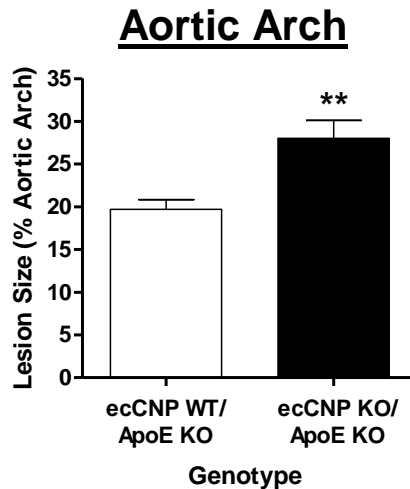
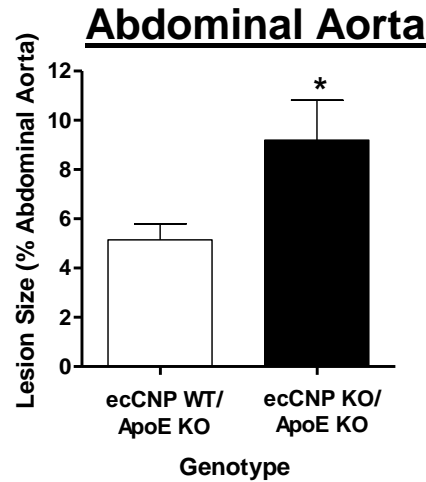
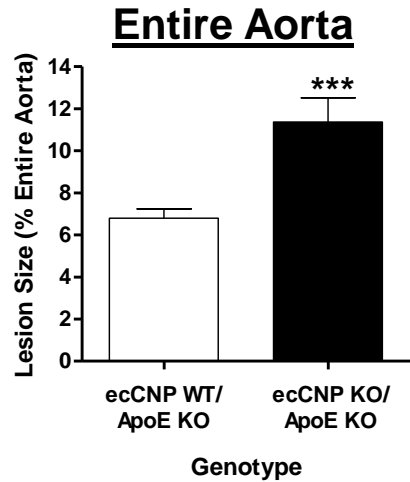
Platelet P-selectin expression



Endothelial P-selectin expression



Accelerated atherogenesis in CNP KO mice

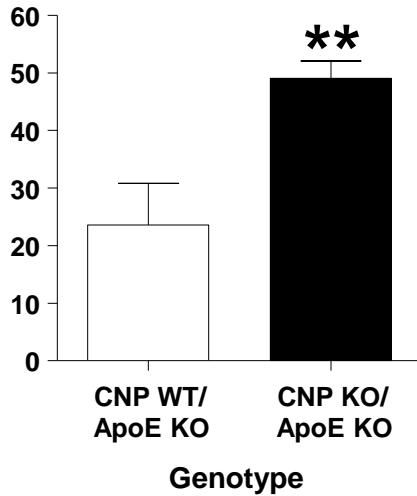


Oil Red O Staining (Lipid)

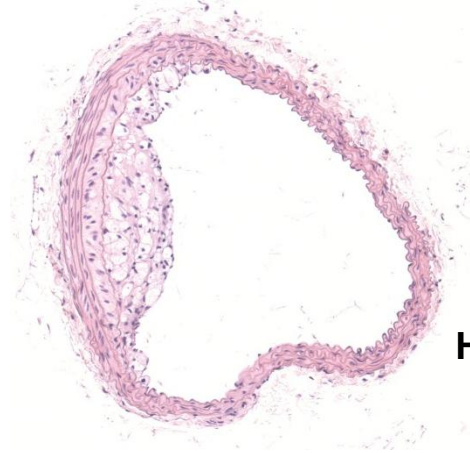
(12 week high-fat diet)

CNP/ApoE double knockout mice have larger atherosclerotic plaques

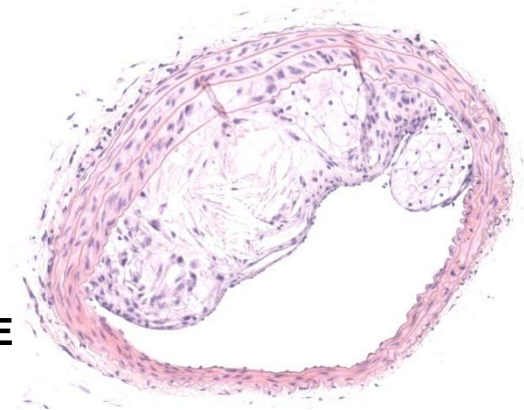
Plaque Area (% of total lumen area)



ecCNP WT/
ApoE KO

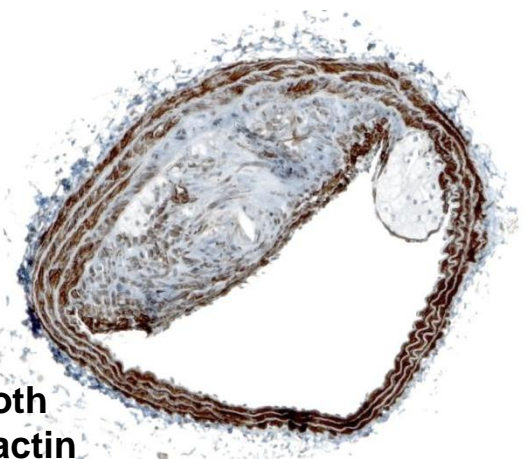
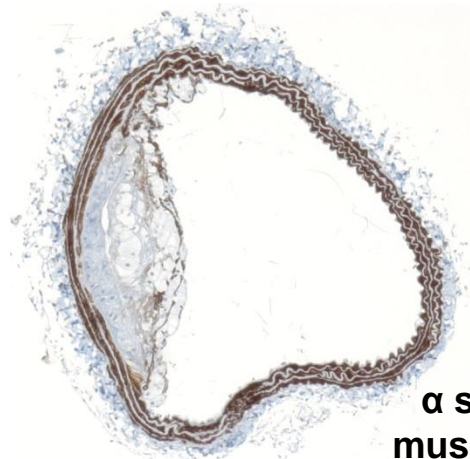
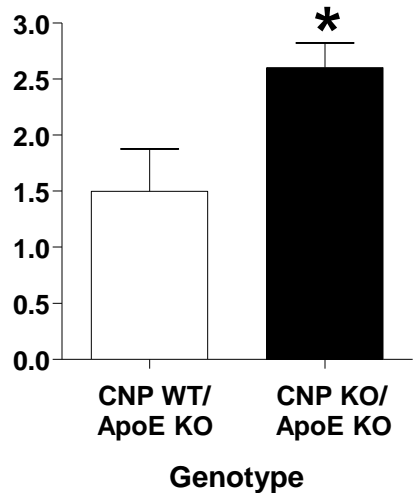


ecCNP KO/
ApoE KO



H & E

Intima/media thickness ratio



α smooth
muscle actin

(Brachiocephalic artery)



Barts and The London
School of Medicine and Dentistry

ecCNP KO phenotyping summary

- ① Achieved selective deletion of CNP in endothelial cells
- ② Vascular phenotype
 - Loss of endothelium-derived CNP results in impaired resistance artery function *in vitro* and hypertension *in vivo*
 - CNP is involved in the regulation of inflammatory cell recruitment & platelet reactivity (increased endothelial & platelet P-selectin expression)
 - CNP is important for the maintenance of vascular integrity and curbing the progression of atherosclerotic disease
- ③ Tissue-specific CNP KO is an ideal model to further delineate the importance of CNP in cardiovascular health & disease
 - Assessment of cardiac function in ecCNP KO mice vs. cardiomyocyte (alphaMHC-Cre) CNP KO (ECG telemetry; coronary vascular reactivity; ischaemia reperfusion (I/R) injury; heart failure)
- ④ Pharmacology infers that the majority of the cytoprotective functions of CNP appear to be mediated via NPR-C
 - Assessing the vascular importance of NPR-B and NPR-C (transgenic approach)
 - Development of small molecule NPR-C agonists

Acknowledgements



wellcometrust



British Heart Foundation



Barts and The London
School of Medicine and Dentistry