Cathepsin K Deficiency Suppresses the Development of Experimental Intimal Hyperplasia in Response to Injury

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All other authors have reported that they have no relationships relevant to the contents of this paper to disclose

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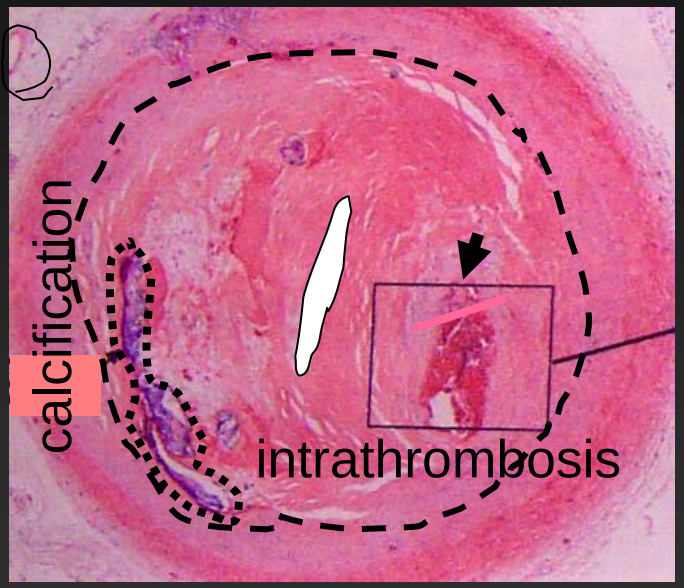
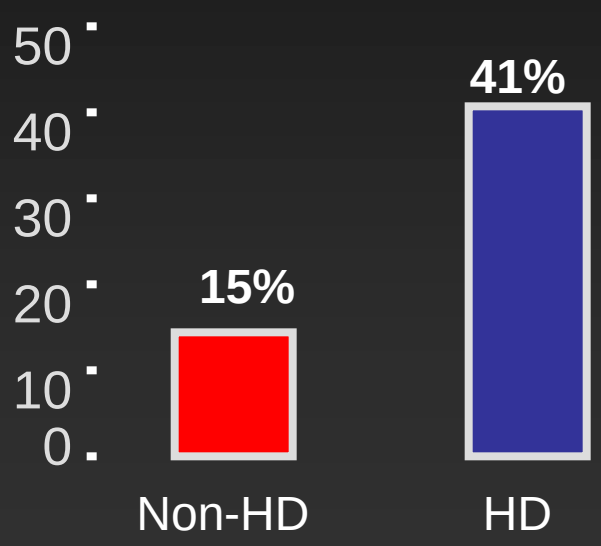
Atherosclerosis-based vascular disease and it complications

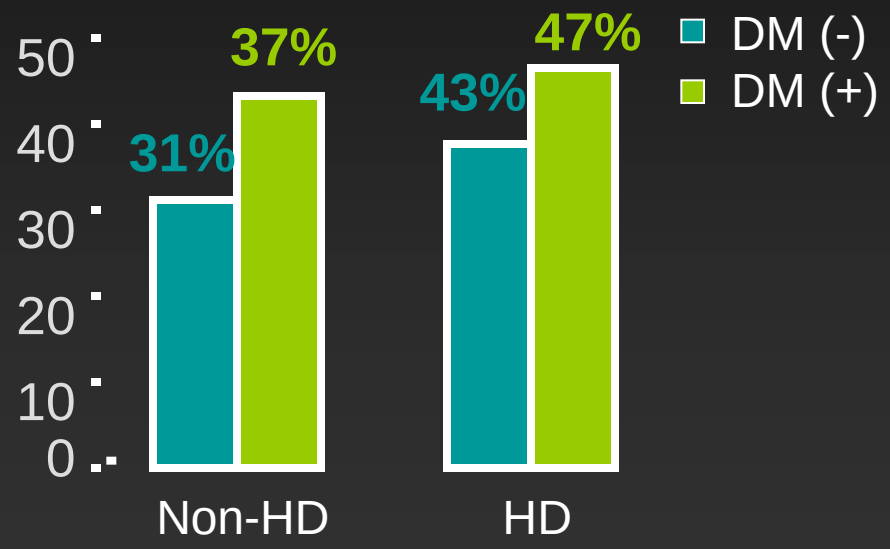


1) Plaque rupture
2) Thrombosis
3) Calcification
4) Restenosis
5) Aneurysm
6) Vasa vasorum

1) Atherosclerosis
2) Vascular injuries
3) Vascular responses

DES-related Restenosis in patients with DM or/and haemodialysis





Otsuka et al. EHJ 2011;32:829-837

Natsuaki M et al. Circ J 2011;75:1616-1625

DES: drug eluting stent; Non-HD, none-haemodialysis

The properties of cysteine protease: cathepsins (Cats)

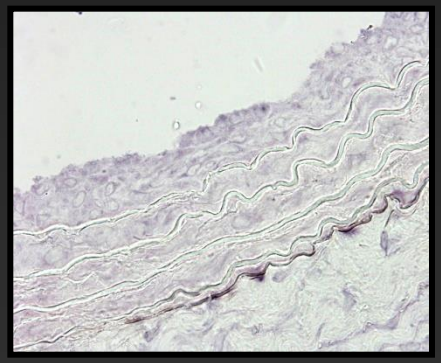
- Cats generally known as functioned in lysosomes, were discovered in the half of the 20th century. There are 11 human Cats (B,C,F,H,K,L,O,S,V,W,and X) that belong to papain subfamily of cysteinyl proteases. Cystatin C (CystC) is one of the major endogenous Cat inhibitors.

- Previously, It has been reported that Cats degrade apoptosis-related molecules such as Bcl-2, Bcl-xL, and Mcl-1, and contribute cell apoptosis.

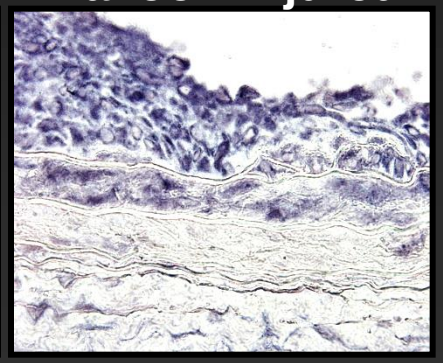
CatK expression in the balloon-injured carotid artery and failing myocardium of rat

In situ hybridization

Control

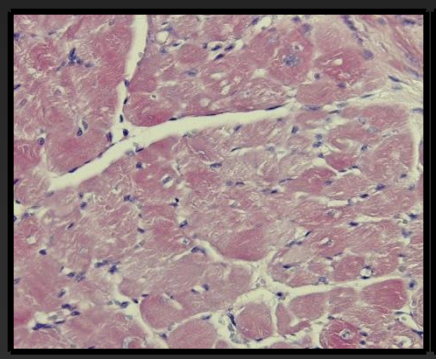


Balloon-injured

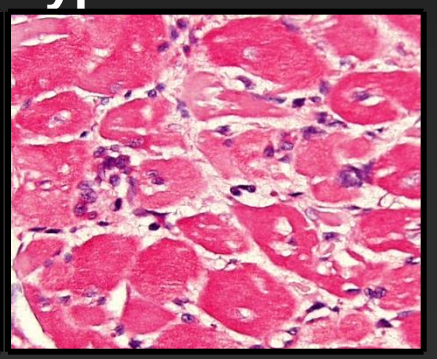


Immunostaining

Control



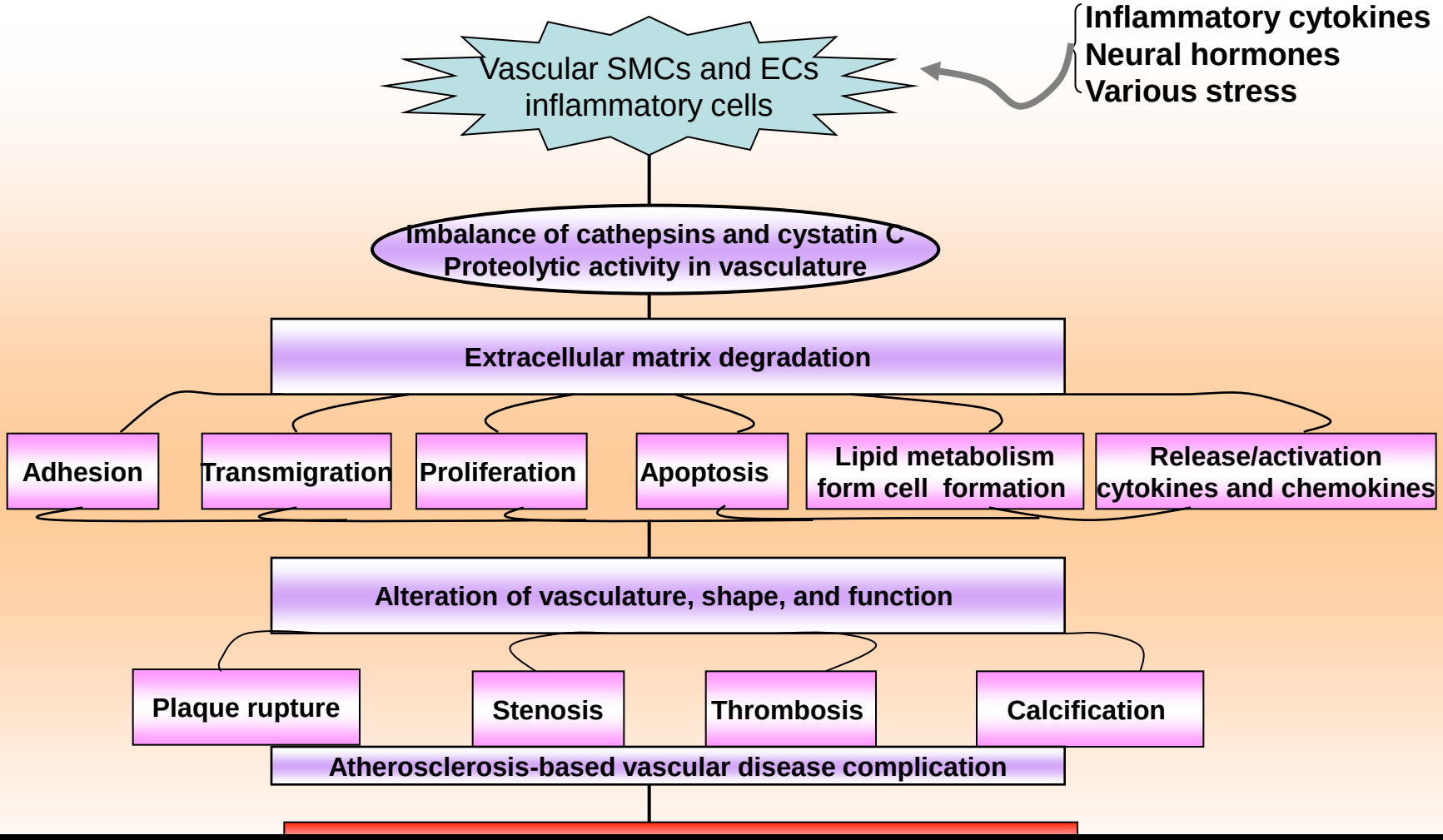
Hypertensive-HF



Rat

Cheng XW, et al. *Am J Path 2004, 2006, 2008; Hypertension 2006.*

Illustration of cathepsin function in pathogenesis of atherosclerosis-based vascular disease and its implications

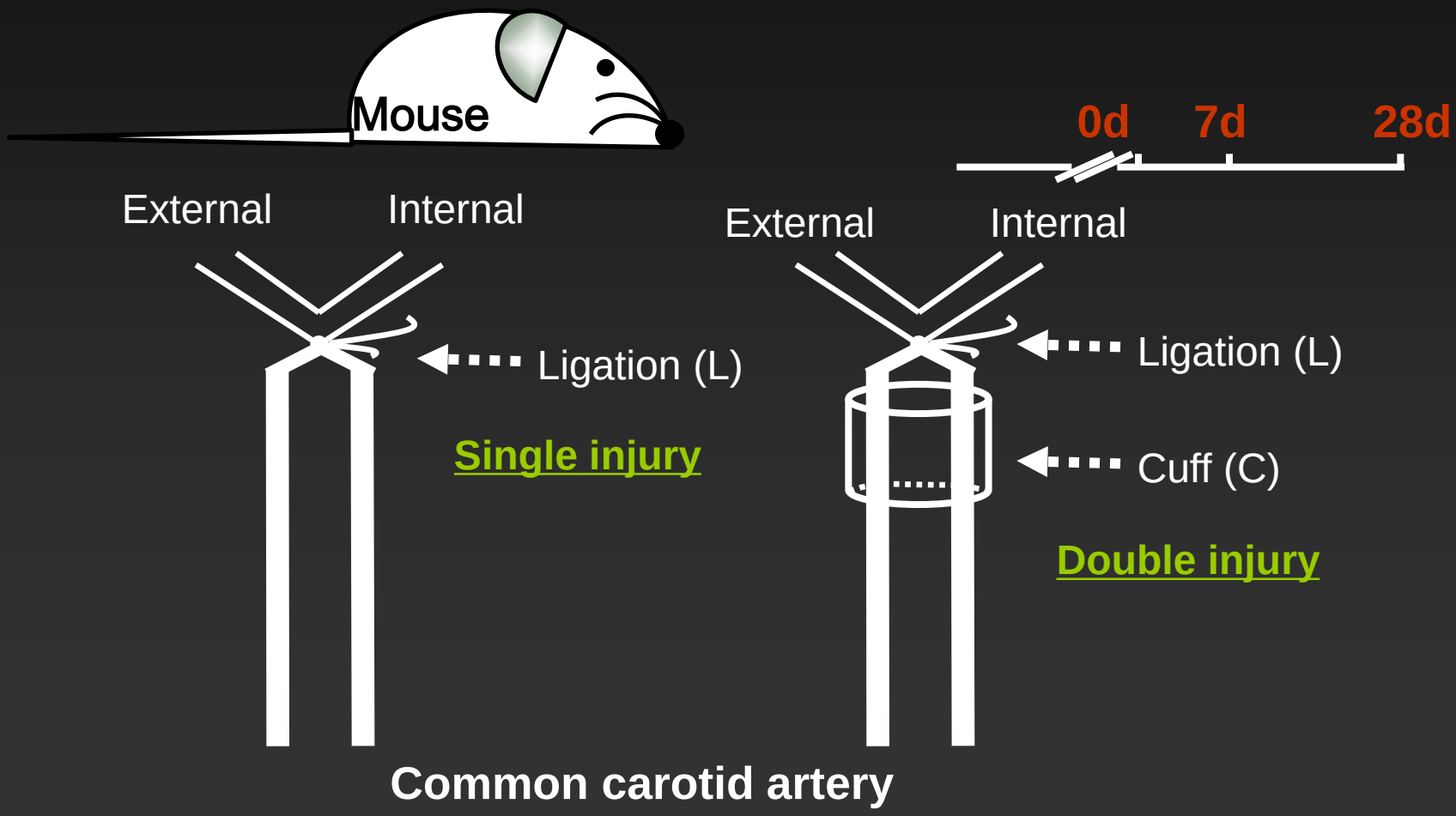


However, we found that the role of individual cathepsin for vascular remodeling and restenosis in response to injury is poorly understood.

Goal

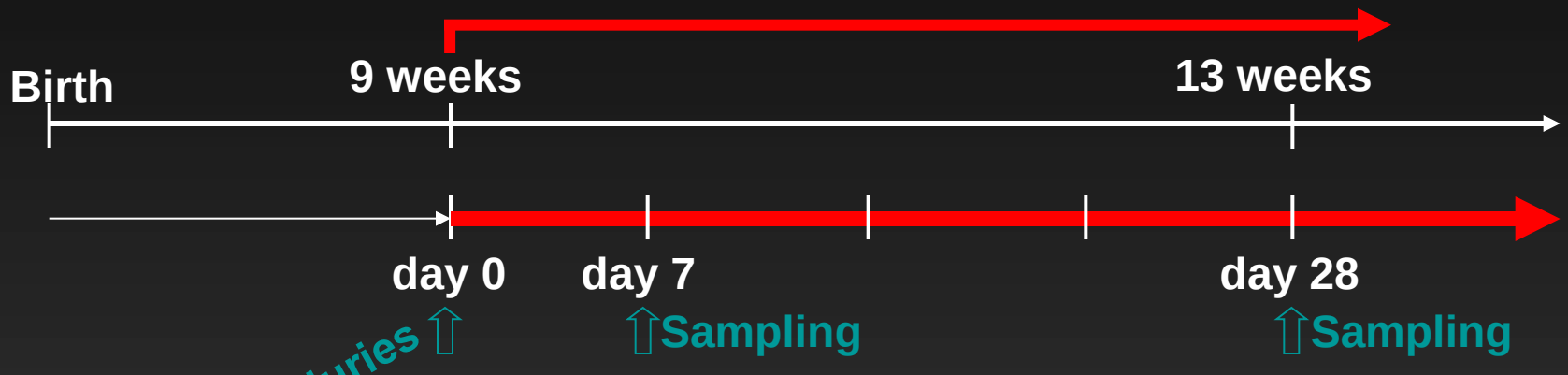
- The present study explored the possibility that CatK deficiency suppresses vascular remodeling and restensois in response to injury, focusing on vascular smooth muscle cell (SMC) apoptosis and proliferation in mouse carotid artery injury model.

Murine carotid artery injury model



Sasaki T, Cheng XW et al. *Lab Invest 2004; ATVB 2006; Atherosclerosis 2009.*

Exp: Protocol (1)

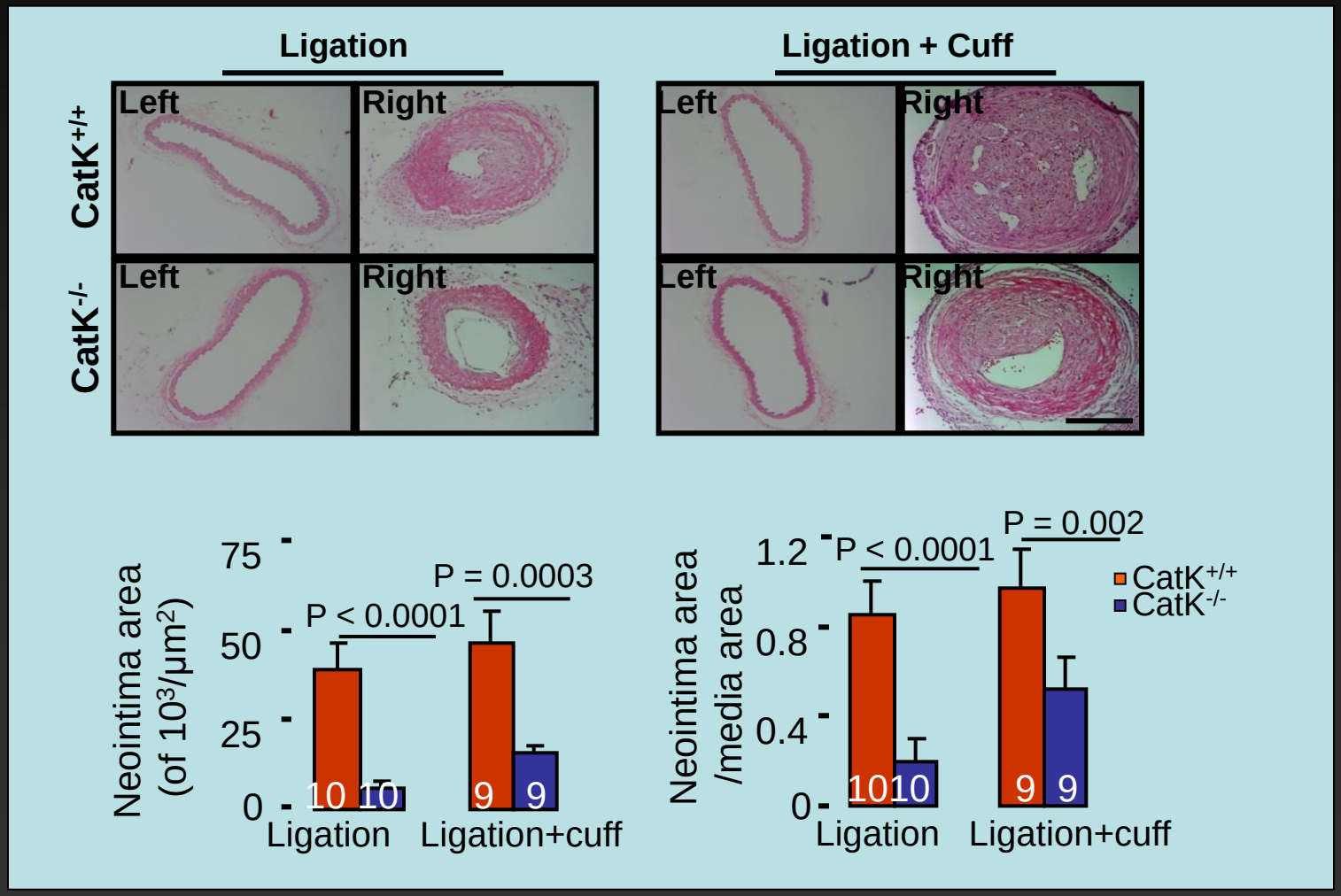


① $CatK^{+/+}$ ligation (*n* = 15)
② $CatK^{-/-}$ ligation (*n* = 16)
③ $CatK^{+/+}$ ligation + cuff (*n* = 14)
④ $CatK^{-/-}$ ligation + cuff (*n* = 13)

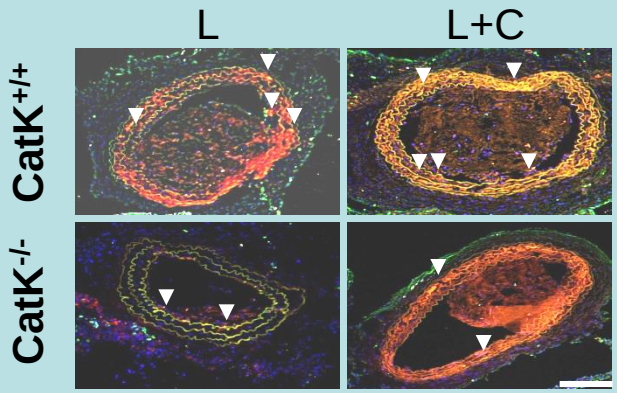
Methods

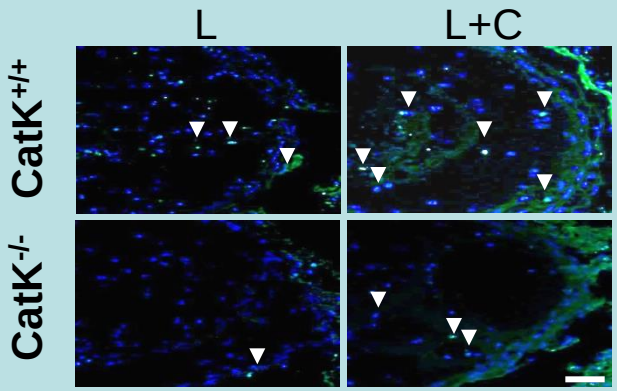
- Morphological analysis
- Immunohistochemistry
- Quantitative real-time PCR
- ELISA etc.

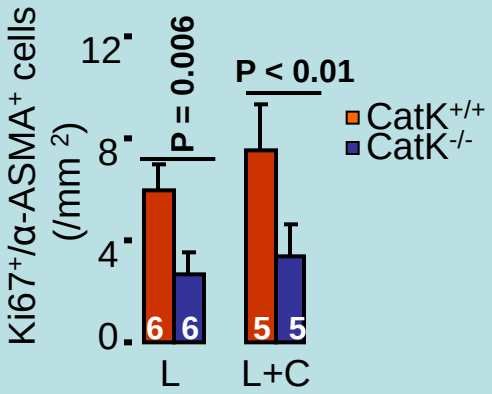
CatK$^{-/-}$ reduces neointimal formation in response to injuries

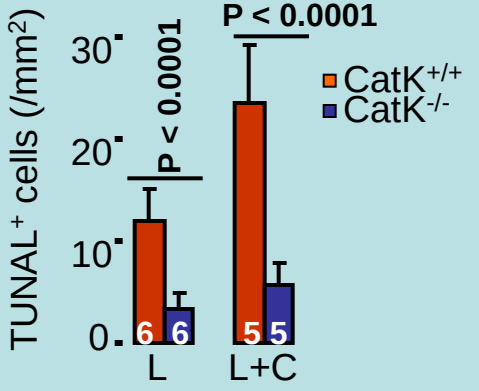


CatK$^{-/-}$ reduces medial SMC proliferation and apoptosis response to injuries



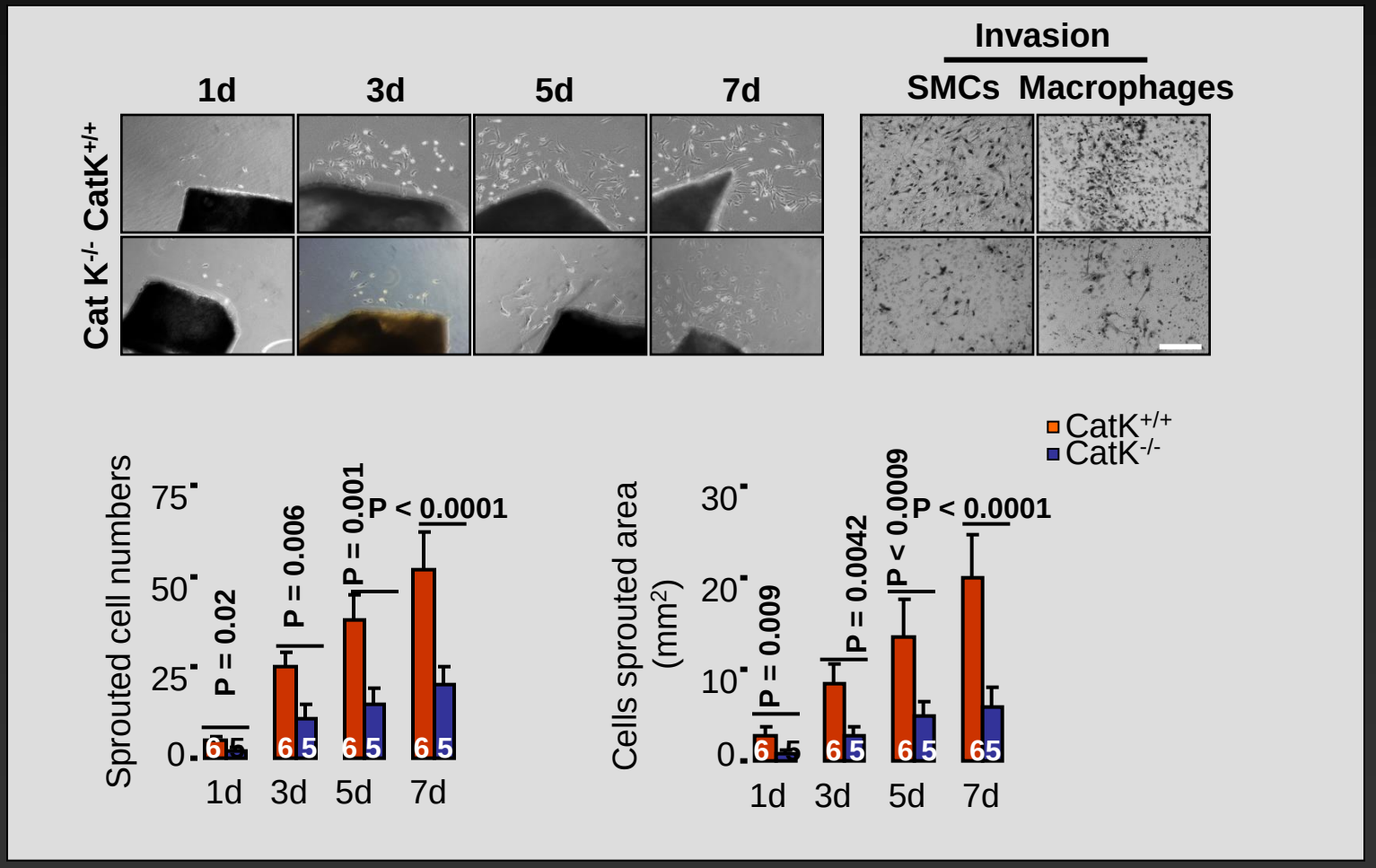




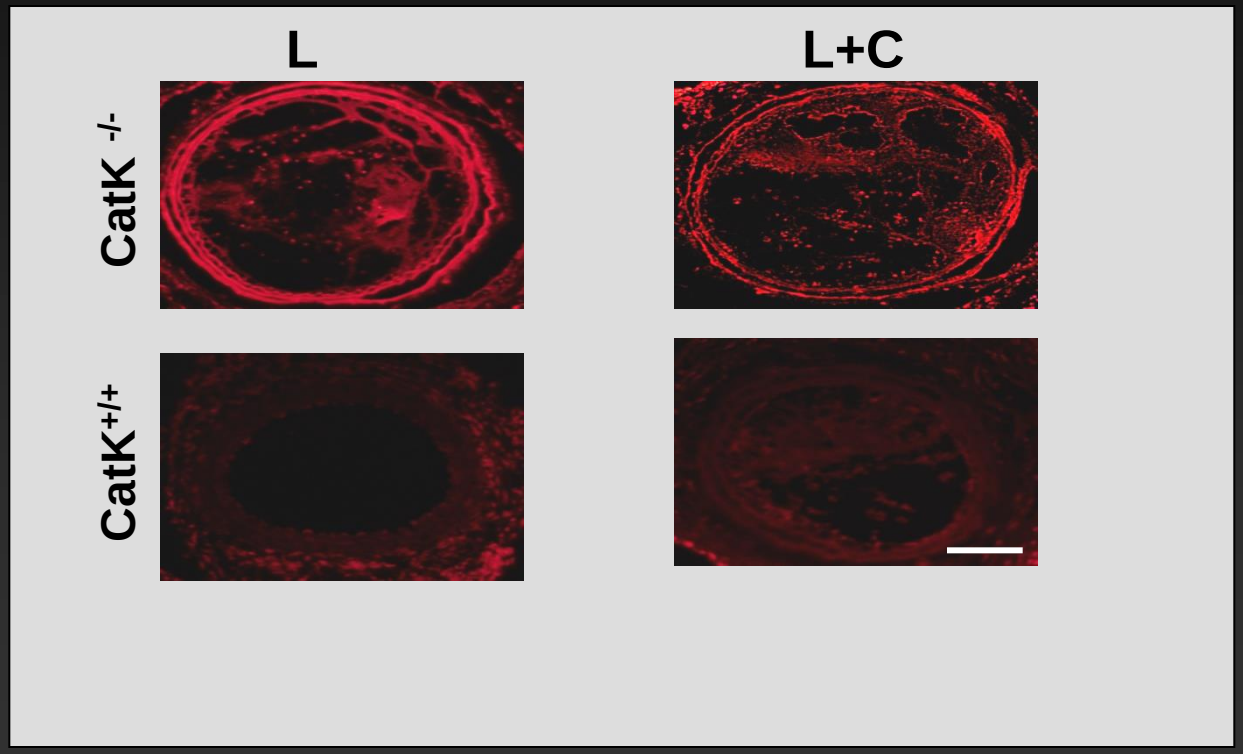


L = ligation
L+C = ligation + cuff

CatK$^{-/-}$ impairs aorta-derived SMC sprouting and invasion of SMC and macrophage

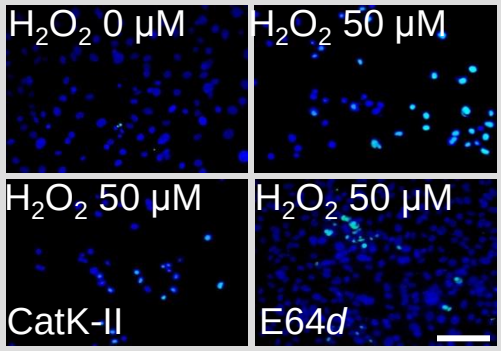


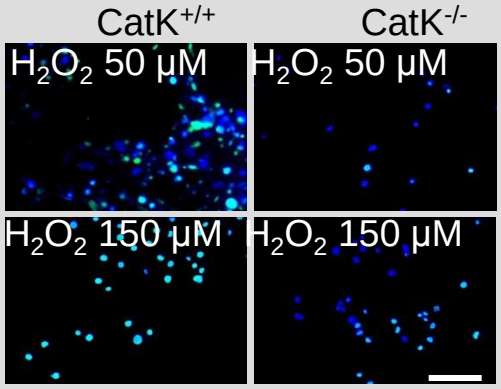
CatK$^{-/-}$ reduces superoxide production in response to both injuries

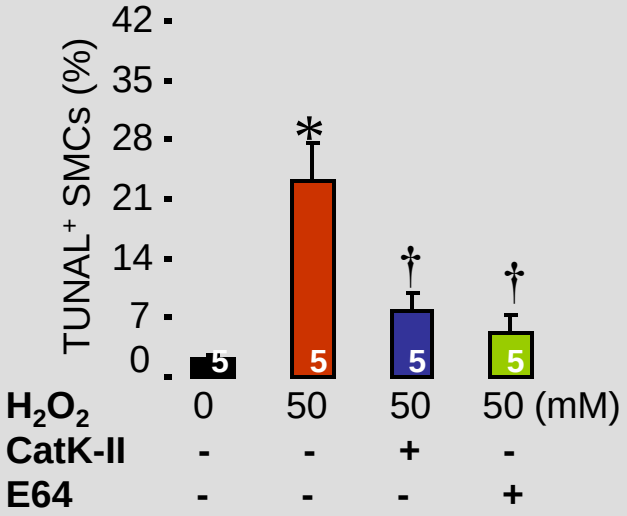


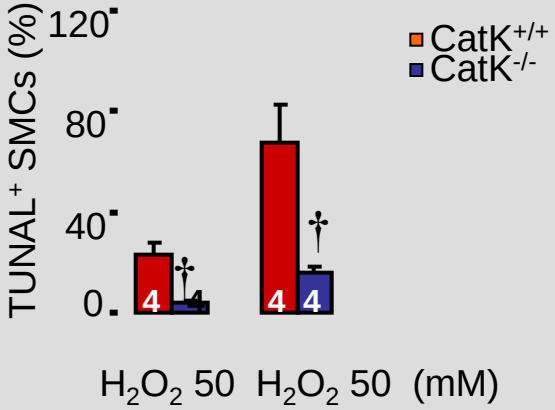
L = ligation
L+C = ligation + cuff

CatK$^{-/-}$ reduces aortic SMC apoptosis induced hydrogen peroxide





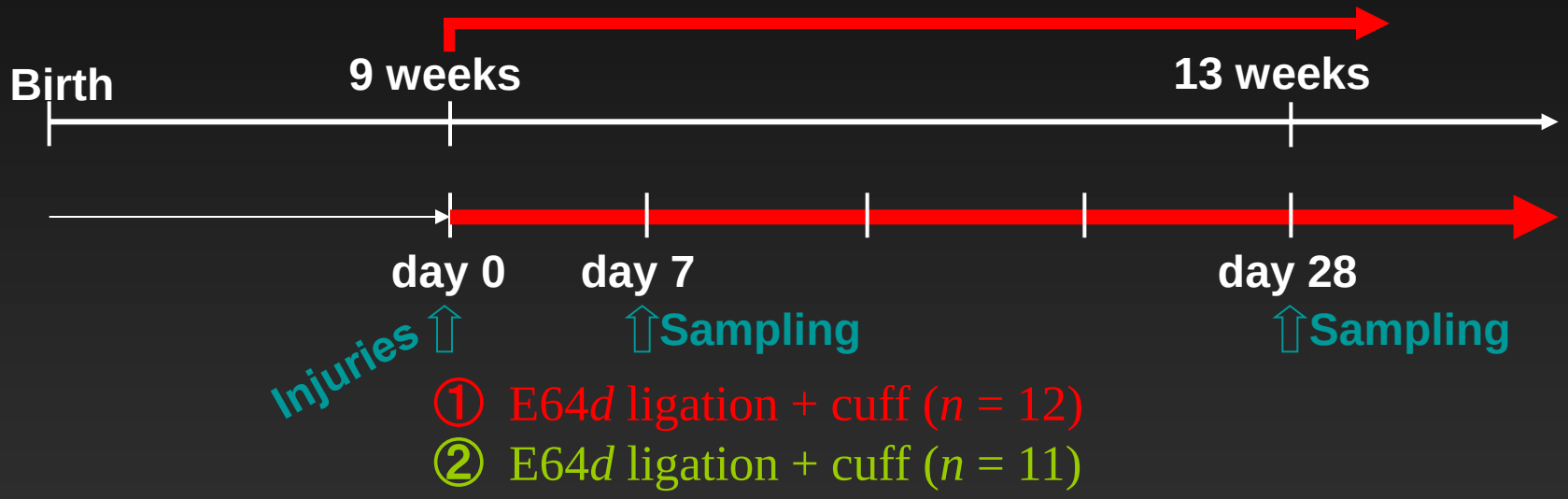




CatK-II = CatK specific inhibitor

* P < 0.001 vs control; † p < 0.05 vs corresponding control

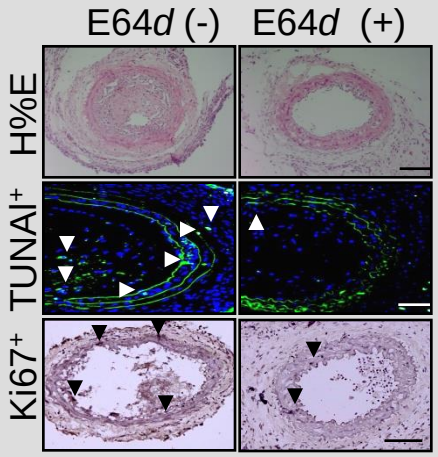
Exp: Protocol (2)

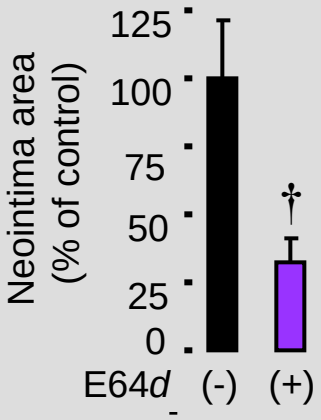


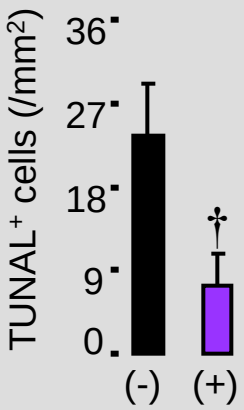
Methods

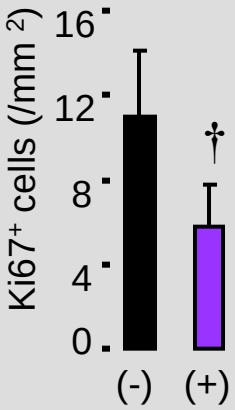
- Morphological analysis
- Immunohistochemistry
- Quantitative real-time PCR
- ELISA etc.

Efficacy of E64*d* on neointimal formation and SMC apoptosis and proliferation





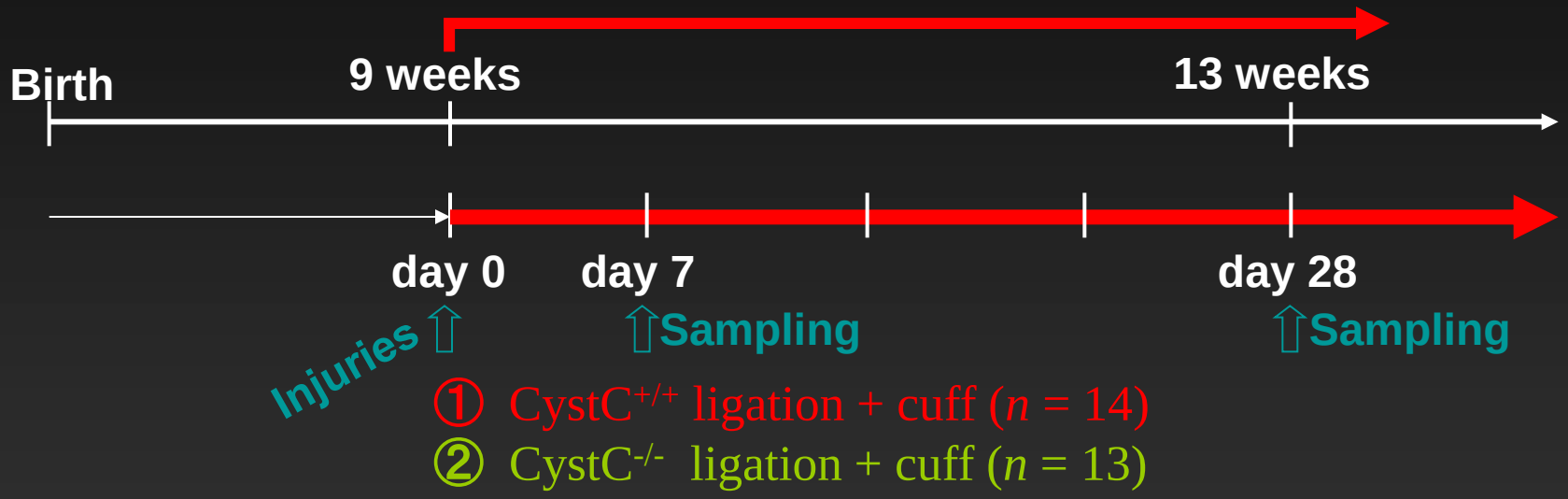




E64*d* = non-specific CatK inhibitor

† $p < 0.05$ vs control

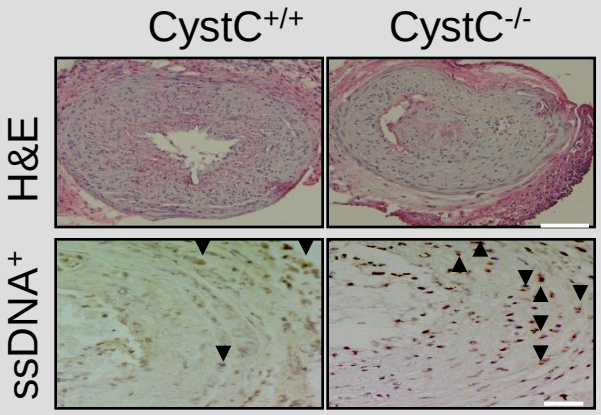
Exp: Protocol (3)

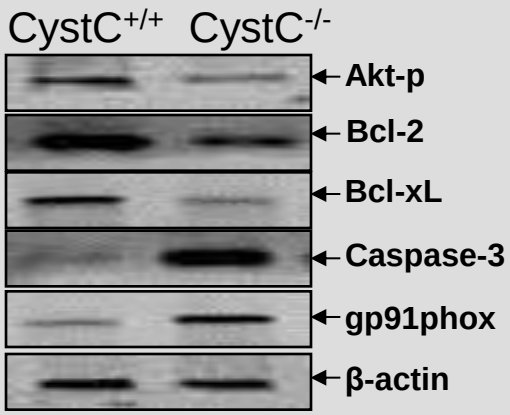


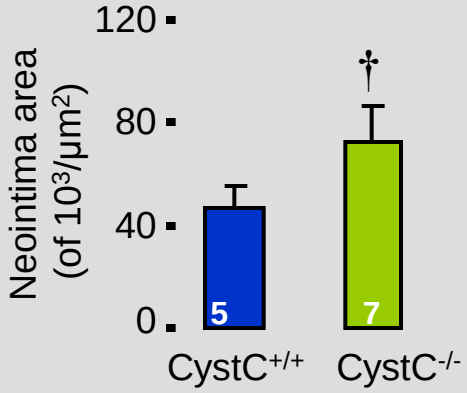
Methods

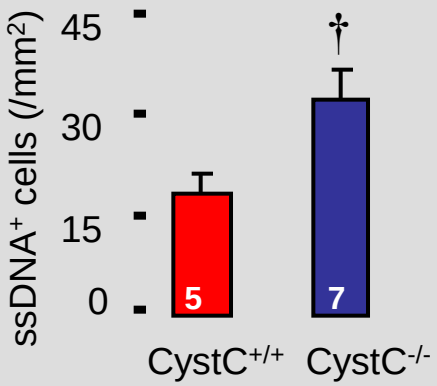
- Morphological analysis
- Immunohistochemistry
- Quantitative real-time PCR
- ELISA etc.

CystC$^{-/-}$ reduces neointimal formation and SMC apoptosis





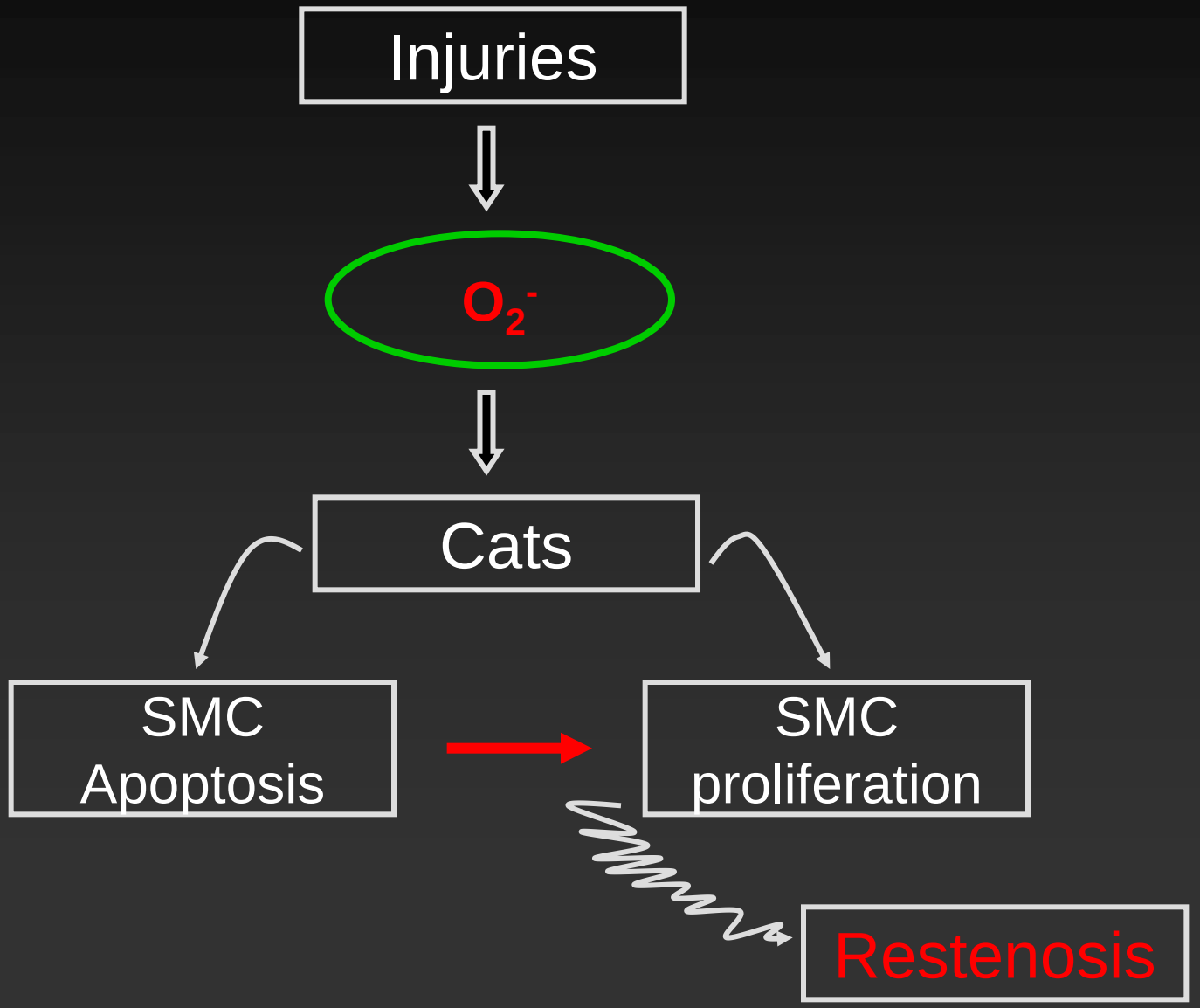




Observations

- On operative day 28, CatK deficiency significantly reduced neointimal formation in both single- and double-injured arteries as compared with corresponding control Cat $K^{+/+}$ mice.
- At day 7, CatK deficiency reduced lesion macrophage content, medial cell proliferation and apoptosis, the mRNA levels of TLR-4, CCL12, and gelatinolytic activities of MMP-2 and -9, and increased the mRNA levels of TIMP-1 and -2.
- E64*d* decreased neointimal lesion formation and medial SMC apoptosis and proliferation.
- Cystatin C deficiency enhanced lesion macrophage content, neointimal lesion, medial cell proliferation and apoptosis.
- CatK deficiency reduced SMC or/and macrophage invasion proliferation, apoptosis.

Proposed mechanism



Conclusion

This study demonstrates an essential role of Cat K in atherosclerotic neointimal formation in response to injury,possibly via the reduction of SMC apoptosis and proliferation associated inflammation and stress, suggesting a novel therapeutic strategy for the control of vascular intervention-related restenosis by regulating Cat K activity.

Circulation Research

7 November 2011

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