Exercise Rescues Vascular Action in Response to Hypoxia in Aged Animal and Human

Department of Cardiology
Nagoya University School of Medicine

Xian Wu Cheng MD, PhD

Disclosures (None)

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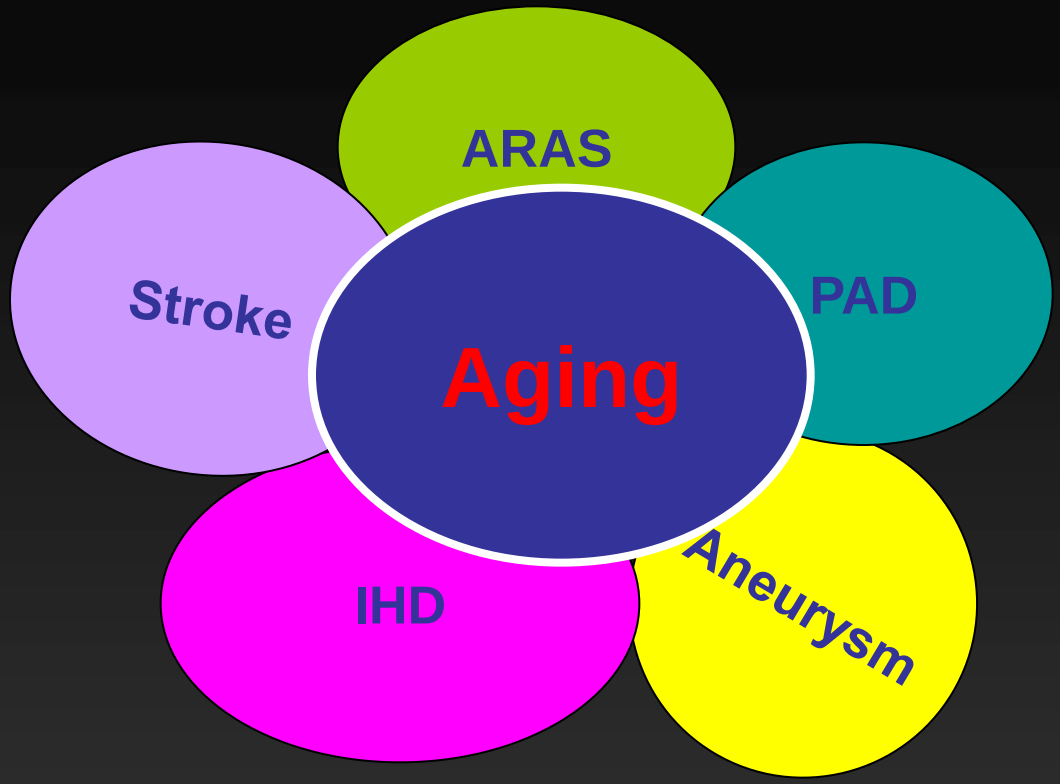


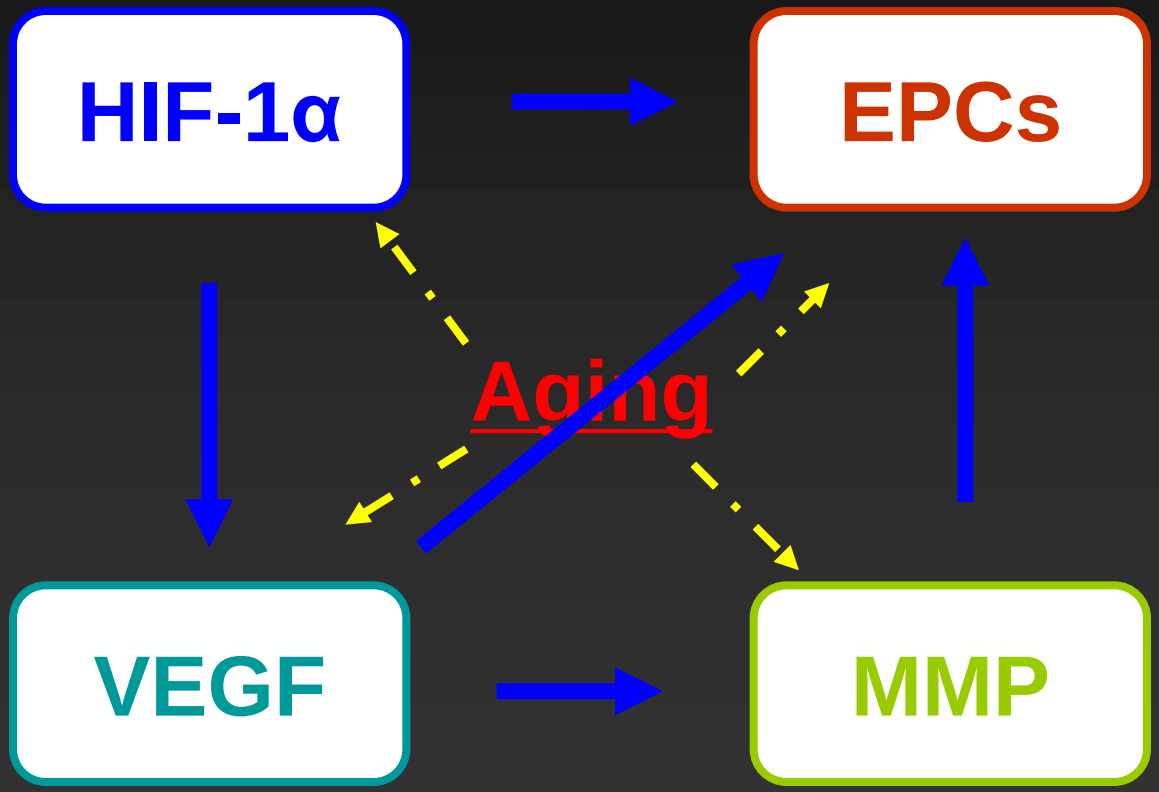
1) Atherosclerosis
2) Vascular aging
3) Ischemia-hypoxia

IHD: Ischemic heart disease
PAD: Peripheral arterial disease
ARAS: Atherosclerotic renal artery stenosis

Anti-vascular aging (anti-aging)

- Exercises
- Pharmacological interventions
- Diet/calorie restriction
- Others

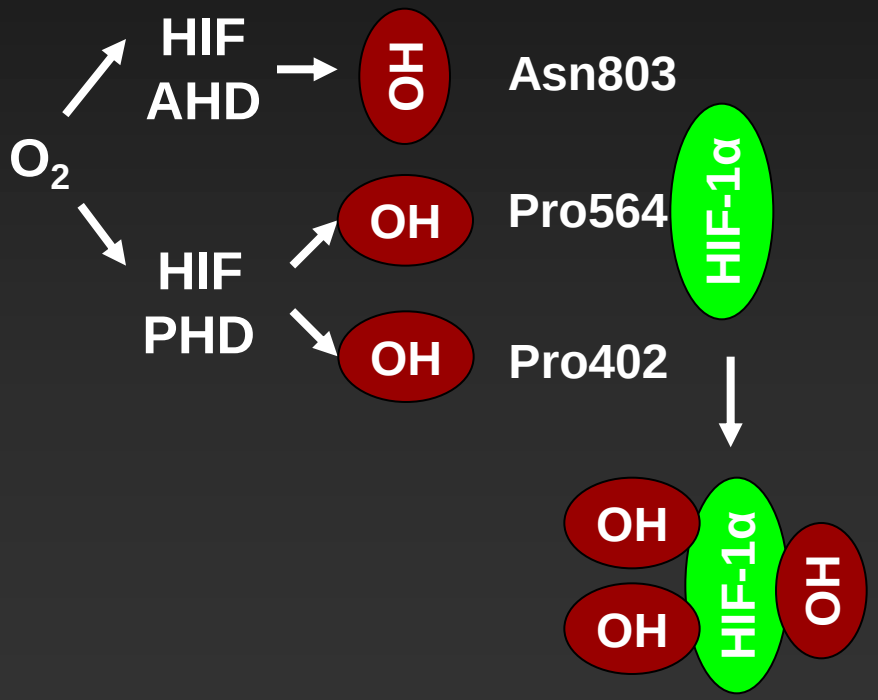
Key player for vascular action response to hypoxia



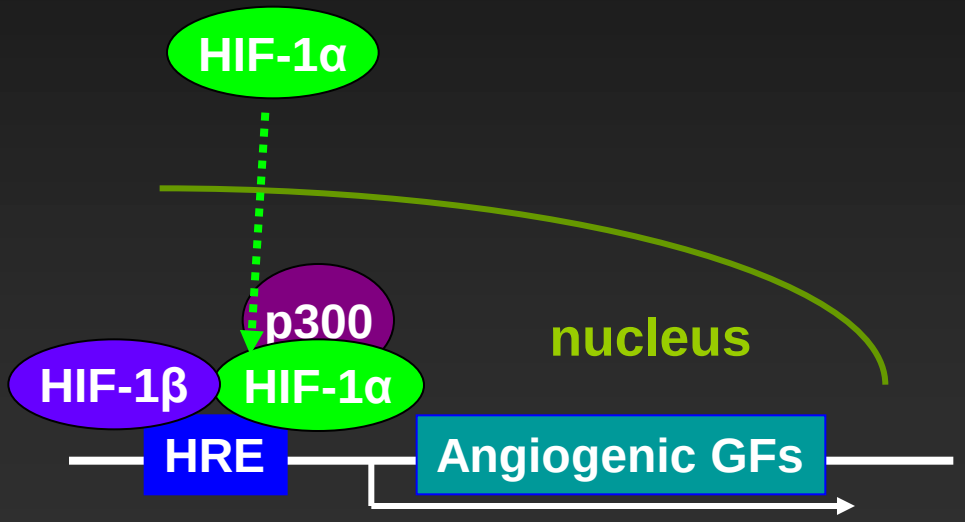
HIF-1α: Hypoxia-induced factor-α; EPC: endothelial progenitor cell
VEGF: vascular endothelial growth factor; MMP: matrix metalloproteinase

HIF-1α metabolism

Normal oxygen condition



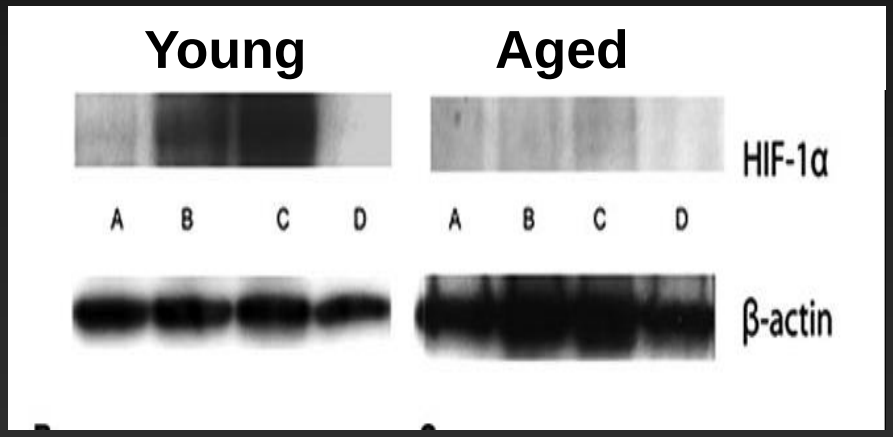
Hypoxic condition

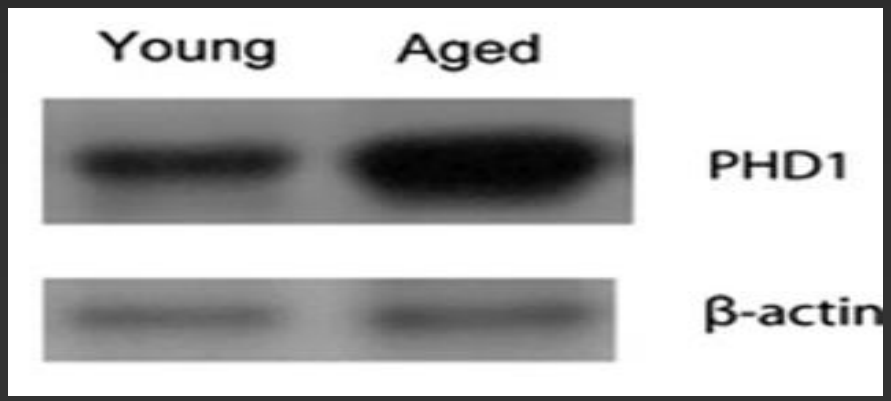


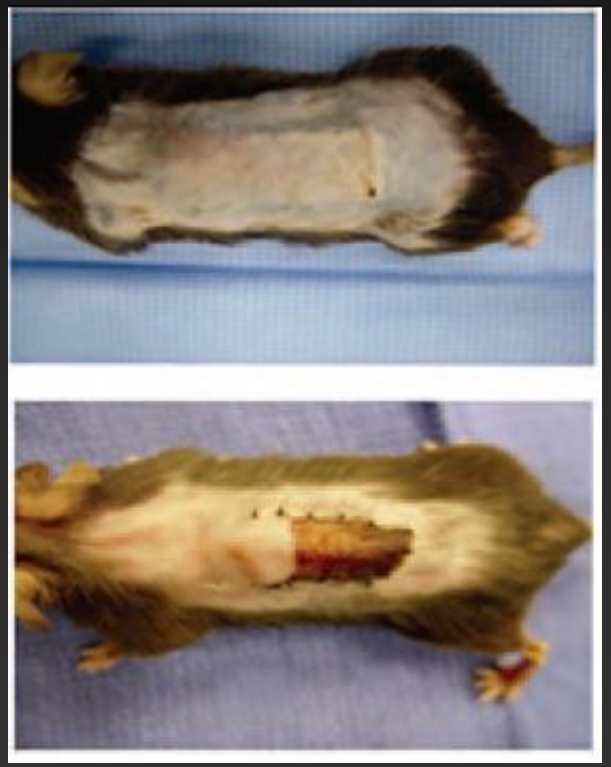
HIF-1α: Hypoxia-induced factor-α
GF: Growth factor
HD: Asparaginyl hydroxylase
PHD: Proly hydroxylase domain

Representative ischemic flaps showing complete healing in the young and necrosis in the aged

Western Blotting







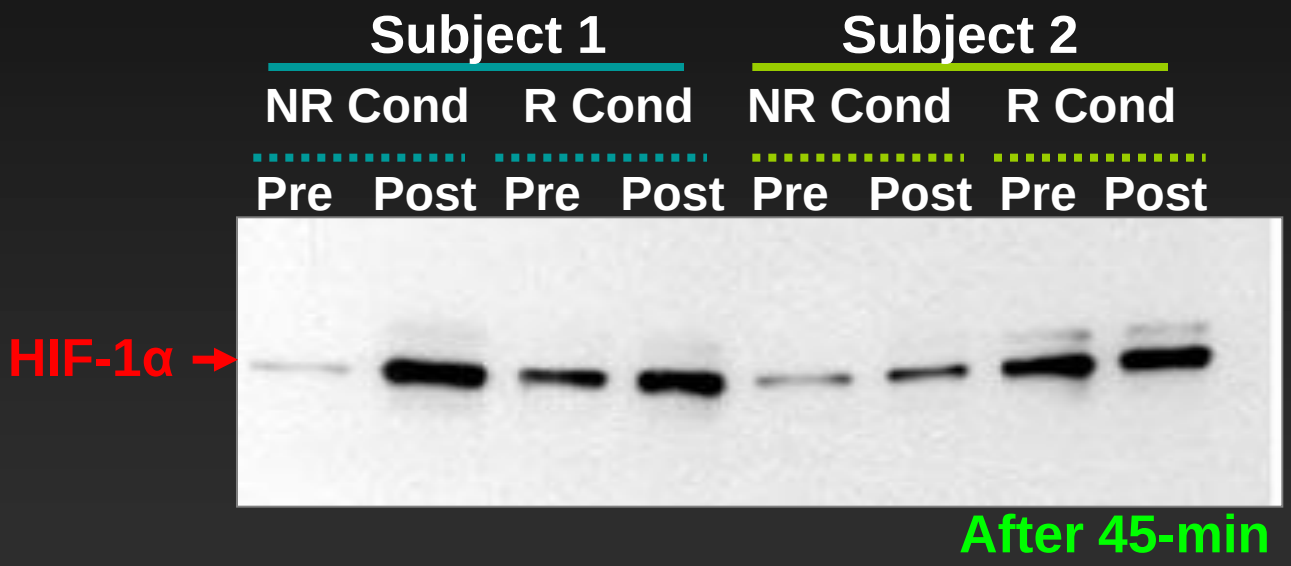
Yong

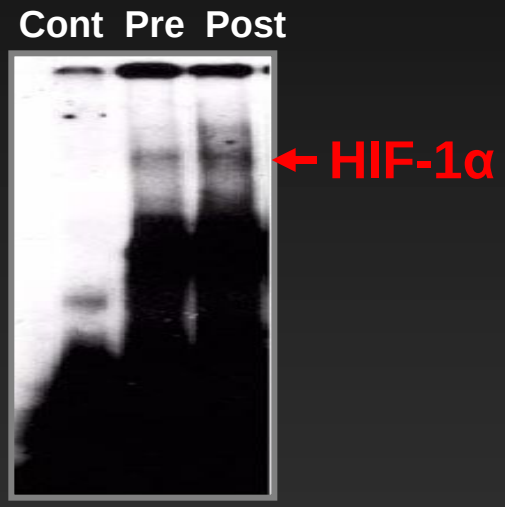
Aged

PHD: Proly hydroxylase

Chang EI et al. Circulation 2007

Exercise induces HIF-1α expression and its DNA binding activity

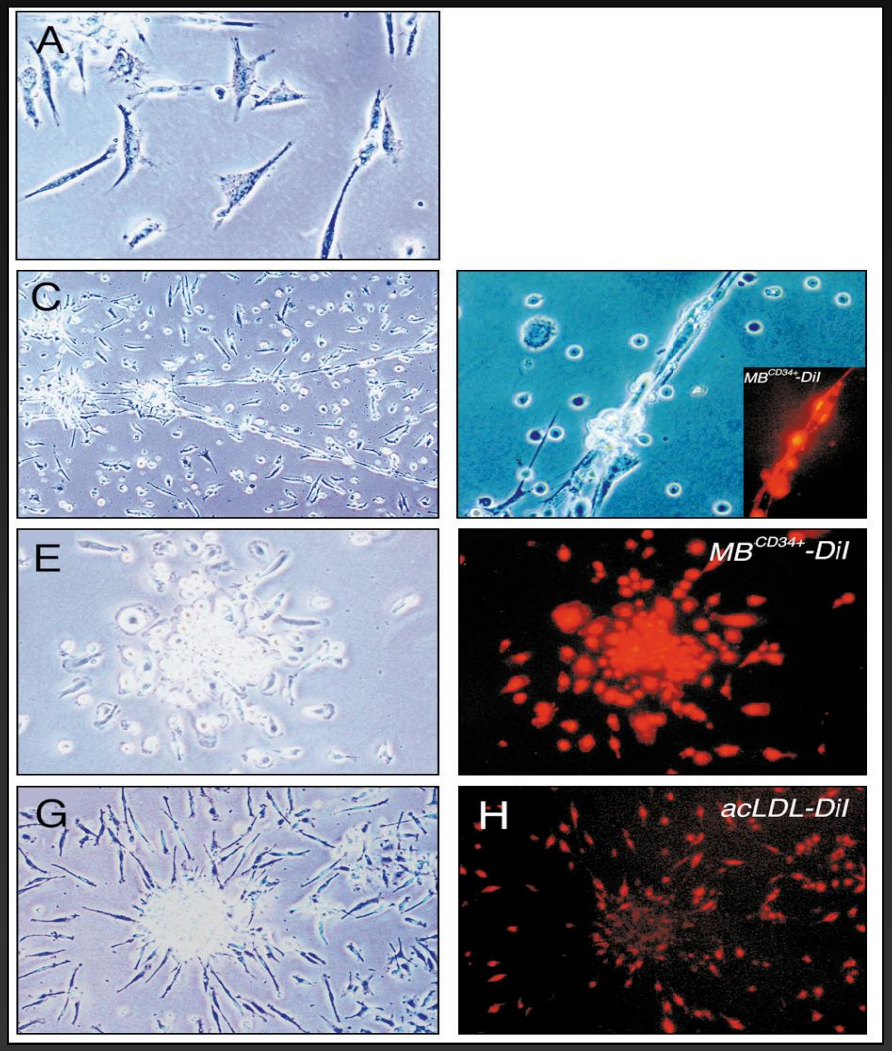




NR Cond: non-restricted blood flow condition
R Cond: restricted blood flow condition
Pre: before 1-legged knee-extension exercise
Post: after 1-legged knee-extension exercise

Ameln et al. FASEB J 2005

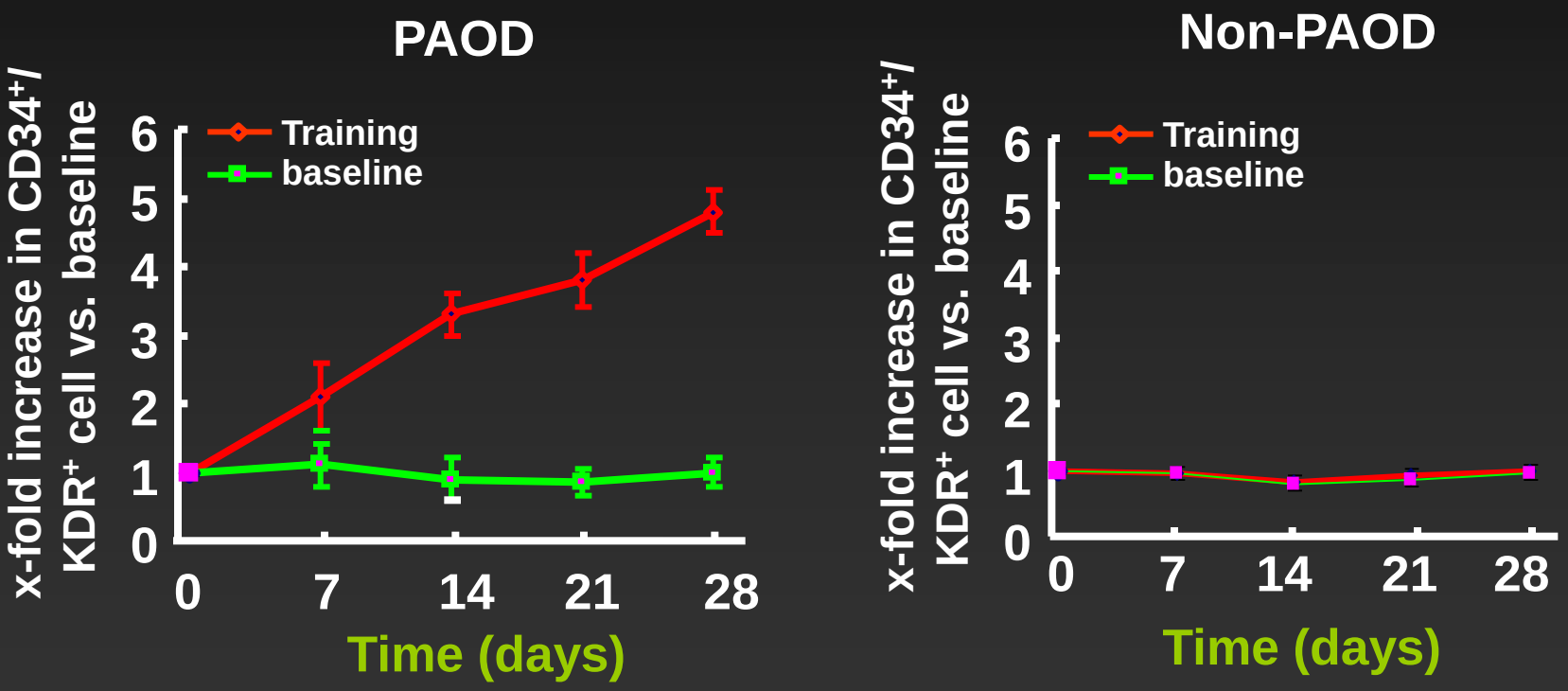
Isolation of putative endothlial progenitor cells (EPCs) in peripheral blood



EPCs Markers:
CD45 (-)
CD31 (+)
CD34 (+)
Flk-1 (+)
Tie-2 (+)

Asahara T and Muroahra T. Science 1997.

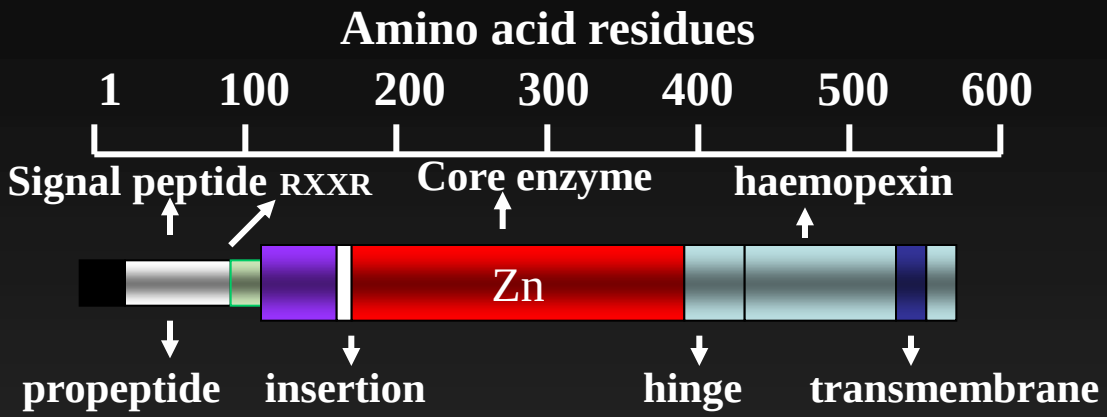
Effects of exercise and ischemia on CPCs mobilization in patients with PAOD

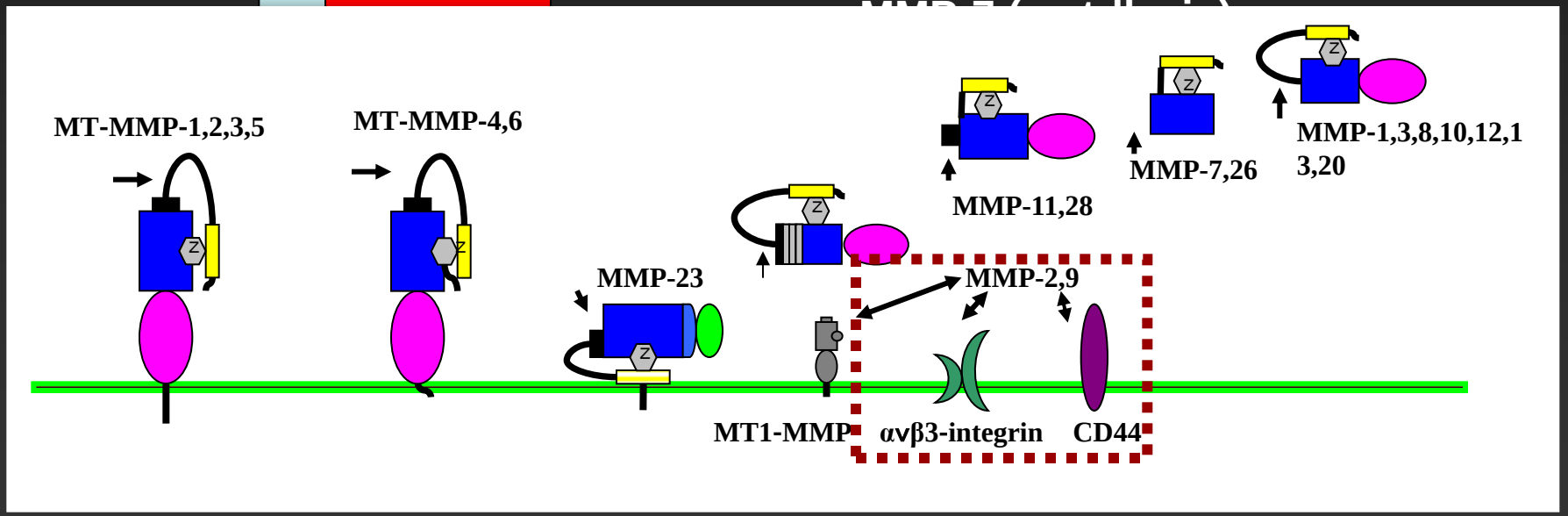


CPC: Circulating progenitor cells
PAOD: Peripheral artery occlusive disease

Sandori et al. Circ 2005

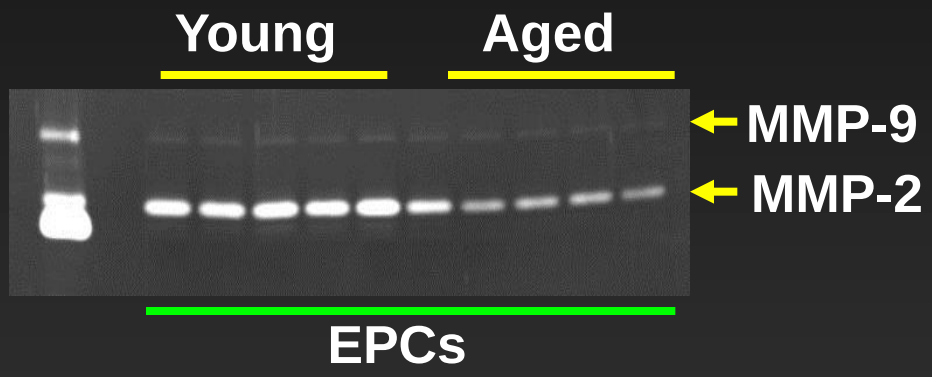
Review on MMP structure and classification

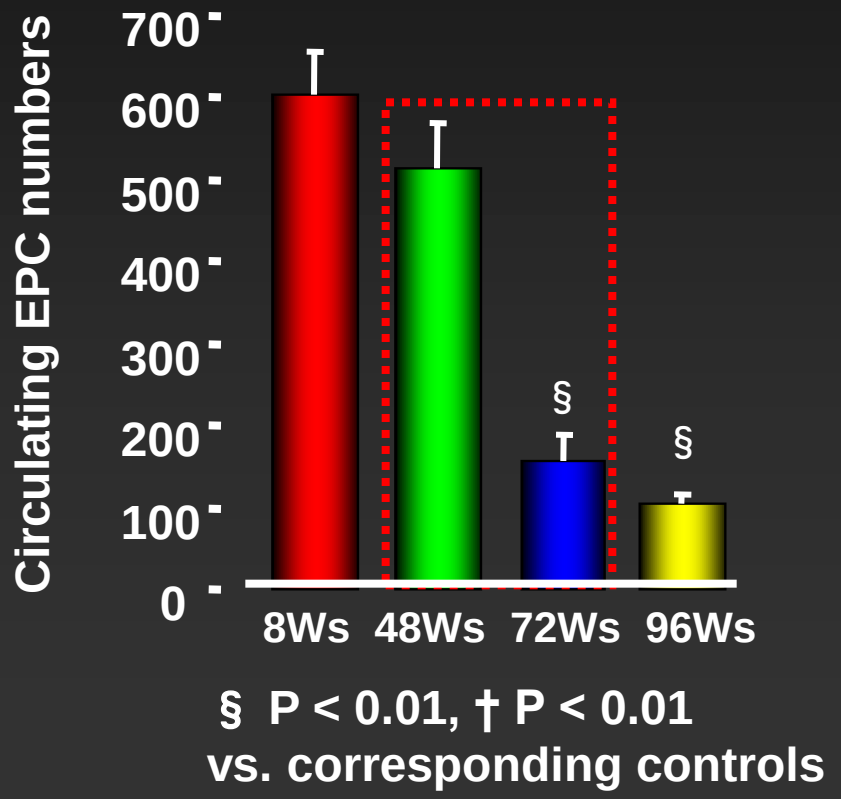




Cheng XW . JCEM (Review) 2007

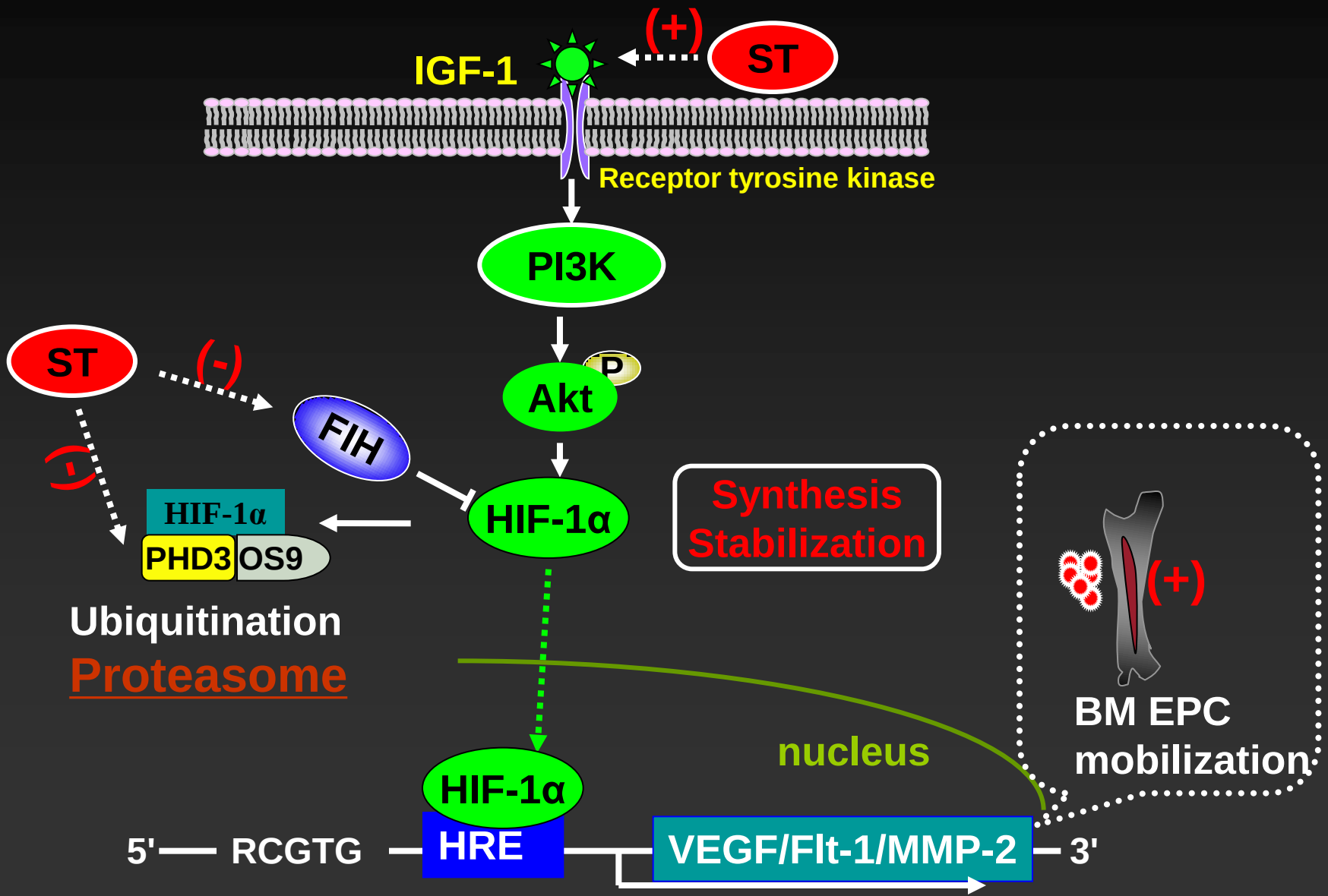
Age impairs MMP-2 expression and EPC recruitment response to ischemia





Cheng XW et al. Circ Res 2007; CEPP 2010

Hypothesis



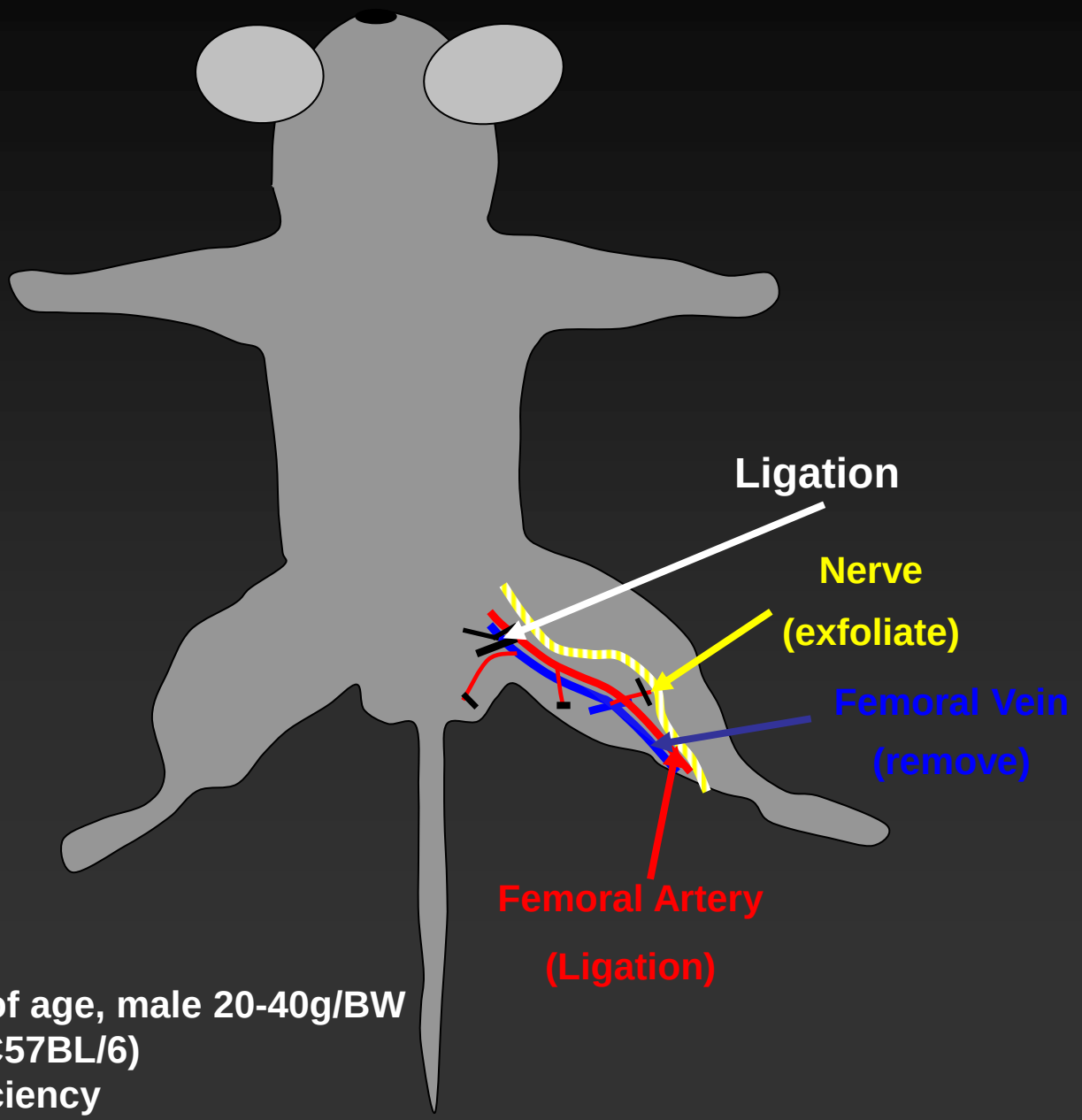
IGF: Insulin-like growth factor-1; FIH: factor inhibiting HIF-1

ST: Swimming training; PI3K: Phosphoinositide-3-kinase; (+): Positive; (-): Negative

Goal

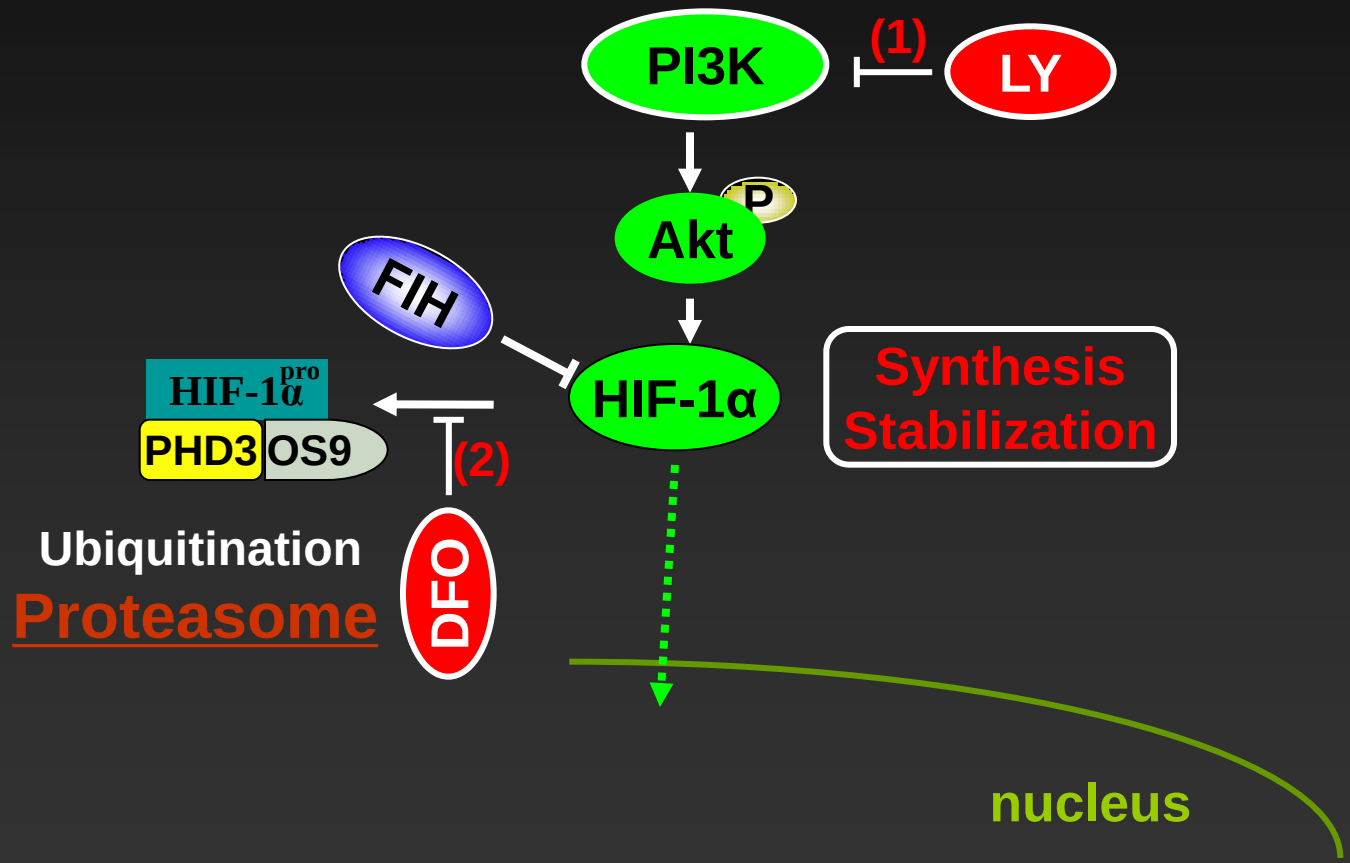
- To clarify the beneficial effects, and the mechanisms of actions, of exercise training in neovascularization of advanced age.

Mouse femoral artery ligation model



Aged mice: 24 months of age, male 20-40g/BW
WT: Wild type (C57BL/6)
MMP-2 KO: MMP-2-deficiency

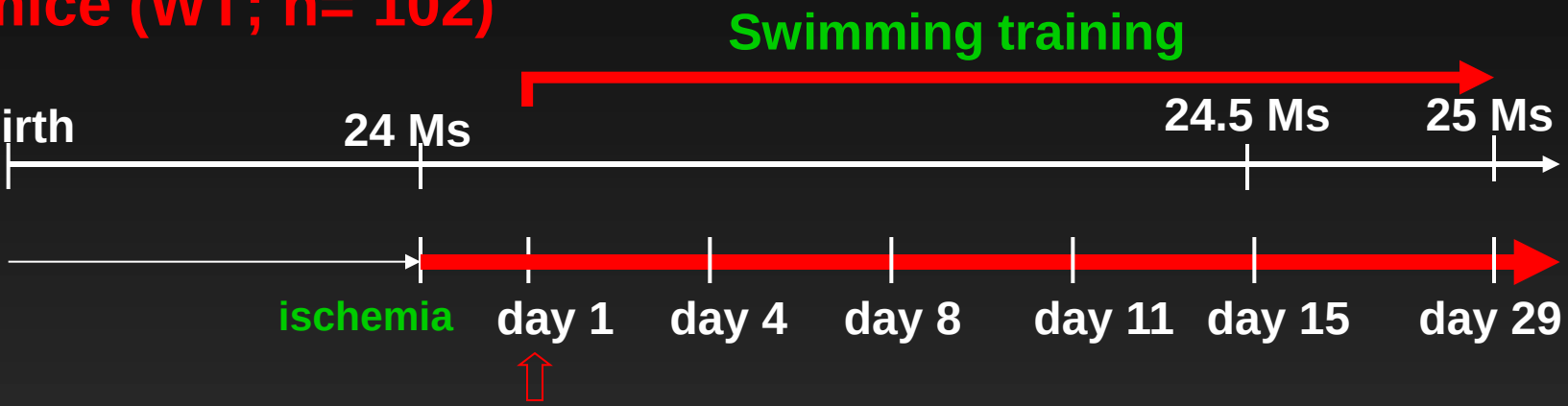
Experiment (1)



FIH: Factor inhibiting HIF-1
LY294002: PI3K inhibitor
DPO: Deferoxamine, HIF-1α stabilizer

Protocol (1)

WT mice (WT; n= 102)

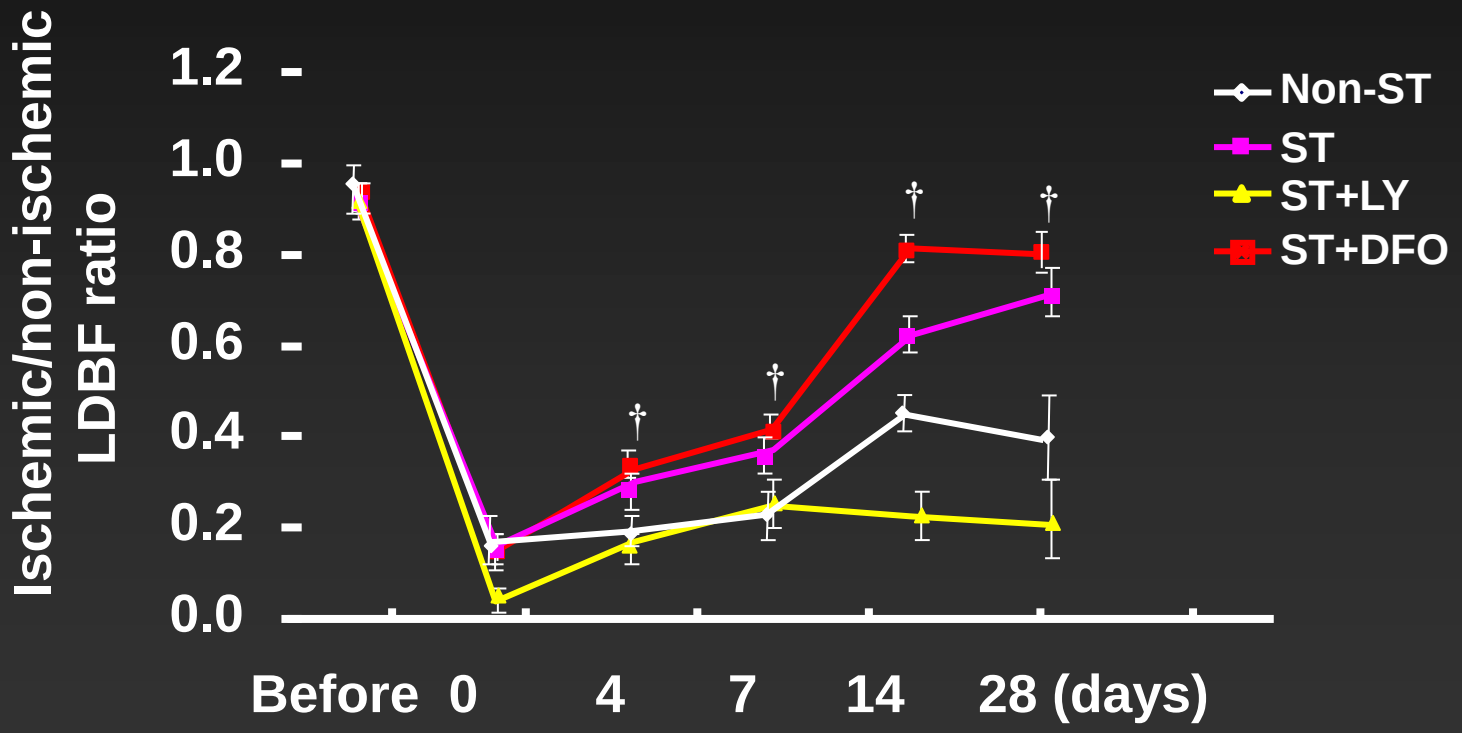


① Non-swimming training (non-ST),
② Swimming training (ST),
③ LY294002 (0.5 mom/kg/d; ST-LY),
④ Deferoxamine (10 mg/kg/d; ST-DFO)

Methods

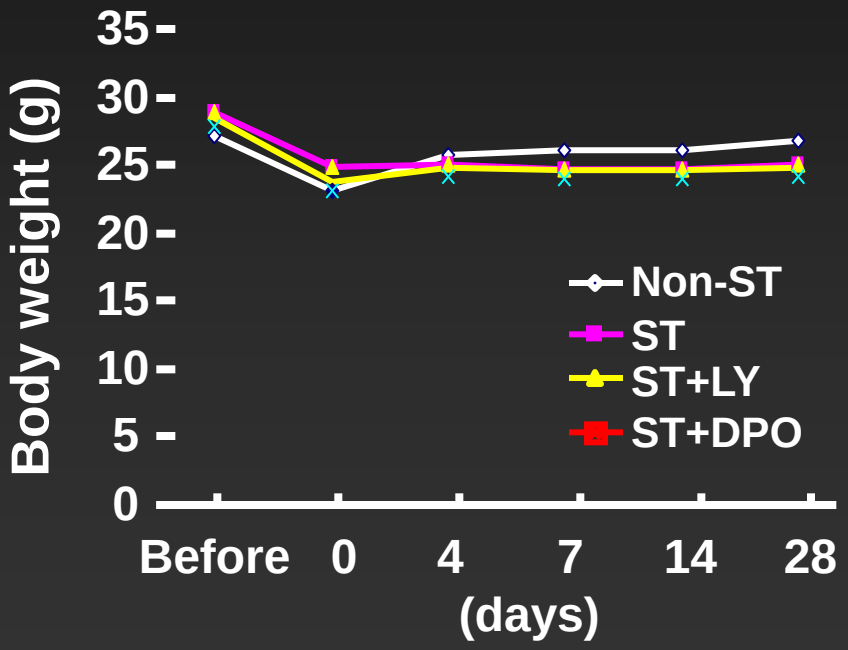
- Laser Doppler blood flow (LDBF) analysis
- Histological and biological analysis
- Bone-marrow transplantation assay
- In vitro stimulation assays

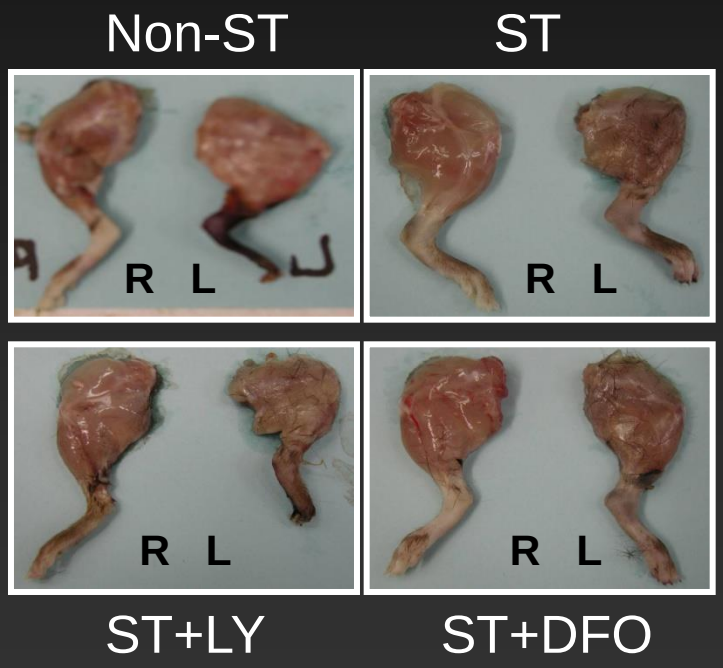
ST improves impaired blood flow perfusion in advanced age



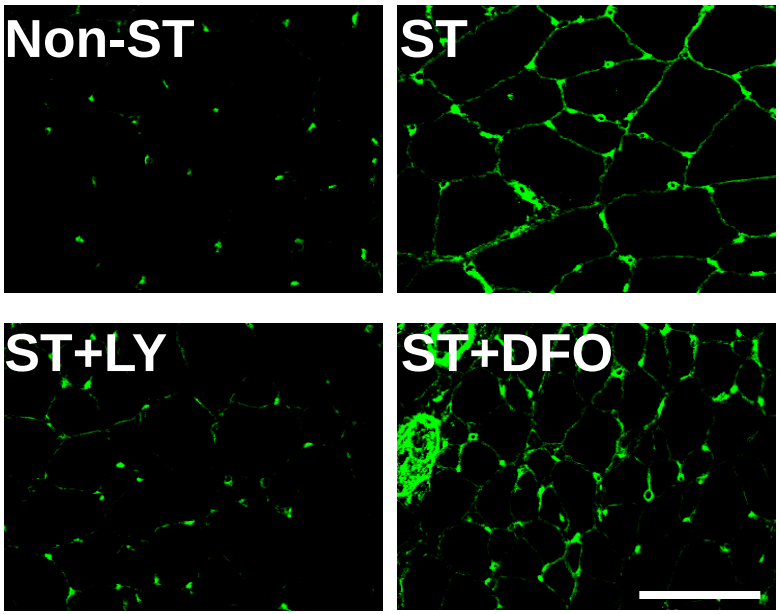
† P < 0.01 vs. corresponding controls

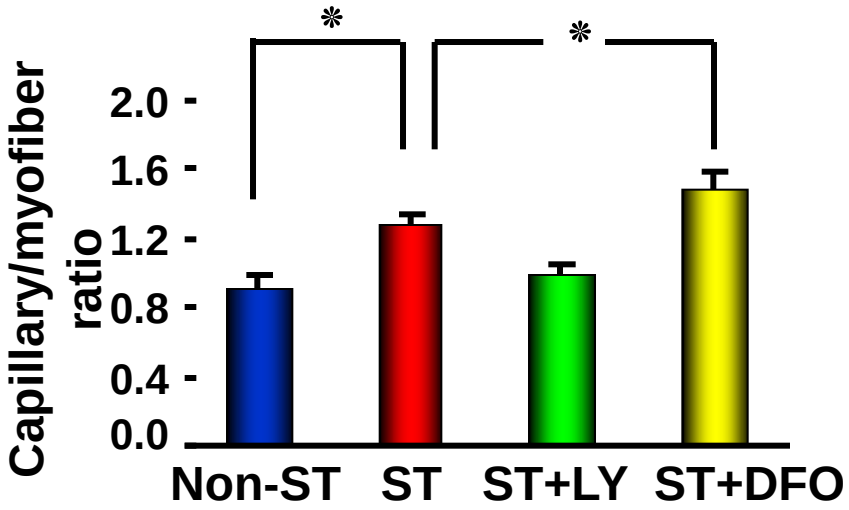
Body weight in four experimental groups





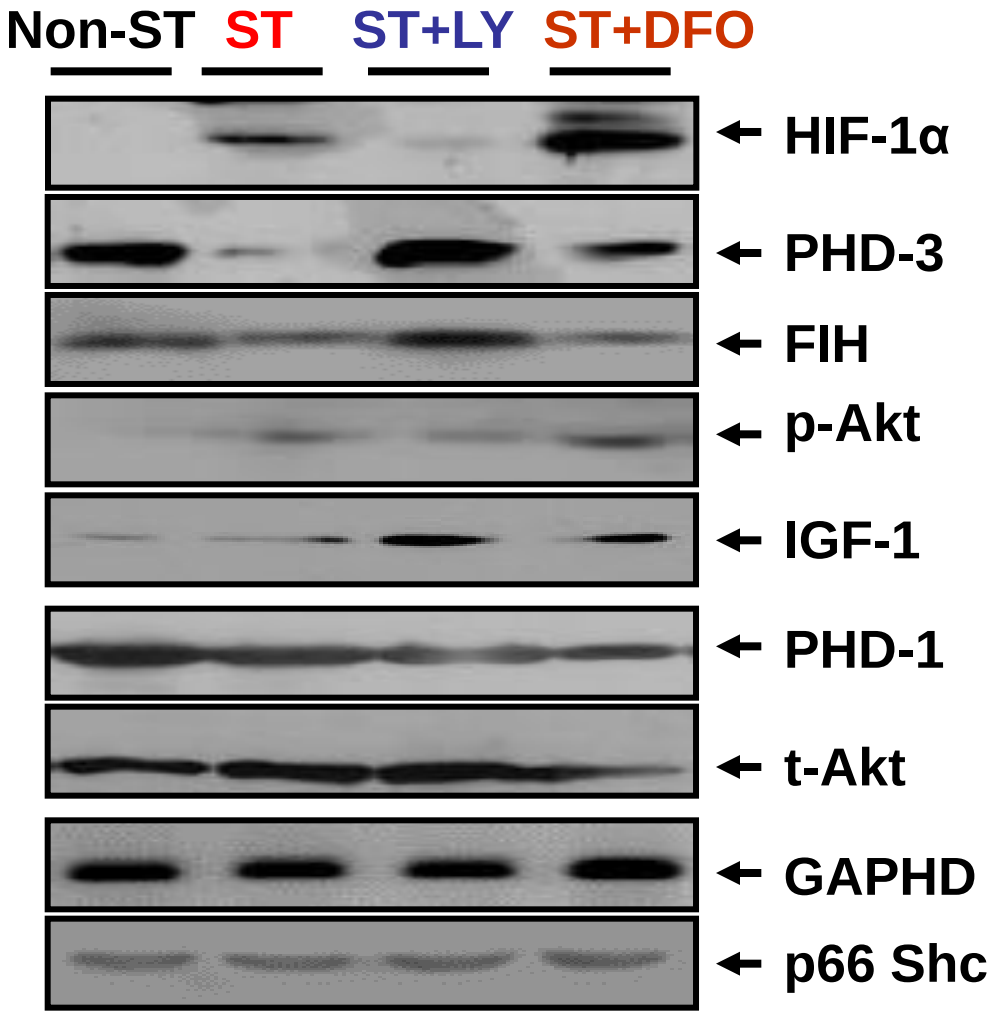
ST restores neovascularization response to hypoxia in the muscle of advanced age



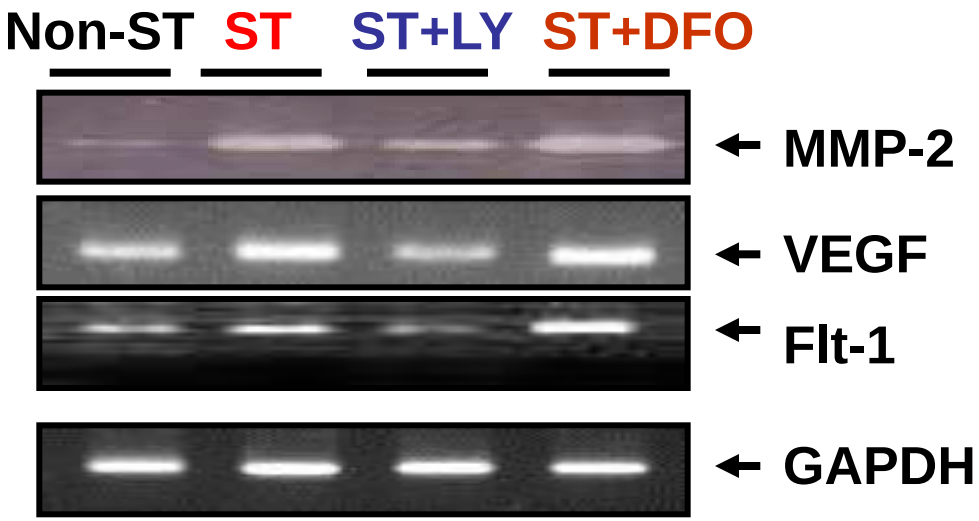


* P < 0.05 vs. corresponding controls

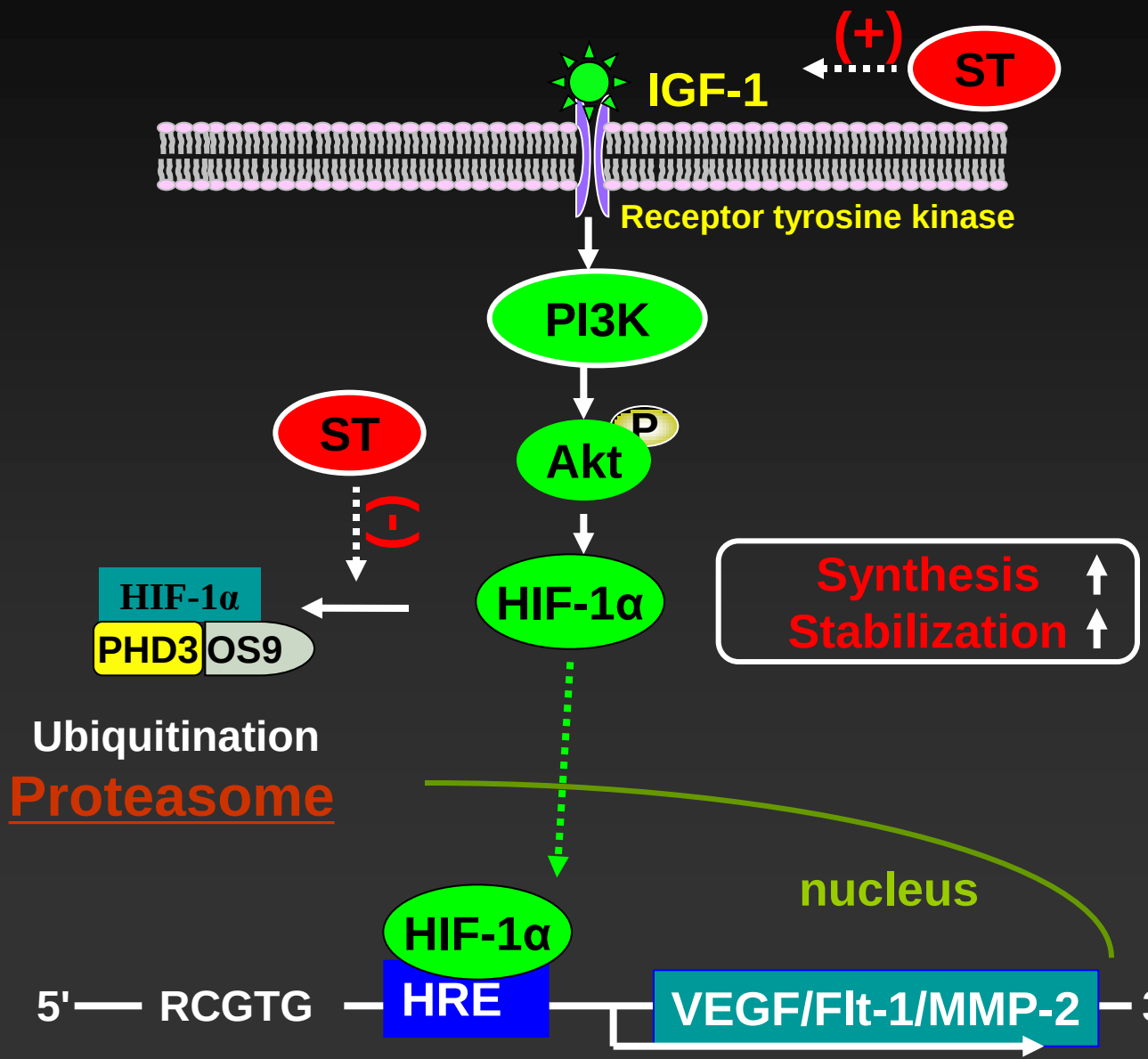
Effects of ST on the levels of HIF-1/p-Akt and PHD3/FIH proteins in ischemic muscles



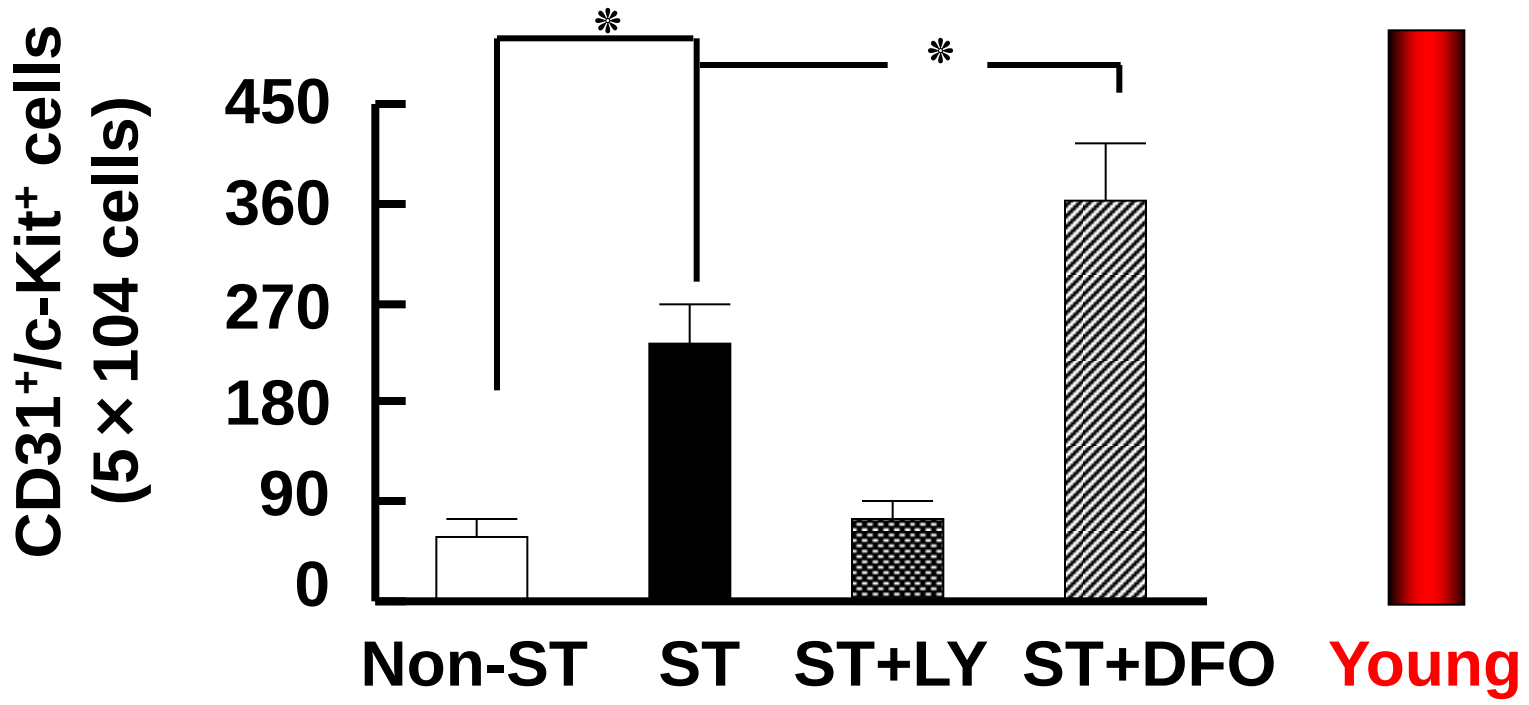
ST increased VEGF/Flt-1 mRNAs and MMP-2 activity in ischemic muscle



ST stimulates IGF-1/PI3K/Akt signaling pathway in ischemic tissue of advanced age

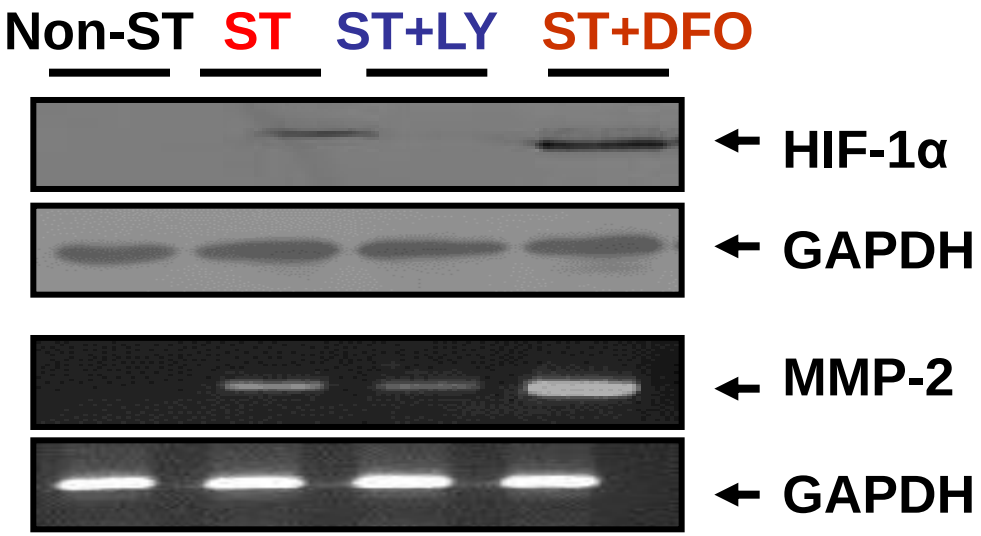


ST enhances BM-derived CD31^{+}/c-Kit^{+} EPC -like mononuclear cell mobilization

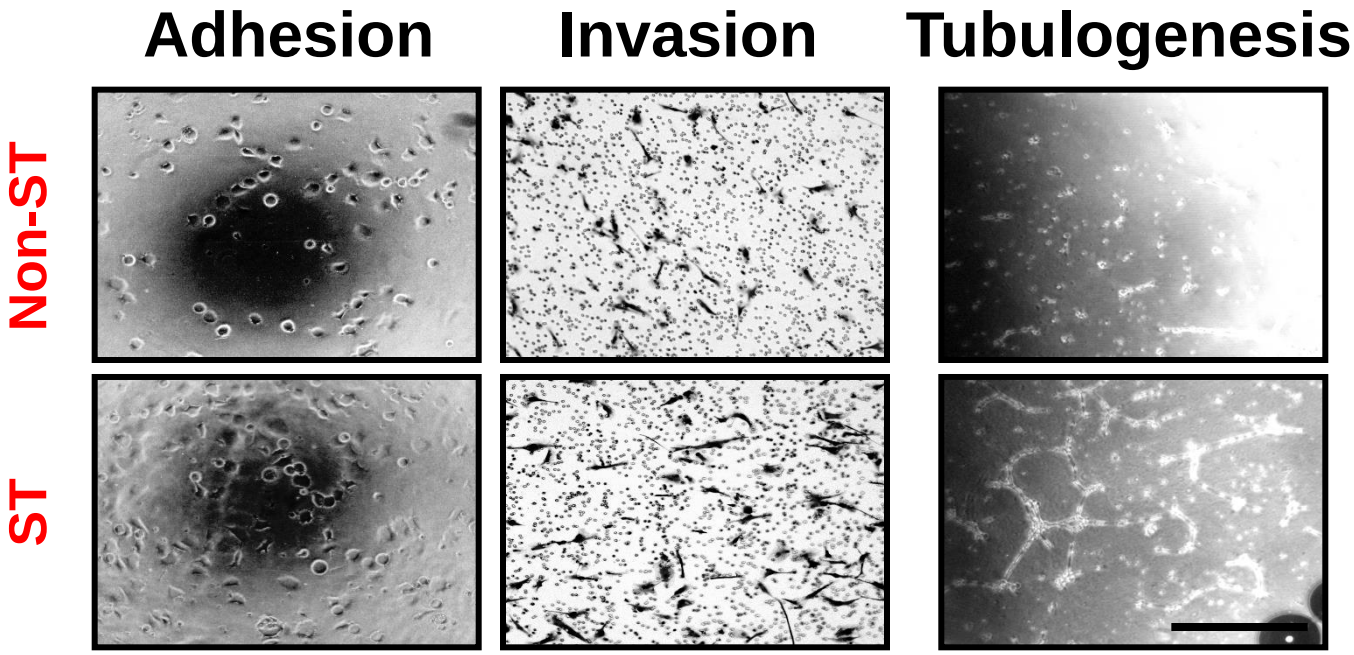


* P < 0.05 vs. corresponding controls

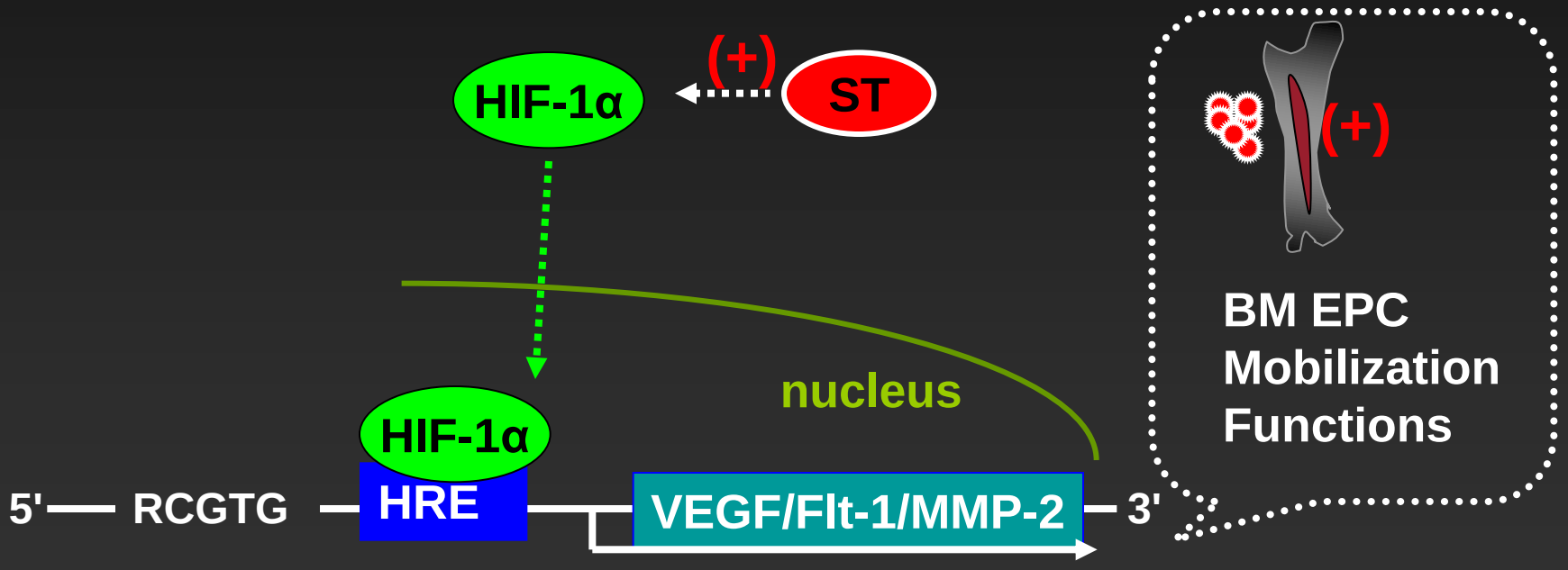
ST stimulates HIF-1α protein production and MMP-2 activity in BM-derived EPCs



ST improves BM-derived EPC function in advanced age

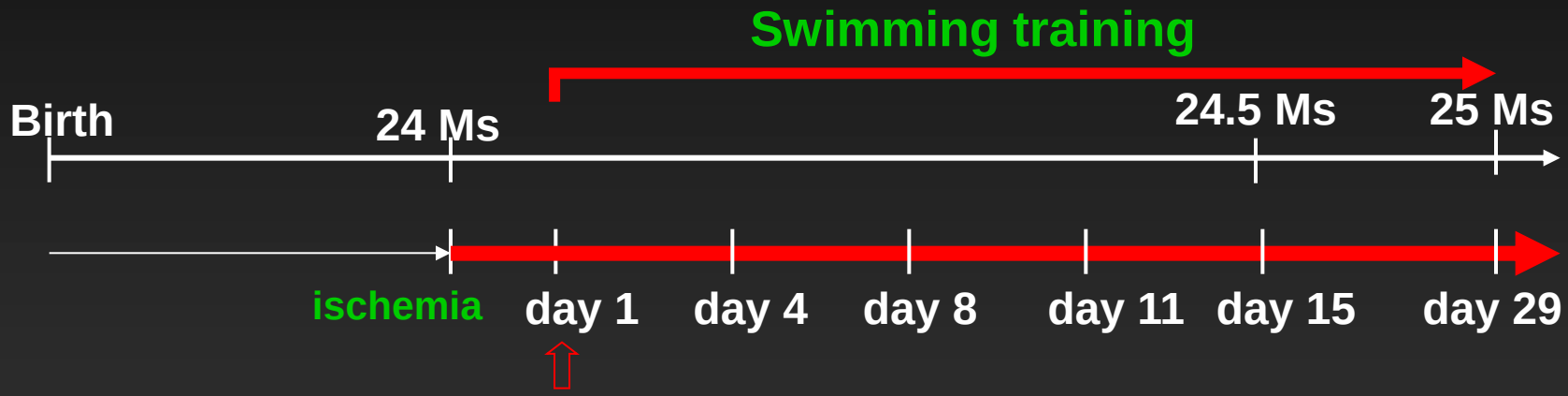


ST may stimulate HIF-1/VEGF/MPP-2 activation in BM-derived EPCs of advanced age



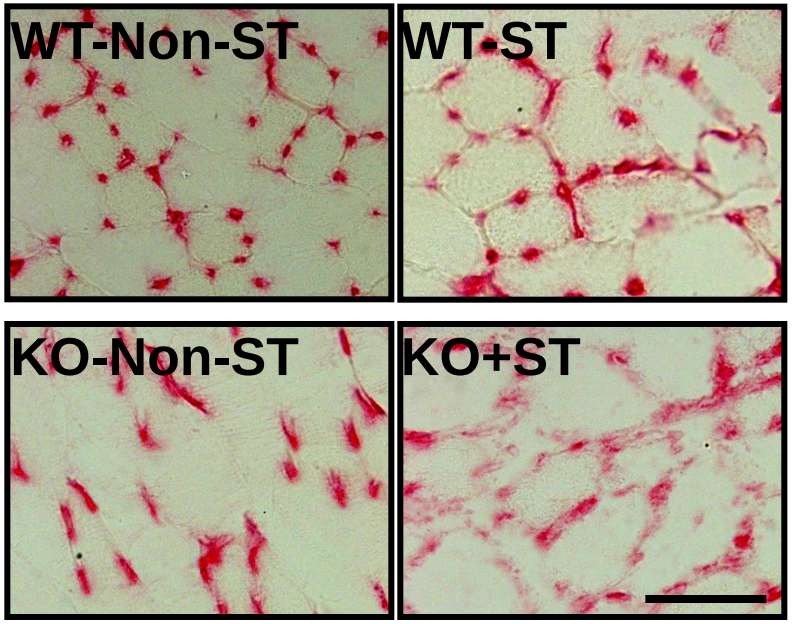
Exp1: Protocol (2)

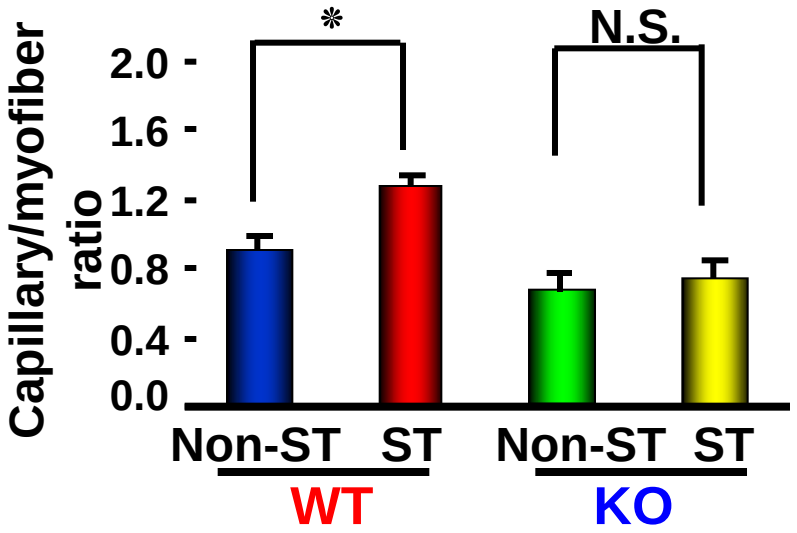
MMP-2$^{-/-}$ mice (KO; n= 48)



① Non-swimming training (KO-non-ST)
② Swimming training (KO-ST)

MMP-2 deficiency impairs ST-induced neovascularization in advanced age

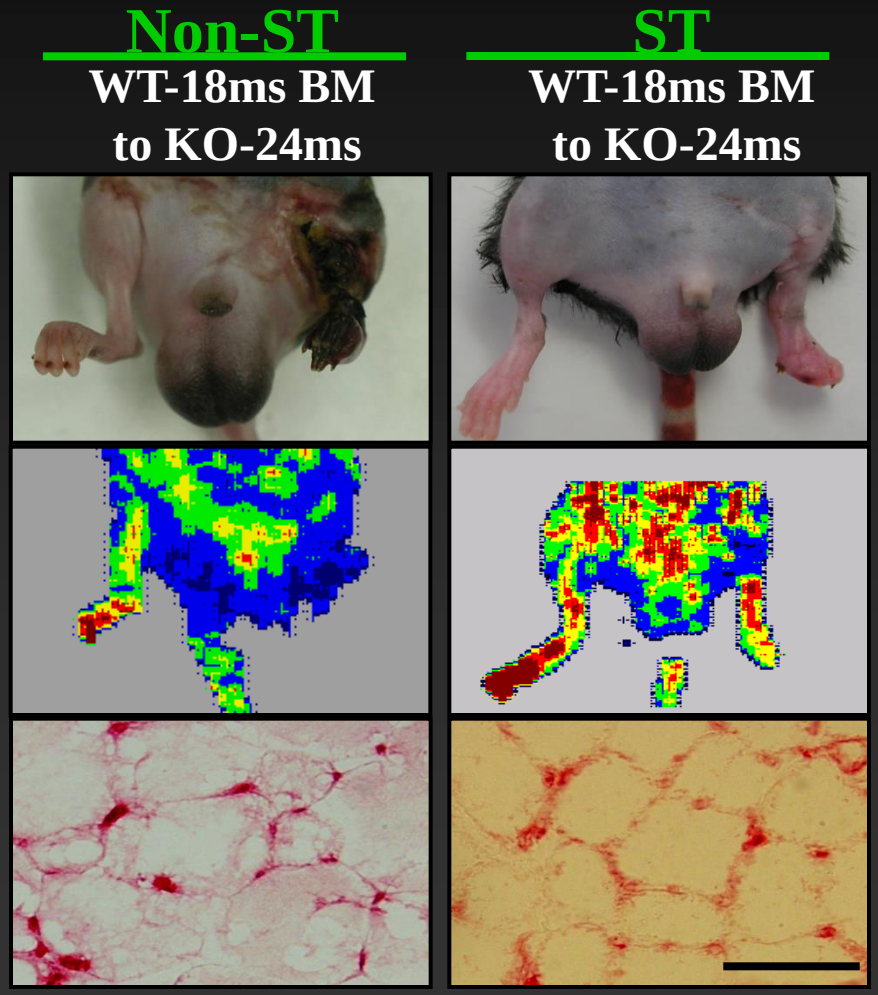




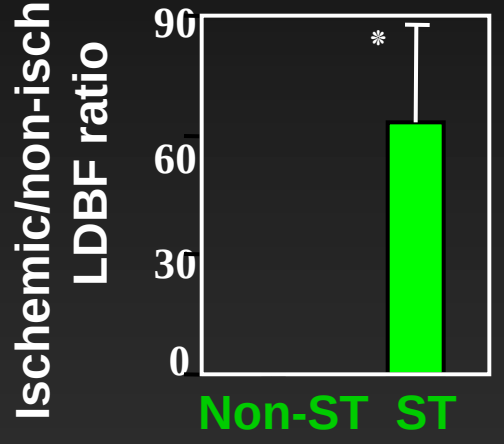
* P < 0.05 vs. corresponding controls

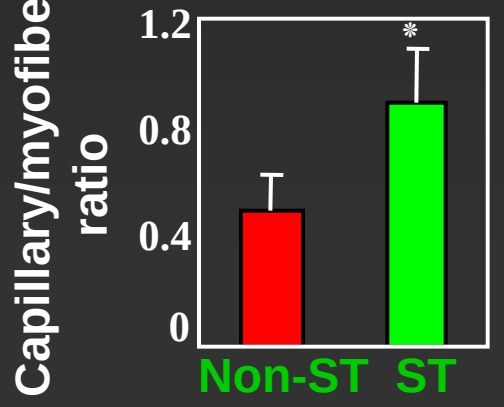
• N.S.: no significant difference

ST improves BMT-mediated blood flow and capillary density in mice of advanced age

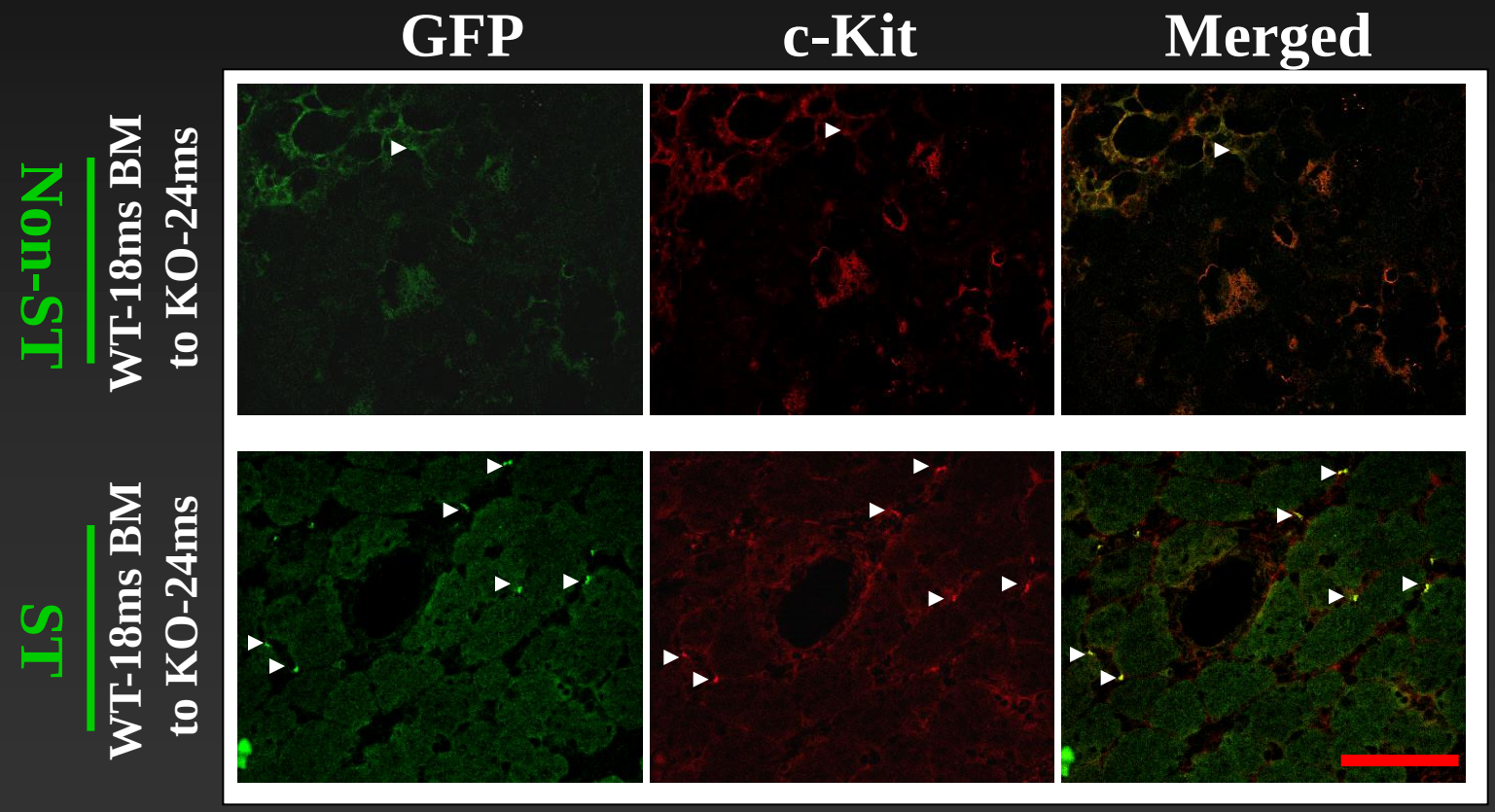


BMT: bone marrow transplantation





ST improves EPC-like c-Kit^{+} cells trafficking to sites of ischemic muscle in KO mice



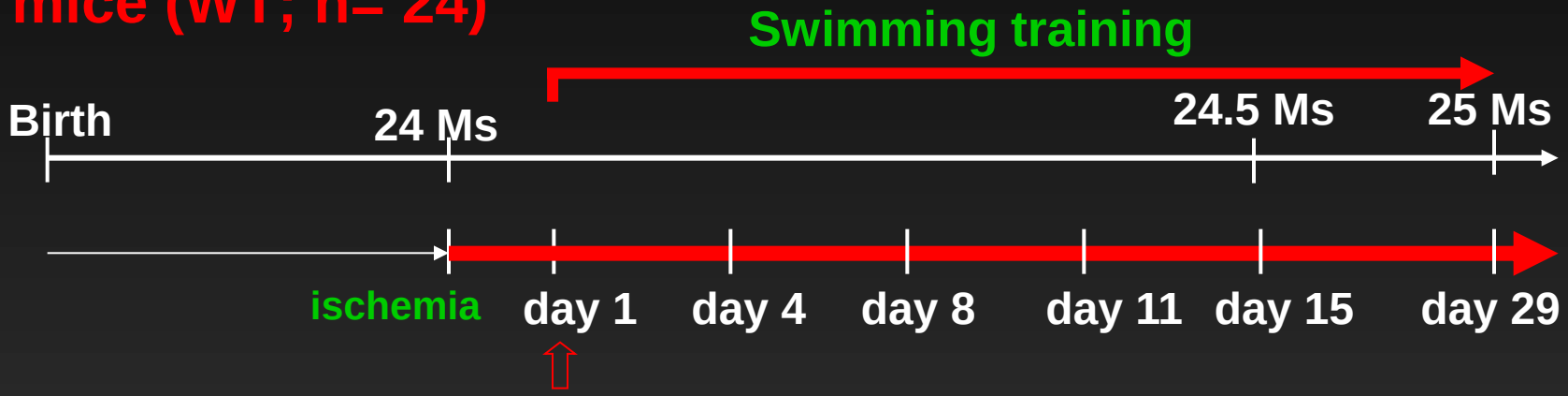
Cheng XW, et al. Circulation. 122:707-16; 2010

Anti-vascular aging (anti-aging)

- Exercises
- Pharmacological interventions
- Diet/calorie restriction
- Others

Protocol (3)

WT mice (WT; n= 24)

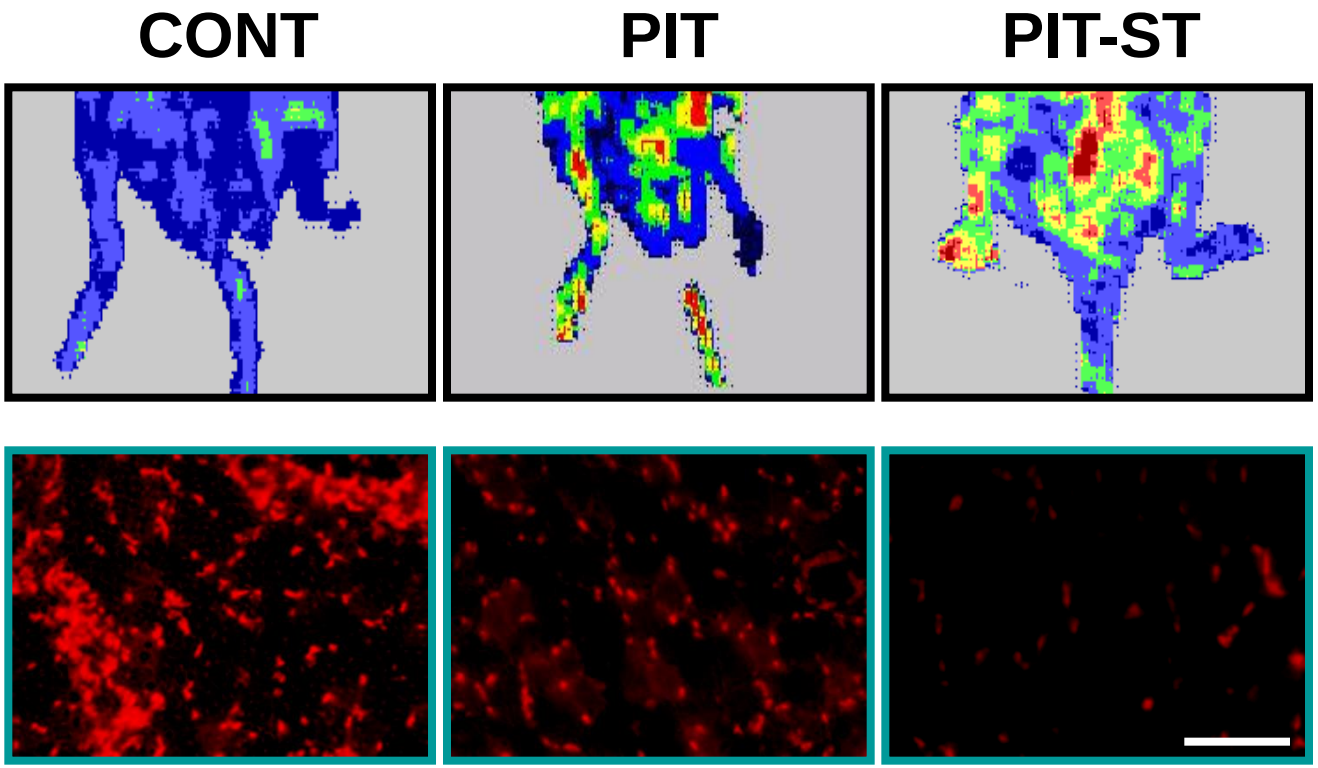


① None treatment (Cont)
② Pitavastatin (PIT)
③ Pitavastatin+swimming training (PIT-ST),

Methods

- Laser Doppler blood flow (LDBF) analysis
- Histological and biological analysis
- Bone-marrow transplantation assay
- In vitro stimulation assays

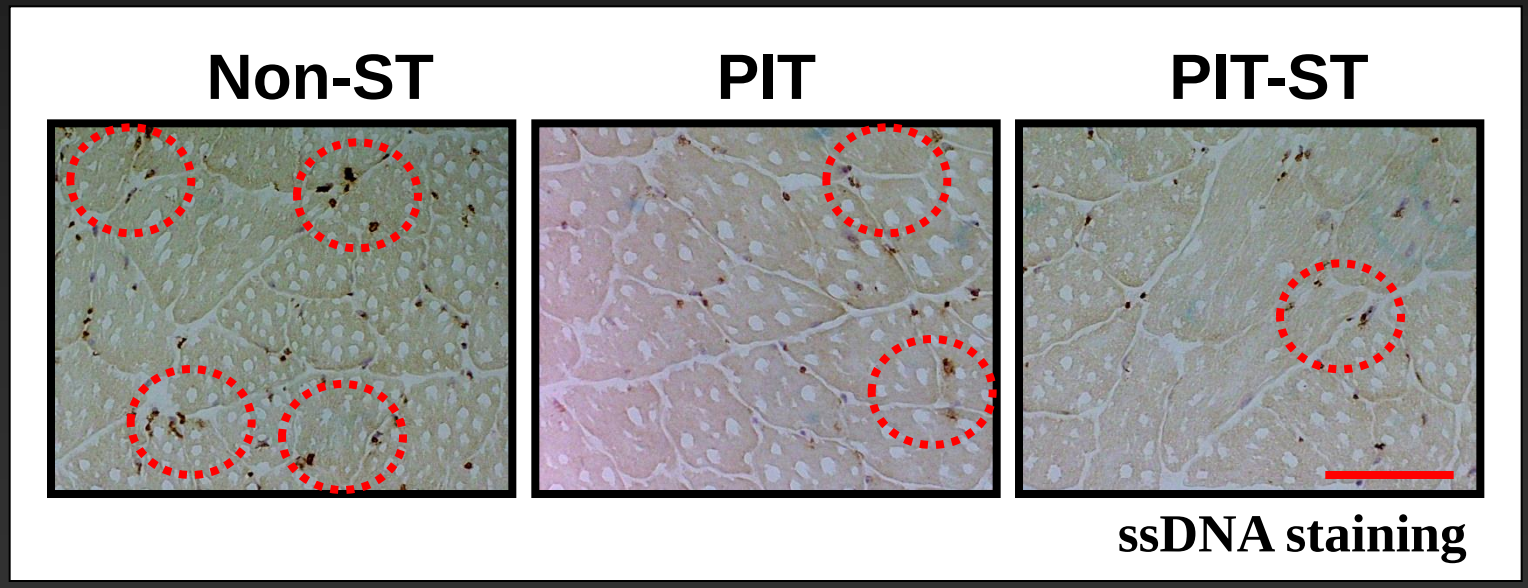
ST enhanced improvement statin-mediated of blood flow in aged mice



DHE: Dihydroethidium staining

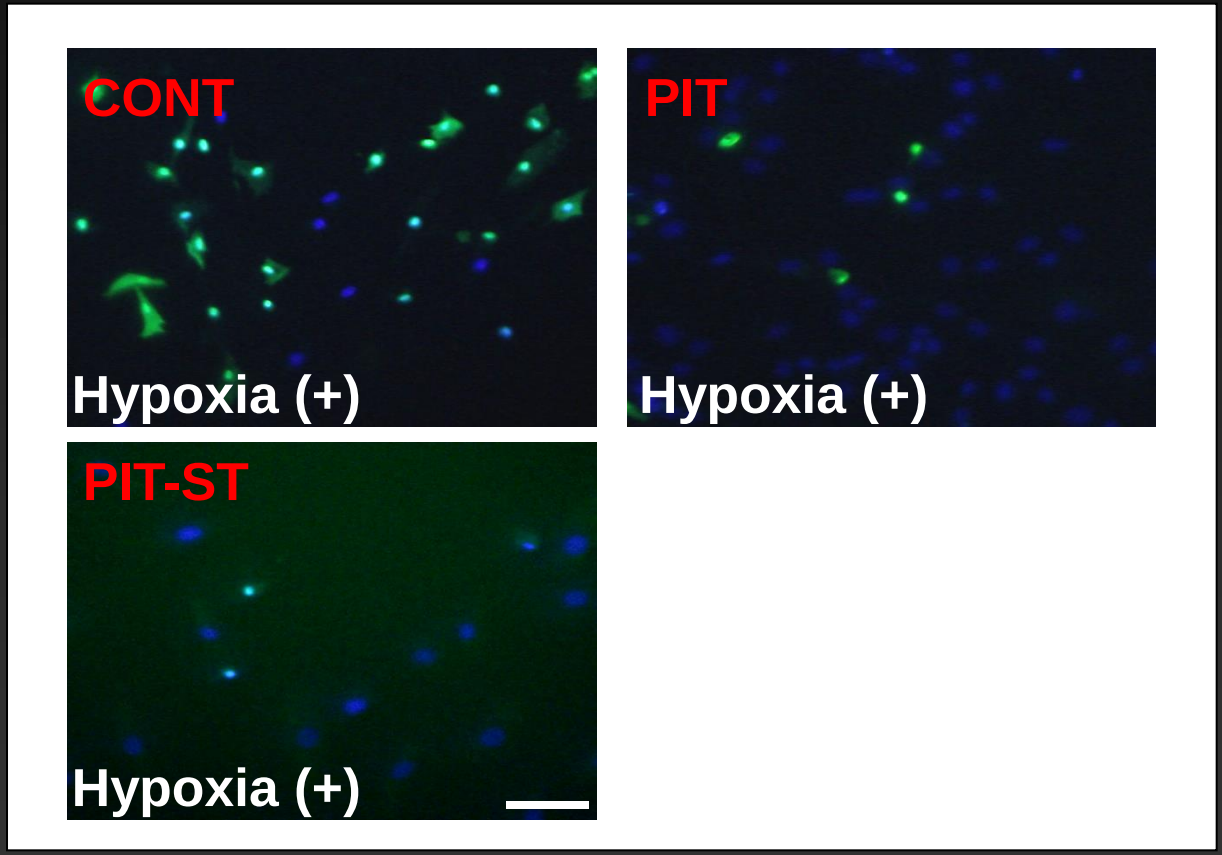
Cheng XW, et al. (un-published data)

ST enhances PIT-mediated anti-apoptotic effect in ischemic muscle of aged mice



Cheng XW, et al. (un-published data)

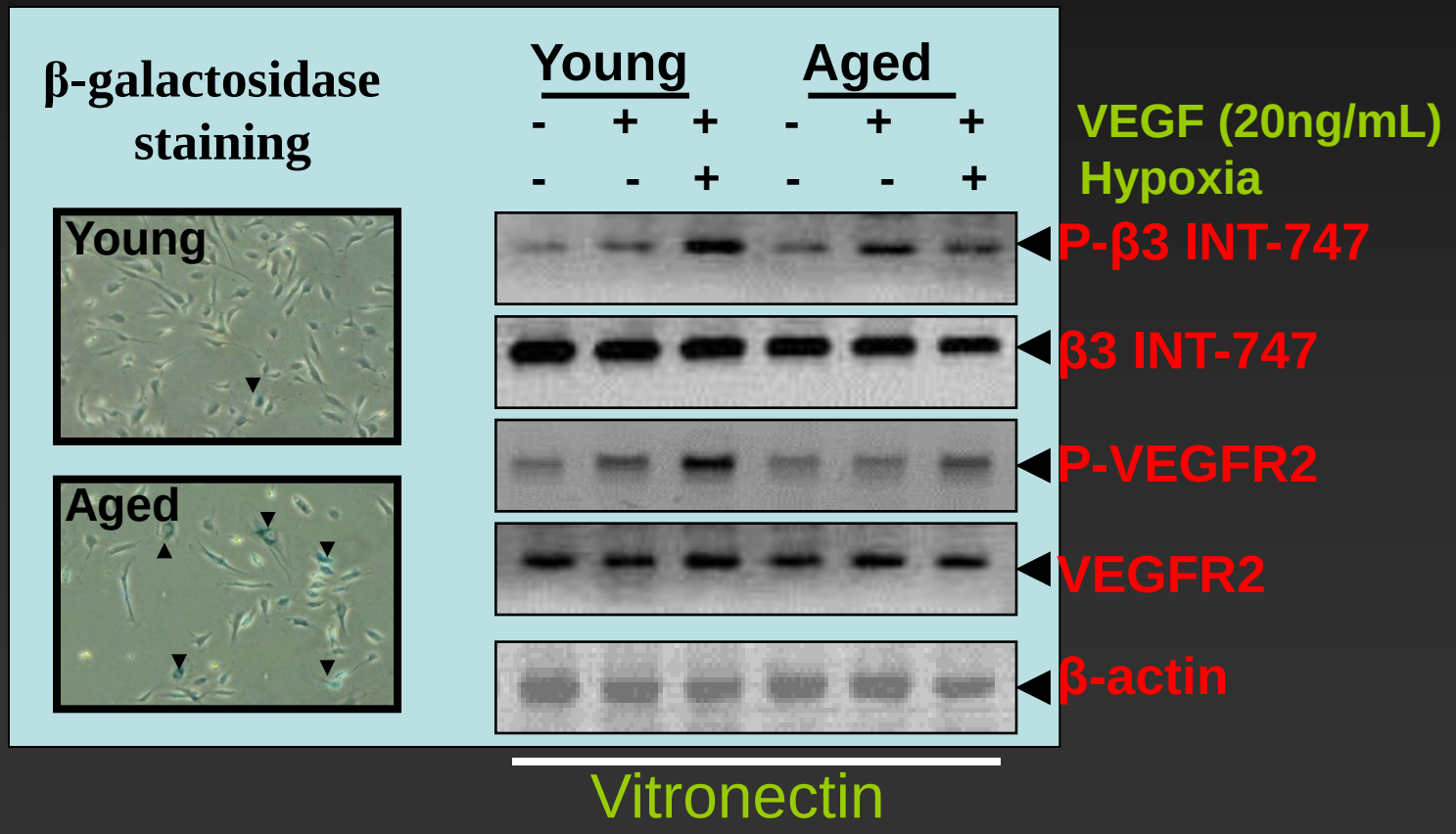
ST enhanced pitavastatin-mediated anti-apoptotic effect in circulating EPCs in aged mice



TUNEL staining for apoptosis

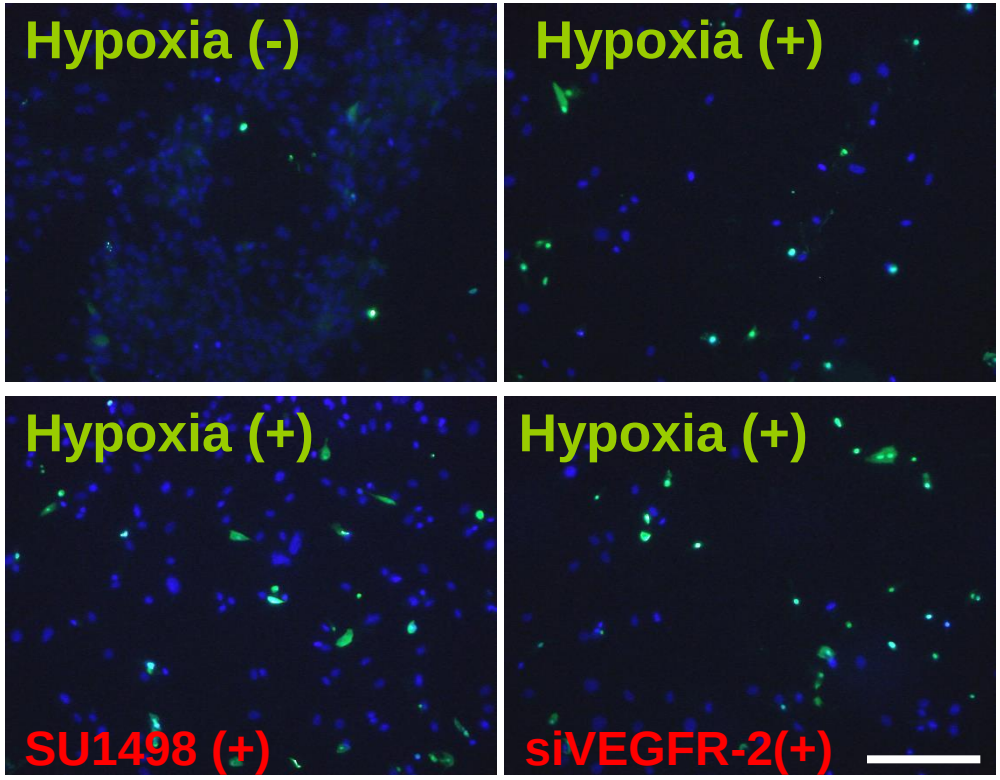
Cheng XW, et al. (un-published data)

Aging impairs VEGF-induced VEGFR2 and INT β3-pho in human EPCs response to hypoxia



Cheng XW, et al. (un-published data)

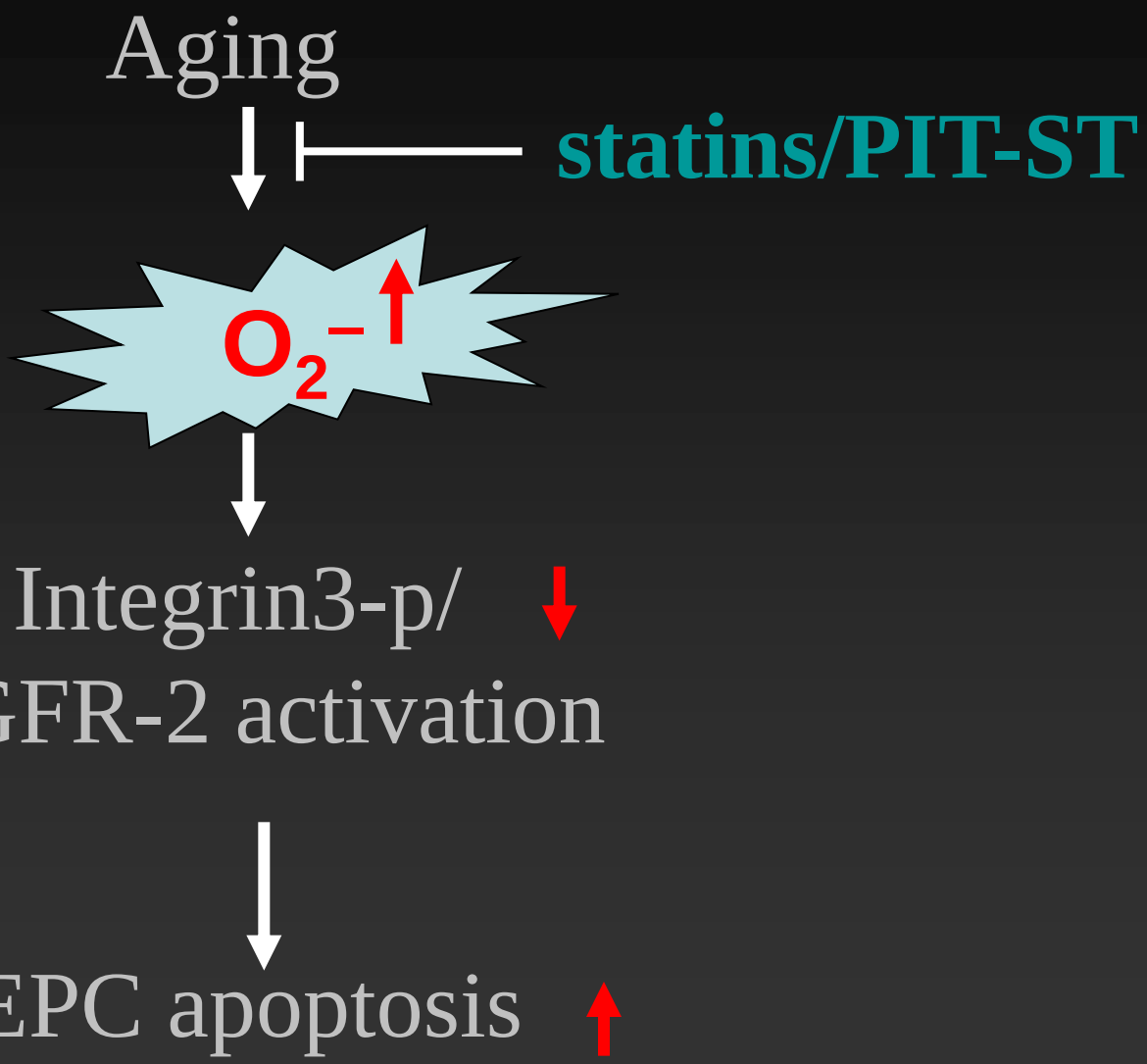
Impairment of VEGFR-2 involved contributes to apoptosis response to hypoxia in aged EPCs



SU1498: VEGFR-2 inhibitor
siVEGFR-2: short-interfering RNA to VEGFR-2

Cheng XW, et al. (un-published data)

Proposed mechanism

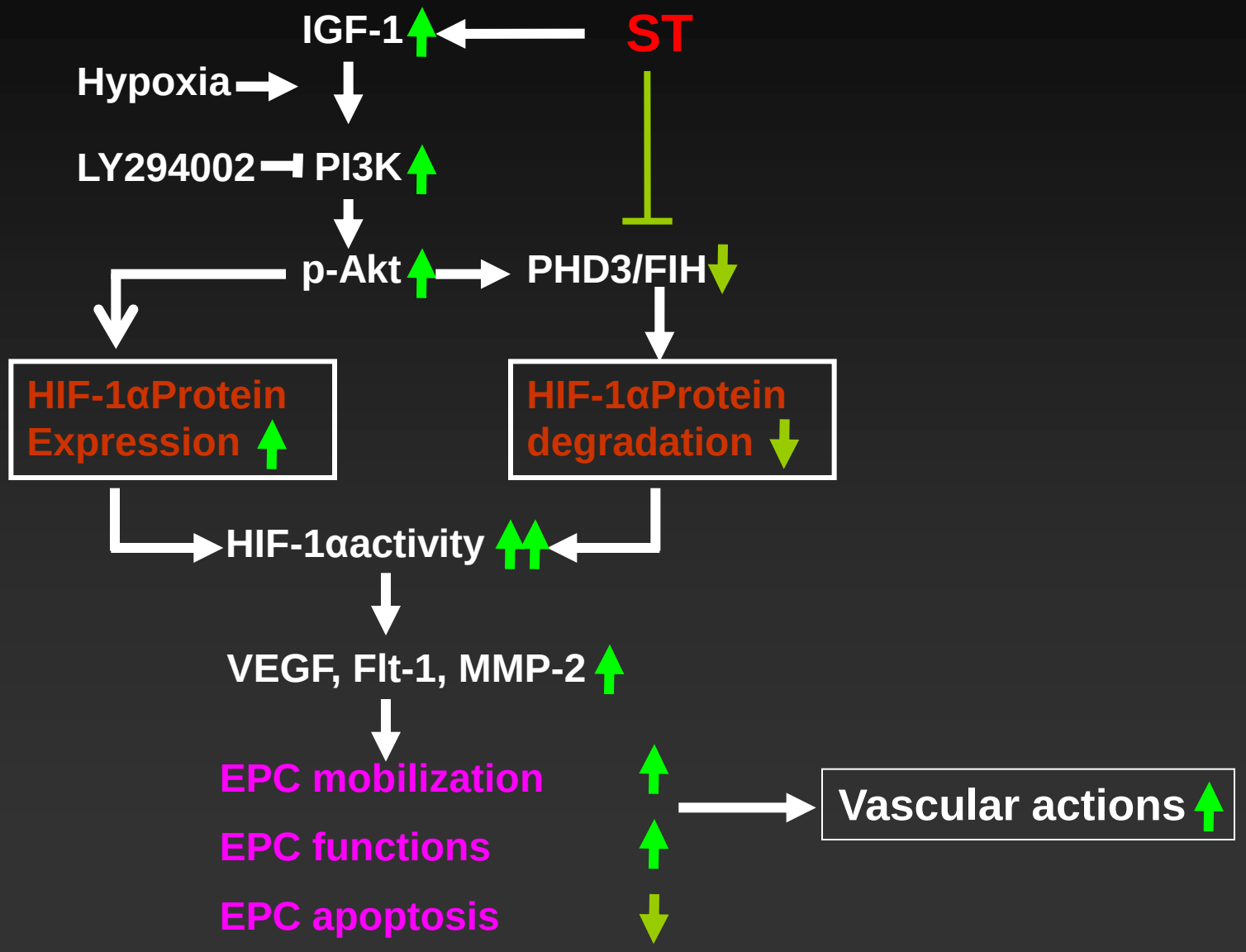


Cheng XW and Murohara T **(unpublished)**

Observations

- ST improved blood flow perfusion and capillary formation in the ischemic muscle of advanced age.
- ST enhanced BM-derived $CD31^+/c\text{-}Kit^+$ EPC-like mononuclear cell recruitment and their intrinsic functions.
- ST increased HIF-1α synthesis and stabilization through stimulation of PI3K/Akt signaling pathway and reduction of PHD3/FIH expression of ischemic muscle of advanced age.
- ST also increased HIF-1 protein production and MMP-2 activity in cultured BM-derived EPC of advanced age.

Proposed mechanisms underlying the improvement of ST-mediated vascular actions in advanced age



Cheng XW, et al. Circulation. 122:707-16. 2010

Conclusion

Our findings suggest that therapeutic interventions with exercise training in advanced age designed to restore the “young” hypoxic response can be recommended as a powerful strategy to prevent age-associated decline in vascular and regeneration and function by recruiting and and improving delivery of EPCs to the vasculature of Ischemic tissues trough PI3K signaling pathway-dependent HIF-1α/VEGF/MMP-2 activation.

ΦAblation group:

Φ PCI Group: Cathepsin K in CAD; On going studies

Yinden Y et al. Cathepsin K in chronic AF; On going studies;

Φ Imaging group:

Oshima S et al. mitochondrial and DCM; JACC Img 2008, EHJ 2009 and on going studies;

Φ HF group:

Hirashiki A et al. Cardiac morphology in DCM/HCM; JCVP2009; CEPP2010 and On going;

Φ Clinical Laboratory:

Takeshita K et al. Notch Signaling; BBRC 2009, Lab Invest 2011 and On going studies;

Φ Basic Research group:

Bando Y et al. DPP4 in DM. Circ Res (2011 submission) and On going studies;

Acknowledgments

Nagoya University

Cardiology

Michitaka Tsuzuki
Toyoaki Murohara

Cardiovascular Research Medicine

Haizhen Song
Kenji Okumura

Geriatrics

Aiko Inoue
Lina Hu
Kae Nakamura
Wazuko Adachi
Masafumi Kuzuya

Hamamatsu University

Anatomy and Neuroscience

Takeshi Sasaki