

The Soluble VEGF Receptor sFlt-1 Contributes to Impaired Neovascularization in Aged Mice

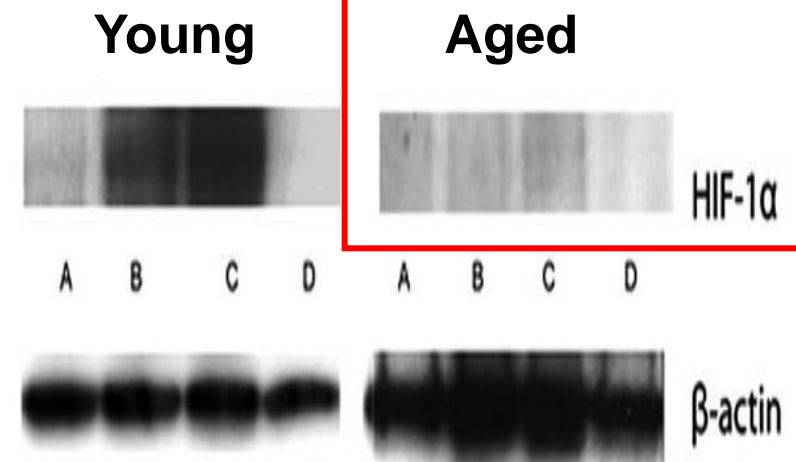
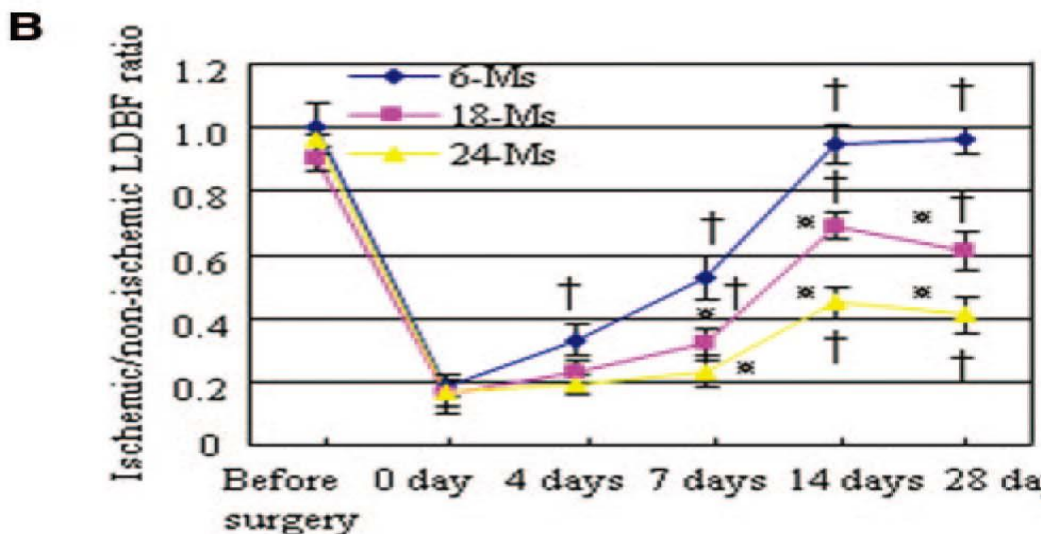
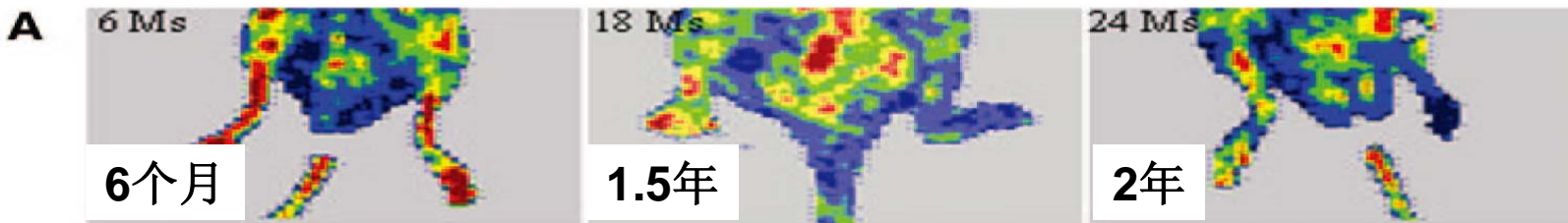
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Department of COI

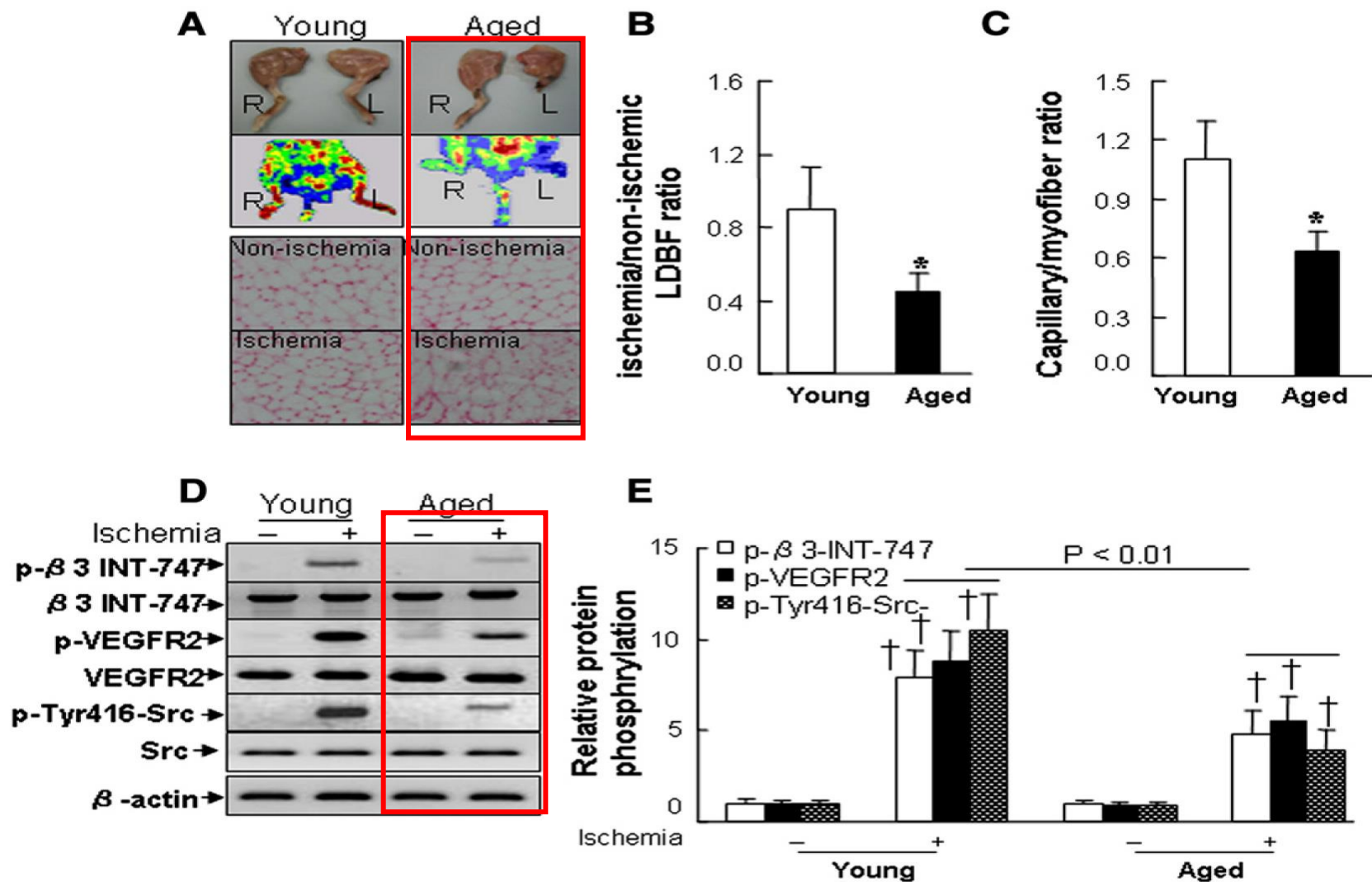
Nagoya University Graduate School of Medicine

Exercise Training Stimulates Ischemia-Induced Neovascularization via Phosphatidylinositol 3-Kinase/Akt-Dependent Hypoxia-Induced Factor-1 α Reactivation in Mice of Advanced Age

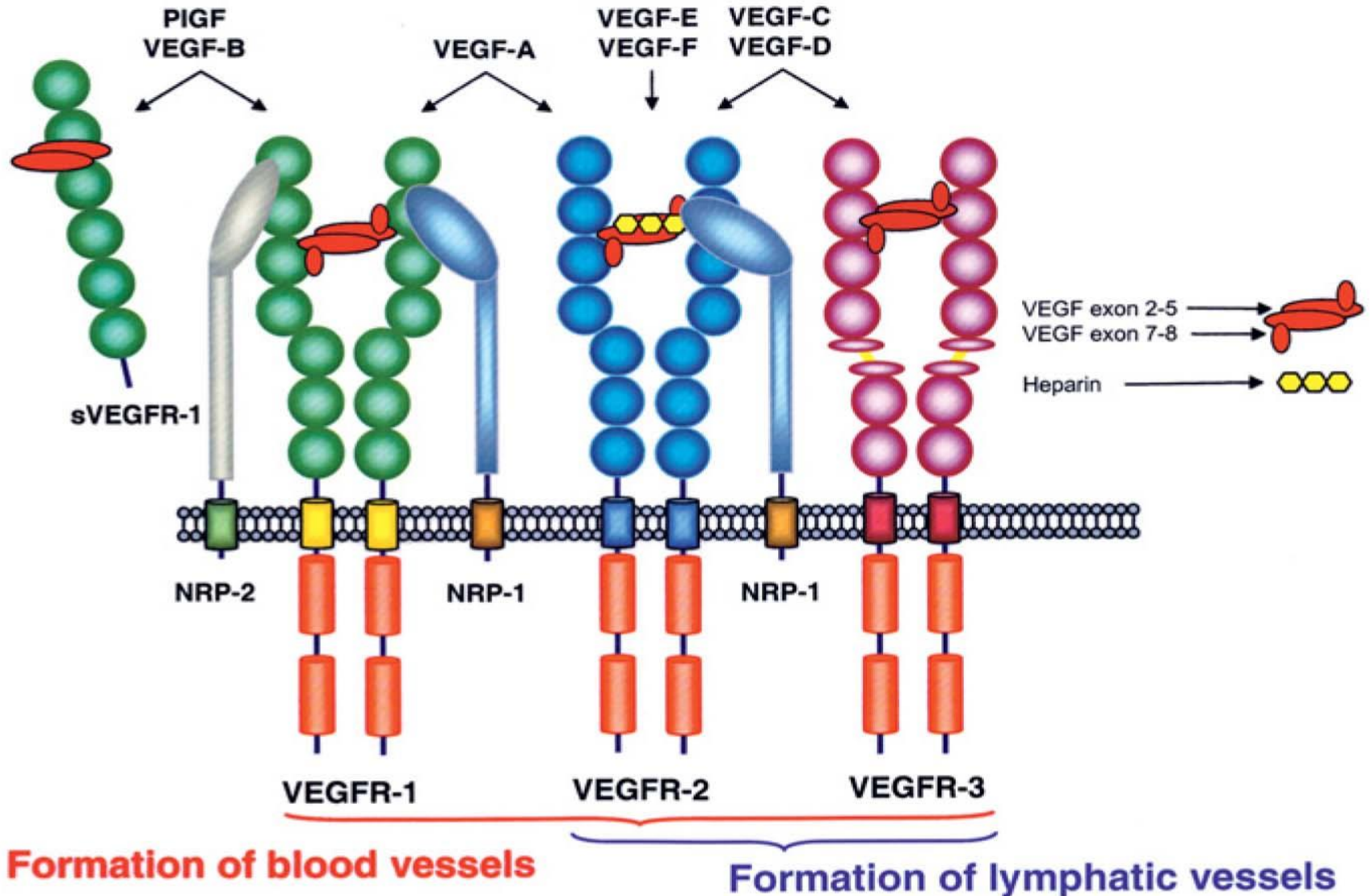
Xian Wu Cheng, Masafumi Kuzuya, Weon Kim, Haizhen Song, Lina Hu, Aiko Inoue, Kae Nakamura, Qun Di, Takeshi Sasaki, Michitaka Tsuzuki, Guo-Ping Shi, Kenji Okumura and Toyoaki Murohara



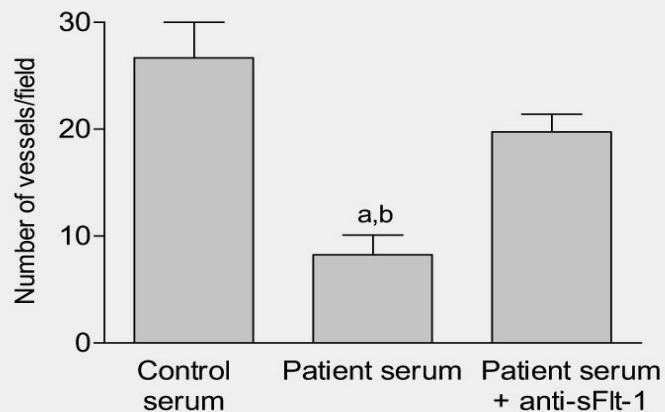
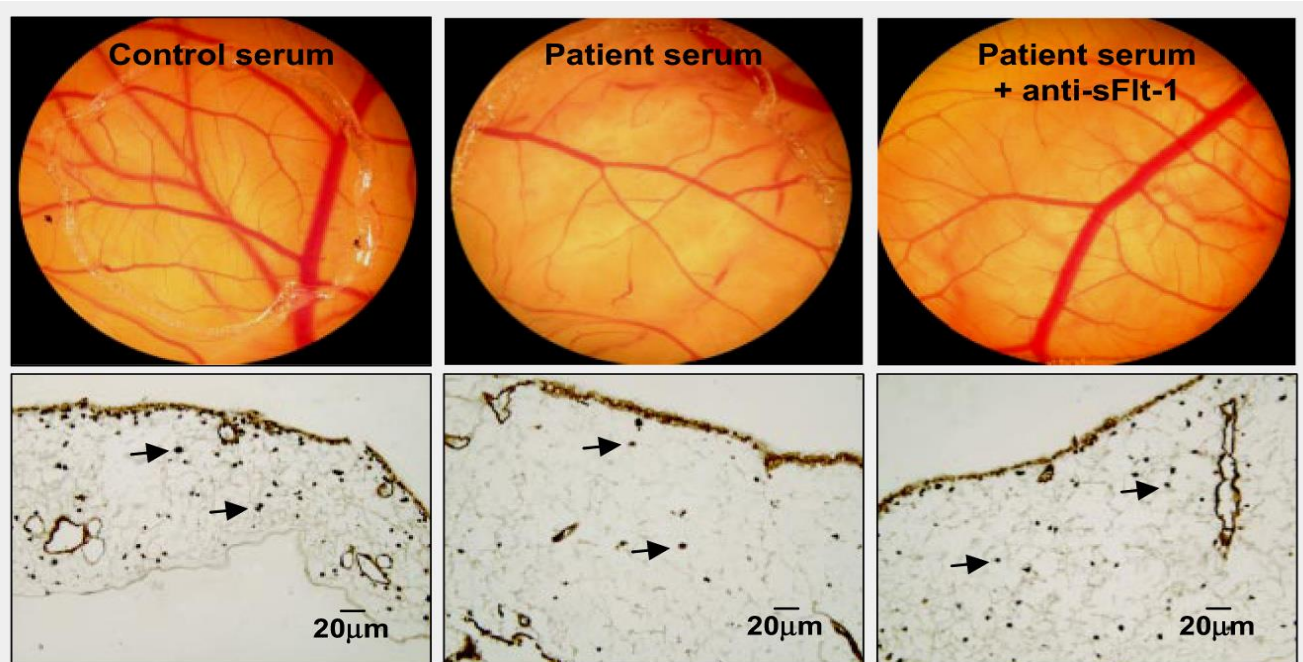
Aging declines ischemic neovascularization via the impairment of the cross-activation of VEGFR2 and $\beta 3$ integrin



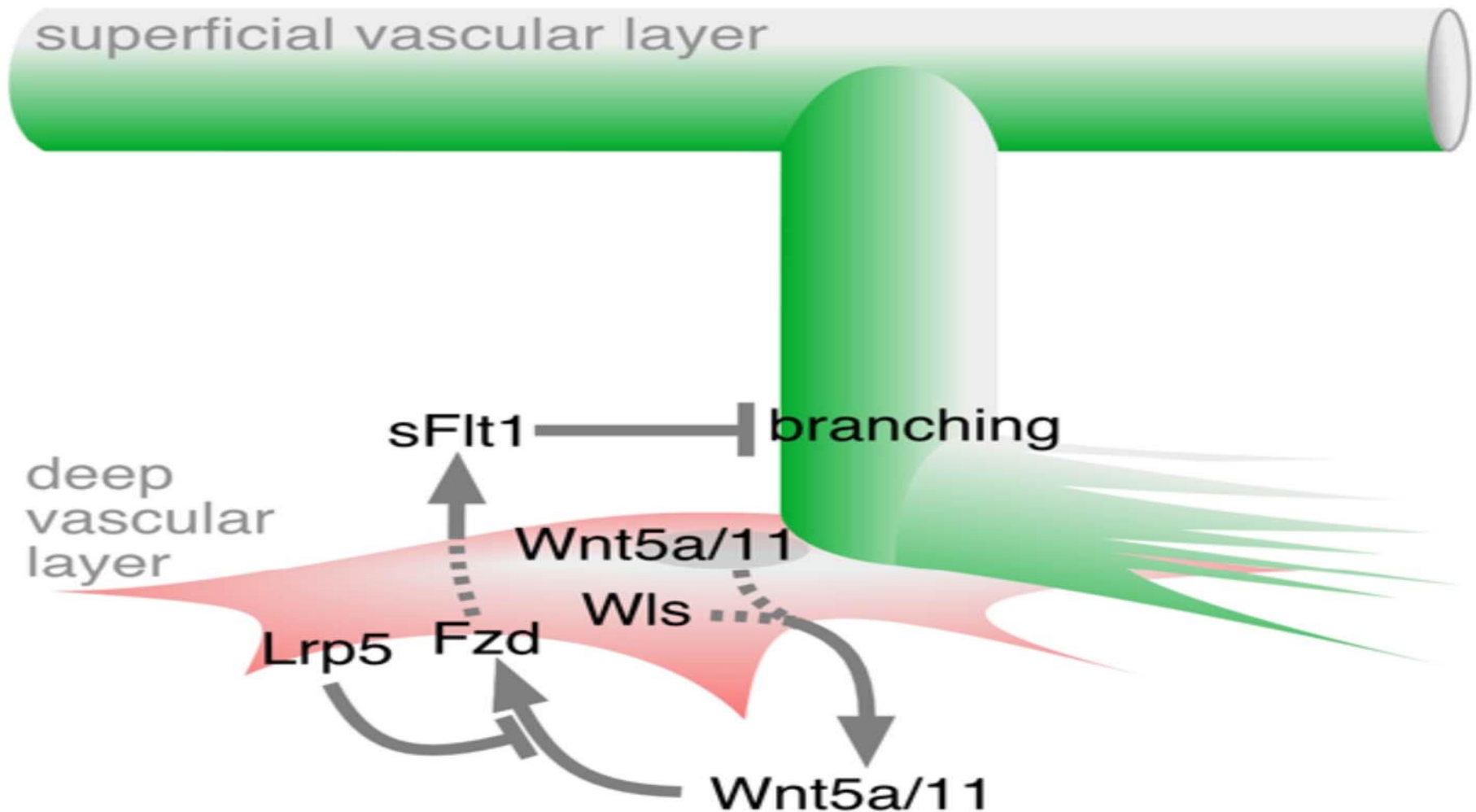
Schematic representation of VEGF family and their receptors



Chorioallantoic membrane assay shows that sFlt1 blocking improved sFlt1 antiangiogenic activity



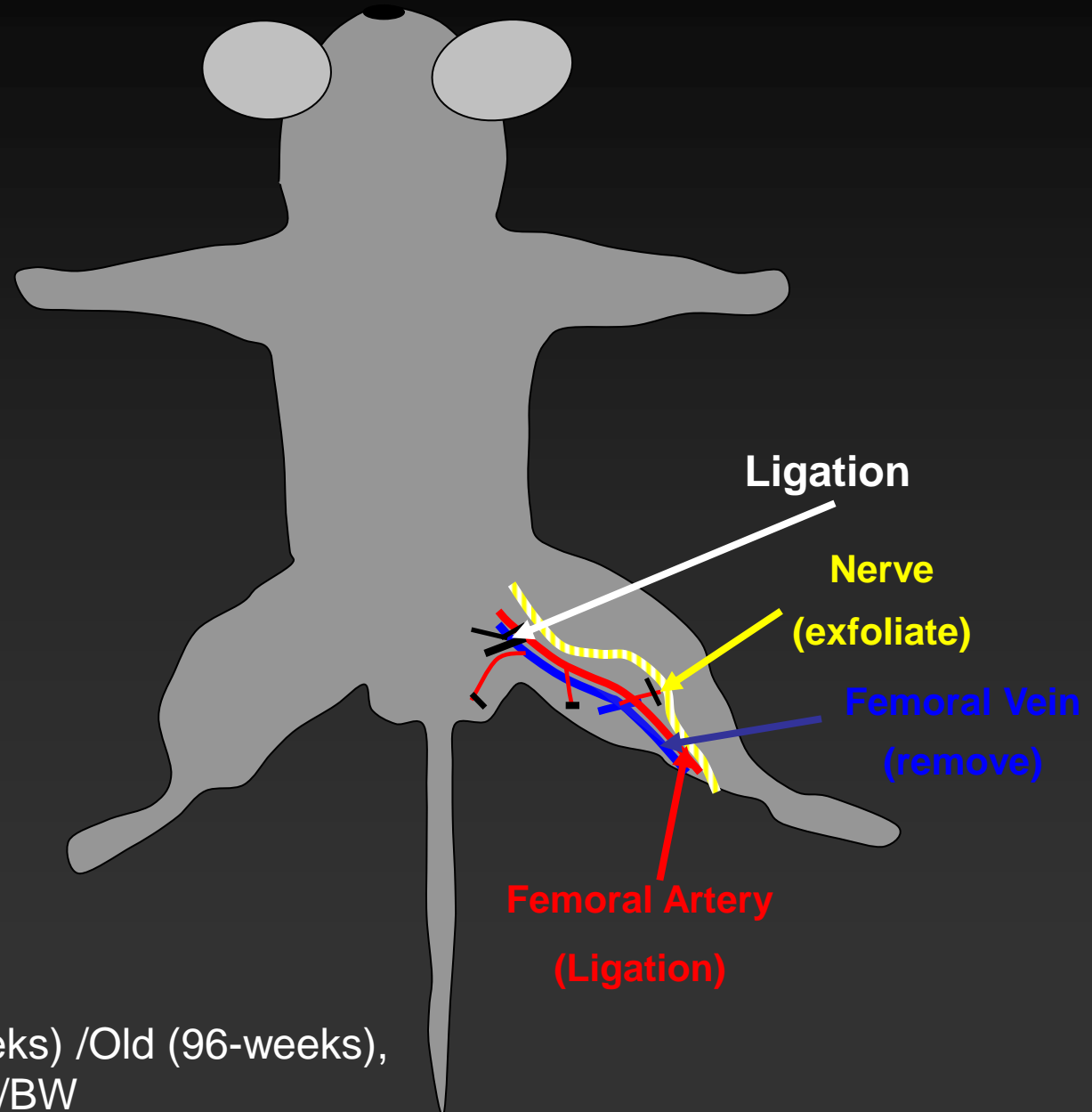
Schematic depicting suppression of angiogenic branching by a Wnt5a/11-Flt1 pathway



Purpose

- ❖ To investigate the effects of aging on ischemia-induced neovascularization and its molecular mechanisms with a focusing on the novel role of sFlt1 in a mouse hindlimb model.

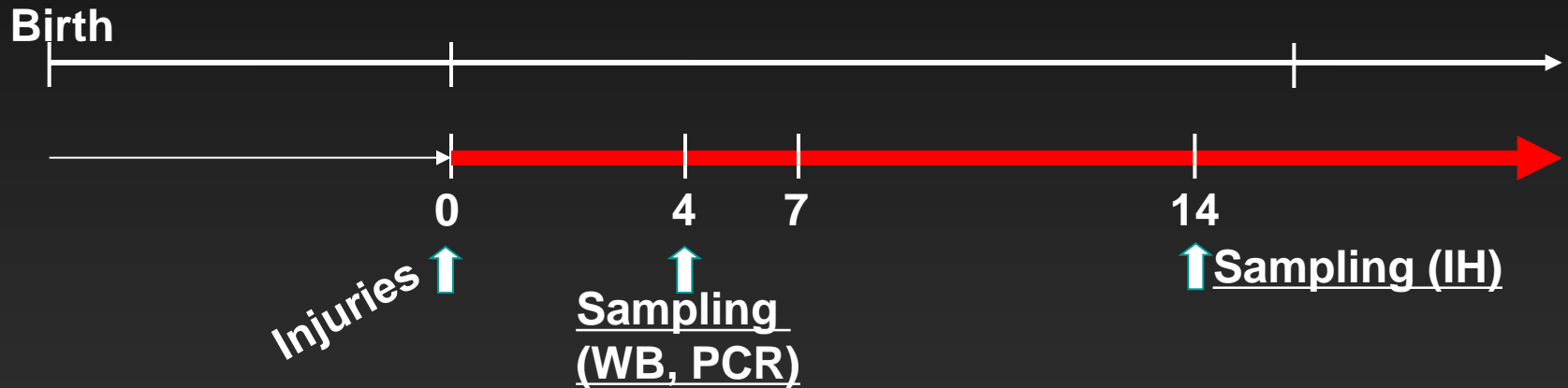
Mouse femoral artery ligation model



Age

: Young (8-weeks) / Old (96-weeks),
male 20-30g/BW

Exp: Protocol



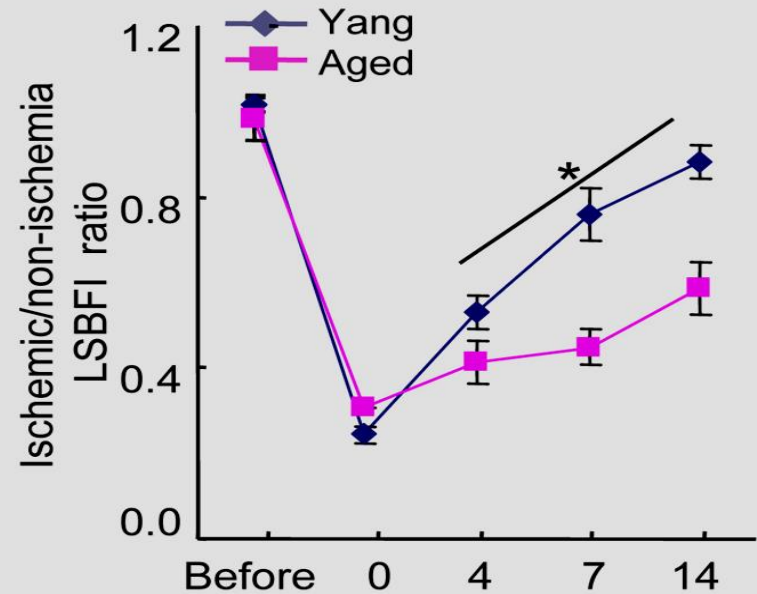
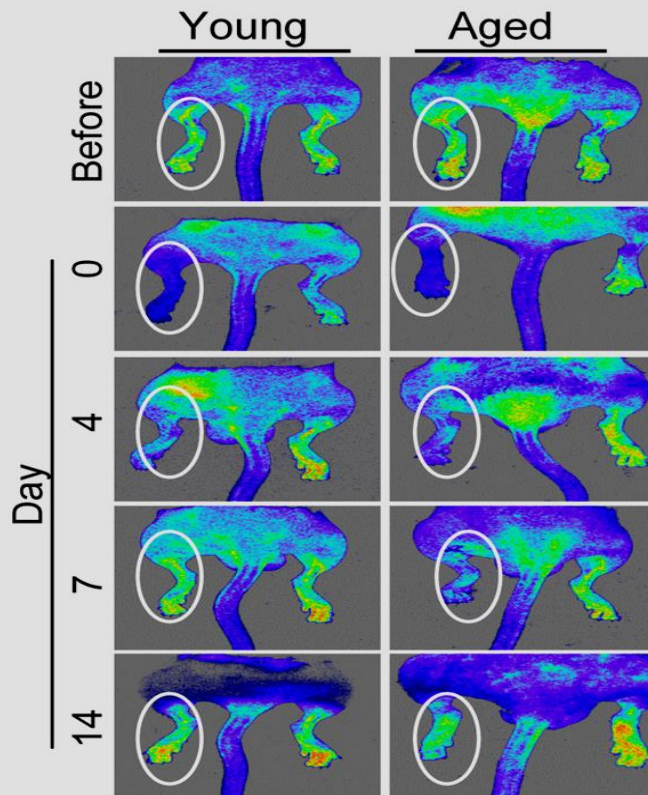
① Young ($n = 24$)

② Aged ($n = 22$)

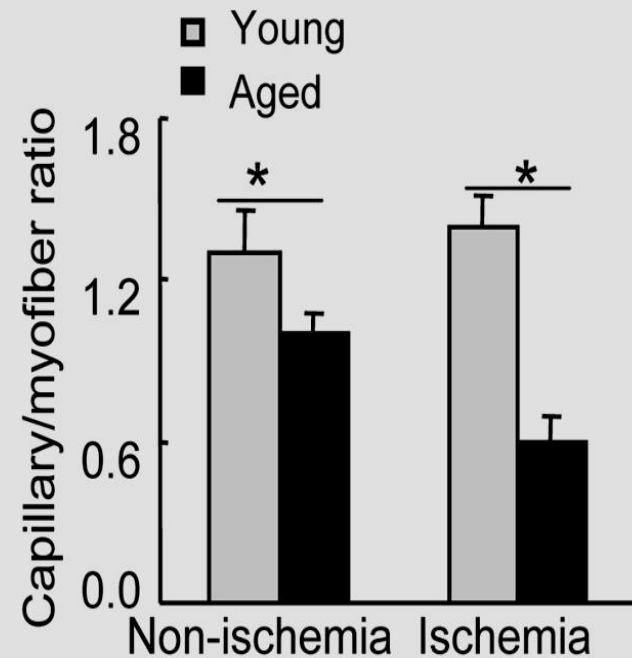
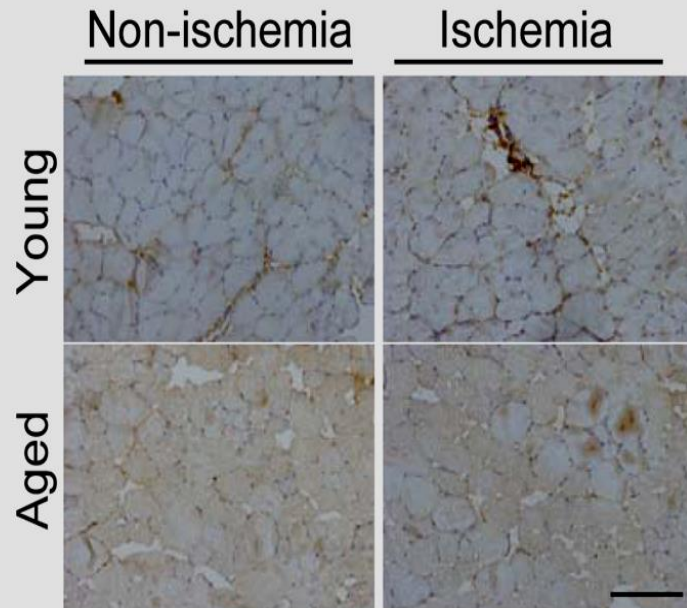
Methods

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

Aging impairs blood flow recovery during the follow-up period

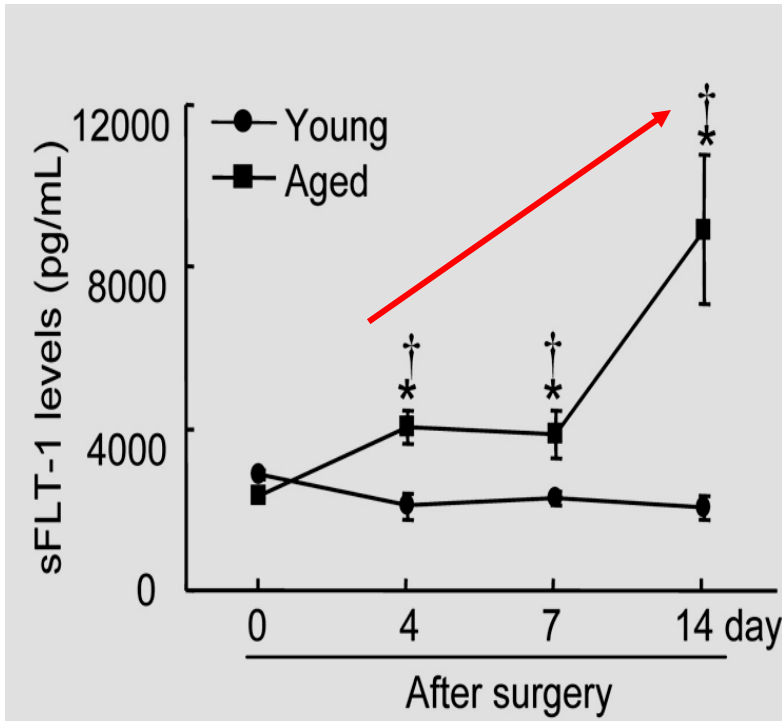


Aging impairs blood capillary formation at 14 after ischemic surgery

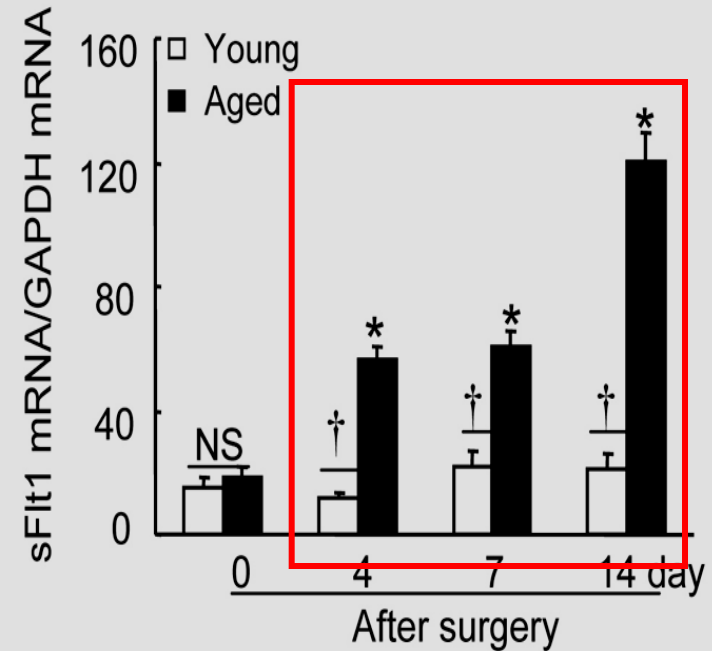


Aging increases the levels of plasma and ischemic muscles sFlt1 in a time-dependent course

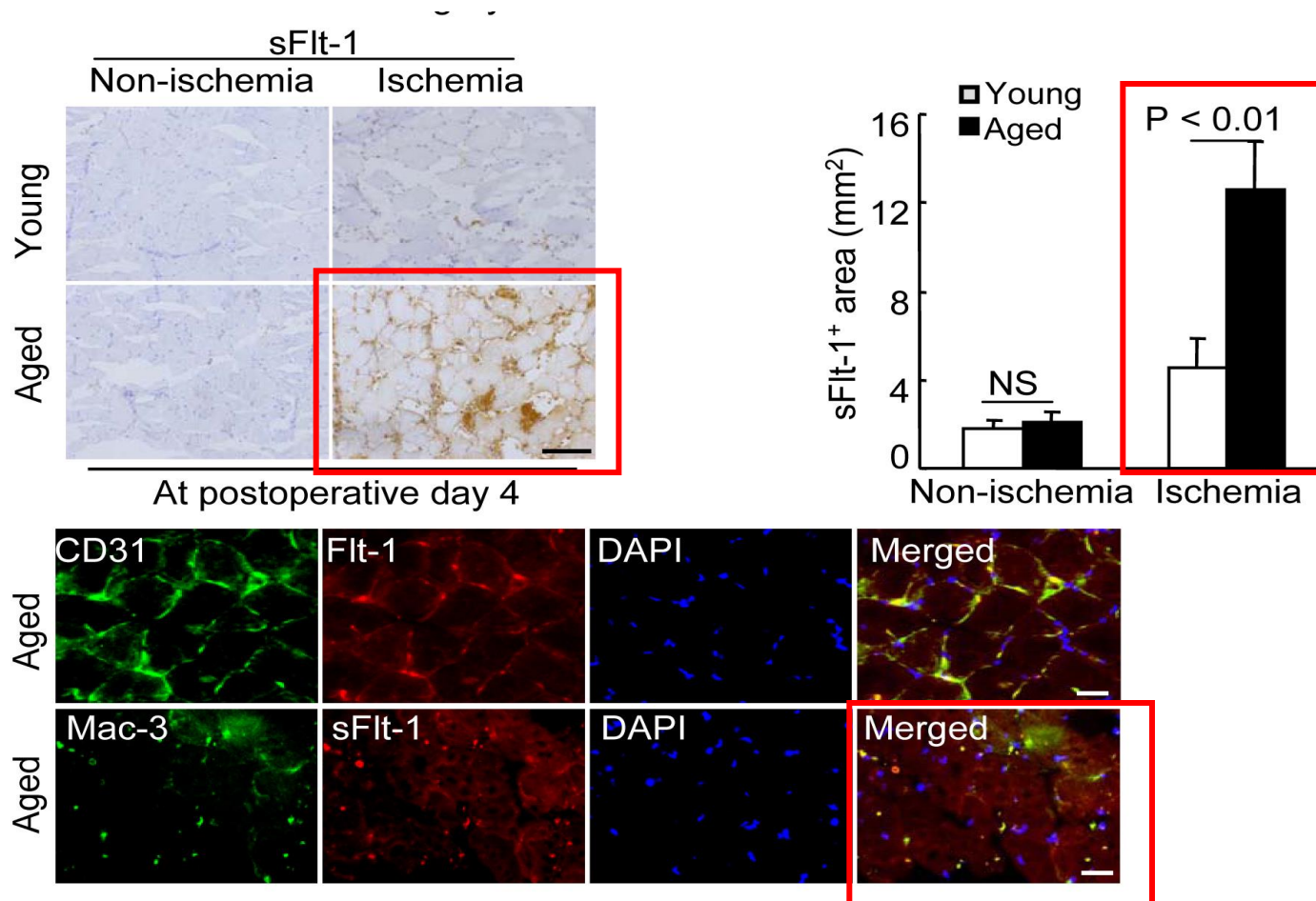
ELISA



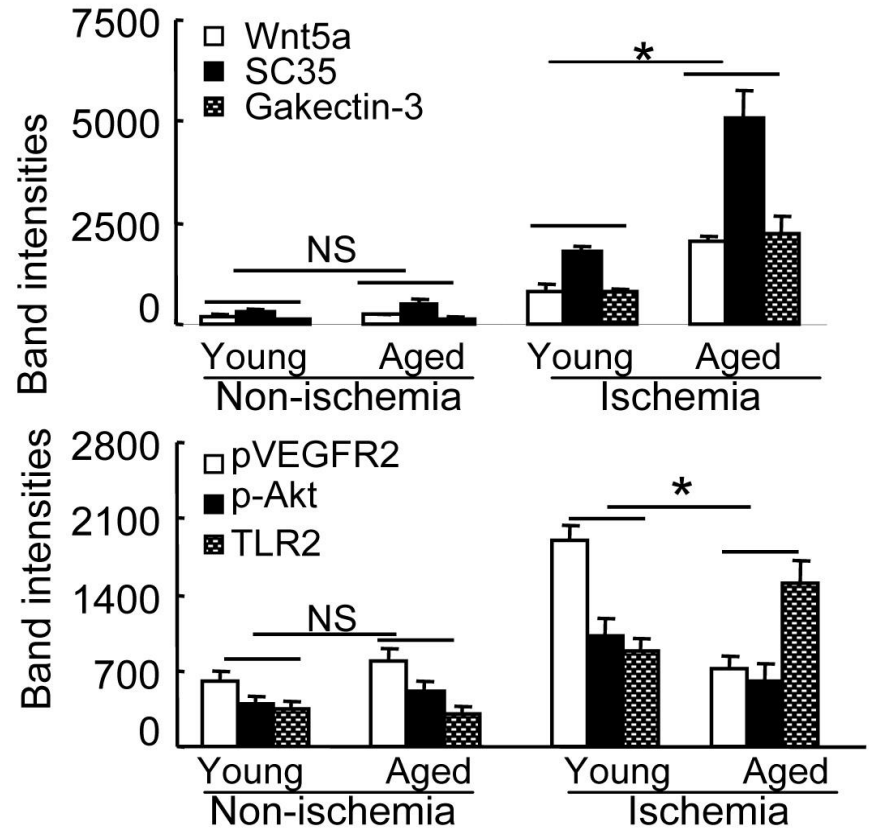
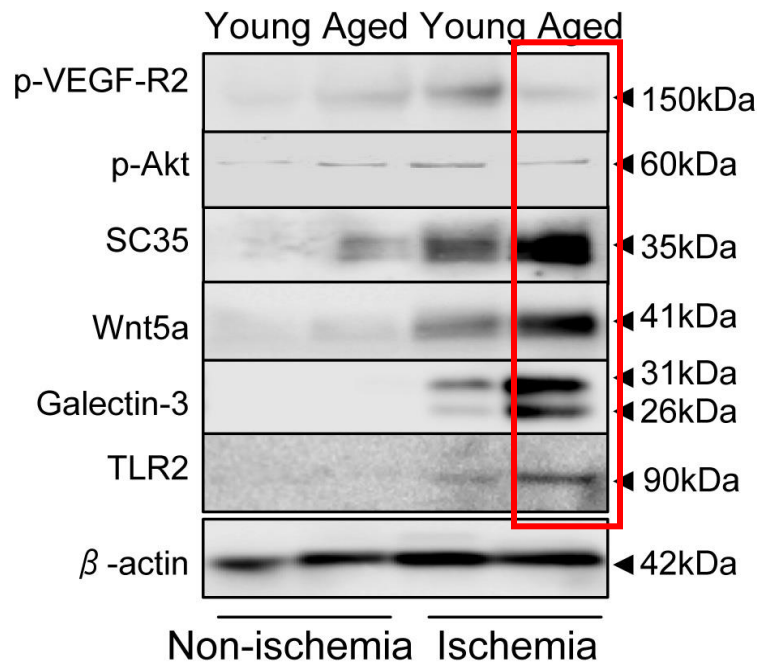
Real-time PCR



Immunostaining and Immunofluorescence show sFlt1 expression in infiltrated macrophages



Aging enhanced the levels of p-Akt, SC35, Galectin-3, Wnt5a, and TLR2 and reduced p-VEGFR2 proteins

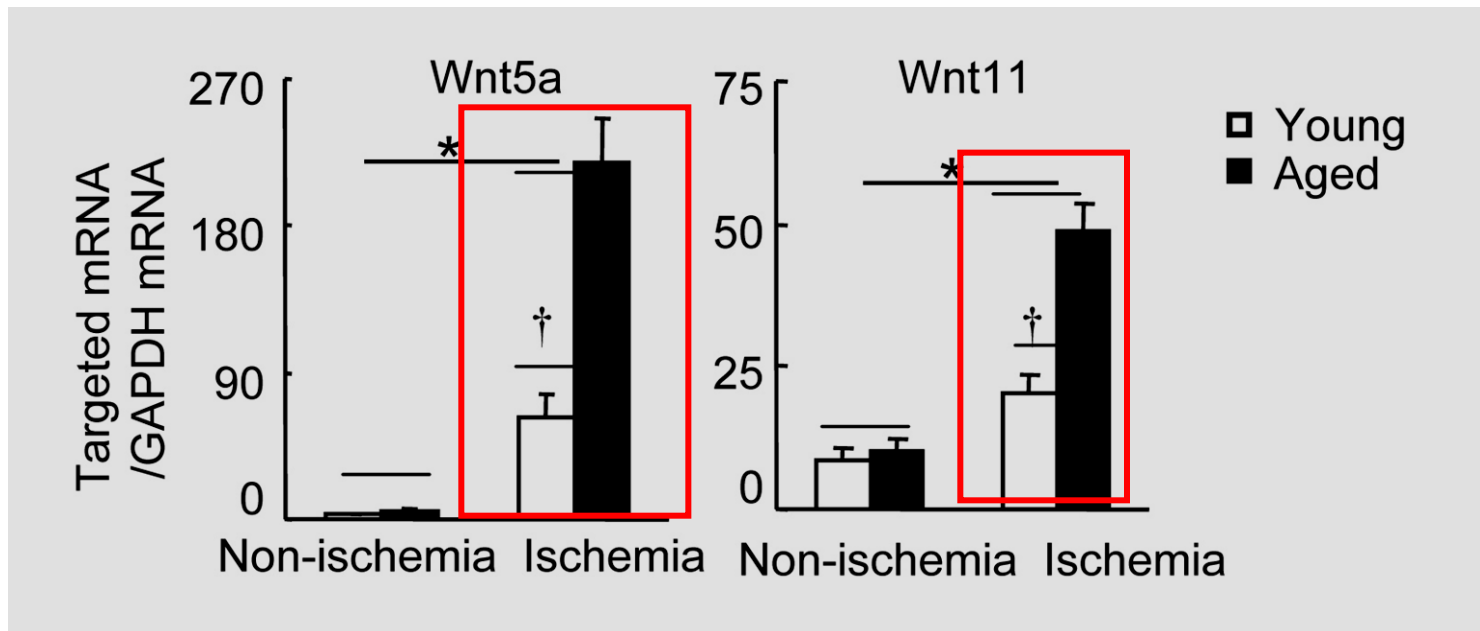


TLR2: toll-like receptor-2

p-VEGFR2: phospho-vascular endothelial growth factor receptor-2

Aging increased the expression of canonical Wnt5a and Wnt11 in the ischemic muscles

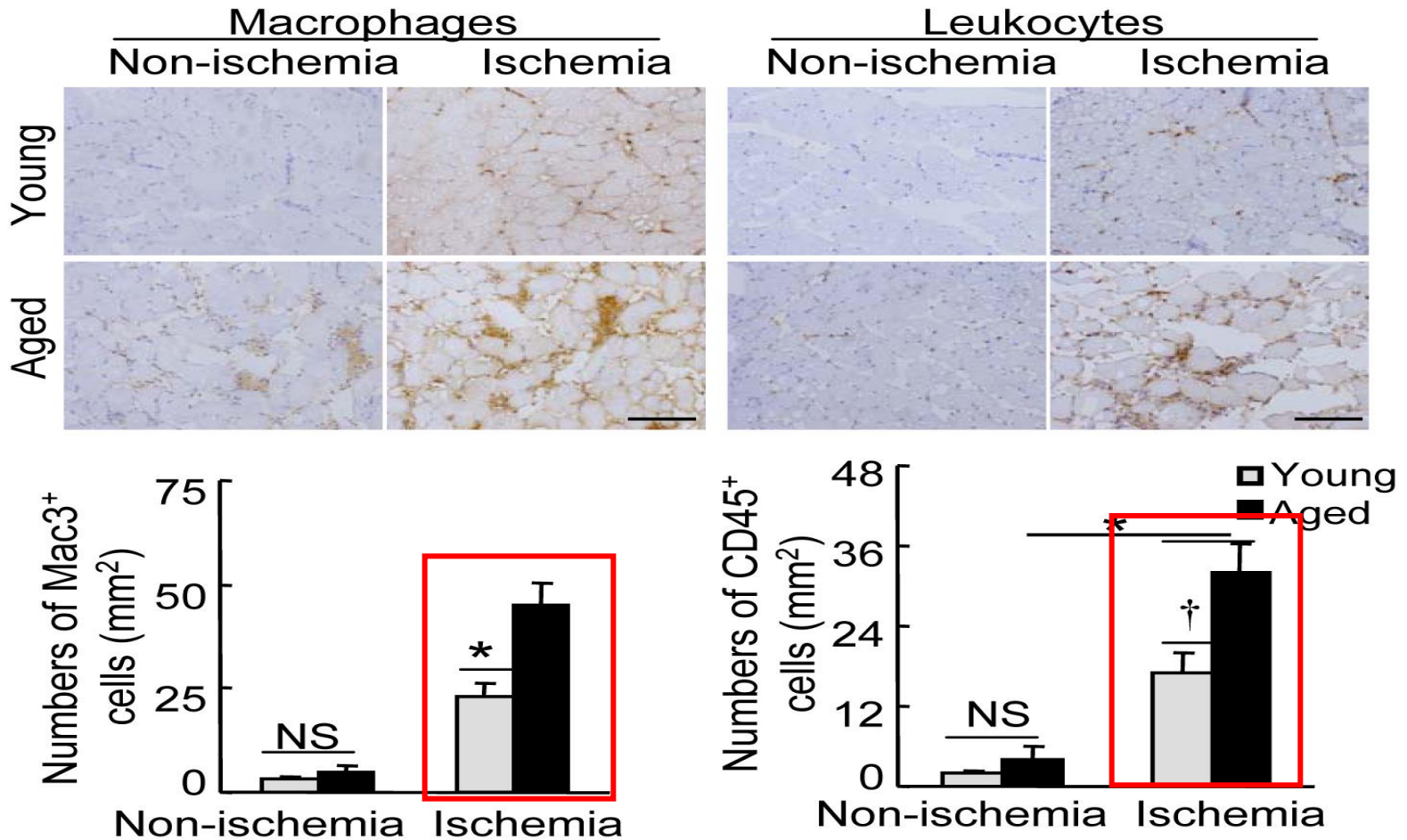
Real-time PCR



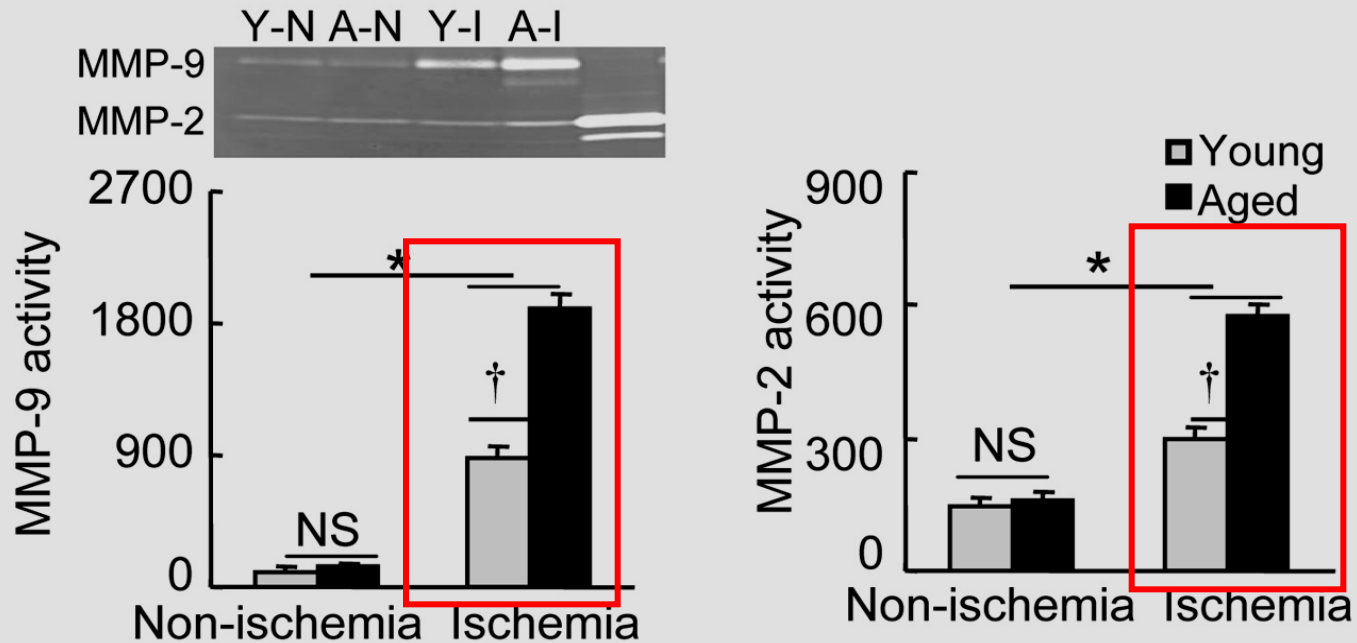
Aging had no effects on the expression of non-canonical Wnt family members

Parameter	Young nonischemia	Aged Nonischemia	Young Ischemia	Aged Ischemia
<i>mWnt3</i>	3.2 ± 1.4	5.6 ± 1.6	$208.9 \pm 43.2^*$	$245.6 \pm 53.8^*$
<i>mWnt3a</i>	4.3 ± 1.0	6.6 ± 1.8	$298.7 \pm 117.1^*$	$216.1 \pm 51.0^*$
<i>mWnt5b</i>	1.3 ± 0.3	1.8 ± 0.9	$49.9 \pm 14.8^*$	$30.4 \pm 4.3^*$
<i>mWnt7a</i>	6.5 ± 2.1	8.0 ± 2.0	$213.0 \pm 82.4^*$	$164.1 \pm 35.1^*$
<i>mWnt7b</i>	3.4 ± 1.3	7.0 ± 5.1	$382.5 \pm 106.0^*$	$302.6 \pm 58.1^*$
<i>mWnt8a</i>	3.2 ± 0.9	2.5 ± 0.7	$23.2 \pm 5.7^*$	$20.7 \pm 6.8^*$
<i>mWnt9b</i>	0.9 ± 0.3	1.1 ± 0.4	$8.2 \pm 2.1^*$	$7.3 \pm 3.1^*$
<i>mWnt10a</i>	13.8 ± 2.3	15.9 ± 2.5	$45.7 \pm 6.0^*$	$49.1 \pm 8.5^*$
<i>mWnt10b</i>	3.2 ± 1.7	1.6 ± 0.4	$309.0 \pm 60.1^*$	$389.3 \pm 107^*$

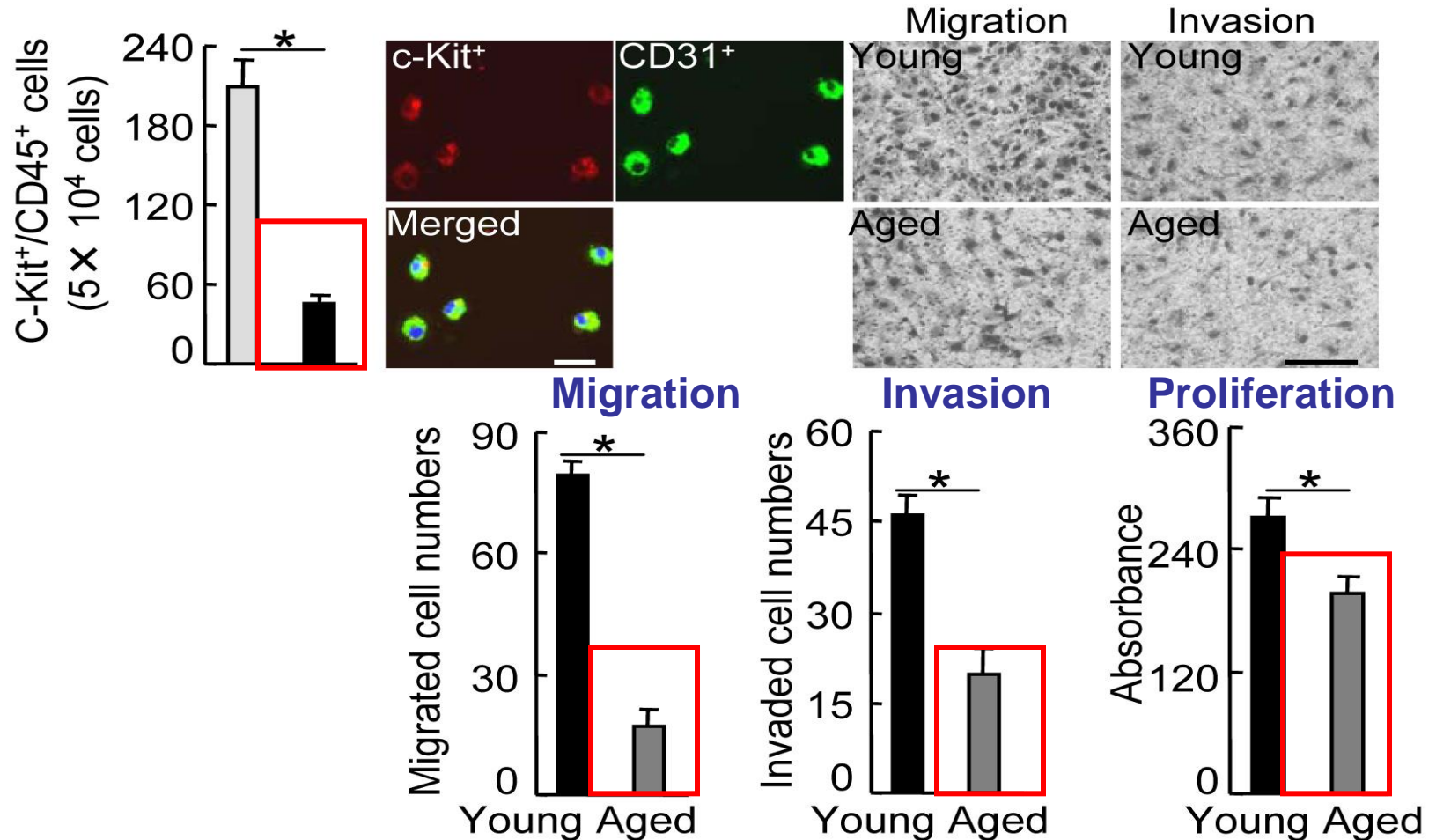
Aging enhances macrophage and leukocyte infiltration in the ischemic muscles



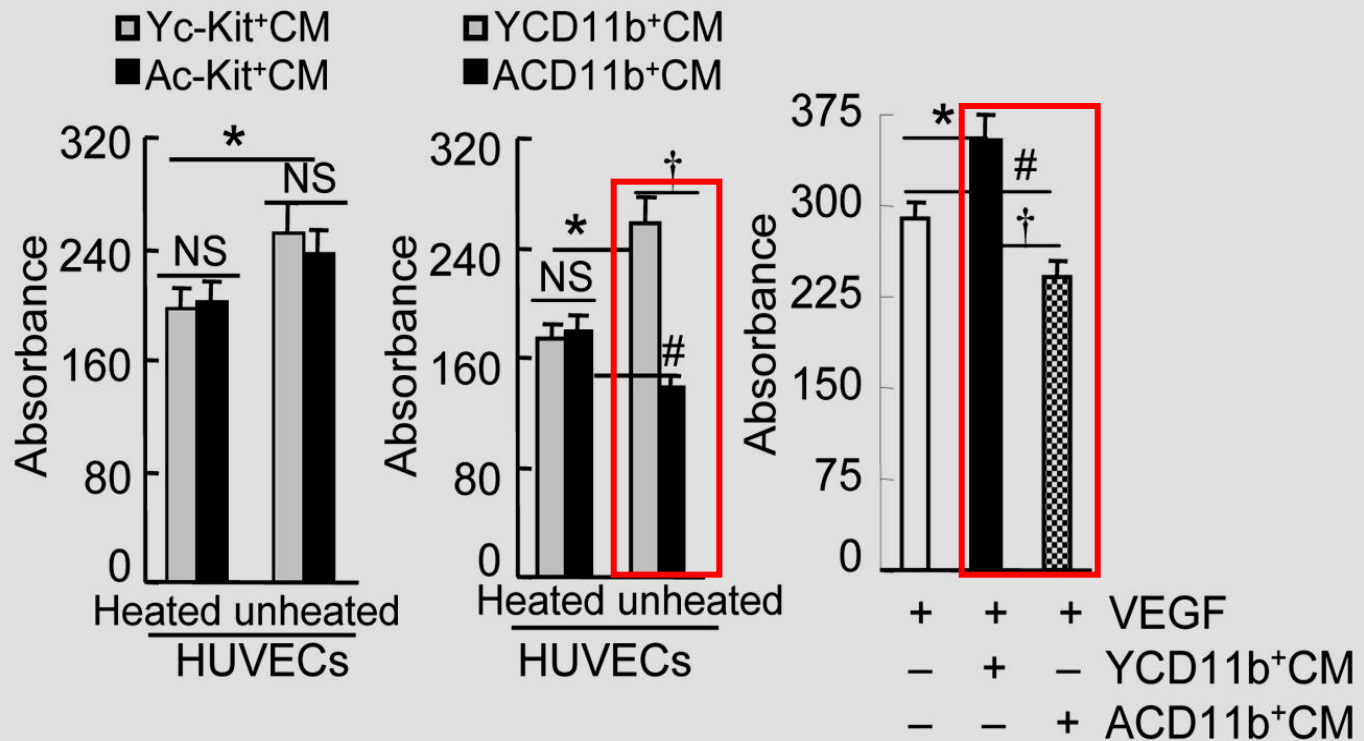
Aging increased the levels of MMP-2 and MMP-9 activities in the ischemic muscles



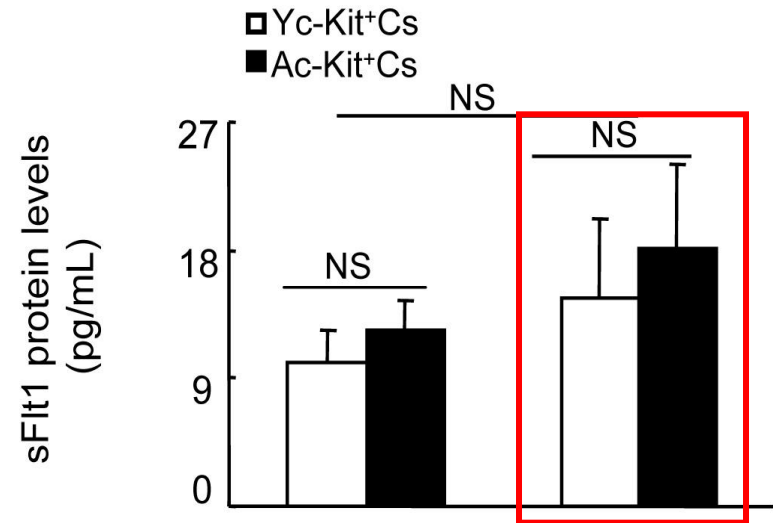
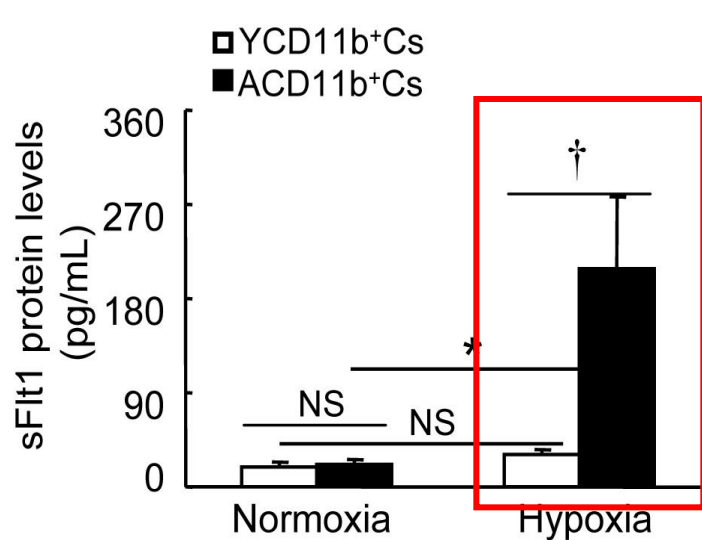
Aging impairs bone marrow CD31⁺/c-Kit⁺ mobilization and function



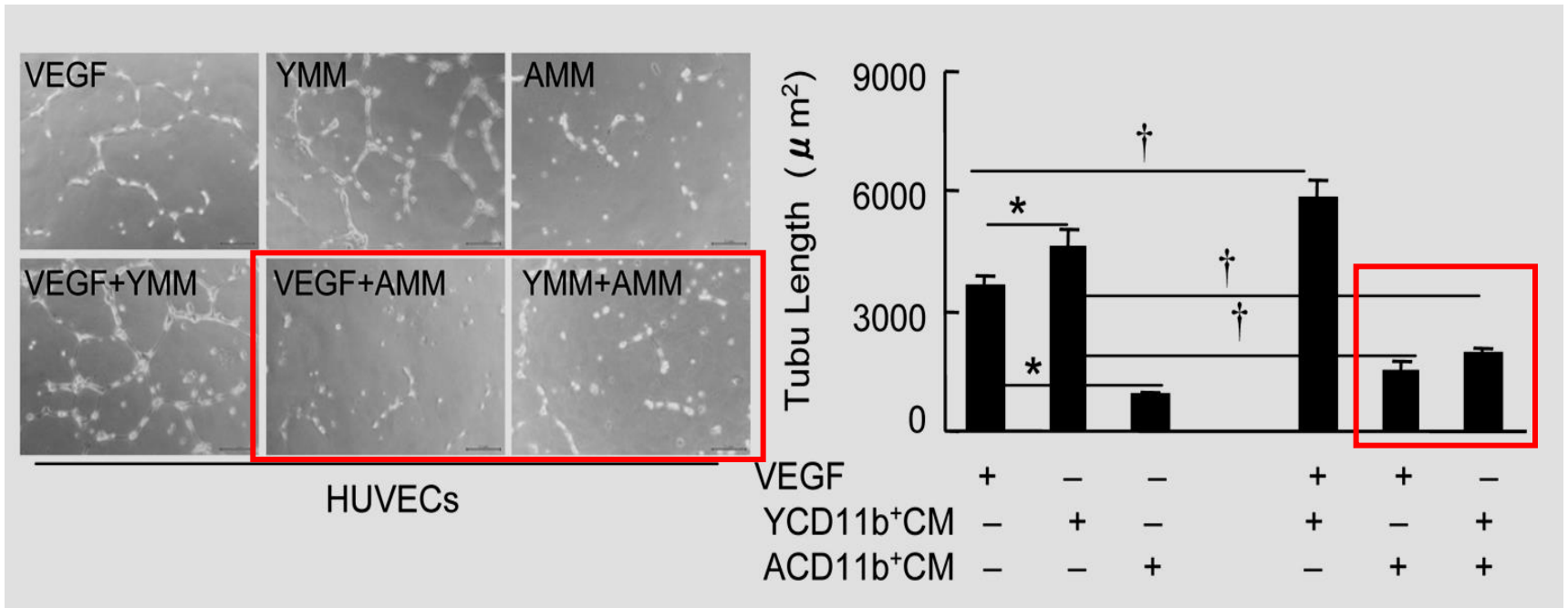
Cultured medium from BM-derived CD11b⁺ cells of aged mice impaired HUVEC proliferation



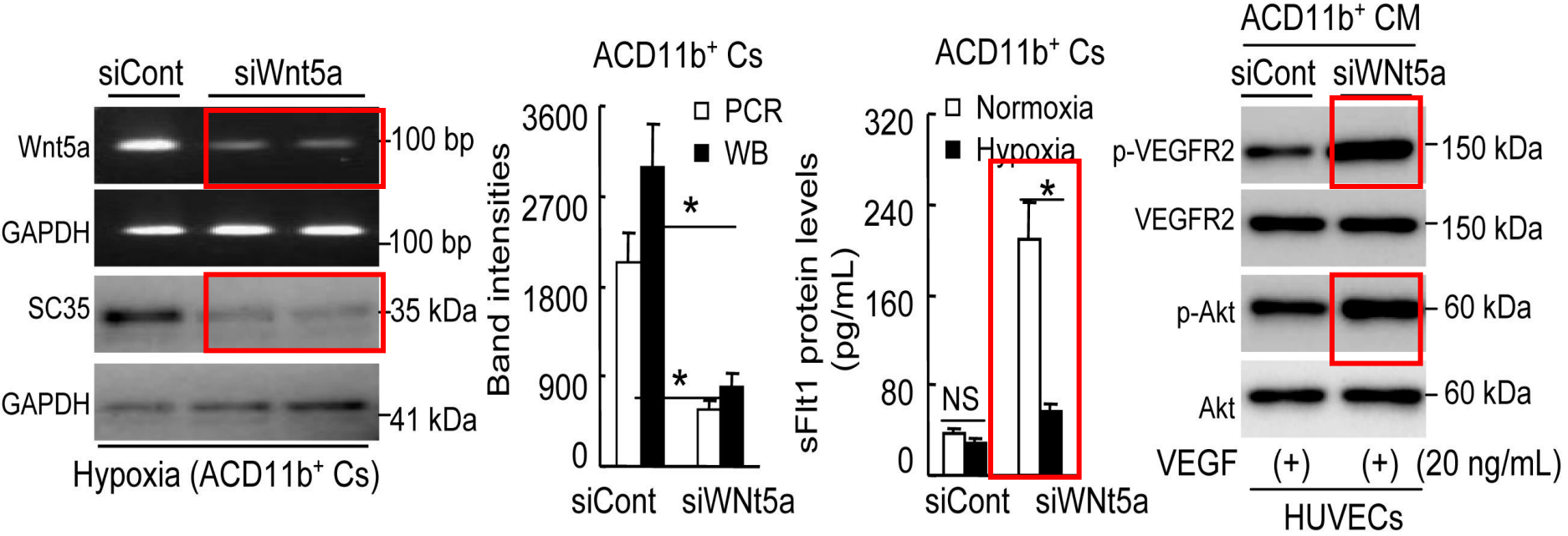
Hypoxia stimulated the expression of sFlt1 and mRNA in aged BM-derived CD11b⁺ cells



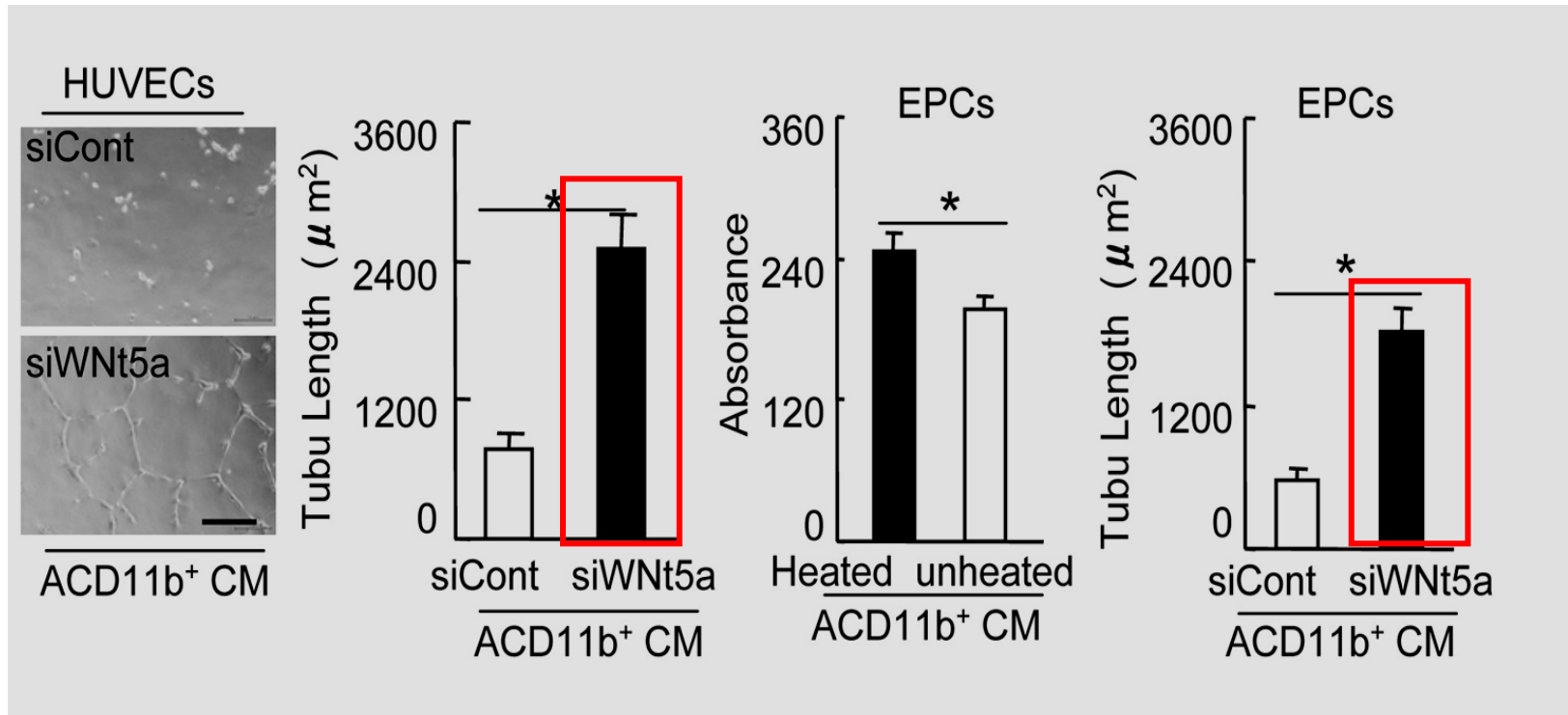
Cultured medium from BM-derived CD11b⁺ cells of Aged mice impaired HUVEC tubulogenesis



siWnt5a reduced SC35 and sFlt1 express and enhanced pAkt and pVEGFR2 levels in aged BM-derived CD11b⁺ cells in response to hypoxia



Cultured medium of aged BM-derived CD11b⁺ cells treated siWnt5a mitigated HUVEC and EPC tubulogenic actions



Proposed mechanism of aging-related impaired neovascularization



Aging and Disease

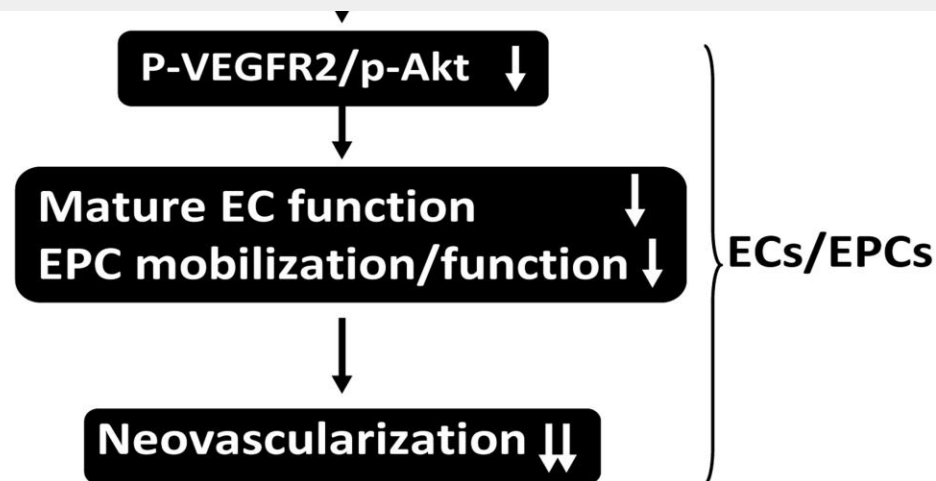
www.aginganddisease.org

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Original Article

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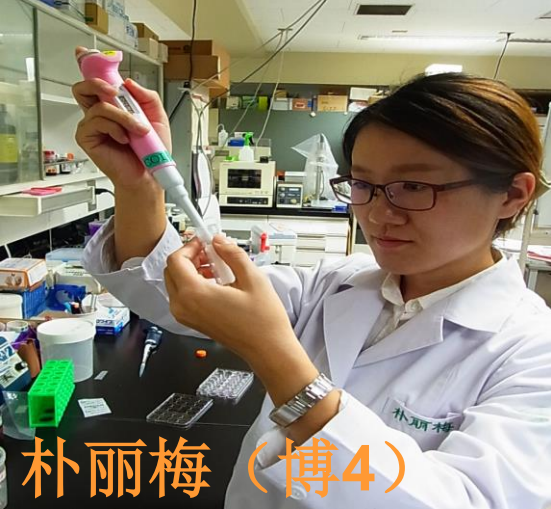
日本循環器学会東海支部

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Conclusion

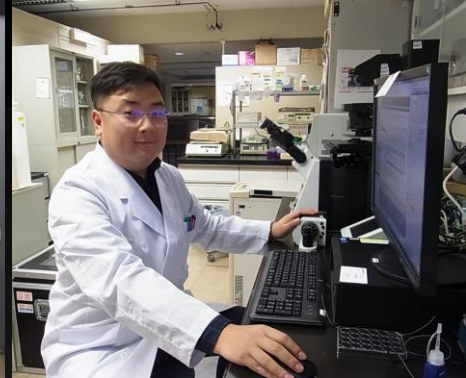
decline neovascularization in
ic stress via the VEGFR2/Akt
n in ECs and EPCs that is
nt5a/SC35 axis activated
d sFlt1 production in



朴丽梅 (博4)



俞成林 (博2) 王海龙 (博1)



孟祥坤 (博1)



许文虎 (博3)



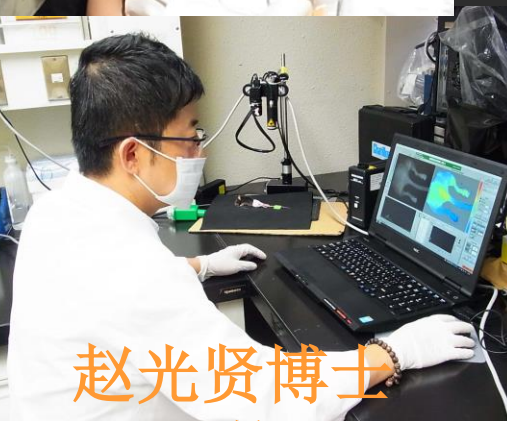
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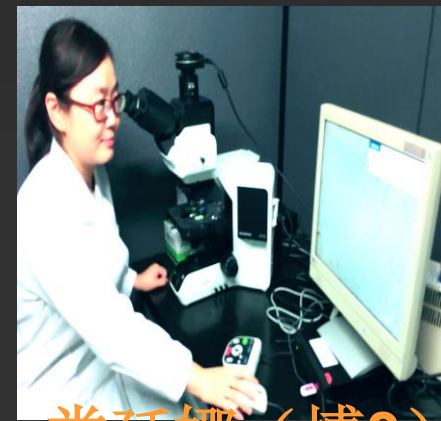
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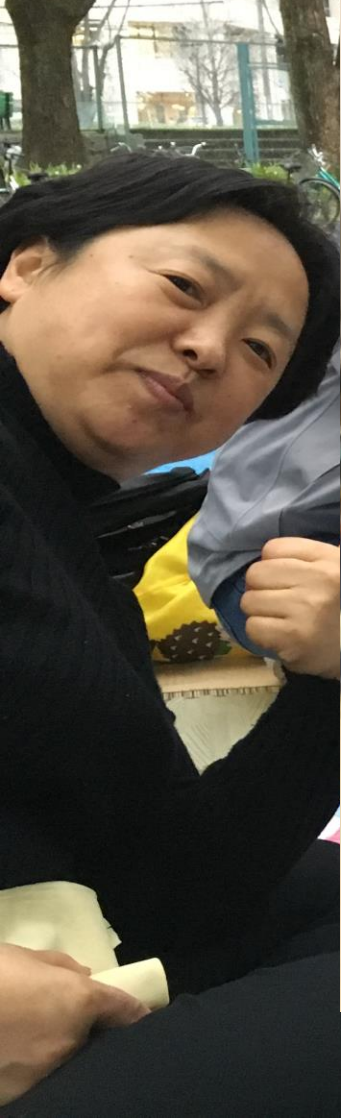
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赵光贤博士 (2017毕业)



类延娜 (博3)



2017年4月1日桜見