



Optimization of Murine Hindlimb Ischemia Model for Preclinical Evaluation of Vascular Regenerative Therapeutics for Peripheral Artery Disease

Nancy Rivera,¹ Sara Alharbi,² Xiaomin Zhang,² Guillermo Ameer,^{1,2} Bin Jiang,^{1,2*}

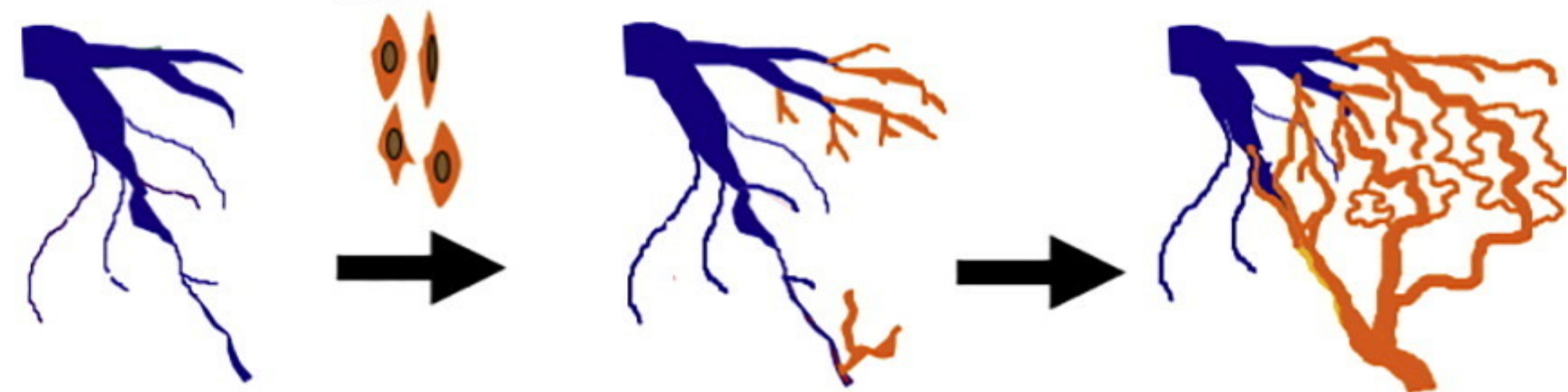
¹Department of Biomedical Engineering, Northwestern University, Evanston, IL

² Department of Surgery, Northwestern University, Chicago, IL

Northwestern Medicine
Feinberg School of Medicine

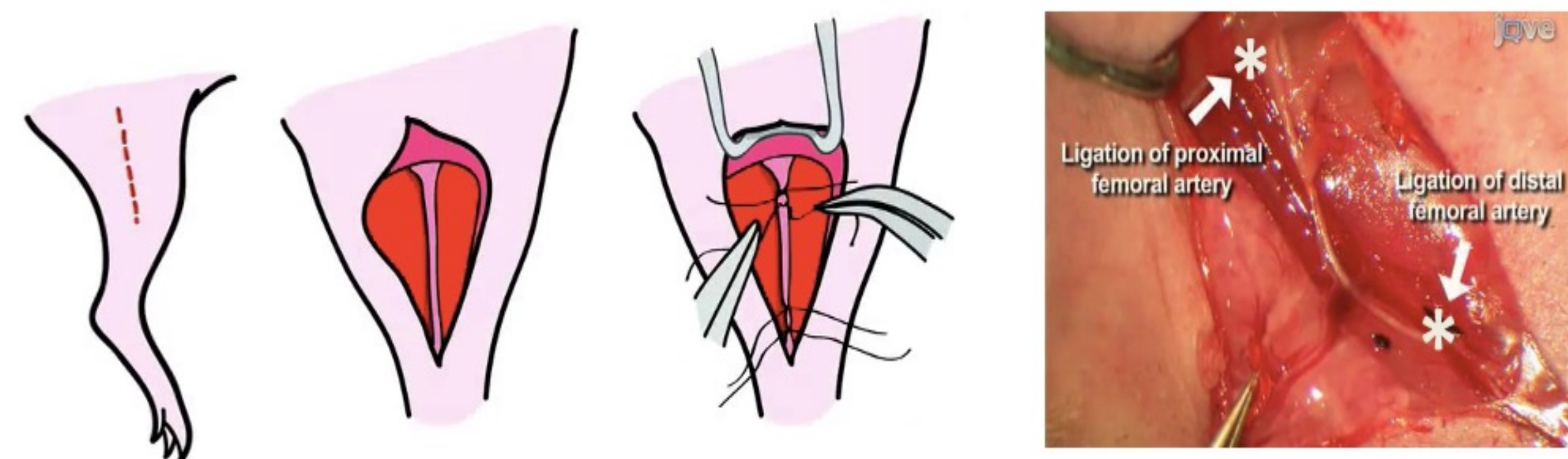
Introduction

- Peripheral arterial disease (PAD) affects 200 million patients worldwide with risks of limb amputation and mortality.
- Transplantation of autologous cells can potentially regenerate vascular tissues and restore limb perfusion.
- Current animal models of PAD only induce acute limb ischemia, which is not representative of the clinical condition, hindering therapeutic development.
- The goal of this study is to optimize the murine hindlimb ischemia model for preclinical evaluation of cell-based regenerative therapies.



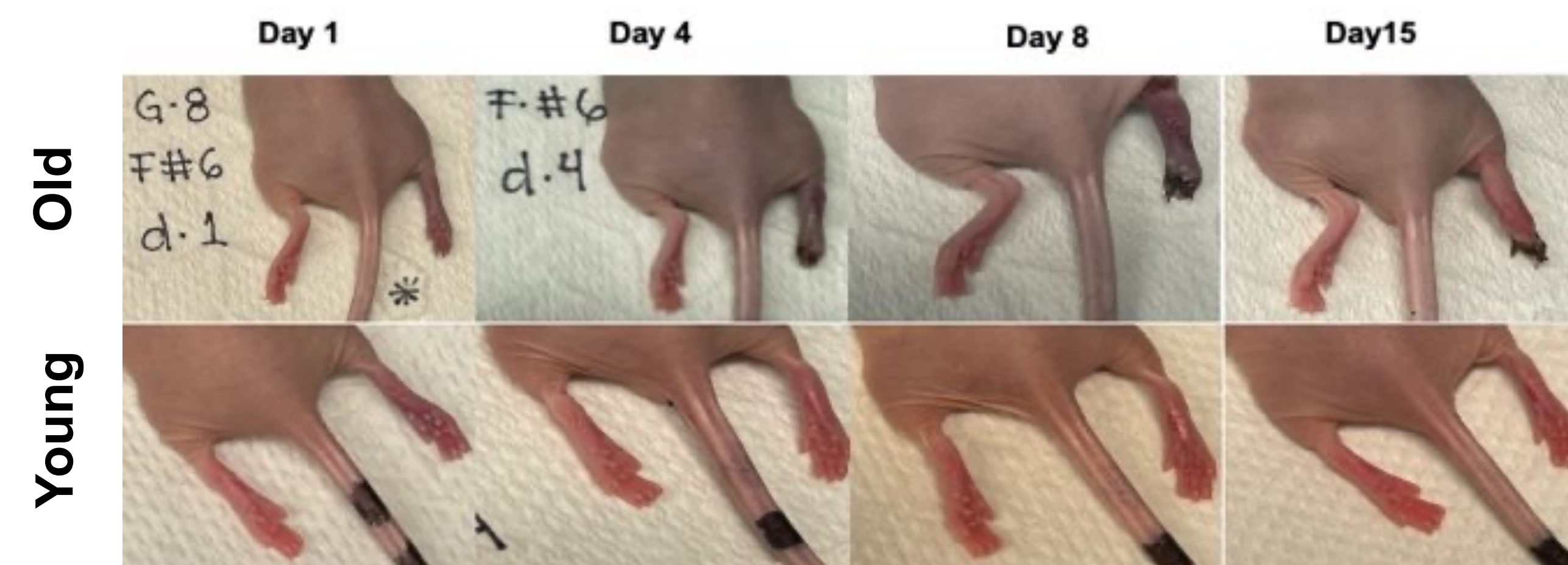
Methods

- Animals:** Male and female nude mice (NU/NU) at 6 or 14 weeks old
- Surgery:** Double-knotted ligations on the right femoral artery to induce ischemia.
- Treatments:** Intramuscular injection of PBS or Matrigel as cell delivery vehicle at the injured leg on day 1
- Imaging:** Laser Doppler Imaging (LDI) to measure blood perfusion at day 0 (after surgery), 1,4,8,15,22,29, and 36.
- Staining:** Histological staining with H&E and Masson's Trichrome; immunohistochemistry of vascular markers.

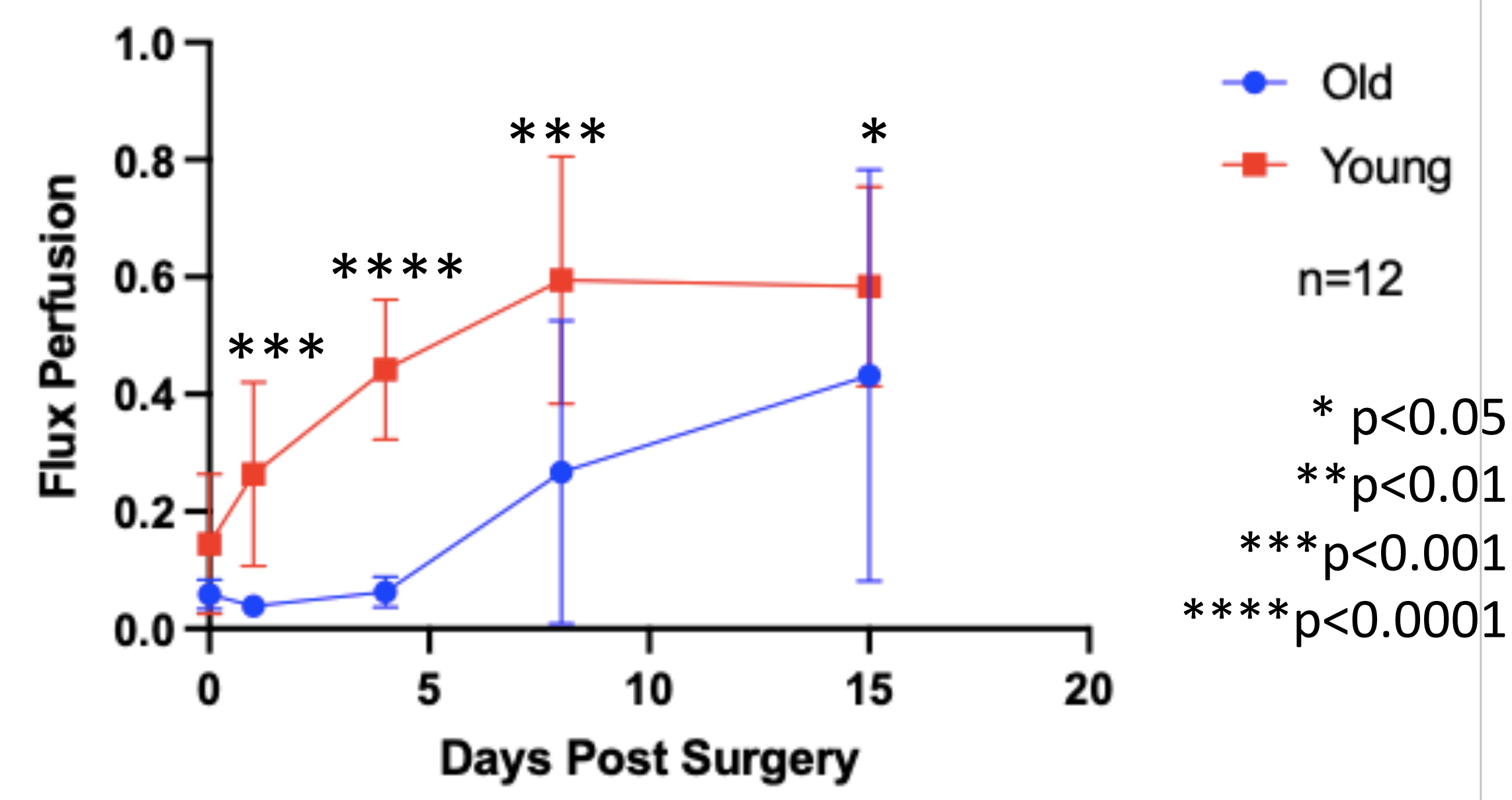
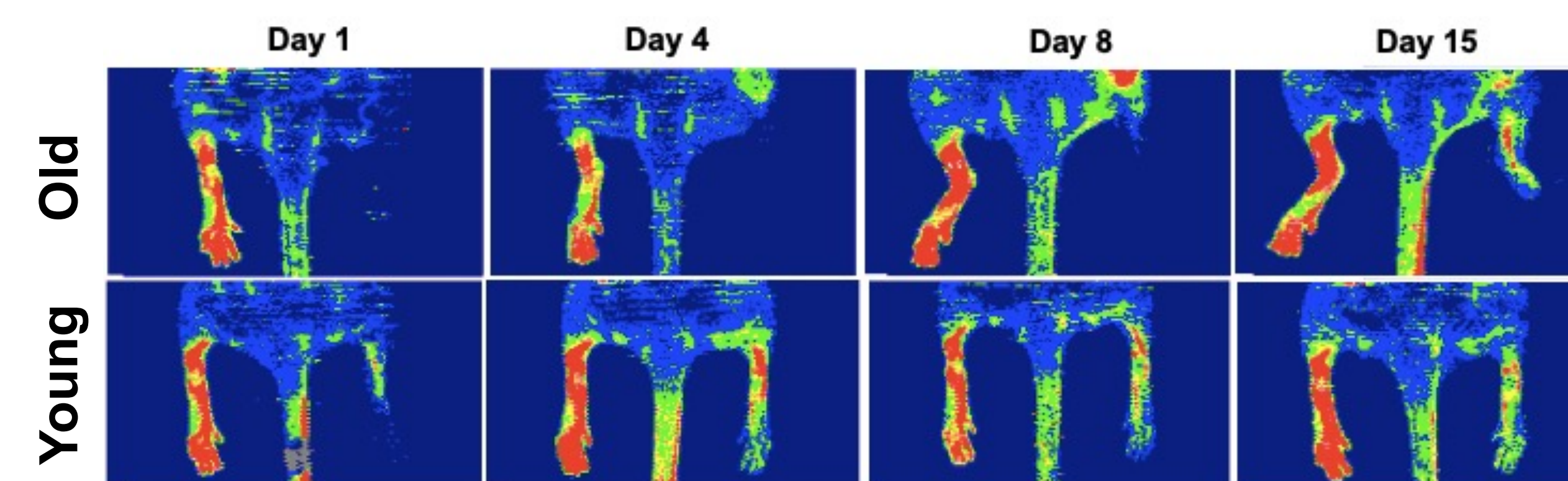


Results

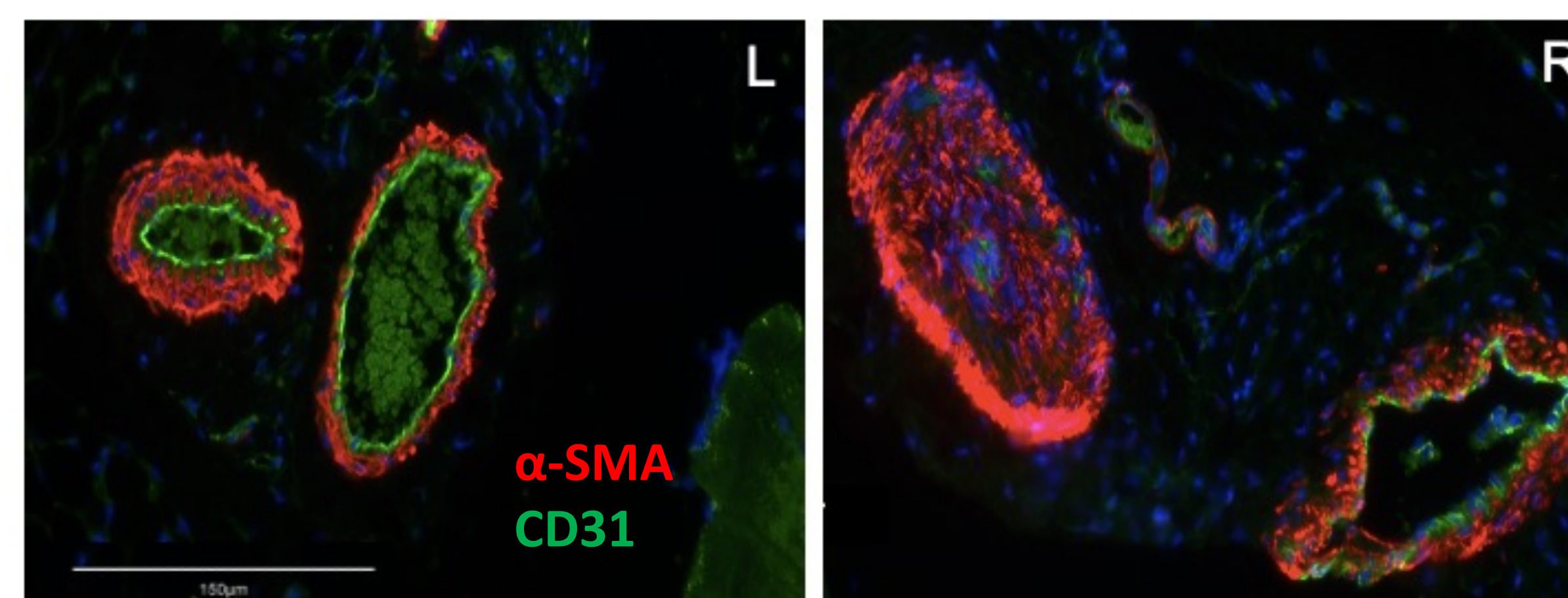
- Hindlimb ischemia induced tissue necrosis and limb loss in the old but not the young mice



- Blood perfusion rate was significantly lower in the old mice



- Ligated femoral artery exhibited occlusion and vascular remodeling



Discussion

Age of the mice influences the surgery outcome

- Old mice exhibited significantly worse surgical outcomes, including increased limb loss due to necrosis (60% of the old mice vs 0% of the young mice), and significantly lower limb perfusion rate at various time points.
- A number of factors could have contributed to the age-related differences, including oxidative stress, senescence, inflammation, and peripheral nerve degeneration.
- An animal model that is more representative of the clinical condition is expected to improve the success rate when translating future preclinical results into clinical therapeutics.

Ongoing work

- Quantitative analysis of vascular density
- Qualitative assessment of fibrosis, inflammation, and muscle atrophy
- Analysis of sex-related differences in vascular regeneration
- Mechanistic investigation of age- and sex-related differences

Conclusions

- Femoral artery ligation in old mice induces chronic limb ischemia, tissue necrosis, and limb loss, consistent with the clinical condition of critical limb ischemia.
- This chronic PAD model with old mice could be used to evaluate cell-based regenerative therapeutics for parameters such as limb salvage and long-term vascular regeneration.

Acknowledgements

This work is supported by the American Heart Association (19TPA34890008).