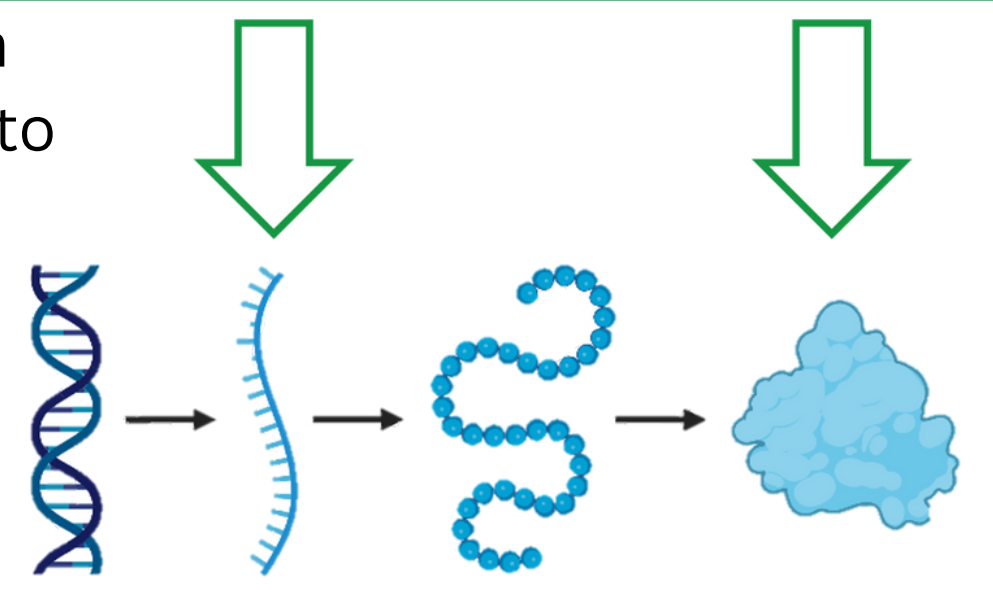


Gene Expression as Biomarkers of Tissue Viability in *Ex Vivo* Normothermic Limb Perfusion (EVNLP)

Abigail Meyers, BS, Varun Koppa, PhD, Jesse Zhan, BS, Payam Sadeghi, MD, Francis Papay, MD, Antonio Rampazzo, MD, PhD, Bahar Bassiri Gharb, MD, PhD

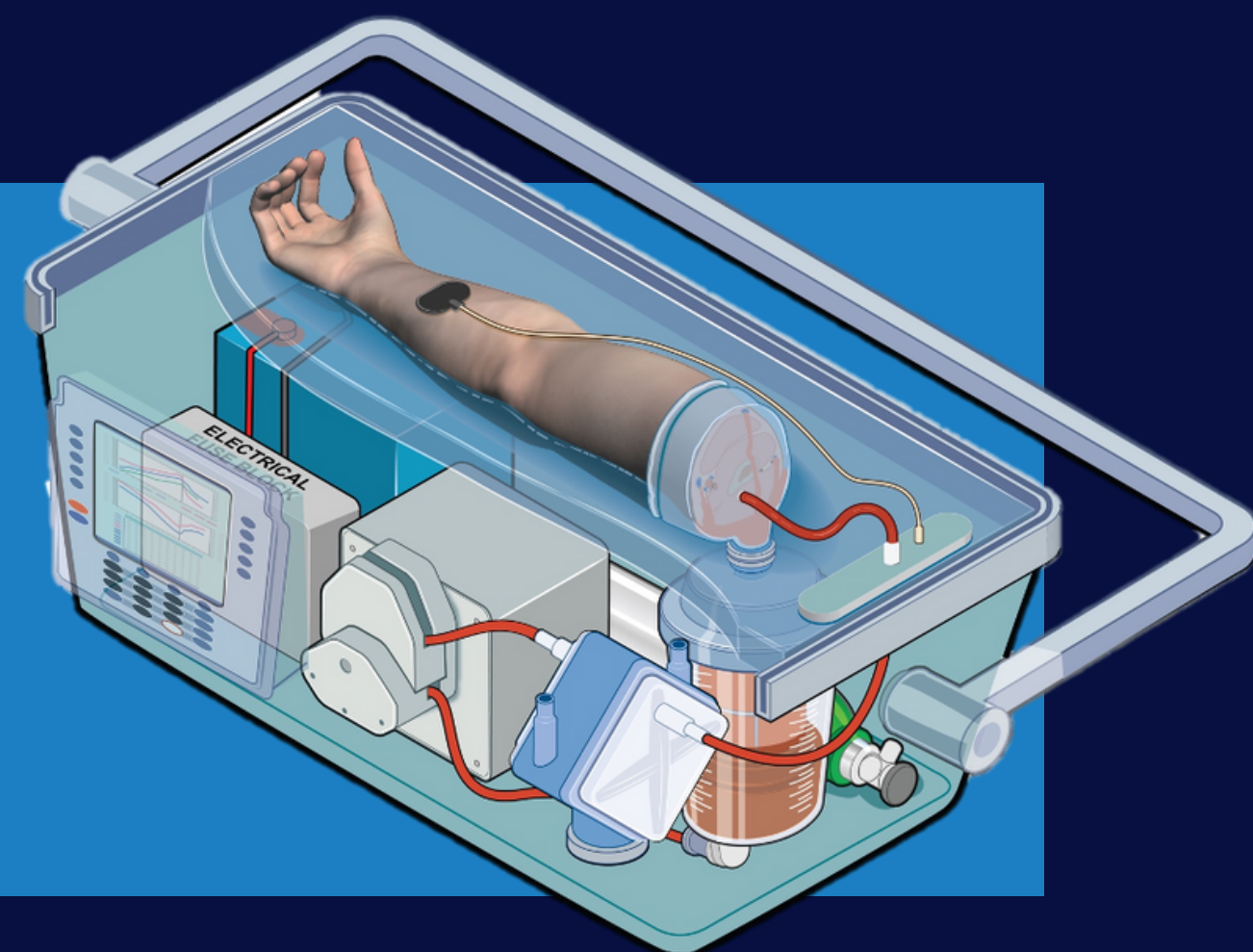
Background

- Muscle viability remains the primary focus of limb preservation
- Ex vivo* normothermic limb perfusion (EVNLP) has been shown to preserve amputated limbs in a near physiologic metabolic state
- Transcriptional changes appear earlier than immuno- and histological
- Genetic analyses are more sensitive than histology

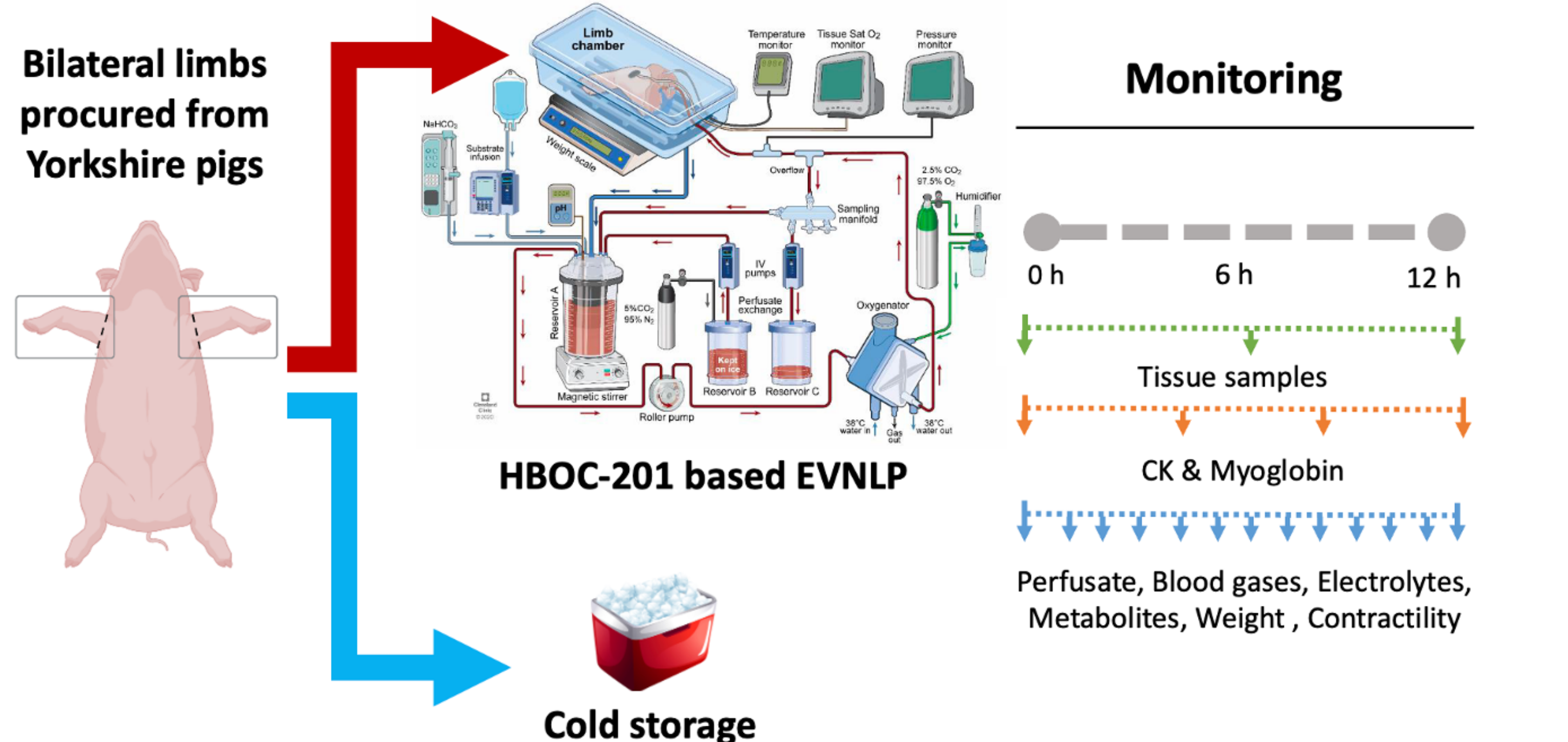


Purpose

- Molecular mechanisms during normothermic perfusion have not been studied
- Genetic biomarkers of tissue and DNA damage and hypoxia during EVNLP would allow us to accurately assess cellular injury and suitability of the limb for transplantation
- Aim to evaluate the effect of *ex vivo* normothermic limb perfusion on the transcriptome

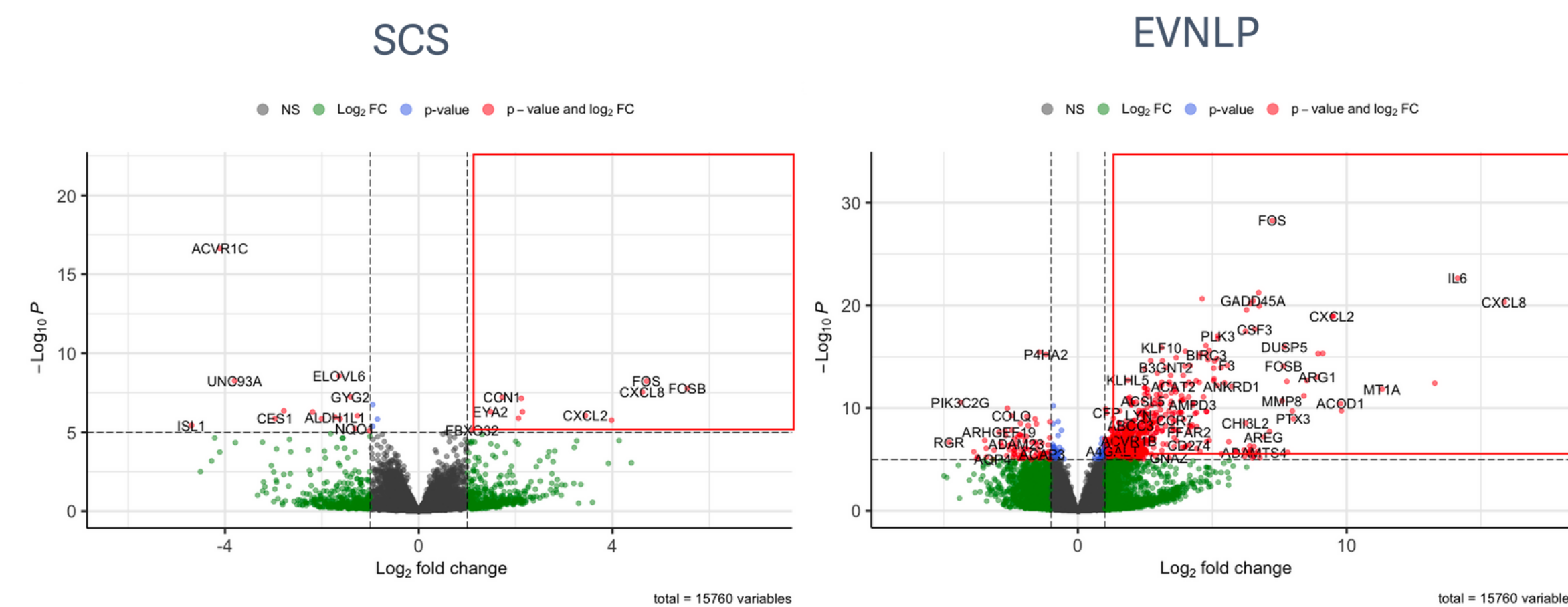


Methods

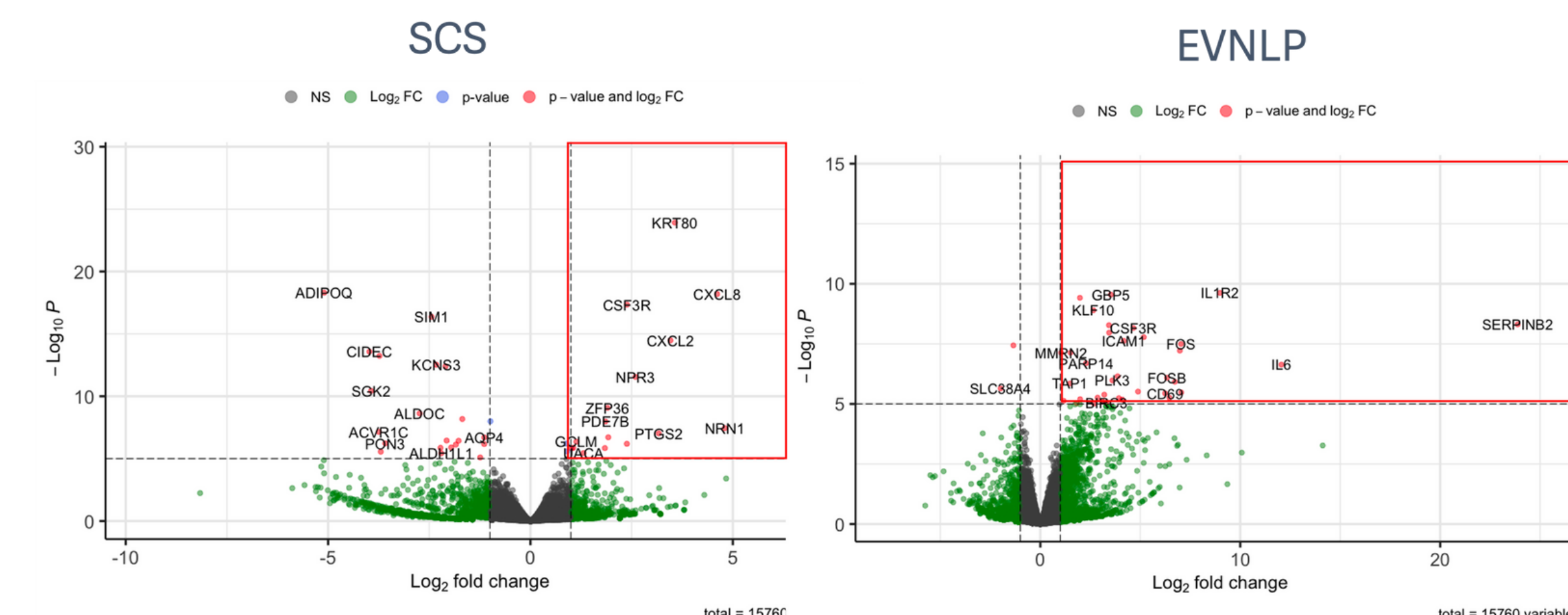


Results

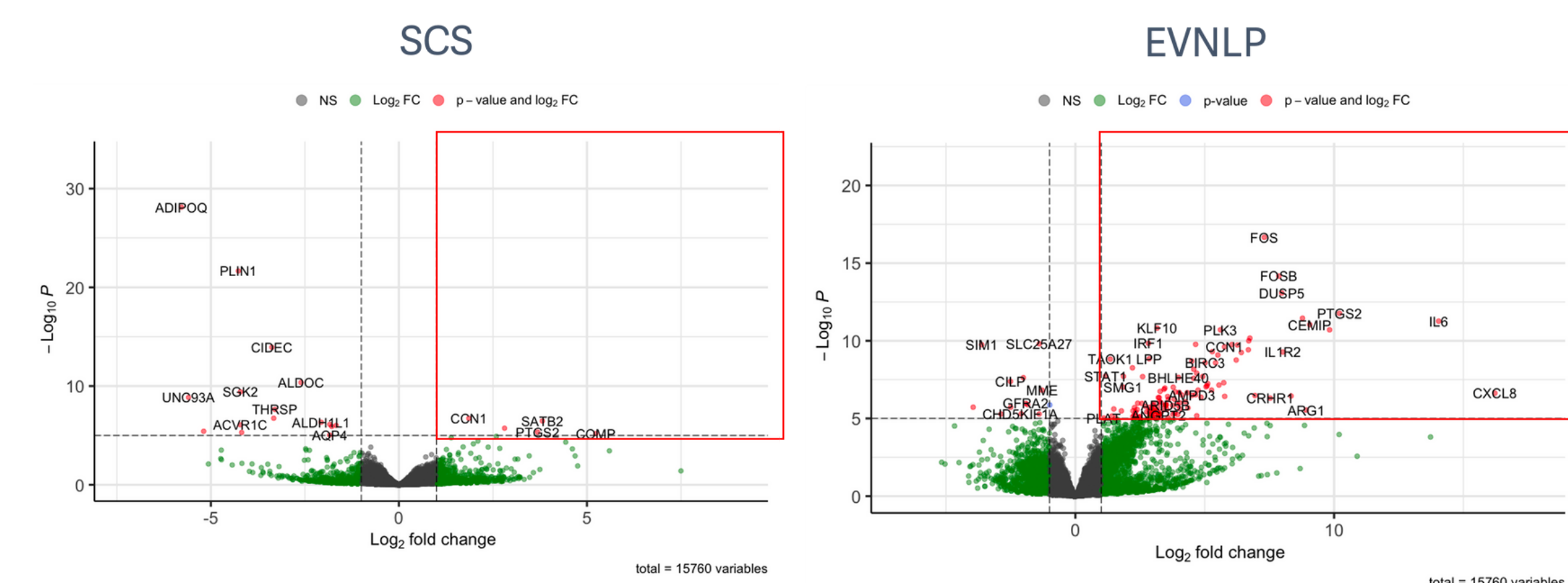
Divergent Genetic Expression



Baseline vs. 6h Timepoint



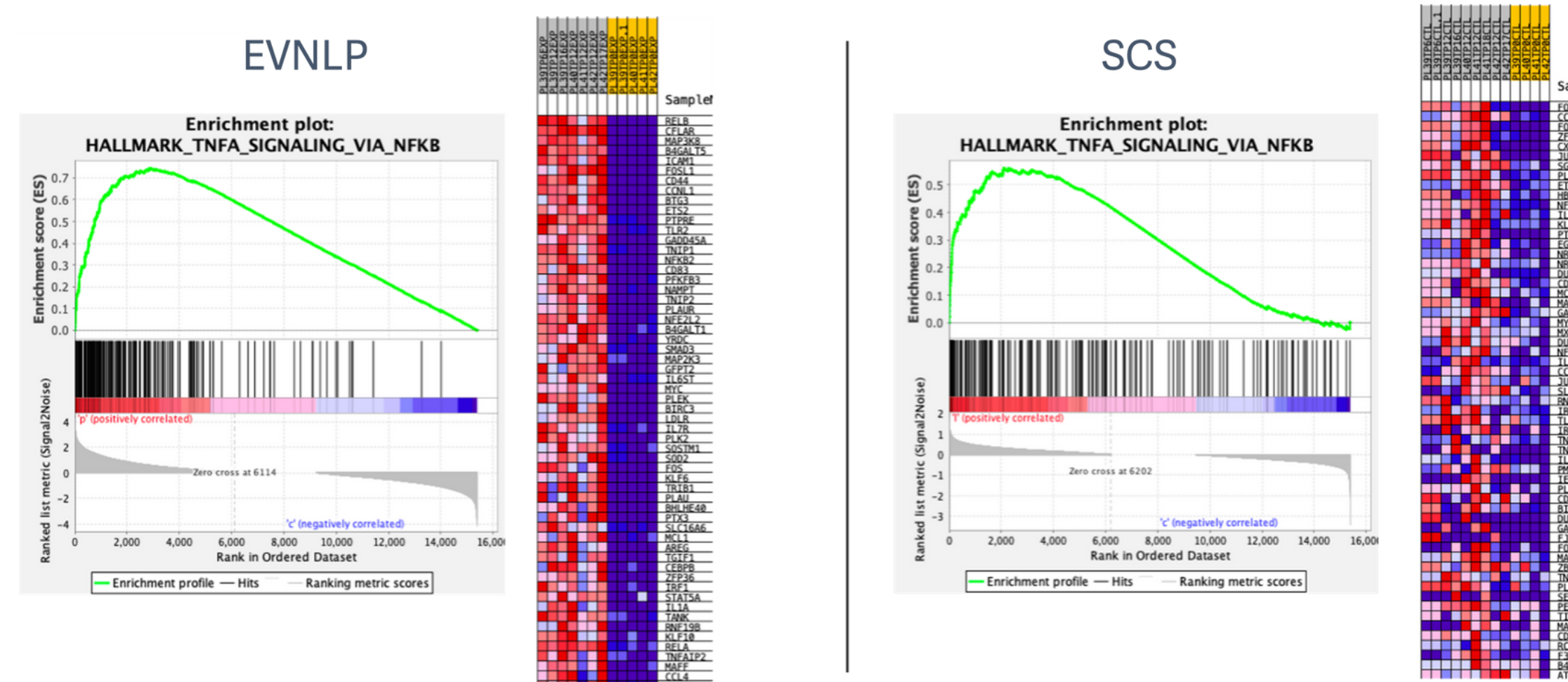
Baseline vs. >16h Timepoint



Pathways Upregulated in EVNLP

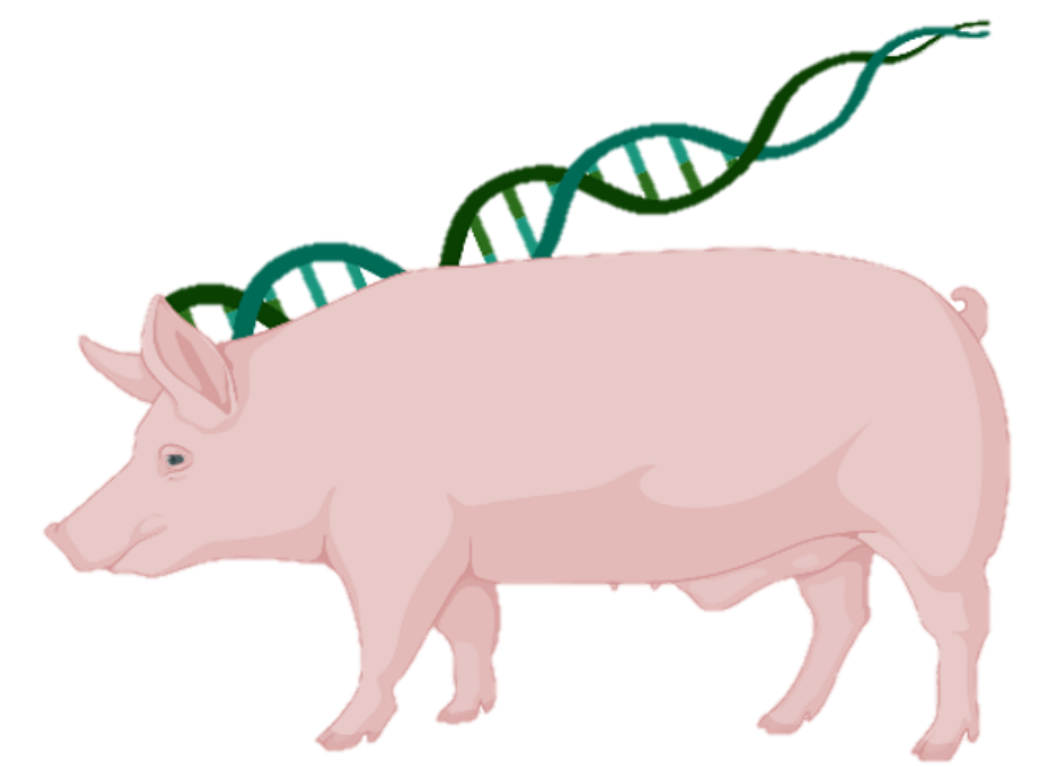
Pathway term	Fold enrichment	Adj. p-value
Wound healing involved in inflammatory response	9.29	2.25E-02
Regulation of translation in response to endoplasmic reticulum stress	8.36	1.19E-02
Inflammatory response to wounding	7.96	3.28E-02
Negative regulation of activated T cell proliferation	7.43	1.72E-02
Exonucleolytic catabolism of deadenylated mRNA	7.43	1.72E-02
Negative regulation of oxidative stress-induced intrinsic apoptotic signaling pathway	5.57	4.13E-02
Lipid droplet disassembly	4.01	2.59E-02
Response to ischemia	4.25	3.33E-02
Response to fluid shear stress	4.25	3.32E-02
NIK/NF-kappa B signaling	4.01	2.59E-02
Wound healing involved in inflammatory response	9.29	2.25E-02

Analysis of Top Expressed Genes



Conclusions

- EVNLP upregulates gene expression compared to SCS
- The transcriptome also changes over time in EVNLP, less in SCS
- Differential expression of genes involved in inflammation, hypoxia, apoptosis, muscle/tissue repair
- Triggering these pathways early during perfusion may be beneficial to these limbs at replantation
- Identification of these molecular markers is the first step to building a rapid test for limb viability



REFERENCES

- Said SA, Ordeñana CX, Rezaei M, et al. Ex-Vivo Normothermic Limb Perfusion With a Hemoglobin-Based Oxygen Carrier Perfusate [published correction appears in Mil Med. 2020 Sep 18;185(9-10):e1900]. Mil Med. 2020;185(Suppl 1):110-120. doi:10.1093/milmed/usz314
- Rezaei, M; Ordenana, CX; Figueroa, BA et al. Ex Vivo Normothermic Perfusion of Human Upper Limbs. Transplantation: August 2022 - Volume 106 - Issue 8 - p 1638-1646 doi: 10.1097/TP.0000000000004045
- Meyers A, Pandey S, Koppa V, et al. Weight Gain is an Early Indicator of Injury in Ex Vivo Normothermic Limb Perfusion (EVNLP) [published online ahead of print, 2022 Oct 28]. Artif Organs. 2022;10.1111/aor.14442. doi:10.1111/aor.14442