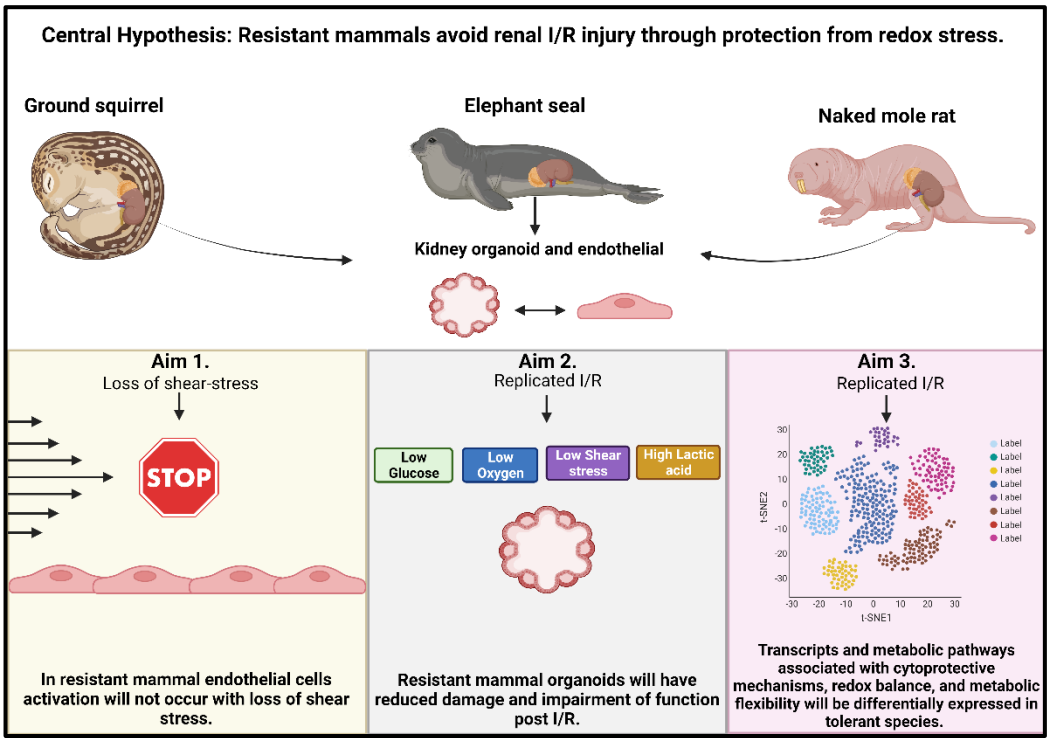
Disorders associated with **ischemia/reperfusion (I/R)** such as myocardial infarction, stroke, embolism, and peripheral vascular disease are some of the leading causes of debilitating disease and death in the Western world. Tissue injury and death are a result of the initial ischemia, subsequent reperfusion after ischemic insult results in the upregulation of oxidant generation and pro-inflammatory signaling further exacerbating tissue injury. For this reason, understanding the underlying mechanisms that may contribute to I/R tolerance is crucial in the identification and development of novel therapeutic strategies. Mammals that possess a natural resistance to I/R injury can provide additional clues to mechanisms that should be targeted. Furthermore, if there are multiple mammal species that possess such pathological resistance that introduces the question: are there multiple molecular mechanisms and physiological responses that protect tissue from I/R injury, and if so, which mechanisms should we prioritize in targeting for translational medicinal applications? For my future research, I will utilize **kidney endothelial cells and organoids** derived from *three distinct mammalian models* that naturally experience I/R without any signs of injury. The power of this research program lies in the utilization of three mammalian species that are not only phylogenetically distinct but also naturally experience different modes of ischemia/reperfusion. **This proposal aims to understand the possibly convergent or divergent mechanisms that allow these unique mammals to avoid (I/R) injury.** *Cardiovascular regulation in deep hibernators.* Hibernators enter bouts of torpor wherein body temperature (T_b) and metabolic rate are greatly reduced. This process is defined as a controlled and coordinated decline in physiological/homeostatic function. Hibernating ground squirrels (GS) can experience a ***≈ 97% reduction in cardiac output (CO)*** during torpor. What is even more interesting, is that during a hibernation season, individuals will periodically exit out of torpor (CO and T_b return to normal) for 12-18 hours before falling back down, this event happens roughly 25 times during a hibernation season. Essentially deep hibernators can experience up to ***25 heart attacks every winter.*** I have also measured kidney function during torpor and have shown a drop in renal clearance that mimics the conditions reminiscent of acute kidney injury in humans. *Extreme diving and hypoxemia in marine mammals.* Northern elephant seals (NES) (*Mirounga angustirostris*) have *a* ***maximum dive time of 119 min***. Previous work has shown that NES will completely utilize blood O_2 stores during routine dives, suggesting that these individuals experience tissue hypoxia every time they dive. We also understand that during dives, blood supply is cut off to the liver and kidney among other peripheral organs without any detrimental effects. *Extreme anoxic tolerance in a mammal.* Naked mole rats (NMR) (*Heterocephalus glaber*) experience extremes in atmospheric gases (hypoxia and hypercapnia) due to their subterranean lifestyle. These environmental extremes have created a phenotype that can tolerate hours of severe hypoxia and ***even 18 minutes of complete anoxia***. However, few scientists have investigated how naked mole rats avoid damage (at the organ level) during subsequent blood oxygenation after a hypoxic event. The mechanisms responsible for the phenotypes presented are underexplored. Also, to our knowledge, there has been very little work comparing these types of mammals in such an integrative fashion. This provides us with a unique opportunity to utilize a comparative biology approach for the benefit of translational medicine. Using a comparative approach, I aim to identify the most feasible



therapeutic targets for I/R injury. The central hypothesis of this proposal is that through the modulation of cytoprotective transcription factors, metabolic fuel switching, and antioxidants unique mammals are able to avoid the consequences of I/R.

Specific Aim 1. The effect of alterations in shear stress on Nox2 activation and peroxynitrite production in endothelial cells derived from resistant mammals. I hypothesize that in resistant mammal endothelial cells activation will not occur with loss of shear stress. We will derive primary endothelial cells from these resistant mammals following established protocols using magnetic beads and FACS. We will then use a cellular shear stress model to better understand if endothelial cell activation occurs under the loss of shear stress in I/R-resistant mammals. *With I/R-resistant mammals, we can determine the phenotypes that protect endothelial cells from I/R*

Specific Aim 2. The effect of simulated ischemia on kidney organoids derived from resistant mammals. I hypothesize that resistant mammal organoids will have reduced damage and impairment of function post I/R. I have already created kidney organoids from two of the three proposed species, using the same protocols, I will derive kidney organoids from the adult stem cells (ASC) from naked mole rats. Kidney organoids will flow adapted for a week prior to replicated I/R. Post-replicated I/R we will measure markers of oxidative stress (e.g., lipid peroxidation). *Using kidney organoids, we will be able to determine if resistant mammals are capable of avoiding ROS damage or if they are better at repairing ROS damage.*

Specific Aim 3. Ascertaining the molecular mechanism underlying I/R tolerance.
I hypothesize that transcripts associated with cytoprotective mechanisms, redox balance, and metabolic flexibility will be differentially expressed in tolerant species. The effects of replicated I/R on molecular pathways will be determined using scRNA-sq and metabolomics. Although seemingly ambitious this work will be done with great assistance. Dr. Sudmant will assist with transcriptomics. For metabolomics, organoid evaluation will take place at the UC Davis Metabolomics Center using the GC-TOF-MS untargeted approach. *By taking an evolutionary approach I can discover the convergent/divergent molecular underpinnings that allow for I/R tolerance in mammals.*