

**Impaired pulmonary endothelial barrier function in sickle cell mice**

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## Supplementary Appendix

### Impaired Pulmonary Endothelial Barrier Function in Sickle Cell Mice

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### Supplementary Methods

**Isolation and culture of mouse lung microvascular endothelial cells (MLMVEC):** Briefly, the conjugation of Dynabeads with mouse CD31, also known as Platelet endothelial cell adhesion molecule (PECAM-1) antibody (Invitrogen, Carlsbad, CA) was prepared per manufacturer's instructions. All procedures were completed under the laminar hood. Solutions were filtered using sterile 0.22- $\mu$ m filters. Culture dishes were coated with 10  $\mu$ g/cm<sup>2</sup> fibronectin for 3-4 hours. Prior to dissection, the incubator was set to 37<sup>o</sup> C. Collagenase Type 1 (0.2%) was prepared with Hanks Balanced salt solution (HBSS), separated into a 15 mL tube (7 mL for each mouse) and warmed to 37°C prior to digestion. Mice were euthanized by inhalation of CO<sub>2</sub> from a pressurized tank in an uncrowded chamber followed by decapitation and transported to a sterile environment. The abdomen and chest were exposed, and the lungs were removed and quickly placed in petri dishes containing a small amount of ice-cold HBSS. The debris and other

non-lung tissues were removed rapidly. The lungs were transferred to a small dish with 500  $\mu$ L HBSS and minced into  $\sim$  125 pieces and transferred to 15 mL tubes containing collagenase. The tubes were then placed in a shaking incubator at 37°C for 45 min with agitation for tissue dissociation. The tissue was then passed through a 70  $\mu$ m Cell strainer and wash the strainer with HBSS and centrifuged x 8 min at 4<sup>0</sup> C. The supernatant was discarded, the pellet suspended in 1 mL base medium (Dulbecco's Modified Eagle medium, DMEM high glucose with 1X Penicillin/Streptomycin) and transferred to a 1.5 mL Eppendorf tube. The CD31 antibody conjugated Dynabeads (10  $\mu$ L) were added to each tube and tumbled for 1 hr at 4<sup>0</sup> C. After 1 hr, each tube was mounted on a Magnetic Particle Concentrator (MPC) from Fisher Scientific for 1-2 min. The supernatant was then removed, the cells were washed with 1 mL isolation medium (DMEM high glucose; 20% FBS with 1X Penicillin/Streptomycin) by pipetting up and down and then remount on MPC. This was repeated five times with isolation medium and at the last wash, the cells were resuspended in endothelial growth medium (EBM-2: Lonza, Allendale, NJ). Cells were seeded into a Fibronectin coated 12-well plate. The culture medium was changed next day and then change half of the medium every other day.

The endothelial cells were cultured in EBM-2 medium and maintained at 37<sup>0</sup> CO<sub>2</sub> incubator and grown to form monolayers with typical cobblestone morphology. The confluent endothelial monolayers were briefly washed with 1X phosphate buffer saline (PBS) and then the Accutase was added to detach the cells. The detached cells were resuspended in fresh EBM-2 medium and one-third of the cells density was seeded into Fibronectin coated 12-well plates. Cells were maintained in culture and were used at 80-90% confluency at passage 2-3 for all experimental studies.