**Aortic Explant Assay**

**Reagents:**

1. Digestion Buffer PLUS pen/strep (optional):
   1. **50mg/mL** Collagenase Type II (Catalog # CLSS Worthington Biochemical Corp.)
   2. **50mg/mL** Soybean Trypsin Inhibitor (Worthington cat # 3571)
   3. **0.354mL** Elastase ES (Worthington 100mg cat # 2279 35H8143)
2. DF10 or ISFM PLUS pen/strep
3. 1x sterile PBS PLUS pen/strep
4. Media: DMEM 4.5 g/L glucose+ Hyclone FBS 1:1 ratio

**Equipment:**

1. Chamber slides: Lab Tek II 154526 OR 6 well-plate with 0.1% gelatin coating
2. Petri dish
3. Glass bead sterilizer
4. Forceps
5. Vannas Scissors

**Protocol:**

1. Autoclave tools
2. Turn on glass bead sterilizer (to be used in between each mouse)
3. Plate chamber slides with desired
4. Euthanize mouse in CO2 chamber
5. Perfuse with 10mL sterile PBS (+ or - p/s) by hand
6. Excise aorta and place in petri dish with PBS + p/s
7. Digest in 1 mL buffer (above) for 10 minutes in 37 degree incubator
8. Peel adventitia from medial layer with forceps under dissection scope
9. Wash 3x in PBS + p/s
10. Cut 1 to 3 mm pieces of aorta horizontally
11. Bring tools and petri dish into cell culture hood
12. Place several pieces of aorta in each well of chamber slides or gelatin coated 6 well
13. Add 100uL media to each well and let it spread out, to just cover the tissue
14. Ensure tissue is somewhere in the middle of chamber
15. Place in 37°C incubator
16. After one week (for DMEM:serum 1:1) or two weeks (DF10), observe endogenous fluorescence under scope