Baylis Flow Protocol

Harvest: Order of mice (eYFP-/-, LysM mice, Myh11 ERT2 Mice)

1. Weigh mouse and take tail for genotyping
2. Draw blood by cardiac puncture
3. Perfuse with 10 mL PBS
4. Remove liver, spleen, Epi fat
   1. Weigh spleen and epi fat
   2. Cut spleen in half (half will be used as a YFP+ control)
   3. Place in PBS with liver on ice
5. Empty peritoneum
6. Remove lungs and weigh (take large single lobe for PFA)
7. Take aorta with BCA and both carotids
   1. Place in FACs buffer on ice
8. Take heart
   1. Weigh heart and take top half for PFA
9. Take kidneys for weights take half for PFA

Digestion:

1. Remove FACs buffer and add liberase cocktail
2. Mince Aortas finely
3. Place in 37 degree for 1.5 hours (Triturate at 60 minutes with 1 mL pipet)
4. Mix with 5 mL FACs buffer
5. Spin the eYFP- control at 500 for 8 minutes
6. Take small aliquot and heat at 80 degrees for 10 minutes
7. Spin the remaining tubes

Live/Dead:

1. Resuspend samples requiring L/D in L/D yellow
   1. Make enough for 14 samples (2 extra)
      1. Make 250ul/sample at 1.1 uL/2mL PBS
      2. 3.5 mL of PBS + 1.925 uL of L/D dye
      3. 25 minutes at 4 C
2. Wash in FACs + Fc block
3. Spin at 400 g for 10 minutes

Surface Stains:

1. Make master mix of antibodies
   1. 300 uL of FACs buffer
      1. CD45: 3.75 uL
      2. CD11b: 1.875 uL
      3. CD86: 3.75 uL
      4. F480: 15 uL
      5. CD11c: 3.75 uL
      6. ~~CD206: 15 uL~~
2. Make FMO mixes
   1. 100 uL
      1. CD45: 1.75
      2. CD11b: 0.625
      3. CD86: 1.25
      4. F480:5
      5. CD11c: 1.25
      6. ~~CD206: 5~~
3. Stain for 20 minutes on ice with agitation
4. Wash with 1 mL FACs buffer

Fix/ Perm – Intracellular Markers:

1. 100 uL of Reagent A
   1. Incubate for 15 minutes at RT
   2. Wash with 2 mL FACs buffer
2. 100 uL of reagent B
   1. Make master mix of B with CD206 for 8 samples
   2. 800 uL of B + 40 uL of CD206
   3. Incubate for 20 minutes at RT
   4. Wash with 2 mL of FACs buffer
3. 100 uL of reagent A
   1. Incubate for 15 minutes at RT
   2. 2 mL of FACs buffer
   3. Spin
   4. Resuspend in FACs
   5. Store until Monday morning at 4C