**Microglia FACS staining after isolation (from UCSD)**

use 5ml Polyproprylene eppendorf tubes

**Antibodies**

anti-mouse CD11b –APC, clone M1/70, eBioscience, cat 17-0112-82

anti-mouse CD45 – Alexa488 (PE) , clone 30-F11, Biolegend, cat 103122

FcBlock (anti-mouse CD16/32, eBioscience, cat 14-0161-82

DAPI 1:10,000, for 45 sec, wash twice with PBS; dilute in H2O

**Protocol**

1. **Resuspend cells staining buffer (300 ul of HBSS+EDTA+BSA)**
   1. Resuspend in a 15ml falcon tube
2. **Add Fc Block (~1:100 dilution) and incubate for 15 min at 4C or in the fridge**
   1. Add this to cells resuspended in the HBSS
3. **Take ~5% of cells for unstained control, put on ice**
   1. Already did this during the isolation during the percoll separation step
4. **Add antibodies (CD11b, CD45, 1:100 dilution) for 20-30 min at 4C**
   1. Add directly to cells in the FC block
   2. Mix by tapping tube; do not vortex
   3. Don’t add to unstained cells!!
5. **Add DAPI, 45sec, then dilute with HBSS+BSA+EDTA**
   1. Add 1:1000 dilution
   2. Add to unstained cells
6. **Put 70um filter on top of a FACS test tube and filter resuspended pellet into the tube**
   1. Push filter hard onto tube
   2. Do this for unstained samples as well
7. **Centrifuge for 10 min at 400g**
   1. Prepare collection tubes during spin time
      1. Coat FACS tubes with 1mL of staining buffer to prevent cells from sticking to walls of tubes
      2. Invert tube several times
8. **Vacuum out supernatant**
   1. Remove most of supernatant; not all
9. **Resuspend pellet in 500-1000 uL staining buffer**
   1. Depending on machine

**\*\*keep cells on ice the whole time\*\***

**Gating Notes**

* 1) live vs dead
* 2) FSC vs SSC
* 3) singles vs. doublets
* 4) CD11B (high) vs. CD45 (low)
* 5) CX3CR1 (optional) 🡪 gate on positive cells

**Misc. Notes**

* \*\*don’t need to compensate with only 2-3 colors
* CD45, CD11B, and CX3CR1 are all surface markers
* Usually get 20-50% live cells after staining
* Better to do staining right after microglia prep rather than waiting O/N for staining

**Processing Notes**

* Can do RNA seq or Atac Seq
* RNA Seq
  + Spin down sample in Eppendorf (not in facs tubes
  + Remove supernatant
  + Add 150 uL of trizol 🡪 resuspend
  + Store in -80C (can keep stored for months)
* ATAC Seq
  + Do transposase reaction and then freeze for processing