Isolation of Bone Marrow Derived Macrophages

1. Dissection
	1. Open mouse and perfuse. Place mouse ON ICE. Use scalpel to cut skin/hair on ventral side of the mouse diagonally down leg. Remove muscles and fat from the hip joint to foot. Repeat on dorsal side (cutting fur across midline and securing the tail will make this easier). Cut off leg AT THE HIP JOINT (Important- do NOT cut through bone) to remove the legs and place ON ICE or in dish of PBS. Clean muscles from bone in PBS or in paper towel (rubbing the bone under the paper towel will expedite this process) with scissors and/or scalpel. Separate the tibia and the femur at the knee joint (cut the ligaments and tendons with scissors- they will be quite firm but distinct from bone).
	2. When bones are mostly clean, cut off small portion on each end. Insert syringe with PBS and inject fluid over a 50mL falcon tube (ON ICE) to remove marrow. Use syringe to scrape the cavity. Inject PBS until the marrow cavity is visibly clear. Repeat for both bones in both legs. Keep marrow from each mouse in its own separate tube- do NOT pool mice. (4 bones will require ~5-10 mL of PBS).
2. Filtration
	1. After collecting marrow, filter the solution through a 100 um filter to a new 50mL falcon tube.
	2. Centrifuge 500g x 10 min at 4°C
	3. Resuspend pellet in 5 mL serum free- BMDM media (DMEM+Hepes+Glucose 1g/L, 1%P/S, no FCS)
3. Ficoll-Paque
	1. Prepare a 15 mL falcon tube with 5 mL Ficoll-Paque.
	2. SLOWLY pipet 5mL of filtered sample in media on top of the Ficoll-Paque to form a separate, distinct layer.
	3. Centrifuge 35 min at 450 g with NO BRAKE
	4. Collect the hazy ring (~2mL at the top of the Ficoll gradient)
	5. Centrifuge again 500g x 10 min at 4°C
4. Plating and Culture
	1. Resuspend pellet in 1 mL BMDM media + 10% FBS
	2. Count cells
	3. Plate up to 10^7 cells in T25 flask with 10mL BMDM media + 10% FBS and 20 ng/mL M-CSF
	4. Next day, transfer cells to T75 flask with 10 more mL BMDM+FBS+M-CSF (to make 20 mL total)
	5. Grow cells for 7 days, adding 10 more mL BMDM+FBS+M-CSF on day 4-5. May mature up to 10 days.
	6. To remove cells for analysis by flow, add 5mL TrypLE for 5 min at 37°C. Rescue with BMDM media+FBS, use vigorous pipetting and cell scraper to remove.

 \* Another optional plating protocol without M-CSF uses “detachment” method instead- Plate cells in T75 or 150mm dish. In 2 hours, aspirate non-attached cells and medium, wash 3x PBS, add medium and culture for 7-10 days.

Materials List:

M-CSF: ebiosciences cat # 14-8983-62

Ficoll-Paque: Sigma-Aldrich cat #GE17-1440-02

TrypLE: Thermo-Fisher cat#12604013

BMDM media: Thermo-Fisher DMEM+ Hepes + 1g/L Glucose cat #12320032

+/- 10% FBS

Cell scraper: Sigma-Aldrich cat#SIAL0008-150EA

Dissection- scissors, scalpel, ice, 50mL tube, PBS, 20mL syringe

100um filter

50 mL, 15 mL tubes, T25, T75 flasks or similar

4 degree centrifuge (large)