**BMDM’s Isolation**

**Day 0:**

Remove fur/skin from mice legs. With the largest “lab” scissors, cut very proximally near the spine/hip girdle to detach the femur without actually cutting it open and losing the marrow. Take both legs in a 50 mL conical tube, in 20 mL standard culture media (DMEM, P/S, L-glut) w/o FBS. Save in 4C overnight at the longest before proceeding to next step.

 **Day 0/1**

Prepare one 0.65 mL mcfuge tube with the bottom cut off per bone (4 per mouse) (~ 1 mm hole diameter or less so the bone itself won’t go through). Use a razor. If it’s close but not quite big enough you can twist the corner of the razor in the hole.

Use 1 6wp well per mouse and label plate. Pour a bit of media from tube into plate well to keep bones submerged.

Thoroughly dissect Tibia and femur. This includes removing the muscles and tendons, the hip girdle and foot. Gently snip away at the ligaments/tendons making each connection and be sure not to break open the tibia or femur. You can just break off the fibula. You should finish with 2 sets of fairly clean tibias/fibulas per mouse.

Pipette 50 uL PBS into 1.7 mL microcentrifuge tubes. Cut ~1mm off the edge of each bone such that the marrow is exposed (it’s “shiny”). Spin all tubes (4 per mouse, 0.65 mL tube with cut bone (cut side down) inside 1.7 mL tube with PBS. 7k rpm, very brief spin. (just let it reach max speed for 20-30 sec. Should yield a pretty substantial brown/red pellet.

Combine tubes from individual mice (resuspend and pipette into one. Can combine by genotype too if more cells needed. Discard any residual bits of muscle that get caught in the pipette tip.

~200-300 uL total suspension per mouse, add 50 uL RBC lysis buffer. Resuspend and let lyse 2-3 mins. Add excess of PBS to dilute/deactivate RBS lysis (just fill the 1.7 mL tube). Spin 900 rpm 7 mins 4C.

**Under hood:**

Make up 10 mL media with 5% MCSF per mouse. (**note: potency of MCSF varies, may need to use 5 or 10 % and learn what “fully differentiated” looks like and potentially do a time course validation with new batches) (Source of MCSF: L929-conditioned media (See Cathryn Martel Protocol)**

Aspirate supernatant from 1.7 mL tubes, resuspend each in 1 mL media thoroughly.

Plate in final volume 10 mL on **bacterial culture** (sterile, non-tissue culture treated) plate, or greiner suspension plate as appropriate, disperse well.

Place in incubator, will take at least 48 hours to start differentiating and fully adhere. Wait at least 3-4 days before changing the media once more to full media w/ 5% MCSF before experiments/treatments, then a few days after that, can replate.

Wash 10 cm plates 2x with pbs, put 2 mL cellstripper for 20 mins in incubator. Then, add 6 mL normal media to neutralize cellstripper. Collect in 50 mL tube. Spin 7 mins 1k rpm. Replate at 300-500k cells/well for 12wp.