**PREPARATION:**

1. Weigh the recipient mice (e.g. ldrl-knock out).
2. Have an irradiation control mice (will be irradiated, but will not receive bone marrow transplant)
3. Have ready a Ketamine and Xylazine cocktail for the transplantation step (20mg/mL ketamine + 4mg/mL Xylazine (a pain killer)
   * 9.6mL saline
   * 0.5mL of 100mg/mL Ketamine
   * 2.5mL of 100mg/mL Xylazine

**BONE MARROW ISOLATION**:

1. Take donor mice (around 8-10 week old). 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.
2. Take both legs in a 50 mL conical tube, in 20mL standard culture media (DMEM, P/S, L-glut) w/o FBS. (Can save in 4C overnight at the longest before proceeding to next step).
3. Prepare one 0.65 mL microtube with the bottom cut off per bone (4 per mouse) (~1mm hole diameter or less so the bone itself won’t go through). Use a razor for this step. If it’s close but not quite big enough you can twist the corner of the razor in the hole.
4. Take one 6-well plate, and use one well per mouse and label each. Pour at least 1-2mL of media from tube into plate well to keep bones submerged.
5. 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.
   1. You can just break off the fibula. You should finish with 2 sets of fairly clean tibias/fibulas per mouse.
6. Pipette 100µL PBS into labeled 1.7mL microtubes. Place the 0.65mL microtube (from step 5) inside the 1.7mL ones. (4 per mice)
7. Cut ~1mm off the edge of each bone such that the marrow is exposed (it’s “shiny”).
8. Place the cut bone (cut side down) inside 0.65mL/1.7mL tube with PBS.
9. Spin at 7k rpm very briefly (just let it reach max speed for 20-30 sec). (Should yield a pretty substantial brown/red pellet)
10. Combine tubes from individual mice (re-suspend and pipette into one tube). Can combine by genotype too if more cells needed. Discard any residual bits of muscle that get caught in the pipette tip.
11. Add 50µL red blood cell (RBC) lysis buffer to the ~200-300µL total suspension per mouse. Resuspend and let lyse for 2-3 mins. Add excess of PBS to dilute/deactivate RBS lysis (just fill the 1.7 mL tube).  Spin 900rpm for 7 mins at 4C.
12. Take the pellet and re-suspense with 1ml of PBS. Take the 10ul of PBS(which contains BM) and measure the cell numbers with hematocytometer. Make the BM concentration ~1-5\*106 cells/100µl (This is not strict number. Generally, with 1 donor mice = can inject more than 20 recipient mice).

**IRRADIATION**

1. Place the recipient mice on irradiation pie and lethally irradiate each mice with 10 Gy.
2. Within 6 hours after irradiation, bone marrow transplant the recipient mice.

**BONE MARROW TRANSPLANTATION**

1. Take the recipient mice and inject 4µL of the Ketamine and Xylazine cocktail per gram of mice body weight (peritoneal). The mice should go to sleep fast. Can inject a whole cage the same time and proceed immediately with the bone marrow injection.
2. Eye inject 100µL (∼1-5\*106) of donor marrow cells via a single injection.
3. Give antibiotic water to the mice that received the bone marrow transplant for at least a week.
   1. Caution to not leave the bottle of water leaking on top of the mice since these will lose some of their body heat.
4. Cover the mice with paper towel as a blanket, because they tend to lose body heat. They should wake up in 1hour, 4 hours max.
5. Allow the donor marrow to repopulate for 4 weeks.
6. The irradiated ‘control’ mice will not receive bone marrow transplantation. Do not need to give antibiotic water to this mice. It should die within a week.