Isolation of Murine Hepatic Immune cells

1. Euthanize the mouse and perfuse it via the left (for brain and liver; for lungs would be the right) cardiac ventricle. To do so insert a 26-gauge needle into the left ventricle, cut the right atrium of the heart with scissors and administer 20 ml of cold PBS.

2. Optional: Insert a 26-gauge needle into the portal vein (thick and dark blue vessel located on the underside of the liver) and slowly inject 5 ml of cold PBS to eliminate the blood. If the procedure is correctly done the liver will become pale.

3. Tease out the liver using forceps and scissors to cut it into small pieces in a 6 well plate. Use two glass slides, with the non polished writing part to further disrupt the liver tissues.

4. In a final volume of 5ml per organ homogenate the liver as much as possible and add Collagenase D and DNAse I.

Collagenase D [Roche Ref: 11 088 882 001 (2.5g – 0.233 U/mg)] --> Stock concentration 500mg/ml.
DNAse I [(Roche Ref: 11 284 932 001 (100mg)] --> Stock concentration 10mg/ml.
Prepare 5ml RPMI with Collagenase D (0.5mg/ml final d=1/1000) and DNAse I (0.01mg/ml final d=1/1000) per organ.

Pipette up and down with 1ml blue tips. Incubate 30min at 37oC. Pipette up and down with 1ml blue tips.

5. Then add EDTA to 2mM final pipette up and down with 1ml blue tips for 5min at RT.

6. Put what is left of the liver pieces in a cell strainer and squeeze it with a syringe head (or through a stainless steel mesh). Filter the suspension and wash once @ 400xg (1400 rpm) for 8 minutes, 4ºC. Remove the supernatant slowly.

7. Carefully ressuspend the pellet in a 33.3% Percoll solution (v/v) – add 1.7 ml Percoll 100% + **3.3 ml of RPMI at RT!** per organ- and transfer it into a 15ml Falcon tube.

8. **Centrifuge @2000 rpm (800 g) with no brake for 30 minutes at RT.** Gently aspirate the supernatant as much as possible, leaving only the cell pellet, avoiding contamination with hepatocytes (white part please avoid!) from the supernantant. Wash the pellet once as the Percoll can inhibit the red blood cell lysis buffer.

9. Ressuspend the pellet in 1 ml red blood cell lysis buffer and incubate at RT for 5 minutes to lyse RBC.

10. Add 5 ml of FACS buffer and centrifuge @1500 rpm (480 g) for 8 minutes with the high-brake setting at 8ºC.

11. Ressuspend the cells in FACS buffer and count the viable ones. They are ready for cell surface phenotyping by flow cytometry. Use 2x106 cells per well (staining). Ressupend in 0.2 ml Facs Buffer.