

Pre-cDC1 versus CD8 α + cDC1

Purpose: Compare the conventional dendritic cell (cDC) subsets CD8 α + cDC1 and pre-cDC1 by RNAsequencing.

Mice:

6 BALB/c females, age-matched

Protocol:

Dendritic Cell Isolation

Harvest spleens from naïve mice. Pool 2 spleens per “mouse” for 3 biological replicates. Create single cell suspensions in Complete Media (**CM**) (RPMI-1640 with 10% FBS, 1% Sodium Pyruvate, 1% MEM NEAA, and 100 U/mL penicillin-streptomycin). Lyse red blood cells with Pharm Lyse (BD), wash cells in PBS, and then count total splenocytes. Isolate splenic Pan-DCs using the Pan DC Isolation Kit from Miltenyi (130-100-875). Count total DCs.

Flow Cytometry Staining

Resuspend cells in flow buffer (PBS + 0.5% FBS) and incubate with anti-mouse Fc Block (Thermo Fisher) for 15 minutes on ice. Stain cells with anti-mouse CD8 α PE-Cyanine7 (clone: 53-6.7) (Invitrogen) and anti-mouse CD24 Pacific Blue (clone: M1/69) (Biolegend) in the dark for 30 minutes on ice. Wash cells with 1 mL PBS, centrifuge, and dump supernatant. Resuspend stained cells in 3 mL of CM.

Fluorescence-Activated Cell Sorting

Pre-cDC1 (CD24^{high}CD8 α -) and CD8 α + cDC1s (CD8 α +) were sorted by the University of Arizona Flow Cytometry Core using a FACS Aria III (BD). Cells were sorted directly into tubes already containing 500 μ L RNeasy lysis buffer (Qiagen).

Samples were kept on ice, transferred* to labeled 1.7 mL Eppendorf tubes, and delivered to the University of Arizona Genomics Core (UAGC) for processing.

*During transfer step there was contamination of mouse1 pre-cDC1 and mouse1 CD8 α + cDC1 samples

