## SUPPLEMENTARY MATERIALS

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## Materials and Methods

### Animals

Mice were bred and maintained under SPF conditions in accredited animal facilities at the University of Oxford. All procedures were conducted according to the Operations of Animals in Scientific Procedures Act (ASPA) of 1986 and approved by the Kennedy Institute of Rheumatology Ethics Committee. Animals were housed in individually ventilated cages at a constant temperature with food and water *ad libitum*. Mice were free of known intestinal pathogens and negative for *Helicobacter* species. C57Bl/6 and Irf5$^{tm1/J}$ (*Irf5*$^{-/-}$) mice were bred in house. C57Bl/6 SJL CD45.1$^{+}$ WT mice were purchased from the University of Oxford BMS. ) Cx3Cr1$^{tm1.1(cre)Jung/J}$, (CX$_3$CR1$^{IRF5+}$, cWT) were acquired from JAX (Jackson Laboratories, UK, JAX stock #025524)) and crossed with C57BL/6-Irf5$^{tm1Ppr}$/J (Irf5$^{fl/fl}$) to generate conditional *Irf5*$^{-/-}$ mice (cKO, CX$_3$CR1$^{IRF5-}$). CX$_3$CR1$^{GFP/GFP}$ CD45.1$^{+}$ mice (B6.129P(Cg)-Ptprc$^{a}$ Cx3cr1$^{tm1Litt/LittJ}$) were maintained in house. B6.SJL-*Ptprc*$^{a}$ (CD45.1$^{+}$ WT mice) were crossed with B6.129P(Cg)-Ptprc$^{a}$ Cx3cr1$^{tm1Litt/LittJ}$ to generate CD45.1$^{+}$ CX$_3$CR1$^{GFP/+}$, and B6.129P-Cx3cr1tm1Litt/J were crossed with Irf5$^{-/-}$ to generate CD45.2$^{+}$ CX$_3$CR1$^{GFP/+}$ Irf5$^{-/-}$ mice. For experiments, corresponding strains of mice were co-housed from weaning.

### Generation of Mixed Bone Marrow Chimaeras

≥ 20 g male C57BL/6 SJL (CD45.1$^{+}$ WT) mice were selected as bone marrow recipients. 2 days before irradiation, mice were fed antibiotic-treated water (Co-trimoxazole, Aspen Pharma), or Baytril, Bayer) which was maintained until 2 weeks post-irradiation (PI). Recipients were lethally irradiated by exposure to 11 Gy split into two equal doses 6 hrs apart in an X-ray irradiator (Gulmay). Within 24 hrs post-irradiation, 4 x10$^{6}$ freshly isolated bone marrow cells at a 1:1 ratio (CD45.1$^{+}$ WT and CD45.2$^{+}$ *Irf5*$^{-/-}$, or CD45.1$^{+}$ CX$_3$CR1$^{GFP/+}$

and CD45.2$^{+}$ CX$_3$CR1$^{GFP/+}$ Irf5$^{-/-}$) were injected i.v. into the tail vein of CD45.1$^{+}$ WT hosts. 6 weeks post-irradiation, reconstitution was assessed by collecting blood leukocytes by tail vein bleed.

### *Helicobacter hepaticus* culture

*Helicobacter hepaticus* (Hh) NCI-Frederick isolate 1A (Hh1a; strain 51449; American Type Culture Collection) was grown at 37°C on blood agar plates containing Campylobacter-selective supplement (skirrow), (Oxoid) under microaerophilic conditions (10% $CO_2$, 10% $H_2$, balance $N_2$) in a vented CampyPak jar (Oxoid). After 2 days of blood agar plates, cultures were harvested using cotton swabs and transferred to liquid culture (Tryptan Soya Broth (Sigma Aldrich) dissolved in 1000 mL MilliQ $H_2O$, and autoclaved) containing 10% FCS + 4 mL Campylobacter-selective supplement (skirrow) (Oxoid). Hh culture was inoculated at 0.05 $OD_{600}$ in a 500 mL vented Erlenmeyer flasks (Corning). Liquid culture was maintained at 37°C shaking at 100 rpm under microaerophilic conditions, and split every 24 hours to 0.05 $OD_{600}$ to maintain stable growth. Hh viability was assessed by labelling with a fluorescent live/dead assay kit (Life Technologies) according to the manufacturer's protocol using a CKX41 fluorescence microscope (Olympus).

### *Helicobacter hepaticus* + αIL10R model of colitis

Mice were infected with 1x10$^8$ colony forming units (cfu) Hh in 200 µL sterile PBS on days 0 and 1 by oral gavage with a 22G curved, blunted needle (Popper & Sons). Mice were injected intraperitoneally once weekly starting on day 0 with 1 mg anti-IL10R blocking antibody (clone 1B1.2) in a volume of 200 µL. Infected mice were monitored weekly for colitis symptoms. Mice were culled by Schedule 1 method three weeks after day of infection, and organs were harvested for analysis.

**Assessment of bacterial colonisation**

Caecal contents were collected after mice were sacrificed. DNA was isolated from faeces using Stool DNA extraction kit (Qiagen) as per manufacturer's instructions. qPCR was performed with primers against the *Hh cdtB* gene using a Viia7 Real-Time PCR system (Applied Biosystems) as described by Maloy *et al.,* (2003) (*58*). In order to construct standard curves, DNA was extracted from Hh cultures using a DNeasy Kit (Qiagen). Primer sequences: *cdt*B Reverse - *TCG TCC AAA ATG CAC AGG TG, cdt*B Forward - *CCG CAA ATT GCA GCA ATA CTT*, *cdt*B Probe - *AAT ATA CGC GCA CAC CTC TCA TCT GAC CAT*.

**qPCR**

For each reaction, 10 ng cDNA was added to 2.5 µL 2X qPCR FAST mastermix (Primerdesign). 0.1 µL primer + Taqman probe mix (Table.M2) was added to each sample, and the reaction volume was topped up to 5 µL with RNase/DNase-free $H_2O$ (Promega). Thermal cycling was carried out using a Viia7 real time PCR system (Applied Biosystems). Thermal cycling: 1x120 s95°C, 40x(5 s 95°C 20 s 60°C).

**Histopathological assessment**

Post-sacrifice, 0.5 cm pieces of caecum, and proximal, mid and distal colon were fixed in PBS + 4% paraformaldehyde (Sigma Aldrich). Fixed tissue was embedded in paraffin blocks, and sectioned using a microtome and stained with Haematoxylin and Eosin (H&E) by the Kennedy Institute of Rheumatology Histology Facility (Kennedy Institute of Rheumatology, University of Oxford).

Sections were scored in a blinded manner by two researchers according to Izcue *et al*., (2008) (*59*).

**Immunofluorescence labelling of colons**

After whole colon excision and longitudinal slicing, colon tissue was washed in PBS, rolled into Swiss rolls, embedded in Optimal Cutting Temperature (OCT) medium (Tissue-Tek), before freezing on dry ice with 2-methylbutane, and stored at -80°C (Bialkowska et al. 2016). 5 µM cryosections were air dried and rehydrated with PBS. The sections were fixed in a 1:1 mixture of methanol:acetone (Merck) and blocked with PBS containing 5% goat serum and 5% mouse serum (blocking solution). Sections were then blocked with biotin/avidin (Invitrogen). Sections were then labelled with primary antibodies (α-F4/80 (recombinant CI:A3-1, Enzo), α-CD11c-biotin (N418, Biolegend), α-CD206 (MR5D3, Bio-rad)) and Sytox blue (Thermo Fisher) performed in blocking solution overnight at 4°C. Secondary antibody labelling was performed with goat α-rat-Alexa Fluor 488, goat α-rabbit-Alexa Fluor 555 and streptavidin-Alexa Fluor 647 (all Thermo Fisher). The sections were stained at RT in the dark for 30 min. Sections were then stained with Hoechst 33342 (Thermo Fisher) for 10min, unless previously stained with Sytox blue overnight. After PBS washes, the tissue was mounted using 5% N-propyl gallate (Merck) in glycerol and imaged using a Zeiss Axio Scope A1 (Zeiss).

**Image analysis and quantification**

The images were analysed using IMARIS (Bitplane). Of each mouse, six individual colon swiss rolls sections were stained in three independent rounds of staining. Within each staining round, intensity thresholds for CD206, CD11c and F4/80 were kept constant. Surfaces were created on F4/80+ cells using the surface tool in Imaris. The size cut-off for

a cell was kept at 6.5 µM constantly. Intensity means for CD11c+ and CD206+ cells were defined on single channel spot analysis and kept as a cut-off standard within one staining cycle. Using an automated Python-script, CD11c+ and CD206+ macrophages were defined within F4/80+ cells as cells that reached the intensity mean threshold for CD206 and CD11c respectively. The number of double-positive cells was counted and quantified as a percentage of total F4/80+ cells. For quantification of macrophage subsets distance to the epithelium, a region of interest within one swiss roll was selected based on tissue architecture where the different regions (epithelium, lamina propria and muscularis) across the depth of the colon were clearly visible. Using the filaments tool, the epithelium and serosal membrane were manually drawn and a channel was created using these filaments. Surfaces were created automatically on these channels, and using the distance transformation tool within the surface statistics, two channels were created that measured the distance of a selected object in relation to their surface. Again, F4/80+ cell surface statistics and defining CD206+ and CD11c+ macrophages were analysed as described above. In addition, each CD206+ and CD11c+ macrophage was set in relation to its minimal distance to the epithelium and the serosal membrane using the distance transformation channels described above. Adding the two minimal distances for each macrophage gives the total distance between the membranes for each point. Distance to membrane was calculated as a percentage of the minimal distance of the respective membrane in relation to the total membrane, allowing correction for the elongation of the epithelium in inflammation.

**Isolation of lamina propria leukocytes**

Colons and/or caeca were harvested from mice, washed in PBS/BSA and content flushed with forceps. Intestines were then opened longitudinally and washed once more before blotting to remove mucus. Gut tissue was then cut into 1 cm long pieces and placed in 50

mL centrifuge tube (Greiner) in ice cold PBS + 0.1% BSA. Colons were incubated 2 times at 200 rpm in 40 mL HBSS + 0.1% BSA + 1% Penicillin-Streptomycin (PS, Lonza) + 5mM EDTA (Sigma-Aldrich) at 37 °C for 10 min before the supernatant was aspirated. Tissue was placed in 40 mL PBS + 0.1% BSA + 1% PS for 5 min. Intestines were then incubated with 20 mL RPMI + 10% FCS +1% PS + 2.5 U/mL Collagenase VIII (Sigma-Aldrich) + 2 U/mL DNAse I (Roche), shaking at 200 rpm for 45 mins - 1 hour at 37 °C. Supernatant was filtered through a 70 µm cell strainer to which 30 mL of ice cold PBS + 0.1% BSA + 1% PS + 5 mM EDTA was added to ablate collagenase/DNase activity. Cells were washed in 30 mL PBS/BSA before filtering once more through a 40 µm cell strainer. The cells were then pelleted by centrifugation at 400 rcf for 10 minutes at 4 °C.

Colonic lamina propria leukocytes (cLPLs) were isolated by resuspending cells in 4 mL P80 (80% P100 (9:1 percoll:10X PBS) + 20% RPMI) percoll in a 15 mL centrifuge tube (Greiner) before overlaying 4 mL P40 (40% P100 + 60% 1X PBS) layer. Cells were spun at 3000 rpm for 20 min at room temperature, slow acceleration, no brake. Mucus and cellular debris were aspirated from the surface of the P40 layer with a Pasteur pipette and pipetted into 40 mL of ice cold PBS + 0.1% BSA. Cells were pelleted by centrifugation at 400 rcf for 10 min and resuspended in 1 mL RPMI + 10% FCS + 1% PS before counting.

**Isolation of blood leukocytes**

Blood was harvested by either tail vein bleed or cardiac puncture. Mice were culled by Schedule 1 method in accordance with the project licence. Prior to cardiac puncture a 1 mL syringe was coated with PBS + 2 mM EDTA. Cardiac puncture was performed with a 27G needle. Tail vein bleeds were performed using a #24 blade scalpel (Swann-Morton Ltd.) Collected blood was placed in 1 mL of sterile 2 mM EDTA/PBS solution in a 15 mL centrifuge tube (Greiner). Tubes were topped up with ice-cold PBS + 0.1% BSA. Cells were pelleted

by centrifugation at 400 rcf for 10 mins at 4 °C and the supernatant discarded. Erythrocytes were then lysed using 10-20X the blood sample volume of ACK lysis buffer (Gibco) for 3 mins. Tubes were then topped up to 15 mL with ice cold PBS + 0.1% BSA to quench the lysis buffer. Cells were washed in PBS/BSA, resuspended in PBS + 0.1% BSA to the desired cell concentration, and stored at 4 °C until required.

**Bone marrow isolation**

Whole hind legs of mice were harvested and stored at 4 °C until processing. Processing was carried out in a class II lamina flow hood. Femurs and tibia muscle tissue was removed using scissors, followed by desiccation in 70% ethanol solution for 3 min. Remaining muscle was cleaned from the bones using a lint-free tissue. Scissors were used to cut the ends of bones. A 27G needle was inserted into the opening, and marrow was flushed into a 50 mL centrifuge tube using 10 mL of sterile, ice cold PBS. Cells were then filtered through a 70 µm cell strainer (Greiner). Red blood cells were lysed with ACK as described above. Cells were resuspended in PBS + 0.1% BSA at 4 °C until required.

**Monocyte isolation**

Splenocytes were prepared as described above. Monocytes were enriched by negative selection using an EasySep™ Mouse Monocyte Isolation Kit (StemCell) according to the manufacturer's instructions. Cells were then resuspended in ice cold PBS + 0.1% BSA for counting and downstream processing.

**T cell restimulation**

A minimum of 1 x$10^6$ freshly isolated cLPLs were incubated in V-bottomed 96 well plates (Corning) in 250 µL Iscove's Modified Dulbecco's Medium (Gibco) supplemented with 10% FCS + 1% P/S + 0.1 µM phorbol 12-myristate 13-acetate (PMA, Sigma Aldrich) + 1µM Ionomycin (Sigma Alrich) + 10 µg/mL GolgiPlug (BD) for 4 hrs at 37 °C. Cells were washed 3 x in 150 µL FACS buffer before extracellular and intracellular staining.

**Flow cytometry**

CompBeads (BD) were used to prepare single stained controls as per manufacturer's instructions to set up fluorophore compensation. Labelled cells were acquired using either an LSR II (BD), or Fortessa X20 (BD) flow cytometer using FACSDiva (BD). Data were analysed using Flowjo (Treestar, Inc.) software.

**Extracellular labelling of cells**

5x$10^5$ - 2x$10^6$ cells were plated on either V-bottomed or U-bottomed 96 well plates. The cells were washed twice with 150 µL FACS buffer (PBS + 0.1 % BSA + 1 mM EDTA + 0.01% Sodium Azide) at 400 rcf for 3 min 4°C. Cells were then Fc blocked for 10 min with αCD16/CD32 (BD) 1/100 in 20 µL FACS buffer at room temperature (RT) followed by washing once in 150 µL FACS buffer. Fixable Viability Dye eFluor®780 (ThermoFisher) and primary extracellular antibodies (**Table. 1**) were added for 20 min at 4 °C in 20 µL FACS buffer in the dark. Labelled cells were then washed twice with 150 µL FACS buffer. Cells were then fixed for 30 mins in 50 µL Cytofix (BD), washed twice with 150 µL FACS buffer, and resuspended in 200 µL FACS buffer before acquisition. For assessment of apoptosis, the eBioscience™ Annexin V Apoptosis Detection Kit APC (ThermoFisher) was used according to manufacturer's instructions in combination with extracellular labelling and fixable viability dye.

## Intracellular labelling of cytokines

Intracellular labelling of cytokines from mouse colons was performed immediately after isolation without further stimulation. Surface markers were labelled as described above. For Cytokine labelling, cells were fixed in 50 µL Cytofix/Cytoperm (BD), washed twice in 150 µL Perm/Wash buffer (BD) at 600 rcf, 4 °C. Intracellular antibodies (**Table. 2**) were incubated in 20 µL Perm/Wash for 20 mins at 4 °C in the dark, after which samples were washed twice in 150 µL Perm/Wash, and once in 150 µL FACS buffer before resuspension in 200 µL FACS buffer for acquisition.

## Intracellular labelling of IRF5

Intracellular labelling of IRF5 was performed immediately after isolation without further stimulation. Surface markers were labelled as described above. For nuclear staining, cells were fixed in 50 µL Fix/Perm (eBioscience) according to manufacturer's instructions, washed twice in 150 µL Perm buffer (eBioscience) at 600 rcf, 4 °C. Intracellular antibodies (**Table. 2**) were incubated in 20 µL Perm for 20 mins at 4 °C, protected from light. Next, samples were washed twice in 150 µL Perm, and once in 150 µL FACS buffer before resuspension in 200 µL FACS buffer for acquisition.

## Cell sorting

Cells were stained with extracellular cytokines as described above, except no fixation step was performed. Cells were then sorted using a FACSAria™ II (BD) at the Kennedy Institute of Rheumatology FACS facility.

**Generation and analysis of “small bulk” RNA-sequencing data from MBMC**

For “small bulk” RNA-sequencing 100 cell samples were sorted through a 100 µm diameter nozzle into 2 µL of lysis buffer and amplified cDNA prepared using the Smart-seq2 protocol (*60*). Libraries were prepared using Nextera XT kits (Illumina) and sequenced to a mean depth of 17M read pairs (Illumina HiSeq 4000). Sequence reads were aligned to the mouse genome with Hisat2 (version 2.1.0) using a “genome_trans” index built from the mm10 release of the mouse genome and Ensembl version 91 annotations (two-pass strategy to discover novel splice sites; with parameters: --dta and --score-min L,0.0,-0.2) (*61*). Mapped reads were counted using featureCounts (Subread version 1.6.3; Ensembl version 91 annotations; default parameters) (*62*). TPMs were estimated with salmon (version 0.11.3, with parameter "--gcBias") using a quasi index built from the full set of Ensembl version 91 transcriptome annotations (parameters "--k=31 --keepDuplicates") (*63*). The median alignment rate was 83.8% (assessed with Picard tools v2.10.9, https://github.com/broadinstitute/picard).

For each of the GSEA analyses genes detected in a least one of the biological replicates (n=3) were pre-ranked by p-value for differential expression (Deseq2, paired design, local fit for dispersion, no independent filtering). Enrichment of GO biological processes (org.Mm.ed.db R package version 3.7.0; with ≥5 and ≤500 genes) was tested using fgsea (*64*) (version 1.8.0). Changes in gene expression across the wildtype monocyte waterfall were identified using DESeq2 (likelihood ratio test, modelling animal and cell-type). Differential expression analyses were performed using DESeq2 (paired tests, local dispersion fit).

**Generation and pre-processing of single-cell RNA-sequencing data**

The inflamed cLP small-bulk RNA-seq and scRNA-seq data were generated from a common set of MBMCs (n=3, cells pooled from all animals for scRNA-seq analysis). Subsequently, in a separate experiment, the steady state blood and cLP scRNA-seq datasets were generated from a second set of MBMCs (n=5, cells pooled from all animals for both analyses). Single-cell RNA-sequencing libraries were generated using the 10x Genomics Single Cell 3' Solution (inflamed colon dataset: version 2; steady state colon and PBMC datasets: version 3) kit and sequenced (inflamed colon dataset: Illumina HiSeq 4000 with an average depth of >200k read per cell; steady state colon and PBMC datasets: NovaSeq 6000 with an average depth of >50k reads per cell). Data analysis was performed using Python3 pipelines (https://github.com/sansomlab/tenx) written using CGAT-core[75]. Read mapping, quantitation and aggregation of sample count matrices was performed with the 10x Genomics Cell Ranger pipeline (inflamed colon dataset: version 2.1.1; steady state datasets: version 3.1.0). For the "cellranger count" step, a custom reference was built using Ensembl annotations (version 91) that included genes with protein coding, lincRNA, macro_lincRNA, immune (IG_*, TR_*), antisense_RNA, and miRNA Ensembl (version 91) biotypes. Within each dataset, *Irf5*$^{-/-}$ and wildtype samples were aggregated. No normalisation was applied during the aggregation step. Cells with barcodes common to more than one sample from the same sequencing batch were removed from the analysis to avoid issues associated with index hopping. For each of the three experiments, the aggregated count matrices were randomly down-sampled in order to normalise the median number of UMIs per-cell between the *Irf5*$^{-/-}$ and wildtype samples ("downsampleMatrix" function from the DropletUtils R package) and genes detected in less than 3 cells removed.

For the inflamed colon dataset, cells with < 500 genes, > 30k UMIs, < 0.3% or > 7.5% mitochondrial UMIs or that were identified as contaminating B cells (n=38) were removed. For the steady state colon dataset, cells with < 1k genes, > 25k UMIs, > 7.5% mitochondrial UMIs, identified as contaminating B, T or stromal cells (n=75), or with high expression of

interferon-induced genes (n=34) were removed. For the steady state PBMC dataset, cells with < 500 genes, > 20k UMIs, > 5% mitochondrial UMIs, with high expression of haemoglobin genes (n=8) or identified as contaminating T cells (n=694) were removed. For each of the datasets either the WT or Irf5$^{-/-}$cells were randomly down sampled to retain an equal number of cells per genotype.

**Analysis of single-cell RNA-sequencing data from the inflamed cLP**

Per-cell UMI counts were normalised, scaled and variation associated with total UMI counts, percentage of mitochondrial counts and cell cycle (all effects; using known G2 and S phase associated genes (*66*)) regressed out with the Seurat R package (version 2.3.4). Significantly variable genes were selected using the "trendVar" function from the R Bioconductor package Scran (minimum mean log-expression 0.05, BH adjusted p-value < 0.05). These genes were used as input for principal component analysis (PCA), and significant PCs (n=30) identified using Seurat ("JackStraw" test, BH adjusted p < 0.05). Graph-based clustering of the significant PCs was performed using Seurat ("original" Louvain algorithm, resolution=1.1). Significant cluster markers conserved between the genotypes were identified by intersecting the results of separate tests for cluster markers within each genotype (Seurat "Findmarkers" function, Wilcoxon tests, BH adjusted p value < 0.05). The UMAP projection was computed using all of the significant PCs ("RunUMAP" function, Seurat). Significant differentially expressed genes between genotype within cluster were identified using the "FindMarkers" function (Wilcoxon tests, BH adjusted p value < 0.05, fold change > 1.5).

For pseudo-time analysis the data was subset to cells identified as Monocytes or Macrophages. Significant variable genes and PCs (n=20) and clusters (resolution=1.2) were recomputed as above. The diffusion map (Figure 6a) was constructed from all of the significant PCs using the R Destiny package[77]. The R package Slingshot (version 1.2.0) was used to fit a minimum spanning tree to the full diffusion map and to infer the global lineage

structure ("*Ly6c2* mono" cluster specified as the root state) [45]. Slingshot was used to construct smoothed curves along the lineages and to compute pseudo-time values for each cell.

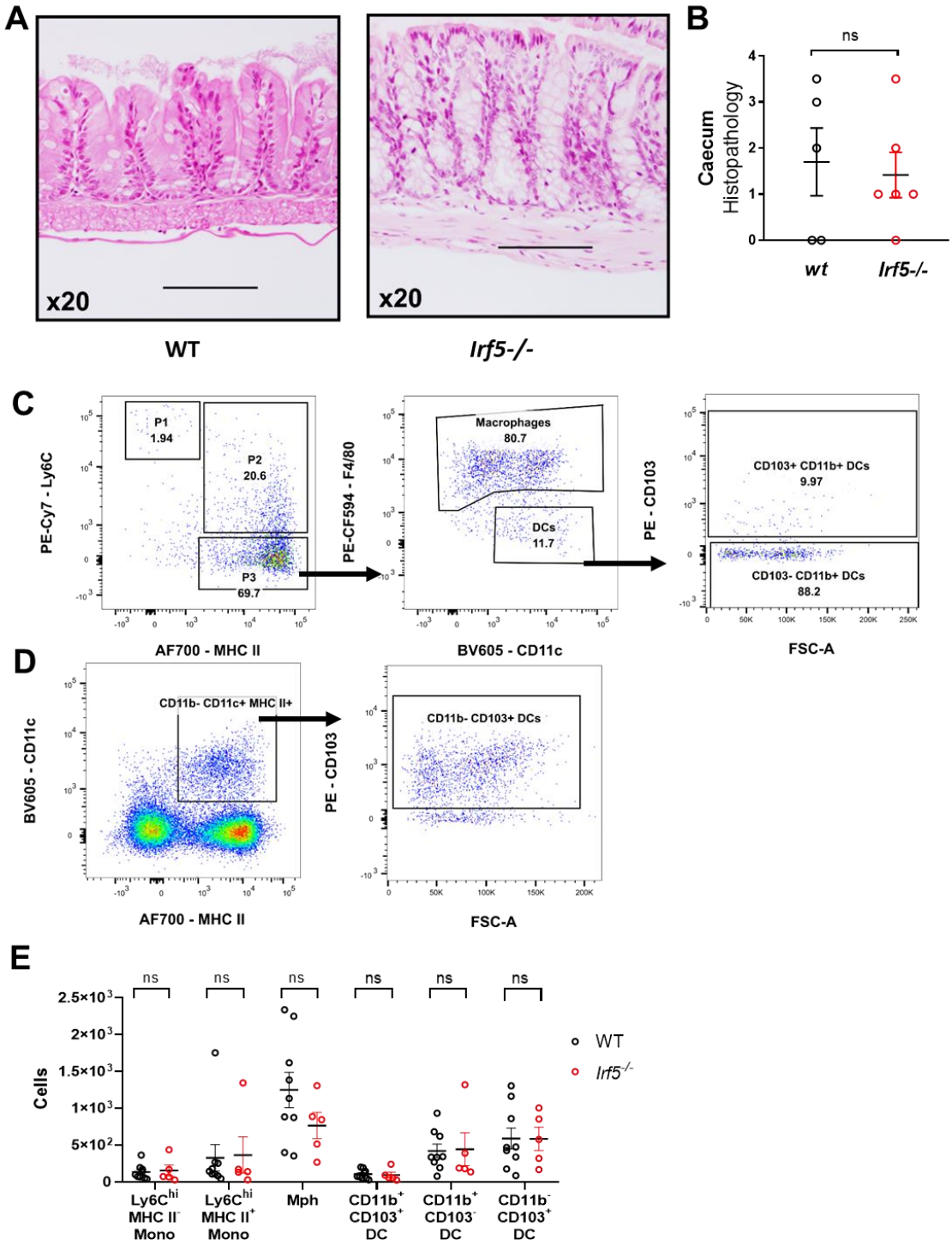
**Analysis of steady state blood and cLP single-cell RNA-sequencing datasets**

These datasets were analysed using Seurat version 3.1.1. Normalisation was performed with sctransform (default parameters), and variation associated with mitochondrial UMI percentage or cell cycle (all effects; using known G2 and S phase associated genes[76]) regressed out. Principal components were calculated using the top 3,000 variable features. Clustering was performed with the Leiden algorithm (cLP dataset: k=30, n=30 top PCs, resolution=1; PBMC dataset: k=30, n=15 PCs, resolution=0.25). Significant cluster markers and genes differentially expressed between genotype within cluster were identified as described for the inflamed cLP dataset.

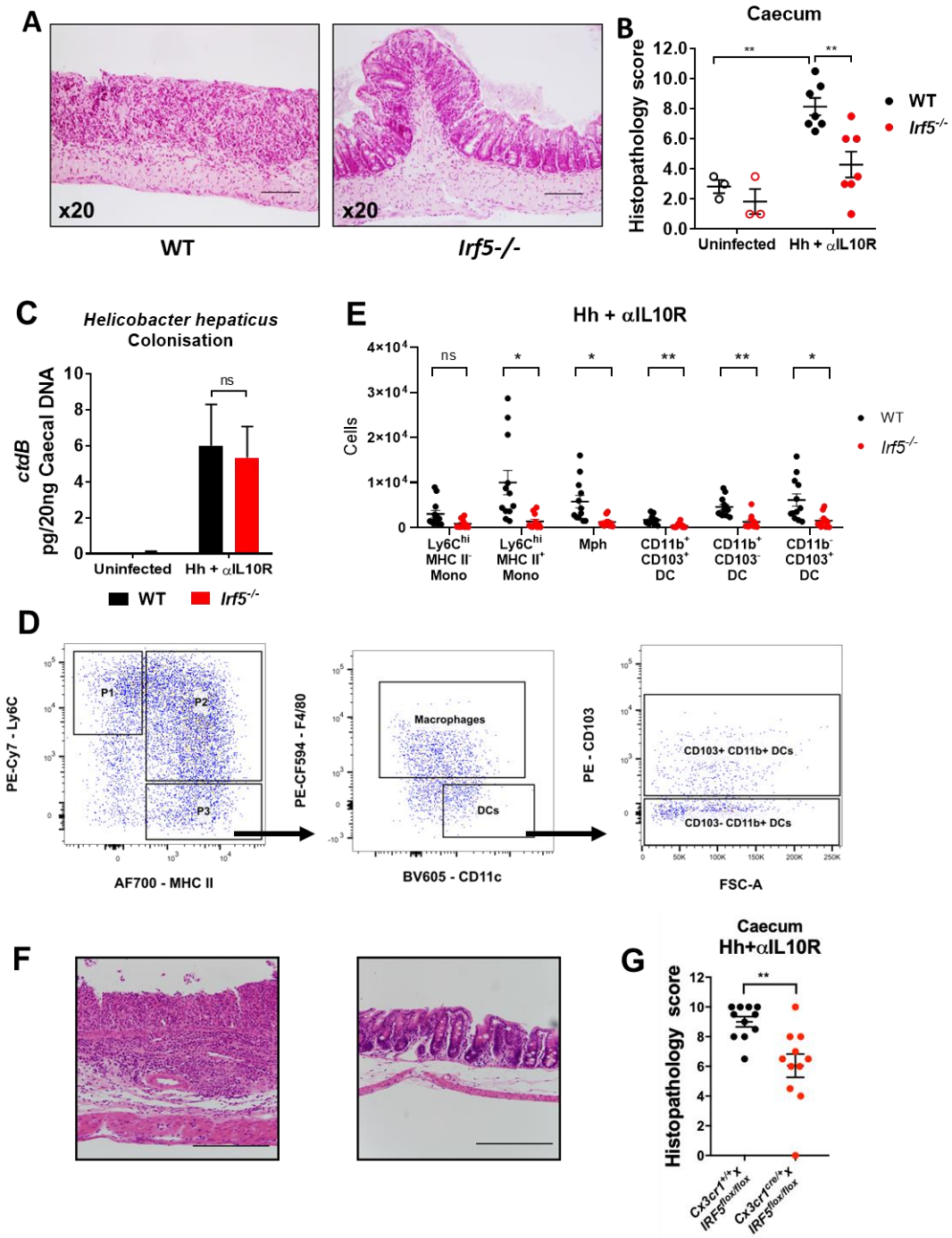
**Statistical analysis**

Data were analysed using Prism V.7 (GraphPad). Statistical tests were performed as indicated in figure legends. Two-sided testing was used in all instances unless indicated. Differences were considered statistically significant when $p \leq 0.05$.

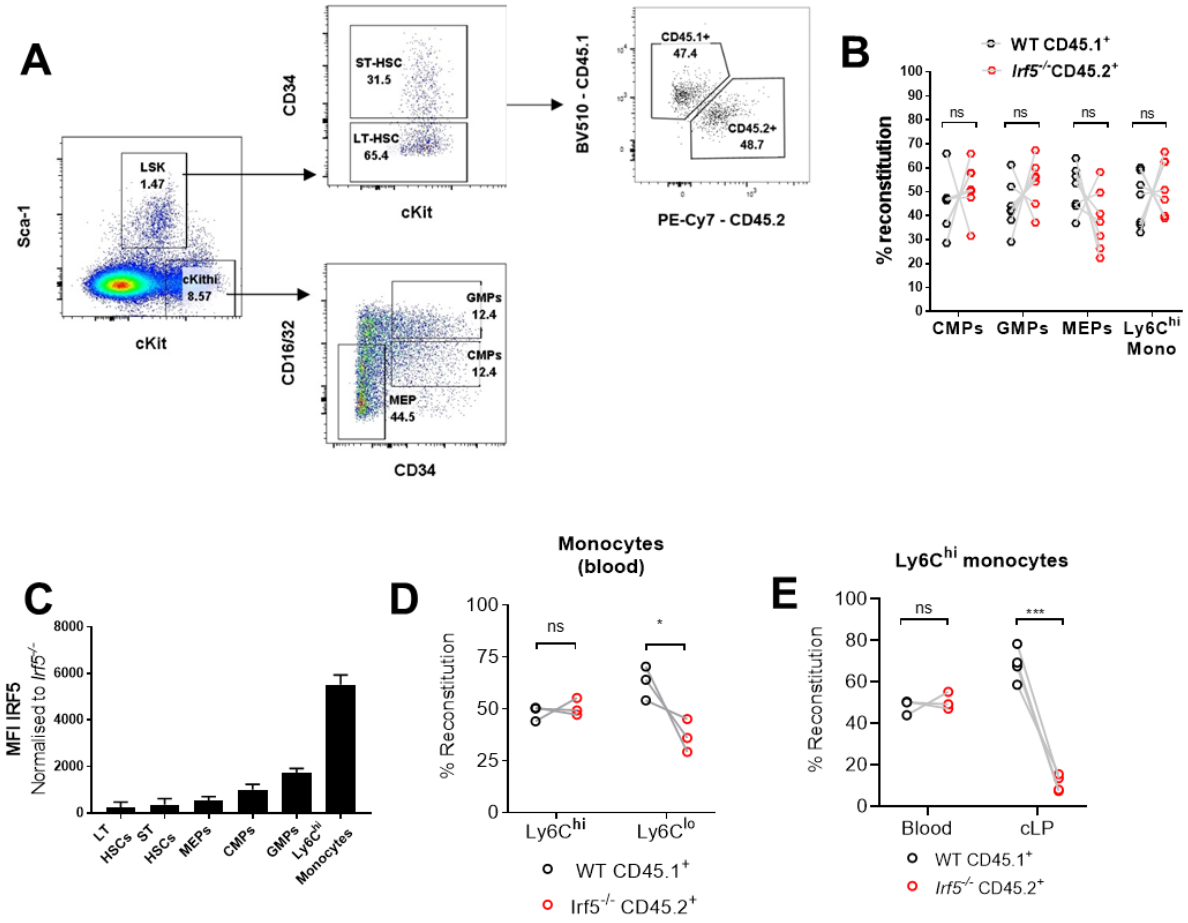
## Supplementary Figures and Tables



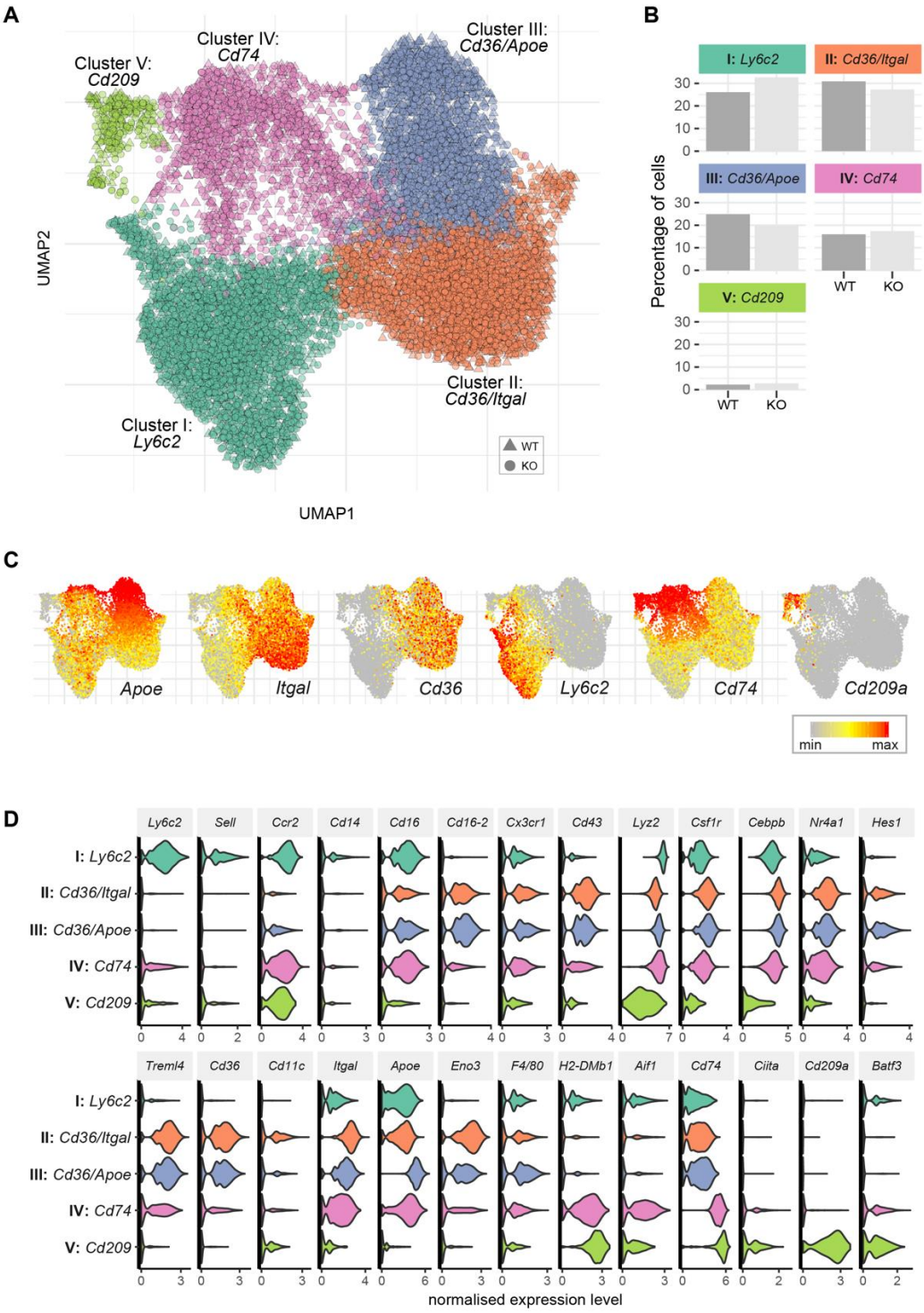

**Supplementary Figure S1: IRF5 deficiency and caecum physiology at steady state. A)** H&E sections of WT (n=5) and *Irf5*$^{-/-}$ (n=5) caecum at steady state. **B)** Histopathology scoring of steady state caeca. **B, C)** Mann-Whitney U test **C)** Gating strategy to identify P1 monocytes, P2 monocytes, macrophages, and CD11b$^{+}$ CD103$^{+}$ DCs and CD11b$^{+}$ CD103$^{-}$ DCs representative of steady state mice. **D)** Gating strategy for CD11b$^{-}$ CD103$^{+}$ DCs. **C, D)** Gating done on LiveCD45$^{+}$Dump$^{-}$ CD11b$^{+}$Ly6G$^{-}$SiglecF and was based on FMO controls. **E)** The absolute number of intestinal MNPs in the cLP of steady state WT (n=9) and *Irf5*$^{-/-}$ (n=5) mice. Two-Way ANOVA with Sidak correction. **B, C, E)** Mann-Whitney U test. Data presented are mean ± SEM, ns = not significant.



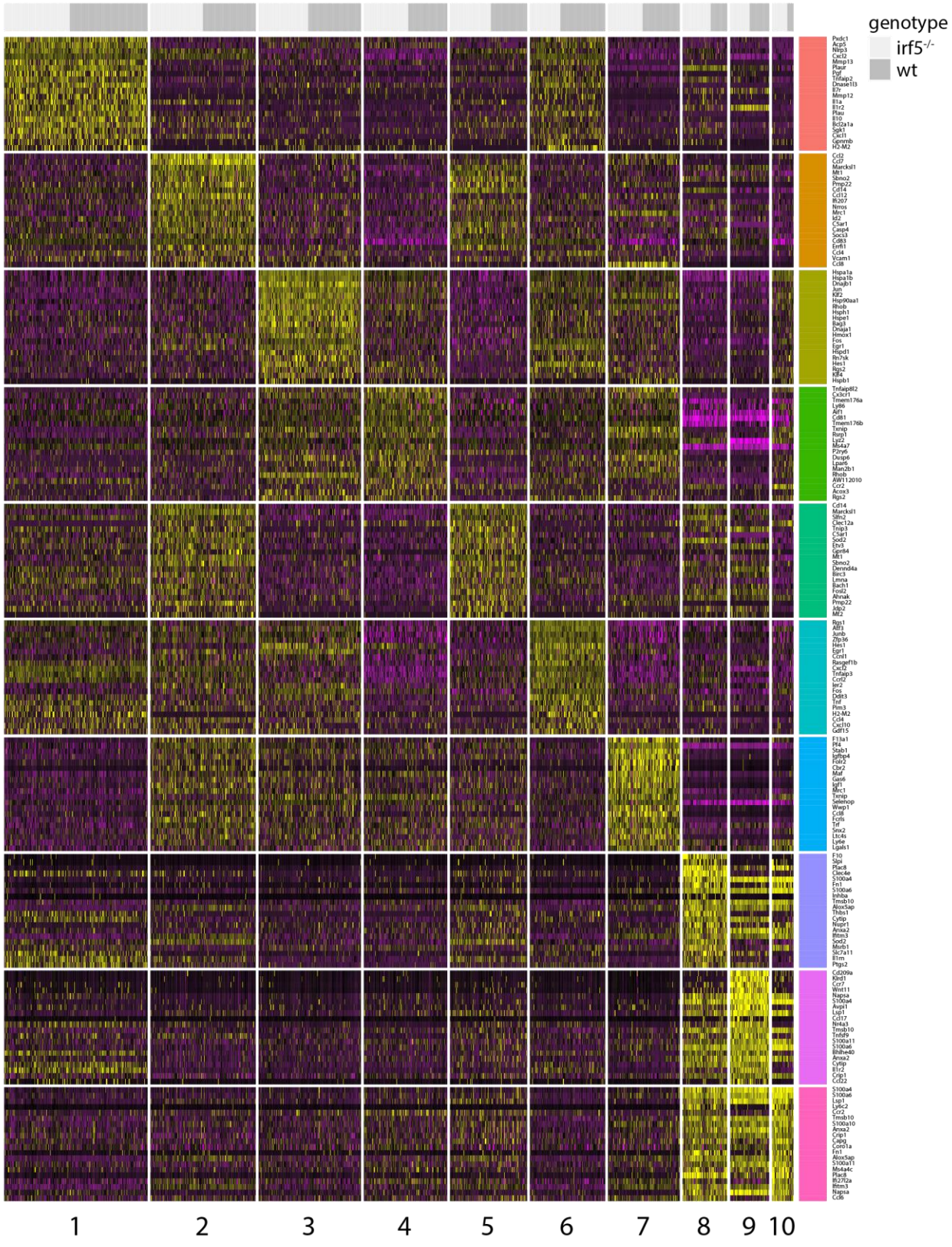

**Supplementary Figure S2: IRF5 deficiency and caecum in inflammation. A)** Representative of two independent experiments H&E-stained sections of WT and *Irf5*$^{-/-}$ caecum at d21 Hh + αIL10R colitis. **B)** Histopathology scoring of inflamed caeca (WT ss n= 3 and Hh n =7, *Irf5*$^{-/-}$ ss n= 3 and Hh n =7). **C)** Quantification of *Helicobacter hepaticus* load at d21 colitis (WT ss n= 3 and Hh n =7, *Irf5*$^{-/-}$ ss n= 3 and Hh n =7. **D)** Gating strategy to identify P1 monocytes, P2 monocytes, macrophages, and CD11b$^{+}$ CD103$^{+}$ DCs and CD11b$^{+}$ CD103$^{-}$ DCs representative of d21 Hh + αIL10R inflamed mice. Gating done on LiveCD45$^{+}$Dump$^{-}$CD11b$^{+}$Ly6G$^{-}$SiglecF and was based on FMO controls. **E)** The absolute number of intestinal MNPs in the cLP of WT (n=12) and *Irf5*$^{-/-}$ (n=11) mice at d21 colitis. Data are pooled from two independent experiments. Two-Way ANOVA with Sidak correction. **F)** Representative H&E stained sections of CX$_3$CR1$^{IRF5+}$ or CX$_3$CR1$^{IRF5-}$ caeca at d21 colitis. **G)** Histopathology scoring of CX$_3$CR1$^{IRF5+}$ and CX$_3$CR1$^{IRF5-}$ mice at d21 Hh + αIL10R colitis (WT n=12, *Irf5*$^{-/-}$ n=12). Data presented are mean ± SEM, ns = not significant, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p < 0.0001$



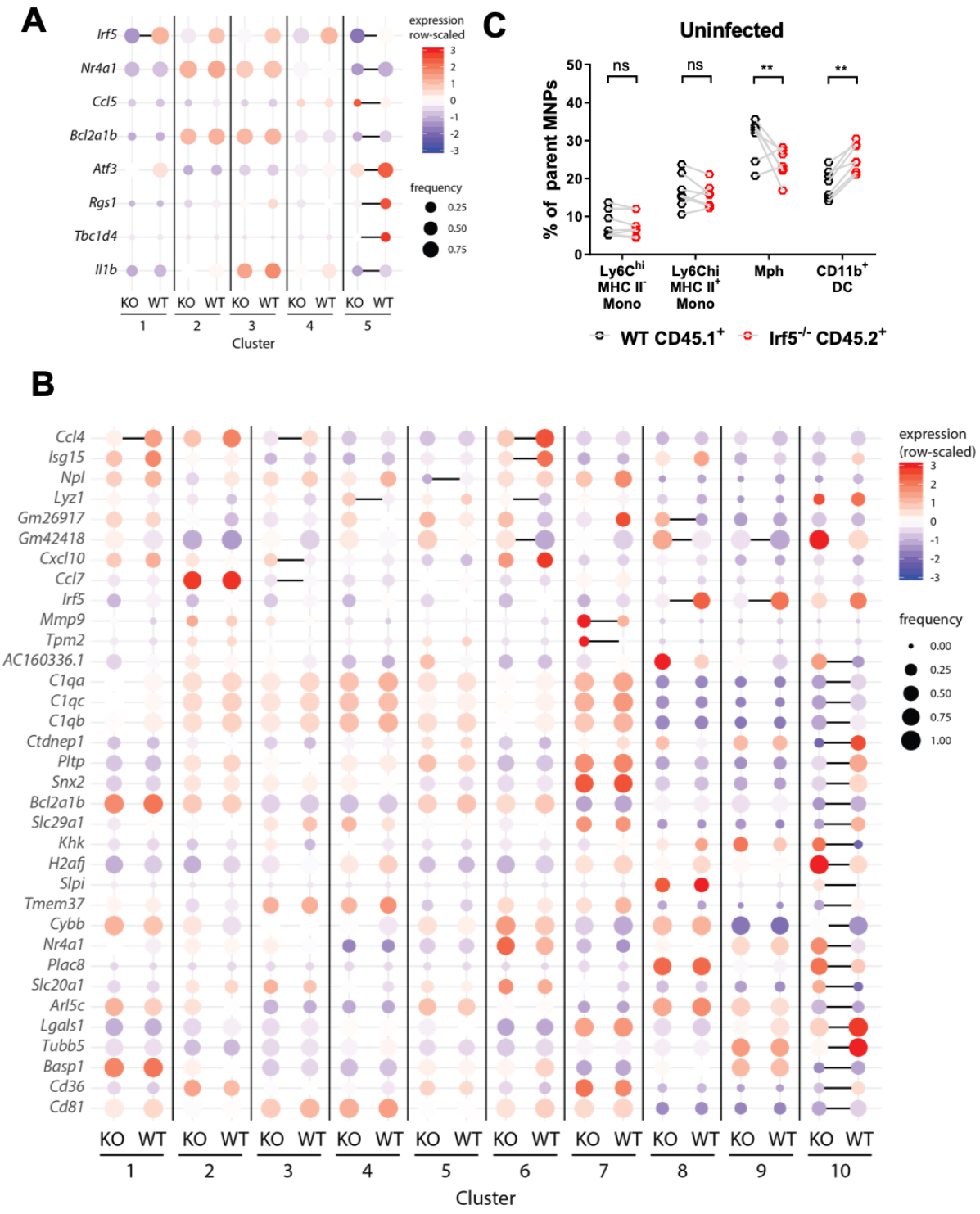

**Supplementary Figure S3: MBMC: monocyte development in the bone marrow and blood. A)** Gating strategy to identify HSPCs in MBMC. **B)** Quantification of HSPC reconstitution (n=7). **C)** IRF5 expression in the bone marrow quantified by intracellular flow cytometry staining. **D)** Reconstitution of blood Ly6C$^{hi}$ and Ly6C$^{lo}$ monocytes (n=3). **E)** Reconstitution of blood and cLP Ly6C$^{hi}$ monocytes (n=3-5).



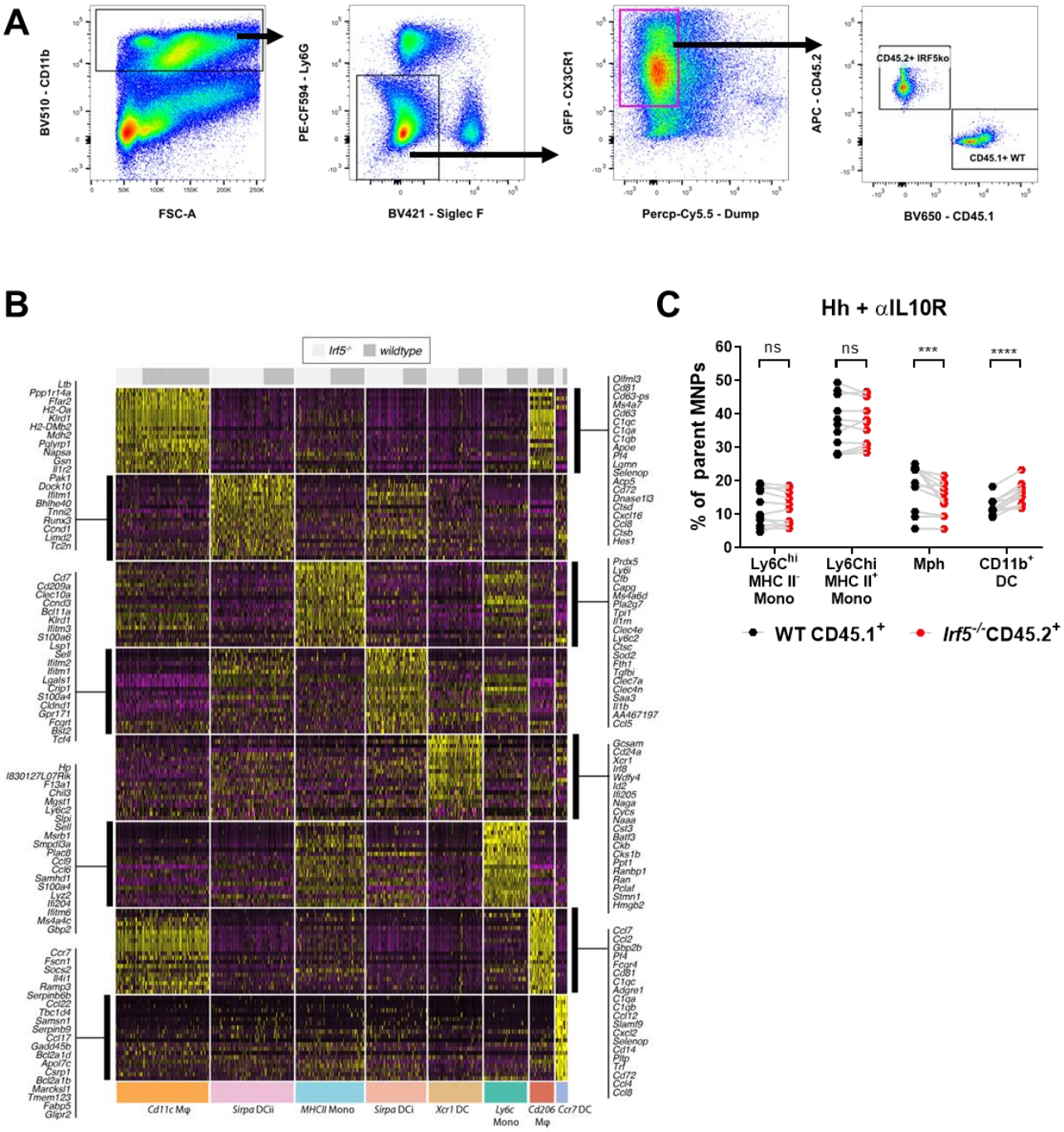

**Supplementary Figure S4: scRNA-seq: monocyte development in blood**. CD45$^{+}$CD11b$^{+}$ SiglecF$^{-}$Ly6G$^{-}$CX$_3$CR1$^{+}$Dump- cells were sorted from the blood of five MBMC animals and subjected to droplet-based single cell transcriptomic analysis. **A)** Graph based clustering[76] of equal numbers of WT and *Irf5*$^{-/-}$ cells (n=12528 total) identified five monocyte clusters. **B)** The bar plots show the percentages of WT and *Irf5*$^{-/-}$ cells that were found in each cluster. **C)** The expression of selected genes that mark the identified populations. **D)** The violin plots show the expression levels (x axes) of selected markers of monocyte sub-populations in each of the identified clusters (y axes).



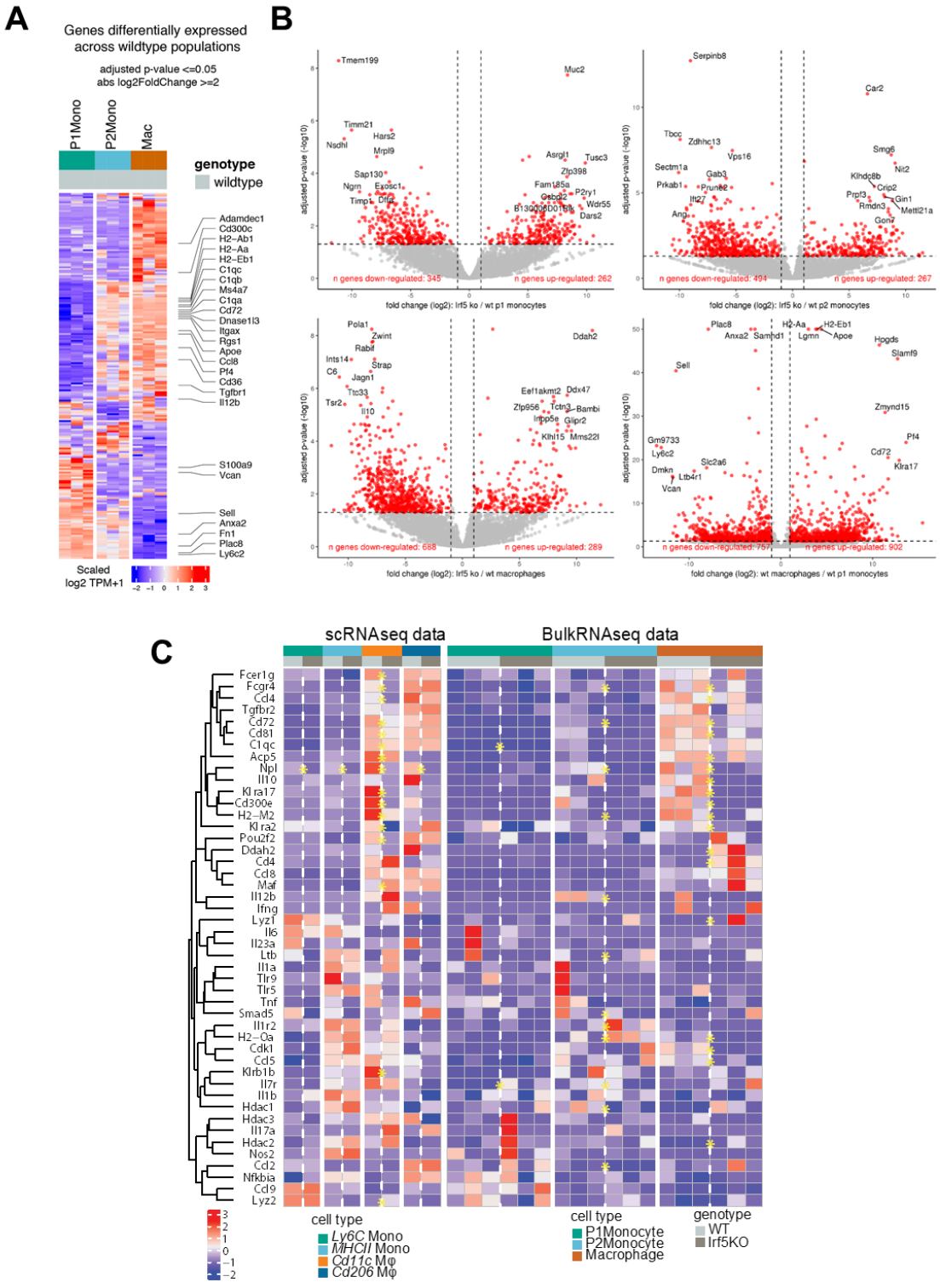

**Supplementary Figure S5: scRNA-seq: MNP populations in cLP at steady state.** The heatmap shows the expression of the top significant conserved cluster marker genes from the MBMC single-cell RNA-sequencing experiment (**Fig 3**) (Seurat analysis, Wilcoxon tests, BH adjusted $p < 0.05$ in separate tests of cells of both genotypes). scRNA-seq: experiment performed once, pool of MNPs isolated from cLP of 5 MBMCs.



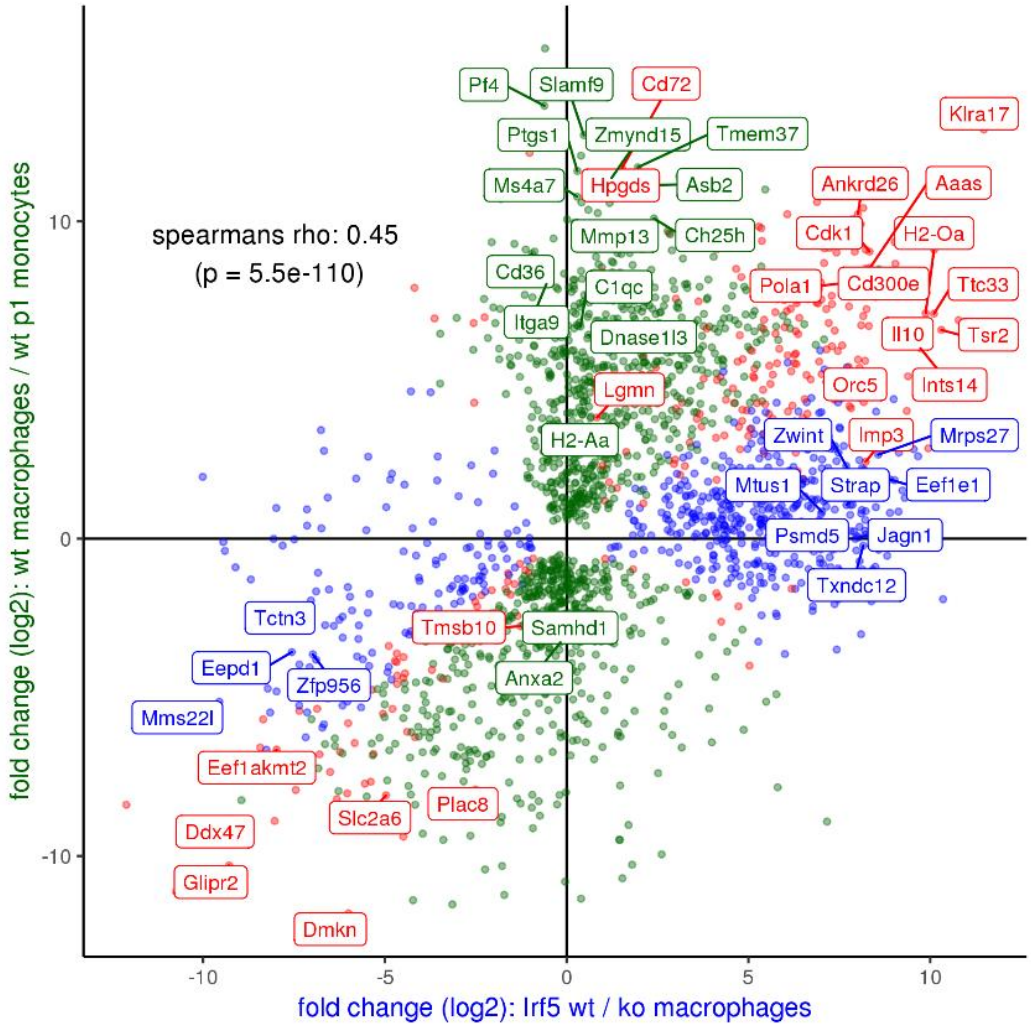

**Supplementary Figure S6: scRNA-seq: IRF5 effect on blood monocytes and cLP MNPs at steady state**. The split dot plots show within cluster differential expression between steady state WT and *Irf5*$^{-/-}$ **A)** blood monocytes (see **Supplementary Fig S4**) and **B)** cLP MNP (see **Fig 4**). Significant differences between the genotypes (|fc| > 1.5, BH adjusted $p < 0.05$, Wilcoxon tests) are indicated by solid black lines. All genes found to be significantly differentially expressed in at least one cluster are shown. Dot size is proportional to the frequency of gene detection in the single cells. Dot colour is proportional to the (row-scaled) expression level of the gene. **C)** The relative compositions of the MNP compartment of WT- and *Irf5*$^{-/-}$-donor derived cLPLs were analysed by flow cytometry in uninfected MBMCs. Data are pooled from two independent experiments. Two-Way ANOVA with Sidak correction. Data presented are mean ± SEM, ns = not significant, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p < 0.0001$.



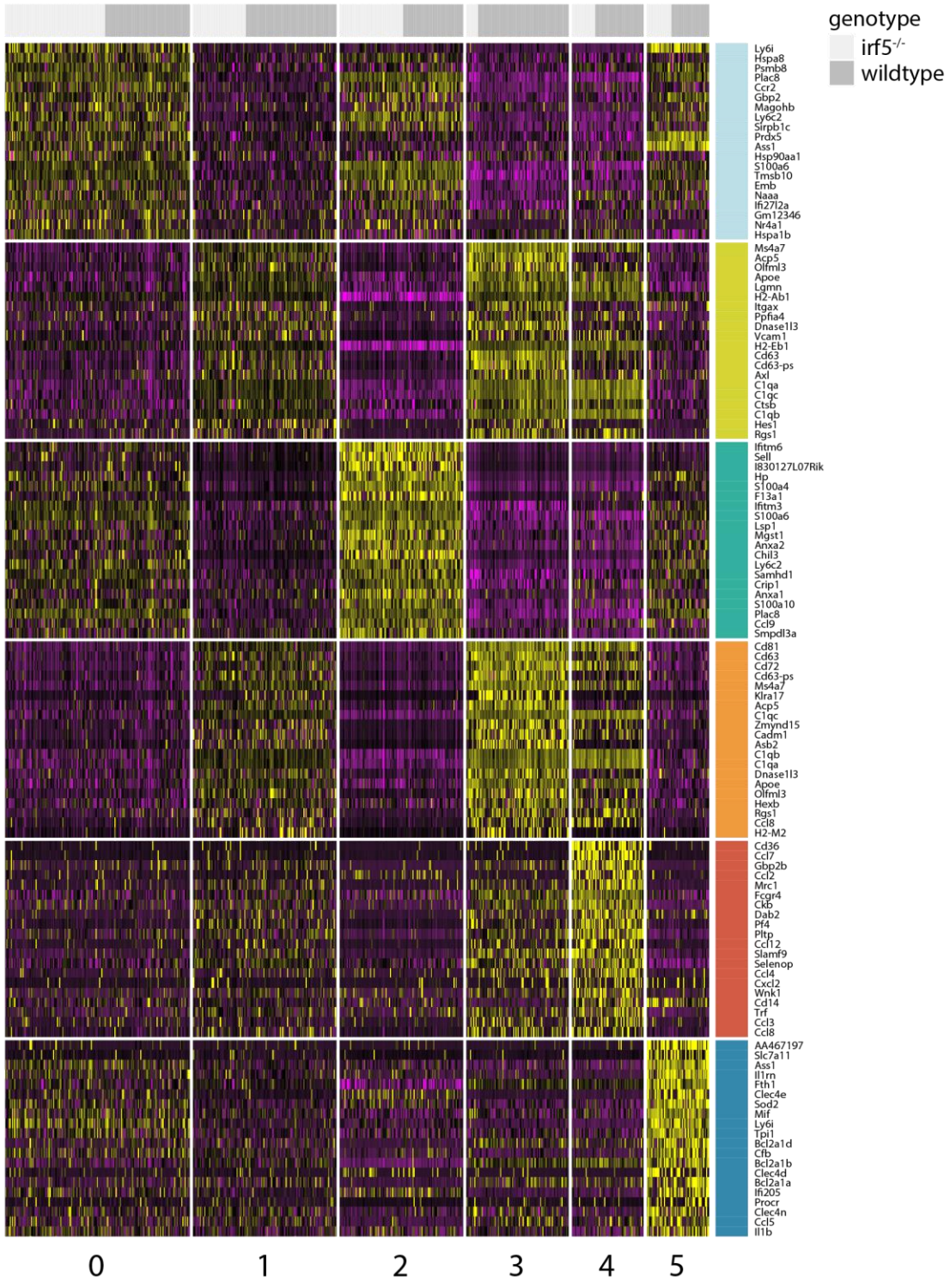

**Supplementary Figure S7: scRNA-seq: MNP populations in inflamed colon. A)** Representative gating strategy for sorting $CX_3CR1^+$ MNPs from MBMCs, gated on singlets, Live, $CD45^+$. **B)** The heatmap shows the expression of the top significant conserved cluster marker genes from the MBMC single-cell RNA-sequencing experiment (Seurat analysis, Wilcoxon tests, BH adjusted $p < 0.05$ in separate tests of cells of both genotypes). scRNAseq: experiment performed once, pool of MNPs isolated from cLP of 3 MBMCs at d21 Hh + αIL10R colitis. **C)** The relative compositions of the MNP compartment of WT- and *Irf5*$^{-/-}$-donor derived cLPLs were analysed by flow cytometry in MBMCs at d21 Hh + αIL10R colitis. Data are pooled from two independent experiments. Two-Way ANOVA with Sidak correction. Data presented are mean ± SEM, ns = not significant, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p < 0.0001$.



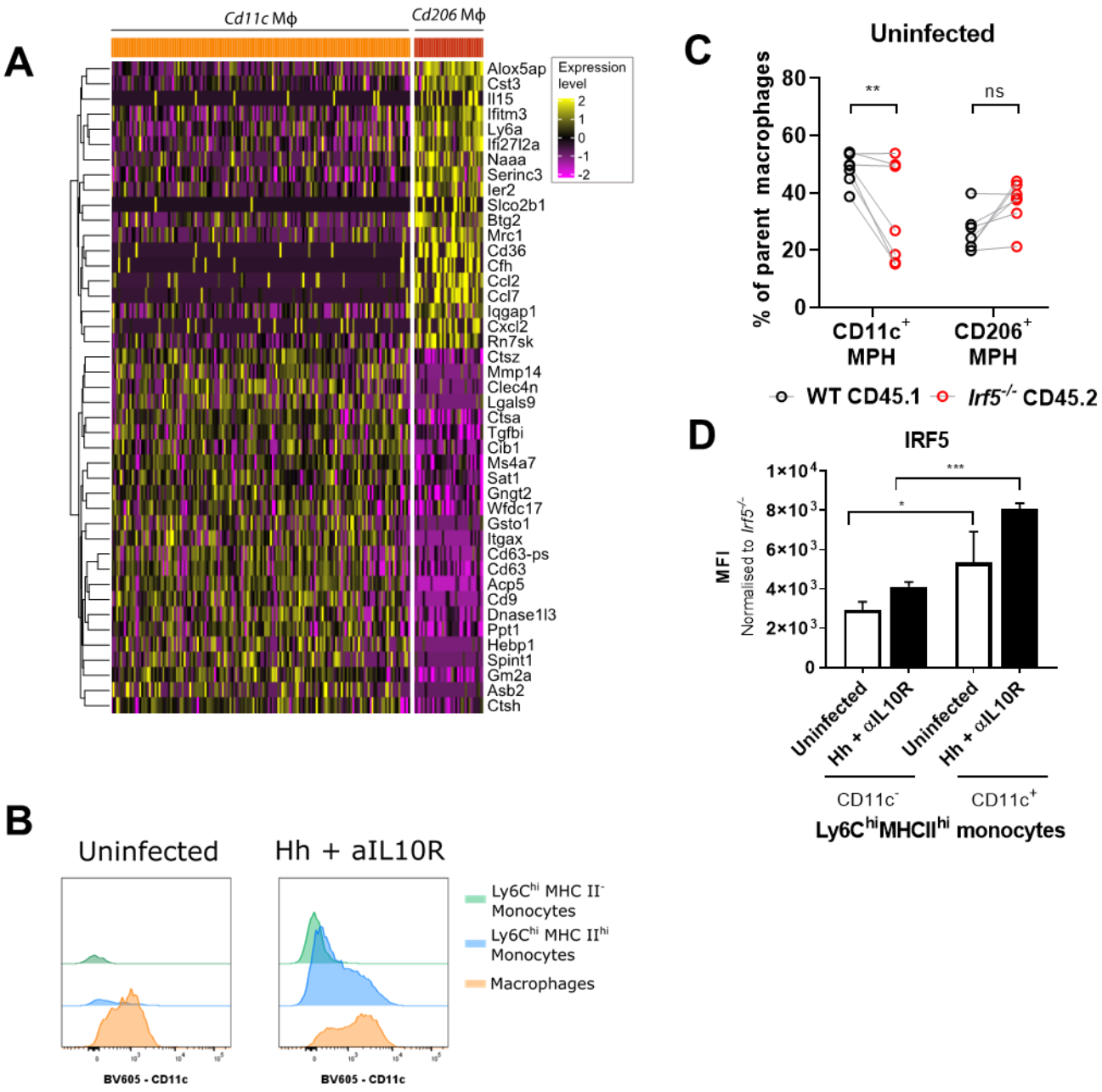

**Supplementary Figure S8: scRNA-seq and bulk RNA-seq: comparison of IRF5 dependent genes.** Small-bulk RNA-seq analysis of MNP populations at d21 Hh + αIL10R colitis (experiment performed once, n=3 chimaeras)**. A)** The heatmap shows the expression of genes with significant variation in expression between WT P1 monocytes, WT P2 monocytes and WT macrophages isolated from the inflamed cLP of the MBMCs (DESeq2, LRT test, BH adjusted $p < 0.05$). **B)** The volcano plots show genes found to be significantly differentially expressed (red dots, separate DESeq2 analyses, BH adjusted $p < 0.05$, $|fc| > 2$) between WT vs *Irf5*$^{-/-}$ P1 monocytes (top left), WT vs *Irf5*$^{-/-}$ P2 monocytes (top right), WT vs *Irf5*$^{-/-}$ macrophages (bottom left), and between WT P1 monocytes vs WT macrophages (bottom right). **C)** The heatmap shows expression of genes found to be significantly differentially expressed (yellow stars, BH adjusted $p < 0.05$) between WT and *Irf5*$^{-/-}$ MNPs in scRNA-seq (Wilcoxon tests) or small bulk RNA-seq (DESeq2 analyses) datasets.




**Supplementary Figure S9: IRF5 in monocyte to macrophage differentiation.** Comparison of genes differentially expressed in WT vs *Irf5*$^{-/-}$ macrophages with those differentially expressed between WT P1 monocytes and WT macrophages isolated from d21 Hh + αIL10R MBMCs. Genes are coloured to indicate significant differential expression in both comparisons (red dots), in only WT vs *Irf5*$^{-/-}$ macrophages (blue dots) or in only WT P1 monocytes vs WT macrophages (green dots) (DESeq2 analyses, BH adjusted $p < 0.05$, see **Supplementary Fig. S6b**).




**Supplementary Figure S10: scRNA-seq: monocyte and macrophage populations in inflamed colon.** The heatmap shows the expression of the top significant marker genes for the clusters shown in **Fig 6** (Seurat analysis, Wilcoxon tests, BH adjusted $p < 0.05$).




**Supplementary Figure S11: scRNA-seq: gene expression in *Cd11c* vs *Cd206* macrophages.** **A)** Heatmap of expression of selected genes in *Cd11c* and *Cd206* macrophages from the inflamed cLP of the MBMCs (see **Fig 5**). All of the genes shown were found to be differentially expressed between the *Cd11c* and *Cd206* clusters (Wilcoxon tests, BH adjusted $p < 0.05$). **B)** Cd11c protein surface expression levels on MNP populations. Representative histograms from 9 steady state and 12 d21 *Hh* + αIL10R experiments. **C)** The frequency of parent WT and *Irf5-/-* macrophages expressing CD11c or CD206 at steady state. Data from two independent experiments**. D)** IRF5 expression in $CD11c^+$ vs $CD11c^-$ Ly6ChiMHCII+ monocytes in MBMC assessed by intracellular flow cytometry. One representative experiment, uninfected n=3, Hh + αIL10R n=4.

**Table.1: List of antibodies used for surface staining**

| Epitope | Colour | Clone | Manufacturer | Cat # | Dilution |
|---|---|---|---|---|---|
| Annexin V | APC | N/A | Biolegend | 640919 | 1/20 |
| B220 | PerCP Cy5.5 | RA3-6B2 | eBio | 45-0452-82 | 1/200 |
| CD3 | PerCP Cy5.5 | 145-2C11 | Biolegend | 100328 | 1/200 |
| CD4 | BV605 | RM4-5 | Biolegend | 100547 | 1/400 |
| CD11b | V500 | M1/70 | BD | 562128 | 1/200 |
| CD11b | BV510 | M1/70 | Biolegend | 101245 | 1/200 |
| CD11c | BV605 | N418 | Biolegend | 117333 | 1/200 |
| CD11c | BV785 | N418 | Biolegend | 117335 | 1/200 |
| CD11c | PerCP-Cy5.5 | N418 | ThermoFisher | 45-0114-82 | 1/200 |
| CD16/32 | APC | 93 | eBioscience | 17-0161-82 | 1/200 |
| CD19 | Percp Cy5.5 | 6D5 | Biolegend | 115533 | 1/200 |
| CD31 | PE | MEC13.3 | Biolegend | 102507 | 1/200 |
| CD34 | FITC | RAM34 | BD | 553733 | 1/200 |
| CD34 | Biotin | RAM34 | ThermoFisher | 13-0341-82 | 1/200 |
| CD45 | V500 | 30-F11 | BD | 561487 | 1/400 |
| CD45 | BV650 | 30-F11 | BD | 563410 | 1/200 |
| CD45.1 | APC | A20 | eBioscience | 17-0453-82 | 1/200 |
| CD45.1 | BV650 | A20 | Biolegend | 110735 | 1/200 |
| CD45.1 | PE | A20 | eBio | 12-0453-82 | 1/200 |
| CD45.1 | PE Cy7 | A20 | eBioscience | 25-0453-82 | 1/200 |
| CD45.2 | AF700 | 104 | Biolegend | 109822 | 1/200 |
| CD45.2 | FITC | 104 | BD Pharmingen | 553772 | 1/200 |
| CD45.2 | PE | 104 | eBio | 12-0454-83 | 1/200 |
| CD45.2 | PE Cy7 | 104 | Biolegend | 109830 | 1/200 |
| CD45.2 | PerCP Cy5.5 | 104 | eBio | 45-0454-82 | 1/200 |
| CD64 | PE | X54-5/7.1 | Biolegend | 139303 | 1/100 |
| CD103 | PE | 2E7 | eBioscience | 12-1031-82 | 1/100 |
| CD138 | Percp Cy5.5 | 281-2 | Biolegend | 142509 | 1/200 |
| CD206 | APC | CO68L2 | Biolegend | 141708 | 1/100 |
| cKit | PB | 2B8 | Biolegend | 105820 | 1/200 |
| F4/80 | APC | BM8 | eBio | 17-4801-82 | 1/200 |
| F4/80 | PerCP-Cy5.5 | BM8 | eBioscience | 45-4801-82 | 1/100 |
| F4/80 | PE | BM8 | Biolegend | 123109 | 1/200 |
| F4/80 | PE/Dazzle 594 | BM8 | Biolegend | 123145 | 1/200 |
| FcεRIα | Percp Cy5.5 | MAR-1 | BioLegend | 134319 | 1/200 |
| Gp38 | PE-Cy7 | 8.1.1 | Biolegend | 127411 | 1/300 |
| IL-7Ra | PerCP Cy5.5 | A7R34 | Biolegend | 135021 | 1/200 |
| Ly6C | PE-Cy7 | HK1.4 | Biolegend | 128017 | 1/200 |
| Ly6C | BV785 | HK1.4 | Biolegend | 128041 | 1/300 |
| Ly6G | PerCP Cy5.5 | 1A8 | Biolegend | 127615 | 1/200 |
| Ly6G | PE-CF594 | 1A8 | BD | 562700 | 1/200 |
| MHC II | AF700 | M5/114.15.2 | eBioscience | 56-5321-80 | 1/200 |
| NK1.1 | PerCP Cy5.5 | PK136 | eBioscience | 45-5941-82 | 1/200 |
| Sca-1 | AF700 | D7 | eBio | 56-5981-82 | 1/200 |

| | | | | | |
|---|---|---|---|---|---|
| Siglec F | BV421 | E50-2440 | BD | 562681 | 1/200 |
| Siglec F | PE | E50-2440 | BD | 552126 | 1/200 |
| Streptavidin | BV605 | N/A | Biolegend | 405229 | 1/300 |
| TCRb | PerCP-Cy5.5 | H57-597 | eBioscience | 45-5961-82 | 1/200 |
| TCRb | AF700 | H57-597 | Biolegend | 109223 | 1/400 |
| Ter119 | PerCP-Cy5.5 | TER-119 | Biolegend | 116227 | 1/200 |

**Table.2: List of antibodies used for intracellular staining**

| Epitope | Colour | Clone | Manufacturer | Cat # | Dilution |
|---|---|---|---|---|---|
| Foxp3 | PE-eFluor 610 | FJK-15S | ThermoFisher | 61-5773-82 | 1/200 |
| Goat anti- rabbit (secondary ab) | AF488 | Polyclonal | Life Technologies | A-11008 | 1/300 |
| Il12p4/p70 | PE | C15.6 | BD | 562038 | 1/100 |
| IL-17A | FITC | 17B7 | eBioscience | 11-7177-81 | 1/200 |
| IFNg | APC | XMG1.2 | eBioscience | 17-7311-81 | 1/200 |
| IRF5 | N/A | Rabbit polyclonal | abcam | 21689 | 1/100 |
| Live/Dead | eF780 | N/A | ThermoFisher | 65-0865-14 | 1/1000 |
| TNFα | Pacific Blue | MP6-XT22 | Biolegend | 506318 | 1/100 |
| Pro-IL-1b | PE | NJTEN3 | ebioscience | 12F114-80 | 1/100 |