

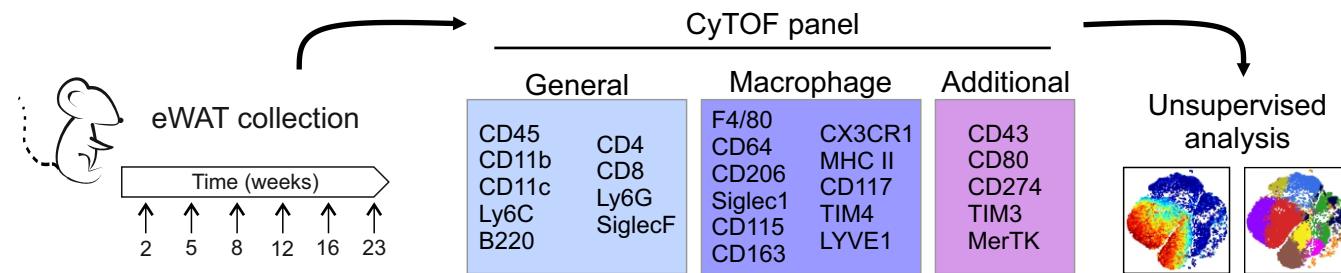
*Supplementary Material for*

**Single-cell proteomics reveals the defined heterogeneity of resident macrophages in white adipose tissue, Félix *et al.***

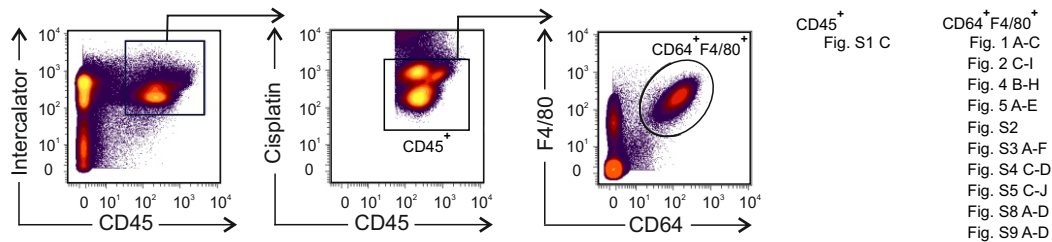


The Supplementary Material for this article includes 10 Supplementary Figures and 1 Supplementary Table.

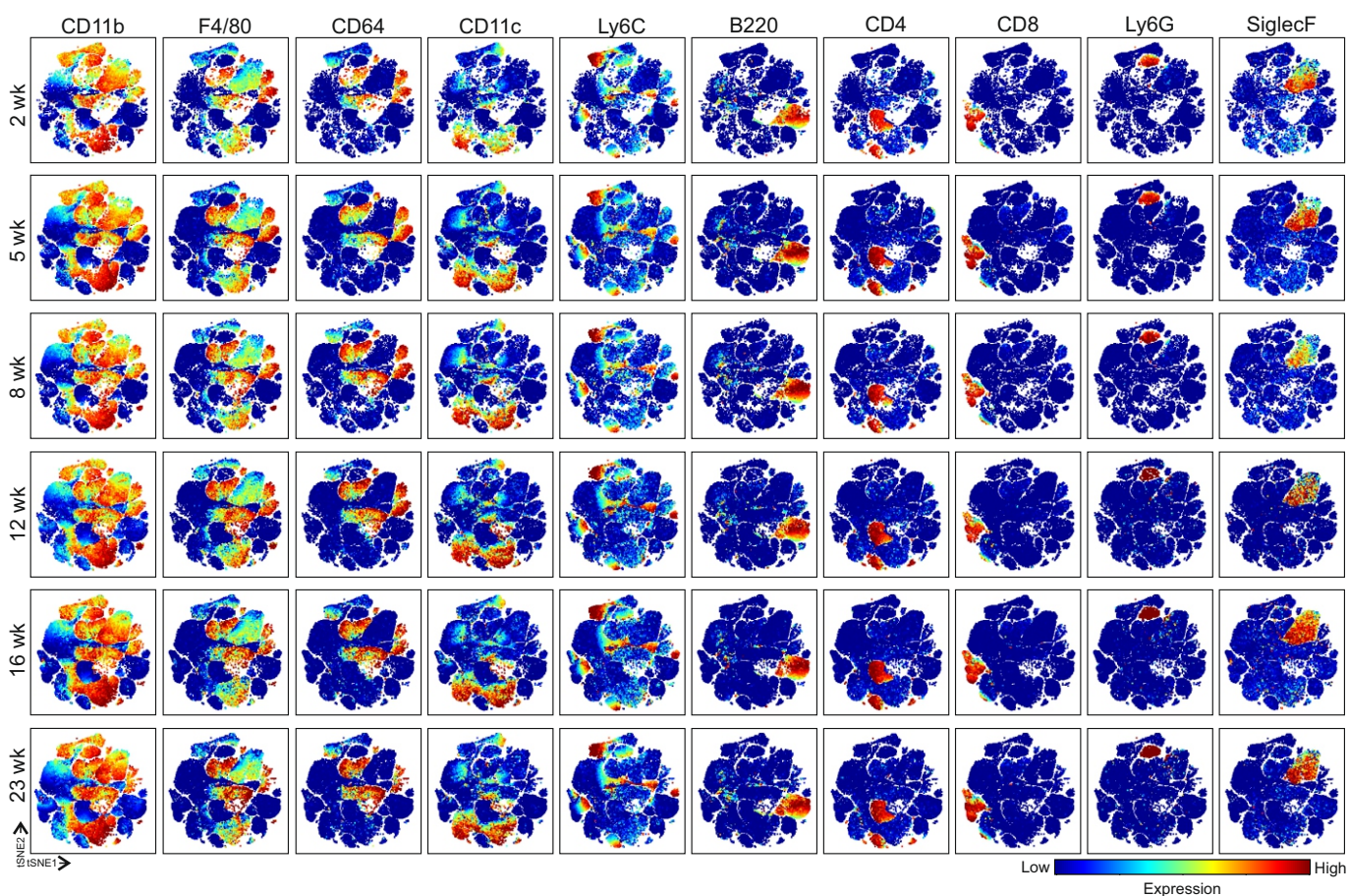
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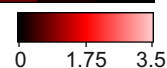
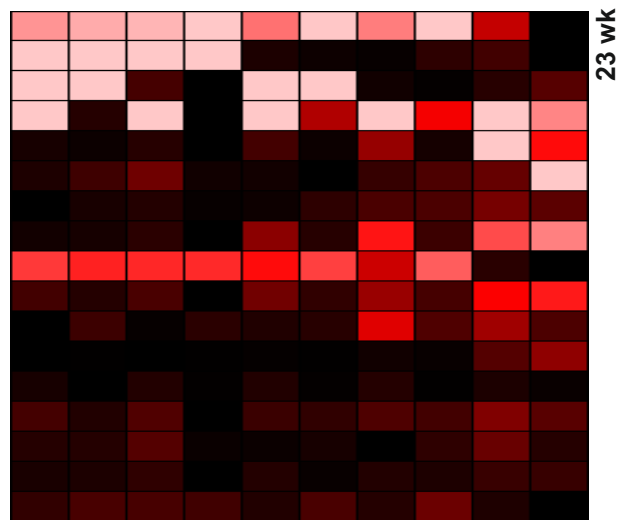
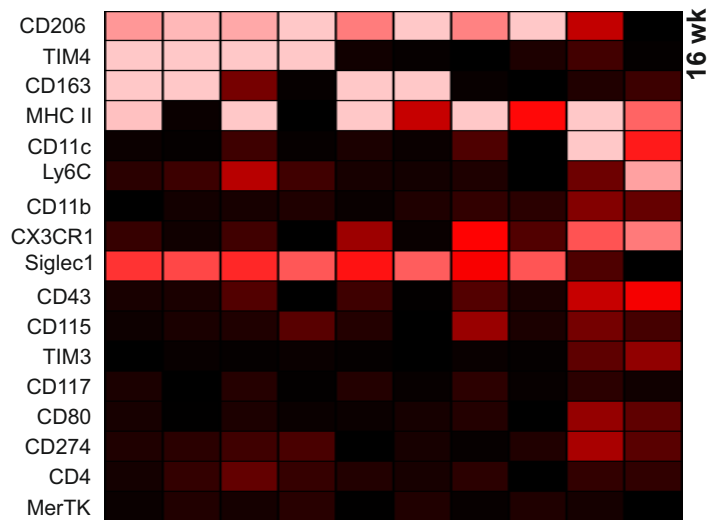
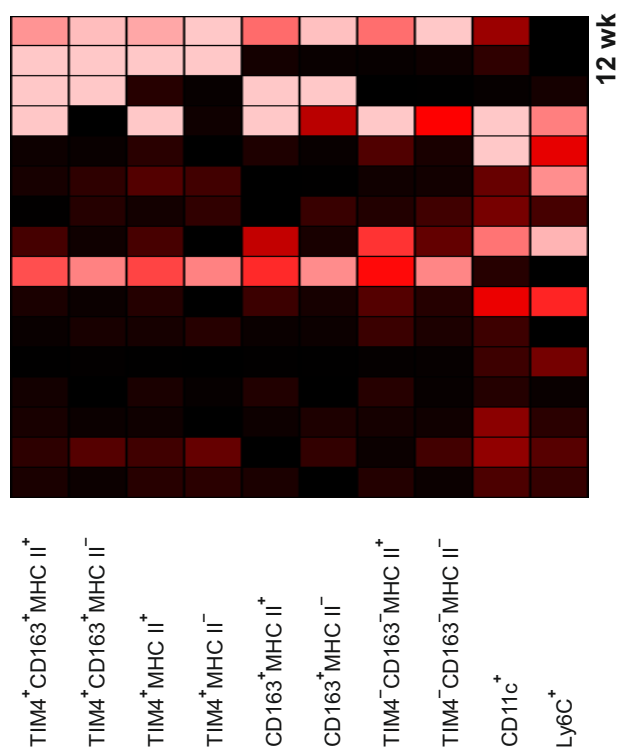
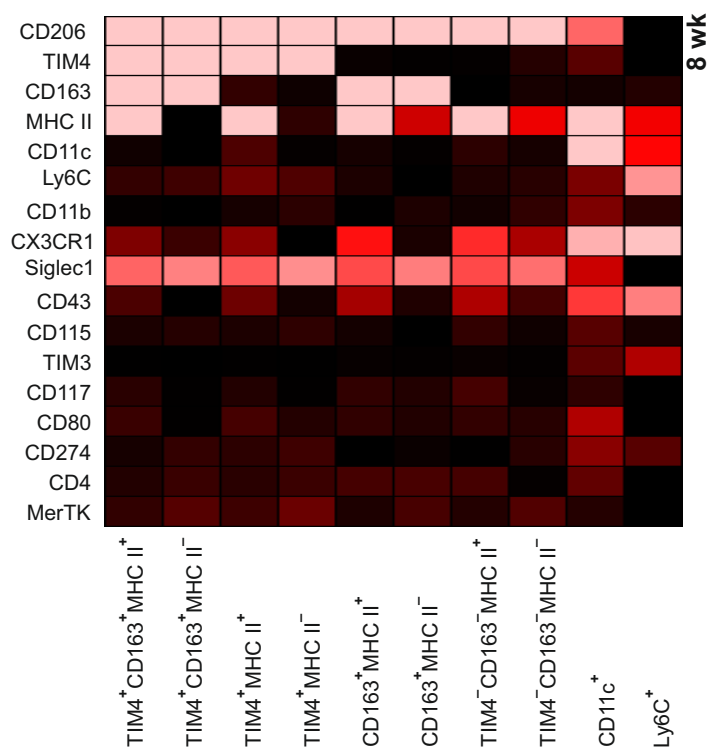
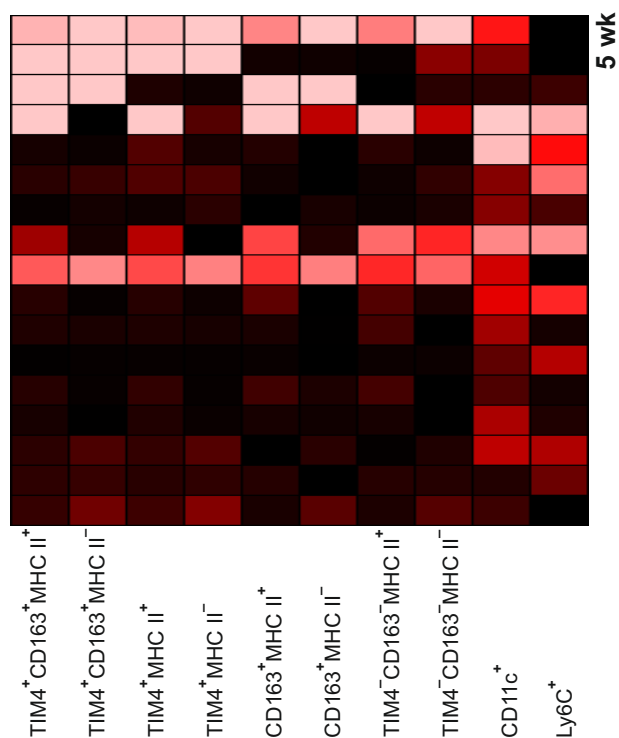
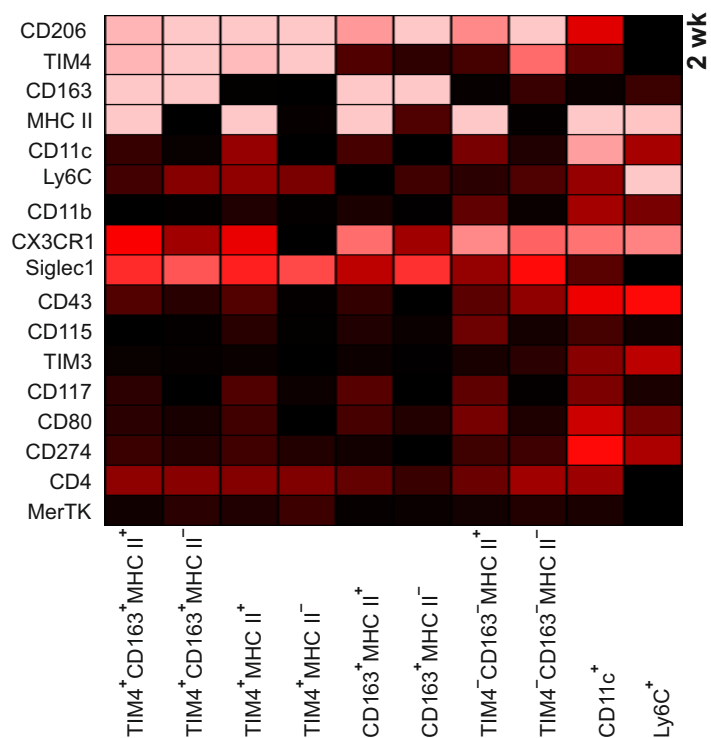
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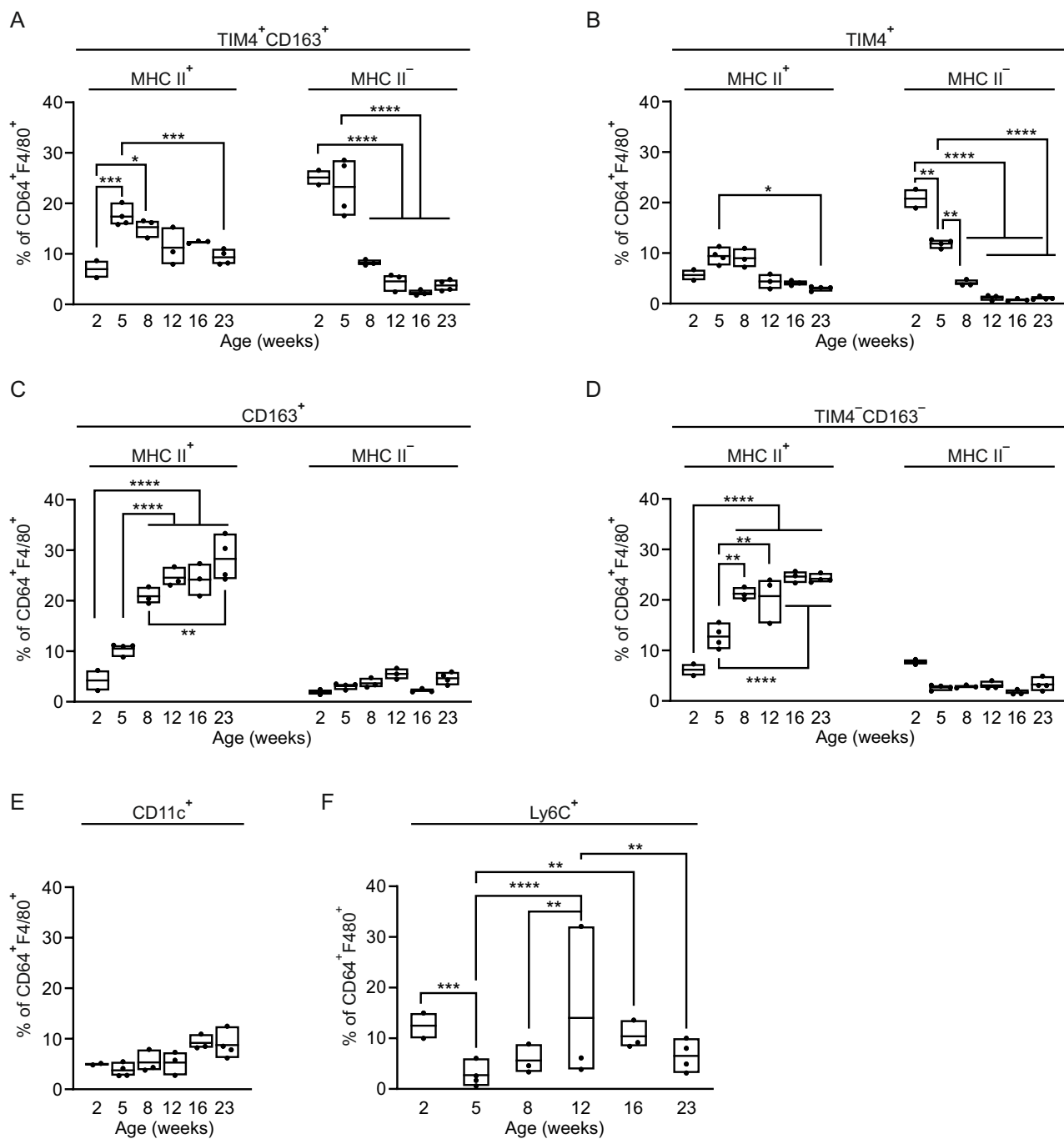
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**Supplementary Figure 1. (A)** Schematic diagram for the experimental design and workflow. **(B)** Representative gating strategy of the mass cytometric data for eWAT single (Intercalation<sup>+</sup>) live (Cisplatin<sup>-</sup>) CD45<sup>+</sup> leukocytes and CD45<sup>+</sup>CD64<sup>+</sup>F4/80<sup>+</sup> macrophages. **(C)** Representative viSNE plots of CD45<sup>+</sup> leukocytes at the indicated time points from wild type (WT) eWAT. Color code indicates the expression level of a given marker from low (blue) to high (red).

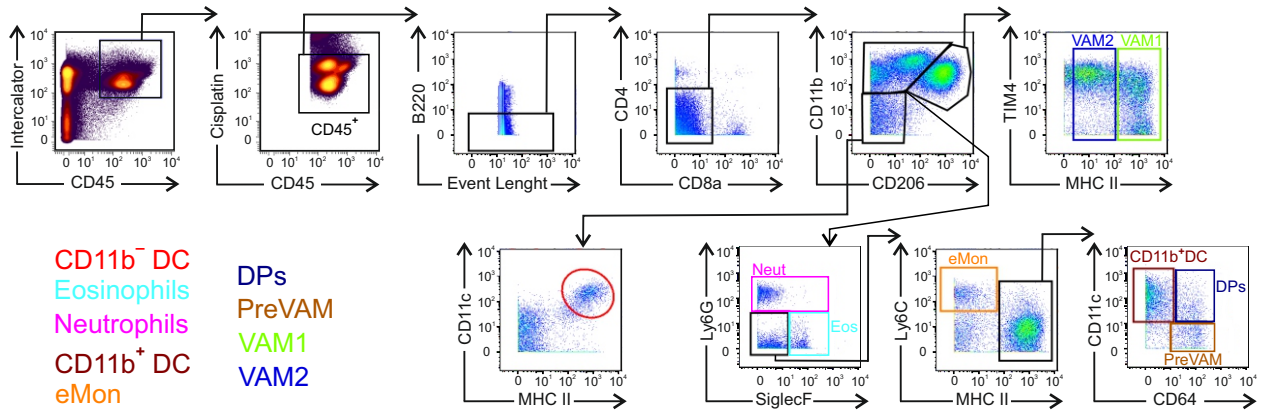


**Supplementary Figure 2.** Representative heat map analyses showing the mean expression of the indicated markers in FlowSOM metaclusters (identified in Figure 1B) in wild type (WT) eWAT at 2, 5, 8, 12, 16, and 23 weeks of age. Color code indicates the expression level of a given marker from low (black) to high (white).

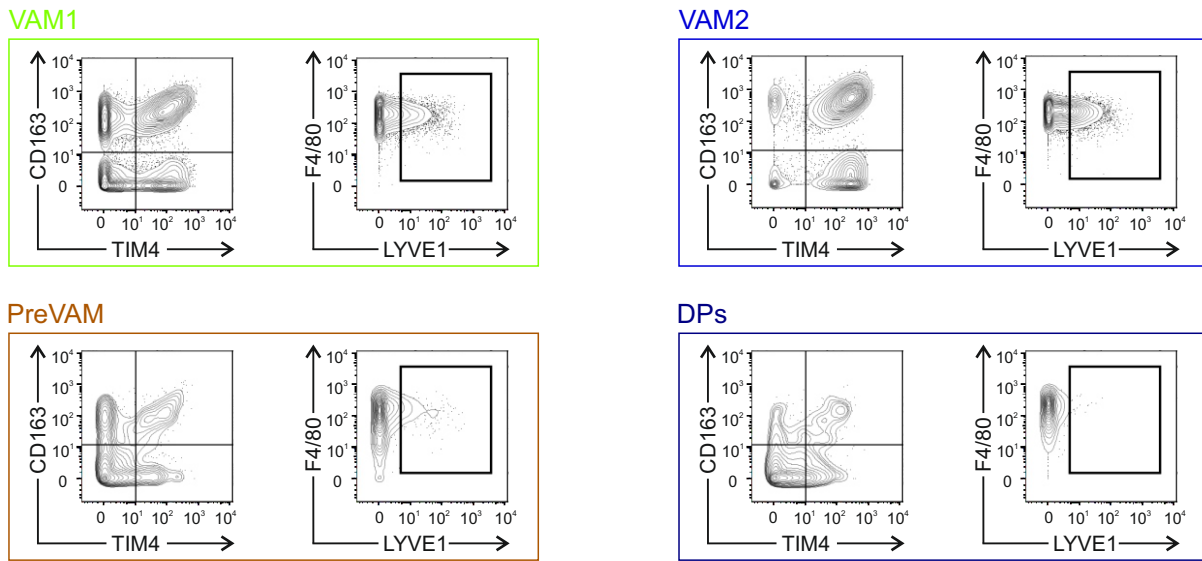


**Supplementary Figure 3. (A-F)** Frequencies of eWAT macrophage subpopulations in wild type (WT) mice based on the FlowSOM analyses at the indicated time points. The quantitative data are shown as mean  $\pm$  SEM (\* $P \leq 0.0332$ , \*\* $P \leq 0.0021$ , \*\*\* $P \leq 0.0002$ , \*\*\*\* $P \leq 0.0001$ , two-way ANOVA with Bonferroni post-hoc test). Each data point represents a pooled eWAT from 7 (2 wk), 3 (5 wk), or 2 (8, 12, 16, and 23 wk) mice. All mass cytometry data are from 1 (2, 12 and 16 wk), 2 (8 wk), 3 (5 wk) or 4 (23 wk) independent experiments.

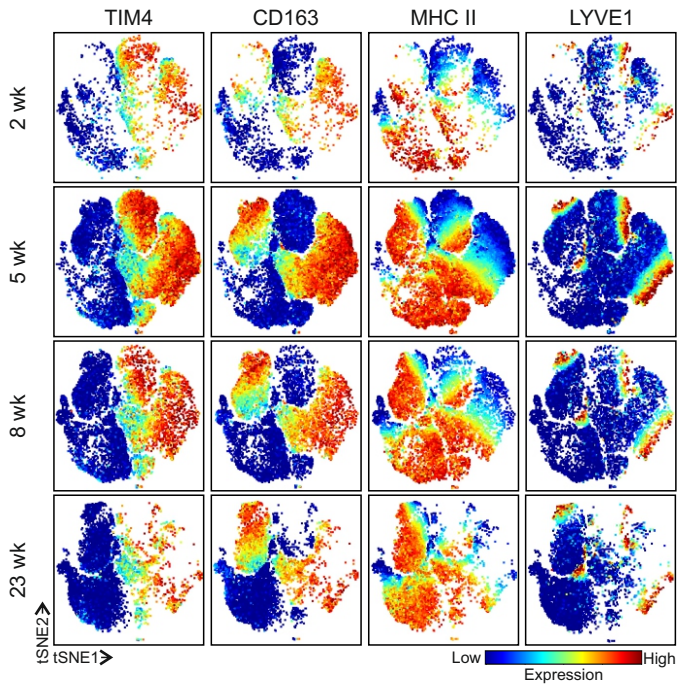
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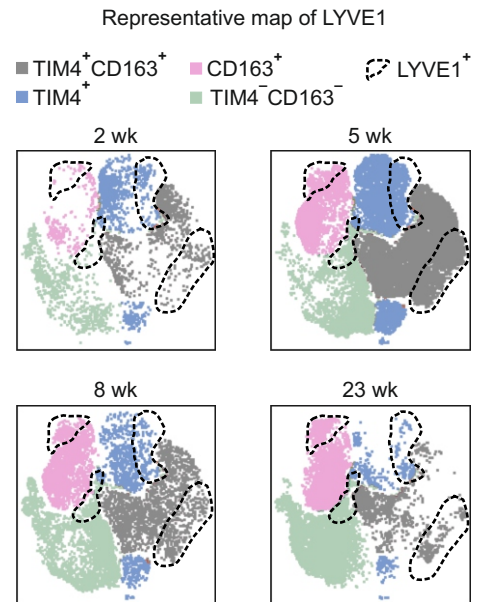
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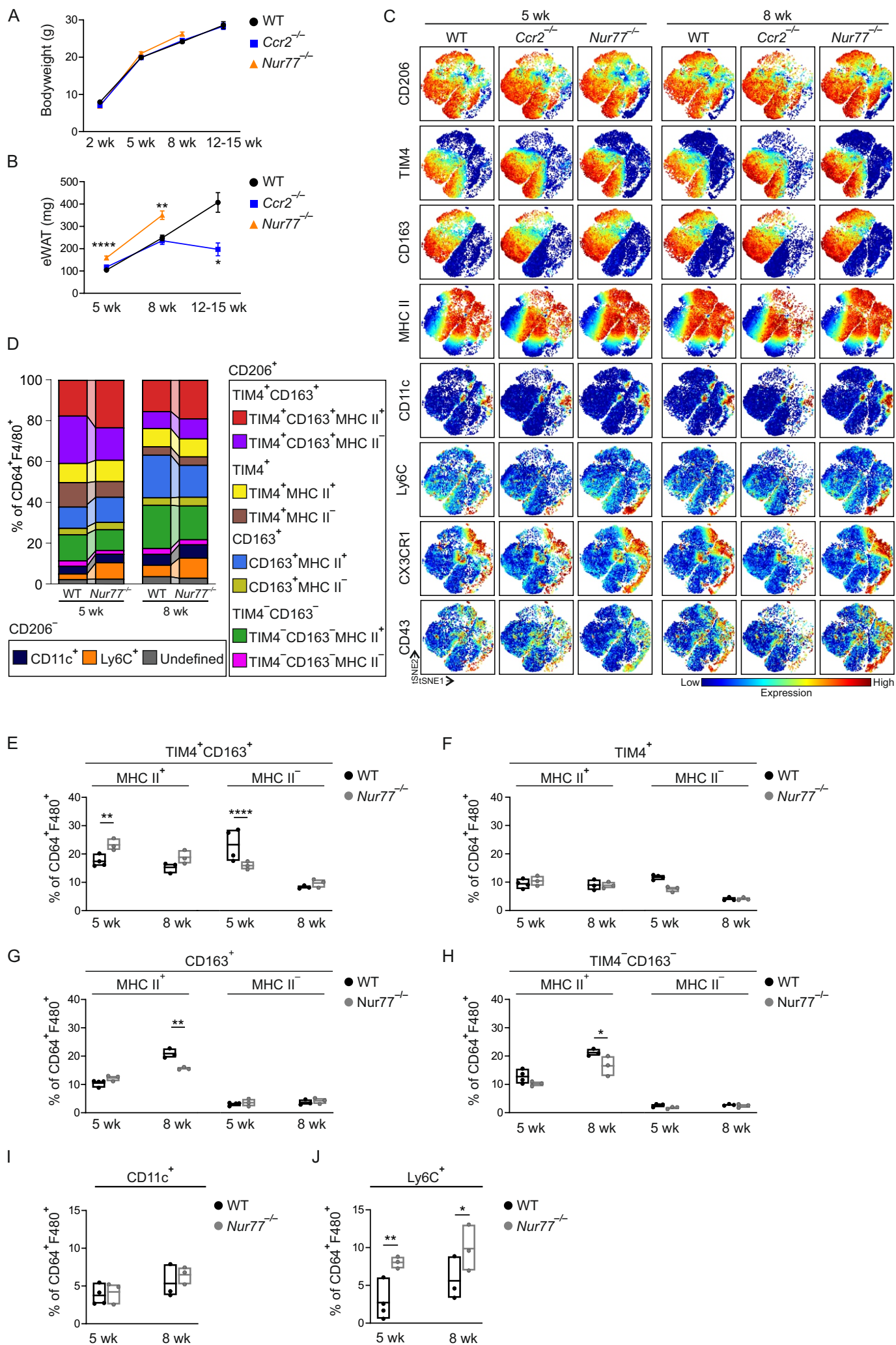
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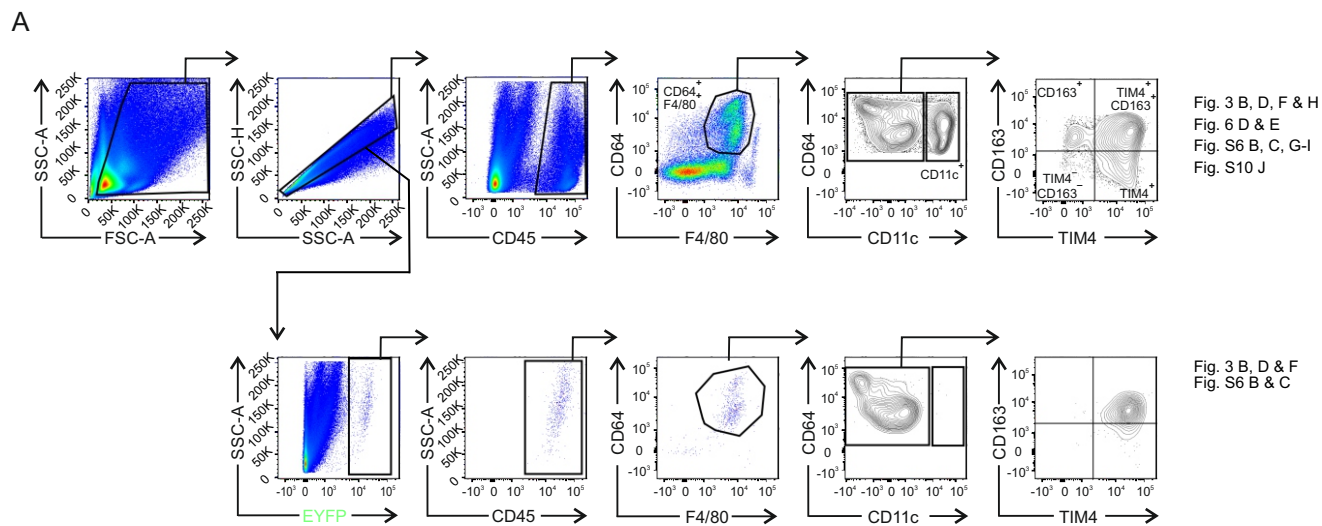
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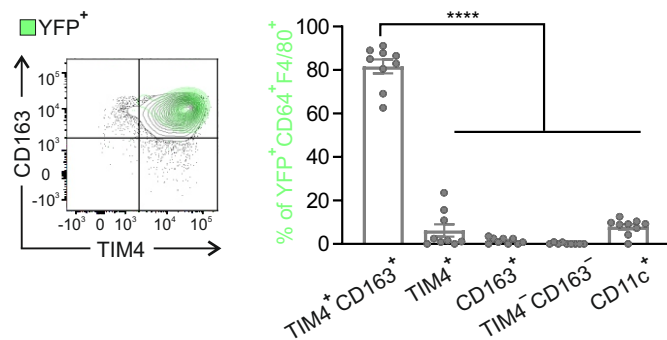
**Supplementary Figure 4. (A)** Representative example showing the gating of our mass cytometric data from eWAT of a 5-week-old wild type (WT) mouse using the strategy described by Silva *et al.* (11) for defining the vascular-associated macrophages (VAMs). **(B)** TIM4, CD163 and LYVE1 gatings on the different VAM populations. Note that each VAM subpopulation includes cells from TIM4<sup>+</sup>CD163<sup>+</sup>, TIM4<sup>+</sup>, CD163<sup>+</sup>, and TIM4<sup>-</sup>CD163<sup>-</sup> macrophage subpopulations and both LYVE1<sup>+</sup> and LYVE1<sup>-</sup> cells. **(C)** Representative eWAT macrophage viSNE plots from WT at the indicated time points showing LYVE1 expression. **(D)** Manual gating of TIM4<sup>+</sup>CD163<sup>+</sup>, TIM4<sup>+</sup>, CD163<sup>+</sup>, and TIM4<sup>-</sup>CD163<sup>-</sup> ATMs on the viSNE map. The areas within the dotted lines (drawn manually based on the data shown in (C) represent LYVE1 positive cells in each main macrophage population.



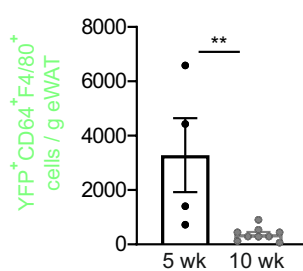
**Supplementary Figure 5. (A)** Bodyweight follow-up of wild type (WT), *Ccr2*<sup>-/-</sup> and *Nur77*<sup>-/-</sup> mice at the indicated time points. **(B)** eWAT weight of WT, *Ccr2*<sup>-/-</sup> and *Nur77*<sup>-/-</sup> mice at indicated time points. **(C)** Representative viSNE plots of eWAT in 5- and 8-week-old WT, *Ccr2*<sup>-/-</sup> and *Nur77*<sup>-/-</sup> mice. Color code indicates the expression level of a given marker from low (blue) to high (red). **(D)** Frequencies of macrophage subpopulations in eWAT based on the FlowSOM analyses of *Nur77*<sup>-/-</sup> mice at 5 and 8 weeks of age. Each FlowSOM metacluster (subpopulation) is represented by an individual color depicted in the columns. **(E-J)** Frequencies of eWAT macrophage subpopulations in *Nur77*<sup>-/-</sup> mice based on the FlowSOM analyses at the indicated time points. The quantitative data are shown as mean ± SEM [*\*P* ≤ 0.0332, *\*\*P* ≤ 0.0021, *\*\*\*P* ≤ 0.0002, *\*\*\*\*P* ≤ 0.0001, non-parametric two-tailed Mann-Whitney test (A and B), or two-way ANOVA with Bonferroni post-hoc test for (E-J)]. Each data point represents a grouped mean value (A and B), a pooled eWAT from 2 (8 wk), or 3 (5 wk) mice (E-J). All mass cytometry data are from 2 (8 wk WT and *Nur77*<sup>-/-</sup>, and 5 wk *Nur77*<sup>-/-</sup>) or 3 (5 wk WT) independent experiments.



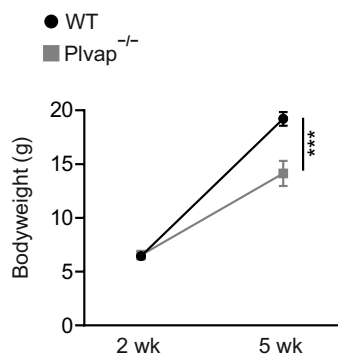
**B** 10 wk *Cx3cr1*<sup>CreERT2</sup> x *R26R-EYFP*



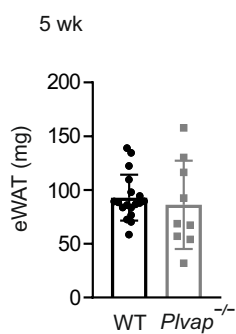
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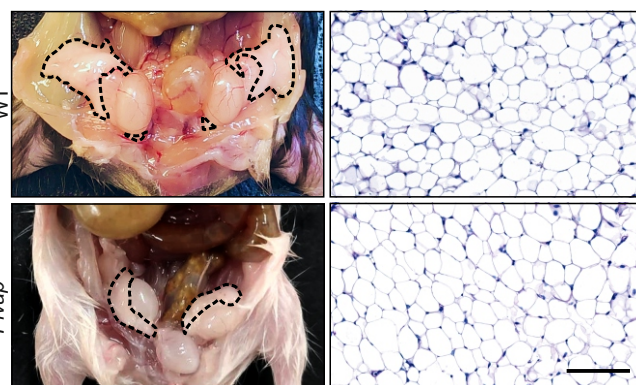
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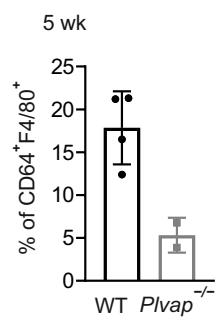
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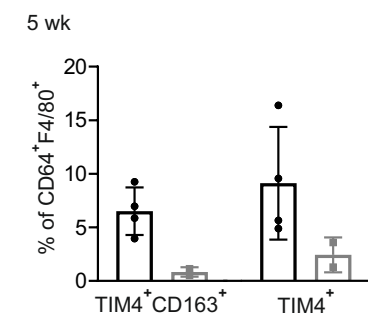
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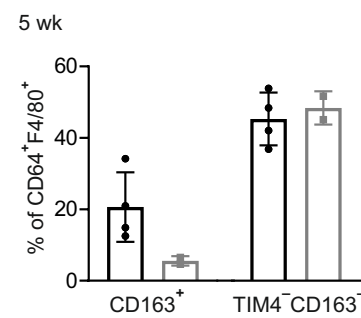
**G**



**H**

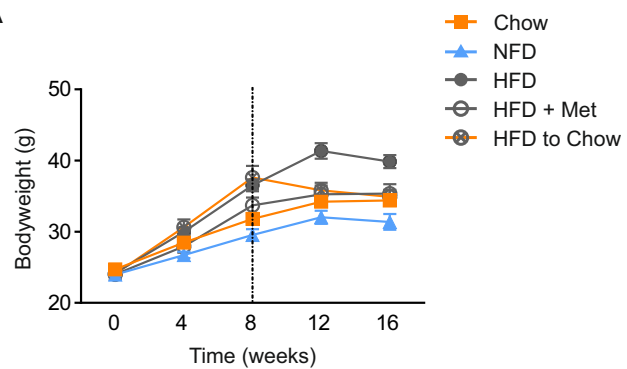


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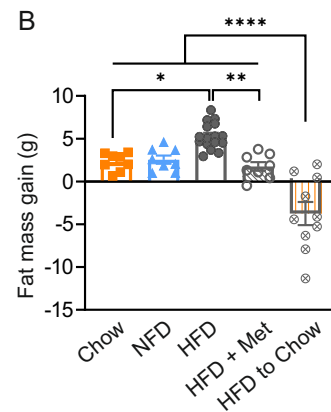


**Supplementary Figure 6.** (A) Representative gating strategy for the flow cytometric data used to identify the four main populations of eWAT macrophages and the strategy for gating YFP positive cells onto the macrophages. (B) Representative FACS plot and quantifications of yolk sac-derived cell in eWAT of 10-week-old *Cx3cr1<sup>CreERT2</sup>;R26R-EYFP* reporter mice induced E13.5 with tamoxifen. FACS plot shows the backgating of the YFP<sup>+</sup> cells (green) on the different ATM populations. Quantifications show the frequency of CD45<sup>+</sup>YFP<sup>+</sup>CD64<sup>+</sup>F4/80<sup>+</sup> cells in each ATM population. (C) Quantifications of CD45<sup>+</sup>YFP<sup>+</sup>CD64<sup>+</sup>F4/80<sup>+</sup> cell counts at 5 and 10 weeks of age in eWAT of *Cx3cr1<sup>CreERT2</sup>;R26R-EYFP* reporter mice induced E13.5 with tamoxifen. (D) Bodyweight (g) of wild type (WT) and *Plvap<sup>-/-</sup>* male mice at the age of 2 and 5 weeks. (E) eWAT weight in 5-week-old WT and *Plvap<sup>-/-</sup>* mice. (F) Representative macroscopic images and PAS-stained histology of eWAT in 5-week-old WT and *Plvap<sup>-/-</sup>* mice. Epididymal fat pads are outlined with a dashed line. Scale bar 100  $\mu$ m. (G) Quantification of macrophages (CD45<sup>+</sup>CD64<sup>+</sup>F4/80<sup>+</sup> cells) in eWAT of 5-week-old *Plvap<sup>-/-</sup>* mice. (H-I) Quantification of TIM4<sup>+</sup>CD163<sup>+</sup>, TIM4<sup>+</sup>, CD163<sup>+</sup> and TIM4<sup>-</sup>CD163<sup>-</sup> ATMs in eWAT of 5-week-old *Plvap<sup>-/-</sup>* mice. The quantitative data are shown as mean  $\pm$  SEM (\* $P \leq 0.0332$ , \*\* $P \leq 0.0021$ , \*\*\* $P \leq 0.0002$ , \*\*\*\* $P \leq 0.0001$ , one-way ANOVA with Bonferroni post-hoc test for B and non-parametric two-tailed Mann-Whitney test for C, D and E). Each data point represents one mouse (B, C, E, G, H and I) or mean value (D). All flow cytometry data are from 2 (G-I for *Plvap<sup>-/-</sup>*) or 3 (B, C, and G-I for WT) independent experiments.

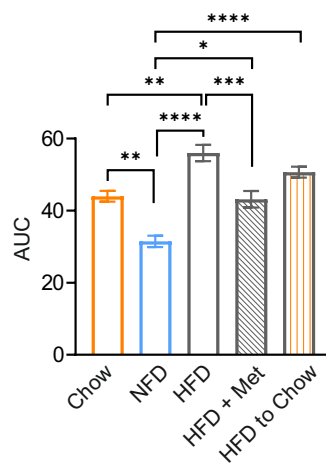
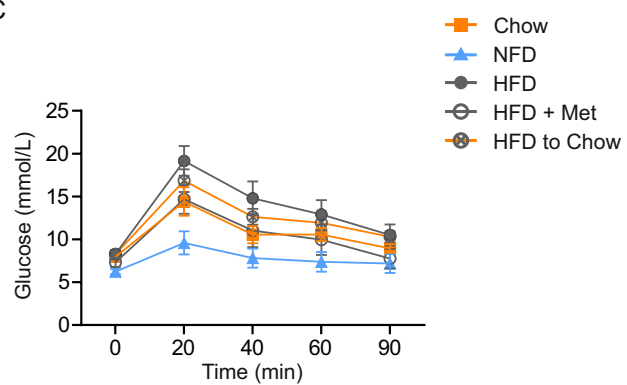
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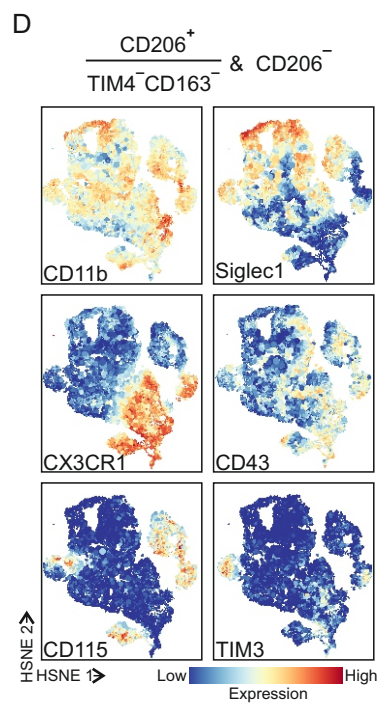
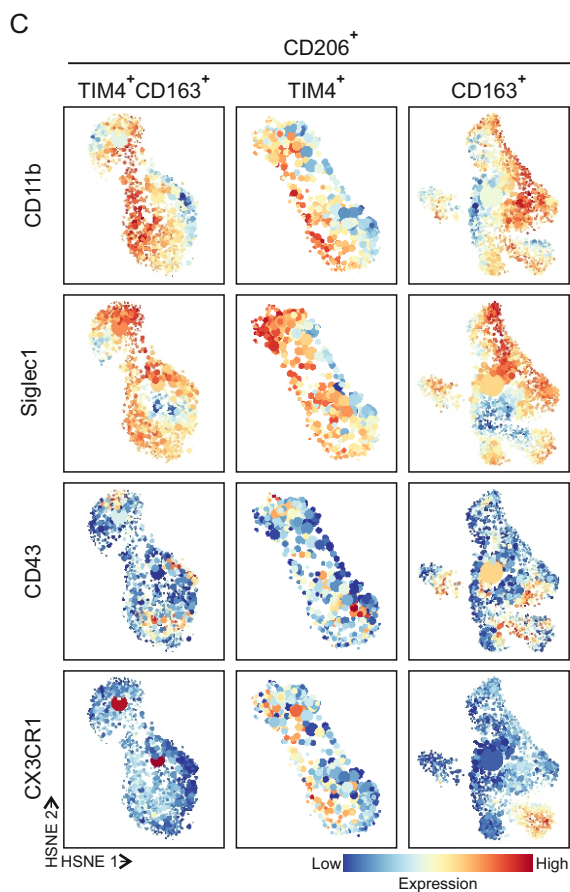
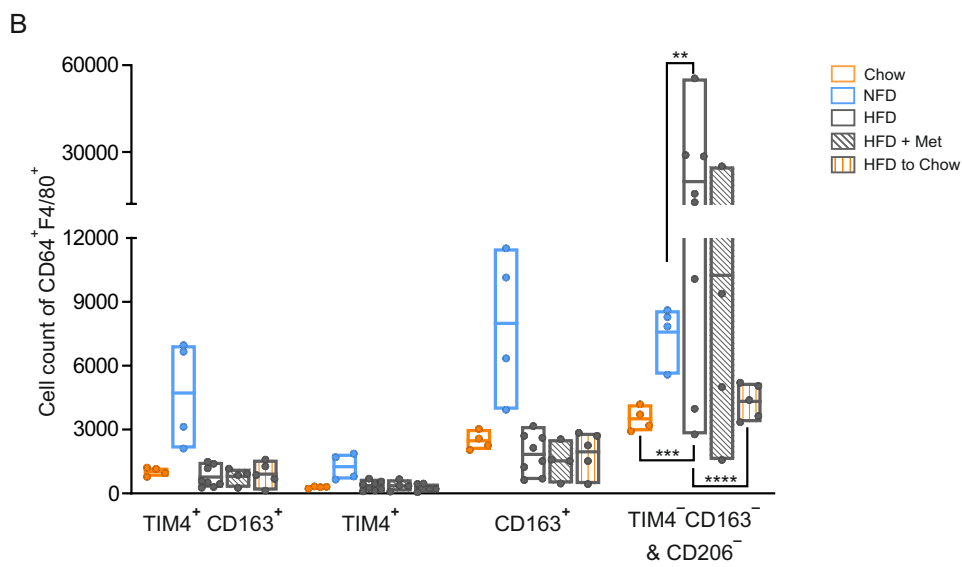
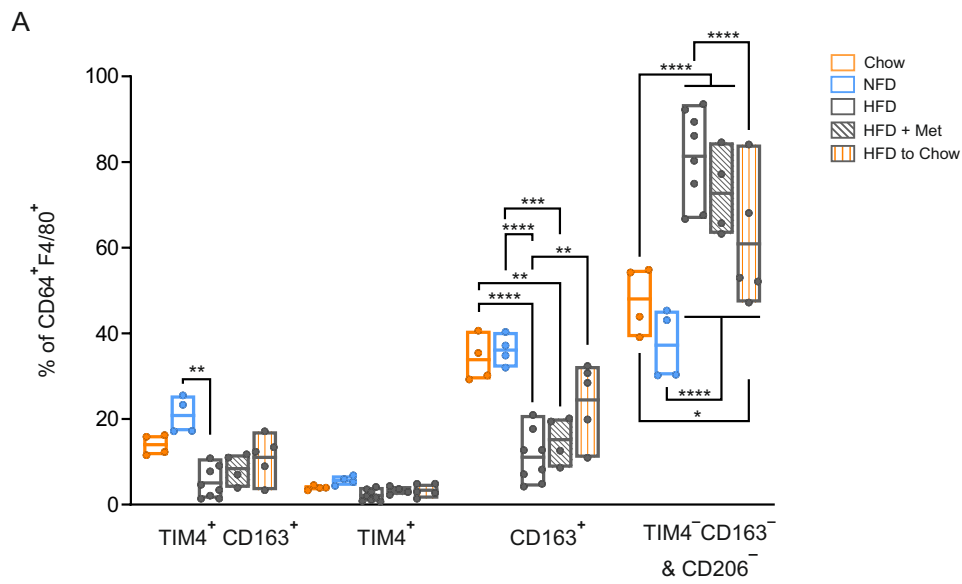
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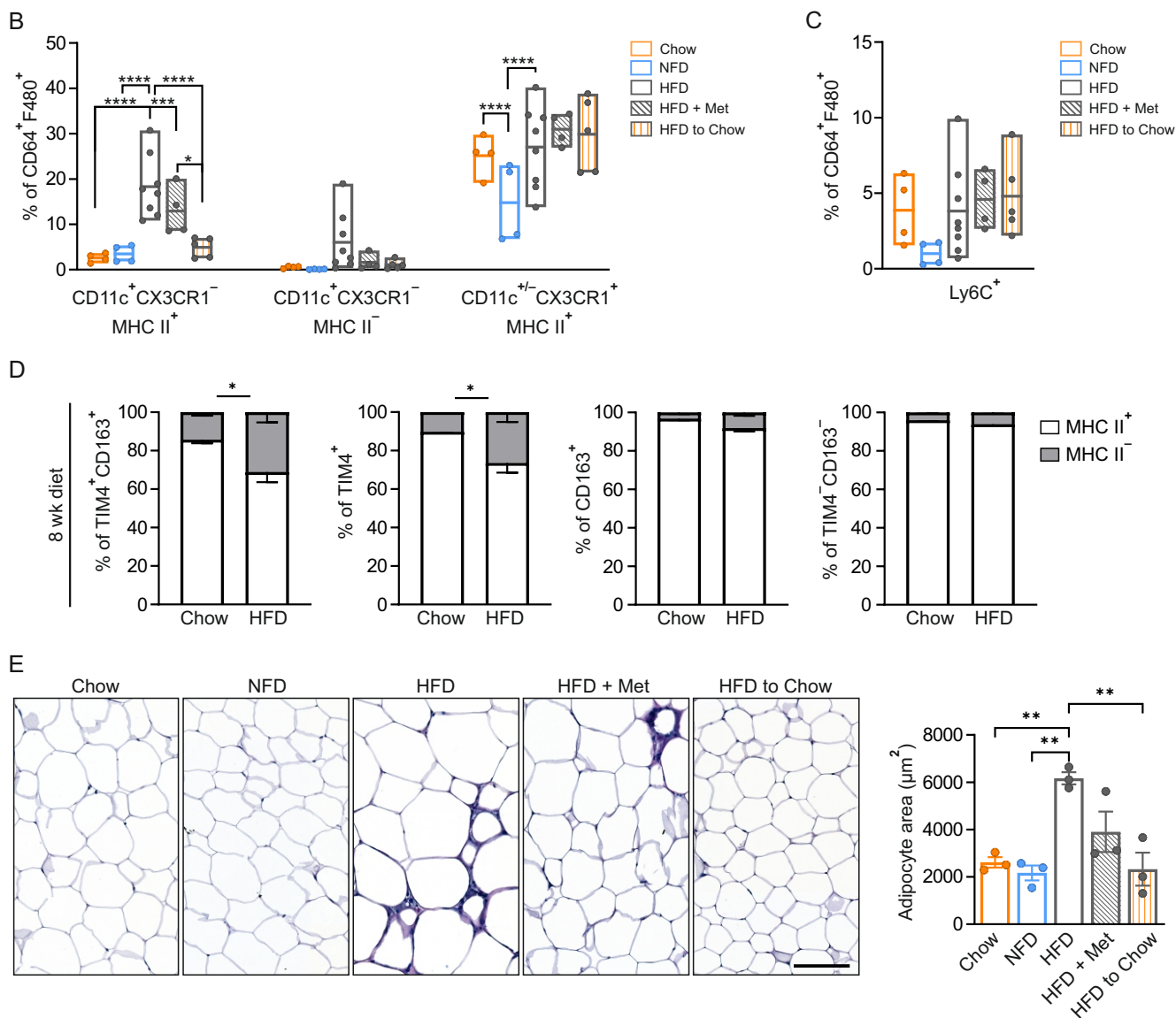
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**Supplementary Figure 7. (A)** Bodyweight gain of wild type (WT) mice in different diet groups. The vertical black line indicates the intervention time in HFD+Met and HFD to Chow groups. **(B)** Quantification of fat mass gain by Echo-MRI between weeks 7 and 15 in the different groups, each dot represents one mouse. **(C)** Glucose tolerance test curves and area under the curve (AUC) values for mice in the different diet groups. The quantitative data are shown as mean  $\pm$  SEM (\* $P \leq 0.0332$ , \*\* $P \leq 0.0021$ , \*\*\* $P \leq 0.0002$ , \*\*\*\* $P \leq 0.0001$ , one-way ANOVA with Bonferroni post-hoc test).



**Supplementary Figure 8. (A)** Frequencies of macrophage subpopulations in eWAT of Chow, NFD, HFD, HFD + Met, and HFD to Chow mice at the end of the 16 wk experiment. **(B)** Cell count of macrophage subpopulations in eWAT of Chow, NFD, HFD, HFD + Met, and HFD to Chow mice at the end of the 16 wk experiment. **(C)** Expression level of the indicated marker from low (blue) to high (red) in a selection of TIM4<sup>+</sup>CD163<sup>+</sup>, TIM4<sup>+</sup>, and CD163<sup>+</sup> HSNEs. **(D)** Expression level of the indicated markers from low (blue) to high (red) in a selection of TIM4<sup>+</sup>CD163<sup>+</sup> and CD206<sup>+</sup> HSNE. In C and D, the clusters are the same as shown in main Figures 4E and F, respectively. The quantitative data are shown as mean  $\pm$  SEM (\* $P \leq 0.0332$ , \*\* $P \leq 0.0021$ , \*\*\* $P \leq 0.0002$ , \*\*\*\* $P \leq 0.0001$ , two-way ANOVA with Bonferroni post-hoc test). One data point represents a pooled eWAT from 2 mice. All mass cytometry data are from 3 (HFD + Met and NFD), 4 (Chow and HFD to Chow) or 6 (HFD) independent experiments (A and B).



**Supplementary Figure 9. (A)** Representative viSNE plots for indicated diet groups from wild type (WT) eWAT of CD45<sup>+</sup>CD64<sup>+</sup>F4/80<sup>+</sup> cells. Color code indicates the expression level of a given marker from low (blue) to high (red). **(B)** Frequencies of the CD11c<sup>+</sup>CX3CR1<sup>-</sup>MHC II<sup>+</sup>, CD11c<sup>+</sup>CX3CR1<sup>-</sup>MHC II<sup>-</sup> and CD11c<sup>+/+</sup>CX3CR1<sup>+</sup>MHC II<sup>+</sup> subpopulations in the indicated experimental groups. **(C)** Frequencies of the Ly6C<sup>+</sup> cells in the indicated experimental groups. **(D)** Frequencies of MHC II<sup>+</sup> and MHC II<sup>-</sup> cells in TIM4<sup>+</sup>CD163<sup>+</sup>, TIM4<sup>+</sup>, CD163<sup>+</sup>, and TIM4<sup>-</sup>CD163<sup>-</sup> ATMs in mice after 8 weeks on Chow or HFD. **(E)** Representative PAS stained eWAT histology and quantification of adipocyte area ( $\mu\text{m}^2$ ) of the indicated experimental groups. Scale bar 100  $\mu\text{m}$ . The quantitative data are shown as mean  $\pm$  SEM [ $*P \leq 0.0332$ ,  $**P \leq 0.0021$ ,  $***P \leq 0.0002$ ,  $****P \leq 0.0001$ , one- (E) or two-way (B-D) ANOVA with Bonferroni post-hoc test]. Each data point represents 1 mouse (E) or a pooled eWAT from 2 (B and C) mice. 8 wk diet experiment (D) data are from n = 5 (HFD) or n = 6 (Chow) mice. All mass cytometry data are from 1 (Chow and HFD 8wk diet), 3 (HFD + Met and NFD), 4 (Chow and HFD to Chow) or 6 (HFD) independent experiments.

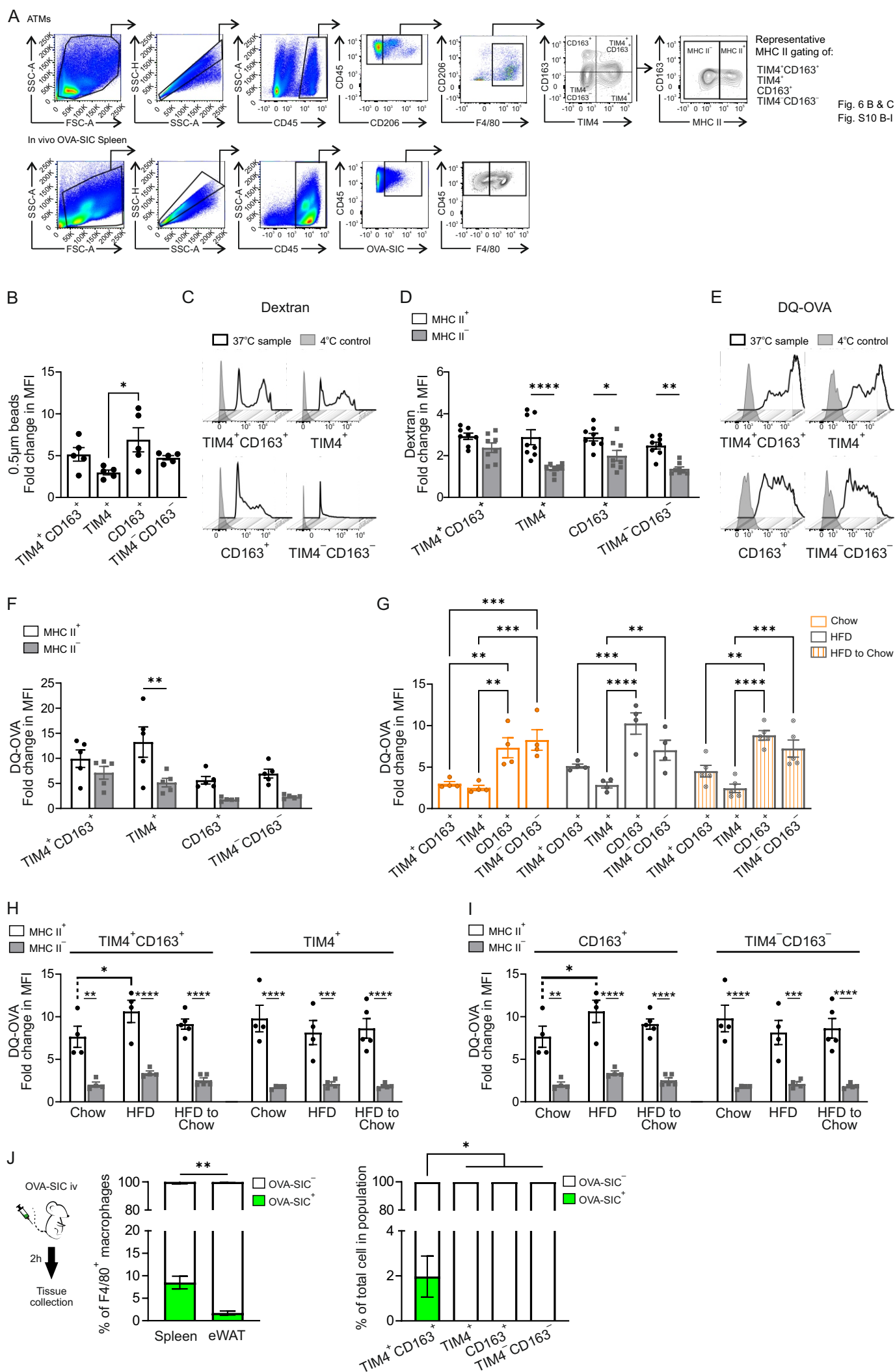


Fig. 6 B & C  
 Fig. S10 B-I

**Supplementary Figure 10. (A)** Representative gating strategy for the flow cytometric data used for gating cells onto the four main macrophage populations and their MHC II<sup>+</sup> and MHC II<sup>-</sup> subtypes, and gating strategy for the spleen CD45<sup>+</sup>F4/80<sup>+</sup> cells. **(B)** Phagocytosis of particles by eWAT macrophages. Fold change in mean fluorescence intensity (MFI) in TIM4<sup>+</sup>CD163<sup>+</sup>, TIM4<sup>+</sup>, CD163<sup>+</sup>, and TIM4<sup>-</sup>CD163<sup>-</sup> eWAT macrophages after *in vitro* phagocytosis of fluorescent 0.5  $\mu$ m beads. **(C)** Representative histograms for dextran in four main ATM populations in 5-week-old wild type (WT) mice. **(D)** *In vitro* endocytosis of dextran by MHC II<sup>+</sup> and MHC II<sup>-</sup> macrophage subpopulations. Fold change in dextran MFI in MHC II<sup>+</sup> and MHC II<sup>-</sup> subsets of TIM4<sup>+</sup>CD163<sup>+</sup>, TIM4<sup>+</sup>, CD163<sup>+</sup>, and TIM4<sup>-</sup>CD163<sup>-</sup> eWAT macrophages. **(E)** Representative histograms for DQ-OVA in four main ATM populations in 5-week-old WT mice. **(F)** *In vitro* antigen processing by MHC II<sup>+</sup> and MHC II<sup>-</sup> macrophage subpopulations. Fold change in DQ-OVA MFI in MHC II<sup>+</sup> and MHC II<sup>-</sup> subsets of TIM4<sup>+</sup>CD163<sup>+</sup>, TIM4<sup>+</sup>, CD163<sup>+</sup>, and TIM4<sup>-</sup>CD163<sup>-</sup> eWAT macrophages. **(G)** *In vitro* antigen processing by eWAT macrophages of Chow, HFD, and HFD to Chow mice. **(H-I)** *In vitro* antigen processing by MHC II<sup>+</sup> and MHC II<sup>-</sup> macrophage subpopulations. Fold change in DQ-OVA MFI in MHC II<sup>+</sup> and MHC II<sup>-</sup> subsets of TIM4<sup>+</sup>CD163<sup>+</sup>, TIM4<sup>+</sup>, CD163<sup>+</sup>, and TIM4<sup>-</sup>CD163<sup>-</sup> eWAT macrophages. **(J)** Experimental setup for intravenously administered ovalbumin immune complex (OVA-SIC) uptake and quantifications of OVA-SIC positive and negative macrophage frequencies in the spleen (CD45<sup>+</sup>F4/80<sup>+</sup> cells) and eWAT (CD45<sup>+</sup>CD64<sup>+</sup>F4/80<sup>+</sup> cells). Quantifications of OVA-SIC positive and negative cell frequencies in TIM4<sup>+</sup>CD163<sup>+</sup>, TIM4<sup>+</sup>, CD163<sup>+</sup>, and TIM4<sup>-</sup>CD163<sup>-</sup> ATM populations of 5-week-old WT mice under *in vivo* settings. The quantitative data are shown as mean  $\pm$  SEM [ $*P \leq 0.0332$ ,  $**P \leq 0.0021$ ,  $***P \leq 0.0002$ ,  $****P \leq 0.0001$ , one-way ANOVA (B and for ATM population comparison in J) and two-way ANOVA with Bonferroni post-hoc test (D, F, G, H and I), and Mann-Whitney test (for spleen and eWAT comparison in J). In quantifications, data are presented as fold change in MFI (at 37 °C versus background at 4 °C), and each dot represents one mouse. OVA-SIC *in vivo* experiment (J) data are from n = 9 mice. All flow cytometry data are from 2 (B, D, F and J) or 3 (G-I) independent experiments.

**Supplementary Table 1 – Antibody list**

| <b>Antibody</b>          | <b>Manufacturer</b> | <b>Catalog number and RRID ID</b>                   |
|--------------------------|---------------------|-----------------------------------------------------|
| anti-APC 176Yb           | Fluidigm            | Cat#: 3176007C                                      |
| anti-B220 160Gd          | Fluidigm            | Cat#: 3160012C                                      |
| anti-CD4 172Yb           | Fluidigm            | Cat#: 3172003C                                      |
| anti-CD8a 168Er          | Fluidigm            | Cat#: 3168003C                                      |
| anti-CD11b APC-Cy-7      | BD Pharmingen™      | Cat#: BD 557657 RRID: <a href="#">AB_396772</a>     |
| anti-CD11b BV786         | BD Pharmingen™      | Cat#: BD 740861 RRID: <a href="#">AB_2740514</a>    |
| anti-CD11b 148Nd         | Fluidigm            | Cat#: 3148003C                                      |
| anti-CD11c BV711         | BioLegend®          | Cat#: 117349 RRID: <a href="#">AB_2563905</a>       |
| anti-CD11c 142Nd         | Fluidigm            | Cat#: 3142003C                                      |
| anti-CD16/32             | BioXCell            | Cat#: BE0307 RRID: <a href="#">AB_2736987</a>       |
| anti-CD19 166Er          | Fluidigm            | Cat#: 3166015C                                      |
| anti-CD43 146Nd          | Fluidigm            | Cat#: 3146009C                                      |
| anti-CD45 PerCP-Cy5,5    | BD Pharmingen™      | Cat#:BD 550994 RRID: <a href="#">AB_394003</a>      |
| anti-CD45 147Sm          | Fluidigm            | Cat#: 3147003C                                      |
| anti-CD45 175Lu          | Fluidigm            | Cat#: 3175010C                                      |
| anti-CD45 89Y            | Fluidigm            | Cat#: 3089005C                                      |
| anti-CD64 BV786          | BD Pharmingen™      | Cat#: BD 741024 RRID: <a href="#">AB_2740644</a>    |
| anti-CD64 PE             | BioLegend®          | Cat#: 139304 RRID: <a href="#">AB_10612740</a>      |
| anti-CD64 151Eu          | Fluidigm            | Cat#: 3151012C                                      |
| anti-CD68 FITC           | BioLegend®          | Cat#: 137005 RRID: <a href="#">AB_10575475</a>      |
| anti-CD80 171Yb          | Fluidigm            | Cat#: 3171008C                                      |
| anti-CD115               | Bio X Cell          | Cat#: BE0213 RRID: <a href="#">AB_2687699</a>       |
| anti-CD115 PE-Cy7        | eBioscience         | Cat#: 25-1152-80 RRID: <a href="#">AB_2573385</a>   |
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| anti-CD117 173Yb         | Fluidigm            | Cat#: 3173004C                                      |
| anti-CD163               | BioRad              | Cat#: MCA342GA RRID: <a href="#">AB_2074558</a>     |
| anti-CD163 APC           | BioLegend®          | Cat#: 155305 RRID: <a href="#">AB_2814059</a>       |
| anti-CD206 A488          | BioRad              | Cat#: MCA2235A488T RRID: <a href="#">AB_2297790</a> |
| anti-CD206 BV650         | BioLegend®          | Cat#: 141723RRID: <a href="#">AB_2562445</a>        |
| anti-CD206 169Tm         | Fluidigm            | Cat#: 3169021C                                      |
| anti-CD274 153Eu         | Fluidigm            | Cat#: 3153016C                                      |
| anti-Chicken Egg Albumin | Sigma-Aldrich       | Cat#: C6534-2ML                                     |
| anti-Cisplatin 195Pt     | Fluidigm            | Cat#: 201064                                        |
| anti-CX3CR1 BV650        | BioLegend®          | Cat#: 149033 RRID: <a href="#">AB_2565999</a>       |
| anti-CX3CR1 164Dy        | Fluidigm            | Cat#: 3164023C                                      |
| anti-F4/80 A488          | eBioscience         | Cat#: 53-4801-82 RRID: <a href="#">AB_469915</a>    |
| anti-F4/80 A700          | BioRad              | Cat#: MCA497A700T RRID: <a href="#">AB_1102556</a>  |
| anti-F4/80 159Tb         | Fluidigm            | Cat#: 3159009C                                      |
| anti-FITC 144Nd          | Fluidigm            | Cat#: 3144006C                                      |
| anti-Intercalator 103Rh  | Fluidigm            | Cat#: 201103A                                       |
| anti-Ly6C BV421          | BD Pharmingen™      | Cat#: BD 562727 RRID: <a href="#">AB_2737748</a>    |
| anti-Ly6C 150Nd          | Fluidigm            | Cat#: 3150010C                                      |
| anti-Ly6C 162Dy          | Fluidigm            | Cat#: 3162014C                                      |
| anti-Ly6G BV510          | BioLegend®          | Cat#: 127633 RRID: <a href="#">AB_2562937</a>       |
| anti-Ly6G 141Pr          | Fluidigm            | Cat#: 3141008C                                      |
| anti-LYVE1 PE            | R&D Systems         | Cat#: FAB2125P RRID: <a href="#">AB_10889020</a>    |
| anti-MerTK APC           | R&D Systems         | Cat#: FAB5912A                                      |

**Supplementary Table 1 – Antibody list**

| <b>Antibody</b>       | <b>Manufacturer</b> | <b>Catalog number and RRID ID</b>                  |
|-----------------------|---------------------|----------------------------------------------------|
| anti-MerTK FITC       | BioLegend®          | Cat#: 151503 RRID: <a href="#">AB_2617034</a>      |
| anti-MHC II APC       | eBioscience         | Cat#: 17-5321-82 RRID: <a href="#">AB_469455</a>   |
| anti-MHC II PE-Cy7    | eBioscience         | Cat#: 25-5321-82 RRID: <a href="#">AB_10870792</a> |
| anti-MHC II 174Yb     | Fluidigm            | Cat#: 3174003C                                     |
| anti-PE 165Ho         | Fluidigm            | Cat#: 3165015C                                     |
| anti-SiglecF APC      | BioLegend®          | Cat#: 155507 RRID: <a href="#">AB_2750236</a>      |
| anti-SiglecF PE-CF594 | BD Pharmingen™      | Cat#: BD 562757 RRID: <a href="#">AB_2687994</a>   |
| anti-Siglec1 170Er    | Fluidigm            | Cat#: 3170018C                                     |
| anti-TER-119 154Sm    | Fluidigm            | Cat#: 3154005C                                     |
| anti-TIM3 162Dy       | Fluidigm            | Cat#: 3162029C                                     |
| anti-TIM4             | BioLegend®          | Cat#: 130002 RRID: <a href="#">AB_1227802</a>      |
| anti-TIM4 PE          | BioLegend®          | Cat#: 130005 RRID: <a href="#">AB_1227807</a>      |