Supplemental Table S1. Antibodies for intestinal immune cell surface stain

Antibody	Fluorophore	Company, Catalogue # (Clone)	RRID	Dilution
Ly6C	Alexa Fluor 488	BioLegend, 128022 (HK1.4)	AB_10639728	1:100
Tim-4	PE	BioLegend, 130005 (RMT4-54)	AB_1227807	1:50
CD64	PE-Dazzle 594	BioLegend, 139320 (X54-5/7.1)	AB_2566559	1:200
CD11b	PerCP-Cy5.5	eBioscience, 45-0112-82 (M1/70)	AB_953558	1:200
CCR2	Brilliant Violet 421	BioLegend, 150605 (SA203G11)	AB_2571913	1:100
CD45	Brilliant Violet 510	BioLegend, 103138 (30-F11)	AB_2563061	1:200
F4/80	Brilliant Violet 605	BioLegend, 123133 (BM8)	AB_2562305	1:100
CD4	APC	BioLegend, 100516 (RM4-5)	AB_312719	1:50
MHC II	AF700	eBioscience, 56-5231-82 (M5/114.15.2)	AB_494009	1:200
CD3	PE-Cy7	eBioscience, 25-0031-81 (145-2C11)	AB_469571	1:200
B220	PE-Cy7	eBioscience, 25-0452-81 (RA3-6B2)	AB_469626	1:200
Ly6G	PE-Cy7	eBioscience, 25-0112-81 (M1/70)	AB_469587	1:200
Live-Dead NIR	APC-Cy7	Invitrogen, L34975	NA	1:200

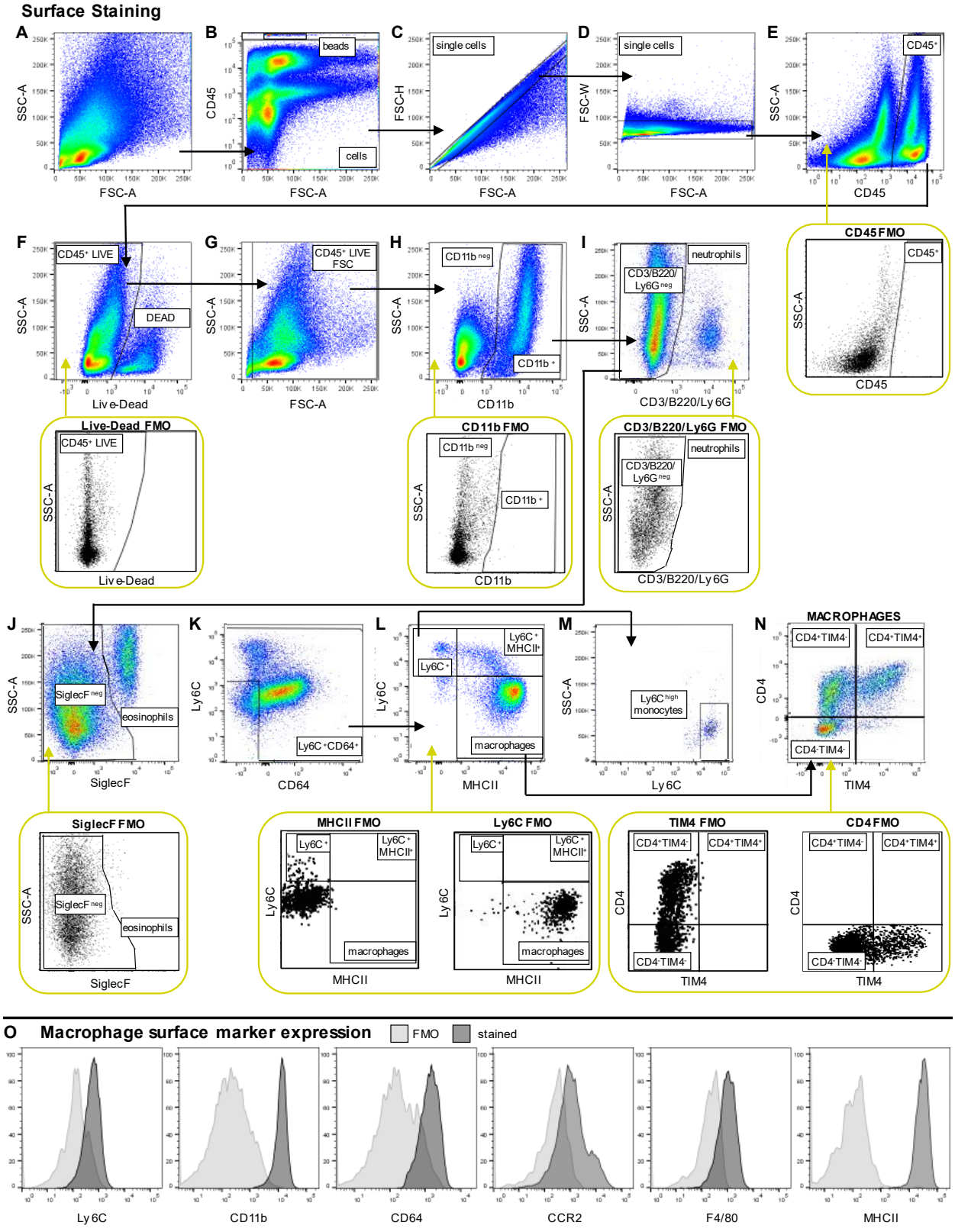
Supplemental Table S2. Antibodies for intestinal immune cell surface stain (to be followed by intracellular staining)

Antibody	Fluorophore	Company, Catalogue # (Clone)	RRID	Dilution
Tim-4	PE	BioLegend, 130005 (RMT4-54)	AB_1227807	1:50
CD64	PE-Dazzle 594	BioLegend, 139320 (X54-5/7.1)	AB_2566559	1:200
CD11b	PE-Cy7	eBioscience, 45-0112-82 (M1/70)	AB_953558	1:200
CD45	Brilliant Violet 510	BioLegend, 103138 (30-F11)	AB_2563061	1:200
CD4	APC	BioLegend, 100516 (RM4-5)	AB_312719	1:50
MHC II	AF700	eBioscience, 56-5231-82 (M5/114.15.2)	AB_494009	1:200
Live-Dead NIR	APC-Cy7	Invitrogen, L34975	NA	1:200

Supplemental Table S3. Antibodies for intracellular intestinal immune cell staining

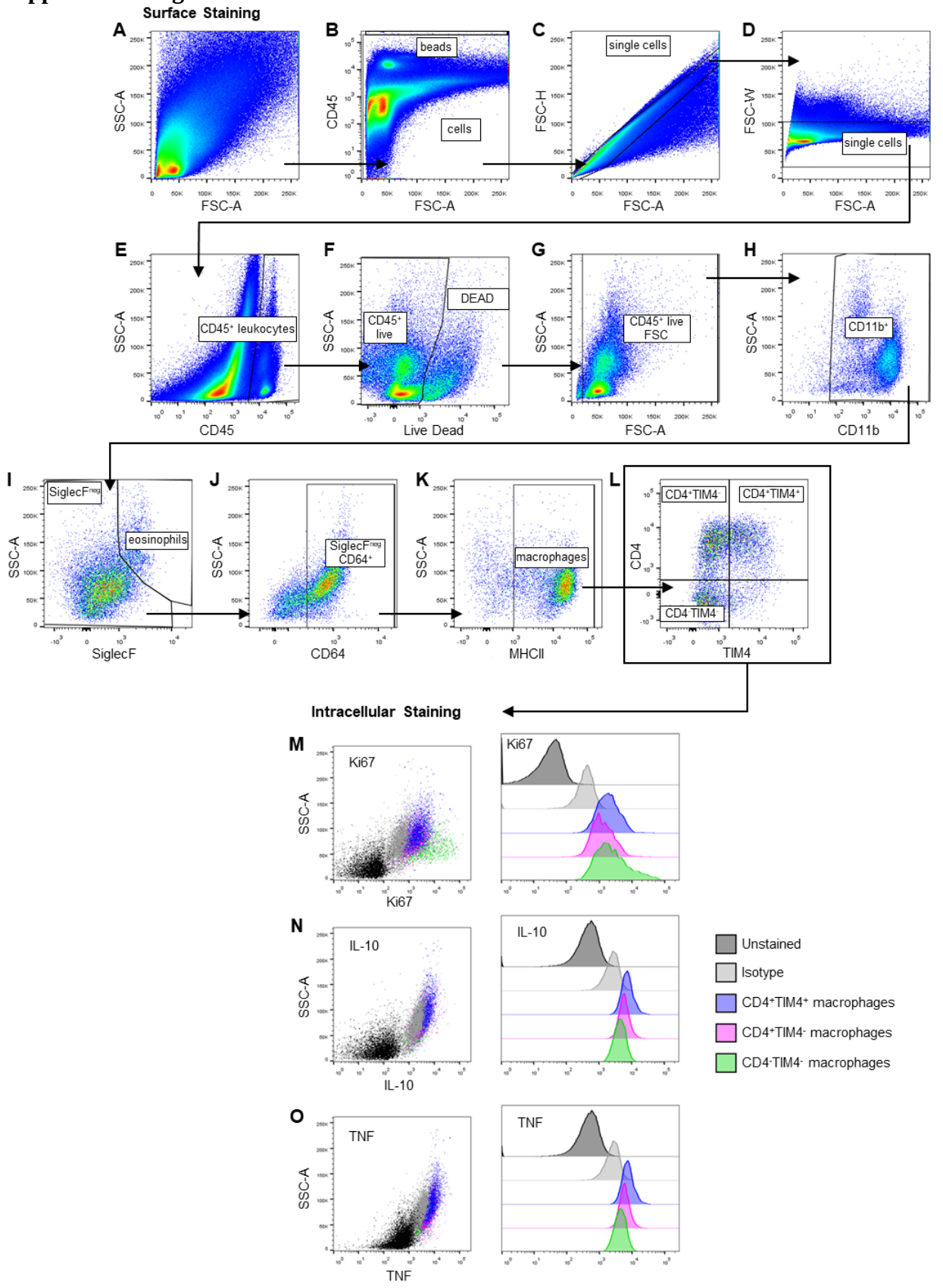
Antibody	Fluorophore	Company, Catalogue #, Clone	RRID	Dilution
TNF	Alexa Fluor 488	eBioscience, 53-7321-82 (MP6-XT22)	AB_469936	1:67
IL-10	PerCP-Cy5.5	eBioscience, 45-7101-80 (JES5-16E3)	AB_996679	1:67
Ki67	Brilliant Violet 605	BioLegend, 652413 (16A8)	AB_2562664	1:67
Rat IgG1, κ	Alexa Fluor 488	eBioscience, 53-4301-80 (eBRG1)	AB_493962	1:67
Rat IgG2b, κ	PerCP-Cy5.5	eBioscience, 45-4031-80 (eB149/10H5)	AB_906266	1:67
Rat IgG2a, κ	Brilliant Violet 605	BioLegend, 400539 (RTK2758)	AB_11126979	1:67

Supplemental Figure S1.

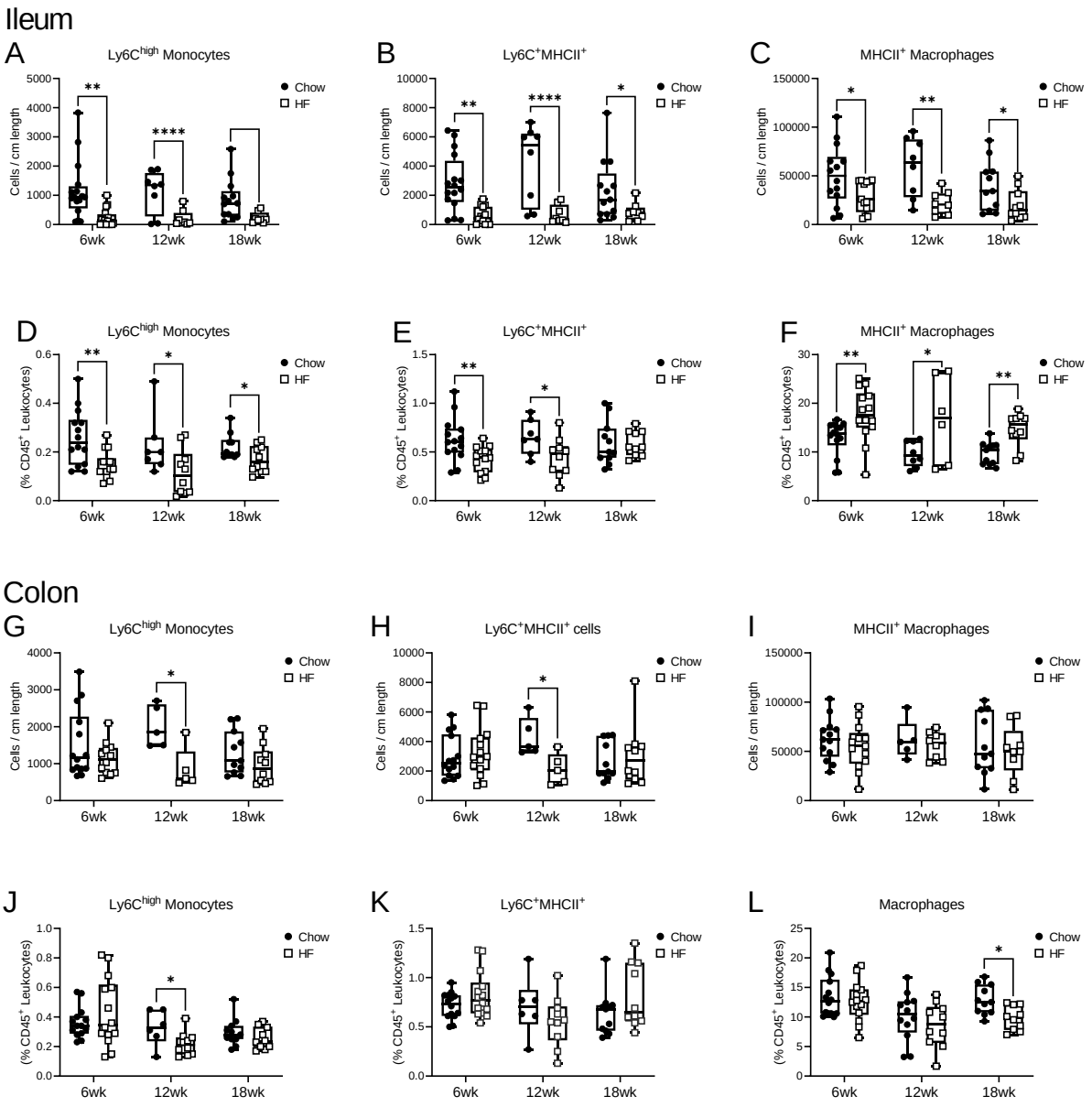


Supplemental Figure S1. Flow cytometry gating to identify intestinal macrophages. The gating strategy to identify intestinal macrophages is adapted from Shaw *et al.* (10.1084/jem.20180019). A representative ileum sample from a chow-fed mouse is shown. A) Captured events. B) Absolute Counting Beads (beads) are separated from collected events (cells) by removing $CD45^{high}$ events (see Methods). C-D) Height and width gates are applied to cells to to exclude aggregates. E) $CD45^{+}$ leukocytes are gated. F) Live-$Dead^{neg}$ cells (i.e., live $CD45^{+}$ cells) are gated. G) Low FSC/SSC events are removed. H) $CD11b^{+}$ myeloid cells are gated. I) A CD3/B220/Ly6G dump gate is used to remove neutrophils ($Ly6G^{+}SSC^{high}$). J) SiglecF is used to remove eosinophils ($SiglecF^{+}SSC^{high}$). K) $Ly6C^{neg}CD64^{neg}$ cells are removed from live $CD45^{+}CD11b^{+}CD3/B220/Ly6G^{neg}SiglecF^{neg}$ cells. L) Cells are gated according to their expression of Ly6C and MHCII: $Ly6C^{+}MHCII^{neg}$ monocytes, $Ly6C^{+}MHCII^{+}$ transitional monocyte-macrophages, $Ly6C^{neg}MHCII^{+}$ macrophages. M) $Ly6C^{high}$ monocytes are live $CD45^{+}CD11b^{+}CD3/B220/Ly6G^{neg}SiglecF^{neg}Ly6C^{high}MHCII^{neg}$ cells; N) live $CD45^{+}CD11b^{+}CD3/B220/Ly6G^{neg}SiglecF^{neg}Ly6C^{neg}MHCII^{+}$ macrophages are divided into subsets based on their expression of CD4 and TIM-4: monocyte-derived $CD4^{-}TIM4^{-}$ and $CD4^{+}TIM4^{-}$ macrophages and tissue-resident $CD4^{+}TIM4^{+}$ macrophages. O) Representative surface marker expression staining of macrophages (gated from plot L) with FMO (fluorescence-minus-one) controls.

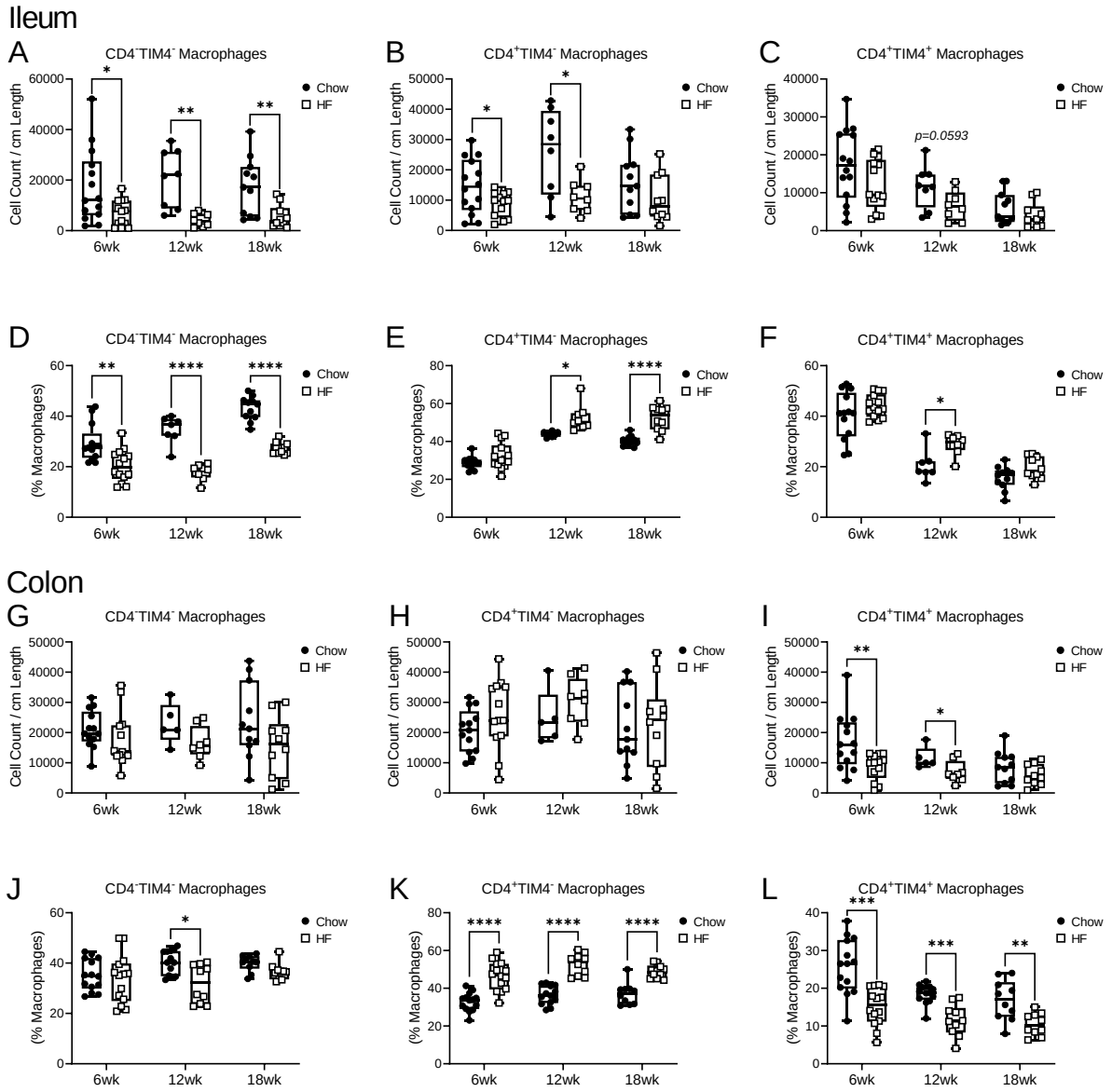
Supplemental Figure S2.



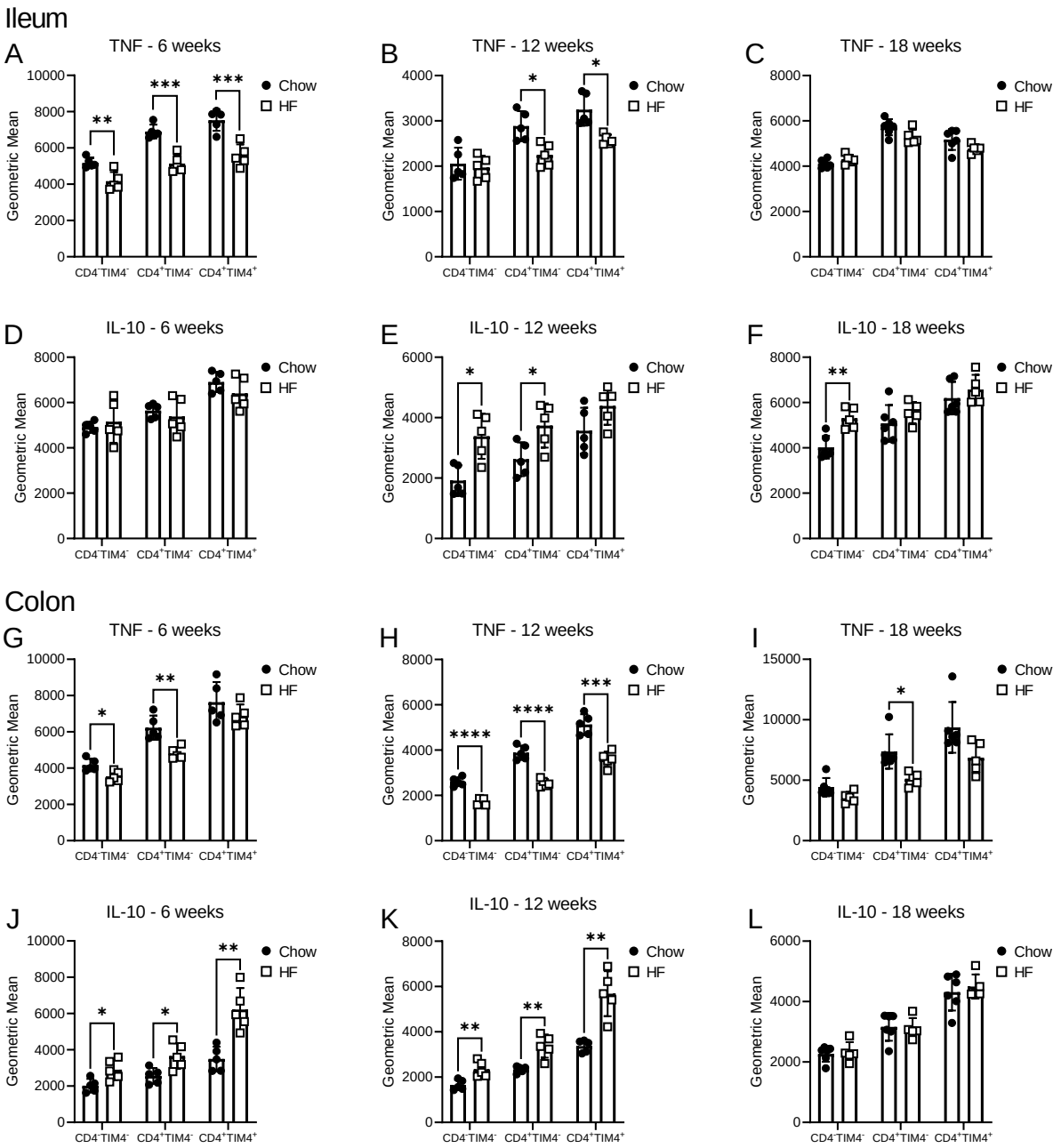
Supplemental Figure S2. Flow cytometry gating for macrophage intracellular staining. The gating strategy to identify intestinal macrophages in Supplemental Figure S2 is adapted from Shaw *et al.* (10.1084/jem.20180019). A representative colon sample from a chow-fed mouse is shown. A) Captured events. B) Absolute Counting Beads (beads) are separated from collected events (cells) by removing $CD45^{high}$ events (see Methods). C-D) Height and width gates are applied to cells to exclude aggregates. E) $CD45^{+}$ leukocytes are gated. F) $Live\text{-}Dead^{neg}$ cells (i.e., live $CD45^{+}$ cells) are gated. G) Low FSC/SSC events are removed. H-K) macrophages are identified as live $CD45^{+}CD11b^{+}SiglecF^{neg}CD64^{+}MHCII^{+}$ cells, and subdivided by their expression of CD4 and TIM-4: monocyte-derived $CD4^{-}TIM4^{-}$ and $CD4^{+}TIM4^{-}$ macrophages and tissue-resident $CD4^{+}TIM4^{+}$ macrophages. Representative plots are shown of unstained, isotype, and fully-stained samples of macrophage intracellular expression of Ki67 (L), IL-10 (M), and TNF (N).



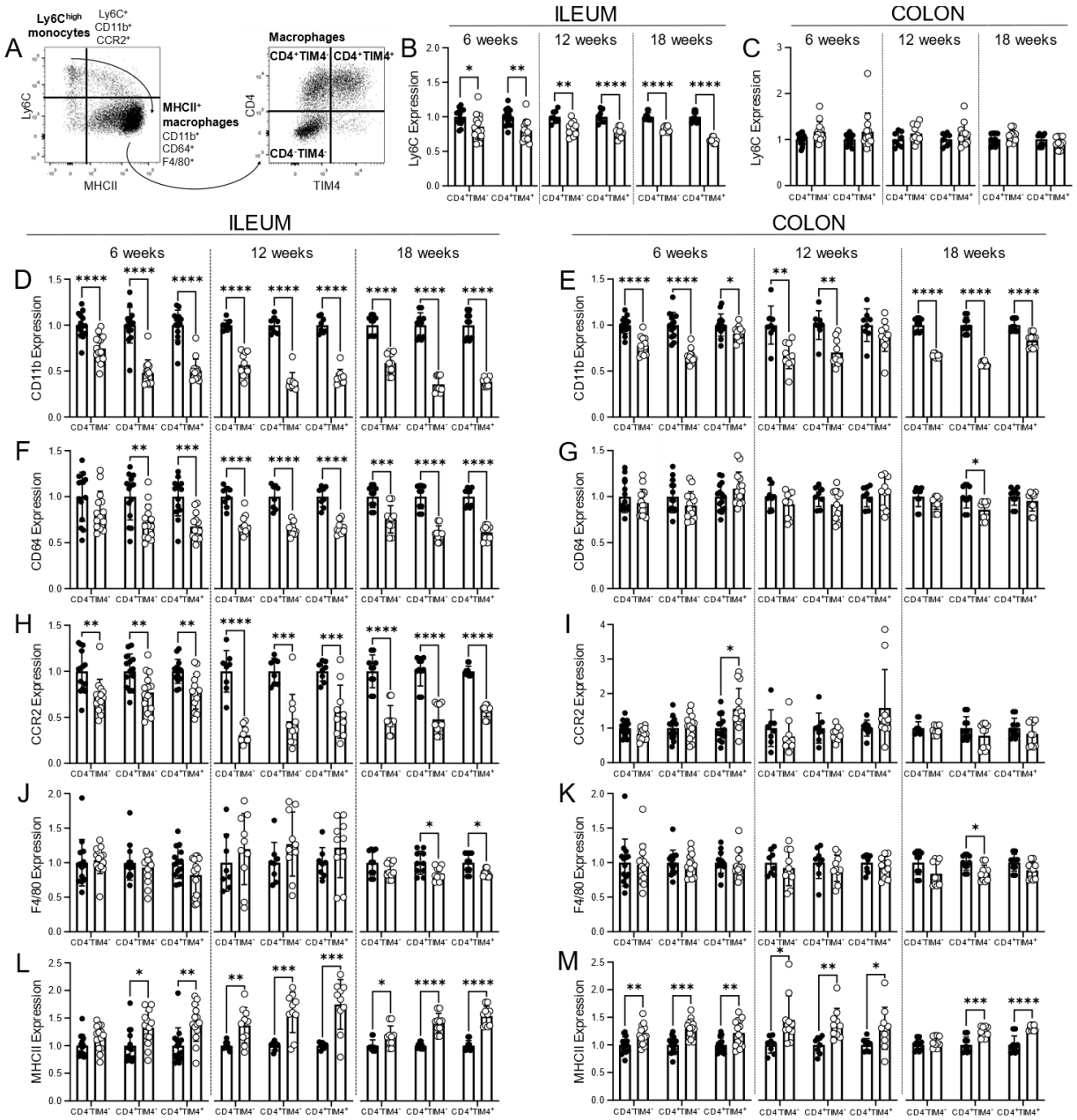
Supplemental Figure S3. Intestinal monocyte and macrophage cell numbers per tissue length and prevalence in chow and HF-fed mice. Intestinal monocyte and macrophage populations were assessed by flow cytometry in the colon and ileum after 6, 12, or 18 weeks of diet allocation to standard chow (Chow) or high fat (HF) diet. Cell numbers adjusted by ileum tissue length of: (A) Ly6C^{high} monocytes, (B) Ly6C^{+}MHCII^{+} cells, (C) total MHCII^{+} macrophages. Ileum prevalence (as a proportion of total CD45^{+} leukocytes) of: (D) Ly6C^{high} monocytes, (E) Ly6C^{+}MHCII^{+} cells, (F) total MHCII^{+} macrophages. Cell numbers adjusted by colon tissue length of: (G) Ly6C^{high} monocytes, (H) Ly6C^{+}MHCII^{+} cells (I) total MHCII^{+} macrophages. Colon prevalence (as a proportion of total CD45^{+} leukocytes) of: (J) Ly6C^{high} monocytes, (K) Ly6C^{+}MHCII^{+} cells, (L) total MHCII^{+} macrophages. Each data point indicates an individual mouse. Data are presented as box and whisker plots, minimum to maximum, with the center line at the median. Data are pooled from 1 to 3 independent experiments of n=4-5 mice per group. Statistical significance was assessed by two-tailed parametric Student's t test or Welch's t test for unequal variances or by non-parametric Mann-Whitney U test between diet groups at each time point by cell population. *$p<0.05$, **$p<0.01$, ****$p<0.0001$.



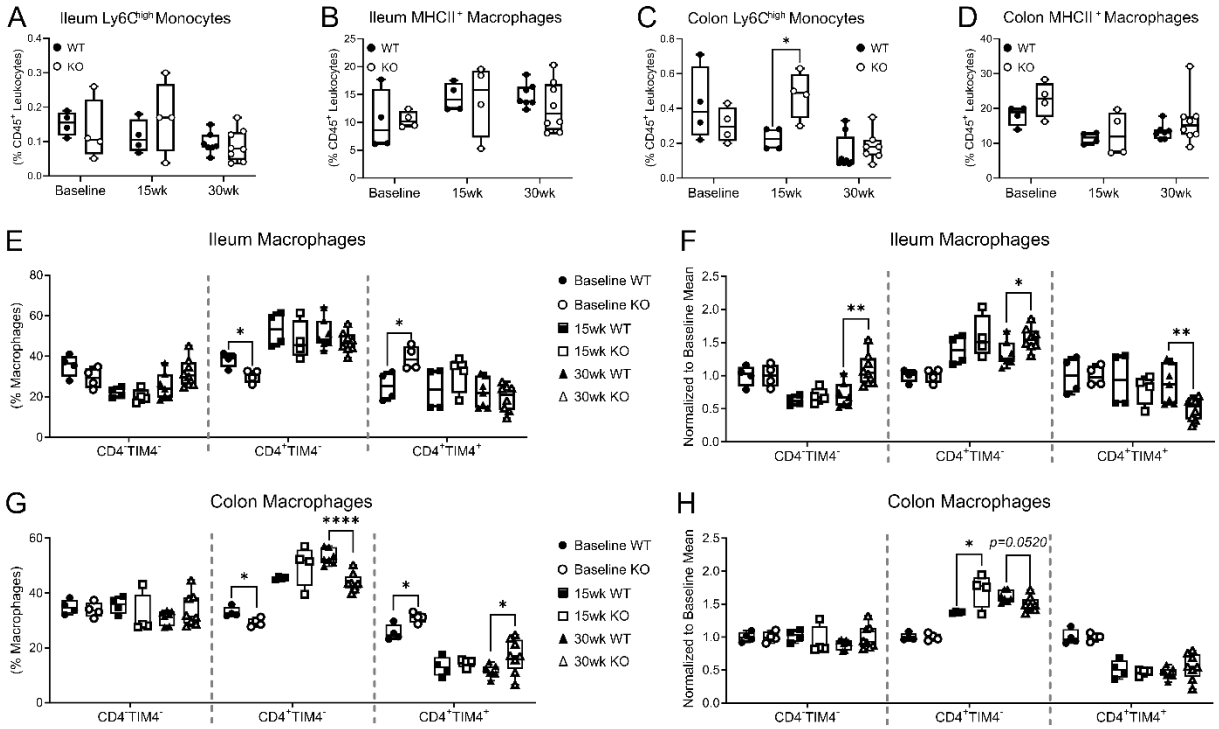
Supplemental Figure S4. Intestinal macrophage cell numbers per tissue length and prevalence in chow and HF-fed mice. Intestinal monocyte and macrophage populations were assessed by flow cytometry in the colon and ileum after 6, 12, or 18 weeks of diet allocation to standard chow (Chow) or high fat (HF) diet. Cell numbers adjusted by ileum tissue length of: (A) $CD4^{-}TIM4^{-}$ macrophages, (B) $CD4^{+}TIM4^{-}$ macrophages, (C) $CD4^{+}TIM4^{+}$ macrophages. Ileum prevalence (as a proportion of total macrophages) of: (D) $CD4^{-}TIM4^{-}$ macrophages, (E) $CD4^{+}TIM4^{-}$ macrophages, and (F) $CD4^{+}TIM4^{+}$ macrophages. Cell numbers adjusted by colon tissue length of: (G) $CD4^{-}TIM4^{-}$ macrophages, (H) $CD4^{+}TIM4^{-}$ macrophages, (I) $CD4^{+}TIM4^{+}$ macrophages. Colon prevalence (as a proportion of total macrophages) of: (J) $CD4^{-}TIM4^{-}$ macrophages, (K) $CD4^{+}TIM4^{-}$ macrophages, and (L) $CD4^{+}TIM4^{+}$ macrophages. Each data point indicates an individual mouse. Data are presented as box and whisker plots, minimum to maximum, with the center line at the median. Data are pooled from 1 to 3 independent experiments of n=4-5 mice per group. Statistical significance was assessed by two-tailed parametric Student's t test or Welch's t test for unequal variances or by non-parametric Mann-Whitney U test between diet groups at each time point by macrophage population. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.



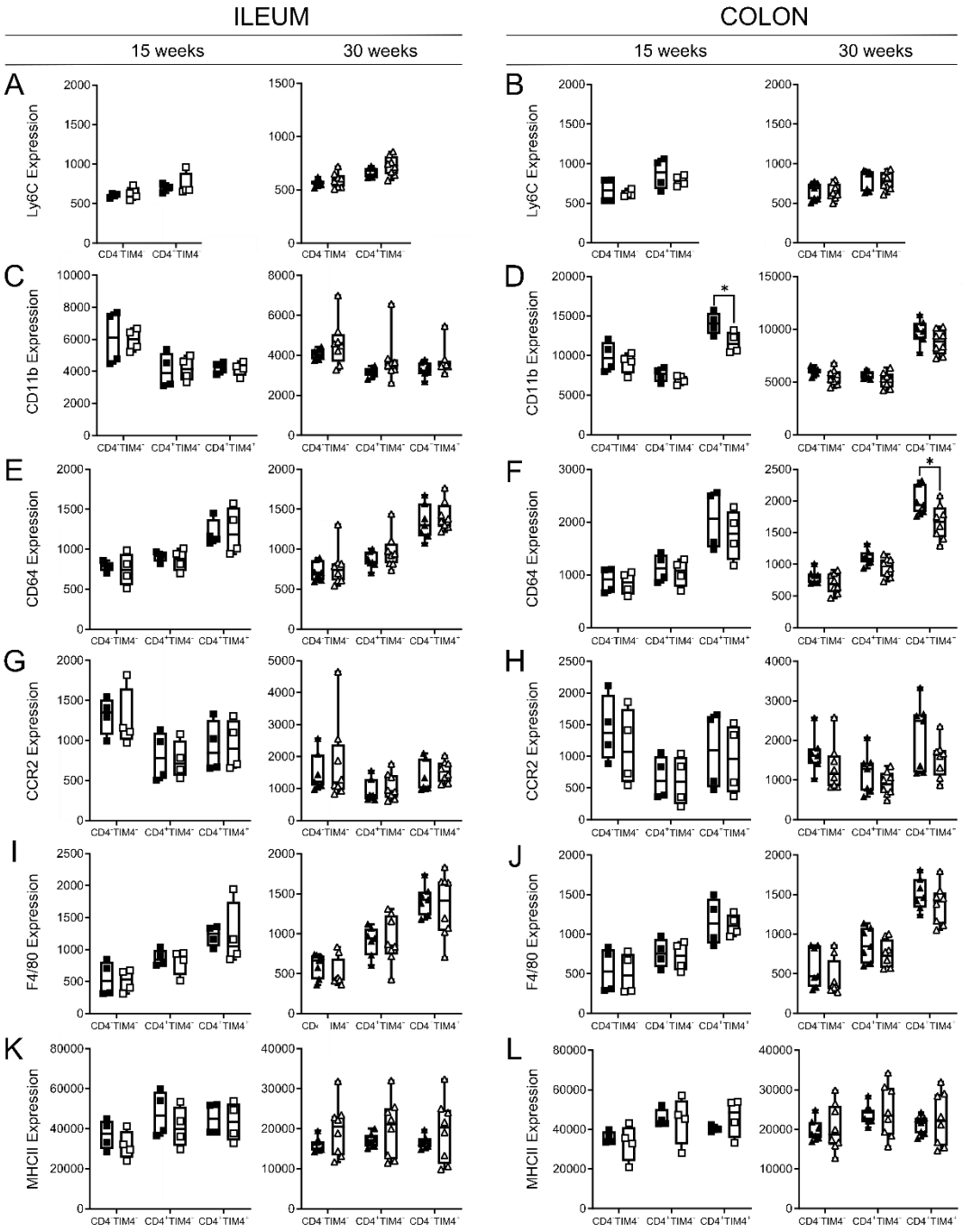
Supplemental Figure S5. TNF and IL-10 expression in ileum and colon macrophages of chow and HF-fed mice. Ileum and colon $CD4^{-}TIM4^{-}$, $CD4^{+}TIM4^{-}$, and $CD4^{+}TIM4^{+}$ macrophage intracellular expression of TNF and IL-10 was assessed by flow cytometry after 6, 12, or 18 weeks of allocation to standard chow (Chow) or high fat (HF) diet. Ileum macrophage intracellular expression of TNF after: (A) 6 weeks, (B) 12 weeks, (C) 18 weeks. Ileum macrophage intracellular expression of IL-10 after: (D) 6 weeks, (E) 12 weeks, (F) 18 weeks. Colon macrophage intracellular expression of TNF after: (G) 6 weeks, (H) 12 weeks, (I) 18 weeks. Colon macrophage intracellular expression of IL-10 after: (J) 6 weeks, (K) 12 weeks, (L) 18 weeks. Each data point indicates an individual mouse. Data are from 1 independent experiment at each time point of n=5-6 mice per group. Data are presented as box and whisker plots, minimum to maximum, where the center line indicates the median. Statistical significance was assessed by two-tailed parametric Student's t test or Welch's t test for unequal variances or by non-parametric Mann-Whitney U test between diet groups at each time point by macrophage population. *$*p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.*



Supplemental Figure S6. Intestinal macrophage surface marker phenotype is altered between chow and HF-fed mice. Flow cytometry analysis of $CD4^{-}TIM4^{-}$, $CD4^{+}TIM4^{-}$, and $CD4^{+}TIM4^{+}$ macrophages in the ileums and colons of female mice fed a standard chow (black-filled circles) or high fat (white-filled circles) diet after 6-, 12-, or 18-weeks diet allocation. (A) surface marker phenotype changes in the monocyte-to-macrophage transition. Macrophage phenotype was assessed by examining surface expression (geometric mean fluorescence intensity) of Ly6C (B-C), CD11b (D-E), CD64 (F-G), CCR2 (H-I), F4/80 (J-K), and MHCII (L-M). Macrophage surface marker phenotype was compared across lengths of diet allocation (i.e., independent experiments) by normalizing the geometric mean data from each mouse to the mean of the chow mouse group for each macrophage population and surface marker in each independent experiment. Geometric mean expression data are also presented as concatenated histograms in Figure 4. Each data point indicates an individual mouse. Data are from 2 or 3 independent experiments of n=4-5 mice per group at each time point. Data are presented with box height at the mean with error bars at ± standard deviation. Statistical significance for each surface marker was assessed between diet groups at each time point by macrophage population by two-tailed parametric Student's t test or Welch's t test for unequal variances or by non-parametric Mann-Whitney U test. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.



Supplemental Figure S7. Intestinal monocyte and macrophage prevalence in HF-fed littermate WT and TNF$^{-/-}$ mice. Littermate wildtype (WT) and TNF$^{-/-}$ (KO) mouse intestinal monocytes and macrophages were assessed by flow cytometry prior to diet allocation (Baseline), and after 15 weeks (15wk) or 30 weeks (30wk) of high fat diet allocation. Ileum prevalence (as a proportion of total CD45$^+$ leukocytes) of: (A) Ly6C^{high} monocytes, (B) total MHCII$^+$ macrophages. Colon prevalence (as a proportion of total CD45$^+$ leukocytes) of: (C) Ly6C^{high} monocytes, (D) total MHCII$^+$ macrophages. (E) ileum prevalence of CD4$^-$TIM4$^-$, CD4$^+$TIM4$^-$, and CD4$^+$TIM4$^+$ macrophages (as a proportion of total macrophages). (F) ileum CD4$^-$TIM4$^-$, CD4$^+$TIM4$^-$, and CD4$^+$TIM4$^+$ macrophages normalized to the mean of the baseline data to adjust for between-genotype differences in prevalence. (G) colon prevalence of CD4$^-$TIM4$^-$, CD4$^+$TIM4$^-$, and CD4$^+$TIM4$^+$ macrophages (as a proportion of total macrophages). (F) colon CD4$^-$TIM4$^-$, CD4$^+$TIM4$^-$, and CD4$^+$TIM4$^+$ macrophages normalized to the mean of the baseline data to adjust for between-genotype differences in prevalence. Each data point indicates an individual mouse. Data are presented as box and whisker plots, minimum to maximum, with the center line at the median. Data in F and H were normalized by dividing each data point from 15- and 30-weeks diet intake to the mean of the respective genotype group at baseline. Data are from independent experiments of WT n=4 and KO n=4 at Baseline or 15 weeks, and WT n=7 and KO n=8 at 30 weeks. Statistical significance was assessed by two-tailed parametric Student's t test or Welch's t test for unequal variances or by non-parametric Mann-Whitney U test between genotypes at each time point by monocyte or macrophage population. *$p<0.05$, **$p<0.01$, ****$p<0.0001$.



Supplemental Figure S8. TNF does not mediate changes to macrophage surface marker phenotype in HF-fed female mice. Flow cytometry analysis of $CD4^{-}TIM4^{-}$, $CD4^{+}TIM4^{-}$, and $CD4^{+}TIM4^{+}$ macrophage populations in the ileums and colons of littermate wildtype (WT) (black-filled data points) and $TNF^{-/-}$ (KO) (white-filled data points) female mice on high fat diet for 15 or 30 weeks. Macrophage phenotype was assessed by examining surface expression (geometric mean fluorescence intensity) of Ly6C (A-B), CD11b (C-D), CD64 (E-F), CCR2 (G-H), F4/80 (I-J), and MHCII (K-L). Geometric mean expression data are presented as concatenated histograms in Figure 7. Each data point indicates an individual mouse. Data are from one experiment of WT n=4 and KO n=4 mice at 15 weeks, and WT n=7 and KO n=8 mice at 30 weeks. Statistical significance was assessed by two-tailed parametric Student's t test or Welch's t test for unequal variances or by non-parametric Mann-Whitney U test between genotypes by macrophage population at each time point. *$p<0.05$.