Figure S1

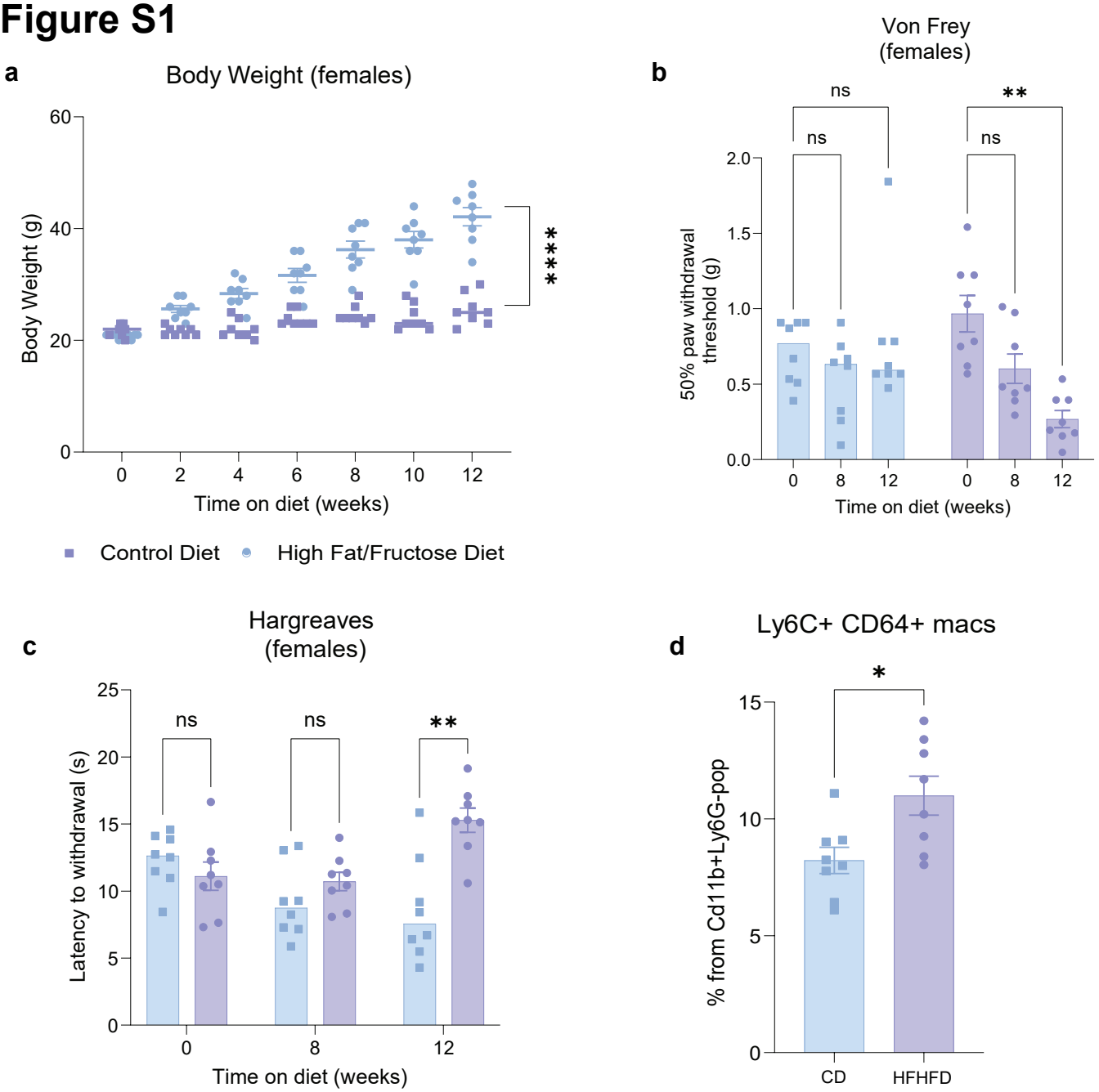


Fig S1. Female mice on HFHFD are equally susceptible to weight gain, behavioral changes, and immune cell infiltration in nerves

a. Body weight measurement over time on HFHFD vs CD feeding from female C57Bl6 mice (p-value <0.0001). **b.** Von Frey up down method was used to assess mechanical sensitivity in female mice fed HFHFD vs CD over 12 weeks (p-value 0 vs 12w = 0.0058). **c.** Hargreaves assay was used to assess heat sensitivity in female mice fed HFHFD vs CD over 12 weeks (p-value = 0.0044). **d.** Proportions of Ly6c+ CD64+ recruited macrophages from Live Cd45+ Cd11b+ cells in sciatic nerves of female mice fed HFHFD vs CD for 12 weeks (p-value = 0.0153).

Figure S2

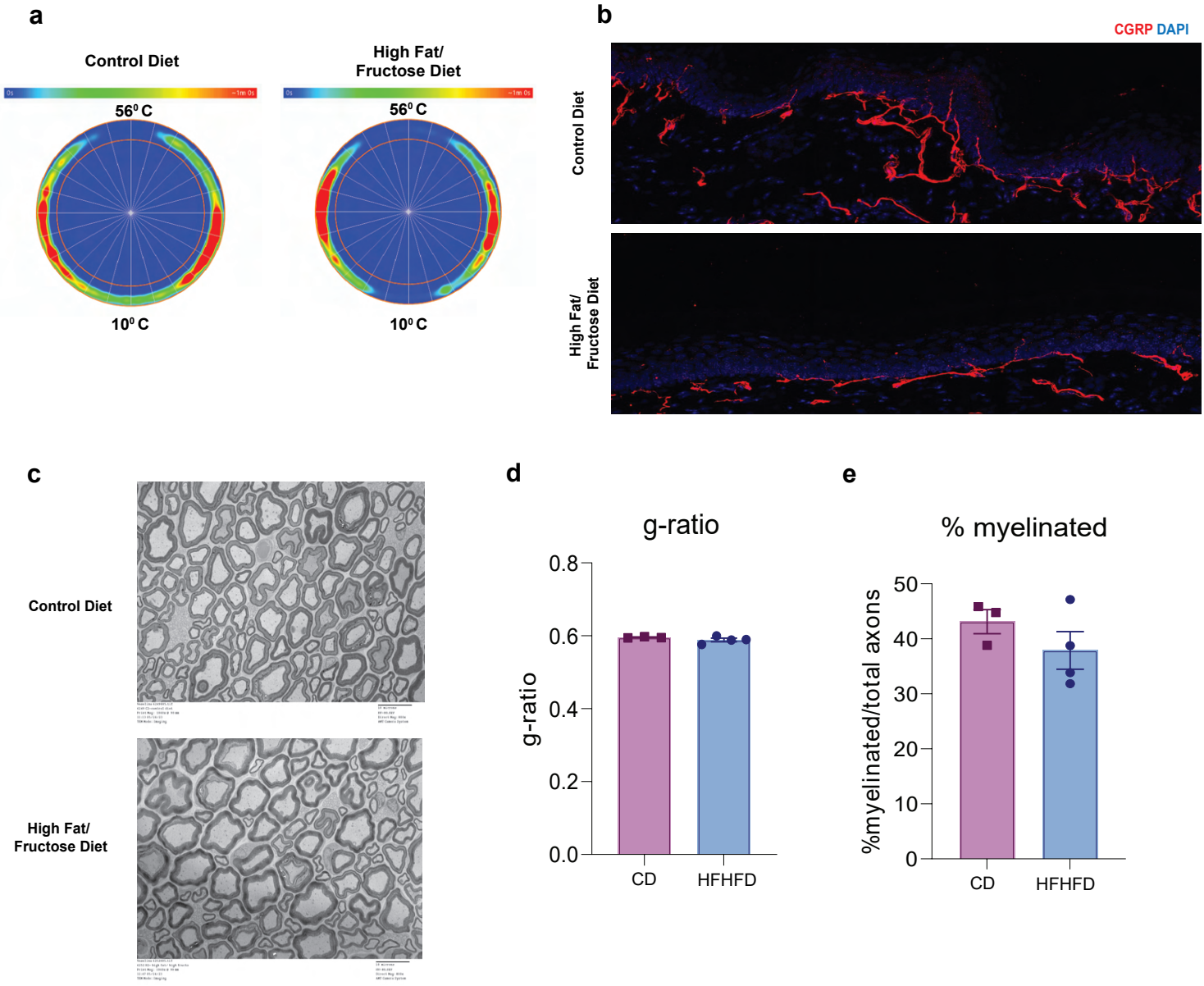


Fig S2. Behavior and histological parameters tested on CD vs HFHFD fed mice
a. Representative heat maps showing the location of the mouse during a 1-hour thermal gradient ring recording. **b.** Representative immunohistochemistry images with CGRP stained in red and DAPI in blue from CD and HFHFD hind paw skin after 24 weeks of feeding. **c.** Representative EM images from sciatic nerves of mice fed CD and HFHFD for 12 weeks. **d.** Quantification of EM images of g-ratio and **e**. percent myelinated fibers. Each dot represents an average of 2 images from each mouse.

Figure S3

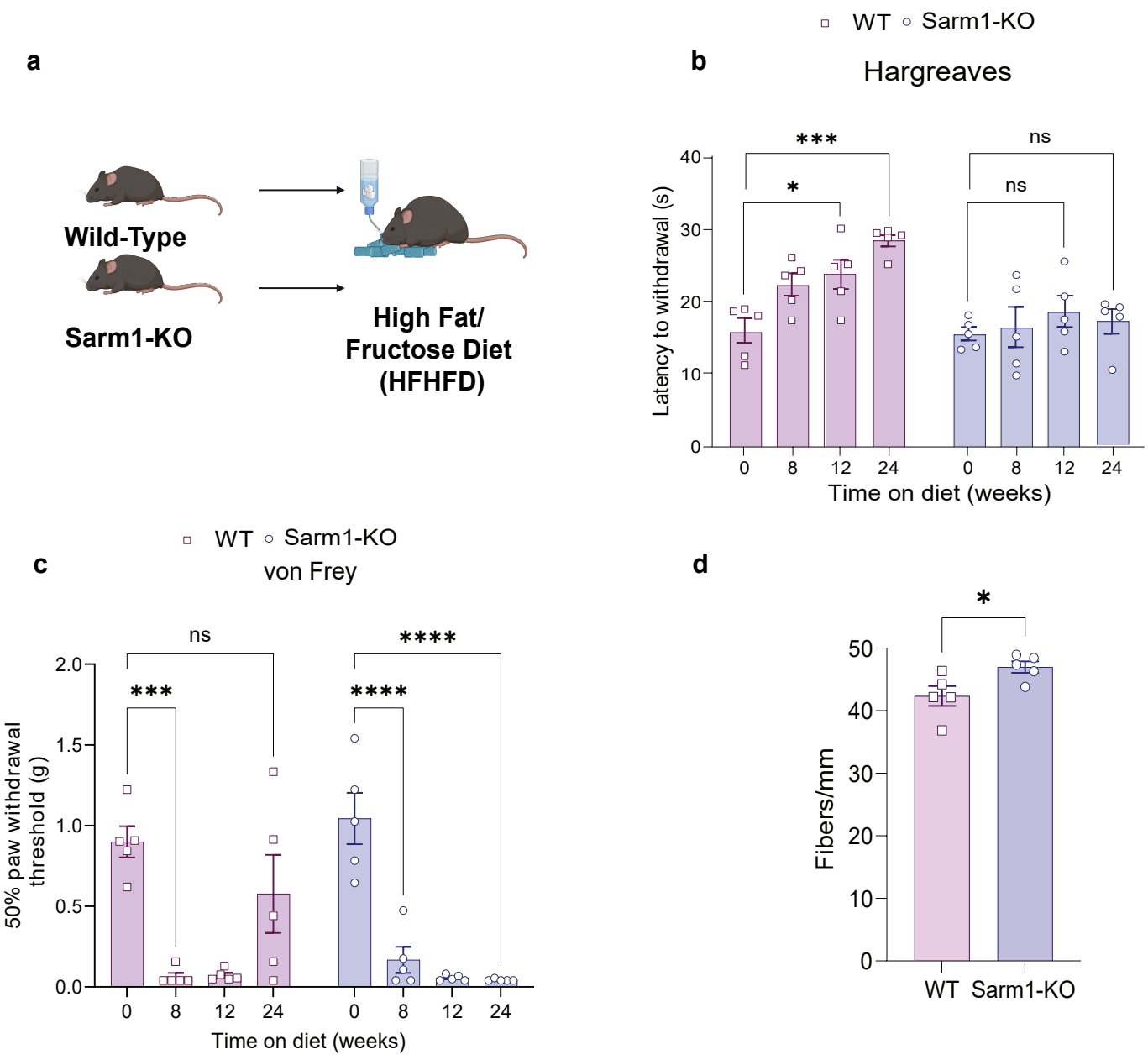


Fig S3. Sarm1 knockdown prevents the loss of heat sensitivity and skin axonal degeneration in HFHFD-fed mice but leads to persistent mechanical allodynia

a. Illustration of the experiment. **b.** Hargreaves test was used to assess thermal sensitivity in WT vs Sarm1-KO male mice fed HFHFD or CD over 24 weeks. **c.** Von Frey filaments were used to assess mechanical sensitivity in WT vs Sarm1-KO male mice fed HFHFD or CD over 24 weeks. **d.** Quantification of IENFD from PGP9.5 staining in hind paws of skin from WT and Sarm1-KO male mice fed HFHFD or CD for 24 weeks (p-value = 0.0353).

Figure S4

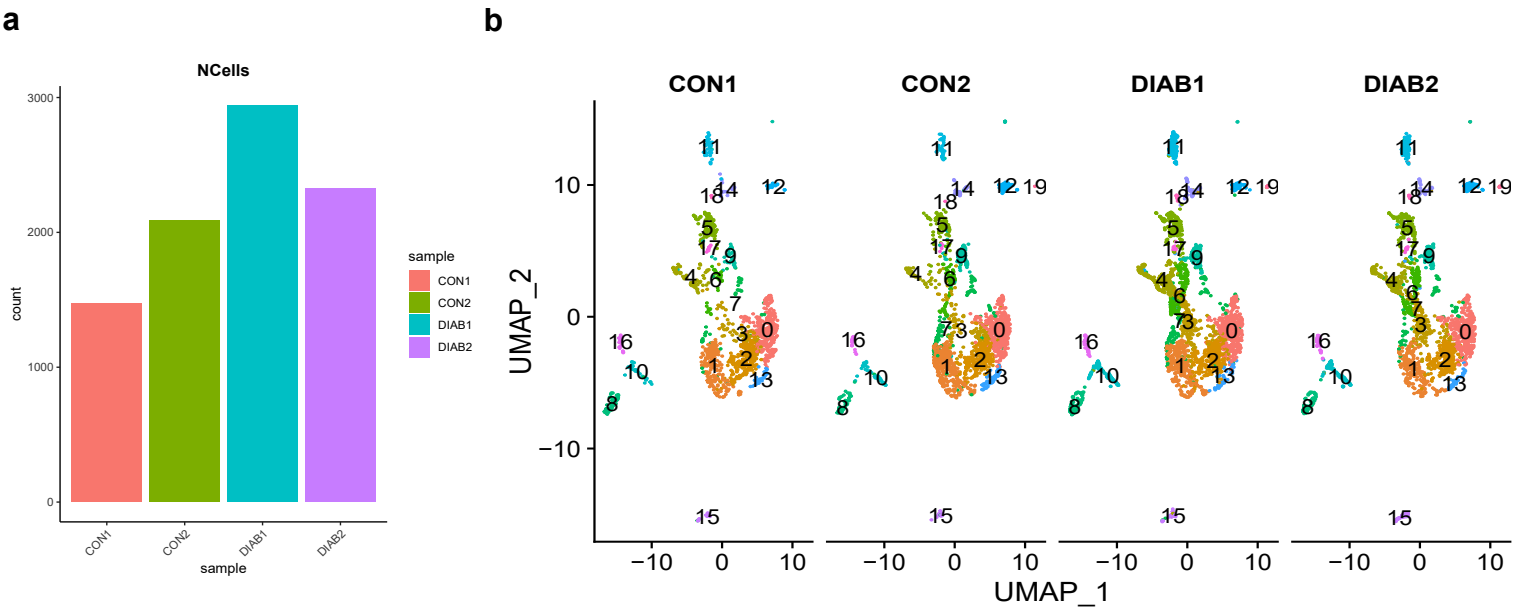


Fig S4. Sciatic nerve immune cell single cell sequencing
a. Number of cells analyzed in each sample post-filtering. **b.** UMAP plot showing similar cell composition in replicates of each condition.

Figure S5

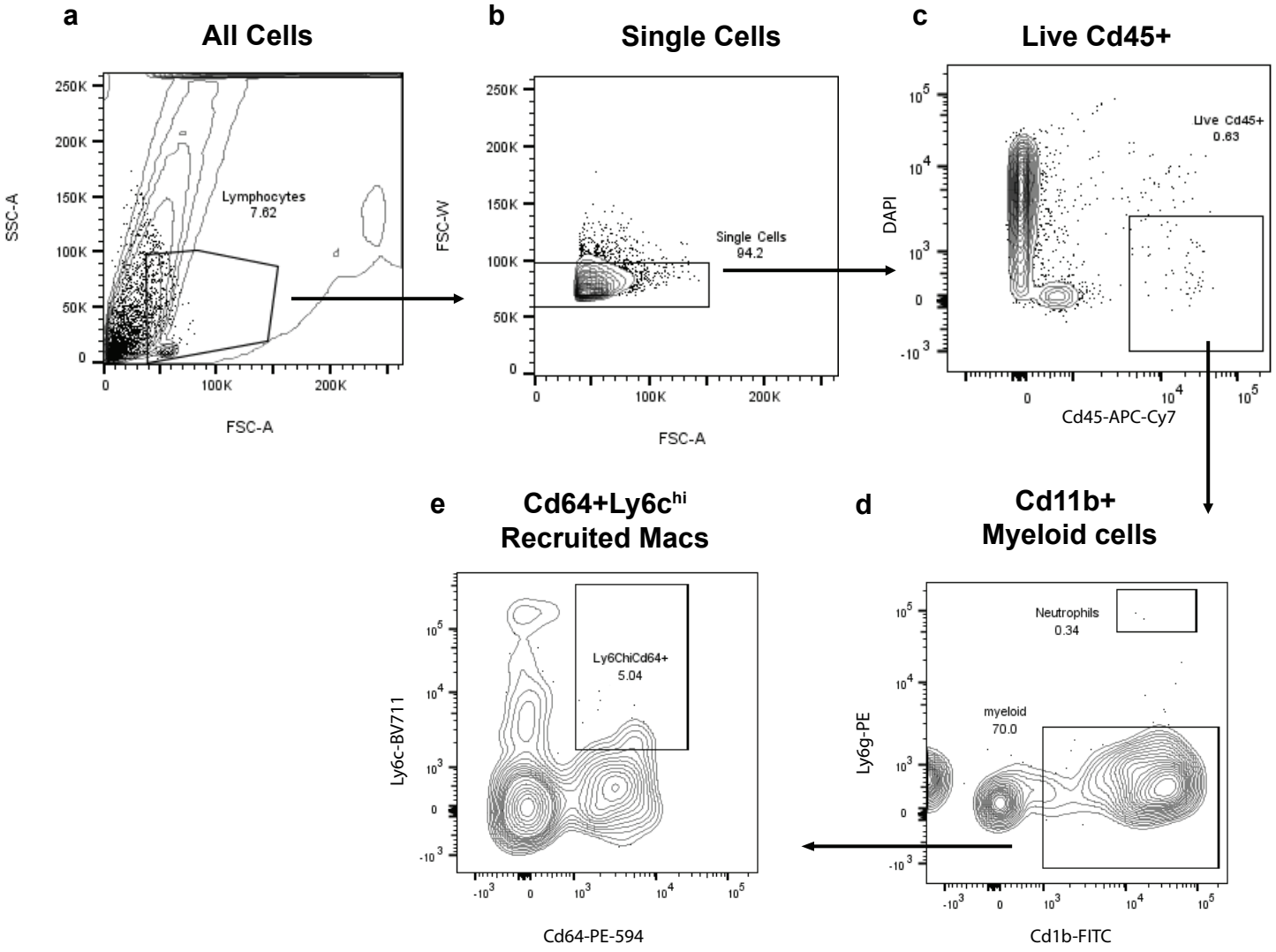
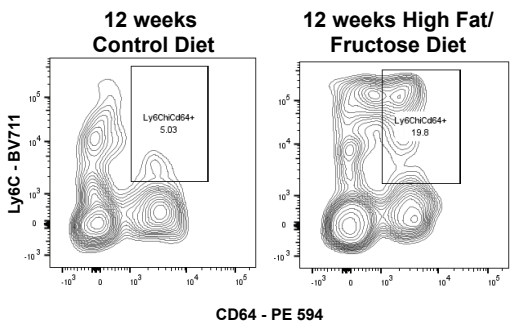


Fig S5. Gating strategy for flow cytometry assessment of recruited macrophages into the sciatic nerve

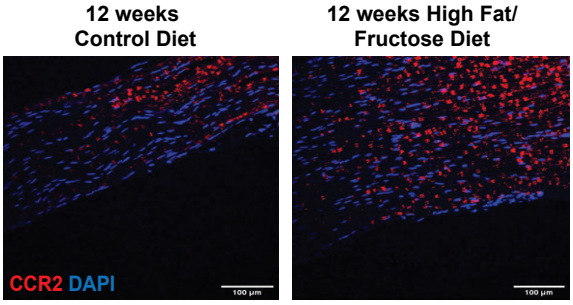
a. FSC-A and SSC-A were used to gate on all cells and exclude aggregates and small debris. **b.** FSC-A and FSC-W were used to gate on single cells. **c.** DAPI and CD45-APC-Cy7 were used to capture Live CD45+ immune cells. **d.** Cd11b-FITC and Ly6g-PE were used to gate on myeloid cells and exclude neutrophils**. e.** Cd64-PE-594 and Ly6c-BV711 were used to identify Ly6C+ Cd64+ recruited macrophages.

Figure S6

a



b



c

GO Biological Process	Fold Enrichment	P-value
Eosinophil chemotaxis	> 100	6.03E-10
T cell extravasation	>100	1.29E-04
Negative regulation of natural killer cell activation	>100	2.23E-04
Regulation of serotonin secretion	>100	2.23E-04
Monocyte chemotaxis	85.07	6.42E-0-
Brown fat cell differentiation	56.44	1.07E-06
Lymphocyte chemotaxis	53.81	1.27E-06
Long -chain fatty acid transport	46.28	2.24E-06
Neutrophil chemotaxis	45.08	5.98E-09
Chemokine -mediated signaling pathway	43.17	1.50E-07

Fig S6. Changes in recruited and resident macrophages in sciatic nerves of mice fed HFHFD vs CD for 12 weeks

a. Representative flow cytometry contour plot of Ly6C and CD64 gate, pre-gated on Live CD45+Cd11b+ showing difference between CD and HFHFD sciatic nerves. **b.** Immunohistochemistry of sciatic nerves showing CCR2 in red, labeling recruited macrophages, and DAPI in blue from mice fed HFHFD vs CD for 12 weeks. **c**. GO pathway analysis from differentially expressed genes in resident resMacs clusters in HFHFD vs CD showing enrichment in chemotaxis pathways.

Figure S7

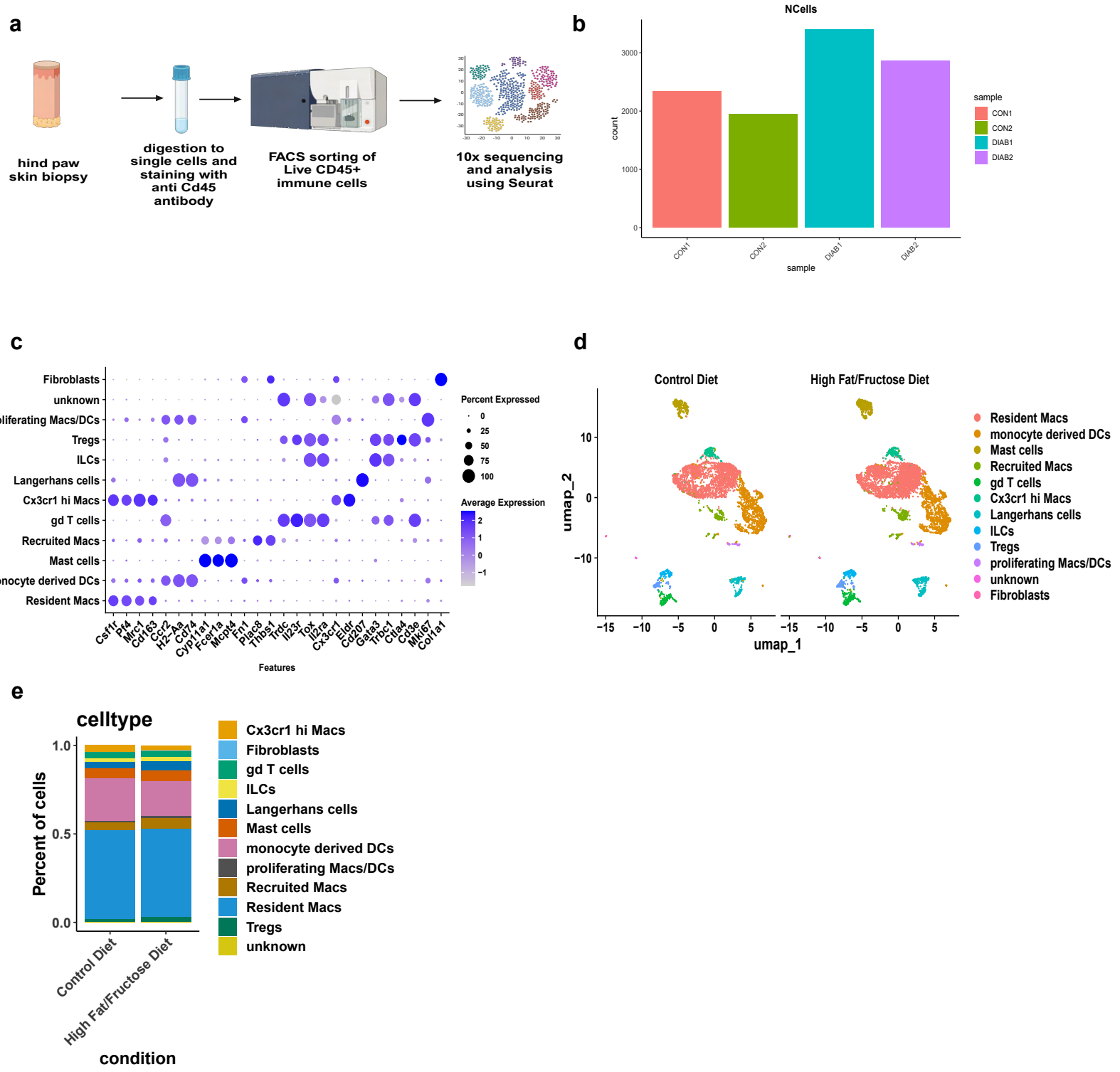


Fig S7. Single cell sequencing of skin immune cells from mice fed HFHFD or CD for 12 weeks shows no change in immune cell composition

a. Workflow for tissue collection, processing, sorting, and sequencing of skin immune cells from male mice fed HFHFD or CD for 12 weeks from 4 mice per group pooled into 2 samples each group. **b.** Number of cells analyzed in each sample post-filtering. **c.** Dot plot of marker genes used to identify different immune cells in dataset. **d.** UMAP plot showing clusters identified in sciatic nerves of CD and HFHFD fed mice from 2 samples per group. **e.** Barplot showing proportions of the different cell types per group.

Figure S8

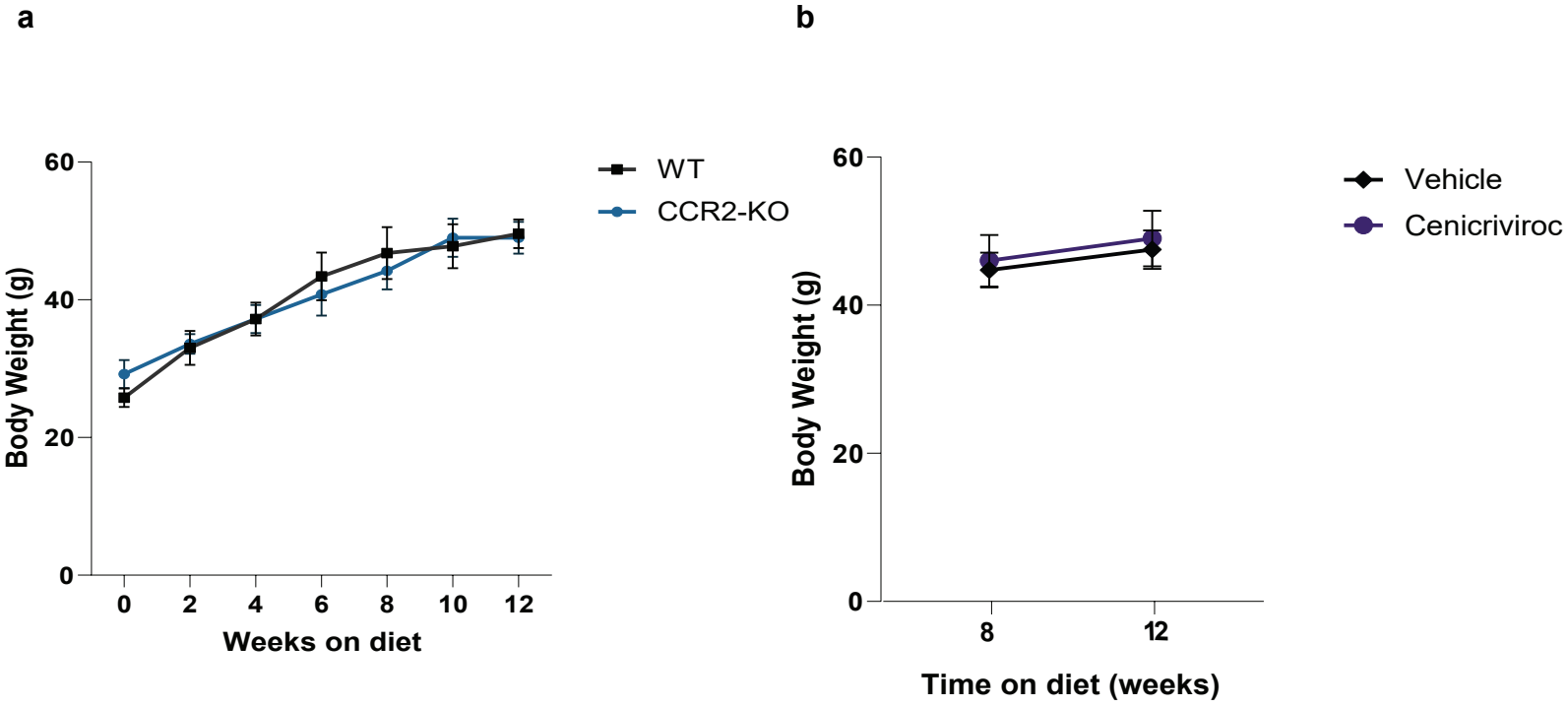


Fig S8. Blocking macrophage recruitment does not affect body weight gain
a. Body weight measurements in age matched CCR2-KO and WT mice over 12 weeks on HFHFD (n=5 mice per group). **b.** Body weight measurement of mice injected with CVC or vehicle before start of injections and at the end of experiment (n=4 per group).