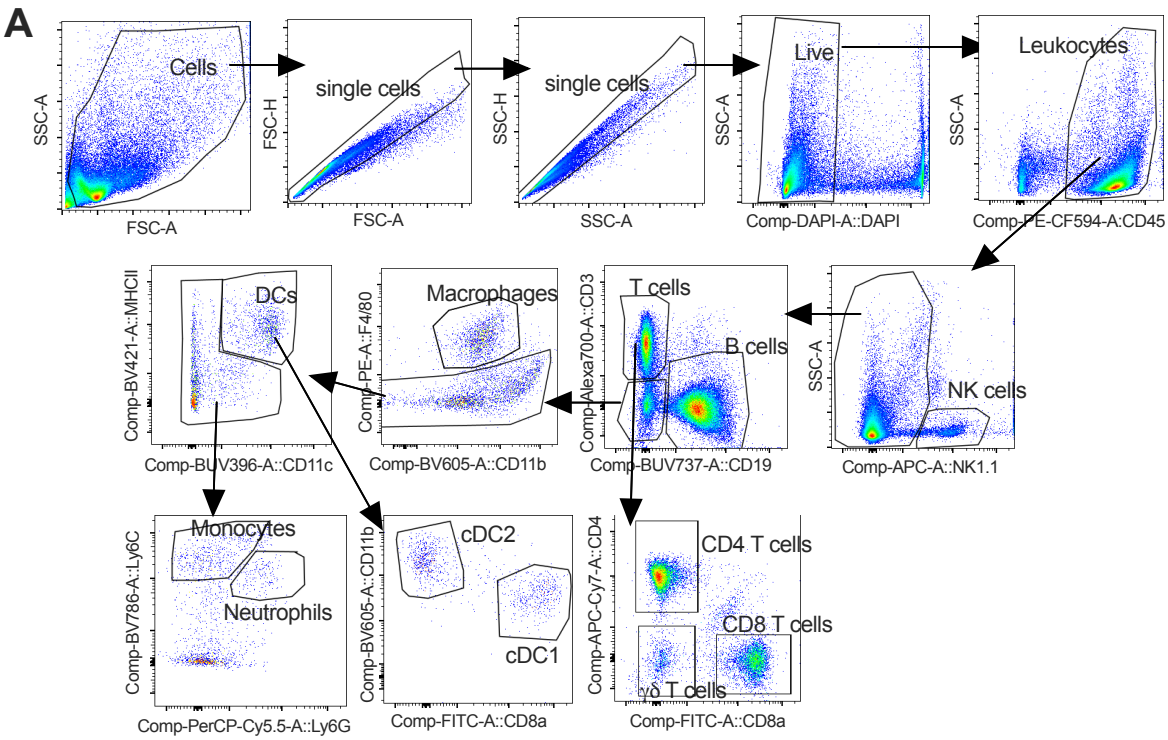
Supplemental Figure 1



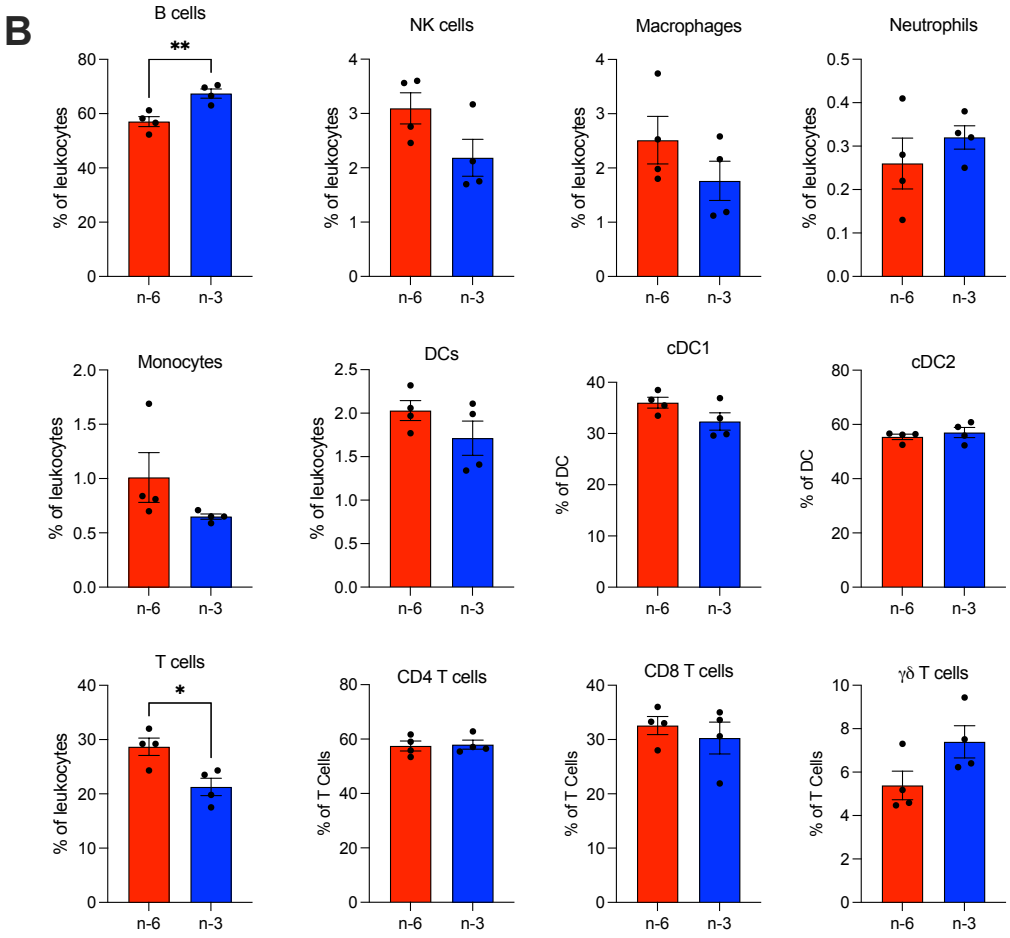


Figure S1. Flow cytometry gating strategy and proportion of splenic immune cell subsets in mice under the different diets. A) Representative gating workflow used to identify major splenic immune cell subsets. B) Mice were maintained on n-6 or n-3 PUFA-enriched diets for 9–10 weeks (n = 4 per group). Spleens were harvested, processed into single-cell suspensions, and stained for flow cytometric analysis of major immune cell populations. Bar graphs show the relative proportions of splenic immune subsets, including B cells, NK cells, macrophages, monocytes, neutrophils, dendritic cells, and T cells. Data are presented as mean ± SEM. Statistical significance was assessed using an unpaired two-tailed Student's t-test. $*P < 0.05$, $**P < 0.01$

Supplemental Figure 2

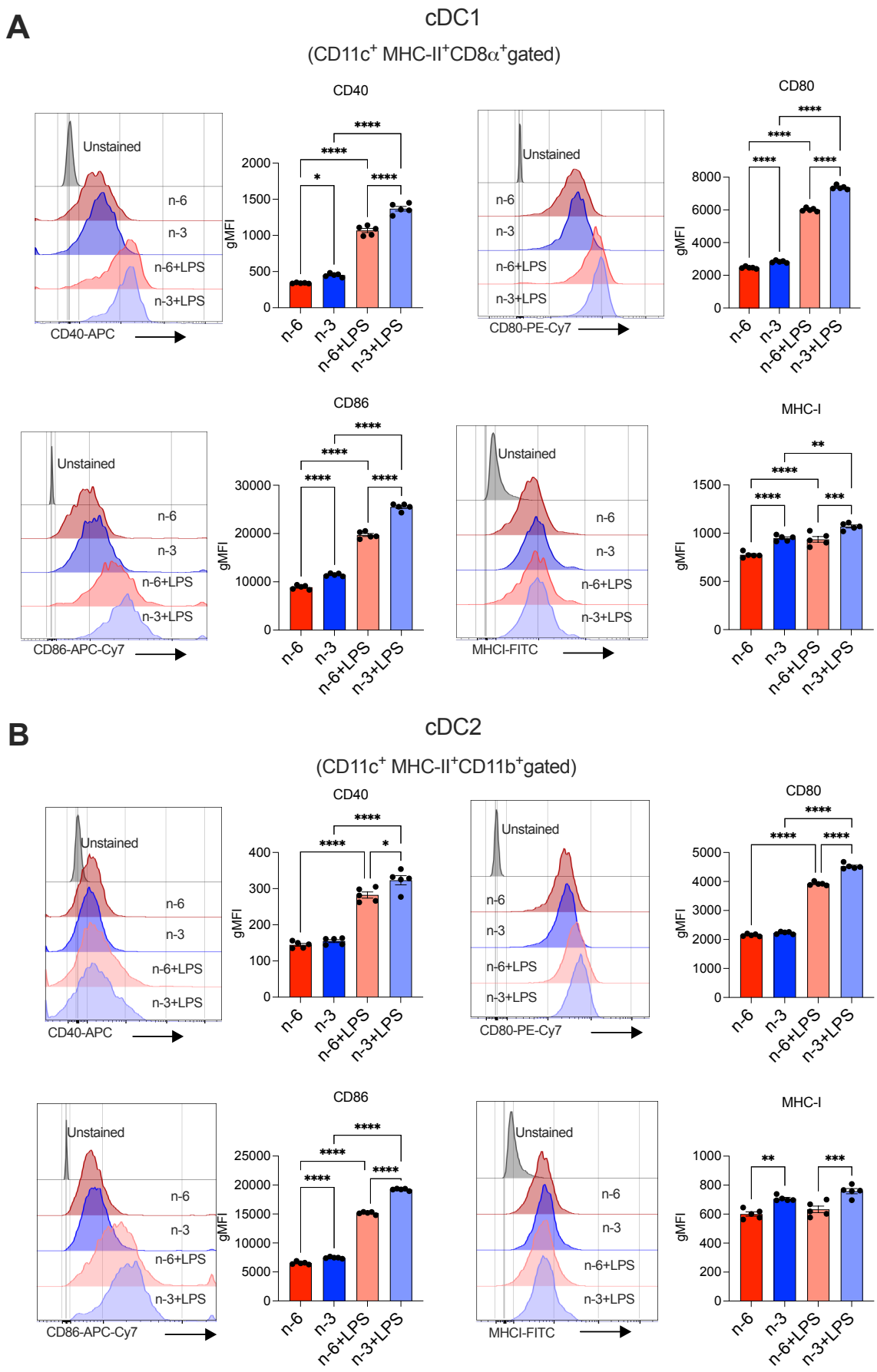


Figure S2. Dietary PUFAs modulate co-stimulatory and MHC-I molecule expression in splenic DC subsets. Mice were maintained on n-6 or n-3 PUFA-enriched diets for 9–10 weeks (n = 5 per group). DCs were isolated from spleen and stimulated with LPS (100 ng/mL) or vehicle control for 6 hours. Cells were then stained for surface expression of CD40, CD80, CD86, and MHC class I. Representative histograms and bar graphs depict expression levels of the indicated markers on: (A) conventional type 1 DCs (cDC1; $CD11c^+$ $MHC\text{-}II^+$ $CD8\alpha^+$) and (B) conventional type 2 DCs (cDC2; $CD11c^+$ $MHC\text{-}II^+$ $CD11b^+$). Data are presented as mean ± SEM and represent at least two independent experiments. Statistical significance was assessed using one-way ANOVA followed by Tukey's multiple comparisons test: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Supplemental Figure 3

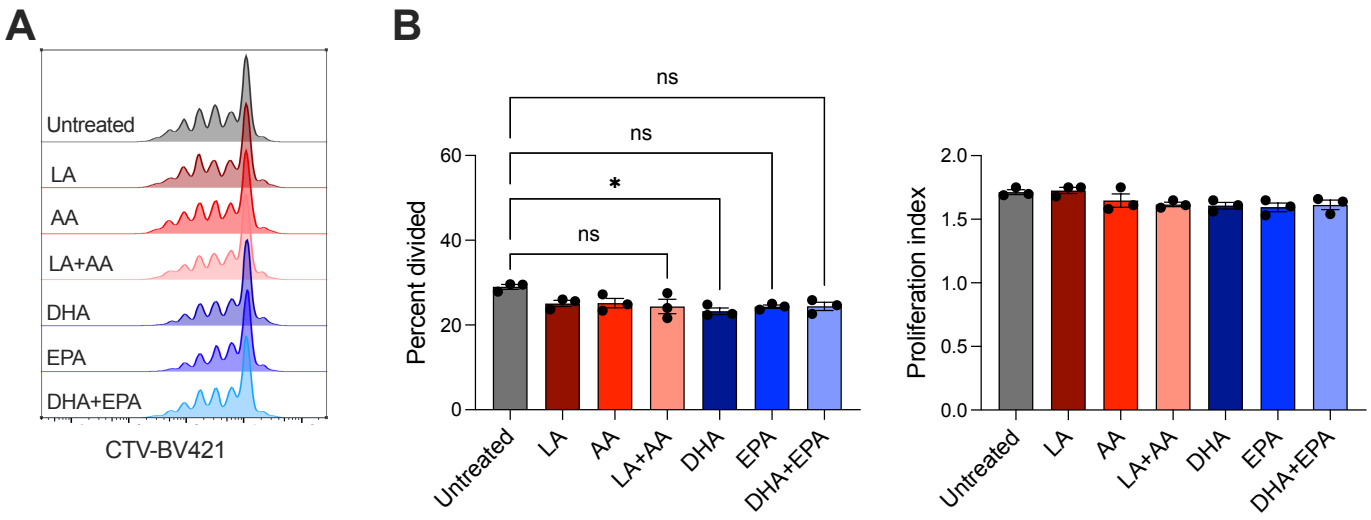


Figure S3. **Effects of ex vivo exposure to defined n-6 and n-3 fatty acids**. Splenic dendritic cells (spDCs) isolated from chow diet–fed mice were treated ex vivo with the indicated purified n-6 (linoleic acid (LA), Adrenic acid (AA) alone or in combination) or n-3 (eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) alone or in combination) as described in the Methods. (A) Representative flow cytometry histograms showing CTV dilution in proliferating $CD8^+$ T cells co-cultured with fatty acid–treated spDCs. (B) Quantification of T cell proliferation parameters, including percent divided and proliferation index. Data represent at least two independent experiments with comparable results and are presented as mean ± SEM. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple-comparisons test: $P < 0.05$.

Supplemental Figure 4

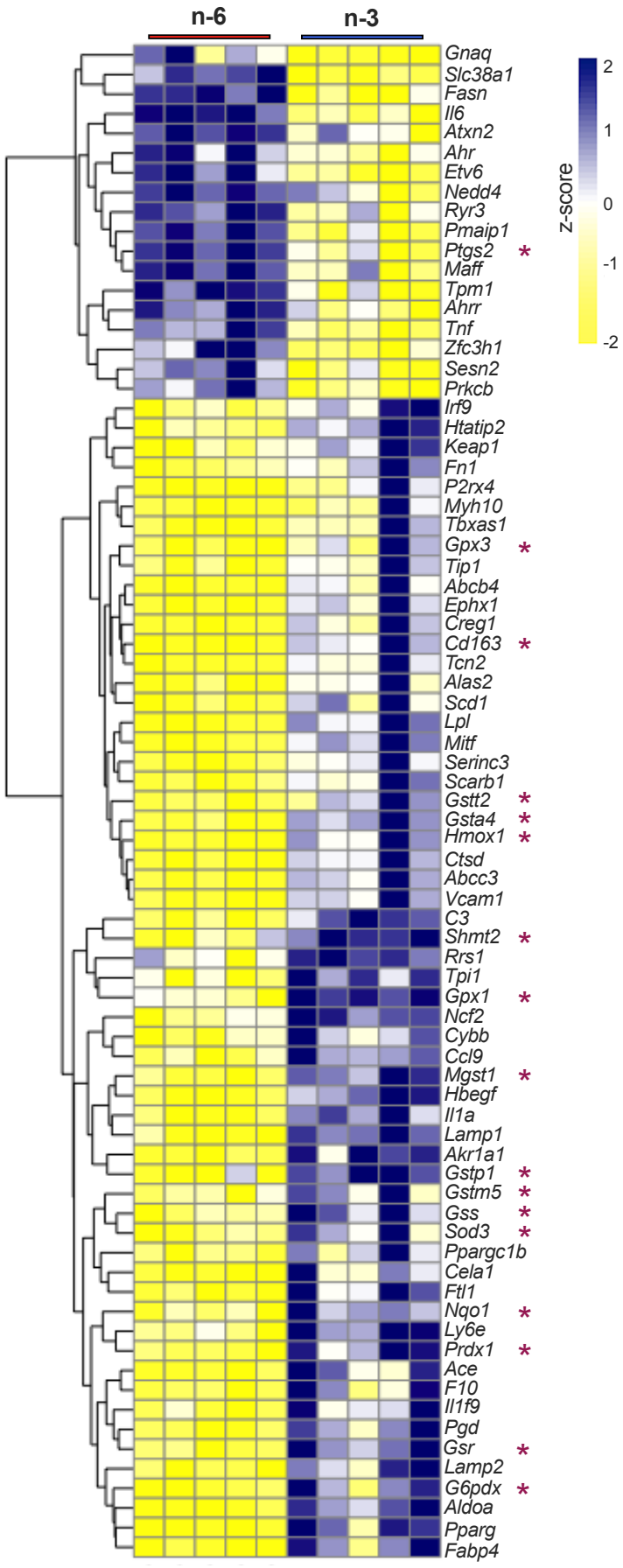


Figure S4. Heat map of Nrf2-regulated genes differentially expressed in splenic DCs according to diet. Heat maps show expression profiles of Nrf2 target genes in spDCs isolated from mice fed n-3 versus n-6 PUFA-enriched diets (n = 5 per group). Genes marked with an asterisk represent those significantly upregulated in n-3–conditioned spDCs that are implicated in glutathione biosynthesis and cellular detoxification (***Gss***, ***Gsr***, ***Shmt2***, ***Mgst1***, ***Gstp1***, ***Gstm5***, ***Gstt2***, and ***Gsta4***), as well as canonical Nrf2 targets involved in redox homeostasis (***Nqo1***, ***Prdx1***, ***Sod3***, ***Gpx1***, and ***Gpx3***). Data reflect relative mRNA expression levels from RNA-seq analysis.

Supplemental Figure 5

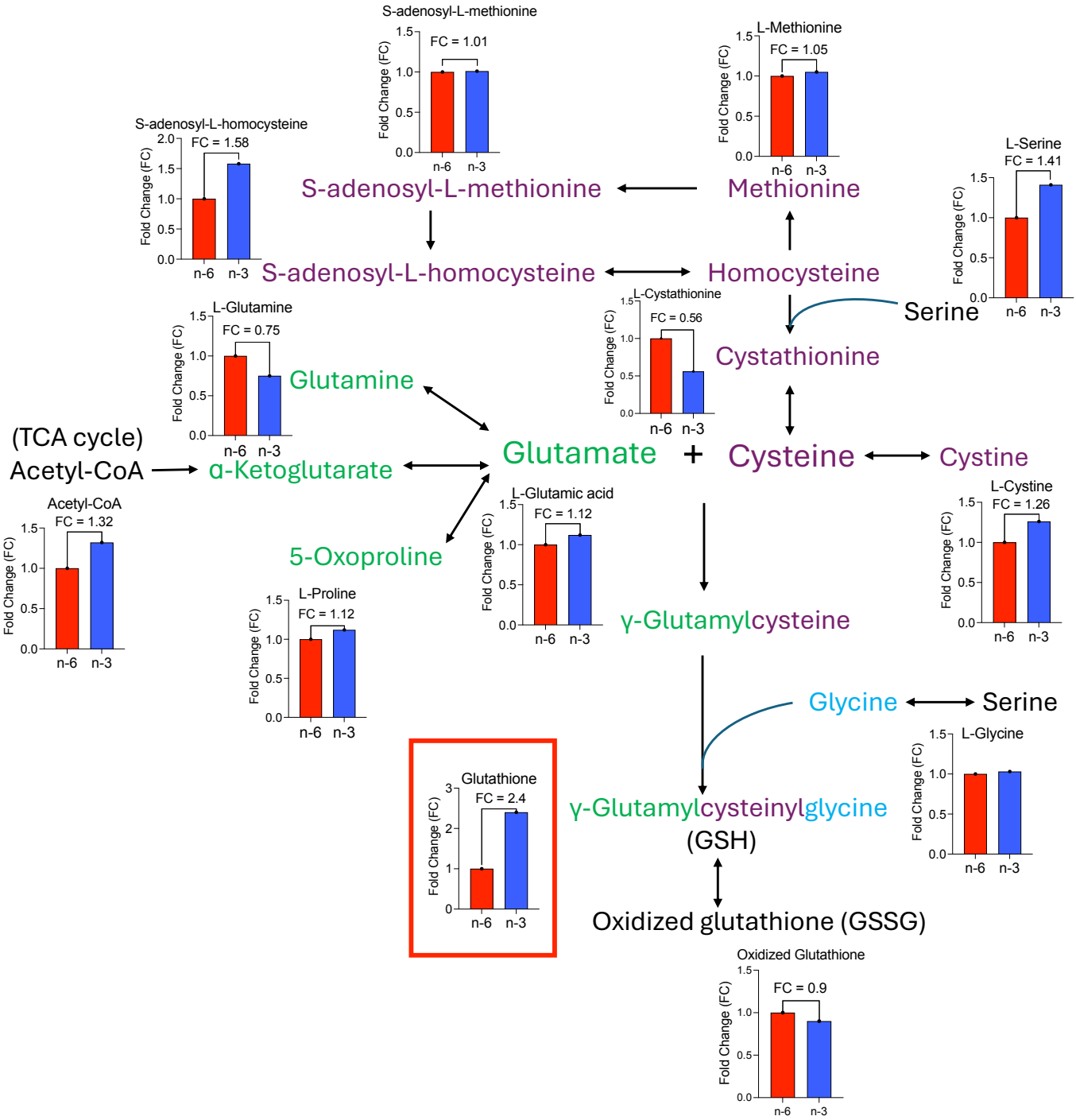


Figure S5. Glutathione–related metabolites in splenic DCs isolated from mice under PUFA-enriched diets. spDCs were isolated from mice fed n-3 (n = 7) or n-6 (n = 8) PUFA-enriched diets. Cells were pooled by group, and targeted polar metabolomic profiling was performed by LC–MS/MS. Bar graphs depict fold changes in the relative abundance of metabolites involved in glutathione biosynthesis and redox regulation, including L-methionine, S-adenosylmethionine, S-adenosylhomocysteine, L-serine, L-glutamine, L-cystathionine, L-cystine, L-proline, glycine, acetyl-CoA, reduced glutathione (GSH), and oxidized glutathione (GSSG). Metabolite levels were normalized to cell number prior to analysis.

Supplemental Figure 6

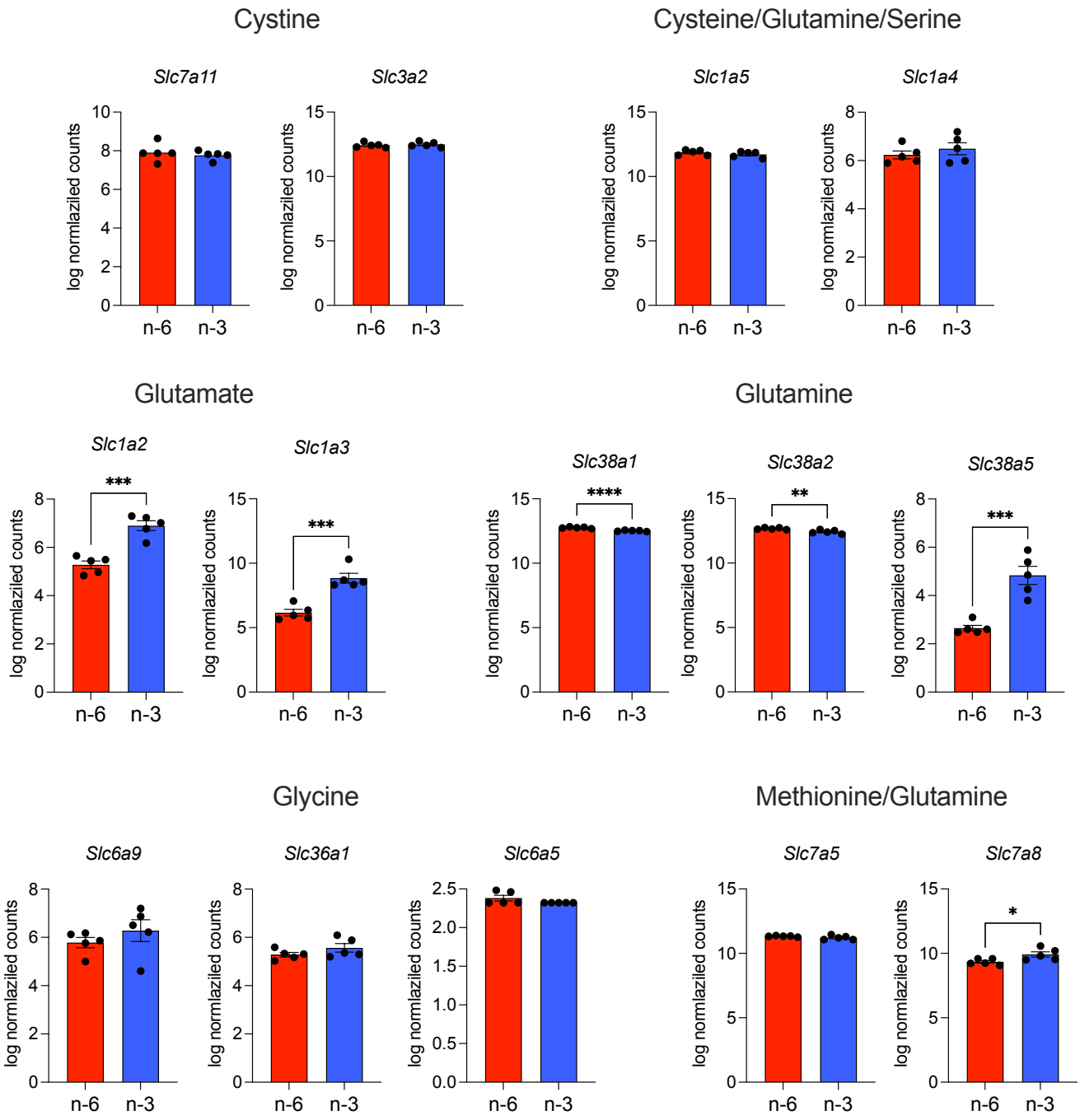
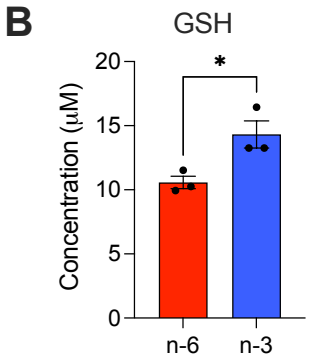


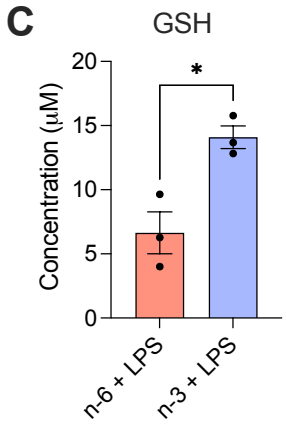
Figure S6: Expression of glutathione-related amino acid transporters in splenic DCs from PUFA-fed mice: RNA-seq data from spDCs isolated from mice receiving either an n-3 or n-6 PUFA-enriched diet (n = 5 per group) are presented as log-normalized counts for transporters associated with the uptake of cystine, cysteine/serine, glutamate, glutamine, glycine, and methionine/glutamine. Statistical significance was determined using unpaired two-tailed Student's *t*-test: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

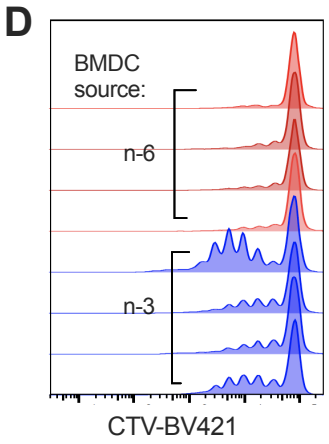
Supplemental Figure 7

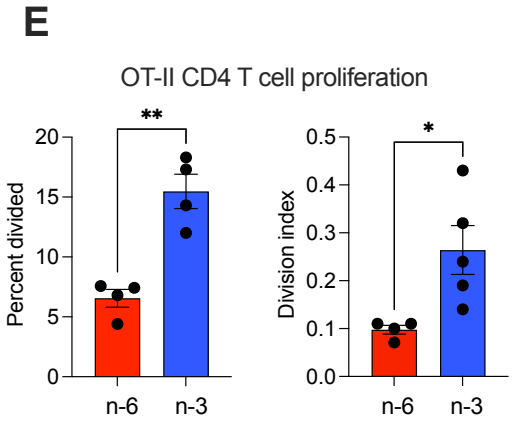
A

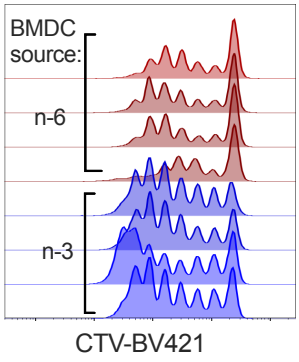
Category	Function	FDR	N	P	Z
Cell Death and Survival	Cell death of myeloid cells	0	27	3.25E-07	-2.109
	Apoptosis of myeloid cells	0	23	8.64E-07	-2.528
	Organismal death	0	245	3.06E-33	-5.554
Lipid	Accumulation	0	38	1.69E-08	-1.685
	Metabolism	0	52	2.55E-08	2.989
Cell-To-Cell Signaling and Inflammatory Response	Migration of phagocytes	0	34	5.7E-09	2.033
	Influx of phagocytes	0	11	0.00000108	2.157
	Interaction of leukocytes	0	43	4.28E-11	2.434
	Activation of phagocytes	0	41	2.44E-09	2.435
	Activation of antigen presenting cells	0	38	1.4E-09	2.744
Cellular Development	Maturation of cells	0	39	0.00000129	2.158
	Differentiation of progenitor cells	0	34	1.18E-08	2.154











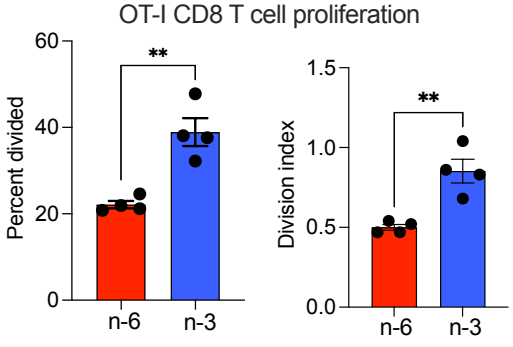


Figure S7. Dietary PUFAs shape the transcriptomic and functional profile of bone marrow–derived dendritic cells (BMDCs). Mice were maintained on n-6 or n-3 PUFA-enriched diets for 9–10 weeks. Bone marrow cells were isolated and differentiated with recombinant GM-CSF to generate BMDCs. $CD11c^+MHC\text{-}II^+$ BMDCs were sorted and analyzed as follows: (A) Ingenuity Pathway Analysis (IPA) of RNA-seq data identifying top significantly altered biological processes in BMDCs from n-6 vs. n-3 diet–fed mice (n = 5 per group). (B–C) Total intracellular GSH levels quantified using a Glutathione Assay Kit in (B) untreated or (C) LPS-stimulated BMDCs from each dietary group (n = 3 per group). (D–G) BMDCs from indicated diets were pulsed with OVA and co-cultured with OT-II ($CD4^+$) or OT-I ($CD8^+$) T cells for 72 h. (D, F) Representative CTV dilution histograms. (E, G) Quantification of T cell proliferation shown as percent divided and division index for OT-II (E) and OT-I (G) T cells (n = 4 per group). (B–G) Data are representative of at least two independent experiments and are presented as mean ± SEM. Statistical significance was assessed using an unpaired two-tailed Student's t-test: $*P < 0.05$, $**P < 0.01$