Supplementary Information

Obesity-related T cell dysfunction impairs immunosurveillance and increases cancer risk

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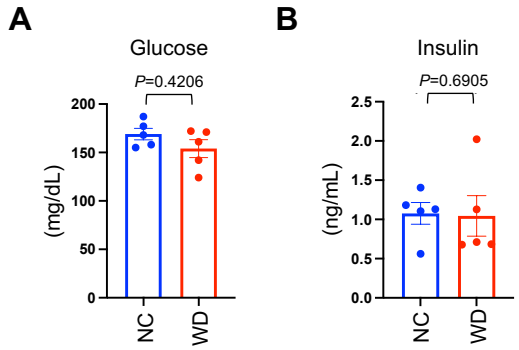
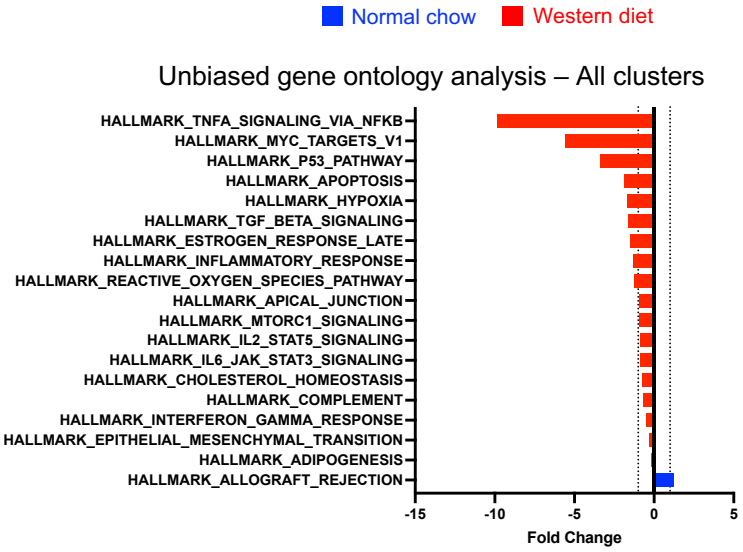
Supplementary Figure S1



Supplementary Figure S1: Mice were placed on either normal chow (NC) or western diet (WD) for 12 weeks. At that time, mice were fasted for 3 hours before serum (A) glucose and (B) insulin levels were measured (NC n=5, WD n=5). For all bar graphs, each point represents an individual mouse with SEM indicated by the error bars. Exact *P* values were calculated by two-sided Mann-Whitney *U* test.

Supplementary Figure S2

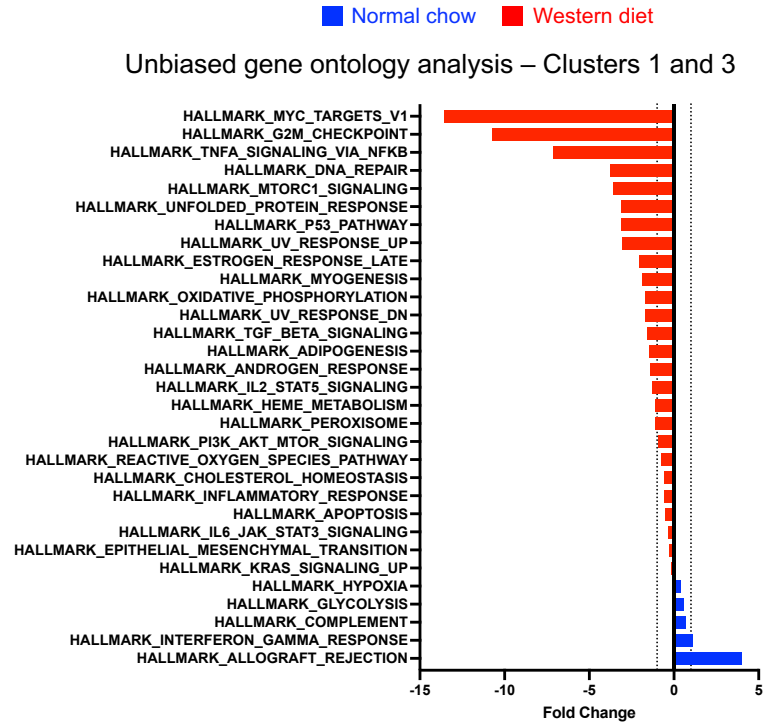
A



B

Pathway	P-value	Fold change
HALLMARK_TNFA_SIGNALING_VIA_NFKB	2.31E-07	-9.810151
HALLMARK_MYC_TARGETS_V1	6.55E-07	-5.5525126
HALLMARK_P53_PATHWAY	4.79E-06	-3.3593437
HALLMARK_APOPTOSIS	3.09E-05	-1.8341408
HALLMARK_HYPOXIA	4.85E-12	-1.6680639
HALLMARK_TGF_BETA_SIGNALING	0.00397	-1.6079465
HALLMARK_ESTROGEN_RESPONSE_LATE	1.23E-05	-1.4649321
HALLMARK_INFLAMMATORY_RESPONSE	7.92E-08	-1.3183318
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	6.55E-07	-1.2065217
HALLMARK_APICAL_JUNCTION	0.00397	-0.9211269
HALLMARK_MTORC1_SIGNALING	1.80E-06	-0.9140131
HALLMARK_IL2_STAT5_SIGNALING	0.00089	-0.8830993
HALLMARK_IL6_JAK_STAT3_SIGNALING	4.79E-06	-0.8526852
HALLMARK_CHOLESTEROL_HOMEOSTASIS	0.01568	-0.7186951
HALLMARK_COMPLEMENT	4.79E-06	-0.6949577
HALLMARK_INTERFERON_GAMMA_RESPONSE	7.49E-05	-0.4698092
HALLMARK_PEROXISOME	0.01568	-0.3370136
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	0.01568	-0.2898063
HALLMARK_ADIPOGENESIS	0.0004	-0.181533
HALLMARK_ALLOGRAFT_REJECTION	2.63E-09	1.21519337

C

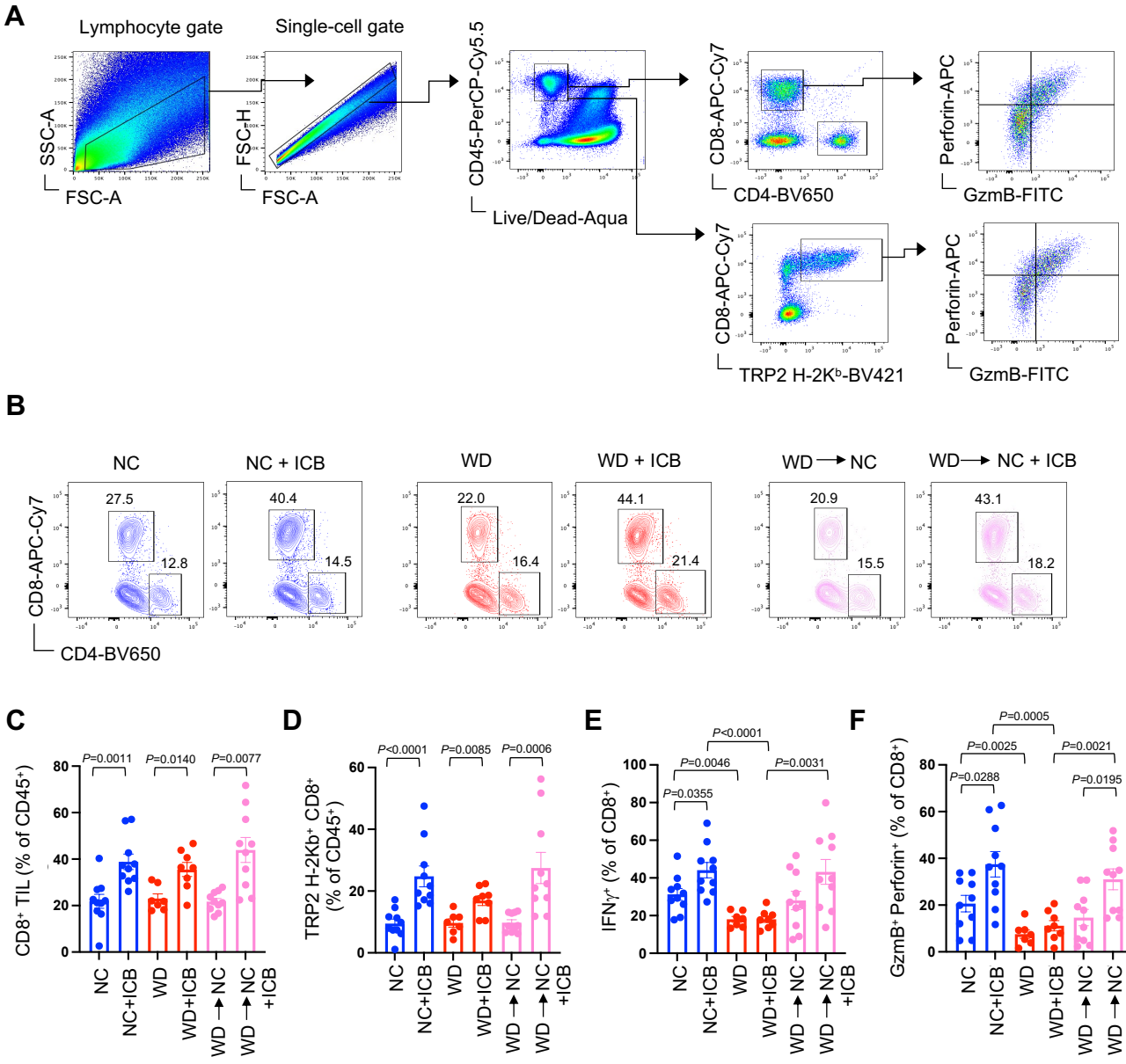


D

Pathway	P-value	Fold change
HALLMARK_MYC_TARGETS_V1	4.79E-06	-13.538083
HALLMARK_G2M_CHECKPOINT	4.79E-06	-10.687153
HALLMARK_TNFA_SIGNALING_VIA_NFKB	1.92E-16	-7.1369088
HALLMARK_DNA_REPAIR	0.0001762	-3.7500101
HALLMARK_MTORC1_SIGNALING	8.96E-16	-3.5771559
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	0.0004018	-3.1061193
HALLMARK_P53_PATHWAY	6.61E-11	-3.1008447
HALLMARK_UV_RESPONSE_UP	2.63E-08	-3.0569757
HALLMARK_ESTROGEN_RESPONSE_LATE	1.23E-05	-2.0373894
HALLMARK_MYOGENESIS	0.0008886	-1.8774308
HALLMARK_OXIDATIVE_PHOSPHORYLATION	0.0004018	-1.699182
HALLMARK_UV_RESPONSE_DN	0.0004018	-1.6851626
HALLMARK_TGF_BETA_SIGNALING	0.0001762	-1.5908114
HALLMARK_ADIPOGENESIS	0.0008886	-1.4762573
HALLMARK_ANDROGEN_RESPONSE	0.0298094	-1.3669536
HALLMARK_IL2_STAT5_SIGNALING	6.61E-11	-1.2469363
HALLMARK_HEME_METABOLISM	0.0156844	-1.083145
HALLMARK_PEROXISOME	0.0298094	-1.0695359
HALLMARK_PI3K_AKT_MTOR_SIGNALING	0.0298094	-0.9343376
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	0.0004018	-0.7667961
HALLMARK_CHOLESTEROL_HOMEOSTASIS	0.0019063	-0.5780767
HALLMARK_INFLAMMATORY_RESPONSE	7.49E-05	-0.5430823
HALLMARK_APOPTOSIS	6.55E-07	-0.5262974
HALLMARK_IL6_JAK_STAT3_SIGNALING	0.0008886	-0.3359172
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	4.79E-06	-0.29115
HALLMARK_KRAS_SIGNALING_UP	1.23E-05	-0.1206735
HALLMARK_HYPOXIA	7.96E-18	0.37638642
HALLMARK_GLYCOLYSIS	0.0019063	0.57180268
HALLMARK_COMPLEMENT	6.61E-11	0.71103851
HALLMARK_INTERFERON_GAMMA_RESPONSE	1.82E-11	1.07935422
HALLMARK_ALLOGRAFT_REJECTION	4.85E-12	3.96311122

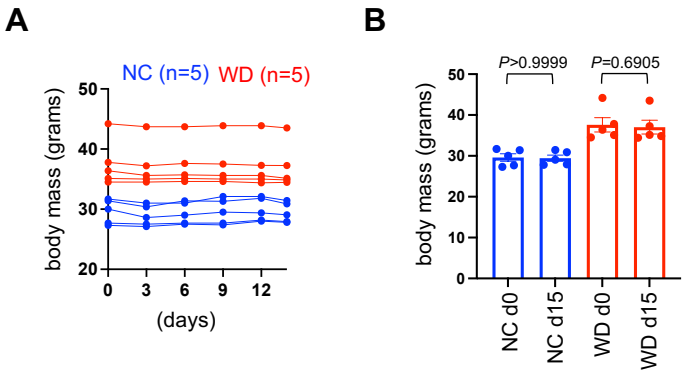
Supplementary Figure S2: Mice were placed on either normal chow (NC) or western diet (WD) for 12 weeks. scRNAseq was performed on CD8$^+$ TIL sorted from day 14 established B16 tumors. (A, B) Unbiased gene ontology was performed on all CD8$^+$ TIL, and significant pathways are shown along with respective *P* values and fold changes. (C, D) Unbiased gene ontology was performed on cytolytic CD8$^+$ TIL clusters 1 and 3, and significant pathways are shown along with respective *P* values and fold changes.

Supplementary Figure S3



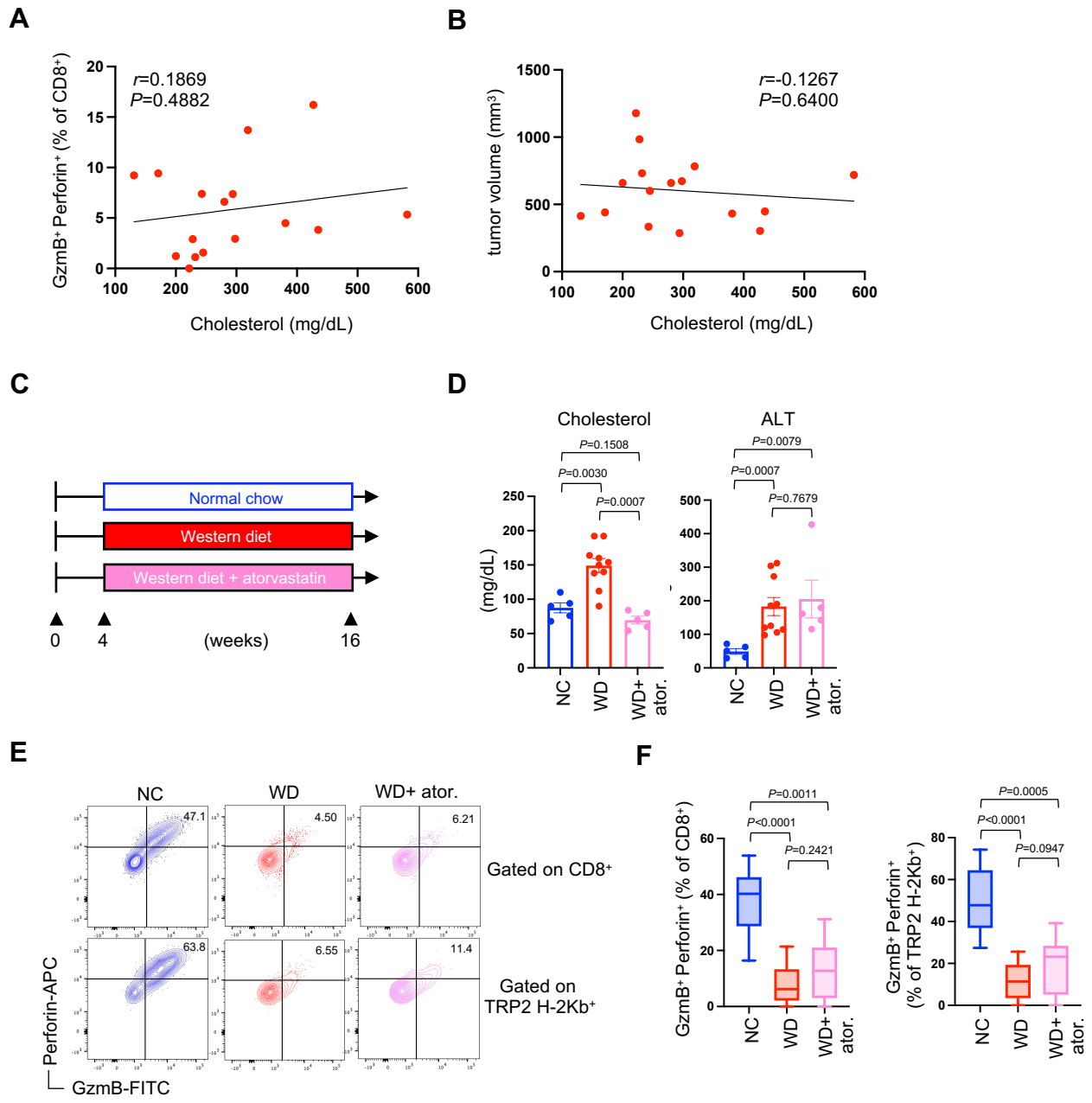
Supplementary Figure S3: (A) Gating strategy for ex vivo analysis of CD8$^+$ TIL, TRP-2-specific CD8$^+$ TIL, and CD4$^+$ TIL from B16 melanoma tumors at day 15. (B) Representative FACS plots show TIL that were gated for CD45$^+$ expression and display the frequency of CD8$^+$ and CD4$^+$ T cells from untreated normal chow (NC) and western diet (WD) mice as well as immune checkpoint blockade (ICB) treated cohorts. Graphs display pooled data from 2 independent experiments showing the frequency of (C) total CD8$^+$ TIL, (D) TRP-2-specific CD8$^+$ TIL, (E) IFNγ^+ CD8$^+$ TIL, and (F) CD8$^+$ TIL co-expressing GzmB and Perforin (NC n=10, NC+ICB n=10, WD n=7, WD+ICB n=8, WD→NC n=10, WD→NC+ICB n=10). For all bar graphs, each point represents an individual mouse with SEM indicated by the error bars. Exact *P* values were calculated by two-sided Mann-Whitney *U* test.

Supplementary Figure S4



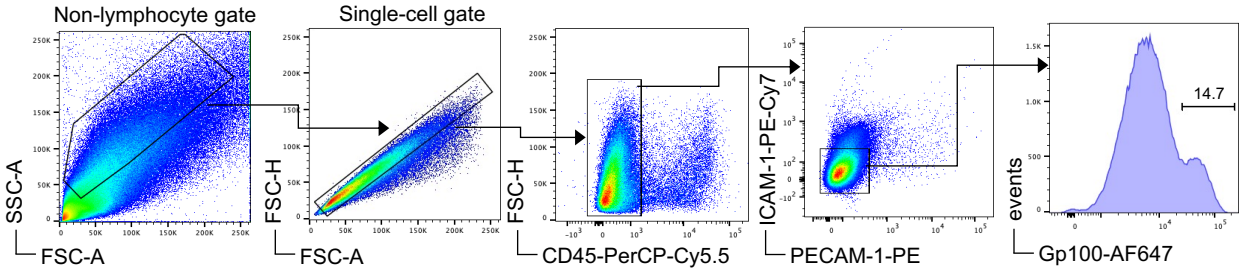
Supplementary Figure S4: Mice were placed on either normal chow (NC) or western diet (WD) for 12 weeks. At this time, mice were weighed and then received a subcutaneous injection of B16 tumor cells in the flank (day 0). (A) Mice were weighed every 3 days until takedown on day 15. (B) Day 15 body mass was calculated by subtracting tumor mass from body mass (NC n=5, WD n=5). For all bar graphs, each point represents an individual mouse with SEM indicated by the error bars. Exact *P* values were calculated by two-sided Mann-Whitney *U* test.

Supplementary Figure S5



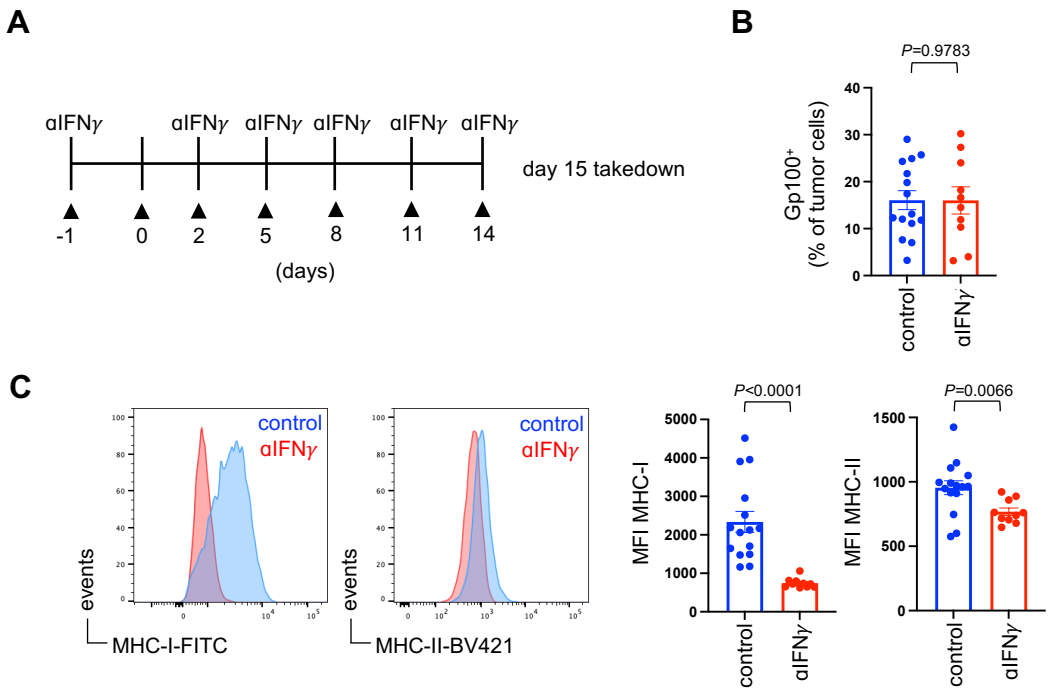
Supplementary Figure S5: Linear regression analysis comparing (A) the frequency of CD8$^+$ TIL co-expressing GzmB and Perforin, or (B) tumor volumes to mouse serum cholesterol in western diet (WD) mice (pooled data from 3 independent experiments, n=16). (C) Mice were placed on either normal chow (NC), WD, or WD containing atorvastatin for 12 weeks. (D) Serum cholesterol and alanine aminotransferase (ALT) levels were measured (NC n=5, WD n=10, WD+ator. n=5). At this time, mice received a subcutaneous injection of B16 tumor cells in the flank. (E) Tumors were harvested at day 16 and representative FACS plots show intracellular expression of GzmB and Perforin from total CD8$^+$ TIL and TRP-2-specific CD8$^+$ TIL. (F) Pooled data from 2 independent experiments show the percent of total CD8$^+$ TIL and TRP-2-specific CD8$^+$ TIL co-expressing GzmB and Perforin (NC n=12, WD n=19, WD+ator. n=8). (A, B) Linear regression analysis was performed by calculating the Pearson correlation value (*r*) and corresponding *P* value. Each point represents an individual mouse. (D, F) For all bar graphs, each point represents an individual mouse with SEM indicated by the error bars. All box-and-whisker plots: The box indicates the 25th and 75th percentile, the line indicates the data median, and the whiskers indicate the minimum and maximum of all individual values. All *n*'s represent an individual mouse. Exact *P* values were calculated by two-sided Mann-Whitney *U* test.

Supplementary Figure S6



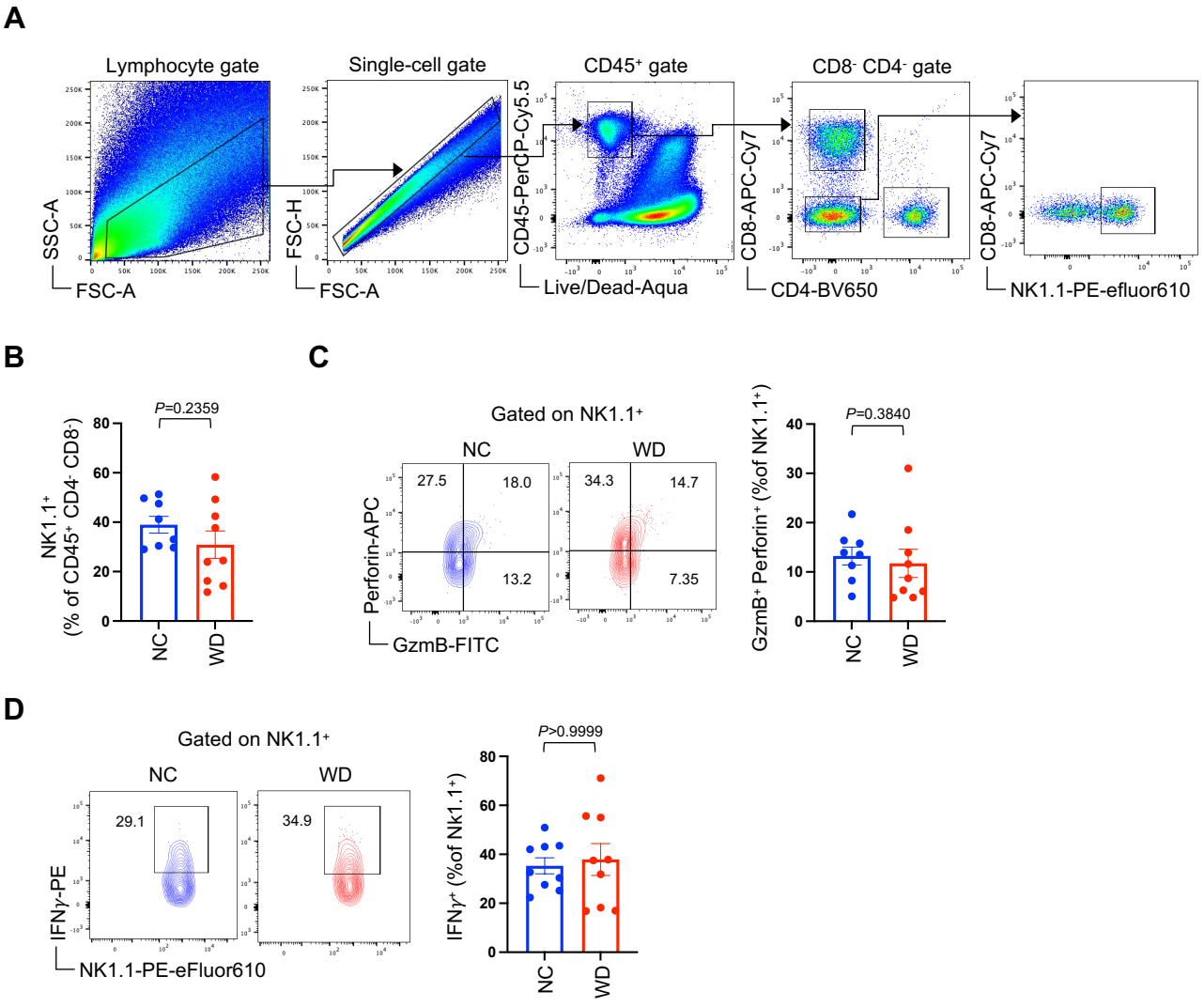
Supplementary Figure S6: Gating strategy for ex vivo Gp100+ tumor cells. Tumor cells were distinguished from leukocytes, stromal endothelial cells, and fibroblasts via lack of CD45, ICAM-1, and PECAM-1 surface expression.

Supplemental Figure S7



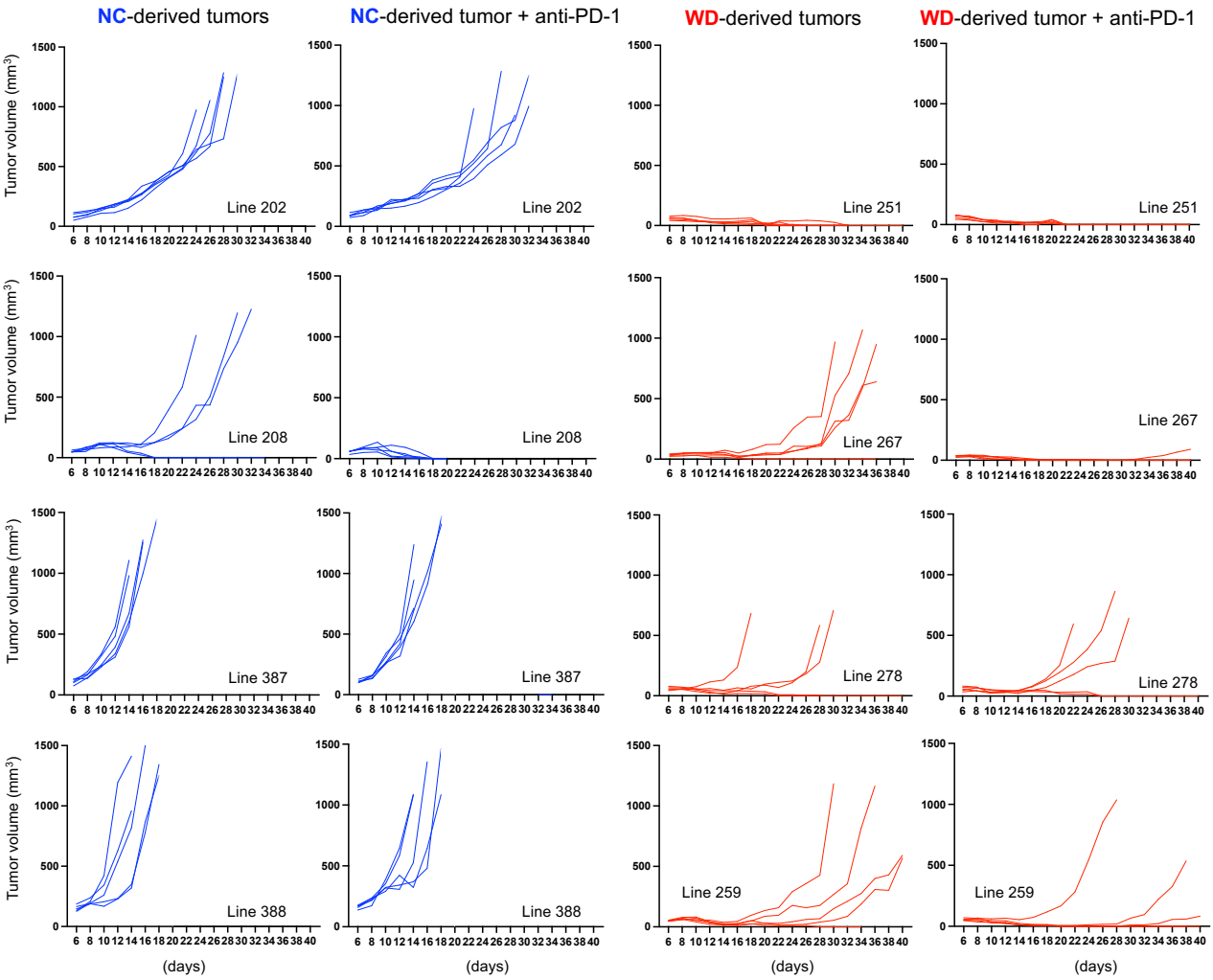
Supplementary Figure S7: (A) B6 mice were treated with either αIFNγ antibody or vehicle control via intraperitoneal injection on days -1, 2, 5, 8, 11, and 14 relative to subcutaneous injection of B16 tumor cells in the flank on day 0. (B) Tumors were harvested on day 15 and the frequency of $Gp100^+$ tumor cells was measured. (C) Representative FACS plots show MHC-I and MHC-II expression on $Gp100^+$ tumor cells, and pooled median fluorescence intensity (MFI) data from 2 independent experiments is graphed (control n=10, αIFNγ n=10). For all bar graphs, each point represents an individual mouse with SEM indicated by the error bars. Exact *P* values were calculated by two-sided Mann-Whitney *U* test.

Supplementary Figure S8



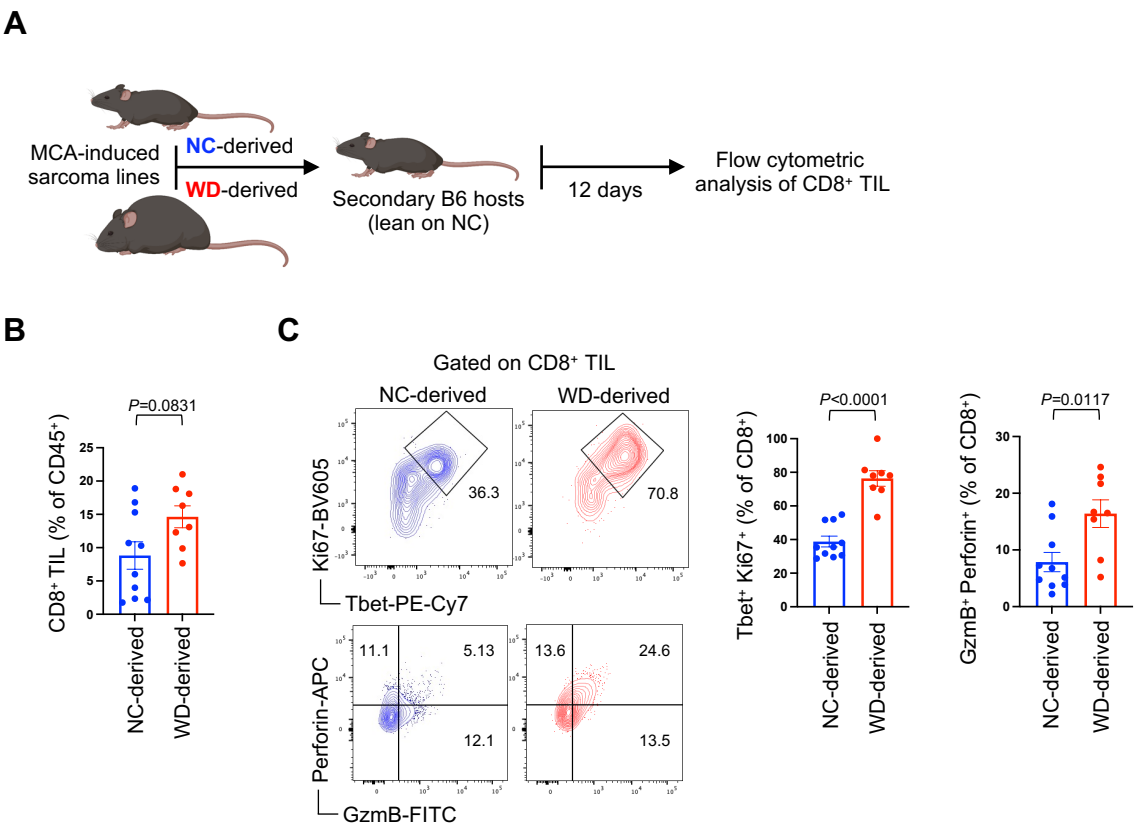
Supplementary Figure S8: B16 tumors were harvested from the indicated mice at day 15. (A) Gating strategy for NK1.1$^+$ natural killer (NK) cells from ex vivo B16 melanoma tumors. (B) Graphs display pooled data from 2 independent experiments showing the frequency of NK cells from normal chow (NC) and western diet (WD) tumors. Representative FACS plots and graphs show (C) GzmB and Perforin co-expression and (D) IFNγ expression by NK cells (NC n=10, WD n=10). For all bar graphs, each point represents an individual mouse with SEM indicated by the error bars. Exact *P* values were calculated by two-sided Mann-Whitney *U* test.

Supplementary Figure S9



Supplementary Figure S9: Individual growth curves for the 4 normal chow (NC)-derived and 4 western diet (WD)-derived sarcoma lines following transplant into 5 secondary B6 recipients left untreated (PBS) and 5 treated with anti-PD-1 on days 7 and 14 (all groups n=5). Each graph represents a unique MCA-induced sarcoma cell line derived from an individual mouse in Figure 5B.

Supplementary Figure S10



Supplementary Figure S10: (A) MCA-induced sarcoma cell lines were generated from mice on normal chow (NC) and mice on western diet (WD) for reimplantation into secondary lean recipients. Two NC-derived (Lines 202 and 387) and two WD-derived (Lines 267 and 278) lines were injected subcutaneously into the flank of 5 secondary recipients each, and tumors were harvested for flow cytometric analysis of $CD8^+$ TIL on day 12, and (B) the frequency of $CD8^+$ TIL was assessed. (C) Representative FACS plots show co-expression of GzmB and Perforin as well as Tbet and Ki67 from $CD8^+$ TIL, and pooled data is graphed (NC-derived n=10, WD-derived n=8). For all bar graphs, each point represents an individual mouse with SEM indicated by the error bars. Exact *P* values were calculated by two-sided Mann-Whitney *U* test. (A) Created using BioRender.com.