Supplemental Figures

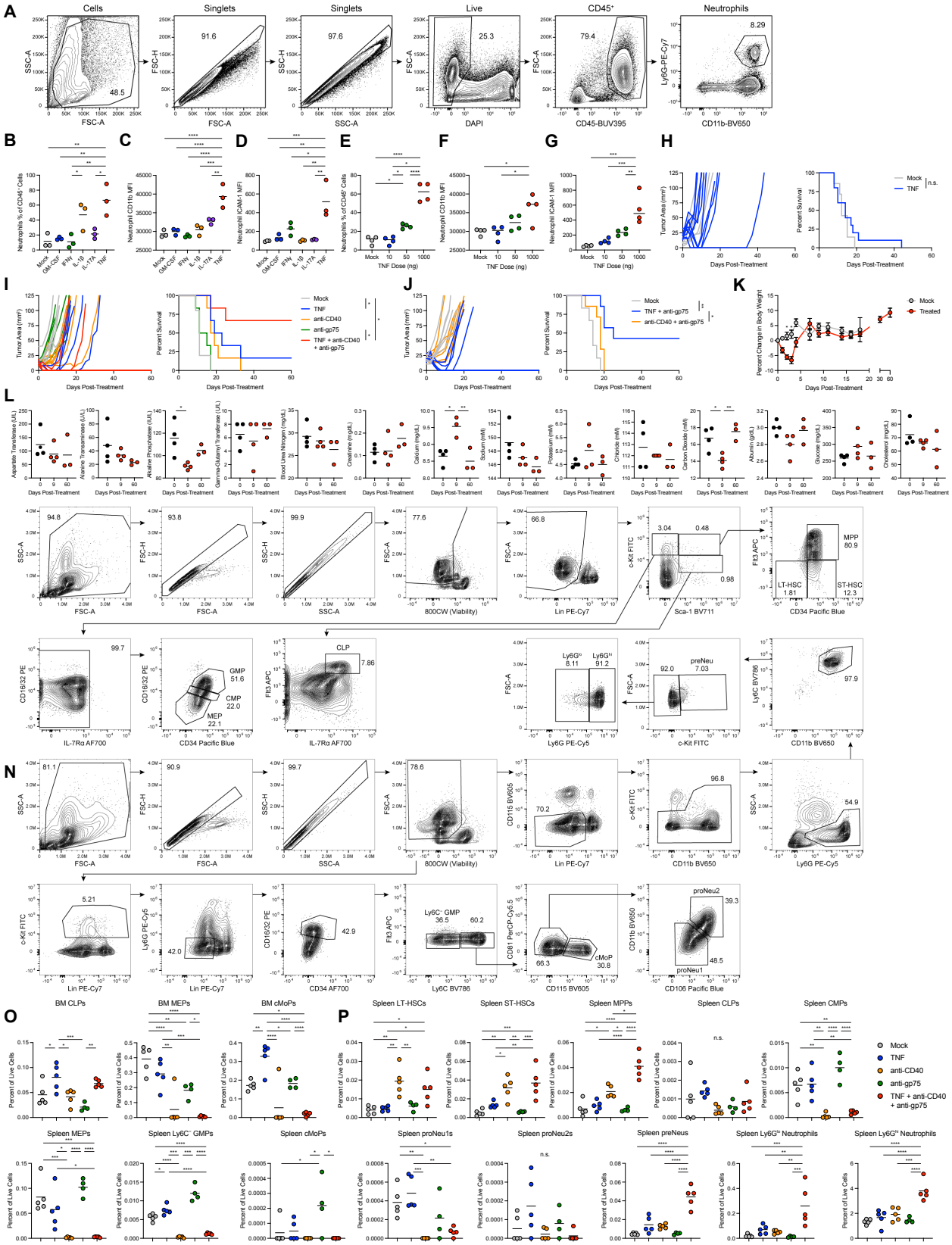

Figure S1: Neutrophil-activating therapy induces neutrophil expansion and recruitment to tumors. Related to Figure 1.

(**A**) Flow cytometry gating strategy for neutrophils. (**B**) Neutrophil infiltration of B16 tumors 24 hours after treatment with the indicated cytokines (n=3). (**C-D**) Expression of CD11b (C) and ICAM-1 (D) on tumor-infiltrating neutrophils 24 hours after administration of the indicated cytokines (n=3). (**E**) Neutrophil infiltration of B16 tumors 24 hours after treatment with the indicated dose of TNF (n=4). (**F-G**) Expression of CD11b (C) and ICAM-1 (D) on tumor-infiltrating neutrophils 24 hours after administration of the indicated dose of TNF (n=4). (**H**) Survival of B16-bearing mice following treatment with 1 μg TNF (mock n=7, TNF n=10). (**I-J**) Survival of B16-bearing mice treated with the indicated factors (I: mock n=5, others n=6; J: mock n=6, others n=7). (**K**) Percent change in mouse body weight following treatment of B16 tumors (n=5, mean +/- SEM shown). (**L**) Mouse blood chemistry values obtained on the indicated days after treatment of B16 tumors with neutrophil-activating therapy (d60 n=3, others n=4). (**M-N**) Flow cytometry gating strategies to identify hematopoietic stem cells, progenitors, and neutrophil precursors. (**O-P**) Frequencies of hematopoietic stem cells, progenitors, and precursor populations in the bone marrow (O) and spleen (P) 24 hours after treatment of B16 with neutrophil-activating therapy (anti-gp75 n=4, others n=5). Statistics: One-way ANOVA with Tukey's multiple comparisons test (B-G, L, O-P), Log-rank test (H), Log-rank test with Bonferroni correction (I-J), Two-way ANOVA with Sidak's multiple comparisons test (K). For all dot plots, the line indicates the mean. Data are representative of 2 (B-G, I-J, O-P) or 1 (K-L) independent experiments or pooled from 4 experiments (H).

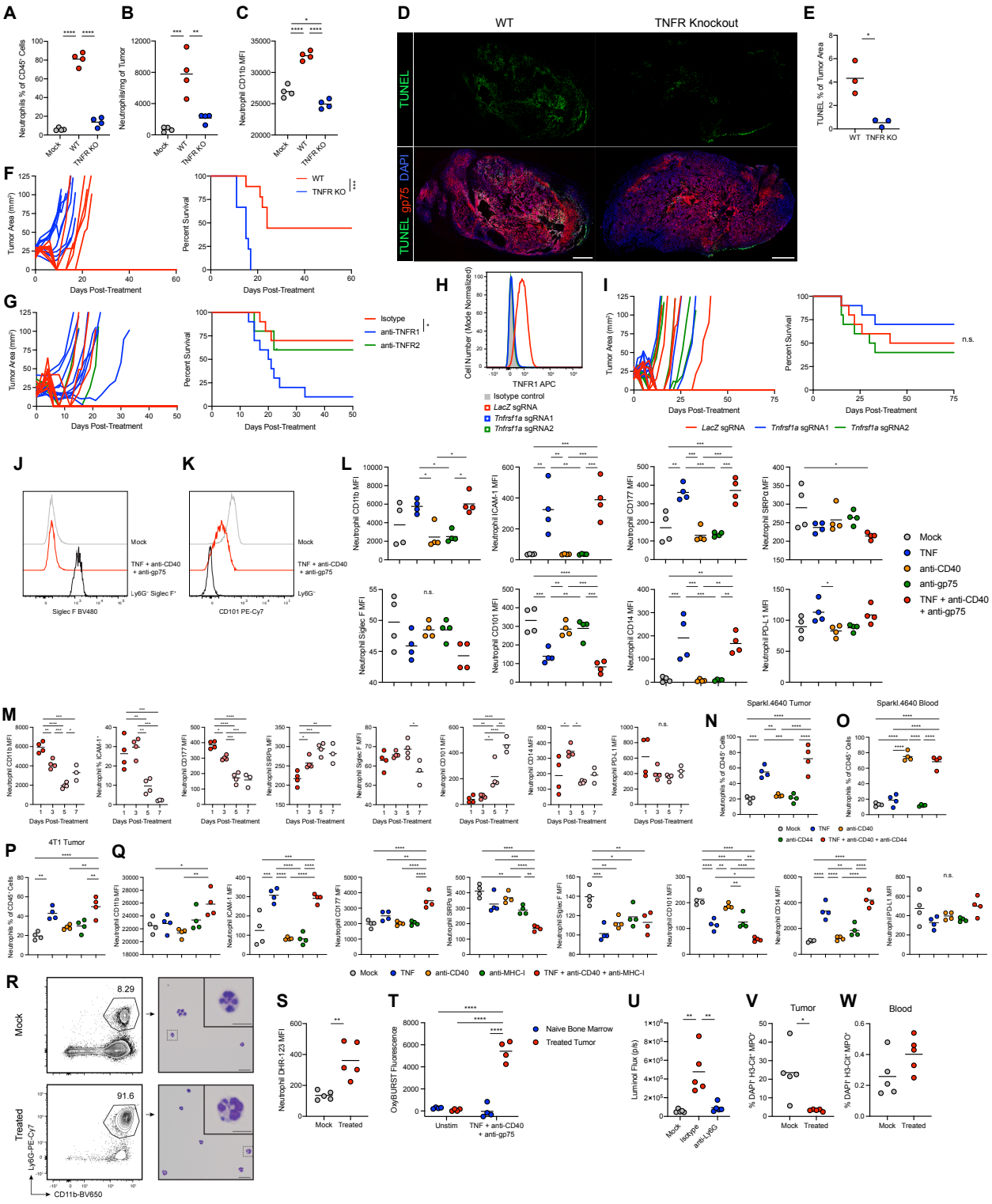

Figure S2: Therapy induces neutrophil activation. Related to Figure 1.

(**A-B**) Tumor-infiltrating neutrophil frequency of CD45$^+$ cells (A) and numbers/mg of tumor (B) 24 hours after treatment of B16 tumors with neutrophil-activating therapy in wild-type (WT) or TNF receptor knockout (TNFR KO) mice (n=4). (**C**) CD11b expression on tumor-infiltrating

neutrophils 4 hours after treatment of B16 tumors with neutrophil-activating therapy in WT or TNFR KO mice (n=4). (**D-E**) Representative immunofluorescence (D) and quantification (E) of TUNEL staining in B16 tumors in WT or TNFR KO mice 24 hours after treatment with neutrophil-activating therapy. Scale bars = 500 μm. (n=3) (**F**) Survival of B16-bearing WT or TNFR KO mice treated with neutrophil-activating therapy (n=9). (**G**) Survival of B16-bearing mice treated with neutrophil-activating therapy following administration of TNFR blocking antibodies (TNFR2 n=5, others n=10). (**H**) TNFR1 staining of B16 cells following knockout of TNFR1 with CRISPR-Cas9 sgRNA targeting *Tnfrsf1a* or the irrelevant *LacZ*. (**I**) Survival of mice bearing TNFR1-knockout B16 following treatment with neutrophil-activating therapy (n=10). (**J**) Representative histograms comparing Siglec F expression in mock or treated tumor-infiltrating neutrophils to a Siglec F expression in $Ly6G^-$ Siglec F^+ cell population in the tumor of mock-treated mice, 4 hours after treatment. (**K**) Representative histograms comparing the expression level of CD101 in mock or treated tumor-infiltrating neutrophils to that of $Ly6G^-$ cells in the tumor of mock-treated mice, 4 hours after treatment. (**L**) Surface marker expression on neutrophils in the blood 4 hours after treatment of B16 tumors with the indicated components (n=4). (**M**) Surface marker expression on neutrophils in the blood at the indicated times post-treatment with the full neutrophil-activating therapy (d7 n=3, others n=4). (**N-O**) Neutrophil frequency in the tumor (N) and blood (O) 24 hours after treatment of Sparkl.4640 with neutrophil-activating therapy (n=4). (**P**) Neutrophil frequency in 4T1 tumors 24 hours after therapy (n=4). (**Q**) Surface marker expression on neutrophils in 4T1 tumors 4 hours after treatment with the indicated components (n=4). (**R**) Representative flow cytometry staining of $CD11b^+Ly6G^+$ cells, gated on single/live/$CD45^+$ cells (left panels), and May-Grünwald-Giemsa staining of sorted $CD45^+CD11b^+Ly6G^+$ cells (right panels), in B16 tumors 24 hours after treatment with neutrophil-activating therapy. Image scale bars = 30 μm. Inset scale bars = 10 μm. (**S**) Tumor-infiltrating neutrophil MFI of the ROS-sensing dye dihydrorhodamine-123 (DHR-123) 4 hours after treatment with neutrophil-activating therapy (n=5). (**T**) Fluorescence of OxyBURST Green H_2HFF BSA following *in vitro* stimulation of neutrophils isolated from treated tumors or naïve bone marrow with neutrophil-activating therapy (n=4). (**U**) Bioluminescence in the tumor emitted from the ROS-sensing molecule luminol during *in vivo* imaging 4 hours after treatment of B16 with neutrophil-activating therapy (n=5). (**V-W**) Percentage of neutrophils staining triple positive for DAPI, citrullinated histone H3 (H3-Cit), and myeloperoxidase (MPO) in the tumor (V) and blood (W) of mice treated with neutrophil-activating therapy (n=5). Statistics: One-way ANOVA with Tukey's multiple comparisons test (A-E, L-Q, U), Log-rank test (F), Log-rank test with Bonferroni correction (G, I), Unpaired two-tailed t test (S, V-W), Two-way ANOVA with Tukey's

multiple comparisons test (T). For all dot plots, the line indicates the mean. Data are representative of 3 (F, S), 2 (A-E, H, J-P, R, T, U-W) or 1 (Q) independent experiments or pooled from 2 experiments (G, I).

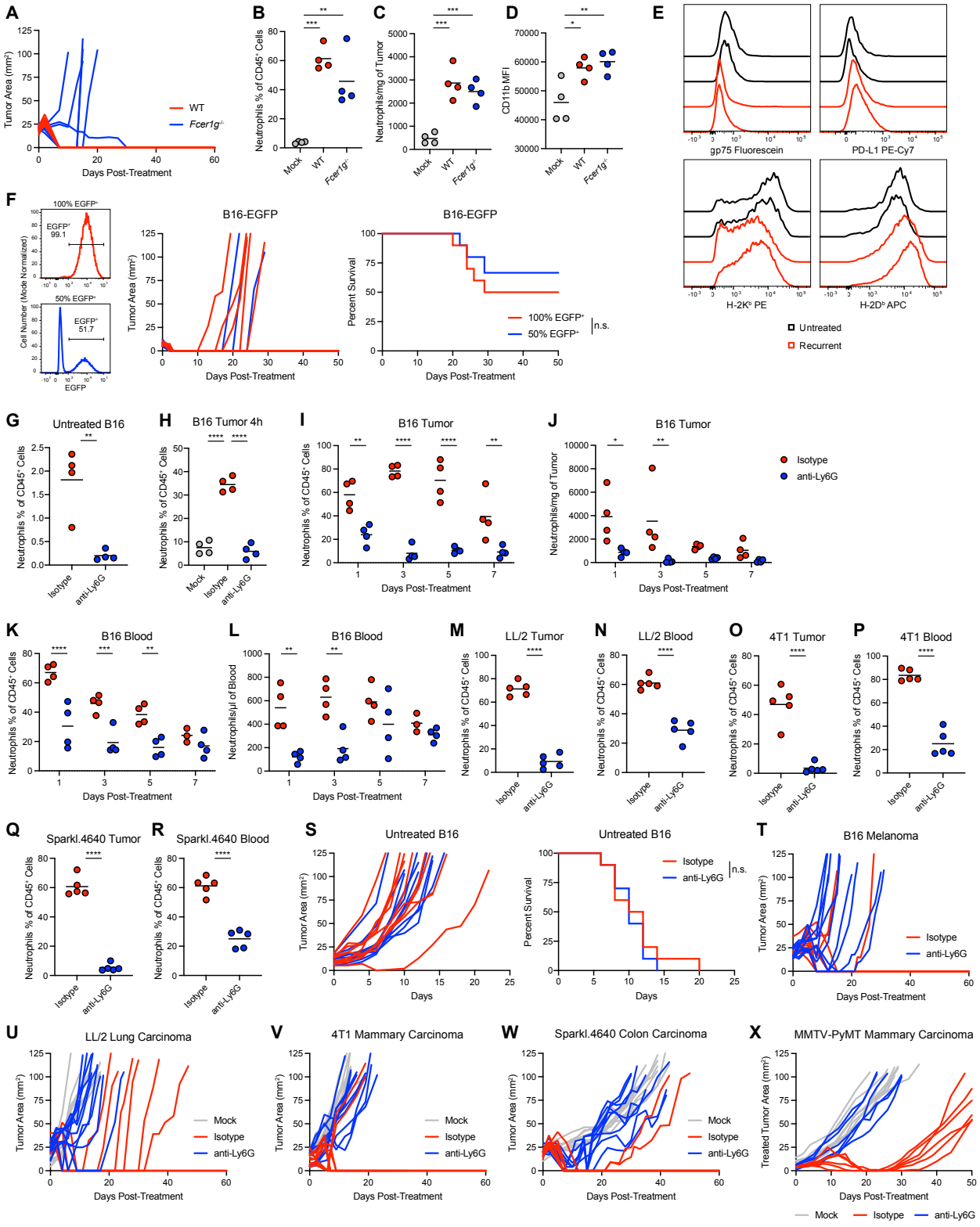

Figure S3: Neutrophil depletion prevents tumor clearance. Related to Figure 2.

(**A**) Tumor growth in WT and *Fcer1g*$^{-/-}$ B16-bearing mice after treatment with neutrophil-activating therapy (n=10). (**B-D**) Neutrophil frequency (B), numbers (C), and CD11b expression (D) in WT versus *Fcer1g*$^{-/-}$ mice, 24 hours after treatment (n=4). (**E**) Expression of gp75, PD-L1, and MHCI molecules on B16 tumor cells from day 0 untreated tumors or tumors that recurred following neutrophil-activating therapy. (**F**) Tumor growth and survival of mice implanted with B16 tumors, in which either 50% or 100% of the B16 cells expressed EGFP on the surface, following treatment with TNF + anti-CD40 + anti-EGFP (n=10). (**G**) Neutrophil frequency in untreated B16 tumors in mice administered anti-Ly6G or isotype control (n=4). (**H**) Neutrophil frequency in B16 tumors 4 hours after treatment with neutrophil-activating therapy in mice administered anti-Ly6G or isotype control (n=4). (**I-L**) Neutrophil frequencies (I, K) and numbers (J, L) in the tumor (I-J) and blood (K-L) of B16-bearing mice at multiple time points after our Ly6G depletion plus neutrophil-activating therapy protocol (n=4). (**M-R**) Neutrophil frequency in the tumor (M, O, Q) and blood (N, P, R) 24 hours after neutrophil-activating treatment of Ly6G-depleted mice bearing LL/2 (M-N), 4T1 (O-P), and Sparkl.4640 (Q-R) tumors. (n=5) (**S**) B16 tumor growth and survival of mice receiving anti-Ly6G or isotype control in the absence of neutrophil-activating therapy (n=10). (**T-W**) Tumor growth in mice bearing B16 (T) (n=10), LL/2 (U) (mock n=8, others n=10), 4T1 (V) (n=10), or Sparkl.4640 (W) (mock n=8, isotype n=10, anti-Ly6G n=9) tumors that received anti-Ly6G or isotype control prior to neutrophil-activating therapy or mock treatment. (**X**) Growth of the treated tumor in MMTV-PyMT mice administered anti-Ly6G or isotype control prior to neutrophil-activating therapy. Isotype control mice were euthanized at day 50 due to tumor burden in distant untreated breasts. (mock n=8, others n=6) Statistics: One-way ANOVA with Tukey's multiple comparisons test (B-D, H), Log-rank test (F, S), Unpaired two-tailed t test (G, M-R), Two-way ANOVA with Tukey's multiple comparisons test (I-L). For all dot plots, the line indicates the mean. Data are representative of 3 (T), 2 (A-D), or 1 (E-S) independent experiments or pooled from 3 (U-W) or 6 (X) experiments.

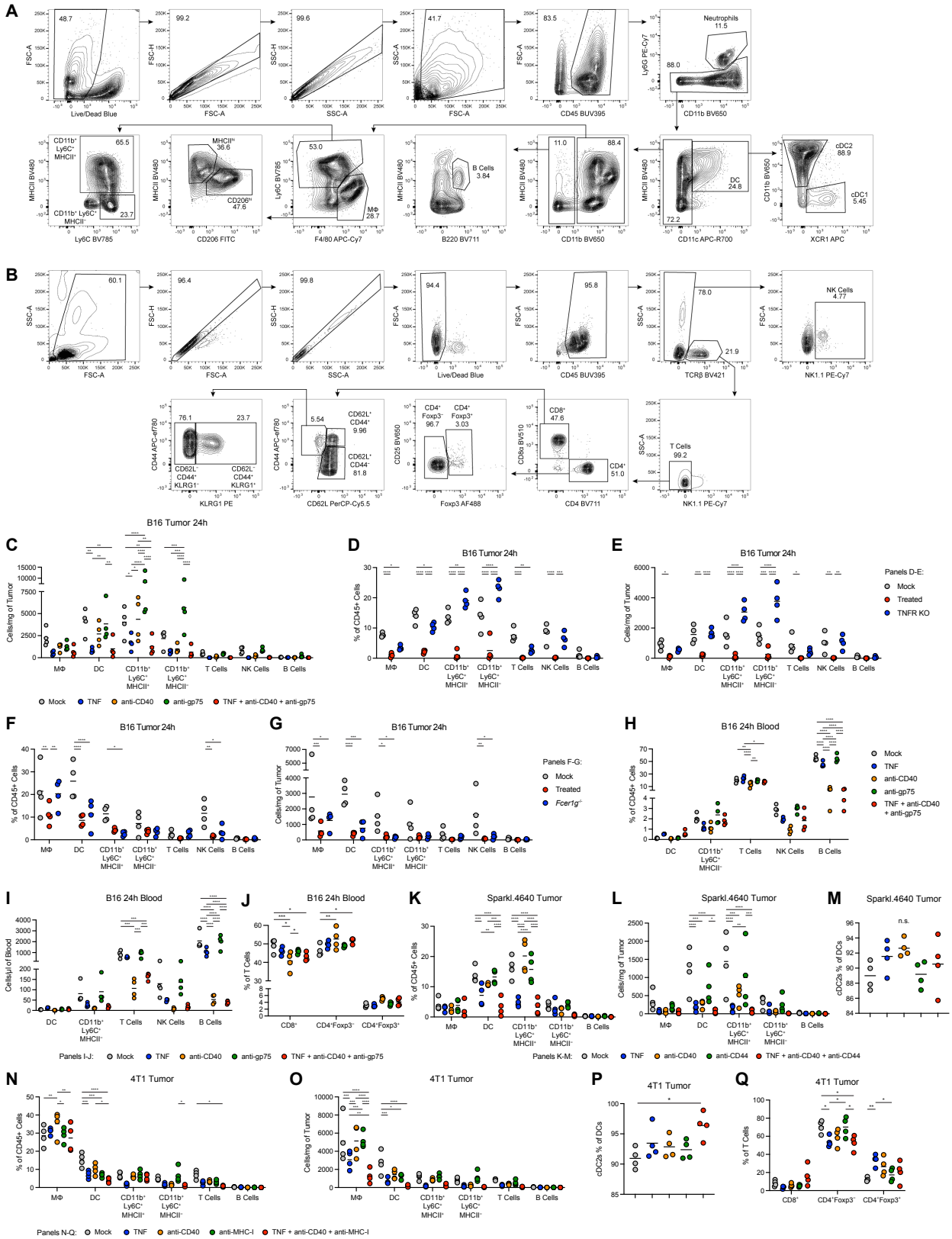
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
B16 Tumor 24h
B16 24h Blood
Sparkl.4640 Tumor
4T1 Tumor
Panels D-E: Mock Treated TNFR KO
Panels F-G: Mock Treated Fcer1g-/-
Panels I-J: Mock TNF anti-CD40 anti-gp75 TNF + anti-CD40 + anti-gp75
Panels K-M: Mock TNF anti-CD40 anti-CD44 TNF + anti-CD40 + anti-CD44
Panels N-Q: Mock TNF anti-CD40 anti-MHC-I TNF + anti-CD40 + anti-MHC-I
Mock TNF anti-CD40 anti-gp75 TNF + anti-CD40 + anti-gp75
Cells/mg of Tumor
% of CD45+ Cells
Cells/μl of Blood
% of T Cells
cDC2s % of DCs
MΦ DC CD11b+ Ly6C+ MHCII+ CD11b+ Ly6C+ MHCII- T Cells NK Cells B Cells
CD8+ CD4+Foxp3- CD4+Foxp3+

Figure S4: Impact of neutrophil-activating therapy on the numbers and frequencies of other immune cells. Related to Figure 3.

(**A-B**) Flow cytometry gating strategy for identifying myeloid and lymphoid subsets. $CD4^+Foxp3^-$ T cells were gated for memory and effector phenotype in the same manner as the $CD8^+$ T cells shown. (**C**) Numbers of other immune cells in B16 tumors 24 hours after treatment with the indicated components (n=4). (**D-E**) Frequencies (D) and numbers (E) of immune cells in B16 tumors 24 hours after treatment of WT or TNFR KO mice with the full neutrophil-activating therapy (n=4). (**F-G**) Frequencies (F) and numbers (G) of immune cells in B16 tumors 24 hours after treatment of WT or *Fcer1g*$^{-/-}$ mice with the full neutrophil-activating therapy (n=4). (**H-I**) Frequencies (H) and numbers (I) of immune cells in the blood of B16-bearing mice 24 hours after treatment with the indicated components (n=4). (**J**) Frequencies of T cell subsets as a percentage of total T cells in the blood 24 hours after treatment of B16 tumors with the indicated components (n=4). (**K-L**) Frequencies (K) and numbers (L) of immune cells in Sparkl.4640 tumors 24 hours after treatment with the indicated components (n=4). (**M**) Percent of $CD11b^+$ cDC2s out of total DCs in Sparkl.4640 tumors 24 hours after treatment (n=4). (**N-O**) Frequencies (N) and numbers (O) of immune cells in 4T1 tumors 24 hours after treatment with the indicated components (n=4). (**P**) Percent of $CD11b^+$ cDC2s out of total DCs in 4T1 tumors 24 hours after treatment (n=4). (**Q**) Percent of T cell subsets out of total T cells in 4T1 tumors 24 hours after treatment (n=4). Statistics: Two-way ANOVA with Tukey's multiple comparisons test (C-L, N-O, Q), One-way ANOVA with Tukey's multiple comparisons test (M, P). For all dot plots, the line indicates the mean. Data are representative of 2 (C-J) or 1 (K-Q) independent experiments.

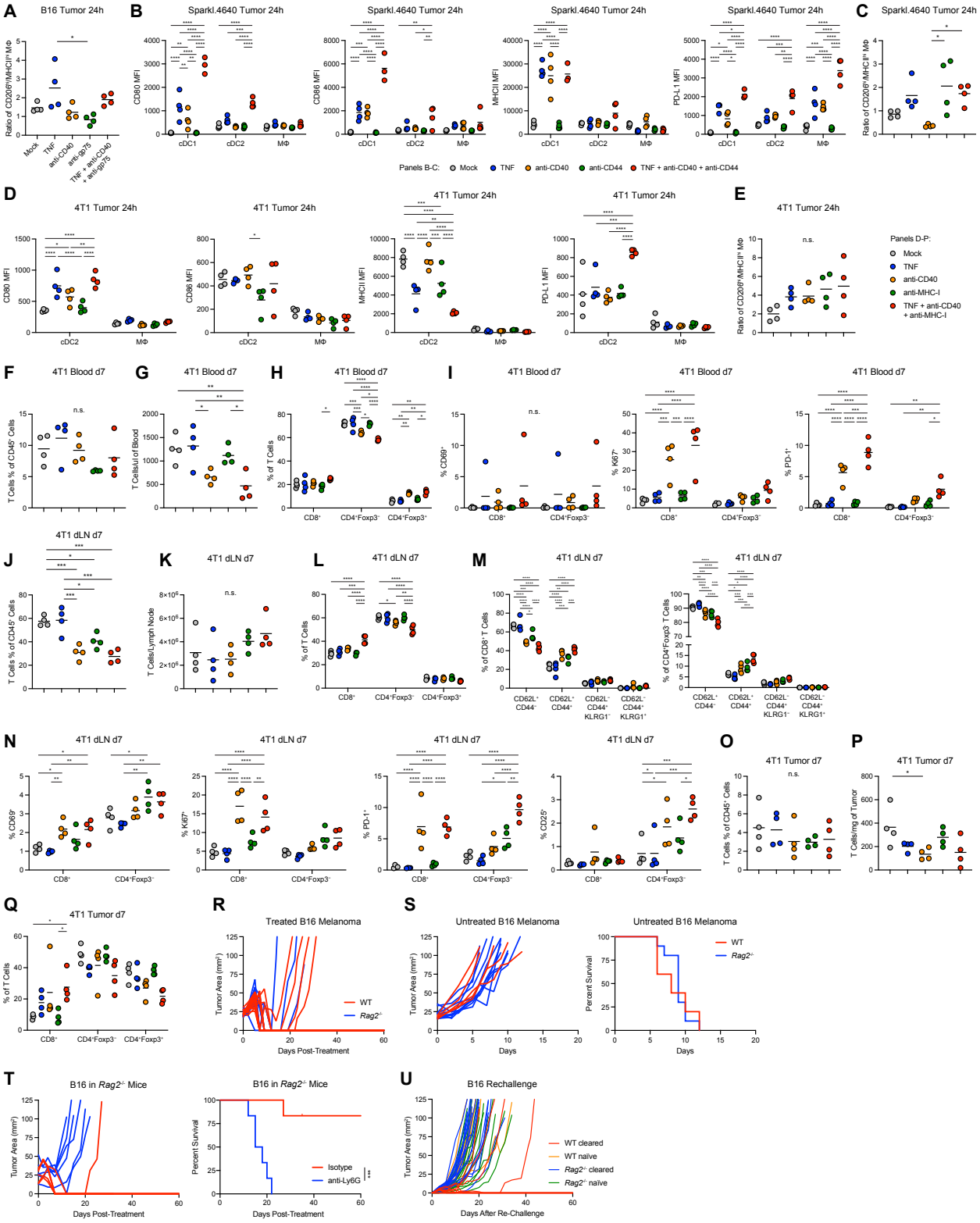

Figure S5: Activation and priming of immune cells induced by neutrophil-activating therapy. Related to Figure 3.

(**A**) Ratio of CD206^{hi} to MHCII^{hi} macrophages in B16 tumors 24 hours after treatment with the indicated components (n=4). (**B**) Expression of activation markers on APC populations in SparkI.4640 tumors 24 hours after treatment with the indicated components (n=4). (**C**) Ratio of CD206^{hi} to MHCII^{hi} macrophages in SparkI.4640 tumors 24 hours after treatment with the indicated components (n=4). (**D**) Expression of activation markers on APC populations in 4T1 tumors 24 hours after treatment with the indicated components (n=4). (**E**) Ratio of CD206^{hi} to MHCII^{hi} macrophages in 4T1 tumors 24 hours after treatment with the indicated components (n=4). (**F-G**) Frequencies (F) and numbers (G) of total T cells in the blood 7 days after treatment of 4T1 (n=4). (**H**) Percent of T cell subsets out of total T cells in the blood 7 days after treatment of 4T1 (n=4). (**I**) Expression of markers on T cell subsets in the blood 7 days after treatment of 4T1 (n=4). (**J-K**) Frequencies (J) and numbers (K) of total T cells in the tumor-draining lymph node (dLN) 7 days after treatment of 4T1 (n=4). (**L**) Percent of T cell subsets out of total T cells in the dLN 7 days after treatment of 4T1 (n=4). (**M**) Memory and effector phenotypes of T cell subsets in the dLN 7 days after treatment of 4T1 (n=4). (**N**) Expression of markers on T cell subsets in the dLN 7 days after treatment of 4T1 (n=4). (**O-P**) Frequencies (O) and numbers (P) of total T cells in the tumor 7 days after treatment of 4T1 (n=4). (**Q**) Percent of T cell subsets out of total T cells in the tumor 7 days after treatment of 4T1 (n=4). (**R**) Growth of B16 in WT or *Rag2*$^{-/-}$ mice following treatment with neutrophil-activating therapy (n=15). (**S**) Growth of B16 in untreated WT or *Rag2*$^{-/-}$ mice (WT n=5, *Rag2*$^{-/-}$ n=10). (**T**) Tumor growth and survival of B16-bearing *Rag2*$^{-/-}$ mice treated with neutrophil-activating therapy after administration of anti-Ly6G or isotype control (n=6). (**U**) Growth of B16 implanted in tumor-naïve or B16-cleared WT or *Rag2*$^{-/-}$ mice 50 days after initial treatment with neutrophil-activating therapy (WT cleared n=14, *Rag2*$^{-/-}$ cleared n=18, WT naïve n=10, *Rag2*$^{-/-}$ naïve n=15). Statistics: One-way ANOVA with Tukey's multiple comparisons test (A, C, E-G, J-K, O-P), Two-way ANOVA with Tukey's multiple comparisons test (B, D, H-I, L-N, Q), Log-rank test (S-T). For all dot plots, the line indicates the mean. Data are representative of 3 (R), 2 (A, U), or 1 (B-Q, T) independent experiments or pooled from 2 experiments (S).

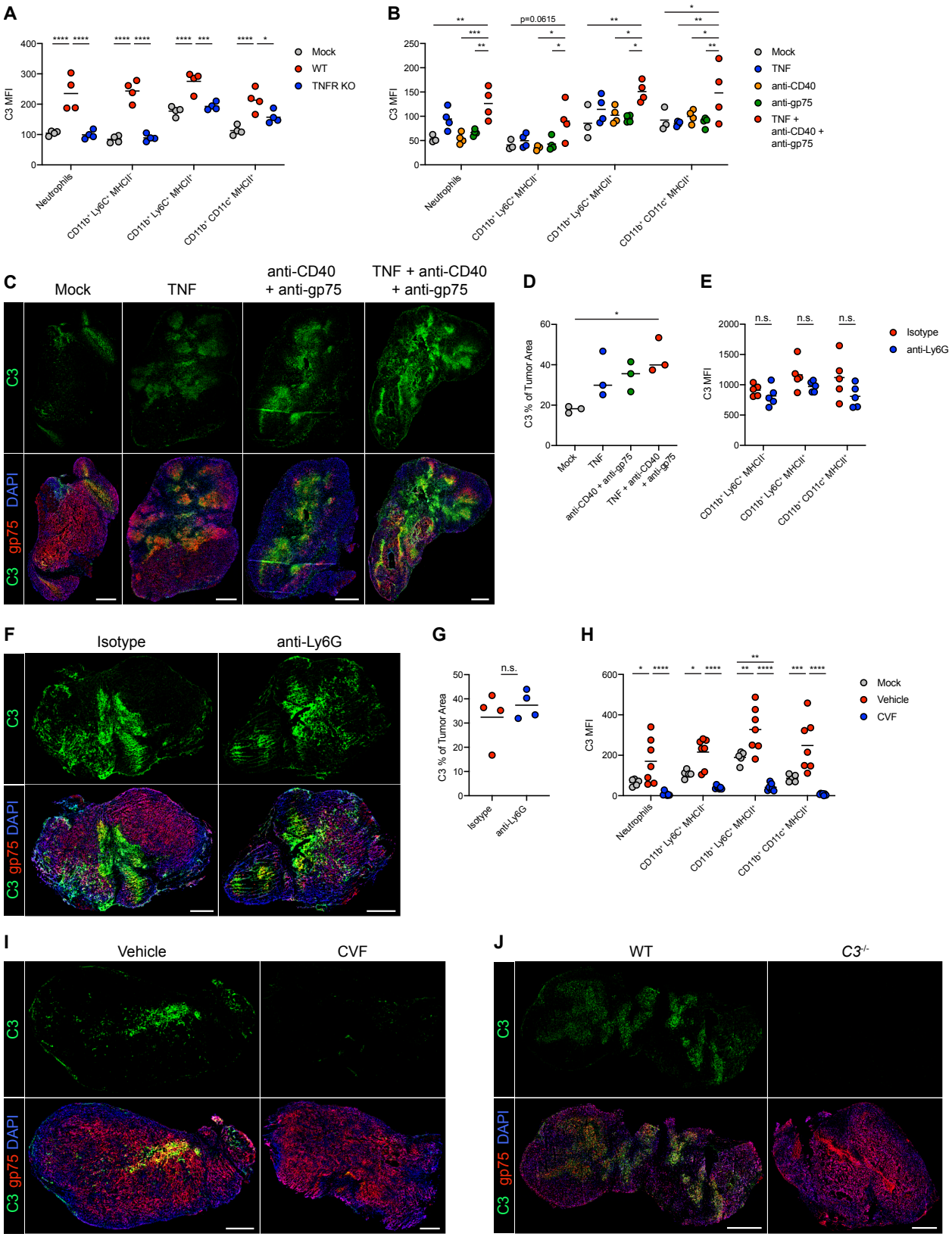
A
C3 MFI
Mock
WT
TNFR KO
Neutrophils
CD11b+ Ly6C+ MHCII-
CD11b+ Ly6C+ MHCII+
CD11b+ CD11c+ MHCII+
B
C3 MFI
p=0.0615
Mock
TNF
anti-CD40
anti-gp75
TNF + anti-CD40 + anti-gp75
C
Mock
TNF
anti-CD40 + anti-gp75
TNF + anti-CD40 + anti-gp75
C3
C3 gp75 DAPI
D
C3 % of Tumor Area
Mock
TNF
anti-CD40 + anti-gp75
TNF + anti-CD40 + anti-gp75
E
C3 MFI
n.s.
Isotype
anti-Ly6G
F
Isotype
anti-Ly6G
C3
C3 gp75 DAPI
G
C3 % of Tumor Area
n.s.
Isotype
anti-Ly6G
H
C3 MFI
Mock
Vehicle
CVF
I
Vehicle
CVF
C3
C3 gp75 DAPI
J
WT
C3-/-
C3
C3 gp75 DAPI

Figure S6: Complement activation is induced by neutrophil-activating therapy. Related to Figure 4.

(**A**) MFI of C3 deposition on myeloid cell populations in B16 tumors 4 hours after treatment of WT or TNFR KO mice with neutrophil-activating therapy (n=4). (**B**) MFI of C3 deposition on myeloid cell populations in B16 tumors 4 hours after treatment with the indicated components (n=4). (**C-D**) Representative immunofluorescence (D) and quantification (E) of C3 staining in B16 tumors 4 hours after treatment with the indicated components. Scale bars = 500 μm. (n=3) (**E**) MFI of C3 deposition on myeloid cell populations in B16 tumors 4 hours after treatment of Ly6G-depleted mice with neutrophil-activating therapy (n=5). (**F-G**) Representative immunofluorescence (F) and quantification (G) of C3 staining in B16 tumors 4 hours after treatment following Ly6G depletion. Scale bars = 500 μm. (n=4) (**H**) MFI of C3 deposition on myeloid cell populations in B16 tumors 4 hours after neutrophil-activating therapy following complement depletion by CVF (mock n=5, others n=7). (**I**) Representative immunofluorescence of C3 staining in B16 tumors 4 hours after neutrophil-activating therapy following administration of CVF. Scale bars = 500 μm. (**J**) Representative immunofluorescence of C3 staining in B16 tumors 4 hours after treatment of WT or *C3*$^{-/-}$ mice. Scale bars = 500 μm. Statistics: Two-way ANOVA with Tukey's multiple comparisons test (A-B, E, H), One-way ANOVA with Tukey's multiple comparisons test (D, G). For all dot plots, the line indicates the mean. Data are representative of 3 (I), 2 (B, E-G), or 1 (A, C-D, J) independent experiments or pooled from 2 experiments (H).

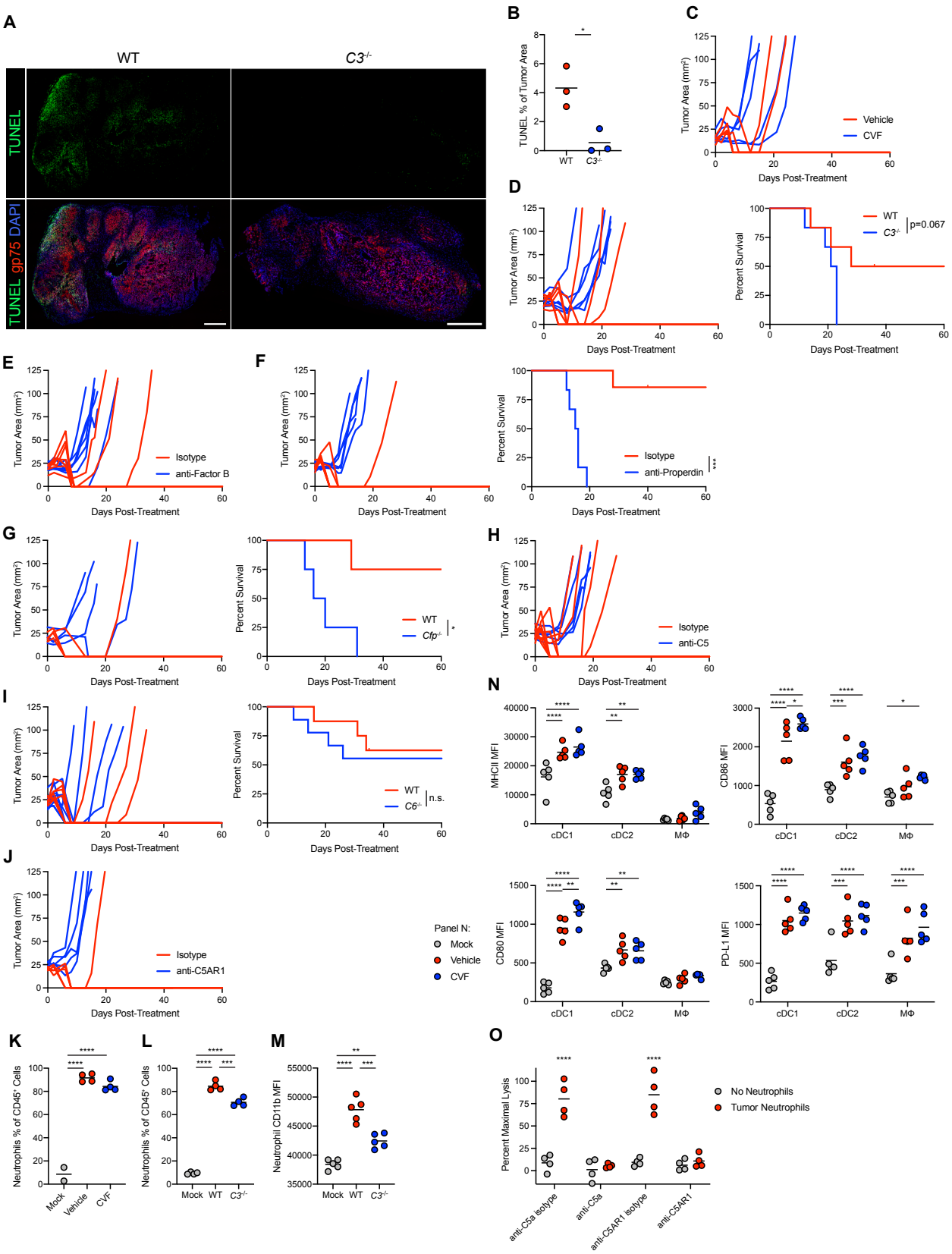
A
WT
C3-/-
TUNEL
TUNEL gp75 DAPI
B
TUNEL % of Tumor Area
WT
C3-/-
C
Tumor Area (mm2)
Vehicle
CVF
Days Post-Treatment
D
Tumor Area (mm2)
Days Post-Treatment
Percent Survival
WT
C3-/-
p=0.067
Days Post-Treatment
E
Tumor Area (mm2)
Isotype
anti-Factor B
Days Post-Treatment
F
Tumor Area (mm2)
Days Post-Treatment
Percent Survival
Isotype
anti-Properdin
***
Days Post-Treatment
G
Tumor Area (mm2)
Days Post-Treatment
Percent Survival
WT
Cfp-/-
*
Days Post-Treatment
H
Tumor Area (mm2)
Isotype
anti-C5
Days Post-Treatment
I
Tumor Area (mm2)
Days Post-Treatment
Percent Survival
WT
C6-/-
n.s.
Days Post-Treatment
J
Tumor Area (mm2)
Isotype
anti-C5AR1
Days Post-Treatment
N
MHCII MFI
CD86 MFI
CD80 MFI
PD-L1 MFI
cDC1
cDC2
MΦ
Panel N:
Mock
Vehicle
CVF
K
Neutrophils % of CD45+ Cells
Mock
Vehicle
CVF
L
Neutrophils % of CD45+ Cells
Mock
WT
C3-/-
M
Neutrophil CD11b MFI
Mock
WT
C3-/-
O
Percent Maximal Lysis
anti-C5a isotype
anti-C5a
anti-C5AR1 isotype
anti-C5AR1
No Neutrophils
Tumor Neutrophils

Figure S7: The alternative complement pathway induces neutrophil activation through C5a. Related to Figure 4.

(**A-B**) Representative immunofluorescence (A) and quantification (B) of TUNEL staining in B16 tumors 24 hours after treatment of WT or *C3*$^{-/-}$ mice. Scale bars = 500 μm. (n=3) (**C**) Growth of B16 tumors in mice depleted of complement with Cobra Venom Factor (CVF) prior to treatment with neutrophil-activating therapy (n=5). (**D**) Tumor growth and survival of B16-bearing WT or *C3*$^{-/-}$ mice following treatment with neutrophil-activating therapy (n=6). (**E**) Growth of B16 in mice treated following administration of anti-Factor B (n=7). (**F**) Tumor growth and survival of B16-bearing mice treated with neutrophil-activating therapy following administration of a blocking antibody targeting properdin (isotype n=7, anti-properdin n=6). (**G**) Tumor growth and survival of B16-bearing WT or *Cfp*$^{-/-}$ mice following treatment with neutrophil-activating therapy (n=4). (**H**) Growth of B16 in mice treated with neutrophil-activating therapy following administration of anti-C5 (mock n=12, anti-C5 n=5). (**I**) Tumor growth and survival of B16-bearing WT or *C6*$^{-/-}$ mice following neutrophil-activating therapy (WT n=8, C6$^{-/-}$ n=9). (**J**) Growth of B16 in mice treated following administration of anti-C5AR1 (n=5). (**K**) Frequency of B16 tumor-infiltrating neutrophils 24 hours after treatment with neutrophil-activating therapy following CVF administration (mock n=2, others n=4). (**L**) Frequency of B16 tumor-infiltrating neutrophils 24 hours after treatment of WT or *C3*$^{-/-}$ mice with neutrophil-activating therapy (n=4). (**M**) MFI of CD11b on B16 tumor-infiltrating neutrophils in *C3*$^{-/-}$ mice 4 hours after neutrophil-activating therapy (n=4). (**N**) Activation marker expression on tumor APCs 4 hours after treatment of complement-depleted mice with neutrophil-activating therapy (n=5). (**O**) Percent lysis of B16 cells co-cultured with neutrophils isolated from treated tumors, stimulated *in vitro* with TNF + anti-CD40 + anti-gp75 in active serum in the presence of blocking antibodies targeting C5a, C5AR1, or isotype controls. Asterisks indicate significance relative to the six samples without elevated levels of lysis (n=4). Statistics: One-way ANOVA with Tukey's multiple comparisons test (B, K-M), Log-rank test (D, F-G, I), Two-way ANOVA with Tukey's multiple comparisons test (N-O). For all dot plots, the line indicates the mean. Data are representative of 2 (C-E, J-O) or 1 (A-B, G) independent experiments or pooled from 2 experiments (F, H-I).

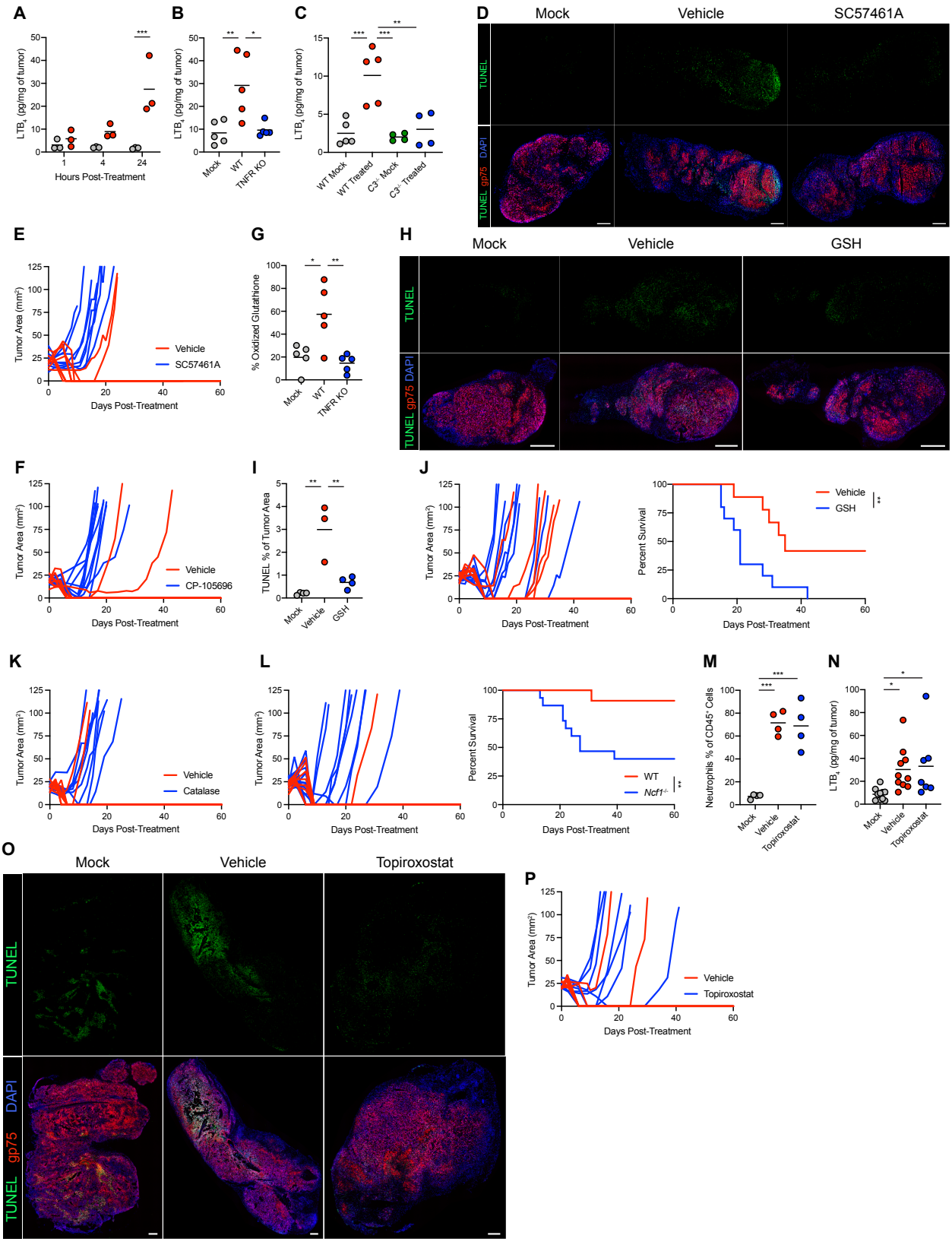

Figure S8: Xanthine oxidase induces cell death downstream of neutrophil infiltration and secretion of LTB_4. Related to Figures 5 and 6.

(**A**) LTB_4 levels in B16 tumors following treatment with neutrophil-activating therapy (n=3). (**B**) LTB_4 levels in B16 tumors 24 hours after treatment of WT or TNFR KO mice with neutrophil-activating therapy (n=5). (**C**) LTB_4 levels in B16 tumors 24 hours after treatment of WT or *C3*$^{-/-}$ mice with neutrophil-activating therapy (WT n=5, *C3*$^{-/-}$ n=4). (**D**) Representative TUNEL immunofluorescence in B16 tumors 24 hours after treatment following administration of the LTA_4H inhibitor SC57461A. Scale bars = 500 μm. (**E-F**) Growth of B16 in mice administered SC57461A (E) (vehicle n=9, SC57461A n=8) or the BLT1 antagonist CP-105696 (F) (vehicle n=9, CP-105696 n=10) prior to treatment with neutrophil-activating therapy. (**G**) Oxidized glutathione percentage of total glutathione in B16 lysates 24 hours after neutrophil-activating therapy in WT or TNFR KO mice (n=5). **(H-I**) Representative immunofluorescence (H) and quantification (I) of TUNEL staining in B16 tumors 24 hours after treatment following GSH administration. Scale bars = 500 μm. (vehicle n=3, others n=4) (**J**) Tumor growth and survival of B16-bearing mice treated following administration of GSH (vehicle n=9, GSH n=10). (**K**) Growth of B16 in mice administered catalase during treatment with neutrophil-activating therapy (n=9). (**L**) Tumor growth and survival of B16-bearing WT or *Ncf1*$^{-/-}$ mice following treatment with neutrophil-activating therapy (WT n=11, *Ncf1*$^{-/-}$ n=15). (**M**) Frequency of neutrophils in B16 tumors 24 hours after treatment with neutrophil-activating therapy following topiroxostat administration (n=4). (**N**) LTB_4 levels in B16 tumors 24 hours after neutrophil-activating therapy following topiroxostat administration (topiroxostat n=7, others n=10). (**O**) Representative TUNEL immunofluorescence in B16 tumors 24 hours after treatment with neutrophil-activating therapy following administration of topiroxostat. Scale bars = 500 μm. (**P**) Growth of B16 in mice administered topiroxostat during treatment with neutrophil-activating therapy (vehicle n=9, topiroxostat n=8). Statistics: Two-way ANOVA with Tukey's multiple comparisons test (A), One-way ANOVA with Tukey's multiple comparisons test (B-C, G, I, M-N), Log-rank test (J, L). For all dot plots, the line indicates the mean. Data are representative of 2 (B, G, J, M, P) or 1 (A, C-D, H-I, O) independent experiments or pooled from 2 experiments (E-F, K-L, N).

Supplemental Table

Table S1: Oligonucleotide sequences, Related to STAR Methods

Name	Sequence	Source	Identifier
Tnfrsf1a sgRNA1	AGACCTAGCAAGATAACCAG	Doench et al., 2016	N/A
Tnfrsf1a sgRNA2	GATGGGGATACATCCATCAG	Doench et al., 2016	N/A
Surface EGFP	ATAGCAGGATCCGCCGCCACCATGGAGACCGAT ACCCTCCTGCTGTGGGTTCTCCTGCTGTGGGT GCCTGGCTCCACCGGTGATATGGTTAGCAAGG GCGAAGAGCTCTTTACTGGCGTCGTGCCAATAC TCGTCGAGCTGGATGGGGACGTTAATGGCCATA AATTCAGCGTGAGCGGCGAGGGGGAGGGGGA CGCCACCTACGGAAAGCTTACTTTGAAGTTTATT TGCACTACAGGCAAGTTGCCTGTGCCTTGGCCT ACACTCGTGACCACACTCACTTACGGGGTGCAG TGTTTTTCTAGGTATCCTGATCACATGAAACAGC ACGACTTTTTCAAGAGCGCAATGCCTGAAGGCTA CGTCCAGGAGAGAACCATATTTTTCAAGGATGAT GGTAACTACAAAACTAGAGCTGAAGTCAAGTTTG AGGGGGACACCCTCGTGAACAGAATTGAATTGA AAGGCATTGATTTCAAGGAGGACGGAAACATTCT CGGACACAAACTGGAATATAATTACAATAGTCATA ACGTCTATATCATGGCAGATAAGCAGAAGAACGG GATTAAAGTCAATTTCAAAATCAGACACAATATCG AGGATGGCTCCGTTCAACTGGCTGATCATTATCA ACAGAACACCCCTATCGGCGACGGACCTGTTTT GCTCCCTGACAATCACTACTTGTCTACCCAGTCC GCTCTCAGCAAAGACCCCAACGAAAAGCGCGAT CACATGGTTCTGCTGGAGTTCGTGACAGCCGCA GGCATAACACTGGGGATGGACGAGCTTTACAAG AATTCTATGGGAGGAGATAGTCAGGAGGTGACC GTCGTGCCTCACTCCCTGCCCTTTAAGGTGGTC GTTATCTCAGCTATACTTGCCCTTGTGGTTTTGA CTGTTATATCCCTGATTATCCTCATCATGCTGTG GCAGAAAAAGCCCCGGTAATCTAGAACTCGT	Synthesized by GeneArt, ThermoFisher Scientific	N/A