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Supplemental information

Cancer genes disfavoring T cell immunity identified via integrated systems approach

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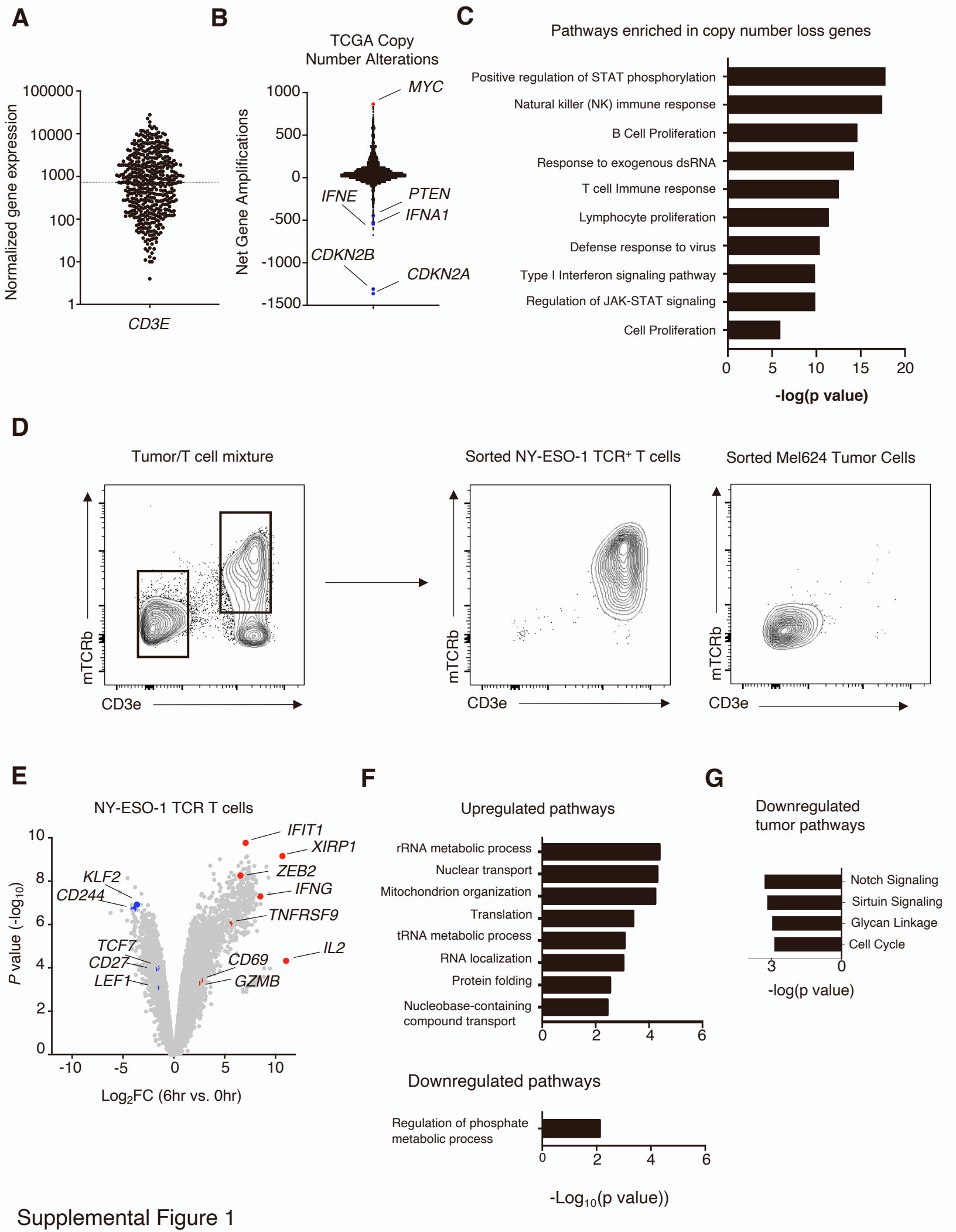
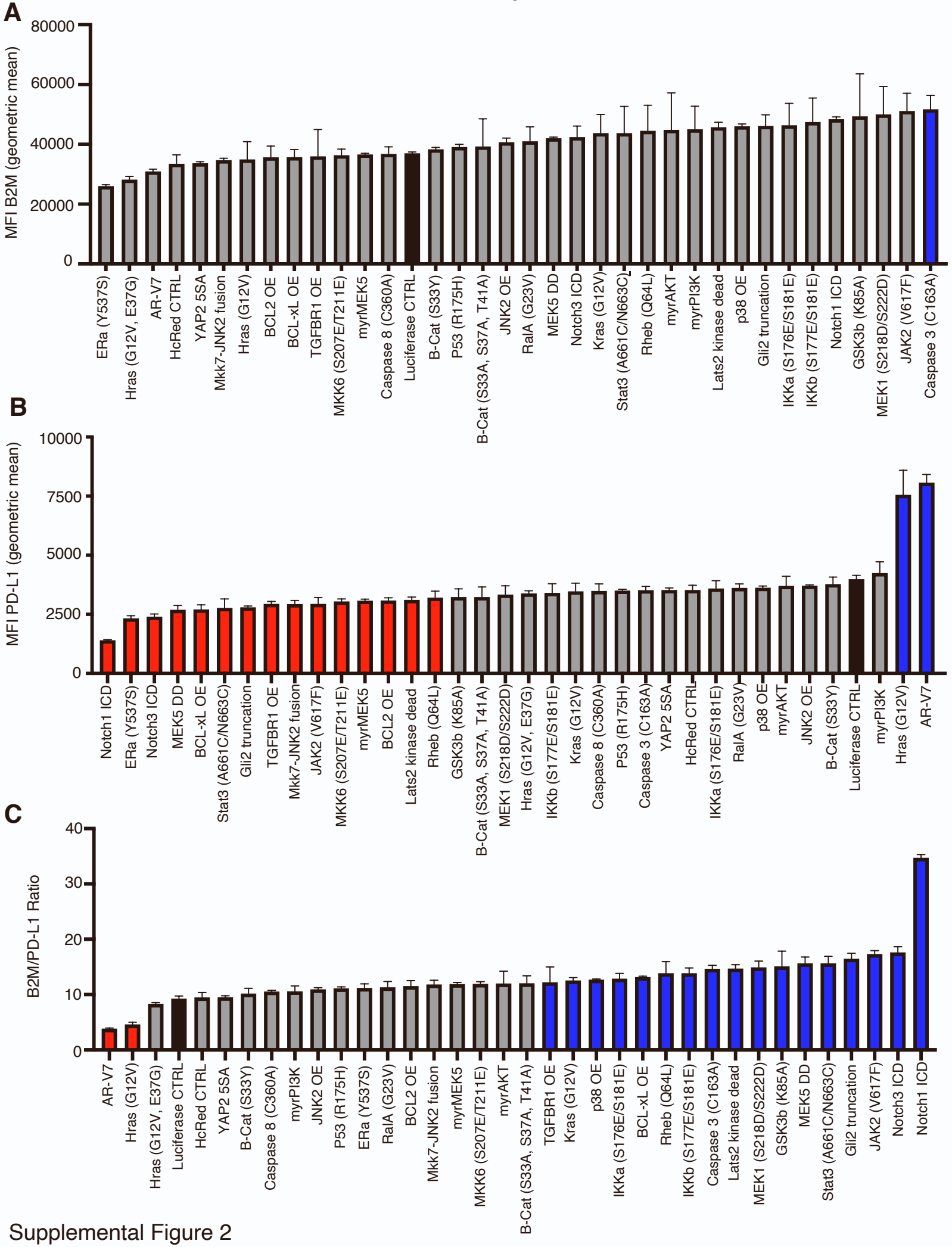
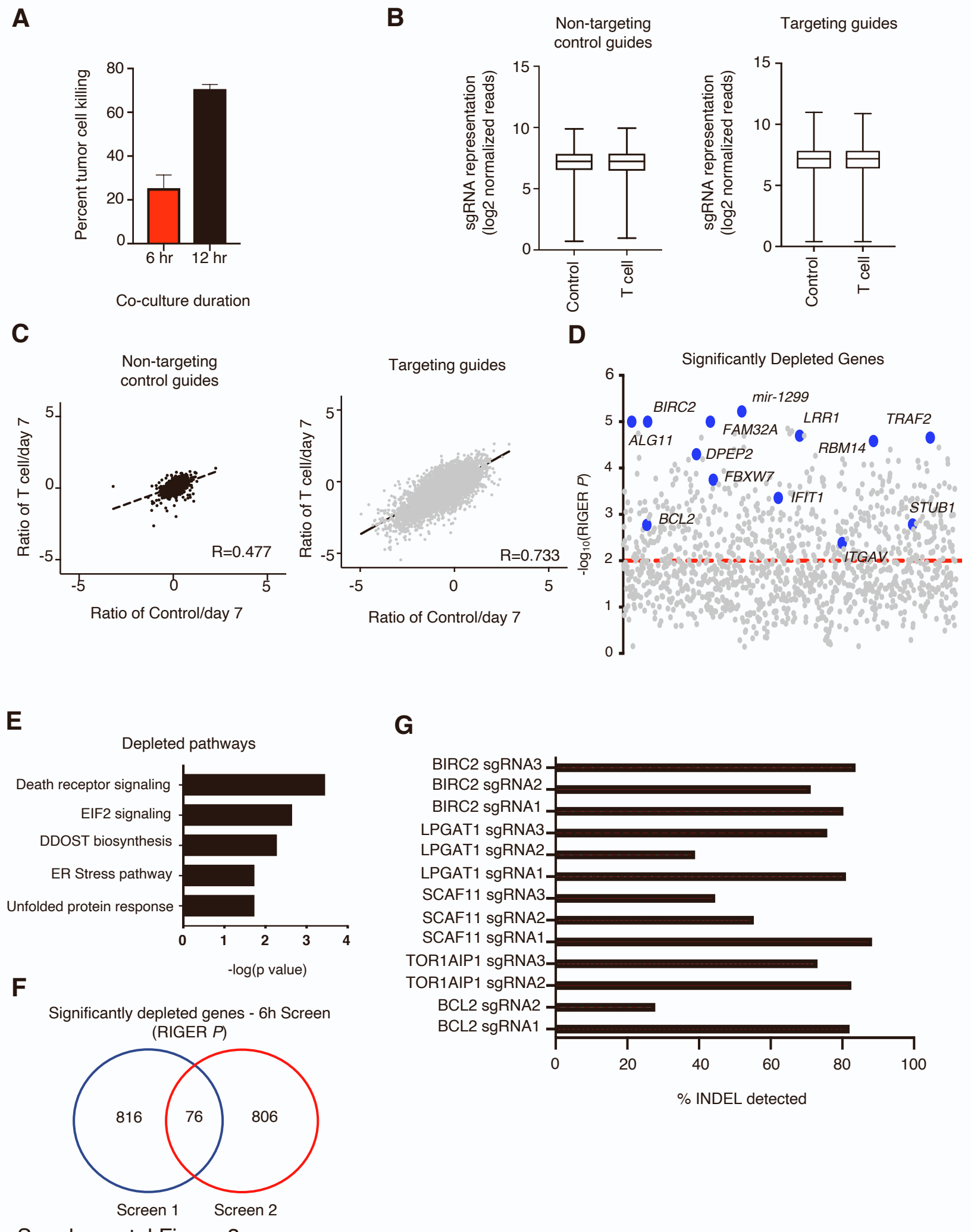


Supplemental Figure 1

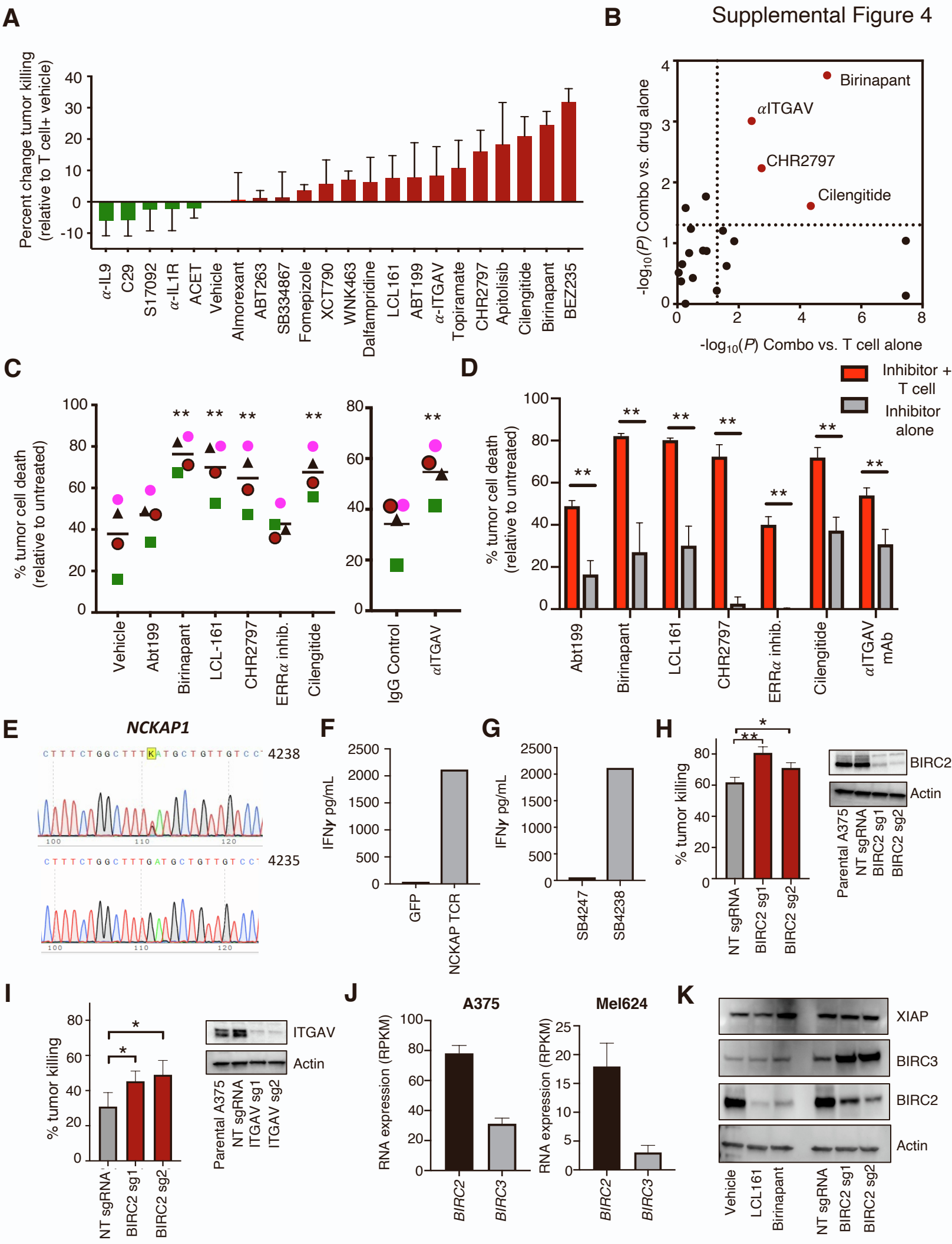


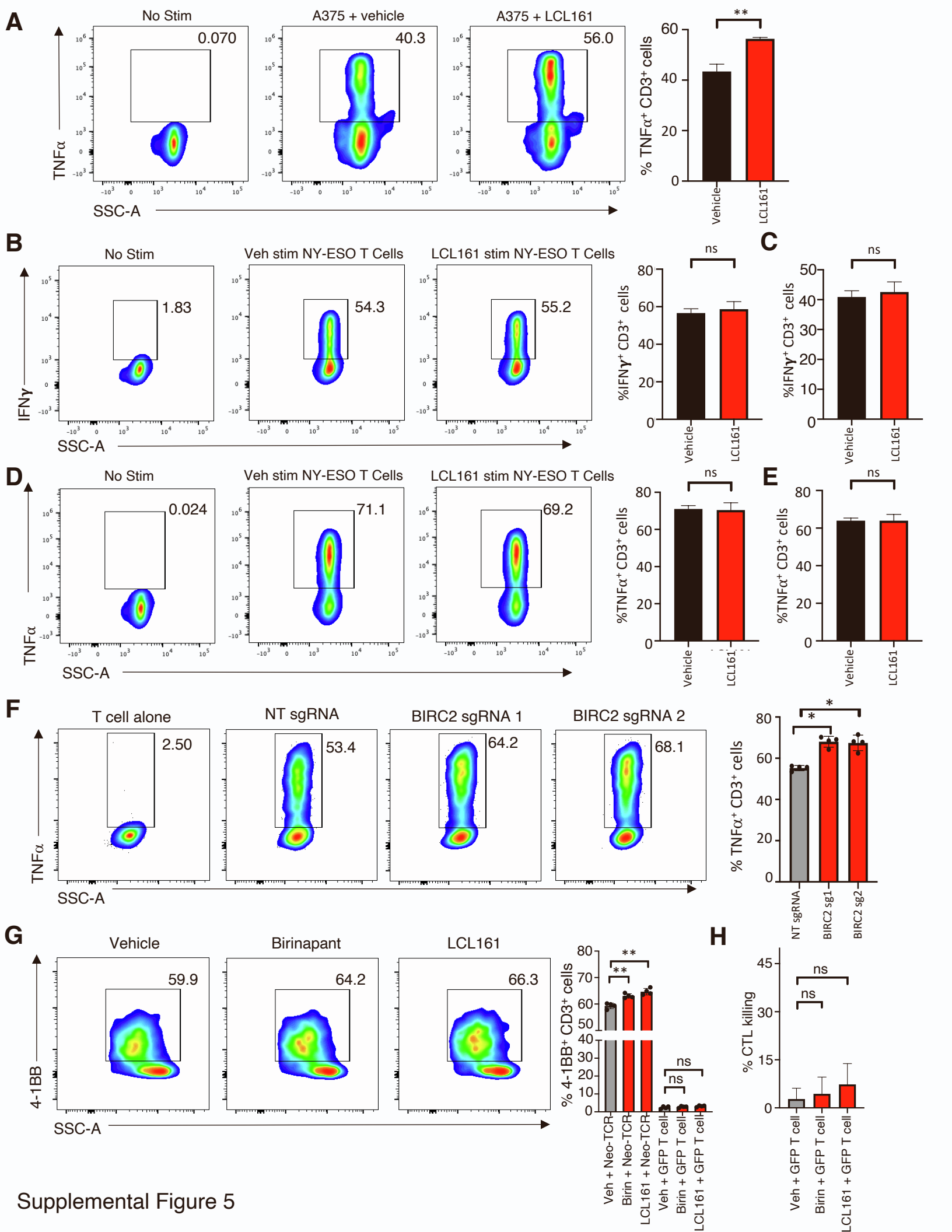
Supplemental Figure 2



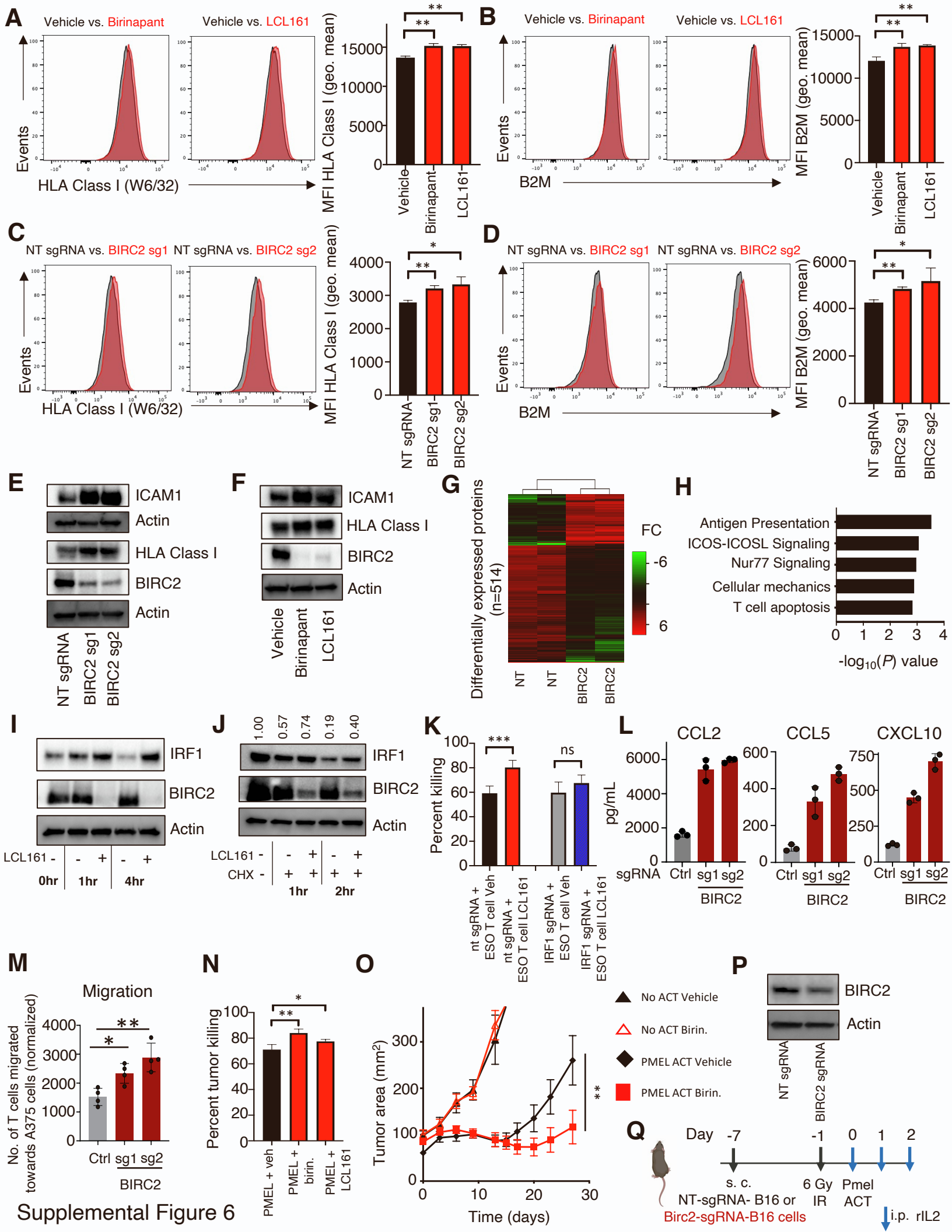
Supplemental Figure 3

Supplemental Figure 4





Supplemental Figure 5



Supplemental Figure 6

Supplemental Figure Titles and Legends

Supplemental Figure 1, related to Figure 1**: Tumor CNA in genes associated with immune regulation. (a)** Individual melanoma samples from TCGA SKCM dataset were analyzed for normalized expression of *CD3E*. Median value indicated with dotted line. **(b)** Tumor samples from across the TCGA PanCancer Atlas Studies were analyzed for copy number alterations (CNAs) in all genes. Net gene amplifications were plotted as the sum of genomic deletions and amplifications, where deletions were imputed as a negative value. Genes with net amplifications are displayed with positive net amplification values, while genes with net deletions are displayed with negative net amplification values. **(c)** 200 most frequently deleted genes (based on net amplifications) were analyzed for pathway enrichment using gene ontology (GO) analysis. Selected enriched pathways are displayed. **(d)** Sorting strategy for isolation of NY-ESO-1 TCR-expressing T cells and Mel624 melanoma cells following co-culture. **(e)** Volcano plot representing differentially expressed genes analyzed by RNA-seq of $CD3^{+}mTCRb^{+}$ T cells that were co-cultured with Mel624 cells for 0 or 6h. Abundance is represented as relative fold change (*x* axis) versus significance (*y* axis). **(f)** Ingenuity Pathway Analysis of T cell genes that were differentially expressed following co-culture with Mel624 melanoma cells. Data is pooled from three independent experiments **(g)** Ingenuity Pathway Analysis (IPA) of Mel624 genes that were significantly downregulated upon ESO T cell co-culture. Data from **(e-g)** is pooled from three independent experiments.

Supplemental Figure 2, related to Figure 2**: Constitutive activation of tumor pro-growth and survival pathways alters immune phenotype**. A375 cells expressing pathway activating genes as described in **Figure 2** were assessed for **(a)** beta-2-microglobulin and **(b)** PD-L1 expression by flow cytometry surface staining. **(c)** Ratio of beta-2-microglobulin/PD-L1 was calculated. Data is representative of two independent experiments and experiments were performed in technical triplicates. Values from control cells (transduced with luciferase only construct) are depicted in black. Second control cells (transduced with HcRed only construct) are also indicated as "HcRed CTRL". Altered cells with significantly lower values are indicated in red, while cells with significantly higher values are indicated in blue. Statistical analysis was performed by one-way ANOVA followed by Dunnett-corrected multiple comparison test.

Supplemental Figure 3, related to Figure 3**: Genome-scale 2CT screen for tumor genes regulated susceptibility to T cell CYT. (a)** Mel624 melanoma cells were co-cultured with ESO T cells at a 1:3 E:T ratio for 6 or 12h and tumor cell survival was measured by flow cytometry. **(b)** Normalized reads of non-targeting control guides and targeted guides were analyzed in GECKOv2-transduced Mel624 cells +/- 6h ESO T cell co-culture. **(c)** Scatterplot demonstrating the effect of T cell selection pressure on the global distribution of sgRNAs after co-culture with ESO T cells. **(d)** RIGER statistical analysis (Luo et al., 2008) was performed to identify top depleted genes. **(e)** Top 250 genes for which sgRNAs were significantly ($p < 0.05$) depleted by RIGER ranking were analyzed by Ingenuity Pathway Analysis (IPA) and enriched pathways were determined. **(f)** Overlap of genes enriched after T cell-based selection in two independent screens. Venn diagrams depict shared and unique most enriched candidates that achieved statistical significant depletion based on RIGER *p* analysis. **(g)** TIDE analysis of INDELs generated

by CRISPR-Cas9 at each exonic target site for the genes validated in A375 cells at day 20 post-transduction.

Supplemental Figure 4, related to Figure 4: Small-molecule screen reveals candidate compounds capable of augmenting specific T cell killing of tumor. **(a)** A375 melanoma cells were co-cultured with ESO T cells along with inhibitors at 500 nM except for α-ITGAV at 0.25 μg/mL and tumor cell elimination was measured by flow cytometry. **(b)** Plot of *p* values (significance threshold $p < 0.05$, dotted lines) between tumor elimination by the combination of inhibitor and ESO T cell treatment versus ESO T cells alone (*x* axis) and tumor elimination by the combination treatment versus inhibitor alone (*y* axis). **(c)** A375 melanoma killing by ESO T cells (1:2 E:T ratio, 16h treatment) was assayed by flow cytometry using ESO T cells generated from four independent healthy donors, with each symbol representing a donor. **(d)** A375 melanoma cells were cultured with inhibitors alone or with inhibitors + ESO T cells and tumor killing was measured by flow cytometry. **(e)** Genomic DNA from SB4238 (NCKAP1 mutant) and SB4235 tumor lines (NCKAP1 wild type) was collected and Sanger Sequencing was performed at the NCKAP1 locus to confirm the presence of a mutation. **(f)** Primary human T cells were transduced with GFP or a NCKAP1 reactive TCR and co-cultured with SB4238 tumor cells for 16h. ELISA was performed to determine IFNγ secretion. **(g)** Primary human T cells were transduced with a NCKAP1 reactive TCR and co-cultured with SB4247 or SB4238 tumor cells for 16h. ELISA was performed to determine IFNγ secretion. **(h)** Cas9 expressing A375 cells were transduced with either control, non-targeted sgRNAs or BIRC2-targeting sgRNAs and western blot analysis was performed for BIRC2 expression. Control or BIRC2-perturbed cell lines were co-cultured with ESO T cells at a 1:2 E/T ratio for 24h and tumor killing was measured by flow cytometry enumeration of live cells. **(i)** Cas9 expressing A375 cells were transduced with either control, non-targeted sgRNAs or ITGAV-targeting sgRNAs and western blot analysis was performed for ITGAV expression. Control or ITGAV-perturbed cell lines were co-cultured with ESO T cells for 24 hours and tumor killing was measured by flow cytometry enumeration of live cells. **(j)** Quantification of BIRC2 and BIRC3 RNA expression in A375 and Mel624 melanoma cells by RNA sequencing analysis. RNA expression for each tumor cell line was measured in biological triplicates, with mean values depicted. **(k)** A375 melanoma cells were treated with vehicle, 5 μM LCL161 or 5 μM birinapant for 16h. Additionally, Cas9 expressing A375 cells were transduced with either control, non-targeted sgRNAs or BIRC2-targeting sgRNAs. Western blot analysis was performed on all samples. Data are representative of four independent experiments **(a-b, d)**, depict pooled results from four independent donors **(c)**, are from a single experiment **(f-g),** summarize three biological replicates **(j)**, or are representative of two independent experiments **(h-I, k)**. Statistical analysis was performed by paired *t* test **(c)**, or Student's *t* test. $** p < 0.01$, $* p < 0.05$.

Supplemental Figure 5, related to Figure 5**: Genetic or pharmacological inhibition of tumor BIRC2 increases T cell tumor recognition and response**. **(a)** ESO T cells were co-cultured with A375 melanoma cells in the presence or absence of 5μM LCL161 for 5h along with brefeldin A and monensin and T cell intracellular production of TNFα was measured by flow cytometry. **(b-d)** ESO T cells were stimulated using PMA/Ionomycin for 6h in the presence of brefeldin A and

monensin and T cell intracellular production of **(b)** IFNγ and **(d)** TNFα was measured by flow cytometry. **(c,e)** ESO T cells were stimulated using αCD3/αCD28 coated plates (2μg/mL) for 6h in the presence of brefeldin A and monensin and T cell intracellular production of **(c)** IFNγ and **(e)** TNFα was measured by flow cytometry. **(f)** A375 melanoma cells transduced with Cas9 and non-targeting (NT) or BIRC2-targeting sgRNAs were co-cultured with ESO T cells for 5h in the presence of brefeldin A and monensin and T cell intracellular production of TNFα was measured by flow cytometry. **(g)** Colon cancer cell line SB4238 was co-cultured with T cells transduced with a TCR reactive against $NCKAP1^{D438Y}$ along with inhibitors at 5μM. After 16h co-culture, T cell upregulation of 4-1BB was measured by flow cytometry. **(h)** Colon cancer cell line SB4238 was co-cultured with T cells transduced with control GFP for 24h and tumor killing was measured by flow cytometry. Data are representative of three independent experiments with at least technical triplicates **(a, b, d, f, g)**, or two independent experiments with technical quadruplicates **(c, e, h)**. Statistical analysis was performed by Student's *t* test. * $p < 0.05$, ** $p < 0.01$, ns: not significant.

Supplemental Figure 6, related to Figure 5 and Figure 6**: Genetic or pharmacological inhibition of tumor BIRC2 increases expression of tumor antigen presentation pathway genes**. **(a-b)** A375 melanoma cells were treated with vehicle or inhibitors at 5μM and **(a)** HLA Class I expression by W6/32 antibody and **(b)** beta-2-microglobulin expression were measured by flow cytometry. **(c-e)** A375 melanoma cells transduced with Cas9 and non-targeting (NT) or BIRC2-targeting sgRNAs were analyzed for expression of **(c)** HLA Class I and **(d)** beta-2-microglobulin by flow cytometry or were **(e)** profiled by western blot analysis. **(f)** A375 melanoma cells treated with vehicle or inhibitors at 5μM were profiled by western blot analysis. **(g-h)** A375 melanoma cells transduced with Cas9 and non-targeting (NT) or BIRC2-targeting sgRNAs were analyzed TMT-proteomics and differentially expressed proteins (greater than +/- 1.5 fold differential expression) were plotted in a heatmap **(g)**. Proteins with increased expression are indicated in red, while proteins with decreased expression are indicated in green. **(h)** Proteins with increased expression were analyzed by pathway analysis and significantly enriched pathways are depicted. **(i)** A375 melanoma cells were treated with vehicle or LCL161 at 5μM for various timepoints and protein expression was profiled by western blot analysis. **(j)** A375 melanoma cells were treated with vehicle or LCL161 at 5μM in the presence of cycloheximide (50 μg/mL) for 1 or 2h and protein expression was measured by western blot analysis. **(k)** A375 melanoma cells transduced with Cas9 and non-targeting (NT) or IRF1-targeting sgRNAs were co-cultured with ESO T cells in the presence of vehicle or LCL161 at 5μM and tumor killing was measured by flow cytometry. **(l-m)** A375 cells transduced with Cas9 and NT sgRNA or BIRC2 targeting sgRNA were assayed for production of **(l)** CCL2, CCL5/RANTES, and CXCL10 and **(m)** ability to induce migration of ESO T cells across a transwell membrane. **(n)** B16 melanoma cells were co-cultured with PMEL-1 TCR T cells at a 1:2 E:T ratio for 16h in the presence of vehicle or inhibitors at 5μM and tumor killing was measured by flow cytometry. **(o)** Subcutaneous tumor growth in mice receiving ACT of PMEL1 T cells along with vehicle or Birinapant (50 mg drug/kg body weight). Mice were treated with Birinapant via IP injection every 48h beginning 24h after PMEL1 T cell infusion for a total of 5 doses. **(p)** B16 melanoma cells were transduced with Cas9 and NT sgRNA or BIRC2-targeting sgRNA and protein expression was measured by western blot

analysis. **(q)** Treatment scheme for experiment depicted in **Figure 6e-f**. Data is representative of three independent experiments **(a-f, i-j, l-m)**, two independent experiments **(k, n, o)** or depicts a single experiment with technical duplicates **(g-h)**. Experiments depicted in (**o)** were conducted with n=5 mice per group for no ACT groups, and 7 mice per group in PMEL treatments. Statistical analysis was performed by Student's *t* test, or Wilcoxon rank sum test **(o)**. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.