*Supplementary Information*

# CRISPR activation screen identifies BCL-2 proteins and B3GNT2 as drivers of cancer resistance to T cell-mediated cytotoxicity

Julia Joung[1,2,3,4,5,8,†], Paul C. Kirchgatterer[1,2,3,4,5], Ankita Singh[1,2,3,4,5], Jang H. Cho[1,2,3,4,5], Suchita P. Nety[1,2,3,4,5], Rebecca C. Larson[6,7], Rhiannon K. Macrae[1,2,3,4,5], Rebecca Deasy[2], Yuen-Yi Tseng[2], Marcela V. Maus[6,7], and Feng Zhang[1,2,3,4,5,†]

[1] Department of Biological Engineering, MIT, Cambridge, MA 02139, USA

[2] Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA

[3] Department of Brain and Cognitive Science, MIT, Cambridge, MA 02139, USA

[4] McGovern Institute for Brain Research at MIT, Cambridge, MA 02139, USA

[5] Howard Hughes Medical Institute, MIT, Cambridge, MA 02139, USA

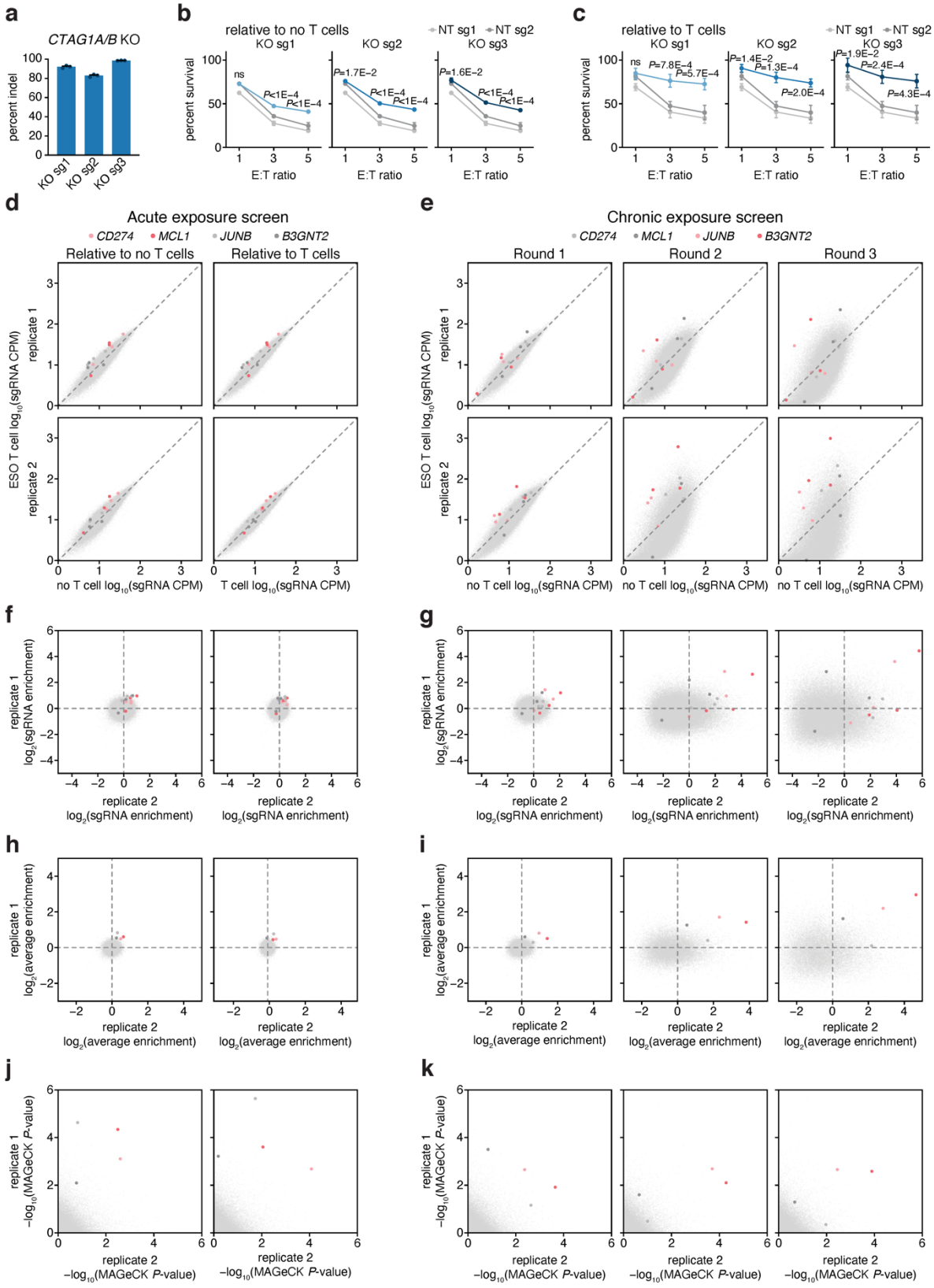
[6] Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

[7] Cellular Immunotherapy Program, Cancer Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

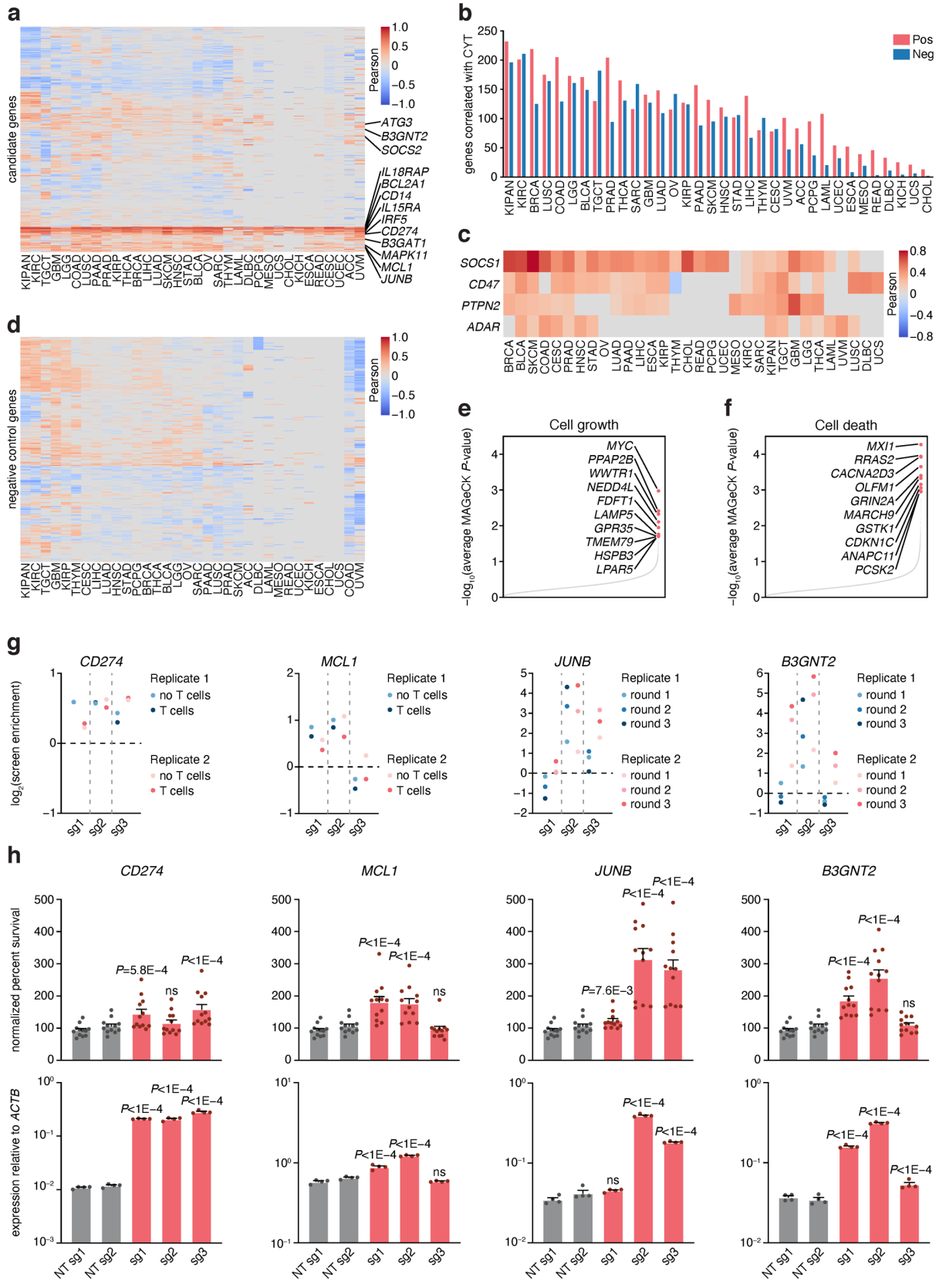
[8] Present address: Whitehead Institute, Cambridge, MA 02142, USA

[†] Correspondence should be addressed to julia@joung.science (J.J.) and zhang@broadinstitute.org (F.Z.).

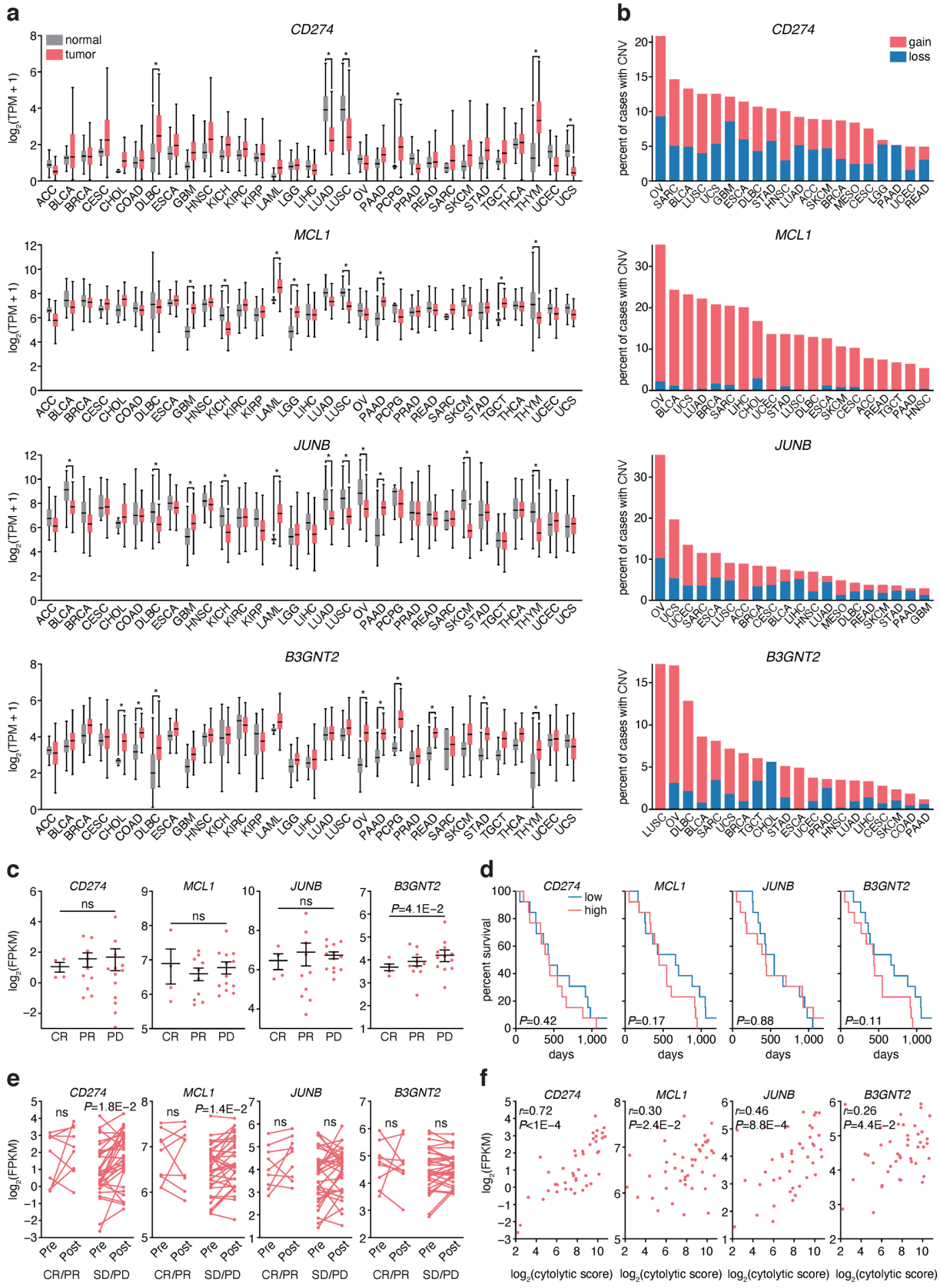
## Supplementary Figures



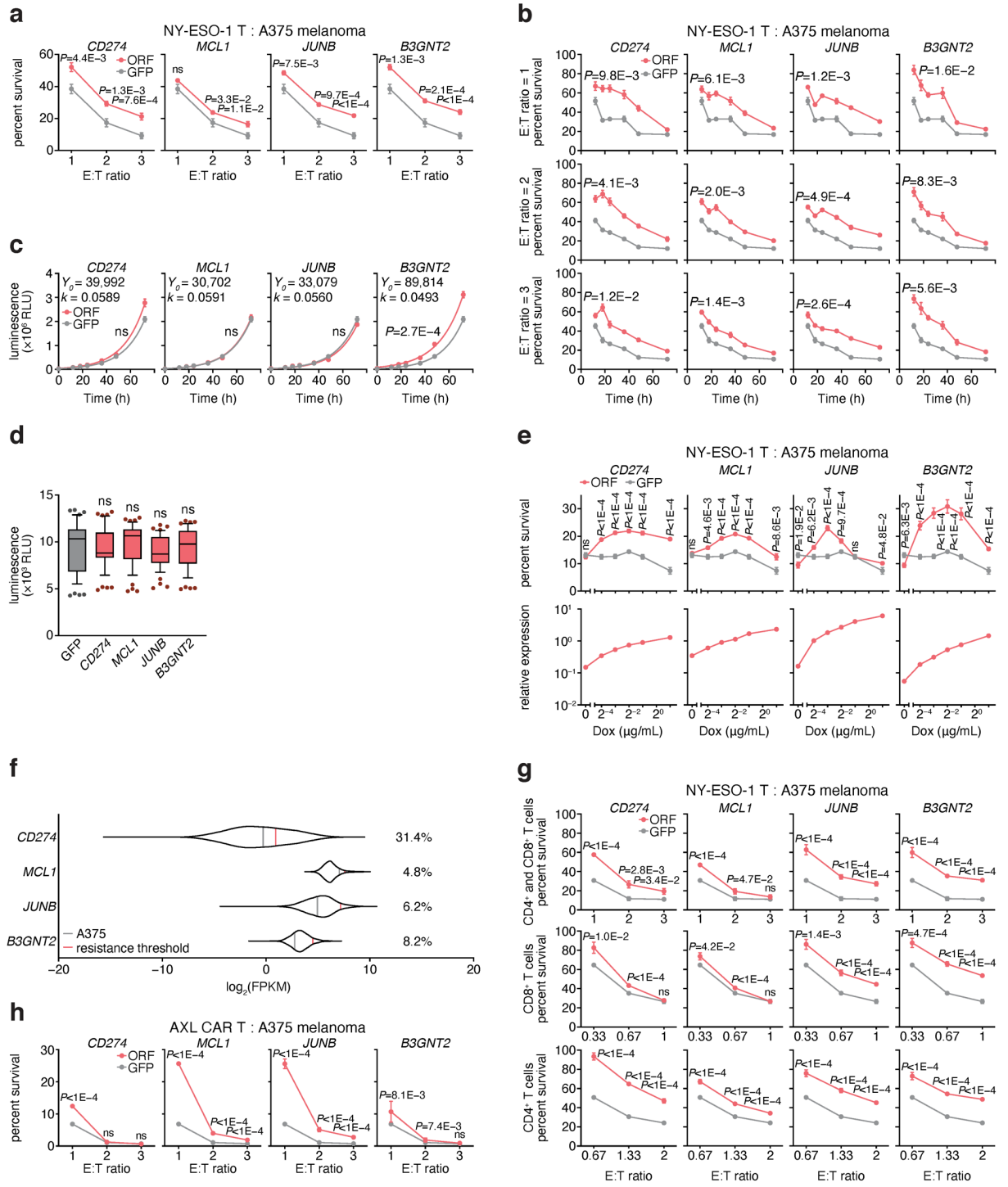
**Supplementary Figure 1 | Establishing a genome-scale CRISPR activation screen for resistance to T cell cytotoxicity.** (**a**) Percent indels generated by different CRISPR knockout (KO) sgRNAs targeting *CTAG1A/B* genes that encode for the NY-ESO-1 antigen. $N = 3$. (**b-c**) Cell survival of NY-ESO-$1^+$ and HLA-$A2^+$ A375 melanoma cells with different *CTAG1A/B* KO sgRNAs that were co-cultured with T cells expressing the NY-ESO-1 T cell receptor (ESO T cells) relative to cells that have not been exposed to T cells (**b**) or cells co-cultured with unmodified T cells (**c**). $N = 8$. NT, non-targeting. Percent survival at different effector to target (E:T) ratios were measured. Two-tailed *t*-tests with adjustments for multiple comparisons were performed. (**d-k**) MAGeCK analysis results of the acute and chronic exposure screens showing normalized sgRNA counts as counts per million (CPM; **d-e**), sgRNA enrichment in the ESO T cell condition relative to control (**f-g**), gene enrichment determined by the average sgRNA enrichment (**h-i**), and MAGeCK analysis *P*-values (**j-k**). The two most enriched genes from each screening strategy are highlighted in red. All values are mean ± s.e.m. ns, not significant. Source data are provided in Source Data 6 and Supplementary Data 1.


a
candidate genes
Pearson
1.0
0.5
0
−0.5
−1.0
ATG3
B3GNT2
SOCS2
IL18RAP
BCL2A1
CD14
IL15RA
IRF5
CD274
B3GAT1
MAPK11
MCL1
JUNB
KIPAN KIRC TGCT GBM LGG COAD LUSC PAAD PRAD KIRP THCA BRCA LUAD SKCM HNSC STAD BLCA OV SARC THYM LAML DLBC PCPG MESO UCS CHOL ESCA READ CESC UCEC ACC UVM
b
genes correlated with CYT
Pos
Neg
250
200
150
100
50
0
KIPAN KIRC BRCA LUSC COAD LGG BLCA TGCT PRAD THCA SARC GBM LUAD OV KIRP PAAD SKCM HNSC STAD LIHC THYM CESC UVM ACC PCPG LAML UCEC ESCA MESO READ DLBC KICH UCS CHOL
c
SOCS1
CD47
PTPN2
ADAR
Pearson
0.8
0.4
0
−0.4
−0.8
BRCA BLCA SKCM COAD CESC PRAD HNSC STAD OV LUAD PAAD LIHC ESCA KIRP THYM CHOL READ PCPG UCEC MESO KIRC SARC KIPAN TGCT GBM LGG THCA LAML UVM LUSC DLBC UCS
d
negative control genes
Pearson
1.0
0.5
0
−0.5
−1.0
KIPAN KIRC TGCT GBM KIRP THYM CESC LIHC LUAD HNSC SCAG BRCA THCA BLCA LGG OV SARC PAAD LUSC PRAD SKCM ACC DLBC LAML MESO READ UCS KICH ESCA CHOL UCS COAD UVM
e
Cell growth
−log10(average MAGeCK P-value)
MYC
PPAP2B
WWTR1
NEDD4L
FDFT1
LAMP5
GPR35
TMEM79
HSPB3
LPAR5
f
Cell death
−log10(average MAGeCK P-value)
MXI1
RRAS2
CACNA2D3
OLFM1
GRIN2A
MARCH9
GSTK1
CDKN1C
ANAPC11
PCSK2
g
CD274
MCL1
JUNB
B3GNT2
log2(screen enrichment)
Replicate 1
no T cells
T cells
Replicate 2
no T cells
T cells
round 1
round 2
round 3
sg1 sg2 sg3
h
CD274
MCL1
JUNB
B3GNT2
normalized percent survival
P=5.8E−4
P<1E−4
ns
P=7.6E−3
expression relative to ACTB
NT sg1 NT sg2 sg1 sg2 sg3

**Supplementary Figure 2 | Validation of four candidate genes for resistance to T cell cytotoxicity.** (**a**) Heatmap showing Pearson's correlation between cytolytic activity and expression of the top 576 candidate genes across patient tumors from TCGA. Only significant (FDR < 0.05) correlations are shown. (**b**) Number of candidate genes with significant (FDR < 0.05) positive or negative Pearson's correlation across patient tumors from TCGA. Out of 34 tumor types, 27 had more genes with positive correlation than negative correlation. (**c-d**) Heatmaps showing significant Pearson's correlations between cytolytic activity and expression of four positive control genes known to promote resistance upon overexpression (**c**), or 291 negative control housekeeping genes (**d**) across patient tumors from TCGA. (**e-f**) Average MAGeCK analysis *P*-values of genes that promote (**e**) or reduce (**f**) A375 cell fitness determined by comparing the distribution of sgRNAs in the CRISPRa screen control conditions to the initial sgRNA library. (**g**) Enrichment of CRISPR activation sgRNAs targeting each candidate gene for different screening biological replicates (bioreps). (**h**) T cell cytotoxicity resistance ($n$ = 12) and transcriptional upregulation ($n$ = 4) in A375 cells upon CRISPR activation of candidate genes. NT, non-targeting. All values are mean ± s.e.m. ns, not significant. Two-tailed *t*-tests were performed. Source data are provided in Source Data 7, Supplementary Data 1, and Supplementary Data 5.

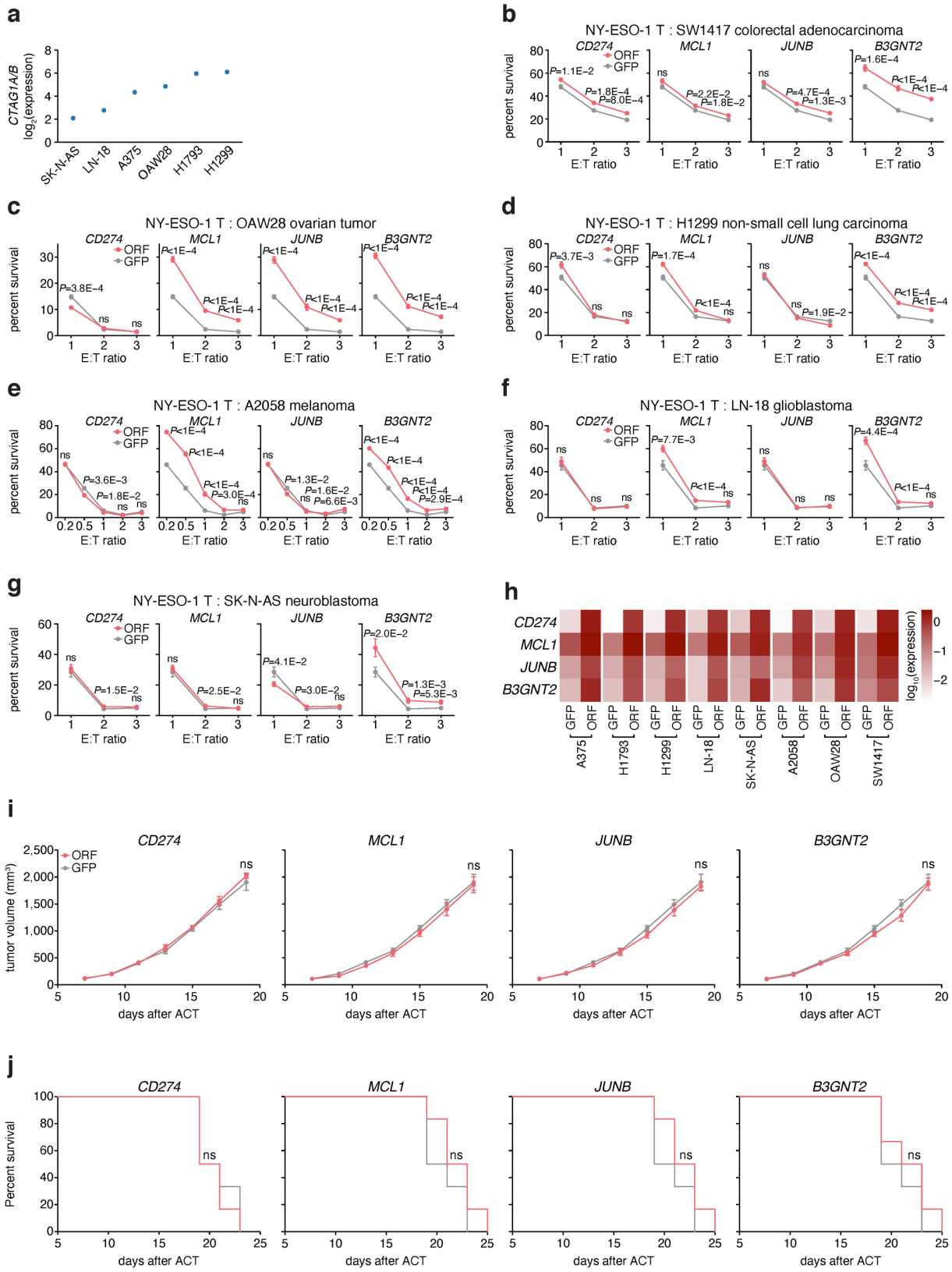

a
b
c
d
e
f
CD274
MCL1
JUNB
B3GNT2
normal
tumor
gain
loss
log2(TPM + 1)
percent of cases with CNV
log2(FPKM)
percent survival
days
low
high
ns
P=4.1E−2
P=0.42
P=0.17
P=0.88
P=0.11
P=1.8E−2
P=1.4E−2
CR
PR
PD
Pre
Post
CR/PR
SD/PD
r=0.72
P<1E−4
r=0.30
P=2.4E−2
r=0.46
P=8.8E−4
r=0.26
P=4.4E−2
log2(cytolytic score)

**Supplementary Figure 3 | Relevance of top four candidate genes in patient tumors.** (**a**) Box plots showing expression of candidate genes in TCGA datasets for 31 types of human cancers. Tumor types with significantly differentially expressed genes (two-tailed *t*-tests with adjustments for multiple comparisons) between tumor ($n$ = 9,736) and matched normal tissues ($n$ = 8,587) are indicated. *$P$ < 0.05. Box plots indicate median (middle line), 25$^{th}$, 75$^{th}$ percentile (box), and whiskers are defined by 1.5 times the interquartile range (*i.e.*, it is the distance between the upper and lower quartiles). (**b**) Focal copy number variation of candidate genes represented as percent of cases for each type of cancer in TCGA. (**c**) Comparison of candidate gene expression and clinical response to anti-PD-1 treatment using patient data from the Hugo et al. dataset [1]. Mean ± s.e.m. is shown. CR ($n$ = 4), PR ($n$ = 10), PD ($n$ = 13). Two-tailed Mann-Whitney tests were performed. (**d**) Overall survival of patients in the Hugo et al. dataset [1]. Patients were stratified based on high (top 50%) or low (bottom 50%) expression of candidate genes. $N$ = 13 for each expression group. Mantel-Cox log-rank tests were performed. (**e**) Comparison of candidate gene expression before (pre) and after (post) anti-PD-1 treatment using matched patient tumor samples from the Riaz et al. dataset [2]. Data from responders (CR or PR, $n$ = 9 per timepoint) and non-responders (SD or PD, $n$ = 33 per timepoint) were grouped together. Pre- and post-immunotherapy expression levels were compared using two-tailed paired *t*-test. (**f**) Pearson correlation between candidate gene expression and cytolytic score [3] prior to treatment using patient data from Riaz et al. [2]. $N$ = 43. Two-tailed Pearson correlation $P$ values are shown. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. Source data are provided in Source Data 8.

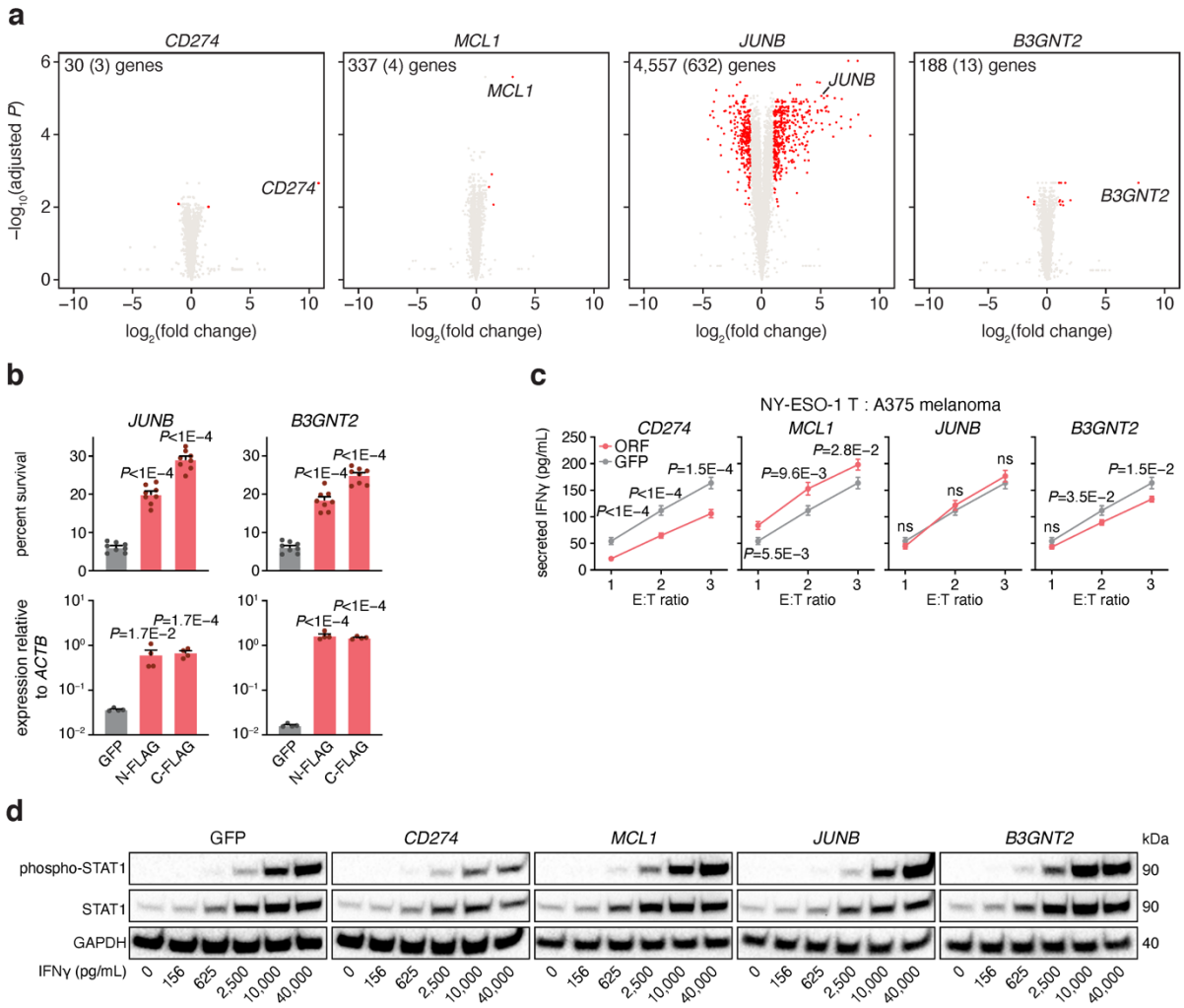



**Supplementary Figure 4 | Candidate genes mediate resistance to T cell cytotoxicity across different co-culture conditions.** (**a**) Cell survival against ESO T cell-mediated cytotoxicity in A375 cells overexpressing candidate gene ORFs. T cells were derived from donors that were not used in the CRISPRa screen. $N = 8$. (**b**) Cell survival against ESO T cell-mediated cytotoxicity in

A375 cells overexpressing candidate gene ORFs over time. Viability was measured using an alternative assay based on secreted *Gaussia* luciferase. $N = 8$. Repeated measures ANOVA with adjustments for multiple comparisons were performed. (**c**) Growth of A375 cells overexpressing candidate gene ORFs in the absence of ESO T cell co-culture over time measured using secreted *Gaussia* luciferase. Data was fitted using the Malthusian exponential growth $Y(t) = Y_0 e^{kt}$, with $t$ = time, $Y$ = luminescence, $k$ = growth rate. $N = 8$. The growth rates were compared using the extra sum-of-squares F test. (**d**) Box plots showing growth of A375 cells overexpressing candidate gene ORFs in the absence of ESO T cell co-culture measured using a luminescent cell viability assay from 5 independent experiments with $n = 44$. Box plots indicate median (middle line), 25$^{th}$, 75$^{th}$ percentile (box), and 10$^{th}$ and 90$^{th}$ percentile (whiskers). Two-tailed *t*-tests were performed. (**e**) Dox-induction of candidate genes in A375 cells. Cell survival against ESO T cell cytotoxicity ($n = 8$) and candidate gene expression relative to *ACTB* control ($n = 4$) were measured at different Dox concentrations. (**f**) Expression of candidate genes in 1,019 cell lines from the Cancer Cell Line Encyclopedia [4]. Expression in A375 cells and the expression threshold above which the candidate gene conferred resistance to T cell cytotoxicity in A375 cells are indicated. The percentage of cell lines with gene expression above the resistance threshold is shown. (**g**) Cell survival of A375 cells overexpressing candidate genes against $CD4^+$, $CD8^+$, or $CD4^+$ and $CD8^+$ T cell cytotoxicity. $N = 8$. (**h**) Cell survival of A375 cells overexpressing candidate genes against T cells expressing the AXL chimeric antigen receptor (CAR). $N = 8$. All values are mean ± s.e.m. ns, not significant. Two-tailed *t*-tests with adjustments for multiple comparisons were performed unless otherwise indicated. Source data are provided in Source Data 9.

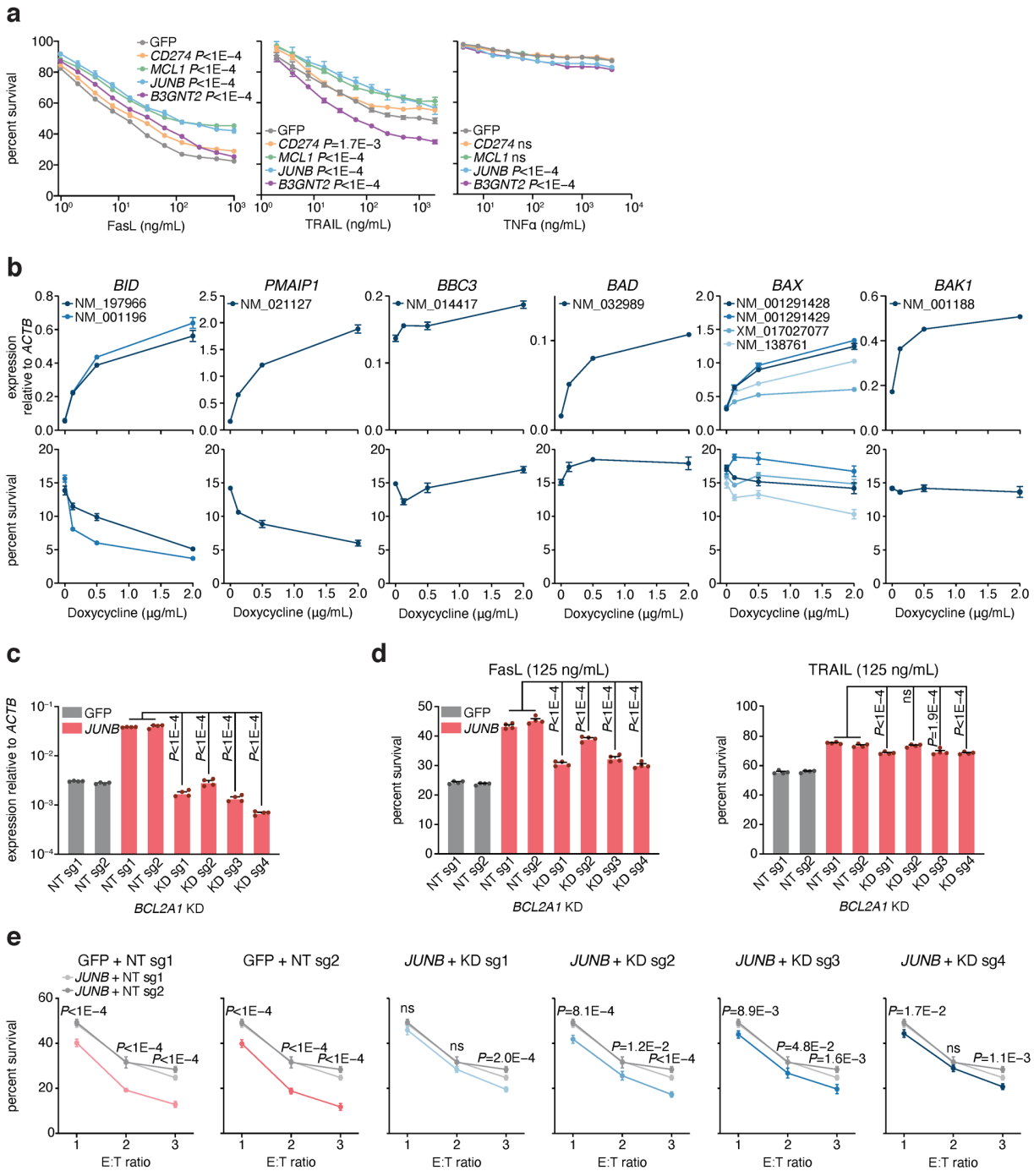

a
CTAG1A/B log2(expression)
SK-N-AS
LN-18
A375
OAW28
H1793
H1299
b
NY-ESO-1 T : SW1417 colorectal adenocarcinoma
CD274
MCL1
JUNB
B3GNT2
ORF
GFP
percent survival
E:T ratio
c
NY-ESO-1 T : OAW28 ovarian tumor
d
NY-ESO-1 T : H1299 non-small cell lung carcinoma
e
NY-ESO-1 T : A2058 melanoma
f
NY-ESO-1 T : LN-18 glioblastoma
g
NY-ESO-1 T : SK-N-AS neuroblastoma
h
log10(expression)
i
tumor volume (mm3)
days after ACT
j
Percent survival
days after ACT

**Supplementary Figure 5 | Candidate genes mediate resistance to T cell cytotoxicity in other cell types and *in vivo*.** (**a**) Expression level of the NY-ESO-1 antigen represented as the average expression of *CTAG1A* and *CTAG1B* genes. RNA-seq expression data for each cell line was obtained from the Cancer Cell Line Encyclopedia [4]. (**b-g**) Resistance to ESO T cell-mediated cytotoxicity in SW1417 (NY-ESO-1$^{-}$, HLA-A2$^{-}$) colorectal adenocarcinoma (**b**), OAW28 (NY-ESO-1$^{+}$, HLA-A2$^{-}$) ovarian cystadenocarcinoma (**c**), H1299 (NY-ESO-1$^{+}$, HLA-A2$^{-}$) non-small cell lung carcinoma (**d**), A2058 (NY-ESO-1$^{-}$, HLA-A2$^{-}$) melanoma (**e**), LN-18 (NY-ESO-1$^{+}$, HLA-A2$^{+}$) glioblastoma (**f**), or SK-N-AS (NY-ESO-1$^{+}$, HLA-A2$^{-}$) neuroblastoma (**g**) cell lines. Cell lines that did not endogenously express HLA-A2 or NY-ESO-1 were transduced with the respective constructs. $N = 8$ (**b**, **c**, **e**) or 12 (**d**, **f**, **g**). Two-tailed *t*-tests with adjustments for multiple comparisons were performed. (**h**) Heatmap showing average expression ($n = 4$) relative to *ACTB* of candidate genes in various cell lines overexpressing the ORF or GFP. (**i-j**) Tumor growth in control mice that did not receive adoptive cell transfer (ACT). $N = 6$. (**i**) Tumor volume is shown. Two-tailed *t*-tests with adjustments for multiple comparisons were performed. (**j**) Overall survival is shown. Mantel-Cox log-rank tests were performed. All values are mean ± s.e.m. ns, not significant. Source data are provided in Source Data 10.



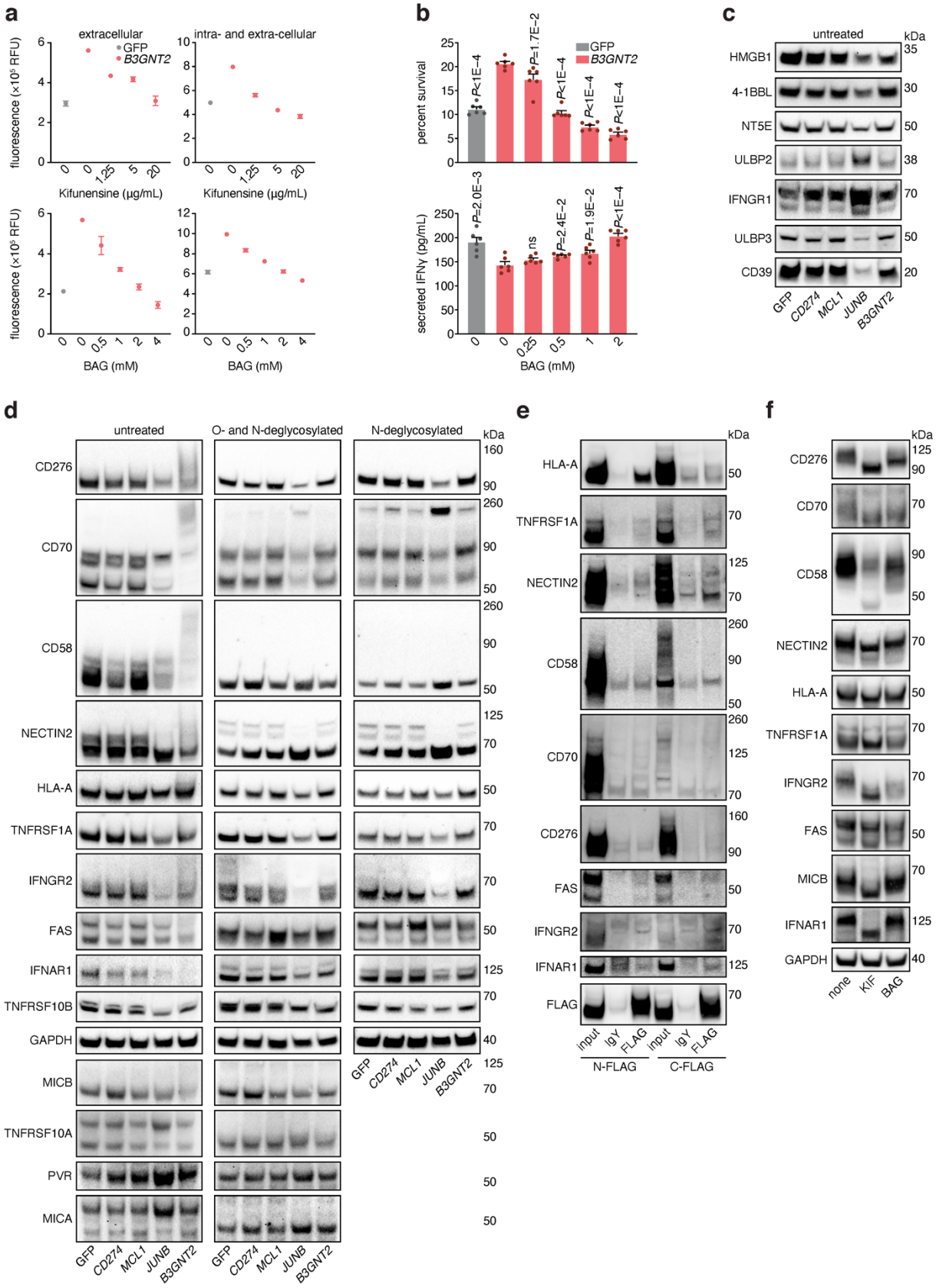

**Supplementary Figure 6 | Effect of candidate gene overexpression on the transcriptome and IFNγ pathways.** (**a**) Volcano plots showing transcriptome changes measured by RNA-seq in A375 cells overexpressing candidate genes. The number of genes that were significantly differentially expressed with *P*-value ≥ 0.01 FDR correction based on two-tailed *t*-tests are indicated. Out of these genes, those with $|\log_2(fold\ change)| \geq 1$ are shown as red dots and the number of genes is indicated in parentheses. $N = 3$. (**b**) T cell cytotoxicity resistance ($n = 8$) and transcriptional upregulation ($n = 4$) in A375 cells overexpressing N- or C-terminal FLAG tagged (N-FLAG or C-FLAG respectively) *JUNB* or *B3GNT2*. Two-tailed *t*-tests were performed. (**c**) IFNγ measured by ELISA in cell culture media of ESO T cells co-cultured with A375 cells overexpressing candidate genes. $N = 16$. Two-tailed *t*-tests with adjustments for multiple comparisons were performed. (**d**) Western blots of phosphorylated or total STAT1 in A375 cells overexpressing candidate genes that have been exposed to different concentrations of IFNγ. Data is representative of two

independent experiments. All values are mean ± s.e.m. ns, not significant. Source data are provided in Source Data 11 and Supplementary Data 6.

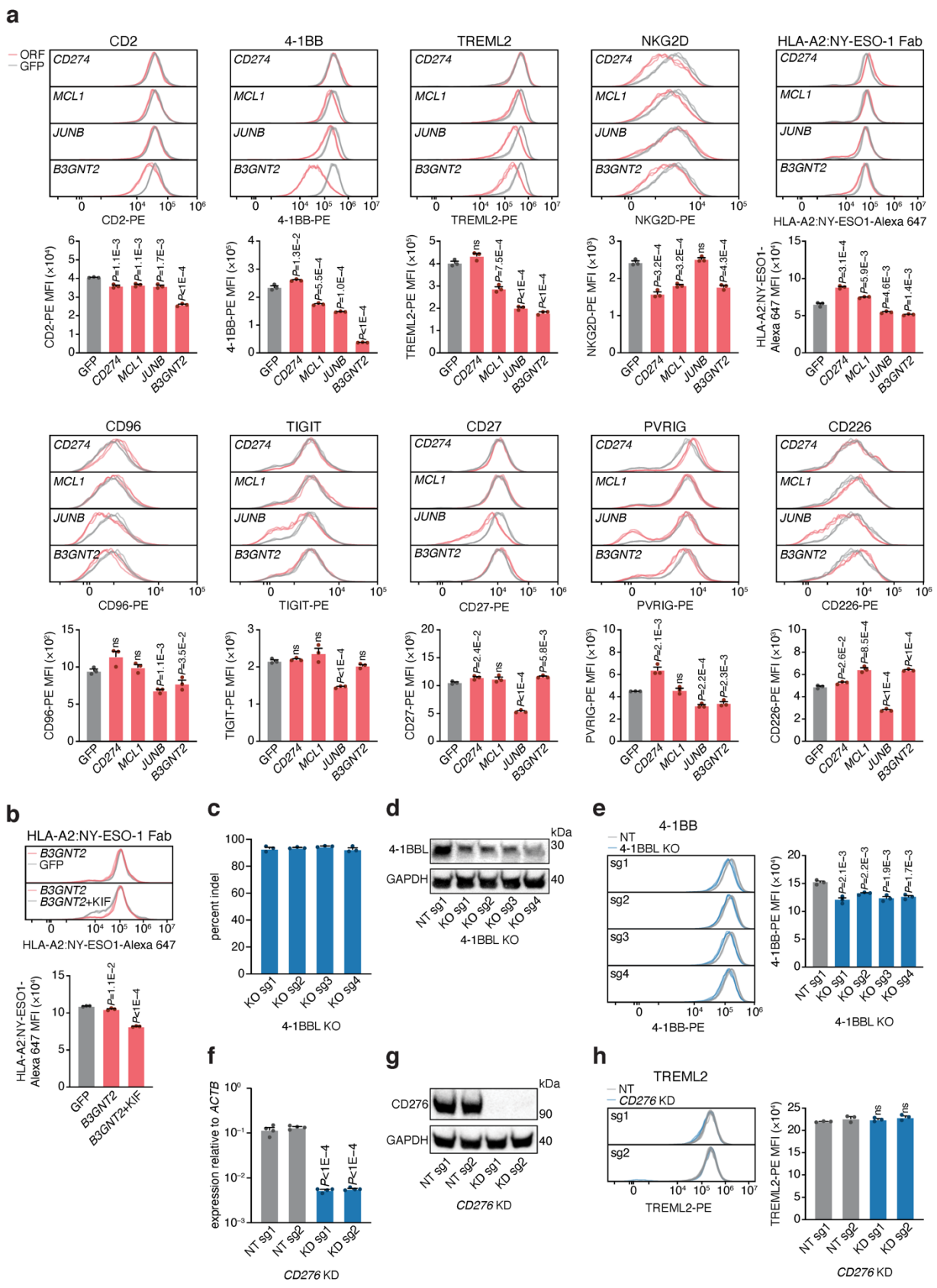



**Supplementary Figure 7 | *MCL1* and *JUNB* mediate survival by resisting FasL- and TRAIL-induced cytotoxicity.** (**a**) Cell survival of A375 cells overexpressing candidate genes against different concentrations of FasL, TRAIL, or TNF$\alpha$. $N$ = 4. Repeated measures ANOVA with adjustments for multiple comparisons were performed. (**b**) Dox-induction of different RefSeq isoforms of genes in the mitochondrial apoptosis pathway in A375 cells overexpressing *MCL1*.

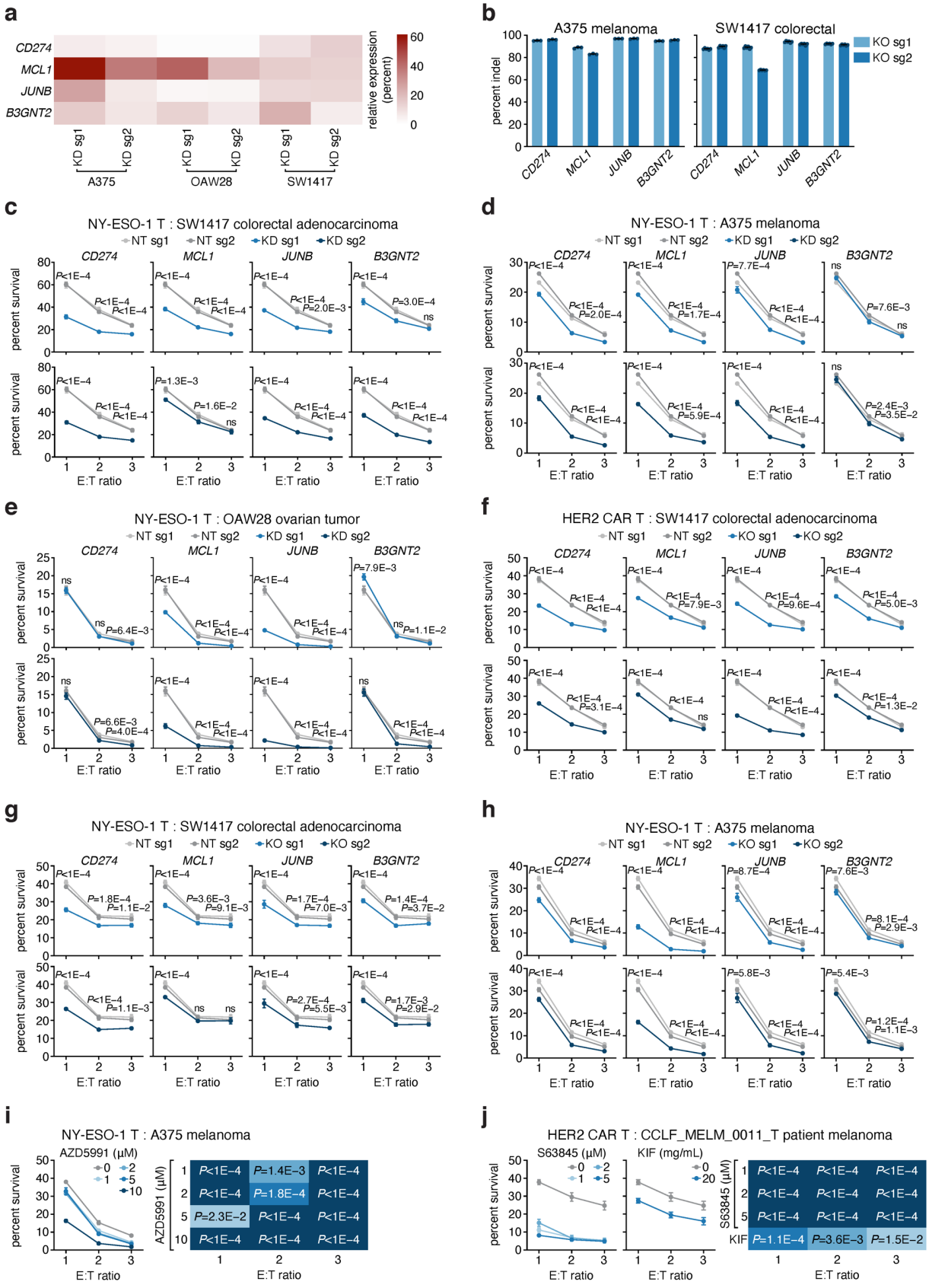
Cell survival against ESO T cell-induced cytotoxicity ($n$ = 8) and expression ($n$ = 4) of *MCL1* interaction partners were measured at different Dox concentrations. (**c**) Expression of *BCL2A1* in A375 cells overexpressing *JUNB* or *B3GNT2* with *BCL2A1* knocked down. $N$ = 4. KD, knockdown. NT, non-targeting. Two-tailed $t$-tests were performed. (**d**) Cell survival against 125 ng/μL of FasL- or TRAIL-induced cell death in A375 cells overexpressing *JUNB* or GFP with *BCL2A1* knocked down. $N$ = 4. Two-tailed $t$-tests were performed. (**e**) Cell survival against ESO T cells in A375 cells overexpressing *JUNB* or GFP with *BCL2A1* knocked down. $N$ = 8. Two-tailed $t$-tests with adjustments for multiple comparisons were performed. All values are mean ± s.e.m. ns, not significant. Source data are provided in Source Data 12.


a
extracellular
intra- and extra-cellular
GFP
B3GNT2
fluorescence (×10⁵ RFU)
Kifunensine (μg/mL)
BAG (mM)
b
GFP
B3GNT2
percent survival
secreted IFNγ (pg/mL)
P<1E−4
P=1.7E−2
P=2.0E−3
ns
P=2.4E−2
P=1.9E−2
BAG (mM)
c
untreated
kDa
HMGB1
4-1BBL
NT5E
ULBP2
IFNGR1
ULBP3
CD39
GFP
CD274
MCL1
JUNB
B3GNT2
d
untreated
O- and N-deglycosylated
N-deglycosylated
CD276
CD70
CD58
NECTIN2
HLA-A
TNFRSF1A
IFNGR2
FAS
IFNAR1
TNFRSF10B
GAPDH
MICB
TNFRSF10A
PVR
MICA
e
HLA-A
TNFRSF1A
NECTIN2
CD58
CD70
CD276
FAS
IFNGR2
IFNAR1
FLAG
input
IgY
FLAG
N-FLAG
C-FLAG
f
CD276
CD70
CD58
NECTIN2
HLA-A
TNFRSF1A
IFNGR2
FAS
MICB
IFNAR1
GAPDH
none
KIF
BAG

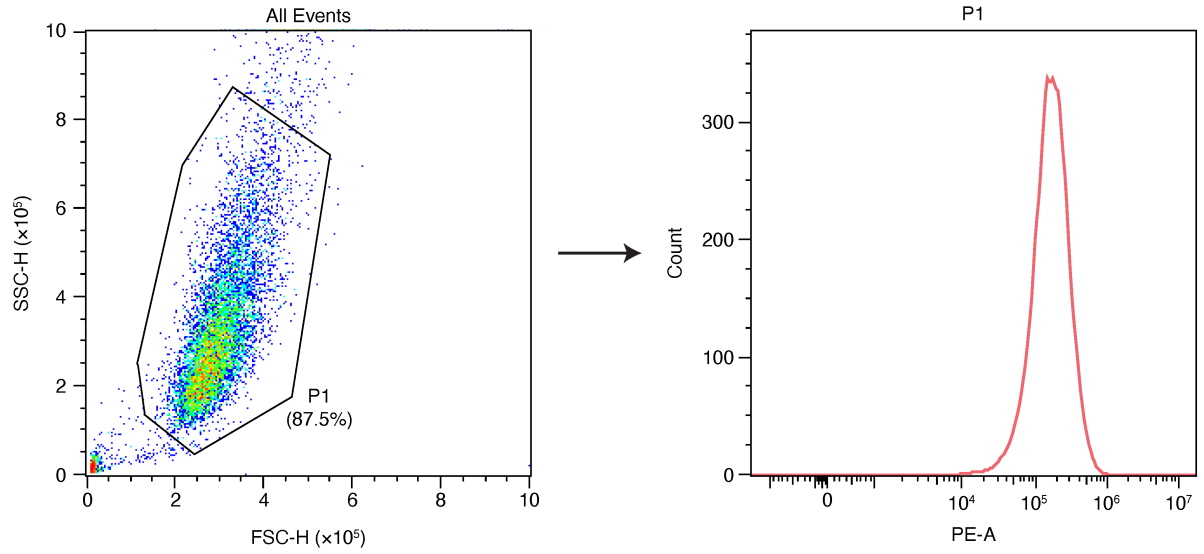
**Supplementary Figure 8 | *B3GNT2* adds poly-LacNAc to tumor ligands and receptors to promote tumor immune evasion.** (**a**) Intra- and extra-cellular poly-LacNAc measured by tomato lectin staining in A375 cells overexpressing *B3GNT2* or GFP that were treated with different concentrations of kifunensine or benzyl-2-acetamido-2-deoxy-α-D-galactopyranoside (BAG) glycosylation inhibitors. $N = 2$. (**b**) Cell survival against T cell cytotoxicity (top) and T cell IFN$\gamma$ secretion (bottom) in A375 cells overexpressing *B3GNT2* or GFP that have been treated with different concentrations of BAG at E:T ratio of 3. BAG was used to pretreat A375 cells and was present during co-culture. $N = 6$. Two-tailed *t*-tests were performed. (**c-d**) Western blots of different tumor ligands and receptors that interact with T cells in A375 cells overexpressing candidate genes. For a subset of the ligands and receptors that were potentially glycosylated (**d**), enzymatic deglycosylation was performed to confirm presence of *N*- or *O*-glycosylation. Data is representative of two independent experiments. (**e**) Co-IP of N- or C-terminal FLAG tagged (N-FLAG or C-FLAG) *B3GNT2* followed by Western blot for different *B3GNT2* target proteins. 2% of the input and IgY IP controls are shown. Data is representative of two independent experiments. (**f**) Western blots of tumor cell surface ligands and receptors that interact with T cells in SW1417 colorectal adenocarcinoma cells. Cells were treated with kifunensine (KIF) or BAG to inhibit *N*- or *O*- glycosylation respectively. Data is representative of two independent experiments. All values are mean ± s.e.m. ns, not significant. Source data are provided in Source Data 13.


a
CD2
4-1BB
TREML2
NKG2D
HLA-A2:NY-ESO-1 Fab
ORF
GFP
CD274
MCL1
JUNB
B3GNT2
CD2-PE
4-1BB-PE
TREML2-PE
NKG2D-PE
HLA-A2:NY-ESO1-Alexa 647
CD2-PE MFI (×10⁴)
4-1BB-PE MFI (×10⁵)
TREML2-PE MFI (×10⁵)
NKG2D-PE MFI (×10⁴)
HLA-A2:NY-ESO1-Alexa 647 MFI (×10⁴)
P=1.1E−3
P=1.1E−3
P=1.7E−3
P<1E−4
P=1.3E−2
P=5.5E−4
P=1.0E−4
P<1E−4
ns
P=7.5E−4
P<1E−4
P<1E−4
P=3.2E−4
P=3.2E−4
ns
P=4.3E−4
P=3.1E−4
P=5.9E−3
P=4.6E−3
P=1.4E−3
CD96
TIGIT
CD27
PVRIG
CD226
CD96-PE
TIGIT-PE
CD27-PE
PVRIG-PE
CD226-PE
CD96-PE MFI (×10³)
TIGIT-PE MFI (×10³)
CD27-PE MFI (×10³)
PVRIG-PE MFI (×10³)
CD226-PE MFI (×10³)
ns
ns
P=1.1E−3
P=3.5E−2
ns
ns
P<1E−4
ns
P=2.4E−2
ns
P<1E−4
P=5.8E−3
P=2.1E−3
ns
P=2.2E−4
P=2.3E−3
P=2.6E−2
P=8.5E−4
P<1E−4
P<1E−4
GFP
b
HLA-A2:NY-ESO-1 Fab
B3GNT2
GFP
B3GNT2
B3GNT2+KIF
HLA-A2:NY-ESO1-Alexa 647
HLA-A2:NY-ESO1-Alexa 647 MFI (×10⁴)
P=1.1E−2
P<1E−4
B3GNT2
B3GNT2+KIF
c
percent indel
KO sg1
KO sg2
KO sg3
KO sg4
4-1BBL KO
d
kDa
4-1BBL
GAPDH
30
40
NT sg1
KO sg1
KO sg2
KO sg3
KO sg4
4-1BBL KO
e
4-1BB
NT
4-1BBL KO
sg1
sg2
sg3
sg4
4-1BB-PE
4-1BB-PE MFI (×10⁴)
P=2.1E−3
P=2.2E−3
P=1.9E−3
P=1.7E−3
NT sg1
KO sg1
KO sg2
KO sg3
KO sg4
4-1BBL KO
f
expression relative to ACTB
P<1E−4
P<1E−4
NT sg1
NT sg2
KD sg1
KD sg2
CD276 KD
g
kDa
CD276
GAPDH
90
40
NT sg1
NT sg2
KD sg1
KD sg2
CD276 KD
h
TREML2
NT
CD276 KD
sg1
sg2
TREML2-PE
TREML2-PE MFI (×10⁴)
ns
ns
NT sg1
NT sg2
KD sg1
KD sg2
CD276 KD

**Supplementary Figure 9 | *B3GNT2* overexpression disrupts binding of T cell ligands and receptors to tumor cells.** (**a**) Histograms (top) and corresponding median fluorescence intensity (MFI; bottom) showing binding of various T cell ligands or receptor proteins to A375 cells overexpressing candidate genes measured by flow cytometry. $N = 3$. (**b**) Histograms (top) and corresponding MFI (bottom) showing binding of an antibody specific for HLA-A2:NY-ESO-1 to A375 cells overexpressing *B3GNT2* or GFP that have been treated with kifunensine (KIF). $N = 3$. (**c**) Percent indels generated by different CRISPR knockout (KO) sgRNAs targeting 4-1BBL (*TNFSF9*) in A375 cells. $N = 3$. (**d**) Protein expression of 4-1BBL after CRISPR KO. Data is representative of two independent experiments. (**e**) Histograms (left) and corresponding MFI (right) showing binding of 4-1BB to A375 cells with different KO sgRNAs targeting 4-1BBL. $N = 3$. (**f**) *CD276* expression in A375 cells with different CRISPR knockdown (KD) sgRNAs. $N = 4$. (**g**) Protein expression of CD276 after CRISPR KD. Data is representative of two independent experiments. (**h**) Histograms (left) and corresponding MFI (right) showing binding of TREML2 to A375 cells with different KD sgRNAs targeting *CD276*. $N = 3$. All values are mean ± s.e.m. NT, non-targeting; ns, not significant. Two-tailed *t*-tests were performed. Source data are provided in Source Data 14.


a
CD274
MCL1
JUNB
B3GNT2
KD sg1
KD sg2
A375
OAW28
SW1417
relative expression (percent)
b
A375 melanoma
SW1417 colorectal
KO sg1
KO sg2
percent indel
c
NY-ESO-1 T : SW1417 colorectal adenocarcinoma
NT sg1
NT sg2
KD sg1
KD sg2
percent survival
E:T ratio
d
NY-ESO-1 T : A375 melanoma
e
NY-ESO-1 T : OAW28 ovarian tumor
f
HER2 CAR T : SW1417 colorectal adenocarcinoma
KO sg1
KO sg2
g
NY-ESO-1 T : SW1417 colorectal adenocarcinoma
h
NY-ESO-1 T : A375 melanoma
i
NY-ESO-1 T : A375 melanoma
AZD5991 (μM)
j
HER2 CAR T : CCLF_MELM_0011_T patient melanoma
S63845 (μM)
KIF (mg/mL)

**Supplementary Figure 10 | Inhibition of candidate genes increases susceptibility of tumors to T cell killing upon knockdown.** (**a**) Heatmap showing expression of candidate genes in different cell lines transduced with CRISPR knockdown (KD) sgRNAs relative to non-targeting (NT) sgRNAs. $N = 4$. (**b**) Percent indels generated by different CRISPR knockout (KO) sgRNAs targeting candidate genes in A375 melanoma ($N = 3$) and SW1417 colorectal adenocarcinoma cells ($N = 6$). (**c-h**) Cell survival against T cell-mediated cytotoxicity with different candidate genes knocked down (**c-e**) or knocked out (**f-h**). Cell lines that did not endogenously express HLA-A2 or NY-ESO-1 were transduced with the respective constructs. $N = 8$. (**c**) SW1417 (NY-ESO-1$^-$, HLA-A2$^-$) colorectal adenocarcinoma against ESO T cells. (**d**) A375 (NY-ESO-1$^+$, HLA-A2$^+$) melanoma against ESO T cells. (**e**) OAW28 (NY-ESO-1$^+$, HLA-A2$^-$) ovarian cystadenocarcinoma against ESO T cells. (**f**) SW1417 (HER2$^+$) colorectal adenocarcinoma against HER2 CAR T cells. (**g**) SW1417 (NY-ESO-1$^-$, HLA-A2$^-$) colorectal adenocarcinoma against ESO T cells. (**h**) A375 (NY-ESO-1$^+$, HLA-A2$^+$) melanoma against ESO T cells. (**i-j**) Cell survival against T cell cytotoxicity in tumor cells treated with small molecule inhibitors targeting MCL1 (S63845 and AZD5991) or B3GNT2 (kifunensine). Heatmaps show statistical analysis results for each condition. $N = 8$. (**i**) A375 (NY-ESO-1$^+$, HLA-A2$^+$) melanoma against ESO T cells. (**j**) CCLF_MELM_0011_T (HER2$^+$) primary patient-derived melanoma model against HER2 CAR T cells. All values are mean ± s.e.m. ns, not significant. Two-tailed *t*-tests with adjustments for multiple comparisons were performed. Source data are provided in Source Data 15.




**Supplementary Figure 11 | Example flow cytometry gating strategy.** Cells were gated on forward scatter (FSC) and side scatter (SSC) signals to discard debris. After eliminating debris, median fluorescence intensity was measured on P1. Example gating strategy is representative of all flow cytometry data presented.

**Supplementary Tables**

**Supplementary Table 1 | List of sgRNAs and primers used in the study**. (**a**) sgRNA ID, perturbation, target sequences, and target genes. (**b**) Primers for indel amplification and quantification.

| **Target gene** | **sgRNA ID** | **Perturbation** | **Target sequence (5' to 3')** |
|---|---|---|---|
| *CTAG1A/B* | sg1 | knockout | GCGGGGTCCGCATGGCGGCG |
| *CTAG1A/B* | sg2 | knockout | CAGAATACAACTCAAGCAGG |
| *CTAG1A/B* | sg3 | knockout | GAATGGATGCTGCAGATGCG |
| *CD274* | sg1 | activation | CTGACCTTCGGTGAAATCGG |
| *CD274* | sg2 | activation | TCAGTTTAGGTATCTAGTGT |
| *CD274* | sg3 | activation | CTATACACAGCTTTATTCCT |
| *MCL1* | sg1 | activation | CATGGAAAGAGCTCGAGCCC |
| *MCL1* | sg2 | activation | CACTCAGAGCCTCCGAAGAC |
| *MCL1* | sg3 | activation | CGGAGCCGCCGTTACGTAAC |
| *JUNB* | sg1 | activation | CCCCTCCTCGAGCGTGGGGA |
| *JUNB* | sg2 | activation | AGGCGGCTCGCGTCACTGTC |
| *JUNB* | sg3 | activation | GCGCGTGTCCTTGTAAACAG |
| *B3GNT2* | sg1 | activation | GCCGCAGGGAGCGCGGGCCC |
| *B3GNT2* | sg2 | activation | GTGGGTCCTGGTACCGGGTG |
| *B3GNT2* | sg3 | activation | CGGAACCCTCCCAAAACTTG |
| *CD274* | sg1 | knockdown | AGCAGCTGGCGCGTCCCGCG |
| *CD274* | sg2 | knockdown | TCGGGAAGCTGCGCAGAACT |
| *MCL1* | sg1 | knockdown | AGCTTCCGGAGGGTTGCGCA |
| *MCL1* | sg2 | knockdown | CCTTTATCACGGTTTTAGGG |
| *JUNB* | sg1 | knockdown | CTGGGACCTTGAGAGCGGCC |
| *JUNB* | sg2 | knockdown | TATCGCGCCAGAGAGGGCGA |
| *B3GNT2* | sg1 | knockdown | CTGCGCCTCACTCCAGGCTC |
| *B3GNT2* | sg2 | knockdown | GGAGTGAGGCGCAGCGGCAG |

| | | | |
|---|---|---|---|
| *BCL2A1* | sg1 | knockdown | TACGCACGAAAGTGACTAGG |
| *BCL2A1* | sg2 | knockdown | ACATGATGATACATGGAGGC |
| *BCL2A1* | sg3 | knockdown | GGCTCACCTTGAAGCTGTTG |
| *BCL2A1* | sg4 | knockdown | TCAAGACTTTGCTCTCCACC |
| *CD276* | sg1 | knockdown | GCGGCTCCGGTGCGTCCCTG |
| *CD276* | sg2 | knockdown | GCGTCCCTGAGTCCCAGAGT |
| *CD274* | sg1 | knockout | ACATGTCAGTTCATGTTCAG |
| *CD274* | sg2 | knockout | GGTTCCCAAGGACCTATATG |
| *MCL1* | sg1 | knockout | AGTCGCTGGAGATTATCTCT |
| *MCL1* | sg2 | knockout | CCAAAAGTCGCCCTCCCGGG |
| *JUNB* | sg1 | knockout | CCGGAGTCTCAAAGCGCCTG |
| *JUNB* | sg2 | knockout | GGGTAAAAGTACTGTCCCGG |
| *B3GNT2* | sg1 | knockout | CAACGCAGGGAACCAAACGG |
| *B3GNT2* | sg2 | knockout | GGTTCCAGTATGCCTCGGGA |
| *TNFSF9* | sg1 | knockout | CCCATCGATCAGCAGAACTG |
| *TNFSF9* | sg2 | knockout | GCCAGCCCGAGACTCCGCGA |
| *TNFSF9* | sg3 | knockout | GGGGGGCCTGAGCTACAAAG |
| *TNFSF9* | sg4 | knockout | TCAACTAGAGCTGCGGCGCG |
| Nontargeting | sg1 | | CTGAAAAAGGAAGGAGTTGA |
| Nontargeting | sg2 | | AAGATGAAAGGAAAGGCGTT |

**Supplementary Table 2 | Primers for indel amplification and quantification**.

| **Target gene** | **sgRNA ID** | **Primer** | **Sequence (5' to 3')** |
|---|---|---|---|
| *CTAG1A/B* | sg1 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTGGGC AGCAAGGGCCTC |
| *CTAG1A/B* | sg1 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT GCTCTCCGGCCCCCT |
| *CTAG1A/B* | sg2 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTTCAG GGCTGAATGGATGCTG |

| | | | |
|---|---|---|---|
| *CTAG1A/B* | sg2 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT TGCCCTCCCCATCTCCC |
| *CTAG1A/B* | sg3 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTGGGC AGCAAGGGCCTC |
| *CTAG1A/B* | sg3 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT GCCCCCACCTCGCCA |
| *CD274* | sg1 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTTGTT TATGTCCTAGCCCCATAC |
| *CD274* | sg1 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT CTTGATGGTCACTGCTTGTCC |
| *CD274* | sg2 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTAAAC GCTGTGCCAATTTTGTAAATG |
| *CD274* | sg2 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT GACAATTAGTGCAGCCAGGTCTA |
| *MCL1* | sg1 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTGGAG TTGGTCGGGGAATCTG |
| *MCL1* | sg1 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT CAACCCGTCGTAAGGTCTCC |
| *MCL1* | sg2 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTAAAA GAAACGCGGTAATCGGAC |
| *MCL1* | sg2 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT GCGCTTCCGCCAATCAC |
| *JUNB* | sg1 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTGGCC TCTCTCTACACGACTAC |
| *JUNB* | sg1 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT CAGCTCCGAAGAGGCGAG |
| *JUNB* | sg2 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTATTG TCCCCAACAGCAACGG |
| *JUNB* | sg2 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTGTGCAGATCGTCCAGGGC |

| | | | |
|---|---|---|---|
| *B3GNT2* | sg1 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTTGCT GGCGATTAAGTCCCTC |
| *B3GNT2* | sg1 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT ATCTGAAAGGTCGGGGTGGT |
| *B3GNT2* | sg2 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTTCTC CAAAAGCAGTAGCCAAG |
| *B3GNT2* | sg2 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT TATATTGGAGAGCCTGCCCG |
| *TNFSF9* | sg1 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTTCTT TTCTCCCAGGGCTGC |
| *TNFSF9* | sg1 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT CTTTGTAGCTCAGGCCCCC |
| *TNFSF9* | sg2 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTTTCC TCGCCTGCCCCT |
| *TNFSF9* | sg2 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT GCCGCAGGTCCAAGAGG |
| *TNFSF9* | sg3 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTACAA AGAGGACACGAAGGAGC |
| *TNFSF9* | sg3 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT CCCCAGCAGCAGAGCG |
| *TNFSF9* | sg4 | Fwd | CTTTCCCTACACGACGCTCTTCCGATCTTTTC CTCCCACAGTTCTGCTGAT |
| *TNFSF9* | sg4 | Rev | GACTGGAGTTCAGACGTGTGCTCTTCCGATCT CGCAGCTCTAGTTGAAAGAAGACA |

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