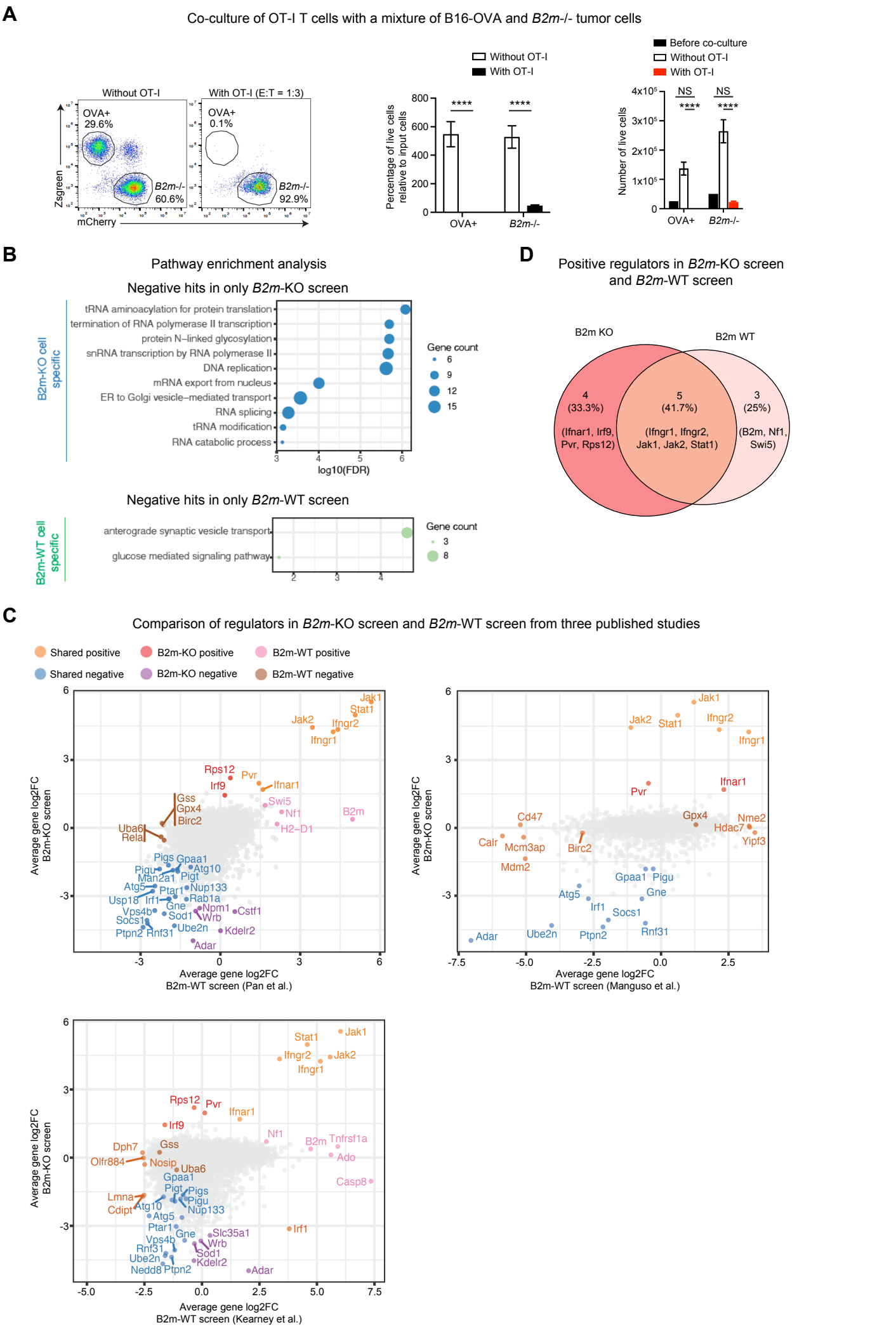
Figure S1


A
Co-culture of OT-I T cells with a mixture of B16-OVA and B2m-/- tumor cells
Without OT-I
With OT-I (E:T = 1:3)
OVA+ 29.6%
B2m-/- 60.6%
OVA+ 0.1%
B2m-/- 92.9%
Zsgreen
mCherry
Without OT-I
With OT-I
Percentage of live cells relative to input cells
OVA+
B2m-/-
Before co-culture
Without OT-I
With OT-I
Number of live cells
NS
****
B
Pathway enrichment analysis
Negative hits in only B2m-KO screen
B2m-KO cell specific
tRNA aminoacylation for protein translation
termination of RNA polymerase II transcription
protein N–linked glycosylation
snRNA transcription by RNA polymerase II
DNA replication
mRNA export from nucleus
ER to Golgi vesicle–mediated transport
RNA splicing
tRNA modification
RNA catabolic process
log10(FDR)
Gene count
Negative hits in only B2m-WT screen
B2m-WT cell specific
anterograde synaptic vesicle transport
glucose mediated signaling pathway
D
Positive regulators in B2m-KO screen and B2m-WT screen
B2m KO
B2m WT
4 (33.3%) (Ifnar1, Irf9, Pvr, Rps12)
5 (41.7%) (Ifngr1, Ifngr2, Jak1, Jak2, Stat1)
3 (25%) (B2m, Nf1, Swi5)
C
Comparison of regulators in B2m-KO screen and B2m-WT screen from three published studies
Shared positive
B2m-KO positive
B2m-WT positive
Shared negative
B2m-KO negative
B2m-WT negative
Average gene log2FC B2m-KO screen
Average gene log2FC B2m-WT screen (Pan et al.)
Average gene log2FC B2m-WT screen (Manguso et al.)
Average gene log2FC B2m-WT screen (Kearney et al.)

Supplementary Fig. S1. Regulators of MHC-I independent and MHC-I dependent T cell-mediated killing.

(**A**) Impact of activated T cells on the survival of *B2m*-/- tumor cells. B16-Ova cells and B16 *B2m*-/- cells were co-cultured for 48h with (E:T = 1:3) or without OT-I T cells. Representative flow cytometry plots are shown for B16-Ova cells and B16 *B2m*-/- cells that were co-cultured with OT-I T cells (E:T ratio of 1:3) or without T cells (left panel). Percentage of live cells relative to input cells (middle panel) and number of live cells (right panel) are shown (n = 5-6/group). Data are representative of two experiments. Data are depicted as the mean ± SEM. To determine statistical significance, a two-way ANOVA with Tukey's multiple comparison test was used. ****$p < 0.0001$.

(**B**) Pathway enrichment analysis of negative regulators (depleted gRNAs) identified only in the *B2m*-KO screen (upper panel) or only the *B2m*-WT screen (lower panel). The dot size indicates the number of genes enriched in the corresponding pathways.

(**C**) Pathway scatter plots comparing enrichment or depletion of gRNAs targeting the indicated genes in the current *B2m*-KO screen (Y-axis) and *B2m*-WT screens from three published studies (X-axis).

(**D**) Venn diagram comparing genes for positive regulators (enriched gRNAs) identified in the current screen with *B2m*-KO cells (light red, left) and a previous co-culture screen of *B2m*-WT B16F10 tumor cells with OT-I T cells (pale red, right) (1).