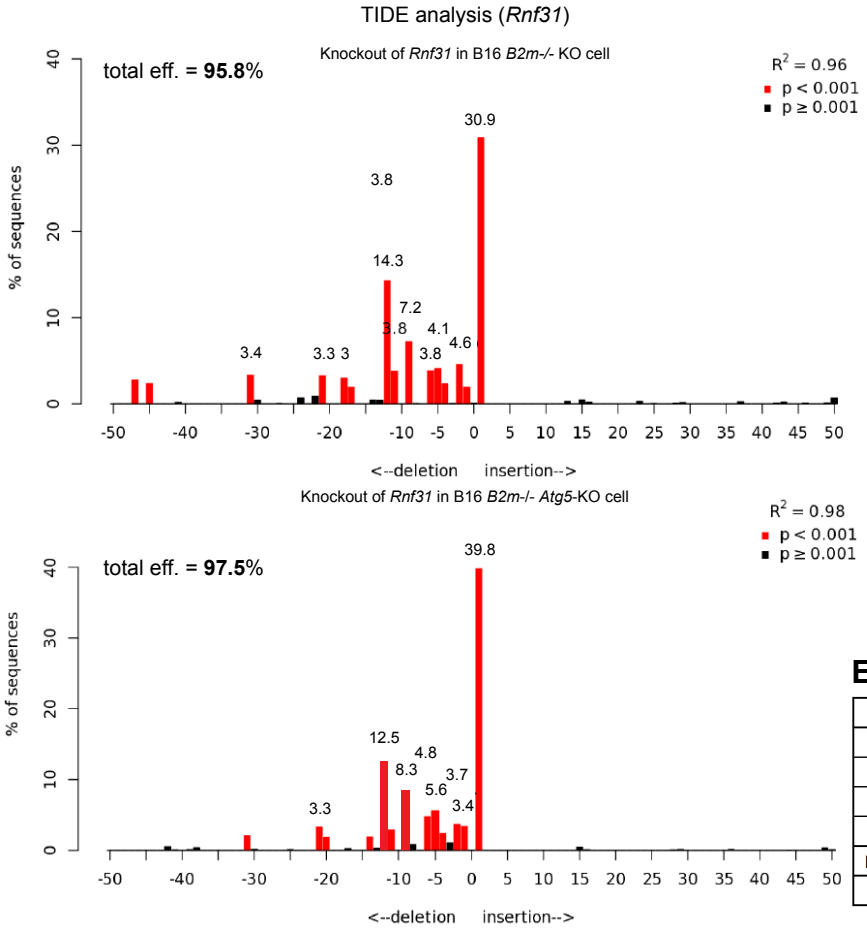
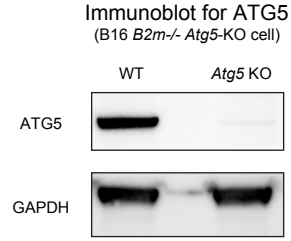
Figure S3

A



B

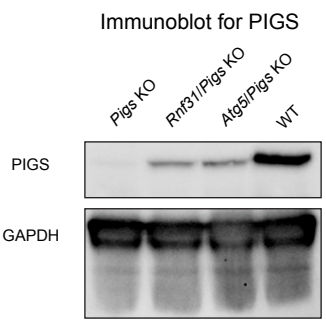


C

TIDE analysis of control gRNA

	Editing efficiency
B16 *B2m-/-* ctrl KO	95.2%

D



E

TIDE analysis of *Rnf31*

	Editing efficiency
B16 *B2m-/- Rnf31*-KO	95.8%
B16 *B2m-/- Rnf31/Atg5*-dKO	97.5%
B16 *Rnf31*-KO	96.8%
B16 *Rnf31/Atg5*-dKO	96.4%
PY8119 *B2m-/- Rnf31/Atg5*-dKO	94.3%
MC38 *B2m-/- Rnf31/Atg5*-dKO	91.3%

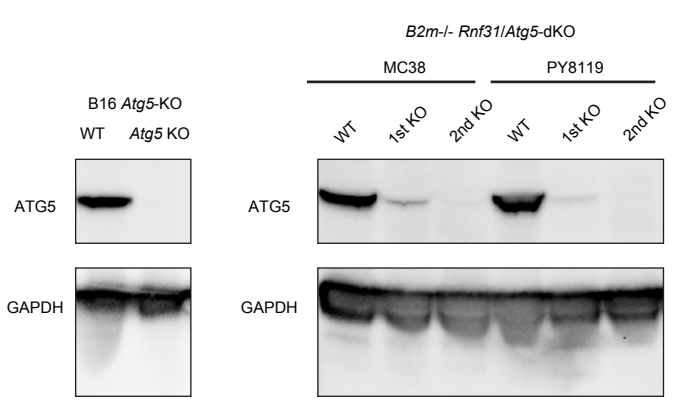
TIDE analysis of *RNF31*

	Editing efficiency
A375 *RNF31*-KO	90.7%
A375 *RNF31/ATG5*-dKO	92.0%

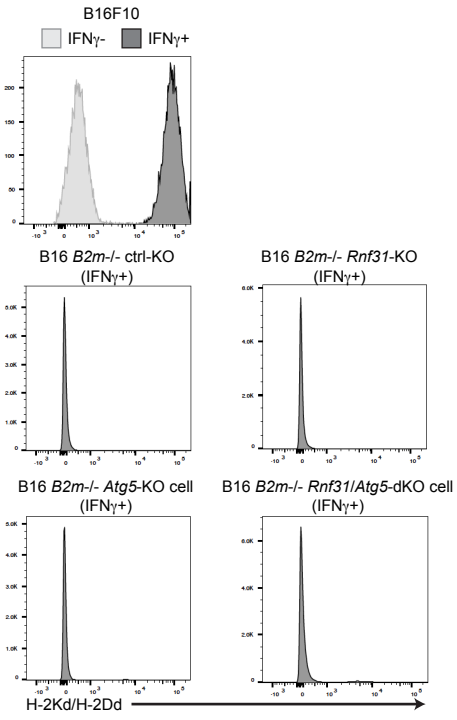
TIDE analysis of *ATG5*

	Editing efficiency
A375 *ATG5*-KO	86.3%
A375 *RNF31/ATG5*-dKO	87.8%

F



G



H

Editing efficiency of *Ifngr1* and *Tnfrsf1a*
(B16 *B2m-/- Rnf31/Atg5*-dKO cell)

	Genes	
	Ifngr1	*Tnfrsf1a*
Ifngr1 KO	90.5%	NA
Tnfrsf1a KO	NA	90.1%
Ifngr1/Tnfrsf1a KO	90.5%	90.2%

Supplementary Fig. S3. Generation of tumor cell lines with multiple gene edits.

(**A**) TIDE analysis of *Rnf31* gene editing efficiency in B16 *B2m*-/- *Rnf31*-KO cells (upper panel) and B16 *B2m*-/- *Rnf31*/*Atg5*-dKO cells (lower panel).

(**B**) Western blot analysis for ATG5 with cell lysates from B16 *B2m*-/- cells (WT) and B16 *B2m*-/- *Atg5*-KO cells. GAPDH is shown as a loading control.

(**C**) Editing efficiency for a control gRNA analysed by TIDE analysis in B16 *B2m*-/- ctrl-KO cells.

(**D**) Western blot analysis for PIGS with cell lysates from B16 *B2m*-/- cells (WT), B16 *B2m*-/- *Pigs*-KO cells, B16 *B2m*-/- *Rnf31*/*Pigs*-dKO cells, and B16 *B2m*-/- *Atg5*/*Pigs*-dKO cells. GAPDH is shown as a loading control.

(**E**) Summary of editing efficiency of *Rnf31*, *Rnf3*, and *Atg5* genes revealed by TIDE analysis for the indicated cell lines.

(**F**) Western blot analysis for ATG5 with cell lysates from B16 *B2m*-/- cells (WT), B16 *B2m*-/- *Atg5*-KO cells, MC38 *B2m*-/- cells (WT), MC38 *B2m*-/- *Rnf31*/Atg5-dKO cells (electroporated once or twice), PY8119 *B2m*-/- cells (WT), and PY8119 *B2m*-/- *Rnf31*/Atg5-dKO cells (electroporated once or twice). GAPDH is shown as a loading control.

(**G**) Histogram of flow cytometry analysis for H-2K^{d}/H-2D^{d} expression. Indicated cell lines were treated with or without IFNγ (50ng/ml) for 48h.

(**H**) Summary of editing efficiency of *Ifngr1* and *Tnfrsf1a* genes revealed by TIDE analysis of indicated cell lines. NA, not applicable.