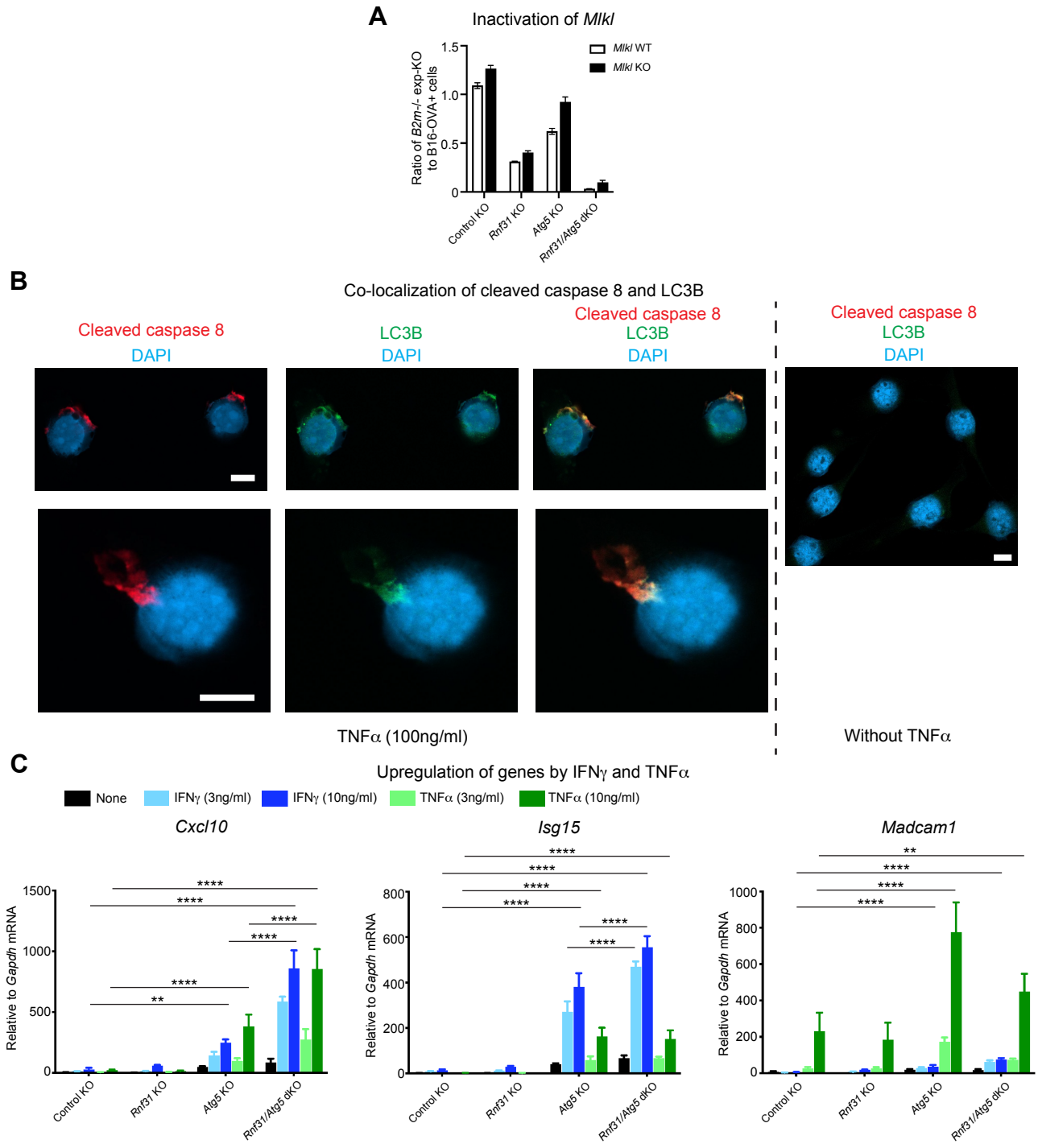
Figure S6


A
Inactivation of Mlkl
Mlkl WT
Mlkl KO
Ratio of B2m-/- exp-KO to B16-OVA+ cells
1.5
1.0
0.5
0
Control KO
Rnf31 KO
Atg5 KO
Rnf31/Atg5 dKO
B
Co-localization of cleaved caspase 8 and LC3B
Cleaved caspase 8
DAPI
LC3B
DAPI
Cleaved caspase 8
LC3B
DAPI
Cleaved caspase 8
LC3B
DAPI
TNFα (100ng/ml)
Without TNFα
C
Upregulation of genes by IFNγ and TNFα
None
IFNγ (3ng/ml)
IFNγ (10ng/ml)
TNFα (3ng/ml)
TNFα (10ng/ml)
Cxcl10
Isg15
Madcam1
Relative to Gapdh mRNA
1500
1000
500
0
800
600
400
200
****
**
Control KO
Rnf31 KO
Atg5 KO
Rnf31/Atg5 dKO

Supplementary Fig. S6. Functional links between autophagy and cytokine signaling pathways.

(**A**) Impact of *Mlkl* inactivation on killing of *Rnf31* or *Atg5* deficient tumor cells (B16F10) by CD8 T cells. B16-Ova and *B2m*-/- exp-KO cells were co-cultured for 48h without or with OT-I T cells (E:T = 1:3). Ratio of *B2m*-/- exp-KO cells to B16-Ova cells is shown (n = 4/group). Data are representative of two experiments. Data are depicted as the mean ± SEM.

(**B**) Co-localization of cleaved caspase 8 and LC3B. Immunofluorescence staining of B16 *Rnf31*-/- cells after 4.5h of treatment with or without 100 ng/ml TNFα. Cleaved caspase 8 (red), LC3B (green) and DAPI (light blue). Scale bar represents 10 μm.

(**C**) RT-qPCR analysis of *Cxcl10*, *Isg15*, and *Madcam1* mRNA levels in B16 *B2m*-/- ctrl-KO cells and B16 *B2m*-/- exp-KO cells (triplicate measurements). 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. ****$p < 0.0001$; **$p < 0.01$.