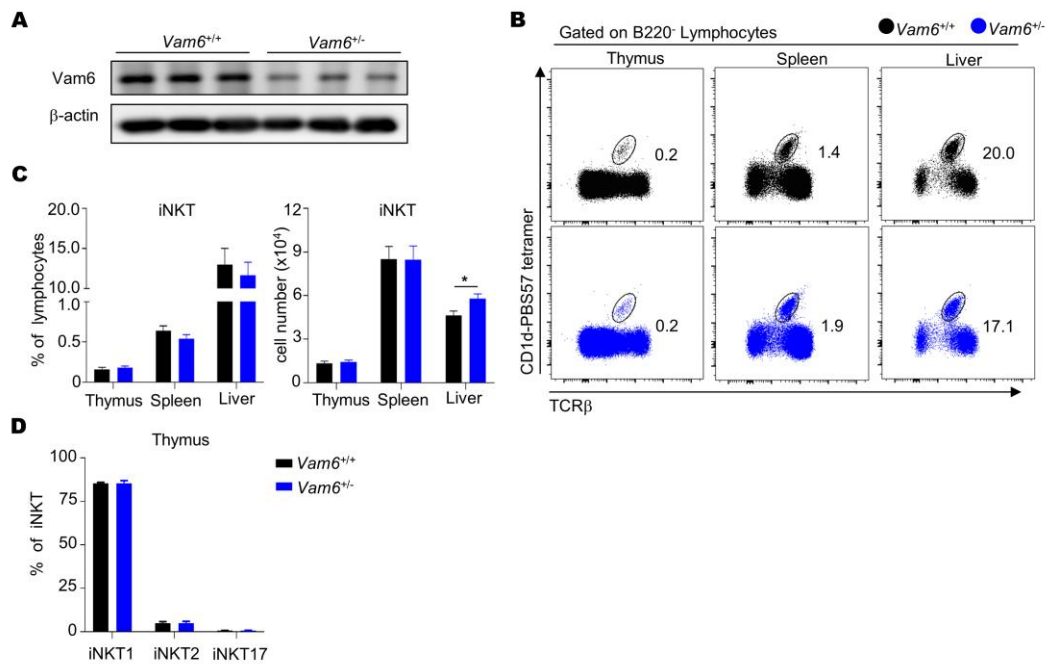


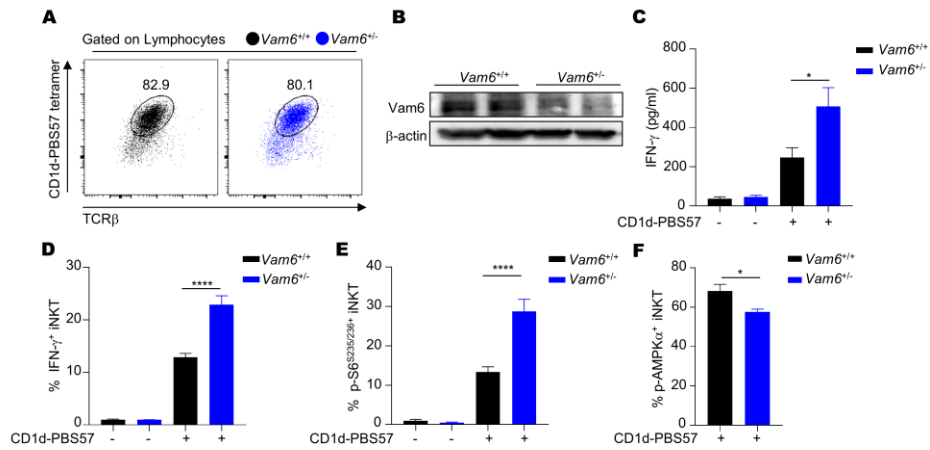
Supplementary Information for

**Vam6 reduces iNKT cell function in tumor via modulating AMPK/mTOR
pathways**

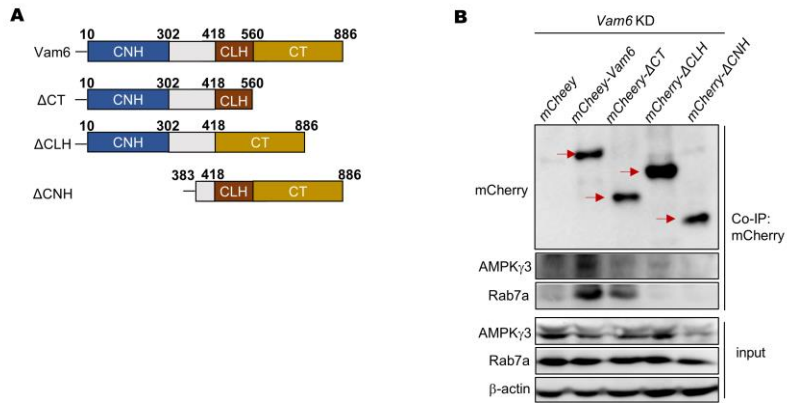
Supplementary



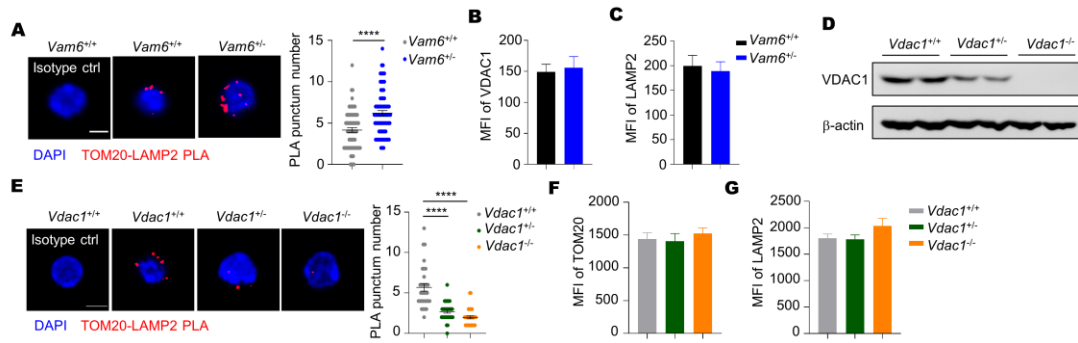
Supplementary Figure 1. Reducing Vam6 expression has no influences on iNKT cell development. **(A)** Expression level of Vam6 in splenic T cells from *Vam6*^{+/+} and *Vam6*^{+/-} mice. n = 3 mice for each group. **(B-D)** Representative flow cytometry dot plots **(B)**, frequencies and absolute numbers **(C)**, and frequencies of subsets **(D)** of iNKT cells in indicated tissues from *Vam6*^{+/+} and *Vam6*^{+/-} mice. n = 5 mice for each group. Data are shown as mean ± SEM, pooled from two independent experiments, and analyzed by two-tailed Mann-Whitney tests in **(C)**. *P < 0.05.



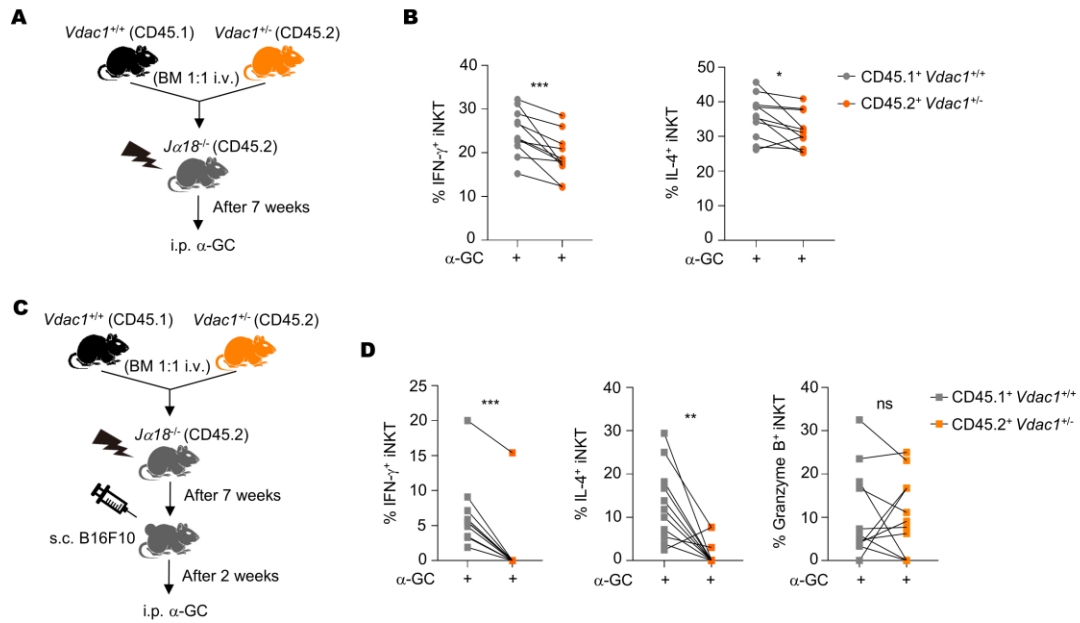
Supplementary Figure 2. Reducing Vam6 expression in expanded iNKT cells influences cytokine production and AMPK/mTORC1 activities. **(A-B)** Purities **(A)** and Vam6 expression level **(B)** in expanded *Vam6*^{+/+} and *Vam6*^{-/-} iNKT cells. **(C-F)** IFN-γ production in supernatants **(C)**, intracellular IFN-γ **(D)**, phosphorylation of S6^{S235/S236} **(E)**, and phosphorylation of AMPK α **(F)** in expanded *Vam6*^{+/+} and *Vam6*^{-/-} iNKT cells stimulated with or without CD1d-PBS57 tetramer. n = 9-10 samples for each group. Data are shown as means ± SEM and pooled from three independent experiments in **(C-F)**. Two-tailed Mann-Whitney test was applied in **(C-F)**. *P < 0.05, ****P < 0.0001.



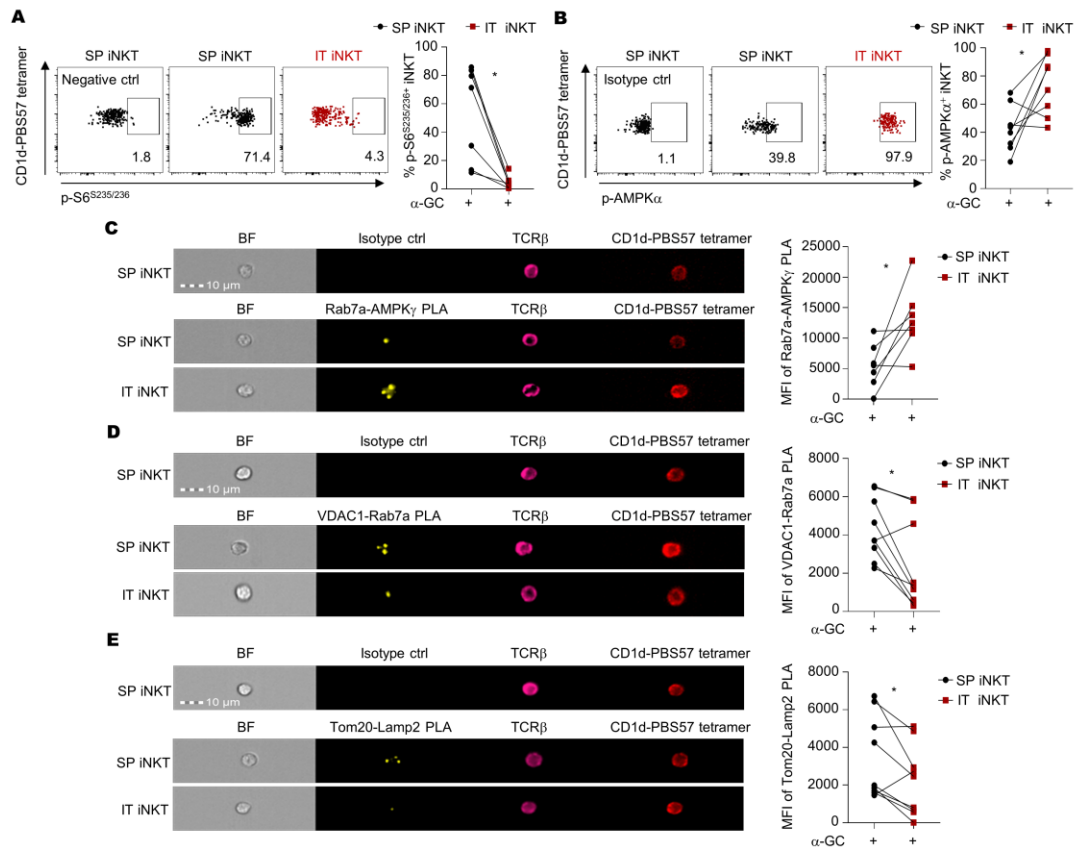
Supplementary Figure 3. Essential region of Vam6 for binding AMPK and Rab7a. **(A)** Diagram showing Vam6 truncations. **(B)** Levels of AMPK γ 3 and Rab7a co-immunoprecipitated with mCherry in NIH-3T3 cells expressing *mCherry*, *mCherry-Vam6*, *mCherry- Δ CT*, *mCherry- Δ CLH*, and *mCherry- Δ CNH*, respectively. Red arrows indicate mCherry, mCherry-Vam6, mCherry- Δ CT, mCherry- Δ CLH, and mCherry- Δ CNH, respectively.



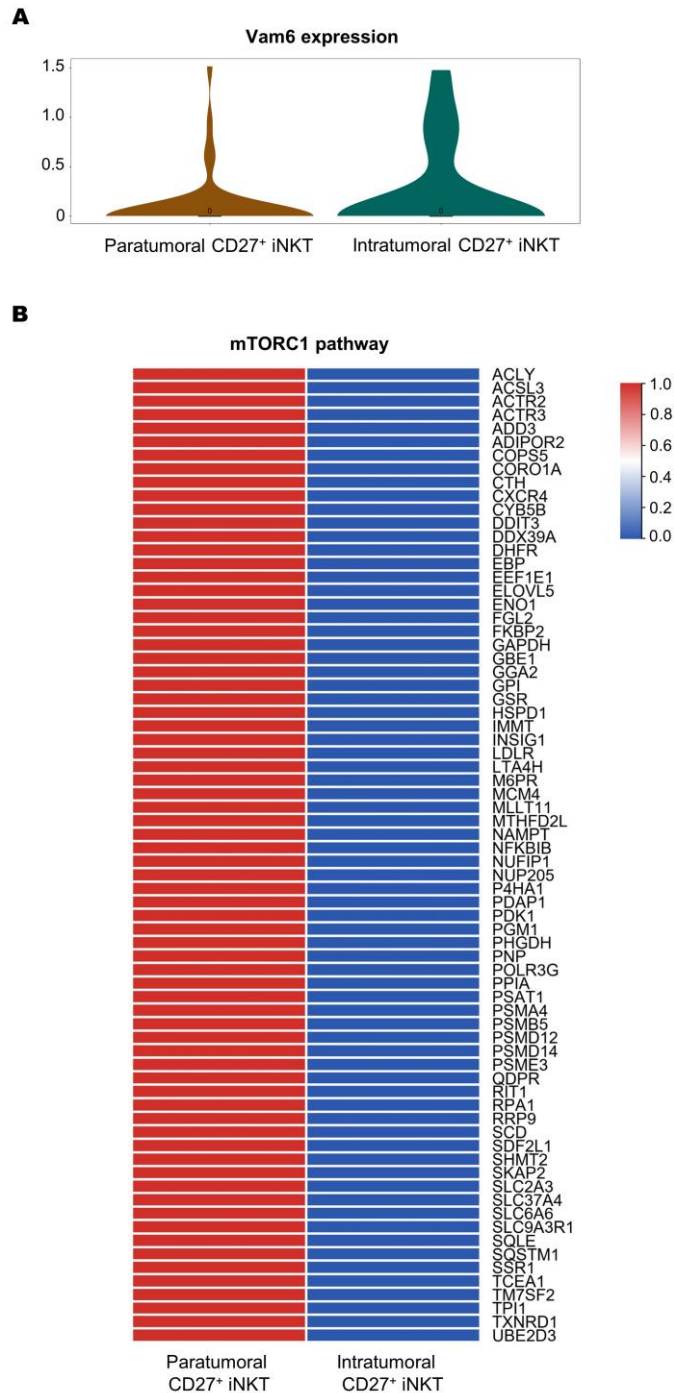
Supplementary Figure 4. Vam6 and VDAC1 control mitochondria-lysosome contact. (A-C) TOM20-LAMP2 PLA puncta (A, n = 62-66 cells for each group), levels of VDAC1 (B, n = 9 replicates for each group), and levels of LAMP2 (C, n = 9 replicates for each group) in *Vam6*^{+/+} and *Vam6*^{+/-} iNKT cells stimulated with anti-CD3 plus anti-CD28 for 4 hours. Scale bar, 3 μm. (D) Expression level of VDAC1 in splenic T cells from *Vdac1*^{+/+}, *Vdac1*^{+/-}, and *Vdac1*^{-/-} mice. n = 2 mice for each group. (E-G) TOM20-LAMP2 PLA puncta (E, n = 32-33 cells for each group), levels of Tom20 (F, n = 12 replicates for each group), and levels of LAMP2 (G, n = 12 replicates for each group) in *Vdac1*^{+/+}, *Vdac1*^{+/-}, and *Vdac1*^{-/-} iNKT cells stimulated with CD1d-PBS57 tetramer for 4 hours. Data are shown as mean ± SEM and pooled from three independent experiments in (A-C) and (E-G). Data were analyzed by two-tailed unpaired Student's t test (A and E) and two-tailed Mann-Whitney tests (B-C and F-G). ****p < 0.0001.



Supplementary Figure 5. VDAC1 inhibits cytokine production in iNKT cells. **(A)** Experimental procedure for **(B)**. **(B)** IFN- γ and IL-4 production in CD45.1⁺ *Vdac1*^{+/+} iNKT cells and CD45.2⁺ *Vdac1*^{+/-} iNKT cells in spleens of chimeric mice, 4 hours after α -GC or PBS injection. $n = 11$ mice. **(C)** Experimental procedure for **(D)**. **(D)** IFN- γ , IL-4, and granzyme B production in CD45.1⁺ *Vdac1*^{+/+} iNKT cells and CD45.2⁺ *Vdac1*^{+/-} iNKT cells in B16F10 tumors of chimeric mice, 4 hours post α -GC injection. $n = 11$ mice. Data were analyzed by two-tailed Wilcoxon matched-pairs signed rank tests. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ns, not significant.



Supplementary Figure 6. Intratumoral iNKT cells show lower mTORC1 activation and VDAC1-Rab7a interaction but higher AMPK activation and Rab7a-AMPK γ interaction. **(A-B)** Phosphorylation of S6^{S235/S236} **(A, n = 7 mice)**, and phosphorylation of AMPK α **(B, n = 8 mice)** in splenic (SP) and intratumoral (IT) iNKT cells from MC38 tumor-bearing mice, 4 hours post α -GC injection. **(C-E)** Median fluorescence intensity (MFI) of Rab7a-AMPK γ PLA **(C, n = 7 mice)**, MFI of VDAC1-Rab7a PLA **(D, n = 9 mice)**, and MFI of TOM20-LAMP2 PLA **(E, n = 9 mice)** detected by image flow cytometry in splenic (SP) and intratumoral (IT) iNKT cells from MC38 tumor-bearing mice, 4 hours post α -GC injection. Data are pooled from three **(B and E)**, or four **(C-D)** independent experiments and were analyzed by two-tailed Wilcoxon matched-pairs signed rank tests. * $P < 0.05$.



Supplementary Figure 7. Published scRNA-seq data show impaired Vam6-mTORC1 axis in CD27⁺ iNKT cells in tumors of CRLM patients. **(A-B)** Vam6 expression **(A)** and heatmap of mTORC1 pathway related genes **(B)** in CD27⁺ iNKT cells from paracarcinoma tissues and primary tumors of CRLM patients (GEO: GSE164522).