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SUPPLEMENTARY INFORMATION

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Metabolic reprogramming of natural killer cells in obesity limits antitumor responses

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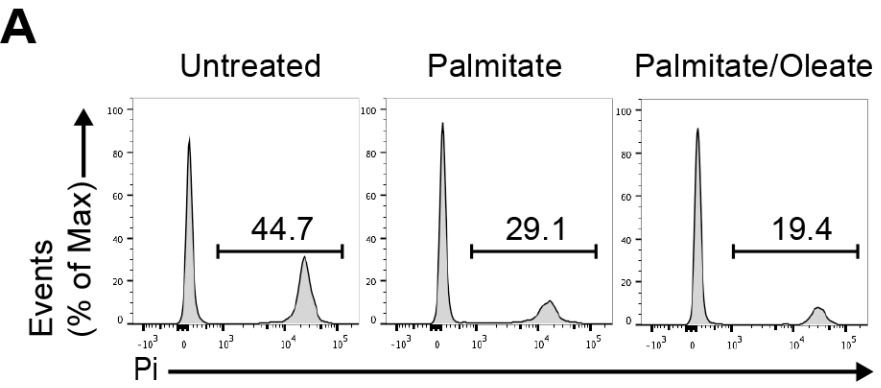
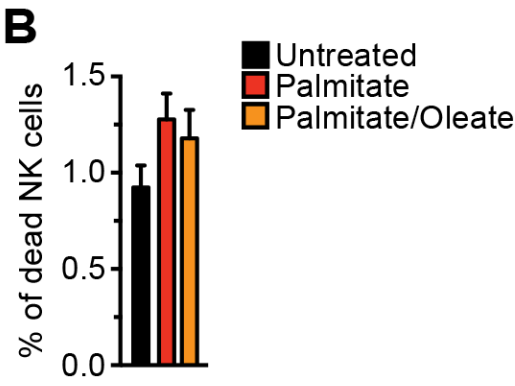
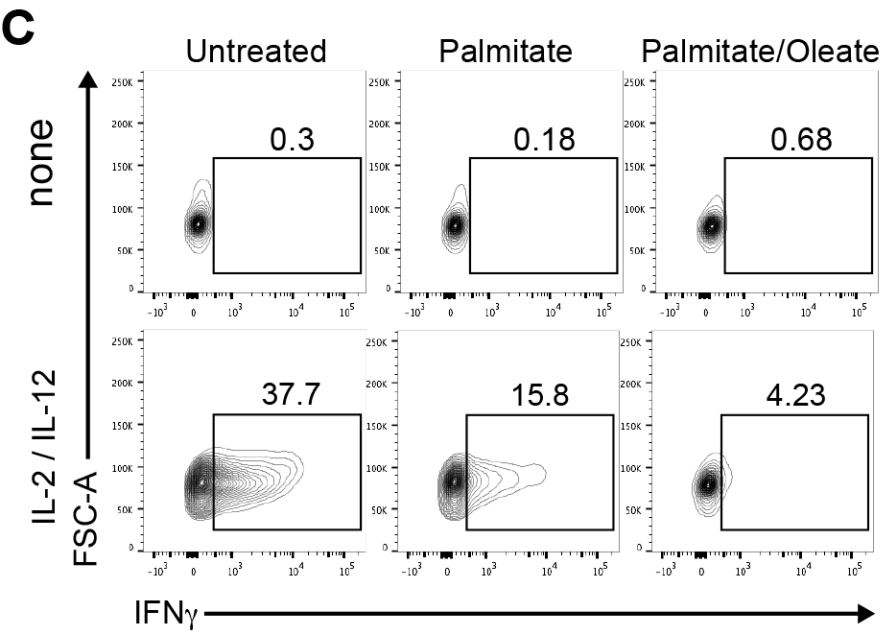
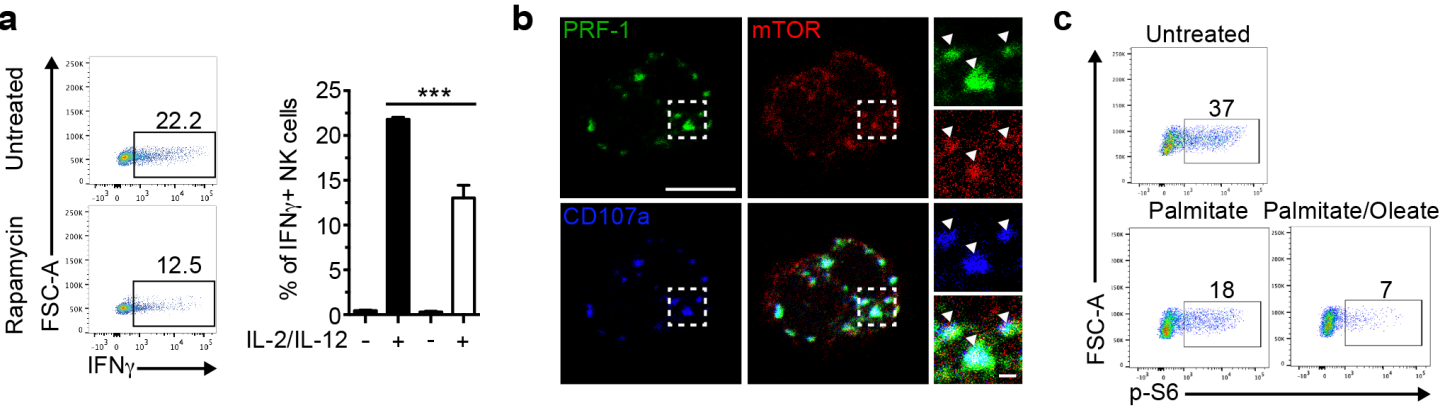


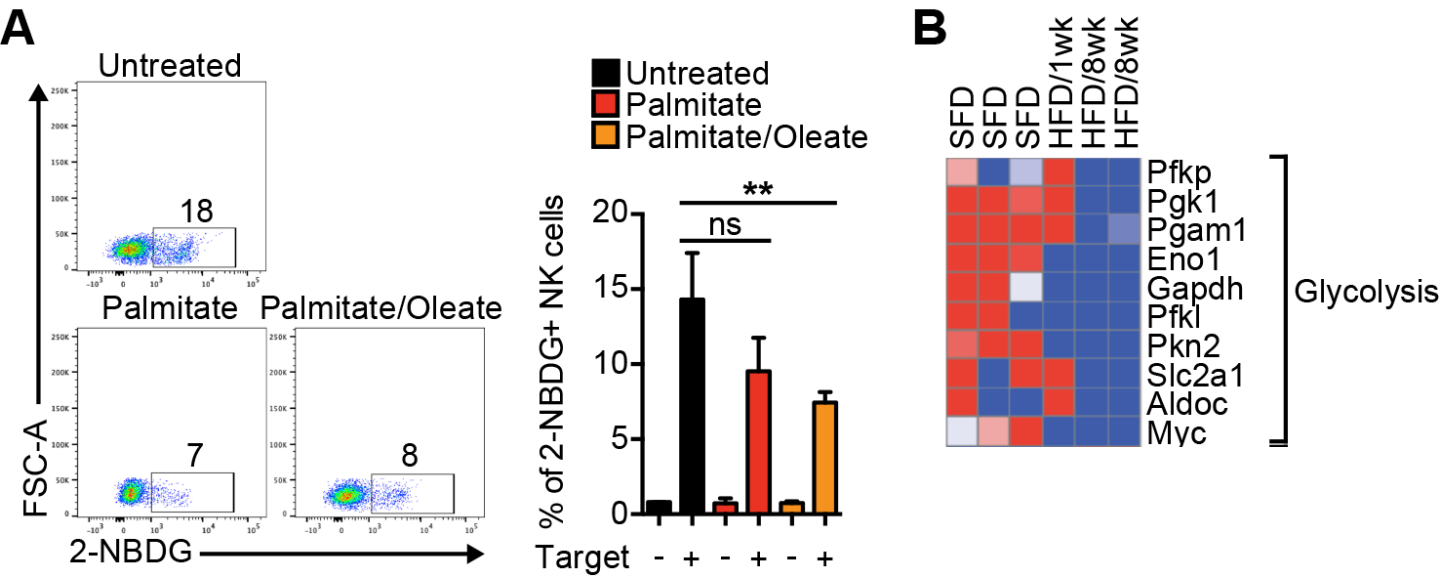




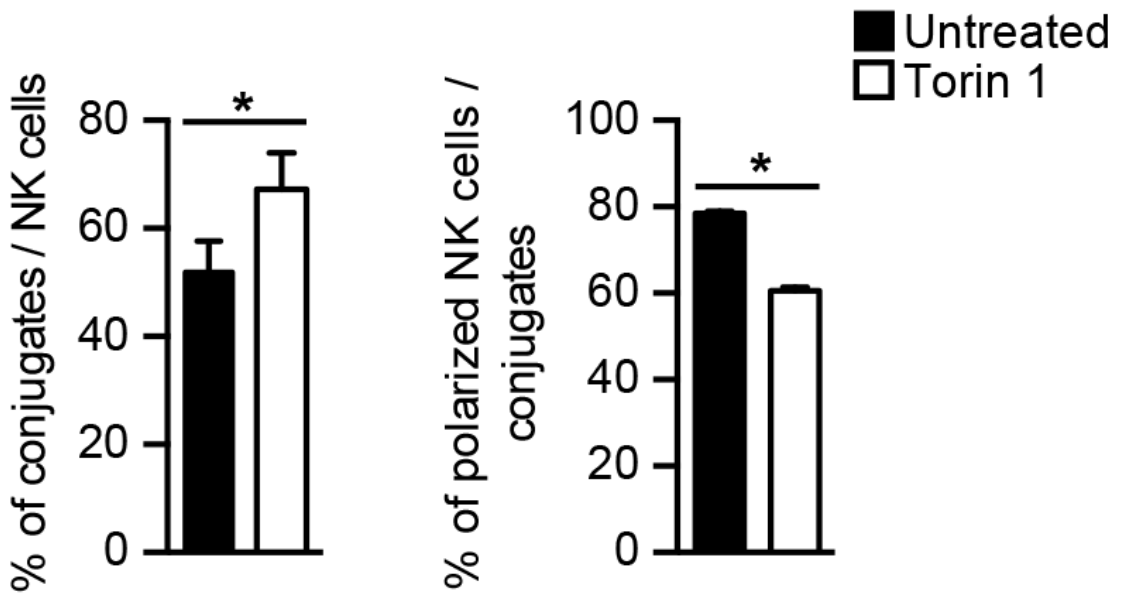
Supplemental Figure 1: Lipid accumulation impairs NK cell function. (A) Histograms of Pi^+ target cells after co-incubation with NK cells treated or not with lipids. Numbers indicate the percentage of dead target cells. (B) Quantification of dead NK cells Pi^+ after co-incubation with target cells. (C) Representative plots of isolated NK cells treated with lipids and stimulated or not with IL-2/IL-12. Numbers above the outlined area indicate the percentage of IFN-g^+ NK cells.



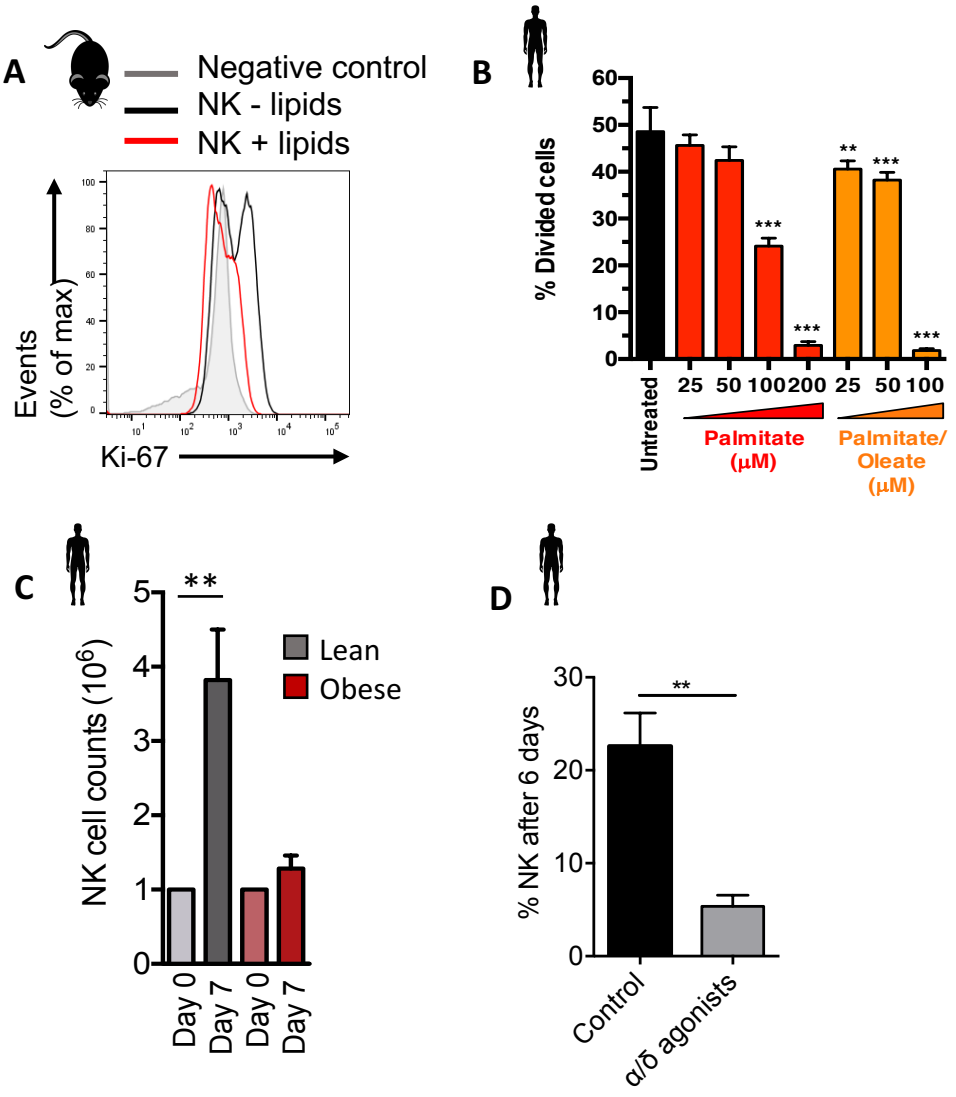
Supplemental Figure 2: mTOR activation is required for NK cell effector function.
(A) Plots and quantification of IFN-g$^+$ NK cells pre-cultured with rapamycin (20 nM) and then stimulated with IL-2/IL-12. Numbers above the outlined area indicate the percentage of IFN-g$^+$ NK cells. (B) Images of YT-indy cells stained for PRF-1, mTOR and CD107a. (C) Plots of p-S6$^+$ stimulated NK cells treated with lipids. Numbers above the outlined area indicate the percentage of p-S6$^+$ NK cells. Mean ± s.d. Statistics have been calculated using a two-way ANOVA followed by a Bonferroni test to analyze each replicate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



Supplemental Figure 3: Lipid accumulation leads to glycolysis downregulation. (A) Histograms and quantification of 2-NBDG^{+} NK cells treated with lipids after co-incubated with target cells. (B) Heat-map visualization of differentially expressed glycolytic and OxPhos related genes. Red indicates genes with increased expression and blue with decreased expression. Mean ± s.d. Statistics have been calculated using a two-way ANOVA followed by a Bonferroni test to analyze each replicate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

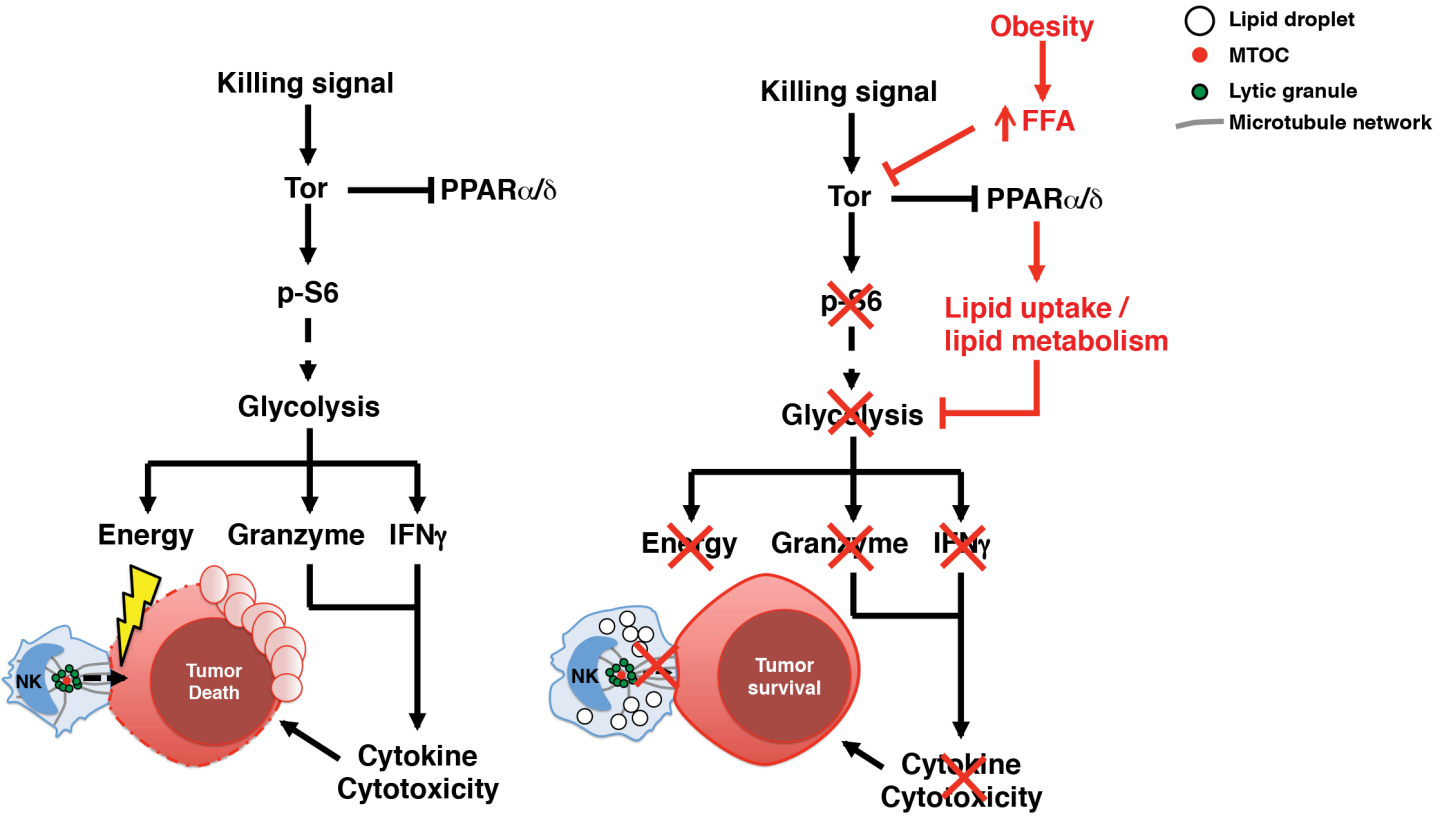


Supplemental Figure 4: mTOR downregulation inhibits lytic granule polarization. Percentage of NK:T conjugates and polarized NK cells per conjugates with untreated NK cells or cultured with Torin 1 (250 mM). Mean ± s.d. of duplicates, Untreated, n=126 cells, Torin 1, n=106 cells. Statistics have been calculated using $\chi 2$ test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



Supplementary Figure 5: FFA, obesity and PPAR agonists inhibits NK cell proliferation.

A) Splenic NK cells from lean mice were cultured with low-dose IL-15 with or without FFA (palmitate + oleate) for 24 h before stimulation with IL-12/IL-15/IL-18 for 18 h. Unstimulated NK cells were used as negative control. Cells were subsequently stained with intranuclear Ki-67. B) Human NK cells from lean blood were isolated and stained with CFSE before culturing with IL-15 with or without FFA (palmitate or palmitate and oleate) for 5 days. CFSE dilution was measured by flow cytometry and % of dividing cells were calculated. C) Primary NK cells were isolated from lean (n=5) and obese (n=5) individuals and cultured at $1x10^6$ cells per well in IL-15 for 7 days. NK cell expansion was measured by counting NK cells on day 0 and day 7. D) Splenic NK cells were cultured with low dose IL-15 in the presence or absence of PPAR agonists for 6 days. NK cell expansion was measured by counting NK cells on day 0 and day 7.



Supplementary Figure 6: Model of metabolic consequences of obesity on NK cell effector functions. Left panel: Upon stimulation, NK cells turn on the Tor pathway and aerobic glycolysis that leads to the transcription and production of granzyme and IFN-g. Increased aerobic glycolysis provides the energy to allow NK cells to direct lytic granules toward the tumor cell synapse. Thus, switching on mTOR and glycolysis allows NK cells to meet the requirements to kill the tumor cells. Right panel: During obesity, the level of free fatty acid (FFA) increases in serum resulting in the activation of the PPARa/d pathway and Tor inhibition. Expression of PPARa/d target genes leads the increase of lipid uptake and lipid metabolism. When activated, NK cells are unable to turn on glycolysis resulting in a lack of energy. Thus, NK cells display a downregulation of granzyme and IFN-g production and are unable to direct the killing machinery toward the tumor cells that they encounter, resulting in tumor survival.