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Supplemental Information

Conditional Deletion of PGC-1α Results in Energetic and Functional Defects in NK Cells

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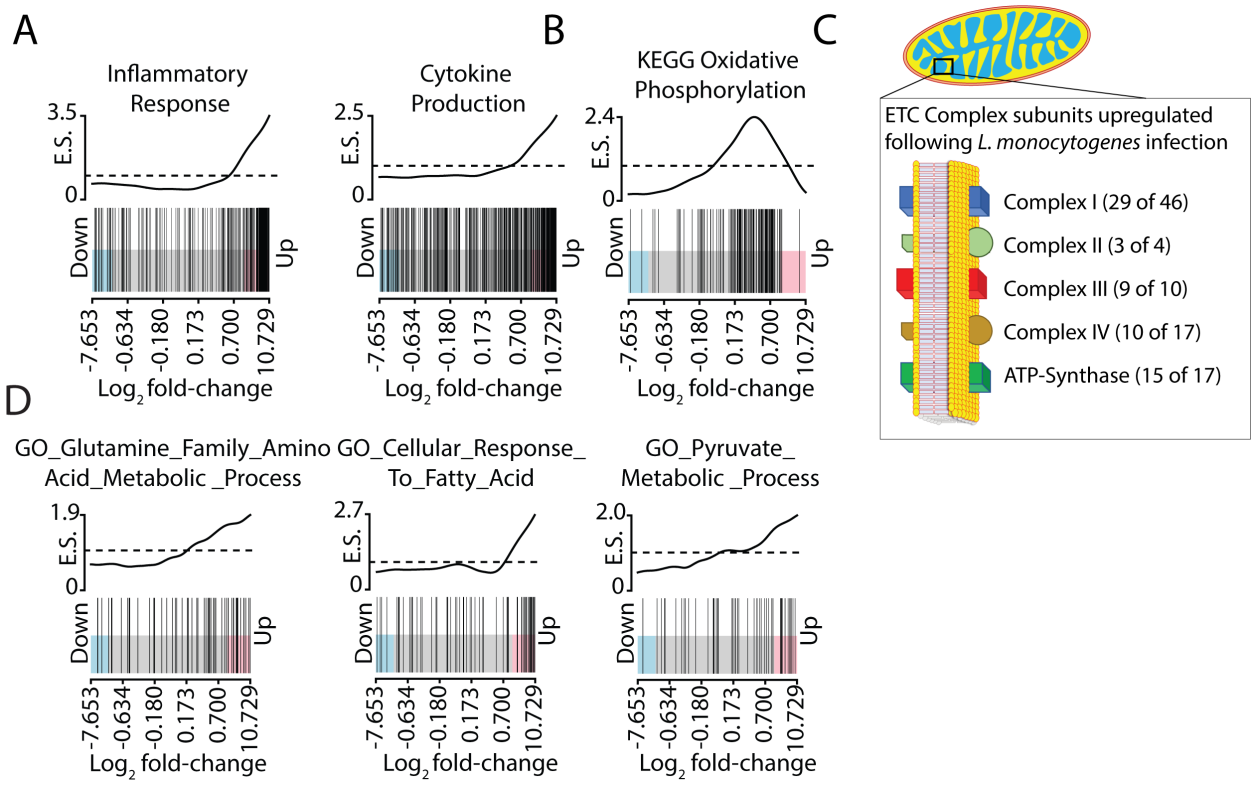
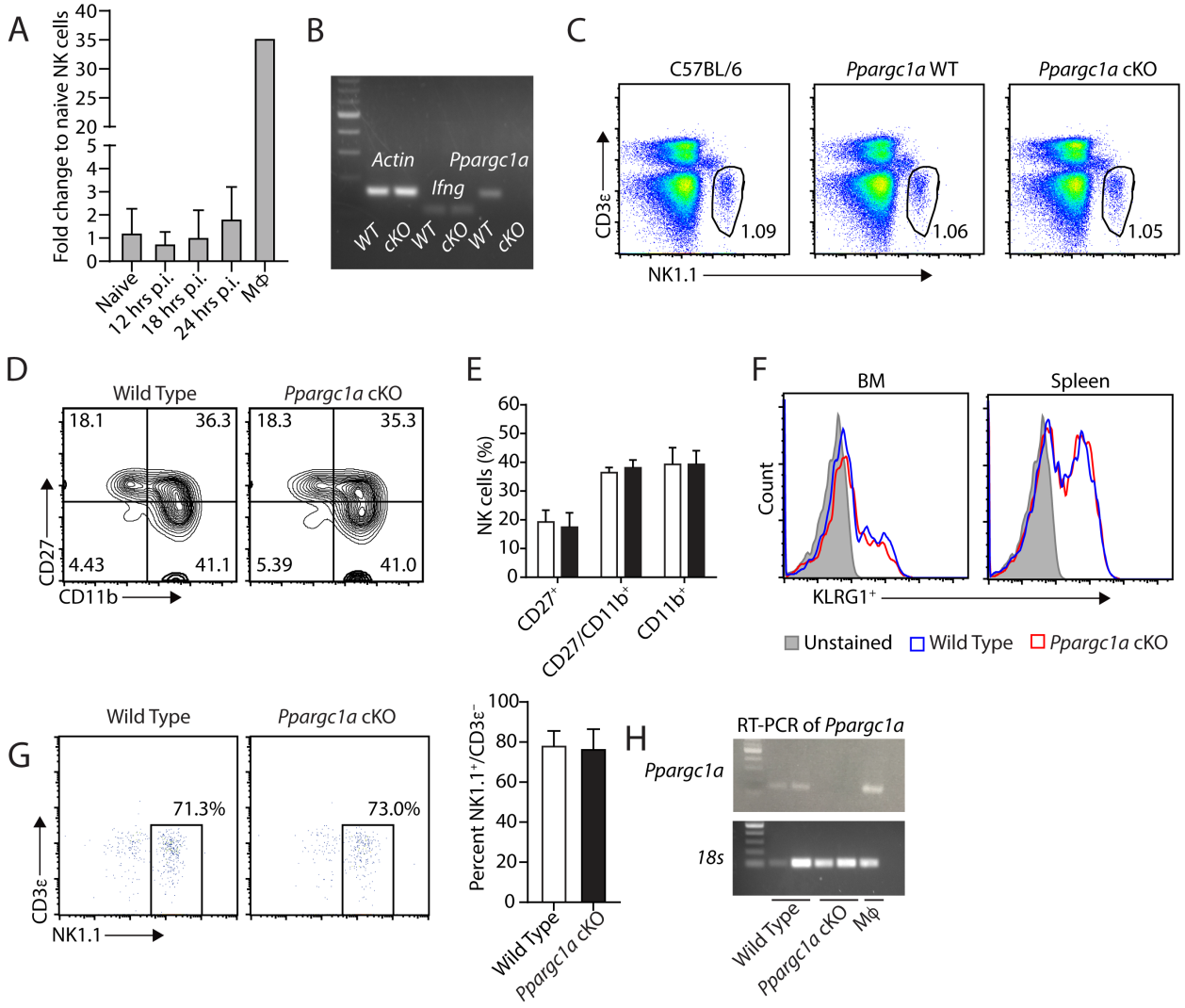
SUPPLEMENTAL INFORMATION

Supplemental Figures and Legends



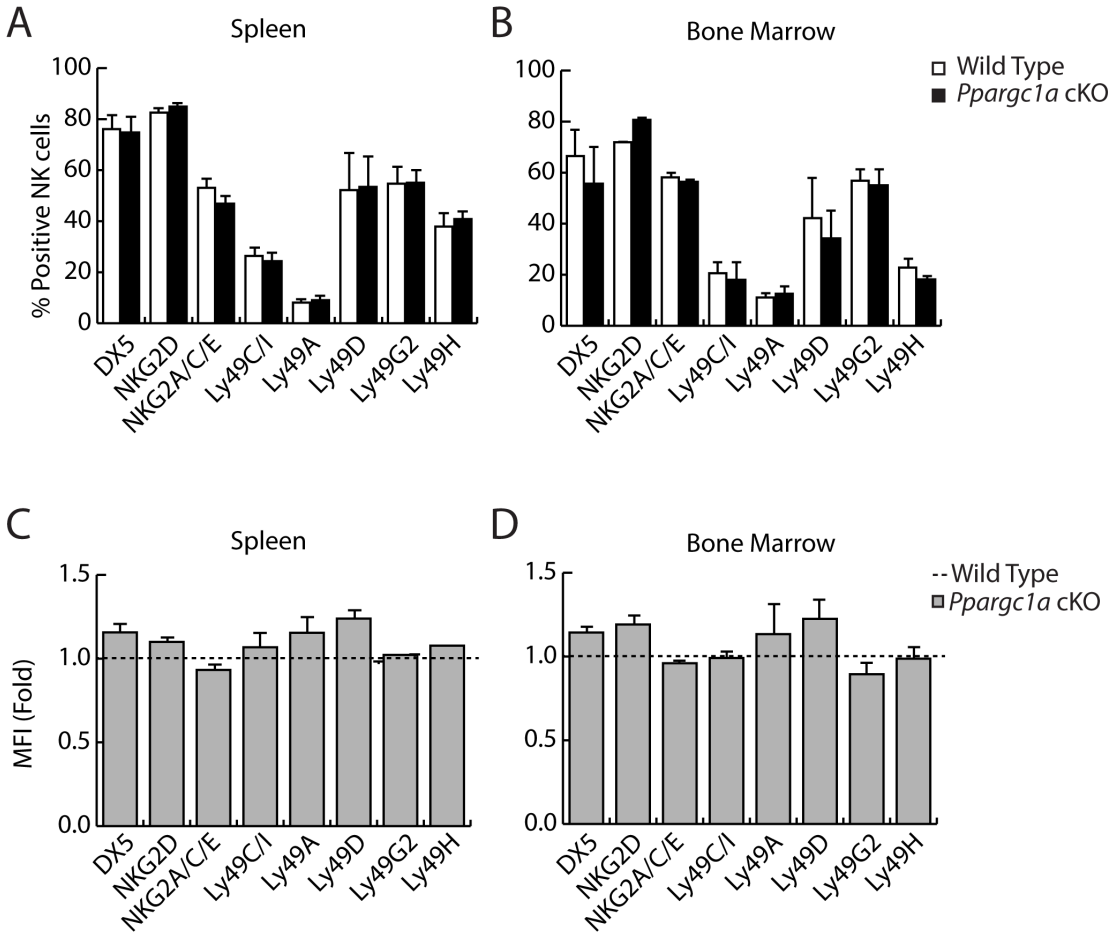
Supplemental Figure 1. GSEA results for OxPhos gene sets upregulated in NK cells following infection, Related to Figure 1. RNA-Sequencing data from *L. monocytogenes* infection experiments performed previously in the lab (Nanbakhsh et al., 2019) were analyzed for differential expression of metabolic genes. Competitive GSEA was performed to identify changes in gene expression in infected versus control mice (n=3 individual mice per experiment group). Enrichment of (A) Hallmark gene sets indicative of immune activation was evaluated, along with enrichment of (B) the KEGG pathway Oxidative Phosphorylation to evaluate global OxPhos regulation. (C) For genes comprising the mitochondrial electron transport chain complexes found in the KEGG data set Oxidative Phosphorylation, individual expression values were examined, and the number of genes that were upregulated more than two-fold out of the total number of genes comprising each ETC complex (I-IV, ATP-Synthase) are shown. (D) Enrichment of gene sets associated with different mitochondrial nutrient utilization pathways was also evaluated. The significance of GSEA results was determined using the FRY variation of rotational gene set testing for differential expression analysis (**Table 1**).



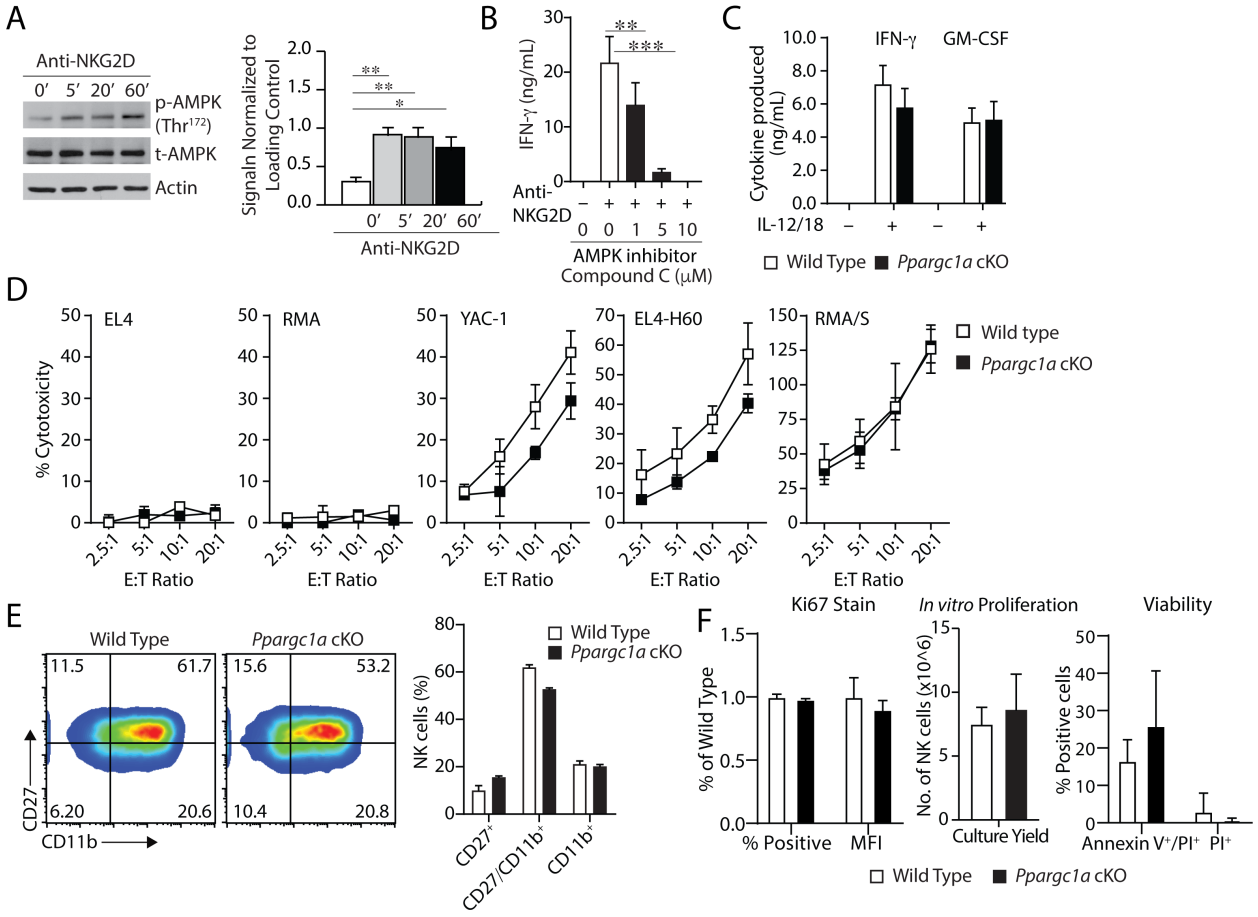
Supplemental Figure 2. NK cell development is unaltered in *Ppargc1a* cKO mice, Related to Figure 1

(A) NK cells were isolated via negative selection from the spleens of naïve or *L. monocytogenes*-infected mice at indicated time points, RNA was extracted, and RT-qPCR was performed to quantify gene expression compared to control RNA from a macrophage cell line. (B) mRNA was isolated from IL-2-cultured NK cells from WT, and *Ppargc1a* cKO mice and RT-PCR was performed using primers for *Ppargc1a* along with *Actinb* and *Ifng* as controls. RT-PCR products were then ran through agarose gels followed by imaging. Lymphocytes were collected from the spleens of C57BL/6 mice as well as WT and *Ppargc1a* cKO littermates and cells were stained for (C) NK cells surface markers along with (D,E) CD27 and CD11b, and population percentages were quantified (n=3 individual mice for each genotype, two independent experiments). (F) Splenic lymphocytes were collected and stained with NK cell surface

markers along with KLRG1 to identify terminally mature NK cell populations. (G) The purity of WT and *Ppargc1a* cKO cells isolated via negative selection for gene expression comparisons (**Fig. 1G**) was evaluated through analysis of NK1.1 and CD3ε expression by flow cytometry (n=3 individual mice for each genotype). (H) *Ppargc1a* gene expression was evaluated in NK cells from WT and *Ppargc1a* cKO mice by RT-PCR and reaction products were resolved on an agarose gel. Products from 18s RT-PCR using the same samples are also shown to demonstrate the presence of template cDNA. Bar graphs present the mean ± SD or mean ± SEM, where multiple experiments are indicated.

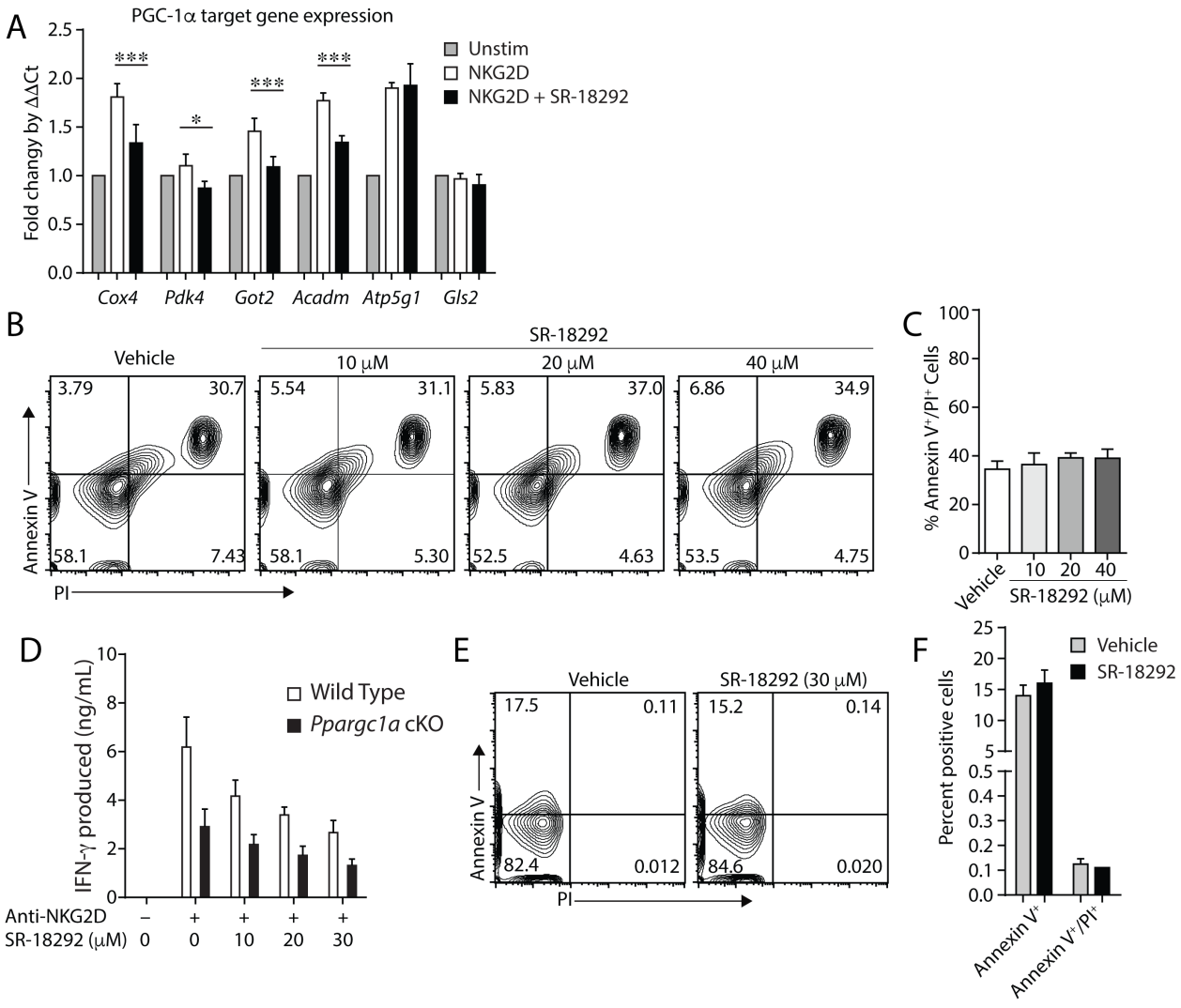


Supplemental Figure 3. Activation and inhibitory receptor expression is unaltered in *Ppargc1a* cKO mice, Related to Figure 2 Lymphocytes were collected from the spleens and bone marrow of WT and *Ppargc1a* cKO mice and surface receptor expression was analyzed via flow cytometry. Data for various activation and inhibitor receptors were quantified both in terms of (A, B) percent positive cells and (C, D) receptor expression level as determined by mean fluorescence intensity (n=3 individual mice for each genotype, three individual experiments). Bar graphs present the mean ± SEM.



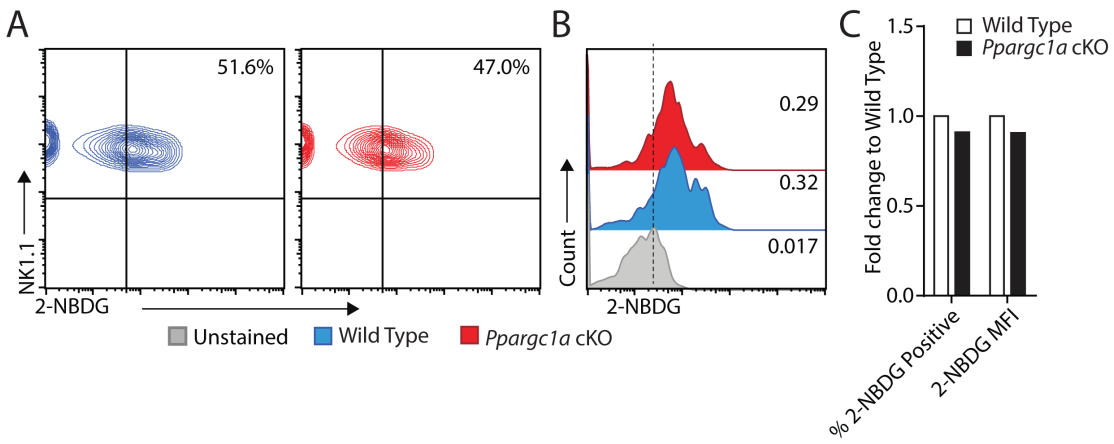
Supplemental Figure 4. AMPK-PGC-1α signaling contributes to NK cell functions *in vitro*, Related to Figure 2. (A) IL-2-cultured NK cells from the spleens of WT mice were collected and seeded in anti-NKG2D activating antibody coated wells for the indicated time points. Lysates were collected and western blot was used to evaluate activation of AMPK by phosphorylation of T172. Data were quantified over multiple experiments (n=5 individual mice, three independent experiments). (B) IL-2-cultured NK cells from the spleens of WT mice were seeded in control or anti-NKG2D activating antibody coated wells for 6 hours in the presence or absence of the AMPK inhibitor Compound C. Supernatants were collected and IFN-γ secretion was measured via ELISA (n=3 individual mice). (C) IL-2-cultured NK cells from WT and *Ppargc1a* cKO mice were activated with IL-12 and IL-18 (1 and 10 ng/mL, respectively), and supernatants were collected after 6 hours for analysis of cytokine secretion via multiplex (n=6 individual mice for each genotype, two independent experiments). (D) IL-2-cultured NK cells from the spleens of WT and *Ppargc1a* cKO mice were incubated with ^{51}Cr-labeled target cells for 4 hours. The supernatant was collected, and cytotoxic potential was evaluated on control cells (EL4, RMA) and cells known to be

susceptible to NK cell-mediated lysis (YAC-1, EL4-H60, RMA/S) (n=2 individual mice for each genotype). (E) IL-2-cultured NK cells were washed of IL-2 and stained in FACS buffer with NK cell surface markers (NK1.1 and CD3ε), along with cell surface proteins denoting different stages of NK cell maturation (CD27 and CD11b). Representative and compiled data are presented (n=3 individual mice for each genotype). (F) IL-2-cultured NK cells were washed of IL-2, counted, and stained with Ki67 to measure proliferation or Annexin V and Propidium Iodide to measure viability (n=3 individual mice for each genotype). Bar graphs present the mean ± SD or mean ± SEM, where multiple experiments are indicated. Statistical significance was calculated using Student's t-test. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.



Supplemental Figure 5. SR-18292 inhibits PGC-1α target gene transcription in NK cells, Related to Figure 4. (A) IL-2-cultured NK cells were collected, and RT-qPCR was used to quantify expression of individual genes following activation with NKG2D in the presence or absence of the PGC-1α inhibitor SR-18292 (30 μM) (n=4 individual mice). (B, C) IL-2-cultured NK cells from the spleens of WT mice were seeded in anti-NKG2D activating antibody coated wells in the presence or absence of indicated amounts of SR-18292. Cells were activated for 6 hours then collected and stained for Annexin V and Propidium Iodide along with NK cell surface markers. Cell viability was determined by measuring Annexin V/Propidium Iodide double-positive cells (n=3 individual mice). (D) IL-2-cultured NK cells from the spleens of WT and *Ppargc1a*-cKO mice were seeded in control or anti-NKG2D activating antibody coated wells for 6 hours in the presence or absence of the PGC-1α inhibitor SR-18292. Supernatants were collected and IFN-γ

secretion was measured via ELISA (n=3 individual mice per genotype). (E, F) Human NK cells were isolated from the PBMCs of healthy donors via negative selection and then seeded in anti-NKG2D activating antibody coated wells in the presence or absence of SR-18292. Cells were activated for 18 hours and stained for FACS analysis. Cell viability was determined by measuring Annexin V single-positive cells and Annexin V/Propidium Iodide double-positive cells (n=2 individual donors). Bar graphs present the mean ± SD or mean ± SEM where multiple experiments are indicated. Statistical significance was calculated using paired Two-way ANOVA corrected for multiple comparisons using the Holm-Sidak method (A). * = $p < 0.05$; *** = $p < 0.001$.



Supplemental Figure 6. Glucose uptake is maintained in PGC-1α-deficient NK cells, Related to Figure 5. (A-C) WT and *Ppargc1a* cKO mice were injected with 2×10^5 B16F10 melanoma cells. Ten days later, lungs were removed and lymphocytes were isolated and incubated with the glucose analog 2-NBDG for 1 hour. Cells were then stained with a live/dead indicator and NK cell surface markers, and 2-NBDG uptake was evaluated. Data were quantified both in terms of percent positive cells and on a per-cell basis as determined by mean fluorescence intensity, and 2-NBDG uptake by *Ppargc1a* cKO NK cells was established as a percentage of uptake by WT NK cells (n=1 individual mouse per genotype).

Supplemental Tables and Legends

Supplemental Table 1. GSEA results of WT NK cell transcriptome following *L. monocytogenes* infection, Related to Figure 1. RNA was collected from WT NK cells from control mice or mice infected with *L. monocytogenes* for 48 hours. RNA sequencing was performed, and changes in gene expression between control and infected NK cells were quantified. Competitive GSEA analysis was used to identify metabolism-associated gene sets whose genes were upregulated in NK cells collected from infected mice compared to control mice. Individual gene sets from the MSigDB c2, Hallmark, and Gene Ontology databases are shown below, along with the enrichment score, the direction of enrichment, and P-Value obtained from competitive enrichment analysis.

c2 Gene sets					
Gene list	NGenes	E.S	Direction	PValue	FDR
REACTOME_MITOCHONDRIAL _PROTEIN_IMPORT	67	2.3	Up	2.02E-07	2.36E-06
PENG_GLUTAMINE_DEPRIVATION_DN	451	1.8	Up	1.17E-06	1.13E-05
WONG_MITOCHONDRIA _GENE_MODULE	237	2.1	Up	0.005169884	0.020188589
MOOTHA_MITOCHONDRIA	492	1.7	Up	0.006424164	0.024187796
GALLUZZI_PERMEABILIZE _MITOCHONDRIA	55	1.6	Up	0.006540834	0.02460748
KEGG_OXIDATIVE_PHOSPHORYLATION	120	2.4	Up	0.007156613	0.026523215
REACTOME_TCA_CYCLE_AND_ RESPIRATORY_ELECTRON_TRANSPORT	129	2.3	Up	0.010105588	0.035478343
REACTOME_RESPIRATORY_ ELECTRON_TRANSPORT	76	2.5	Up	0.013731344	0.046086249
KEGG_ALANINE_ASPARTATE_ AND_GLUTAMATE_METABOLISM	28	1.8	Up	0.039166237	0.10972579
Hallmark Gene sets					
Gene list	NGenes	E.S.	Direction	P-Value	FDR
HALLMARK_INFLAMMATORY _RESPONSE	244	3.5	Up	2.83E-16	2.83E-15
HALLMARK_GLYCOLYSIS	239	2.1	Up	6.18E-07	2.21E-06
HALLMARK_OXIDATIVE_	227	2.3	Up	0.000437715	0.001094288

PHOSPHORYLATION					
GO Gene sets					
Gene list	NGenes	E.S.	Direction	P-value	FDR
GO_CYTOKINE_ACTIVITY	117	4.1	Up	2.92E-15	1.15E-12
GO_INNATE_IMMUNE_RESPONSE	620	2.7	Up	1.03E-13	3.16E-11
GO_REGULATION_OF_CYTOKINE_ SECRETION_INVOLVED_ IN_IMMUNE_RESPONSE	23	4.1	Up	9.37E-09	7.04E-07
GO_CYTOKINE_SECRETION	66	3.0	Up	4.03E-06	0.00011828
GO_INFLAMMATORY_RESPONSE	481	2.9	Up	6.31E-12	1.34E-09
GO_DEFENSE_RESPONSE_TO_BACTERIUM	206	3.0	Up	6.64E-11	1.11E-08
GO_POSITIVE_REGULATION_OF_ ACUTE_INFLAMMATORY_RESPONSE	24	4.9	Up	2.31E-10	3.03E-08
GO_POSITIVE_REGULATION_ OF_CYTOKINE_PRODUCTION	448	2.5	Up	2.36E-10	3.04E-08
GO_LONG_CHAIN_FATTY _ACID_BINDING	17	3.2	Up	0.000142391	0.002303669
GO_PYRUVATE_METABOLIC_PROCESS	53	2.0	Up	0.000187068	0.002897211
GO_MITOCHONDRIAL_ MEMBRANE_ORGANIZATION	119	1.6	Up	0.000211895	0.003201273
GO_RESPONSE_TO_LIPID	922	2.0	Up	0.000604139	0.007538282
GO_CELLULAR_RESPONSE _TO_FATTY_ACID	70	2.7	Up	0.001260774	0.013779102
GO_MITOCHONDRIAL_TRANSLATION	111	2.6	Up	0.001327153	0.01427674
GO_MITOCHONDRIAL_ TRANSMEMBRANE_TRANSPORT	69	2.1	Up	0.001351704	0.01444004
GO_MITOCHONDRIAL_TRANSPORT	212	1.5	Up	0.001662305	0.016936276
GO_PROTEIN_LOCALIZATION _TO_MITOCHONDRION	82	1.6	Up	0.002290085	0.022195097
GO_FATTY_ACID_HOMEOSTASIS	15	2.6	Up	0.002647074	0.024797207
GO_INNER_MITOCHONDRIAL_ MEMBRANE_PROTEIN_COMPLEX	104	2.7	Up	0.004361493	0.037081601
GO_POSITIVE_REGULATION_OF_ MITOCHONDRION_ORGANIZATION	182	1.3	Up	0.006238116	0.04842789
GO_PROTEIN_TARGETING	60	1.8	Up	0.007530304	0.055722439

_TO_MITOCHONDRION					
GO_POSITIVE_REGULATION _OF_LIPID_TRANSPORT	62	1.8	Up	0.010875769	0.07270959
GO_RESPONSE_TO_FATTY_ACID	102	2.2	Up	0.011618423	0.076758799
GO_GLUTAMINE_FAMILY_AMINO _ACID_METABOLIC_PROCESS	55	1.9	Up	0.01639513	0.096800364
GO_MITOCHONDRIAL _PROTEIN_COMPLEX	133	2.6	Up	0.016991254	0.098681359
GO_OXIDATIVE_PHOSPHORYLATION	81	2.5	Up	0.037989095	0.16934085

Abbreviates used: NGenes – number of genes; E.S. – enrichment score; FDR – false discovery rate.

Supplemental Table 2. List of primers used to evaluate gene expression, Related to Figure 1, Figure 5, Supplemental Figure 2, and Supplemental Figure 5.

Primers used for RT-PCR Analysis		
Gene	Forward Primer	Reverse Primer
Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
18s rRNA	CTCAACACGGGAAACCTCAC	CGCTCCACCAACTAAGAACG
Pdk1	TCCCCCGATTCAGGTTCAC	CCCGGTCACTCATCTTCACA
Pdk2	CACCGGACTCTAAGCCAGTT	ACGGGGTCATCTCCATAGGT
Pdk3	TCCTGGACTTCGGAAGGGATA	GAAGGGCGGTTCAACAAGTTA
Pdk4	ATCTAACATCGCCAGAATTAAACC	GGAACGTACACAATGTGGATTG
Cpt1a	CTCCGCCTGAGCCATGAAG	CACCAGTGATGATGCCATTCT
Cpt1b	TTGCCCTACAGCTGGCTCATTTCC	GCACCCAGATGATTGGGATACTGT
Acadm	GATCGCAATGGGTGCTTTTGATAGAA	AGCTGATTGGCAATGTCTCCAGCAAA
Lcad	TCTTTTCCTCGGAGCATGACA	GACCTCTCTACTCACTTCTCCAG
vLcad	GGCCAAGCTGGTGAAACACAAGAA	ACAGAACCACCACCATGGCATAGA
Glul	CAGAGACCAACTTGAGGCACA	CTCCATTCCAAACCAGGGGT
Got1	GCGCCTCCATCAGTCTTTG	ATTCATCTGTGCGGTACGCTC
Got2	TGCAGCTCCAAGGTTATCGC	CCACGGACGCTATCTCCTTC
Gpt1	AGGTCCTGGCCCTCTGTGTC	CCTTCTCTTGGCATCCTCTGG
Gpt2	AACCATTCACTGAGGTAATCCGA	GGGCTGTTTAGTAGGTTTGGGTA
Gls1	GGGAATTCACTTTTGTCACGA	GACTTCACCCTTTGATCACC
Gls2	TCAGGCATTCCGAAAGAAGTTT	CAGAAGGGGATCTTCGTGTGG
Cox4	GAGAGCCATTTCTACTTCGGT	GCAGACAGCATCGTGACAT
Tfam	CCAAAAAGACCTCGTTCAGC	ATGTCTCCGGATCGTTTCAC
Atp5g1	AGTTGGTGTGGCTGGATCA	GCTGCTTGAGAGATGGGTTC
Slc1a5	GGTCTCCTGGATTATGTGGTACG	AGCACAGAATGTATTTGCCGAG
Mfn2	AGAGGCAGTTTGAGGAGTGC	ATGATGAGACGAACGGCCTC
Ifng	ACAGCAAGGCGAAAAAGGATG	TGGTGGACCACTCGGATGA
Gzmb	CCACTCTCGACCCTACATGG	GGCCCCCAAAGTGACATTTATT
Ppargc1a	CCAACCAGTACAACAATGAGC	CTCCATCTGTCAGTGCATCA

Transparent Methods

Mice and cell lines. *Ppargc1a*$^{fl/fl}$ mice and wild type C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME), while *Ncr1*^{iCre} mice were a generous gift from Dr. Eric Vivier (Narni-Mancinelli et al., 2011). *Ppargc1a*$^{fl/fl}$ mice were bred with *Ncr1*^{iCre} mice to obtain *Ppargc1a*$^{fl/fl}$-*Ncr1*$^{WT/Cre}$ mice. These mice were bred to get littermate control mice (*Ppargc1a*$^{fl/fl}$-*Ncr1*$^{WT/WT}$) (WT) and experimental (*Ppargc1a*$^{fl/fl}$-*Ncr1*$^{WT/Cre}$) (*Ppargc1a*-cKO) mice in which PGC-1α is deleted in NK cells. Animals were housed at the Biological Resource Center in pathogen-free conditions at the Medical College of Wisconsin. Female littermate mice between the ages of 8 to 12 weeks were used. All animal protocols (AUA00001512) and the experiments carried out under them were approved and oversaw by the Institutional Animal Care and Use Committees (IACUC) of the Medical Collge of Wisconsin, Milwaukee, WI. Cell lines were purchased from ATCC (Rockville, MD) include EL4, RMA, RMA/S, and YAC-1 and were maintained in RPMI-1640 medium containing 10% heat-inactivated FBS (HyClone Laboratories, Logan, UT). *H60*-expressing EL4 stable cell lines were previously generated (Regunathan et al., 2005). Verification of RMA and RMA/s was performed through analysis of levels of MHC-Class I (H2-K^b and H2-D^b). The expression of H60 was used to confirm EL4-H60 cell lines. YAC-1 background was verified through lack of H-2^b and the presence of H-2^a. All cell lines were regularly tested and are negative for mycoplasma.

Media, antibodies and recombinant proteins. Culture media was RPMI 1640 from Gibco (Waltham, MA) supplemented with 10% heat-inactivated FBS (HyClone Laboratories, Logan, UT), penicillin/streptomycin (Corning, Corning NY), Pyruvate (Corning, Corning NY) and BME (Millipore-Sigma, St. Louis, MO). Chemical addition was included with the media, as indicated. Compound C was purchased from Millipore Sigma (St. Louis, MO). SR 18292 was purchased from Cayman Chemical (Ann Arbor, MI). CD3ε (17A2), NK1.1 (PK136), CD49b (DX5), CD27 (LG.7F9), CD11b (M1/70), KLRG1 (2F1), NCR1 (29A1.4), NKG2D (CX5), Ki67 (SolA15), NKG2D activating antibody (A10), NK1.1 activating antibody (PK136), Ly49H activating antibody (3D10), Human NKG2D (1D11), Ly49H (3D10), NKG2A/C/E (20d5), CD122 (5H4 or TM-b1), IFN-γ (XMG1.2), Mitotracker Green FM, TMRE, and LIVE/DEAD^{TM} Fixable Violet antibodies and dyes were from Thermo-Fisher Scientific (Waltham, MA); Ly49D (4E5) and CD132 (TUGm2), and 2B4 activating antibody were from Biolegend (San Diego, CA); Ly49A (A1), Ly49G2 (4D11), and Ly49C/I (5E6), were from BD Biosciences (Franklin Lakes, NJ). Recombinant IL-2 was provided by the NCI (NCI-BRB-Preclinical Repository, Maryland, and MD).

Recombinant IL-12 was from Peprotech (Rocky Hill, NJ), and IL-18 was from Thermo-Fisher Scientific (Waltham, MA).

Murine NK cell cultures. Cells from spleens of indicated mice were prepared by gently grinding the dissected organs with micro slides (VWR, Radnor, PA). Spleens were harvested from indicated mice and filtered through nylon wool columns to isolate lymphocyte populations. Cells were cultured in 1000 units IL-2 for four days at which point adherent NK cells were collected and re-cultured in 1000 units IL-2. These cells were then split an additional time on day five and cells were utilized on day 6 for hypoxia experiments while all other experiments were performed after seven days of culture. Cells undergoing this procedure are referred to as IL-2-cultured NK cells.

Human NK cell analyses. De-identified healthy human peripheral blood mononuclear cells (PBMC) were obtained from volunteer donors from Versiti (Milwaukee, WI) for the isolation of primary NK cells. Acquisition and utilization of human PBMCs was performed under an approved protocol (PRO00031019) from the Institutional Review Board (IRB) of the Medical College of Wisconsin and the Froedtert Hospital. Human NK cells were isolated from the peripheral blood of healthy donors. Samples were layered on top of Lymphoprep reagent (STEMCELL Technologies, Vancouver, Canada) at a 1:1 ratio, and gradient centrifugation was used to separate the lymphocyte layer. Lymphocytes were collected and washed, and NK cells were isolated using the human NK cell negative selection kit (STEMCELL Technologies, Vancouver, Canada). Cells were then rested overnight in cell culture media supplemented with 100 units of IL-2/mL. Cells were then washed and resuspended in 10 units of IL-2/mL and activated with plate-bound anti-human NKG2D antibody (10 μg/mL) ± SR-18292 or co-cultured with ^{51}Cr-labeled K562 target cells ± SR-18292. Supernatants were collected from NKG2D-stimulated cells, and a multiplex kit (Bio-Rad, Hercules, CA) was used to quantify cytokine/chemokine production. Supernatants were collected from K562-stimulated cells, and the amount of ^{51}Cr released into the media was used to quantify cell-mediated cytotoxicity. For Seahorse analysis, NK cells were freshly isolated and immediately activated with plate-bound anti-human NKG2D antibody (10 μg/mL) ± SR-18292 for 18 hours. A set of cells was then utilized for viability analysis by flow cytometry, while media was replaced with unbuffered RPMI on the second set of cells and mitochondrial parameters were quantified using a Seahorse instrument as described below.

Cell separation, flow cytometry, and cell sorting. Bone marrow cells were flushed and a single cell suspension was made by passing cells through a syringe and needle. Cells from spleens were prepared by gently grinding the dissected organs with micro slides (VWR, Radnor, PA). To collect lung NK cells, lungs were perfused with 10 mL of PBS and separated into lobes. Lung pieces were then digested and dissociated using the Miltenyi Lung Dissociation Kit and the Miltenyi GentleMACS Octo Dissociator (Miltenyi Biotec, Bergisch Gladbach, Germany). Lung-derived CD3ε$^-$NK1.1$^+$ NK cells were either analyzed from total lymphocyte populations for mitochondrial mass and membrane potential analyses or FACS-sorted (CD3ε$^-$NK1.1$^+$) and used for gene expression analyses. Freshly isolated or cultured NK cells used for flow cytometry were surface stained for twenty minutes in PBS containing 1% BSA (Millipore-Sigma, St. Louis, MO) and specified antibodies at 1:200. For cell viability analysis, human or mouse NK cells were collected following treatment under specified conditions and stained using the FITC-Annexin V Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ). To quantify proliferation, IL-2-cultured NK cells were washed and surface-stained for NK1.1 and CD3ε, and intracellularly-stained for Ki67 using the FoxP3/Transcription Factor Staining Buffer Set (Thermo-Fisher Scientific, Waltham, MA). Subsequent flow cytometry analyses were conducted with a MACSQuant Analyzer 10 (Miltenyi Biotec, Bergisch Gladbach, Germany) and analyzed with FlowJo software (FlowJo LLC, Ashland, OR). For cell isolation, NK cells were either isolated using a negative selection kit (STEMCELL Technologies, Vancouver, Canada) or through flow sorting with a FACSMelody Cell Sorter (BD Biosciences, Franklin Lakes, NJ).

B16F10 lung metastasis model. B16F10 melanoma cells growing in log phase were harvested and resuspended in PBS. 2×10^5 cells were injected into mice intravenously. 10-14 days post-injection as specified, the recipient mice were sacrificed. The lungs were perfused with 10 mL PBS and resected for subsequent imaging or *ex vivo* analysis. For nodule counts, lungs were placed in dishes with PBS and counts were performed blinded. Averages were obtained from two counts from two individuals. For H&E staining, lung sections were fixed in zinc-formalin. Samples were then frozen and sectioned for imaging.

***L. monocytogenes* infection.** Frozen stocks of the 10403S strain of *Listeria monocytogenes* were thawed and diluted in PBS and 2×10^4 CFU of bacteria were then injected into littermate WT and *Ppargc1a* cKO mice. 24-48 hours later, spleens were removed and NK cells were collected for RT-qPCR analysis. Livers were collected for CFU assays. Livers were resuspended in 5 mL RPMI and ground into single-cell suspensions using a tissue homogenizer. Serial dilutions of 1:5, 1:10, 1:50, and 1:100 were then made of

individual livers and dilutions were spotted in triplicate onto agar combined with Brain-Heart Infusion broth. Colonies were counted 24 hours later and reported numbers are averages of all 12 spots corrected for dilution factor.

Western Blotting. IL-2-cultured NK cells were lysed in ice-cold RIPA buffer (Boston BioProducts, Ashland, MA) with phosphatase inhibitor cocktail, PhosSTOP (Roche Diagnostics GmbH, Mannheim, Germany), and proteinase inhibitor cocktail (Millipore-Sigma, St Louis, MO). Lysates were incubated for 30 minutes on ice, centrifuged at 15,000 *g* for 10 min at 4°C. For Western blotting, cell lysates were separated by SDS-PAGE, transferred to PVDF membrane, and probed with specified primary antibodies followed by secondary Abs conjugated with horseradish peroxidase. The signal was detected by autoradiography films (LabScientific Inc., Livingston, NJ).

RT-PCR. Total RNA was extracted from *ex vivo* isolated or FACS-sorted cells using TRIzol Reagent (Thermo-Fisher Scientific, Waltham, MA). Total RNA was collected from IL-2-cultured NK cells using the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany). Reverse transcription was conducted using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). qPCR was performed with a QuantStudio 6 Flex Real-Time PCR instrument (Thermo-Fisher Scientific, Waltham, MA) with SYBR Green-based detection (MidSci). The transcript levels of β-Actin or 18s rRNA were used as a control. Primers used for the RT-PCR reactions in this study can be found in **Supplementary Table 2**.

RNA sequencing. RNA-Sequencing results were analyzed from a data set collected previously in the lab and published by Nanbakhsh et al. (Nanbakhsh et al., 2019). The methodology for collection and analytical techniques used in this study are detailed in the text and figure legends. Analyses include competitive GSEA to test for enrichment of gene sets in established databases including the Hallmark, MSigDB c2, and Gene Ontology databases, as well as our curated PGC-1α target gene set (**Table 2**). We also used the FRY variation of rotational gene set testing (Wu et al., 2010), to determine significance of enrichment of individual gene sets.

***In vitro* and *in vivo* cytotoxicity assays**. For *in vitro* cytotoxicity, EL4, EL4-H60, RMA, RMA/S, YAC-1, or B16F10 target cells were incubated with 50 μCui ^{51}Chromium for 1 hour. Following incubation, ^{51}Chromium (^{51}Cr)-labeled target cells were washed and co-cultured with IL-2-cultured NK cells at varied

effector to target (E:T) ratios in 96-well plates for 4 hours with or without SR18292 as indicated. Supernatants were then collected, and the amount of ^{51}Cr released from target cells was measured using a gamma counter. Percent specific lysis was calculated using amounts of absolute, spontaneous, and experimental ^{51}Cr-release from target cells. For *in vivo* cytotoxicity, splenocytes were collected from C57BL/6J (Self), B6.b2$^{mtm1Unc/J}$ (Missing Self), and BALB/cJ (Non-Self) mice and cells from all three mice were labeled with CFSE dye. Missing Self and Non-Self cells were also marked with Cell Trace Red (CTR) or Cell Trace Violet, respectively (all dyes from Thermo-Fisher Scientific, Waltham, MA). Cells from the three backgrounds were then combined in a 1:1:1 ratio, and 7.5×10^6 cells of the mixture were injected intravenously into either experimental mice or mice injected with NK cell depleting or IgG control antibodies 24 hours prior. Eighteen hours later, splenocytes were harvested from injected mice and the percentages of labeled cells recovered were obtained via flow cytometry. Deviation from the original 1:1:1 ratio was used to determine the killing of missing-self and non-self target cells.

Cytokine production measurements. IL-2-cultured NK cells were harvested and 2.5×10^5 cells were stimulated with plate-bound anti-NKG2D (2.5 μg/mL) antibody (ThermoFisher) or IL-12 and 18 (1 and 10 ng/mL, respectively) in 96-well plate for 6 hours. Activation was performed in the presence or absence of chemical supplementation as indicated. After 6 hours, supernatants were collected, and amount of cytokines released into the supernatant was measured using ELISA or the multiplex platform from Bio-Rad (Bio-Rad, Hercules, CA). For hypoxia experiments, cell cultures were divided on Day 6, and 3×10^6 cells were placed in flasks in 1% O2 while the remaining cells remained in normoxic conditions. Twenty-four hours later, cells from both conditions were collected and activated in normoxic conditions with plate-bound anti-NKG2D activating antibody (2.5 μg/mL) for 6 hours, and cytokine/chemokine release was measured using a multiplex kit (Bio-Rad, Hercules, CA). For *ex vivo* evaluation of IFN-γ production, NK cells were isolated from spleens using a negative selection kit (STEMCELL Technologies, Vancouver, Canada) and seeded onto anti-NKG2D activating antibody coated plates for 6 hours. Cells were then harvested and stained with surface antibodies and fluorochrome-labeled IFN-γ (XMG1.2) using the BD Cytofix/Cytoperm Kit (BD Biosciences, Franklin Lakes, NJ).

Mitochondrial mass and membrane potential quantification. Mitochondrial mass and membrane potential were quantified using Mitotracker Green FM and TMRE, respectively. Lung lymphocytes were collected as described and resuspended in culture media containing Mitotracker Green FM and TMRE (50

nM) for 25 minutes. NK1.1 and CD3ε surface antibodies along with LIVE/DEAD^{TM} Fixable Violet were then added to cells at 1:200 or 1:1000 respectively, and cells were stained for an additional ten minutes. Cells were washed twice and resuspended with PBS containing 1% BSA for subsequent analysis.

Seahorse measurements. The bioenergetic function of murine and human NK cells was measured using a Seahorse XF-96 Extracellular Flux Analyzer (Agilent, Santa Clara, CA). 1×10^5 cells were seeded in V3 PET Cell Culture Microplates with unbuffered RPMI pH adjusted to 7.4. Basal levels of oxygen consumption and extracellular acidification were obtained to determine basal OxPhos and glycolytic rates. To identify additional mitochondrial parameters, injection with the ATP-Synthase inhibitor oligomycin (1 μg/mL), the mitochondrial uncoupler carbonylcyanide p-(trifluoromethoxy) phenylhydrazone (FCCP; 3 μmol/L), and the complex III inhibitor Antimycin A (10 μmol/L), were used to measure maximal and ATP-linked oxygen consumption rates. Maximal glycolysis was determined after oligomycin addition.

HPLC metabolite analysis. Metabolite analysis was performed on IL-2-cultured NK cells. HPLC analysis was performed using an Agilent 1100 chromatograph and variable wavelength detector. Following 6-hour culture with or without plate-bound anti-NKG2D activating antibody (2.5 μg/mL), metabolites were extracted in a potassium phosphate buffer for oxidized species or a potassium hydroxide buffer for reduced species. Samples were resuspended in appropriate buffers with tetrabutylammonium bisulfate as an ion-pairing reagent. Samples were then injected onto a Kinetix C18 column (100 × 4.6 mm, 2.6 μM particle size) (Phenomenex, Torrence, CA), and reverse-phase chromatography was performed by eluting samples with a methanol mobile phase using a gradient method. Metabolites were detected using a variable wavelength detector to measure absorbance at 254 nm. Concentrations and ratios of various metabolites were then determined through peak integration performed by Agilent ChemStation software, followed by comparison to values obtained for known standards of each metabolite. ATP, ADP, NAD, and NADH standards were obtained from Millipore-Sigma (St. Louis, MO).

Statistics. Data are presented as Mean ± SD or as Mean ± SEM when multiple experiments are represented. Statistical analyses were conducted using Prism software (GraphPad, La Jolla, CA). Depending on the number of parameters being evaulated, statistical significance was calculated using Student's t-tests corrected for multiple comparisons using the Holm-Sidak method where indicated or two-

way ANOVA corrected for multiple comparisons using the Holm-Sidak method where indicated. The significance is indicated as *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.