Supporting Information

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SI Materials and Methods

NK Cell Stimulation and Flow Cytometry. Splenocytes were stimulated with plate-bound anti-NK1.1 or anti-NKp46 or PMA/ionomycin for 5–6 h or incubated with IL-15 for 24 h before analysis of CD107a for NK cell degranulation or intracellular staining of IFNγ, granzyme B, and perforin. Surface and intracellular staining were performed as previously described (1). The following antibodies were used: NK1.1, CD3, TCRβ, CD45.1, CD45.2, Ly49H, Ly49D, Ly49F, Ly49G2, Ly49A, Ly49CI, NKG2A, KLRG1, NKp46, NKG2D, CD122, CD11b, CD43, CD27, B220, CD61, CD51, CD44, annexin V, Ki67, CD107a, IFNγ, granzyme B, perforin, T-bet, Eomes, and phospho-mTOR (p-mTOR) (Ser2448) (eBioscience, BD, or Cell Signaling). Cells were acquired on a BD FACSAria using FACSDIva software (BD Biosciences) and analyzed with FlowJo software (Tristar).

Adoptive Transfer, Lentiviral Transduction, and MCMV Infection. Sorted NK1.1$^+$ CD3$^-$ NK cells (>98%) were injected into *Rag2*$^{-/-}\gamma C^{-/-}$ mice intravenously. CFSE labeling of cells was performed according to the manufacturer's instructions (Invitrogen). In Fig. 4*D*, 5×10^4 pfu of a salivary gland stock of MCMV (Smith strain) was injected intraperitoneally. For in vivo reconstitution of OPN-i expression, in vitro cultured HSCs (Lin$^-$Scal1$^+$cKit$^+$) were infected with the indicated GFP-expressing lentiviral vectors for 2 d before i.v. injection of sorted GFP$^+$ cells into sublethally irradiated (550 rads) *Rag2*$^{-/-}\gamma C^{-/-}$ hosts. Viral particles were propagated by infection of 293T cells followed by concentration and titering for optimal multiplicity of infection in the infection assays that follow.

In Vitro and in Vivo Cytotoxicity Assays. In vitro cytotoxic assays were performed as previously described (1, 2). Following administration of sorted NK cells (2.5×10^5), *Rag2*$^{-/-}\gamma C^{-/-}$ mice were injected i.v. with (5×10^4) B16F10 melanoma cells on day 10 before enumeration of lung metastasis nodules on day 25, as described previously (3).

Analysis of *Spp1* mRNA and OPN Protein Expression. Quantitative real-time PCR was performed and analyzed as described (4) with the TaqMan gene expression assays [*Spp1* (Mm00436767_m1) and *Rps18* (Mm02601777_g1)]. Western analysis of OPN and actin protein levels was performed as described previously (5). Band intensity was quantified using ImageJ software, version 1.45b (National Institutes of Health).

Microarray. Splenic NK cells were sort purified (>95%) 10 d posttransfer into *Rag2*$^{-/-}\gamma C^{-/-}$ mice. RNA was prepared using RNeasy Plus Micro kit (Qiagen) before amplifying, labeling, and hybridizing to MOA430 2.0 chips (Affymetrix) at the Dana-Farber Cancer Institute Microarray Core Facility.

1. Leavenworth JW, et al. (2010) Analysis of the cellular mechanism underlying inhibition of EAE after treatment with anti-NKG2A F(ab′)2. *Proc Natl Acad Sci USA* 107(6): 2562–2567.
2. Leavenworth JW, Wang X, Wenander CS, Spee P, Cantor H (2011) Mobilization of natural killer cells inhibits development of collagen-induced arthritis. *Proc Natl Acad Sci USA* 108(35):14584–14589.
3. Werneck MB, Lugo-Villarino G, Hwang ES, Cantor H, Glimcher LH (2008) T-bet plays a key role in NK-mediated control of melanoma metastatic disease. *J Immunol* 180(12): 8004–8010.
4. Leavenworth JW, Verbinnen B, Yin J, Huang H, Cantor H (2015) A p85α–osteopontin axis couples the receptor ICOS to sustained Bcl-6 expression by follicular helper and regulatory T cells. *Nat Immunol* 16:96–106.
5. Shinohara ML, et al. (2006) Osteopontin expression is essential for interferon-alpha production by plasmacytoid dendritic cells. *Nat Immunol* 7(5):498–506.

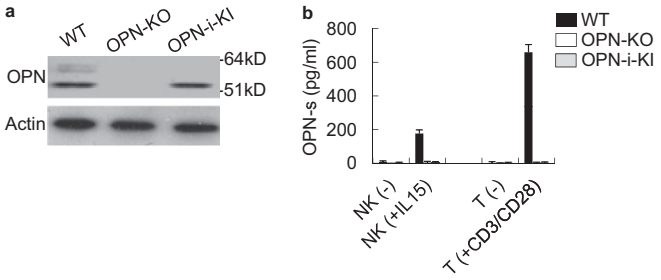


Fig. S1. Analysis of OPN protein expression in OPN-i-KI cells. (*A*) Purified NK cells from the indicated mouse strains were cultured with IL-15 (100 ng/mL) for 24 h. Western analysis of OPN and actin expression is shown. (*B*) Secreted OPN protein measured by ELISA from supernatants of purified NK and T cells from each mouse strain after stimulation with (+) or without (–) indicated reagents for 24 h.

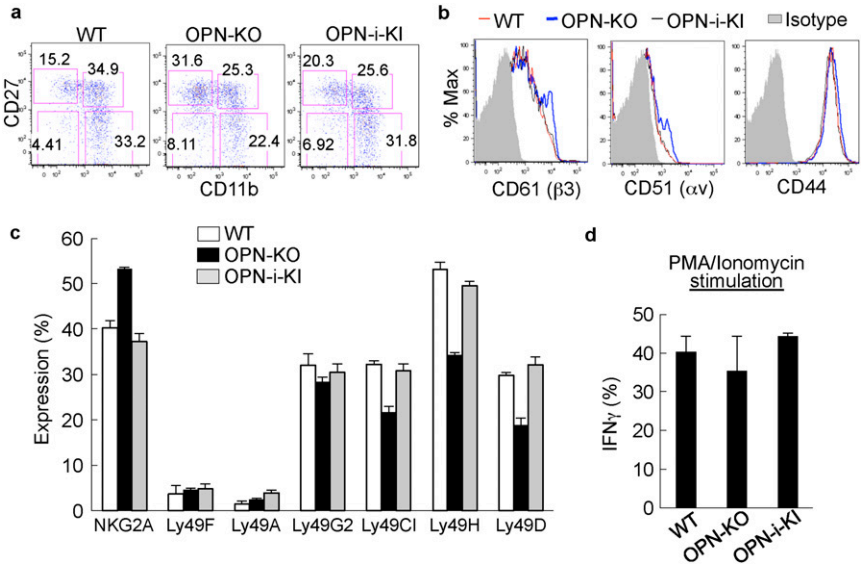


Fig. S2. Reduced mature NK cells from OPN-i–deficient mice. (*A*) Representative FACS plots show splenic NK ($NK1.1^+TCR\beta^-$) subsets from the indicated mouse stains defined according to surface expression of CD11b and CD27. (*B*) Histogram overlays of CD61, CD51, and CD44 expression are shown. (*C*) Percent of NK cells expressing the indicated NKG2A and Ly49 receptors in naïve WT, OPN-KO, and OPN-i–KI mice ($n = 3$ mice per group). (*D*) NK cells from the indicated mice were stimulated with PMA and ionomycin for 5 h. Percents of NK cells expressing IFNγ are shown. Bars indicate mean ± SEM.

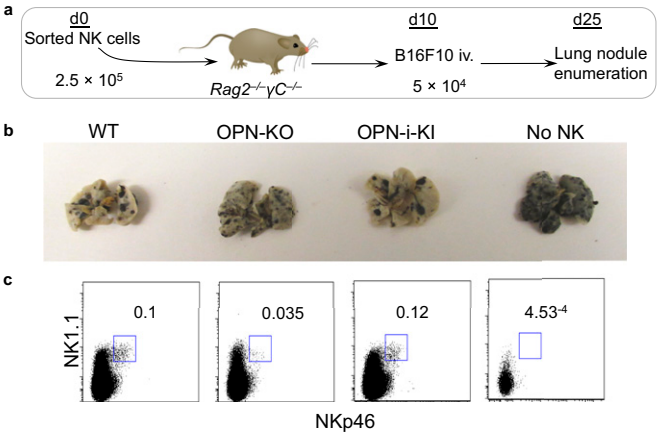


Fig. S3. OPN-i–expressing NK cells prevent B16F10 melanoma metastasis. (*A*) Schematic of NK cell transfer and B16F10 melanoma model of tumor metastasis. Sorted NK cells from indicated mice were transferred into $Rag2^{-/-}\gamma C^{-/-}$ mice followed by inoculation of B16F10 melanoma cells i.v. 10 d posttransfer. At 15 d after tumor challenge, recipient mice were killed and the lungs dissected. (*B*) Representative pictures of lungs are shown. (*C*) FACS plots show splenic NK cells ($NK1.1^+NKp46^+$) from these mice.

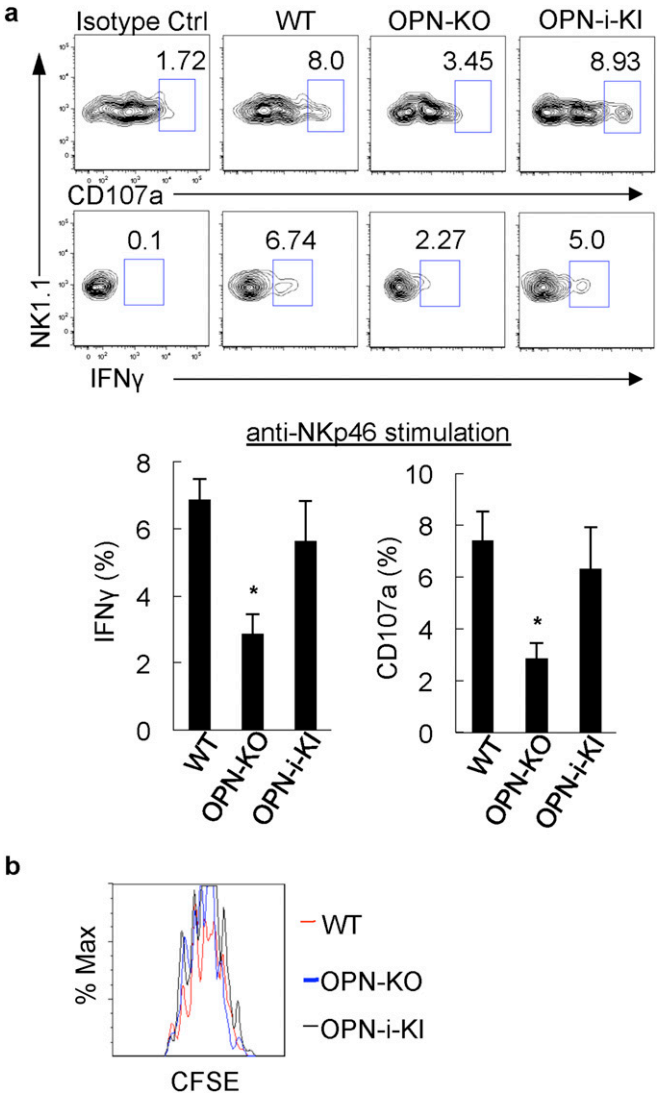


Fig. S4. OPN-i–deficient NK cells proliferate normally but display impaired activity. (*A*) NK cells from WT, OPN-KO, and OPN-i–KI mice were labeled with CFSE before transfer into *Rag2*$^{-/-}\gamma C^{-/-}$ hosts. Cells recovered 3 d later from spleen were stimulated for 5 h with plate-bound anti-NKp46 antibody followed by flow cytometry analysis of CD107a and IFNγ expression. Representative plots show expression of CD107a and IFNγ in NK (NK1.1^{+}CD3^{-}) cells. Percent of CD107a and IFNγ-expressing NK cells (n = 3 mice per group) are graphed. Values are shown as mean ± SEM. (*B*) NK cells from *A* were analyzed for CFSE levels and representative histogram overlay is shown.

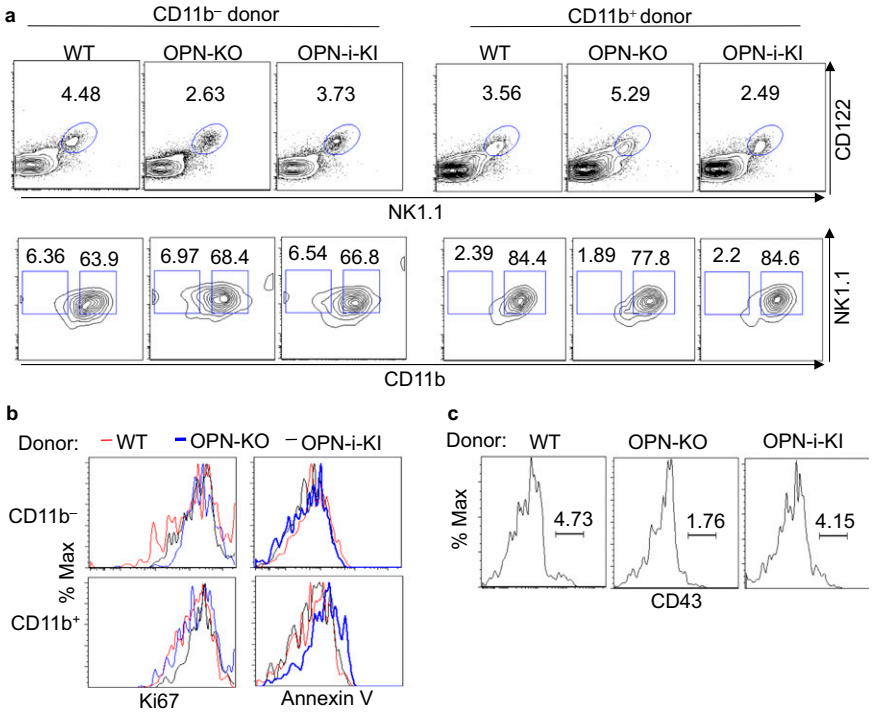
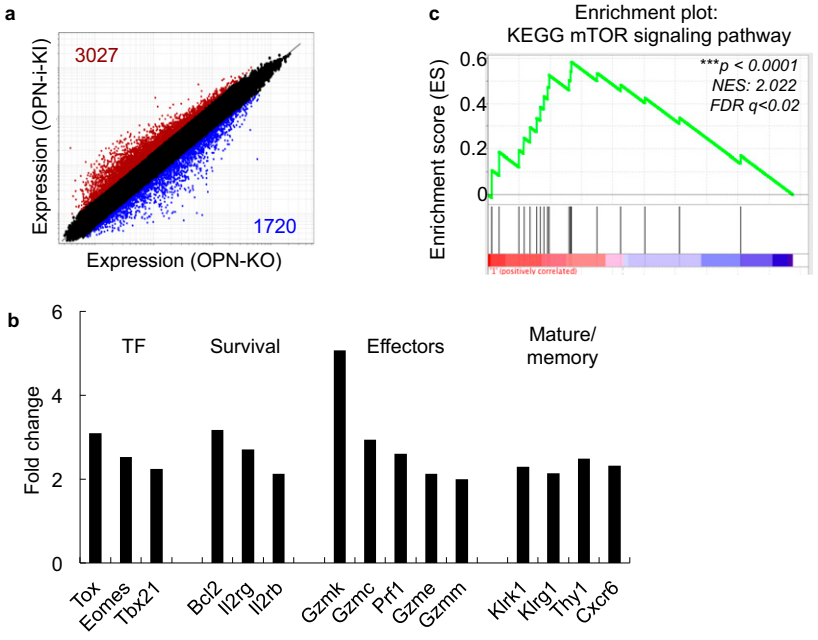


Fig. S5. Analysis of NK cell homeostatic expansion at day 8 after transfer into *Rag2*$^{-/-}\gamma C^{-/-}$ hosts. (*A*) Sorted CD11b^{-} immature and CD11b^{+} mature NK cells from WT, OPN-KO, and OPN-i–KI mice were transferred into *Rag2*$^{-/-}\gamma C^{-/-}$ hosts, separately. FACS plots show percent of NK1.1^{+}CD122^{+} splenic NK cells and CD11b expression in NK cells at day 8 after transfer. (*B*) Histogram overlays show expression of Ki67 and annexin V on transferred NK cells (as in *A*) from WT (red line), OPN-KO (blue line), and OPN-i–KI (black line) mice. (*C*) Expression of CD43 in NK cells at day 8 after transfer of CD11b^{-} immature NK cells from the indicated mice in *A*.



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Genes upregulated in OPN-i-KI NK cells

KEGG pathway	No. of genes	% of input	P Value	Fold Enrichment	FDR
mmu04660:T cell receptor signaling pathway	29	1.258	3.94E-07	2.872	4.84E-04
mmu04910:Insulin signaling pathway	31	1.345	1.14E-06	2.625	0.0014
mmu04650:Natural killer cell mediated cytotoxicity	28	1.215	2.74E-06	2.682	0.0034
mmu04150:mTOR signaling pathway	16	0.694	2.86E-05	3.463	0.0351
mmu05220:Chronic myeloid leukemia	19	0.824	4.95E-05	2.922	0.0608
mmu05200:Pathways in cancer	49	2.126	7.68E-05	1.773	0.0942

Fig. S6. Microarray analysis of NK cells at day 10 after transfer into *Rag2*$^{-/-}\gamma C^{-/-}$ hosts. (*A*) Multiplot of genes expressed in sorted OPN-i–NK cells relative to OPN-KO NK cells from *Rag2*$^{-/-}\gamma C^{-/-}$ hosts 10 d after transfer (as in Fig. 4*A*). Genes up-regulated (red) and down-regulated (blue) in OPN-i–KI NK cells with a twofold difference are indicated. (*B*) Expression of transcripts encoding Eomes and T-bet TF and associated with NK cell survival, effector function, maturation, and memory formation in OPN-i–KI NK cells relative to their expression in OPN-KO NK cells, both sorted from *Rag2*$^{-/-}\gamma C^{-/-}$ hosts at day 10 posttransfer. (*C*) GSEA of genes up-regulated in OPN-i–KI NK cells (denoted as red in *A*). Upward deflection of the green line indicates enrichment of the mTOR pathway signature within the OPN-i–KI NK population ($P < 0.0001$). (*D*) List of KEGG pathways enriched from genes up-regulated in transferred OPN-i–KI cells (denoted as red in *A*). No values are given when the FDR was above 0.1. Table was generated using NIAID DAVID and KEGG databases. Genes associated with T-cell receptor pathway are not T-cell specific. FDR, false discovery rate; NES, normalized enrichment score.