SUPPLEMENTAL MATERIAL

Ebihara et al., http://www.jem.org/cgi/content/full/jem.20091573/DC1

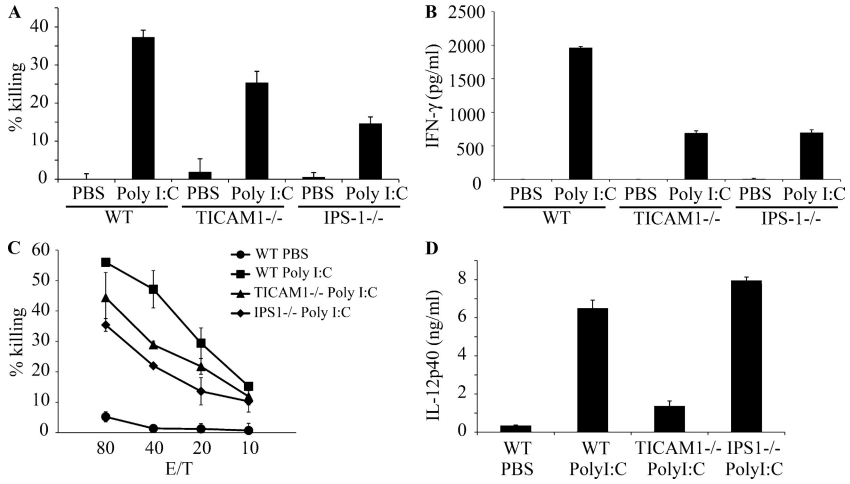

Figure S1. KO mice results suggest that both IPS-1 and TICAM-1 in BMDC participate in polyI:C-driven NK activation. (A and B) IPS-1 and TICAM-1 in BMDC participate in polyI:C-driven NK activation. 2.5 × 10^5 BMDCs prepared from WT, TICAM1$^{-/-}$, and IPS1$^{-/-}$ mice were incubated with 5 × 10^5 NK cells in the presence or absence (PBS) of 50 µg/ml polyI:C for 24 h. Then, the supernatants were harvested for IFN-γ ELISA (B). To determine NK cytotoxicity, ^{51}Cr-labeled B16D8 cells were added to the culture and, 4 h later, released ^{51}Cr was measured (A). One representative of three similar experiments is shown. (C) Both IPS-1 and TICAM-1 participate in in vivo polyI:C-induced NK activation. WT, IPS-1$^{-/-}$, and TICAM-1$^{-/-}$ mice were i.p. injected with 250 µg polyI:C. After 24 h, NK cells were harvested by DX5-MACS beads from spleen and used as effector cells in a cytotoxic assay with ^{51}Cr-labeled B16D8 targets. Cytotoxic activity of NK cells was measured under the indicated E/T ratios 4 h after the E/T mixing. One representative of the three similar experiments is shown. (D) Increasing serum level of IL-12p40 is dependent on TICAM-1. 250 µg polyI:C was i.p. injected into a series of mice as in B. 8 h after injection of polyI:C, blood serum was collected to determine the levels of IL-12p40 by ELISA. Although it is not depicted, IL-12p70 was not detected in these samples by ELISA. Data in A–D represent mean ± SD.

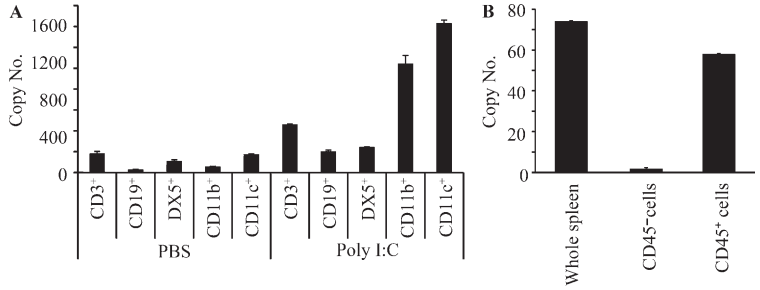

Figure S2. In vivo polyI:C response of INAM in LN cells. (A) Up-regulation of INAM expression in LN cells by polyI:C injection. WT C57BL/6 mice were i.p. injected with 100 µg polyI:C or control buffer. After 24 h, inguinal, axillary, and mesenteric LN were harvested. Cell populations with indicated markers were separated by FACS sorting, and the INAM mRNA level of each population was determined by real-time PCR. (B) CD45$^+$ cells express INAM. Splenocytes were separated into CD45$^-$ and CD45$^+$ cells after the polyI:C injection as in A. The INAM mRNA levels of the two populations were determined by real-time PCR. Representative data from one of three experiments are shown. Data in A and B represent mean ± SD.

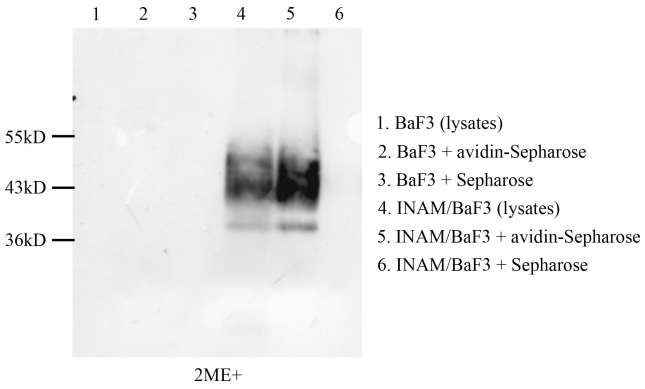

Figure S3. INAM is expressed on cell surface. Membrane proteins of Flag-tagged INAM-expressing BaF3 (INAM/BaF3) and control BaF3 were biotinylated and solubilized. Biotinylated proteins were immunoprecipitated by Avidin-Sepharose or control Sepharose. After electrophoresis on SDS-PAGE, INAM was detected by anti-Flag M2 mAb.

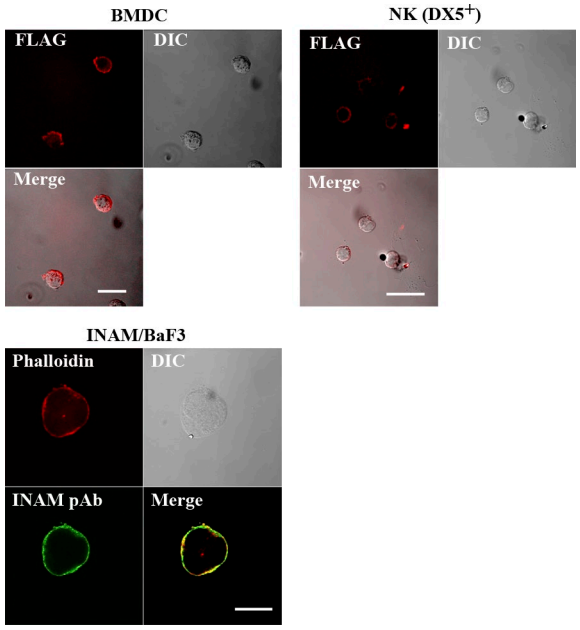

Figure S4. Confocal analysis of surface-expressed INAM. WT BMDC (left) or NK cells (right) were infected with INAM-expressing vector and stained with anti-FLAG mAb (Alexa Fluor 568). Stable Ba/F3 transfectants expressing INAM (bottom) were permeabilized and stained with phalloidin and anti-INAM pAb, followed by Alexa Fluor 488–conjugated secondary antibody. Cells were analyzed on a confocal laser-scanning microscope (LSM 510 META). Bars, 20 µm.

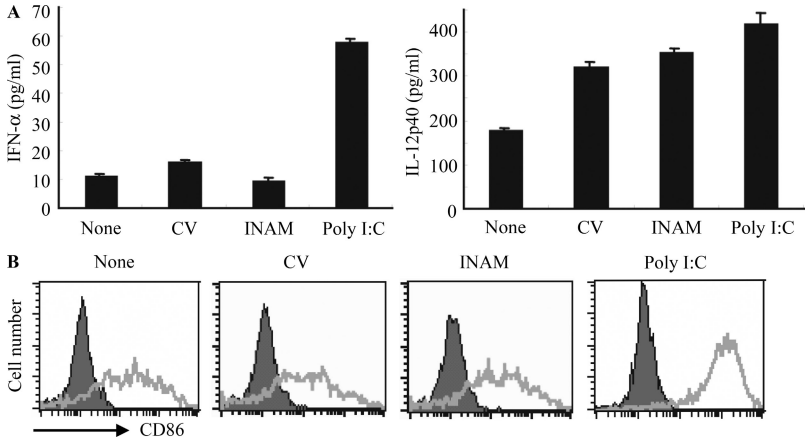

Figure S5. INAM-overexpressing BMDC did not induce cytokine responses and maturation. WT BMDCs were transfected with control lentivirus (CV) or INAM-expressing lentivirus (INAM-virus) and cultured for 24 h. (A) ELISA of IFN-α and IL-12p40 in the culture supernatants. Data shown are means ± SD of triplicate samples from one experiment representative of three. (B) Flow cytometry for CD86 in the transfected BMDC. PolyI:C stimulation (10 μg/ml) was used for positive control.

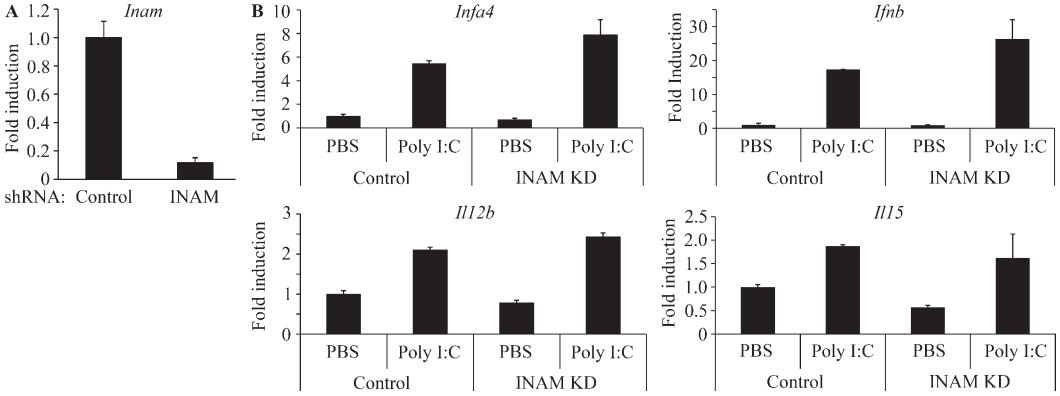

Figure S6. The effect of gene silencing of INAM on the polyI:C-mediated cytokine inducing profile in BMDC. (A) Gene silencing of INAM in BMDC. 5×10^5 WT BMDCs were infected with INAM shRNA-generating lentivirus or control lentivirus. After 36 h, the levels of INAM mRNA expression were assessed by real time PCR. Data show one of three similar experiments. (B) Effect of BMDC INAM on cytokine expression. INAM in 5×10^5 WT BMDCs was silenced as in A. Then, control or INAM-silenced BMDC were stimulated with 10 μg/ml polyI:C for 8 h. RNA was harvested from BMDC with RNeasy and the levels of indicated mRNA were determined by real-time PCR. Data show one of two similar experimental results. Data in A and B represent mean ± SD.

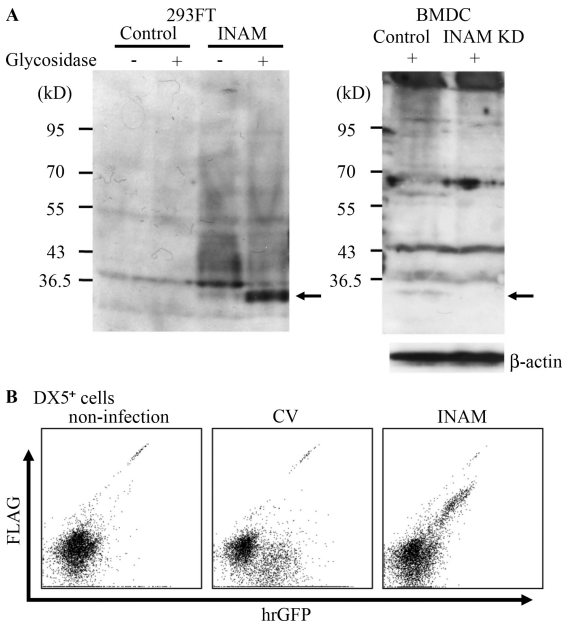

Figure S7. Detection of the INAM protein in DCs and NK cells. (A) Detection of the endogenous INAM protein in BMDC. 5×10^6 BMDCs were transduced with INAM-shRNA or control shRNA–expressing lentivirus. 48 h later, these cells were lysed and treated with *N*-glycosidase F for 2 h at 37°C. All cell lysates were subjected to SDS-PAGE and immunoblotted by rabbit anti-INAM pAb. The cell lysates from 293FT cells transfected with pEFBOS or pEFBOS/INAM were used as negative and positive control, respectively. Arrows indicate the band for INAM. Mr markers are shown to the left. One of three similar experiments is shown. (B) $DX5^+$ NK cells express GFP and FLAG, markers for INAM. 5×10^5 $DX5^+$ cells were transduced with control or INAM-expressing lentivirus for 48 h. Then, these cells were permeabilized and stained with rabbit anti-FLAG pAb and PE-anti rabbit IgG. Levels of FLAG and hrGFP, reflecting INAM expression, were measured by FACSCalibur. Experiments were performed more than six times with different conditions and representative data are shown.

Table S1. TICAM-1-inducible genes encoding putative membrane or GPI-anchored proteins

Official symbol	Other aliases	UniGene ID	Fold induction (poly I:C stimulation/nonstimulation)			
			WT	MyD88$^{-/-}$	TLR3$^{-/-}$	TICAM-1$^{-/-}$
Aplnr	APJ, Agtrl1, msr/apj	Mm.29368	2.074101377	0.79485698	0.24913528	0.296911294
Fam26f	INAM, A630077B13Rik	Mm.34479	15.57360865	8.048081457	0.939239821	1.221297574
Clec4e	Clecsf9, Mincle	Mm.248327	5.65851862	7.142025946	2.761541794	2.087684899
Ly6i	Ly-6M, AI789751	Mm.358339	5.679941154	26.36364231	0.734513568	1.09611157
Slamf8	Blame, SBBI42	Mm.179812	6.814581008	5.127202394	1.802731559	1.122849288
Tmem171	Gm905, MGC117733	Mm.28264	12.42279971	7.454421156	2.274145126	3.051240138
Pvrl4	1200017F15Rik, Prr4	Mm.263414	5.02297837	4.096701442	1.627391239	1.961829994
Vcam1	CD106	Mm.76649	4.742423155	4.572993249	0.948952117	0.554171652
Tnfsf10	APO-2L, TL2, Trail	Mm.1062	41.9745751	30.22262268	6.007858781	2.631939934