Web Fig. 1. Cell surface expression of NKG2D on cell populations from KARAP-mutant and KARAP-Tg mice. The indicated cell populations were analyzed by flow cytometry for cell surface expression of NKG2D by staining with a mAb. The histograms show electronic gating on the respective cell populations. The MFI of the gated positive cells is indicated above each histogram. (a) Analysis of NKG2D cell surface expression in homozygous KARAP-mutant mice (/), heterozygotes (+/) or wild-type littermates (+/+). (b) Analysis of CD8+ T cells from KARAP-Tg mice and nontransgenic littermates. ND, not done; NS, not stimulated.

Web Fig. 2. NKG2D-dependent NK cell activation in the absence of KARAP. (a) Freshly isolated NK cells from poly(IC)-treated KARAP-mutant mice (/, open bars) or wild-type littermates (+/+, solid bars) were stimulated with RMA lymphoma cells transduced or not with the NKG2D ligands Rae-1 or H-60 (left panel) or with the indicated plate-bound antibodies (right panel). Accumulation of IFN- was evaluated by intracellular cytokine staining. A representative experiment is shown ($n = 3$). (b) The cytotoxicity of freshly isolated NK cells from poly(IC)-treated KARAP-mutant mice (/, open squares) or wild-type littermates (+/+, closed squares) against RMA lymphoma cells transfected or not with Rae-1 or H-60 as indicated. The effector cells were incubated with a control antibody (upper panels) or a mAb to NKG2D (lower panels). A representative experiment is shown ($n = 4$).

Fig 1

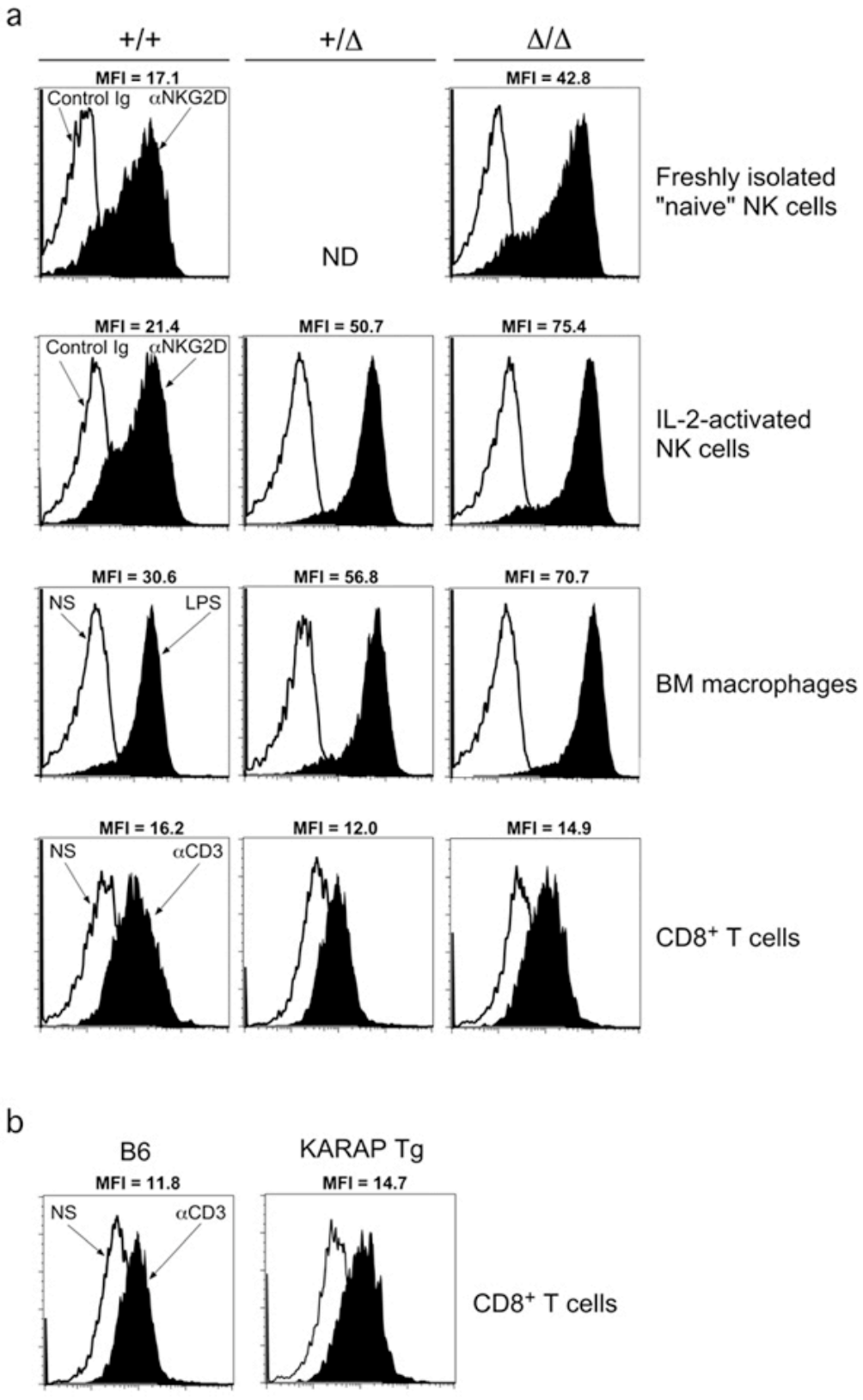
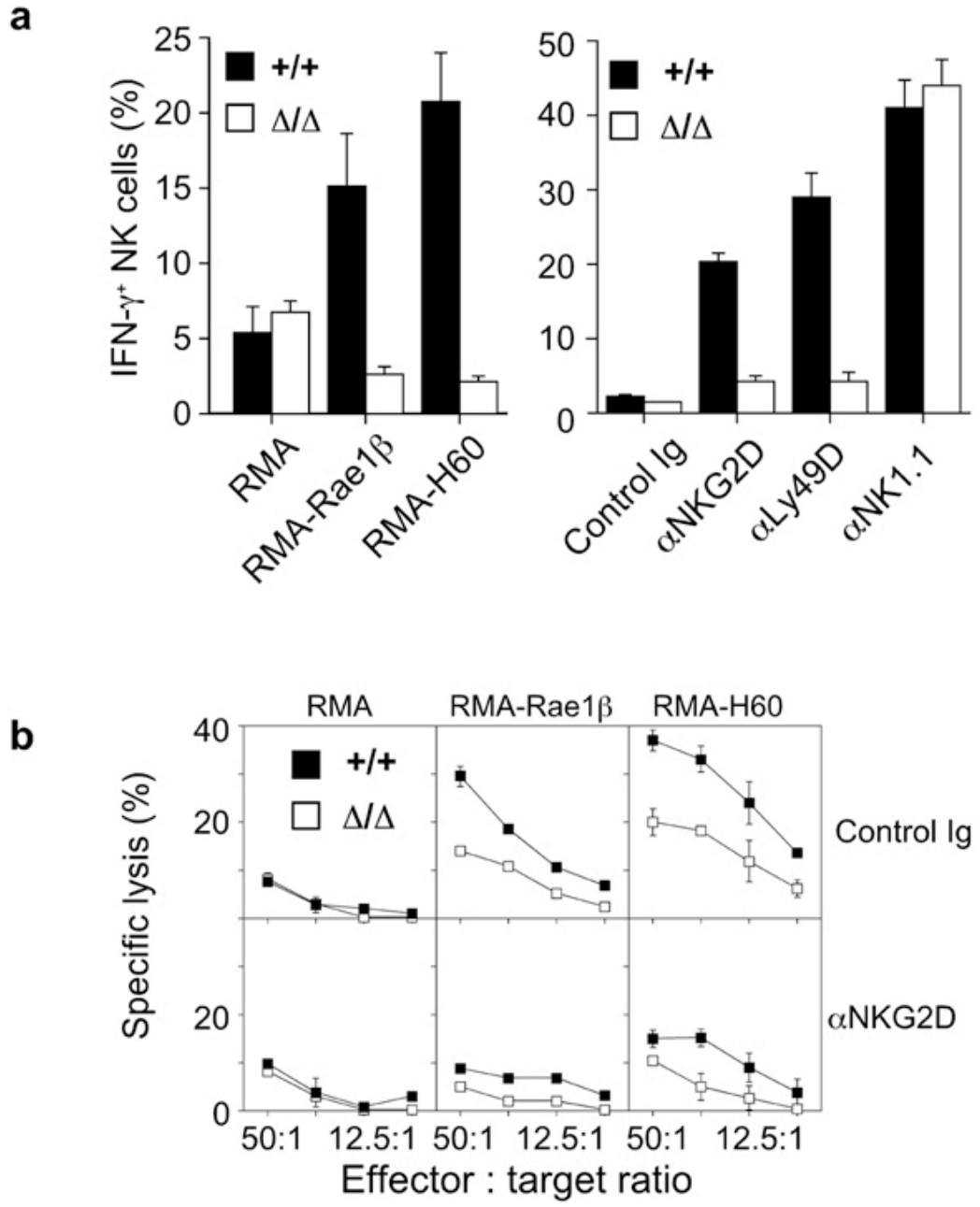

a
+/+
+/Δ
Δ/Δ
MFI = 17.1
Control Ig
αNKG2D
MFI = 42.8
Freshly isolated "naive" NK cells
ND
MFI = 21.4
Control Ig
αNKG2D
MFI = 50.7
MFI = 75.4
IL-2-activated NK cells
MFI = 30.6
NS
LPS
MFI = 56.8
MFI = 70.7
BM macrophages
MFI = 16.2
NS
αCD3
MFI = 12.0
MFI = 14.9
CD8+ T cells
b
B6
KARAP Tg
MFI = 11.8
NS
αCD3
MFI = 14.7
CD8+ T cells

Fig 2


a
IFN-γ+ NK cells (%)
+/+
Δ/Δ
RMA
RMA-Rae1β
RMA-H60
Control Ig
αNKG2D
αLy49D
αNK1.1
b
RMA
RMA-Rae1β
RMA-H60
Specific lysis (%)
Control Ig
αNKG2D
50:1
12.5:1
Effector : target ratio