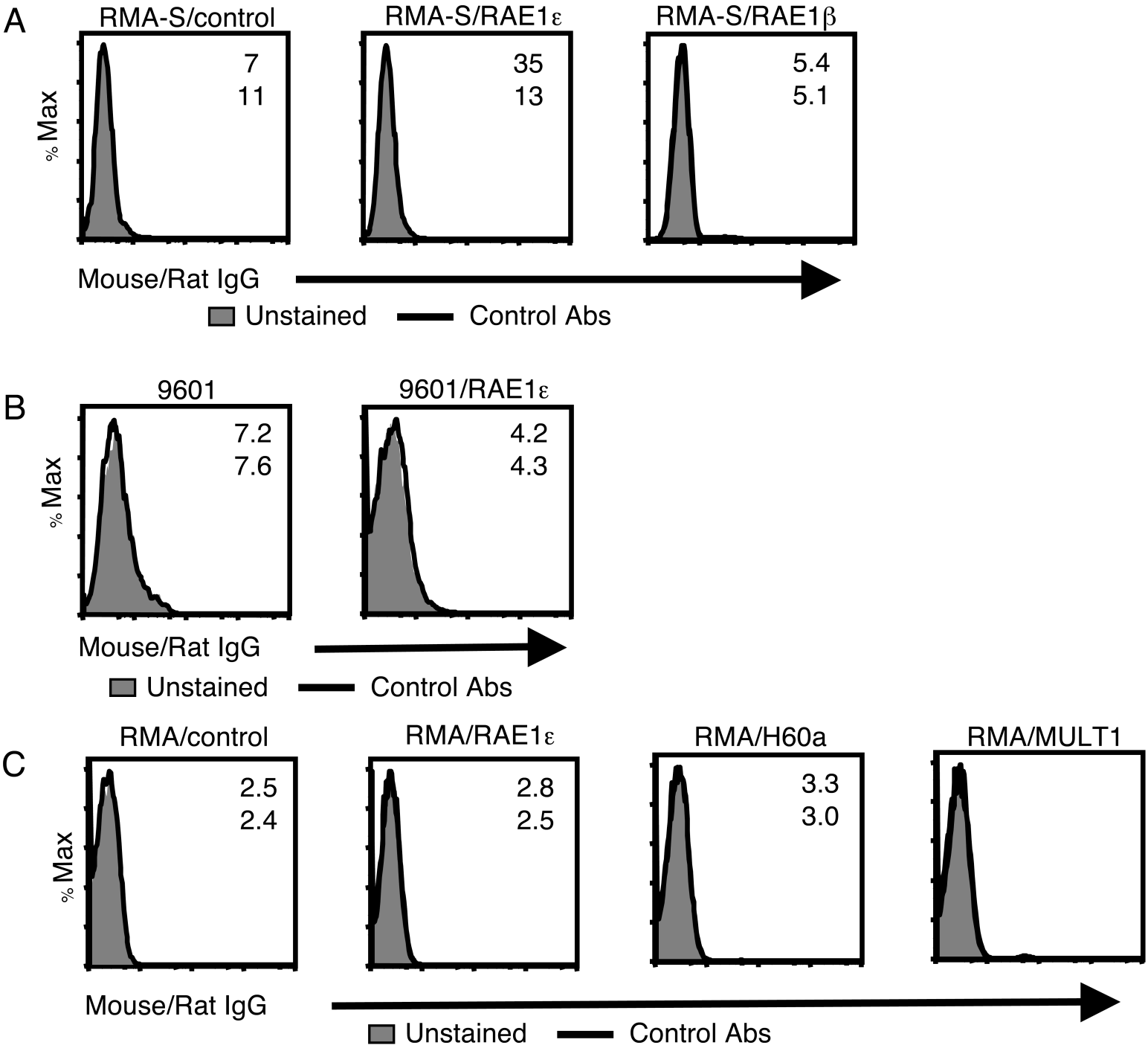
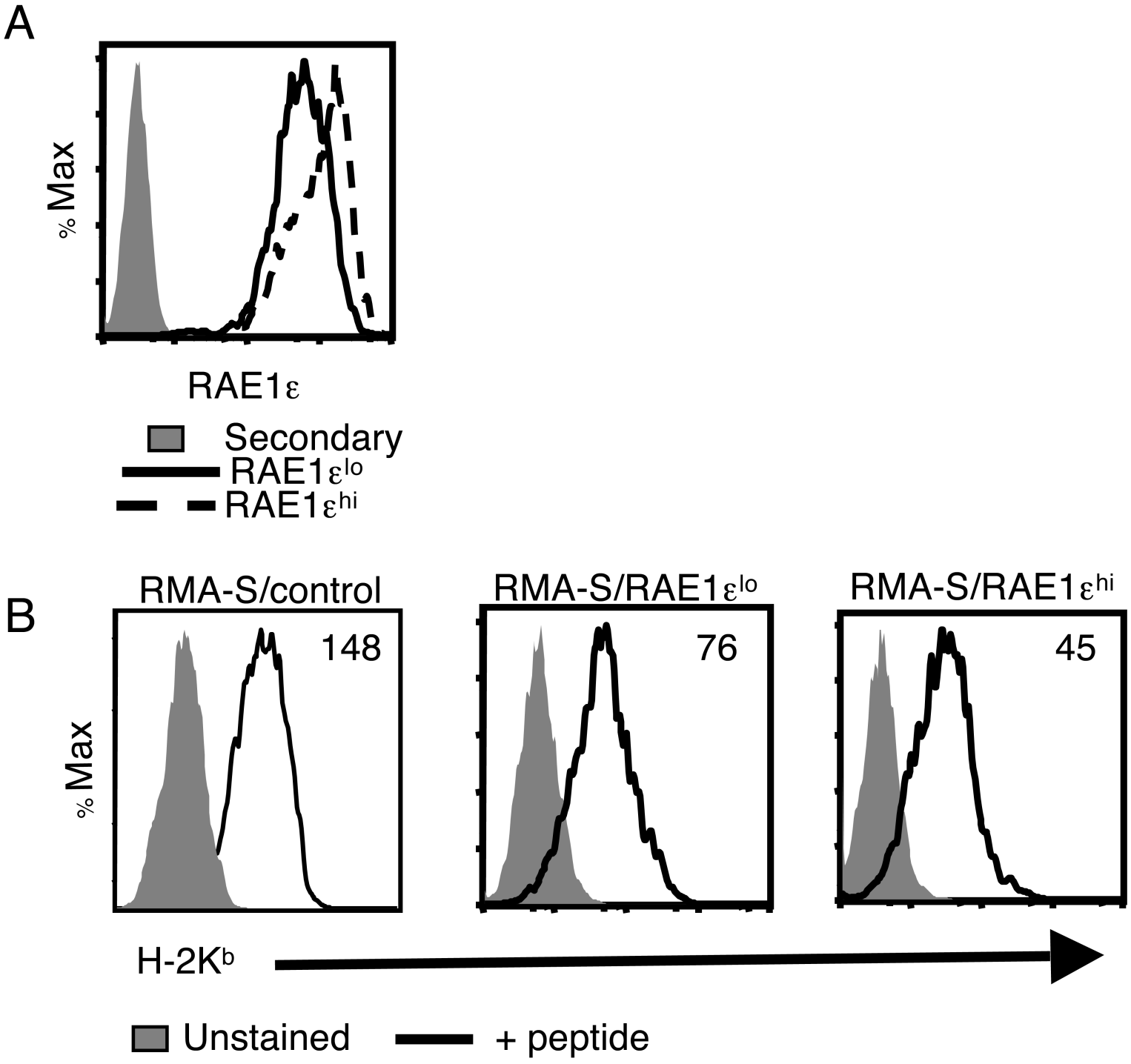
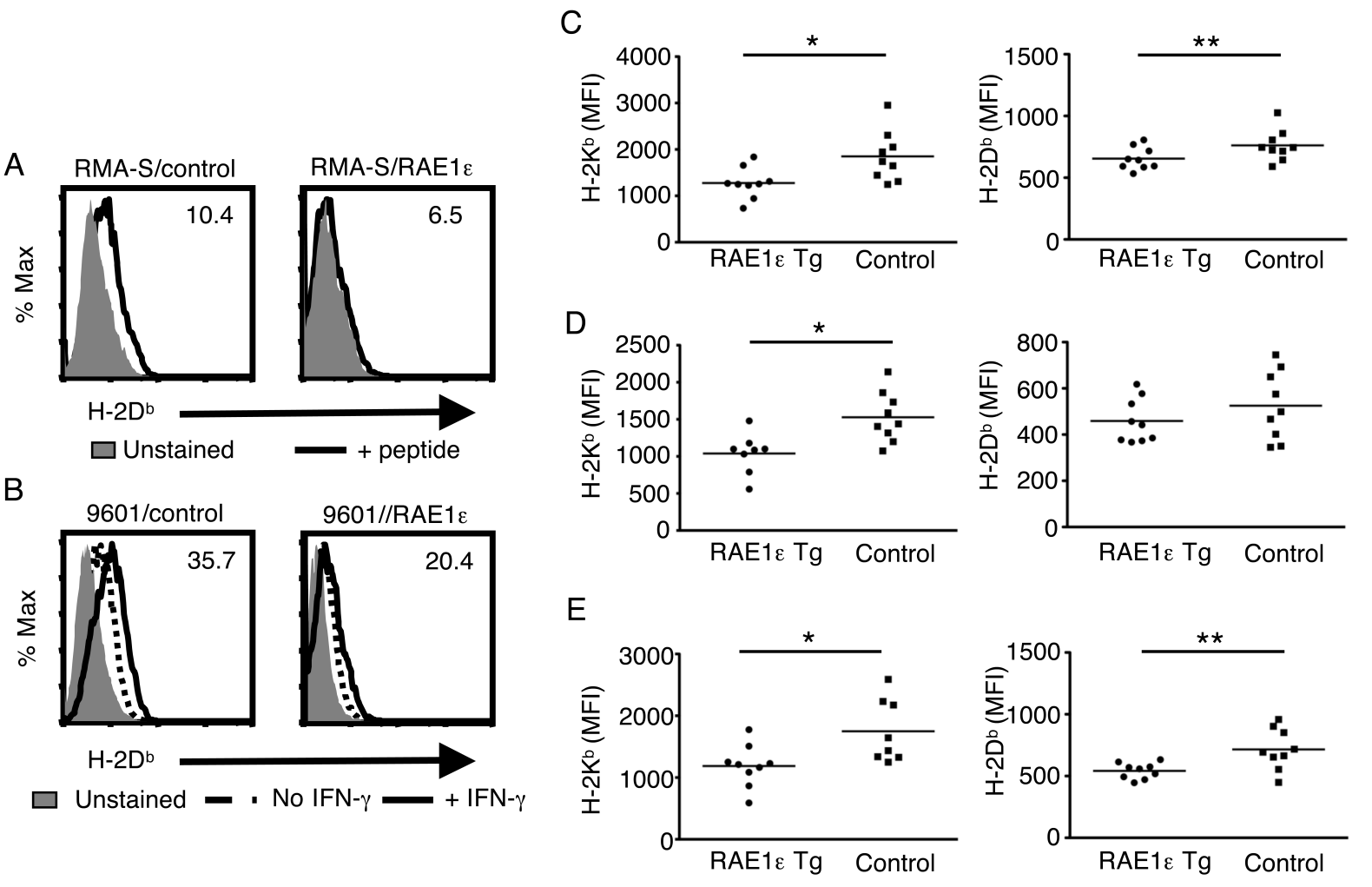
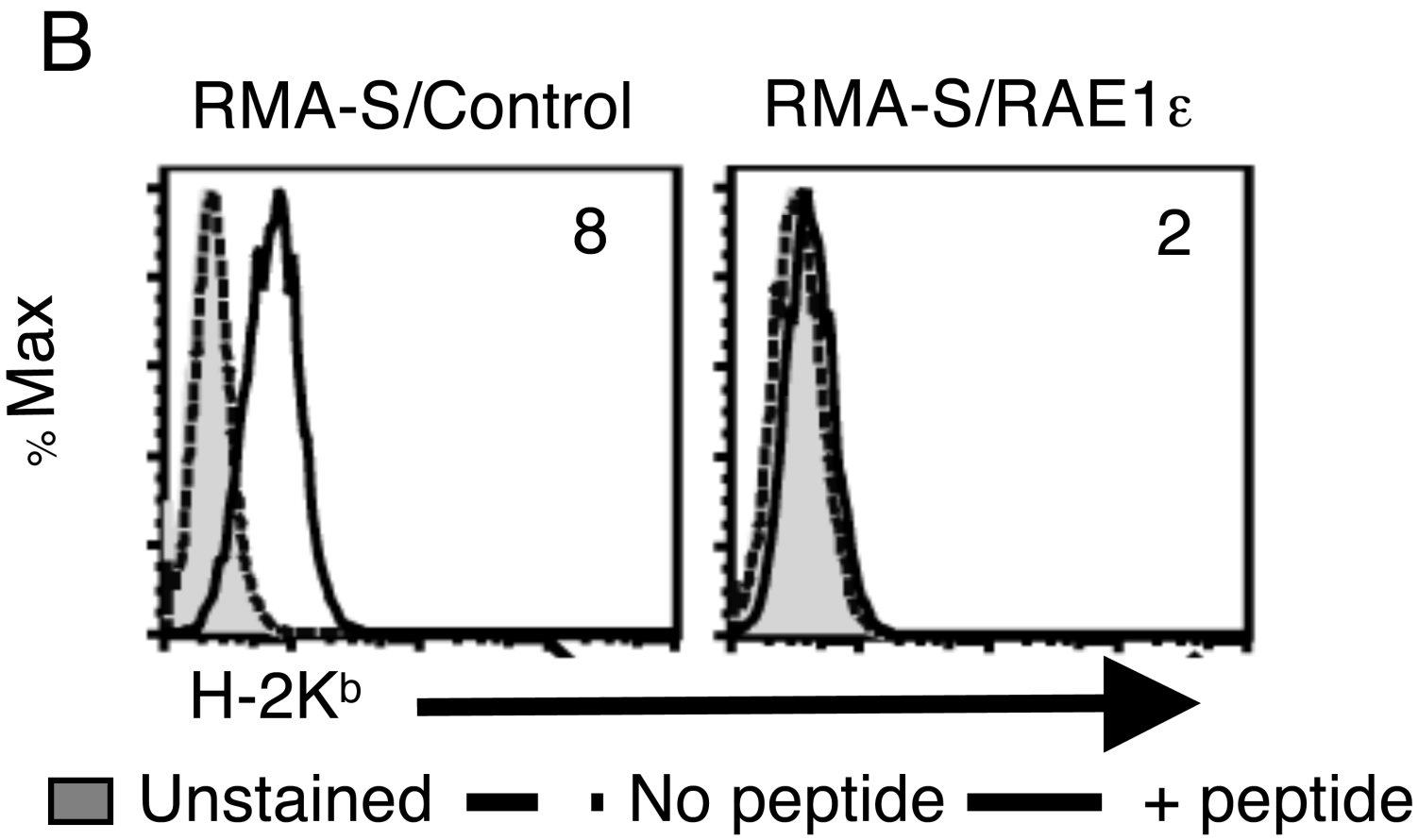
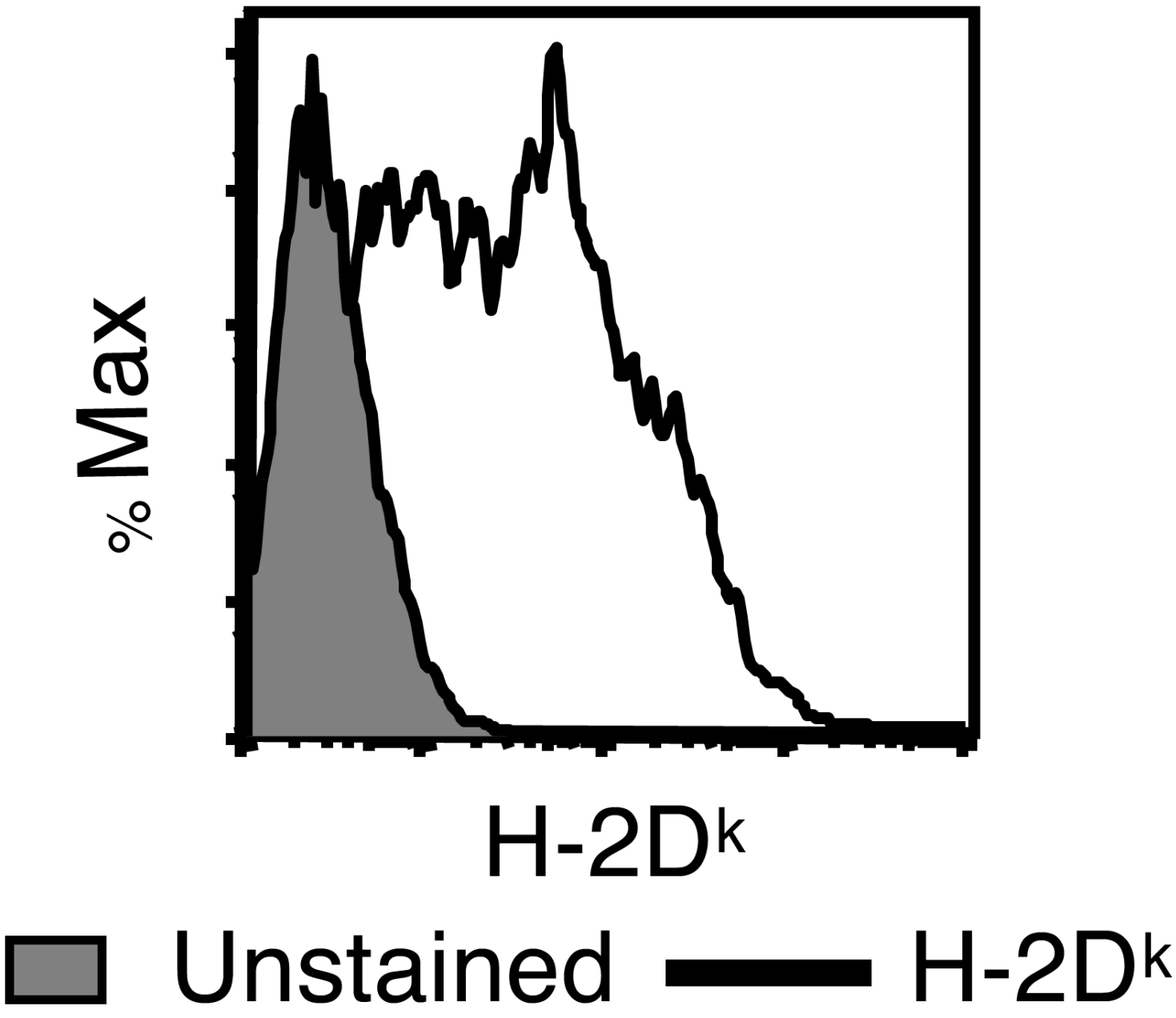
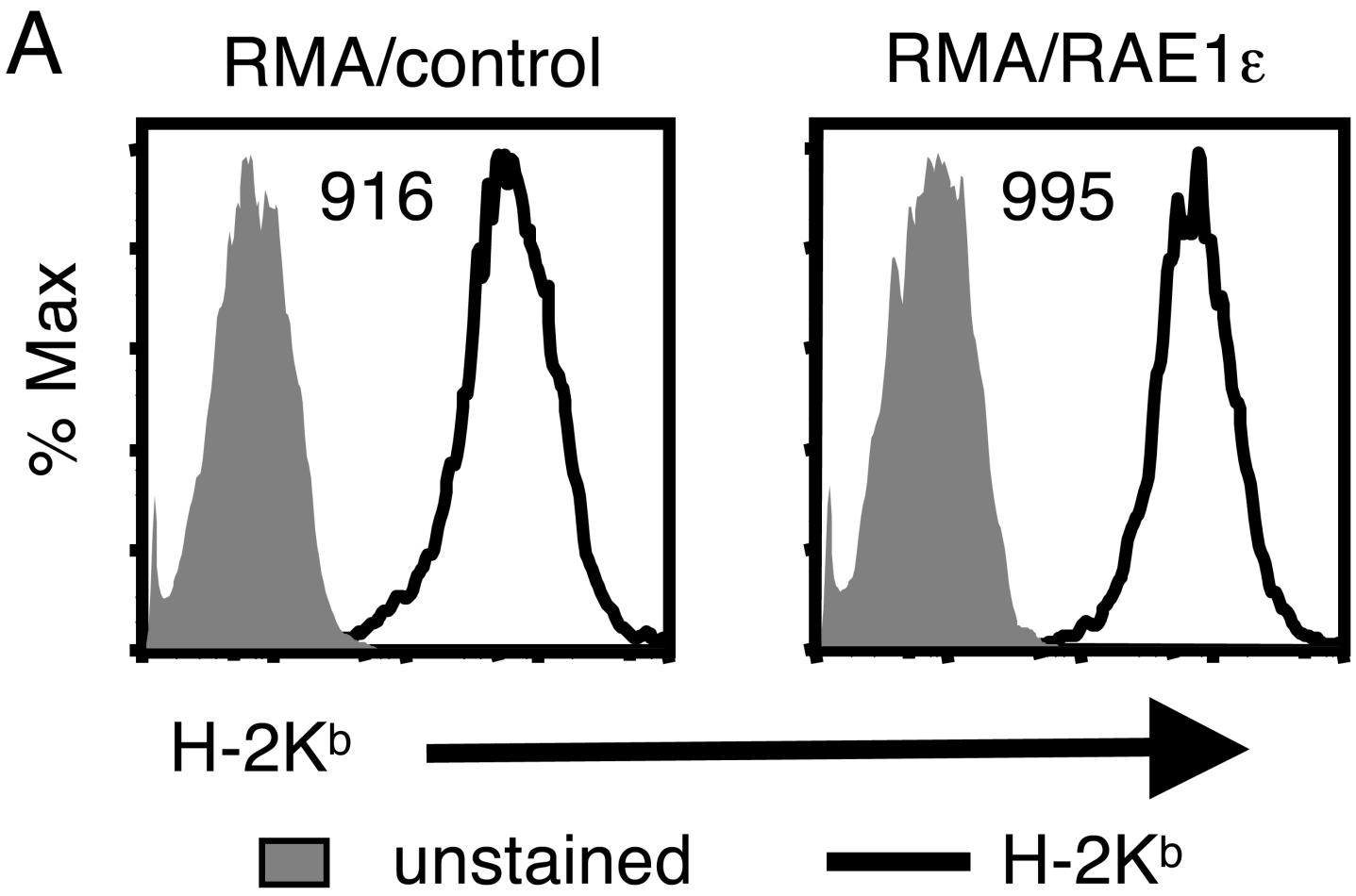

A
RMA-S/control
RMA-S/RAE1ε
RMA-S/RAE1β
7
11
35
13
5.4
5.1
% Max
Mouse/Rat IgG
Unstained
Control Abs
B
9601
9601/RAE1ε
7.2
7.6
4.2
4.3
% Max
Mouse/Rat IgG
Unstained
Control Abs
C
RMA/control
RMA/RAE1ε
RMA/H60a
RMA/MULT1
2.5
2.4
2.8
2.5
3.3
3.0
% Max
Mouse/Rat IgG
Unstained
Control Abs


A
% Max
RAE1ε
Secondary
RAE1εlo
RAE1εhi
B
RMA-S/control
RMA-S/RAE1εlo
RMA-S/RAE1εhi
148
76
45
% Max
H-2Kb
Unstained
+ peptide


A
RMA-S/control
RMA-S/RAE1ε
10.4
6.5
% Max
H-2Db
Unstained
+ peptide
B
9601/control
9601//RAE1ε
35.7
20.4
% Max
H-2Db
Unstained
No IFN-γ
+ IFN-γ
C
H-2Kb (MFI)
H-2Db (MFI)
RAE1ε Tg
Control
D
H-2Kb (MFI)
H-2Db (MFI)
RAE1ε Tg
Control
E
H-2Kb (MFI)
H-2Db (MFI)
RAE1ε Tg
Control


B
RMA-S/Control
RMA-S/RAE1ε
8
2
% Max
H-2Kb
Unstained
No peptide
+ peptide


% Max
H-2D^k
Unstained
H-2D^k


A
RMA/control
RMA/RAE1ε
916
995
% Max
H-2K^b
unstained
H-2K^b

SUPPORTING INFORMATION FIGURE 1. Minimal background staining on RMA-S, RMA and 9601 cell lines with control antibodies. (A-C) RMA-S (A), 9601 (B) or 9601 (C) cell lines were stained with a directly conjugated control mouse IgG (H-2^b control) or control rat IgG (NKG2D ligand control) followed by a conjugated anti-rat IgG secondary. The MFIs of unstained cells (top number) and cells stained with control antibodies (bottom number) are shown.

SUPPORTING INFORMATION FIGURE 2. Inverse relationship between RAE1ε and MHC class I expression. (A) RMA-S/RAE1ε^{hi} and RMA-S/RAE1ε^{lo} cells were stained with a RAE1ε-specific or secondary antibody and analyzed by flow cytometry. (B) RMA/control, RMA-S/RAE1ε^{hi} and RMA-S/RAE1ε^{lo} cells were cultured with or without SIINFEKL peptide (1μM) for 16 hours and stained with a H-2K^b-specific antibody. The MFIs of H-2K^b staining are shown.

SUPPORTING INFORMATION FIGURE 3. Ectopic expression of RAE1ε results in decreased MHC class I surface expression. (A) The RMA-S/Control and RMA-S/RAE1ε cell lines were cultured with or without SIINFEKL peptide (1μM) for 16 hours, stained with a H-2D^b-specific antibody and analyzed by flow cytometry. (B) The 9601/control and 9601/RAE1ε cell lines were cultured with or without IFN-γ (1000U/ml) for 48 h, stained with a H-2D^b-specific antibody, and analyzed by flow cytometry. (C) Total PBMC from RAE1ε transgenic (n=9) and non-transgenic (n=9) mice were stained with H-2K^b-specific (left panel) or H-2D^b-specific (right panel) antibodies and analyzed by flow cytometry. (D) PBMC from (C) gated on CD3ε$^+$ cells. (E) PBMC from (C) gated on B220$^+$ cells. *$p<0.01$, **$p<0.05$.

SUPPORTING INFORMATION FIGURE 4. MHC class I expression on target cells. The target cells used in Fig. 2E were stained with a H-2K^b-specific antibody and analyzed by flow cytometry.

SUPPORTING INFORMATION FIGURE 5. MHC class I expression on RAW 264.7 cells. Untransfected RAW 264.7 cells were stained with an H-2D^k antibody.

SUPPORTING INFORMATION FIGURE 6. Ectopic expression of RAE1ε does not decrease total MHC class I expression. RMA/control and RMA/RAE1ε cells were fixed, permeabilized, and stained with a H-2K^b-specific antibody and analyzed by flow cytometry. The MFIs of H-2K^b staining are shown.

Supplementary Materials and Methods

Cell line generation

RMA-S/RAE1ε^{hi} and RMA-S/RAE1ε^{lo} cell lines were isolated by sorting for different levels of EGFP expression using a FACSVantage SE cell sorter (BD Biosciences).

Antibodies

Control mouse IgG2b (clone 27-35) and rat IgG2a (clone # R35-95) were purchased from BD Biosciences. H-2D^{b}-specific (clone #KH95), CD3ε-specific (clone #500A2), and B220-specific (clone # RA3-6B2) antibodies were purchased from BD Biosciences.

Intracellular staining

Cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) and permeabilized with 0.01 Saponin (Sigma-Aldrich). They were then stained with antibody and analyzed on a FACScan (BD Biosciences.