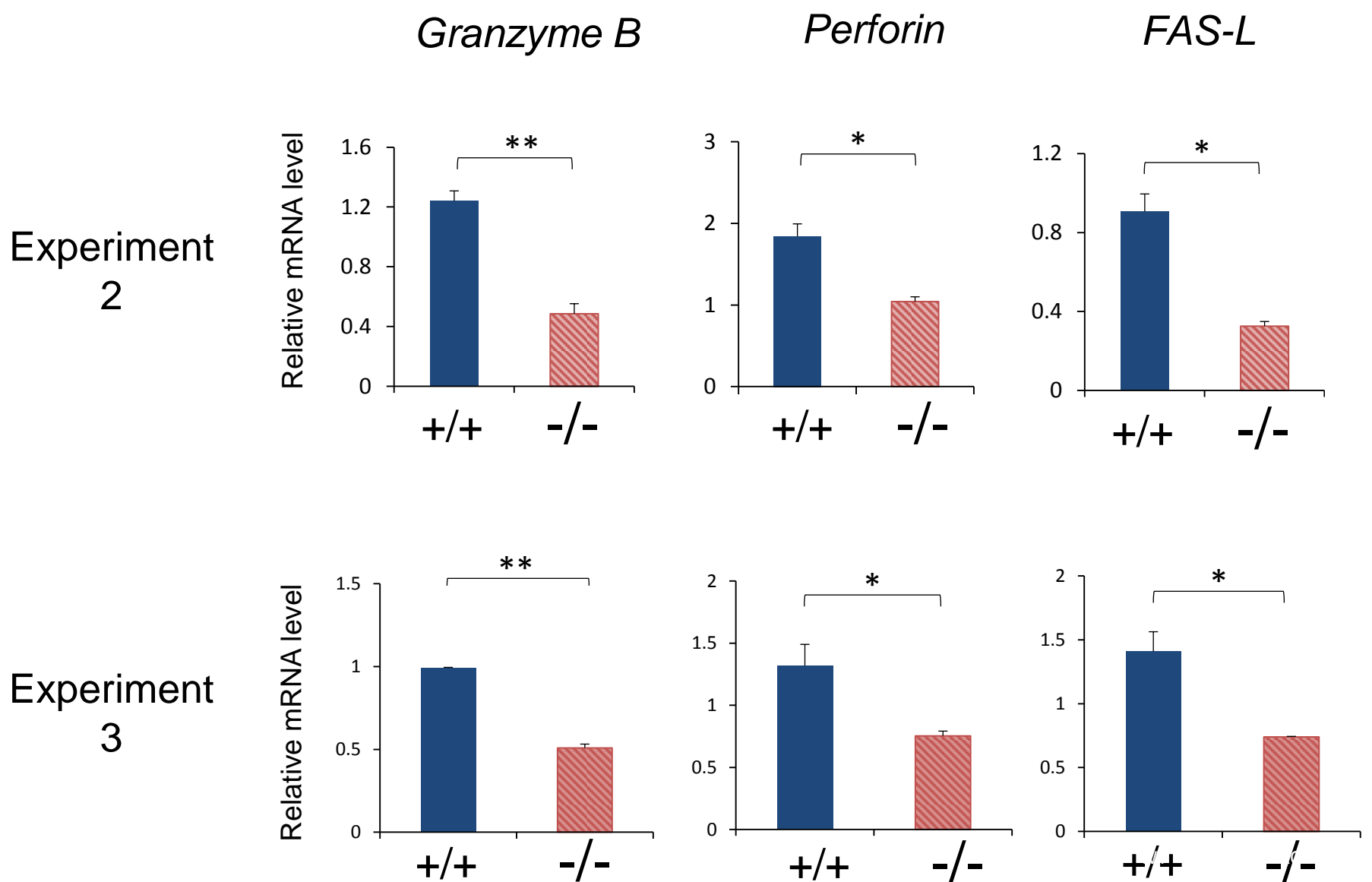
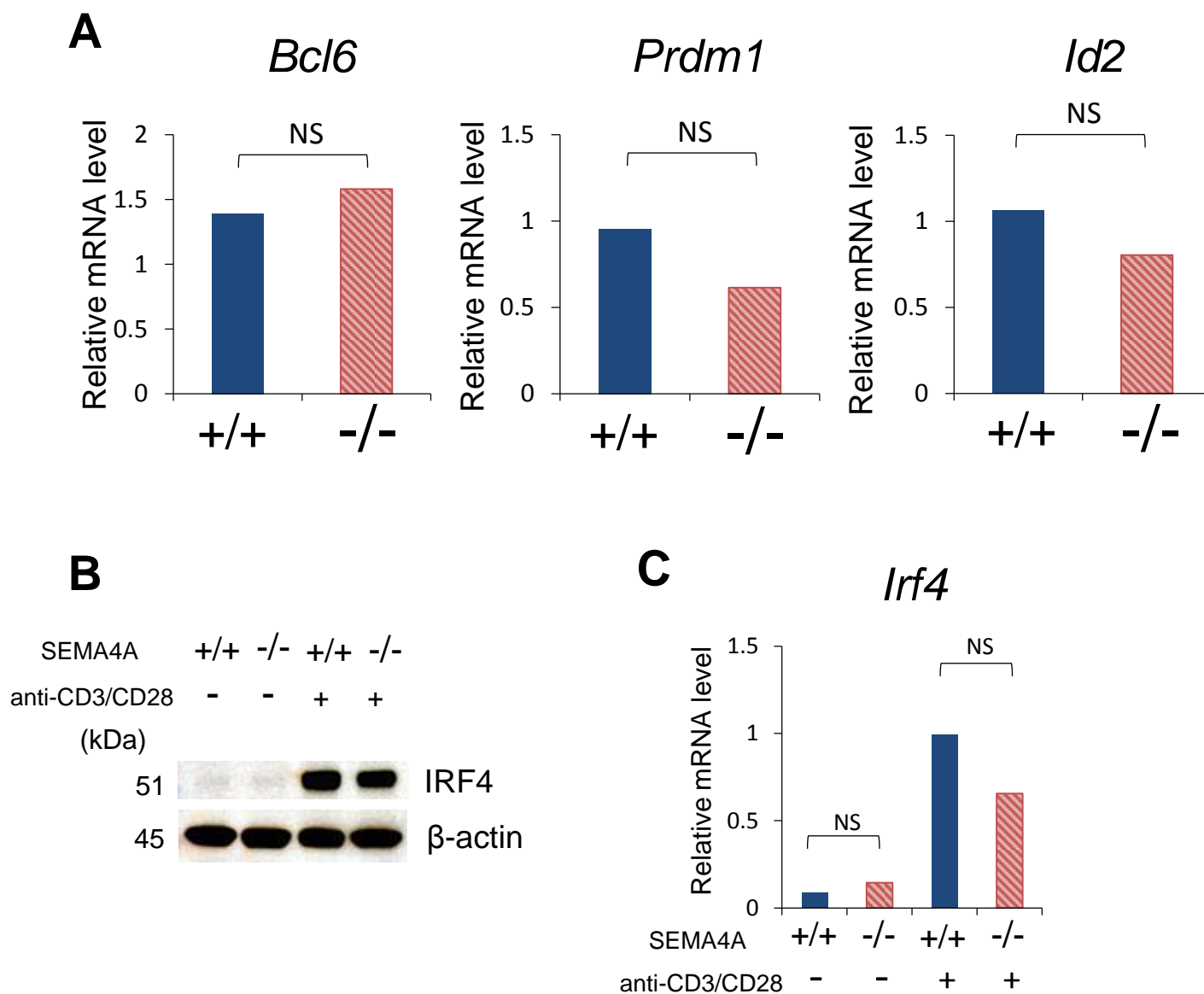


# Supplemental Figure. 1



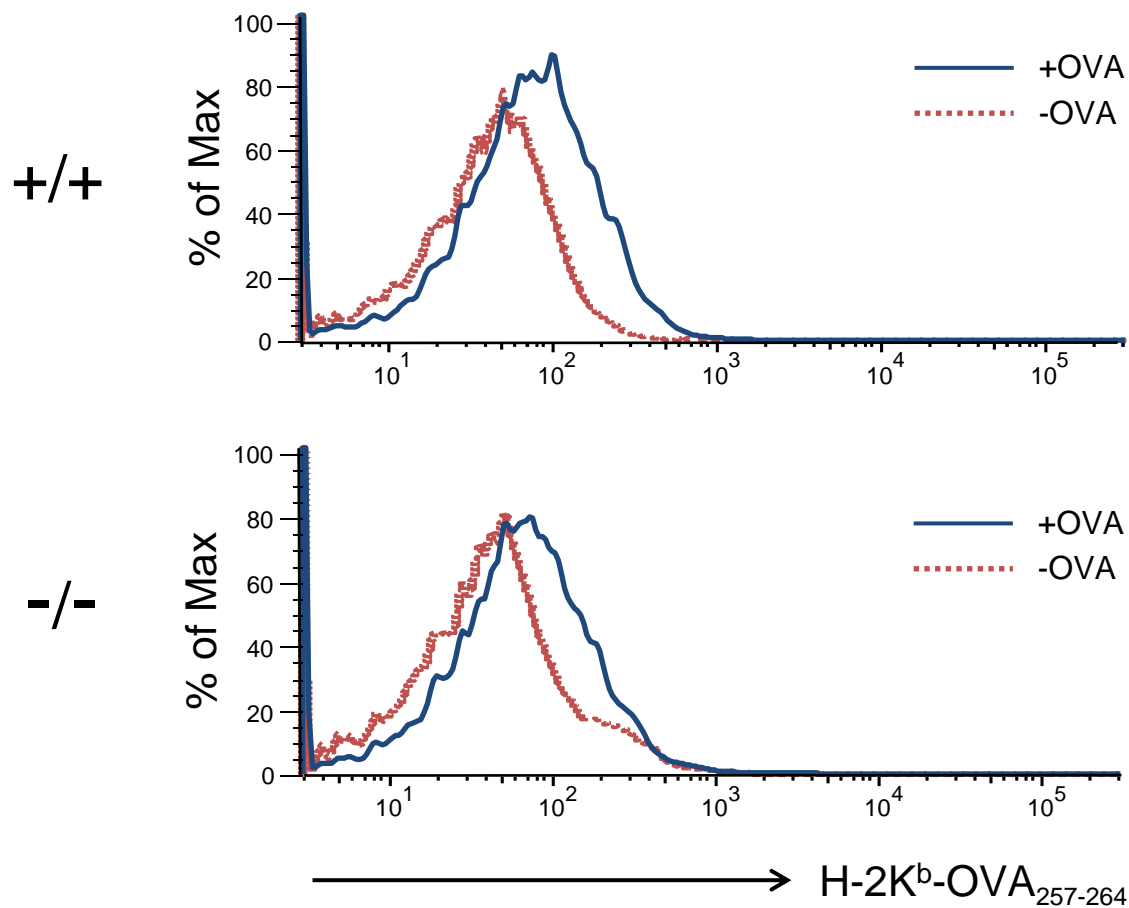
**Supplemental Figure 1.** Reduced mRNA expression levels of *Granzyme B*, *Perforin*, and *FAS-L* in *SEMA4A*<sup>-/-</sup> CD8<sup>+</sup> T cells were reproducible in two experiments in addition to that shown in Fig. 2F. CD8<sup>+</sup> T cells prepared from wild-type (+/+, blue closed bar) or *SEMA4A*<sup>-/-</sup> (-/-, red hatched bar) mice were cultured with anti-CD3 (1 µg/ml) and anti-CD28 (2 µg/ml) for 2 days. Relative expression levels of mRNAs encoding *Granzyme B*, *Perforin*, or *FAS-L* were determined by quantitative real-time PCR, with *GAPDH* as the normalization control. Data are represented as means ± s.e.m. Statistical analysis used Student's t-test (n = 3 from each group). \*, p < 0.05; \*\*, p < 0.01.

# Supplemental Figure. 2



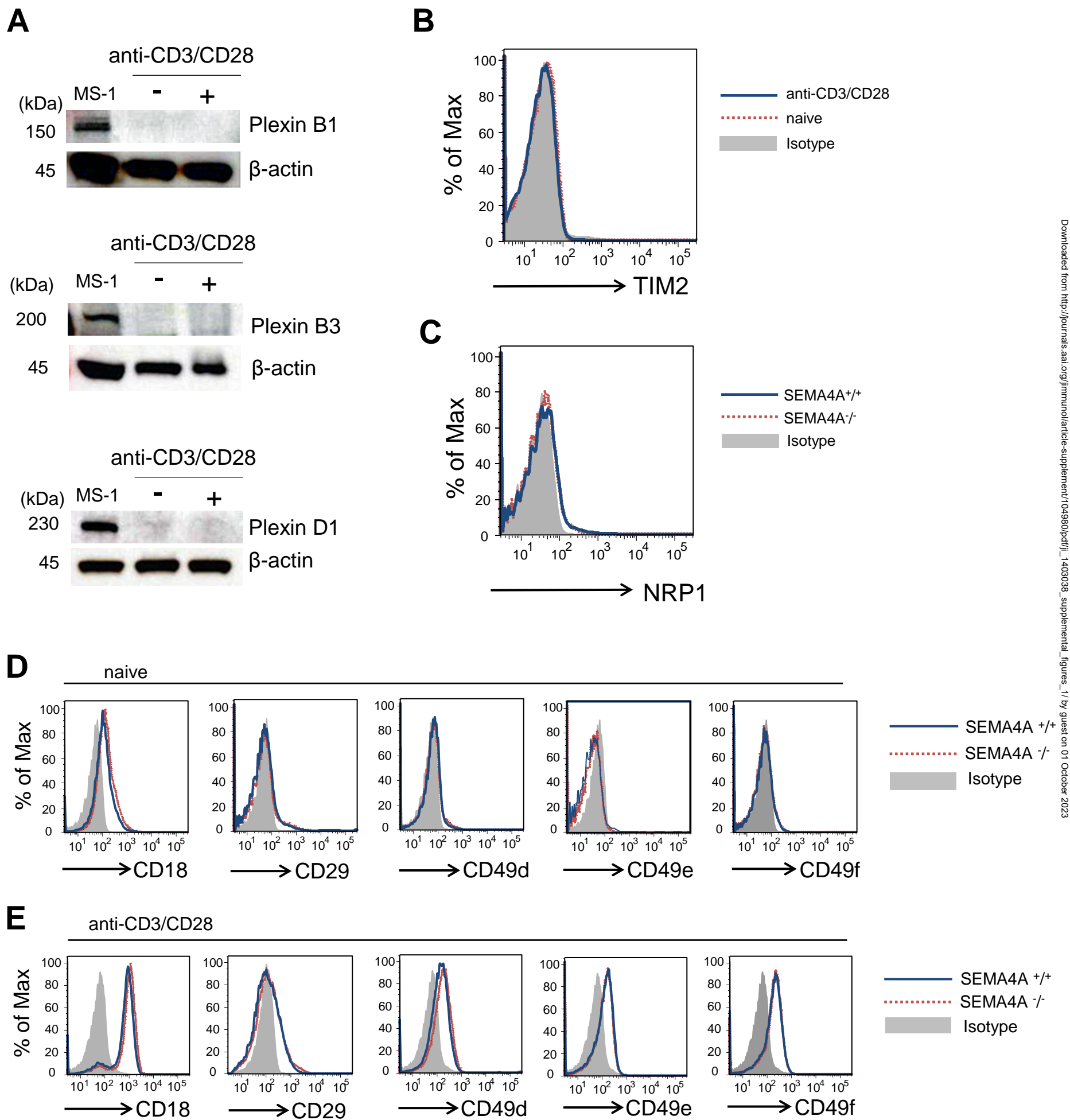
**Supplemental Figure 2.** (A) CD8<sup>+</sup> T cells prepared from wild-type (+/+, blue closed bar) or SEMA4A<sup>-/-</sup> (-/-, red hatched bar) mice were cultured with anti-CD3 (1  $\mu$ g/ml) and anti-CD28 (2  $\mu$ g/ml) for 2 days. mRNAs were prepared from these CD8<sup>+</sup> T cells. Relative levels of *Bcl6*, *Prdm1*, or *Id2* were determined by quantitative real-time PCR, with *GAPDH* as the normalization control. Data are represented as means  $\pm$  s.e.m. Statistical analysis used Student's t-test ( $n = 3$  from each groups). NS, not significant,  $p > 0.05$ . (B) Whole-cell extracts prepared from CD8<sup>+</sup> T cells cultured for 2 days with or without anti-CD3 (1  $\mu$ g/ml) and anti-CD28 (2  $\mu$ g/ml) were probed by western blotting with anti-IRF4 or anti- $\beta$ -actin. (C) CD8<sup>+</sup> T cells prepared from wild-type (+/+, blue closed bar) or SEMA4A<sup>-/-</sup> (-/-, red hatched bar) mice were cultured with or without anti-CD3 (1  $\mu$ g/ml) and anti-CD28 (2  $\mu$ g/ml) for 2 days. mRNAs were prepared from these activated CD8<sup>+</sup> T cells. Relative expression levels of *Irf4* were determined by quantitative real-time PCR, with *GAPDH* as the normalization control. Data are represented as means  $\pm$  s.e.m. Statistical analysis used Student's t-test ( $n = 3$  from each groups). NS, not significant,  $p > 0.05$ .

# Supplemental Figure. 3



**Supplemental Figure 3.** SEMA4A<sup>-/-</sup> DCs did not exhibit impaired cross-presentation capacity. Splenic DCs derived from wild-type (+/+) or SEMA4A<sup>-/-</sup> (-/-) mice were incubated for 15 hours with or without OVA. Subsequently, DCs were stained with anti-CD11c and anti-H-2K<sup>b</sup>-OVA<sub>257-264</sub>. Expression of H-2K<sup>b</sup>-OVA<sub>257-264</sub> in CD11c-positive cells is presented in the histogram.

# Supplemental Figure. 4



**Supplemental Figure 4.** (A) Whole-cell extracts were prepared from wild-type CD8<sup>+</sup> T cells cultured for 2 days with or without anti-CD3 (1 µg/ml) and anti-CD28 (2 µg/ml), and then probed by western blotting with the indicated antibodies. MS-1, a cell line established from pancreatic islet endothelial cells, was used as a positive control. (B) CD8<sup>+</sup> T cells prepared from wild-type mice were cultured with anti-CD3 (1 µg/ml) and anti-CD28 (2 µg/ml) for 2 days, and then stained with anti-TIM2. Wild-type CD8<sup>+</sup> T cells were also stained with the isotype-matched control antibody (Isotype, grey-filled histogram). (C) CD8<sup>+</sup> T cells prepared from wild-type (+/+, blue closed bar) or SEMA4A<sup>-/-</sup> (-/-, red hatched bar) mice were stained with anti-NRP1. Wild-type CD8<sup>+</sup> T cells were also stained with the isotype-matched control antibody (Isotype, grey-filled histogram). (D) Expression levels of indicated integrin molecules in CD8<sup>+</sup> T cells prepared from wild-type (SEMA4A<sup>+/+</sup>, blue solid line) or SEMA4A<sup>-/-</sup> (red dotted line) mice was examined by flow cytometry. Wild-type CD8<sup>+</sup> T cells were also stained with an isotype-matched control antibody (Isotype, grey-filled histogram). (E) CD8<sup>+</sup> T cells prepared from wild-type (SEMA4A<sup>+/+</sup>, blue solid line) or SEMA4A<sup>-/-</sup> (red dotted line) mice were cultured with anti-CD3 (1 µg/ml) and anti-CD28 (2 µg/ml) for 2 days. The expression levels of the indicated integrin molecules on the resultant cells were analyzed by flow cytometry. CD8<sup>+</sup> T cells prepared from wild-type mice were also stained with the isotype-matched control antibody (Isotype, grey-filled histogram).