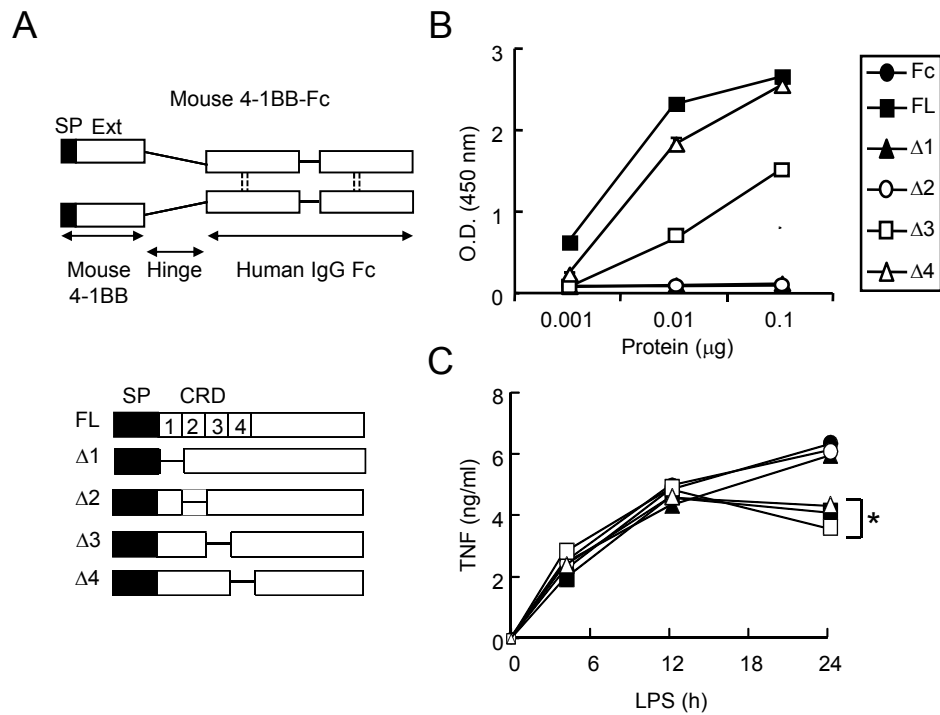
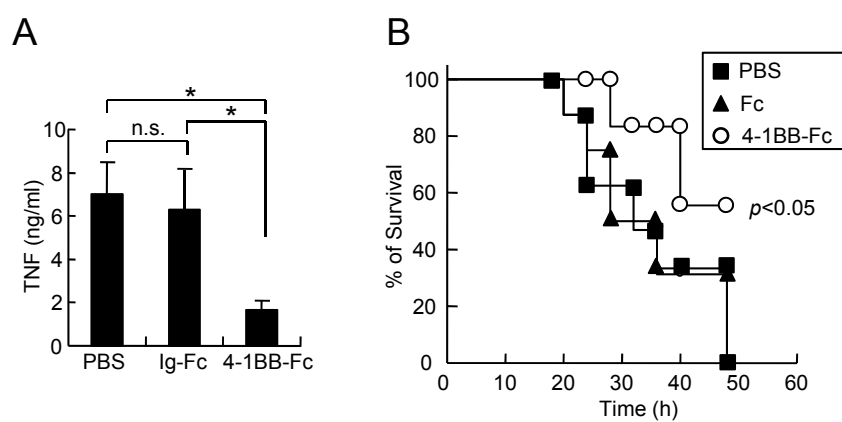


Supplemental Figure 1



Supplemental Figure 1. Essential CRD regions for binding of 4-1BB-Fc and inhibition of sustained TNF production. (A) Schematic representation of the recombinant 4-1BB-Fc proteins. The entire extracellular region (EXT) of mouse 4-1BB (full length, FL) is shown with its signal peptide (SP), and different CRDs (1, 2, 3, and 4). The 4-1BB-Fc mutant proteins with deletion of CRD1 (Δ1), CRD2 (Δ2), CRD3 (Δ3) or CRD4 (Δ4) are shown. (B & C) Binding and inhibition of sustained TNF production by 4-1BB-Fc molecules with different CRDs. Binding of 4-1BB-Fc molecules to 4-1BBL was analyzed by ELISA (B). (C) Macrophages were stimulated with LPS (0.1 μg/ml), and different types of 4-1BB-Fc and Fc of human IgG (5 μg/ml) were added after 4 hours. Culture supernatants were obtained at the indicated times, and TNF concentrations were analyzed by ELISA.

Supplemental Figure 2



Supplemental Figure 2. Fc fragment does not ameliorate the LPS-induced sepsis in mice. Wildtype mice were administered with PBS, Fc fragment in PBS (50 μ g), or 4-1BB-Fc in PBS (50 μ g) intraperitoneally, and injected with LPS (450 μ g per mouse). Blood samples were collected after 2 hours by retro-orbital bleeding, and TNF concentration was determined by ELISA (A). Survival of mice was monitored. Data are shown as the mean \pm SD. $n=5$. P values are shown. *, $p<0.05$. n.s., not significant.