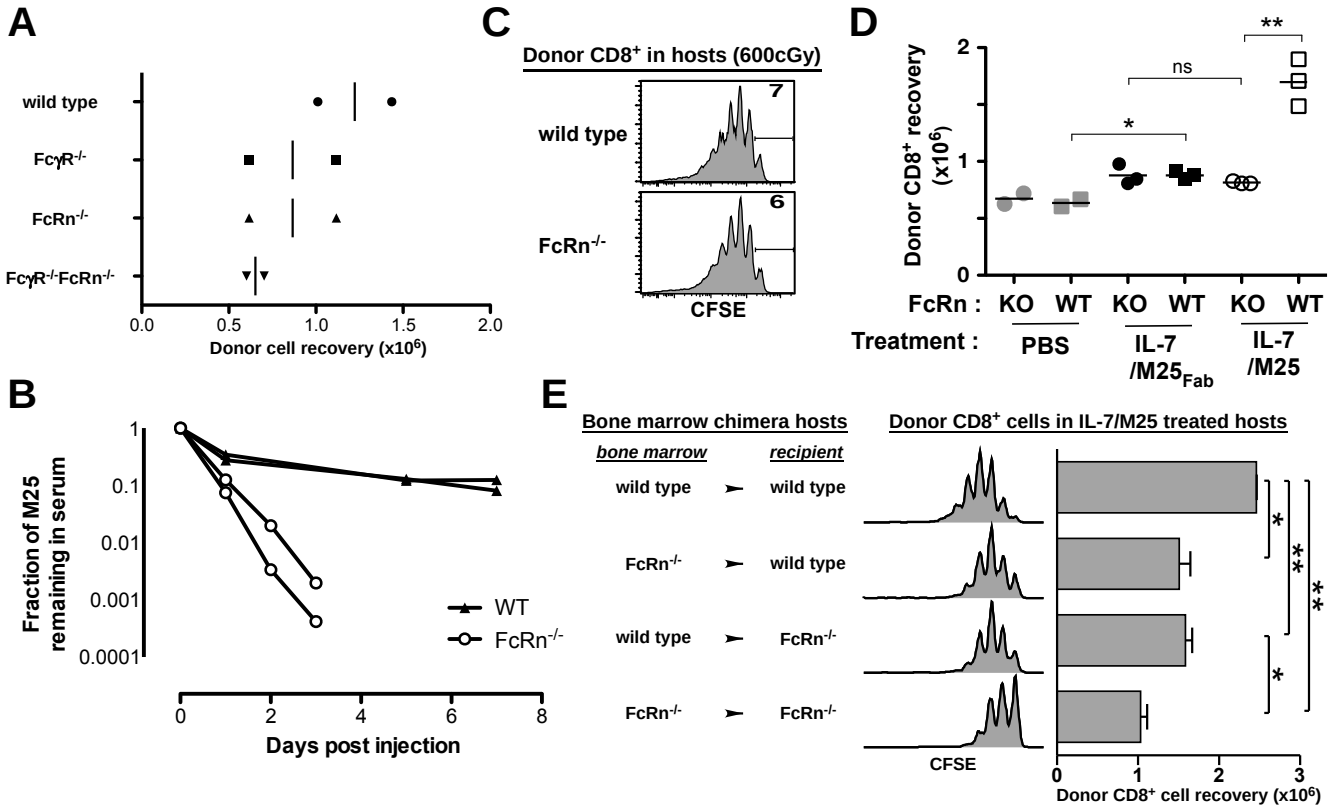
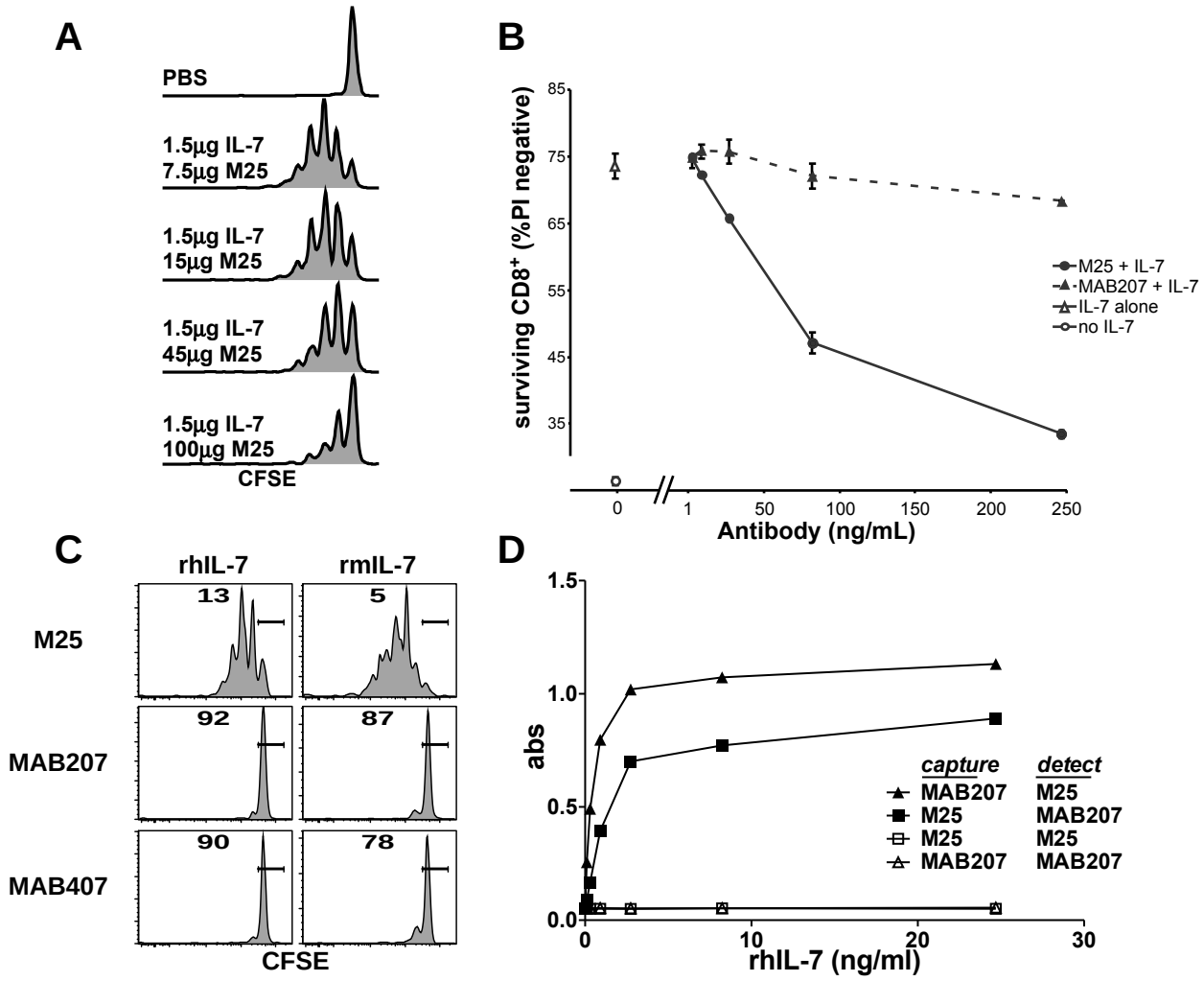


Supplemental Figure 1. The potency of IL-7/M25 depends on host expression of FcRn. (A) Donor cell recoveries from the experiment as described in Figure 2A. (B) Wild type and FcRn$^{-/-}$ mice were injected with 200µl PBS containing 25µg biotin-conjugated M25 and 5µg IL-7 via the tail vein. Within 5mins of injection, and every day for the following 7 days, mice were anesthetized by isoflurane inhalation, 80-100µL of blood was drawn from the retroorbital plexus and separated serum was stored at -20ºC for simultaneous analysis of all samples. Biotinylated M25 content of serially diluted serum samples was determined by ELISA, using rat anti-mouse IgG (Jackson ImmunoResearch) capture and detection by streptavidin-conjugated horseradish peroxidase. Shown are the biotin-M25 contents normalized to the T=5mins value for each individual mouse from one such experiment. (C) 3x10^6 CFSE-labeled B6.CD45.1$^+$ LN cells were adoptively transferred to irradiated (600 cGy) CD45.2$^+$ hosts as indicated. The CFSE profiles of CD45.1$^+$ CD8$^+$ lymphocytes at d7 are shown here. (D) CD8$^+$ cells were purified from B6.CD45.1$^+$ donor LN, CFSE-labeled and 3.5x10^6 transferred to each CD45.2$^+$ host, either FcRn-/- (circles) or wild type (squares), on d0. Hosts received *i.p.* injections every other day of PBS alone or 1.5µg rhIL-7 plus either M25 (7.5µg) or M25$_{Fab}$ (7.5µg equivalent). The d7 recoveries of CD45.1$^+$ CD8$^+$ cells from host LN and spleen are presented with horizontal bars indicating the average of 2-3 separately analyzed mice per group. (E) 10^7 bone marrow cells isolated from the hind long bones and pelvis of wild type (CD45.1$^+$) or FcRn$^{-/-}$ (CD45.2$^+$) mice and were injected *i.v.* into recipients of the same or reciprocal genotype that had been lethally irradiated (1050cGy) one day earlier. Bone marrow chimeras were rested 7 weeks prior to the transfer (*i.v.*) of 1.5x10^6 purified and CFSE-labeled CD90.1$^+$ CD8$^+$ LN cells on d0, followed by *i.p.* injections of PBS or rhIL-7/M25 (3.75µg/22.5µg) on d1, 3, 5 and analysis on d7. Depicted here are the CFSE profiles of CD8$^+$ CD90.1$^+$ cells from host LN (middle) as well as the average recoveries of CD8$^+$ CD90.1$^+$ cells from host LN and spleen, error bars indicate SEM (N=4). Data are representative of 2-3 experiments. *P<.05, **P<.005 and ns indicates not significant



Supplemental Figure 2. M25 blocks the effect of IL-7 *in vivo* and *in vitro* and M25, but not other IL-7 binding mAb, form IL-7/mAb complexes with potent *in vivo* activity. (A) $5x10^6$ CFSE-labeled B6.CD45.1$^+$ LN cells were adoptively transferred to B6.CD45.2$^+$ hosts on d0. Injections of PBS alone or rhIL-7 (1.5µg) with M25 (7.5, 15, 45, or 100µg) were administered *i.p.* on d1, 3, and 5. Shown are the CFSE profiles of CD45.1$^+$ CD8$^+$ cells from host LN on d7. (B) $4.5x10^5$ wild type LN cells were cultured at 37°C in RPMI complete media (⊙), media supplemented with 1ng/mL rhIL-7 alone (▲), or media supplemented with 1ng/mL rhIL-7 and indicated concentrations of mAbs M25 (●) or MAB207 (▲). After 48hrs in culture, samples were stained for CD4, CD8 and live cells (propidum iodide negative) and analyzed by flow cytometry. Error bars indicate SD (N=2). (C**)** $2x10^6$ CFSE labeled B6.CD90.1$^+$ LN cells were injected *i.v.* into B6.CD90.2$^+$ hosts. On d1, 3, and 5, host mice received *i.p.* injections of 1.5µg IL-7 + 7.5µg mAb, as indicated, and donor cell proliferation in host LN and spleen was analyzed on d7. CFSE histograms shown here are gated on CD90.1$^+$ CD8$^+$ lymphocytes. (D**)** ELISA of titrated concentrations of rhIL-7 using capture mAbs and biotin-conjugated detection mAbs as shown followed by streptavadin-HRP (Jackson ImmunoResearch) and developed with 1-Step™ Ultra TMB-ELISA (Thermo Scientific) according to manufacturers instructions. Shown are the absorbance at 450nm for duplicate samples +/- SEM. Data are representative of 2-3 experiments.