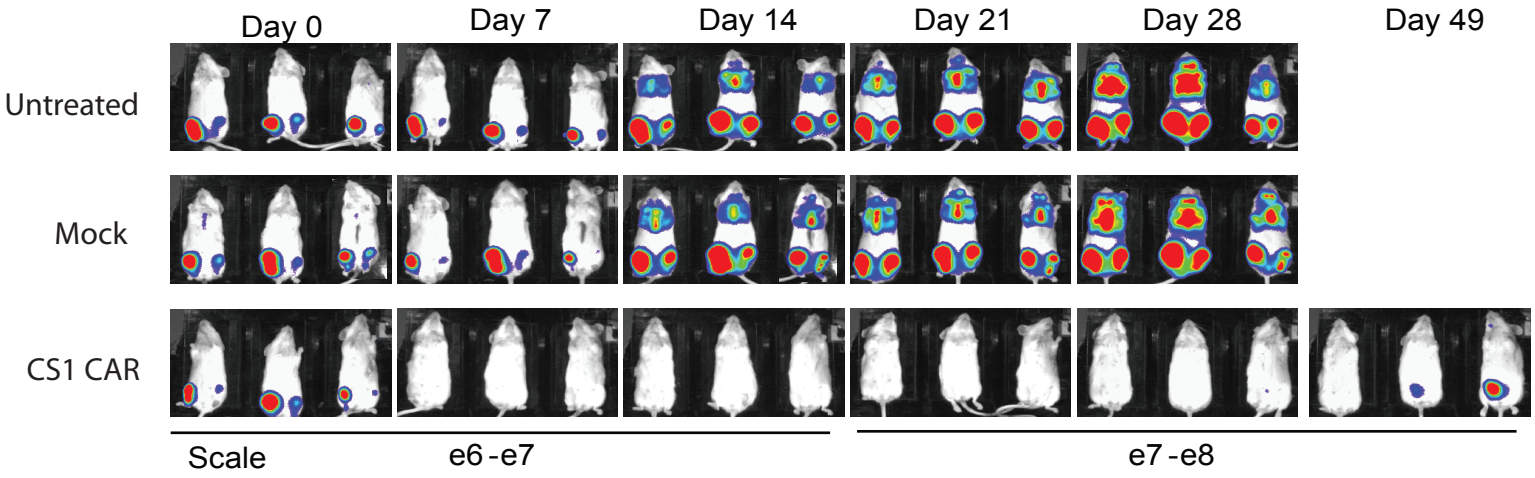
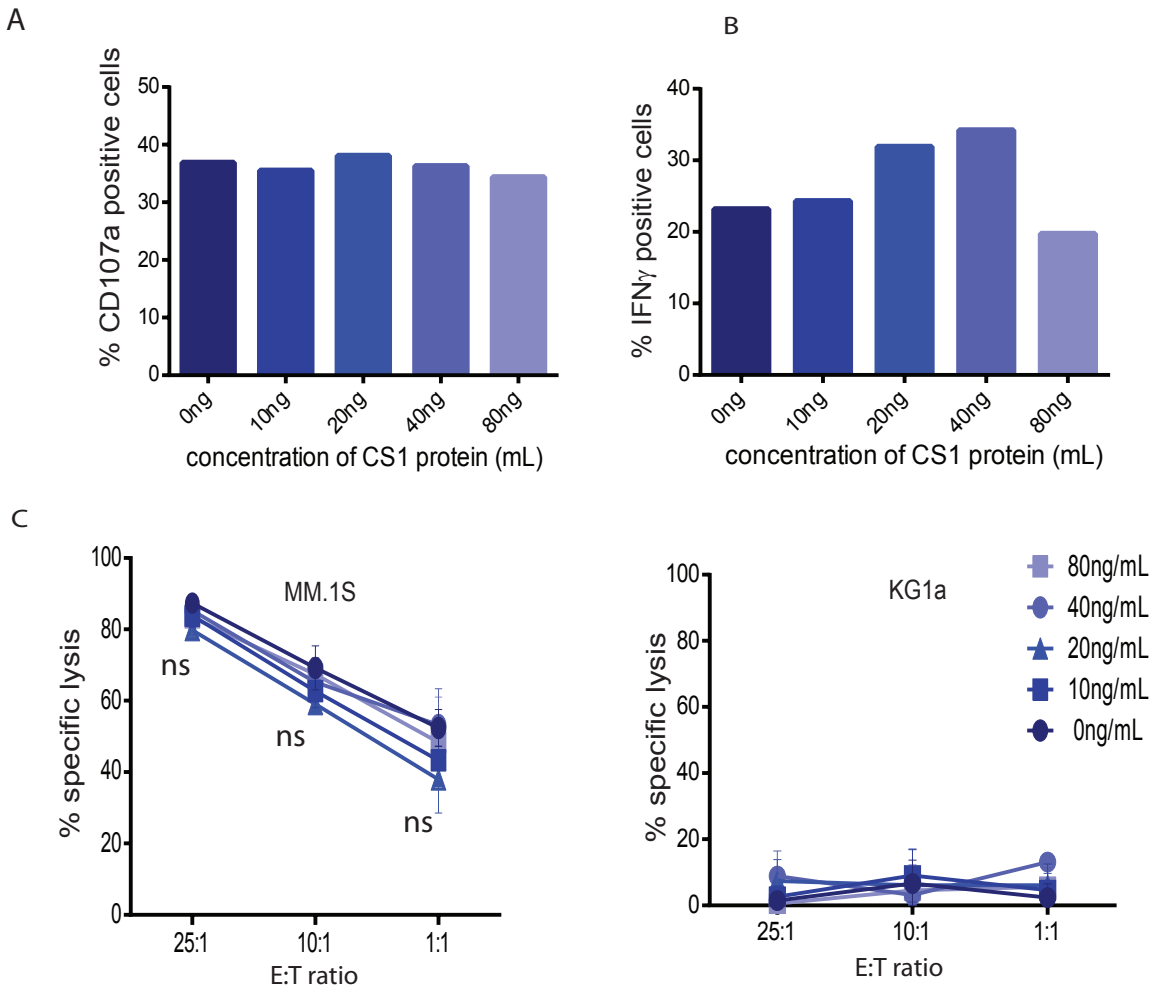
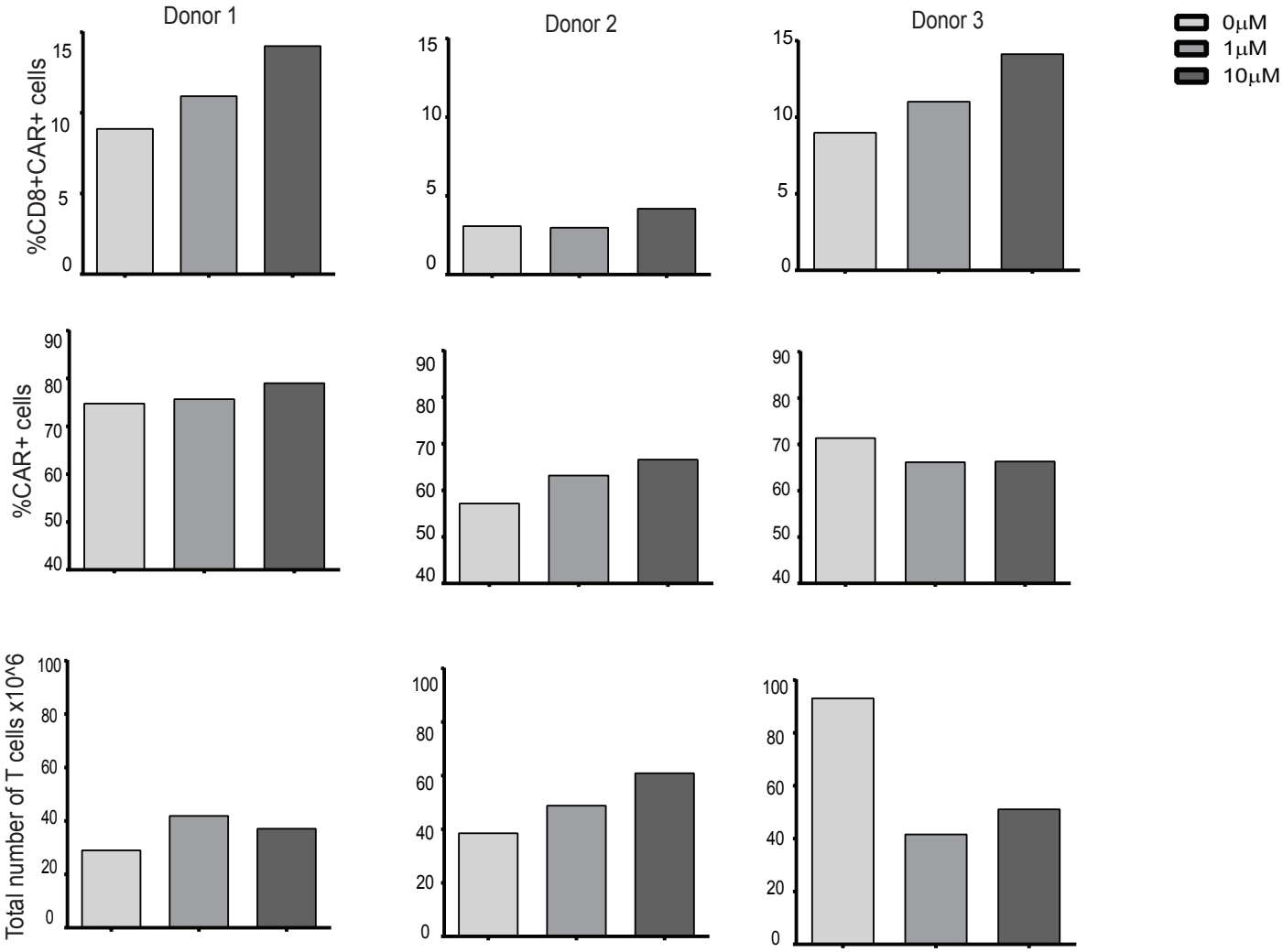


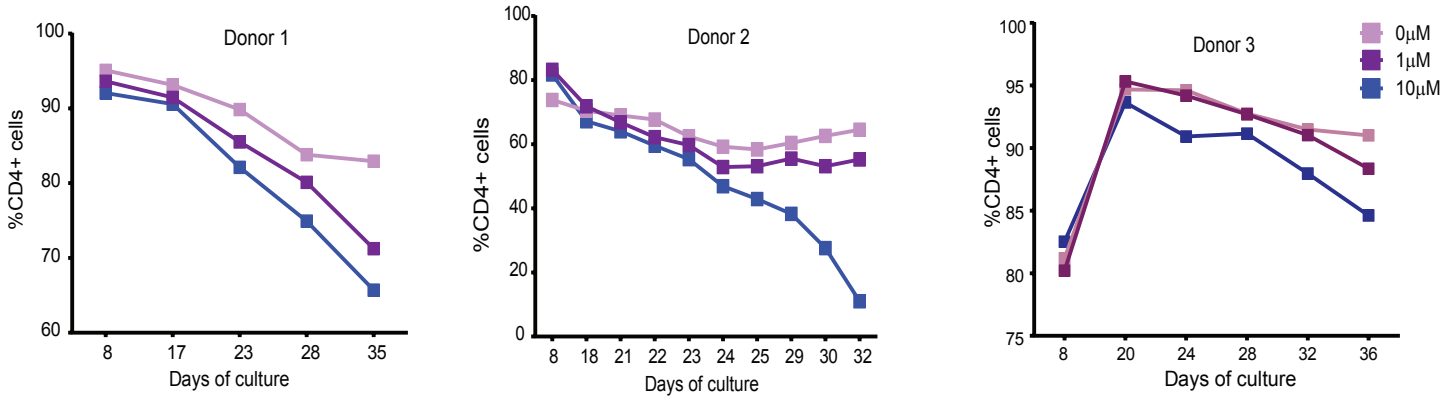
Supplemental Figure 1. High dose CS1 CAR T cells were not able to prevent tumor relapse A total of 2 x 10^6 ffluc-GFP MM.1S cells were intratibially (i.t.) injected into NSG mice. Five days following tumor inoculation, mice were injected i.v. with dosed 3 x 10^6 CAR T cells or non-transduced mock cells. Tumor signals were monitored with Xenogen imaging once a week.



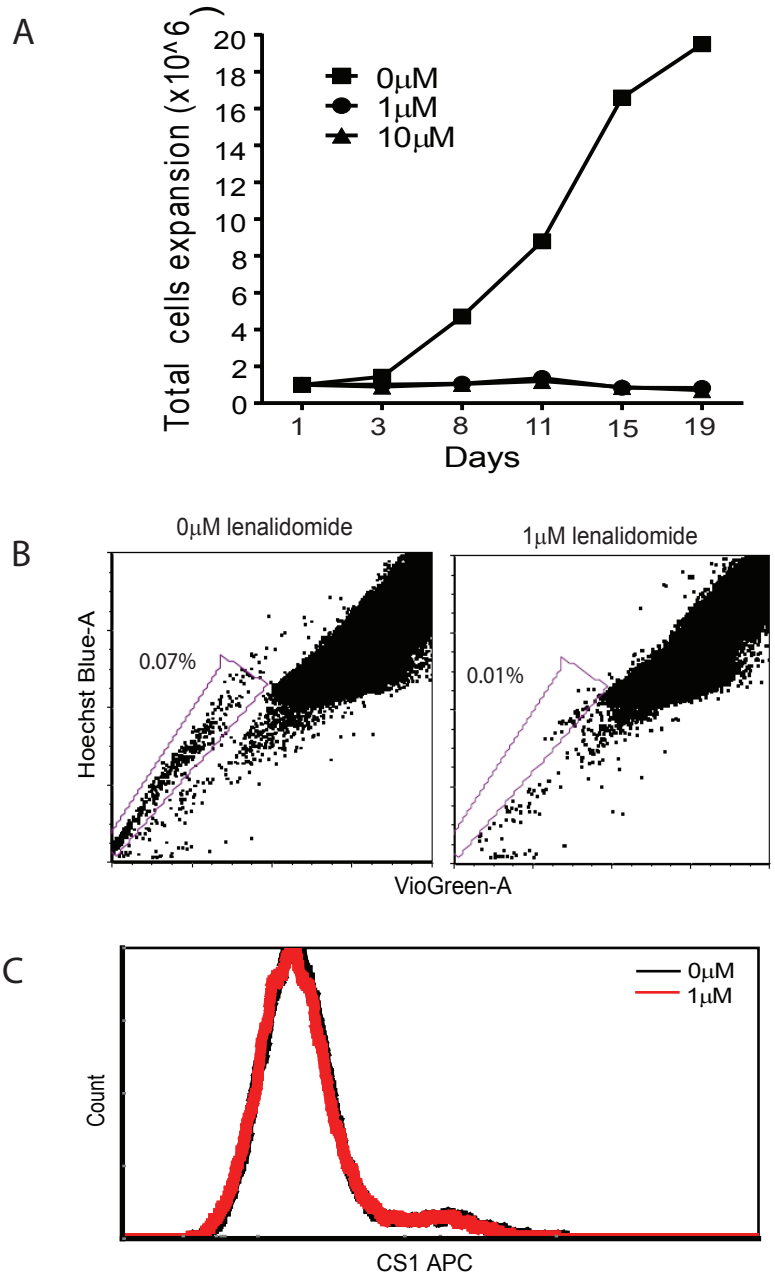
Supplemental Figure 2. Soluble CS1 does not interfere with CS1 CAR function (A) Expanded CS1 and BCMA CAR T cells were co-cultured with MM.1S cells at a 1:1 ratio in medium containing Golgi plug and CD107a for six hours at 37°C in the presence of CS1 protein at concentrations of 0, 10, 20, 40, and 80 ng/mL. Percentages of CD107a+ cells per CAR+ cells are presented. (B) In separate culture, 5 x 10^5 CS1 T cells were activated overnight with 5 x 10^5 MM.1S cells in the presence of brefeldin A. The cell mixture was then stained using anti-CD8, anti-CD3, anti-CD4, Erbitux, and streptavidin PE followed by intracellular staining with antibody against INFγ. IFNγ-positive cells (CAR gated) are presented. (C) CS1 CAR T cells were co-cultured with luciferase expressing MM.1S for 4 hours in the presence of CS1 protein at concentrations of 0, 10, 20, 40, and 80 ng/mL. Specific lysis was analyzed after adding substrate luciferin. Myeloid leukemic cells KG1a were used as a negative control. Two-way ANOVA test was used.



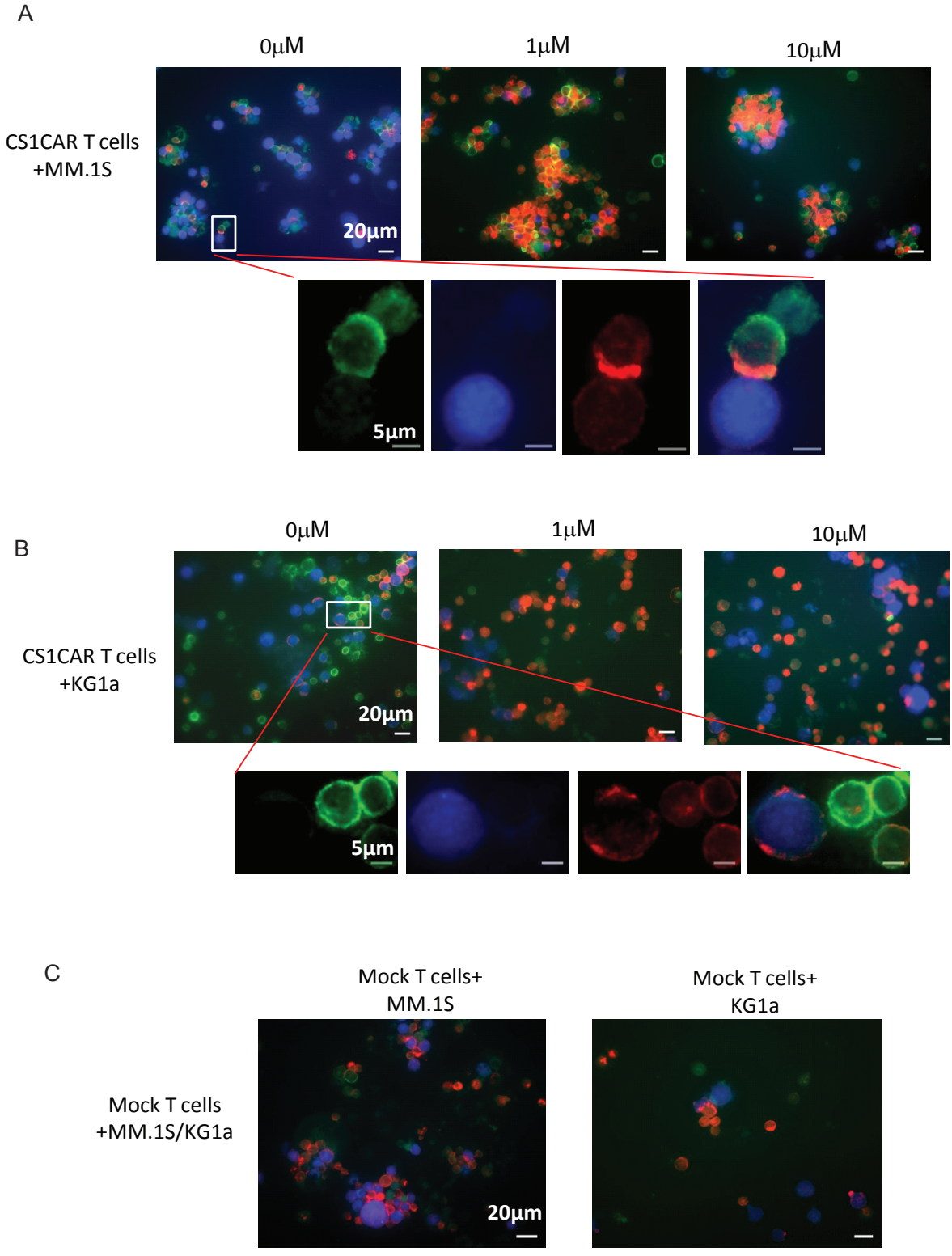
Supplemental Figure 3. Lenalidomide preferentially expanded CD8+CAR+ T cells in a dose-dependent manner Central memory T cells isolated from three different donors were activated and transduced with lentivirus encoding CS1 CAR and expanded in vitro in the presence of 0, 1, and 10 μM lenalidomide. Growth of total cell number and percentages of positive cells for CD8+CAR+ and CAR+ T cells on day 20 of culture are presented.



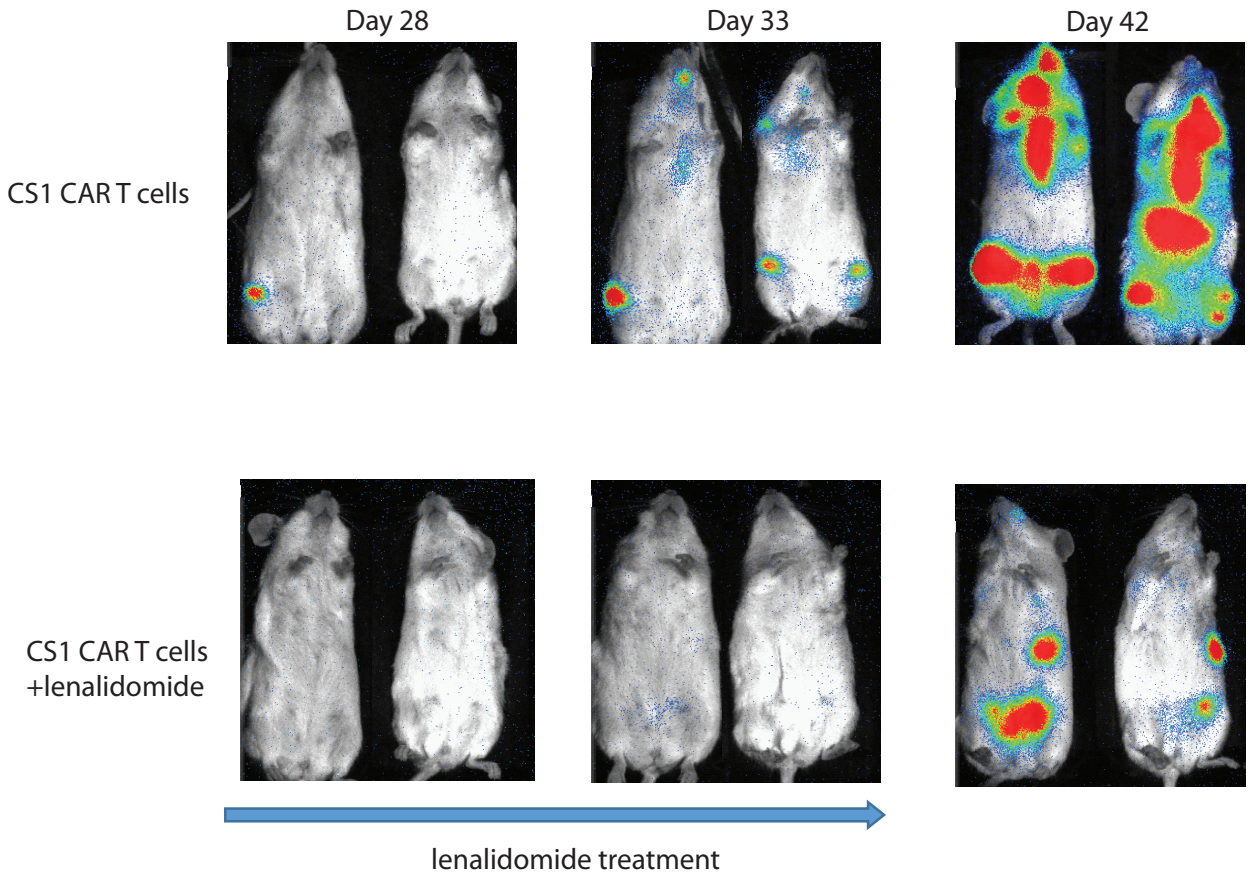
Supplemental Figure 4. Lenalidomide decreased CD4+ T cell expansion in a dose-dependent manner Central memory T cells isolated from three different donors were activated and transduced with lentivirus encoding CS1 CAR and expanded in vitro in the presence of 0, 1, and 10 μM lenalidomide. Percentages of CD4+ positive cells in the cultures at different time points are presented.



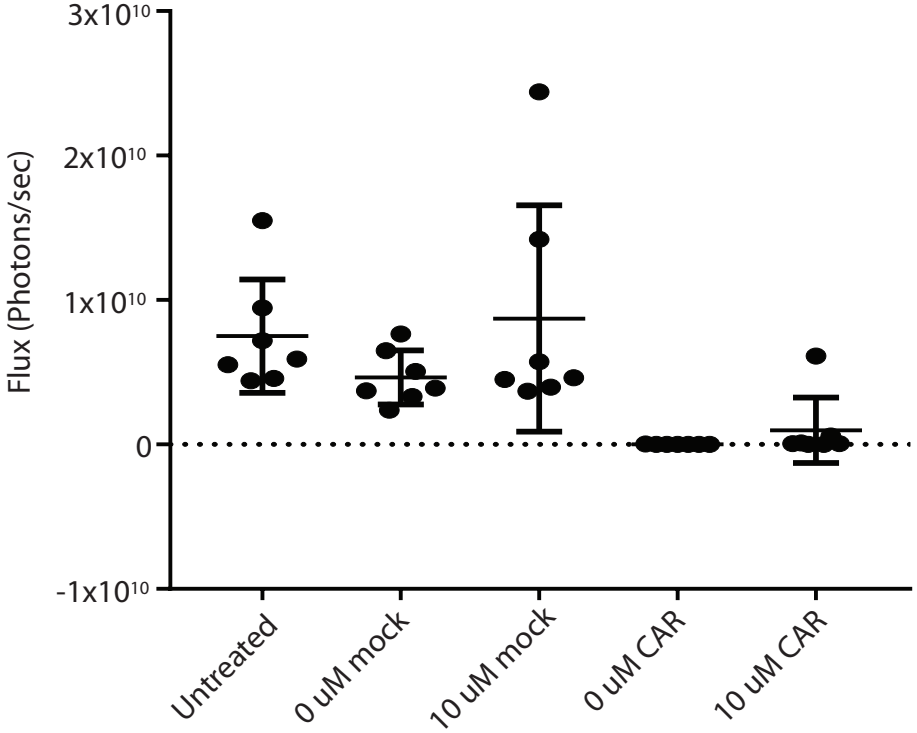
Supplemental Figure 5. Lenalidomide eradicated MM-initiating cells without downregulation of CS1 expression on MM cells MM.1s cells were treated with different concentrations of lenalidomide (0, 1, and 10 μM) (A) Growth of total cell number was determined by Guava Viacount at different time points. (B) In a separate experiment, MM.1s cells were treated with lenalidomide at concentrations of 0 and 1 μM for 48 hours. Following treatment, cells were stained with Hoechst dye at a final concentration of 5 μg/mL. After 2 hours incubation, cells were labeled with 5 μg/mL propidium iodide for 5 min at 4°C. Analysis was conducted with flow cytometry. Percentages of side population in 0 and 1 μM lenalidomide-treated MM.1S cells are depicted. (C) CS1 expression on MM.1s after 48 hours treatment with 0 and 1 μM lenalidomide.



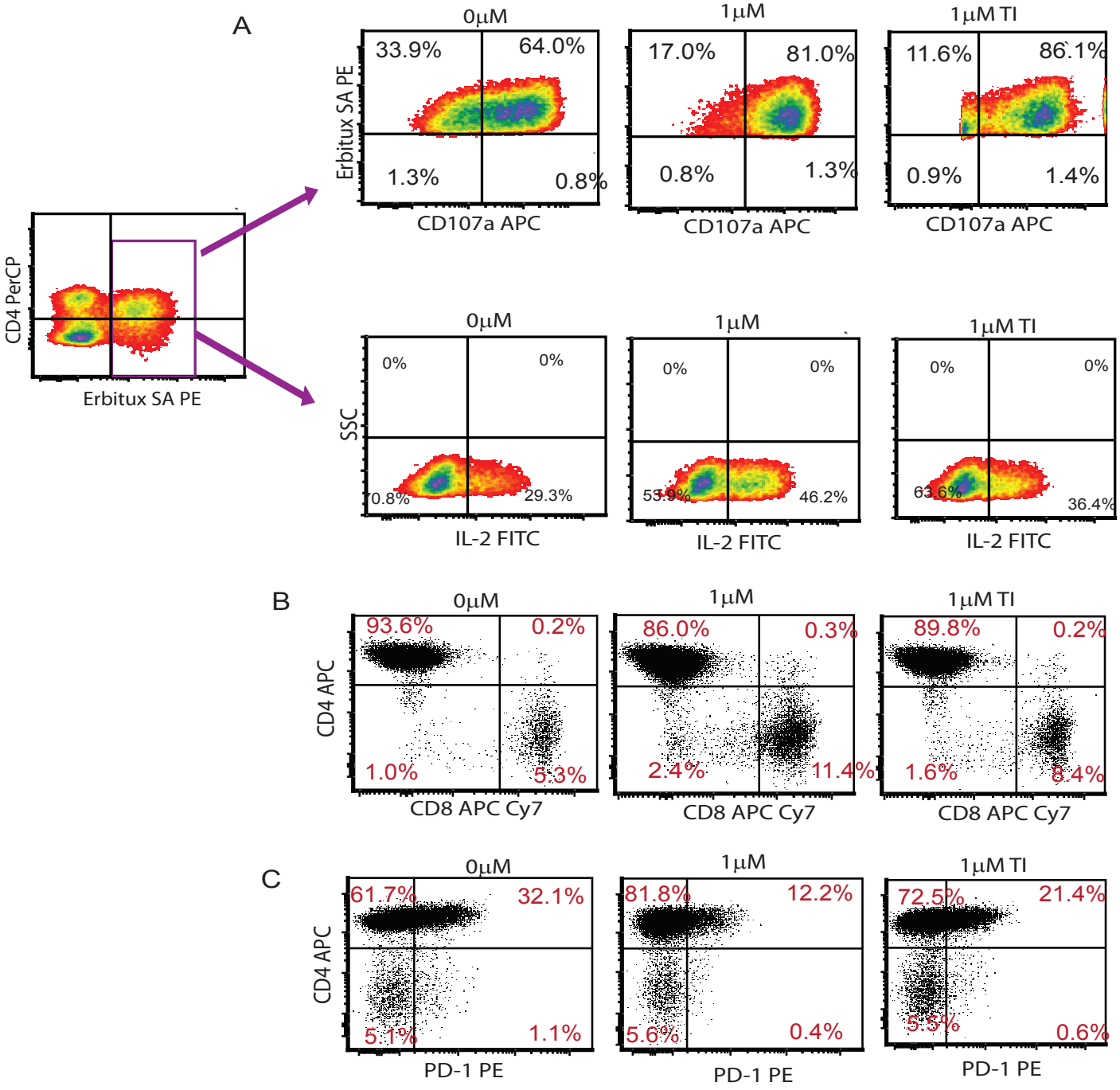
Supplemental Figure 6. Lenalidomide improves immune synapse formation between CS1 CAR T cells and MM cells Tumor cells labeled with CellTracker Blue were co-cultured with the same number of CS1 CAR T cells that had been treated with different concentrations of lenalidomide. After 2 hours incubation, co-cultures were washed and fixed with 4% paraformaldehyde (PFA). CAR T cells were stained with Fab antibody followed by secondary antibody conjugated with Alexa 488. The F-actin was labeled in red. The images from MM.1S and CS1 CAR T cells (A), KG1a and CS1 CAR T cells (B), and non-transduced mock T cells with MM.1S cells and KG1a cells (C) co-culturing are presented.



Supplemental Figure 7. Lenalidomide improved the anti-MM activity of CS1 CAR T cells A total of 2 x 10^6 ffluc GFP MM.1S cells were intratibially (i.t.) injected into NSG mice. Five days following tumor inoculation, mice received a single injection (i.v.) of dosed 1 x 10^6 CAR T cells and 5-7.5 mg/kg lenalidomide i.p injection daily for 30 days. Tumor signals at the indicated time points are presented.



Supplemental Figure 8. Comparison of anti-MM activity of CS1 CAR T cells pre-treated with and without lenalidomide A total of 2 x 10^6 ffluc GFP MM.1S cells were intratibially (i.t.) injected into NSG mice. Five days following tumor inoculation, mice were injected i.v. with dosed 1 x 10^6 CAR T cells that had been treated with 10 μM lenalidomide. Non-treated CS1 CAR T cells and non-transduced mock cells were used as controls. Tumor signals on day 21 are presented. Representative data from two experiments are presented.



Supplemental Figure 9. Lenalidomide enhances CS1 CAR T cell function only when continuous combinatorial therapy is applied Central memory T cells were activated and transduced with lentivirus encoding CS1 CAR and expanded in vitro in the presence of 0 and 1μM lenalidomide for 2 weeks. We then split the cultures into two groups, one continued getting 1μM lenalidomide every other day and another culture stopped lenalidomide as therapeutic interruption (TI). Two weeks later, (A) intracellular IL-2 and degranulation marker CD107a were analyzed upon stimulation with MM.1S cells. (B) Percentages of CD8+ T cells and (C) PD1 expression on the T cells were determined with flow cytometry and presented.