

Figure S4

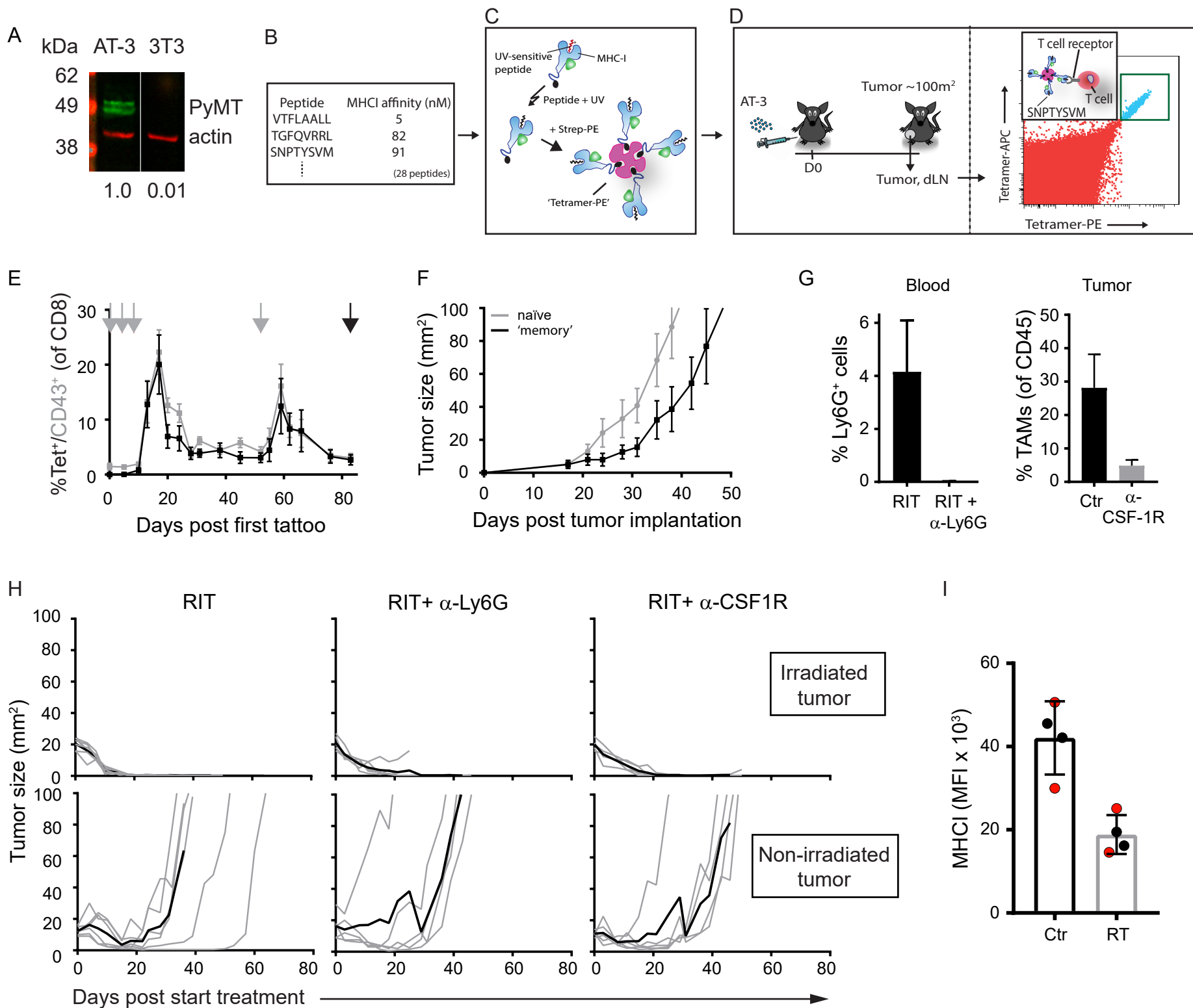


Figure S4. Related to Figure 4. Identification of an AT-3 tumor-specific (CD8) T cell epitope, and targeting either Ly6G or CSF1R does not improve RIT-mediated control of non-irradiated tumor. (A) PyMT and actin immunoblot of AT-3 or 3T3 cell lysates. Note that the left and right lanes are from the same blot and the relative PyMT expression levels are shown below the image. (B) A total of 28 PyMT peptides that are predicted to bind MHC-I with the indicated affinity were synthesized; note that only the three peptides with the highest affinity are shown. (C) Peptide-MHC multimers ("tetramers") were generated for the 28 peptides as shown. (D) CD8⁺ T cells from the AT-3 tumor and dLN were analyzed for tetramer-binding, identifying SNPTYSVM as a tumor antigen. (E-F; related to Figure 4A-D) Mice (6 per group) received SNPTYSVM vaccination (for details see Figure 4A) on days 0, 3, 6 and 52 (gray arrows). The CD8⁺ T cell response was then followed in the peripheral blood with H-2Kb/SNPTYSVM tetramers (black symbols), and the CD43 mAb (gray symbols). On day 83 (the black arrow), the mice were implanted with AT-3 tumor cells. (F) Time course of the tumor size of "memory" mice (i.e. the mice shown in Fig. S4E that received DNA vaccination at the times indicated by the black arrow), and age-matched naïve mice (shown in gray lines) after implantation with 2×10^5 AT-3 tumor cells in the fourth mammary fat pad. (G: related to Figure 4G) Percentage of Ly6G⁺ (left) and TAMs (right) measured in the blood and tumor tissue of mice treated with RIT in the absence or presence of anti-Ly6G or anti-CSF-1R mAbs. (H) Time course of the size of the irradiated and non-irradiated tumors in mice treated as indicated. The gray and black lines represent individual and average data, respectively, for the mice shown in Figure 4G. (I) MHC I expression on tumor/stromal cells (CD45⁻) from mice bearing irradiated (RT) and non-irradiated (Ctr) AT-3 tumors. Data shown is from Day 3 (red circles) and Day 8 (black circles) after treatment (2 mice per timepoint).