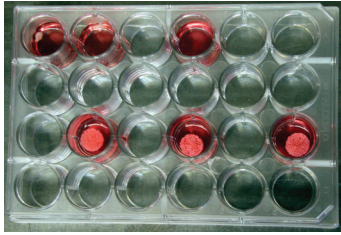
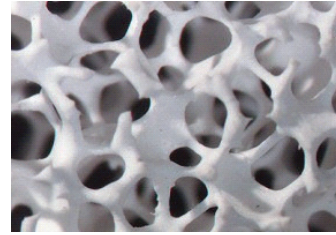
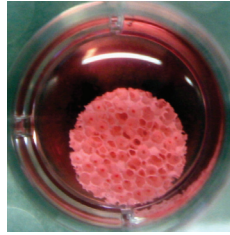


Supplementary Figure 1: Controlling lack of HP 15 hours after in vivo transfer of naïve 2C T cells. CFSE-labeled naïve 2C T cells (5×10^6) were transferred into a Rag2^{-/-}. After 15 hours, inguinal LNs were harvested, homogenized and analyzed by flow cytometry for CFSE dilution of the transferred CD8⁺, 1B2⁺ 2C T cells. Shown are results representative of two individual experiments.

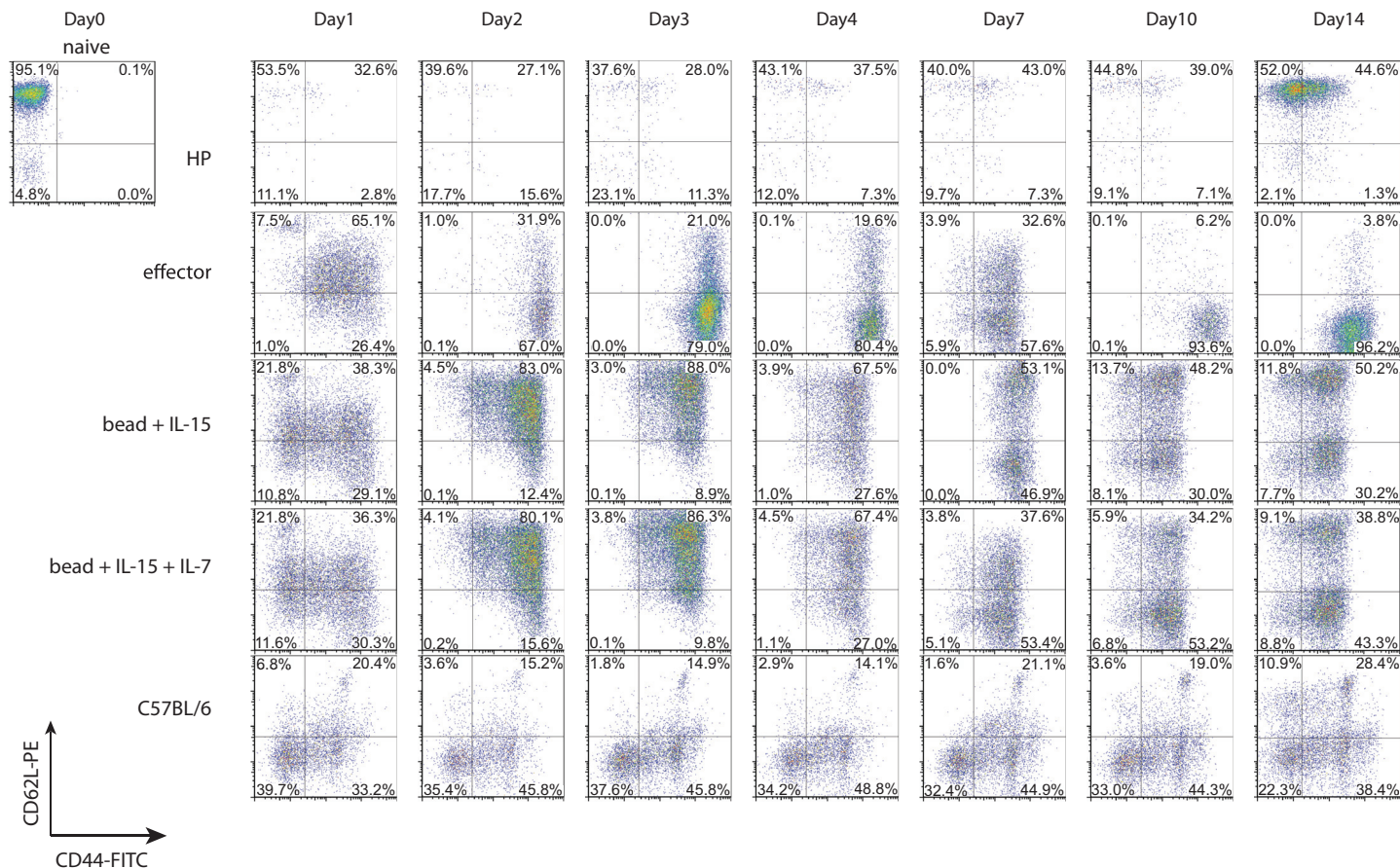
Sponceram discs in 24 well plate



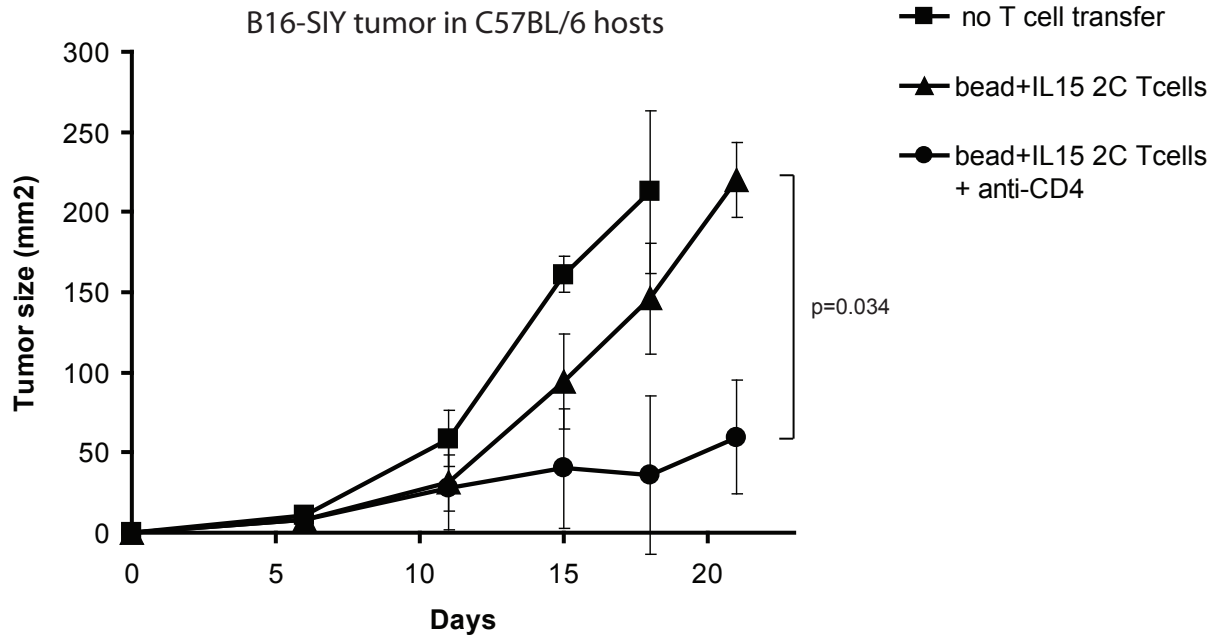
Sponceram magnification



Supplementary Figure 2: Three-dimensional culture system. Collagen-coated Sponceram® discs were loaded with FRCs (day -2) or DCs (day -1) before adding naïve CFSE-labeled 2C T cells. Represented are the discs in culture medium. A microscopical picture of the structure provided by the company is shown in the magnification.



Supplementary Figure 3: Kinetic analysis of 2C T cell phenotypes during HP and in vitro stimulations. Flow cytometry analysis of CD62L and CD44 is shown for CD8⁺, 1B2⁺ lymphoid cells at the indicated days. Day 0 shows naïve T cells. HP represents analysis from the blood of Rag2^{-/-} mice that received 3-5x10⁶ naïve 2C T cells on day 0. The effector group shows naïve T cells in vitro stimulated with P815.B7.1 (at a ratio 1:5). The bead groups include T cells stimulated in vitro with a 3 times excess of anti-CD3/CD28 coated beads in the presence of 200 ng/ml IL-15, 400 ng/ml IL-15RFcIg in combination with 50 n/ml IL-7 when indicated. Aliquots of cryopreserved C57BL/6 splenocytes were used as a control for performing the surface staining on various days.



Supplementary Figure 4: Tumor growth control in wild type C57BL/6 is mediated by in vitro generated memory-like T cells in synergism with CD4 T cell depletion. On day 0, C57BL/6 mice were inoculated s.c. with 5×10^5 B16-SIY tumor cells (stably gene modified to express the SIY peptide presented in H2-Kb which is also recognized by the 2C TCR). On day 1, 1×10^6 bead + IL-15 stimulated 2C T cells were transferred i.v.. Where indicated, mice received 100ug anti-CD4 depleting antibody i.v. on days 0, 3, 7 and 10. Results are shown as mean \pm SEM. Differences between the indicated groups are significant ($P=0.034$) as calculated by Wilcoxon rank sum test.