

**Supplementary Figure 1**. Biodistribution of 4-1BB-raptor conjugates. Aptamer-sense siRNA was transcribed in the presence of 250 μCi α<sup>32</sup>P-ATP and annealed with the antisense oligonucleotide as described in Methods. C57BL/6 mice were transferred with OT-I cells and activated by OVA/LPS injection. 4 hours after peptide administration mice were injected with 1 nmole (2.2x10<sup>6</sup> cpm) of either 4-1BB-raptor or scrambled aptamer-raptor conjugates. **A**. Spleen, lymph node, and bone marrow cells were pooled 24 hours later and 4-1BB-expressing cells were purified using 4 -1BB antibody coated microbeads (Miltenyi Biotec, Cambridge, MA). Fractions of cells retained on columns and passed through were collected, counted and <sup>32</sup>P radioactivity was determined by liquid scintillation using a Wallac micro-beta scintillation counter (Perkin Elmer, Waltham, MA). **B.** 24 hours after aptamer injection animals were sacrificed and tissue samples were homogenized, washed, and analyzed for <sup>32</sup>P radioactivity.



**Supplementary Figure 2**. <u>4-1BB aptamer-targeted delivery of raptor siRNA to OT-I cells</u>. **A**. Biodistribution. Peptide/LPS vaccinated OT-I transferred mice were administered 1nmole of <sup>32</sup>P-labeled 4-1BB-raptor conjugate (2.2x10<sup>6</sup> cpm) via the tail vein 4h after vaccination (see legends for Fig. S1). 24h later splenocytes were isolated, CD45.1+ (OT-I), CD45.1- host CD8+ cells, CD4+ cells, CD19+ B cells, and F4/80 macrophages were purified using Milteny magnetic microbeads and radioactivity was measured. B. mTORC1 inhibition. Peptide/LPS vaccinated mice were treate with rapamycin, 4-1BB-GFP or 4-1BB raptor conjugates. Splenocytes were isolated after 48 hours and analyzed by multiparameter flow cytometry for mTORC1 activity (Phospho-S6) in CD8+CD45.1+ cells(OT-I), CD8+CD45.1- cells (host CD8+ T cells), CD4+FoxP3- cells (conventional CD4+ T cells), CD4+FoxP3+ cells (Treg) and CD19+ cells (B cells). Results were normalized to percent of mTORC1+ cells in mock-treated mice (2 mice per group).



**Supplementary Figure 3**. <u>CD4 and CD8 T cells contribute to protective immunity</u>. C57BL/6 mice were vaccinated with GM-CSF-expressing irradiated B16/F10 tumor cells (GVAX) and treated with rapamycin or with either 4-1BB-GFP or 4-1BB-raptor conjugates. At day 40 mice were challenged with  $10^6$  B16/F10 melanoma cells and survival was determined (5 mice per group). At day 39, 42 and 45, 100 µg of GK1.5 anti-CD4 Ab or 2.43 anti-CD8 Ab were administered i.p., condition that lead to a 99% reduction in CD4+ and CD8+ cells, respectively, in the spleen for at least 7 days (data not shown). Statistical analysis of groups treated with GVAX + 4-1BB-raptor: Isotype Ab versus anti-CD4 Ab, P=0.0640; Isotype Ab versus CD8 Ab, P=0.0052.