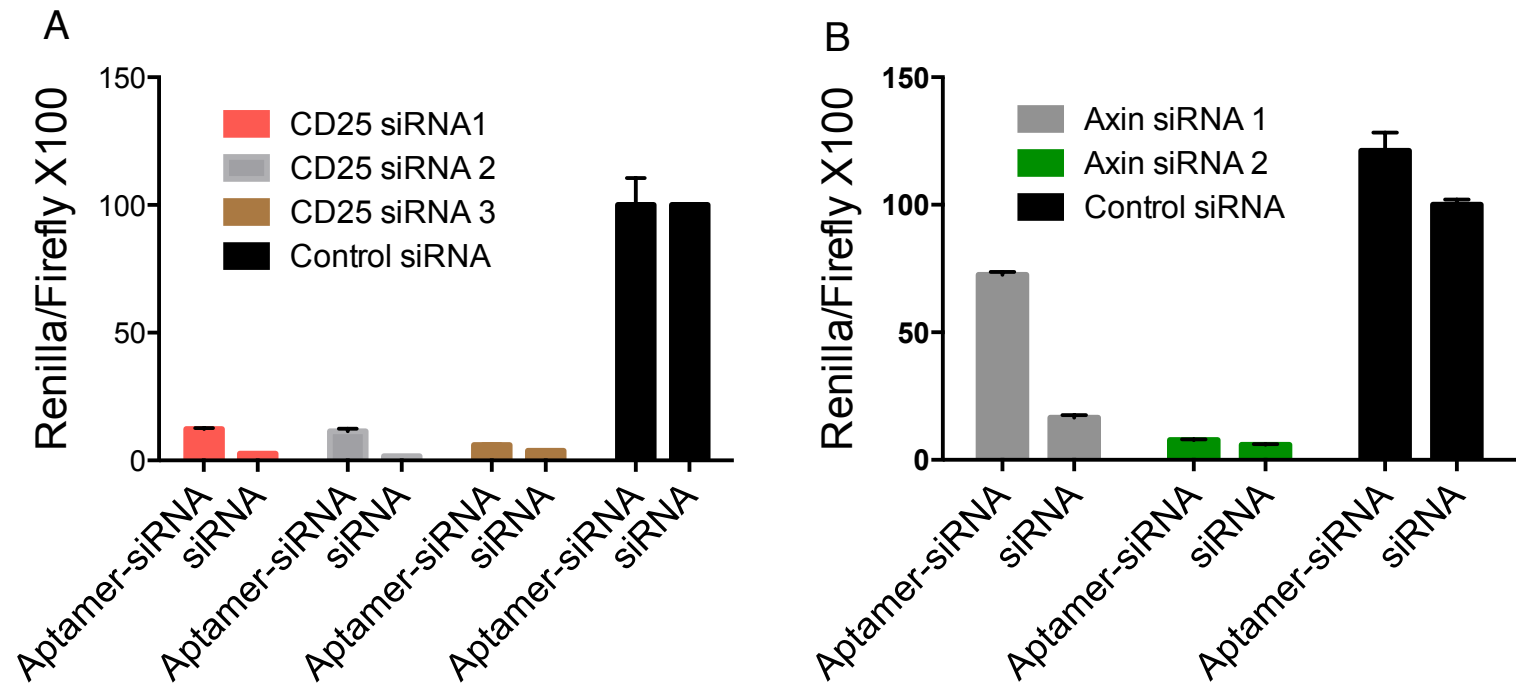


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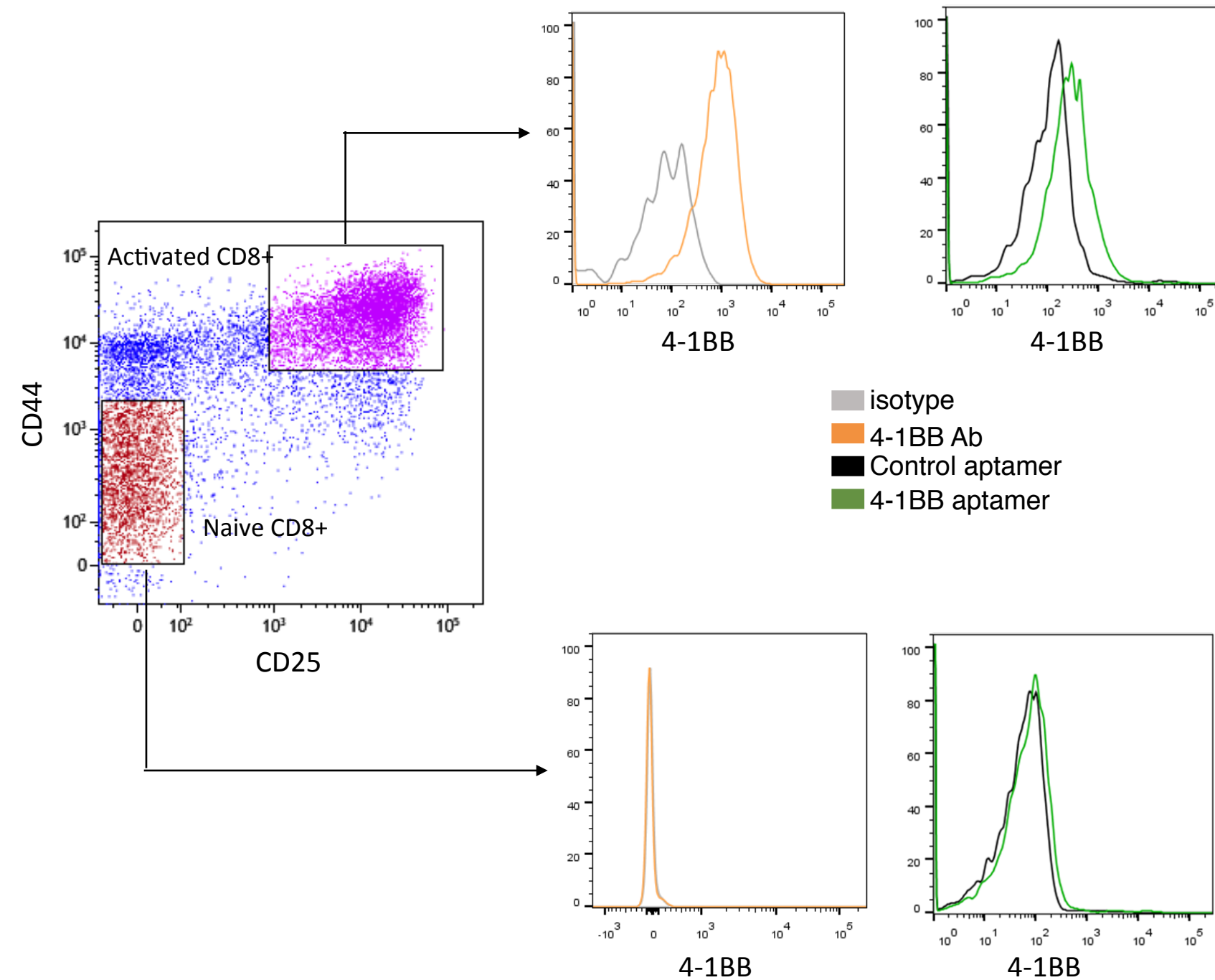
## **Supplemental Information**

### **Aptamer-Targeted Attenuation of IL-2 Signaling in CD8<sup>+</sup> T Cells Enhances Antitumor Immunity**

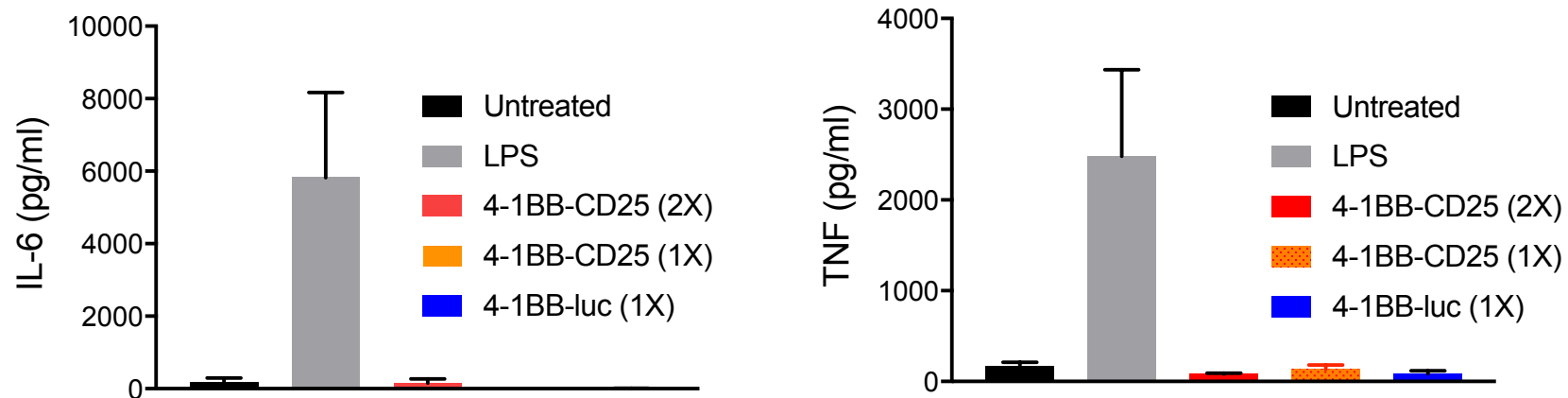
**Anugraha Rajagopalan, Alexey Bereznoy, Brett Schrand, Yvonne Pupilampu-Dove, and Eli Gilboa**



**Figure S1** : Activity of unconjugated and 4-1BB aptamer conjugated siRNAs. siRNA activity was measured in the  $\Psi$ CHECK<sup>®</sup> assay (Fig. 1a and Methods). **a.** CD25 siRNAs. **b.** Axin siRNAs.

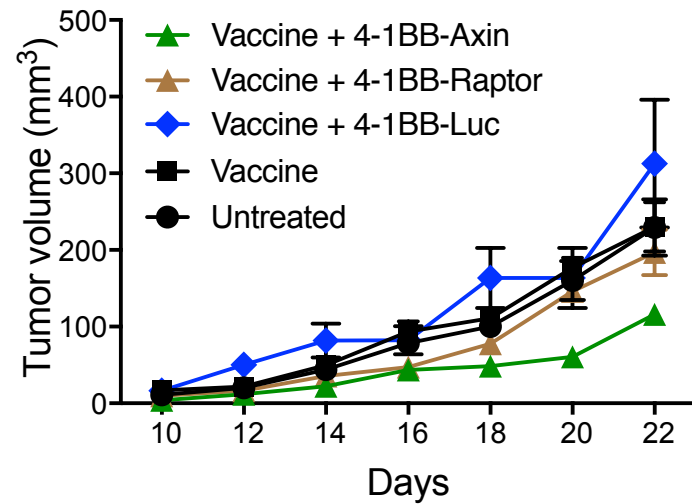


**Figure S2. Specificity of 4-1BB aptamer binding to 4-1BB expressing T cells.** Splenocytes from C57BL/6 mice were activated using anti-CD3 antibody. After 24 hours, they were mixed 1:1 with naïve splenocytes. Cells were stained with fluorophore labeled anti-CD8, anti-CD44, anti-CD25 and anti-4-1BB antibodies, and with fluorophore labeled 4-1BB aptamers as described in Methods. 4-1BB expression on activated and naive CD8+ T cells was determined by gating on CD44<sup>high</sup> CD25<sup>high</sup> and CD44<sup>low</sup> CD25<sup>low</sup> populations, respectively.

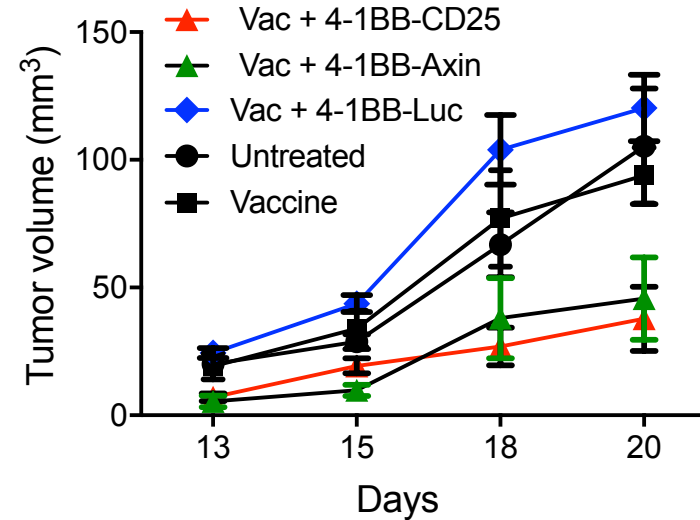


**Figure S3 :** Serum cytokine levels in mice administered with 4-1BB aptamer-siRNA conjugates, Mice were administered with 20  $\mu$ g of LPS, 1 or 2 nanomoles of conjugate/injection (1X and 2X, respectively) as described in Methods and serum levels of IL-6 and TNF measured 24 hours later.

A.



B.



**Figure S4.** Enhancing vaccine-induced tumor immunity in 4T1 tumor bearing mice vaccinated with MHC class II and B7-1 expressing irradiated 4T1 tumor cells and treated with 4-1BB aptamer-targeted siRNA conjugates. A. Comparison of Axin and Raptor siRNAs. Treatment started at day 7 post tumor implantation. B. Comparison of Axin and CD25 siRNA. Treatment started at day 7 post tumor implantation. For details see Methods and legends to Fig. 4.