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Supplemental Data

Assembling OX40 Aptamers on a Molecular Scaffold to Create a Receptor-Activating Aptamer

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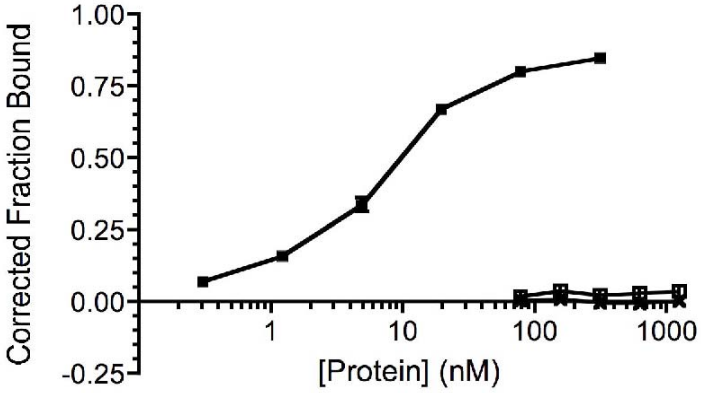

Figure S1. Aptamer 9.8 Binds to the Extracellular Portion of Murine OX40
The constant region (Fc) of human IgG and protein G were part of the selection since the selection target consisted of a fusion protein of the extracellular portion of murine OX40 to the human IgG Fc that was immobilized through binding to protein G coated beads. We therefore verified the binding specificity of aptamer 9.8 to the extracellular portion of murine OX40: Binding affinities to murine OX40 human IgG Fc fusion protein (■) were compared to human IgG (○) as well as protein G (∗) to demonstrate aptamer specificity to the extracellular portion of OX40 (n=3, error bars represent SEM).

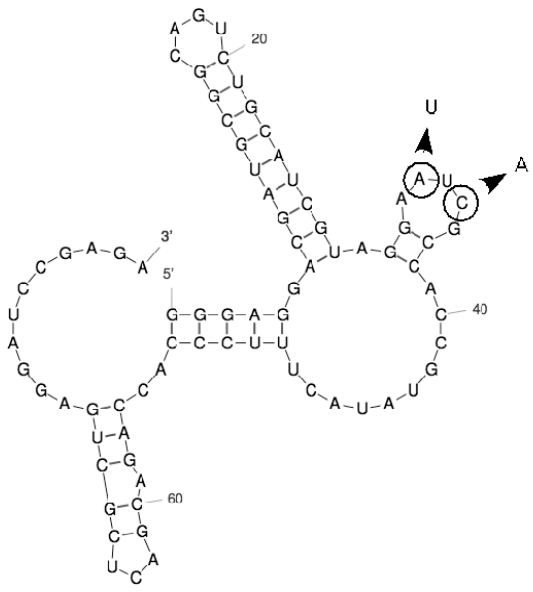

Figure S2. Predicted Structure of Aptamer Clone 9.8 and Point Mutant Aptamer Design

Lowest free energy predicted secondary structure of the OX40 aptamer was determined using the structure prediction program M-Fold by Michael Zuker (http://bioweb.pasteur.fr/sequanal/interfaces/mfold.html). The two nucleotides that are altered in the control mutant aptamer are circled.

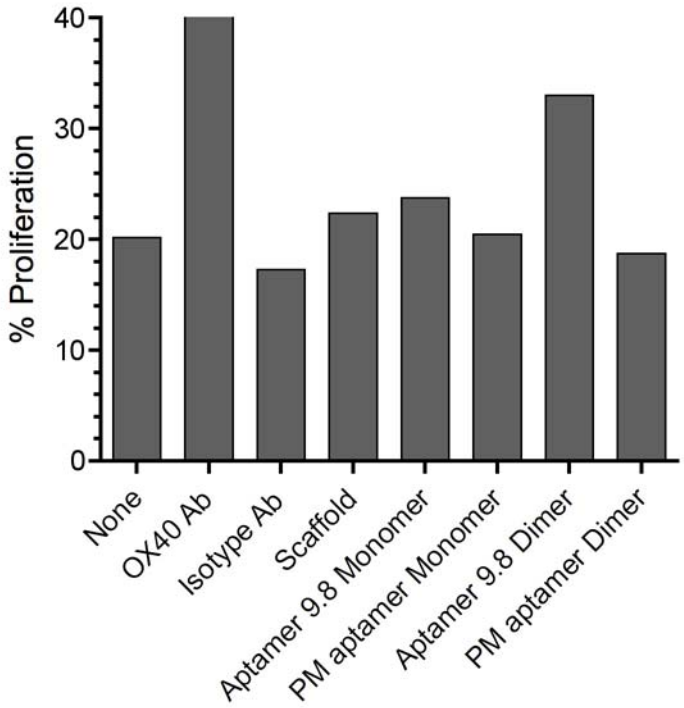

Figure S3. Monomeric Aptamer 9.8 Is Unable to Induce OX40 Function
50 µg of *Staphyloccocal enterotoxin B* were administered to female Balb/c mice intraperitoneally. Auxillary, inguinal and mesenteric lymph nodes were harvested after 24 hours. Cells were teased into single cell suspension and labelled with carboxyfluoroscein succinimidyl ester (CFSE). 10^5 cells were cultured for 72 hours in the presence of 0.5ng/mL *Staphyloccocal enterotoxin B*. Experimental groups also included 33nM OX40 agonistic antibody (OX86), isotype control, 66nM of aptamer dimer, point mutant aptamer dimer or equivalent amounts of aptamer monomer or scaffold. Groups were set up in five replicates and pooled for analysis. Cell proliferation data was collected using flow cytometry using a FACScalibur and evaluated using CellQuest software.

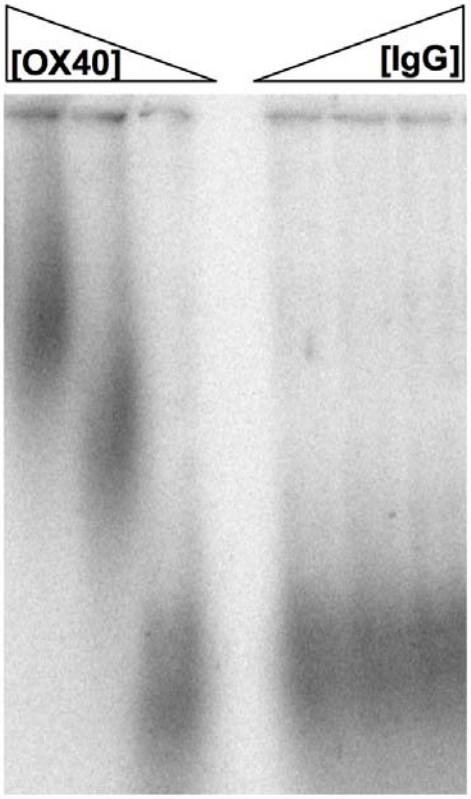

Figure S4. The Dimerized Aptamer Is Capable of Interacting with Two OX40 Proteins

A fixed amount of 5'labeled, gel purified aptamer 9.8 dimer was incubated with either increasing concentrations of murine OX40 Fc fusion protein (0, 75nM or 150nM) or equal concentrations of human IgG in buffer containing 150mM NaCl, 2mM CaCl2, 20mM Hepes (pH 7.4) and 0.01% BSA. RNA-protein complexes were then separated on a 6% native polyacrylamide gel containing 0.3X TBE, 20mM CaCl2 at 10W at RT.

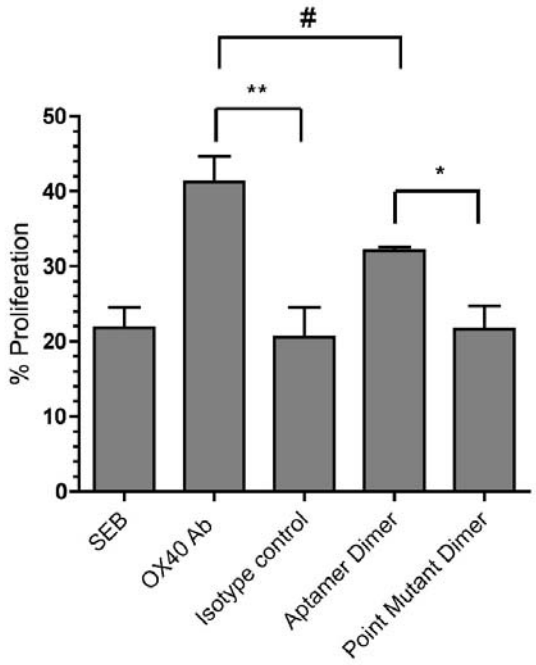

Figure S5. RNA Aptamer Dimers Are Capable of Enhancing Staphylococcal Enterotoxin B Induced Proliferation

The aptamer dimer's effect on proliferation of SEB primed lymph node cells was assessed by flow-cytometric analysis of the CFSE labeled cells. Mean percentage of proliferating cells of 3 independent experiments. Error bars indicate SEM; *P<0.05, **P<0.01, # not significant.

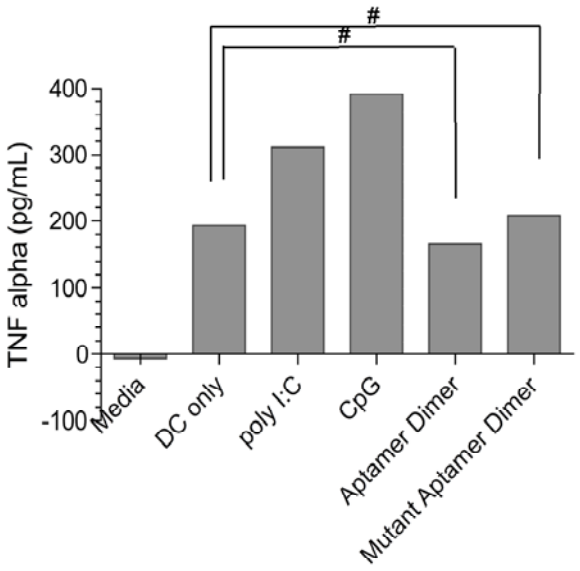

Figure S6. Secretion of the Cytokine TNFα Is Not Induced upon Receptor Activating Aptamer-Mediated OX40 Activation but Is Enhanced upon Toll Like Receptor Activation through CpG Oligonucleotides

To rule out that the observed in vitro effects on T-cells could stem from toll like receptor activation, we evaluated the concentration of the cytokine TNFα in the supernatants of treated DCs. To this end, bone marrow derived DCs were generated (see methods for details). After 6 days of culture in the presence of IL4 and GMCSF, 66 nM aptamer dimer, point mutant aptamer dimer, 1µM CpG or 20µg/ml polyI:C were added to the cells. After overnight culture, supernatants were harvested. The concentration of TNFα in these supernatants was determined using the OptEIA mouse TNFα ELISA kit (BD biosciences); n=3, error bars represent SEM; # n.s.

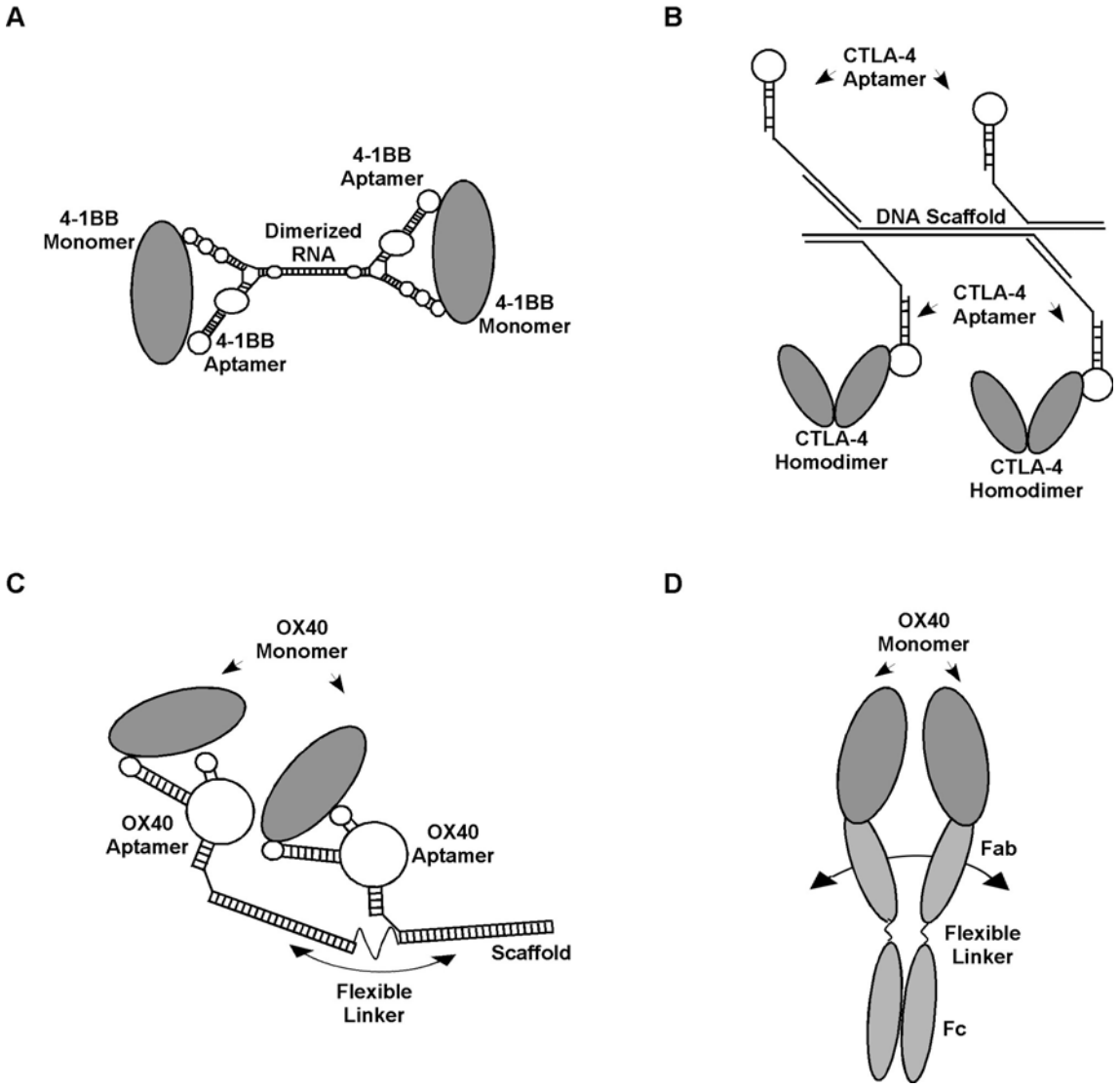

Figure S7. Schematic of Different Aptamer Multimerization Approaches
(A)Two 4-1BB aptamers are dimerized through the addition of complementary single-stranded overhangs, which are annealed to one another to form a linker that is ca. 65Å in length and fairly rigid.
(B) Four CTLA-4 aptamers were annealed to a complementary scaffold to create a tetravalent compound. The tetravalent molecule is capable of binding multiple CTLA-4 homodimers, leading to increased avidity.
(C) Two OX40 aptamers are annealed to a scaffold consisting of a tandem-repeat of a 20-nucletotide randomized region, that are separated by a flexible polyethylene linker. This scaffold-based approach is more flexible than the rigid approach.
(D) Depiction of the interaction between an agonistic antibody specific to OX40 and its molecular target. The antibody induces receptor function by dimerization of receptor monomers. The presence of the hinge region allows flexibility of the Fab arms as well as rotational freedom of the individual Fab relative to the Fc portion.