

Supplemental Figure 1: Fab affinities for CTLA-4. Reference subtracted SPR sensorgrams of analytes XTX100 (A), internally generated ipilimumab-analog (B), and clinical-grade ipilimumab (C) Fabs at serial dilutions of 0.781 to 100 nM flowed over immobilized recombinant human CTLA-4. Assay was performed at 37 °C. Calculated k_a , k_d , and K_D are reported.

Supplemental Figure 2: Affinity of anti-CTLA-4 mAb for FcγRIIIa. Reference subtracted SPR sensorgrams of analytes ATX110 (A), XTX100 (B), and XTX101 (C) at serial dilutions of 15.6 to 250 nM flowed over immobilized recombinant human FcγRIIIa. Assay was performed at 25 °C. Calculated k_a , k_d , and K_D are reported.

Supplemental Figure 3: In vitro activation of XTX101 with recombinant enzymes. XTX101 was incubated with 20 nM of recombinant human MMP-1, -2, -7, -9, -10, or -14 at 37 °C for specified amounts of time. Samples were analyzed by reducing CE-SDS, and results are shown as the average ± SD of 2 independent experiments. Calculated catalytic efficiencies are shown in Supplemental Table 3.

Supplemental Figure 4: XTX101 is activated by multiple human MMPs. XTX101 was incubated with 30 ng of indicated recombinant human MMPs overnight at 37 °C. Samples were subsequently assessed for activity by CTLA-4 binding ELISA. Technical replicates were performed, and the average K_D +/- standard error (SE) are reported.

Supplemental Figure 5: XTX101 Monotherapy in MB49 Tumor Model. For the TGI study, B-hCTLA-4 mice bearing syngeneic MB49 murine bladder carcinoma tumors (~150 mm³) were treated i.v. with a single dose of XTX100 (black), XTX101 (red), or isotype (gray) mAb at indicated dose levels (n=8 per group). Tumor volume was measured twice weekly and plotted as mean ± SEM. **** p <0.0001, compared to isotype control.

Supplemental Figure 6: Pharmacodynamic effect of XTX101 Monotherapy in MB49 Tumor Model. Immunophenotypic analyses by flow cytometry of live CD45⁺ CD3⁺ CD4⁺ Foxp3⁺ Tregs and live CD45⁺ CD3⁺ CD8⁺ T cells from spleens were measured on Day 7.