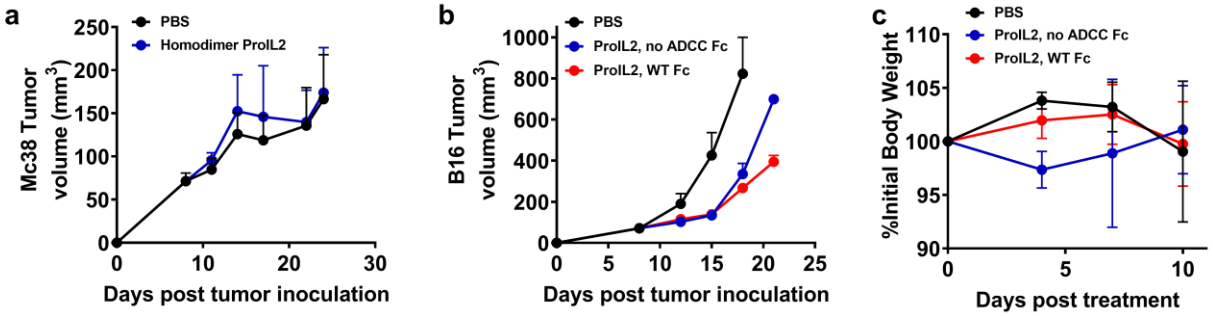
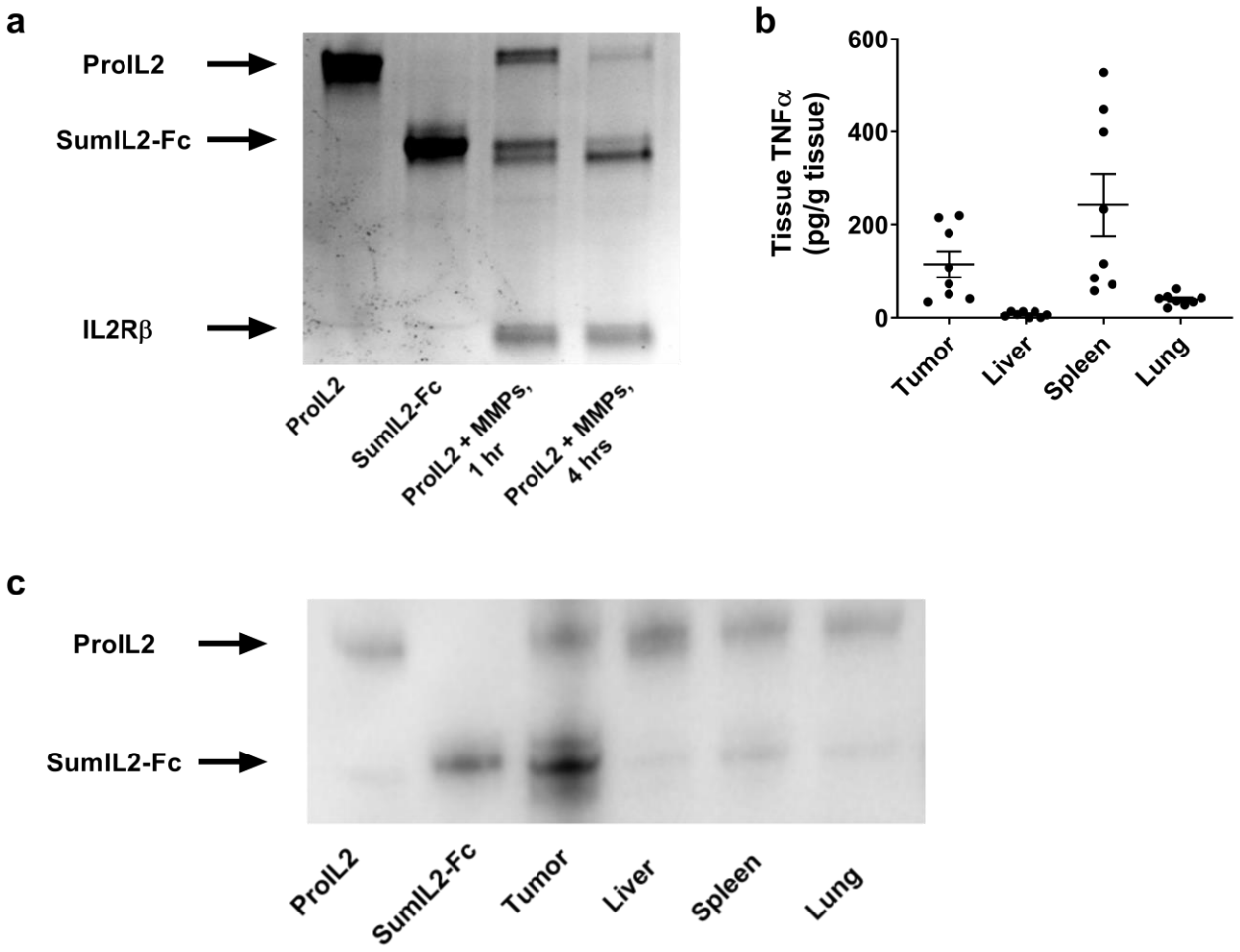
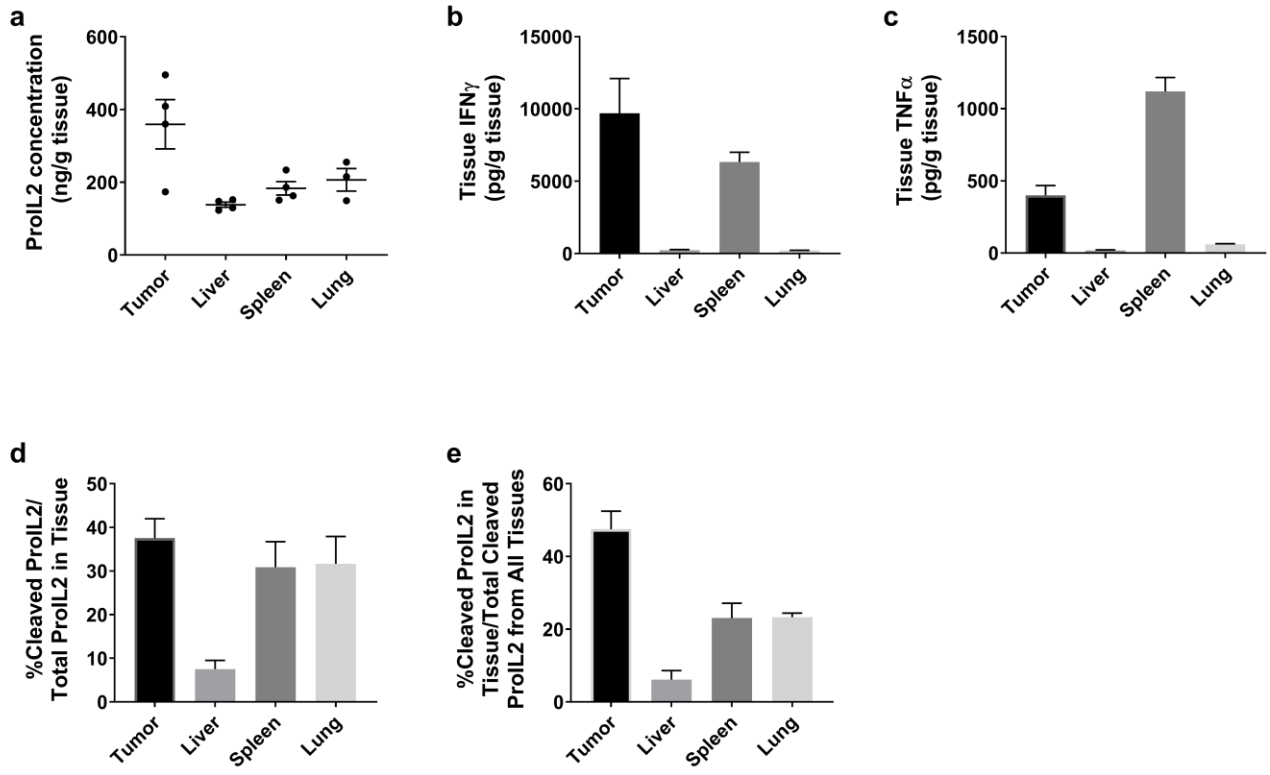


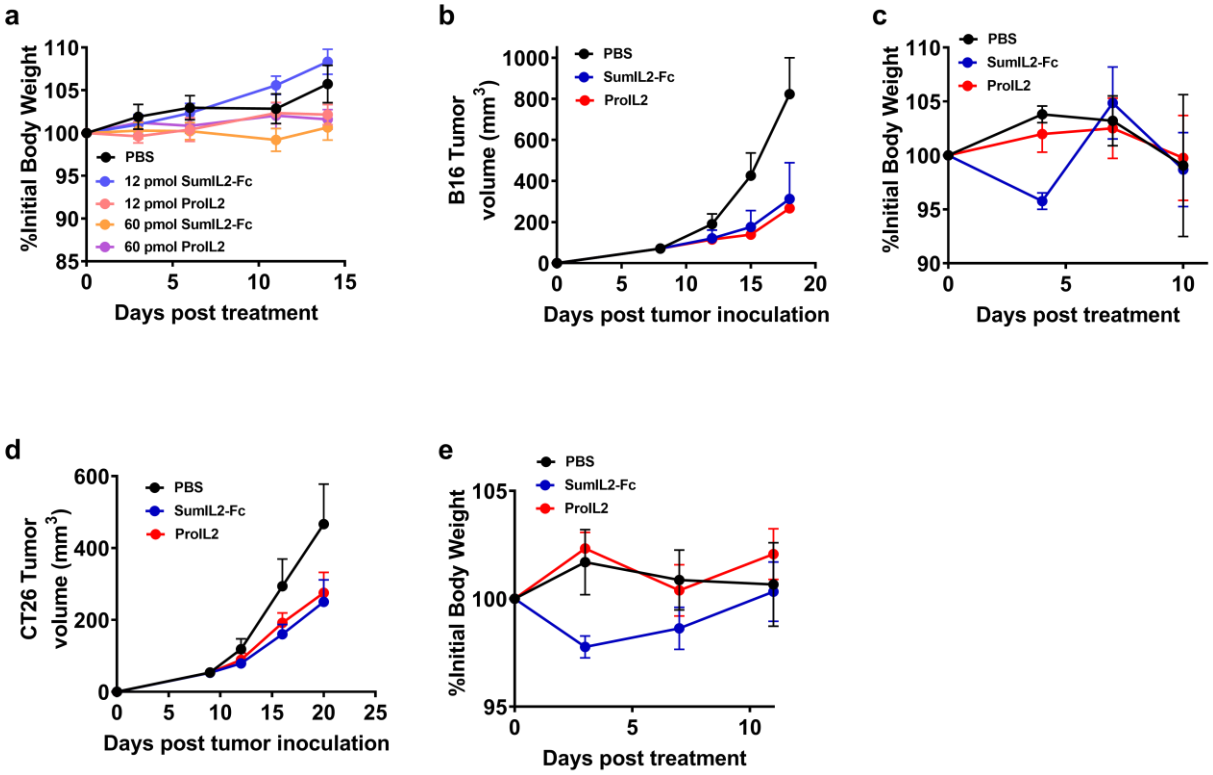
Supplementary Figure 1. Testing and optimizing ProIL2 designs. a MC38 s.c. tumor bearing mice were injected i.p. with PBS or a homodimeric form of ProIL2 (300 pmol) one time on day 9 post tumor inoculation; tumor growth was measured. **b-c** B16 s.c. tumor bearing mice were injected i.p. with PBS, the best ProIL2 candidate with a no ADCC mutant Fc, or the best ProIL2 candidate with a WT hIgG1 Fc (300 pmol) one time on day 9 post tumor inoculation; tumor growth and body weight were measured. Data represent the mean ± s.e.m of replicates.



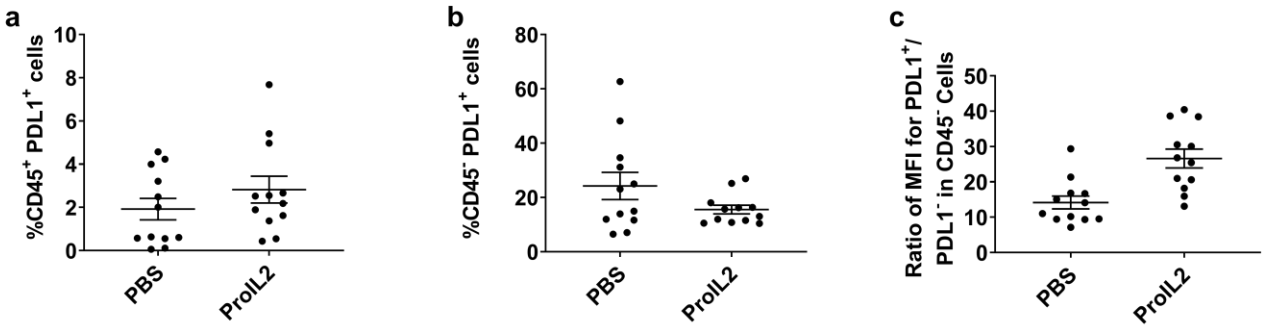
Supplementary Figure 2. Cleavage and Biodistribution of ProIL2 *in vitro* and *in vivo*. a ProIL2 was incubated with human MMP2, MMP9, and MMP14 for 1 or 4 hours, then run on non-reducing SDS-PAGE. ProIL2 and SumIL2-Fc were also run as controls. **b-c** 200 pmol ProIL2 was injected i.p. into CT26 s.c. tumor bearing mice, and the labeled tissues were collected and homogenized 24 hours after treatment. **b** Cytometric Bead Array was used to quantify the amount of TNFα in each tissue homogenate. **c** hIgG immunoprecipitation of equivalent weights of tissue homogenate was performed with Protein A binding beads, and then run on Western Blot with hIgG binding antibody. Data represent the mean ± s.e.m of replicates.



Supplementary Figure 3. Biodistribution of ProIL2 in B16 tumor bearing mice. 300 pmol ProIL2 was injected i.p. into B16 s. c. tumor bearing mice, and the labeled tissues were collected and homogenized 24 hours after treatment. **a** hIgG ELISA was used to quantify the amount ProIL2 in each homogenate, normalized by total tissue weight. 1 representative experiment is shown. **b-c** Cytometric Bead Array was used to quantify the amount of IFNγ or TNFα in each tissue homogenate. **d-e** hIgG immunoprecipitation of equivalent weights of tissue homogenate was performed with Protein A binding beads, and then run on Western Blot with hIgG binding antibody. **d** Relative gel band intensity per lane was quantified. **e** Western Blot relative gel band intensities were quantified, and within each mouse, the total percentage of cleaved ProIL2 between each tissue was compared and quantified. Data represent the mean ± s.e.m of replicates.



Supplementary Figure 4. ProIL2 has equivalent antitumor efficacy to SumIL2-Fc without toxicity in multiple tumor models. a MC38 s.c. tumor bearing mice were injected i.p. with one dose of the labeled treatment on day 9 post tumor inoculation; body weight was measured. **b-c** B16 s.c. tumor bearing mice were injected i.p. with PBS, SumIL2-Fc or ProIL2 (300 pmol) one time on day 9 post tumor inoculation; tumor growth and body weight were measured. **d-e** CT26 s.c. tumor bearing mice were injected i.p. with PBS, SumIL2-Fc or ProIL2 (300 pmol) one time on day 9 post tumor inoculation; tumor growth and body weight were measured. Data represent the mean ± s.e.m of replicates.



Supplementary Figure 5. ProIL2 can synergize with anti-PDL1 therapy. B16 s.c. tumor bearing mice were injected i.p. with PBS or ProIL2 (300 pmol) one time on day 9 post tumor inoculation. 72 hours after treatment, mice were euthanized and tumors were extracted, digested in collagenase/DNAse, and resuspended as single cells. **a-b** Flow cytometry was used to quantify the total percentage of $CD45^+/PDL1^+$ cells or $CD45^-/PDL1^+$ cells in each tumor. **c** In $CD45^-$ cells, the MFI of $PDL1^+$ and $PDL1^-$ cells were compared. Data represent the mean ± s.e.m of replicates.