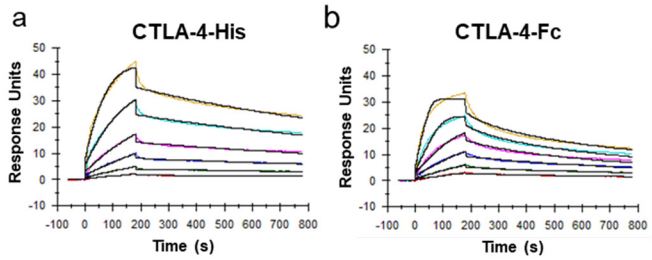
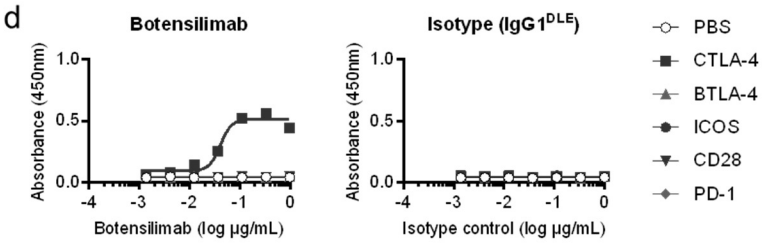
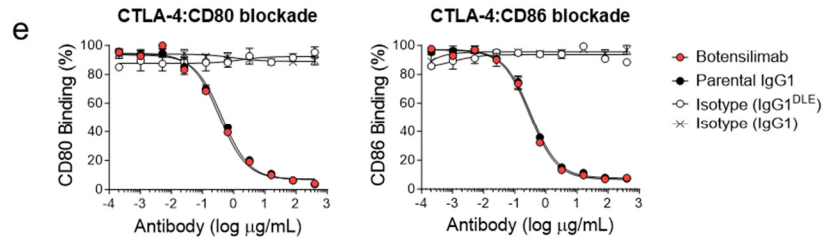
Supplementary Figure S7

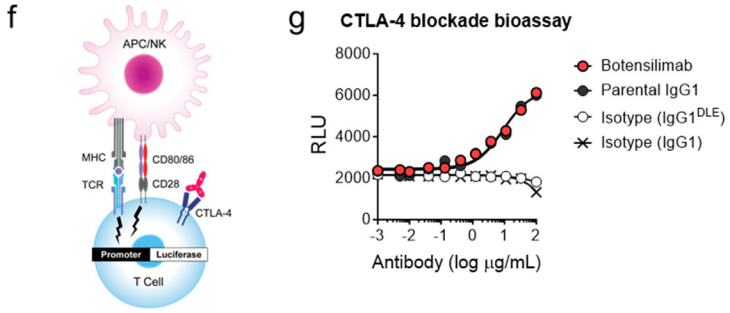


c

Human recombinant proteins	Botensilimab K_D (nM)	Parental IgG1 K_D (nM)	Ipilimumab K_D (nM)
CTLA-4-His	0.87	0.33	0.89
CTLA-4-Fc	0.42	0.45	0.37







Supplementary Figure S7. Botensilimab binds selectively to CTLA-4 and completely blocks CTLA-4 interactions with CD80 and CD86 to enhance T cell activation. Binding affinity of botensilimab to human CTLA-4 determined by surface plasmon resonance. Representative sensorgram showing binding of botensilimab to recombinant human **(a)** CTLA-4-His and **(b)**

CTLA-4-Fc. Concentrations: 30 (yellow), 15 (cyan), 7.5 (pink), 3.75 (blue), 1.875 (green) and 0.93 (red) nM. Fitted binding curves in black. **(c)** Binding association (Ka), binding dissociation (Kd) and affinity measurements (K_D) for botensilimab, parental IgG1 and ipilimumab to human CTLA-4-His and CTLA-4-Fc by surface plasmon resonance. Data are average of two individual experiments. **(d)** Botensilimab binds selectively to CTLA-4, but not other CD28 family members. Binding of botensilimab or an Fc-enhanced isotype control antibody ($IgG1^{DLE}$) to recombinant CD28 family members immobilized to an assay plate measured using an enzyme-linked immunosorbent assay. Binding was detected using a horseradish peroxidase labelled secondary antibody and absorbance measured at 450 nm. **(e)** Ability of botensilimab to block cell-expressed CTLA-4 binding to CD80-Fc and CD86-Fc. CTLA-4 expressing Chinese hamster ovarian cells were incubated with botensilimab, parental IgG1 (an IgG1 variant of botensilimab), $IgG1^{DLE}$, or IgG1 isotype controls, followed by incubation with 0.2 µg/mL fluorescent-labelled recombinant CD80-Fc or CD86-Fc. The mean fluorescence intensity (MFI) of CD80- or CD86-bound cells was assessed by flow cytometry. **(f)** Illustration of the CTLA-4 ligand blocking bioassay (Promega) showing target cells (Jurkat cells expressing CTLA-4 and an IL2-dependent luciferase reporter) co-cultured with antigen-presenting cells (APC) engineered to express CD80, CD86 and an antigen-independent T cell activator. αCTLA-4 blocks CTLA-4 interactions with CD80 and CD86 to re-establish T cell receptor (TCR) and CD28 pathway activated luminescence. **(g)** Dose response of luciferase activity induced by botensilimab, parental IgG1, $IgG1^{DLE}$, or IgG1 isotype controls. Luciferase activity measured as relative luminesce units (RLU).