Supplementary Table S1: Proteins for BLI Octet study

Protein	Vendor	Cat. No.
Human CD25	Acro Biosystems	ILA-H52H9
Human CD122	Acro Biosystems	CD2-H5221
Mouse CD25	Acro Biosystems	ILA-M52H9
Mouse CD122	Sino Biological	50792-M08H-20
Cynomolgus CD25	Sino Biological	90265-C08H-50
Rhesus/Cynomolgus CD122	Sino Biological	90328-C08H-50

Supplementary Table S2: Flow cytometry markers for STAT5 signaling study

Marker	Fluorochrome	Vendor	Cat. No.
pSTAT5	AF647	BD Biosciences	562076
CD3	PerCP-Cy5.5	BioLegend	300430
CD4	AF700	BioLegend	300526
CD8	APC-Cy7	BioLegend	301016
CD25	BV421	BioLegend	356114
CD56	AF488	BD Biosciences	557699
FOXP3	PE-CF594	BD Biosciences	563955

Supplementary Table S3: Flow cytometry markers for TILs study

Antibody	Vendor	Catalogue
CD25-PE	Biolegend	102008
CD4-APC	Biolegend	100412
FoxP3-Alexa Fluor488	Biolegend	320012
CD45-Percp Cy5.5*	Biolegend	103132
CD8-Percp Cy5.5*	Biolegend	100734
NK1.1-PE	Biolegend	108708
CD3-FITC	BD Biosciences	555274

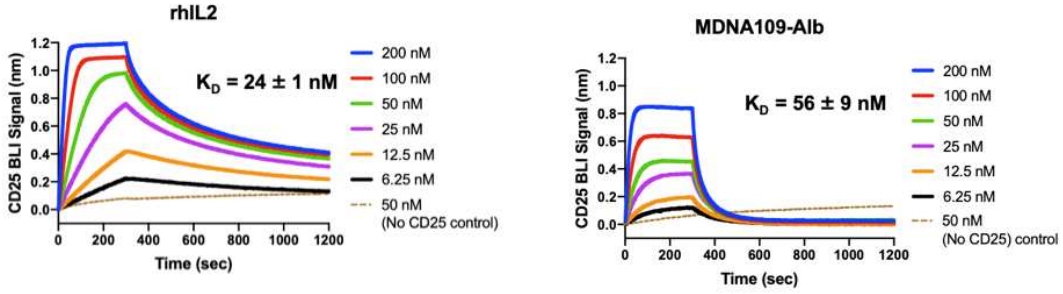
*used in separate panels.

Supplementary Table S4: Flow cytometry markers for memory T cell study

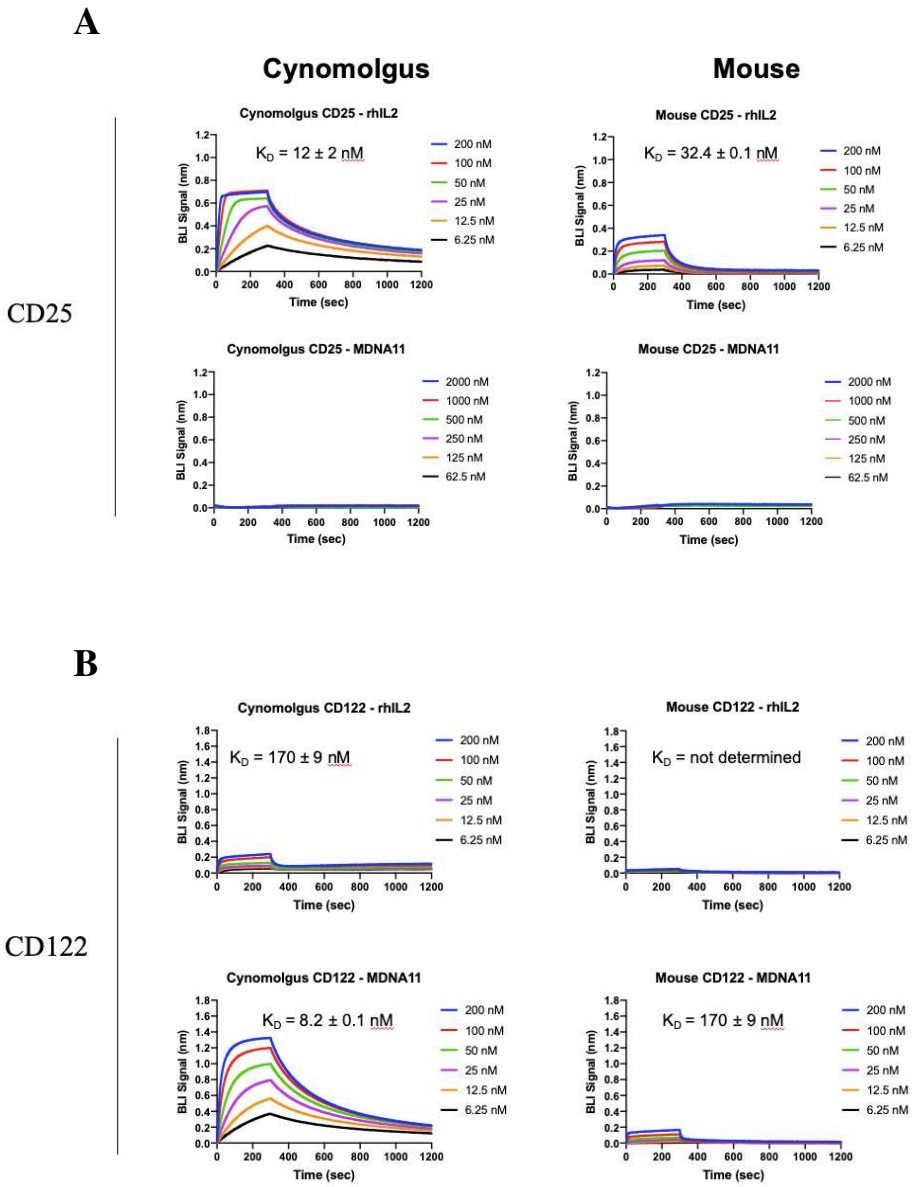
Antibody	Vendor	Catalogue
CD44-BV421	Biolegend	103040
CD62L-APC	Biolegend	104412
CD3-FITC	BD Biosciences	555274
CD8a-PerCP Cy5.5	Biolegend	100734
CD4-APC-Cy7	Biolegend	100412

Supplementary Table S5: Immunophenotyping antigens and cell populations for NHP study

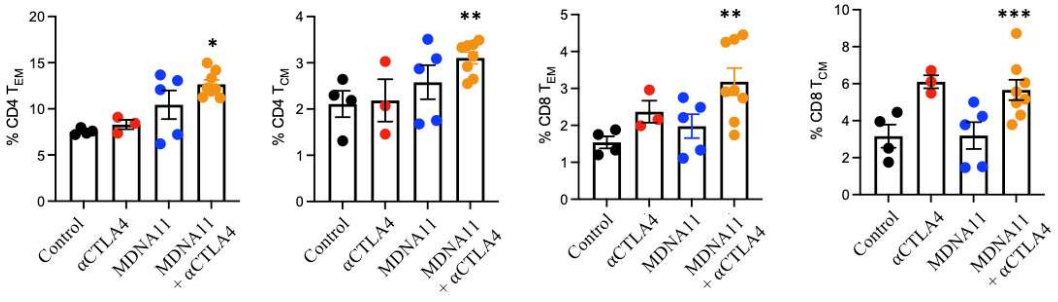
Panel	Antigen Marker(s)	Cell Population Identified
1	CD45+/CD14-/CD20+	B-lymphocytes
	CD45+/CD14-/CD20-/CD159a-/CD3+	T-lymphocytes
	CD45+/CD14-/CD20-/CD159a-/CD3+/CD4+/CD8-	T-helper lymphocytes
	CD45+/CD14-/CD20-/CD159a-/CD3+/CD4-/CD8+	T-cytotoxic lymphocytes
	CD45+/CD14-/CD20-/CD3-/CD159a+	Natural-killer cells
2	CD45+/CD4+/CD25+/FoxP3+	CD4+ Tregs
	CD45+/CD4+/CD25+	Activated T-helper lymphocytes
	CD45+/CD4+/CD25-	Non-Activated T-helper lymphocytes
3	CD45+/CD8+/CD25+/FoxP3+	CD8+ Tregs
	CD45+/CD8+/CD25+	Activated T-cytotoxic lymphocytes
	CD45+/CD8+/CD25-	Non-Activated T-cytotoxic lymphocytes[a]
4	LD-/CD45+/CD3+/CD4+/CD8-/Ki67+	Ki67+ T-helper lymphocytes (Panel 5)
	LD-/CD45+/CD3+/CD4-/CD8+/Ki67+	Ki67+ T-cytotoxic lymphocytes (Panel 5)
	LD-/CD45+/CD3+/CD4+/CD8-/FoxP3+/Ki67+	Ki67+ CD4+ Tregs
	LD-/CD45+/CD3-/CD56+/Ki67+	Ki67+ CD56+ cells



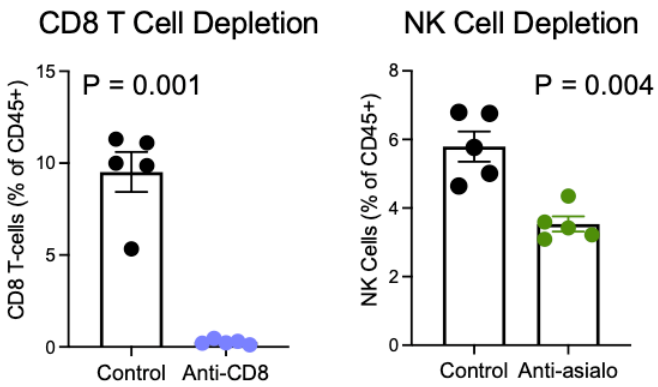
Supplementary figure S1: Role of F42A and E62A mutations in MDNA11 on CD25 binding. A version of MDNA11 without the F42A and E62A mutations (MDNA109-Alb) binds CD25 with similar affinity as rhIL-2.



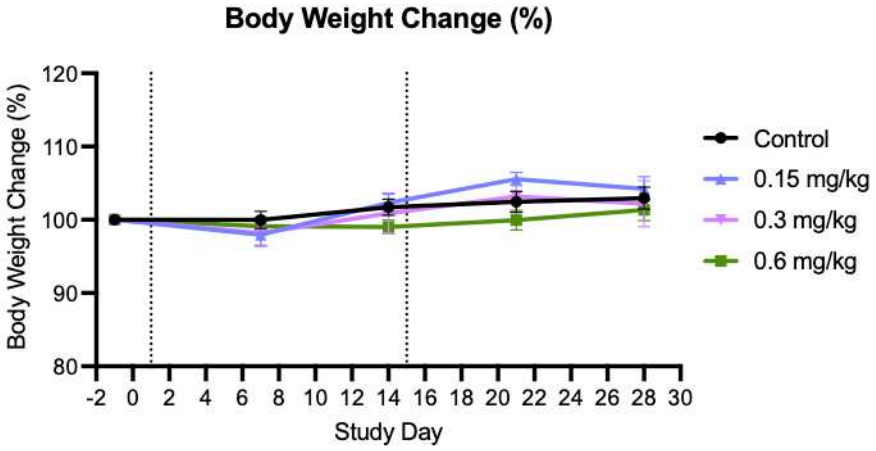
Supplementary figure S2: Binding of rhIL-2 and MDNA11 to cynomolgus and mouse IL-2 receptor. (A) Binding to CD25. (B) Binding and CD122. Studies were performed using BLI Octet.



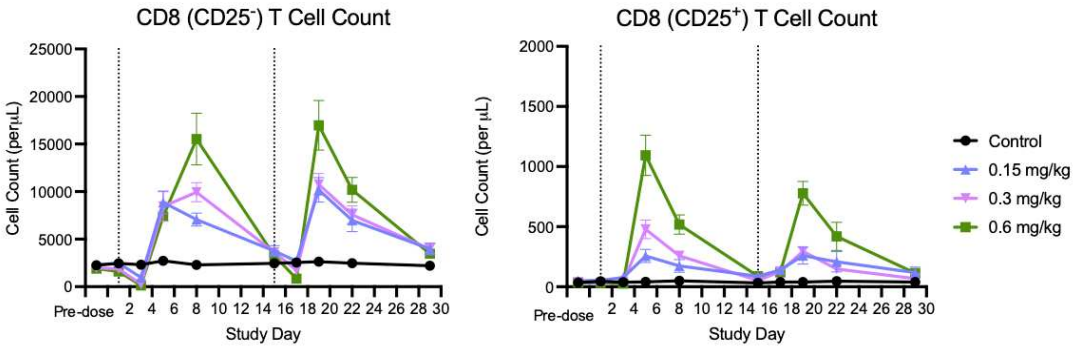
Supplementary figure S3: Quantification of memory T cells in CT26 tumor bearing mice treated with MDNA11 and anti-CTLA4. Data show mean ± SEM. Analysis performed on blood collected on Study Day 97 after mice had confirmed to be resistant to CT26 re-challenge on Day 49. Effector ($CD44^{hi}/CD62L^{lo/-}$) and central ($CD44^{hi}/CD62L^{hi}$) memory T cells in both CD4 and CD8 lineages were analyzed by flow cytometry.



Supplementary figure S4: Quantification of CD8 T cells and NK cells in mice treated with anti-CD8 and anti-asialo GM1 antibodies. Blood samples collected on Study Day 23 (3 days post second depletion) were analyzed by flow cytometry. Data show mean ± SEM. Statistical analysis was performed using the Student's t-test.



Supplementary figure S5: Change in body weight of cynomolgus monkeys following treatment with MDNA11. Data show mean ± SEM. Vertical dotted lines indicate dosing occasions on Study Day 1 and 15.



Supplementary figure S6: Response of naïve and activated CD8 T cells to MDNA11 in NHP. CD25 was used to distinguish between naïve ($CD25^-$) and activated ($CD25^+$) cells. Data show mean ± SEM. Vertical dotted lines indicate dosing occasions on Study Day 1 and 15. Note the difference on scale of y-axis.