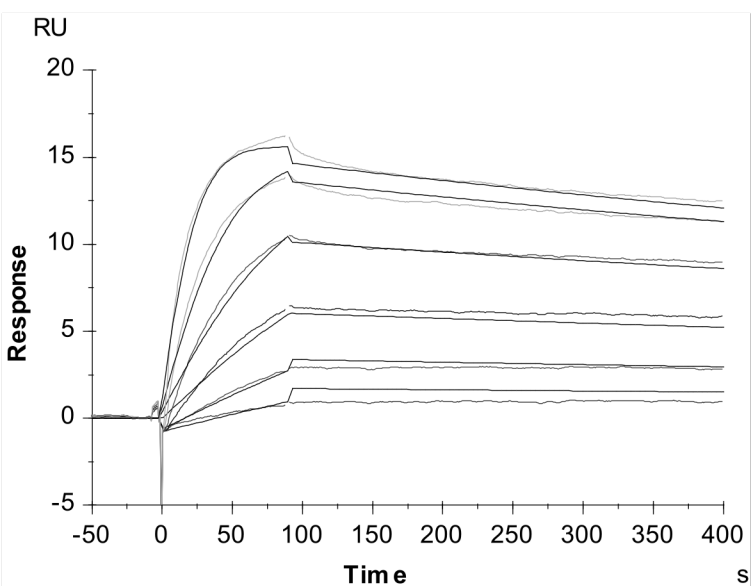


Supplementary Figure 1

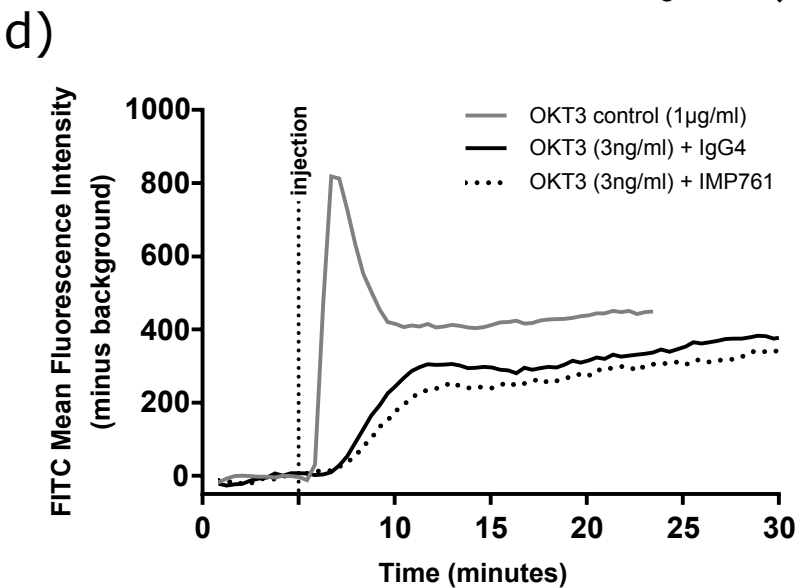
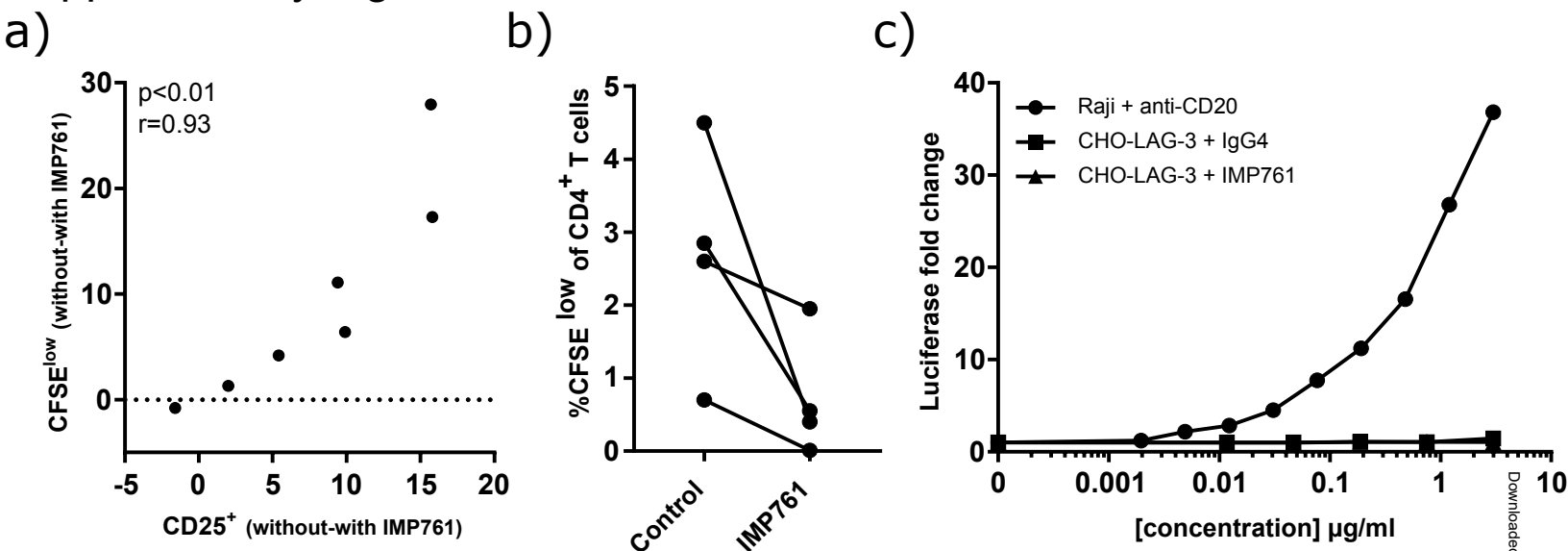


IMP761 binding affinity to LAG-3Ig.

Representative plot (out of three different experiments) of the label-free interaction analysis between LAG-3Ig protein and six different concentrations of IMP761 (0.078 – 2.5 nM).

Data are fitted using the kinetic global fit (Langmuir 1:1) model. Resonance Unit (RU) = 0 represents the baseline and Time = 0 second represents the assay start.

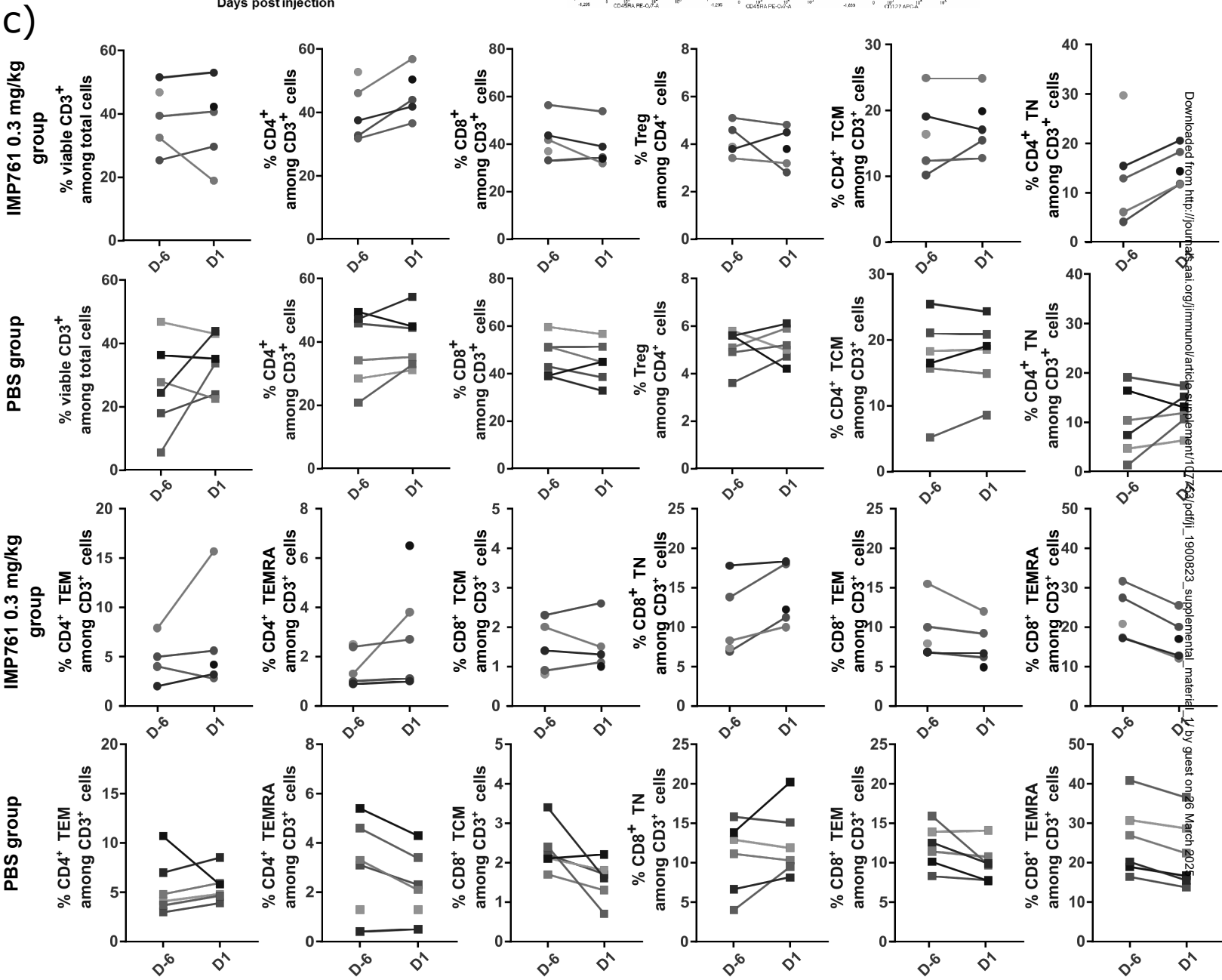
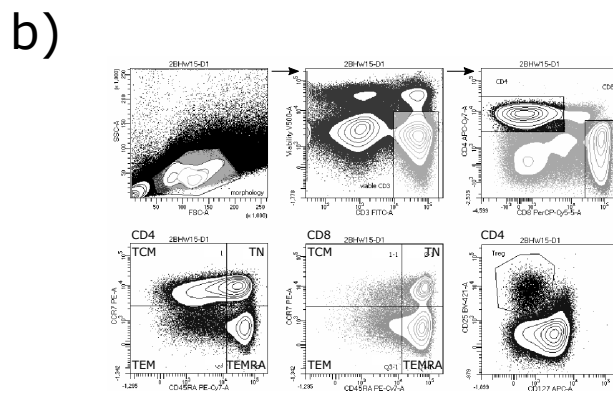
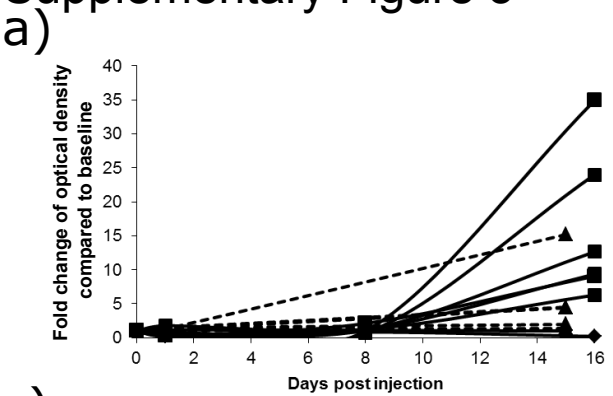
Supplementary Figure 2



Correlation of IMP761 capacity to inhibit CD8⁺ T cells proliferation and activation, capacity of IMP761 to inhibit CD4⁺ T cells proliferation, absence of IMP761-induced antibody-dependent cellular cytotoxicity and cellular calcium concentration measurement after IMP761 injection.

- a) Percentage of inhibition of proliferation (as measured by CFSE^{low} staining) compared to the percentage of inhibition of activation (as measured by CD25⁺ staining) when adding IMP761 to CEF-activated CD8⁺ T cells. CD8⁺ T cells were gated as CD14⁻CD4⁻CD8⁺ cells. A non-parametric Spearman correlation test was performed to calculate the p and r values.
- b) CFSE^{low} % of CD4⁺ T cell incubated for six days with CEF peptide pool in the absence or presence of 300 ng/ml IMP761 in four different individuals.
- c) Luciferase fold change of Fc γ RIIIa-expressing Jurkat/NFAT-luc cells after incubation with LAG-3-expressing CHO cells in presence of different concentration of IMP761 (or IgG4 isotype control) for six hours. Incubation of Fc γ RIIIa-expressing Jurkat/NFAT-luc with Raji cells and anti-CD20 was used as a positive control for induced ADCC. Representative example of three experiments.
- d) Representative example of FITC Mean fluorescence intensity (minus background) of LAG-3-expressing Jurkat cells labeled with a Fluo-4 NW Calcium Assay Kit after injection of suboptimal concentration (3 ng/ml) of anti-CD3 (OKT3) in presence or not of IMP761 or IgG4 control at 600 ng/ml. anti-CD3 at 1 $\mu\text{g/ml}$ was used as positive control (left). Area Under the Curve of the cellular calcium concentration measurements over time (calcium flux AUC) as measured by the Fluo-4 NW Calcium Assay Kit of anti-CD3 activated (3 ng/ml: black circles and lines or 10 ng/ml: grey circles and line) LAG-3-expressing Jurkat cells in presence of IMP761 or IgG4 control (600 ng/ml) (right).

Supplementary Figure 3



Kinetics of anti-IMP761 antibody detection in non-human primate serums and evolution of peripheral T cells percentage after injection of IMP761/PBS.

a) Fold change of optical density compared to baseline of anti-IMP761 detected in the serum of cynomolgus macaques injected with PBS control (solid lines and diamonds), 0.03 mg/kg (dotted lines and triangles) or 0.3 mg/kg (solid lines and squares) IMP761 at different time-points post injection. b) CD4⁺ and CD8⁺ T cell subset gating strategy. Central memory T cells (TCM) were gated as viable CD3⁺CD4⁺/CD8⁺CCR7⁺CD45RA⁻. Naïve T cells (TN) were gated as viable CD3⁺CD4⁺/CD8⁺CCR7⁺CD45RA⁺. Effector Memory T cells (TEM) were gated as viable CD3⁺CD4⁺/CD8⁺CCR7⁺CD45RA⁻. Effector Memory RA T cells (TEMRA) were gated as viable CD3⁺CD4⁺/CD8⁺CCR7⁺CD45RA⁺. Regulatory T cells (Treg) were gated as viable CD3⁺CD4⁺C25⁺CD127^{low}. c) Longitudinal percentages (day -6 and day +1 injection) of CD4⁺ and CD8⁺ T cell subsets (TCM, TN, TEM, TEMRA, Tregs) in animals injected with PBS (circles) or 0.3 mg/ml IMP761 (squares).

Supplementary Table 1

		IDR1 erythema diameter (mm)					IDR2 erythema diameter (mm)								
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	IDR1 AUC	IDR2 AUC	IDR2 vs IDR1 % difference	IDR2 vs IDR1 % difference group median	
IMP761	0.3 mg/kg	2BHW15	7.58	7.43	5.33	4.2	5	6.7	7.48	6.85	-	26	18	-31	-91.5
		1BJF15	5.23	5.25	-	-	-	-	-	-	-	8	0	-100	
		1BLM15	6.08	6.88	4.75	-	-	5.13	-	-	-	15	3	-83	
		1XY15	6.28	9.13	5.23	6.1	6.83	5	5.53	6.28	-	27	14	-47	
		2BKG15	7.98	12.3	5.63	6.65	6.95	-	-	-	-	36	0	-100	
		1BJG15	10.5	11.9	10.5	9.93	9.25	-	-	-	-	47	0	-100	
	0.03 mg/kg	1BAK14	6.95	6.7	6.3	-	-	6	-	-	-	20	6	-70	-66
		1BBH14	10.2	10.3	8.23	-	-	8.99	10.08	9.25	8	29	36	27	
		1BCP14	11	13.3	11.3	10.5	9.93	8.41	-	-	-	58	8	-85	
		1BJ15	10.2	9.4	7.83	6.85	-	9.05	-	-	-	103	9	-91	
		1BN15	11.3	13.6	7.38	-	-	6.8	8.1	6.68	6.5	32	28	-13	
		1CG15	9.19	7.2	7.18	-	-	6.86	-	-	-	24	9	-62	
PBS	-	1BHP15	8.58	10	7.85	8.35	7.6	7.53	8.5	7.78	-	38	20	-47	-59
		1BJB15	10.8	9.53	7.7	-	-	-	4.9	-	-	23	5	-78	
		1BLC15	11.2	13.9	7.24	5.81	-	6.38	11.02	-	-	33	14	-56	
		1BLG14	7.03	8.08	7.63	-	-	8.99	8.23	7.85	-	23	25	10	
		1EM15	7.59	6	6.6	-	-	7.76	-	-	-	20	8	-62	
		1F15	8.75	9.7	7.98	-	-	8.45	-	-	-	26	8	-68	

Daily erythema diameter measurements of tuberculin-specific responses at 24 hours post PPD-tuberculin challenge up to erythema disappearance.

IDR: Intradermal reaction; AUC: Area Under the Curve