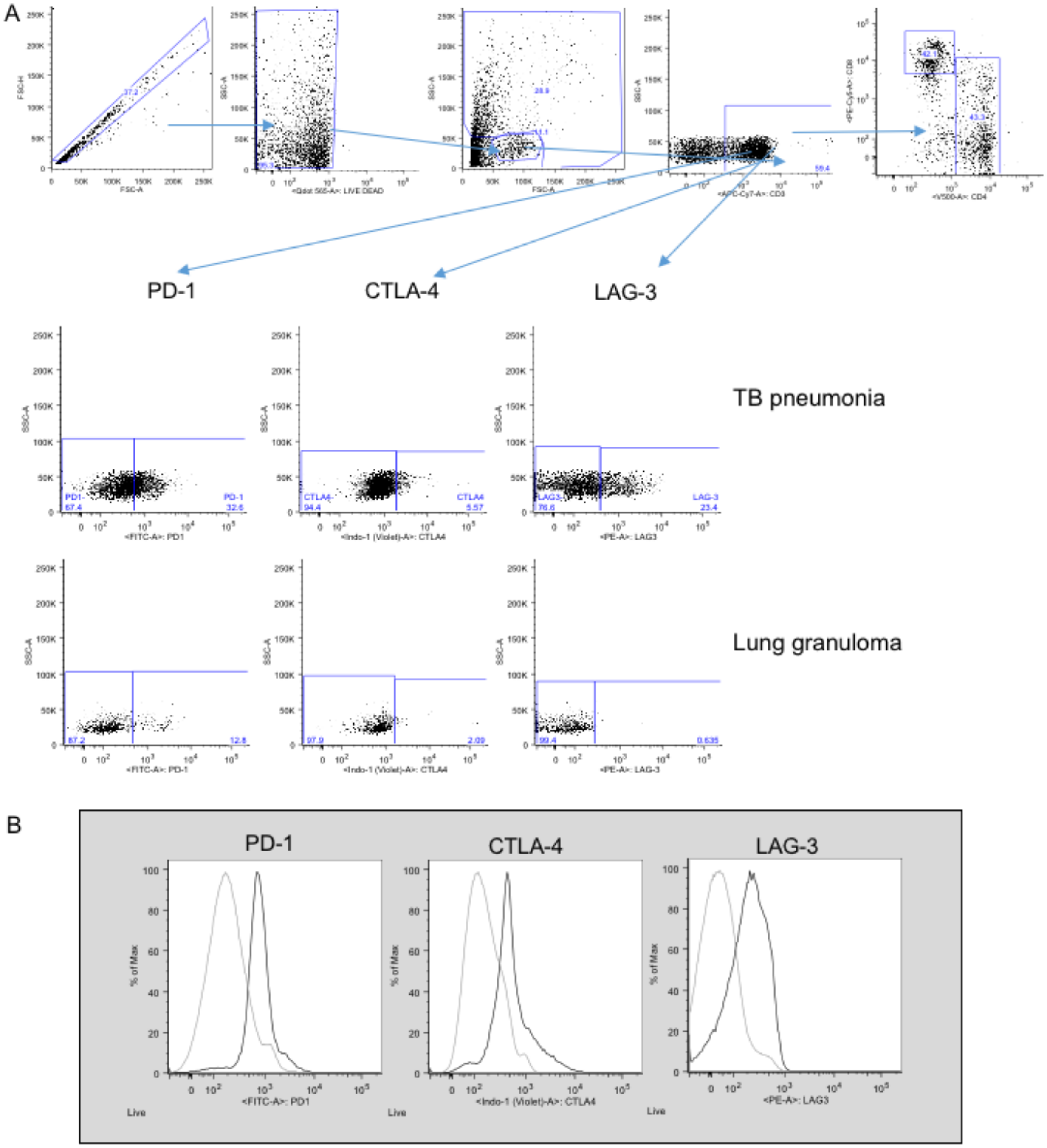
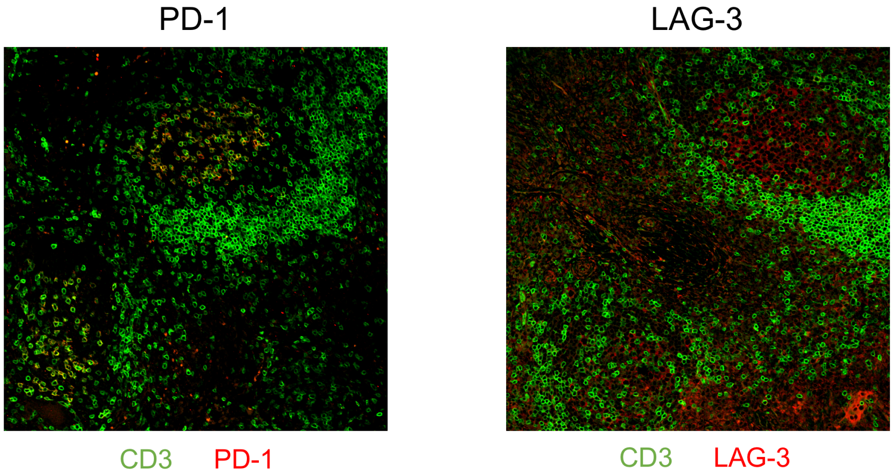
Supplementary Information

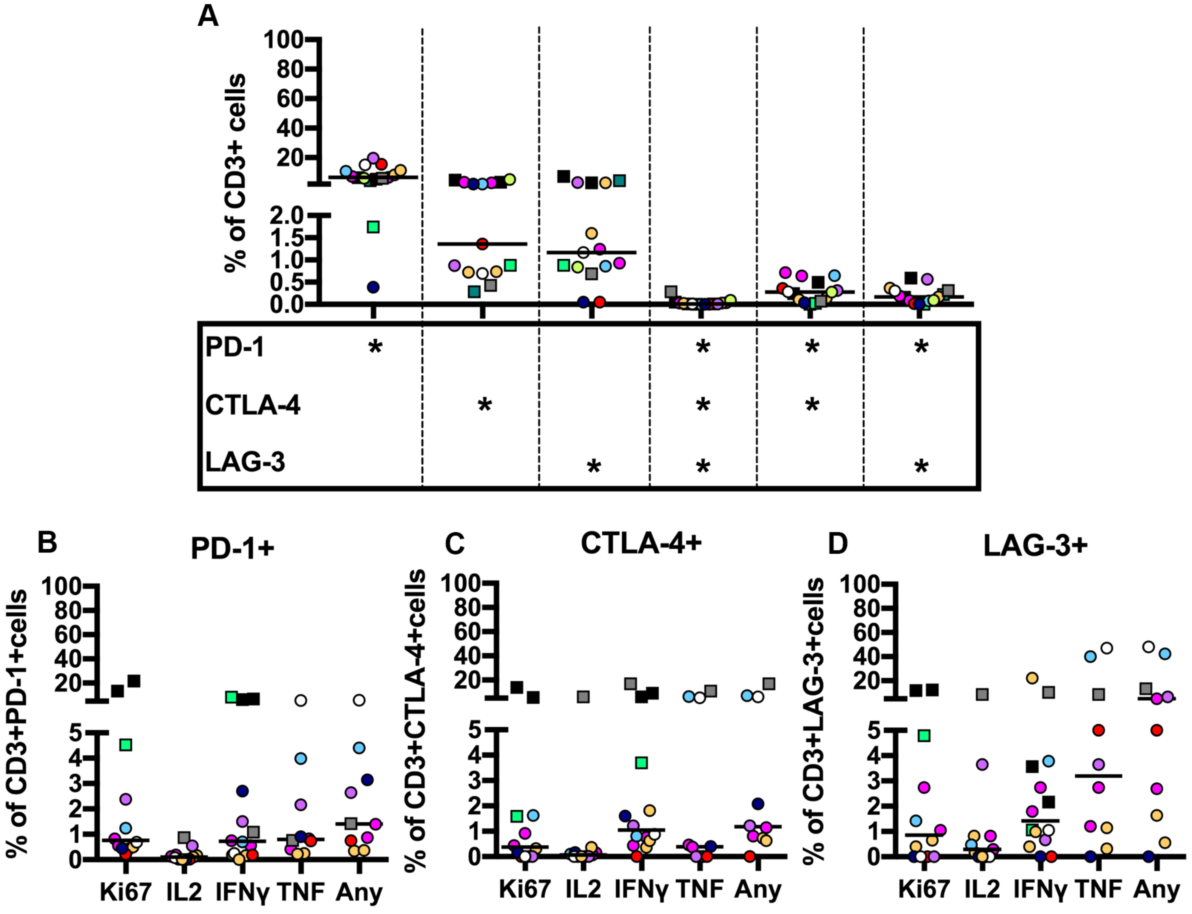


Supp Fig 1. Gating strategy and positive control examples for flow cytometry antibodies against inhibitory receptors in single cell suspensions.

(A) Flow cytometry gating strategy example from TB pneumonia sample excised from Mtb-infected cynomolgus macaque. (B) Testing of flow cytometry antibodies in PBMCs isolated from Mtb-infected cynomolgus macaque stimulated by PDBU & ionomycin for 3 hours at 37°C.



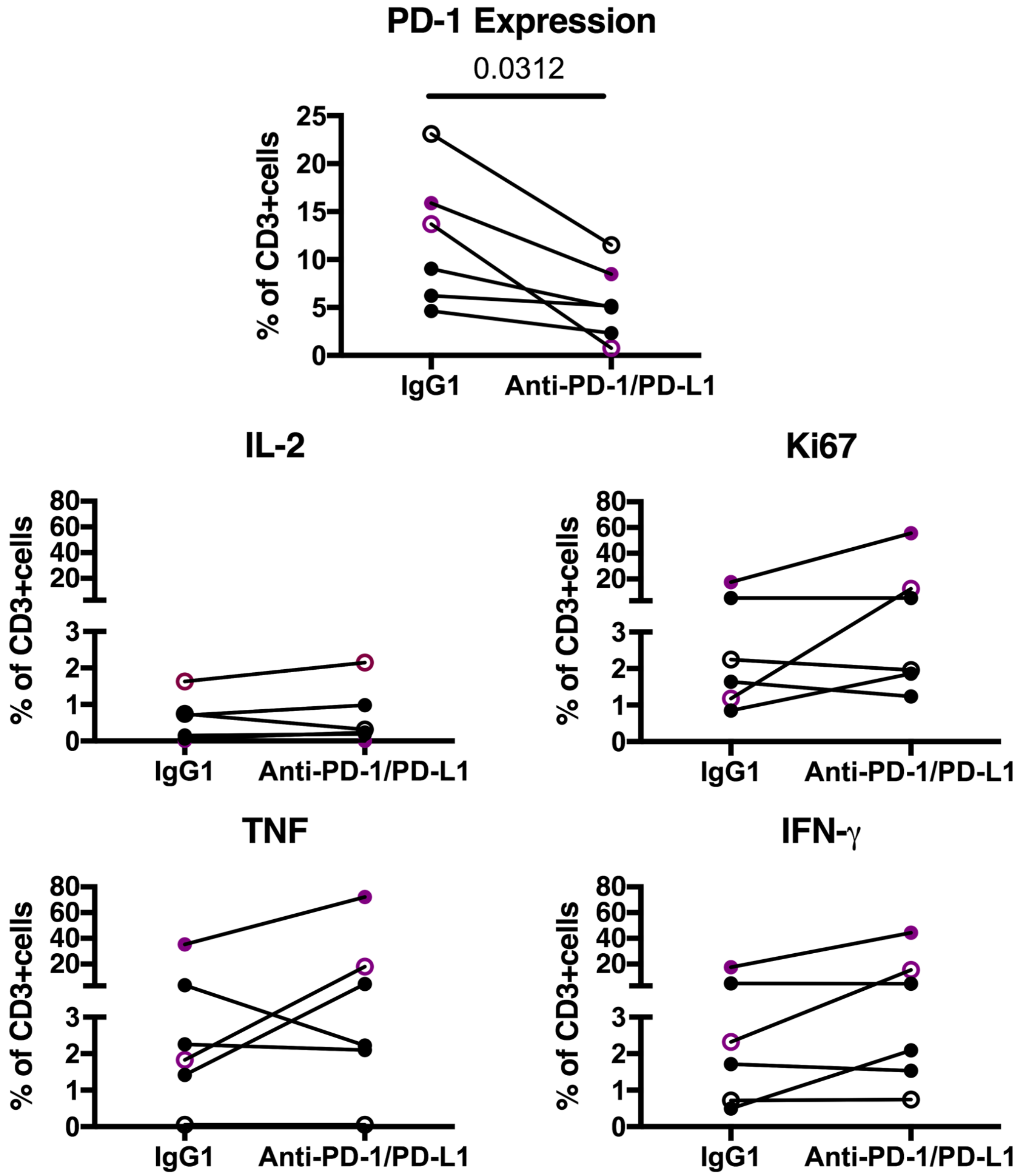
Supp Fig 2. Positive control examples for immunofluorescence antibodies against PD-1 and LAG-3 in paraffin-embedded samples, using lymph node sections as positive controls.



Supp Fig 3. Frequency of inhibitory receptor expression in thoracic lymph nodes infected with Mtb and corresponding frequency of cytokine expression in inhibitory receptor-positive T cells.

Mtb-infected lymph nodes defined as having positive CFU and/or granulomas present in histopathology at time of necropsy. (A) Frequencies of expression of inhibitory receptors on CD3+ T cells from Mtb-infected lymph nodes. Frequencies of cytokine or proliferation marker expression in CD3+ cells from Mtb-infected lymph nodes also expressing (B) PD-1, (C) CTLA-4, or (D) LAG-3. Each point indicates a lymph node, each color corresponds to a NHP (12

NHPs), circles indicate cynomolgus macaque, squares indicate rhesus macaques. Lines at medians.



Supp Fig 4. PD-1 and PD-L1 neutralization assay.

PD-1 activity in lung samples and PBMCs from Mtb-infected macaques can be blocked by anti-PD-1 and anti-PD-L1 antibodies, but cytokine and proliferation frequencies do not increase in response to decreased PD-1 levels and under stimulation with Mtb-specific antigens ESAT-6 and CFP-10. Lines connect matched IgG isotype controls and anti-PD-1/anti-PD-L1 treated samples, each color corresponds to a NHP (2 NHPs), filled circles indicate lung sample, open circles indicate PBMC sample. P-values indicated on figure, Wilcoxon matched-pairs signed rank test.

Supp Table 1. Frequencies of cells expressing inhibitory receptors PD-1, CTLA-4, or LAG-3 by flow cytometry. (A) Individual inhibitory receptor expression, or (B) inhibitory receptor co-expression in individual granulomas, complex TB disease, or Mtb-infected LNs from Mtb-infected cynomolgus or rhesus macaques, or in both combined macaque species.

(A)			***PD-1 (%)***		***CTLA4 (%)***		***LAG3 (%)***	
			Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**
CD3	Gran	Cyno	22.9	21.9	2.08	2.33	0.879	1.857
		Rhesus	9.17	12.26	1.83	4.6265	5.12	16.625
		Both	16.55	18.2575	2.015	3.01	1.53	4.4265
	Complex[a]	Cyno	32.6	36.752	1.1	0.751	0.231	0.165
		Rhesus	2.27	2.28	1.12	0.96	5.96	11.48
		Both	32.1	36.011	1.1	0.8555	0.231	5.853
	LymphNode	Cyno	9.53	8.675	1.685	2.31675	1.0475	1.29315
		Rhesus	5.43	3.205	0.878	3.74	2.85	4.933
		Both	6.74	5.87	1.36	2.604	1.17	2.109
CD4	Gran	Cyno	25.2	27.1	2.83	4.3205	0.709	1.377
		Rhesus	10.145	13.6125	3.075	26.54075	4.99	13.025
		Both	25	23.6	2.83	4.308	1.11	2.925
	Complex[a]	Cyno	50.6	64.37	1.49	0.95	1.5	1.771
		Rhesus	7.91	0	0.626	0	5.63	0
		Both	44.75	59.25	1.47	1.2305	1.54	2.62825
	LymphNode	Cyno	11.35	10.0025	2.32	2.5665	0.653	1.40375
		Rhesus	10.1	8.48	13.1	69.2774	2.45	3.3865
		Both	10.3	6.2	2.86	5.117	0.988	1.921
CD8	Gran	Cyno	10.845	16.3425	0.591	2.39775	0.3465	1.525275
		Rhesus	9.7	9.505	0.8875	2.8315	7.23	24.46
		Both	9.995	13.6325	0.6445	1.95	1.18	4.6715

Complex[a]	Cyno	24.4	24.866	0.354	0.463	0.152	1.1365
	Rhesus	1.39	0	1.21	0	1.08	0
	Both	21.85	24.577	0.365	0.819	0.2245	1.098175
LymphNode	Cyno	4.25	7.3425	1.16	1.473	0.79	2.22025
	Rhesus	3.39	3.47	1.05	5.699	0.785	1.472
	Both	3.39	3.72	1.13	2.353	0.785	1.697

(B)			***C+L+P+ (%)***[b]		***C+L-P+ (%)***[c]		***C-L+P+ (%)***[d]	
			Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**
CD3	Gran	Cyno	0.0906	0.478	0.698	1.7222	0	0.551
		Rhesus	0.593	0.94215	0	0.4965	2.83	4.2205
		Both	0.1675	0.607	0.6	1.7525	0.169	1.4825
	Complex[a]	Cyno	0.0257	0.109	0.391	0.765	0.0793	0.145
		Rhesus	0.04905	0.0117	0.073	0.146	0.465	0.71
		Both	0.0317	0.077525	0.234	0.75215	0.11	0.4568
	LymphNode	Cyno	0.0178	0.027665	0.295	0.5105	0.13225	0.257475
		Rhesus	0.0132	0.1594	0.0719	0.3584	0.225	0.372175
		Both	0.0132	0.03189	0.28	0.4251	0.171	0.2366
CD4	Gran	Cyno	0	0.305	1.21	2.0265	0.138	0.435
		Rhesus	0.425	1.08	0.2995	1.2105	0.705	6.3015
		Both	0	0.568	1.11	1.88	0.248	0.671
	Complex[a]	Cyno	0.0714	0.276	0.712	0.922	0.393	0.9324
		Rhesus	0.0894	0	0	0	2.19	0
		Both	0.0795	0.215975	0.4935	0.9473	0.5525	1.4173
	LymphNode	Cyno	0.0342	0.07337	0.3	0.661475	0.0811	0.20175
		Rhesus	0.252	0.329	0.529	6.350445	0.0799	0.859515
		Both	0.0407	0.24808	0.305	0.733	0.0799	0.1857
CD8	Gran	Cyno	0	0	0.02515	0.6245	0.02515	0.6745
		Rhesus	0	0.477	0	0.14975	3.355	8.04375
		Both	0	0	0	0.46825	0.11665	1.9175
	Complex[a]	Cyno	0	0	0.0758	0.1348	0.0758	0.08118
		Rhesus	0.315	0	0.0899	0	0.18	0
		Both	0	0.23625	0.08285	0.113625	0.07965	0.14316
	LymphNode	Cyno	0.003925	0.018225	0.1385	0.15215	0.07735	0.299995
		Rhesus	0	0.22365	0.259	0.4333	0.092	0.11405
		Both	0	0.0273	0.172	0.2448	0.0861	0.15851

a - Complex refers to more severe forms of TB disease, including clusters of granulomas, consolidations, or TB pneumonia

b – C+L+P+ refers to CTLA-4+LAG-3+PD-1+ co-expression

c – C+L-P+ refers to CTLA-4+LAG-3-PD-1+ co-expression
d – C-L+P+ refers to CTLA-4-LAG-3+PD-1+ co-expression

Supp Table 2. Table of parameter ranges used to create both biorepositories of 4500 unique granulomas. All ranges, with the exception of the exhaustionThreshold, were performed within the ranges outlined by previous versions of GranSim. Our methods section details how we assigned the ranges for the exhaustionThreshold.

Parameter Name	Description	Range	Units	Reference
growthExtMtbBound	Upper bound of number of external Mtb used in growth function	[180,240]	Number of bacteria	(1-6)
growthRateIntMtb	Fractional growth rate of intracellular bacteria	[0.001,0.005]	Unitless	(1-6)
growthRateExtMtb	Fractional growth rate of extracellular bacteria	[0.001,0.003]	Unitless	(1-6)
deathRateExtMtbCaseated	Upper bound on the number of external Mtb used in growth function	[1,2]	Number of bacteria	(1-6)
Core				
estBoundFactorTNF	Adjustment for coarse grained internalized fraction estimate	[0.4,0.5]	Unitless	(1-6)
estBoundFactorIL10	Adjustment for coarse grained internalized fraction estimate	[0.4,0.6]	Unitless	(1-6)
estConsRateTNF	Scaling Factor for coarse grained TNF dynamics	[5e-4,9e-4]	Unitless	(1-6)
estConsRateIL10	Scaling Factor for coarse grained IL10 dynamics	[2e-4,6e-4]	Unitless	(1-6)
estIntPartitionTNF	Scaling Factor for coarse grained internalization of bound TNFR1	[9,13]	Unitless	(1-6)
nrKillingCaseation	Number of killings for a compartment to become caseated	[7,13]	Number	(1-6)

caseationHealingTime	Time it takes for a caseated compartment to heal	[1700,2600]	Timesteps	(1-6)
sourceDensity	Density of vascular sources on the gridspace	[0.002,0.05]	Unitless	(1-6)
diffusivityTNF	TNF diffusivity	[4e-08, 6e-08]	Cm^2/second	(1-6)
diffusivityChemokines	Chemokine diffusivity	[4e-08,6e-08]	Cm^2/second	(1-6)
diffusivityIL10	IL10 diffusivity	[4e-08,6e-08]	Cm^2/second	(1-6)
ChemokinekDeg	Chemokine degradation rate constant	[0.0005,0.005]	1/second	(1-6)
kDeg	TNF degradation rate constant	[0.0005,0.005]	1/second	(1-6)
Ikdeg	Degradation rate constant for IL10	[0.0003,0.003]	1/second	(1-6)
IC50ChemokineIL10	IC50 of IL10 inhibition of chemokine secretion	[1,10]	Molecules/mL	(1-6)
thresholdApoptosisTNF	TNF threshold for TNF-induced apoptosis	[1000, 5000]	Unitless	(1-6)
kApoptosis	Rate of apoptosis happening	[1e-07, 2e-6]	1/second	(1-6)
saturationApoptosisTNF	Signal saturation of number of internal bound TNFR1 Molecules	[5000, 9000]	Molecules	(1-6)
minChemotaxis	Minimum of Chemotaxis sensitivity range	[1, 50]	Molecules	(1-6)
maxChemotaxis	Maximum of Chemotaxis sensitivity range	[100,1000]	Molecules	(1-6)
maxIL10Inhibition	Coarse grained TNF/IL10 dose dependence parameter beta	[0.05, 0.3]	Log10(ng/mL)	(1-6)
Mac				
initDensity	Initial density of macrophages on the gridspace	[0.005,0.03]	Unitless	(1-6)
movementRest	Time required for a resting macrophage to move one micro-compartment	[1,10]	Timesteps	(1-6)
movementAct	Time required for an activated macrophage to move one micro-compartment	[10,50]	Timesteps	(1-6)

movementInf	Time required for an infected macrophage to move one micro-compartment	[100,200]	Timesteps	(1-6)
dTNF	Secretion rate of TNF by a macrophage	[1.3,1.7]	Molecules/second	(1-6)
dCCL2	Secretion rate of CCL2 by a macrophage	[4,8]	Molecules/second	(1-6)
dCCL5	Secretion rate of CCL5 by a macrophage	[4,8]	Molecules/second	(1-6)
dIL10Act	Secretion rate of IL10 by an activated macrophage	[0.2, 0.4]	Molecules/second	(1-6)
halfSatIL10	Half saturation for TNF induction of IL10 in an activated macrophage	[170,210]	Number/cell	(1-6)
thresholdNFkBTNF	TNF threshold for NFkB activation	[75,115]	Molecules	(1-6)
kNFkB	Rate of NFkB activation	[0.7e-5, 1e-5]	Fraction	(1-6)
probKillExtMtbRest	Probability of a resting macrophage to kill extracellular bacteria	[0.05, 0.3]	Unitless	(1-6)
fKillExtMtbRest	Fractional increase of a resting macrophage to kill extracellular bacteria when STAT1 or NFkB pathways are on	[0.3,0.5]	Unitless	(1-6)
nrExtMtbNFkB	Number of extracellular bacteria for NFkB activation in an infected macrophage	[150, 250]	Bacteria	(1-6)
nrIntMtbCInf	Number of intracellular bacteria necessary for an infected macrophage to become chronically infected	[8,12]	Bacteria	(1-6)
nrIntMtbBurstCInf	Number of intracellular bacteria necessary for a chronically	[13,20]	Bacteria	(1-6)

	infected macrophage to burst			
nrExtMtbUptakeAct	Number of extracellular bacteria an activated macrophage can uptake and kill	[3,7]	Bacteria	(1-6)
Stat1ActivationTime	Time a macrophage is Stat1 activated	[400,460]	Timesteps	(1-6)
nfkbActivationTime	Time a macrophage is NFkB activated	[13,17]	Timesteps	(1-6)
Stat3ActivationTime	Time a macrophage is Stat3 activated	[75,125]	Timesteps	(1-6)
thresholdSTAT3IL10	Threshold of IL10 to IL10R1 for STAT3 signaling	[5,15]	Unitless	(1-6)
kSTAT3IL10	Rate constant of bound IL10 to IL10R1 for STAT3 signaling	[5e-4, 1.5e-3]	Unitless	(1-6)
probHealCaseation	Rate constant for wound healing	[0.005, 0.05]	Unitless	(1-6)
T cell				
maxAge	Maximum age of a T cell	[400,460]	Timesteps	(1-6)
exhaustionThreshold	Threshold of Exposure Events for an individual T cell before it becomes exhausted	[200, 10000] (5236 during generation of 2nd biorepository)	Count	Estimated
probMoveToMac	Probability of a T cell moving into a compartment already containing a macrophage	[0.01, 0.2]	Unitless	(1-6)
probMoveToTcell	Probability of a T cell moving into a compartment already containing another T cell	[0.01, 0.2]	Unitless	(1-6)
maxDivisions	Maximum number of times a T cell can create a daughter cell	[3,5]	Timesteps	(1-6)
γ-producing T cells				
dTNF	Secretion rate of TNF by γ-producing T cell	[0.1, 0.2]	Molecules/second	(1-6)
maxTimeReg	Time span during which a γ producing T cell remains down-regulated	[30,40]	Timesteps	(1-6)

probApoptosisFasFasL	Probability of Fas/FasL induced apoptosis by a γ-producing T cell	[0.01,0.03]	Unitless	(1-6)
probTNFProducer	Probability that a γ-producing T cell can produce TNF	[0.04, 0.1]	Unitless	(1-6)
probIFNProducer	Probability that a γ-producing T cell can produce IFNγ	[0.3, 0.4]	Unitless	(1-6)
probIFNMooreExtend	Probability a macrophage will be IFNγ/STAT1 activated in the extended Moore Neighborhood.	[0.2, 0.3]	Unitless	(1-6)
Cytotoxic T cells				
dTNF	Secretion rate of TNF by a cytotoxic T cell	[0.01, 0.02]	Molecules/second	(1-6)
maxTimeReg	Time span during which a cytotoxic T cell remains down-regulated	[30,40]	Timesteps	(1-6)
probKillMac	Probability of a cytotoxic T cell killing a chronically infected mac	[0.005, 0.015]	Unitless	(1-6)
probKillMacCleanly	Probability of a cytotoxic T cell killing a chronically infected mac cleanly	[0.6, 0.9]	Unitless	(1-6)
probTNFProducer	Probability that a cytotoxic T cell is producing TNF	[0.05, 0.09]	Unitless	(1-6)
Regulatory T cells				
dIL10	Secretion of IL10 by a regulatory T cell	[0.7, 0.8]	Molecules/second	(1-6)
probTregDeactivate	Probability of successful downregulation by a regulatory T cell	[0.01, 0.02]	Unitless	(1-6)
factorDeactIL10	Factor when a regulatory T cell is making IL10 to scale probTregDeactivate	[1,3]	Unitless	(1-6)

Supporting Information References

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