Supplemental information: Regulation of macrophage phenotype and plasticity by complex activation signals

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We constructed mathematical models comprising minimal nonlinear Ordinary Differential Equation (ODE) networks. Our models were developed from previously described models of T cell subset specialization,[1–3] adding additional connectivities and species to account for the complex kinetics of CD86 and CD206 after stimulation. We constructed 70 models with different topologies. A representative set of the studied topologies, models 1-6, are illustrated in Figure 2 and Supplementary Fig. S4. A model described by the mutual-inhibition self-activation (MISA) motif (Model 2) fits the data better than one in which the M1- and M2-associated pathways are activated independently (Model 1). However, the MISA is nevertheless insufficient to capture the complex kinetics of CD86 and CD206 expression after co-stimulation.

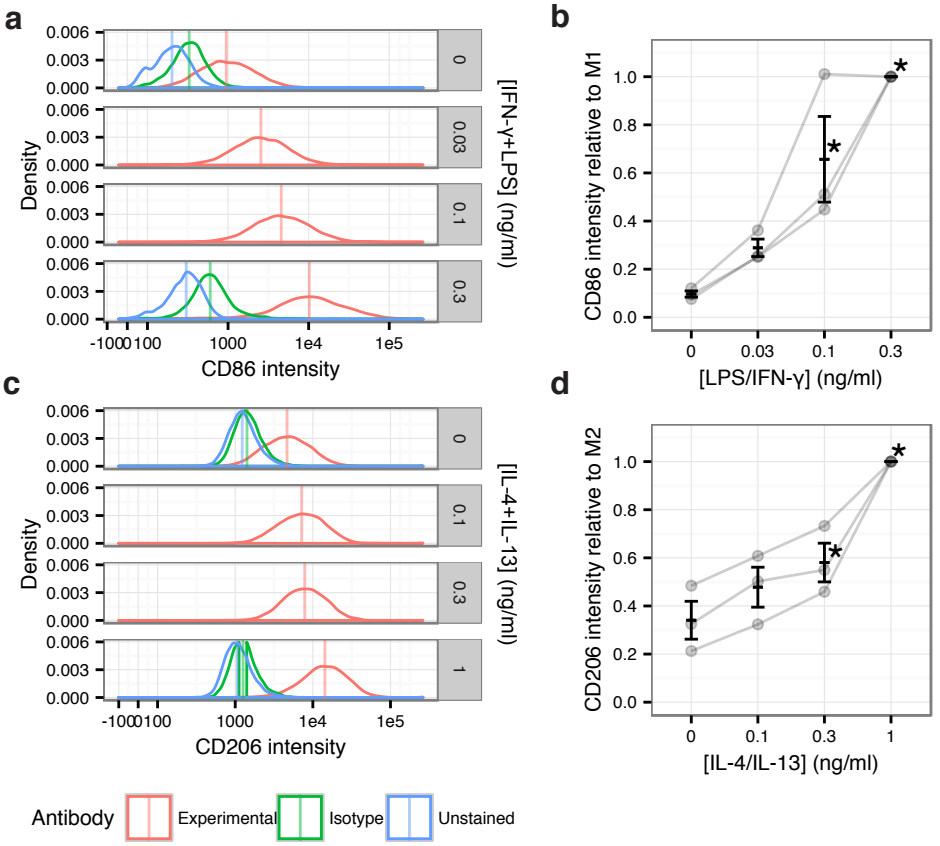
Models that extend the MISA topology by introducing a new species Y that interacts with the M1- and M2-associated pathways, and representing unknown processes downstream of LPS/IFN and/or IL-4/IL-13 signalling events improve the model score (see AICc below) (Models 3, 5, 6). Specifically, we found that an incoherent feed-forward loop on M1, mediated by an additional species Y, was necessary to capture the decay of CD86 expression in costimulated cells at 96 hours.[4] An activating link between Y and M2 was also consistent with the increased expression of CD206 of costimulated cells at later times. In particular, cooperative activation of M2 from Y and M2 improved the overall fit. To discover extended topologies, we were guided by the features of the temporal data and the literature on macrophage activation, as discussed in the main text.

Model quality was assessed based on optimization of parameters by fitting to the 96-hour time course data (Fig. 2) of four timepoints (24, 48, 72, 96 h) for four different stimulation conditions ({0.3,0}, {0.3,1}, {0,0}, {0,1} ng/ml {LPS/IFN-γ, IL-4/IL-13}). The number of replicates was between three and five for each timepoint, giving 72 experimental data points. The error metric used was the sum of squared residuals (RSS) with normalized mean weighting. Parameter estimation was performed by minimizing the RSS of the model predicted CD86 and CD206 values to the normalized mean-weighted experimental values. The Matlab Optimization Toolbox and the trust-region-reflective algorithm were used to perform 1,000 individual fits. Parameters were initialized from a lognormal distribution with a mean and variance of 2, and were constrained to be positive. Parameters were optimized to the normalized timecourse data, and thus are expressed in arbitrary concentration and time units. Initial fits were performed using 400 trust-region-reflective iterations, or until convergence, using normalized unweighted experimental values. A second fit was then performed to the normalized mean-weighted experimental values. All models were assessed using the AICc criterion, a scoring metric for model selection that includes penalties for increasing the number of fitted parameters.[5]

To replicate the cell-to-cell variability in the flow cytometry data, individual cells were given static parameters drawn from a distribution. Cell populations with between 3000 and 10000 cells were simulated, and model parameters for each cell were drawn from a lognormal distribution centered on the optimized parameters and with a variance of one percent of the mean. The resulting CD86 and CD206 expression levels for all models in Figure 2 and fitted parameters (Supplementary Fig. S4) showed single-peaked distributions shifting with dosage, in qualitative agreement with the experimental density plots.

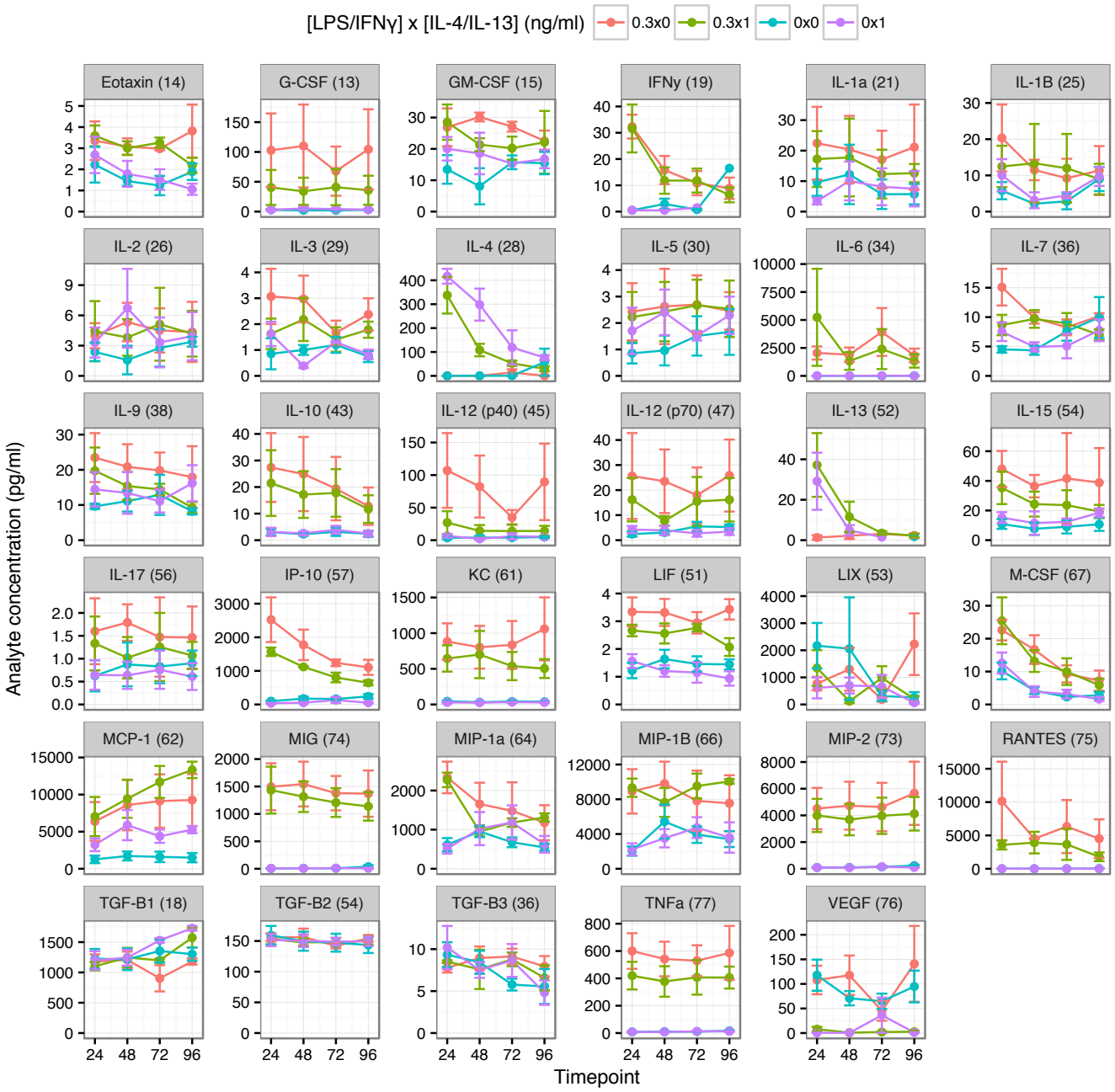
Supplemental Figures

Supplementary Figure S1



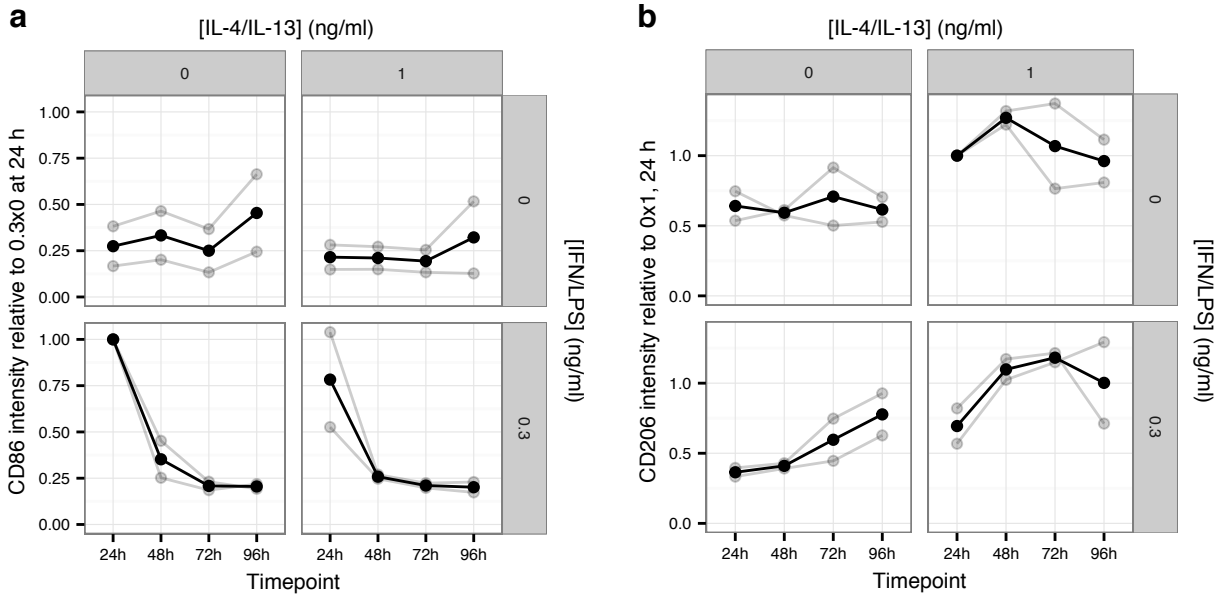
Expression of phenotypic markers CD86 and CD206 in response to LPS/IFN-γ and IL-4/IL-13 stimuli is dose-dependent. (A) Representative flow cytometry histograms of CD86 intensity after 48 hours of treatment with indicated dose of LPS/IFN-γ. (B) Average median normalized CD86 expression $\pm$ SEM as a function of increasing LPS/IFN-γ dose. Data are normalized to 0.3 ng/ml treatment condition. Asterisk indicates difference vs. untreated, $p < 0.05$; $n = 3$. (C) Representative flow cytometry histograms of CD206 intensity after 48 hours of treatment with indicated dose of IL-4/IL-13. (D) Average median normalized CD206 intensity $\pm$ SEM as a function of increasing IL-4/IL-13 dose. Data are normalized to 1 ng/ml treatment condition. Asterisk indicates difference vs. untreated by two-sided t test, $p < 0.05$; $n = 3$.

Supplementary Figure S2



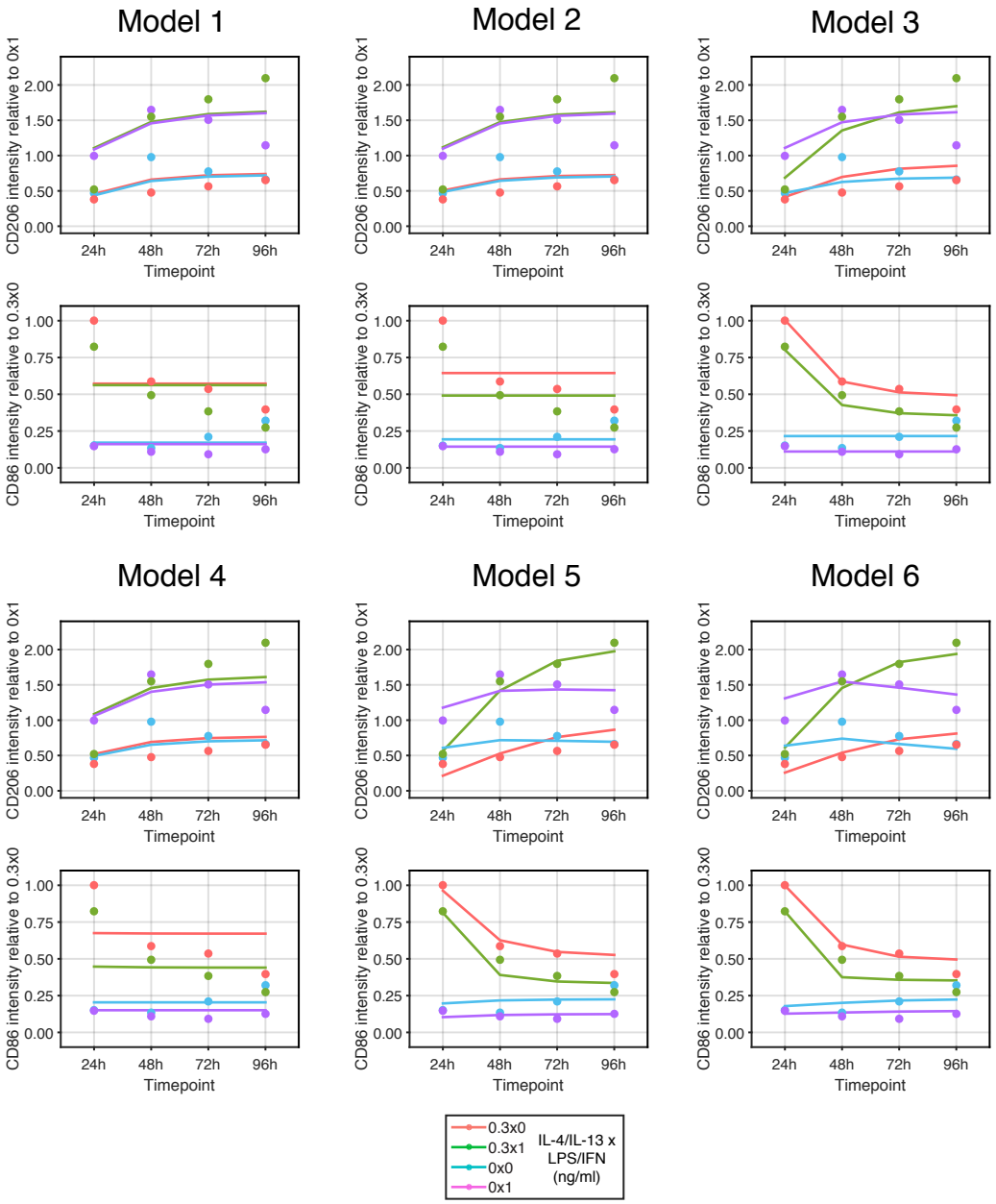
Cytokines present in culture media of macrophages exposed to LPS/IFN-γ ± IL-4/IL-13 for the indicated time in hours, assessed by multiplex ELISA. Data presented as mean ± SEM, n=3.

Supplementary Figure S3



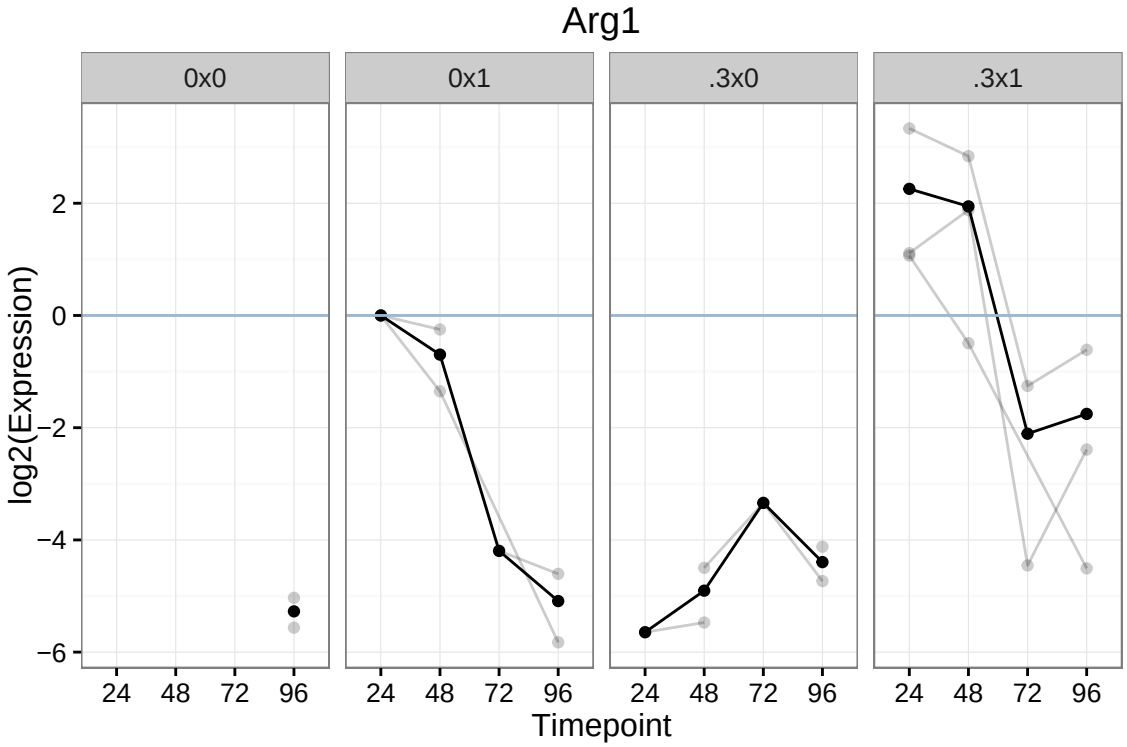
Pulse-chase experiment. Cells were treated with LPS/IFN-γ or IL-4/IL-13 at t=0. Media was replaced with untreated media at t=24 hours. Cells were collected at t=24, 48, 72, and 96 hours for analysis of CD86 and CD206 expression by flow cytometry to observe how marker expression evolved over time in the absence of continued stimulus. Median normalized fluorescence intensity from each experiment (n=2) is plotted in gray and the mean is plotted in black.

Supplementary Figure S4



Simulated timecourse experiment from each model shown in Fig. 3. Points mark experimental data, as shown in Fig. 3B.

Supplementary Figure S5



qRT-PCR timecourse data for *Arg1* expression, relative to the IL-4/IL-13-only condition at 24 hours. Gray points are individual observations and gray lines connect points from the same experiment; the black points and line represent the average. Missing points indicate missing data due to signals below limit of quantitation. Headings describe stimulation condition as ng/ml concentration of LPS/IFN-γ x IL-4/IL-13. Timepoint is in hours.

Supplementary Equations

The parameters of these equations are described in Supplementary Table S2 and fitted numeric values are given in Supplementary Table S3.

Equation S1. Model 1: Self-activation

$$\frac{d[M1]}{dt} = k_1 \frac{S1}{S1 + K_{ind1}} + k_3 \frac{[M1]}{[M1] + K_{act}} + k_5 - d_1[M1]$$

$$\frac{d[M2]}{dt} = k_2 \frac{S2}{S2 + K_{ind2}} + k_4 \frac{[M2]}{[M2] + K_{act}} + k_6 - d_2[M2]$$

$$\frac{d[CD86]}{dt} = g_1[M1] - d_4[CD86]$$

$$\frac{d[CD206]}{dt} = g_2[M2] - d_5[CD206]$$

Equation S2. Model 2: MISA

$$\frac{d[M1]}{dt} = \frac{k_1 \frac{S1}{S1+K_{ind1}} + k_3 \frac{[M1]}{[M1]+K_{act}}}{1 + \left([M2]/K_{rep2}\right)^n} + k_5 - d_1[M1]$$

$$\frac{d[M2]}{dt} = \frac{k_2 \frac{S2}{S2+K_{ind2}} + k_4 \frac{[M2]}{[M2]+K_{act}}}{1 + \left([M1]/K_{rep1}\right)^n} + k_6 - d_2[M2]$$

$$\frac{d[CD86]}{dt} = g_1[M1] - d_4[CD86]$$

$$\frac{d[CD206]}{dt} = g_2[M2] - d_5[CD206]$$

Equation S3. Model 3: MISA with IFFL and inhibition between Y and M1

$$\frac{d[M1]}{dt} = \frac{k_1 \frac{S1}{S1+K_{ind1}} + k_3 \frac{[M1]}{[M1]+K_{act}}}{\left(1 + \left([M2]/K_{rep2}\right)^n\right)\left(1 + \left([Y]/K_Y\right)^n\right)} + k_5 - d_1[M1]$$

$$\frac{d[M2]}{dt} = \frac{k_2 \frac{S2}{S2+K_{ind2}} + k_4 \frac{[M2]}{[M2]+K_{act}}}{\left(1 + \left([M1]/K_{rep1}\right)^n\right)} + k_6 + k_7 \frac{[Y]}{[Y]+K_{CY}} - d_2[M2]$$

$$\frac{d[Y]}{dt} = k_8 \frac{S1}{S1 + K_{ind1}} - d_3[Y1]$$

$$\frac{d[CD86]}{dt} = g_1[M1] - d_4[CD86]$$

$$\frac{d[CD206]}{dt} = g_2[M2] - d_5[CD206]$$

Equation S4. Model 4: MISA with cooperative IFFL

$$\frac{d[M1]}{dt} = \frac{k_1 \frac{S1}{S1+K_{ind1}} + k_3 \frac{[M1]}{[M1]+K_{act}}}{\left(1+\left([M2]/K_{rep2}\right)^n\right)} + k_5 - d_1[M1]$$

$$\frac{d[M2]}{dt} = \frac{k_2 \frac{S2}{S2+K_{ind2}} + k_4 \frac{[M2]}{[M2]+K_{act}} + k_7 \frac{[M2][Y]}{(K_{CM2}+[M2])(K_{CY}+[Y])}}{(1+([M1]/K_{rep1})^n)} + k_6 - d_2[M2]$$

$$\frac{d[Y]}{dt} = k_8 \frac{S1}{S1+K_{ind1}} - d_3[Y1]$$

$$\frac{d[CD86]}{dt} = g_1[M1] - d_4[CD86]$$

$$\frac{d[CD206]}{dt} = g_2[M2] - d_5[CD206]$$

Equation S5. Model 5: MISA with cooperative IFFL and inhibition between Y and M1

$$\frac{d[M1]}{dt} = \frac{k_1 \frac{S1}{S1+K_{ind1}} + k_3 \frac{[M1]}{[M1]+K_{act}}}{\left(1+\left([M2]/K_{rep2}\right)^n\right)(1+([Y]/K_Y)^n)} + k_5 - d_1[M1]$$

$$\frac{d[M2]}{dt} = \frac{k_2 \frac{S2}{S2+K_{ind2}} + k_4 \frac{[M2]}{[M2]+K_{act}} + k_7 \frac{[M2][Y]}{(K_{CM2}+[M2])(K_{CY}+[Y])}}{(1+([M1]/K_{rep1})^n)} + k_6 - d_2[M2]$$

$$\frac{d[Y]}{dt} = k_8 \frac{S1}{S1+K_{ind1}} - d_3[Y1]$$

$$\frac{d[CD86]}{dt} = g_1[M1] - d_4[CD86]$$

$$\frac{d[CD206]}{dt} = g_2[M2] - d_5[CD206]$$

Equation S6. Model 6: MISA with cooperative IFFL, inhibition between Y and M1, and inhibition on Y

$$\frac{d[M1]}{dt} = \frac{k_1 \frac{S1}{S1+K_{ind1}} + k_3 \frac{[M1]}{[M1]+K_{act}}}{\left(1+\left([M2]/K_{rep2}\right)^n\right)(1+([Y]/K_Y)^n)} + k_5 - d_1[M1]$$

$$\frac{d[M2]}{dt} = \frac{k_2 \frac{S2}{S2+K_{ind2}} + k_4 \frac{[M2]}{[M2]+K_{act}} + k_7 \frac{[M2][Y]}{(K_{CM2}+[M2])(K_{CY}+[Y])}}{(1+([M1]/K_{rep1})^n)} + k_6 - d_2[M2]$$

$$\frac{d[Y]}{dt} = \frac{k_8 \frac{S1}{S1+K_{ind1}}}{1+(S2/K_{ind2})^n} - d_3[Y1]$$

$$\frac{d[CD86]}{dt} = g_1[M1] - d_4[CD86]$$

$$\frac{d[CD206]}{dt} = g_2[M2] - d_5[CD206]$$

Supplementary Tables

Supplementary Table S1. qPCR primers

Gene	Direction	Sequence	Amplicon length (bp)
Arg1	F	CTCTGTCTTTTAGGGTTACGG	152
	R	CTCGAGGCTGTCCTTTTGAG	
Chi3l3	F	AGTGCTGATCTCAATGTGGATTC	142
	R	TAGGGGCACCAATTCCAGTC	
Gapdh	F	GTCAAGCTCATTTCCTGGTATGAC	131
	R	TCTCTTGCTCAGTGTCCTTGC	
Hprt	F	TGGACAGGACTGAAAGACTTGCTCG	81
	R	CCTTGAGCACACAGAGGGCCAC	
Il10	F	CCCACTTCCCAGTCGGCCAG	300
	R	GGAGAAATCGATGACAGCGCCTC	
Kdm6b	F	GGTTCACTTCGGCTCAACTTAG	75
	R	CTCCACCGTATGTTCACCGC	
Ldha[A]	F	TGTCTCCAGCAAAGACTACTGT	155
	R	GACTGTACTTGACAATGTTGGGA	
Mrc1	F	TGTTTTGGTTGGGACTGACC	269
	R	TGCAGTAACTGGTGGATTGTC	
mVPA1[6]	F	GGAGCCCAGTGTAGAAGAGCA	87
	R	AGCCAGCGAACCATATCCTGA	
Nos2	F	TTGGGTCTTGTTCACTCCAC	211
	R	TGTATTGTTGGGCTGAGAACAG	
Retnla	F	GCCAATCCAGCTAACTATCCC	187
	R	AGTCAACGAGTAAGCACAGG	
Sdha[B]	F	CTTGAATGAGGCTGACTGTG	87
	R	ATCACATAAGCTGGTCCTGT	
Tnfa	F	CCCACGTCGTAGCAAACCACCA	172
	R	TCGGGGCAGCCTTGTCCCTT	

[A] RTPrimerDB[7] 3720; [B] RTPrimerDB 3875

Supplementary Table S2. Model parameters

Parameter	Meaning
k_1	Maximum stimulation rate of M1 cascade under induction with S1
k_2	Maximum stimulation rate of M2 cascade under induction with S2
k_3	Maximum stimulation rate of M1 cascade under self-activation
k_4	Maximum stimulation rate of M2 cascade under self-activation
k_5	Basal rate of M1 activation
k_6	Basal rate of M2 activation
k_7	Maximum rate of M2 stimulation from Y and M2 cooperative stimulation
k_8	Maximum rate of Y production under S1 induction
K_Y	Level of Y to reach half-maximum inhibition of M1
K_{CY}	Level of Y to reach half-maximum cooperative activation of M2
K_{CM2}	Level of M2 to reach half-maximum cooperative activation of M2
K_{rep1}	Level of M1 to reach half-maximum inhibition of M2
K_{rep2}	Level of M2 to reach half-maximum inhibition of M1
K_{ind1}	Level of S1 to reach half-maximum induction of M1
K_{ind2}	Level of S2 to reach half-maximum induction of M2
K_{act}	Level of M1 or M2 to reach half-maximum self-activation

Parameter	Meaning
d_1	M1 decay rate
d_2	M2 decay rate
d_3	Y decay rate
d_4	CD86 decay rate
d_5	CD206 decay rate
g_1	CD86 production rate
g_2	CD206 production rate
n	Hill coefficient

Supplementary Table S3. Model parameter values

Model	1	2	3	4	5	6
k_1	0.4341	0.5871	10.421	0.7595	2.2698	2.8511
k_2	1.1124	1.6691	1.4815	1.4998	0.8939	1.0146
k_3	0.8322	0.791	1.0512	0.6856	1.1102	0.7833
k_4	1.6865	0.311	0.9216	0.4332	1.5412	1.1121
k_5	0.0456	0.0679	0.0421	0.0968	0.0332	0.1067
k_6	0.0172	0.5813	0.3239	0.541	0.0831	0.1589
k_7	N/A	N/A	0.1977	4.3814	2.8281	2.2411
k_8	N/A	N/A	0.1096	0.1594	0.7288	0.1206
K_{M2}	N/A	N/A	N/A	9.9178	1.3292	0.0012
K_{CY}	N/A	N/A	0.3438	8.4118	5.4604	5.4965
K_Y	N/A	N/A	N/A	N/A	0.0209	0.9182
K_{rep1}	N/A	2.8882	2.2441	1.9004	2.4051	1.0306
K_{rep2}	N/A	14.228	1.4216	2.0858	1.0162	1.195
K_{ind1}*	1	1	1	1	1	1
K_{ind2}*	0.3	0.3	0.3	0.3	0.3	0.3
K_{act}*	1	1	1	1	1	1
d_1*	1	1	1	1	1	1
d_2*	1	1	1	1	1	1
d_3*	N/A	N/A	0.05	0.05	0.05	0.05
d_4*	0.05	0.05	0.05	0.05	0.05	0.05
d_5*	0.05	0.05	0.05	0.05	0.05	0.05
g_1*	1	1	1	1	1	1
g_2*	1	1	1	1	1	1
n	N/A	2	N/A	2	2	2
Free parameters	6	8	11	12	13	13

Representative best fit parameter values for each model from optimization. Parameters are in arbitrary units of concentration and time, relative to the rate of degradation of the M1 species (d_1), which is approximated to be 1 [/hr] according to the half-life of STAT1.[8] The parameters were optimized to normalized CD86 and CD206 expression levels. Parameters with an asterisk were fixed to constrain parameter space during optimization. Fixed values were chosen based on initial parameter searches. Alternative constraints yielded different quantitative values, but the same ordering of model scores according to the AICc. The threshold parameters for induction, K_{ind1} and K_{ind2} are based on the dose-response of CD86 and CD206 under the single-stimulus conditions. K_{act} is approximated from the experimental data condition with no induction stimulus at 24 hours. A Hill coefficient of 2 was used for all parameter sets. Parameter sets estimated using a Hill coefficient of 1 produced AICc scores equivalent or worse than the AICc scores using a Hill coefficient of 2.

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