Supporting Information File S1

A mechanistic integrative computational model of macrophage polarization: implications in human pathophysiology

Chen Zhao, Adam C. Mirando, Richard J. Sové, Thalyta X. Medeiros, Brian H. Annex, Aleksander S. Popel

Figure S1

Figure S1. Complete model diagram with all nodes and reactions. Reaction rates are labeled (*v#*) and correspond to the rate laws described in detail in Table S1. For the names of model nodes (reaction species): 'X/Y' – complex formed by X and Y, 'pX' – phosphorylated form of species X, 'X_n' – species X in nucleus, 'mX' – mRNA of species X, 'aX' – species X in its activated form, 'Deg' – degradation, dashed arrows – inhibition. Reaction descriptions, parameters, rates and differential equations for all model nodes are summarized in Tables S1 and S2.

41 **Figure S2**

43 **Figure S2. Additional model calibration data of IFN-γ-driven pathway.** Comparisons between model

44 simulations and literature experimental data of IFN-γ-induced (A-C) STAT1 activation at 5 ng/ml (1), 10

45 ng/ml (2), and 20 ng/ml of IFN-γ (3, 4), (D-E) upregulation of SOCS1 and SOCS3 (data is mRNA

46 expression) (5), (F) increase in IRF-1 expression at 6 h (6), and (G) increase in iNOS expression at 12 h

47 (7). (H) Data and simulated expression level of miR-3473b at 24 h after mimic transfection (8). (I)

48 Hypoxia in combination with IFN-γ can significantly induce HIF-2α expression (for IFN-γ alone,

49 simulation suggested a mild decrease while data suggested an insignificant increase in HIF-2α) (9). (A-I)

50 All experimental data are measured in macrophage cell lines and values are for protein levels unless noted

51 otherwise. Y-axes show normalized expression respectively (A-E: simulations and data are normalized to

52 the maximum expression; F, H: normalized to the no-treatment/time 0 expression; G: normalized to the

53 expression at 12 h; I: normalized to the expression under IFN-γ treatment with hypoxia). (D-E) For

54 induction of SOCS1/3, data in terms of SOCS1/3 mRNA expression are compared with simulation

55 (SOCS1/3 protein level), given that SOCS proteins are highly labile (direct protein measurements are

56 scarce) and that Wormald et al. reported a tight temporal correlation between signaling-induced

57 expression of SOCS1/3 protein and mRNA (10). S – simulation, D – literature data, Utr – untreated, Trd –
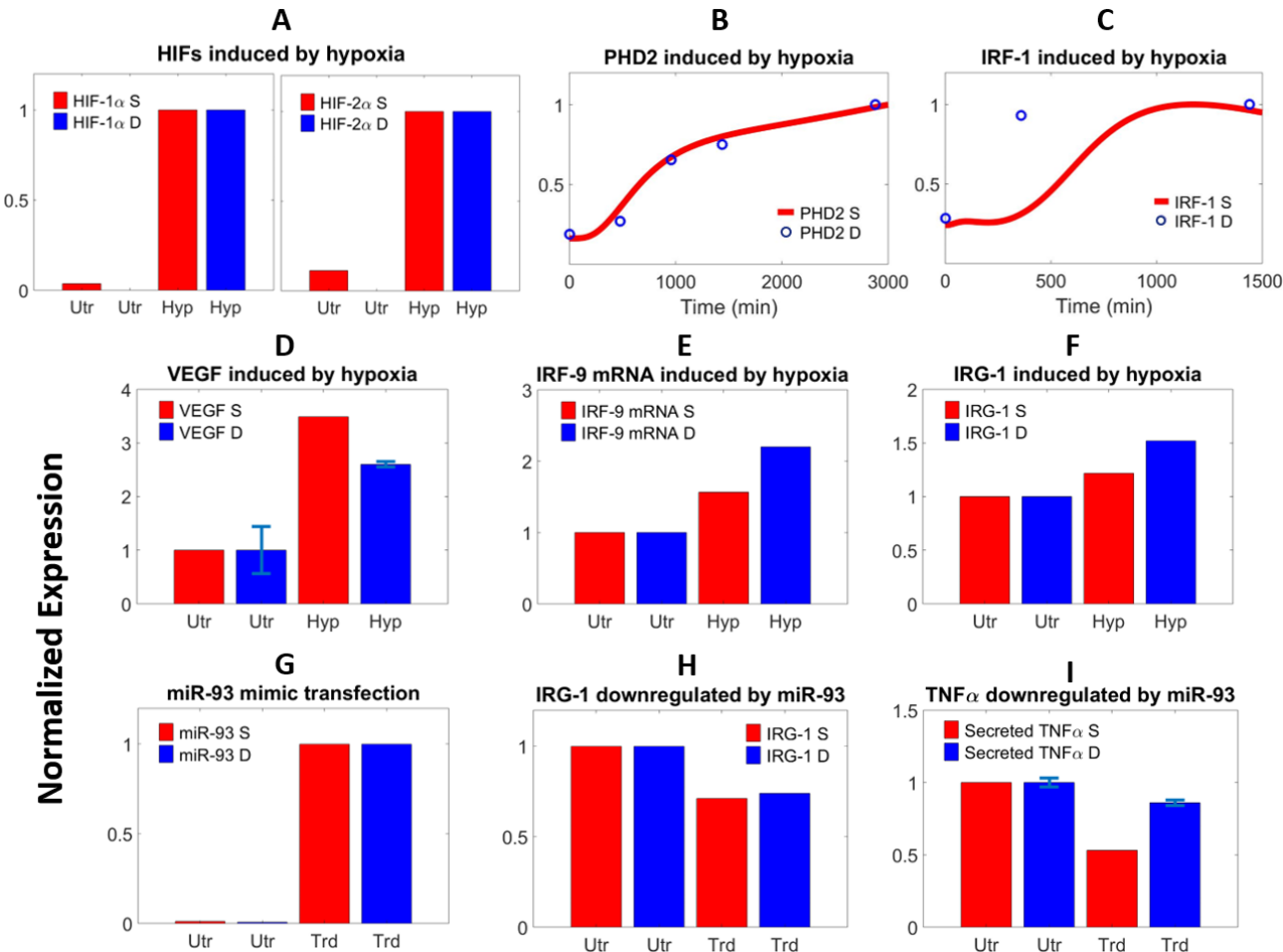
58 IFN-γ treated, Hyp – hypoxia.

Figure S3
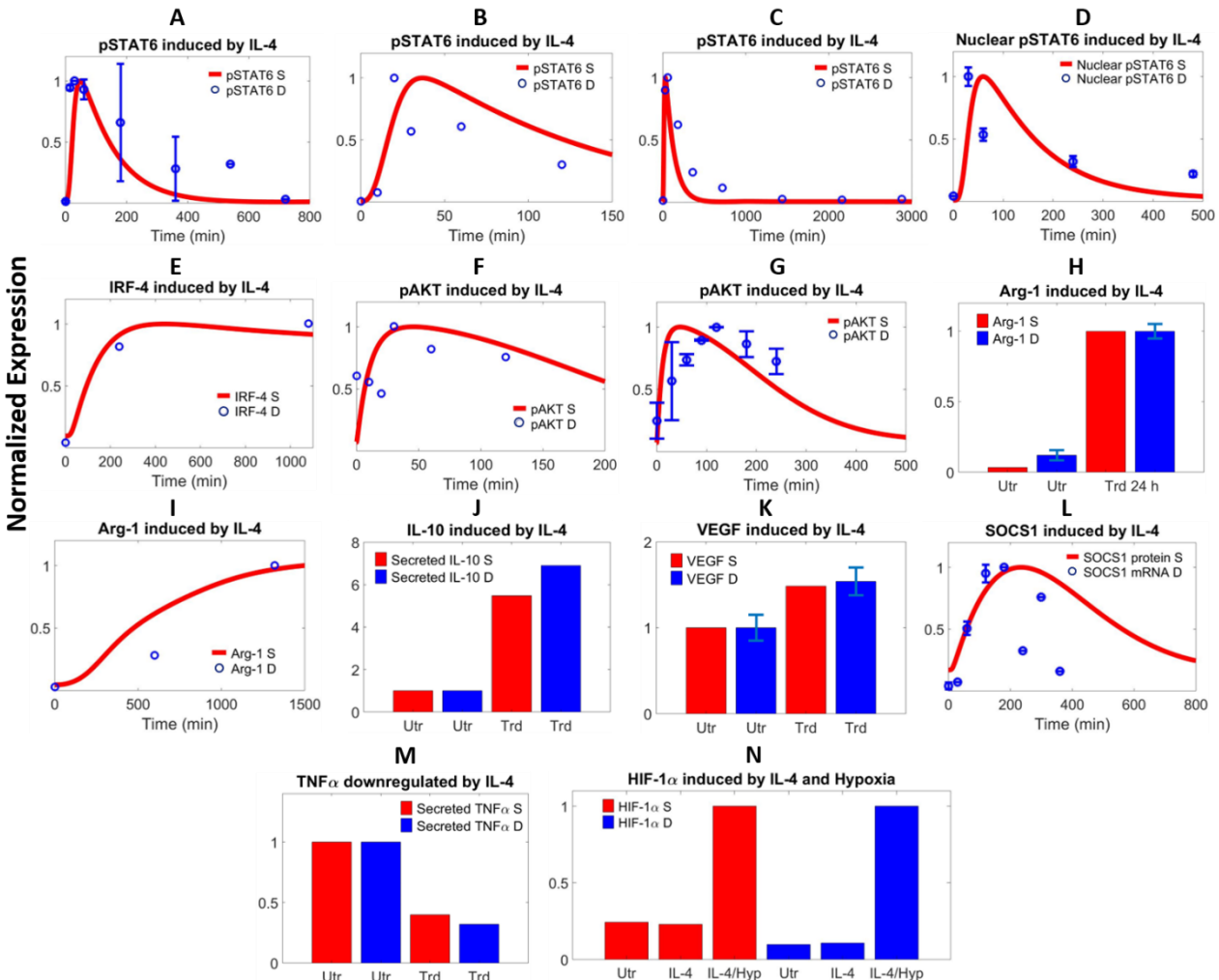
Figure S3. Additional model calibration data of IL-4-driven pathway. Comparisons between model simulations and published experimental data of IL-4-induced (A-C) STAT6 activation at 10 ng/ml (5, 11, 12), 50 ng/ml (13) and 100 nM IL-4 (14), (D) phosphorylated STAT6 in nucleus (15), (E) increase in IRF-4 expression (16), (F-G) AKT activation at 50 ng/ml (17) and 20 ng/ml IL-4 (18), (H-I) increase in Arg-1 production (15, 19), (J) increase in IL-10 production at 24 h (20), (K) increase in VEGF production (21), (L) SOCS1 induction (5), and (M) inhibition of TNFα production at 24 h (20). (N) Simulation and data show that hypoxia can stabilize HIF-1α protein while IL-4 stimulation has no effect (9). (A-N) All experimental data are measured in macrophage cell lines and values are for protein levels unless noted otherwise. Y-axes show normalized expression respectively (A-G, I, L: simulations and data are normalized to the maximum expression; J, K, M: normalized to the no-treatment/time 0 expression; H: normalized to the expression at 24 h; N: normalized to the expression under IL-4 treatment with hypoxia). (H) For Arg-1 production, data in terms of Arg-1 activity (formation of urea from arginine) is compared with simulation (Arg-1 protein level). (K) For VEGF production, data in terms of intracellular VEGF level is compared with simulation (secreted VEGF level). (L) For SOCS1 induction, data in terms of SOCS1 mRNA level is compared with simulation (SOCS1 protein level), given the reasoning stated in Figures S2D-E. S – simulation, D – literature data, Utr – untreated, Trd – IL-4 treated, Hyp – hypoxia.

Figure S4

Figure S4. Additional model calibration data of hypoxia-driven pathway. Comparisons between model simulations and literature experimental data of hypoxia-induced (A) stabilization of HIF-1α and HIF-2α at 18 h (22), (B) PHD upregulation (23), (C) IRF-1 induction (24), (D) VEGF production at 24 h (25), (E) de-suppression of IRF-9 (26) and (F) downstream upregulation of IRG-1 at 12 h (26). (G) Data and simulated expression level of miR-93 before and after 24 h of mimic transfection, which leads to downregulation of (H) IRG-1 abundance at 24 h (26). (I) Transfection of miR-93 mimic decreases TNFα production at 12 h under hypoxia (26). (A-I) All experimental data are measured in macrophage cell lines (except for B, which is in Hela cells) and values are for protein levels unless noted otherwise. Y-axes show normalized expression respectively (A: simulation and data are normalized to the expression under hypoxia; B, C: normalized to the maximum expression; D, E, F, H: normalized to the no-treatment expression; G: normalized to the miR-93 expression at 24 h after transfection; I: normalized to the expression under 12 h of hypoxia without miR-93 mimic). (D) For VEGF production, data in terms of intracellular VEGF level is compared with simulation (secreted VEGF level). (F and H) For IRG-1 regulation, data in terms of IRG-1 mRNA level is compared with simulation (IRG-1 protein level). S – simulation, D – literature data, Utr – untreated, Trd – treated with miR-93 mimic, Hyp – hypoxia.
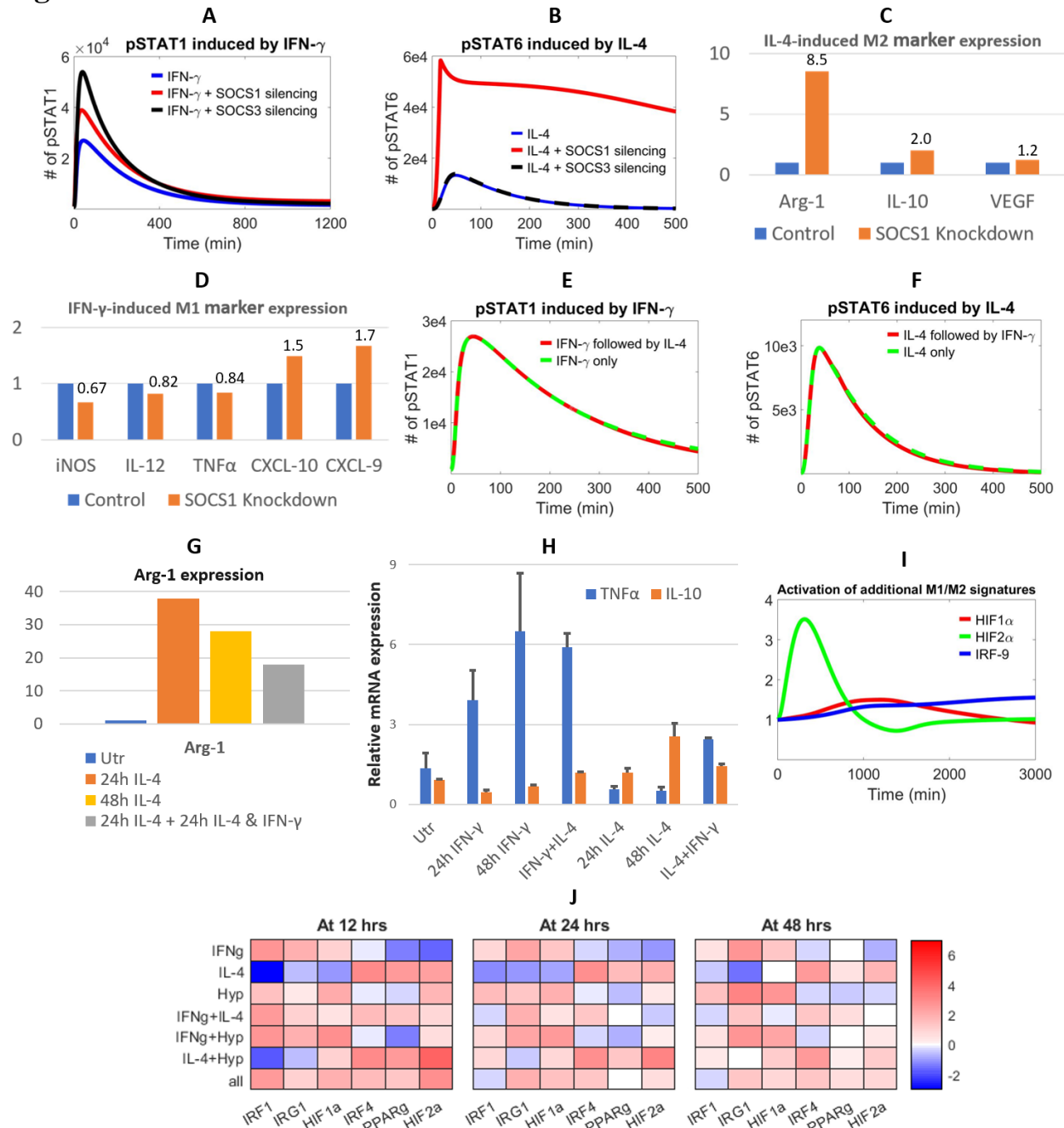
Figure S5

Figure S5. Additional *in silico* investigation of pathway feedback within M1-M2 network. (A)
Silencing of SOCS1 or SOCS3 in macrophages can promote activation of STAT1 by IFN-γ. (B) SOCS1
silencing, but not SOCS3 silencing, can markedly boost STAT6 activation by IL-4. (A-B) Silencing is
modeled as 0x initial level with 0x production. Knockdown of SOCS1 (modeled as 0.3x initial level with
0.3x production) (C) promotes IL-4-induced M2 marker expression and (D) differentially influences M1
marker expression in response to IFN-γ. In the scenarios of IFN-γ stimulation followed by the addition of
IL-4 (at 4 h), or IL-4 stimulation followed by the addition of IFN-γ (at 1 hr), there is no obvious change of
(E) STAT1 or (F) STAT6 activation. (G) The addition of a second stimulus IFN-γ (at 24 h post IL-4
stimulation) can antagonize the expression pattern of Arg-1 induced by IL-4. (H) RT-qPCR analysis

105 (results presented as mean $\pm$ SEM, n=3) of TNFα and IL-10 gene expression in THP-1 cells stimulated

106 with 24 and 48 h of IFN-γ (or IL-4), and 24 h of IFN-γ (or IL-4) then another 24 h of IFN-γ plus IL-4

107 (labeled as IFNγ+IL-4 or IL-4+IFNγ). (I) Temporal expression profiles of HIFs and IRF-9 when

108 macrophages are stimulated with IFN-γ and IL-4 simultaneously. (J) Temporal relative protein expression

109 patterns of six M1-M2 signature transcription factors in macrophages under seven different stimulation

110 conditions ('A+B' means simultaneous stimulation, 'all' means IFN-γ+IL-4+hypoxia, all expression

111 levels are normalized to the untreated/time 0 levels and then log2 transformed). (A-J) All simulation

112 results are protein levels (except CXCL10 is mRNA level). (C,D,G,I) Y-axes show relative expression

113 (normalized to untreated/control/time 0 levels). Simulated treatment doses are 10 ng/ml IFN-γ and 10

114 ng/ml IL-4 for (A-D), 10 ng/ml IFN-γ and 20 ng/ml IL-4 for (E-F), 20 ng/ml IFN-γ and 20 ng/ml IL-4 for

115 (G), 10 ng/ml IFN-γ and 5 ng/ml IL-4 for (I-J). Utr – untreated, Hyp – hypoxia (2% oxygen for J).

116

117 **Figure S6**

118
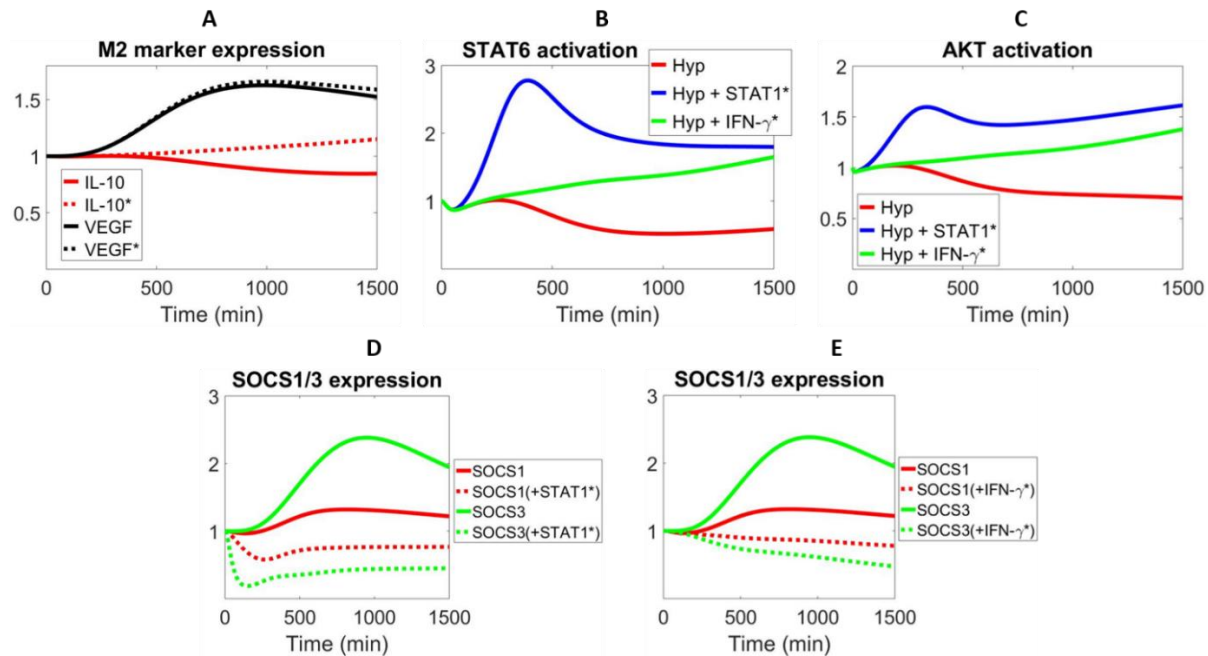
119 **Figure S6. Temporal response of M2 markers and transcription factors under hypoxia.** (A)

120 Inhibition of IFN-γ production under hypoxia (dashed lines) can upregulation the expression of M2

121 markers IL-10 and VEGF (marginal effect), compared to hypoxia alone (solid lines). Simulations show

122 stronger temporal activation of (B) pSTAT6 and (C) pAKT under hypoxia in combination with IFN-γ

123 inhibition or STAT1 inhibition, compared to hypoxia alone. SOCS1 and SOCS3 expression are reduced

124 under hypoxia with either (D) STAT1 or (E) IFN-γ inhibition (dashed lines), compared to hypoxia alone

125 (solid lines). STAT1* means inhibition of STAT1 activation, IFN-γ* means inhibition of IFN-γ

126 production. Inhibition of IFN-γ is simulated by setting its production rate to 10% of the original value;

127 STAT1 inhibition is simulated as a 90% decrease in the binding rate between STAT1 and activated IFN-γ

128 receptor complex. (A-E) Expression levels are normalized to their respective t=0 values (e.g. normoxia,

129 unstimulated). Hyp – hypoxia. All simulation results are protein levels.

Figure S7
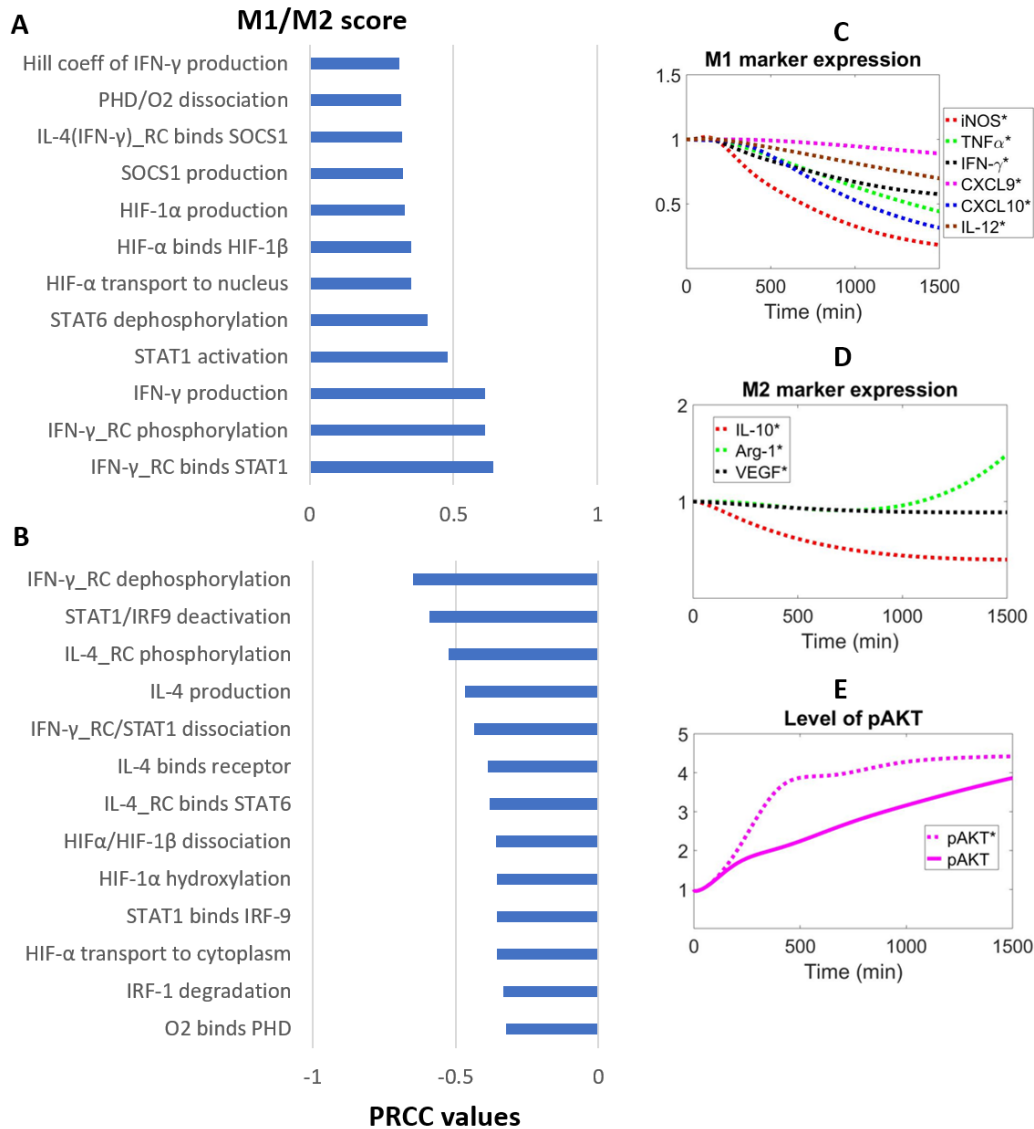
Figure S7. Parameter sensitivities under high IL-4 production. (A-B) Sensitivity indices (top 25 positive and negative PRCC values with p<0.05) of model parameters that control M1 and M2 marker expression in terms of the M1/M2 score under high IL-4 production (10x). In the parameter descriptions, 'X_RC' means receptor complex formed by ligand X, receptor and JAK, 'X/Y' means complex formed by X and Y. (C-D) Simulated relative time-course expression (dashed lines) of M1 and M2 markers when macrophages are subjected to AKT inhibition (simulated as a 90% decrease in the AKT activation rate) under high IL-4 production. (E) Under the scenario of high IL-4 production, inhibition of STAT6 triggers increased activation of AKT as a compensatory mechanism to further upregulate M2 marker expression. (C-E) Marker expression levels are normalized to their respective t=0 values (e.g. normal IL-4 production, unstimulated). All simulation results are protein levels (except CXCL10 is mRNA level). (A-B) More details about the parameters listed can be found in Table S1 using the labels (positive – *ka37, kr70, kf17, k127, k61, kf64, kf63, k33, k45, k37, kf42, kf44*; negative – *kr42, k99, kf8, k1, kr44, kf7, kf13, kr64, k71, kf95, kr63, k78, kf70*; order is from top to bottom as displayed).

Figure S8

Parameter distribution
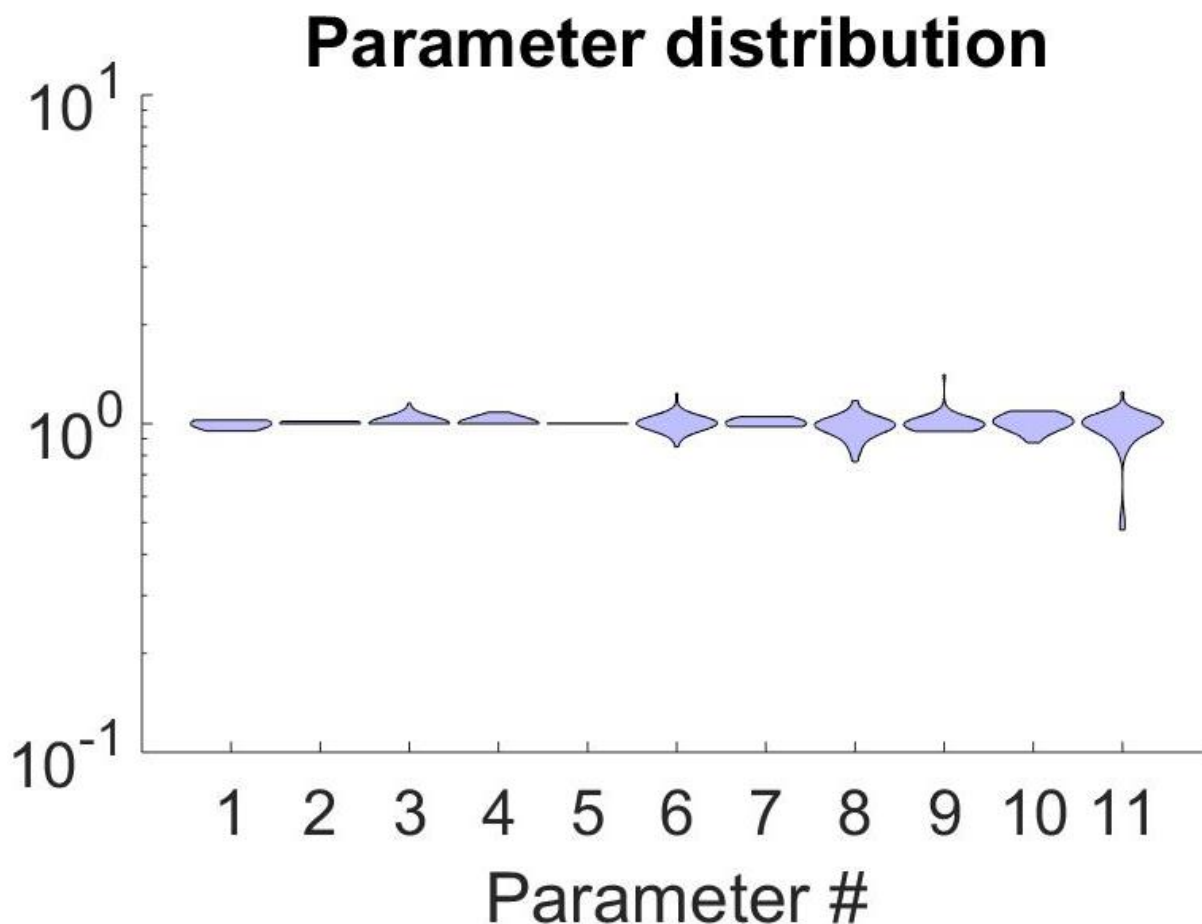
Figure S8. Parameter distribution after bootstrapping. Parameter estimate distributions (represented by violin plots) of the top 11 most sensitive parameters. The 11 parameters were re-estimated 50 times following the resampling procedures described in the Materials and Methods section (all parameter values are normalized to their respective original values for display, y-axis in log scale). During bootstrapping, parameter values are allowed to vary from 0.1x to 10x (of their original values). Parameter descriptions – #1. IFN-γ receptor dephosphorylation rate; 2. forward binding rate of activated IFN-γ receptor complex with STAT1; 3. deactivation rate of STAT1/IRF9 complex in nucleus; 4. IFN-γ receptor phosphorylation rate; 5. reverse binding (e.g. dissociation) rate of activated IFN-γ receptor complex with STAT1; 6. forward binding rate of IL-4 (or IFN-γ) receptor complex with SOCS1; 7. dephosphorylation rate of STAT6; 8. degradation rate of SOCS1/3-bound IL-4 (or IFN-γ) receptor complex; 9. rate of IL-4 receptor phosphorylation; 10. STAT1 activation rate; 11. forward binding rate of IFN-γ receptor complex with SOCS3. The 11 parameters are chosen based on the overall ranking of their absolute PRCC values (from high to low) derived from the sensitivity analysis in three scenarios (IL-4 stimulation, IFN-γ stimulation, and hypoxia).

Table S1

No.	Reaction descriptions	Reaction flux details (v=)	Parameter values	Refs.
v1	IL-4 production	*k1*	*k1*=0.2 min⁻¹	Fitted
v2	IL-4R production	*k2*	*k2*=0.16 min⁻¹	Fitted
v3	IL-4R constitutive degradation	*k3**[IL4R]	*k3*=0.007 min⁻¹	Fitted
v4	JAK production	*k4*	*k4*=50 min⁻¹	Fitted
v5	JAK constitutive degradation	*k5**[JAK]	*k5*=0.0005 min⁻¹	(27)
v6	IL-4R pre-associates with JAK	*kf6**[JAK]*[IL4R]-*kr6**[IL4R/JAK]	*kf6*=4e-6 min⁻¹, *kr6*=0.0018 min⁻¹	Fitted
v7	IL-4 binds receptor	*kf7**[IL4]*[IL4R/JAK]-*kr7**[IL4/R/JAK]	*kf7*=2e-7 min⁻¹, *kr7*=0.01 min⁻¹	(28)
v8	IL-4 receptor complex phosphorylation	*kf8**[IL4/R/JAK]-*kr8**[pIL4/R/JAK]	*kf8*=0.6 min⁻¹, *kr8*=0.1 min⁻¹	Fitted
v9	Internalization of phosphorylated IL-4 receptor complex	*k9**[pIL4/R/JAK]	*k9*=0.2 min⁻¹	Fitted
v10	Shuttling of IL-4 receptor complex to lysosomes	*k10**[pIL4/R/JAK_i]	*k10*=1 min⁻¹	Fitted
v11	Degradation of IL-4 and IL-4R in lysosomes	*k11**[IL4/R_lyso]	*k11*=0.3 min⁻¹	Fitted
v12	Recycling of IL-4R	*k12**[IL4/R_lyso]	*k12*=1 min⁻¹	Fitted
v13	Binding of STAT6 with ligand-activated IL-4 receptor complex	*kf13**[STAT6]*[pIL4/R/JAK_i]-*kr13**[pIL4/R/JAK/STAT6]	*kf13*=0.1 min⁻¹, *kr13*=10 min⁻¹	Fitted
v14	Activation of STAT6 by phosphorylation	*k14**[pIL4/R/JAK/STAT6]	*k14*=8 min⁻¹	Fitted
v15	Phosphorylation of AKT	*k15**[AKT]*(1-[PTEN]/([PTEN]+ka15))*([pIL4/R/JAK]/([pIL4/R/JAK]+kb15))	*k15*=1.16 min⁻¹, *ka15*=5000, *kb15*=4	Fitted
v16	AKT dephosphorylation	*k16**[pAKT]	*k16*=0.015 min⁻¹	(29)
v17	Internalized IL-4 receptor complex binds SOCS1	*kf17**[pIL4/R/JAK_i]*[SOCS1]-*kr17**[IL4/R/JAK/SOCS1_i]	*kf17*=0.008 min⁻¹, *kr17*=0.2 min⁻¹	Fitted
v18	SOCS1 inhibits IL-4 signaling and shuttles IL-4 and receptor to lysosomes	*k10**[IL4/R/JAK/SOCS1_i]		*
v19	SOCS1 sequesters JAK from the IL-4 receptor complex	*k19**[IL4/R/JAK/SOCS1_i]	*k19*=0.03 min⁻¹	Fitted
v20	Shuttling of internalized IL-4 and receptor to lysosomes	*k10**[IL4/R]		*
v21	SOCS1 targets JAK for degradation	*k21**[SOCS1/JAK]	*k21*=0.1 min⁻¹	Fitted
v22	Internalized IL-4 and receptor associate with JAK to reactivate signaling	*kf6**[IL4/R]*[JAK]-*kr6**[pIL4/R/JAK_i]		*
v23	Internalized IL-4 receptor complex binds SOCS3	*kf23**[pIL4/R/JAK_i]*[SOCS3]-*kr17**[IL4/R/JAK/SOCS3_i],	*kf23*=0.0004 min⁻¹	Fitted *

v24	SOCS3 inhibits IL-4 signaling and shuttles IL-4 and receptor to lysosomes	k10*[IL4/R/JAK/SOCS3_i]		*
v25	SOCS3 sequesters JAK from the IL-4 receptor complex	k19*[IL4/R/JAK/SOCS3_i]		*
v26	SOCS3 targets JAK for degradation	k26*[SOCS3/JAK]	k26=0.01 min⁻¹	Fitted
v27	Surface IL-4 receptor complex binds SOCS1	kf17*[IL4/R/JAK]*[SOCS1]-kr17*[IL4/R/JAK/SOCS1]		*
v28	SOCS1-mediated shuttling of surface IL-4 and receptor to lysosomes	k28*[IL4/R/JAK/SOCS1]	k28=0.1 min⁻¹	Fitted
v29	Surface IL-4 receptor complex binds SOCS3	kf23*[IL4/R/JAK]*[SOCS3]-kr17*[IL4/R/JAK/SOCS3]		*
v30	SOCS3-mediated shuttling of surface IL-4 and receptor to lysosomes	k28*[IL4/R/JAK/SOCS3]		*
v31	pSTAT6 dimerization	kf31*[pSTAT6]*[pSTAT6]-kr31*[pSTAT6D]	kf31=0.002 min⁻¹, kr31=1 min⁻¹	Fitted
v32	Activated STAT6 dimer transports to nucleus	k32*[pSTAT6D]	k32=0.4 min⁻¹	Fitted
v33	Dephosphorylation of nuclear STAT6 dimer	k33*[pSTAT6D_n]	k33=0.01 min⁻¹	(30)
v34	Dissociation of STAT6 dimer in nucleus	kr31*[STAT6D_n]		*
v35	Nuclear export of STAT6	k35*[STAT6_n]	k35=0.05 min⁻¹	(31)
v36	Dephosphorylation of STAT6	k33*[pSTAT6]		*
v37	IFN-γ production	k37*([HIF1α/β_n]+0.2*[HIF2α/β_n])*(1-[pSTAT6D_n]/([pSTAT6D_n]+ka37))	k37=0.0288 min⁻¹, ka37=100	Fitted
v38	IFNGR production	k38	k38=1.22 min⁻¹	Fitted
v39	IFNGR constitutive degradation	k39*[IFNGR]	k39=0.006 min⁻¹	(32)
v40	IFNGR pre-associates with JAK	kf6*[IFNGR]*[JAK]-kr40*[IFNGR/JAK]	kr40=0.002 min⁻¹	Fitted *
v41	IFN-γ binds receptor	kf41*[IFNγ]*[IFNGR/JAK]-kr7*[IFNγ/R/JAK]	kf41=1.6e-7 min⁻¹	(33, 34)*
v42	IFN-γ receptor complex phosphorylation	kf42*[IFNγ/R/JAK]-kr42*[pIFNγ/R/JAK]	kf42=3 min⁻¹, kr42=10 min⁻¹	Fitted
v43	IFN-γ receptor complex binds SOCS1	kf17*[IFNγ/R/JAK]*[SOCS1]-kr17*[IFNγ/R/JAK/SOCS1]		*
v44	STAT1 binds ligand-activated IFN-γ receptor complex	kf44*[STAT1]*[pIFNγ/R/JAK]-kr44*[pIFNγ/R/JAK/STAT1]	kf44=0.03 min⁻¹, kr44=3 min⁻¹	Fitted
v45	STAT1 activation by phosphorylation	k45*[pIFNγ/R/JAK/STAT1]	k45=2 min⁻¹	Fitted
v46	Shuttling of IFN-γ receptor complex to lysosomes	k46*[pIFNγ/R/JAK]	k46=0.1 min⁻¹	Fitted

v47	Degradation of IFN-γ and IFNGR in lysosomes	k47*[IFNγ/R_lyso]	k47=1 min^{-1}	Fitted
v48	Recycling of IFNGR	k12*[IFNγ/R_lyso]		*
v49	SOCS1 inhibits IFN-γ signaling and shuttles IFN-γ and receptor to lysosomes	k46*[IFNγ/R/JAK/SOCS1]		*
v50	SOCS1 sequesters JAK from the IFN-γ receptor complex	k19*[IFNγ/R/JAK/SOCS1]		*
v51	IFN-γ and receptor bind JAK to reactivate signaling	kf6*[IFNγ/R]*[JAK]-kr40*[IFNγ/R/JAK],		*
v52	IFN-γ receptor complex binds SOCS3	kf52*[IFNγ/R/JAK]*[SOCS3]-kr17*[IFNγ/R/JAK/SOCS3]	kf52=0.004 min^{-1}	Fitted *
v53	SOCS3 inhibits IFN-γ signaling and shuttles IFN-γ and receptor to lysosomes	k46*[IFNγ/R/JAK/SOCS3]		*
v54	SOCS3 sequesters JAK from the IFN-γ receptor complex	k19*[IFNγ/R/JAK/SOCS3]		*
v55	pSTAT1 dimerization	kf55*[pSTAT1]*[pSTAT1]-kr31*[pSTAT1D]	kf55=0.1 min^{-1}	Fitted *
v56	Activated STAT1 dimer transports to nucleus	k56*[pSTAT1D]	k56=1 min^{-1}	Fitted
v57	Dephosphorylation of nuclear STAT1 dimer	k57*[pSTAT1D_n]	k57=0.03 min^{-1}	(35)
v58	Dissociation of STAT1 dimer in nucleus	kr31*[STAT1D_n]		*
v59	Nuclear export of STAT1	k59*[STAT1_n]	k59=0.1 min^{-1}	Fitted
v60	Dephosphorylation of STAT1	k57*[pSTAT1]		*
v61	HIF-1α production is promoted by TNFα signaling and downregulated by miR-93	k61*([TNFα]+ka61)*(1.4-[miR93]/([miR93]+kb61))	k61=0.0187 min^{-1}, ka61=800, kb61=1000	Fitted
v62	HIF-2α production is promoted by PPARγ	k62*([aPPARG]/([aPPARG]+ka62))	k62=178 min^{-1}, ka62=10000	Fitted
v63	HIF-1α transport to nucleus	kf63*[HIF1α]-kr63*[HIF1α_n]	kf63=0.005 min^{-1}, kr63=0.018 min^{-1}	(36)
v64	HIF-2α transport to nucleus	kf63*[HIF2α]-kr63*[HIF2α_n]		*
v65	Nuclear HIF-1α binds HIF-1β	kf64*[HIF1α_n]*[HIF1β_n]-kr64*[HIF1α/β_n]	kf64=5e-7 min^{-1}, kr64=0.03 min^{-1}	Fitted; (36)
v66	Nuclear HIF-2α binds HIF-1β	kf64*[HIF2α_n]*[HIF1β_n]-kr64*[HIF2α/β_n]		*
v67	PHD production	k67*(0.0001+[HIF1α/β_n]^2/([HIF1α/β_n]^2+ka67)*[HIF2α/β_n]^2/([HIF2α/β_n]^2+kb67))	k67=602 min^{-1}, ka67=32600, kb67=25400	Fitted
v68	PHD degradation	k68*[PHD]	k68=0.0008 min^{-1}	(27)
v69	Itaconate influences PHD activation	kf69*[PHD]*[Itaconate]-kr69*[aPHD]	kf69=8e-9 min^{-1}, kr69=0.07 min^{-1}	Fitted
v70	PHD binds oxygen	kf70*[aPHD]*[O$_2$]-kr70*[O$_2$/aPHD]	kf70=7.14e-9 min^{-1}, kr70=10.8 min^{-1}	Fitted; (36)
v71	HIF1α hydroxylation	k71*[O$_2$/aPHD]*[HIF-1α]	k71=1.33e-5 min^{-1}	Fitted

$v72$	HIF1α de-ubiquitination	$k72*[\text{HIF1α\_OH}]$	$k72$=0.03 min^{-1}	Fitted
$v73$	Degradation of hydroxylated HIF-1α	$k73*[\text{HIF1α\_OH}]$	$k73$=0.6 min^{-1}	Fitted
$v74$	HIF2α hydroxylation	$k74*[\text{O}_2/\text{aPHD}]*[\text{HIF-2α}]$	$k74$=5e-6 min^{-1}	Fitted
$v75$	HIF2α de-ubiquitination	$k72*[\text{HIF2α\_OH}]$		*
$v76$	Degradation of hydroxylated HIF-2α	$k73*[\text{HIF2α\_OH}]$		*
$v77$	STAT1 and STAT6 regulate IRF-1 production	$k77*(([\text{pSTAT1D\_n}]+[\text{pSTAT1D/IRF9\_n}])/[\text{pSTAT6D\_n}])/(([\text{pSTAT1D\_n}]+[\text{pSTAT1D/IRF9\_n}])/[\text{pSTAT6D\_n}]+ka77)$	$k77$=106 min^{-1}, $ka77$=50	Fitted
$v78$	IRF-1 degradation	$k78*[\text{IRF1}]$	$k78$=0.022 min^{-1}	(37)
$v79$	IFN-γ (through IRF-1) inhibits miR-3473 production	$k79*(1-[\text{IRF1}]^2/([\text{IRF1}]^2+ka79))$	$k79$=14.8 min^{-1}, $ka79$=40000	Fitted
$v80$	Degradation of miR-3473	$k80*[\text{miR3473}]$	$k80$=0.0012 min^{-1}	(38)
$v81$	miR-3473 binds PTEN mRNA	$kf81*[\text{miR3473}]*[\text{mPTEN}]-kr81*[\text{miR3473/mPTEN}]$	$kf81$=0.0001 min^{-1}, $kr81$=0.06 min^{-1}	(39)
$v82$	Degradation of miR3473-bound PTEN mRNA	$k82*[\text{miR3473/mPTEN}]$	$k82$=0.1 min^{-1}	Fitted
$v83$	Production of PTEN mRNA	$k83$	$k83$=0.64 min^{-1}	Fitted
$v84$	PTEN translation	$k84*[\text{mPTEN}]$	$k84$=2.32 min^{-1}	Fitted
$v85$	PTEN degradation	$k85*[\text{PTEN}]$	$k85$=0.0003 min^{-1}	(27)
$v86$	PTEN mRNA constitutive degradation	$k86*[\text{mPTEN}]$	$k86$=0.003 min^{-1}	(40)
$v87$	miR-93 production is downregulated by IFN-γ (represented by STAT1) and TNFα signaling	$k87*(1-[\text{TNFα}]*[\text{pSTAT1D\_n}]^2/([\text{TNFα}]*[\text{pSTAT1D\_n}]^2+ka87))$	$k87$=6050 min^{-1}, $ka87$=1000	Fitted
$v88$	miR-93 degradation	$k88*[\text{miR93}]$	$k88$=0.0018 min^{-1}	(38)
$v89$	miR-93 binds IRF9 mRNA	$kf81*[\text{miR93}]*[\text{mIRF9}]-kr81*[\text{miR93/mIRF9}]$		*
$v90$	Degradation of miR93-bound IRF9 mRNA	$k82*[\text{miR93/mIRF9}]$		*
$v91$	IRF9 mRNA production	$k91$	$k91$=0.5 min^{-1}	Fitted
$v92$	IRF9 mRNA constitutive degradation	$k86*[\text{mIRF9}]$		*
$v93$	IRF9 translation	$k93*[\text{mIRF9}]$	$k93$=1.18 min^{-1}	Fitted
$v94$	IRF9 degradation	$k94*[\text{IRF9}]$	$k94$=0.004 min^{-1}	Fitted
$v95$	IRF9 binds activated STAT1 dimer in cytoplasm	$kf95*[\text{IRF9}]*[\text{pSTAT1D}]-kr95*[\text{pSTAT1D/IRF9}]$	$kf95$=0.00013 min^{-1}, $kr95$=1 min^{-1}	Fitted
$v96$	Nuclear translocation of STAT1/IRF9 complex	$k96*[\text{pSTAT1D/IRF9}]$	$k96$=0.1 min^{-1}	Fitted
$v97$	IRF9 binds activated STAT1 dimer in nucleus	$kf95*[\text{IRF9\_n}]*[\text{pSTAT1D\_n}]-kr95*[\text{pSTAT1D/IRF9\_n}]$		*
$v98$	IRF9 translocation to nucleus	$k96*[\text{IRF9}]-kr98*[\text{IRF9\_n}]$	$kr98$=0.01 min^{-1}	Fitted
$v99$	Deactivation of STAT1/IRF9 complex in nucleus	$k99*[\text{pSTAT1D/IRF9\_n}]$	$k99$=0.4 min^{-1}	Fitted

v100	IRF4 production regulated by STAT6 and AKT	$k100$*[pSTAT6D_n]/([pSTAT6D_n]+$ka100$)*[pAKT]/([pAKT]+$kb100$)	$k100$=6840 min^{-1}, $ka100$=30000, $kb100$=20000	Fitted
v101	IRF4 degradation	$k101$*[IRF4]	$k101$=0.0002 min^{-1}	(27)
v102	STAT6 promotes PPARγ production	$k102$*[pSTAT6D_n]/([pSTAT6D_n]+$ka102$)	$k102$=107 min^{-1}, $ka102$=1000	Fitted
v103	AKT promotes PPARγ activation	$k103$*[PPARG]*[pAKT]/([pAKT]+$ka103$)	$k103$=50 min^{-1}, $ka103$=10000	Fitted
v104	PPARγ deactivation	$k104$*[aPPARG]	$k104$=50 min^{-1}	Fitted
v105	Degradation of activated PPARγ	$k105$*[aPPARG]	$k105$=0.0018 min^{-1}	(41)
v106	Degradation of PPARγ	$k105$*[PPARG]		*
v107	IRF9 and IRF1 promotes IRG-1 production	$k107$*[IRF1]*([IRF9_n]+[pSTAT1D/IRF9_n])^2	$k107$=4.77e-12 min^{-1}	Fitted
v108	IRG1 degradation	$k108$*[IRG1]	$k108$=6.2e-4 min^{-1}	(27)
v109	IRG1 promotes itaconate production	$k109$*[IRG1]^2	$k109$=0.025 min^{-1}	Fitted
v110	Itaconate degradation	$k110$*[Itaconate]	$k110$=0.005 min^{-1}	Fitted
v111	HIF1/2 and AKT induce VEGF production	$k111$*([HIF1α/β_n]*[HIF2α/β_n]+$ka111$)*([pAKT]+$kb111$)	$k111$=3e-9 min^{-1}, $ka111$=29200, $kb111$=42700	Fitted
v112	Removal of secreted VEGF	$k112$*[VEGF]	$k112$=0.001 min^{-1}	Fitted
v113	iNOS production is dependent on HIF1, IRF1 and type I IFN signaling (represented by IRF9)	$k113$*(0.02+[HIF1α/β_n]/([HIF1α/β_n]+$ka113$))*([IRF1]/([IRF1]+$kb113$))*([IRF9T]+15000)	$k113$=0.036 min^{-1}, $ka113$=4000, $kb113$=9000	Fitted
v114	iNOS degradation	$k114$*[iNOS]	$k114$=0.006 min^{-1}	(42)
v115	ARG1 production is promoted by STAT6, IRF4 and HIFs while downregulated by TNFα	$k115$*([pSTAT6D_n]*[IRF4]+$ka115$)*([HIF1α/β_n]+2*[HIF2α/β_n])^2*(1.2-[TNFα]^2/([TNFα]^2+$kb115$))	$k115$=3.76e-9 min^{-1}, $ka115$=1.2e7, $kb115$=100000	Fitted
v116	ARG1 degradation	$k116$*[ARG1]	$k116$=0.0006 min^{-1}	(43, 44)
v117	IRF1 and type I IFNs (represented by IRF9) can induce TNFα production	$k117$*[IRF1]^2/([IRF1]^2+$ka117$)*(30000+[IRF9T])^2	$k117$=7.2e-10 min^{-1}, $ka117$=1.6e6	Fitted
v118	Removal of secreted TNFα	$k118$*[TNFα]	$k118$=0.0008 min^{-1}	Fitted
v119	IL-10 production is promoted by AKT, type I IFNs (represented by IRF9) and STAT3 (represented by secreted IL-10)	$k119$*[pAKT]*(2000+[IL10])*([IRF9T]+15000)	$k119$=6.22e-13 min^{-1}	Fitted
v120	Removal of secreted IL-10	$k120$*[IL10]	$k120$=0.0015 min^{-1}	Fitted
v121	IRF1 promotes IL-12 production	$k121$*[IRF1]^2	$k121$=5e-8 min^{-1}	Fitted
v122	Removal of secreted IL-12	$k122$*[IL12]	$k122$=0.00033 min^{-1}	Fitted
v123	STAT1 induces CXCL9 synthesis	$k123$*[pSTAT1D_n]^2	$k123$=1.67e-6 min^{-1}	Fitted
v124	Removal of secreted CXCL9	$k124$*[CXCL9]	$k124$=0.0002 min^{-1}	Fitted

$v125$	STAT1/IRF9 complex induces CXCL10 mRNA production	$k125$*[pSTAT1D/IRF9_n]^2	$k125$=1e-6 min^{-1}	Fitted
$v126$	CXCL10 mRNA degradation	$k126$*[mCXCL10]	$k126$=0.0035 min^{-1}	(40)
$v127$	STAT1 and STAT6 induce SOCS1 production	$k127$*[pSTAT1D_n]/([pSTAT1D_n]+$ka127$)*[pSTAT6D_n]/([pSTAT6D_n]+$kb127$)	$k127$=13342 min^{-1}, $ka127$=6000, $kb127$=500	Fitted
$v128$	Degradation of SOCS1	$k128$*[SOCS1]	$k128$=0.004 min^{-1}	(45)
$v129$	STAT1 induces SOCS3 production	$k129$*[pSTAT1D_n]/([pSTAT1D_n]+$ka129$)	$k129$=6571 min^{-1}, $ka129$=6000	Fitted
$v130$	Degradation of SOCS3	$k130$*[SOCS3]	$k130$=0.05 min^{-1}	Fitted

163

164 **Table S1. Complete list of model reactions and parameter values.** Reactions are formulated

165 mechanistically based on literature evidence (the labels *v#* here match with the labels in Figure S1 and

166 Table S2). As shown in the last column, numerical values of model parameters (~140 in total) are either

167 estimated from relevant published data (e.g. experimental measurements, prior models) or estimated

168 computationally through whole-model optimization ('fitted'). Certain parameter values are shared by

169 more than one reaction fluxes (marked by * in the last column).

170

171

172

173

174

175

176

177

178

179

180

181

Table S2

Name of model node	Initial condition (in # of molecules)	Equation (d[x]/dt=)	Initial condition Refs.
VEGF	5223	*v111-v112*	Estimated from (46)
IFNγ	358	*v37-v41*	Estimated from (47)
IFNGR/JAK	20376	*v40-v41*	Total IFNGR estimated from (48, 49)
IFNGR	106	*-v40+v48*	Fitted
IFNγ/R	1	*v50+v54-v51*	Fitted
IFNγ/R/JAK	0	*v41-v42-v43+v51-v52*	Fitted
pIFNγ/R/JAK	0	*v42-v44*	Fitted
STAT1	397262	*-v44+v59+v60*	Total STAT1 estimated from (49, 50)
pIFNγ/R/JAK/STAT1	84	*v44-v45*	Fitted
pSTAT1	41	*v45-v60-2*v55*	Fitted
pSTAT1D	81	*v55-v56-v95*	Fitted
pSTAT1D_n	80	*v56-v57-v97*	Fitted
STAT1D_n	84	*v57+v99-v58*	Fitted
STAT1_n	1673	*2*v58-v59*	Fitted
SOCS1	3169	*v127-v128-v17+v18+v21-v27+v28-v43+v49*	Total SOCS1 estimated from (51)
IFNγ/R/JAK/SOCS1	9	*v43-v49-v50*	Fitted
IFNγ/R_lyso	1	*v46-v47-v48+v49+v53*	Fitted
SOCS1/JAK	3	*v50+v19-v21*	Fitted
IRF9	2674	*v93-v94-v95-v98*	Total IRF9 estimated from (49)
IRF9_n	27001	*v98-v97+v99*	Fitted
pSTAT1D/IRF9	26	*v95-v96*	Fitted
pSTAT1D/IRF9_n	203	*v96-v99+v97*	Fitted
SOCS3	1735	*v129-v130-v23+v24+v26-v29+v30-v52+v53*	Total SOCS3 estimated from (51)
IFNγ/R/JAK/SOCS3	3	*v52-v53-v54*	Fitted
SOCS3/JAK	8	*v54+v25-v26*	Fitted
IL4	288	*v1-v7*	Estimated from (52)
IL4R	16	*v2-v6+v12*	Fitted
IL4/R	0	*v19+v25-v22*	Fitted
IL4R/JAK	3478	*v6-v7*	Total IL4R estimated from (49)
IL4/R/JAK	0	*v7-v8*	Fitted
pIL4/R/JAK	0	*v8-v9*	Fitted
pIL4/R/JAK_i	0	*v9-v10-v13+v14-v17+v22-v23*	Fitted
IL4/R/JAK/SOCS1_i	0	*v17-v18-v19*	Fitted

IL4/R_lyso	0	$v10-v11-v12+v18+v20+v24+v28+v30$	Fitted
IL4/R/JAK/SOCS3_i	0	$v23-v24-v25$	Fitted
IL4/R/JAK/SOCS1	2	$v27-v28$	Fitted
IL4/R/JAK/SOCS3	0	$v29-v30$	Fitted
STAT6	59874	$v35+v36-v13$	Total STAT6 estimated from (49, 51)
pIL4/R/JAK/STAT6	0	$v13-v14$	Fitted
pSTAT6	26	$v14-v36-2*v31$	Fitted
pSTAT6D	1	$v31-v32$	Fitted
pSTAT6D_n	39	$v32-v33$	Fitted
STAT6D_n	0	$v33-v34$	Fitted
STAT6_n	16	$2*v34-v35$	Fitted
PTEN	64899	$v84-v85$	Estimated from (49)
IRF4	16460	$v100-v101$	Estimated from (49)
IL10	571	$v119-v120$	Estimated from (53)
JAK	99297	$v4-v5-v6+v10+v18-v22+v24+v28+v30-v40+v46+v49+v53$	Total JAK estimated from (49)
ARG1	875428	$v115-v116$	Estimated from (49)
miR3473	1180	$v79-v80-v81+v82$	Estimated from (54)
mPTEN	8	$v83-v86-v81$	Total mPTEN estimated from (55)
miR3473/mPTEN	6	$v81-v82$	Fitted
iNOS	538	$v113-v114$	Fitted
HIF1α	640	$v61-v63-v71+v72$	Total HIF1α estimated from (56)
HIF1α_n	178	$v63-v65$	Fitted
HIF1α/β_n	44	$v65$	Fitted
HIF1α_OH	42	$v71-v72-v73$	Fitted
HIF2α	878	$v62-v64-v74+v75$	Total HIF2α estimated from (56)
HIF2α_n	244	$v64-v66$	Fitted
HIF2α/β_n	61	$v66$	Fitted
HIF2α_OH	22	$v74-v75-v76$	Fitted
HIF1β_n	14897	$-v65-v66$	Total HIF1β estimated from (49)
PPARG	1437	$v102-v103+v104-v106$	Total PPARG estimated from (57)
aPPARG	782	$v103-v104-v105$	Fitted
IRF1	614	$v77-v78$	Estimated from (49)
PHD	5470	$v67-v68-v69$	Total PHD estimated from (49)
O$_2$	1.204e8	*Constant*	Estimated from (56)
O$_2$/aPHD	3088	$v70-v71-v74$	Fitted
aPHD	38846	$v69-v70+v71+v74$	Fitted

TNFα	619	*v117-v118*	Estimated from (58-60)
CXCL9	54	*v123-v124*	Fitted
mIRF9	9	*v91-v92-v89*	Total mIRF9 estimated from (61)
miR93/mIRF9	5	*v89-v90*	Fitted
miR93	841	*v87-v88-v89+v90*	Fitted
IL12	58	*v121-v122*	Estimated from (62)
mCXCL10	12	*v125-v126*	Estimated from (61)
AKT	198060	*-v15+v16*	Total AKT estimated from (49)
pAKT	11942	*v15-v16*	Fitted
IRG1	3522	*v107-v108*	Estimated from (49)
Itaconate	6.214e7	*v109-v110*	Estimated from (63)
IRF9T (repeated assignment)	29904	*[IRF9T]=[IRF9]+[IRF9_n]+ [pSTAT1D/IRF9]+[pSTAT1D/IRF9_n]*	Fitted

183

184 **Table S2. Differential equations and initial conditions of all model nodes.** A summary of the model

185 equations and initial conditions in terms of absolute copy numbers of all 80 model nodes (proteins,

186 RNAs, complexes, etc.) derived from the 34 functionally unique species. The initial levels (and also the

187 steady state levels) of 31 out of 34 unique species are estimated and calibrated with respect to literature

188 data (using both direct measurements and indirect observations), and the model is simulated in control

189 condition without external stimulation until equilibrium to obtain the initial levels (e.g. copy numbers) of

190 all 80 model nodes. A cell volume of 1 pL is assumed when doing unit conversion calculations for O_2 and

191 itaconate (64, 65). To reduce model complexity, it is assumed that transcription factors and enzymes in

192 Hill-type reactions are not consumed, mRNAs are not consumed during translation, and oxygen level is

193 constant during each simulation run.

194

195

196

197

198

199

200

201

202

Table S3

Description of Data Used in Calibration	PMIDs of Sources
IFN-γ module (in response to IFN-γ treatments unless noted otherwise below)	
Surface-bound IFN-γ	2953810
Phosphorylation of JAK	12667213
Phosphorylation of STAT1	16473883, 26882544, 26299368, 28280036, 10490990
Expression of IRF-1	17293456, 18802049
Expression of iNOS	18655171, 9667738
TNFα secretion	8802049
IL-12 secretion	25950470
CXCL-9 secretion	25950470
CXCL-10 mRNA expression	25918247
Expression of miR-3473b	25092892
Itaconate expression	26829557
PTEN expression upon miR-3473b overexpression	25092892
HIF1α expression upon IFN-γ treatment and hypoxia	20194441
SOCS1 mRNA expression	17093501
SOCS3 mRNA expression	17093501
HIF2α expression upon IFN-γ treatment and hypoxia	20194441
IL-4 module (in response to IL-4 treatments unless noted otherwise below)	
STAT6 phosphorylation	27731330, 17093501, 26894960, 26883801, 27464342, 25175012
Phosphorylated STAT6 in nucleus	23913966
IRF-4 expression	29871928, 23287596, 20580461
AKT activation	26894960, 27731330, 27507812
PPARγ expression	29203644, 24385430
Arg-1 expression	23287596, 27117406, 23913966
Arg-1 activity	22348056
IL-10 secretion	28903394, 21753147
VEGF secretion	28842601
VEGF expression (intracellular)	28903394
TNFα secretion	28903394, 21753147
HIF2α expression upon IL-4 treatment and hypoxia	20194441
SOCS1 mRNA expression	17093501
HIF1α expression upon IL-4 treatment and hypoxia	20194441
Hypoxia module (in response to hypoxia unless noted otherwise below)	
HIF1a stabilization	24301659, 16533170, 20644254, 19454749
HIF2a stabilization	16533170, 20644254, 19454749
iNOS expression	28211523
Arg-1 expression	28211523

TNFα secretion	22566835
IFN-γ secretion	19234213
VEGF secretion	17065555
VEGF expression (intracellular)	28903394
miR-93 expression	28356443
IFN-γ secretion upon miR-93 overexpression and hypoxia	28356443
PHD2 expression	12912907
IRF-1 expression	11313373
IRF-9 mRNA expression	28356443
IRG-1 mRNA expression	28356443
IRG-1 mRNA expression upon miR-93 overexpression	28356443
TNFα secretion upon miR-93 overexpression and hypoxia	28356443

204

205 **Table S3. Summary of literature sources used in model calibration.** Listed here are the descriptions

206 and PMIDs of all the literature sources from which the model calibration datasets were extracted.

207

208

209

210

211

212

213

214

215

216

217

218

219

220

221

222

Protocol S1: Additional information regarding model formulation and analysis

Model Formulation

Macrophage polarization is a complex, dynamic multi-pathway process with numerous feedbacks and cross-talks, which also makes its a highly suitable topic for systems biology modeling. Given the many relevant pathways and the large number of mechanistic details that govern macrophage polarization, it makes sense to approach this problem in a stepwise manner (e.g. first build a "beginner model", then gradually enrich the model with more pathways of high importance in disease contexts). After careful analysis of literature knowledge, we ended up choosing three pathways (IFN-γ, IL-4, hypoxia) to model in the beginning step (as presented in this paper), since IFN-γ and IL-4 are representative inducers of M1 and M2 phenotypes and many experimental studies have tried to elucidate their downstream signaling and gene regulation mechanisms (which provided a wealth of quantitative data that can be used to calibrate our "beginner model"), and also because that hypoxia is a key signature in the disease areas that we are interested in (namely peripheral arterial disease and cancer) while its direct impact on macrophage polarization and connections with other macrophage pathways have not been systematically characterized before.

Model Calibration

During model calibration, values of some model parameters are derived directly from literature data and previous models (as shown in Table S1); for the remaining parameters with no literature reference, we put in tentative values first and then hand-tuned the entire model extensively (by adjusting parameter values and observing model response) until the model simulations achieved good visual agreements with all the respective calibration datasets (from the sources listed in Table S3) simultaneously. In the meantime, the initial conditions of "unique" species in the model have to stay within the allowed ranges (0.5x-2x of concentration values estimated from literature as listed in Table S2, except for total HIF-1α and HIF-2α which we set that their resting concentrations per cell should be less than a few nanomolar according to (56)). Then we performed preliminary sensitivity analysis (for three cases, IL-4 or IFN-γ or hypoxia stimulation) and collectively identified 101 parameters that have statistically significant ($p < 0.05$) PRCC values. Among the 101 parameters, 82 had no literature reference and global optimization using

254   *patternsearch* in MATLAB was then performed for those 82 parameter values (with 0.5x-2x as

255   the allowed ranges) with respect to all the calibration datasets (along with the initial condition

256   checks in every iteration) to generate the final parameter values. We rounded the final values to

257   three significant digits for all parameters (as listed in Table S1).

258   For the initial condition checks, we simulated the model for 100000 minutes to obtain species

259   endpoint values (and check if they are within appropriate ranges as described above) and use

260   these endpoint values as new initial conditions to generate simulations and calculate squared

261   errors with respect to the literature data in every iteration of *patternsearch* optimization. Since

262   we selected this very long time span, in each iteration these new initial conditions obtained

263   would represent a set of species equilibrium states (of macrophages under normoxia without

264   externally added stimuli) that can be compared with quantitative literature data.

265

Model Sensitivity Analysis and Uncertainty Quantification

267   For model sensitivity analysis, we used Latin Hypercube Sampling method with parameter

268   ranges of 0.5x-2x to calculate the PRCC values (with $p=0.05$ as the cutoff for statistical

269   significance) based on the algorithm and code published in (66). The output of interest in PRCC

270   calculations are "M1/M2 scores", which are the multiplication of six M1 markers

271   ([iNOS]*[IFNγ]*[TNFα]*[IL12]*[CXCL9]*[mCXCL10]; [IFNγ] is removed when calculating

272   PRCCs in scenarios of IFN-γ stimulation) divided by the multiplication of three M2 markers

273   ([ARG1]*[VEGF]*[IL10]). For uncertainty quantification, each of the 50 re-sampled datasets

274   has 229 individual datapoints that covered all the experimental conditions used in model

275   calibration. For each datapoint, we assumed its value is within a distinct normal distribution with

276   a mean and a standard deviation (whenever possible, the mean and standard deviations are

277   calculated from the corresponding literature data that we gathered; for datapoints that only had

278   one value and no repeat, we considered that single value as the mean and assumed that the

279   standard deviation equals to 10% of that value). Then the 229 datapoint values in each re-

280   sampled dataset were compiled in order by generating random numbers from the 229 normal

281   distributions. The 50 re-sampled datasets were then fed into the optimization algorithm to obtain

282   50 sets of new parameter estimates (as described in Materials and Methods).

283

284

References for Supporting Information

1.   Majoros A, Platanitis E, Szappanos D, Cheon H, Vogl C, Shukla P, et al. Response to interferons and antibacterial innate immunity in the absence of tyrosine-phosphorylated STAT1. EMBO Rep. 2016;17(3):367-82.

2.   Kim HS, Kim DC, Kim HM, Kwon HJ, Kwon SJ, Kang SJ, et al. STAT1 deficiency redirects IFN signalling toward suppression of TLR response through a feedback activation of STAT3. Sci Rep. 2015;5:13414.

3.   Li X, Zhang Z, Li L, Gong W, Lazenby AJ, Swanson BJ, et al. Myeloid-derived cullin 3 promotes STAT3 phosphorylation by inhibiting OGT expression and protects against intestinal inflammation. J Exp Med. 2017;214(4):1093-109.

4.   Ting LM, Kim AC, Cattamanchi A, Ernst JD. Mycobacterium tuberculosis inhibits IFN-gamma transcriptional responses without inhibiting activation of STAT1. J Immunol. 1999;163(7):3898-906.

5.   Dickensheets H, Vazquez N, Sheikh F, Gingras S, Murray PJ, Ryan JJ, et al. Suppressor of cytokine signaling-1 is an IL-4-inducible gene in macrophages and feedback inhibits IL-4 signaling. Genes Immun. 2007;8(1):21-7.

6.   Vila-del Sol V, Punzon C, Fresno M. IFN-gamma-induced TNF-alpha expression is regulated by interferon regulatory factors 1 and 8 in mouse macrophages. J Immunol. 2008;181(7):4461-70.

7.   Ohata T, Fukuda K, Murakami A, Ohigashi H, Sugimura T, Wakabayashi K. Inhibition by 1'-acetoxychavicol acetate of lipopolysaccharide- and interferon-gamma-induced nitric oxide production through suppression of inducible nitric oxide synthase gene expression in RAW264 cells. Carcinogenesis. 1998;19(6):1007-12.

8.   Wu C, Xue Y, Wang P, Lin L, Liu Q, Li N, et al. IFN-gamma primes macrophage activation by increasing phosphatase and tensin homolog via downregulation of miR-3473b. J Immunol. 2014;193(6):3036-44.

9.   Takeda N, O'Dea EL, Doedens A, Kim JW, Weidemann A, Stockmann C, et al. Differential activation and antagonistic function of HIF-{alpha} isoforms in macrophages are essential for NO homeostasis. Genes Dev. 2010;24(5):491-501.

10.   Wormald S, Zhang JG, Krebs DL, Mielke LA, Silver J, Alexander WS, et al. The comparative roles of suppressor of cytokine signaling-1 and -3 in the inhibition and desensitization of cytokine signaling. J Biol Chem. 2006;281(16):11135-43.

11.   Covarrubias AJ, Aksoylar HI, Yu J, Snyder NW, Worth AJ, Iyer SS, et al. Akt-mTORC1 signaling regulates Acly to integrate metabolic input to control of macrophage activation. Elife. 2016;5.

12.   Yao Y, Wang Y, Zhang Z, He L, Zhu J, Zhang M, et al. Chop Deficiency Protects Mice Against Bleomycin-induced Pulmonary Fibrosis by Attenuating M2 Macrophage Production. Mol Ther. 2016;24(5):915-25.

13.   Rex J, Albrecht U, Ehlting C, Thomas M, Zanger UM, Sawodny O, et al. Model-Based Characterization of Inflammatory Gene Expression Patterns of Activated Macrophages. PLoS Comput Biol. 2016;12(7):e1005018.

14.   Zhu L, Yang T, Li L, Sun L, Hou Y, Hu X, et al. TSC1 controls macrophage polarization to prevent inflammatory disease. Nat Commun. 2014;5:4696.

15.   Sheldon KE, Shandilya H, Kepka-Lenhart D, Poljakovic M, Ghosh A, Morris SM, Jr. Shaping the murine macrophage phenotype: IL-4 and cyclic AMP synergistically activate the arginase I promoter. J Immunol. 2013;191(5):2290-8.

16.   El Chartouni C, Schwarzfischer L, Rehli M. Interleukin-4 induced interferon regulatory factor (Irf) 4 participates in the regulation of alternative macrophage priming. Immunobiology. 2010;215(9-10):821-5.

17.   Kimura T, Nada S, Takegahara N, Okuno T, Nojima S, Kang S, et al. Polarization of M2 macrophages requires Lamtor1 that integrates cytokine and amino-acid signals. Nat Commun. 2016;7:13130.

334 18.   McCormick SM, Gowda N, Fang JX, Heller NM. Suppressor of Cytokine Signaling (SOCS)1
335 Regulates Interleukin-4 (IL-4)-activated Insulin Receptor Substrate (IRS)-2 Tyrosine Phosphorylation in
336 Monocytes and Macrophages via the Proteasome. J Biol Chem. 2016;291(39):20574-87.
337 19.   Zanin RF, Braganhol E, Bergamin LS, Campesato LF, Filho AZ, Moreira JC, et al. Differential
338 macrophage activation alters the expression profile of NTPDase and ecto-5'-nucleotidase. PLoS One.
339 2012;7(2):e31205.
340 20.   Zhang W, Xu W, Xiong S. Macrophage differentiation and polarization via phosphatidylinositol 3-
341 kinase/Akt-ERK signaling pathway conferred by serum amyloid P component. J Immunol.
342 2011;187(4):1764-77.
343 21.   Lee C, Bae SS, Joo H, Bae H. Melittin suppresses tumor progression by regulating tumor-
344 associated macrophages in a Lewis lung carcinoma mouse model. Oncotarget. 2017;8(33):54951-65.
345 22.   Fang HY, Hughes R, Murdoch C, Coffelt SB, Biswas SK, Harris AL, et al. Hypoxia-inducible factors
346 1 and 2 are important transcriptional effectors in primary macrophages experiencing hypoxia. Blood.
347 2009;114(4):844-59.
348 23.   Berra E, Benizri E, Ginouves A, Volmat V, Roux D, Pouyssegur J. HIF prolyl-hydroxylase 2 is the
349 key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. EMBO J. 2003;22(16):4082-
350 90.
351 24.   Carta L, Pastorino S, Melillo G, Bosco MC, Massazza S, Varesio L. Engineering of macrophages to
352 produce IFN-gamma in response to hypoxia. J Immunol. 2001;166(9):5374-80.
353 25.   Mei Y, Thompson MD, Shiraishi Y, Cohen RA, Tong X. Sarcoplasmic/endoplasmic reticulum Ca2+
354 ATPase C674 promotes ischemia- and hypoxia-induced angiogenesis via coordinated endothelial cell and
355 macrophage function. J Mol Cell Cardiol. 2014;76:275-82.
356 26.   Ganta VC, Choi MH, Kutateladze A, Fox TE, Farber CR, Annex BH. A MicroRNA93-Interferon
357 Regulatory Factor-9-Immunoresponsive Gene-1-Itaconic Acid Pathway Modulates M2-Like Macrophage
358 Polarization to Revascularize Ischemic Muscle. Circulation. 2017;135(24):2403-25.
359 27.   Cambridge SB, Gnad F, Nguyen C, Bermejo JL, Kruger M, Mann M. Systems-wide proteomic
360 analysis in mammalian cells reveals conserved, functional protein turnover. J Proteome Res.
361 2011;10(12):5275-84.
362 28.   Bankaitis KV, Fingleton B. Targeting IL4/IL4R for the treatment of epithelial cancer metastasis.
363 Clin Exp Metastasis. 2015;32(8):847-56.
364 29.   Gao T, Furnari F, Newton AC. PHLPP: a phosphatase that directly dephosphorylates Akt,
365 promotes apoptosis, and suppresses tumor growth. Mol Cell. 2005;18(1):13-24.
366 30.   Hanson EM, Dickensheets H, Qu CK, Donnelly RP, Keegan AD. Regulation of the
367 dephosphorylation of Stat6. Participation of Tyr-713 in the interleukin-4 receptor alpha, the tyrosine
368 phosphatase SHP-1, and the proteasome. J Biol Chem. 2003;278(6):3903-11.
369 31.   Chen HC, Reich NC. Live cell imaging reveals continuous STAT6 nuclear trafficking. J Immunol.
370 2010;185(1):64-70.
371 32.   Londino JD, Gulick DL, Lear TB, Suber TL, Weathington NM, Masa LS, et al. Post-translational
372 modification of the interferon-gamma receptor alters its stability and signaling. Biochem J.
373 2017;474(20):3543-57.
374 33.   Ping Z, Qi J, Sun Y, Lu G, Shi Y, Wang X, et al. Crystal structure of the interferon gamma receptor
375 alpha chain from chicken reveals an undetected extra helix compared with the human counterparts. J
376 Interferon Cytokine Res. 2014;34(1):41-51.
377 34.   Nickoloff BJ. Binding of 125I-gamma interferon to cultured human keratinocytes. J Invest
378 Dermatol. 1987;89(2):132-5.
379 35.   Zhu W, Mustelin T, David M. Arginine methylation of STAT1 regulates its dephosphorylation by T
380 cell protein tyrosine phosphatase. J Biol Chem. 2002;277(39):35787-90.
381 36.   Zhao C, Isenberg JS, Popel AS. Transcriptional and Post-Transcriptional Regulation of
382 Thrombospondin-1 Expression: A Computational Model. PLoS Comput Biol. 2017;13(1):e1005272.

383   37.   Eckhardt I, Weigert A, Fulda S. Identification of IRF1 as critical dual regulator of Smac mimetic-
384   induced apoptosis and inflammatory cytokine response. Cell Death Dis. 2014;5:e1562.
385   38.   Marzi MJ, Ghini F, Cerruti B, de Pretis S, Bonetti P, Giacomelli C, et al. Degradation dynamics of
386   microRNAs revealed by a novel pulse-chase approach. Genome Res. 2016;26(4):554-65.
387   39.   Wang X, Li Y, Xu X, Wang YH. Toward a system-level understanding of microRNA pathway via
388   mathematical modeling. Biosystems. 2010;100(1):31-8.
389   40.   Sharova LV, Sharov AA, Nedorezov T, Piao Y, Shaik N, Ko MS. Database for mRNA half-life of 19
390   977 genes obtained by DNA microarray analysis of pluripotent and differentiating mouse embryonic
391   stem cells. DNA Res. 2009;16(1):45-58.
392   41.   Waite KJ, Floyd ZE, Arbour-Reily P, Stephens JM. Interferon-gamma-induced regulation of
393   peroxisome proliferator-activated receptor gamma and STATs in adipocytes. J Biol Chem.
394   2001;276(10):7062-8.
395   42.   Kolodziejski PJ, Koo JS, Eissa NT. Regulation of inducible nitric oxide synthase by rapid cellular
396   turnover and cotranslational down-regulation by dimerization inhibitors. Proc Natl Acad Sci U S A.
397   2004;101(52):18141-6.
398   43.   Setty BA, Jin Y, Houghton PJ, Yeager ND, Gross TG, Nelin LD. Hypoxic Proliferation of
399   Osteosarcoma Cells Depends on Arginase II. Cell Physiol Biochem. 2016;39(2):802-13.
400   44.   Schimke RT. The Importance of Both Synthesis and Degradation in the Control of Arginase Levels
401   in Rat Liver. J Biol Chem. 1964;239:3808-17.
402   45.   Zimmer J, Weitnauer M, Boutin S, Kublbeck G, Thiele S, Walker P, et al. Nuclear Localization of
403   Suppressor of Cytokine Signaling-1 Regulates Local Immunity in the Lung. Front Immunol. 2016;7:514.
404   46.   Eubank TD, Roda JM, Liu H, O'Neil T, Marsh CB. Opposing roles for HIF-1alpha and HIF-2alpha in
405   the regulation of angiogenesis by mononuclear phagocytes. Blood. 2011;117(1):323-32.
406   47.   Yu Y, Cui Y, Zhao Y, Liu S, Song G, Jiao P, et al. The binding capability of plasma phospholipid
407   transfer protein, but not HDL pool size, is critical to repress LPS induced inflammation. Sci Rep.
408   2016;6:20845.
409   48.   Crouse JA, Mitchell WM. Interferon-gamma receptor: mRNA half-life, receptor mass, and
410   abundance on A431 human epidermoid carcinoma cells. J Interferon Res. 1992;12(1):23-5.
411   49.   Azimifar SB, Nagaraj N, Cox J, Mann M. Cell-type-resolved quantitative proteomics of murine
412   liver. Cell Metab. 2014;20(6):1076-87.
413   50.   Precious BL, Carlos TS, Goodbourn S, Randall RE. Catalytic turnover of STAT1 allows PIV5 to
414   dismantle the interferon-induced anti-viral state of cells. Virology. 2007;368(1):114-21.
415   51.   Brenes A, Afzal V, Kent R, Lamond AI. The Encyclopedia of Proteome Dynamics: a big data
416   ecosystem for (prote)omics. Nucleic Acids Res. 2018;46(D1):D1202-D9.
417   52.   Mukherjee S, Chen LY, Papadimos TJ, Huang S, Zuraw BL, Pan ZK. Lipopolysaccharide-driven Th2
418   cytokine production in macrophages is regulated by both MyD88 and TRAM. J Biol Chem.
419   2009;284(43):29391-8.
420   53.   Yang HL, Zhou WJ, Chang KK, Mei J, Huang LQ, Wang MY, et al. The crosstalk between
421   endometrial stromal cells and macrophages impairs cytotoxicity of NK cells in endometriosis by
422   secreting IL-10 and TGF-beta. Reproduction. 2017;154(6):815-25.
423   54.   Bissels U, Wild S, Tomiuk S, Holste A, Hafner M, Tuschl T, et al. Absolute quantification of
424   microRNAs by using a universal reference. RNA. 2009;15(12):2375-84.
425   55.   Schwanhausser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, et al. Global quantification of
426   mammalian gene expression control. Nature. 2011;473(7347):337-42.
427   56.   Tuckerman JR, Zhao Y, Hewitson KS, Tian YM, Pugh CW, Ratcliffe PJ, et al. Determination and
428   comparison of specific activity of the HIF-prolyl hydroxylases. FEBS Lett. 2004;576(1-2):145-50.
429   57.   Simicevic J, Schmid AW, Gilardoni PA, Zoller B, Raghav SK, Krier I, et al. Absolute quantification
430   of transcription factors during cellular differentiation using multiplexed targeted proteomics. Nat
431   Methods. 2013;10(6):570-6.

432 58.   Xaus J, Comalada M, Valledor AF, Lloberas J, Lopez-Soriano F, Argiles JM, et al. LPS induces
433 apoptosis in macrophages mostly through the autocrine production of TNF-alpha. Blood.
434 2000;95(12):3823-31.
435 59.   Barbera-Cremades M, Gomez AI, Baroja-Mazo A, Martinez-Alarcon L, Martinez CM, de Torre-
436 Minguela C, et al. P2X7 Receptor Induces Tumor Necrosis Factor-alpha Converting Enzyme Activation
437 and Release to Boost TNF-alpha Production. Front Immunol. 2017;8:862.
438 60.   Cudejko C, Wouters K, Fuentes L, Hannou SA, Paquet C, Bantubungi K, et al. p16INK4a deficiency
439 promotes IL-4-induced polarization and inhibits proinflammatory signaling in macrophages. Blood.
440 2011;118(9):2556-66.
441 61.   Marinov GK, Williams BA, McCue K, Schroth GP, Gertz J, Myers RM, et al. From single-cell to cell-
442 pool transcriptomes: stochasticity in gene expression and RNA splicing. Genome Res. 2014;24(3):496-
443 510.
444 62.   Su Z, Yang R, Zhang W, Xu L, Zhong Y, Yin Y, et al. The synergistic interaction between the
445 calcineurin B subunit and IFN-gamma enhances macrophage antitumor activity. Cell Death Dis.
446 2015;6:e1740.
447 63.   Michelucci A, Cordes T, Ghelfi J, Pailot A, Reiling N, Goldmann O, et al. Immune-responsive gene
448 1 protein links metabolism to immunity by catalyzing itaconic acid production. Proc Natl Acad Sci U S A.
449 2013;110(19):7820-5.
450 64.   Krombach F, Munzing S, Allmeling AM, Gerlach JT, Behr J, Dorger M. Cell size of alveolar
451 macrophages: an interspecies comparison. Environ Health Perspect. 1997;105 Suppl 5:1261-3.
452 65.   Swanson JA, Lee M, Knapp PE. Cellular dimensions affecting the nucleocytoplasmic volume ratio.
453 J Cell Biol. 1991;115(4):941-8.
454 66.   Marino S, Hogue IB, Ray CJ, Kirschner DE. A methodology for performing global uncertainty and
455 sensitivity analysis in systems biology. J Theor Biol. 2008;254(1):178-96.

456