

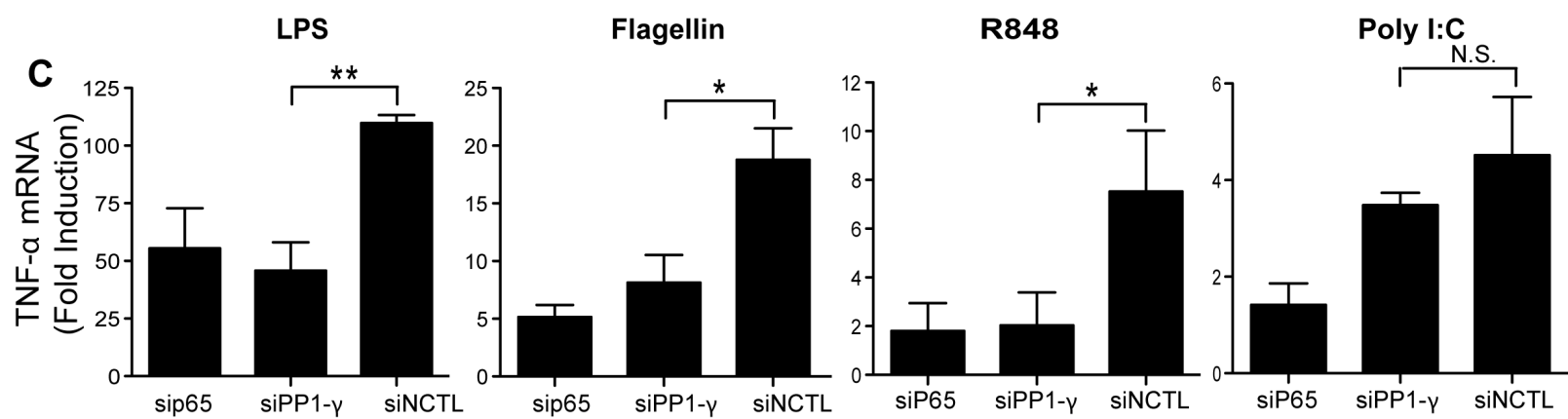
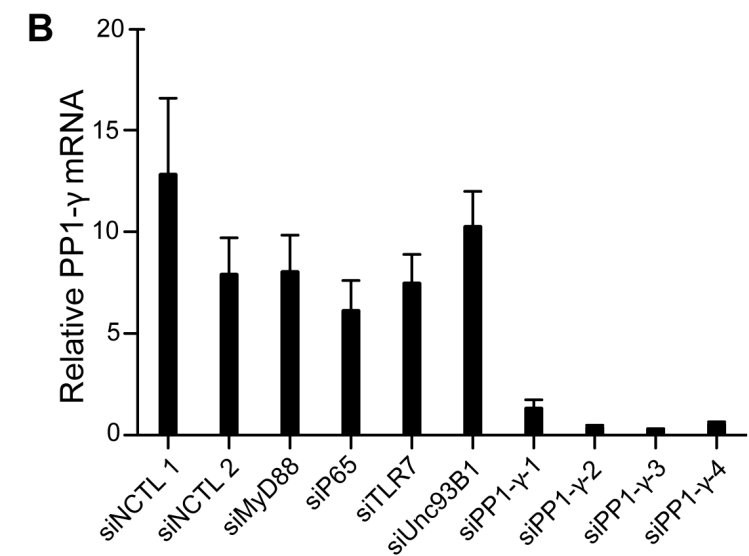
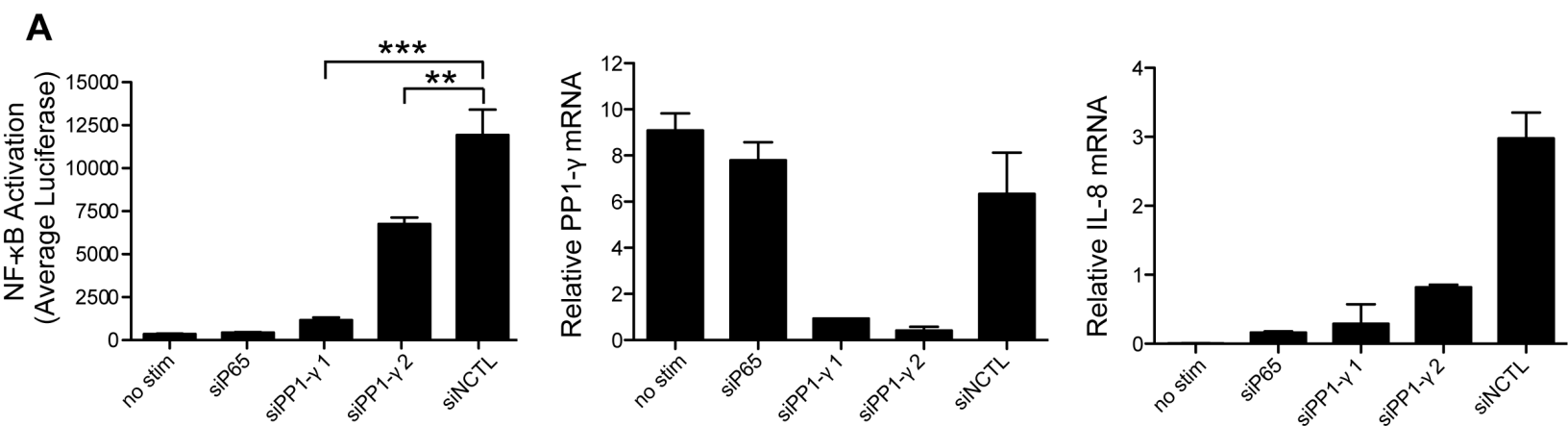
Figure S1. Silencing of PP1- γ impairs induction of MyD88-dependent proinflammatory cytokines. A) HEK293T/NF- κ B-luc cells stably expressing TLR7 (HEK293T/TLR7/NF- κ B-luc) were reverse transfected with the indicated siRNAs. Forty-eight hours post-transfection, cells were stimulated for 16 h with R848 (10 μ M), and NF- κ B activation was measured by luciferase, and relative levels of PP1- γ or IL-8 were evaluated by RT-PCR. Also see [Figure 1C](#). B) HEK293T/TLR7/NF- κ B-luc cells were transfected with the indicated siRNAs and stimulated with R848 (3 μ M) for 12 hours. Total cellular RNA was collected from each sample and used to measure relative levels of PP1- γ mRNA by RT-PCR. C) HEK293T/NF- κ B cells stably expressing TLR3, TLR4, or TLR7 were reverse transfected with the indicated siRNAs. Forty-eight hours post-transfection, cells were stimulated for 3 h with LPS (TLR4, 100 ng/mL), Flagellin (TLR5, 100 ng/mL), R848 (TLR7, 10 μ M), or poly I:C (TLR3; 50 μ g/mL). For evaluation of TLR5 signaling, HEK293T/NF- μ B cells stably expressing TLR7 were used. After ligand treatment, the relative levels of TNF- α mRNA were evaluated by RT-PCR. Also see [Figure 1E](#). Data in (A–B) are representative of at least three independent experiments, data in (C) is representative of at least two independent experiments; by two-tailed student's t test, $P \leq 0.05 = *$, $P \leq 0.01 = **$, N.S. = not significant. Bar graphs are presented as the mean relative mRNA levels \pm SD from a representative experiment.

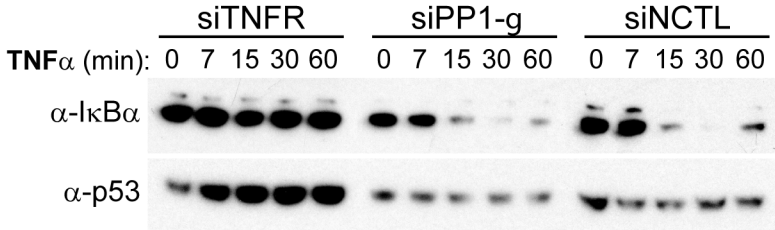
Figure S2. PP1- γ silencing does not impair NF- κ B signaling events downstream of TNFR. HEK293T/TLR7/NF- κ B-luc cells were reverse transfected with the indicated siRNAs. Seventy-two hours later, cells were stimulated with TNF- α (10 ng/mL) for the indicated timepoints, and whole cell lysates were collected and evaluated by SDS-PAGE and immunoblotting with the indicated antibodies. Data shown are representative of at least three independent experiments.

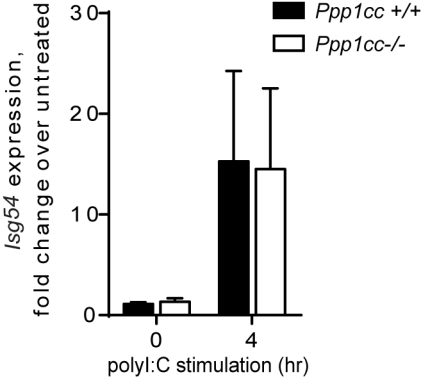
Figure S3. TLR3 signaling is unchanged in *Ppp1cc*-deficient macrophages. Expression of *Isg54* was measured after stimulation with polyI:C for the indicated amount of time. The graph shows the mean \pm SEM of three mice for each genotype.

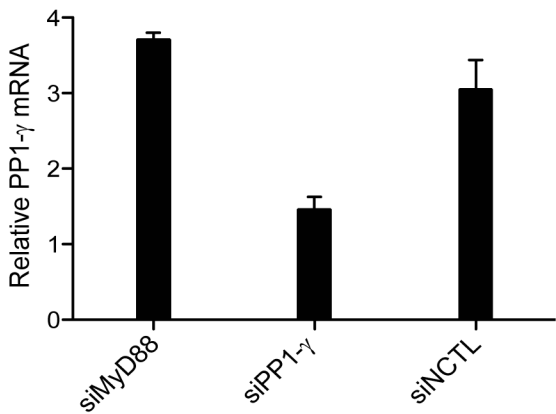
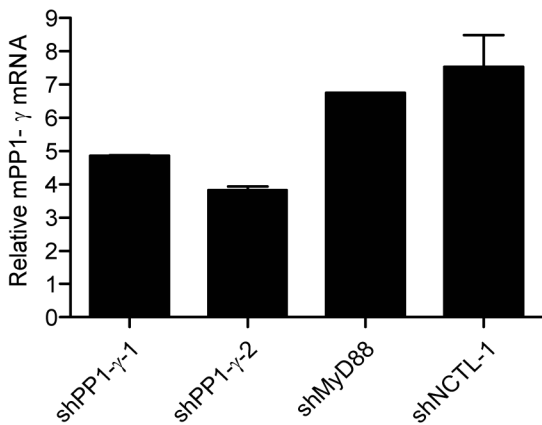
Figure S4. Silencing of PP1- γ in THP-1 or RAW cell lines. A) THP-1 monocytic cells were transfected with siRNAs. Cells were stimulated with R848 (10 μ M) for the indicated time points, and relative levels of PP1- γ mRNA were evaluated by RT-PCR to confirm silencing of PP1- γ . Also see [Figure 7A](#). B) RAW264.7 cells were transduced with the indicated shRNAs and stable cell lines were established as described. RNA was isolated from stable cell lines and relative levels of PP1- γ mRNA were evaluated by RT-PCR to confirm silencing of PP1- γ . Also see [Figure 7C–E](#).

Table S1. Candidate gene hits from the secondary confirmation screen are listed in descending order of NF- κ B luciferase reporter activation. The Z primes of the first and second runs of the screen were 0.72 and 0.62, respectively. The negative and positive controls in are in red.







A**B**

Gene_Symbol	Entrez Gene ID	Run1 AVG	Run1 STDEV	Run2 AVG	Run2 STDEV	Combined Average	Combined STDEV	pVAL
IKK SE	POS CONT	280.1071	33.76854595	144.6697248	29.89887941	212.3884338	95.76871676	1.80E-04
Map3k3	26406	164.91837	6.150547766	189.559633	2.309401077	177.2390002	17.42400606	1.29E-10
RELA	POS CONT	175.5357	15.74440417	101.8509174	12.18824374	138.6933159	52.10301953	3.55E-05
TNFRSF10A	8797	44.193878	13.1196874	50.97018349	12.63749258	47.58203052	4.791571878	1.31E-05
TIRAP	114609	24.47449	11.85252729	24.07798165	8.673908024	24.27623572	0.280373598	4.08E-04
CARD14	79092	10.77551	3.494069866	33.91972477	6.222106344	22.34761749	16.36543107	5.17E-03
CPM	1368	0.9795918	0.322681394	39.96788991	8.419441219	20.47374087	27.56888995	5.43E-02
Ppp1cc	19047	23.147959	5.273220203	9.678899083	1.7902685	16.41342913	9.524063734	1.97E-03
JADE2	23338	5.2653061	2.052686567	26.3853211	16.45172684	15.82531361	14.93410581	4.73E-02
MAVS	57506	12.938776	2.078696075	15.12155963	4.308947992	14.03016757	1.543461455	9.31E-06
Sf3b4	107701	1.0408163	0.332011819	22.70183486	35.49319409	11.87132559	15.31665309	0.216582
DDX41	51428	14.607143	6.426451978	6.876146789	2.001885877	10.74164482	5.466639745	5.23E-03
STAR3	10948	2.7857143	1.593187984	7.44266055	2.447580507	5.114187418	3.292958283	0.014499
CDC25B	994	1.5510204	0.694477532	7.782110092	11.52150738	4.66656525	4.406045769	0.203841
Grhl1	195733	2.1683673	0.782172422	5.745412844	2.994513542	3.956890095	2.529353128	3.32E-02
DDX6	1656	4.2193878	0.831053385	1.793577982	0.300799932	3.006482868	1.715306541	1.08E-02
NRSN2	80023	1.9693878	0.776315371	3.894495413	1.361069289	2.931941584	1.361256679	1.43E-02
FLJ33069	137835	1.6734694	0.35229952	3.589449541	2.644487787	2.631459465	1.354802559	7.54E-02
Stx5a	56389	1.1071429	0.35480283	3.672018349	1.677375606	2.389580603	1.813640853	0.080909
Lsm14a	67070	3	1.544022883	1.435779817	0.99392143	2.217889908	1.106707699	7.08E-02
PAK2	5062	2.3826531	1.444841749	1.866972477	0.515750845	2.124812769	0.364641238	3.37E-02
PPM1A	5494	2.6938776	0.438656842	1.431192661	0.661611448	2.062535106	0.892853049	2.05E-02
LPL	4023	1.8214286	0.732493872	2.22706422	1.443846962	2.024246396	0.286827718	5.11E-02
PGBD3	267004	3.1428571	3.939744691	0.823394495	0.517752898	1.983125819	1.640107767	0.26262
APOC3	345	1.6989796	1.377752568	2.043577982	0.797140718	1.871278787	0.243667858	7.48E-02
PHLPP1	23239	0.8469388	0.101015254	2.73853211	0.175154891	1.792735443	1.361256679	0.109762
NR4A1	3164	2.1734694	0.615524098	1.183486239	0.22031087	1.678477813	0.700023798	4.81E-02
Rab14	68365	1.1632653	0.216621542	2.190366972	0.160899577	1.676816139	0.726270553	2.97E-02
NR4A1	3164	1.0714286	0.215013342	2.165137615	0.60267153	1.618283093	0.76369081	7.16E-02
SOS2	6655	1.0204082	0.175557659	2.201834862	0.915441267	1.611121513	0.83539483	0.110984
HERC6	55008	1.3265306	0.566303548	1.80733945	0.347737486	1.566935031	0.339983189	3.72E-02
USP33	23032	1.255102	0.393442007	1.582568807	0.528751395	1.418835424	0.231553971	7.40E-02
ARPC1A	10552	1.0306122	0.170339919	1.793577982	0.569916055	1.412095113	0.539498246	0.109915
ABCC1	4363	0.7857143	0.226267778	1.972477064	0.727533155	1.379095675	0.839168008	0.202981
TCF7	6932	0.9744898	0.248485626	1.589449541	0.645529822	1.281969669	0.434842206	0.196491
Hnt	235106	1.2295918	0.560729528	1.325688073	0.729208724	1.277639955	6.80E-02	0.215489
FASN	2194	1.2908163	0.551240823	1.236238532	0.165710017	1.263527429	3.86E-02	0.138238
CCDC6	8030	1.005102	0.361777508	1.46559633	0.519375995	1.235349186	0.325618635	0.209782
SENP2	59343	1.4030612	0.61255661	0.924311927	0.212450205	1.163686576	0.338526875	0.293176
Brf1	72308	1.0663265	0.62733667	1.110091743	0.114342013	1.088209137	0.030946679	0.373429
Sybl1	20955	1.0102041	0.185927216	1.155963303	0.489946052	1.083083692	0.103067334	0.365129
EEF1G	1937	0.7959184	0.123577565	1.298165138	0.278119383	1.047041752	0.355142097	0.426678
Glim	170823	0.877551	0.158956754	1.20412844	0.43712973	1.04083973	0.230925108	0.440545
Eef1g	67160	1.2142857	0.352890132	0.857798165	0.392784856	1.03604194	0.252074763	0.453658
VECTOR ONLY	NEG CONT	1.081633	0.259753511	0.935779817	0.467079709	1.008706235	0.10313353	0.5
D5C2	1824	0.9795918	0.373344836	1.009174312	0.576839802	0.994383074	2.09E-02	0.4781
HNRPA0	10949	1.2857143	0.680748133	0.69266055	0.162310147	0.989187418	0.419352318	0.475112
CCDC6	8030	0.8112245	0.20297318	1.128440367	1.042028456	0.969832428	0.224305498	0.460467
BLXL4	26235	1.1530612	0.758770277	0.775229358	0.174954553	0.964145291	0.267167475	0.442257
RAN	5901	0.8622449	0.385195634	0.97706422	0.518409512	0.919654559	0.081189521	0.361242
EBP	10682	1.1887755	0.512884118	0.644495413	0.106562967	0.916635462	0.384864148	0.36235
SMARCAL1	50485	1.1989796	0.459183673	0.610091743	0.122285927	0.904535667	0.416406591	0.343351
PITX1	5307	1.1377551	0.52492372	0.548165138	6.24E-02	0.84296012	0.416903062	0.271126
TRGV7	6981	0.627551	0.224412479	1.050458716	0.809231173	0.839004868	0.299040899	0.305322
DDX18	8886	0.9744898	0.325838978	0.573394495	0.246343515	0.773942146	0.283617207	0.140261
C9orf114	51490	0.7602041	0.235284675	0.752293578	0.410902673	0.75624883	5.59E-03	0.108583
ACADL	33	0.8469388	0.212414898	0.653669725	7.24E-02	0.75030425	0.136661856	4.87E-02
C15	716	0.9438776	0.322842694	0.550458716	0.168334666	0.747168133	0.278189126	0.104774
Sox9	20682	1.1479592	1.064408489	0.323394495	2.74E-02	0.73567684	0.583055283	0.272575
CILP	8483	1.0510204	0.867846995	0.378440367	0.214814119	0.714730388	0.475585908	0.221415
DYX1C1	161582	0.9744898	0.420683814	0.394495413	8.70E-02	0.684492604	0.410117961	0.103071
SYMPK	8189	0.8622449	0.273994318	0.46559633	0.148191882	0.663920614	0.280472892	4.60E-02
C9orf114	51490	0.9183673	0.339863837	0.380733945	6.02E-02	0.649550646	0.380164224	6.35E-02
AFAP1L1	134265	0.8418367	9.33E-02	0.424311927	0.103083509	0.633074331	0.295234623	2.23E-02
USP33	23032	0.7295918	0.179757963	0.48853211	0.208719797	0.609061973	0.170454967	1.42E-02
DDX58	23586	0.5102041	2.89E-02	0.672018349	0.309613206	0.591111215	0.114419965	1.16E-02
RASGRP3	25780	0.7704082	0.231717297	0.385321101	6.40E-02	0.577864632	0.272297673	1.51E-02
Cik2	12748	0.7806122	8.72E-02	0.373853211	5.62E-02	0.577232728	0.287622071	1.07E-02
PPP1R3B	79660	0.7806122	0.371247246	0.364678899	0.1323933	0.572645572	0.294109289	0.029573
TFB2M	64216	0.5816327	0.103392409	0.483944954	0.183084422	0.532788804	6.91E-02	2.31E-03
SRP9	6726	0.6989796	0.202288037	0.362385321	9.98E-02	0.530682456	0.238008091	6.90E-03
FASTK	10922	0.6479592	0.108470876	0.405963303	4.64E-02	0.526961243	0.171116928	2.25E-03
A2M	2	0.5510204	5.77E-02	0.48853211	0.220740249	0.519776259	0.044185899	2.13E-03
PRKACA	5566	0.5306122	5.77E-02	0.316513761	0.05270241	0.423563003	0.151390489	4.34E-04
ARHGAP1	392	0.5510204	7.26E-02	0.27293578	4.20E-02	0.411978094	0.196635526	6.32E-04
TBX21	30009	0.5102041	0	0.30733945	6.97E-02	0.408771766	0.143446957	3.23E-04

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