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Supplementary Information for

Non-coding RNA *MaIL1* is an integral component of the TLR4-TRIF pathway

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www.pnas.org/cgi/doi/10.1073/pnas.1920393117

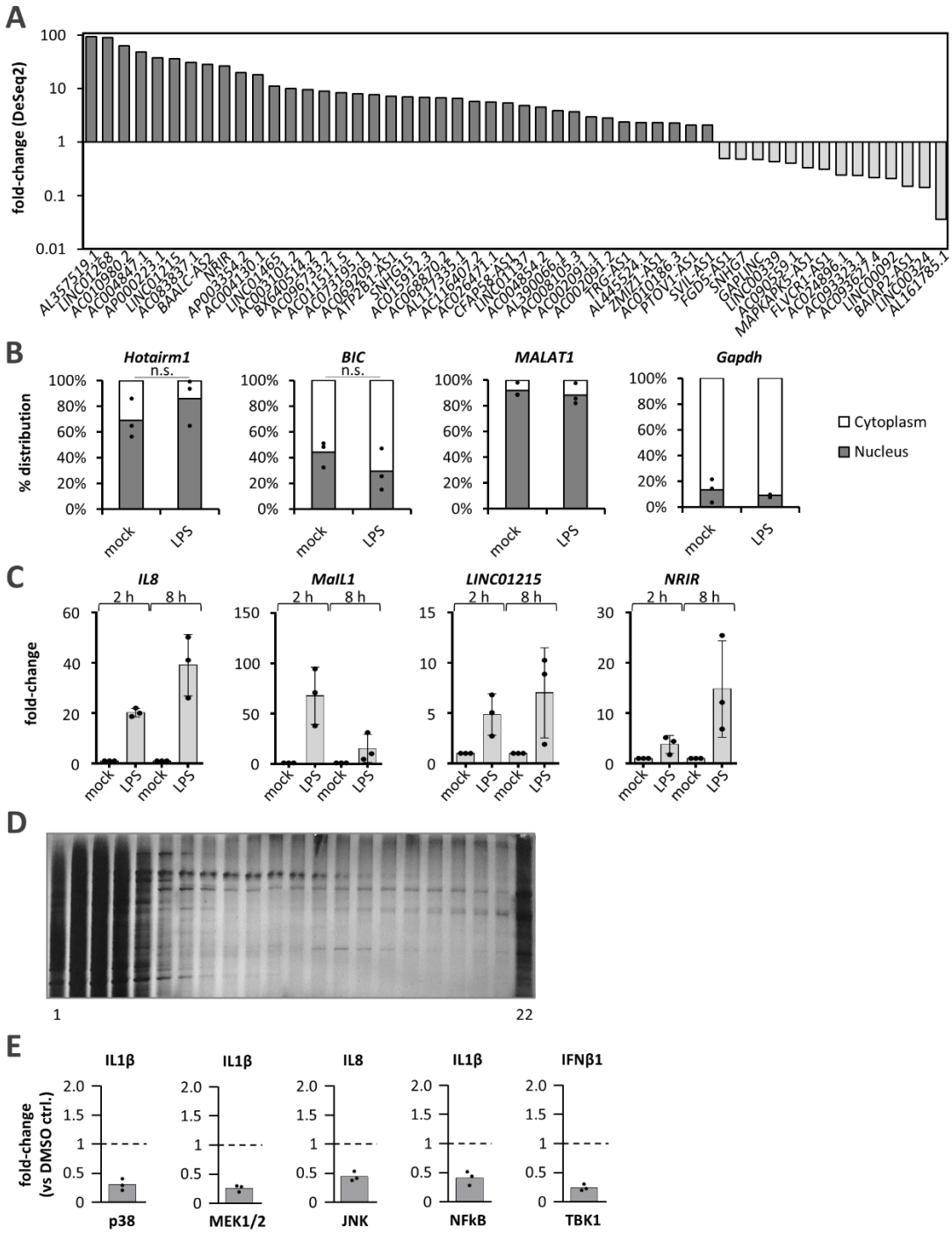


Fig. S1. Immune-pathway dependence and subcellular localization of macrophage lncRNAs. **A)** Significantly regulated lncRNAs after 8 h LPS-stimulation (DeSeq2-determined fold-changes; $P_{adj} \leq 0.05$). **B)** qRT-PCR analysis of percent-distribution of *Hotairm1*, *BIC*, *MALAT1* and *Gapdh* RNA in cytoplasm and nucleus in mock and 8 h LPS treated primary macrophages. **C)** qRT-PCR analysis of *IL8*, *MaIL1*, *LINC01215* and *NRIR* expression in 2 and 8 h LPS- compared to mock-treated primary macrophages. **D)** Representative silver-stained SDS gel illustrating protein content and differential protein sedimentation, using samples from Fig. 2A. **E)** qRT-PCR analysis of *IL1*β, *IL8* or *IFN*β*1* mRNA suppression by MAPK, NFκB and TBK1 inhibitors.

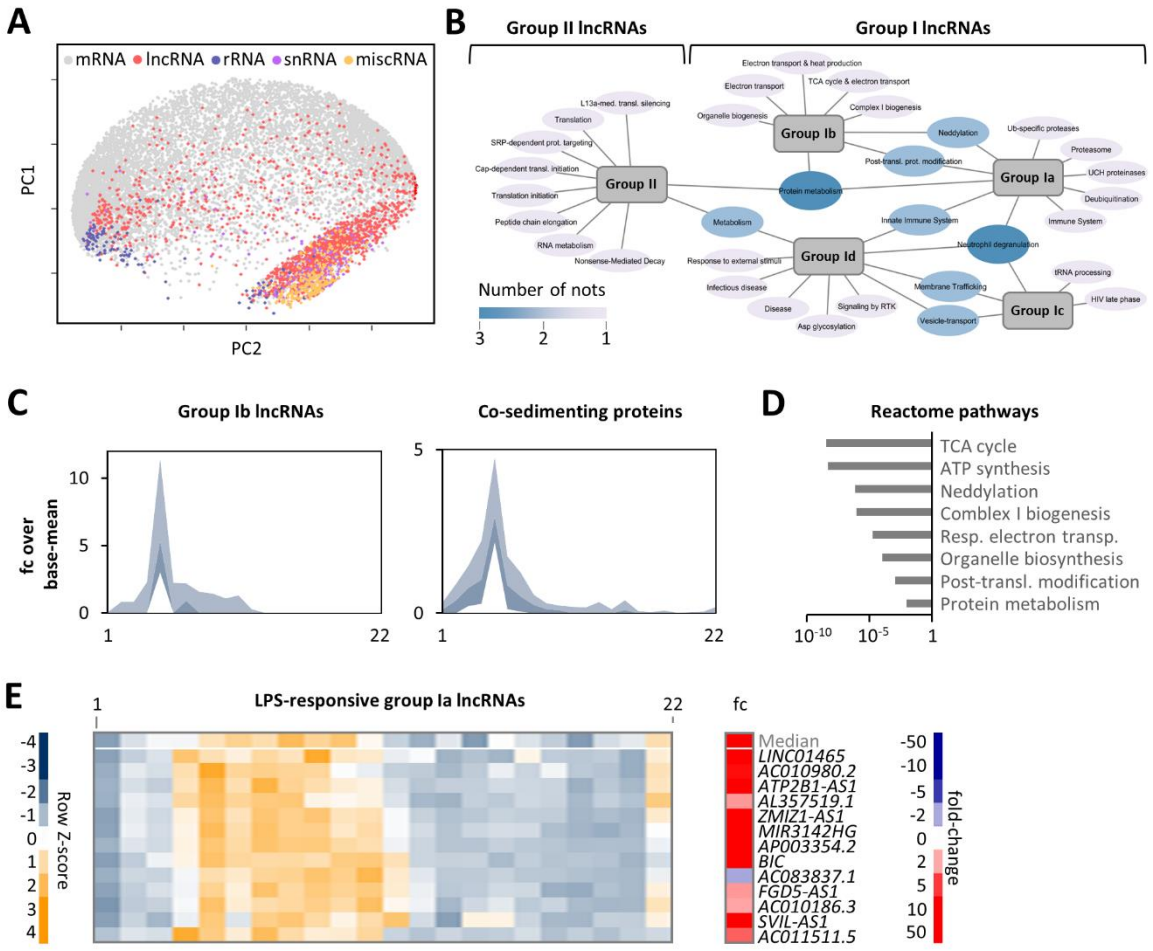


Fig. S2. Co-sedimentation of group I and group II lncRNAs and proteins. A) PCA analysis of gradient RNA-Seq data (RNA classes highlighted). **B)** Top-enriched pathways among proteins co-sedimenting with the indicated lncRNA sub-groups. **C)** Fold-change plots (inner two quartiles shown, relative to base-mean) showing group Ib lncRNAs (left) and co-sedimenting proteins (right). **D)** Reactome pathway analysis of proteins from A). **E)** Z-score heatmap showing group Ia lncRNA abundances in 10-60 % glycerol gradient fractions. Heatmap is limited to lncRNAs differentially regulated (≥ 2 or ≤ 0.5, $P_{adj} \leq 0.05$) upon LPS-treatment (see Fig. 1), as color coded to the right (fc = fold-change in LPS vs. mock comparison).

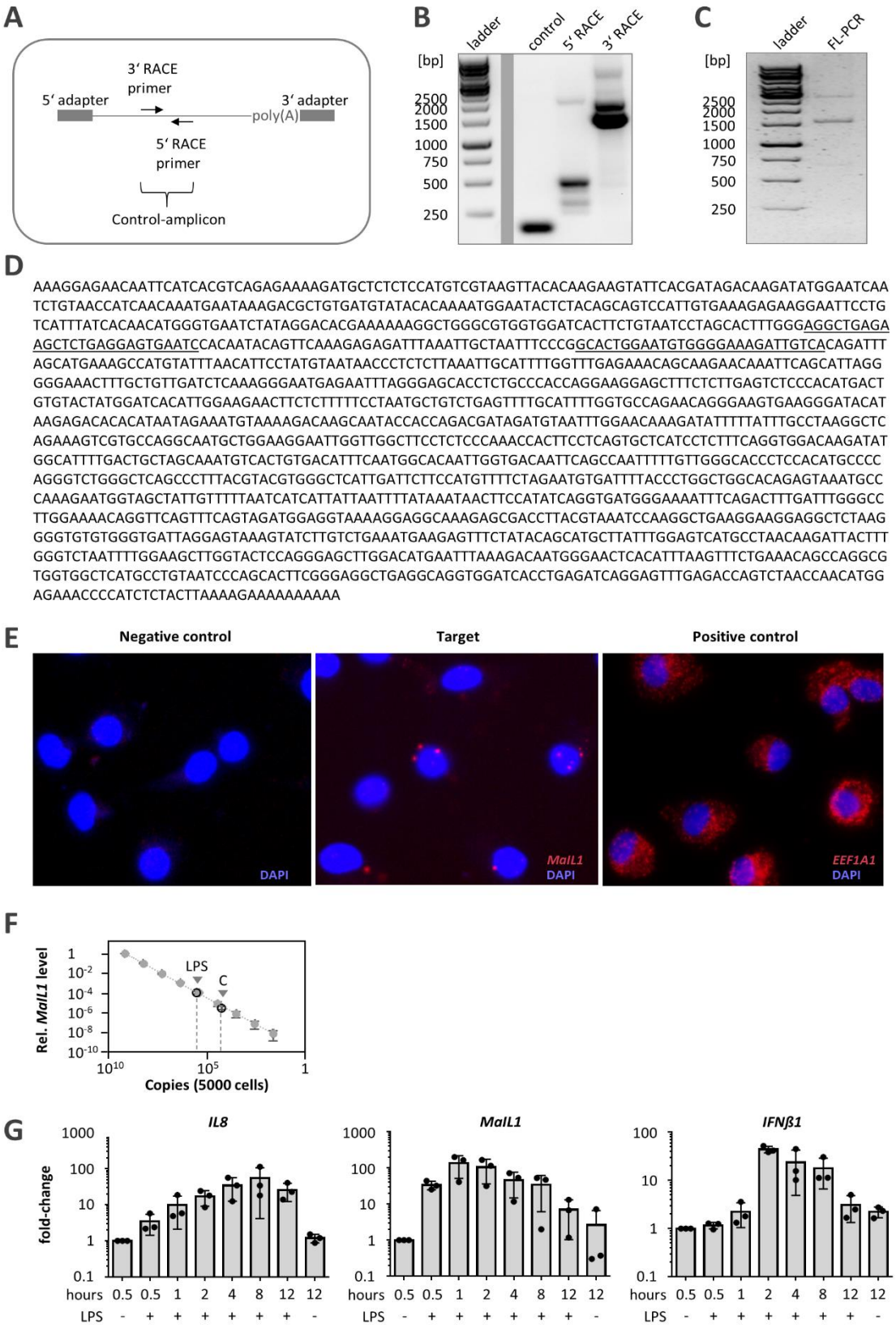


Fig. S3. *MalL1* transcript characterization. A) Layout of the RACE-PCR experiment. RACE-PCR is performed with a gene-specific and a 5' or 3' RACE-adapter primer. Control reaction is performed

by combining 5’ and 3’ RACE gene-specific primer. **B)** Agarose gel analysis of amplicons from control-reaction, 5’ and 3’ RACE. **C)** Full-length *MaIL1* amplicon, obtained using primers annealing to the 5’ and 3’ RACE-PCR inferred cDNA ends. **D)** Sequence of the RACE-PCR determined spliced *MaIL1* full-length cDNA (gene-specific RACE-primers highlighted. **E)** Representative images of RNA-FISH negative control, *MaIL1* (Target) and *EEF1A1* (Positive control) experiments. Same brightens and contrast settings were applied to negative control and *MaIL1* images. **F)** Copy-number determination by qRT-PCR (standard dilution series and RNA from 5000 macrophages stimulated with LPS or mock-treated for 8 h; *MaIL1* levels relative to undiluted standard sample). **G)** Time-course analysis of *IL8*, *MaIL1* and *IFNβ1* expression in primary LPS-stimulated macrophages (three different donors), relative to 0.5 h mock-treatment.

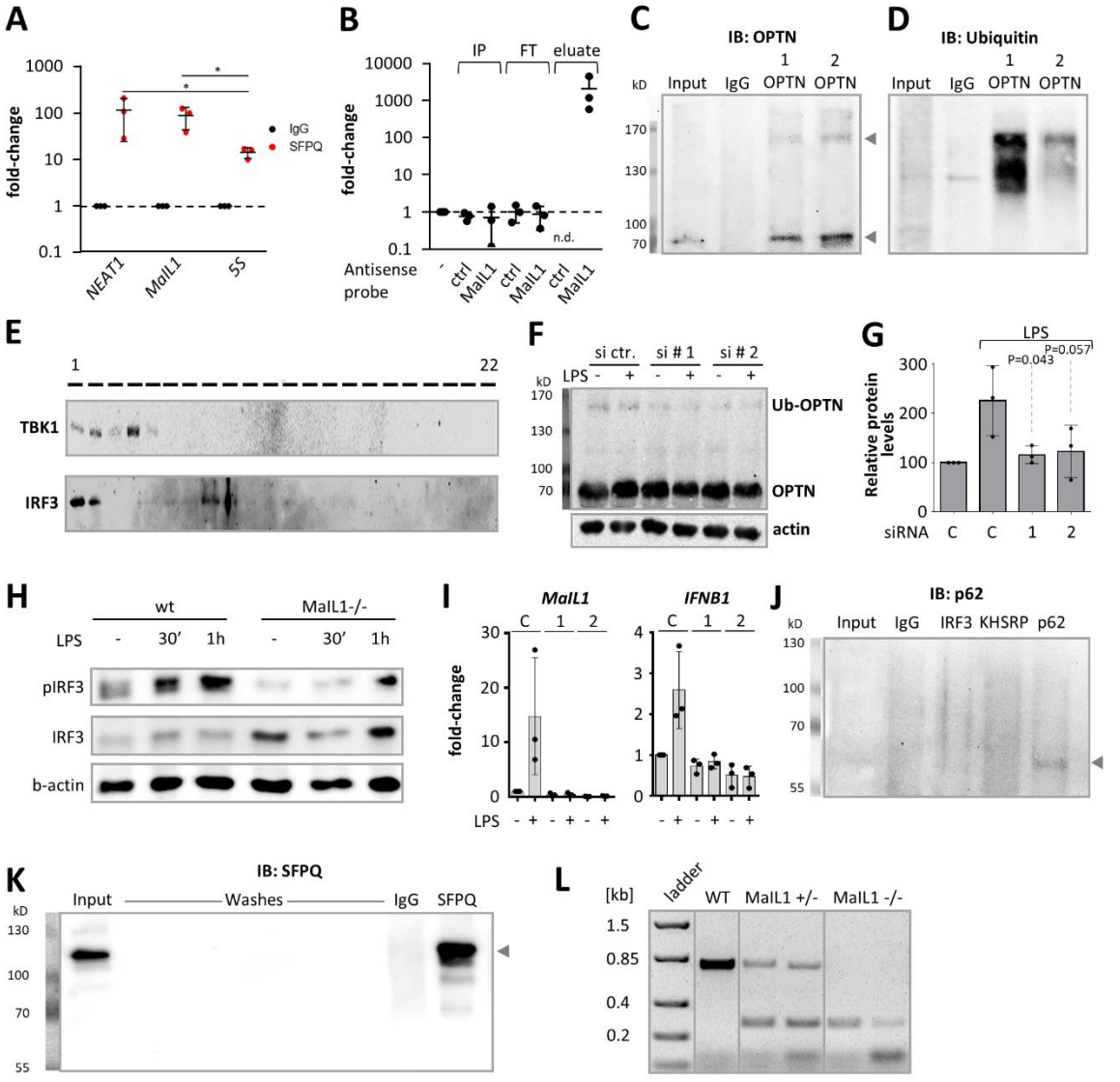


Fig. S4. Mechanistic implications of *MaIL1* in macrophage IRF3 signaling. A) qRT-PCR analysis of relative *NEAT1*, *MaIL1* and *5S* RNA enrichment in eluates from SFPQ compared to control CoIPs. **B)** qRT-PCR analysis of *MaIL1* lncRNA expression relative to untreated lysates in RAP-MS input (IP), flow-through (FT) and eluate fractions using control (ctrl) or *MaIL1* antisense (MaIL1) oligonucleotide pools. **C)** Western blot analysis of OPTN CoIP input and eluate fractions (IgG: control IgG CoIP eluate; 1 OPTN and 2 OPTN: eluates from CoIPs with two different anti-OPTN IgGs). Immunoblot developed with anti-OPTN IgG. **D)** Same as C), but immunoblot developed with anti-ubiquitin IgG. **E)** Western blot analysis of 10-60 % glycerol gradient fractions (see Fig. 4A). Two blots, probed for total TBK1 and total IRF3 protein. **F)** Representative Western blot corresponding to quantifications shown in panel G). **G)** Western blot quantification of non-ubiquitin-associated OPTN band intensity in primary macrophages, displayed as percentage relative to control-siRNA and mock treatment, normalized to actin. **H)** Representative Western blot showing total and phospho-IRF3 levels in wild-type and MaIL1 KO THP1 cells. **I)** qRT-PCR analysis of *MaIL1* and *IFNB1* expression in a control (C) THP1 clone and two MaIL1 knockout (1 and 2) THP1 clones, 16 h post LPS treatment, compared to mock treatment. **J)** and **K)** Representative Western blots showing successful p62 and SFPQ immuno-precipitation, respectively. **L)** Agarose gel showing genomic PCR products for wild-type (WT) and *MaIL1* partly (MaIL1 +/-) and fully (MaIL1 -/-) deficient THP1 cell clones. Marker band sizes are indicated (kb = kilobase). Where applicable, standard deviations and individual data points derived from at least three independent

experiments are shown. P values (** = ≤ 0.01, * = ≤ 0.05, n.s. = not significant) were determined using a one-way ANOVA test.

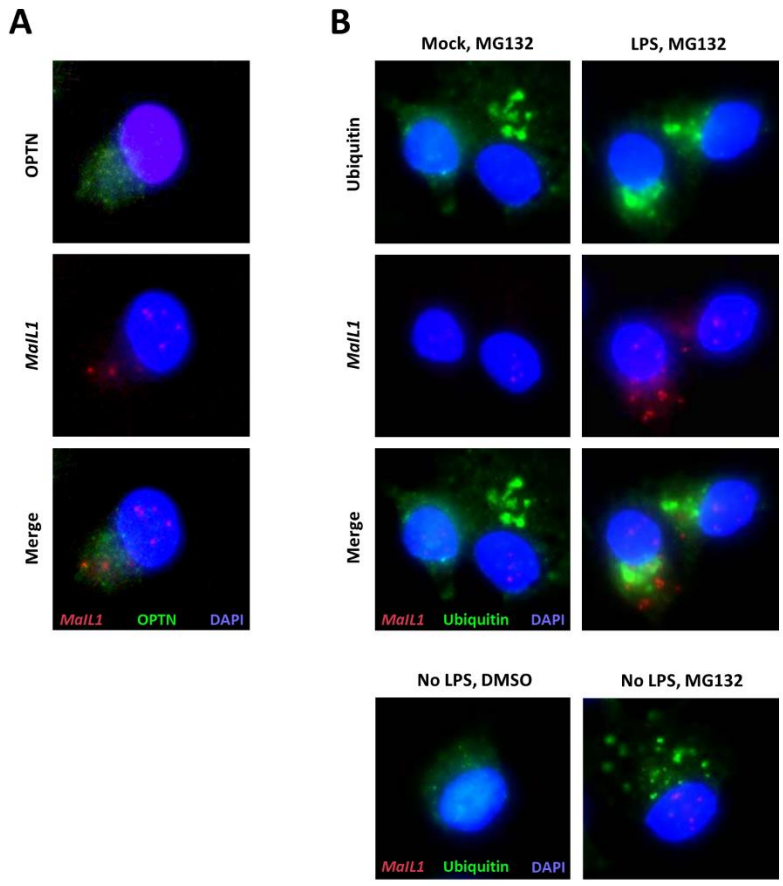


Fig. S5. Localization of *MaIL1* to OPTN and Ubiquitin-occupied compartments. A) RNA-FISH and immunofluorescence analysis of *MaIL1* and OPTN subcellular localization in 8 h LPS treated primary human macrophages. Nucleus counterstained with DAPI. **B)** Top: RNA-FISH and immunofluorescence analysis of *MaIL1* and Ubiquitin subcellular localization in 8 h mock or LPS treated primary human macrophages, pre-treated with 10 µM MG132. Bottom: *MaIL1* and Ubiquitin staining in primary human macrophages treated with DMSO or 10 µM MG132 for 12 h. Nucleus counterstained with DAPI.

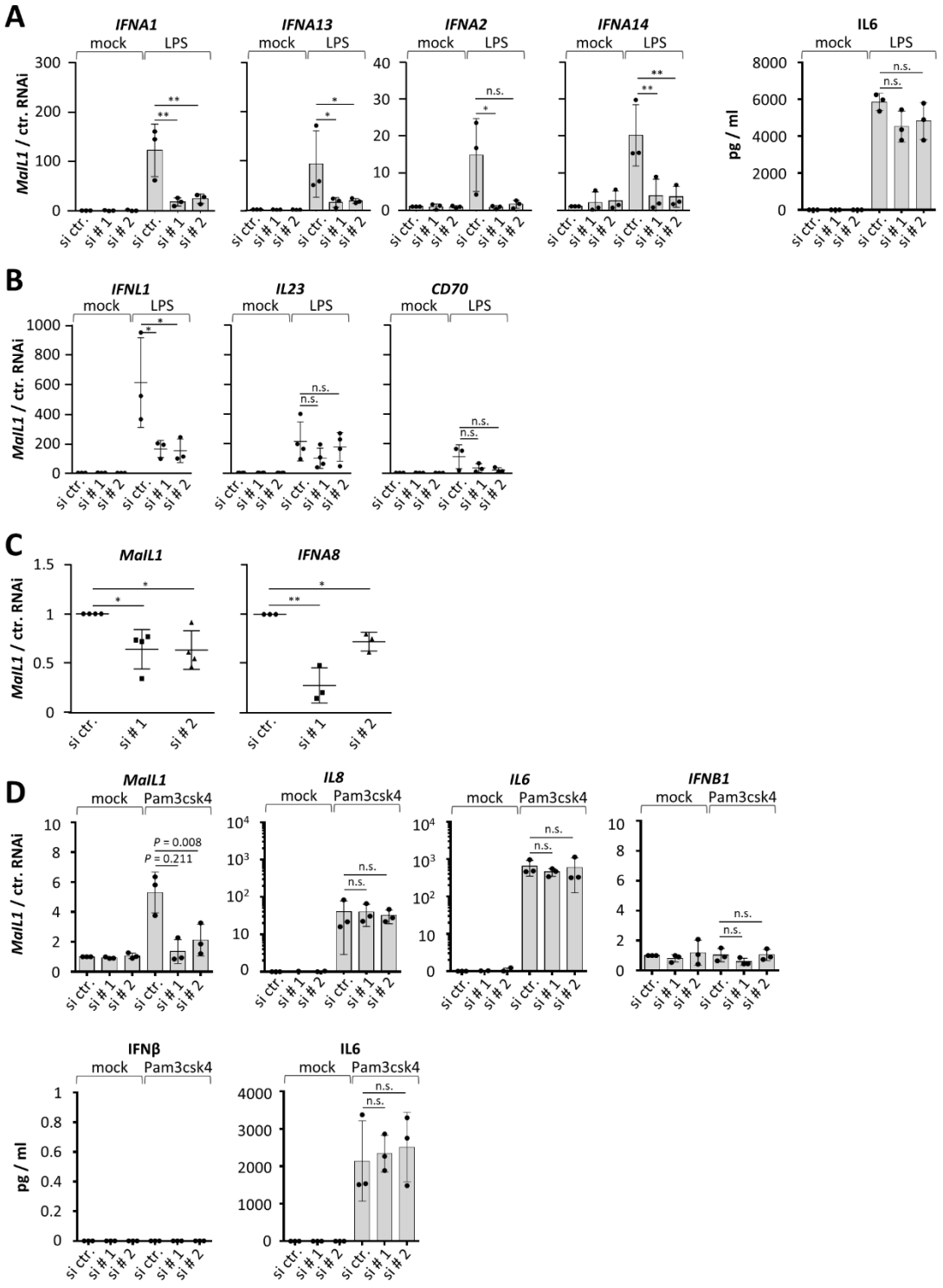


Fig. S6. Dependence of immune-gene activation on *MaIL1*. A) Left: qRT-PCR analysis of *IFNA1, IFNA13*, *IFNA2* and *IFNA14* mRNA expression in 8 h LPS or mock-treated primary human macrophages upon control or *MaIL1* knockdown (with siRNA # 1 and 2). Fold-changes relative to control siRNA and mock-treatment. Right: IL6 quantification by ELISA in the same samples. **B)** qRT-PCR analysis of *IFNL1*, *IL23* and *CD70* mRNA expression in 8 h LPS or mock-treated primary human macrophages upon control or *MaIL1* knockdown (with siRNA # 1 and 2). Fold-changes

relative to control siRNA and mock-treatment **C)** qRT-PCR analysis verifying *MaIL1* knockdown and effect on *IFNA8* mRNA expression in 8 h polyI:C treated macrophages. Fold-changes relative to control siRNA treatment. **D)** Top: qRT-PCR analysis of *MaIL1, IL8*, *IL6* and *IFNB1* mRNA expression in 8 h Pam3csk4 or mock-treated primary human macrophages upon control or *MaIL1* knockdown (with siRNA # 1 and 2). Fold-changes relative to control siRNA and mock-treatment. Bottom: IFNβ and IL6 quantification by ELISA in the same samples. Where applicable, standard deviations and individual data points derived from at least three independent experiments (with cells from different donors) are shown. P values (** = ≤ 0.01, * = ≤ 0.05) were determined using a one-way ANOVA test.

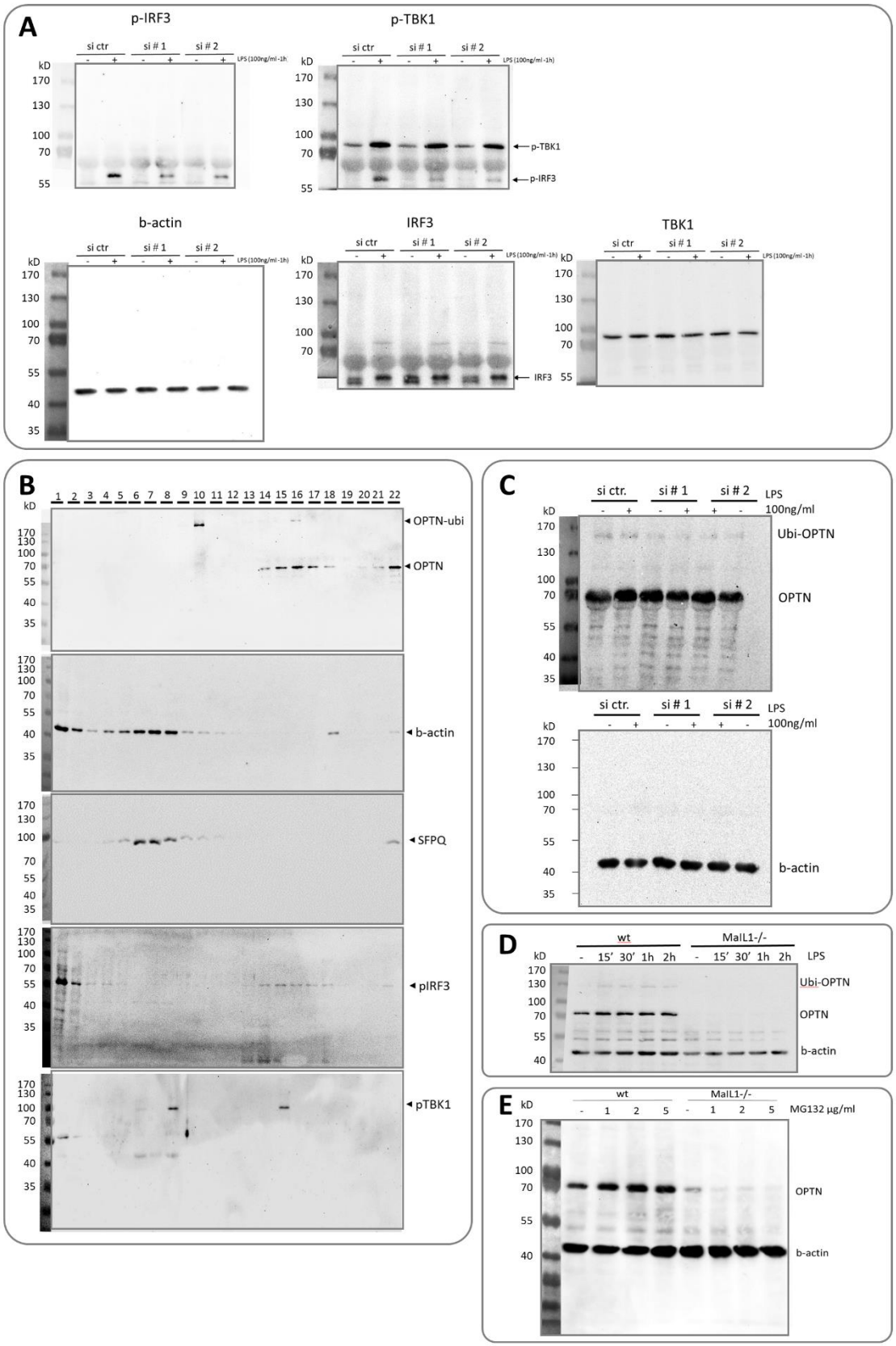


Fig. S7. Relevant Western blot full-scans. A) Fig. 5B blots. **B)** Glycerol gradient blots. **C)** Representative Fig. 5C blots. **D)** Fig. 5F (left) blot. **E)** Fig. 5F (right) blot.

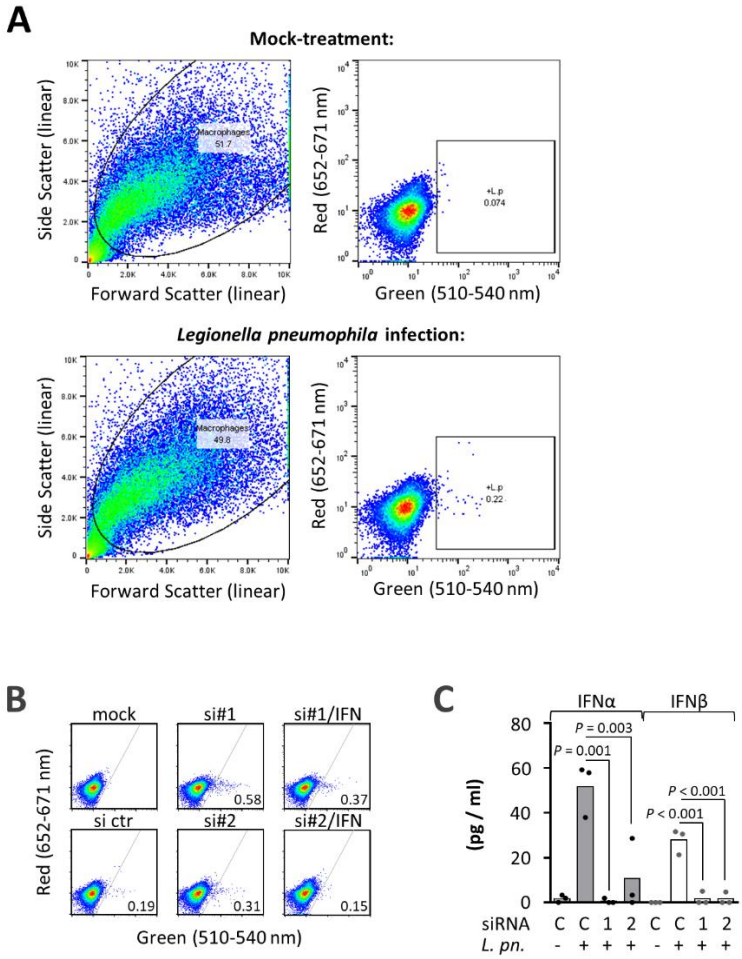
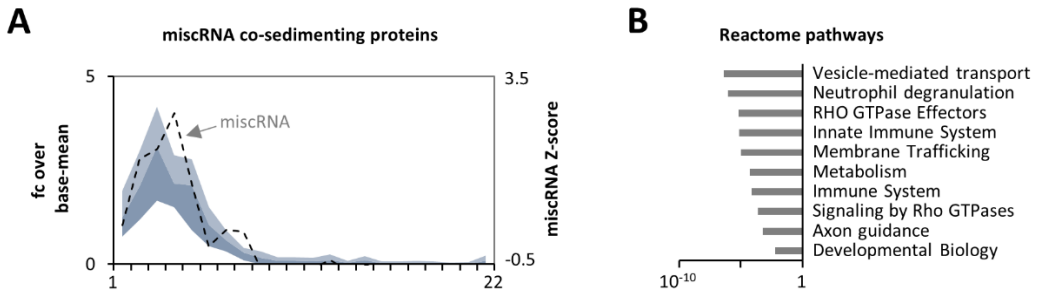


Fig. S8. FACS gating strategy. A) Cell gate in forward- and side-scatter (FSC/SSC) plot (left) was applied to red/green auto-fluorescence plot (right). Top: mock-treated cells. Bottom: GFP-*Legionella pneumophila* treated cells. **B)** Representative FACS plots (*L. pneumophila* infected macrophage cultures, treated with control or *MaIL1* siRNA 1 and 2 and IFNβ (IFN). Mean percentages of infected cells are shown). **C)** IFNα and IFNβ ELISA with supernatants from mock- or *L. pneumophila* treated macrophages (MOI 0.1, 24 h), transfected with control (C) or *MaIL1* siRNA (1 or 2). *L. pn.* = *Legionella pneumophila*. Three independent replicates (macrophages from different donors) and one-way ANOVA test.



C

Top 10 miscRNA co-sedimenting proteins

Protein name	R^2
Dipeptidyl peptidase 1 (CTSC)	0.965
Enoyl-CoA hydratase, mitochondrial (ECHS1)	0.952
Twinfilin-2 (TWF2)	0.946
Actin-related protein 2/3 complex subunit 5 (ARPC5)	0,946
Cytochrome c oxidase subunit 2 (COX2)	0.945
Methionine adenosyltransferase 2 subunit beta	0.939
Fructose-bisphosphate aldolase A (ALDOA)	0.938
Acyl-Coenzyme A dehydrogenase (ACADM)	0.938
Dihydropyrimidinase-related protein 2 (DPYSL2)	0.937
cDNA, FLJ95265 (highly similar to acetyl-Coenzyme A acyltransferase 2, ACAA2)	0.935

Fig. S9. miscRNA co-sedimenting proteins. A) Fold-change plots (inner two quartiles shown, relative to base-mean) showing proteins co-sedimenting with miscRNAs. Dashed line illustrates miscRNA sedimentation profile. **B)** Reactome pathway analysis of proteins from A). **C)** Top 10 proteins co-sedimenting with miscRNAs (according to R^2 value, in descending order).

Table S1. PCR oligonucleotides used in the present study.

Target	Oligo name	Oligo sequence
hMaIL1 (qRT-PCR)	OBS-0865	Fwd: AGCTCTGAGGAGTGAATCCAC
	OBS-0866	Rev: ACATGGCTTTCATGCTAAATCTGTG
hNEAT1 (qRT-PCR)	OBS-0678	Fwd: ACTCTTCTTGTGAGCTCACTCC
	OBS-0679	Rev: ACAATACCGACTCCAACAGCC
LINC001215 (qRT-PCR)	OBS-0889	Fwd: ATTCTCCTGCCTCACAAGTGC
	OBS-0890	Rev: TCTGCTCTATGTCTGCACTGG
hNRIR (qRT-PCR)	OBS-2323	Fwd: ACCTTGATCTTGGACTTCCTAG
	OBS-2324	Rev: ACTGGATGAGACAGAATGCTG
hU6 (qRT-PCR)	OBS-0712	Fwd: GCTTCGGCAGCACATATACTAAAAT
	OBS-0713	Rev: ATATGGAACGCTTCACGAATTTG
hIL1β (qRT-PCR)	OBS-0720	Fwd: ATGGAGCAACAAGTGGTGTTCTC
	OBS-0721	Rev: TCAACACGCAGGACAGGTACAG
hIFIT1 (qRT-PCR)	OBS-0729	Fwd: ATGCAGGAAGAACATGACAACC
	OBS-0730	Rev: TCTGGACACTCCATTCTATAGCG
hCD80 (qRT-PCR)	OBS-0768	Fwd: AACCGGACCATCTTTGATATCATC
	OBS-0769	Rev: TCGTATGTGCCCTCGTCAGA
hIFNA8 (qRT-PCR)	OBS-2200	Fwd: ACTTGACCAGCAGCTGAATG
	OBS-2201	Rev: TCATGATTTCTGCTCTGACAACC
hIFNA1 (qRT-PCR)	OBS-2202	Fwd: CAGGAGGACCTTGATGCTC
	OBS-2203	Rev: TCTGCTGGATCAGCTCATGG
hIFNB1 (qRT-PCR)	OBS-2204	Fwd: AACATGACCAACAAGTGTCTCC
	OBS-2205	Rev: TGTCCTTGAGGCAGTATTCAAG
hIFNA2 (qRT-PCR)	OBS-2259	Fwd: TGAAGGACAGACATGACTTTGG
	OBS-2260	Rev: AGATGAGTCCTTTGTGCTGAAG
hIFNA13 (qRT-PCR)	OBS-2261	Fwd: ATGAGCAGAATCTCTCCTTCCTC
	OBS-2262	Rev: TGAAGATCTGCTGGATCAGCTC
hIFNA14 (qRT-PCR)	OBS-2263	Fwd: ATGAATGAGGACTCCATCCTG
	OBS-2264	Rev: ATCTCATGATTTCTGCTCTGAC
hIFNL1 (qRT-PCR)	OBS-2206	Fwd: ACTTCCAAGCCCACCACAAC
	OBS-2207	Rev: ACAGGAGAGCTGCAACTCCAG
IL23A (qRT-PCR)	OBS-1580	Fwd: CTCTGCTCCCTGATAGCCCT
	OBS-1581	Rev: GGGACTGAGGCTTGGAATCT
hCD70 (qRT-PCR)	OBS-2208	Fwd: TAGCTGAGCTGCAGCTGAATC
	OBS-2209	Rev: CCTGGATGTGTACCATGTAGA
hCD80 (qRT-PCR)	OBS-768	Fwd: AACCGGACCATCTTTGATATCATC
	OBS-769	Rev: TCGTATGTGCCCTCGTCAGA
hIL-8 (qRT-PCR)	OBS-0017	Fwd: ACTGAGAGTGATTGAGAGTGGAC
	OBS-0018	Rev: AACCCTCTGCACCCAGTTTTC
hIL-6 (qRT-PCR)	OBS-0015	Fwd: AATTCGGTACATCCTCGACGG
	OBS-0016	Rev: TTGGAAGGTTCAGGTTGTTTTCT
hGAPDH (qRT-PCR)	OBS-0430	Fwd: CCACATCGCTCAGACACCAT
	OBS-0431	Rev: CGCAACAATATCCACTTTACCAGAG
h5S (qRT-PCR)	OBS-2418	Fwd: TCTACGGCCATACCACCCTG
	OBS-2419	Rev: AGCCTACAGCACCCGGTATTC
MaIL1 RACE-PCR	OBS-1042	Fwd: AGGCTGAGAAGCTCTGAGGAGTGAATCC
	OBS-1043	Rev: TGACAATCTTTCCCCACATTCCAGTGC
MaIL1 full length PCR	OBS-1191	Fwd: ATCGGATTCGAAAGCTCAGAGAAAAGATGC
	OBS-1182	Rev: TCCGATGCGGCCGCTTTTTTTTTTCTTTTAAGTAGAG
MaIL1 genomic PCR	OBS-1044	ACCACCATTTAGTTAAATCACACCAG
	OBS-1045	AAGAGTCATTTGGATAGTGCTTACC

Table S2. RAP-MS antisense 80-mer oligonucleotide pools used in the present study.

Target	Oligo ID	Oligo sequence
Control (random)	Ctrl_1	CACTATGGAAAGGCGGCTTCAGCTGCCAATCTTGGATCCCAGCGAAAGCAGGAACCGATGTGGCCAGAGGAATACGTCCG
	Ctrl_2	GATTTCGGTCTGTACGGCTATAATTTGGTGAGTTAAACTGGATCAGCAGTTTGCTAGACGATTTGGCACAGTTAGTCACA
	Ctrl_3	TTACATGGTCCTAATCGGCTGCACCGCTCGGGCGGACCAAAACCCTTGAGCACAAAAATTAAGCAGAGGCTAGTCGCGTG
	Ctrl_4	GCTGTTACCTTCCACGCCGGCTGGCAACAACGATTATCATCCGCTACTGGGCAGGAAGAAAGTGTTTAAAAGAAGTCTTG
	Ctrl_5	GCTGCCACTTGCTAACCCCATTGCGGAATCCTATTATACGATCGGACAGCCTTGTGTTCGTGGAGACGGCACATTAGGCT
	Ctrl_6	TGCACAATTGATGTTCCGATGCAGGATAACAACTGGATCACGTATGTATTGTACCAAAGCATGTACATCGCAAGTCCCCG
	Ctrl_7	GACGCCTAGACGTATACTAGCAAAACGACATCTTAATTGAATTATTTATCCTACGCGCGCCTCCTAGATGACATGAATAT
	Ctrl_8	GCTTGTGCTGGTATTGCCCCGTGTGTGCTATTAGAAGCGGAGAAATCAGGGACAGTGTCTCGTCGGCCCACCTGCACACA
	Ctrl_9	GCTAGTGCAAAACACCCAAGCGACCCTGACAGTGCGAATTGGCGAGCCTTAAGCTCTTTCGTTTGCTGACGAGCGTTGCT
	Ctrl_10	AGCAAACACGTCGAGCAAATACGTATGCCGCACATTTGACCACGCAGCCACATACCCCTGGAAGGAAGAACGCATACTAG
MalL1	KB15_1	CCCCACATTCCAGTGCCGGGAAATTAGCAATTTAAATCTCTCTTTGAACTGTATTGTGGATTCACTCCTCAGAGCTTCTC
	KB15_2	CTGTTTCTCAAACCAAAATGCAATTTAAGAGAGGGTTATTACATAGGAATGTTAAATACATGGCTTTCATGCTAAATCTG
	KB15_3	TCCTTCCTGGTGGGCAGAGGTGCTCCCTAAATTCTCATTCCCTTTGAGATCAACAGCAAAGTTTCCCCCTAATGCTGAAT
	KB15_4	CCAAAATGCAAAACTCAGACAGCATTAGGAAAAAGAGAAGTTCTTCCAATGTGATCCATAGTACACAGTCATGTGGGAGA
	KB15_5	TTACATCTATCGTCTGGTGGTATTGCTTGTCTTTTACATTTCTATTATGTGTGTCTCTTATGTATCCCTTCACTTCCCTG
	KB15_6	GGTTTGGGAGAGGAAGCCAACCAATTCCTTCCAGCATTGCCTGGCACGACTTTCTGAGCCTTAGGCAAATAAAAATATCT
	KB15_7	CAATTGTGCCATTGAAATGTCACAGTGACATTTGCTAGCAGTCAAAATGCCATATCTTGTCCACCTGAAAGAGGATGAGC
	KB15_8	GGAAGAATCAATGAGCCCACGTACGTAAAGGGCTGAGCCCAGACCCTGGGGCATGTGGAGGGTGCCCAACAAAAATTGGC
	KB15_9	TTTTACCTCCATCTACTGAAACTGAACCTGTTTTCCAAGGCCCAAATCAAAGTCTGAAATTTTCCCATCACCTGATATGG
	KB15_10	ACAAGATACTTTACTCCTAATCACCCACACACCCCTTAGAGCCTCCTTCCTTCAGCCTTGGATTTACGTAAGGTCGCTCT
	KB15_11	CTGGAGTACCAAGCTTCCAAAATTAGACCCAAAGTAATCTTGTTAGGCATGACTCCAAATAAGCATGCTGTATAGAAACT

Table S3. Patient characterization.

No	gender	age	infection
1	m	53	control
2	m	49	control
3	m	38	control
4	w	71	control
5	w	49	control
6	m	62	control
7	m	28	control
8	m	29	control
9	w	27	control
10	m	64	control
11	w	25	infection (bacterial)
12	m	45	infection (bacterial)
13	w	64	infection (bacterial)
14	w	19	infection (fungal)
15	w	64	infection (fungal)
16	w	42	infection (fungal)
17	w	68	infection (nd)
18	w	59	infection (nd)
19	w	52	infection (nd)
20	w	58	infection (nd)
21	w	73	infection (nd)
22	w	61	infection (polymicrobial)

nd = causative pathogen not determined (i.e. culture negative)

Supporting Datasets:

Dataset S1 (separate file). LncRNA regulation and subcellular distribution upon mock or LPS treatment.

Dataset S2 (separate file). Glycerol gradient distribution of recorded lncRNAs (RNA-Seq).

Dataset S3 (separate file). Glycerol gradient distribution of recorded proteins (mass-spectrometry).

Supplementary Methods

Cell culture and infection assays

Leukocytes from buffy coats were purified using Lymphoprep gradient medium (Stemcell Technologies, # 07851), followed by Miltenyi MACS bead-based enrichment of monocytes (CD14-beads), T cells (CD4-beads / CD8-beads / CD45RO-beads), B cells (CD19-beads), NK cells (CD56-beads), granulocytes (CD66b-beads), according to the manufacturer's instructions. Macrophages were generated by plating monocytes at 4*10e5 cells per ml of X-Vivo 15 medium (Lonza) or RPMI (RNA-FISH experiments), containing 5 % FCS (Biochrom) and 15 ng / ml recombinant human GM-CSF (Preprotech). Cells were stimulated and sampled 7 days post onset of differentiation. THP1 cells were grown in RPMI 1640 medium (Thermo Fisher), 1% penicillin / streptomycin solution (Thermo Fisher), 10% FBS (Biochrom). THP1-cells were differentiated with 20 nM PMA (Sigma- Aldrich) for 24 h.

Cells were stimulated with LPS (*Salmonella enterica* serovar Typhimurium, Sigma) or Pam3csk4 (Invivogen) at 100 ng / ml for the indicated durations. M-Tridap and Resiquimod (both Invivogen) were used at 1 µg / ml and poly(I:C) (Invivogen) at 20 µg / ml. For pathway inhibition cells were pre-incubated with the following inhibitors (50 µM final concentration) for 2 hours or the indicated time intervals: NFκB Activation Inhibitor CAS 545380-34-5 (NFκB inhibitor, Merck Millipore), U0126 (MEK1/2 MAPK inhibitor, Merck Millipore), SB203580 (p38 MAPK inhibitor, Merck Millipore), Amlexanox (TBK1 inhibitor, Abcam), JNK inhibitor II (Merck Millipore), MG132 (Merck Millipore).

For bacterial infection with *Legionella pneumophila* strain Corby, modified for constitutive GFP-expression (P(mip)-gfp plasmid, (60)), bacteria were grown on charcoal-plates, diluted in PBS (OD_{600} = 1) and added to macrophage cultures (MOI of 0.1, assuming $2x10^9$ bacteria per ml at OD1). For synchronized cell activation, macrophages were pre-treated with 10 ng LPS / ml. To synchronize the infection, culture plates were centrifuged for 10 min at 250 g. For human IFNβ (Preprotech) co-stimulation 1 ng / ml was used as a final concentration. Cells were incubated at 37 °C with 5 % CO2 atmosphere.

Western blot (antibodies and dilutions)

Blots were blocked with TBST buffer containing 5 % milk powder and 3 % BSA. Antibodies were used at a 1:500 (anti-OPTN and anti-pIRF3) or 1:1000 (all other antibodies) dilution in TBST buffer, containing 3 % BSA. Primary antibodies: anti-SFPQ (mouse IgG, Abcam, ab11825), anti-TBK1 (rabbit IgG, Cell Signaling, 3504), anti-p-TBK1 (Ser172, rabbit IgG, Cell Signaling, 5483), anti-IRF3 (rabbit IgG, Cell Signaling, 11904), anti-p-IRF3 (Ser386, rabbit IgG, Abcam, ab76493), anti-Actin (mouse IgG, Santa Cruz, sc-47778), anti-OPTN (C1, mouse IgG, Santa Cruz, sc-271549), anti-OPTN (C2, mouse IgG, Santa Cruz, sc-166576), anti-Ubiquitin (mouse IgG, Enzo, BML-PW8810-0100). Control antibodies (CoIP experiments): anti-FLAG (mouse IgG, Sigma-Aldrich, F1804), rabbit IgG (Cell Signaling, 7074). Secondary antibodies: anti-mouse-HRP (goat IgG, Santa Cruz, sc-2005), anti-rabbit-HRP (mouse IgG, Cell Signaling, 5127S).

RNA-FISH

Monocyte-derived macrophages, grown in chamber slides (Nunc™ Lab-Tek™ II CC2™ Chamber Slide System) at a density of 0.5 x 10^6 / ml (0.25 $x10^6$ cells / chamber), were stimulated. Subsequently, medium was removed and cells were washed once with PBS, followed by fixation in 4 % paraformaldehyde / PBS for 30 min at 4 °C. Cells were washed twice and stored in ice cold PBS until further processing. RNA-FISH was performed using the QuantiGene® ViewRNA ISH Cell Assay (Affymetrix). Volumes of all components were adapted to the chamber slide format. After fixation and two washes with PBS for 1 min at RT, cells were permeabilized with detergent solution for 5 min at RT, rinsed with PBS twice and treated with protease (1:4,000, provided with the kit) for 10 min at RT. After rinsing three times with PBS, FISH probes (diluted 1:40 in probe set diluent) were added, followed by 3 h incubation at 37°C for probe set hybridization. A probe homologous

to EF1α served as positive control for the hybridization, whereas the probe set diluent without probe ("no probe" control) served as control for background staining (Fig. S3E). Probes were designed by Affymetrix (Homo sapiens EEF1A1 Catalog nr. VA1-10418; Homo sapiens ENSG00000254281 Catalog nr. VPRWEK4 (sequence provided by L.N.S.). Alternating washing and hybridization steps, including the Preamplifier, Amplifier and Label Probe hybridization steps, were performed according to the manufacturer's instructions. Nuclei were counterstained with Roti®-Mount FluorCare DAPI (Carl Roth®) which also served as mounting medium. Images were acquired at an Olympus DP 80 microscope equipped with UV light at a 600x magnification. The DAPI signal was detected at 345 nm and the probe signal at 550 nm.

Glycerol gradient ultracentrifugation

Gradients were poured by layering solutions of increasing glycerol concentration into 40 ml ultracentrifugation tubes on a dry-ice / ethanol slurry (38.4 ml final gradient volume). Solutions were poured in 5 % increments, starting at 10 % and ending at 60 % glycerol in buffer (10 mM Tris [pH 8], 150 mM NaCl, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5% Triton, 0.5 mM EDTA, 1 mM DTT). Gradients were stored at -80 °C and thawed over-night at 4 °C before use. For total cell lysate generation, 10^8 macrophages were washed once with PBS, resuspended in 400 µl gradient buffer, and passed through a 26 G needle (five times) followed by disruption of nuclei using a Dounce homogenizer (10 strokes). Debris was pelleted by brief centrifugation (30 seconds, 8000 g) and supernatant (~ 300 µl) was supplemented with 2 µl recombinant RNasin RNase inhibitor (Promega) and layered onto thawed gradient. Gradients were centrifuged at 50200 rcf (Servall S-34 rotor), acceleration level 1 and brakes off, for 20 h at 4 °C using a Servall Evolution ultracentrifuge (Hitachi). Gradients were fractionated from the top in 900 µl steps to obtain 44 fractions. The last fraction was the pellet, resuspended in 900 µl gradient buffer. Protein and RNA were recovered by extraction with an equal volume of PCI. For RNA-recovery, the aqueous phase was extracted a second time with PCI, followed by RNA precipitation with 30:1 ethanol / 3M sodium acetate and DNaseI digestion as described above. For protein recovery, the phenol phase was mixed 1:1 with distilled water and extracted again, followed by protein-precipitation using ice-cold acetone and centrifugation (5 min, max speed). Pellets were air-dried and resuspended in 8 M urea (mass-spec grade).

RNA affinity chromatography

RAP-MS was performed as previously described by the Guttman lab (https://www.guttmanlab.caltech.edu/RAP_MS_Protocol_April2015.pdf), with $8x10^7$ UV-crosslinked cells per sample, grown in X-Vivo 15 medium (Lonza), without SILAC isotopes. Crosslinking was performed at 800 mJ / cm^2, on an ice-bath, using an HL-2000 HybriLinker (UVP). For lysate generation, the whole cell lysate protocol was followed, using $16x10^7$ cells. Lysates were split up 1:1 for *MaIL1* and control captures, to obtain an equivalent of $8x10^7$ cells per capture. Different from the Guttman lab protocol, RNA was purified by PCI extraction. For Western blot analysis, proteins were pelleted with acetone after the Benzonase elution step. For mass-spectrometry, instead of Benzonase elution, proteins were digested "on-bead". Relative protein enrichment was determined using Proteome Discover, comparing RAP-MS captures carried out with *MaIL1* antisense and random control antisense oligo pools, respectively. RAP-MS oligonucleotide antisense pools were generated by segmenting RACE-PCR refined *MaIL1* cDNA sequence into 80mers with 13-18 nt spacing. Sequences were searched against the GRCh38 genome using EMSEMBL BLAT and sequences with more than 1 hit were removed. Remaining sequences were analyzed by RepeatMasker (http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker) and sequences mapping to repeat regions were removed. Remaining sequences were reverse-complement transformed. Sequences with ≥ 5 Ts in a row were removed to prevent from poly(A) RNA capture. A random sequence of the same size as the *MaIL1* cDNA was generated (http://www.bioinformatics.org/sms2/random_dna.html) and used for control probe generation. Sequences (Table S2) were synthesized at Metabion AG and 3' mono-biotinylated using terminal transferase (New England Biolabs) and Biotin-11-ddUTP (Jena Bioscience) according to the manufacturer's instructions.

Mass spectrometry setup and conditions

Mass spectrometric analysis of digested samples was performed using an Orbitrap Velos Pro mass spectrometer (ThermoScientific). An Ultimate nanoRSLC-HPLC system (Dionex), equipped with a custom end-fritted 50cm x 75µm C18 RP column filled with 2.4 µm beads (Dr. Maisch GmbH) was connected online to the mass spectrometer through a Proxeon nanospray source. 1-15 µL (depending on peptide concentration and sample complexity) of the tryptic digest were injected onto a 300µm ID x 1cm C18 PepMap pre-concentration column (Thermo Scientific). Automated trapping and desalting of the sample was performed at a flowrate of 6 µL / min using water / 0.05% formic acid as solvent.

Separation of the tryptic peptides was achieved with the following gradient of water / 0.05% formic acid (solvent A) and 80% acetonitrile / 0.045% formic acid (solvent B) at a flow rate of 300 nL / min: holding 4% B for five min, followed by a linear gradient to 45%B within 30 min and linear increase to 95% solvent B in additional 5 min. The column was connected to a stainless steel nanoemitter (Proxeon, Denmark) and the eluent was sprayed directly towards the heated capillary of the mass spectrometer using a potential of 2300 V. A survey scan with a resolution of 60000 within the Orbitrap mass analyzer was combined with at least three data-dependent MS/MS scans with dynamic exclusion for 30 s either using CID with the linear ion-trap or using HCD combined with orbitrap detection at a resolution of 7500.

Bronchoalveolar lavage (BAL) and patient selection

Bronchoalveolar lavage fluid (BALF) samples were obtained from patients at the Department of Infectious Diseases and Respiratory Medicine (Charité University Hospital Berlin). All patients underwent bronchoscopy including bronchoalveolar lavage (BAL) on clinical indication and had provided oral and written informed consent. The study was approved by the 'Ethics committee of Charité - Universitätsmedizin Berlin', 10117 Berlin, Germany (EA2/086/16). Bronchoscopy was performed with a flexible fiberoptic bronchoscope and BAL was performed by instillation of 150 ml pre-warmed sterile 0.9% NaCl solution. In patients with focal abnormalities in chest imaging, BAL was performed in the corresponding pulmonary segment; in patients without radiological abnormalities or diffuse infiltrates, BAL was performed in the right middle lobe or the lingula. Diagnosis of infection was based on culture results, clinical signs of infection, chest imaging, lab results and response to antimicrobial therapy and cellular analyses of BALF. The diagnosis was made by a board-certified pulmonologist. Only patients with non-mycobacterial infection were selected for the infection group and all of the selected patients showed elevated neutrophil frequency in BALF. Control patients underwent bronchoscopy and BAL because of idiopathic coughing or for the exclusion of pulmonary tuberculosis or pulmonary involvement of systemic diseases, such as rheumatoid arthritis. All of the control patients showed no obvious abnormalities in chest imaging and BAL cellular pattern. For patient characterization see Table S3. BALF was processed directly after bronchoscopy. It was centrifuged one time at 400 x g and supernatants were stored at -80 °C for up to two years. For liver reference tissue collection cirrhosis was an exclusion criterion. Non-diseased tissue from tumor resections was processed. All patients had provided oral and written informed consent and the study was approved by the local ethics committee (Marburg FB20 Ethikkomission Az.: Studie 14/17).

Bioinformatics

Demultiplexed reads were imported into the CLC genomics workbench (Qiagen), and upon TruSeq adapter- and quality-trimming, mapped to the GRCh38 annotation with standard settings (mismatch cost = 2; insertion cost = 3; deletion cost = 3; length fraction = 0.8; similarity fraction = 0.8). Differential expression analysis was done using DeSeq2 (63), based on uniquely mapped reads.

For subcellular localization analysis RPKM values were corrected to account for the different RNA content of nucleus and cytoplasm, as described in the Supplementary Methods (SI Appendix). To this end the subcellular % distribution of Gapdh mRNA was determined by qRT-PCR and applied to the following equation to calculate the correction factor (*CF*) to be applied to all cytoplasmic RPKMs:

$$CF_{RPKM_{cytoplasm}} = \frac{\left(\frac{RPKM_{nucleus} \times \%_{cytoplasm}}{1 - \%_{cytoplasm}}\right)}{RPKM_{cytoplasm}} = \frac{\left(\frac{715.03 \times 0.8876}{1 - 0.8876}\right)}{625.46} = \underline{9.02}$$

On multiplication of cytoplasmic RPKMs with the CF, cytoplasmic distribution in % was calculated by division of the corrected cytoplasmic RPKM and the sum of corrected cytoplasmic and the nuclear RPKM of a given gene.

RNA-Seq data from glycerol gradient fractions were normalized to *Escherichia coli* spike-in RNA. Human GRCh38 (GENCODE) and *E. coli* K12 MG1655 (NCBI: GCF_000005845.2_ASM584v2) annotation- and genome-files were fused and upon read mapping separate tables of uniquely mapping reads were created for both organisms. Human RPKMs were calculated based on the human read table and subsequently normalized to the percentage of *E. coli* reads in each sample. Row Z-scores were calculated using these normalized abundance values. RNA-Seq data can be accessed through the NCBI GEO pipeline (accession GSE101409).

Mass-spectrometry data analysis was performed using Proteome Discoverer 2.2 (ThermoScientific) with SEQUEST search engine. Uniprot databases were used. For gradient samples, abundance values were normalized to the relative protein content in each fraction (determined using the BCA-method and silver-gel quantification). Gradient proteomics data are provided in Dataset S3.

Hierarchical clustering was done using Cluster (Eisen lab) and Heatmaps were generated using JAVA TreeView. Pathway enrichment analysis was performed using ConsensusPathDB. Pathway networks were visualized using Cytoscape version 3.7.2. PCA analysis, was performed based on RPKMs using the R-script prcomp (stats) and the rgl package.

Glycerol gradient step-by-step protocol (Leon N Schulte lab)

Gradient preparation:

1. Prepare a dry-ice ethanol slurry and wait for the ethanol to cool down
2. Prepare buffered Glycerol solutions (10 mM Tris [pH 8], 150 mM NaCl, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 % Triton, 0.5 mM EDTA, 1 mM DTT) with the following percentages:

   10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 %

   To this end prepare 60 % and 0 % Glycerol solution and combine according to the following table, to obtain e.g. 5 ml of each solution:

Final concentration	60 % Glycerol	0 % Glycerol
55%	4.58	0.42
50%	4.17	0.83
45%	3.75	1.25
40%	3.33	1.67
35%	2.92	2.08
30%	2.50	2.50
25%	2.08	2.92
20%	1.67	3.33
15%	1.25	3.75
10%	0.83	4.17

3. Place 40 ml ultracentrifugation screw cap tube into ethanol/dry-ice slurry and add 3.5 ml 60 % glycerol solution. Wait until solution is completely frozen.
4. Successively, add remaining glycerol solutions (ending with 10 %) in 3.5 ml steps, as described in step 3.
5. Close ultracentrifugation tube and store gradient at – 80 °C

Loading, ultracentrifugation and fractionation:

1. Thaw gradient overnight, standing upright, at 4 °C to allow for continuous gradient formation.
2. Harvest cells (e.g. 10^8 macrophages), wash once with PBS and resuspend in 400 µl of lysis buffer (10 mM Tris [pH8], 150 mM NaCl, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 % Triton, 0.5 mM EDTA, 1 mM DTT)
3. Incubate cell lysate on ice for 10 min
4. Pass lysate through a 26 Gauge needle 5 times
5. Break nuclei using a dounce homogenizer with 8-10 strokes
6. Add 2 µl of recombinant RNase inhibitor (Promega)
7. Centrifuge Lysate briefly (e.g. 30 seconds, 8000 g) to pellet cellular debris
8. Carefully layer the supernatant onto glycerol gradient
9. Balance gradient(s) and subject to ultra-centrifugation. This step needs to be optimized depending on the centrifuge model (e.g. by A260 absorbance measurement or tracking of U6 snRNA and Gapdh mRNA sedimentation patterns by qRT-PCR). For a Servall Evolution Ultracentrifuge (Hitachi) centrifuge at 50.200 rcf (Servall S-34 rotor), acceleration level 1 and brakes off, for 20 h at 4 °C.

10. Carefully collect 900 µl fractions from the top of the gradient (2 ml tubes)
11. From each fraction remove 100 µl for western blotting / SDS page (e.g. load 15 µl per pocket for silver staining)

Protein / RNA recovery

- To each fraction add 800 µl PCI ("for RNA")
- Mix vigorously by vortexing
- Centrifuge for 30 min at 15 °C and max. speed

  RNA recovery:
- Transfer 700 µl aqueous supernatants to new 1 ml tubes
- Add 700 µl PCI ("for RNA"), mix by vortexing and centrifuge as above
- Transfer 600 µl aqueous supernatants to new 2 ml tubes
- To precipitate the RNA add 1 µl Glycoblue and 1.4 ml ice-cold ethanol / 3M sodiumacetate (30:1)
- Invert tubes several times and incubate at -20 °C overnight
- Pellet by centrifugation at max. speed for 15 min (4 °C)
- Wash with 500 µl 70 % ethanol and air dry pellets
- Perform DNaseI digestion according to available protocols and purify RNA by PCI extraction as above (without double-extraction).
- Dissolve RNA pellets water. Keep the same volume for all fractions. Don't adjust RNA concentrations.

  Protein recovery:
- For protein precipitation add 800 µl of H2O to the PCI phase and vortex (This step is critical as it removes excess glycerol, which will otherwise inhibit protein precipitation!)
- Centrifuge for 30 min at 15 °C and max. speed
- Carefully remove and discard the aqueous phase
- Add 1.2 ml ice cold acetone to the lower phase (phenol phase), mix vigorously by pipetting
- Incubate over night at -20 °C
- Centrifuge for 30 min at max. speed (4 °C)
- (optional: wash pellet with ice-cold acetone)
- Air-dry pellets
- Resuspend pellets in 0.5-1 ml mass-spec grade 8 M urea (keep the same volume for all fractions)

Considerations for sample analysis:

For qRT-PCR analysis use one-step kits and same RNA volumes rather than equal concentrations in all reactions, to account for the different RNA content of each fraction.

For downstream RNA-Seq analysis supplement RNA from all fractions with fixed amount of spike-in-RNA (e.g. *E. coli* total RNA) for normalization of reads according to the different RNA content of each fraction.

For proteomics analysis supplement protein samples with fixed amount of spike-in protein (e.g. cytochrome C) or normalize obtained protein abundances according to protein content in each fraction (BCA measurement or Western blot analysis of endogenous marker proteins).