Figure S1

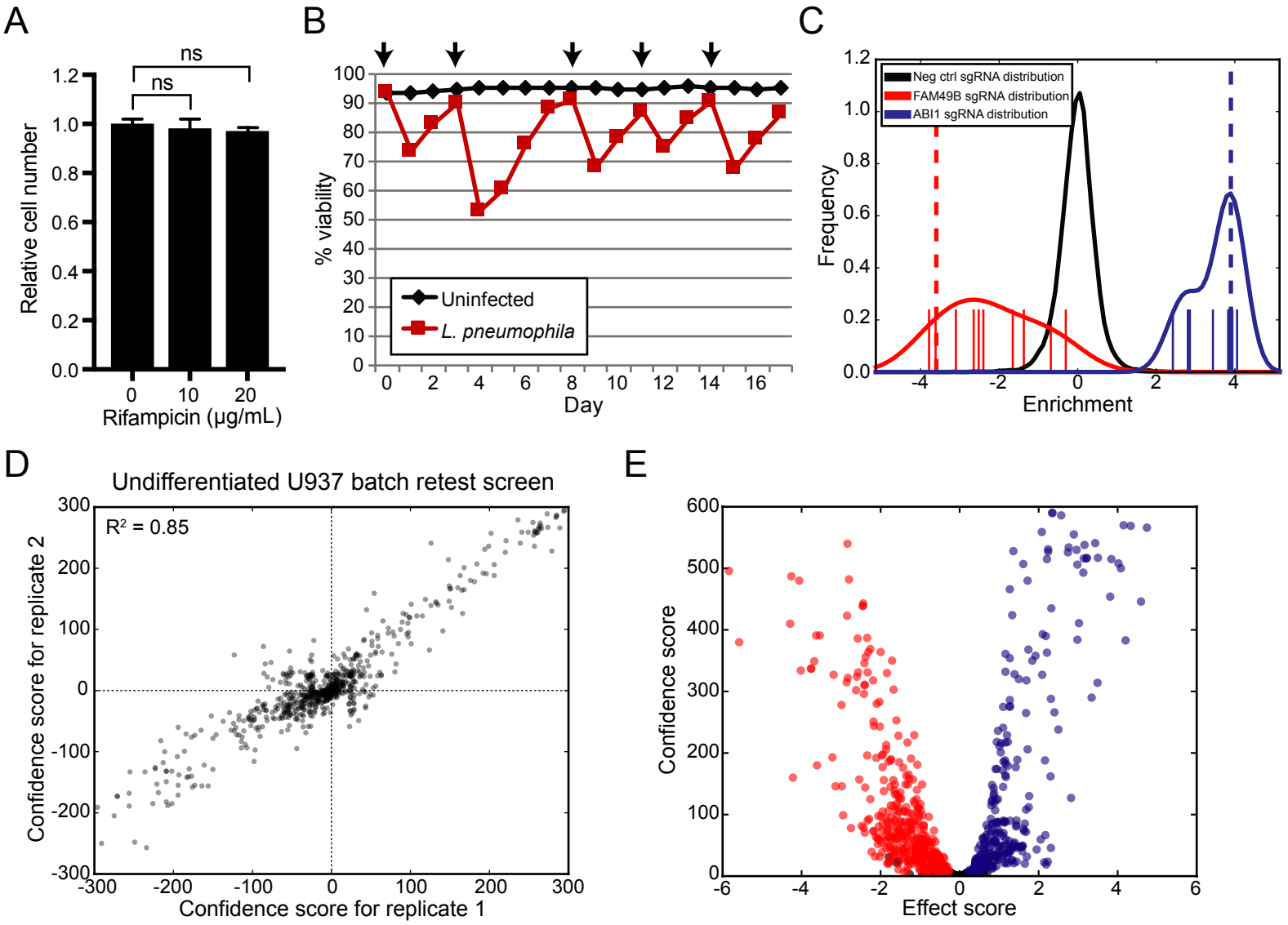

A
ns
ns
Relative cell number
Rifampicin (µg/mL)
B
% viability
Day
Uninfected
L. pneumophila
C
Neg ctrl sgRNA distribution
FAM49B sgRNA distribution
ABI1 sgRNA distribution
Frequency
Enrichment
D
Undifferentiated U937 batch retest screen
R² = 0.85
Confidence score for replicate 2
Confidence score for replicate 1
E
Confidence score
Effect score

Figure S1. Quality metrics of CRISPR screens in undifferentiated U937 cells, Related to Figure 1

(A) U937 cells were treated with rifampicin for 24 h and cell numbers were quantified by flow cytometry and compared between treated and untreated cells (*n* = 3 technical replicates, two-tailed Student's *t* test, ns = not significant; error bars, s.d.).

(B) Viability of U937 cells was measured daily during the screen by FSC/SSC on a flow cytometer. Arrows mark days of *L. pneumophila* infection. Red, cells infected by *L. pneumophila*; black, uninfected control population.

(C) Distribution of sgRNAs for top enriched (ABI1) and depleted (FAM49B) gene knockouts from genome-wide screen. The distribution of the 10 sgRNAs per gene (red and blue) is compared against the distribution of the negative control sgRNAs shown in black. Dotted lines represent effect scores for the indicated genes calculated by the casTLE analysis.

(D) Correlation of signed confidence scores between replicates in undifferentiated U937 batch retest screen.

(E) Volcano plot of confidence score vs. effect size for genes in the undifferentiated U937 batch retest screen. Blue dots indicate genes whose knockout protect host cells from *L. pneumophila* infection and red dots indicate genes whose knockout sensitize host cells to *L. pneumophila* infection where the 95% credible interval does not cross 0.

Figure S2

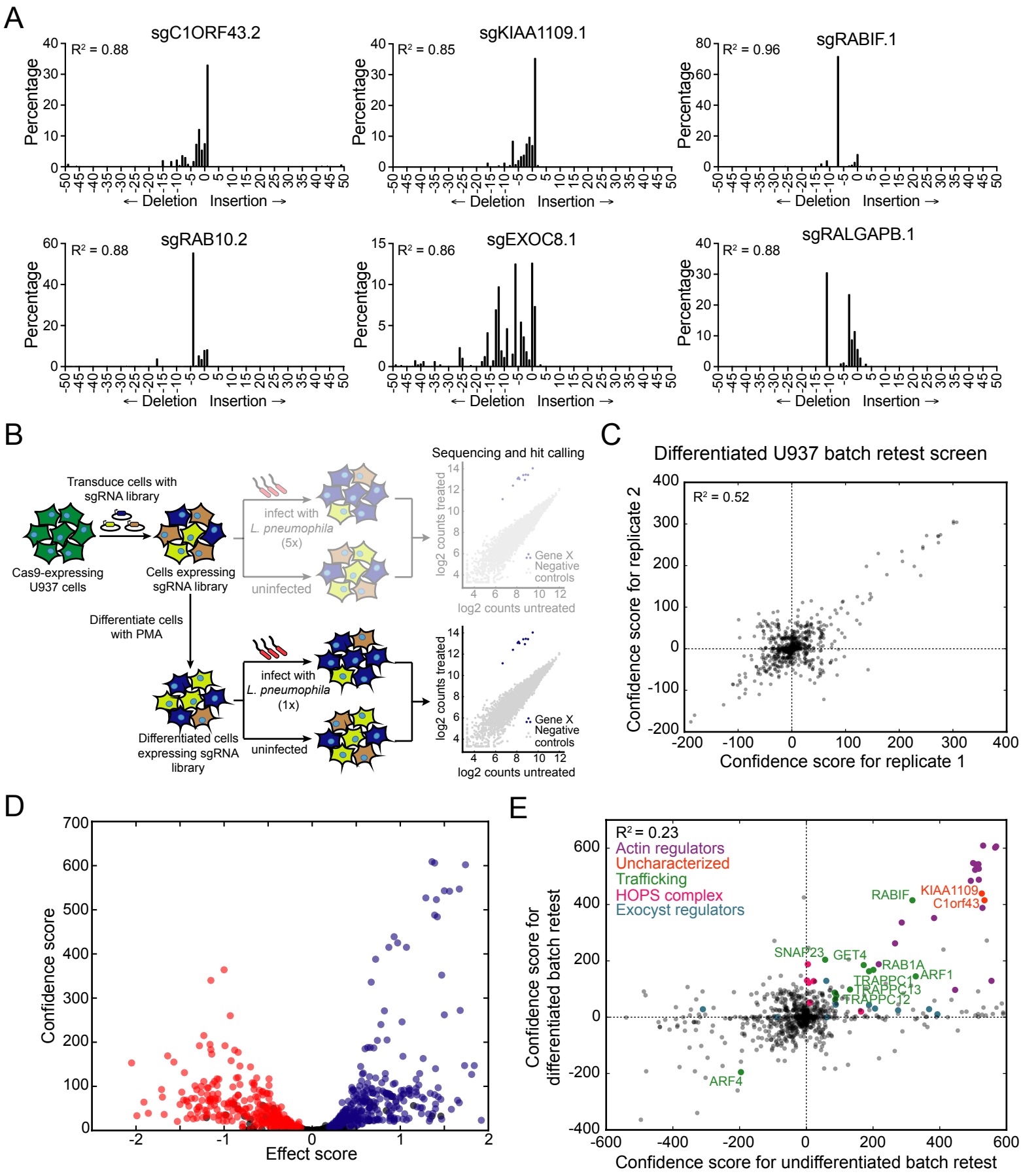

A
sgC1ORF43.2
sgKIAA1109.1
sgRABIF.1
sgRAB10.2
sgEXOC8.1
sgRALGAPB.1
R² = 0.88
R² = 0.85
R² = 0.96
R² = 0.86
Percentage
← Deletion Insertion →
B
Transduce cells with sgRNA library
Cas9-expressing U937 cells
Cells expressing sgRNA library
infect with L. pneumophila (5x)
uninfected
Differentiate cells with PMA
Differentiated cells expressing sgRNA library
infect with L. pneumophila (1x)
uninfected
Sequencing and hit calling
log2 counts treated
log2 counts untreated
Gene X
Negative controls
C
Differentiated U937 batch retest screen
R² = 0.52
Confidence score for replicate 2
Confidence score for replicate 1
D
Confidence score
Effect score
E
R² = 0.23
Actin regulators
Uncharacterized
Trafficking
HOPS complex
Exocyst regulators
RABIF
KIAA1109
C1orf43
SNAP23
GET4
RAB1A
ARF1
TRAPPC1
TRAPPC13
TRAPPC12
ARF4
Confidence score for differentiated batch retest
Confidence score for undifferentiated batch retest

Figure S2. Batch retest screen in differentiated U937 macrophage-like cells reveals conserved hits across cell states, Related to Figure 2

(A) TIDE indel analysis for selected sgRNAs.

(B) Schematic comparing batch retest screens performed in undifferentiated and differentiated U937 cells. The undifferentiated U937 screen was performed as in the genome-wide screen, while in the differentiated screen, U937 cells containing the sgRNA library were first differentiated into macrophage-like cells by PMA treatment before one round of *L. pneumophila* infection.

(C) Correlation of signed confidence scores between replicates in PMA-differentiated U937 batch retest screen.

(D) Volcano plot of confidence score vs. effect size for genes in PMA-differentiated U937 batch retest screen. Blue dots indicate genes whose knockout protect host cells from *L. pneumophila* infection and red dots indicate genes whose knockout sensitize host cells to *L. pneumophila* infection where the 95% credible interval does not cross 0.

(E) Correlation of signed confidence scores between batch retest screens in undifferentiated and differentiated U937 cells. Colored dots indicate genes belonging to various processes.

Figure S3

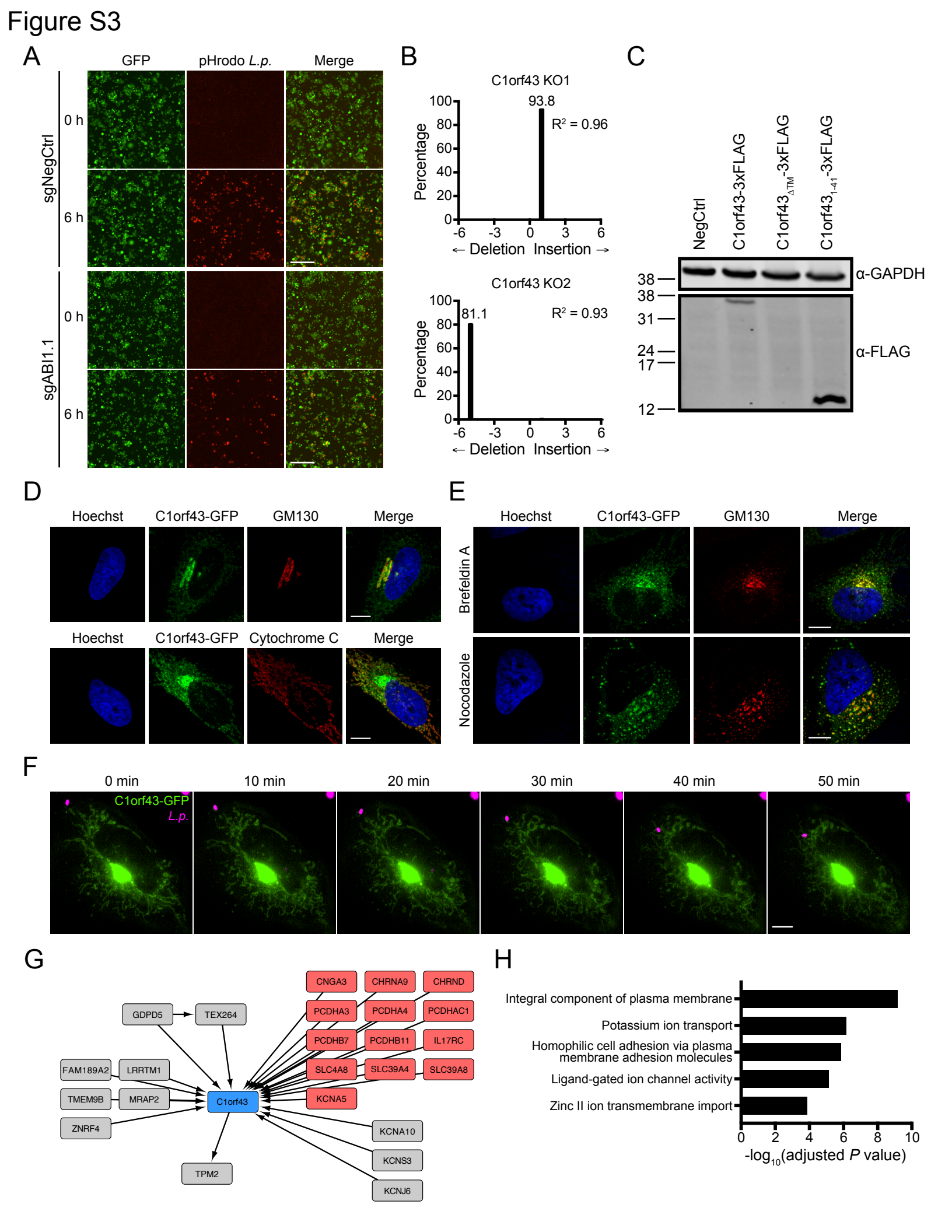

A
GFP
pHrodo L.p.
Merge
sgNegCtrl
sgABI1.1
0 h
6 h
B
C1orf43 KO1
93.8
R² = 0.96
Percentage
Deletion
Insertion
C1orf43 KO2
81.1
R² = 0.93
C
NegCtrl
C1orf43-3xFLAG
C1orf43ΔTM-3xFLAG
C1orf431-41-3xFLAG
α-GAPDH
α-FLAG
38
31
24
17
12
D
Hoechst
C1orf43-GFP
GM130
Merge
Cytochrome C
E
Brefeldin A
Nocodazole
F
0 min
10 min
20 min
30 min
40 min
50 min
C1orf43-GFP
L.p.
G
GDPD5
TEX264
FAM189A2
LRRTM1
TMEM9B
MRAP2
ZNRF4
C1orf43
TPM2
CNGA3
CHRNA9
CHRND
PCDHA3
PCDHA4
PCDHAC1
PCDHB7
PCDHB11
IL17RC
SLC4A8
SLC39A4
SLC39A8
KCNA5
KCNA10
KCNS3
KCNJ6
H
Integral component of plasma membrane
Potassium ion transport
Homophilic cell adhesion via plasma membrane adhesion molecules
Ligand-gated ion channel activity
Zinc II ion transmembrane import
-log10(adjusted P value)

Figure S3. C1orf43 primarily localizes to the Golgi and mitochondria and interacts with plasma membrane proteins, Related to Figure 3

(A) Examples of immunofluorescence microscopy images of differentiated U937 knockout cells (GFP+) infected with pHrodo Red-labeled Δ*dotA L. pneumophila* at MOI = 10 at 0 h and 6 h after infection. Scale bar, 200 μm.

(B) TIDE indel analysis for U937 C1orf43 knockout clones.

(C) Expression of C1orf43 variants was analyzed by immunoblot. U937 cells were lentivirally infected with constructs encoding the indicated C-terminal 3xFLAG-tagged C1ORF43 variants. Full-length C1orf43 and a truncated C1orf43 containing only the first 41 amino acids ($C1orf43_{1\text{-}41}$) were detected, but a C1orf43 isoform lacking the transmembrane domain ($C1orf43_{\Delta TM}$) was not.

(D) Immunofluorescence microscopy of HeLa FcγRII cells stably expressing C1orf43-GFP. Cells were stained with anti-GM130 (red, top) or anti-Cytochrome C (red, bottom) and Hoechst dye (blue). Scale bars, 10 μm.

(E) Localization of C1orf43-GFP in HeLa FcγRII cells upon treatment with Golgi-fragmenting drugs. Cells stably expressing C1orf43-GFP were treated with 10 ug/mL brefeldin A for 30 min or 5 μg/mL nocodazole for 2 h and stained with anti-GM130 (red) and Hoechst dye (blue). Scale bars, 10 μm.

(F) Live-cell fluorescence microscopy of HeLa FcγRII cells stably expressing C1orf43-GFP during infection with WT *L. pneumophila* (magenta). Images were taken every 1 min. Scale bar, 10 μm. Corresponding video can be found in Video S1.

(G) BioPlex protein-protein interaction network for C1orf43. Arrows point from the bait node to the prey node. Red nodes are annotated as integral components of the plasma membrane in GO.

(H) Adjusted *P* values using Fisher's Exact Test for select non-redundant enriched GO terms among C1orf43 BioPlex interactome.

Figure S4

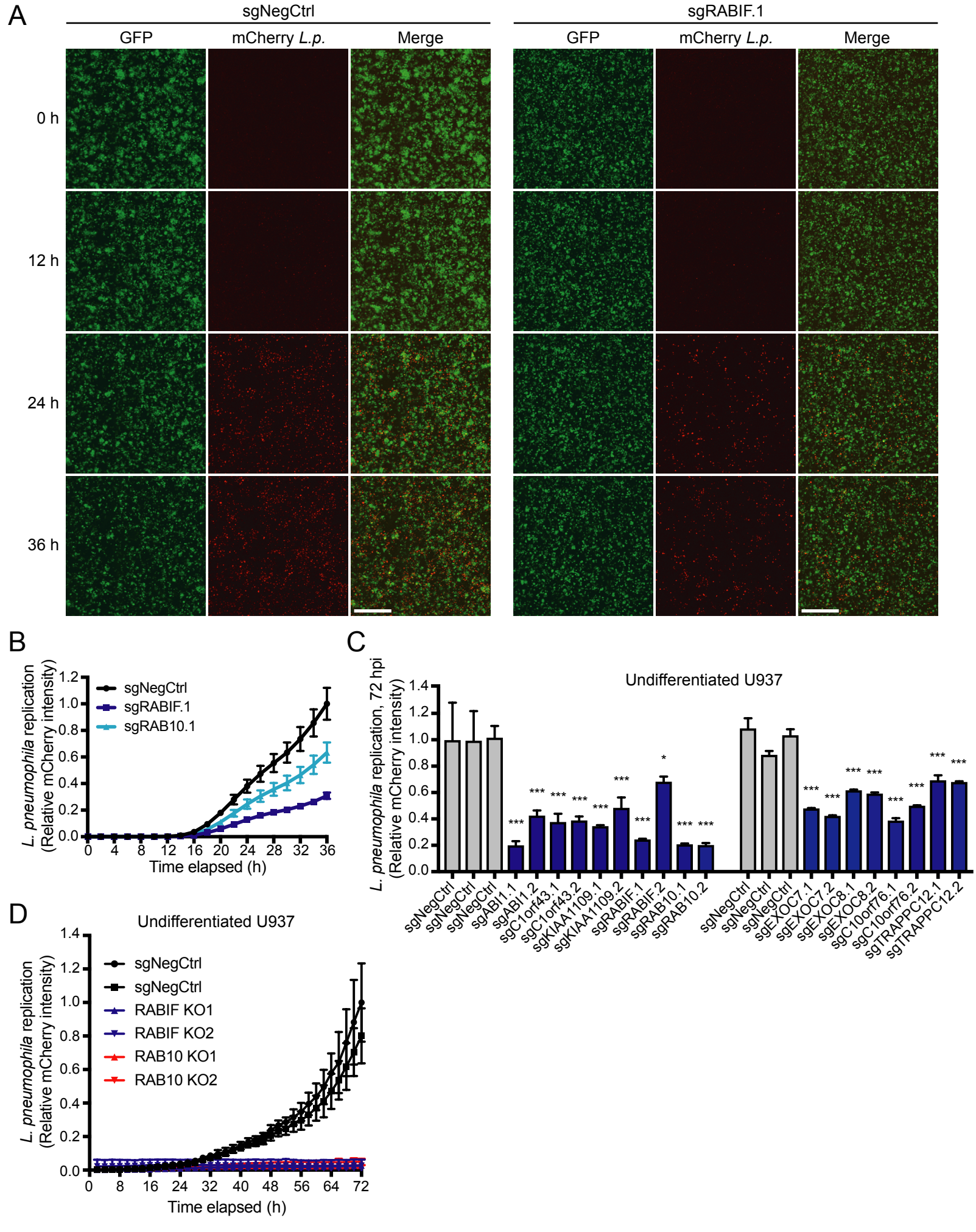

A
sgNegCtrl
sgRABIF.1
GFP
mCherry L.p.
Merge
0 h
12 h
24 h
36 h
B
L. pneumophila replication
(Relative mCherry intensity)
sgNegCtrl
sgRABIF.1
sgRAB10.1
Time elapsed (h)
C
Undifferentiated U937
L. pneumophila replication, 72 hpi
(Relative mCherry intensity)
sgNegCtrl
sgABI1.1
sgABI1.2
sgC1orf43.1
sgC1orf43.2
sgKIAA1109.1
sgKIAA1109.2
sgRABIF.1
sgRABIF.2
sgRAB10.1
sgRAB10.2
sgEXOC7.1
sgEXOC7.2
sgEXOC8.1
sgEXOC8.2
sgC10orf76.1
sgC10orf76.2
sgTRAPPC12.1
sgTRAPPC12.2
D
Undifferentiated U937
L. pneumophila replication
(Relative mCherry intensity)
sgNegCtrl
sgNegCtrl
RABIF KO1
RABIF KO2
RAB10 KO1
RAB10 KO2
Time elapsed (h)

Figure S4. Gene knockouts cause a defect in *L. pneumophila* intracellular replication, Related to Figure 4

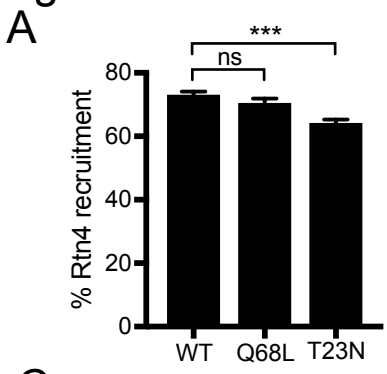
(A) Examples of immunofluorescence microscopy images of differentiated U937 knockout cells (GFP+) infected with mCherry-expressing *L. pneumophila* at MOI = 4 at 12 h intervals. Scale bars, 500 μm.

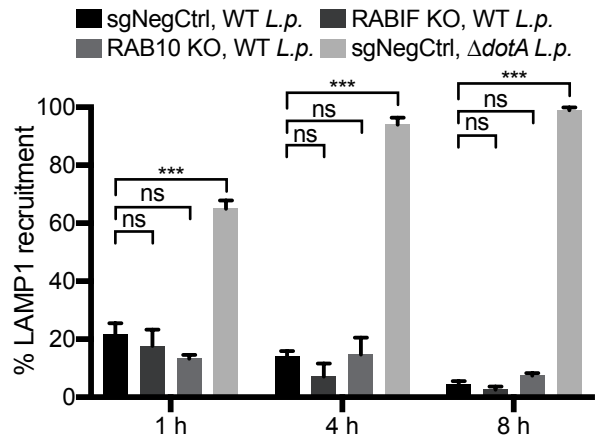
(B) Examples of quantification of *L. pneumophila* replication over time for select sgRNAs. Differentiated U937 knockout cells were infected with mCherry-expressing *L. pneumophila* at MOI = 1. Total mCherry fluorescence intensity per well was measured by Incucyte every 2 h for 36 h. Data represent mean ± s.d. for 3 technical replicates.

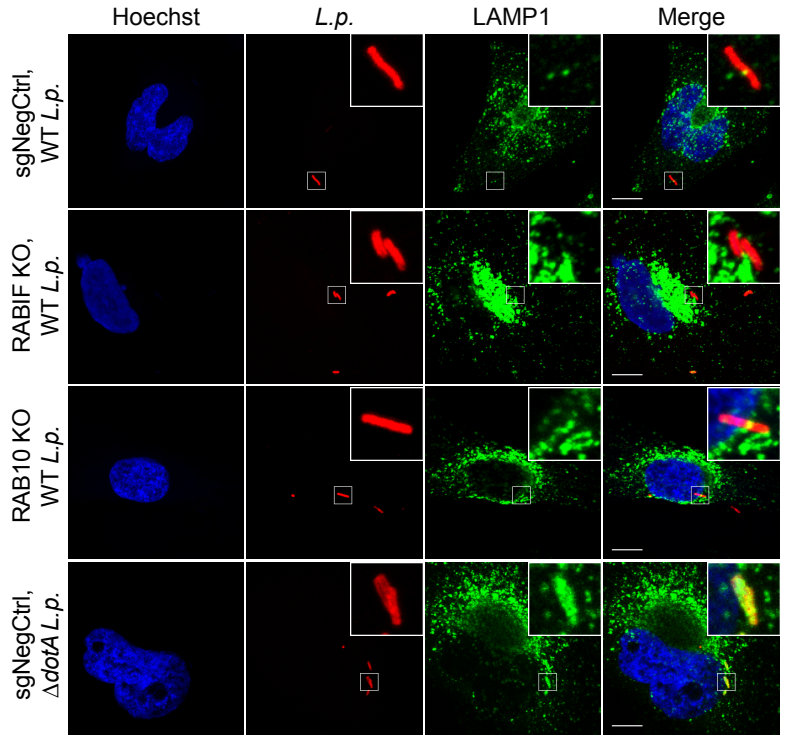
(C) *L. pneumophila* replication analysis in undifferentiated U937 cells. U937 knockout cells were infected with mCherry-expressing *L. pneumophila* at MOI = 4. Total mCherry fluorescence intensity per well was measured by Incucyte at 72 h as a combined metric of *L. pneumophila* uptake and intracellular replication. Knockout cells were compared to three negative controls with the most stringent significance value reported (n = 3 technical replicates, one-way ANOVA, Dunnett's multiple comparison test, *P<0.05, ***P<0.001; error bars, s.d.). Data shown are representative of 3 independent experiments.

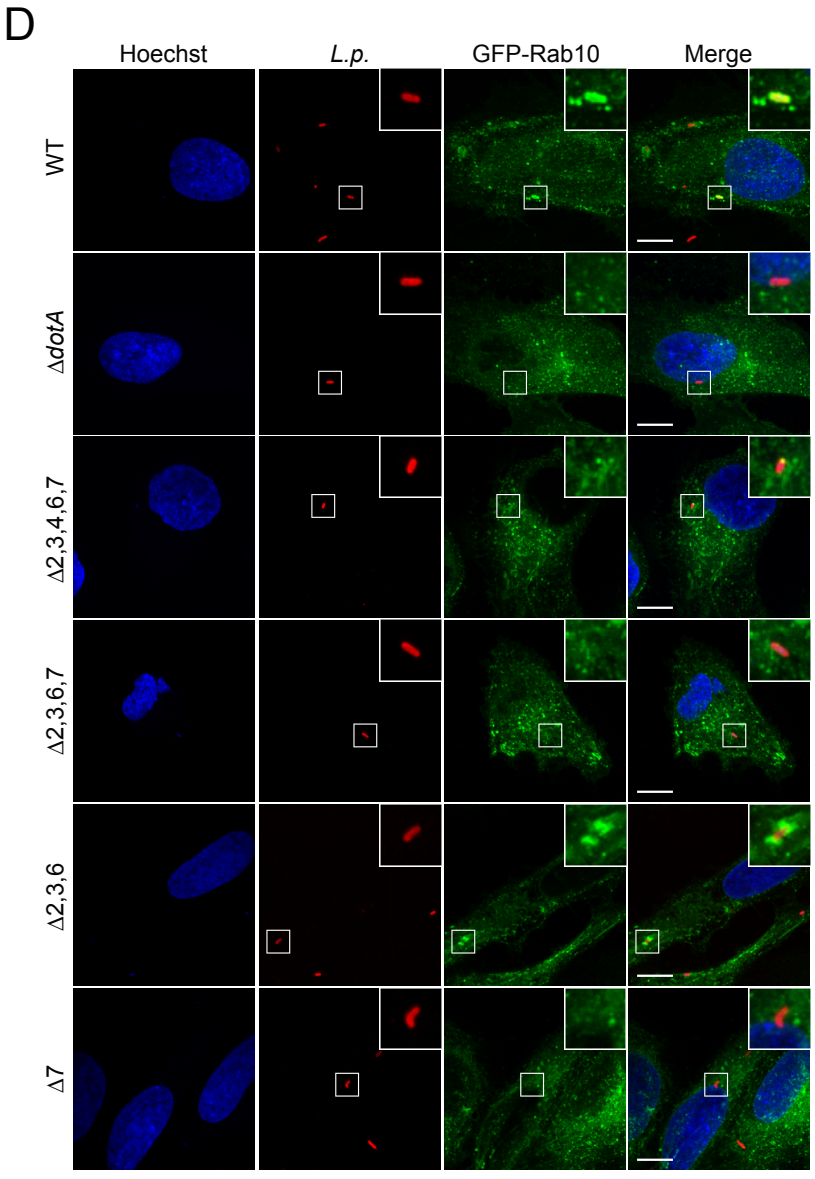
(D) *L. pneumophila* replication analysis in undifferentiated U937 *RABIF* and *RAB10* clonal knockout cells. Undifferentiated U937 clonal knockout cells were infected with mCherry-expressing *L. pneumophila* at MOI = 4. Total mCherry fluorescence intensity was measured by Incucyte every 2 h for 72 h and compared between knockout cells and negative control cells. Data represent mean ± s.d. of 4 technical replicates and are representative of 2 independent experiments.

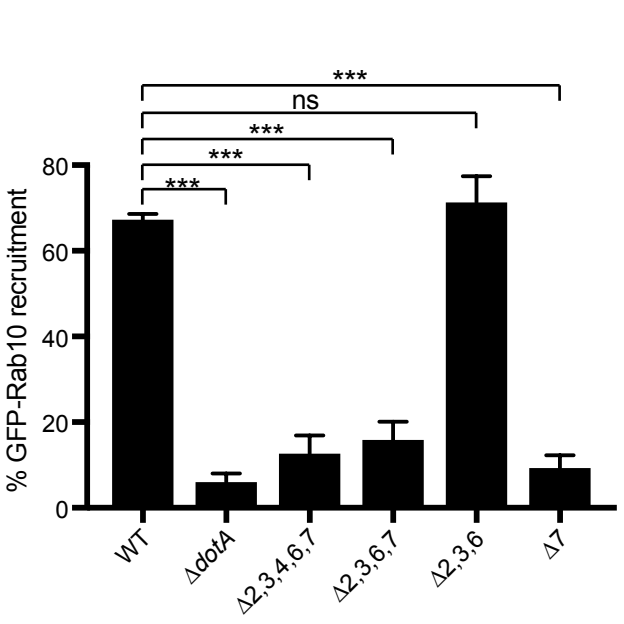
Figure S5











F

Genes in cluster 7

Gene ID	Gene name
lpg2508	*sdjA*
lpg2509	*sdeD*
lpg2510	*sdcA*
lpg2511	*sidC*
lpg2523	*lem26*
lpg2525	*mavK*
lpg2527	*lnaB*
lpg2552	*LecE*
lpg2556	*legK3*

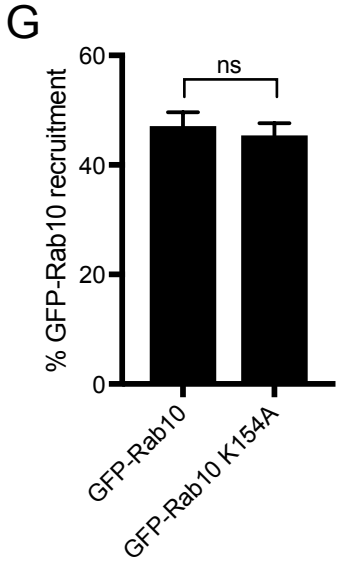


Figure S5. RAB10 regulates ER recruitment to the LCV and is itself recruited to the LCV, Related to Figures 4 and 5

(A) HeLa FcγRII cells stably overexpressing WT GFP-Rab10, GFP-Rab10 Q68L, or GFP-Rab10 T23N were infected with WT *L. pneumophila* at MOI = 1 for 4 h, fixed and immunostained for *L. pneumophila* and RTN4. The percentage of intracellular *L. pneumophila* co-localizing with RTN4 was quantified and Rab10 mutants were compared to WT Rab10 (*n* = 3 independent experiments with at least 100 LCVs analyzed per experiment, two-tailed Student's *t* test, ns = not significant, ***P<0.001; error bars, s.d.).

(B) HeLa FcγRII *RABIF* and *RAB10* clonal knockout cells or negative control cells were infected with WT or Δ*dotA L. pneumophila* at MOI = 3 for 1, 4 or 8 h, fixed, and immunostained for *L. pneumophila* (red) and LAMP1 (green). Nuclei were stained with Hoechst dye (blue). Scale bar, 10 μm.

(C) Quantification of the percentage of intracellular *L. pneumophila* co-localizing with LAMP1. Knockout cells were compared to negative control cells for infection with WT *L. pneumophila* (*n* = 3 technical replicates with at least 50 LCVs analyzed per replicate, two-tailed Student's *t* test with Holm-Sidak multiple comparisons correction, ns = not significant, ***P<0.001; error bars, s.d.).

(D) HeLa FcγRII cells stably expressing GFP-Rab10 were infected with the indicated *L. pneumophila* effector cluster deletion strains at MOI = 3 for 1 h, fixed, and immunostained for *L. pneumophila* (red). Nuclei were stained with Hoechst dye (blue). Scale bar, 10 μm.

(E) Quantification of the percentage of intracellular *L. pneumophila* co-localizing with GFP-Rab10 for the indicated *L. pneumophila* effector cluster deletion strains. Mutant *L. pneumophila* strains were compared to WT *L. pneumophila* (*n* = 3 technical replicates with at least 50 LCVs analyzed per replicate, two-tailed Student's *t* test, ns = not significant, ***P<0.001; error bars, s.d.).

(F) List of *L. pneumophila* genes in gene cluster 7.

(G) HeLa FcγRII cells stably expressing WT GFP-Rab10 or GFP-RAB10 K154A mutant were infected with WT *L. pneumophila* at MOI = 3 for 1 h, fixed, and immunostained for *L. pneumophila*. The percentage of intracellular *L. pneumophila* co-localizing with WT or mutant GFP-Rab10 was quantified and compared (*n = 3* technical replicates with at least 50 LCVs analyzed per replicate, two-tailed Student's *t* test, ns = not significant; error bars, s.d.).