Science **Supporting Online Material**

Drosophila RNAi Screen Reveals CD36 Family Member Required for Mycobacterial Infection

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Materials and Methods

Tissue Culture Conditions

S2 cells [Schneider's Line S2 cells/SL2; (*S1*)] were grown at 25°C in Schneider's media (SM; Gibco) with 10% heat inactivated (h.i.) fetal bovine serum (FBS; JRH Biosciences) except during RNAi treatment when FBS was omitted (*S2*). HEK293 cells were grown in Dulbecco's Modified Eagle Medium (DMEM; Gibco) with 10% h.i. FBS at 37°C in a humidified 5% CO_2 atmosphere. Cells were grown in either 384-well or 96-well optically clear plates (Costar).

Bacterial Strains and Growth Conditions

A clinical isolate of *M. fortuitum* (EJR154) was used. *M. smegmatis* is strain mc2-155. For all infection experiments, bacteria were grown at 37°C to log phase in Middlebrook 7H9 broth with 0.5% Tween, BBL Middlebrook ADC Enrichment, and 0.2% glycerol. 50 μg/ml kanamycin was added when selecting plasmids in EJR154. Prior to infections, bacteria were washed twice in PBS and resuspended in SM for S2 infections or RPMI complete for BMM infections. In order to obtain a single cell suspension, bacteria were centrifuged at 1500 rpm for 5' (Eppendorf centrifuge 5804) and supernatants harvested. OD_{600} was used to estimate bacterial number (1 $OD_{600} = 5 \times 10^8$/ml). These estimates were similar to bacterial number determined by CFUs.

Bone Marrow Macrophages

BMMs were isolated from C57/BL6 mice according to the method of Celada *et al.* (*S3*), except that the growth media contained FBS rather than horse serum. Six days after harvest, 7.5×10^4 cells were plated per well in 96 well plates in RPMI with 0.2 mM L-glutamine, 1 mM Na pyruvate, and 0.1% (v/v) β-mercaptoethanol (RMPI complete) with 10% FBS. On day 7, cells were incubated with RPMI complete with either cytochalasin B (20 μM) or solvent control (ethanol) for 10' before the addition of 1.8×10^5 EJR154 containing *map24::GFP* or *map49::GFP*. Macrophages were co-cultured with bacteria for 4h, then washed extensively in PBS, and incubated for an additional 24h in the presence of 25 μM amikacin. Cells were fixed and stained with TRITC-phalloidin (50 μg/ml; Sigma) or 5 μg/ml Hoechst 33342 (Sigma) and images were acquired using the AutoScope described in (*S2*).

RNAi Screen

Details regarding the RNAi library can be found in (*S4*). It has previously identified genes involved in viability (*S4*), cytokinesis (*S2*), *Drosophila* C virus infection (*S5*), and Wnt signaling (*S6*). RNAi treatment was performed as described (*S2*), except that 2 x10^4 S2 cells were plated in each well and antibiotics were omitted from the media. Three days after RNAi treatment, cells were infected with 1.25 x 10^5 bacteria (EJR154 containing *map24::gfp*) prepared as described above. To inhibit growth of extracellular bacteria, 2h post-infection, amikacin was added (final concentration 25 µM). Two days later, cells were fixed with 4% formaldehyde in PBS and stained with 5 µg/ml Hoechst 33342. Two 20X images from each well were acquired using the AutoScope. For each dsRNA, the number of *Drosophila* nuclei and bacterial clusters were determined in order to calculate the percent infection. This allowed us to distinguish those dsRNAs that specifically disrupt infection from those that reduce the overall GFP signal because of their effect on *Drosophila* cell number. In order to quantitate percent infection, the number of bacterial clusters and *Drosophila* nuclei were determined using MetaMorph software (Universal Imaging). We confirmed that automated quantitation closely paralleled manual quantitation and visual scoring. We found that those dsRNAs that decreased the number of *Drosophila* cells also caused more variable infection. In determining which dsRNAs were hits, we classified the wells based upon the number of *Drosophila* nuclei into three groups (<600, 600-1000, and >1000) and determined the S.D. for each group. Since the S.D. was larger in the category with the fewest *Drosophila* cells, effectively we required a greater fold effect for those dsRNAs, and therefore, attempted to select dsRNAs that had profound affects on the infection, as opposed to more general effects on the host cells. Those dsRNAs that decreased infection by 2 S.D. in the primary screen were reamplified and retested. With the exception of dsRNAs targeting Actin, we did not amplify and retest dsRNAs that had predicted overlaps of > 21 base pairs with more than five mRNAs in addition to their intended target. Table S3 provides details regarding amplicons used in this study, and additional information is available at flyrnai.org.

Phagocytosis Assays

1 x 10^5 S2 cells per well were treated with dsRNAs in 96 well plates for three days. After RNAi treatment, cells were pre-incubated in 50 µl PBS containing 20 µg/ml Hoechst 33342 for 10', followed by the addition of FITC-*E. coli* (50 µg/well; Vybrant Phagocytosis Assay kit, Molecular Probes) or FITC-*S.aureus* (25 µg/well; Molecular Probes) in 100 µl PBS. Bacteria were spun onto cells at 1000 rpm for 3'. 45' later, media containing bacteria was aspirated, followed by addition of 250 µg/ml Trypan blue (EM Science) in 1X SSC (pH4.4) for 1'. Immediately after aspirating Trypan blue, Hoechst and FITC signals were detected in an Analyst GT plate reader. Percent uptake was calculated as described in fig. S3. To generate a standard curve, cells that had not been treated with RNAi were pre-incubated in 50 µl PBS containing 20 µg/ml Hoechst 33342 for 10' in the presence of 16 µM cytochalasin B or solvent (ethanol) control, prior to adding bacteria as described above.

Antibiotic Protection Experiments

1.1 x10^5 S2 cells were treated with 750 ng dsRNA in 96 well plates. *M. smegmatis* was prepared for infection as described above. Three days after RNAi treatment, 6 x 10^5 *M. smegmatis* were added to each well. Plates were spun for 3' at 1000 rpm. After 45', media containing 250 μg/ml amikacin was added for 1h to kill extracellular bacteria. S2 cells were washed twice, lysed in PBS with 0.1% TritonX-100, and the released bacteria were plated on LB. CFU were calculated after three days of growth at 37°C.

Expression Constructs

cDNA encoding CG7228 was obtained from the Berkeley *Drosophila* Genome Project (clone ID RE21078). The PmlI-NheI fragment containing CG7228 was moved into the EcoRV-XbaI sites of pPacPL (*S7*). From pPacPL, the BamHI-NotI fragment containing CG7228 was inserted into the BamHI-NotI sites of pECFP-N1(Clontech). In this cloning step, the ECFP fusion protein is removed from the vector, leaving a full-length, untagged CG7228 under control of the CMV promoter. Human SR-BI and SR-BII expression constructs were provided by James S. Owen (University College London). The murine CD36 expression construct was provided by Kathryn J. Moore (Massachusetts General Hospital). A murine LIMP II construct, provided by K. Akasaki (Fukuyama University), did not detectably alter bacterial uptake (data not shown).

HEK293 Experiments

HEK293 cells (1 x 10^4) were grown overnight in 96 well plates followed by transfection using Effectene (Qiagen) with 150 ng vector (pcDNA3.1) or plasmids encoding CG7228, CD36, SR-BI, or SR-BII. Two days later, cells were infected with *M. fortuitum map24::GFP* for 3h, washed once with PBS, and then incubated for an additional day in the presence of amikacin (50 μg/ml). Cells were fixed and stained as described in the RNAi screen. For uptake of FITC-*E.coli* and FITC-*S.aureus*, two days after transfection, cells were incubated for 2 h in 150 μl PBS containing 25 μg FITC-*E.coli* or 25 μg FITC-*S.aureus* with 6.7 μg/ml Hoechst 33342, followed by quenching with Trypan blue as described for the S2 cell phagocytosis assay. Images were immediately acquired on the Autoscope.

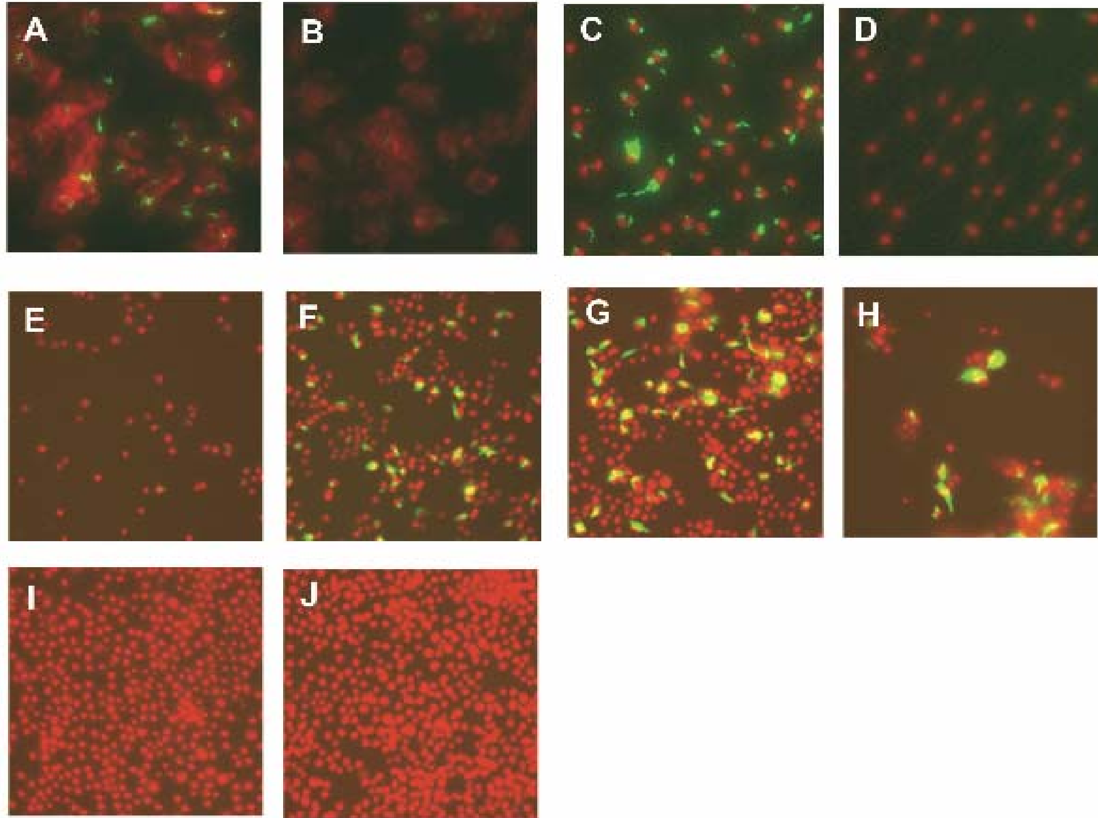

Fig. S1. *map24* and *map49* are induced during intracellular growth by *M. fortuitum*. Bone marrow macrophages (BMM) were infected with *M. fortuitum* containing *map24::GFP* (**A** and **B**) or *map49::GFP* (**C** and **D**). 24 h later, GFP expression (in green) was detected

(**A and C**) unless BMM were preincubated with cytochalasin B (**B and D**), demonstrating that repborter expression depends upon intracellular growth of the bacteria. In A and B, phalloidin staining is in red, whereas Hoechst 33342 staining is shown in red in C and D. *Drosophila* S2 cells were infected with the same bacterial strain at a MOI of 20 (**E-H**). After 6h (**E**), little GFP expression was detected. By 24 h (**F**), GFP is apparent (in green). The bacterial clusters increase in size at 48 h (**G**), and by 72 h (**H**), the bacteria disrupted the monolayer of fly cells. The monolayer remained intact if infected by the non-pathogenic species *M. smegmatis* (**I**) or uninfected (**J**). S2 nuclei were visualized with Hoechst 33342 and are represented in red (**E-J**). Images are 20X. Adjustments to contrast and brightness were applied equally to panels A and B, panels C and D, and panels E through J.

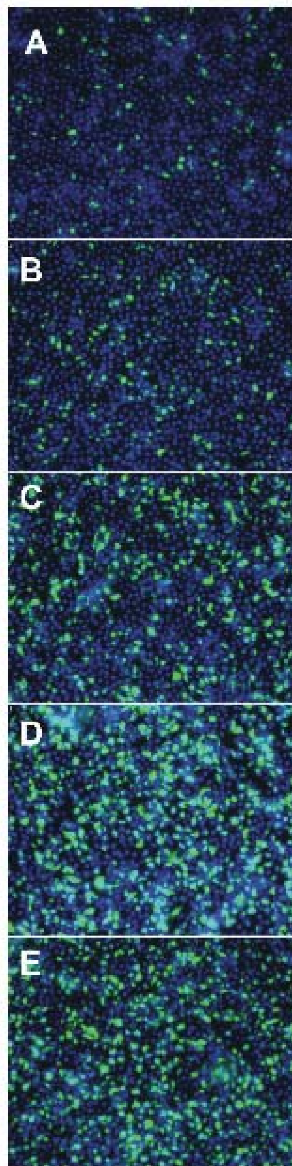

Fig. S2. Infection of *Drosophila* S2 cells increased with rising multiplicity of infection (MOI). S2 cells (5 x 10^4) were plated in 384-well plates and infected the following day with *M. fortuitum* containing *map24::GFP* (**A**-**D**) at an MOI of 1 (**A**), 2 (**B**), 5 (**C**), or 10

(**D**). At an MOI of 5, 47% of the *Drosophila* cells were infected (based upon >1000 *Drosophila* cells counted manually). Results were indistinguishable with *M. fortuitum* containing *map49::GFP*, which is shown at an MOI of 10 in E.

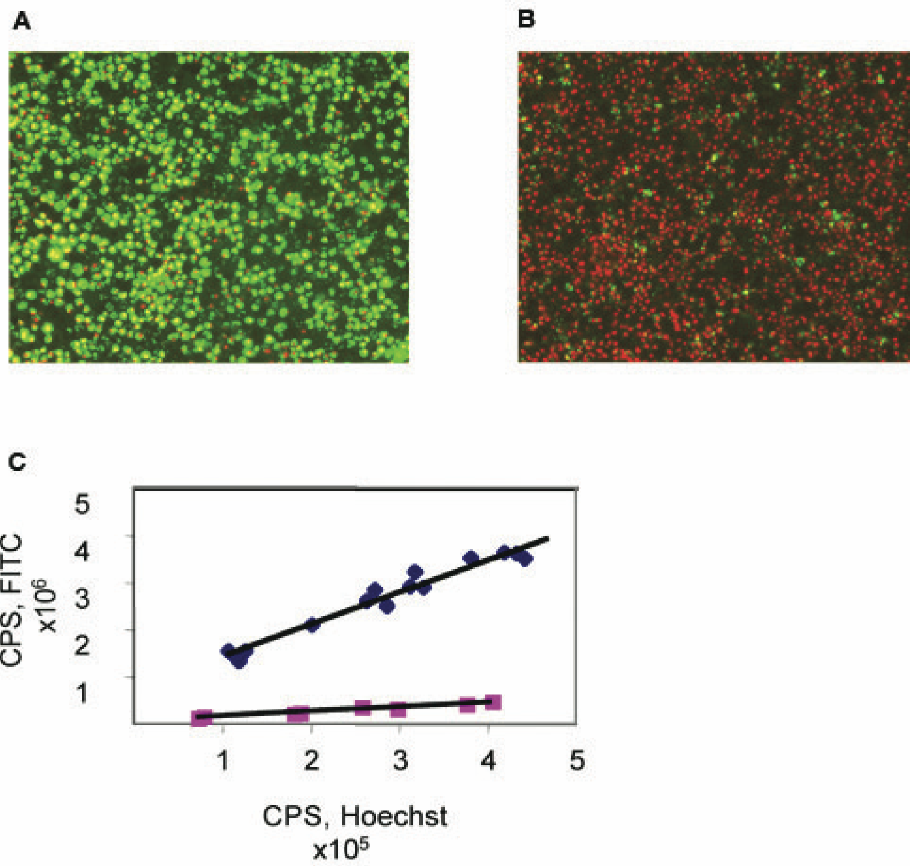

Fig. S3. FITC-conjugated *E.coli* were used to measure phagocytosis. FITC-*E.coli* were incubated with S2 cells in the presence of Hoechst 33342 for 45', followed by the addition of Trypan blue to quench extracellular fluorescence. **A**, in the absence of cytochalasin B, cells internalize bacteria. Internalization is blocked by the addition of

cytochalasin B (**B**), demonstrating the efficacy of quenching by Trypan blue. The fluorescent intensity of FITC (a measure of bacterial uptake) and Hoechst (a measure of *Drosophila* cell number) was determined using an Analyst GT plate reader. In order to normalize data based upon the number of S2 cells in each well, which might vary after three days of RNAi treatment, standard curves were generated for each experiment. The linear relationship between counts per second (CPS) of Hoechst signal and FITC signal is shown (**C**), both in the presence (pink) or absence (blue) of cytochalasin. In the presence of cytochalasin, residual signal can result from incomplete quenching of bacteria that are bound, but not internalized in S2 cells. The Hoechst values from each well were compared to the standard curves, in order to calculate the predicted FITC signal, in the presence (FITCcyto) or absence (FITCpred) of cytochalasin B. The % uptake was determined by comparing the experimentally observed FITC (FITCobs) signal to the predicted values from the standard curve, using the following formula: % uptake = (FITCobs-FITCcyto)/(FITCpred-FITCcyto) x 100.

Table S1. Cellular factors required for *M. fortuitum* infection in S2 cells.[1]

CG	Name	% Infection[2]	Z- score	Description[3]
Cytoskeleton Organization and Biogenesis				
CG12530	Cdc42	6.0 +/- 0.3	9.8	Ras small GTPase, Rho type
CG30294	Act57B[4]	8.3 +/- 0.8	8.8	Actin
CG4027	Act5C[4]	9.9 +/- 2.0	8.2	Actin
CG12051	Act42A[4]	16.1 +/- 2.4	5.6	Actin
CG8978	Sop2	8.6 +/- 1.8	8.7	Arp 2/3 protein complex
CG7558	Arp66B	13.1 +/- 0.5	6.8	Arp 2/3 protein complex
CG9901	Arp14D	13.1 +/- 2.1	6.8	Arp 2/3 protein complex
CG5972	Arc-p20	13.7 +/- 1.8	6.6	Arp 2/3 protein complex
CG9881	p16-ARC	15.0 +/- 2.2	6.0	Arp 2/3 protein complex
CG10954	Arc-p34	15.6 +/- 2.3	5.8	Arp 2/3 protein complex
CG10540		12.8 +/- 1.2	7.0	F-actin capping protein complex
CG17158	Cpb	16.6 +/- 1.6	5.4	F-actin capping protein complex
CG14782		14.5 +/- 1.1	6.3	Guanyl-nucleotide exchange factor activity
CG4254	Tsr	17.7 +/- 3.3	4.9	Actin binding, cofilin type
CG9749	Abi	22.1 +/- 2.1	3.1	SH3 domain
CG9553	Chic	24.7 +/- 3.8	2.0	Profilin domain
CG4931	Sra-1	#[5]	*3.0*	Rho Interactor Activity
Vesicle Trafficking				
CG6625	Snap	2.9 +/- 1.2	11.1	NSF attachment protein
CG11027	Arf102F	7.9 +/- 1.0	9.0	ADP-ribosylation factor
CG8385	Arf79F	18.4 +/- 1.7	4.6	ADP-ribosylation factor
CG12210	Syb	14.9 +/- 1.3	6.1	SNAP receptor activity
CG31136	Syx1A	18.8 +/- 2.8	4.5	SNAP receptor activity
CG1515	l(1)G0155	23.5 +/- 1.8	2.5	SNAP receptor activity
CG5081	Syx7	#[5]	*3.1*	SNAP receptor activity
CG3269	Rab2	19.1 +/- 4.0	4.3	Ras small GTPase, Rab type
CG3664	Rab5	4.2 +/- 0.8	10.6	Ras small GTPase, Rab type
CG5915	Rab7	14.0 +/- 0.9	6.5	Ras small GTPase, Rab type
CG8287	Rab8	18.3 +/- 3.4	4.7	Ras small GTPase, Rab type
CG5771	Rab11	5.5 +/- 1.4	10.0	Ras small GTPase, Rab type

CG40304	Rab21	17.2 +/- 2.0	5.1	Ras small GTPase, Rab type
CG7961	AlphaCop	5.4 +/- 1.2	10.1	COPI vesicle coat
CG6223	BetaCop	16.6 +/- 2.6	5.4	COPI vesicle coat
CG14813	DeltaCOP	22.3 +/- 3.0	3.0	COPI vesicle coat
CG1528	GammaCop	14.7 +/- 2.5	6.2	COPI vesicle coat
CG3948	ZetaCOP	17.7 +/- 2.2	4.9	COPI vesicle coat
CG1250	Sec23	18.2 +/- 1.5	4.7	COPII vesicle coat
CG7073	Sar1	21.2 +/- 1.0	3.5	COPII vesicle coat
CG8055		7.7 +/- 1.2	9.1	Snf7
CG8843	Sec5	19.3 +/- 3.2	4.3	Exocyst
CG5341	Sec6	22.0 +/- 1.3	3.1	Exocyst
CG3885		21.3 +/- 2.9	3.4	Exocyst
CG15811	Rop	21.9 +/- 2.9	3.2	SNAP receptor binding
CG4755	RhoGAP92B	17.4 +/- 1.2	5.0	Rho GTPase activating protein
CG9375	Ras85D	19.1 +/- 2.0	4.3	Ras small GTPase, Ras type
<u>Lipid Metabolism</u>				
CG3523	Fatty Acid Synthase	2.1 +/- 0.7	11.4	Fatty acid biosynthesis
CG11198		11.8 +/- 0.9	7.4	Fatty acid biosynthesis
CG8522	HLH106	9.7 +/- 0.9	8.2	Fatty acid biosynthesis
CG10367	Hmgcr	10.1 +/- 2.0	8.1	Hydroxymethylglutaryl-CoA reductase activity
CG7923	Fad2	#[5]	*2.5*	Stearoyl-CoA 9-desaturase activity
<u>Proteolysis and Peptidolysis</u>				
CG12284	Th	0.7 +/- 0.4	12	Ubiquitin-protein ligase activity
CG11624	Ubi-p63E	1.1 +/- 0.7	11.8	Ubiquitin
CG11700		3.3 +/- 2.1	10.9	Ubiquitin
CG17331		13.3 +/- 1.7	6.8	Proteasome core complex
CG10107		21.3 +/- 1.4	3.4	Peptidase C48, SUMO/Sentrin/Ubl1
CG2960	RpL40	22.8 +/- 2.2	2.8	Ribosomal protein L40e
CG10682	Vihar	24.7 +/- 1.4	2.0	Ubiquitin conjugating enzyme activity
CG6233	Ufd1-like	#[5]	*2.8*	Ubiquitin fusion degradation protein, UFD1
<u>Proton Transport</u>				
CG1088	Vha26	18.0 +/- 1.9	4.8	Hydrogen-transporting ATPase V1 domain
CG17369	Vha55	22.9 +/- 2.2	2.8	Hydrogen-transporting ATPase V1 domain
CG3762	Vha68-2	23.6 +/- 2.4	2.5	Hydrogen-transporting ATPase V1 domain
<u>Establishment or Maintenance of Chromatin Structure</u>				
CG6121		12.2 +/- 1.2	7.2	Histone acetyltransferase activity

CG7776	E(Pc)	16.3 +/- 1.9	5.5	
CG9696	Dom	16.7 +/- 2.3	5.4	DEAD/DEAH box helicase, N-terminal
CG10712	Chro	20.3 +/- 1.3	3.9	Chromo domain
CG7752	Z4	20.6 +/- 2.6	3.7	DNA binding
Signal Transduction				
CG8556	Rac2	11.8 +/- 0.9	7.4	Ras small GTPase, Rho type
CG15507	Kay	21.3 +/- 2.8	3.4	Fos transforming protein
CG8770	Gbeta76C	#[5]	*3.1*	Heterotrimeric G-protein complex
Other				
CG7228		2.7 +/- 0.6	11.2	CD36 antigen
CG8610	Cdc27	10.6 +/- 1.4	7.9	Cell cycle
CG6058	Ald	19.5 +/- 2.3	4.2	Fructose bisphosphate aldolase activity
CG8743		24.1 +/- 4.4	2.3	Calcium ion transport
CG7769	DDB1	#[5]	*4.0*	Damaged DNA binding
CG9467		21.9 +/- 1.6	3.2	Potassium ion transport
Function Unknown				
CG11990		17.6 +/- 1.6	5.0	
CG15415		20.1 +/- 1.5	3.9	
CG12770	Vps28	20.3 +/- 2.3	3.8	
CG3911		20.1 +/- 1.7	3.9	
CG14542		21.8 +/- 3.9	3.2	
CG13038		#[5]	*3.3*	
CG14657		#[5]	*2.4*	

[1 2] Percent infection was determined as described in Methods and represents the average of two sites from three independent wells for each dsRNA. Cells were treated with dsRNAs for 3 d except where noted. For 384 untreated wells, the average infection was 29.5 +/- 2.4, which was used to calculate z-scores.

[3] Genes were categorized based upon GeneOntology index (GO) biological function and additional information is annotated based upon GO molecular function, cellular component, or protein domains as reported in FlyBase (flybase.org). The vesicle trafficking category includes genes annotated in GO as vesicle trafficking or intracellular protein transport.

[4] dsRNAs targeting all six alleles of actin were identified in the primary screen. Each of these dsRNAs were predicted to have significant overlap with all of the actin alleles, so only three were resynthesized for repeated testing.

#[5] Several dsRNAs did not have z-scores of ≥ 2 after 3 d of RNAi treatment, but did after 4 d and 5 d of RNAi. For those, the z-score is in italics and is not directly comparable to the 3d scores.

[6] DRSC numbers correspond to amplicons targeting putative ORFs that are not annotated in FlyBase (see flyrnai.org).

Table S2. Cellular factors required for phagocytosis of *E.coli* in S2 cells.

CG	Name	%Uptake[1]	Description[2]
GFP[3]		86 +/- 13	
Cytoskeleton Organization and Biogenesis			
CG12530	Cdc42	38 +/- 14	Ras small GTPase, Rho type
CG30294	Act57B	43 +/- 9	Actin
CG4027	Act5C	42 +/- 17	Actin
CG8978	Sop2	59 +/- 11	Arp 2/3 protein complex
CG7558	Arp66B	50 +/- 11	Arp 2/3 protein complex
CG9901	Arp14D	50 +/- 18	Arp 2/3 protein complex
CG5972	Arc-p20	64 +/- 14	Arp 2/3 protein complex
CG9881	p16-ARC	57 +/- 15	Arp 2/3 protein complex
CG10954	Arc-p34	53 +/- 14	Arp 2/3 protein complex
CG10540		49 +/- 5	F-actin capping protein complex
CG17158	Cpb	50 +/- 12	F-actin capping protein complex
CG4254	Tsr	51 +/- 11	Actin binding, cofilin type
CG9749	Abi	42 +/- 10	SH3 domain
CG4931	Sra-1	44 +/- 12	Rho Interactor Activity
Vesicle Trafficking			
CG6625	Snap	0 +/- 7	NSF attachment protein
CG11027	Arf102F	2 +/- 8	ADP-ribosylation factor
CG8385	Arf79F	39 +/- 11	ADP-ribosylation factor
CG12210	Syb	53 +/- 8	SNAP receptor activity
CG31136	Syx1A	45 +/- 11	SNAP receptor activity
CG5081	Syx7	60 +/- 12	SNAP receptor activity
CG3664	Rab5	63 +/- 17	Ras small GTPase, Rab type
CG5915	Rab7	46 +/- 12	Ras small GTPase, Rab type
CG8287	Rab8	24 +/- 9	Ras small GTPase, Rab type
CG5771	Rab11	5 +/- 9	Ras small GTPase, Rab type
CG40304	Rab21	45 +/- 24	Ras small GTPase, Rab type
CG7961	AlphaCop	7 +/- 7	COPI vesicle coat
CG6223	BetaCop	15 +/- 6	COPI vesicle coat
CG1481	DeltaCOP	25 +/- 6	COPI vesicle coat
CG1528	GammaCop	9 +/- 7	COPI vesicle coat
CG3948	ZetaCOP	12 +/- 6	COPI vesicle coat
CG1250	Sec23	40 +/- 13	COPII vesicle coat
CG7073	Sar1	59 +/- 13	COPII vesicle coat

CG8055		55 +/- 8	Snf7
CG8843	Sec5	70 +/- 12	Exocyst
CG5341	Sec6	62 +/- 10	Exocyst
CG15811	Rop	37 +/- 6	SNAP receptor binding
CG4755	RhoGAP92B	46 +/- 14	Rho GTPase activating protein
<u>Lipid Metabolism</u>			
CG3523	Fatty Acid Synthase	8 +/- 9	Fatty acid biosynthesis
CG11198		38 +/- 9	Fatty acid biosynthesis
CG8522	HLH106	10 +/- 7	Fatty acid biosynthesis
CG10367	Hmgcr	24 +/- 13	Hydroxymethylglutaryl-CoA reductase activity
CG7923	Fad2	47 +/- 14	Stearoyl-CoA 9-desaturase activity
<u>Proteolysis and Peptidolysis</u>			
CG12284	Th	12 +/- 10	Ubiquitin-protein ligase activity
CG11624	Ubi-p63E	15 +/- 6	Ubiquitin
CG11700		21+/- 11	Ubiquitin
CG17331		39 +/- 9	Proteasome core complex
CG10682	Vihar	48 +/- 10	Ubiquitin conjugating enzyme activity
<u>Signal Transduction</u>			
CG8556	Rac2	68 +/- 15	Ras small GTPase, Rho type
CG8770	Gbeta76C	71 +/- 10	Heterotrimeric G-protein complex
<u>Other</u>			
CG8610	Cdc27	32 +/- 8	Cell cycle
<u>Unknown</u>			
CG11990		59 +/- 15	
CG15415		45 +/- 11	
CG3911		55 +/- 15	
CG14657		65 +/- 16	

[1]Uptake of Fluorescein (FITC)-conjugated *E.coli* was determined by using Trypan blue to quench fluorescence from uninternalized bacteria. In order to normalize data for variations in *Drosophila* cell number, the percent uptake was determined by comparing the FITC signal to a standard curve of untreated well (see fig. S3). Data are the average of three independent experiments performed in triplicate (p value < .01 based upon Student's *t*-test).

[2] Genes were categorized based upon GO annotation as described in table S1.

[3] dsRNA targeting GFP was used as a negative control.

Table S3. dsRNA amplicons used in this study. Gene name, FBgn#, DRSC amplicon number, primers used to amplify the dsRNAs, expected sizes of the dsRNAs, as well as the number of potential off-targets and the maximum length of the off-target region are listed. The final column indicates whether the amplicon was previously identified as affecting cell viability in Boutros *et al*., 2004. Additional amplicon information can be obtained at flyrnai.org.

Predicted Targeted Gene	FBGN	DRSC Amplicon ID	'S' Primer	'R' Primer	Amp. Length	Number of Potential Secondary Targets	Max Secondary Target Overlap	Viability Hit in Boutros, *et al.*, 2004 (*S4*)
Rab5	FBgn0014010	DRSC00777	TCCTGGCCAGCCGTGT	GGCAACCACTCCACGCA	264	1	23	NO
CG3523	FBgn0027571	DRSC00268	GCCTGTCTTGGTGTAAGTG	AGCGTCGTCCTGGCAC	503	0	0	NO
Sra-1	FBgn0038320	DRSC15679	CATTGAGGGCTCCTTCGT	TGTCGGTGCGAGAGACA	515	0	0	NO
sec5	FBgn0031537	DRSC00714	GGCAGATCCAGGCTGATG	TCCCATTGGCGATAAACTG	504	0	0	NO
cpb	FBgn0011570	DRSC00809	TCCATCTCCTCGACCATCT	GAGCACGGCAAGGACTAT	503	0	0	NO
Sop2	FBgn0001961	DRSC03438	GCAGTGGCACACAGCTAT	GCGTCAAGTGGTCGCC	516	0	0	NO
aret	FBgn0000114	DRSC03485	CTTTCCCGCTGGTACTCC	TCACCATCGCCTGGACTA	474	1	22	NO
Arc-p34	FBgn0032859	DRSC02113	AATGTGACATAGCCAATGTTGT	CCAAGCCTGATCGTGTCA	208	0	0	NO
Act57B	FBgn0000044	DRSC04042	AGGATTCCATTCCCAGGAAG	CCGTCCTGCTGACTGAG	510	5	62	NO
Rab2	FBgn0014009	DRSC05017	GACACCTTCCTGGATCTTCT	CTATCACACGCTCTTATTACC	317	0	0	NO
CG10540	FBgn0034577	DRSC04080	ACCTCCTTGGCCACCTG	GTCGCTTCTACGACCCG	494	0	0	NO
dom	FBgn0020306	DRSC04558	TTCCGTCTTGCATCGTTTT	ATAAATCTAGAGACGTTAAACCT	511	0	0	NO
CG8055	FBgn0033385	DRSC07061	TGGTTGTCGCCTTTTTCTTT	GCACGACATGATGGATGAC	248	0	0	YES
CG11198	FBgn0033246	DRSC07249	CTGCCTCTACGGCTTCC	CACCAGCCGGCTTATGA	514	0	0	NO
Vps28	FBgn0021814	DRSC06168	AGGAACTGGCGGACCTG	GCCGATCTATACGCAATCA	497	0	0	NO
Syb	FBgn0003660	DRSC07559	GAGCAGCACAACGGCTAT	ACAATGCAGCCCAGAAGAA	248	0	0	NO
sec6	FBgn0034367	DRSC06932	ACGATGGTGATGATGTAGTGA	GGCTTGGAGGGCAATGAA	488	0	0	NO
alphaCop	FBgn0025725	DRSC08706	AGGAAGCTAAGCTTGTCAAA	GGACGAGTCTGGAGTGTTT	513	0	0	NO
Ufd1-like	FBgn0036136	DRSC10607	GCCGCCAACAGTTCCAG	CCCTCGGCGCTGGAC	510	0	0	NO
CG10107	FBgn0035713	DRSC09698	GGATTAAAGGTTGCTTTACGG	GGTGCAAAAGTGGACCAAG	500	0	0	NO
Cdc27	FBgn0012058	DRSC11112	CCATCGGCCGATTGTTTC	GATGATGGGCAAAAAGCTAAA	519	1	21	YES
Rop	FBgn0004574	DRSC08693	CCCATGGCCAGATCCTGT	GAAGCTGGACGCCTACAA	498	0	0	NO
Arp66B	FBgn0011744	DRSC09669	GATCCGGAGACGTGTGTC	CGAGGGCTATGTGATCGG	505	0	0	NO
Syx7	FBgn0033583	DRSC11763	TTGAGCTCCGGAGAATCTT	GGACTTACAGCATATGGAGAAT	247	0	0	NO
Gbeta76C	FBgn0004623	DRSC11174	TCCATGTCGTGACCGAAG	CGCCAACAAGGTGCAGAT	486	0	0	NO
Rab8	FBgn0015796	DRSC11261	CGGTCAATTCGCACTTGTT	CACTGCCGGCCAGGAG	191	0	0	NO
CG3885	FBgn0036718	DRSC10384	GACTAATAACGTCCATCTGAAAA	CCACGGTCAGTCGGTTTA	579	0	0	NO
Snap	FBgn0011712	DRSC11285	TGATGAGCTTGAACTCCCT	CCAAGTCTATTCAGCACTATG	318	0	0	NO
Arf79F	FBgn0010348	DRSC11606	TAGCGATTAGCGTTCTTCA	CTGCCAAATGCAATGAACG	161	0	0	NO
Chro	FBgn0044324	DRSC11630	CAACTCTTCCCGGGTCAC	GTCCCGTGCGGTATCTTT	500	0	0	NO

th	FBgn0003691	DRSC11404	GCCACCGTATCGATATAGAG	CCAACGACTCGACGCT	502	0	0	YES
Z4	FBgn0037066	DRSC11848	TTTGCTTCCCGGTTTGGG	CAAGTGCGGCACTTGTTC	508	0	0	NO
Abi	FBgn0020510	DRSC14099	TGCTGCTGCGATCGTC	ATCGACTACTCGATGTTGG	517	0	0	NO
Vha26	FBgn0015324	DRSC12371	GGCCAGCAGCTCAACAC	GCGCGAGGACCATGTG	298	1	21	NO
sec23	FBgn0037357	DRSC12387	GGAATGGGGGCTGCATG	ACGACGAGCTGAAGCAC	508	0	0	NO
CG9467	FBgn0037758	DRSC16498	CTCCCGACTTTGTGCCATA	CGCCACTATCGAAAGAATC	515	0	0	NO
Vha55	FBgn0005671	DRSC16896	ATGGGGATCTGGGTGATGG	GCCCGTGGCCAGAAGAT	512	0	0	NO
DDB1	FBgn0027049	DRSC16639	AGAGGCACTATTGCGAAGT	AATAATTCCCCGCTCCATTC	514	0	0	NO
sar1	FBgn0038947	DRSC17049	GGTTGTTAGCTGATACAGTCC	GACGCGTCTGGAAGGAC	227	0	0	NO
Rab7	FBgn0015795	DRSC16810	CTCCAACTCTAACGCATTCT	GACACTGCTGGTCAGGAAC	345	0	0	NO
CG14782	FBgn0025381	DRSC18568	GTCGCCGGCTGTTGTG	AGTACAACAAGCAGCATATCA	509	0	0	NO
deltaCOP	FBgn0028969	DRSC18760	CCGCCTTGGATTTGGTGT	TCCCGAGTACAGCCACT	407	0	0	NO
CG14542	FBgn0039402	DRSC14886	CACAGCCTGTGCTTTCTG	AGATTATCGCGGATATCAAGA	545	0	0	NO
Ald	FBgn0000064	DRSC14109	AACGGCGGCGGGAAC	AGATCCTGAAGAAGAAGGGA	506	1	22	NO
kay	FBgn0001297	DRSC16977	GCCGCCGGCTTGAGA	CGCGAATACCTCAAATACG	495	0	0	NO
CG6121	FBgn0026080	DRSC18661	GAACTCTGTCATTACGTAGAAG	CCTACAACTCCGCAGACA	513	0	0	NO
l(1)G0155	FBgn0026664	DRSC17970	GCTCTGCAGCGACAATTT	CGAGGCGCGTCTCCT	591	0	0	NO
betaCop	FBgn0008635	DRSC20312	ACTCTGGGTGGCATAGGTT	GGACACTGGCAAGTACAGG	502	0	0	NO
p16-ARC	FBgn0031437	DRSC00730	GACAGCACGCGGACAAT	GCCAAAAACACGTCCAGC	496	0	0	NO
chic	FBgn0000308	DRSC03507	TGTGTTGTCTTCATGCAGTG	CAAAAGAGGAGCTCTCCAAA	166	0	0	NO
Arc-p20	FBgn0031781	DRSC02917	GAACCGTTTGAGGAACTCC	ACATTGAAGCCCTACCTGAC	495	0	0	NO
CG7228	FBgn0031969	DRSC03033	ACTTTCCTCCGACGCAATA	GGCGTTAACATTGCCAATAAA	508	0	0	NO
Vha68-2	FBgn0020367	DRSC02721	GGTCACCTCCGGTGATGT	CCCTGAAGGACATTAACGAG	138	0	0	NO
HmgZ	FBgn0010228	DRSC04620	CCTCGGAGGACTCTTTCTT	GGAGCAGAAGGCCATCAA	152	0	0	NO
tsr	FBgn0011726	DRSC04718	CATTTCTGGATATCTTCTAGAAAC	TGGTGTAACTGTGTCTGATGT	168	0	0	NO
Act42A	FBgn0000043	DRSC04835	GCCTCCATGCCGAGGAA	CTTACTGAGGCTCCTTTGAA	500	5	35	NO
iota;Try	FBgn0015001	DRSC07634	TGGCCACCAAAGGACCTC	CCCTGGCAGGTGTCTATTC	520	0	0	NO
Rac2	FBgn0014011	DRSC11262	GGACATAGCACGGATCGTA	CGGAGCGGTGGGAAAG	504	2	32	NO
vihar	FBgn0027936	DRSC09778	CGTAGAAGGCGTCTAGATAT	TCAGCCCCGAGCAAAGT	570	1	21	YES
CG3911	FBgn0035992	DRSC10388	GCTGAGTATGGCACTGTAG	CAAGCAGCTGGAGCGAA	271	0	0	NO
Fad2	FBgn0029172	DRSC11165	CACACGGAAAAATATGAGGAA	GGGCTCATCGCACATTCA	512	2	23	NO
CG13038	FBgn0040795	DRSC09919	GCTACCCGGCACGTAGA	CTCTGGTTTTGGCCAAACC	245	0	0	NO
zetaCOP	FBgn0040512	DRSC11412	CCGTCGCAGATCTCGTC	GCATCCTGGCCAAGTACTA	322	0	0	NO
HLH106	FBgn0015234	DRSC11182	GCGGTGTCAGTAGATTGTG	AGTTCCTGGATATGGCTATTG	493	0	0	YES
Ras85D	FBgn0003205	DRSC16814	TCTTGCCTGCTCGTTGTT	ACCTGCCTGCTGGACATC	246	0	0	NO
Rab11	FBgn0015790	DRSC16809	TGTGAGTATGTTCTGGAATGC	AAAACAATTAAAGCGCAAATCT	339	2	23	NO
Hmgcr	FBgn0001205	DRSC16704	GGTAGTCAAAGTGTTCATAAGG	TCATCGCCTTGGTAGTTAAAT	519	0	0	NO
Syx1A	FBgn0013343	DRSC15359	TCTCGCCCTGCGACTC	ACTCGGCCATCCTGTCC	513	0	0	NO

gammaCop	FBgn0028968	DRSC16955	GTCCATCTGGCAACGACC	TCGGTAGAGCGTCTGATG	492	0	0	NO
Arf102F	FBgn0013749	DRSC17195	GTTCTCTTTCAGCTTCAGTTAT	GGCTTGGATGCTGCTGG	250	0	0	NO
CG11700	FBgn0029856	DRSC17794	ACCAAGTGAAGGGTCGATT	CCTGCGTCTGCGGGG	456	3	61	YES
Act5C	FBgn0000042	DRSC17723	CCGCAAGCCTCCATTCC	GAGGCCCCGCTGAACC	500	5	77	NO
Arp14D	FBgn0011742	DRSC19332	CGTATCGTTCTTGAGTACCC	GTGGCTACGCTTTCAATCA	404	0	0	NO
Cdc42	FBgn0010341	DRSC20228	CCTCGTCGAATACATTTTTCA	ACGGAGCCGTGGGTAAG	544	1	21	NO
CG8743	FBgn0036904	DRSC11032	CCAGTCGGCCGTCAAAG	TCCTACATTTTCGATCCACATA	493	0	0	NO
CG11990	FBgn0037657	DRSC14462	GCTCCCGCTATTACACCC	CCATCAAGGCAAAGCGTC	496	0	0	NO
RpL40	FBgn0003941	DRSC00782	CGCAGGTTGTTGGTGTGT	GCCCTCGCTCAGGATTC	129	0	0	YES
CG17331	FBgn0032596	DRSC02603	GCGGCCAGGCTAGCA	ATCCCAACGCGGGTCC	280	0	0	YES
CG14657	FBgn0037282	DRSC12219	TGTTCTATCGTTGATAGCCAC	GCTTTGGCTTGCGGACC	130	0	0	NO
RhoGAP92B	FBgn0038747	DRSC15637	GACTTCGCCGATGAAAAAG	GGGTCTGCTGCGAGTG	508	0	0	NO
Rab21	FBgn0039966	DRSC20968	ATCCGTATCCGGATTCTGG	TGGTGCTGCGCTACATG	506	0	0	NO
E(Pc)	FBgn0000581	DRSC05281	AGTGGGCAGGACTAGTGC	ACTGCGTTGTCGTCGTAA	500	0	0	NO
CG11624	FBgn0003943	DRSC08703	GGGGGATTCCCTCCTTATCT	AGCAGCTTGAGGATGGAC	426	3	44	NO
CG15415	FBgn0031549	DRSC00430	GGAGCTTCCAACTCCTTTTT	CGCTTTGAGTCCCTAAAATC	506	0	0	YES
		DRSC01346	GTAAACGGGCTCGAGGTTA	GAAGCAGCGACCAGGAC	210	4	25	NO
		DRSC17648	CTAGCAGCAATCCACTTGAA	CGACCTGCTGCTGATGTT	172	0	0	NO

References and Notes

S1. Description of the S2 cell line is available on line at flyrnai.org.
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