

***Drosophila* RNAi Screen Reveals CD36 Family Member Required for Mycobacterial Infection**

Jennifer A. Philips,^{1*} Eric J. Rubin,³ Norbert Perrimon^{1,2*}

¹Department of Genetics, ²Howard Hughes Medical Institute, Harvard Medical School, 77 Louis Pasteur Avenue, Boston, MA 02115, USA. ³Department of Immunology and Infectious Disease, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA.

*To whom correspondence should be addressed. E-mail: jphilips@partners.org (J.A.P.) or perrimon@receptor.med.harvard.edu (N.P.).

Certain pathogens, such as *Mycobacterium tuberculosis*, survive within the hostile intracellular environment of a macrophage. To identify host factors required for mycobacterial entry and survival within macrophages, we performed a genomewide RNAi screen in *Drosophila* macrophage-like cells, using *Mycobacterium fortuitum*. We identified factors required for general phagocytosis, as well as those needed specifically for mycobacterial infection. One specific factor, Peste (Pes), is a CD36 family member required for uptake of mycobacteria, but not *E. coli* or *S. aureus*. Moreover, mammalian class B scavenger receptors (SRs) conferred uptake of bacteria to nonphagocytic cells, with SR-BI and SR-BII uniquely mediating uptake of *M. fortuitum*, which suggests a conserved role for class B SRs in pattern recognition and innate immunity.

About one-third of the world's population is infected by *M. tuberculosis*, and it is responsible for more deaths yearly than any other bacterial pathogen. In addition, other pathogenic mycobacteria, including *M. fortuitum*, are capable of causing infection in humans (1). While macrophages play a central role in host defense, recognizing and destroying pathogens, pathogenic mycobacteria are able to survive within this hostile environment. Mycobacteria can escape phagosomal-lysosomal fusion (2) and grow in a variety of evolutionarily divergent phagocytic cells, including mammalian macrophages, fish monocytes (3), fly hemocytes (4), and amoeba (5). Thus, mycobacteria appear to target evolutionarily conserved molecules for intracellular survival and growth. Although several factors involved in phagosomal maturation arrest have been studied (6), there has been no systematic, genetic approach for identifying host factors required for mycobacterial survival. Here we describe a model of infection using *Drosophila* S2 cells, a macrophage-like cell line (7–9) that is readily amenable to RNA interference (RNAi). This allowed us to conduct a systematic functional genomic screen to identify host factors required for uptake and growth of mycobacteria.

M. fortuitum has several properties making it a useful model mycobacterium. Like *M. tuberculosis*, it restricts interferon-gamma-induced nitric oxide production and limits phagosomal fusion with lysosomes (10), suggesting common virulence properties with other mycobacteria. In addition, *M. fortuitum* infects *Diptera* in nature (11) so flies may have innate mechanisms to combat infection. Practically, *M. fortuitum* grows relatively rapidly at 25°C, the temperature at which S2 cells grow, thus facilitating development of a robust assay of intracellular growth. To detect intracellular growth, we tested constructs in which green fluorescent protein (GFP) expression is under control of the *map24* and *map49* promoters that are induced when the fish pathogen *Mycobacterium marinum* infects macrophages (12). We found that these promoters could also be used to efficiently detect intracellular growth of *M. fortuitum* (fig. S1). By 24 h post-infection of S2 cells, expression of *map24* and *map49* was induced (figs. S1 and S2; and Fig. 1A).

In mammalian cells, recruitment of the Arp2/3 complex is required for phagocytosis (13), while expression of a dominant negative version of Rab5 causes internalized *Mycobacterium avium* to be delivered to the lysosome (14). Thus, we reasoned that dsRNAs targeting Arp14D and Rab5 would decrease infection in *Drosophila* cells. Indeed, we found that these dsRNAs blocked intracellular growth of *M. fortuitum* (Fig. 1A). Using this assay, we performed a genomewide RNAi screen in which each unique dsRNA was tested for its ability to disrupt infection based upon visual inspection and automated image analysis (15) (Fig. 1B). We found 6/7 Arp2/3 components, 5/7 COPI components, and all six alleles of actin, suggesting a false negative rate of less than 20%. Upon repeat testing of the candidates identified in the primary screen, 86 dsRNAs decreased infection by 2 S.D. ($P < 0.05$; tables S1 and S3). These dsRNAs target genes involved in basic cellular processes, the largest categories of which are predicted to have a role in vesicle trafficking and actin cytoskeletal organization (Fig. 1C).

Because GFP expression requires internalization of bacteria, we expected to find dsRNAs that block general phagocytosis. Indeed, we found that 54 dsRNAs caused a significant decrease in phagocytosis as measured by the uptake of fluorescent *Escherichia coli* ($P < 0.01$; table S2 and fig. S3). Approximately two-thirds of these dsRNAs target factors involved in the actin cytoskeleton and vesicle trafficking (Fig. 1C). Nearly all of the actin cytoskeletal components that affected *M. fortuitum* infection appeared to be generally required for phagocytosis, including Cdc42, the Arp2/3 complex, actin capping proteins, and cofilin, all molecules previously implicated in phagocytosis (13). The majority of vesicle trafficking genes also affected phagocytosis, with exceptions such as CG1515 (predicted to have SNAP receptor activity) and Rab2.

However, the requirement for many genes for infection could not be explained by their role in phagocytosis. For example, dsRNA that targets *chickadee* (*chic*), which encodes a Profilin, caused a small increase in phagocytosis (118% of GFP treated well; $P < 0.05$), similar to what has been reported in hemocytes heterozygous for *chic* (*chic*^{01320/+}; 9). Hence, Chic is unlikely to act at the level of uptake and may play a unique role in mycobacterial infection. Some dsRNAs, such as those that target Rab5 and Rac2, caused a mild defect in phagocytosis relative to their severe defect in the *M. fortuitum* infection, indicating that they may have additional roles later in infection. Finally, there were categories of host factors that were not required for bacterial uptake in general but were nonetheless needed for infection with *M. fortuitum*. These include components of the vacuolar ATPase and chromatin factors, along with many that do not fit into distinct categories (Fig. 2C).

We further characterized one host factor that appeared to be specifically required for mycobacterial uptake. The dsRNA that targeted CG7228, a member of the CD36 family of scavenger receptors (SR), blocked infection by *M. fortuitum* (Fig. 2A), but appeared to be dispensable for phagocytosis in general. dSR-CI, a class C scavenger receptor, makes a small contribution toward the uptake of *E. coli* and *Staphylococcus aureus* in S2 cells (8), but we could not detect a similar contribution from CG7228 (Fig. 2B). However, CG7228 was required for uptake of *M. smegmatis*, as were more general phagocytosis factors (Fig. 2C). In addition, CG7228 is required for uptake of *L. monocytogenes* (16). The fact that CG7228 is required for infection with *M. fortuitum* and for uptake of *M. smegmatis* and *L. monocytogenes*, whereas it makes no contribution toward the uptake of *E. coli* or *S. aureus*, suggests that it functions in pattern recognition, detecting some component of *Mycobacteria* and *Listeria*. Based upon its role in bacterial infection, we have named it *peste* (*pes*).

To determine whether *Pes* was sufficient to confer uptake of mycobacteria, we expressed it in human embryonic kidney 293 (HEK293) cells that are refractory to infection with *M. fortuitum*. HEK293 cells transfected with *Pes* could be infected with *M. fortuitum* (Fig. 3A), showing that the *Drosophila* SR can mediate uptake of mycobacteria in human cells. *Pes* also caused a small increase in the uptake of *E. coli* and *S. aureus* when transfected in HEK293 cells (Fig. 3B and 3C), although it did not seem to be required for their uptake in S2 cells. This apparent discrepancy may be explained by genetic redundancy provided by PGRP-Lc (7), dSR-CI (8), and potentially other SRs that mediate uptake of *E. coli* and *S. aureus* in S2 cells.

While in *Drosophila* there are more than ten class B SRs, in humans and mice there are four-- CD36, SR-BI, its splice isoform SR-BII, and LIMP-II. We tested the ability of these molecules to mediate infection with *M. fortuitum* and found that human SR-BI and SR-BII (17) resulted in an even greater level of infection than *Pes* when transfected in HEK293 cells. In contrast, expression of murine CD36 caused little, if any, increase in infection with *M. fortuitum* (Fig. 3A). However, cells transfected with CD36 were capable of taking up *E. coli*, and to a lesser extent *S. aureus*. SR-BI and SR-BII seemed more indiscriminate in terms of their bacterial recognition, as they were able to mediate uptake of all bacteria tested (Fig. 3B and 3C). Whereas SR-BI and SR-BII have been extensively studied for their role in cholesterol transport in liver and steroidogenic tissue, their role in macrophages is less clear (18). We suggest that these receptors may be important in host defense against a broad range of bacteria, including *M. fortuitum*, *E. coli*, and *S. aureus*, and could play a role in the non-opsonic uptake of *M. tuberculosis*. Consistent with this idea, fucoidin, a non-specific inhibitor that blocks both class A SRs and SR-BI (19), interferes with binding and uptake of *M. tuberculosis* in monocyte-derived macrophages (20). CD36 may play a partially overlapping role recognizing bacteria, but is unable to mediate uptake of *M. fortuitum*. As CD36 augments TLR2 signaling in response to a subset of TLR2 ligands (21), we speculate that CD36, SR-BI, and SR-BII serve partially overlapping roles in both bacterial uptake and TLR signaling, which may represent an evolutionarily conserved strategy in host defense, linking phagocytosis to antimicrobial signaling.

References and Notes

1. D. E. Griffith, R. J. Wallace, Jr., *Semin. Respir. Infect.* **11**, 301 (1996).
2. D. G. Russell, *Nat. Rev. Mol. Cell. Biol.* **2**, 569 (2001).
3. S. H. El-Etr, L. Yan, J. D. Cirillo, *Infect. Immun.* **69**, 7310 (2001).
4. M. S. Dionne, N. Ghori, D. S. Schneider, *Infect. Immun.* **71**, 3540 (2003).

5. J. D. Cirillo, S. Falkow, L. S. Tompkins, L. E. Bermudez, *Infect. Immun.* **65**, 3759 (1997).
6. I. Vergne, J. Chua, S. B. Singh, V. Deretic, *Annu. Rev. Cell. Dev. Biol.* **20**, 367 (2004).
7. M. Ramet, P. Manfrulli, A. Pearson, B. Mathey-Prevot, R. A. Ezekowitz, *Nature* **416**, 644 (2002).
8. M. Ramet *et al.*, *Immunity* **15**, 1027 (2001).
9. A. M. Pearson *et al.*, *Microbes Infect.* **5**, 815 (2003).
10. T. R. Da Silva *et al.*, *Infect. Immun.* **70**, 5628 (2002).
11. O. Fischer *et al.*, *Med. Vet. Entomol.* **15**, 208 (2001).
12. L. Ramakrishnan, N. A. Federspiel, S. Falkow, *Science* **288**, 1436 (2000).
13. S. Greenberg, S. Grinstein. *Curr. Opin. Immun.* **14**, 136 (2002)
14. V. A. Kelley, J. S. Schorey, *Mol. Biol. Cell.* **14**, 3366 (2003).
15. For further details, see materials and methods available as SOM.
16. H. Agaisse, *et al.*, submitted to *Science*.
17. J. V. Mulcahy, D. R. Riddell, J.S. Owen, *Biochem. J.* **377**, 741 (2004).
18. D. Rhainds, L. Brisette, *Int. J. Biochem. Cell Biol.* **36**, 39 (2004).
19. J. Husemann, J. D. Loike, T. Kodama, S. C. Silverstein, *J. Neuroimmunol.* **114**, 142 (2001).
20. S. Zimmerli, S. Edwards, J. D. Ernst, *Am. J. Respir. Cell Mol. Biol.* **15**, 760 (1996).
21. K. Hoebe *et al.*, *Nature* **433**, 523 (2005).
22. We thank C. Cosma and L. Ramakrishnan (University of Washington) for generously providing *map24* and *map49* plasmids. Expression constructs for CD36, SR-BI and SR-BII, and LIMP-II were kindly provided by K. Moore (Massachusetts General Hospital), J. Owen (University College London), and K. Akasaki (Fukuyama University), respectively. S. De Bruin and N. Glikzman (Universal Imaging) provided MetaMorph software training. E. Troy, L. Burrack, and D. Higgins assisted with BMM experiments. We are indebted to the *Drosophila* RNAi Screening Center for providing expertise and the facilities for screening, and to the Perrimon and Rubin laboratory members for assistance, in particular S. Cherry. D. Higgins, B. Mathey-Prevot, L. Burrack, and M. Gibson provided useful comments on the manuscript. This work was supported by the NIH. N. P. is an Investigator of the Howard Hughes Medical Institute.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1116006/DC1

Materials and Methods

Figs. S1 to S3

Tables S1 to S3

References and Notes

13 June 2005; accepted 5 July 2005

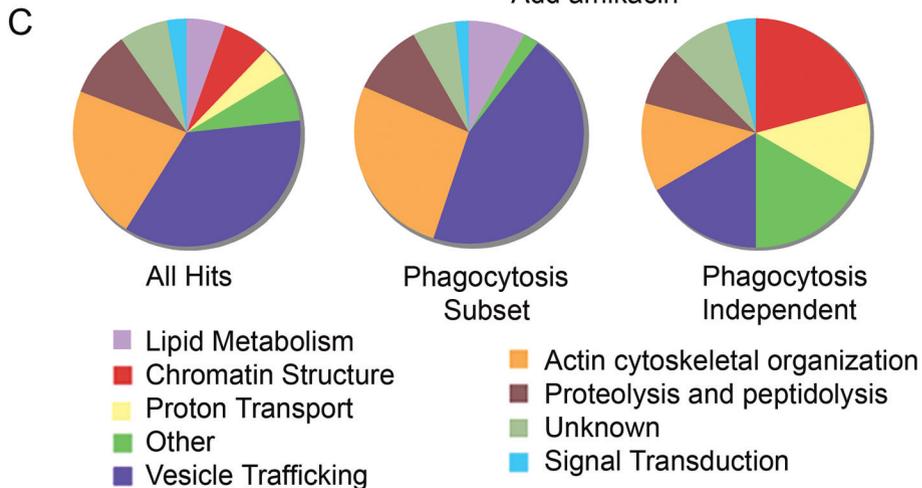
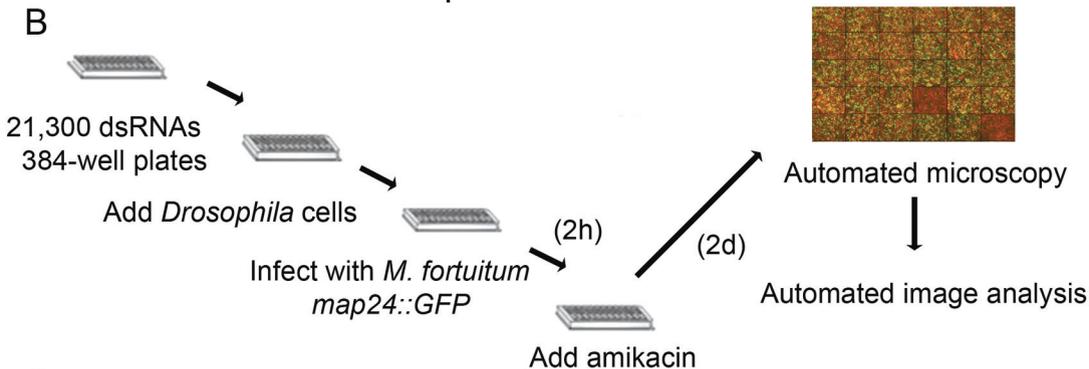
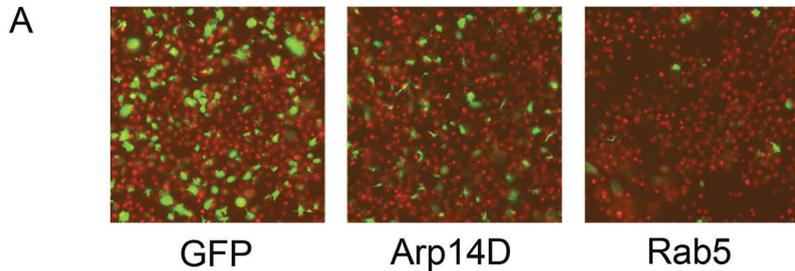
Published online 14 July 2005; 10.1126/science.1116006

Include this information when citing this paper.

Fig. 1. Host factors required for *M. fortuitum* infection identified by RNAi. After treatment with dsRNAs, S2 cells were infected with *M. fortuitum map24::GFP*. dsRNA targeting Arp14D or Rab5 decreased infection compared to controls treated with dsRNA targeting GFP (A). This assay was used as the basis of a genome-wide screen described in Methods (B). A composite image of 35 wells is shown. Pie charts based upon GeneOntology (GO) index biological function (C) show the categories of host factors that reproducibly decreased infection after 3d of dsRNA treatment, as well as the subsets that are required for phagocytosis and those that are apparently phagocytosis independent.

Fig. 2. CG7228 is required in S2 cells for infection by *M. fortuitum* and uptake of *M. smegmatis* but not *E. coli* or *S. aureus*. (A) dsRNA against CG7228 blocked infection by *M. fortuitum* compared to controls treated with dsRNA against GFP. (B) uptake of FITC-conjugated *E. coli* (black bars) and *S. aureus* (grey bars) is blocked by dsRNA toward dSR-CI, Arp14D, Cdc42, and CG3523, but not CG7228. Data show the percent uptake as compared to untreated cells (see fig. S3). The average of six experiments in which each dsRNA was tested in triplicate is shown with error bars denoting S.D. (**P* < 0.05 compared to GFP treated wells based upon a Students *t*-test.) (C) Antibiotic protection experiments demonstrate the role of CG7228, CG3523, and Cdc42 in the uptake of *M. smegmatis*. Data are normalized to GFP; error bars indicate S.D. from a representative experiment performed in triplicate.

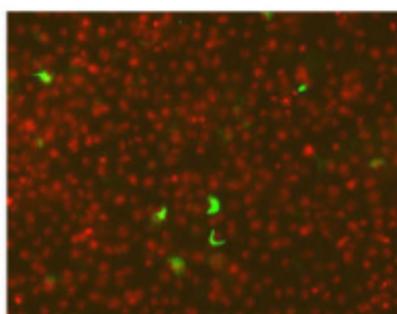
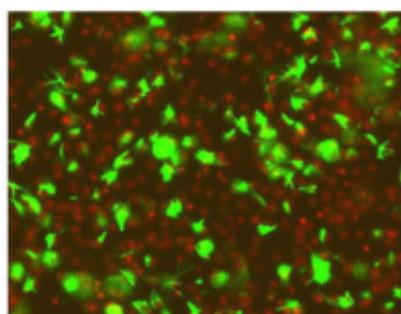
Fig. 3. Class B SRs mediate uptake of *M. fortuitum*, *E. coli*, and *S. aureus*. HEK293 cells transfected with vector (pcDNA3.1) or expression constructs for CG7228 (Pes), CD36, SR-BI, or SR-BII were (A) infected with *M. fortuitum map24::GFP* or incubated with (B) FITC-*E. coli* or (C) FITC-*S. aureus*. In (A) green represents GFP expression from the *map24* promoter, whereas in (B) and (C) green shows FITC signal from internalized bacteria. Nuclei are red. Images are 40X.



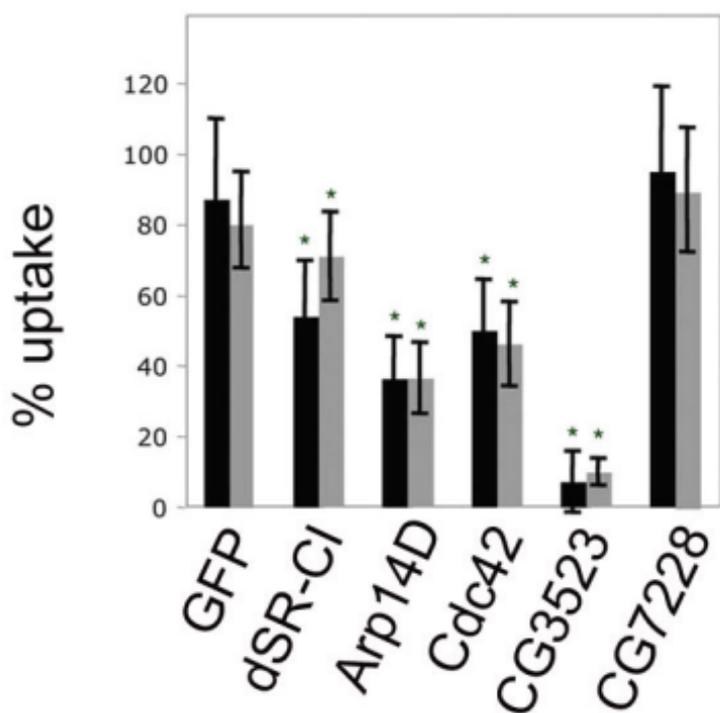
A

GFP

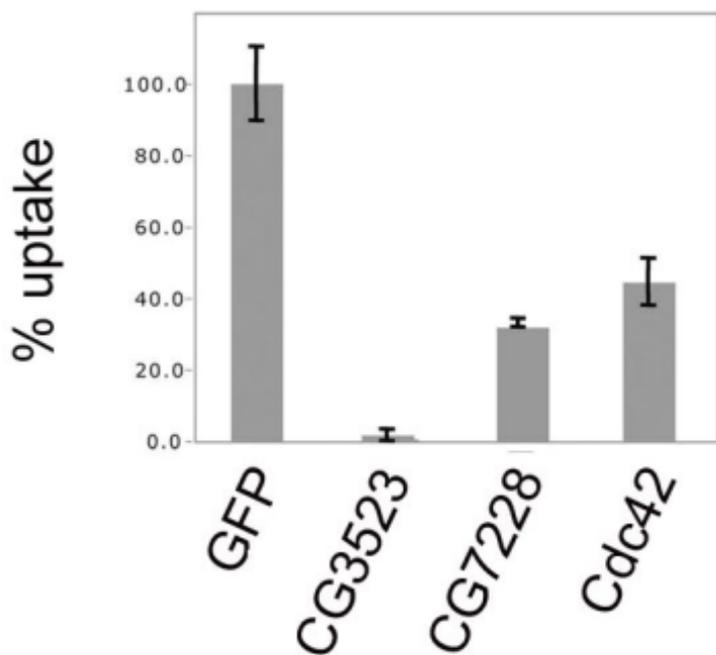
CG7228



B



C



vector

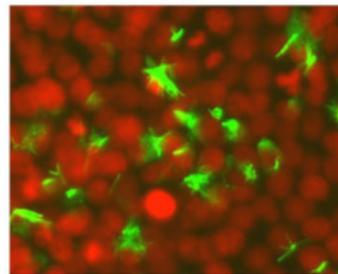
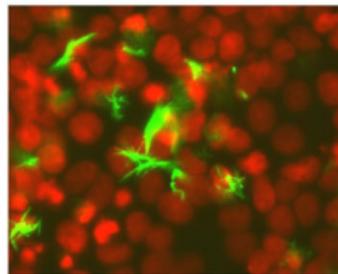
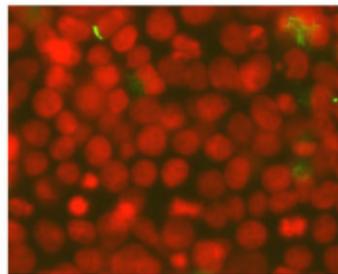
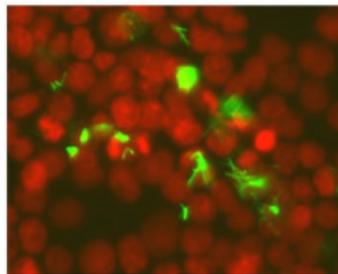
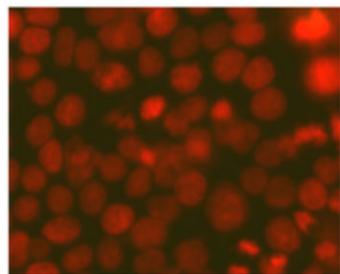
CG7228

CD36

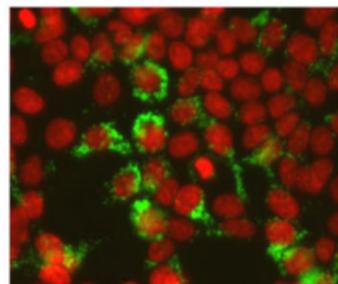
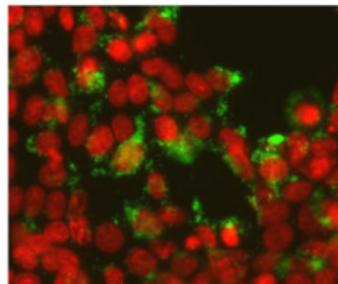
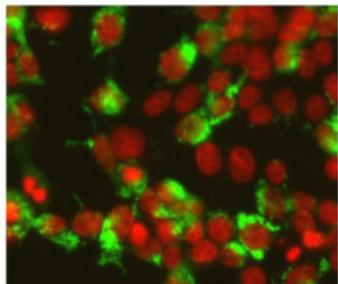
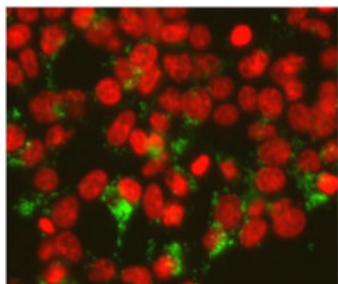
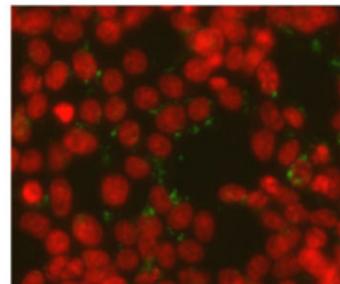
SR-BI

SR-BII

A



B



C

