Tam et al., http://www.jcb.org/cgi/content/full/jcb.201003053/DC1

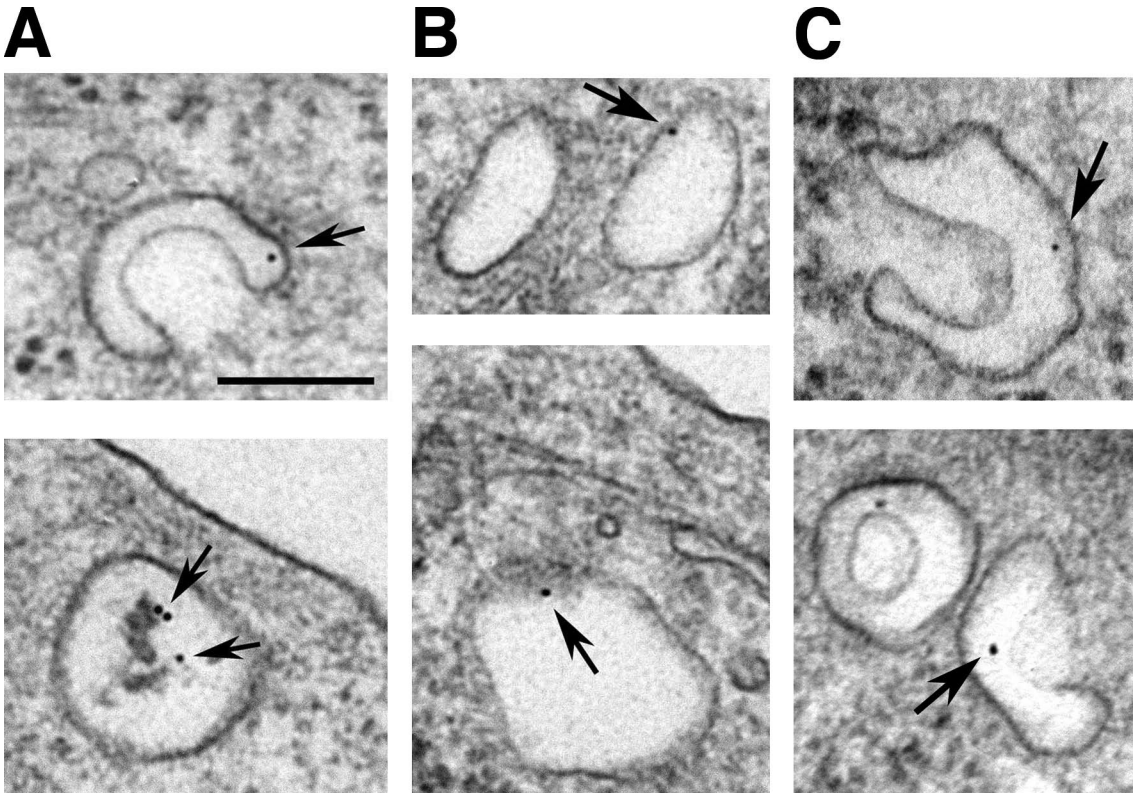


Figure S1. **Large BSA-gold–positive endosomes are detected in HeLa cells transfected with different siRNA duplex oligonucleotides and injured with SLO.** (A–C) EM images of BSA-gold–containing large endosomes detected in cells treated with control siRNA followed by SLO wounding in the presence of Ca^{2+} (A), ASM siRNA followed by SLO wounding in the presence of Ca^{2+} (B), and ASM siRNA followed by SLO wounding in the presence of $Ca^{2+}$and 10 µg/ml rhASM (C). Arrows point to BSA-gold within endosomes. Bar, 200 nm.

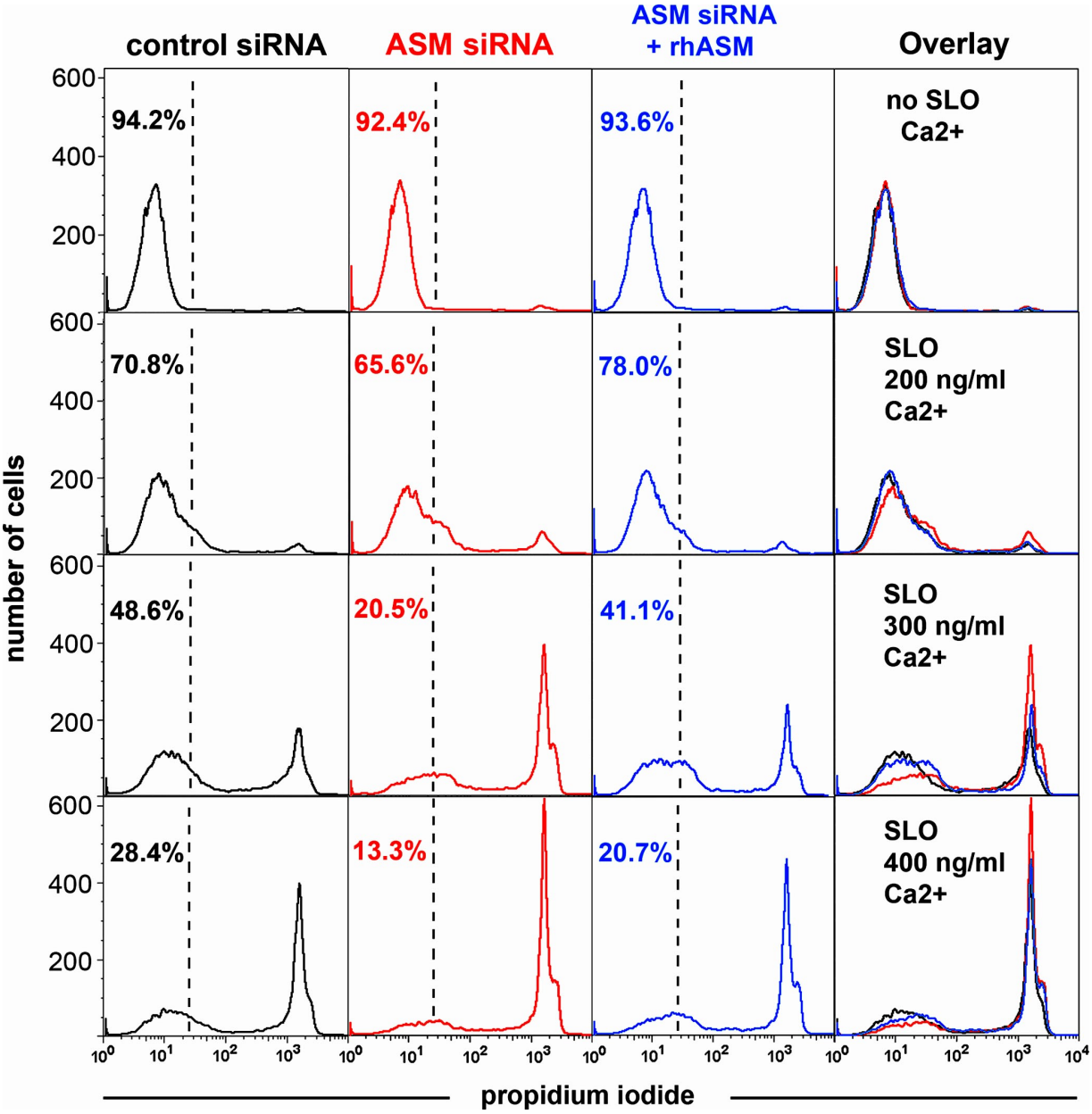
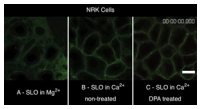
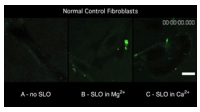


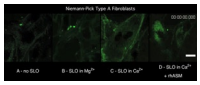
Figure S2. **Transcriptional silencing of ASM causes a defect in plasma membrane repair that is proportional to the extent of cell permeabilization and is reversed by the addition of rhASM.** FACS quantification of PI staining in HeLa cells pretreated with control siRNA, ASM siRNA, or ASM siRNA followed by rhASM. Ca^{2+}-dependent repair of SLO permeabilization was less efficient in cells treated with ASM siRNA when compared with cells treated with control siRNA. This phenotype was reversed by the addition of rhASM during the wounding procedure. Percentages correspond to resealed (PI negative) cells in the gated region denoted by the dashed lines. The results shown in this figure are representative of several independent experiments.



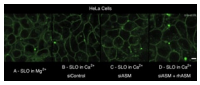
Video 1. **Inhibition of ASM activity with DPA allows rapid and sustained influx of FM1-43 into SLO-permeabilized NRK cells.** NRK cells were preincubated with 100 ng/ml SLO at 4°C and transferred to a live imaging chamber at 37°C, followed by the addition of FM1-43 and time-lapse confocal imaging for 4 min at 1 frame/3 s. (A) Ca^{2+}-free DME and FM1-43 were added 10 s before the beginning of the video. (B) Ca^{2+}-containing DME and FM1-43 were added 10 s before the beginning of the video to nontreated cells. (C) Ca^{2+}-containing DME and FM1-43 were added 10 s before the beginning of the video to DPA-treated cells. This video is displayed at 8 frames/s. Bar, 18 μm.



Video 2. **Normal human fibroblasts contain the influx of FM1-43 after SLO permeabilization.** Normal control fibroblasts were preincubated or not with 600 ng/ml SLO at 4°C and transferred to a live imaging chamber at 37°C, followed by the addition of FM1-43 and time-lapse confocal imaging for 4 min at 1 frame/3 s. (A) No SLO was added to cells in Ca^{2+}-containing DME. (B) Ca^{2+}-free DME and FM1-43 were added to SLO-treated cells 10 s before the beginning of the video. (C) Ca^{2+}-containing DME and FM1-43 were added to SLO-treated cells 10 s before the beginning of the video. This video is displayed at 8 frames/s. Bar, 18 μm.



Video 3. **NPA fibroblasts are defective in plasma membrane repair after SLO permeabilization, but the addition of rhASM restores their ability to contain FM1-43 influx.** NPA fibroblasts were preincubated or not with 600 ng/ml SLO at 4°C and transferred to a live imaging chamber at 37°C, followed by the addition of FM1-43 and time-lapse confocal imaging for 4 min at 1 frame/3 s. (A) No SLO was added. (B) Ca^{2+}-free DME and FM1-43 were added to SLO-treated cells 10 s before the beginning of the video. (C) Ca^{2+}-containing DME and FM1-43 were added to SLO-treated cells 10 s before the beginning of the video. (D) Ca^{2+}-containing DME, FM1-43, and rhASM at 10 μg/ml were added to SLO-treated cells 10 s before the beginning of the video. This video is displayed at 8 frames/s. Bar, 18 μm.



Video 4. **HeLa cells treated with ASM siRNA show accelerated FM1-43 influx after SLO permeabilization, and this defect is rescued with the exogenous addition of rhASM.** HeLa cells were preincubated with 600 ng/ml SLO at 4°C and transferred to a live imaging chamber at 37°C, followed by the addition of FM1-43 and time-lapse confocal imaging for 4 min at 1 frame/3 s. (A) Ca^{2+}-free DME and FM1-43 were added 10 s before the beginning of the video. (B) Ca^{2+}-containing DME and FM1-43 dye were added 10 s before the beginning of the video to cells treated with control siRNA. (C) Ca^{2+}-containing DME and FM1-43 were added 10 s before the beginning of the video to cells treated with ASM siRNA. (D) Ca^{2+}-containing DME, FM1-43, and exogenous rhASM at 10 μg/ml were added 10 s before the beginning of the video to cells treated with ASM siRNA. This video is displayed at 8 frames/s. Bar, 18 μm.