

# Supplemental Materials

*Molecular Biology of the Cell*

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**Figure S1.** Secondary RNAi screen reveals host genes that affect actin-based motility.

(A) Phalloidin-stained samples used to measure the frequency of TagBFP-expressing *L. monocytogenes* (magenta) with actin tails (green, see arrows). Scale bar, 10  $\mu\text{m}$  (inset, 5  $\mu\text{m}$ ).

(B) Pooled data from two biological replicates of the screen. Each dot represents the average frequency of bacteria with actin tails per well. 3 wells/siRNA were done in each run of the screen. Significance determined relative to the NT siRNA control using a Kruskal-Wallis ANOVA with an uncorrected Dunn's test. For visibility, the data in (B) are split into two graphs and the NT data duplicated. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Figure S2.** Secondary RNAi screen reveals host genes reduce host monolayer integrity.

(A, B) Phalloidin-stained samples were imaged (left) and then a binary mask was applied (right) to quantify the percentage of surface area covered by cells (i.e. % confluency) from intact

(A) and disrupted (B) monolayers. Scale bar, 10  $\mu\text{m}$ . (C) Pooled data from two biological replicates of the screen. Each dot represents the average percentage of confluency per well. 3 wells/siRNA were done in each run of the screen. Significance determined relative to the NT siRNA control using a Kruskal-Wallis ANOVA with an uncorrected Dunn's test. For visibility, the data in (C) are split into two graphs and the NT data duplicated. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Table S1.** Primary siRNA screen results

**Table S2.** List of siRNAs used in the RNAi screens



