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Supplemental material

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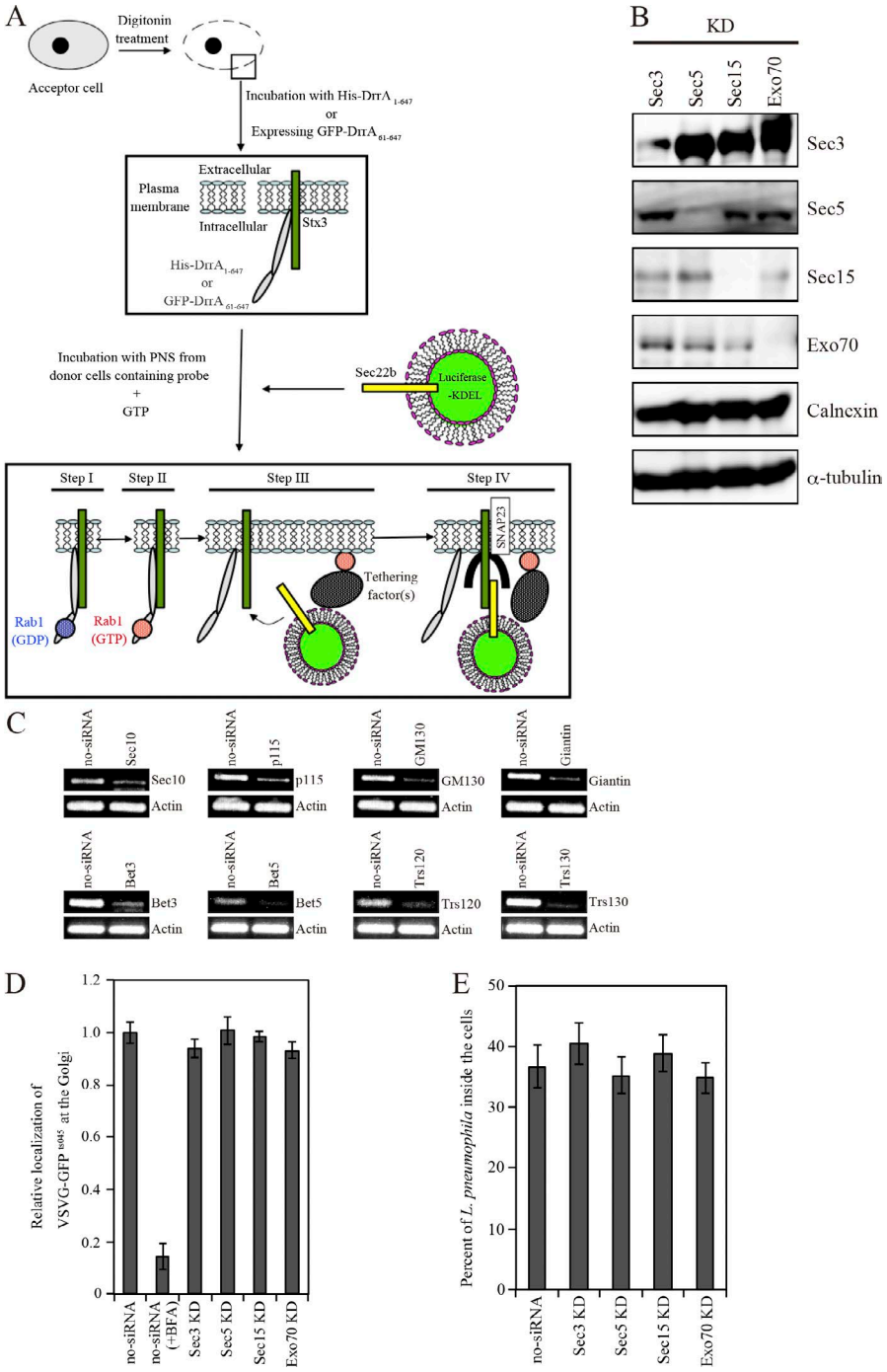

Figure S1. **Scheme of semi-intact assay and siRNA efficiency. (A)** Step I: recombinant or expressed DrrA is distributed to the PM of acceptor cells, and DrrA proteins interact with both Rab1 (GDP-form) and Stx3. Step II: DrrA activates Rab1 through its GEF activity. Steps III and IV: Activated Rab1 recruits ER-derived vesicles to the PM through the host tethering factors and facilitates the formation of noncanonical SNARE paring. For fusion between tethered vesicles and the PM, ATP is necessary. Although we could not exclude the possibility that recombinant His_6-DrrA localized to endosomes, our previous paper showed that recombinant His_6-DrrA protein was distributed predominantly in the PM (Arasaki et al., 2012). **(B)** HEK293-FcγRII cells were transfected with siRNAs targeting the indicated proteins. 72 h after transfection, cell lysates were prepared, and equal amounts of the lysates were analyzed by immunoblot using indicated antibodies. Calnexin and α-tubulin levels were also used to assess equal protein loading. KD, knockdown. **(C)** HEK293-FcγRII cells were transfected with or without siRNAs targeting the indicated proteins. 72 h after transfection, RNA was prepared from these cells and subjected to RT-PCR with specific primers for the indicated proteins. **(D)** HeLa-FcγRII cells were transfected with siRNA targeting Sec3, Sec5, Sec15, or Exo70. 48 h after transfection, cells were additionally transfected with VSVG-GFP^{ts045} and incubated at 40°C for 24 h and then shifted to 32°C for 30 min to allow transport of VSVG-GFP^{ts045} from the ER to the Golgi. Brefeldin A (BFA) treatment was used as a negative control. At least 30 cells expressing VSVG-GFP^{ts045} were scored in each experiment. Values are the mean ± SD of three independent experiments. The ratio of Golgi distribution of VSVG-GFP^{ts045} was measured in each condition and compared with mock treatment. **(E)** HeLa-FcγRII cells were transfected with siRNA targeting Sec3, Sec5, Sec15, or Exo70. 72 h after transfection, cells were infected with wild-type *Legionella* for 30 min at MOI 10 and fixed, and extracellular and intracellular *Legionella* were stained. The graph shows the percentage of *Legionella* inside the cells. At least 30 infected cells were scored in each experiment. Values are the mean ± SD of three independent experiments.

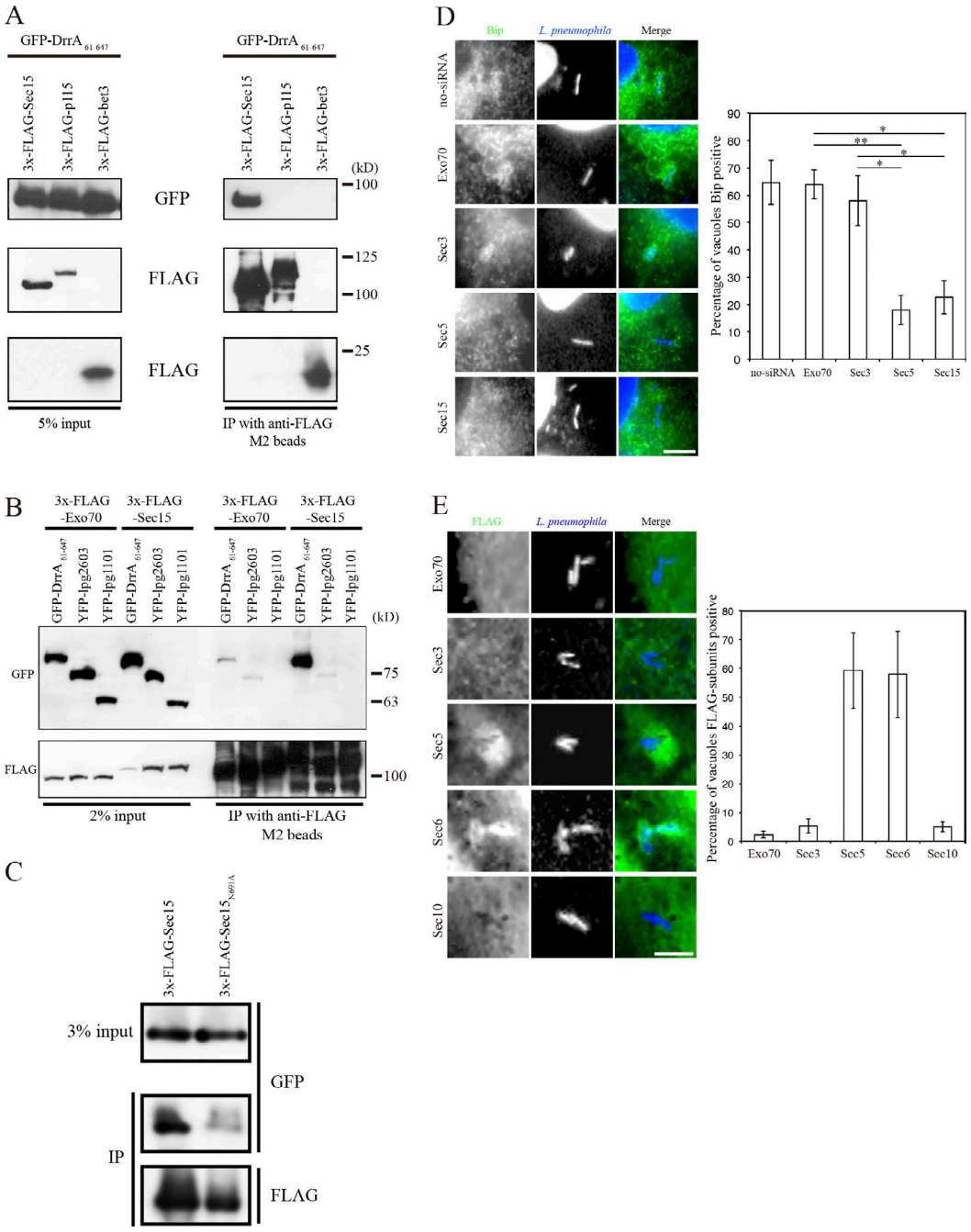

Figure S2. **Specificity of Sec15–DrrA interaction and of the exocyst components in LCV recruitment as well as LCV–ER association. (A)** HEK293-FcγRII cells were cotransfected with GFP-DrrA$_{61–647}$ and 3x-FLAG-Sec15, -p115, or -bet3. 24 h after transfection, cell lysates were prepared, and the 3x-FLAG proteins were precipitated from the lysates using anti-FLAG M2 agarose. The precipitated proteins were analyzed by antibodies against GFP and FLAG. IP, immunoprecipitation. **(B)** HEK293-FcγRII cells were cotransfected with 3x-FLAG-Exo70 or -Sec15 and GFP-DrrA$_{61–647}$, YFP-lpg2603, or YFP-lgp1101. 24 h after transfection, cell lysates were prepared, and the 3x-FLAG proteins were precipitated from the lysates using anti-FLAG M2 agarose. The precipitated proteins were analyzed by antibodies against GFP and FLAG. **(C)** HEK293-FcγRII cells were cotransfected with plasmids encoding 3x-FLAG-Sec15 (wild-type) or -Sec15$_{N691A}$ and GFP-Rab11 (S20V; a GTP-locked form; Kim et al., 2015). 24 h after transfection, cell lysates were prepared, and the 3x-FLAG proteins were precipitated from the lysates using anti-FLAG M2 agarose. The precipitated proteins were analyzed by Western blotting using antibodies against GFP and FLAG. **(D)** HeLa-FcγRII cells were transfected with siRNA targeting Exo70, Sec3, Sec5, or Sec15. 72 h after transfection, cells were infected with wild-type *Legionella* for 4 h at MOI 10 and fixed, and extracellular and intracellular *Legionella* were stained. After staining, cells were permeabilized and stained with an antibody against Bip. Intracellular *Legionella* were detected by Hoechst 33342. Bar, 2 µm. The graph shows the percentage of Bip-positive vacuoles. Values are the mean ± SD of three independent experiments, in each of which 50 vacuoles were scored. *P < 0.05 and **P < 0.01 compared with no-siRNA. **(E)** HeLa-FcγRII cells were transfected with 3x-FLAG-Exo70, -Sec3, -Sec5, -Sec6, or -Sec10. 24 h after transfection, cells were infected with wild-type *Legionella* for 1 h at MOI 10 and fixed, and extracellular *Legionella* was stained. After staining, cells were permeabilized and stained with an antibody against FLAG. Intracellular *Legionella* were detected by Hoechst 33342. Bar, 2 µm. The graph shows the percentage of FLAG-positive vacuoles. Values are the mean ± SD of three independent experiments, in each of which 100 vacuoles were scored.

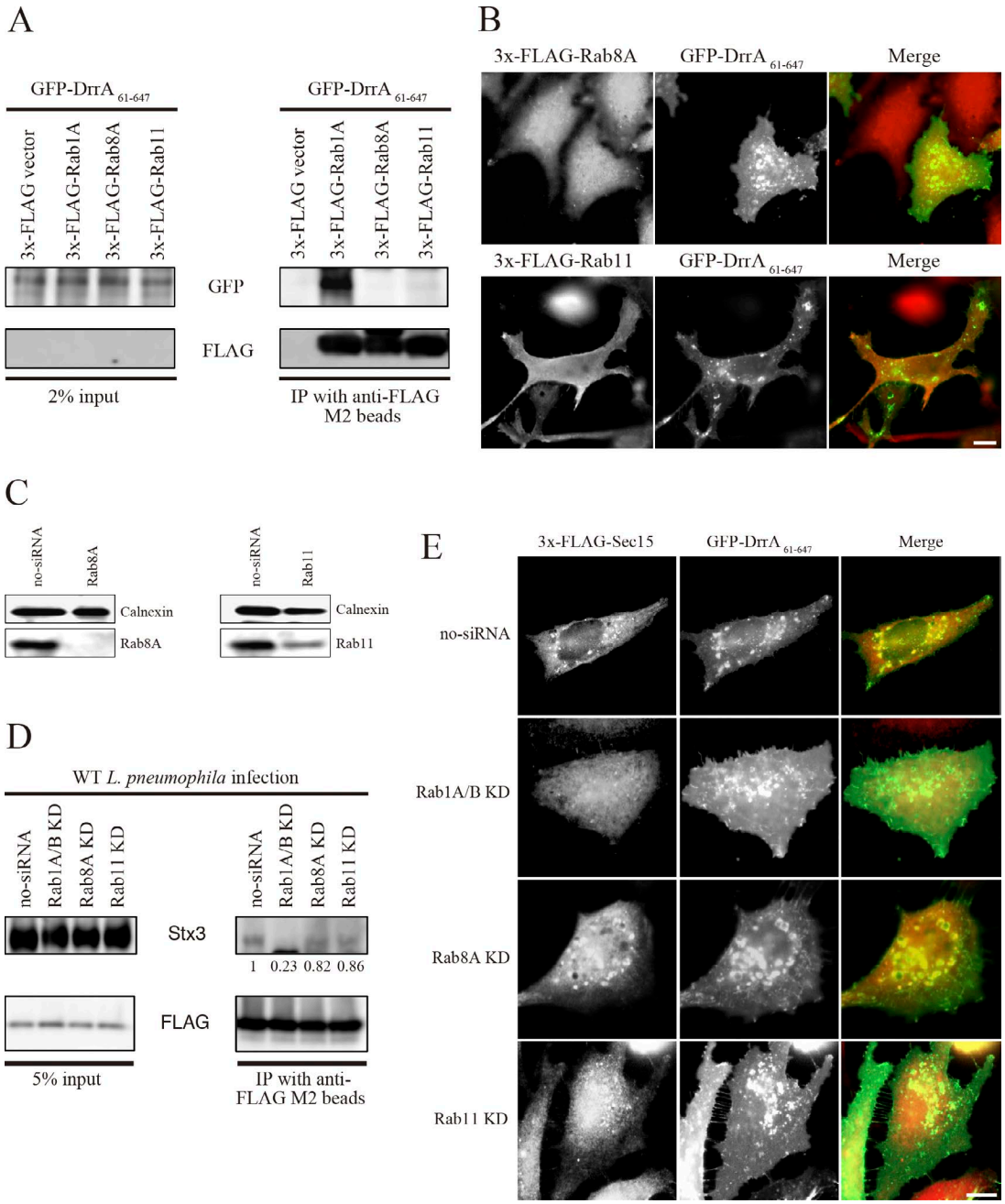

Figure S3. **DrrA–Rab1 complex specifically facilitates exocyst-dependent tether. (A)** HEK293-FcγRII cells were cotransfected with plasmids encoding 3xFLAG-vector, -Rab1A, -Rab8A, or -Rab11 and GFP-DrrA$_{61–647}$. 24 h after transfection, cell lysates were prepared, and the 3x-FLAG proteins were precipitated from the lysates using anti-FLAG M2 agarose. The precipitated proteins were analyzed by antibodies against GFP and FLAG. IP, immunoprecipitation. **(B)** HeLa-FcγRII cells were cotransfected with 3xFLAG-Rab8A or -Rab11 and GFP-DrrA$_{61–647}$. 24 h after transfection, cells were fixed and stained with an antibody against FLAG. Bar, 5 µm. **(C)** HeLa-FcγRII cells were transfected with siRNA targeting Rab8A or Rab11. 72 h after transfection, cell lysates were prepared, and equal amounts of the lysates were analyzed by Western blotting using indicated antibodies. Calnexin level was used to assess equal protein loading. **(D)** 3x-FLAG-Sec22b–expressing HEK293-FcγRII cells were transfected with or without siRNA targeting Rab1A/B, Rab8A, or Rab11. 72 h after transfection, cells were infected with wild-type *Legionella* for 1 h at MOI 50. After infection, cell lysates were prepared, and 3x-FLAG-Sec22b was precipitated from the lysates using anti-FLAG M2 agarose. The precipitated proteins were analyzed by Western blotting using antibodies against Stx3 and FLAG. Values below the Stx3 strip represent the average of the Stx3/FLAG intensity ratio (n = 3) normalized to that in no-siRNA. KD, knockdown. **(E)** HeLa-FcγRII cells were transfected with siRNA targeting Rab1A/B, Rab8A, or Rab11. 48 h after transfection, cells were additionally transfected with a plasmid encoding 3xFLAG-Sec15 for 24 h, fixed, and stained with an antibody against FLAG. Bar, 5 µm.

References

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