Supplemental material

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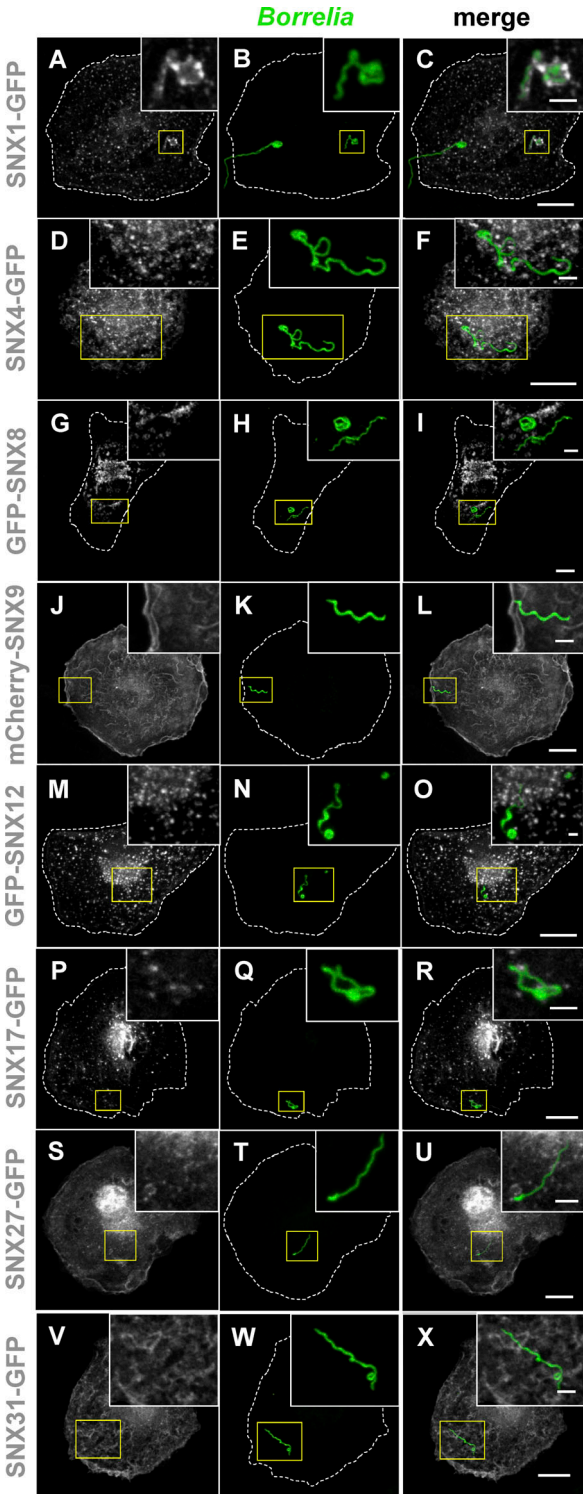


Figure S1. **Localization of SNX isoform constructs in macrophages with internalized borreliae.** Confocal micrographs of macrophages expressing GFP- or mCherry-fused constructs, as indicated, of SNX1 (A–C), SNX4 (D–F), SNX8 (G–I), SNX9 (J–L), SNX12 (M–O), SNX17 (P–R), SNX27 (S–U), and SNX31 (V–X) with internalized borreliae stained by specific antibody (B, E, H, K, N, Q, T, and W), and respective merges (C, F, I, L, O, R, U, and X). Box indicates area of magnified inset. Scale bars: 10 µm, and 1 µm for insets. Related to Fig. 1.

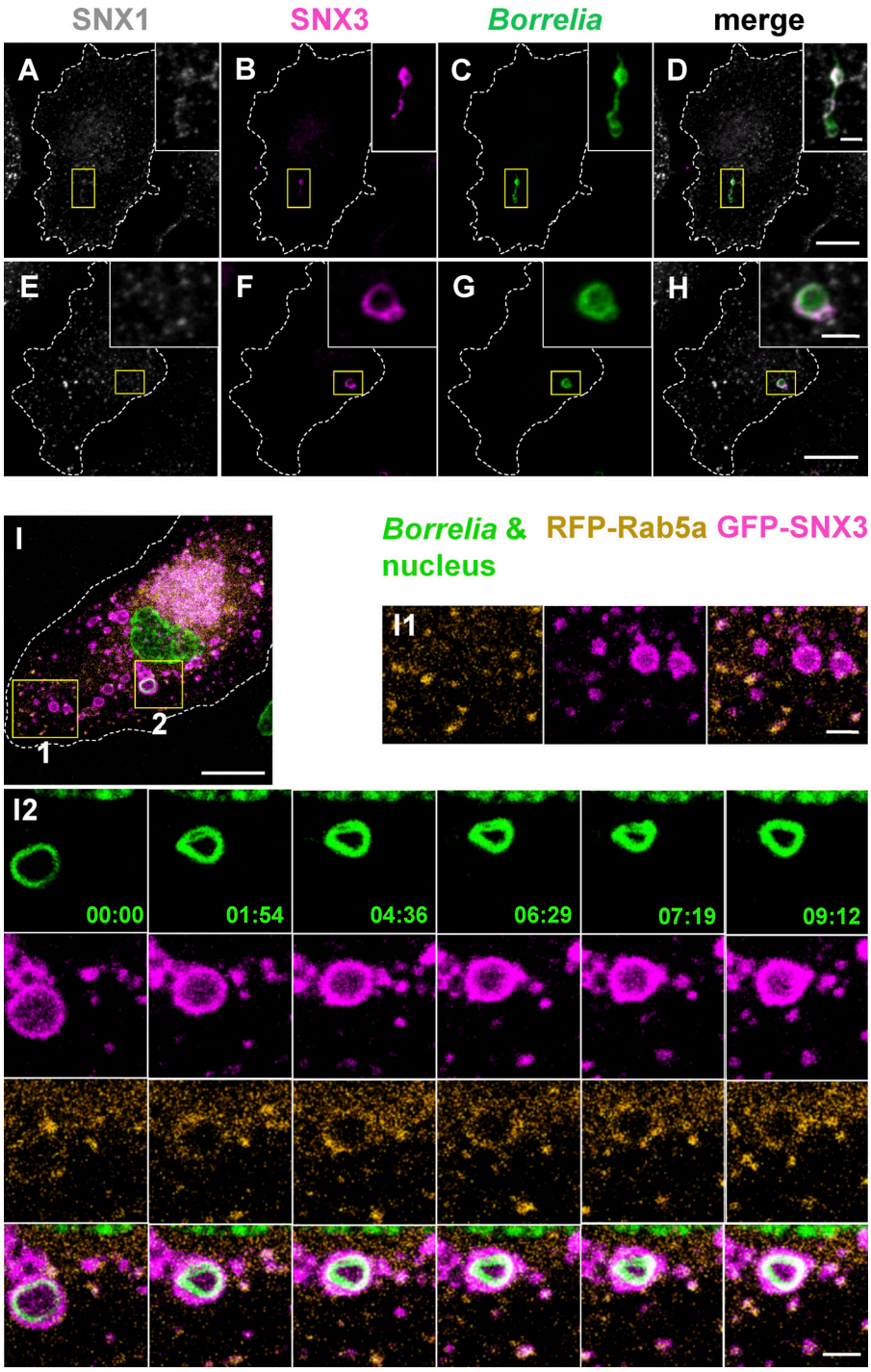


Figure S2. **Localization of endogenous SNX1 and SNX3 at borreliae-containing phagosomes and of GFP-SNX3 at RFP-Rab5a vesicles. (A–H)** Confocal micrographs of macrophages stained for endogenous SNX1 (A and E) and SNX3 (B and F), using specific primary antibodies, with internalized borreliae stained by *B. burgdorferi*–specific antibody (C and G), with merges (D and H), with *B. burgdorferi* cell in A–D partially elongated, and *B. burgdorferi* cell in E–H compacted. Yellow boxes indicate areas shown enlarged as insets. Scale bars: 10 µm. **(I)** SNX3 is present at Rab5a-positive vesicles. Still image from live cell video of macrophage expressing RFP-Rab5a and GFP-SNX3, with borreliae and nucleus stained with Hoechst 33342 (Video 3). Yellow boxes indicate detail region shown enlarged on the right (I1) or as a gallery of still images (I2), with time since start of experiment indicated in minutes:seconds. Scale bars: 10 µm in I and 1 µm in I1 and I2. Related to Fig. 1.

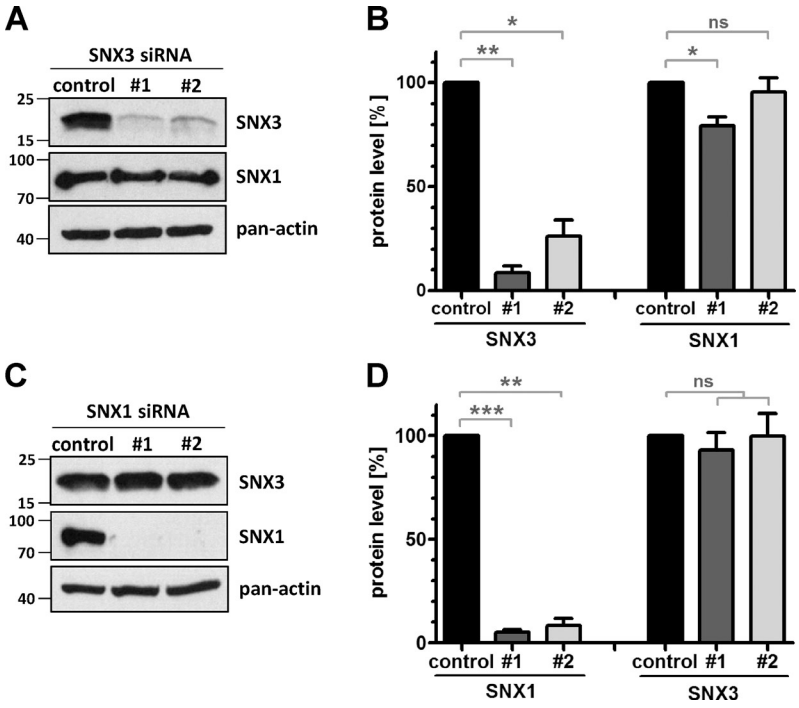


Figure S3. **siRNA-mediated knockdown of SNX1 and SNX3. (A and C)** Western blots of lysates from macrophages treated with control siRNA or each time with two individual siRNAs for SNX3 (A) and SNX1 (C), as indicated. Lysates were probed for SNX1 and SNX3 and for pan-actin as loading control. **(B and D)** Evaluation of protein levels for lysates from siRNA-treated cells from A and C. Note efficient knockdown of SNX1 and SNX3 with respective siRNAs, with only slight concomitant reduction of the other isoform. *n* = 3, one-way ANOVA; *, P < 0.05; **, P < 0.01; ***, P < 0.001. Error bars: mean ± SEM. Related to Fig. 2.

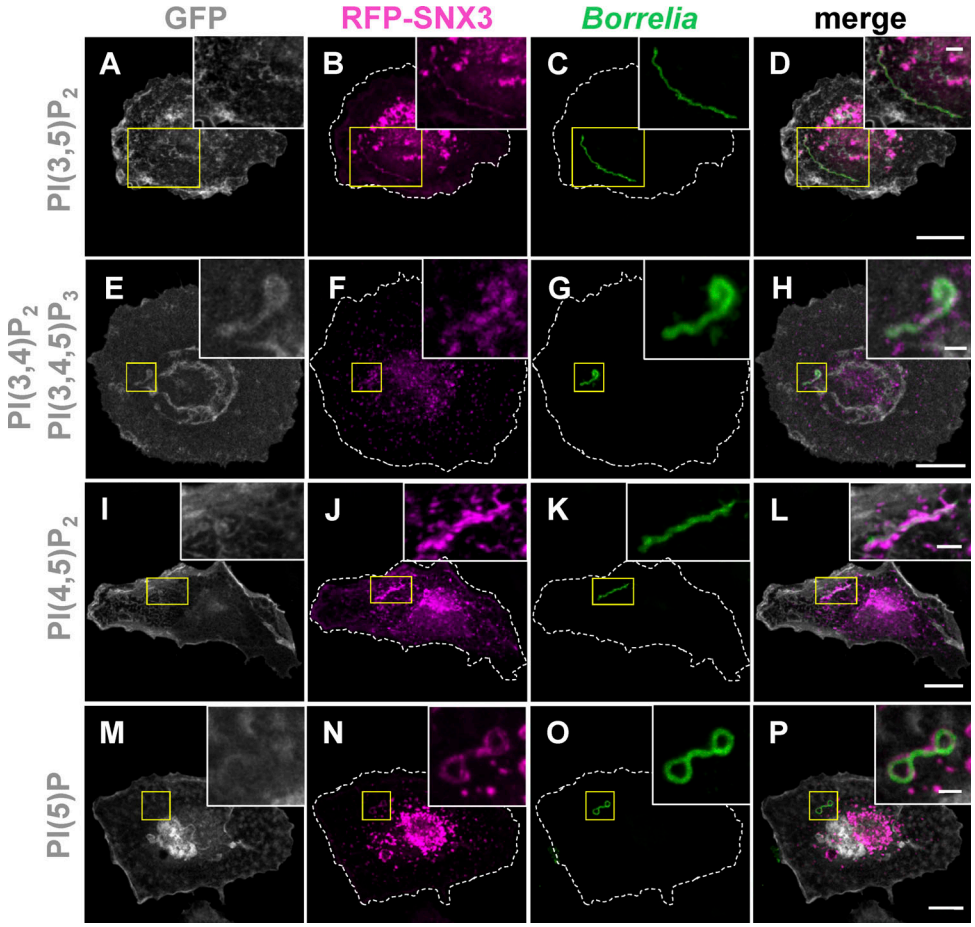


Figure S4. **Localization of phosphoinositols at *B. burgdorferi*–containing phagosomes. (A–P)** Confocal micrographs of macrophages coexpressing fluorescent probes for $PI(3,5)P_2$ (A), $PI(3,4,5)P_3$ (E), $PI(4,5)P_2$ (I), or PI(5)P (M) and coexpressing RFP-SNX3 (B, F, J, and N), with internalized borreliae stained by specific antibody (C, G, K, and O). White boxes indicate areas shown enlarged as insets. Scale bars: 10 µm, and 1 µm for insets. Related to Fig. 3.

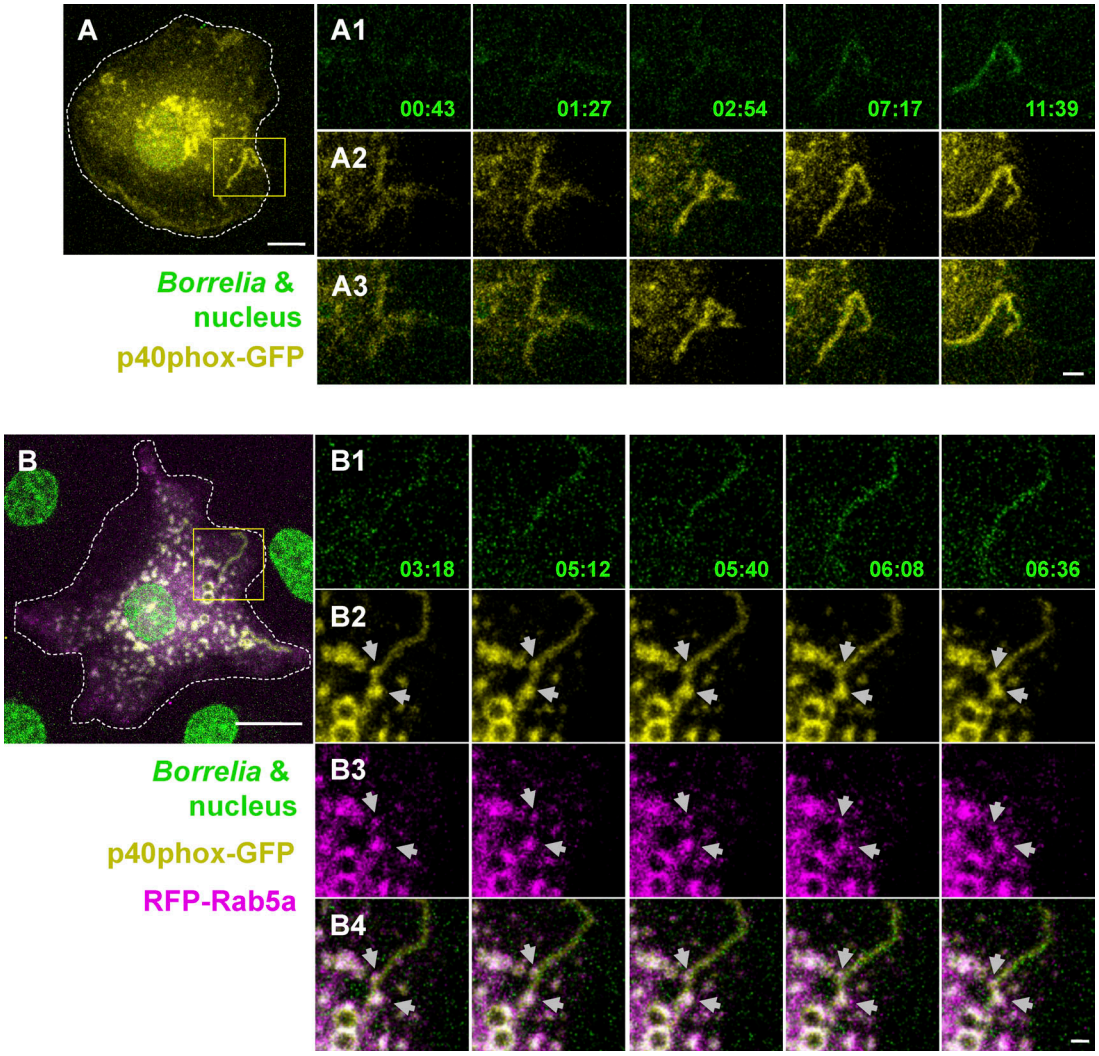


Figure S5. **Source of PI(3)P at borreliae phagosomes. (A and B)** Still images from confocal time-lapse videos of macrophage with internalized borreliae (see Videos 5 and 6). Borreliae were stained by Hoechst 33342, also staining the macrophage nucleus (A1 and B1); PI(3)P sensor p40phox-GFP is shown in yellow (A2 and B2), and RFP-Rab5a shown in magenta (B3) with merges (A3 and B4). Yellow boxes in A and B indicate areas of detail images. Note gradual enrichment of the PI(3)P sensor at internalized borreliae, with only occasional contact of PI(3)P-positive vesicles (A2 and A3), which are positive for RFP-Rab5a (B2–B4), as indicated by arrows. Scale bars: 10 µm, and 1 µm for insets. Related to Fig. 3.

A

accession	description	OS	coverage [%]	peptides [n]	unique peptides [n]	MW [kDa]
O60493	Sorting nexin-3	SNX3	54.3	8	8	18.8
Q9UMY4	Sorting nexin-12	SNX12	9.3	2	2	19.7
Q07065	Cytoskeleton-associated protein 4	CKAP4	7.6	3	3	66.0
Q15286	Ras-related protein Rab-35	RAB35	7.5	1	1	23.0
O00182	Galectin-9	LGALS9	6.2	2	2	39.5
Q12965	Unconventional myosin-Ie	MYO1E	5.4	5	2	127.0
Q12840	Kinesin heavy chain isoform 5A	KIF5A	0.7	1	1	117.3
P33176	Kinesin-1 heavy chain	KIF5B	0.7	1	1	109.6
O60282	Kinesin heavy chain isoform 5C	KIF5C	0.7	1	1	109.4

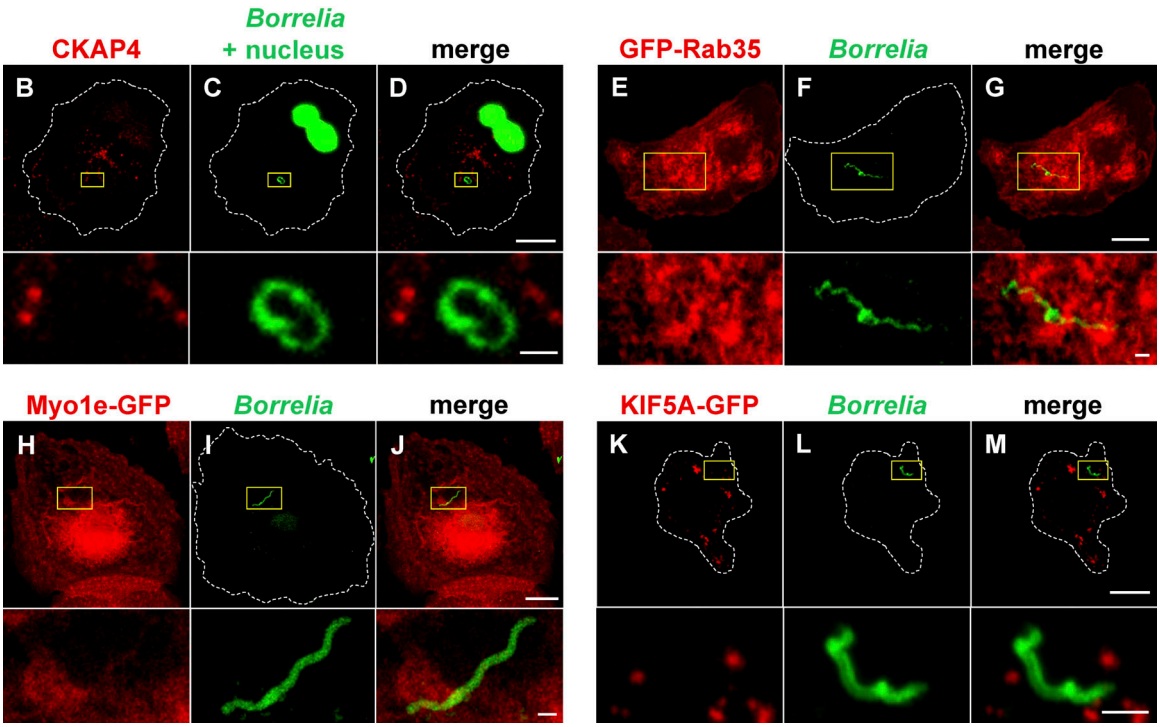


Figure S6. **Evaluation of potential SNX3-binding proteins identified by mass spectrometry. (A)** Table of candidate proteins identified by mass spectrometry analysis of anti-GFP immunoprecipitates from lysates of macrophages expressing GFP-SNX3-C, listing accession numbers, protein names and acronyms, percent coverage, number of peptides, number of unique peptides, and molecular weight (MW) in kilodaltons. OS, original species (human). **(B–M)** Fluorescence micrographs of macrophages stained for CKAP4 (B–D) or expressing GFP-Rab35 (E–G), Myo1e-GFP (H–J), or KIF5A-GFP (K–M) and coincubated with borreliae stained with Hoechst 33342 (B–D) or *B. burgdorferi*–specific antibody (E–M), with merges. Yellow boxes indicate detail regions shown enlarged below panels. Scale bars: 10 µm for larger panels and 1 µm for detail regions. For images of GFP-SNX12, see Fig. S1, M–O. Related to Fig. 6.

A

accession	description	OS	coverage [%]	peptides [n]	unique peptides [n]	MW [kDa]
Q9NP72	Ras-related protein Rab-18	RAB18	26.7	4	4	23.0
O00161	Synaptosomal-associated protein 23	SNAP23	11.4	2	2	23.3
P61026	Ras-related protein Rab-10	RAB10	11.0	2	1	22.5
P61006	Ras-related protein Rab-8a	RAB8A	10.6	2	1	23.7
O75955	Flotillin-1	FLOT1	6.6	2	2	47.3
Q14254	Flotillin-2	FLOT2	3.3	1	1	47.0

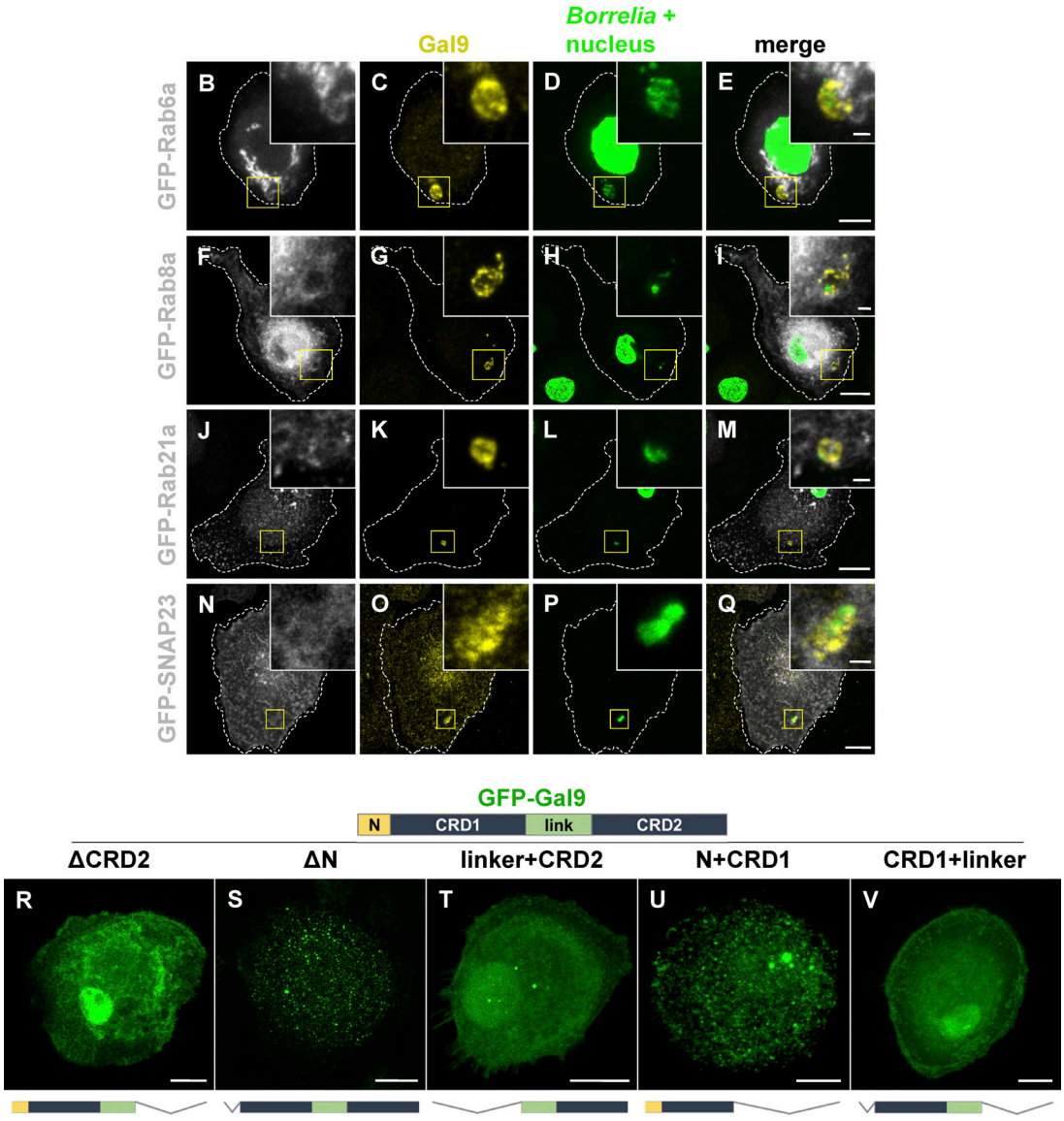
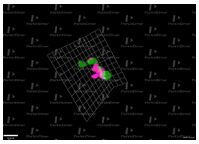
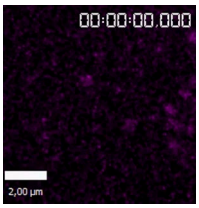


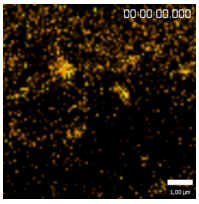
Figure S7. **Evaluation of potential galectin-9–binding proteins identified by mass spectrometry. (A)** Table of candidate proteins identified by mass spectrometry analysis of anti-GFP immunoprecipitates from lysates of macrophages expressing GFP-galectin-9, listing accession numbers, protein names and acronyms, percent coverage, number of peptides, number of unique peptides, and molecular weight (MW) in kilodaltons. **(B–Q)** Fluorescence micrographs of macrophages expressing GFP-Rab6a (B), GFP-Rab8a (F), GFP-Rab21a (J), or GFP-SNAP23 (N), stained for galectin-9 (C, G, K, and O), with internalized borreliae and nuclei stained by Hoechst 33342 (D, H, L, and P), with merges (E, I, M, and Q). Scale bars: 10 µm for larger panels and 1 µm for detail regions. For images of GFP-flotillin-2, see Fig. 7 I. **(R–V)** Fluorescence micrographs of macrophages expressing indicated galectin-9 truncation constructs, with the schematic domain structure beneath each panel. Scale bars: 10 µm. Related to Fig. 7.



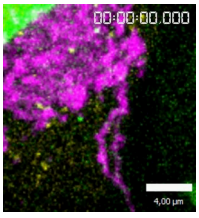
Video 1. **Endogenous SNX3 is enriched at borreliae phagosomes.** Primary human macrophages infected with borreliae showing endogenous SNX3 (magenta) at phagosomes (green). Spirochetes and SNX3 were visualized using rabbit and goat polyclonal antibodies. Rotation video of nine confocal micrographs arranged in a z-stack. Video replay frame rate: 4 frames/s. Related to Fig. 1.



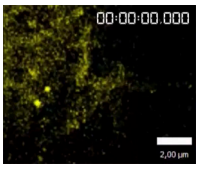
Video 2. **SNX3 decorates *B. burgdorferi*–containing phagosome during the compaction of the spirochete.** Primary human macrophage expressing RFP-SNX3 (gray) was coincubated with GFP-expressing borreliae (green) cells and imaged by live-cell spinning disk microscopy. Time-lapse video of subcellular region shows the progressive compaction of internalized *B. burgdorferi*. Note the enrichment of RFP-SNX3 at borreliae phagosomes (gallery of still images in Fig. 1 I). Time-lapse acquisition parameter: 150 ms for the green channel and 200 ms for the red fluorescence channel. Video replay frame rate: 3 frames/s. Related to Fig. 1.



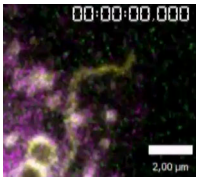
Video 3. **SNX3 localizes to Rab5a-positive vesicles and becomes enriched at phagosomes during their compaction.** Primary human macrophage expressing GFP-SNX3 (magenta) and RFP-Rab5a (gold) was coincubated with borreliae, visualized by Hoechst 33342 staining of their DNA (green) and imaged by live-cell spinning disk microscopy. Time-lapse video of subcellular region shows an internalized *B. burgdorferi* spirochete. Note that SNX3 is present as a coat at the phagosome and is subsequently contacted by Rab5a-positive vesicles (gallery of still images in Fig. S2 I). Time-lapse acquisition parameter: 150 ms for the blue and 200 ms for the green and red fluorescence channel. Video replay frame rate: 6 frames/s. Related to Fig. S2.



Video 4. **Galectin-9 is present at vesicles that contact borreliae phagosomes during their compaction.** Primary human macrophage expressing RFP-SNX3 (magenta) and GFP-Gal9 (yellow) was coincubated with *B. burgdorferi* cells, visualized by Hoechst 33342 staining of their DNA (green), and imaged by live-cell spinning disk microscopy. Time-lapse video of subcellular region shows enrichment of SNX3 at a *B. burgdorferi*–containing phagosome, which is contacted by galectin-9 vesicles (gallery of still images in Fig. 6 G). Time-lapse acquisition parameter: 150 ms for the blue channel and 200 ms for the green and red fluorescence channel. Video replay frame rate: 4 frames/s. Related to Fig. 6.



Video 5. **Gradual enrichment of PI(3)P at *B. burgdorferi*–containing phagosome.** Primary human macrophage expressing the PI(3)P sensor p40phox-GFP (yellow) was coincubated with borreliae stained by Hoechst 33342 (green) and imaged by live-cell spinning disk microscopy. Time-lapse video of subcellular region shows an internalized *B. burgdorferi* spirochete. Note the gradual enrichment of the PI(3)P sensor at internalized borreliae. Time-lapse acquisition parameter: 200 ms for the blue and 200 ms for the green fluorescence channel. Video replay frame rate: 4 frames/s. Related to Fig. S5.



Video 6. **Gradual enrichment of PI(3)P at *B. burgdorferi*–containing phagosome, with phagosome-contacting Rab5a vesicles.** Primary human macrophage expressing PI(3)P sensor p40phox-GFP (yellow) and RFP-Rab5a (magenta) coincubated with borreliae stained by Hoechst 33342 (green) was imaged by live-cell spinning disk microscopy. Time-lapse video of subcellular region shows an internalized *B. burgdorferi* spirochete. Note gradual enrichment of p40phox at internalized borreliae, with only occasional contact of PI(3)P-positive vesicles, which are also positive for RFP-Rab5a. Time-lapse acquisition parameter: 200 ms for the blue and 200 ms for the green and red fluorescence channel. Video replay frame rate: 4 frames/s. Related to Fig. S5.

Provided online are two tables in a PDF. Table S1 shows values for specified parameters following various treatments. Table S2 shows siRNAs, oligonucleotides, plasmids, and antibodies used in this study.