

**Table S1.** Values for specified parameters following various treatments

Accumulation of fluorophore-labeled SNX fusion constructs at internalized borreliae ( <b>Figure 1B</b> )		
Overexpression of	Accumulation at internalized borreliae (Mean ± SEM)	
SNX1-GFP	48.33% ± 4.41%	
GFP-SNX3	73.33% ± 10.14%	
SNX4-GFP	11.67% ± 2.89%	
GFP-SNX8	20.00% ± 11.55%	
mCherry-SNX9	3.33% ± 1.67%	
GFP-SNX12	5.00% ± 2.89%	
SNX17-GFP	16.67% ± 1.67%	
SNX27-GFP	1.67% ± 1.67%	
SNX31-GFP	11.67% ± 4.41%	
Effect of double knockdown of SNX1 and SNX3 on the morphology of internalized borreliae ( <b>Figure 2B</b> )		
siRNA target	Morphology of internalized borreliae	Quantification (Mean ± SEM)
Non-specific control	compacted	70.40% ± 6.17%
SNX1 #1		60.13% ± 10.35%
SNX3 #1		27.67% ± 5.25%
SNX1 #1 + SNX3 #1		37.20% ± 6.18%
Fluorescence intensity of DQ-BSA/borreliae phagosomes after SNX3 siRNA treatment ( <b>Figure 2I</b> )		
siRNA target	Quantification (Mean ± SEM)	
Non-specific control	1311 a.u. ± 51.96 a.u.	
SNX3 #1	1058 a.u. ± 51.90 a.u.	
SNX3 #2	1135 a.u. ± 76.17 a.u.	
Fluorescence intensity of DQ-BSA after SNX3 siRNA treatment ( <b>Figure 2J</b> )		
siRNA target	Quantification (Mean ± SEM)	
Non-specific control	1525 a.u. ± 17.18 a.u.	
SNX3 #1	1587 a.u. ± 23.89 a.u.	
SNX3 #2	1470 a.u. ± 17.11 a.u.	

survival assay after SNX3 knockdown - Bacteria number [ $10^4$ /ml] (Figure 2K)						
	days					
siRNA target	7	8	9	10		
Non-specific control	2.21 ± 0.20	3.27 ± 1.53	11.93 ± 5.53	19.23 ± 6.69		
SNX3	2.01 ± 0.89	5.38 ± 3.24	18.04 ± 7.53	44.40 ± 13.41		
SNX1 expression level after SNX3 knockdown (Figure S3B)						
siRNA target	Quantification SNX1 protein level (Mean ± SEM)		Corresponding SNX3 protein level (Mean ± SEM)			
Non-specific control	100% ± 0%		100% ± 0%			
SNX3 #1	8.77% ± 3.12%		79.37% ± 4.38%			
SNX3 #2	26.23% ± 7.61%		95.47% ± 7.06%			
SNX3 expression level after SNX1 knockdown (Figure S3D)						
siRNA target	Quantification SNX3 protein level (Mean ± SEM)		Corresponding SNX1 protein level (Mean ± SEM)			
Non-specific control	100% ± 0%		100% ± 0%			
SNX1 #1	5.23% ± 1.08%		93.30% ± 8.14%			
SNX1 #2	8.39% ± 3.51%		99.73% ± 11.14%			
Compaction after Wortmannin treatment (Figure 3Q)						
Wortmannin concentration	Morphology of internalized borreliae		Quantification (Mean ± SEM)			
control	compacted		49.90% ± 9.27%			
1 μM			21.63% ± 2.15%			
SNX3 accumulation at <i>Borrelia</i> phagosomes after Wortmannin treatment (Figure 3T)						
Wortmannin concentration	Quantification (Mean ± SEM)					
control	62.43% ± 5.41%					
1 μM	2.97% ± 1.63%					
Effect of SNX3 rescue after knockdown on the morphology of internalized borreliae (Figure 4D)						
siRNA target	overexpression of	Morphology of internalized borreliae	Quantification (Mean ± SEM)			
Non-specific control	GFP control	compacted	71.27% ± 4.78%			
	siRNA insensitive GFP-SNX3		60.13% ± 4.83%			
	siRNA insensitive GFP-SNX3-Y71A		47.70% ± 7.39%			

SNX3 #1	GFP control	compacted	34.70% ± 6.35%
	siRNA insensitive GFP-SNX3		64.63% ± 2.81%
	siRNA insensitive GFP-SNX3-Y71A		31.67% ± 3.35%

**Accumulation of GFP-labeled SNX deletion constructs at internalized borreliae (Figure 5B)**

Overexpression	Accumulation at internalized borreliae (Mean ± SEM)
GFP-SNX3-FL	61.67% ± 10.93%
GFP-SNX3-ΔN	41.67% ± 4.41%
GFP-SNX3-PX+C	38.33% ± 3.33%
GFP-SNX3-ΔC	11.67% ± 7.27%
GFP-SNX3-N+α	0%
GFP-SNX3-C	0%

**Effect of rescue by different SNX3 constructs after knockdown on the morphology of internalized borreliae (Figure 5S1)**

siRNA target	Morphology of internalized borreliae	overexpression construct	Quantification (Mean ± SEM)
Non-specific control	compacted	GFP-C1	67.50% ± 3.64%
		GFP-SNX3-FL	58.08% ± 0.78%
		GFP-SNX3-ΔN	64.83% ± 7.61%
		GFP-SNX3-PX+C	62.50% ± 7.51%
		GFP-SNX3-ΔC	37.28% ± 6.12%
		GFP-SNX3-N+α	41.9% ± 4.33%
		GFP-SNX3-C	42.85% ± 4.01%
SNX3	compacted	GFP-C1	34.03% ± 2.85%
		GFP-SNX3-FL	68.23% ± 1.76%
		GFP-SNX3-ΔN	55.23% ± 6.65%
		GFP-SNX3-PX+C	56.48% ± 6.98%
		GFP-SNX3-ΔC	41.45% ± 6.55%
		GFP-SNX3-N+α	36.48% ± 5.70%
		GFP-SNX3-C	40.65% ± 3.72%

**Effect of double knockdown of SNX1 and SNX3 on the morphology of internalized borreliae (Figure 6I)**

siRNA target	Morphology of internalized borreliae	Quantification (Mean ± SEM)
Non-specific control	compacted	70.20% ± 5.06%

SNX3 #1		35.67% ± 2.04%
Gal9 #1		39.03% ± 2.84%
SNX3 #1 + Gal9 #1		39.23% ± 9.37%

**Fluorescence intensity of DQ-BSA/borreliae phagosomes after Gal9 siRNA treatment (Figure 7G)**

siRNA target	Quantification (Mean ± SEM)
Non-specific control	1311 a.u. ± 51.96 a.u.
Gal9 #1	1074 a.u. ± 60.87 a.u.
Gal9 #2	1048 a.u. ± 57.07 a.u.

**Fluorescence intensity of DQ-BSA after Gal9 siRNA treatment (Figure 7H)**

siRNA target	Quantification (Mean ± SEM)
Non-specific control	1525 a.u. ± 17.18 a.u.
Gal9 #1	1591 a.u. ± 21.37 a.u.
Gal9 #2	1590 a.u. ± 33.30 a.u.

**Effect of rescue by different Gal9 constructs after knockdown on the morphology of internalized borreliae (Figure 8B)**

siRNA target	Morphology of internalized borreliae	Overexpression construct	Quantification (Mean ± SEM)
Non-specific control	Compacted	GFP-C1	54.32% ± 2.80%
		GFP-Gal9-FL	54.69% ± 3.12%
		GFP-Gal9-ΔCRD2	44.40% ± 5.02%
		GFP-Gal9-ΔN	52.19% ± 5.54%
		GFP-Gal9-linker+CRD2	53.28% ± 5.03%
		GFP-Gal9-N+CRD1	41.13% ± 8.34%
		GFP-Gal9-CRD1+linker	51.57% ± 8.91%
Gal9	Compacted	GFP-C1	36.56% ± 1.62%
		GFP-Gal9-FL	56.17% ± 3.08%
		GFP-Gal9-ΔCRD2	43.84% ± 1.30%
		GFP-Gal9-ΔN	56.57% ± 5.00%
		GFP-Gal9-linker+CRD2	35.24% ± 3.66%
		GFP-Gal9-N+CRD1	35.78% ± 4.64%
		GFP-Gal9-CRD1+linker	41.89% ± 4.60%

Effect of rescue by different Gal9 CRD mutants after knockdown on the morphology of internalized borreliae (Figure 8D)					
siRNA target	Morphology of internalized borreliae	Overexpression construct	Quantification (Mean ± SEM)		
Non-specific control	Compacted	GFP-C1	58.52% ± 3.98%		
Gal9	Compacted	GFP-C1	40.46% ± 0.46%		
		GFP-Gal9-FL	59.32% ± 4.32%		
		GFP-Gal9-A46V	51.08% ± 3.46%		
		GFP-Gal9-N137A	53.36% ± 5.74%		
		GFP-Gal9-R252A	50.00% ± 4.55%		
		GFP-Gal9-A46V+N137A	57.81% ± 1.29%		
Effect of SNX3 knockdown on Rab5a and galectin-9 fluorescence intensity at borreliae phagosomes (Figure 9C+D)					
Protein	siRNA target	Fluorescence intensity at borreliae phagosomes (Mean ± SEM)			
Rab5a	Control	38078 a.u. ± 5603 a.u.			
	SNX3	17497 a.u. ± 2468 a.u.			
Galectin-9	Control	2837 a.u. ± 291.2 a.u.			
	SNX3	1685 a.u. ± 144.5 a.u.			
Effect of SNX3 knockdown on phagosomes size (Figure 9E)					
siRNA target	Volume of borreliae phagosomes (Mean ± SEM)				
control	2.80 ± 0.31 μm <sup>3</sup>				
SNX3	2.65 ± 0.25 μm <sup>3</sup>				

**Table S2. siRNAs, oligonucleotides, plasmids, and antibodies used in this study**

siRNAs	
Name/target	Sequence (5' → 3')
SNX3 #1	GCGUCAGCUUCCUUUUAGA-(dTdT)
SNX3 #2	CUCAU AUG CUC AGU UUGU-(dTdT)
SNX1 #1	AAGAACAA GACCA AGAGGCCAC-(dTdT)
SNX1 #2	GAACAAGACCAAGAGGCCAC-(dTdT)
Gal9 #1	GGACUUCAGAUCACUGU-(dTdT)
Gal9 #2	GGAAGACACACAUGCCUUUCC-(dTdT)

Oligonucleotides			
#	Name	Sequence (5' → 3')	Restriction site
P01	SNX3_fw	AAAAAAAGGTACCCCGCGGAGACCGTGGCTGACACCC	KpnI
P02	SNX3_ΔC_rev	TTTTTGATCCTCAATCTATTATTCATCTGTAAAAAC	BamHI
P03	SNX3_N+α_rev	TTTTTTTTGGATCCTCAGCTGGGGGGTCCGTAGG	BamHI
P04	SNX3_ΔN_fw	AAAAAAAGGTACCCCAACCTGAATGACGCCTACGGACCC	KpnI
P05	SNX3_rev	TTTTTGATCCTCAGGCATGTCTTATTTAGATGG	BamHI
P06	SNX3_PX+C_fw	AAAAAAAGGTACCCCAACTCCTCGAGATCGATGTGAGC	KpnI
P07	SNX3_C_fw	AAAAAAAGGTACCCAAAAGCTATACTCCATCT	KpnI
P08	SNX3_C_rev	TTTTTGATCCTCAGGCATGTCTTATTT	BamHI
P09	Gal9_fw	AAAAAACTCGAGCCGCCCTCAGCGGTTCCCAGGCTCCCTAC	Xhol
P10	Gal9_rev	TTTTTGATCCTCATGTCTGCACATGGTCAGCTGGATGTC	KpnI
P11	SNX3_PX_fw	GAAAGAACCTACTGTTAGAAGAACGCCAGTGACTTG	-
P12	SNX3_PX_rev	CAAAGTCACTGGCTCTTCTTAACAGTAGATTCTTC	-
P13	SNX3_si-insens_fw	GAAAGCGTTTGCGGCAGCTCCATTAGAGGAGATG	-
P14	SNX3_si-insens_rev	CATCTCCTCTGAATGGAAGCTGCCGAAAAACGCTTC	-
P15	SNX3_GST_fw	AAAAAAAGGATCCCGCGGAGACCGTGGCTGACACCC	BamHI
P16	SNX3_GST_rev	AAAAAAAGAATTCTCAGGCATGTCTTATTTAGATGG	EcoRI
P17	KIF5A_stop_fw	CGGCATGGACGAGCTGTACAAGTGATCCATGAATGGAGC	-
P18	KIF5A_stop_rev	GCTCCATTGATGGACTTGTACAGCTCGTCCATGCCG	-

P19	Gal9_CRD1_fw	AAAAAACTCGAGCCTTTCTGGGACTATTCAAGGAGG	Xhol
P20	Gal9_CRD1_rev	TTTTTGGTACCTCAGAAGCTGATGTAGGACAGCTGC	KpnI
P21	Gal9_linker_fw	AAAAAACTCGAGCCCAGAACCCCCCACAG	Xhol
P22	Gal9_linker_rev	TTTTTGGTACCTCAAGGCATCGGATAGGCCGG	KpnI
P23	Gal9_si-insens_fw	CAGGACGGACTACAATAACAGTCATGGGACC	-
P24	Gal9_si-insens_rev	GGTCCCATTGACTGTTATTGTAGTCCGTCTG	-
P25	Gal9_A46V_fw	GGAACCAGGTTGTTGAACTTCAGACTGG	-
P26	Gal9_A46V_rev	AAAGTTCACACAAACCTGGTCCACTGGCAGC	-
P27	Gal9_N137A_fw	ATCTCCGTCGCTGGCTCTGTGCAGCTGTCTA	-
P28	Gal9_N137A_rev	TGCACAGAGGCCAGCGACGGAGATGGTGTCCAC	-
P29	Gal9_R252A_fw	CAGTGCTCAGGCGTTCCACATCAACCTGTGCT	-
P30	Gal9_R252A_rev	TTGATGTGGAACGCCTGAGCACTGGGCAGGAC	-

Antibodies and staining reagents				
Antibody	Company	Species	Dilution IF	Dilution WB
anti-SNX3 (C16)	Santa Cruz	goat polyclonal	1/100	-
anti-SNX3	Proteintech	rabbit polyclonal	1/200	1/200
anti-SNX1	BD Biosciences	mouse monoclonal	1/100	1/1000
anti-Gal9	Proteintech	rabbit polyclonal	1/200	1/500
anti- <i>Borrelia burgdorferi</i>	Antibodies online	rabbit polyclonal	1/1000	1/1000
anti- <i>Borrelia burgdorferi</i> Bss42	Novus Biologicals	mouse monoclonal	1/1000	1/1000
anti-GFP	Novus Biologicals	rabbit polyclonal	-	1/5000
anti-pan-actin	Merck Millipore	mouse monoclonal	-	1/5000
anti-mouse-HRP	GE Healthcare	sheep polyclonal	-	1/5000
anti-rabbit-HRP	GE Healthcare	donkey polyclonal	-	1/5000
anti-goat-HRP	abcam	rabbit polyclonal	-	1/5000
anti-rabbit-AlexaFluor488/568/647	Thermo Scientific	donkey or goat	1/200	-
anti-mouse-AlexaFluor488/568/647	Thermo Scientific	donkey or goat	1/200	-
anti-goat-AlexaFluor488/568/647	Thermo Scientific	donkey	1/200	-

Phalloidin AlexaFluor488/568/647	Thermo Scientific	-	1/50	-
<b>Plasmids</b>				
Name	Provided by/ purchased from	Reference		
GFP-SNX3	S. Grinstein	Braun et al, Cell Microbiol. 2010 Sep 1;12(9):1352-67		
RFP-SNX3	J. Gruenberg	Pons et al, PLoS Biol. 2008 Sep 2;6(9):e214		
Rab5a-RFP	S. Grinstein			
SNX1-GFP	S. Grinstein			
SNX4-GFP	L. Binkle			
GFP-SNX8	K. Sandvig	Dyve et al, Biochem Biophys Res Commun. 2009 Dec 4;390(1):109-14		
mCherry-SNX9	V. Haucke	Posor et al, Nature. 2013 Jul 11;499(7457):233-7		
GFP-SNX12	J. Gruenberg	Pons et al, PLoS One. 2012;7(6):e38949		
SNX17-GFP	R. Boettcher	Böttcher et al, Nat Cell Biol. 2012 May 6;14(6):584-92		
SNX27-GFP	R. Boettcher	Tseng et al, J Mol Biol. 2014 Sep 9;426(18):3180-3194		
SNX31-GFP	R. Boettcher	Tseng et al, J Mol Biol. 2014 Sep 9;426(18):3180-3194		
PX-p40phox-GFP	Addgene #19010	Derived from Addgene, deposited by M. Yaffe		
PHD-Ing2x3-GFP	Addgene #21589	Derived from Addgene, deposited by J. Yuan		
MLNx2-GFP	T. Balla	Hammond et al, PLoS One. 2015 Oct 13;10(10):e0139957		
P4M-SidM-GFP	T. Balla	Hammond et al, J Cell Biol. 2014 Apr 14;205(1):113-26		
TAPP1-PH-GFP	T. Balla	Kimber et al, Biochem J. 2002 Feb 1;361(Pt 3):525-36		
PH-PLC $\delta$ 1-GFP	T. Balla	Várnai & Balla, J Cell Biol. 1998 Oct 19;143(2):501-10.		
PH-AKT-GFP	T. Balla	Sason et al, Mol Biol Cell. 2009 Jan;20(1):544-55		
EGFP-C1	Clontech			
EGFP-C2	Clontech			
pGEX-2T	GE Healthcare			
GFP-SNX3 siRNA-insensitive		This work		
GFP-SNX3-Y71A		This work		
GFP-SNX3-Y71A siRNA-insensitive		This work		
GST-SNX3		This work		

GST-SNX3-Y71A		This work
GFP-SNX3-ΔN		This work
GFP-SNX3-PX+C		This work
GFP-SNX3-N+α		This work
GFP-SNX3-ΔC		This work
GFP-SNX3-C		This work
GFP-SNX3-ΔN siRNA-insensitive		This work
GFP-SNX3-PX+C siRNA-insensitive		This work
GFP-SNX3-ΔC siRNA-insensitive		This work
GFP-Gal9		This work
KIF5A-GFP-CIBN	Addgene #102252	Derived from Addgene, deposited by B. Cui
KIF5A-GFP		This work
Myo1e-GFP	M. Krendel	Ouderkirk & Krendel, Exp Cell Res. 2014 Apr 1;322(2):265-76
GFP-Rab35	A. Echard	Klinkert & Echard, Traffic. 2016 Oct;17(10):1063-77
GFP-Gal9-ΔCRD2		This work
GFP-Gal9-ΔN		This work
GFP-Gal9-linker+CRD2		This work
GFP-Gal9-N+CRD1		This work
GFP-Gal9-CRD1+linker		This work
GFP-Gal9-ACRD2 siRNA-insensitive		This work
GFP-Gal9-ΔN siRNA-insensitive		This work
GFP-Gal9-N+CRD1 siRNA-insensitive		This work
GFP-Gal9-CRD1+linker siRNA-insensitive		This work
GFP-Gal9-A46V siRNA-insensitive		This work
GFP-Gal9-N137A siRNA-insensitive		This work
GFP-Gal9-R252A siRNA-insensitive		This work
GFP-Gal9-A46V+N173A siRNA-insensitive		This work
flotillin-1-mCherry	A. Echard	Baumann et al., J Biol Chem 2012 Nov;287(47): 39664-72

flotillin-2-mCherry	A. Echard	Baumann et al., J Biol Chem 2012 Nov;287(47): 39664-72
GFP-Rab6a	J. Kremerskothen	Rzomb et al., Infect Immun. 2003 Oct;71(10):5855-70
GFP-Rab8a	D. Sheff	Henry & Sheff, Mol Biol Cell 2008 May;19(5):2059-68
GFP-Rab10	K. Simons	Schucke et el., Traffic 2007 Jan;8(1): Epub 2006
GFP-Rab18	Addgene #49550	Derived from Addgene, deposited by M. Scidmore
GFP-Rab21a	A.T. Jones	Simpson et al., J Cell Sci 2004 Sept;117:6297-6311
GFP-SNAP23	M. Coppolino	Williams et al., Mol Biol Cell 2014 Jul;25(13):2061-70