Table 1S Histomorphometric analysis of 9-wk-old *Mx1-cre;Ctsk*$^{fl/fl}$ mice and their control littermates

Parameters	Male		Female	
	Ctsk$^{fl/fl}$ (n=6)	*Mx1;Ctsk*$^{fl/fl}$ (n=5)	*Ctsk*$^{fl/fl}$ (n=4)	*Mx1;Ctsk*$^{fl/fl}$ (n=5)
BV/TV (%)	9.38±2.28	16.68±5.40*	5.01±2.54	16.42±2.39*
Tb.Th (μm)	36.93±8.14	40.27±6.63	26.10±1.95	39.45±2.24*
Tb.N (/mm)	2.55±0.31	4.06±0.76*	1.88±0.83	4.15±0.42*
Tb.Sp (μm)	361±52	213±54*	588±263	204±29*
MS/BS (%)	30.82±4.24	33.09±3.09	27.74±4.57	30.55±4.74
MAR (μm/day)	2.15±0.29	2.92±0.43*	2.80±0.23	3.72±0.25*
BFR/BS ($\mu m^3/\mu m^2$/year)	241±42	352±60*	286±64	418±86*
BFR/BV (%/year)	1324±326	1731±288	2095±397	1975±382
BFR/TV (%/year)	139±20	292±87*	116±58	334±66*
Ob.S/BS (%)	12.19±7.64	19.01±7.12	27.52±10.62	26.16±6.77
N.Ob/T.Ar (/mm^2)	49.02±22.90	126.04±45.70*	81.27±33.41	197.55±45.70*
N.Ob/B.Pm (/mm)	10.14±5.79	15.42±5.50	23.48±9.64	24.11±6.14
OV/TV (%)	0.06±0.09	0.25±0.22	0.09±0.10	0.55±0.36*
OS/BS (%)	4.78±6.10	8.71±7.02	6.49±4.45	15.91±8.57
O.Th (μm)	2.71±0.85	3.33±0.47	3.13±0.76	4.08±0.48
Oc.S/BS (%)	1.70±0.93	5.43±2.98*	1.59±0.42	3.69±0.51*
N.Oc/T.Ar (/mm^2)	3.04±1.32	15.05±8.85*	2.06±1.02	10.42±1.05*
N.Oc/B.Pm (/mm)	0.62±0.30	1.80±1.02*	0.55±0.13	1.26±0.12*
ES/BS (%)	0.78±0.42	1.38±0.95	0.46±0.08	0.82±0.34

Results are mean ± SD. *$p<0.05$ versus *Ctsk*$^{fl/fl}$ mice, unpaired *t* test

Table 2S Histomorphometric analysis of 9-wk-old male *CD11b;Ctsk*$^{fl/fl}$ mice and their control littermates

Parameters	*CD11b;Ctsk*$^{+/+}$ (n=7)	*CD11b;Ctsk*$^{fl/fl}$ (n=7)
BV/TV (%)	12.23±1.85	15.79±3.73*
Tb.Th (μm)	33.29±2.64	39.36±5.21*
Tb.N (/mm)	3.66±0.38	3.99±0.63
Tb.Sp (μm)	242±30	217±45
MS/BS (%)	30.98±4.38	36.86±16.16
MAR (μm/day)	1.46±0.30	2.55±0.74*
BFR/BS ($\mu m^3/\mu m^2$/year)	169±59	317±104*
BFR/BV (%/year)	924±299	1690±927
BFR/TV (%/year)	132±48	269±90*
Ob.S/BS (%)	5.24±2.45	10.92±6.11*
N.Ob/T.Ar (/mm^2)	31.19±13.81	68.88±42.53*
N.Ob/B.Pm (/mm)	4.30±1.85	8.34±4.39*
OV/TV (%)	0.04±0.04	0.10±0.09
OS/BS (%)	2.23±1.66	4.00±3.46
O.Th (μm)	2.36±0.35	3.07±0.56
Oc.S/BS (%)	0.91±0.65	2.31±1.23*
N.Oc/T.Ar (/mm^2)	2.63±1.62	6.67±3.96*
N.Oc/B.Pm (/mm)	0.36±0.23	0.79±0.41*
ES/BS (%)	0.47±0.34	0.81±0.36

Results are mean ± SD. *p<0.05 versus *CD11b;Ctsk*$^{+/+}$, unpaired *t* test

Table 3S Cortical bone μCT analysis of 9-wk-old male *Mx1;Ctsk*$^{fl/fl}$ mice and their control littermates

Parameters	*Ctsk*$^{fl/fl}$ (n=5)	*Mx1;Ctsk*$^{fl/fl}$ (n=5)
Total cross-sectional volume (mm^3)	1.40±0.08	1.55±0.19
Cortical volume (mm^3)	0.56±0.06	0.65±0.10
Marrow volume (mm^3)	0.83±0.07	0.90±0.11
Cortical thickness (mm)	0.17±0.02	0.18±0.01

Results are mean ± SD.

Table 4S Cortical bone μCT analysis of 9-wk-old male *CD11b;Ctsk*$^{fl/fl}$ mice and their control littermates

Parameters	*CD11b;Ctsk*$^{+/+}$ (n=6)	*CD11b;Ctsk*$^{fl/fl}$ (n=6)
Total cross-sectional volume (mm^3)	1.20±0.13	1.40±0.14*
Cortical volume (mm^3)	0.50±0.07	0.65±0.11*
Marrow volume (mm^3)	0.70±0.08	0.75±0.05
Cortical thickness (mm)	0.18±0.02	0.20±0.01

Results are mean ± SD. *$p<0.05$ versus *CD11b;Ctsk*$^{+/+}$ mice, unpaired *t* test

Table 5S Cortical bone μCT analysis of 13-week-old male *Osx;Ctsk*$^{fl/fl}$ mice and their control littermates

Parameters	*Osx;Ctsk*$^{+/+}$ (n=5)	*Osx;Ctsk*$^{fl/fl}$ (n=5)
Total cross-sectional volume (mm^3)	1.37±0.04	1.27±0.13
Cortical volume (mm^3)	0.61±0.04	0.60±0.05
Marrow volume (mm^3)	0.76±0.03	0.67±0.10
Cortical thickness (mm)	0.19±0.01	0.20±0.01

Results are mean ± SD.

Table 6S Histomorphometric analysis of 13-week-old *Osx;Ctsk*$^{fl/fl}$ mice and their control littermates

Parameters	Male		Female	
	Osx;Ctsk$^{+/+}$ (n=6)	*Osx;Ctsk*$^{fl/fl}$ (n=7)	*Osx;Ctsk*$^{+/+}$ (n=4)	*Osx;Ctsk*$^{fl/fl}$ (n=7)
BV/TV (%)	12.72±5.39	8.85±3.38	4.51±2.30	4.79±2.23
Tb.Th (μm)	33.28±7.01	30.95±5.16	27.84±4.96	30.57±3.88
Tb.N (/mm)	3.73±0.98	2.78±0.67	1.55±0.55	1.55±0.62
Tb.Sp (μm)	252±86	348±99	687±276	687±210
MS/BS (%)	19.86±8.28	27.32±5.97	36.68±4.20	35.77±4.79
MAR (μm/day)	1.38±0.41	1.48±0.22	1.95±0.13	1.97±0.26
BFR/BS ($\mu m^3/\mu m^2$/year)	107±75	146±29	261±29	257±44
BFR/BV (%/year)	581±395	861±212	1971±400	1676±258
BFR/TV (%/year)	94±80	88±30	83±30	85±44
Ob.S/BS (%)	8.93±5.55	7.60±3.87	19.37±9.13	19.36±8.41
N.Ob/T.Ar (/mm^2)	59.22±44.72	37.56±21.21	51.42±24.58	53.70±35.75
N.Ob/B.Pm (/mm)	7.46±4.25	6.76±3.39	17.76±9.80	17.05±6.89
OV/TV (%)	0.07±0.06	0.05±0.04	0.06±0.06	0.05±0.03
OS/BS (%)	2.70±1.84	2.86±1.84	7.32±5.82	6.55±4.50
O.Th (μm)	3.41±0.41	2.86±0.51	2.90±0.36	2.80±0.60
Oc.S/BS (%)	1.58±1.16	2.04±0.97	1.47±0.65	2.20±1.36
N.Oc/T.Ar (/mm^2)	4.88±4.41	3.63±0.97	1.75±1.17	2.70±2.35
N.Oc/B.Pm (/mm)	0.61±0.43	0.66±0.14	0.54±0.26	0.82±0.45
ES/BS (%)	0.66±0.42	0.71±0.63	0.91±0.64	0.81±0.53

Results are mean ± SD.

Table 7S Sequences of oligonucleotides for qRT-PCR analysis

Gene	Forward primer	Reverse primer
Alp	CTTGACTGTGGTTACTGCTGATCA	GTATCCACCGAATGTGAAAACGT
Type I collagen	CCCAAGGAAAAGAAGCACGTC	ACATTAGGCGCAGGAAGGTCA
Dmp1	ATGACTGTCAGGACGGCTAC	AGTTATAGTGAACTCTCTAC
Runx2	AGTCCCAACTTCCTGTGCTCC	CGGTAACCACAGTCCCATCTG
Osteopontin	CTCCAATCGTCCCTACAGTCG	CCAAGCTATCACCTCGGCC
RANKL	CAAGCTCCGAGCTGGTGAAG	CCTGAACTTTGAAAGCCCCA
OPG	AAGAGCAAACCTTCCAGCTGC	CACGCTGCTTTCACAGAGGTC
Ctsk	AGGCATTGACTCTGAAGATGCT	TCCCCACAGGAATCTCTCTG
Sphk1	TGAGGTGGTGAATGGGCTAATGGA	AACAGCAGTGTGCAGTTGATGAGC
Sphk2	TGGGCTGTCCTTCAACCTCATACA	AGTGACAATGCCTTCCCACTCACT
Efnb2	TCTGTGTCATCGGTTGGCTACGTT	ACAGACGCACAGGACACTTCTCAA
Wnt10b	AGGCTTCTCCTTCCGTTCAGTTGT	ATTCCCACCCTTCCTGCTGAAGAA
Bmp6	AGAAGGGCACTCTTTCAGGTTCCA	TCACACCACCGAGAGTCAACACAA
Gapdh	TGCACCACCAACTGCTTAG	GGATGCAGGGATGATGTTC

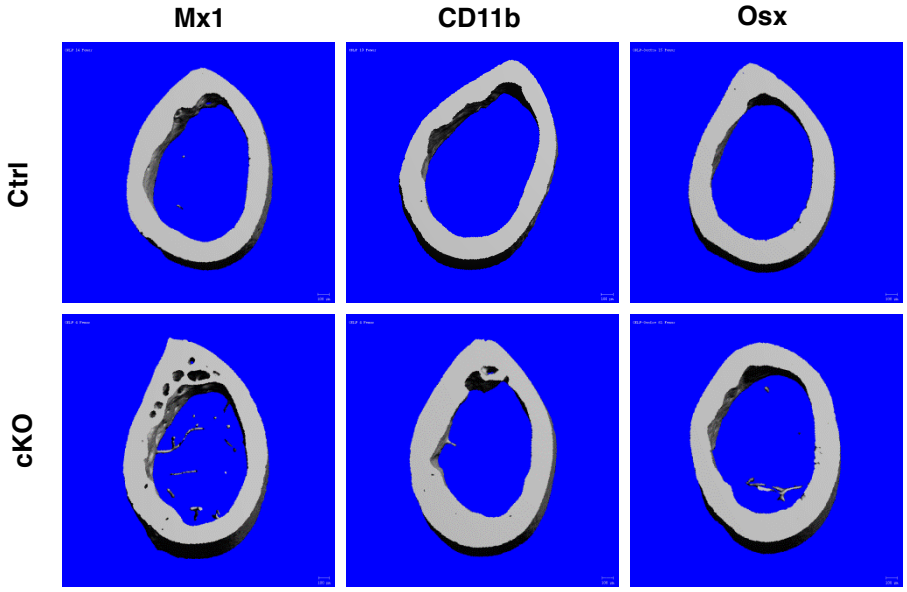

Figure 1S μCT images of cortical bone in three conditional knockouts (cKO) and their controls (Ctrl). Deletion of *Ctsk* in osteoclasts (CD11b) increases total cross-sectional volume (1.20±0.05 vs 1.40±0.06, $p<0.05$) and cortical bone volume (0.50±0.03 vs 0.65±0.05, $p<0.05$).

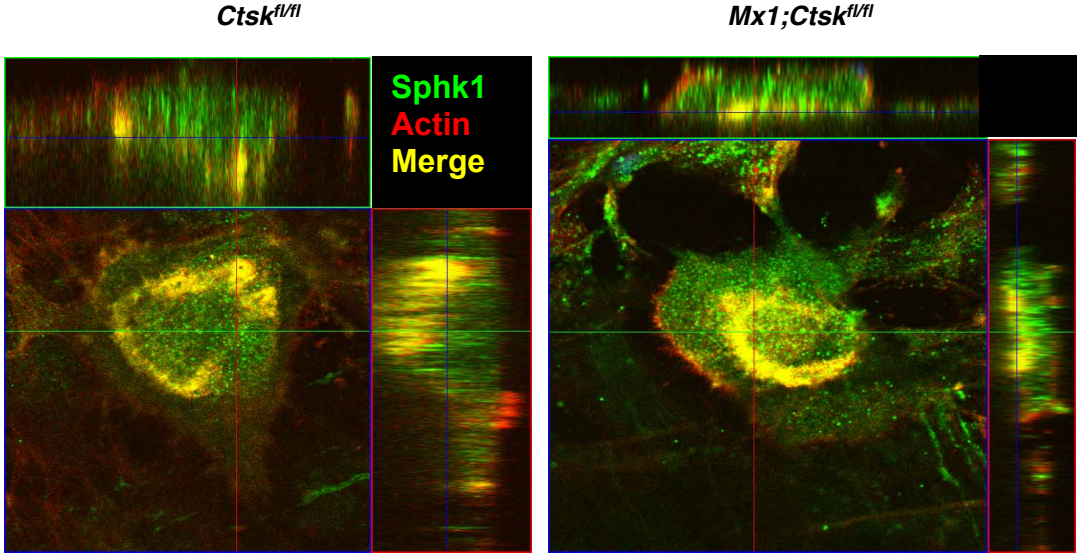

Figure 2S Defective bone resorption in *Mx1;Ctsk*$^{fl/fl}$ osteoclasts. High magnification of Z-axis images of individual podosome showing shallower resorption pit in *Mx1;Ctsk*$^{fl/fl}$ osteoclasts grown on dentin slices. The localization of Sphk1 (green) and actin (red) were analyzed by confocal microscope.

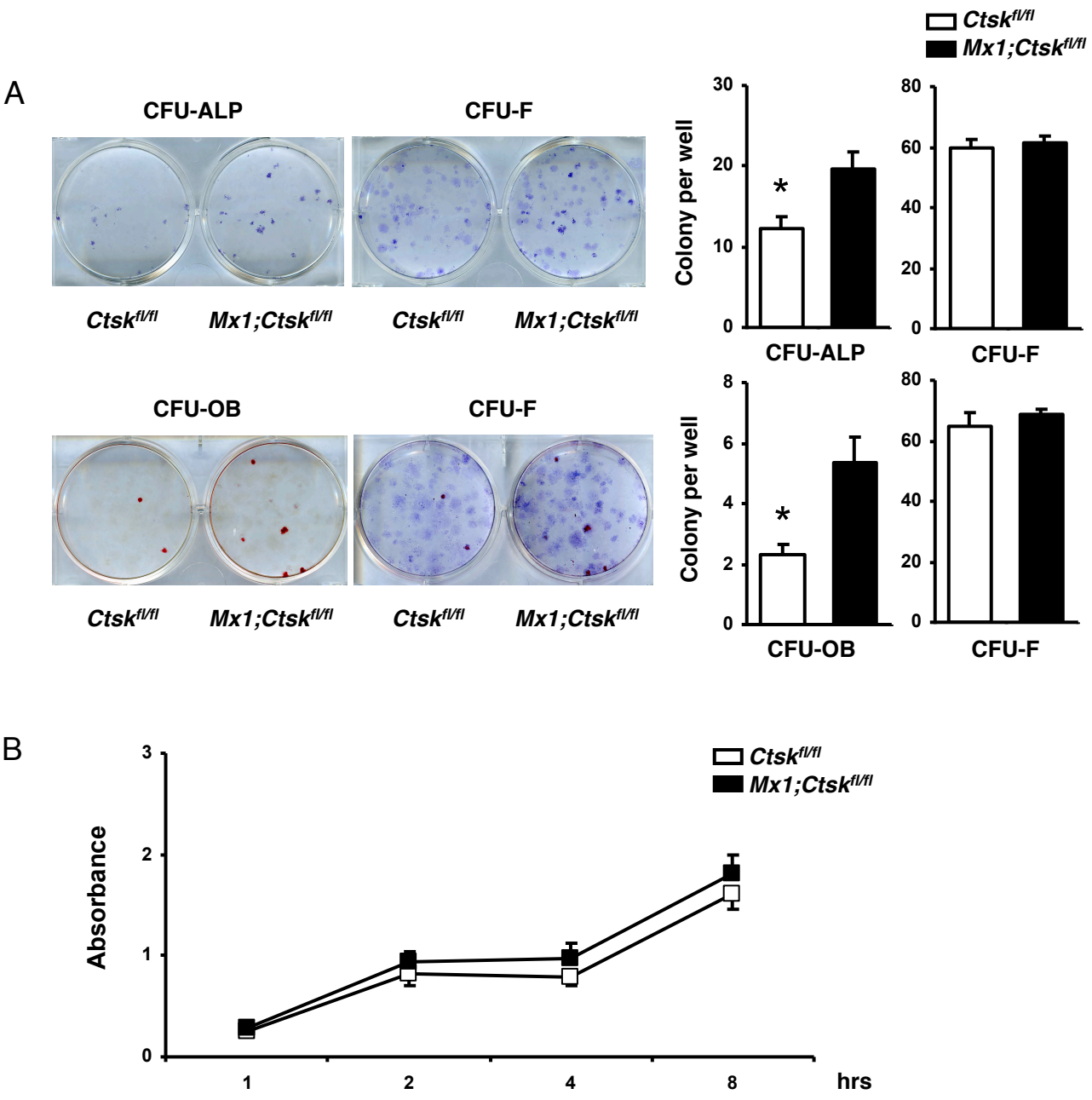

Figure 3S Deletion of *Ctsk* in hematopoietic cells increases osteoblast precursors and differentiation but not proliferation. (A) Cultured osteoblasts derived from long bones of *Mx1;Ctsk*$^{fl/fl}$ mice and control littermates were stained for ALP and alizarin red. *Mx1;Ctsk*$^{fl/fl}$ osteoblasts contain an increased number of CFU-ALP and CFU-OB but not CFU-F. Results are mean ± SEM. *$p < 0.05$ versus controls. (B) Time course of cell proliferation assessed by BrdU labeling showed no changes in proliferation of *Mx1;Ctsk*$^{fl/fl}$ osteoblasts.

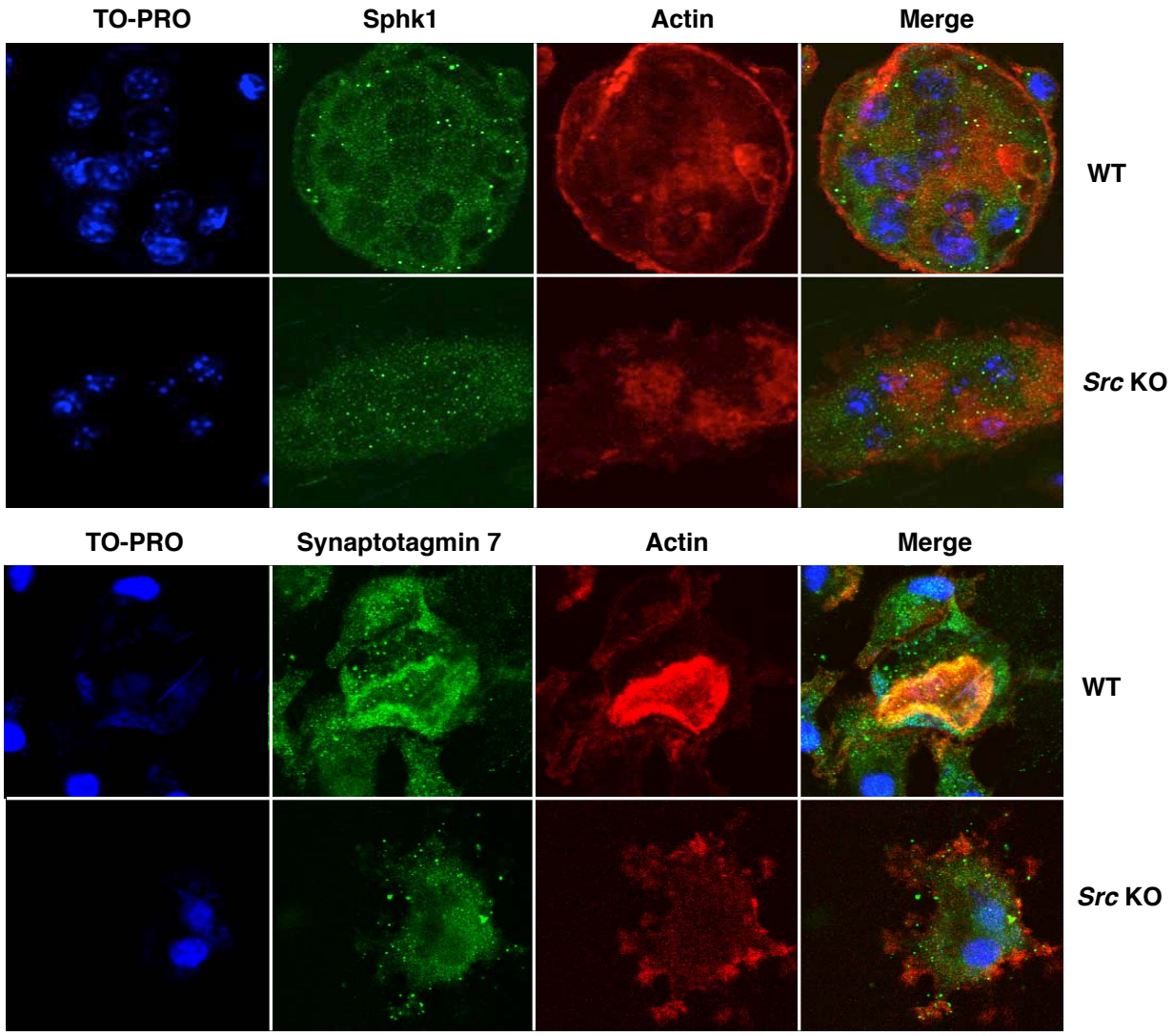

Figure 4S Absence of Src does not alter Sphk1 and Synaptotagmin 7 expression. Osteoclasts were generated by co-culture with osteoblasts on collagen gel in the presence of vitamin D_3 and PGE_2. Cells were replated onto dentin slices and cultured for 48 h and immunolabeled for Sphk1 and synaptotagmin 7 (green) and actin (red). Cells were visualized by scanning confocal immunofluorescence microscopy.