

Supplementary Materials for

Aberrant Rac1-cofilin signaling mediates defects in dendritic spines, synaptic function, and sensory perception in fragile X syndrome

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This PDF file includes:

Fig. S1. Validation of somatosensory cortex synapse enrichment.

Fig. S2. Subcutaneous administration of hydroxypropyl- β -cyclodextrin (vehicle) has little to no effect on cofilin signaling.

Fig. S3. Mature *Fmr1* KO mice exhibit normal Rac1 signaling at somatosensory cortex synapses.

Fig. S4. Mature *Fmr1* KO mice exhibit normal cofilin phosphorylation and activity at somatosensory cortex synapses.

Table S1. Summary of statistics for Fig. 1.

Table S2. Summary of statistics for Fig. 2.

Table S3. Summary of statistics for Fig. 3.

Table S4. Summary of statistics for Fig. 4.

Table S5. Summary of statistics for Fig. 5.

Table S6. Summary of statistics for Fig. 6.

Table S7. Summary of statistics for fig. S2.

Table S8. Summary of statistics for figs. S3 and S4.



Figure S1. Validation of somatosensory cortex synapse enrichment. Representative western blot from a 1-week-old wild-type (WT) mouse showing enrichment of presynaptic (VAMP2 and SV2) and postsynaptic (PSD95) markers as well the absence of a nuclear marker (Histone H3) in somatosensory cortex synaptosome fractions versus total lysates.



Figure S2. Subcutaneous administration of hydroxypropyl- β -cyclodextrin (vehicle) has little to no effect on cofilin signaling. (A to C) Representative Western blots (top) and summary data (bottom) from somatosensory synaptosomes of 1- to 2-month-old wild-type mice, either untreated (left) or treated with hydroxypropyl- β -cyclodextrin (vehicle; right). Synaptosomes from vehicle-treated animals exhibited no change in the abundance of phosphorylated (p-) cofilin (A), p-LIMK1 (B), and p-Slingshot1 (C) relative to those from untreated animals (n = 7 animals per group).



Figure S3. Mature *Fmr1* KO mice exhibit normal Rac1 signaling at somatosensory cortex synapses. (A to E) Representative Western blots (top) and summary data (bottom) from somatosensory lysates (A) or synaptosomes (B to E) of 4-week-old WT and *Fmr1* KO mice showing relative abundance of active, phosphorylated or total proteins in the Rac1 signaling pathway: (A) Rac1-GTP, (B) p-PAK1, (C) p-LIMK1, (D) p-Slingshot1, and (E) F-actin/G-actin ratio (n = 5 - 12 animals per group). (F to J) Representative western blots (top) and summary data (bottom) from somatosensory lysates (F) or synaptosomes (G to J) of 2- to 5-month-old WT and *Fmr1* KO mice showing relative abundance of active, phosphorylated or total proteins in the Rac1 signaling pathway, as in (A to E) (n = 4 - 10 animals per group). Data are mean ± SEM. *p < 0.05.

Synaptosomes (2 - 5 months)



Figure S4. Mature *Fmr1* KO mice exhibit normal cofilin phosphorylation and activity at somatosensory cortex synapses. (A) Representative wWstern blots and summary data of cofilin phosphorylation in somatosensory synaptosomes from 2- to 5-month-old WT and *Fmr1* KO mice (9 - 14 mice per group). Membranes were probed with antibodies to p-cofilin and total cofilin.

Figure	N	Mean ± SEM	p value	Statistical Test
1A	WT (p-Cofilin) = 12 animals	1.000 ± 0.2277	_	Two-tailed
	KO (p-Cofilin) = 12 animals	1.764 ± 0.2814	*. 0.046	unpaired t-
	WT (Cofilin) = 12 animals	1.000 ± 0.05896	_	test, WT vs
	KO (Cofilin) = 12 animals	1.080 ± 0.05266	0.327	KO
1B	WT (p-Cofilin) = 7 animals	1.000 ± 0.1544	-	
	KO (p-Cofilin) = 7 animals	1.115 ± 0.2765	0.729	
	WT (Cofilin) = 7 animals	1.000 ± 0.1190	-	
	KO (Cofilin)= 7 animals	0.9104 ± 0.1131	0.5952	
1C	WT (p-Cofilin) = 12 animals	1.000 ± 0.1458	-	
	KO (p-Cofilin) = 10 animals	1.958 ± 0.4158	*, 0.030	
	WT (Cofilin) = 12 animals	1.000 ±0.0918	-	
	KO (Cofilin)= 10 animals	0.7626 ± 0.0823	0.073	
1D	WT (p-Cofilin) = 10 animals	1.000 ± 0.1355	-	
	KO (p-Cofilin) = 9 animals	1.035 ± 0.2629	0.9038	
	WT (Cofilin) = 10 animals	1.000 ± 0.1106	-	
	KO (Cofilin)= 9 animals	1.087 ± 0.1015	0.5710	
1E	WT (F/G actin) = 5 animals	1.000 ± 0.4336	-	
	KO (F/G actin) = 6 animals	3.495 ± 0.7284	*, 0.021	
1G	WT = 3 animals / 12 dendrites	100.0 ± 4.957	-	
(% Filopodia)	KO = 3 animals / 13 dendrites	126.2 ± 5.838	***, 0.0009	
1G	WT = 3 animals / 12 dendrites, 154	100.0 ± 7.941	-	
(% Density)	segments, 660 spines			
	KO = 3 animals / 13 dendrites, 182	131.1 ± 11.82	*, 0.0429	
	segments, 933 spines			

Table S1. Summary of statistics for Fig. 1.

Table S2. Summary of statistics for Fig. 2.

Figure	N	Mean ± SEM	p value	Statistical
				lest
2A	WT (Rac1-GTP) = 4 animals	1.000 ± 0.0396	-	Two-tailed
	KO (Rac1-GTP) = 4 animals	1.673 ± 0.0396	****,<0.0001	unpaired t-
	WT (Rac1) = 4 animals	1.000 ± 0.0327	-	test, WT vs KO
	KO (Rac1) = 4 animals	1.065 ± 0.0320	0.207	
2B	WT (p-PAK1) = 12 animals	1.000 ± 0.0904	-	
	KO(p-PAK1) = 15 animals	1.791 ± 0.3295	*, 0.047	
	WT (PAK1) = 12 animals	1.000 ± 0.0865	-	
	KO (PAK1) = 15 animals	1.109 ± 0.1615	0.327	
2C	WT (p-PAK4) = 8 animals	1.000 ± 0.1862	_	
	KO(p-PAK4) = 7 animals	1.133 ± 0.3728	0.745	
	WT $(PAK4) = 8$ animals	1.000 ± 0.1111	-	
	KO (PAK4) = 7 animals	1.126 ± 0.1913	0.567	
2D	WT (p-LimK1) = 10 animals	1.000 ± 0.0929	_	
	KO(p-LimK1) = 9 animals	2.047 ± 0.4434	*, 0.020	
	WT (LimK1) = 10 animals	1.000 ± 0.0730	-	
	KO(LimK1) = 9 animals	0.9765 ± 0.1525	0.887	
2E	WT (p-SSH1) = 8 animals	1.000 ± 0.1109	_	
	KO(p-SSH1) = 5 animals	1.764 ± 0.3759	*, 0.037	
	WT (SSH1) = 8 animals	1.000 ± 0.1424	_	
	KO(SSH1) = 5 animals	1.148 ± 0.3567	0.673	

Table S3.	Summary	of	statistics	for	Fig.	3.

Figure	N	Mean ± SEM	p value	Statistical Test
			(p < 0.05)	
3A	WT vehicle (p-LimK1) = 13 animals	1.000 ± 0.2463	-	Two-way ANOVA
	KO vehicle (p- LimK1) = 12 animals	2.1853 ± 0.3478	*, 0.012	with Tukey's post-
	WT FRAX486 (p- LimK1) = 13 animals	0.9886 ± 0.2433	0.999	test (WT vehicle vs
	KO FRAX486 (p- LimK1) = 11 animals	0.9751 ± 0.1843	*, 0.016	KO vehicle, WT
	WT vehicle (LimK1) = 13 animals	1.000 ± 0.1247	-	vehicle vs WT
	KO vehicle (LimK1) = 12 animals	0.9795 ± 0.1466	0.999	FRAX486 and KO
	WT FRAX486 (LimK1) = 13 animals	1.0094 ± 0.1750	0.999	vehicle vs KO
	KO FRAX486 (LimK1) = 11 animals	1.1066 ± 0.2149	0.953	FRAX486)
3B	WT vehicle (p-SSH1) = 12 animals	1.000 ± 0.3312	-	
	KO vehicle (p-SSH1) = 10 animals	2.3299 ± 0.2033	**, 0.003	
	WT FRAX486 (p-SSH1) = 8 animals	1.3092 ± 0.2307	0.847	
	KO FRAX486 (p-SSH1) = 10 animals	1.1526 ± 0.2792	*, 0.013	
	WT vehicle (SSH1) = 12 animals	1.000 ± 0.3930	-	
	KO vehicle (SSH1) = 10 animals	0.8570 ± 0.3334	0.986	
	WT FRAX486 (SSH1) = 8 animals	0.7212 ± 0.1630	0.910	
	KO FRAX486 (SSH1) = 10 animals	0.7692 ± 0.1949	0.999	
3C	WT vehicle (p-Cofilin) = 14 animals	1.0000 ± 0.2270	-	
	KO vehicle (p-Cofilin) = 14 animals	2.0817 ± 0.2463	**, 0.006	
	WT FRAX486 (p-Cofilin) = 15 animals	0.9440 ± 0.2045	0.998	
	KO FRAX486 (p-Cofilin) = 9 animals	0.7251 ± 0.2293	**, 0.002	
	WT vehicle (Cofilin) = 14 animals	1.0000 ± 0.2013	-	
	KO vehicle (Cofilin) = 14 animals	1.0703 ± 0.2069	0.995	
	WT FRAX486 (Cofilin) = 15 animals	0.8696 ± 0.1850	0.966	
	KO FRAX486 (Cofilin) = 9 animals	1.0339 ± 0.2354	0.999	
3D	WT vehicle (F/G actin) = 10 animals	1.0000 ± 0.1430	-	
	KO vehicle (F/G actin) = 9 animals	1.9193 ± 0.3043	*, 0.012	
	WT FRAX486 (F/G actin) = 10 animals	1.0028 ± 0.1529	0.999	
	KO FRAX486 (F/G actin) = 7 animals	1.0919 ± 0.1543	*, 0.048	

Table S4. Summary of statistics for Fig. 4.

4B (cultured neurons) 3 independent experiments No virus (F/G actin) = 14 wells GFP (F/G actin) = 13 wells UT-Cofilin (F/G actin) = 13 wells Cofilin S3A (F/G actin) = 13 wells Cofilin S3A (F/G actin) = 15 wells Cofilin S3A (F/G actin) = 15 wells 1.000 ± 0.1266 0.9950 ± 0.1625 0.9988	Figure	Ν	Mean ± SEM	p value	Statistical Test
4B (cultured neurons) 3 independent experiments No virus (FG actin) = 13 wells 1.000 ± 0.1266 - One-way ANOVA Mo virus (FG actin) = 13 wells 1.007 ± 0.1602 0.988 0.998 virus vs. Cofilin S3A, (FG actin) = 15 wells 0.998 ± 0.1625 0.988 post-test (No virus vs. Cofilin S3A, No virus vs. Cofilin S3D (F/G actin) = 15 wells 0.4280 ± 0.0750 0.988 Cofilin S3A, No virus vs. Cofilin S3A, No virus vs. Cofilin S3D (F/G actin) = 15 wells 0.988 ± 0.1421 0.988 Cofilin S3A, No virus vs. Cofilin S3A, No virus vs. Cofilin S3A, No virus vs. Cofilin S3D 4D (Spine length) WT (GFP) = 10 animals, 21 neurons, 1286 spines, 196 segments of 10 µm KO (GFP) es animals, 15 neurons, 765 spines, 119 segments of 10 µm KO (S3A) = 6 animals, 15 neurons, 765 spines, 123 segments of 10 µm KO (S3A) = 6 animals, 15 neurons, 887 spines, 123 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (GFP) - same as above 0.7354 ± 0.0129 . 0.092 ± 0.0520 (GFP) wr KO (S3A), and WT (WT. Cof.) WT (WT. Cof.) KO (WT. Cof.) 0.6046 ± 0.0146 . 0.033 0.541 * (OO) WT (WT. Cof.) KO (WT. Cof.) 0.898 . 0.6356 ± 0.0173 ****, <0.0001 WT (WT. Cof.) KO (WT. Cof.) WT (WT. Cof.) KO (WT. Cof.) WT (WT. Cof.) WT (WT. Cof.) WT (WT. Cof.) KO (WT. Cof.) WT (WT. Cof.) WT (WT. Cof.) KO (GFP) 0.8109 . 0.222 . 0.0730 0.999 ****, <0.0001 . 0.398 4C (%Stubby/ Mushroom) WT (GFP) - same as above KO (GSA) 0.7223 ± 0.0220 . 0.723 **				(p < 0.05)	
(cutured neurons) No virus (F/G actin) = 14 wells 1.0000 ± 0.1266 - with Tukey's (coliin S3A) GFP (F/G actin) = 15 wells 1.0107 ± 0.1602 0.986 virus vs Cofilin (coliin S3D (F/G actin) = 13 wells 0.4280 ± 0.0750 *, 0.042 0.986 virus vs Cofilin (coliin S3D (F/G actin) = 15 wells 1.0338 ± 0.1421 0.986 virus vs Cofilin S3A, No virus vs (spine spines, 196 segments of 10 µm 1.1442 ± 0.0532 - Two-way ANOVA with Tukey's post-test (WT (GFP) = 8 animals, 20 neurons, 1381 1.3326 ± 0.0494 *, 0.043 virus vs Cofilin S3A) (spine length) WT (Cof.) = 7 animals, 15 neurons, 765 1.0087 ± 0.0403 0.370 (GFP) vs KO (GFP) vs KO (GFP) vs WT (GFP) vs KO 0.9462 ± 0.0364 *, 0.047 (WT (Cof.), vs KO (WT (Cof.) kSO (GFP) vs KO (GFP) vs KO </th <th>4B</th> <th>3 independent experiments</th> <th></th> <th></th> <th>One-way ANOVA</th>	4B	3 independent experiments			One-way ANOVA
neurons) GFP (F/G actin) = 13 wells Cofilin GFA actin) = 15 wells 1.1017 ± 0.1602 0.986 post-test virus vs cofilin S3A (F/G actin) = 15 wells Cofilin S3A (F/G actin) = 13 wells Cofilin S3D (F/G actin) = 15 wells 0.986 ± 0.1625 0.998 S3A, No virus vs GFP, No virus vs Cofilin S3A, No virus vs Cofilin S3D) 4D (Spine length) WT (GFP) = 10 animals, 21 neurons, 1286 spines, 196 segments of 10 µm WT (WT. Cof.) = 7 animals, 15 neurons, 765 spines, 170 segments of 10 µm WT (WT. Cof.) = 7 animals, 15 neurons, 765 spines, 119 segments of 10 µm WT (WT. Cof.) = 7 animals, 15 neurons, 877 spines, 123 segments of 10 µm WT (S3A) = 6 animals, 15 neurons, 887 spines, 123 segments of 10 µm WT (S3A) = 6 animals, 17 neurons, 887 spines, 123 segments of 10 µm WT (WT. Cof.) 0.9462 ± 0.0364 *, 0.047 (GFP) vs KO (GFP) vs KO (GFP) vs KO (GFP) vs KO 4E WT (GFP) – same as above 0.7354 ± 0.0129 - - (KO (GFP) 0.6509 ± 0.0221 * * 0.0331 4F WT (GFP) – same as above 0.7354 ± 0.0129 - - (KO (GFP) 0.6509 ± 0.0221 * 0.0031 - (KO (GFP) 0.6509 ± 0.0221 * 0.0031 - (KO (GFP) 0.6509 ± 0.0221 * 0.0031 - (KO (GFP) <t< th=""><th>(cultured</th><th>No virus (F/G actin) = 14 wells</th><th>1.0000 ± 0.1266</th><th>-</th><th>with Tukey's</th></t<>	(cultured	No virus (F/G actin) = 14 wells	1.0000 ± 0.1266	-	with Tukey's
4E WT (GFP) = same as above 0.9350 ± 0.1625 0.0814 ± 0.01625 0.988 0.9850 ± 0.1625 virus vs Coffilm S3A, No virus vs Coffilm S3D (F/G actin) = 15 wells 4D WT (GFP) = 10 animals, 21 neurons, 1286 1.0938 ± 0.1421 0.988 GFP, No virus vs Coffilm S3A, No virus vs Coffilm S3A, No virus vs Coffilm S3D 4D WT (GFP) = 10 animals, 21 neurons, 1286 1.1442 ± 0.0532 - - KO (GFP) = 8 animals, 20 neurons, 1381 1.3326 ± 0.0494 *, 0.043 0.370 spines, 170 segments of 10 µm 1.2496 ± 0.0519 *, 0.043 0.370 Spines, 171 segments of 10 µm 1.2496 ± 0.0519 *, 0.019 (GFP) vs (KO (GFP) vs (KO (GFP) vs (KO (GFP) ws (KD) vitts vs Coffilm S3A, 16 neurons, 887 *, 0.043 *, *, 0.047 (GFP) vs (KO (GFP) vs (KO (GFP) vs (KO (SA)) = 7 animals, 17 neurons, 1119 spines, 132 segments of 10 µm 0.9298 ± 0.0520 *****, <0.0001 (WT (Cof.), KO (WT. Cof.) 0.6509 ± 0.0221 ** ****, <0.0001 width) WT (WT. Cof.) 0.6509 ± 0.0221 ****, <0.0001 *****, <0.0001 WT (Cof.) (GFP) (Spine head WT (GFP) = same as above 0.7354 ± 0.0129 - *****, <0.000	neurons)	GFP (F/G actin) = 13 wells	1.1017 ± 0.1602	0.986	post-test (No
40 Cofilin S3A (F/G actin) = 13 wells Cofilin S3D (F/G actin) = 15 wells 0.4280 ± 0.0750 1.0938 ± 0.1421 *, 0.042 9.988 S3A, No virus vs Cofilin S3A, No virus vs Cofilin S3A, No vi		WT-Cofilin (F/G actin) = 15 wells	0.9950 ± 0.1625	0.998	virus vs Cofilin
Cofilin S3D (F/G actin) = 15 wells 1.0938 ± 0.1421 0.988 GFP, No virus vs Cofilin S3A, No virus vs S0D 4D (Spine length) WT (GFP) = 10 animals, 21 neurons, 1286 spines, 196 segments of 10 µm WT (WT. Cof.) = 7 animals, 15 neurons, 1381 spines, 170 segments of 10 µm WT (WT. Cof.) = 7 animals, 15 neurons, 765 spines, 119 segments of 10 µm KO (WT. Cof.) = 9 animals, 15 neurons, 911 spines, 123 segments of 10 µm KO (S3A) = 6 animals, 16 neurons, 887 spines, 132 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 817 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm WT (WT. Cof.) KO (WT. Cof.) WT (S3A) = 6 anime segment of 10 µm WT (WT. Cof.) KO (WT. Cof.) KO (WT. Cof.) WT (S3A) = 6 anime segment of 10 µm WT (WT. Cof.) KO (S3A) = 7 animals, 17 neurons, 1119 spines, 123 segment of 10 µm WT (WT. Cof.) KO (S3A) = 7 animals, 17 neurons, 1119 spines, 123 segment of 10 µm WT (WT. Cof.) WT (S3A) = 6 anime segment of 10 µm WT (WT. Cof.) KO (WT. Cof.) WT (S3A) = 6 anime segment of 10 µm WT (WT. Cof.) WT (S3A) = 6 anime segment of 10 µm WT (WT. Cof.) WT (S3A) = 6 anime segment of 10 µm WT (WT. Cof.) WT (S3A) = 6 anime segment of 10 µm WT (WT. Cof.) WT (S3A) = 6 anime segment of 10 µm WT (WT. Cof.) WT (S3A) = 6 anime segment of 10 µm WT (WT. Cof.		Cofilin S3A (F/G actin) = 13 wells	0.4280 ± 0.0750	*, 0.042	S3A, No virus vs
4D WT (GFP) = 10 animals, 21 neurons, 1286 1.1442 ± 0.0532 - Two-way ANOVA (Spine length) spines, 196 segments of 10 µm 1.3326 ± 0.0494 *, 0.043 with Tukey's post-test WT (WT. Coft) = 7 animals, 15 neurons, 765 1.0087 ± 0.0403 0.370 (GFP) wK (GFP) wK (GFP) wK (GFP) ws WT spines, 123 segments of 10 µm WT (WT. Coft) = 7 animals, 15 neurons, 911 1.2496 ± 0.0519 *, 0.019 (GFP) ws WT wT (SA) = 6 animals, 16 neurons, 887 0.9462 ± 0.0364 *, 0.047 (GFP) ws WT (WT. Coft), KO (Signe head WT (GFP) - same as above 0.7354 ± 0.0129 - (WT. Coft) (GFP) vs KO (Signe head WT (GFP) - same as above 0.7354 ± 0.0129 - (WT. Coft) (GFP) width) WT (GFP) - same as above 0.7354 ± 0.0129 - (WT. Coft) (WT. Coft) (BFP) + 0.031 width ratio) WT (GFP) - same as above 1.6570 ± 0.0709 - - (WT. Coft) (WT. Coft) 0.899 width ratio) WT (GFP) - same as above 0.6856 ± 0.0177 ****, <0.0001 *****, <0.0001 ****, <0.0001		Cofilin S3D (F/G actin) = 15 wells	1.0938 ± 0.1421	0.988	GFP, No virus vs
4D WT (GFP) = 10 animals, 21 neurons, 1286 1.1442 \pm 0.0532 - Two-way ANOVA (Spine length) WT (GFP) = 8 animals, 20 neurons, 1381 1.3326 \pm 0.0494 *, 0.043 with Tukey's pairs, 170 segments of 10 µm WT (WT. Cof.) = 7 animals, 15 neurons, 765 1.0087 \pm 0.0403 0.370 with FW (GFP) vs K0 spines, 170 segments of 10 µm K0 (GFT) = 8 animals, 15 neurons, 765 1.0087 \pm 0.0403 0.370 wWT (GFP) vs K0 WT (WT. Cof.) = 9 animals, 15 neurons, 887 spines, 132 segments of 10 µm 0.9462 \pm 0.0364 *, 0.019 WT (C. Cof.) K0 (GFP) vs K0 WT (GFP) - same as above 0.7354 \pm 0.0129 - ****, <0.0001 (WT. Cof.) (GFP) vs K0 width) WT (GFP) - same as above 0.7354 \pm 0.0129 - * 0.009 WT (WT. Cof.) 0.6104 \pm 0.0139 0.541 * 0.0001 (WT. Cof.) WT (WT. Cof.) 0.6366 \pm 0.0173 * * 0.001 WT. Cof.) WT (GFP) - same as above 1.6570 \pm 0.0709 - 0.031 * * WT (GFP) - same as above					Cofilin S3A, No
					virus vs Cofilin
4D WT (GFP) = 10 animals, 21 neurons, 1286 1.1442 ± 0.0532 - Two-way ANOVA (Spine length) KO (GFP) = 8 animals, 20 neurons, 1381 1.3326 ± 0.0494 *, 0.043 with Tukey's post-test WT (WT. Cof.) = 7 animals, 15 neurons, 765 1.0887 ± 0.0403 0.370 (GFP) wS (O (GFP) wS (O (GFP) ws (VT (GFP) vs (VT (GFP) vs (VT (CSA)) = 6 animals, 15 neurons, 887 wT (S3A) = 6 animals, 16 neurons, 887 *, 0.047 *, 0.019 (WT Cof.) KO (SA), and WT (WT. Cof.) 0.9462 ± 0.0364 *, 0.047 (GFP) vs (VT (WT. Cof.) KO (SA), and WT 4E WT (GFP) - same as above 0.7354 ± 0.0129 - - (WT. Cof.) (WT. Cof.) WT (WT. Cof.) 0.6911 ± 0.0139 0.541 *, 0.001 (WT. Cof.) (WT. Cof.) (WT. Cof.) (WT. Cof.) (WT. Cof.) (WT. Cof.) (WT (GFP) - same as above 1.6570 ± 0.0709 - . . width ratio) WT (WT. Cof.) 0.6366 ± 0.0191 *, 0.0001 					S3D)
(Spine length) spines, 196 segments of 10 µm KO (GFP) = 8 animals, 20 neurons, 1381 spines, 170 segments of 10 µm WT (WT. Cof.) = 7 animals, 15 neurons, 765 spines, 119 segments of 10 µm KO (WT. Cof.) = 9 animals, 15 neurons, 911 spines, 123 segments of 10 µm WT (S3A) = 6 animals, 16 neurons, 887 spines, 132 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 811 spines, 132 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (GFP) - same as above 0.7354 ± 0.0129 0.7354 ± 0.0129 	4D	WT (GFP) = 10 animals, 21 neurons, 1286	1.1442 ± 0.0532	-	Two-way ANOVA
length) KO (GFP) = 8 animals, 20 neurons, 1381 1.3326 ± 0.0494 *, 0.043 post-test wT (GFP) = 8 animals, 15 neurons, 765 1.0087 ± 0.0403 0.370 (WT (GFP) vs KO (GFP), WT (GFP) vs WT (GPP) vs WT (S3A), WT (SFP) vs WT (S3A), WT (SSA), WT (SSA) 0.370 (SFP) vs WT (SAA), WT (GFP) vs WT (SAA), WT (SSA) 0.9462 ± 0.0364 *, 0.019 (WT. Cof.), KO (GFP) vs WT (SAA), WT (SSA) 0.9462 ± 0.0364 *, 0.047 (SAA), and WT (WT. Cof.), KO (GFP) vs KO (SAA) VWT (S3A) = 7 animals, 16 neurons, 887 spines, 132 segments of 10 µm 0.9462 ± 0.0364 *, 0.047 (SAA), and WT (WT. Cof.), KO (GFP) vs KO (SAA) Spines, 151 segments of 10 µm 0.9298 ± 0.0520 *****, <0.0001 (WT. Cof.) vs KO (WT. Cof.) 0.6509 ± 0.0221 - Width WT (GFP) - same as above 0.7354 ± 0.0129 - *, 0.033 (WT. Cof.) (WT. Cof.) 0.6461 ± 0.0139 0.541 WVT (WT. Cof.) 0.6356 ± 0.0191 *, 0.031 *, 0.031 ****, <0.0001 WT (SA) WT (GFP) - same as above 1.6570 ± 0.0799 *****, <0.0001 *****, <0.0001 *****, <0.0001 WT (SA) 0.819 *****, <0.0001 0.8398 *****, <0.0001	(Spine	spines, 196 segments of 10 μ m			with Tukey's
4F WT (GFP) - same as above 0.7354 ± 0.019 - (WT. Cof.) - (Spine head width) WT (GFP) - same as above 0.7354 ± 0.019 * 0.031 4F WT (GFP) - same as above 0.6356 ± 0.0191 * 0.031 WT (S3A) 0.6356 ± 0.0191 * 0.001 (WT. Cof.) width WT (GFP) - same as above 0.7354 ± 0.0129 - (WT. Cof.) (Spine head width) WT (GFP) - same as above 0.7354 ± 0.0129 - (WT. Cof.) (Signe head width) WT (GFP) - same as above 0.6356 ± 0.0191 * 0.033 WT (GFP) = same as above 1.6570 ± 0.0709 - . . . width WT (GFP) = same as above 0.6365 ± 0.0191 * .0.001 . WT (GFP) = same as above 1.6570 ± 0.0709 - (%Stubby/ WT (GFP) = same as above 0.6582 ± 0.0177 .0.388 (%Thin/Filopdia) KO (GFP) 0.66106 ± 0.0218 - </th <th>length)</th> <th>KO (GFP) = 8 animals, 20 neurons, 1381</th> <th>1.3326 ± 0.0494</th> <th>*, 0.043</th> <th>post-test</th>	length)	KO (GFP) = 8 animals, 20 neurons, 1381	1.3326 ± 0.0494	*, 0.043	post-test
WT (WT. Cof.) = 7 animals, 15 neurons, 765 spines, 119 segments of 10 µm KO (WT. Cof.) = 9 animals, 15 neurons, 911 spines, 123 segments of 10 µm WT (S3A) = 6 animals, 16 neurons, 887 spines, 132 segments of 10 µm WT (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm WT (WT. Cof.) 0.9462 ± 0.0364 0.9298 ± 0.0520 *, 0.047 ****, <0.0001 (GFP) vs KO (S3A), and WT (WT. Cof.) vs KO (WT. Cof.) 4E (Spine head width) WT (GFP) = same as above KO (GFP) 0.7354 ± 0.0129 0.6509 ± 0.0221 - ***, 0.009 4F (Length- width ratio) WT (GFP) = same as above KO (S3A) 0.6356 ± 0.0191 0.6566 ± 0.0146 *, 0.033 0.987 4G (%Stubby/ Mushroom) WT (GFP) = same as above KO (S3A) 0.7823 ± 0.0204 1.6570 ± 0.0779 - ****, <0.0001 4H (%T. Cof.) WT (GFP) = same as above KO (S3A) 0.7823 ± 0.0204 1.5550 ± 0.0177 - ****, <0.0001 4G (%Stubby/ Mushroom) WT (GFP) = same as above KO (GFP) 0.7823 ± 0.0177 0.7823 ± 0.0204 1.5550 ± 0.0177 - ****, <0.0001 4H (%Thin/ Filopodia) WT (GFP) = same as above KO (WT. Cof.) 0.8405 ± 0.0177 0.788 ± 0.0173 - ****, <0.0001 WT (GFP) = same as above KO (WT. Cof.) 0.8105 ± 0.0173 *****, <0.0001 WT (GFP) = same as above KO (WT. Cof.) 0.7823 ± 0.0177 *****, <0.0001 WT (GFP) = same as abo		spines, 170 segments of 10 μ m			(WT (GFP) vs KO
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		WT (WT. Cof.) = 7 animals, 15 neurons, 765	1.0087 ± 0.0403	0.370	(GFP), WT (GFP)
KO (WT. Cof.) = 9 animals, 15 neurons, 911 spines, 123 segments of 10 µm WT (S3A) = 6 animals, 16 neurons, 887 spines, 132 segments of 10 µm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 µm 1.2496 ± 0.0519 *, 0.047 (GFP) vs KO (S3A), and WT 4E WT (GFP) - same as above 0.7354 ± 0.0129 - - (Spine head width) WT (GFP) - same as above 0.7354 ± 0.0129 - - WT (WT. Cof.) 0.6609 ± 0.0221 **, 0.009 (WT. Cof.) (WT. Cof.) WT (WT. Cof.) 0.6609 ± 0.0221 **, 0.009 0.541 WT (WT. Cof.) 0.6604 ± 0.0146 *, 0.033 0.7188 ± 0.0249 0.987 KO (GFP) 2.2756 ± 0.0709 - - - width ratio) WT (GFP) - same as above 1.6570 ± 0.0709 - KO (S3A) 0.7354 ± 0.0129 - - (Length- width ratio) WT (GFP) - same as above 1.6570 ± 0.0709 - KO (S3A) 0.7354 ± 0.0129 - - - WT (GFP) - same as above 0.7352 ± 0.0709 - - (%Stubby/ Mushroom) WT (GFP) - same as above 0.7823 ± 0.0204 - (%Stubby/ KO (GFP) 0.		spines, 119 segments of 10 μm			vs WT (S3A), WT
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		KO (WT. Cof.) = 9 animals, 15 neurons, 911	1.2496 ± 0.0519	*, 0.019	(GFP) vs WT
WT (S3A) = 6 animals, 16 neurons, 887 spines, 132 segments of 10 μm KO (S3A) = 7 animals, 17 neurons, 1119 spines, 151 segments of 10 μm 0.9298 ± 0.0520 **.**, <0.0001 (GFP) vs KO (S3A), and WT WT. Cof.) vs KO WT. Cof.) vs KO WT. Cof.) 4E (Spine head width) WT (GFP) - same as above KO (GFP) 0.7354 ± 0.0129 0.6509 ± 0.0221 - **, 0.009 - **, 0.009 4F (Length- width ratio) WT (GFP) - same as above KO (S3A) 0.6361 ± 0.0146 0.6365 ± 0.0191 *, 0.033 0.541 4F (Length- width ratio) WT (GFP) - same as above KO (WT. Cof.) 1.6570 ± 0.0709 2.2756 ± 0.0868 - ****, <0.0001 4G (%Stubby/ MUShroom) WT (GFP) - same as above KO (S3A) 0.7822 ± 0.0779 1.5550 ± 0.0773 - ****, <0.0001 4G (%Stubby/ MUShroom) WT (GFP) - same as above KO (S3A) 0.7823 ± 0.0204 1.5550 ± 0.0773 - ****, <0.0001 4H (%Thin/ Filopodia) WT (GFP) - same as above KO (WT. Cof.) 0.2107 ± 0.0175 0.3811 ± 0.0222 - ****, <0.0001 4H WT (GFP) - same as above (%Thin/ KO (GFP) 0.2107 ± 0.0175 0.3811 ± 0.0220 - ****, <0.0001 WT (WT. Cof.) WT (WT. Cof.) 0.2107 ± 0.0175 0.3881 ± 0.0220 - ****, <0.0001 WT (GFP) - same as above (%Thin/ KO (GFP) 0.2108 ± 0.0130 0.399 0.399 KO (S3A) 0.8109 ± 0.0222 **		spines, 123 segments of 10 μm			(WT. Cof.), KO
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		WT (S3A) = 6 animals, 16 neurons, 887	0.9462 ± 0.0364	*, 0.047	(GFP) vs KO
KO (S3A) = 7 animals, 17 neurons, 1119 0.9298 ± 0.0520 $^{\text{true}}$, <0.0001 (W1. Cot.) vs KO (WT. Cof.) gpine head width) WT (GFP) – same as above 0.7354 ± 0.0129 - (Spine head width) WT (WT. Cof.) 0.6091 ± 0.0221 - KO (S3A) 0.6911 ± 0.0139 0.541 WT (WT. Cof.) 0.6046 ± 0.0146 *, 0.033 WT (S3A) 0.7188 ± 0.0249 0.987 KO (S3A) 0.6336 ± 0.0191 *, 0.001 WT (GFP) – same as above 1.6570 ± 0.0709 - (Length- KO (GFP) 2.2756 ± 0.0868 ****, <0.0001 width ratio) WT (WT. Cof.) 2.2876 ± 0.0173 ****, <0.0001 WT (S3A) 1.5550 ± 0.0773 ****, <0.0001 WT (GFP) – same as above 0.7823 ± 0.0204 - (%Stubby/ KO (GFP) 0.6616 ± 0.0218 ****, <0.0001 Mushroom) WT (WT. Cof.) 0.7892 ± 0.0133 0.269 KO (S3A) 0.8109 ± 0.0222 ****, <0.0001 WT (GFP) – same as above 0.2127 ± 0.021 - (%Thin/ KO (GFP) 0.3417 ± 0.0175 ****, <0.		spines, 132 segments of 10 μm		****	(S3A), and WI
spines, 151 segments of 10 μm (W1. Col.) 4E WT (GFP) – same as above 0.7354 ± 0.0129 - (Spine head width) WT (GFP) – same as above 0.6509 ± 0.0221 **, 0.009 WT (WT. Cof.) 0.6041 ± 0.0139 0.541 (Some head width) WT (WT. Cof.) 0.6046 ± 0.0146 *, 0.033 WT (S3A) 0.7188 ± 0.0249 0.987 (Length- width ratio) WT (GFP) – same as above 1.6570 ± 0.0709 - (Length- width ratio) WT (WT. Cof.) 2.2756 ± 0.0868 *****, <0.0001 KO (WT. Cof.) 2.2876 ± 0.1141 *****, <0.0001 WT (S3A) 1.4411 ± 0.0607 0.398 KO (S3A) 1.5550 ± 0.0773 *****, <0.0001 WT (GFP) – same as above 0.7823 ± 0.0204 - (%Stubby/ KO (GFP) 0.6106 ± 0.0218 *****, <0.0001 MUT (S3A) 0.8405 ± 0.0130 0.2999 KO (WT. Cof.) 0.8405 ± 0.0136 0.269 KO (S3A) 0.8405 ± 0.0136 0.269 KO (S3A) 0.8405 ± 0.0133 0.999		KO (S3A) = 7 animals, 17 neurons, 1119	0.9298 ± 0.0520	^^^^, <0.0001	(WT. Cof.) vs KO
4E WT (GFP) - same as above 0.7354 ± 0.0129 - (Spine head width) KO (GFP) 0.6509 ± 0.0221 **, 0.009 WT (WT. Cof.) 0.6911 ± 0.0139 0.541 KO (WT. Cof.) 0.6046 ± 0.0146 *, 0.033 WT (S3A) 0.7188 ± 0.0249 0.987 KO (S3A) 0.6356 ± 0.0191 *, 0.001 WT (WT. Cof.) 1.6570 ± 0.0709 - KO (GFP) 2.2756 ± 0.0868 *****, <0.0001 WT (WT. Cof.) 1.6022 ± 0.0709 0.899 KO (WT. Cof.) 2.2876 ± 0.1141 *****, <0.0001 WT (S3A) 1.4411 ± 0.0607 0.398 KO (S3A) 1.5550 ± 0.0773 *****, <0.0001 WT (WT. Cof.) 0.7823 ± 0.0204 - (%Stubby/ KO (GFP) 0.6106 ± 0.0218 *****, <0.0001 WT (S3A) 0.8405 ± 0.0136 0.269 KO (S3A) 0.8109 ± 0.0222 *****, <0.0001 WT (GFP) – same as above 0.2127 ± 0.0220 - (%Stubby/ WT (GFP) – same as above 0.2127 ± 0.0220 -		spines, 151 segments of 10 μm			(WT. Cof.)
42 WT (GFP) - Same as above 0.734 ± 0.0129 - (Spine head width) KO (GFP) 0.6509 ± 0.0221 **, 0.009 WT (WT. Cof.) 0.6611 ± 0.0139 0.541 KO (WT. Cof.) 0.6046 ± 0.0146 *, 0.033 WT (S3A) 0.7188 ± 0.0249 0.987 KO (S3A) 0.6561 ± 0.0709 - VT (GFP) - same as above 1.6570 ± 0.0709 - (Length- width ratio) WT (WT. Cof.) 2.2756 ± 0.0868 *****, <0.0001 WT (S3A) 1.4411 ± 0.0607 0.398 KO (S3A) 1.45550 ± 0.0773 *****, <0.0001 WT (SFP) - same as above 0.7823 ± 0.0204 - (%Stubby/ Mushroom) WT (WT. Cof.) 0.6106 ± 0.0218 *****, <0.0001 WT (S3A) 0.8109 ± 0.0222 *****, <0.0001 WT (S3A) 0.8109 ± 0.0222 *****, <0.0001 WT (S3A) 0.8109 ± 0.0222 ****, <0.0001 WT (S3A) 0.8109 ± 0.0222 *****, <0.0001 WT (SA) 0.8109 ± 0.0222 *****, <0.0001 WT (SA) 0.8109 ± 0.0222 *****, <0.0001 WT (GFP) - same as above 0.21	46		0 7254 + 0 0120		
(spine nead width) KO (GFP) 0.6303 ± 0.0221 ,0.009 width) WT (WT. Cof.) 0.6911 ± 0.0139 0.541 KO (WT. Cof.) 0.6046 ± 0.0146 *,0.033 WT (S3A) 0.7188 ± 0.0249 0.987 KO (S3A) 0.6356 ± 0.0191 *,0.001 4F WT (GFP) – same as above 1.6570 ± 0.0709 - (Length- KO (GFP) 2.2756 ± 0.0868 *****, <0.0001 width ratio) WT (WT. Cof.) 1.6622 ± 0.0709 0.899 KO (WT. Cof.) 1.28276 ± 0.1141 *****, <0.0001 WT (S3A) 1.4411 ± 0.0607 0.398 KO (S3A) 1.5550 ± 0.0773 *****, <0.0001 WT (S3A) 1.5550 ± 0.0773 *****, <0.0001 WG (S3A) 0.6106 ± 0.0218 *****, <0.0001 Mushroom) WT (WT. Cof.) 0.6106 ± 0.0218 *****, <0.0001 WT (S3A) 0.8405 ± 0.0136 0.269 KO (S3A) 0.8109 ± 0.0222 *****, <0.0001 WT (S3A) 0.8109 ± 0.0222 ****, <0.0001 WT (S3A) 0.8109 ± 0.0222 *****, <0.0001 WT (S7D) 0.210	4E (Spipe bood	KO (CED)	0.7334 ± 0.0129	** 0.000	
Withiny WT (WT. Col.) 0.6041 ± 0.0136 0.0311 KO (WT. Col.) 0.6041 ± 0.0146 *, 0.033 WT (S3A) 0.7188 ± 0.0249 0.987 KO (S3A) 0.6356 ± 0.0191 *, 0.031 4F WT (GFP) – same as above 1.6570 ± 0.0709 - KO (GFP) 2.2756 ± 0.0868 *****, <0.0001 width ratio) WT (WT. Cof.) 1.6022 ± 0.0709 0.899 KO (WT. Cof.) 2.2876 ± 0.1141 *****, <0.0001 WT (S3A) 1.4411 ± 0.0607 0.398 KO (S3A) 1.5550 ± 0.0773 *****, <0.0001 WT (GFP) – same as above 0.7823 ± 0.0204 - (%Stubby/ KO (GFP) 0.6106 ± 0.0218 *****, <0.0001 Mushroom) WT (WT. Cof.) 0.6106 ± 0.0218 *****, <0.0001 WT (S3A) 0.8109 ± 0.0222 *****, <0.0001 WT (SA) 0.8109 ± 0.0222 *****, <0.0001 WT (SA) 0.8109 ± 0.0222 *****, <0.0001 WT (GFP) – same as above 0.2127 ± 0.0201 - (%Thin/ KO (GFP) 0.3417 ± 0.0175 *****, <0.0001 WT (S3A)	(Spille flead		0.0309 ± 0.0221 0.6011 ± 0.0130	, 0.009	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	width)	KO(WT, Cof)	0.0911 ± 0.0139 0.6046 ± 0.0146	* 0.033	
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4F WT (GFP) - same as above 1.6570 ± 0.0709 - (Length- width ratio) KO (GFP) 2.2756 ± 0.0868 *****, <0.0001 WT (WT. Cof.) 1.6022 ± 0.0709 0.899 KO (WT. Cof.) 2.2876 ± 0.1141 *****, <0.0001 WT (S3A) 1.4411 ± 0.0607 0.398 KO (S3A) 1.5550 ± 0.0773 *****, <0.0001 4G WT (GFP) - same as above 0.7823 ± 0.0204 - (%Stubby/ KO (GFP) 0.6568 ± 0.0177 *****, <0.0001 Mushroom) WT (WT. Cof.) 0.7892 ± 0.0193 0.999 KO (WT. Cof.) 0.6106 ± 0.0218 *****, <0.0001 WT (S3A) 0.8109 ± 0.0222 *****, <0.0001 WT (S3A) 0.8109 ± 0.0222 *****, <0.0001 WT (S3A) 0.8109 ± 0.0222 *****, <0.0001 WT (WT. Cof.) 0.2127 ± 0.0201 - (%Thin/ KO (GFP) 0.2108 ± 0.0133 0.999 KO (WT. Cof.) 0.2108 ± 0.0133 0.999 KO (WT. Cof.) 0.3811 ± 0.0220 *****, <0.0001		KO(S3A)	0.7100 ± 0.0249 0.6356 ± 0.0101	* 0.031	
(Length- width ratio) (VT (GFP) 1.0010 ± 0.0103 *****, <0.0001	4F	WT (GEP) – same as above	1.6570 ± 0.0709	, 0.001	
width ratio) WT (WT. Cof.) 1.6022 ± 0.0009 0.899 KO (WT. Cof.) 2.2876 ± 0.1141 ****, <0.0001 WT (S3A) 1.4411 ± 0.0607 0.398 KO (S3A) 1.5550 ± 0.0773 ****, <0.0001 4G WT (GFP) - same as above 0.7823 ± 0.0204 - (%Stubby/ KO (GFP) 0.6568 ± 0.0177 ****, <0.0001 Mushroom) WT (WT. Cof.) 0.7892 ± 0.0193 0.999 KO (S3A) 0.8405 ± 0.0136 0.269 WT (S3A) 0.8109 ± 0.0222 ****, <0.0001 WT (S3A) 0.8109 ± 0.0221 ****, <0.0001 WT (GFP) - same as above 0.2127 ± 0.0201 - (%Thin/ WT (GFP) - same as above 0.2127 ± 0.0201 - (%Thin/ KO (GFP) 0.3417 ± 0.0175 ****, <0.0001 Filopodia) WT (WT. Cof.) 0.2108 ± 0.0193 0.999 KO (WT. Cof.) 0.3881 ± 0.0220 *****, <0.0001 WT (S3A) 0.1595 ± 0.0136 0.355 KO (WT. Cof.) 0.3881 ± 0.0214 *****, <0.0001	(Length-	KO (GFP)	22756 ± 0.0868	**** <0 0001	
Width rate() WT (WT. Cof.) 1.0022 ± 0.0703 0.003 KO (WT. Cof.) 2.2876 ± 0.1141 *****, <0.0001 WT (S3A) 1.4411 ± 0.0607 0.398 KO (S3A) 1.5550 ± 0.0773 *****, <0.0001 WT (GFP) - same as above 0.7823 ± 0.0204 - (%Stubby/ KO (GFP) 0.6568 ± 0.0177 *****, <0.0001 Mushroom) WT (WT. Cof.) 0.7892 ± 0.0193 0.999 KO (WT. Cof.) 0.6106 ± 0.0218 *****, <0.0001 WT (S3A) 0.8405 ± 0.0136 0.269 KO (S3A) 0.8109 ± 0.0222 *****, <0.0001 4H WT (GFP) - same as above 0.2127 ± 0.0201 - (%Thin/ KO (GFP) 0.2108 ± 0.0193 0.999 Filopodia) WT (WT. Cof.) 0.2108 ± 0.0193 0.999 KO (WT. Cof.) 0.2108 ± 0.0193 0.999 KO (WT. Cof.) 0.3881 ± 0.0220 *****, <0.0001 WT (S3A) 0.1595 ± 0.0136 0.355 KO (S3A) 0.1595 ± 0.0136 0.355	width ratio)		1.6022 ± 0.0000	0.800	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	wiatilitatioj	KO(WT, Cof)	2.2876 ± 0.0703	**** ~0 0001	
Image: With (GSA)Image: With (GSA)Image: With (GSA)4GWT (GFP) - same as above 0.7823 ± 0.0204 -(%Stubby/KO (GFP) 0.6568 ± 0.0177 *****, <0.0001Mushroom)WT (WT. Cof.) 0.7892 ± 0.0193 0.999 KO (WT. Cof.) 0.6106 ± 0.0218 *****, <0.0001WT (S3A) 0.8405 ± 0.0136 0.269 KO (S3A) 0.8109 ± 0.0222 *****, <0.00014HWT (GFP) - same as above 0.2127 ± 0.0201 -(%Thin/KO (GFP) 0.3417 ± 0.0175 *****, <0.0001Filopodia)WT (WT. Cof.) 0.2108 ± 0.0193 0.999 WT (S3A) 0.1595 ± 0.0136 0.355 KO (WT. Cof.) 0.1595 ± 0.0136 0.355 KO (S3A) 0.1808 ± 0.0214 *****, <0.0001		WT (S3A)	1.4411 ± 0.0607	0 398	
4GWT (GFP) - same as above 0.7823 ± 0.0204 -(%Stubby/ Mushroom)KO (GFP) 0.6568 ± 0.0177 *****, <0.0001		KO (S3A)	1.4411 ± 0.0007 1.5550 ± 0.0773	**** <0.0001	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	4G	WT (GEP) - same as above	0.7823 ± 0.0204	, <0.0001	
Mushroom)WT (WT. Cof.) KO (WT. Cof.) 0.7892 ± 0.0193 0.6106 ± 0.0218 0.999 ****, <0.0001	(%Stubby/	KO (GEP)	0.6568 ± 0.0204	**** <0.0001	
KO (WT. Cof.) 0.6106 ± 0.0218 $****, <0.0001$ WT (S3A) 0.8405 ± 0.0136 0.269 KO (S3A) 0.8109 ± 0.0222 $****, <0.0001$ 4HWT (GFP) - same as above 0.2127 ± 0.0201 -(%Thin/KO (GFP) 0.3417 ± 0.0175 $****, <0.0001$ Filopodia)WT (WT. Cof.) 0.2108 ± 0.0193 0.999 KO (WT. Cof.) 0.3881 ± 0.0220 $****, <0.0001$ WT (S3A) 0.1595 ± 0.0136 0.355 KO (S3A) 0.1808 ± 0.0214 $****, <0.0001$	Mushroom)	WT (WT Cof)	0.7892 ± 0.0193	0.999	
WT (S3A) 0.8405 ± 0.0136 0.269 KO (S3A) 0.8109 ± 0.0222 *****, <0.0001 4H WT (GFP) - same as above 0.2127 ± 0.0201 - (%Thin/ KO (GFP) 0.3417 ± 0.0175 *****, <0.0001 Filopodia) WT (WT. Cof.) 0.2108 ± 0.0193 0.999 KO (WT. Cof.) 0.3881 ± 0.0220 *****, <0.0001 WT (S3A) 0.1595 ± 0.0136 0.355 KO (S3A) 0.1808 ± 0.0214 *****, <0.0001		KO (WT Cof)	0.6106 ± 0.0218	**** <0 0001	
KO (S3A) 0.8109 ± 0.0222 *****, <0.0001		WT (S3A)	0.8405 ± 0.0210	0.269	
4H WT (GFP) - same as above 0.2127 ± 0.0201 - (%Thin/ KO (GFP) 0.3417 ± 0.0175 ****, <0.0001		KO (S3A)	0.8109 ± 0.0222	**** <0.0001	
(%Thin/ Filopodia)KO (GFP) 0.3417 ± 0.0175 ****, <0.0001	4H	WT (GFP) – same as above	0.2127 ± 0.0201	_	
Filopodia) WT (WT. Cof.) KO (WT. Cof.) 0.2108 ± 0.0193 0.999 WT (S3A) KO (S3A) 0.1595 ± 0.0136 0.355 WT (S3A) 0.1808 ± 0.0214 ****, <0.0001	(%Thin/	KO (GFP)	0.3417 ± 0.0175	**** <0.0001	
KO (WT. Cof.) 0.3881 ± 0.0220 ****, <0.0001	Filopodia)	WT (WT, Cof.)	0.2108 ± 0.0193	0.999	
WT (S3A) 0.1595 ± 0.0136 0.355 KO (S3A) 0.1808 ± 0.0214 *****, <0.0001		KO (WT. Cof.)	0.3881 ± 0.0220	****. <0.0001	
KO (S3A) 0.1808 ± 0.0214 ****, <0.0001		WT (S3A)	0.1595 ± 0.0136	0.355	
		KO (S3A)	0.1808 ± 0.0214	****. <0.0001	
4I WT (GFP) – same as above 6.8827 + 0.3208 –	41	WT (GFP) – same as above	6.8827 ± 0.3208	_	
(Spine KO (GFP) 8.1235 + 0.3485 * 0.046	(Spine	KO (GFP)	8.1235 ± 0.3485	*. 0.046	
density) WT (WT, Cof.) 6.1092 + 0.3035 0.583	densitv)	WT (WT, Cof.)	6.1092 ± 0.3035	0.583	
KO (WT. Cof.) 8.3008 + 0.3801 ***. 0.0005		KO (WT. Cof.)	8.3008 ± 0.3801	***. 0.0005	
WT (S3A) 6.7197 + 0.3395 0.999		WT (S3A)	6.7197 ± 0.3395	0.999	
6.5762 ± 0.2974 ** 0.009		KO (S3A)	6.5762 ± 0.2974	**. 0.009	

Figure	N	Mean ± SEM	p value	Statistical Test
			(p < 0.05)	
5B	WT vehicle = 13 animals	0.669 ± 0.054	-	Two-way ANOVA
(NMDA/AM	KO vehicle = 11 animals	1.531 ± 0.288	**, 0.0014	with Tukey's post-
PA ratio)	WT FRAX486 = 11 animals	0.727 ± 0.042	-	test (WT vehicle vs.
	KO FRAX486 = 6 animals	0.503 ± 0.710	0.8297	KO vehicle, KO
5D	WT vehicle = 10 animals	25.65 ± 2.273	-	vehicle vs KO
(mEPSC	KO vehicle = 10 animals	21.60 ± 1.317	0.4386	FRAX486)
amplitude)	WT FRAX486 = 11 animals	26.28 ± 1.369	-	
	KO FRAX486 = 9 animals	29.36 ± 2.521	*, 0.0365	
5D	WT vehicle = 10 animals	1.014 ± 0.131	-	Two-way ANOVA
(mEPSC	KO vehicle = 10 animals	0.650 ± 0.095	0.1577	with Tukey's post-
frequency)	WT FRAX486 = 11 animals	0.471 ± 0.111	*, 0.0119	test (WT vehicle vs
	KO FRAX486 = 9 animals	0.655 ± 0.141	0.9999	KO vehicle, WT
5E	WT vehicle = 15 animals	0.606 ± 0.068	-	vehicle vs WT
(NMDA/AM	KO vehicle = 7 animals	0.431 ± 0.065	0.3704	FRAX486, KO
PA ratio)	WT FRAX486 = 11 animals	0.571 ± 0.048	0.9821	vehicle vs KO
	KO FRAX486 = 10 animals	0.599 ± 0.089	0.4678	FRAX486)
5E	WT vehicle = 18 animals	29.21 ± 1.273	-	
(mEPSC	KO vehicle = 7 animals	27.79 ± 1.251	0.9301	
amplitude)	WT FRAX486 = 16 animals	22.67 ± 1.379	**, 0.004	
	KO FRAX486 = 12 animals	22.14 ± 1.635	0.1254	
5E	WT vehicle = 18 animals	2.472 ± 0.426	-	
(mEPSC	KO vehicle = 7 animals	2.454 ± 0.212	0.9999	
frequency)	WT FRAX486 = 16 animals	2.873 ± 0.761	0.9452	
	KO FRAX486 = 12 animals	1.920 ± 0.391	0.9505	

Table S5. Summary of statistics for Fig. 5.

Table S6. Summary of statistics for Fig. 6.

Figure	Ν	Mean ± SEM	p value	Statistical Test
			(p < 0.05)	
6B	WT (familiar) = 13 animals	42.05 ± 3.276	-	Two-tailed unpaired
	WT (novel) = 13 animals	58.96 ± 2.663	***, 0.0005	t test for WT
	KO (familiar) = 7 animals	48.54 ± 4.097	-	(familiar) vs WT
	KO (novel) = 7 animals	51.46 ± 4.097	0.6259	(novel) and KO
6C	WT (familiar) = 10 animals	47.50 ± 2.435	-	(familiar) vs KO
	WT (novel) = 10 animals	52.60 ± 2.449	0.1569	(novel)
6D	WT (familiar) = 7 animals	52.54 ± 4.100	-	
	WT (novel) = 7 animals	47.46 ± 4.100	0.3989	
6E	WT (familiar) = 7 animals	38.13 ± 2.872	-	
	WT (novel) = 7 animals	61.87 ± 2.872	****, <0.0001	
	KO (familiar) = 8 animals	41.08 ± 3.631	-	
	KO (novel) = 8 animals	58.92 ± 3.631	**, 0.003	
6F	WT-vehicle (familiar) = 15 animals	43.57 ± 2.705	-	
	WT-vehicle (novel) = 15 animals	56.43 ± 2.705	**, 0.0022	
	KO-vehicle (familiar) = 10 animals	51.79 ± 3.446	-	
	KO-vehicle (novel) = 10 animals	48.71 ± 2.997	0.5089	
	WT-FRAX486 (familiar) = 9 animals	41.44 ± 4.219	-	
	WT- FRAX486 (novel) = 9 animals	58.83 ± 4.344	*, 0.0111	
	KO- FRAX486 (familiar) = 10 animals	40.19 ± 3.188	-	
	KO- FRAX486 (novel) = 10 animals	59.81 ± 3.188	***, 0.0003	

Table S7. Summary	of	statistics	for	fig.	S2.
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Figure	N	Mean ± SEM	p value	Statistical
			(p < 0.05)	Test
Supp. 2A	WT untreated (p-Cofilin) = 7 animals	1.000 ± 0.1259	-	Two-tailed
	WT vehicle (p-Cofilin) = 7 animals	1.060 ± 0.2010	0.8031	unpaired t test
	WT untreated (Cofilin) = 7 animals	1.000 ± 0.1360	_	for WT
	WT vehicle (Cofilin) = 7 animals	0.8786 ± 0.1109	0.5023	untreated vs
Supp. 2B	WT untreated (p-LimK1) = 7 animals	1.000 ± 0.1525	-	WT vehicle
	WT vehicle (p-LimK1) = 7 animals	1.116 ± 0.2125	0.6659	treated
	WT untreated (LimK1) = 7 animals	1.000 ± 0.1458	-	animals
	WT vehicle (LimK1) = 7 animals	0.9594 ± 0.1327	0.8402	
Supp. 2C	WT untreated (p-SSH1) = 7 animals	1.000 ± 0.1953	-	
	WT vehicle (p-SSH1) = 7 animals	1.028 ± 0.2255	0.9270	
	WT untreated (SSH1) = 7 animals	1.000 ± 0.1571	-	
	WT vehicle (SSH1) = 7 animals	0.8207 ± 0.1127	0.3718	

Table S8. Summary of statistics for figs. S3 and S4.

N	Mean ± SEM	p value	Statistical Test
WT (Rac1-GTP) = 8 animals	1.000 ± 0.1437	-	Two-tailed unpaired <i>t</i> test for
KO (Rac-GTP) = 7 animals	1.052 ± 0.1341	0.7984	WT vs KO
WT (Rac1) = 8 animals	1.000 ± 0.01174	-	
KO (Rac1) = 7 animals	0.9846 ± 0.0082	0.3136	
WT (p-PAK1) = 8 animals	1.000 ± 0.0937	-	
KO (p-PAK1) = 8 animals	0.9798 ± 0.1460	0.9089	
WT (PAK1) = 8 animals	1.000 ± 0.0786	-	
KO (PAK1) = 8 animals	0.9904 ± 0.1071	0.9435	
WT (p-LIMK1) = 10 animals	1.000 ± 0.1349	-	
KO (p-LIMK1) = 9 animals	1.050 ± 0.3280	0.8849	
WT (LIMK1) = 10 animals	1.000 ± 0.1205	-	
KO (LIMK1) = 9 animals	1.253 ± 0.1664	0.2291	
WT (p-SSH1) = 8 animals	1.000 ± 0.0716	-	
KO (p -SSH1) = 7 animals	3.127 ± 0.9302	*, 0.0293	
WT (SSH1) = 8 animals	1.000 ± 0.0795	-	
KO (SSH1) = 7 animals	0.9207 ± 0.1603	0.6537	
WT (F/G actin) = 5 animals	1.000 ± 0.0543	-	
KO(F/G actin) = 5 animals	1.156 ± 0.1955	0.4592	
WT (Rac1-GTP) = 7 animals	1.000 ± 0.1767	-	
KO (Rac-GTP) = 5 animals	1.213 ± 0.1235	0.3944	
WI (Rac1) = 7 animals	1.000 ± 0.3615	-	
KO(Rac1) = 5 animals	1.054 ± 0.0246	0.2852	
WT (p-PAK1) = 4 animals	1.000 ± 0.2757	-	
KO(p-PAK1) = 6 animals	0.7542 ± 0.2850	0.5723	
WI (PAK1) = 4 animals	1.000 ± 0.0708	-	
KO(PAK1) = 6 animals	0.9089 ± 0.0372	0.2461	
VVI (p-LIVIKI) = 8 animals	1.000 ± 0.2764	-	
KO(p-LINK1) = 5 animals	0.8735 ± 0.1535	0.7419	
VVI (LIIVIKI) = 8 animals	1.000 ± 0.1722	-	
M(T (n SSH1) = 0 animals	1.000 ± 0.0910	0.0034	
VV = (p-33 - 1) = 9 animals	1.000 ± 0.0703	-	
(P-3SH1) = 7 animals W(T (SSH1) = 0 animals	1.000 ± 0.1002	0.2003	
KO(SSH1) = 7 animals	1.000 ± 0.1092 1.070 ± 0.2167	0 7750	
	NWT (Rac1-GTP) = 8 animals KO (Rac-GTP) = 7 animalsWT (Rac1) = 8 animals KO (Rac1) = 7 animalsWT (p-PAK1) = 8 animals KO (p-PAK1) = 8 animals WT (p-AK1) = 8 animals WT (PAK1) = 8 animals WT (PAK1) = 8 animals 	NMean \pm SEMWT (Rac1-GTP) = 8 animals1.000 \pm 0.1437KO (Rac-GTP) = 7 animals1.052 \pm 0.1341WT (Rac1) = 8 animals1.000 \pm 0.01174KO (Rac1) = 7 animals0.9846 \pm 0.0082WT (p-PAK1) = 8 animals0.9798 \pm 0.1460WT (PAK1) = 8 animals0.9798 \pm 0.1460WT (PAK1) = 8 animals0.9904 \pm 0.1071WT (p-LIMK1) = 10 animals1.000 \pm 0.03280WT (p-LIMK1) = 10 animals1.000 \pm 0.1205KO (p-LIMK1) = 9 animals1.000 \pm 0.1205KO (LIMK1) = 9 animals1.000 \pm 0.0716KO (p-SSH1) = 7 animals1.000 \pm 0.0716KO (p-SSH1) = 7 animals1.000 \pm 0.0795KO (SSH1) = 7 animals1.000 \pm 0.0795KO (SSH1) = 7 animals1.000 \pm 0.1767KO (Rac-GTP) = 5 animals1.000 \pm 0.1767KO (Rac-GTP) = 5 animals1.000 \pm 0.2757KO (Rac1) = 5 animals1.000 \pm 0.2757KO (p-PAK1) = 4 animals1.000 \pm 0.2757KO (p-PAK1) = 4 animals0.9089 \pm 0.0372WT (p-LIMK1) = 8 animals1.000 \pm 0.2764KO (p-LIMK1) = 5 animals1.000 \pm 0.2764KO (p-LIMK1) = 5 animals1.000 \pm 0.2764KO (p-LIMK1) = 8 animals1.000 \pm 0.2764KO (p-SSH1) = 7 animals1.000 \pm 0.0783WT (p-SSH1) = 9 animals1.000 \pm 0.0783WT (p-SSH1) = 9 animals1.000 \pm 0.0783KO (p-SSH1) = 7 animals1.000 \pm 0.0783KO (p-SSH1) = 7 animals1.000 \pm 0.1692KO (SSH1) = 7 animals1.079 \pm 0.2167 <th>NMean \pm SEMp valueWT (Rac1-GTP) = 8 animals1.000 ± 0.1437-KO (Rac-GTP) = 7 animals$1.052 \pm 0.1341$$0.7984$WT (Rac1) = 8 animals$1.000 \pm 0.0082$$0.3136$WT (Pact) = 7 animals$0.9846 \pm 0.0082$$0.3136$WT (p-PAK1) = 8 animals$1.000 \pm 0.0937$-KO (p-PAK1) = 8 animals$0.9798 \pm 0.1460$$0.9089$WT (PAK1) = 8 animals$0.9798 \pm 0.1460$$0.9089$WT (PAK1) = 8 animals$0.9094 \pm 0.1071$$0.9435$WT (p-LIMK1) = 10 animals$1.000 \pm 0.0380$$-$KO (p-LIMK1) = 9 animals$1.000 \pm 0.1205$-KO (p-LIMK1) = 9 animals1.000 ± 0.0716-KO (p-SSH1) = 8 animals1.000 ± 0.0716-KO (p-SSH1) = 7 animals1.000 ± 0.0795-KO (p-SSH1) = 7 animals1.000 ± 0.0795-KO (SSH1) = 7 animals1.000 ± 0.0795-KO (F/G actin) = 5 animals1.000 ± 0.0543-KO (Rac1) = 5 animals1.000 ± 0.0795-KO (Rac1) = 5 animals1.000 ± 0.0767-KO (Rac1) = 5 animals1.000 ± 0.0767-KO (Rac1) = 5 animals1.000 ± 0.0783-KO (p-PAK1) = 4 animals1.000 ± 0.0783-MT (PAK1) = 4 animals$1.000 \pm 0.$</th>	NMean \pm SEMp valueWT (Rac1-GTP) = 8 animals 1.000 ± 0.1437 -KO (Rac-GTP) = 7 animals 1.052 ± 0.1341 0.7984 WT (Rac1) = 8 animals 1.000 ± 0.0082 0.3136 WT (Pact) = 7 animals 0.9846 ± 0.0082 0.3136 WT (p-PAK1) = 8 animals 1.000 ± 0.0937 -KO (p-PAK1) = 8 animals 0.9798 ± 0.1460 0.9089 WT (PAK1) = 8 animals 0.9798 ± 0.1460 0.9089 WT (PAK1) = 8 animals 0.9094 ± 0.1071 0.9435 WT (p-LIMK1) = 10 animals 1.000 ± 0.0380 $-$ KO (p-LIMK1) = 9 animals 1.000 ± 0.1205 -KO (p-LIMK1) = 9 animals 1.000 ± 0.0716 -KO (p-SSH1) = 8 animals 1.000 ± 0.0716 -KO (p-SSH1) = 7 animals 1.000 ± 0.0795 -KO (p-SSH1) = 7 animals 1.000 ± 0.0795 -KO (SSH1) = 7 animals 1.000 ± 0.0795 -KO (F/G actin) = 5 animals 1.000 ± 0.0543 -KO (Rac1) = 5 animals 1.000 ± 0.0795 -KO (Rac1) = 5 animals 1.000 ± 0.0767 -KO (Rac1) = 5 animals 1.000 ± 0.0767 -KO (Rac1) = 5 animals 1.000 ± 0.0783 -KO (p-PAK1) = 4 animals 1.000 ± 0.0783 -MT (PAK1) = 4 animals $1.000 \pm 0.$

<u> </u>	$MT (\Gamma (O a atia) = 0 a a imple$	4 000 0 4007	
3J	W I (F/G actin) = 9 animais	1.000 ± 0.1697	-
	KO (F/G actin) = 10 animals	1.054 ± 0.2098	0.9599
4A	WT (p-Cofilin) = 13 animals	1.000 ± 0.0925	-
	KO (p-Cofilin) = 9 animals	1.052 ± 0.1864	0.7860
	WT (Cofilin) = 13 animals	1.000 ± 0.0933	-
	KO (Cofilin)= 9 animals	1.295 ± 0.1668	0.1097