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Supplementary Materials for

Rapamycin Prevents Seizures After Depletion of STRADA in a Rare Neurodevelopmental Disorder

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencetranslationalmedicine.org/cgi/content/full/5/182/182ra53/DC1)

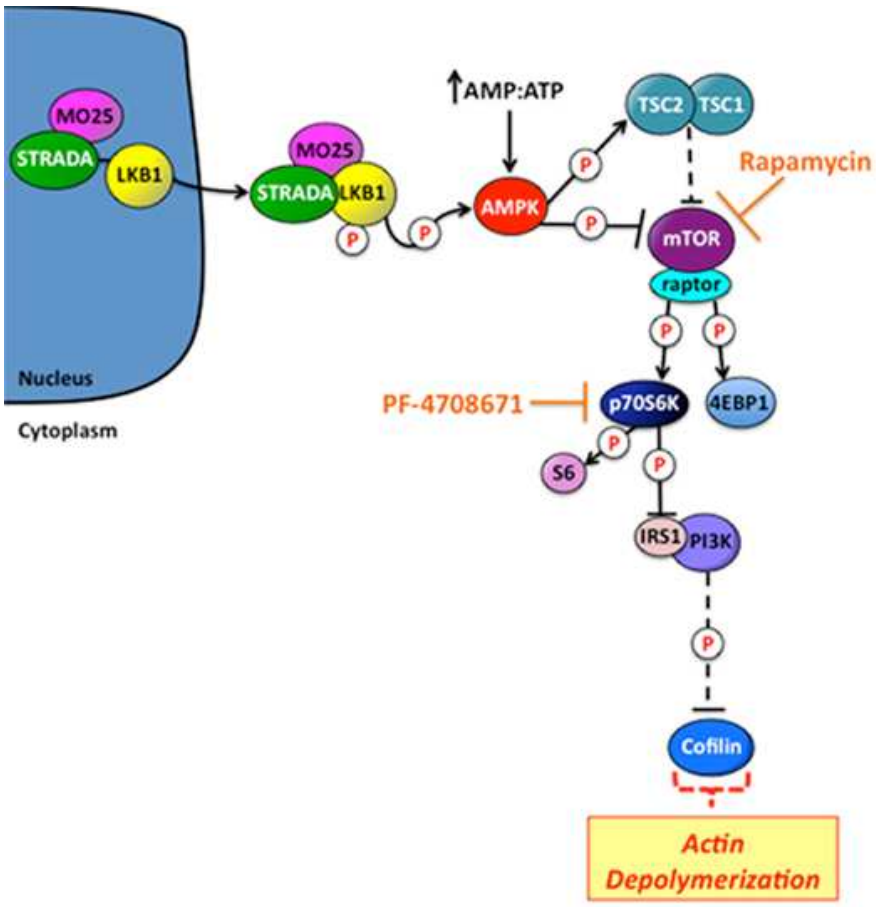

Fig. S1. The pathway schematic illustrates STRADA signaling through mTORC1. STRADA and scaffolding protein MO25 bind LKB1 and shuttle it out of the nucleus and activate its kinase activity toward AMPK. AMPK, activated by LKB1 phosphorylation as well as a high ratio of AMP:ATP indicating low cellular energy, phosphorylates and activates TSC2. TSC2 complexes with TSC1 to inhibit mTOR signaling. mTOR can form two complexes, the rapamycin-sensitive mTORC1 or the rapamycin-insensitive mTORC2. mTORC1 phosphorylates and activates 4EBP1 and p70S6K, which phosphorylates S6 and IRS1 (*16*). Downstream of IRS1, cofilin phosphorylation has an inverse relationship to mTORC1 activity. Thus, depletion of STRADA would be expected to result in disinhibited mTORC1 and consequently, enhanced phosphorylation of S6, 4EBP1, and IRS1, and diminished phosphorylation of cofilin. Importantly, rapamycin inhibits mTORC1 and PF-4708671 (p70S6Ki) inhibits p70S6K.

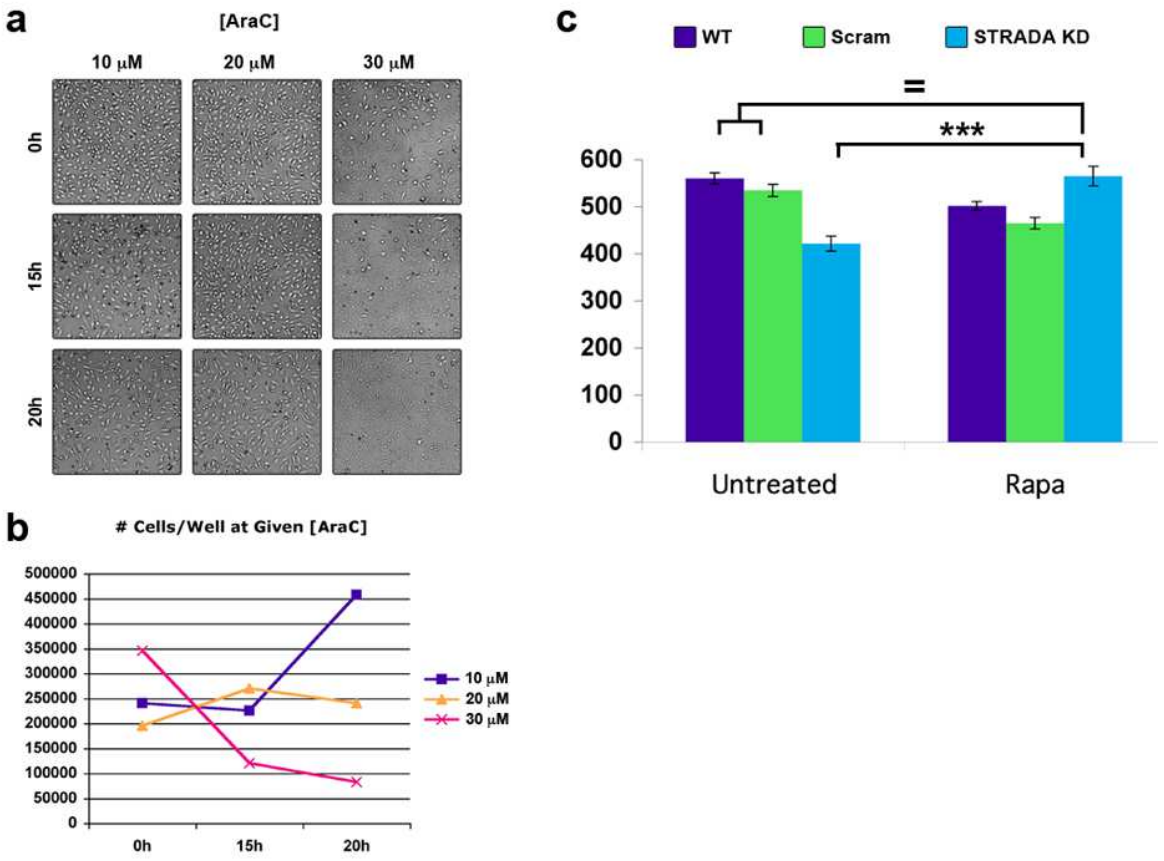

Fig. S2. Ara-C mitotic inhibition demonstrates migration deficit with STRADA depletion and rescue with rapamycin. (**A, B**) The mitotic inhibitor arabinofuranosyl cytosine (Ara-C) was used to inhibit mitosis of migrating cells and thus eliminate this as a variable affecting gap closure in the wound-healing migration assay. Scale bar, 250 μm. Effective non-lethal Ara-C dose was established through microscopic visualization (**A**) and Trypan Blue (Sigma) cell counts (**B**) at 0h, 15h, and 20h (each following a 24-hour pre-treatment) of Ara-C treatment at indicated doses, and 20 μM was determined to be the optimal effective anti-proliferative, non-apoptotic Ara-C dose. (**C**) Wound-healing migration assays were performed with 24-hour pre-treatment with Ara-C on wildtype untransfected (WT), Scrambled, and STRADA knockdown mNPCs. With Ara-C treatment, STRADA knockdown mNPCs exhibited migration defect, rescued fully with rapamycin treatment. n = 3 wells, 90 measures per condition at each timepoint, ***$P < 0.001$, $=P > 0.05$.

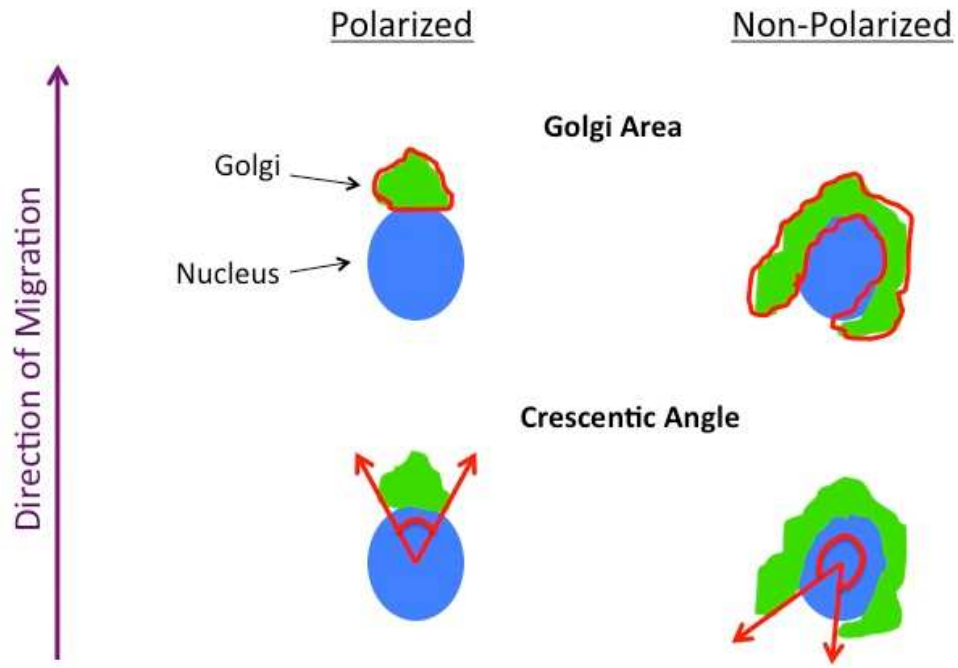

Fig. S3. Golgi compaction indicates polarity in migrating cells. Polarized migrating cells exhibit Golgi that is compacted forward of the nucleus, in the direction of migration. Left, this is indicated by reduced Golgi area and reduced crescentic angle subtended by Golgi around the nucleus. In contrast, non-polarized cells (right) exhibit increased Golgi area and increased angle around the nucleus.

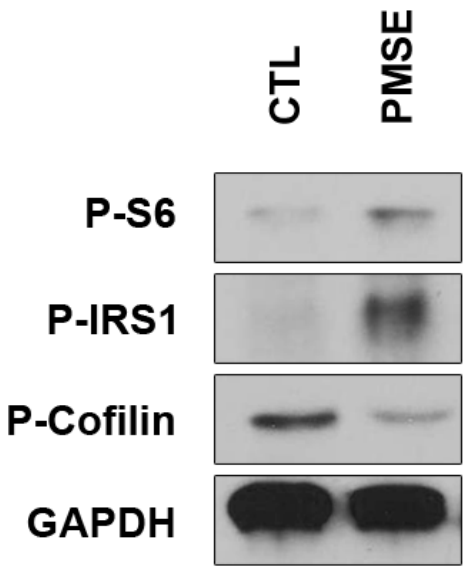

Fig. S4. PMSE patient fibroblasts display evidence of aberrant mTORC1 activation. PMSE patient fibroblasts (PMSE; *STRADA*$^{-/-}$) exhibit enhanced phosphorylation of S6 and IRS1, and diminished phosphorylation of cofilin, relative to control (CTL; *STRADA*$^{+/+}$) fibroblasts, supporting the mechanism established in STRADA knockdown mNPCs. GAPDH serves as an internal loading control.

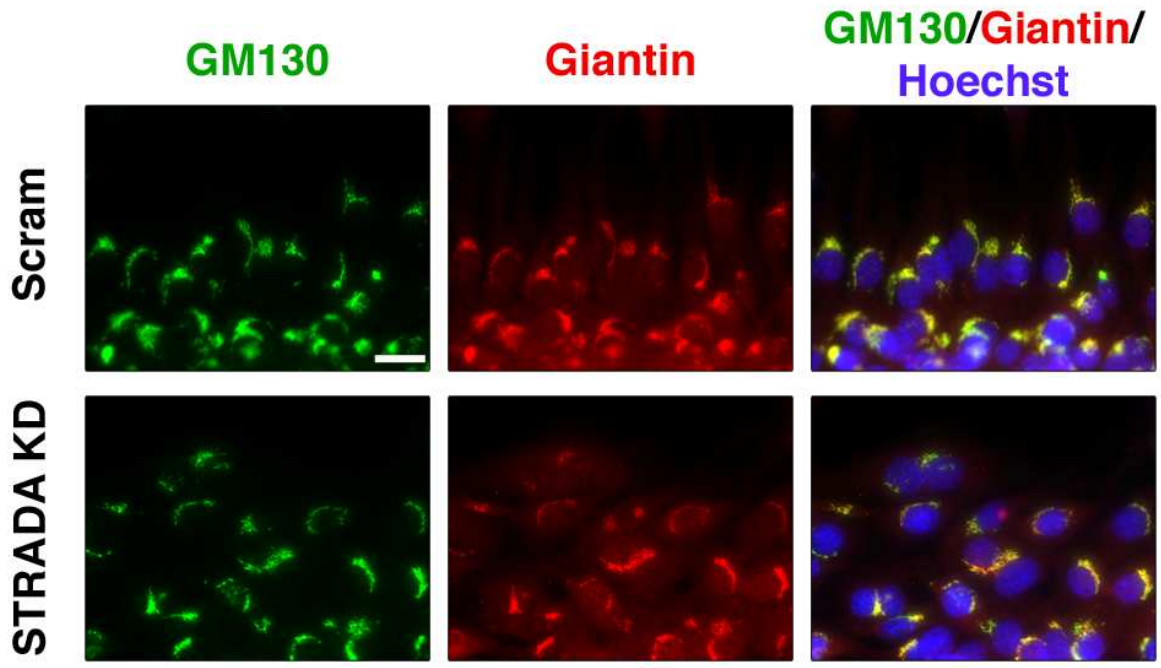

Fig. S5. Co-immunolabeling with giantin antibody confirms Golgi-specific GM130 stain. To ensure Golgi specificity of our GM130 stain, we co-labeled for GM130 (green, in the first column) and a second Golgi marker, giantin (red, in the second column), and ensured co-localization of the two markers (yellow, in the third column overlay). Scale bar, 50 μm.

Table S1. Summary of quantitative and statistical measures corresponding to figures in the text.

Corresponding Figure	Cell Type	Condition	Treatment	Mean	S.E.M.	Unit Measured	n	Comparison	p-value
1D	mNPCs	Scram	Untreated	313.62	3.67	μm	3 wells, 90 measures	Scram vs. STRADA KD-untreated	<0.00001
		STRADA KD	Untreated	244.77	4.42	μm	3 wells, 90 measures	STRADA KD-untreated vs. STRADA KD-Rapa	0.000016
			Rapamycin (50 nM)	268.4	9.03	μm	3 wells, 90 measures		
1E	mNPCs	Scram	Untreated	135.99	3.61	μm	3 wells, 90 measures	Scram vs. STRADA KD-untreated	<0.00001
		STRADA KD	Untreated	113.66	2.9	μm	3 wells, 90 measures	Scram vs. STRADA KD-p70S6Ki	0.74
			p70S6Ki (10 μM)	134.35	2.51	μm	3 wells, 90 measures		
2B	mNPCs	Scram	Untreated	508.59	26.63	μm	20 cells	Scram vs. STRADA KD	<0.00001
		STRADA KD	Untreated	688.74	64.13	μm	15 cells		
2C	mNPCs	STRADA KD	Untreated	397.46	23.89	μm	20 cells	STRADA KD-untreated vs. STRADA KD-Rapa	0.0001
		STRADA KD	Rapamycin (50 nM)	348.31	21.98	μm	20 cells		
2D	mNPCs	Scram	Untreated	784.88	92.93	degrees^2	20 cells	Scram vs. STRADA KD	<0.00001
		STRADA KD	Untreated	2050.1	129.39	degrees^2	15 cells		
2E	mNPCs	STRADA KD	Untreated	2254.38	111.08	degrees^2	20 cells	STRADA KD-untreated vs. STRADA KD-Rapa	<0.00001
		STRADA KD	Rapamycin (50 nM)	1481.8	84.12	degrees^2	20 cells		
2J	mNPCs	Scram		78.83	1.33	μm^2	640 cells	Scram vs. STRADA KD-untreated	<0.00001
		STRADA KD	Untreated	98.8	1.95	μm^2	600 cells	STRADA KD-untreated vs. STRADA KD-Rapa	0.00204
		STRADA KD	Rapamycin (50 nM)	88.66	2.51	μm^2	300 cells	STRADA KD-untreated vs. STRADA KD-p70S6Ki	<0.00001
		STRADA KD	p70S6Ki (10 μM)	77.87	1.94	μm^2	360 cells	Scram vs. STRADA KD-p70S6Ki	0.67
2K	mNPCs	Scram		98.56	1.86	degrees	640 cells	Scram vs. STRADA KD-untreated	<0.00001
		STRADA KD	Untreated	112.82	2.35	degrees	600 cells	STRADA KD-untreated vs. STRADA KD-Rapa	0.037
		STRADA KD	Rapamycin (50 nM)	104.93	2.56	degrees	300 cells	STRADA KD-untreated vs. STRADA KD-p70S6Ki	0.033
		STRADA KD	p70S6Ki (10 μM)	104.86	2.8	degrees	360 cells		
3E	mNPCs *in vivo*	STRADA KD	Vehicle Control (saline)	26.35	5.37	% GFP+ cells in CP	5 animals	Vehicle Control vs. Rapamycin-treated	0.000051
		STRADA KD	Rapamycin (5 mg/kg)	67.78	2.8	% GFP+ cells in CP	6 animals		
4F	Hu Fibroblasts	CTL		231.71	6.97	μm	3 wells, 90 measures	CTL vs. PMSE-untreated	0.0019
		PMSE	Untreated	201.55	6.14	μm	3 wells, 90 measures	PMSE-untreated vs. PMSE-Rapa	<0.00001
		PMSE	Rapamycin (100 nM)	269.29	9.54	μm	3 wells, 90 measures	PMSE-untreated vs. PMSE-p70S6Ki	<0.00001
		PMSE	p70S6Ki (10 μM)	285.75	7.58	μm	3 wells, 90 measures		
S2	mNPCs	WT	AraC only	560.05	11.66	μm	3 wells, 90 measures	WT-AraC vs. Scram AraC	0.14
		Scram	AraC only	534.61	13.13	μm	3 wells, 90 measures	Scram-AraC vs. STRADA KD-AraC	<0.00001
		STRADA KD	AraC only	421.47	15.68	μm	3 wells, 90 measures	STRADA KD-AraC vs. STRADA KD-AraC+Rapa	<0.00001
		STRADA KD	AraC+Rapamycin (50 nM)	564.65	20.83	μm	3 wells, 90 measures	Scram-AraC vs. STRADA KD-AraC+Rapa	0.2

Table S2. Clinical features of historical control PMSE patients. CPS, complex partial seizures; GTCS, generalized tonic-clonic seizures; EPC, epilepsia partialis continua; SE, status epilepticus; DQ, developmental quotient.

Cases	Ages	Seizure Onset before age 7 mos	Patients with seizures at least monthly	Seizure Types	Infantile Spasms (%)	Mortality	Average DQ
16	7 mos-28 yrs	100%	100%	CPS, GTCS, EPC, SE, Seizure Clusters	2 (12%)	6 (38%)	0.2

Table S2. Clinical features of historical control PMSE patients. CPS, complex partial seizures; GTCS, generalized tonic-clonic seizures; EPC, epilepsia partialis continua; SE, status epilepticus; DQ, developmental quotient.

Movie S1. Control mNPCs treated with scrambled shRNA display a uniform linear pattern of migration.

Movie S2. STRADA-depleted mNPCs exhibit disrupted pathfinding.

Movie S3. Rapamycin rescues pathfinding defect in STRADA-depleted mNPCs.