Supporting Information

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SI Materials and Methods

RNA Isolation and Real-Time Quantitative PCR. Total cellular RNA was isolated by using TRIzol (15596026; Life Technologies) according to the manufacturer's instructions. RNA quantification and purity were assessed by using a Nanodrop spectrophotometer. One microgram of total RNA was used for cDNA synthesis with qScript cDNA Supermix kit (95048-100; Quanta Biosciences). Real-time quantitative PCR (RT-qPCR) reactions were performed in triplicate on an ABI 7500 Fast Real-time PCR System with SYBR Green PCR master mix (no. 4309155; Applied Biosystems, Life Technologies). Standard curves were run for all primer pairs to ensure high efficiencies (>97%; $R^2 > 0.98$) and a single shoulder-free peak upon melt curve analysis. Primer sequences are provided in Table S1. GAPDH levels were used for normalization. Similar results were obtained with Rpl29 (not tested in macaque) and Eef1A.

Cell Culture and Transfection. *Primary hippocampal cell culture.* Hippocampal neurons were isolated from embryonic day 18 (E18) rat hippocampus. Freshly dissociated primary hippocampal neurons were immediately transfected with siRNA (Invitrogen) or plasmid DNA by using the Amaxa Nucleofector system (Lonza) per manufacturer's protocol (nucleofection program O-003 and Lonza kit VAPG-1003). The following siRNA sequences were used: Invitrogen, AM4620 Silencer FAM-labeled Negative Control 1 siRNA, 5′-AGUACUGCUUACGAUACGGTT-3′ and 3′-CCGUAUCGUAAGCAGUACUTT-5′; Invitrogen, 199229 Silencer Prkcz (PKM-ζ siRNA 1), 5′-CCCUGACAUGAAUACUGAAtt-3′ and 3′-UUCAGUAUUCAUGUCAGGGtt-5′; Invitrogen, s130022 Silencer Select Prkcz (PKM-ζ siRNA 2), 5′-GGUUGUUCCUGGUCAUCGAtt-3′and 3′-UCGAUGACCAGGAACAACCgg-5′.

For immunofluorescence experiments, neurons were cotransfected with pmaxGFP (Lonza) where indicated. Cells were electroporated at a density of 2.0×10^7 cells per mL with 10 nM Silencer Select siRNA (Invitrogen) or 100 nM Silencer siRNA (Invitrogen), and 1 μg of pmaxGFP. Control or transfected neurons were plated on coverslips or cell culture plates coated with 0.1 mg/mL poly-L-lysine in 95% MEM (Cellgro), 5% (vol/vol) FBS, and 0.6% (wt/vol) D-glucose. Cells were plated at a density of ∼1,500 cells per cm^2 for coverslips or ∼26,000 cells per cm^2 for RNA and protein analysis. Culture medium was replaced after 2–3 h with maintenance medium composed of Neurobasal medium (Gibco) supplemented with 2% (vol/vol) B-27 (Gibco), penicillin–streptomycin, 2 mM L-glutamine, and 10 mM Hepes buffer (pH 7.2). Half the volume of culture medium was replaced with fresh maintenance medium 24 h after transfection when applicable. Hippocampal neurons were maintained up to 3 d in culture.

Cell lines. HEK293 cells were maintained in high-glucose DMEM (Cellgro), 10% (vol/vol) FBS, penicillin–streptomycin, 1 mM sodium pyruvate, 2 mM L-glutamine, nonessential amino acids, and 10 mM Hepes buffer (pH 7.2). HEK293 cells were transfected by using Lipofectamine 2000 (Invitrogen) following manufacturer's instructions. Stable-transfected cell lines were established by antibiotic selection with 2 μg/mL blasticidin.

Immunofluorescence. Hippocampal neurons plated on coverslips (12 mm, 1.5 thickness; Fisher Scientific) were fixed in 4% paraformaldehyde (Electron Microscopy Services) for 15 min at room temperature and washed three times for 5 min each in PBS. Coverslips were treated with 50 mM ammonium chloride for 15 min, washed three times for 5 min each in PBS, and incubated for 30 min in 10% horse serum and 0.05% saponin in PBS (blocking solution). Primary antibodies diluted in blocking solution were incubated for 1 h at room temperature. Coverslips were washed three times in PBS plus 0.1% Tween 20, incubated with secondary antibody for 30 min at room temperature, and washed three times with PBS plus 0.1% Tween 20 before mounting onto glass slides with ProLong Gold (P36930; Invitrogen). A χ^2 test was used to determine significance of phenotypic changes in neuron polarization. A list of primary and secondary antibodies and concentrations used can be found in Table S3.

Western Blotting and Coimmunoprecipitation. Proteins were extracted from tissues or neurons in culture by sonication in radioimmunoprecipitation assay buffer (20 mM Tris, pH 7.2, 170 mM NaCl, 1% NaDeoxycholate, 1% Triton X-100, 0.1% SDS, 5 mM EGTA, 5 mM EDTA) supplemented with protease and phosphatase inhibitor (78443; Pierce). Lysates for coimmunoprecipitation were prepared by lysing hippocampal tissue in 10 mM Tris, pH 7.2, 150 mM NaCl, 1% Triton X-100, 5 mM $MgCl_2$, 2 mM EGTA, 6 mM 2-mercaptoethanol, and sonication. Lysates were clarified by centrifugation and precleared by incubating with Protein A agarose beads (Repligen) for 2 h. After removing agarose beads, lysate was incubated with either anti-pan atypical protein kinase C (aPKC) (SC-216; Santa Cruz) or anti-PKM-ζ/PKC-ζ (C24E6/no. 9368; Cell Signaling) overnight at 4 °C and then incubated for 2 h with Protein A beads. For coimmunoprecipitation of tagged overexpressed proteins, lysis was performed by sonication in 25 mM Hepes, pH 7.5, 150 mM NaCl, 0.25% NaDeoxycholate, 1% Nonidet P-40, 10% (vol/vol) glycerol supplemented with protease, and phosphatase inhibitor. Lysates were clarified by centrifugation and incubated for 2 h at 4 °C with anti-FLAG M2 magnetic beads (M8823; Sigma). Immunoprecipitated complexes were washed three times with lysis buffer, resuspended in 1× LDS sample buffer (Invitrogen), and boiled. Samples were analyzed by Western blotting. A list of primary and secondary antibodies and concentrations used can be found in Table S3. Signal was detected by using the Odyssey Imaging System (LI-COR).

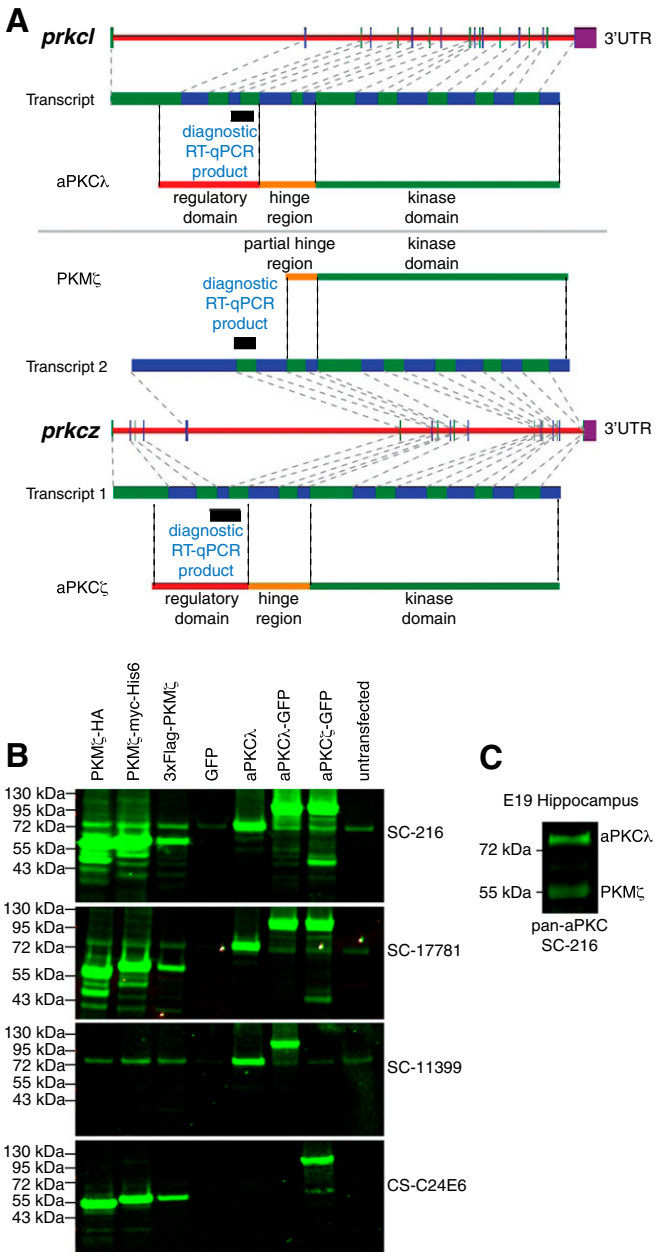

Fig. S1. Schematic representation of genes, transcripts, and proteins of the aPKC family. (*A*) Isoform-selective primers were used to determine mRNA levels of specific isoforms by RT-qPCR. (*B*) Validation of antibodies in HEK293 overexpressing tagged aPKC constructs. PKM-ζ–HA, PKM-ζ–myc–His6, 3xFlag–PKM-ζ, GFP alone, aPKCλ, aPKC-λ–GFP, and aPKC-ζ–GFP were transfected in HEK293. Lysates were resolved by SDS/PAGE and immunoblotted with Santa Cruz SC-216, SC-17781, and SC-11399 and Cell Signaling C24E6. C24E6 recognizes overexpressed aPKC-ζ and PKM-ζ, but not endogenous aPKCs or aPKC-λ. (*C*) Hippocampal lysates were resolved by SDS/PAGE and probed with Santa Cruz SC-216 antibody, detecting both aPKC-λ and PKM-ζ in E19 rat hippocampus.

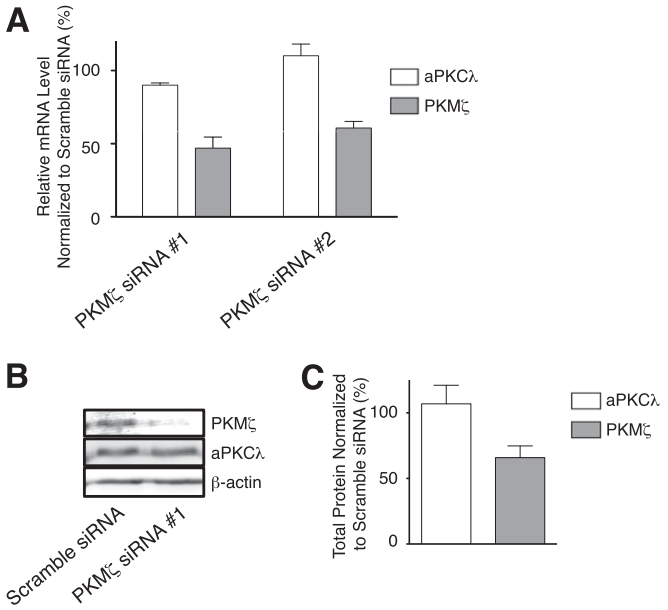

Fig. S2. Transfection of neurons with PKM-ζ–specific siRNA sequences selectively reduces PKM-ζ, but not aPKC-λ, levels. (*A*) RT-qPCR at day 3 in vitro (DIV3) revealed specific knockdown of PKM-ζ transcripts by two siRNA sequences (PKM-ζ mRNA levels 47.0 ± 5.4% and 60.8 ± 3.2% of scramble control, respectively; mean ± SEM of three independent biological replicates). mRNA expression was normalized to GAPDH. Data are presented as mean ± SEM of three independent biological replicates. (*B* and *C*) Representative Western blot for PKM-ζ and aPKC-λ in cell lysates from hippocampal neurons transfected with scramble or PKM-ζ siRNA 1 (*B*) and quantification of signal intensity normalized to β-actin (mean ± SEM of three independent biological replicates) (*C*).

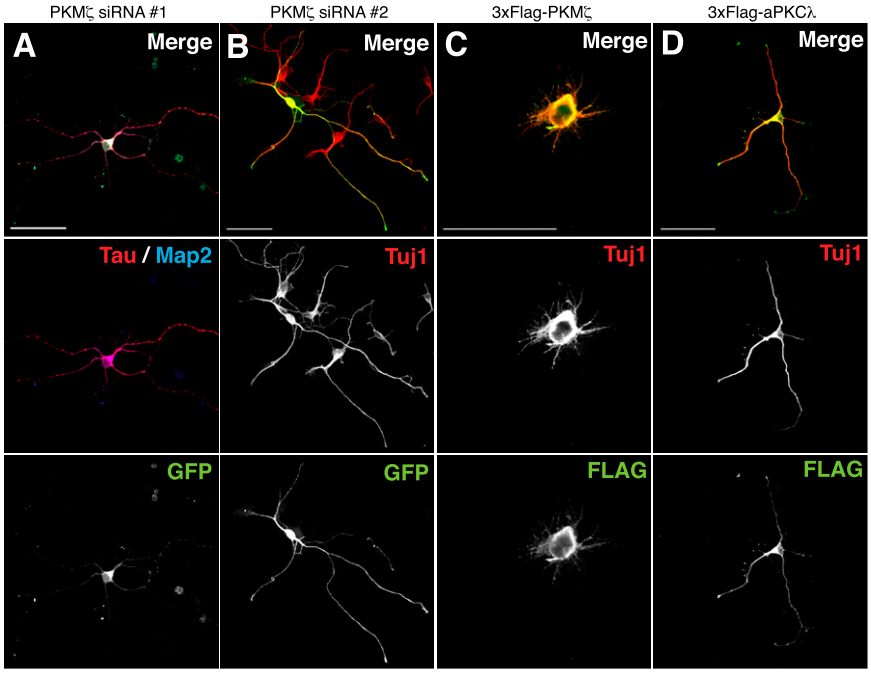

Fig. S3. Additional images of transfected neurons fixed and stained on DIV3. (*A* and *B*) Neurons treated with PKM-ζ siRNA and stained for Tau and Map2 (*A*) or Tuj1 (*B*) exhibit a multiple axon phenotype. (*C* and *D*) Hippocampal neurons overexpressing Flag-tagged PKM-ζ (*C*) or aPKC-λ (*D*) were stained for Tuj1 and Flag. (Scale bars: 50 μm.)

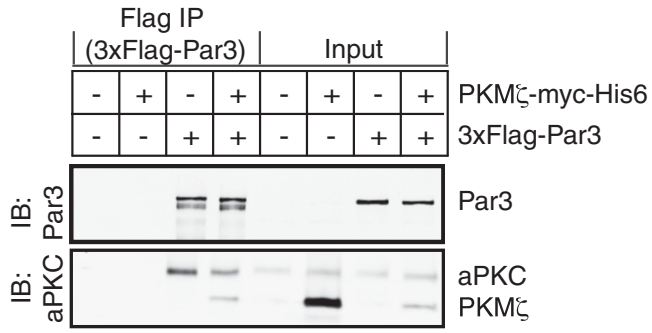

Fig. S4. Overexpression of PKM-ζ–myc–His6 and 3xFlag–Par3 in HEK293 confirms interaction by Flag pull-down and coimmunoprecipitation. PKM-ζ–myc–His6, 3xFlag–Par3, or both were expressed in HEK293 cells as indicated (+). Flag antibodies were used for immunoprecipitation. Immunoprecipitates were resolved by SDS/PAGE, and immunoblots were probed with Par3 and aPKC antibodies.

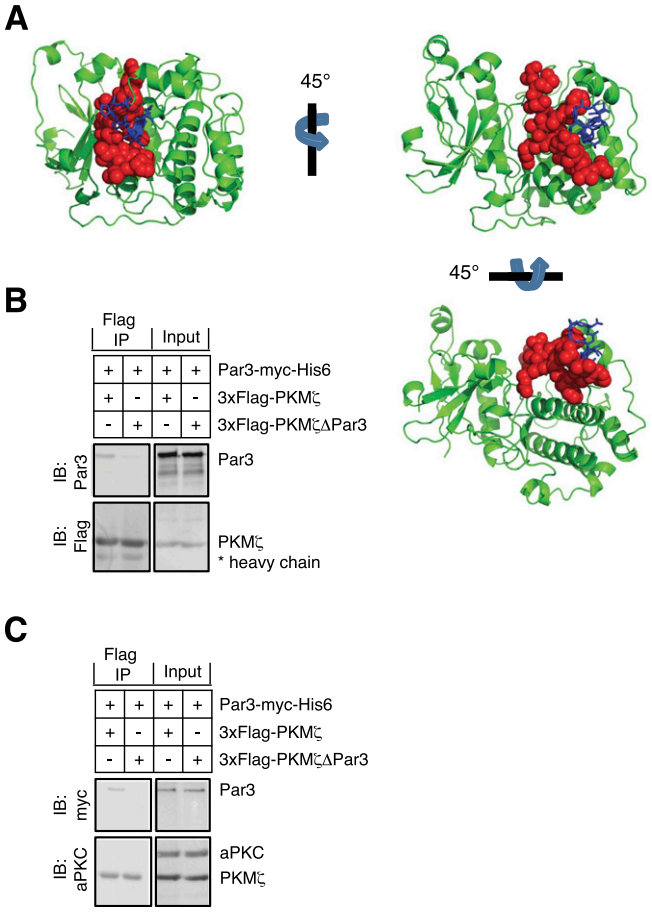

Fig. S5. Deletion of predicted Par3-interacting loop in PKM-ζ disrupts PKM-ζ–Par3 interaction. (*A*) I-TASSER predicted structure of PKM-ζ (green) bound to Par3 (red) based on published structure and interaction of aPKC-λ and Par3. Homologous amino acid sequence comprising Par3-interacting loop (IITDNPD) is highlighted in blue. (*B* and *C*) (PKM-ζΔPar3) abolished the ability of this protein to bind Par3 (*B* and *C*). Par3–myc–His6 and 3xFlag–PKM-ζ or PKM-ζ with IITDNPD deletion (3xFlag–PKM-ζΔPar3) were expressed in HEK293 cells as indicated (+). Flag antibodies were used for immunoprecipitation. Immunoprecipitates were resolved by SDS/PAGE, and immunoblots were probed with Par3 and Flag or myc and aPKC antibodies as indicated.

Table S1. RT-qPCR primers

Gene	Sequence
Rat	
GAPDH	AGGTCGGTGTGAACGGATTTG
	GGGGTCGTTGATGGCAACA
Eef1A	TGCCAATTTCTGGTTGGAATG
	GGGTGACTTTCCATCCCTTGA
Rpl29	CAAGTCCAAGAACCACACCAC
	GCAAAGCGCATGTTCCTCAG
aPKC-λ	TGAGCAGCCATTCACCATGA
	GGGAACACGTGGATCAGGAG
aPKC-ζ	GTCAGGGCAGGGACGAAGT
	GGCGGTAGATGGACTTGTCTTCT
PKM-ζ	GCTCCTTAAAGGGACGGAAGAT
	GGCTCCACGGCGGTAGAT
Macaque	
GAPDH	TGCACCACCAACTGCTTAGC
	GGCGTGGACTGTGGTCATGAG
Eef1A	TCGGGCAAGTCCACCACTAC
	CCAAGACCCAGGCATACTTGA
aPKC-λ	GGGATGCCTTGTCCAGGAG
	GCTTGGCTTGGAAAGTGTGG
aPKC-ζ	CTGCCGTGTCCAGGAGAA
	AAGCGCTTGGCTTGGAAGA
PKM-ζ	GCTGAGGAGGCAGGAGAAGA
	CGCTTGGCTTGGAAGAGAT

Table S2. List of Plasmids used

Plasmid	Backbone
Par3(712-837)-GAL4AD	pGAD-GH (Clontech)
Par3(813-837)-GAL4AD	pGAD-GH (Clontech)
Par3(2-710)-GAL4AD	pGAD-GH (Clontech)
empty-DBD	pGBT9 (Clontech)
PKMζ-GAL4DBD	pGBT9 (Clontech)
PKMζ-myc-His6	pcDNA6-*myc*-His A (Invitrogen)
PKMζΔIITDNPD-myc-His6 (PKMζΔPar3-myc-His6)	pcDNA6-*myc*-His A (Invitrogen)
Par3-myc-His6	pcDNA6-*myc*-His A (Invitrogen)
3xFlag-PKMζ	pFLAG3x
3xFlag-PKMζ (siRNA-resistant)	pFLAG3x
3xFlag-PKMζΔIITDNPD (3xFlag-PKMζΔPar3)	pFLAG3x
3xFlag-aPKCλ	pFLAG3x
3xFlag-Par3	pFLAG3x
PKMζ-HA	pcDNA3 (Invitrogen)
aPKCλ	pcDNA3 (Invitrogen)
GFP	pEGFP-C2 (Clontech)
aPKCλ-GFP	pEGFP-C2 (Clontech)
aPKCζ-GFP	pEGFP-C2 (Clontech)

Table S3. Antibodies and concentrations

Target	Antibody no.	Host species	Source	Use
pan-aPKC	SC-216	Rabbit	Santa Cruz	W (1:1,000), IP
pan-aPKC	SC-17781	Mouse	Santa Cruz	W (1:100), IF (1:50)
aPKC-λ	SC-11399	Rabbit	Santa Cruz	W (1:250), IF (1:200)
aPKC-ζ/PKM-ζ	C24E6/9368	Rabbit	Cell Signaling	IP
Par3	SC-98509	Rabbit	Santa Cruz	W (1:500)
Par3	07-330	Rabbit	Millipore	W (1:1,000), IF (1:200)
Tuj1	mab5544	Mouse	Millipore	IF (1:1,000)
Tuj1	5568P	Rabbit	Cell Signaling	IF (1:100)
Tau	mab3420	Mouse	Millipore	IF (1:750)
Map2	AB5622	Rabbit	Chemicon	IF (1:1,000)
FLAGM2	F1804	Mouse	Sigma	W (1:1,000), IF (1:100)
Beta-actin	A1978	Mouse	Sigma	W (1:2,000)
Anti-mouse IR680	926-32222	Donkey	LI-COR	2°W (1:15,000)
Anti-mouse IR800	926-32212	Donkey	LI-COR	2°W (1:15,000)
Anti-rabbit IR680	926-32223	Donkey	LI-COR	2°W (1:15,000)
Anti-rabbit IR800	926-32213	Donkey	LI-COR	2°W (1:15,000)
Anti-mouse Alexa Fluor 350	A11045	Goat	Invitrogen	2°IF (1:1,000)
Anti-mouse Alexa Fluor 568	A10037	Donkey	Invitrogen	2°IF (1:1,000)
Anti-rabbit Alexa Fluor 405	A31556	Goat	Invitrogen	2°IF (1:1,000)
Anti-rabbit Alexa Fluor 647	A31573	Donkey	Invitrogen	2°IF (1:1,000)

IF, immunofluorescence; IP, immunoprecipitation; W, Western blot.