SUPPLEMENTAL MATERIALS

Table S1: ***Alternatively spliced variants of Cdc42 mRNA are found from fish to mammals.*** Sequence comparison for indicated species shows conservation of *Cdc42* alternative splicing from fish to primates.

Species (common name)	NCBI Accession # *prenyl-Cdc42*	NCBI Accession # *palm-Cdc42*	CaaX motif prenyl-Cdc42	CCaX motif palm-Cdc42
Saccharomyces cerevisiae (yeast)	NM_001182116.1	-	CAIL	-
Caenorhabditis elegans (worm)	NM_063197.7	-	CNIL	-
Saccoglossus kowalevskii (worm)	NM_001168041.1	-	CVLL	-
Acanthaster planci (star fish)	XM_022244898.1	-	CSLL	-
Danio rerio (zebra fish)	NM_200632.2	NM_001018120.2	CVLL	CCIF
Xenopus tropicalis (frog)	NM_001017070.3	NM_001008026.1	CRLL	CCIF
Xenopus laevis (frog)	NM_001085899.1	XM_018224258.1	CMLL	CCIF
Nanorana parkeri (frog)	XM_018568983.1	XM_018568984.1	CRLL	CCIF
Callorhinchus milii (shark)	XM_007897764.1	XM_007897763.1	CVLL	CCIF
Python bivittatus (snake)	XM_015889191.1	XM_015889192.1	CVLL	CCIF
Alligator mississippiensis (alligator)	XM_014597185.2	XM_014597186.2	CVLL	CCIF
Gallus gallus (chicken)	NM_205048.1	XM_015296827.1	CVLL	CCIF
Chelonia mydas (turtle)	XM_007065922.1	XM_007065923.1	CVLL	CCIF
Mus musculus (mouse)	NM_009861.3	NM_001243769.1	CVLL	CCIF
Homo sapiens (human)	NM_001039802.1	NM_044472.2	CVLL	CCIF

Table S2: ***Normalized counts for prenyl-Cdc42 and palm-Cdc42 mRNAs from subcellular transcriptome datasets of different neuron types.*** Raw data of published and unpublished subcellular transcriptome datasets were performed by RNA-seq analysis pipeline (STAR, HTSeq and EdgeR) to obtain the normalized counts. Datasets were from cell body and axon-enriched transcriptomes of: embryonic DRG neurons (Minis et al., 2014), embryonic motor neurons (Briese et al., 2016), adult DRG neurons (unpublished data), and cell body-/neurite-enriched transcriptome of embryonic cortical neurons (Taliaferro et al., 2016).

	Cell Body Compartment (FKPM ± SEM)		Axon/Neurite Compartment (FKPM ± SEM)	
Neuron (species)	***Palm-Cdc42***	***Prenyl-Cdc42***	***Palm-Cdc42***	***Prenyl-Cdc42***
Embryonic DRG (mouse)	109.3 ± 23.9	271.2 ± 16.1	47.6 ± 2.3	384.7 ± 6.1
Embryonic motor (mouse)	105.2 ± 10.0	191.4 ± 14.4	18.0 ± 5.6	96.7 ± 40.0
Adult DRG (mouse)	13.0 ± 1.8	67.8 ± 6.6	4.7 ± 2.1	46.6 ± 19.3
Embryonic cortical (mouse)	428.1 ± 145.9	945.6 ± 7.3	343.6 ± 62.7	908.0 ± 44.8

Supplemental Figure S1

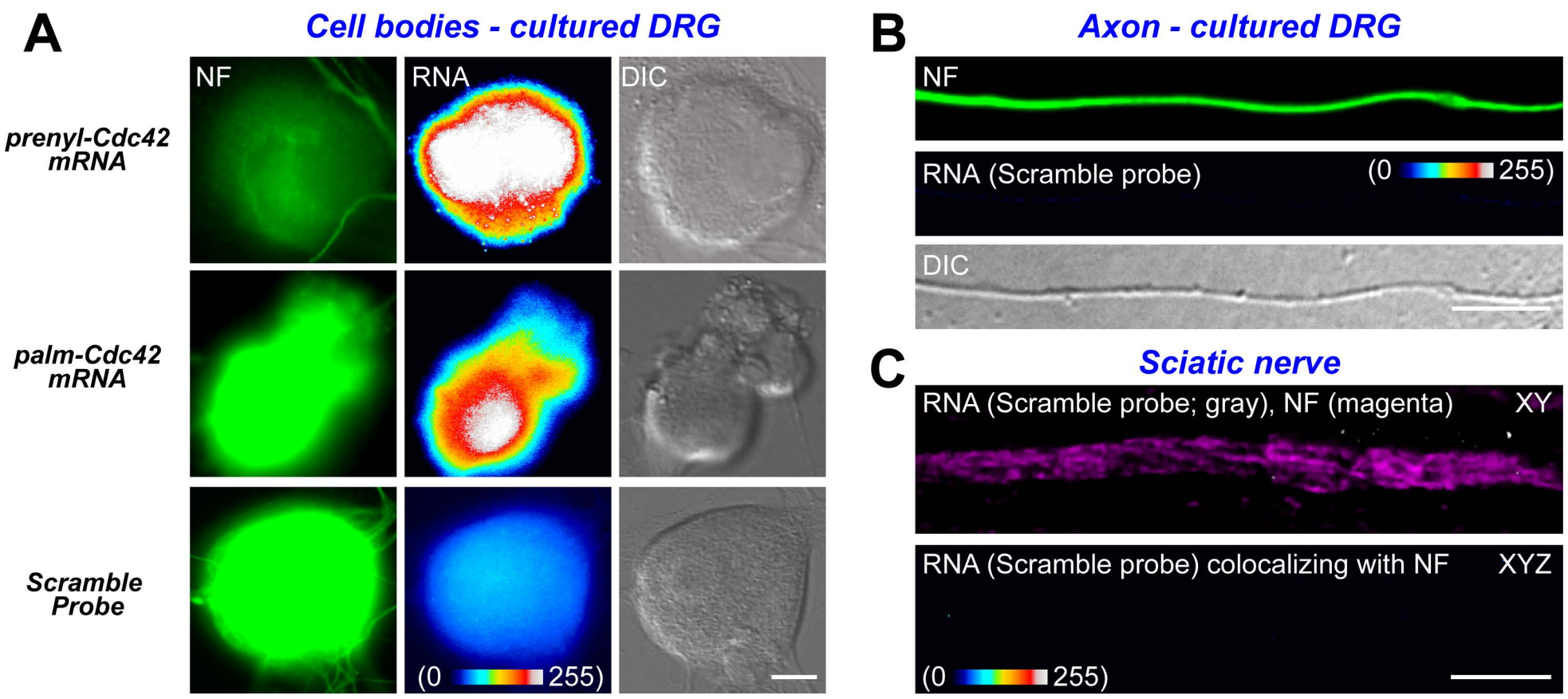

Figure S1: ***Prenyl-Cdc42 mRNA is localizes to axonal processes.***

A, Representative smFISH/IF images for cell bodies of DRG cultures from experiment in Figure 1D [scale bare = 10 µm].

B, Representative smFISH/IF images for scrambled RNA smFISH probe for axons of cultured DRGs in Figure 1D [scale bar = 10 µm].

C, Representative smFISH/IF images for scrambled RNA smFISH probe for sciatic nerve from experiment shown in Figure 1G [Scale bar = 10 µm].

Supplemental Figure S2

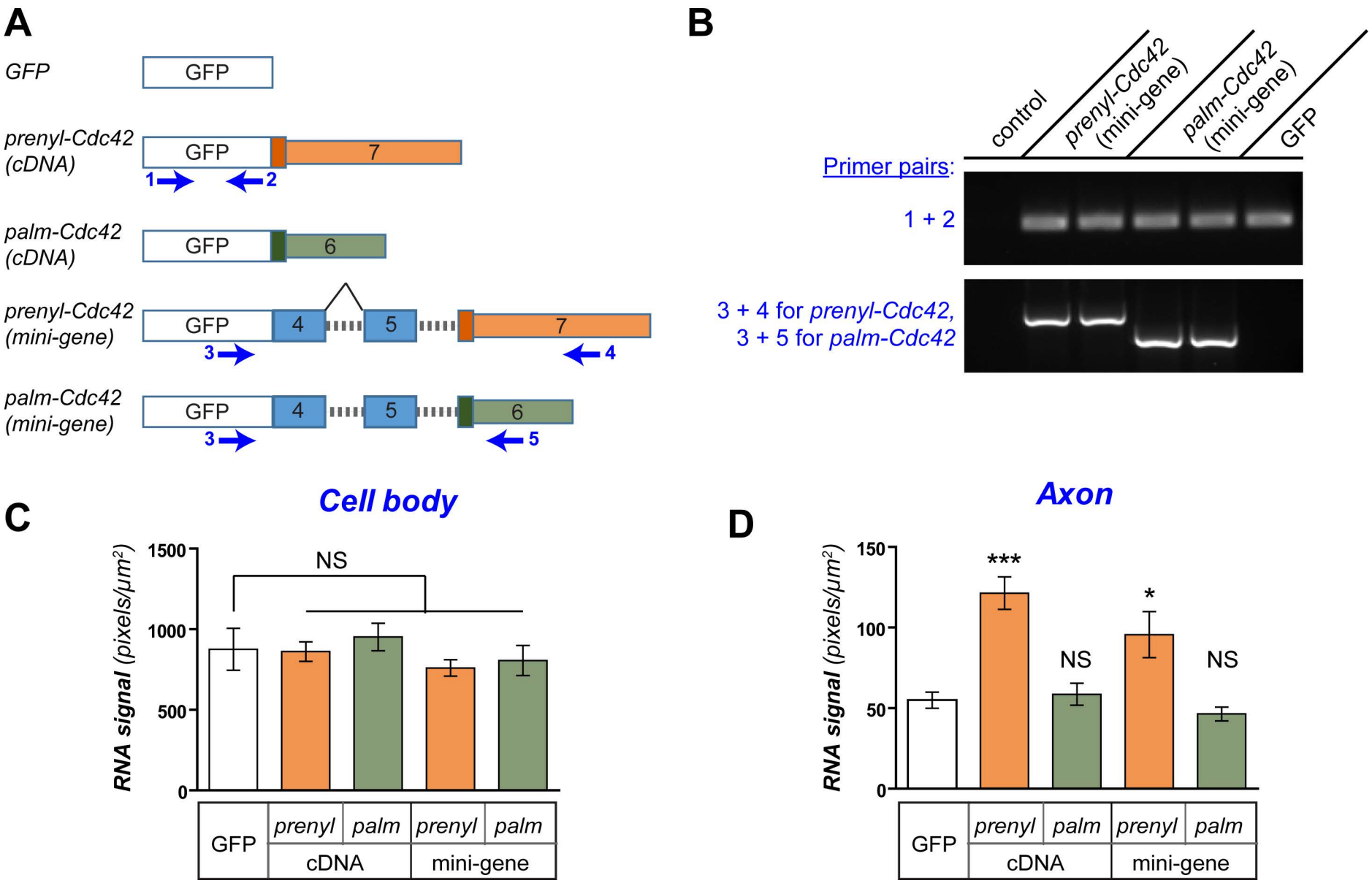

Figure S2: ***Axonal localization of prenyl-Cdc42 does not require splicing.***

A, Schematics for GFP-Cdc42 constructs designed to mimic pre-mRNA splicing differences between *prenyl-Cdc42* and *palm-Cdc42* mRNAs ('mini-genes') vs. mature mRNA ('cDNA'). Sites for annealing of primers 1-5 (blue text) are shown below each RNA schematic.

B, Representative ethidium-stained agarose gel for RT-PCR to detect splice variants using primers outlined in A as indicated. The two lanes for the *CDC42* mini-genes show replicate transfections. Amplification with primers 1 + 2 shows relatively equivalent expression across the transfections. Amplification with primers 3 + 4 and 3 + 5 shows anticipated splice variants for mini-gene constructs. Control is untransfected DRGs.

C-D, Quantification of GFP mRNA signals from smFISH for cell bodies (**C**) and axons (**D**) is shown as mean ± SEM. GFP was used as a negative control (N ≥ 30 neurons in 3 independent experiments; * $p < 0.05$, *** $p < 0.001$ by by Kruskal-Wallis test with multiple comparisons).

Supplemental Figure S3

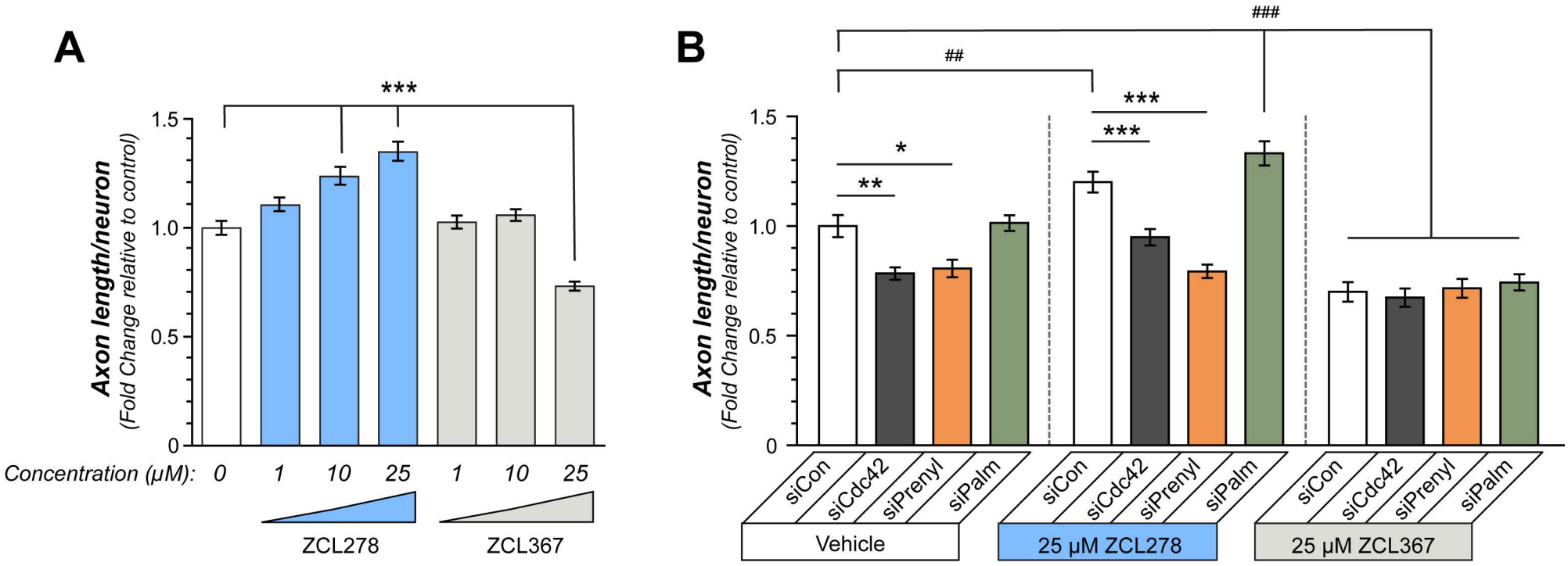

Figure S3: ***Axon growth enhancement by prenyl-Cdc42 requires Cdc42 activity.***

A, Total axon length per neuron for DRG cultures after 48 hours exposure to ZCL278 and ZCL367 shown as mean ± SEM relative to vehicle (control; N ≥ 200 neurons in 3 independent experiments; *** P < 0.005 by one-way ANOVA with pair-wise comparison and Tukey post-hoc tests).

B, Total axon length for DRGs transfected with indicated siRNAs and treated with ZCL278 or ZCL367 is shown as mean ± SEM relative to siCon + vehicle (N ≥ 150 neurons in three independent experiments; * p < 0.05, ** p < 0.01, and *** p < 0.005 for comparison to siCon within vehicle, ZCL278, and ZCL367 groups and # p < 0.05, ## p < 0.01, ### p < 0.005 group-wise comparisons of ZCL278 or ZCL367 groups to vehicle group for each corresponding siRNAs by by Kruskal-Wallis test with multiple comparisons; ZCL367 + pan-siCdc42, siPrenyl or siPalm are not significantly different than ZCL367 plus siCon).

Supplemental Figure S4

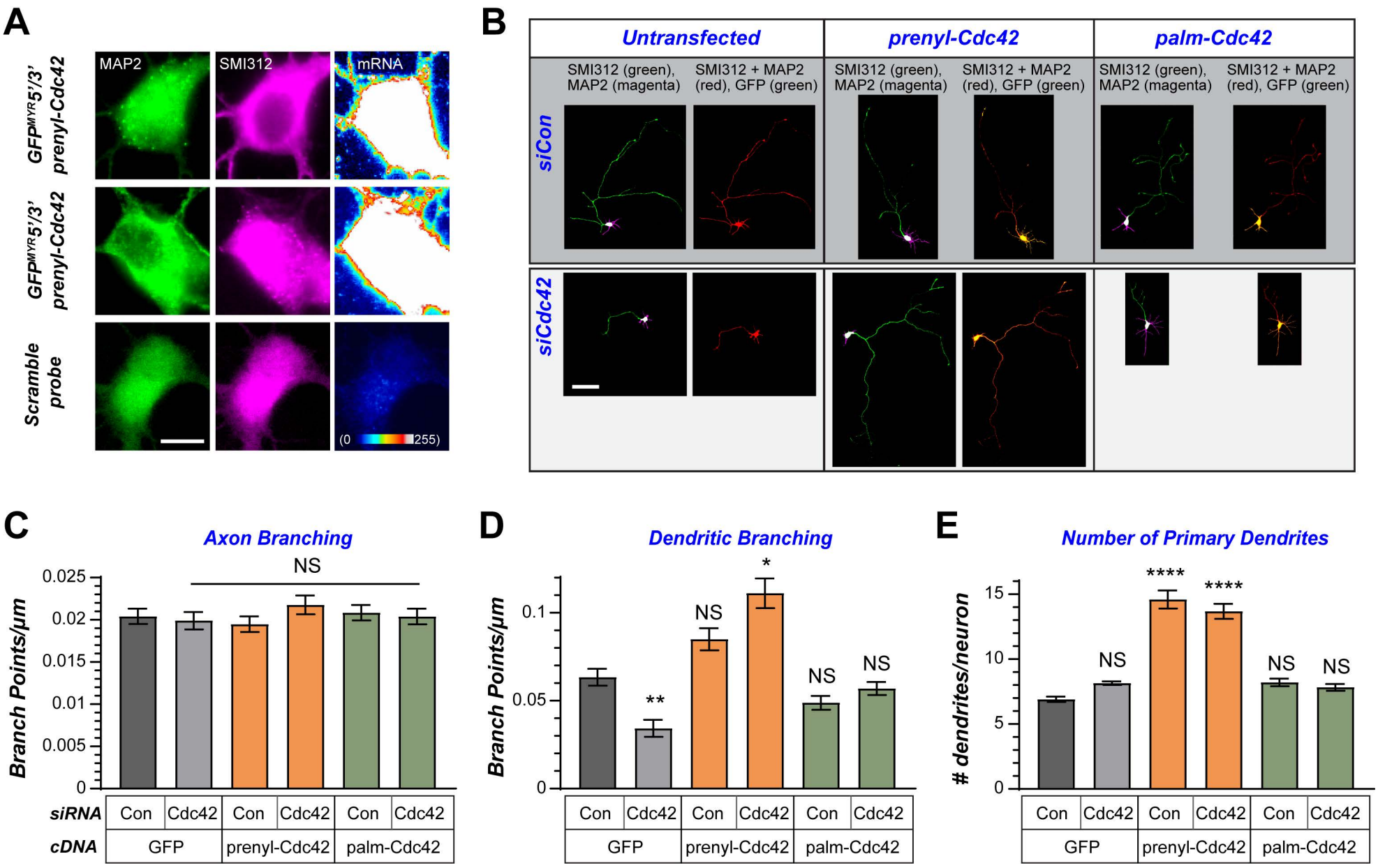

Figure S4: ***Differential localization and functions for Cdc42 mRNA isoforms in polarized cortical neurons.***

A, Representative smFISH/IF images for cell bodies of cortical neurons corresponding to the axon and dendrite images from Figure 5A are shown [scale bar = 20 µm].

B, Representative IF images for DIV9 cortical neurons co-transfected with cotransfected with control or pan-*Cdc42* siRNAs plus GFP, GFP-prenyl-Cdc42, or GFP-palm-Cdc42 mRNAs is shown as Figure 5E-F [Scale bar = 100 µm].

C-E, Quantification of axon branching (**C**), dendrite branching (**D**), and number of primary dendrites per neuronal soma (**E**) is shown for neurons co-transfected as in Figure 5E-F (n ≥ 100 neurons in 3 independent transfections: * $p \leq 0.05$, **$p \leq 0.01$, **** $p \leq 0.001$ and NS = not significant vs. control siRNA + GFP by Kruskal-Wallis test with multiple comparisons).