

Supplemental Figures for

Hümmert et al.

CDC42-effector proteins regulate higher order structure of septins required for CNS myelin integrity

Supplemental Figure S1.

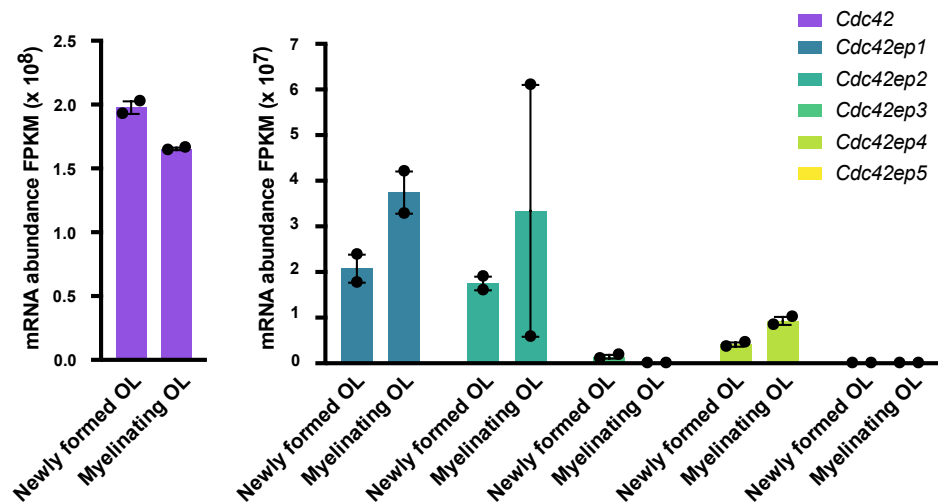
Oligodendrocytes express Cdc42, Cdc42ep1 and Cdc42ep2 according to bulk RNA-Seq data of immunopanned cells.

Supplemental Figure S2.

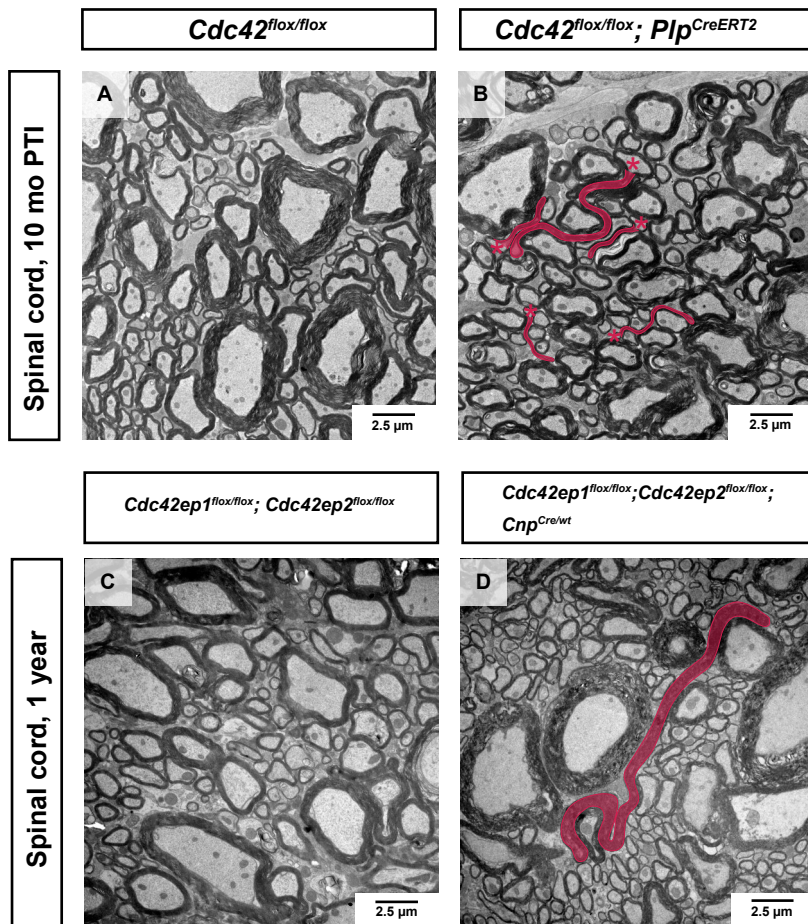
Myelin outfoldings in the spinal cord when oligodendrocytes lack *Cdc42* or both *Cdc42ep1* and *Cdc42ep2*.

Supplemental Figure S3.

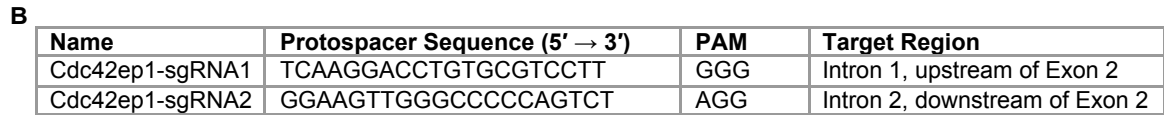
Generation of the Cdc42ep1^{flox} allele using CRISPR/Cas9.



Supplemental Figure S1. Oligodendrocytes express *Cdc42*, *Cdc42ep1* and *Cdc42ep2* according to bulk RNA-Seq data of immunopanned cells. RNA-Seq of cells immunopanned from mouse cortices shows that *Cdc42*, *Cdc42ep1*, and *Cdc42ep2* transcripts are detected in both newly formed oligodendrocytes and myelinating oligodendrocytes. Re-analysis of data from (Zhang et al., 2014). Mean \pm SEM; datapoints represent individual experiments. FPKM, fragments per kilobase of transcript per million fragments mapped.



Supplemental Figure S2. Myelin outfoldings in the spinal cord when oligodendrocytes lack *Cdc42* or both *Cdc42ep1* and *Cdc42ep2*. A-D Representative electron micrographs of cross-sectioned spinal cords show that myelin outfoldings are the main pathology in spinal cords of *Cdc42^{flox/flox}; Plp^{CreERT2}* mice (icKO 10 mo PTI, **B**) and *Cdc42ep1^{flox/flox}; Cdc42ep2^{flox/flox}; Cnp^{Cre/wt}* mice (dcKO at age 1 year, **D**) compared to respective control mice (**A,C**). This phenotype was not quantified; shown are electron micrographs from one mouse per condition representative of n=3 mice per condition. For quantification of myelin outfoldings in optic nerves see **Figure 2D,4I**. Myelin outfoldings highlighted in red; asterisks indicate associated axons.



Supplemental Figure S3. Generation of the *Cdc42ep1^{fllox}* allele using CRISPR/Cas9. A Scheme showing the targeted region of the *Cdc42ep1* gene on mouse chromosome 15 (black), homology-directed repair (HDR) template (green), introns (stippled lines), exons 2 and 3 (grey), open reading frame (bordeaux), and loxP sites (orange). Exon 2 comprises the translation initiation site (ATG, turquoise); exon 3 contains the translation termination site (Stop, blue). Genotyping primer numbers are in purple colour and refer to primer sequences given in the methods section. Scale bar, 1000 bp.

B Sequences of two single guide RNAs (sgRNAs) that were designed to target intronic regions flanking exon 2 of the *Cdc42ep1* gene. *Cdc42ep1*-sgRNA1 targets intron 1 upstream of exon 2; *Cdc42ep1*-sgRNA2 targets intron 2 downstream of exon 2. Protospacer sequences (5' → 3') and corresponding protospacer adjacent motifs (PAM) are indicated.

C Sequence of the *Cdc42ep1* HDR template, a 3524 bp double-stranded DNA fragment containing two loxP sequences flanking exon 2 of *Cdc42ep1*. LoxP sites are highlighted in bold blue lettering; Exon 2 and the 5'-end of Exon 3 are in bold black lettering. Coding sequences are underlined.