Science Signaling AAAS

www.sciencesignaling.org/cgi/content/full/4/170/ra26/DC1

Supplementary Materials for

Thioredoxin Mediates Oxidation-Dependent Phosphorylation of CRMP2 and Growth Cone Collapse

Akifumi Morinaka, Mayumi Yamada, Rurika Itofusa, Yosuke Funato, Yuta Yoshimura, Fumio Nakamura, Takeshi Yoshimura, Kozo Kaibuchi, Yoshio Goshima, Mikio Hoshino, Hiroyuki Kamiguchi, Hiroaki Miki*

*To whom correspondence should be addressed. E-mail: hmiki@protein.osaka-u.ac.jp

Published 26 April 2011, *Sci. Signal.* **4**, ra26 (2011)
DOI: 10.1126/scisignal.2001127

The PDF file includes:

Fig. S1. Immunolocalization of ectopically expressed proteins in growth cones.
Fig. S2. TRX knockdown does not inhibit serum-induced biochemical changes.
Fig. S3. Inhibition of the radial migration of cortical neurons by CRMP2 C504S.
Fig. S4. IAA treatment blocks CRMP2 oligomer formation.
Fig. S5. RT-PCR analyses of MICAL1 to 3.
Fig. S6. Rescue of H_2O_2 generation by ectopically expressed MICAL.
Fig. S7. TRX promotes GSK-3β–dependent CRMP2 phosphorylation in vitro.
Fig. S8. Sema3A stimulation does not alter the degree of Akt or GSK-3β phosphorylation.
Fig. S9. Alignment of the amino acid sequence of the region of human CRMP2 containing Cys^{504} with the corresponding sequences.
Fig. S10. Characterization of the rabbit anti-CRMP2 antibody and the rabbit anti-TRX antibody.

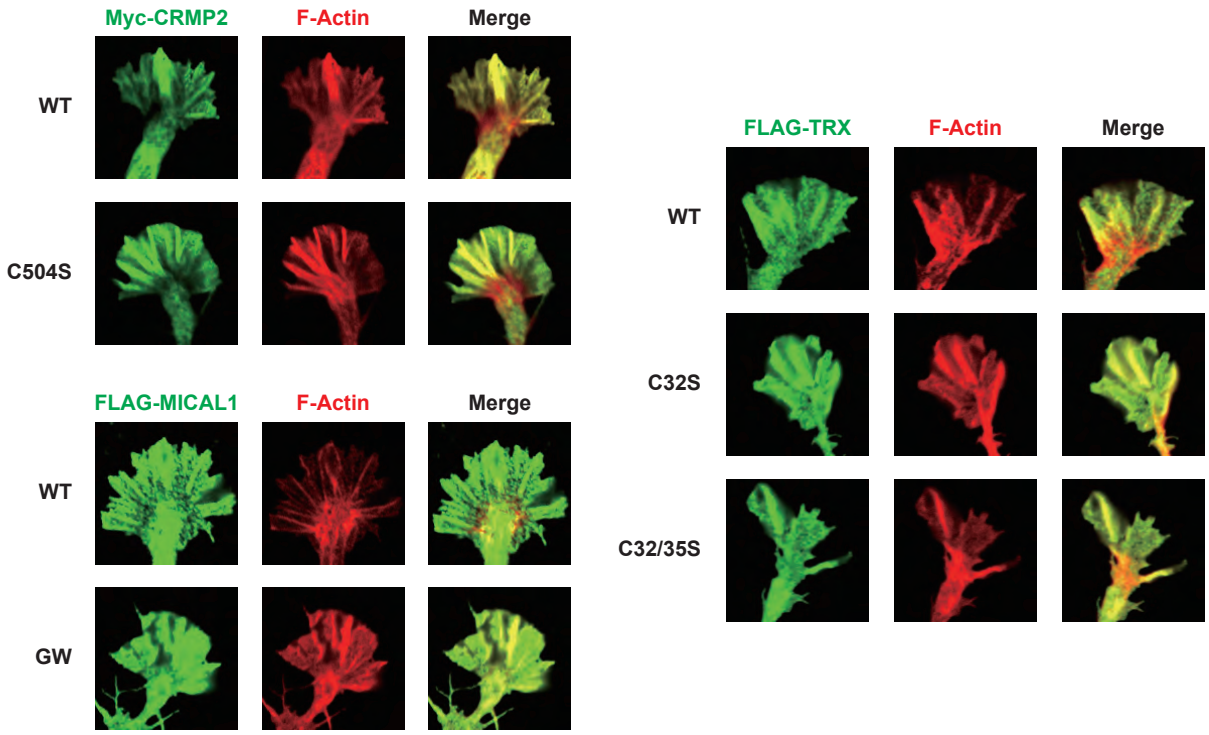

fig. S1. Immunolocalization of ectopically expressed proteins in growth cones.

DRG neurons were transfected with the indicated constructs and subjected to immunocytochemical staining with indicated antibodies (green) and phalloidin to visualize actin filaments (red).

Morinaka fig. S1

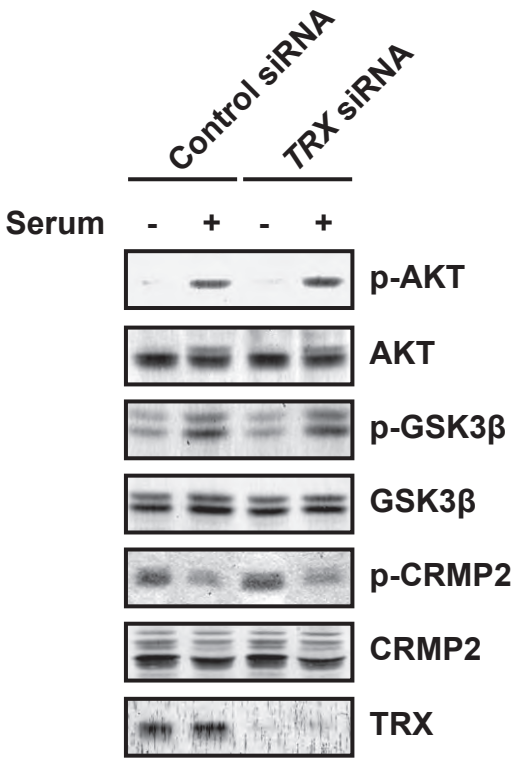

fig. S2. TRX knockdown does not inhibit serum-induced biochemical changes.
DRG neurons were transfected with the indicated siRNAs. After 3 hours of starvation, they were stimulated with serum for 30 minutes and analyzed by immunoblotting with indicated antibodies.

Morinaka fig. S2

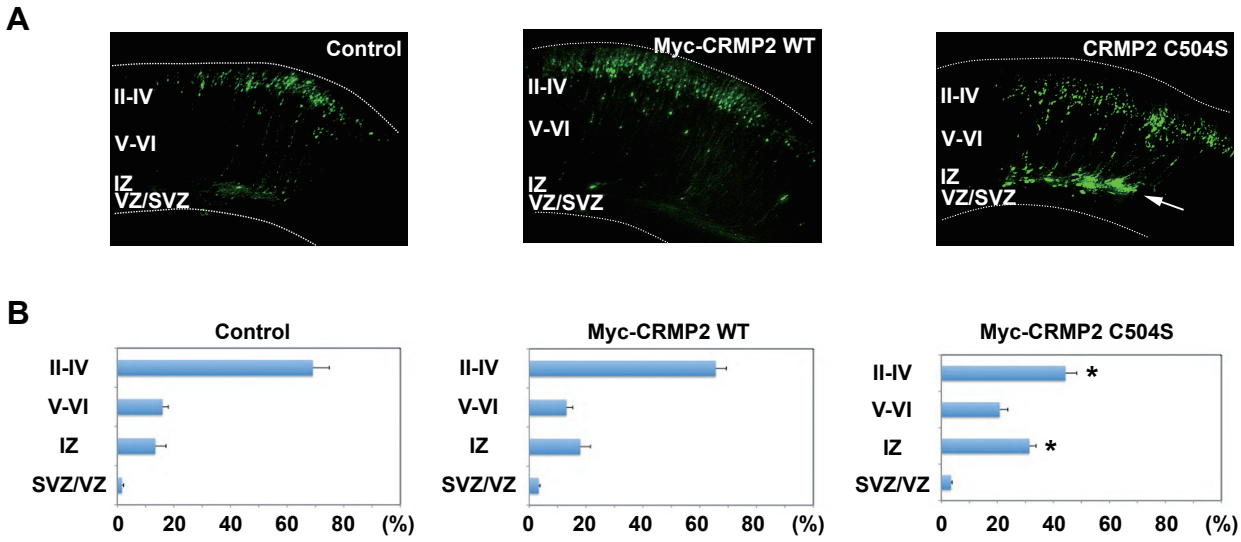

fig. S3. Inhibition of the radial migration of cortical neurons by CRMP2 C504S.
(**A**) Embryonic brains at E14.5 were electroporated with the indicated constructs plus GFP, followed by fixation at P0. Frozen sections were examined. White dotted lines represent pial and ventricular surfaces. A white arrow indicates abnormal accumulation of cells in the IZ. Scale bar, 200 μm. (**B**) Data are mean ± SEM (n = 5). Asterisks (*) indicate significant difference ($p < 0.05$) from the control. II-IV, layers II-IV of the cortical plate; V-VI, layers V-VI of the cortical plate; IZ, intermediate zone; VZ, ventricular zone; SVZ, subventricular zone.

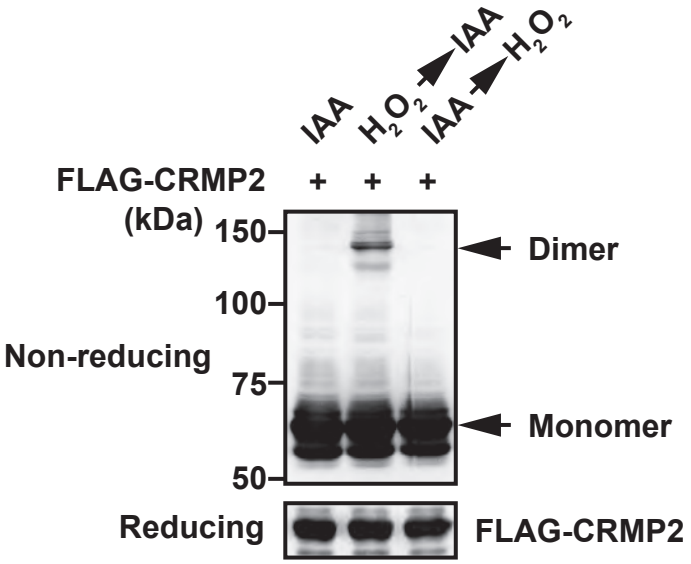

fig. S4. IAA treatment blocks CRMP2 oligomer formation.

Lysates from COS-7 cells transfected with the FLAG-CRMP2-expressing plasmids were treated with H_2O_2 either before or after IAA treatment and immunoblotted under non-reducing or reducing conditions.

Morinaka fig. S4

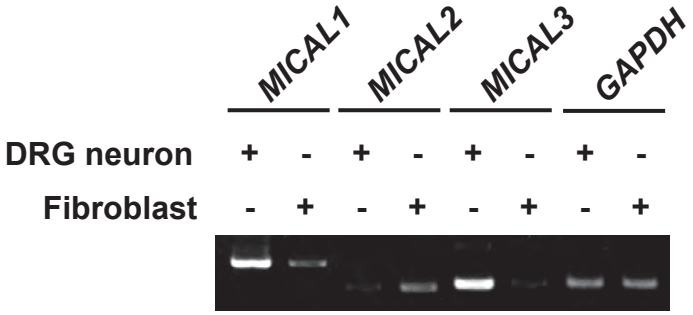

fig. S5. RT-PCR analyses of MICAL1 to 3.

RT-PCR analyses of MICAL1 to 3 were performed. Total RNAs obtained from DRG neurons (100 ng) and fibroblasts (250 ng) were used as templates.

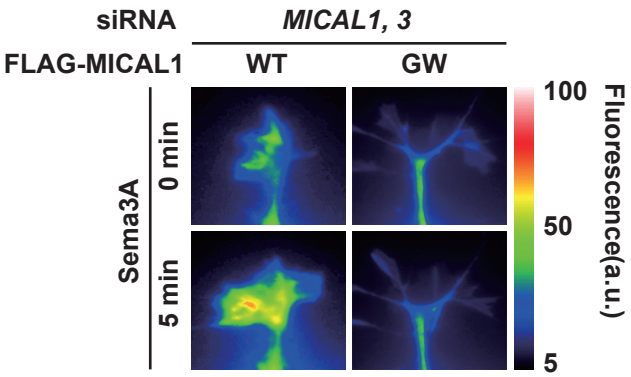

fig. S6. Rescue of H_2O_2 generation by ectopically expressed MICAL.

DRG neurons were transfected with the indicated siRNAs and constructs, together with the GFP-HyPer plasmid, and then stimulated with Sema3A. Representative fluorescent images are pseudo-colored and shown with a calibration bar (a.u.: arbitrary unit).

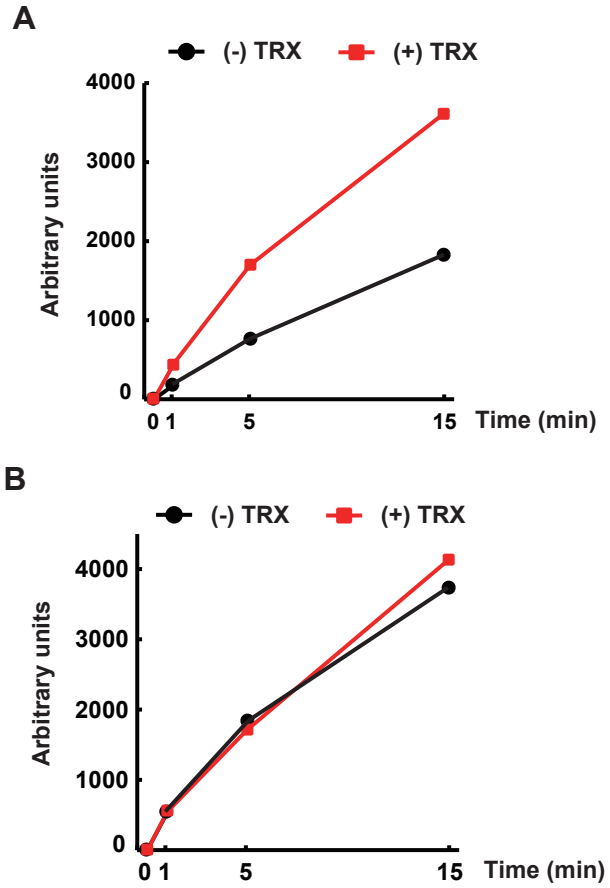

fig. S7. TRX promotes GSK-3β-dependent CRMP2 phosphorylation in vitro.

(**A**) Recombinant CRMP2 pre-phosphorylated by CDK5 was incubated with TxR, NADPH, H_2O_2 and GSK3β in the presence of [γ-^{32}P]ATP for the time indicated. His-TRX was added (red squares) or excluded (black circles). Proteins were then subjected to SDS-PAGE and autoradiography. Signals of CRMP2 at each time point are presented as a graph. (**B**) Recombinant CRMP2 was subjected to a kinase assay with CDK5/p25 as described in (A).

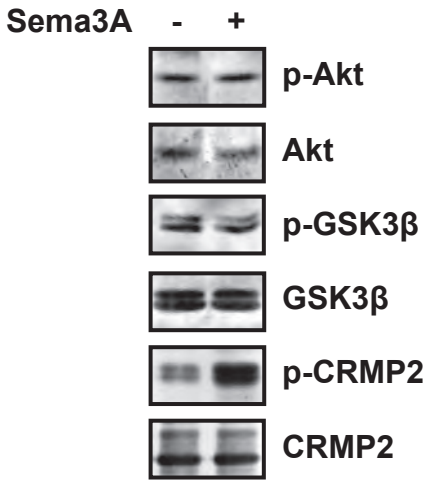

fig. S8. Sema3A stimulation does not alter the degree of Akt or GSK-3β phosphorylation. DRG neurons were stimulated with Sema3A for 30 minutes and analyzed by immunoblotting with indicated antibodies.

Morinaka fig. S8

```
                                  Cys504  Thr509  Thr514      Ser522
H. sapiens       : 497 GLYDGPVCEVSVTPKTVTPASSAKTS----PAKQ 526
M. musculus      : 497 GLYDGPVCEVSVTPKTVTPASSAKTS----PAKQ 526
R. rattus        : 497 GLYDGPVCEVSVTPKTVTPASSAKTS----PAKQ 526
X. laevis        : 497 GLYDGPVCEVSVTPKAVTPASSAKTS----PAKQ 526
D. rerio         : 497 GLYDGPVCEVSVTPKAVTPASSAKTS----PAKQ 526
D. melanogaster  : 504 GSMAKRFAELDIQIPVQEPISAMLAGNLAMPAEG 538
C. elegans       : 801 ASSQQQKPQQNGHHKNSGDFDRNRTK-------- 826
```

fig. S9. Alignment of the amino acid sequence of the region of human CRMP2 containing Cys^{504} with the corresponding sequences.

The human (*Homo sapiens*), mouse (*Mus musculus*), rat (*Rattus rattus*), frog (*Xenopus laevis*), zebrafish (*Danio rerio*), fruit fly (*Drosophila melanogaster*), and nematode (*Caenorhabditis elegans*) sequences are shown. Cys^{504}, GSK3β phosphorylation sites (Thr^{509}, Thr^{514} and Ser^{518}) and CDK5 phosphorylation sites (Ser^{522}) are shown in red, blue, and orange, respectively.

Morinaka fig. S9

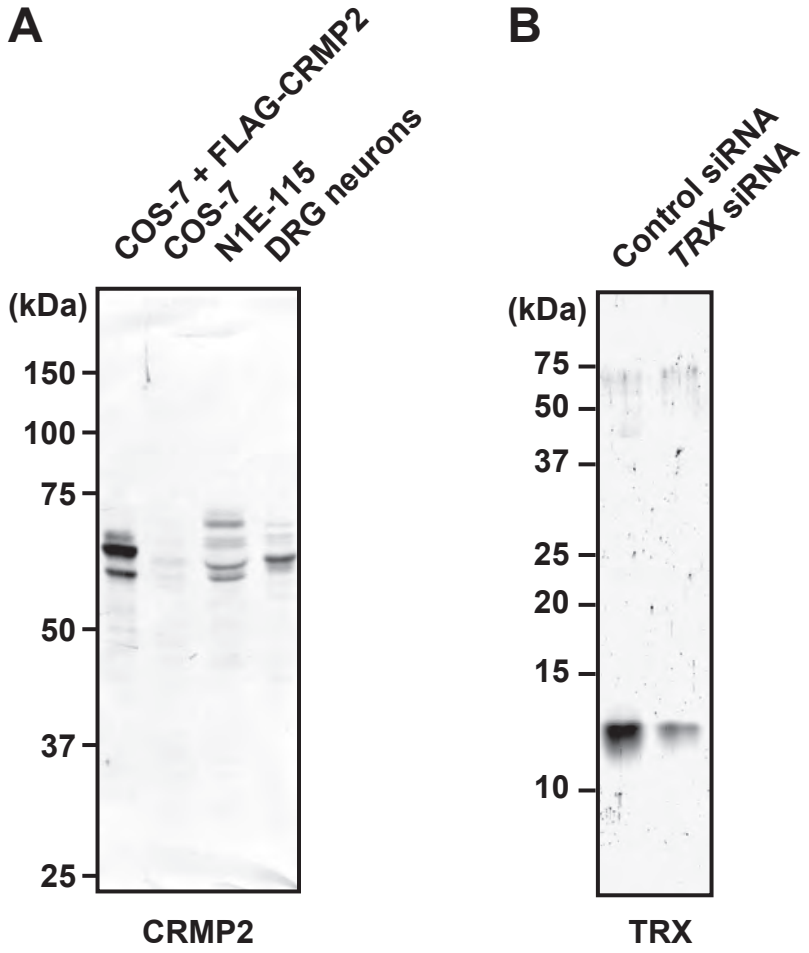

fig. S10. Characterization of the rabbit anti-CRMP2 antibody and the rabbit anti-TRX antibody (**A**) Lysates of indicated cells were subjected to immunoblotting analyses with the rabbit anti-CRMP2 antibody generated in this study. (**B**) Lysates of DRG neurons transfected with the indicated siRNAs were subjected to immunoblotting analyses with the rabbit anti-TRX antibody used in immunocytochemical staining.