Supplementary Material for

Stk25 mediates TrkA and CCM2 dependent death in pediatric tumor cells of neural origin*

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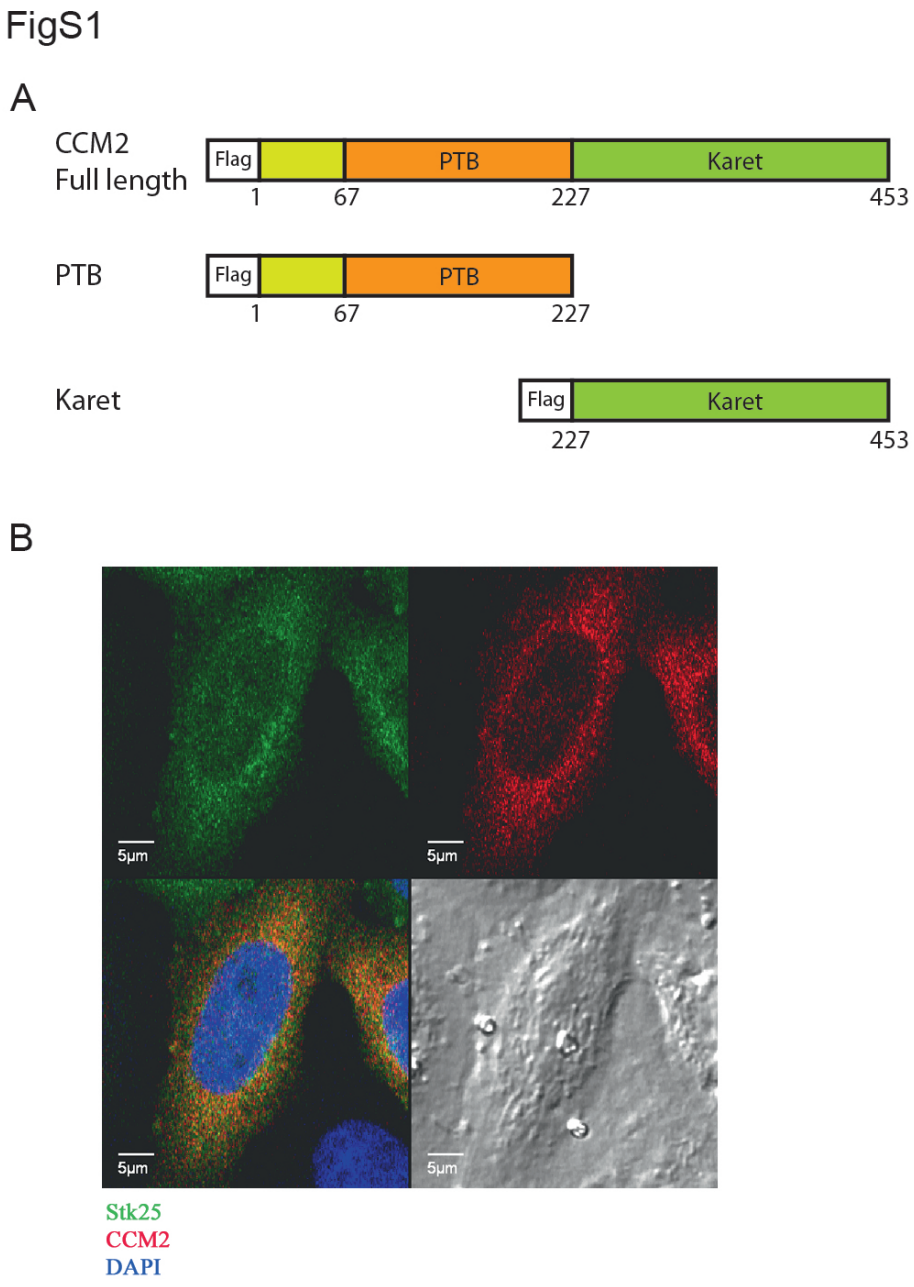
(Supplementary Table 2 is provided as a separate Excel file)

Supplementary Figures

Supplementary Figure 1. (A) Schematic representation of the FLAG tagged CCM2 constructs used in this study. **(B)** Confocal microscopy images of HeLa cells co-transfected with Stk25-GFP (green) and CCM2-RFP (red). Nuclei are stained with DAPI and visualized in blue. Scale bar 5 µm.



Supplementary Figure 2. (**A**) Stk24 and Stk25 protein expression in MB-TrkA cells transduced with the indicated shRNAs. ERK1/2 levels are used to verify equal loading. Relative quantification of (**B**) Stk24 and (**C**) Stk25 transcripts measured by quantitative PCR in shRNA-transduced MB-TrkA cells. (**D**) MB-TrkA cells were transduced with either murine Stk25wt-GFP or murine Stk25K49R-GFP constructs together with or without shRNA targeting the 3'UTR of human Stk25. Expression of exogenous constructs was assessed by Western blot with anti GFP antibody. ERK blots were used to confirm equal loading. (**E)** and (**F)** FACS analyses of cell death in the indicated cell types after 72 hr with or without NGF. n=3, Average + Standard deviation, *** indicates p< 0.001, ** indicates p < 0.01, t test. (**G**) Representative blots showing Stk24 and Stk25 expression upon long NGF time course in MB-TrkA cells. ERK levels are used to verify equal loading. Stk24 and Stk25 expression quantification from three independent experiments is shown in the graph. Average + Standard deviation. (**H**) Viability of Hek cells measured with XTT assay 24 hours after transfection with the indicated transgenes. n=3, Average + Standard deviation, *** indicates p< 0.001, ** indicates p < 0.01, * indicates p < 0.05 t test.

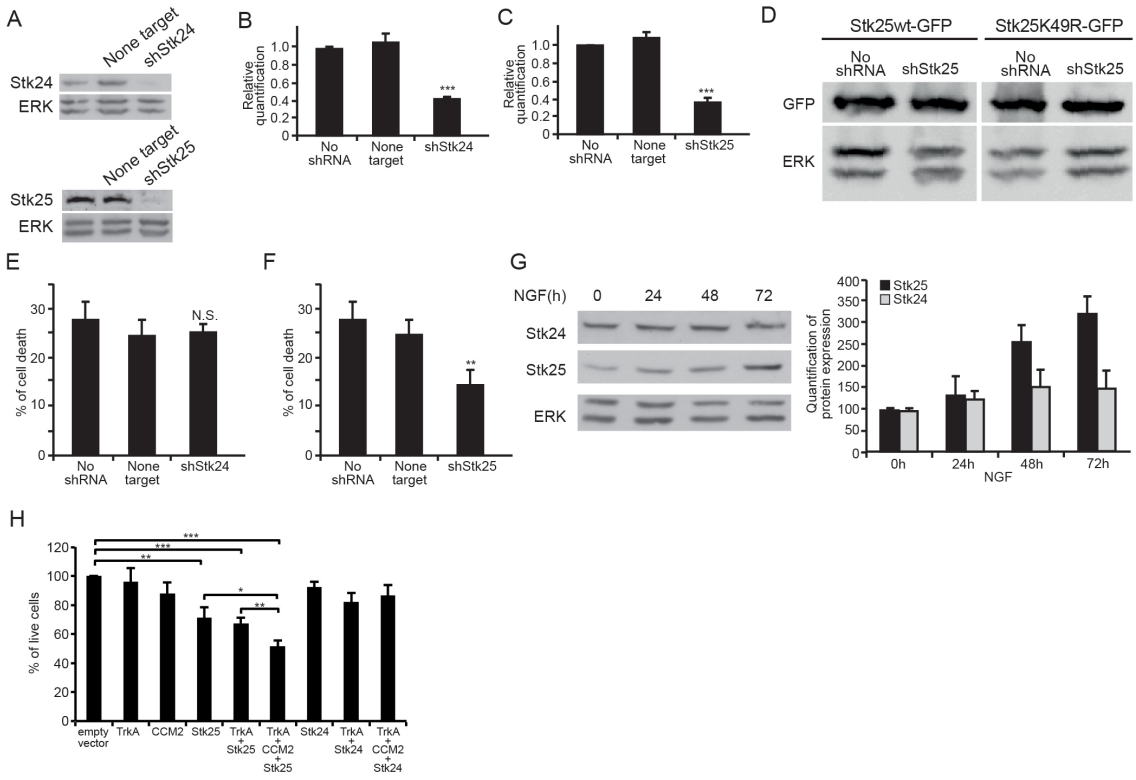


Table S1: High Confidence Interactors of CCM2 Domains

*CCM2 full length, or either the PTB domain or the Karet domain alone were expressed stably in a HEK293 cell derivative, and used to perform affinity purification coupled to mass spectrometry (at least 4 biological replicates were performed). Data was analyzed using the SAINT software tool, and stringent cut-offs, both for the SAINT scoring and for the minimal number of spectra were applied (see Methods for details). The complete list of the proteins which passed the filters is listed in the table, along with the total spectral counts across all repeats, the average spectral counts per biological repeat, the normalized spectral counts (obtained by dividing the averaged spectra by the length of the protein in amino acids *100), and the SAINT score, averaged across all replicates. Note that peptides corresponding to MST4 and CCM3 were detected with the full length CCM2 protein, but that they were associated with low SAINT scores and did not pass the stringent filtering; these are shown in grey.*

Bait	Prey	total spectra	n repeats	Averaged spectra	Normalized spectra	Averaged SAINT
PTB	ICAP1	85	4	21.3	10.6	1
PTB	CCM1	229	4	57.3	7.8	1
Full	ICAP1	278	9	30.9	15.4	1
Full	CCM1	829	9	92.1	12.5	1
Full	MST4	12	9	1.3	0.3	0.19
Full	CCM3	46	9	5.1	2.4	0.42
Karet	MST4	253	4	63.3	15.2	1
Karet	CCM3	182	4	45.5	21.5	1
Karet	STK24	188	4	47.0	10.6	1
Karet	STK25	153	4	38.3	9.0	1

***Methods**: Mass spectrometry data was searched with Mascot 2.2 against the human complement of RefSeq 29, using the following search parameters: fully tryptic cleavage with up to two missed cleavages allowed, methionine oxidation allowed as a variable modification, 3 Da tolerance for the precursor ions and 0.6 Da for the fragment ions. Identifications were transferred into a ProHits relational database (1) and filtered for significance using SAINT (2).*

1. *Liu, G., Zhang, J., Larsen, B., Stark, C., Breitkreutz, A., Lin, Z. Y., Breitkreutz, B. J., Ding, Y., Colwill, K., Pasculescu, A., Pawson, T., Wrana, J. L., Nesvizhskii, A. I., Raught, B., Tyers, M., and Gingras, A. C. (2010) Nature biotechnology **28**, 1015-1017*
2. *Choi, H., Larsen, B., Lin, Z. Y., Breitkreutz, A., Mellacheruvu, D., Fermin, D., Qin, Z. S., Tyers, M., Gingras, A. C., and Nesvizhskii, A. I. (2011) Nature methods **8**, 70-73*

Table S3: CCM2 Phosphorylation Sites

Peptide Sequence	Site	No. Peptides with unambiguous site localization	Highest Mascot Score (Corresponding Ascore)
GIITDpSFGR	S384	96	50.54 (39.83)
TAQDPGISPSQpSLCAESSR	S168	7	109.2 (40.71)
TAQDPGISPSQSLCAEpSSR	S173	6	82.09 (13.19)
RPLHpTVVLSLPER	T44	2	39.89 (94.00)
RPLHTVVLpSLPER	S48	1	38.77 (79.88)

***Methods**: 5ug of recombinant STK25 was incubated at 30°C for two hours with 10ug of GST-CCM2 and a kinase assay buffer (20mM MOPS, pH 7.2, 25mM β-glycerophosphate, 5mM EGTA, 1mM $NaVO_4$, 1mM DTT, 10mM $MgCl_2$, 10mM $MnCl_2$ and 2mM ATP). Following the assay the sample was diluted two-fold with 200mM ammonium bicarbonate. DTT was added to 5mM and the sample placed at 95°C for 10min to reduce cysteine residues. After cooling to room temperature, cysteines were alkylated using 10mM iodoacetamide and the sample left for 30 minutes in the dark. Excess iodoacetamide was quenched with 5mM DTT. 500ng of trypsin was added and the sample digested overnight at 37°C. The following morning the sample was acidified to pH 2.5 with formic acid and desalted using 75ul of POROS 20 R2 resin (Applied Biosystems) packed in a microspin column (Pierce) and subsequently vacuum-centrifuged to dryness. The sample was resuspended in 60% (v/v) acetonitrile (ACN)/25% (v/v) lactic acid/2.5% (v/v) trifluoroacetic acid (TFA) and loaded onto an equilibrated 200ul spin tip packed with 3mg of Titansphere® titanium dioxide (GL Sciences). After washing sequentially with: 1) 60% (v/v) ACN/25% (v/v) lactic acid/2.5% (v/v) TFA; 2) 80% (v/v) ACN, 0.1% (v/v) TFA; 3) 0.1% (v/v) TFA; and 4) H_2O, phosphopeptides were eluted with 5% (v/v) NH_4OH and immediately acidified to pH 2.7 with 10% TFA. The enriched fraction was then desalted with POROS 20 R2 resin as above. The sample was subsequently vacuum-centrifuged to dryness and resuspended in 6ul of 5% formic acid for LC/MS-MS. LC-MS/MS was performed using an Eksigent nano Ultra HPLC and a ThermoFisher Orbitrap Elite Mass spectrometer. Samples were loaded using an autosampler directly onto a home-made 75um ID x 10cm packed tip column filled with C18 particles (3um, Reprosil, Dr. Maish). Digested peptides were separated using a linear gradient of 100% water (0.1% formic acid) to 35% acetonitrile (0.1% formic acid) over 90mins at 200nl/min. A data dependent mode was used to acquire an MS scan (60,000 resolution) followed by 10 MS/MS CID scans at low resolution. Peptides were dynamically excluded for 15 sec after being selected for MS/MS. MS/MS spectra were queried against the RefSeq human database (version 45) using Mascot version 2.3.02 (Matrix Science). Cysteine residues were searched as a fixed modification of +57.0215 Da, methionine residues with a variable modification of +15.9949 Da, and serine, threonine, and tyrosine residues with a variable modification of +79.9663. Peptides were queried using tryptic cleavage constraints with a maximum of two missed cleavages sites. The mass tolerances were 12 ppm for parent masses and 0.6 Da for fragment masses. Site assignment was determined using Ascore (Beausoleil et al., 2006: Nat Biotechnol. 24:1285-92.) as implemented in Scaffold PTM (version 1.0.3).*