

Disease-associated scaffold protein CNK2 modulates PSD size and influences trafficking of new synaptic binding partner TNIK

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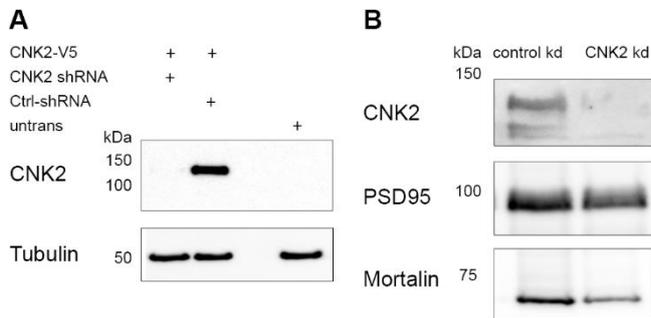
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Supplement:**Supplement Figure 1: shRNA mediated knockdown of CNK2**

(A) Western blot of shRNA mediated knockdown of V5 tagged CNK2 expressed in COS-7 cells. Western blot was probed with anti-V5 antibody. Tubulin serves as loading control. (B) Synaptosome preparation of primary neurons (DIV 23) infected with lentivirus transducing shRNA (DIV 3) to knockdown endogenous CNK2. Control knockdown (left lane) CNK2 knockdown (right lane) tested by western blot with antibody detecting endogenous CNK2 (upper panel). PSD-95 (middle panel) and Mortalin (lower panel) serve as loading control.

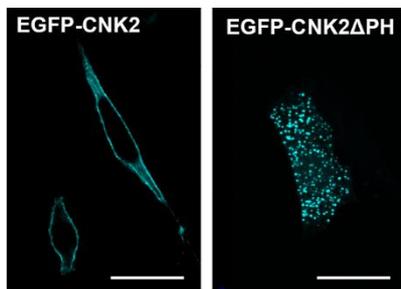
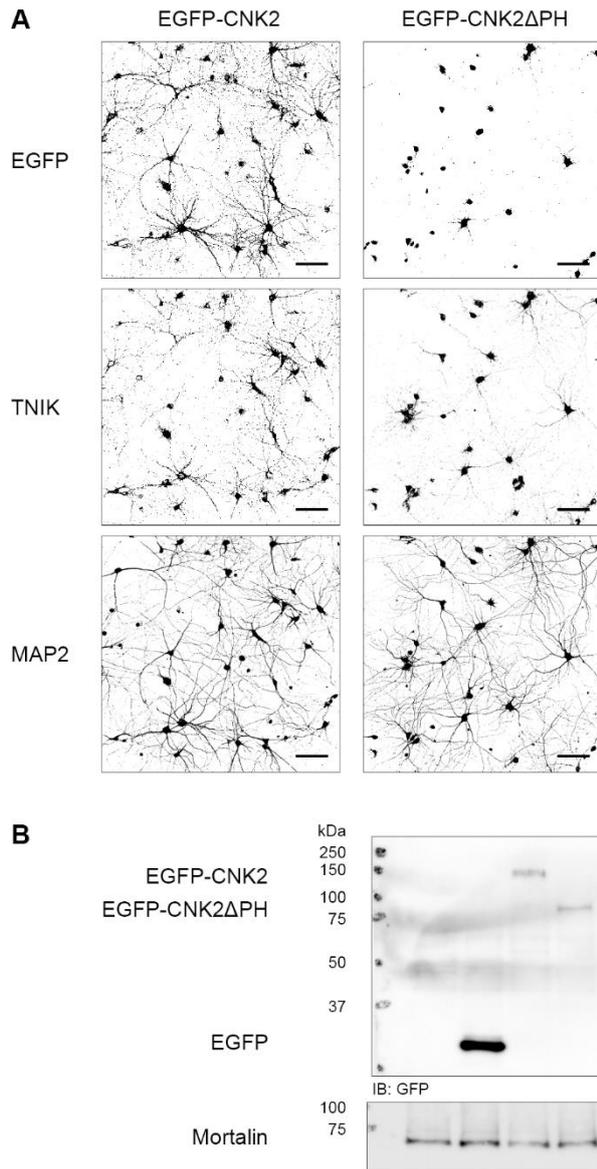
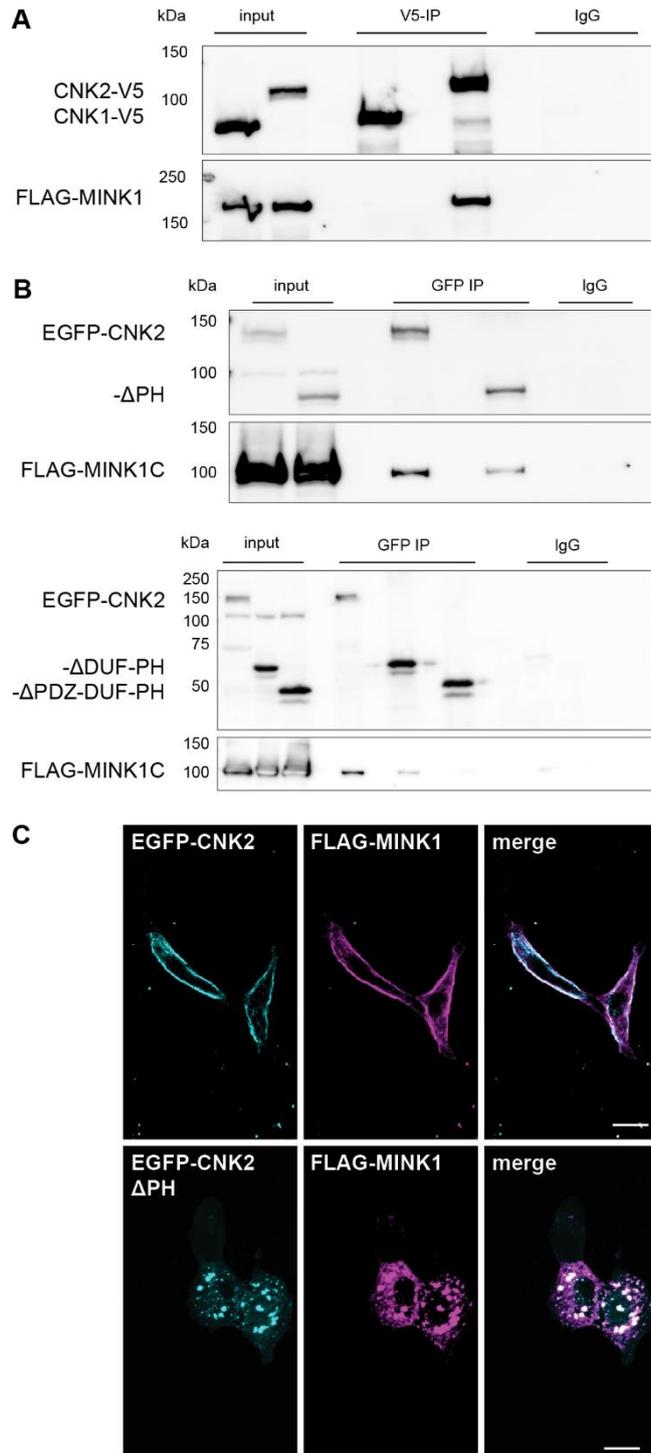
**Supplement Figure 2: The CNK2 variant CNK2 Δ PH does not bind to the membrane**

Image of COS-7 cells overexpressing EGFP-CNK2 (left) or EGFP- CNK2 Δ PH (right) Scale bar: 20 μ m



Supplement Figure 3: CNK2 Δ PH is mis-localised in neurons

(A) Overview images of neurons (DIV 23) expressing EGFP-CNK2 or EGFP-CNK2 Δ PH following viral-mediated gene delivery. Lower panel depicts MAP2, indicating the general dendritic structure of neurons. Thresholds are set identical for each panel (EGFP/TNIK/MAP2). Scale bar: 100 μ m. (B) Western blot of whole cell lysates from cultured hippocampal neurons infected with EGFP, EGFP-CNK2 or EGFP-CNK2 Δ PH; proteins detected by western blot with anti-GFP antibody. Mortalin as loading control.



Supplement Figure 4: CNK2 interacts with MINK1 and regulates its localisation

(A) CNK2 specifically interacts with MINK1. Co-immunoprecipitation experiment with CNK1-V5, CNK2-V5 and FLAG-MINK1 overexpressed in COS-7 cells. Proteins were immunoprecipitated with either anti-V5 (mouse) antibody or normal mouse IgG as a negative control. (B) Co-immunoprecipitation experiments of EGFP-CNK2 variants overexpressed in COS-7 cells together with FLAG-MINK1C (aa 534-1301). Proteins were immunoprecipitated with either anti-GFP (mouse) antibody or normal mouse IgG as negative control. Proteins were detected by WB. Input control

(lysate) is on the left. (C) Immunofluorescence experiment in COS-7 cells expressing FLAG-MINK1 together with EGFP-CNK2 (upper panel) or EGFP-CNK2 Δ PH (lower panel); Left lane shows EGFP-tagged CNK2 variants (cyan), middle lane shows FLAG-MINK1 (magenta), and right lane shows merged channels. Scale bar: 10 μ m

Gene Name	Gene ID	Interaction domain (AA)	Clones detected
Arhgap39	223666	1-319	2
Cytohesin1	19157	14-100	7
Cytohesin4	72318	6-235	5
Magi3	99470	63-656	1
Mink1	50932	430-533	4
Rlf	109263	989-1496	1
Samd12	320679	41-161	3
Sox5	20678	351-582	1
Tnik	665113	337-598	1

Supplement Table 1: Yeast-two-hybrid screen of cDNAs from adult mouse brain

The bait full-length CNKSR2 (mus musculus, Gene ID: 245684, aa 1-1032), cloned into pB27 (N-LexA-bait-C fusion) was used with an adult mouse brain cDNA library

Supplement Fig. 5: full blots

Figure 1A

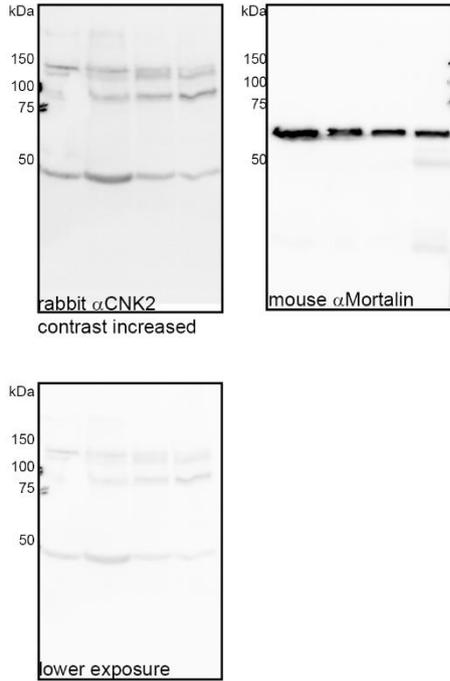


Figure 4B

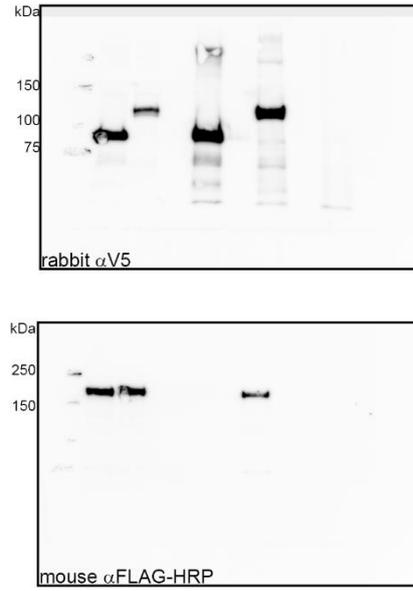


Figure 4D

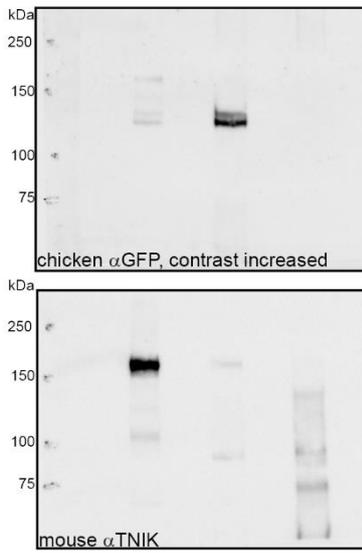
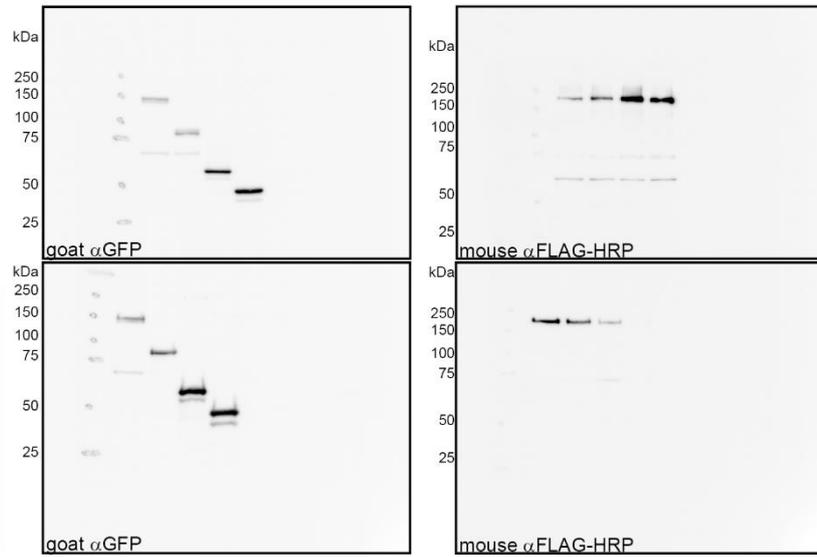
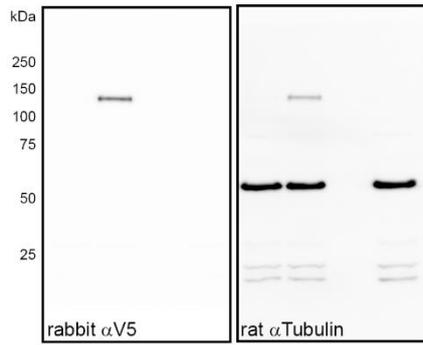


Figure 5B

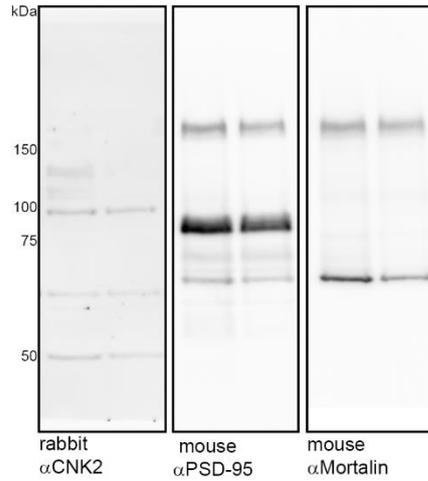


Supplement Fig. 5: full blots

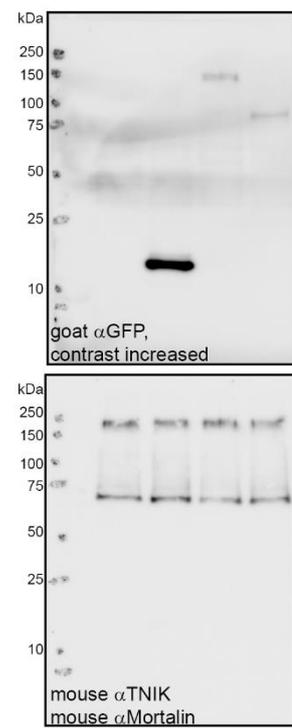
Supplement Figure 1A



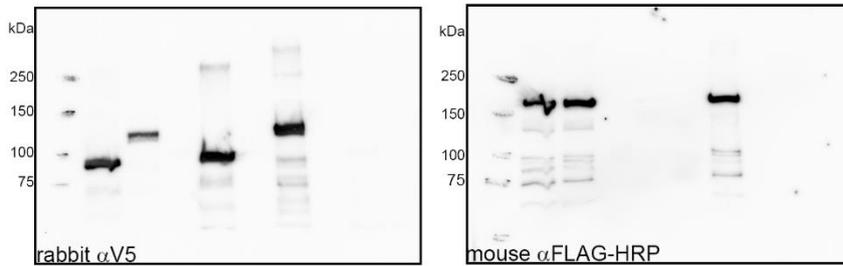
Supplement Figure 1B



Supplement Figure 3B



Supplement Figure 4A



Supplement Figure 4B

