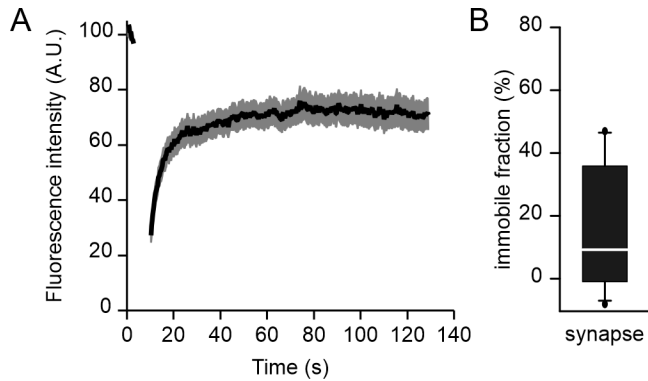
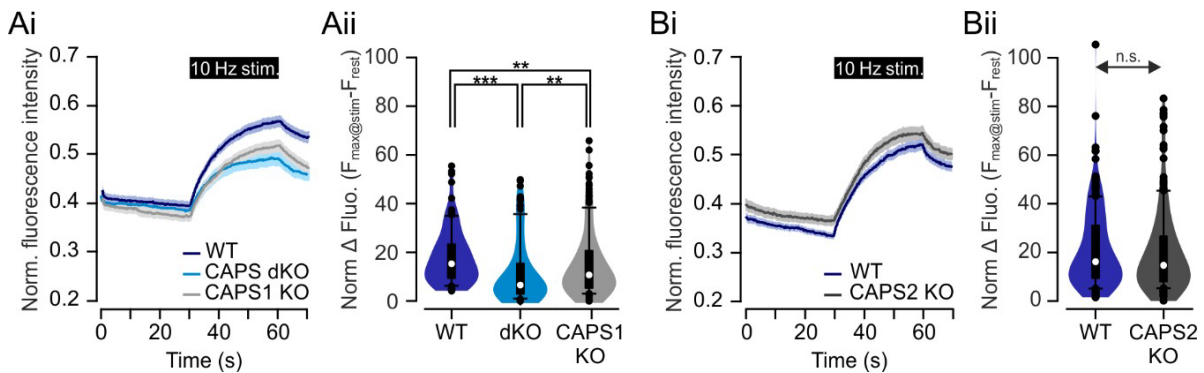


# Localization of the priming factors CAPS1 and CAPS2 in mouse sensory neurons is determined by their N-termini

## Supplementary Material



**Supplementary Figure 1: mRFP fluorescence recovery after bleaching is much faster and complete than any CAPS protein.** WT DRG neurons were transfected with a lentivirus encoding for mRFP before being co-cultured with WT SC neurons. After 9 DIV in culture, active synapses were stained with Syt1-C2 and FRAP experiment was performed as in Figure 7. **(A)** Average fluorescence recovery of the mRFP at synapses over time. Data are mean  $\pm$  SEM. **(B)** Immobile CAPS fraction displayed as box plot.  $N_{\text{experiment}} = 1$ ,  $N_{\text{synapses}} = 11$ .



**Supplementary Figure 2 CAPS1 but not CAPS2 deletion impairs synaptic transmission.** **(A)** CAPS dKO and CAPS1 KO effect on synaptic transmission in DRG neurons. WT, CAPS dKO, or CAPS1 KO DRG neurons were isolated from E18/19 mice, infected with SypHy and co-cultured with WT SC neurons. **(Ai)** Normalized average SypHy signal at synapses in response to 10 Hz field electrode stimulation are shown for WT (blue), CAPS dKO (light blue), and CAPS1 KO neurons (light grey). Data are mean  $\pm$  SEM. **(Aii)** Violin plot of the maximum normalized fluorescence intensity increase in SypHy elicited during 10 Hz stimulation. Experiments were performed on a minimum of three independent cultures for every genotype and the number of measured synapses was 119, 143, and 171 for WT, CAPS dKO, and CAPS1 KO neurons, respectively. \*\* $p < 0.01$  or \*\*\*  $p < 0.001$  ANOVA on rank with Dunn's post-hoc test. **(B)** CAPS2 KO effect on synaptic transmission in DRG neurons. WT or CAPS2 KO DRG neurons were isolated from P6 to P8 mice, infected with SypHy and co-cultured with WT SC neurons. **(Bi)** Normalized average SypHy fluorescence intensity measured at synapses in response to electrical stimulation for WT neurons (blue) and CAPS2 KO neurons (dark grey). Data are mean  $\pm$  SEM. **(Bii)** Violin plot of the maximum normalized fluorescence intensity increase in SypHy elicited by 10 Hz electrical stimulation. Experiments were performed on a minimum of three independent cultures for every genotype. 241 and 184 synapses were measured in WT and CAPS2 KO neurons, respectively. n.s. = not significant.

**A** CAPS1

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1 MLDPSSEEE SDEILLEERG KDVLGSAASG ARLSPSRTSE GSAGSAGMGG
51 SGAGAGVGAG GGGGSGASSG GGAGGLQPSS RAGGRPSSP SPSVSEKEK
101 EELERLOKEE EERKKRLQLY VFVMRCIAYP FNAKOPTDMA RROOKISKOO
151 LOTVKDRFOA FLNGETOIVA DEAFMNAVOS YVEVFLKSDR VARMVQSGGC
201 SANDSREVEFK KHLEKRVSL PEIDGLSKET VLSSWMAKFD AIYRGEEDPR
251 KOQARMTASA ASELILSKEQ LYEMFONILG IKKFEHOLLY NACOLDNPDE
301 QAAQIRRELD GRLQMADQIA RERKFPKFVS KEMENMYIEE LKSSVNLLMA
351 NLESMPVSKG GEFKLOLKR SHNASIIDMG EESENQLSKS DVLLSFSLEV
401 VIMEVOGLKS LAPNRIVYCT MEVEGGEKLO TDQAEASKPT WGTQGDFSTT
451 HALPAVKVKL FTESTGVLAL EDKELGRVIL HPTPNSPKOS EWHKMTVSKN
501 CPDQDLKIKL AVRMDKPQNM KHSGYLWTIG KNVWKRWKKR FFVLVOVSOY
551 TFAMCSYREK KAEPOELLQL DGYTVDYTDP QPLEGGRAF FNAVKEGDTV
601 IFASDDEODR ILWVOAMYRA TGOSHKPVP TOVOKLNAK GNPOLDAPI
651 SOFSGLKDAD RAQKHGMDEF ISSNPCNFDH ASLFEMVQRL TLDHRLNSY
701 SCLGWFSPGO VFVLDEYCAR NGVRGCHRHL CYLRDLLERA ENGAMIDPTL
751 LHYSFAFCAS HVHGNRPDGI GTVTVEEKER FEEIKERLRV LLENQITHFR
801 YCFPFGRPEG ALKATLSLLE RVLMKDIVTP VPOEEVKTVI RKCLEOAALV
851 NYSRLSEYAK IEENQKDAEN VGRLITPAKK LEDTIRLAEL VIEVLOQNEE
901 HHAEPHVDKG EAFAWSDLM VEHAETFLSL FAVDMDALE VOPPDTWDSF
951 PLFOLLNDFL RTDYNLCNGK FHKHLODLFA PLVVRYVDLM ESSIAOSIHR
1001 GFERESWEPV KSLTSNLPNV NLPVNLPKV PNLPVNIPLG IPOMPTSAP
1051 SWMAIYDAD NGSGTSEDLF WKLDALQTFI RDLHWPEEEF GKHLEORLKL
1101 MASDMIESCV KRTRIAFEVK LOKTSRSTDF RVPOSICTMF NVMVDAKAOS
1151 TKLCSMEMGQ EHQYHSKIDE LIETVKEMI TLLVAKFVTI LEGVLAKLSR
1201 YDEGTLFSSF LSFTVKAASK YVDVPKPGMD VADAYVTFVR HSODVLRDKV
1251 NEEMYIERLF DOWYNSSMNI ICTWLTDRMD LQLHIYQLKT LIRMVKKTYR
1301 DFRLOGVLDS TLNSKTYETI RNRLTVEEAT ASVSEGGGLO GISMKDSDEE
1351 DEEDD

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**Supplementary Figure 3 CAPS1 proteins are detected after IP by nano-LC-MS/MS.**

**(A)** Protein sequence of mouse CAPS1 (SwissProt: Q80TJ1) is shown in black. Amino acids identified by MS/MS fragmentation are shown in red, covering 54% of the protein sequence. **(B)** Example of collision induced damage (CID) MS/MS fragmentation spectra (blue; y-ions, red; b-ions, green; +NH<sub>3</sub>, +H<sub>2</sub>O, +2H, parent) derived from the underlined tryptic mouse CAPS1 peptide in A analyzed by nano-LC ESI-MS/MS

