SUPPLEMENTAL DATA

DILEUCINE AND PDZ BINDING MOTIFS MEDIATE SALM1 TRAFFICKING IN HIPPOCAMPAL NEURONS

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SUPPLEMENTAL METHODS

Immunofluoresence-HeLa cells or hippocampal neurons were transiently transfected with Myc-SALM1 or Myc-SALM2 constructs using calcium phosphate precipitation (Clontech, Mountainview, CA) as described in the Experimental Procedures. GFP cDNA was cotransfected with the SALM2 constructs in neurons to visualize their morphology. Transfections were performed at 11-12 days *in vitro* (DIV) and immunocytochemistry was performed at 14-15 DIV.

For hippocampal neurons, surface staining was performed for 20 min at room temperature with anti-Myc monoclonal antibody (1:500, clone #9E10, hybridoma purchased from ATCC, Manassas, VA). Neurons were washed with neurobasal media, fixed with 4% paraformaldehyde, blocked for 10 min with 10% normal goat serum in PBS and stained with Alexa Fluor 555 antibodies (Suppl. Fig. 1). For total staining, neurons were permeabilized with 0.25% Triton X-100. Phalloidin (Invitrogen) or primary antibodies (1:500, SALM1; 1:200, transferrin receptor (Zymed/Invitrogen); 1:200, EEA1 (BD Transduction Laboratories) were incubated for 1 hr at room temperature and then stained with Alexa Fluor 555 and 444 secondary antibodies. Images were acquired using an LSM710 microscope with a 40x 1.3 NA oil objective. Surface expression of transfected SALM proteins was quantified using Metamorph. Images were thresholded and integrated pixel intensity of surface puncta on axons and dendrites was examined in 10 μm regions.

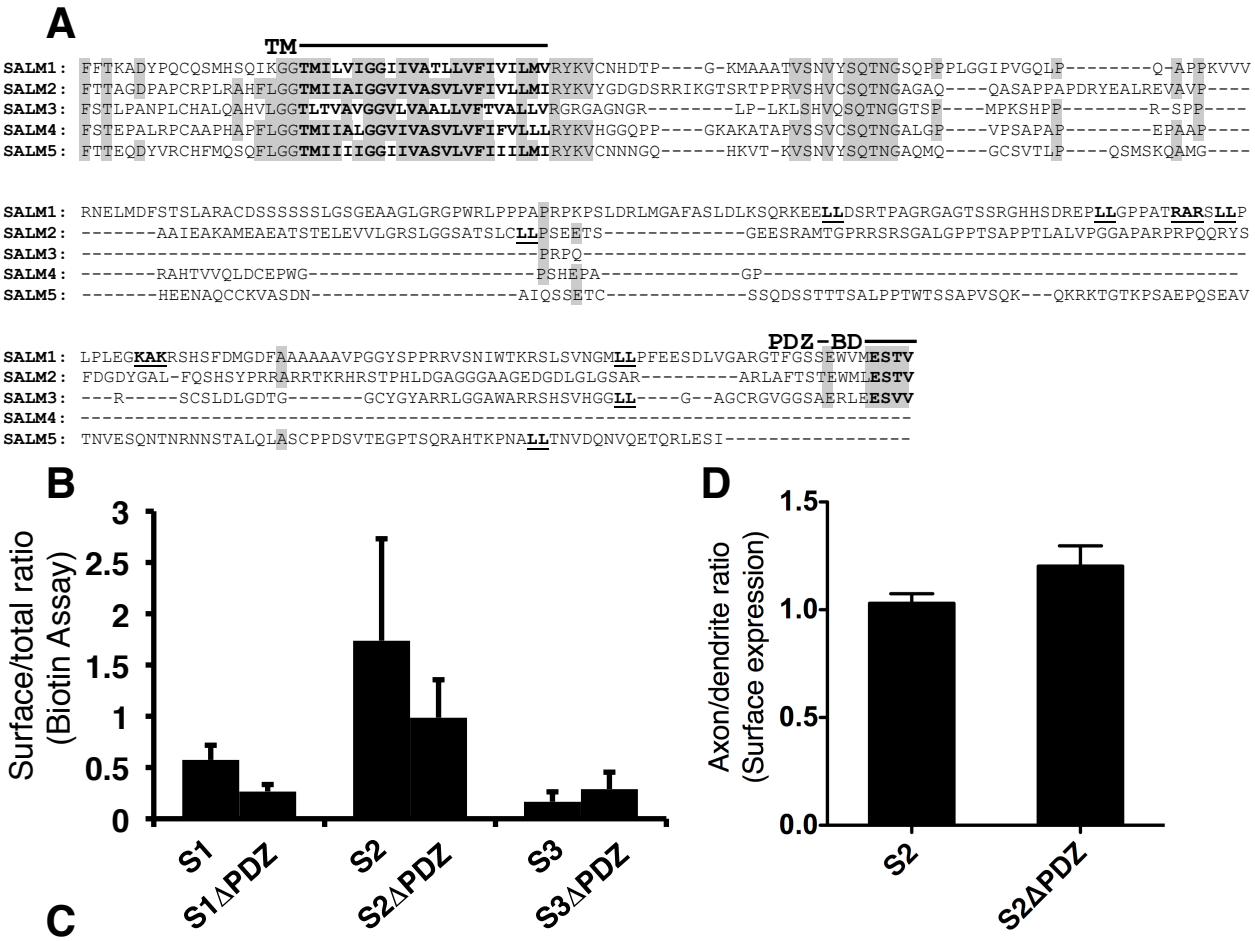
For HeLa cells, total staining was performed after cells were fixed with 4% paraformaldehyde at room temperature, and washed with PBS. They were permeabilized with 0.1% Triton X-100/PBS for five min, blocked with 10% normal goat serum in PBS, and incubated with primary antibodies for 1 hr. N-terminal SALM1 antibody (1:500) was incubated with KDEL (1:400, Stressgen/Enzo Life Sciences, Plymouth Meeting, PA) or GM130 (1:100, BD Transduction Laboratories, San Jose, CA) antibodies and stained with Alexa Fluor 568 (KDEL and GM130) or Alexa Fluor 444 (SALM1) secondary antibodies (Invitrogen/Molecular Probes) for 30 min. Images were acquired using an LSM710 microscope with a 63x 1.4 NA oil objective.

SUPPLEMENTAL FILES and FIGURE LEGENDS

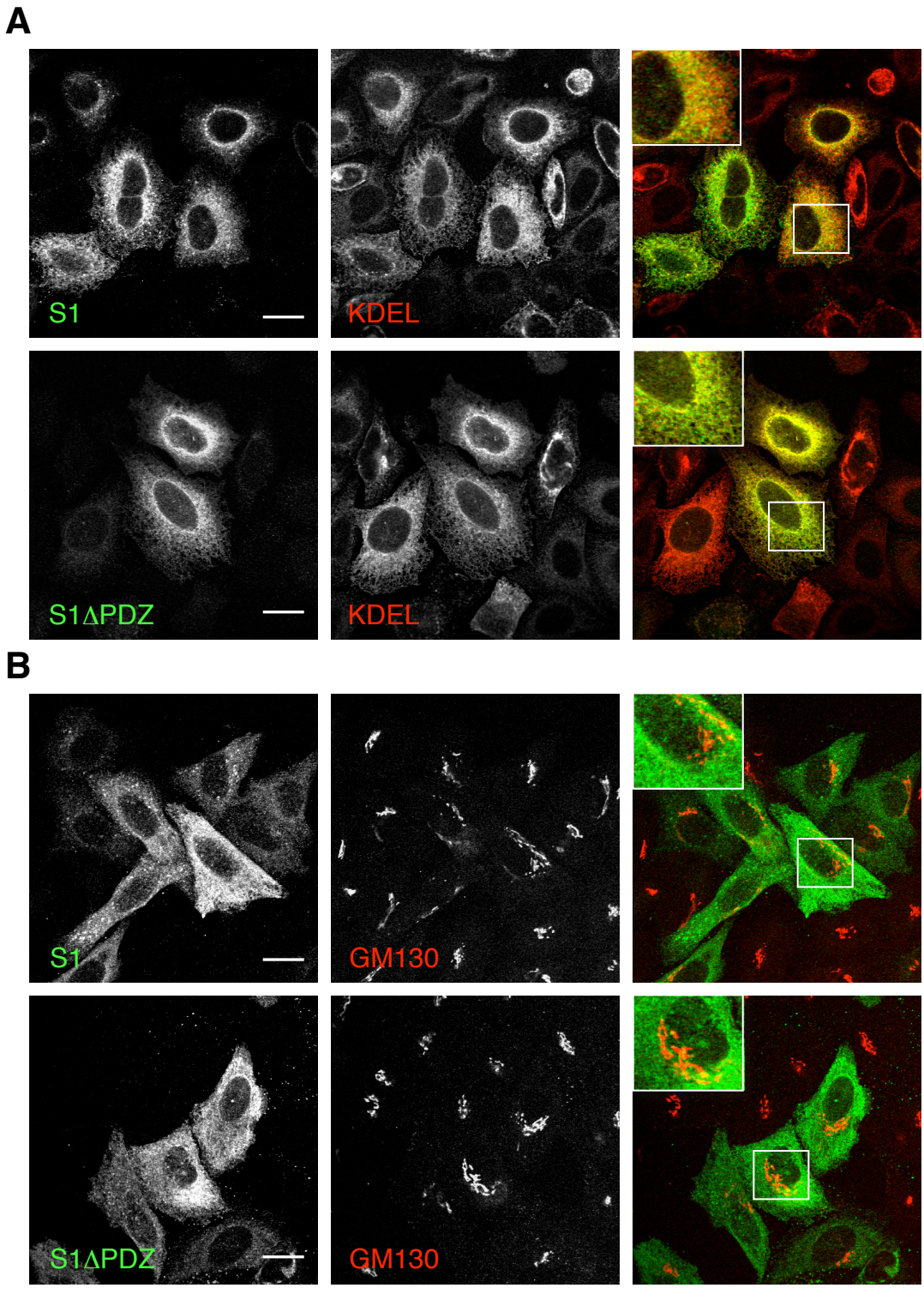
Supplemental Figure 1. **Putative ER retention motifs in the SALMs.** A, Sequence alignment of SALMs 1-5. Potential dileucine and/or ER retention motifs are underlined. (TM = Transmembrane domain, PDZ-BD = PDZ binding domain). B, Quantification of the ratio of biotinylated surface to total levels of Myc-SALM1 (S1, 0.57$\pm$0.14, n=4), Myc-SALM1ΔPDZ (S1ΔPDZ, 0.27$\pm$0.07, n=4), Myc-SALM2 (S2, 1.74$\pm$0.99, n=4), Myc-SALM2ΔPDZ (S2ΔPDZ, 0.99$\pm$0.37, n=4), SALM3 (S3, 0.17$\pm$0.10, n=5) or SALM3ΔPDZ (S3ΔPDZ, 0.29$\pm$0.17, n=5) shown in Fig. 1A. There is a decrease in surface levels of SALM1ΔPDZ compared to SALM1, while both SALM2 constructs have higher surface expression levels than SALM1 or SALM3. C, Hippocampal neurons were transfected with Myc-SALM2 (S2) or Myc-SALM2ΔPDZ (S2ΔPDZ). SALM2 surface staining was detected with anti-Myc antibodies. SALM2 and SALM2ΔPDZ were detected on the surface of both axons and dendrites. Large arrows indicate dendritic arbors, while small arrows indicate axonal processes. Scale bar = 25 μm. D, Quantification of surface expression (integrated intensity) of SALM2 (1.03$\pm$0.04, n=8) and SALM2ΔPDZ (1.19$\pm$0.09, n=8) in axons and dendrites of transfected neurons shows no significant difference in the ratios.

Supplemental Figure 2. **SALM1ΔPDZ ER localization.** HeLa cells were transfected with Myc-SALM1 or Myc-SALM1ΔPDZ, and then permeabilized and immunolabeled. A polyclonal SALM1 antibody directed against the N-terminus (S1-NT) was used to detect the intracellular expression of the SALM1 constructs (green) and monoclonal antibodies against the ER marker KDEL or the Golgi marker GM130 (red) were used to visualize the ER and Golgi as shown in A and B, respectively. SALM1 and SALM1ΔPDZ co-localize with the ER marker, but not GM130 (insets show high magnification). Scale bar = 20 μm.

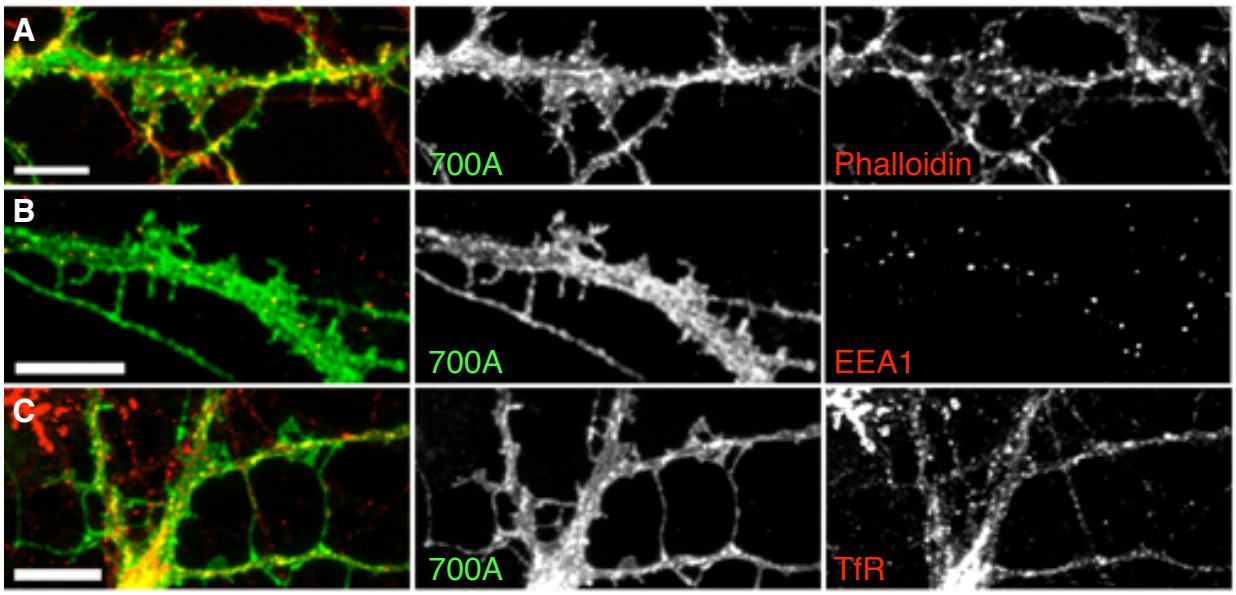
Supplemental Figure 3. **SALM1 700A localization in dendrites and processes.** A-C, Hippocampal neurons were transfected with Myc-SALM1 700A at DIV 12 and then permeabilized and immunolabeled after 48 hrs. Polyclonal SALM1 antibodies were used to stain the total expression of 700A in dendrites and processes (green). Phalloidin (A) was used to stain F-actin and monoclonal antibodies were used to detect EEA1 (B) or transferrin receptor (C), shown in red. While the phalloidin staining indicates that actin is present in both the dendrites and processes, both the endosomal markers EEA1 and transferrin receptor are mainly limited to the dendrites. Scale bar = 10 μm.


A
TM
SALM1: FFTKADYPQCQSMHSQIKGGTMILVIGGIIVATLLVFIVILMVRYKVCNHDTP----G-KMAAATVSNVYSQTNGSQPPLGGIPVGQLP--------Q-APPKVVV
SALM2: FTTAGDPAPCRPLRAHFLGGTMIIAIGGVIVASVLVFIVLLMIRYKVYGDGDSRRIKGTSRTPPRVSHVCSQTNGAGAQ----QASAPFAPDRYEALREVAVP----
SALM3: FSTLPANPLCHALQAHVLGGTLTVAVGGVLVAALLVFTVALLVRGRGAGNGR--------LP-LKLSHVQSQTNGGTSP----MPKSHPP--------R-SPP----
SALM4: FSTEPALRPCAAPHAPFLGGTMIIALGGVIVASVLVFIFVLLLRYKVHGGQPP----GKAKATAPVSSVCSQTNGALGP----VPSAPAP--------EPAAP----
SALM5: FTTEQDYVRCHFMQSQFLGGTMIIIIGGIIVASVLVFIIILMIRYKVCNNNGQ------HKVT-KVSNVYSQTNGAQMQ----GCSVTLP----QSMSKQAMG----
SALM1: RNELMDFSTSLARACDSSSSSSLGSGEAAGLGRGPWRLPPPAPRPKPSLDRLMGAFASLDLKSQRKEELLDSRTPAGRGAGTSSRGHHSDREPLLGPPATRARSLLP
SALM2: -------AAIEAKAMEAEATSTELEVVLGRSLGGSATSLCLLPSEETS-------------GEESRAMTGPRRSRSGALGPPTSAPPTLALVPGGAPARPRPQQRYS
SALM3: ----------PRPQ----------
SALM4: -------RAHTVVQLDCEPWG----------PSHEPA----------GP----------
SALM5: -------HEENAQCCKVASDN----------AIQSSETC----------SSQDSSTTTSALPPTWTSSAPVSQK---QKRKTGTKPSAEPQSEAV
PDZ-BD
SALM1: LPLEGKAKRSHSFDMGDFAAAAAAVPGGYSPPRRVSNIWTKRSLSVNGMLLPFEESDLVGARGTFGSSEWVMESTV
SALM2: FDGDYGAL-FQSHSYPRRARRTKRHRSTPHLDGAGGGAAGEDGDLGLGSAR---------ARLAFTSTEWMLESTV
SALM3: ---R-----SCSLDLGDTG-------GCYGYARRLGGAWARRSHSVHGGLL----G--AGCRGVGGSAERLESVV
SALM4: ----------
SALM5: TNVESQNTNRNNSTALQLASCPPDSVTEGPTSQRAHTKPNALLTNVDQNVQETQRLESI----------
B
Surface/total ratio (Biotin Assay)
3
2.5
2
1.5
1
0.5
0
S1
S1ΔPDZ
S2
S2ΔPDZ
S3
S3ΔPDZ
D
Axon/dendrite ratio (Surface expression)
1.5
1.0
0.5
0.0
S2
S2ΔPDZ
C
S2
S2ΔPDZ

Suppl. Fig. 1



Suppl. Fig. 2



Suppl. Fig. 3