Supplemental Material

Supplemental Table 1.

Two databases were compared to find proteins that were 1) present in the PSD and 2) contain putative NLSs. Proteins are listed here with their SwissProt ID in the middle column, and the amino acid sequence of the putative NLS (or multiple NLSs) in the last column. The following databases were compared: PSD database (PPID: http://defiant.inf.ed.ac.uk:8000, (Husi and Grant, 2001) and putative NLS database: (http://cubic.bioc.columbia.edu/predictNLS/ (Cokol et al., 2000).

Description of custom-made, isoform-specific importin-α rabbit polyclonal antibodies.

Rabbit polyclonal antibodies were generated against importin α isoform-specific peptides. The following peptides were synthesized and injected into rabbits (ABR, Rockford, IL):

Importin α 1: TAEETEEEVMSDGGFHEAQINNMEMAPG

Importin α 2: DQNVVPETTSEGFAFQVQDGAPGTFNF

Importin α 3: YNFDPTANLQTKEFNF

Importin α 4: TFGFNSSTNVPTEGFQF

Importin α 6: VELINEEAAMFDSLLMDSYVSSTTGES

These peptide sequences were chosen because they are unique to each isoform and because they are externally exposed in the crystal structure of mouse importin α (Kobe, 1999), and thus likely to be accessible for IP and immunocytochemistry. We tested the specificity of the antisera using multiple assays. First, we transfected individual FLAG-tagged importin α isoforms into HEK cells and processed them for immunocytochemistry

(ICC) with both anti-FLAG and anti-importin α peptide antibodies. Second, to test the specificity of the antibodies in immunoprecipitation (IP) assays, we transfected HEK cells with FLAG-tagged importin α isoforms, performed IPs with each isoform specific importin α antibody, resolved the IPs by SDS-PAGE and immunoblotted with anti-FLAG antibodies. To test the specificity of the antibodies by immunoblotting, we expressed FLAG-tagged importin α isoforms in HEK cells, immunoprecipitated with anti-FLAG antibodies, followed by SDS-PAGE and immunoblotting with isoform-specific antibodies. In a final assay, we tested the specificity of each antibody in siRNA experiments using custom oligos specifically designed to knock-down each importin α isoform in HEK cells (using SMARTselection-designed siRNAs from Dharmacon, Boulder, CO). 48 hours after knockdown, cells were lysed and then probed for each importin α isoform using each of the isoform-specific antibodies.

Supplemental Figure 1.

A) To confirm that our GST-NR1 C-terminal constructs were functional, we performed GST pulldowns in brain lysates and immunoblotted with anti-calmodulin antibodies, Calmodulin has been shown to bind the C0 cassette (which is present in all NR1 splice variants) at low salt concentrations in a calcium-dependent manner (Ehlers MD, 1996). Both GST-NR1 constructs bound calmodulin in calcium-containing buffers whereas no binding occurred in buffers in which calcium was chelated with EDTA, as previously described (Ehlers MD). B) FLAG-tagged importin α expressed in HEK cells is pulled down by GST-NR1-1a. GST-NR1-1a C terminal constructs can pull down FLAG-importin α1 transfected into HEK cells as detected by FLAG antibodies whereas GST alone cannot. Lysates were washed in modified RIPA buffer.

Supplemental Figure 2.

Activity triggers nuclear translocation of importin α but promotes neither cleavage of NR1 nor nuclear translocation of NR1. A) Lysates from unstimulated (US) and 2x100 Hz stimulated (S) hippocampal slices were immunoblotted with an anti-NR1-C1 cassette antibody. No lower molecular weight cleavage products were observed in stimulated slices, and there was no decrease in the 110kD full-length NR1 band. * indicates bottom of gel. B) Representative photomicrographs of cultured cortical neurons silenced with tetrodotoxin (TTX) for 4 h, stimulated with glutamate for 5 min (40 μM), fixed 25 min later and processed for immunocytochemistry with anti-C0 or anti-C1 NR1 antibodies. Scale bar = 20μM. C) Quantification of NR1 C0 and C1 cassette mean pixel intensity in the nucleus, revealing no nuclear NR1 in unstimulated or stimulated neurons.

Supplemental Figure 3.

Cultured cortical neurons (21 DIV) were silenced for 4 h with tetrodotoxin (TTX, 1μM), then incubated with 40mM glutamate in feeding media (or vehicle) for 5 min followed by 3 washes with original conditioned media, and incubated with conditioned medium at 37^{o} for 20min. Cultures were then fixed and stained with anti-importin α antibodies. The nuclear to cytoplasmic ratio of the mean pixel intensity of importin α immunoreactivity was measured by manually outlining the nucleus (based on absence of MAP2 staining) to determine nuclear pixel intensity, and subtracting this value from the entire cell soma to obtain somatic cytoplasmic value. The nuclear to cytoplasmic ratio of importin $\alpha 1$ immunoreactivity was significantly higher following glutamate stimulation in cortical cultured neurons ($p < 0.0001$, independent samples t-test).

Supplemental Figure 4.

The NLS and flanking serines in NR1 is conserved across species. Primary amino acid sequences of NR1 from 7 different species were aligned using ClustalW; from EMBL http://www.ebi.ac.uk/Tools/clustalw2/index.html). The region encoding or corresponding to the C1 cassette including the bipartide NLS (Pinkstaff et al. 2001), and residues flanking the NLS that are PKC (blue)/PKA (brown) targets of phosphorylation, are conserved. Numbers label last amino acid listed for each species.

Supplemental Figure 5.

Importin α can immunoprecipitate (IP) NR1 and this interaction is regulated by activity. 21 DIV cortical neurons were silenced with tetrodotoxin (TTX, 1μM) for 6h, incubated with 40μM glutamate or vehicle for 5 min, lysed, and processed for IP with anti-importin α antibodies followed by immunoblotting (IB) for NR1. Importin α binds NR1 in TTX silenced neurons; binding is reduced following glutamate stimulation. IgG bands shows equal loading between glutamate and TTX treated neurons in IPs.

Supplemental Figure 6.

A. Immunoblotting (IB) with anti-phospho-Ser 896 and Ser-890 NR1 antibodies of GST-NR1 C-terminal constructs confirms phosphorylation after *in-vitro* PKC phosphorylation. Immunoblotting (IB) with anti-phospho-Ser 897, 896 and 890 NR1 antibodies of GST-NR1-1a C-terminal construct shows increase in phosphorylation only in serine 897 after PKA *in-vitro* phosphorylation.

B. PKA activation has no effect on binding between NR1 and importin α (TTX = 0.06 ± 0.003 a.u.; fsk = 0.06 ± 0.002 a.u. p = ns, independent samples t-test. n=6).

Supplemental Figure 7.

Lysates from hippocampal slices were probed with anti-phospho ser890 NR1 antibodies. 2x100Hz, but not 1x100Hz, stimulation increases phosphorylation at this site (2x100 Hz = 277 ± 7.5 % of US; 1x100Hz = 112 ± 7.0% of US. $p<0.05$, paired t-test. n= 4).

Supplemental Figure 8.

Quantification of the mean importin α pixel intensity in NR1-containing spines. A) in basal, TTX-silenced and glutamate stimulated (5 and 15 min) neurons (*$p < 0.05$, **$p < 0.01$, ANOVA and LSD multiple comparisons test); n=number of cells and B) Quantification of the mean importin α pixel intensity in NR1-containing spines in basal, TTX-silenced, glutamate stimulated, glutamate plus inhibitors of PKC (chel) (*$p < 0.05$ or lower, from all other conditions, student's t test); n=number of cells.

Cokol M, Nair R, Rost B (2000) Finding nuclear localization signals. EMBO Rep 1:411-415.

Ehlers MD ZS, Bernhadt JP, Huganir RL. (1996) Inactivation of NMDA receptors by direct interaction of calmodulin with the NR1 subunit. Cell 84:745-755.

Husi H, Grant SG (2001) Isolation of 2000-kDa complexes of N-methyl-D-aspartate receptor and postsynaptic density 95 from mouse brain. J Neurochem 77:281-291.

Kobe B (1999) Autoinhibition by an internal nuclear localization signal revealed by the crystal structure of mammalian importin alpha. Nat Struct Biol 6:388-397.

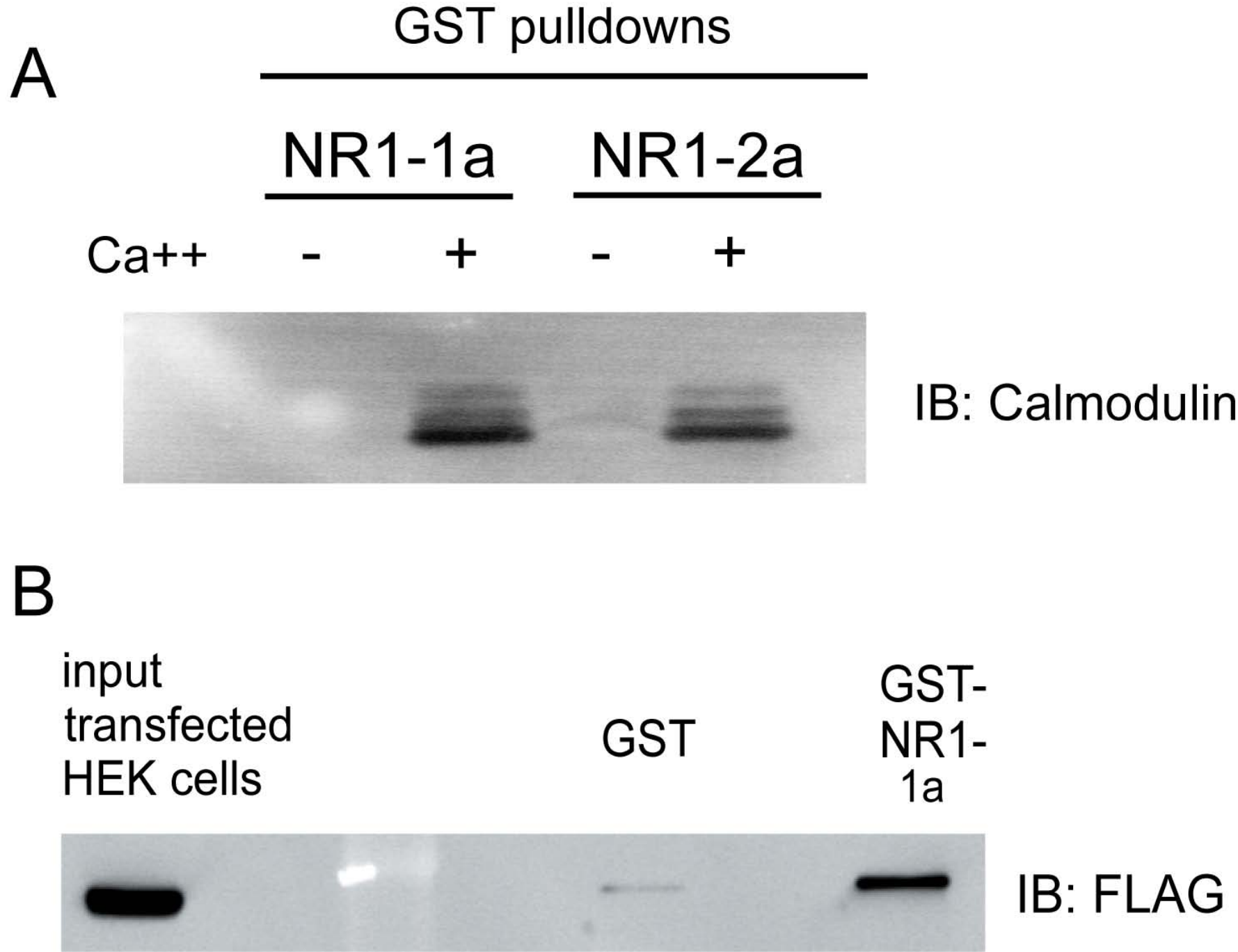
Pinkstaff JK, Chappell SA, Mauro VP, Edelman GM, Krushel LA (2001) Internal initiation of translation of five dendritically localized neuronal mRNAs. Proc Natl Acad Sci U S A 98:2770-2775.

Supplemental Table 1. Comparison of NLS database with PSD database

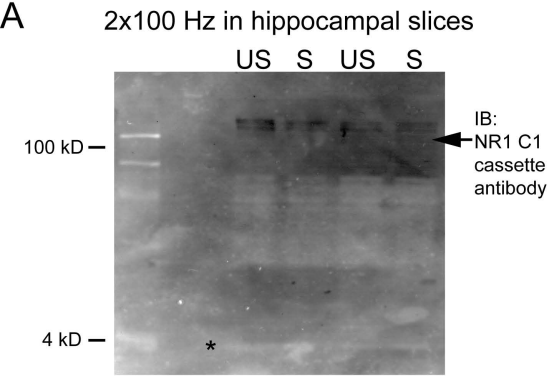
Protein	SwissProt ID	NLS sequence
acetylcholine receptor protein, beta-2 ch	P17787	RLRLRRRQRER
Muscarinic acetylcholine receptor M3	P20309	KKKRRK
ALR	O14687	KRRQRR, KKRKRK, KKKQQRRGRKR
ANKYRIN G.	Q12955	KKRKHRKRSRDRKKKS
CREB1 (cAMP-response element binding protein)	P16220	RRKKKE
CREB5 (cAMP Response element binding protein)	Q02930	RRRVVDEDPDERRRKF, RCRQKR
Voltage-dependent P/Q-type calcium channel alpha-1	O00555	RRHRRRKE
Voltage-dependent L-type calcium channel alpha-1C	Q13936	RKFKKRKE
Voltage-dependent L-type calcium channel alpha-1D	Q01668	RKFKKRKE
Voltage-gated L-type calcium channel alpha-1 subunit	O95226	RKFRRRKEK
DELTA-CATENIN	O00379	KKKKKKK
ALPHA2(E)-CATENIN	Q12795	PLVKREKQ
Drebrin (Developmentally regulated brain protein).	Q16643	EHRRK
Estrogen receptor beta (ER-beta).	Q92731	DKNRRKS
c-fos	P01100	RRERNKMAAAKCRNRRR
G9A (euchromatic histone-lysine N-methyltransferase 2)	Q14349	RKAKKKWRKDSPWVKPSRKRRKREPPR

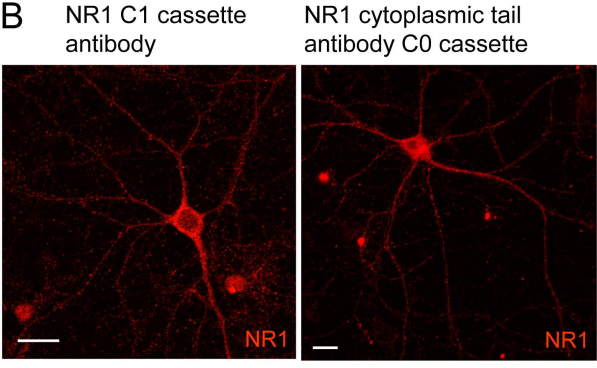
GMF-beta (Glia maturation factor beta)	P17774	RKFRFRKE
GNAS1	O75685	RWFQHRRNRRRR
HSP 86 (Heat shock protein HSP 90-alpha)	P07900	KKKKKKIK
HCG V (PROTEIN PHOSPHATASE 1 regulatory subunit)	O60927	LRKRKP
MAPK	O60491	LRKRKL
N-Methyl-D-Aspartate receptor subunit	Q12867	KKKATFRAITSTLASSFKRRR
Nitric-oxide synthase, endothelial	P29474	RRKRKE
ATP-sensitive inward rectifier potassium channel 1	P48048	PKKRAKT
PESCADILLO	O00541	KKREKYLYQKIMFGKRRK
UBIQUITIN-LIGASE E3-ALPHA	O60708	KKRRK
Voltage-gated sodium channel aplha subunit	O95788	RRNRRKKRK, RRSVKRN
HPK/GCK-LIKE KINASE HGK (Mitogen-activated protein kinase kinase kinase kinase 4)	O95819	RRLEEQQRREREAR, RRREQEEKRRLEELERRRK

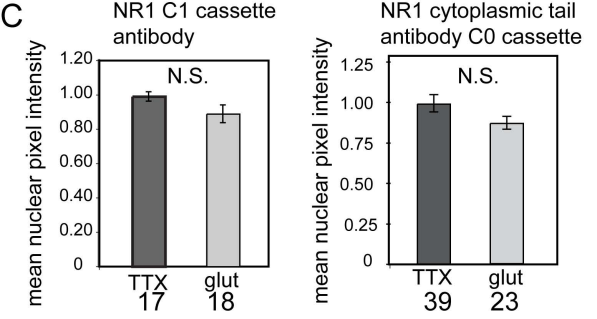
Supplemental Figure 1



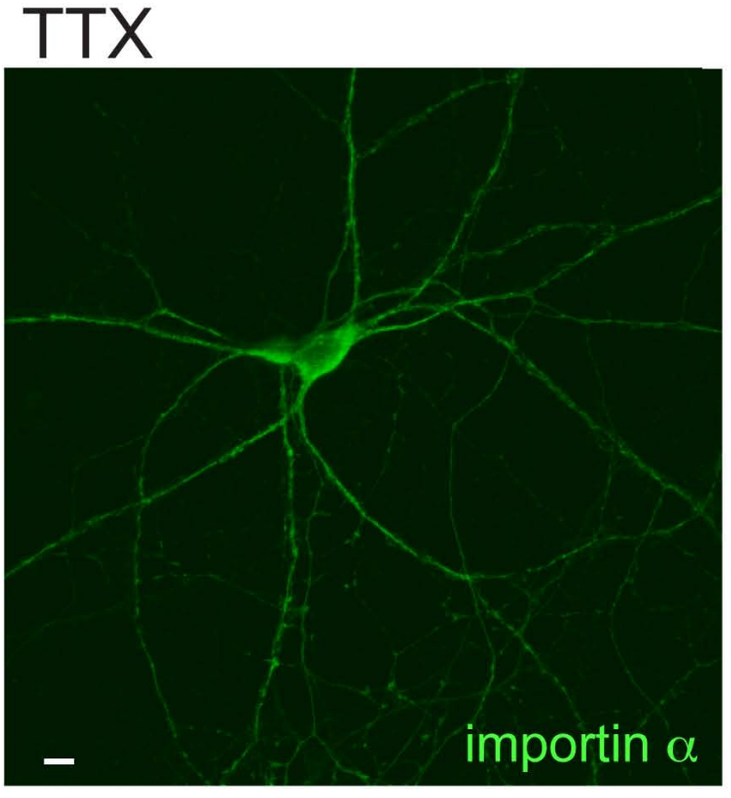
Supplemental Figure 2

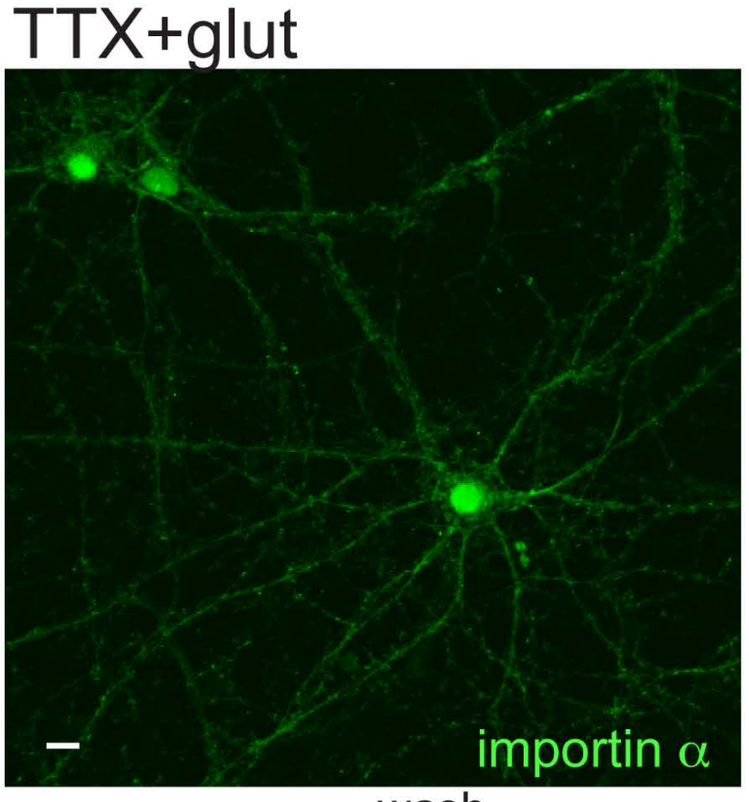


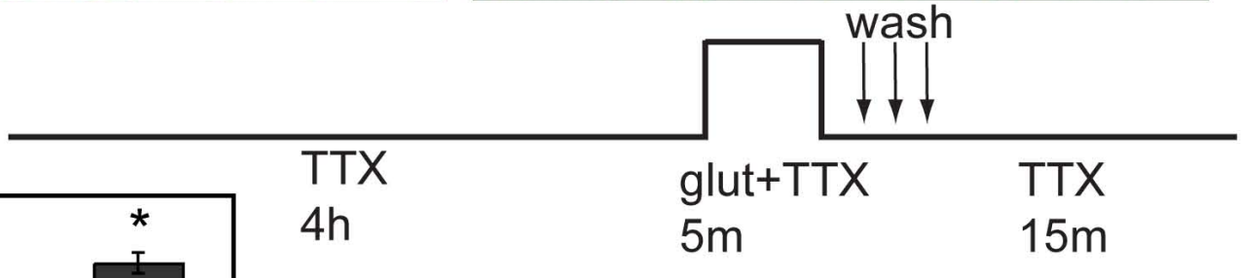


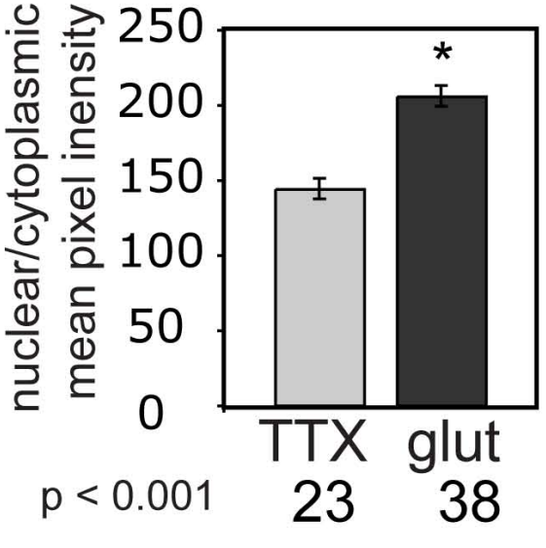


Supplemental Figure 3







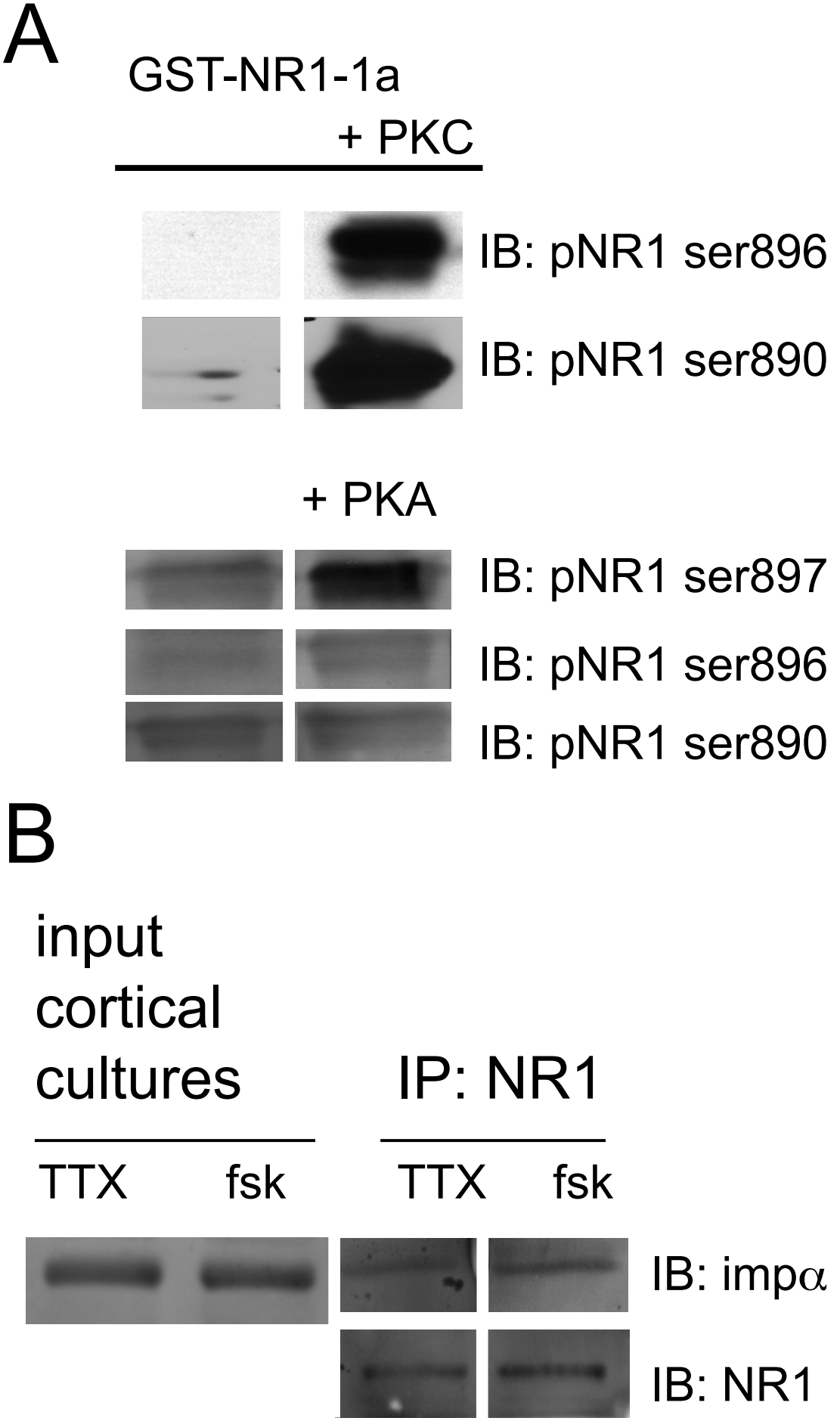


Supplemental Figure 4

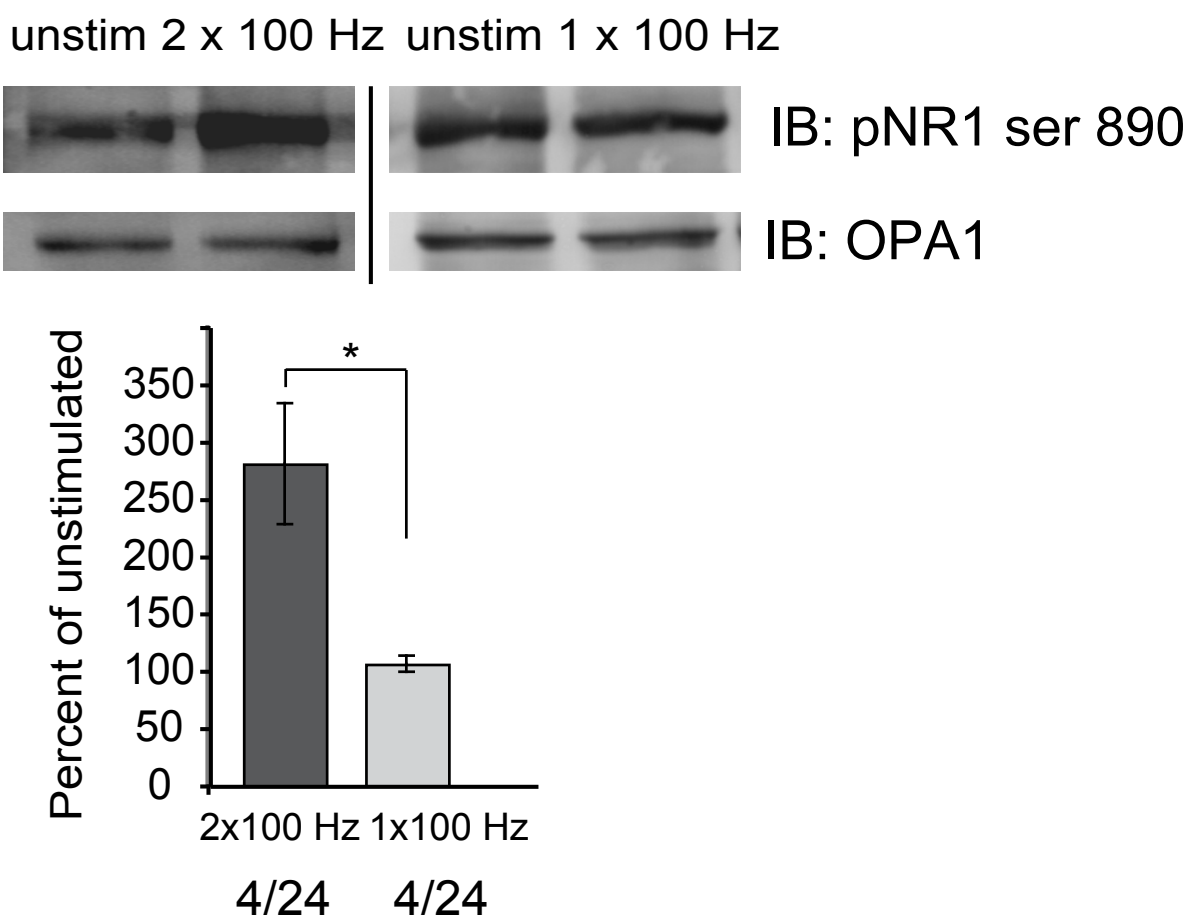
mouse	DRKSGRAEPDP**KKK**ATFRAITSTLA**SSFKRRRSS**KDT - 900
rat	DRKSGRAEPDP**KKK**ATFRAITSTLA**SSFKRRRSS**KDT - 900
human	DRKSGRAEPDP**KKK**ATFRAITSTLA**SSFKRRRSS**KDT - 900
dog	DRKSGRAEPDP**KKK**ATFRAITSTLA**SSFKRRRSS**KDT - 900
chicken	DRKSGRAEPDP**KKK**ATFRSITSTLA**SSFKRRRSS**KDT - 898
zebra finch	DRKSGRAEPDP**KKK**ATFRSI TSTLA**SSFKRRRSS**KDT - 898
zebrafish	DRKSGRAEPDP**KKK**ASFRSISTNLA**S**NI**KRRRSS**KDT - 916

Supplemental Figure 5

Supplemental Figure 6

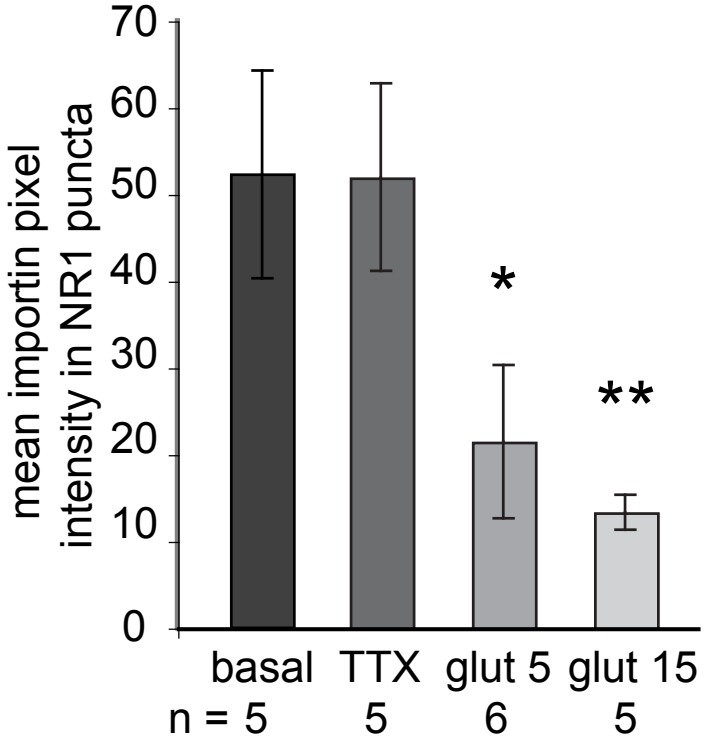


Supplemental Figure 7



Supplemental Figure 8

A



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