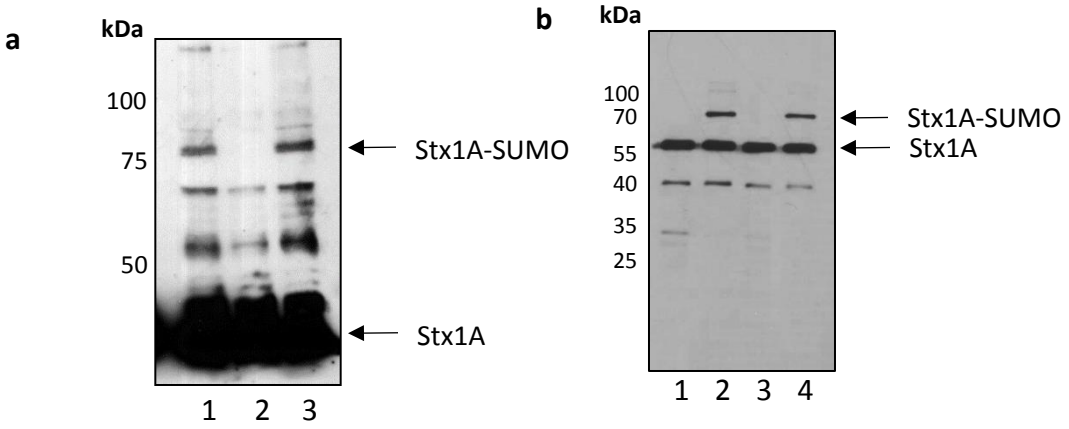
SUMOylation of Syntaxin1A regulates presynaptic endocytosis

Tim J. Craig, Dina Anderson, Ashley J. Evans Fatima Girach and Jeremy M. Henley

School of Biochemistry, Medical Sciences Building, University of Bristol, University Walk, Bristol, BS8 1TD, UK

Supplementary Information

Figure S1 – Mutation of individual sites preserves Stx1A SUMOylation



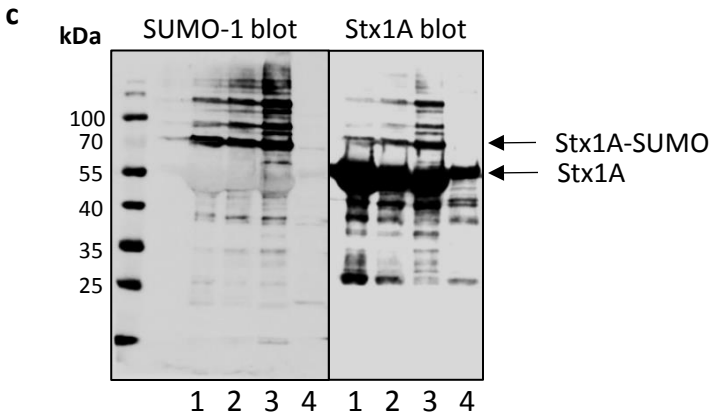
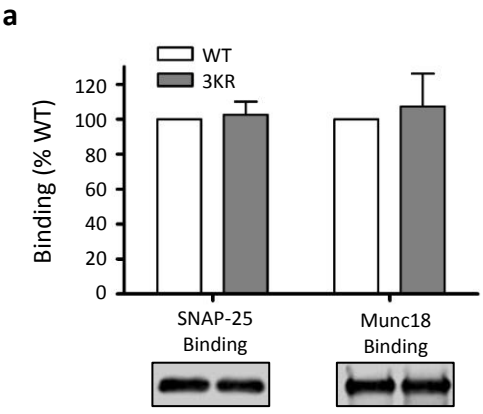
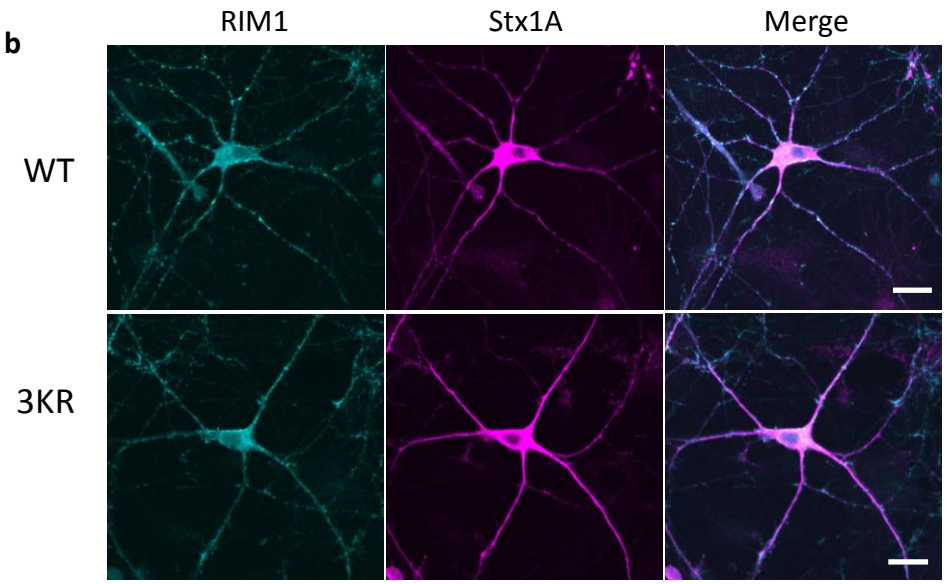
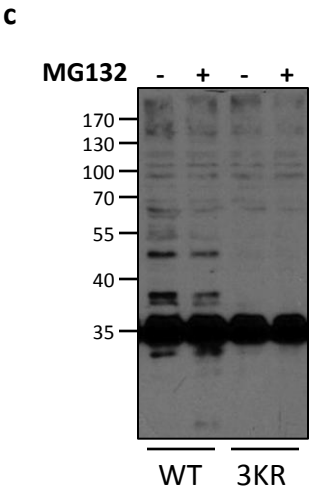
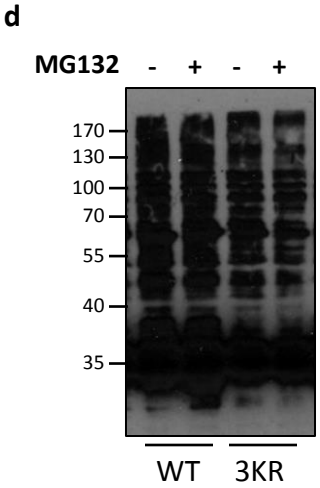


Figure S2 – 3KR mutation displays normal binding, distribution and does not affect ubiquitination









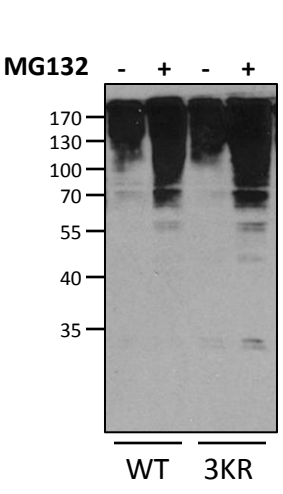
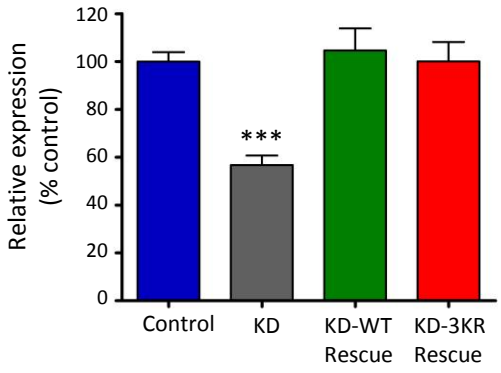


Figure S3 – Efficacy of Stx1 shRNA

a



b

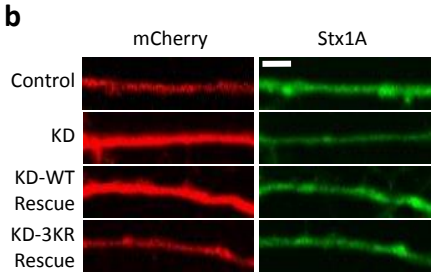


Figure S4 – The size of the SV pool is unchanged in 3KR rescue neurones

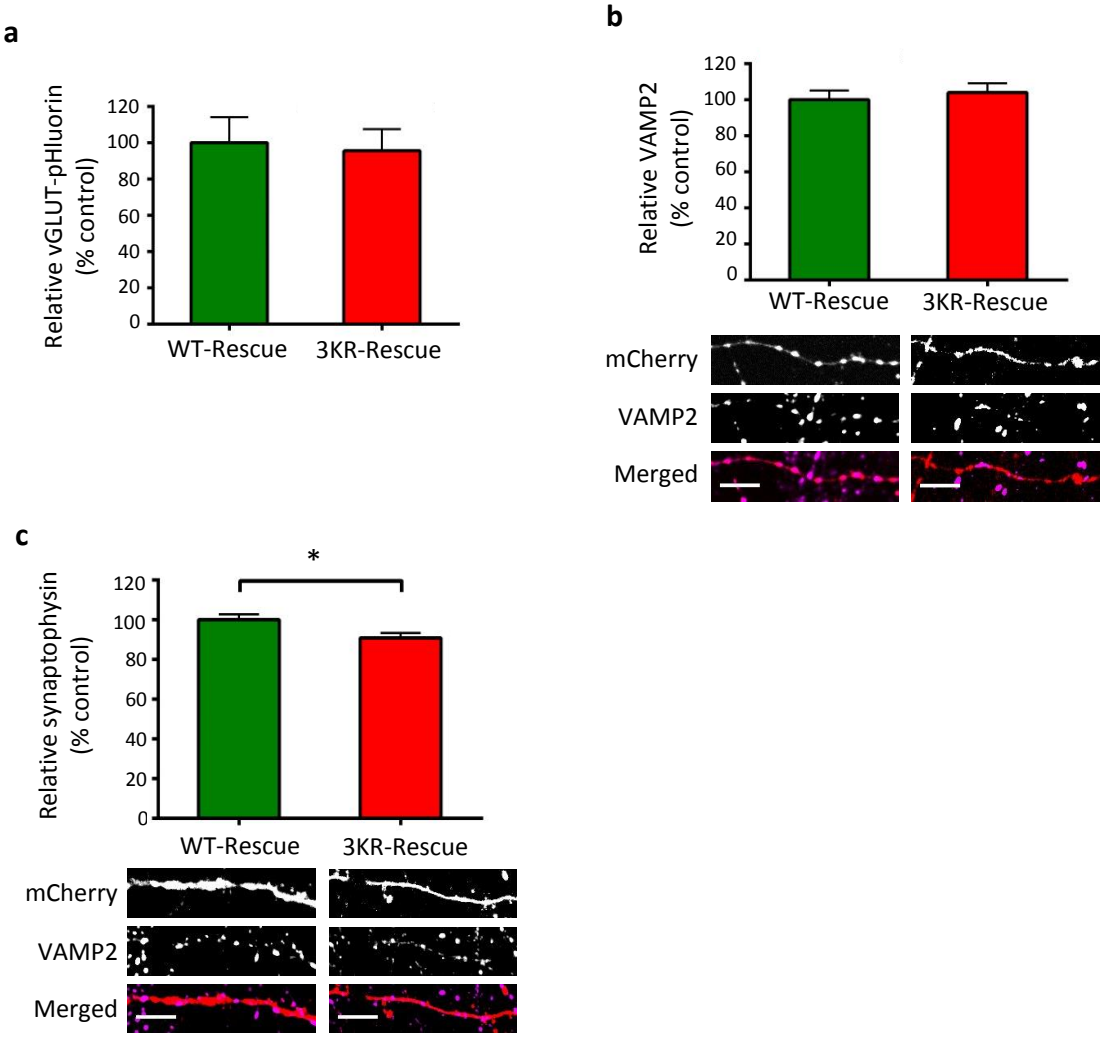
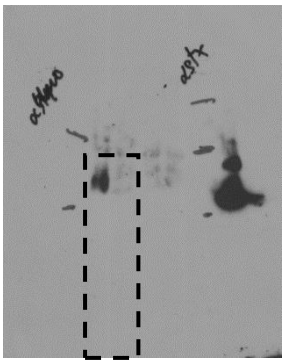
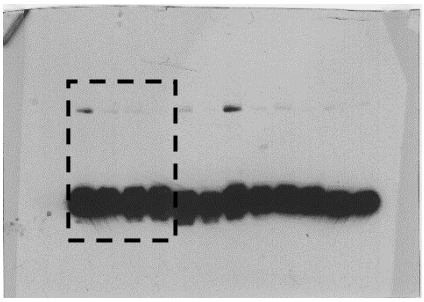


Figure S5– Uncropped blots

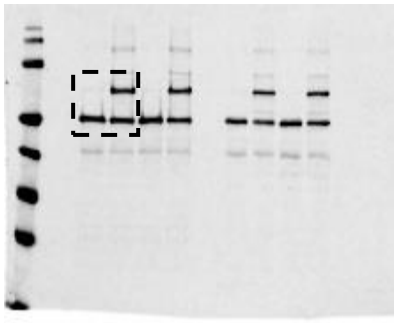
a



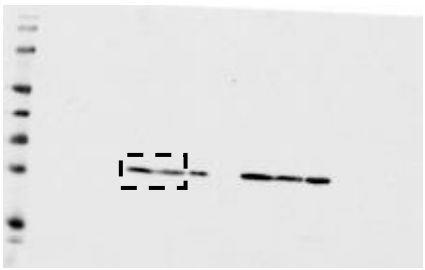
b



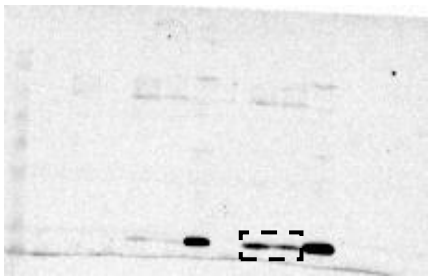
c



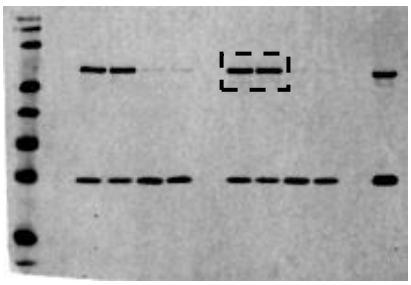
d



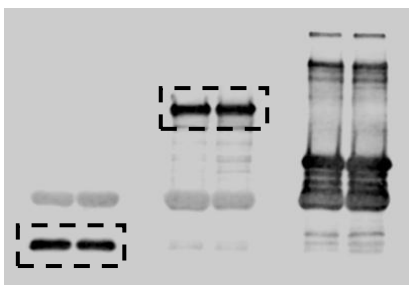
e



f



g



SNAP-25 Munc18

Supplementary Figure Titles and Legends

Figure S1– Mutation of individual sites preserves Stx1A SUMOylation

a: HEK293 cells SUMOylation assay of WT and K204R Stx1A with YFP-SUMO1. Cells transfected with 1: Stx1A WT, Ubc9 + YFP-SUMO1, 2: Stx1A WT, Ubc9 + YFP-SUMO1 ΔGG, 3: Stx1A K204R, Ubc9 + YFP-SUMO1.

b: *E.Coli* SUMOylation assay of WT + K204R Stx1A. Lane 1: Stx1A WT + SENP1 treatment, 2: Stx1A WT, 3: Stx1A K204R + SENP1 treatment, 4: Stx1A K204R.

c: *E.Coli* SUMOylation assay of K252R, K253R and K256R Stx1A. Lane 1: WT Stx1A, 2: K252R Stx1A, 3: K253R Stx1A, 4: K256R Stx1A. Note that although SUMOylation appears much lower for K256R, this is due to a greatly decreased expression level (see Stx1A blot).

Figure S2 – 3KR mutation displays normal binding, distribution and does not affect ubiquitination

a: Affinity pulldown from cortical neuronal lysate using GST-Stx1A 3KR, blotted to SNAP-25 and Munc18. Graph shows quantification (n=4), normalised to the wild-type binding, with no significant difference in binding detected. Blots below are from representative experiments.

b: Immunostaining of Stx1A-HA transfected neurones, showing similar distribution of WT and 3KR Stx1A. Neurones were immunostained with anti-HA (magenta) and co-stained with anti-RIM1 (cyan). Scale bar = 20 µm.

c-e: Lysates of HEK cells transfected with Stx1A WT or 3KR, treated with 20 µM MG132 for 4 hours where indicated. **c:** Stx1A blot, **d:** long exposure Stx1A blot, **e:** Ubiquitin blot.

Figure S3 – Efficacy of Stx1 shRNA

a: Quantification of Stx1A levels detected by immunostaining with HPC-1 of hippocampal neurones transfected with Stx1 shRNA and rescue constructs. Values are normalised to the average control values for each set of experiments. Control n=25, KD n=20, KD-WT rescue n=17, KD-3KR rescue n=15, from at least 3 separate experiments. ***, $p < 0.001$ (1 way ANOVA).

b: Representative images of neuronal processes used for quantification. mCherry is used as a transfection marker. Scale bar = 10 µm.

Figure S4 – 3KR mutation does not change the synaptic vesicle pool size

a: Quantification of total vGLUT-pHluorin fluorescence, taken as an average of the signal at 10 time-points following NH_4Cl treatment. These data are from the same cells as the data in **Fig3a,b,c**. 3KR-rescue signals (n=16) are averaged to the mean of the WT-rescue (n=13) signals.

b: Confocal imaging of Syntaxin1a knockdown-rescue with either WT or 3KR mutant, with VAMP2 used as a synaptic vesicle pool marker. Graph: quantification of VAMP2 signals (n=30 for both). Representative mCherry, VAMP2 and merged images are shown below quantification. Scale bar = 10µm.

c: Confocal imaging of Syntaxin1a knockdown-rescue with either WT or 3KR mutant, with synaptophysin used as a synaptic vesicle pool marker. Graph: quantification of VAMP2 signals (n=30 for both). *, $p < 0.05$. Representative mCherry, VAMP2 and merged images are shown below quantification. Scale bar = 10µm.

Figure S5 – Uncropped blots

a: Full blot from Fig 1b. Cropped area is indicated with a dashed box.

b: Full blot from Fig 1g. Cropped area is indicated with a dashed box.

c: Full Stx1a blot from Fig 2b. Cropped area is indicated with a dashed box.

d: Full SNAP-25 blot from Fig 2b. Cropped area is indicated with a dashed box.

e: Full VAMP2 blot from Fig 2b. Cropped area is indicated with a dashed box.

f: Full Munc18 blot from Fig 2b. Cropped area is indicated with a dashed box.

g: Full Munc18/SNAP-25 blot from Fig S1c. Cropped area is indicated with a dashed box.