Plk2 promotes synaptic destabilization through disruption of N-cadherin adhesion complexes during homeostatic adaptation to hyperexcitation

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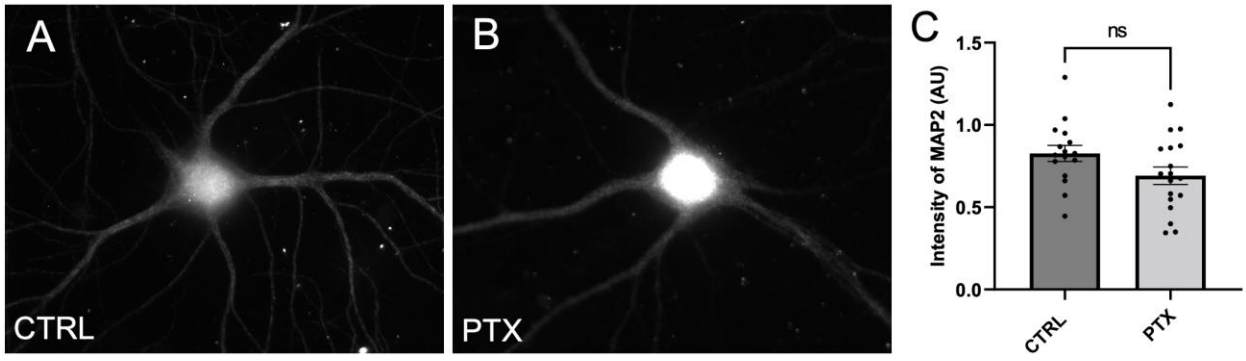
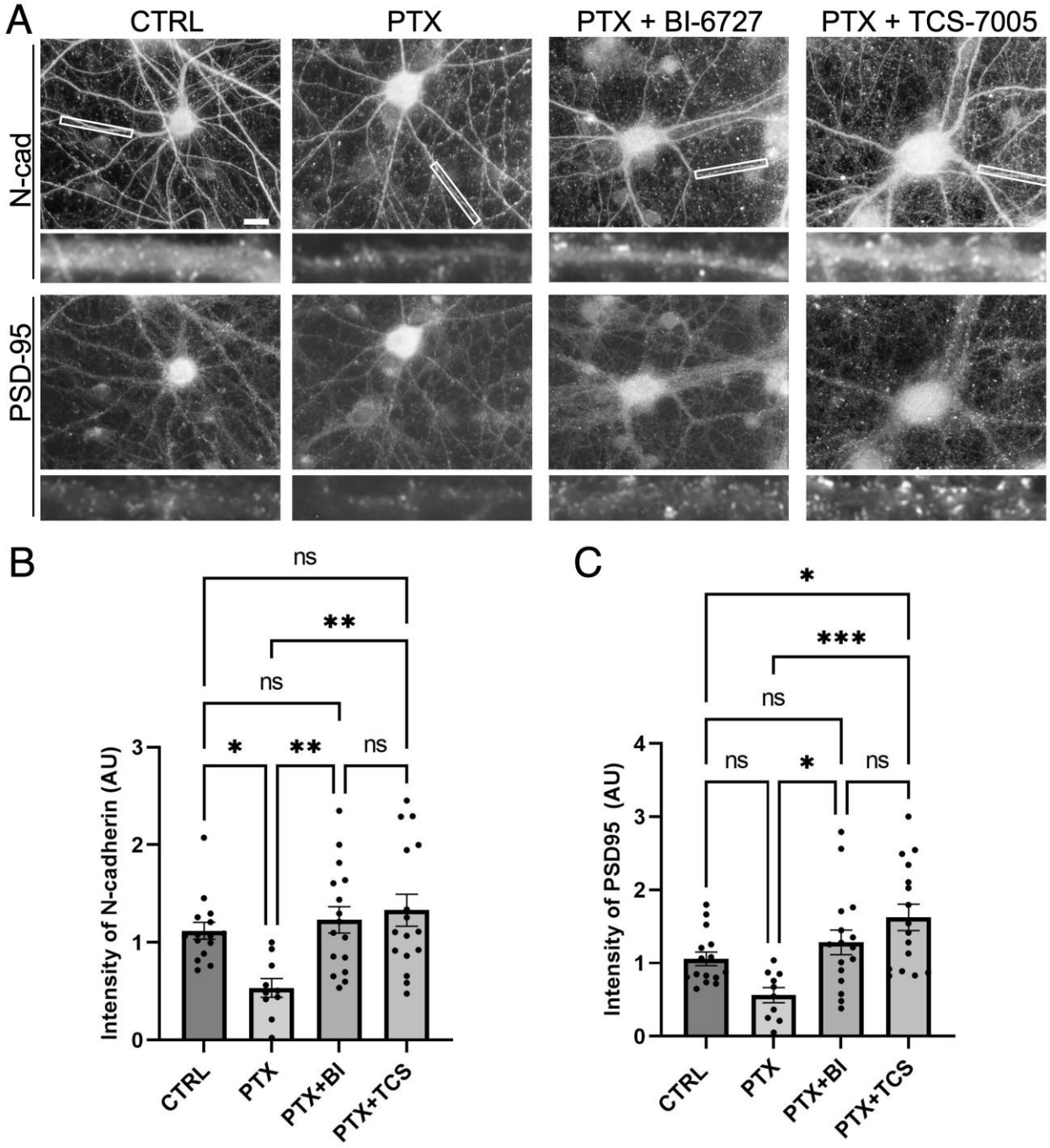
SUPPLEMENTARY MATERIAL

Supplementary Figures

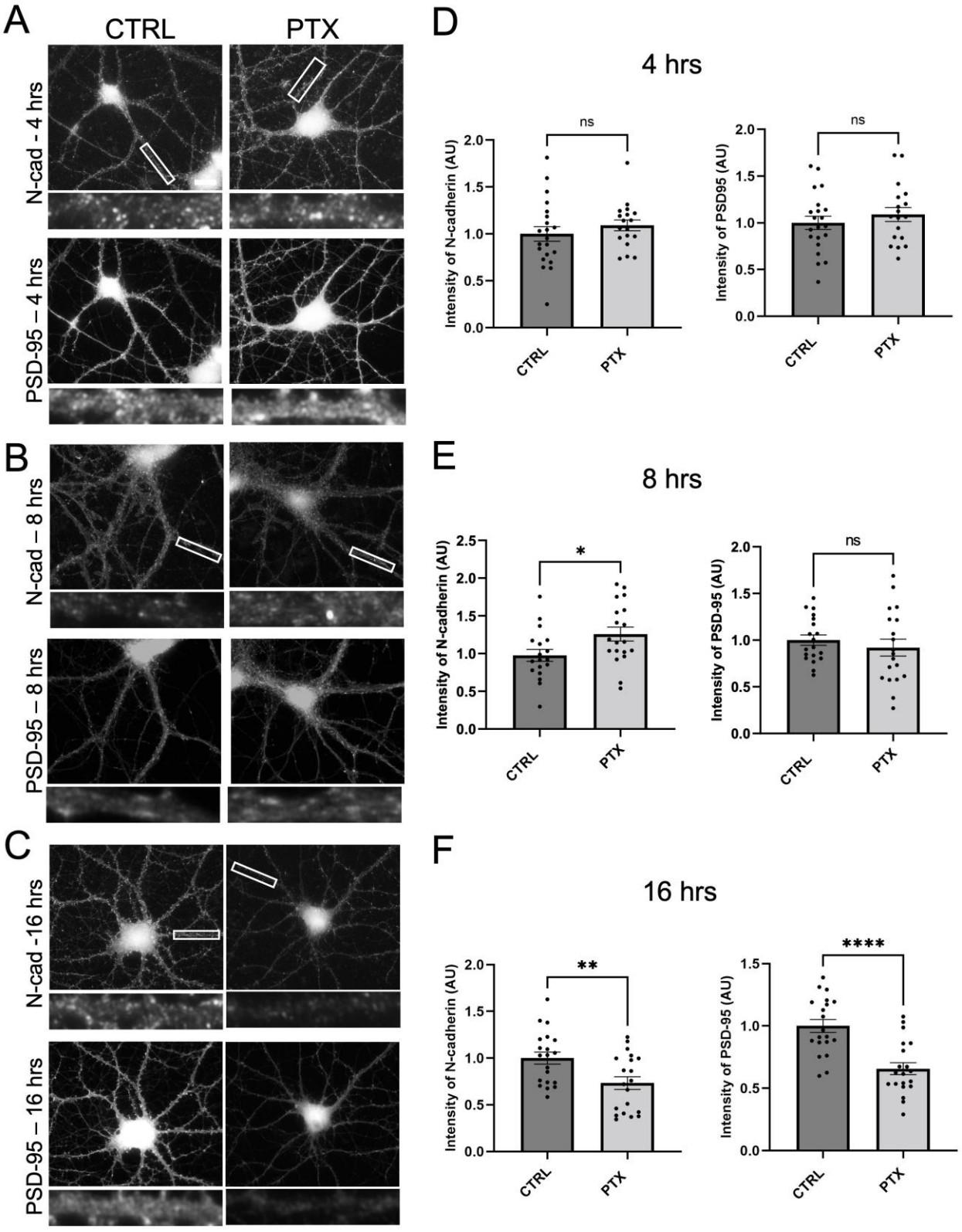


Supplementary Figure 1. Hyperexcitation does not affect intensity of MAP2 in dendrites. (A-B). Primary hippocampal neurons were stained with MAP2 as a negative control under basal conditions (CTRL) and with picrotoxin treatment (PTX). **(C)** Quantification of MAP2 intensity in secondary dendrites (n.s., non-significant; p=0.0689, t=1.882, DF=32, n=16-18 neurons, unpaired Student's t-test).



Supplementary Figure 2. Loss of N-cadherin and PSD-95 during hyperexcitation. (A) Double immunostaining of endogenous N-cadherin and PSD-95 under conditions as indicated at top of images. Higher magnification views of representative secondary dendrites (boxed regions) are shown below each neuron. Scale, 10 μm. **(B-C)** Quantification of (B) N-cadherin (p= 0.0016,

F=5.811, DF= 3) (n=10-16 neurons) and (C) PSD-95 (p= 0.0003, F=7.491, DF=3) (n=10-16 neurons); one-way ANOVA and Tukey's multiple comparison test; *p<0.05, **p<0.01, ***p<0.001, ns=nonsignificant.


A
CTRL
PTX
N-cad - 4 hrs
PSD-95 – 4 hrs
B
N-cad – 8 hrs
PSD-95 – 8 hrs
C
N-cad -16 hrs
PSD-95 – 16 hrs
D
4 hrs
ns
Intensity of N-cadherin (AU)
Intensity of PSD95 (AU)
CTRL
PTX
E
8 hrs
*
ns
Intensity of PSD-95 (AU)
F
16 hrs
**
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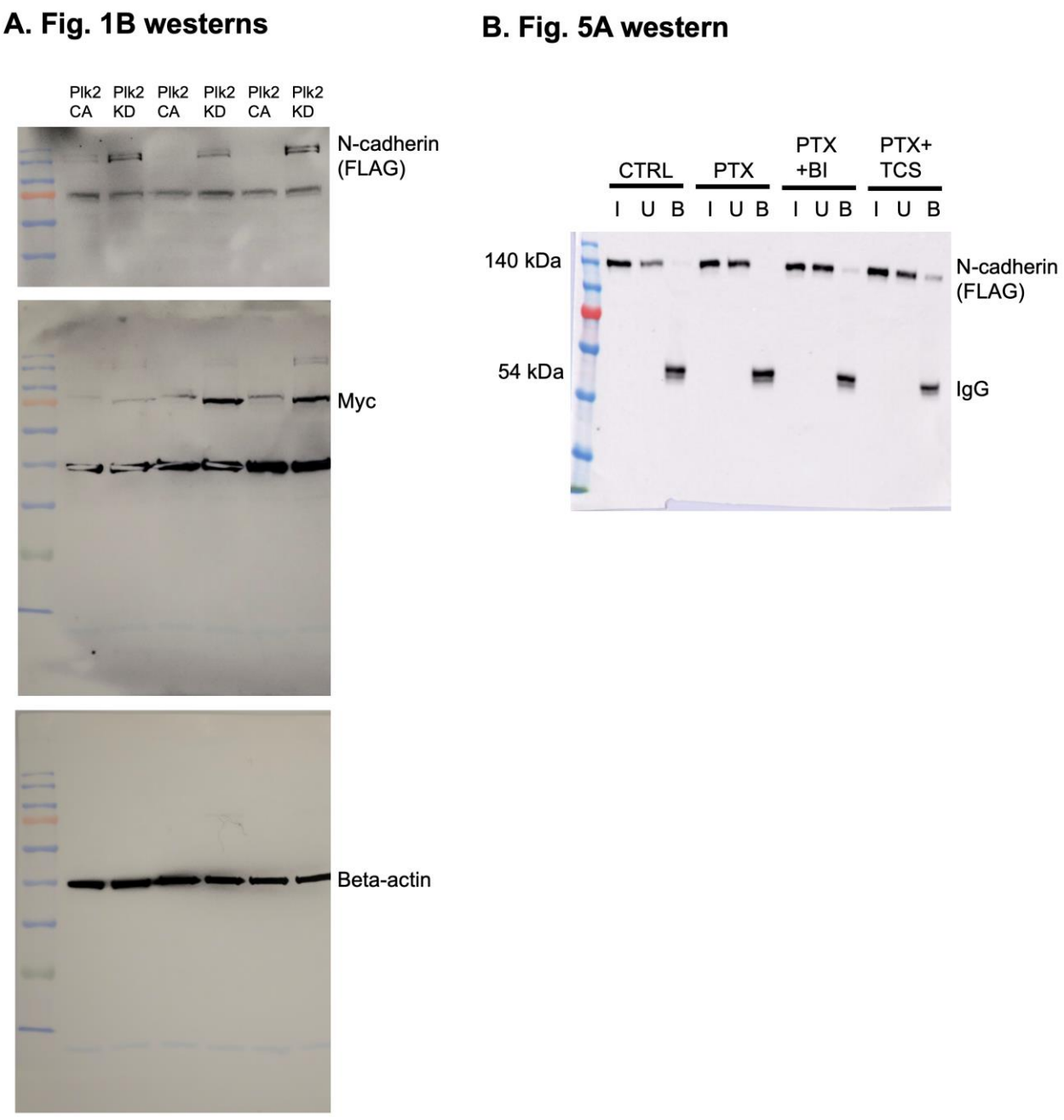
Supplementary Figure 3. Time course of N-cadherin and PSD-95 levels during hyperexcitation. (A-C) Double immunostaining of endogenous N-cadherin and PSD-95 in hippocampal neurons left unstimulated (CTRL) or treated with PTX for (A) 4 hrs, (B) 8 hrs, and (C) 16 hrs. Higher magnification views of representative secondary dendrites (boxed regions) are shown below each neuron. Scale, 10 μm. **(D-F)** Quantification of images from (A-C) (N-cadherin: p= 0.3664, t=0.9145, DF=37, PSD-95: p= 0.3934, t=0.8636, DF=37 for n=18-21 neurons for 4 hrs, N-cadherin: p= 0.0275, t=2.300, DF=35, PSD-95: p=0.4555, t= 0.7544, DF=36 18-19 neurons for 8 hrs, N-cadherin: p=0.0068, t=2.864, DF= 38, PSD-95: p<0.0001,t=4.866, DF=38 20 neurons for 16 hrs); unpaired student t-tests, *p<0.05, **p<0.01, ****p<0.0001, ns=nonsignificant.



Supplementary Figure 4. Full Western blots of cropped images used in the study. (A) Western blots from Fig. 1B. Immunoblots of FLAG-N-cadherin and myc-tagged Plk2-KD or -CA co-transfected in COS-7 cells. Loading control is β-actin (n=6 cell culture preparations). **(B)** Western blot from Fig. 5A. Cultured hippocampal neuron lysates were prepared following treatments as

shown at top, immunoprecipitated for APP, and probed for N-cadherin. I, input; U, unbound; B, bound. CTRL, unstimulated; BI, BI-6727; TCS, TCS-7005 (n=5 culture preparations).