Early Changes in Transcriptomic Profiles in Synaptodendrosomes Reveal Aberrant Synaptic Functions in Alzheimer's Disease

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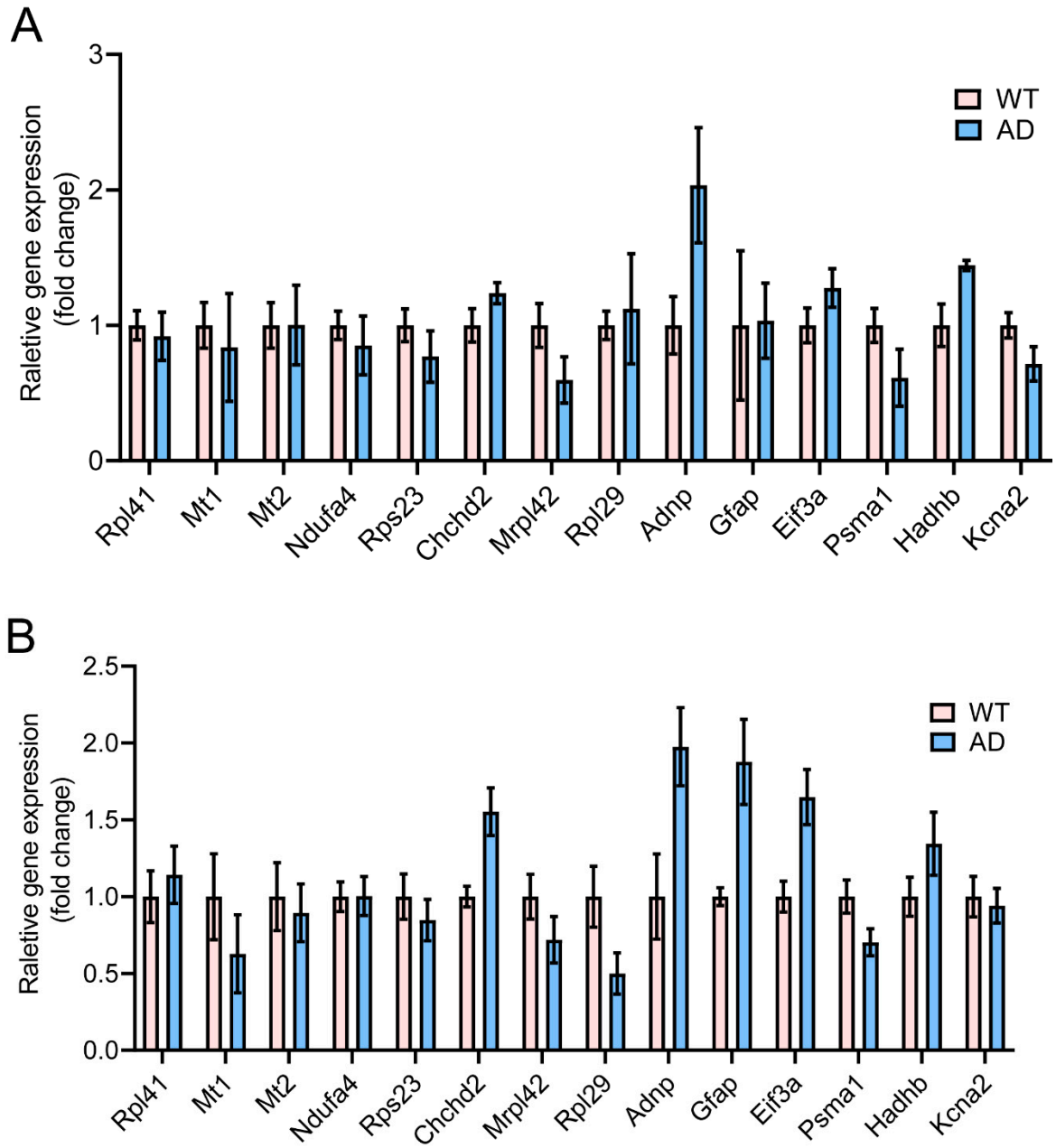
This document includes:

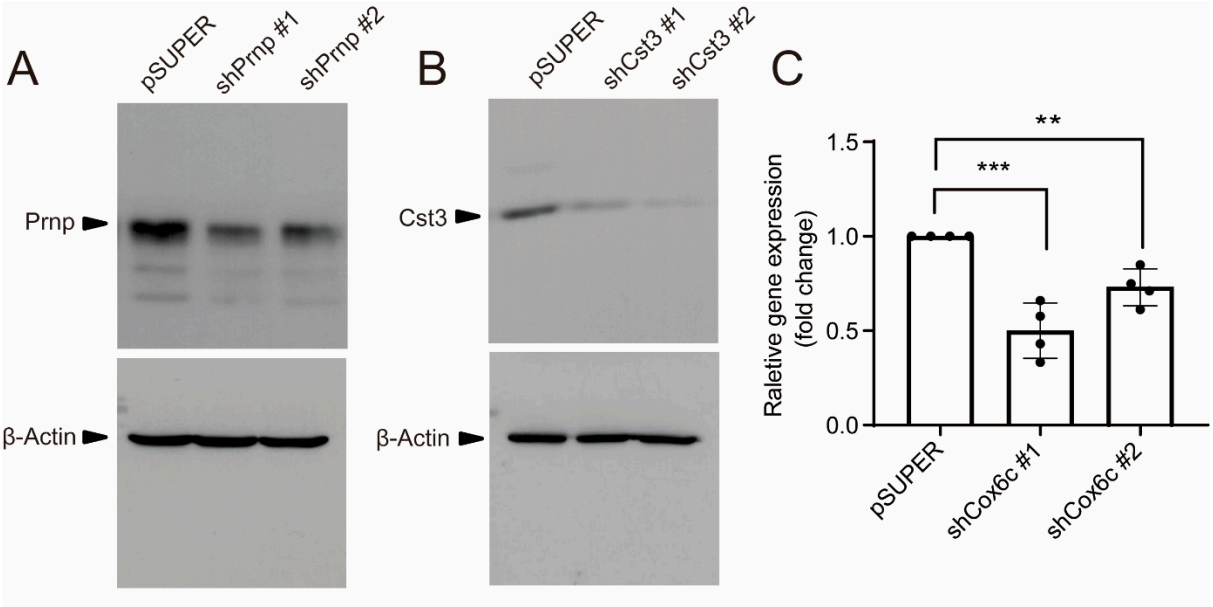
Supplementary Figure S1 to S3 and Supplementary table S1 and S2

Supplementary Figure S1



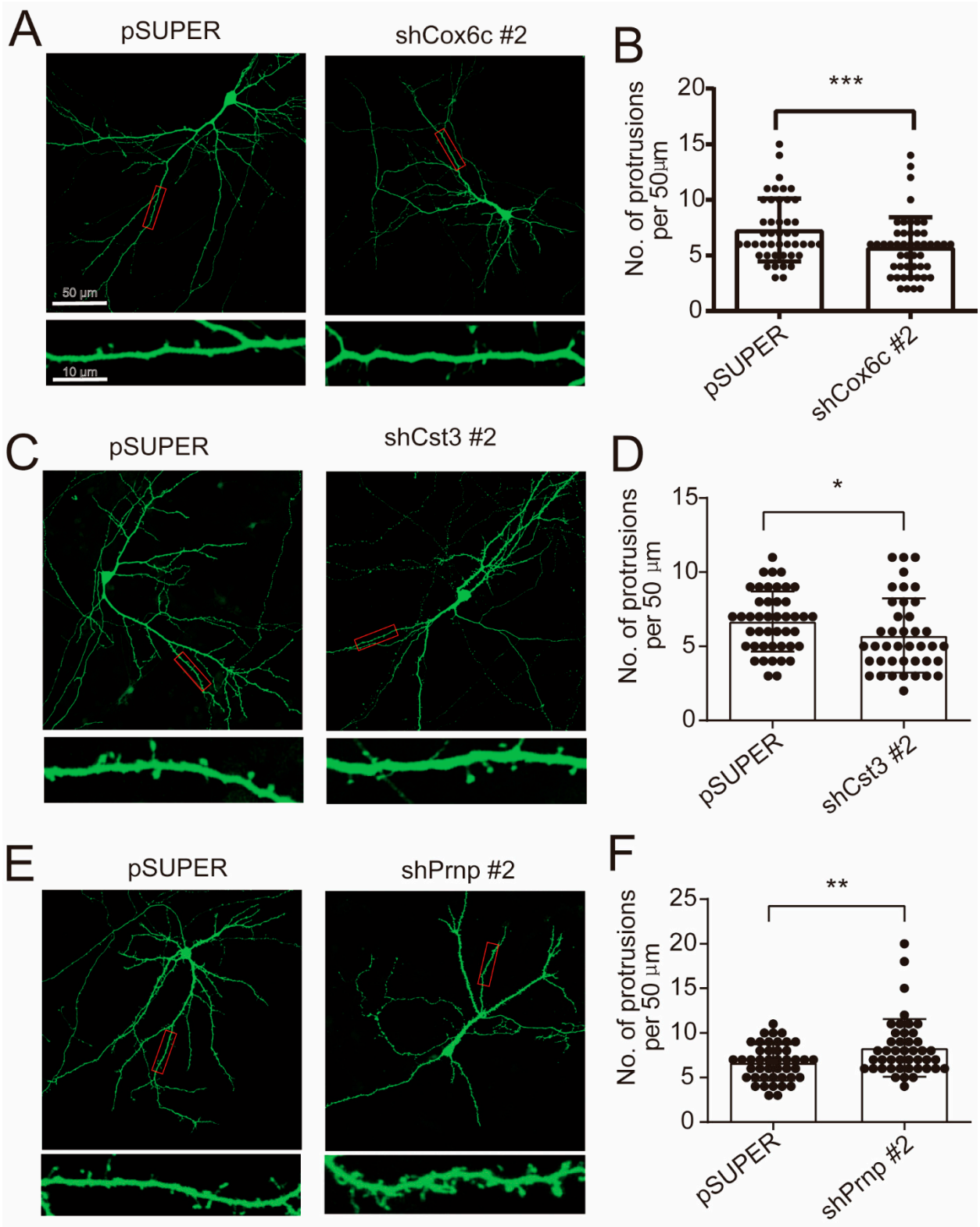
Supplementary Figure S1: Validation of candidate gene expression. We used quantitative RT-PCR to investigate the expression of candidate genes. (**A**) Differentially expressed genes were validated in synaptodendrosomes from 3-month old AD and WT mice. (**B**) Differentially expressed genes were validated in synaptodendrosomes from 6-month old AD and WT mice. All analyses were performed in 3 independent experiments (mean ± SEM; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; two-way ANOVA; $n \geqslant 6$ animals).

Supplementary Figure S2



Supplementary Figure S2: Validation of knockdown efficiency of shRNAs. We designed 2 shRNAs against a specific gene. The knockdown efficiency was examined in cultured hippocampal neuron. (**A**) Western blot analysis of shPrnp #1 and shPrnp #2 efficiency. (**B**) Western blot analysis of shCst3 #1 and shCst3 #2 efficiency. (**C**) Due to the lack of commercially available Cox6c antibody to detect its expression in neurons, qPCR was used to validate the reduction of *Cox6c* transcript upon shRNA-mediated knockdown. Western blotting was performed in 2 independent experiments. qPCR was performed in 2 independent experiments with duplicates (mean ± SEM; $^{**}p < 0.01$, $^{***}p < 0.001$; two-way ANOVA; $n \geqslant 4$).

Supplementary Figure S3



Supplementary Figure S3: Examination of neuronal morphology by shRNA-mediated knockdown of DEGs. We designed 2 shRNAs against a specific gene. The effect of the second shRNAs were shown here. (**A, C, E**) Rat hippocampal neurons were transfected at 12 days in vitro (DIV) with pSUPER-GFP plus pSUPER-shCox6c, pSUPER-shCst3, and pSUPER-shPrnp, respectively. Confocal images (40x) were collected at DIV 14. Scale bar: top, 50 µm; bottom, 10 µm. Representative images of GFP in transfected neurons are shown, along with magnified images of

the dendritic protrusions. (**B, D, E**) Quantitative analysis of dendritic spine density in Cox6c, Cst3 and Prnp-knockdown neurons, respectively. A total of 20–25 cells from 3 independent experiments (mean ± SEM; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; two-tailed Student-t test).