

Supplemental Information

TRBP maintains mammalian embryonic neural stem cell properties by enhancing the Notch signaling pathway as a novel transcriptional coactivator

Sung-Hyun Byun, Juwan Kim, Dasol Han, Mookwang Kwon, Jae Youl Cho, Hui Xuan Ng, Samuel J. Pleasure, and Keejung Yoon

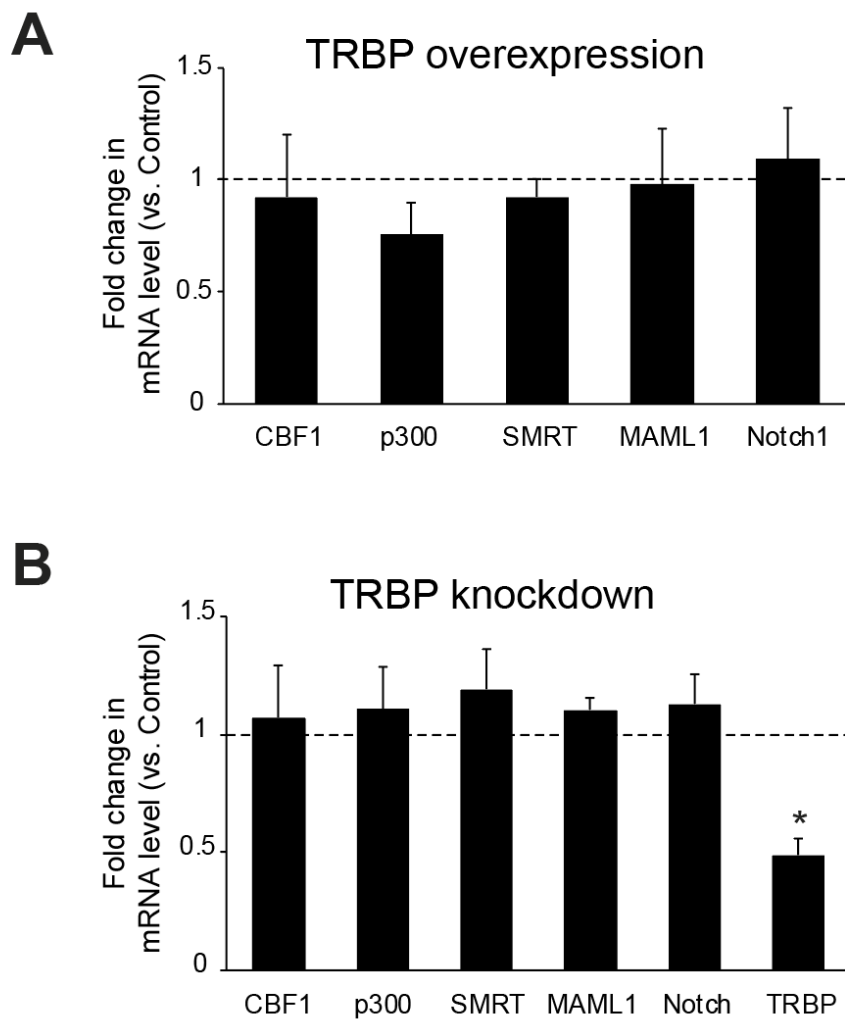


Figure S1. TRBP does not affect gene expression levels of Notch coactivational members. mRNA expression levels of each indicated Notch coactivational member were measured by qRT-PCR two days after E14.5 primary neural progenitor cells were transduced with retroviral vectors expressing (A) TRBP or (B) shRNA against TRBP. Error bars represent s.d. Student's *t*-test was used to determine statistical significance. * $P < 0.05$.

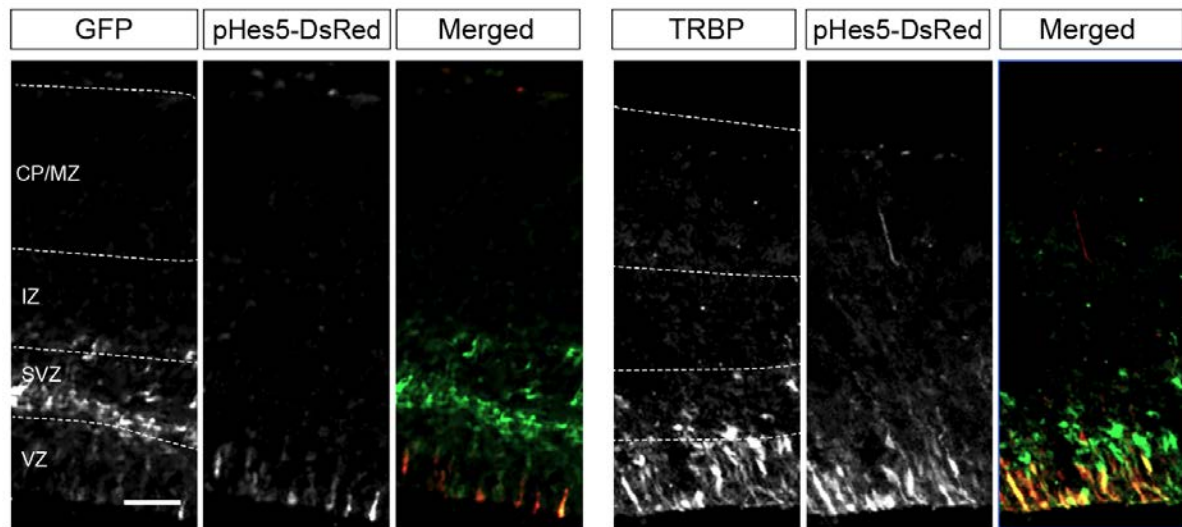


Figure S2. TRBP increases Hes5 promoter activity *in vivo*. Double immunolabeling of E14.5 brain sections electroporated *in utero* with the indicated plasmids at E13.5 using anti-GFP and anti-DsRed as primary antibodies, and Alexa dye-conjugated secondary antibodies to visualize effector gene expression (green) and Hes5 reporter activity (red). Scale bar, 50 μm .

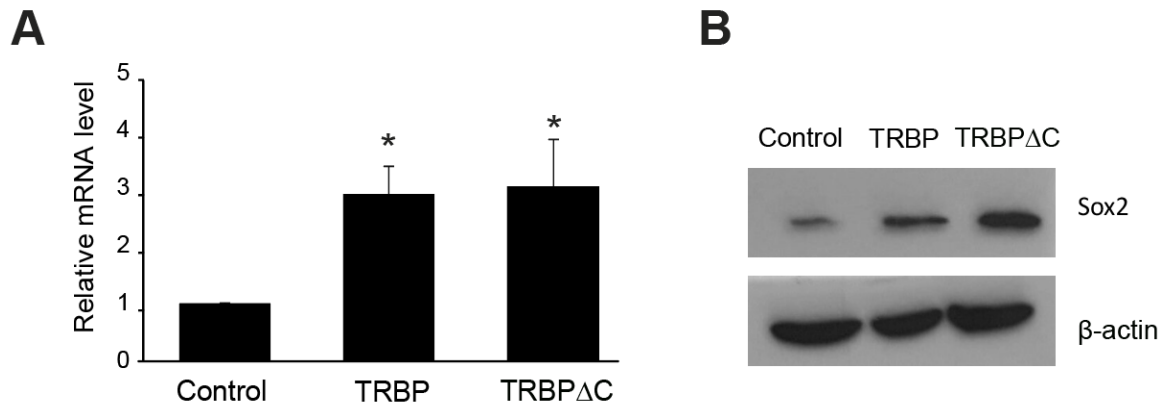


Figure S3. The Dicer-binding defective TRBP mutant behaves like wild-type TRBP in neural progenitor cells. (A) Hes5 mRNA expression levels measured by qRT-PCR two days after E14.5 primary neural progenitor cells were transduced with retroviral vectors expressing TRBP or TRBPΔC. (B) Western blot analysis of primary neural progenitor cell lysates infected with TRBP- or TRBPΔC-expressing retrovirus for Sox2 proteins. Error bars represent s.d. Student's *t*-test was used to determine statistical significance. * $P < 0.05$.

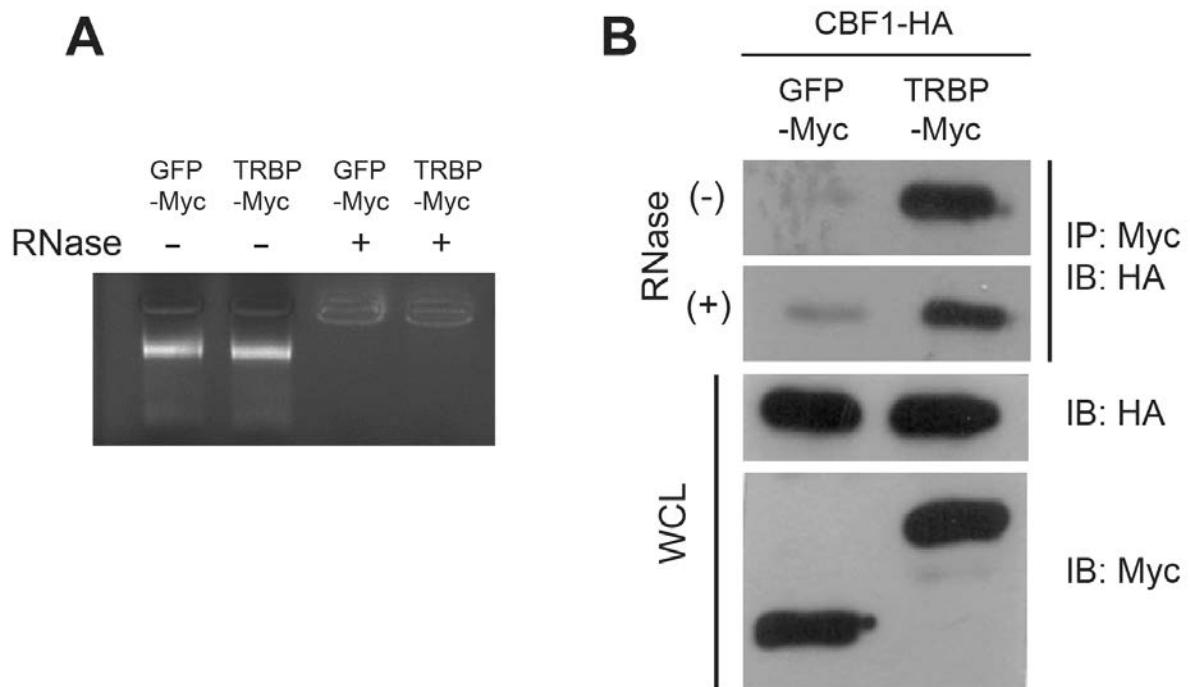


Figure S4. TRBP interacts with CBF1 in an RNA-independent manner. (A) Cellular RNA removal by RNase treatment of HEK 293T cell lysates prior to co-IP was assessed by agarose gel electrophoresis. (B) Co-IP assays were used to examine binding between TRBP and CBF1 with or without RNase pretreatment of cell lysates.