

Figure S1. Immunofluorescence analysis of TFAP2A and TFAP2B in human fetal retina. Tissue sections from human fetal retina at 10-11 weeks gestation were immunostained with mouse monoclonal anti-TFAP2A or rabbit anti-TFAP2B antibodies followed by donkey anti-mouse or donkey anti-rabbit secondary antibodies conjugated with Alexa 555 or Alexa 488. Sections were counterstained with DAPI to label the nuclei. Photographs were taken with a Zeiss LSM 510 confocal microscope equipped with a 40X objective for TFAP2A and 20X objective for TFAP2B. Abbreviations: ONBL, outer neuroblastic layer; GCL, ganglion cell layer.

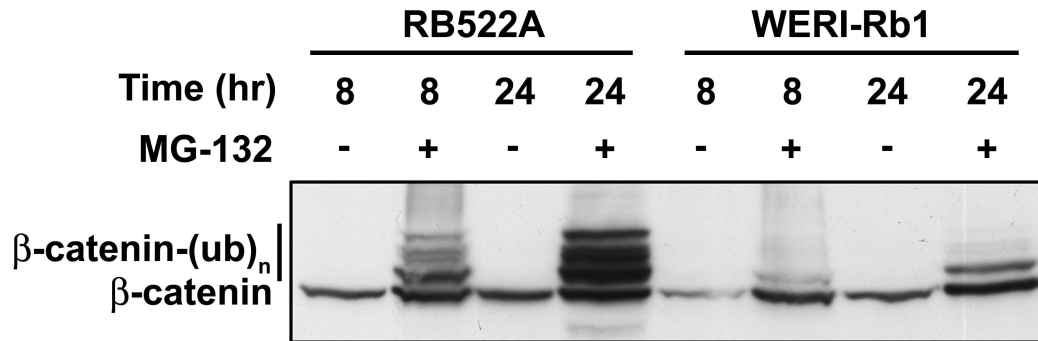


Figure S2. MG-132 treatment of retinoblastoma cells. RB522A and WERI-Rb1 cells were treated with MG-132 for 8 hrs and 24 hrs, as indicated. Proteins were separated in an 8% polyacrylamide-SDS gel and transferred to a nitrocellulose membrane. The membrane was immunostained with anti- β -catenin antibody. The signal was detected using the ECL reagent. The multiple bands shown in the lanes treated with MG-132 are poly-ubiquitinated β -catenin.

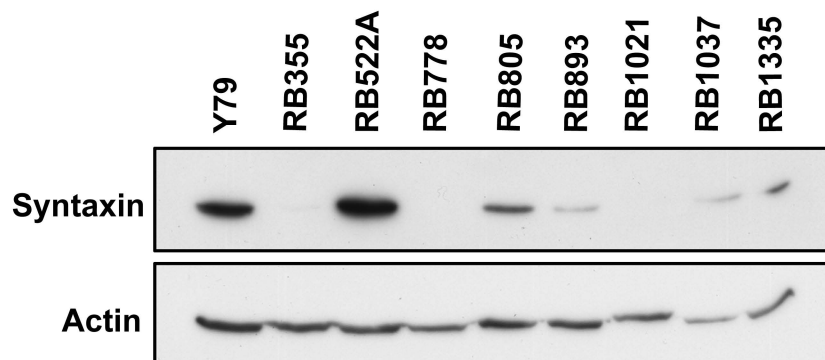


Figure S3. Western blot analysis of syntaxin using protein lysates from retinoblastoma cell lines, as indicated. Proteins were separated in a 8% polyacrylamide-SDS gel and transferred to a PVDF membrane. The membrane was sequentially immunostained with anti-syntaxin and anti-actin antibodies. The signal was detected using the ECL reagent.

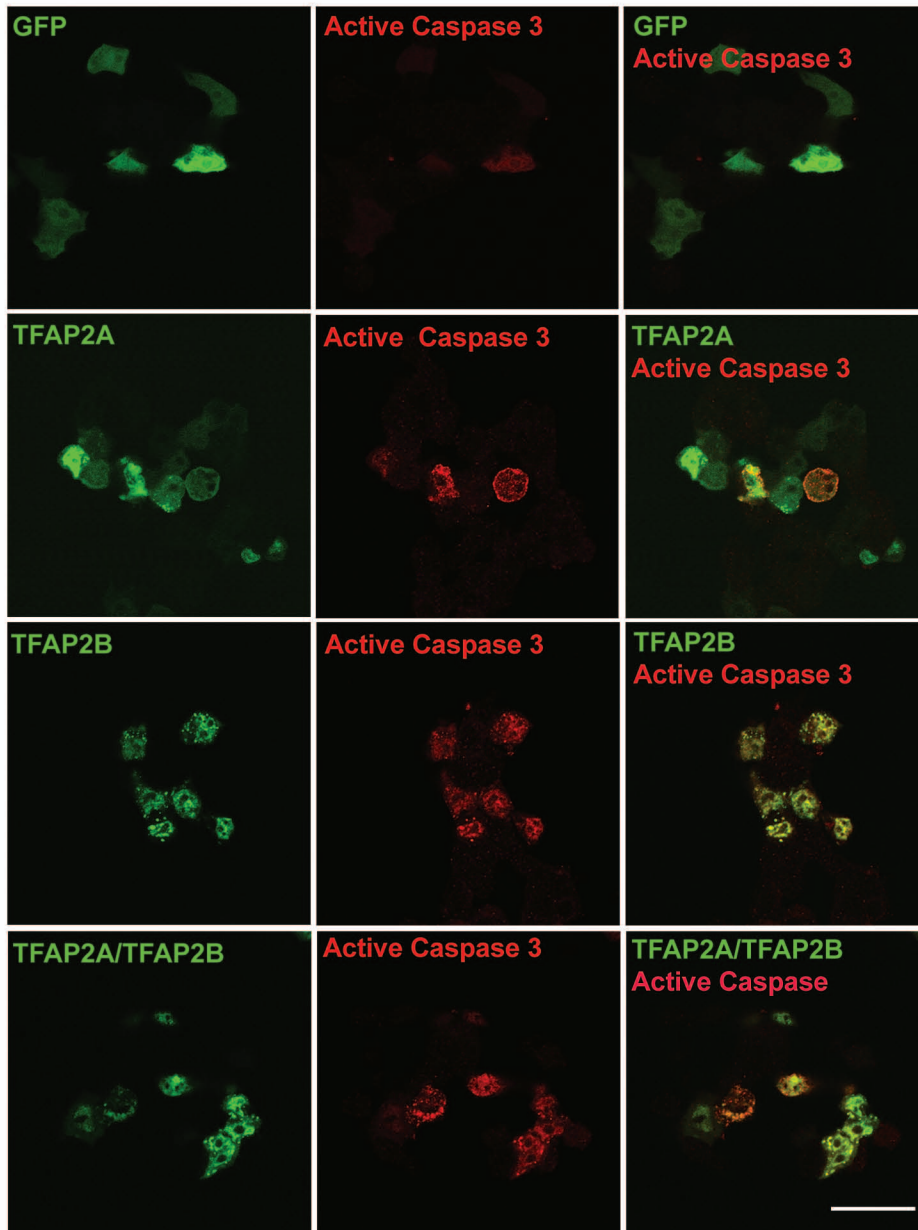


Figure S4. TFAP2A and TFAP2B induce retinoblastoma cell death through apoptosis. RB522A cells were transfected with pEGFP-C1, pEGFP-C1-TFAP2A, pEGFP-C1-TFAP2B or both pEGFP-C1-TFAP2A and pEGFP-C1-TFAP2B. Cleaved Caspase 3 was visualized in RB522A cells by immunostaining with anti-active Caspase 3 antibody followed by donkey anti-rabbit secondary antibody linked to Alexa 555. GFP-positive cells were detected by epifluorescence. Merged pictures show co-localization of GFP or GFP-TFAP2, and active Caspase 3. Photographs were taken with a Zeiss LSM 510 confocal microscope equipped with a 40X objective. Scale bar = 25 μ m.

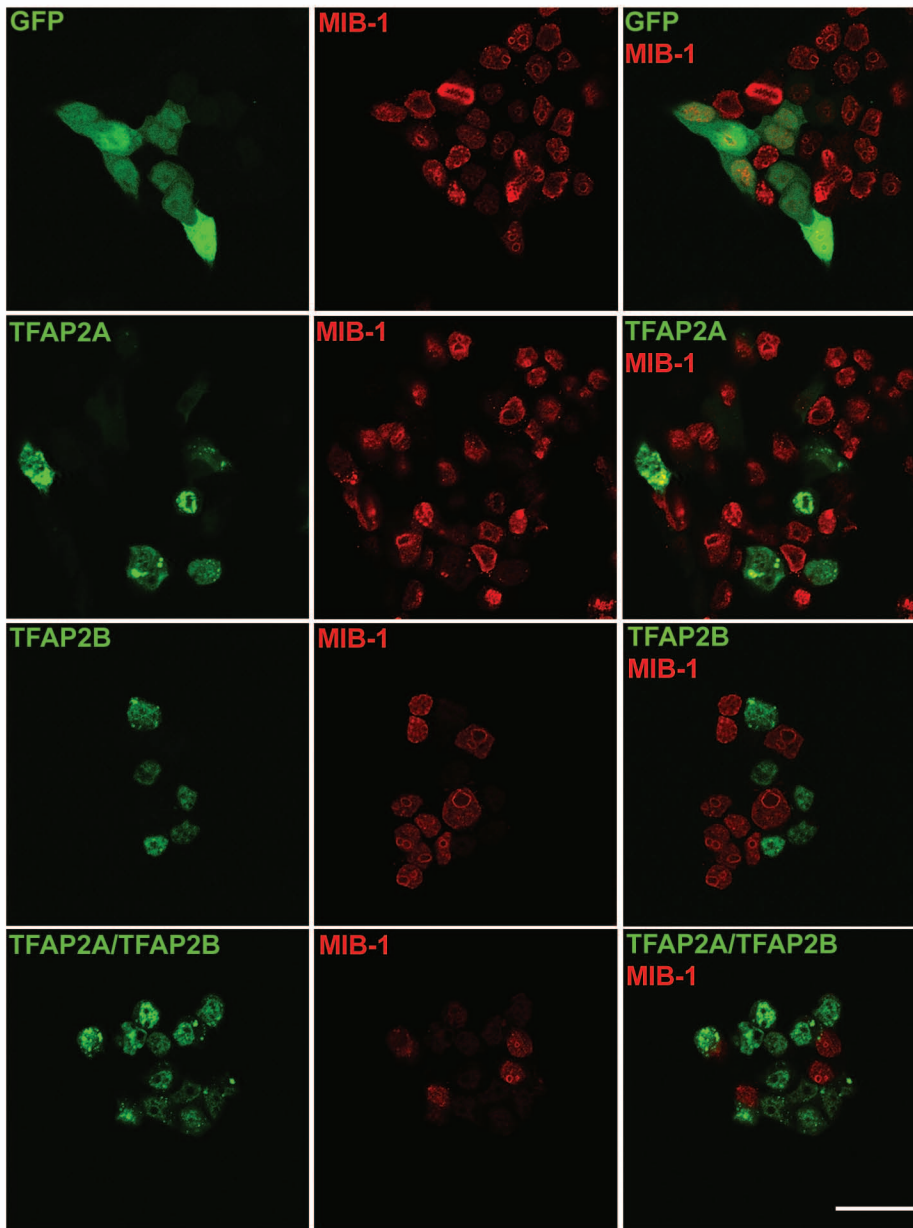


Figure S5. TFAP2A and TFAP2B inhibit retinoblastoma cell proliferation. RB522A cells were transfected with pEGFP-C1, pEGFP-C1-TFAP2A, pEGFP-C1-TFAP2B or both pEGFP-C1-TFAP2A and pEGFP-C1-TFAP2B. The Ki-67 proliferation marker was visualized by staining with MIB-1 antibody followed by donkey anti-mouse secondary antibody linked to Alexa 555. Merged pictures show co-localization of GFP or GFP-TFAP2 with Ki-67 antigen. Scale bar = 25 μ m.

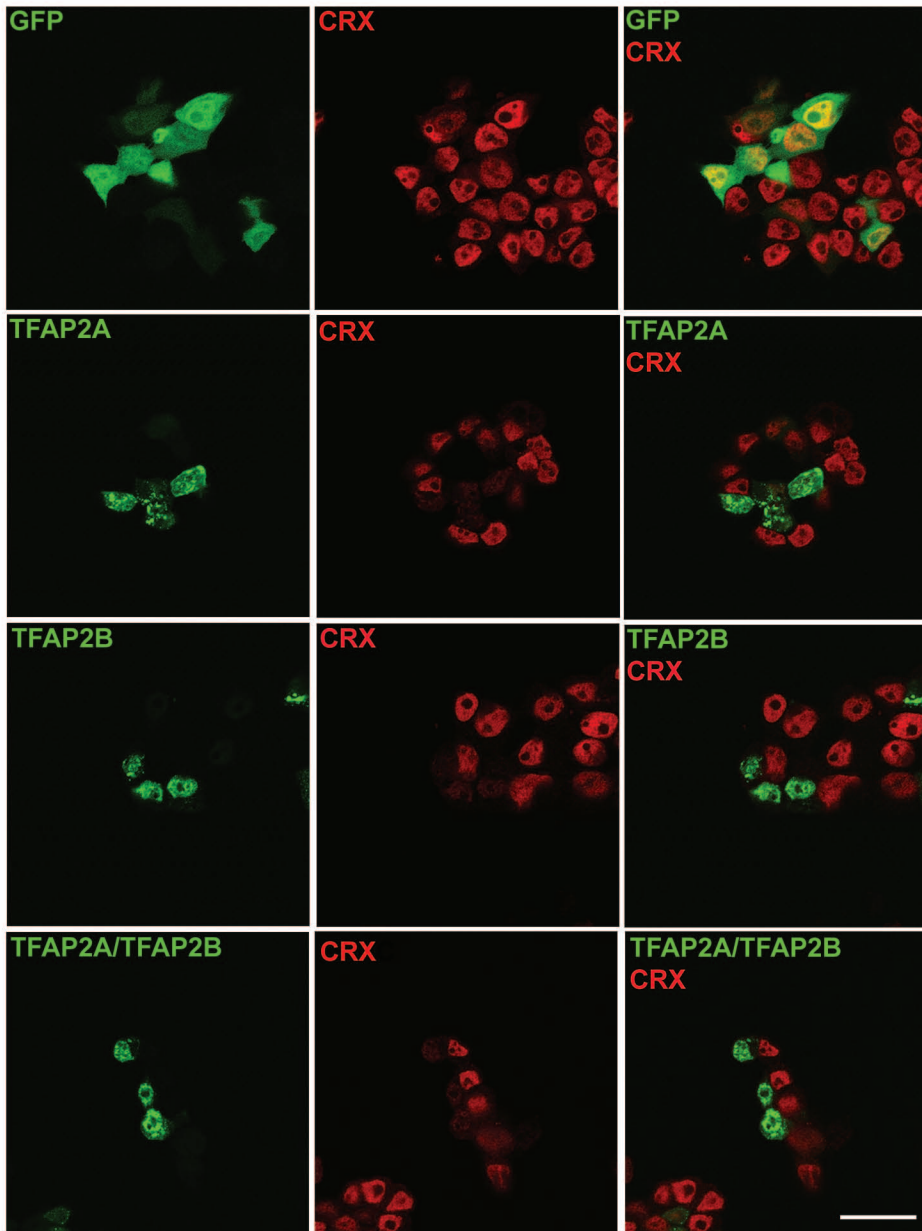


Figure S6. Reduction in CRX staining in retinoblastoma cells expressing TFAP2A and TFAP2B. RB522A cells were transfected with pEGFP-C1, pEGFP-C1-TFAP2A, pEGFP-C1-TFAP2B or both pEGFP-C1-TFAP2A and TFAP2B. CRX was visualized by staining with anti-CRX antibody followed by donkey anti-mouse secondary antibody linked to Alexa 555. Merged pictures show co-localization of GFP or GFP-TFAP2, and CRX. Scale bar = 25 μ m.