Figure S5

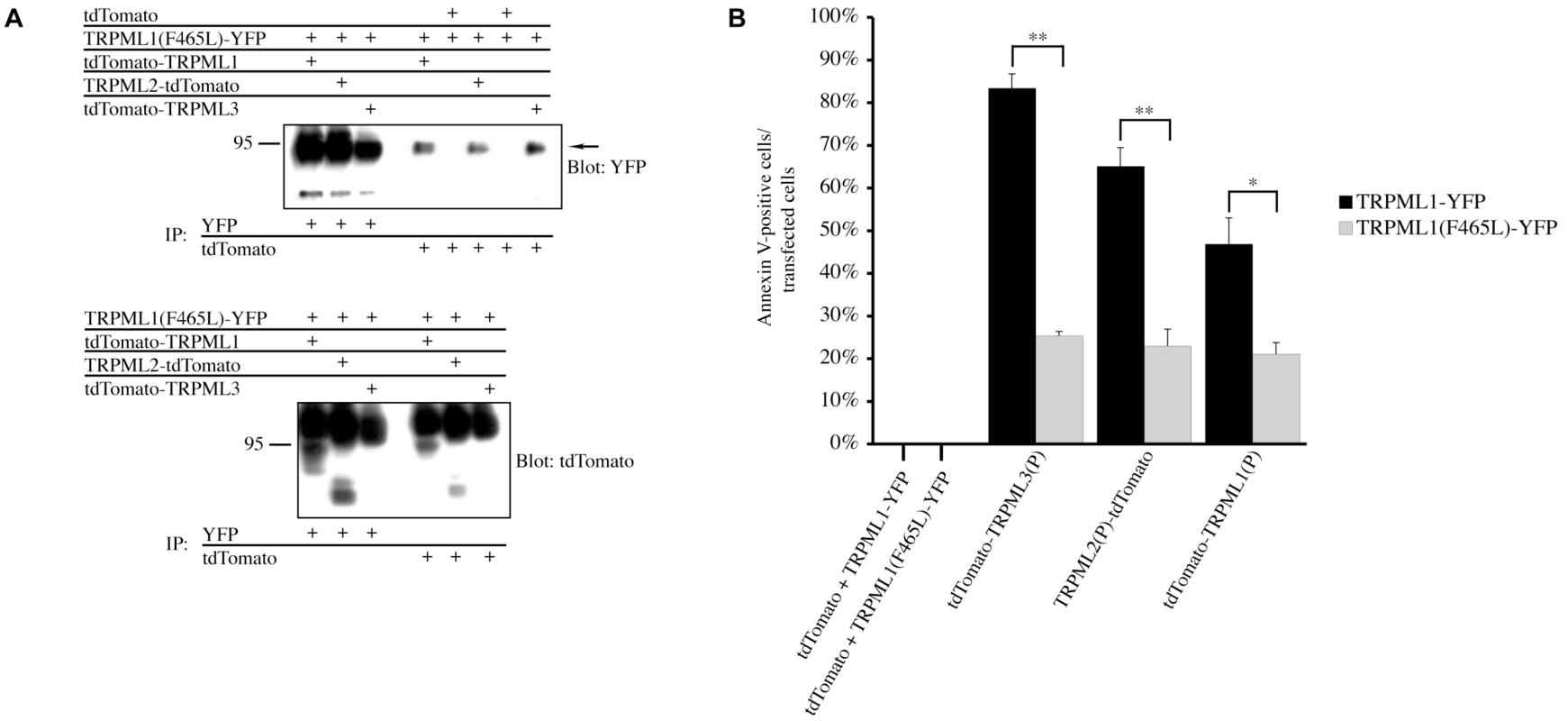

Fig. S5. TRPML1(F465L) physically interacts with WT-TRPMLs and attenuates the cytotoxic effects of TRPML(P) channel expression. (**A**) TRPML1(F465L)-YFP co-IPs with WT-TRPML channels. HEK cells were co-transfected with the indicated expression constructs (above top and bottom panels) and YFP- or tdTomato-tagged constructs were then immunoprecipitated (as indicated below panels). Immunoprecipitates were separated on gels followed by immunoblotting against the indicated targets (to the right of panels). Bands corresponding with the monomeric TRPML1(F465L)-YFP isoform are demarcated with an arrow to the right of the YFP-probed blot. A proteolytic isoform of TRPML1(F465L)-YFP is also detected in some immunoprecipitates. Right side of top panel and left side of bottom panel, tdTomato-tagged WT-TRPMLs co-IP with TRPML1(F465L)-YFP. Left side of top panel and right side of bottom panel, positive controls. (**B**) HeLa cells were co-transfected with TRPML1-YFP or TRPML1(F465L)-YFP in combination with the indicated tdTomato-tagged expression constructs. Alexa Fluor 647-AnnexinV staining was performed in order to detect early apoptotic cells. The histogram indicates the mean percentage of cells exhibiting both YFP-derived and tdTomato-derived fluorescence, under each condition, that also stained positively for Alexa Fluor 647-AnnexinV. n=3 independent experiments of at least 44 cells per assay. *, $p<0.05$; **, $p<0.01$.