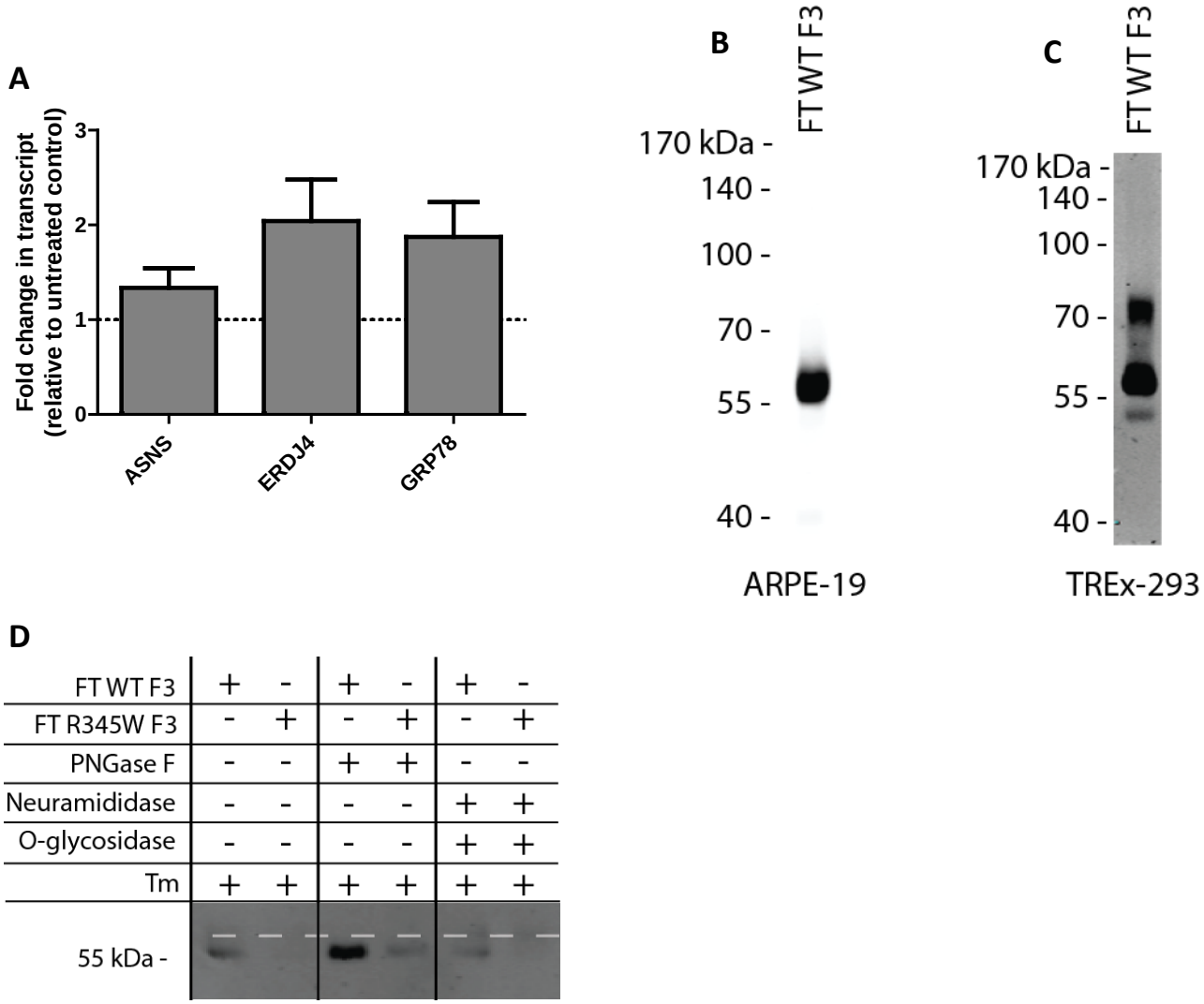
SUPPLEMENTAL FIGURE 2



Supplemental Figure 2. qPCR analysis of ARPE-19 cells treated with DTT and Western blot analysis of secreted F3. (A) ARPE-19 cells seeded for 24 h, followed by treatment with 500 μM DTT. After 1 h of treatment, cells were harvested and RNA was extracted. Transcripts were normalized to the housekeeping gene, RPLP2 and are presented as levels relative to transcripts from untreated cells. Representative data of 6 biological replicates, n = 2. (B, C) The main secreted F3 species from ARPE-19 and TREx-293 cells (an HEK-based cell line) migrates at ~55 kDa. (B) ARPE-19 cells were infected with adenovirus at an MOI of 5 for 48 h, after which media was replaced for 24 h and F3 was IP'd using anti-FLAG M1 agarose beads. Beads were washed and bound protein was eluted followed by Western blotting for F3. (C) TREx-293 cells were transfected with a construct encoding for FT WT F3, and a similar IP approach as described in (A) was utilized for enriching for secreted FT WT F3. (D) F3 secreted after treatment with Tm is not susceptible to glycosidase cleavage. ARPE-19 cells were infected with adenovirus at an MOI of 25 for 48 h, followed by media replacement with OptiMEM containing Tm (1 μg/mL) for 24 h. Conditioned media was denatured and treated with PNGaseF (1 μL), or neuramididase (1 μL) and O-glycosidase (1 μL) for 1 h at 37°C. The samples were analyzed by Western blotting. The migration of WT F3 originating from untreated cells is designated by the dashed white line, n = 2.