Supplemental Figure 4. Western blot analysis of TREx-293 cell lysates and TREx-293/ARPE-19 secreted protein. (A-C) TREx-293 cells handle N249Q differently than ARPE-19 cells (cf Fig. 4A, C). F3 was IP’d from TREx-293 cell lysates (A) and conditioned media (B), followed by quantification (C). Data shown are representative of ≥ 2 independent experiments. Note: the secreted WT F3 lane shown in ‘A’ was also used as a comparison in Supplemental Fig. 2C. (D) Secreted N249Q/R345W F3 run under native conditions is not recognized by the ProSci fibulin-3 antibody. Conditioned media from ARPE-19 cells infected with adenovirus encoding for FT F3 variants (MOI 25) was run under native conditions and separated on a 4-16% Bis Tris gel. As with the anti-FLAG tag antibody, the ProSci fibulin-3 antibody does not recognize FT N249Q/R345W F3, but is able to detect the other FT F3 variants.