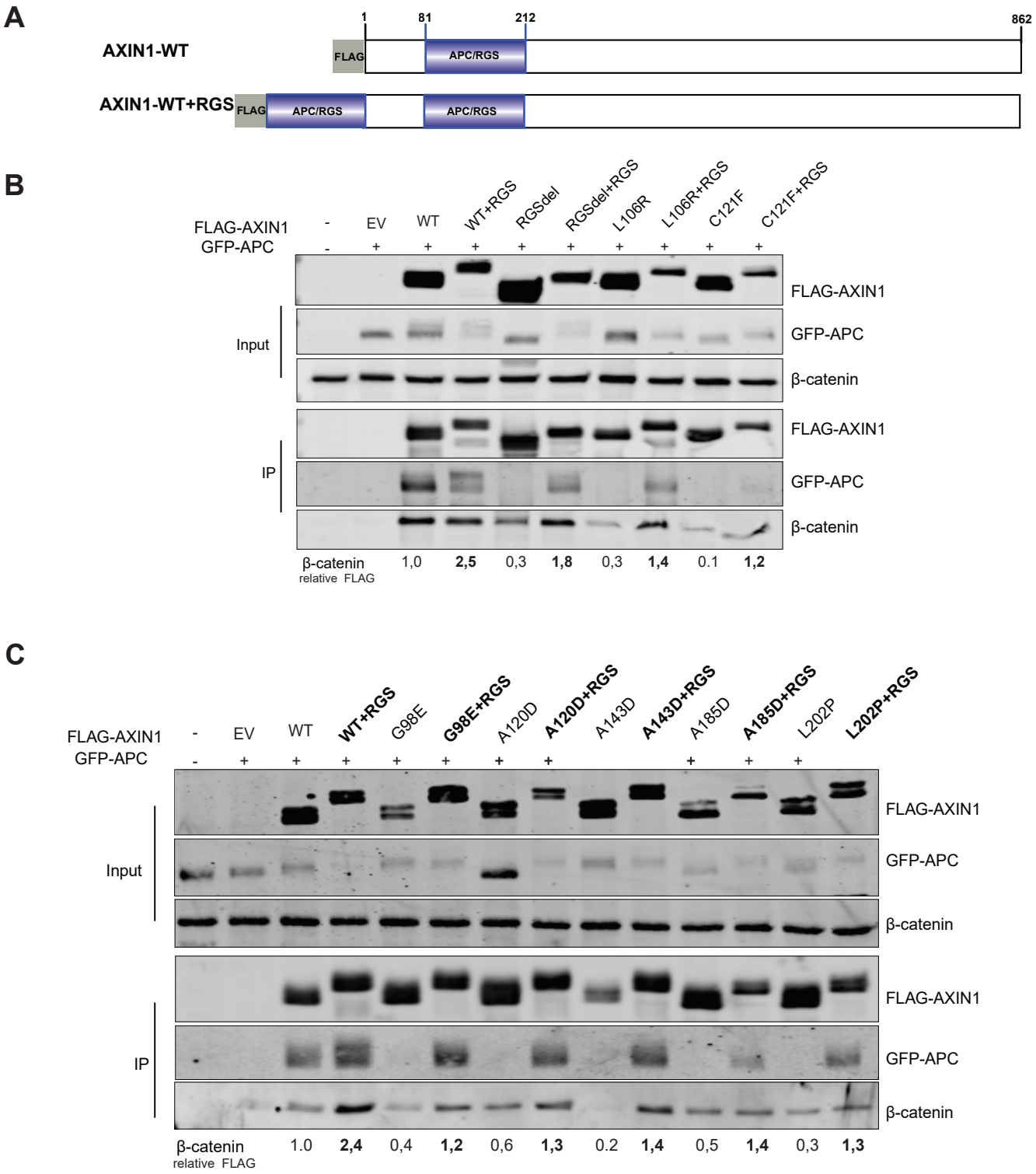
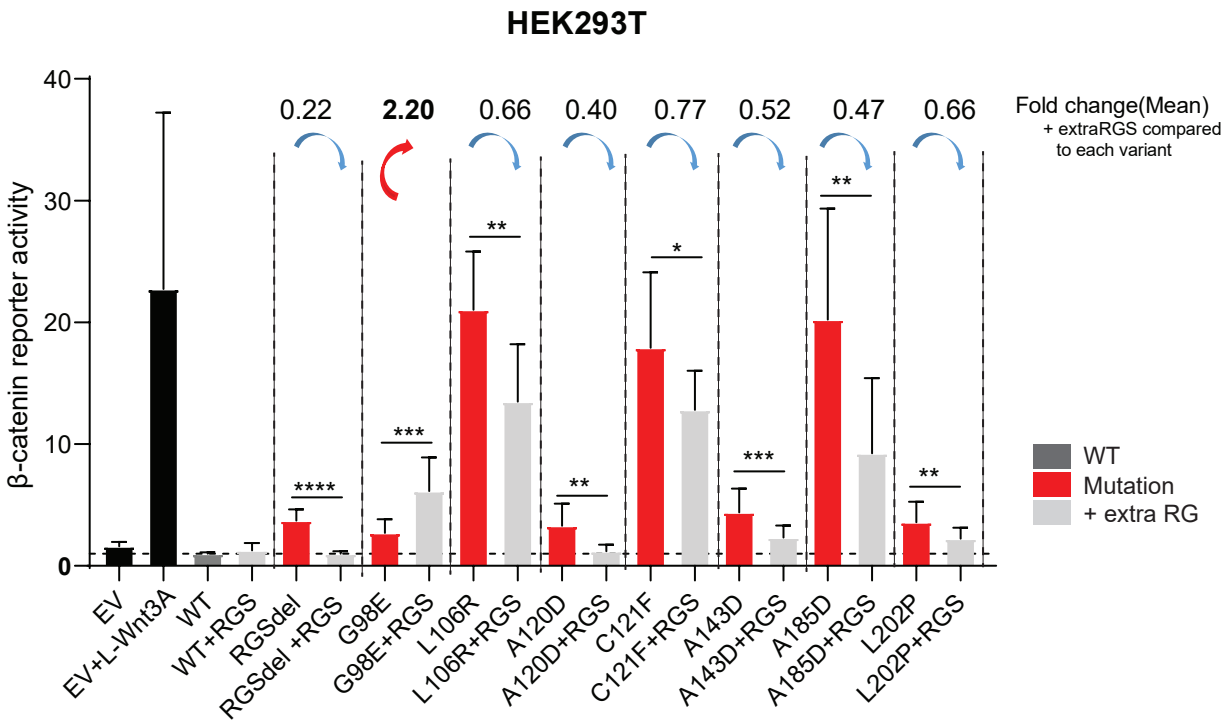


Supplementary Fig.12 Page 1

D



Supplementary Fig.13 Page 2

Addition of an extra RGS/APC domain restores APC and β-catenin binding to selected RGS/APC domain variants, and (partially) restores β-catenin regulation. (A) Schematic diagram showing the extra RGS/APC inserted before the tankyrase domain and directly following the N-terminal FLAG-tag. (B,C) Immunoprecipitation experiment showing that the extra RGS/APC domain results in restoration of binding to co-transfected GFP-APC and endogenous β-catenin for all investigated variants. (D) A β-catenin reporter assay investigating the β-catenin regulatory properties of AXIN1 RGS/APC variants with/without an extra RGS/APC domain. The extra RGS domain restores β-catenin regulation of ΔRGS-AXIN1 and all moderate variants (A120D, A143D and L202P), with the exception of G98E that shows an unexplained increase in signaling. Three variants with a stronger impact on β-catenin signaling (L106R, C121F and A185D), all show a significant reduction in β-catenin reporter activity, but no restoration to wild-type levels (in triplicate, n=2 independent experiments). All data shown as mean ± SD. Statistical significance for all experiments was analyzed using a Mann-Whitney test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).