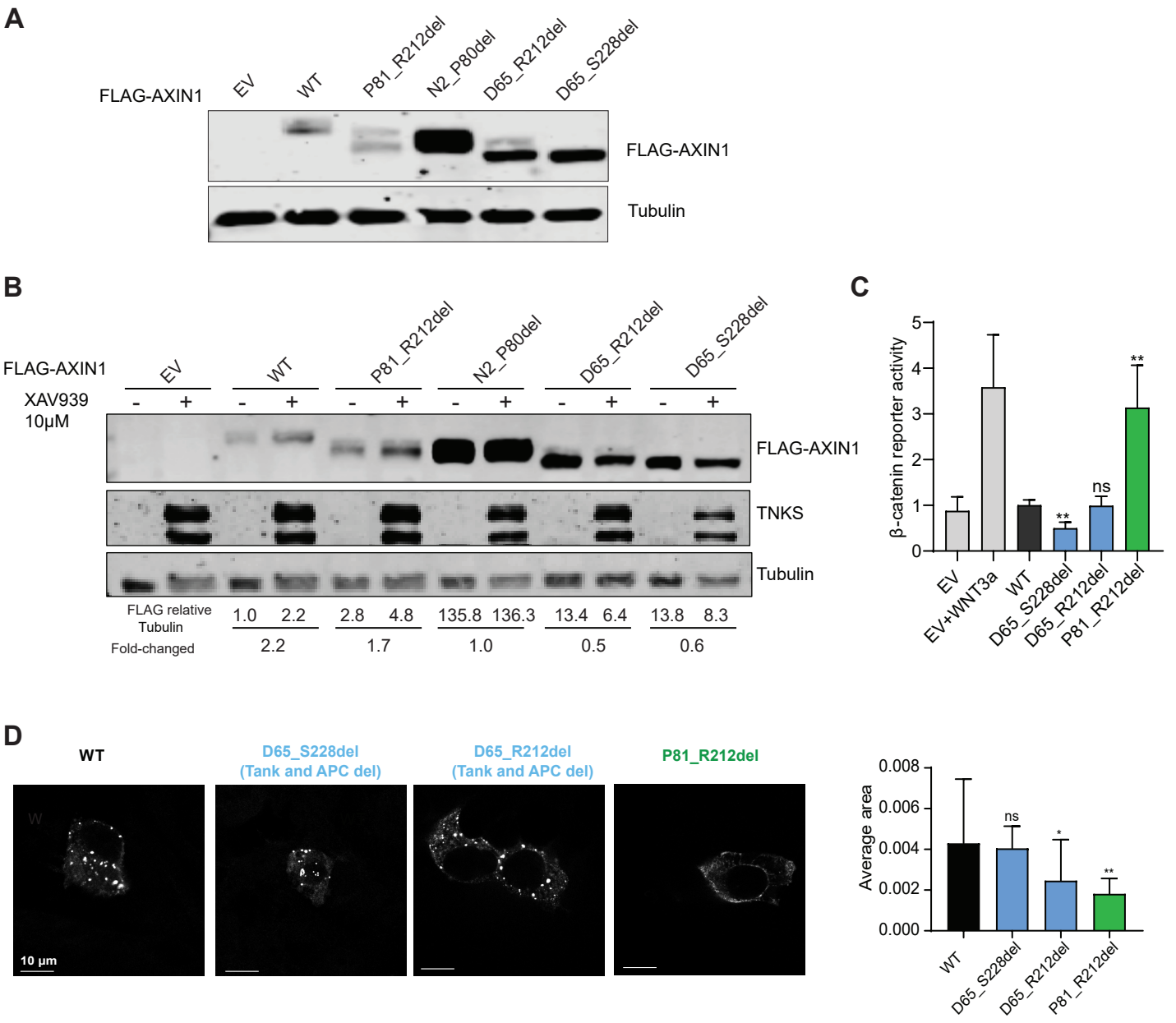

Supplementary Fig.10 An accurate removal of the RGS/APC binding domain is required to correctly determine the functional consequences of losing this domain. The P81_R212del and N2_P80del variants correctly delete the RGS/APC and tankyrase domains, respectively. The D65_R212del and D65_S228del variants, besides removing the RGS/APC domain, also remove amino acids essential for tankyrase binding. (A) Deleting the entire or the C-terminal part of the tankyrase domain leads to higher baseline levels of AXIN1. (B) Applying the tankyrase inhibitor XAV939 for 24hrs increased wild-type and P81_R212del protein levels, but had no effect or even reduced those of others. Accumulation of TNKS1/2 protein levels is used as positive control for the effect of XAV939. (C) A β-catenin reporter assay shows that the accurate removal of the RGS/APC domain leads to increased signaling, while simultaneous removal of part of the tankyrase domain has no effect. (D) Expression of wild-type AXIN1 and both variants lacking part of the tankyrase domain, lead to the formation of clear intracellular puncta. Accurate removal of the RGS/APC domain yields fewer and smaller puncta, and shows a generally more diffuse cytoplasmic pattern. Average dot size was determined using ImageJ software on at least 6 independent cells. Data shown as mean ± SD. Statistical significance for all experiments was analyzed using a Mann-Whitney test (*$P < 0.05$, **$P < 0.01$).