Supplementary Fig.8 β-catenin reporter analysis of AXIN1 R395P and R395H HEK293T knockin clones. Using gene editing we generated HEK293T clones endogenously expressing the R395P and R395H AXIN1 variants. Three AXIN1 containing chromosomes are present in the hypotriploid HEK293T cell line. The R395H clone is correctly modified on all chromosomes, while the 3 independent R395P clones carry two chromosomes correctly expressing R395P and one chromosome carrying a R395Afs*17 mutation. This latter variant lacks most important functional domains and is barely expressed at RNA level (see cDNA sequence analysis and Figure 7C), meaning that it can be considered as a knock-out chromosome. All clones were compared with a homozygous R395Afs*17 clone. (A) Immunoblot analysis of the generated clones using AXIN1 antibodies targeting both N- and C-terminal epitopes (cat.# 3323S and cat. #2087S, Cell Signaling Technology). For the C-terminal blot one irrelevant lane was removed. (B) Genomic and cDNA sequencing of all obtained clones. cDNA sequence analysis of the R395P clones reveals a much lower peak intensity for the R395Afs*17 expressing chromosome, most likely resulting from nonsense-mediated mRNA decay. (C) At baseline, all R395P expressing clones show a comparable increase in β-catenin reporter activity as the homozygous R395Afs*17 clone. Following siRNA-mediated AXIN2 knockdown, all knock-out and R395P expressing clones show a prominent increase in signaling, while wild-type cells are barely affected. This result is in accordance with a loss-of-function behavior of R395P AXIN1. If it would possess a dominant-negative activity, it would be expected to dominantly interfere with endogenous AXIN2 and other components of the breakdown complex, and would lead to higher reporter activity at baseline. The low level β-catenin reporter activity for the R395H variant at baseline and following AXIN2 knockdown, indicates that this variant is functional in correctly regulating β-catenin signaling also at endogenous level. Values are presented relative to the WRE/CMV-Renilla ratios obtained for the siControl-WT, which is arbitrarily set to 1. The data are shown as mean ± SD. Statistical significance for all experiments was determined using a Mann-Whitney test (**$P < 0.01$).