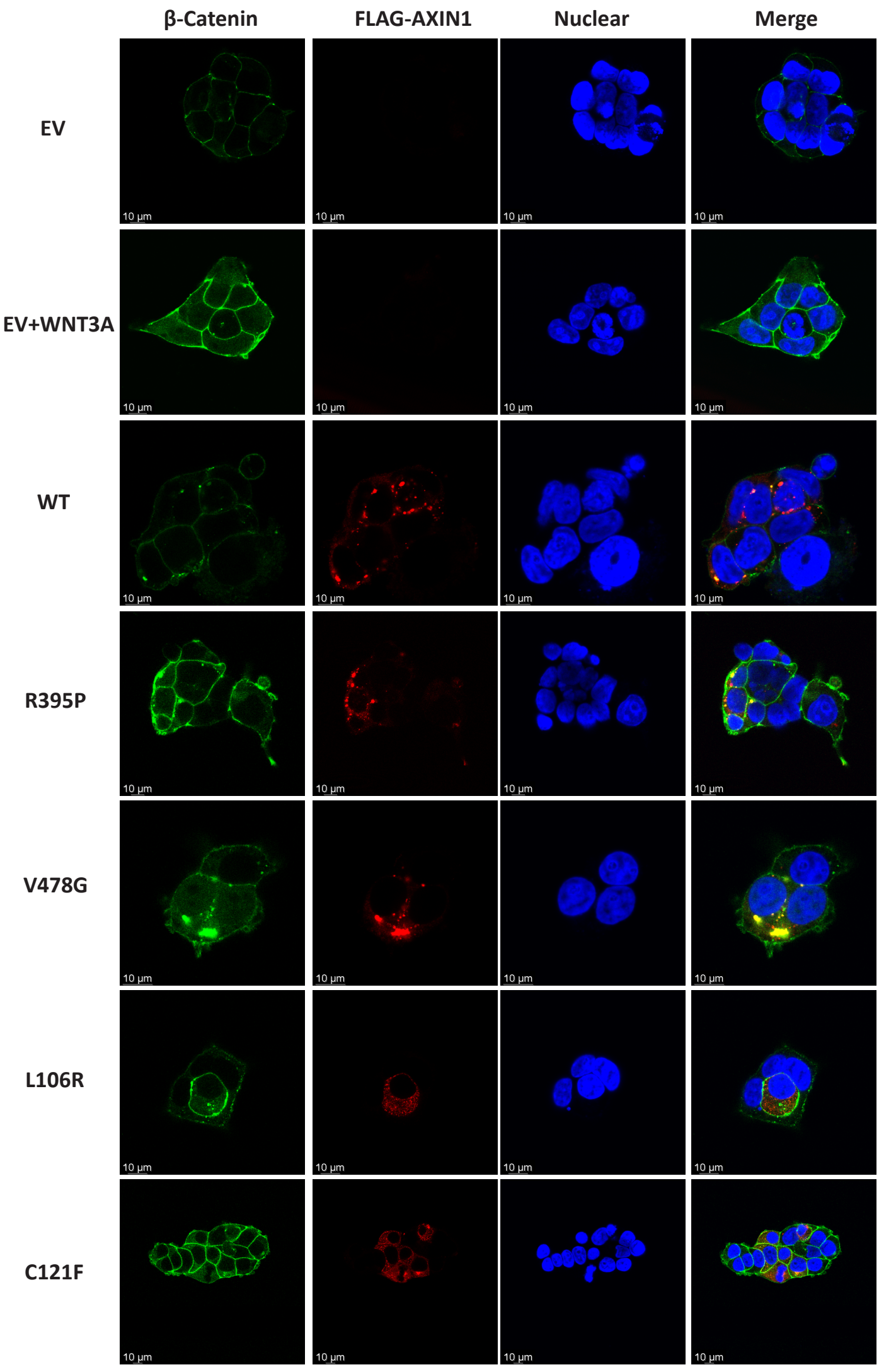
β-Catenin
FLAG-AXIN1
Nuclear
Merge
EV
EV+WNT3A
WT
R395P
V478G
L106R
C121F
10 μm

Supplementary Figure 1

Image shows an immunofluorescence experiment to demonstrate the endogenous location of β-catenin, following the indicated treatments. To obtain this image, HEK293T cells were grown on glass slides in a 12-well plate, and either left untreated, exposed to 1/10 diluted L-Wnt3A conditioned medium, or transfected with 80 ng of the indicated AXIN1 variants. The next day, the cells were fixed for 10 minutes with -20°C methanol and double-stained with the indicated antibodies (Mouse Anti-β-Catenin (cat.#610154, BD Biosciences) and FLAG-(D6W5B) Rabbit mAb (cat.#14793, Cell Signaling Technology)). Exposure to Wnt3A yields a clear increase in nuclear localized β-catenin. Similar staining patterns are observed in cells transfected with the more defective R395P and V478G variants. The image for the latter V478G variant provides an internal control, as the upper non-transfected cell shows a negative nucleus, while the other two transfected cells clearly show a nuclear β-catenin staining. Nuclear β-catenin is less obvious but occasionally weakly detectable in cells transfected with the partially defective L106R and C121F variants. These results confirm that overexpression of (partially) defective AXIN1, facilitates the translocation of β-catenin into the nucleus.