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Supplemental information

Wg/Wnt-signaling-induced nuclear translocation of β-catenin is attenuated by a β-catenin peptide through its interference with the IFT-A complex

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Supplemental Data:

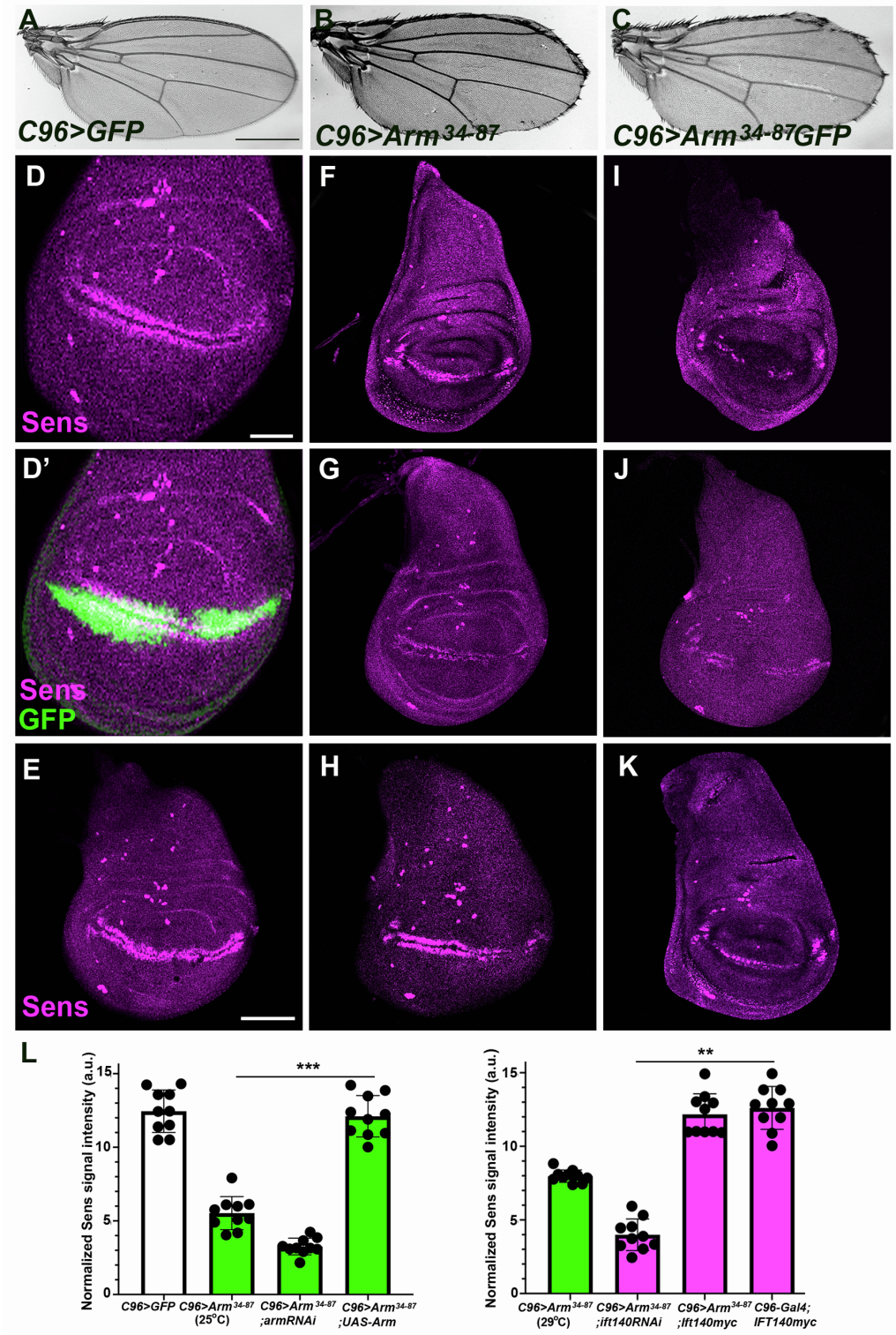

Figure S1 (supplement to Figure 1). Effects of Arm$^{34-87}$ or Arm$^{34-87}$GFP peptide expression on wing development.

(**A-C**) Adult wings: *C96-Gal4, UAS-GFP (C96>GFP)* as wild-type control (**A**). *C96-Gal4, UAS-Arm$^{34-87}$*: note partial wing margin loss caused by Arm$^{34-87}$ expression (**B**), or *C96-Gal4, UAS-Arm$^{34-87}$GFP* (**C**): note very similar defects and wing margin loss (cf to panel B).

(D'-D'') Control wing imaginal disc highlighting future wing-blade stained with Sens (magenta) expression (**D,D'**) at the D/V boundary, and GFP (green, **D'**) demonstrating the *C96-Gal4* expression domain (overlapping the future wing margin region).

(**E-K**) All full wing discs displaying Sens expression (in magenta, reared at 25°C): (**E**) *C96-Gal4* driver alone (control), note expression of Sens covering the entire wing margin. (**F-K**) Examples of *C96-Gal4, UAS-Arm*$^{34-87}$: note partial loss of Sens staining in all discs. Also note variable effects from wing to wing, showing consistent Sens staining loss but in different regions of the future wing margin. This si due the variability of the Gal4-driver. Scale bar represents 100μm in D-D', and 50μm in E-K.

(**L**) Quantification of the Sens signal at the D/V boundary of wing imaginal discs (n>10 discs), from the genotypes shown in main Figure 1. Please see also Suppl. Fig. S4A for outline how quantification was performed. Student's *t*-test was used for signal intensity quantification (** p<0.01; *** p<0.001).

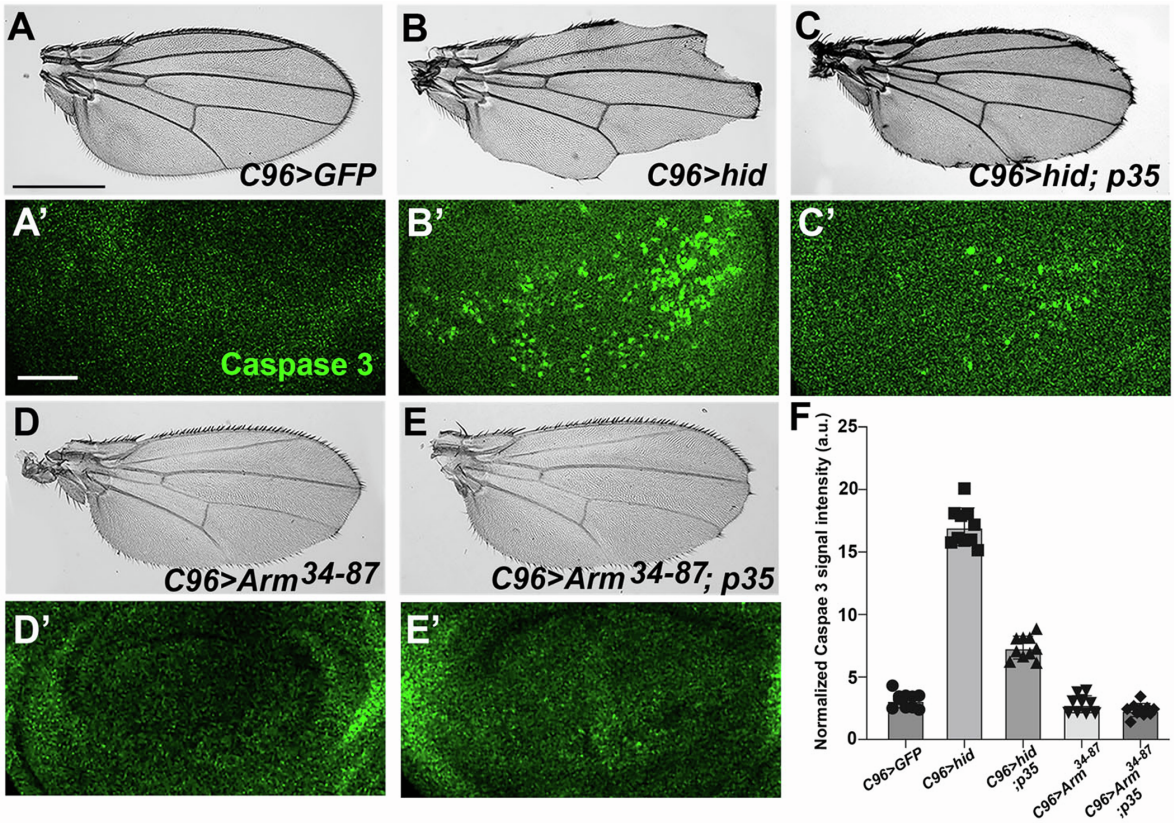

Figure S2 (Suppl. to Figure 1): Expression of the Arm$^{34-87}$ peptide does not induce cell death.

(**A**) *C96>GFP* as a control. (**B**) Expression of Hid causes loss of wing margin. (**C**) Co-expression of Hid with the cell death inhibitor p35 largely rescued the wing margin loss phenotype.

(**A'-C'**) Hid expression by *C96-Gal4* induces caspase activation and cell death marked by cleaved Caspase 3 staining (**B'**; compare to *C96>GFP* as a control, **A'**; note green signal above background). Caspase 3 detection (green) induced by Hid is suppressed by p35 co-expression (**C'**).

Wing notching induced by *C96>Arm$^{34-87}$* expression (**D**) is not suppressed by p35 co-expression (**E**).

(**D'-E'**) Arm$^{34-87}$ expression does not induce cell death, as detected via cleaved Caspase 3 (**D'**, cf to control in **A'**). p35 co-expression has no effect on lack of Caspase 3 staining (**E'**). Scale bar represents 100μm in A-C, D-E and 50μm in A'-C', D'-E'.

(**F**) Quantification of the Caspase3 signal at the D/V boundary of wing imaginal discs (n=10 discs).

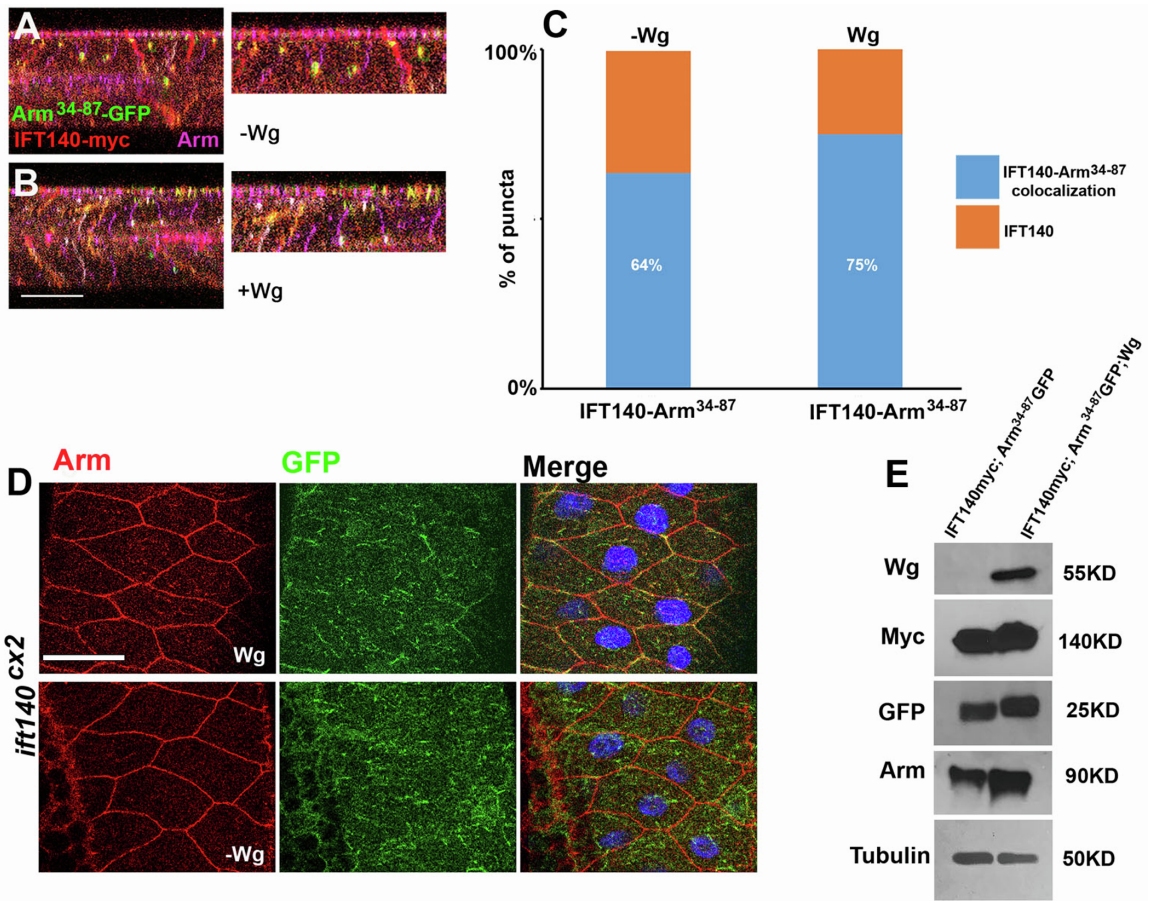

Figure S3 (Suppl. to Figure 2): Co-localization of IFT140, Arm, and Arm$^{34-87}$ in *Drosophila* salivary gland cells.

Salivary glands (SGs) were stained for Arm$^{34-87}$-GFP (green), IFT140-myc (red), and endogenous Arm (magenta).

(**A-B**) Z-sections of SG tissue. Note punctate staining displaying overlap within the cytoplasmic sub-apical region of SG cells. (**A**) Without expression of Wg, endogenous Arm/β-catenin mainly localizes to the junctional regions of the membrane. Note punctate staining of Arm$^{34-87}$-GFP overlapping with IFT140-myc. (**B**) Upon Wg expression, Arm/β-catenin is stabilized and triple positive puncta, staining for Arm$^{34-87}$-GFP, IFT140-myc and endogenous Arm, are detected in the cytoplasm. (**C**) Quantification of the number of punctae containing IFT140 alone (orange) or Arm$^{34-87}$ with IFT140 (blue) (n>150 punctae per genotype from five different salivary glands). Note that Arm$^{34-87}$ and IFT140 cytoplasmic co-localization does not depend on Wg-signaling.

(**D**) Salivary glands mutant for *ift140*, in the *ift140^{cx2}* allele, were stained with Arm (red), Arm$^{34-87}$-GFP (green), and Hoechst (blue). Note that co-localization of full-length Arm and Arm$^{34-87}$-GFP, as induced by Wg expression (seen above in panel **B**, and main Figure 2A-B), is lost in the *ift140^{cx2}* background. This suggests that wild-type IFT-A complexes can form multimers of unknown stoichiometry. Note also that full-length Arm is not detected in the cytoplasm upon Wg-induction in the absence of IFT-A. Scale bar represents 50μm.

(**E**) Western blot showing levels of Wg, IFT140 myc, Arm^{34-87} and Arm expression under the control of the salivary gland specific *C805-Gal4* driver. Expression level of these proteins were checked using anti-Wg, anti-Myc, anti-GFP and anti-Arm antibody. γ-Tubulin was used as a loading control.

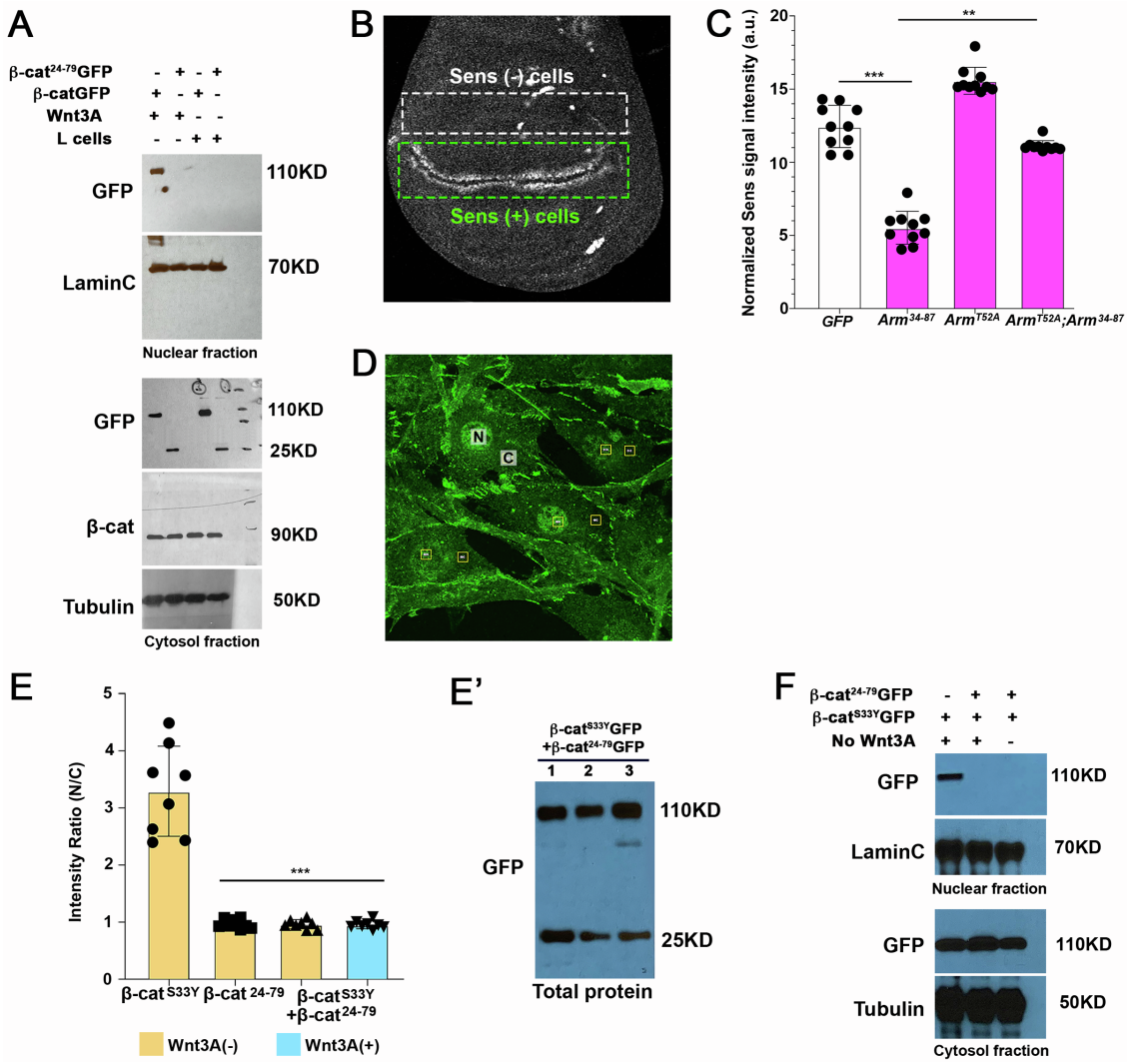

Figure S4 (supplement to Figure 3 and 4). Intensity/levels of nuclear β-catenin accumulation in MEFs and quantification of Sens intensity in immunostaining of wing imaginal discs.

(**A**) β-cat$^{24-79}$-GFP peptide blocks nuclear translocation of endogenous β-catenin. Full gel Western blots (including marker lane) of panels shown in main Figure 3I.

(**B**) Example of quantification of Sens signal intensity in a *C96>GFP* wing disc context. To establish an appropriate quantification of the signal, we normalized Sens signal intensity by subtracting the signal in the Sens negative (-) cells from the signal obtained in Sens positive region, "(+) cells".

(**C**) Quantification of the Sens signal at the D/V boundary of the *C96>Arm$^{34-87}$* wing imaginal discs with *C96>Arm^{T52A}* or *C96>Arm$^{34-87}$; >Arm^{T52A}* wing imaginal discs (n=10 discs). Student's *t*-test was applied for statistical analysis (** p<0.01; *** p<0.001)

(**D**) Example of quantification β-catenin accumulation in the nucleus in MEFs, in the context of defining the intensity ratio of nuclear and cytoplasmic β-catenin. Note indicated squares over nuclear and cytoplasmic regions. One cell example has the respective regions marked as N for nuclear and C for cytoplasmic (see panel **E** for actual values of an experiment).

(**E**) Quantification of nuclear β-cat^{S33Y} (stable point mutant) or the β-cat$^{24-79}$ fragment and β-cat^{S33Y}; β-cat$^{24-79}$ immunofluorescence signal in MEFs is shown as ratio of nuclear (N) vs cytoplasmic (C) signal. Y-axis denominates the ratio of intensity values of selected regions (area of 4.705µm x 4.843µm) within nuclei and cytoplasm of individual cells, see sample rectangles in panel **D** (membrane-associated β-catenin was purposely excluded). Mean ± s.d. of values, obtained in randomly selected cells, averages from 8 to 10 independent experiments (*n*) are shown; Student's *t*-test: *** $p < 0.001$, as comparted to the control βcat^{S33Y} transfection experimental set. (**E'**) Western blot of three independent experiments with co-transfected β-cat^{S33Y}-GFP and the β-cat$^{24-79}$GFP fragment (lanes 1-3) stained for the GFP tag (shown as total protein extracts), documenting comparable transfection efficiency for both transgenes.

(**F**) Western blots of nuclear and total cytosol fraction of β-cat^{S33Y}-GFP or β-cat^{S33Y}-GFP with the β-cat$^{24-79}$-GFP peptide with and without treatment of Wnt3A. γ-tubulin and LaminC were used as loading controls for cytoplasm and nuclei, respectively. Note nuclear accumulation of "active", stable form of β-catenin/β-cat^{S33Y}-GFP (without Wnt treatment) and lack of its nuclear localization in the presence of the β-cat$^{24-79}$-GFP peptide.

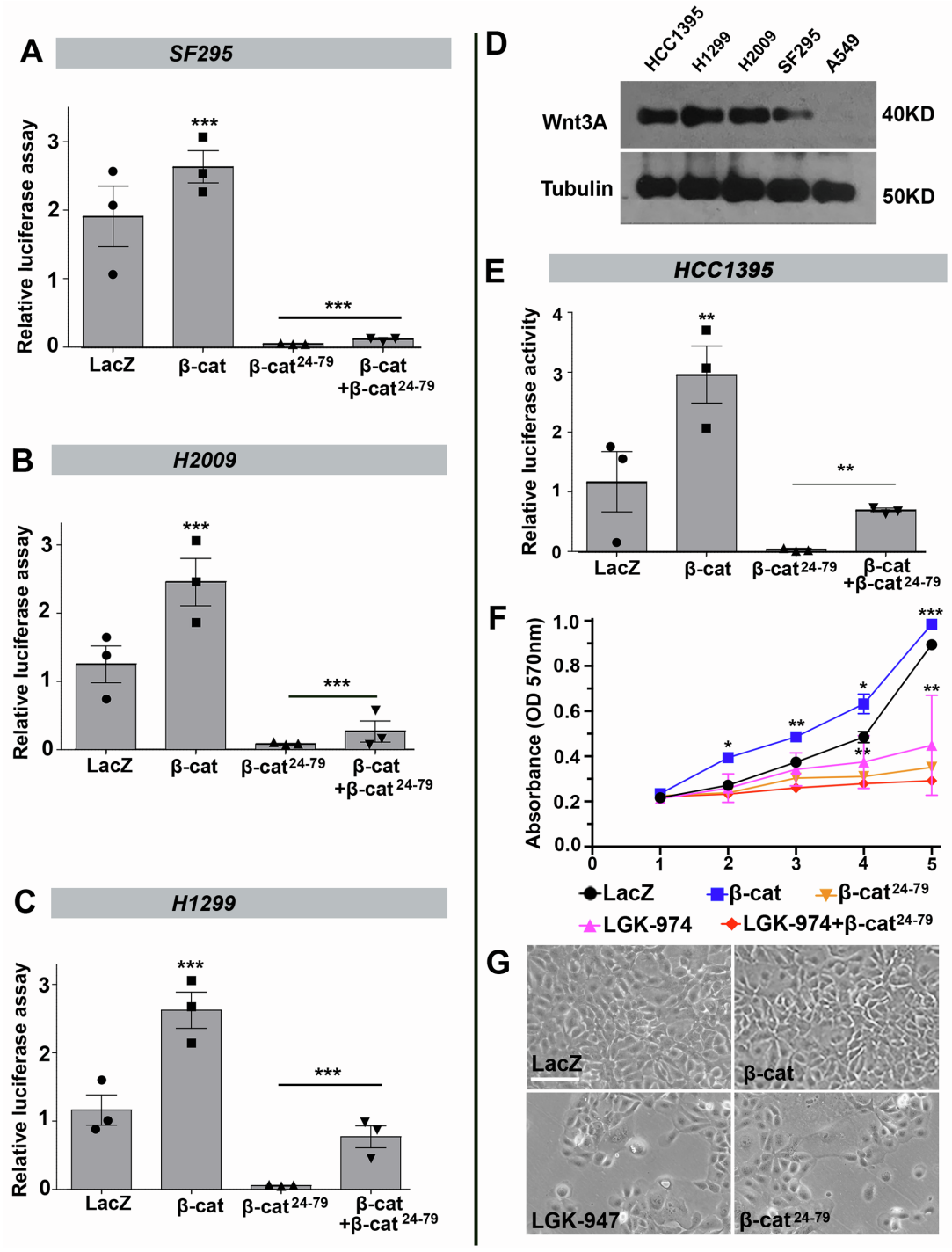

Figure S5 (Suppl. to Figure 4). The *β*-catenin$^{34-87}$ peptide strongly inhibits Wnt signaling in human cancer cells.

(**A-C**) Assay for Wnt-signaling activity in SF295 brain tumor cell line (**A**) and the two lung cancer lines H2009, and H1299 (**B and C,** respectively) transfected with either control LacZ, β-catenin, β-cat$^{24-79}$ peptide, or β-catenin plus β-cat$^{24-79}$ peptide, respectively. The relative luciferase activity indicates the ratio of TOP-Flash Wnt-reporter firefly luciferase and renilla luciferase activities. Overexpression of β-catenin increases the relative luciferase activity, whereas transfection with the

β-cat$^{24-79}$peptide, or β-catenin plus the β-cat$^{24-79}$peptide causes a marked reduction of Wnt-signaling activity in all tumor cells (***p <0.001, three independent assays, student's t-test).

(**D**) Detection of endogenous Wnt3A expression in cancer cell lines (HCC1395, H1299, H2009, and SF295, with A549 line as a negative control). Note high levels of "endogenous" (autocrine) Wnt3a in HCC1395, H2009 and H1299.

(**E-G**) β-cat$^{24-79}$ blocks Wnt-signaling and reduces proliferation in HCC1395 cancer cells. (**E**) Relative luciferase assay as described in panel A-C. Note that co-transfection of *wt* β-catenin increases the relative luciferase activity (compared to LacZ control) and that transfection of the β-cat$^{24-79}$peptide markedly reduces reporter activity (** p <0.001, three independent assays). (**F-G**) Proliferation of HCC1395 cells is suppressed by the β-cat$^{24-79}$ peptide. Cells were transfected with LacZ (control), β-catenin, β-cat$^{24-79}$ or treated with LGK-974, or LGK-974 together with β-cat$^{24-79}$. LGK-974 blocks Wnt secretion via its effects on Porcupine (which is required for Wnt protein secretion). Proliferation was assessed via the cell proliferation assay (see Methods). Note markedly reduced proliferation in β-cat$^{24-79}$ transfected cells and β-cat$^{24-79}$ transfected cells together with LGK-974 (quantif. in **F**; *p <0.01; **p <0.001; ***p <0.0001, three independent assays) and also visible as cell density in growing cells (**G)**, with photographs taken at 72hrs after transfection. Scale bar represents 500μm.