

Figure S1: PTK7 inhibits excess Wnt-PCP activity to rescue activin-induced CE in AC explants.

A. Embryos at the one-cell stage were injected with RNA encoding the constitutively-activated Alk4 receptor (250pg), Wnt11 (400pg) PTK7 (1ng) or the LRP6 (2ng) proteins. Embryos were also co-injected with the *PTK7-MO* (15ng). Blastula-stage ACs were grown to neurula st. 17 for CE cell movement scoring. These results are described graphically in (C). We compared the effects of either wild-type LRP6 or PTK7 proteins co-injected with the activated-activin receptor (Alk4). Uninjected control AC explants are all round; they do not undergo CE elongation (n=17). In Alk4 injected ACs, only 10% do not elongate and 40% undergo extremely strong elongations (n=34). In Alk4/Wnt11 co-injected ACs (n=34), 40% of the ACs do not elongate (four-fold increase vs Alk4 alone) and the percent of strongly elongating ACs was reduced to less than 15% (three-fold decrease vs Alk4 alone). LRP6 co-injection reversed this effect (n=33), less than 10% of the Alk4/Wnt11 ACs did not elongate and over 30% elongated strongly. A nearly identical effect was seen for PTK7 co-injection (n=29); about 15% of the Alk4/Wnt11 ACs did not elongate and over 30% strongly elongated. Thus, in this assay, ectopic PTK7 and LRP6 proteins acted identically to inhibit excessive Wnt-PCP activity. Co-expression of Alk/Wnt11 ACs with the *PTK7-MO* (n=28) gave an additive effect, further inhibiting Wnt-PCP activity, since none of the explants in this group underwent strong elongation. Co-expression of Wnt11 and the *LRP6-MO* also gave a similar effect (Tahinci et al., 2007).

B. Graphic description of the statistical distribution of CE in the different injected AC groups. Explant length/width ratio was determined by Image Pro Plus 5, and explants were divided into three groups, strong-long elongations (blue), intermediate-medium elongations (red) and round-non-elongated (yellow).

Figure S2: PTK7 and LRP6 proteins inhibit Wnt-PCP dependent jun-phosphorylation in cultured cells.

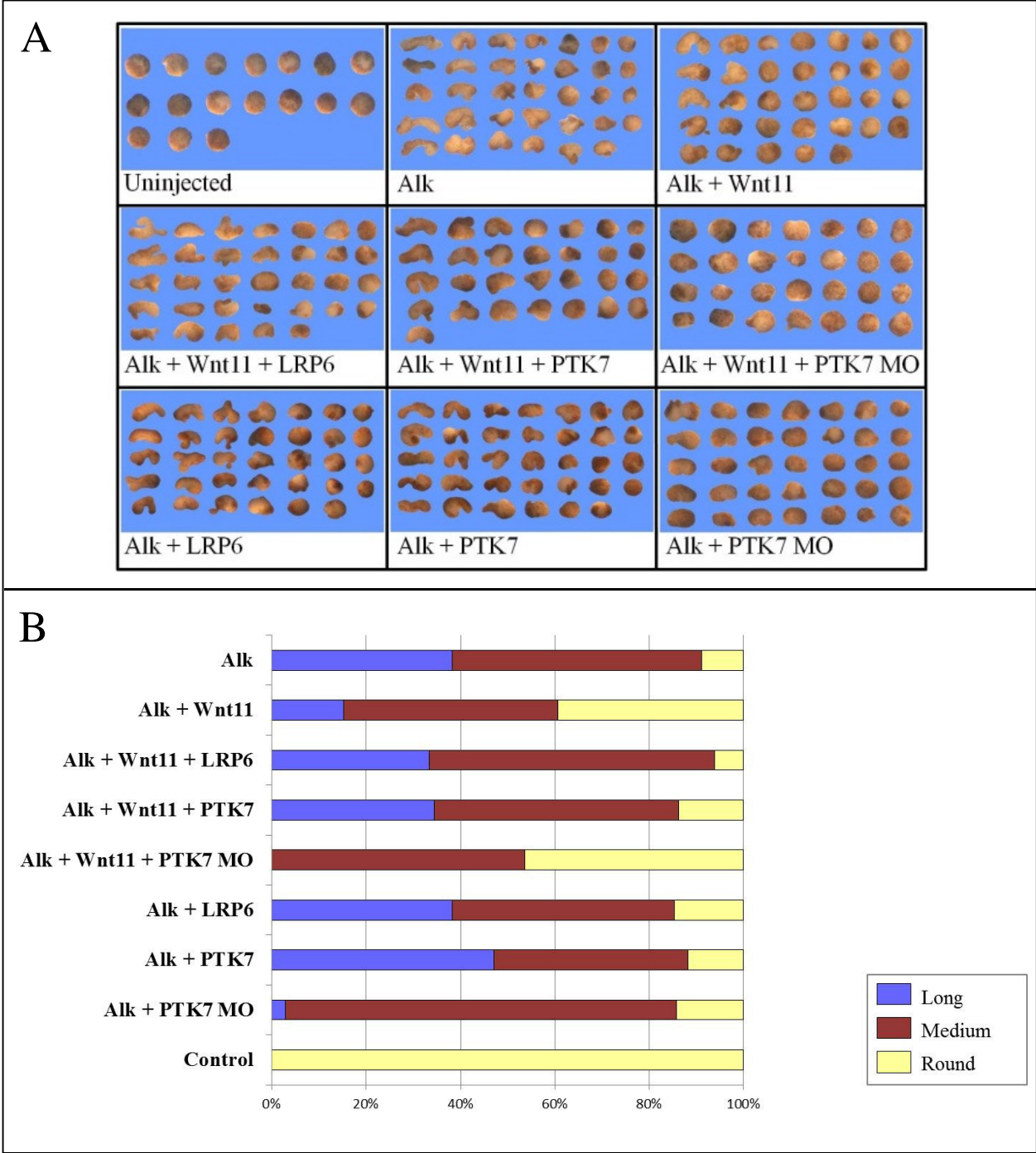
A. Phosphorylated-jun induced by UV irradiation are down regulated by Wnt-PCP inhibitory proteins. HEK293 cells were transfected with the *dnWnt11* (0.75µg) or *dnNXfz7-fun* (0.75µg) expression plasmids. 48h after transfection, cells were irradiated by UV and harvested after 30 min. Total cell lysates (100 µg/each) were analyzed by electrophoresis and immunoblotting with a p-jun specific antibody.

B. Phosphorylated-jun induced by UV irradiation is down regulated by dnWnt11, constitutively active LRP6 and PTK7 proteins. HEK293 cells were transfected with the dnWnt11 expression (1µg), LRPΔE1-4-myc (1µg) or the PTK7-HA expression plasmids (1µg) as indicated. 48h after transfection, cells were irradiated by UV and harvested after 30 min. Lysates were analysed as described in (A).

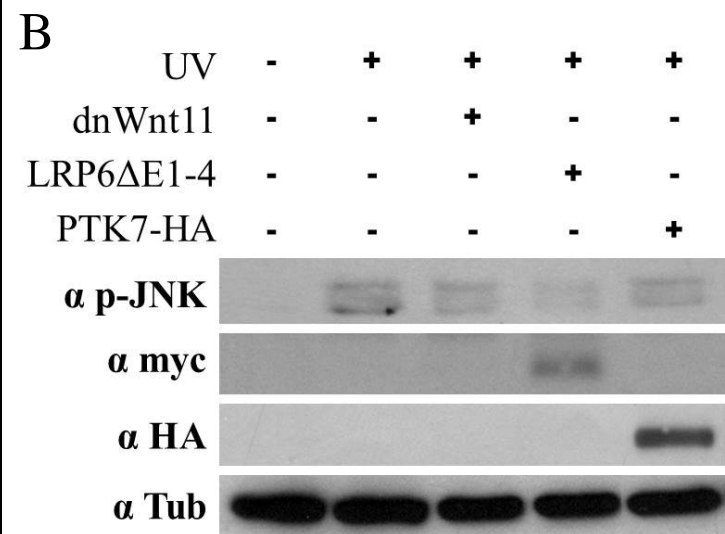
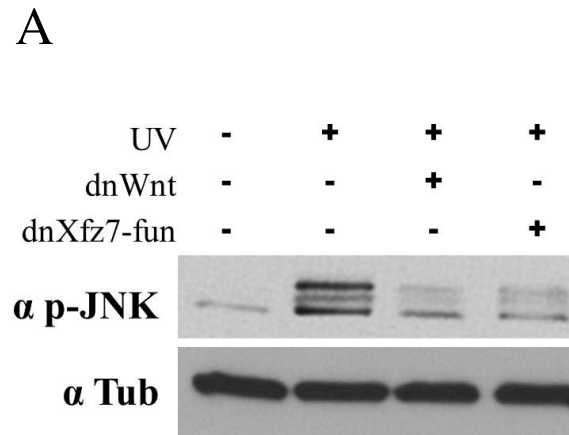
Figure S3: Wild type and truncated-activated LRP6 proteins physically interact

HEK293 cells were transfected with the *VSVG-LRP6*, *LRP6ΔE1-4-myc* or *Xiro3-myc* expression plasmids. Whole cell extracts were prepared 48h after transfection for co-IP with myc antibody and recombinant protein G Agarose beads (Invitrogen). The co-IP proteins were immunoblotted and detected by VSVG or myc antibodies. Cells transfected with Xiro3-myc were used for a tag control. The right panel shows the IP proteins and the left panel shows the total protein expression in the cells (WB input).

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3

