

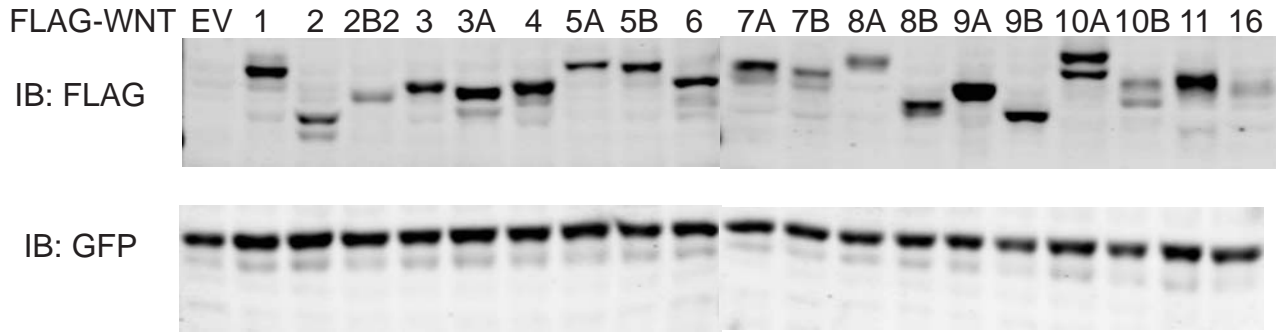
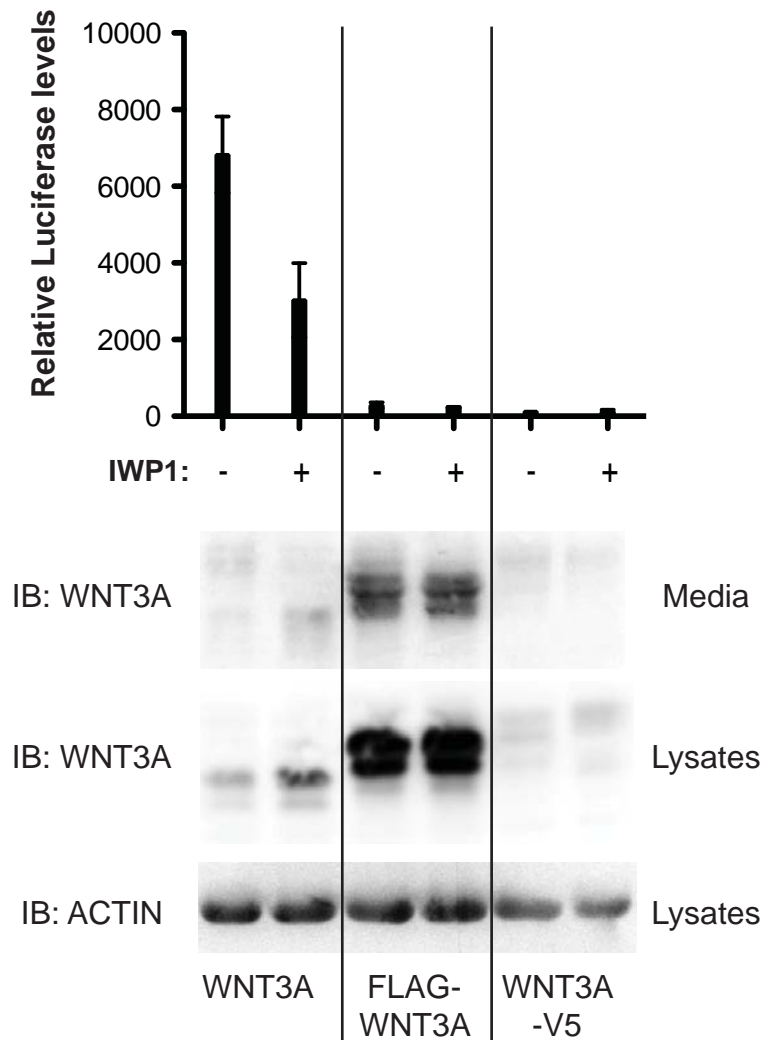
A**Hela cells****B****WNT Signaling in STF HEK293**

Fig. S1. Analysis of FLAG-tagged Wnts. **(A)** N-terminal FLAG-tagged Wnts are well expressed. HeLa cells were transfected with 10 ng of the indicated FLAG-tagged Wnt plasmid in a 12-well dish. Lysates were analyzed by SDS-PAGE and immunoblotting with anti-FLAG antibody. **(B)** FLAG-tagged WNT3A is much less active than untagged Wnts and its robust secretion is insensitive to IWP. HEK293 cells with an integrated Super8XTopFlash reporter (STF cells (Coombs et al., 2010)) were transfected with 100 ng of the various WNT3A plasmids as indicated and treated with either DMSO or IWP1 (2 μ M) overnight. Lysates and conditioned media were analyzed by SDS-PAGE and immunoblotted with an anti-WNT3A monoclonal antibody. Based on protein abundance and TOPflash activity, the specific activity of the FLAG-tagged WNT3A is approximately 2500-fold lower than that of the untagged WNT3A.

WNT mRNA levels in HEK293 cells

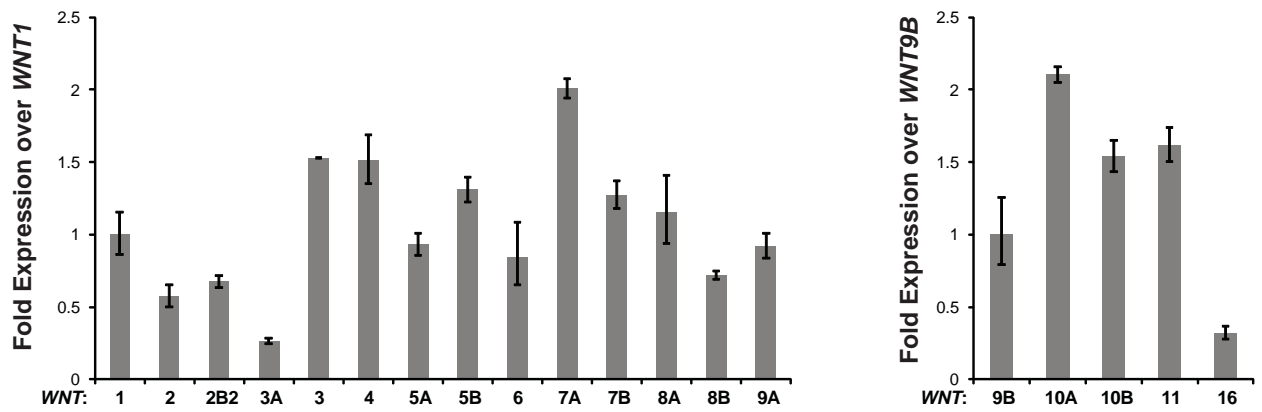


Fig. S2. *WNT* mRNA levels in HEK293 cells. 293 cells were transfected with plasmids expressing V5-tagged Wnts and expression was monitored by qRT-PCR of isolated mRNA. All Wnts produce relatively similar amounts of mRNA transcripts with little variation. The experiment was performed in two different sets: The first set displays fold expression over *WNT1* mRNA, while the second set displays fold expression over *WNT9B* mRNA. mRNA levels were normalized to GAPDH.