Supplemental methods

Detection of β−galactosidase- TOPGAL mice harbor a DNA construct containing artificial LEF/TCF binding sites linked to β-galactosidase coding sequence and are widely used to monitor Wnt/β-catenin signaling activity. To measure modulation of Wnt/β-catenin signaling activity, cells isolated from the TOPGAL mice with treated with ethanol (EtOH), 300 nM RA or 100 nM retinoid antagonist (ANT) in presence and absence of Wnt3a for 24 hours. Cultures were washed with ice cold PBS and incubated with 1 µM fluorescein digalactoside (FDG) (Molecular Probes, Eugene, OR) that is a fluorogenic substrate for β-galactosidase for 2.5 min at 37°C to detect β-galactosidase activity. Images were captured with a fluorescence microscope. Fluorescein positive cells represent cells in which Wnt/β-catenin signaling was activated.

Supplemental figure legend

Figure 1. RA enhances and retinoid antagonist inhibits Wnt/β-catenin signaling activity stimulated by Wnt3a. A-L, epiphyseal chondrocytes isolated from TOPGAL Wnt/β-catenin reporter mice were treated with ethanol (EtOH, A, D, G and J), 300 nM RA (B, E, H and K) or 100 nM retinoid antagonist (ANT) (C, F, I and L) in absence (A-F) or presence (G-L) of 100 ng/ml of rWnt3a for 24 hours. Cultures were incubated with fluorescein β−galactodidase substrate, and fluorescence (A-C, G-I) and phase contrast (D-F, J-L) images were captured.