Expanded View Figures

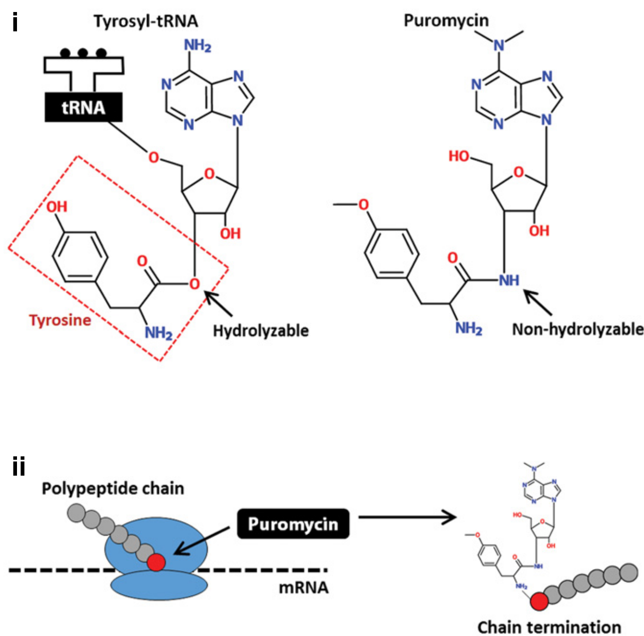

Figure EV1. Depiction of the SUnSET technique.
(i) Puromycin is structurally similar to tyrosyl-tRNA, but contains a non-hydrolyzable amide bond where tyrosine is normally cleaved from the tRNA complex and incorporated into a polypeptide chain. (ii) Puromycin is added to a growing polypeptide chain and terminates elongation of the peptide chain synthesis.

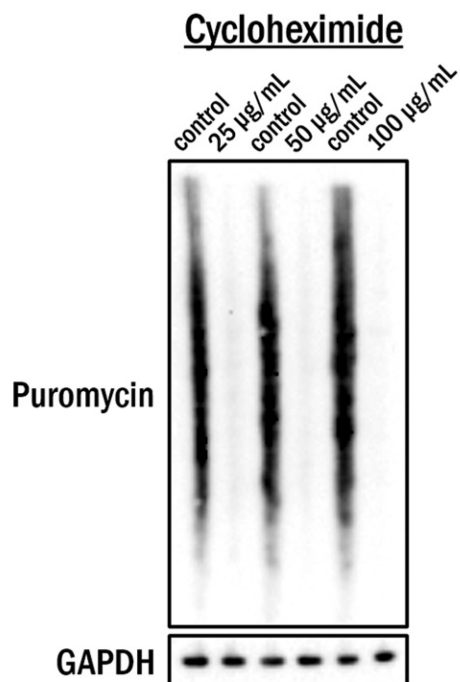

Figure EV2. Inhibition of translation by cycloheximide assessed by SUnSET assay.
HEK 293T cells were treated with indicated concentrations of cycloheximide or solvent (water) for 4 h prior to incubation with 0.5 μM puromycin for 15 min. Cell lysates were subjected to Western blot analysis for detection of incorporated puromycin. GAPDH was used as a loading control.

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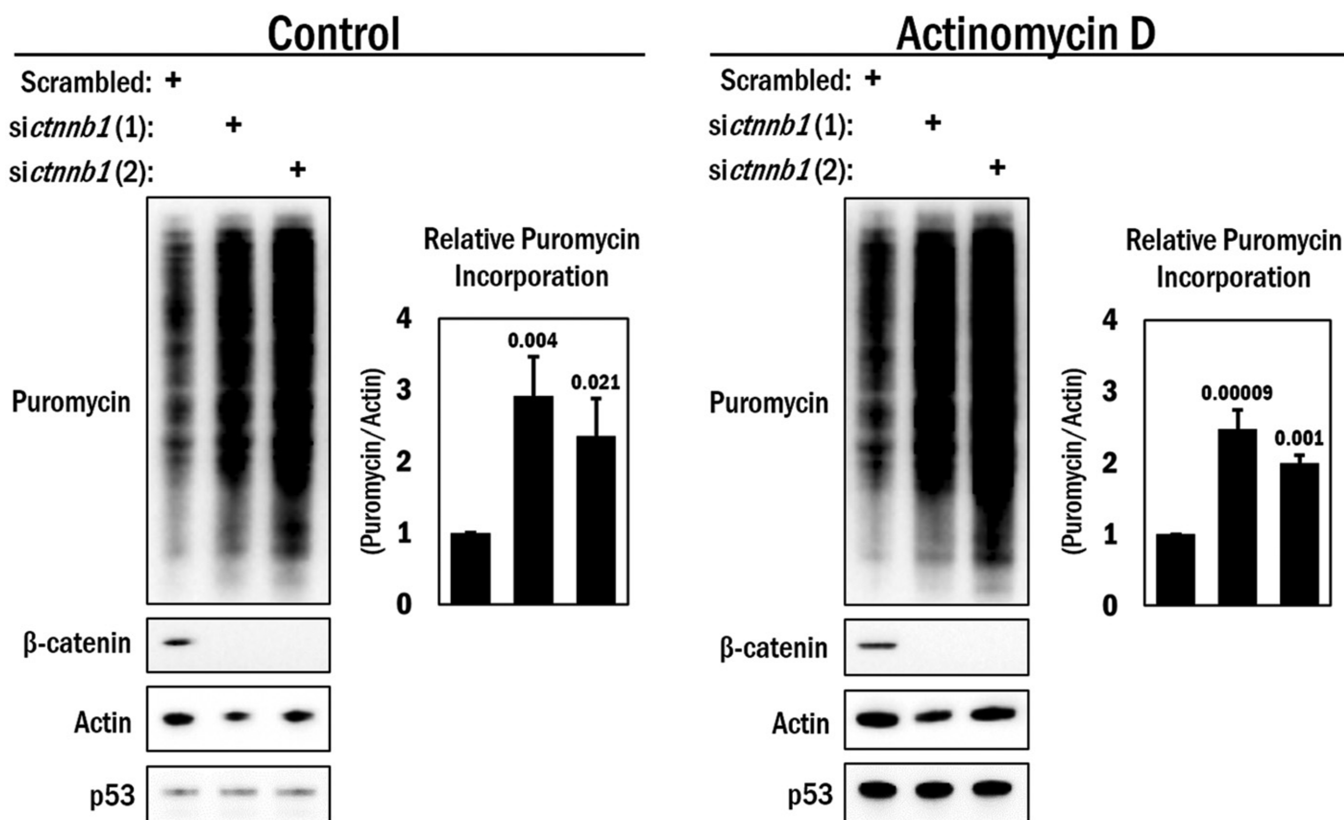

Figure EV3. De-repression of β-catenin-mediated translation repression is independent of transcription.

A10 cells were transfected with siRNAs targeting *ctnnb1* (β-catenin) or scrambled RNA (control) and 36 h later treated with either actinomycin D (0.5 μg/ml) or DMSO for 4 h. Before harvesting, the cells were pulsed with 0.5 μM puromycin for 15 min and subjected to Western blot analysis for detection of the puromycin incorporated peptides. β-Catenin blots indicated efficacy of siRNA. Actin was used as a loading control, and p53 was used as a control for actinomycin D activity. The average puromycin/actin ratio is shown in the graphs to the right ($n = 3$). A one-way ANOVA and Tukey post hoc test were performed, with P-values indicated above the error bars.

Source data are available online for this figure.

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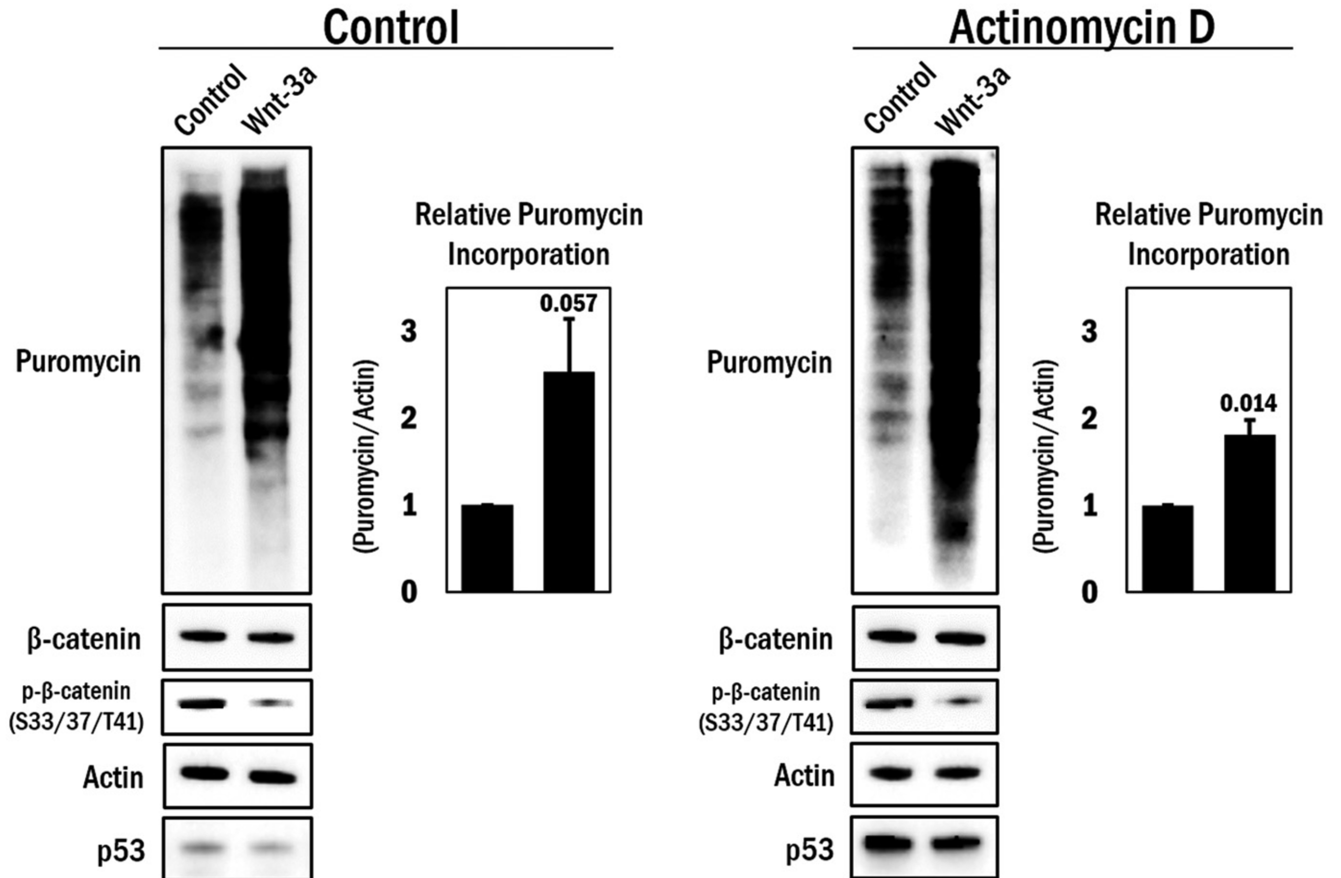

Figure EV4. De-repression of the β-catenin-mediated translation repression by Wnt stimulation is independent of transcription.

A10 cells were serum deprived overnight prior to 1 h pre-treatment with either actinomycin D (0.5 μg/ml) or its solvent (DMSO), and the cells were stimulated with Wnt-3a (100 ng/ml) or its solvent (0.1% BSA in PBS) for 4 h. Before harvesting for Western blot analysis, the cells were further treated with 0.5 μM puromycin for 15 min. Total β-catenin and phospho-β-catenin (S33/37/T41) levels indicate effective Wnt-3a stimulation. P53 was used as a control for actinomycin D activity, and actin was used as a loading control. The average puromycin/actin ratio was graphed ($n = 3$). Error bars indicate standard deviation. An independent samples t-test was performed (two-tailed), with the P-value indicated above the error bar.

Source data are available online for this figure.

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