Supporting Information

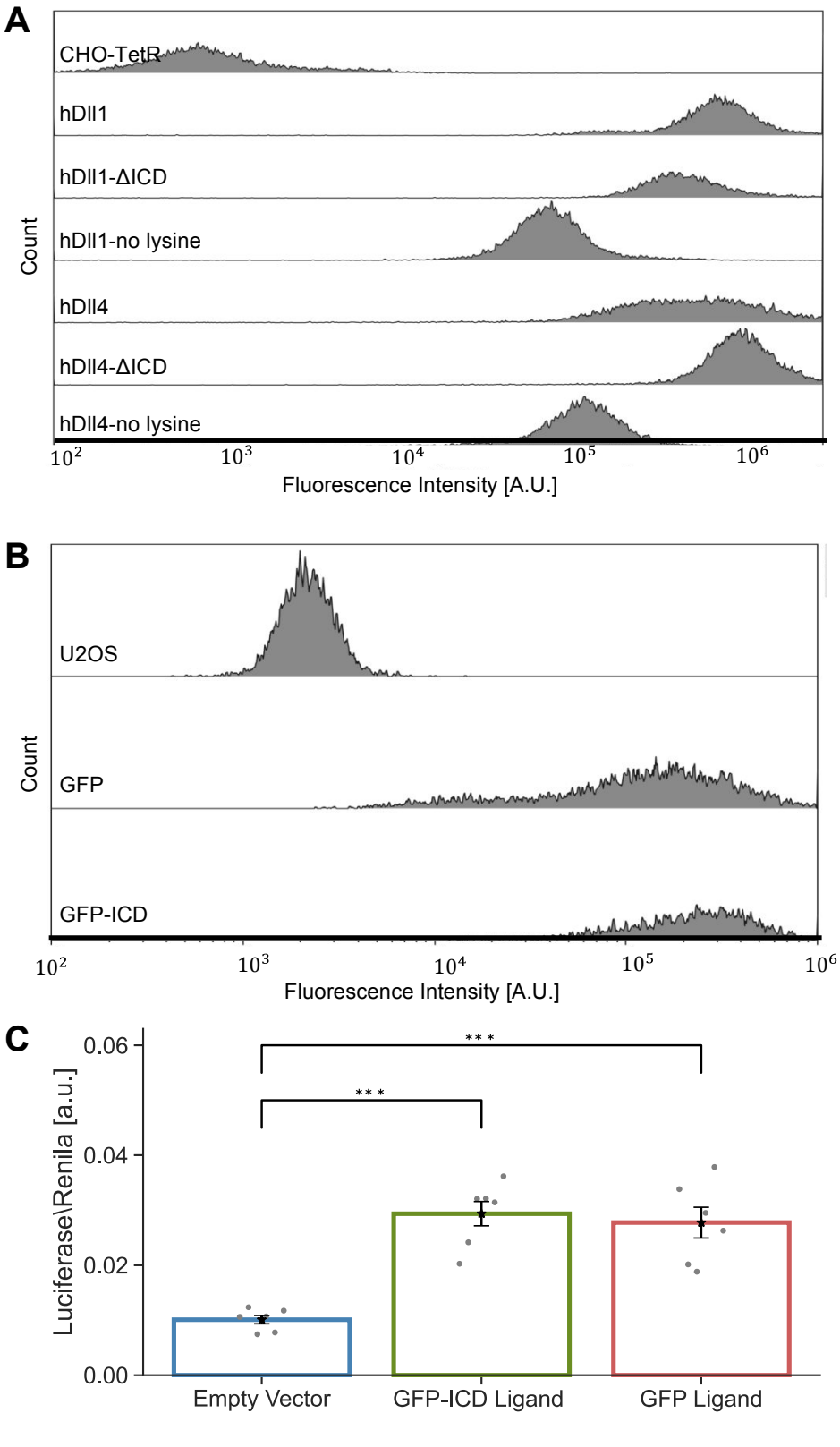

Figure S1. FACS analysis of fluorescently tagged ligands. (A) Comparison of the fluorescence of CHO-TetR cells expressing different ligand variants tagged with mCherry (as indicated for each row). Control cells (CHO-TetR) that do not express fluorescent protein are shown in the top row. **(B)** Comparison of the fluorescence of U2OS cells expressing either the GFP or GFP-ICD ligand of the aa-synNotch system (as indicated). Control cells (U2OS) that do not express fluorescent protein are shown in the top row. **(C)** Luciferase activity assay with U2OS cells expressing αGFP-mCherry receptors co-cultured with U2OS cells expressing either GFP or GFP-ICD ligands Data points show mean values from n=6 from 3 independent experiments. Error bars represent S.E.M. ***p<0.001.

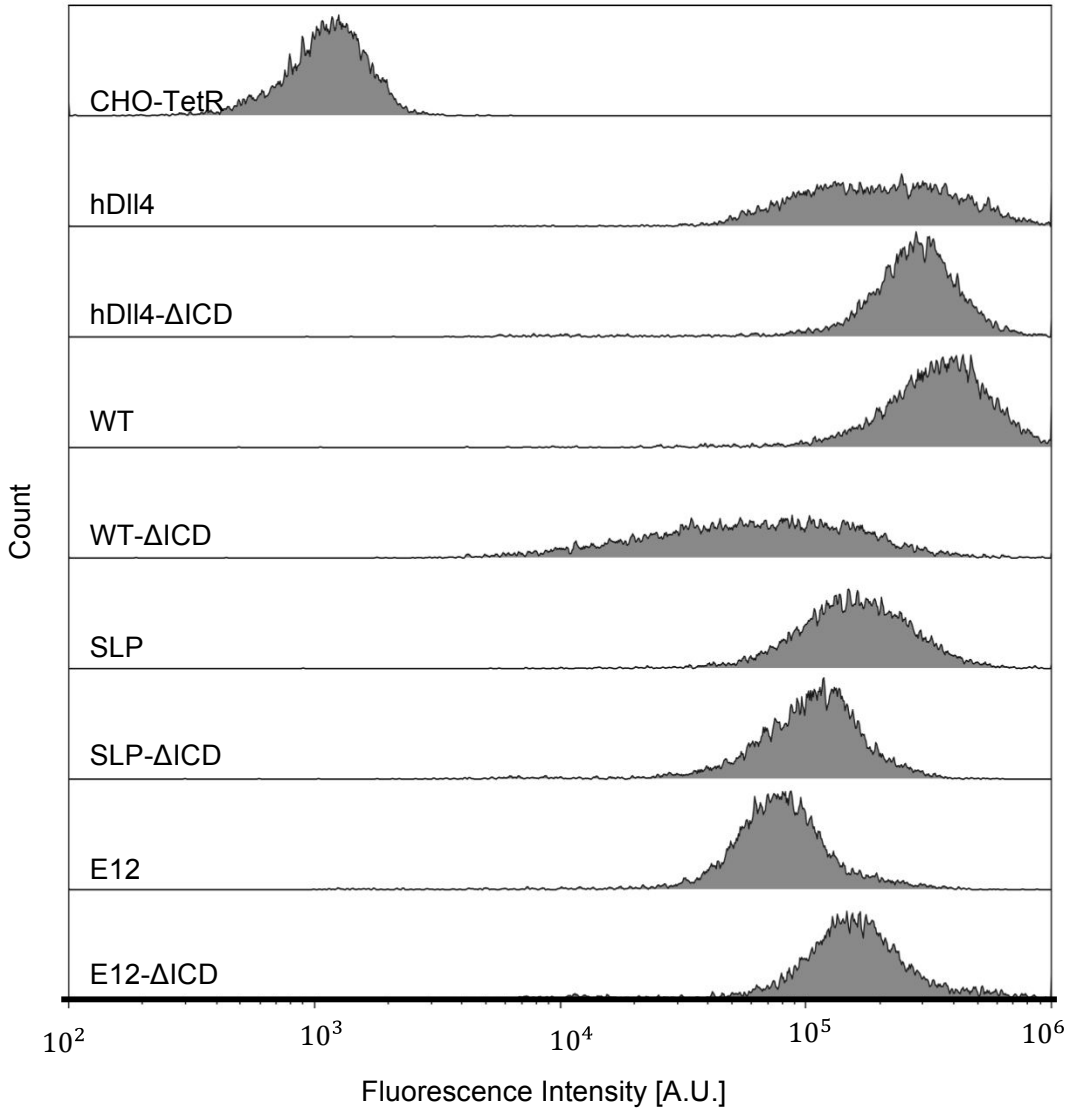

Figure S2. FACS analysis of fluorescently tagged ligands. Comparison of the fluorescence of CHO-TetR cells expressing different ligand variants tagged with mCherry (as indicated for each row). Control cells (CHO-TetR) that do not express fluorescent protein are shown in the top row.

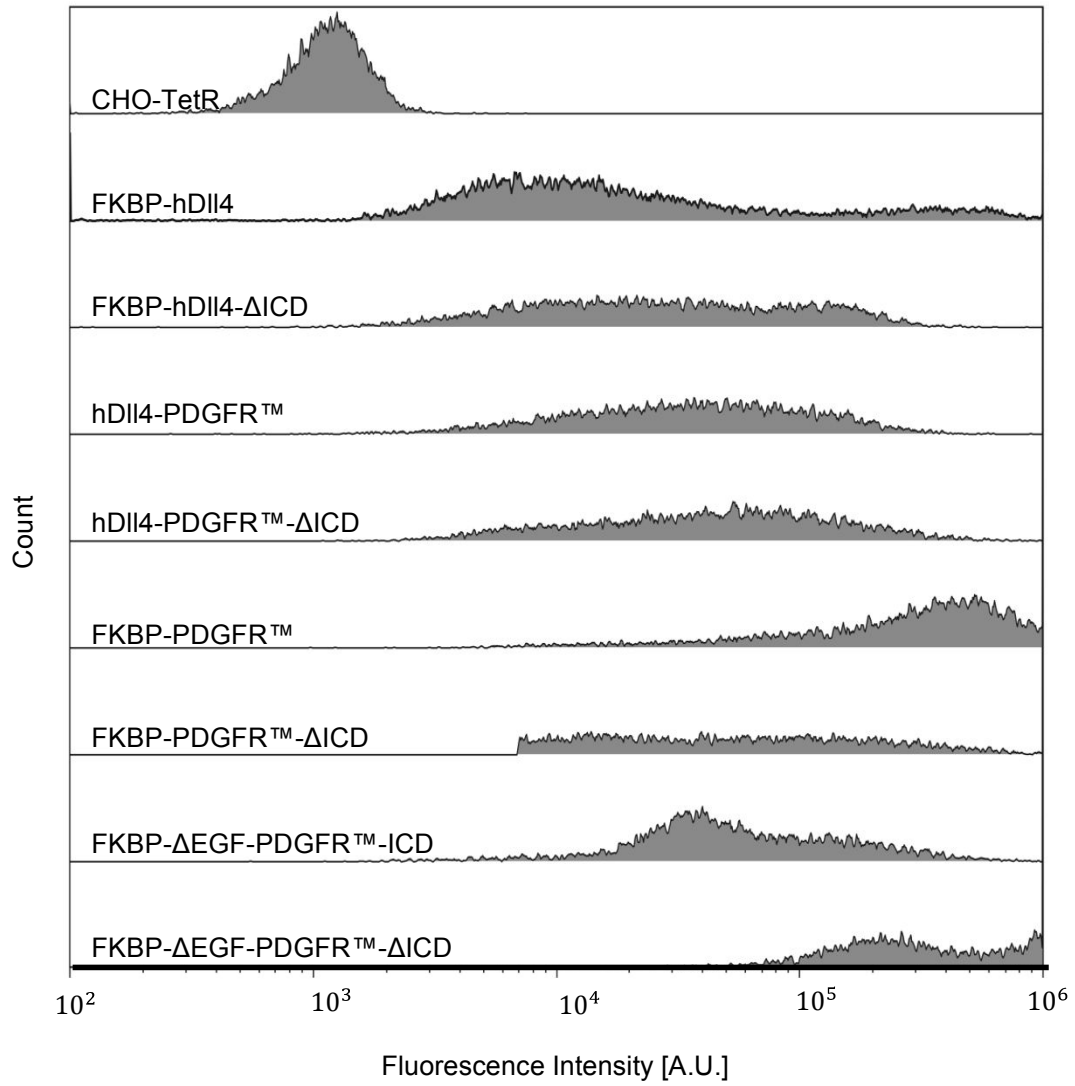

Figure S3. FACS analysis of fluorescently tagged ligands. Comparison of the fluorescence of CHO-TetR cells expressing different ligand variants tagged with mCherry (as indicated for each row). Control cells (CHO-TetR) that do not express fluorescent protein are shown in the top row.

Supplementary Methods

Flow Cytometry

CHO and U2OS cells were seeded in 6-well plates at approximately 70% confluence 24 hours before FACS. Cells were treated with 100ng/ml doxycycline right after seeding. Directly prior to FACS, cells were trypsinized, spun at 1000 rpm for 5 min, and resuspended in 200 μL of FACS buffer. FACS buffer consisted of PBS with 1% FBS serum and 5mM EDTA. Flow cytometry was performed using a Cytoflex5L flow cytometer (Beckman Coulter). Kaluza software was used to analyze the data.