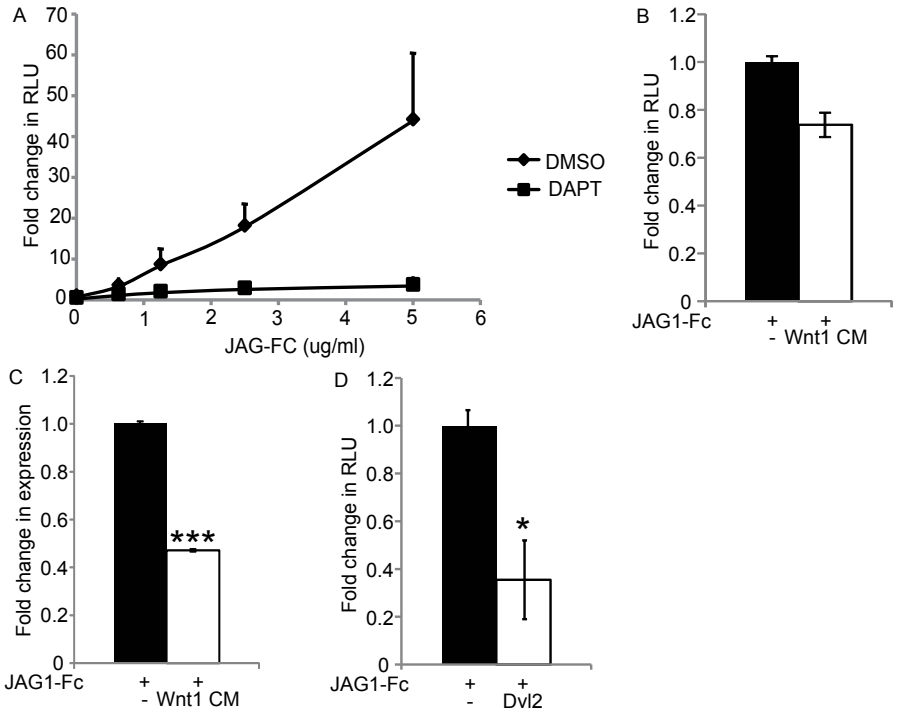

Fig. S1. Dishevelled inhibits Notch signalling. (**A**) SHEP-RBPJκ-Luc cells were cultured on plastic treated with different concentrations of JAG1-Fc ligand for 24 hours before analysing luciferase activity. DAPT (4 μM) was added as indicated to inhibit γ-secretase function. Data are presented as mean fold change (±s.e.m.) in relative luciferase units (RLU) compared with control cells cultured on plastic treated with 0 μg/ml Jag1-Fc ligand. Immobilised JAG1-Fc induced luciferase expression in a dose-dependent manner that was abolished by DAPT treatment. (**B**,**C**) SHEP-RBPJκ-Luc cells were cultured for 24 hours on plastic treated with 2 μg/ml JAG1-Fc and treated with conditioned medium from control or Wnt1-expressing SHEP cells for the last 12 hours before lysis. (B) Data are presented as mean fold change (±s.e.m.) in relative luciferase units (RLU) compared with control cells. Jag1-Fc-induced luciferase expression was lower after treatment with Wnt1-conditioned medium (two-tailed t-test). (C) qRT-PCR analysis of Notch target, *HES1*, in SHEP-RBPJκ-Luc cells. Expression was normalised to *HPRT1*. Data are presented as mean fold change (±s.e.m.) in normalised expression values relative to control cells. *HES1* expression was significantly reduced in cells treated with Wnt1-conditioned medium (two-tailed t-test). (**D**) Control or Dvl2-expressing SHEP-RBPJκ-Luc cells were cultured on plastic treated with the soluble ligand JAG1-Fc. Data are presented as mean fold change (±s.e.m.) in ligand-induced reporter activity [measured in relative luciferase units (RLU)] compared with control cells. Notch signalling was significantly lower in Dvl2-expressing cells than controls (two-tailed t-test). $^{\dagger}P$=0.052, *P<0.05, ***P<0.001.

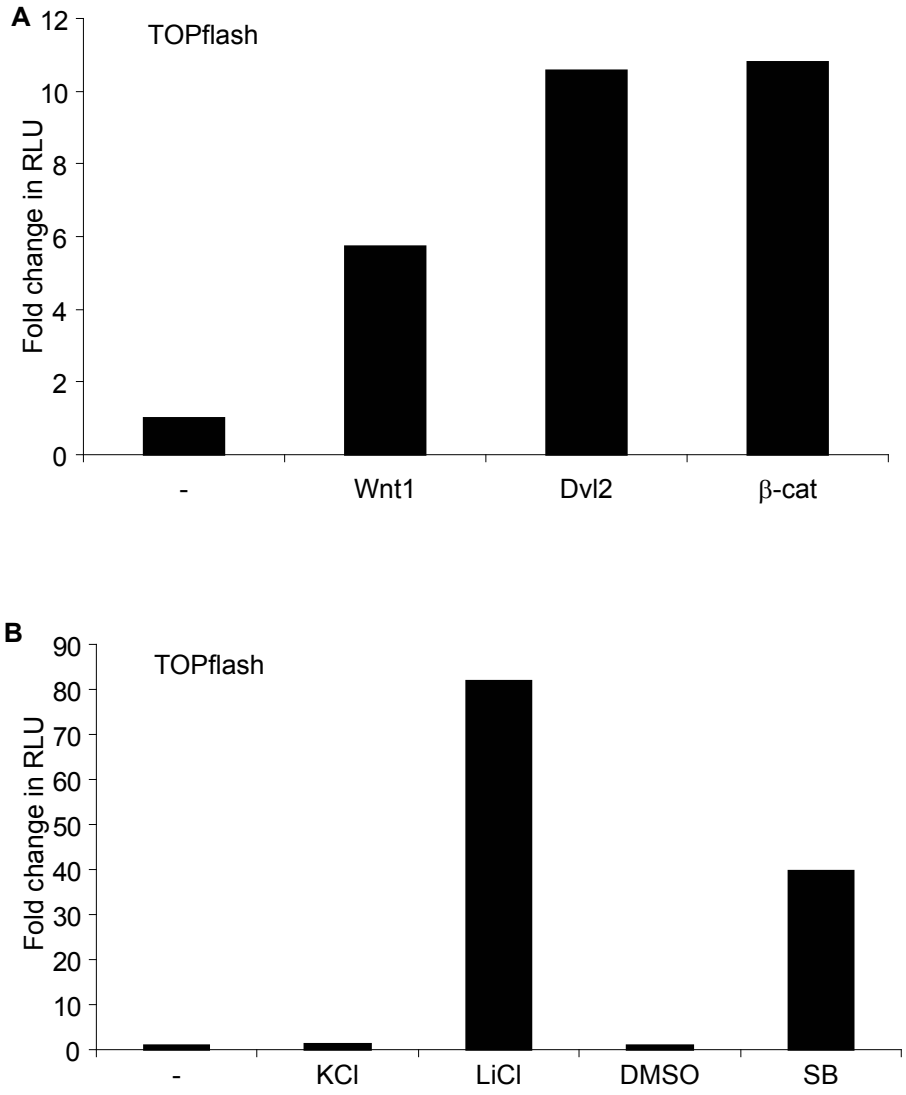

Fig. S2. Activation of Wnt signalling by Wnt pathway components and GSK3β inhibitors. HEK 293T cells were transfected with the Wnt reporter construct (TOPflash) and a *Renilla* luciferase control construct (pRL-CMV). Wnt signalling was activated by co-transfection of vectors encoding Wnt pathway components or drug treatments, as indicated. Data are presented as mean fold increase in relative luciferase units (RLU), relative to the corresponding negative control. (**A**) Expression of Wnt pathway components activates signalling. Fold increase in RLU in response to expression of mWnt1, mDvl2, S45F mβ-catenin (β-cat) or empty vector control. (**B**) GSK3β inhibitors activate Wnt signalling. Cells were treated overnight with 20 mM LiCl or 10 μM SB216763 (SB) to inhibit GSK3β, and the respective controls of 20 mM KCl or DMSO are shown. These are the same conditions used to active Wnt signalling as in Fig. 1.

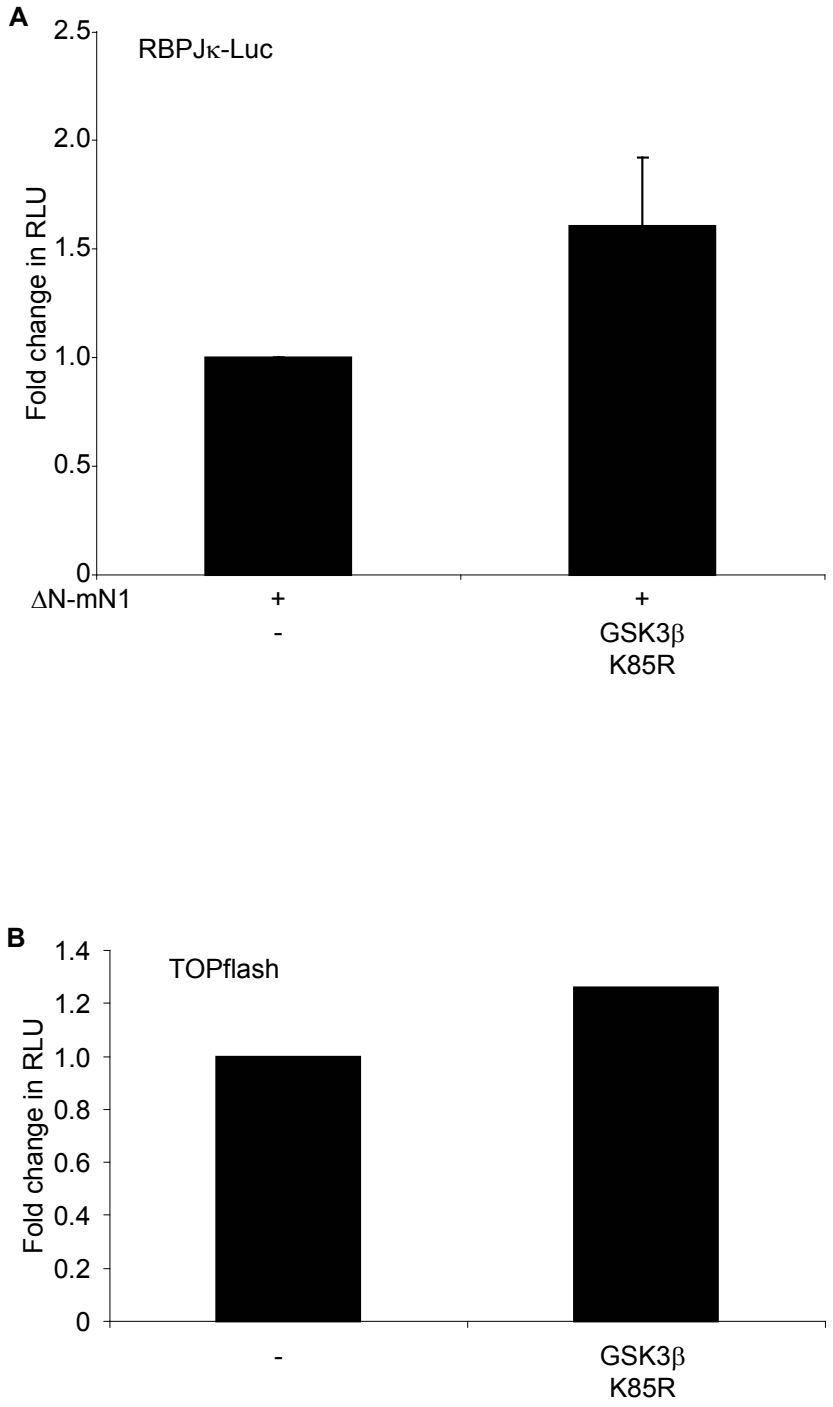

Fig. S3. GSK3β K85R does not inhibit Notch signalling. (**A**) GSK3β K85R expression does not inhibit Notch signalling. CHO-K1 cells were transfected with the RBPJκ-reporter construct (RBPJκ-Luc) and a *Renilla* luciferase control construct (pRL-CMV). Notch and Wnt signalling were activated by expression of ΔN-mN1 and GSK3β K85R. Data are presented as mean fold change (±s.e.m.) in relative luciferase units (RLU) compared with ΔN-mN1 alone. GSK3β K85R did not inhibit ΔN-mN1 activity ($P>0.05$). (**B**) Activation of Wnt signalling by GSK3β K85R. Cells were transfected with the Wnt reporter construct (TOPflash) and pRL-CMV. Wnt signalling was activated by expression of GSK3β K85R. Data are presented as mean fold increase in relative luciferase units (RLU), relative to the negative control.

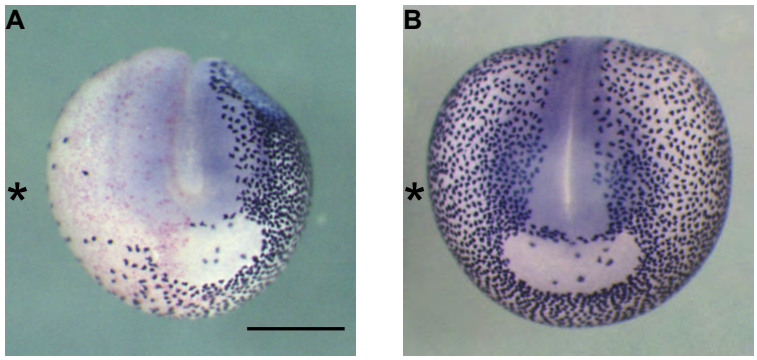

Fig. S4. Dishevelled rescues the XNICD phenotype in vivo. (**A**,**B**) Anterior views of the same *Xenopus* embryos that are shown in Fig. 2F,H. The injected side is marked with an asterisk. Scale bar: 500 μm.

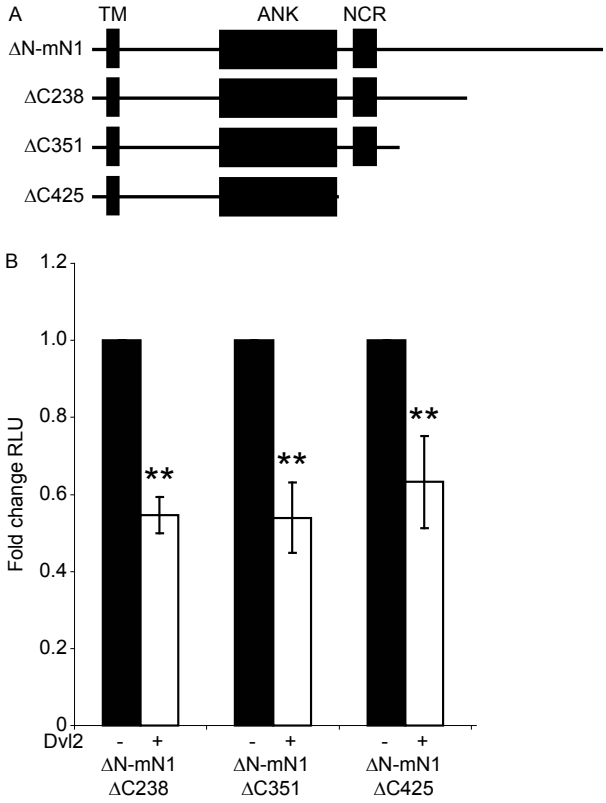

Fig. S5. Dishevelled inhibits ΔN-mN1 C-terminal deletion mutants. (**A**) Schematic of the Notch constructs used in the C-terminal deletion study. TM, transmembrane domain; ANK, Ankyrin repeats. (**B**) CHO-K1 cells were transfected with the RBPJκ-reporter construct (RBPJκ-Luc) and a *Renilla* luciferase control construct (pRL-CMV). Notch and Wnt signalling were activated by expression of mDvl2 with ΔN-mN1 or one of the ΔN-mN1 deletion constructs: ΔC238, ΔC351 and ΔC425. Data are presented as mean fold change (±s.e.m.) in relative luciferase units (RLU), relative to each construct expressed alone. Dishevelled significantly inhibited each Notch construct (**P<0.01, one-way ANOVA and Tukey's post-hoc test).

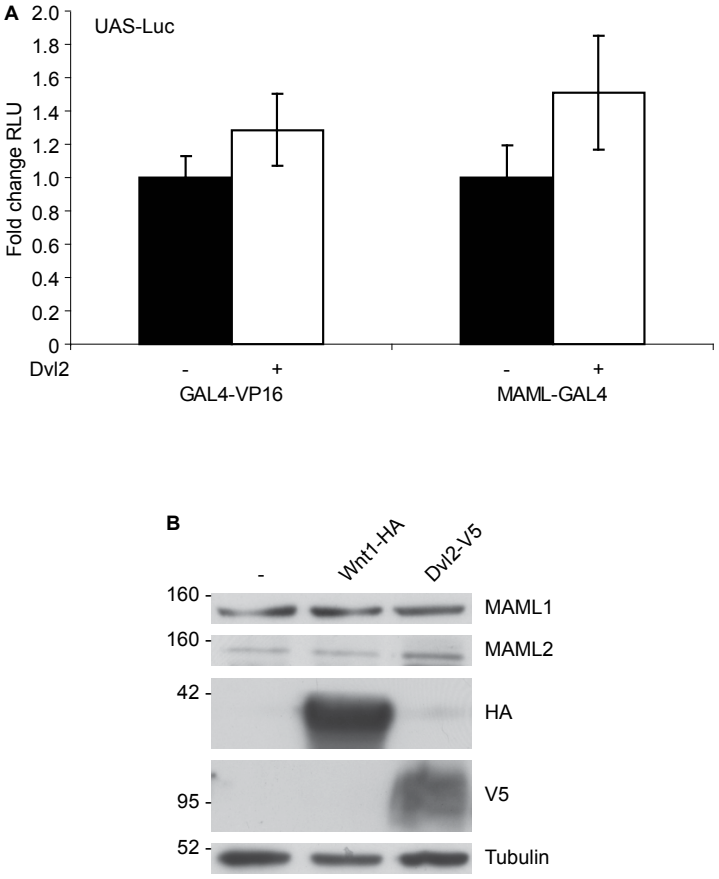

Fig. S6. Dishevelled does not inhibit MAML. (**A**) CHO-K1 cells were transfected with the GAL4-reporter construct (UAS-Luc) and a *Renilla* luciferase control construct (pRL-CMV). The transcriptional reporter was activated by co-expression of either GAL4-VP16 or MAML-GAL4. Data are presented as mean fold change (±s.e.m.) in relative luciferase units (RLU), relative to each construct expressed alone. Dishevelled co-expression did not inhibit the activity of either construct ($P>0.05$). (**B**) Wnt signalling does not reduce the expression of MAML. Cells were transfected with expression constructs encoding either Wnt1-HA or Dvl2-V5. Lysates were analysed by immunoblotting to examine the expression of endogenous MAML1 and MAML2. HA, V5 and Tubulin are shown as controls. The position of molecular weight markers are shown (in kDa).

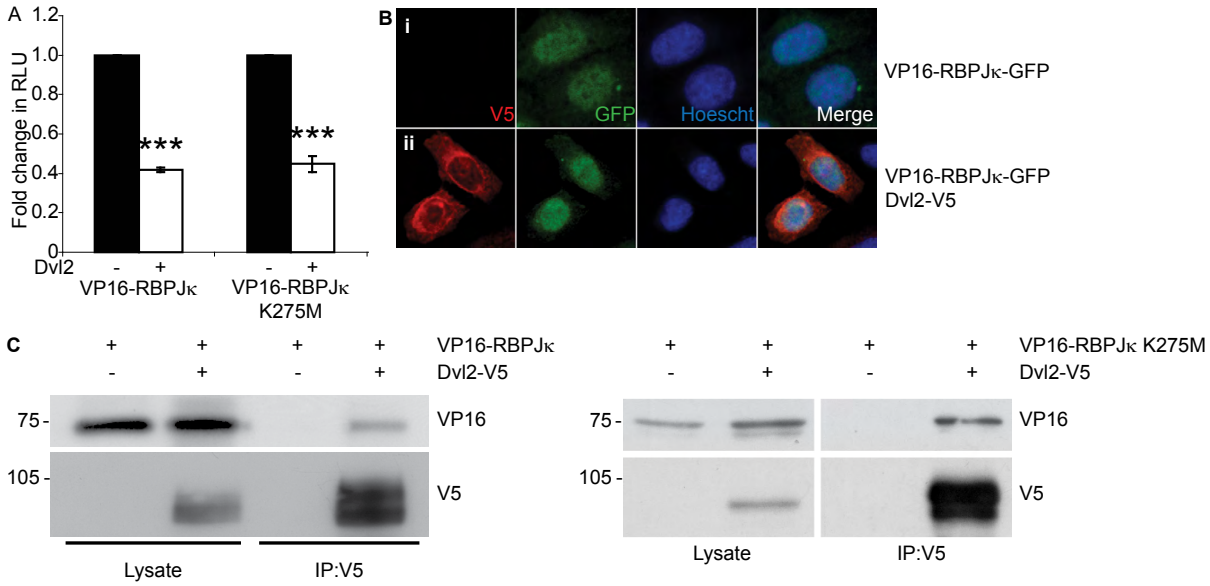

Fig. S7. Dishevelled inhibits CSL transcription factors. (**A**) Dishevelled inhibits a VP16-RBPJκ molecule that cannot bind NICD. CHO-K1 cells were transfected with RBPJκ-Luc and pRL-CMV. Notch and Wnt signalling were activated by co-transfection of vectors encoding VP16-RBPJκ or VP16-RBPJκ-K275M and mDvl2, as indicated. Data are presented as mean fold change (±s.e.m.) in relative luciferase units (RLU), relative to each RBPJκ molecule alone. mDvl2 inhibited both VP16-RBPJκ constructs (***P<0.001, one-way ANOVA and Tukey's post-hoc test). (**B**) Dishevelled does not alter the nuclear localisation of RBPJκ. Cells expressing VP16-RBPJκ-GFP and mDvl2-V5, as indicated, were fixed and immunostained for GFP (green) and V5 (red) epitopes. (**C**,**D**) Dishevelled binds RBPJκ and a form of RBPJκ that cannot bind NICD. CHO-K1 cells expressing mDvl2-V5 and VP16-RBPJκ (C) or VP16-RBPJκ-K275M (D) were subjected to immunoprecipitation (IP) using V5 antibody. IP samples were analysed by western blotting with V5 and VP16 antibodies, alongside total lysates. Positions of molecular weight markers (in kDa) are shown.

Table S1. Molecular cloning primers used

Primer name	Sequence
N1 5210F	TAGTAAGCTTGTGAAGAGTGAGCCGGTGGA
N1 5589R	TGCTGCTGAGTCCACTGTCT
N1 5970F	AGGGTGTCTTCCAGATCCTGCTCCG
N1 6400R	TACGCTCGAGGTTGTACTCATCCAAAAGCCGCACG
mβ-cat S45FF	ACCACCACAGCTCCTTTCCTGAGTGGCAAGGGC
mβ-cat S45FR	GCCGTTGCCACTCAGGAAAGGAGCTGTGGTGGT
mβ-cat 1914F	GAGATAGTAGAAGGGTGTACTGG
mβ-cat 2555R	CCTACTCGAGGTCAGTATCAAACCAGG
RBPJκ K275MF	CGGGCAGACTGTCATGCTTGTGTGCTCAGTG
RBPJκ K275MR	CACTGAGCACACAAGCATGACAGTCTGCCCG
hN4 4357F	TACCAAGCTTCCCCTGCTGCCTGGACCA
hN4 4563R	AGGCCGTCGAGTGAAACCA
RBPJκ *Bgl*IIF	CAGTAAGATCTATGCCCTCCGGTTTTCCT
RBPJκ *Bgl*IIR	CTGAAGATCTGGACACCACGGTTGCTGT
T7	TAATACGACTCACTATAGGG
BGHR	TAGAAGGCACAGTCGAGG
mDvl2 132R	GATCGGCGCCCGCCATGGTCTCGCT
mDvl2 1654F	GATCGCCGGCTGTGAGAGTTAC
mDvl2 793F	ACTGAGCAGAGCAGTGCCT
mDvl2 1668R	CACGCTCGAGGTAACTCTCACAGCCACCA
mMAML1 176F	GATCGGCCATGGAGGCCATCGCGGGCGCTGGAGGC
mMAML1 548R	AGTTGTGTGAAACAGAGT

Table S2. qRT-PCR primers used

Gene	Forward primer sequence	Reverse primer sequence
HES1	AAAGATAGCTCGCGGCATT	TGCTTCACTGTCATTTCCAGA
HEY1	TATCGGAGTTTGGGATTTCG	GCATCTAGTCCTTCAATGATG CT
HPRT1	TGACCTTGATTTATTTTGCATA CC	CGAGCAAGACGTTCAGTACT
a-tubulin	GCAGGAAAGCATGTCCCCA	TGGCAGCATCTTCCTTTCCA
esr1	CAGCACCAGCTCACAGTGCA	GCGCATCTTTTCCACAATGG
rpl8	TACGCCACCGTTATCTCCCA	CGACCACCACCAGCAACAAC

Table S3. Primary antibodies used

Antigen	Source
β-gal	Promega, Madison, USA
Renilla luciferase	Millipore, Billerica, USA
Cleaved-Notch1 (NICD Val 1744)	Rockland Immunochemicals, Gilbertsville, USA
Dvl2	Cell Signaling Technology, Danvers, USA
GFP	Invitrogen, Carlsbad, USA
LaminB1	Abcam, Cambridge, UK
myc (4A6 clone)	Millipore
RBPJκ (clone T6709)	Institute of Immunology Co, Tokyo, Japan
VP16	Santa Cruz Biotechnology California, USA
V5	Invitrogen
Tubulin	Keith Gull, University of Manchester, UK