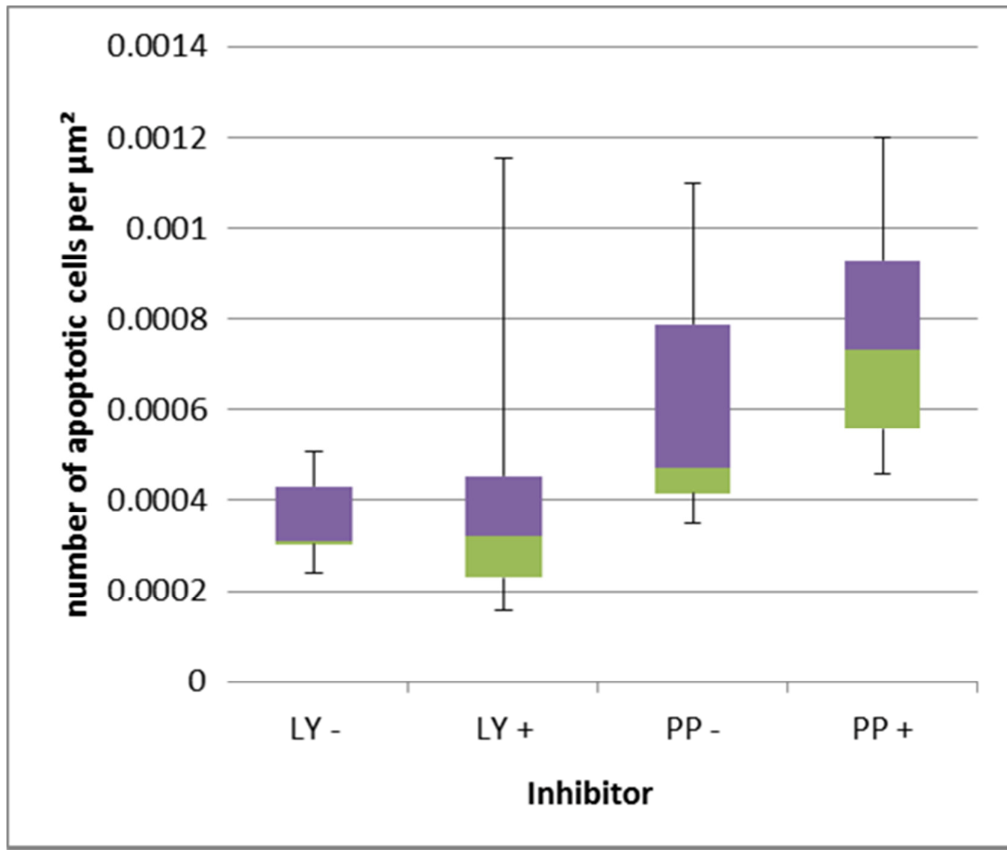


**Supplementary Figure 1. Small molecular inhibitors do not induce apoptosis in mouse PSM explants after culture (see also Figure 4).** Box plot showing spread of TUNEL staining measurements in mouse E10.5 PSM explants treated with LY411575 (LY) or Pyrvinium pamoate (PP) compared to untreated controls. "+" indicates treated half and "-" indicates control half (n=3 explants per drug, 9 technical replicates). p=0.289 for LY, p=0.159 for PP. The line in the box shows the median.

**Supplementary Table 1:** Primers used to generate anti-RNA intronic probes

| Gene | Forward primer | Reverse primer |
|---|---|---|
| ***mDll1(i)*** | 5'-GGTGCCTGACCTGCCTACAGAA-3' | 5'-GAGACTCCCCAGTATCACCAGGTGG-3' |
| ***mDll3(i)*** | 5'-GTGCCCCAGAGCCTTTACTACCC-3' | 5'-CGGTGGTCAGGGAAGCTCACTG-3' |
| ***mNotch1(i)*** | 5'-CACGCTTTGGGTAGATAGGAG-3' | 5'-GGAAGGTCAGAAGAGAGAGG-3' |
| ***cDll1(i)*** | 5'-CGAAGCCGCAGTCGTCCTCCT-3' | 5'-TTGGCTGTTTCGTAGCTC-3' |
| ***cNotch1(i)*** | 5'-ACGGCTTCTGCGCAAACCTG-3' | 5'-TGCATCCGATACCCAGGCTGC-3' |

**Supplementary Table 2:** Primers used for quantitative RT-PCR analysis

| Gene | Forward primer | Reverse primer |
|---|---|---|
| ***Dll1*** | 5'-TCAGATAACCCTGACGGAGGC-3' | 5'-AGGTAAGAGTTGCCGAGGTCC-3' |
| ***Notch1*** | 5'-GCCGCAAGAGG CTTGAGAT-3' | 5'-GGAGTCCTGGCATCGTTGG-3' |
| ***Hes7*** | 5'-GAAGCCGTTGGTGGAGAAG-3' | 5'-GGCTTCGCTCCCTCAAGTAG-3' |
| ***β-actin*** | 5'-GGCTGTATTCCCCTCCATCG-3' | 5'-CCAGTTGGTAACAATGCCATGT-3' |
| ***GAPDH*** | 5'-ATGAATACGGCTACAGCAACAGG-3' | 5'- CTCTTGCTCAGTGTCCTTGCTG-3' |

**Supplementary Table 3:** Details of antibodies used in immunohistochemistry

| Antibody | Species | Dilution used in immunochemistry | | Origin of antibody |
|---|---|---|---|---|
| | | Sections | Wholemount | |
| anti-Notch1 (mN1A) | mouse | 1:20 | 1:25 | BD Biosciences |
| anti-Dll1 (PGPM-1F9) | rat | 1:50 | 1:50 | Kind gift from E.Kremmer |
| anti-Cleaved Notch1 (NICD) (Val1744) (D3B8) | rabbit | 1:200 | | Cell Signalling Technologies |

**Supplementary Table 4:** ANOVA analysis of significant differences in variance

| | Df | Sum Sq. | Mean Sq. | F value | Pr(>F) | Significance |
|---|---|---|---|---|---|---|
| ***Gene*** | 3 | 5.257 | 1.7525 | 13793.64 | <2e-16 | *** |
| ***Sample*** | 15 | 0.323 | 0.0215 | 169.52 | <2e-16 | *** |
| ***Gene: Sample*** | 45 | 0.278 | 0.0062 | 48.59 | <2e-16 | *** |
| ***Gene:Sample:Fix.cult*** | 45 | 0.128 | 0.0028 | 22.38 | <2e-16 | *** |
| ***Sample:Fix.cult*** | 15 | 0.053 | 0.0036 | 27.98 | <2e-16 | *** |
| ***Gene:Fix.cult*** | 3 | 0.047 | 0.0157 | 123.5 | <2e-16 | *** |
| ***Fix.cult*** | 1 | 0.021 | 0.0207 | 162.83 | <2e-16 | *** |
| ***Residuals*** | 251 | 0.032 | 0.0001 | | | |

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

**Supplementary Table 5.** Fisher’s F-test for differences between sample variances in RT-qPCR fix and culture analysis

| | Normalised to GAPDH | | | | Normalised to β-actin | | | |
|---|---|---|---|---|---|---|---|---|
| | **Dll1** | **Notch1** | **Hes7** | **β-actin** | **Dll1** | **Notch1** | **Hes7** | **GADPH** |
| **Dll1** | - | 0.014 | 0.0001229 | <0.001 | - | 0.006904 | 0.0006761 | <0.001 |
| **Notch1** | - | - | 0.1075 | <0.001 | - | - | 0.4196 | <0.001 |
| **Hes7** | - | - | - | <0.001 | - | - | - | <0.001 |

Fisher’s F-test for differences between the total variance for each gene across all fix/culture caudal PSM explant pairs carried out in R and the outcomes are shown. The values in the table indicate the significance of the deviation of the relevant variance ration from unity. Values were normalised to either *mGAPDH* or *mβ-actin*

**Supplemental Experimental Procedures**

*Apoptosis Assay*

The ApopTag kit (Chemicon) was used to perform a version of the TUNEL assay on chick PSM explants with the following modifications. Samples were prepared for the assay by proteinase K treatment and fixation (4% formaldehyde in PBS, 2 mM EGTA, 0.1% glutaraldehyde) prior to washing in PBST. Equilibration buffer was then added to the explants and left for 15 min at room temperature. Incubation in working strength terminal transferase (TdT) was carried out overnight at 4 °C and the reaction stopped with stop/wash buffer by incubation at 37 °C for 40 min. Samples were then washed for a minimum of six times in TBST before heat inactivating the enzyme at 65 °C for 20 min. Samples were then incubated in blocking reagent (20% Blocking Reagent (Roche), 20% heat inactivated goat serum in TBST) for 2 h at room temperature before addition of 150mU/ml antidigoxigenin-AP antibody (Roche) and incubation overnight at 4 °C. Explants were then washed three times for 1 h in MABT (0.1 M maleic acid, 0.15 M NaCl, 10% Tween 20, pH 7.5) followed by two 10 minute washes in NTMT (100 mM NaCl, 100 mM Tris HCl pH 9.5, 50 mM MgCl2, 1% Tween 20). Samples were then incubated in NBT/BCIP colour reagent (0.027% 50 mg/ml NBT (Promega), 0.014% 50 mg/ml BCIP (Promega) in NTMT) for a minimum of 5 min at room temperature until sufficient signal had developed. Samples were then sectioned at 10µm, and imaged using a Leica DM5500B microscope using a Hamamatsu camera. Labelled cells in the PSM were counted manually using the 20x objective. The number of dots per section were counted and divided by the total area to produce a ratio of apoptosis per section. The results were then analysed by paired T-Test. Results were also shown graphically by means of a boxplot to show the spread of the data, with the box displaying the middle half of the data.