

## Supplementary Figure Legend

**Figure S1.** Multiple sequence alignment of Notch3 3'UTR in five species. Red color indicates the seed match region of miR-206 with Notch3 3'UTR.

**Figure S2.** HeLa cells were co-transfected with the luciferase reporters containing the mutated miR-206 binding site in the mNotch3 (A) or hNotch3 (B) 3'UTR, along with a miR-206 expression plasmid (50, 100, 200 ng). Luciferase activities were measured 24 hr post-transfection, which was normalized to the  $\beta$ -gal controls. A non-related miRNA cel-67 served as control.

**Figure S3.** (A) Western blot to determine the level of hNotch3 protein in HeLa cells transfected with miR-206 (4  $\mu$ g, 6-cm plate) or cel-64 (4  $\mu$ g, 6-cm plate). Two different experimental results are shown. The protein level of hNotch3 was consistently repressed by miR-206 in both experiments. In cel-64 transfected cells, the level of hNotch3 appeared to be decreased in exp.1 but increased in exp.2, which may be due to experimental variations. The result of exp.2 is presented in the main Fig. 2C. (B) Real-time PCR analysis of miR-206 expression in *SHP*<sup>+/+</sup> (wild-type) and *SHP*<sup>-/-</sup> (SHP knockout) mouse embryonic fibroblasts (MEFs).

**Table S1. Primer sequences used in this study.**

## Multiple sequence alignment of Notch3 3'UTR in five species

miR-206:3'.....GUAAGGU..-5'.

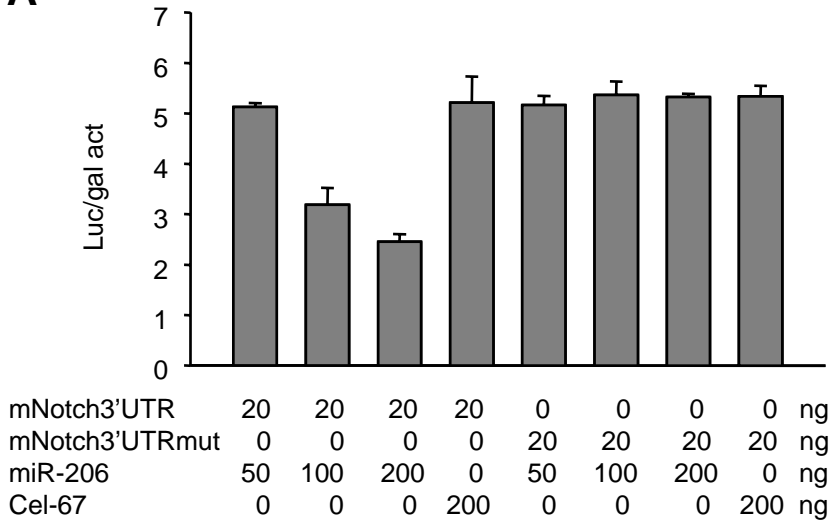
```
1 GGGAGTGTGGGAGCCTCCT-CCCCACCCCA----CCTCCCTCACTTCACTGCATTCCAAGATGGGACATGTTCCATAGCCTTGCTGGGGAAGGGCCCACTGCCAACTC
2 GGGAGTGTGGGAGCCTCCT-CCCCACCCCA----CCTCCCTCACTTCACTGCATTCCAAGATGGGACATGTTCCATAGCCTTGCTGGGGAAGGGCCCACTGCCAACTC
3 GGGACATTTGGGAGCCTCCTTCCCTCCCCATTCCCCTTCTCACTTCACTGCATTCCAGATAAGACGTGT-----AGACTCACTGGGAAAAGGGGTC-TTGTCTGCTC
4 GGGACACTTGGGAGCCTCCCCCGCCTCCCCATCCTCCTTCTCACTTCACTGCATTCCAGACAAGACTTGT-----AGACTCATTGGGAAAAGGGGTC-TTCTCTGCC
5 GTGAGACTTGGGAGCCTCTT-TCCTATCCCA----CCTCCCTCACTTCACTGCATTCCAGATGGAACCTTGTCTATAGCTTTGCTGGGGAAGGGCC-CTTTTGGCCC
```

1. Human
2. Pantroglodytes
3. Mouse
4. Rat
5. Canis familiaris

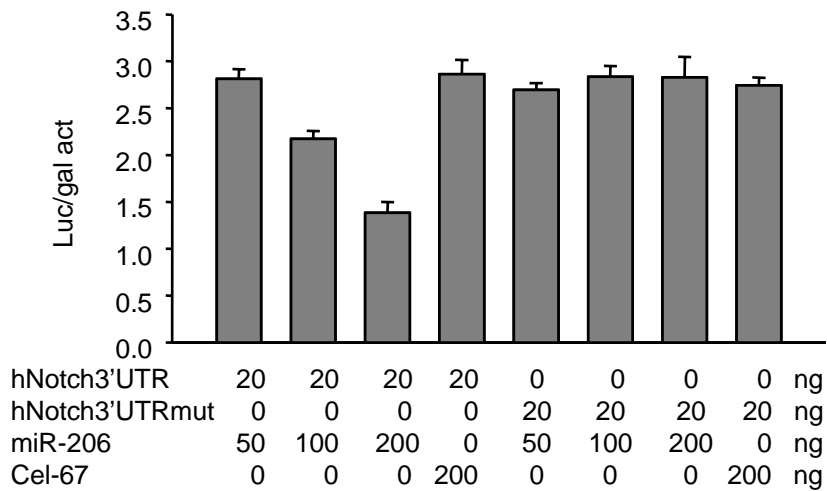
The result shows that the seed region of miR-206 is very conserved in the Notch3 3'UTR of the five species. Same results are predicted using miRanda, TargetScan and PicTar.

**A**

Mouse Notch3 3'UTR mutation

**B**

Human Notch3 3'UTR mutation



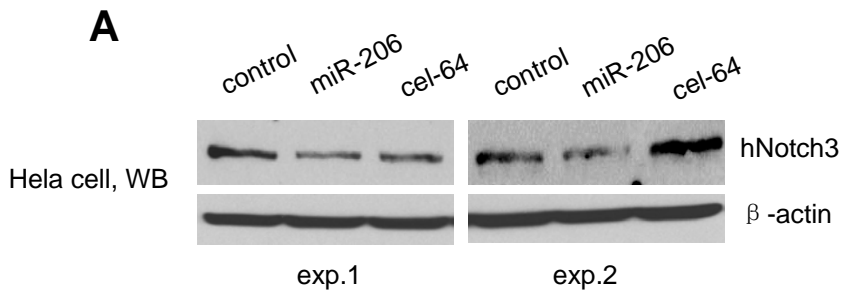
Mutation primers:

mouse: CCTTCCTCACTTCACTGC**ATTCCA**GATAAGACGTGTAGACTC

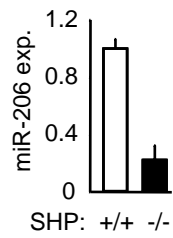
5'CCTTCCTCACTTCACTGC**AcTttA**GATAAGACGTGTAGACTC 3'  
 3'GGAAGGAGTGAAGTGACGTgAaaTCTATTCTGCACATCTGAG 5'

human: CCTCCCTCACTTCACTGC**ATTC**AGATGGGACATGTTCCATAG

5'CCTCCCTCACTTCACTGC**ATctgA**AGATGGGACATGTTCCATAG 3'  
 3'GGAGGGAGTGAAGTGACGTAgacTCTACCCTGTACAAGGTATC 5'



**B** miR-206 expression in MEFs (q-PCR)



## Primer sequences used for deletion constructs

### mNotch-3 UTR Luc constructs

Forward	5'-AGAGCTCCTAAGGGATCTGGGACCTCCTTTTGA
Reverse	5'-TCAAGCTTCAGCTACAAAGGACTGTGACCAGGTGC
mNotch-3'UTRMuR	5'-TCAAGCTTCTCCCAAATGTCCCCTGACCCCA

### hNotch-3 UTR Luc constructs

Forward	5'-GCCAAGCTGGATTCTGTGTACCTAGT
Reverse	5'-CAGCTCCTCTTCTTGTAGTCCCACA
hNotch-3'UTRMuF	5'-ATTCCAGATGGGACATGTTCC

### Notch3 N3IC construct

N3IC-F	5'- ACT CGA GAT GGT TGC CAG GCG AAA GCG AGA AC
N3IC-R	5' AAA GTC GAC TAT GCA TAG TCT GGT ACG TCA TAT GGA TAG GCC ATC ACC TGC CTC TTG GG