SUPPLEMENTARY INFORMATION

Supplemental Figures

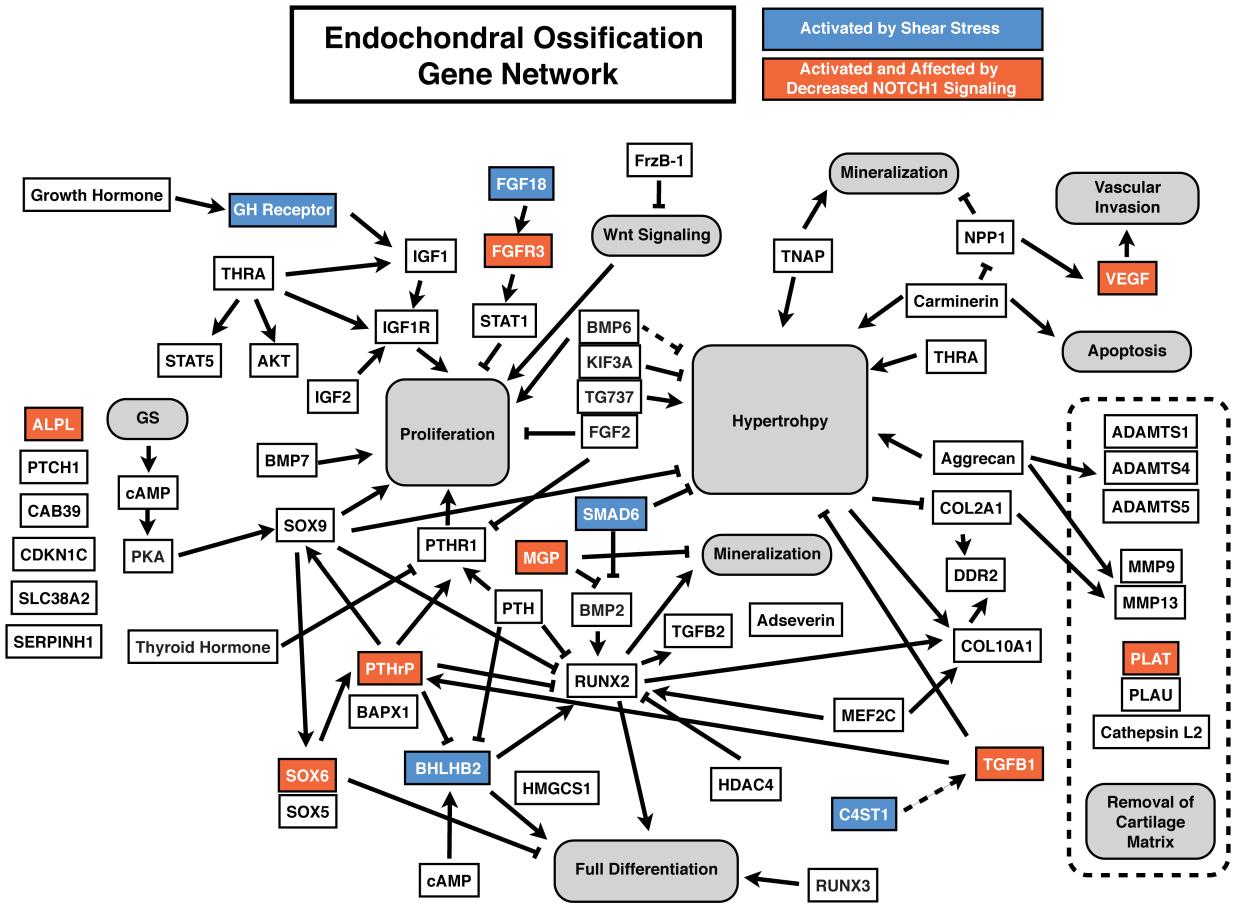


Figure S1. Endochondral ossification gene network is affected by shear stress and NOTCH1 signaling. Genes highlighted in blue were activated by flow (≥ 2 fold, condition 2 vs. condition 1) and those in orange were both activated by flow (≥ 2 fold, condition 2 vs. condition 1) and affected by NOTCH1 signaling (≥ 0.5 fold, condition 3 vs. condition 4).

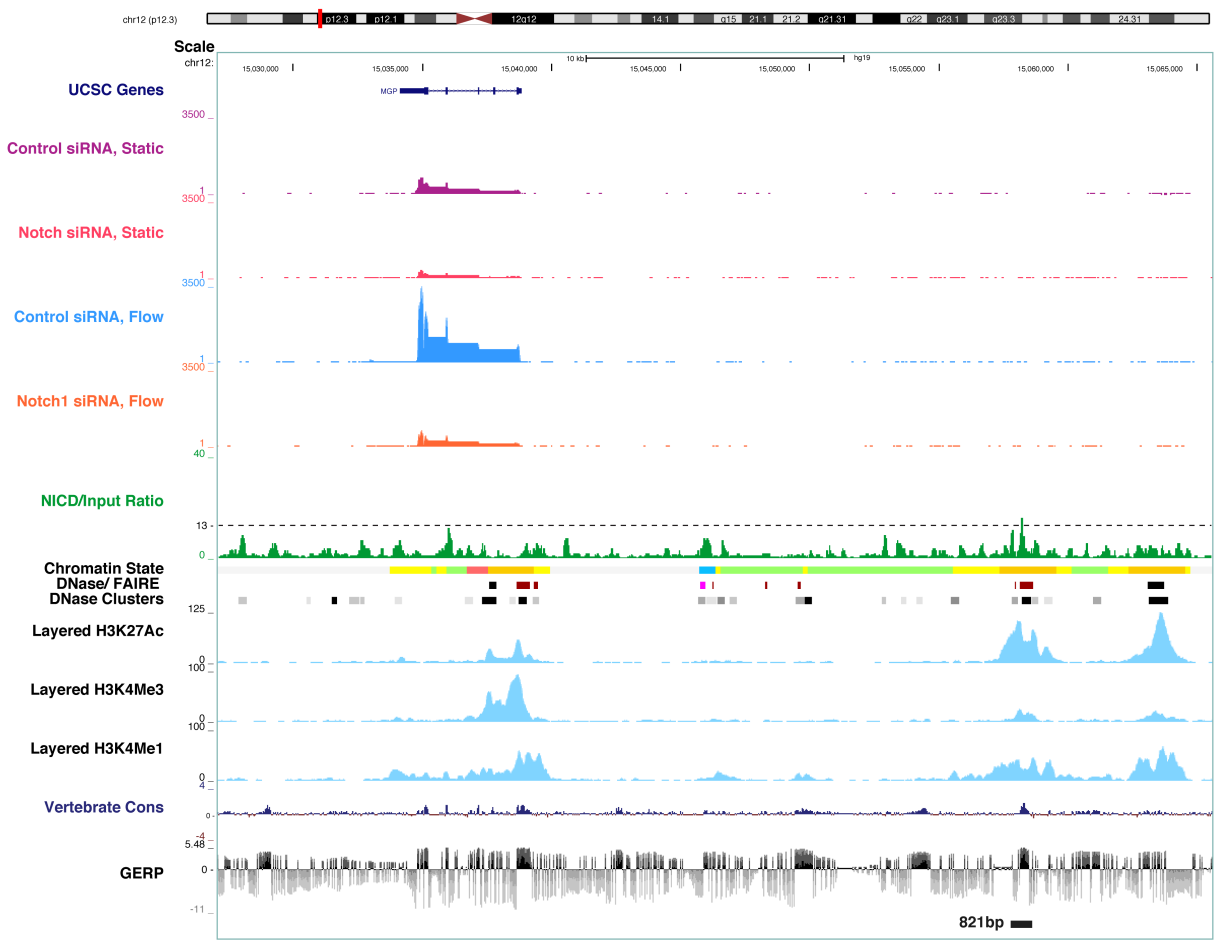
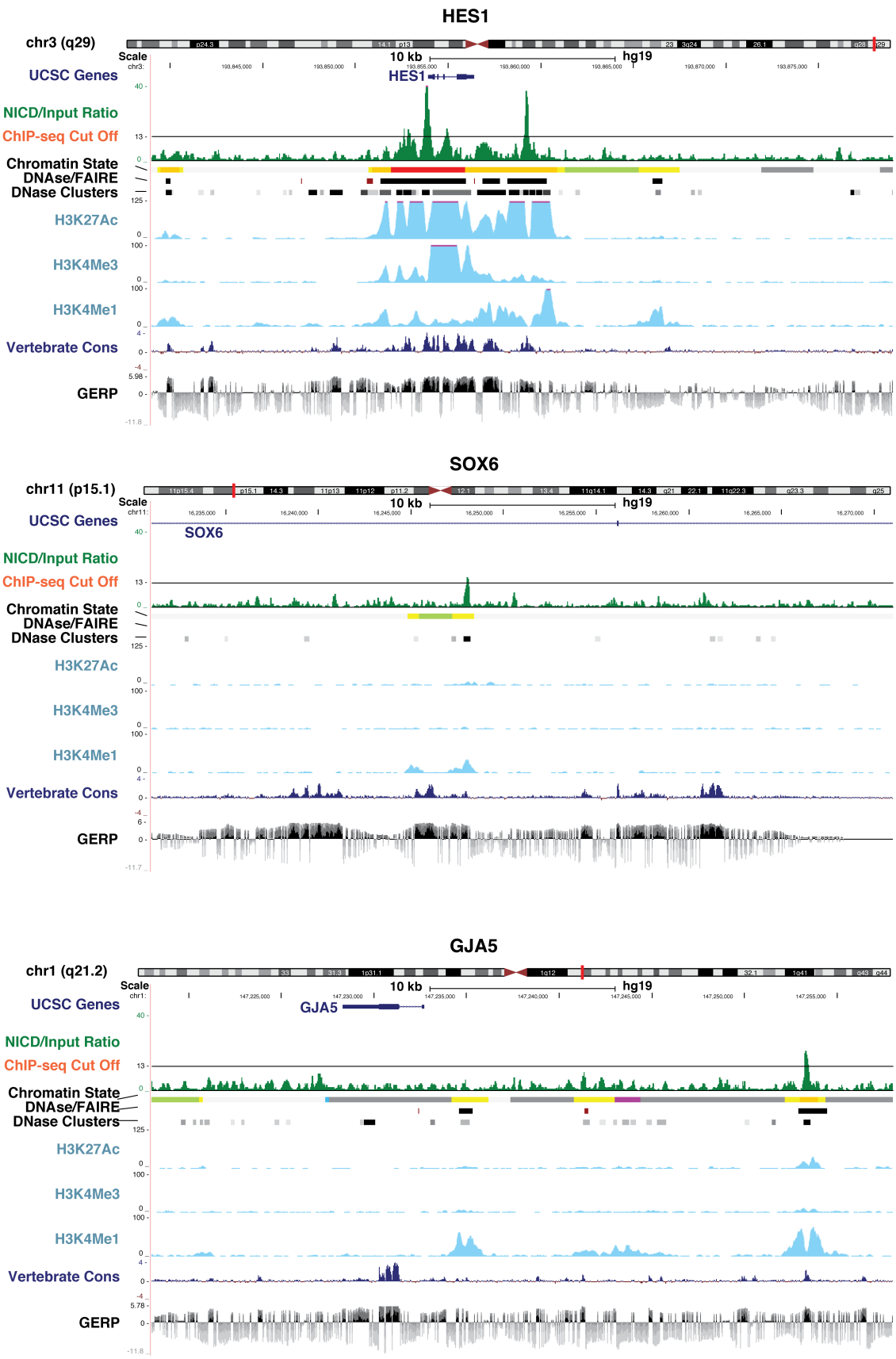


Figure S2. MGP RNA-seq Data. (**A)** 30 kb overview of genomic region containing the MGP gene locus. *UCSC Genes* shows MGP gene exons as tall filled boxes, UTRs as short filled boxes and introns as thin lines[1]. mRNA-seq reads are plotted as a histogram in four conditions for HAVECs. *NICD/Input Ratio* shows a histogram of NICD-myc ChIP-seq results with a threshold of 13 as the cutoff between background and real called peaks. Data from HUVEC cell lines in UCSC genome browser (*http://genome.ucsc.edu*, assembly NCBI37/mm9) and the ENCODE project are labeled as follows[1,2]: *Chromatin State* colors indicate types of regulatory regions such as strong enhancer (orange), weak/poised enhancer (yellow), insulator (blue), transcriptional transition/elongation (green) and active promoter regions (red); *DNAse/FAIRE* displays open chromatin and transcription factor binding sites with black bars representing peaks identified by both DNase1 hypersensitivity and FAIRE assay and red bars representing lower significance peaks; *DNAse Clusters* indicate open chromatin (grey boxes) and intensity of signal strength is indicated by the box darkness; *H3K27Ac* indicates active regulatory elements, *H3K4Me3* is often found near promoters and *H3K4Me1* is often found near regulatory elements. *Vertebrate conservation* shows measurements of sequence conservation using the *phyloP* method from human to zebrafish with blue peaks showing more conservation. Genomic Evolutionary Rate Profiling (GERP) shows evolutionary constraint with positive scores indicating a substitution deficit.


HES1
chr3 (q29)
Scale
10 kb
hg19
UCSC Genes
HES1
NICD/Input Ratio
ChIP-seq Cut Off
Chromatin State
DNAse/FAIRE
DNase Clusters
H3K27Ac
H3K4Me3
H3K4Me1
Vertebrate Cons
GERP
SOX6
chr11 (p15.1)
Scale
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SOX6
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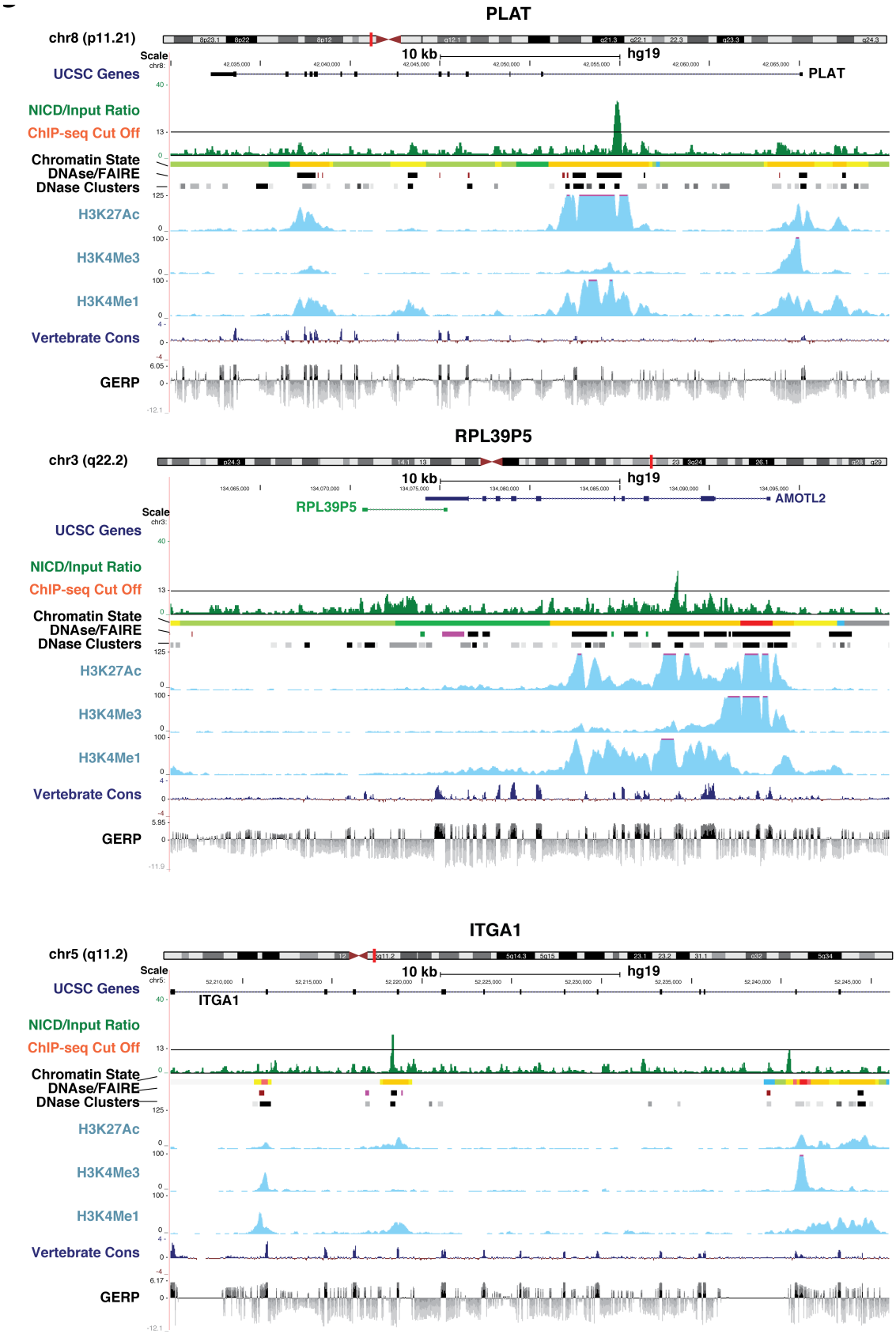
Vertebrate Cons
GERP
GJA5
chr1 (q21.2)
Scale
10 kb
hg19
UCSC Genes
GJA5
NICD/Input Ratio
ChIP-seq Cut Off
Chromatin State
DNAse/FAIRE
DNase Clusters
H3K27Ac
H3K4Me3
H3K4Me1
Vertebrate Cons
GERP

Figure S3. Potential HES1, SOX6 and GJA5 enhancer regions with NICD ChIP-seq. 30 kb overview of genomic region containing the gene locus. *NICD/Input Ratio* shows a histogram of NICD-myc ChIP-seq results with a threshold of 13 as the cutoff between background and real called peaks. Data from HUVEC cell lines in UCSC genome browser (*http://genome.ucsc.edu*, assembly NCBI37/mm9) and the ENCODE project are labeled as follows[1,2]: *Chromatin State* colors indicate types of regulatory regions such as strong enhancer (orange), weak/poised enhancer (yellow), insulator (blue), transcriptional transition/elongation (green) and active promoter regions (red); *DNAse/FAIRE* displays open chromatin and transcription factor binding sites with black bars representing peaks identified by both DNase1 hypersensitivity and FAIRE assay and red bars representing lower significance peaks; *DNAse Clusters* indicate open chromatin (grey boxes) and intensity of signal strength is indicated by the box darkness; *H3K27Ac* indicates active regulatory elements, *H3K4Me3* is often found near promoters and *H3K4Me1* is often found near regulatory elements. *Vertebrate conservation* shows measurements of sequence conservation using the *phyloP* method from human to zebrafish with blue peaks showing more conservation. Genomic Evolutionary Rate Profiling (GERP) shows evolutionary constraint with positive scores indicating a substitution deficit.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Scale
10 kb
hg19
UCSC Genes
PLAT
NICD/Input Ratio
ChIP-seq Cut Off
Chromatin State
DNAse/FAIRE
DNase Clusters
H3K27Ac
H3K4Me3
H3K4Me1
Vertebrate Cons
GERP
RPL39P5
chr3 (q22.2)
RPL39P5
AMOTL2
ITGA1
chr5 (q11.2)
ITGA1

Figure S4. Potential PLAT, RPL39P5 and ITGA1 enhancer regions with NICD ChIP-seq. 30 kb overview of genomic region containing the gene locus. *NICD/Input Ratio* shows a histogram of NICD-myc ChIP-seq results with a threshold of 13 as the cutoff between background and real called peaks. Data from HUVEC cell lines in UCSC genome browser (*http://genome.ucsc.edu*, assembly NCBI37/mm9) and the ENCODE project are labeled as follows[1,2]: *Chromatin State* colors indicate types of regulatory regions such as strong enhancer (orange), weak/poised enhancer (yellow), insulator (blue), transcriptional transition/elongation (green) and active promoter regions (red); *DNAse/FAIRE* displays open chromatin and transcription factor binding sites with black bars representing peaks identified by both DNase1 hypersensitivity and FAIRE assay and red bars representing lower significance peaks; *DNAse Clusters* indicate open chromatin (grey boxes) and intensity of signal strength is indicated by the box darkness; *H3K27Ac* indicates active regulatory elements, *H3K4Me3* is often found near promoters and *H3K4Me1* is often found near regulatory elements. *Vertebrate conservation* shows measurements of sequence conservation using the *phyloP* method from human to zebrafish with blue peaks showing more conservation. Genomic Evolutionary Rate Profiling (GERP) shows evolutionary constraint with positive scores indicating a substitution deficit.

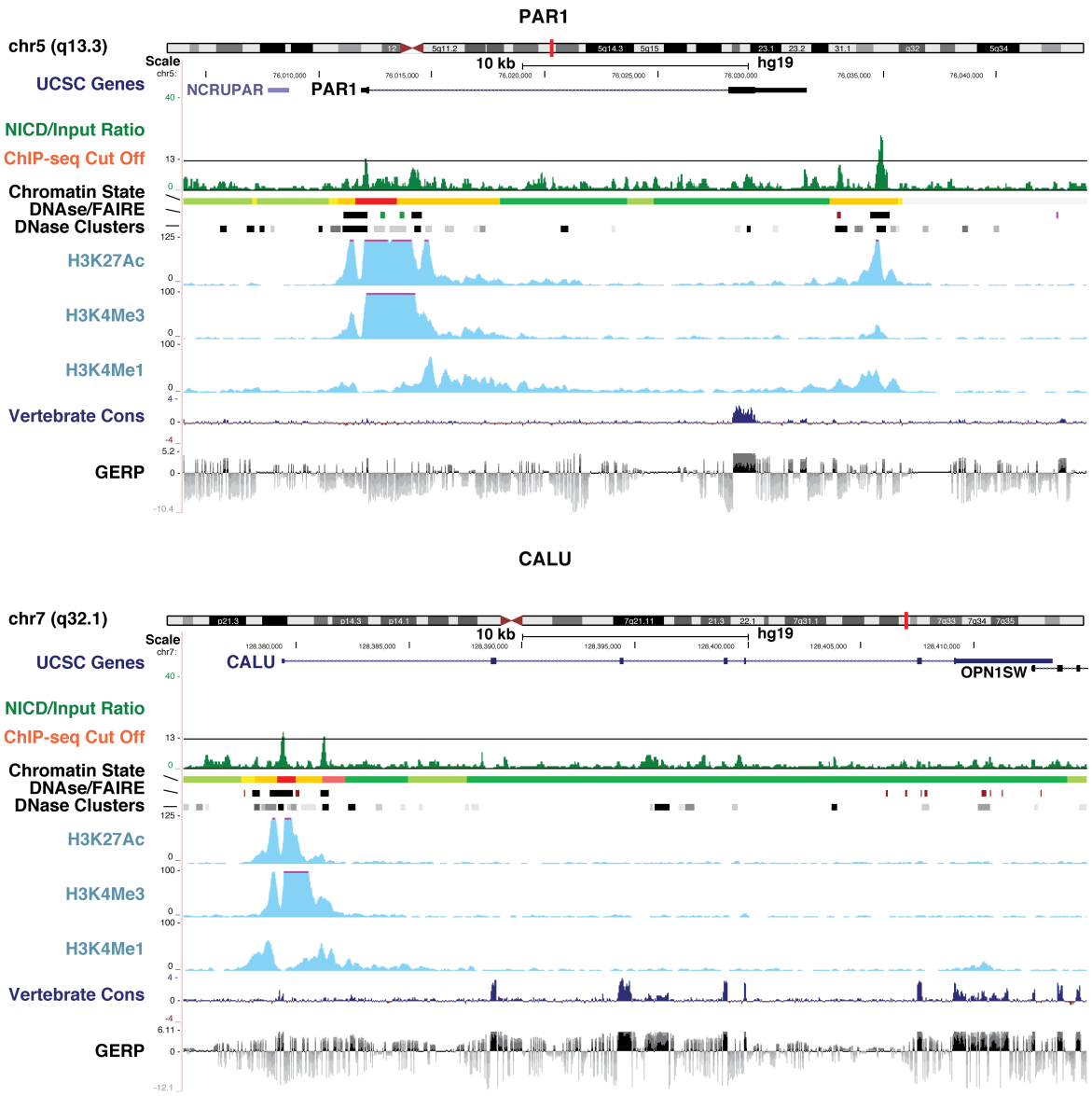

Figure S5. Potential PAR1 and CALU enhancer regions with NICD ChIP-seq. 30 kb overview of genomic region containing the gene locus. *NICD/Input Ratio* shows a histogram of NICD-myc ChIP-seq results with a threshold of 13 as the cutoff between background and real called peaks. Data from HUVEC cell lines in UCSC genome browser (*http://genome.ucsc.edu*, assembly NCBI37/mm9) and the ENCODE project are labeled as follows[1,2]: *Chromatin State* colors indicate types of regulatory regions such as strong enhancer (orange), weak/poised enhancer (yellow), insulator (blue), transcriptional transition/elongation (green) and active promoter regions (red); *DNAse/FAIRE* displays open chromatin and transcription factor binding sites with black bars representing peaks identified by both DNase1 hypersensitivity and FAIRE assay and red bars representing lower significance peaks; *DNAse Clusters* indicate open chromatin (grey boxes) and intensity of signal strength is indicated by the box darkness; *H3K27Ac* indicates active regulatory elements, *H3K4Me3* is often found near promoters and *H3K4Me1* is often found near regulatory elements. *Vertebrate conservation* shows measurements of sequence conservation using the *phyloP* method from human to zebrafish with blue peaks showing more conservation. Genomic Evolutionary Rate Profiling (GERP) shows evolutionary constraint with positive scores indicating a substitution deficit.

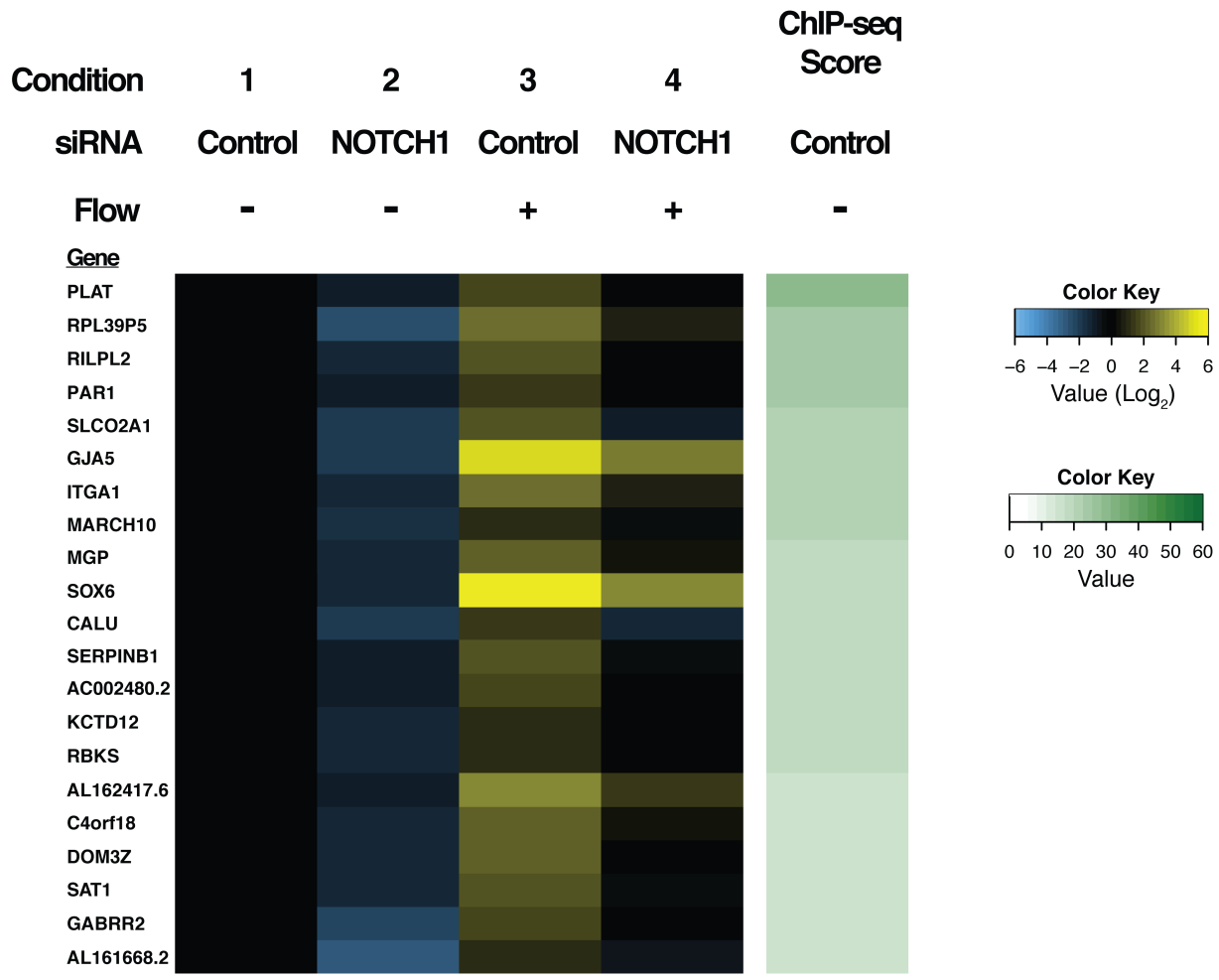

Figure S6. Heat map and clustering analysis of gene expression and ChIP-seq scores that indicate potential direct targets of NOTCH1. The expression of each gene was normalized to condition 1 (control siRNA and no flow) and then Log_2 transformed. Displayed genes were selected based on at least 2 fold downregulation upon NOTCH1 siRNA knockdown in both static and flow conditions or at least 2 fold upregulation in flow control siRNA condition compared to static flow as well as a ChIP-seq score above 13.

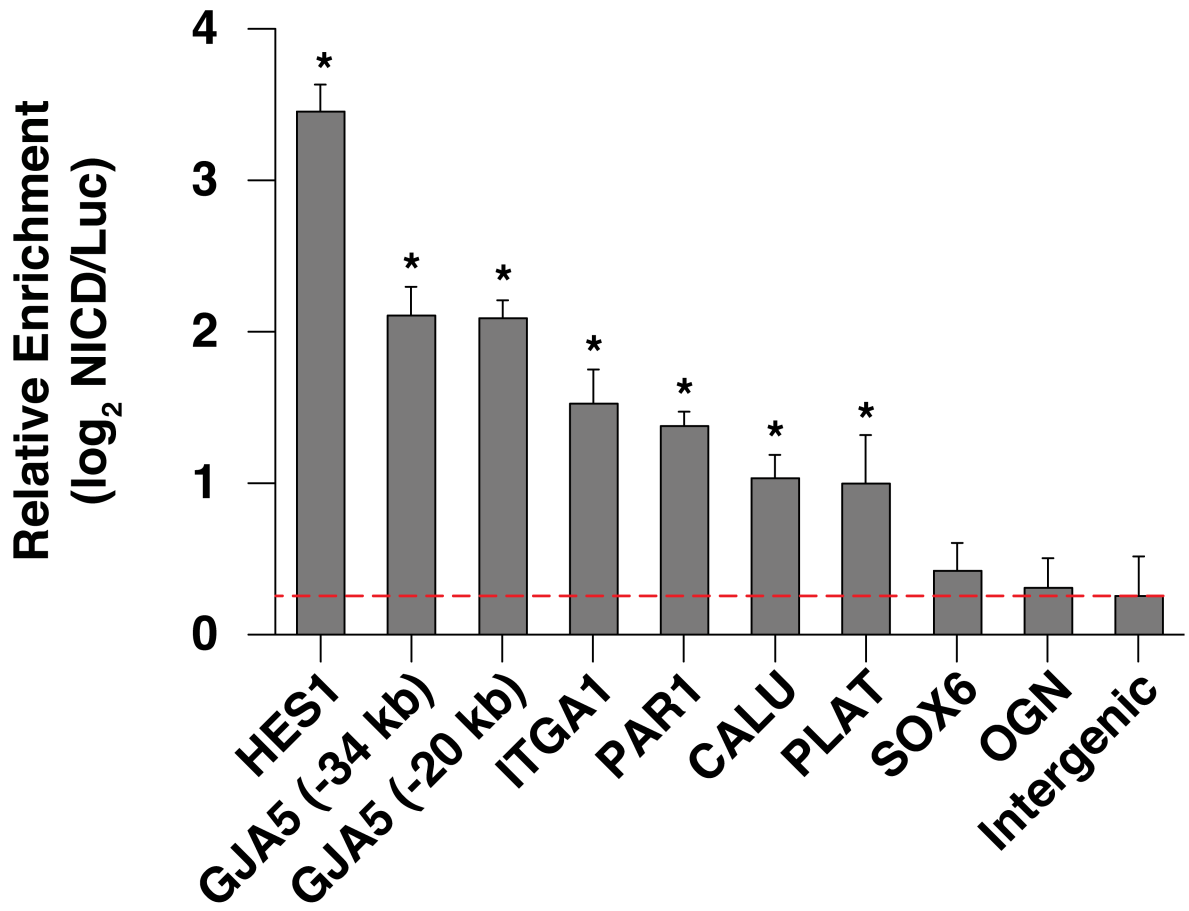

Figure S7. ChIP qRT-PCR with NICD1-myc tag in human aortic valve endothelial cells. Relative enrichment of NICD1-myc pull-down compared to Luciferase-myc pull-down is shown for each site: positive control is HES1 NOTCH1/CSL binding site and negative control is a non-specific intergenic region. Negative control enrichment level indicated by dashed red line. Error bars indicate standard deviation. Significant enrichment over corresponding negative control intergenic site is indicated (*, $p<0.05$).

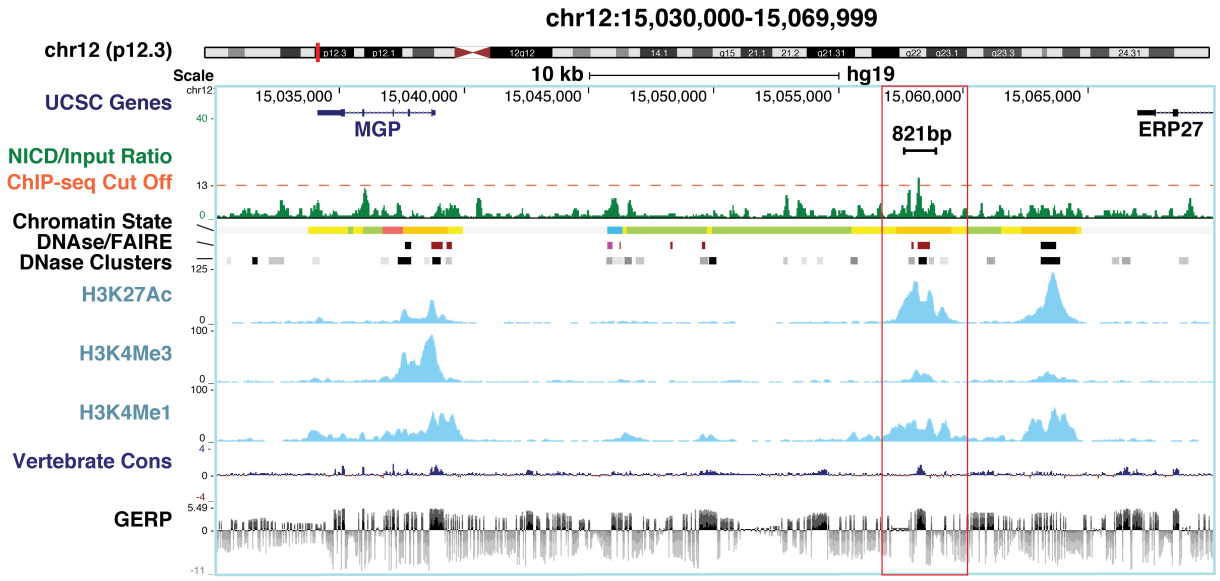

Figure S8. Overview of genomic region containing the MGP gene locus. NICD/Input Ratio is derived from our NICD-myc ChIP-seq results. All other components are data from HUVEC cell lines pulled from the UCSC genome browser (*http://genome.ucsc.edu*, assembly NCBI37/mm9) and the ENCODE project[1,2]. *NICD/Input Ratio:* histogram of NICD-myc ChIP-seq results with a threshold of 13 as the cutoff for peak calling; *Chromatin State*: strong enhancer (orange), weak/poised enhancer (yellow), insulator (blue), transcriptional transition/elongation (green), active promoter regions (red); *DNAse/FAIRE:* DNase1 hypersensitivity and FAIRE assay peaks (black), lower significance peaks (red); *DNAse Clusters;* open chromatin (grey boxes), intensity of signal strength is indicated by the box darkness; *H3K27Ac:* active regulatory elements; *H3K4Me3:* putative promoters; *H3K4Me1:* putative regulatory elements; *Vertebrate conservation:* peak intensity corresponds to conservation from human to zebrafish. *Genomic Evolutionary Rate Profiling (GERP):* evolutionary constraint with positive scores indicating a substitution deficit. The region boxed in orange indicates the putative endothelial enhancer region of MGP where NICD1 appears to bind and the black bar indicates the 821 bp cloned for further enhancer studies.

CSL Site #3 CSL Site #1 CSL Site #2

Wt TGTTGGAGTCCCGGATTCCAAGGGCTTTTGGGAACAGATTTGTGAGAAAAGAGTGAAAAG

3X Mut TGTTGGAGTCGACGATTCCAAGGGCTTTGTCGAACAGATTTGGTCGAAAAGAGTGAAAAG

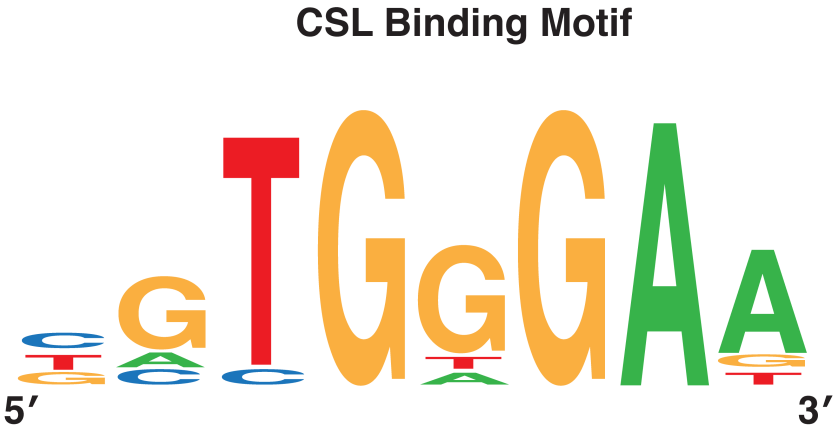

Figure S9. Wildtype and 3X Mut MGP Endothelial Enhancer DNA Sequence. High confidence CSL binding sites are labeled in red (site #1 and #2) and the lower confidence binding site is shown in orange (site #3). Mutations used to generate the 3x Mut construct are indicated in blue. CSL Motif is adapted from Ong et al. 2006 with permission from *J Biol Chem* [3].

REFERENCES

1. Kent, W. J. *et al.* The human genome browser at UCSC. *Genome Res.* **12,** 996–1006 (2002).
2. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* **489,** 57–74 (2012).
3. Ong, C.-T. *et al.* Target selectivity of vertebrate notch proteins. Collaboration between discrete domains and CSL-binding site architecture determines activation probability. *J. Biol. Chem.* **281,** 5106–5119 (2006).

Sequences

ChIP-seq primers

Custom Taqman probes (Life Technologies) were designed with the following seuquences as target: GAPDH (GRCh37/hg19) chr12:6,643,459-6,643,659; HES1 CSL (GRCh37/hg19) chr3:193,853,706-193,854,009; HES1 Non-CSL (GRCh37/hg19) chr3:193,845,818-193,846,315. Custom PrimeTime qRT-PCR primers (Integrated DNA Technologies, Coralville, IA) were designed with the following sequences: **MGP CSL**- Primer 1- 5'-GGG CTT TTG GGA ACA GAT TTG-3', Primer 2- 5'-GTT CAG GAA GTT AGG GCA GG-3', Probe- 5'-/56-FAM/AGT GAA AAG /ZEN/GAA GCA AGC AGG AGG A/3IABkFQ/-3'; **MGP non-CSL**, Primer 1- 5'-GAA GGG AAG AGG CTA AGT CAG-3', Primer 2- 5'-GTC AAC ATC ACATTATCA CCC AC-3', Probe-5'-/56-FAM/CCC TCC CAT /ZEN/GAA CAC CTA ACATTA CCA C/3IABkFQ/-3'. **OGN**, Primer 1- 5'-CAG AAT GTG AAA CTG TGG ACG-3', PrimeTime Primer 2- 5'-ACA TGG CAA GGA ACT GAG G-3', Probe- 5'-/56-FAM/TC TTG CTA G/Zen/C TGT TGA CTG ACG GC/3IABkFQ/-3'. **ITGA1**, Primer 1- 5'-TGT TGC CAT CAC TCC AGC-3', Primer 2- 5'-GGG AAT GTG GAG GTT TCA GAG-3', Probe- 5'-/56-FAM/TG ACC TGT T/Zen/G ACT CAC TTC CAG TTC C/3IABkFQ/-3'. **PLAT**, Primer 1- 5'-TGT TTG GTC TGT TGG CTG TAG-3', Primer 2- 5'-ACT ATT GTT CCC GTT TTC CCC-3', Probe- 5'-/56-FAM/CA TCA TTC A/Zen/C AGG TTG TTT GGC AGG G/3IABkFQ/-3'. **SOX6**, Primer 1- 5'-AGG AAA TGC CCG ACA CAG, Primer 2- 5'-CTA ACC TTC ATG GCT GAG ATC AT, Probe- 5'-/56-FAM/AC AGA GGA G/Zen/C ATG TGT GCA AAA CAG /3IABkFQ/. **CALU**, Primer 1- 5'-GCT ACG AGG AAA GGT AAG TAC G, Primer 2- 5'-CAA GAC CGG GGA CAG AAA G, Probe- 5'-/56-FAM/CG AAT AAA G/Zen/A TGA GGC AGT GGT GAA GGG /3IABkFQ/. **GJA5 Site 1**, Primer 1- 5'-TGG GAG GCT ATC AGC TCA G, Primer 2- 5'-CAC AGC CGG GTT ATC AGA AG, Probe- 5'-/56-FAM/CC CAC ACC A/Zen/G CTT CCT CTG CTA TT/3IABkFQ/. **GJA5 Site 2**, Primer 1- 5'-GGG AGG CTA TCA GCT CAG AG, Primer 2- 5'-CAC AGC CGG GTT ATC AGA AG, Probe 5'-/56-FAM/CC CAC ACC A/Zen/G

CTT CCT CTG CTA TT/3IABkFQ/. **SAT1**, Primer 1- 5'-CTG AAG GGT GGC AGA ATA CTC, Primer 2- 5'-GTG CCT TTA GCT TGA, Probe 5'-/56-FAM/CT TAC GTC A/Zen/T GGT GGC ATA TTT TGG CA/3IABkFQ/. **PAR1**, Primer 1- 5'-CCA AGA GAC AAT TCA GAA CAG C, Primer 2- 5'-TGC AAT CAA CAG TCC CCA TG, Probe 5'-/56-FAM/TC CTG TAA A/Zen/G TGA AAC TCA AGC GCC A/3IABkFQ/. **Intergenic**, Primer 1- 5'-AAT ATG GCT CTG TTC CAC CC, Primer 2- 5'-GAA CAA TAT GAA ATC TGG GCA CC, Probe- 5'-CCT TAA ACT CTG ACT GCT GGT GAG CTG.

Cloning Sequences

MGP-Wt-800 bp For, 5′- AATGCCATAAGGGTCCTTCC-3′; MGP-Wt-800 bp Rev, 5′-TGAAGAAGCGAGCCACATC-3′; MGP-3XMut-800 bp For 5′-AAGTGGTCAGGTGTAGTACAGAGATCTGGGGCAATCATTGCATGTTGGAGTC**GAC**GATTCC AAGGGCTTT**GTC**GAACAGATTTG**GTC**GAAAAGAGTGAAA-3.

siRNA Sequences

NOTCH1 siRNA- Ambion 4392422 s9633

Control siRNA- Ambion 4390843

Lentiviral NICD Sequence-

Notch 1- UniProtKB/Swiss-Prot: P46531.4 aa1769- 2555

atgcctgagggcttcaaagtgtctgaggccagcaagaagaagcggcgggagcccctcggcgaggactccgtgggcctcaagccc ctgaagaacgcttcagacggtgccctcatggacgacaaccagaatgagtgggggacgaggacctggagaccaagaagttccgg ttcgaggagcccgtggttctgcctgacctggacgaccagacagaccaccggcagtggactcagcagcacctggatgccgctgacct gcgcatgtctgccatggcccccacaccgccccagggtgaggttgacgccgactgcatggacgtcaatgtccgcgggcctgatggctt caccccgctcatgatcgcctcctgcagcgggggcggcctggagacgggcaacagcgaggaagaggaggacgcgccggccgtc

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