## SUPPLEMENTARY TABLES

| Construct | E14.5 | | | E17.5 | | |
|---|---|---|---|---|---|---|
| | Embryos analysed | Fingertips | Digits | Embryos analysed | Skulls | Growth Plates |
| i1 | 9 | 0 | 1 | 8 | **3** | **7** |
| i2 | 9 | 0 | 0 | 10 | 1 | **3** |
| i3 | 7 | 0 | 0 | 5 | **2** | **4** |
| i4 | 6 | 0 | 0 | 11 | **2** | **10** |
| i5 | 5 | **3** | **3** | 7 | **3** | **5** |
| i6 | 12 | 0 | **9** | 7 | 1 | **7** |
| i7 | 7 | **2** | **3** | 6 | 1 | **6** |
| i8 | 7 | 1 | **4** | 10 | **5** | **10** |
| i9 | 5 | 0 | 0 | 6 | 1 | **5** |

**Supplementary Table 1: Tissue-specific activity of enhancer elements.**

Each stage shows total number of embryos analyzed for each construct as well as those displaying positive staining for the corresponding tissue. Positive scoring is indicated in bold.

| Construct | Genomic Position | Size | Guide | Sequence |
|---|---|---|---|---|
| Del(2-9), Dup(int) | Chr1:75,015,710-75,091,187 | 75kb | N2-L1 | gagacacgtggagaattcgc-agg |
|  |  |  | N2-R1 | gttacccacactactacgtt-agg |
| Del(4-6) | Chr1:75,050,992-75,063,567 | 13kb | N4-L2 | ggacacgactttcataacac-tgg |
|  |  |  | N4-R2 | aatttcgggtagggcgttgg-agg |
| Del(7-9) | Chr1:75,063,567-75,091,187 | 24kb | N4-R2 | aatttcgggtagggcgttgg-agg |
|  |  |  | N2-R1 | gttacccacactactacgtt-agg |
| Del(4-9) | Chr1:75,050,992-75,091,187 | 37kb | N4-L2 | ggacacgactttcataacac-tgg |
|  |  |  | N2-R1 | gttacccacactactacgtt-agg |
| Dup(syn) | Chr1:74,989,792-75,055,634 | 65kb | N9-L9 | agcgtggggcttttaaccgt-ggg |
|  |  |  | N9-R6 | ttagacacaccagtatacgg-agg |
| Dup(csp) | Chr1:75,005,921-75,060,430 | 54kb | N10-L4 | ggggcaatctgatatagtgg-ggg |
|  |  |  | N10-R5 | tggcccctgacccgtaggat-tgg |

**Supplementary Table 2: Genomic rearrangements generated using CRISPR/Cas9 genome editing.**

Two sgRNAs flanking the target region were used to generate the genomic rearrangement.

| Construct | Genomic Position | Size | Primer | Sequence |
|---|---|---|---|---|
| centromeric homologous arm | | | | |
| SB-HR-L1 | Chr1:75,055,877-75,058,875 | 3kb | HR3a1-f-SalI | tata***gtcgac***caaagtccttgtaaggaacagcagt |
| | | | HR3a1-r-ClaI | tata***atcgat***gacatgcctctgctgtacatagttt |
| SB-HR-L2 | Chr1:75,058,877-75,060,875 | 2kb | HR3a2-f-F3-ClaI | tata***atcgat***tacaagctttacgaagttcctattcttcaaatagtataggaacttcagcaactcaggaagaattcctaacac |
| | | | HR3a2-r-F3-SacII | tata***ccgcgg***gtagaagttcctatactatttgaagaataggaacttcttgcagccctcctatagaaaatgga |
| telomeric homologous arm | | | | |
| SB-HR-R | Chr1:75,060,877-75,063,875 | 3kb | HR3b-f-XhoI | tata***ctcgag***tctataagaacacacaacaatgtgccag |
| | | | HR3b-r-NotI | tata***gcggccgc***actgttctgggtgaaccagaaatctt |

**Supplementary Table 3: Homologous arms cloned for the insertion of the SB cassette.**

Cloning of the centromeric arm (total size 5kb) was performed in two steps (constructs SB-HR-L1 and SB-HR-L2). Restrictions sites are shown in italic/bold.

| Element | Vista ID | Primer forward, reverse | Genomic Position (mm9) | Size (bp) |
|---|---|---|---|---|
| i1 | mm1142 | ctcagtgtctcaaccacttgaa, ctctgccatgacttcttgtgta | chr1:75,008,008-75,012,847 | 4840 |
| i2 | mm1143 | ggtgggattaatctctcgactg, ggtgatgaacagcagtatggaa | chr1:75,023,290-75,026,536 | 3247 |
| i3 | mm1148 | tctcccagaccaaaatgcttat, aaccttgccctcatgaagttta | chr1:75,046,263-75,049,025 | 2763 |
| i4 | mm1144 | cagactggagttcacagagtgc, actcaggcacaagtctagcaca | chr1:75,051,762-75,053,663 | 1902 |
| i5 |  | cctctgtgctcttgagttagactac, cctccttgctagttcttacctaaaga | chr1:75,053,880-75,055,928 | 2045 |
| i6 | mm1145 | tccttgagagactccagaaagg, tcccccatatcagatgtttacc | chr1:75,059,085-75,064,020 | 4936 |
| i7 | mm1146 | gtactgggaaaaatggcaagag, ctgaaagggggttagaaggact | chr1:75,068,299-75,072,430 | 4132 |
| i8 |  | ttgaggcagaaggattgtcata, agccagaggtcaacatttgagt | chr1:75,075,786-75,080,268 | 4483 |
| i9 | mm1439 | gctgagatgaatgacagtgagg, gtcacacctgatgatctgcatt | chr1:75,085,302-75,089,234 | 3933 |

**Supplementary Table 4: Genomic regions tested for enhancer activity.**

| Primer | Sequence |
|---|---|
| *Gapdh*-F | GGGAAGCCCATCACCATCTT |
| *Gapdh*-R | CGGCCTCACCCCATTTG |
| *Ihh*-F | GCCGACCGCCTCATGAC |
| *Ihh*-R | CATGACAGAGATGGCCAGTGA |
| *Nhej1*-F (exon3+4) | TGAAGACAGAGCCATTTGAAGA |
| *Nhej1*-R (exon3+4) | GCTTTCCATCACCAACAGCA |
| *Nhej1*-F (exon2+3) | CATTGCTTCGGATGAAGGACC |
| *Nhej1*-R (exon2+3) | TCAATCGACTTCGGCTCAG |
| *Cnppd1*-F | CCTATTCGCCGACTCCAGAAA |
| *Cnppd1*-R | CATTCGTCATTGAAGACCTCCTC |
| *Fam134a*-F | CACAAACATGACAAGAGAAAGCG |
| *Fam134a*-R | AGCTCAGAGTCTGTAATAGCCA |

**Supplementary Table 5: qPCR primer sequences.**

| Viewpoint | 1st primer (5’ – 3’) | Genomic Position | 2nd primer (5’ – 3’) | Genomic Position |
|---|---|---|---|---|
| *Ihh* promoter | ACAGCTGGGGACCCTATAC | chr1:74,998,765-74,998,783 | CCCGTCAGGAGGACAATC | chr1:75,059,837-75,059,854 |

**Supplementary Table 6: 4C-seq primer sequences.**

For reference, the mm9 mouse genome was used.