Supplemental Information

Decreased Enhancer-Promoter Proximity

Accompanying Enhancer Activation

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Supplementary Text and Figures

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Supplementary Figures

Figure S1. FISH and Shh expression during NPC differentiation. Related to Figure 1.

A) Violin plots representing Shh-SBE6 inter-probe distances after FISH in ESCs and in NPCs differentiated for 3 (D3) and 7 (D7) days of differentiation. Data are from the second (rep 2) and third (rep 3) biological replicates of the experiment shown in Figure 1. * p < 0.05, ** p <0.01. B) Kernel density plots showing Shh mRNA expression in single NPCs relative to Gapdh and normalised to expression in ESC at D3 and D7 of differentiation for a second biological replicate. Statistical data relating to FISH data from this figure are included in Table S1.

Figure S2. Chromosome conformation capture of the Shh regulatory region. Related to Figure 2.

A) 5C heat-maps from ESCs and NPCs for the Shh regulatory region (mm9, chr5:28604000-29780000) with 16kb binning and smoothing (biological replicate of experiment in Figure 2). Difference 5C plots are shown above the main 5C plots. B) 5C heat maps from the control Usp22 locus.

Figure S3. Inducing Shh expression in ESCs does not perturb pluripotency. Related to Figure 3.

A) qRT-PCR showing mean (± SEM) log2 mRNA levels of Shh, Oct4 and Nestin relative to Gapdh and normalized to eGFP expressing ESCs, in NPCs and in ESCs expressing tShh-Vp64, tSBE6-Vp64, tSBE2-Vp64 and tSBE(6+2)-Vp64. Data are from one biological replicate. B) As in (A) but for genes from the Shh TAD and the flanking TADs. Data in A and B relate to main Figure 3. C) Virtual 4C visualisation of 5C data from main Figure 3E, for ESCs and for ESCs expression SBE(6+2)-Vp64 with SBE6 and SBE2 viewpoints. Genome co-ordinates (Mb) are from the mm9 assembly of the mouse genome.

Figure S4. Chromatin and transcription profiling of the Shh regulatory region. Related to Figure 3.

A) Normalised read counts for H3K27me3 ChIP-seq across the Shh regulatory region. The position of Shh enhancers (SBE2-6 and ZRS) as well as the position of genes in the region is shown below the ChIP track. Genome co-ordinates are from the mm9 assembly of the mouse genome. NCBI GEO accession number for these data: GSE89512. Data are from Illingworth et al., 2015; GEO accession GSE69955. B) Log2 ratio H3K27ac ChIP: input of MNase digested chromatin, hybridised to an array covering the
Shh regulatory region. Mapping is aligned to the genome browser track shown in (A). Data are from; ESCs expressing control eGFP, or TALE-Vp64 fusions targeting the Shh promoter (tShh-Vp64), SBE6 (tSBE6-Vp64) or SBE2 (tSBE2-Vp64). Dotted lines represent location of the Shh promoter, SBE6 and SBE2. NCBI GEO accession number for these data: GSE89512. C) Normalised read counts for 4SU-seq during a time course of NPC differentiation from day 0 to day 7. Tracks are aligned to the genome co-ordinates in (A). NCBI GEO accession number for these data: GSE115774.

**Figure S5. Chromosome conformation capture of the Shh regulatory region in wild type, tSBE6-PARP1 and tSBE6-PARP1 + tNE-CTCF ESCs. Related to Figure 6.**

Biological replicate for experiment in Figure 6G. 5C heatmaps of the Shh regulatory region (mm9, chr5: 28604000-29780000) with 16kb binning and smoothing. Difference 5C plots are shown above the main 5C plots.
Table S1, Mann-Whitney U significance tests for inter-probe FISH distances across the Shh regulatory domain during NPC differentiation. Related to Figure 1

Table showing the p-values of each biological replicate for differences between the inter-probe distances from ESCs against the data from various days of NPC differentiation. Values in bold are significant (p<0.05). Also shown are the % alleles (probe pairs) where probes are considered co-localised (i.e. distances of <200nm) for data from ESCs and NPCs. 40 to 100 loci were analysed per experiment. Unless otherwise indicated, imaged were captured using structured illumination microscopy (SIM).

| Probe pairs       | Day of NPC differentiation | p      | Imaging mode | % of probe pair <200nm in ESCs | % of probe pair <200nm in NPCs |
|-------------------|----------------------------|--------|--------------|-------------------------------|-------------------------------|
| Shh-SBE6          | 3                          | 0.55   | SIM          | 26.3                          | 25.4                          |
|                   |                             | 0.018  | SIM          | 11.5                          | 7.5                           |
|                   |                             | 0.002  | SIM          | 11.5                          | 9.7                           |
| Shh-SBE6          | 4                          | 0.011  | SIM          | 26.3                          | 18.1                          |
| Shh-SBE6          | 5                          | <0.001 | SIM          | 26.3                          | 13.5                          |
| Shh-SBE6          | 6                          | <0.001 | SIM          | 26.3                          | 11.0                          |
|                   | 7                          | 0.033  | SIM          |                               |                               |
|                   |                             | 0.020  | SIM          |                               |                               |
|                   |                             | 0.003  | SIM          |                               |                               |
|                   |                             | 0.002  | SIM          |                               |                               |
|                   |                             | <0.001 | SIM          | 26.3                          | 19.2                          |
|                   |                             | <0.001 | SIM          | 11.5                          | 4.3                           |
|                   |                             | <0.001 | SIM          | 25.3                          | 9.1                           |
|                   |                             | <0.001 | SIM          | 7.8                           | 3.8                           |
|                   |                             | <0.001 | SIM          | 15.8                          | 3.9                           |
|                   |                             | <0.001 | SIM          | 26                            | 3.6                           |
|                   |                             | <0.001 | SIM          | 13.6                          | 4.9                           |
| Shh-SBE4          | 7                          | 0.018  | SIM          | 23.8                          | 6.6                           |
|                   |                             | 0.015  | SIM          | 10.6                          | 5.4                           |
|                   |                             | <0.001 | SIM          |                               |                               |
| Shh-SBE2/3        | 7                          | 0.946  | SIM          | 17.5                          | 18                            |
|                   |                             | 0.644  | SIM          |                               |                               |
|                   |                             | 0.529  | SIM          |                               |                               |
|                   |                             | 0.493  | SIM          |                               |                               |
| Shh-ZRS           | 7                          | 0.057  | SIM          |                               |                               |
|                   |                             | 0.656  | SIM          |                               |                               |
|                   |                             | 0.394  | SIM          |                               |                               |
|                   |                             | 0.388  | SIM          |                               |                               |
| Shh-Control       | 7                          | 0.680  | SIM          | 26.3                          | 26.3                          |
|                   |                             | 0.432  | SIM          | 13.6                          | 13.6                          |

Unless otherwise indicated, images were captured using structured illumination microscopy (SIM).
Table S2. Mann-Whitney U significance tests for Shh-SBE6 inter-probe distances between eGFP and TALE-Vp64/TALE-Vp128 transfected cells. Related to Figure 3.

*p*-values of each biological replicate of the data from Figures 3C and D are shown for the differences between the inter-probe distances from ESCs expressing a control eGFP construct and the data from ESCs expressing TALE-Vp64/Vp128 constructs. Values in bold are significant (*p*<0.05). Also shown are the % alleles (probe pairs) where probes are considered co-localised (i.e. distances of <200nm).

| TALE-Vp64/Vp128 constructs | Shh-SBE6 p value | % of probe pair <200nm in eGFP control | % probe pairs <200nm in TALE-fusions |
|-----------------------------|------------------|---------------------------------------|-------------------------------------|
| tShh-Vp128                  | 0.440            | 26.6                                  | 35.8                                |
|                             | 0.073            | 31.5                                  | 8.9                                 |
| tSBE6-Vp128                 | 0.001            | 31.5                                  | 7.5                                 |
| tSBE2-Vp128                 | 0.0021           | 22.2                                  | 1.8                                 |
|                             | <0.001           | 31.5                                  | 0                                   |
| tSBE(6+2)-Vp64              | 0.045            | 8.4                                   | 5.6                                 |
|                             | 0.013            | 28.3                                  | 9.6                                 |
|                             | <0.001           | 32.9                                  | 3.6                                 |
|                             | <0.001           | 25.7                                  | 2.9                                 |
|                             | <0.001           | 29.5                                  | 3.5                                 |
|                             | <0.001           | 31.1                                  | 18.4                                |
|                             | <0.001           | 25.7                                  | 2.9                                 |
|                             | <0.001           | 30.8                                  | 4.1                                 |
| tSBE(6+2)-Δ                 | 0.228            | 28.3                                  | 28.3                                |

Table S3. Mann-Whitney U significance tests for Shh-SBE6 inter-probe distances across the Shh regulatory domain after expression of TALE-LDB1. Related to Figure 3.

Table showing the *p*-values of each biological replicate for differences between the inter-probe distances between the data from ESCs expressing a control eGFP construct and the data from ESCs expressing TALE-LDB1 constructs. Data are from Figures 3H and I. Values in bold are significant (*p*<0.05). Also shown are the % of alleles (probe pairs) where probes are considered as co-localised (i.e. distances of <200nm) for data from ESCs and cells expressing the various TALE-LDB1 fusions.

| Probe pairs | TALE-LDB1 constructs | p value | % of probe pair <200nm in ESCs | % of probe pair <200nm in TALE-LDB1 |
|-------------|----------------------|---------|-------------------------------|-------------------------------------|
| Shh-SBE6    | tShh-LDB1 + tSBE6-LDB1 | 0.022   | 36.3                          | 54.4                                |
|             | tShh-LDB1 + tSBE2-LDB1 | 0.457   | 17.7                          | 21.2                                |
|             |                       | 0.167   | 22.6                          | 17.2                                |
| Shh-SBE2/3  | tShh-LDB1 + tSBE2-LDB1 | 0.017   | 22.1                          | 34.8                                |
|             | <0.001               | 18.6    | 45.5                          |                                     |
|             | <0.001               | 10.5    | 35.1                          |                                     |
| SBE6-SBE2/3 | tShh-LDB1+tSBE2-LDB1  | 0.895   | 29.1                          | 19.4                                |
|             | 0.198                | 28.8    | 29.5                          |                                     |
|             | tSBE6-LDB1+tSBE2-LDB1 | 0.018   | 23.7                          | 48.8                                |
**Table S4, Mann-Whitney U significance tests for Shh-SBE6 inter-probe distances after expression of TALE-VWA or SIX3. Related to Figure 4.**

*p*-values of each biological replicate of the data from Figure 4 are shown for the differences between the inter-probe distances from ESCs expressing a control eGFP construct and those from ESCs expressing TALE-VWA or Six3 constructs. Values in bold are significant (*p*<0.05). Also shown are the % alleles (probe pairs) where probes are considered co-localised (i.e. distances of <200nm).

| TALE-constructs | Shh-SBE6 *p* value | % probe pairs <200nm in eGFP control | % probe pairs <200nm in TALE-fusions |
|-----------------|--------------------|--------------------------------------|-------------------------------------|
| tShh-VWA        | 0.359              | 17.4                                 | 20.4                                |
| tSBE(6+2)-VWA   | 0.04 <0.001        | 17.4                                 | 4.3                                 |
| tShh-SIX3       | 0.594              | 22.6                                 | 29.6                                |
| tSBE2-SIX3      | 0.012 <0.001 <0.001| 22.6                                 | 21.4 20.6 8.3                       |

| TALE-constructs | Shh-SBE6 *p* value |
|-----------------|--------------------|
| tSBE(6+2)-Vp64 + tNE-CTCF | 0.626 0.602 |
| tSBE(6+2)-Vp64 + tNE-Δ | 0.596 0.168 |
| tSBE(6+2)-VWA + tNE-Δ | 0.627 |

**Table S5, Mann-Whitney U significance tests for Shh-SBE6 inter-probe distances after expression of TALEs-that target NE. Related to Figure 5.**

*p*-values of each biological replicate are shown for the differences between the Shh-SBE6 inter-probe distances from ESCs expressing a control eGFP construct and from ESCs expressing various TALE-constructs. Data from Figure 5.
Table S6. Mann-Whitney U significance tests for Shh-SBE6 inter-probe distances after TALE-fusion expression and after treatment with olaparib. Related to Figure 6.

*p*-values of each biological replicate are shown for differences between the inter-probe distances from ESCs expressing a control eGFP construct against the data from ESCs expressing TALE constructs, or from NPCs treated with olaparib. Data relate to Figure 6C-F. Values in bold are significant (p<0.05).

| TALE-fusion constructs | Shh-SBE6 *p* value |
|------------------------|--------------------|
| tSBE6-PARP1           | 0.001              |
| tSBE6-PARP1 + tNE-CTCF | 0.106              |
| tSBE2-PARP1           | <0.001             |
| tSBE2-PARP1 + tNE-CTCF | 0.883              |
| tSBE2-SIX3            | 0.012              |
|                       | <0.001             |
|                       | <0.001             |
| tSBE2-SIX3 + Olaparib | 0.997              |
|                       | 0.629              |
|                       | 0.204              |
| tSBE2-PARP1+Olaparib  | 0.816              |
|                       | 0.680              |
|                       | 0.417              |
| NPC (d7) + Olaparib   | 0.799              |
|                       | 0.546              |
| tShh-DEL              | 0.003              |
| tSBE2-DEL             | <0.001             |
|                       | <0.001             |
| tSBE2-DEL + Olaparib  | <0.001             |
|                       | <0.001             |

Table S7. Mann-Whitney U significance tests for Shh-SBE6 inter-probe FISH distances across the Shh regulatory domain after recruitment of wild-type and mutant PARP1. Related to Figure 6.

*p*-values for the differences between the Shh-SBE6 inter-probe distances from ESCs expressing a control eGFP construct and from ESCs expressing various TALE-PARP1 fusion constructs, either wild-type PARP1, or catalytically inactive mutant PARP1s (E988K or D890K+M899N). Data relate to Figure 6H. Values in bold are significant (p<0.05).

| Probe pairs      | TALE-fusion constructs | *p* value |
|------------------|------------------------|----------|
| Shh-SBE6         | tSBE6-PARP1            | <0.001   |
|                  | tSBE2-PARP1            | <0.001   |
|                  | tSBE6-E988K            | 0.168    |
|                  |                        | 0.337    |
|                  | tSBE6-D890V+M899N      | 0.794    |
|                  |                        | 0.226    |
|                  | tSBE2-E988K            | 0.756    |
|                  |                        | 0.447    |
|                  | tSBE2-D890V+M899N      | 0.984    |
|                  |                        | 0.7414   |
**Table S8: Enhancer and FISH probe co-ordinates. Related to STAR Methods section 3D-FISH**

The table lists the genome co-ordinates (bp) on chromosome 5 (mm9 assembly of the mouse genome) of Shh and its enhancers, and the fosmid probes used for FISH.

| Locus | Gene/enhancer | start (bp) | end (bp) | fosmid ID | Clone name |
|-------|---------------|------------|----------|-----------|------------|
| CTRL  | 28411516      | 28449628   | G135P60453C9 | WI1-2806K8 |
| Shh   | 28783380-28793641 | 28754458   | 28795879 | G135P64333A4 | WI1-574018 |
| SBE6  | 28889688-28890461 | 28887686   | 28924744 | G135P67311F4 | WI1-442E17 |
| SBE4  | 29111752-29112865 | 29107140   | 29147593 | G135P600205H10 | WI1-2751A06 |
| SBE2/3| 29202018-29219606 | 29195832   | 29239355 | G135P603171G8 | WI1-1275C09 |
| ZRS   | 29638200-29643599 | 29611727   | 29653695 | G135P600929F6 | WI1-1047E14 |

**Table S9: Table of PCR primers for qRT-PCR and Chip qPCR. Related to STAR Methods section**

**qRT-PCR primers**
- Gapdh Fw: ATCACCATCTTCCAGGAGCGAG
- Gapdh Rv: GACCTTTTTGGCTCCACCCTTC
- Oct4 (Pou5f1) Fw: CGAGAACAATGAGAACCTTC
- Oct4 (Pou5f1) Rv: CCTTCTCTAGCCCAAGCTGAT
- Nestin Fw: GATCGCTCAGATCCTGGAAG
- Nestin Rv: AGGTGTCTGCAAGCGAGAGT
- Shh Fw: ACGATTTAAGGAACTCACCC
- Shh Rv: TTGTCTTTTGCAACCTCTGAG
- 9530036O11Rik Fw: GCCTGAAACACACAGAATGC
- 9530036O11Rik RV: GATGGGAGAACTCAGCCAAG
- Parg Fw: GAAGAAGTGGCTGGAACCTCCT
- Parg Rv: GTTTCCGAACCTCTCTGCTCT

**ChIP qPCR primers**
- tNE Fw: CAAGAACAACATGGGTCTAGGG
- tNE Rv: TGAATTGGAACATTTCTTCAGG
- tShh Fw: AAGGCTGGAGAGCCTTGAG
- tShh Rv: TAAAGCCACACGACCCAG
- Actin Fw: CCTCGATGCTGACCCACTCCT
- Actin Rv: GACACTGCCCAATCGCTCT
Table S10: 5C sequencing technical and biological replicates reads. Related to STAR Methods section

| Experiment                                      | No. of reads | No. of used reads | No. of used reads Shh reg. region |
|-------------------------------------------------|--------------|-------------------|----------------------------------|
| ESC biological replicate 1 (tSBE-Vp64 experiments) | 45,024,284   | 23,168,046        | 13,752,662                       |
| ESC biological replicate 1                       | 1,745,058    | 1,368,704         | 844,392                          |
| ESC biological replicate 2                       | 3,606,454    | 2,830,120         | 1,751,368                        |
| NPC biological replicate 1-technical replicate 1  | 3,984,330    | 1,627,144         | 795,658                          |
| NPC biological replicate 1-technical replicate 2  | 9,814,008    | 4,168,546         | 2,267,024                        |
| NPC biological replicate 2-technical replicate 1  | 2,079,276    | 894,214           | 411,396                          |
| NPC biological replicate 2-technical replicate 2  | 92,081,330   | 33,796,970        | 15,038,310                       |
| NPC biological replicate 2-technical replicate 3  | 16,481,938   | 5,648,428         | 2,705,028                        |
| tSBE6-Vp64 biological replicate 1                | 90,452,930   | 50,035,502        | 30,790,810                       |
| tSBE2-Vp64 biological replicate 1                | 1.01E+08     | 44,074,854        | 21,016,670                       |
| tSBE(6+2)-Vp64 biological replicate 1-technical replicate 2 | 79,792,180   | 32,329,736        | 16,415,488                       |
| tSBE6-PARP1 biological replicate 1               | 2,066,022    | 1,002,156         | 562,782                          |
| tSBE6-PARP1 biological replicate 2               | 1,664,928    | 1,255,928         | 728,276                          |
| tSBE6-PARP1 + tNE-CTCF biological replicate 1     | 2,478,062    | 1,873,946         | 1,173,160                        |
| tSBE6-PARP1 + tNE-CTCF biological replicate 2     | 1,604,560    | 1,231,786         | 706,104                          |