

Supplementary Figures

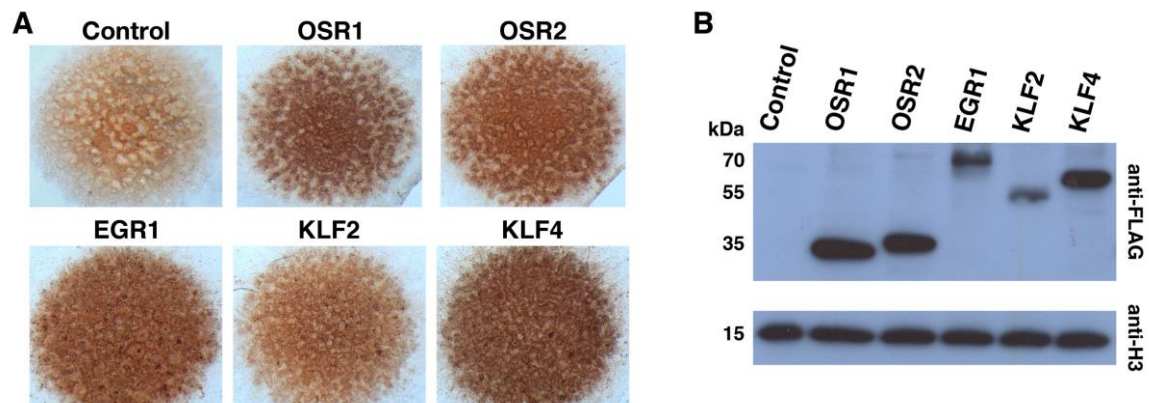


Fig. S1. Detection of the 3F-tagged TFs within the chMM cultures. (A) Immunohistochemistry with an anti-FLAG antibody to detect the infection level of retroviral RCAS-BP(A) particles carrying each of the TF CDS fused at their 3'-end with the 3F tag. (B) Western blot analysis of the 3F-tagged recombinant TFs overexpressed in chMM cultures. TFs were detected by using an anti-FLAG antibody. Protein amount in each loaded sample was controlled by using an anti-H3 antibody recognizing histone proteins H3.

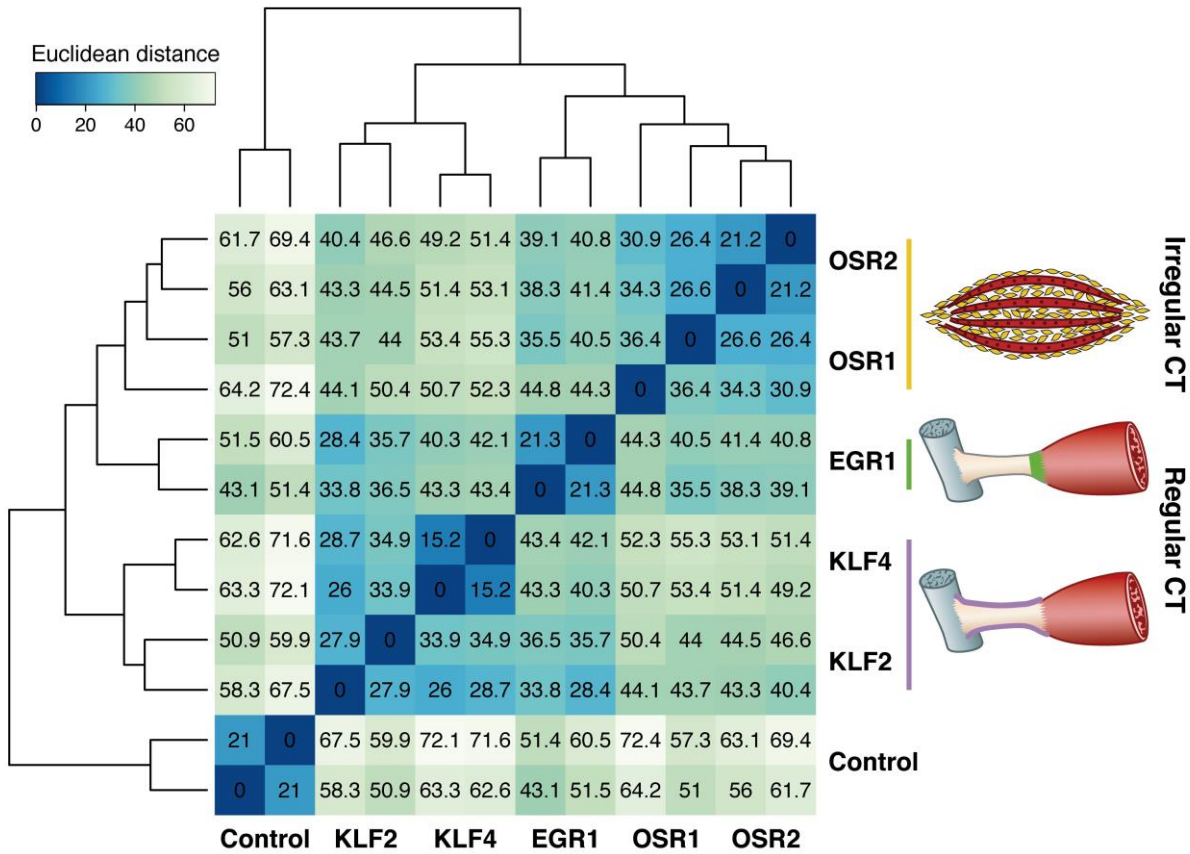


Fig. S2. Sample-to-sample distance across TF-overexpressing chMM cultures. Euclidean distances were calculated across all biological replicates and conditions.

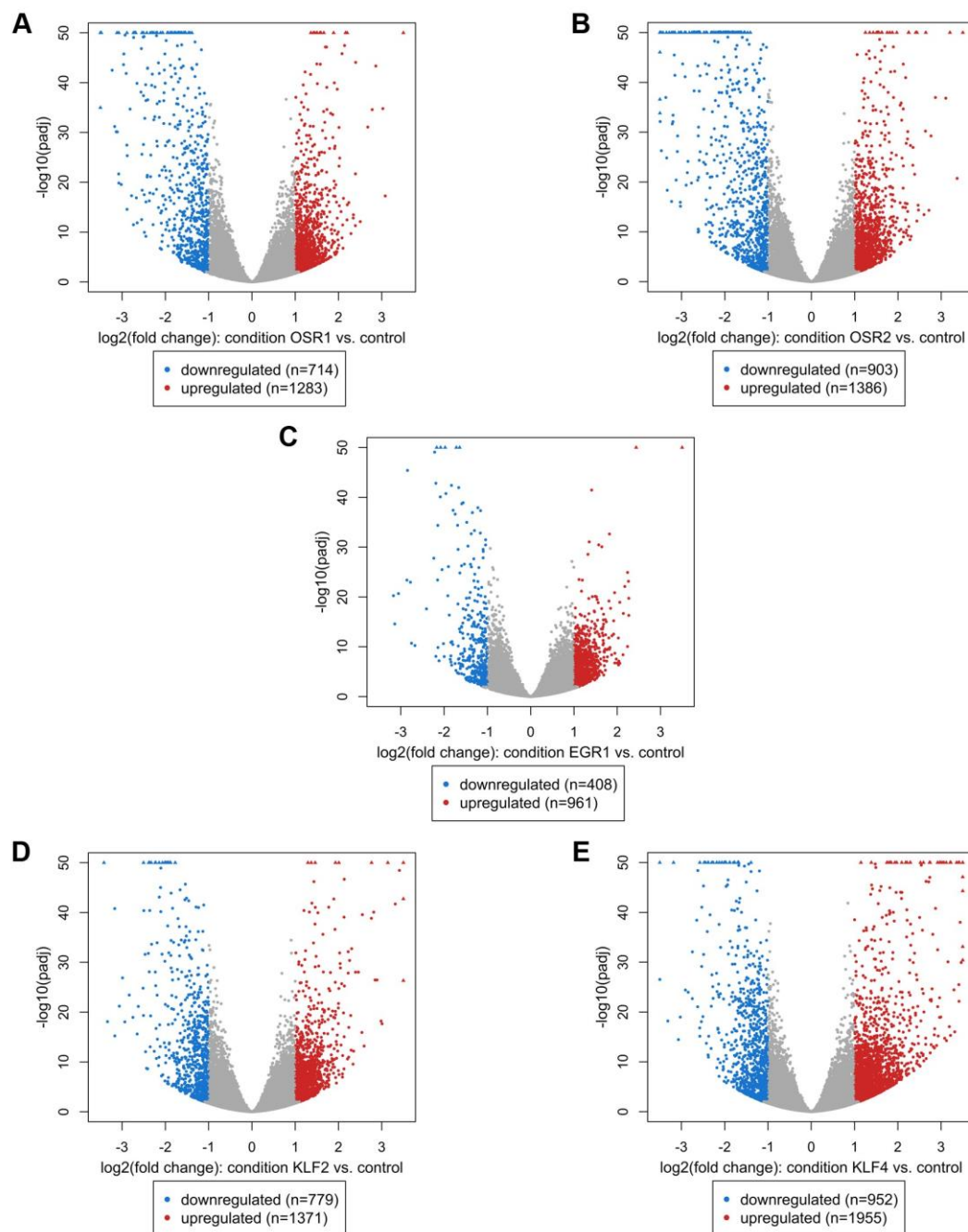


Fig. S3. DE genes detected in TF-overexpressing chMM cultures. Volcano plots of the 10,712 DE genes detected in the chMM cultures overexpressing OSR1 (A), OSR2 (B), EGR1 (C), KLF2 (D) and KLF4 (E). (A) 1,997 DE genes detected upon OSR1 overexpression. (B) 2,289 DE genes detected upon OSR2 overexpression. (C) 1,369 DE genes detected upon EGR1 overexpression. (D) 2,150 DE genes detected upon KLF2 overexpression. (E) 2,907 DE genes detected upon KLF4 overexpression.

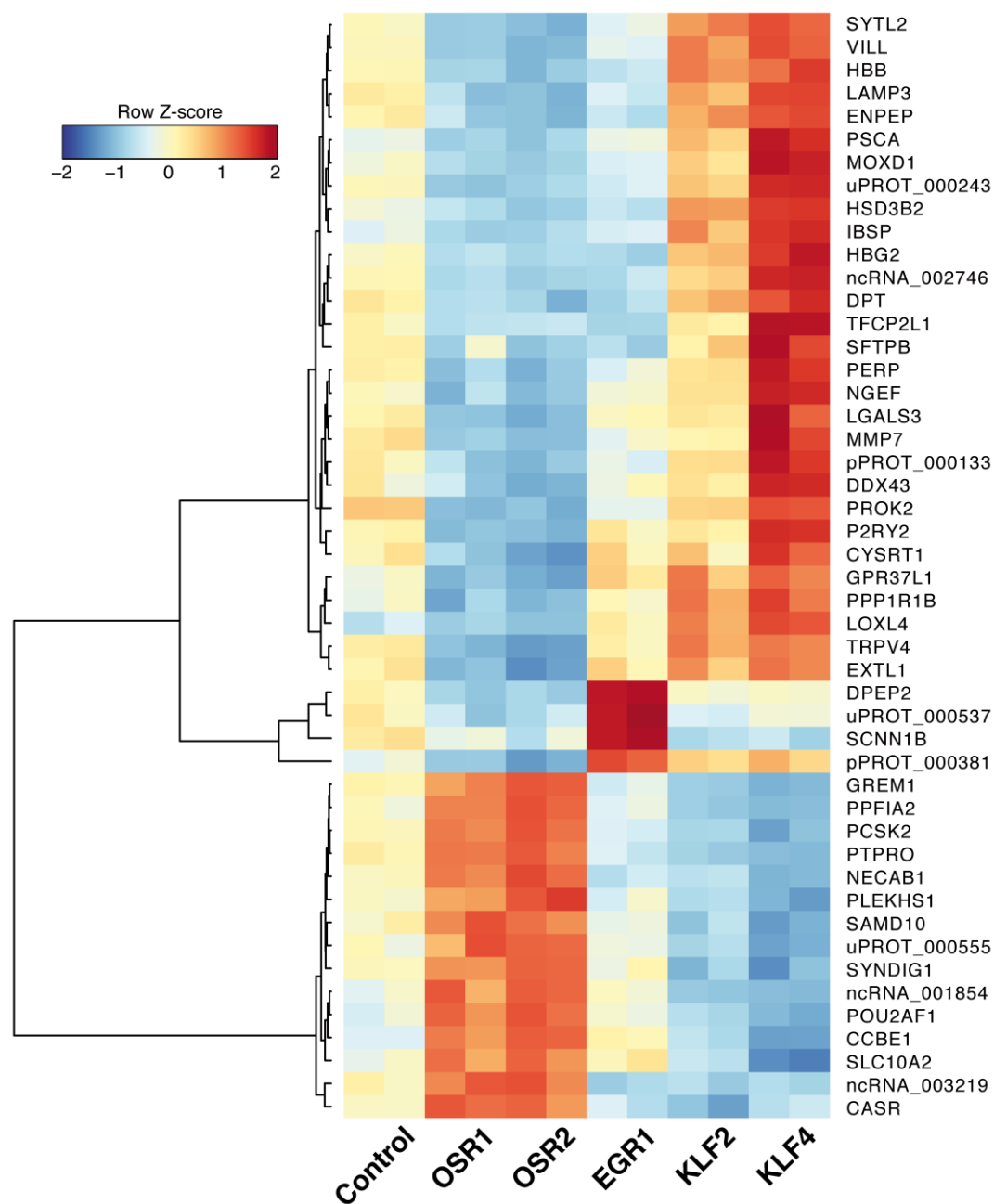


Fig. S4. Consistency among the TF regulatory patterns. Heatmap of the 48 shared DE genes regulated in opposite directions across all chMM cultures.

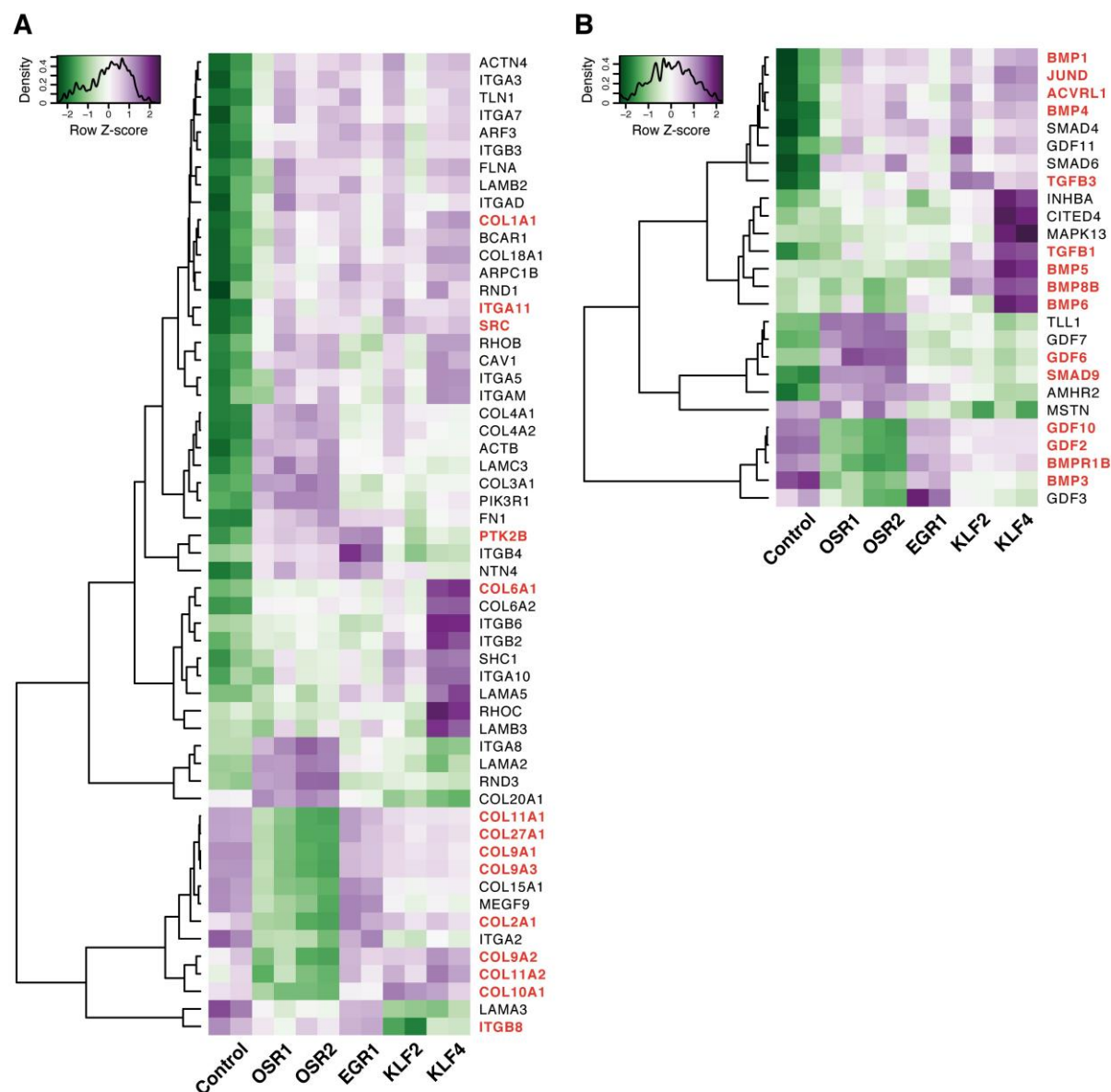


Fig. S5. DE genes associated with the Integrin and TGF- β signalling pathways. (A) Heatmap of the 56 DE genes associated with the Panther Integrin signalling pathway. (B) Heatmap of the 26 DE genes associated with the Panther TGF- β signalling pathway. Genes depicted in red are associated with GO terms related to cartilage and bone development.

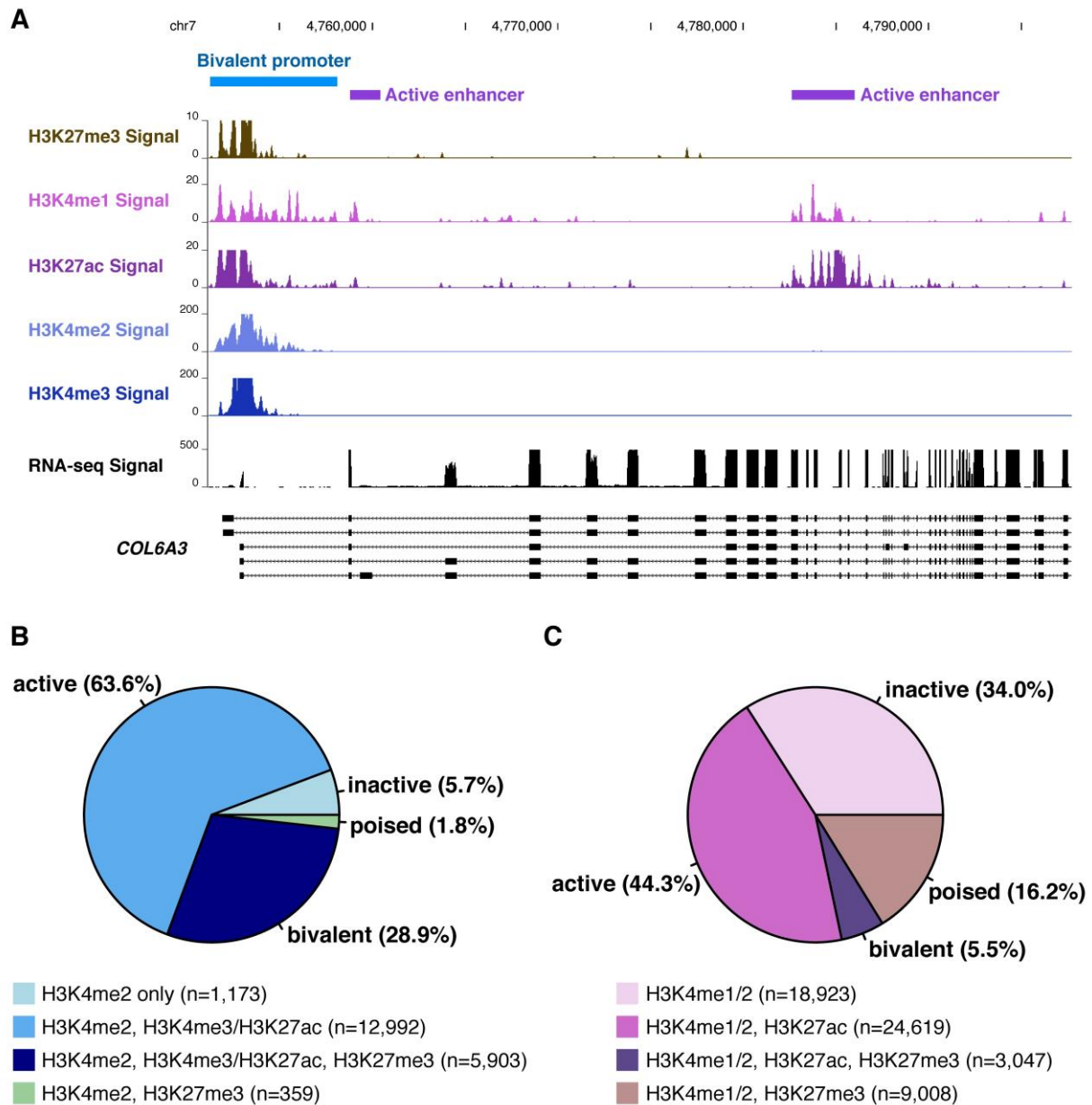


Fig. S6. Chromatin regulatory landscape in chMM cultures. (A) Chromatin landscape in the vicinity of *COL6A3* gene in chMM cultures. Five covalent histone tail modifications were investigated genome-wide in chMM cultures infected with retroviruses carrying no recombinant protein: H3K4me2 (blue), H3K4me3 (dark blue), H3K4me1 (pink), H3K27ac (dark purple) and H3K27me3 (brown). (B) Promoter regulatory domains. 20,427 promoters were identified and divided into four chromatin states: inactive (light blue), poised (green), active (blue) and bivalent (dark blue). (C) Enhancer regulatory domains. 55,597 enhancers were identified and divided into four chromatin states: inactive (light pink), poised (brown), active (purple) and bivalent (dark purple).

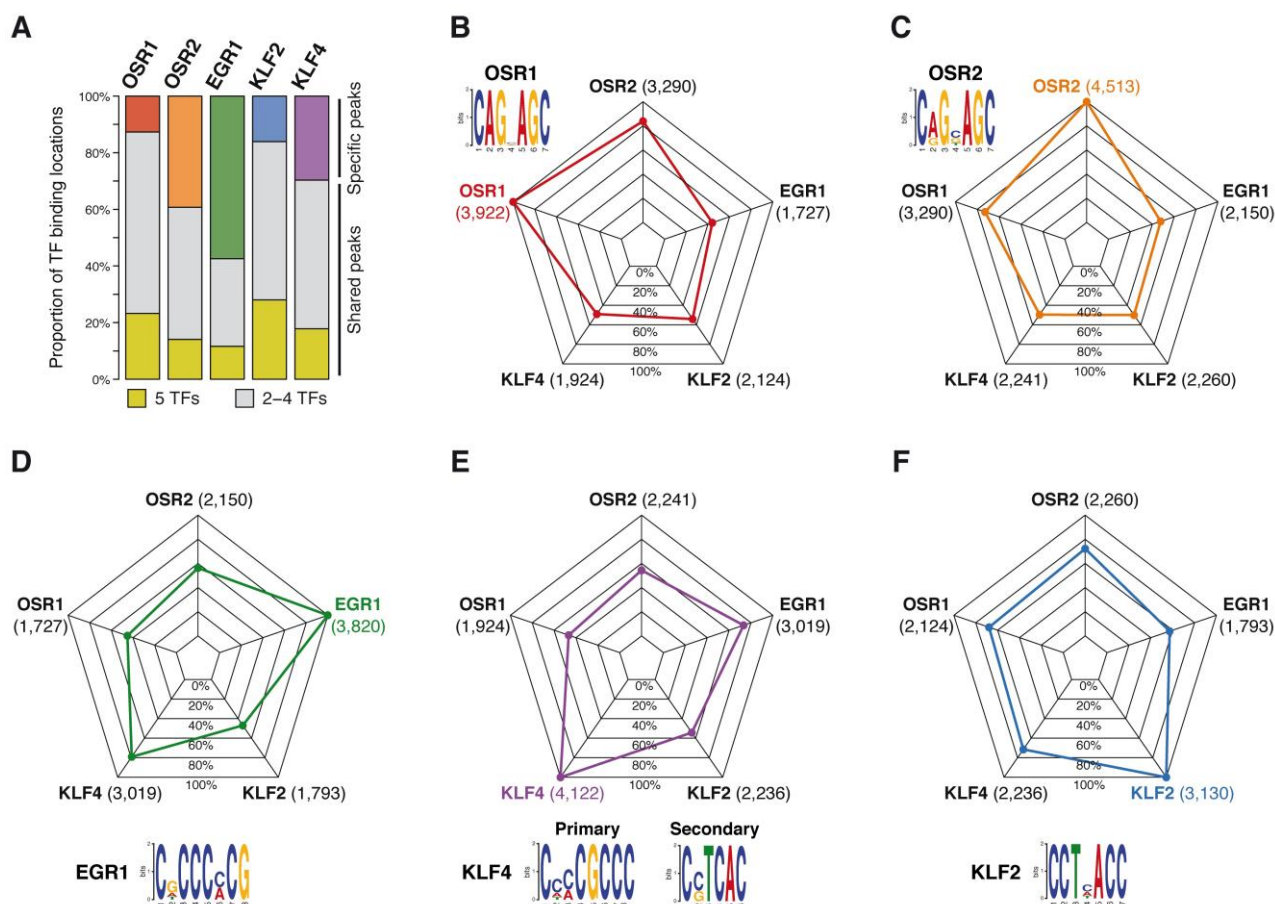


Fig. S7. Genome-wide TF binding patterns. (A) Proportion of TF binding regions specific to a single TF and shared by multiple TFs. (B-F) Pairwise comparisons of shared occupancy among the five TFs for OSR1 (B), OSR2 (C), EGR1 (D), KLF4 (E) and KLF2 (F).

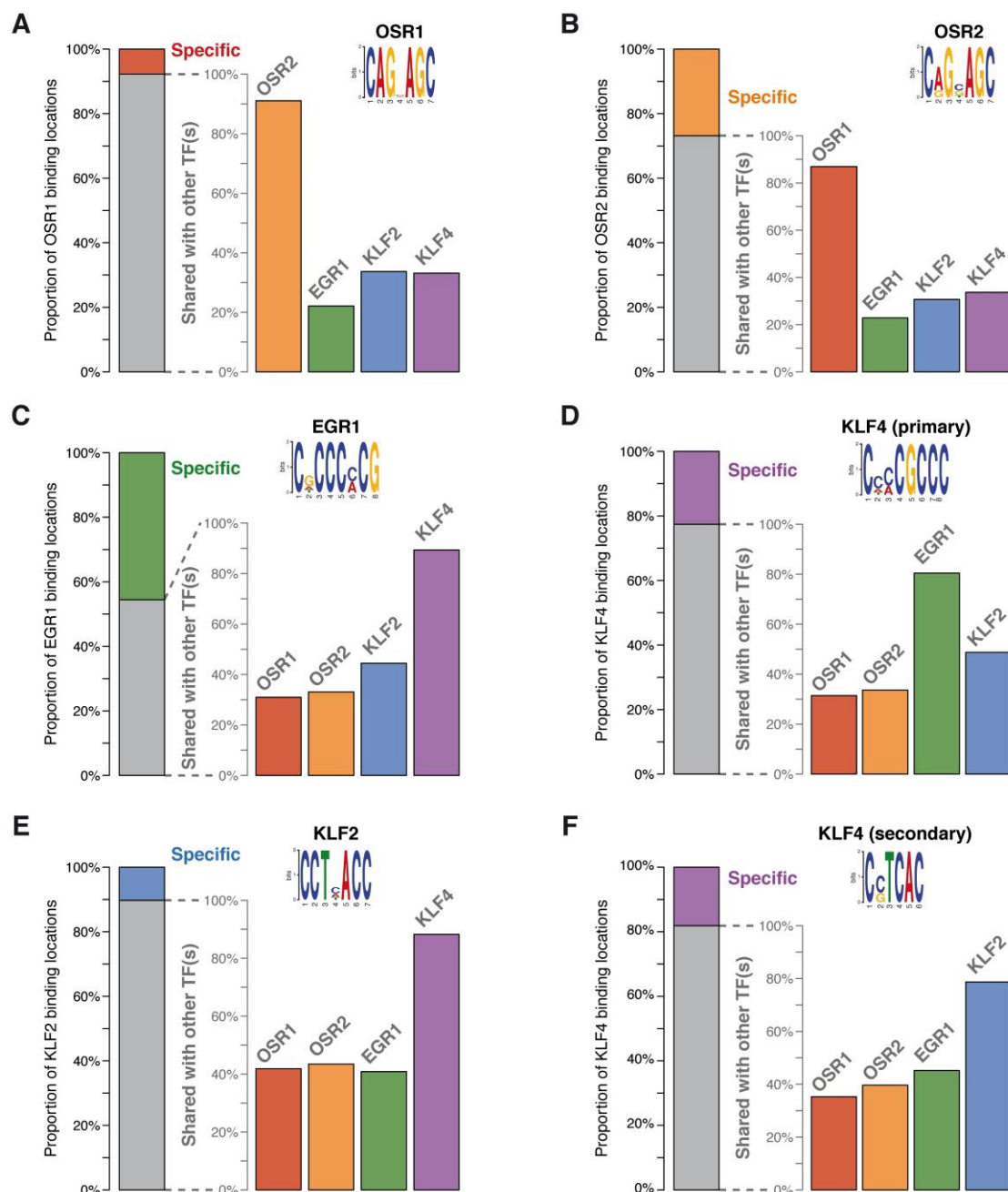


Fig. S8. TF occupancy among shared target genes. (A,B) Pairwise comparisons of TF occupancy among the 318 direct target genes shared by OSR1 and OSR2 for OSR1 (A) and OSR2 (B) binding locations. (C,D) Pairwise comparisons of TF occupancy among the 317 direct target genes shared by EGR1 and KLF4 for EGR1 (C) and KLF4 (D) binding locations. (E,F) Pairwise comparisons of TF occupancy among the 313 direct target genes shared by KLF2 and KLF4 for KLF2 (E) and KLF4 (F) binding locations.

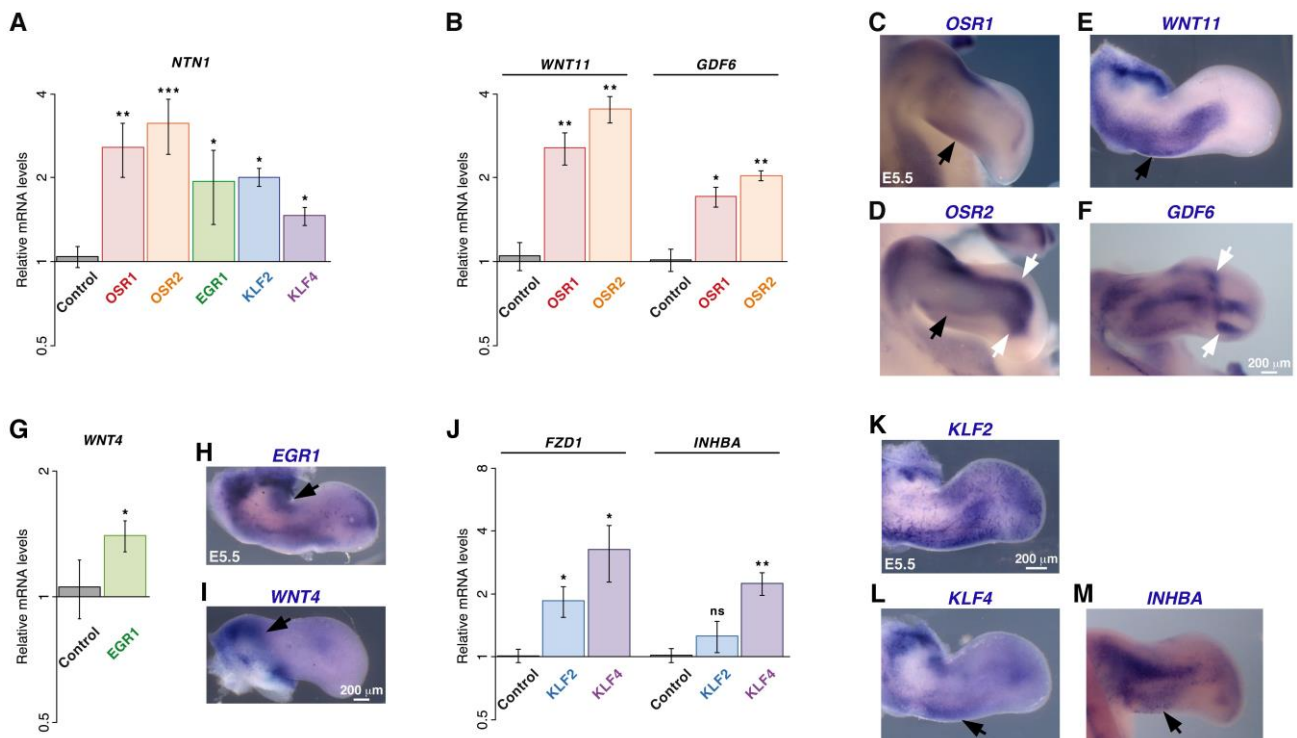


Fig. S9. Validation of selected candidate target genes. (A) Quantitative RT-PCR analysis of *NTN1* expression in TF-overexpressing chMM cultures. (B) Quantitative RT-PCR analysis of *WNT11* and *GDF6* expression in *OSR1*- and *OSR2*-overexpressing chMM cultures. (C-F) Whole-mount in situ hybridization to forelimbs of E5.5 chick embryos with *OSR1* (C), *OSR2* (D), *WNT11* (E) and *GDF6* (F) probes (blue). Black arrows indicate *OSR1*, *OSR2* and *WNT11* overlapping expression domains, while white arrows indicate *OSR2* and *GDF6* overlapping expression domains. (G) Quantitative RT-PCR analysis of *WNT4* expression in *EGR1*-overexpressing chMM cultures. (H,I) Whole-mount in situ hybridization to forelimbs of E5.5 chick embryos with *EGR1* (H) and *WNT4* (I) probes (blue). Areas of overlapping expression are indicated by arrows. (J) Quantitative RT-PCR analysis of *FZD1* and *INHBA* expression in *KLF2*- and *KLF4*-overexpressing chMM cultures. (K-M) Whole-mount in situ hybridization to forelimbs of E5.5 chick embryos with *KLF2* (K), *KLF4* (L) and *INHBA* (M) probes (blue). Areas of overlapping expression are indicated by arrows. (A,B,G,J) Quantitative RT-PCR graphs depict relative mRNA levels: mean \pm SEM; two-tailed Mann-Whitney U test: ns, non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

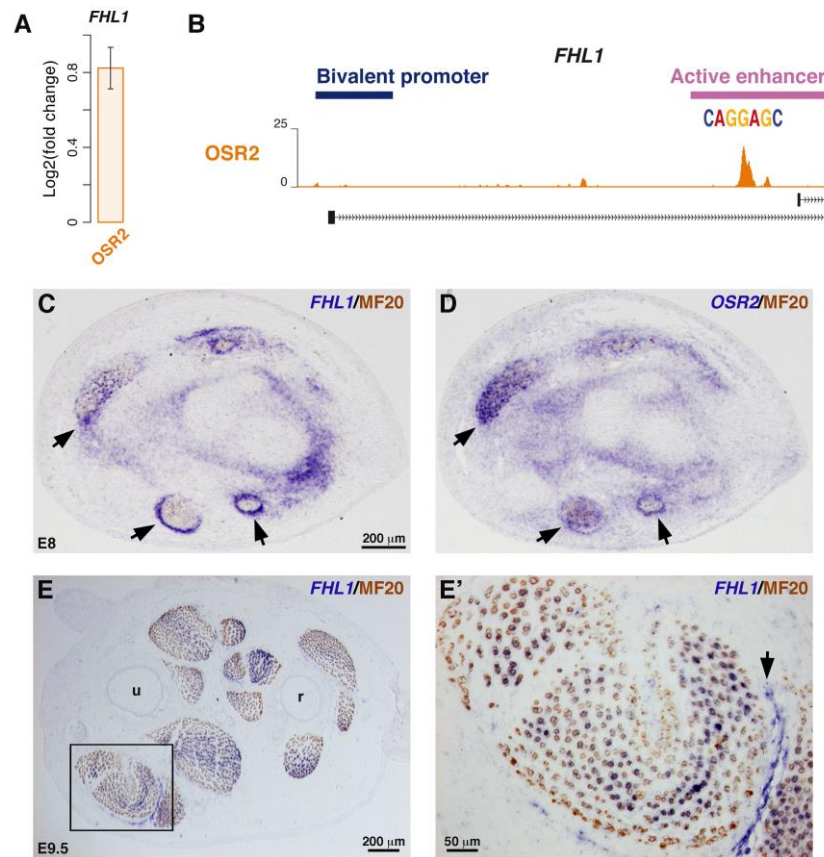


Fig. S10. *FHL1* as direct target of *OSR2*. (A) *FHL1* expression levels in *OSR2*-overexpressing chMM cultures determined by RNA-seq. (B) Binding site and motif for *OSR2* identified by ChIP-seq within an intronic enhancer of *FHL1* gene. (C-E') In situ hybridization to forelimbs of E8 (C,D) and E9.5 (E,E') chick embryos followed by immunohistochemistry with the MF20 antibody (brown). (C,D) Adjacent and transverse limb sections were hybridized with *FHL1* (C) and *OSR2* (D) probes (blue). Areas of overlapping expression are indicated by arrows. (E,E') Transverse limb sections were hybridized with *FHL1* probes (blue). *FHL1* is expressed in MF20⁺ myotubes, but also in CT (arrow). (E') is a higher magnification of the boxed area in (E). u, ulna; r, radius.

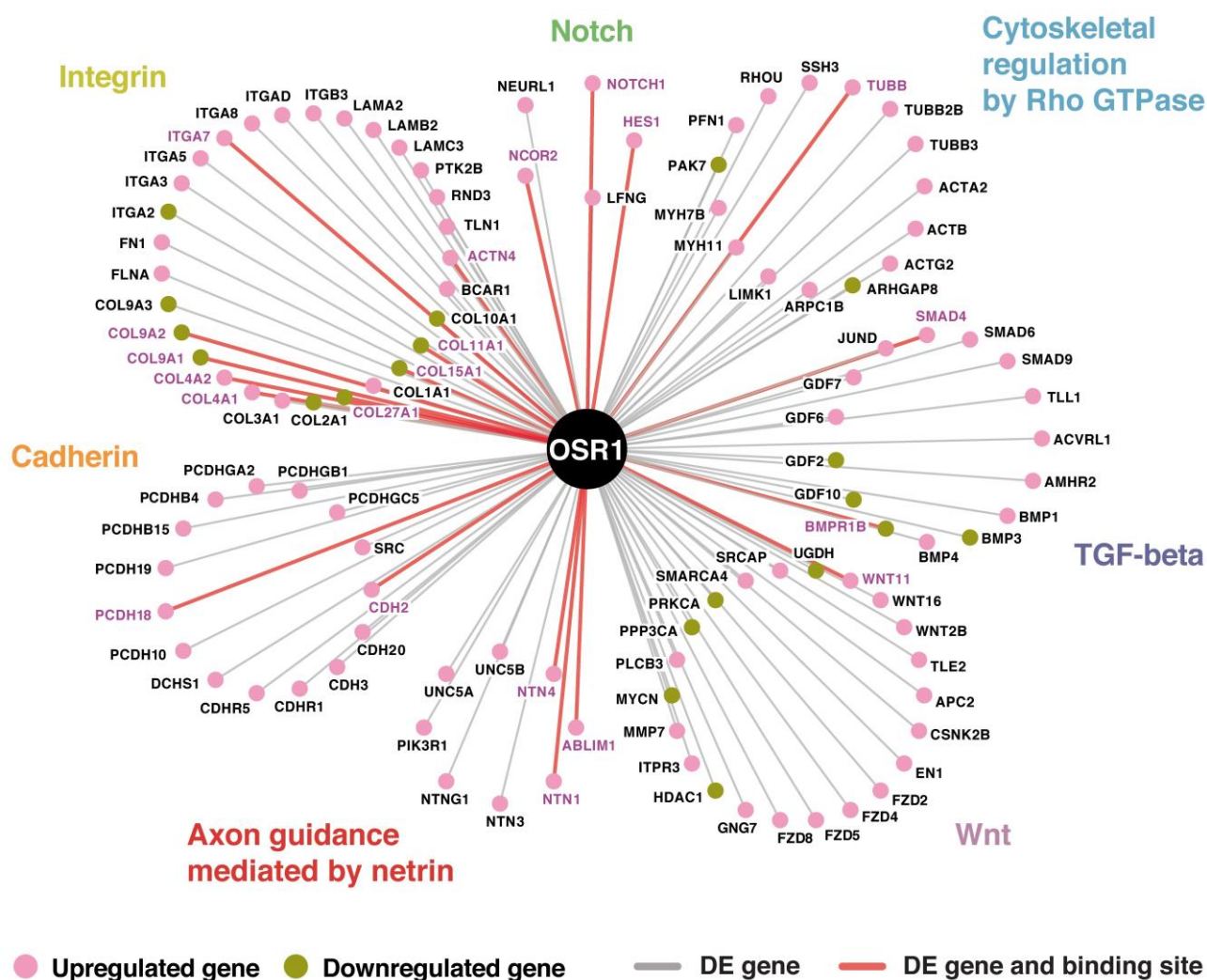


Fig. S11. Molecular signatures regulated by OSR1. Circular representation of DE genes identified in chMM cultures upon OSR1 overexpression that are associated with the selected Panther signalling pathways. Downregulated genes are depicted as brown nodes and upregulated genes as pink nodes. Connections in red correspond to direct interactions between OSR1 and its target genes, while indirect interactions are depicted in grey.

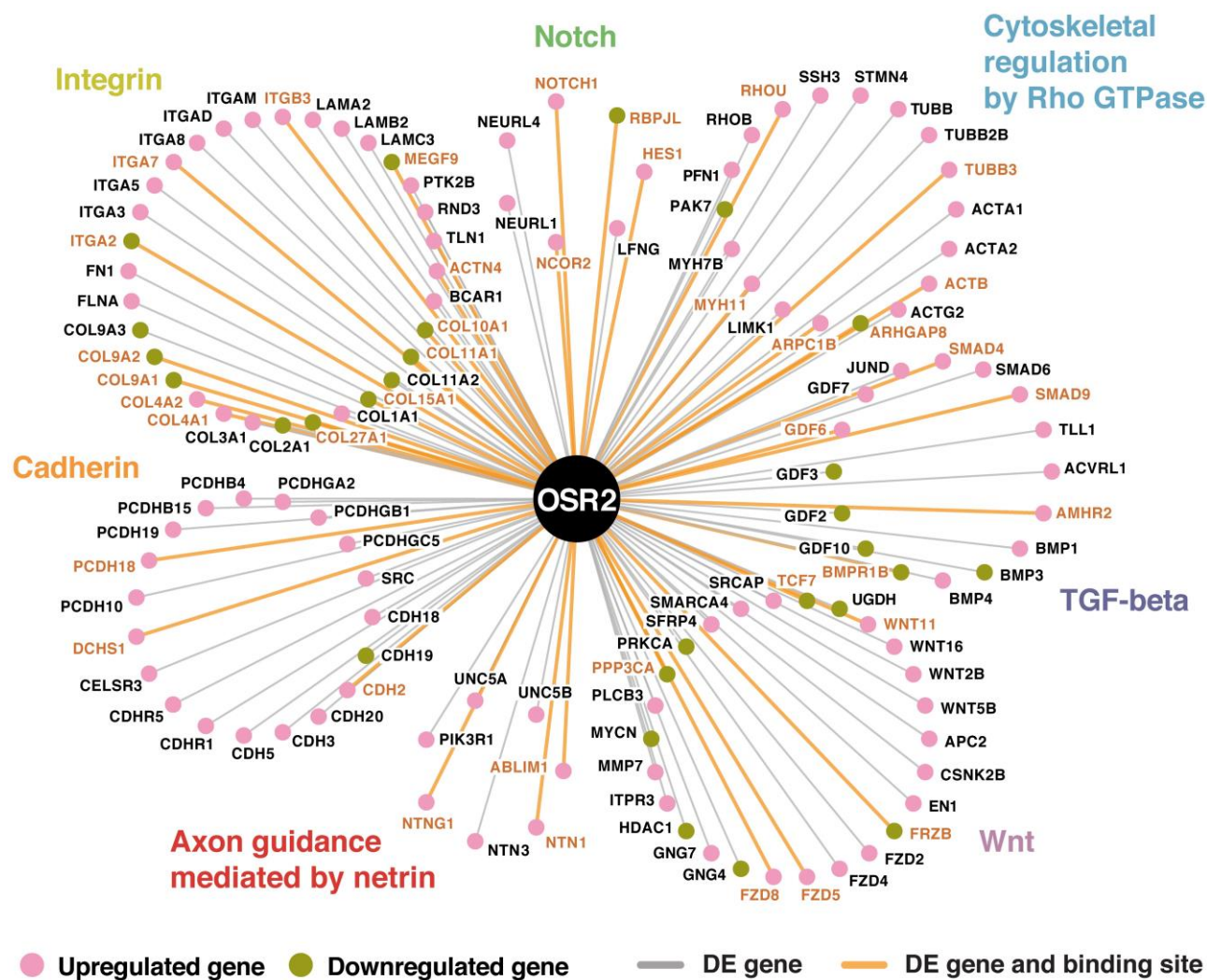


Fig. S12. Molecular signatures regulated by OSR2. Circular representation of DE genes identified in chMM cultures upon OSR2 overexpression that are associated with the selected Panther signalling pathways. Downregulated genes are depicted as brown nodes and upregulated genes as pink nodes. Connections in orange correspond to direct interactions between OSR2 and its target genes, while indirect interactions are depicted in grey.

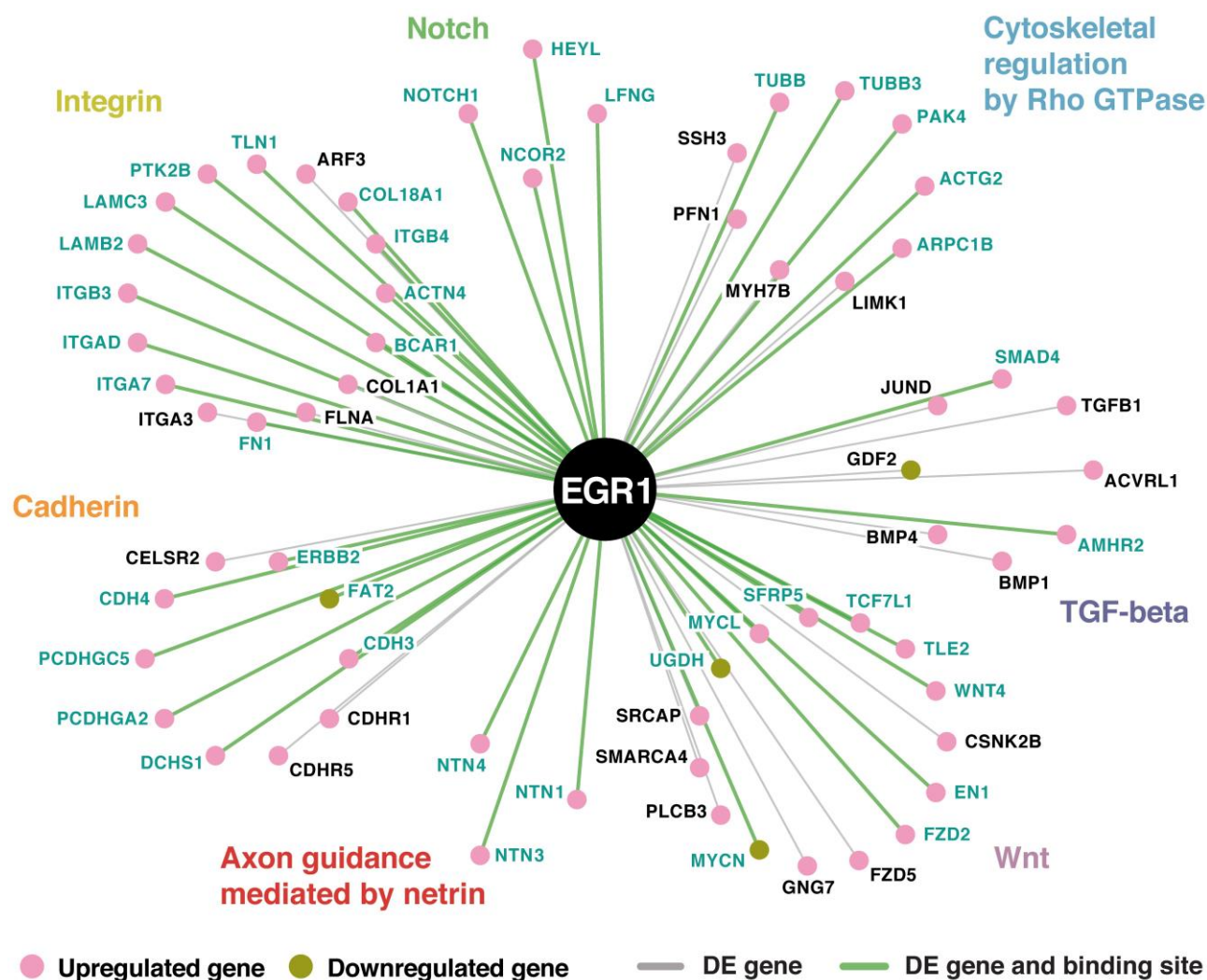


Fig. S13. Molecular signatures regulated by EGR1. Circular representation of DE genes identified in chMM cultures upon EGR1 overexpression that are associated with the selected Panther signalling pathways. Downregulated genes are depicted as brown nodes and upregulated genes as pink nodes. Connections in green correspond to direct interactions between EGR1 and its target genes, while indirect interactions are depicted in grey.

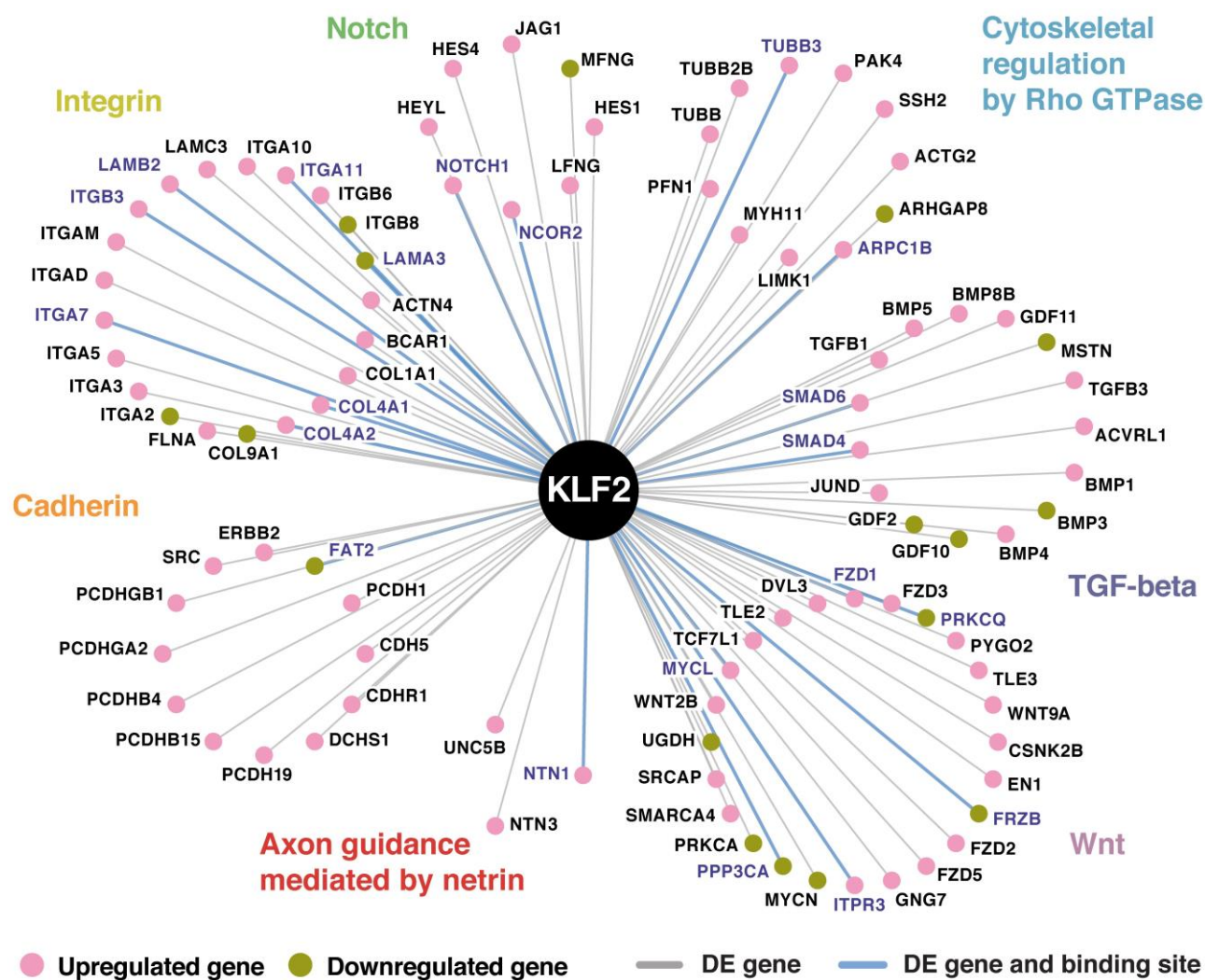


Fig. S14. Molecular signatures regulated by KLF2. Circular representation of DE genes identified in chMM cultures upon KLF2 overexpression that are associated with the selected Panther signalling pathways. Downregulated genes are depicted as brown nodes and upregulated genes as pink nodes. Connections in blue correspond to direct interactions between KLF2 and its target genes, while indirect interactions are depicted in grey.

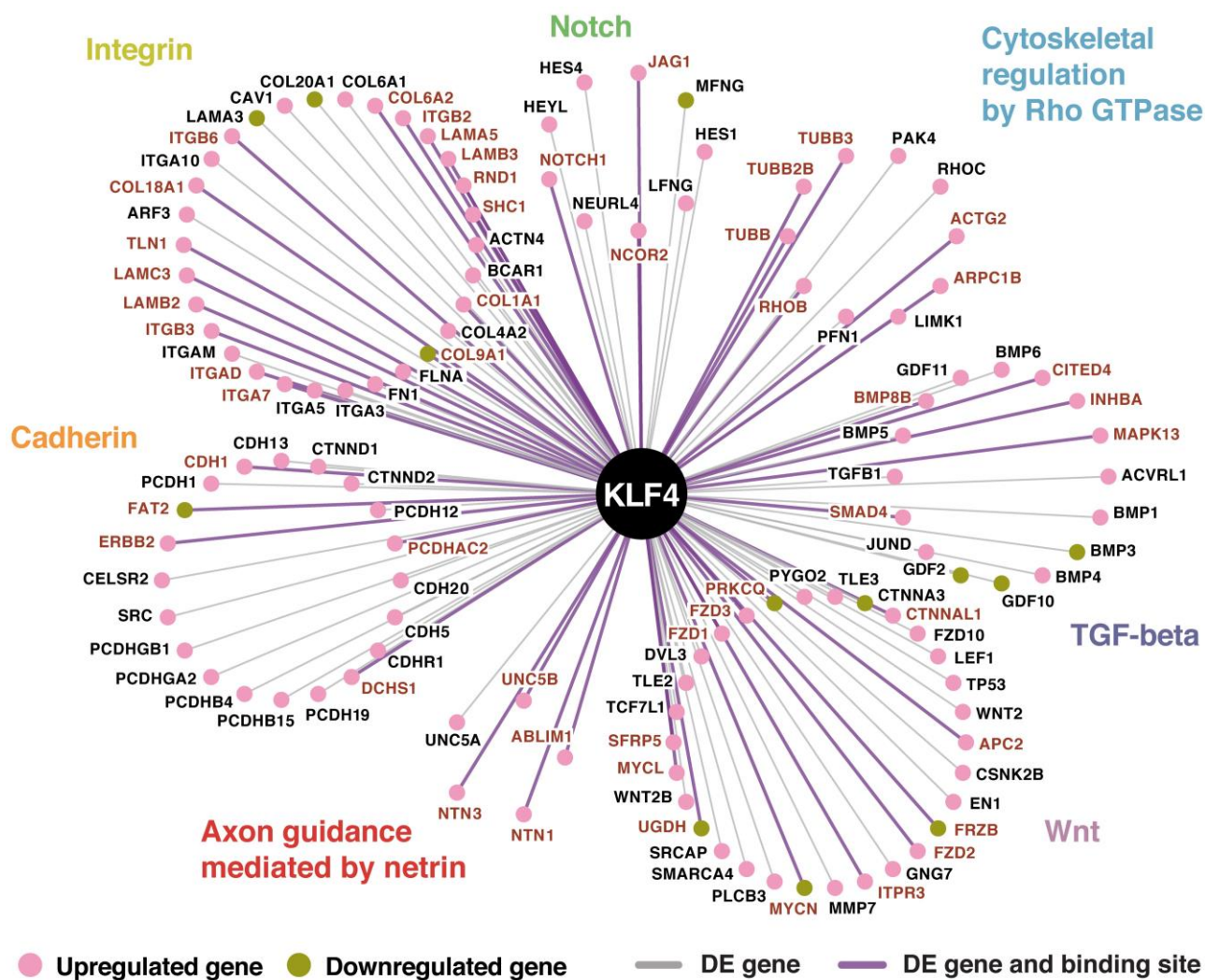


Fig. S15. Molecular signatures regulated by KLF4. Circular representation of DE genes identified in chMM cultures upon KLF4 overexpression that are associated with the selected Panther signalling pathways. Downregulated genes are depicted as brown nodes and upregulated genes as pink nodes. Connections in purple correspond to direct interactions between KLF4 and its target genes, while indirect interactions are depicted in grey.

Supplementary Tables

Table S1. Table combining RNA-seq and ChIP-seq data.

[Click here to Download Table S1](#)

Table S2. List of primers.

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Table S3. RNA-seq mapping and assignment metrics.

[Click here to Download Table S3](#)

Table S4. Histone modification ChIP-seq metrics.

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Table S5. Transcription Factor ChIP-seq metrics.

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