Supplemental Figures SF1-SF8

Precocious Chondrocyte Differentiation Disrupts Skeletal Growth in Kabuki Syndrome Mice

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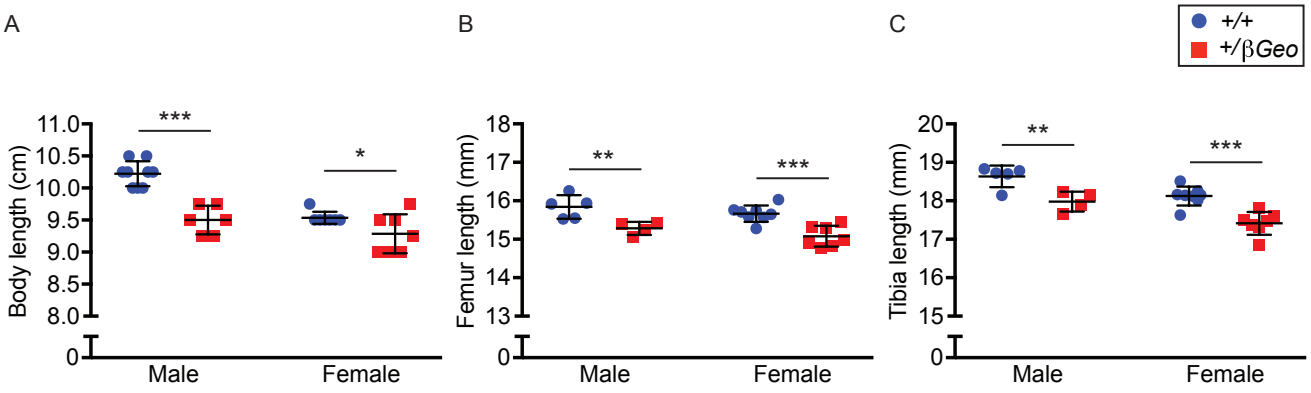
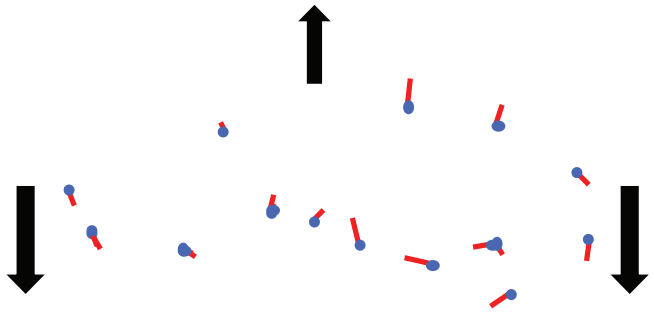
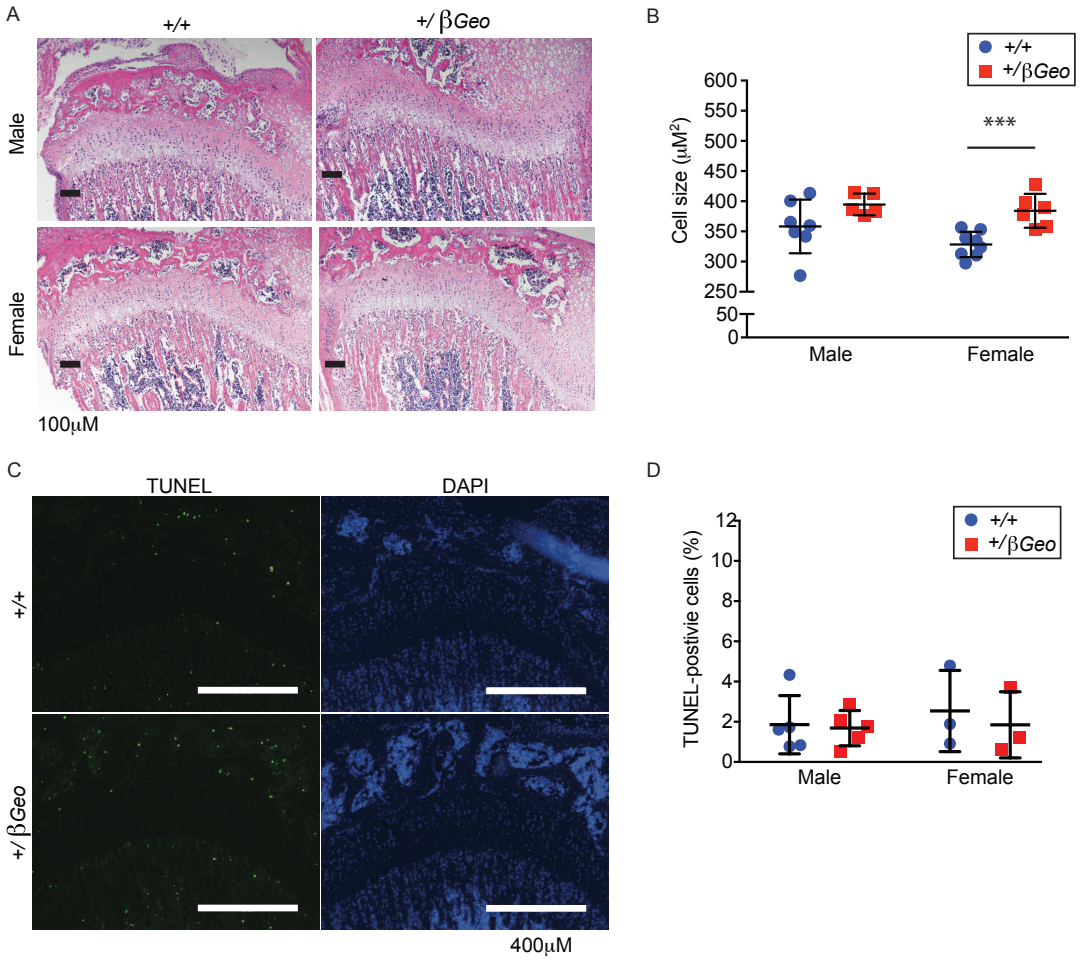


Supplemental Figure 1: Kabuki syndrome 1 mice have consistent skeletal growth retardation at 18 weeks. (A) Quantification of body length in 18 week old *Kmt2d*$^{+/+}$ male (n=9) and female (n=7) mice and *Kmt2d*$^{+/\beta Geo}$ male (n=6) and female (n=7) mice. Quantification of femur **(B)** and tibia **(C)** length in 18 week old *Kmt2d*$^{+/+}$ male (n=5) and female (n=8) mice and *Kmt2d*$^{+/\beta Geo}$ male (n=4) and female (n=7) mice. Data represent mean ± standard deviation. One-sided unpaired Student's t-test; *P<0.05, **P<0.01, ***P<0.001.

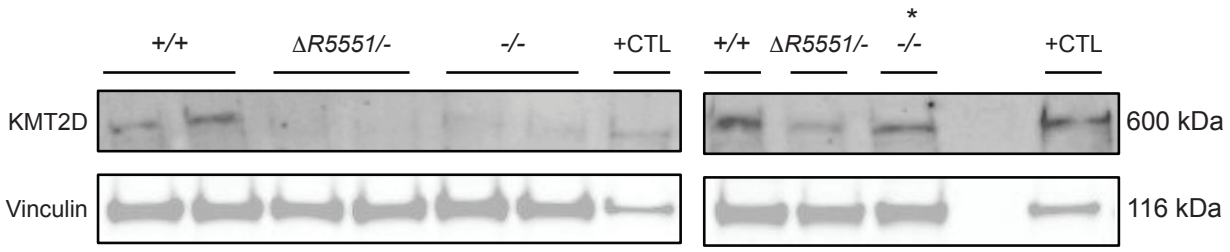


PC1-Left Lateral View

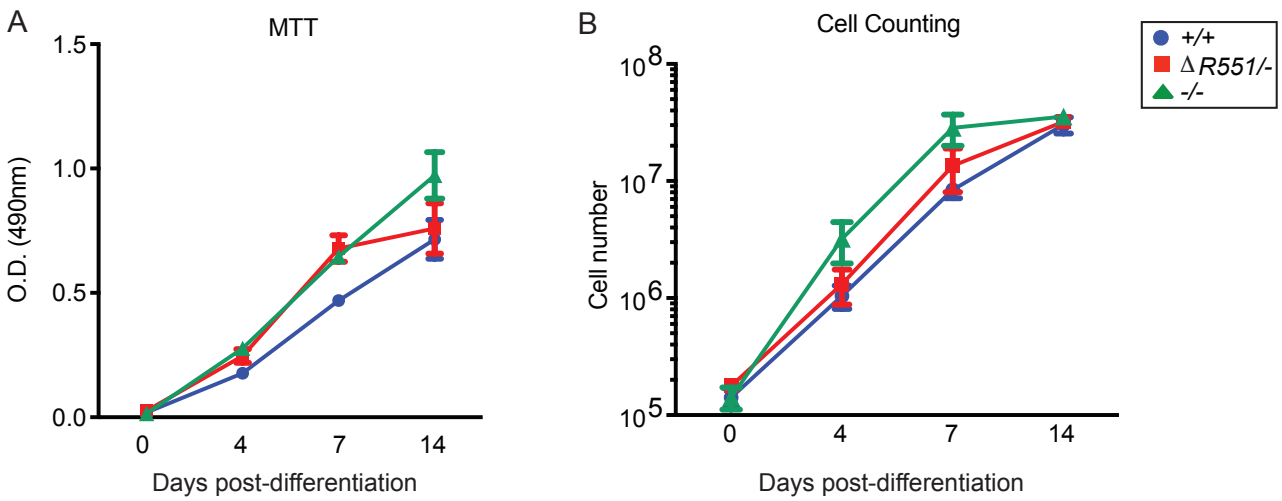
Supplemental Figure 2: *Kmt2d*$^{+/\beta Geo}$ mice exhibit ventral bowing, brachycephaly, and dorsal expansion of the skull compared to *Kmt2d*$^{+/+}$ mice. Blue points represent landmarks and show mean form of the sample in left lateral view. Red vectors show displacement of landmarks from the mean form in *Kmt2d*$^{+/\beta Geo}$ mice. Thick black arrows illustrate overall direction of displacement, suggesting ventral bowing, dorsal expansion, and brachycephaly. n=21 *Kmt2d*$^{+/+}$ mice, n=13 *Kmt2d*$^{\beta geo/+}$ mice.



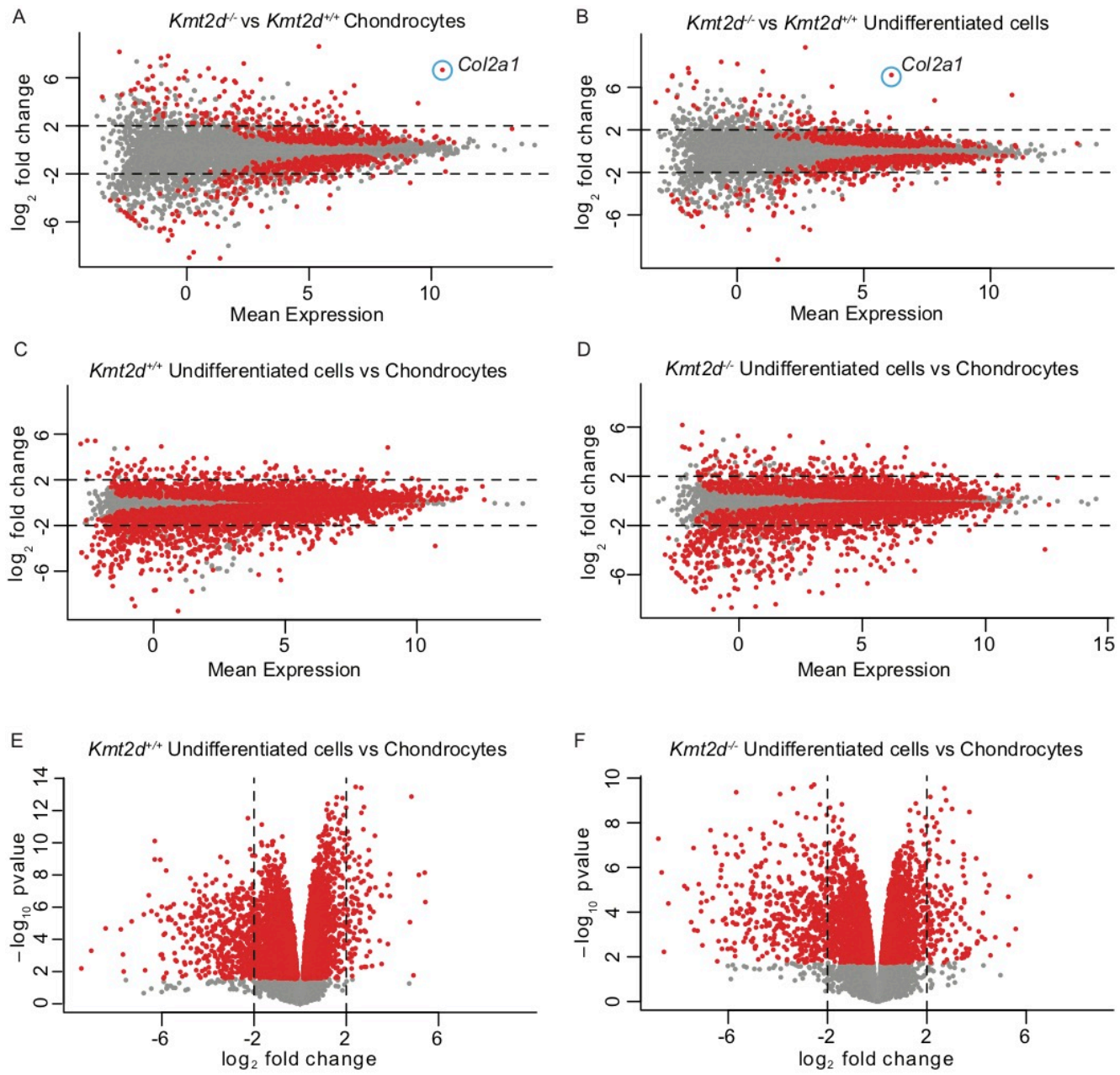
Supplemental Figure 3: Further characterization of Kabuki syndrome 1 proximal tibia growth plates. (A) Low magnification proximal tibia growth plate images from *Kmt2d*$^{+/+}$ and *Kmt2d*$^{+/\beta Geo}$ mice; images correspond to representative images in Figure 3A. **(B)** Female but not male *Kmt2d*$^{+/\beta Geo}$ mice exhibit increased hypertrophic chondrocyte cell size compared to *Kmt2d*$^{+/+}$ mice. Hypertrophic chondrocytes from H and E stained proximal tibia growth plate sections were measured and analyzed for cell size using Image J (n=15 *Kmt2d*$^{+/+}$ mice; 7 males and 8 females; n=11 *Kmt2d*$^{+/\beta Geo}$ mice; 5 males and 6 females). Data represent mean ± standard deviation; one-sided unpaired Student's t-test; ***P<0.001. **(C)** TUNEL staining and **(D)** quantification showing no difference in cell death between *Kmt2d*$^{+/\beta Geo}$ and *Kmt2d*$^{+/+}$ growth plates (n=8 *Kmt2d*$^{+/+}$ mice; 5 males and 3 females; n=8 *Kmt2d*$^{+/\beta Geo}$ mice; 5 males and 3 females). Data represent mean ± standard deviation. Two-sided unpaired Student's t-test. P not significant.



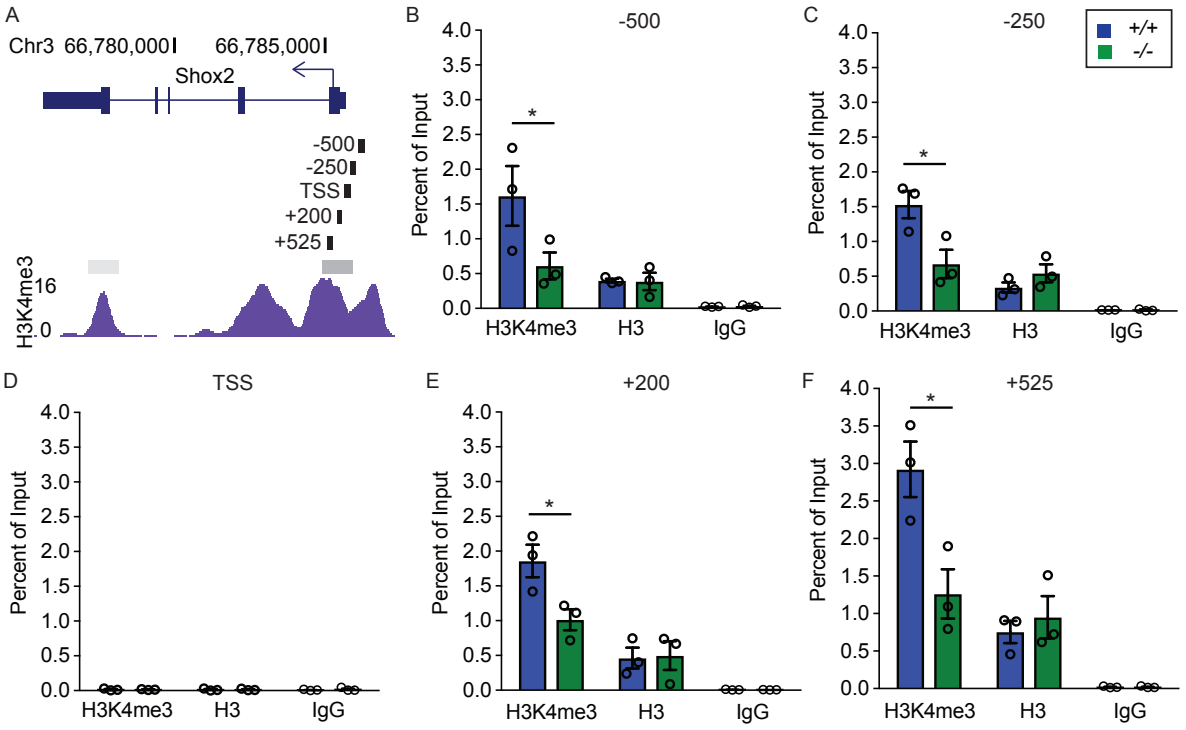
Supplemental Figure 4: KMT2D protein expression in *Kmt2d*$^{+/+}$, *Kmt2d*$^{\Delta R5551/-}$, and *Kmt2d*$^{-/-}$ stable cell lines. *Kmt2d*$^{+/+}$ stable cell lines express KMT2D (600kDa), similar to the positive control cell lysate (+CTL). *Kmt2d*$^{\Delta R5551/-}$ and *Kmt2d*$^{-/-}$ express little to no KMT2D protein, suggesting a hypomorphic and/or loss of function mutation mechanism. The *Kmt2d*$^{-/-}$ clonal cell line indicated with an asterisk continues to express KMT2D protein at similar levels to *Kmt2d*$^{+/+}$ stable cell lines and therefore was not used in experimental analyses. Vinculin was used as loading control.



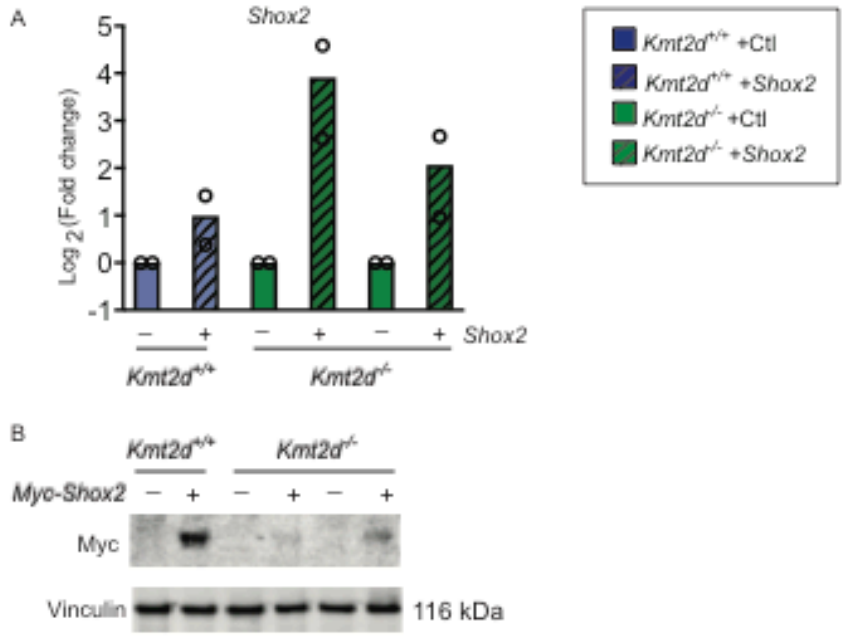
Supplemental Figure 5. Cell proliferation trends toward being increased in *Kmt2d*$^{-/-}$ chondrocytes compared to *Kmt2d*$^{+/+}$ chondrocytes at day 7 and day 14 post-induction of differentiation. Cellular proliferation was measured using the **(A)** MTT assay and **(B)** cell counting both prior to differentiation and at 4, 7, and 14 days after induction of differentiation. Data represent mean ± standard error of the mean; the experiment was performed two times with 3 independent stable cell lines per time point (n=6) for *Kmt2d*$^{+/+}$ and *Kmt2d*$^{\Delta R5551/-}$ and with 2 independent stable cell lines per time point (n=4) for *Kmt2d*$^{-/-}$; mixed model ANOVA with Tukey's adjustment method within each time point; P values not significant.



Supplemental Figure 6: The number of genes required to undergo chondrocyte differentiation greatly exceeds the number altered upon loss of *Kmt2d*. MA plots comparing differentially expressed genes in *Kmt2d*$^{-/-}$ and *Kmt2d*$^{+/+}$ **(A)** chondrocytes and **(B)** undifferentiated mesenchymal cells. **(C, D)** MA plots and **(E, F)** volcano plots comparing differentially expressed genes in **(C, E)** *Kmt2d*$^{+/+}$ and **(D, F)** *Kmt2d*$^{-/-}$ cells over the course of differentiation from mesenchymal cells to chondrocytes. Chondrocytes were differentiated for 7 days.



Supplemental Figure 7. H3K4me3 levels are depleted surrounding the *Shox2* transcription start site in *Kmt2d*$^{-/-}$ cells whereas levels of H3 remain constant. (A) Schematic indicating the genomic location of the primers used for quantitative PCR following chromatin immunoprecipitation (ChIP-qPCR) in relation to the levels and known peak of H3K4me3 at the 5' end of the *Shox2* gene in mouse embryonic limb bud from the ENCODE data set. The *Shox2* gene and its orientation, exons, and 5' and 3' untranslated regions are indicated with genomic coordinates above. Black vertical bars indicate positions of amplicons amplified by primer sets, and the adjacent numbers indicate the approximate midpoint of each amplicon in relation to the transcription start site (TSS) of *Shox2.* Solid gray horizontal bars indicate peaks of H3K4me3 with the darker gray bar indicating the peak of interest. The purple plot indicates relative H3K4me3 enrichment. ChIP-qPCR revealed depletion of H3K4me3 in *Kmt2d*$^{-/-}$ cells compared to *Kmt2d*$^{+/+}$ cells both **(B, C)** upstream and **(E, F)** downstream of the transcription start site. Levels of H3 remained constant in *Kmt2d*$^{-/-}$ and *Kmt2d*$^{+/+}$ cells **(B, C)** upstream and **(E, F)** downstream of the transcription start site. Neither H3K4me3 nor H3 was found at the **(D)** transcription start site in the presumed nucleosome-free region. Anti-IgG antibody was used as a negative control. Data represent mean ± standard error of the mean; n=3 per group; one-sided unpaired Student's t-test; *P<0.05.



Supplemental Figure 8. Increased *Shox2* transcript levels and Myc-SHOX2 protein overexpression in individual *Kmt2d*$^{-/-}$ and *Kmt2d*$^{+/+}$ stable cell lines. (A) *Shox2* overexpression by qRT-PCR with primers specific to *mShox2*. Blue bars represent *Kmt2d*$^{+/+}$ stable cell lines and green bars represent *Kmt2d*$^{-/-}$ stable cell lines. Solid bars represent cells infected with control lentivirus, and hatched bars represent cells infected with lentivirus overexpressing *Shox2.* The experiment was performed in duplicate (n=2 per group). **(B)** Western blot using anti-Myc antibody reveals Myc-SHOX2 overexpression in *Kmt2d*$^{+/+}$ cells and in one *Kmt2d*$^{-/-}$ cell line. Vinculin serves as a loading control. Ctl, control Lentiviral-ORF particles.

Supplemental Methods

Histology and histomorphometry

For hypertrophic chondrocyte cell size measurement, hypertrophic chondrocytes from H and E stained proximal tibia growth plate sections were measured for cell size using ImageJ. Average cell size was determined by measuring the area of 60 cells (fifteen hypertrophic chondrocytes per section and four sections per mouse). Cells were chosen from approximately the same level within the hypertrophic zone for comparison.

Terminal Transferase dUTP Nick End Labelling (TUNEL) Assay

TUNEL assays were performed according to the manufacturer's protocol (Promega, G3250). Images were collected using the EVOS FL Auto Imaging System (Thermo Fisher Scientific). The green fluorescent TUNEL positive cells were counted as a percentage of the total number of DAPI-stained cells in the growth plate. A cell counting tool in ImageJ was employed to count one section per mouse with total five male and six female mice.

MTT cell proliferation assay

ATDC5 stable cell lines (*Kmt2d*$^{+/+}$, *Kmt2d*$^{\Delta R5551/-}$, *Kmt2d*$^{-/-}$) were maintained and differentiated as described in the Methods section of the main text and seeded at 1×10^3 cells/well in 96-well plates for the MTT assay. At the indicated time points, 10 µL of cell proliferation reagent (MTS) was added to the medium and incubated at 37° C, 5% CO_2 for 1.5 hrs, as per manufacturer's recommendation (Promega). Absorbance was measured at 490 nm using a Biotek synergy 2 plate reader.

Cell counting assay

ATDC5 stable cell lines (*Kmt2d*$^{+/+}$, *Kmt2d*$^{\Delta R5551/-}$, *Kmt2d*$^{-/-}$) were maintained and differentiated as described in the Methods section of the main text and seeded at 6.3×10^3 cells/well in 24-well plates for cell counting. At the indicated time points, cells were trypsinized and stained with a 0.4 % trypan blue solution (Corning). Cells were manually counted using a hemocytometer.

Quantitative PCR (qPCR) Primer Sequences

The primer sequences (all mouse) are: *Gapdh*: 5'-TGGCCTTCCGTGTTCCTAC-3' and 5'-GAGTTGCTGTTGAAGTCGCA-3'; *Sox9*: 5'-CGGAACAGACTCACATCTCTCC-3' and 5'-GCTTGCACGTCGGTTTTGG-3'; *Col2a1*: 5'-CGGTCCTACGGTGTCAGG-3' and 5'-GCAGAGGACATTCCCAGTGT-3'; *Col10a1*: 5'-CATAAAGGGCCCACTTGCTA-3' and 5'-

CAGGAATGCCTTGTTCTCCT-3'; *Shox2*: 5'-TGGAACAACTCAACGAGCTGGAGA-3' and 5'-TTCAAACTGGCTAGCGGCTCCTAT-3'.