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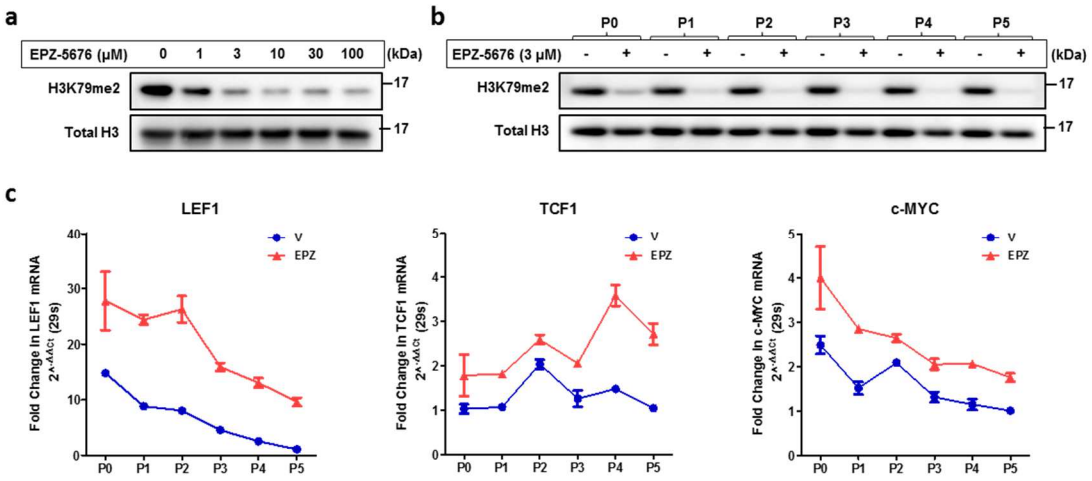
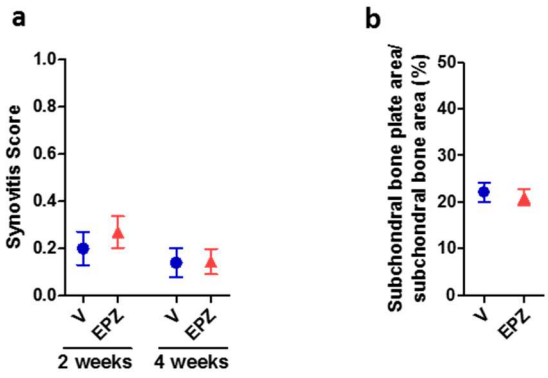
Title of file for HTML: Supplementary Information
Description: Supplementary Figures and Supplementary Tables

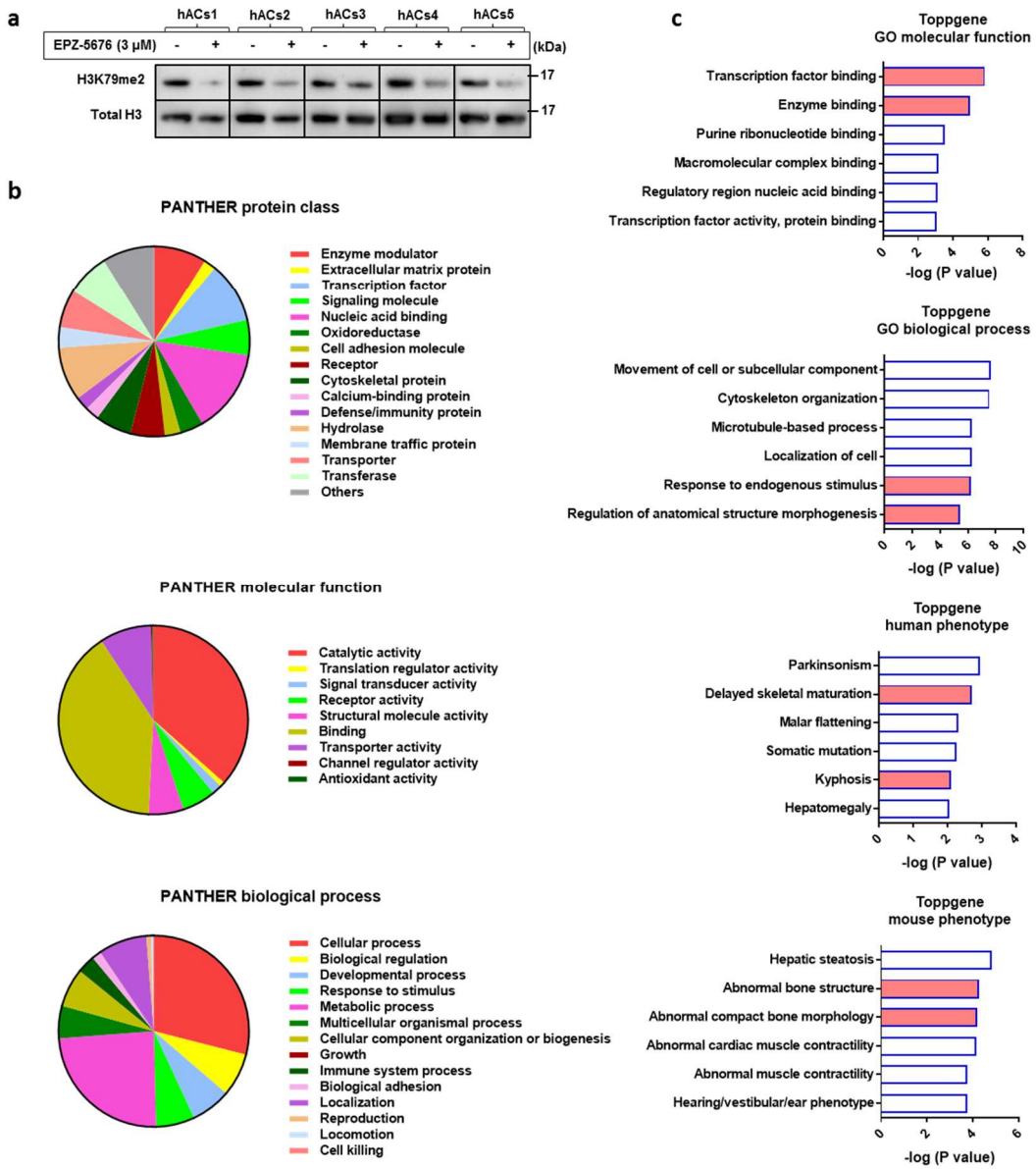
Type of file: PDF
Title of file for HTML: Peer Review File



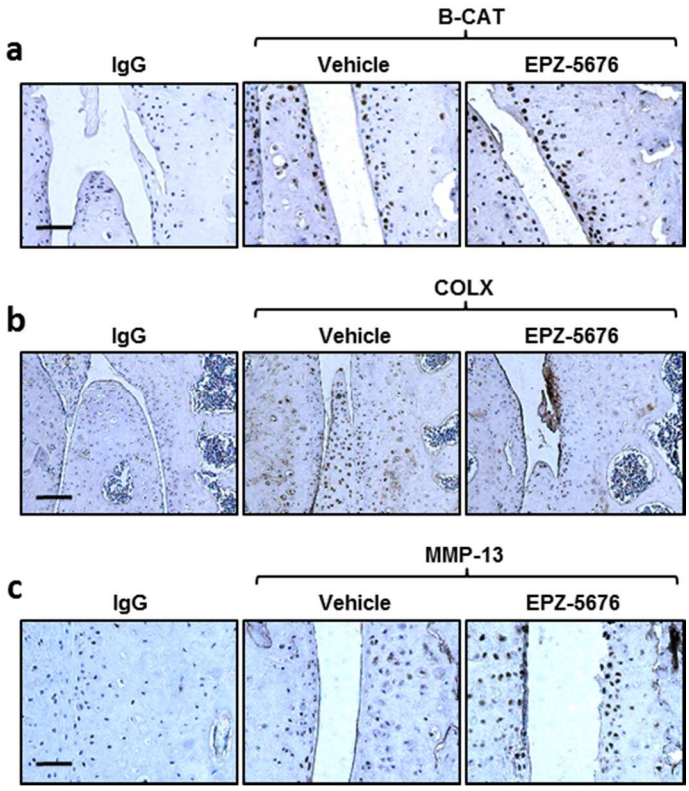
Supplementary Figure 1 | DOT1L inhibition in a chondrocyte dedifferentiation assay. (**a,b**) Immunoblot analysis showing decreased methylated H3K79 levels (H3K79me2) in human articular chondrocytes: treated with EPZ-5676 (EPZ) at the indicated concentrations or vehicle (V) for 4 days (**a**) or treated with 3 µM EPZ from passage 0 (P0) to P5 (**b**). The image is representative of one experiment. (**c**) Messenger RNA expression levels determined by quantitative PCR for *LEF1*, *TCF1* and *c-MYC* in human articular chondrocytes from P0 until P5 treated with EPZ-5676 or vehicle. Data are from one experiment with technical triplicates. Error bars indicate mean ± s.e.m.



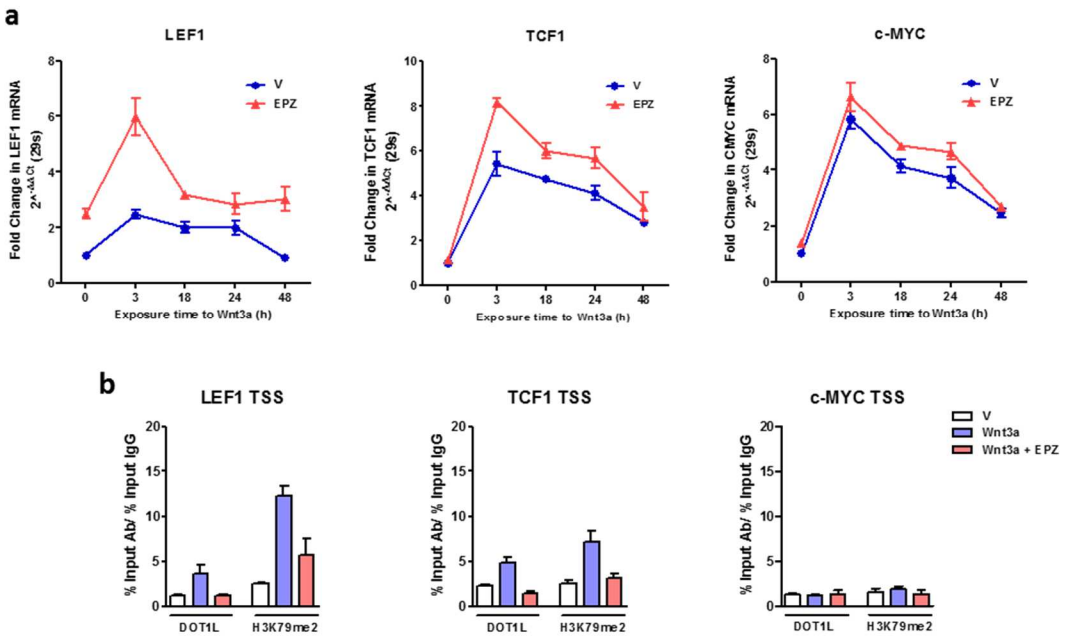
Supplementary Figure 2 | Severity of synovitis and extent of subchondral bone remodeling in DOT1L inhibitor-injected mice. C57/Bl6 wild-type mouse knees were injected with EPZ (5 mg/kg) or vehicle and sacrificed after 2 or 4 weeks. Knees were sectioned and stained with Hematoxylin-Safranin O. Synovitis was scored (see Methods) and is shown in (**a**)**.** Subchondral bone plate histomorphometry at the medial and lateral side of the tibia at 4 weeks was performed (see Methods) and is shown in (**b**). One experiment was performed with n=10 and 5 respectively. Error bars indicate mean ± s.e.m.



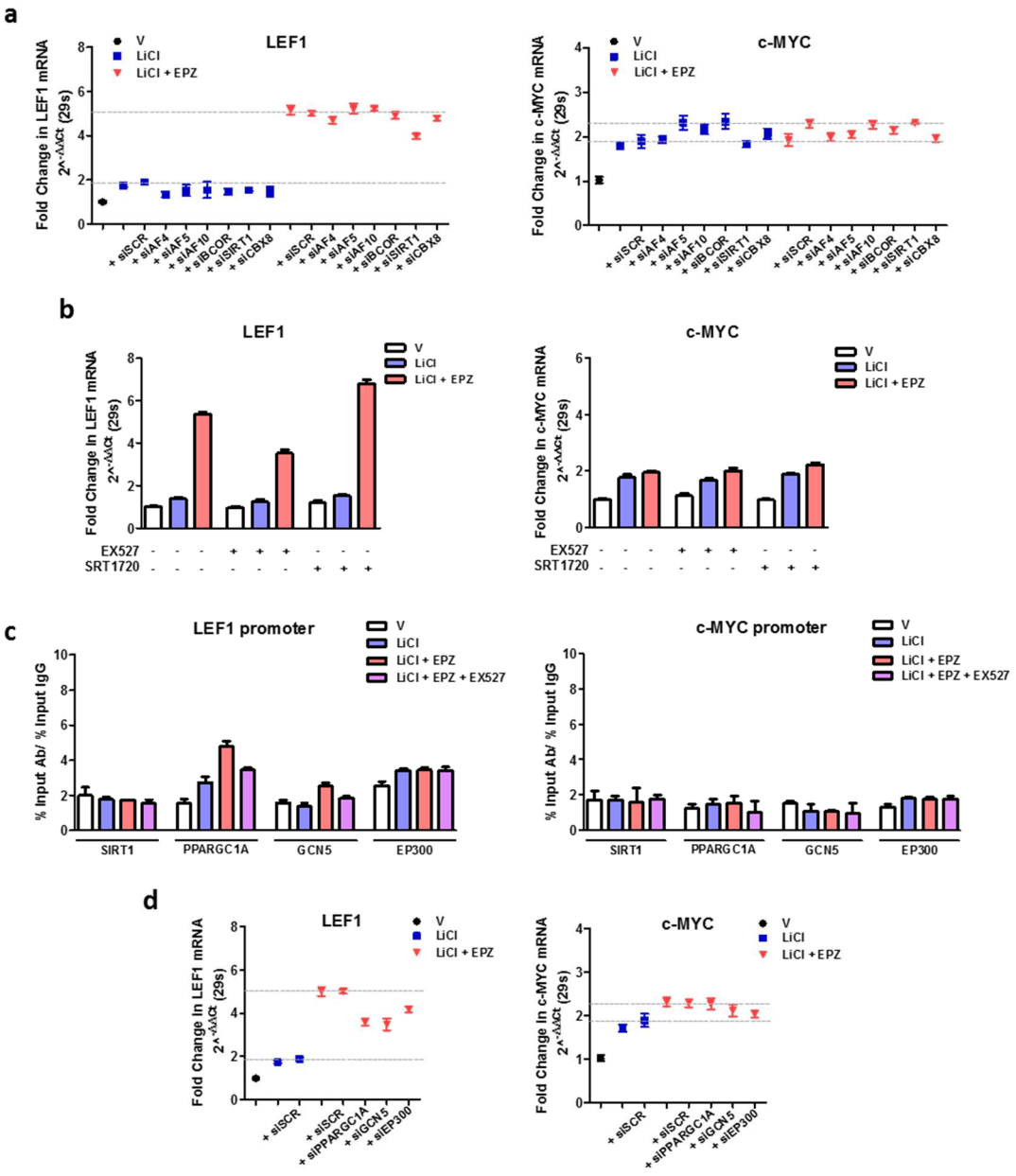
Supplementary Figure 3 | Transcriptome network analysis of DOT1L inhibition in primary human articular chondrocytes. (**a**) Immunoblot analysis of methylated H3K79 protein levels in 3 μM EPZ-5676 or vehicle-treated human articular chondrocytes (during 4 days) from 5 different donors (hACs1-hACs5). (**b**) Pie charts representing different categories of transcripts regulated by DOT1L using the "GO-slim" and Protein class ontology of the PANTHER Database. **(c)** Top six gene set enrichments as analysed by Toppgenesuite in the indicated categories.



Supplementary Figure 4 | *In vivo* DOT1L inhibition triggers articular chondrocyte hypertrophy. (**a,b,c**) Immunohistochemistry of beta-catenin (B-CAT) (**a**), type-X Collagen (COLX) (**b**) and matrix metalloproteinase-13 (MMP-13) (**c**) in the articular cartilage of C57/Bl6 wild-type mice after injection of EPZ-5676. DOT1L inhibition did not result in detectable changes in B-CAT immunostaining. Hypertrophy markers COLX and MMP-13 are increased after EPZ-5676 treatment, particularly in the vicinity of the lesions. The images are representative of 3 different animals. Scale bar, 50 µm (**a**, **c**) or 100 µm (**b**).

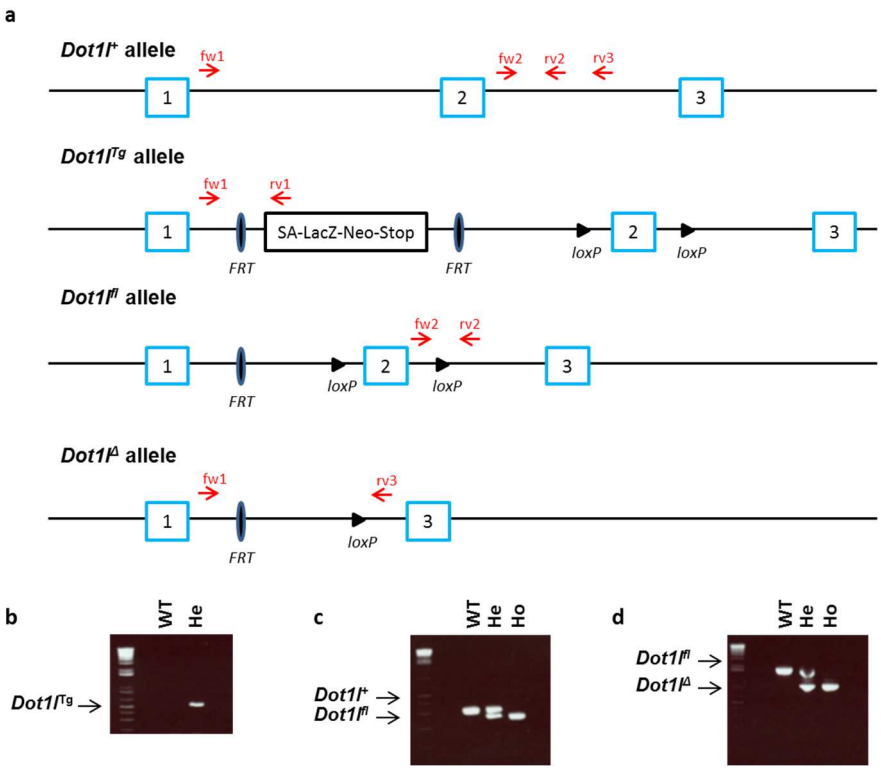


Supplementary Figure 5 | Effects of recombinant Wnt3a on DOT1L-inhibited chondrocytes. (**a**) *LEF1*, *TCF1* and *c-MYC* expression measured by quantitative PCR in human articular chondrocytes treated with EPZ-5676 and recombinant Wnt3a. Data are from one experiment with three technical replicates. (**b**) ChIP-qPCR analysis of DOT1L and methylated H3K79 on the transcriptional start site (TSS) of Wnt target genes. Data are from 2 experiments. Error bars indicate mean ± s.e.m.



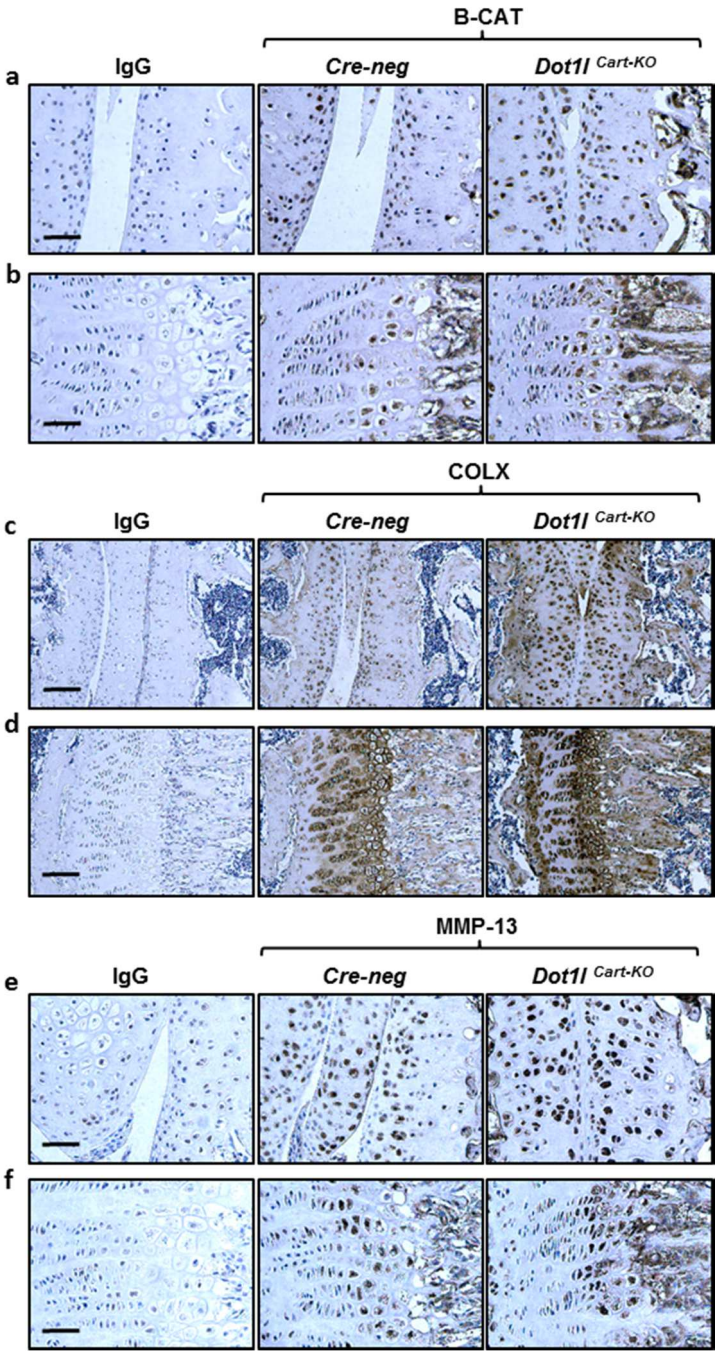
Supplementary Figure 6 | Effects of SIRT1 and associated transcriptional activators on *LEF1* and *c-MYC* expression upon DOT1L inhibition. All experiments were performed in healthy human articular chondrocytes, treated as indicated with DOT1L inhibitor EPZ-5676, Wnt activator LiCl, SIRT1 antagonist EX527 or SIRT1 agonist SRT1720. All data are presented as mean ± s.e.m. (**a**) Expression levels of *LEF1* and *c-MYC* Wnt target genes measured by quantitative PCR in chondrocytes transfected with indicated specific or scrambled siRNA (siSCR). Data are from one experiment with technical triplicates. (**b**) *LEF1* and *c-MYC* expression measured by quantitative PCR in the presence of a SIRT1 agonist or antagonist. Data from 2 experiments each with technical triplicates. (**c**) ChIP-qPCR analysis of SIRT1,

PPARGC1A, GCN5 and EP300 binding on the *LEF1* and *c-MYC* promoter and (**d**) *LEF1* and *c-MYC* expression after siRNA transfection with indicated specific or scrambled siRNA. Data from 2 biologically independent experiments.

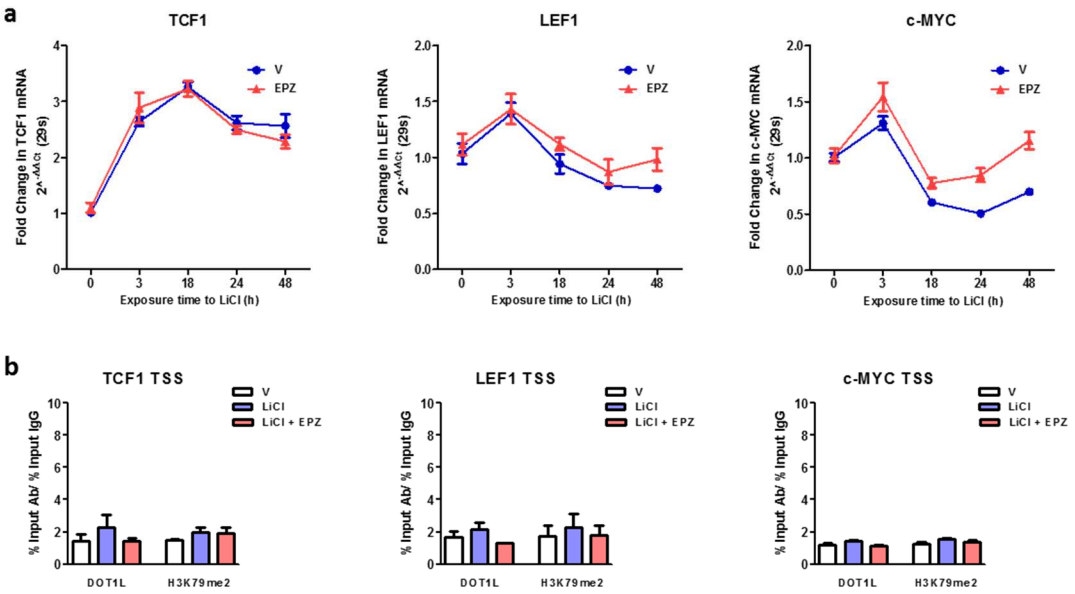


Supplementary Figure 7 | Generation of cartilage-specific *Dot1l*$^{\Delta}$ knockout mice. (**a**) *Dot1l*$^{Tg/+}$ mice (Knockout Mouse Project (KOMP) (CSD29070)) were crossed with FLP-transgenic mice to obtain floxed Dot1l mice (*Dot1l*$^{fl/+}$). These mice were bred to *Col2*-Cre mice to generate conditional cartilage-specific *Dot1l*$^{\Delta}$ knockout mice. Loss of exon 2 (*Dot1l*$^{\Delta/\Delta}$) led to a non-functional frame shift mutation. (**b-d**) Genotyping of *Dot1l*$^{+}$, *Dot1l*^{Tg}, *Dot1l*^{fl} and *Dot1l*$^{\Delta}$ knockout mice. Loss of exon 2 (*Dot1l*$^{\Delta/\Delta}$) led to a non-functional frame shift mutation. (**b-d**) Genotyping of *Dot1l*$^{+}$, *Dot1l*^{Tg}, *Dot1l*^{fl} and *Dot1l*$^{\Delta}$ alleles. (WT: wildtype, He: heterozygous, Ho: homozygous knockout).

(fw1: GAAGACTGTCTAGAGACTTAGGGTTGTGGGGCA,
rv1: CCAACTGACCTTGGGCAAGAACAT pair: *Dot1l*^{Tg} (503bp);
fw2: GAGGCATCTCTCCATGACCCTTGTCTGGGAA,
rv2: AGGAACCACAGGATGCTTCA pair: *Dot1l*$^{+}$ (313bp) and *Dot1l*^{fl} (265bp);
fw1, rv3: AAGGGTGTGGGTGGGCCTTCTTAGGTTAGGTATGT pair: *Dot1l*^{fl} (1524bp) and *Dot1l*$^{\Delta}$ (841bp)).

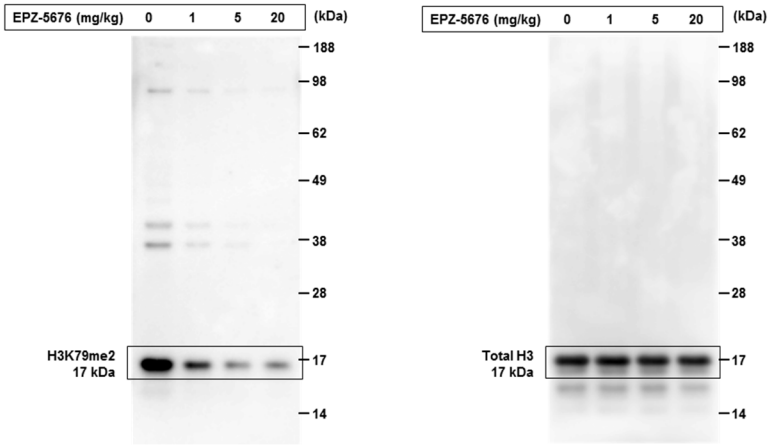


Supplementary Figure 8 | Loss of DOT1L accelerates chondrocyte hypertrophy in cartilage-specific *Dot1l* knockout mice. (**a-f**) Immunohistochemistry of B-CAT (**a,b**), COLX (**c,d**) and MMP13 (**e,f**) in the articular cartilage (**a,c,e**) and the growth plate (**b,d,f**) of 4-week-old *Cre-neg* and *Dot1l*$^{Cart\text{-}KO}$ mice. DOT1L loss did not result in detectable changes in B-CAT immunostaining. Hypertrophy markers COLX and MMP-13 are increased in *Dot1l*$^{Cart\text{-}KO}$ mice. The images are representative of 3 different animals. Scale bar, 50 µm (**a**,**b,e,f**) or 100 µm (**c,d**).

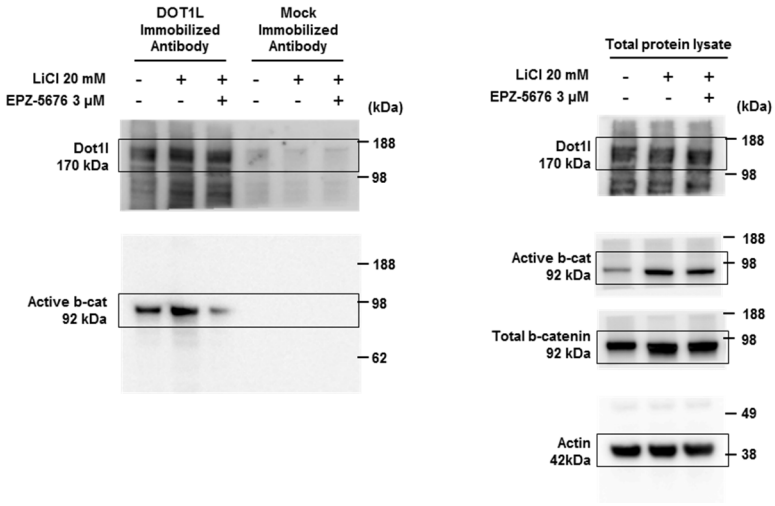


Supplementary Figure 9 | The effect of DOT1L inhibition on Wnt target genes is absent in osteoblasts. (**a**) *LEF1*, *TCF1* and *c-MYC* expression measured by quantitative PCR in human osteoblasts treated with EPZ-5676 and LiCl. Data are from one experiment with three technical replicates. (**b**) ChIP-qPCR analysis of DOT1L and methylated H3K79 on the transcriptional start site (TSS) of Wnt target genes in osteoblasts. Data are from two biologically independent experiments. Error bars indicate mean ± s.e.m.

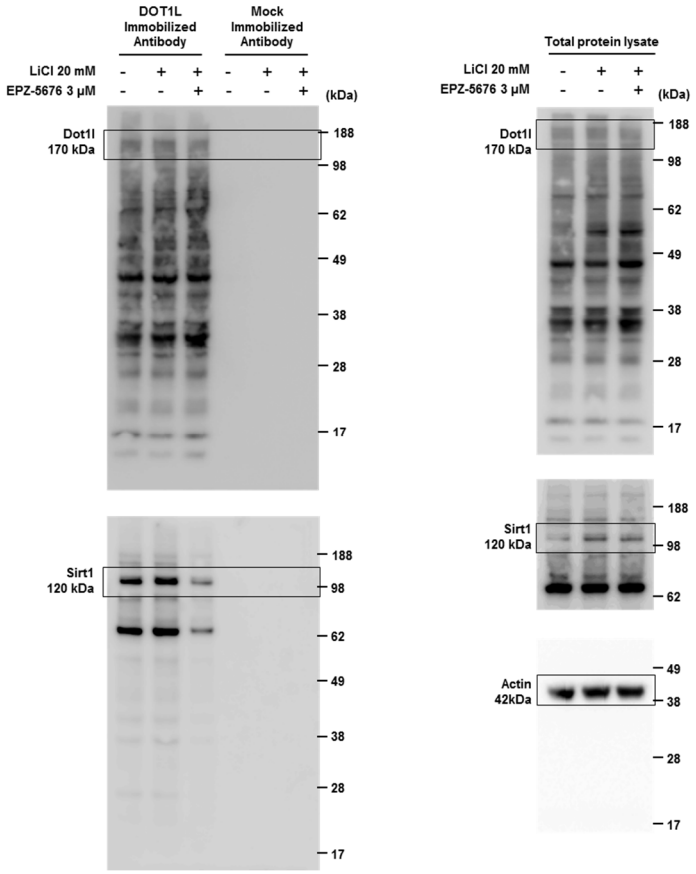
Uncropped blots Fig. 1c



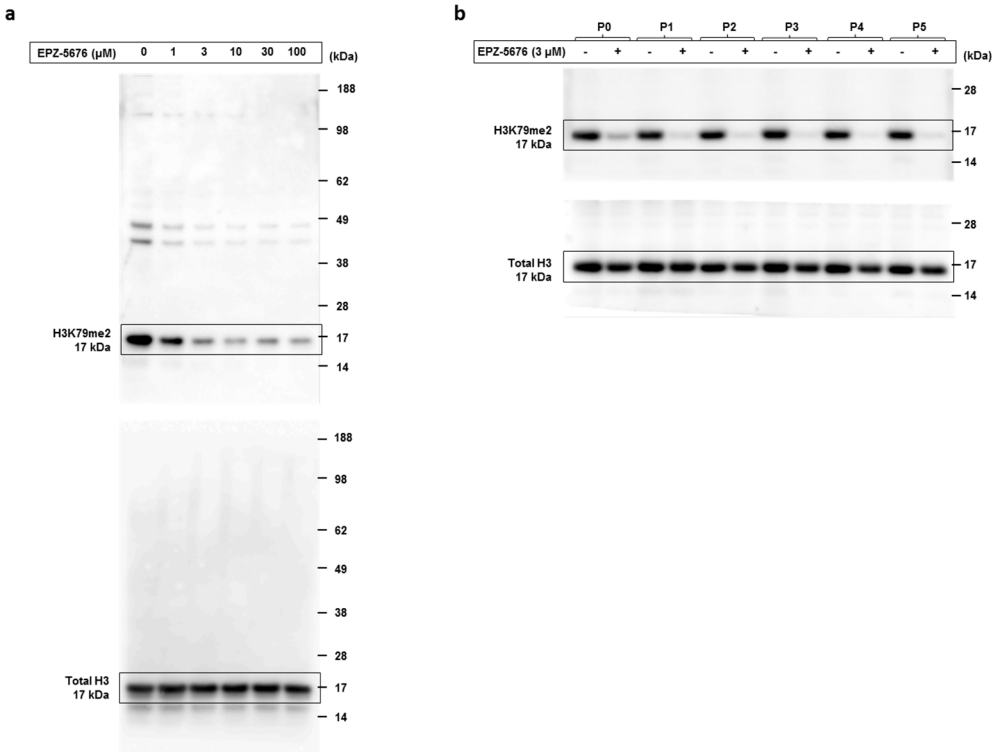
Uncropped blots Fig. 2b



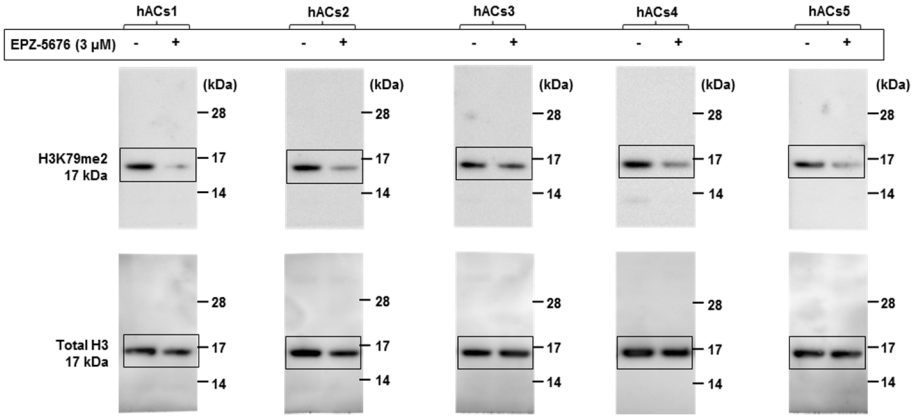
Uncropped blots Fig. 4e



Uncropped blots Supplementary Fig. 1



Uncropped blots Supplementary Fig. 3a



Supplementary Figure 10 | Unprocessed original scans. Uncropped images of scanned western blots and gels shown in the Figures are provided.

Supplementary Table 1 | Top down- and upregulated genes upon DOT1L inhibition that were previously linked with cartilage biology.

Gene	Biological relevance	Log ratio microarray
Top downregulated		
RGS5	Chondrocyte differentiation	-0,54782278
COL6A1	KO mice develop osteoarthritis	-0,541920087
SCUBE3	TGFβ receptor ligand	-0,476171007
FHL1	Downstream target of Wnt	-0,400055228
GDF10	Bmp family member	-0,392016034
LOXL2	Collagen formation, chondrocyte differentiation	-0,372004916
COL14A1	Plays an adhesive role by integrating collagen bundles	-0,308844443
ACAN	Extracellular matrix	-0,305167395
IGFBP5	IGF pathway	-0,304701203
Top upregulated		
CCL7	Upregulated in osteoarthritis	1,038689032
CCL8	Upregulated in osteoarthritis	0,796655267
MMP1	Degradation of extracellular matrix proteins	0,65190844
DKK1	Wnt modulator	0,573072224
SFRP1	Wnt modulator	0,570064988
CTHRC1	Inhibit collagen matrix synthesis	0,56757143
IGF1	IGF pathway	0,516760007
EMILIN2	Wnt modulator	0,41962033
LEF1	Wnt direct target gene	0,404441205
GPNMB	New gene associated with osteoarthritis	0,32814179
DACT1	Wnt antagonist	0,309393924

Supplementary Table 2 | siRNA catalog numbers.

siRNA	Catalog number
siAF4	Qiagen, S104147437
siAF5	Qiagen, S104203101
siAF10	Qiagen, S100066444
siBCOR	Qiagen, S100311423
siCBX8	Qiagen, S100339255
siDOT1L	Invitrogen, AM51331
siGCN5	Qiagen, S100426118
siP300	Qiagen, S102622592
siPPARGC1A	Qiagen, S100101024
siSCR	Qiagen, S103650318
siSIRT1	Qiagen, S100098434

Supplementary Table 3 | Human primers used for qPCR.

Primer name	Sequence
ACAN-Fw	ACAAGGTCTCACTGCCCAAC
ACAN-Rv	AATGGAACACGATGCCTGTC
CCL7-Fw	ATGAAAGCCTCTGCAGCACT
CCL7-Rv	CCAGCCTCTGCTTAGGGATT
CCL8-Fw	GTTTCTGCAGCGCTTCTGTG
CCL8-Rv	CACGTTAAAGCAGCAGGTGA
CHI3L2-Fw	CCCTTATCACTGGCCACAAC
CHI3L2-Rv	CCACCTTCTCTGATGGCATT
c-MYC-Fw	TTTCGGGTAGTGGAAAACCA
c-MYC-Rv	GCTCGAATTTCTTCCAGATATCCTC
COL1A1-Fw	AAGAGGAAGGCCAAGTCGAG
COL1A1-Rv	CACACGTCTCGGTCATGGTA
COL2A1-Fw	GCACCTGCAGAGACCTGAAA
COL2A1-Rv	GTCTCGCCAGTCTCCATGTT
COL6A1-Fw	ATCGACAACCTGAGGGACAG
COL6A1-Rv	TGATAGCGCAGTCGGTGTAG
COL14A1-Fw	TTCAAGGCCTTATGCCAGAC
COL14A1-Rv	ACTCTGGGCTTTGGATCCTT
CTHRC1-Fw	TCGAGCGCCTCTGAGATTAT
CTHRC1-Rv	CTTTGAATCCATCCCGACCT
DACT1-Fw	AGTCGCCTGGAGGAGAAGTT
DACT1-Rv	GCTCTTGCAACTGATTCAACAA
DKK1-Fw	CGGGAATTACTGCAAAAATGG
DKK1-Rv	ACCCATCCAAGGTGCTATGA
EDNRA-Fw	AATGGCAGCTTGAGAATTGC
EDNRA-Rv	TGAAGAGGGAACCAGCAAAG
EMILIN2-Fw	GGCTTTAGAGGGGGAGATTG
EMILIN2-Rv	TTGGGGACAAAGTCTTCCTG
FHL1-Fw	TGGCTCTGGAGCTAATTTGG
FHL1-Rv	GGCCATCCTTTTGCACATAC
FRZB-Fw	GAGGAGCTGCCAGTGTACGA
FRZB-Rv	ACTGCTTGCCCCTCTACAGTT
GDF10-Fw	GTCCACATGCACAGGCTCTAT
GDF10-Rv	TGCATGGAAGTCAGGTTGAA
GPNMB-Fw	TTCCTGGGATTTCTGCTCCT
GPNMB-Rv	TGTGCTCCCTCATGTAAGCA
HEY2-Fw	TGGGGAGCGAGAACAATTAC
HEY2-Rv	CCTCTCCTTTTCTTTCTTGCCATA
IGF1-Fw	CAGCAGTCTTCCAACCCAAT

IGF1-Rv	ACAGCGCCAGGTAGAAGAGA
IGFBP5-Fw	GGTTTGCCTCAACGAAAAGA
IGFBP5-Rv	AGTAGGTCTCCTCGGCCATC
ITGB3-Fw	GTGTGCCTGGTGCTCTGATG
ITGB3-Rv	ACTCACTGGGAACTCGATGG
LEF1-Fw	CAGTCATCCCGAAGAGGAAG
LEF1-Rv	AGGGCTCCTGAGAGGTTTGT
LOXL2-Fw	CCTGGGGAGAGGACATACAAT
LOXL2-Rv	AGCTTGCAGCTGGAGATGTG
MATN3-Fw	GACAGAACAGGGTCCCATCA
MATN3-Rv	TGAGAGCCTAGGGCACACTT
MMP1-Fw	TTCGGGGAGAAGTGATGTTC
MMP1-Rv	GTCGGCAAATTCGTAAGCAG
MMP3-Fw	GGCCAGGGATTAATGGAGAT
MMP3-Rv	TGAAAGAGACCCAGGGAGTG
NOTCH3-Fw	GTGTGCAAATGGAGGTCGTT
NOTCH3-Rv	CCACTGAACTCTGGCAGACA
RGS5-Fw	TCAGTTGGTGACCTTGTCATTC
RGS5-Rv	TTGTTCTGCAGGAGTTTGTCC
RIPK4-Fw	CTTCGGGCAGGTGTACAAGG
RIPK4-Rv	TTCTTCCAAAAGCTCCATGC
S29-Fw	CCAGCAGCTCTACTGGAGTCA
S29-Rv	GCCTATGTCCTTCGCGTACT
S100B-Fw	AGGGAGACAAGCACAAGCTG
S100B-Rv	GAAGTCACATTCGCCGTCTC
SCUBE3-Fw	CGGACACAACTGTCTGGATG
SCUBE3-Rv	GTTGTCGCTGAGGAAGAAGC
SERPINA1-Fw	GGGAAACTACAGCACCTGGA
SERPINA1-Rv	CCCCATTGCTGAAGACCTTA
SFRP1-Fw	CAAGCCCCAAGGCACAAC
SFRP1-Rv	TTCATCCTCAGTGCAAACTCG
SLIT3-Fw	CCAGGATCACCAAGATGGAC
SLIT3-Rv	CGCTCTAGCTGCTTCAGGTC
SOX9-Fw	GGTGCTCAAAGGCTACGACT
SOX9-Rv	GTAATCCGGGTGTCCTTCT
TCF1-Fw	CACCCATCCTCAAAGAGCTG
TCF1-Rv	ATGTTGTGCTGCTGCAGGTA

Supplementary Table 4 | Human primers used for ChIP-qPCR.

Primer name	Sequence
c-MYCpromoter-Fw	CCGCCTGCGATGATTTATAC
c-MYCpromoter-Rv	GCTCCCTCTCAAACCCTCTC
c-MYC-TSS-Fw	GGCACTTTGCACTGGAACTT
c-MYC-TSS-Rv	CTTTCAGAGAAGCGGGTCCT
LEF1promoter-Fw	AGCAATTGGCAGCCCTATTT
LEF1promoter-Rv	TGCAAACCAGTCTGCTGAAC
LEF1-TSS-Fw	AGAAAAACCGAAGCGAAAGG
LEF1-TSS-Rv	AACGAAACGTCCACTTCCTG
TCF1promoter-Fw	AGTAAGCGGGGTCAGGAGTT
TCF1promoter-Rv	CCACTCGGCATAGCCTTAAA
TCF1TSS-Fw	AGCTCGCGGAGCCGCTCTGC
TCF1-TSS-Rv	GGGGCGCGGGGCGCTGGGCG