**Figure Legends**

Figure 1. Dnmt3b expression in murine and human knee joint cartilage. (A) Representative images showing that Dnmt3a is not expressed in chondrocytes from 3 mo WT articular cartilage [Dnmt3a expression in pancreas tissue (positive control)], while robust expression of Dnmt3b in chondrocytes of 3 mo WT articular cartilage and lower expression in underlying growth plate cartilage (n=3); the magnified images of the dashed and solid boxed area of articular cartilage in separate panels; (B) Representative images showing Dnmt3b expression in 3 mo (n=3) versus 27 mo (n=3) WT murine knee articular cartilage; (C) Representative images showing Dnmt3b expression in 14 wk old murine articular cartilage following MLI surgery (n=3) or cartilage from sham control knees (n=3); (D) Representative images showing Dnmt3b expression in 14 wk old murine articular cartilage in mice fed a high fat diet (HFD) (n=3) or controls (Ctrl) fed a normal diet (n=3). (E) Two representative images showing DNMT3B expression in human healthy articular cartilage (n=11) or osteoarthritic cartilage tissue sections (n=71). (F) Reduced DNMT3B expression in human primary OA chondrocytes compared to healthy chondrocytes (n=3). (G) Induction of human primary chondrocytes (n = 3) with IL-1β results in decreased expression of DNMT3B mRNA and protein levels. All scale bars, 100 µm.

Figure S1. Dnmt3b expression in embryonic and adult murine knee joint cartilage. Immunohistochemical staining shows Dnmt3b protein expression in chondrocytes from C57BL/6 WT murine articular cartilage knee joints at embryonic time point E18.5 and post-natal time points P7, P28 and P56 (n=3). *, hypertrophic chondrocyte; **, bone marrow cells. All scale bars, 100 µm.

Figure S2. Altered anabolic and catabolic gene expression in IL-1β-treated human primary chondrocytes. Reduced COL2A1 and increased MMP13 expression following IL-1β treatment (48 h) of human primary articular chondrocytes isolated from total knee replacement surgeries (n=3).

Figure S3. IL-1β regulation of Dnmt3b is mediated in part by NF-κB. (A) Sequence alignment and conservation of an NF-κB binding site (red) in the promoter region of the Dnmt3b gene of different
species. (B) Reduced luciferase expression in IL-1β stimulated (24 h) chondrogenic murine ATDC-5 cells transfected with a luciferase reporter plasmid containing the Dnmt3b promoter sequence when compared to control (Ctrl) untreated cells. Reduced luciferase activity is attenuated when ATDC-5 cells were transfected with a reporter plasmid containing a mutated NF-κB binding site (ATCTGGCTCC) (n=3). (C, D) Pull-down of genomic DNA with an NF-κB antibody (ChIP assay) shows interaction of NF-κB with its binding site in the Dnmt3b promoter region by qPCR (C) and semi-quantitative gel electrophoresis (D) utilizing specific primers to amplify the Dnmt3b promoter region containing the NF-κB binding site (n=3). (E, F) Dnmt3b mRNA and protein levels were reduced in murine WT primary articular chondrocytes (extracted from 3 mo murine knee joints) following IL-1β induction (48 h) (n=3).

Figure S4. Ablation of Dnmt3b in articular chondrocytes in vitro alters cell homeostasis. Reduced expression of (A) Dnmt3b mRNA and (B) Dnmt3b protein following transfection with 20nM Dnmt3b siRNA for 48 h in murine primary chondrocytes isolated from 3 mo WT mice (n=3). (C) Dnmt3b siRNA treatment resulted in increased expression of the catabolic/hypertrophic chondrocyte markers Col10a1, Runx2, and Mmp13, and decreased expression of the anabolic marker, Col2a1 in murine primary chondrocytes (n=3). (D) Representative images showing reduced Dnmt3b expression resulted in increased alkaline phosphatase (ALP) activity in murine primary chondrocytes, but not to the extent resulting from BMP-2 treatment (positive control) (n=3). (E, F) Dnmt3b siRNA treatment affected the balance of TGF-β and BMP signaling as shown by decreased phospho(p)-Smad2 expression and increased p-Smad1/5 expression in murine primary chondrocytes, respectively (n=3).

Figure 2. Dnmt3b loss-of-function mice develop accelerated OA. (A) Representative alcian blue / hematoxylin / orange G (ABH/OG) staining of tissue sections of knee joints harvested from 5 mo and 8 mo Dnmt3b loss-of-function (LOF) mice and Cre + control (Ctrl) mice (n=5). The magnified images of the boxed regions are shown respectively. (B) OARSI scoring system was used to quantify the ABH/OG stained tissue sections (n=5). (C) Articular cartilage area was quantified by histomorphometry (n=5). (D)
Representative microCT images of knee joints from 8 mo Dnmt3b LOF and Ctrl mice (n=5). (E) Subchondral bone volume and (F) subchondral bone trabecular connective density were calculated from the microCT images (n=5). Arrows in 5 mo old Dnmt3b LOF mice show areas of proteoglycan loss and cartilage fibrillation, respectively. Arrows in 8 mo old Dnmt3b LOF mice show osteophyte formation and an area of proteoglycan loss in articular cartilage, respectively. Yellow arrows in microCT images denote osteophyte formation. Scale bars, 100 µm.

Figure S5. Ablation of Dnmt3b in articular chondrocytes in vivo. (A) Representative fluorescence microscopy shows recombination efficiency in 2 mo Agc1CreERT2; mT/mG mice followed by tamoxifen injection for 5 days (n=3). (B) Dnmt3b protein knock-down in 3 mo articular chondrocytes of Dnmt3b LOF mice compared to Cre + control (Ctrl) littermates (n=3). Scale bars, 100 µm.

Figure S6. Expression of Dnmt as well as anabolic and catabolic genes in Dnmt3b LOF cartilage tissue. (A) qPCR analysis of Dnmts and Tets in 3 mo articular cartilage isolated from Dnmt3b LOF and Cre+ control (Ctrl) mice (n=3). (B) Tet activity analysis in chondrocytes from 3 mo Dnmt3b LOF or Ctrl mice (n=3). (C)Col2a1 (anabolic) and Col10a1, Runx2 and Mmp13 (catabolic / hypertrophic) marker expression in articular chondrocytes from 3 mo Dnmt3b LOF and Cre + control (Ctrl) mice (n=3).

Figure S7. Analysis of apoptosis and reactive oxygen species in Dnmt3b LOF cartilage. (A) Representative TUNEL staining and analysis of knee joint articular cartilage tissue sections from 5 mo Dnmt3b LOF and Cre+ control (Ctrl) mice by fluorescence microscopy (n=5); (B) Quantification of apoptotic cell numbers from the TUNEL-stained fluorescent images (n=5). (C) Reactive oxygen species (ROS) analysis of 2 mo Dnmt3b LOF articular chondrocytes (i.e. chondrocytes from Dnmt3bff mice treated with Ad5-Cre for 48 h) compared to control (Ctrl) chondrocytes (Dnmt3b LOF chondrocytes treated with Ad5-GFP for 48 h) (n=3).

Figure 3. Altered epigenomic and transcriptomic signatures in Dnmt3b LOF chondrocytes. gDNA and RNA isolated from control and Dnmt3b LOF cells for RNA-seq and methylC-seq analysis (n=3). (A)
PCA plot of samples based on RNA-seq data; (B) Heatmap display of top 25 significantly differentially expressed genes between Dnmt3b LOF and control; (C) Word cloud representing gene frequency of enriched function categories from all differentially expressed genes; (D) Global methylation distribution of Dnmt3b LOF and control samples showing there is no global difference; (E) Methylation difference of DMR versus genome as background; (F) Significant overlap of genes nearby DMRs and differentially expressed genes, p-values calculated by hypergeometric test.

Figure S8. Analysis of RNA-seq and methylC-seq data. (A) Heatmap of sample-to-sample distances using the rlog-transformed values showing more similarity of RNA-seq signal observed in each group; (B) An MA-plot of gene expression changes. The log2 fold change for a particular comparison is plotted on the y-axis and the average of the counts normalized by size factor is shown on the x-axis. Each gene is represented with a dot. Genes with an adjusted p value below a threshold (here 0.1, the default) are shown in red; (C) Enrichment of differentially expressed genes show enriched function related to cell cycle process, bone development etc; (D) Genes from differentially expressed list are related with TGF/BMP pathway network, green indicates down-regulation and red indicates up-regulation; (E) Genomic feature distributions of DMRs; (F) Enriched transcription factor binding sites found in DMRs using Homer software. n=3.

Figure 4. Mitochondria function and cellular homeostasis in Dnmt3b LOF chondrocytes. Primary articular chondrocytes were isolated from 2 mo Dnmt3bf/f mice and infected with Ad5-Cre (Dnmt3b LOF) or Ad5-GFP (Ctrl) for 48 h. (A) Mitochondrial respiration was measured by the Seahorse XF Extracellular Flux Analyzer. Basal respiration and maximal respiration, as measured by the oxygen consumption rate (OCR) are shown (n=8). (B) TCA metabolite (succinate, fumarate) and NADH analysis by HPLC-MS (n=3). (C) Mitochondrial metabolism analysis was measured in 2 mo WT cells treated with either 1mM diethyl succinate or vehicle for 48 h by the Seahorse XF Extracellular Flux Analyzer (n=8). (D) Mitochondrial respiration analysis in BMP-2-treated 2 mo WT chondrocytes in the presence or absence antimycin A + rotenone for 48 h (n=8).
Figure S9. Chondrocyte gene expression in Dnmt3b LOF cells. Primary articular chondrocytes were isolated from 2 mo Dnmt3b\textsuperscript{f/f} mice and infected with Ad5-Cre (Dnmt3b LOF) or Ad5-GFP (Ctrl) for 48 h. Expression of anabolic (Col2a1) or catabolic/hypertrophic genes (Col10a1, Runx2, Mmp13) is shown (n=3).

Figure S10. Chondrocyte gene expression under succinate treatment. Primary articular chondrocytes from 2 mo WT mice were treated with 1mM diethyl succinate or vehicle for 48 h. (A) Cellular succinate levels in murine chondrocytes (n=3). (B) Chondrocyte gene expression in response to succinate treatment was analyzed by qPCR (n=3).

Figure S11. Chondrocyte gene expression under antimycin A and rotenone (A&R) treatment. Primary articular chondrocytes form 2 mo WT mice were treated with 0.1 µM antimycin A and rotenone for 48 h. (A) Effect of antimycin A and rotenone treatment on chondrocyte proliferation and apoptosis (n=3). (B) Analysis of chondrocyte gene expression in response to BMP-2 treatment + / - antimycin A + rotenone (n=3).

Figure 5. Dnmt3b gain-of-function mice are protected from cartilage degeneration following surgical induction of OA. MLI or sham surgeries were performed on Dnmt3b gain-of-function (GOF) mice or Cre + control (Ctrl) mice. (A) Alcian blue / hematoxylin / orange G stained sections of Ctrl or Dnmt3b GOF knee joints 12 weeks following sham surgery. Representative images of histological sections from Ctrl or Dnmt3b GOF mice at 8 wk or 12 wk following MLI surgery (n=5). The magnified images of the boxed regions are shown respectively. (B) Quantitation of histological assessment by OARSI scoring (n=5). (C) Histomorphometric analysis of Ctrl or Dnmt3b GOF cartilage (n=5). Scale bars, 100 µm.

Figure 6. Mitochondria function and cellular homeostasis in Dnmt3b GOF chondrocytes. (A) Gene expression in chondrocytes isolated from 10 wk Ctrl or Dnmt3b GOF chondrocytes (n=3). (B) Mitochondrial respiration in primary articular chondrocytes isolated from 2 mo Col2a1Cre; Rosa-rtTA\textsuperscript{f/+};
*Dnmt3b-tg* mice, treated with vehicle (Ctrl) or doxycycline (*Dnmt3b* GOF) for 48 h (n=3). Mitochondrial respiration was measured by the Seahorse XF Extracellular Flux Analyzer.

Figure S12. Generation of *Dnmt3b* gain-of-function (GOF) transgenic mice. (A) Schematic representation of the strategy utilized to generate doxycycline (DOX)-inducible *Dnmt3b* over-expression in murine cartilage tissue. (B) Genotyping strategy, utilizing three different primer pairs, to confirm recombination. Mouse line #9 was used for breeding and subsequent experimental analyses.

Figure S13. Over-expression of *Dnmt3b* in articular chondrocytes in vivo. (A) Representative fluorescence microscopy shows recombination efficiency in 10 wk *Col2a1Cre; Rosa-rtTA*+/−; *H2BGFP* mice (n=3). Scale bar, 100 µm. (B) Confirmation of Dnmt3b protein over-expression in primary articular chondrocytes from 10 wk *Dnmt3b* GOF or Cre + control (Ctrl) mice by Western blotting using Dnmt3b antibodies (n=3).
Figure S1

E18.5  P7  P28  P56

Dnmt3b
**Figure S3**

(A) Mouse: -182 GGGGGCCAGGGAGGCTCCGAGGAGGCA -153

Human: -169 AGGGGCAGGGCGAGGCTCCGAGCGATTC -140

Dog: -386 CAAGGCTGTCGGGAGGCAGGGGCAGG -357

Horse: -90 AGGCATTGGCCGGAGGTTTGGGGATGTGC -61

(B) Dnmt3b promoter activity

(C) Signal relative to input

(D) Gel electrophoresis

(E) Dnmt3b mRNA expression

(F) Western blot

Legend:

- Ctrl: Control
- IL-1β: Interleukin-1β
- Positive Control
- Negative Control
- Anti-NFκB
Figure S4

A

Relative Gene Expression

Col10a1

Runx2

Mmp13

B

Dnmt3b

β-Actin

C

Col2a1

Col10a1

Runx2

Mmp13

D

Ctrl

Dnmt3b siRNA

BMP2

Dnmt3b siRNA

E

pSmad3

Smad3

β-Actin

F

pSmad1/5

Smad1/5

β-Actin
Figure S5

A  

mT/mG  

Agc1CreER;mT/mG

B

<table>
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<tr>
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**Figure S6**

A

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B

TET activity (normalized to Ctrl)

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<tr>
<td>Runx2</td>
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<td>3</td>
</tr>
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<td>Mmp13</td>
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C

Relative Gene Expression

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<tr>
<td>Mmp13</td>
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Figure S7

A) Ctrl vs. Dnmt3b LOF

B) % TUNEL Positive Cells

C) ROS (relative to Ctrl)
Fig. S8

A

mean expression

log fold change

Dnmt3b LOF 3
Dnmt3b LOF 1
Dnmt3b LOF 2
Ctrl 3
Ctrl 1
Ctrl 2

0 2 4 6 8

B

-0.3 -0.2 -0.1 0.0 0.1 0.2 0.3

1e+00 1e+02 1e+04 1e+06

mean expression

Enrichment of differentially expressed genes

C

cell cycle process
mitotic cell cycle
skeletal system development
ossification
bone development
regulation of osteoblast differentiation
synapse organization
regulation of ossification
osteoblast differentiation

-log10 P-value

E

Intron Exon Intergenic Promoter

F

FoxO1-related mTOR pathway, energy metabolism

Chondrogenesis related

Sox4

Sox9

Pax3:Fkhr

Nfatc2

p=1e-45

p=1e-40

p=1e-6

p=1e-14
Figure S9

Relative Gene Expression

<table>
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<tr>
<th></th>
<th>Ctrl</th>
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<tbody>
<tr>
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<tr>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>Mmp13</td>
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</table>

* Indicates statistical significance.
Figure S10

A

Succinate Conc (ng/ml)

Veh  | Succinate
---   |---
0     | 2.5
1     | 2
1.5   | 1.5
2     | 1

B

<table>
<thead>
<tr>
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<th>Veh</th>
<th>Succinate</th>
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<tbody>
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<tr>
<td>Mmp13</td>
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Figure S12

A

Rosa26 → loxP → STOP → loxP → rtTA

+ Col2a1-Cre

Rosa26 → rtTA

+ DOX

tetO → Dnmt3b

B

Primers b, b’

1Kb

500bp

Primers c, c’

1Kb

500bp

Primers d, d’

1Kb

500bp