

Table SI. Primer sequences for genotyping.

Primer type	Sequence (5'-3')
Kdm6a-Flox	
Forward	TCTCTATGCACTGGATGTAT
Reverse	AAAAGATGTTAAGGGTTGTT
R26-CAG-LSL-tdTomato	
Wild-type forward	AAG GGA GCT GCA GTG GAG TA
Wild-type reverse	CCG AAA ATC TGT GGG AAG TC
Mutant forward	GGC ATT AAA GCA GCG TAT CC
Mutant reverse	CTG TTC CTG TAC GGC ATG G
Col2a1-CreERT2	
Wild-type	GAGTGA CTGCGGTTGGAAA
Common	GGAGGAAGACAAGTAGAGGAGG
Mutant	TGCGAACCTCATCACTCGTTGC

Kdm6a-Flox is a conditional knockout strain where essential exons of the Kdm6a gene are flanked by loxP sites. R26-CAG-LSL-tdTomato is a Cre-dependent reporter strain in which a loxP-flanked transcriptional stop cassette prevents the expression of the tdTomato fluorescent protein. Upon Cre-mediated recombination, tdTomato is expressed, permanently labeling Cre-active cells and their progeny. For the R26-CAG-LSL-tdTomato reporter allele, a four-primer strategy was used. Two independent primer pairs (wild-type forward/reverse and mutant forward/reverse) were used to separately amplify the wild-type and mutant DNA fragments, which avoids potential primer competition and yields optimal band clarity. Col2a1-CreERT2 expresses CreERT2 specifically in chondrocytes, driven by the Col2a1 promoter. CreERT2, tamoxifen-inducible Cre recombinase; Col2a1, collagen type II $\alpha 1$ chain; Kdm6a, lysine demethylase 6A.

Table SII. Cell quality control metrics of the single-cell RNA sequencing sample at 10 days post-fracture.

Sample	Number of cells before filtering	Number of cells after filtering	Cell retention rate after filtering, %	Median genes per cell before filtering	Median genes per cell after filtering
WT3	14,355	12,790	89.10	1,500	1,452
WT2	13,932	11,623	83.43	1,644	1,620
WT1	14,591	12,527	85.85	1,720	1,668
FX3	16,023	13,221	82.51	1,736	1,697
FX2	11,832	9,584	81.00	1,769	1,784
FX1	15,467	13,642	88.20	1,556	1,497

FX, Kdm6a conditional knockout mice; WT, wild-type.

Table SIII. Cell quality control metrics of the single-cell RNA sequencing sample at 21 days post-fracture.

Sample	Number of cells before filtering	Number of cells after filtering	Cell retention rate after filtering, %	Median genes per cell before filtering	Median genes per cell after filtering
WT3	8,369	5,514	65.89	791	939
WT2	9,995	7,211	72.15	793	890
WT1	7,691	5,364	69.74	909	1,043
FX3	10,030	7,702	76.79	975	1,035
FX2	9,414	7,253	77.04	1,076	1,191
FX1	9,087	6,540	71.97	834	924

FX, Kdm6a conditional knockout mice; WT, wild-type.