

Figure S1. Immunohistochemistry to assess for LOXL2 and LOX protein expression in healing fractures. Fractures were generated by external blunt trauma to the femur after its stabilization by insertion of an intramedullary pin. Bones were retrieved at different time points. Immunohistochemical staining of sections made from healing day 10 assayed with LOXL2 antibody showed intense positive signals in proliferating chondrocytes (white arrow), with less staining of cells lining bone (osteoblasts, black arrows). LOX immunostaining was present but weak in chondrocytes on day 10, and stronger in cells lining bone on day 14. Scale bar = 10 μm ; photographed at 20x. Data are representative images from three animals per group. Primary antibody concentration was 4 $\mu\text{g/ml}$ for LOXL2 and 3 $\mu\text{g/ml}$ for LOX.

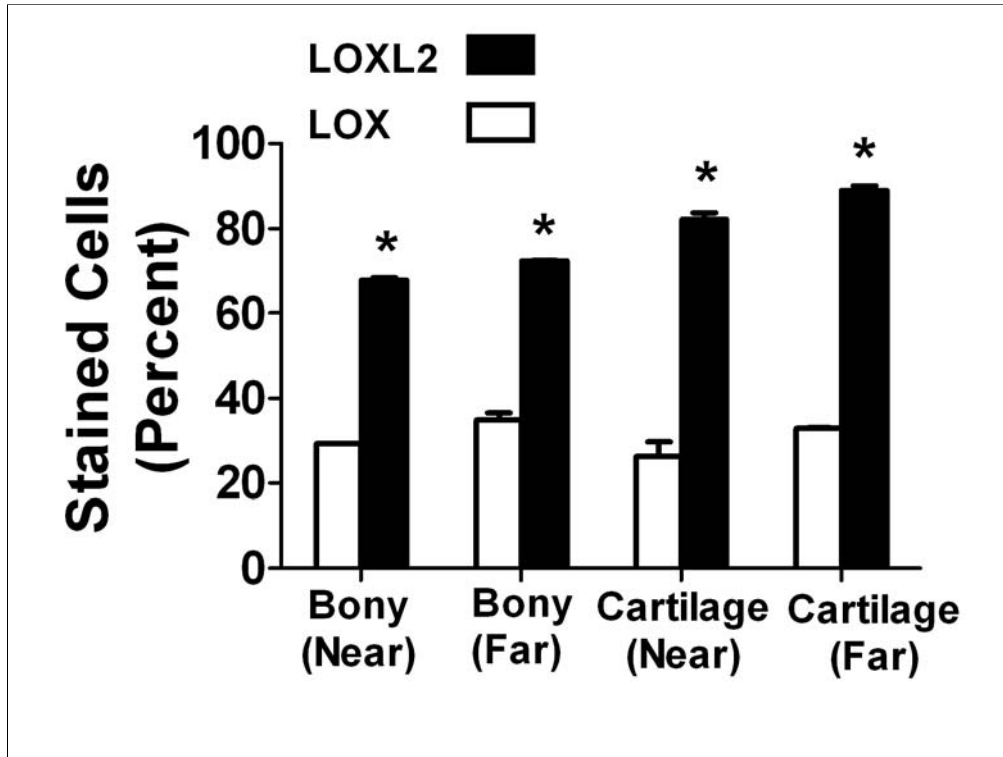


Figure S2. More chondrocytes expressed LOXL2 than LOX on day 10 of healing. Sections (three) from three mice (n=3) per antibody were evaluated by immunohistochemistry and histomorphometric analyses. Four 0.09 mm² areas on each slide were evaluated for LOX and LOXL2 positive chondrocytes, respectively. The four areas were a bony area near the fracture (within 0.25 mm), a bony area far (1.3 – 1.5 mm) from the fracture, cartilage area near (within 0.25 mm) the fracture and cartilage area far (1.3 – 1.5 mm) from the fracture. Total stained and unstained chondrocytes were counted based on morphology. Data are expressed as the percent positive chondrocytes in each of the four areas; *, p< 0.01; paired t-test.

Sequences producing significant alignments:








| Accession | Description | Max score | Total score | Query coverage | E value | Max ident | Links |
|---|---|---------------------------|-----------------------------|--------------------------------|-------------------------|---------------------------|---|
| Transcripts | | | | | | | |
| NM_033325.1 | Mus musculus lysyl oxidase-like 2 (Loxl2), mRNA | 42.1 | 42.1 | 100% | 0.004 | 100% |  |
| XM_001477924.1 | PREDICTED: Mus musculus similar to RIKEN cDNA 1110014J01 gene (LOC100041622), mRNA | 28.2 | 28.2 | 66% | 67 | 100% |  |
| XM_001472971.1 | PREDICTED: Mus musculus RIKEN cDNA 3110054G05 gene (3110054G05Rik), mRNA | 28.2 | 28.2 | 66% | 67 | 100% |  |
| XM_001478109.1 | PREDICTED: Mus musculus hypothetical protein LOC100047418 (LOC100047418), mRNA | 28.2 | 28.2 | 66% | 67 | 100% |  |
| NM_029101.3 | Mus musculus RIKEN cDNA 1110014J01 gene (1110014J01Rik), mRNA | 28.2 | 28.2 | 66% | 67 | 100% |  |
| NM_001081372.1 | Mus musculus liver carboxylesterase N-like (LOC382044), mRNA | 28.2 | 28.2 | 66% | 67 | 100% |  |
| XM_918183.2 | PREDICTED: Mus musculus similar to RIKEN cDNA 1110014J01 gene, transcript variant 1 (LOC639318), mRNA | 28.2 | 28.2 | 66% | 67 | 100% |  |
| Genomic sequences [show first] | | | | | | | |
| NT_166318.1 | Mus musculus chromosome 12 genomic contig, strain C57BL/6J | 36.2 | 173 | 100% | 0.28 | 100% | |
| NW_001030504.1 | Mus musculus chromosome 12 genomic contig, alternate assembly (based on Celera assembly) | 36.2 | 141 | 95% | 0.28 | 100% | |

Figure S3. Blast analysis against the sense sequence of LOXL2 shRNA clone 11. Data show no similarity with any other lysyl oxidase isoform, and insufficient similarity for knockdown with unrelated transcripts.

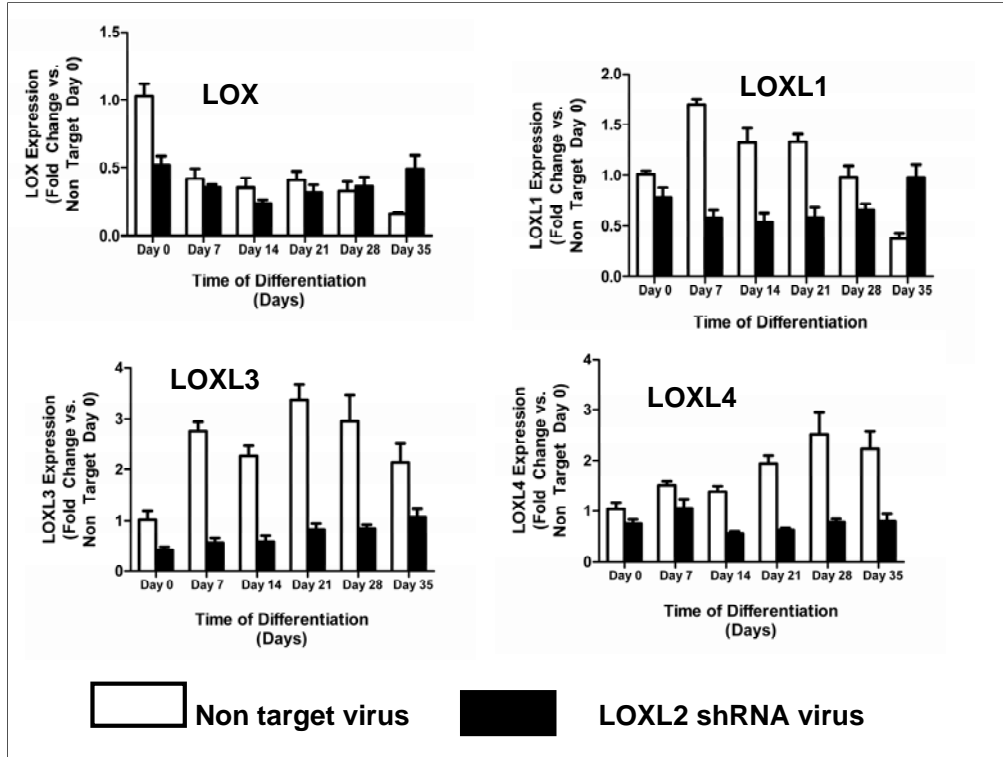


Figure S4. Lysyl oxidase isoforms gene expressions in ATDC5 cells infected with LOXL2 shRNA lentivirus. A lentivirus knock down of LOXL2 was introduced in differentiating ATDC5 cells. RNA was extracted at day 0, 7, 14, 21 and 28, and then subjected to qPCR for expression of LOX, LOXL1, LOXL3, LOXL4. LOX was not significantly down-regulated, whereas LOXL1, LOXL3, and LOXL4 were down regulated (two way ANOVA, $p < 0.05$; two independent experiments; three independent cultures per experiment).