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Supplemental Information

**Treatment of Intervertebral Disk Disease
by the Administration of mRNA Encoding
a Cartilage-Anabolic Transcription Factor**

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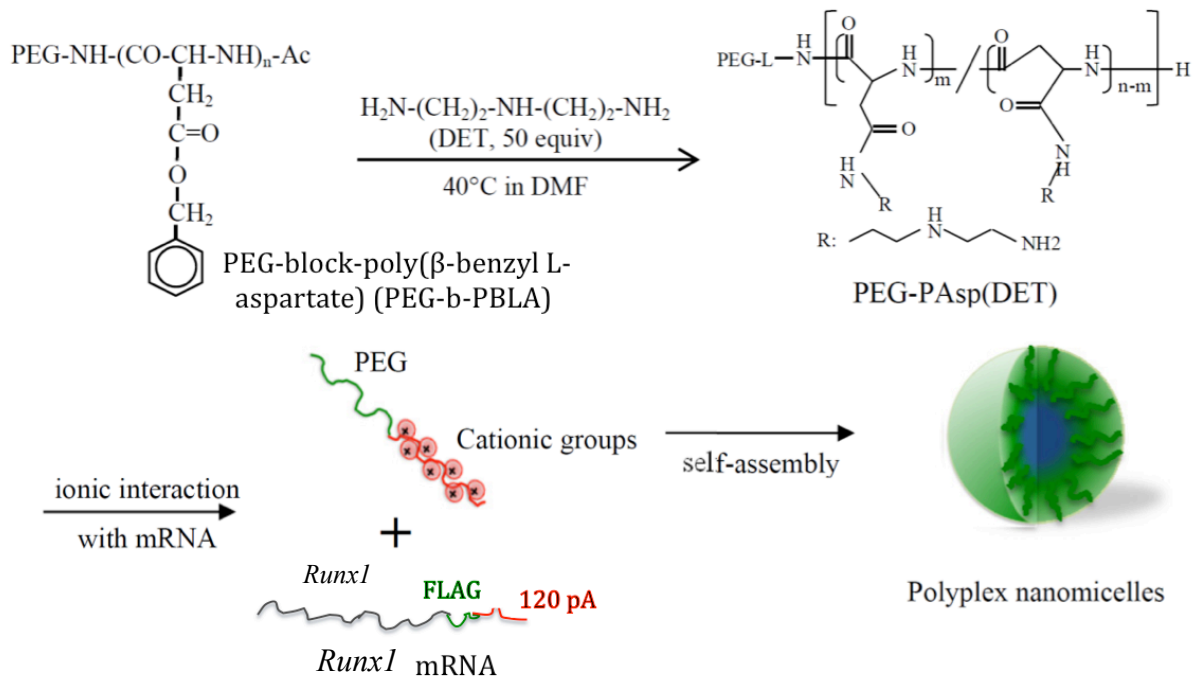
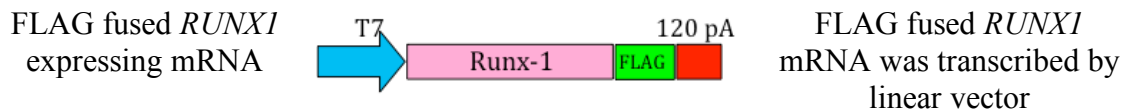
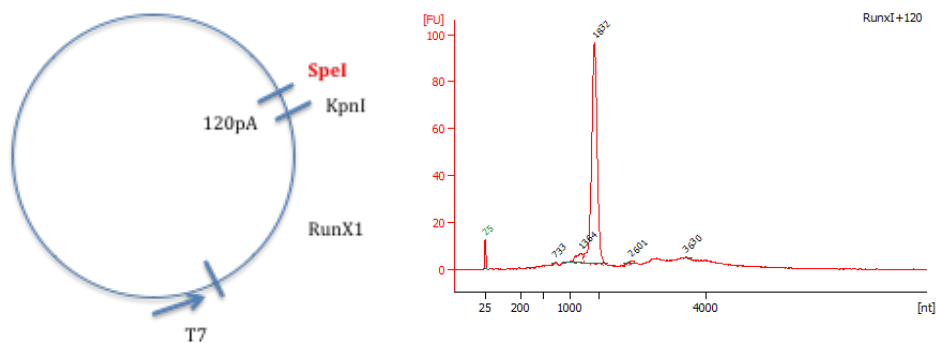
A**B****C**

Fig. S1. (A) PEG- Poly{N-[N'-(2-aminoethyl)-2-aminoethyl]aspartamide} block copolymer (PEG-PAsp[DET]) was synthesized and self-assembled with mRNA to form polyplex nanomicelles. (B) The Runx1-FL sequence was sub-cloned into a pSP73 vector, containing a 120 bp chemically synthesized poly(d(A/T)) fragment downstream of the cDNA region. (C) The vectors were linearized with SpeI for IVT, and all transcribed mRNAs were analyzed for size and purity with the Agilent RNA 6000 Nano Assay on a BioAnalyzer 2100.

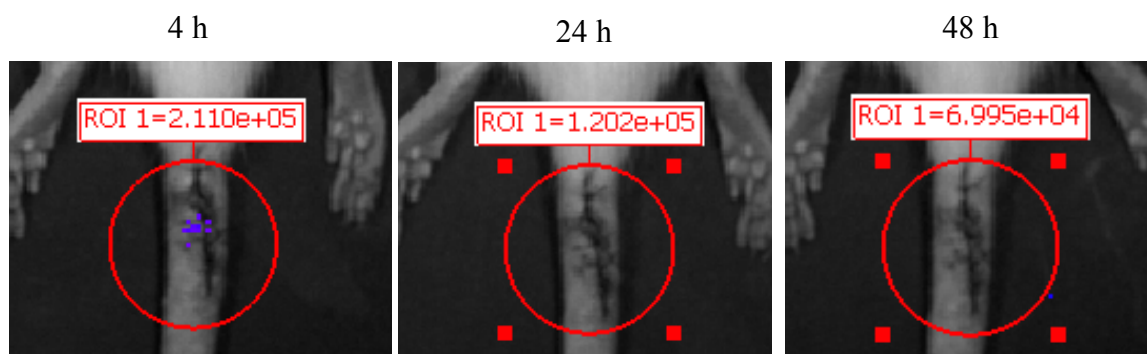


Fig. S2. IVIS images of *Luc* mRNA in the form of naked mRNA without nanomicelles.

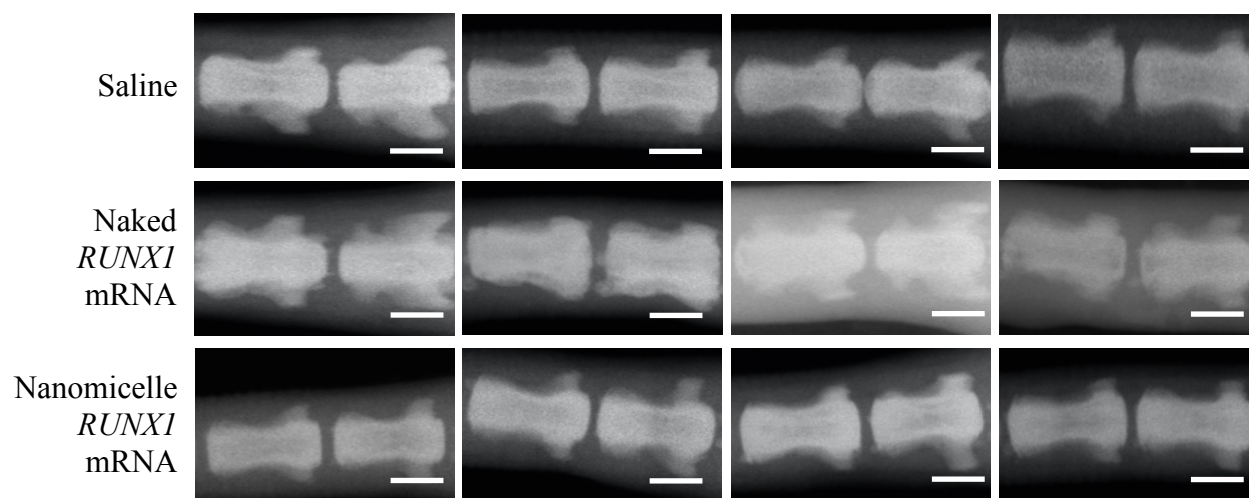


Fig. S3. All other radiographic images of coccygeal disk at 4 weeks post-injection (bar= 4 mm).

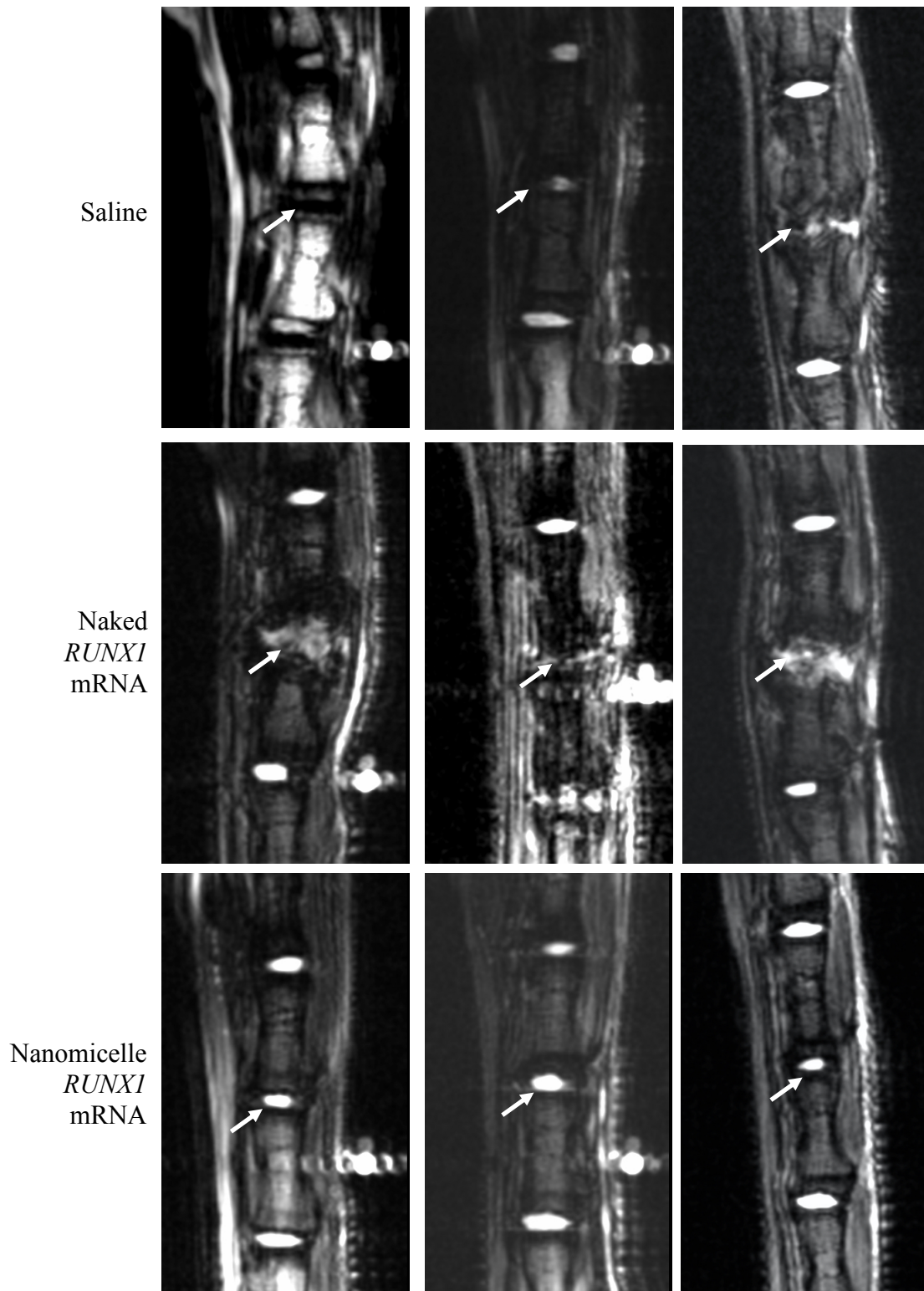


Fig. S4. All other MRI-T2 images of coccygeal disk at 4 weeks post-injection. Arrows indicate the micro-injected disks.

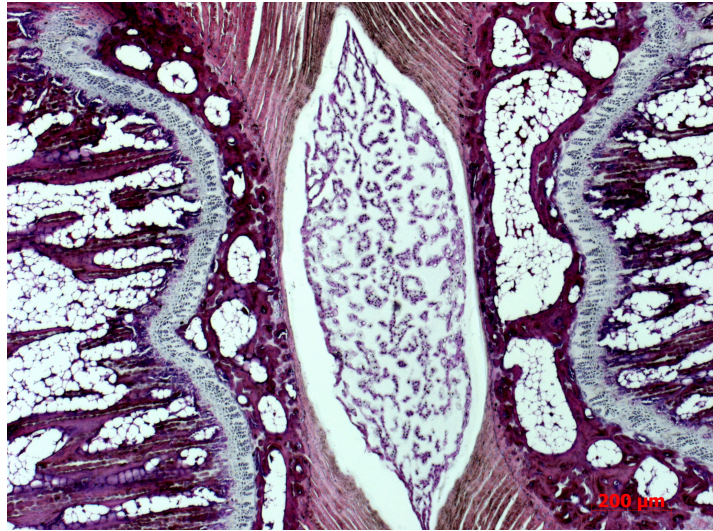
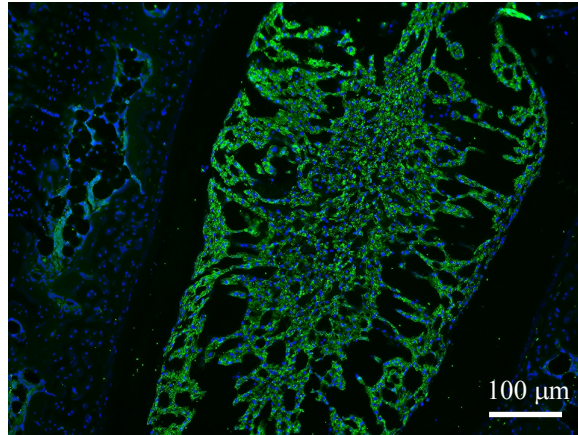


Fig. S5. H&E staining of Intact disk.

Collagen
type II



Aggrecan

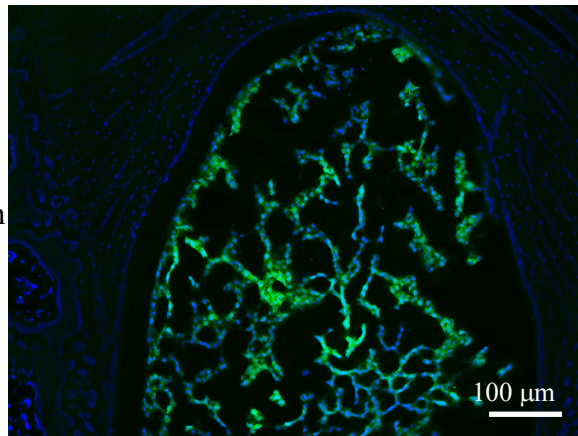


Fig. S6. Type II Collagen and aggrecan IHC of Intact disk.

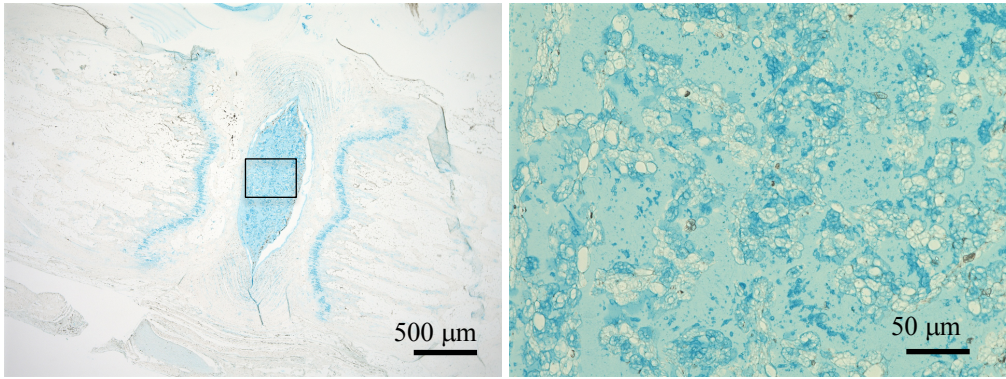


Fig. S7. Alcian blue staining of intact disk.

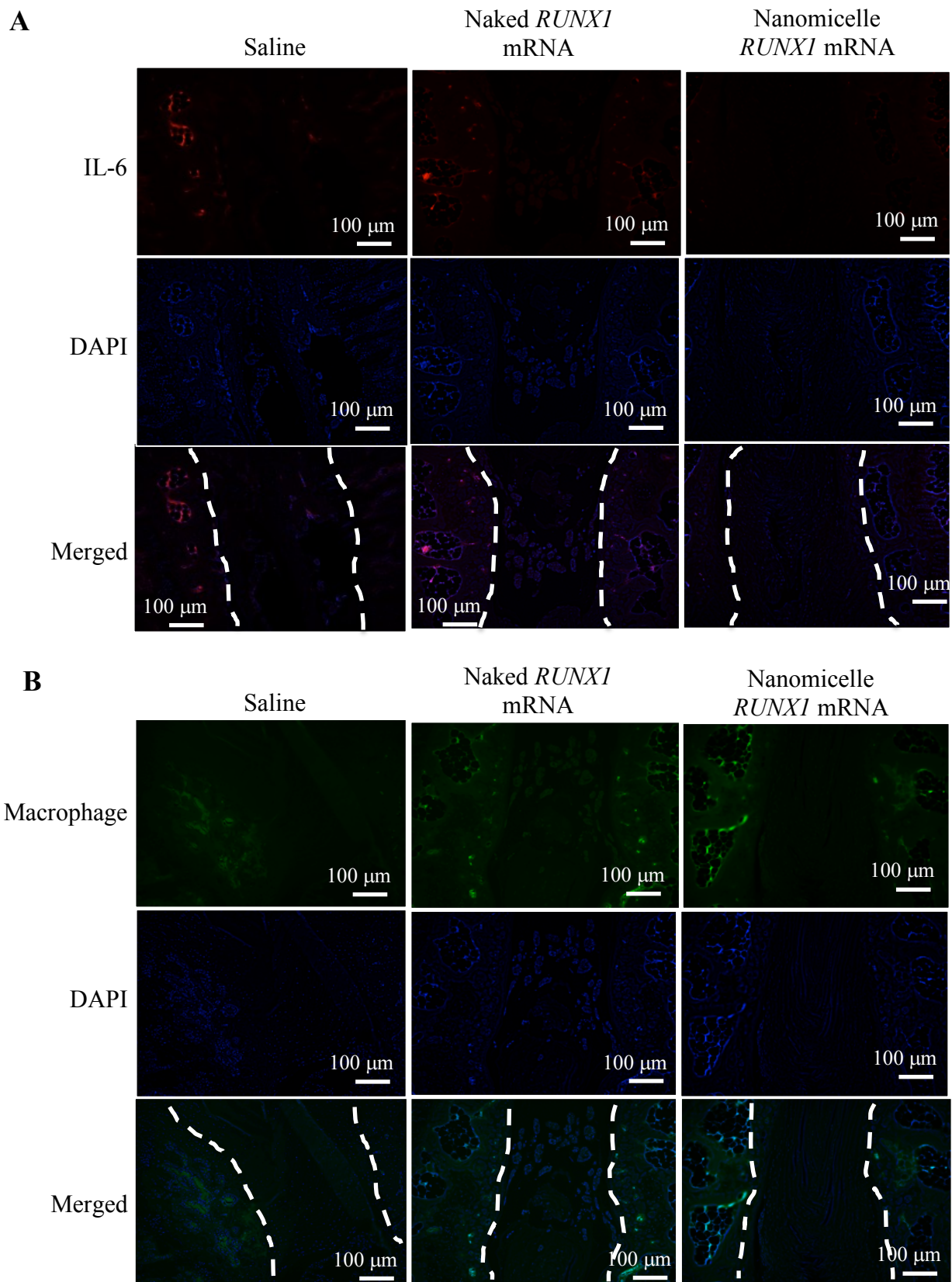
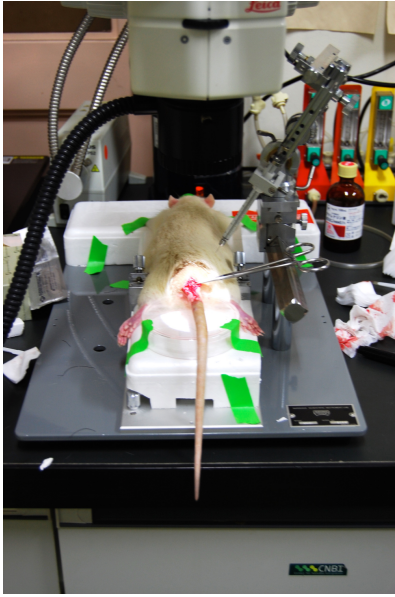
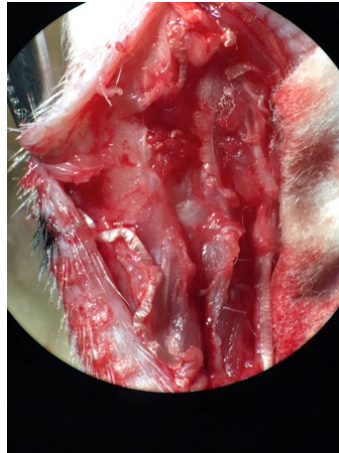


Fig. S8. IL-6 and macrophage IHC staining of disks removed at 2 weeks post-injection. **(A)** IL-6 IHC, the 2nd Ab was labeled with Alexa-647. **(B)** Macrophage IHC, the 2nd Ab was labeled with Alexa-488. Dotted lines indicate the disk area. Bar = 100 μ m.

A**B**

Pre-injection



micro-injection

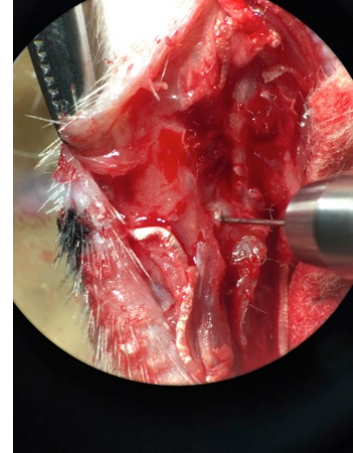
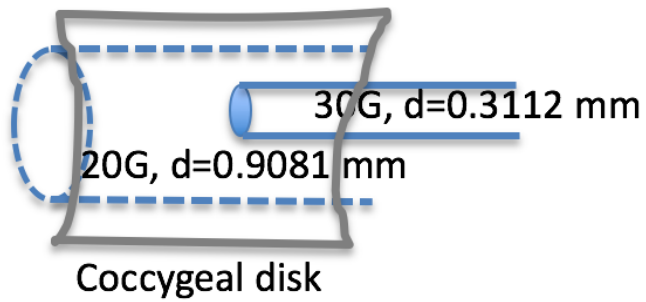
**C**

Fig. S9. (A) Rat coccygeal disks were operated on under the assistance of a stereotaxic instrument and microscope. (B) Gene mixtures were micro-injected with 30G micro-injection needle at $1 \mu\text{l}/\text{min}$ at a 1 mm depth. (C) Illustration of disk punctured with 20G needle, subsequently micro-injected with 30G needle through the larger track.