iScience, Volume *27*

Supplemental information

Regenerative potential of mouse neonatal intervertebral disc depends on collagen crosslink density

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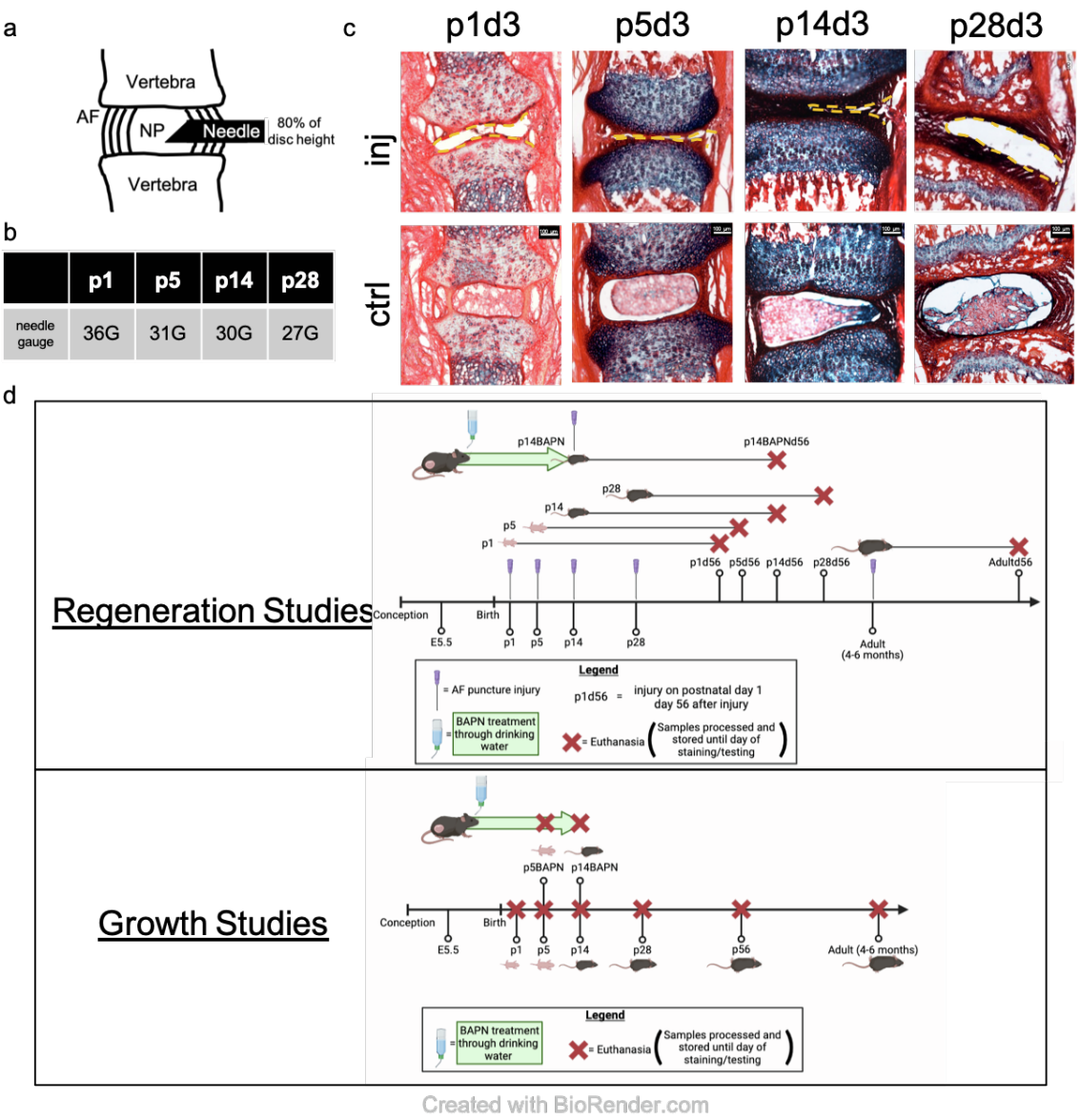
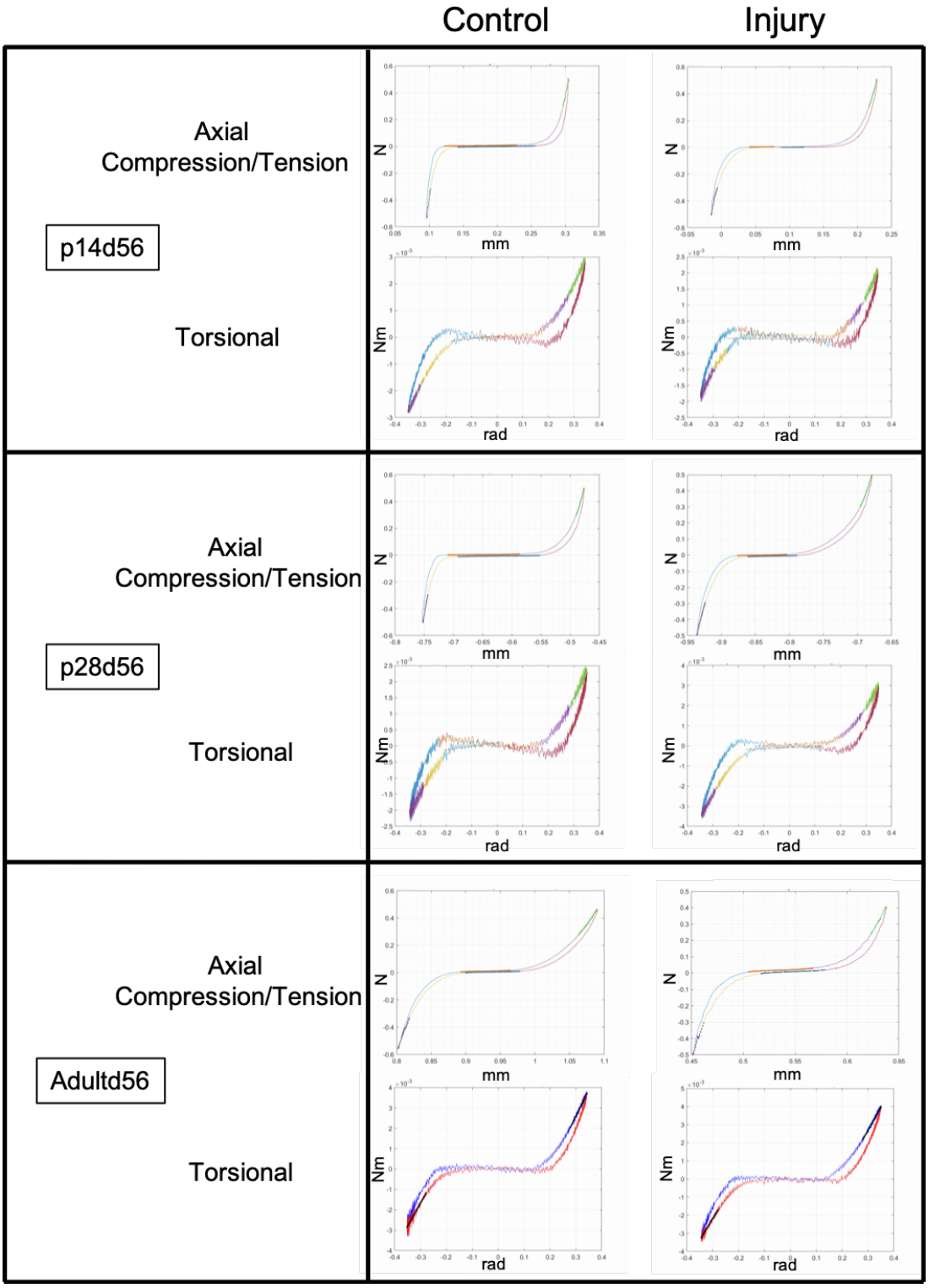


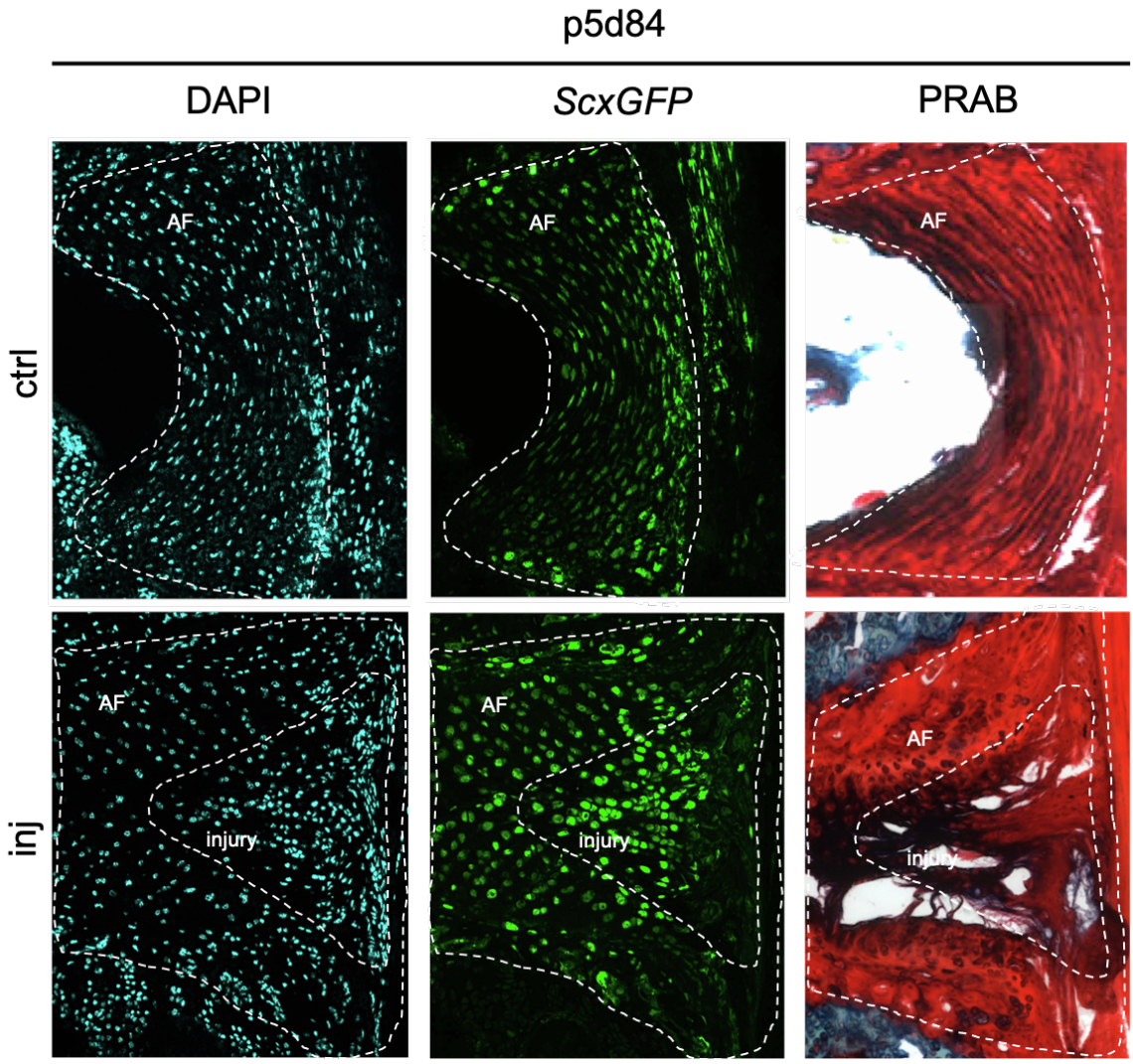
Supplemental Figure 1 Related to Figures 1-7. Injuries are comparable across postnatal p5, p14, and p28 ages but not p1. (A) Diagram illustrating puncture injury technique highlighting the use of needles approximately 80% of the IVD disc height and puncturing ≥50% of the IVD depth (B) Needle sizes used for each postnatal age (C) Representative Picrosirius Red Alcian Blue images of injured (Top) and control (Bottom) IVDs of postnatal day 1 mice - 3 days after injury (p1d3), p5d3, p14d3, and p28d3 mice under brightfield light. Yellow dashed lines outline injury sites. Scale bars represent 100 μm. (D) Timeline for injuries in p1, p5 mice, p14, p28, adult, and p14-BAPN mice (top) and timeline depicting age and/or BAPN treatment of mice used in growth studies (bottom).

Collagen Disorganization Grading Scale	
3	extensive darkening of birefringence **and** lamellar disruption
2	minor darkening of birefringence **and** disruption of lamellae
2	extensive darkening of birefringence **or** lamellar disruption adjacent to
1	minor darkening of birefringence **or** disruption of lamellae
0	bright birefringence **and** no disruption

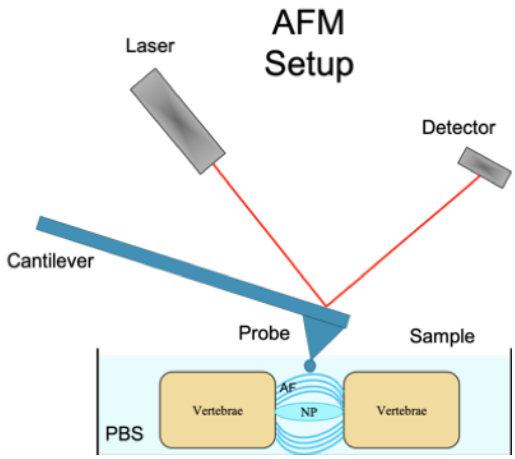
Supplemental Figure 2. Related to Figures 1,3, and 6. Collagen disorganization grading scale used to evaluated polarized light images.



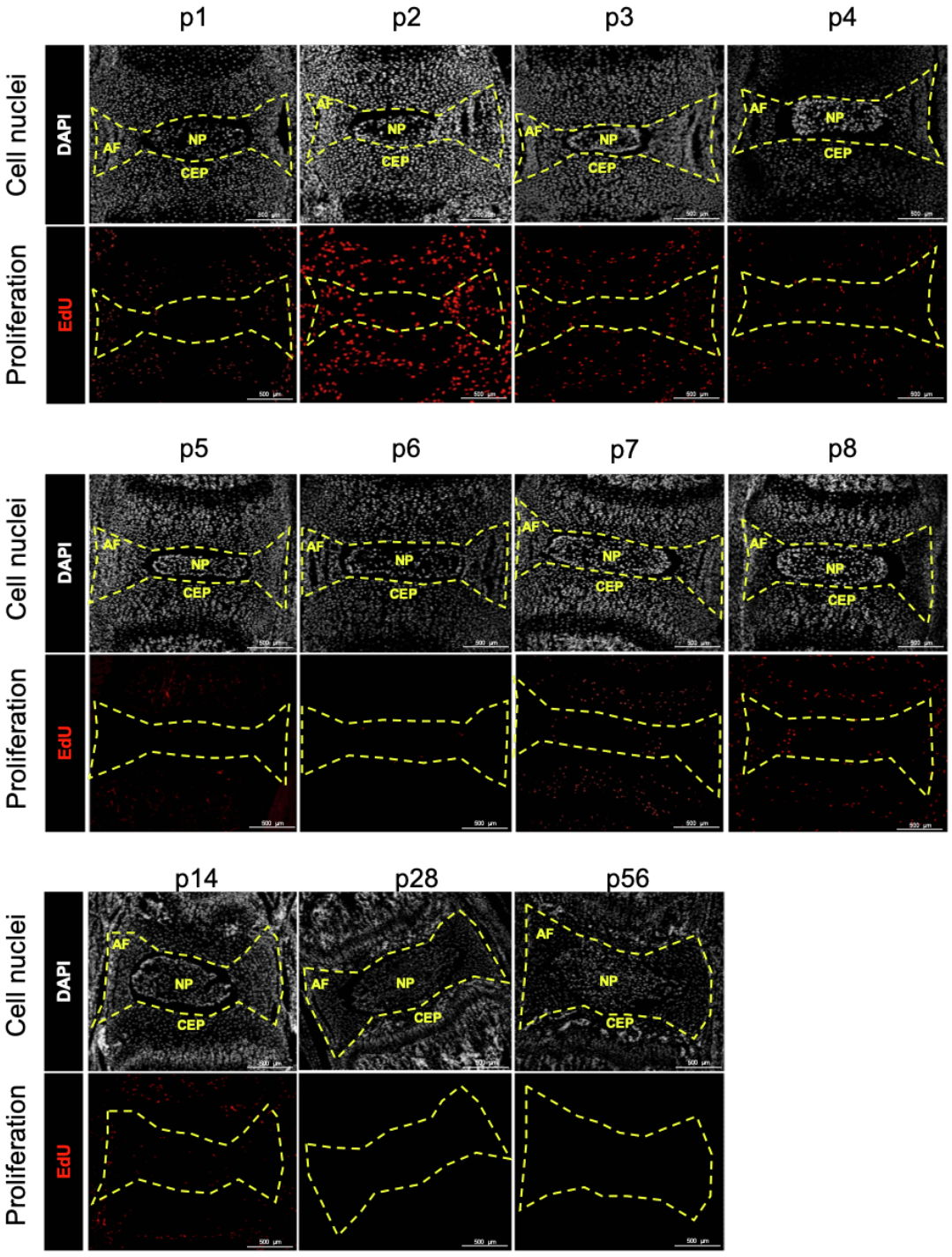
Supplemental Figure 3. Related to Figure 2. Representative force/displacement curves for biomechanical testing on control and injured p14d56 (top), p28d56 (middle), and adultd56 (bottom) specimens.



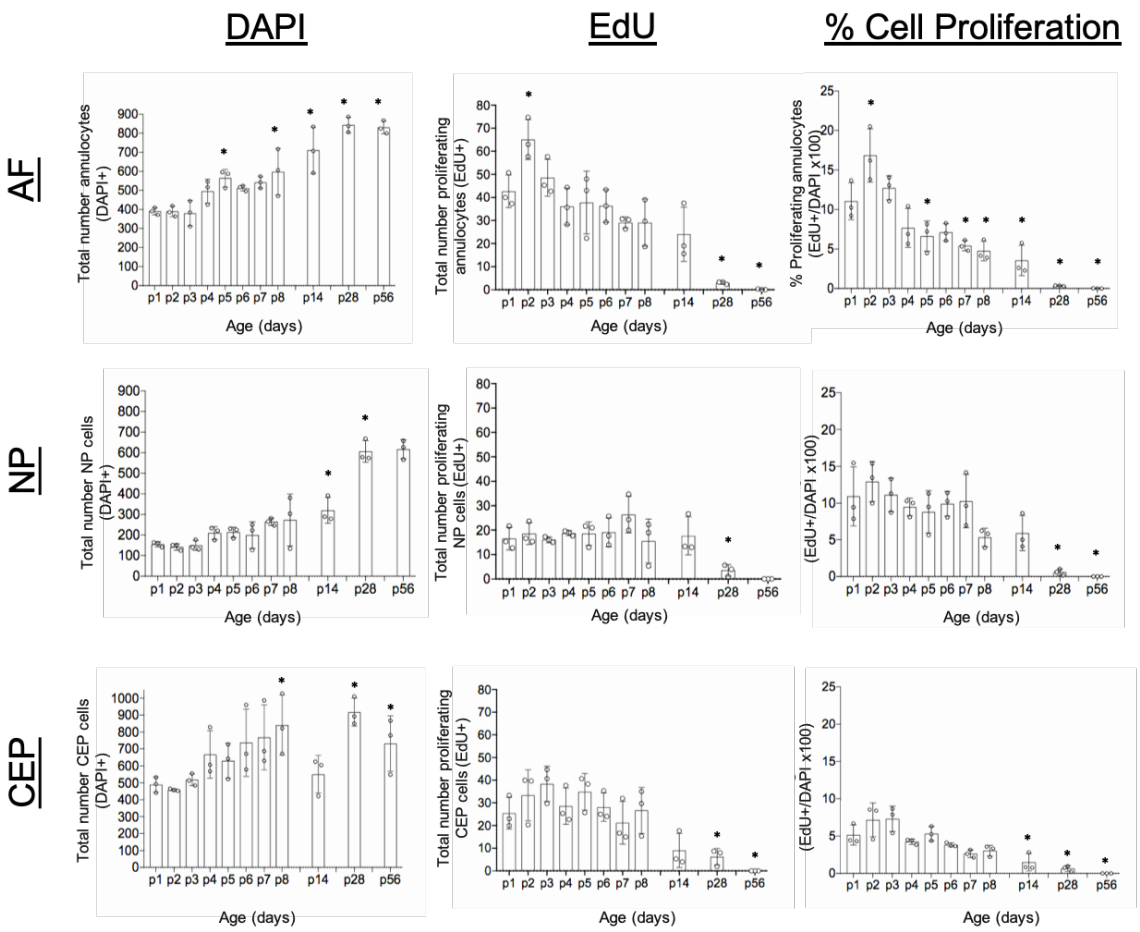
Supplemental Figure 4. Related to Figure 3. p5d84 maintains highly cellular matrix deposition within injury sites organized structure near injury sites. Control IVDs (top) and injured IVDs (bottom). DAPI (left) ScxGFP (middle), and Picrosirius Red Alcian Blue (PRAB) (right) images of p5d84 IVDs.



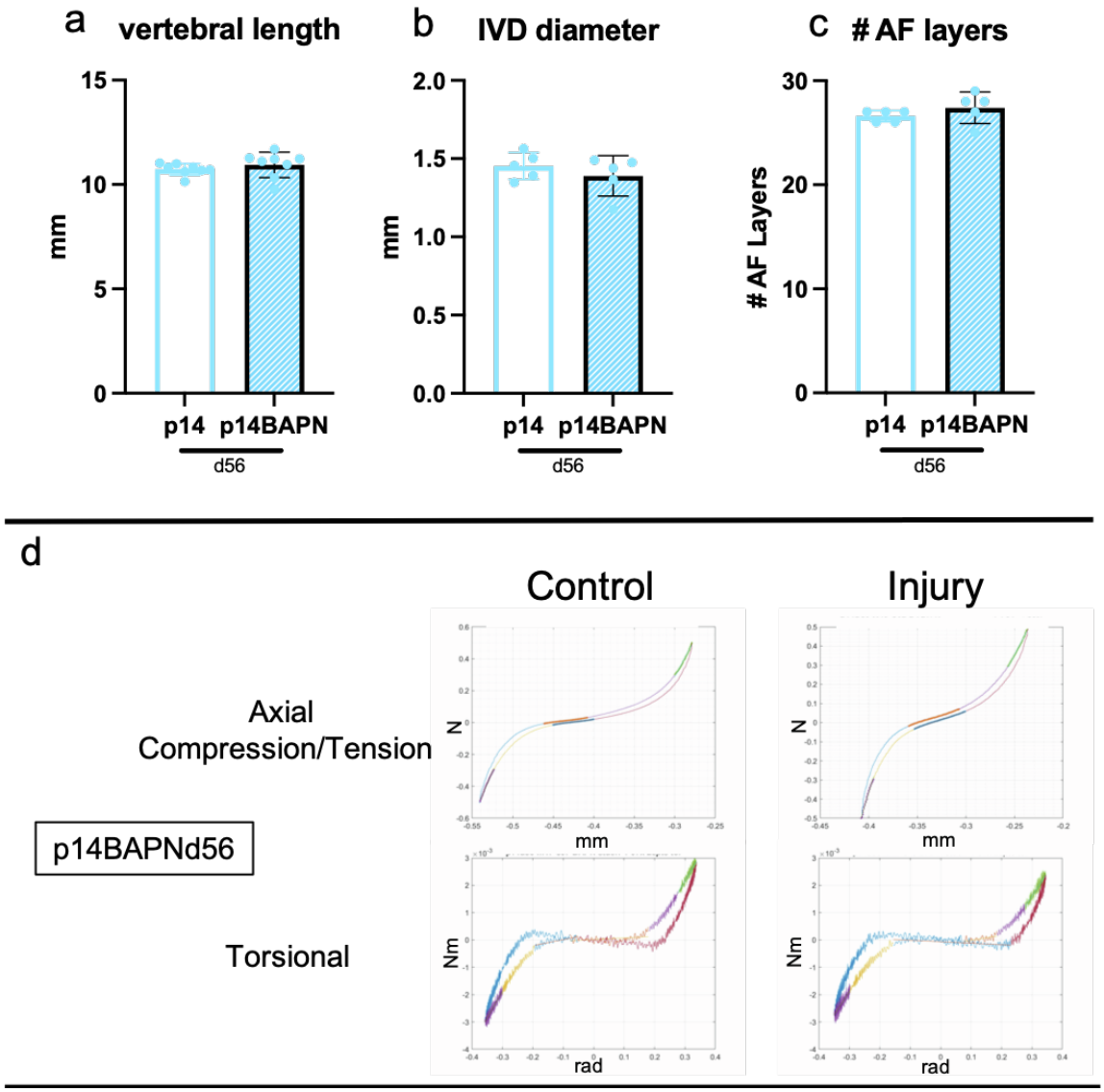
Supplemental Figure 5. Related to Figure 4. Sample setup for AFM nano-indentation testing of outer AF tissue



Supplemental Figure 6. Related to Figure 4. Representative images of EdU detection in postnatal IVDs using mid-sagittal sections of coccygeal IVDs (yellow outline).



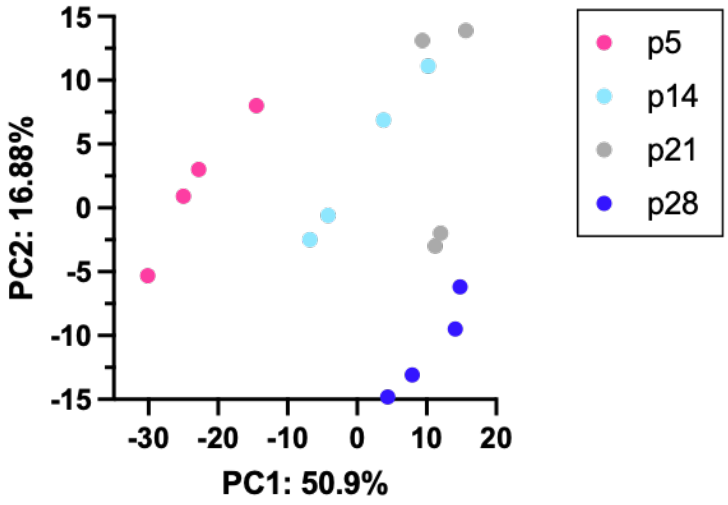
Supplemental Figure 7. Related to Figure 4. Quantification of total number of cells (DAPI), total number of proliferating cells (EdU), and % Cell proliferation in AF (top), NP (middle), and collagenous endplate (CEP) (bottom).



Supplemental Figure 8. Related to Figure 5 and 6. BAPN treatment does not change other growth parameters but does change functional regeneration after injury. Quantification of (A) vertebral length, (B) IVD diameter, and (C) Number of AF lamellar layers in uninjured control IVDs from p14d56 and p14BAPNd56 mice. (D) Representative force/displacement curves for biomechanical testing on control and injured p14BAPNd56 specimens.

p14 vs p28			
	Go Term	P_{Adj}	Combined Score
Depleted in p28	**extracellular matrix organization (GO:0030198)**	**0.003307**	47.44
	collagen fibril organization (GO:0030199)	**0.01623**	64.85
	protein localization to synapse (GO:0035418)	0.04944	218.03
	extracellular structure organization (GO:0043062)	**0.07997**	27.56
	external encapsulating structure organization (GO:0045229)	**0.07997**	27.27
	neuron differentiation (GO:0030182)	0.1007	27.77
	embryonic digestive tract development (GO:0048566)	0.1269	83.98
	eye morphogenesis (GO:0048592)	0.1269	83.98
	regulation of NMDA receptor activity (GO:2000310)	0.1269	65.25
	regulation of smoothened signaling pathway (GO:0008589)	0.1269	45.22
Enriched in p28	osteoclast differentiation (GO:0030316)	0.0001201	332.76
	blood vessel morphogenesis (GO:0048514)	0.0001201	155.46
	neutrophil degranulation (GO:0043312)	**0.0001201**	50.36
	neutrophil activation involved in immune response (GO:0002283)	**0.0001201**	49.34
	neutrophil mediated immunity (GO:0002446)	**0.0001201**	48.59
	extracellular matrix organization (GO:0030198)	0.0006805	47.19
	cellular response to cytokine stimulus (GO:0071345)	**0.0006805**	39.43
	regulation of cell migration (GO:0030334)	0.0007268	40.48
	extracellular structure organization (GO:0043062)	0.001018	50.42
	external encapsulating structure organization (GO:0045229)	0.001018	49.92

Supplemental Figure 9. Related to Figure 7. Top 10 depleted and enriched pathways in p28 AF cells compared to p14 AF cells.



Supplemental Figure 10. Related to Figure 7. Bulk sequencing of p5, p14, p21, and p28 cells revealed PCA plot highlighting p5, p14, and p28 gene expression profiles are distinct but not p21 is not.