Science Advances
AAAS

Supplementary Materials for

Nuclear factor κB overactivation in the intervertebral disc leads to macrophage recruitment and severe disc degeneration

Kevin G. Burt *et al.*

Corresponding author: Nadeen O. Chahine, noc7@columbia.edu

Sci. Adv. **10**, eadj3194 (2024)
DOI: 10.1126/sciadv.adj3194

This PDF file includes:

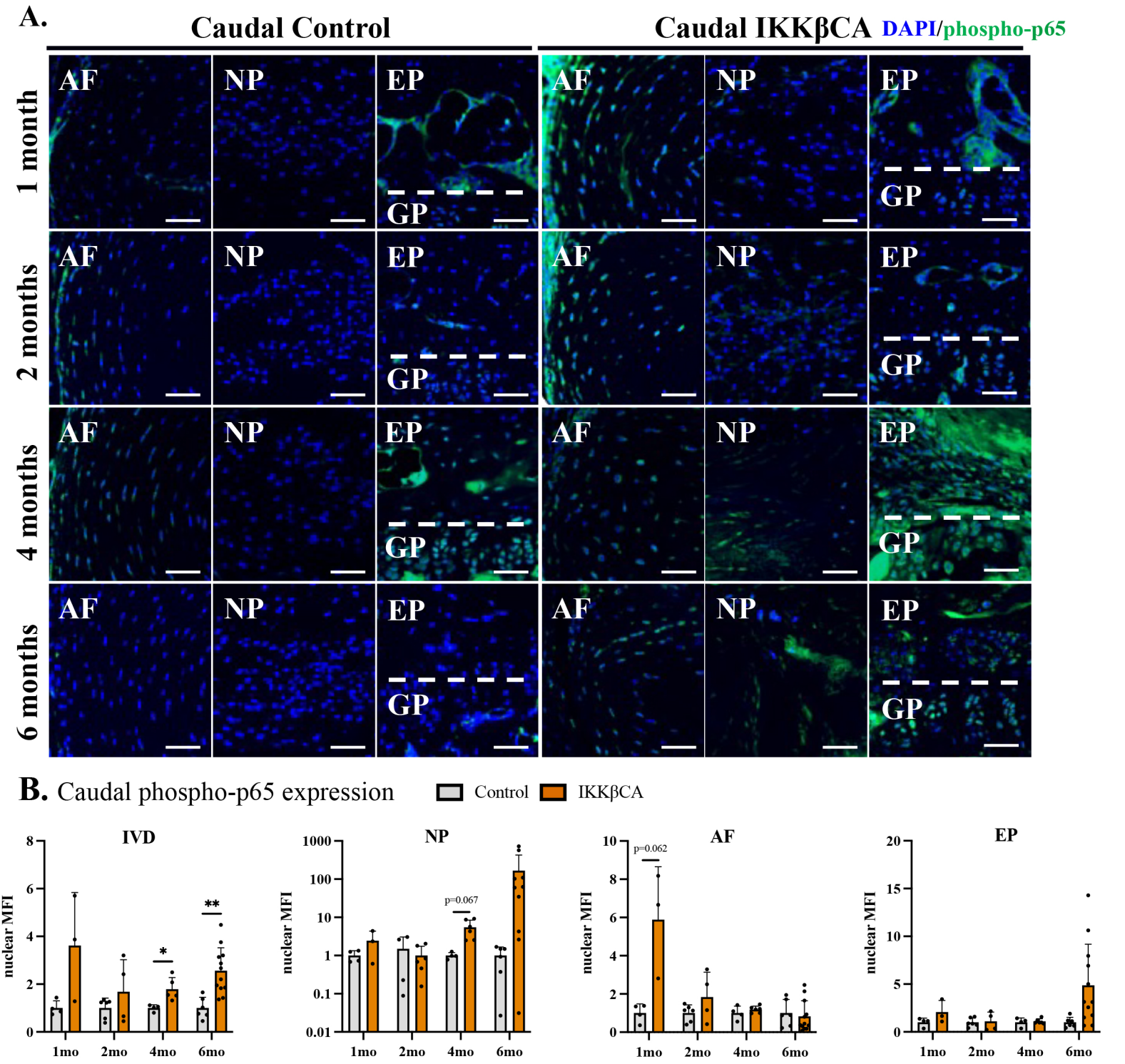

Fig. S1. NF-κB activation within IKKβCA caudal IVDs. (**A**) Representative IF staining for phosphorylated p65 (green) within mid sagittal sections of control (*AcanCre*$^{-/-}$*;Ikk2ca*$^{fl/fl}$) and IKKβCA caudal IVDs. Scale bar = 50μm. (**B**) Nuclear MFI quantification (normalized to control within time point) of phosphorylated p65. *p<0.05, **p<0.01 (n=3-6 mice/group, 1-2 discs/mouse).

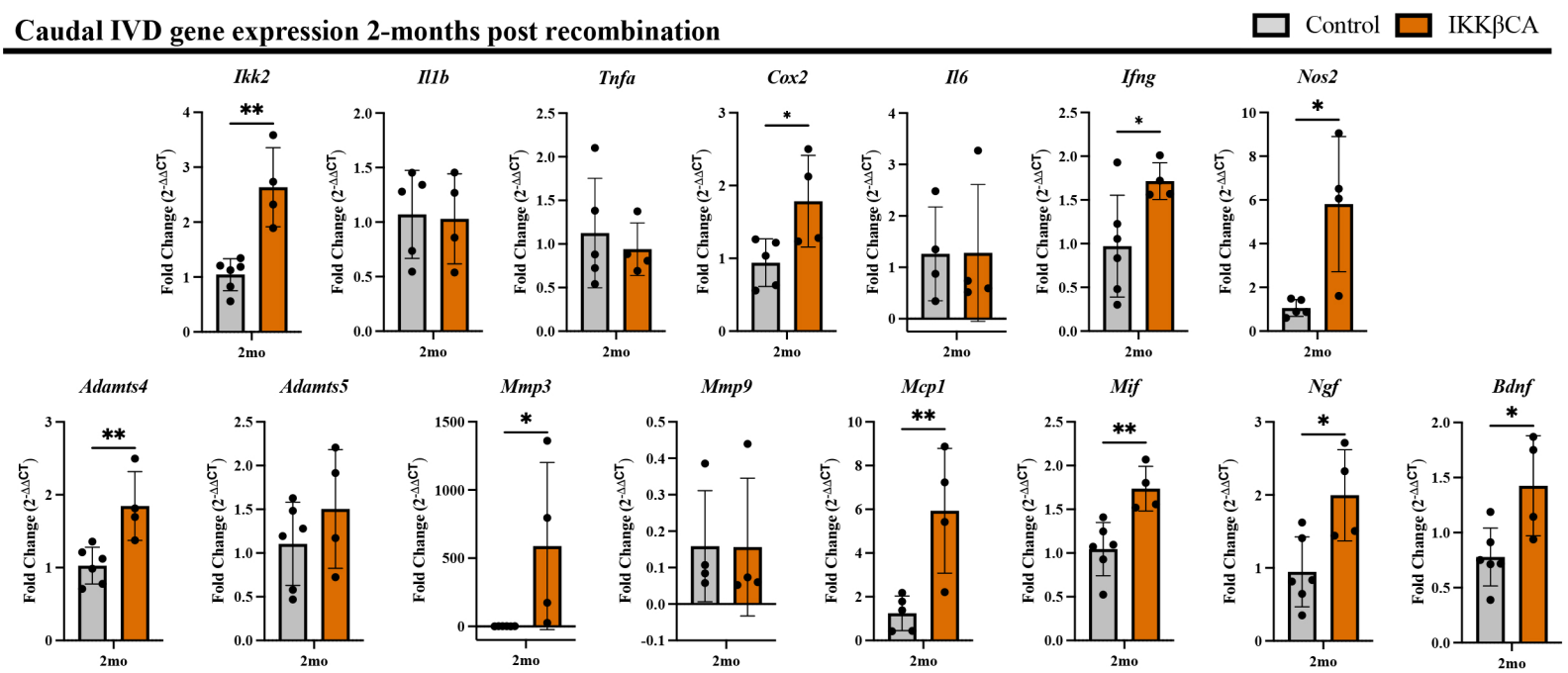

Fig. S2. IKKβ over-expression upregulates caudal IVD inflammatory cytokine, chemokine, catabolic enzyme, and neurotrophic factor gene expression. Gene expression changes (relative to control) from total RNA isolated from control (*AcanCre*$^{-/-}$*:Ikk2ca*$^{fl/fl}$) and IKKβCA whole caudal IVDs containing NP, AF, and EP, 2-months post recombination. *p<0.05, **p<0.01, ***p<0.001. (n=3 mice/genotype per timepoint, 1-2 discs/mouse).

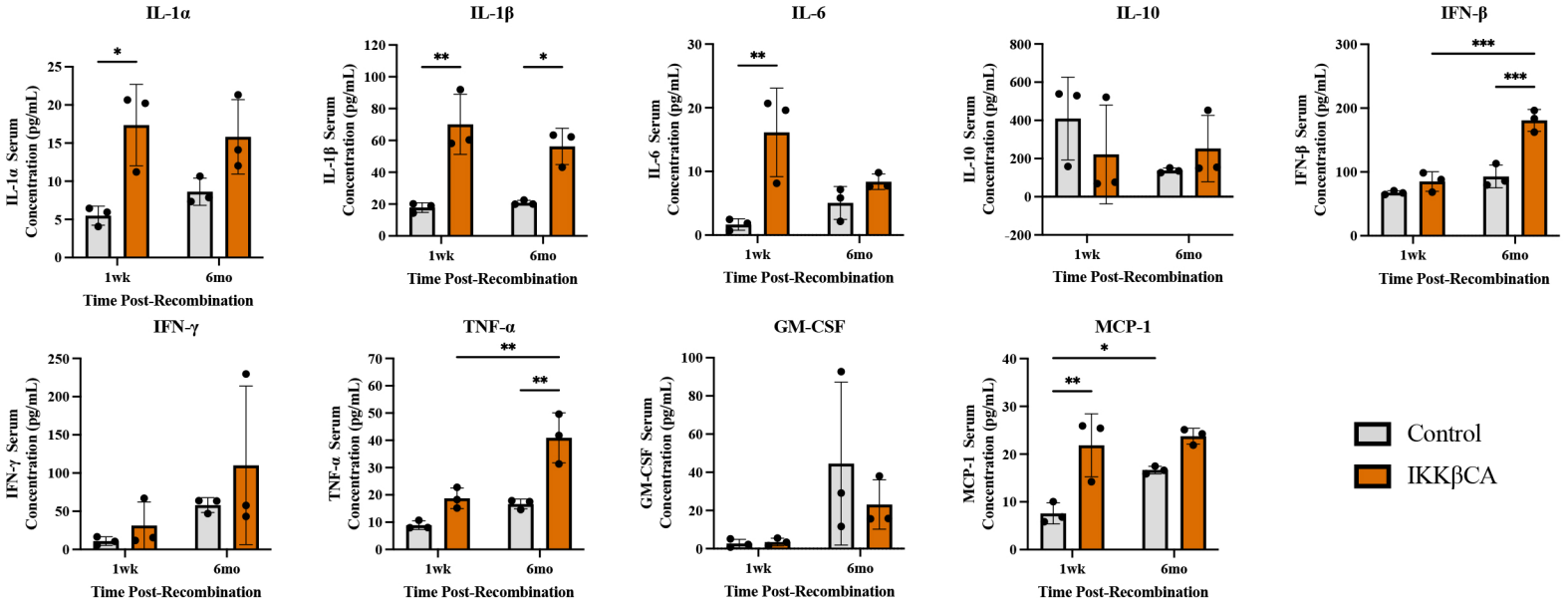

Fig. S3. IKKβ over-expression upregulates circulating inflammatory cytokine and chemokines in the serum. Cytokine and chemokine levels within serum isolated from control (*AcanCre*$^{-/-}$*;Ikk2ca*$^{fl/fl}$) and IKKβCA mice 1 week and 6 months post recombination. *p<0.05, **p<0.01, ***p<0.001 (n=3/genotype per timepoint).

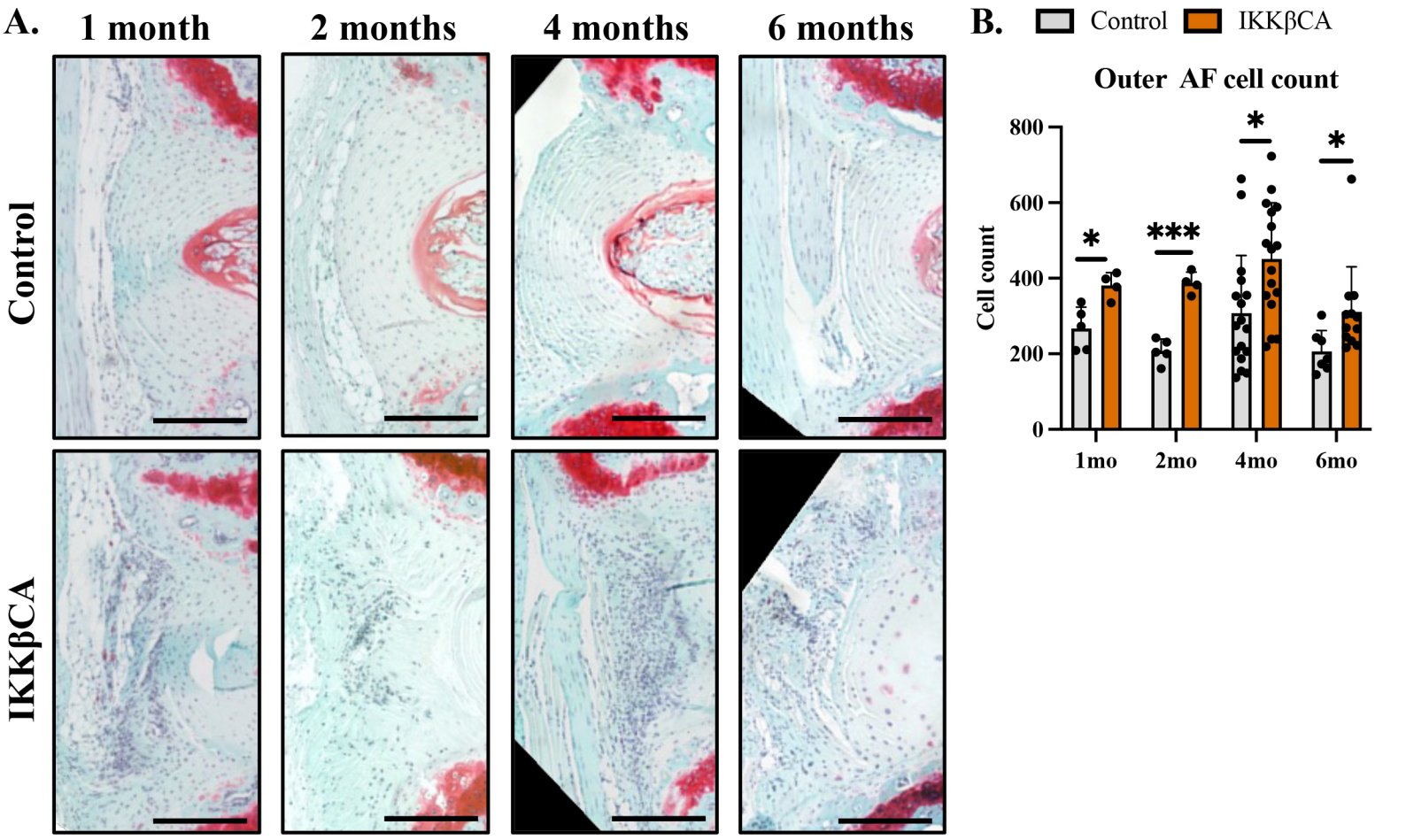

Fig. S4. IKKβ over-expression increases cellularity presence within the caudal AF. (**A**) Representative images of safranin-O stained mid sagittal sections of control (*AcanCre*$^{-/-}$*;Ikk2ca*$^{fl/fl}$) and IKKβCA caudal IVDs 1-, 2-, 4-, and 6-months post recombination. Scale bar = 250μm. (**B**) Quantification of outer AF cellularity using haematoxylin nuclear stain (n=4-6 mice/genotype and timepoint, 1-4 IVDs/mouse).

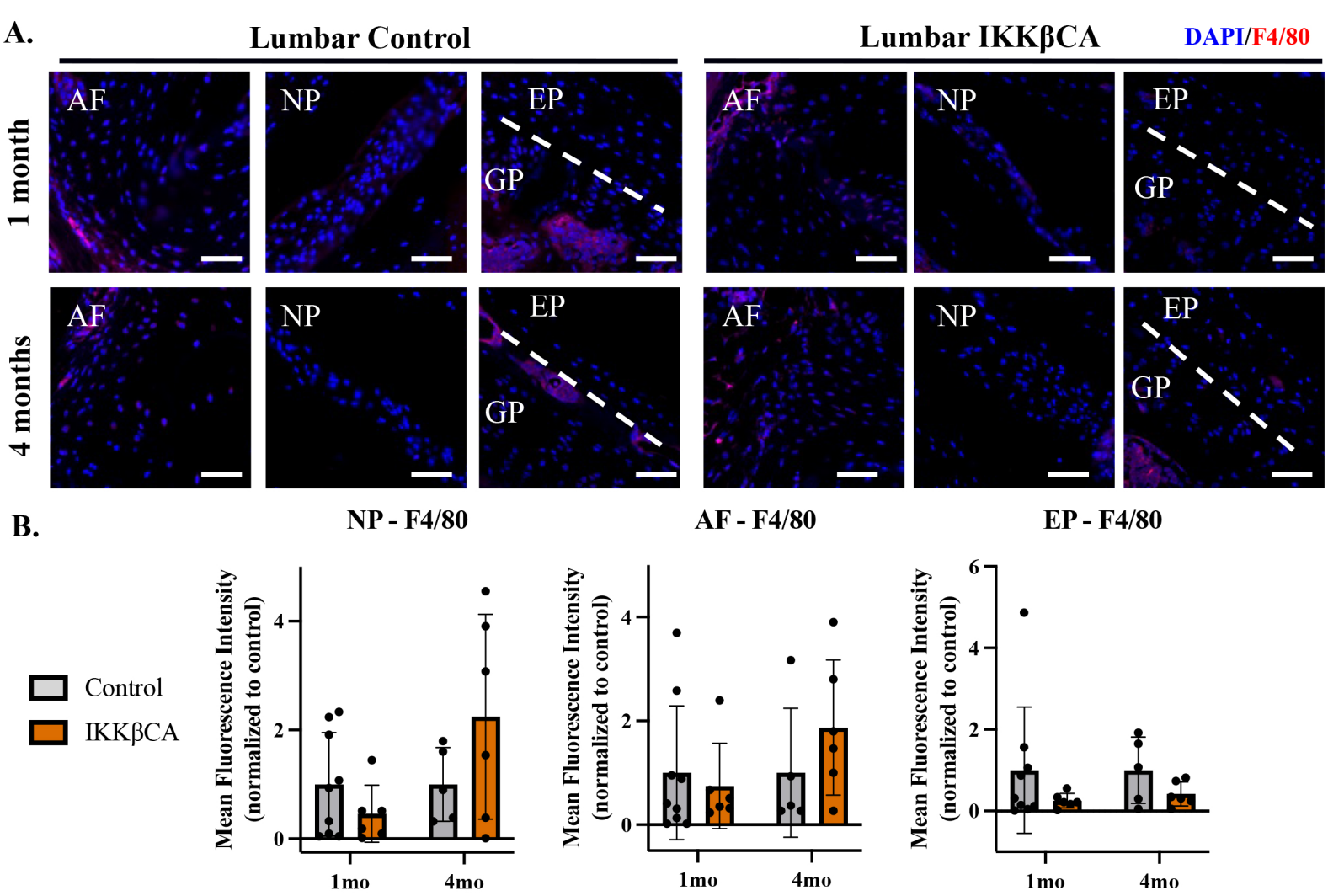

Fig. S5. Macrophage presence within the lumbar IVDs. (**A**) Representative images of IF staining for F4/80, in mid sagittal sections of control (*AcanCre*$^{-/-}$*:Ikk2ca*$^{fl/fl}$) and IKKβCA lumbar IVDs at 1- and 4-months post recombination. Scale bar = 100μm. (**B**) MFI quantification of F4/80 expression within individual NP, AF, and EP compartments. Letters (a,b,c) indicating statistically significant ($p<0.05$) different groupings. (n=3-6 mice/genotype, 1-2 discs/mouse).

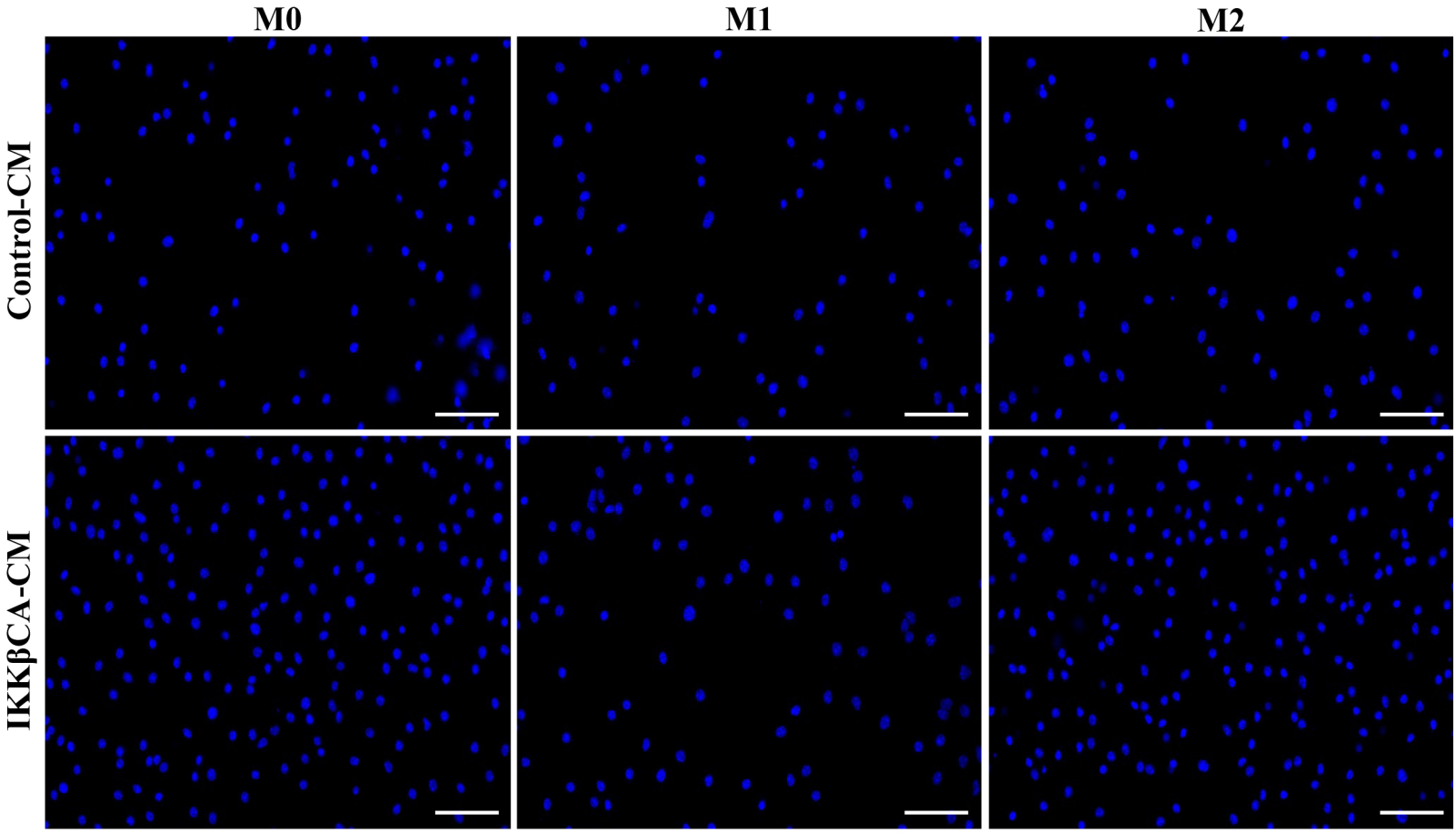

Fig. S6. Caudal IKKβCA-CM increases macrophage migration *in vitro*. Representative images of DAPI (nuclear) stained M0, M1, and M2 macrophages on transwell membranes following caudal IKKβCA-CM stimulation. Cell counting used for quantification of migration through transwell membranes. Scale bar = 100μm (n=3).

Table S1: FWD and REV murine primers (IDT) for rt-qPCR.

Gene Target, murine	Forward Primer	Reverse Primer
Glyceraldehyde-3-phosphate dehydrogenase (***Gapdh***)	AAC AGC AAC TCC CAC TCT TC	CCT GTT GCT GTA GCC GTA TT
inhibitor of nuclear factor kappa B kinase subunit beta (***Ikk2***)	CTG AAG ATC GCC TGT AGA AA	TCC ATC TGT AAC CAG CTC CAG
interleukin-6 (***Il6***)	CTT CCA TCC AGT TGC CTT CT	CTC CGA CTT GTG AAG TGG TAT AG
tumor necrosis factor alpha (***Tnfa***)	TTG CTC TGT GAA GGG AAT GG	GGC TCT GAG GAG TAG ACA ATA AAG
interleukin-1 beta (***Il1b***)	ATG GGC AAC CAC TTA CCT ATT T	GTT CTA GAG AGT GCT GCC TAA TG
nitric oxide synthase 2 (***Nos2***)	TCT CCC TTT CCT CCC TTC TT	CTT CAG TCA GGA GGT TGA GTT T
interferon gamma (***Ifng***)	GGC CAT CAG CAA CAA CAT AAG	GTT GAC CTC AAA CTT GGC AAT AC
Prostaglandin-Endoperoxide Synthase 2 (***Ptgs2***/***Cox2***)	GAA GAT TCC CTC CGG TGT TT	CCC TTC TCA CTG GCT TAT GTA G
Cluster of differentiation 38 (**CD38**)	TCT CTC TCT CTC TCT CTC TCT CT	TCA GCT GTG CTG AGG ATT TAG
Cluster of differentiation 206 (**CD206**)	GGA ATC AAG GGC ACA GAG TTA	TTC CAT CTG CTC CAC AAT CC
monocyte chemoattractant protein-1 (***Mcp1***)	CTC GGA CTG TGA TGC CTT AAT	TGG ATC CAC ACC TTG CAT TTA
macrophage inhibitory factor (***Mif***)	GTT CCA CCT TCG CTT GAG T	CAT CGC TAC CGG TGG ATA AA
Matrix Metallopeptidase 3 (***Mmp3***)	GGA CCA GGG ATT AAT GGA GAT G	TGA GCA GCA ACC AGG AAT AG
Matrix Metallopeptidase 9 (***Mmp9***)	CTG GAA CTC ACA CGA CAT CTT	TCC ACC TTG TTC ACC TCA TTT
A Disintegrin-Like And Metalloprotease With Thrombospondin Type 1, Motif 4 (***Adamts4***)	GGC AGA GAA GGG ATG ATG TAA TAG	CCC AAC ATC ACC CAG GTA ATA A
A Disintegrin-Like And Metalloprotease With Thrombospondin Type 1, Motif 5 (***Adamts5***)	GTG CTG TGT TTG CCA TCT TC	GCA CTG CCT TGT TCT GTT TC
nerve growth factor (***Ngf***)	CAG TGA GGT GCA TAG CGT AAT	CTC CTT CTG GGA CAT TGC TAT C
brain derived neurotrophic factor (***Bdnf***)	CAA GAG TCC CGT CTG TAC TTT AC	GAC TAG GGA AAT GGG CTT AAC A

Table S2: Primary and secondary fluorescent antibodies used for immunohistochemistry.

Antigen	Company	Cat #	Dilution
[1º] mCherry	Sicgen	AB0040-200	1:250
[1º] Phosphorylated p-65	Abcam	AB86299	1:500
[1º] F4/80	Bio-rad	MCA497GA	1:100
[1º] IKKβ	Sigma	07-1479	1:500
[1º] CD38	Fisher	PIMA516871	1:100
[1º] CD206	Fisher	PIPA595840	1:100
[2º] Donkey anti-goat (AF 594)	Thermo Fisher	A-11058	1:250
[2º] Donkey anti-rabbit (AF 594)	Abcam	AB150064	1:200
[2º] Donkey anti-rat (AF 488)	Abcam	Ab150153	1:200