

APPENDIX

Supplemental Figure 1: Expression of Notch and Notch ligands in NVR mouse at

E18.5. Immunohistochemistry was performed for Notch1, Notch 2, Notch 4, HES1, DDL4 and JAGGED1 in the TMJ condyles of *CBF:H2B-Venus* reporter mouse strain (NVR) at E18.5.

Supplemental Figure 2: Generation of fibrocartilage stem cell (FCSC) line with

ligand independent constitutively activated Notch. (a) PCR analysis following enzymatic restriction digest confirming Notch intracellular domain (NICD) in bacterial colonies. Lane 3 represents positive NICD colony. (b) Rat FCSCs following 48hrs transduction with GFP NCID plasmid lentivirus.

Supplemental Figure 3: Expression of Notch receptors are increased in TNF-alpha

induced TMJ arthritis mouse model. (a) TNF-alpha was injected unilaterally into the TMJ intra-articular space of 6 week-old mice twice 3 days apart to induce inflammatory TMJ arthritis, while saline was injected into the contralateral TMJ as a control. TMJ condyles were examined by immunohistochemistry after 2 and 14 days. (b) Immunohistochemistry was performed for Notch1, Notch 2 and Notch 4 in TMJ condyles following exposure to saline control and TNF-alpha.

Supplemental Figure 4: Expression of Notch ligands are increased in TNF-alpha

induced TMJ arthritis mouse model. (a) TNF-alpha was injected unilaterally into the TMJ intra-articular space of 6 week-old mice twice 3 days apart to induce inflammatory TMJ arthritis, while saline was injected into the contralateral TMJ as a control. TMJ condyles were examined by immunohistochemistry after 2 and 14 days. (b)

Immunohistochemistry was performed for DLL4, JAGGED1 and HES1 in TMJ condyles following exposure to saline control and TNF-alpha.

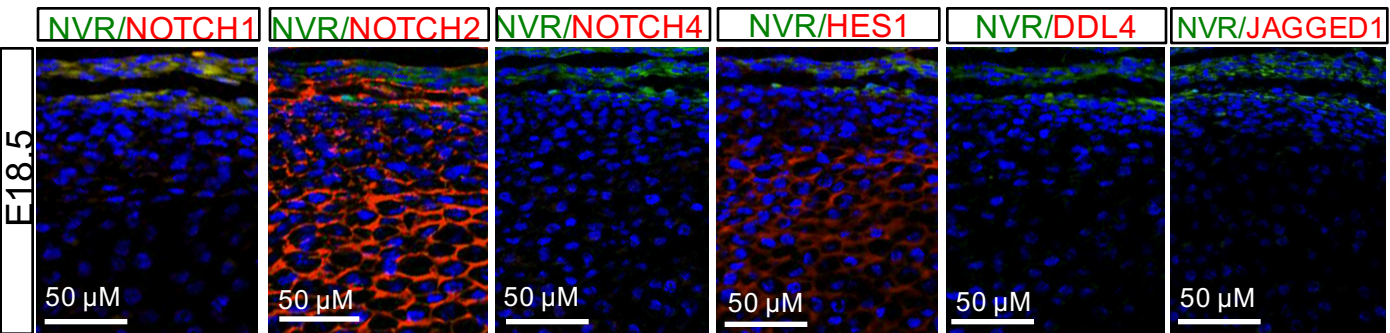
Supplemental Table 1: PCR Primers

Gene	FWD or REV	Sequence
<i>Rn Gapdh</i>	Forward	CCG CAT CTT CTT GTG CAG TG
	Reverse	GGT AAC CAG GCG TCC GAT AC
<i>Rn Acan</i>	Forward	CCA TGT GTG GGT GAC AAG GA
	Reverse	GCT CCA TTT GTG TGG CGA AG
<i>Rn Sox9</i>	Forward	TCT ACT CCA CCT TCA CCT ACA T
	Reverse	CTG TGT GTA GAC GGG TTG TT
<i>Rn Col2a1</i>	Forward	CAT AGG GCC TGT CTG TTT CTT
	Reverse	CCA TTC AGT GCA GAT CCT AGA G
<i>Rn Runx2</i>	Forward	GGACCGACACAGCCATATAAA
	Reverse	GCCTCATTCCCTAACCTGAAA
<i>Rn Ocn</i>	Forward	ATC TAT GGC ACC ACC GTT TAG
	Reverse	CAT ACT TTC GAG GCA GAG AGA G
<i>Rn Pparγ</i>	Forward	CTG GCC TCC CTG ATG AAT AAA G
	Reverse	AGG CTC CAT AAA GTC ACC AAA G
<i>Rn Notch1</i>	Forward	CTC AAC ACA CTG GGC TCT TT
	Reverse	ATT CTG ACA TGG GTT GGA GAT G
<i>Rn Notch2</i>	Forward	CTTACAGCTTCCCTCTGTCATC
	Reverse	CTCTGAGCAACCCTAGCTTATC
<i>Rn Notch3</i>	Forward	GCAAGCACTCACACACAATAAA
	Reverse	CTCTACTCTCTCTCTCCCTTCTTC
<i>Rn Notch4</i>	Forward	GGCAACAAAAGCACGAGGAG
	Reverse	GTGTAGTCCCGTCGTCTGTG
<i>Rn Dll1</i>	Forward	CTCTTCTGCAACCAGGGTAAG
	Reverse	CAGGAAGCCCAAGGTCAAATA
<i>Rn Dll3</i>	Forward	GTCCCAGGTTCCCACATTCC
	Reverse	CCAGACGATGGCAGAGAAGG
<i>Rn Dll4</i>	Forward	GCCTTCCTTCTGCATTGTTTAC
	Reverse	CCTCCTCCTTCTCCTCTTCTT
<i>Rn Jagged1</i>	Forward	AGAGAGAGAGAGAGAGAGAGAGA
	Reverse	CCAGAGAAGGGCCAAGATAAC
<i>Rn Jagged2</i>	Forward	ATGCCACACAGACCCTACCA
	Reverse	CAGCCCTCCTGACCCTATCT
<i>Rn Hes1</i>	Forward	ATT CTG ACA TGG GTT GGA GAT G

	Reverse	CCA GAA TGT CTG CCT TCT CC
<i>Rn Hes3</i>	Forward	ACAAAGGAAGGTCCACAAGAG
	Reverse	AGAGGAGAGAGAGAGAGAGAGA
<i>Rn Hes5</i>	Forward	GCTCAGTCCCAAGGAGAAA
	Reverse	GAGGAGATGCGTCAGGTG
<i>Rn Hes6</i>	Forward	GGGAGGACACTACAGAAAGAAG
	Reverse	CTCATGCTCAGCCTCAAGAT
<i>Rn Hes7</i>	Forward	GGAGAAAGGAACAGGGAAAGAA
	Reverse	GAAAGGACAGGGAACTGGTATC
<i>Rn Hey1</i>	Forward	GGC TAT GGA CTA TCG GAG TTT G
	Reverse	TGG GAT GCG TAG TTG TTG AG
<i>Rn Hey2</i>	Forward	AGATCTCCACAGCAGCAATAAA
	Reverse	GTTGCCAAGCTGCCTTAAAC
<i>Hs Notch1intra</i>	Forward	CTCACCTGGTGCAGACCCAG
	Reverse	GCACCTGTAGCTGGTGGCTG

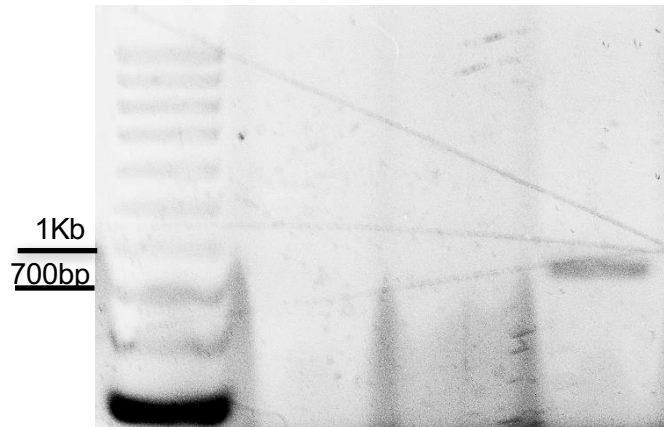
Supplemental Table 2: Antibodies used for immunohistochemistry

Primary Antibody	Source
Goat anti- Notch 1	R&D #AF1057 (1:100)
Rabbit anti- Notch 2	Abcam #ab8926 (1:100)
Rabbit anti-Notch 4	Homemade #Rb2-2AFR (1:100)
Goat anti- DLL4	R&D #AF1389 (1:100)
Goat anti-Jagged 1	R&D #AF599 (1:100)
Rabbit anti-Hes 1	Santa Cruz #H-140 (1:100)

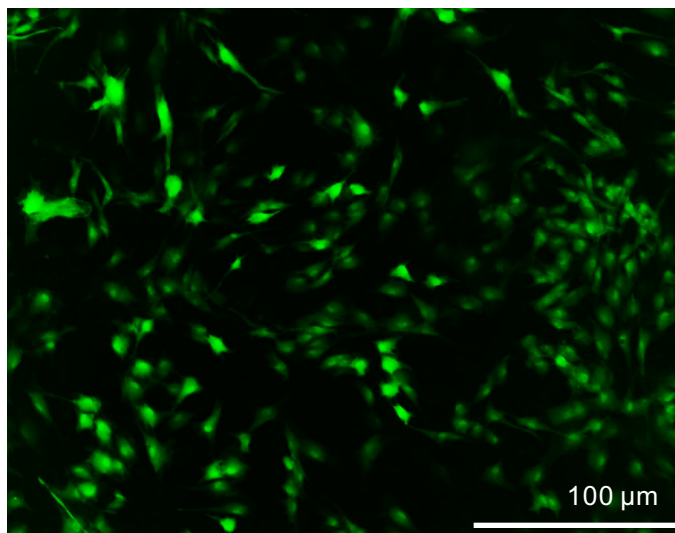


Supplemental Figure 1

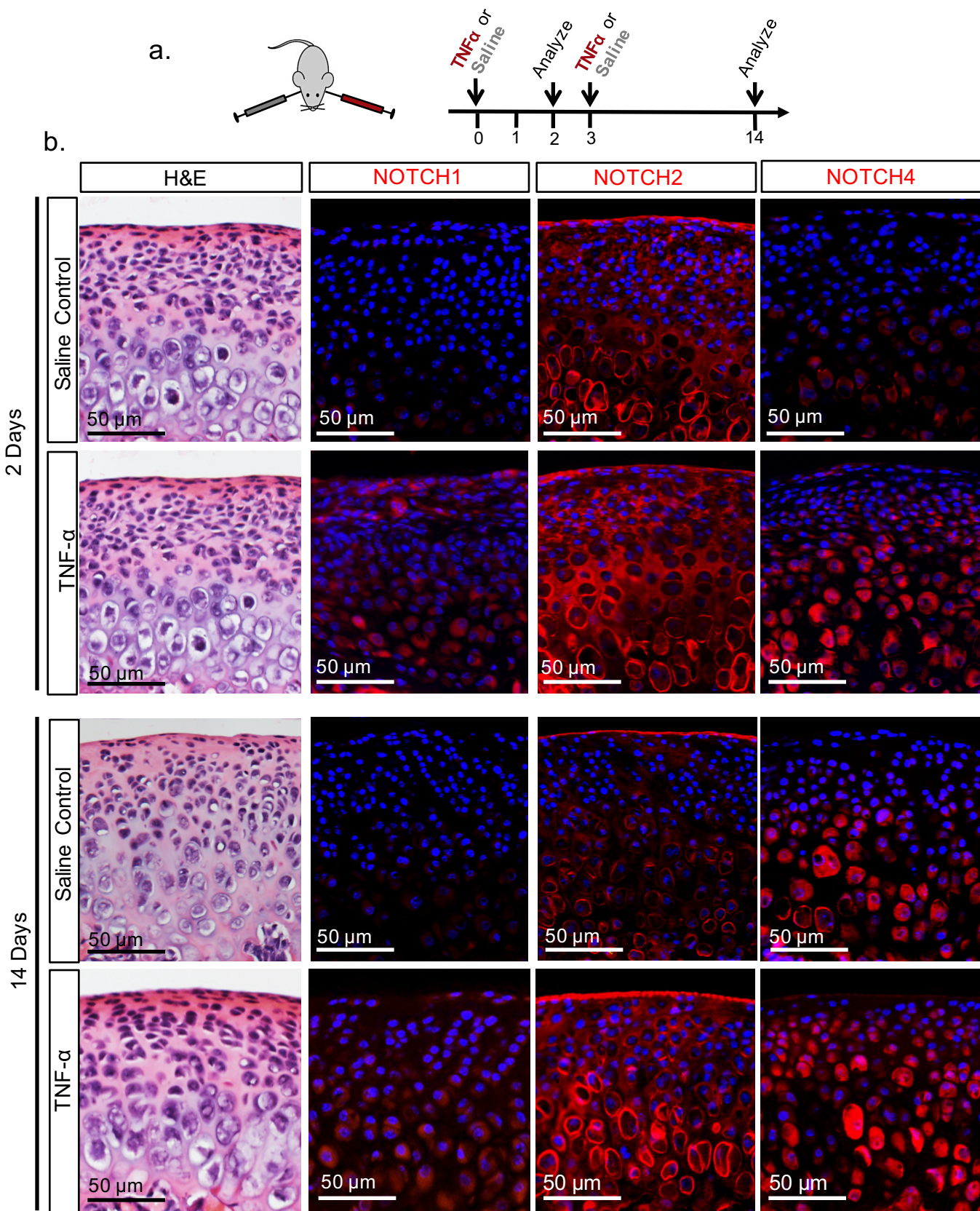
a. MW 1 2 3



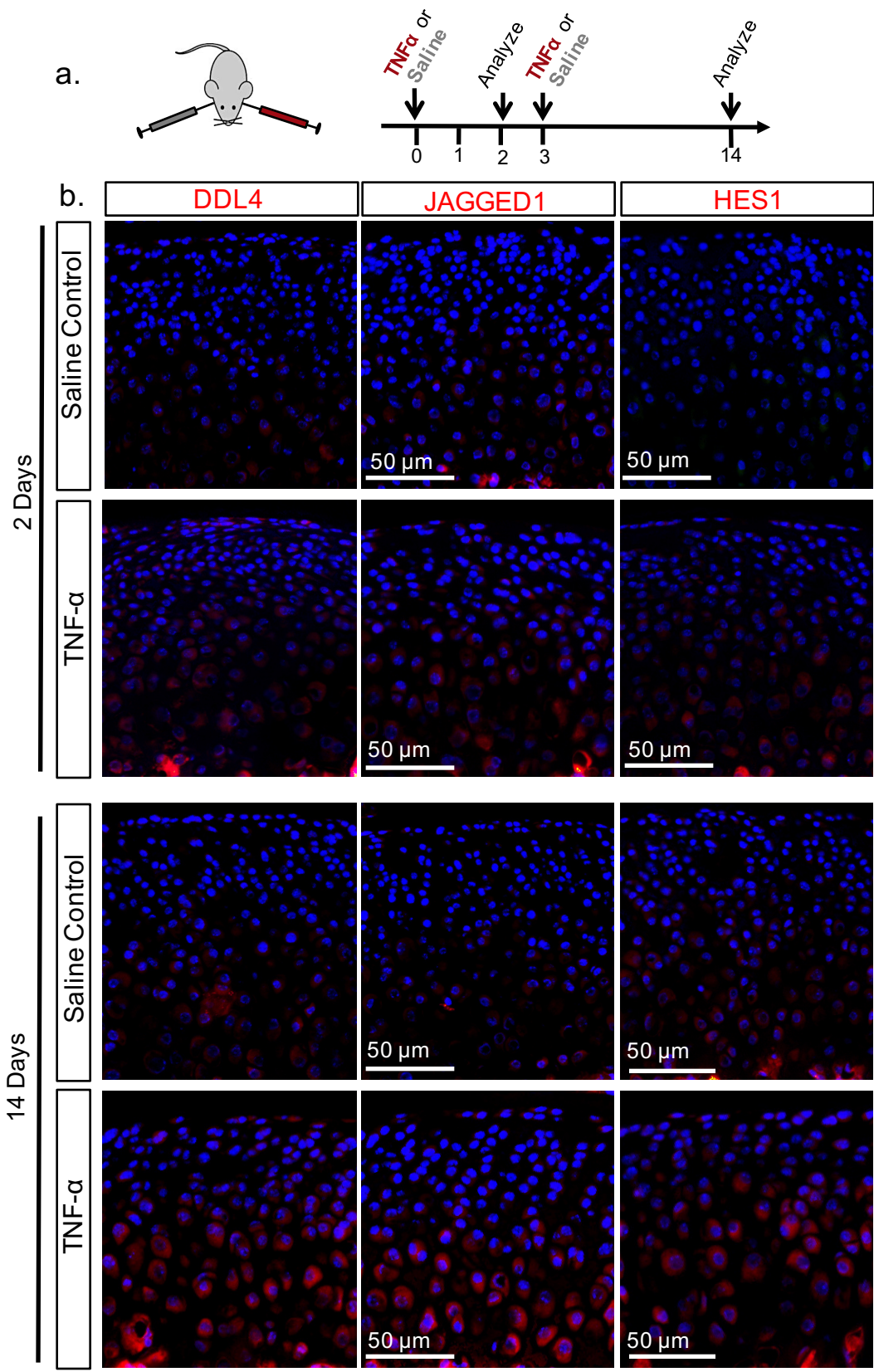
b.



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4