

Figure S1. Loading controls for mouse embryonic tissues analyzed in Figure 1. Western blot analysis using a C-terminal specific Jag1 antibody that recognizes all protein isoforms. Samples assessed include wild type E14.5 and E16.5 lenses, E14.5 liver, E16.5 heart and E9.5 whole mouse embryos (probed with rabbit anti-Jag1). Blot reprobed with mouse anti beta-actin (B-actin) as loading control.

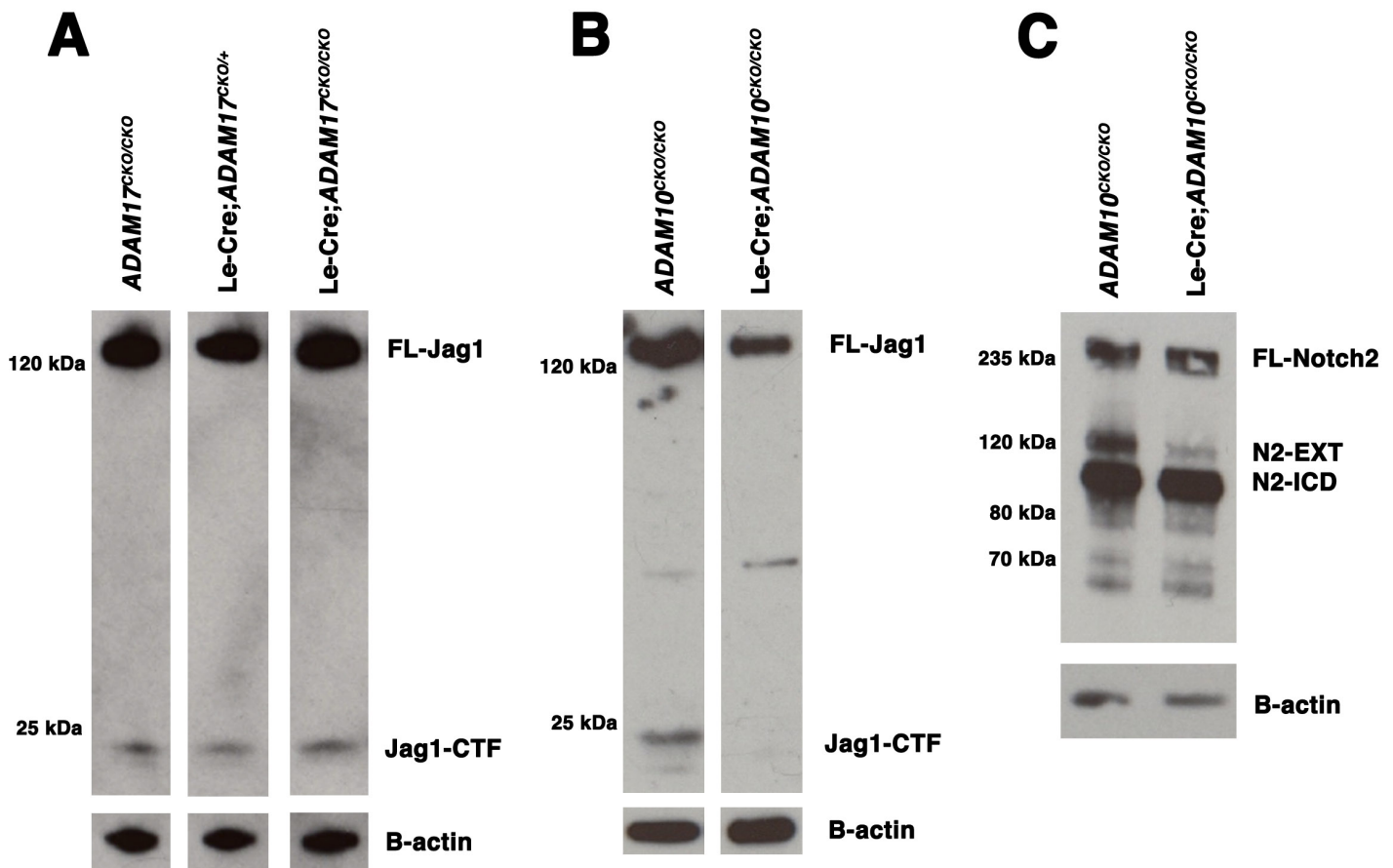


Figure S2. ADAM10, but not ADAM17, is necessary for Jag1-CTF production in the mouse lens.

(A) Western blot analysis of lens protein lysates from E14.5 Le-Cre;ADAM17 allelic series. Jag1-CTF generation is unaffected by removal of ADAM17. (B) Analogous Western blot assessment of Jag1 protein isoforms in lens protein lysates from E14.5 Le-Cre;ADAM10^{CKO/CKO} embryos. There is an obvious loss of the Jag1-CTF after ADAM10 removal. Blots in panels A,B probed with goat anti-Jag1 antibody (C) E14.5 Le-Cre;ADAM10^{CKO/CKO} mutants also display a clear decrease in the extracellular-truncated form of the Notch2 receptor, N2-EXT. In all panels, anti Beta-actin reprobing (B-Actin) served as a loading control. n = 3 biologic replicates/genotype in A. n = 1 biologic replicate/genotype in B,C, but independently performed (lysates loaded and probed by Western 3 times = technical replicates).

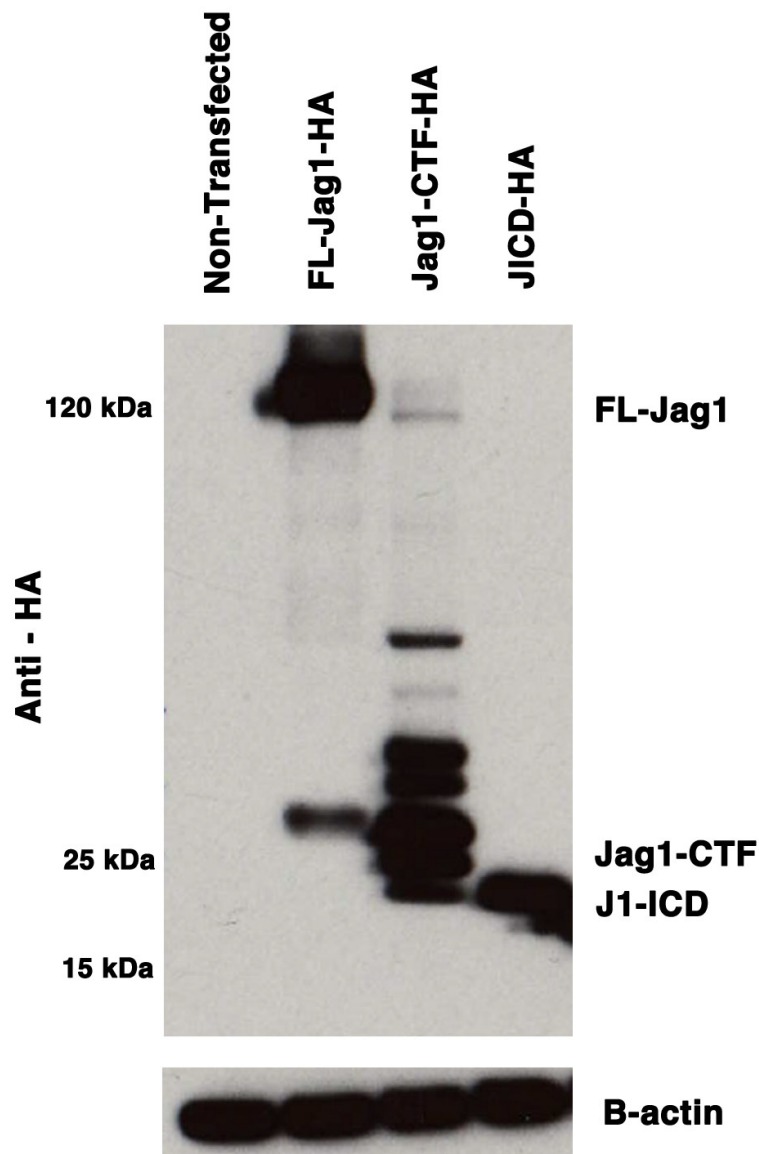


Figure S3. Anti-HA antibody Western blot.

B3 lens cells were transiently transfected with constructs for each rat epitope-tagged Jag1 isoform, then cell lysates harvested for analysis. Each lane was loaded with 50 ug of total protein. Non-transfected B4 lysate has no signal. B3 cell lysates after FL-Jag1-HA transfection contained Jag1-CTF-HA isoform, but not a J1-ICD-HA product. By contrast, B3 lysates from Jag1-CTF-HA transfections contained the J1-ICD-HA isoform, similar in size to in J1-ICD-HA after transfection of this isoform. Anti Beta-actin (B-actin) reprobing served as loading control. Blot is representative of n = 3 independent transfection to Western experiments.

Table S1. qPCR primer pairs obtained from the Harvard PrimerBank.

Gene	Forward primer 5'->3'	Reverse primer 3'->5'
<i>β-ACTIN</i>	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
<i>ADAM10</i>	ATGGGAGGTCAGTATGGGAATC	ACTGCTCTTTTGGCACGCT
<i>ADAM17</i>	GTGGATGGTAAAAACGAAAGCG	GGCTAGAACCCTAGAGTCAGG
<i>JAG1</i>	CAACCGTGCCAGTGACTATTTCTGC	TGTTCCCGTGAAGCCTTTGTTACAG
<i>JAG2</i>	AACGATACCACCCGAATGAGG	GCTGCCACAGTAGTTCAGGTCTTTG
<i>NOTCH1</i>	GAGGCGTGCCAGACTATGC	CTTGTACTCCGTGAGCGTGA
<i>NOTCH2</i>	CAACCGCAATGGAGGCTATG	GCGAAGGCACAATCATCAATGTT
<i>NOTCH3</i>	CGTGGCTTCTTTCTACTGTGC	CGTTCACCGGATTTGTGTCAC
<i>HES1</i>	TCAACACGACACCGGATAAAC	GCCGCGAGCTATCTTTCTTCA
<i>HES5</i>	GATTCCTCTGTGTGGGTGGATG	GATTTTATTATGGCGGCTTCGG
<i>PAX6</i>	TTTGCCCGAGAAAGACTAGC	CATTTGGCCCTTCGATTAGA