Supplementary Information for
Notch-Jagged signaling complex defined by an interaction mosaic.

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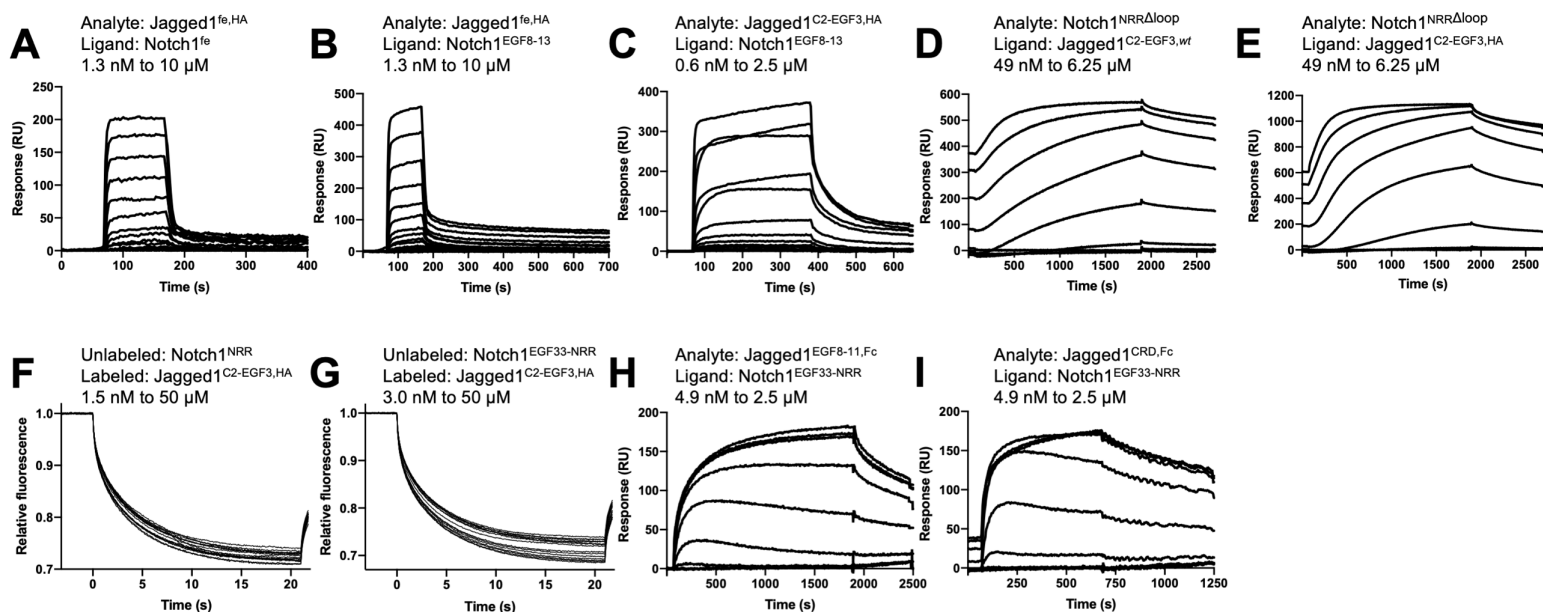Email: r.a.scheltema@uu.nl (R.A.S.), twitter@xlinkx_pd; b.j.c.janssen@uu.nl (B.J.C.J.).

This PDF file includes:

> Figures S1 to S7
> Table S1
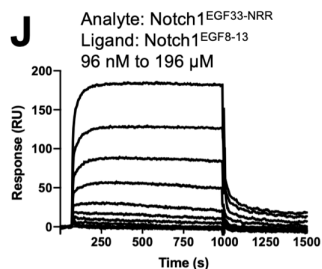
Other supplementary materials for this manuscript include the following:
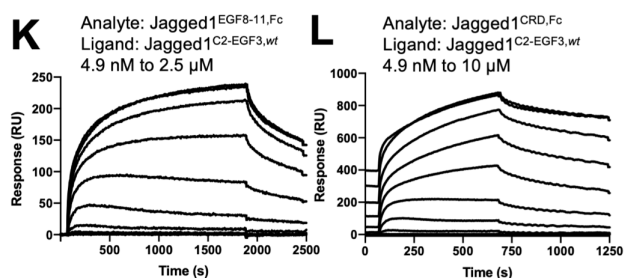
> Datasets S1 to S3

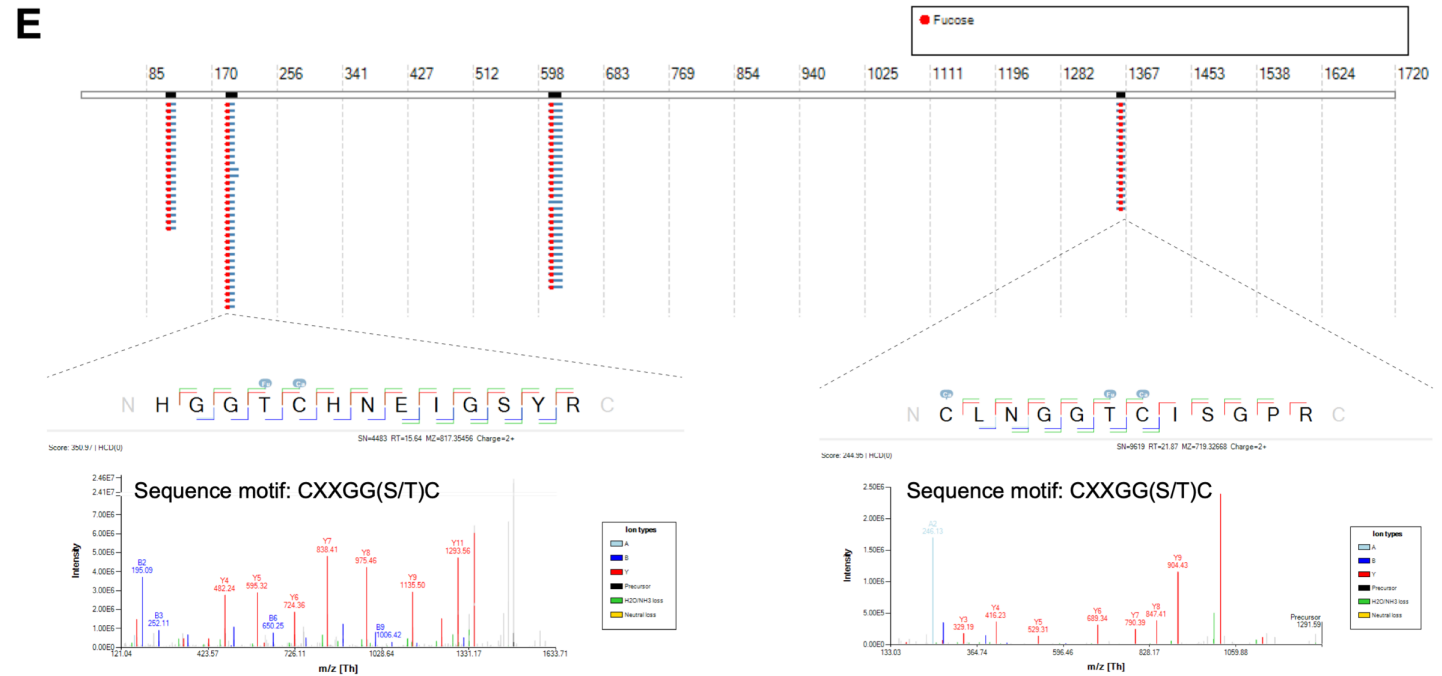Sensorgrams and MST traces related to Fig. 2

A Analyte: Jagged1^{fe,HA}
Ligand: Notch1^{fe}
1.3 nM to 10 μM

B Analyte: Jagged1^{fe,HA}
Ligand: Notch1$^{EGF8-13}$
1.3 nM to 10 μM

C Analyte: Jagged1$^{C2-EGF3,HA}$
Ligand: Notch1$^{EGF8-13}$
0.6 nM to 2.5 μM

D Analyte: Notch1^{NRRΔloop}
Ligand: Jagged1$^{C2-EGF3,wt}$
49 nM to 6.25 μM

E Analyte: Notch1^{NRRΔloop}
Ligand: Jagged1$^{C2-EGF3,HA}$
49 nM to 6.25 μM

F Unlabeled: Notch1^{NRR}
Labeled: Jagged1$^{C2-EGF3,HA}$
1.5 nM to 50 μM

G Unlabeled: Notch1$^{EGF33-NRR}$
Labeled: Jagged1$^{C2-EGF3,HA}$
3.0 nM to 50 μM

H Analyte: Jagged1$^{EGF8-11,Fc}$
Ligand: Notch1$^{EGF33-NRR}$
4.9 nM to 2.5 μM

I Analyte: Jagged1^{CRD,Fc}
Ligand: Notch1$^{EGF33-NRR}$
4.9 nM to 2.5 μM

Sensorgram related to Fig. 3

J Analyte: Notch1$^{EGF33-NRR}$
Ligand: Notch1$^{EGF8-13}$
96 nM to 196 μM
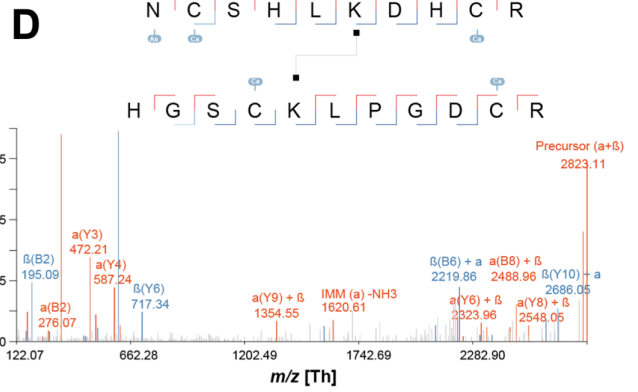
Sensorgrams related to Fig. 4

K Analyte: Jagged1$^{EGF8-11,Fc}$
Ligand: Jagged1$^{C2-EGF3,wt}$
4.9 nM to 2.5 μM

L Analyte: Jagged1^{CRD,Fc}
Ligand: Jagged1$^{C2-EGF3,wt}$
4.9 nM to 10 μM

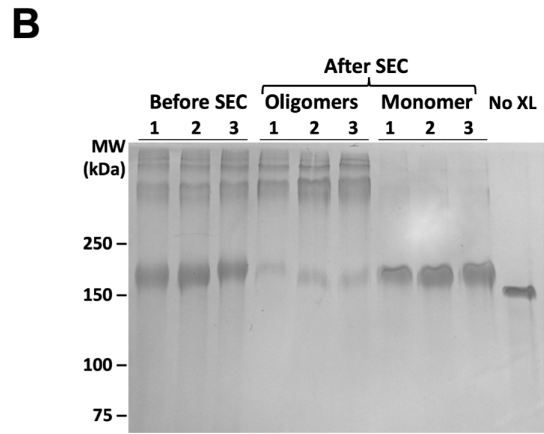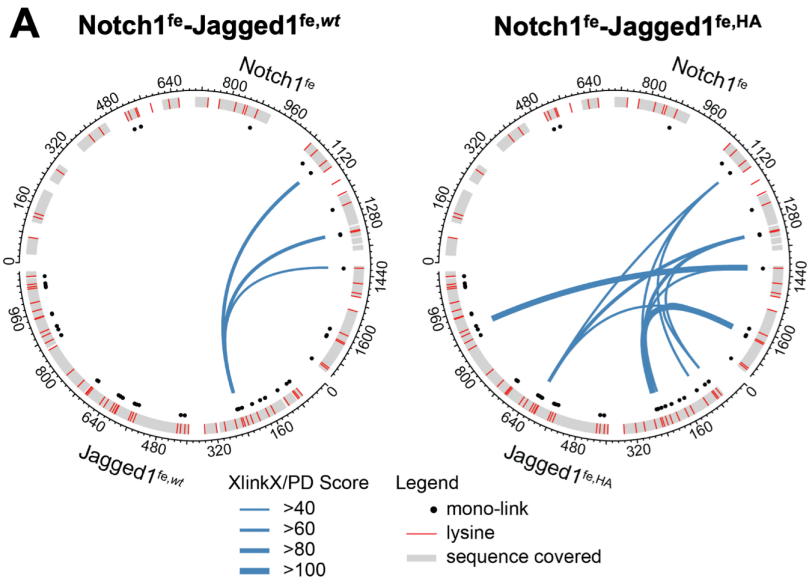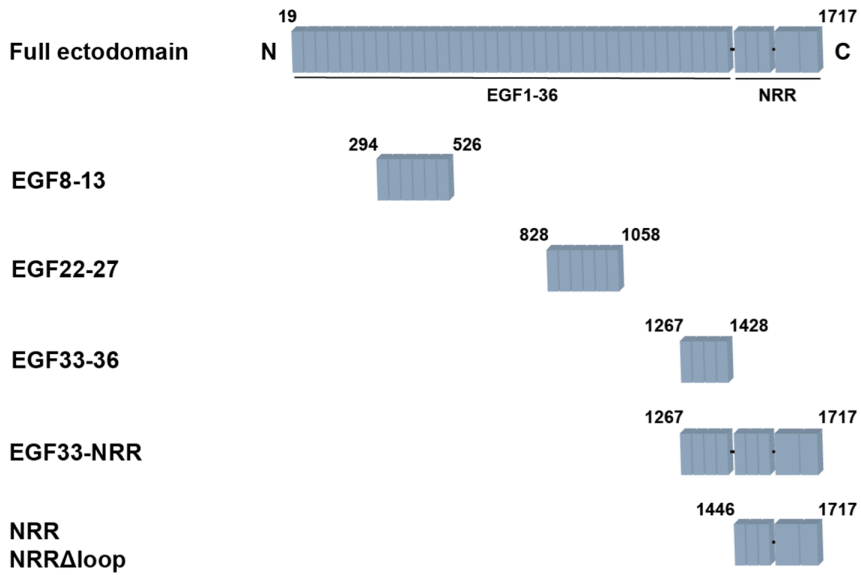**Fig. S1. Sensorgrams and MST traces.** (*A-I*) Sensorgrams and MST traces related to Fig. 2, with sensorgrams of Jagged1^{fe,HA} binding to Notch1^{fe} (*A*) and to Notch1$^{EGF8-13}$ (*B*), Jagged1$^{C2-EGF3,HA}$ binding to Notch1$^{EGF8-13}$ (*C*), Notch1^{NRRΔloop} binding to Jagged1$^{C2-EGF3,wt}$ (*D*) and to Jagged1$^{C2-EGF3,HA}$ (*E*), MST traces of Notch1^{NRR} binding to Jagged1$^{C2-EGF3,HA}$ (*F*) and Notch1$^{EGF33-NRR}$ binding to Jagged1$^{C2-EGF3,HA}$ (*G*), sensorgrams of Jagged1$^{EGF8-11,Fc}$ binding to Notch1$^{EGF33-NRR}$ (*H*) and Jagged1^{CRD,Fc} binding to Notch1$^{EGF33-NRR}$ (*I*). (*J*) Sensorgram related to Fig. 3, with Notch1$^{EGF33-NRR}$ binding to Notch1$^{EGF8-13}$. (*K* and *L*) Sensorgrams related to Fig. 4, with Jagged1$^{EGF8-11,Fc}$ binding to

Jagged1$^{C2-EGF3,wt}$ (*K*) and Jagged1^{CRD,Fc} binding to Jagged1$^{C2-EGF3,wt}$ (*L*). The concentration range used in the experiment is indicated in all panels.

A

Notch1^fe-Jagged1^fe,wt

Notch1^fe-Jagged1^fe,HA

XlinkX/PD Score
>40
>60
>80
>100

Legend
● mono-link
— lysine
▓ sequence covered

B

After SEC

Before SEC   Oligomers   Monomer   No XL
1 2 3   1 2 3   1 2 3

MW
(kDa)

250

150

100

75

C

Oligomeric Jagged1^fe,HA

C2-EGF3   EGF8-11   CRD

D

N C S H L K D H C R

H G S C K L P G D C R

Precursor (a+ß)
2823.11

β(B2)
195.09

a(Y3)
472.21

a(Y4)
587.24

β(Y6)
717.34

a(Y9) + ß
1354.55

IMM (a) -NH3
1620.61

ß(B6) + a
2219.86

a(B8) + ß
2488.96

ß(Y10) - a
2686.05

a(Y6) + ß
2323.96

a(Y8) + ß
2548.05

a(B2)
276.07

Intensity [a.u.]

m/z [Th]

E

● Fucose

85 170 256 341 427 512 598 683 769 854 940 1025 1111 1196 1282 1367 1453 1538 1624 1720

N H G G T C H N E I G S Y R C

SN=4483 RT=15.64 MZ=817.35456 Charge=2+

Sequence motif: CXXGG(S/T)C

B2
195.09

B3
252.11

Y4
482.24

Y5
595.32

Y6
724.36

B6
650.25

Y7
838.41

Y8
975.46

B9
1006.42

Y9
1135.50

Y11
1293.56

Intensity

m/z [Th]

Ion types
A
B
Y
Precursor
H2O/NH3 loss
Neutral loss

N C L N G G T C I S G P R C

SN=9619 RT=21.87 MZ=719.32668 Charge=2+

Sequence motif: CXXGG(S/T)C

A2
246.13

Y3
329.19

Y4
416.23

Y5
529.31

Y6
689.34

Y7
790.39

Y8
847.41

Y9
904.43

Precursor
1291.59

Intensity

m/z [Th]

Ion types
A
B
Y
Precursor
H2O/NH3 loss
Neutral loss

4

Fig. S2. Additional information related to cross-linking mass-spectrometry experiments. (*A*) Circular plots indicating the inter-links, by XlinkX/Proteome Discoverer score, and mono-links identified in the cross-linking experiment of Notch1^{fe}-Jagged1^{fe,wt} (left) and Notch1^{fe}-Jagged1^{fe,HA} (right). The sequence covered in the peptide identification is indicated. (*B*) Coomassie-stained SDS-PAGE showing the cross-linked oligomeric and monomeric Jagged1 fractions purified by size exclusion chromatography in triplicate. The monomer fractions are well separated from the oligomeric fractions. (*C*) Overview of the detected distance constraints from the XL-MS experiments for oligomeric Jagged1^{fe,HA}. The detected distance constraints for monomeric Jagged1^{fe,HA} are shown in Fig. 4*G*. (*D*) Example mass spectrum of an identified cross-link. (*E*) Example of several identified core O-fucose residues on Notch1 shows that are our Notch1 protein is fucosylated. Positive identifications were made by an open peptide search and filtered based on the precursor mass difference, presence of glycan diagnostic ions, and conformance to the expected sequence motif. The blue stretches indicate where peptides were detected carrying a core fucose, with the red dot showing the precise position. The insets show representative spectra where the peptide was identified with high confidence, with a precursor mass difference indicative of a core fucose. A list of the identifications can be found in Dataset S3.
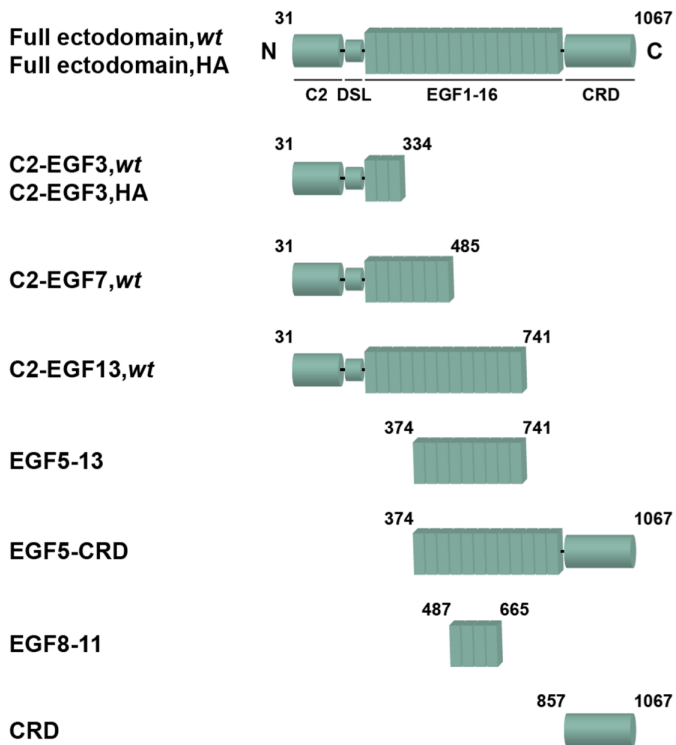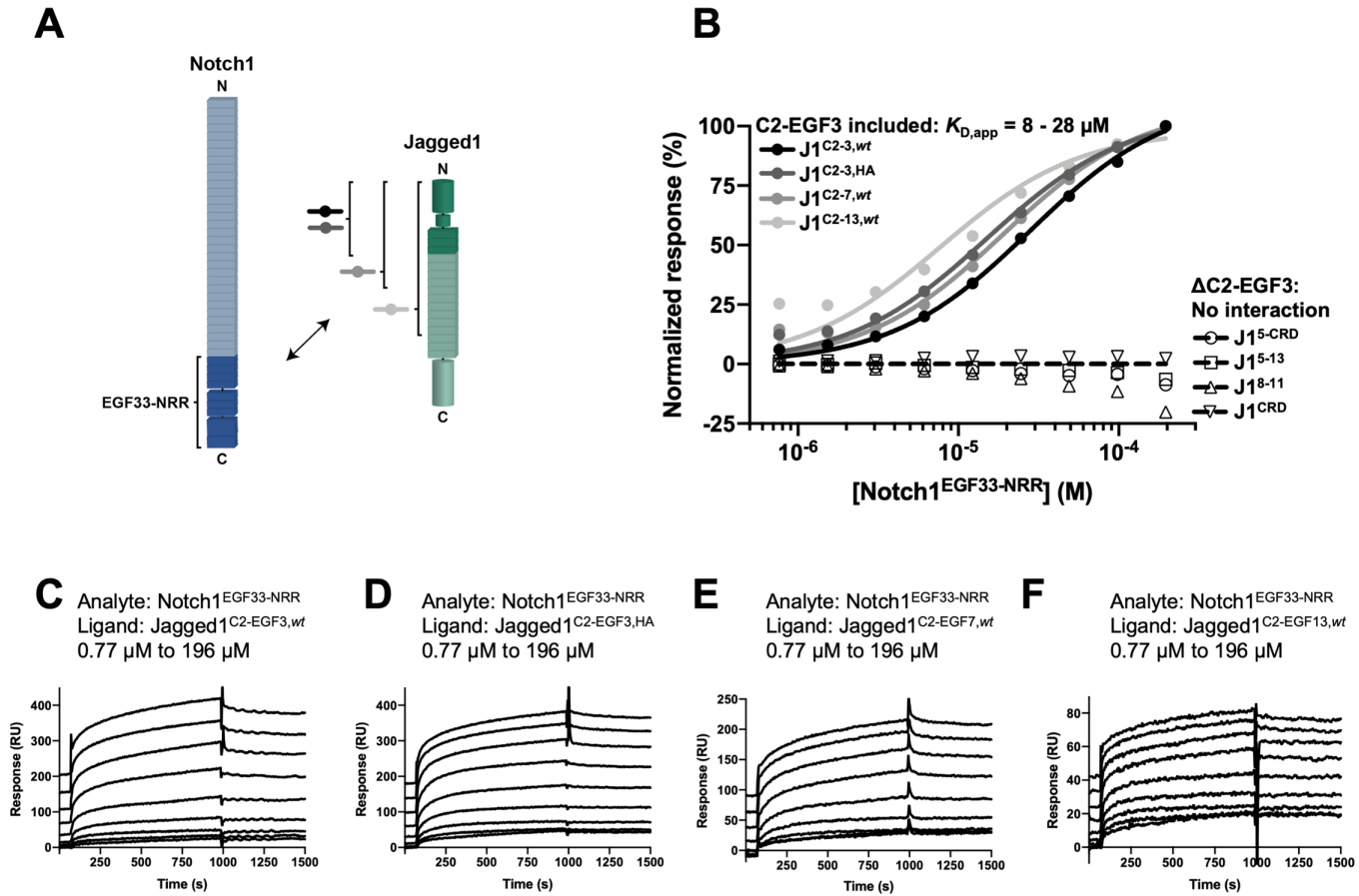
Notch1

Full ectodomain

19   1717

N   C

EGF1-36   NRR

EGF8-13 — 294 – 526

EGF22-27 — 828 – 1058

EGF33-36 — 1267 – 1428

EGF33-NRR — 1267 – 1717

NRR
NRRΔloop — 1446 – 1717

Jagged1

Full ectodomain,*wt*
Full ectodomain,HA

31   1067

N   C

C2   DSL   EGF1-16   CRD

C2-EGF3,*wt*
C2-EGF3,HA — 31 – 334

C2-EGF7,*wt* — 31 – 485

C2-EGF13,*wt* — 31 – 741

EGF5-13 — 374 – 741

EGF5-CRD — 374 – 1067

EGF8-11 — 487 – 665

CRD — 857 – 1067

**Fig. S3. Domain composition and main constructs generated.**

Fig. S4. The C2-EGF3 domain of Jagged1 is necessary and sufficient for Notch1 EGF33-NRR interaction. (*A*) Schematic representation of the interactions reported in panels (*B-F*). (*B*) SPR equilibrium binding plots of Notch1[EGF33-NRR] to Jagged1[C2-EGF3,*wt*] (black), Jagged1[C2-EGF3,HA] (dark grey), Jagged1[C2-EGF7,*wt*] (grey), Jagged1[C2-EGF13,*wt*] (light grey), Jagged1[EGF5-CRD] (open circle), Jagged1[EGF5-13] (open square), Jagged1[EGF8-11] (open triangle) and Jagged1[CRD] (open inverted triangle). (*C-F*) Corresponding SPR sensorgrams, with Notch1[EGF33-NRR] binding to Jagged1[C2-EGF3,*wt*] (*C*), to Jagged1[C2-EGF3,HA] (*D*), to Jagged1[C2-EGF7,*wt*] (*E*), and to Jagged1[C2-EGF13,*wt*] (*F*).

7
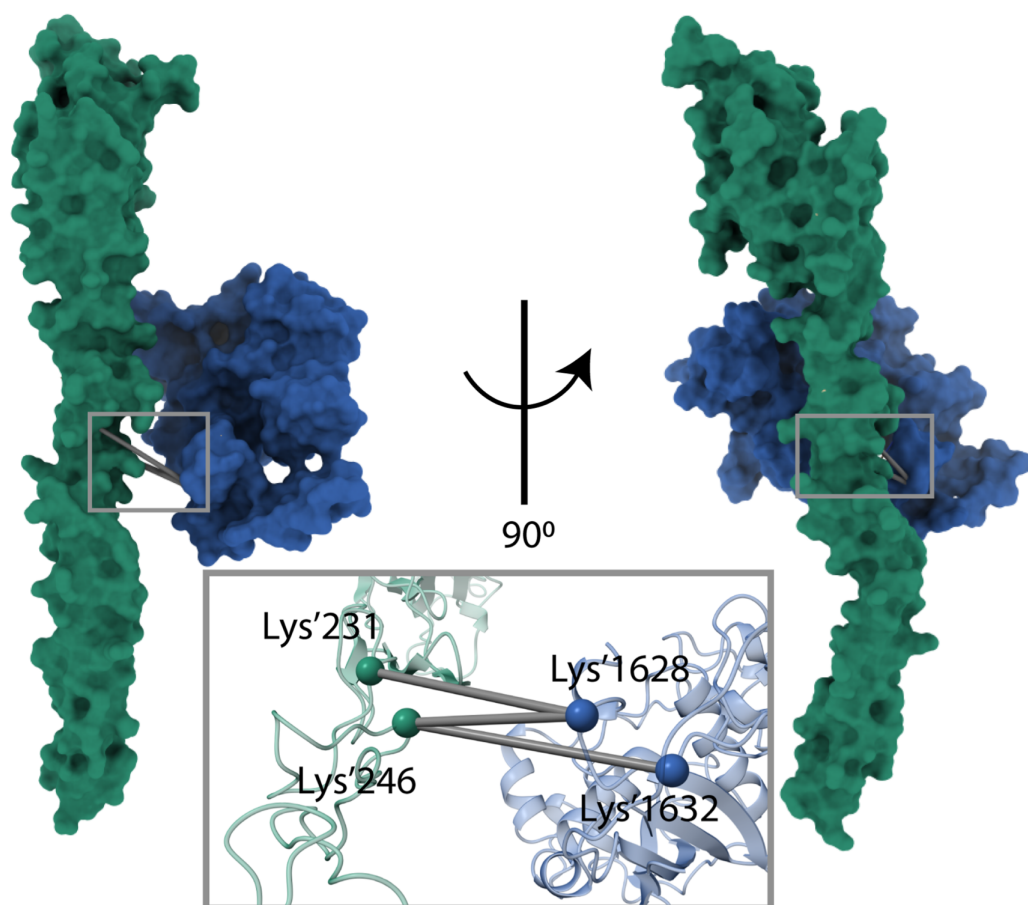
Fig. S5. Exploded model of the Notch1 NRR - Jagged1 C2-EGF3 complex. Docking of the Notch1 NRR - Jagged1 C2-EGF3 complex using the structure of Notch1 NRR described here (blue) and that of Jagged1 C2-EGF3 (green; PDB: 5UK5) and based on cross-links obtained by XL-MS. The two structures are slightly separated from each other to indicate the cross-links.

A

Asn1587

Asn1489

B

Asp1538

His1539

Tyr1535

Asp1546

Tyr1532
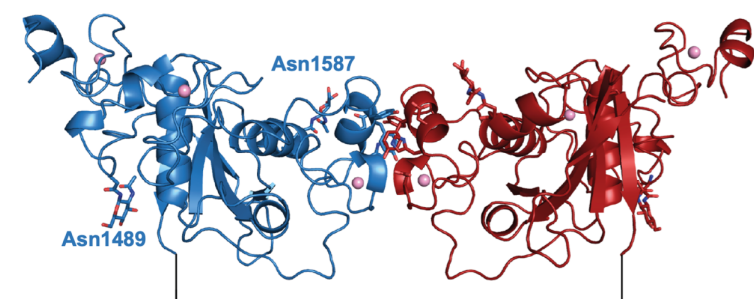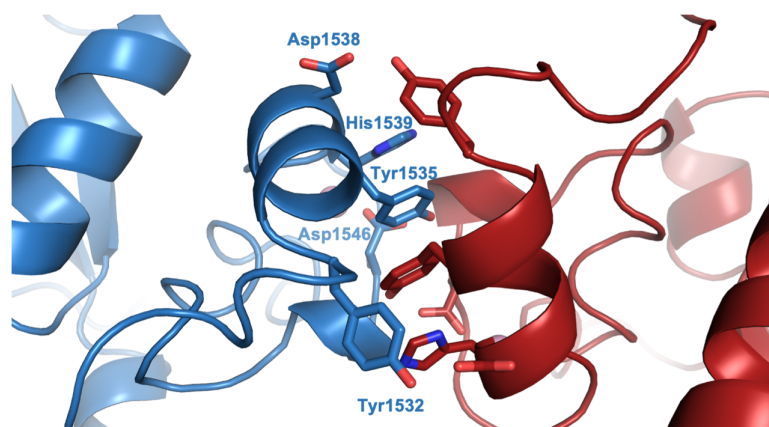
C

S1-cleaved mouse Notch1 NRR	
Data collection	
Space group	$P\,4_2\,2_1\,2$
Cell dimensions	
a, b, c (Å)	65.72, 65.72, 161.68
α, β, γ (°)	90, 90, 90
Resolution (Å)	60.89 - 2.06 (2.30 - 2.06)
No. observed reflections	195430 (8934)
No. unique reflections	15144 (757)
R_{merge}	0.105 (1.431)
Mean I/σI	14.6 (1.7)
$CC_{1/2}$	0.999 (0.770)
Completeness (spherical, %)	66.9 (12.6)
Completeness (ellipsoidal, %)	91.5 (64.6)
Ellipsoidal resolution limits (Å) [direction]	2.44 [a*] 2.44 [b*] 2.06 [c*]
Redundancy	12.9 (11.8)
Refinement	
Resolution (Å)	60.89 - 2.06
R_{work}/R_{free} (%)	19.53 / 23.39
Average B-factors (Å^2)	
Protein	57.7
Glycans/ions	94.5
Waters	46.6
R.M.S. deviations	
Bond lengths (Å)	0.0084
Bond angles (°)	1.52
Ramachandran (%)	
Favored	95.61
Allowed	4.39
Outliers	0
Molprobity score	2.06

Fig. S6. Structure of the S1-cleaved mouse Notch1 NRR. (*A*) Proposed orientation of the Notch1 NRR dimer with respect to the cell surface. (*B*) Key residues at the dimerization interface are indicated. (*C*) Data collection and refinement statistics. Highest resolution shell in parentheses.
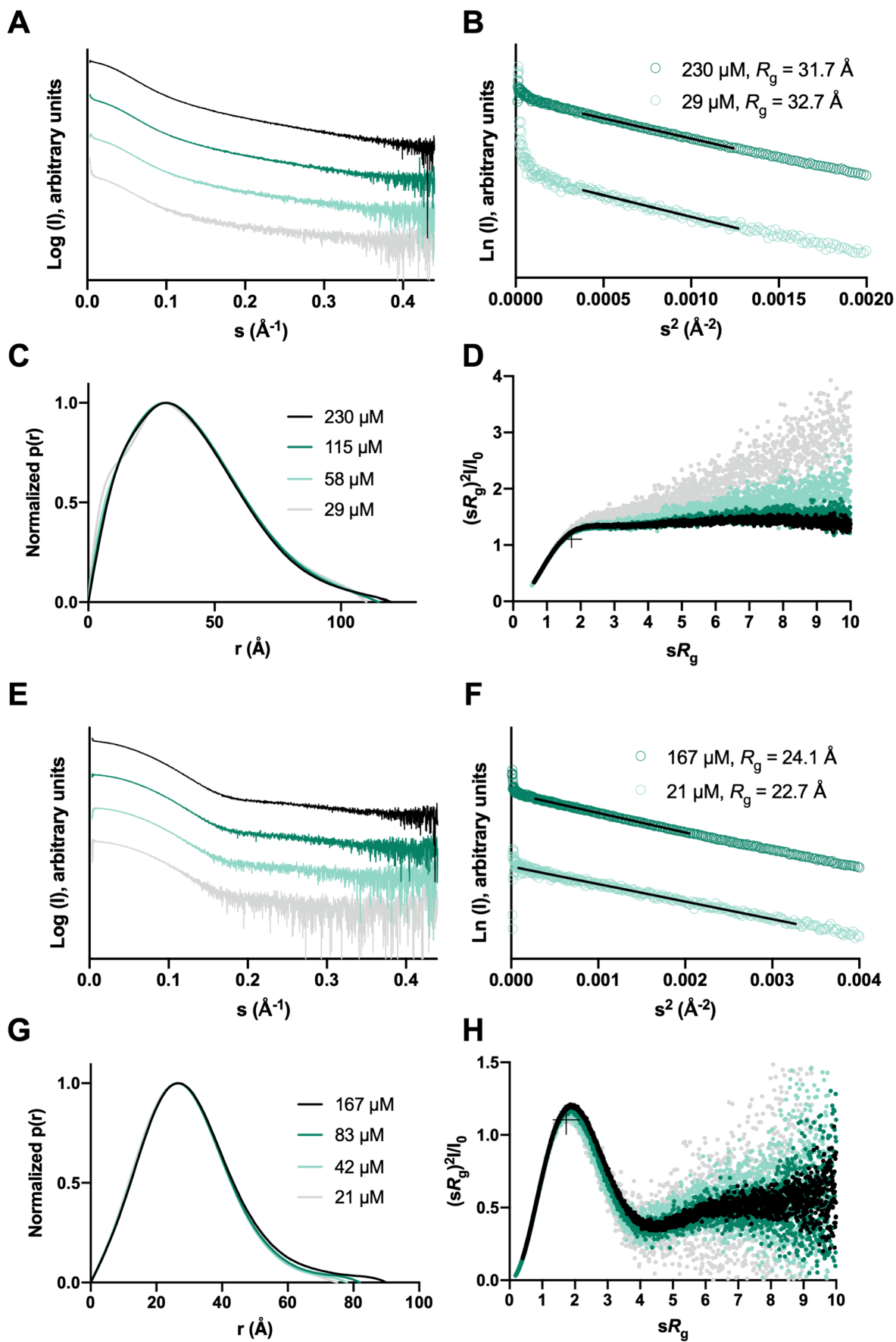
Fig. S7. Jagged1^{EGF8-11} and Jagged1^{CRD} have distinct structural properties. (*A-D*) Structural analysis of Jagged1$^{EGF8-11}$ from batch SAXS, including Log (I) versus s plot (*A*), Guinier plot with black lines indicating the fits used to derive the R_g (*B*), pair distance distribution function (*C*) and dimensionless Kratky plot with crosshairs indicating the peak position for a globular protein (*D*). (*E-H*) Structural analysis of Jagged1^{CRD} from batch SAXS, including Log (I) versus s plot (*E*), Guinier plot with black lines indicating the fits used to derive the R_g (*F*), pair distance distribution function (*G*) and dimensionless Kratky plot with crosshairs indicating the peak position for a globular protein (*H*).

Table S1. Description of the files uploaded to the PRIDE repository.

Filename	Description
20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_MT1.raw 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_MT2.raw 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_MT3.raw	Triplicate measurements of the crosslinked and PhoX enriched fraction for the mouse Notch1 - mouse high-affinity mutant Jagged1 full ectodomain complex.
20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_MT_Asn-_AspSites.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_MT_evidence.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_MT_peptides.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_MT_PhoX H2OSites.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_MT_PhoX TrisSites.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_MT.pdf	MaxQuant output tables and annotated spectra.
20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_MT_Crosslinks.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_MT_CSMs.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_MT_CSMs.pdf	Proteome Discoverer XlinkX/PD output tables and annotated spectra.
20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_WT1.raw 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_WT2.raw 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_WT3.raw	Triplicate measurements of the crosslinked and PhoX enriched fraction for the mouse Notch1 - mouse wild-type Jagged1 full ectodomain complex.
20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_WT_Asn-_AspSites.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_WT_evidence.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_WT_peptides.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_WT_PhoX H2OSites.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_WT_PhoX TrisSites.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_WT.pdf	MaxQuant output tables and annotated spectra.
20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_WT_Crosslinks.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_WT_CSMs.txt 20190316_L1_Ag6_Klyko001_SA_NOTCH_PhoX_WT_CSMs.pdf	Proteome Discoverer XlinkX/PD output tables and annotated spectra.
20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_MT1.raw 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_MT2.raw 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_MT3.raw	Triplicate measurements of the flow-through (i.e. not cross-linked & mono-linked) fraction for the mouse Notch1 - mouse high-affinity mutant Jagged1 full ectodomain complex.
20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_MT_Asn-_AspSites.txt 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_MT_evidence.txt 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_MT_peptides.txt 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_MT_PhoX H2OSites.txt 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_MT_PhoX TrisSites.txt 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_MT.pdf	MaxQuant output tables and annotated spectra.
20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_WT1.raw 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_WT2.raw 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_WT3.raw	Triplicate measurements of the flow-through (i.e. not cross-linked & mono-linked) fraction for the mouse Notch1 - mouse wild-type Jagged1 full ectodomain complex.
20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_WT_Asn-_AspSites.txt 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_WT_evidence.txt 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_WT_peptides.txt 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_WT_PhoX H2OSites.txt 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_WT_PhoX TrisSites.txt 20190923_L1_Ag1_Klyko001_SA_JagNotch_PhoX_FT_WT.pdf	MaxQuant output tables and annotated spectra.

Dataset S1 (separate file). This annotated excel file contains the Crosslinks table of XlinkX/PD, broken up in inter- and intra-links. The original Crosslinks and CSM tables can be found in the PRIDE repository.

Dataset S2 (separate file). This annotated excel file contains the Site specific tables of MaxQuant for the PhoX:Tris and PhoX;H2O monolinks. The original tables plus the Evidence and Peptide tables can be found in the PRIDE repository.

Dataset S3 (separate file). Fucosylated peptides from Notch1, identified by open search. The position numbering is according to Uniprot.