The structural and functional significance of lipid-binding properties of human Notch ligands



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8.1 Chapter 1 Supplementary

8.1.1 Table of knockout mouse models of the Notch pathway

Genotype	Phenotype	References
Notch1 (-/-)	Homozygous embryonic lethal (E9.5). Severe defects in angiogenic vascular remodelling. Impaired somitogenesis, neurogenesis.	(Krebs et al., 2000; Swiatek et al., 1994; Takeshita et al., 2007)
Notch2 (-/-)	Homozygous embryonic lethality (E11.5) with widespread pyknosis and apoptosis. Developmental abnormalities characteristic of Alagille syndrome (hypomorph).	(Hamada et al., 1999; McCright et al., 2002)
Notch3 (-/-)	Normal embryonic development, viable and fertile. Minor vascular defects and defects in thymus development.	(Kitamoto et al., 2005; Krebs et al., 2003)
Notch4 (-/-)	Normal embryonic development, viable and fertile adults. Minor defects in vasculature, e.g., delayed vessel growth in the retina, slightly elevated blood pressure. Delayed tumour onset and reduced tumour perfusion.	(Costa et al., 2013; James et al., 2014; Krebs et al., 2000; Takeshita et al., 2007)
Jag1 (-/-)	Embryonic lethality (E10.5) in homozygous mutants with cranial haemorrhages and defects in yolk sac vasculature remodelling. Heterozygous mutants exhibit eye dysmorphology similar to phenotypes of Coloboma mice. Endothelial-specific deletion leads to embryonic lethality and cardiovascular defects.	(High et al., 2008; Y. Xue et al., 1999)
Jag2 (-/-)	Homozygous neonatal lethality (E10.5). Mutants die at birth from completely penetrant cleft palate with soft tissue syndactyly.	(J. Xu et al., 2010)
Dll1 (-/-)	Homozygous embryonic lethality (E10-12) due to loss of segment polarity and defective maintenance of somite borders.	(de Angelis et al., 1997)

Dll3 (-/-)	~20% embryonic lethality. Developmental defects in segmentation clock progression and skeletal deformities.	(Bochter et al., 2022; G. Chapman et al., 2011; Hoyne et al., 2011; N. Zhang et al., 2002)
Dll4 (+/-)	Haploinsufficiency results in embryonic lethality (E8.5-10.5) due to major defects in vasculature, including defective arteriogenesis and vascular remodelling.	(Duarte et al., 2004; Gale et al., 2004; Krebs et al., 2004)

8.1.2 Sequence analysis of human, *Drosophila*, *C. elegans* Notch receptors and ligands

A) Human Notch 1

EGF1	RGPR	C	SQPGET	C	LNGGK	C	EAANGTEA	C	V	C	GGA**F**VGPR	C	QD
EGF2	PNP	C	LSTP	C	KNAGT	C	HVVDRRGVADYA	C	S	C	ALG**F**SGPL	C	LT
EGF3	PL**DN**A	C	LTNP	C	RNGGT	C	DLLTLTEYK	C	R	C	PPG**W**SGKS	C	QQ
EGF4	ADP	C	ASNP	C	ANGGQ	C	LPFEAS**Y**I	C	H	C	PPS**F**HGPT	C	RQ
cbEGF5	**D**V**NE**	C	GQKPGL	C	RHGGT	C	H**N**EVGS**Y**R	C	V	C	RATHTGPN	C	ER
EGF6	PYVP	C	SPSP	C	QNGGT	C	RPTGDVTHE	C	A	C	LPG**F**TGQN	C	EE
cbEGF7	**N**I**DD**	C	PGNN	C	KNGGA	C	V**D**GVNT**Y**N	C	R	C	PPE**W**TGQY	C	TE
cbEGF8	**D**V**DE**	C	QLMPNA	C	QNGGT	C	H**N**THGG**Y**N	C	V	C	VNG**W**TGED	C	SE
cbEGF9	**N**I**DD**	C	ASAA	C	FHGAT	C	H**D**RVAS**F**Y	C	E	C	PHG**R**TGLL	C	HL
EGF10	**N**DA	C	ISNP	C	NEGSN	C	DTNPVNGKAI	C	T	C	PSG**Y**TGPA	C	SQ
cbEGF11	**D**V**DE**	C	SLGANP	C	EHAGK	C	I**N**TLGS**F**E	C	Q	C	LQG**Y**TGPR	C	EI
cbEGF12	**D**V**NE**	C	VSNP	C	QNDAT	C	L**D**QIGE**F**Q	C	I	C	MPG**Y**EGVH	C	EV
cbEGF13	**N**T**DE**	C	ASSP	C	LHNGR	C	L**D**KINE**F**Q	C	E	C	PTG**F**TGHL	C	QY
cbEGF14	**D**V**DE**	C	ASTP	C	KNGAK	C	L**D**GPNT**Y**T	C	V	C	TEG**Y**TGTH	C	EV
cbEGF15	**D**I**DE**	C	DPDP	C	HYGS	C	K**D**GVAT**F**T	C	L	C	RPG**Y**TGHH	C	ET
cbEGF16	**N**I**NE**	C	SSQP	C	RHGGT	C	Q**D**RDNA**Y**L	C	F	C	LKG**T**TGPN	C	EI
cbEGF17	**N**L**DD**	C	ASSP	C	DSGT	C	L**D**KIDG**Y**E	C	A	C	EPG**Y**TGSM	C	NI
cbEGF18	**N**I**DE**	C	AGNP	C	HNGGT	C	E**D**GING**F**T	C	R	C	PEG**Y**HDPT	C	LS
cbEGF19	**E**V**NE**	C	NSNP	C	VHGA	C	R**D**SLNG**Y**K	C	D	C	DPG**W**SGTN	C	DI
cbEGF20	**N**N**NE**	C	ESNP	C	VNGGT	C	K**D**MTSG**Y**V	C	T	C	REG**F**SGPN	C	QT
cbEGF21	**N**I**NE**	C	ASNP	C	LNQGT	C	I**D**DVAG**Y**K	C	N	C	LLP**Y**TGAT	C	EV
EGF22	VLAP	C	APSP	C	RNGGE	C	R**Q**SEDYESFS	C	V	C	PTG**W**QGQT	C	EV
cbEGF23	**D**I**NE**	C	VLSP	C	RHGAS	C	Q**N**THGG**Y**R	C	H	C	QAG**Y**SGRN	C	ET

cbEGF24	DIDD	C	RPNP	C	HNGGS	C	TDGINTAF	C	D	C	LPGFRGTF	C	EE
cbEGF25	DINE	C	ASDP	C	RNGAN	C	TDCVDSYT	C	T	C	PAGFSGIH	C	EN
EGF26	NTPD	C	TESS	C	FNGGT	C	VDGINSFT	C	L	C	PPGFTGSY	C	QH
cbEGF27	DVNE	C	DSQP	C	LHGGT	C	QDGCGSYR	C	T	C	PQGYTGPN	C	QN
EGF28	LVHW	C	DSSP	C	KNGGK	C	WQTHTQYR	C	E	C	PSGWTGLY	C	DV
EGF29	PSVS	C	EVAAQRQGVDVARL	C	QHGGL	C	VDAGNTHH	C	R	C	QAGYTGSY	C	ED
EGF30	LVDE	C	SPSP	C	QNGAT	C	TDYLGGYS	C	K	C	VAGYHGVN	C	SE
cbEGF31	EIDE	C	LSHP	C	QNGGT	C	LDLPNTYK	C	S	C	PRGTQGVH	C	EI
cbEGF32	NVDD	C	NPPVDPVSRSPK	C	FNNGT	C	VDQVGGYS	C	T	C	PPGFVGER	C	EG
cbEGF33	DVNE	C	LSNP	C	DARGTQN	C	VQRVNDFH	C	E	C	RAGHTGRR	C	ES
EGF34	VING	C	KGKP	C	KNGGT	C	AVASNTARGFI	C	K	C	PAGFEGAT	C	EN
EGF35	DART	C	GSLR	C	LNGGT	C	ISGPRSPT	C	L	C	LGPFTGPE	C	QF
EGF36	PASSP	C	LGGNP	C	YNQGT	C	EPTSESPFYR	C	L	C	PAKFNGLL	C	HI

B) Human Notch 2

EGF1	HALQ	C	RDGYEP	C	VNEGM	C	VTYHNGTGY	C	K	C	PEGFLGEY	C	QH
EGF2	RDP	C	EKNR	C	QNGGT	C	VAQAMLGKAT	C	R	C	ASGFTGED	C	QY
EGF3	STSHP	C	FVSRP	C	LNGGT	C	HMLSRDTYE	C	T	C	QVGFTGKE	C	QW
EGF4	TDA	C	LSHP	C	ANGST	C	TTVANQFS	C	K	C	LTGFTGQK	C	ET
cbEGF5	DVNE	C	DIPGH	C	QHGGT	C	LNLPGSYQ	C	Q	C	PQGFTGQY	C	DS
EGF6	LYVP	C	APSP	C	VNGGT	C	RQTGDFTFE	C	N	C	LPGFEGST	C	ER
cbEGF7	NIDD	C	PNHR	C	QNGGV	C	VDGVNTYN	C	R	C	PPQWTGQF	C	TE
cbEGF8	DVDE	C	LLQPNA	C	QNGGT	C	ANRNGGYG	C	V	C	VNGWSGDD	C	SE
cbEGF9	NIDD	C	AFAS	C	TPGST	C	IDRVASFS	C	M	C	PEGKAGLL	C	HL
EGF10	DDA	C	ISNP	C	HKGAL	C	DTNPLNGQYI	C	T	C	PQGYKGAD	C	TE
cbEGF11	DVDE	C	AMANSNP	C	EHAGK	C	VNTDGAFH	C	E	C	LKGYAGPR	C	EM
cbEGF12	DINE	C	HSDP	C	QNDAT	C	LDKIGGFT	C	L	C	MPGFKGVH	C	EL
cbEGF13	EINE	C	QSNP	C	VNNGQ	C	VDKVNRFQ	C	L	C	PPGFTGPV	C	QI
cbEGF14	DIDD	C	SSTP	C	LNGAK	C	IDHPNGYE	C	Q	C	ATGFTGVL	C	EE
cbEGF15	NIDN	C	DPDP	C	HHGQ	C	QDGIDSYT	C	I	C	NPGYMGAI	C	SD
EGF16	QIDE	C	YSSP	C	LNDGR	C	IDLVNGYQ	C	N	C	QPGTSGVN	C	EI
cbEGF17	NFDD	C	ASNP	C	IHGI	C	MDGINRYS	C	V	C	SPGFTGQR	C	NI
cbEGF18	DIDE	C	ASNP	C	RKGAT	C	INGVNGFR	C	I	C	PEGPHHPS	C	YS
EGF19	QVNE	C	LSNP	C	IHGN	C	TGGLSGYK	C	L	C	DAGWVGIN	C	EV
cbEGF20	DKNE	C	LSNP	C	QNGGT	C	DNLVNGYR	C	T	C	KKGFKGYN	C	QV
cbEGF21	NIDE	C	ASNP	C	LNQGT	C	FDDISGYT	C	H	C	VLPYTGKN	C	QT
EGF22	VLAP	C	SPNP	C	ENAAV	C	KESPNFESYT	C	L	C	APGWQGQR	C	TI
cbEGF23	DIDE	C	ISKP	C	MNHGL	C	HNTQGSYM	C	E	C	PPGFSGMD	C	EE

cbEGF24	DIDD	C	LANP	C	QNGGS	C	MDGVNTFS	C	L	C	LPGFTGDK	C	QT
cbEGF25	DMNE	C	LSEP	C	KNGGT	C	SDYVNSYT	C	K	C	QAGFDGVH	C	EN
cbEGF26	NINE	C	TESS	C	FNGGT	C	VDGINSFS	C	L	C	PVGFTGSF	C	LH
cbEGF27	EINE	C	SSHP	C	LNEGT	C	VDGLGTYR	C	S	C	PLGYTGKN	C	QT
EGF28	LVNL	C	SRSP	C	KNKGT	C	VQKKAESQ	C	L	C	PSGWAGAY	C	DV
EGF29	PNVS	C	DIAASRRGVLVEHL	C	QHSGV	C	INAGNTHY	C	Q	C	PLGYTGSY	C	EE
EGF30	QLDE	C	ASNP	C	QHGAT	C	SDFIGGYR	C	E	C	VPGYQGVN	C	EY
cbEGF31	EVDE	C	QNQP	C	QNGGT	C	IDLVNHFK	C	S	C	PPGTRGLL	C	EE
cbEGF32	NIDD	C	ARGPH	C	LNGGQ	C	MDRIGGYS	C	R	C	LPGFAGER	C	EG
cbEGF33	DINE	C	LSNP	C	SSEGSLD	C	IQLTNDYL	C	V	C	RSAFTGRH	C	ET
EGF34	FVDV	C	PQMP	C	LNGGT	C	AVASNMPDGFI	C	R	C	PPGFSGAR	C	QS
EGF35	S	C	GQVK	C	RKGEQ	C	VHTASGPR	C	F	C	PSPRD	C	ES
EGF36	G	C	ASSP	C	QHGGS	C	HPQRQPPYYS	C	Q	C	APPFSGSR	C	EL

C) Human Notch 3

EGF1	AAPP	C	LDGSP	C	ANGGR	C	TQLPSREAA	C	L	C	PPGWVGER	C	Q
EGF2	LEDP	C	HSGP	C	AGRGV	C	QSSVVAGTARFS	C	R	C	PRGFRGPD	C	S
EGF3	LPDP	C	LSSP	C	AHGAR	C	SVGPDGRFL	C	S	C	PPGYQGRS	C	RS
cbEGF4	DVDE	C	RVGEP	C	RHGGT	C	LNTPGSFR	C	Q	C	PAGYTGPL	C	EN
EGF5	PAVP	C	APSP	C	RNGGT	C	RQSGDLTYD	C	A	C	LPGFEGQN	C	EV
cbEGF6	NVDD	C	PGHR	C	LNGGT	C	VDGVNTYN	C	Q	C	PPEWTGQF	C	TE
EGF7	DVDE	C	QLQPNA	C	HNGGT	C	FNTLGGHS	C	V	C	VNGWTGES	C	SQ
cbEGF8	NIDD	C	ATAV	C	FHGAT	C	HDRVASFY	C	A	C	PMGKTGLL	C	H
EGF9	LDDA	C	VSNP	C	HEDAI	C	DTNPVNGRAI	C	T	C	PPGFTGGA	C	DQ
cbEGF10	DVDE	C	SIGANP	C	EHLGR	C	VNTQGSFL	C	Q	C	GRGYTGPR	C	ET
cbEGF11	DVNE	C	LSGP	C	RNQAT	C	LDRIGQFT	C	I	C	MAGFTGTY	C	EV
cbEGF12	DIDE	C	QSSP	C	VNGGV	C	KDRVNGFS	C	T	C	PSGFSGST	C	QL
cbEGF13	DVDE	C	ASTP	C	RNGAK	C	VDQPDGYE	C	R	C	AEGFEGTL	C	DR
cbEGF14	NVDD	C	SPDP	C	HHGR	C	VDGIASFS	C	A	C	APGYTGTR	C	ES
EGF15	QVDE	C	RSQP	C	RHGGK	C	LDLVDKYL	C	R	C	PSGTTGVN	C	EV
cbEGF16	NIDD	C	ASNP	C	TFGV	C	RDGINRYD	C	V	C	QPGFTGPL	C	NV
cbEGF17	EINE	C	ASSP	C	GEGGS	C	VDGENGFR	C	L	C	PPGSLPPL	C	LP
EGF18	PSHP	C	AHEP	C	SHGI	C	YDAPGGFR	C	V	C	EPGWSGPR	C	SQSL
EGF19	ARDA	C	ESQP	C	RAGGT	C	SSDGMGFH	C	T	C	PPGVQGRQ	C	E
EGF20	LLSP	C	TPNP	C	EHGGR	C	ESAPGQLPV	C	S	C	PQGWQGPR	C	QQ
cbEGF21	DVDE	C	AGPAP	C	GPHGI	C	TNLAGSFS	C	T	C	HGGYTGPS	C	DQ
cbEGF22	DIND	C	DPNP	C	LNGGS	C	QDGVGSFS	C	S	C	LPGFAGPR	C	AR
cbEGF23	DVDE	C	LSNP	C	GPGT	C	TDHVASFT	C	T	C	PPGYGGFH	C	EQ

EGF24	DLPD	C	SPSS	C	FNGGT	C	VDGVNSFS	C	L	C	RPGYTGAH	C	QH
EGF25	EADP	C	LSRP	C	LHGGV	C	SAAHPGFR	C	T	C	LESFTGPQ	C	QT
EGF26	LVDW	C	SRQP	C	QNGGR	C	VQTGAY	C	L	C	PPGWSGRL	C	DI
EGF27	RSLP	C	REAAAQI GVRLEQL	C	QAGGQ	C	VDEDSSHY	C	V	C	PEGRTGSH	C	EQ
EGF28	EVDP	C	LAQP	C	QHGGT	C	RGYMGGYM	C	E	C	LPGYNGDN	C	ED
cbEGF29	DVDE	C	ASQP	C	QHGGS	C	IDLVARYL	C	S	C	PPGTLGVL	C	EI
cbEGF30	NEDD	C	GPGPPLD SGPR	C	LHNGT	C	VDLVGGFR	C	T	C	PPGYTGLR	C	EA
cbEGF31	DINE	C	RSGA	C	HAAHTRD	C	LQDPGGGFR	C	L	C	HAGFSGPR	C	QT
EGF32	VLSP	C	ESQP	C	QHGGQ	C	RPSPGPGGGLTFT	C	H	C	AQPFWGPR	C	ER
EGF33	VARS	C	RELQ	C	PVGVP	C	QQTPRGPR	C	A	C	PPGLSGPS	C	RSFPG SPPGA
EGF34	SNAS	C	AAAP	C	LHGGS	C	RPAPLAPFFR	C	A	C	AQGWTGPR	C	EA

D) Human Notch 4

EGF1	RGLL	C	GSFPEP	C	ANGGT	C	LSLSLGQGT	C	Q	C	APGFLGET	C	Q
EGF2	FPDP	C	QNAQL	C	QNGGS	C	QALLPAPLGLPS SPSPLTPSFL	C	T	C	LPGFTGER	C	QAK
EGF3	LEDP	C	PPSF	C	SKRGR	C	HIQASGRPQ	C	S	C	MPGWTGEQ	C	Q
EGF4	LRDF	C	SANP	C	VNGGV	C	LATYPQIQ	C	H	C	PPGFEGHA	C	ER
cbEGF5	DVNE	C	FQDPGP	C	PKGTS	C	HNTLGSFQ	C	L	C	PVGQEGPR	C	EL
EGF6	RAGP	C	PPRG	C	SNGGT	C	QLMPEKDSTFHL	C	L	C	PPGFIGPD	C	EV
cbEGF7	NPDN	C	VSHQ	C	QNGGT	C	QDGLDTYT	C	L	C	PETWTGWD	C	SE
cbEGF8	DVDE	C	ETQGPPH	C	RNGGT	C	QNSAGSFH	C	V	C	VSGWGGTS	C	EE
cbEGF9	NLDD	C	IAAT	C	APGST	C	IDRVGSFS	C	L	C	PPGRTGLL	C	H
EGF10	LEDM	C	LSQP	C	HGDAQ	C	STNPLTGSTL	C	L	C	QPGYSGPT	C	HQ
cbEGF11	DLDE	C	LMAQQGPSP	C	EHGGS	C	LNTPGSFN	C	L	C	PPGYTGSR	C	EA
cbEGF12	DHNE	C	LSQP	C	HPGST	C	LDLLATFH	C	L	C	PPGLEGQL	C	EV
cbEGF13	ETNE	C	ASAP	C	LNHAD	C	HDLLNGFQ	C	I	C	LPGFSGTR	C	EE
cbEGF14	DIDE	C	RSSP	C	ANGGQ	C	QDQPGAFH	C	K	C	LPGFEGPR	C	QT
cbEGF15	EVDE	C	LSDP	C	PVGAS	C	LDLPGAFF	C	L	C	PSGFTGQL	C	
EGF16	EVPL	C	APNL	C	QPKQI	C	KDQKDKAN	C	L	C	PDGSPG	C	AP
EGF17	PEDN	C	T	C	HHGH	C	QRSS	C	V	C	DVGWTGPE	C	EA
EGF18	ELGG	C	ISAP	C	AHGGT	C	YPQPSGYN	C	T	C	PTGYTGPT	C	SE
EGF19	EMTA	C	HSGP	C	LNGGS	C	NPSPGGYY	C	T	C	PPSHTGPQ	C	QT
EGF20	STDY	C	VSAP	C	FNGGT	C	VNRPGTFS	C	L	C	AMGFQGPR	C	EGK
EGF21	LRPS	C	ADSP	C	RNRAT	C	QDSPQGPR	C	L	C	PTGYTGGS	C	QT

EGF22	LM**D**L	C	AQKP	C	PRNSH	C	L**Q**TGPS**F**H	C	L	C	LQG**W**TGPL	C	NL
EGF23	PLSS	C	QKAALSQGI DVSSL	C	HNGGL	C	V**D**SGPS**Y**F	C	H	C	PPG**F**QGSL	C	QD
EGF24	HV**N**P	C	ESRP	C	QNGAT	C	MAQPSG**Y**L	C	Q	C	APG**Y**DGQN	C	SK
EGF25	**E**L**D**A	C	QSQP	C	HNHGT	C	TPKPGG**F**H	C	A	C	PPG**F**VGLR	C	EG
EGF26	**D**V**DE**	C	LDQP	C	HPTGTAA	C	HSLANA**F**Y	C	Q	C	LPGHTGQW	C	EV
EGF27	**E**I**D**P	C	HSQP	C	FHGGT	C	EATAGSPLGFI	C	H	C	PKG**F**EGPT	C	SH
EGF28	RAPS	C	GFHH	C	HHGGL	C	LPSPKPGFPPR	C	A	C	LSG**Y**GGPD	C	LTPP
EGF29	APKG	C	GPPSP	C	LYNGS	C	SETTGLGGPGFR	C	S	C	PHSSPGPR	C	QK

E) *Drosophila* Notch

EGF1	VAAS	C	TSVG	C	QNGGT	C	VTQLNGKTY	C	A	C	DSH**Y**VGDY	C	EH
EGF2	RNP	C	NSMR	C	QNGGT	C	QVTFRNGRPGIS	C	K	C	PLG**F**DESL	C	EI
EGF3	AVP**N**A	C	DHVT	C	LNGGT	C	QLKTLEEYT	C	A	C	ANG**Y**TGER	C	ET
EGF4	KNL	C	ASSP	C	RNGAT	C	TALAGSSSFT	C	S	C	PPG**F**TGDT	C	SY
EGF5	**D**IE**E**	C	QSNP	C	KYGGT	C	V**N**THGS**Y**Q	C	M	C	PTG**Y**TGKD	C	DT
EGF6	KYKP	C	SPSP	C	QNGGI	C	RSNGLS**Y**E	C	K	C	PKG**F**EGKN	C	EQ
cbEGF7	**N**Y**DD**	C	LGHL	C	QNGGT	C	I**D**GISD**Y**T	C	R	C	PPN**F**TGRF	C	QD
cbEGF8	**D**V**DE**	C	AQRDHPV	C	QNGAT	C	T**N**THGS**Y**S	C	I	C	VNG**W**AGLD	C	SN
cbEGF9	**N**T**DD**	C	KQAA	C	FYGAT	C	I**D**GVGS**F**Y	C	Q	C	TKG**K**TGLL	C	HL
EGF10	**D**DA	C	TSNP	C	HADAI	C	DTSPINGSYA	C	S	C	ATG**Y**KGVD	C	SE
cbEGF11	**D**I**DE**	C	DQGSP	C	EHNGI	C	V**N**TPGS**Y**R	C	N	C	SQG**F**TGPR	C	ET
cbEGF12	**N**I**NE**	C	ESHP	C	QNEGS	C	L**D**DPGT**F**R	C	V	C	MPG**F**TGTQ	C	EI
cbEGF13	**D**I**DE**	C	QSNP	C	LNDGT	C	H**D**KING**F**K	C	S	C	ALG**F**TGAR	C	QI
cbEGF14	**N**I**DD**	C	QSQP	C	RNRGI	C	H**D**SIAG**Y**S	C	E	C	PPG**Y**TGTS	C	EI
cbEGF15	**N**I**ND**	C	DSNP	C	HRGK	C	I**D**DVNS**F**K	C	L	C	DPG**Y**TGYI	C	QK
EGF16	QI**NE**	C	ESNP	C	QFDGH	C	Q**D**RVGS**Y**Y	C	Q	C	QAGTSGKN	C	EV
cbEGF17	**N**V**NE**	C	HSNP	C	NNGAT	C	I**D**GINS**Y**K	C	Q	C	VPG**F**TGQH	C	EK
cbEGF18	**N**V**DE**	C	ISSP	C	ANNGV	C	I**D**QVNG**Y**K	C	E	C	PRG**F**YDAH	C	LS
cbEGF19	**D**V**DE**	C	ASNP	C	VNEGR	C	E**D**GINE**F**I	C	H	C	PPG**Y**TGKR	C	EL
cbEGF20	**D**I**DE**	C	SSNP	C	QHGGT	C	Y**D**KLNA**F**S	C	Q	C	MPG**Y**TGQK	C	ET
cbEGF21	**N**I**DD**	C	VTNP	C	GNGGT	C	I**D**KVNG**Y**K	C	V	C	KVP**F**TGRD	C	ES
EGF22	KM**D**P	C	ASNR	C	KNEAK	C	TPSSNFLDFS	C	T	C	KLG**Y**TGRY	C	DE
cbEGF23	**D**I**DE**	C	SLSSP	C	RNGAS	C	L**N**VPGS**Y**R	C	L	C	TKG**Y**EGRD	C	AI
cbEGF24	**N**T**DD**	C	ASFP	C	QNGGT	C	L**D**GIGD**Y**S	C	L	C	VDG**F**DGKH	C	ET
cbEGF25	**D**I**NE**	C	LSQP	C	QNGAT	C	S**Q**YVNS**Y**T	C	T	C	PLG**F**SGIN	C	QT
EGF26	**N**DE**D**	C	TESS	C	LNGGS	C	I**D**GING**Y**N	C	S	C	LAG**Y**SGAN	C	QY
EGF27	KL**N**K	C	DSNP	C	LNGAT	C	HEQNNE**Y**T	C	H	C	PSG**F**TGKQ	C	SE

EGF28	YV**D**W	C	GQSP	C	ENGAT	C	S**Q**MKHQ**F**S	C	K	C	SAG**W**TGKL	C	DV
EGF29	QTIS	C	QDAADRKG LSLRQL	C	NNGT	C	K**D**YGNSHV	C	Y	C	SQG**Y**AGSY	C	QK
cbEGF30	**E**I**DE**	C	QSQP	C	QNGGT	C	R**D**LIGA**Y**E	C	Q	C	RQG**F**QGQN	C	EL
cbEGF31	**N**I**DD**	C	APNP	C	QNGGT	C	H**D**RVMN**F**S	C	S	C	PPGTMGII	C	EI
cbEGF32	**N**K**DD**	C	KPGA	C	HNNGS	C	I**D**RVGG**F**E	C	V	C	QPG**F**VGAR	C	EG
cbEGF33	**D**I**NE**	C	LSNP	C	SNAGTLD	C	V**Q**LVNN**Y**H	C	N	C	RPGHMGRH	C	EH
EGF34	KV**D**F	C	AQSP	C	QNGGN	C	NIRQSGHH	C	I	C	NNG**F**YGKN	C	EL
EGF35	SGQ**D**	C	DSNP	C	RVGN	C	VVADEG**F**GYR	C	E	C	PRGTLGEH	C	EI
EGF36	**D**TL**DE**	C	SPNP	C	AQGAA	C	E**D**LLGD**Y**E	C	L	C	PSK**W**KGKR	C	DI

F) *C. elegans* LIN-12

EGF1	HIGS	C	LGLI	C	GRNGH	C	HAGPVNGTQT SYW	C	R	C	DEG**F**GGEY	C	EQ
gap	QCDVSKCGADEKCVFDKDYRMETCVCKDCDINGNSLLKPSCPSGYGGDDCK												
EGF2	TQGW	C	YPSV	C	MNGGQ	C	IGAGNRAK	C	A	C	PDG**F**KGER	C	EL
cbEGF3	**D**V**NE**	C	EENKNA	C	GNRST	C	M**N**TLGT**Y**I	C	V	C	PQG**F**LPPD	C	LKPGNT STVEF
EGF4	KQPV	C	FLEISADH PDGRSMY	C	QNGGF	C	DKASSK	C	Q	C	PPG**Y**HGST	C	ELLE
EGF5	KE**D**S	C	ASNP	C	SHGV	C	ISFSGG**F**Q	C	I	C	DDG**Y**SGSY	C	QE
EGF6	GK**DN**	C	VNNK	C	EAGSK	C	I**N**GVNS**Y**F	C	D	C	PPE**R**TGPY	C	
EGF7	**E**KM**D**	C	SAIPDI	C	NHGT	C	I**D**SPLSEKAF E	C	Q	C	EPG**Y**EGIL	C	EQ
cbEGF8	**D**K**NE**	C	LSENM	C	LNNGT	C	V**N**LPGS**F**R	C	D	C	ARG**F**GGKW	C	DE
EGF9	PL**N**M	C	QDFH	C	ENDGT	C	MHTSDHSPV	C	Q	C	KNG**F**IGKR	C	EKECPI GF
EGF10	GGVR	C	DLRLEIGI	C	SRQGG K	C	F**N**GGKCLSGF	C	V	C	PPD**F**TGNQ	C	EVNRKN GKSSL
EGF11	SE**N**L	C	LSDP	C	MNNAT	C	I**D**VDAHIGYA	C	I	C	KQG**F**EGDI	C	ER
EGF12	HK**D**L	C	LENP	C	SNGGV	C	H**Q**HRES**F**S	C	D	C	PPG**F**YGNG	C	EQE
EGF13	KMFR	C	LKST	C	QNGGV	C	I**N**EEEKGRK	C	E	C	SYG**F**SGAR	C	EE

G) *C. elegans* GLP-1

EGF1	MGG**E**	C	GREGA	C	SVNGK	C	Y**N**GKLIETYW	C	R	C	KKG**F**GGAF	C	E
Gap	RECDLDCKRGEKCIYDVYGENPTCICQDCEDETPPTERTQKGCEEGYGGPDCKTPLFS												
EGF2	GV**N**P	C	DSDP	C	NNGL	C	YPFYGG**F**Q	C	I	C	NNG**Y**GGSY	C	EE

EGF3	GI**D**H	C	AQNE	C	AEGST	C	V**N**SVYN**Y**Y	C	D	C	PIGKSGRY	C		
EGF4	**E**RT**E**	C	ALMGNI	C	NHGR	C	IPNR**D**EDKN**F**R	C	V	C	DSG**Y**EGEF	C	NK	
cbEGF5	**D**K**NE**	C	LIEET	C	VNNST	C	F**N**LHGD**F**T	C	T	C	KPG**Y**AGKY	C	EE	
EGF6	AI**D**M	C	KDYV	C	QNDGY	C	AHDSNQMPI	C	Y	C	EQG**F**TGQR	C	EIECPS GF	
EGF7	GGIH	C	DLPLQRPH	C	SRSNGT	C	Y**N**DGRCINGF	C	V	C	EPD**Y**IGDR	C	EINRKD FKFP	
EGF8	**D**IQS	C	KYNP	C	VNNAT	C	I**D**LKNSGYS	C	H	C	PLG**F**YGLN	C	E	
EGF9	QHLL	C	TPTT	C	ANGGT	C	EGVNGVIR	C	N	C	PNG**F**SGDY	C	EIK	
EGF10	**D**RQL	C	SRHP	C	KNGGV	C	K**N**TGY	C	E	C	QYG**Y**TGPT	C	EE	

H) Human JAGGED 1

EGF1	**N**RAI	C	RQG	C	SPKHGS	C	KLPGD	C	R	C	QYG**W**QGLY	C	D	
Atypical EGF2	K	C	IPHPG	C	VHGI	C	NEPWQ	C	L	C	ETN**W**GGQL	C	DK	
EGF3	**D**L**N**Y	C	GTHQP	C	LNGGT	C	S**N**TGPDKYQ	C	S	C	PEG**Y**SGPN	C	EI	
EGF4	AEHA	C	LSDP	C	HNRGS	C	KETSLG**F**E	C	E	C	SPG**W**TGPT	C	ST	
cbEGF5	**N**I**DD**	C	SPNN	C	SHGGT	C	Q**D**LVNG**F**K	C	V	C	PPQ**W**TGKT	C	QL	
cbEGF6	**D**A**NE**	C	EAKP	C	VNAKS	C	K**N**LIAS**Y**Y	C	D	C	LPG**W**MGQN	C	DI	
cbEGF7	**N**I**ND**	C	LGQ	C	QNDAS	C	R**D**LVNG**Y**R	C	I	C	PPG**Y**AGDH	C	ER	
cbEGF8	**D**I**DE**	C	ASNP	C	LNGGH	C	Q**N**EINR**F**Q	C	L	C	PTG**F**SGNL	C	QL	
EGF9	**D**I**D**Y	C	EPNP	C	QNGAQ	C	Y**N**RASD**Y**F	C	K	C	PED**Y**EGKN	C	SH	
Gap	LKDH	C	RTTP	C	EVIDS	C	TVAMASND							
Atypical EGF10	TPEGVR	Y	ISSNV	C	GPHGK	C	KS**Q**SGGK**F**T	C	D	C	NKG**F**TGTY	C	HE	
cbEGF11	**N**I**ND**	C	ESNP	C	RNGGT	C	I**D**GVNS**Y**K	C	I	C	SDG**W**EGAY	C	ET	
cbEGF12	**N**I**ND**	C	SQNP	C	HNGGT	C	R**D**LVND**F**Y	C	D	C	KNG**W**KGKT	C	HS	
EGF13	RDS**Q**	C	DEAT	C	NNGGT	C	Y**D**EGDA**F**K	C	M	C	PGG**W**EGTT	C	NIA	
EGF14	RNSS	C	LPNP	C	HNGGT	C	VVNGES**F**T	C	V	C	KEG**W**EGPI	C	AQ	
cbEGF15	**N**T**ND**	C	SPHP	C	YNSGT	C	V**D**GDNW**Y**R	C	E	C	APG**F**AGPD	C	RI	
cbEGF16	**N**I**NE**	C	QSSP	C	AFGAT	C	V**D**EING**Y**R	C	V	C	PPGHSGAK	C	QE	

I) Human JAGGED2

EGF1	KEAV	C	KQG	C	NLLHGG	C	TVPGE	C	R	C	SYG**W**QGRF	C	D
Atypical EGF2	**E**	C	VPYPG	C	VHGS	C	VEPWQ	C	N	C	ETN**W**GGLL	C	DK
EGF3	**D**L**N**Y	C	GSHHP	C	TNGGT	C	I**N**AEPDQYR	C	T	C	PDG**Y**SGRN	C	EK
EGF4	AEHA	C	TSNP	C	ANGGS	C	HEVPSG**F**E	C	H	C	PSG**W**SGPT	C	AL
cbEGF5	**D**I**DE**	C	ASNP	C	AAGGT	C	V**D**QVDG**F**E	C	I	C	PEQ**W**VGAT	C	QL

cbEGF6	DANE	C	EGKP	C	LNAFS	C	KNLIGGYY	C	D	C	IPGWKGIN	C	HI
cbEGF7	NVND	C	RGQ	C	QHGGT	C	KDLVNGYQ	C	V	C	PRGFGGRH	C	EL
cbEGF8	ERDE	C	ASSP	C	HSGGL	C	EDLADGFH	C	H	C	PQGFSGPL	C	EV
EGF9	DVDL	C	EPSP	C	RNGAR	C	YNLEGDYY	C	A	C	PDDFGGKN	C	SV
Gap	PREP	C	PGGA	C	RVIDG	C	GSDAGPG						
Atypical EGF10	MPGT	A	ASGV	C	GPHGR	C	VSQPGGNFS	C	I	C	DSGFTGTY	C	HE
cbEGF11	NIDD	C	LGQP	C	RNGGT	C	IDEVDAFR	C	F	C	PSGWEGEL	C	DT
cbEGF12	NPND	C	LPDP	C	HSRGR	C	YDLVNDFY	C	A	C	DDGWKGKT	C	HS
EGF13	REFQ	C	DAYT	C	SNGGT	C	YDSGDTFR	C	A	C	PPGWKGST	C	AVA
EGF14	KNSS	C	LPNP	C	VNGGT	C	VGSGASFS	C	I	C	RDGWEGRT	C	TH
cbEGF15	NTND	C	NPLP	C	YNGGI	C	VDGVNWFR	C	E	C	APGFAGPD	C	RI
cbEGF16	NIDE	C	QSSP	C	AYGAT	C	VDEINGYR	C	S	C	PPGRAGPR	C	QE

J) Human DLL1

EGF1	TEPI	C	LPG	C	DEQHGF	C	DKPGE	C	K	C	RVGWQGRY	C	D
Atypical EGF2	E	C	IRYPG	C	LHGT	C	QQPWQ	C	N	C	QEGWGGLF	C	NQ
EGF3	DLNY	C	THHKP	C	KNGAT	C	TNTGQGSYT	C	S	C	RPGYTGAT	C	EL
EGF4	GIDE	C	DPSP	C	KNGGS	C	TDLENSYS	C	T	C	PPGFYGKI	C	EL
EGF5	SAMT	C	ADGP	C	FNGGR	C	SDSPDGGYS	C	R	C	PVGYSGFN	C	EK
EGF6	KIDY	C	SSSP	C	SNGAK	C	VDLGDAYL	C	R	C	QAGFSGRH	C	DD
cbEGF7	NVDD	C	ASSP	C	ANGGT	C	RDGVNDFS	C	T	C	PPGYTGRN	C	SA
EGF8	PVSR	C	EHAP	C	HNGAT	C	HERGHRYV	C	E	C	ARGYGGPN	C	QF

K) Human DLL3

EGF1	APLV	C	RAG	C	SPEHGF	C	EQPGE	C	R	C	LEGWTGPL	C	T
Gap					VPVSTSS	C	LSPRGPSSATTG	C	LVP				
EGF2	GPGP	C	DGNP	C	ANGGS	C	SETPRSFE	C	T	C	PRGFYGLR	C	EV
EGF3	SGVT	C	ADGP	C	FNGGL	C	VGGADPDSAYI	C	H	C	PPGFQGSN	C	EK
EGF4	RVDR	C	SLQP	C	RNGGL	C	LDLGHALR	C	R	C	RAGFAGPR	C	EH
EGF5	DLDD	C	AGRA	C	ANGGT	C	VEGGGAHR	C	S	C	ALGFGGRD	C	RE
EGF6	RADP	C	AARP	C	AHGGR	C	YAHFSGLV	C	A	C	APGYMGAR	C	EF

L) Human DLL4

EGF1	QQPI	C	LSG	C	EQNGY	C	SKPAE	C	L	C	RPGWQGRL	C	N
Atypical EGF2	E	C	IPHNG	C	RHGT	C	STPWQ	C	T	C	DEGWGGLF	C	DQ

EGF3	**DLNY**	C	THHSP	C	KNGAT	C	SNSGQRSYT	C	T	C	RPG**Y**TGVD	C	EL
EGF4	**E**LS**E**	C	DSNP	C	RNGGS	C	K**D**QEDG**Y**H	C	L	C	PPG**Y**YGLH	C	EH
EGF5	STLS	C	ADSP	C	FNGGS	C	RERNQGANYA	C	E	C	PPN**F**TGSN	C	EK
EGF6	KV**D**R	C	TSNP	C	ANGGQ	C	L**N**RGPSRM	C	R	C	RPG**F**TGTY	C	EL
EGF7	HVS**D**	C	ARNP	C	AHGGT	C	HDLENGLM	C	T	C	PAG**F**SGRR	C	EV
EGF8	SI**D**A	C	ASSP	C	FNRAT	C	YTDLSTDTFV	C	N	C	PYG**F**VGSR	C	EF

M) *Drosophila* Serrate

EGF1	**E**EAI	C	KAG	C	DPVHGK	C	DRPGE	C	E	C	RPG**W**RGPL	C	N
Atypical EGF2	**E**	C	MVYPG	C	KHGS	C	NGSAWK	C	V	C	DTN**W**GGIL	C	DQ
EGF3	**D**L**N**F	C	GTHEP	C	KHGGT	C	E**N**TAPDKYR	C	T	C	AEGLSGEQ	C	EI
Atypical EGF4	VEHP	C	ATRP	C	RNGGT	C							
Disordered	TLKTSNRTQAQVYRTSHGRSNMGRPVRRSSSMRSLDHLRPEGQALNGSSSPGLVSLGSLQLQ												
Atypical EGF4							Q**Q**LAPD**F**T	C	D	C	AAG**W**TGPT	C	EI
cbEGF5	**N**I**DE**	C	AGGP	C	EHGGT	C	I**D**LIGG**F**R	C	E	C	PPE**W**HGDV	C	QV
cbEGF6	D**VNE**	C											
Disordered	EAPHSAGIAANALLTTTATAIIGSNLSSTALLAALTSAVASTSL												
cbEGF6			AIGP	C	INAKE	C	R**N**QPGS**F**A	C	I	C	KEG**W**GGVT	C	AE
cbEGF7	**N**L**DD**	C	VGQ	C	RNGAT	C	I**D**LVND**Y**R	C	A	C	ASG**F**KGRD	C	ET
cbEGF8	**D**I**DE**	C	ATSP	C	RNGGE	C	V**D**MVGK**F**N	C	I	C	PLG**Y**SGSL	C	EE
EGF9	AK**EN**	C	TPSP	C	LEGH	C	L**N**TPEG**Y**Y	C	H	C	PPDRAGKH	C	EQ
Atypical EGF10	LRPL	C	SQPP	C	NEG	C							
Disordered	FANVSLATSATTTTTTTTTATTTRKMAKPSGLPCSGHGS												
Atypical EGF10							CEMSDVGTF	C	K	C	HVGHTGTF	C	EH
cbEGF11	**N**L**NE**	C	SPNP	C	RNGGI	C	L**D**GDGD**F**T	C	E	C	MSG**W**TGKR	C	SE
EGF12	RATG	C	YAGQ	C	QNGGT	C	MPGAPDKALQPH	C	R	C	APG**W**TGLF	C	AE
EGF13	AI**DQ**	C	RGQP	C	HNGGT	C	ESGAGW**F**R	C	V	C	AQG**F**SGPD	C	RI
cbEGF14	**N**V**NE**	C	SPQP	C	QGGAT	C	I**D**GIGG**Y**S	C	I	C	PPGRHGLR	C	EI

N) *Drosophila* Delta

EGF1	HIPK	C	AKG	C	EHGH	C	DKPNQ	C	V	C	QLG**W**KGAL	C	N
Atypical EGF2	**E**	C	VLEPN	C	IHGT	C	NKPWT	C	I	C	NEG**W**GGLY	C	NQ
EGF3	**D**L**N**Y	C	TNHRP	C	KNGGT	C	F**N**TGEGLYT	C	K	C	APG**Y**SGDD	C	EN
EGF4	**E**IYS	C	DADVNP	C	QNGGT	C	I**D**EPHTKTGYK	C	H	C	ANG**W**SGKM	C	EE
EGF5	KVLT	C	SDKP	C	HQGI	C	R**N**VRPGLGSKGQGYQ	C	E	C	PIG**Y**SGPN	C	DL
EGF6	QL**DN**	C	SPNP	C	INGGS	C	QPSGK	C	I	C	PAG**F**SGTR	C	ET
cbEGF7	**N**I**DD**	C	LGHQ	C	ENGGT	C	I**D**MVNQ**Y**R	C	Q	C	VPG**F**HGTH	C	SS
EGF8	KV**D**L	C	LIRP	C	ANGGT	C	L**N**LNND**Y**Q	C	T	C	RAG**F**TGKD	C	SV
cbEGF9	**D**I**DE**	C	SSGP	C	HNGGT	C	M**N**RVNS**F**E	C	V	C	ANG**F**RGKQ	C	DE

O) *C. elegans* LAG-2

EGF1	**D**PRK	C	S	C	ENDGI	C	VSSMIHPSQPNQTSSNEQLI	C	E	C	TNG**F**TGTR	C	E
Gap	IFGFNQFQLTAP												
EGF2	RP**D**A	C	SVKDA	C	LNGAK	C	FP**N**GPKV**F**	C	S	C	AVG**F**IGEF	C	EI

P) *C. elegans* APX-1

EGF1	SNPI	C	AGG	C	SNRGR	C	VAPNQ	C	S	C	ADG**F**NGTR	C	E
EGF2	Q	C	LPRAG	C	VNGD	C	V**N**ETPNT	C	K	C	RDG**F**IGDR	C	DI
EGF3	**D**IKI	C	SLEKP	C	ANGGI	C	SIDSSSSTGYK	C	H	C	PFE**F**VGSQ	C	KTPL
EGF4	SKVR	C	SAEHV	C	KNGGA	C	ISMDDTNIQ	C	K	C	RRG**F**SGKF	C	EIG
Incomplete EGF5	**N**HG**D**	C	SAMR	C	SAGET	C	**Q**ISGD**F**AI	C					

Q) *C. elegans* ARG-1

EGF1	**N**IKF	C	SHQNP	C	ANGGV	C	STDYK	C	Q	C	PSE**F**LGPR	C	ETPV
EGF2	SKVH	C	TLERV	C	KHGGT	C	VSV**N**RTNVQ	C	K	C	IRG**F**LGSL	C	EI

R) *C. elegans* DSL-2

EGF1	TKS**Q**	C	SMMCA	C	ENDAP	C	YSST**D**PRTSREYVY	C	E	C	LTG**F**EGKY	C	QN

S) *C. elegans* DSL-6

EGF1	TPM**D**	C	S	C	KNNGK	C	V**D**SAETPI	C	E	C	TRG**F**KGDT	C	EE

Table 8-1. Bioinformatics analysis of the EGF domains of Notch receptors and canonical ligands in human, Drosophila and *C. elegans*. Schematics of each receptor and canonical ligand are shown in Chapter 1 — Introduction (Figure 1-3). The amino acid sequences of each receptor/ ligand are shown as one EGF domain per row. The conserved cysteines (yellow highlight) are aligned in columns. The Ca2+ binding EGFs (cbEGF, green text) predicted by the Ca2+ binding consensus D/N/E-x-D/N-D/N/E/Q-xm-D/N/Q∗-xn-Y/F (where ∗ indicates possible β-hydroxylation, and m/n are variables) are labelled with the Ca2+ consensus residues highlighted in red if the complete consensus is present or shown to bind Ca2+. Consensus hydrophobic packing residues are shown (green highlight), with substitutions of this conserved residue indicated (cyan highlight).

8.1.3 Glycosylation of Notch receptors and ligands

Type	Enzyme	Donor Substrate	Target Glycosylation Site
O-fucosylation			
Protein O-fucosyltransferase 1	OFUT1 (*Drosophila*) POFUT1 (mammals)	GDP-fucose	Addition of O-fucose to Ser/Thr within the consensus sequence C^2-X_{4-5}-(S/T)-C^3.
N-acetylglucosaminyl transferase	Fringe (*Drosophila*) LFng, MFng, RFng (mammals)	UDP-GlcNAc	Addition of N-acetylglucosamine (GlcNAc) to O-fucose on Notch EGF repeats using a β-1,3-linkage.
β-1,4-galactosyltransferase 1	B4GALT1 (mammals)	UDP-galactose	Addition of a galactose to GlcNAc-fucose-O disaccharide on Notch EGF to form a trisaccharide.
β-galactoside α-2,3-sialyltransferase	ST3GALT (mammals)	CMP-sialic acid	Addition of a sialic acid to galactose-GlcNAc-fucose-O trisaccharide on Notch EGF to form a tetrasaccharide.
O-glucosylation			
Protein O-glucosyltransferase 1	Rumi (*Drosophila*) POGLUT1, POGLUT2, POGLUT3 (mammals)	UDP-Glc	POGLUT1 adds O-glucose to Ser within the consensus sequence C^1-X-S-X-(P/A)-C^2. POGLUT2/3 have distinct target site from POGLUT1 and add O-glucose onto a consensus C^3-X-N-T-X-G-S-F-X-C^4.
Glucoside xylosyltransferase	Shams (*Drosophila*) GXYLT1 and 2 (mammals)	UDP-xylose	Addition of a xylose to O-glucose on Notch EGF repeats to form a disaccharide.
Xyloside xylosyltransferases	Xxylt (*Drosophila*) XXYLT1 (mammals)	UDP-xylose	Addition of a xylose to xylose-glucose-O disaccharide on Notch EGF to form a xylose-xylose-glucose-O trisaccharide.

O-GlcNAcylation			
EGF domain-specific O-linked GlcNAc transferase	EOGT (*Drosophila*) EOGT1 (mammals)	UDP-GlcNAc	Addition of an O-GlcNAc to the putative consensus sequence C^5-X-X-G-**X**-(S/T)-G-X-X-C^6, where the amino acid preceding the S/T (the bold 'X') is usually an aromatic residue.
β-1,4 galactosyltransferase	B4GALT1 (mammals)	UDP-galactose	Addition of a galactose to O-GlcNAc on Notch EGF to form a disaccharide.
α-2,3/6-sialyltransferase	ST3/6GALT (mammals)	CMP-sialic acid	Addition of a sialic acid to galactose-GlcNAc-O disaccharide on Notch EGF to form a trisaccharide.
Other types of glycosylation			
Mannoside acetylglucosaminyl-transferase 3	MGAT3/GnT-III	UDP-GlcNAc	Addition of a β-1,4-linked GlcNAc to the mannosyl core of N-linked glycans to generate a bisecting GlcNAc.
Protein N-acetylgalactosaminyl-transferase 11	GALNT11	UDP-GalNAc	Addition of O-GalNAc to S/T residues. O-glycosylate human Notch1 at EGF6, EGF36 and a site in the juxta-membrane region close to the S2 site.

Table 8-2. Glycosyltransferases modifying Notch receptors and ligands. O-linked glycans are attached to a specific serine (S) or threonine (T) residue in the consensus sequences, catalysed by distinct enzymes in the ER and can be further extended in the Golgi. Modified S/T residue is underlined in the consensus sequence.

8.2 Chapter 2 Supplementary

8.2.1 Primers

A) Primers for mutagenesis of DLL4-NE3

Plasmid: pcDNA3.1(+)			
pcDNA3.1(+)_Fwd	5' GCGGTTTTGGCAGTACATCAAT 3'		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
45.5	22	66.8	Very Weak
~2360 bp			
pcDNA3.1(+)_Rev	5' TAGAGCCCCAGCTGGTTCTTT 3'		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
52.4	21	66.0	Weak
~2360 bp			
E40K_Fwd (GAG-AAG)	5' TTCATCAACAAGCGCGGCGTACTGGCCA 3'		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
72.2	18	74.7	Moderate
Right Arm: ~1900 bp		Overlap Length: 20 bp	
E40K_Rev	5' ACGCCGCGCTTGTTGATGAACTCCTGCAGCTG 3'		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
52.4	21	64.9	Very Weak

Left Arm: ~470 bp		Overlap Annealing Temp: 59.3 °C	
R41S_Fwd (CGC-AGC)	5’ ATCAACGAGAGCGGCGTACTGGCCAGTG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
72.2	18	70.4	Moderate
Right Arm: ~1900 bp		Overlap Length: 20 bp	
R41S_Rev	5’ AGTACGCCGCTCTCGTTGATGAACTCCTGCAG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
52.4	21	64.9	Very Weak
Left Arm: ~470 bp		Overlap Annealing Temp: 59.3 °C	
G47W_Fwd (GGG-TGG)	5’ TGGCCAGTTGGCGGCCTTGCGAGCCC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
82.4	17	76.0	Moderate
Right Arm: ~1880 bp		Overlap Length: 18 bp	
G47W_Rev	5’ AAGGCCGCCAACTGGCCAGTACGCCGCG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
72.2	18	72.0	Moderate
Left Arm: ~490 bp		Overlap Annealing Temp: 60.5 °C	
P49S_Fwd (CCT-TCT)	5’ CAGTGGGCGGTCTTGCGAGCCCGGCTGCC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
77.8	18	75.9	Weak
Right Arm: ~1880 bp		Overlap Length: 18 bp	
P49S_Rev	5’ TCGCAAGACCGCCCACTGGCCAGTAC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
70.0	20	71.2	Moderate
Left Arm: ~490 bp		Overlap Annealing Temp: 60.5 °C	
D128N_Fwd (GAC-AAC)	5’ GCGCCAGGAGACAACCTGCGGCCAGAGGCCT 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
72.2	18	71.9	Very Strong
Right Arm: ~1640 bp		Overlap Length: 18 bp	
D128N_Rev	5’ CAGGTTGTCTCCT GGCGCGTGCCA 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
72.2	18	73.8	Strong
Left Arm: ~730 bp		Overlap Annealing Temp: 60.5 °C	
P135A_Fwd (CCA-GCA)	5’ CCAGAGGCCTTGGCACCAGATGCACTCATCAGC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
55.0	20	64.7	Moderate
Right Arm: ~1620 bp		Overlap Length: 18 bp	
P135A_Rev	5’ TGGTGCCAAGGCCTCTGGCCGCAG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
72.2	18	71.8	Very Strong
Left Arm: ~750 bp		Overlap Annealing Temp: 60.5 °C	
A124P_Fwd (GCG-CCG)	5’ AAGCTTGGCACCCGCCAGGAGACGACCTGC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
72.2	18	69.8	Strong
Right Arm: ~1650 bp		Overlap Length: 18 bp	
A124P_Rev	5’ CTGGCGGGTGCCAAGCTTCGATGATGAG 3’		

GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
52.4	21	67.0	None
Left Arm: ~720 bp		Overlap Annealing Temp: 60.5 °C	
D127E_Fwd (GAC-GAG)	5’ GCCAGGAGAGGACCTGCGGCCAGAGGCC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
77.8	18	73.0	Very Strong
Right Arm: ~1640 bp		Overlap Length: 18 bp	
D127E_Rev	5’ CGCAGGTCCTCTCCTGGCGCGTGCCAA 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
66.7	18	74.1	Strong
Left Arm: ~730 bp		Overlap Annealing Temp: 62.8 °C	
P131T_Fwd (CCA-ACA)	5’ CTGCGGACAGAGGCCTTGCCACCAG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
66.7	18	67.2	Moderate
Right Arm: ~1630 bp		Overlap Length: 18 bp	
P131T_Rev	5’ AGGCCTCTGTCCGCAGGTCGTCTCCTGG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
72.2	18	69.7	Strong
Left Arm: ~740 bp		Overlap Annealing Temp: 60.5 °C	
V91L_Fwd (GTC-CTC)	5’ TCCTTCGCTCTCCGGGACGACAGTAGCGGC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
67	30	67	Strong
Right Arm: ~1630 bp		Overlap Length: 18 bp	
V91L_Rev	5’ GTCCCGGAGAGCGAAGGAGTTGGTGCCC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
68	28	68	Weak
Left Arm: ~740 bp		Overlap Annealing Temp: 58-60 °C	
S96R_Fwd (AGC-CGC)	5’ ACGACAGTCGCGGCGGGGGGCGCAACCCTC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
77	30	74	Moderate
Right Arm: ~1630 bp		Overlap Length: 21 bp	
S96R_Rev	5’ GCCCCCCGCCGCGACTGTCGTCCCGGAC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
82	28	73	Moderate
Left Arm: ~740 bp		Overlap Annealing Temp: 70 °C	
G98R_Fwd (GGG-CGG)	5’ GCGGCCGGGGGCGCAACCCTCTCCAAC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
78	27	68	Moderate
Right Arm: ~1630 bp		Overlap Length: 21 bp	
G98R_Rev	5’ GAGGGTTGCGCCCCCGGCCGCTACTGTC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
75	28	69	Moderate
Left Arm: ~740 bp		Overlap Annealing Temp: 73-74 °C	
R100G_Fwd (CGC-GGC)	5’ CGGGGGGGGCAACCCTCTCCAACTGCCCTT 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
70	30	72	Very Strong

Right Arm: ~1630 bp		Overlap Length: 20 bp	
R100G_Rev	5’ GGAGAGGGTTGCCCCCCCCGCCGCTACTG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
76	29	70	Moderate
Left Arm: ~740 bp		Overlap Annealing Temp: 68-69 °C	
L164F_Fwd (CTC-TTC)	5’ CAAACCAGCACCTTCACAAGGCTGCGCTAC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
57	30	66	Strong
Right Arm: ~1630 bp		Overlap Length: 22 bp	
L164F_Rev	5’ GCCTTGTGAAGGTGCTGGTTTGCTCATCC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
55	29	62	Moderate
Left Arm: ~740 bp		Overlap Annealing Temp: 62 °C	
L12_ERGV_del_Fwd	5’ GTTCATCAACCTGGCCAGTGGGCGGCCTTG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
63	30	62	Very strong
Right Arm: ~1900 bp		Overlap Length: 20 bp	
L12_ERGV_del_Rev	5’ CACTGGCCAGGTTGATGAACTCCTGCAGC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
59	29	58	Strong
Left Arm: ~470 bp		Overlap Annealing Temp: 65.6 °C	
L56_DDLR_del_Fwd	5’ CGCGCCAGGACCAGAGGCCTTGCCACCAG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
72	29	65	Moderate
Right Arm: ~1640 bp		Overlap Length: 19 bp	
L56_DDLR_del_Rev	5’ GGCCTCTGGTCCTGGCGCGTGCCAAGC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
74	27	65	Strong
Left Arm: ~730 bp		Overlap Annealing Temp: 76.8 °C	
L56_RPEA_del_Fwd	5’ GACGACCTGTTGCCACCAGATGCACTCATC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
57	30	59	Moderate
Right Arm: ~1640 bp		Overlap Length: 19 bp	
L56_RPEA_del_Rev	5’ CTGGTGGCAACAGGTCGTCTCCTGGCGCG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
69	29	65	Strong
Left Arm: ~730 bp		Overlap Annealing Temp: 67 °C	
T7 promoter Forward	5’ TAATACGACTCACTATAGGG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
40	20	50	None
For sequencing only			
BGH Reverse	5’ TAGAAGGCACAGTCGAGG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
56	18	55	None
For sequencing only			

B) Primers for mutagenesis of JAG1-NE3

Plasmid: pHL-sec			
pHLsec_Fwd	5’ GGCGGCTCTAGAGCCTCTGC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
70	20	63	Strong
~1300 bp			
pHLsec_Rev	5’ CCGGGAGATCATGAGGGTGTC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
62	21	61	None
~1300 bp			
N143Q_Fwd (AAT-CAG)	5’ ATTCCAGTCAGGACACCGTTCAACCTGAC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
50	29	57	Strong
Right Arm: ~730 bp		Overlap Length: 21 bp	
N143Q_Rev	5’ GAACGGTGTCCTGACTGGAATCCCACGCCTC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
61	31	62	Weak
Left Arm: ~570 bp		Overlap Annealing Temp: 65.2 °C	

C) Primers for mutagenesis of JAG2-NE3

Plasmid: pHL-sec			
pHLsec_Fwd	5’ GGCGGCTCTAGAGCCTCTGC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
70	20	63	Strong
~1330 bp			
pHLsec_Rev	5’ CCGGGAGATCATGAGGGTGTC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
62	21	61	None
~1330 bp			
F218A_Fwd (TTC-GCC)	5’ GCAACGACTTTGCCGGCCACTACACCTGCGAC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
63	32	63	Moderate
Right Arm: ~540 bp		Overlap Length: 23 bp	
F218A_Rev	5’ GTAGTGGCCGGCAAAGTCGTTGCGGGGCC 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
69	29	64	Very strong
Left Arm: ~810 bp		Overlap Annealing Temp: 74.1 °C	
E239K_Fwd (GAG-AAG)	5’ GATGGGCAAGAAGTGCAAGGAAGCTGTGTG 3’		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
53	30	63	Weak
Right Arm: ~480 bp		Overlap Length: 20 bp	
E239K_Rev	5’ CCTTGCACTTCTTGCCCATCCAGCCGTC 3’		

GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
61	28	67	Weak
Left Arm: ~870 bp		Overlap Annealing Temp: 66.9 °C	

D) Primers for making full-length DLL4 plasmids

Plasmid: pcDNA3.1(+)			
pcDNA3.1(+)_Fwd	5' GCGGTTTTGGCAGTACATCAAT 3'		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
45.5	22	66.8	Very Weak
NE3_BlpI_Rev	5' TCGCTGAGCTCCAGCTCACAGTCC 3'		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
63	24	66.0	Weak
~1320 bp			

E) Primers for sequencing S2 cell lines

Plasmid: pEXS2-2 (Expres2ion® Biotechnologies, Horsholm, Denmark)			
pEXS2-2_Fwd	5' GGTAAAAGTTTTGAACGCG 3'		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
42	19	54	Very weak
For sequencing only			
pEXS2-2_Rev	5' CGCGCTTGAAAGGAGTGTG 3'		
GC content (40-60%)/%	**Length (18-30 bases)/bp**	**Tm (65~75°C)/°C**	**2° Structure**
58	19	59	None
For sequencing only			
$NOTCH1_{E11-13}$: 877 bp		$NOTCH1_{E7-13}$: 1342 bp	

F) Primers for RT-qPCR in HUVECs

Gene	**Primer**	**Oligo sequence (5' to 3')**	**Target sequence length (bp)**
hGAPDH_1	Forward	GTCTCCTCTGACTTCAACAGCG (920…941)	131
	Reverse	ACCACCCTGTTGCTGTAGCCAA (1029…1050)	
hDLL4	Forward	CTGCGAGAAGAAAGTGGACAGG (1194…1215)	139
	Reverse	ACAGTCGCTGACGTGGAGTTCA (1311…1332)	
hJAG1	Forward	GAATGGCAACAAAACTTGCAT (642…662)	71
	Reverse	AGCCTTGTCGGCAAATAGC (694…712)	
hHEY1	Forward	CATACGGCAGGAGGGAAAG (313…331)	125
	Reverse	GCATCTAGTCCTTCAATGATGCT (415…437)	
hHEY2	Forward	CCCGCCCTTGTCAGTATC (10069…10086)	60

	Reverse	TTGTTTGTTCCACTGCTGGT (10109…10128)	
hHEYL	Forward	GTCCCCACTGCCTTTGAG (208…225)	68
	Reverse	ACCGTCATCTGCAAGACCTC (256…275)	
hHELT	Forward	CCGTTCAGTACCTGAGAGCACT (612…633)	125
	Reverse	CACCAGGTTCTTCATGCACTCG (715…736)	
hHES1	Forward	GAAGCACCTCCGGAACCT (255…272)	111
	Reverse	GTCACCTCGTTCATGCACTC (346…365)	
hHES2	Forward	CAGCTTAAGGGGCTCATCCT (103…122)	76
	Reverse	GGACGTCTGCCTTCTCTAGC (159…178)	
hHES3	Forward	CGCATCAATGTGTCACTGG (22…40)	76
	Reverse	ATTTGCGCTTCCGGATCT (80…97)	
hHES4	Forward	CTGGACGCCCTCAGAAAA (308…325)	87
	Reverse	GCTCCGCAGGTGTCTCAC (377…394)	
hHES5	Forward	TCAGCTACCTGAAGCACAGC (197…216)	67
	Reverse	TAGTCCTGGTGCAGGCTCTT (340…359)	
hHES6	Forward	AGCCCCTGGTGGAGAAGA (222…239)	113
	Reverse	CAGCACTTCGGCGTTCTC (317…334)	
hHES7	Forward	GCAGCCTGGAAGAGCTGA (89…106)	77
	Reverse	TTTCTCCAGCTTCGGGTTC (147…165)	
hBHLHE40	Forward	TGAAAGCACTAACAAACCTAATTGA (311…335)	91
	Reverse	TTTCTCCCTGACAGCTCACC (382…401)	
hBHLHE41	Forward	AGCGGCCTGGAGAAGTATCT (1108…1127)	69
	Reverse	GATGCCGGGGTATAGCAG (1159…1176)	
hEFNB2	Forward	GCAAGTTCTGCTGGATCAACCAG (1200…1222)	135
	Reverse	GCTGTTGCCGTCTGTGCTAGAA (1313…1334)	
hGAPDH_2	Forward	GATTTGGTCGTATTGGGCGC (105…124)	237
	Reverse	CGCCCCACTTGATTTTGGAG (322…341)	

Table 8-3. Primers used for mutagenesis, sequencing, and RT-qPCR. Overlapped regions in primer pairs used for mutagenesis are underlined, with the mutated codon/ anti-codon highlighted in yellow. The primer properties (GC content, Tm) were calculated by SnapGene and its secondary structure was assessed using Sigma-Aldrich OligoEvaluatorTM. (F) The nucleotide positions of the qPCR primers in the open reading frame (ORF) of the specific gene are indicated by numbers in the bracket.

8.2.2 Buffers & Chemicals

(A) 10x TBE buffer (pH 8.3)

Component	Concentration (mM)	Amount (for 1L)
Tris-HCl	900	108 g
Boric Acid	900	55 g
0.5M EDTA (pH 8)	30	40 mL

(B) PBS/EDTA buffer for TC use (pH ~7.4, autoclaved)

	Component	Concentration (mM)	Amount (for 1L)
PBS tablets (Sigma-Aldrich)	Phosphate buffer	10	5 tablets
	KCl	2.7	
	NaCl	137	
	0.5M EDTA (pH 8)	1	2 mL

(C) PBS buffer for ligand uptake assay (pH ~7.4, autoclaved)

	Component	Concentration (mM)	Amount (for 1L)
PBS tablets (Sigma-Aldrich)	Phosphate buffer	10	5 tablets
	KCl	2.7	
	NaCl	137	

(D) 5X Resolving buffer for SDS-PAGE (pH 8.8)

Component	Concentration	Amount (for 200mL)
Tris-HCl	1.9 M	46 g
SDS (20% stock solution)	0.5%	5 mL

(E) 5X Stacking buffer for SDS-PAGE (pH 6.8)

Component	Concentration	Amount (for 200mL)
Tris-HCl	1.25 M	30.25 g
SDS (20% stock solution)	0.5%	5 mL

(F) Acrylamide gel for SDS-PAGE (for 2 gels)

10% Resolving Gel		4% Stacking Gel	
Component	**Amount**	**Component**	**Amount**
40% Acrylamide	5 mL	40% Acrylamide	1 mL
5X Resolving buffer	4 mL	5X Stacking buffer	2 mL
H_2O	11 mL	H_2O	7 mL
10% APS (fresh)	300 µL	10% APS (fresh)	200 µL
TEMED (add last)	30 µL	TEMED (add last)	20 µL

(G) 1X Loading dye for SDS-PAGE

Component	Concentration	Amount (for 50mL)
Non-reducing dye		
Tris-HCl	50 mM	0.303 g
SDS (20% stock solution)	3%	7.5 mL
Glycerol	10%	5 mL
Bromophenol blue (BPB)	-	Trace amount
Extra component for reducing dye		
β-mercaptoethanol	5%	2.5 mL

(H) 5X Running/electrophoresis buffer for SDS-PAGE (pH ~8.3)

Component	Concentration	Amount (for 1L)
Tris-HCl	150 mM	18.75 g
SDS (20% stock solution)	0.5%	25 mL
Glycine	1.25 M	90 g

(I) Blotting buffer for Western Blot (freshly made, prechilled to ice-cold)

Component	Concentration	Amount (for 1L)
Tris-HCl	25 mM	3.03 g
SDS (20% stock solution)	0.1%	5 mL
Glycine	190 mM	14.4 g
Methanol	20%	200 mL

(J) PBST buffer for Western Blot

	Component	Concentration (mM)	Amount (for 1L)
PBS tablets (Sigma-Aldrich)	Phosphate buffer	10	5 tablets
	KCl	2.7	
	NaCl	137	
	100% Triton	0.1%	1 mL

(K) HBS buffer for TC use (pH 7.4, autoclaved)

Component	Concentration (mM)	Amount (for 1L)
HEPES	20	4.766 g
NaCl	150	8.766 g
$CaCl_2$ (1.0M stock solution)	0.9	0.9 mL

(L) HBS buffer for ELISA and liposome aggregation assay (pH 7.4)

Component	Concentration (mM)	Amount (for 1L)
HEPES	20	4.766 g
NaCl	200	11.688 g
$CaCl_2$ (1.0M stock solution)	5	5 mL

(M) HBS/BSA buffer for liposome aggregation assay (pH 7.4, freshly made)

Component		Concentration	Amount
HBS buffer	HEPES	20 mM	10 mL
	NaCl	200 mM	
	$CaCl_2$ (1.0M stock solution)	5 mM	
Fatty-acid free BSA (Sigma)		3% w/v	0.3 g

(N) ELISA washing buffer (pH 7.5)

Component	Concentration (mM)	Amount (for 1L)
HEPES	20	4.766 g
NaCl	200	11.688 g
$CaCl_2$ (1.0M stock solution)	5	5 mL
Tween-20	0.05% (v/v)	0.5 mL

(O) Citrate buffer for ELISA (pH 4.0)

Component	Concentration (mM)	Amount (for 500mL)
Citric Acid Monohydrate	90	9.46 g

(P) HRP substrate solution for ELISA (freshly made)

Component	Concentration	Amount
ABTS powder	0.33 mg/mL	0.004 g
H_2O_2 (30% w/w stock)	0.025%	10 µL
Citrate buffer	-	12 mL

(Q) FACS buffer (filtered with 0.22µm filter)

Component	Concentration (mM)	Amount (for 50mL)
PBS buffer	-	49 mL
Sodium azide (NaN_3)	0.1% w/v	0.05 g
FBS	2% v/v	1 mL

(R) DAPI staining solution for FACS (freshly made)

Component	Concentration	Amount
PBS buffer	-	4 mL
DAPI (5mg/mL stock)	1 ng/µL	0.8 µL

(S) DAPI staining solution for ligand uptake assay (freshly made)

Component	Concentration	Amount
HBSS buffer	-	4 mL
DAPI (5mg/mL stock)	1 ng/µL	0.8 µL

Table 8-4. Composition of buffer solutions.

8.2.3 PCR mix and programme

(A) KOD Polymerase PCR Mix

Component	Volume (µL)
Template DNA (10 ng/µL)	2
MQ H_2O	31
10x KOD Buffer	5
25 mM $MgSO_4$	3
2 mM dNTPs	5
10 µM Forward Primer	1.5
10 µM Reverse Primer	1.5
KOD Polymerase	1

(B) PCR Programme

Step	Temperature (°C)	Time (s)	
Initiation	95	120	
Denaturation	95	20	30 cycles
Annealing	55	10	
Elongation	70	20 per 1 kb	
Terminal elongation	70	600	
Keep	4		

Table 8-5.(A) PCR mix in a standard 50µL reaction volume. (B) PCR programme consisting of the initiation, denaturation, annealing and elongation steps. Finished reactions were kept at 4°C if to be used imminently, or -20°C for longer storage.

8.2.4 Restriction digestion and ligation

(A) Double Digestion Mix

Component	Volume (µL)	
	WT Plasmid Vector	**Overlap Extension PCR Product**
DNA	15	30
10x CutSmart Buffer	5	5
EcoRI-HF	1	1
XhoI	1	1
MQ H_2O	28	13

(B) Ligation Mix (Molar Concentration $_{Insert\ DNA}$: Molar Concentration $_{Vector}$ ≥ 3:1)

	Volume (µL)	
Component	**Ligation Mixture**	**Control**
Vector DNA	3	3
Insert DNA	5	-
MQ H_2O	-	5
5x DNA Dilution Buffer	2	2
2x T4 Ligation Buffer	10	10
T4 Ligase	1	1

Table 8-6. (A) Restriction digestion mix in a standard 50µL reaction volume. (B) Ligation mix in a standard 20µL reaction volume.

8.2.5 Agarose gel

Component	**Amount**	
	1% (w/v) Agarose Gel	**1.5% (w/v) Agarose Gel**
1x TBE Buffer	50 mL	50 mL
Agarose	0.5 g	0.75 g
SYBR Safe	5 µL	5 µL

Table 8-7. Agarose gel preparation.

8.2.6 Transformation

(A) Selection Plates: 1.5% agar (w/v)

Component	**Amount**
MQ H_2O	100 mL
Agar	1.5 g
2xYT Broth Capsules	3
50 mg/mL Ampicillin stock	200 µL (100 µg/mL)
50 mg/mL Kanamycin stock	100 µL (50 µg/mL)

(B) 2xYT Medium

Component	**Amount**
MQ H_2O	100 mL
2xYT Broth Capsules	3
50 mg/mL Ampicillin stock	200 µL (100 µg/mL)
50 mg/mL Kanamycin stock	100 µL (50 µg/mL)

Table 8-8. Agar plates for colony selection (A) and medium for E. coli expansion (B). Appropriate antibiotic selection reagent was used depending on the plasmid transformed (a final concentration of 100 µg/mL ampicillin or 50 µg/mL kanamycin).

8.2.7 Information for various sizes of cell culture plates and flasks

Culture Plates/flasks	*Surface area (cm^2)*	*Washing buffer (PBS/HBS, mL)* *Approx. volume*	*Trypsin (diluted 1:15 in PBS, mL)* *Approx. volume*	*Growth medium (mL)* *Approx. volume*
6-well	9.6	1-3	1	1-3
12-well	3.5	1-2	0.4	1-2
24-well	1.9	0.5-1.0	0.2-0.3	0.5-1.0
48-well	1.1	0.2-0.4	0.1-0.2	0.2-0.4
96-well	0.32	0.2	0.03-0.05	0.2
T-25	25	2-3	0.5-1	3-5
T-75	75	3-5	1-3	8-15
T-175	175	7-10	5-7	35-50

Table 8-9. Information for various sizes of cell culture plates and flasks. The trypsin treatment and growth medium volume will vary with cell type, this table only provides a typical range.

8.2.8 Protein sequences

Human $DLL4_{N\text{-}EGF3\text{-}Fc\text{-}His}$

MAAASRSASGWALLLLVALWQQRAAGSGVFQLQLQEFINERGVLASGRPCEPGCRTFFRVCLKH
FQAVVSPGPCTFGTVSTPVLGTNSFAVRDDSSGGGRNPLQLPFNFTWPGTFSLIIEAWHAPGDD
LRPEALPPDALISKIAIQGSLAVGQNWLLDEQTSTLTRLRYSYRVICSDNYYGDNCSRLCKKRN
DHFGHYVCQPDGNLSCLPGWTGEYCQQPICLSGCHEQNGYCSKPAECLCRPGWQGRLCNECIPH
NGCRHGTCSTPWQCTCDEGWGGLFCDQDLNYCTHHSPCKNGATCSNSGQRSYTCTCRPGYTGVD
CELELRTLEVLFQGPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKAT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKHHHHHH*

Human $DLL4_{Full\text{-}Length}$

MAAASRSASGWALLLLVALWQQRAAGSGVFQLQLQEFINERGVLASGRPCEPGCRTFFRVCLKH
FQAVVSPGPCTFGTVSTPVLGTNSFAVRDDSSGGGRNPLQLPFNFTWPGTFSLIIEAWHAPGDD
LRPEALPPDALISKIAIQGSLAVGQNWLLDEQTSTLTRLRYSYRVICSDNYYGDNCSRLCKKRN
DHFGHYVCQPDGNLSCLPGWTGEYCQQPICLSGCHEQNGYCSKPAECLCRPGWQGRLCNECIPH

NGCRHGTCSTPWQCTCDEGWGGLFCDQDLNYCTHHSPCKNGATCSNSGQRSYTCTCRPGYTGVD
CELELSECDSNPCRNGGSCKDQEDGYHCLCPPGYYGLHCEHSTLSCADSPCFNGGSCRERNQGA
NYACECPPNFTGSNCEKKVDRCTSNPCANGGQCLNRGPSRMCRCRPGFTGTYCELHVSDCARNP
CAHGGTCHDLENGLMCTCPAGFSGRRCEVRTSIDACASSPCFNRATCYTDLSTDTFVCNCPYGF
VGSRCEFPVGLPPSFPWVAVSLGVGLAVLLVLLGMVAVAVRQLRLRRPDDGSREAMNNLSDFQK
DNLIPAAQLKNTNQKKELEVDCGLDKSNCGKQQNHTLDYNLAPGPLGRGTMPGKFPHSDKSLGE
KAPLRLHSEKPECRISAICSPRDSMYQSVCLISEERNECVIATEV*

Human JAG1$_{N\text{-}EGF3\text{-}Fc\text{-}His}$

MRSPRTRGRSGRPLSLLLALLCALRAKVCGASGQFELEILSMQNVNGELQNGNCCGGARNPGDR
KCTRDECDTYFKVCLKEYQSRVTAGGPCSFGSGSTPVIGGNTFNLKASRGNDRNRIVLPFSFAW
PRSYTLLVEAWDSSNDTVQPDSIIEKASHSGMINPSRQWQTLKQNTGVAHFEYQIRVTCDDYYY
GFGCNKFCRPRDDFFGHYACDQNGNKTCMEGWMGPECNRAICRQGCSPKHGSCKLPGDCRCQYG
WQGLYCDKCIPHPGCVHGICNEPWQCLCETNWGGQLCDKDLNYCGTHQPCLNGGTCSNTGPDKY
QCSCPEGYSGPNCEIGTLEVLFQGPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKATPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKH
HHHHH*

Human JAG1$_{Full\text{-}Length}$

MRSPRTRGRSGRPLSLLLALLCALRAKVCGASGQFELEILSMQNVNGELQNGNCCGGARNPGDR
KCTRDECDTYFKVCLKEYQSRVTAGGPCSFGSGSTPVIGGNTFNLKASRGNDRNRIVLPFSFAW
PRSYTLLVEAWDSSNDTVQPDSIIEKASHSGMINPSRQWQTLKQNTGVAHFEYQIRVTCDDYYY
GFGCNKFCRPRDDFFGHYACDQNGNKTCMEGWMGPECNRAICRQGCSPKHGSCKLPGDCRCQYG
WQGLYCDKCIPHPGCVHGICNEPWQCLCETNWGGQLCDKDLNYCGTHQPCLNGGTCSNTGPDKY
QCSCPEGYSGPNCEIAEHACLSDPCHNRGSCKETSLGFECECSPGWTGPTCSTNIDDCSPNNCS
HGGTCQDLVNGFKCVCPPQWTGKTCQLDANECEAKPCVNAKSCKNLIASYYCDCLPGWMGQNCD
ININDCLGQCQNDASCRDLVNGYRCICPPGYAGDHCERDIDECASNPCLNGGHCQNEINRFQCL
CPTGFSGNLCQLDIDYCEPNPCQNGAQCYNRASDYFCKCPEDYEGKNCSHLKDHCRTTPCEVID
SCTVAMASNDTPEGVRYISSNVCGPHGKCKSQSGGKFTCDCNKGFTGTYCHENINDCESNPCRN
GGTCIDGVNSYKCICSDGWEGAYCETNINDCSQNPCHNGGTCRDLVNDFYCDCKNGWKGKTCHS
RDSQCDEATCNNGGTCYDEGDAFKCMCPGGWEGTTCNIARNSSCLPNPCHNGGTCVVNGESFTC
VCKEGWEGPICAQNTNDCSPHPCYNSGTCVDGDNWYRCECAPGFAGPDCRININECQSSPCAFG
ATCVDEINGYRCVCPPGHSGAKCQEVSGRPCITMGSVIPDGAKWDDDCNTCQCLNGRIACSKVW

CGPRPCLLHKGHSECPSGQSCIPILDDQCFVHPCTGVGECRSSSLQPVKTKCTSDSYYQDNCAN
ITFTFNKEMMSPGLTTEHICSELRNLNILKNVSAEYSIYIACEPSPSANNEIHVAISAEDIRDD
GNPIKEITDKIIDLVSKRDGNSSLIAAVAEVRVQRRPLKNRTDFLVPLLSSVLTVAWICCLVTA
FYWCLRKRRKPGSHTHSASEDNTTNNVREQLNQIKNPIEKHGANTVPIKDYENKNSKMSKIRTH
NSEVEEDDMDKHQQKARFAKQPAYTLVDREEKPPNGTPTKHPNWTNKQDNRDLESAQSLNRMEY
IV*

Human JAG1$_{\text{Full-Ectodomain-Fc}}$ (R&D, 1277-JG)

SGQFELEILSMQNVNGELQNGNCCGGARNPGDRKCTRDECDTYFKVCLKEYQSRVTAGGPCSFG
SGSTPVIGGNTFNLKASRGNDRNRIVLPFSFAWPRSYTLLVEAWDSSNDTVQPDSIIEKASHSG
MINPSRQWQTLKQNTGVAHFEYQIRVTCDDYYYGFGCNKFCRPRDDFFGHYACDQNGNKTCMEG
WMGPECNRAICRQGCSPKHGSCKLPGDCRCQYGWQGLYCDKCIPHPGCVHGICNEPWQCLCETN
WGGQLCDKDLNYCGTHQPCLNGGTCSNTGPDKYQCSCPEGYSGPNCEIAEHACLSDPCHNRGSC
KETSLGFECECSPGWTGPTCSTNIDDCSPNNCSHGGTCQDLVNGFKCVCPPQWTGKTCQLDANE
CEAKPCVNAKSCKNLIASYYCDCLPGWMGQNCDININDCLGQCQNDASCRDLVNGYRCICPPGY
AGDHCERDIDECASNPCLNGGHCQNEINRFQCLCPTGFSGNLCQLDIDYCEPNPCQNGAQCYNR
ASDYFCKCPEDYEGKNCSHLKDHCRTTPCEVIDSCTVAMASNDTPEGVRYISSNVCGPHGKCKS
QSGGKFTCDCNKGFTGTYCHENINDCESNPCRNGGTCIDGVNSYKCICSDGWEGAYCETNINDC
SQNPCHNGGTCRDLVNDFYCDCKNGWKGKTCHSRDSQCDEATCNNGGTCYDEGDAFKCMCPGGW
EGTTCNIARNSSCLPNPCHNGGTCVVNGESFTCVCKEGWEGPICAQNTNDCSPHPCYNSGTCVD
GDNWYRCECAPGFAGPDCRININECQSSPCAFGATCVDEINGYRCVCPPGHSGAKCQEVSGRPC
ITMGSVIPDGAKWDDDCNTCQCLNGRIACSKVWCGPRPCLLHKGHSECPSGQSCIPILDDQCFV
HPCTGVGECRSSSLQPVKTKCTSDSYYQDNCANITFTFNKEMMSPGLTTEHICSELRNLNILKN
VSAEYSIYIACEPSPSANNEIHVAISAEDIRDDGNPIKEITDKIIDLVSKRDGNSIEGRMDPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPEL*

Human JAG2$_{\text{N-EGF3-Fc-His}}$

MFMPSSFSYSSWATCWLLCCLIILAKNCGRLRPNSRSAAAIAMRAQGRGRLPRRLLLLLALWVQ
AARPMGYFELQLSALRNVNGELLSGACCDGDGRTTRAGGCGHDECDTYVRVCLKEYQAKVTPTG
PCSYGHGATPVLGGNSFYLPPAGAAGDRARARARAGGDQDPGLVVIPFQFAWPRSFTLIVEAWD
WDNDTTPNEELLIERVSHAGMINPEDRWKSLHFSGHVAHLELQIRVRCDENYYSATCNKFCRPR
NDFFGHYTCDQYGNKACMDGWMGKECKEAVCKQGCNLLHGGCTVPGECRCSYGWQGRFCDECVP

YPGCVHGSCVEPWQCNCETNWGGLLCDKDLNYCGSHHPCTNGGTCINAEPDQYRCTCPDGYSGR
NCEKAGTLEVLFQGPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKAT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKHHHHHH*

Human NOTCH1$_{\text{EGF11-13-BirA-His}}$

MKLCILLAVVAFVGLSLGQDVDECSLGANPCEHAGKCINTLGSFECQCLQGYTGPRCEIDVNEC
VSNPCQNDATCLDQIGEFQCICMPGYEGVHCEVNTDECASSPCLHNGRCLDKINEFQCECPTGF
TGHLCQYDGSGLEVLFQGPGSLHHILDAQKMVWNHRGHHHHHHHH*

Human NOTCH1$_{\text{EGF7-13-BirA-His}}$

MKLCILLAVVAFVGLSLGENIDDCPGNNCKNGGACVDGVNTYNCRCPPEWTGQYCTEDVDECQL
MPNACQNGGTCHNTHGGYNCVCVNGWTGEDCSENIDDCASAACFHGATCHDRVASFYCECPHGR
TGLLCHLNDACISNPCNEGSNCDTNPVNGKAICTCPSGYTGPACSQDVDECSLGANPCEHAGKC
INTLGSFECQCLQGYTGPRCEIDVNECVSNPCQNDATCLDQIGEFQCICMPGYEGVHCEVNTDE
CASSPCLHNGRCLDKINEFQCECPTGFTGHLCQYDGSGLEVLFQGPGSLHHILDAQKMVWNHRG
HHHHHHHH*

Human NOTCH2$_{\text{EGF11-13-BirA-His}}$

MKLCILLAVVAFVGLSLGEDVDECAMANSNPCEHAGKCVNTDGAFHCECLKGYAGPRCEMDINE
CHSDPCQNDATCLDKIGGFTCLCMPGFKGVHCELEINECQSNPCVNNGQCVDKVNRFQCLCPPG
FTGPVCQIDGSGLEVLFQGPGSLHHILDAQKMVWNHRGHHHHHHHH*

8.2.9 Buffers for chromatography

Protein	Buffer	Component	Concentration
JAG1 from HEK293T/S	Dilution/Washing	Tris	50 mM
		NaCl	200 mM
		pH	9.0
	Elution	Tris	50 mM
		NaCl	200 mM
		Imidazole	300 mM
		pH	9.0
DLL4 from HEK293F	Dilution	MES	20 mM
		NaCl	200 mM
		pH	6.5
	Washing	MES	20 mM
		NaCl	200 mM
		Imidazole	50 mM
		pH	6.5
	Elution	MES	20 mM
		NaCl	200 mM
		Imidazole	300 mM
		pH	6.5
NOTCH1/2 from S2	Washing	Tris	50 mM
		NaCl	200 mM
		$CaCl_2$	1 mM
		Imidazole	2.5 mM
		pH	8.5
	Elution	Tris	50 mM
		NaCl	200 mM
		$CaCl_2$	1 mM
		Imidazole	250 mM
		pH	8.5

Table 8-10. Buffers for IMAC. All buffers were filtered by a 0.22μm PVDF membrane (Durapore®) before use.

Protein	Component	Concentration
JAG1/2 from HEK293T/S	Tris	50 mM
	NaCl	200 mM
	pH	7.5
DLL4 from HEK293F	Tris	50 mM
	NaCl	200 mM
	pH	7.5
NOTCH1/2 from S2	Tris	10 mM
	NaCl	200 mM
	$CaCl_2$	1 mM
	pH	7.5

Table 8-11. Gel filtration buffers for SEC. All buffers were filtered by a 0.22µm PVDF membrane (Durapore®) before use.

8.2.10 SDS-PAGE

Component	Stacking gel	Resolving gel
Acrylamide	4-6%	8-16%
APS	0.2%	0.15%
TEMED	0.2%	0.15%
SDS	0.1%	0.1%
Tris-HCl	0.25 M	0.38 M
pH	6.8	8.8

Table 8-12. SDS-PAGE gel preparation. APS: ammonium persulfate; TEMED: tetramethyl-ethylenediamine; SDS: sodium dodecyl-sulphate. See Appendix 8.2.2 for details.

8.2.11 Liposome compositions

	Lipid #1 (PC-rich)			
Component	POPC	DOPS	PE-CF	
Ratio	70	25	5	
	Lipid #2 (SM-containing)			
Component	POPC	SM	DOPS	PE-CF
Ratio	30	40	25	5
	Lipid #3 (PIP_2-containing)			
Component	POPC	DOPS	$PI(4,5)P_2$	PE-CF
Ratio	70	20	5	5
	Lipid #4 (Ganglioside-rich)			
Component	POPC	DOPS	Ganglioside	PE-CF
Ratio	30	25	40	5

Table 8-13. Lipid stock compositions. POPC: 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine; DOPS: 1,2-dioleoyl-sn-glycero-3-phospho-L-serine; SM: sphingomyelin (brain, porcine); $PI(4,5)P_2$: L-α-phosphatidylinositol-4,5-bisphosphate (brain, porcine); Ganglioside: total ganglioside extract (brain, porcine); PE-CF: 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(carboxyfluorescein) (excitation/emission 490/515nm).

8.2.12 Blocking medium for ligand/receptor uptake assays

Blocking medium for HEK293 cells			
Component	Complete DMEM	Goat serum	BSA
Concentration	-	10% v/v	1% w/v
Blocking medium for HUVECs			
Component	Complete EGM-2	Goat serum	BSA
Concentration	-	10% v/v	1% w/v

Table 8-14. Blocking medium compositions. Blocking medium was freshly made, sterilised by filtration through a 0.22µm pore size membrane (Millex-GP syringe filter, Millipore), and warmed up to 37°C before use.

8.3 Chapter 3 Supplementary

8.3.1 Summary table of cancer database search results of DLL4 and JAG1 (updated in August 2024)

DLL4	Region	Missense mutations	JAG1	Region	Missense mutations
Signal peptide	1-26	S5F (1) G26V (1)	**Signal peptide**	1-33	L14P (2) G30W (1) G30V (1)
C2 domain			**C2 domain**		
β1	28-38	Q35R (1)	**β1**	34-43	Q34E (1) F35L (1) E38D (1) I39T (1) S41F (5)
β1-β2	39-55	E40K (1) R41S (1) G47W (1) P49S (1) P52S (1)	**β1-β2**	44-72	G47R (1) G52W (1) A58V (1) E70D (1) D72N (1)
β2	56-64	R55P (1) R55Q (1) R59C (1)	**β2**	73-81	T73A (1) T73I (1) C78Y (1)
β2-β3 ***(site 1 binding interface with Notch EGF12)***	65-76	F65V (1) F65L (1) V69I (1) C74Y (2)	**β2-β3** ***(site 1 binding interface with Notch EGF12)***	82-94	V86F (2) G89E (1) G90R (2)
β3	77-81	T78N (1) T81M (1)	**β3**	95-98	G97V (1) S98F (1)
β3-β4	82-102	V91L (1) S96R (1) G98R (1)	**β3-β4**	99-119	P100R (2) P100L (1) G103W (1)

		R100G (18)			K110N (1) G114D (1) D116Y (1)
β4	103-107	L103I (1) L103V (1) Q104P (4)	**β4**	120-124	P123S (1) F124L (1)
β4-β5	108-113	-	**β4-β5**	125-130	P129Q (1)
β5	114-123	H123R (1)	**β5**	131-140	S131F (1) T133R (1) L135F (1) A138V (1) W139S (1) D140G (1)
β5-β6	124-138	A124P (1) D127E (1) D128N (2) P131T (1) P135A (1)	**β5-β6**	141-150	S141F (1) S142N (1) D144G (2) T145N (2) T145I (1) V146I (2) S150N (1)
β6	139-148	S148F (3)	**β6**	151-159	I151V (1) E153G (1) H157Y (1)
β6-β7	149-155	V151L (1) G152C (1) Q153K (1) N154I (1)	**β6-β7**	160-163	M160T (1) I161M (1) P163H (1)
β7	156-161	L156R (1) L156S (2) L157T (1) T161A (1) T161I (1)	**β7**	164-172	W167C (1) T169A (2) T169M (2)
β7-β8	162-165	S162R (5) L164F (1)	**β7-β8**	173-177	V176I (2) A177V (1)
β8	166-172	R168S (1) R168C (1) R168H (2) R172L (1) N178S (1)	**β8**	178-184	Y181H (1) R184H (2)
DSL ***(site 2 binding interface with Notch1 EGF11)***	173-217	D182G (1) D182E (1) N183S (1) R186H (1) R191C (2) V199E (1) C200R (1) C200G (10) N205S (1) P210S (1) E215K (1)	**DSL** ***(site 2 binding interface with Notch1 EGF11)***	185-229	V185G (1) D188G (1) D189V (1) Y191H (2) Y191S (1) Y192C (1) C212S (1) D213N (1) Q214P (1) G216D (1) N217T (1) K218R (1) K218N (1)

					C220Y (1) C220F (1) C220R (1) E228K (4)
EGF1	218-251	P220S (2) I221F (1) I221T (1) S224L (1) H227Y (1) G231S (2) L240F (1) R242H (1) R248G (1) R248Q (1) C250Y (1)	**EGF1**	230-263	H242D (1) K246T (1) P248S (1) G249S (1) G256D (1) G256V (1) Y261C (1)
EGF2	252-282	E252K (2) R260C (3) R260H (1) G262S (1) G262D (1) T271A (2) E274G (1) E274D (2) G277E (1) G278C (1) G278D (1)	**EGF2 (atypical)**	264-294	C265Y (1) P267Q (1) P269L (1) V272I (6) G274S (1) Q281H (1) C284G (1) E285D (1) W288L (1) G290S (1) D294E (1) D294V (1)
EGF3	284-322	P293T (1) N296Y (1) S303N (1) P314Q (1) G315S (1) G315V (1)	**EGF3**	296-334	L297V (1) L297P (1) H303Y (1) L307I (1) G310R (1) S313I (1) G316D (1) G316S (1) C322S (1)
EGF4	324-360	E327K (1) R334H (3) N335I (1) S338N (1) D344N (3) D344Y (1) P352L (1) C359R (1)	**EGF4**	336-372	A336D (1) H338L (1) A339S (1) P344L (1) L356M (1) L356V (1) E359Q (1) G365S (1) G368D (1) T370I (1) S372C (1) S372A (1)
EGF5	362-400	A367V (1) G374R (1) R378W (3) R378Q (4) R380C (1) A384G (1) A384D (2) P392T (1) P392L (1) G396D (1) C399G (1)	**EGF5 (Ca^{2+}-binding)**	374-410	I375F (1) D377G (1) C378R (1) P380S (1) S384F (4) G386R (1) G386W (2) G386V (1) G395E (1) G395V (1) G395R (2)

					F396Y (1) K397N (1) C400Y (1) P401H (1) G406V (1) T408M (1)
EGF6	402-438	D404E (1) C406R (2) S408N (1) G414R (1) R420L (1) R420Q (1) P422S (1) S423G (1) R424S (1) R427C (1) R427H (1) R427H (2) R429S (1) P430T (1) T433M (2) C437Y (2)	**EGF6 (Ca^{2+}-binding)**	412-448	D412Y (2) E415K (1) C416R (1) E417D (1) S426F (1) K428T (1) A432S (1) S433I (1) S433T (1) D437N (1) G441S (1) M443I (1) Q445K (2) D448Y (1)
EGF7	440-476	V441I (1) D443N (1) R446C (2) R446H (1) R446L (1) N447K (1) P448S (1) C449F (1) A450T (3) H451Y (2) G452S (2) G452D (1) C455Y (1) H456R (1) D457E (1) E459K (1) G461R (1) T465A (1) P467S (1) A468V (2) G469C (1) S471F (1) R473Q (1) R474H (1)	**EGF7 (Ca^{2+}-binding)**	450-485	C458R (1) N460K (1) A462T (2) R465Q (1) G470C (1) G470D (1) R472C (1) I474M (1) D482G (1) D482N (1) H483L (1)
EGF8	480-518	R478W (4) T479I (2) S486L (1) T501I (1) D502Y (3) R516C (5) R516H (1) R516S (1) E518G (1)	**EGF8 (Ca^{2+}-binding)**	487-523	D487N (1) A492P (1) A492T (1) N498S (1) G499W (1) C502R (2) R508K (2) R508I (1) F509I (1) T515I (1) S518F (1) C522F (2)
Link	519-529	P520L (2) V521M (2)	**EGF9**	525-561	L524V (2) D525E (2)

		V521L (1) P524Q (1) S526N (1) F527S (1) P528S (1) P528L (2)			P531R (1) N532Y (1) P533T (1) P533L (2) G537S (1) C540Y (1) R543C (1) C551Y (1) P552H (1) E553K (1) G557S (1) N559S (1) C560Y (1)
			Link	562-585	L563M (1) D565E (1) H566N (1) H566Y (1) H566Q (1) H566S (1) R568S (1) T569K (1) T570A (1) C572Y (1) E573K (1) T579S (1) A583V (1) S584F (1)
			EGF10	586-627	D586N (2) G590W (1) V591G (1) R592W (1) R592Q (1) S596F (1) N597K (1) V598I (1) G600V (1) H602N (1) H602Y (1) G603R (1) K606N (1) T622M (2) G623R (1) G623E (2) H627N (2)
			EGF11 (Ca^{2+}-binding)	629-665	N629T (1) N629I (1) D632Y (1) N636I (1) R639K (1) D646N (4) S650F (1) I654T (1) G658S (1) G658D (2) W659R (1) A662T (2) E665K (1)
			EGF12 (Ca^{2+}-binding)	667-703	D670N (1) Q673K (1) G680S (1)

					T681R (1) R683H (2) D684N (2) D684Y (1) N695H (1) G696A (1) G696R (1) G696E (1) W697G (1) K698I (1) T701P (2)
			EGF13	705-741	R705P (1) C709R (2) E711G (2) A712V (1) T713M (1) G717S (2) G718D (1) Y721C (1) E723D (1) E723K (1) M730T (1) C731G (1) P732H (1) G734S (1) W735L (1) G737E (1) T738A (1) N741H (1) A743V (1)
			EGF14	744-780	R744Q (4) N751K (1) G756W (1) G756E (1) G757C (1) G757D (2) V760A (1) G763S (1) G763D (1) E764K (2) S765F (1) T767M (2) V769I (3) C770Y (1) E772V (1) G773S (2) G773D (1) E775A (1) P777S (1) I778M (1)
			EGF15 (Ca^{2+}-binding)	782-818	S787N (2) P788H (1) P788R (1) P790S (1) P790T (1) C791Y (1) N793D (1) G795S (1) C797Y (1) G800E (1)

					N802S (2) R805W (3) E807K (2) P810T (1) G811S (1) C817Y (1)
			EGF16 (Ca^{2+}-binding)	820-856	N820K (1) Q825R (1) S827L (1) G832R (1) G832E (1) A833V (1) R843Q (1) C846F (1) P848A (1) P848L (1) P848S (2) H850Q (1) H850R (1) G852R (1) A853T (1)
			CRD	857-1067	R861I (1) G867W (1) P871R (10) D872G (1) D878V (1) L886M (1) G888V (1) G888R (1) G888E (1) R889W (2) R889Q (4) A891T (6) S893L (1) R900Q (1) P901H (1) C902R (1) E910K (2) P912F (1) P919S (1) H928N (1) V933G (1) C936Y (1) C936W (1) R937Q (1) P943T (1) P943L (1) T946K (1) S950C (1) D956Y (1) N957K (1) C958G (1) A959V (1) N960D (1) N966K (3) M969I (1) M969T (1) M970L (1) P972T (1) T976M (3)

					E977Q (1) H978Y (1) C980Y (2) L986F (1) N987S (1) L989F (1) V992I (1) S993F (1) I1000V (1) A1001T (1) E1003K (1) S1007L (1) N1009I (1) I1012L (1) I1012V (1) H1013L (1) S1017F (1) E1019K (1) R1022W (4) R1022Q (1) R1022L (1) P1027Q (1) I1028L (1) G1130R (1) K1034N (1) I1035V (1) D1037Y (1) D1037N (5) R1042C (1) D1043N (1) S1047L (2) L1048V (1) I1049M (2) V1052I (1) R1056S (1) R1060Q (1) R1060P (2) K1063E (1) N1064S (1)
Trans-membrane helical	530-550	V532I (2) G535D (1) L542Q (1) L545R (1) G546D (1) V550M (1)	**Trans-membrane helical**	1068-1093	W1081R (1) A1088T (1) A1088S (2)
Cytoplasmic	551-685	A551S (1) A551D (1) V552M (1) R556W (3) R556Q (1) R558G (1) R558Q (1) R558P (1) R559W (2) P560L (1) D562N (2) G563S (1) E566G (1) M568I (1) N569K (1)	**Cytoplasmic**	1094-1218	R1094W (1) R1094Q (1) R1096W (1) R1096Q (1) R1097W (2) R1097Q (1) P1099L (1) G1100A (1) S1101G (1) S1105L (1) R1116W (2) Q1121K (1) N1124K (1) P1125H (1) I1126T (2)

		S572L (1) D573N (1) P581S (1) A582D (1) N587H (1) K591N (2) T612A (1) R624W (2) R624Q (2) G625R (1) G625E (1) T626A (1) T626I (1) P632S (5) P632H (1) P632L (1) D635N (1) D635A (1) D635G (1) K636N (1) E640D (1) K641M (1) A642V (1) R645W (1) P651T (1) R654W (1) R654Q (1) I655M (1) S660F (1) P661S (1) D663N (1) R676K (1) V685I (1)			E1127K (1) G1130R (2) A1131T (1) I1136M (1) E1140K (1) S1147C (1) N1153K (1) S1154P (1) V1156A (1) E1158K (1) E1158G (1) D1160N (4) Q1165H (1) K1167N (1) R1169W (1) P1174L (2) A1175T (1) Y1176H (1) T1177M (1) D1180N (2) P1185H (1) N1187H (1) G1188S (2) T1189M (2) P1190L (1) P1194T (1) P1194Q (1) W1196R (1) T1197R (1) D1204Y (1) R1213Q (2) M1214I (5) Y1216C (1)

Table 8-15. Cancer-related missense mutations in DLL4 and JAG1. Missense mutations recorded in the COSMIC database are shown as black and additional missense mutations recorded in the GDC data portal are shown as blue. Numbers in the parentheses indicate the number of independent cases or tumour samples in which each mutation was found. Website URLs: https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=DLL4; https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=JAG1; https://portal.gdc.cancer.gov/genes/ENSG00000128917; https://portal.gdc.cancer.gov/genes/ENSG00000101384.

8.3.2 Secretion tests of DLL4 variants

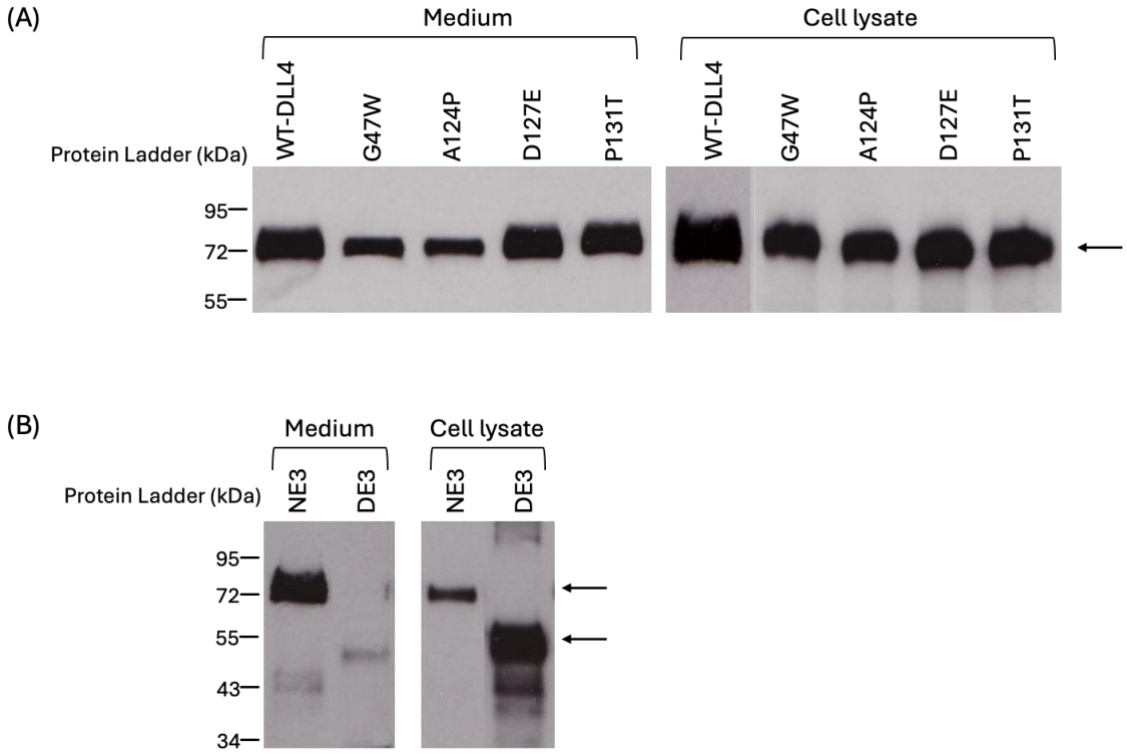


Figure 8-1. (A) Secretion tests of DLL4 cancer database variants in HEK293T. Western blot analysis of medium and cell lysate samples of DLL4 cancer database variants expressed using HEK293T cells. Note the thinner bands of G47W and A124P compared to WT-DLL4. Proteins were separated by reducing SDS-PAGE and detected by anti-His-HRP antibody. The theoretical monomeric MW of $DLL4_{NE3\text{-}Fc\text{-}His}$ is ~64kDa, whilst experimentally, the monomer migrated to a higher MW position ~72kDa. Target bands with desired MW were indicated by arrows. **(B) Secretion tests of DLL4 DSL-EGF3 (DE3) fragment in HEK293F.** Western blot analysis of medium and cell lysate samples of DLL4-DE3 fragment expressed using HEK293F cells. Note that little DE3 was detected in the medium, suggesting non-secretion. Proteins were separated by reducing SDS-PAGE and detected by anti-His-HRP antibody. The theoretical monomeric MW of $DLL4_{DE3\text{-}Fc\text{-}His}$ is ~47kDa, whilst experimentally, the monomer migrated to a higher MW position ~55kDa. Target bands with desired MW were indicated by arrows.

8.3.3 Cell surface expression of FL-DLL4 variants

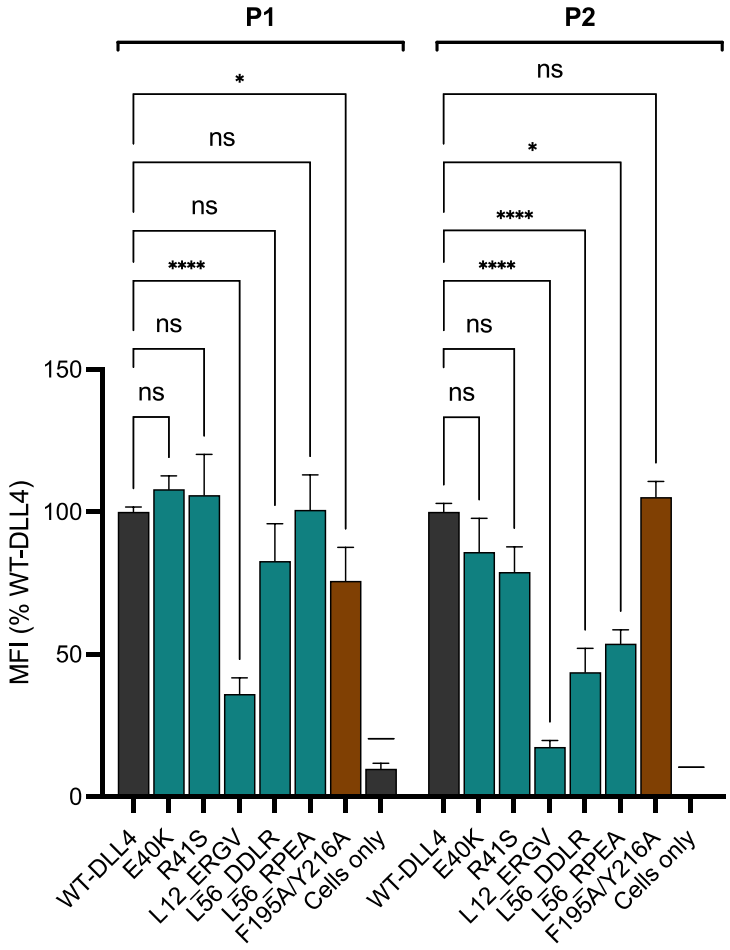


Figure 8-2. Cell surface expression of FL-DLL4 variants in HEK293T cells. FL-DLL4-expressing HEK293T cells were assessed for their ligand surface expression levels. 30,000 cells were measured per reading in flow cytometry, and representative histograms were shown in Chapter 3 (Figure 3-12). Due to a bimodal distribution, the two cell populations (P1 and P2) were analysed separately. The median fluorescence intensity (MFI) of each cell population was normalised as percentage of WT-DLL4 surface expression and presented as mean ± SD, representing combined results of n≥3 independent experiments. E40K, R41S, and F195A/Y216A showed similar surface expression levels to WT-DLL4. The three C2-loop shortening variants all showed statistically significant decrease in surface expression compared with WT-DLL4. Untransfected HEK293T cells, 'Cells only', were used as a negative control. Statistical analysis was performed using Kruskal-Wallis test followed by Dunn's multiple comparison test. ns (not statistically significant) $P>0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, ****P≤0.0001.

8.4 Chapter 4 Supplementary

8.4.1 qPCR primers check

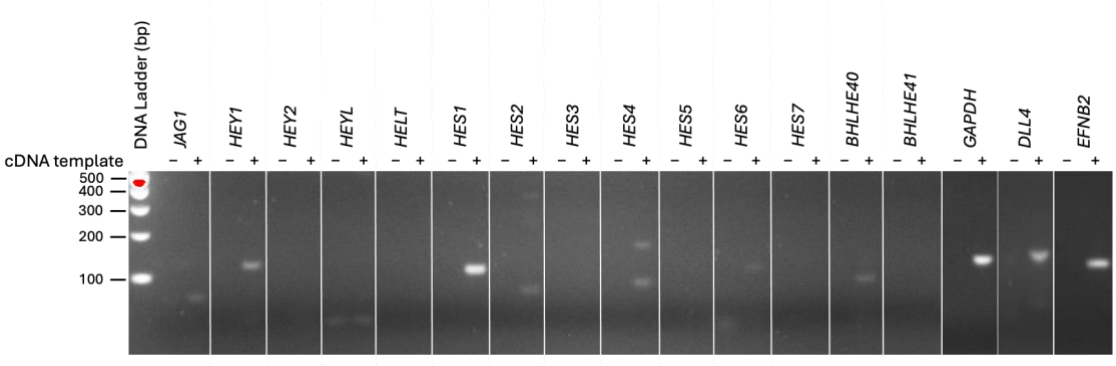


Figure 8-3. Expression of Notch downstream target genes in HUVECs. The expression of a variety of Notch target genes in WT-DLL4-treated (+) HUVECs was analysed by RT-PCR. No template negative controls (−) were tested alongside to check that the primer pairs do not self-anneal. The target genes were amplified by qPCR primers (see Appendix 8.2.1 for primer details) and visualised by agarose gel electrophoresis. Note that some genes tested were not expressed or expressed at non-detectable levels in HUVECs. *HES4* qPCR primers resulted in two bands, suggesting non-specificity in annealing sequences.

8.4.2 RT-qPCR assay optimisation

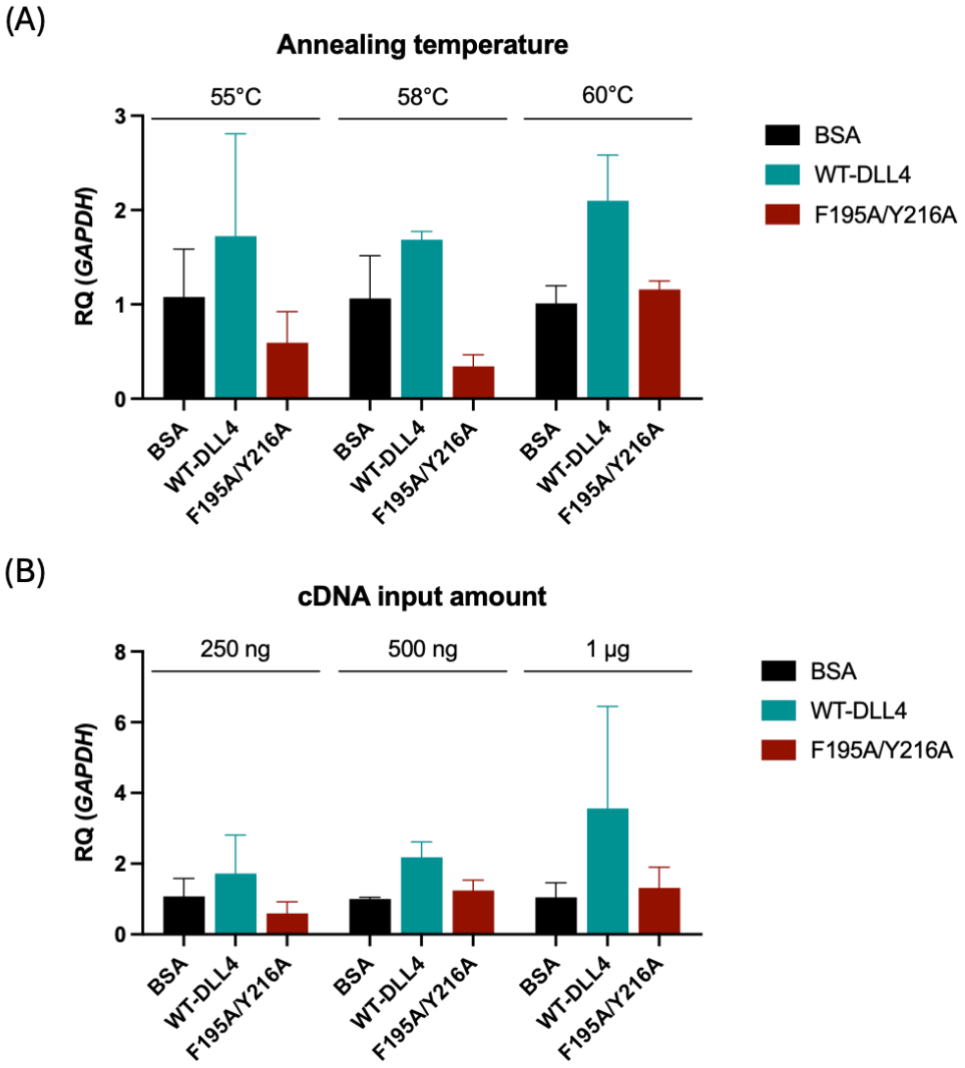


Figure 8-4. Optimisation of qPCR assay conditions. HUVECs were incubated with 5ng/µL WT-DLL4 (teal), F195A/Y216A (brown), or BSA (black) for 24 hours. qPCR was performed under different reaction conditions: (A) ~500ng cDNA template was added per reaction. The annealing step of PCR was performed at 55°C, 58°C, or 60°C. (B) The annealing step of PCR was performed at 60°C. A varying amount of input cDNA, from 250ng to 1µg, was added per reaction. The expression level of *HEY1*, normalised by the endogenous control *GAPDH*, were quantified using the $\Delta\Delta C_T$ method as an RQ value, indicating a fold change relative to the BSA sample. The RQ values of all BSA samples were calibrated to 1-fold. Data was presented as mean ± SD of triplicate measurements.

8.5 Chapter 5 Supplementary

8.5.1 $NOTCH2_{EGF11-13}$ purification

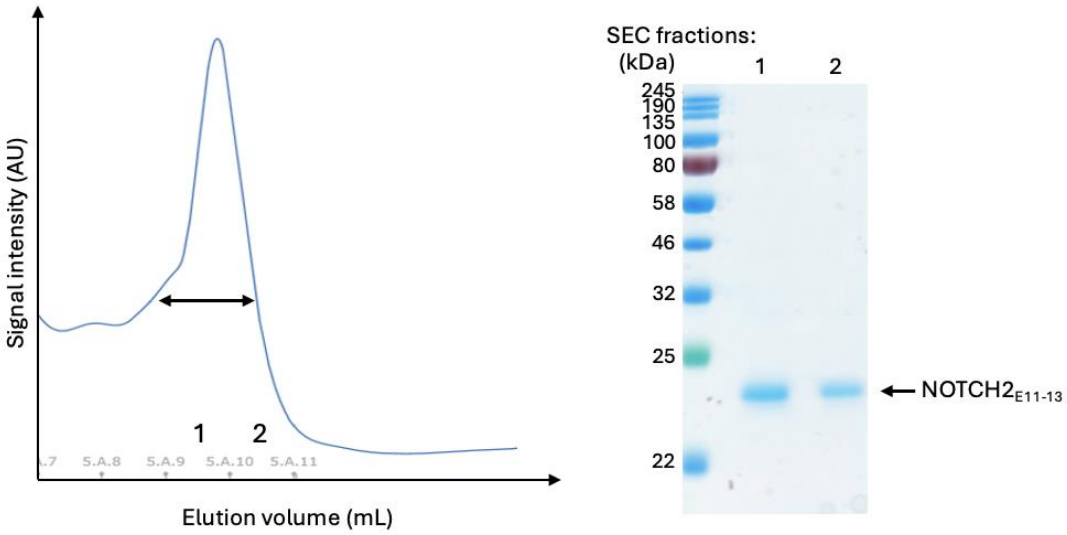


Figure 8-5. Purification of $NOTCH2_{E11-13}$ from S2 cell line (cited from Dr Yao Meng's thesis). (Left) The size exclusion chromatogram of $NOTCH2_{E11-13}$. The protein elution peak is indicated by a double-headed arrow. (Right) Non-reducing SDS-PAGE to check the identity and purity of the eluted protein. The target band with desired MW was indicated by an arrow. The theoretical MW of $NOTCH2_{E11-13-BirA-His}$ is ~18 kDa, whilst experimentally, the protein band migrated to a higher MW position ~24 kDa. The clear target band suggests homogenous product with >90% purity.

8.5.2 Receptor uptake ability of FL-DLL4 variants

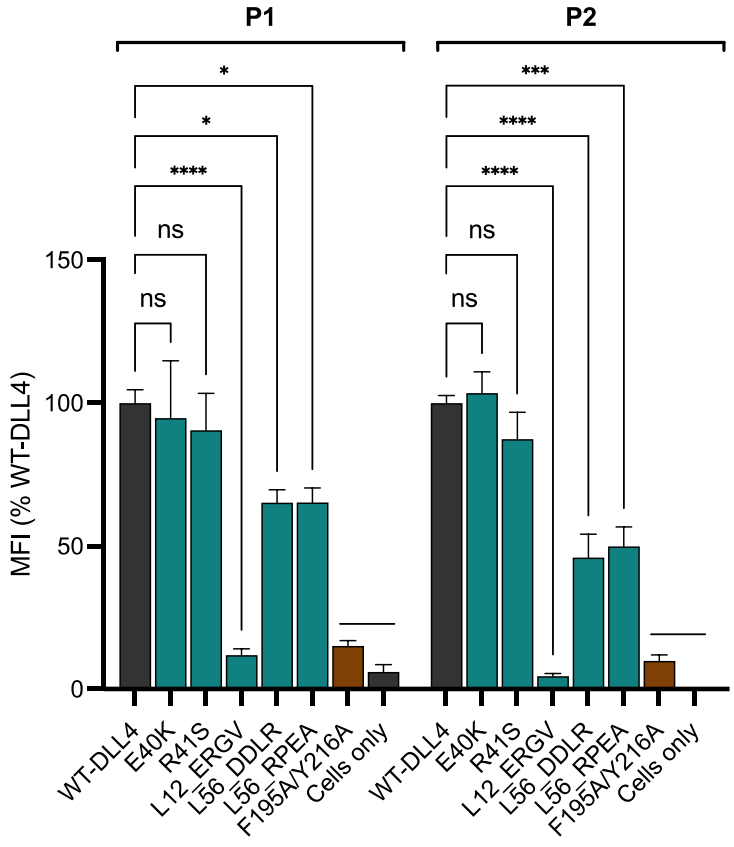


Figure 8-6. NOTCH1$_{E7-13}$ receptor uptake by FL-DLL4 variants expressing in HEK293T cells. FL-DLL4-expressing HEK293T cells were assessed for their receptor uptake ability. 30,000 cells were measured per reading in flow cytometry, and representative histograms were shown in Chapter 5 (Figure 5-9). Due to a bimodal distribution, the two cell populations (P1 and P2) were analysed separately. The median fluorescence intensity (MFI) of each cell population was normalised as percentage of WT-DLL4 receptor uptake and presented as mean ± SD, representing combined results of n=3 independent experiments. E40K and R41S both showed similar receptor uptake ability to WT-DLL4. The three C2-loop shortening variants all showed statistically significant decrease in receptor uptake compared with WT-DLL4. The receptor-binding-deficient F195A//Y216A variant and untransfected HEK293T cells (i.e., 'Cells only') were used as negative controls. Statistical analysis was performed using Kruskal-Wallis test followed by Dunn's multiple comparison test. ns (not statistically significant) P>0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P≤0.0001.

8.5.3 Liposome aggregation assays

8.5.3.1 Representative FACS raw data

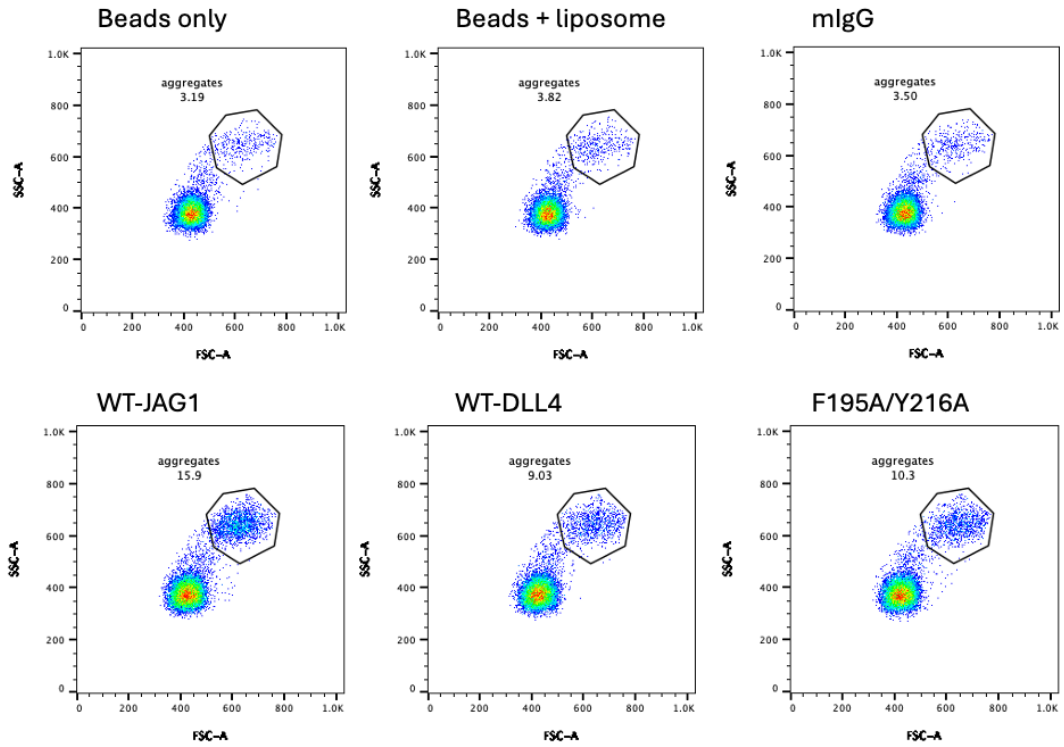


Figure 8-7. Liposome aggregation assays of purified Notch ligands. A set of representative FACS raw data for SM-rich liposome binding. WT-DLL4$_{NE3}$, DLL4-F195A/Y216A$_{NE3}$, or WT-JAG1$_{NE3}$ were incubated with SM-rich liposomes. 'Beads only', 'beads + liposomes', and mIgG were used as negative controls. Bead population was gated for size, complexity, and fluorescence, after which the aggregated beads population was shown as a percentage (circled). SSC: side scatter; FSC: forward scatter.

8.5.3.2 Liposome size and homogeneity checked by DLS

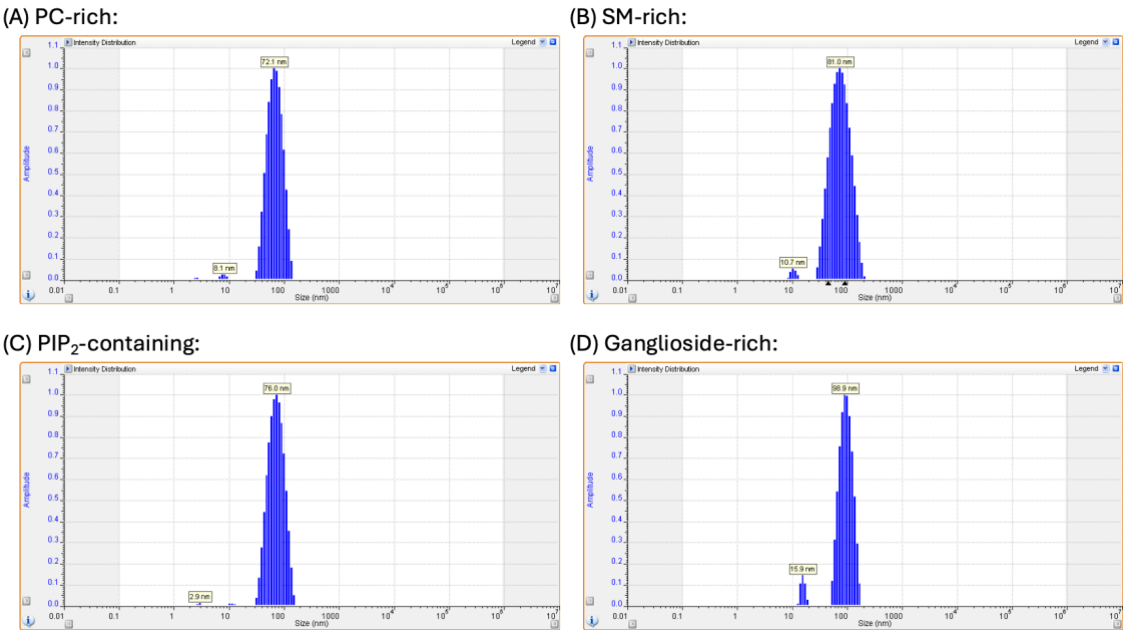


Figure 8-8. Size and homogeneity of liposomes. Size and homogeneity of the (A) PC-rich liposomes (POPC:DOPS:PE-CF = 70:25:5), (B) SM-rich liposomes (POPC:SM:DOPS:PE-CF = 30:40:25:5), (C) PIP_2-containing liposomes (POPC:DOPS:PI(4,5)P_2:PE-CF = 70:20:5:5), and (D) ganglioside-rich liposomes (POPC:DOPS:Ganglioside:PE-CF = 30:25:40:5) were assessed by DLS after extrusion through a 100 nm pore width membrane. The PC-rich liposomes were ≥90% homogeneous with an average diameter of 72.1 nm. The SM-rich were ≥90% homogeneous with an average diameter of 81.0 nm. The PIP_2-containing liposomes were ≥90% homogeneous with an average diameter of 76.0 nm. The ganglioside-rich liposomes were ≥90% homogeneous with an average diameter of 98.9 nm.

8.6 Chapter 6 Supplementary

8.6.1 Additional analysis of $N143Q_{NE3}$

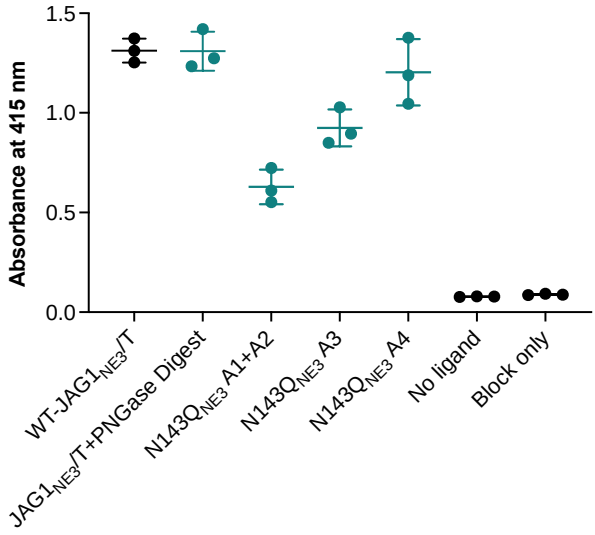


Figure 8-9. Epitope recognition of different $N143Q_{NE3}$ SEC fractions analysed by 65D MAb. The $N143Q_{NE3}$ A1+A2 fraction had poor epitope recognition, and the A3 fraction also had a lower binding affinity for 65D MAb than the A4 fraction. The A4 fraction, which was collected as the soluble, purified $N143Q_{NE3}$ protein for further analysis, had similar 65D binding affinity as the $WT\text{-}JAG1_{NE3}$.

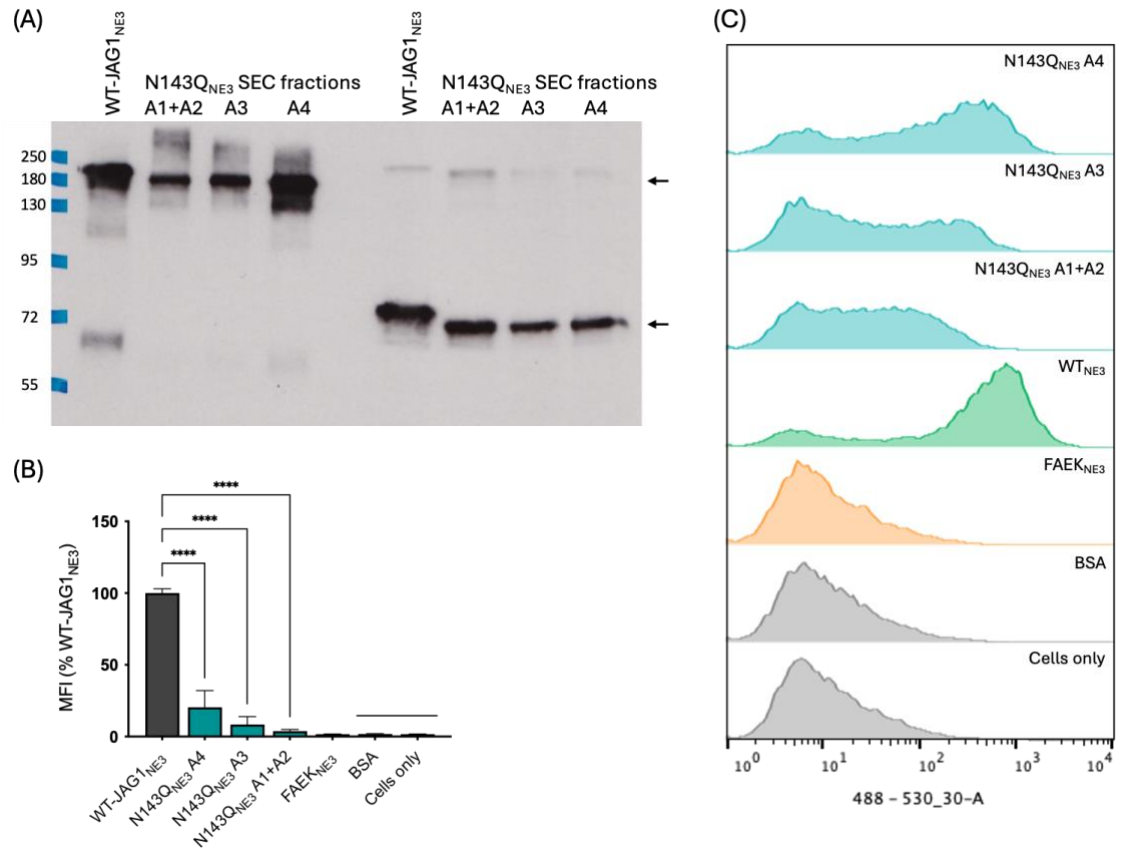


Figure 8-10. Notch activation by different N143Q$_{NE3}$ SEC fractions. (A) The different fractions of N143Q$_{NE3}$ obtained from SEC were analysed by Western blot using anti-His-HRP antibody. All N143Q$_{NE3}$ fractions tested were immunoreactive, showing a single band with a MW slightly lower than WT-JAG1$_{NE3}$. (B) Notch activation by different fractions of N143Q$_{NE3}$ was assessed by CHO-K1 hN1G4^{esn} reporter cells. YFP expression levels were quantified as median fluorescence intensity (MFI). Data, presented as mean ± SD, represent combined results of n≥3 independent experiments, in which 10,000 cells were measured in each reading. BSA and 'cells only' were used as the background control. Statistical analysis was performed using Welch's t-test. ****$P \leq 0.0001$. (C) A representative histogram showing YFP expression levels measured by flow cytometry. Note the more leftward shift of the N143Q$_{NE3}$ A1+A2 and A3 fractions compared to the N143Q$_{NE3}$ A4 fraction.

8.6.2 ELISA-type assay to check ligand immobilisation

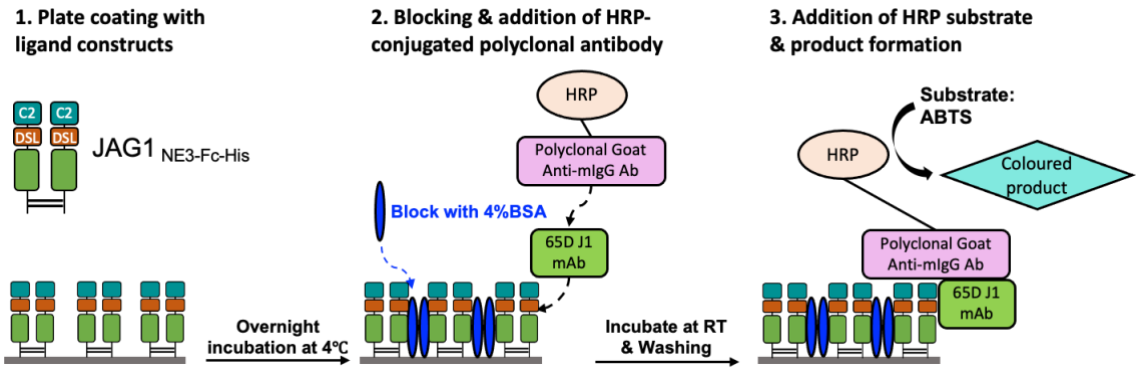


Figure 8-11. ELISA-type assay to check ligand immobilisation. The 96-well TC plate was coated with purified ligand constructs such as $JAG1_{NE3\text{-}Fc\text{-}His}$ at 4°C overnight. The plate was then washed with ELISA buffer and blocked with 4% BSA for 1hr. After blocking, anti-JAG1 65D antibody (0.2 ng/µL) was added and incubated for 1hr, followed by HRP-conjugated anti-mIgG secondary antibody (1:1000) and incubated for another 1hr. After washing steps to remove unbound antibodies, HRP substrate solution (ABTS) was added and the absorbance at a wavelength of 415 nm was measured immediately using a plate reader.

8.6.3 Notch activation control experiments for protein G precoating

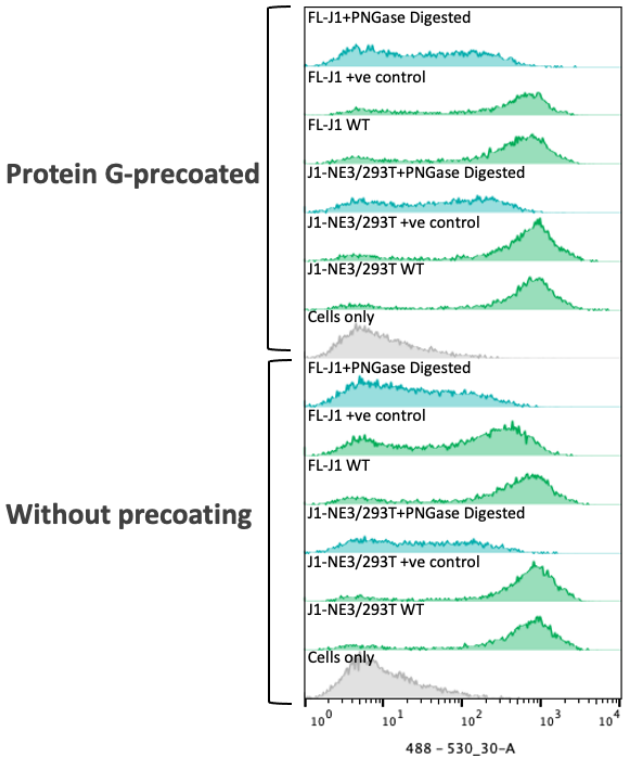


Figure 8-12. A representative histogram showing Notch activation by PNGase F digested $JAG1_{NE3}$ and FL-J1 with or without protein G-precoating. Notch activation by

WT-JAG1$_{NE3}$ and FL-J1, their PNGase F digested samples, and the positive controls were assessed by CHO-K1 hN1G4^{esn} reporter cells using flow cytometry. Without protein G-precoating, both the PNGase F digested FL-J1 and its positive control resulted in lower Notch activation compared to their equivalent samples with protein G-precoating, whereas protein G alone had no effect on the activity of JAG1$_{NE3}$ or the cells.

8.6.4 Liposome aggregation assays of deglycosylated JAG1$_{NE3}$

8.6.4.1 Liposome size and homogeneity checked by DLS

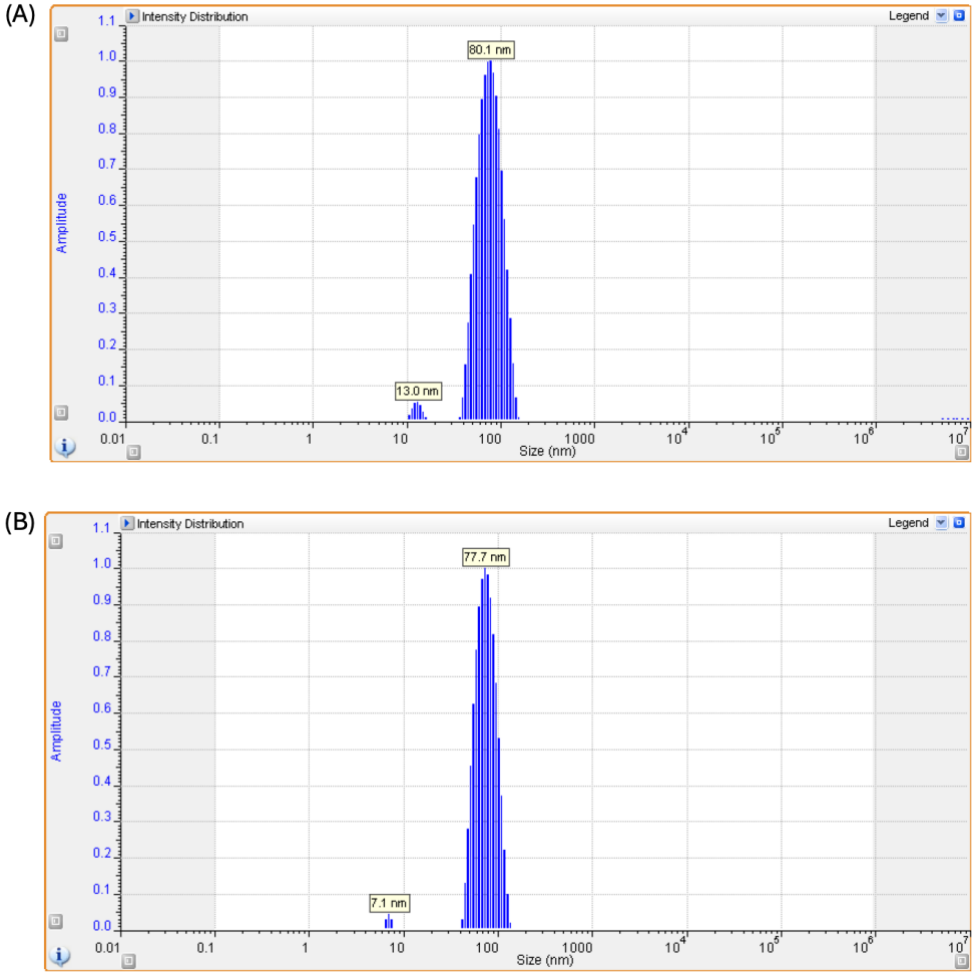


Figure 8-13. Size and homogeneity of liposomes. Size and homogeneity of the (A) PC-rich liposomes (POPC:DOPS:PE-CF = 70:25:5) or (B) SM-rich liposomes (POPC:SM:DOPS:PE-CF = 30:40:25:5) were assessed by DLS after extrusion through a 100 nm pore width membrane. The PC-rich liposomes were ≥90% homogeneous with an average diameter of 80.1 nm. The SM-rich were ≥90% homogeneous with an average diameter of 77.7 nm.

8.6.4.2 Representative FACS raw data

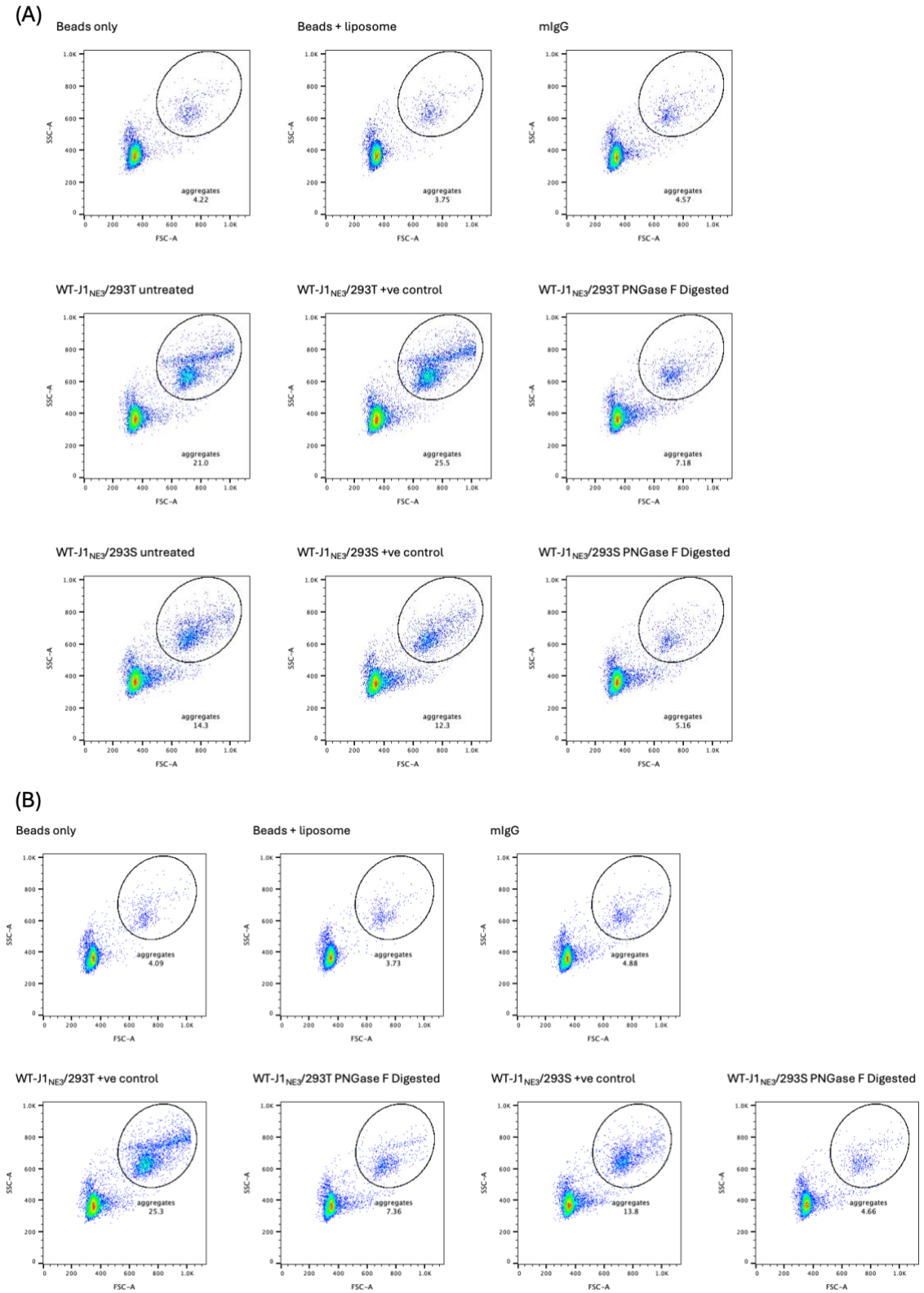


Figure 8-14. Liposome aggregation assays of PNGase F digested WT-JAG1$_{NE3}$. A set of representative FACS raw data for (A) PC-rich or (B) SM-rich liposome binding. WT-JAG1 or PNGase F digested JAG1-coated protein G beads were incubated with PC- or SM- rich

liposomes. Bead population was gated for size, complexity, and fluorescence, after which the aggregated beads population was shown as a percentage (circled). SSC: side scatter; FSC: forward scatter.

8.7 Chapter 7 Supplementary

8.7.1 Notch activation by WT-DLL4$_{NE3}$ and WT-JAG1$_{NE3}$

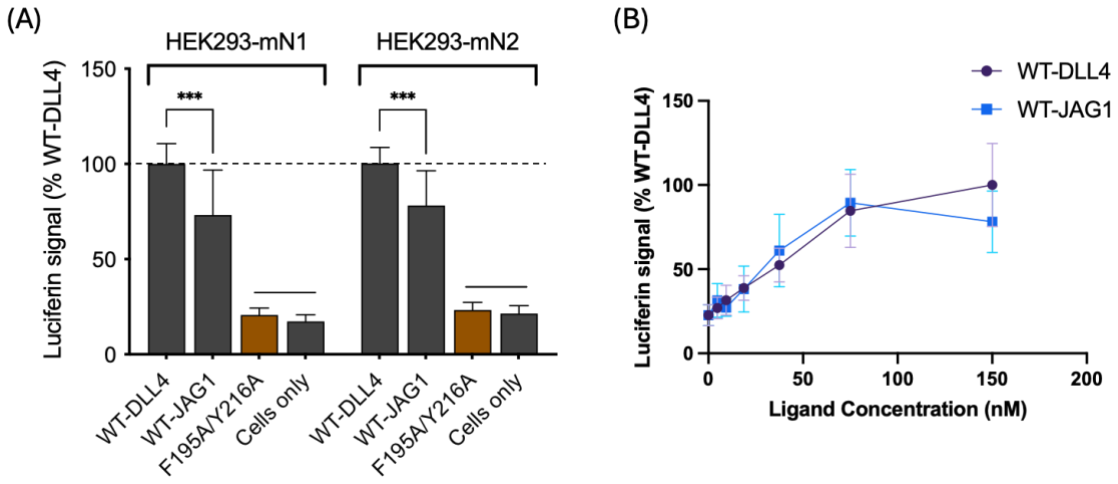


Figure 8-15. Notch activation by purified WT-DLL4$_{NE3}$ and WT-JAG1$_{NE3}$. (A) Notch activation was assessed by HEK293 mN1- or mN2- NLuc/CLuc-RBPJ reporter cells at high ligand concentrations (150nM). F195A/Y216A and 'cells only' were tested alongside as negative controls. The luminescence intensity readout was used to quantify the amount of NICD released upon Notch activation. The data was normalised as percentage of WT-DLL4 activity and presented as mean ± SD, representing combined results of n=3 independent experiments, in which 6 technical repeats were measured in each experiment. Statistical analysis was performed in each case using Welch ANOVA followed by Dunnett's T3 multiple comparison test. ***$P \leq 0.001$. (B) Serial dilution titration tests of ligand activities, assessed by HEK293 mN2-NLuc/CLuc-RBPJ reported cells. Notch activation by WT-JAG1 was similar to the activity of WT-DLL4 at low ligand concentrations (≤75nM), but lower than WT-DLL4 activity at 150nM.

8.7.2 Surface electrostatic potential of Notch ligand C2 domains

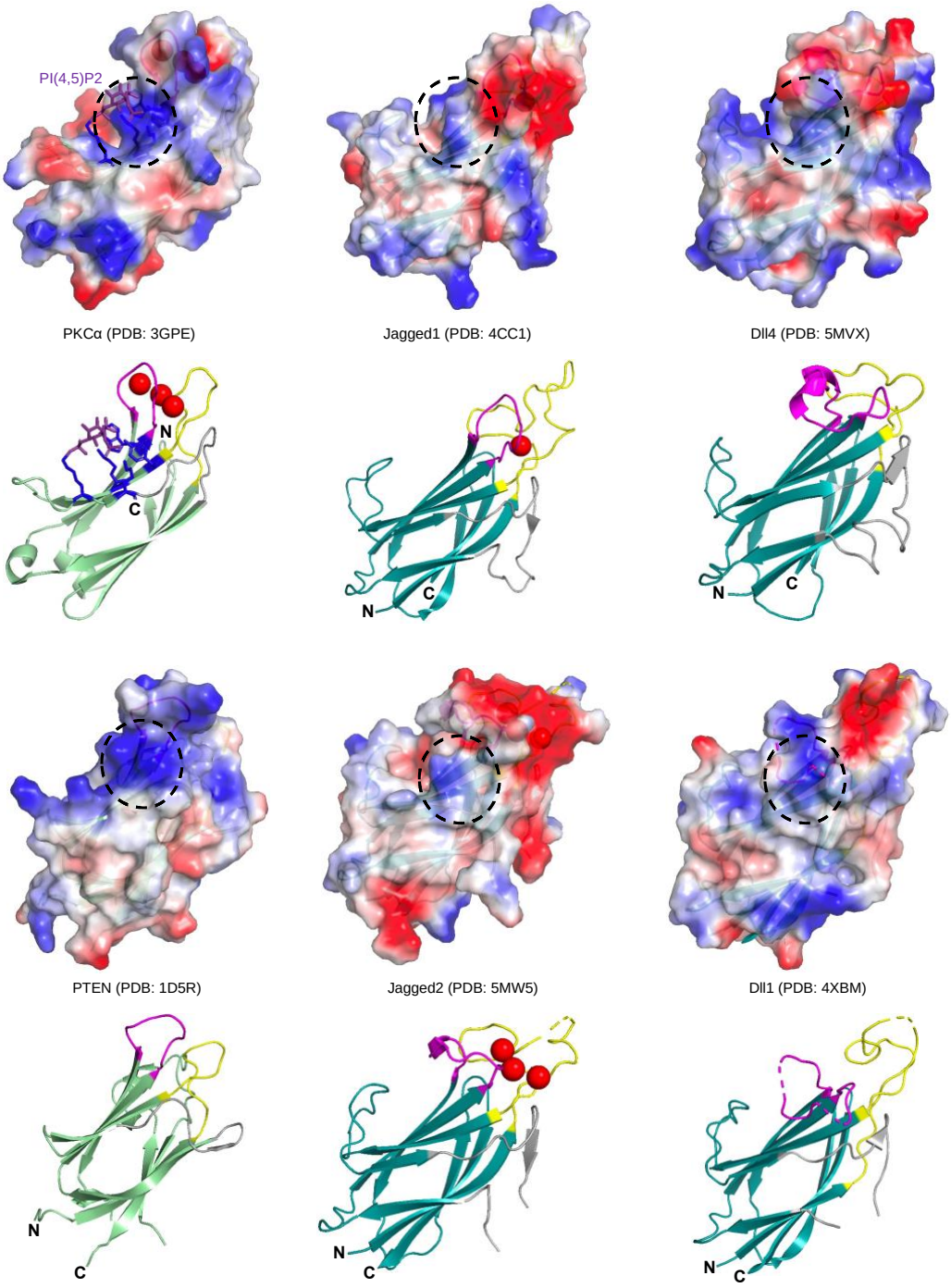


Figure 8-16. Electrostatic properties of C2 domains in Notch ligands and other proteins. Surface electrostatic potential of the C2 domains of Notch ligands, PKCα, and PTEN. (Note that the electrostatic surfaces of Jagged1/2 and PKCα were calculated in the absence of Ca^{2+}). The proposed cationic β-groove made of a cluster of basic residues, which was originally identified in PKCα as a conserved motif to specifically bind phosphatidylinositol (POPI), is highlighted with a black dotted circle (Corbalan-Garcia & Gómez-Fernández, 2014; Guerrero-Valero et al., 2009)

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