

SUPPLEMENTAL MATERIAL

SUPPLEMENTAL TABLE I. Summary of identified *NOTCH1* variants

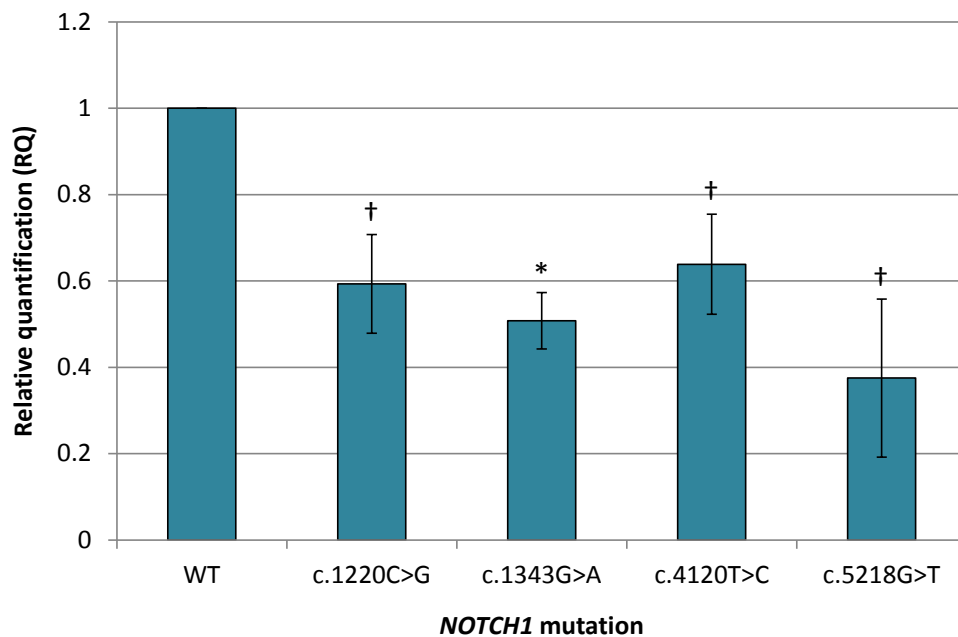
Exon	Coding variant	Protein variant	Variant type	Protein domain
7	c.1220C>G	p.P407R	Missense	EGF 10
8	c.1343G>A	p.R448Q	Missense	EGF 11
8	c.1345T>C	p.C449R	Missense	EGF 11
8	c.1367G>A	p.C456Y	Missense	EGF 11
10	c.1649dupA	p.Y550*	Frameshift	EGF 14
25	c.4120T>C	p.C1374R	Missense	EGF 35
26	c.4663G>T	p.E1555*	Nonsense	LNR 3
26	c.4739dupT	p.M1580lfs*30	Frameshift	-
28	c.5218G>T	p.A1740S	Missense	TM
32	c.6049_6050delTC	p.S2017Tfs*9	Frameshift	ANK 4

Key to abbreviations: EGF, epidermal growth factor-like repeat domain; LNR, Lin-12 NOTCH repeat domain; TM, transmembrane domain; ANK, ankyrin repeat domain.

SUPPLEMENTAL TABLE II. Predicted pathogenicity of identified *NOTCH1* variants

Variant	MutationTaster2	PolyPhen-2	SIFT Protein
p.P407R	Disease causing (prob: 0.999)	Probably damaging (0.968)	Tolerated (0.51)
p.R448Q	Disease causing (prob: 0.999)	Probably damaging (0.984)	Tolerated (0.29)
p.C449R	Disease causing (prob: 0.999)	Probably damaging (1.000)	Damaging (0)
p.C456Y	Disease causing (prob: 0.999)	Probably damaging (1.000)	Damaging (0)
p.Y550*	Disease causing (prob: 1)	n/a	n/a
p.C1374R	Disease causing (prob: 0.999)	Probably damaging (0.999)	Damaging (0)
p.E1555*	Disease causing (prob: 1)	n/a	n/a
p.M1580lfs*30	Disease causing (prob: 1)	n/a	n/a
p.A1740S	Polymorphism (prob: 0.980)	Benign (0.134)	Tolerated (0.42)
p.S2017Tfs*9	Disease causing (prob: 1)	n/a	n/a

SUPPLEMENTAL FIGURE I. Real-time PCR of transient transfections



Real-time quantitative PCR of cells transiently transfected with mutagenized *NOTCH1* cDNA constructs. All missense mutations tested demonstrate reduced *NOTCH1* transcript level by comparison to cells transfected with a full-length wild-type construct (WT). Relative quantification of gene expression is calculated relative to the WT baseline value (set to 1) and normalized to *GAPDH* and *ACTB*. Graphs represent the mean of three independent experiments with error bars indicating SEM. **Key:** * $p < 0.01$; † $p < 0.05$