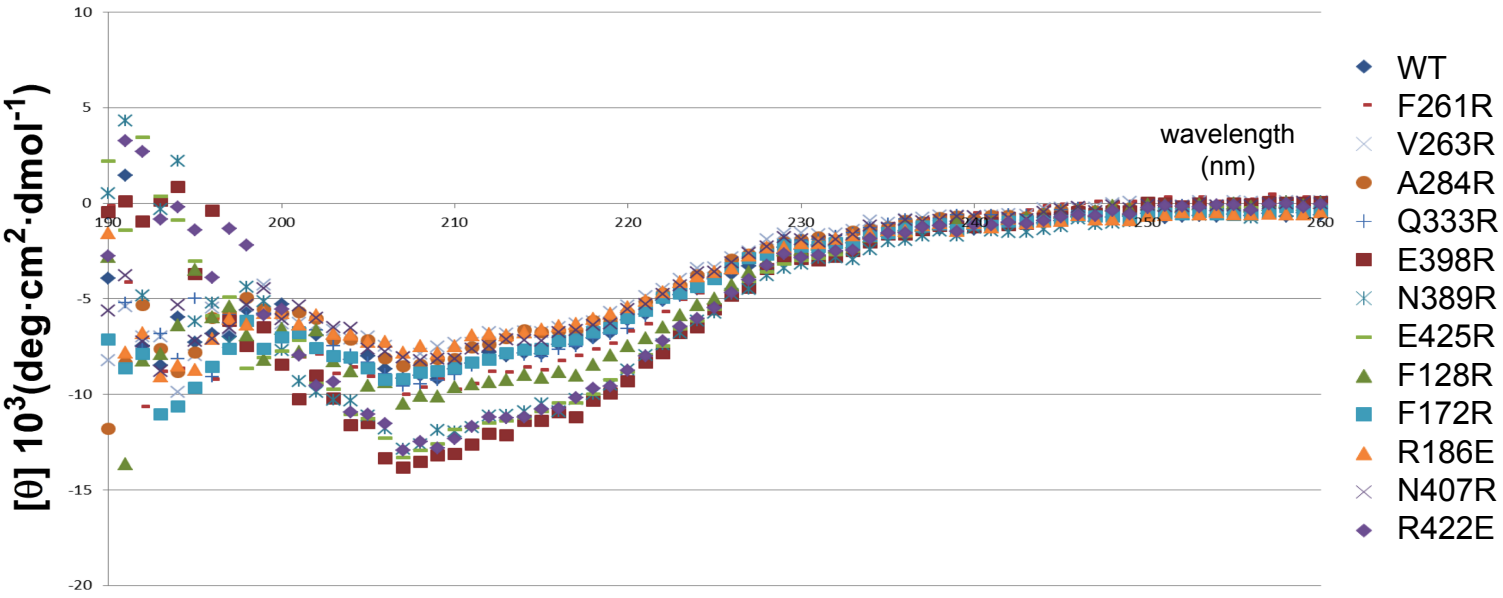

		Helix1	Helix2	Strand1	Strand2	Turns	Unordered	Total	NRMSD	(mg/ml)
	WT	0.00	0.07	0.21	0.12	0.25	0.35	1.00	0.11	1.4
NTD MUTS	F128R	0.00	0.06	0.25	0.13	0.24	0.31	0.99	0.11	1.5
	F172R	0.00	0.07	0.24	0.14	0.24	0.31	1.00	0.06	1.3
	R186E	0.00	0.06	0.25	0.13	0.23	0.32	0.99	0.09	1.2
BTD MUTS	F261R	0.00	0.05	0.26	0.13	0.22	0.33	0.99	0.10	1.5
	V263R	0.00	0.06	0.26	0.13	0.23	0.31	0.99	0.08	1.2
	A284R	0.00	0.07	0.24	0.14	0.24	0.30	0.99	0.08	1.3
	Q333R	0.00	0.06	0.26	0.13	0.22	0.32	0.99	0.09	1.4
CTD MUTS	E398R	0.00	0.05	0.24	0.12	0.23	0.35	0.99	0.06	1.9
	N389R	0.00	0.08	0.20	0.13	0.22	0.36	0.99	0.10	1.8
	E425R	0.00	0.06	0.26	0.12	0.21	0.33	0.98	0.07	1.7
	N407R	0.00	0.06	0.25	0.13	0.23	0.32	0.99	0.08	1.2
	R422E	0.01	0.06	0.23	0.13	0.22	0.32	0.97	0.07	1.7

Figure S1. **CD analysis of CSL mutants**. Wild-type and mutant CSL proteins were purified to homogeneity from bacteria, and analyzed by CD in a buffer containing 50mM sodium phosphate pH 6.5 and 75mM NaCl. *Top*, far UV circular dichroism data for wild-type CSL and 12 mutants, showing overall similar spectra. *Bottom*, CD data were analyzed on Dichroweb with CDSSTR using reference set 4, showing overall similar secondary structure content for wild-type and mutant CSL proteins. NRMSD values and protein concentrations (mg/ml) are also shown.

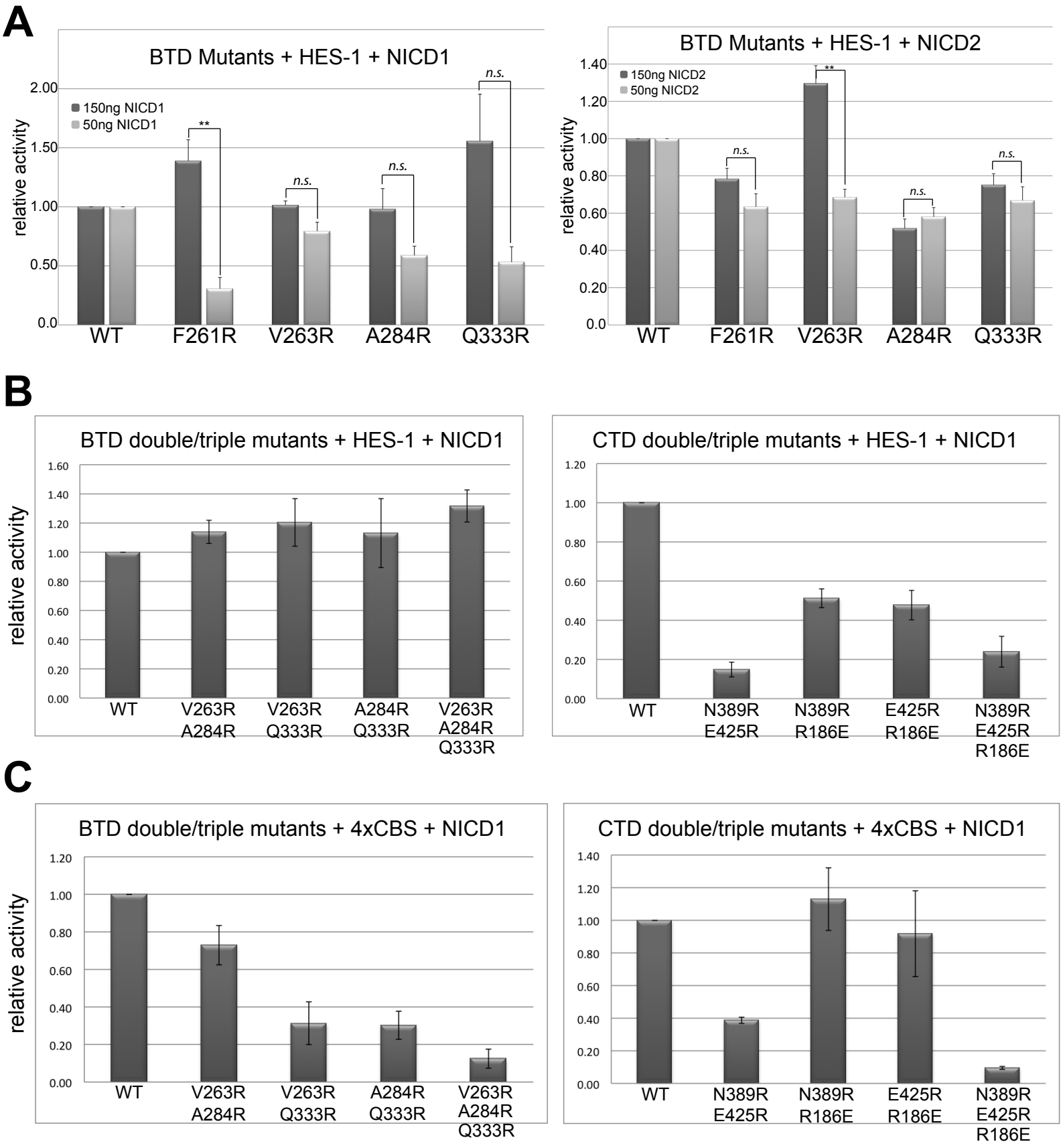

Figure S2. **The effect of NICD expression levels and CSL double/triple mutants on reporter activity.** (A) Figure shows the differences in HES-1 reporter activity for the BTD mutants when either 50ng or 150ng of NICD1/2 are used for transfection. (B,C) Figure shows the cumulative effects of double- and triple-mutations in the BTD and CTD of CSL using the HES-1 (panel B) and 4xCBS (panel C) reporters with NICD1. The data shown are derived from at least three independent experiments performed in duplicate and represent the means ± SEM (standard error of the mean). Statistical significance was assessed using paired student t-tests with *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; and ns, not significant.