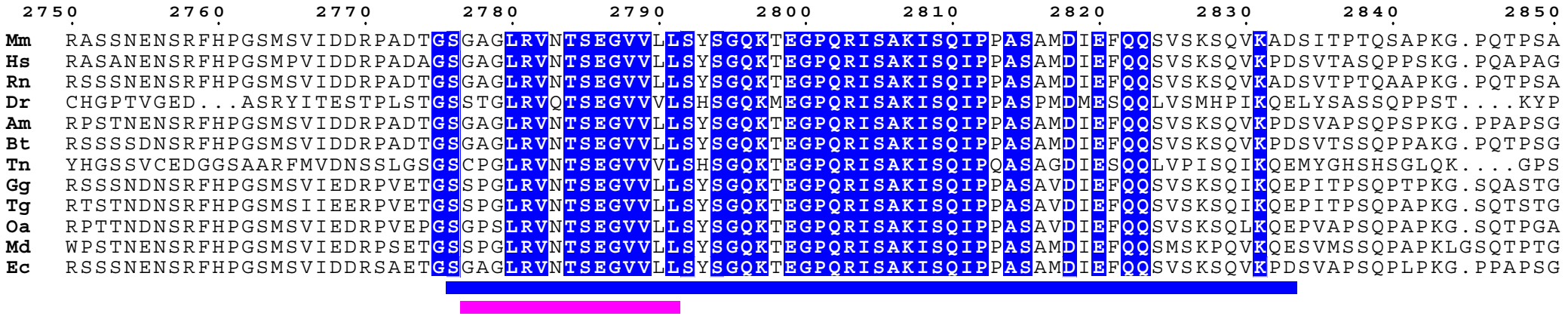

Figure S1. **Sequence alignment of MINT orthologs**. Figure shows sequence alignment for orthologous MINT proteins corresponding to the region that is necessary for interacting with CSL. Numbering corresponds to the *M. musculus* (Mm) ortholog used in this study. Absolutely conserved residues between all orthologs are colored blue. The blue and magenta bars beneath the sequence correspond to construct (2776-2833) used in this study and the region identifed by Oswald et al. (2002) to be necessary for interacting with CSL, respectively.

Fig. S1

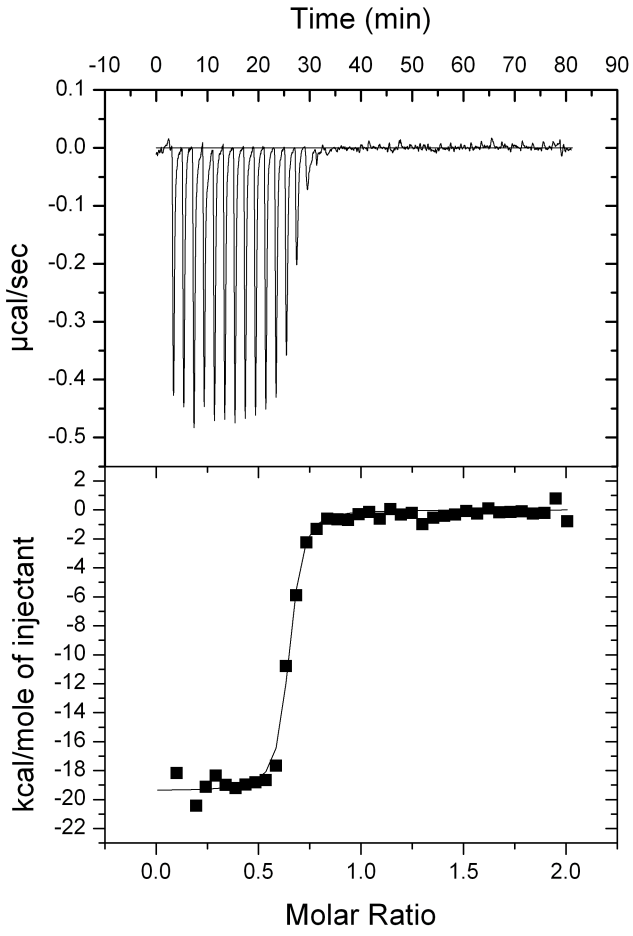

Figure S2. **CSL-MINT control ITC binding experiment**. Figure shows representative thermogram (raw heat signal and nonlinear least squares fit to the integrated data) of the complex formed by CSL and MINT (2776-2833), with CSL in the syringe and MINT in the cell.

Fig. S2

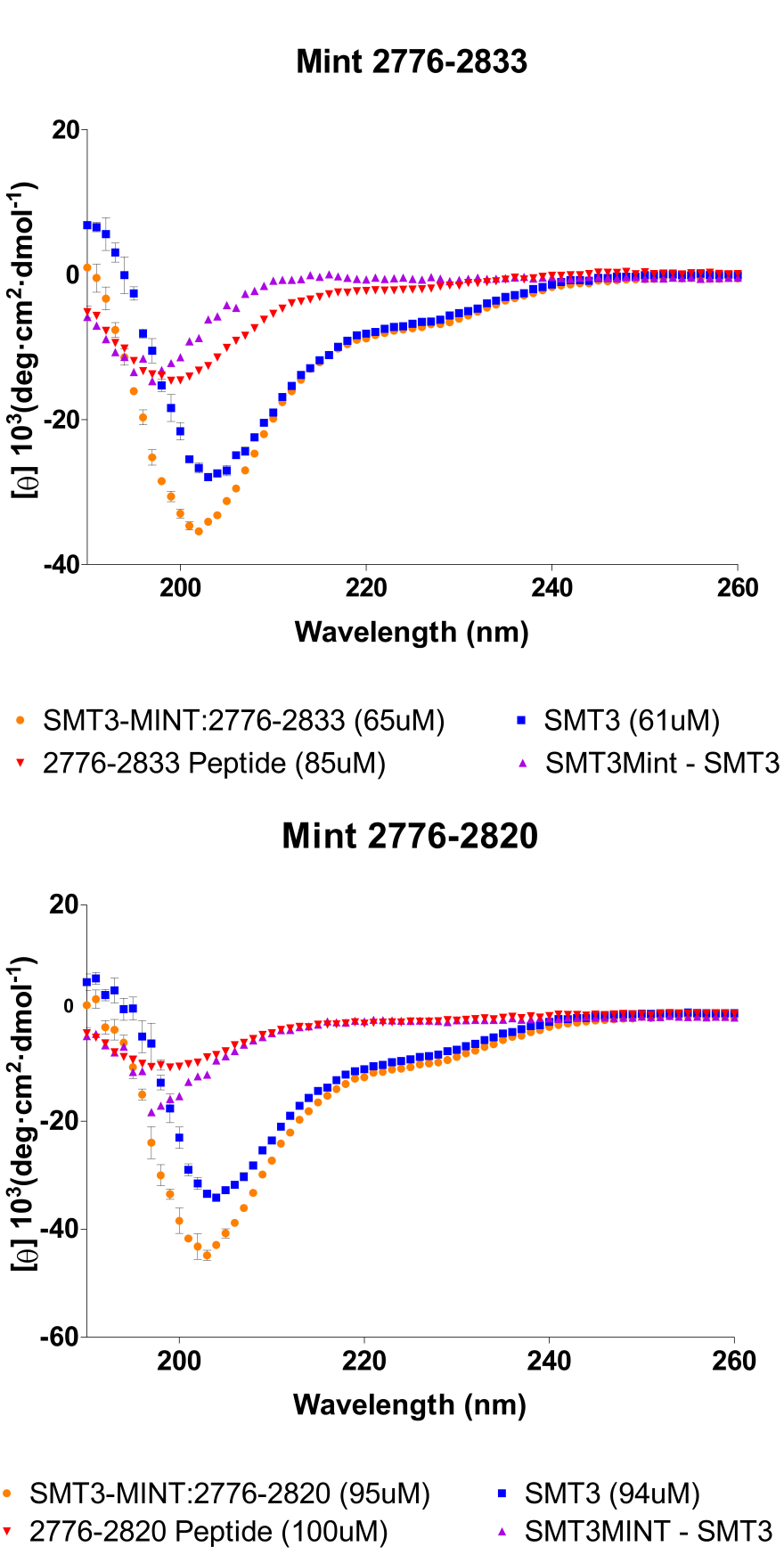

Figure S3. **Comparison of CD for MINT peptides and corresponding SMT3-MINT fusion proteins**. Experiments were performed as described in the Experimental Procedures. CD data are shown for MINT constructs (2776-2833), top, and (2776-2820), bottom. Construct concentrations for CD analysis are denoted. MINT and SMT3-MINT fusions proteins were analyzed in a buffer containing 50mM NaPi pH 6.5 and 150mM NaF. [SMT3MINT - SMT3] represent the calculated spectra from subtracting the collect SMT3 spectra from the correpsonding SMT3-MINT fusion protein spectra.

Fig. S3

	MINT		K (M^{-1})			Kd (μM)			ΔG (kcal/mol)			ΔH (kcal/mol)			TΔS (kcal/mol)		
CSL	2752 - 2833		1.87E+08	±	1.01E+07	0.005	±	0.00028	-11.3	±	0.032	-14.8	±	0.992	-3.52	±	1.007
	smt 2776 - 2833		1.21E+08	±	2.00E+06	0.008	±	0.0013	-11.0	±	0.010	-18.0	±	0.626	-7.0	±	0.626
	2776 - 2833		9.39E+07	±	1.19E+07	0.011	±	0.0014	-10.9	±	0.078	-14.6	±	0.154	-3.7	±	0.164
	smt 2776 - 2820		3.77E+08	±	1.09E+08	0.003	±	0.0007	-11.7	±	0.165	-19.3	±	0.545	-7.6	±	0.698
	2776 - 2820		5.80E+07	±	1.17E+07	0.018	±	0.0038	-10.6	±	0.124	-10.0	±	1.241	0.6	±	1.280
	smt 2801 - 2833		5.49E+05	±	7.53E+04	1.845	±	0.2698	-7.8	±	0.084	-7.4	±	1.209	0.4	±	1.169
	2801 - 2833		1.13E+05	±	1.41E+03	8.850	±	0.1107	-6.9	±	0.007	-11.3	±	0.120	-4.4	±	0.148
	smt 2776 - 2800		2.45E+05	±	1.87E+04	4.104	±	0.3294	-7.3	±	0.047	-4.9	±	0.607	2.4	±	0.591
	smt 2776 - 2812		1.39E+08	±	4.92E+07	0.008	±	0.0023	-11.1	±	0.196	-14.0	±	1.051	-2.9	±	1.246
	smt 2791 - 2812		9.43E+04	±	1.28E+04	10.746	±	1.5570	-6.8	±	0.084	-10.6	±	1.260	-3.8	±	1.346
	smt 2791 - 2820		8.21E+04	±	1.83E+04	12.654	±	3.1515	-6.7	±	0.141	-12.8	±	1.948	-6.1	±	2.075
	smt 2801 - 2820		1.71E+05	±	7.35E+04	6.504	±	2.3590	-7.1	±	0.240	-19.0	±	2.170	-11.9	±	2.232
BTD	2776 - 2833		2.46E+04	±	3.02E+03	41.044	±	4.8050	-6.0	±	0.071	-14.9	±	3.573	-8.9	±	3.529
	2801 - 2833		9.11E+04	±	3.16E+04	11.804	±	3.5800	-6.7	±	0.196	-8.2	±	1.606	-1.4	±	1.797
	smt 2776 - 2812		NBD			NBD			NBD			NBD			NBD		
	smt 2791 - 2812		NBD			NBD			NBD			NBD			NBD		
	smt 2791 - 2820	**	5.25E+04	±	3.05E+04	23.338	±	11.4040	-6.2	±	0.445	-12.3	±	4.685	-6.1	±	4.629
	smt 2801 - 2820		7.35E+04	±	2.38E+04	14.458	±	3.9470	-6.6	±	0.180	-7.6	±	1.450	-1.0	±	1.612
	smt 2776 - 2800		NBD			NBD			NBD			NBD			NBD		
CTD	2801 - 2833		NBD			NBD			NBD			NBD			NBD		
	2776 - 2833		1.68E+04	±	2.21E+03	60.330	±	7.8390	-5.8	±	0.078	-7.7	±	1.864	-2.0	±	1.946
	smt 2776 - 2800		8.82E+03	±	2.50E+03	120.942	±	40.3150	-5.4	±	0.186	-12.7	±	3.187	-7.4	±	3.368
BTD-CTD	2776 - 2833		1.04E+06	±	1.18E+05	0.969	±	0.1130	-8.2	±	0.069	-14.2	±	1.635	-6.0	±	1.672

Supplemental Table I.

All experiments were performed at 25°C, except for the BTD/SMT(2791-2820) complex, which was performed at 15°C (**). Values are the mean of at least three independent experiments and errors represent the standard deviations of multiple experiments. NBD = no binding detected.