

**Supporting Information**

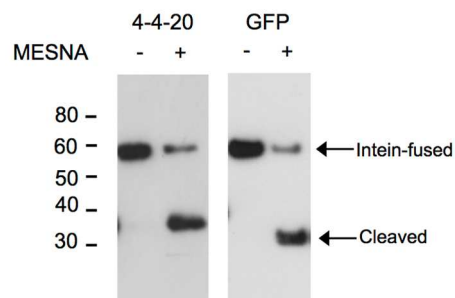
**An Evolved Mxe GyrA Intein for Enhanced Production of Fusion  
Proteins**

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**Supplemental Table 1:** Intein mutations and surface display levels for directed evolution round 2.

Amino Acid	21	33	50	51	74	105	107	110	112	114	117	118	124	129	144	158	160	164	168	190	191	Fold Increase <sup>a</sup>	Statistical Significance <sup>b</sup>
WT	I	I	L	F	N	I	R	F	V	C	F	A	F	Y	H	D	R	A	S	I	T	1.0±.1	
F1-01									A	R	T			C	R							1.1±.1	NS
F1-12		T			D					R							Q					1.8±.1	**
F1-16				S					A				L	C							M	1.8±.3	**
F2-02									A	R	T				R				G			1.4±.2	NS
F2-05	T			S						G	L						Q					1.8±.2	**
F2-08				S			S	A	G	T							Q		G			1.4±.1	*
F2-18		T	P	L					H	L	T						Q					1.5±.2	*
F5-03				L	D		S		R	L		L										1.4±.1	*
F5-06							C	S	G	T		C	R	G	Q							1.4±.3	NS

- Fold increase relative to the wild-type intein as fusions to 4-4-20, mean ± S.D from three independent yeast colonies.
- Statistical analysis was performed by an unpaired student's t-test, with double asterisks representing  $p < 0.01$ , single asterisks representing  $p < 0.05$ , and NS designating that differences are non-significant ( $p > 0.05$ ).



**Supplemental Figure 1:** MESNA release of 4-4-20 and GFP with the wild-type intein.

The catalytic activity of the wild-type intein was examined by reacting secreted 4-4-20 and GFP with MESNA for 20 h. Anti-FLAG Western blotting demonstrates ~75% release for both 4-4-20 and GFP in the presence of MESNA.