SUPPLEMENTAL MATERIAL

Supplemental Methods

Thermo Scientific™ Pierce™ Bicinchoninic Acid (BCA) Protein Assay. The protein concentrations of MBP-PI2 and MBP, purified from amylose affinity chromatography, were estimated using the Pierce™ BCA Protein Assay kit (Thermo Scientific) according to manufacturer's instructions. BSA standards and purified proteins were plated in duplicate in a 96-well plate and the absorbance was measured using the BioTek Epoch 2 Microplate Spectrophotometer at A_{562nm} to produce a BSA standard curve (Fig. S2). Protein concentrations were estimated using protein absorbance values and the equation generated from the best fit line of the BSA standard curve. The estimated concentrations were then used to determine the parameters of downstream experiments.

Trypsin activity optimization assay. 1 mg/mL stock solutions of trypsin (MP Biomedicals, Cat no. 9002-07-7), trypsin inhibitor (Sigma-Aldrich, Cat no. 9035-81-8), and BSA were prepared in elution buffer. All total reaction volumes were 20 µL. Reactions requiring BSA were calculated to contain 0.13 mg/mL BSA. Trypsin, trypsin inhibitor, and elution buffer were added to the appropriate reaction vessels in the indicated ratios with respect to BSA. Trypsin and trypsin inhibitor control reactions were incubated for 1 hour at room temperature to allow for proper inhibition, while all other reaction vessels were kept on ice. Following incubation, BSA was added to appropriate reaction vessels and incubated for another 1.5 hour. 10 µL of each reaction was added to 10 µL Bio-Rad 2X Laemmli sample buffer with 5% BME, then heated at 95°C for 5 minutes. 15 µL of each prepared sample was analyzed by SDS-PAGE (Fig. S3) according to the SDS-PAGE protocol described in the main text.

Supplemental Tables

TABLE S1. BLAST nucleotide sequence alignment results

Query	Subject	Max Score	Total Score	Query Cover	E value	Percent Identity	Accession Length
pMAL-c2X-PI2	pMAL-c2X-LLMZ161	983	983	53%	0.0	99.45%	578
pMAL-c2X	pMAL-c2X-PI2	928	928	50%	0.0	99.02%	1004
pMAL-c2X-PI2	pMAL-c2X (original empty vector sequence)	928	928	50%	0.0	99.02%	6645
pMAL-c2X	pMAL-c2X (original empty vector sequence)	1788	1788	96%	0.0	99.78%	6645

TABLE S2A. NanoDrop™ 2000c Spectrophotometer readings

Sample	MW (kDa)	ε ($M^{-1}cm^{-1}$)	A260/280	A280	Conc. (mg/ml)	Vol. (mL)
MBP rep 1	-	-	0.63	0.381	0.381	1.0
MBP rep 2	-	-	0.55	0.348	0.348	1.0
MBP rep 3	-	-	0.57	0.361	0.361	1.0
MBP-PI2 rep 1	-	-	0.63	0.355	0.355	0.3
MBP-PI2 rep 2	-	-	0.64	0.281	0.281	0.3
MBP-PI2 rep 3	-	-	0.67	0.291	0.291	0.3
MBP-PI2 rep 1	66.5	82250	0.63	0.355	0.287*	0.3
MBP-PI2 rep 2	66.5	82250	0.64	0.281	0.227*	0.3
MBP-PI2 rep 3	66.5	82250	0.67	0.291	0.235*	0.3

TABLE S2B. Averaged NanoDrop™ 2000c readings and protein yield

Sample	Average A280	Average conc. (mg/ml)	Vol. (mL)	Average yield (mg)
MBP-β-gal-α	0.363	0.363	1.0	**0.363**
MBP-PI2	0.309	*0.250	0.3	**0.075**

**Manually calculated from previous readings using MW and ε*

TABLE S3. ExPASy PeptideCutter predicts factor Xa and trypsin cleavage sites in MBP-β-gal-α and MBP-PI2.

Open reading frame	Protease	Cleavage site sequence	No. predicted cleavage sites	Predicted mass of cleavage product (kDa)
MBP-β-gal-α	Factor Xa	Ile-Glu-Gly-Arg	1	25.9
MBP-PI2	Factor Xa	Ile-Glu-Gly-Arg	1	17.1
MBP-β-gal-α	Trypsin	C'-Lys or C'-Arg without N'-Pro	20	multiple
MBP-PI2	Trypsin	C'-Lys or C'-Arg without N'-Pro	27	multiple
MBP-β-gal-α	Thrombin	Leu-Val-Pro-Arg-Gly-Ser	0	N/A
MBP-PI2	Thrombin	Leu-Val-Pro-Arg-Gly-Ser	0	N/A
MBP-β-gal-α	TEV protease	Glu-Asn-Leu-Tyr-Phe-Gln-Gly/Ser	0	N/A
MBP-PI2	TEV protease	Glu-Asn-Leu-Tyr-Phe-Gln-Gly/Ser	0	N/A

Supplemental Figures

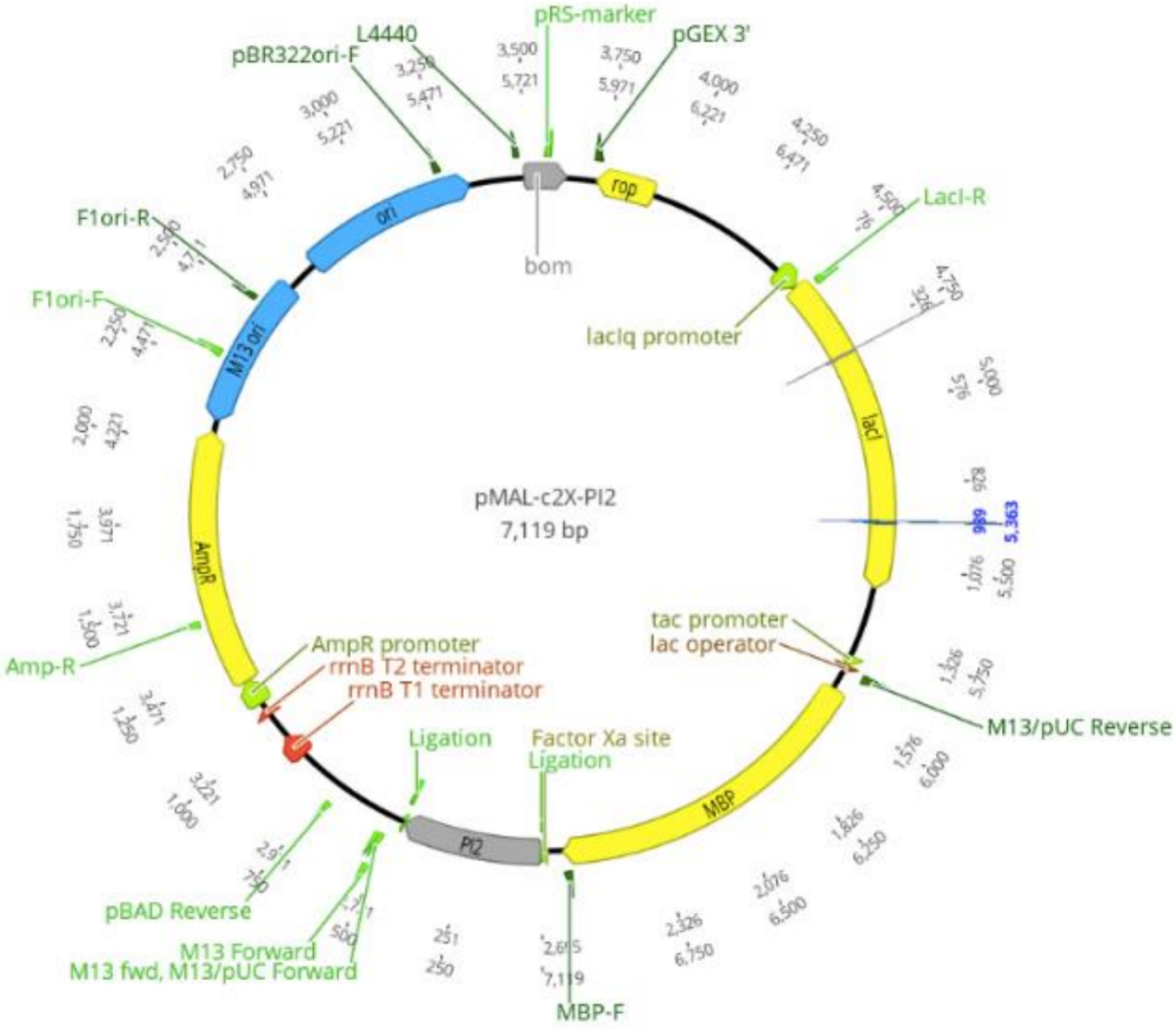
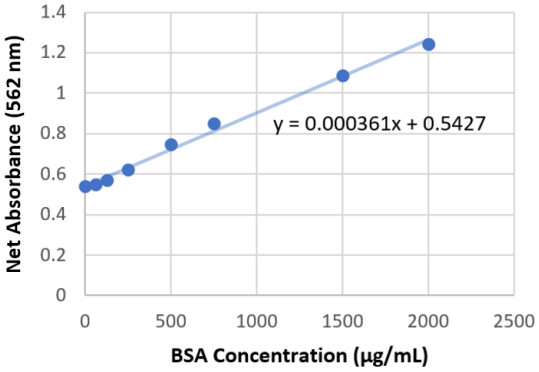


FIG. S1 Vector map of pMAL-c2x-PI2. Downstream of the *lac* operon promoter is the MBP tag coloured in yellow, *pi2* coloured in grey, and the factor Xa cleavage site is seen as the upstream ligation site relative to *pi2*. *Adapted from Grewal et al. 2020* (4).

A

BSA Concentration (µg/mL)	Absorbance at 562nm	
	Replicate 1	Replicate 2
2000	1.249	1.239
1500	1.076	1.099
750	0.847	0.853
500	0.746	0.748
250	0.629	0.616
125	0.578	0.565
62.5	0.545	0.555
0	0.549	0.531

B



C

Purified Protein	Absorbance at 562nm	
	Replicate 1	Replicate 2
MBP-PI2	0.796	0.833
MBP-β-gal-α	0.643	0.624

D

Purified Protein	Concentration (µg/mL)	Vol. (mL)	Yield (mg)
MBP-PI2	752.91	0.3	0.25152
MBP-β-gal-α	251.52	1.0	0.225873

FIG. S2 BCA assay to determine the concentration of purified MBP-PI2 and MBP proteins. A) The absorbance values of BSA standards at 562 nm. B) BSA standard curve. Net absorbance is the average value of two replicate absorbance readings at 562 nm. The equation of the best fit line (solid blue line) was generated from a linear regression. C) The absorbance values of purified MBP-PI2 and MBP proteins. D) The concentration and yield of MBP-PI2 and MBP obtained from the BSA standard curve.

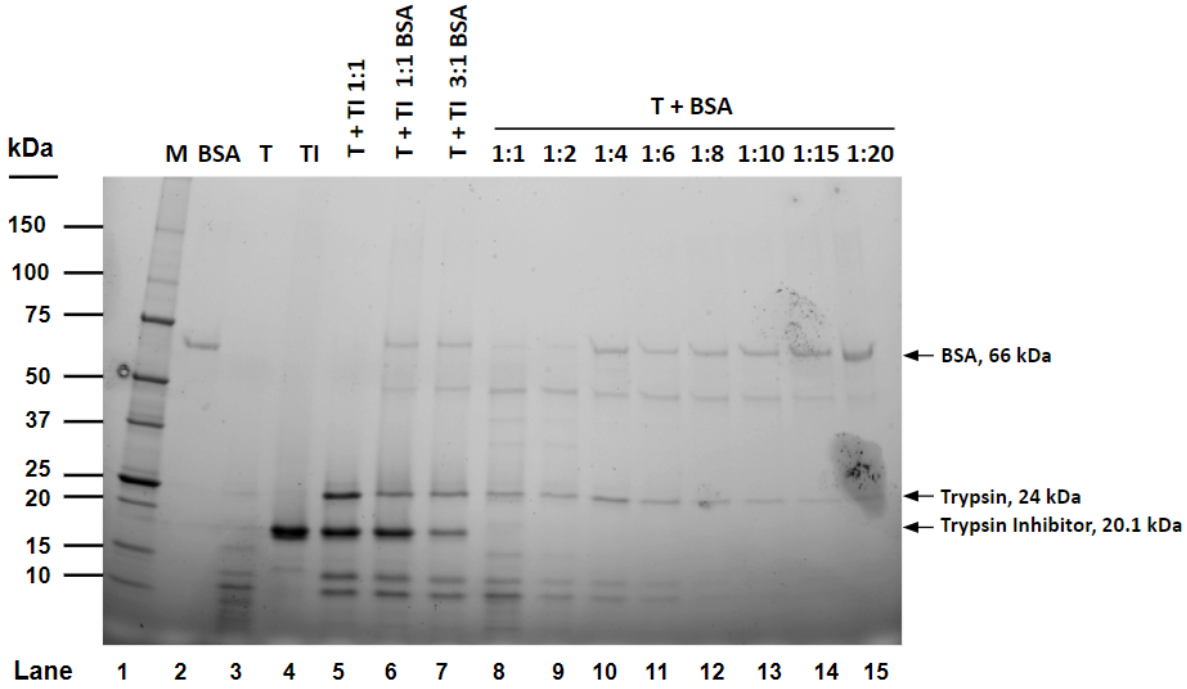


FIG. S3 Trypsin Optimization Assay. Coomassie blue-stained SDS-PAGE showing optimization assay for trypsin (T) and trypsin inhibitor (TI) using BSA. Lane 1: Protein ladder. Lane 2: BSA. Lane 3: T. Lane 4: TI. Lane 5: T and TI at 1:1 ratio. Lane 6: T and TI at 1:1 ratio with BSA. Lane 7: T and TI at 3:1 ratio with BSA. Lanes 8-15: T and BSA at several ratios. Arrows indicate BSA (66 kDa), trypsin (24 kDa) and trypsin inhibitor (20.1 kDa).

>pMAL_c2X_PI2 insert nucleotide sequence

NNNNNNNNNNNNNNNNNNTAGANGANNGTTNNTCGCCGGGTACATGTTCGCTTTCGGTT
CGTCAGATTCACCTTCGCAAACGAATTTACCGTTTTTACCGAAGTAGTAGCAACCTTTGTA
ACCGGTGCAGCAGGTGGTGCAACCGGTCGGGTAGATCAGAGATTTACCTTCAGAACGC
GGGCATTTAGAGTACGCGATGTGCGGGTCGCAGTTCAGCGGGCACGCTTTCGGTTTTTT
CGGGTCAGACTGACCTTCGCAGATGAACGCACCGTTCGCAGAGTAGTAGTTGCAACCTT
TGTAACCCGCGCAGCAGTTGGTGCAGATACGGTTTTCCGGAGAACCTTCAGATCTCGGG
CAGATACCGAAACCCAGGTTACCGCATTCCAGGGTGCACGCTTTCGCGTCAACGTGTTC
CATCGCAGAAACCAGAACCAGCAGACCCAGAACGATCAGCAGGTACGCAACGAAGTTAA
CTTCTTTGTGAACGTCCATATCTGAAATCCTTCCCTCGATCCCGAGGTTGTTGTTATTGTT
ATTGTTGTTGTTGTTCGAGCTCGAATTAGTCTGCGCGTCTTTCAGGGCTTCATCGACAGT
CTGACGACCGCTGGCGGCGTTGATCACCGCAGTACGCACGGCATACCAGAAAGCGGAC
ATCTGCGGGATGTTCGGCATGATTTCACCTTTCTGGGCGTTTTCCATAGTGGCGGCAATA
CGTGGATCTTTCGCCAACTCTTCCTCGTAAGACTTCAGCGCTNCNNNNCCCAGCGGTTT
GTCTTTATTAACCGCTTCCAGACCTTCATCAGTCAGCAGATAGTTTTCGAGGAACTCTTTT
GCCAGCTCTTTGTTCGGACTGGCGGCGTTAATACCTGCGCTCAGCACGCCAACGAACGG
TTTGGATGGTTGACCCTTGAAGGTCGGCAGTACCGTTACACCATAATTCACTTTGCTGGT
GTCGATGTTGGACCATGCCCACGGGCCGTTGATGGTCATCGCTGTTTCGCCTTTNNN

>pMAL-c2X-PI2 amino acid sequence (3'5' Frame 1)

XKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEF
LENYLLTDEGLEAVNKDKPLXXXALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYA
VRTAVINAAS**GR**QTVDEALKDAQTNSSSNNNNNNNNNLG**IEGR**ISDMDVHKEVNFVAYLLI
VLGLLVLVSAMEHVDAKACTLECGNLGFGICPRSEGSPENRICTNCCAGYKGCNYYSANGAF
ICEGQSDPKKPKACPLNCDPHIAYSKCPRSEGKSLIYPTGCTTCCTGYKGCYYFGKNGKFVC
EGESDEPKANMYPAXNXXLXXXXXX

FIG. S4. pMAL-c2X-PI2 DNA and corresponding amino acid sequence. The Sanger sequencing results of the pMAL-c2X-PI2 plasmid followed by the ExPASy generated amino acid sequence with the primary (IEGR) and secondary (GR) factor Xa cut sites highlighted.