Batch ID	Plate Location	Construct ID	Cloned AA Seq	Vector	MW (kDa)	pI
TOC011	A01	HMGB1_N-GST-His-thrombin	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLP YYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMD PMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDG SSMGSSHHHHHHSSGLVPRGSGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSK KCSERWKTMSAKEKGKFEDMAKADKARYEREMKTYIPPKGETKKKFKDPNAPKR PPSAFFLFCSEYRPKIKGEHPGLSIGDVAKKLGEMWNNTAADDKQPYEKKAAKLK EKYEKDIA	pET28-GST-LIC	45.3/ 17.5	8.94/ 9.56
TOC011	A02	HMGB1_N-His-TEV	MHHHHHHSSGRENLYFQGGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKC SERWKTMSAKEKGKFEDMAKADKARYEREMKTYIPPKGETKKKFKDPNAPKRPP SAFFLFCSEYRPKIKGEHPGLSIGDVAKKLGEMWNNTAADDKQPYEKKAAKLKEK YEKDIA	pET28-MHL	17.4/ 19.6	9.56/ 9.55
TOC011	A03	HMGB1_N-His-Sumo-TEV	mkkhhhhhhhssglvprgsmsdsevnqeakpevkpevkpethinlkvsdgsseiffkikkttplr rlmeafakrqgkemdslrflydgiriqadqtpedldmednd iieahreqiggtenlyfqgGKMSS YAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEKGKFEDMAKADKA RYEREMKTYIPPKGETKKKFKDPNAPKRPPSAFFLFCSEYRPKIKGEHPGLSIGDVA KKLGEMWNNTAADDKQPYEKKAAKLKEKYEKDIA	pET28-MKH8SUMO	31.9/ 17.4	9.16/ 9.56
TOC011	A04	HMGB1_FL_N-GST-His-thrombin	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLP YYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMD PMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDG SSMGSSHHHHHHSSGLVPRGSMGKGDPKKPRGKMSSYAFFVQTCREEHKKKHP DASVNFSEFSKKCSERWKTMSAKEKGKFEDMAKADKARYEREMKTYIPPKGETK KKFKDPNAPKRPPSAFFLFCSEYRPKIKGEHPGLSIGDVAKKLGEMWNNTAADDK QPYEKKAAKLKEKYEKDIAAYRAKGKPDAAKKGVVKAEKSKKKKEEEEDEEDEEDE EEEEDEEDEDEEEDDDDE	pET28-GST-LIC	52.8/ 25.0	6.08/ 5.6
TOC011	A05	HMGB1_FL_N-His-TEV	MHHHHHHSSGRENLYFQGMGKGDPKKPRGKMSSYAFFVQTCREEHKKKHPDA SVNFSEFSKKCSERWKTMSAKEKGKFEDMAKADKARYEREMKTYIPPKGETKKK FKDPNAPKRPPSAFFLFCSEYRPKIKGEHPGLSIGDVAKKLGEMWNNTAADDKQ PYEKKAAKLKEKYEKDIAAYRAKGKPDAAKKGVVKAEKSKKKKEEEEDEEDEEDEE EEEDEEDEDEEEDDDDE	pET28-MHL	27.1/ 25.0	6.07/ 5.60
TOC011	A06	HMGB1_FL_N-His-Sumo-TEV	mkkhhhhhhhssglvprgsmsdsevnqeakpevkpevkpethinlkvsdgsseiffkikkttplr rlmeafakrqgkemdslrflydgiriqadqtpedldmednd iieahreqiggtenlyfqgMGKGD PKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEKGK FEDMAKADKARYEREMKTYIPPKGETKKKFKDPNAPKRPPSAFFLFCSEYRPKIKG EHPGLSIGDVAKKLGEMWNNTAADDKQPYEKKAAKLKEKYEKDIAAYRAKGKPD AAKKGVVKAEKSKKKKEEEEDEEDEEDEEEEEDEEDEDEEEDDDDE	pET28-MKH8SUMO	39.4/ 25.0	5.91/ 5.60

Features of constructs are colour coded as GST-tag, His-tag, Sumo-tag, TEV cleavage site, thrombin cleavage site and HMGB1.

Constructs A01, A02 and A03 span a C-terminally truncated version of HMGB1: aa. 11-160
Constructs A04, A05 and A06 spans the full-length HMGB1: aa. 1-215

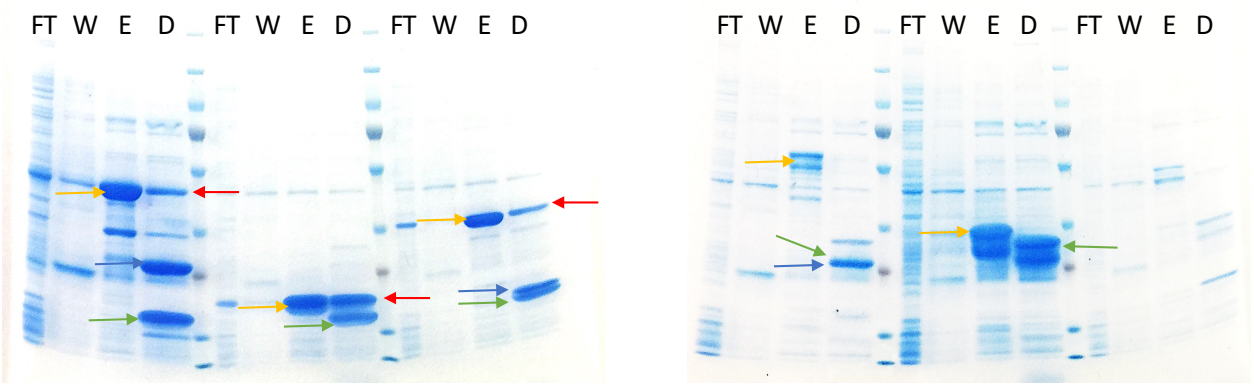
Protein Expression:

Each construct transformed into *E. coli* BL21 codon plus RIL. Resulting starter culture used to inoculate 2 L LB culture. Cultures grown at 37 ˚C until OD_{600} ~ 0.6 and inoculated with 0.2 mM IPTG before growing overnight at 18 ˚C. Cells were then harvested by centrifugation.

Protein Purification:

HMGB1 cell pellets resuspended in ~ 200 mL 20 mM Hepes pH 7.4, 150 mM NaCl supplemented with benzonase and 1 x protease inhibitor mix for each construct. Resuspensions lysed by

sonication and then clarified by centrifugation before rocking with ~ 5 mL Ni-NTA resin per construct at 4 ˚C for 1 hour (FT). Beads were washed with 200 mL resuspension buffer supplemented with 15 mM imidazole (W). Protein eluted with resuspension buffer supplemented with 300 mM imidazole (E). Eluted protein digested with TEV (A02/3/5/6) or thrombin (A01/4) overnight at 4 ˚C with dialysis with snakeskin MWCO 3000 against resuspension buffer (D).



Eluted protein

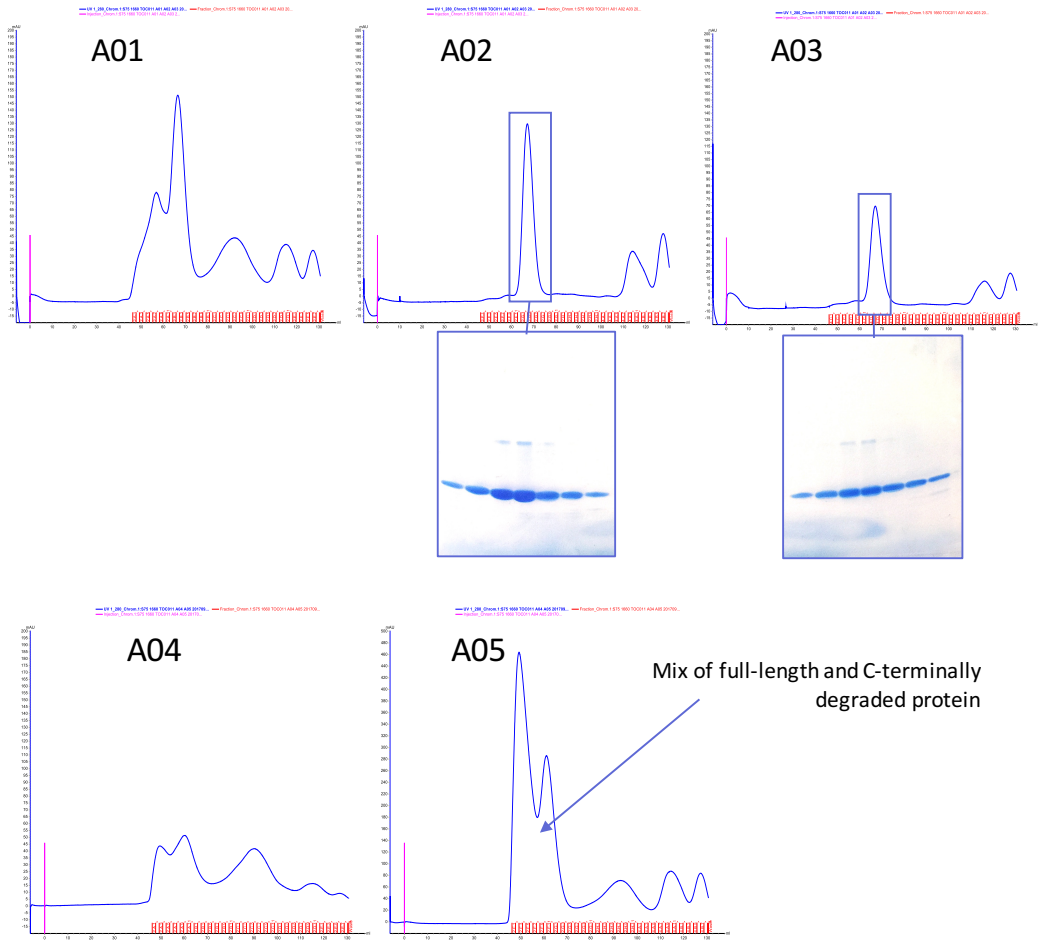
Cleaved protein

Cleaved tag

Uncleaved protein

A01: high expression, incomplete cleavage
A02: high expression, ~ 50 % protein not cleaved by TEV
A03: high expression, incomplete cleavage, tag and protein ~ same size
A04: low expression, incomplete cleavage, tag and protein ~ same size
A05: high expression, complete cleavage, 2 species present - degradation?
A06: no expression

Protein dialysed (fresh buffer) and digested for further 6 hours before samples rocked with ~ 2 mL Ni-NTA resin per construct at 4 ˚C for 1 hour. Cleaved protein flow through then concentrated to ~ 5 mL and run on S75 16/60 column in 20 mM Hepes pH 7.4, 150 mM NaCl at 1 ml/min.



Conclusions:

Full-length (1-215) HMGB1 has lower expression and is more unstable the C-terminally truncated constructs (11-160).

Final yield of A02 – 5.8 mg/mL 50 µL x 12, A03 – 3.5 mg/mL 50 µL x 10. Protein flash frozen in liquid nitrogen and stored at – 80 ˚C.