Pumilio protects Xbp1 mRNA from regulated Ire1-dependent decay

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Supplementary Figure 1 - The 3'UTRs of Xbp1^{unspliced} and Xbp1^{spliced} differ in their stability effect
a) The stability of the GFP reporters fused with the 3'UTRs of Xbp1^{spliced} or Xbp1^{unspliced} was assessed by RT-PCR, using primers specific for *gfp* and *rp49* mRNAs (control). The levels of mRNA reporter were normalized to those of *rp49* mRNA, and averages and standard deviations from three independent experiments are plotted. The 3'UTR of Xbp1^{unspliced} shows a higher stability effect of the reporter half-life (2 fold stabilization) relative to the spliced form. Data are presented as mean ± SD. Spliced (green, n=2); Unspliced (blue, n=2). n = 2 biological independent experiments.
b) Immunoblot from adult *Drosophila* eyes expressing TAP-PumHD (sGMR-GAL4; UAS-TAP-PumHD) and the 2 parental control lines. Both input and pull-down samples were run and probed with anti-TAP and anti-Tubulin (loading control) antibodies. n = 2 independent experiments.

c) RT-PCR results from S2 cells treated with PumRNAi or LacZ RNAi (NTC - non-target control) to assay for the efficiency and specificity of PumRNAi treatments, using primers specific for *pumilio (pum)* or the loading control *rp49*. RNA samples were treated (+RT) or not (-RT) with reverse transcriptase.

M – molecular weight markers. $n \ge 3$ of independent experiments.



Supplementary Figure 2 - hIRE1a KR domain phosphorylates hPUM1 in vitro

a) Schematic representation of domains and putative phosphorylation sites present in Human PUM1. **b**) *in vitro* radioactive kinase assay with γ -[³²P]ATP of purified hPUM1 forms incubated with hIRE1 α KR domain. The specificity of phosphorylation was monitored by treatment with λ PP or incubation with specific inhibitors of IRE1 kinase activity (Apy29 and compound#18). Arrows express hPUM1 phosphorylated forms corresponding to the expected MW of each purified protein. Treatment with kinase inhibitors decreased the amount of detected radioactive protein bands. n≥2 of independent experiments. Source data file is provided.



Supplementary Figure 3 - Validation of in vitro phosphorylation hPUM1-C and dPUM-D3 a) In vitro phosphorylation hPUM1-C domain and dPUM-D3 domain by hIRE1a KR using the control kinase-dead Ire1 (hIRE1KD, D688N). Assays were conducted as described before. Arrows denote phosphorylated forms of hPUM-C (Blue arrow) and dPUM-D3 purified proteins (red arrow). Auto-phosphorylation of hIRE1a KR is denoted by a black arrow. n=3 of independent experiments.

b) Phostag immunoblot of phosphorylation of hPUM-C with an antibody specific for phosphorylated IRE1(anti-rabbit Phospho-IRE1). The specificity of phosphorylation was monitored by treatment with λPP or incubation with specific inhibitors of IRE1a kinase activity (Apy29 and compound #18) and a reaction lacking ATP (first lane). The phosphorylation state of hIRE1a KR is decreased in control reactions as expected compared to the reactions containing hIRE1a+ATP or hIRE1a+hPUM-C+ATP. An additional control was made by conducting a kinase assay of hPUM-C without incubation with the hIRE1 α KR domain. M – Protein molecular weight marker lane. n \ge 2 of independent experiments. Source data file is provided.



Supplementary Figure 4 – PUM1 protects human XBP1 from regulated Ire1-dependent decay

a) In vitro transcripts of human XBP1 were incubated with non-phosphorylated (0P) and phosphorylated (3P) forms of purified hIRE1 α KR. Human PUM1 FL protects XBP1 RNA from Ire1 dependent non-canonical decay, but does not impair IRE1 dependent XBP1 splicing. n = 3 of independent experiments.

b) RT-qPCRs for human XBP1s and the XBP1s target SYVN1 from human MDA-MB-231 cells treated with Thapsigargin (Tg) or vehicle control (DMSO) after transfection with a non-targeting control (siNTC) siRNA, or siRNAs for PUM1 and PUM2 (siPUM1+2). Knock-down efficiency of siPUM1+2 was evaluated by RT-qPCR for PUM1 and PUM2. n= 2 biological independent experiments. Source data file is provided.

Supplementary Table 1

Pum D1-V5-H6 n pIZ	FW dom.1 + pcma PUM Rev. dom.1 + pcma PUM	CGAATTTAAAGCTTCAAAATGAAGTTTTTGGGTG GCCCTCTAGACTCTCTGCAGGTGATGGTTG	
r umD3-V5-H6 n plz	Fw pcmb + dom. 3 PUM Rev. Fw pcmb + dom. 3 PUM	CGAATTTAAAGCTTCAAAATGTATGGCGTAGCACCA GCCCTCTAGACTTCCTGGCTGTGGGGGCAC	ATGG
r um D1D3-V5-H6 n plz	FW dom.1 + pcma PUM Rev. Fw pcmb + dom. 3 PUM	CGAATTTAAAGCTTCAAAATGAAGTTTTTGGGTG GCCCTCTAGACTTCCTGGCTGTGGGGCAC	
u milioV5-H6 pET26b	Nde1-pumilio Fw EcoR1-V5His6Pum	GGATTCCATATGAAGTTTTTGGGTGGTAACGATG GGAATTCCAGTCAGATAAACTCAATGGTG	
umD3-V5-H6	Nde1-D3	GGATTCCATATGTATGGCGTAGCACC	
n pET26b	EcoR1-V5His6Pum	GGAATTCCAGTCAGATAAACTCAATGGTG	
umHD-V5-H6	Nde1-PumHD FW	GGATTCCATATGAGATCTCGCCTTCTCGAAG	
	ECOR1-V5HIS6Pum	GGAATTCCAGTCAGATAAACTCAATGGTG	
ET26b	EcoR1-V5His6Pum	GCCCTCTAGACTTCCTGGCTGTGGGGGCAC	
bp1-3'UTR	Xbp1-BamHI	CGGGATCCTTTATATTTTGAATATTTTTAG	
	Xbp1-3UTR un FW Xbp1-3UTR sp FW		
	Xbp1-revGH	gctctagaGcGTGACACTATAGAACTCGAG	
PumPRE Mutants	PumS3-R	TAATCATAAGCCTATGTGGTTAGAG	
n pRM-GFP	PumS3-Fmut Pum s1 Fw	CTCTTATAacaAacaATTGTGCTCAAAG GCTTTATATATGATACTGTAGTTAGAGCG	
	Pum s1rev mut	CTTCGATTATGAATTTATGTTGTGCCTATC	
ew reporter	RV xbp1 HA	ggc tcg cat aat ctg gaa cat cgt acg ga t aat aaG ATA TCT	GCG AGC AGA CTT TCG GC
bp1-HA-GFP	FW xbp1 HA	tat ccg tac gat gtt cca gat tat gcg ag c ctc tGA CCC CGG	FCG CCA CCA TGG TGA GC
	eGfp-stop -fw eGFP-stop-rev	GGACICAGAICICGAGGGIACCGAICIG GGTACCCTCGAGATCTGAGTCCTTACTTGT	
	eGFP-ecoR5 Fw	GGGATATCGTGAGCAAGGGCGAGGAGCTG	
	eGfp-stop -fw eGFP-stop-rev	GGACTCAGATCTCGAGGGTACCGATCTG GGTACCCTCGAGATCTGAGTCCTTACTTGT	
	eGFP-ecoR5 Fw	GGGATATCGTGAGCAAGGGCGAGGAGCTG	
Pumilio			
phosphomutants	EwmutT5374		
537 A. \$540 A. \$544 A	RevmutT537A-S540A-S544A	CTC TGC AGG TGC TGG TTG ACG TGC ACC AGG TGC	GCG ATT GAA GCC
1007 A , 3 040 A , 3 044 A			
S902A	SITmutD3 B FW	CGTTGACGGGTCGCCGCGACGCCTTCGACCGCAGCACC	0
	SITmutD3 B rev	GGTGCTGCGGTCGAAGGCGTCGCGGCGACCCGTCAAC	G
	Mut Dom1 nFW	GCC GAT TCG ACT TGC GCC AAA GTG GTT G	
	MutDom1 nREV	GGG GGC CAG TAT GCC CGC ATT GCC GCT	
	MutDom3A nFW MutDom3A nREV	CCC GCC CAG CAG GGT GCC GAG AAT C	
		aDCD animous used	T
		qPCR primers used	Target
	GFP-FW1	qPCR primers used	Target gfp
	GFP-FW1 GFP-Fw2 Xbp1 intron	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA	Target gfp gfp Unspliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACCGCAAG	Target gfp gfp Unspliced Unspliced /spliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp PP40-Er1	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACCGCAGA CACAACTTCCCAGAGTGAGCGCCAG AGATCGTGAGAGAGCGCAGCAAGC	Target gfp Unspliced Unspliced /spliced Unspliced /Spliced PD40
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACCGCAAG CACAACTTCCCAGAGTGAGGCCAG AGATCGTGAAGAGCGCACCAAGC GCACCAGGAACTTCTGAAGCGCACAAGC GCACCAGGAACTTCTGAATCCGG	Target gfp Unspliced Unspliced /spliced Unspliced /Spliced RP49 RP49 RP49
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTGGATGCTGCAGA CCAGACATCGCGTGACCGCAAG CACAACTTCCCAGAGGGCCAG AGATCGTGAAGAGAGCGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCGCTTGACGTCGAACTCTTCGT	Target gfp Unspliced Unspliced/spliced Unspliced/Spliced RP49 RP49 Spliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACCGCAAG CACAACTTCCCAGAGGCCAG AGATCGTGAAGAGAGCGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCGCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC	Target gfp Unspliced Unspliced/spliced Unspliced/Spliced RP49 RP49 Spliced Unspliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACCGCAAG CACAACTTCCCAGAGGCCAGG GCACCAGGAACATCTTCAGAGGCCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCGCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCAACCTTGGACCGCAGGG CCAACCTTGGACCGCAGGG	Target gfp Unspliced Unspliced /spliced Unspliced /spliced RP49 RP49 Spliced Unspliced spliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw2 xbp1 S_Fw3	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACCGCAAG CACAACTTCCCAGAGGCCACAAGC GGACCAGGAACTTCTTGAATCCGG GCCCTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCAACCTTGGATCCCAAGCTGACCC CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA	Target gfp Unspliced Unspliced (spliced Unspliced /Spliced RP49 RP49 Spliced Unspliced spliced spliced spliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw2 xbp15_Fw3 rp49_Fw3	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACGCAAG CACAACTTGCCAGAGGCCACAAG GACACGTGAACAGCGAGGCCACAAGC GCACCAGGAACTTCTTGAATCCGG GCGCTGACGCAAGCGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCGCTGACGCAAGCCACCAAGC GCACCAGGAACTCCTTCGT CTGCAGCATCCAAAGCTGACCC CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCAGGGTA CCCAAGATCGTGAAGAAGCGCACCCAAGC	Target gfp Unspliced (spliced Unspliced /spliced Unspliced /Spliced RP49 RP49 Spliced Unspliced spliced spliced spliced rp49
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw2 xbp15_Fw3 rp49_Fw3 rp49_rev4	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACCGCAAG CACAACTTCCCAGAGGCCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCCCTGGACGCAAGCCGCAAGC GCACCAGGAACTTCTTGAATCCGG GCGCTGACACCAAGCCGCACCCAAGC GCACCATGCAAAGCTGACCC CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCAGGTA CCCAAGATCGTGAAGAAGCGCACCAAGC GCCAAGTCGTGAAGAAGCGCACCAAGC GCCAAGTCGTGAAGAAGCGCACCAAGC	Target gfp gfp Unspliced Unspliced /spliced Unspliced /Spliced RP49 RP49 Spliced Spliced unspliced spliced spliced spliced p49 rp49
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp15_Fw3 rp49_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCCGTGACCGCAAG CACAACTTTCCAGAGGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCCCCGAGCACCAAGC GCACCAGGACTTCTTGAATCCGG GCGCTTGAACGCAAGCTGACCC CCCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCAGG CCCAAGTCGTGAAGAAGCGCACCAAGC GCCAAGTTGGTGAAGAAGCGCACCAAGC GCCAAGTTGGTGAAGAAGCGCCACCAAGC GCCAAGTTGGTGAAGAAGCGCACCAAGC GCCCAGTTGTGCACCAGGAACTTCTTGAATCC TGTCACCGATCTGGTTCTTCAGGC	Target gfp gfp Unspliced Unspliced /spliced Unspliced/Spliced RP49 Spliced Unspliced spliced Unspliced Spliced Hspliced Unspliced Spliced Hsc3
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw2 xbp1s_Fw3 rp49_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acr2/5wcPCP	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCCGTGACGCAGA CCAGACATCGCGTGACGCAAG AGATCGTGAAGAGCGACCAAG GGCTCAGCTGAACGCAAGC GCACACTTTCCAGAGGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCCCCAGGACTTCTTGAATCCGG GCCACCATGGACCTCAAAGCTGACCC CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCAGGGTA CCAACCTTGGATCTGCCGCAGCGCACCAAGC GCACGTTGTGACACAGGAAAGCCCACCAAGC GCACGTTGTGCACCAGGAAACTTCTTGAATCC TGTCACCGATCTGGTTCTTCAGGC GTCCCATGACCAAGGCAAAGCCATC CAATCATCGCCGAAGGAATCTTC	Target gfp gfp Unspliced Unspliced /spliced Unspliced /Spliced RP49 RP49 Spliced spli
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw2 xbp15_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCCTGACCGCAAG AGATCGTGAAGAGCGACCCAAGC GCACACTTTCCAGAGCCACAAGC GCACCAGGAACTCTTCGAAGCCCAGA GCACCACGGACTCTTGGATCTCGG GCGCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CAACCTTGGATCTGCCGCA CAACCTTGGATCTGCCGCA CCAAGATCGTGAAGAAGCGCACCAAGC GCACGTTGTGCACCAGGAACTTCTTGAATCC TGTCACCGATCTGGTTCTTCAGGC GTCCCATGACCAAGGAACAACCATC CATCACTGCCGAGAACTTG CTGCAGCGCTCGCAACTTC CATCACTGCCGAGAACTTG CTGCAGCTTCGTATACCATCG	Target gfp Unspliced Unspliced /spliced Unspliced/Spliced RP49 RP49 Spliced Unspliced spliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw2 xbp15_Fw3 rp49_Fw3 rp49_Fw3 rp49_rev4 Hsc3_rev Acat2-tw-qPCR	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACCGCAGA CCAGACATCGCGTGACCGCAGG CACACTTTCCAAGAGAGCGCCAG AGATCGTGAAGAGCGCACCAAGC GCCCCAGGAACTTCTTGATACCGGG GCCCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCAACCTTGGATCTGCCGCAGGG CCAACCTTGGATCTGCCGCAGGGTA CCCAAGATCGTGAAGAAGCGCACCAAGC GCCCATGGTGCTGCACCAGGGACCTTCTTGAATCC TGTCACCGATCTGGTTCTTCAGGC GTCCCATGACCAAGGACAACCATC CATCACTGCCCAGGAACTCTG CATCACTGCCCAGGAACCTC CATCACTGCCAGAGACTCTG CATCACTGCCAGAGAATCTG CTGAGCTTCTGTATACCCTCG	Target gfp Unspliced Unspliced/spliced Unspliced/Spliced RP49 RP49 Spliced Unspliced spliced spliced spliced spliced spliced spliced spliced spliced spliced rp49 rp49 Acat2 Acat2
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw3 rp49_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTGGATGCTGCAGA CCAGACATCGCGTGACCGCAAG CACAACTTCCCAGAGTGAGGCCAG AGATCGTGAAGAGAGCGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCCCTTGACGTCGAACTCTTCGAT CTGCAGCATCCAAAGCTGACCC CCAACCTTGGATCTGCCGCAGGG CCCAACCTTGGATCTGCCGCAGGG CCCAAGCTTGGTGTCACCC CCCAAGCTTGGATCTGCCCCAGGG CCCAAGCTTGGACCGCAGGG CCCAAGCTTGGACCGCAGGGA CCCAAGCTTGGACCGCAGGGACACCCAAGC GCCCATGGACTGTGAACACAGGCACCCAAGC GCCCATGACCAAGGACAACCATC CATCACTGCCGAGGAATCTG CTGAGCTTCTGTATACCCTCG	Target gfp Unspliced Unspliced /spliced Unspliced/Spliced RP49 RP49 Spliced Unspliced spliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp15_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-rv-qPCR	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTGGATGCTGCAGA CCAGACATCGCGTGACGCGCAGA CCACACTTCCCAGAGGCCCCAAGG GACACGTGAACGAGAGGCCACCAAGC GCCCCAGGAACTTCTTGAATCCGG GCCCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCCAACCTTGGATCTGCCGCAGGG CCCAACCTTGGATCTGCCCCAGGG CCCAAGCTTGGATCTGCCCCAGGGACCCCAGC GCCCATGTGAACGTGACCC CCCAAGCTTGGATCTGCCCCAGGG CCCAAGGTCGTGAAGAGCGCACCAAGC GCCCATGTGAACTGTGCGCAGGGAACTCTTTGAATCC TGTCACCGATCTGGTTCTTCAGGC GTCCCATGACCAAGGAACACCATC CATCACTGCCGAGAATCTG CTGAGCTTCTGTATACCCTCG	Target gfp unspliced Unspliced /spliced Unspliced /Spliced RP49 RP49 Spliced Unspliced /spliced spliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp15_Fw2 xbp1S_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-rw-qPCR	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACGCGAGA CCACACTTTCCAGAGGCCCCAAG GACACGTGAACGAGAGCGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCGCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCCAAGCATCGACACCAAGG CCAACCTTGGATCTGCCGCAGGG CCCAAGCTTGGACCGCAGGG CCCAAGCTTGGATCTGCCGCAGGGTA CCCCAAGATCGTGAACACCAGC GGCCCATCAGGACCACGAGGACACCAAGC GCCCATGACCAAGGACAACCATC CATCACTGCCGAGAATCTG CTGAGCTTCTGTATACCCTCG	Target gfp gfp Unspliced Unspliced / Spliced RP49 RP49 Spliced Unspliced spliced spliced ypiced Hsc3 Hsc3 Acat2 Acat2 Bases End Modification 3'
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp15_Fw2 xbp1S_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-rw-qPCR Name pum1site-wt-3BioTEG pum1site-wt	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACGCGAGG CCACACTTTCCAGAGGCGCAGCAAGG GACACGTGAACGAGAGCGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCGCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCAACCTTGGATCTGCCGCAGGG CCCAAGCTTGGATCTGCCGCAGGG CCCAAGCTTGGATCTGCCGCAGGGTA CCCCAGGATCTGGTGACCAGGAACCATC CACCTTGGACCGAGGACAACCATC CATCACTGCCGAGAGACATCTG CTGCACGATCTGGTACCCCGGAGACTCTG CCCAAGGATCTGGTCTTCAGGC GTCCCATGACCAAGGACAACCATC CATCACTGCCGAGAATCTG CTGAGCTTCTGTATACCCTCG	Target gfp gfp Unspliced Unspliced /spliced Unspliced/Spliced RP49 RP49 Spliced Unspliced spliced spl
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp15_Fw2 xbp15_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-fw-qPCR Acat2-rw-qPCR Mame pum1site-wt-3BioTEG pum1site-wt pum1site-mt	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACGCGAGG CACAACTTTCCAGAGGCGCAGCAAGG GACACGTGAACAGGAGGCCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCGCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCCCA CCAACCTTGGATCTGCCCCA CCAACCTTGGATCTGCCCCA CCAAGATCGTGAAGAAGCGCACCAAGC GCCCATGGATCTGGTGAAGAAGCGCACCAAGC GCCCATGGATCTGGTGTCTTCAGGC GTCCCATGACCAAGGACAACCATC CATCACTGCCGAGAATCTG CTGAGGCTTCTGTATACCCTCG	Target gfp gfp Unspliced Unspliced /spliced RP49 Spliced Unspliced spliced spliced Unspliced spliced Hsc3 Hsc3 Acat2 Bases End Modification 3' 27 Biotin-TEG 27
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw2 xbp1s_Fw3 rp49_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-tw-qPCR Acat2-tw-qPCR Acat2-tw-qPCR Mame pum1site-wt-3BioTEG pum1site-wt	qPCR primers used GGCTACGTCCAGGAGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACGCGAAG CACAACTTTCCAGAGTGAGGCCAG AGATCGTGAAGAAGCGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCGCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCAGGG GCACCATGGATCTGCCGCA CCAAGATCGTGAAGAAGCGCACCAAGC GCCCATGACCAGGACAACCATC CATCACTGCCGAGAGACACCATC CATCACTGCGCAGAGACACCATC CATCACTGCGCAGAGACACCATC CATCACTGCCGAGAACTCTG CTGAGCTTCTGTATACCCTCG EMSA assays RNA oligos Sequence GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG	Target gfp gfp Unspliced (spliced Unspliced (Spliced RP49 Spliced Unspliced spliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw3 rp49_Fw3 rp49_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-fw-qPCR Acat2-rv-qPCR	qPCR primers used GGCTACGTCCAGGAGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCCGTGACGCGAGA CCAGACATCGCGTGACCGCAAG GACACTTTCCAGAGGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCCCCAGGACTTCTTGAATCCGG GCCCCAGGACTTCTTGAATCCGG GCCAACCTTGGATCTGCCGCAC CCAACCTTGGATCTGCCGCAC CCAACCTTGGATCTGCCGCAC CCAACCTTGGATCTGCCGCAC CCAACCTTGGATCTGCCGCAC CCAACCTTGGATCTGCCGCAC CCAACCTTGGATCTGCCGCAC CCAACCTTGGATCTGCCGCAC CCAACCTTGGATCTGCCGCACCACAGC GCCCATCACAAGGACAACCATC CATCACTGCCTGGAGATCTG CTGAGCTTCGTATAACCTCG EMSA assays RNA oligos Sequence GAUAGGCUGUACAUAAAUUCAUAAUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG Reagents/Kits Source or reference	Target gfp gfp Unspliced Unspliced /spliced RP49 Spliced unspliced spliced spliced unspliced spliced gfp Maca12 Aca12 Aca12 27 Biotin-TEG 27 Identifiers Additional information
	GFP-FW1 GFP-FW2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp15_FW3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-rw-qPCR Acat2-rw-qPCR Acat2-rw-qPCR Name pum1site-wt-3BioTEG pum1site-wt pum1site-mut Designation Amersham [™] Hybon N+	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAAACATCGCGTGACGCGAGA CACAACTTCCCAGAGGCCCCAAGG GACTCGTGAAGAGAGCGCACCAAGC GCCCCAGGAACTTCTTGAATCCGG GCCCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCCAACCTTGGATCTGCCGCAGGG CCCAACCTTGGATCTGCCGCAGGG CCCAAGATCGTGAACGCCACCCAAGC GCCCATGGATCTGCCGCAGGGAACTCTTGGATCC TGTCACCGATCTGGTTCTTCAGGC GTCCCATGACCAGGAAACCATC CATCACTGCCGAGGAACCCATC CATCACTGCCGAGGAATCTG CTGAGCTTCTGTATACCCTCG EMSA assays RNA oligos Sequence GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCACAACAUAAAUUCAUAAUCG GAUAGGCACAACAUAAAUUCAUAAUCG	Target gfp gfp Unspliced /spliced Unspliced /Spliced RP49 RP49 RP49 spliced unspliced /spliced unspliced spliced
	GFP-FW1 GFP-Fw2 Xbp1 FlUns Xbp1 FlUns Xbp1 FlUns Xbp1 FlUns PP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp15_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-fw-qPCR Acat2-rw-qPCR Mame pum1site-wt-3BioTEG pum1site-wt pum1site-mut Designation Amersham [™] Hybon N+ Ef(1 Strentaulfin-HDP Amorsham [™]	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTGGATGCTGCAGA CCACAACTTCCCAGAGGCCGCAAG CACAACTTCCCAGAGGCCCCAAG GGCTCAGCTGAACGCGAGGCCCCAG GGCTCAGCATCCCAAGGCGCACCAAGC GCCCCAGGAACTTCTTGAATCCGG GCCCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCCAACCTTGGATCTGCCGCAGGG CCCAACCTTGGATCTGCCCCAGGGGTA CCCCAGGTGTGAAGACGCCACCAAGC GCCCATGGATCTGCGCAGGGAACTCTTGGATCC TGTCACCGATCTGGTTCTTCAGGC GTCCCATGACCAGGAACCATC CATCACTGCCGAGAATCTG CTGAGCTTCTGTATACCCTCG EMSA assays GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GE Healthcare GE Healthcare	Target gfp gfp Unspliced /spliced Unspliced /Spliced RP49 RP49 RP49 spliced unspliced /spliced spliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp15_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-fw-qPCR Acat2-rw-qPCR Mame pum1site-wt pum1site-wt pum1site-mut Designation Amersham [™] Hybon N+ ECL Streptavidin-HRP Amersham [™] 1xTBS 1% Casein blocker	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCACACTTCCCAGAGGCCCCAAG CACAACTTCCCAGAGGCCCCAAG GACACGTGAACGCGAAGCGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCGCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCAACCTTGGATCTGCCGCAGGG CCCAACCTTGGATCTGCCGCAGGGTA CCCCAAGATCGTGAACGCACCAAGC GCACGTTGTGACGTGACCC CCCAAGATCGTGAACCGCAGGG CCCAAGATCGTGAACCGCAGGGA CCCAAGATCGTGACCAGGAGACTCTTCTGAATCC TGTCACCGATCTGGTTCTTCAGGC GTCCCATGACCAAGGACAACCATC CATCACTGCCGAGAGATCTG CTGAGCTTCTGTATACCCTCG EMSA assays GAUAGGCUGUACAUAAAUUCAUAAUCGUAUAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GE Healthcare GE Healthcare BIO-RAD	Target gfp gfp gfp Unspliced /spliced Unspliced /Spliced Unspliced /Spliced RP49 RP49 RP49 RP49 spliced spliced unspliced spliced spliced spliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw3 rp49_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-rw-qPCR Acat2-rw-qPCR Mame pum1site-wt-3BioTEG pum1site-wt pum1site-mt pum1	qPCR primers used GGCTACGTCCAGGAGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCGTGACGCGAAG CACAACTTTCCAGAGGCGCACCAAGC GGCTCAGCTGAAGAGCGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCGCTTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCAGGG GCACCAGGATCCAAGGACAACCATC CCAAGATCGTGACCACGAGAGC GTCCACGATCTGGTGTCTTCAGGC GTCCACGGACCACGAGAGACCATC CACCTTGGATCTGCTGCTGG GTCACCGATCTGGTTCTTCAGGC GTCCATGACCAAGAACACATC CACCTTGGATCAGGACAACCATC CACCATGCCAGAGAACCATC CACCATGCCAACAQAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GE Healthcare GCE Healthcare GU-RAD	Target gfp gfp Unspliced /spliced Unspliced/Spliced RP49 Spliced spliced <
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw3 rp49_Fw3 rp49_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-fw-qPCR Acat2-rv-qPCR Mame pum1site-wt-3BioTEG pum1site-wt pum1site-mut Designation Amersham TM Hybon N+ ECL Streptavidin-HRP Amersham TM IxTB5 1% Casein blocker	qPCR primers used GGCTACGTCCAGGAGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCCGTGACCGCAAG CACAACTTTCCAGAGGCACCAAGC GCACCAGGAACTCTTGGATGCTGCAGG GCACCAGGAACTCTTGGATGCCCC CCAGCACTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCAGG CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCAGG CCCAAGTTGTGAAGAAGCGCACCAAGC GCCAACTTGGATCTGCCGCAGG CCCAAGATCGTGAAGAAGCGCACCAAGC GCCAAGTTGGTGAAGAAGCGCACCAAGC GCCAACTTGGATCTGCCGCAGGAACTTC CATCACTGCCAAGGACAACCATC CATCACTGCCGAGAATCTG CTGAGCTTCTGATATACCTTG CTGAGCTTGTGTGAAGAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GE Healthcare GC Healthcare BIO-RAD	Target gfp gfp Unspliced /spliced Unspliced /Spliced RP49 RP49 RP49 spliced spliced spliced spliced spliced spliced spliced p49 rp49 Hsc3 Hsc3 Acat2 Bases End Modification 3' 27 Biotin-TEG 27 Identifiers Additional informatt RPN 2222B RPN 1231 (1:1000) #1610782
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw3 rp49_Fw3 rp49_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-fw-qPCR Acat2-rv-qPCR Mame pum1site-wt-3BioTEG pum1site-wt pum1site-mut Designation Amersham ^{Tw} Hybon N+ ECL Streptavidin-HRP Amersham ^{Tw} 1xT85 1% Casein blocker T7 primer T7 primer T7 primer	qPCR primers used GGCTACGTCCAGGAGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCCGTGACCGCAAG CACAACTTTCCAGAGGCACCAAGC GCACCAGGAACTCTTGGATGCTGCAGG GCACCAGGAACTTCTTGAATCCGG GCGCTTGACGTCGACCC CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCAGG CCCAACGTTGGATCTGCCGCA CCAAGATTGTGAAGAAGCGCACCAAGC GCACGTTGGTCACCAGGAACTTCTTGAATCC TGTCACCGATCTGGTTCTTCAGGC GTCCCATGACCAAGGACAACCATC CATCACTGCCGAGGAATCTG CTGAGCTTGTGTACAAGGACAACCATC CATCACTGCCAGGAATCTG CTGAGCTTGTATACCCTCG BGAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACACAACAUAAAUUCAUAAUCG	gfp gfp gfp Unspliced /spliced Unspliced /Spliced Unspliced/Spliced RP49 RP49 RP49 Spliced spliced spliced 27 27 <td< td=""></td<>
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw3 rp49_Fw3 rp49_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-fw-qPCR Acat2-rv-qPCR Mame pum1site-wt-3BioTEG pum1site-wt pum1site-mut Designation Amersham ^{Tw} Hybon N+ ECL Streptavidin-HRP Amersham ^{Tw} 1xT85 1% Casein blocker T7 primer T7-mai Pum Fw T7-mai Pum Fw	qPCR primers used GGCTACGTCCAGGAGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCCGTGACCGCAAG CACAACTTTCCAGAGGCCACCAAGC GCACCAGGAACACGAGAGCGCACCAAGC GCACCAGGAACTCTTGAATCCGG GCGCTGACGTCGAACTCTTCGT CTGCAGCATCCAAAGCTGACCC CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCAGG CCAACCTTGGATCTGCCGCAGGCACCCAAGC GCACGTTGTGACACAGGAACCTCTTGAATCC TGTCACCGATCAGGTCATCAAGGACAACCATC CATCACTGCCGAGGAATCTG CTGAGCTTGTGTACAAGGACAACCATC CATCACTGCCGAGGAATCTG CTGAGCTTGTGTAACAAGGACAACCATC GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAACUCAUAAUUCGUAAUAUCG GGE Healthcare	Target gfp gfp Unspliced /spliced Unspliced /Spliced RP49 RP49 RP49 spliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw3 rp49_Fw3 rp49_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-rv-qPCR A	qPCR primers used GGCTACGTCCAGGAGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCCGTGACCGCAAG CACAACTTTCCAGAGGCACCAAGC GCACCAGGAACACGAGAGCGCACCAAGC GCACCAGGAACTTCTTGAATCCGG GCGCCTGACCGCAAGC CCAGCATTCAAAGCTGACCC CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCAGG CCAACCTTGGATCTGCCGCAC CAACCTTGGATCTGCCGCAGG CCCAAGATCGTGAAGAAGCGCACCAAGC GCACGTTGGTGAAGAAGCGCACCAAGC GCACGTTGGTGAAGAAGCGCACCAAGC GCACGTTGGATCTGCCGCAGGGTA CCCAAGATCGGTCTTCAGGC GTCACCGATCTGGTCTTCAGGC GTCACCGACGAAGAACACCATC CATCACTGCCGAGGAATCTG CTGAGCTTGTATACCCTCG BCAAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACACAUAAAUUCAUAAUCG GAUAGGCUGUACACACAUAAAUUCAUAAUCG GC Healthcare BIO-RAD	gfp gfp gfp unspliced /spliced Unspliced /Spliced Unspliced /Spliced RP49 RP49 RP49 RP49 spliced spliced 27 27 27 27 spliced
	GFP-FW1 GFP-Fw2 Xbp1 intron Xbp1 FUns Xbp1 RUnsSp RP49-Fc1 RP49-Fc2 Rev2 xbp1 spl/Uns Intron fw xbp1 xbp1 spliced fw xbp1s_Fw3 rp49_Fw3 rp49_Fw3 rp49_Fw3 rp49_rev4 Hsc3_forw Hsc3_rev Acat2-fw-qPCR Acat2-rv-qPCR Acat2-rv-qPCR Acat2-rv-qPCR Acat2-rv-qPCR Acat2-rv-qPCR Acat2-fw-qPCR Acat2-rv-qPCR A	qPCR primers used GGCTACGTCCAGGAGCGCACCATCTT AGACCCCAACGAGAAGCG GGGTCAGCTTTGGATGCTGCAGA CCAGACATCGCCTGACCGCAAG CACAACTTTCCAAGAGCGAAGCCACCAAGC GCACCAGGAACTCTTCGAACCCCAGG GCAGCATTCACAAGCTGACCC CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACCTTGGATCTGCCGCA CCAACGTTGGATCTGCCGCA CCAACGTTGGTCACCAGGAACTTCTGAATCC TGTCACCGATCTGGTTCTTCAGGC GTCCCATGACCAAGGACAACCATC CATCACTGCCGAGAACTTG CTGAGCTTGTGTAACCCTCG EMSA assays RNA oligos Sequence GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACAUAAAUUCAUAAUCG GAUAGGCUGUACACUAAAUUCAUAAUCG GAUAGGCUGUACACUAAAUUCAUAAUCG GAUAGGCUGUACACUAAAUUCAUAAUCG GAUAGGCUGUACACUACAUAAUUCAUAAUCG GAUAGGCUGUACACUACAUAAAUUCAUAAUCG <t< td=""><td>gfp gfp gfp Unspliced /spliced Unspliced /Spliced RP49 RP49 RP49 spliced spliced pa9 p49 rp49 rp49 rp49 rp49 rp49 rp49 rp40 rp49 rp43 Spliced identifiers Acat2 Identifiers Additional informati RPN 1231 (1:1000) #1610782</td></t<>	gfp gfp gfp Unspliced /spliced Unspliced /Spliced RP49 RP49 RP49 spliced spliced pa9 p49 rp49 rp49 rp49 rp49 rp49 rp49 rp40 rp49 rp43 Spliced identifiers Acat2 Identifiers Additional informati RPN 1231 (1:1000) #1610782

Designation	Source or reference	Identifiers	Additional
			information
Antibodies			
V5 Tag Antibody (mouse monoclonal)	Invitrogen	(R960-25); RRID: AB 2556564	1:1000-1:5000
TAP-tag Antibody (Rabbit-pAb)	GenScript	#A00683	1:2000
ELAV (Anti-Rat)	Developmental Studies Hybridoma Bank (DSHB),	DSHB Cat# 1ea AB_528217	(1:400)
anti-alpha-tubulin (mouse monoclonal)	Developmental Studies Hybridoma Bank (DSHB),	DSHB Cat# 12G10 RRID:AB_1157911	(1:50)
Anti-HA (mouse monoclonal)	Biolegend Clone [16B12]	Covance Catalog# MMS-101R	(1:2000)
Anti-HA (rat monoclonal)	Chromotek Clone [7C9]	HA antibody [7C9] RRID: AB_2631399	(1:1000)
Anti-GFP (rat monoclonal)	Chromotek Clone [3H9]	3h9-100 RRID: AB_10773374	(1:1000)
Ire1 Phospho	Genentech	NA	(1: 1000)
Anti-mouse IgG HRP Anti-Rabbit IgG HRP Anti-Rat IgG HRP	GE Healthcare Sigma Sigma	NXA931 A8275 A9037	(1:5000) (1:10000) (1:2000)
Cy3-conjugated donkey anti-rabbit IgG (H+L)	Jackson ImmunoResearch Iaboratories	#711-165-152	(1:400)
Cy3 AffiniPure Donkey Anti-Rat IgG (H+L)	Jackson ImmunoResearch laboratories	#712-165-150	(1:400)
Cy3-conjugated donkey anti-mouse	Jackson ImmunoResearch laboratories	#715-165-150	(1:400)
Alexa Fluor® 488 AffiniPure Donkey Anti-Mouse IgG (H+L)	Jackson ImmunoResearch Iaboratories	715-545-151	(1:400)
Goat anti-Rabbit IgG Dye 650	Advansta	R-05761-250	(1:400)
Alexa Fluor® 647 AffiniPure Donkey Anti-Rat IgG (H+L)	Jackson ImmunoResearch laboratories	712-605-153	(1:400)
Alexa Fluor® 647 AffiniPure Donkey Anti-Mouse IgG (H+L)	Jackson ImmunoResearch Iaboratories	715-605-151	(1:400)
Goat anti-Mouse IgG Dye 650	Advansta	R-05764-250	(1:400)

Reagents/Kits

Supplementary Table 2

NucleoBond ® Xtra Midi kit for transfection-grade plasmid	MACHEREY-NAGEL	MN740410	
NZYMiniprep	nzvtech	MB01002	
NZY Total RNA isolation kit	nzytech	MB13402	
TBIzol™Reagent	Invitrogen	#15596026	
RiboMAXTM Large Scale RNA Production System	Promega	#P1300	
RNA Clean & Concentrator ™ - 5	Zymo Research	B1015	
RNasin® Ribonuclease Inhibitor	Promega	N2511	
Amersham [™] Hybond PVDF Blotting Membrane	GE Healthcare	# 10600021	
Amersham™ ECL Chemiluminescent HRP Substrate	GE Healthcare	RPN2232	
ATP, [γ-32P]- 3000Ci/mmol, 250μCi , 5mCi/mL	PerkinElmer	PELSBLU502H250UC	
Amersham Hyperfilm™ ECL	GE Healthcare	#28906836	
Microseal B Adhesive Seals	BIO-RAD	MSB-1001	
iCycler iQ® PCR Plates 96-well	BIO-RAD	# 2239441	
Effectene Transfection Reagent	QIAGEN	# 1054250	
Penicillin/streptomycin	ThermoFisher Scientific	Cat#15140122	
FBS Hyclone	Biowest	SV30160.03	
Schneider's medium	Biowest	L0207-500	
NZYColour Protein Marker II	nzytech	MB09002	
cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail	Roche	4693159001	
Phos-tag™ Acrylamide NARD institute	Wako-Chem Europe GmbH	AAL-107	
Halt Protease & Phosphatase Inhitor Cocktail, EDTA free	Thermo Scientific	#78441	
40% Acryllamide/Bis Solution 29:1	BIO-RAD	#1610146	
4–20% Mini-PROTEAN® TGX™ Precast Protein Gel	BIO-RAD	#456-1085	
7% Mini-PROTEAN® TGX™ Precast Protein Gel	BIO-RAD	#456-1025	
Slide-A-Lyzer MINI Dialysis devices	Thermo Scientific	#88404	
Amicon Ultra Centrifugal filter Devices	Merck	10K, 30K, 50K	
Rabbit IgG agarose beads	Sigma	A2909	
Ni-NTA Resin	G biosciences	#186-939	
V5 tagged Protein Purification Kit	MBL	code: 3317	
NZY I aq DNA polymerase	nzytech	MB00101	
Physion High-Fidelity DNA Polymerase	Fermentas ThermoEisber Scientific	#EP0501 E-530S	
NZYMutagenesis kit	nzytoch	MB01201	
Green Safe Premium	nzytech	MB01201	
GeneRuler 1kB DNA Ladder	ThermoScientific	#SM0311	
	100	100010	
14 Polynucleotide Kiñase	NEB	M02015 M02025	
14 DINA Liyase Jambda nhosnbatase	NEB	NU2023 P0753S	
Amgen Compound #18	Genentech	107555	
Subitinib	Sigma-Aldrich	PZ0012	
Apy29	MedChemTronica	HY-17537	
4µ8c	Sigma-Aldrich	SML0949	
Actinomycin D	Sigma	A1410	
Vectashield Mounting medium	Vector Laboratories,	VectorLabs: H-1200	
with DAPI	RRID:SCR_000821		
Software			
FIJI http://fiji.sc/	http://fiji.sc/	RRID:SCR 002285)	
GraphPad Prism	https://www.graphpad.com/	free trial	
https://astatsa.com/OneWay_Anova_with_TukeyHSD/	astatsa.com	2016 Navendu Vasavada	
Image Lab Software for Mac Version 6.1	Bio-RAD	SOFT-LIT-170-9690-ILSMAC-V-6-1	
CFX Manager™ Software	Bio-RAD	1845001	
Microsoft Excel version16.55	©2021Microsoft		

Supplementary Table 3 - MIQE checklist

ITEM TO CHECK	IMPORTANCE	CHECKLIST
EXPERIMENTAL DESIGN	F	Neg targeting central (NTC) deBNA against Dumilia yergus central
Number within each group	E	3-6 biological replicates and 3 technical replicates
Assay carried out by core lab or investigator's lab?	D	Investigator's lab
SAMPLE	D	
Description	E	Dmel-2 cells (S2 derivative):control and samples treated with dsRNAs
Volume/mass of sample processed Microdissection or macrodissection	E	n/a (cell culture)
Processing procedure	E	Investigator's lab
If frozen - how and how quickly? If fixed - with what, how quickly?	E	cells processed immediately for RNA isolation, using RNA kit
Sample storage conditions and duration (especially for FFPE samples)	E	na
NUCLEIC ACID EXTRACTION Procedure and/or instrumentation	E	kit RNA extraction
Name of kit and details of any modifications	E	NZY Total RNA isolation kit (nzytech) / Quick-RNA Miniprep (Zymo Research)
Source of additional reagents used	D	1ul Turbo DNA (Ambion) per sample
Contamination assessment (DNA or RNA)	E	qPCR on RNA after turbo Dnase treatment control in qPCR (no RT)
Nucleic acid quantification	E	Nanodrop One Nanodrop One
Purity (A260/A280)	D	1.8 to 2.0
Yield RNA integrity method/instrument	E	1-2µg total RNA RNA quality evaluated with electrophoresis
RIN/RQI or Cq of 3' and 5' transcripts	E	N/A (used gel electrophoresis)
Inhibition testing (Cq dilutions, spike or other)	E	Dilutions (standard curve efficiencies)
REVERSE TRANSCRIPTION	F	PovortAid H Minus First Strand cDNA Synthesis Kit (Thorme/Formentas)
Amount of RNA and reaction volume	E	0,5 to 1 µg RNA for RT, and 500 ng to 1 µg noRT 20 µl reaction volume
Priming oligonucleotide (if using GSP) and concentration Reverse transcriptase and concentration	E	Random hexamers from 500 ng/µl stock - 50 ng/µl in annealing, 25 ng/µl final in 20 µl
Temperature and time	E	42°C for 45 minutes
Manufacturer of reagents and catalogue numbers Cos with and without RT	D D*	Inermo Scientific (K1632) 25ng of RNA sample after turboDNase treatment. C
Storage conditions of cDNA	D	stored at -20°C
If multiplex, efficiency and LOD of each assay.	E	n/a
Sequence accession number	E	Provided in qPCR primer information table
Amplicon length	Ē	Provided in qPCR primer information table
In silico specificity screen (BLAST, etc) Pseudogenes, retropseudogenes or other homologs?	E D	NCBI Primer-BLAST ; Flyrnai.org
Sequence alignment	D	
Secondary structure analysis of amplicon Location of each primer by exon or intron (if applicable)	E	Provided in qPCR primer information table
What splice variants are targeted?	E	Provided in qPCR primer information table
qPCR OLIGONUCLEOTIDES Primer sequences	E	Provided in qPCR primer information table
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Table 1. MIQE checklist for authors, reviewers and editors. All essential information (E) must be submitted with the manuscript. Desirable information (D) should be submitted if available. If using primers obtained from RTPrimerDB, information on qPCR target, oligonucleotides, protocols and validation is available from that source.

*: Assessing the absence of DNA using a no RT assay is essential when first extracting RNA. Once the sample has been validated as RDNA-free, inclusion of a no-RT control is desirable, but no longer essential.

**: Disclosure of the probe sequence is highly desirable and strongly encouraged. However, since not all commercial pre-designed assay vendors provide this information, it cannot be an essential requirement. Use of such assays is advised against.