Figure S1

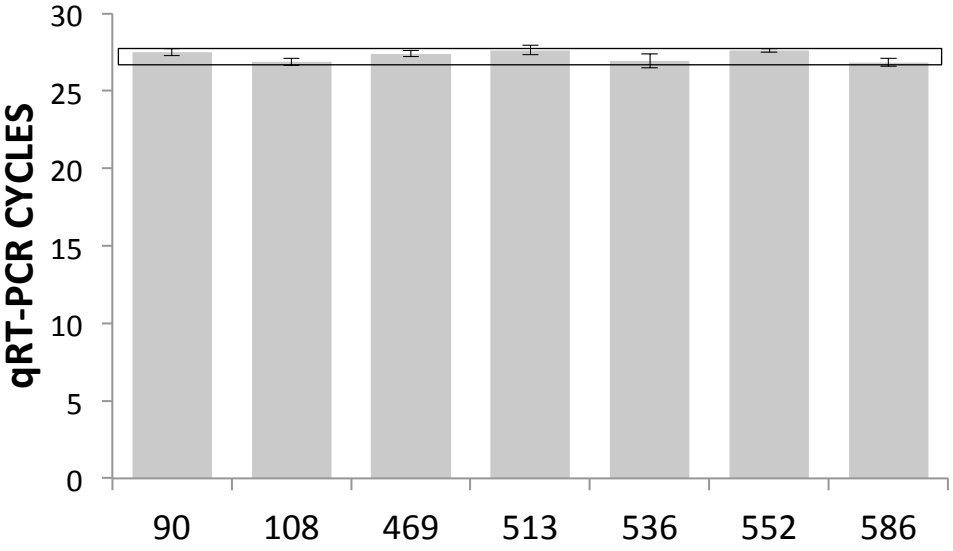


Figure S2

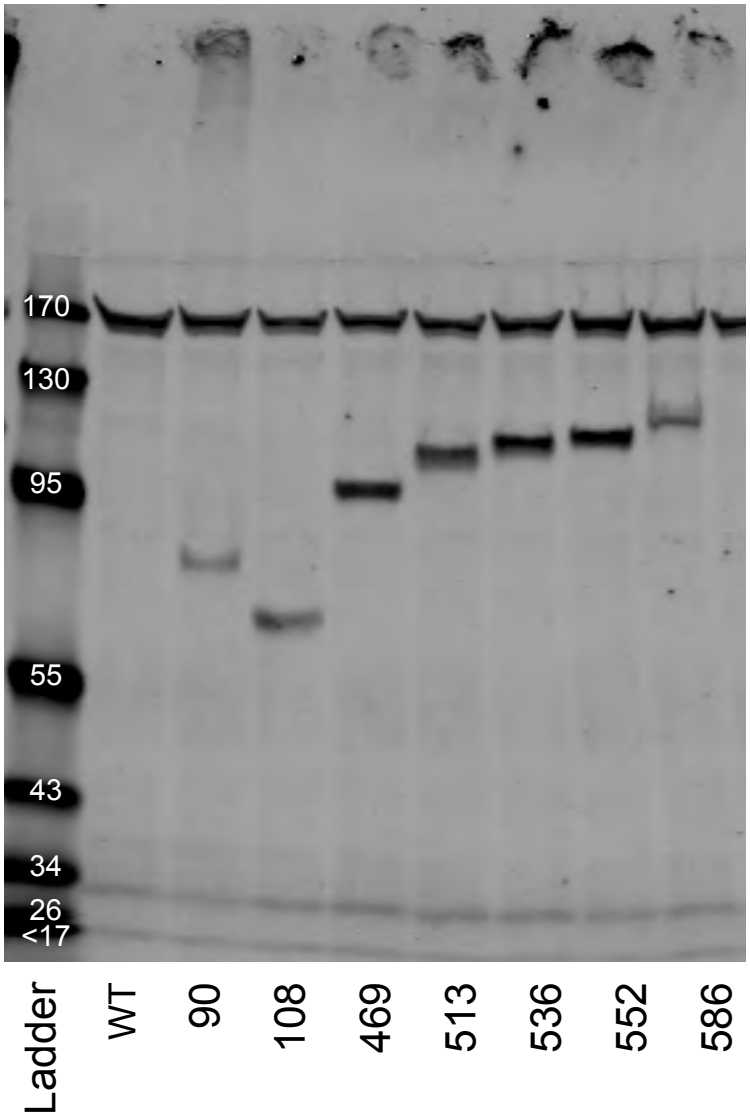


Figure S3

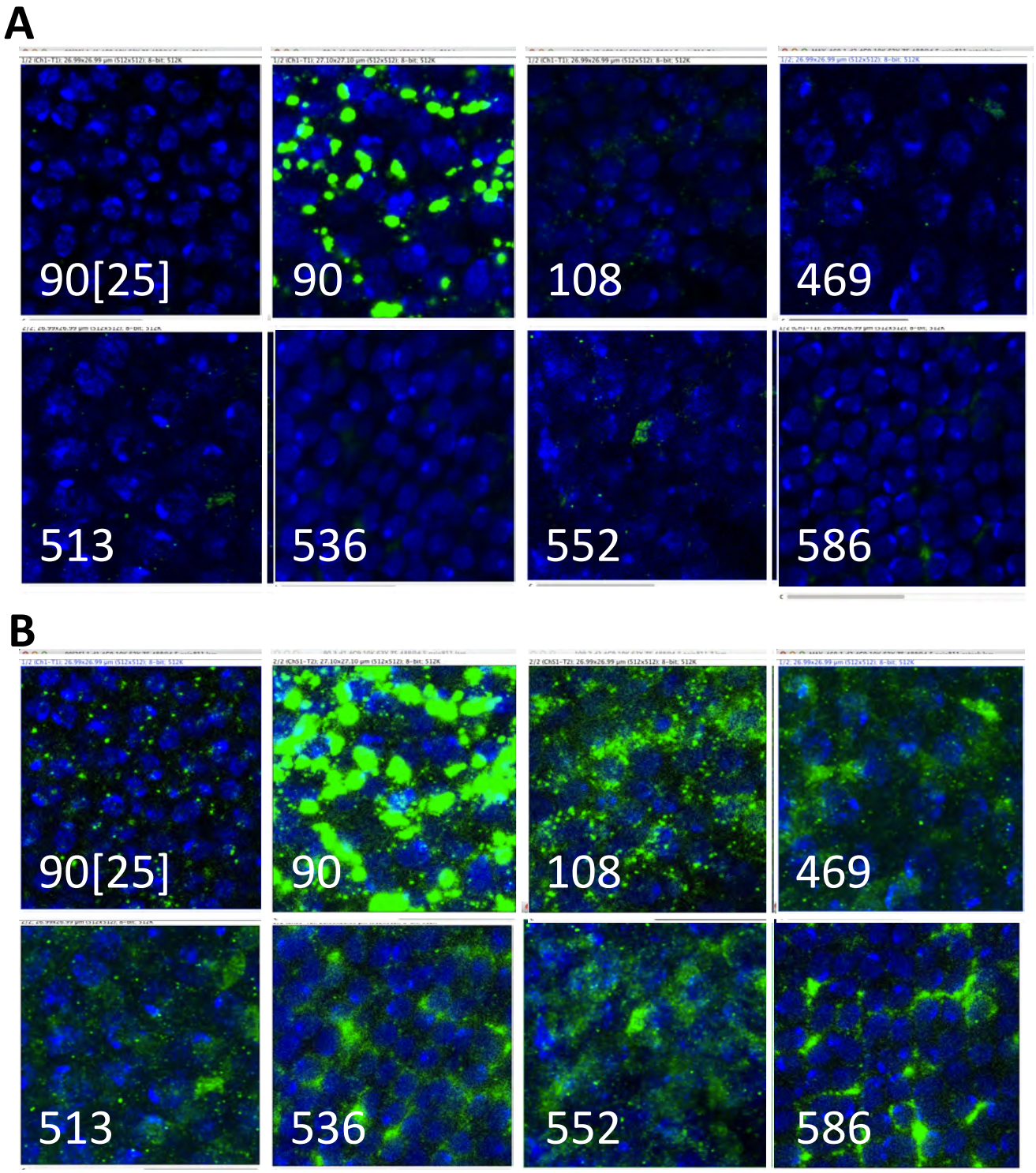

A
90[25]
90
108
469
513
536
552
586
B
90[25]
90
108
469
513
536
552
586

Figure S4

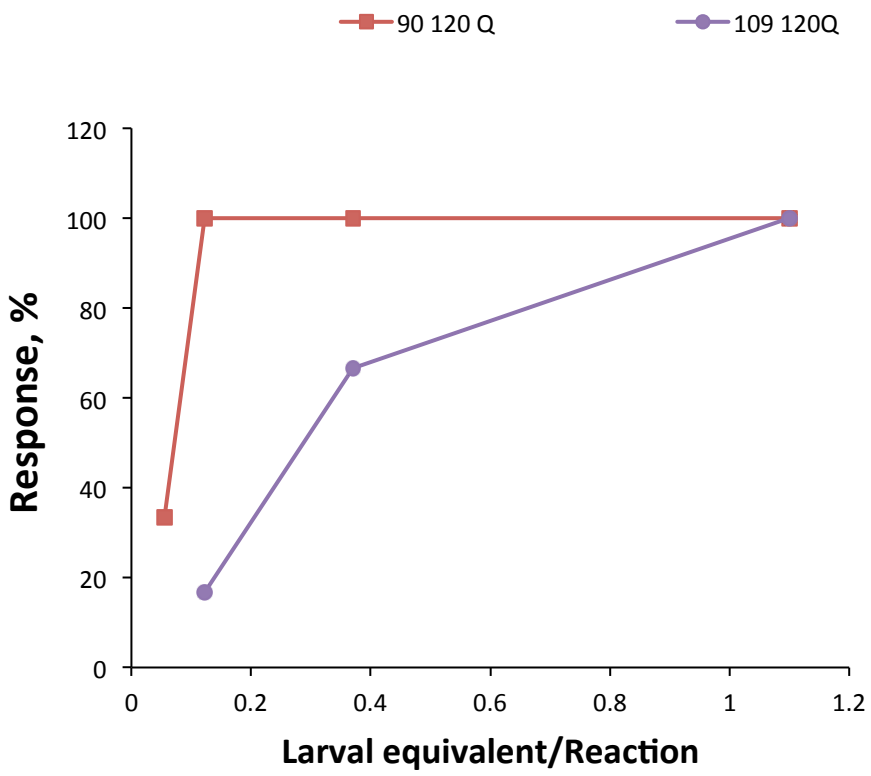

90 120 Q
109 120Q
120
100
80
60
40
20
0
Response, %
0
0.2
0.4
0.6
0.8
1
1.2
Larval equivalent/Reaction

FIGURE S5

Sequences

A

SEQUENCE OF THE 90 (EXON 1) FRAGMENT

142**90(exon 1)**

```
atggcgaccctggaaaagctgatgaaggccttcgagtccctcaaaagcttccaacagcaa
M  A  T  L  E  K  L  M  K  A  F  E  S  L  K  S  F  Q  Q  Q
cagcagcaacagcaacaacagcagcaacagcaacaacagcagcaacagcagcaacagcag
Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q
caacagcagcaacagcaacaacagcagcaacagcaacaacagcagcaacagcaacaacag
Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q
cagcaacagcaacaacagcagcaacagcaacaacagcagcaacagcaacaacagcagcaa
Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q
cagcaacaacagcagcaacagcaacaacagcagcaacagcaacaacagcagcaacagcaa
Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q
caacagcagcaacagcaacaacagcagcaacagcaacaacagcagcaacagcaacaacag
Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q
cagcaacagcaacaacagcagcaacagcaacaacagcagcaacagcaacaaccgccacca
Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  Q  P  P  P
cctccccctccacccccacctcctcaacttcctcaacctcctccacaggcacagcctctg
P  P  P  P  P  P  P  P  Q  L  P  Q  P  P  P  Q  A  Q  P  L
ctgcctcagccacaacctcctccacctccacctccacctcctccaggaccagctgtggct
L  P  Q  P  Q  P  P  P  P  P  P  P  P  P  P  G  P  A  V  A
gaggagcctctgcaccgaccataaggtacctctaga
E  E  P  L  H  R  P  *
```

B

TERMINAL SEQUENCES OF THE FRAGMENTS

108

```
ctgacaatatgaggtacctctaga
L  T  I  *
```

469

```
gccttaacataggtcgacggtacctctaga
A  L  T  *
```

513

```
tcagtggattaagtcgagggtacctctaga
S  V  D  *
```

536

```
cacagctcctaagtcgagggtacctctaga
H  S  S  *
```

552

```
ctgaatgattaagtcgagggtacctctaga
L  N  D  *
```

586

```
gtgttagactaagtcgagggtacctctaga
V  L  D  *
```

FIGURE S6

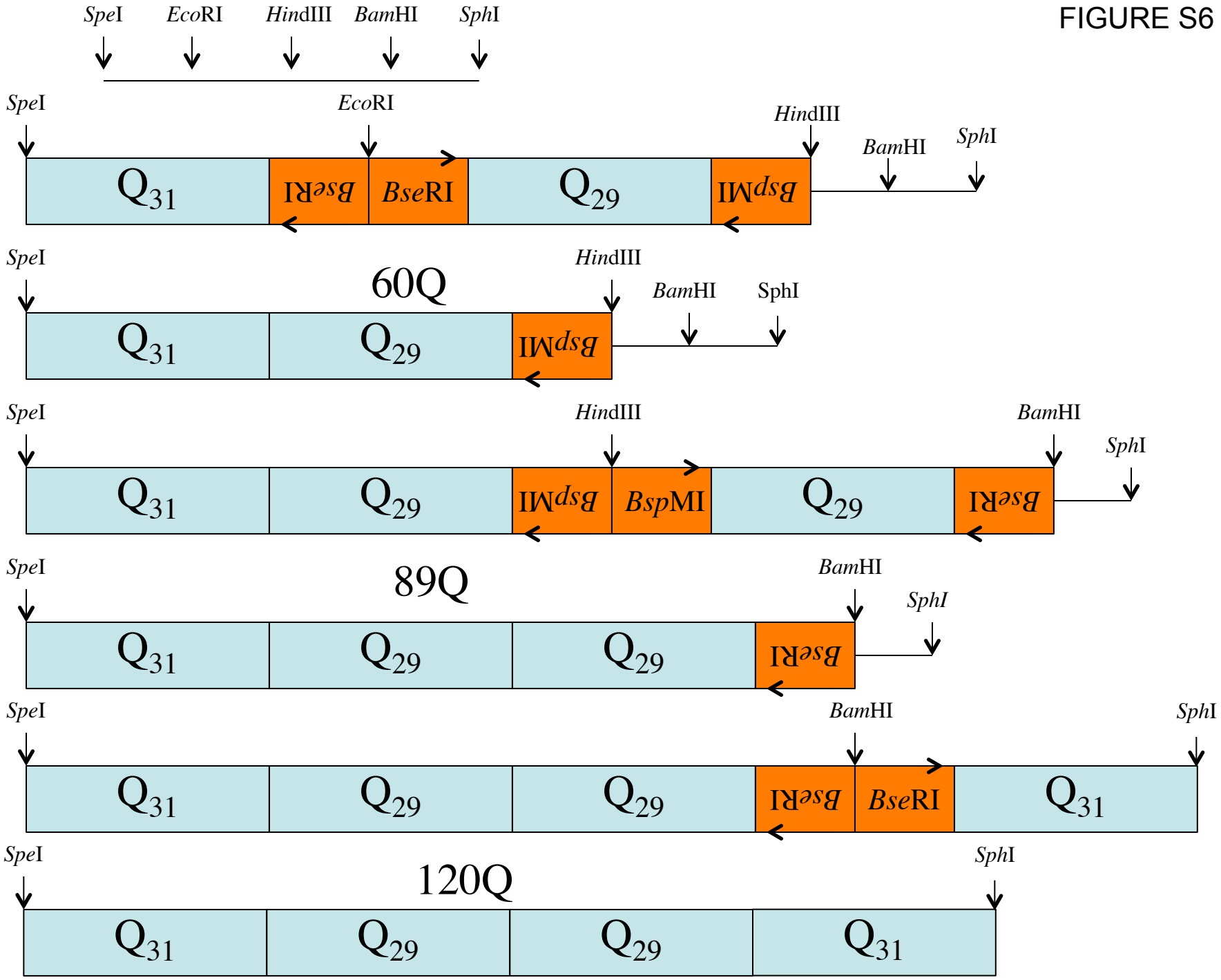
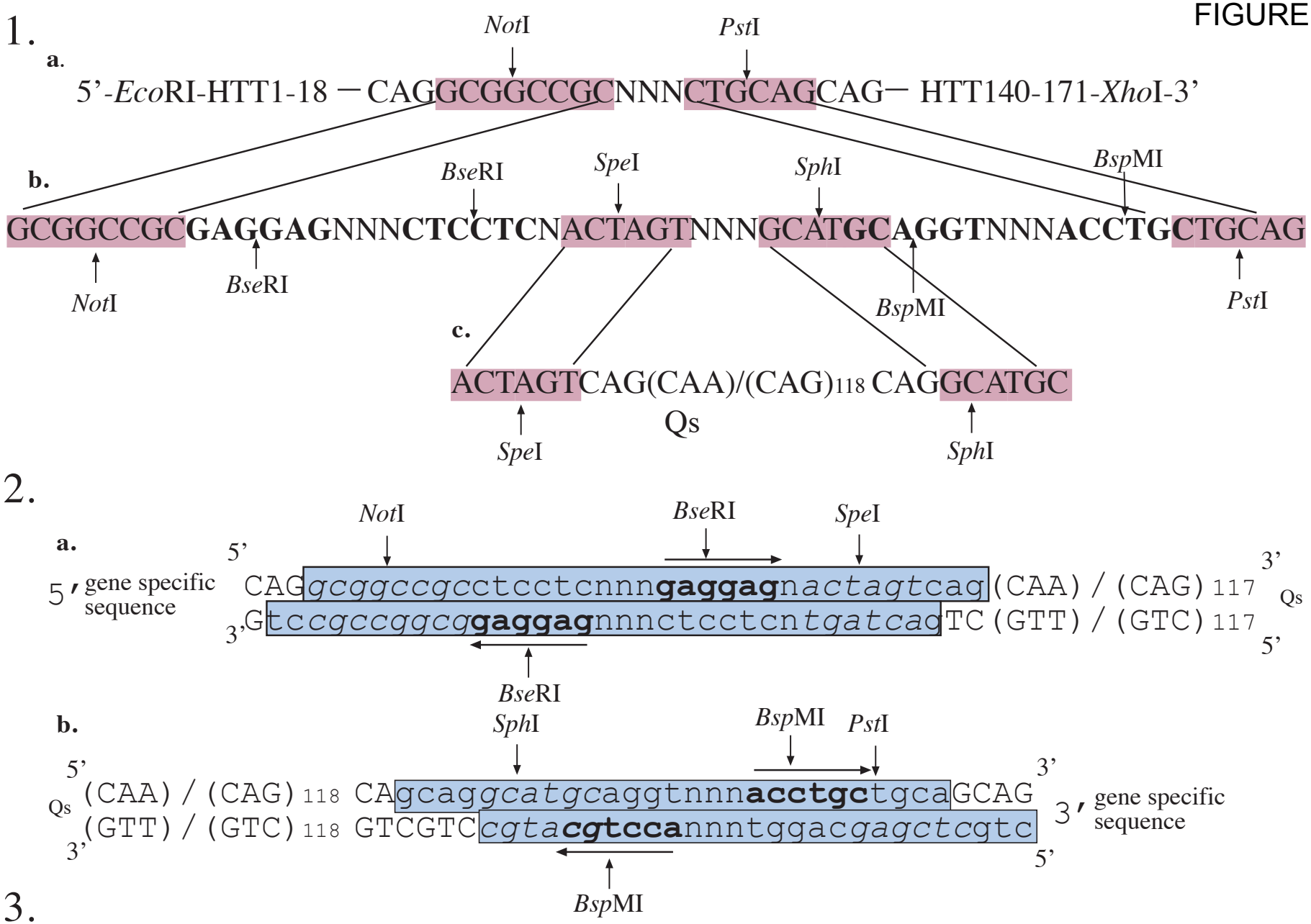

SpeI
EcoRI
HindIII
BamHI
SphI
Q31
BseRI
BseRI
Q29
BspMI
60Q
BspMI
BspMI
BspMI
BseRI
89Q
BseRI
BseRI
BseRI
Q31
120Q
Q31
Q29
Q29
Q31

FIGURE S7


1.
a.
NotI
PstI
5'-EcoRI-HTT1-18 — CAGGCGGCCGCNNNCTGCAGCAG— HTT140-171-XhoI-3'
b.
BseRI
SpeI
SphI
BspMI
GCGGCCGCGAGGAGNNNCTCCTCNACTAGTNNNGCATGCAGGTNNNACCTGCTGCAG
NotI
BseRI
BspMI
PstI
c.
ACTAGTCAG(CAA)/(CAG)118 CAGGCATGC
Qs
SpeI
SphI
2.
a.
NotI
BseRI
SpeI
5'
3'
5' gene specific sequence
CAGgcggccgcctcctcnnngaggagnactagtcag(CAA)/(CAG)117
3'GtccgccggcggaggagnnnctcctcntgatcagTC(GTT)/(GTC)117
Qs
5'
BseRI
b.
SphI
BspMI
PstI
5'
3'
Qs
(CAA)/(CAG)118 CAgcaggcatgcaggtnnnacctgctgcaGCAG
(GTT)/(GTC)118 GTCGTCcgtacgtccannntggacgagctcgtc
3' gene specific sequence
3'
5'
BspMI
3.
5'-EcoRI-HTT1-18 — CAG[(CAA)/(CAG)]118 CAG— HTT140-171-XhoI-3'

Supplemental figure legends

Figure S1. RNA expression from targeted HTT transgenes. Each HTT transgene is inserted in the same chromosomal location (51D) and orientation. RNA from heads of 3 day old adult flies expressing the different transgenic HTT fragments was quantified by qRT-PCR. The levels of expression are statistically indistinguishable and all within a single PCR cycle (indicated by the box); thus, mRNAs are expressed at the same level.

Figure S2. Human HTT is not proteolytically processed in Drosophila. Crosses were Y/*w*; UAS-FRAGMENT males X *w*; +; tub-GAL80^{ts} *da*-GAL4 virgins (the same as in Figure 4.). Representative western of adult males after 3 days of induction at 29°C probed with 4C9 antibody. No evidence of discrete bands of any size other than the expressed peptide sizes is seen. The signal in the 90 lane is weak, presumably due to all of the protein having been incorporated into insoluble species that were unable to enter the gel.

Figure S3. High-resolution images of fragment accumulation in eye discs. Images taken with a Zeiss 63x objective are presented here. In (A), the confocal settings are similar to those used for optimum exposure in Figure 5. The bottom set of images (B) shows the same images as the top, but with the maximum brightness and contrast in imageJ adjusted from 255 to ~20 to make the diffuse huntingtin more visible. This reveals the relative level of signal between the different fragments and allows one to visualize the distribution of the various fragments. (Green = HTT, blue = DAPI).

Figure S4. Titration of seeding potential. The limit of detection for the 90 and 108misfolded HTT fragments was tested by gradually reducing the amount of larvae homogenate, while keeping the quantity of A11 antibody in the immunopurification the same. The 108 is about 25% as potent in seeding amyloid as the 90 fragment. Other fragments do not achieve statistical significance for seeding above background levels.

Figure S5. Sequences of the constructs used in this research. Fragment DNA was inserted using the phiC31 targeted insertion system (Bischof et al., 2007) into the multi-cloning site of the pUASTattB vector (see GenBank: EF362409.1 for numbered sequence), between the *Eco*RI restriction site (GAATTC, ending at nucleotide 5245) and either the *Xho*I or the *Xba*I site (TCTAGA, beginning at nucleotide 5280). The sequence of the 90$^{ex1\ 120Q}$ fragment is listed in its entirety in (A). All of the other fragments begin with the sequence listed in 90$^{ex1\ 120Q}$, and then continue with the human Htt mRNA sequence (NCBI Reference Sequence: NM_002111.6) until the engineered stop codon as detailed in (B). The termini of the fragment constructs have sequences that differ at the DNA level from the human cDNA sequence (although the amino acid matches to the human protein are unchanged). Stocks were sequenced to verify the fidelity of the inserts. No mutations were detected in the fly lines presented.

Figure S6. Strategy for construction of the 120Q cassette. A series of 4 oligonucleotides were cloned into a pBSK vector with the multiple cloning site replaced with *Sph*I, *Eco*RI, *Hin*DIII, *Bam*HI, and *Spe*I. Use of class IIS restriction enzymes allow the 'scarless' healing of ligated fragments (described in methods).

Figure S7. Strategy for assembling an HTT fragment containing a 120Q cassette. The multiple cloning site of pBSK was replaced with a *Eco*RI, *Not*I, *Pst*I, and *Xho*I sites (1a) and. was further modified by cloning an oligonucleotide containing *Not*I, *Bse*RI facing left, *Bse*RI facing right, *Spe*I, *Sph*I, *Bsp*MI facing left, *Bsp*MI facing right, and *Pst*I sites into the *Not*I and *Pst*I sites (1b). The *Spe*I-Q_{120}-*Sph*I fragment from Fig. S6 was cloned into this plasmid (1c). The HTT coding sequences 5' and 3' from the site of the polyQs were PCR amplified from a full-length human HTT cDNA clone with 23Qs (a kind gift from P. J. Muchowski, UCSF) using primers that generated unique restriction sites (*Eco*RI-5'-*Not*I and *Pst*I-3'-*Xho*I respectively) (2a). The 5' part of the target gene is sealed to the left side of the Q-repeat coding sequence by deleting the spacer with *Bse*RI digestion followed by ligation (2a) and then repeating with *Bsp*MI digestion/ligation (2b). The resulting HTT gene with 120 Q's ends at aa 171 with an *Eco*RI site just before the start codon and a *Xho*I site at the end (3). Shorter fragments were made by PCR from the 171 clone. Using this *Eco*RI/*Xho*I cassette, all fragments were inserted into the pUAST-attB Drosophila transformation vector. The longer fragments were constructed by insertion of additional sequences at the *Xho*I site to extend the fragments.