Table 1. Overview of humanised murine and hamster PrP transgenic flies generated in the project

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Signal peptide</th>
<th>GPI-anchor</th>
<th>Disease association</th>
<th>Spontaneous misfolding</th>
<th>Chromosome</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo3F4WT UAS-GAL4</td>
<td>Murine</td>
<td>Murine</td>
<td>None</td>
<td>No</td>
<td>2</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>Mo3F4FFI UAS-GAL4</td>
<td>Murine</td>
<td>Murine</td>
<td>FFI</td>
<td>Yes</td>
<td>2</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>Mo3F4CJD UAS-GAL4</td>
<td>Murine</td>
<td>Murine</td>
<td>CJD</td>
<td>Yes</td>
<td>2</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>HaWT UAS-GAL4</td>
<td>Hamster</td>
<td>Hamster</td>
<td>None</td>
<td>No</td>
<td>2</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>HaFFI UAS-GAL4</td>
<td>Hamster</td>
<td>Hamster</td>
<td>FFI</td>
<td>Yes</td>
<td>2</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>HaCJD UAS-GAL4</td>
<td>Hamster</td>
<td>Hamster</td>
<td>CJD</td>
<td>Yes</td>
<td>2</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>Mo3F4WT UAS-GAL4</td>
<td>Insect</td>
<td>Murine</td>
<td>None</td>
<td>No</td>
<td>2</td>
<td>Transgenesis vector constructed in this project*</td>
</tr>
<tr>
<td>HaWT UAS-GAL4</td>
<td>Insect</td>
<td>Hamster</td>
<td>None</td>
<td>No</td>
<td>2</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>HaCJD UAS-GAL4</td>
<td>Insect</td>
<td>Hamster</td>
<td>CJD</td>
<td>Yes</td>
<td>2</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>HaCJD LexA-LexAop</td>
<td>Hamster</td>
<td>Hamster</td>
<td>CJD</td>
<td>Yes</td>
<td>3</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>HaWT UAS-GAL4 ; HaCJD LexAop-LexA</td>
<td>Hamster</td>
<td>Hamster</td>
<td>CJD</td>
<td>Yes</td>
<td>2+3</td>
<td>Characterised in this project*</td>
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</tbody>
</table>

*All fly lines generated in collaboration with Dr. Alana Thackray
Table 2. Overview of ovine PrP transgenic flies used or generated in the project

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Signal peptide</th>
<th>GPI-anchor</th>
<th>Disease association</th>
<th>Spontaneous misfolding</th>
<th>Chromosome</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRQ(GPI) UAS-GAL4</td>
<td>Insect</td>
<td>Ovine</td>
<td>None</td>
<td>No</td>
<td>2</td>
<td>Thackray et al., 2012</td>
</tr>
<tr>
<td>VRQ(cyt) UAS-GAL4</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>No</td>
<td>2</td>
<td>Thackray et al., 2014a</td>
</tr>
<tr>
<td>VRQ(ΔGPI) UAS-GAL4</td>
<td>Insect</td>
<td>None</td>
<td>Scrapie (GSS)</td>
<td>Yes</td>
<td>2</td>
<td>Thackray et al., 2014b</td>
</tr>
<tr>
<td>VRQ(GPI) LexAop-LexA</td>
<td>Insect</td>
<td>Ovine</td>
<td>None</td>
<td>No</td>
<td>3</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>VRQ(ΔGPI) LexAop-LexA</td>
<td>Insect</td>
<td>None</td>
<td>Scrapie (GSS)</td>
<td>Yes</td>
<td>3</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>VRQ(GPI) UAS-GAL4; VRQ(GPI)</td>
<td>Insect</td>
<td>Ovine</td>
<td>None</td>
<td>No</td>
<td>2 + 3</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>LexAop-LexA</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VRQ(GPI) UAS-GAL4; VRQ(ΔGPI)</td>
<td>Insect</td>
<td>None</td>
<td>Scrapie (GSS)</td>
<td>Yes</td>
<td>2 + 3</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>LexAop-LexA</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*All fly lines generated in collaboration with Dr. Alana Thackray
Table 3. Overview of human PrP transgenic flies generated in the project

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Signal peptide</th>
<th>GPI anchor</th>
<th>Disease association</th>
<th>Spontaneous misfolding</th>
<th>Chromosome</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>M129 UAS-GAL4</td>
<td>Insect</td>
<td>Human</td>
<td>None</td>
<td>No</td>
<td>2</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>V129 UAS-GAL4</td>
<td>Insect</td>
<td>Human</td>
<td>None</td>
<td>No</td>
<td>2</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>M129 UAS-GAL4</td>
<td>Insect</td>
<td>Human</td>
<td>None</td>
<td>No</td>
<td>3</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>V129 UAS-GAL4</td>
<td>Insect</td>
<td>Human</td>
<td>None</td>
<td>No</td>
<td>3</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>M129 UAS-GAL4; M129 UAS-GAL4</td>
<td>Insect</td>
<td>Human</td>
<td>None</td>
<td>No</td>
<td>2 + 3</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>V129 UAS-GAL4; V129 UAS-GAL4</td>
<td>Insect</td>
<td>Human</td>
<td>None</td>
<td>No</td>
<td>2 + 3</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>M129 UAS-GAL4; V129 UAS-GAL4</td>
<td>Insect</td>
<td>Human</td>
<td>None</td>
<td>No</td>
<td>2 + 3</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>V129 UAS-GAL4; M129 UAS-GAL4</td>
<td>Insect</td>
<td>Human</td>
<td>None</td>
<td>No</td>
<td>2 + 3</td>
<td>Characterised in this project*</td>
</tr>
</tbody>
</table>

*All fly lines generated in collaboration with Dr. Alana Thackray
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Signal peptide</th>
<th>GPI-anchor</th>
<th>Disease association</th>
<th>Spontaneous misfolding</th>
<th>Chromosome</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>N138 UAS-GAL4</td>
<td>Insect</td>
<td>Cervid</td>
<td>None</td>
<td>No</td>
<td>2</td>
<td>Characterised in this project*</td>
</tr>
<tr>
<td>S138 UAS-GAL4</td>
<td>Insect</td>
<td>Cervid</td>
<td>None</td>
<td>No</td>
<td>2</td>
<td>Characterised in this project*</td>
</tr>
</tbody>
</table>

*All fly lines generated in collaboration with Dr. Alana Thackray
Figure 1. Plasmid maps of pUASTattB and pJFRC-MUH plasmids.

**Figure legends:** The PrP insert was incorporated after restriction enzyme digest corresponding to the restriction sites of the plasmid. Both plasmids carried an attB site for homologous recombination with the attP site in the *Drosophila* genome, ampicillin resistance genes that were transcribed after transgene incorporation and a white+ mini gene that acted as a marker for *Drosophila* transgenesis and caused red-eye colouration in successfully manipulated flies. Plasmid maps shown were adjusted from the Addgene plasmid repository.
Figure 2. *Drosophila* transgenesis using *phiC31* site-specific recombination.

**Figure legends:** Homologous recombination between the attP site located on chromosome 2 or 3 depending on the recipient fly line used and the attB site located in the plasmid. The phiC31 integrase was delivered in the form of mRNA.
Figure 3. Process of fly balancing and stable stock maintenance.

Fly balancing

- chr 2
- PrP
- White+ mini gene
- +

- chr 2
- If
- CyO

- Balanced curly winged fly with white+ mini gene that induces red eye colouration

Stable stock maintenance

- chr 2
- PrP
- CyO
- White+ mini gene

- chr 2
- PrP
- CyO

- chr 2
- PrP
- CyO
- PrP

- Stable stock

Combination of two balancer chromosomes of the same genotype is lethal
**Table 5.** Genotypes of *Drosophila* transgenic for murine or hamster PrP carrying mutations associated with inherited human prion diseases FFI and CJD.

<table>
<thead>
<tr>
<th>PrP donor species</th>
<th>PrP transgene</th>
<th>Mutation</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo3F4WT</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mo3F4FF1</td>
<td>D177N</td>
<td>FFI</td>
<td></td>
</tr>
<tr>
<td>Mo3F4CJD</td>
<td>E199K</td>
<td>CJD</td>
<td></td>
</tr>
<tr>
<td><strong>Hamster</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HaWT</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>HaFF1</td>
<td>E178N</td>
<td>FFI</td>
<td></td>
</tr>
<tr>
<td>HaCJD</td>
<td>E200K</td>
<td>CJD</td>
<td></td>
</tr>
</tbody>
</table>

**Figure legends:** Mouse codon numbering is -1 compared to human due to a natural single codon deletion at the N-terminus. Mouse PrP was engineered to contain the L108M, V111M substitutions creating the 3F4 epitope. Hamster PrP carries the 3F4 epitope naturally.

WT: wild type; Mox: murine; Ha: hamster
Figure 4. PCR analysis of pBSKSII-PrP plasmid.

Samples:

Track 1 – PBSKSII-Mo3F4WT
Track 2 – PBSKSII-Mo3F4FFI
Track 3 – PBSKSII-Mo3F4CJD
Track 4 – Negative control (no template)
Track 5 – PBSKSII-HaWT
Track 6 – PBSKSII-HaFFI
Track 7 – PBSKSII-HaCJD

100 ng of purified plasmid DNA was subjected to PCR with mouse primers MoFI and MoRI (samples 1,2,3 and 4) or hamster primers HaFI and HaRI (samples 5,6,7). The samples were run on a 1% agarose gel at 80V for 50 min. Molecular weight markers are shown on the left hand side.
Figure 5. PCR analysis of mouse 3F4 and hamster pUAST-PrP transgenic flies (hamster and mouse specific primers).

Samples:
Track 1 – Mo 3F4 WT transgenic flies
Track 2 – Mo 3F4 FFI transgenic flies
Track 3 – Mo 3F4 CJD transgenic flies
Track 4 - Ha WT transgenic flies
Track 5 - Ha FFI transgenic flies
Track 6 – Ha CJD transgenic flies
Track 7 – Negative control – no PCR template

PCR with 2 μl fly head homogenate (5 fly heads in 25 μl PK-lysis buffer) with mouse (MoFI and MoRI) or hamster (HaFI and HaRI) specific primers. PCR: 40 cycles. The products were run on a 1% agarose gel at 80V for 60 min. Molecular weight markers are shown on the left hand side.
Figure 6. PCR verification of mouse and hamster PrP transgenic *Drosophila* (murine/hamster signal peptide) – pUAST primers.

Samples:
Track 1 – Mouse 3F4 wild type fly
Track 2 – Mouse 3F4 FFI fly
Track 3 – Mouse 3F4 CJD fly
Track 4 – Negative control (no template)
Track 5 – Hamster wild type fly
Track 6 – Hamster FFI fly
Track 7 – Hamster CJD fly

PCR with 2 µl fly head homogenate (5 fly heads in 25 µl PK-lysis buffer) with pUAST-F and pUAST-R primers. PCR: 40 cycles. Samples run on a 1% agarose gel at 80V for 50 min. Molecular weight marker shown on the left hand side.
Figure 7. Hamster forward primer (HaPDF1) binding site from the first step of semi-nested PCR to create a PrP transgene carrying an insect signal peptide sequence.

Figure legends: In this PCR step, the first part of the insect signal sequence is synthesised at the N-terminal part of the mature hamster WT PrP sequence. The same mechanism took place in the case of the mouse (MoPD1F) primer.

Figure 8. Semi-nested PCR to generate hamster and mouse PrP transgene with insect signal peptide - the first step.

Samples:
Track 1 – Hamster WT
Track 2 – Hamster CJD
Track 3 – Negative control (no template)
Track 4 – Mouse 3F4 WT
Track 5 – Mouse 3F4 CJD

The PCR products (25 ng) of a hamster and a mouse PrP transgenes were used as templates to generate HaPrP or Mo3F4PrP with an insect signal peptide sequence using HaPD1F and HaRI primers for hamster PrP and MoPD1F and MoRI primers for mouse 3F4 transgene. The samples were run on 1% agarose gel at 80V for 90 min. Molecular weight markers are shown on the left hand side.
Figure 9. Forward primer (PD2F) in the second step of the semi-nested PCR to create murine and hamster WT PrP with insect signal peptide.

Insect signal peptide
ATG GCC AGC AAA GTC TCG ATC CTT CTG CTA ACC GTC CAT CTT CTG GCT CAG ACC TTC GCC CAG
GCG GAA TTC CAT GCG AGC AAA GTC TCG ATC CTT CTG CTA ACC GTC CAT CTT CTG G PD2F primer
HaPD1F primer GTC CAT CTT CTG GCT CAG ACC TTC GCC CAG AAG AAG CAG AAG CCT G
EcoRI restriction site

Figure legends: In the second step of PCR to generate the full-length insect signal peptide, the PD2F primer binds to the previously generated part of an insect signal peptide sequence (by HaPD1F primer); PD2F primer is universal for both murine and hamster PrP and carries an EcoRI restriction enzyme site.

Figure 10. Semi-nested PCR to generate a hamster and mouse 3F4 PrP transgene with insect signal peptide – the second step.

Samples:
Track 1 – Hamster WT
Track 2 – Hamster CJD
Track 3 – Negative control (no template)
Track 4 – Mouse 3F4 WT
Track 5 – Mouse 3F4 CJD

The PCR product (5 µl) from the first step of the semi-nested PCR was used as a template to generate HaPrP and Mo3F4PrP with an insect signal peptide sequence using PD2F and HaRI (hamster) or MoRI (mouse 3F4) primers. The samples were run on a 1% agarose gel at 80V for 50 min. Molecular weight markers are shown on the left hand side.

Figures 9 & 10
Figure 11. Restriction enzyme digest of Mo3F4WT transgene with insect signal peptide and pUAST plasmid with ovine PrP insert.

Samples:
Track 1 – Mouse 3F4 WT
Track 2 – Mouse 3F4 WT
Track 3 – Mouse 3F4 WT
Track 4 – Blank track
Track 5 - pUAST plasmid with ovine PrP insert (faint band clearly visible on the light box)
Track 6 - pUAST plasmid with ovine PrP insert (faint band clearly visible on the light box)
Track 7 - pUAST plasmid with ovine PrP insert (faint band clearly visible on the light box)

The PCR products from the second step of semi-nested PCR were digested by XhoI and EcoRI (buffer H) for 90 min and run on a 1% low melting point agarose gel at 80V for 90 min. Molecular weight markers are shown on the left hand side.
Figure 12. PCR verification of a large-scale prep of pUAST-Mo3F4WT (insect signal peptide) transformed bacteria.

Samples:
Track 1 – Mouse 3F4 WT (culture 1)
Track 2 – Mouse 3F4 WT (culture 2)
Track 3 – negative control (no template)
Track 4 – positive control – 5 µl PCR product from 2nd round of semi-nested PCR

PCR with PD2F and MoRI primers. The products were run on a 1% agarose gel at 80V for 90 min. Molecular weight markers are shown on the left hand side.
Figure 13. PCR verification of a transgene presence in HaWT and HaCJD PrP (insect signal peptide) transgenic flies.

Samples:

Track 1 – HaWT M1
Track 2 – HaWT M2
Track 3 – HaWT M3
Track 4 – HaWT M4
Track 5 – HaWT M5
Track 6 – negative control (no template)
Track 7 – HaCJD M2
Track 8 – HaCJD M3
Track 9 – HaCJD M4
Track 10 – HaCJD M5
Track 11 – purified pUAST plasmid 500 ng

M1-M5 represent multiple fly lines generated and tested. PCR with 2 μl fly head homogenate (5 fly heads in 25 μl PK-lysis buffer) with pUAST-F and -R primers; 40 cycles, the products were run on a 1% agarose gel at 80V for 50 min. Molecular weight markers are shown on the left hand side.
Figure 14. PCR of chromosome 3 hamster CJD flies with pJFRC19 specific primers.

Samples:

Track 1 – HaCJD fly head homogenate (AMTC line 7)
Track 2 – HaCJD fly head homogenate (AMTC line 7b)
Track 3 – negative control (no template)
Track 4 – positive control pJFRC-VRQm purified plasmid 200 ng

PCR with 2 μl fly head homogenate (5 fly heads in 25 μl PK-lysis buffer) with pJFRC19 forward and reverse primers; 40 cycles, 50°C annealing temperature. The products were run on a 1% agarose gel at 80V for 50 min. Molecular weight markers are shown on the left hand side.

Figure 15. Dual PrP fly balancing and assembly process.
Figure legends: The resulting fly genotype was achieved using double balancers that carry phenotypic markers on both chromosome 2 and 3. Using the phenotypic markers as trackers, the correct fly genotype can be established in the final cross. The combination of markers MKRS and TM6C is lethal, as well as combinations of two CyO or two TM6B alleles.
**Figure 16. Verification of ovine PrP transgenic flies with PrP on chromosome 3.**

**Samples:**

Track 1 – VRQ(GPI) transgenic fly  
Track 2 – VRQ(GPI) transgenic fly  
Track 3 – negative control (no template)  
Track 4 – positive control 100 ng pJFRC19-MUH-VRQ(GPI) purified plasmid  
Track 5 – VRQ(ΔGPI) transgenic fly  
Track 6 – VRQ(ΔGPI) transgenic fly  
Track 7 – negative control (no template)  
Track 8 - positive control 100 ng pJFRC19-MUH-VRQ(ΔGPI) purified plasmid

PCR with 2 µl fly head homogenate (5 fly heads in 25 µl PK-lysis buffer) with VRQm-F, VRQm-R, VRQs-F, VRQs-R primers; 40 cycles. The products were run on a 1% agarose gel at 80V for 50 min. Molecular weight markers are shown on the left hand side.
Figure 17. PCR of chromosome 3 VRQ(GPI) flies with pJFRC specific primers.

Samples:

Track 1 – VRQ(GPI)/TM6C fly head homogenate
Track 2 – VRQ(GPI)/TM6C fly head homogenate
Track 3 – VRQ(GPI)/TM6C fly head homogenate
Track 4 – negative control (no template)
Track 5 – VRQ(GPI)-pJFRC purified plasmid 400 ng
Track 6 – VRQ(GPI)-pJFRC purified plasmid 200 ng

PCR with 0.5 μl fly head homogenate (5 fly heads in 25 μl PK-lys buffer) with pJFRC19 forward and reverse primers; 40 cycles. The products were run on a 1% agarose gel at 80V for 50 min. Molecular weight markers are shown on the left hand side.
Figure 18. PCR of chromosome 3 VRQ(ΔGPI) flies with pJFRC specific primers.

Samples:

Track 1 – VRQ(ΔGPI)/TM6C fly head homogenate
Track 2 – VRQ(ΔGPI)/TM6C fly head homogenate
Track 3 – VRQ(ΔGPI)/TM6C fly head homogenate
Track 4 – negative control (no template)
Track 5 – VRQ(ΔGPI)-pJFRC purified plasmid 400 ng
Track 6 – VRQ(ΔGPI)-pJFRC purified plasmid 200 ng

PCR with 0.5 μl fly head homogenate (5 fly heads in 25 μl PK-lysis buffer) with pJFRC19 forward and reverse primers; 40 cycles. The products were run on a 1% agarose gel at 80V for 50 min. Molecular weight markers are shown on the left hand side.
Figure 19. PCR of chromosome 2 human PrP flies with pUAST specific primers.

Samples:

Track 1 – V129 human PrP transgenic fly (sample 1)
Track 2 – V129 human PrP transgenic fly (sample 1b)
Track 3 – M129 human PrP transgenic fly (sample 3)
Track 4 – M129 human PrP transgenic fly (sample 3b)

PCR with 0.5 μl fly head homogenate (5 fly heads in 25 μl PK-lysis buffer) with pUAST forward and reverse primers; 40 cycles, annealing temperature 55°C. The products were run on a 1% agarose gel at 80V for 50 min. Molecular weight markers are shown on the left hand side.
Figure 20. PCR of chromosome 3 human V129 and M129 flies with pJFRC19 specific primers.

Samples:

Track 1 – V129 human PrP fly head homogenate (line 2)
Track 2 – V129 human PrP fly head homogenate (line 2b)
Track 3 – M129 human PrP fly head homogenate (line 4)
Track 4 – M129 human PrP fly head homogenate (line 4b)

PCR with 0.5 µl fly head homogenate (5 fly heads in 25 µl PK-lysis buffer) with pJFRC19 forward and reverse primers; 40 cycles, 50°C annealing temperature. The products were run on a 1% agarose gel at 80V for 50 min. Molecular weight markers are shown on the left hand side.
Figure 21. PCR of chromosome 2 cervid PrP flies with pUAST specific primers.

Samples:

Track 1 – N138 cervid PrP fly head homogenate (line 5)
Track 2 – N138 cervid PrP fly head homogenate (line 5b)
Track 3 – S138 cervid PrP fly head homogenate (line 6)
Track 4 – S138 cervid PrP fly head homogenate (line 6b)
Track 5 – negative control – no template
Track 6 – 400 ng of pUAST purified plasmid

PCR with 0.5 μl fly head homogenate (5 fly heads in 25 μl PK-lysis buffer) with pUAST forward and reverse primers; 40 cycles, annealing temperature 55°C. The products were run on a 1% agarose gel at 80V for 50 min. Molecular weight markers are shown on the left hand side.
Table 7. Classification of *Drosophila* driver systems.

<table>
<thead>
<tr>
<th>Effector</th>
<th>Function</th>
<th>Target</th>
<th>Origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAL4</td>
<td>Transcriptional activator</td>
<td>UAS</td>
<td><em>S. cerevisiae</em></td>
<td>(Brand and Perrimon, 1993)</td>
</tr>
<tr>
<td>GAL80</td>
<td>Transcriptional repressor by direct binding to GAL4</td>
<td>GAL4</td>
<td><em>S. cerevisiae</em></td>
<td>(Ma and Ptashne, 1987)</td>
</tr>
<tr>
<td>LexA</td>
<td>Transcriptional activator</td>
<td>lexAop</td>
<td><em>Escherichia coli</em></td>
<td>(Brent and Ptashne, 1980, Brent and Ptashne, 1981)</td>
</tr>
<tr>
<td>Flp</td>
<td>Recombinase that excises DNA sequences flanked by two identical (homotypic) FRT sites</td>
<td>FRT</td>
<td><em>S. cerevisiae</em></td>
<td>(Golic and Lindquist, 1989, McLeod et al., 1986, Bischof and Basler, 2008)</td>
</tr>
<tr>
<td>Cre</td>
<td>Recombinase that excises DNA sequences flanked by two homotypic loxP sites</td>
<td>loxP</td>
<td>Bacteriophage P1</td>
<td>(Bischof and Basler, 2008, Abremski and Hoess, 1984)</td>
</tr>
<tr>
<td>PhC31</td>
<td>Integrase that mediates DNA integration in the genome. Unlike Cre and Flp, targets heterotypic att sites</td>
<td>attB/attP</td>
<td>Streptomyces bacteriophage PhC31</td>
<td>(Bischof et al., 2007, Thorpe and Smith, 1998)</td>
</tr>
<tr>
<td>VP16</td>
<td>Transactivation domain, insensitive to GAL80 repression</td>
<td>Multiple transcription factors</td>
<td>Herpes simplex virus</td>
<td>(Croston et al., 1992, Hall and Struhl, 2002, Triezenberg et al., 1988)</td>
</tr>
</tbody>
</table>

Adapted from (del Valle Rodríguez et al., 2012)
Table 8. Full list of fly driver lines used in the project.

<table>
<thead>
<tr>
<th>Driver fly line</th>
<th>System(s)</th>
<th>Target</th>
<th>Dual</th>
</tr>
</thead>
<tbody>
<tr>
<td>elav-GAL4</td>
<td>UAS-GAL4</td>
<td>Pan-neuronal</td>
<td>No</td>
</tr>
<tr>
<td>57C10-GAL4</td>
<td>UAS-GAL4</td>
<td>Pan-neuronal</td>
<td>No</td>
</tr>
<tr>
<td>repo-GAL4</td>
<td>UAS-GAL4</td>
<td>Glial cells</td>
<td>No</td>
</tr>
<tr>
<td>57C10-LexA</td>
<td>LexA-LexAop</td>
<td>Pan-neuronal</td>
<td>No</td>
</tr>
<tr>
<td>57C10-GAL4 ;</td>
<td>LexA-LexAop</td>
<td>Pan-neuronal and glial cells</td>
<td>Yes</td>
</tr>
<tr>
<td>repo-LexA</td>
<td>UAS-GAL4 ; LexA-LexAop</td>
<td>Pan-neuronal and glial cells</td>
<td></td>
</tr>
<tr>
<td>57C10-GAL4 ;</td>
<td>LexA-LexAop</td>
<td>Pan-neuronal and glass multiple</td>
<td></td>
</tr>
<tr>
<td>GMR-LexA</td>
<td>UAS-GAL4 ; LexA-LexAop</td>
<td>reporter (GMR) in the eye</td>
<td></td>
</tr>
<tr>
<td>57C10-GAL4 ;</td>
<td>LexA-LexAop</td>
<td>Pan-neuronal and sensory neurons of</td>
<td></td>
</tr>
<tr>
<td>iav-LexA</td>
<td>UAS-GAL4 ; LexA-LexAop</td>
<td>chordotonal organs</td>
<td>Yes</td>
</tr>
<tr>
<td>57C10-GAL4 ;</td>
<td>LexA-LexAop</td>
<td>Pan-neuronal and unspecified interneurons</td>
<td>Yes</td>
</tr>
<tr>
<td>71A10-LexA</td>
<td>UAS-GAL4 ; LexA-LexAop</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57C10-GAL4 ;</td>
<td>LexA-LexAop</td>
<td>Pan-neuronal and basin interneurons</td>
<td>Yes</td>
</tr>
<tr>
<td>72F11-LexA</td>
<td>UAS-GAL4 ; LexA-LexAop</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57C10-GAL4 ;</td>
<td>LexA-LexAop</td>
<td>Pan-neuronal and basin interneurons</td>
<td>Yes</td>
</tr>
<tr>
<td>20B01-LexA</td>
<td>UAS-GAL4 ; LexA-LexAop</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table legends: The single fly driver lines express under the control of one driver system and dual fly driver lines combine two different expression systems that allow for expression of two independent transgenes (if placed under the respective promoter)
Figure 22. Bicinchoninic acid assay (BCA) with PrP transgenic and 51D fly head homogenates.

![Graph showing BCA assay results for different samples.]

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Absorbance (A490)</th>
<th>SD</th>
<th>Mean interpolated conc. µg/ml</th>
<th>Mean fly head protein content</th>
</tr>
</thead>
<tbody>
<tr>
<td>51D 5 heads</td>
<td>0.379</td>
<td>0.0032</td>
<td>1313.6</td>
<td></td>
</tr>
<tr>
<td>51D 2.5 heads</td>
<td>0.237</td>
<td>0.0021</td>
<td>646.3</td>
<td>1.32 µg(±0.0151)</td>
</tr>
<tr>
<td>51D 1.25 heads</td>
<td>0.165</td>
<td>0.0017</td>
<td>307.9</td>
<td></td>
</tr>
<tr>
<td>51D 0.63 heads</td>
<td>0.119</td>
<td>0.0025</td>
<td>94.1</td>
<td></td>
</tr>
<tr>
<td>51D 0.31 heads</td>
<td>0.110</td>
<td>0.0025</td>
<td>47.1</td>
<td></td>
</tr>
<tr>
<td>ARQ 5 heads</td>
<td>0.396</td>
<td>0.0021</td>
<td>1393.5</td>
<td></td>
</tr>
<tr>
<td>ARQ 2.5 heads</td>
<td>0.269</td>
<td>0.0025</td>
<td>794.3</td>
<td>1.39 µg(±0.0098)</td>
</tr>
<tr>
<td>ARQ 0.125 heads</td>
<td>0.176</td>
<td>0.0055</td>
<td>362.0</td>
<td></td>
</tr>
<tr>
<td>ARQ 0.63 heads</td>
<td>0.129</td>
<td>0.0042</td>
<td>138.7</td>
<td></td>
</tr>
<tr>
<td>ARQ 0.31 heads</td>
<td>0.117</td>
<td>0.0023</td>
<td>82.4</td>
<td></td>
</tr>
</tbody>
</table>

Difference between genotypes: 1.32 µg(±0.0151)  vs 1.39 µg(±0.0098)

Statistics:
- Two-tailed t-test: P = 0.0339
- One-way ANOVA: P = 0.5377

**Figure legends:** 51D-UAS x elav-GAL4 and ARQ-UAS x elav-GAL4 fly head homogenates were prepared by homogenising 10 fly heads in 12 µl AEBSF lysis buffer. After sonication on ice and centrifugation, 10 µl of each supernatant was taken out and diluted to achieve 5 fly head equivalent in 5 µl of a solution in the first well. The dilution medium was 50mM sodium acetate. The homogenate titrations were performed in triplicates. Bovine serum albumin (BSA) standard two-fold dilution started at 2 mg/ml per well.

Plate reading: 490 nm, incubation at 37 °C for 90 min
Figure 23. 57C10-LexA expression of fluorescent proteins driven pan-neuronally in *Drosophila* larvae.

(A) LexA driven pan-neuronal expression of membrane-targeted GFP (green) in *Drosophila* larval CNS
(B) LexA driven pan-neuronal expression of membrane-targeted td-Tomato (red) in *Drosophila* larval CNS

Dorsal view with anterior side to the left; Scale bars = 25 μm

Figure 24. Repo-LexA expression in glial cells (GFP) and 57C10-GAL4 pan-neuronal expression (Tomato Red) in *Drosophila* larvae.

(A) 57C10-GAL4 pan-neuronal expression of membrane targeted td-Tomato (red) in *Drosophila* larval CNS
(B) Repo-LexA GFP expression in glial cells (apart from midline glia) in *Drosophila* larval CNS
(C) Repo-LexA and 57C10-GAL4 expression combined in *Drosophila* larval CNS
(D) Detailed repo-LexA GFP expression in peripheral glia in *Drosophila* larval CNS

Dual fluorescent protein transgenic flies; dorso-lateral view with anterior side to the left; Scale bars = 25 μm

Figures 23 & 24
Figure 25. iav-LexA expression in sensory neurons of chordotonal organs (GFP) and 57C10-GAL4 pan-neuronal expression (Tomato Red) in Drosophila larvae

(A) 57C10-GAL4 pan-neuronal expression of membrane targeted td-Tomato (red) in Drosophila larval CNS
(B) iav-LexA GFP expression in sensory neurons of chordotonal organs in Drosophila larval CNS
(C) iav-LexA and 57C10-GAL4 expression combined in Drosophila larval CNS
(D) Detailed iav-LexA GFP expression in peripheral nerves in Drosophila larval CNS

Dual fluorescent protein transgenic flies; Dorsal view with anterior side to the left; Scale bars = 25 μm

Figure 26. 71A10-LexA expression in unspecified classes of interneurons (GFP) and 57C10-GAL4 pan-neuronal expression (Tomato Red) in Drosophila larvae

(A) 57C10-GAL4 pan-neuronal expression of membrane targeted td-Tomato (red) in Drosophila larval CNS
(B) 71A10-LexA GFP expression in interneurons in Drosophila larval CNS
(C) 71A10-LexA and 57C10-GAL4 expression combined in Drosophila larval CNS

Dual fluorescent protein transgenic flies; Dorso-lateral view with anterior side to the left; Scale bars = 25 μm
Figure 27. 72F11-LexA expression driven in basin interneurons (GFP) and 57C10-GAL4 pan-neuronal expression (Tomato Red) in *Drosophila* larvae.

(A) 57C10-GAL4 pan-neuronal expression of membrane targeted td-Tomato (red) in *Drosophila* larval CNS

(B) 72F11-LexA GFP expression in ‘basin’ (postsynaptic partners of the chordotonal and class IV sensory organs) interneurons in *Drosophila* larval CNS

(C) 72F11-LexA and 57C10-GAL4 expression combined in *Drosophila* larval CNS

Dual fluorescent protein transgenic flies; Dorsal view with anterior side to the left; Scale bars = 25 μm

Figure 28. 20B01-LexA expression driven in basin interneurons (GFP) and 57C10-GAL4 pan-neuronal expression (Tomato Red) in *Drosophila* larvae.

(A) 57C10-GAL4 pan-neuronal expression of membrane targeted td-Tomato (red) in *Drosophila* larval CNS

(B) 20B01-LexA GFP expression in ‘basin’ (postsynaptic partners of the chordotonal sensory organs) interneurons in *Drosophila* larval CNS

(C) 20B01-LexA and 57C10-GAL4 expression combined in *Drosophila* larval CNS

Dual fluorescent protein transgenic flies; Dorsal view with anterior side to the left; Scale bars = 25 μm
Figure 29. PrP transgenic fly driver crossing (GAL4 and LexA drivers)

**elav-GAL4 cross**

- **chr 2**: UAS-PrP, CyO
- **chr 2**: Elav-GAL4 driver
- **PrP** pan-neuronally expressed under the control of elav-GAL4 driver

**repo-LexA cross**

- **chr 2**: +, LexAop-PrP, TM6C
- **chr 3**: +, CyO
- **PrP** expressed in glial cells under the control of repo-LexA driver

**Figure legends:** The PrP expression is triggered in the progeny of PrP transgenic fly by crossing with either GAL4 driver, in the case of the UAS controlled fly or LexA driver, if the fly is LexAop controlled. The crosses with other Gal4 and LexA drivers are based on the same mechanism but the expression of PrP is targeted to different tissues or cell types.
Figure 30. Western blot detection of murine 3F4 PrP transgenic flies expressing PrP under elav-GAL4 control (chromosome 2).

Samples:
Track 1 – Ovine VRQ(GP)-UAS x elav-GAL4 (positive control)
Track 2 – 51D-UAS x elav-GAL4 (negative control)
Track 3 – Mouse 3F4 WT-UAS x elav-GAL4
Track 4 – Mouse 3F4 FFI-UAS x elav-GAL4
Track 5 – Mouse 3F4 CJD-UAS x elav-GAL4
Track 6 – VRQ recombinant protein 25 ng (positive control)

Head homogenates from 5 male head equivalents in 10 µl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 10 µl of Laemmli buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 µl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 31. Western blot detection of hamster PrP transgenic flies expressing PrP under elav-GAL4 control (chromosome 2).

Samples:

Track 1 – Ovine VRQ(GPI)-UAS x elav-GAL4 (positive control)
Track 2 – 51D-UAS x elav-GAL4 (negative control)
Track 3 – Hamster WT-UAS x elav-GAL4
Track 4 – Hamster FFI-UAS x elav-GAL4
Track 5 – Hamster CJD-UAS x elav-GAL4
Track 6 – VRQ recombinant protein 25 ng (positive control)

Head homogenates from 5 male head equivalents in 10 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 10 μl of Laemmli buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 μl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 32. Capture-detector ELISA of mouse 3F4 and hamster WT PrP transgenic flies driven by elav-GAL4 from chromosome 2.

Samples:
Mo3F4WT: mouse 3F4 wild type-UAS x elav-GAL4
HaWT: hamster wild type-UAS x elav-GAL4
51D: 51D-UAS x elav-GAL4 (negative control)

Head homogenates from 10 male fly head equivalents in 10 µl AEBSF lysis buffer (the volume was made up to 50 µl with PBS). CD-ELISA standard: VRQ recombinant protein starting at 1000 ng/ml (in 2-fold dilution steps in PBS). All samples were prepared in triplicates. Capture antibody: anti-PrP monoclonal antibody 245 (biotinylated); detector antibody: SAF32; detection system: PNPP/alkaline phosphatase – exposure time 90 minutes, measured at OD

Approximate PrP content was calculated per 1 fly head (51D background subtracted):

Mouse 3F4 WT/elav-GAL4 = 5.8 ng (±0.0241)
Hamster WT/elav-GAL4 = 6.7 ng (±0.009)

Statistics:
Comparison of mouse 3F4 WT and hamster WT PrP transgenic fly expression levels:
Two-tailed t-test: P = 0.0042 (PrP expression level was significantly elevated in Hamster WT PrP expressing flies)

Comparison of PrP expression levels in contrast to the negative control 51D fly:
Two-tailed t-test: comparison of mouse 3F4 WT and hamster WT with 51D negative control flies resulted in P < 0.0001 (PrP expression levels were significantly different from the negative control 51D flies)
Figure 33: Insect signal peptide hamster WT and CJD PrP transgenic flies.

Samples:

Track 1 – Hamster WT-UAS (hamster signal peptide) x elav-GAL4 (positive control)
Track 2 – Hamster WT-UAS (insect signal peptide) x elav-GAL4 (line M1)
Track 3 – Hamster WT-UAS (insect signal peptide) x elav-GAL4 (line M2)
Track 4 – Hamster CJD-UAS (hamster signal peptide) x elav-GAL4 (positive control)
Track 5 – Hamster CJD-UAS (insect signal peptide) x elav-GAL4 (line M1)
Track 6 – Hamster CJD-UAS (insect signal peptide) x elav-GAL4 (line M2)

Head homogenates from 4 male head equivalents in 5 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 5 μl of Laemmli buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 μl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Ponceau S staining was used as a loading control. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 34: Hamster PrP transgenic flies with hamster signal peptide (chromosome 3).

Samples:

Track 1 – Hamster CJD-UAS x 57C10-GAL4; repo-LexA  
Track 2 – Hamster CJD-LexAop x 57C10-GAL4; repo-LexA

Head homogenates from 5 male head equivalents in 5 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 5 μl of Laemmli buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 μl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Ponceau S staining was used as a loading control. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 35. Dual PrP transgenic fly crossing with GAL4/LexA dual driver.

Dual PrP transgenic fly simultaneously expressing two types of PrP (no phenotypic markers present)

Figure legends: The dual PrP transgenic fly that is created according to the crossing scheme above expresses two types of PrP from both chromosome 2 and 3. The repo-LexA fly driver is used as an example; the situation is the same in the case of iav-LexA or GMR-LexA where the expression is targeted to different tissue/cell types. The crossing scheme was the same in the case of crossing of the dual drivers with fluorescent protein transgenic flies (these replaced the PrP transgenic flies).
Figure 36: Dual hamster PrP transgenic WT/CJD double driven (GAL4/LexA) fly PrP expression.

Samples:

Track 1 – Hamster WT-UAS x 57C10-GAL4/repo-LexA
Track 2 – Hamster CJD-LexAop x 57C10-GAL4/repo-LexA
Track 3 – Hamster WT-UAS/HaCJD-LexAop x 57C10-GAL4/ repo-LexA

Track 4 – Hamster WT-UAS x 57C10-GAL4/GMR-LexA
Track 5 – Hamster CJD-LexAop x 57C10-GAL4/GMR-LexA
Track 6 – Hamster WT-UAS/HaCJD-LexAop x 57C10-GAL4/ GMR-LexA

Track 7 – Hamster WT-UAS x 57C10-GAL4/iav-LexA
Track 8 – Hamster CJD-LexAop x 57C10-GAL4/iav-LexA
Track 9 – Hamster WT-UAS/HaCJD-LexAop x 57C10-GAL4/ iav-LexA

Head homogenates from 2 female head equivalents in 5 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 5 μl of Laemmli buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 μl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Ponceau S staining was used as a loading control. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 37. Western blot detection of pan-neuronal PrP expression in ovine PrP transgenic flies driven by elav-GAL4 driver.

Samples:

Track 1 – VRQ(GP1)-UAS x elav-GAL4  
Track 2 – VRQ(ΔGP1)-UAS x elav-GAL4  
Track 3 – VRQ(cyt)-UAS x elav-GAL4  
Track 4 – 51D-UAS x elav-GAL4 (negative control)  
Track 5 – ARQ+RFP-UAS x elav-GAL4 (positive control)

Head homogenates from 5 male head equivalents in 10 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 10 μl of Laemmli buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 μl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 38. Capture-detector ELISA of ovine PrP transgenic flies.

<table>
<thead>
<tr>
<th>OD_{415}</th>
<th>51D 10 heads</th>
<th>VRQ(GPl) 20 heads</th>
<th>VRQ(ΔGPl) 10 heads</th>
<th>VRQ(Cyt) 10 heads</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>3.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Samples:

51D: 51D-UAS x elav-GAL4 (negative control)
VRQ(GPl): VRQ(GPl)-UAS x elav-GAL4
VRQ(ΔGPl): VRQ(ΔGPl)-UAS x elav-GAL4
VRQ(cyt): VRQ(cyt)-UAS x elav-GAL4

Head homogenates from 10 or 20 male fly head equivalents in 10 μl AEBSF lysis buffer (the volume was made up to 50 μl with PBS). CD-ELISA standard: VRQ recombinant protein starting at 1000 ng/ml (in 2-fold dilution steps in PBS). All samples were prepared in triplicates. Capture antibody: anti-PrP monoclonal antibody 245 (biotinylated); detector antibody: SAF32; detection system: PNPP/alkaline phosphatase – exposure time 90 minutes, measured at OD_{415}

Approximate PrP content was calculated per 1 fly head (51D background subtracted):

VRQ(GPl)-UAS/elav-GAL4 = 0.2 ng (±0.00159)
VRQ(ΔGPl)-UAS/elav-GAL4 = 1.5 ng (±0.31359)
VRQ(cyt)-UAS/elav-GAL4 = 0.1 ng (±0.00239) (not quantifiable by ELISA due to a different conformation as seen in Thackray, et al., 2014b)

Statistics:

Comparison of ovine PrP transgenic fly expression levels:
Two-tailed t-test: VRQ (GPl), vs. VRQ(ΔGPl): P < 0.0001, VRQ(GPl) vs. VRQ(cyt): P = 0.003, VRQ(ΔGPl) vs. VRQ(cyt): P < 0.0001 (PrP contents in different topological variants of PrP expressed in the fly are significantly different in all cases)

Comparison of PrP expression levels of all genotypes to negative 51D flies:
Two-tailed t-test: All VRQ (GPl), VRQ(ΔGPl) and VRQ(cyt) PrP contents are significantly elevated above the level of 51D negative control flies (P < 0.0001).
Table 9. Phenotypes of the progeny from ovine PrP transgenic flies crossed with 57C10-LexA pan-neuronal driver fly line.

<table>
<thead>
<tr>
<th>PrP genotype</th>
<th>curly wings, stubble</th>
<th>curly wings, non-stubble</th>
<th>straight wings, stubble</th>
<th>straight wings, non-stubble</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRQ(GPI)</td>
<td>53</td>
<td>46</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td>VRQ(ΔGPI)</td>
<td>46</td>
<td>39</td>
<td>49</td>
<td>0</td>
</tr>
</tbody>
</table>

Table legends: After the cross of chromosome 3 ovine PrP-LexAop transgenic flies with a 57C10-LexA pan-neuronal driver, the progeny of interest (straight-wings, non-stubble) were absent as shown in this table. The male to female ratio was 1:1. Absolute count of progeny hatching and collected over the course of 3 days is shown here.

Figure 39. Fluorescent markers associated with PrP expression – lethality tracing.

(A) The PrP positive larvae can be distinguished by td-Tomato red fluorescence in the CNS without yellow fluorescence in the eye
(B) The PrP negative flies exhibit a combination of yellow (YFP) expression in the eye and td-Tomato red fluorescence in CNS
(C) Another PrP negative fly phenotype exhibits solely yellow (YFP) expression in the eye

The last possible phenotype occurring in the progeny are flies with no fluorescence (not shown) that have no PrP expression. The right genotype (A) was selected based on these phenotypic features.

Dorsal view with anterior side to the right. Scale bars = 25 μm
Table 10. Results of the lethality tracing experiment for VRQ(GPI)-LexAop/57C10-LexA flies.

<table>
<thead>
<tr>
<th>Categories observed</th>
<th>VRQ(GPI)-LexAop/57C10-LexA 1</th>
<th>Negative 1</th>
<th>VRQ(GPI)-LexAop/57C10-LexA 2</th>
<th>Negative 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of larvae collected</td>
<td>24</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Number of larvae after 5 days</td>
<td>18</td>
<td>11</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Adults hatched</td>
<td>6</td>
<td>11</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Probability to hatch</td>
<td>25%</td>
<td>55%</td>
<td>40%</td>
<td>100%</td>
</tr>
<tr>
<td>Probability to hatch compared to control flies</td>
<td>45%</td>
<td>-</td>
<td>40%</td>
<td>-</td>
</tr>
<tr>
<td>Larvae disturbing agar surface</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Ability to fly</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure legends Two test sets of PrP expressing larvae were set up with their respective controls (non-PrP transgenic flies based on their fluorescent phenotype) and the probability to hatch was calculated based on day-to-day observation. The observed behaviour differences of test and control larvae were the ability of larvae to disturb the agar surface and ability to fly after hatching.
Figure 40. Western blot detection of pan-neuronally expressed PrP in VRQ(GPI)-LexAop transgenic fly under 57C10-LexA control.

Samples:

Track 1 – VRQ(GPI)-UAS x elav-GAL4 (positive control)
Track 2 – VRQ(GPI)-LexAop (chromosome 3) x 57C10-LexA
Track 3 – AttP2-LexAop (chromosome 3) x 57C10-LexA (negative control)
Track 4 – 25 ng VRQ recombinant protein (positive control)

Head homogenates from 5 male head equivalents in 10 µl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 10 µl of Laemmli buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 µl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 41. Western blot detection of VRQ-LexAop expression levels of ovine PrP transgenic flies driven by various LexA subset specific drivers.

Results:

Track 1 – VRQ(GPI)-UAS chromosome 2 x elav-GAL4 (control)
Track 2 – VRQ(ΔGPI)-UAS chromosome 2 x elav-GAL4 (control)
Track 3 – VRQ(GPI)-LexAop chromosome 3 x 71A10-LexA
Track 4 – VRQ(GPI)-LexAop chromosome 3 x 72F11-LexA
Track 5 – VRQ(GPI)-LexAop chromosome 3 x iav-LexA
Track 6 – VRQ(GPI)-LexAop chromosome 3 x repo-LexA
Track 7 – VRQ(GPI)-LexAop chromosome 3 x GMR-LexA

Head homogenates from 5 male head equivalents in 10 µl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 10 µl of Laemmli buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 µl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Ponceau S staining was used as a loading control. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 42. Capture-detector ELISA of ovine PrP transgenic flies (chromosome 3).

![Graph showing CD-ELISA - LexA driven (chr 3) ovine PrP transgenic flies](image)

**Samples:**

AttP2-iv: AttP2-LexAop (chromosome 3) x iav-LexA (negative control)  
VRQ(GPl)-iv: VRQ(GPl)-LexAop (chromosome 3) x iav-LexA  
VRQ(GPl)-repo: VRQ(GPl)-LexAop (chromosome 3) x repo-LexA

Head homogenates from 10 male fly head equivalents in 10 µl AEBSF lysis buffer (the volume was made up to 50 µl with PBS). CD-ELISA standard: VRQ recombinant protein starting at 1000 ng/ml (in 2-fold dilution steps in PBS). All samples were prepared in duplicates. Capture antibody: anti-PrP monoclonal antibody 245; detector antibody: SAF32; detection system: PNPP/alkaline phosphatase – exposure time 90 minutes, measured at OD415

Approximate PrP content was calculated per 1 fly head (attP2 background subtracted):

VRQ(GPl)-LexAop/iv-LexA = 0.3 ng (±0. 0.02937)  
VRQ(GPl)-LexAop/repo-LexA = 5.0 ng (±0.019287)

**Statistics:**

Comparison of ovine PrP (chromosome 3 - LexA) transgenic fly expression levels:  
Two-tailed t-test: VRQ(GPl)-iv vs. VRQ(GPl)-repo: P < 0.0001 (contents of PrP expressed in the fly are significantly different between iav-LexA and repo-LexA driven flies)

Comparison of PrP expression levels of both genotypes to negative 51D flies:  
Two-tailed t-test: Both VRQ(GPl)-iv (P = 0.0027) and VRQ(GPl)-repo (P < 0.0001) PrP contents are significantly elevated above the level of 51D negative control flies.
Figure 43. Dual PrP transgenic VRQ(GPI) ; VRQ(ΔGPI) double driven (GAL4 ; LexA) fly PrP expression testing.

Samples:

Track 1 – 51D-UAS ; nos-AttP2-LexAop (negative control)
Track 2 – VRQ(GPI)-UAS
Track 3 – VRQ(ΔGPI)-LexAop
Track 4 – VRQ(GPI)-UAS ; VRQ(GPI)-LexAop
Track 5 – VRQ(GPI)-UAS ; VRQ(ΔGPI)-LexAop

(The driver fly line genotypes can be seen next to the figure)

Head homogenates from 5 male head equivalents in 5 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 5 μl of Laemml buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 μl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Ponceau S staining was used as a loading control. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 44: Insect signal peptide human PrP transgenic flies (chromosome 2).

Samples:

Track 1 – Human V129-UAS (chromosome 2) x elav-GAL4 (line 1)
Track 2 – Human V129-UAS (chromosome 2) x elav-GAL4 (line 1b)
Track 3 – Human M129-UAS (chromosome 2) x elav-GAL4 (line 3)
Track 4 – Human M129-UAS (chromosome 2) x elav-GAL4 (line 3b)

Head homogenates from 5 male head equivalents in 10 µl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 10 µl of Laemmli buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 µl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Ponceau S staining was used as a loading control. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 45: Insect signal peptide human PrP transgenic flies (chromosome 2 and chromosome 3).

Samples:

Track 1 – Human V129-UAS (chromosome 2) x elav-GAL4
Track 2 – Human V129-UAS (chromosome 3) x elav-GAL4
Track 3 – Human M129-UAS (chromosome 2) x elav-GAL4
Track 4 – Human M129-UAS (chromosome 3) x elav-GAL4

Head homogenates from 2 (one female and one male) head equivalents in 5 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 5 μl of Laemmli buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 μl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Ponceau S staining was used as a loading control. Molecular mass markers (kDa) are shown on the left-hand side.

Figure 45
Figure 46: Insect signal peptide human PrP transgenic flies (chromosome 2, chromosome 3 and dual PrP flies).

Samples:

Track 1 – 51D-UAS x elav-GAL4 (negative control)
Track 2 – Human M129-UAS (chromosome 2) x elav-GAL4
Track 3 – Human M129-UAS (chromosome 3) x elav-GAL4
Track 4 – Human M129-UAS/M129-UAS (chromosome 2 + 3) x elav-GAL4
Track 5 – Human M129-UAS/V129-UAS (chromosome 2 + 3) x elav-GAL4
Track 6 – Human V129-UAS/M129-UAS (chromosome 2 + 3) x elav-GAL4
Track 7 – Human V129-UAS/V129-UAS (chromosome 2 + 3) x elav-GAL4

Head homogenates from 5 male fly head equivalents in 5 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 5 μl of Laemmli buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 μl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Ponceau S staining was used as a loading control. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 47. Capture-detector ELISA human PrP transgenic flies (chromosome 2, chromosome 3 and dual PrP flies).

![Graph showing CD-ELISA results for human PrP transgenic flies.]

**Samples:**
- **51D:** 51D-UAS x elav-GAL4 (negative control)
- **M129 ch2:** human M129-UAS (chromosome 2) x elav-GAL4
- **M129 ch3:** human M129-UAS (chromosome 3) x elav-GAL4
- **V129 ch2:** human V129-UAS (chromosome 2) x elav-GAL4
- **V129 ch3:** human V129-UAS (chromosome 3) x elav-GAL4
- **M129/M129:** human M129-UAS/M129-UAS (chromosome 2 + 3) x elav-GAL4

Head homogenates from 10 male fly head equivalents in 10 µl AEBSF lysis buffer (the volume was made up to 50 µl with PBS). CD-ELISA standard: VRQ recombinant protein starting at 1000 ng/ml (in 2-fold dilution steps in PBS). All samples were prepared in triplicates. Capture antibody: anti-PrP monoclonal antibody 245; detector antibody: SAF32; detection system: PNPP/alkaline phosphatase – exposure time 90 minutes, measured at OD$_{415}$

Approximate PrP content was calculated per 1 fly head (51D background subtracted):
- **M129 ch2 = 4.5 ng (±0.09405)**
- **M129 ch3 = 3.2 ng (±0.02121)**
- **V129 ch2 = 3.9 ng (±0.00919)**
- **V129 ch3 = 2.0 ng (±0.01061)**
- **M129/M129 = 5.2 ng (±0.02616)**

**Statistics:**

**Comparison of human PrP transgenic fly expression levels:**
Two-tailed t-test: M129 ch2 vs. M129 ch3: P = 0.0178, M129 ch2 vs. V129 ch2: P = 0.0762, M129 ch2 vs. V129 ch3: P = 0.0048, M129 ch2 vs. M129/M129: P = 0.0572, M129 ch3 vs. V129 ch2: P = 0.0035, M129 ch3 vs. V129 ch3: P = 0.0014, M129 ch3 vs. M129/M129: P = 0.0009, V129 ch2 vs. V129 ch3: P = 0.0002, V129 ch2 vs. M129/M129: P = 0.0015, V129 ch3 vs. M129/M129: P = 0.0003 (contents of PrP expressed in the fly are significantly different between genotypes, apart from the M129 ch2 and M129/M129 dual PrP expressing flies where there is an elevation in PrP levels that is not statistically different)

**Comparison of PrP expression levels of all genotypes to negative 51D flies:**
Two-tailed t-test: All M129 ch2 (P = 0.0015), M129 ch3 (P = 0.0002), V129 ch2 (P < 0.0001), V129 ch3 (P < 0.0001) and M129/M129 (P < 0.0001). PrP contents are significantly elevated above the level of 51D negative control flies.
Figure 48. Insect signal peptide cervid PrP transgenic flies (chromosome 2).

Samples:

Track 1 – Cervid N138-UAS (chromosome 2) x elav-GAL4 (line 5)
Track 2 – Cervid N138-UAS (chromosome 2) x elav-GAL4 (line 5b)
Track 3 – Cervid S138-UAS (chromosome 2) x elav-GAL4 (line 6)
Track 4 – Cervid S138-UAS (chromosome 2) x elav-GAL4 (line 6b)

Head homogenates from 5 male head equivalents in 10 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 10 μl of Laemmli buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 μl in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Ponceau S staining was used as a loading control. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 49. Western blot detection of PrP expression levels of 51D (elav-GAL4 and repo-GAL4 driven) flies.

Samples:
Track 1 – 51D-UAS x elav-GAL4  
Track 2 – 51D-UAS x repo-GAL4  
Track 3 – VRQ(GP)-UAS x elav-GAL4

The repo-GAL4 driver used for this cross triggers PrP expression in glial cells, whereas the elav-GAL4 driver triggers PrP expression pan-neuronally.

Head homogenates from 5 male head equivalents in 10 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 10 μl of Laemmli Buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Molecular mass markers (kDa) are shown on the left-hand side.
Figure A. Climbing ability assay of 51D control flies with or without scrapie inocula 0–40 days old (figure adapted from Thackray et al., 2014a)

**Figure legends:** The 51D-UAS x elav-GAL4 crossed control flies were assessed for locomotor activity by a negative geotaxis climbing assay following exposure at the larval stage (third instar) to VRQ/VRQ scrapie-infected (closed circles, continuous line) or scrapie-free (open circles, broken line) sheep brain homogenates. The mean PI ± S.D. is shown for three groups of 15 flies of each genotype per time point (45 flies in total for each group). Figure adapted from Thackray et al., 2014a
### Figure B

51D and ovine PrP transgenic flies with or without scrapie exposure subjected to protein misfolding cyclic amplification (PMCA) followed by PK-resistance western blot detection (results adapted from Thackray et al., 2018)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Age of flies (days)</th>
<th>PMCA result</th>
</tr>
</thead>
<tbody>
<tr>
<td>51D-UAS x elav-GAL4</td>
<td>Normal VRQ/VRQ brain homogenate</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(scrapie-free)</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>51D-UAS x elav-GAL4</td>
<td>Scrapie positive VRQ/VRQ brain homogenate PG127</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>VRQ(GPI)-UAS x elav-GAL4</td>
<td>Normal VRQ/VRQ brain homogenate</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(scrapie-free)</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>VRQ(GPI)-UAS x elav-GAL4</td>
<td>Scrapie positive VRQ/VRQ brain homogenate PG127</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>+</td>
</tr>
</tbody>
</table>

**Figure legends:** 51D-UAS x elav-GAL4 and VRQ(GPI)-UAS x elav-GAL4 PrP transgenic *Drosophila* were exposed at the larval stage to PG127 scrapie-infected or prion-free control sheep brain material. At various times after hatching, head homogenate was prepared from harvested flies and used as seed in PMCA reactions. Western blot detection of PK-resistant PrP27–30 in PMCA reaction products seeded with control or PG127-exposed *Drosophila* head homogenate. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 50. Position of three neuromuscular junctions (NMJs) analysed in a dissected *Drosophila* larva.

**Figure legends:** Confocal image of three analysed NMJs of aCC motor neurons (cyan) in abdominal hemi segments A4 and A5 on the left and right side (A4R, A4L and A5R) of the dorsal line (purple) were analysed in every immunostained dissected larva. Anti-HRP immunostaining labels presynaptic neuronal membrane of all bouton types (cyan). Each NMJ innervates dorsal acute muscle 1 (DA1) as shown by a yellow rectangle.
Figure 51. Gross morphological classes of neuromuscular junctions (NMJs) observed in *Drosophila* larvae.

**Figure legends:** Confocal images of dissected larvae revealing NMJs of aCC motor neurons innervating dorsal acute muscle 1 (DA1) in abdominal hemi segments A4 and A5 on the left and right side (A4R, A4L, and A5R) of the dorsal line.

Anti-HRP immunostaining labels the presynaptic neuronal membrane of all bouton types. Based on their appearance, the NMJs of all genotypes and treatment groups were categorized into three groups: 1) linear, 2) non-linear and 3) irregular NMJ. N≥25 NMJs analysed per treatment group.
Table 11. Gross morphology NMJ analysis quantified for all genotypes and treatment groups of *Drosophila* larvae.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Linear NMJs [%]</th>
<th>Non-linear NMJs [%]</th>
<th>Irregular NMJs [%]</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>51D control</td>
<td>53.3</td>
<td>33.3</td>
<td>13.3</td>
<td>30</td>
</tr>
<tr>
<td>51D prion inoculated</td>
<td>61.1</td>
<td>22.2</td>
<td>16.7</td>
<td>36</td>
</tr>
<tr>
<td>VRQ(GPI) control</td>
<td>44.1</td>
<td>38.2</td>
<td>17.6</td>
<td>34</td>
</tr>
<tr>
<td>VRQ(GPI) prion inoculated</td>
<td>51.7</td>
<td>20.7</td>
<td>27.6</td>
<td>29</td>
</tr>
<tr>
<td>VRQ(ΔGPI) control</td>
<td>55.6</td>
<td>38.9</td>
<td>5.6</td>
<td>36</td>
</tr>
<tr>
<td>VRQ(ΔGPI) prion inoculated</td>
<td>33.3</td>
<td>63.3</td>
<td>3.3</td>
<td>30</td>
</tr>
<tr>
<td>VRQ(cyt) control</td>
<td>20.0</td>
<td>16.0</td>
<td>64.0</td>
<td>25</td>
</tr>
<tr>
<td>VRQ(cytI) prion inoculated</td>
<td>26.5</td>
<td>11.8</td>
<td>61.8</td>
<td>34</td>
</tr>
</tbody>
</table>

**Table legends:** The gross morphology categories in each treatment group were quantified and the category that represents the biggest proportion of NMJs per each genotype is shown in bold. Quantification of each group is indicated as the percentage from the total number of NMJs analysed.
Figure 52. Analysis of neuronal architecture of larval neuromuscular junctions (NMJs) (aCC motor neuron).

Figure legends:

**Left**: the cable length (blue), NMJ spread (yellow) and number of branches (white) of an aCC motor neuron were quantified in each NMJ.

**Centre**: Number of boutons (white) and satellite boutons (blue) were counted; area of each bouton was measured (yellow).

**Right**: The active zones in each bouton were manually counted using the CellCounter plug-in in Fiji (ImageJ) programme.
Figure 53. Neuromuscular junction DA1 muscle area.

Figure legends:

Neuromuscular junction analysis of DA1 muscle area in PrP transgenic *Drosophila* larvae:

(A) genotype comparison

(B) treatment group comparison

Mean DA1 muscle area was established as indicated in Figure 50. Error bars = SEM; not significant = no stars and/or no brackets

P ≤ 0.05 = *, P ≤ 0.01 = **, P ≤ 0.001 = ***, P ≤ 0.0001 = ****. Outliers were excluded (ROUT Q = 1.000%)
Figure 54. Neuromuscular junction total synaptic bouton count.

![Figure 54](image)

**Figure legends:**

Neuromuscular junction analysis of total synaptic bouton count in PrP transgenic *Drosophila* larvae:

(A) genotype comparison

(B) treatment group comparison

Error bars = SEM; not significant = no stars and/or no brackets

P ≤ 0.05 = *; P ≤ 0.01 = **; P ≤ 0.001 = ***; P ≤ 0.0001 = ****. Outliers were excluded (ROUT Q = 1.000%)
Figure 55. Neuromuscular junction synaptic bouton count normalised to average DA1 muscle area and single bouton area.

Figure legends:

Neuromuscular junction analysis of synaptic bouton count normalised to average DA1 muscle area in PrP transgenic *Drosophila* larvae:

(A) treatment group comparison

(B) single bouton area treatment group comparison (bouton area normalised to the number of boutons)

Bouton area [μm$^2$]. Error bars = SEM; not significant = no stars and/or no brackets

$P \leq 0.05 = ^{*}; P \leq 0.01 = ^{**}; P \leq 0.001 = ^{***}; P \leq 0.0001 = ^{****}$. Outliers were excluded (ROUT Q = 1.000%)
Figure 56. Neuromuscular junction satellite bouton count.

Figure legends:

Neuromuscular junction analysis of satellite bouton count in PrP transgenic *Drosophila* larvae:

(A) genotype comparison

(B) treatment group comparison

Error bars = SEM; not significant = no stars and/or no brackets

$P \leq 0.05 = *; P \leq 0.01 = **; P \leq 0.001 = ***; P \leq 0.0001 = ****$. Outliers were excluded (ROUT Q = 1.000%)
Figure 57. Neuromuscular junction analysis of raw active zone count.

Figure legends:

Neuromuscular junction analysis of raw active zone count in PrP transgenic *Drosophila* larvae:

(A) genotype comparison

(B) treatment group comparison

Error bars = SEM; not significant = no stars and/or no brackets

P ≤ 0.05=*; P ≤ 0.01= **; P ≤ 0.001=***; P ≤ 0.0001=****. Outliers were excluded (ROUT Q = 1.000%)
Figure 58. Neuromuscular junction analysis of active zone count normalised to average DA1 muscle area.

Figure legends:

Neuromuscular junction analysis of active zone count normalised to average DA1 muscle area in PrP transgenic *Drosophila* larvae:

(A) genotype comparison

(B) treatment group comparison

Error bars = SEM; not significant = no stars and/or no brackets

P ≤ 0.05 = *, P ≤ 0.01 = **; P ≤ 0.001 = ***; P ≤ 0.0001 = ****. Outliers were excluded (ROUT Q = 1.000%)
Figure 59. Western blot detection of PrP expression in PrP transgenic *Drosophila* 2\textsuperscript{nd} instar larvae.

**Samples:**

Track 1 – Mouse 3F4 WT-UAS x elav-GAL4
Track 2 – Mouse 3F4 FFI-UAS x elav-GAL4
Track 3 – Mouse 3F4 CJD-UAS x elav-GAL4
Track 4 – 51D-UAS x elav-GAL4 (negative control)
Track 5 – VRQ recombinant protein 25 ng (positive control)

Homogenates from 5 larvae equivalents in 10 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 10 μl of Laemmli Buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 60. Western blot detection of PrP expression level comparison between 5 day and 16 day old flies maintained at 20°C.

**Samples:**

Track 1 – Mouse 3F4 WT-UAS x elav-GAL4 (5 days)
Track 2 – Mouse 3F4 FFI-UAS x elav-GAL4 (5 days)
Track 3 – Mouse 3F4 CJD-UAS x elav-GAL4 (5 days)
Track 4 – 51D-UAS x elav-GAL4 (negative control) (5 days)
Track 5 – Mouse 3F4 WT-UAS x elav-GAL4 (16 days)
Track 6 – Mouse 3F4 FFI-UAS x elav-GAL4 (16 days)
Track 7 – Mouse 3F4 CJD-UAS x elav-GAL4 (16 days)
Track 8 – 51D-UAS x elav-GAL4 (negative control) (16 days)

Head homogenates from 5 male head equivalents in 10 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 10 μl of Laemmli Buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 61. Western blot detection of PrP expression in 5 day old flies maintained at different temperatures (20°C or 25°C).

Samples:

Track 1 – Mouse 3F4 WT-UAS x elav-GAL4 (20°C)
Track 2 – Mouse 3F4 FFI-UAS x elav-GAL4 (20°C)
Track 3 – Mouse 3F4 CJD-UAS x elav-GAL4 (20°C)
Track 4 – 51D-UAS x elav-GAL4 (negative control) (20°C)
Track 5 – Mouse 3F4 WT-UAS x elav-GAL4 (25°C)
Track 6 – Mouse 3F4 FFI-UAS x elav-GAL4 (25°C)
Track 7 – Mouse 3F4 CJD-UAS x elav-GAL4 (25°C)
Track 8 – 51D-UAS x elav-GAL4 (negative control) (25°C)

Head homogenates from 5 male head equivalents in 10 µl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 10 µl of Laemmli Buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 62. Western blot detection of PK-treatment of VRQ/VRQ normal and scrapie positive ovine brain homogenate (various times of PK-treatment).

**Samples:**

Track 1 – 1 µl of 10% VRQ/VRQ NBH  
Track 2 – 2 µl of 10% VRQ/VRQ NBH + 32 µg/ml PK / 45 minutes  
Track 3 – 2 µl of 10% VRQ/VRQ NBH + 32 µg/ml PK / 60 minutes  
Track 4 – 1 µl of 10% VRQ/VRQ 0005  
Track 5 – 2 µl of 10% VRQ/VRQ 0005 + 32 µg/ml PK / 45 minutes  
Track 6 – 2 µl of 10% VRQ/VRQ 0005 + 32 µg/ml PK / 60 minutes  
Track 7 – 2 µl of 10% VRQ/VRQ 0005 + 32 µg/ml PK / 90 minutes  
Track 8 – 2 µl of 10% VRQ/VRQ 0005 + 32 µg/ml PK / 120 minutes  
Track 9 – 25 ng of VRQ recombinant protein

NBH = normal brain homogenate; 0005 = scrapie positive homogenate

Ovine brain homogenates (diluted in PBS up to 9 µl) treated with 1 µl PK (to the final concentration specified) were incubated at 37°C (for the specified time). The reaction was stopped by addition of 10 µl 2X Laemmli buffer and incubation at 80°C for 5 minutes. Samples were subjected to SDS-PAGE (12% gel; 180V/50min).

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 63. Western blot detection of PK-treatment of VRQ/VRQ normal and scrapie positive murine brain homogenate.

Samples:

Track 1 – 1 µl of 10% C57BL6 (prion negative) – no PK-treatment
Track 2 – 2 µl of 10% C57BL6 (prion negative) + 32 µg/ml PK
Track 3 – 1 µl of 10% ME7 (scrapie prion positive) – no PK-treatment
Track 4 – 2 µl of 10% ME7 (scrapie prion positive) + 32 µg/ml PK
Track 5 – 1 µl of 10% CD1 (prion negative) – no PK-treatment
Track 6 – 2 µl of 10% CD1 (prion negative) + 32 µg/ml PK
Track 7 – 1 µl of 10% RML (scrapie prion positive) – no PK-treatment
Track 8 – 2 µl of 10% RML (scrapie prion positive) + 32 µg/ml PK
Track 9 – 25 ng of VRQ recombinant protein

Murine brain homogenates (diluted in PBS up to 9 µl) treated with 1 µl PK (to the final concentration specified) were incubated at 37°C for 60 minutes. The reaction was stopped by addition of 10 µl 2X Laemmli buffer and incubation at 80°C for 5 minutes. Samples were subjected to SDS-PAGE (12% gel; 180V/50min).

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Ponceau S staining used as a loading control. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 64. Western blot detection of PK-digested mouse 3F4 PrP transgenic fly head homogenates.

Samples:

Track 1 – Mouse 3F4 WT-UAS x elav-GAL4 – No PK treatment
Track 2 – Mouse 3F4 WT-UAS x elav-GAL4 + 1 μg/ml PK
Track 3 – Mouse 3F4 WT-UAS x elav-GAL4 + 2 μg/ml PK
Track 4 – Mouse 3F4 FFI-UAS x elav-GAL4 – No PK treatment
Track 5 – Mouse 3F4 FFI-UAS x elav-GAL4 + 1 μg/ml PK
Track 6 – Mouse 3F4 FFI-UAS x elav-GAL4 + 2 μg/ml PK
Track 7 – Mouse 3F4 CJD-UAS x elav-GAL4 – No PK treatment
Track 8 – Mouse 3F4 CJD-UAS x elav-GAL4 + 1 μg/ml PK
Track 9 – Mouse 3F4 CJD-UAS x elav-GAL4 + 2 μg/ml PK

Head homogenates from 1 male head equivalent in 9 μl lysis buffer (without AEBSF) were treated with 1 μl PK (to the final concentration specified) and incubated at 37°C for 30 minutes. The reaction was stopped by addition of 10 μl 2X Laemmli buffer and incubation at 80°C for 5 minutes. Samples were subjected to SDS-PAGE (12% gel; 180V/50min).

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 65. Western blot detection of PK-digested hamster PrP transgenic fly head homogenates.

Samples:

Track 1 – Hamster WT-UAS x elav-GAL4 – No PK treatment
Track 2 – Hamster WT-UAS x elav-GAL4 + 4 µg/ml PK
Track 3 – Hamster WT-UAS x elav-GAL4 + 5 µg/ml PK
Track 4 – Hamster FFI-UAS x elav-GAL4 – No PK treatment
Track 5 – Hamster FFI-UAS x elav-GAL4 + 4 µg/ml PK
Track 6 – Hamster FFI-UAS x elav-GAL4 + 5 µg/ml PK
Track 7 – Hamster CJD-UAS x elav-GAL4 – No PK treatment
Track 8 – Hamster CJD-UAS x elav-GAL4 + 4 µg/ml PK
Track 9 – Hamster CJD-UAS x elav-GAL4 + 5 µg/ml PK

Head homogenates from 1 male head equivalent in 9 µl lysis buffer (without AEBSF) were treated with 1 µl PK (to the final concentration specified) and incubated at 37°C for 30 minutes. The reaction was stopped by addition of 10 µl 2X Laemmli buffer and incubation at 80°C for 5 minutes. Samples were subjected to SDS-PAGE (12% gel; 180V/50min).

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Molecular mass markers (kDa) are shown on the left-hand side.
Figure 66. Comparison of ovine PrP expression levels between two GAL4 pan-neuronal drivers: elav-GAL4 and 57C10-GAL4.

Samples:
Track 1 – 51D-UAS x elav-GAL4 (negative control)
Track 2 – VRQ(GPI)-UAS x elav-GAL4
Track 3 – VRQ(ΔGPI)-UAS x elav-GAL4
Track 4 – VRQ(cyt)-UAS x elav-GAL4
Track 5 – 51D-UAS x 57C10-GAL4 (negative control)
Track 6 – VRQ(GPI)-UAS x 57C10-GAL4
Track 7 – VRQ(ΔGPI)-UAS x 57C10-GAL4
Track 8 – VRQ(cyt)-UAS x 57C10-GAL4

Both drivers, elav-GAL4 and 57C10-GAL4, trigger pan-neuronal PrP expression.
Head homogenates from 5 male head equivalents in 10 μl AEBSF lysis buffer per track were subjected to SDS-PAGE (12% gel; 180V/50min) after addition of 10 μl of Laemmli Buffer.

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Molecular mass markers (kDa) are shown on the left-hand side.
Table 12. Comparison of PrP expression levels of ovine PrP transgenic flies driven by the elav-GAL4 or the 57C10-GAL4 driver line analysed by the ImageJ programme.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Band density (mm² of a peak)</th>
<th>Relative density</th>
<th>Comparison elav x 57C10</th>
</tr>
</thead>
<tbody>
<tr>
<td>elav-VRQ(GPI)</td>
<td>6942.581</td>
<td>1</td>
<td>x</td>
</tr>
<tr>
<td>elav-VRQ(ΔGPI)</td>
<td>19892.957</td>
<td>2.865</td>
<td>y</td>
</tr>
<tr>
<td>elav-VRQ(cyt)</td>
<td>12573.844</td>
<td>1.811</td>
<td>z</td>
</tr>
<tr>
<td>57C10-VRQ(GPI)</td>
<td>7687.53</td>
<td>1.107</td>
<td>111% of x</td>
</tr>
<tr>
<td>57C10-VRQ(ΔGPI)</td>
<td>26188.007</td>
<td>3.772</td>
<td>132% of y</td>
</tr>
<tr>
<td>57C10-VRQ(cyt)</td>
<td>15842.522</td>
<td>2.282</td>
<td>126% of z</td>
</tr>
</tbody>
</table>

**Table legends:** The band density (measured as an area of the peak in mm²), relative band density (densities compared to the first value obtained) and percentage were calculated using ImageJ. The relative density was calculated using the elav-VRQ(GPI) as a standard with relative density of 1 and other band densities were then compared to this standard one. Each ovine PrP genotype crossed with the elav-GAL4 driver was then used as a standard to compare the same genotype of ovine PrP transgenic flies crossed with 57C10-GAL4 driver. The results show the difference in band intensity between elav-GAL4 driven flies and 57C10-GAL4 driven flies as seen in Figure 66.
Figure 67: Western blot detection of PK-digested 30 days old dual PrP transgenic flies VRQ(GPI) ; VRQ(ΔGPI) that co-express PrP under the control of 57C10-GAL4 ; GMR-LexA dual driver.

Samples:

Track 1 – VRQ(GPI)-GAL4/57C10-GAL4 ; VRQ(ΔGPI)-LexAop/GMR-LexA, no PK treatment
Track 2 – VRQ(GPI)-GAL4/57C10-GAL4 ; VRQ(ΔGPI)-LexAop/GMR-LexA, 2 μg/ml PK treatment
Track 3 – VRQ(GPI)-GAL4/57C10-GAL4 ; VRQ(ΔGPI)-LexAop/GMR-LexA, 4 μg/ml PK treatment

Head homogenates from 2 male (30 days old) head equivalents in 9 μl lysis buffer (without AEBSF) were treated with 1 μl PK (to the final concentration specified) and incubated at 37°C for 30 minutes. The reaction was stopped by addition of 10 μl 2X Laemmlli buffer and incubation at 80°C for 5 minutes. Samples were subjected to SDS-PAGE (12% gel; 180V/50min).

The western blot (45mA/hour), membrane probed with anti-PrP monoclonal antibody Sha31 (diluted 1:2000 in 1% milk/TBST) and secondary antibody HRP at the same dilution; both incubated for 1 hour. The protein bands were visualised using ECL staining and ChemiDoc Imaging System. Ponceau S staining was used as a loading control. Molecular mass markers (kDa) are shown on the left-hand side.
Table 13. Dual PrP transgenic flies subjected to protein misfolding cyclic amplification (PMCA) assay to detect the presence or absence of prion-induced misfolding.

<table>
<thead>
<tr>
<th>Driver</th>
<th>Genotype and expression pattern</th>
<th>Age of flies (days)</th>
<th>PMCA result</th>
</tr>
</thead>
<tbody>
<tr>
<td>57C10-GAL4 ; 71A10-LexA</td>
<td>VRQ(GPI) – pan-neuronal</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>VRQ(ΔGPI) – basin-4-interneurons</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>57C10-GAL4 ; 72F11-LexA</td>
<td>VRQ(GPI) – pan-neuronal</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>VRQ(ΔGPI) – interneurons</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>57C10-GAL4 ; iav-LexA</td>
<td>VRQ(GPI) – pan-neuronal</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>VRQ(ΔGPI) – chordotonal organs</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>57C10-GAL4 ; repo-LexA</td>
<td>VRQ(GPI) – pan-neuronal</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>VRQ(ΔGPI) – glial cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>-</td>
</tr>
</tbody>
</table>

Table legends: Fly head homogenates prepared from 20 fly heads harvested at 5 or 30 days after hatching were analysed for the presence or absence of misfolded material.

All genotypes tested (crosses with 71A10, 72F11, iav and repo dual drivers):
- 51D-UAS ; nos-AttP2-LexAop
- VRQ(GPI)-UAS (chromosome 2)
- VRQ(ΔGPI)-LexAop (chromosome 3)
- VRQ(GPI)-UAS ; VRQ(GPI)-LexAop (chromosome 2 + 3)
- VRQ(GPI)-UAS ; VRQ(ΔGPI)-LexAop (chromosome 2 + 3)

Positive control tested (crossed with elav-GAL4 driver):
- VRQ(GPI)-UAS ovine scrapie infected (VRQ/VRQ 0005) – 5 / 30 days old flies

After the PMCA (substrate = VRQ ovine PrP transgenic mice brain homogenate), the western blot detection of PK-resistant Prp27-30 PK-resistant material was performed. The assay has not work as no PK-resistant material was detected in any of the samples tested.
Table 14. Dual PrP transgenic *Drosophila* subjected to real-time quaking-induced conversion (RT-QuIC) to detect the presence or absence of prion-induced misfolding.

<table>
<thead>
<tr>
<th>Driver</th>
<th>Genotype and expression pattern</th>
<th>Age of flies (days)</th>
<th>RT-QuIC result</th>
</tr>
</thead>
<tbody>
<tr>
<td>57C10-GAL4 ; GMR-LexA</td>
<td>VRQ(GPI) – pan-neuronal VRQ(ΔGPI) – eye</td>
<td>5 30</td>
<td>- -</td>
</tr>
<tr>
<td>57C10-GAL4 ; iav-LexA</td>
<td>VRQ(GPI) – pan-neuronal VRQ(ΔGPI) – chordotonal organs</td>
<td>5 30</td>
<td>- -</td>
</tr>
<tr>
<td>57C10-GAL4 ; repo-LexA</td>
<td>VRQ(GPI) – pan-neuronal VRQ(ΔGPI) – glial cells</td>
<td>5 30</td>
<td>- -</td>
</tr>
</tbody>
</table>

**Table legends**: Fly head homogenates prepared from 10 fly heads harvested at 5 or 30 days after hatching were analysed for the presence or absence of misfolded material by RT-QuIC assay.

*All genotypes tested (crosses with GMR, iav and repo dual drivers):*
- 51D-UAS ; nos-AttP2-LexAop
- VRQ(GPI)-UAS (chromosome 2)
- VRQ(ΔGPI)-LexAop (chromosome 3)
- VRQ(GPI)-UAS ; VRQ(GPI)-LexAop (chromosome 2 + 3)
- VRQ(GPI)-UAS ; VRQ(ΔGPI)-LexAop (chromosome 2 + 3)

*Positive control tested (crossed with elav-GAL4 driver):*
- VRQ(GPI)-UAS ovine scrapie infected (VRQ/VRQ 0005) – 5 / 30 days old flies

During RT-QuIC (substrate = recombinant hamster PrP), the incorporation of Thioflavin T in protein aggregates is measured. The assay has not worked as no positive aggregates were detected in any of the samples tested. There were some false positive results (as seen in Table 15)
Table 15. List of samples that tested positive in real-time quaking-induced conversion (RT-QuIC) detection of prion-induced misfolding in *Drosophila* system.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age of flies (days)</th>
<th>RT-QuIC result</th>
</tr>
</thead>
<tbody>
<tr>
<td>51D-UAS ; Attp2-LexAop x 57C10-GAL4 ; GMR-LexA</td>
<td>5</td>
<td>10⁻¹ dilution positive after lag time of 91 hours</td>
</tr>
<tr>
<td>VRQs-LexAop x 57C10-GAL4 ; GMR-LexA</td>
<td>5</td>
<td>10⁻¹ dilution positive after lag time of 95.5 hours</td>
</tr>
<tr>
<td>VRQ(GPI)-UAS ; VRQ[(GPI]-LexAop x 57C10-GAL4 ; GMR-LexA</td>
<td>5</td>
<td>10⁻¹ dilution positive after lag time of 91 hours</td>
</tr>
<tr>
<td>51D-UAS ; Attp2-LexAop x 57C10-GAL4 ; iav-LexA</td>
<td>5</td>
<td>10⁻¹ dilution positive after lag time of 65.5 hours</td>
</tr>
<tr>
<td>VRQ(GPI)-UAS ; VRQ(GPI)-LexAop x 57C10-GAL4 ; repo-LexA</td>
<td>30</td>
<td>10⁻¹ dilution positive after lag time of 13 hours (positive just in 1 of 3 repeats)</td>
</tr>
<tr>
<td>51D-UAS x elav-GAL4 (normal brain homogenate exposed)</td>
<td>30</td>
<td>10⁻¹ dilution positive after lag time of 90 hours</td>
</tr>
<tr>
<td>51D-UAS x elav-GAL4 (scrapie positive homogenate exposed)</td>
<td>30</td>
<td>10⁻¹ dilution positive after lag time of 90 hours</td>
</tr>
</tbody>
</table>

**Table legends:** Fly head homogenates prepared from 10 fly heads harvested at 5 or 30 days after hatching were analysed for the presence or absence of misfolded material RT-QuIC assay. Typically, a sporadic CJD control (10⁻³ dilution of 10% [w/v] brain homogenate) is positive within 6.5 hours. Apart from one sample that showed positive result after 13 hours but failed to react in the same way in the two following repeats, none of the samples can be considered positive.
Figure 68. Survival assay of dual ΔGPI/wild type and dual wild type PrP transgenic Drosophila.

Figure legends: Age-matched pre-mated female flies were set up for a survival assay in triplicates. The numbers of dead flies were recorded three times a week at the same time of day for 62 days. Flies were maintained at 22°C on a standard cornmeal media.

57C10-GAL4 ; repo-LexA crosses: N = 6 female flies per tube (x3)
57C10-GAL4 ; GMR-LexA crosses: N = 10 female flies per tube (x3)
57C10-GAL4 ; iav-LexA crosses: N = 10* female flies per tube (x3)

*57C10-GAL4 ; iav-LexA x VRQ(GPI)-UAS;VRQ(ΔGPI)-LexAop group was set up in triplicates of 9 females

Survival curve generated and statistics (unpaired two-tailed t-test) performed in GraphPad Prism 6
Figure 69. Survival assay of dual PrP transgenic *Drosophila* and their controls.

**Figure legends:** Age-matched pre-mated female flies were set up for a survival assay in triplicates. The numbers of dead flies were recorded three times a week at the same time of a day for 62 days. Flies were maintained at 22°C on a standard cornmeal media.

**57C10-GAL4 ; repo-LexA crosses:** N = 6 female flies per tube (x3)
**57C10-GAL4 ; GMR-LexA crosses:** N = 10 female flies per tube (x3)
**57C10-GAL4 ; iav-LexA crosses:** N = 10* female flies per tube (x3)

*57C10-GAL4 ; iav-LexA x VRQ(GPI)-UAS;VRQ(ΔGPI)-LexAop group was set up in triplicates of 9 females

Survival curve generated and statistics (unpaired two-tailed t-test) performed in GraphPad Prism 6