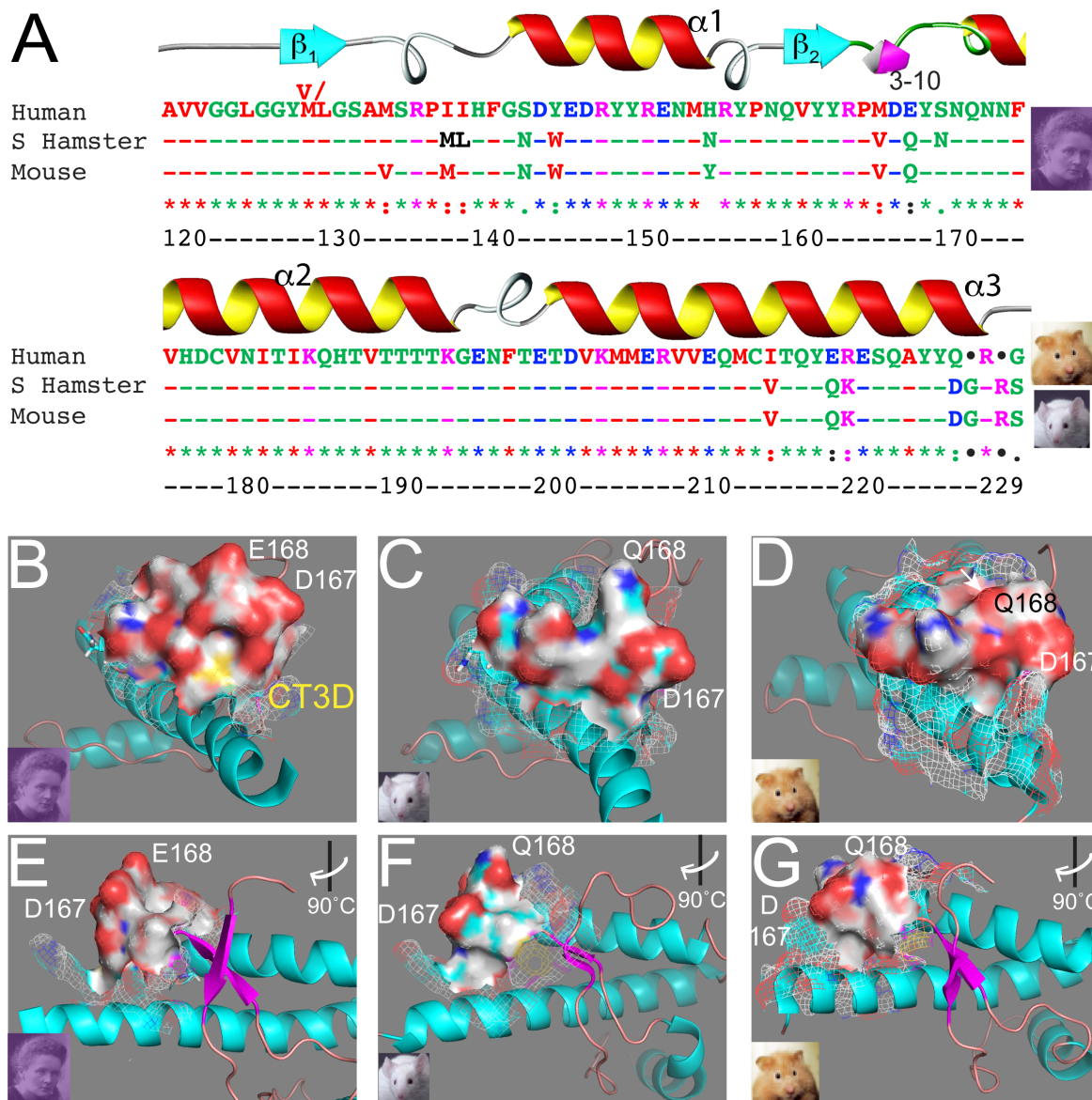
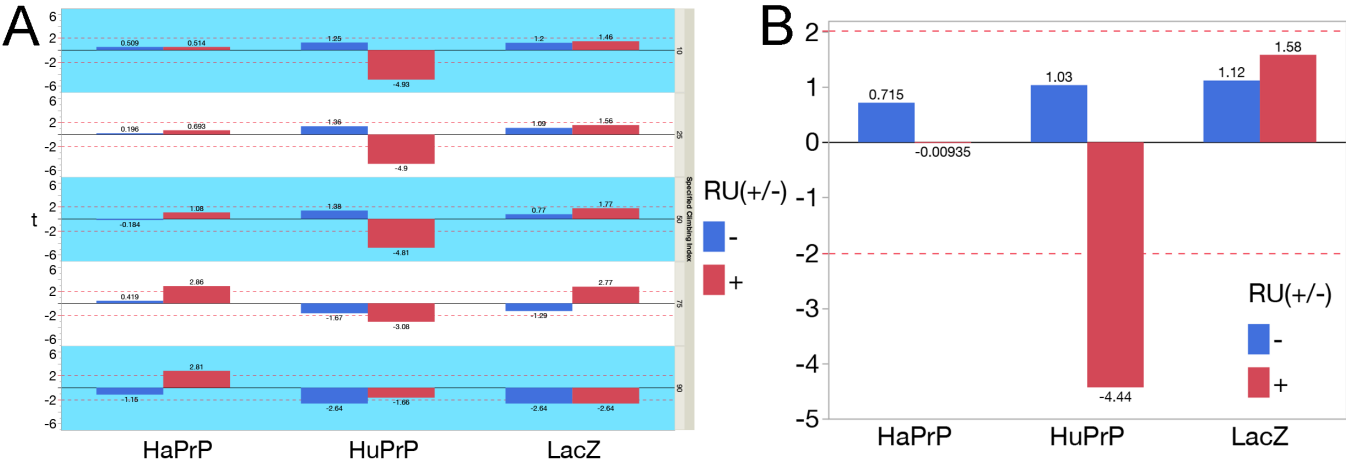
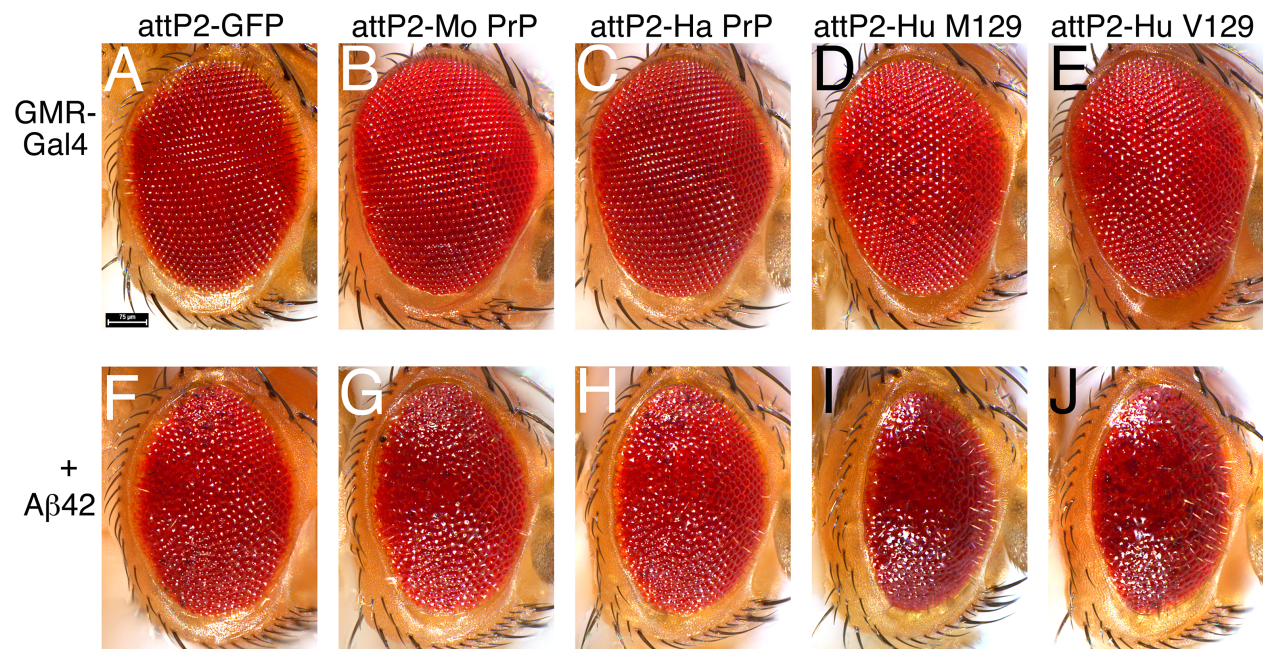




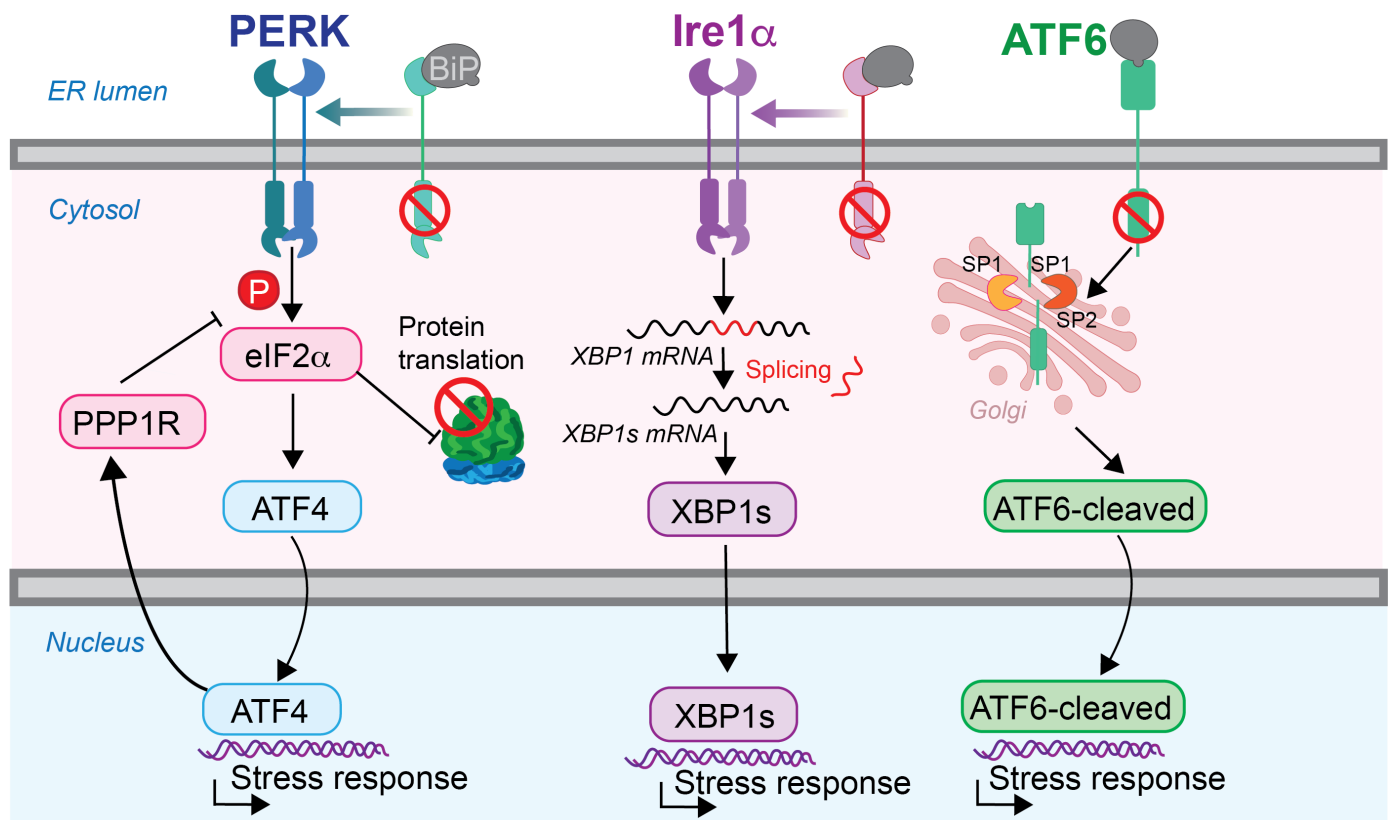
**Fig. S1. Sequence / structure differences between human and animal PrP.** **A**, Sequence alignment of the C-terminal globular domain of PrP from human, Syrian hamster, and mouse. Amino acid numbering corresponds to human PrP throughout to avoid confusion. The alignment shows high overall conservation with most variation clustered in the  $\beta 2$ - $\alpha 2$  loop and distal helix 3. **B-G**, Surface and Mesh views for the  $\beta 2$ - $\alpha 2$  loop, front view (B-D) and side view (E-G). In human PrP, the loop is vertical and tall, with two acidic residues sticking upwards (B and E). In mouse PrP, the loop is not as tall, with D167 shifted to a lower position (C and F). In hamster PrP, the loop is flat and closer to helix 3 (D and G).



**Fig. S2. Statistical analysis of locomotor activity.** **A**, Single-sample t-test analysis of age-dependent climbing index. HuPrP with RU has a significant negative effect on age-specific climbing index. Bars represent t-score. **B**, Area under the curve. Dotted red-line represents the critical value of 2.015 for t-test with 5 degrees of freedom ( $p < 0.05$ ). Single-sample t-test analysis of area under curve for the climbing index. HuPrP with RU has a significant negative effect on area under the climbing index curve. Bars represent t-score. Dotted red-line represents the critical value of 2.015 for t-test with 5 degrees of freedom



**Fig. S3. Human PrP enhances the toxicity of A $\beta$ 42.** A-J, Micrographs of fresh eyes expressing mCD8-GFP, hamster PrP, mouse PrP, human PrP-M129, or human PrP-V129 alone (A-E) or in combination with A $\beta$ 42 (F-J) in the eye under the control of *GMR-Gal4* at 25°C. **A**, Control eyes from flies expressing mCD8-GFP (*GMR-Gal4 / UAS-mCD8-GFP-attP2*). **B and C**, Eyes from flies expressing mouse or hamster PrP (*GMR-Gal4 / UAS-mouse PrP-attP2* and *GMR-Gal4 / UAS-hamster PrP-attP2*) are normal. **D and E**, Eyes from flies expressing human PrP (*GMR-Gal4 / UAS-human PrP-M129-attP2* and *GMR-Gal4 / UAS-human PrP-V129-attP2*) show mild disorganization. These phenotypes are weak because the expression of PrP constructs is lower at 25°C. **F**, The eyes from flies co-expressing GFP and A $\beta$ 42 (*GMR-Gal4 / UAS-mCD8-GFP-attP2 / UAS-A $\beta$ 42*) are disorganized and have necrotic spots. **G and H**, The eyes from flies co-expressing rodent PrP with A $\beta$ 42 (*GMR-Gal4 / UAS-mouse PrP-attP2 / UAS-A $\beta$ 42* and *GMR-Gal4 / UAS-hamster PrP-attP2 / UAS-A $\beta$ 42*) are similar to those in F. **I and J**, The eyes from flies co-expressing human PrP and A $\beta$ 42 (*GMR-Gal4 / UAS-human PrP-M129-attP2 / UAS-A $\beta$ 42* and *GMR-Gal4 / UAS-human PrP-V129-attP2 / UAS-A $\beta$ 42*) are smaller and highly disorganized (glassy).



**Fig. S4. Summary of the three UPR branches.** PERK, Ire1 and ATF6 are three sensors of ER stress that are activated when the chaperone BiP releases the sensors due to an increase of misfolded proteins in the ER. Each sensor activates a different branch of the UPR consisting of different mechanisms of activation and downstream effectors. The overall consequence is acutely blocking translation and increasing the ER biosynthetic and folding capacities.

**Table S1. Predicted age at specified climbing indices ( $\alpha = 0.05$ ).**

| Genotype | RU(+/-) | Climbing Index | Predicted Age (Days) | Std Error | Lower 0.95 | Upper 0.95 |
|----------|---------|----------------|----------------------|-----------|------------|------------|
| HaPrP    | -       | 90             | 1.09                 | 1.27      | -1.58      | 3.76       |
| HaPrP    | -       | 75             | 6.38                 | 0.38      | 5.58       | 7.18       |
| HaPrP    | -       | 50             | 11.86                | 0.35      | 11.12      | 12.61      |
| HaPrP    | -       | 25             | 17.42                | 0.48      | 16.41      | 18.42      |
| HaPrP    | -       | 10             | 22.99                | 0.90      | 21.10      | 24.88      |
| HaPrP    | +       | 90             | 4.00                 | 1.84      | 0.12       | 7.88       |
| HaPrP    | +       | 75             | 10.23                | 0.55      | 9.07       | 11.39      |
| HaPrP    | +       | 50             | 14.76                | 0.40      | 13.91      | 15.62      |
| HaPrP    | +       | 25             | 18.94                | 0.52      | 17.83      | 20.05      |
| HaPrP    | +       | 10             | 23.01                | 0.90      | 21.12      | 24.90      |
| HuPrP    | -       | 90             | --                   | --        | --         | --         |
| HuPrP    | -       | 75             | 3.09                 | 3.20      | -3.66      | 9.85       |
| HuPrP    | -       | 50             | 15.47                | 0.44      | 14.54      | 16.40      |
| HuPrP    | -       | 25             | 20.99                | 0.43      | 20.07      | 21.90      |
| HuPrP    | -       | 10             | 25.77                | 0.78      | 24.13      | 27.41      |
| HuPrP    | +       | 90             | 0.72                 | 0.03      | 0.64       | 0.79       |
| HuPrP    | +       | 75             | 0.87                 | 0.03      | 0.81       | 0.93       |
| HuPrP    | +       | 50             | 1.21                 | 0.02      | 1.16       | 1.26       |
| HuPrP    | +       | 25             | 1.80                 | 0.05      | 1.69       | 1.91       |
| HuPrP    | +       | 10             | 2.56                 | 0.10      | 2.35       | 2.77       |
| LacZ     | -       | 90             | --                   | --        | --         | --         |
| LacZ     | -       | 75             | 3.68                 | 1.13      | 1.30       | 6.07       |
| LacZ     | -       | 50             | 14.06                | 0.34      | 13.35      | 14.77      |
| LacZ     | -       | 25             | 20.16                | 0.35      | 19.43      | 20.90      |
| LacZ     | -       | 10             | 25.60                | 0.64      | 24.24      | 26.95      |
| LacZ     | +       | 90             | --                   | --        | --         | --         |
| LacZ     | +       | 75             | 10.07                | 0.63      | 8.74       | 11.41      |
| LacZ     | +       | 50             | 16.36                | 0.43      | 15.44      | 17.27      |
| LacZ     | +       | 25             | 21.59                | 0.53      | 20.48      | 22.71      |
| LacZ     | +       | 10             | 26.56                | 0.94      | 24.58      | 28.53      |

**Table S2. Climbing Index prediction formulas and parameters.**

| Prediction Model                    | Prediction formula                                  | Genotype | RU(+/-) | $\theta 1$ | q2   | q3   |
|-------------------------------------|-----------------------------------------------------|----------|---------|------------|------|------|
| 3-parameter logistic                | $\text{Log}((q1/\text{Climbing Index})/q2)/q3$      | HaPrP    | -       | 101.05     | 0.1  | 0.2  |
|                                     |                                                     | HaPrP    | +       | 94.19      | 0.02 | 0.27 |
|                                     |                                                     | HuPrP    | -       | 76.96      | 0.01 | 0.24 |
|                                     |                                                     | LacZ     | -       | 79.92      | 0.03 | 0.21 |
|                                     |                                                     | LacZ     | +       | 89.09      | 0.02 | 0.23 |
| 3-parameter 1st order decay kinetic | $\text{Log}((\text{Climbing Index} - q3)/q1)/(-q2)$ | HuPrP    | +       | 209.74     | 1.18 | -0.3 |

**Table S3. ANOVA analysis for XBP-GFP expression.****Comparisons for all pairs using Tukey-Kramer HSD****Connecting Letters Report**

| Level    |   | Mean (Mean signal) | Mean (Integrated density) |
|----------|---|--------------------|---------------------------|
| Abeta42  | A | 12.749299          | 1298165.5                 |
| PrP-V129 | B | 10.634235          | 966270.2                  |
| Control  | C | 6.193575           | 540884.8                  |

Levels not connected by same letter are significantly different.

**Ordered Differences Report (mean signal)**

| Level    | - Level  | p-Value (mean signal) | p-Value (Integrated density) |
|----------|----------|-----------------------|------------------------------|
| Abeta42  | Control  | <.0001                | <.0001                       |
| PrP-V129 | Control  | <.0001                | 0.0005                       |
| Abeta42  | PrP-V129 | 0.0004                | 0.0008                       |

**Table S4. Summary of interactions with the UPR-PERK components**

| 27°C<br>Stock#     | UAS-<br>LacZ<br>3955   | GD-<br>Ire1 $\alpha$ i<br>39561 | TRiP-<br>Ire1 $\alpha$ i<br>62156 | KK-<br>XBP1i<br>109312 | TRiP-<br>XBP1i<br>25990           | UAS-<br>XBP1<br>60730            | UAS-<br>mXBP1s<br>Casas-Tinto<br>2011 | KK-<br>PERKi<br>110278    | TRiP-<br>PERKi<br>42499  | UAS-<br>PERK<br>76248 |
|--------------------|------------------------|---------------------------------|-----------------------------------|------------------------|-----------------------------------|----------------------------------|---------------------------------------|---------------------------|--------------------------|-----------------------|
| <b>GMR</b>         | N0000                  | N0000                           | N0000                             | N0000                  | N0000                             | N0000                            | N0000                                 | N0000                     | N0000                    | E3332                 |
| <b>GMR<br/>PrP</b> | E23000                 | E3311                           | E2201                             | E3300                  | E2300                             | N0000                            | N0000                                 | S3300                     | S3300                    | E3332                 |
|                    | KK-<br>ATF4i<br>109014 | TRiP-<br>ATF4i<br>25985         | UAS-<br>ATF4<br>FlyORF            | UAS-<br>ATF4<br>81650  | TRiP-<br>eIF2 $\alpha$ i<br>44449 | KK-<br>eIF2 $\alpha$ i<br>104562 | KK-<br>PPP1R15<br>107545              | TRiP-<br>PPP1R15<br>33011 | TRiP-<br>4E-BPi<br>80427 | UAS-<br>4E-BP<br>9147 |
| <b>GMR</b>         | N0000                  | N0000                           | N0000                             | E1210                  | E1101                             | E0110                            | E0100                                 | N0000                     | N0000                    | N0000                 |
| <b>GMR<br/>PrP</b> | S3300                  | S2200                           | N0000                             | E3332                  | E1312                             | E0210                            | L0003                                 | L0003                     | S3300                    | E0110                 |

Scoring: N-No effect; S: suppressor; E: enhancer; L: lethal. Effect scored 0 (no effect) – 3 (robust change)

for: size-organization-pigmentation-lethality.

**Table S5. Eye phenotypes of human PrP mutants**

| 27°C<br>HuPrP | UAS-<br>CD8-GFP | V129<br>(WT) | N159D<br>(dog) | D167S<br>(horse) | N159D<br>D167S-2x | N159D<br>D167S, N174S-3x |
|---------------|-----------------|--------------|----------------|------------------|-------------------|--------------------------|
| <b>GMR</b>    | N0000           | E1200        | E1200          | E0100            | E01000            | E0100                    |

Scoring: N-No effect; S: suppressor; E: enhancer. Effect scored 0 (no effect) – 3 (robust change) for: size-organization-pigmentation-lethality.

**Table S6. Two-wayANOVA analysis for mushroom bodies****Comparisons for all pairs using Tukey-Kramer HSD****Connecting Letters Report**

| Level       |    | Mean      |
|-------------|----|-----------|
| Control-D40 | A  | 9888.5951 |
| Control-D1  | B  | 6754.0952 |
| 3X-D40      | C  | 5530.1558 |
| V129-D1     | CD | 5278.4658 |
| 2X-D1       | CD | 5246.9839 |
| N159D-D1    | CD | 5102.4767 |
| D167S-D1    | CD | 5046.6443 |
| D167S-D40   | CD | 5022.8778 |
| 3X-D1       | CD | 4815.1017 |
| 2X-D40      | D  | 4566.5551 |
| N159D-D40   | E  | 3179.4648 |
| V129-D40    | E  | 2471.1883 |

Levels not connected by the same letter are significantly different.

**Ordered Differences Report: T-test corrected**

| Subject 1    | Subject 2    | P-value | Holm p-value (0.05) | Significant (Y/N) |
|--------------|--------------|---------|---------------------|-------------------|
| Control, D1  | 2x, D1       | 0.0001  | 0.000746269         | Y                 |
| Control, D1  | 3x, D1       | 0.0001  | 0.000769231         | Y                 |
| Control, D40 | 2x, d40      | 0.0001  | 0.000806452         | Y                 |
| Control, D40 | 3x, D40      | 0.0001  | 0.000833333         | Y                 |
| Control, D40 | Control, D1  | 0.0001  | 0.000847458         | Y                 |
| D167S, D1    | Control, D1  | 0.0001  | 0.000862069         | Y                 |
| D167S, d40   | Control, D40 | 0.0001  | 0.000909091         | Y                 |
| N159D, D1    | Control, D1  | 0.0001  | 0.000925926         | Y                 |
| N159D, D40   | 2x, D40      | 0.0001  | 0.000980392         | Y                 |
| N159D, D40   | 3x, D40      | 0.0001  | 0.001020408         | Y                 |
| N159D, D40   | Control, D40 | 0.0001  | 0.00106383          | Y                 |
| N159D, D40   | D167S, D40   | 0.0001  | 0.001111111         | Y                 |
| N159D, D40   | N159D, D1    | 0.0001  | 0.001136364         | Y                 |
| V129, D1     | Control, D1  | 0.0001  | 0.001162791         | Y                 |

|            |              |        |             |   |
|------------|--------------|--------|-------------|---|
| V129, D40  | 2x, D40      | 0.0001 | 0.001282051 | Y |
| V129, D40  | 3x, D40      | 0.0001 | 0.001351351 | Y |
| V129, D40  | Control, D40 | 0.0001 | 0.001428571 | Y |
| V129, D40  | D167S, D40   | 0.0001 | 0.001515152 | Y |
| V129, D40  | V129, D1     | 0.0001 | 0.001612903 | Y |
| 3x, D40    | 2x, D40      | 0.004  | 0.001666667 | N |
| 3x, D40    | 3x, D1       | 0.0071 | 0.001785714 | N |
| V129, D40  | N159D, D40   | 0.0282 | 0.001851852 | N |
| D167S, D40 | 3x, D40      | 0.059  | 0.002       | N |
| V129, D1   | 3x, D1       | 0.0663 | 0.002083333 | N |
| D167S, D40 | 2x, D40      | 0.0821 | 0.002173913 | N |
| 3x, D1     | 2x, D1       | 0.0867 | 0.002272727 | N |
| 2x, D40    | 2x, D1       | 0.087  | 0.002380952 | N |
| N159D, D1  | 3x, D1       | 0.2297 | 0.002941176 | N |
| V129, D1   | D167S, D1    | 0.4034 | 0.004545455 | N |
| d167s, D1  | 3x, D1       | 0.404  | 0.005       | N |
| V129, D1   | N159D, D1    | 0.4612 | 0.00625     | N |
| d167s, D1  | 2x, D1       | 0.4701 | 0.007142857 | N |
| N159D, D1  | 2x, D1       | 0.545  | 0.008333333 | N |
| N159D, D1  | D167S, D1    | 0.8339 | 0.0125      | N |
| V129, D1   | 2x, D1       | 0.9    | 0.016666667 | N |
| D167S, D40 | D167S, D1    | 0.9328 | 0.025       | N |