

# **Supplementary data 5**

qRT-PCR

## **Section 3.4**

qRT-PCR validation of selected GPCR transcripts

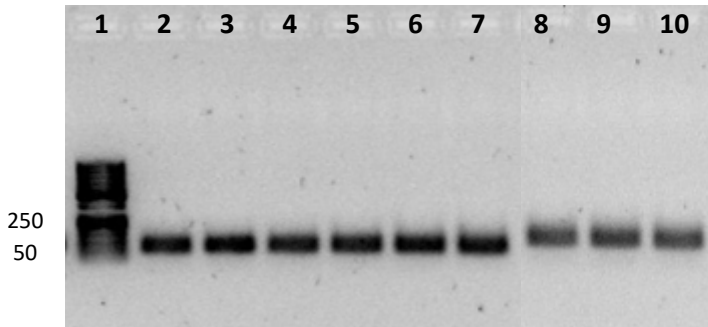
**Table S1. Primers used for qRT-PCR analysis.** Nucleotide sequences for the 17 selected GPCR genes and three candidate reference genes (*rpt6*, *rpl3*, and *rpl40*).

| No. | Target        | Forward primer (5'-3')   | Reverse primer (5'-3')  |
|-----|---------------|--------------------------|-------------------------|
| 1   | c33640_g1_i1  | AGTTATGGTGTCTGCCGGG      | CACCACAATGTTACGCTGG     |
| 2   | c105758_g1_i1 | TGGACCTGAGTTCGATGTTAAGG  | AATGTTGACGCTGGAGTTAATGG |
| 3   | c49038_g1_i1  | TCAGTGCTCTCCCGATCTCA     | GCTCTCTCACAGACAGACCG    |
| 4   | c79514_g1_i1  | CAACCTCAGTCTCCGTGTCC     | GATGATCTTCTGGCCCCGAG    |
| 5   | c67458_g3_i1  | TGCAATCTGAGTGTGACGGA     | GTCAACAAAACGATCAGATGCCA |
| 6   | c81140_g1_i1  | CACCTACTCCACCCACAACC     | AAAGCTCCTACCATGACCGC    |
| 7   | c69296_g1_i2  | TAATTATGGCCGTTTGAACCTGCA | AGCATGAAACTTCGTTGGCTT   |
| 8   | c83185_g2_i1  | ACCATGACACGCAGGAAGAG     | ACAGATGGGAGCGTACATGC    |
| 9   | c49216_g1_i1  | AGCGACATCACGGCCAATAA     | TGACTGTCCTGGCGATGATG    |
| 10  | c27743_g1_i2  | TGCTGTGTTGGACTCCGTAC     | GGAGTTGGACACCGCGAATA    |
| 11  | c78009_g2_i1  | TTATCCCCGCAATCATGGCA     | ACTGCTGACTCTGGTGAACG    |
| 12  | c75240_g1_i1  | CTCAAAGTGAGGATGGCGG      | GCAAACGATGAACAAGACGGA   |
| 13  | c69183_g1_i1  | CCCGCAAGTTCAGGTCAGAA     | TGGTCGTGGTGGTGGTATTG    |
| 14  | c89048_g1_i1  | CCCAGCACGGTCAATCTGAT     | CCCAGGACACAAGATACGAGT   |
| 15  | c107219_g1_i1 | TAACAGGCGCGTTTGTGTG      | ATGGTACAGTGGTTTCCGCA    |
| 16  | c109355_g1_i1 | ACAACACTGACTGTGCGTTCA    | TGAACTGTTGACTGCCTCCA    |
| 17  | c106522_g1_i1 | ACAGCAACTCCTCCGACTTG     | CACCACCTGTACGACCAAGG    |
| 18  | <i>rpt6</i>   | TGCTGGAGCTCTTGAACCAG     | TTCTTCCCGGACGCAAAAGA    |
| 19  | <i>rpl3</i>   | AAGACTCACAAGGGTCTGCG     | GCGGTGGTGATAGCCTTTCT    |
| 20  | <i>rpl40</i>  | GGTGGAGCCTTCTGACACAA     | ATCCTCCAGCTGTTTGCCAG    |

**Table S2. qRT-PCR efficiency parameters for target GPCRs and candidate housekeeping genes.** PCR efficiency (%) and coefficient of determination ( $R^2$ ) values determined via serial dilution curves for the 17 selected GPCR genes and three candidate reference genes (*rpt6*, *rpl3*, and *rpl40*).

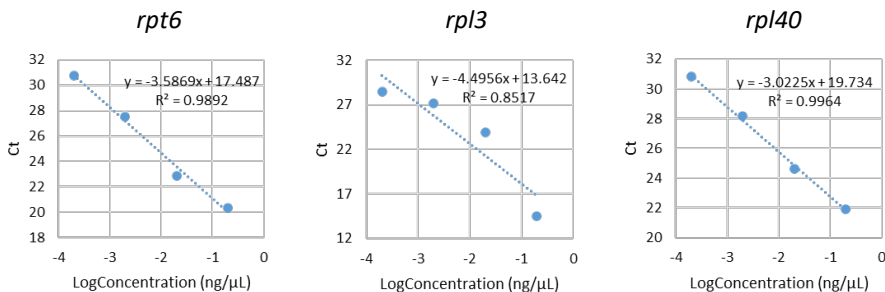
| Final conc. (ng/ $\mu$ L) $\rightarrow$ |               | 0.2      | 0.02     | 0.002    | 0.0002       |       |                    |       |
|-----------------------------------------|---------------|----------|----------|----------|--------------|-------|--------------------|-------|
| Log(conc.) $\rightarrow$<br>No.         |               | 0.30103  | -0.69897 | -1.69897 | -2.69897     | Slope | PCR efficiency (%) | $R^2$ |
| 1                                       | c33640_g1_i1  | 22.68052 | 26.35223 | 29.30643 | 32.93479     | -3.37 | 97.96              | 0.998 |
| 2                                       | c105758_g1_i1 | 20.96756 | 26.41071 | 28.68993 | 33.93903     | -4.12 | 74.89              | 0.978 |
| 3                                       | c49038_g1_i1  | 25.53357 | 29.38338 | 32.86033 | 36.95241     | -3.77 | 84.08              | 0.999 |
| 4                                       | c79514_g1_i1  | 15.80130 | 18.97537 | 21.94205 | 25.52259     | -3.21 | 104.75             | 0.999 |
| 5                                       | c67458_g3_i1  | 22.89668 | 26.93247 | 29.34429 | 32.91149     | -3.25 | 103.29             | 0.992 |
| 6                                       | c81140_g1_i1  | 18.90472 | 25.13814 | 28.93733 | 29.59621     | -3.59 | 90.00              | 0.892 |
| 7                                       | c69296_g1_i2  | 22.40883 | 26.38447 | 29.43135 | 33.71491     | -3.70 | 86.43              | 0.996 |
| 8                                       | c83185_g2_i1  | 30.26957 | 34.77948 | 36.98285 | Undetermined | -3.36 | 98.57              | 0.962 |
| 9                                       | c49216_g1_i1  | 20.83548 | 25.61298 | 28.90946 | 31.93220     | -3.66 | 87.64              | 0.988 |
| 10                                      | c27743_g1_i2  | 16.18811 | 26.84823 | 30.41549 | 33.92405     | -5.68 | 50.01              | 0.914 |
| 11                                      | c78009_g2_i1  | 16.02609 | 24.61998 | 26.16649 | 30.16526     | -4.40 | 68.83              | 0.908 |
| 12                                      | c75240_g1_i1  | 19.30168 | 25.74225 | 27.93463 | 31.58863     | -3.91 | 80.33              | 0.955 |
| 13                                      | c69183_g1_i1  | 17.41033 | 22.91591 | 23.94181 | 27.05188     | -3.00 | 115.72             | 0.926 |
| 14                                      | c89048_g1_i1  | 14.22723 | 24.2135  | 27.73789 | 30.94253     | -5.37 | 53.58              | 0.915 |
| 15                                      | c107219_g1_i1 | 12.10072 | 14.71237 | 17.35781 | 20.91320     | -2.91 | 120.72             | 0.994 |
| 16                                      | c109355_g1_i1 | 13.50759 | 16.91715 | 18.90938 | 19.70821     | -2.06 | 205.90             | 0.926 |
| 17                                      | c106522_g1_i1 | 13.55193 | 16.9506  | 19.83420 | 21.85745     | -2.78 | 128.93             | 0.988 |
| 18                                      | <i>rpt6</i>   | 20.33907 | 22.89569 | 27.50129 | 30.76015     | -3.59 | 90.02              | 0.989 |
| 19                                      | <i>rpl3</i>   | 14.53249 | 23.94285 | 27.20511 | 28.43042     | -4.50 | 66.89              | 0.852 |
| 20                                      | <i>rpl40</i>  | 21.93397 | 24.59916 | 28.16891 | 30.81913     | -3.02 | 114.21             | 0.996 |

## A. Agarose gel analysis

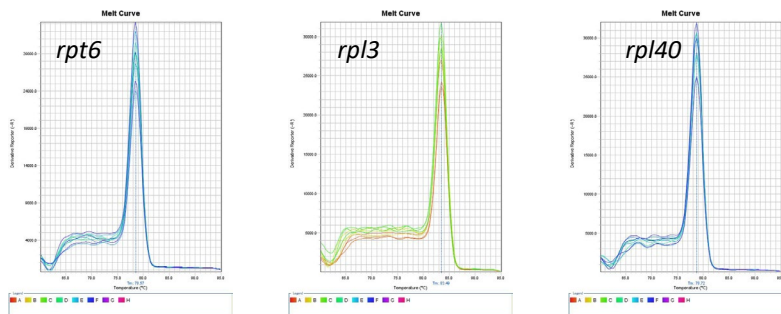


| Lane | Target          | tissue |
|------|-----------------|--------|
| 1    | GeneRuler 50 bp |        |
| 2    | <i>rpl3</i>     | PT     |
| 3    | <i>rpl3</i>     | AT     |
| 4    | <i>rpl3</i>     | MT     |
| 5    | <i>rpl40</i>    | PT     |
| 6    | <i>rpl40</i>    | AT     |
| 7    | <i>rpl40</i>    | MT     |
| 8    | <i>rpt6</i>     | PT     |
| 9    | <i>rpt6</i>     | AT     |
| 10   | <i>rpt6</i>     | MT     |

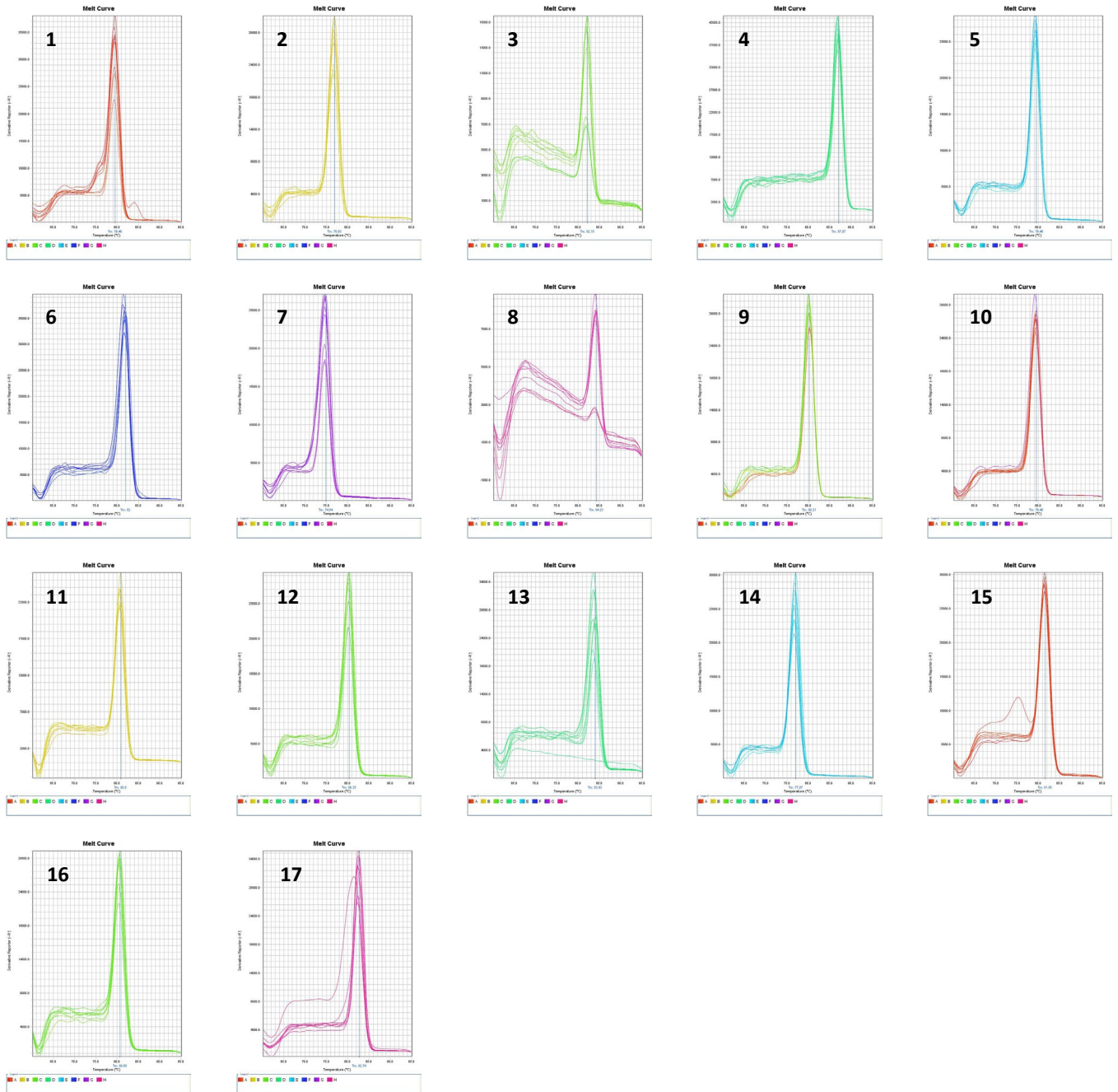
## B. PCR efficiencies



## C. Melt curves

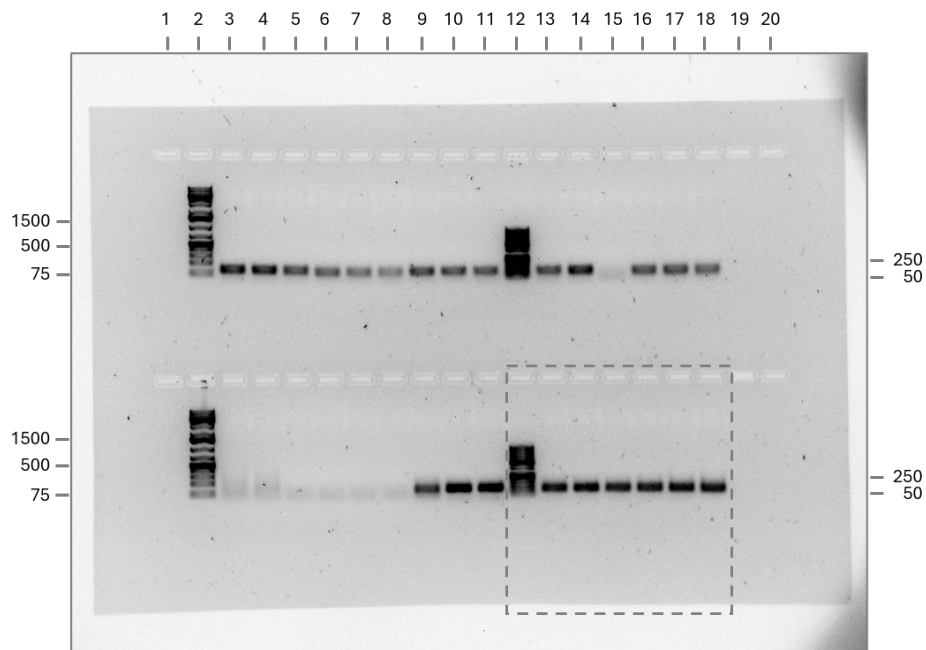


**Figure S1. Preliminary validation of candidate housekeeping genes (*rpt6*, *rpl3*, and *rpl40*) for qRT-PCR. (A)** An 1.2% agarose gel electrophoresis showing PCR amplification products across three distinct tissue types (sample details provided in the adjacent table). Molecular weight marker: GeneRuler 50 bp DNA Ladder (Thermo Scientific). **(B)** Regression curve and quantitative PCR efficiency for each target gene. **(C)** Melt curve analysis confirming single-product amplification specificity.



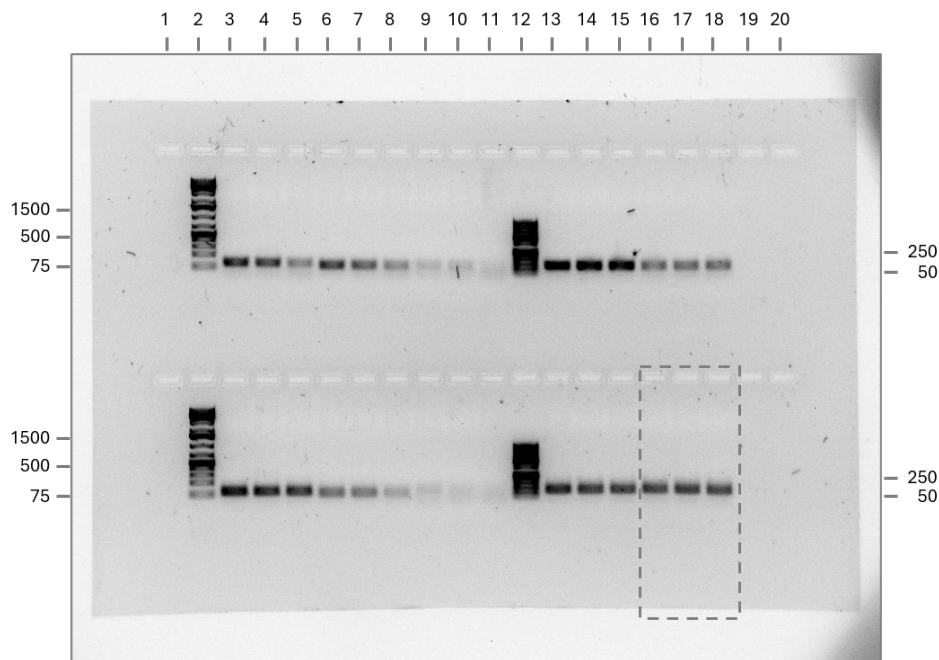
**Figure S2. Melt curve analysis of qRT-PCR products.** Single, sharp dissociation peaks confirm amplification specificity and the absence of non-specific products or primer-dimers for the 17 target GPCR genes.

A. Agarose gel analysis (*rpl3* and *rpl40*)



| Upper lane | Target              | Tissue | Lower lane | Target              | Tissue |
|------------|---------------------|--------|------------|---------------------|--------|
| 1          | Blank               | n/a    | 1          | Blank               | n/a    |
| 2          | GeneRuler 1 kb Plus | n/a    | 2          | GeneRuler 1 kb Plus | n/a    |
| 3          | 10                  | PT     | 3          | 15 (old)            | PT     |
| 4          | 10                  | AT     | 4          | 15 (old)            | AT     |
| 5          | 10                  | MT     | 5          | 15 (old)            | MT     |
| 6          | 11                  | PT     | 6          | 16 (old)            | PT     |
| 7          | 11                  | AT     | 7          | 16 (old)            | AT     |
| 8          | 11                  | MT     | 8          | 16 (old)            | MT     |
| 9          | 12                  | PT     | 9          | 17                  | PT     |
| 10         | 12                  | AT     | 10         | 17                  | AT     |
| 11         | 12                  | MT     | 11         | 17                  | MT     |
| 12         | GeneRuler 50 bp     | n/a    | 12         | GeneRuler 50 bp     | n/a    |
| 13         | 13                  | PT     | 13         | <i>rpl3</i>         | PT     |
| 14         | 13                  | AT     | 14         | <i>rpl3</i>         | AT     |
| 15         | 13                  | MT     | 15         | <i>rpl3</i>         | MT     |
| 16         | 14                  | PT     | 16         | <i>rpl40</i>        | PT     |
| 17         | 14                  | AT     | 17         | <i>rpl40</i>        | AT     |
| 18         | 14                  | MT     | 18         | <i>rpl40</i>        | MT     |
| 19         | Blank               | n/a    | 19         | Blank               | n/a    |
| 20         | Blank               | n/a    | 20         | Blank               | n/a    |

## B. Agarose gel analysis (*rpt6*)



| Upper lane | Target              | Tissue | Lower lane | Target              | Tissue |
|------------|---------------------|--------|------------|---------------------|--------|
| 1          | Blank               | n/a    | 1          | Blank               | n/a    |
| 2          | GeneRuler 1 kb Plus | n/a    | 2          | GeneRuler 1 kb Plus | n/a    |
| 3          | 1                   | PT     | 3          | 6                   | PT     |
| 4          | 1                   | AT     | 4          | 6                   | AT     |
| 5          | 1                   | MT     | 5          | 6                   | MT     |
| 6          | 2                   | PT     | 6          | 7                   | PT     |
| 7          | 2                   | AT     | 7          | 7                   | AT     |
| 8          | 2                   | MT     | 8          | 7                   | MT     |
| 9          | 3                   | PT     | 9          | 8                   | PT     |
| 10         | 3                   | AT     | 10         | 8                   | AT     |
| 11         | 3                   | MT     | 11         | 8                   | MT     |
| 12         | GeneRuler 50 bp     | n/a    | 12         | GeneRuler 50 bp     | n/a    |
| 13         | 4                   | PT     | 13         | 9                   | PT     |
| 14         | 4                   | AT     | 14         | 9                   | AT     |
| 15         | 4                   | MT     | 15         | 9                   | MT     |
| 16         | 5                   | PT     | 16         | <i>rpt6</i>         | PT     |
| 17         | 5                   | AT     | 17         | <i>rpt6</i>         | AT     |
| 18         | 5                   | MT     | 18         | <i>rpt6</i>         | MT     |
| 19         | Blank               | n/a    | 19         | Blank               | n/a    |
| 20         | Blank               | n/a    | 20         | Blank               | n/a    |

**Figure S3. Uncropped original DNA gel images corresponding to Figure S1-A.**

**(A)** Full, uncropped agarose gel image showing PCR products generated for *rp13* and *rp140*. All lanes, including molecular weight markers, are presented for complete transparency. PCR samples were electrophoresed on a 1.2% agarose gel containing 1X SYBR Safe DNA gel stain and visualized using the Alphamager system (Alpha Innotech) according to the manufacturer's instructions. **(B)** Additional uncropped gel panel showing PCR products for *rpt6*. Gel electrophoresis and image acquisition were performed identically to the conditions described for panel A. Lanes enclosed by dashed lines correspond to the cropped regions shown in Figure S1-A.