

**Figure 1b)** Luciferase reporter assay

| REV        | 0        | 5        | 0        | 1        | 3        | 5        |
|------------|----------|----------|----------|----------|----------|----------|
| PET-1      | 0        | 0        | 5        | 5        | 5        | 5        |
| mTph2p-Luc | 1.097446 | 0.779761 | 1.950424 | 1.237107 | 0.753468 | 0.718678 |
|            | 0.975763 | 0.64916  | 1.815218 | 1.35221  | 0.718678 | 0.620305 |
|            | 0.975843 | 0.70125  | 1.652753 | 1.302005 | 0.691085 | 0.562721 |
|            | 0.950947 | 0.651832 | 2.00262  | 1.294153 | 0.653895 | 0.681488 |
| hTph2p-Luc | 1.224194 | 0.14193  | 1.35893  | 0.2129   | 0.266118 | 0.319312 |
|            | 0.799906 | 0.425791 | 2.159418 | 0.266118 | 0.279421 | 0.177415 |
|            | 1.075124 | 0.266114 | 1.14381  | 0.74517  | 0.212895 | 0.031933 |
|            | 0.900776 | 0.106446 | 2.002041 | 0.239505 | 0.298047 | 0.212904 |

**Figure 1c)** In vitro ChIP-qPCR

| <i>mTph2p amplicon</i>   |            |            |            |            |            |
|--------------------------|------------|------------|------------|------------|------------|
| REV                      | 0          | 1          | 2          | 5          | 5          |
| PET-1                    | 5          | 5          | 5          | 5          | 0          |
| REV                      | 1.2634E-07 | 16.4938489 | 20.7329887 | 67.8302164 | 100        |
|                          | 1.81989623 | 18.6209683 | 26.6092546 | 64.6176415 | 100        |
|                          | 0.2980975  | 1.83891678 | 13.2585968 | 63.9492791 | 100        |
|                          | 100        | 28.9172    | 31.31661   | 8.333117   | 0.00184    |
| PET-1                    | 100        | 80.6641759 | 57.6343173 | 32.5335464 | 0.48258541 |
|                          | 100        | 37.2419366 | 44.1351498 | 46.9761375 | 3.47944256 |
| <i>hTph2p amplicon 1</i> |            |            |            |            |            |
| REV                      | 0          | 0.2        | 0.3        | 0.5        | 1          |
| REV                      | 1.167851   | 4.671404   | 6.629298   | 79.27841   | 100        |
|                          | 5.129635   | 20.80497   | 26.06164   | 90.75192   | 100        |
|                          | 0.579892   | 4.496667   | 15.55015   | 104.6085   | 100        |
| <i>hTph2p amplicon 2</i> |            |            |            |            |            |
| REV                      | 0          | 1          | 2          | 5          | 5          |
| PET-1                    | 5          | 5          | 5          | 5          | 0          |
| REV                      | 15.76722   | 19.27764   | 24.23225   | 23.48807   | 100        |
|                          | 5.366034   | 25.70285   | 54.90464   | 109.8093   | 100        |
|                          | 2.895588   | 17.80063   | 41.89936   | 102.4557   | 100        |
|                          | 1.251672   | 6.381326   | 20.80497   | 98.62327   | 100        |
| PET-1                    | 100        | 88.2703    | 23.73355   | 21.99123   | 0.769327   |
|                          | 100        | 79.55365   | 80.3851    | 87.96491   | 10.65794   |
|                          | 100        | 72.19646   | 44.44213   | 49.65462   | 4.210105   |
|                          | 100        | 42.19079   | 37.76181   | 58.64175   | 23.9816    |

**Figure 1e)** Site-directed mutagenesis study, luciferase reporter assay

| REV   | minus    | plus     | minus    | plus     |
|-------|----------|----------|----------|----------|
| PET-1 | minus    | minus    | plus     | plus     |
| WT    | 1.033079 | 0.680232 | 1.732511 | 1.38125  |
|       | 0.961299 | 0.615234 | 2.224331 | 1.541368 |
|       | 1.107363 | 0.691839 | 1.922657 | 1.281129 |

**Figure 1f)**

|           |          |          |          |          |
|-----------|----------|----------|----------|----------|
| RRE mut 1 | 0.999121 | 0.938596 | 2.001192 | 1.556379 |
|           | 1.076509 | 0.868943 | 1.705886 | 1.974551 |
|           | 1.030779 | 0.942261 | 1.506082 | 1.339442 |
| RRE mut 2 | 1.011106 | 0.932698 | 1.557847 | 1.467823 |
|           | 1.120525 | 0.916507 | 1.805745 | 1.725666 |
|           | 0.916907 | 1.014384 | 1.982207 | 1.811685 |

**Figure 1g)**

|           |          |          |          |          |
|-----------|----------|----------|----------|----------|
| PRE mut 3 | 1.037427 | 0.790557 | 1.044156 | 0.72142  |
|           | 0.951905 | 0.629664 | 0.882797 | 0.902821 |
|           | 1.009352 | 0.756599 | 0.854193 | 0.833716 |
| PRE mut 4 | 0.984055 | 0.726734 | 0.943691 | 0.876275 |
|           | 1.055578 | 0.811361 | 1.085791 | 0.806622 |
|           | 1.066737 | 0.587328 | 0.708406 | 0.865277 |

**Figure 1h)** Luciferase reporter assay: REV vs. RORa on mTph2p promoter

| REV        | minus    | plus     | minus    | plus     |
|------------|----------|----------|----------|----------|
| RORa       | minus    | minus    | plus     | plus     |
| mTph2p-Luc | 0.983258 | 0.830093 | 0.924639 | 0.696819 |
|            | 1.02559  | 0.759689 | 0.931648 | 0.62768  |
|            | 0.997338 | 0.761569 | 1.054178 | 0.70639  |
|            | 0.993815 | 0.768999 | 0.934185 | 0.728112 |

**Figure 1i)** Luciferase reporter assay: REV vs. RORa on mBmal1 promoter

| REV         | minus    | plus     | minus    | plus     |
|-------------|----------|----------|----------|----------|
| RORa        | minus    | minus    | plus     | plus     |
| mBmal1p-Luc | 1.097446 | 0.809977 | 1.950424 | 1.237107 |
|             | 0.975763 | 0.758636 | 1.815218 | 1.35221  |
|             | 0.975843 | 0.780994 | 1.652753 | 1.302005 |
|             | 0.950947 | 0.646915 | 2.00262  | 1.294153 |

**Figure 2a)** In vivo dorsal raphe ChIP assay: REV or PET-1 binding affinities

|       |       |       |        |       |
|-------|-------|-------|--------|-------|
| CT    | 00    | 06    | 12     | 18    |
|       | 7.692 | 9.851 | 14.724 | 3.938 |
| REV   | 5.311 | 9.938 | 15.006 | 3.938 |
|       | 8.026 | 7.044 | 16.118 | 2.855 |
| CT    | 00    | 06    | 12     | 18    |
|       | 3.361 | 5.861 | 4.179  | 6.265 |
| PET-1 | 5.357 | 6.2   | 6.498  | 4.326 |
|       | 3.127 | 5.5   | 3.945  | 4.23  |

**Figure 2b)** Real-time qPCR results normalized by Tbp

|                    |            |            |            |            |            |            |            |            |            |
|--------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Circadian time (t) | 04         | 08         | 12         | 16         | 20         | 24         | 04         | 08         | 12         |
| <i>Tph2</i>        | 1.06402393 | 0.88719773 | 0.86018029 | 0.93422457 | 1.21260629 | 0.90680158 | 1.03077223 | 0.8407182  | 0.89963242 |
|                    | 1.00765788 | 1.18017738 | 0.91431027 | 0.92722043 | 1.83337964 | 1.04748181 | 1.21173257 | 1.04769755 | 0.95861113 |
|                    | 1.26861424 | 0.97727397 | 0.91383966 | 0.96247688 | 1.11385709 | 1.06314779 | 1.04726611 | 1.03941447 | 0.93730795 |
|                    | 1.04726611 | 0.97727397 | 0.91383966 | 0.96247688 | 3.42999478 | 1.46314779 | 1.13501677 | 1.06653301 | 0.96236171 |
|                    | 1.03077223 | 0.8407182  | 0.89963242 | 0.96257599 | 3.15617873 | 1.38231597 | 1.26146108 | 1.18674249 | 0.87923717 |
|                    | 1.00765788 | 1.18017738 | 0.91431027 | 1.35476287 | 1.83337964 | 1.33049673 | 1.16878674 | 1.04407994 | 1.06889421 |
| <i>Pet-1</i>       | 1.39681199 | 1.44409739 | 1.4136482  | 1.48202771 | 1.20518309 | 1          | 1.08445795 | 0.98282235 | 0.54250083 |
|                    | 1.39916498 | 1.16508229 | 1.29639931 | 1.46255701 | 1.56193038 | 1.31766368 | 1.28492012 | 1.4470726  | 1.45987134 |
|                    | 1.4214911  | 1.08840035 | 1.25816016 | 1.49002696 | 0.88448283 | 1.3235212  | 1.23541851 | 1.44657441 | 1.53725251 |
| <i>Rev-erba</i>    | 0.798203   | 1.584964   | 0.99791    | 0.5375316  | 0.20568    | 0.295288   | 0.619983   | 1.646939   | 0.82297    |
|                    | 0.933279   | 1.291193   | 0.56114    | 0.3842967  | 0.207296   | 0.245897   | 1.041994   | 1.530359   | 0.91504    |
|                    | 1.349809   | 1.300165   | 1.02838    | 0.3462773  | 0.183575   | 0.31855    | 1.256732   | 1.209979   | 0.67709    |
|                    |            |            |            | 0.8197096  | 0.374042   | 0.186079   |            | 1.474991   |            |
| <i>Ddc</i>         | 0.923937   | 0.939019   | 0.894929   | 0.981597   | 0.75817627 | 1.225526   | 1.002826   | 1.041184   | 0.694155   |
|                    | 1.033322   | 0.991676   | 0.320696   | 0.88832553 | 0.76770415 | 1.207674   | 0.670355   | 0.762154   | 0.665068   |
|                    | 0.963853   | 1.140985   | 0.867625   | 0.92625432 | 0.77229495 | 0.844605   | 1.189264   | 0.839915   | 0.644777   |

**Figure 2c)** HPLC-ECD: Quantification of 5-HT

|                      |          |         |          |          |       |         |
|----------------------|----------|---------|----------|----------|-------|---------|
| CT                   | 00       | 04      | 08       | 12       | 16    | 20      |
|                      | 17.82911 | 26.4704 | 16.86736 | 11.612   | 13.53 | 13.2396 |
| 5-HT (pg/mg protein) | 16.70359 | 28.1973 | 14.23986 | 11.432   | 10.72 | 12.0844 |
|                      | 15.33681 | 25.6794 | 13.35124 | 11.003   | 8.607 | 10.5649 |
|                      | 17.54067 | 23.5838 |          | 13.18209 |       |         |

**Figure 3a)** CRISPR/Cas9 KO efficiency

|                     |   |         |   |          |              |          |         |
|---------------------|---|---------|---|----------|--------------|----------|---------|
| <b>REV-ERBA cKO</b> | 0 | Control | 0 | 89.24731 | Rev-erba cKO | 95.65217 | 90.2439 |
| <b>PET-1 cKO</b>    | 0 | Control | 0 | 92.30769 | Pet-1cKO     | 100      | 100     |

**Figure 3b)** Tph2p mRNA quantification

|      |          |        |          |        |           |        |        |              |        |
|------|----------|--------|----------|--------|-----------|--------|--------|--------------|--------|
|      |          | WT     |          |        | Pet-1 cKO |        |        | Rev-erba cKO |        |
| CT00 | 1        | 1.1548 | 0.921907 | 0.142  | 0.3369    | 0.3202 | 1.2187 | 1.1902       | 1.1635 |
| CT12 | 0.247516 | 0.5805 | 0.5903   | 0.1628 | 0.5476    | 0.4731 | 1.0948 | 0.8671       | 0.8053 |

**Figure 3c)** 5-HT contents (pg/mg protein)

|      |          |          |          |          |              |          |          |           |          |
|------|----------|----------|----------|----------|--------------|----------|----------|-----------|----------|
|      |          | WT       |          |          | Rev-erba cKO |          |          | Pet-1 cKO |          |
| CT00 | 1.014997 | 1.208124 | 0.776879 | 1.127174 | 1.056036     | 1.489904 | 0.215297 | 0.15492   | 0.121316 |
| CT12 | 0.202235 | 0.225335 | 0.291631 | 0.824636 | 0.532722     | 0.982361 | 0.190716 | 0.154384  | 0.050497 |

**Figure 3d)** Rev-erba cKO in vivo ChIP assay

|            |        |        |        |        |              |        |
|------------|--------|--------|--------|--------|--------------|--------|
| anti-REV   |        | WT     |        |        | Rev-erba cKO |        |
| CT00       | 8.634  | 7.692  | 8.026  | 1.233  | 4.684        | 3.988  |
| CT12       | 19.973 | 14.724 | 15.006 | 4.584  | 4.472        | 3.469  |
| anti-PET-1 |        | WT     |        |        | Rev-erba cKO |        |
| CT00       | 3.361  | 5.357  | 3.127  | 16.144 | 10.821       | 9.378  |
| CT12       | 4.179  | 6.498  | 3.945  | 18.568 | 9.139        | 14.322 |

**Figure 4b)** Percent immobility for forced swim test

|         |       |      |       |       |              |       |       |       |
|---------|-------|------|-------|-------|--------------|-------|-------|-------|
|         |       |      |       |       | Control      |       |       |       |
| CT22-01 | 14.6  | 12.5 | 25    | 16.25 | 10.83        | 17.5  | 0     | 4.17  |
| CT10-13 | 45.8  | 35.8 | 41.3  | 27.9  | 51.7         | 46.25 | 60.83 | 57.92 |
|         |       |      |       |       | Rev-erba cKO |       |       |       |
| CT22-01 | 20.83 | 20   | 6.25  | 5     | 1.25         | 27.5  | 0.83  | 12.08 |
| CT10-13 | 4.17  | 35   | 19.58 | 26.25 | 23.33        | 10.4  | 18.33 | 21.67 |
|         |       |      |       |       | Pet1 cKO     |       |       |       |

|                |       |       |       |       |       |       |       |       |       |
|----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| <i>CT22-01</i> | 45.83 | 36.67 | 35    | 29.58 | 28.33 | 27.5  | 43.75 | 57.08 | 44.58 |
| <i>CT10-13</i> | 44.17 | 39.58 | 22.08 | 37.5  | 44.58 | 49.17 | 71.67 | 72.92 | 57.5  |

**Figure 4c)** Percent immobility for tail suspension test

|                |       |       |       |       |                     |      |       |       |       |
|----------------|-------|-------|-------|-------|---------------------|------|-------|-------|-------|
|                |       |       |       |       | <b>Control</b>      |      |       |       |       |
| <i>CT22-01</i> | 14.58 | 12.5  | 1.67  | 16.67 | 10.42               | 1.25 | 4.58  | 3.75  |       |
| <i>CT10-13</i> | 24.17 | 51.25 | 29.17 | 41.67 | 32.92               | 36.7 | 40    | 36.67 |       |
|                |       |       |       |       | <b>Rev-erba cKO</b> |      |       |       |       |
| <i>CT22-01</i> | 19.17 | 29.58 | 8.33  | 20    | 0.83                | 0.42 | 31.67 | 32.92 |       |
| <i>CT10-13</i> | 4.58  | 5     | 10    | 5.42  | 10.42               | 8.75 | 2.5   | 12.5  |       |
|                |       |       |       |       | <b>Pet1 cKO</b>     |      |       |       |       |
| <i>CT22-01</i> | 37.08 | 31.25 | 37.92 | 39.58 | 38.75               | 42.5 | 57.92 | 63.33 | 21.25 |
| <i>CT10-13</i> | 47.08 | 26.67 | 42.08 | 47.92 | 49.17               | 42.5 | 58.33 | 69.17 | 67.08 |

**Figure 5b)** Quantification of 5-HT contents at ZT04 after treatment of drugs

|                      |          |          |            |
|----------------------|----------|----------|------------|
|                      | Vehicle  | SR8278   | GSK4112    |
| 5-HT (pg/mg protein) | 24.14627 | 30.43606 | 21.8642314 |
|                      | 23.90748 | 26.82183 | 21.7916933 |
|                      | 24.83403 | 28.21092 | 21.48005   |

**Figure 5c)** Percent immobility for forced swim test

|                |       |       |       |       |                |       |       |       |       |
|----------------|-------|-------|-------|-------|----------------|-------|-------|-------|-------|
|                |       |       |       |       | <b>Control</b> |       |       |       |       |
| <i>CT22-01</i> | 31.67 | 22.92 | 20    | 25.83 | 14.58          | 13.75 | 9.17  | 15.42 |       |
| <i>CT10-13</i> | 44.58 | 40    | 42.08 | 45.83 | 33.75          | 34.58 | 32.08 | 18.33 |       |
|                |       |       |       |       | <b>SR8278</b>  |       |       |       |       |
| <i>CT22-01</i> | 29.58 | 5     | 17.08 | 4.58  | 5.83           | 6.25  |       |       |       |
| <i>CT10-13</i> | 18.75 | 15.83 | 1.25  | 22.5  | 17.5           | 14.17 |       |       |       |
|                |       |       |       |       | <b>GSK4112</b> |       |       |       |       |
| <i>CT22-01</i> | 28.75 | 38.33 | 47.5  | 40    | 52.92          | 45.42 | 36.25 | 51.25 | 25    |
| <i>CT10-13</i> | 73.75 | 62.5  | 49.17 | 39.58 | 55.83          | 49.17 | 62.08 | 27.08 | 30.42 |

**Figure 5d)** Percent immobility for tail suspension test

|                |       |       |       |        |                |       |       |       |       |
|----------------|-------|-------|-------|--------|----------------|-------|-------|-------|-------|
|                |       |       |       |        | <b>Control</b> |       |       |       |       |
| <i>CT22-01</i> | 27.92 | 21.67 | 12.08 | 18.33  | 13.33          | 17.92 | 1.25  | 13.75 |       |
| <i>CT10-13</i> | 37.92 | 44.58 | 33.75 | 25.417 | 48.75          | 42.08 | 45    | 29.17 |       |
|                |       |       |       |        | <b>SR8278</b>  |       |       |       |       |
| <i>CT22-01</i> | 16.67 | 2.08  | 17.5  | 7.5    | 9.17           | 7.92  |       |       |       |
| <i>CT10-13</i> | 15.42 | 0     | 8.75  | 15     | 21.25          | 1.25  |       |       |       |
|                |       |       |       |        | <b>GSK4112</b> |       |       |       |       |
| <i>CT22-01</i> | 24.58 | 53.33 | 28.33 | 64.58  | 62.08          | 60.42 | 61.25 | 80.83 | 32.5  |
| <i>CT10-13</i> | 52.08 | 36.25 | 73.33 | 21.25  | 34.58          | 79.58 | 17.5  | 51.67 | 57.92 |