

## **SUPPLEMENTARY INFORMATION**

“Wakefulness Induced by TAAR1 Partial Agonism is Mediated Through Dopaminergic Neurotransmission”

Sunmee Park<sup>1</sup>, Jasmine Heu<sup>1</sup>, Marius C. Hoener<sup>2</sup>, Thomas S. Kilduff<sup>1</sup>

<sup>1</sup>Center for Neuroscience, Biosciences Division, SRI International, Menlo Park, CA

<sup>2</sup>Neuroscience, Ophthalmology and Rare Diseases DTA, pRED, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland

### **This file includes:**

Figures S1 to S5

Tables S1 to S2

## Supplementary Figures

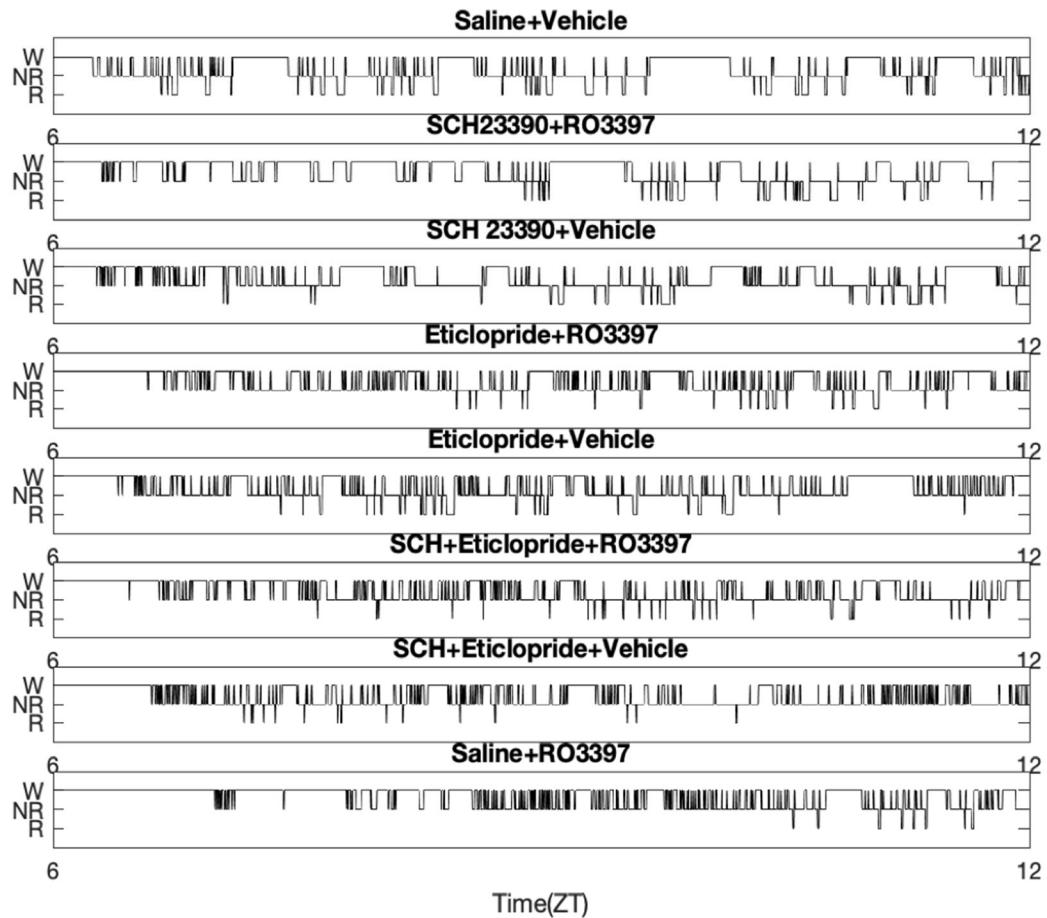


**Figure S1. Example hypnograms of each treatment condition.** Hypnograms of male C57BL/6J mice recorded from ZT6 to ZT12. Each hypnogram represents one mouse from each treatment group, illustrating the variability in distribution of sleep/wake states (Wake, NREM, and REM) across the 6-hour recording period after dosing at ZT6.

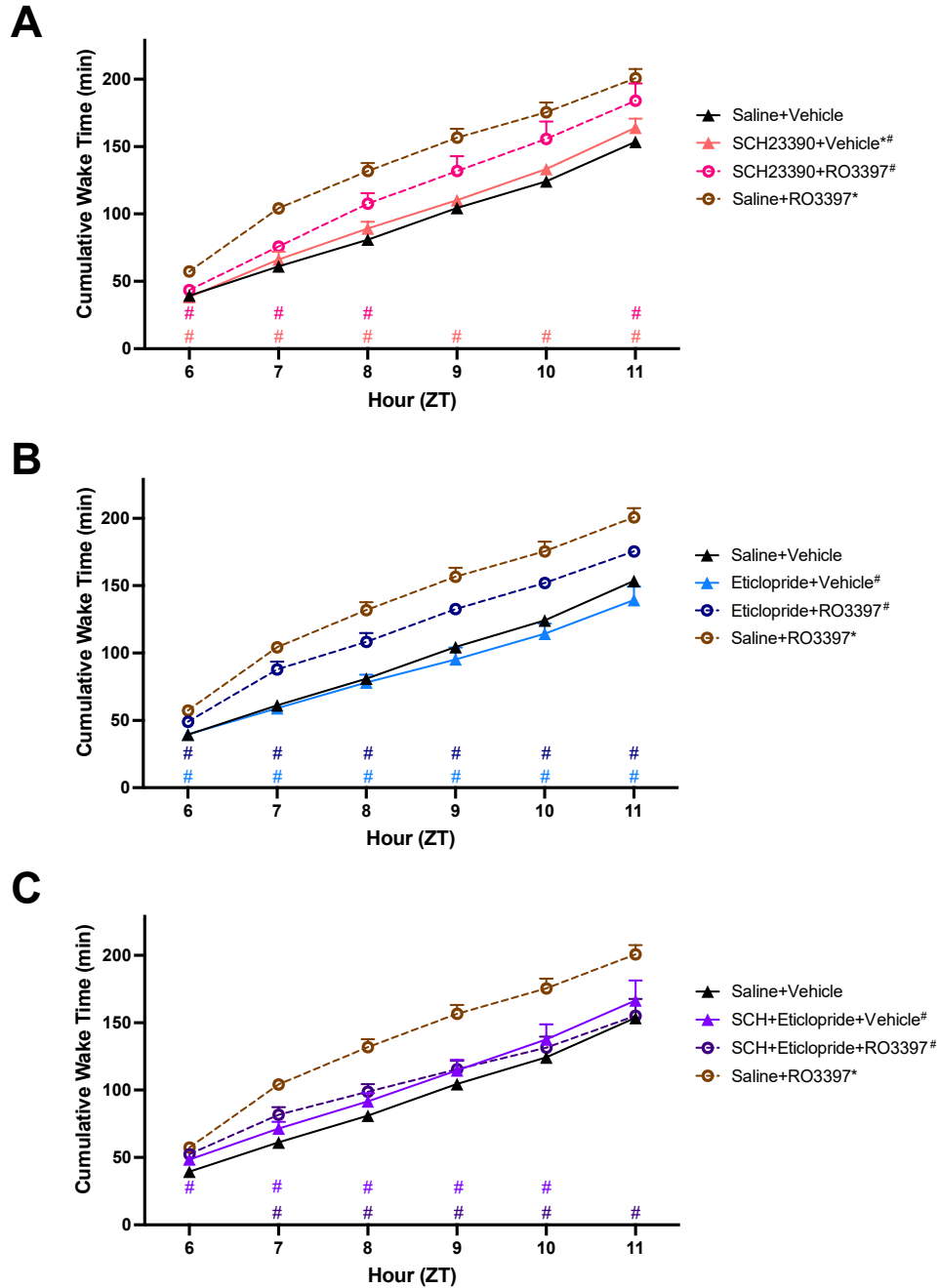


**Figure S2. Cumulative Wake time for the first 6 hours after the second dosing.** For ease of visualization, data are split into three subgroups in which the results from the negative (Sal+Veh) and positive (Sal+RO3397) control treatments are repeated in each graph. **A-C.** Cumulative Wake time (mean+SEM). Colored symbols indicate statistical significance for that hour compared to Sal+Veh(\*) or Sal+RO3397(#) based on RM-ANOVA. \*, #  $p < 0.05$ ;

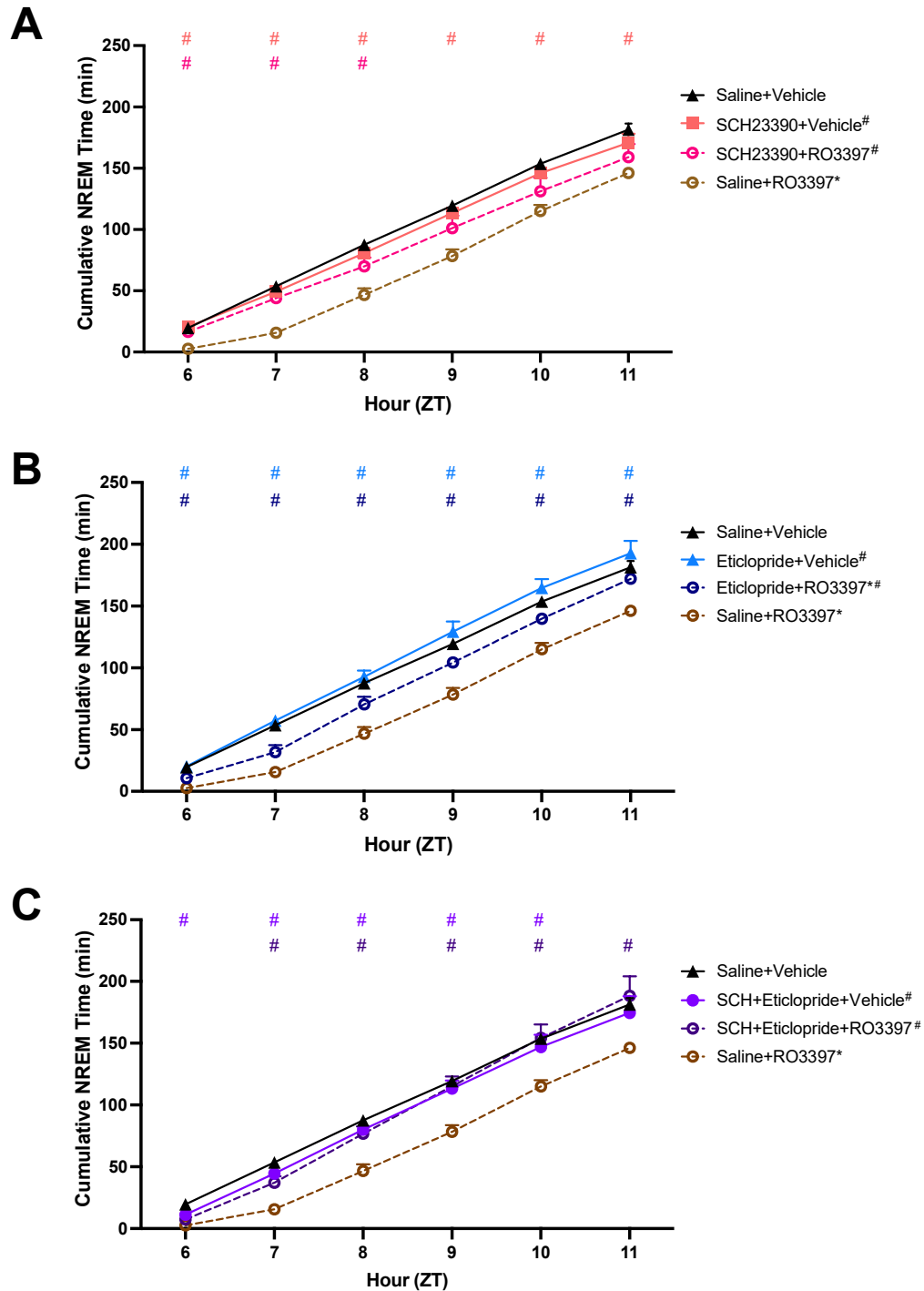


**Figure S3. Cumulative NREM time for the first 6 hours after the second dosing.** For ease of visualization, data are split into three subgroups in which the results from the negative (Sal+Veh) and positive (Sal+RO3397) control treatments are repeated in each graph. **A-C.** Cumulative NREM time (mean+SEM). Colored symbols indicate statistical significance for that hour compared to Sal+Veh(\*) or Sal+RO3397(<sup>#</sup>) based on RM-ANOVA. \*, <sup>#</sup> p < 0.05;

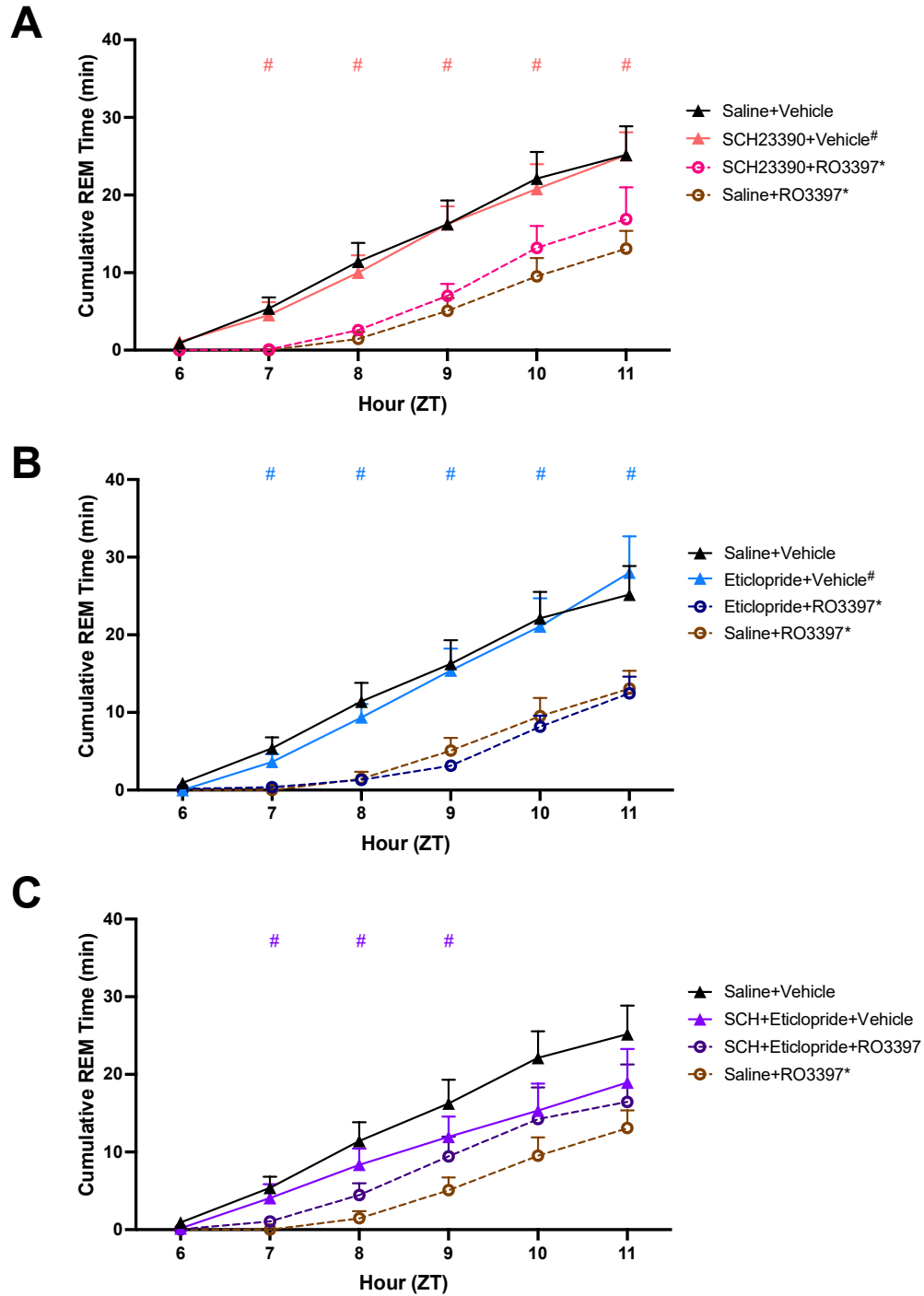


**Figure S4. Cumulative REM time for the first 6 hours after the second dosing.** For ease of visualization, data are split into three subgroups in which the results from the negative (Sal+Veh) and positive (Sal+RO3397) control treatments are repeated in each graph. **A-C.** Cumulative REM time (mean+SEM). Colored symbols indicate statistical significance for that hour compared to Sal+Veh(\*) or Sal+RO3397(#) based on RM-ANOVA. \*, #  $p < 0.05$ ;

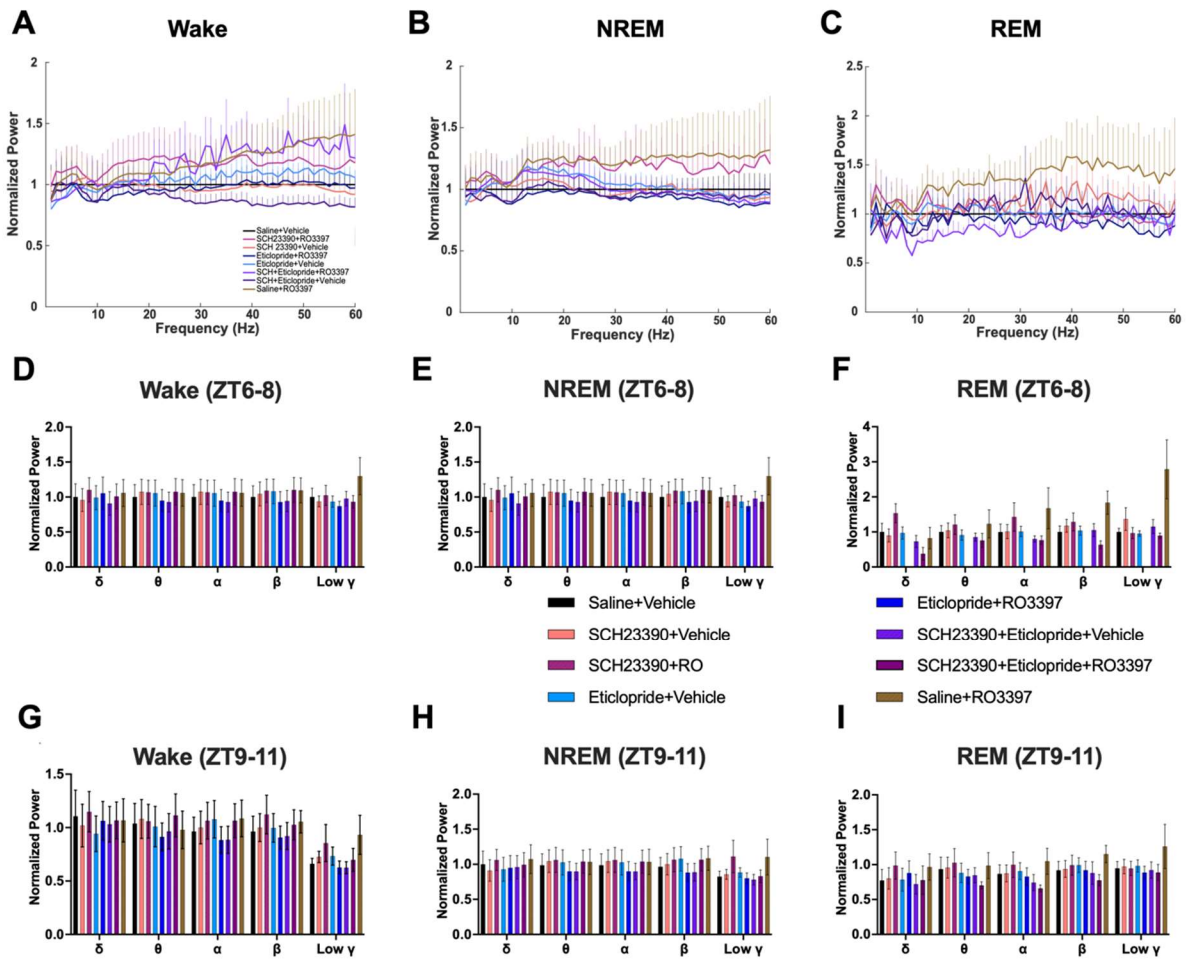


**Figure S5. EEG spectral power (0-60 Hz) for each treatment during the first 6 post-dosing hours. A-C.** 6-hour average spectral power for (A) Wake, (B) NREM sleep and (C) REM sleep. **D-F.** Normalized EEG power binned into the conventional bandwidths for each treatment during the first 3 post-dosing hours (ZT6-8) for (D) Wake, (E) NREM sleep and (F) REM sleep. **G-I.** Normalized EEG power binned into the conventional bandwidths for each treatment during the second 3 post-dosing hours (ZT9-11) for (G) Wake (H) NREM sleep and (I) REM sleep.

**Table S1.** Statistics underlying Figures and Supplementary Figures.

| Figure/Panel<br>(Time bin) | Data Structure      | Type of test                   | F value | p value | Variable                                        |
|----------------------------|---------------------|--------------------------------|---------|---------|-------------------------------------------------|
| <b>1B-D</b>                | normal distribution | 2-way ANOVA (Condition)        | 3.919   | 0.0019  | ZT6-ZT11 hourly wake time (min)                 |
| 1B                         | normal distribution | Tukey's multiple comparison    |         | <0.0001 | Saline+Vehicle vs. Saline+RO3397                |
| 1C                         | normal distribution | Tukey's multiple comparison    |         | 0.0060  | Eticlopride+RO3397 vs. Saline+RO3397            |
| 1D                         | normal distribution | Tukey's multiple comparison    |         | 0.0075  | SCH23390+eticlopride+RO3397 vs. Saline+RO3397   |
| <b>1E-1G</b>               | normal distribution | 2-way ANOVA (Condition x Time) | 5.235   | 0.0002  | ZT6-8, ZT9-11 3-hour bin total wake time (min)  |
| 1E,1F,1G                   | normal distribution | Tukey's multiple comparison    |         | <0.0001 | Saline+Vehicle vs. Saline+RO3397                |
| 1E                         | normal distribution | Tukey's multiple comparison    |         | 0.0002  | SCH23390+Vehicle vs. Saline+RO3397              |
| 1F                         | normal distribution | Tukey's multiple comparison    |         | <0.0001 | Eticlopride+Vehicle vs. Saline+RO3397           |
| 1G                         | normal distribution | Tukey's multiple comparison    |         | 0.0005  | SCH23390+eticlopride+Vehicle vs. Saline+RO3397  |
| 1G                         | normal distribution | Tukey's multiple comparison    |         | 0.0086  | SCH23390+eticlopride+RO3397 vs. Saline+RO3397   |
| <b>2A-C</b>                | heterogeneous dist  | Brown-Forsythe ANOVA test      | 15.990  | <0.0001 | ZT6-12 NREM latency                             |
|                            |                     | Welch's ANOVA                  | 12.850  | <0.001  | ZT6-12 NREM latency                             |
| 2A,B,C                     | normal distribution | Dunnett's multiple comparison  |         | 0.0005  | Saline+Vehicle vs. Saline+RO3397                |
| 2A                         | normal distribution | Dunnett's multiple comparison  |         | 0.0003  | SCH23390+Vehicle vs. Saline+RO3397              |
| 2A                         | normal distribution | Dunnett's multiple comparison  |         | 0.0004  | SCH23389+RO3397 vs. Saline+RO3397               |
| 2B                         | normal distribution | Dunnett's multiple comparison  |         | 0.0003  | Eticlopride+Vehicle vs. Saline+RO3397           |
| 2C                         | normal distribution | Dunnett's multiple comparison  |         | 0.0076  | SCH23390+eticlopride+Vehicle vs. Saline+RO3397  |
| <b>2D-F</b>                | normal distribution | 2-way ANOVA (Condition)        | 2.391   | 0.0350  | ZT6-NT12 hourly NREM time (min)                 |
| 2D,E,F                     | normal distribution | Tukey's multiple comparison    |         | 0.0003  | Saline+Vehicle vs. Saline+RO3397                |
| 2D                         | normal distribution | Tukey's multiple comparison    |         | 0.0164  | SCH23390+Vehicle vs. Saline+RO3397              |
| 2E                         | normal distribution | Tukey's multiple comparison    |         | 0.0013  | Eticlopride+Vehicle vs. Saline+RO3397           |
| 2E                         | normal distribution | Tukey's multiple comparison    |         | 0.0023  | Eticlopride+RO3397 vs. Saline+RO3397            |
| 2F                         | normal distribution | Tukey's multiple comparison    |         | 0.0256  | SCH23390+eticlopride+RO3397 vs. Saline+RO3397   |
| <b>2G-I</b>                | normal distribution | 2-way ANOVA (Condition x Time) | 4.179   | 0.0012  | ZT6-9, ZT9-12 3-hour bin total NREM time (min)  |
| 2G,2H,2I                   | normal distribution | Tukey's multiple comparison    |         | 0.0004  | Saline+Vehicle vs. Saline+RO3397                |
| 2G                         | normal distribution | Tukey's multiple comparison    |         | 0.0060  | SCH23390+Vehicle vs. Saline+RO3397              |
| 2H                         | normal distribution | Tukey's multiple comparison    |         | <0.0001 | Eticlopride+Vehicle vs. Saline+RO3397           |
| 2I                         | normal distribution | Tukey's multiple comparison    |         | 0.0078  | SCH23390+eticlopride+Vehicle vs. Saline+RO3397  |
| 2I                         | normal distribution | Tukey's multiple comparison    |         | 0.0246  | SCH23390+eticlopride+RO3397 vs. Saline+RO3397   |
| <b>3A-C</b>                | heterogeneous dist  | Brown-Forsythe ANOVA test      | 10.550  | <0.0001 | ZT6-12 REM latency                              |
|                            |                     | Welch's ANOVA                  | 8.901   | <0.0001 | ZT6-12 REM latency                              |
| 3A,B,C                     | normal distribution | Dunnett's multiple comparison  |         | 0.0019  | Saline+Vehicle vs. Saline+RO3397                |
| 3A                         | normal distribution | Dunnett's multiple comparison  |         | 0.0013  | SCH23390+Vehicle vs. Saline+RO3397              |
| 3B                         | normal distribution | Dunnett's multiple comparison  |         | 0.0106  | Eticlopride+Vehicle vs. Saline+RO3397           |
| 3C                         | normal distribution | Dunnett's multiple comparison  |         | 0.0466  | SCH23390+eticlopride+Vehicle vs. Saline+Vehicle |
| 3C                         | normal distribution | Dunnett's multiple comparison  |         | 0.0233  | SCH23390+eticlopride+Vehicle vs. Saline+RO3397  |
| <b>3D-F</b>                | normal distribution | 2-way ANOVA (Condition x Time) | 2.458   | 0.0307  | NT6-NT12 NREM hourly time (min)                 |
| 3D,E,F                     | normal distribution | Tukey's multiple comparison    |         | 0.0167  | Saline+Vehicle vs. Saline+RO3397                |
| 3D                         | normal distribution | Tukey's multiple comparison    |         | 0.0073  | SCH23390+Vehicle vs. Saline+RO3397              |
| 3E                         | normal distribution | Tukey's multiple comparison    |         | 0.0146  | Eticlopride+Vehicle vs. Saline+RO3397           |
| <b>3G-I</b>                | normal distribution | 2-way ANOVA (Condition x Time) | 2.473   | 0.0299  | ZT6-9, ZT9-12 3-hour bin total NREM time (min)  |
| 3G,H,I                     | normal distribution | Tukey's multiple comparison    |         | 0.0470  | Saline+Vehicle vs. Saline+RO3397                |
| <b>4A-C</b>                | normal distribution | 2-way ANOVA (Condition x Time) | 2.490   | 0.0290  | ZT6-ZT12 Raw Temperature                        |
| 4B                         | normal distribution | Tukey's multiple comparison    |         | 0.0170  | Eticlopride+Vehicle vs. Saline+Vehicle          |
| 4C                         | normal distribution | Tukey's multiple comparison    |         | 0.0130  | SCH23390+eticlopride+Vehicle vs. Saline+RO3397  |
| 4C                         | normal distribution | Tukey's multiple comparison    |         | 0.0320  | SCH23390+eticlopride+RO3397 vs. Saline+RO3397   |
| <b>4D-F</b>                | normal distribution | 2-way ANOVA (Condition x Time) | 9.660   | <0.0001 | ZT6-ZT12 LMA (counts/min)                       |
| 4D,E,F                     | normal distribution | Tukey's multiple comparison    |         | 0.0467  | Saline+Vehicle vs. Saline+RO3397                |
| 4E                         | normal distribution | Tukey's multiple comparison    |         | 0.0001  | Eticlopride+Vehicle vs. Saline+Vehicle          |
| 4E                         | normal distribution | Tukey's multiple comparison    |         | 0.0004  | Eticlopride+RO3397 vs. Saline+Vehicle           |
| 4E                         | normal distribution | Tukey's multiple comparison    |         | 0.0014  | Eticlopride+Vehicle vs. Saline+RO3397           |
| 4E                         | normal distribution | Tukey's multiple comparison    |         | 0.0061  | Eticlopride+RO3397 vs. Saline+RO3397            |
| 4F                         | normal distribution | Tukey's multiple comparison    |         | 0.0002  | SCH23390+eticlopride+Vehicle vs. Saline+Vehicle |
| 4F                         | normal distribution | Tukey's multiple comparison    |         | 0.0001  | SCH23390+eticlopride+RO3397 vs. Saline+Vehicle  |

| Figure/Panel<br>(Time bin) | Data Structure      | Type of test                   | F value | p value | Variable                                       |
|----------------------------|---------------------|--------------------------------|---------|---------|------------------------------------------------|
| 4F                         | normal distribution | Tukey's multiple comparison    |         | 0.0001  | SCH23390+eticlopride+RO3397 vs. Saline+Vehicle |
| 4F                         | normal distribution | Tukey's multiple comparison    |         | 0.0030  | SCH23390+eticlopride+Vehicle vs. Saline+RO3397 |
| 4F                         | normal distribution | Tukey's multiple comparison    |         | 0.0013  | SCH23390+eticlopride+RO3397 vs. Saline+RO3397  |
| <b>Fig.S1</b>              | normal distribution | 2-way ANOVA (Condition x Time) | 2.882   | <0.0001 | ZT6-11 Cumulative Wake Time                    |
| Fig.S1A,B,C                | normal distribution | Tukey's multiple comparison    |         | <0.0001 | Saline+Vehicle vs. Saline+RO3397               |
| Fig.S1A                    | normal distribution | Tukey's multiple comparison    |         | <0.0001 | SCH23390+Vehicle vs. Saline+RO3397             |
| Fig.S1A                    | normal distribution | Tukey's multiple comparison    |         | 0.0464  | SCH23390+RO3397 vs. Saline+RO3397              |
| Fig.S1B                    | normal distribution | Tukey's multiple comparison    |         | <0.0001 | Eticlopride+Vehicle vs. Saline+RO3397          |
| Fig.S1B                    | normal distribution | Tukey's multiple comparison    |         | 0.0033  | Eticlopride+RO3397 vs. Saline+RO3397           |
| Fig.S1C                    | normal distribution | Tukey's multiple comparison    |         | 0.0025  | SCH23390+eticlopride+Vehicle vs. Saline+RO3397 |
| Fig.S1C                    | normal distribution | Tukey's multiple comparison    |         | 0.0016  | SCH23390+eticlopride+RO3397 vs. Saline+RO3397  |
| <b>Fig.S2</b>              | normal distribution | 2-way ANOVA (Condition x Time) | 5.474   | 0.0010  | ZT6-11 Cumulative NREM Time                    |
| Fig.S2A                    | normal distribution | 2-way ANOVA (Condition x Time) |         | 0.0001  | SCH23390+Vehicle vs. Saline+RO3397             |
| Fig.S2A                    | normal distribution | Tukey's multiple comparison    |         | 0.0401  | SCH23390+RO3397 vs. Saline+RO3397              |
| Fig.S2B                    | normal distribution | Tukey's multiple comparison    |         | 0.0001  | Eticlopride+Vehicle vs. Saline+RO3397          |
| Fig.S2B                    | normal distribution | Tukey's multiple comparison    |         | 0.0007  | Eticlopride+RO3397 vs. Saline+RO3397           |
| Fig.S2C                    | normal distribution | Tukey's multiple comparison    |         | 0.0025  | SCH23390+eticlopride+Vehicle vs. Saline+RO3397 |
| Fig.S2C                    | normal distribution | Tukey's multiple comparison    |         | 0.0050  | SCH23390+eticlopride+RO3397 vs. Saline+RO3397  |
| 3H (ZT19-ZT24)             | normal distribution | 2-way ANOVA (Condition x Time) | 4.761   | <0.0001 | ZT19-ZT24 Normalized NR Beta Power             |
| 3H (ZT1-ZT6)               | normal distribution | 2-way ANOVA (Condition x Time) | 2.543   | 0.0008  | ZT1-ZT6 Normalized NR Beta Power               |
| 3H (ZT7-ZT12)              | normal distribution | 2-way ANOVA (Condition x Time) | 1.589   | 0.0632  | ZT7-ZT12 Normalized NR Beta Power              |
| 4A (ZT19-ZT24)             | normal distribution | 2-way ANOVA (Condition x Time) | 2.660   | 0.0027  | WT mice Cumulative W Time (min)                |
| 4B (ZT19-ZT24)             | normal distribution | 2-way ANOVA (Condition x Time) | 2.780   | 0.0003  | HET mice Cumulative W Time (min)               |
| 4C (ZT19-ZT24)             | normal distribution | 2-way ANOVA (Condition x Time) | 1.040   | 0.4274  | KO mice Cumulative W Time (min)                |
| 4D (ZT19-ZT24)             | normal distribution | 2-way ANOVA (Condition x Time) | 1.980   | 0.0266  | WT mice Cumulative NR Time (min)               |
| 4E (ZT19-ZT24)             | normal distribution | 2-way ANOVA (Condition x Time) | 2.870   | 0.0002  | HET mice Cumulative NR Time (min)              |
| 4F (ZT19-ZT24)             | normal distribution | 2-way ANOVA (Condition x Time) | 1.010   | 0.4548  | KO mice Cumulative NR Time (min)               |

**Table S2.** Basal sleep/wake parameters of male C57BL6/J mice treated with a dopaminergic antagonist followed by TAAR1 agonist or vehicle.

| Treatment Condition          | N | Time (min)                             |               | Number of bouts                         |                             | Mean Bout Duration (min)               |             |
|------------------------------|---|----------------------------------------|---------------|-----------------------------------------|-----------------------------|----------------------------------------|-------------|
|                              |   | ZT6-8                                  | ZT9-11        | ZT6-8                                   | ZT9-11                      | ZT6-8                                  | ZT9-11      |
| <b>WAKE</b>                  |   |                                        |               |                                         |                             |                                        |             |
| Saline+Vehicle               | 7 | 81.0 ± 3.4                             | 72.5 ± 2.6    | 28.4 ± 3.0                              | 29.0 ± 3.0                  | 3.0 ± 0.5                              | 2.6 ± 0.3   |
| SCH23390+Vehicle             | 7 | 89.2 ± 5.0 <sup>††</sup>               | 74.9 ± 5.6    | 36.0 ± 2.8                              | 25.6 ± 1.5                  | 2.4 ± 0.2                              | 2.9 ± 0.3   |
| SCH23390+RO3397              | 7 | 107.6 ± 7.8                            | 76.5 ± 7.2    | 37.0 ± 3.3                              | 31.3 ± 4.8 <sup>†††</sup>   | 2.9 ± 0.3                              | 2.6 ± 0.4   |
| Eticlopride+Vehicle          | 7 | 78.0 ± 5.9 <sup>†††</sup>              | 61.3 ± 5.4    | 52.9 ± 4.2 <sup>™</sup>                 | 46.7 ± 4.6 <sup>†††</sup>   | 1.4 ± 0.2 <sup>†††</sup>               | 1.3 ± 0.1   |
| Eticlopride+RO3397           | 7 | 108.3 ± 6.6                            | 67.2 ± 7.6    | 56.4 ± 5.8 <sup>™™</sup>                | 45.7 ± 5.1 <sup>†††</sup>   | 2.0 ± 0.3 <sup>†††</sup>               | 1.6 ± 0.4   |
| SCH23390+Eticlopride+Vehicle | 7 | 91.6 ± 6.1 <sup>††</sup>               | 74.9 ± 10.4   | 57.3 ± 8.7 <sup>™™</sup>                | 52.71 ± 4.14 <sup>†††</sup> | 1.8 ± 0.3 <sup>†††</sup>               | 1.4 ± 0.2   |
| SCH23390+Eticlopride+RO3397  | 7 | 98.8 ± 5.7 <sup>††</sup>               | 56.4 ± 8.4    | 57.0 ± 4.8 <sup>™™</sup>                | 45.9 ± 6.3 <sup>†††</sup>   | 1.8 ± 0.2 <sup>†††</sup>               | 1.2 ± 0.1   |
| Saline+RO3397                | 7 | 131.9 ± 5.9 <sup>™™</sup>              | 68.9 ± 3.1    | 33.0 ± 3.6 <sup>™</sup>                 | 35.3 ± 5.7                  | 4.3 ± 0.6                              | 2.1 ± 0.3   |
| 2-way ANOVA                  |   | F <sub>(7,48)</sub> = 3.919; p = 0.002 |               | F <sub>(7,48)</sub> = 21.19; p < 0.0001 |                             | F <sub>(7,48)</sub> = 9.56; p < 0.0001 |             |
| <b>NREM Sleep</b>            |   |                                        |               |                                         |                             |                                        |             |
| Saline+Vehicle               | 7 | 87.6 ± 2.5                             | 93.8 ± 3.1    | 48.9 ± 4.6                              | 49.0 ± 6.0                  | 1.9 ± 0.3                              | 2.1 ± 0.3   |
| SCH23390+Vehicle             | 7 | 80.8 ± 3.8 <sup>††</sup>               | 90.0 ± 6.1    | 53.1 ± 4.1                              | 43.6 ± 3.1                  | 1.6 ± 0.2                              | 2.1 ± 0.1   |
| SCH23390+RO3397              | 7 | 69.8 ± 7.3                             | 89.2 ± 4.6    | 47.6 ± 4.0                              | 48.1 ± 5.3                  | 1.5 ± 0.2                              | 2.0 ± 0.3   |
| Eticlopride+Vehicle          | 7 | 92.6 ± 5.2 <sup>†††</sup>              | 100.1 ± 6.4   | 72.7 ± 5.3 <sup>††††</sup>              | 68.4 ± 3.8                  | 1.3 ± 0.1                              | 1.5 ± 0.1   |
| Eticlopride+RO3397           | 7 | 70.4 ± 6.1                             | 101.7 ± 8.6   | 65.7 ± 3.9 <sup>†††</sup>               | 67.4 ± 5.2                  | 1.0 ± 0.1 <sup>†</sup>                 | 1.5 ± 0.1   |
| SCH23390+Eticlopride+Vehicle | 7 | 80.1 ± 5.0 <sup>††</sup>               | 94.5 ± 10.2   | 71.9 ± 6.1 <sup>††††</sup>              | 72.7 ± 6.0 <sup>*</sup>     | 1.1 ± 0.1                              | 1.3 ± 0.2   |
| SCH23390+Eticlopride+RO3397  | 7 | 76.8 ± 5.9 <sup>†</sup>                | 111.6 ± 11.02 | 61.7 ± 7.6 <sup>††</sup>                | 68.4 ± 5.6                  | 1.3 ± 0.2                              | 1.7 ± 0.3   |
| Saline+RO3397                | 7 | 46.7 ± 5.3 <sup>™™</sup>               | 99.5 ± 3.0    | 32.4 ± 3.3                              | 55.7 ± 5.5                  | 1.4 ± 0.1                              | 1.9 ± 0.2   |
| 2-way ANOVA                  |   | F <sub>(7,48)</sub> = 2.391; p = 0.035 |               | F <sub>(7,48)</sub> = 8.347; p < 0.0001 |                             | F <sub>(7,48)</sub> = 2.50; p = 0.0283 |             |
| <b>REM Sleep</b>             |   |                                        |               |                                         |                             |                                        |             |
| Saline+Vehicle               | 7 | 11.4 ± 2.4                             | 13.8 ± 2.1    | 12.0 ± 3.3                              | 15.1 ± 2.2                  | 1.0 ± 0.2                              | 0.8 ± 0.1   |
| SCH23390+Vehicle             | 7 | 10.0 ± 2.2                             | 15.1 ± 1.2    | 9.0 ± 2.1                               | 14.6 ± 1.6                  | 1.0 ± 0.1                              | 1.0 ± 0.1   |
| SCH23390+RO3397              | 7 | 2.6 ± 0.7                              | 14.3 ± 3.9    | 2.6 ± 0.7                               | 14.1 ± 4.2                  | 1.1 ± 0.3                              | 1.0 ± 0.1   |
| Eticlopride+Vehicle          | 7 | 9.4 ± 1.7                              | 18.6 ± 3.2    | 11.4 ± 2.1                              | 24.6 ± 4.5                  | 0.8 ± 0.1                              | 0.8 ± 0.1   |
| Eticlopride+RO3397           | 7 | 1.3 ± 0.5 <sup>*</sup>                 | 11.1 ± 1.9    | 1.7 ± 0.7                               | 17.9 ± 2.4                  | 0.4 ± 0.0 <sup>†</sup>                 | 0.5 ± 0.1   |
| SCH23390+Eticlopride+Vehicle | 7 | 8.4 ± 2.1                              | 10.6 ± 3.2    | 15.4 ± 3.6                              | 18.4 ± 5.9                  | 0.4 ± 0.0 <sup>†</sup>                 | 0.5 ± 0.1   |
| SCH23390+Eticlopride+RO3397  | 7 | 4.5 ± 1.5                              | 12.0 ± 3.6    | 7.3 ± 2.6                               | 16.7 ± 5.4                  | 0.4 ± 0.0 <sup>†</sup>                 | 0.6 ± 0.1   |
| Saline+RO3397                | 7 | 1.4 ± 0.9 <sup>*</sup>                 | 11.6 ± 1.5    | 1.9 ± 0.8                               | 12.9 ± 1.5                  | 0.6 ± 0.2                              | 0.86 ± 0.07 |
| 2-way ANOVA                  |   | F <sub>(7,48)</sub> = 2.473; p = 0.030 |               | F <sub>(7,48)</sub> = 2.204; p = 0.050  |                             | F <sub>(7,48)</sub> = 7.80; p < 0.0001 |             |

\*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p < 0.0001 vs. Saline+Vehicle

<sup>†</sup>p < 0.05; <sup>††</sup>p < 0.01; <sup>†††</sup>p < 0.001; <sup>††††</sup>p < 0.0001 vs. Saline+RO3397