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| Supplemental Table 1. Detailed information for statistical analysis |
| Figure No. | Result from statistical analysis |
| 1C | Control shRNA (81.15±8.69, n=17) and *Aqp4* shRNA (29.24±7.072, n=15), \*\*\*P<0.001, Mann-Whitney test (two-tailed). |
| 1D | Cultured astrocytes (R2=0.428, n=3) and hippocampal slices (R2=0.682, n=2), nonlinear regression. |
| 1I | Control shRNA (58.07±4.71, n=11), *Lrrc8a* shRNA (44.56±6.22, n=9), *Lrrc8c* shRNA (77.90±14.80, n=8) and *Lrrc8a/c* shRNA (54.45±8.70, n=9), NS>0.05, ordinary one-way ANOVA with Tukey’s multiple comparisons test (F (3,33)=2.35, P=0.0899). |
| 1L | WT (61.41±7.75, n=14), BEST1 KO (63.36±8.90, n=10), NS>0.05, unpaired t-test (two-tailed). |
| 1O | Control shRNA (913.74±140.77, n=11), *Best1* shRNA (1350.22±280.80, n=10), NS>0.05, unpaired t-test (two-tailed). |
| 2F | Naive (77.30±12.79, n=10), 7g Control shRNA (72.31±7.12, n=14), 7g *Ttyh1* shRNA (68.69±10.26, n=10), 7g *Ttyh2* shRNA (73.12±9.97, n=12), 7g *Ttyh3* shRNA (75.97±9.15, n=8), 14g Control shRNA (67.77±8.80, n=8), 14g *Ttyh1/2* shRNA (51.64±6.65, n=10), 14g *Ttyh1/3* shRNA (56.17±9.35, n=10) and 14g *Ttyh2/3* shRNA (45.86±8.23, n=10) , NS>0.05, ordinary one-way ANOVA with Tukey’s multiple comparisons (F (8,83)=1.468, P=0.1814). |
| 2G | Naive (77.30±12.79, n=10), 21g Control shRNA (66.37±7.81, n=11), 21g *Ttyh1/2/3* shRNA (20.96±3.96, n=13), 21g *Ttyh1/2/3* shRNA + 7g Ttyh1 shRNA insensitive clone (61.40±5.85, n=18), NS>0.05, \*\*\*P<0.001, ordinary one-way ANOVA with Tukey’s multiple comparisons (F (3,48)=10.35, P<0.0001). |
| 2I | Control shRNA (66.37±7.81, n=11), *Ttyh1/2/3* shRNA (20.96±3.96, n=13), \*\*\*\*P<0.0001, unpaired t-test (two-tailed). |
| 2J | Control shRNA (5.54±0.68, n=11), *Ttyh1/2/3* shRNA (5.94±0.71, n=13), NS>0.05, unpaired t-test (two-tailed).  |
| 2K | Control shRNA (58.07±4.71, n=11), *Lrrc8a* shRNA (44.56±6.22, n=9), NS>0.05, unpaired t-test (two-tailed). |
| 2L | Control shRNA (5.65±0.64, n=11), *Lrrc8a* shRNA (8.73±0.48, n=9), \*\*<0.01, unpaired t-test (two-tailed). |
| 3C | Naive (47.11±14.26, n=18), TTYH2 only (105.62±24.39, n=5), AQP4 only (95.88±24.70, n=22), AQP4+TTYH1 (244.67±23.68, n=21), AQP4+TTYH2 (279.54±23.68, n=23), AQP4+TTYH3 (223.62±23.18, n=13), NS>0.05, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001, Kruskal-Wallis test with Dunn’s multiple comparisons (P<0.0001). |
| 3F | TTYH1+AQP4 (304.71±42.14, n=15), LRRC8A+AQP4 (49.10±19.76, n=10), \*\*\*\*P<0.0001, Mann-Whitney test (two-tailed). |
| 3I | HOS(Tris-Cl) (28.48±20.20, n=8), HOS(NaCl) (251.95±25.56, n=10), \*\*\*\*P<0.0001, unpaired t-test (two-tailed). |
| 3L | Control shRNA (177.63±30.45, n=10), *Lrrc8a* shRNA (34.57±7.30, n=11), TTYH1 + AQP4 + *Lrrc8a* shRNA (182.06±22.84, n=11), \*\*\*P<0.001, ordinary one-way ANOVA with Tukey’s multiple comparisons (F(2, 29)=15.09, P<0.0001). |
| 3O | Control shRNA (46.94±6.45, n=9), *m/h Lrrc8a* shRNA (39.24±8.62, n=9), NS>0.05, unpaired t-test (two-tailed). |
| 3R | TTYH1 + AQP4 + Control shRNA (182.64±21.80, n=10), TTYH1 + AQP4 + *m/h Lrrc8a* shRNA (164.19±8.94, n=13), ns>0.05. unpaired t-test (two-tailed). |
| 4B | Astrocyte with HOS (Tris-Cl) (7.02±10.00, n=8), TTYH1+AQP4+*m/h control* shRNA expressing HEK293T cells with HOS (Tris-Cl) (11.35±4.72, n=11), TTYH1+AQP4+*m/h Lrrc8a* shRNA expressing HEK293T cells with HOS (NaCl) (25.64±2.19, n=9), naïve HEK293T cells with HOS (Tris-Cl) (67.42±6.49, n=8), naïve HEK293T cells with HOS (NaCl) (47.44±5.09, n=14), NS>0.05, \*\*\*\*P<0.0001, ordinary one-way ANOVA with Tukey’s multiple comparisons test (F(4,42)=17.06, P<0.0001). |
| 4C | Astrocyte with HOS (Tris-Cl) (0.38±0.04, n=8), TTYH1+AQP4 expressing HEK 293T cells with HOS (Tris-Cl) (0.34±0.02, n=11), naïve HEK293T cells with HOS (NaCl) (0.38±0.03, n=9), NS>0.05, Kruskal-Wallis test with Dunn’s multiple comparisons’s test (P=0.5202). |
| 4D | SCN- of Naïve HEK293T cells with HOS (NaCl) (1.59±0.07, n=11), SCN- of TTYH1 + AQP4 + *m/h Lrrc8a* shRNA expressing HEK293T cells with HOS (NaCl) (1.84±0.06, n=8), I- of Naïve HEK293T cells with HOS (NaCl) (1.41±0.06, n=9), I- of TTYH1 + AQP4 + *m/h Lrrc8a* shRNA expressing HEK293T cells with HOS (NaCl) (1.47±0.03, n=8), Br- of Naïve HEK293T cells with HOS (NaCl) (1.22±0.06, n=9), Br - of TTYH1 + AQP4 + *m/h Lrrc8a* shRNA expressing HEK293T cells with HOS (NaCl) (1.24±0.03, n=8), F- of Naïve HEK293T cells with HOS (NaCl) (0.58±0.06, n=8), F- of TTYH1 + AQP4 + *m/h Lrrc8a* shRNA expressing HEK293T cells with HOS (NaCl) (0.58±0.03, n=8), \* P<0.05, unpaired t-test (two-tailed). |
| 4E | Astrocyte (0.32±0.01, n=24), AQP4+TTYH1 (0.31±0.01, n=21), AQP4+TTYH2 (0.33±0.02, n=23), AQP4+TTYH3 (0.31±0.02, n=13), NS>0.9999, Kruskal-Wallis test with Dunn’s multiple comparisons (P=0.8139). |
| 4H | TTYH1+AQP4 (243.17±19.57, n=10), TTYH2+AQP4 (231.75±11.93, n=8), TTYH3+AQP4 (225.27±24.93, n=10), NS>0.05, Kruskal-Wallis test with Dunn’s multiple comparisons test (P=0.9855). |
| 4K | Control (56.67±8.03, n=10), DCPIB (-1.26±1.56, n=8), \*\*\*\*P<0.0001, Mann-Whitney test (two-tailed). |
| 4M | Control of TTYH1 +AQP4 (227.08±28.98, n=10), DCPIB of TTYH1+AQP4 (76.46±21.22, n=10), \*\*\*\*<0.0001, paired t-test; Control of TTYH2 +AQP4 (270.14±25.92, n=17), DCPIB of TTYH2+AQP4 (43.38±14.70, n=17), \*\*\*\*<0. 0001, paired t-test; Control of TTYH3 +AQP4 (210.12±32.31, n=10), DCPIB of TTYH3+AQP4 (42.44±24.88, n=10), \*\*\*\*<0. 0001, paired t-test (two-tailed). |
| 5C | Control (57.52±6.27, n=8), Genistein (5.89±4.15, n=8), \*\*P<0.01, Willcoxon matched-pairs signed rank test (two-tailed). |
| 5D | Control of TTYH1 +AQP4 (319.87±47.13, n=10), Genistein of TTYH1+AQP4 (23.44±24.35, n=10), \*\*\*\*<0.0001, paired t-test (two-tailed); Control of TTYH2 +AQP4 (302.49±53.62, n=12), Genistein of TTYH2+AQP4 (58.46±28.51, n=9), \*\*\*<0. 001, unpaired t-test (two-tailed); Control of TTYH3 +AQP4 (275.03±35.19, n=10), Genistein of TTYH3+AQP4 (84.70±35.88, n=15), \*\*<0. 01, unpaired t-test (two-tailed). |
| 5G | Control (50.92±6.34, n=7), PD98059 (7.37±6.25, n=9), \*\*\*P<0.001, Mann-Whitney test (two-tailed). |
| 5J | Control (175.56±33.16, n=7), PD98059 (29.90±27.04, n=9), \*\*P<0.01, Mann-Whitney test (two-tailed). |
| 5M | Naïve HEK293T cells (251.95±25.56, n=10), Genistein (15.36±8.79, n=4) , PD98059 (168.86±27.66, n=7), NS>0.05, \*\*P<0.01, Kruskal-Wallis test with Dunn’s multiple comparisons’s test (P=0.0005). |
| 5N | Genistein in Astrocyte with HOS (Tris-Cl) (88.19±7.62, n=8), Genistein in TTYH1+AQP4 expressing HEK293T cells with HOS (Tris-Cl) (91.00±9.35, n=10), Genistein in naive HEK293T cells with HOS (NaCl) (93.90±3.49, n=4), PD98059 in Astrocyte with HOS (Tris-Cl) (86.46±11.47, n=9), PD98059 in TTYH1+AQP4 expressing HEK293T cells with HOS (Tris-Cl) (88.52±10.38, n=9), PD98059 in naive HEK293T cells with HOS (NaCl) (32.98±10.98, n=7), NS>0.05, \*\*P<0.01, Two-way ANOVA with Tukey’s multiple comparisons test (F(2,41)=4.121, P=0.0234). |
| 5S | Astrocyte (0.28±0.03, n=6), TTYH1+AQP4 (0.21±0.02, n=8), TTYH2+AQP4 (0.29±0.02, n=10), TTYH3+AQP4 (0.20±0.0.01, n=8), NS>0.05, \*P=0.04 Kruskal-Wallis test with Dunn’s multiple comparisons (P=0.0022). |
| 8D | AQP4 only (95.88±24.69, n=22), TTYH1+AQP4 (284.84±16.80, n=57), △1 (268.47±43.83, n=9), △2A (98.52±27.08, n=7), △2A1 (232.92±40.61, n=8), △2A2 (167.62±33.28, n=11), △2A2 (167.62±33.28, n=11), △2A3 (146.52±32.24, n=8), △2A4 (88.19±15.73, n=15), △2B (235.67±38.00, n=9), △2C (82.06±38.18, n=8), △2C1 (245.04±36.56, n=6), △2C2 (74.64±33.27, n=9), R165A (91.38±29.57, n=10), △2C3 (135.31±27.08, n=16), △2C4 (193.84±49.16, n=11), △2D (286.5±42.38, n=7), △2E (294.19±45.91, n=7) ), △4A (283.14±38.66, n=8), △4B (75.43±34.92, n=8), △YY in 4B (153.49±39.96, n=15), △4C (244.80±49.86, n=9), △4D (313.53±69.99, n=11), △4E (188.54±36.80, n=8), △4F (177.54±14.42, n=9), △4G (199.79±61.11, n=8), NS>0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001, Kruskal-Wallis test with Dunn’s multiple comparisons (P<0.0001). |
| 8I | TTYH2-WT+AQP4 (251.78±3.47, n=4), TTYH2-R164A+AQP4 (152.50±21.92, n=9), \*\*P<0.01, Mann-Whitney test (two-tailed). |
| 8K | WT-TTYH1 shRNA insensitive clone + *Ttyh1/2/3* shRNA (61.40±5.85, n=18), R165A-TTYH1 shRNA insensitive clone + *Ttyh1/2/3* shRNA (16.21±4.58, n=10), \*\*\*\*P<0.0001, unpaired t-test (two-tailed). |
| 9C | WT(9.01±5.22, n=11), V161C (2.66±4.44, n=8), L162C (1.26±4.87, n=8), S163C (7.12±2.94, n=10), V164C (28.85±3.79, n=9), R165C (36.58±6.40, n=14), M166C (11.30±2.54, n=5), E167C (18.33±5.68, n=9), L168C (2.30±6.25, n=8), V169C (14.80±1.58, n=8), A170C (6.67±5.28, n=10), \*\*\* P<0.001, ordinary one-way ANOVA with Holm-Sidak’s multiple comparisons test (F(10,87)=5.495, P<0.0001). |
| 9E | WT:R165A [4:0] (241.39±21.94, n=12), WT:R165A [3:1] (226.35±22.94, n=10), WT:R165A [2:2] (168.36±18.73, n=16), WT:R165A [1:3] (154.47±29.03, n=9), WT:R165A [0:4] (79.90±20.26, n=11), linear regression (R2=0.942, P=0.006) |
| 10C | Control shRNA (10.69±2.92, n=112), *Ttyh1* shRNA (2.92±0.25, n=94), \*\*\*\*P<0.0001, unpaired t-test (two-tailed). |
| 10F | Naïve (3281.35±661.49, n=10), Control shRNA (3347.35±36.62, n=10), *Ttyh1/2/3* shRNA (1660.49±384.75, n=12), \*P<0.05, ordinary one-way ANOVA with Dunnett’s multiple comparisons test (F(2,29)=4.309, P=0.0230). |
| 11G | Control (38.43±7.42, n=12), DCPIB (10.24±4.20, n=7), Genistein (11.14±6.40, n=7), \*P<0.05, \*\*P<0.01, Kruskal-Wallis test with Dunn’s multiple comparisons’s test (P=0.0036). |
| 11L | Control shRNA (39.10±4.29, n=10), *Ttyh1/2/3* shRNA (5.47±3.32, n=10), Glial rescue (Tam) (27.50±6.97, n=9), NS>0.05, \*P<0.05, \*\*P<0.01, Kruskal-Wallis test with Dunn’s multiple comparisons’s test (P=0.0022). |