**Supplementary File 1.** Statistical analyses used.

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| **Figure** | **Sample sizeNo. of experiments** | **Statistical test** | **P-value** |
| 1C | Excitatory= 46, inhibitory =445 dissociations | Wilcoxon rank-sum | Excitatory vs inhibitory p=0.35 |
| 1E,F | Control = 34 shArl13b\_1 = 43 shArl13b\_2 = 404 dissociations | Kruskal-Wallis with Dunn’s multiple comparisons test | E: Control vs shArl13b\_1 p<0.001 Control vs shArl13b\_2 p<0.001F: Control vs shArl13b\_1 p<0.001 Control vs shArl13b\_2 p<0.001 |
| 1 H,I | Control = 32 shArl13b\_1 = 28 4 dissociations | Wilcoxon rank-sum test | H: Control vs shArl13b\_1 p=0.52I: Control vs shArl13b\_1 p=0.29 |
| 2C,D | (24h) Control = 25shArl13b\_1 = 24 (48h) Control = 32 shArl13b\_2 = 19 shIft88/shCep164 = 39 4 dissociations | LMM with Dunnett-type correction for multiple comparisons | C: (24h) Control vs shArl13b\_1 p<0.001 (48h) Control vs shArl13b\_2 p=0.001Control vs shIft88/shCep164 p=0.001D: (24h) Control vs shArl13b\_1 p=0.07(48h) Control vs shArl13b\_2 p=0.011Control vs shIft88/shCep164 p=0.016 |
| 2F,G | (24h) Control = 17shArl13b\_1 = 22 4 dissociations(48h) Control = 25 shArl13b\_2 = 19 shIft88/shCep164 = 23 3 dissociations | LMM with Dunnett-type correction for multiple comparisons | F: (24h) Control vs shArl13b\_1 p=0.25(48h) Control vs shArl13b\_2 p=0.50Control vs shIft88/shCep164 p=0.64G: (24h) Control vs shArl13b\_1 p=0.86(48h) Control vs shArl13b\_2 p=0.57Control vs shIft88/shCep164 p=0.98 |
| 3A,D,E | Control = 24 shArl13b\_1 = 24 shArl13b\_2 = 23 >5 dissociations | Kruskal-Wallis with Dunn’s multiple comparisons test | A: Control vs shArl13b\_1 p= 0.007Control vs shArl13b\_2 p=0.007D: Control vs shArl13b\_1 p=0.080Control vs shArl13b\_2 p=0.71E: Control vs shArl13b\_1 p=1Control vs shArl13b\_2 p=0.097 |
| 3B,C | Control = 24 shArl13b\_1 = 24 shArl13b\_2 = 23 >5 dissociations | Kruskal-Wallis with Bonferroni correction | B: Control vs shArl13b\_1 p<0.001Control vs shArl13b\_2 p<0.001C: Control vs shArl13b\_1 p=1Control vs shArl13b\_2 p=0.25 |
| 4A | Control = 32 shArl13b\_1 = 25 >5 dissociations | Wilcoxon rank-sum test | Control vs shArl13b\_1 p=0.032 |
| 4B | Control =18shArl13b\_1 = 20 3 dissociations | ANOVA for repeated measures | Control vs shArl13b\_1:30 pA p=1.0050 pA p=0.99100 pA p=0.82200 pA p=0.84300 pA p=0.88400 pA p=0.99 |
| 5B | n=150 neurons per layer3 animals. |  |  |
| 5D | n= GAD67+ = 115ChAT+ = 31, PV+ = 100, SOM+ = 110,VIP+=1503 animals. |  |  |
| 5F | n = 515 neurons 4 dissociations. |  |  |
| 6B,C | (6h) Control = 23 L-796,778 = 10 MK-4256 = 17 (18h)Control = 35, L-796,778 = 33MK-4256 = 17 (24h) Control = 87 L-796,778 = 17 MK-4256 =40 ≥3 dissociations | LMM with Dunnett-type correction for multiple comparisons | B: (6h) Control vs L-796-778 p=0.96Control vs MK-4256 p=1.00(18h) Control vs L-796-778 p=0.78Control vs MK-4256 p= 0.073(24h) Control vs L-796-778 p= 0.003Control vs MK-4256 p< 0.001C: (6h )Control vs L-796-778 p=0.76Control vs MK-4256 p=0.60(18h)Control vs L-796-778 p=0.02Control vs MK-4256 p=1.00(24h) Control vs L-796-778 p= 0.02Control vs MK-4256 p= 0.28 |
| 6E | GFP control = 35 GFP + L-796,778 = 46 shIft88/shCep164/GFP = 36 shIft88/shCep164/GFP + L-796,778 = 31 3 dissociations | Kruskal-Wallis with Dunn’s multiple comparisons test | Control GFP vs GFP + L-796-778 p= 0.01Control GFP vs shIft88/shCep164 p= 0.01Control GFP vs shIft88/shCep164 + L-796-778 p= 0.0022GFP + L-796-778 vs shIft88/shCep164 p<0.0001GFP + L-796-778 vs shIft88/shCep164 + L-796-778 p<0.0001shIft88/shCep164 vs shIft88/shCep164 + L-796-778 p=0.45 |
| Figure 1 – Supp.1A,B | Control = 19shArl13b\_2 = 263 dissociations | Wilcoxon rank-sum test | A: Control vs shArl13b\_2 p=<0.0001B: Control vs shArl13b\_2 p=0.0048 |
| Figure 1 – Supp.1D,E | Control = 21shArl13b\_2 = 14 shIft88/shCep164 =173 dissociations | Kruskal-Wallis with Dunn’s multiple comparisons test | D: Control vs shArl13b\_2 p=0.74Control vs shIft88/shCep164 p=0.82E: Control vs shArl13b\_2 p=0.52Control vs shIft88/shCep164 p=0.13 |
| Figure 1 – Supp.1F,G | (24h)Control = 23shIft88/shCep164 = 253 dissociations(48h)Control = 43, shCep164/GFP = 20,shIft88/GFP = 17,shIft88/shCep164/GFP = 233 dissociations. | (24h)Wilcoxon rank-sum test(48h)Kruskal-Wallis with Dunn’s multiple comparisons test | F: (24h)Control vs shIft88/shCep164 p=0.033(48h)Control vs shCep164/GFP p= 0.16Control vs shIft88/GFP p= 0.0035Control vs shIft88/shCep164/GFP p= <0.0001shCep164/GFP vs shIft88/GFP p= 0.14shIft88/GFP vs shIft88/shCep164/GFP p= 0.032shCep164/GFP vs shIft88/shCep164/GFP p= 0.00018G: (24h)Control vs shIft88/shCep164 p=0.24(48h)Control vs shCep164/GFP p= 0.29Control vs shIft88/GFP p= 0.017Control vs shIft88/shCep164/GFP p= <0.0001shCep164/GFP vs shIft88/GFP p= 0.19shIft88/GFP vs shIft88/shCep164/GFP p= 0.024shCep164/GFP vs shIft88/shCep164/GFP p= 0.00016 |
| Figure 2 – Supp.1A | (24h)Control = 25shArl13b\_1 = 24 (48h) Control = 32 shArl13b\_2 = 19 shIft88/shCep164 = 39 4 dissociations | LMM with Dunnett-type correction for multiple comparisons |  (24h) Control vs shArl13b\_1 p=0.017(48h) Control vs shArl13b\_2 p=0.14Control vs shIft88/shCep164 p=0.04 |
| Figure 2 – Supp.1B | (24h) Control = 17 shArl13b\_1 = 22 4 dissociations(48h) Control = 25 shArl13b\_2 = 19 shIft88/shCep164 = 23 3 dissociations | LMM with Dunnett-type correction for multiple comparisons | (24h) Control vs shArl13b\_1 p=0.99(48h) Control vs shArl13b\_2 p=0.99Control vs shIft88/shCep164 p=0.96 |
| Figure 5 – Supp.1B | NeuN+ = 1,520GAD67+ = 2536 animals |  |  |
| Figure 6 – Supp.1A | (6h) Control = 23 L-796,778 = 10 MK-4256 = 17 (18h)Control = 35 L-796,778 = 33 MK-4256 = 17 (24h) Control = 87 L-796,778 = 17 MK-4256 =40 ≥3 dissociations | LMM with Dunnett-type correction for multiple comparisons | (6h) Control vs L-796-778 p=0.96Control vs MK-4256 p=0.99 (18h) Control vs L-796-778 p=0.22Control vs MK-4256 p=0.29(24h) Control vs L-796-778 p=0.04Control vs MK-4256p=0.002 |
| Figure 6 – Supp.1B,C | Control = 87 0.5 μM L-796,778 = 26 1 μM L-796,778 = 25 2 μM L-796,778 = 17 0.125 μM MK-4256 = 19 0.5 μM MK-4256 = 15 1 μM MK-4256 = 40 ≥3 dissociations. | LMM with Dunnett-type correction for multiple comparisons | B: Shank3 intensity:Control vs 0.5 μM L-796,778 p =0.96Control vs 1 μM L-796,778 p =0.78Control vs 2 μM L-796,778 p =0.004Control vs 0.125 μM MK-4256 p =0.37Control vs 0.5 μM MK-4256 p =0.37Control vs 1 μM MK-4256 p =0.001VGlut1 intensity:Control vs 0.5 μM L-796,778 p =0.19Control vs 1 μM L-796,778 p =0.47Control vs 2 μM L-796,778 p = 0.037Control vs 0.125 μM MK-4256 p=0.40Control vs 0.5 μM MK-4256 p=0.99Control vs 1 μM MK-4256 p =0.002C: DensityControl vs 0.5 μM L-796,778 p=0.25Control vs 1 μM L-796,778 p=0.98Control vs 2 μM L-796,778 p=0.026Control vs 0.125 μM MK-4256 p=0.42Control vs 0.5 μM MK-4256 p=0.89Control vs 1 μM MK-4256 p=0.28 |
| Figure 6 – Supp.1D | Control = 63 2 μM L-796,778 = 7 1 μM MK-4256 = 33 ≥3 dissociations | LMM with Dunnett-type correction for multiple comparisons | Control vs 2 μM L-796,778 p=0.21Control vs 1 μM MK-4256 p=0.32 |