| Figure/Panel | Statistical test | Post-hoc test | Source of variation | Test statistic | *p* -value |
|---|---|---|---|---|---|
| Fig.1B | Kolmogorov-Smirnov test | — | — | $D$ = 0.109 | 0.0634 |
| Fig.1C | Kolmogorov-Smirnov test | — | — | $D$ = 0.0652 | 0.332 |
| Fig.1E | Kolmogorov-Smirnov test | — | — | $D$ = 0.170 | 0.00224 |
| Fig.1F | Kolmogorov-Smirnov test | — | — | $D$ = 0.192 | <0.0001 |
| Fig.1H | Kolmogorov-Smirnov test | — | — | $D$ = 0.172 | <0.0001 |
| Fig.1I | Kolmogorov-Smirnov test | — | — | $D$ = 0.193 | 0.00289 |
| Fig.1K | Kolmogorov-Smirnov test | — | — | $D$ = 0.143 | 0.027 |
| Fig.1L | Kolmogorov-Smirnov test | — | — | $D$ = 0.0900 | 0.0689 |
| Fig.2A (Cortex) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 16) = 0.5627 | 0.4641 |
| | | | Age | $F$ (1, 16) = 15.68 | 0.0011 |
| | | | Genotype | $F$ (1, 16) = 1.366 | 0.2596 |
| Fig.2A (Hippocampus) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 16) = 0.3952 | 0.5385 |
| | | | Age | $F$ (1, 16) = 15.21 | 0.0013 |
| | | | Genotype | $F$ (1, 16) = 1.962 | 0.1804 |
| Fig.2B (Cortex) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 21) = 21.33 | 0.0001 |
| | | | Age | $F$ (1, 21) = 9.139 | 0.0065 |
| | | | Genotype | $F$ (1, 21) = 2.714 | 0.1143 |
| Fig.2B (Hippocampus) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 18) = 36.78 | <0.0001 |
| | | | Age | $F$ (1, 18) = 46.88 | <0.0001 |
| | | | Genotype | $F$ (1, 18) = 1.017 | 0.3267 |
| Fig.2D (Cortex) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 16) = 93.79 | <0.0001 |
| | | | Age | $F$ (1, 16) = 3.965 | 0.0638 |
| | | | Genotype | $F$ (1, 16) = 26.37 | <0.0001 |
| Fig.2D (Hippocampus) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 16) = 74.27 | <0.0001 |
| | | | Age | $F$ (1, 16) = 201.8 | <0.0001 |
| | | | Genotype | $F$ (1, 16) = 2.341 | 0.1456 |
| Fig.3A (Cortex) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 17) = 0.6808 | 0.4207 |
| | | | Age | $F$ (1, 17) = 706.7 | <0.0001 |
| | | | Genotype | $F$ (1, 17) = 122.5 | <0.0001 |
| Fig.3A (Hippocampus) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 17) = 1.085 | 0.3121 |
| | | | Age | $F$ (1, 17) = 292.6 | <0.0001 |
| | | | Genotype | $F$ (1, 17) = 127.5 | <0.0001 |
| Fig.3C (Cortex) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 12) = 3.984 | 0.0692 |
| | | | Age | $F$ (1, 12) = 896.3 | <0.0001 |
| | | | Genotype | $F$ (1, 12) = 1.230 | 0.2892 |
| Fig.3C (Hippocampus) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 12) = 0.2069 | 0.6573 |
| | | | Age | $F$ (1, 12) = 517.2 | <0.0001 |
| | | | Genotype | $F$ (1, 12) = 82.76 | <0.0001 |
| Fig.4B (CDKL5) | Unpaired two-tailed *t* -test | — | — | $t$ (4) = 5.120 | 0.0069 |
| Fig.4B (PSD-95) | Unpaired two-tailed *t* -test | — | — | $t$ (10) = 2.101 | 0.0619 |
| Fig.4B (SYN) | Unpaired two-tailed *t* -test | — | — | $t$ (10) = 2.447 | 0.0344 |
| Fig.4B (VGAT) | Unpaired two-tailed *t* -test | — | — | $t$ (10) = 1.899 | 0.0867 |

| Figure/Panel | Statistical test | Post-hoc test | Source of variation | Test statistic | *p* -value |
|---|---|---|---|---|---|
| Fig.4B (VGLUT1) | Unpaired two-tailed *t* -test | — | — | $t$ (4) = 0.8254 | 0.4555 |
| Fig.5A | Two-way ANOVA | Tukey | Interaction | $F$ (1, 12) = 0.4730 | 0.5047 |
| | | | Age | $F$ (1, 12) = 7.535 | 0.0178 |
| | | | Genotype | $F$ (1, 12) = 39.53 | <0.0001 |
| Fig.5C | Two-way ANOVA | Tukey | Interaction | $F$ (1, 12) = 0.3885 | 0.5447 |
| | | | Age | $F$ (1, 12) = 5.254 | 0.0408 |
| | | | Genotype | $F$ (1, 12) = 35.56 | <0.0001 |
| Fig.6C (Cortex) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 11) = 0.1673 | 0.6904 |
| | | | Age | $F$ (1, 11) = 3.358 | 0.0941 |
| | | | Genotype | $F$ (1, 11) = 0.8900 | 0.3657 |
| Fig.6C (Hippocampus) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 11) = 2.578 | 0.1367 |
| | | | Age | $F$ (1, 11) = 5.354 | 0.041 |
| | | | Genotype | $F$ (1, 11) = 0.2085 | 0.6569 |
| Fig.6D (3M) | Two-way RM ANOVA | Sidak | Interaction | $F$ (4, 20) = 1.286 | 0.3089 |
| | | | Layer | $F$ (1.607, 8.036) = 193.3 | <0.0001 |
| | | | Genotype | $F$ (1, 5) = 0.2327 | 0.6499 |
| Fig.6D (12M) | Two-way RM ANOVA | Sidak | Interaction | $F$ (4, 24) = 2.481 | 0.0709 |
| | | | Layer | $F$ (2.216, 13.30) = 222.4 | <0.0001 |
| | | | Genotype | $F$ (1, 6) = 0.7112 | 0.4314 |
| Fig.7C (Cortex) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 12) = 3.782 | 0.0756 |
| | | | Age | $F$ (1, 12) = 0.5924 | 0.4564 |
| | | | Genotype | $F$ (1, 12) = 0.09479 | 0.7635 |
| Fig.7C (Hippocampus) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 12) = 0.7799 | 0.3945 |
| | | | Age | $F$ (1, 12) = 9.179 | 0.0105 |
| | | | Genotype | $F$ (1, 12) = 0.07544 | 0.7883 |
| Fig.7D (3M) | Two-way RM ANOVA | Sidak | Interaction | $F$ (4, 24) = 1.563 | 0.2162 |
| | | | Layer | $F$ (1.619, 9.714) = 33.31 | <0.0001 |
| | | | Genotype | $F$ (1, 6) = 1.891 | 0.2182 |
| Fig.7D (12M) | Two-way RM ANOVA | Sidak | Interaction | $F$ (4, 24) = 3.059 | 0.036 |
| | | | Layer | $F$ (2,530. 15,18) = 100.7 | <0.0001 |
| | | | Genotype | $F$ (1, 6) = 1.659 | 0.2452 |
| Fig.8A (Body size - Cortex) | Unpaired two-tailed *t* -test | — | — | $t$ (18) = 4.624 | 0.0002 |
| Fig.8A (Body size - Hippocampus) | Unpaired two-tailed *t* -test | — | — | $t$ (18) = 5.868 | <0.0001 |
| Fig.8A (Cell Density - Cortex) | Unpaired two-tailed *t* -test | — | — | $t$ (18) = 4.962 | 0.0001 |
| Fig.8A (Cell Density - Hippocampus) | Unpaired two-tailed *t* -test | — | — | $t$ (18) = 2.054 | 0.0548 |
| Fig.8B | Two-way ANOVA | Tukey | Interaction | $F$ (1, 18) = 22.10 | 0.0002 |
| | | | Age | $F$ (1, 18) = 28.68 | <0.0001 |
| | | | Genotype | $F$ (1, 18) = 4.113 | 0.0576 |
| Fig.8C | Unpaired two-tailed *t* -test | — | — | $t$ (11) = 7.460 | <0.0001 |
| Fig.8E | Two-way ANOVA | Tukey | Interaction | $F$ (1, 12) = 6.937 | 0.0218 |
| | | | Age | $F$ (1, 12) = 42.51 | <0.0001 |
| | | | Genotype | $F$ (1, 12) = 91.04 | <0.0001 |
| Fig.8F | Unpaired two-tailed *t* -test | — | — | $t$ (7) = 3.789 | 0.0068 |
| Fig.10B | Two-way ANOVA | Tukey | Interaction | $F$ (1, 28) = 83.44 | <0.0001 |
| | | | Age | $F$ (1, 28) = 1.548 | 0.2238 |
| | | | Genotype | $F$ (1, 28) = 13.93 | 0.0009 |
| Fig.10C | Two-way ANOVA | Tukey | Interaction | $F$ (1, 28) = 10.36 | 0.0033 |
| | | | Age | $F$ (1, 28) = 0.03946 | 0.844 |
| | | | Genotype | $F$ (1, 28) = 3.487 | 0.0724 |

| Figure/Panel | Statistical test | Post-hoc test | Source of variation | Test statistic | *p* -value |
|---|---|---|---|---|---|
| Supplementary Fig.1A | Two-way ANOVA | Sidak | Interaction | $F$ (1, 13) = 1.089 | 0.3157 |
| | | | Sex | $F$ (1, 13) = 5.564 | 0.0346 |
| | | | Genotype | $F$ (1, 13) = 46.96 | <0.0001 |
| Supplementary Fig.1B | Two-way ANOVA | Sidak | Interaction | $F$ (1, 13) = 5.241 | 0.0394 |
| | | | Sex | $F$ (1, 13) = 21.16 | 0.0005 |
| | | | Genotype | $F$ (1, 13) = 333.1 | <0.0001 |
| Supplementary Fig.2A (+/+) | Two-way RM ANOVA | Sidak | Interaction | $F$ (4, 24) = 0.6856 | 0.6089 |
| | | | Layer | $F$ (1.585, 9.512) = 85.62 | <0.0001 |
| | | | Genotype | $F$ (1, 6) = 0.2524 | 0.6333 |
| Supplementary Fig.2A (+/-) | Two-way RM ANOVA | Sidak | Interaction | $F$ (4, 24) = 4.715 | 0.006 |
| | | | Layer | $F$ (2.377, 14.26) = 295.4 | <0.0001 |
| | | | Genotype | $F$ (1, 6) = 2.701 | 0.1514 |
| Supplementary Fig.2B (+/+) | Two-way RM ANOVA | Sidak | Interaction | $F$ (4, 24) = 0.5951 | 0.6696 |
| | | | Layer | $F$ (1.720. 10.32) = 37.25 | <0.0001 |
| | | | Genotype | $F$ (1, 6) = 0.2387 | 0.6425 |
| Supplementary Fig.2B (+/-) | Two-way RM ANOVA | Sidak | Interaction | $F$ (4, 24) = 3.271 | 0.0283 |
| | | | Layer | $F$ (2.303, 13.82) = 83.52 | <0.0001 |
| | | | Genotype | $F$ (1, 6) = 12.03 | 0.0133 |
| Supplementary Fig.2D | Two-way RM ANOVA | Sidak | Interaction | $F$ (1, 6) = 2.280 | 0.1818 |
| | | | Brain Region | $F$ (1, 6) = 5.722 | 0.0539 |
| | | | Genotype | $F$ (1, 6) = 0.4211 | 0.5404 |
| Supplementary Fig.2E | Two-way RM ANOVA | Sidak | Interaction | $F$ (4, 24) = 1.790 | 0.1638 |
| | | | Layer | $F$ (1.997, 11.98) = 94.85 | <0.0001 |
| | | | Genotype | $F$ (1, 6) = 0.7053 | 0.4332 |
| Supplementary Fig.3A (Cortex) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 31) = 8.909 | 0.0055 |
| | | | Age | $F$ (1, 31) = 24.06 | <0.0001 |
| | | | Genotype | $F$ (1, 31) = 48.43 | <0.0001 |
| Supplementary Fig.3A (Hippocampus) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 31) = 15.77 | 0.0004 |
| | | | Age | $F$ (1, 31) = 14.12 | 0.0007 |
| | | | Genotype | $F$ (1, 31) = 63.97 | <0.0001 |
| Supplementary Fig.3B (Cortex) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 31) = 9.309 | 0.0046 |
| | | | Age | $F$ (1, 31) = 28.76 | <0.0001 |
| | | | Genotype | $F$ (1, 31) = 9.508 | 0.0043 |
| Supplementary Fig.3B (Hippocampus) | Two-way ANOVA | Tukey | Interaction | $F$ (1, 31) = 1.399 | 0.2459 |
| | | | Age | $F$ (1, 31) = 38.27 | <0.0001 |
| | | | Genotype | $F$ (1, 31) = 11.12 | 0.0022 |

**Table S2. Summary of statistical analyses.** Test statistics are reported as $D$ for the Kolmogorov-Smirnov test, $F$ (DFn, DFd) for ANOVA, and $t$ (df) for $t$ -tests, where DFn and DFd indicate the numerator and denominator degrees of freedom, respectively, and df indicates the degrees of freedom. Abbreviations: RM = repeated measures.