

# Cyclin D2-knock-out mice with attenuated dentate gyrus neurogenesis have robust deficits in long-term memory formation

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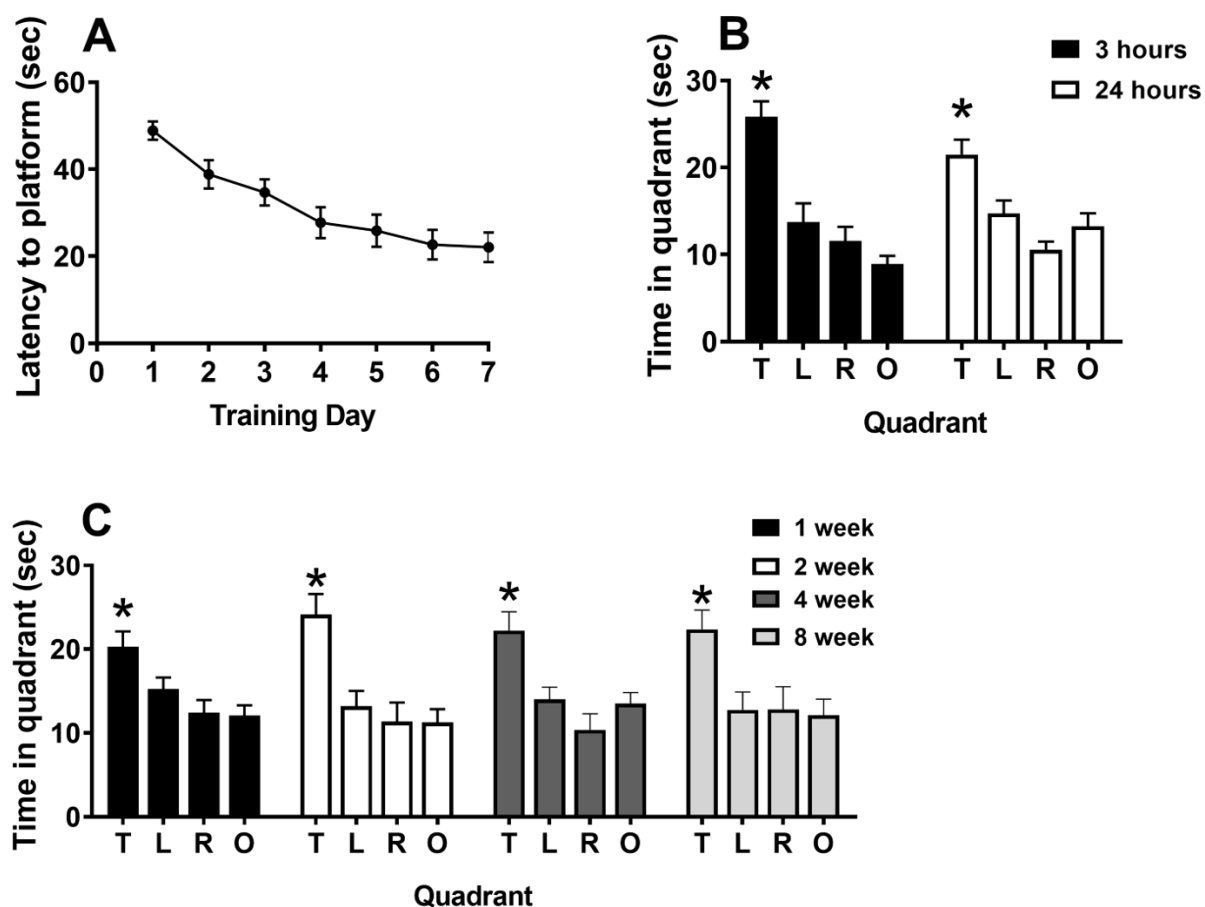
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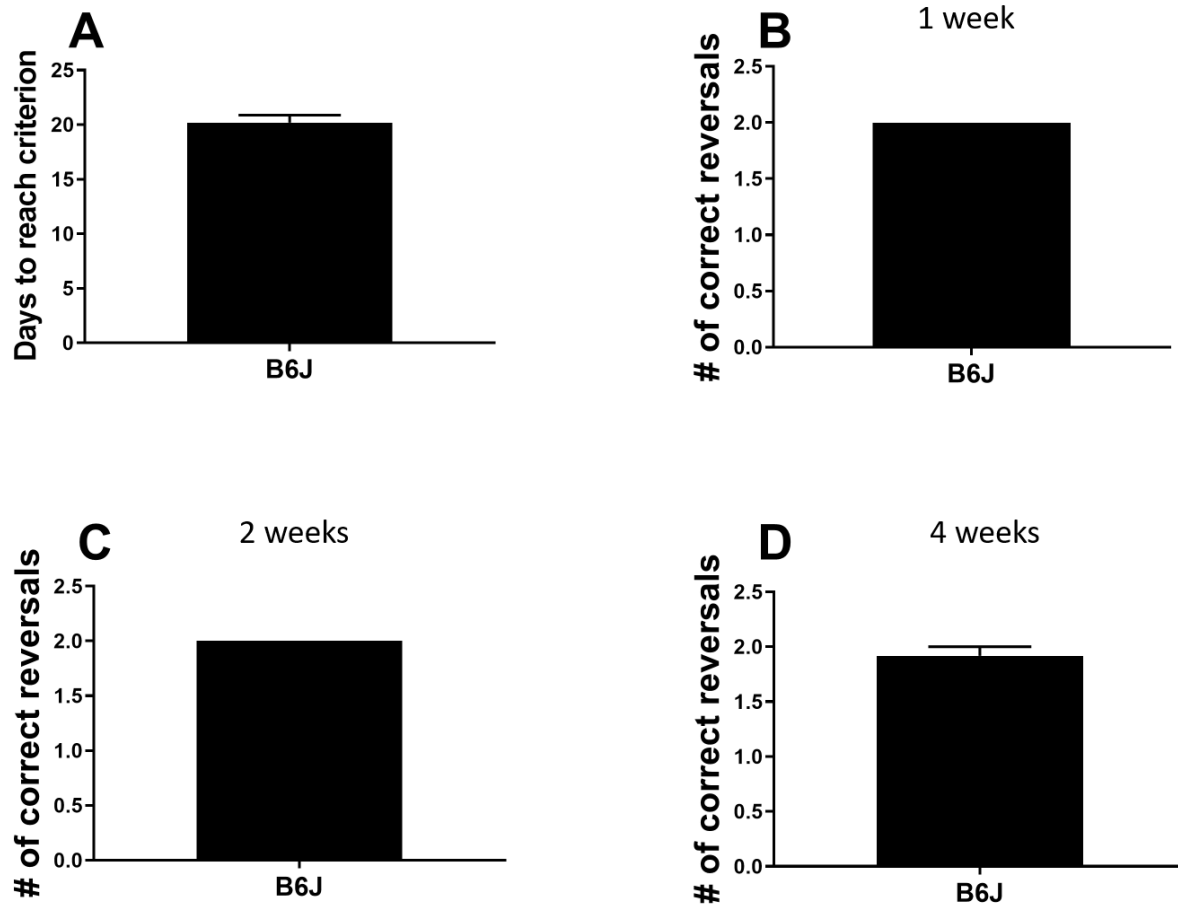
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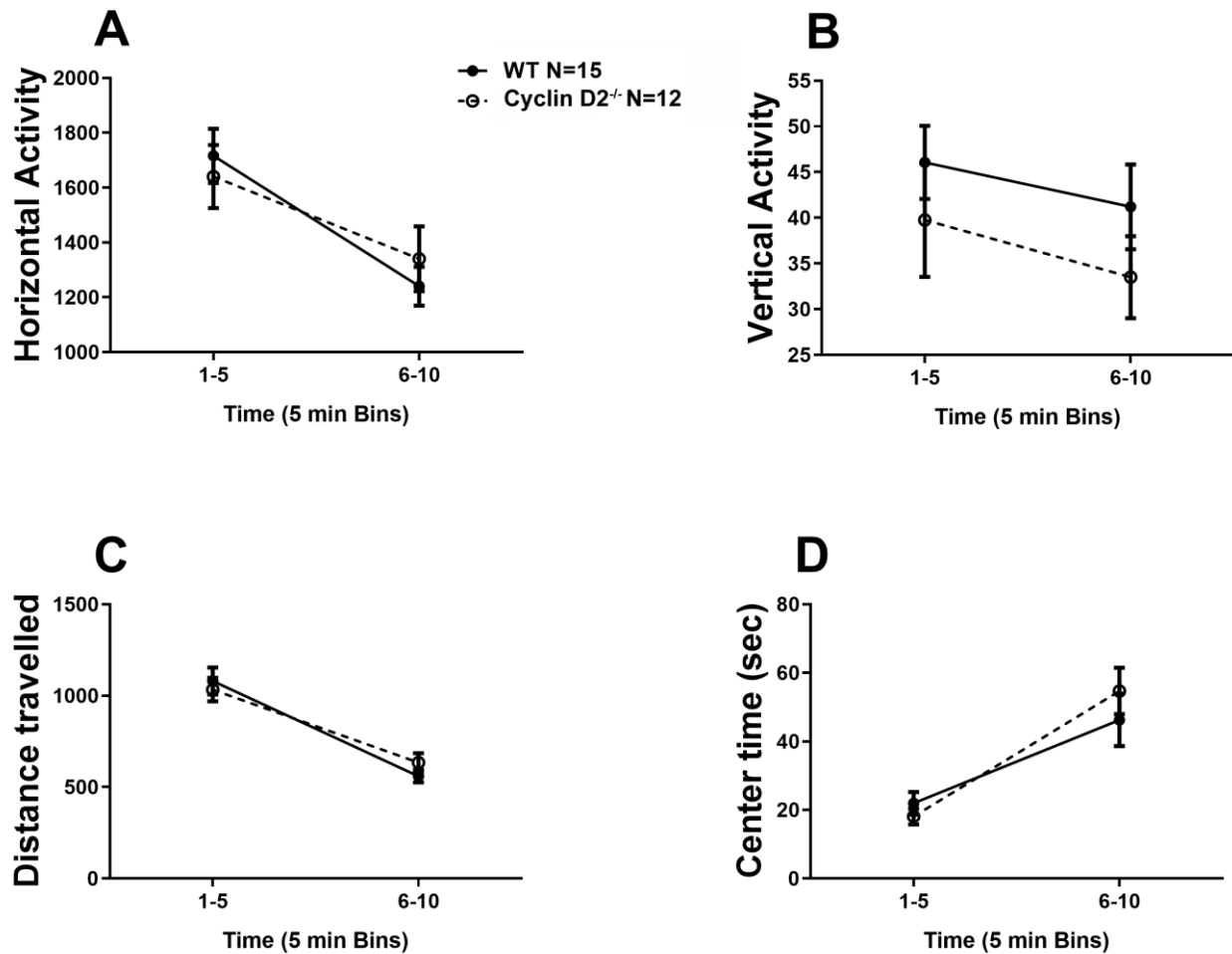
**Supplemental Figure 1**

**C57BL/6J mice demonstrate ability to retain long-term spatial memory over 8 weeks.** C57BL/6J mice (N = 16) were used to validate the modification of Morris water maze to test long-term memory over the course of several weeks. **A**, C57BL/6J mice learned where the platform was over the course of 7 training days, exhibited by a decreasing latency to find the platform. **B**, Mice show intact memory when probed at 3 hours and 24 hours after the final training session, spending significantly more time in the target quadrant compared to all others. **C**, When tested over longer intervals of 1, 2, 4 and 8 weeks, C57BL/6J mice demonstrated long-term spatial memory of the platform location. Data shown as mean  $\pm$  SEM. \* indicates  $p < .05$  target quadrant versus other quadrants.



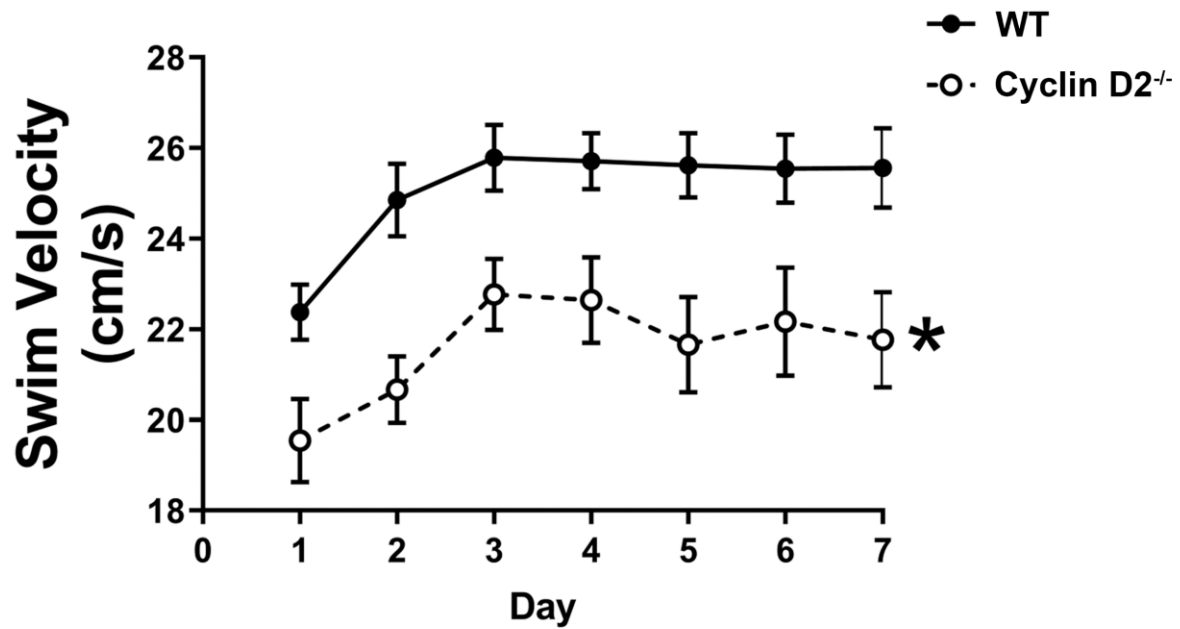
**Supplemental Figure 2**

**B6J mice demonstrate long-term memory in pattern separation task.** C57BL/6J (B6J) mice ( $N = 12$ ) underwent training in an operant touchscreen assay and long-term memory of pattern separation paradigm was probed. **A**, B6J took an average of 20 daily sessions to acquire task, evaluated by the criterion of two reversals in a single session, across 3 sessions in a 4-session block. **B-D**, After the task was successfully acquired, long-term spatial memory was assessed by conducting probe trials at 1-week, 2-week and 4-week time points. Mice were tested in a single 60-minute session and the number of reversals performed was recorded. Across all time points, B6J mice are able to perform  $\sim 2$  reversals during each probe session. Data shown as mean  $\pm$  SEM.



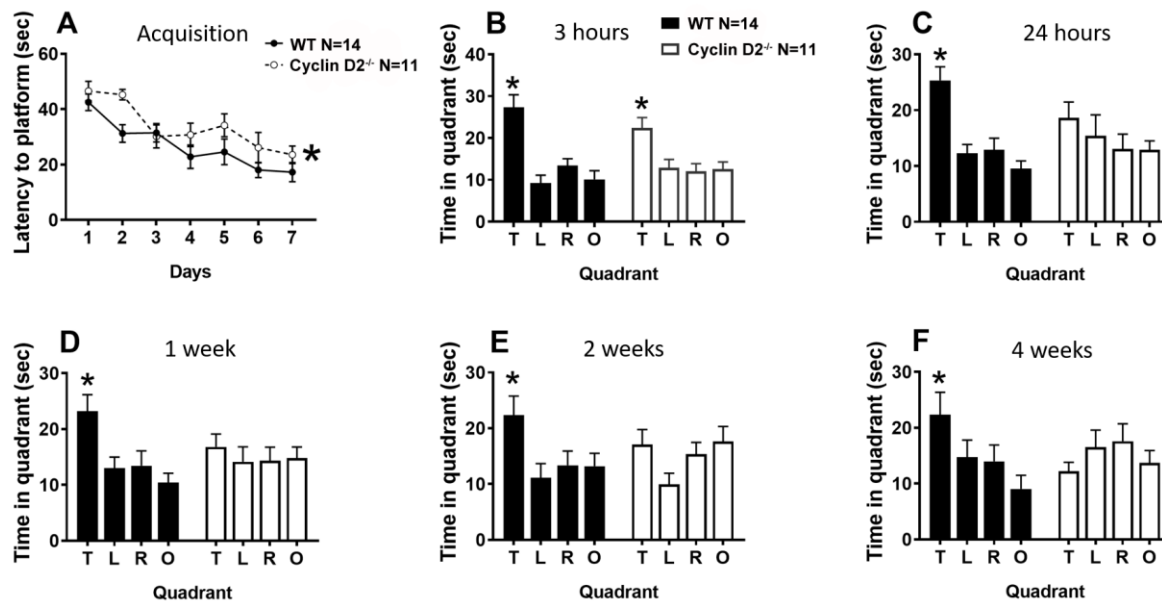
### Supplemental Figure 3

**Cyclin D2<sup>-/-</sup> mice do not have altered locomotor activity.** A-D, Locomotive activity was assessed using the open field assay; mice were placed in a novel arena for 10 minutes and horizontal, vertical, total activity as well as center time was recorded. No genotype differences were found between WT (N = 15) and cyclin D2<sup>-/-</sup> mice (N = 12). Data shown as mean ± SEM.



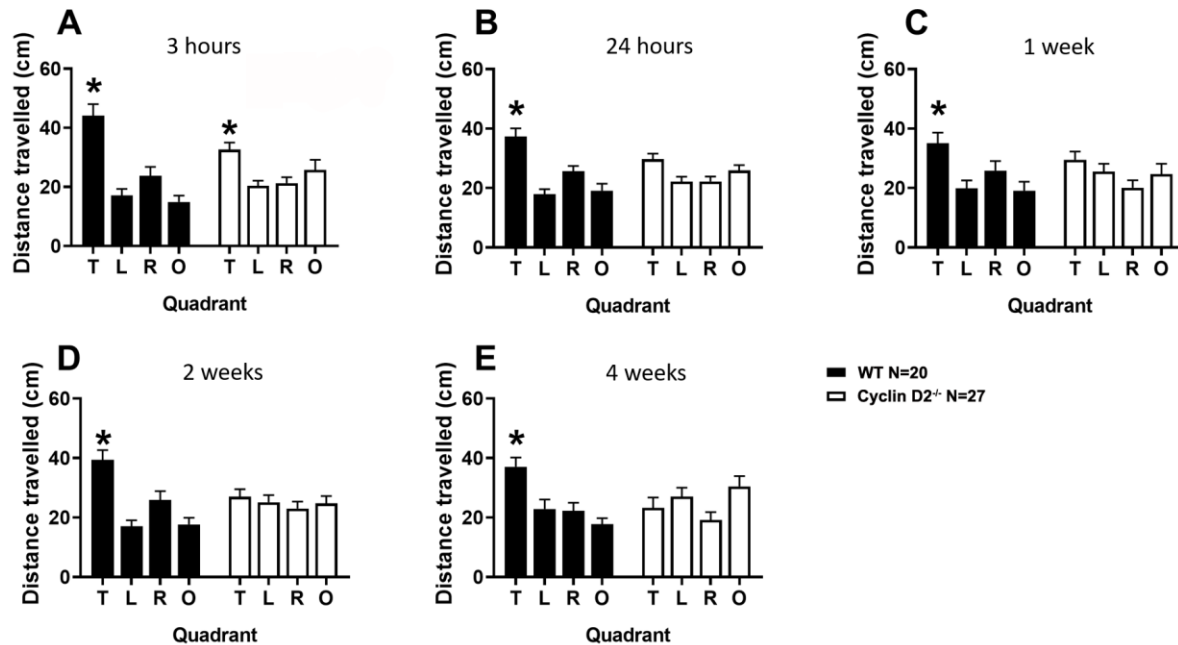
**Supplemental Figure 4**

**Cyclin D2<sup>-/-</sup> mice showed decreased swim velocity in acquisition of Morris water maze task.** Swim velocity was recorded during water maze training sessions, and cyclin D2<sup>-/-</sup> mice (N = 27) demonstrated slower swim speeds compared to WT (N = 20). Data shown as mean  $\pm$  SEM. \* indicates  $p < .05$ , main effect of genotype in repeated measures two-way ANOVA.



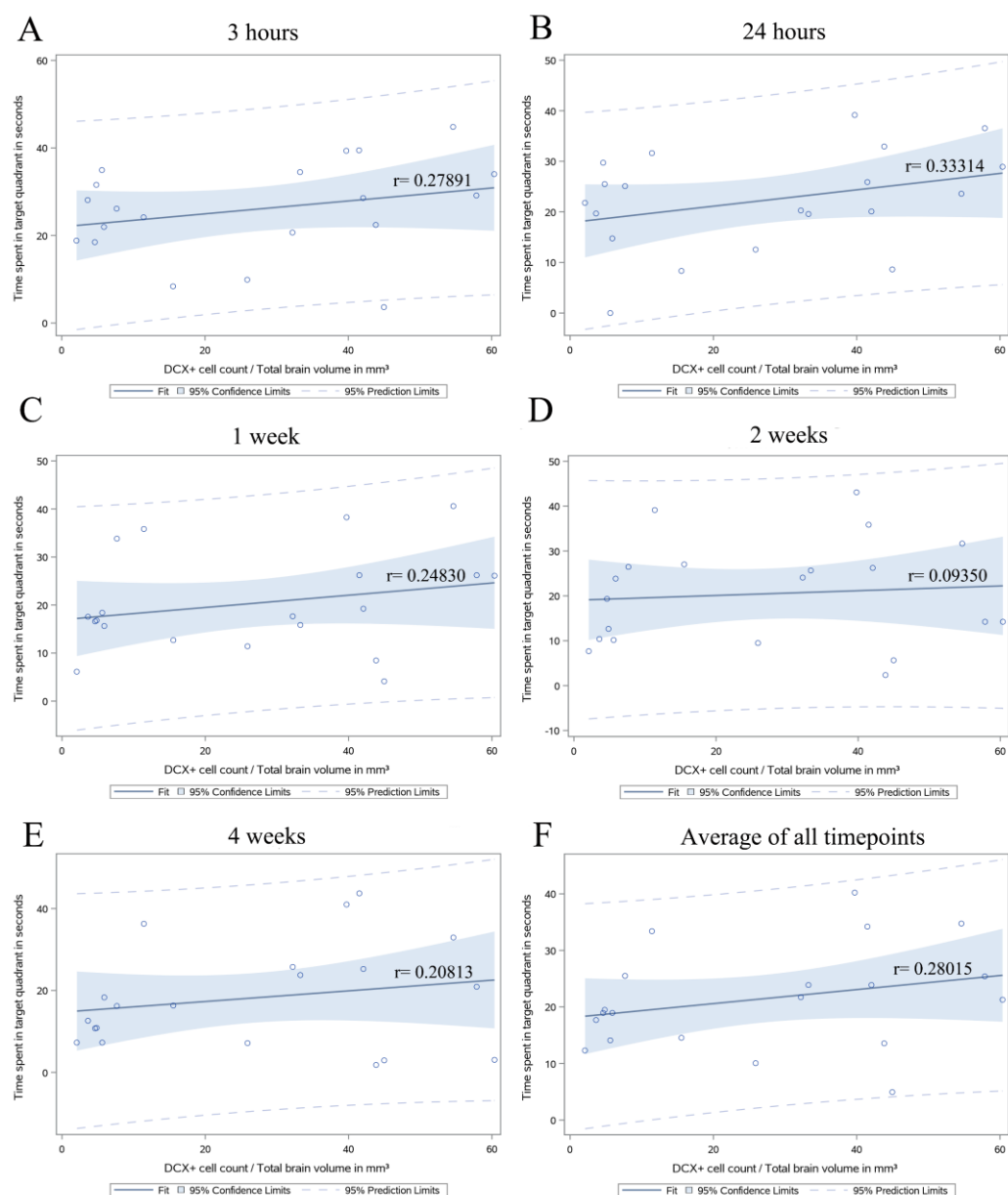
**Supplemental Figure 5**

**Independent cohort replicates long-term spatial memory deficit in cyclin D2<sup>-/-</sup> mice.** **A**, Cyclin D2<sup>-/-</sup> mice (N = 11) were able to acquire the location of the platform over the course of 7 training days. WT mice (N = 14), again, performed significantly better in latency to platform. **B**, Both WT and cyclin D2<sup>-/-</sup> had intact short-term memory during a probe trial 3 hours after the last training session. **C**, At 24 hours, cyclin D2<sup>-/-</sup> showed a deficit in spatial memory of the platform location. WT demonstrated intact memory by spending significantly more time in the target quadrant compared to the other quadrants. **D-F**, Cyclin D2<sup>-/-</sup> mice did not show intact long-term memory of the platform location when probed at 1-week, 2-weeks, and 4-weeks, whereas WT do show intact long-term memory of the platform location. Data shown as mean  $\pm$  SEM. \* indicates  $p < .05$  target quadrant versus other quadrants.



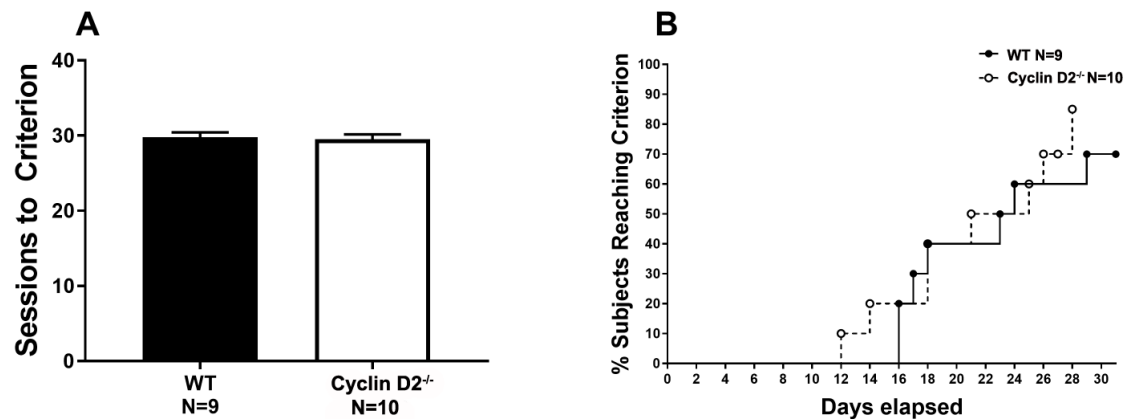
**Supplemental Figure 6**

**Cyclin D2<sup>-/-</sup> mice demonstrate long-term memory deficits in the Morris water maze when slower swim speed is accounted for.** To ensure that phenotype observed was not due to reduced swim speed, Morris water maze data was analyzed by looking at the distance travelled within each quadrant. **A**, Three hours after the last training session, WT and cyclin D2<sup>-/-</sup> mice both swam around more in the target quadrant compared to the other quadrants, suggesting both groups had intact short-term memory. **B-E**, The long-term spatial memory deficits cyclin D2<sup>-/-</sup> mice displayed were reinforced in this analysis; WT travelled more in the target quadrant at the 24-hours, 1-week, 2-week and 4-week probes compared to the cyclin D2<sup>-/-</sup> mice. Data shown as mean  $\pm$  SEM. \* indicates  $p < .05$  target quadrant versus other quadrants.



**Supplemental Figure 7**

**Dentate neurogenesis adjusted for differences in total brain volume moderately correlates with time spent in the target quadrant 24 hours after Morris water maze acquisition learning. A,** A weak correlation was found between the number of DCX+ cells normalized to total brain volume and time spent in target quadrant 3 hours after acquisition. **B,** A moderate correlation ( $r = 0.33314$ ,  $F_{(1,18)} = 2.25$ ,  $p = 0.1512$ ) between DCX+ cell count adjusted for total brain volume and time spent in target quadrant was seen 24 hours after acquisition. **C-F,** The correlation between DCX+ cell count adjusted for total brain volume was weaker at later time points and across all tested time points.



### Supplemental Figure 8

**Cyclin D2<sup>-/-</sup> mice have intact visual discrimination in a non-hippocampal pairwise touchscreen assay.** **A**, Subjects were trained in operant touchscreen chambers and then a non-hippocampal, non-spatial task was employed. WT (N = 9) and cyclin D2<sup>-/-</sup> mice (N = 10) acquired the task in a similar number of sessions, reaching the criterion of 80% accuracy in two consecutive days in an average of 29 sessions. **B**, The proportion of individual subjects that completed acquisition at each training day (survival curve) was not significantly different between genotype. Data shown as mean ± SEM.

### Supplemental Table 1

Statistical data of Supplemental Figures

| <u>Figure</u> | <u>Group</u>     | <u>Metric</u>                     | <u>One Way ANOVA</u> |                      | <u>Bonferroni Post Hoc</u> |                      |                      |
|---------------|------------------|-----------------------------------|----------------------|----------------------|----------------------------|----------------------|----------------------|
|               |                  |                                   | <u>F Statistic</u>   | <u>p value</u>       | <u>T vs. L</u>             | <u>T vs. R</u>       | <u>T vs. O</u>       |
| S1, B         | C57BL/6J<br>N=16 | 3-hr Probe,<br>Time in Quadrant   | F (3,60) = 19.98     | <b>p &lt; .0001*</b> | <b>p &lt; .0001*</b>       | <b>p &lt; .0001*</b> | <b>p &lt; .0001*</b> |
| S1, B         |                  | 24-hr Probe,<br>Time in Quadrant  | F (3,60) = 10.58     | <b>p &lt; .0001*</b> | <b>p = .0043*</b>          | <b>p &lt; .0001*</b> | <b>p = .0004*</b>    |
| S1, C         |                  | 1-week Probe,<br>Time in Quadrant | F (3,60) = 6.504     | <b>p = .0007*</b>    | <b>p = .0568†</b>          | <b>p = .0013*</b>    | <b>p = .0007*</b>    |
| S1, C         |                  | 2-week Probe,<br>Time in Quadrant | F (3,60) = 9.003     | <b>p &lt; .0001*</b> | <b>p = .0012*</b>          | <b>p = .0001*</b>    | <b>p = .0001*</b>    |
| S1, C         |                  | 4-week Probe,<br>Time in Quadrant | F (3,60) = 8.133     | <b>p = .0001*</b>    | <b>p = .0054*</b>          | <b>p &lt; .0001*</b> | <b>p = .0028*</b>    |
| S1, C         |                  | 8-week Probe,<br>Time in Quadrant | F (3,60) = 4.623     | <b>p = .0056*</b>    | <b>p = .0126*</b>          | <b>p = .0132*</b>    | <b>p = .0072*</b>    |

| <u>Figure Panel</u> | <u>Group</u>     | <u>Metric</u>                           | <u>Mean</u> | <u>SEM</u> | <u>Range</u> |
|---------------------|------------------|-----------------------------------------|-------------|------------|--------------|
| S2, A               | C57BL/6J<br>N=12 | Days to Criterion                       | 20.20       | 0.6799     | 18-24        |
| S2, B               |                  | # of correct reversals,<br>1-week probe | 2           | 0          |              |
| S2, C               |                  | # of correct reversals<br>2-week probe  | 2           | 0          |              |
| S2, D               |                  | # of correct reversals<br>4-week probe  | 1.917       | 0.08333    | 1-2          |

| Figure Panel | Test       | Sample Size                            | Metric                        | Time Point | Statistical Test                | Statistic                                                                              | p value                                        | posthoc test                    | p value   |
|--------------|------------|----------------------------------------|-------------------------------|------------|---------------------------------|----------------------------------------------------------------------------------------|------------------------------------------------|---------------------------------|-----------|
| S3, A        | Open Field | WT N=15, Cyclin D2 <sup>-/-</sup> N=12 | Horizontal Activity Over Time | 1-5        | Two Way Repeated Measures ANOVA | Genotype F (1, 25) = 0.0099<br>Time F (1, 25) = 30.71<br>Interaction F (1, 25) = 1.578 | p = .9215<br><b>p &lt; .0001*</b><br>p = .2207 | WT vs. Cyclin D2 <sup>-/-</sup> | p > .9999 |
|              |            |                                        |                               | 6-10       |                                 |                                                                                        |                                                |                                 | p = .9642 |
| S3, B        |            |                                        | Vertical Activity Over Time   | 1-5        | Two Way Repeated Measures ANOVA | Genotype F (1, 25) = 1.488<br>Time F (1, 25) = 2.226<br>Interaction F (1, 25) = 03446  | p = .2339<br>p = .1482<br>p = .8542            | WT vs. Cyclin D2 <sup>-/-</sup> | p = .7214 |
|              |            |                                        |                               | 6-10       |                                 |                                                                                        |                                                |                                 | p = .5323 |

|       |  |  |                          |      |                                 |                                                                                        |                                              |                                 |           |
|-------|--|--|--------------------------|------|---------------------------------|----------------------------------------------------------------------------------------|----------------------------------------------|---------------------------------|-----------|
| S3, C |  |  | Total Activity Over Time | 1-5  | Two Way Repeated Measures ANOVA | Genotype F (1, 25) = .0401<br>Time F (1, 25) = 94.72<br>Interaction F (1, 25) = 1.652  | p =.8429<br><b>p &lt;.0001*</b><br>p =.2105  | WT vs. Cyclin D2 <sup>-/-</sup> | p > .9999 |
|       |  |  |                          | 6-10 |                                 |                                                                                        |                                              |                                 | p = .7478 |
| S3, D |  |  | Center Time over Time    | 1-5  | Two Way Repeated Measures ANOVA | Genotype F (1, 25) = 0.1327<br>Time F (1, 25) = 39.51<br>Interaction F (1, 25) = 1.620 | p = .7188<br><b>p &lt;.0001*</b><br>p =.2148 | WT vs. Cyclin D2 <sup>-/-</sup> | p > .9999 |
|       |  |  |                          | 6-10 |                                 |                                                                                        |                                              |                                 | p = .5851 |

| <u>Figure</u> | <u>Test</u>                      | <u>Sample Size</u>                     | <u>Metric</u> | <u>Time Point</u> | <u>Statistical Test</u>         | <u>Statistic</u>                                                                    | <u>p value</u>                                      | <u>posthoc test</u>                        | <u>p value</u>    |
|---------------|----------------------------------|----------------------------------------|---------------|-------------------|---------------------------------|-------------------------------------------------------------------------------------|-----------------------------------------------------|--------------------------------------------|-------------------|
| S4            | Acquisition of Morris Water Maze | WT N=20, Cyclin D2 <sup>-/-</sup> N=27 | Swim Velocity | Day 1             | Two Way Repeated Measures ANOVA | Day F (6,270) = 6.318<br>Genotype F (1,45) = 12.8<br>Interaction F (6,270) = 0.3247 | <b>p &lt;.0001*</b><br><b>p =.0008*</b><br>p =.9237 | Bonferroni WT vs. Cyclin D2 <sup>-/-</sup> | p = .2010         |
|               |                                  |                                        |               | Day 2             |                                 |                                                                                     |                                                     |                                            | <b>p = .0090*</b> |
|               |                                  |                                        |               | Day 3             |                                 |                                                                                     |                                                     |                                            | p = .1407         |
|               |                                  |                                        |               | Day 4             |                                 |                                                                                     |                                                     |                                            | p = .1268         |
|               |                                  |                                        |               | Day 5             |                                 |                                                                                     |                                                     |                                            | <b>p = .0160*</b> |
|               |                                  |                                        |               | Day 6             |                                 |                                                                                     |                                                     |                                            | p = .0642†        |
|               |                                  |                                        |               | Day 7             |                                 |                                                                                     |                                                     |                                            | <b>p = .0248*</b> |

| Figure | Test                             | Sample Size                            | Metric                   | Time Point        | Statistical Test                | Statistic                                                                            | p value                                            | posthoc test                               | p value   |
|--------|----------------------------------|----------------------------------------|--------------------------|-------------------|---------------------------------|--------------------------------------------------------------------------------------|----------------------------------------------------|--------------------------------------------|-----------|
| S5, A  | Acquisition of Morris Water Maze | WT N=14, Cyclin D2 <sup>-/-</sup> N=11 | Latency to Platform      | Day 1             | Two Way Repeated Measures ANOVA | Day F (6,138) = 11.91<br>Genotype F (1,23) = 6.941<br>Interaction F (6,138) = 0.9106 | <b>p&lt;.0001*</b><br><b>p =.0148*</b><br>p =.4893 | Bonferroni WT vs. Cyclin D2 <sup>-/-</sup> | p > .9999 |
|        |                                  |                                        |                          | Day 2             |                                 |                                                                                      |                                                    |                                            | p=.0656†  |
|        |                                  |                                        |                          | Day 3             |                                 |                                                                                      |                                                    |                                            | p > .9999 |
|        |                                  |                                        |                          | Day 4             |                                 |                                                                                      |                                                    |                                            | p=.9636   |
|        |                                  |                                        |                          | Day 5             |                                 |                                                                                      |                                                    |                                            | p=.5086   |
|        |                                  |                                        |                          | Day 6             |                                 |                                                                                      |                                                    |                                            | p = .9255 |
|        |                                  |                                        |                          | Day 7             |                                 |                                                                                      |                                                    |                                            | p > .9999 |
| Figure | Metric                           |                                        | Group                    | One Way ANOVA     |                                 |                                                                                      | Bonferroni Post Hoc                                |                                            |           |
|        |                                  |                                        |                          | F Statistic       | p value                         | T vs. L                                                                              | T Vs. R                                            | T vs. O                                    |           |
| S5, B  | 3-hr Probe, Time in Quadrant     |                                        | WT                       | F (3, 52) = 14.25 | <b>p &lt; .0001*</b>            | <b>p &lt; .0001*</b>                                                                 | <b>p = .0001*</b>                                  | <b>p &lt; .0001*</b>                       |           |
|        |                                  |                                        | Cyclin D2 <sup>-/-</sup> | F (3, 40) = 6.281 | <b>p = .0014*</b>               | <b>p = .0047*</b>                                                                    | <b>p = .0020*</b>                                  | <b>p = .0032*</b>                          |           |
| S5, C  | 24-hr Probe,                     |                                        | WT                       | F (3, 52) = 13.36 | <b>p &lt; .0001*</b>            | <b>p &lt; .0001*</b>                                                                 | <b>p &lt; .0001*</b>                               | <b>p &lt; .0001*</b>                       |           |

|       |                                   |                          |                    |                   |                   |                   |                   |
|-------|-----------------------------------|--------------------------|--------------------|-------------------|-------------------|-------------------|-------------------|
|       | Time in Quadrant                  | Cyclin D2 <sup>-/-</sup> | F (3, 40) = 0.9229 | p = .4385         | p > .9999         | p = .5001         | p = .4596         |
| S5, D | 1-week Probe,<br>Time in Quadrant | WT                       | F (3, 52) = 5.632  | <b>p = .0020*</b> | <b>p = .0111*</b> | <b>p = .0156*</b> | <b>p = .0011*</b> |
|       |                                   | Cyclin D2 <sup>-/-</sup> | F (3, 40) = 0.2668 | p = .8490         | p > .9999         | p > .9999         | p > .9999         |
| S5, E | 2-week Probe,<br>Time in Quadrant | WT                       | F (3, 52) = 3.345  | <b>p = .0260*</b> | <b>p = .0161</b>  | p = .0721†        | p = .0654†        |
|       |                                   | Cyclin D2 <sup>-/-</sup> | F (3, 40) = 2.198  | p = .1032         | p = .1153         | p > .9999         | p > .9999         |
| S5, F | 4-week Probe,<br>Time in Quadrant | WT                       | F (3, 52) = 2.988  | <b>p = .0393*</b> | p = .2907         | p = .2070         | <b>p = .0139*</b> |
|       |                                   | Cyclin D2 <sup>-/-</sup> | F (3, 40) = 0.9332 | p = .4336         | p = .7252         | p = .4433         | p > .9999         |

| Figure | Metric                              | Group                    | One Way ANOVA       |                      | Bonferroni Post Hoc  |                      |                      |
|--------|-------------------------------------|--------------------------|---------------------|----------------------|----------------------|----------------------|----------------------|
|        |                                     |                          | F Statistic         | p value              | T vs. L              | T Vs. R              | T vs. O              |
| S6, A  | 3-hr Probe,<br>Distance travelled   | WT                       | F (3, 76) = 21.38   | <b>p &lt; .0001*</b> | <b>p &lt; .0001*</b> | <b>p &lt; .0001*</b> | <b>p &lt; .0001*</b> |
|        |                                     | Cyclin D2 <sup>-/-</sup> | F (3, 104) = 5.060  | <b>p = .0026*</b>    | <b>p = .0022*</b>    | <b>p = .005*</b>     | p = .1617            |
| S6, B  | 24-hr Probe,<br>Distance travelled  | WT                       | F (3, 76) = 16.55   | <b>p &lt; .0001*</b> | <b>p &lt; .0001*</b> | <b>p = .0009*</b>    | <b>p &lt; .0001*</b> |
|        |                                     | Cyclin D2 <sup>-/-</sup> | F (3, 104) = 4.179  | <b>p = .0078*</b>    | <b>p = .0093*</b>    | <b>p = .0097*</b>    | p = .3933            |
| S6, C  | 1-week Probe,<br>Distance travelled | WT                       | F (3, 76) = 5.634   | <b>p = .0015*</b>    | <b>p = .0026*</b>    | p = .1168            | <b>p = .0015*</b>    |
|        |                                     | Cyclin D2 <sup>-/-</sup> | F (3, 104) = 1.857  | p = .1415            | p = .9784            | p = .0623            | p = .7020            |
| S6, D  | 2-week Probe,<br>Distance travelled | WT                       | F (3, 76) = 15.04   | <b>p &lt; .0001*</b> | <b>p &lt; .0001*</b> | <b>p = .0019*</b>    | <b>p &lt; .0001*</b> |
|        |                                     | Cyclin D2 <sup>-/-</sup> | F (3, 104) = 0.4573 | p = .7127            | p > .9999            | p = .7383            | p > .9999            |
| S6, E  | 4-week Probe,<br>Distance travelled | WT                       | F (3, 76) = 9.032   | <b>p &lt; .0001*</b> | <b>p = .0016*</b>    | <b>p = .0010*</b>    | <b>p &lt; .0001*</b> |
|        |                                     | Cyclin D2 <sup>-/-</sup> | F (3, 104) = 2.421  | p = .0702†           | p > .9999            | p > .9999            | p = .3215            |

| Figure | Test                                       | Sample Size                                 | Metric                 | Statistical Test                             | Statistic       | P value   |
|--------|--------------------------------------------|---------------------------------------------|------------------------|----------------------------------------------|-----------------|-----------|
| S8, A  | Touchscreen-<br>Pairwise<br>Discrimination | WT N=9,<br>Cyclin D2 <sup>-/-</sup><br>N=10 | Sessions to Criteria   | Unpaired Two-<br>Tailed<br>T-Test            | T (17) = 0.2937 | p = .7726 |
| S8, B  |                                            |                                             | % Animals<br>Completed | Gehan Breslow<br>Wilcoxon Chi<br>Square Test | X = 0.1463      | p = .7021 |