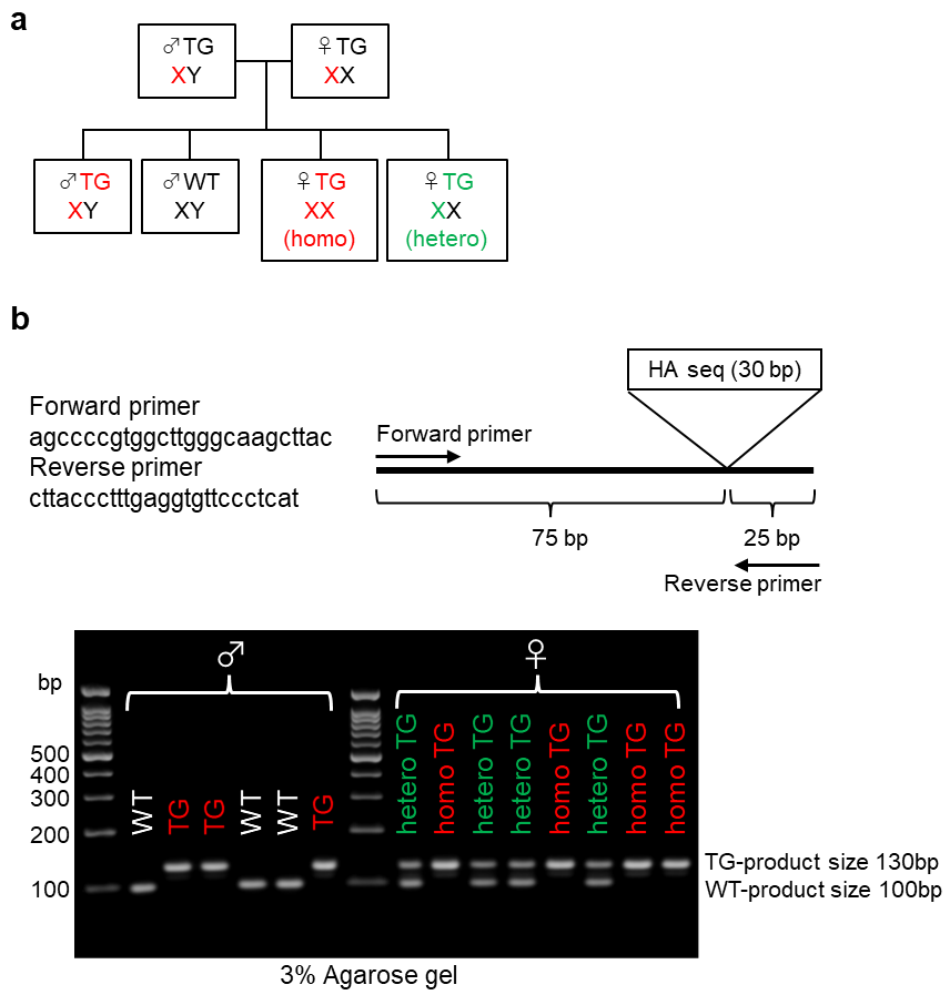
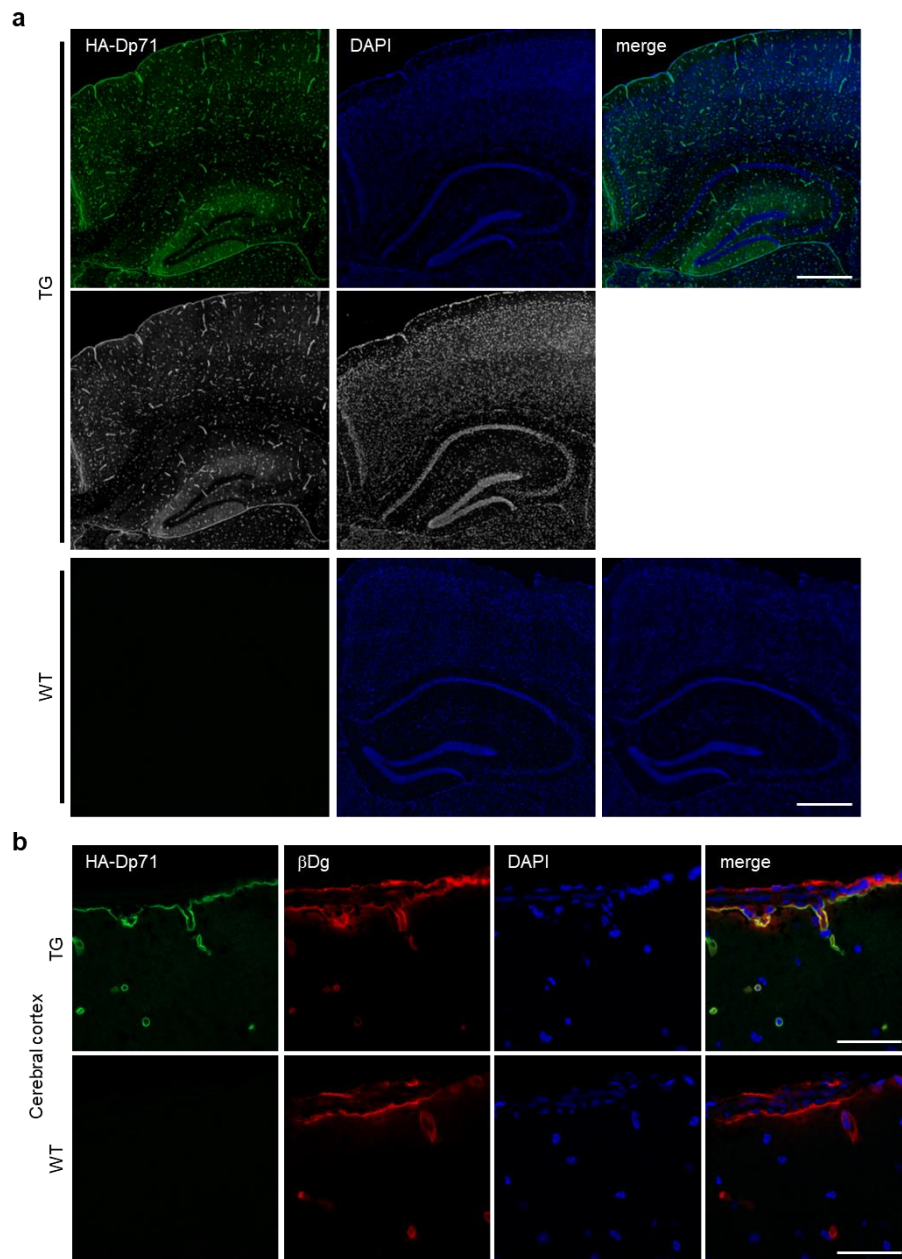


**Suppl. Table S1:** List of antibodies used for western blot and immunostaining

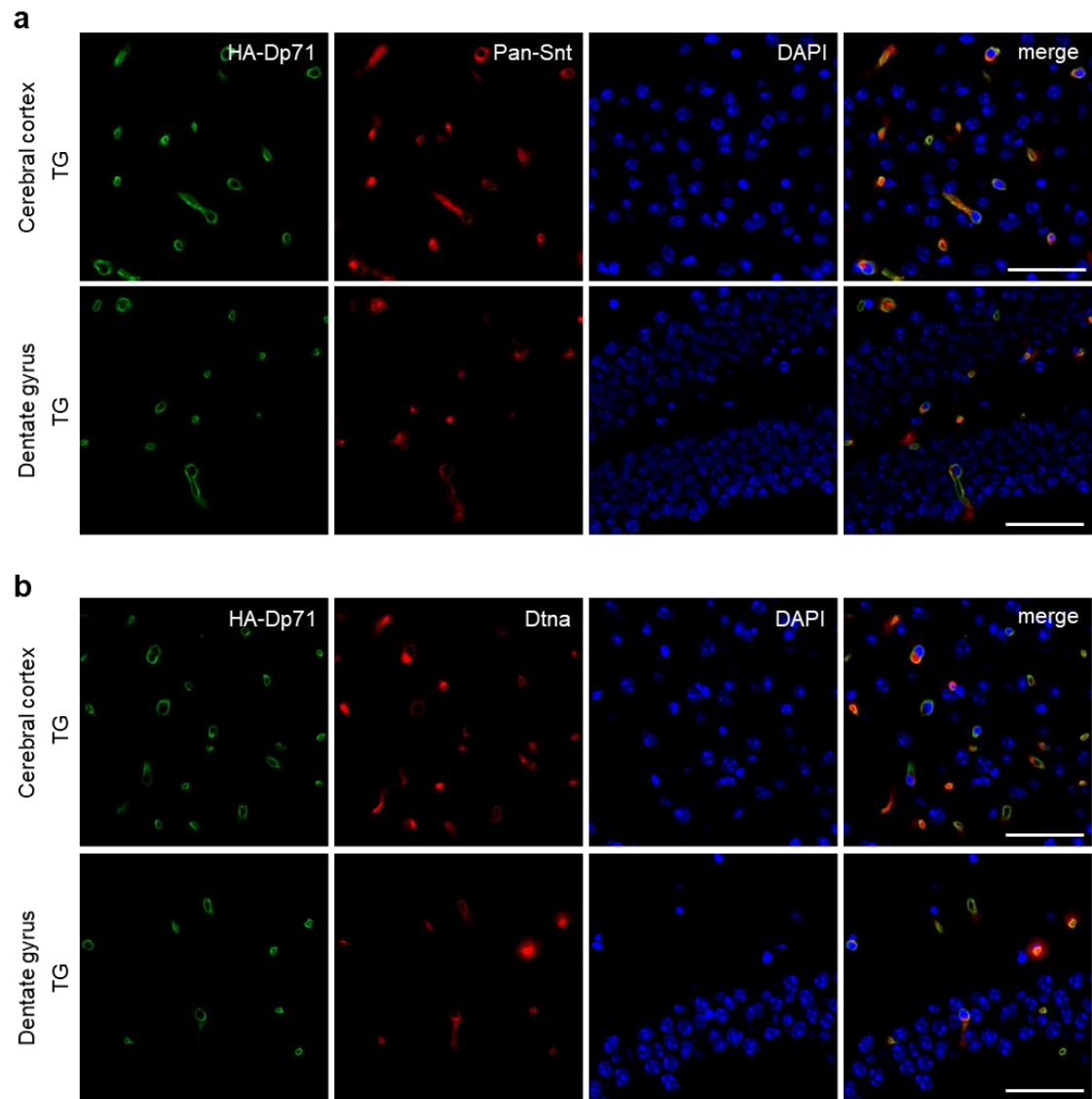
| Protein                                 | Antibody                     | Host   | Company                                                       | Dilution          | Figure                                                                                        |
|-----------------------------------------|------------------------------|--------|---------------------------------------------------------------|-------------------|-----------------------------------------------------------------------------------------------|
| HA-tag                                  | C29F4, #3724                 | Rabbit | Cell Signaling Technology                                     | 2000(WB), 800(IF) | 1b,1c,1d,1e,2a,2b,2c,S2a,S2b,S3a,S3b,3a,3b,3c,3d,3e,4a,4b,4c,5a,5b,5c,5d,5e,S5,S6,6a,6c,6f,S7 |
| HA-tag                                  | 3F10, 11867423001            | Rat    | Roche                                                         | 200(IF)           | 4d                                                                                            |
| HA-tag                                  | 6E2, #2367                   | Mouse  | Cell Signaling Technology                                     | 200(IF)           | 6b,6e                                                                                         |
| Pan-Dystrophin                          | 12715-1-AP                   | Rabbit | Proteintech Japan                                             | 3000(WB), 800(IF) | 1b,1e,4d,S4a,S4b                                                                              |
| Dystrophin (Dys2)                       | Dys2, Dy8/6C5                | Mouse  | Novocastra by Leica Biosystems                                | 200(WB)           | 1e                                                                                            |
| Dp71f                                   | original                     | Rabbit | PMID: 32996569; Hum Mol Genet . 2020 Nov 25;29(19):3312-3326. | 1000(WB)          | 1e                                                                                            |
| Dystrophin (Dys1)                       | Dys1, Dy4/6D3                | Mouse  | Novocastra by Leica Biosystems                                | 20(IF)            | 3e,4c,S6b                                                                                     |
| Actin                                   | 60008-1-Ig                   | Mouse  | Proteintech Japan                                             | 2000(WB)          | 1b,1c,5a                                                                                      |
| Prox1                                   | 5G10, NBP1-30045SS           | Mouse  | Novus Biologicals                                             | 200(IF)           | 2a                                                                                            |
| GFAP                                    | 2E1, sc-33673                | Mouse  | Santa Cruz Biotechnology                                      | 200(IF)           | 2a,2b,5b                                                                                      |
| $\beta$ -Dysroglycan                    | 43DAG1/8D5, NCL- $\beta$ -DG | Mouse  | Leica Biosystems                                              | 100(IF)           | 2c,S2b,3c,4b,5a,5b,S5,S7                                                                      |
| Syntrophin                              | 1351, NB600-1294             | Mouse  | Novus biologicals                                             | 200(IF)           | S3a                                                                                           |
| Dystrobrein (Dtna)                      | C-6, sc-271874               | Mouse  | Santa Cruz Biotechnology                                      | 500(WB), 100(IF)  | S3b,6a,6c,6f                                                                                  |
| Gephyrin                                | 147021                       | Mouse  | Synaptic Systems                                              | 500(IF)           | 3a,3b,4a,S4a,5c,5d,S5,6d,6g                                                                   |
| PSD95                                   | P78352                       | Mouse  | UC Davis/NIH NeuroMab Facility                                | 500(IF)           | 3d,S4b,5a,5c,5e,S5,6g                                                                         |
| TuJ1                                    | MAB1195                      | Mouse  | R&D Systems                                                   | 1000(WB)          | 5a                                                                                            |
| MAP2                                    | AP20, sc-32791               | Mouse  | Santa Cruz Biotechnology                                      | 200(IF)           | 5b                                                                                            |
| VGAT                                    | F-2, sc-393373               | Mouse  | Santa Cruz Biotechnology                                      | 200(IF)           | 5c,S5                                                                                         |
| BNP1 (VGLUT1)                           | A-8, sc-377425               | Mouse  | Santa Cruz Biotechnology                                      | 200(IF)           | 5c,S5                                                                                         |
| DTNB                                    | 12045-1-AP                   | Rabbit | Proteintech Japan                                             | 1000(WB), 200(IF) | 6a,6b,6d,6e,6g                                                                                |
| GAD67                                   | 1G10.2, MAB5406              | Mouse  | Millipore                                                     | 400(IF)           | S6a                                                                                           |
| HRP conjugated anti-mouse IgG           | 1031-05                      | Goat   | Southern Biotechnology Associates                             | 7000(WB)          |                                                                                               |
| HRP conjugated anti-mouse IgM           | 1021-05                      | Goat   | Southern Biotechnology Associates                             | 7000(WB)          |                                                                                               |
| HRP conjugated anti-rabbit IgG          | 4050-05                      | Goat   | Southern Biotechnology Associates                             | 7000(WB)          |                                                                                               |
| VeriBlot for IP Detection Reagent (HRP) | ab131366                     |        | Abcam                                                         | 1500(WB)          |                                                                                               |
| TrueBlot(R) ULTRA: Anti-Ms Ig HRP       | 18-8817-31                   | Rat    | Rockland Immunochemicals                                      | 1500(WB)          |                                                                                               |
| Alexa Fluor 488 anti-rabbit             | A11034                       | Goat   | Life Technologies                                             | 1000(IF)          |                                                                                               |
| Alexa Fluor 568 anti-mouse              | A11004                       | Goat   | Life Technologies                                             | 1000(IF)          |                                                                                               |
| Alexa Fluor 647 anti-mouse              | A21236                       | Goat   | Life Technologies                                             | 1000(IF)          |                                                                                               |
| Alexa Fluor 647 anti-rabbit             | A21245                       | Goat   | Life Technologies                                             | 1000(IF)          |                                                                                               |
| Alexa Fluor 488 anti-rat                | ab150157                     | Goat   | Abcam                                                         | 500(IF)           |                                                                                               |



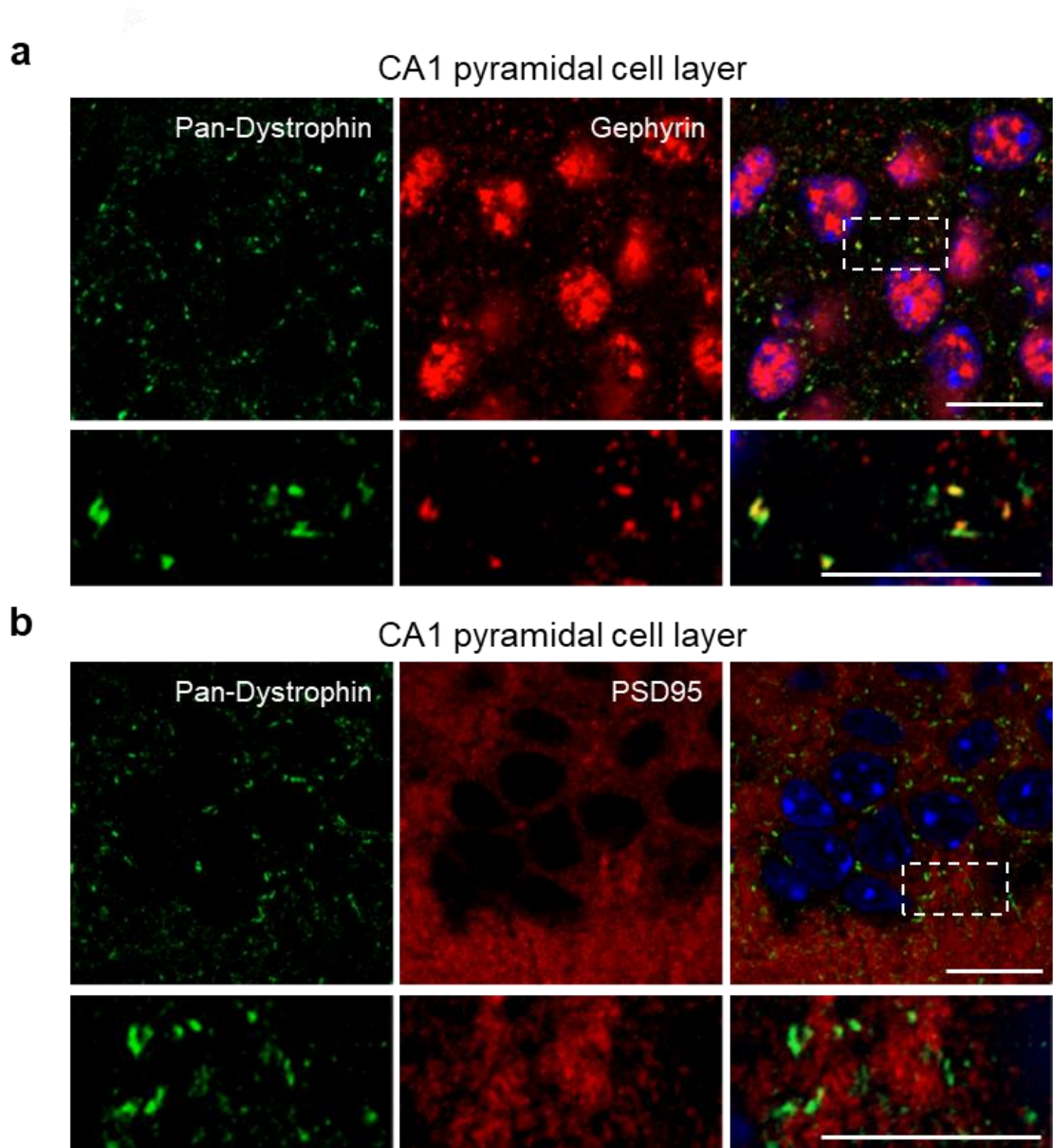
**Suppl. Fig. 1 a** Female transgenic (TG) mice were generated by intercrossing male TG mice with female heterozygous TG mice. **b** PCR genotyping performed using DNA extracted from tails of the offspring. PCR shows heterozygous female mice with single HA sequence (30 bp) inserted allele and single non-inserted allele, or homozygous female mice with both HA sequence inserted allele



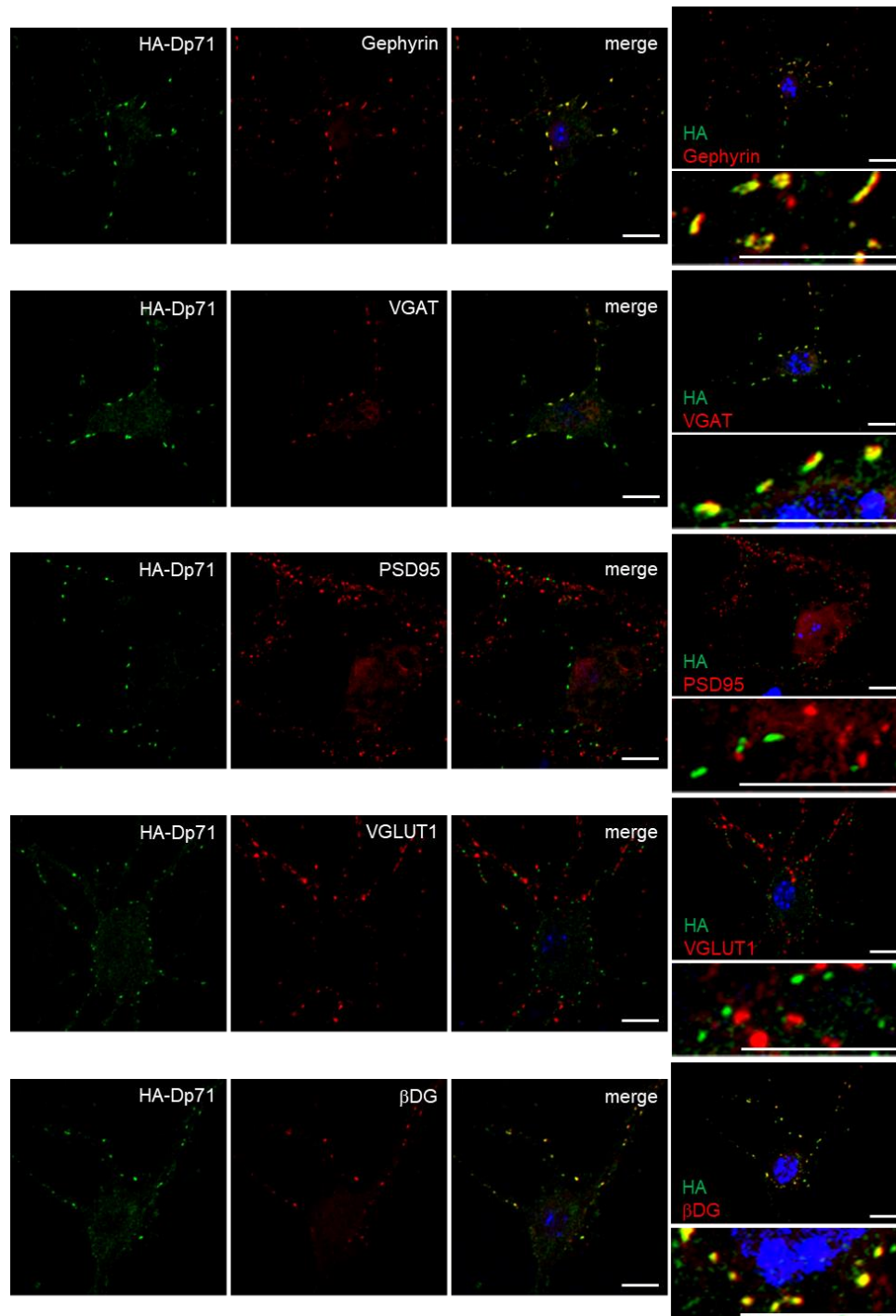
**Suppl. Fig. 2 a** Confocal images for HA-Dp71 and DAPI in the cerebral cortex and hippocampal region acquired by the Zeiss LSM 900 equipped with a computer-driven motorized stage (Carl Zeiss, Oberkochen, Germany). Sixteen image tiles were taken with a 10  $\times$  objective to cover the targeted region in the transgenic (TG) or wild-type (WT) mouse brain sections and the tiles were obtained apposing each other with 10% overlapping. The stitched images were created by the software ZEN 3.2 (Carl Zeiss, Oberkochen, Germany). Scale bar, 500  $\mu$ m. **b** Confocal images for HA-Dp71,  $\beta$ Dg and DAPI in the cerebral cortex of TG or WT mouse brain sections. WT section was used as control to validate signal specificities for HA-Dp71 expression in the TG sections. Scale bar, 50  $\mu$ m



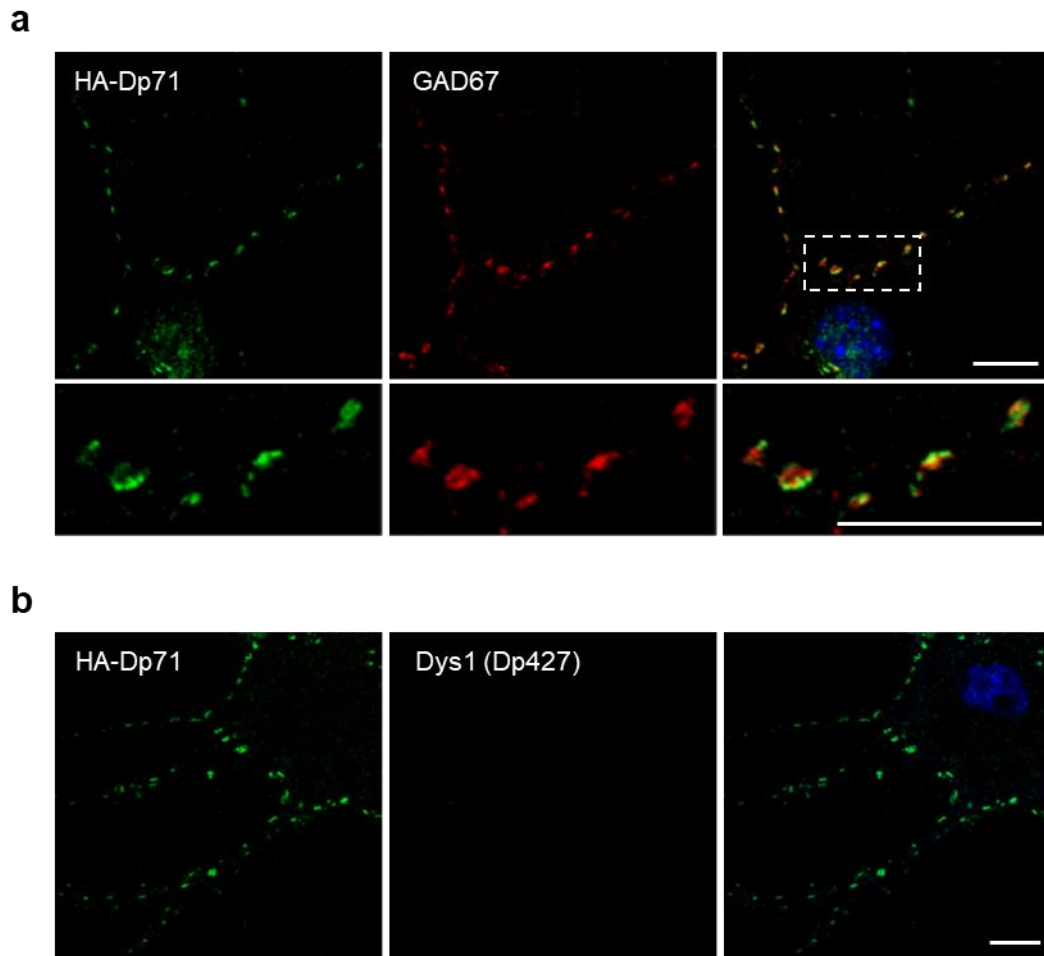
**Suppl. Fig. 3 a** Confocal images for HA-Dp71, Pan-Snt and DAPI in the cerebral cortex and hippocampal dentate gyrus of the transgenic (TG) mouse brain section. **b** Confocal images for HA-Dp71, Dtna and DAPI in the cerebral cortex and hippocampal dentate gyrus of the TG mouse brain section. Scale bar, 50  $\mu$ m



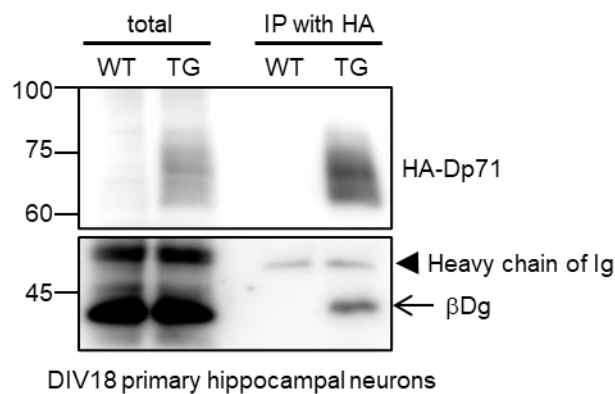
**Suppl. Fig. 4** Double-immunostaining of Pan-Dystrophin with Gephyrin (**a**), or PSD95 (**b**) in the hippocampal CA1 pyramidal cell layer of the TG mouse brain sections. Anti-Gephyrin antibody revealed strong cross-reactions against nuclear component which should be ignored. Fine synaptic particles were specific for Gephyrin. Lower panels show cropped and magnified images of boxed area in the upper panels (a, b). Blue, DAPI nuclear staining. Scale bar, 10  $\mu$ m



**Suppl. Fig. 5** Double immunostaining of HA-Dp71 with various synapse markers, Gephyrin (inhibitory postsynapse), VGAT (inhibitory presynapse), PSD95 (excitatory postsynapse), VGLUT1 (excitatory presynapse), and  $\beta$ Dg in the primary cultured neurons at DIV18. Representative single z-plane images for HA-Dp71 (green), synapse marker (red), and merged were shown on the left side. The maximum intensity projection from multiple z-plane images processed by ZEN software were shown on the right side. Blue, DAPI nuclear staining. Scale bar, 10  $\mu$ m



**Suppl. Fig. 6 a** Double immunostaining of HA-Dp71 with GAD67, an inhibitory presynapse marker, in the primary cultured neurons at DIV18. **b** Double immunostaining of HA-Dp71 with anti-Dys1 antibody, which potentially recognizes Dp427, in the primary cultured neurons at DIV18. Lower panels show cropped and magnified images of boxed area in the upper panels (a). Blue, DAPI nuclear staining. Scale bar, 10  $\mu$ m



**Suppl. Fig. 7** Wild-type (WT) or HA-Dp71 transgenic (TG) mouse embryo-derived hippocampal cultured neurons were maintained for 18 days and subjected to co-immunoprecipitation after PFA-cross-linking as described previously [Ref]. Immunoprecipitates using anti-HA beads were analyzed by western blot detected with anti-HA and  $\beta$ Dg antibodies. IP, immunoprecipitation; Ig, immunoglobulin used for IP

**Ref.** Fujimoto T, Yaoi T, Tanaka H, Itoh K (2020) Dystroglycan regulates proper expression, submembranous localization and subsequent phosphorylation of Dp71 through physical interaction. Hum Mol Genet 29(19):3312-3326. <https://doi.org/10.1093/hmg/ddaa217>