Non-cell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology.

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Supplementary Information

Supplementary Methods

Primary cerebellar cultures

Cerebellum from 2-3 P8 rat pups were dissected and digested with 1 ml of 0.25% trypsin (Worthington) and 100 μg DNase (Sigma) for 15 min at 37C. Trypsin was inactivated with DMEM containing 10% FBS (GIBCO) and cerebella were triturated with polished glass Pasteur pipettes. Dissociated cells were washed with DMEM containing 10% FBS and 300,000 cells were plated onto 12 mm poly-L-Lysine coated coverslips. The next day, medium was discarded and replaced with Neurobasal medium containing B27 supplement (GIBCO).

Golgi staining

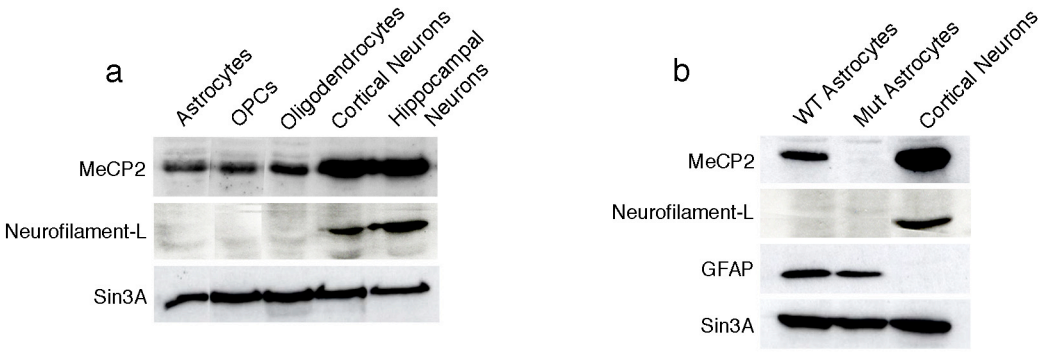
Golgi staining was performed on wild-type and symptomatic RTT littermates (Jaenisch mouse model) as previously described [1]. Images of dendritic arbors were collected using Zeiss Axiovert S100 inverted microscope and Image J software. Dendritic arbors that could be clearly distinguished from neighboring dendritic arbors were manually traced to estimate the extent of branching. To ensure that all dendrites belonging to a single arbor were included in a trace, images were collected as z-stacks using a 25x objective, and only the in focus regions of a dendrite were considered.

Statistical analysis

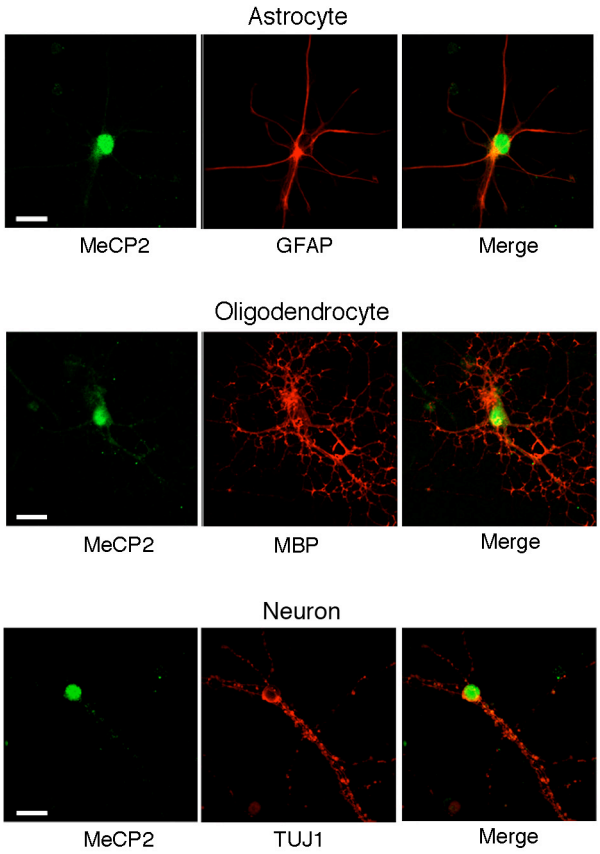
All data were analyzed by unpaired two-tailed t-tests. Statistical analyses were performed using Excel version 11.5.1. Data was considered significant if $p < 0.05$.

References

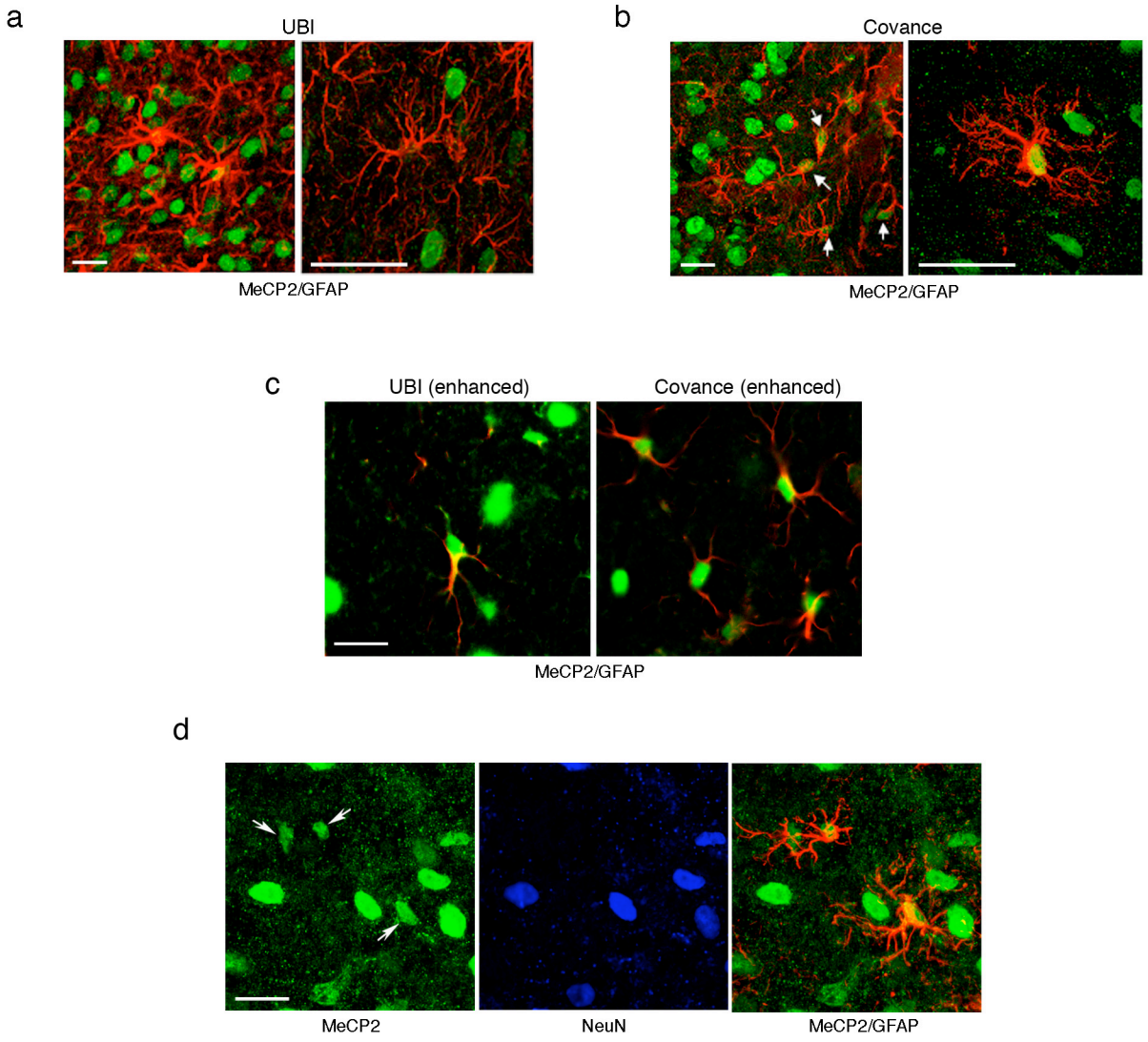
1. Luikart, B.W. et al. TrkB has a cell-autonomous role in the establishment of hippocampal Schaffer collateral synapses. *J Neurosci* **25**, 3774–86 (2005).



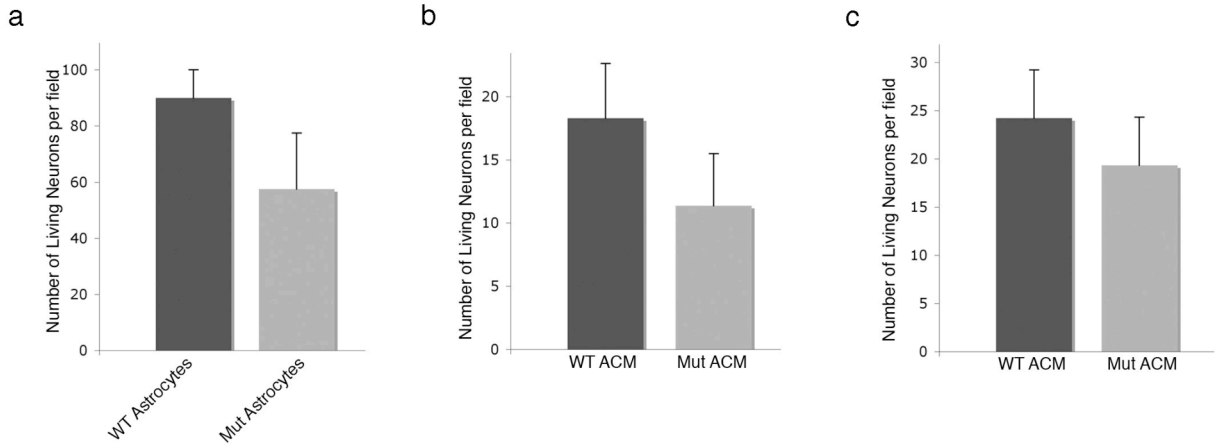
Supplementary Figure S1. *The presence of MeCP2 in enriched primary glial cultures from rat or mouse brains is not neuronal.* Western blot analysis of protein extracts from (a) rat glial and neuronal cultures. (b) mouse astrocyte cultures from WT and RTT brains or from primary neuronal cultures. Note the presence of Neurofilament-L in the neuronal cultures and its absence in the glial cultures. Sin3A serves as a loading control.



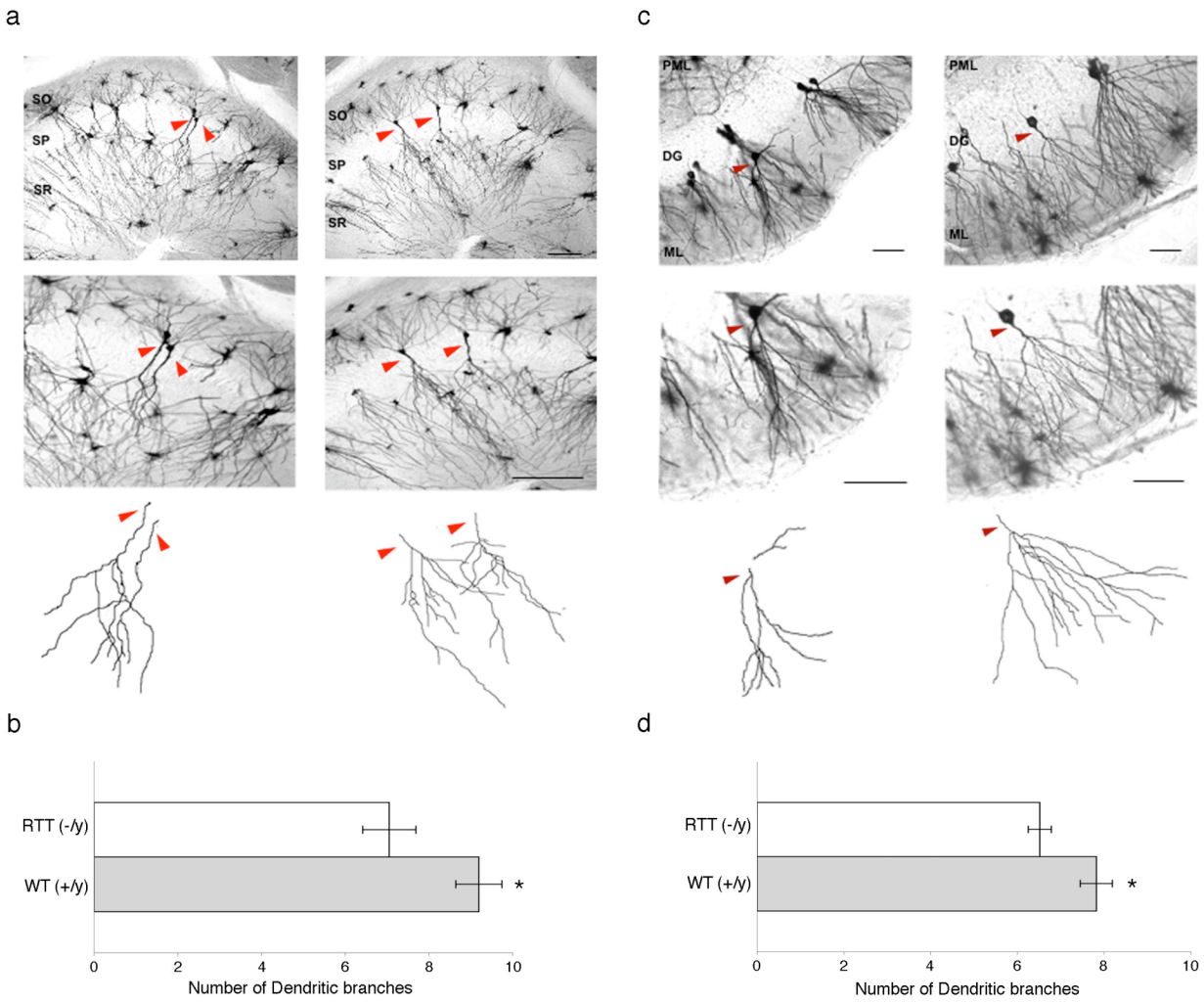
Supplementary Figure S2. *MeCP2 protein is present in nuclei of glia in primary cerebellar cultures.* Immunostaining indicates the presence of MeCP2 protein (green) in different glial types or neurons (red). GFAP and MBP as in Figure 1. TUJ1 indicates neuronal-specific β-tubulin. Calibration bar, 20 μm.



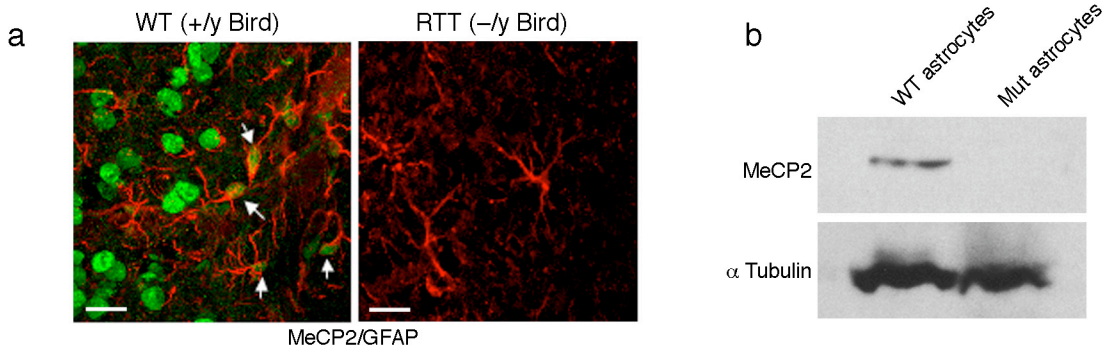
Supplementary Figure S3. *Detection of MeCP2 in glia is antibody- and/or enhancement system-dependent*. Immunohistochemical analysis of normal mouse brains using (a) commercially available anti-MeCP2 antibody (UBI), (b) home made anti-MeCP2 antibody (Covance). MeCP2, green; GFAP, red (c) Biotin/Strepavidin enhancement system with UBI or Covance anti-MeCP2 antibodies (MeCP2, green; GFAP, red), (d) Biotin/Strepavidin enhancement system with Covance anti-MeCP2 antibody (green). NeuN (blue), neuronal marker; GFAP (red), astrocytic marker. Arrows indicate astrocytic MeCP2. Note the difference in the levels of neuronal and astrocytic MeCP2. Calibration bars, 40 μm.



Supplementary Figure S4. *Neuronal survival is reduced when cultured with mutant astrocytes or their conditioned media*. Bar graphs represent the number of hippocampal neurons, in at least 10 fields in different experiments, after 6 DIV in the presence of (a) WT or mutant astrocytes, (b) WT or mutant ACM, (c) WT or mutant ACM (Bird mouse model).



Supplementary Figure S5. *Hippocampal neurons of RTT brains in vivo have fewer dendritic branches than wild-type brains*. Golgi-stained brain sections from 6-week-old symptomatic (RTT) or normal (WT) mice. (a) Images and traces of apical dendrites of CA3 pyramidal neurons. Arrows designate the cell in each field to which the underlying trace corresponds. SO = stratum oriens, SP = stratum pyramidale, SR = stratum radiatum. Calibration bar, 200 μm. (b) Histogram showing that pyramidal neurons of RTT brains have fewer dendritic branches relative to pyramidal neurons of WT brains. n=31 RTT and 30 WT neurons traced. Number of animals in each group is 4. p=.02. Error bars are S.E.M. (c) Images and traces of dentate granule neurons. Arrows as in panel a. ML = molecular layer, PML = polymorphic layer, DG = dentate gyrus. Calibration bar, 100 μm. (d) Histogram showing that MeCP2 dentate granule neurons of RTT brains have fewer dendritic branches relative to dentate granule neurons of WT brains. n=55 RTT and 48 WT total neurons traced. Number of animals in each group is 4. p=.002. Error bars are S.E.M.



Supplementary Figure S6. *MeCP2 is detected in astrocytes of wild-type but not MeCP2-null mice of the Bird model.* (a) Co-immunostaining of brain sections from 6-week-old wild-type (+/y) and RTT (-/y) mice for MeCP2 (green) and for the astrocytic marker GFAP (red). Arrows indicate the presence of MeCP2 in nuclei of astrocytes of wild-type (WT) brains. Calibration bars, 40 mm. (b) Western blot analysis confirms the presence of MeCP2 in WT and its absence in MeCP2-null (Mut) astrocytes. α-tubulin serves as loading control.