Supplemental Information for

Selective loss of parvalbumin-positive GABAergic interneurons in the cerebral cortex of maternally stressed *Gad1*-heterozygous mouse offspring

Running title: Prenatal stress affects *Gad1*-heterozygotes

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Supplementary Figure Legends

Supplementary Figure 1.

Alteration of physical and metabolic parameters by maternal stress (MS) in mothers and fetuses. (a) Elevation of maternal body weight ($GAD67^{+/+}$) during MS (E 15.5 to E 17.5) was significantly suppressed (* $P < 0.05$, ** $P < 0.01$, *t*-test; control, n = 8; stress, n = 9). (b) Fetal body weight ($GAD67^{+/GFP}$) immediately after MS was significantly lower than that of controls. (*** $P < 0.001$, *t*-test; control, n = 22; stress, n = 23). (c) Maternal serum CORT levels ($GAD67^{+/+}$) in the stressed group were significantly higher than those of the control. (* $P < 0.05$, *t*-test; control, n = 7; stress, n = 5). (d) $GAD67^{+/GFP}$ fetuses in stressed mothers ($GAD67^{+/+}$) exhibited significantly higher CORT levels than those in the control condition (*** $P < 0.001$, *t*-test; control, n = 9; stress, n = 7). Error bars represent the s.e.m. Graphs are reproduced from our previously published data, in which the CORT levels were measured by radioimmunoassay.[29]

Supplementary Figure 2.

TUNEL staining of the maternally stressed $GAD67^{+/GFP}$ fetal brain. (a) Fluorescence immunostaining of TUNEL (red) and GFP (green) in the control (left) and stressed (middle) conditions, in addition to a positive control treated with DNase (right). (b, c) Higher magnification of the dotted area indicated in (a) showing the corticostriatal junction (CSJ, b) and medial ganglionic eminence (MGE, c). Arrowheads, TUNEL-positive cells in CSJ. Few TUNEL-positive cells were present in the control and stressed MGE. Note the marked increase in TUNEL-positive cells in the DNase-treated section. Control, n = 3 sections from two pups; stress, n = 4 sections from two pups; DNase I treatment, n = 3 sister sections from control and stress groups. Bars = 200 μm.

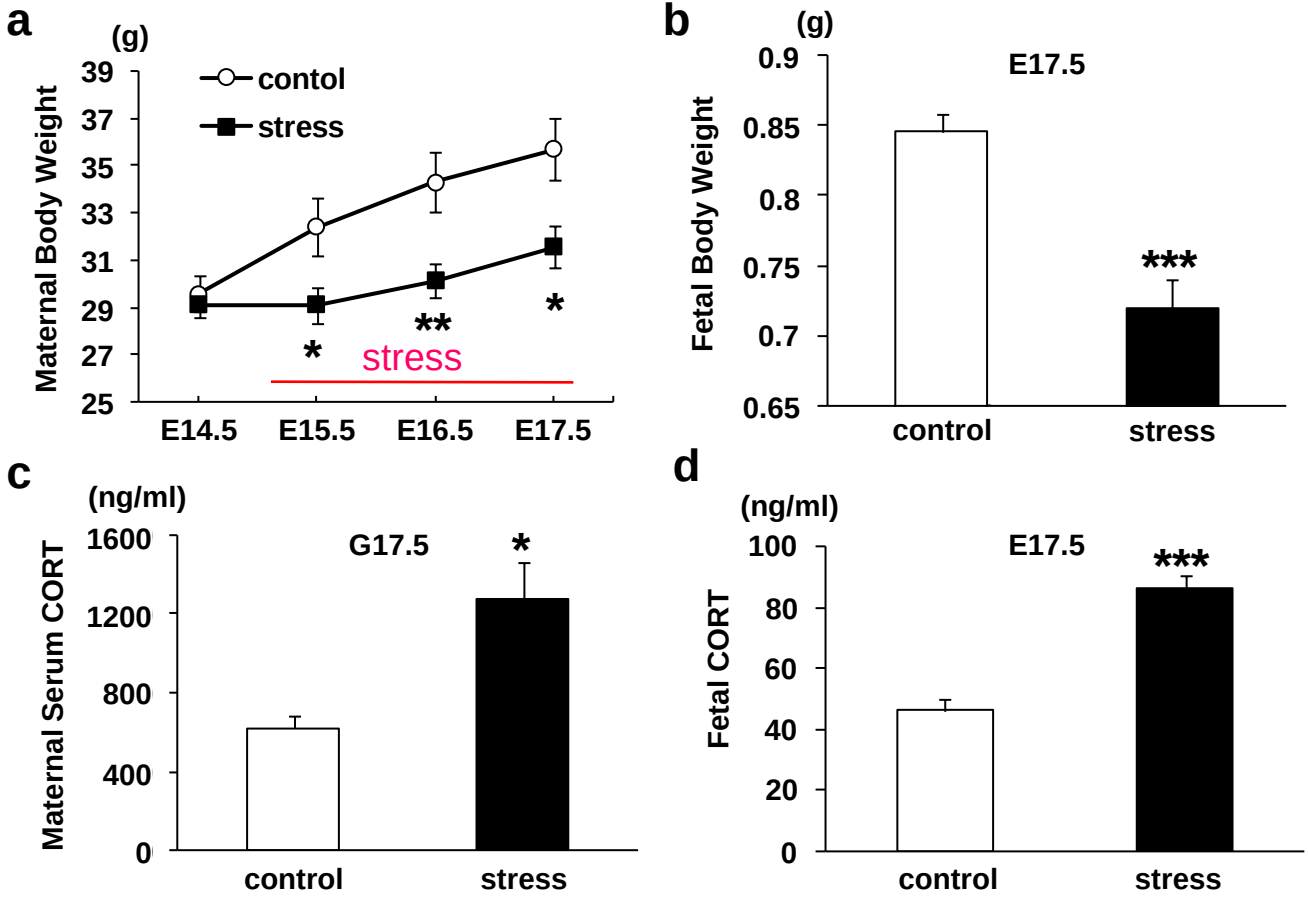
Supplementary Figure 3.

Loss of PV-positive GABAergic interneurons in several brain areas of maternally stressed GAD67$^{+/GFP}$ mice at P21. (a) Double-immunostaining image of PV (red) and GFP (green) in the hippocampus (HIP), motor cortex (M1) and somatosensory cortex (S1) of control and stressed pups. Bars = 200 μm. (b) Quantitative analysis of the density of PV and GFP double-positive cells. (c) Quantitative analysis of the density of PV negative and GFP positive cells from the same sections of (b). * $P < 0.05$, *t*-test; HIP, control, n = 5 sections from three brains; stress, n = 4 sections from four brains; M1, control, n = 5 sections from three brains; stress, n = 4 sections from four brains; S1, control, n = 4 sections from three brains; stress, n = 5 sections from four brains. Error bars represent the s.e.m.

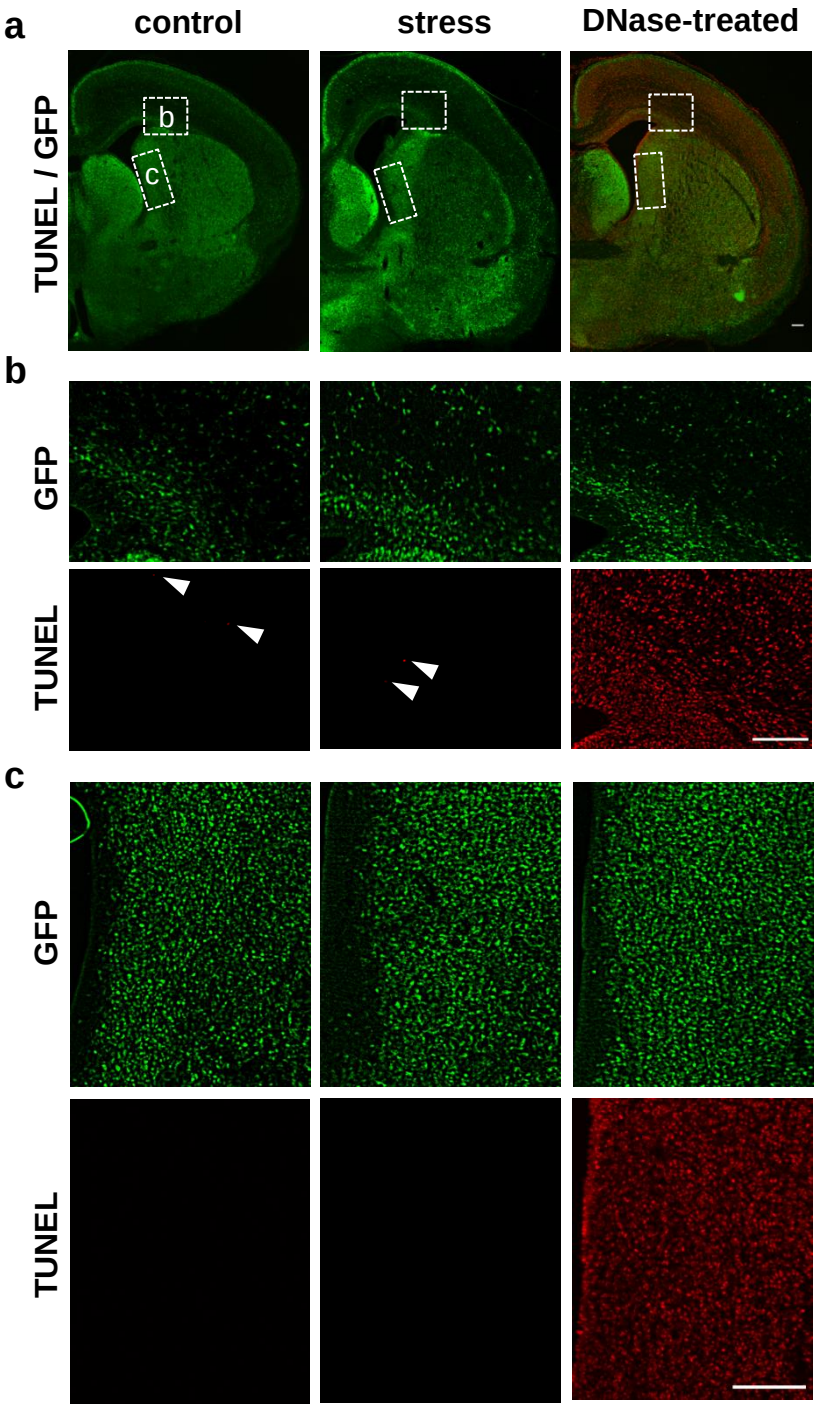
Supplementary Figure 4.

Unchanged densities of PV-positive GABAergic interneurons in several brain areas of maternally stressed GAD67$^{+/+}$ mice at P21. (a) Double-immunostaining images of PV (red) and GAD67 (green) from coronal sections of the control and stressed brain (left panels). Highlighted images of the outlined area of the mPFC (middle panels) and M1 (right panels). Bars = 200 μm (left panel) or 100 μm (middle and right panels). (b) Representative double-immunofluorescence images of sagittal sections of the brain (left panels). Highlighted images of outlined area for HIP (middle panels) and S1 (right panels). Bars = 200 μm (left panel) or 100 μm (middle and right panels). (c) Quantitative analysis of the density of PV and GAD67 double-positive cells (yellow) in mRFP, HIP, M1 and S1. There were no significant differences between the control and stressed pups in any area. (d) Quantitative analysis of the density of PV-negative and GAD67-positive (green) cells from the same sections of (c). There were no significant differences between control and stressed pups in any area. mPFC: control, n = 8 sections from four brains; stress, n = 6 sections from three brains. HIP: control, n = 6 sections from four brains; stress, n = 4 sections from three brains. M1: control, n = 8 sections from four brains; stress, n = 6 sections from three brains. S1: control, n = 6 sections from four brains; stress, n = 4 sections from three brains. Error bars indicate the s.e.m.

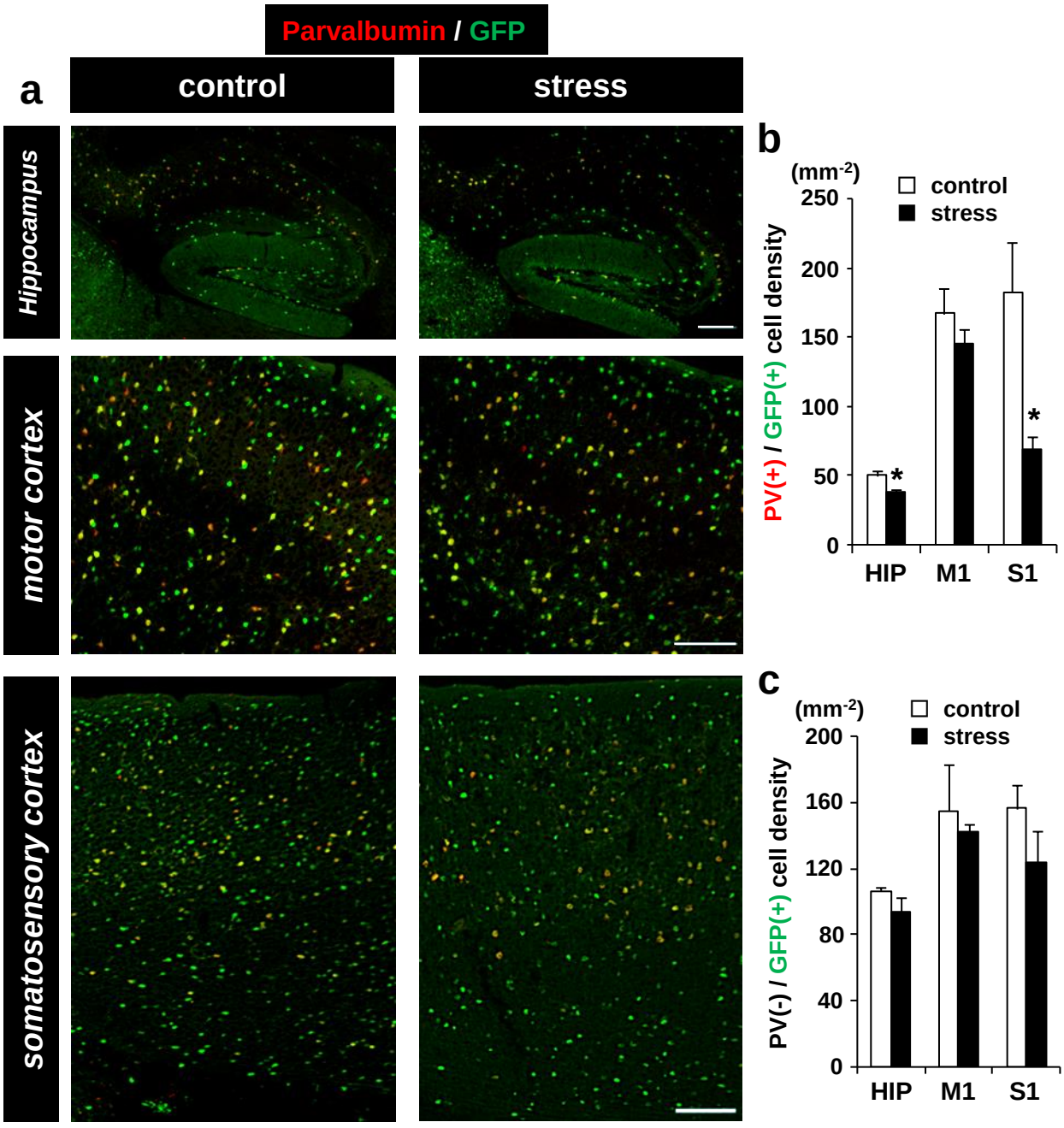
Supplementary Figure 1. Uchida T *et al.*



Supplementary Figure 2. Uchida T et al.



Supplementary Figure 3. Uchida T *et al.*



Supplementary Figure 4. Uchida T *et al*.