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Supplemental information

Prenatal low-dose methylmercury exposure causes premature neuronal differentiation and autism-like behaviors in a rodent model

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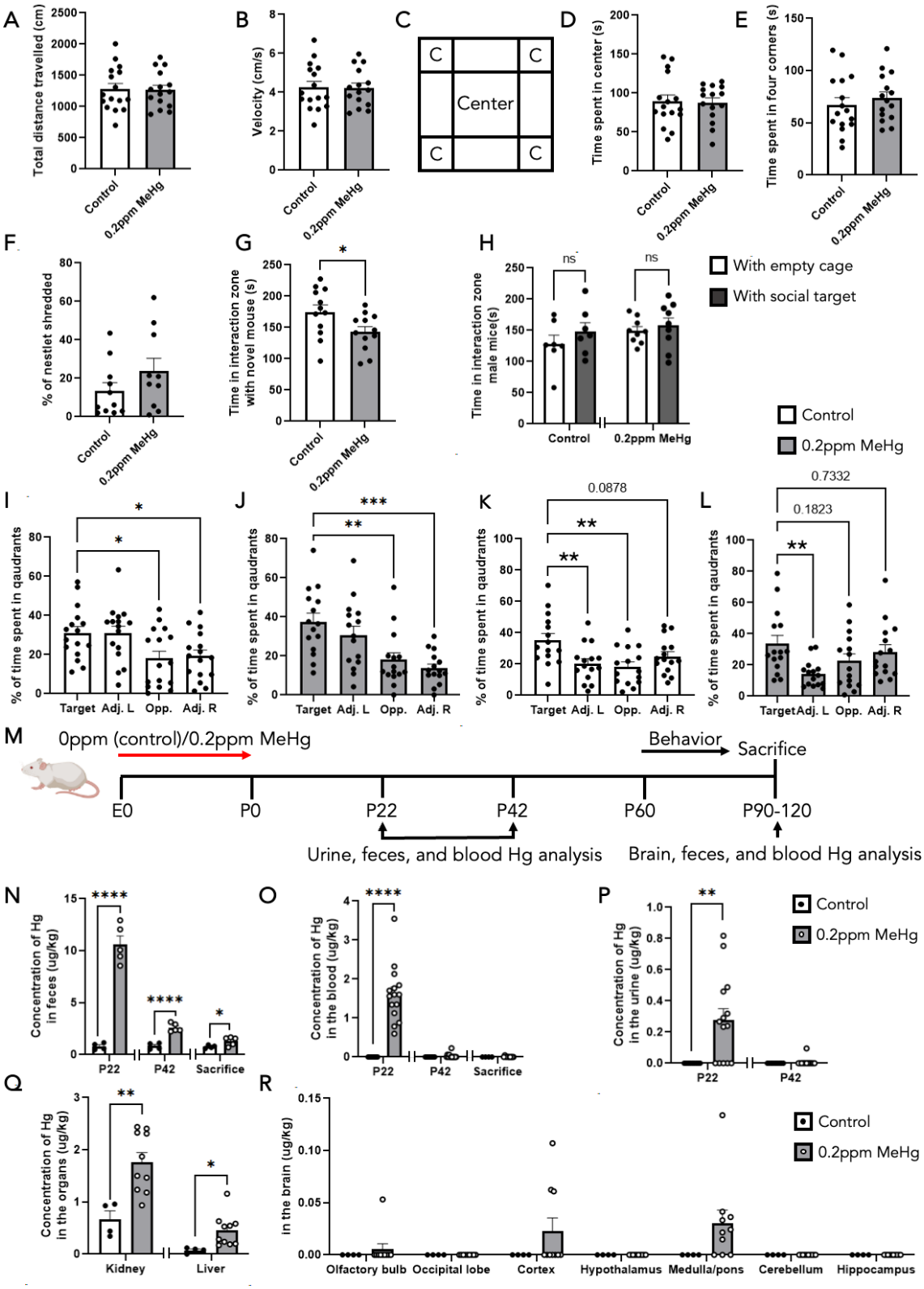

Figure S1. Impact of prenatal MeHg exposure on behaviour and Hg measurements. Related to Figure 1. (A) Open-field data for total distance travelled (cm). n=15-16 animals/group. (B) Open-field data for mean velocity (cm/s). n=15-16 animals/group. (C) Schematic of open-field boundaries used to assess anxiety. (D-E) quantitative analysis

of time spent in the (D) center and (E) four corners of the open-field. n=15-16 animals/group. (F) Percentage of nestlet shredded after 1.5h for control and 0.2ppm MeHg groups, n=10-11 animals/group (G) Time spent in interaction zone with novel mouse for female mice, n=12 animals/group, student t-test, * $p<0.05$. (H) Time spent in interaction zone male mice, n=7 animals/group, student t-test, ns (non-significant). (I) The percentage of time spent in the 4 quadrant zones for control mice was analyzed at day 11 as a measurement of memory. n=16 animals/group, one-way ANOVA with post hoc test, *$p<0.05$. (J) The percentage of time spent in the 4 quadrant zones for MeHg-treated mice was analyzed at day 11 as a measurement of memory. n=15 animals/group, one-way ANOVA with post hoc test, **$p<0.01$, ***$p<0.001$. (K) The percentage of time spent in the 4 quadrant zones for control mice was analyzed at day 14 as a measurement of reversal learning. n=15 animals/group, one-way ANOVA with post hoc test, **$p<0.01$. (L) The percentage of time spent in the 4 quadrant zones for MeHg-treated mice was analyzed at day 14 as a measurement of reversal learning. n=15 animals/group, one-way ANOVA with post hoc test, **$p<0.01$. (M) Schematic of prenatal exposure of MeHg to pregnant mice, created with BioRender.com. Pregnant mice were exposed to either control (0ppm MeHg) or 0.2ppm MeHg. Treatment was administered through drinking water to pregnant mice starting at E0 until P0. Feces and blood were collected at P22, P42, and sacrificed for Hg analysis. Urine was collected at P22 and P42 for Hg analysis. Once behavioural testing was complete the brain, liver, and kidneys were dissected at sacrifice (P90-120) for Hg analysis. (N) Quantitative analysis of Hg concentration in the feces collected at P22, P42, and sacrifice. n=4-5 cages/group, student t-test, *$p<0.05$, ****$p<0.0001$. (O) Quantitative analysis of Hg concentration in the blood collected at P22, P42, and sacrifice. n=4-15 animals/group, student t-test, ****$p<0.0001$. (P) Quantitative analysis of Hg concentration in the urine collected at P22 and P42. n=9-15 animals/group, student t-test, **$p<0.01$. (Q) Quantitative analysis of Hg concentration in the kidneys and liver collected at sacrifice. n=4-7 animals/group, student t-test, *$p<0.05$, **$p<0.01$. (R) Quantitative analysis of Hg concentration in various brain regions collected at sacrifice. n=4-7 animals/group.

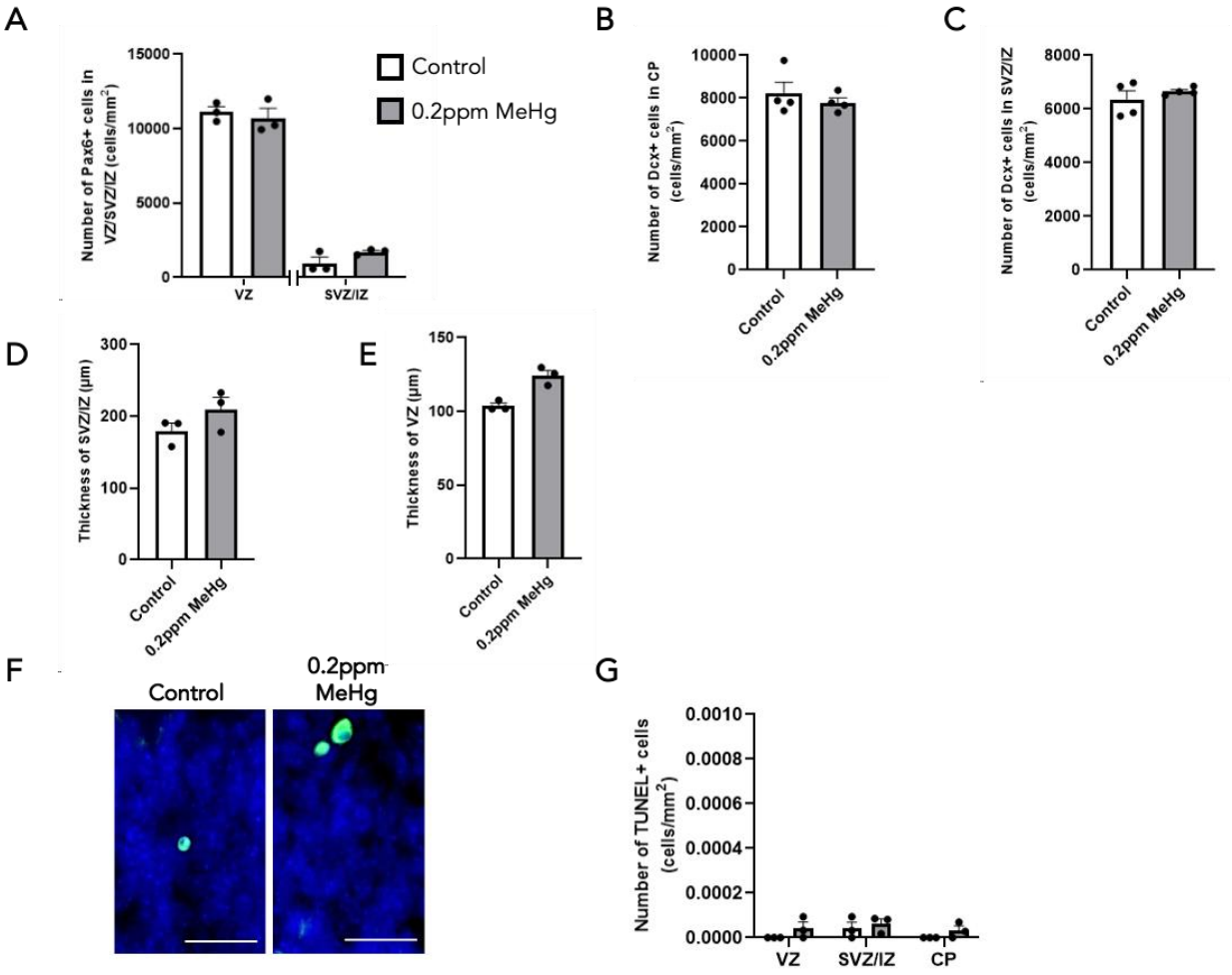

Figure S2. Prenatal MeHg exposure impacts E15 cortex development. Related to Figure 2. (A) Quantitative analysis of the total number of Pax6^{+} cortical precursors within the VZ/SVZ/IZ, determined from sections similar to those shown in (Figure 2B). n=3 embryos/group. (B-C) Quantitative analysis of the number of DCX^{+} immature neurons within CP (B) and SVZ/IZ (C), determined from sections similar to those shown in (Figure 2G). n=4 embryos/group. (D) Quantitative analysis of thickness for the (D) SVZ/IZ and (E) VZ, determined from coronal sections similar to those shown in (Figure 2G), n=3 embryos/group. **Prenatal exposure to 0.2 ppm MeHg did not increase apoptotic cell death in E15 cortex *in vivo.*** (F) Images of E15 cerebral cortex sections from embryos receiving 0ppm and 0.2ppm MeHg treatment were detected for TUNEL+ cells (green), and counterstained for Hoechst (blue). Scale bar: 25 µm. (G) Quantitative analysis of the number of TUNEL+ cells within VZ, SVZ/IZ, and CP, determined from sections similar to those shown in (E). n=3 embryos/group.

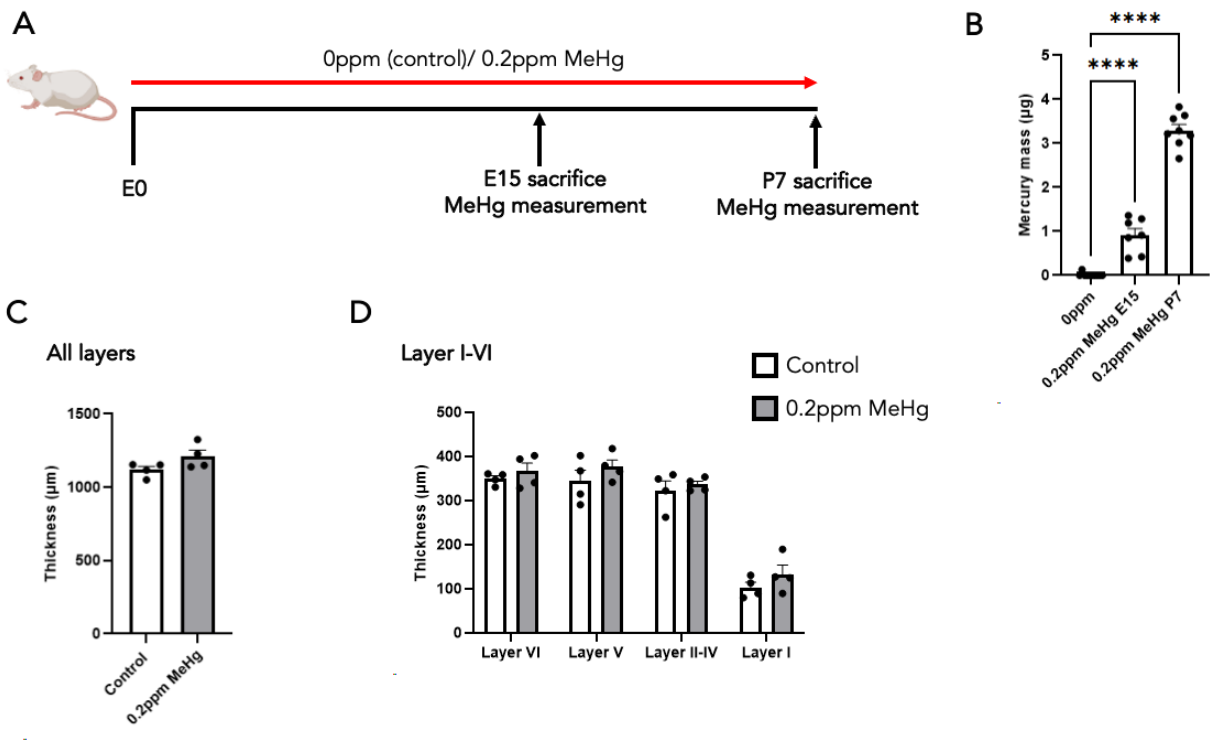

Figure S3. Perinatal MeHg exposure impacts P7 cortex development. **Related to Figure 3.** (A) Schematic of MeHg exposure to pregnant mice, created with BioRender.com. 0ppm (control) and 0.2ppm MeHg were administered through drinking water to pregnant mice starting at E0 until E15 or P7, at which point brains were collected for mercury measurements. (B) Quantitative analysis of mercury mass from hemisphere measurements collected at E15 or P7, n=7-10 embryos/group, one-way ANOVA with post hoc test, ****p<0.0001. (C-D) Quantitative analysis of thickness for all cortical layers (layer I-layer VI), determined from sections similar to those shown in (Figure 3I, L), n=4 embryos/group.

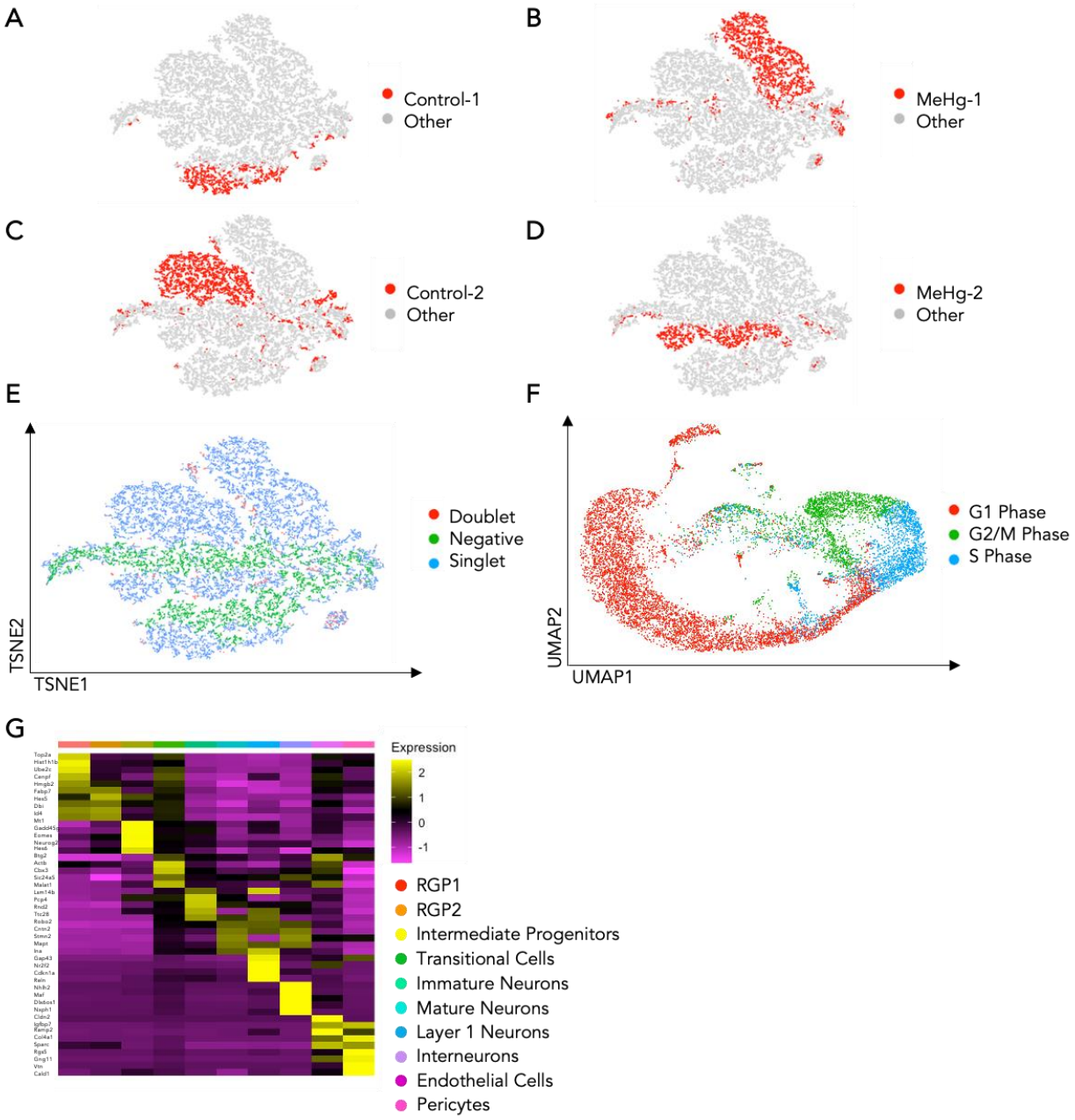

Figure S4. Demultiplexing scRNA-seq dataset from E13.5 cortex. Related to Figure 4. (A-D) T-distributed stochastic neighbor embedding (t-SNE) visualisations, colored by treatment condition, control-1 (0ppm barcode-1) (A), 0.2ppm MeHg barcode-1 (B), control-2 (0ppm barcode-2) (C), 0.2ppm MeHg barcode-2 (D). (E) t-SNE visualisations, colored by singlet, doublet, or negative barcode. (F) Visualization of cells after PCA and UMAP visualisation, colored by cell cycle phase. (G) Heatmaps of top five differentially up-regulated genes relative to all other clusters at E13.5 cortex over ten cell clusters.

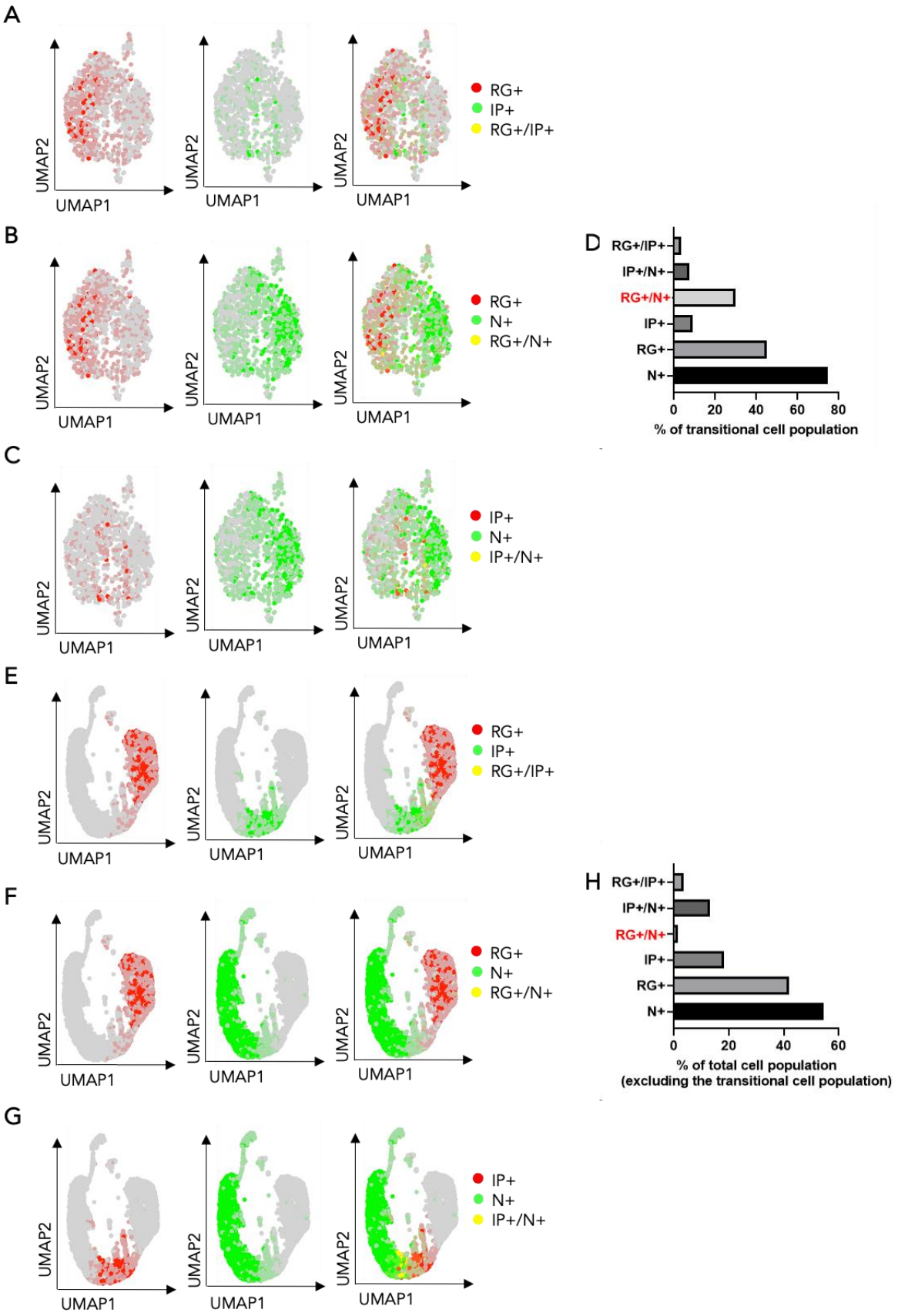
A
UMAP2
UMAP1
RG+
IP+
RG+/IP+
B
RG+
N+
RG+/N+
C
IP+
N+
IP+/N+
D
RG+/IP+
IP+/N+
RG+/N+
IP+
RG+
N+
0
20
40
60
80
% of transitional cell population
E
RG+
IP+
RG+/IP+
F
RG+
N+
RG+/N+
G
IP+
N+
IP+/N+
H
RG+/IP+
IP+/N+
RG+/N+
IP+
RG+
N+
0
20
40
60
% of total cell population
(excluding the transitional cell population)

Figure S5. Asymmetric division of RGP to neurons is dominant in the transitional population. Related to Figure 5. (A-C, E-G) Visualization of cells after PCA and UMAP visualisation colored by co-expression of groups of canonical cell-type markers. Yellow indicates co-expression. (A-C) Transitional cell population. (D) Percentage of cells in the transitional cell cluster displaying co-expression of major cell-type markers. (E-G) Total cell population with transitional population removed. (H) Percentage of cells in the total cell population with transitional population removed displaying co-expression of major cell-type markers.

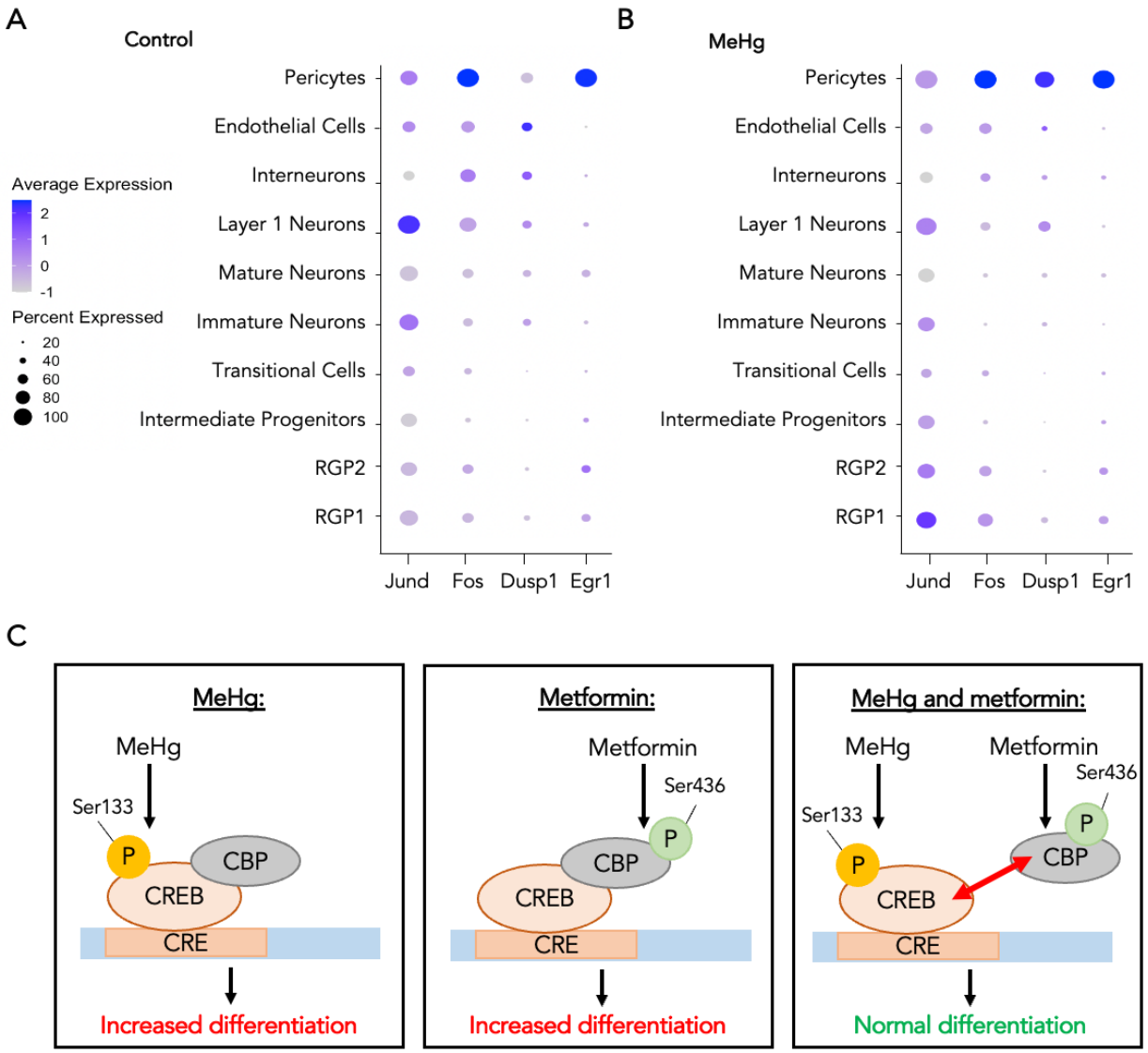

Figure S6. Prenatal MeHg exposure specifically increases CREB target gene expression in radial glial cells. Related to Figure 6. (A-B) Dot plot of CREB target gene (*Jund*, *Fos, Dusp1*, and *Egr1*) expression from scRNA-seq data in pericytes cells, endothelial cells, interneurons, layer 1 neurons, mature neurons, immature neurons, transitional cells, intermediate progenitors, RGP2, and RGP1 cell populations for control (0ppm) (A) and 0.2ppm MeHg (B). (C) Proposed model for MeHg and MeHg+metformin exposure. MeHg alone increases pS133-CREB and represses p-aPKC, which results in increased neuronal differentiation. Metformin alone promotes pS436-CBP, which causes increased neuronal differentiation shown in a previous publication[26]. Co-treatment with MeHg and metformin causes repulsion between pS133-CREB and pS436-CBP, alleviating MeHg-induced neuronal differentiation.

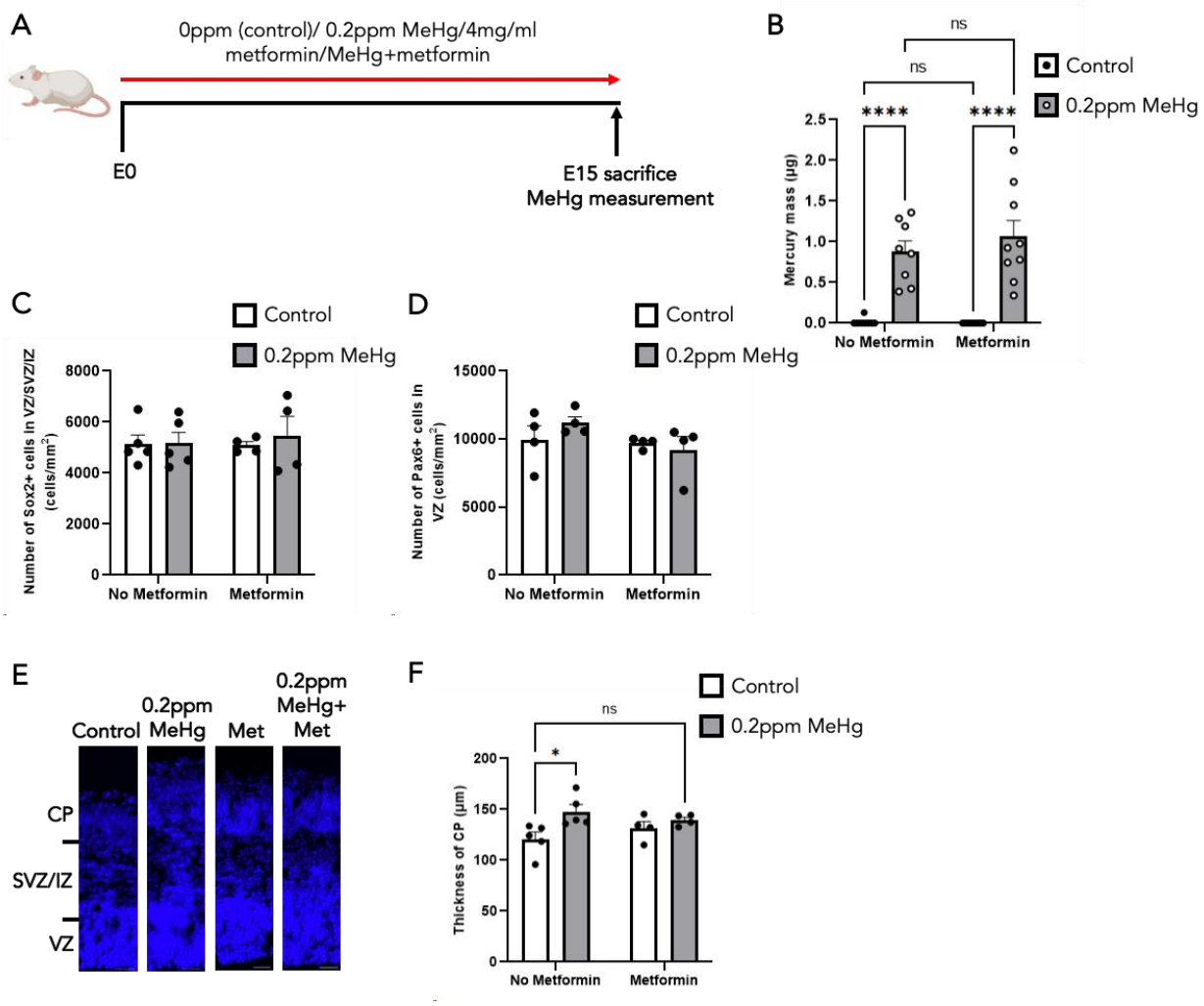

Figure S7. Metformin treatment does not alter MeHg up taken by the brain. Related to Figure 7. (A) Schematic of prenatal exposure of MeHg to pregnant mice, created with BioRender.com. Pregnant mice were exposed to four conditions: i) control (0ppm MeHg + 0µM metformin), ii) 0.2ppm MeHg, iii) 4mg/ml metformin, iv) 0.2ppm MeHg + 4mg/ml metformin administered through drinking water starting at E0 until E15, at which point brains were collected for mercury measurements. **(**B) Quantitative analysis of mercury mass from hemisphere measurements collected at E15, n=8-10 embryos/group, Two-way ANOVA (group × metformin interaction $F(1, 32) = 0.7741$, $P=0.3855$, group $F(1, 32) = 69.74$, $P<0.0001$, metformin $F(1, 32) = 0.5694$, $P=0.4560$) with post hoc test, ****$P<0.0001$, ns (non-significant). **Metformin and MeHg treatments do not alter total cortical precursor populations.** (C) Quantitative analysis of the number of $Sox2^+$ cortical precursors within VZ/SVZ/IZ, determined from sections similar to those shown in (Figure 7L). n=4-5 embryos/group. (D) Quantitative analysis of the number of $Pax6^+$ cortical precursors within VZ, determined from sections similar to those shown in (Figure 7I). n=4 embryos/group. (E-G) Quantitative analysis of thickness for the VZ and CP. (E) Images of E15 cerebral cortex sections from embryos receiving 0ppm and 0.2ppm MeHg treatment, counterstained for Hoechst (blue). Scale bar: 40µm. (F) Two-way ANOVA (MeHg × metformin interaction $F(1, 14) = 2.401$, $P = 0.1436$, MeHg $F(1, 14) = 7.810$, $P = 0.0143$, metformin $F(1, 14) = 0.04262$, $P = 0.8394$, n =18) with post hoc test, *$p<0.05$, ns (non-significant), determined from coronal sections similar to those shown in E.