

Supplementary Material

A single-cell transcriptomic landscape of cadmium-hindered brain development in mice

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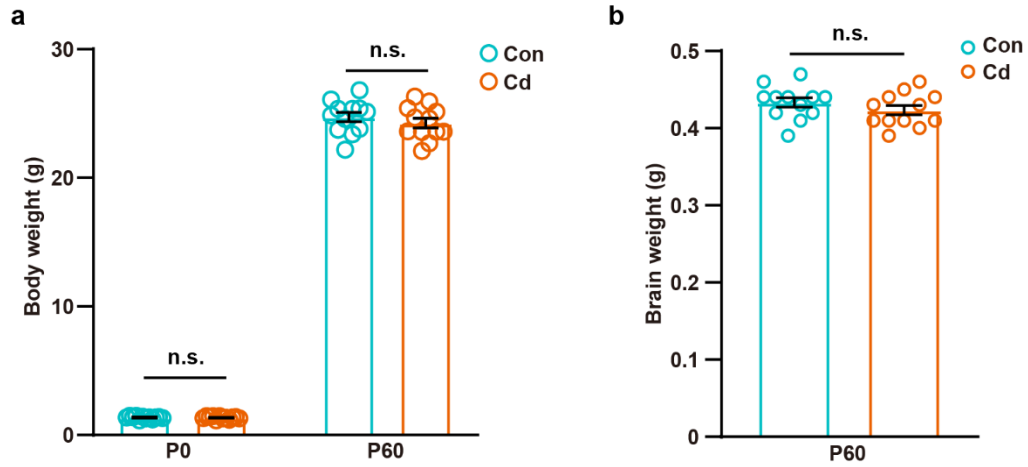
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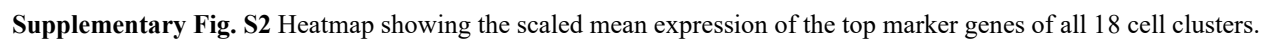
The Supplementary Material includes:

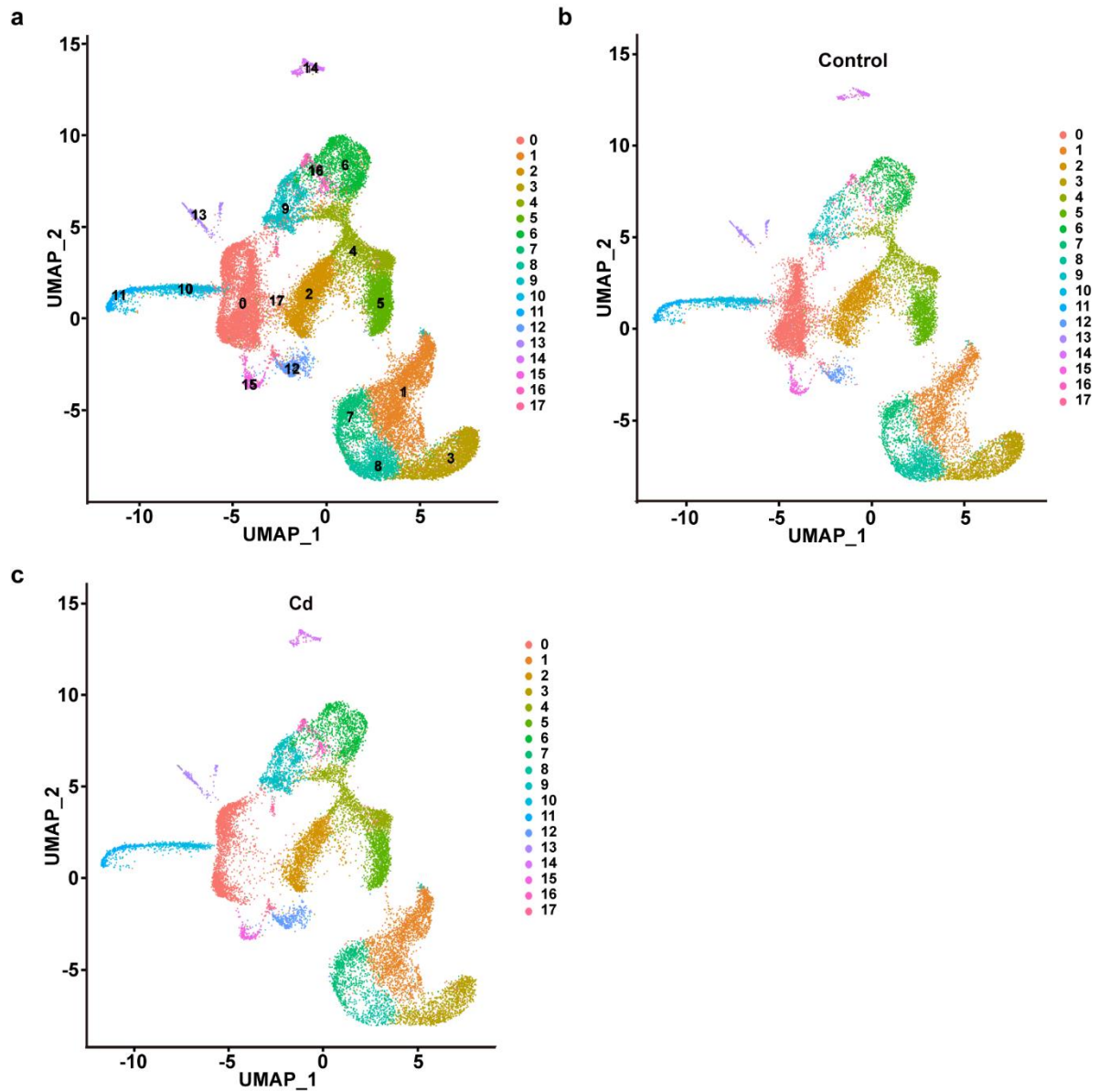
Figure S1 to S9

Table S1 to S8



Supplementary Fig. S1 Body weights and brain weights of the offspring following maternal Cd exposure. **a** The body weights of the offspring at P0 and P60. **b** The brain weights of the offspring at P60. P, postnatal day. Data of 12 offspring from 6 pregnant mice in each group are expressed as the mean $\pm$ SEM. Two-tailed Student's *t* test.

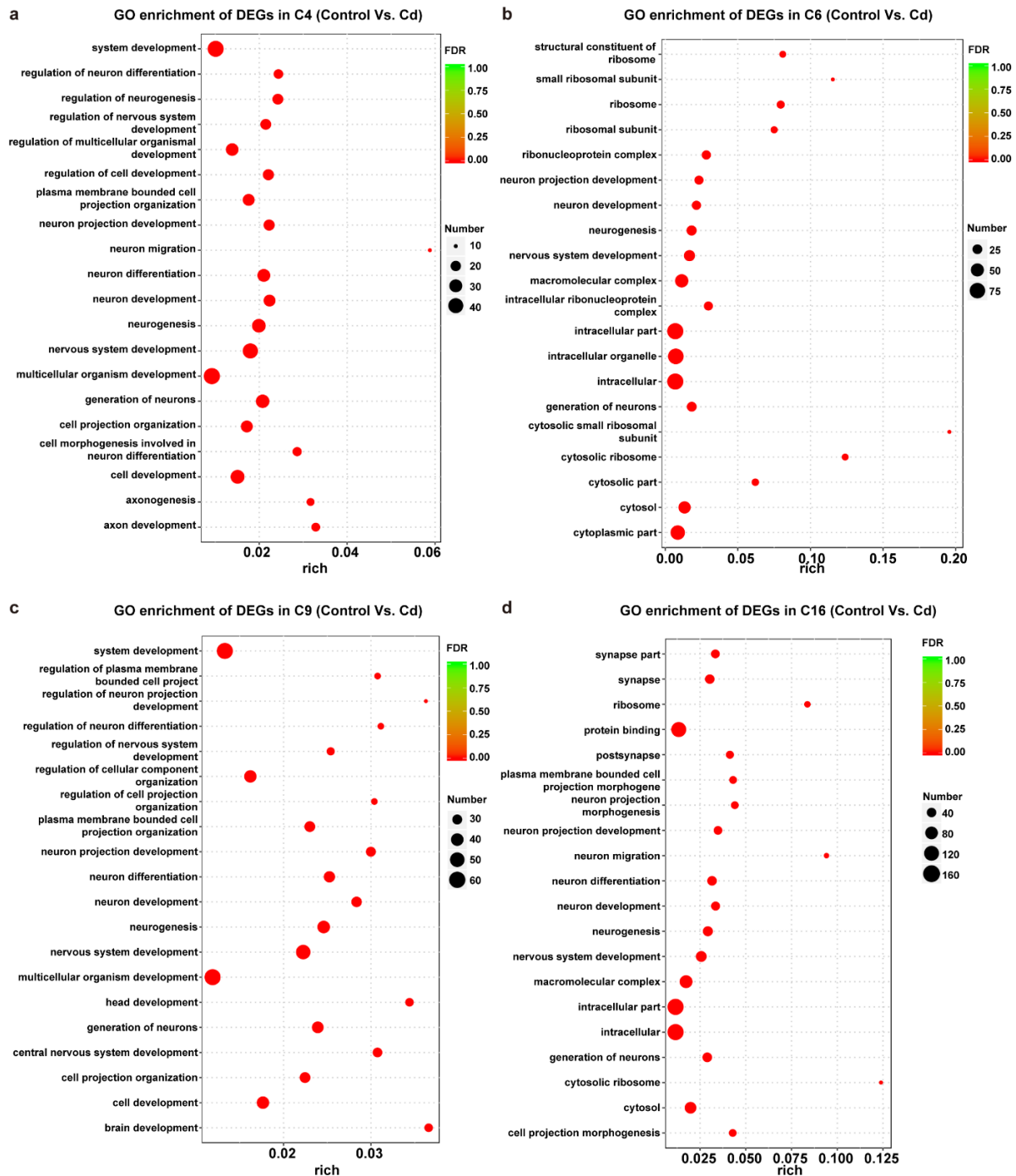




Supplementary Fig. S3 SnRNA-seq analysis of cell clusters in the E15.5 mouse cortex. **a** UMAP plots of 34540 cells from the combination of four independent experimental sets from control and Cd-exposed mice. **b, c** Separated UMAP plots of cells from control (**b**) and Cd- (**c**) exposed mice.

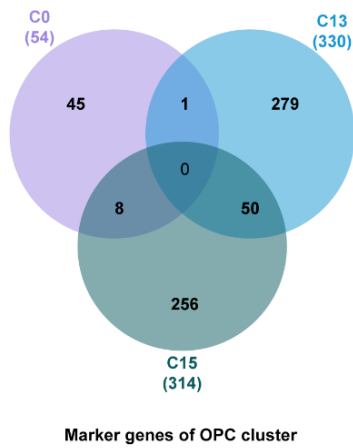


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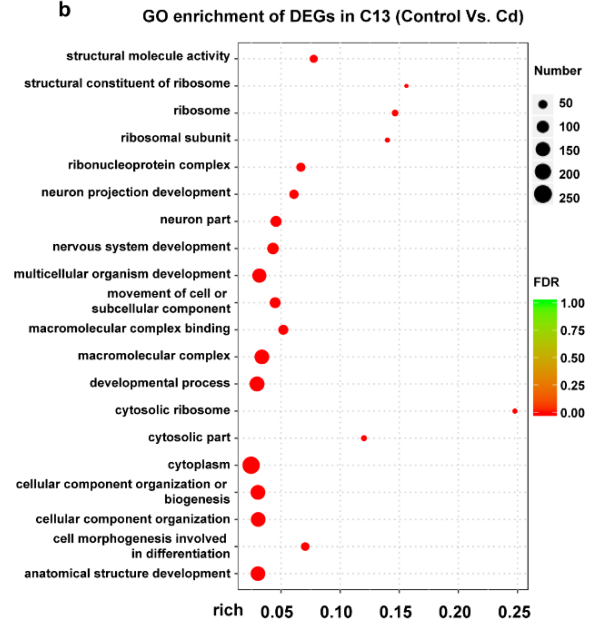


Supplementary Fig. S5 GO analysis of DEGs in the C4, C6, C9, and C16 NPC clusters among control and Cd-exposed mice.

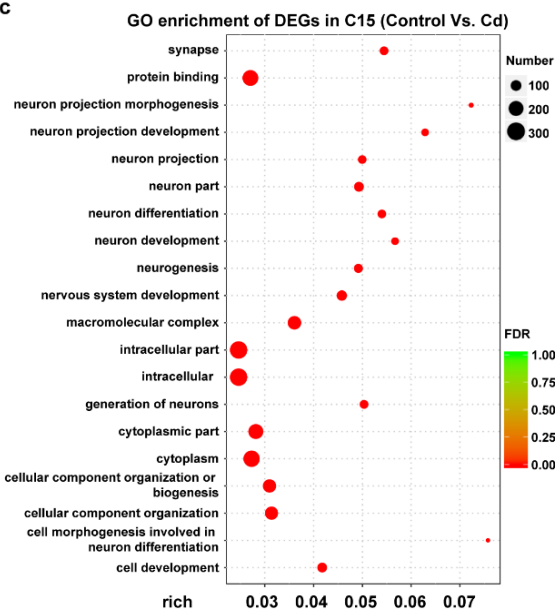
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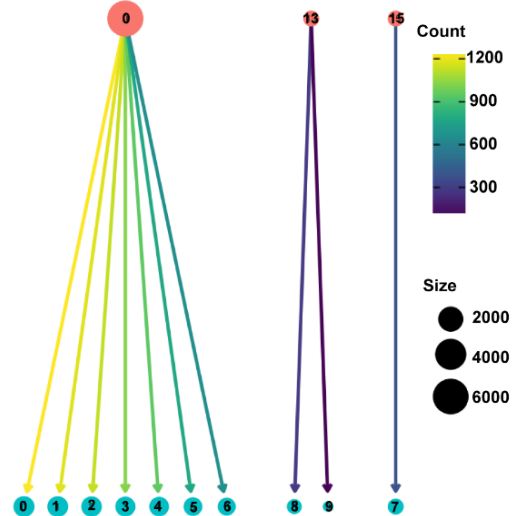
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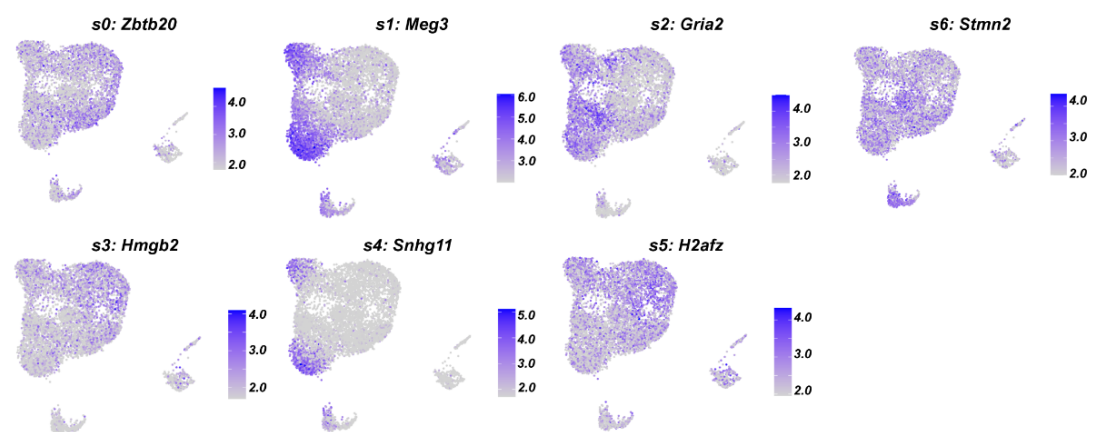
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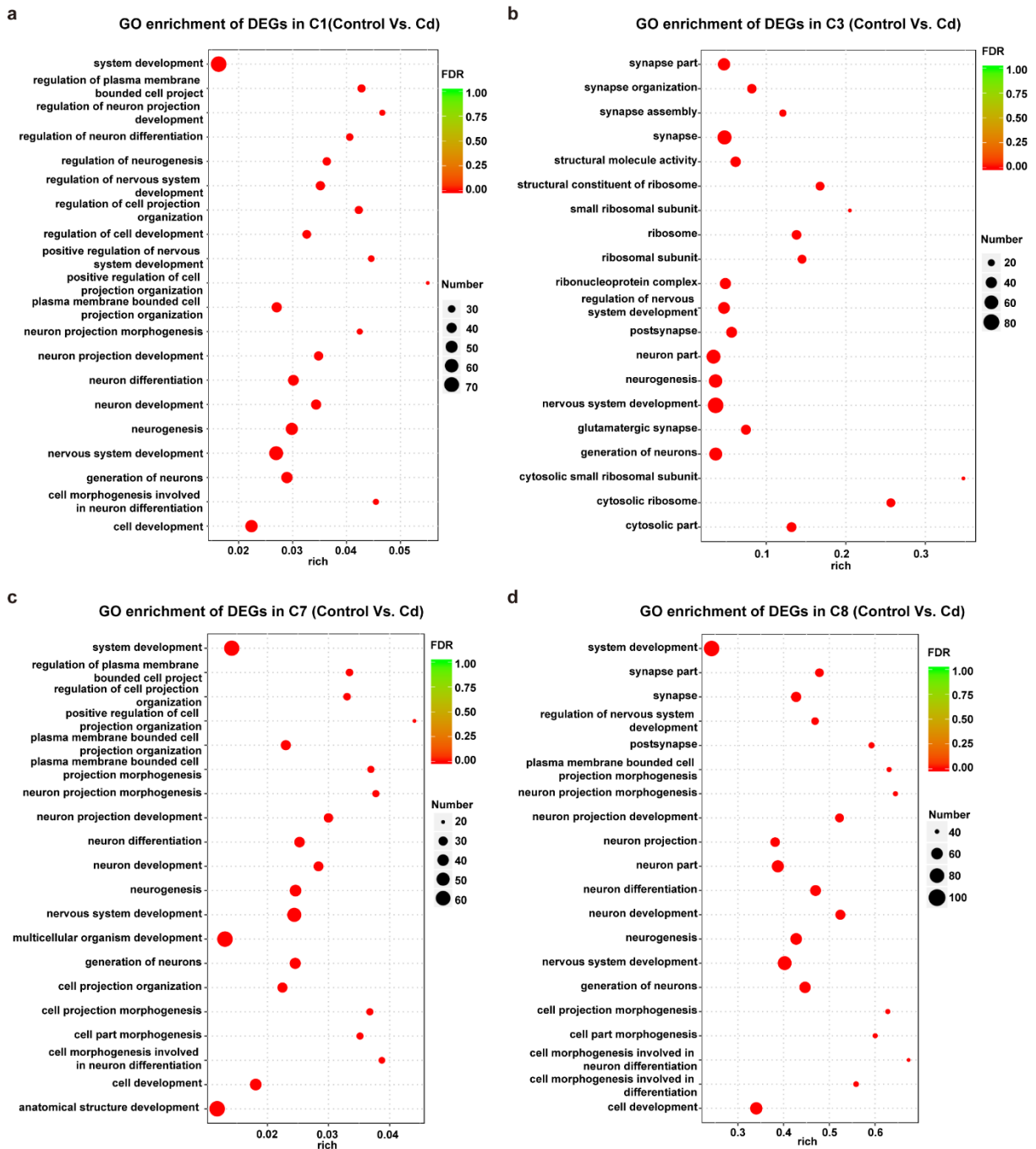
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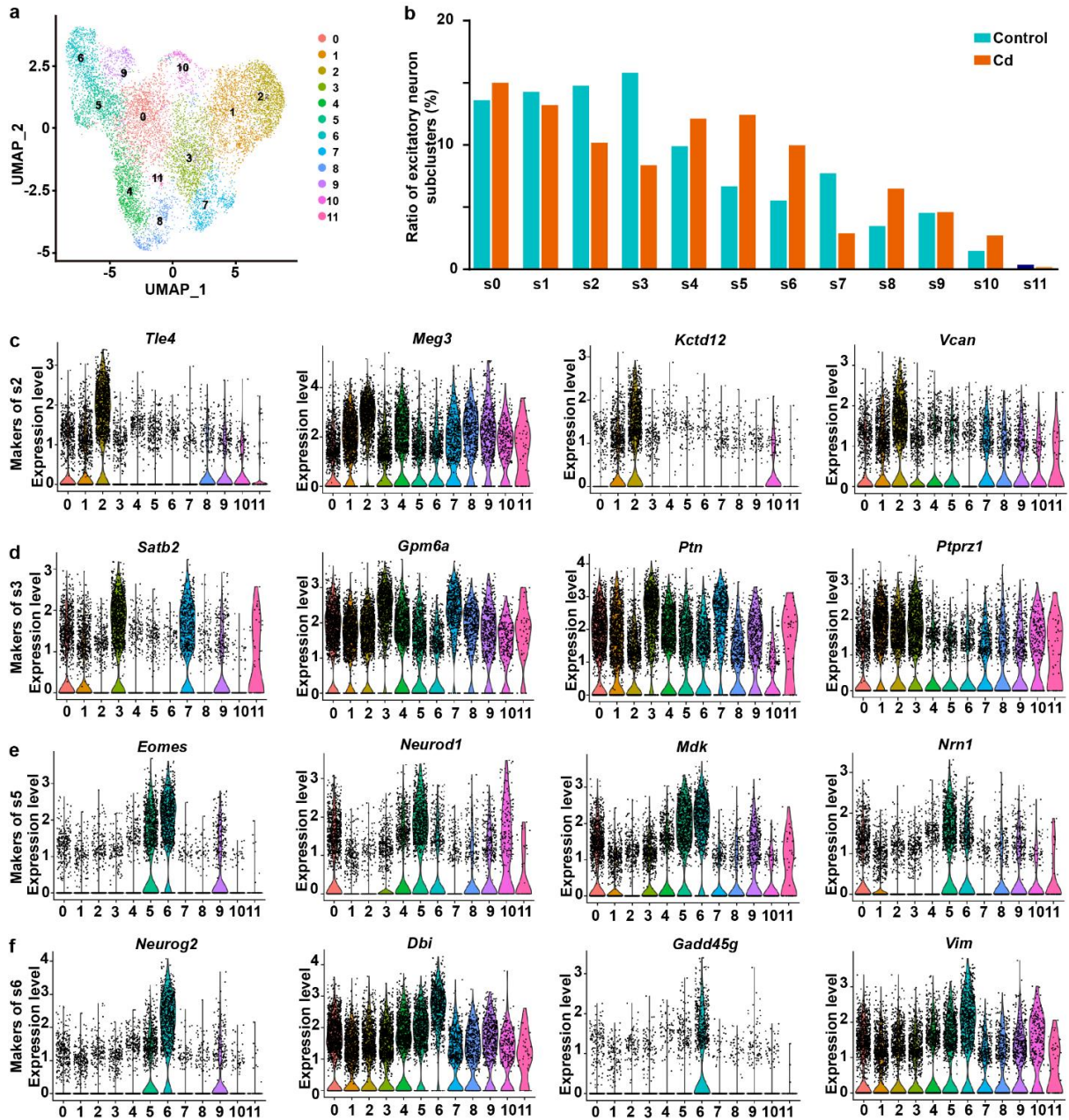
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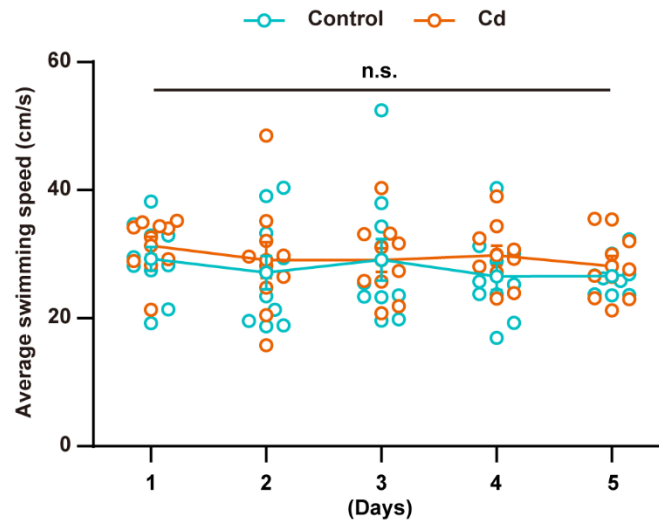
Supplementary Fig. S6 Analysis of the features of OPC clusters. **a** Reclustering of the OPC population. **b** Venn analysis of the marker genes in the three OPC clusters. **c, d** GO analysis of DEGs in the OPC clusters C13 and C15 among control and Cd-exposed mice. **e** Selected feature plots of the top marker genes in the OPC subclusters. S0, subcluster 0; S1, subcluster 1; S2, subcluster 2; S6, subcluster 6; S3, subcluster 3; S4, subcluster 4; S5, subcluster 5.



Supplementary Fig. S7 GO analysis of DEGs in the excitatory neuron clusters C1, C3, C7, and C8 among control and Cd-exposed mice.



Supplementary Fig. S8 Reclustering of the excitatory neuron population. **a** Feature plots showing the distribution of excitatory neuron subclusters. **b** Cell distribution percentages of excitatory neuron subcluster in control and Cd-exposed mice. **c-f** Violin plots showing the expression and distribution of the representative top marker genes in the S2, S3, S5, and S6 excitatory neuron subclusters. S2, subcluster 2; S3, subcluster 3; S5, subcluster 5; S6, subcluster.



Supplementary Fig. S9 The average swimming speed during the training phase of the Morris water maze test in control and Cd-exposed mice. Data of 10 offspring from 6 pregnant mice in each group are expressed as the mean $\pm$ SEM.

Supplementary Table S1 Number and ratio of all cell clusters.

Cell cluster		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
No. of maker gene		54	117	65	262	56	103	352	128	212	471	17	55	205	330	378	314	358	369
Control	No. of cells	4056	1994	1897	1846	1193	1434	940	833	1231	428	841	329	223	182	242	203	131	36
	Ratio	0.225	0.111	0.105	0.102	0.066	0.079	0.052	0.046	0.068	0.024	0.047	0.018	0.012	0.010	0.013	0.011	0.007	0.002
	No. of cells	2874	2311	1653	1213	1502	1227	1264	842	411	1069	501	320	413	218	158	196	237	92
	Ratio	0.174	0.140	0.100	0.074	0.091	0.074	0.077	0.051	0.025	0.065	0.030	0.019	0.025	0.013	0.010	0.012	0.014	0.006

Supplementary Table S2 Markers used in cell population classification.

Cell population (Clusters)	Marker Gene Name	Clusters
NPCs (0, 4, 6, 9, 16)	<i>Pax6</i>	9, 16
	<i>Sfrp1</i>	9, 16
	<i>Sox2</i>	4, 5, 6, 9
	<i>Nestin</i>	X
OPCs (0, 13, 15)	<i>Pdgfra</i>	13
	<i>Olig1</i>	X
	<i>Pmp2</i>	X
	<i>Vcan</i>	3, 15
	<i>Ptprz1</i>	0, 3, 8, 9
	<i>Pax7</i>	X
	<i>Mash1</i>	X
	<i>Neurod2</i>	1, 3, 7, 8
Excitatory neurons (1, 3, 7, 8)	<i>Rbfox1</i>	9, 8
	<i>Slc17a7</i>	X
	<i>Satb2</i>	8
	<i>Camk2a</i>	X
	<i>Neurod6</i>	1, 3, 7, 8
	<i>Pou3f2</i>	1, 8, 16
	<i>Stmn2</i>	3, 7, 8, 12, 15
	<i>Eif1b</i>	7, 8, 16
	<i>Meis2</i>	2, 6
	<i>Gad1</i>	X
Interneurons (2, 5)	<i>Gad2</i>	2, 4, 5, 6
	<i>Pde4dip</i>	5
	<i>Aqp4</i>	X
Astrocytes (12, 17)	<i>Gfap</i>	X
	<i>Aldh1l1</i>	X
	<i>Slco1c1</i>	X
	<i>Slc1a2</i>	12, 17
	<i>Slc4a4</i>	X
	<i>Ptprc</i>	14
Microglia (14)	<i>Iba1</i>	X
	<i>P2ry12</i>	14
	<i>Csf1r</i>	14

X, low expression.

Supplementary Table S3 Typical marker genes and DEGs in NPC cell clusters (control vs. Cd).

Cell clusters	Typical marker genes	Typical DEGs
4	<i>Dlx6os1, Dlx1, Adarb2, Dlx2, Arx, Nrnx3, Ccnd2, Cdca7, Rnd3, Abracl, Sox2, Ascl1, Sp9</i>	<i>Chchd2, Map1b, Mapt, Dynll1, Dcx, Ncam1, Hes5, Ckb, Nfix, Hbb-bs, Ccnd2</i>
6	<i>Hist1h1b, Top2a, Hmgb2, Ube2c, Cenpf, Mki67, Prc1, Pclaf, Tpx2, Cenpa, Sox2, Ascl1</i>	<i>Map1b, Chchd2, Mki67, Dynll1, Mapt, Dcx, Map2, Ncam1, Stmn2, Hes5, Pou2f1</i>
9	<i>Fabp7, Dbi, Vim, Mt3, Ddah1, Hes5, Phgdh, Ptn, H2afz, Hmgb2, Sfrp1, Pax6, Notch1, Hes1, Neurog2</i>	<i>Tnc, Ptporz1, Npm1, Fabp7, H2afz, Slc1a3, Ptma, Rpl35a, Dtna, Naca, Aldh1l1</i>
16	<i>Tpx2, Pcalf, Hmgb2, Cks2, Mki67, H2afz, Ube2c, Top2a, Cenpf, Hist1h1b, Pax6, Sfrp1, Prc1, Lockd</i>	<i>Fau, Dcx, Mapt, Gria2, Rps15a, Gng5, Zbtb20, Ubb, Serf2, Mki67, Pou3f2, Ndufc2</i>

Supplementary Table S4 Typical marker genes and DEGs of the OPC clusters.

Cell clusters	Typical marker genes	Typical DEGs (Control vs. Cd).
0	<i>Lars2, Fabp7, Meg3, Snhg11, Gria2, Kcnq1ot1, Ntm, Hnrnpa2b1, Fus, Gap43, Ptprz1, Map2</i>	<i>H2afz, Hmgb2, Mapt, Ckb, Dcx, Ncam1, Map2, Gap43, Dbi, Meg3, Gria2</i>
13	<i>Dcn, Col3a1, Sparc, Col1a2, Col1a1, Lgals1, Vtn, Apod, Col4a1, Cdkn1c, Serpinh1, Apoe, Akap12, Pdgfra</i>	<i>Chchd2, Mapt, Ncam1, Lgals1, Apod, Dbi, H2afz, Gng5, Gria2, Map1b</i>
15	<i>Reln, Nhlh2, Ndnf, Cacna2d2, Lhx1, Calb2, Emx2, Cdkn1a, Nr2f2, Pcp4, Pdgfra, Vcan</i>	<i>H3f3b, Stmn1, Chchd2, Ndnf, H3f3a, Meg3, Fau, Snhg11, Map1b, Mapt</i>

Supplementary Table S5 Typical marker genes of OPC subclusters.

Cell subclusters	Typical marker genes
0	<i>AY036118, Zbtb20, Gm3764, Qk, Pax6, Top2a, Ptprz1, Slc1a3, Mki67, Pou3f2</i>
1	<i>Snhg11, Meg3, Pfkp, Ntng1, Ntm, Kcnq1ot1, Gria2, Nrnx3, Grm5, Cadps</i>
2	<i>Gria2, Arpp21, Tmem108, Opcml, Ptprd, Mef2c, Zeb2, Tiam2, Khdrbs2, Nin</i>
3	<i>Top2a, Creb5, H2afz, Hmgb2, Pax6, Dbi, Cenpf, Slc1a3, Mki67, Zbtb20</i>
4	<i>Meg3, Malat1, Snhg11, Kcnq1ot1, Sorbs2, Lrfr5, Ube3a, Ptprd, Nrnx3, Tmem108</i>
5	<i>Rpl35a, H2afz, Hmgb2, H2afv, Ckb, Ran, Pkm, Abrac1, Ube2c, Dek</i>
6	<i>Tubb2b, Tmsb4x, Tmsb10, Stmn2, Mllt11, Gm17750, Svbp, Rab3a, Neurod2, Ttr</i>
7	<i>Reln, Nhlh2, Ndnf, Cacna2d2, Lhx1, Emx2, Calb2, Pdgfa, Pcp4, Nr2f2</i>
8	<i>Dcn, Col3a1, Colla2, Lgals1, Colla1, Vtn, Apod, Gja1, Anxa2, S100a11</i>
9	<i>Igfbp7, Cldn5, Egfl7, Flt1, Cd93, Itga1, Slc38a5, Kdr, Higd1b, Ecscr</i>

Supplementary Table S6 Typical marker genes and DEGs of neuron subpopulations.

Cell populations	Cell clusters	Typical marker genes	Typical DEGs (Control vs. Cd).
Excitatory neurons	1	<i>Neurod1, Igfbpl1, Eomes, Rnd2, Neurog2, Lhx2, Nrn1, Sstr2, Unc5d, Neurod6, Neurod2, Pou3f2</i>	<i>Gria2, Chchd2, Pou3f2, Dcx, Ncam1, H2afz, Pou3f3, Mapt, Neurod2, Rbfox1</i>
	3	<i>Sox5, Id2, Fezf2, Csrp2, Neurod6, Rprm, Dab1, Tmem108, Mef2c, Stmn2, Neurod2, Rbfox1</i>	<i>Chchd2, Ssbp2, Meg3, Map1b, Ncam1, Mapt, Gria2, Stmn4, H2afz, Pou3f2, Satb2, Rbfox1</i>
	7	<i>Ppp1r14c, Tfap2d, Fam19a1, Basp1, Adgrl3, Rtn1, Stmn1, Fam19a2, Nr2f1, Neurod2, Eif1b, Stmn2, Neurod6,</i>	<i>Map1b, Gria2, Chchd2, H2afz, Ncam1, Mapt, Dcx, Satb2, Rbfox1, Neurod6, Hmgb2, Pou3f2</i>
	8	<i>Syt4, Satb2, Eif1b, Mef2c, Ttc28, Neurod6, Gpm6a, Id2, Plxna4, Neurod2, Stmn2, Pou3f1, Rbfox1, Pou3f2</i>	<i>Map1b, Mef2c, Gpm6a, Ncam1, Mapt, Satb2, Sox5, Map9, Rbfox1, Pou3f2, Neurod1</i>
Interneurons	2	<i>Ebfl, Isl1, Six3, Foxp1, Zfhx3, Gucylal, Zfp503, Meis2, Bcl11b, Gad2</i>	<i>Dcx, Tsix, Dynll1, H2afz, Ran, Ncam1, Gria2, Mef2c, Gng5, Mapt, Hmgb2, Dbi, Neurod6, Neurod2</i>
	5	<i>Maf, Npy, Arx, Nxph1, Lhx6, Erbb4, Nrnx3, Eph5, Mafb, Pde4dip, Dcx, Gad2</i>	<i>Chchd2, H2afz, Ncam1, Tsix, Mapt, Dcx, H2afv, Mef2c, Meg3, Hmgb2, Ckb, Dbi, Gria2</i>

Supplementary Table S7 Typical marker genes of excitatory neuron subclusters.

Cell subclusters	Typical marker genes
0	<i>Sox11, Nrpl, Igfbpl1, Tcf4, Unc5d, Sema6d, Meis2, Eph5, Rnd2, Insm1, Robo2, Ctnbp2, Frmd4a, Pou3f2</i>
1	<i>Fezf2, Sox5, Ldb2, Dab1, Ssbp2, Ptporz1, Csrp2, Dyncl1l, Opcml, Bcl11b, Stmn2, Neurod6</i>
2	<i>Tle4, Hs3st4, Kctd12, Meg3, Sybu, Sox5, Neto2, Rprm, Can, Tmem108, Stmn2, Mapt, Ephb1</i>
3	<i>Satb2, Tmsb4x, Gpm6a, Ttc28, Ptn, Hs6st2, Rbm24, Ube2ql1, Nrip1, Neurod6, Ptporz1, Pou3f1</i>
4	<i>Tfap2d, Thsd7a, Eph5, Zbtb20, Nr2f1, Adgrl3, Sorbs2, Mir124-2hg, Cdh4, Nr2f2, Sema3c, Meis2</i>
5	<i>Mfap4, Eomes, Neurod1, Mdk, Nrn1, Sstr2, Lhx2, Igsf8, Unc5d, Pcp4, Eph4, Meis2, Hmgb2, Pou3f2</i>
6	<i>Neurog2, Eomes, Mdk, Dbi, Gadd45g, Btg2, Igsf8, Vim, Gng5, Ezr, Hmgb2, Sema5a</i>
7	<i>Gucyl1a1, Mef2c, Syt4, Stmn2, Satb2, Inhba, Syt1, Nr4a2, Ppp1r14c, Eif1b, Map1b, Neurod6, Rbfox1</i>
8	<i>Fam19a1, Nr2f2, Nnat, Gabra2, Fam19a2, Nrp2, Bhlhe22, Nefm, Nefl, Rprm, Gap43, Map1b, Mapt</i>
9	<i>Nrxn3, Dlx6os1, Arx, Maf, Gad2, Sp9, Nxph1, Dlx1, Sox2, Meg3, Eph5, Eph4, Meis2</i>
10	<i>Crym, Nrp2, Snca, Nrpl, Lmo4, Gabra2, Rnf182, Zbtb20, S100a10, Neurod1, Mapt</i>
11	<i>Alas2, Slc25a37, Hba-a2, Hba-a1, Hbb-bt, Hbb-bs, Bpgm, Snca, Epb41, Fbxw7</i>

Supplementary Table S8 Primer sequences for real-time PCR analyses.

Gene	Forward primer	Reverse primer
<i>Pdgfra</i>	GGAGACTCAAGTAACCTTGAC	TCAGTTCTGACGTTGCTTTCAA
<i>Vcan</i>	AGGCGTCTACCGATGTGATGT	TGGCTGCCCTGTAGTGAAAC
<i>Ptprz1</i>	GGAGAAGAACAGAACATCGTCC	TCATTGCTCTGGTAATAGCCCA
<i>Dcx</i>	GACCTGACCCGATCCTTGTCT	TGTTGGCAGATGTCTTTACGTTG
<i>Neurod2</i>	AAGCCAGTGTCTCTTCGTGG	GCCTTGGTCATCTTGCGTTT
<i>Neurod6</i>	TTAACACTACCGTTTGACGAGTC	TGTTTTGGAAAGCTCTCTGGTT
<i>Tubb3</i>	CGCCATGTTTCAGACGCAAG	CTCGGACACCAGGTCGTTCA
<i>Syn1</i>	GCCCAGATGGTTCGACTACAC	TGTTGGTTGTCTACCTTGACCT
<i>Syn2</i>	CAGCATCAACTCTCTGGAGTCT	GGGGTAGTATGTCTGCTCAATGA
<i>Dlg4 (Psd95)</i>	ACCAGAAGAGTATAGCCGATTCG	GGTCTTGTCGTAGTCAAACAGG
<i>Gapdh</i>	ATACGGCTACAGCAACAGGG	GCCTCTCTTGCTCAGTGTCC