Supplementary Information for:

Environmental enrichment ameliorates perinatal brain injury and promotes functional white matter recovery

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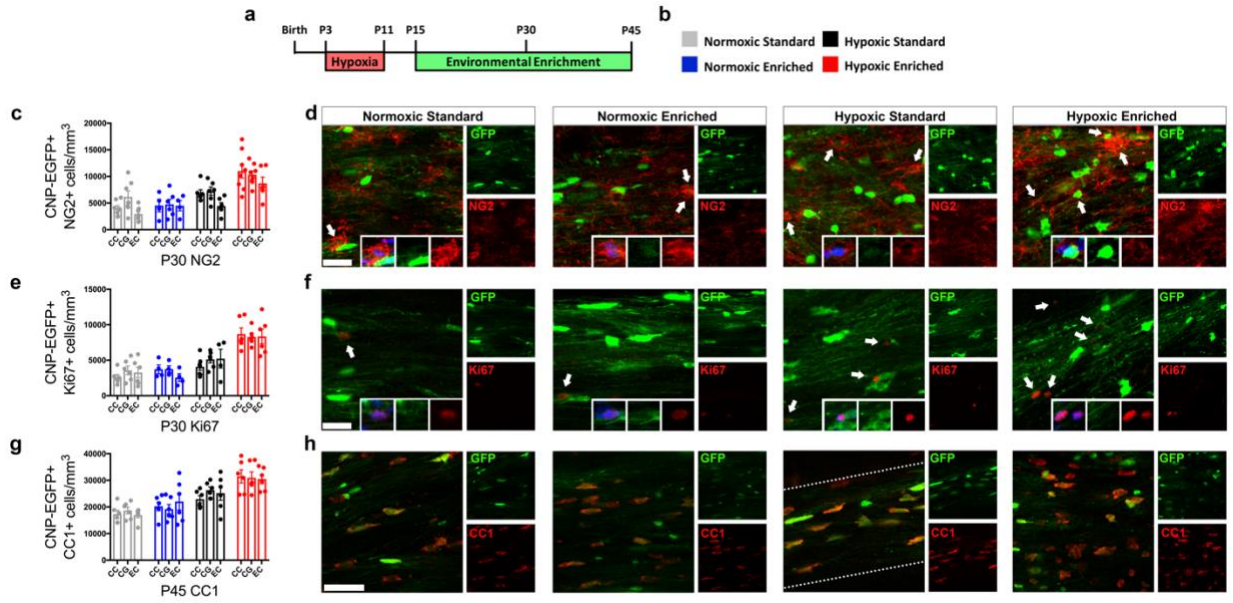
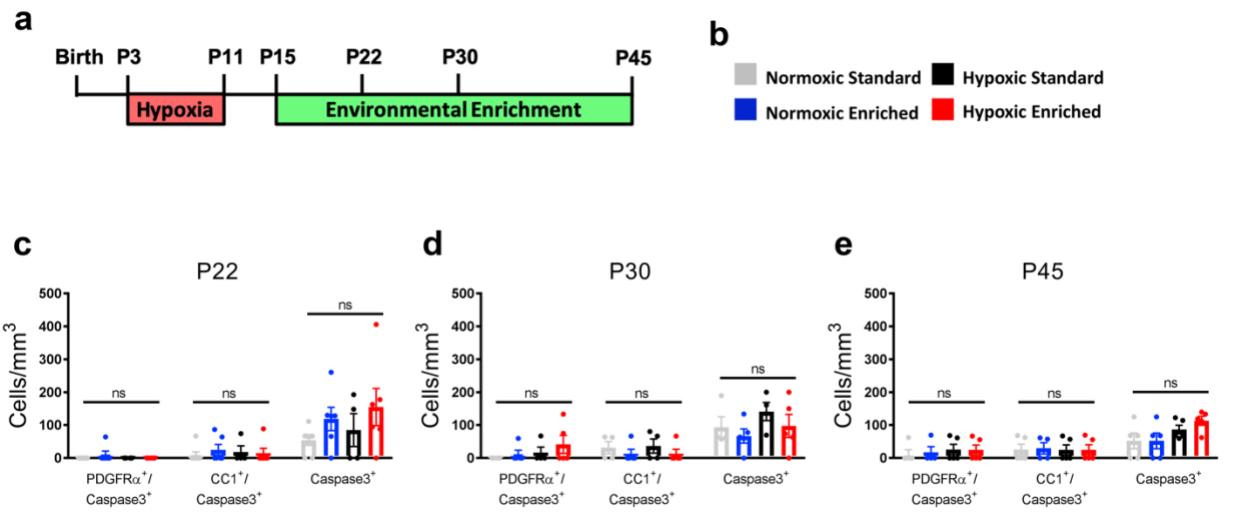
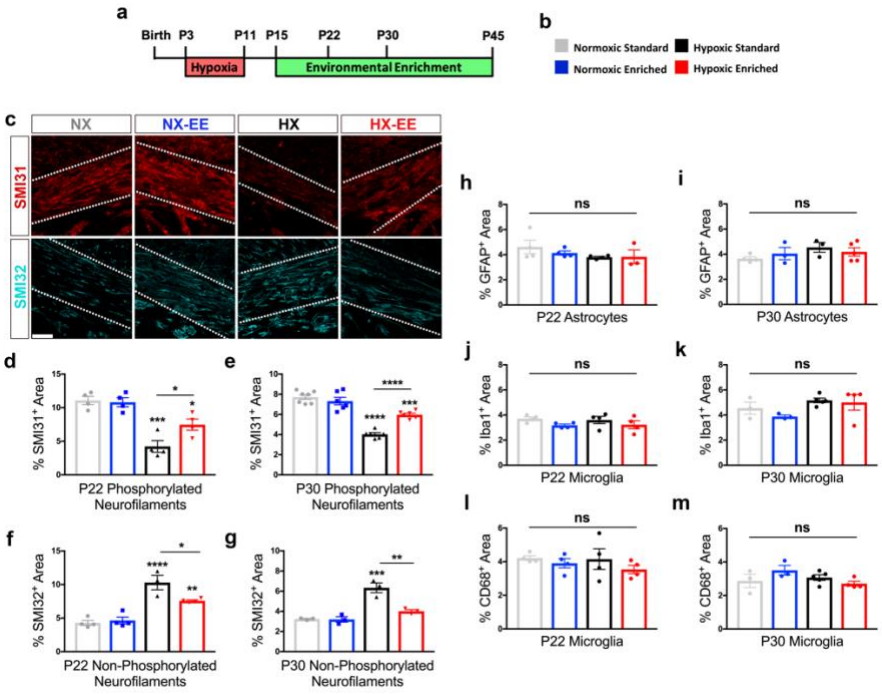


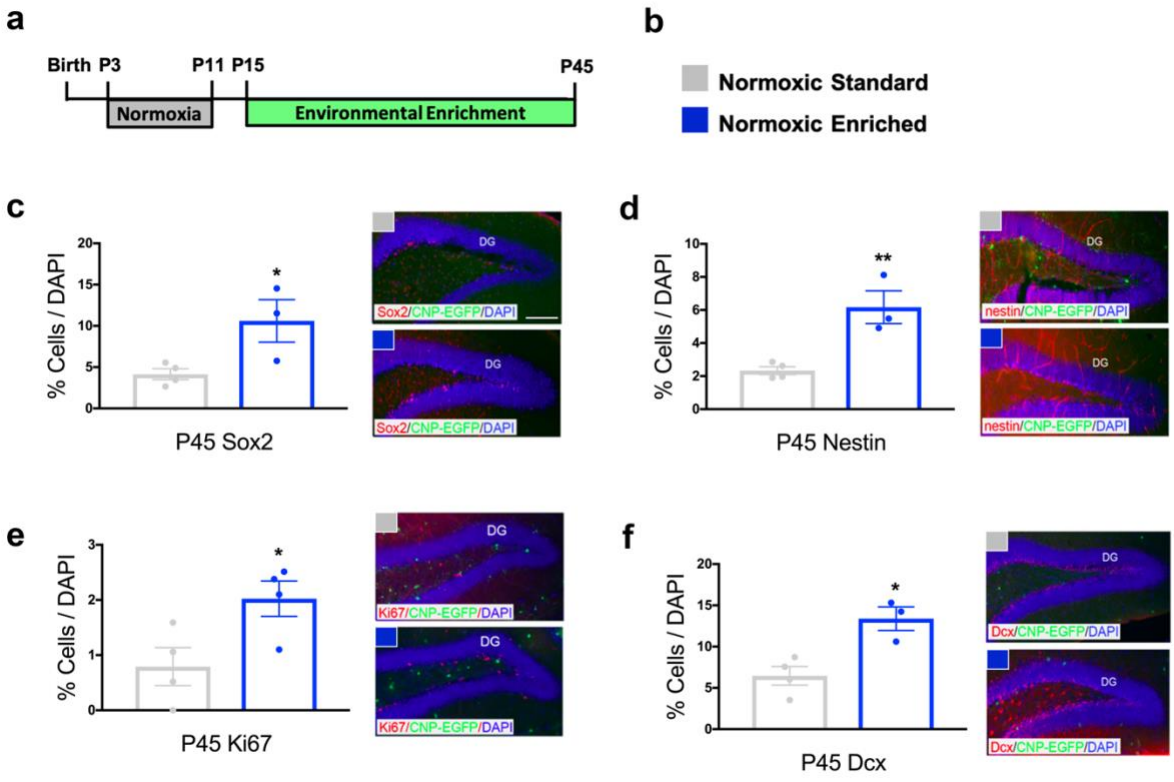
Supplementary Figure 1. Region-specific breakdown and immunohistochemical labeling of OL lineage and proliferation markers from the subcortical WM of CNP-EGFP mice. (a) Experimental paradigm**. (b)** Experimental groups**. (c)** WM region-specific quantification of EGFP+NG2+ OPCs at P30 (CC = corpus callosum, CG = cingulum, EC = external capsule) (NX, *n* = 7; NX-EE, *n* = 6; HX, *n* = 6; HX-EE, *n* = 8). **(d)** Representative images of EGFP- (green) and NG2- (red) expressing OPCs for all groups. Green fluorescence is much reduced in OPCs compared with OL. Arrows and insets are provided to assist in the identification of co-labeled cells (DAPI labeled in blue is included in inset images). Scale bar = 30μm. **(e)** WM region-specific quantification of EGFP+Ki67+ proliferating OPCs at P30 (NX, *n* = 6; NX-EE, *n* = 5; HX, *n* = 7; HX-EE, *n* = 6). **(f)** Representative images of EGFP- (green) and Ki67- (red) expressing proliferating OPCs. Scale bar = 30μm. **(g)** WM region-specific quantification of EGFP+CC1+ OLs at P45 (*n* = 6 for all groups) **(h)** Representative images of EGFP- (green) and CC1- (red) expressing OLs. Scale bar = 50μm. Dotted white lines illustrate the WM/GM border. Data expressed as mean ± SEM. Source data are provided as a Source Data file.



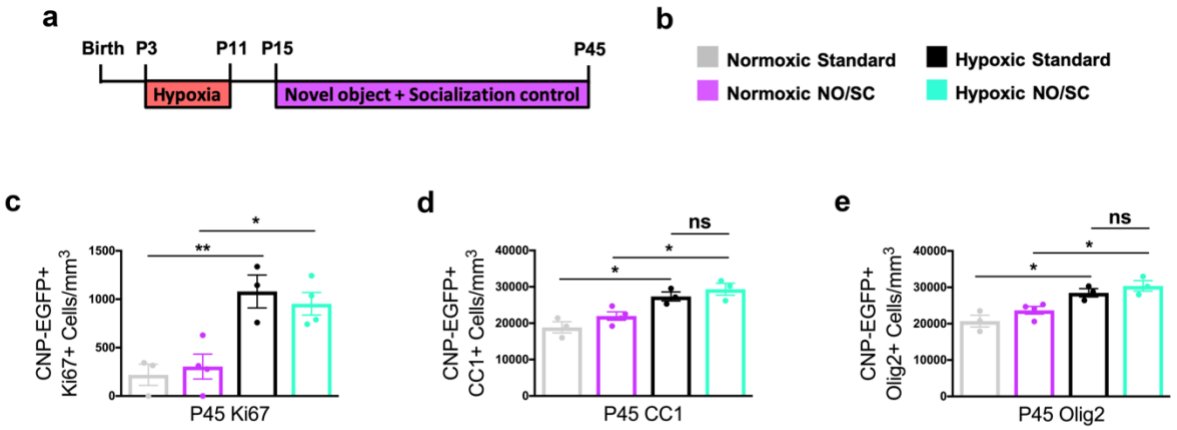
Supplementary Figure 2. Environmental enrichment has no effect on caspase3-mediated apoptosis in the subcortical white matter. (a) Experimental paradigm**. (b)** Experimental groups**. (c-e)** Quantification of PDGFRα+Cleaved Caspase3+, CC1+Cleaved Caspase3+, or total Cleaved Caspase3+ cells at P22 (NX, *n* =7; NX-EE, *n* = 6; HX, *n* = 4; HX-EE, *n* = 6) **(c)**, P30 (NX, *n* =4; NX-EE, *n* = 5; HX, *n* = 4; HX-EE, *n* = 5) **(d)**, and P45 (NX, *n* =5; NX-EE, *n* = 4; HX, *n* = 5; HX-EE, *n* = 5) **(e)**. Data expressed as mean ± SEM. One-way ANOVA followed by a Tukey's post hoc test for multiple comparisons was used to compare differences between groups. Source data are provided as a Source Data file.



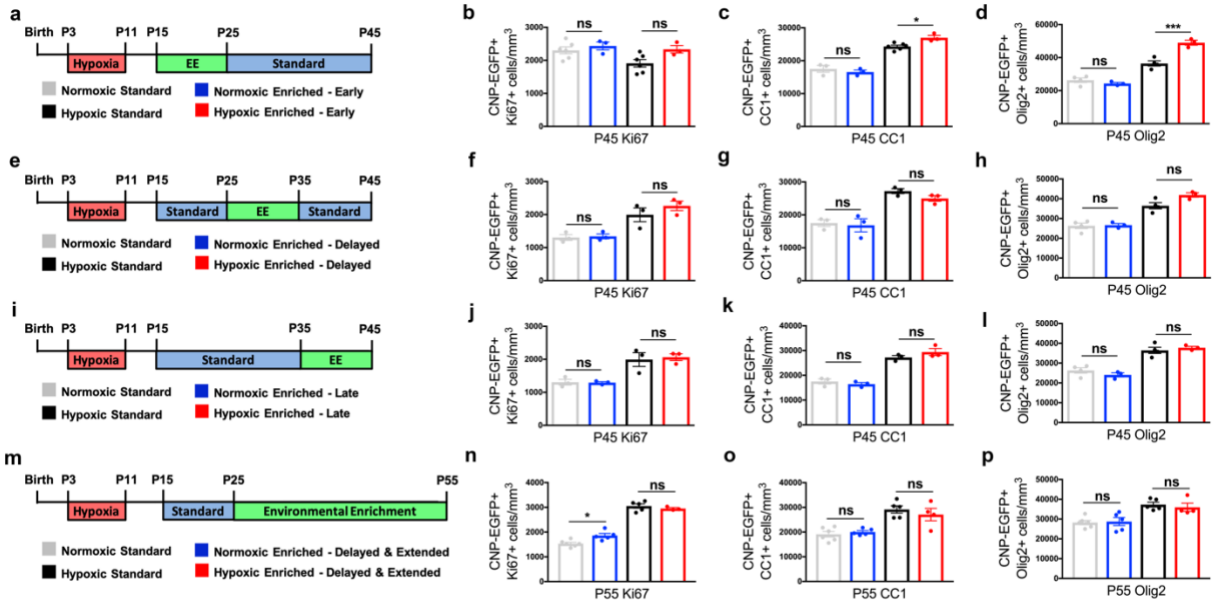
Supplementary Figure 3. Other cell types involved in environmental enrichment-induced recovery from perinatal brain injury. (a) Experimental paradigm**. (b)** Experimental groups**. (c)** Representative images of phosphorylated neurofilaments (SMI31, red) and non-phosphorylated neurofilaments (SMI32, blue) in the subcortical white matter for all groups. Dotted white lines illustrate the WM/GM border. Scale bar = 50µm. **(d-e)** Quantification of phosphorylated neurofilament immunofluorescence (% SMI31+ area) at P22 (*n* = 4 per group) (*** *p* = 0.0002, * *p* = 0.024, * *p* = 0.044) **(d)** and P30 (NX, *n* =7; NX-EE, *n* = 6; HX, *n* = 6; HX-EE, *n* = 6) (*** *p* = 0.0002, **** *p* < 0.0001) **(e)**. **(f-g)** Quantification of non-phosphorylated neurofilament immunofluorescence (% SMI32+ area) at P22 (NX, *n* =4; NX-EE, *n* = 4; HX, *n* = 3; HX-EE, *n* = 4) (**** *p* < 0.0001, ** *p* = 0.004, * *p* = 0.025) **(f)** and P30 (*n* = 3 per group) (*** *p* = 0.0003, ** *p* = 0.002) **(g)**. **(h-i)** Quantification of astrocyte immunofluorescence (% GFAP+ area) at P22 (NX, *n* =4; NX-EE, *n* = 4; HX, *n* = 4; HX-EE, *n* = 3) **(h)** and P30 (NX, *n* =4; NX-EE, *n* = 3; HX, *n* = 3; HX-EE, *n* = 5) **(i)**. **(j-k)** Quantification of microglia immunofluorescence (% IBA1+ area) at P22 (*n* = 4 per group) **(j)** and P30 (NX, *n* =3; NX-EE, *n* = 3; HX, *n* = 5; HX-EE, *n* = 4) **(k)**. **(l-m)** Quantification of phagocytic microglia immunofluorescence (% CD68+ area) at P22 (*n* = 4 per group) **(l)** and P30 (NX, *n* =3; NX-EE, *n* = 3; HX, *n* = 5; HX-EE, *n* = 4) **(m)**. Data expressed as mean ± SEM. One-way ANOVA followed by a Tukey's post hoc test for multiple comparisons was used to compare differences between groups. Source data are provided as a Source Data file.



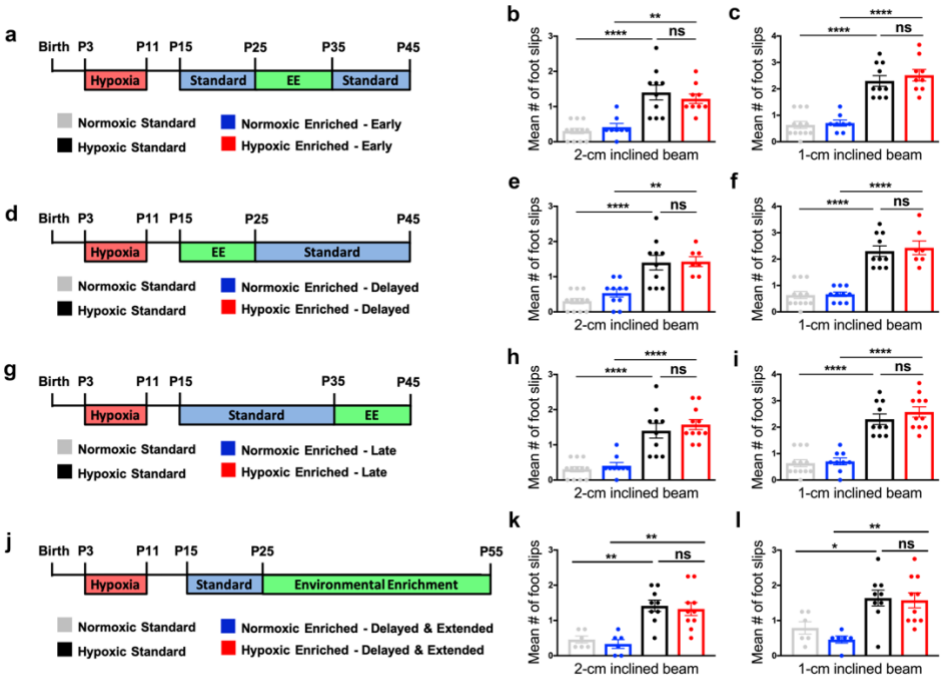
Supplementary Figure 4. Environmental enrichment promotes hippocampal stem cell proliferation and neurogenesis under normal physiological conditions (NX). (a) Experimental paradigm**. (b)** Experimental groups**. (c-f)** Quantification of Sox2+ cells (NX, *n* = 4; NX-EE, *n* = 3; * *p = 0.03*) **(c)**, Nestin+ cells (NX, *n* = 4; NX-EE, *n* = 3; ** *p = 0.007*). Scale bar = 100µm. **(d)**, Ki67+ cells (NX, *n* = 4; NX-EE, *n* = 4; * *p = 0.03*) **(e)**, and Dcx+ cells (NX, *n* = 4; NX-EE, *n* = 3; * *p = 0.01*) **(f)** in the dentate gyrus of the hippocampus at P45. Representative images for each cell population are shown. Data are expressed as the mean ± SEM percentage of cells per total DAPI labeled cells. A two-tailed, unpaired t-test was used to compare differences for each marker. Source data are provided as a Source Data file.



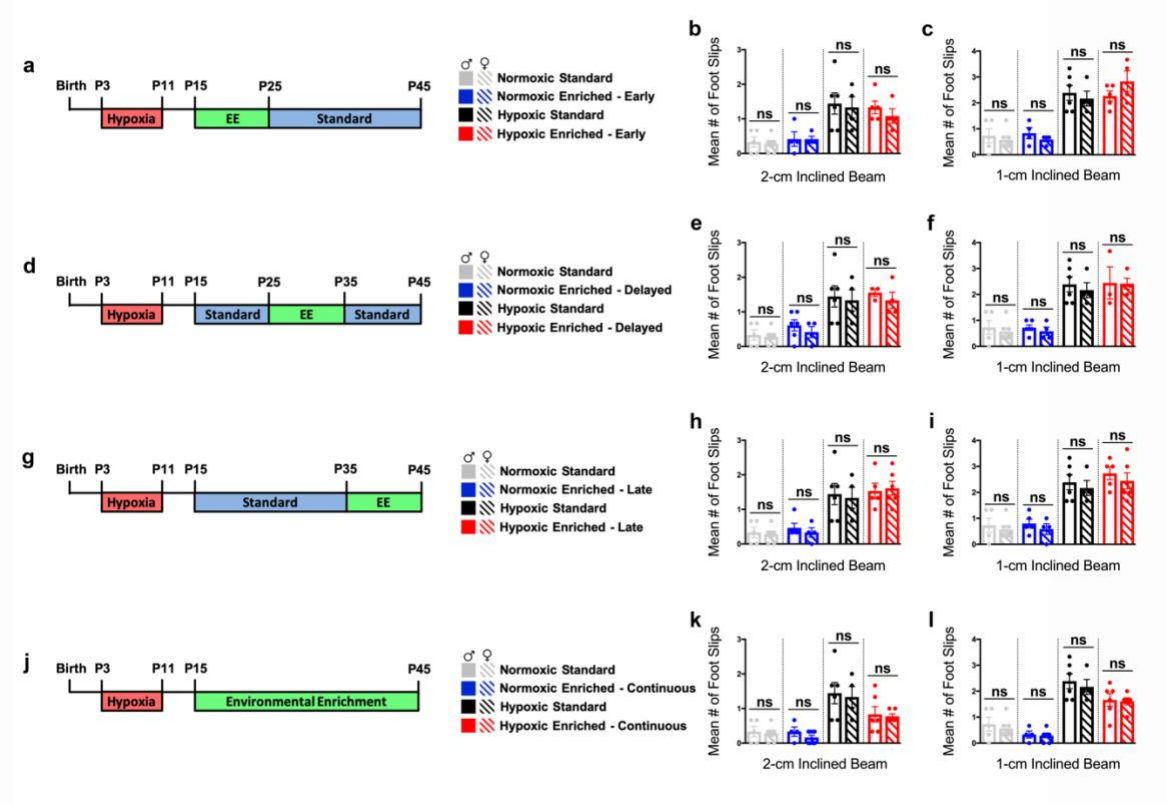
Supplementary Figure 5. Novel object exposure and increased socialization has no effect on OL recovery after perinatal brain injury. (a) Experimental paradigm. Mice were placed in large social groups and exposed to novel objects, without access to a running wheel. **(b)** Experimental groups (NX, NX Novel Object and Socialization Control (NX-NO/SC), HX, HX Novel Object and Socialization Control (HX-NO/SC). **(c)** Quantification of EGFP+Ki67+ cells at P45 (* p = 0.017; ** p = 0.007) (NX, n = 3; NX-NO/SC, n = 4; HX, n = 3; HX NO/SC, n = 4). **(d)** Quantification of EGFP+CC1+ OLs at P45 (From left to right: * p = 0.011, * p = 0.016, ns = not significant) (NX, n = 3; NX-NO/SC, n = 4; HX, n = 3; HX-NO/SC, n = 3). **(e)** Quantification of EGFP+Olig2+ cells at P45 (From left to right: * p = 0.012, * p = 0.019) (NX, n = 3; NX-NO/SC, n = 4; HX, n = 3; HX-NO/SC, n = 3). Data expressed as mean ± SEM. One-way ANOVA followed by a Tukey's post hoc test for multiple comparisons was used to compare differences between groups. Source data are provided as a Source Data file.



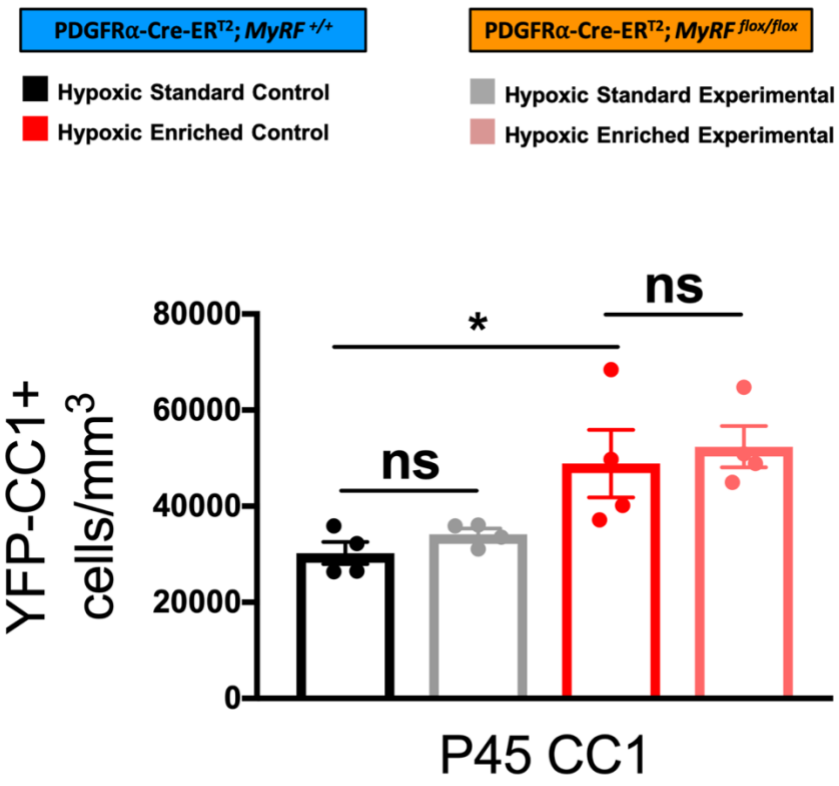
Supplementary Figure 6. Alternative paradigms of non-continuous environmental enrichment did not improve WM recovery after perinatal brain injury. (a) Early intervention paradigm exposed mice to EE from P15-P25 only. **(b)** Quantification of EGFP+Ki67+ OPCs at P45 following early EE (NX, *n* = 6; NX-EE, *n* = 3; HX, *n* = 6; HX-EE, *n* = 3). **(c)** EGFP+CC1+ OLs at P45 following early EE (NX, *n* = 3; NX-EE, *n* = 3; HX, *n* = 6; HX-EE, *n* = 3) (* *p* = 0.04). **(d)** EGFP+Olig2+ cells following early EE (NX, *n* = 4; NX-EE, *n* = 3; HX, *n* = 4; HX-EE, *n* = 3) (*** *p* = 0.0004). **(e)** Delayed intervention paradigm: mice were exposed to EE from P25-P35. **(f)** Quantification of EGFP+Ki67+ OPCs following delayed EE (*n* = 3 per group). **(g)** EGFP+CC1+ OLs following delayed EE (*n* = 3 per group). **(h)** EGFP+Olig2+ cells at P45 following delayed EE (NX, *n* = 4 animals; NX-EE, *n* = 3 animals; HX, *n* = 4 animals; HX-EE, *n* = 3 animals). **(i)** Late intervention paradigm exposed mice to EE from P35 to P45. **(j)** Quantification of EGFP+Ki67+ OPCs following late EE (*n* = 3 per group). **(k)** EGFP+CC1+ OLs following late EE (*n* = 3 per group). **(l)** EGFP+Olig2+ cells at P45 following late EE (*n* = 3 per group). **(m)** Delayed and extended intervention paradigm exposed mice to EE from P25-P55. **(n)** Quantification of EGFP+Ki67+ OPCs at P45 following early EE (NX, *n* = 6; NX-EE, *n* = 5; HX, *n* = 5; HX-EE, *n* = 3) (* *p* = 0.02). **(o)** EGFP+CC1+ OLs at P45 following early EE (NX, *n* = 6; NX-EE, *n* = 5; HX, *n* = 5; HX-EE, *n* = 4). **(p)** EGFP+Olig2+ cells following early EE (NX, *n* = 6; NX-EE, *n* = 5; HX, *n* = 5; HX-EE, *n* = 4). Data expressed as mean ± SEM. One-way ANOVA followed by a Tukey's post hoc test for multiple comparisons was used to compare differences between groups. Source data are provided as a Source Data file.



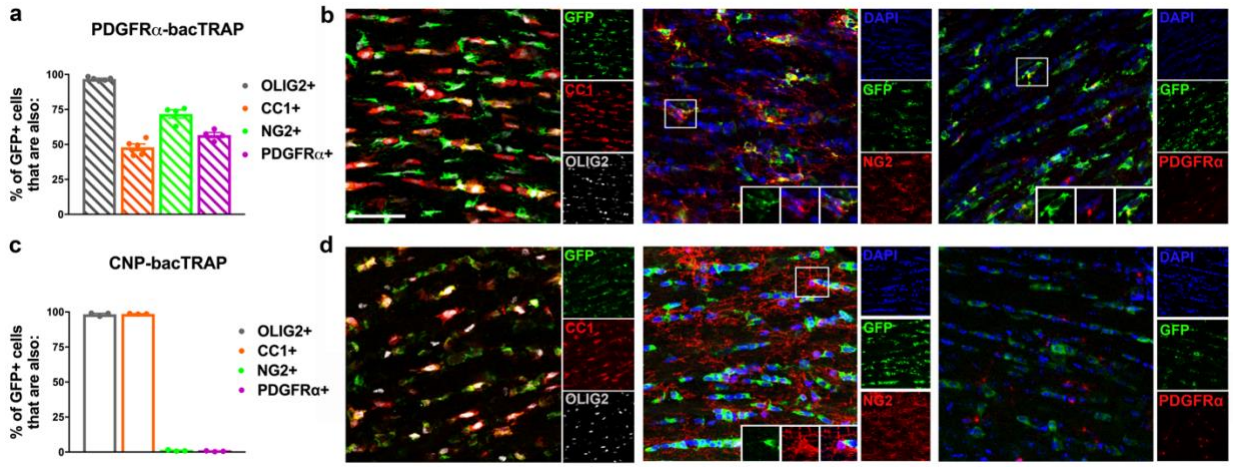
Supplementary Figure 7. Number of foot slips on inclined beam task for alternative paradigms of environmental enrichment. (a) Early intervention paradigm exposed mice to EE from P15-P25 only. **(b-c)** Quantification of foot slips on 2-cm inclined beam (**** $p < 0.0001$, ** $p = 0.003$) **(b)** and 1-cm inclined beam (**** $p < 0.0001$) **(c)** at P45 following early EE (NX, $n = 12$; NX-EE, $n = 8$; HX, $n = 10$; HX-EE, $n = 9$). **(d)** Delayed intervention paradigm: mice were exposed to EE from P25-P35. **(e-f)** Quantification of foot slips on 2-cm inclined beam (**** $p < 0.0001$, ** $p = 0.001$) **(e)** and 1-cm inclined beam (**** $p < 0.0001$) **(f)** at P45 following delayed EE (NX, $n = 12$; NX-EE, $n = 10$; HX, $n = 10$; HX-EE, $n = 7$). **(g)** Late intervention paradigm exposed mice to EE from P35 to P45. **(h-i)** Quantification of foot slips on 2-cm inclined beam (**** $p < 0.0001$) **(h)** and 1-cm inclined beam (**** $p < 0.0001$) **(i)** at P45 following late EE (NX, $n = 12$; NX-EE, $n = 9$; HX, $n = 10$; HX-EE, $n = 11$). **(j)** Delayed and extended intervention paradigm exposed mice to EE from P25-P55. **(k-l)** Quantification of foot slips on 2-cm inclined beam (From left to right: ** $p = 0.003$, ** $p = 0.001$) **(k)** and 1-cm inclined beam (* $p = 0.05$, ** $p = 0.005$) **(l)** at P45 following early EE (NX, $n = 6$; NX-EE, $n = 6$; HX, $n = 9$; HX-EE, $n = 10$). Data expressed as mean ± SEM. One-way ANOVA followed by a Tukey's post hoc test for multiple comparisons was used to compare differences between groups. Source data are provided as a Source Data file.



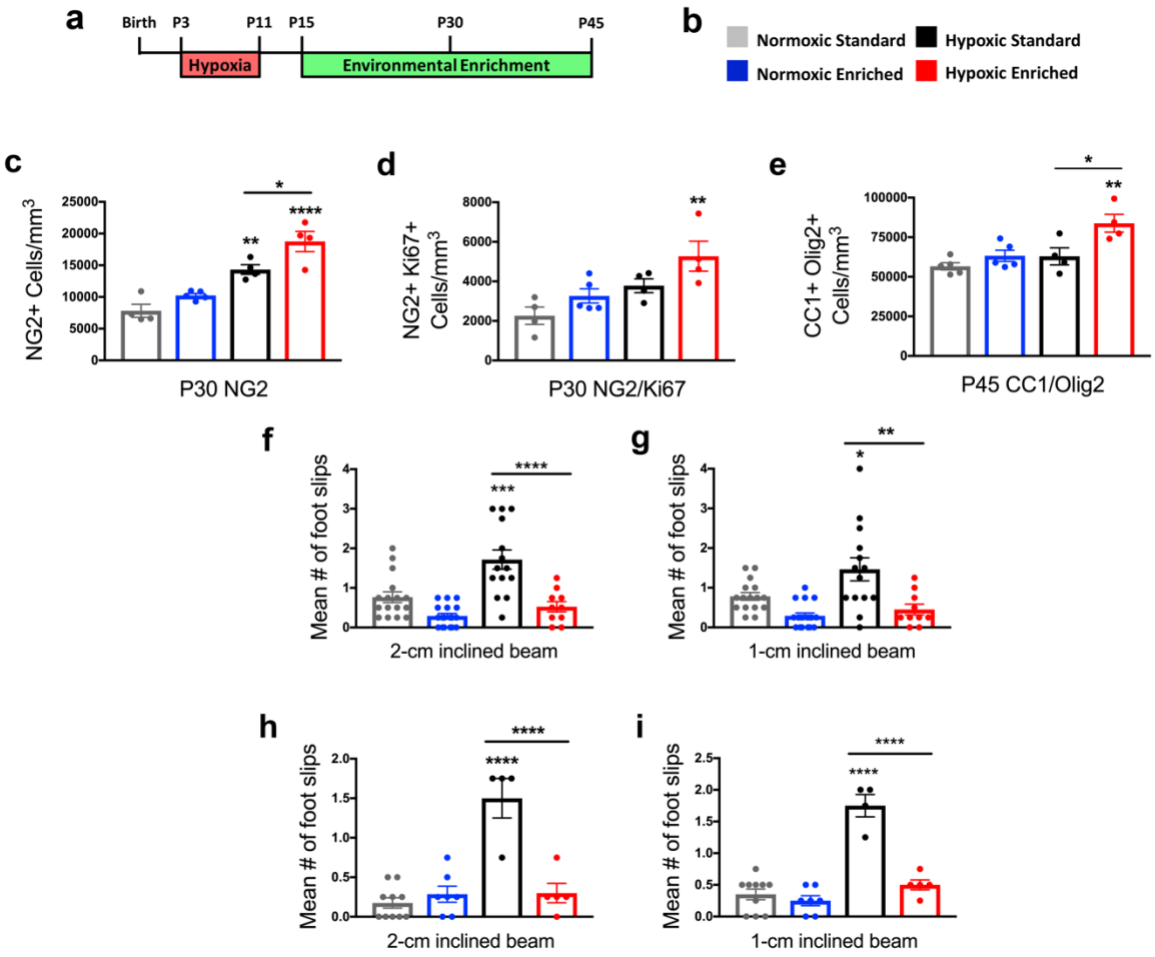
Supplementary Figure 8. Sex comparison of inclined beam task for alternative paradigms of environmental enrichment. (a) Early EE intervention paradigm. **(b-c)** Quantification of foot slips on 2-cm inclined beam **(b)** and 1-cm inclined beam **(c)** following early EE, with males and females analyzed separately (NX, *n* = 12 (7 females, 5 males); NX-EE, *n* = 8 (4 females, 4 males); HX, *n* = 10 (4 females, 6 males); HX-EE, *n* = 9 (4 females, 5 males)). **(d)** Delayed EE intervention paradigm. **(e-f)** Quantification of foot slips on 2-cm inclined beam **(e)** and 1-cm inclined beam **(f)** following delayed EE, with males and females analyzed separately (NX, *n* = 12 (7 females, 5 males); NX-EE, *n* = 10 (4 females, 6 males); HX, *n* = 10 (4 females, 6 males); HX-EE, *n* = 7 (4 females, 3 males)). **(g)** Late EE intervention paradigm. **(h-i)** Quantification of foot slips on 2-cm inclined beam **(h)** and 1-cm inclined beam **(i)** following late EE, with males and females analyzed separately (NX, *n* = 12 (7 females, 5 males); NX-EE, *n* = 9 (4 females, 5 males); HX, *n* = 10 (4 females, 6 males); HX-EE, *n* = 11 (6 females, 5 males)). **(j)** Continuous EE intervention paradigm. **(k-l)** Quantification of foot slips on 2-cm inclined beam **(k)** and 1-cm inclined beam **(l)** following continuous EE, with males and females analyzed separately (NX, n = 12 (7 females, 5 males); NX-EE, n = 12 (8 females, 4 males); HX, n = 10 (4 females, 6 males); HX-EE, n = 12 (6 females, 6 males)). Data expressed as mean ± SEM. One-way ANOVA followed by a Tukey's post hoc test for multiple comparisons was used to compare differences between groups. Source data are provided as a Source Data file.



Supplementary Figure 9. Quantification of total YFP(-) CC1(+) OLs at P45. *n* = 4 mice per group. * *p* = 0.044; ns = not significant. Data expressed as mean ± SEM. One-way ANOVA followed by a Tukey's post hoc test for multiple comparisons was used to compare differences between groups. Source data are provided as a Source Data file.



Supplementary Figure 10. Characterization of oligodendrocyte lineage cells in PDGFRα-bacTRAP and CNP-bacTRAP mice. (a) Characterization of *PDGFRα-bacTRAP* mouse subcortical white matter. Data represent the mean ± SEM of GFP-expressing cells that also express: Olig2 (OL-lineage cells; *n* = 5), CC1 (OLs; *n* = 5), NG2 (OPCs; *n* = 4), or PDGFRα (OPCs; *n* = 4). **(b)** Representative images of corpus callosum from *PDGFRα-bacTRAP* mice immunohistochemically labeled for: GFP (PDGFRα$_+$-OPC ribosomes; green), CC1 (red), and Olig2 (white), DAPI (nuclei; blue), GFP (green), and NG2 (red), or DAPI (nuclei; blue), GFP (green), and PDGFRα (red). Inset images show a magnified view of GFP colocalization with NG2 or PDGFRα-expressing cells. Immunohistochemical labeling was performed once using established protocols. **(c)** Characterization of *CNP-bacTRAP* mouse subcortical white matter. Data represent the mean ± SEM of GFP-expressing cells that also express: OLIG2 (OL-lineage cells), CC1 (OLs), NG2 (OPCs), or PDGFRα (OPCs; *n* = 3 mice). **(d)** Representative images of corpus callosum from *CNP-bacTRAP* mice immunohistochemically labeled for: GFP (CNP$_+$-oligodendrocyte ribosomes; green), CC1 (red), and OLIG2 (white), DAPI (nuclei; blue), GFP (green), and NG2 (red), or DAPI (nuclei; blue), GFP (green), and PDGFRα (red). Inset images show a magnified view of an NG2-expressing cell, juxtaposed to (but not co-localized to) a GFP-expressing cell. Immunohistochemical labeling was performed once using established protocols. Scale bar = 50μm. Source data are provided as a Source Data file.



Supplementary Figure 11. Replication of major findings in CNP-bacTRAP and PDGFRα-bacTRAP mice. (a) Experimental paradigm. *CNP-bacTRAP* and *PDGFRα-bacTRAP* mice were exposed to hypoxic conditions (HX) from postnatal day 3 (P3) to P11, followed by environmental enrichment (EE) from P15 to P45. Mice were sacrificed for analysis at P30 and P45. **(b)** Experimental groups. **(c)** Quantification of NG2+ OPCs at P30 in *CNP-bacTRAP* mice (* $p = 0.04$, ** $p = 0.003$, **** $p < 0.0001$) (NX, $n = 4$; NX-EE, $n = 5$; HX, $n = 4$; HX-EE, $n = 4$). **(d)** Quantification of NG2+Ki67+ proliferating OPCs at P30 in *CNP-bacTRAP* mice (** $p = 0.005$) (NX, $n = 4$; NX-EE, $n = 5$; HX, $n = 4$; HX-EE, $n = 4$). **(e)** Quantification of CC1+OLIG2+ OLs at P45 in *CNP-bacTRAP* mice (* $p = 0.02$, ** $p = 0.002$) (NX, $n = 5$; NX-EE, $n = 5$; HX, $n = 4$; HX-EE, $n = 4$). **(f-g)** Quantification of foot slips in *CNP-bacTRAP* mice for 2-cm beam (*** $p = 0.0002$, **** $p < 0.0001$) **(f)**, and 1-cm beam (* $p = 0.02$, ** $p = 0.001$) **(g)** at P45 (NX, $n = 16$; NX-EE, $n = 18$; HX, $n = 14$; HX-EE, $n = 10$). **(h-i)** Quantification of foot slips in *PDGFRα-bacTRAP* for 2-cm beam **(h)**, and 1-cm beam **(i)** at P45 (**** $p < 0.0001$) (NX, $n = 10$; NX-EE, $n = 7$; HX, $n = 4$; HX-EE, $n = 5$). Data expressed as mean ± SEM. One-way ANOVA followed by a Tukey's post hoc test for

multiple comparisons was used to compare differences between groups. Source data are provided as a Source Data file.