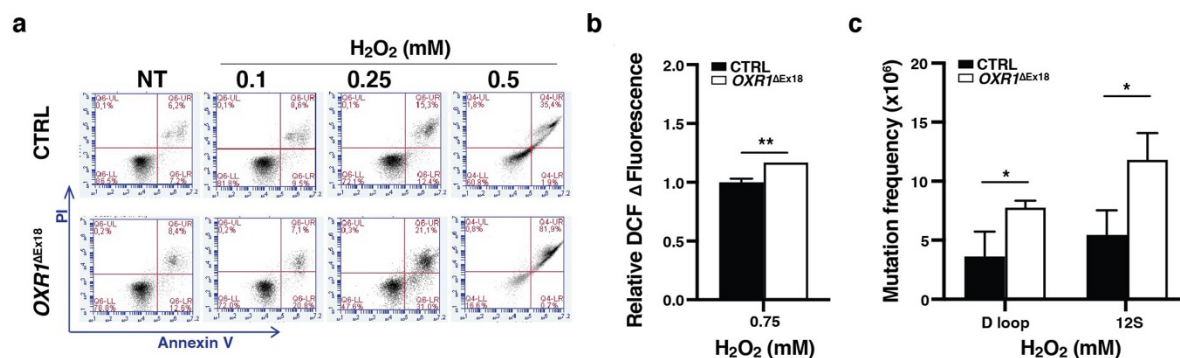


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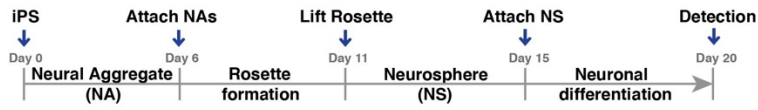
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 GGA AGA AGC CAT TCT TGT AAA ACG TTT GGG AAT CGT ACA CTT TCT AAG AAG GAA GAT
 TTC TTT ATC CAA GAT ATT GAA ATC TGG GCT TTT GAA TAA

Supplementary Figure 1. Donor splice site mutation in the *OXR1* gene causes TLDC domain depletion. **a**, PCR amplified cDNA fragment spanning exon 17-19. The shorter (185bp) product in the patient sample indicates the deletion of exon 18. **b**, The absence of exon 18 results in an open reading frame shift and consequently a premature stop codon “TGA” in exon 19. The underlined part is exon 18, while the rest is exon 19.

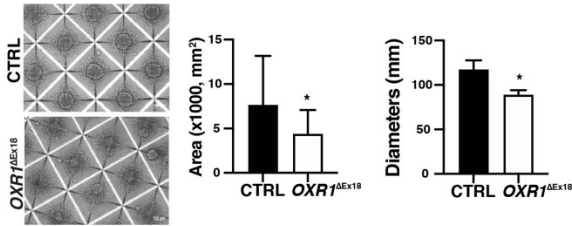


Supplementary Figure 2. Loss of OXR1 increases cell death, general ROS level and mitochondrial DNA mutation frequency in lymphoblasts (LB). **a**, Representative results of FITC-Annexin V/PI double staining by flow cytometry (FCM). **b**, Elevated amounts of ROS generated in patient LB cells (*OXR1*^{ΔEx18}) after H₂O₂ exposure, as compared with the changes in control (CTRL). Intracellular ROS level was measured by DCFDA staining via FCM analysis. **c**, Increased mitochondrial DNA mutation frequency in D-loop and 12S regions in *OXR1*^{ΔEx18} under normal culturing condition. **b-c**, n=3 independent experiments, * $p < 0.05$, ** $p < 0.01$, *t*-test.

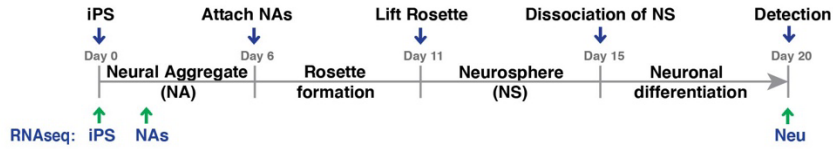
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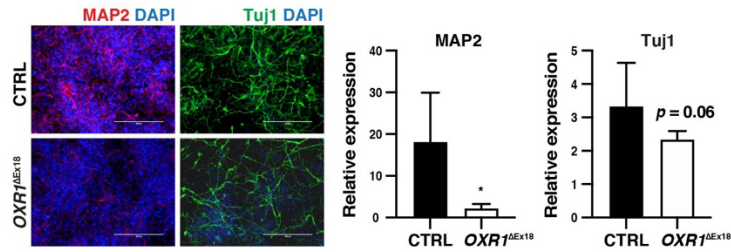
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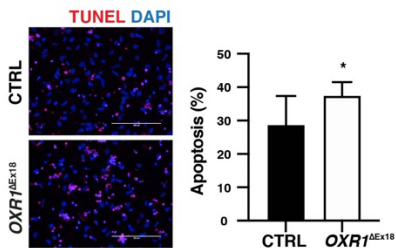
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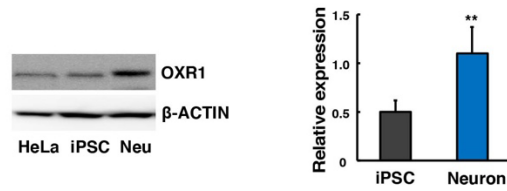
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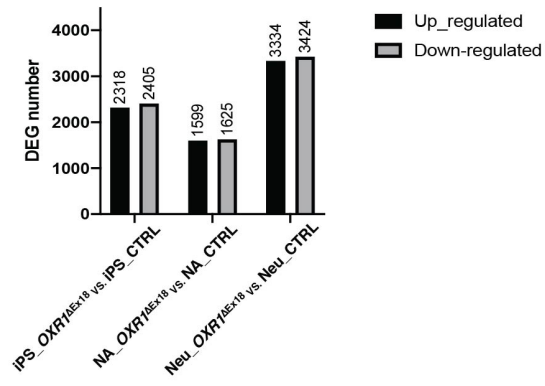
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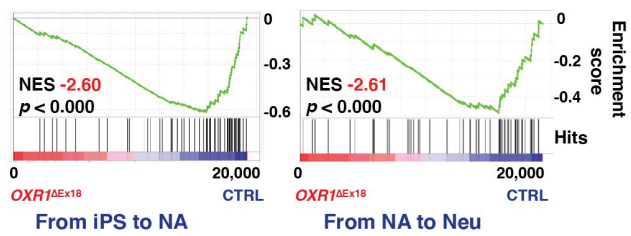


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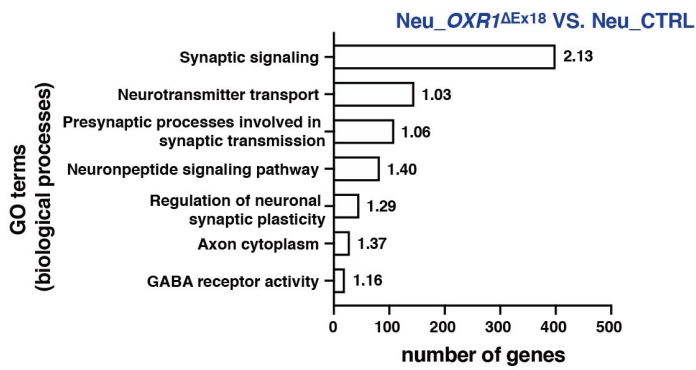


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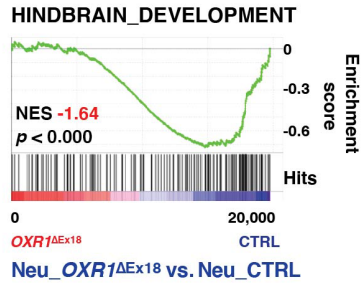
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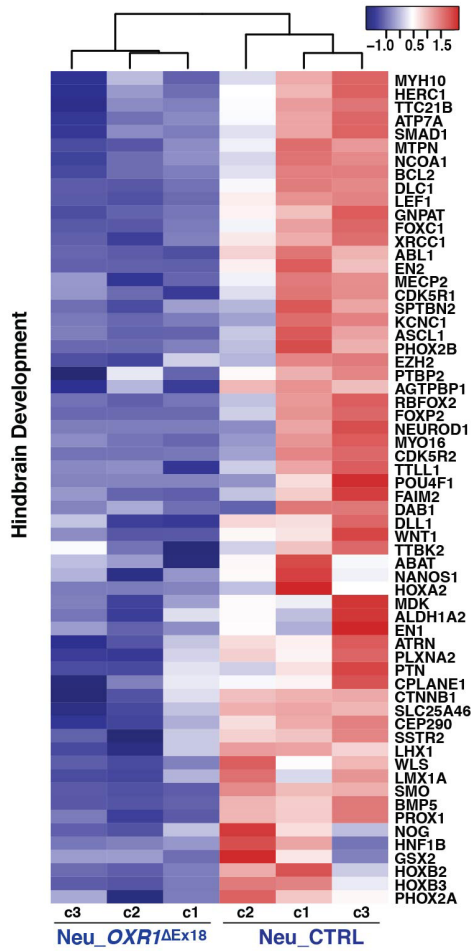
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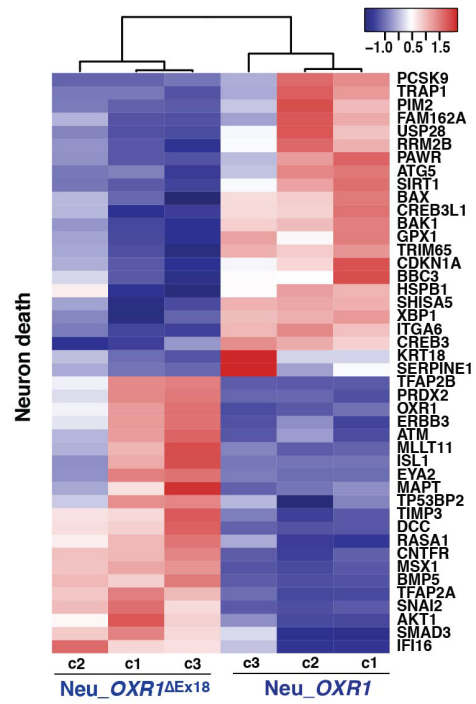
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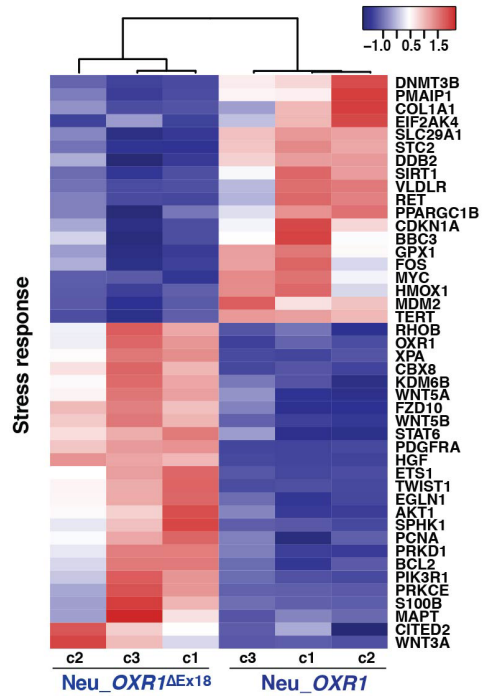
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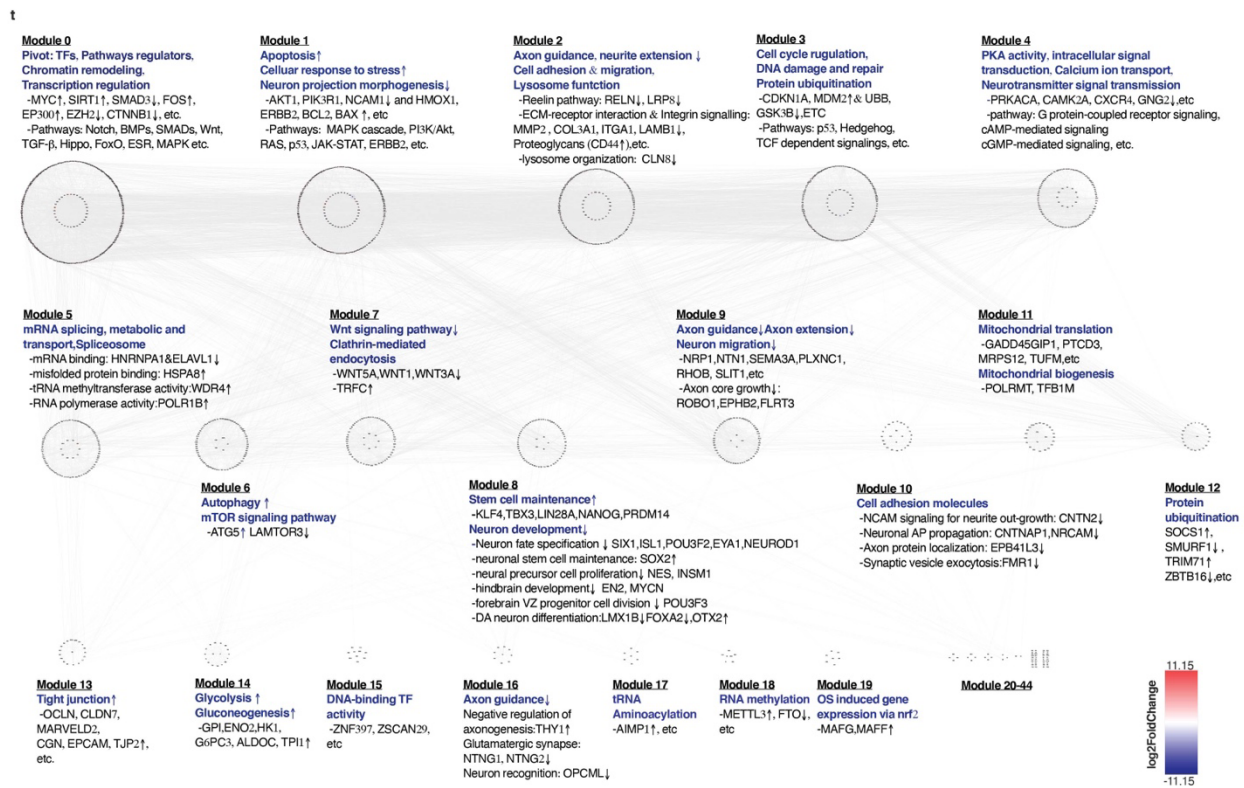


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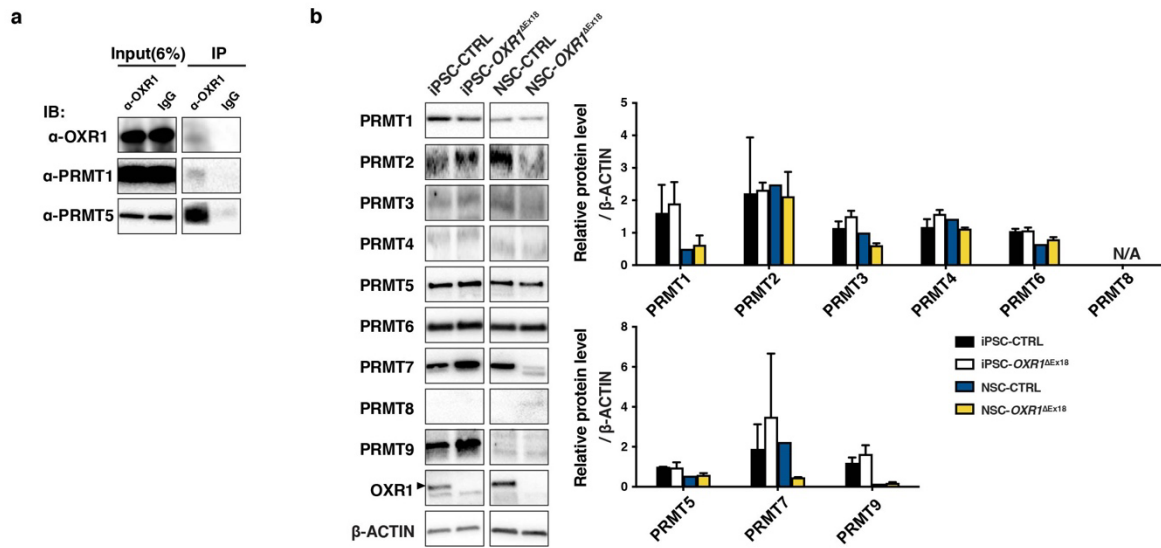
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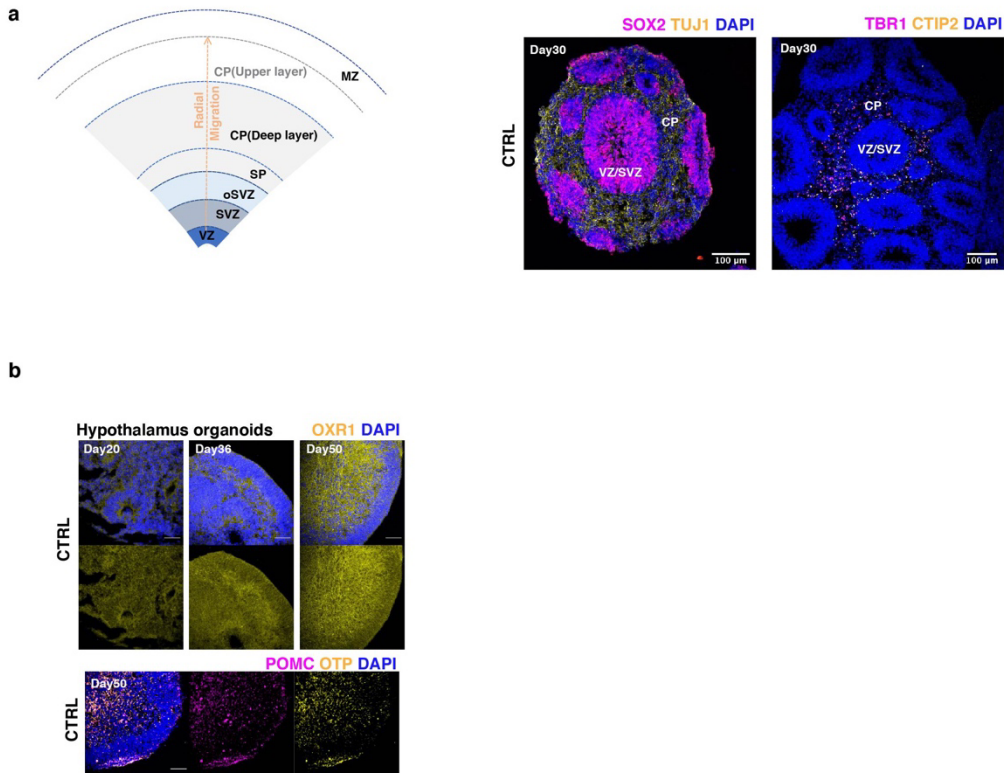


Supplementary Figure 3. Generation and characterization of 2D neuronal differentiation models derived from induced pluripotent stem cells. **a**, Genotyping of generated iPSC from healthy control clones (CTRL) and OXR1-deficient iPSC clones (*OXR1*^{ΔEx18}). After total RNA and cDNA were prepared, PCR was performed using primer spanning exon 18 region. The shorter (185bp) PCR products were detected in all three *OXR1*^{ΔEx18} iPSC clones. **b**, Immunoblot of OXR1 in CTRL and *OXR1*^{ΔEx18} iPSC showing complete lack of OXR1 protein in patient sample. **c**, Karyotype analysis of fibroblast cells and iPSC clones. Only qualified iPSC clones were selected in further experiments. **d**, Immunofluorescence of pluripotent transcription factors OCT4, SOX2, NANOG, and cell surface marker SSEA4 in iPSC. **e**, Gene expression of endogenously expressed pluripotency transcription factors in both iPSC and fibroblasts from CTRL and *OXR1*^{ΔEx18} by qPCR analysis. The gene expression levels were comparable with human embryonic stem (ES) cells. **e**, iPSC were differentiated into the three germ layers *in vitro* by using STEMdiff™ Trilineage Differentiation Kit. Immunocytochemistry was performed to identify the differentiation of ectodermal (SOX1 and Nestin), mesodermal (Brachyury), or endodermal (SOX17) lineages. **g**, Schematic representation of experimental procedure for neurite outgrowth. **h**, Phase contrast images of NAs generated in AggreWell at 48 h after aggregation (left) showing irregularities in the morphology of *OXR1*^{ΔEx18}. Quantification of diameters (middle) and areas (right) of NAs at 96 h after aggregation determined by ImageJ. Four hundred cells of each group from two independent cultures were counted. **i**, Schematic representation of experimental procedure for

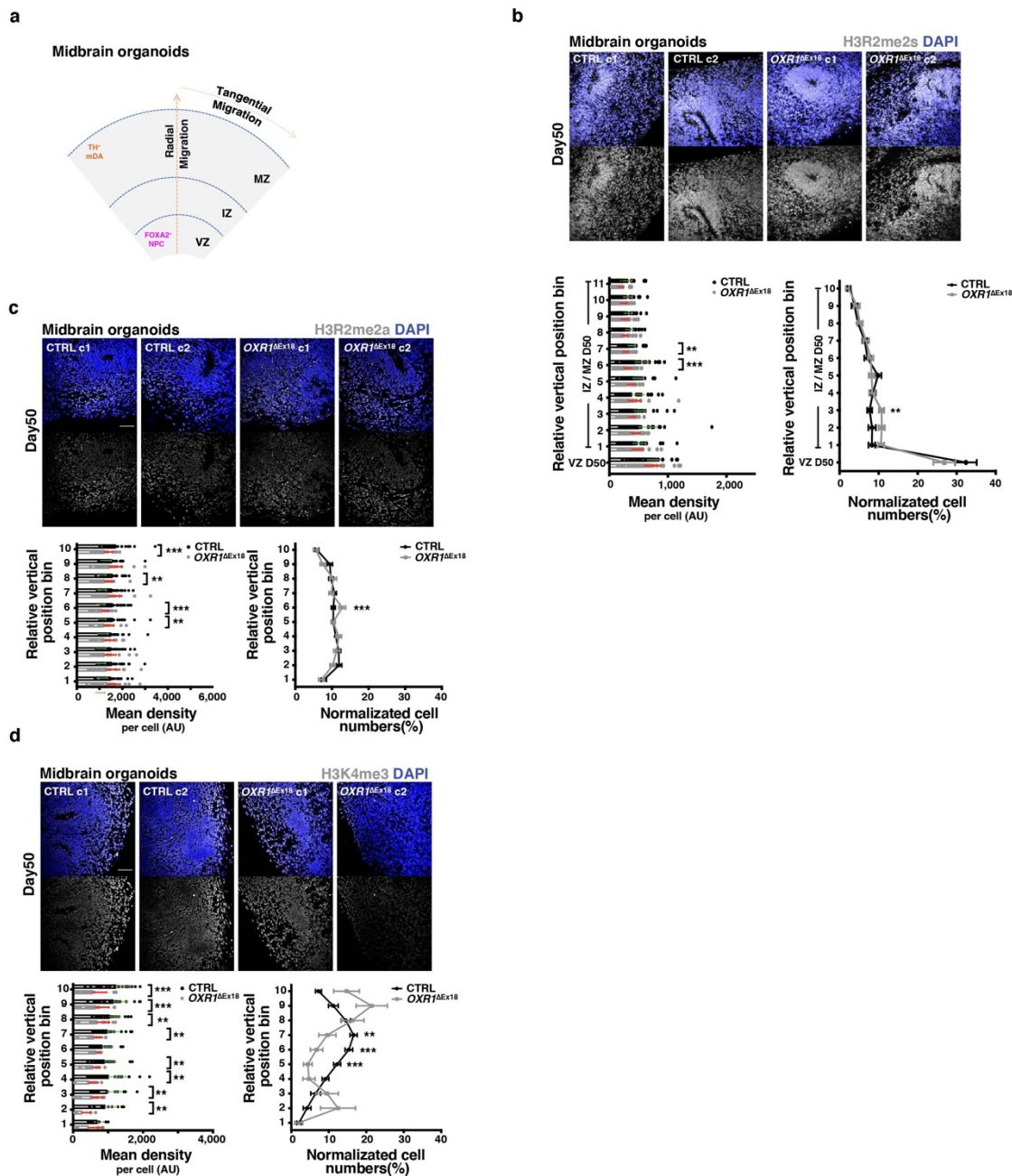
neuronal differentiation and RNA sequence (RNAseq). Neu, neuron. **j**, Immunofluorescence images(left) and quantifications(right) of the fluorescence intensity of MAP2 and Tuj1 from CTRL and *OXR1*^{ΔEx18}. The scale bars, 200 μm. **k**, Representative images of TUNEL staining of apoptotic cells during neural differentiation (left) and quantification of TUNEL staining in control and *OXR1*^{ΔEx18} neuronal cells (right). About 1,300 cells of each group from 8 different microscopic fields were counted. The scale bars, 200 μm. **l**, Western blot images(right) and quantifications(left) of OXR1 expression, Neuron vs. undifferentiated iPS, n=3. **h, j-l**, n=3, Data are means+SD, * $p < 0.05$, ** $p < 0.01$, *t*-test. **m**, Bar chart showing the number of Down-regulated and Up-regulated differentially expressed genes (DEGs) between patient (*OXR1*^{ΔEx18}) and control (CTRL) at each differentiation stage. Thresholds at logFC ≥ 0.5 (Down-DEGs) and ≤ -0.5 (Up-DEGs), significant at adjusted-*p* value < 0.05 . **n**, Gene set enrichment analysis (GSEA) showing significant reduction in neuron fate commitment following neuronal differentiation in *OXR1*^{ΔEx18} groups when compared to CTRL. NES, normalized enrichment score; *p*, nominal *p* value. **o**, Bar charts of the top ranked (upregulated) GO Terms of biological processes relevant to neural development, between *OXR1*^{ΔEx18} and CTRL at neuron stage, identified by GSEA. **p**, Impaired hindbrain development in *OXR1*^{ΔEx18} neurons. GSEA showing downregulated of hindbrain development in Neu_*OXR1*^{ΔEx18}, as compared with Neu_CTRL. **q**, Heatmaps of leading-edge genes of hindbrain development by GSEA in the comparison of Neu_*OXR1*^{ΔEx18} vs. Neu_CTRL. **r-s**, Heatmaps of top 40 leading-edge genes related to neuron death(**r**) or stress response(**s**) in the comparison of Neu_*OXR1*^{ΔEx18} vs. Neu_CTRL. **t**, Topologic biological network analysis of the protein and protein interactions of 1539 DEGs between Neu_*OXR1*^{ΔEx18} and Neu_CTRL. Each module consists of a set of genes that are both highly connected in protein interaction networks and highly correlated in biological databases and group in a circle. For instance, Module 0 functioned as a pivot for the whole network characterized by central transcription factors, binding proteins, and core enzymatic components (HDAC1, SIRT1, EP300, EZH2, etc) crucial for regulating transcription, chromatin organization and several substantial pathways for cellular activities (Notch, BMPs, SMADs and Wnt, etc). Coordinated with Module 1 and 3, Module 0 regulated cell stress responses. Module 8 conferred a lead role in neurogenesis. Module 2, 9, 10 and 16 showed that OXR1-depletion impaired axon guidance at multiple levels, from the axon genesis, axon core growth, to neurite extension, migration, recognition, then to synapse formation. The boundary color of a node(gene) represents its module. Node size represents “degree” describing the relative importance of a single node within the sub-network. Hub genes, highly connected nodes, lie in the inner circle of each module identified by Cytohubba. Node color reflects whether its mRNA is up or down regulated in Neu_*OXR1*^{ΔEx18} in comparison with Neu_CTRL. Color descending from red to blue, indicates log₂ (Fold Change) value from large to small. Visualization by Cytoscape 3.8.1. iPS, induced pluripotent stem cell; NA, neural aggregates; Neu, neurons.



Supplementary Figure 4. OXR1 interacts with PRMT1 and PRMT5. **a**, Immunoprecipitation of OXR1 in U2OS cells demonstrating that OXR1 bound to PRMT1 and PRMT5 respectively. 6% of extracted protein was loaded as input control. **b**, Western blot analysis of PRMTs in iPS and NSC. Quantifications of type I (upper) and type II/III (down) of PRMTs. β -ACTIN was used as loading control. Results are shown as means \pm SD, *t*-test. iPS, induced pluripotent stem cells; NSC, neural stem cells.

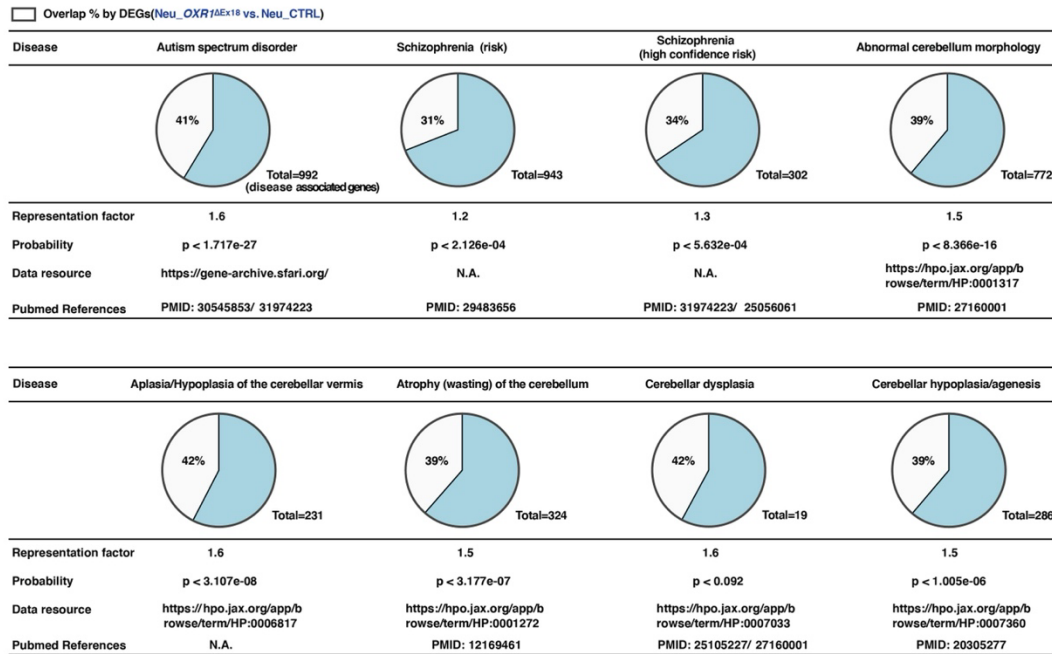


Supplementary Figure 5. Generation and characterization of cerebral organoids and hypothalamus organoids derived from iPS. a, A schematic of cortical layer structure of cerebral organoids or cortical organoids at \geq Day 75(left). The dotted arrow in orange indicating the radial migration of neural progenitor cells from VZ/SVZ. The SP like region is usually interspersed between SVZ and CP. Representative confocal images of cortical region at Day30 (right). The progenitor zone consists of VZ and SVZ, stained with SOX2. TBR1 and CTIP2 marked the deep layer neurons of CP, and the nuclei were visualized using DAPI. VZ, ventricular zone; SVZ, subventricular zone; oSVZ, outer SVZ; SP, subplate; CP, cortical plate ; MZ, marginal zone. **b,** Representative tiling confocal images showing the development of hypothalamus organoids of CTRL immunostained with anti-OXR1 antibodies at Days 20, 35, and 50(upper). OXR1 level increased along with neuronal maturation. Representative tiling confocal images showing control hypothalamus organoids contained POMC⁺ peptidergic neurons and OTP⁺ hypothalamic neurons at Day50 (bottom).



Supplementary Figure 6. OXR1 impacts midbrain development by shaping histone arginine and lysine methylations. **a**, A schematic of midbrain organoid, the dotted arrow in orange indicating the radial migration of neural progenitor cells from VZ, through IZ, to reach MZ, and tangential migration of TH⁺ neurons at the outer surface of MZ. **b-d**, Representative images, and quantifications of the distribution of H3R2me2s⁺ (**b**), H3R2me2a⁺ (**c**), and H3K4me3⁺ (**d**) cells in midbrain organoids at Day50. The dense radial developing structures from apical surface are labelled as VZ. The outside VZ structure (IZ/MZ) from the outer boundary of VZ to basal surface is evenly divided into 10 bins, from 1 to 10. Quantifications

include the average expression level per cell(lower left) and the normalized abundance within each bin and in VZ(lower right) . The normalized abundance was calculated as (the number of positive cells in a bin/ the total number of positive cells). Values represent mean \pm SEM, ** $p < 0.01$; *** $p < 0.001$; Student's t test. In H3R2me2s⁺ (**b**), CTRL, n = 23; *OXR1* ^{Δ Ex18}, n =33, from two independent batches; in H3R2me2a⁺(**c**), CTRL, n = 31; *OXR1* ^{Δ Ex18}, n = 21, from two independent batches; in H3K4me3⁺ (**d**), CTRL, n = 31; *OXR1* ^{Δ Ex18}, n = 21, from two independent batches. VZ, ventricular zone; IZ, intermediate zone; MZ, mantle zone, NPC, neural progenitor cells.



Supplementary Figure 7. Intersection-comparisons of *OXR1* deficiency disease and other neurological developmental diseases. The representation factors and relevant probability are calculated by http://nemates.org/MA/progs/overlap_stats.html. A representation factor > 1 indicates more overlap than expected of two independent gene sets, the set of differentially expressed genes (DEGs) of Neu_ *OXR1*^{ΔEx18} vs. Neu_CTRL and the disease associated gene set of the neurological developmental disease as indicated.