

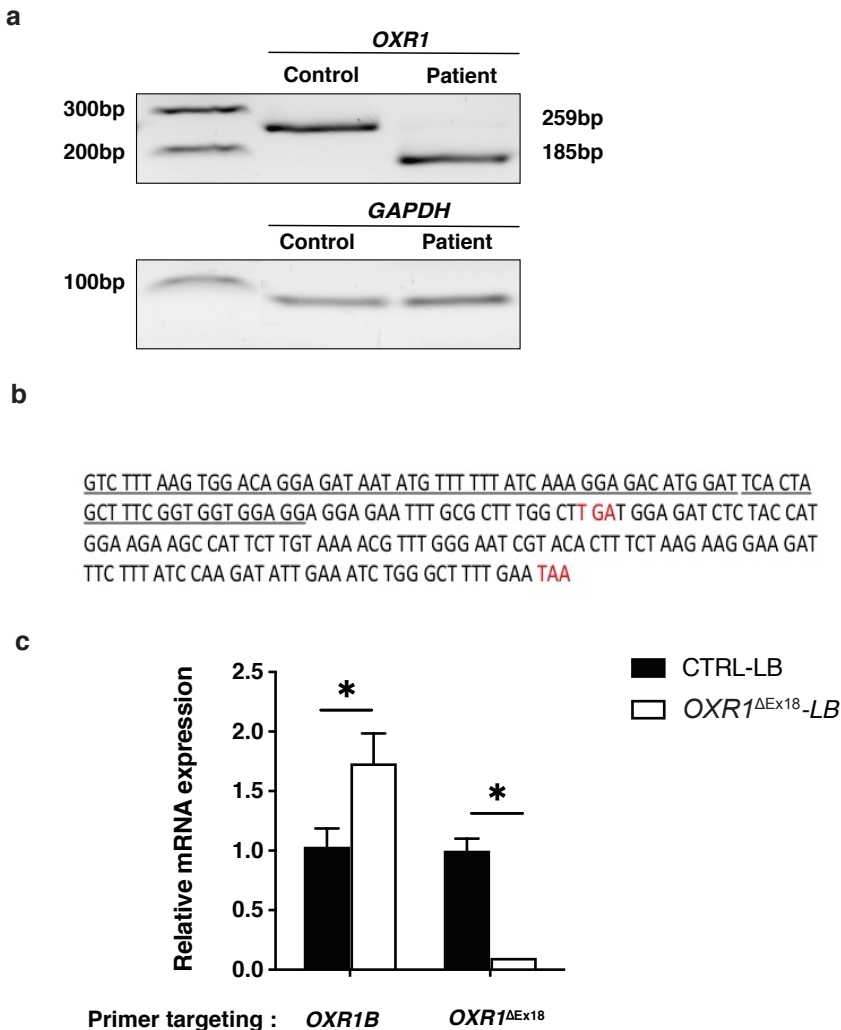


Figure S1. Donor splice site mutation in the *OXR1* gene causes TLDc domain depletion. **a**, PCR amplified cDNA fragment spanning exon 17-19. The shorter (185bp) product in the patient sample indicates the deletion of exon 18. **b**, The absence of exon 18 results in an open reading frame shift and consequently a premature stop codon “TGA” in exon 19. The underlined part is exon 18, while the rest is exon 19. **c**, Quantitative real-time PCR analysis of *OXR1* in the patient ($OXR1^{\Delta Ex18}$) and control (CTRL) lymphoblasts. Primer pair ($OXR1^{\Delta Ex18}$) is specific to the patient’s exon-skipping sequence. Primer pair (*OXR1B*) targets *OXR1* isoform B at the N-terminus.

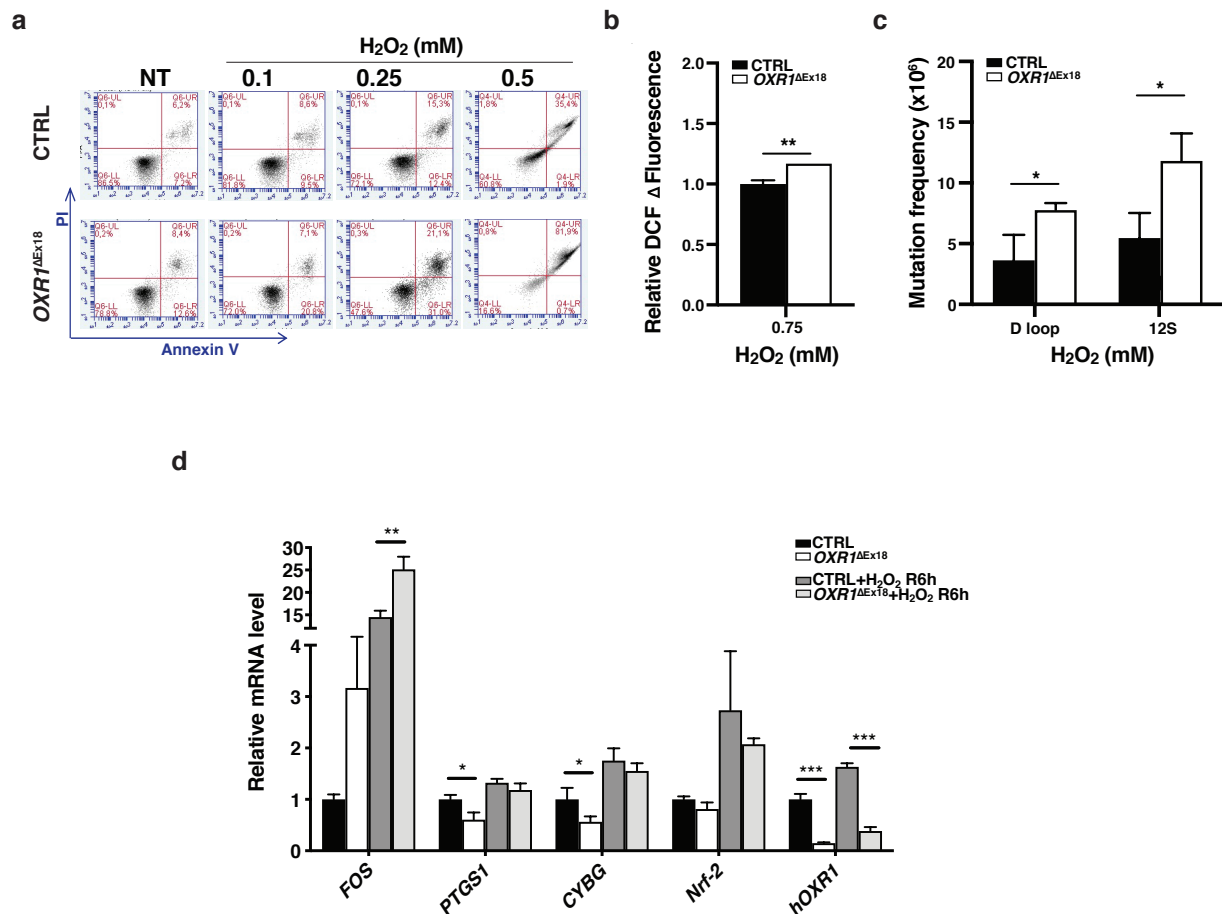
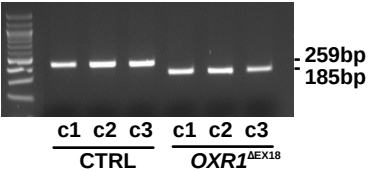


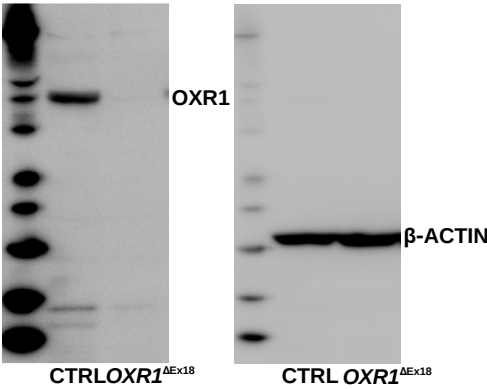
Figure S2. Loss of OXR1 increases cell death, general ROS level and mitochondrial DNA mutation frequency in lymphoblasts (LB). **a**, Representative results of FITC-Annexin V/PI double staining by flow cytometry (FCM). **b**, Elevated amounts of ROS generated in patient LB cells (*OXR1*^{ΔEx18}) after 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 the normal culturing condition. **b-c**, n=3 independent experiments, * $p < 0.05$, ** $p < 0.01$, t -test. **d**, Quantitative real-time PCR analysis of oxidative stress response genes in the patient (*OXR1*^{ΔEx18}) and control (CTRL) lymphoblasts exposed to H₂O₂ and recovery for 6 hours (R6h). n=3 independent samples. * $p < 0.05$, *** $p < 0.001$, multiple t tests with the Holm-Sidak method to correct multiple comparisons.

Figure.S3

a



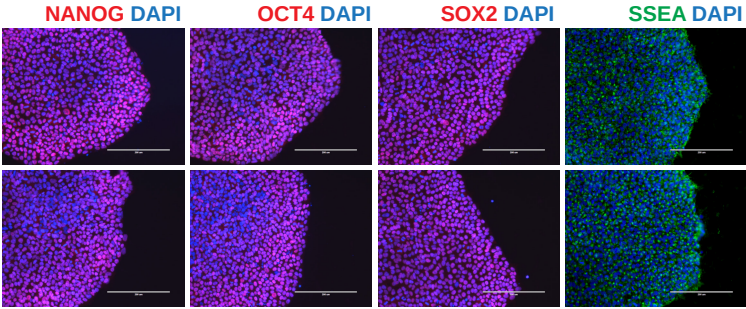
b



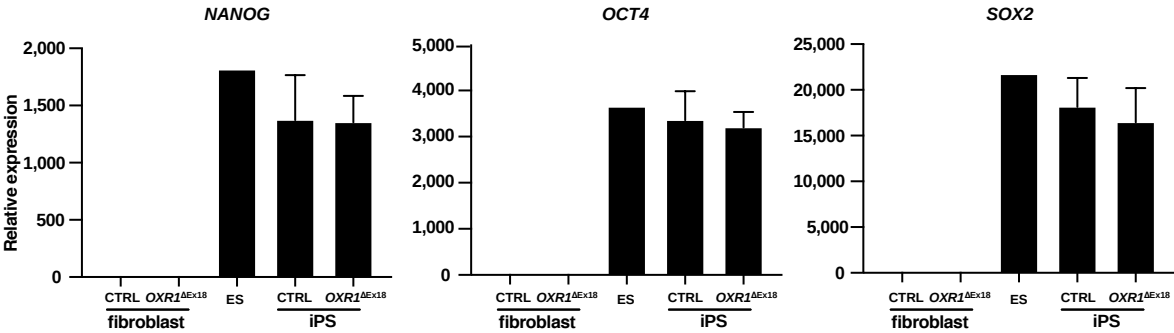
c

Karyotype										Median ratios																							
Sample	Gender	CV%	Threshold		21C	22C	23C	4C	5C	6C	7C	8C	9C	10C	11C	12C	13C	14C	15C	16C	17C	18C	19C	20C	21C	22C	Aut	X	Y				
AG-F_1	Female	5.92	0.88-1.12	0.82-1.18	0.97	1.06	0.98	0.97	0.97	0.96	1.01	1.03	1.05	1.08	1.07	1.04	1.02	0.94	1.08	1.02	1.07	1.00	1.08	1.04	1.05	1.05	1.05	0.94	1.23	0.73			
AG-F_2	Female	6.67	0.87-1.13	0.80-1.20	1.02	1.04	0.94	1.02	0.97	0.98	1.05	1.00	1.03	1.05	1.03	1.04	1.02	1.08	1.04	0.99	0.99	0.90	1.05	0.98	1.00	1.05	1.00	1.00	1.21	0.81			
Oxr-F_1	Female	4.99	0.90-1.10	0.85-1.15	0.98	0.92	0.95	1.00	0.97	0.94	0.99	0.99	0.97	1.03	0.98	0.99	0.99	1.00	1.05	0.98	0.98	1.02	0.98	0.98	1.00	0.95	1.07	1.21	0.76				
Oxr-F_2	Female	5.60	0.89-1.11	0.83-1.17	0.98	1.02	1.01	0.96	0.99	1.03	0.99	1.01	0.99	1.00	0.99	1.07	1.01	1.01	1.08	1.02	0.96	0.96	1.02	1.00	1.09	1.00	0.99	1.00	1.24	0.77			
AG-c10_1	Female	6.35	0.87-1.13	0.81-1.19	1.01	1.04	1.00	0.98	0.96	0.97	1.01	1.01	1.05	1.06	1.02	0.98	0.98	1.04	1.06	1.00	1.01	1.00	1.04	1.00	0.95	0.98	0.94	1.01	1.16	0.77			
AG-c10_2	Female	6.75	0.87-1.13	0.80-1.20	1.00	0.99	1.02	0.96	0.96	0.98	0.97	0.94	1.06	1.05	1.06	1.01	1.02	1.04	1.06	0.95	0.95	1.00	1.02	1.01	0.93	0.96	0.92	1.01	1.15	0.71			
AG-c27_1	Female	6.44	0.87-1.13	0.81-1.19	0.99	0.99	0.99	0.97	0.96	0.99	0.99	0.98	0.99	1.04	1.02	0.97	0.98	0.93	1.03	0.96	0.98	0.93	1.06	0.98	1.00	0.97	0.91	1.07	1.13	0.77			
AG-c27_2	Female	5.68	0.89-1.11	0.83-1.17	1.07	1.02	1.00	1.06	1.02	1.00	0.98	1.01	1.09	1.08	1.04	1.07	1.03	1.02	0.98	1.00	0.98	1.04	1.00	1.02	1.01	1.01	0.96	0.97	1.13	0.75			
AG-c15_1	Female	6.84	0.86-1.14	0.79-1.21	0.94	0.99	0.97	1.06	1.01	0.99	0.96	1.05	0.99	1.02	1.01	1.08	0.98	1.01	1.02	0.97	0.93	1.02	0.97	1.01	0.95	1.03	0.98	1.02	1.14	0.73			
AG-c15_2	Female	6.55	0.87-1.13	0.80-1.20	0.99	0.96	0.98	1.06	0.98	1.04	1.04	1.00	0.99	1.02	0.99	1.05	0.96	1.03	1.07	0.93	0.98	0.96	1.06	1.02	0.96	0.98	0.90	1.01	1.13	0.71			
Oxr-c2_1	Female	6.08	0.88-1.12	0.82-1.18	1.01	1.02	0.99	1.00	0.97	1.03	0.96	0.93	1.04	1.06	1.03	1.03	0.96	1.07	1.02	0.85	0.97	0.97	1.07	0.98	1.09	1.02	1.04	1.02	1.26	0.74			
Oxr-c2_2	Female	6.80	0.87-1.13	0.80-1.20	0.93	1.01	0.98	1.02	1.00	0.99	0.92	0.96	1.04	0.98	1.05	1.06	0.91	1.08	1.01	0.86	0.98	0.89	1.07	0.98	1.04	0.97	1.02	0.96	1.20	0.77			
Oxr-c10_1	Female	6.73	0.87-1.13	0.80-1.20	0.99	1.01	1.00	1.02	1.03	0.96	0.96	1.01	1.03	1.05	1.00	0.98	1.02	1.03	0.99	0.96	0.96	0.96	1.04	0.94	1.06	0.96	0.92	1.04	1.22	0.68			
Oxr-c10_2	Female	7.28	0.85-1.15	0.78-1.22	0.97	1.00	1.00	0.99	1.04	0.96	0.95	0.96	1.05	0.98	0.99	0.97	1.05	1.07	1.00	0.99	0.97	0.94	1.04	0.95	1.12	0.96	0.93	1.02	1.19	0.78			

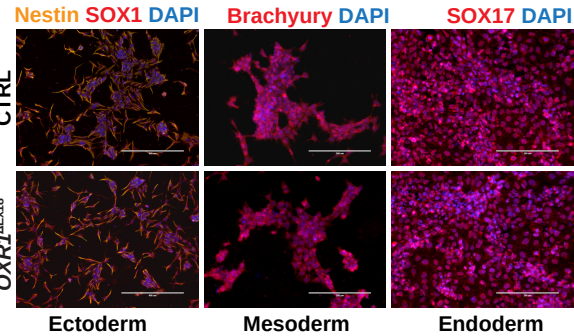
d



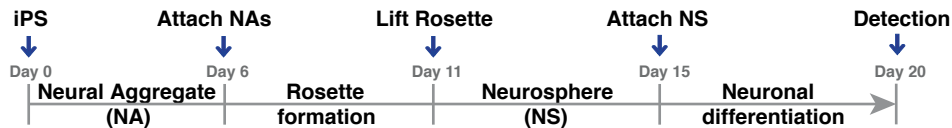
e



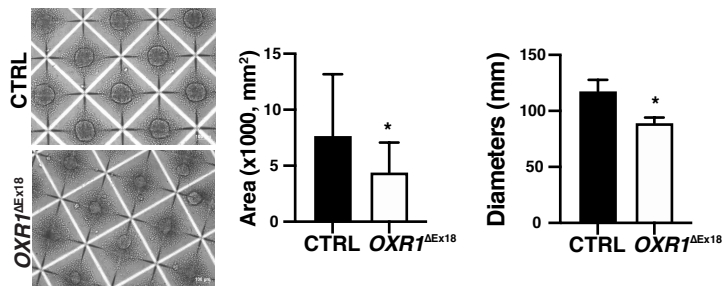
f



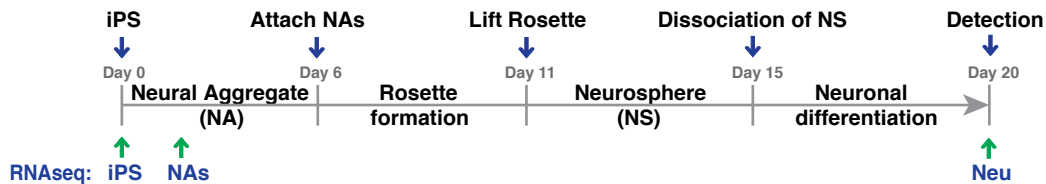
g



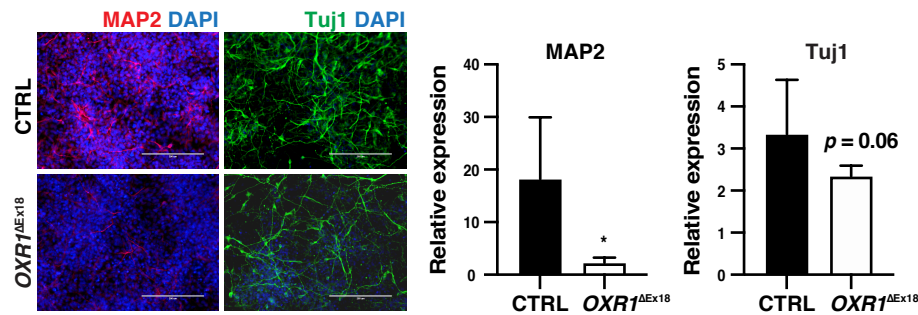
h



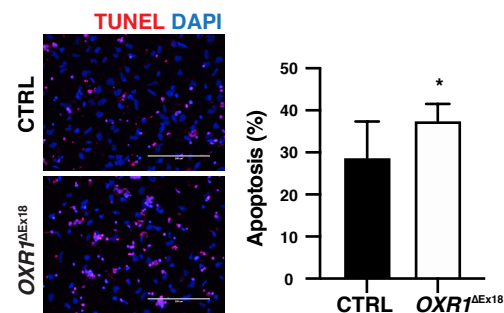
i



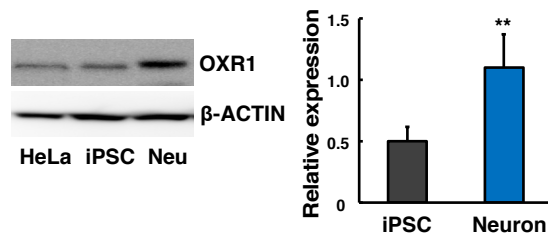
j



k



l

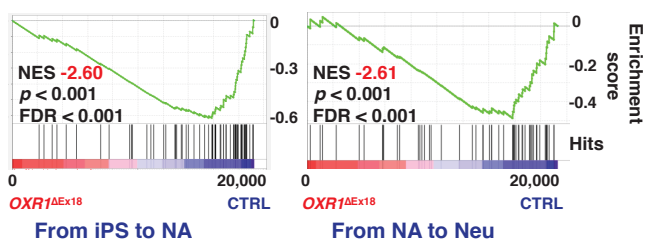


m

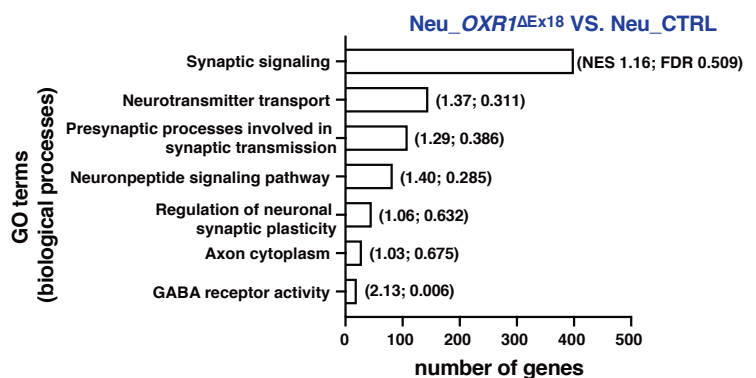
COMPARISON	log2FC > 0.5		log2FC > 1		log2FC > 1.5	
Total number of genes : 29568	UP	DOWN	UP	DOWN	UP	DOWN
iPS_ OXR1 Δ Ex18 vs iPS_CTRL	2318	2405	1579	647	721	368
NA_ OXR1 Δ Ex18 vs NA_CTRL	1599	1625	1039	1369	690	990
Neu_ OXR1 Δ Ex18 vs Neu_CTRL	3334	3424	2841	2544	2041	1752

n

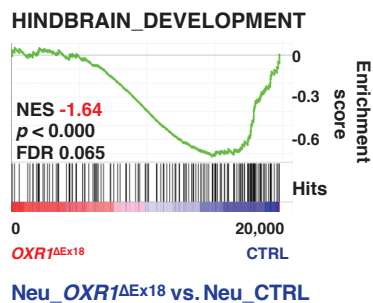
NEURON_FATE_COMMITMENT



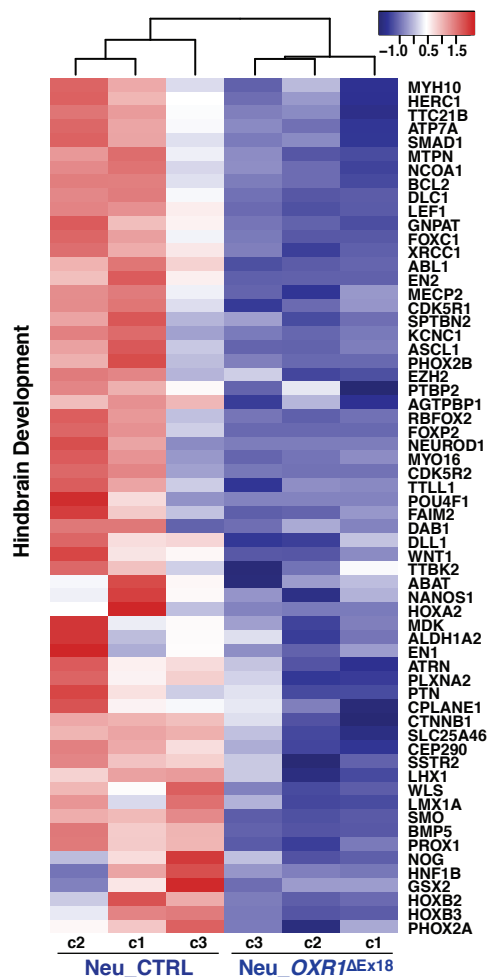
o



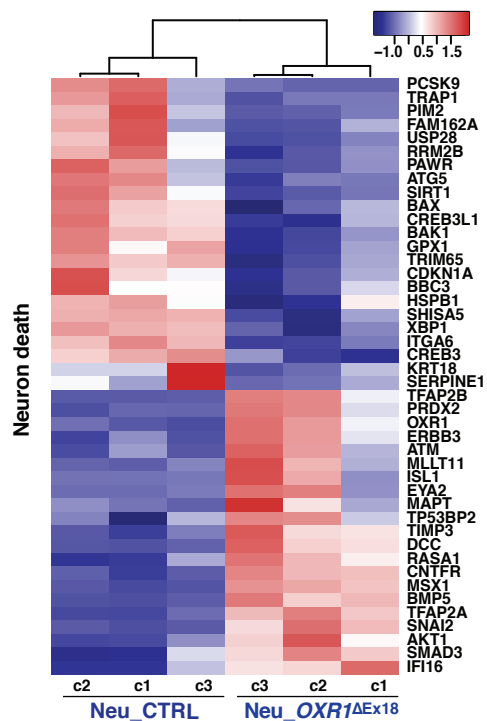
p



q



r



s

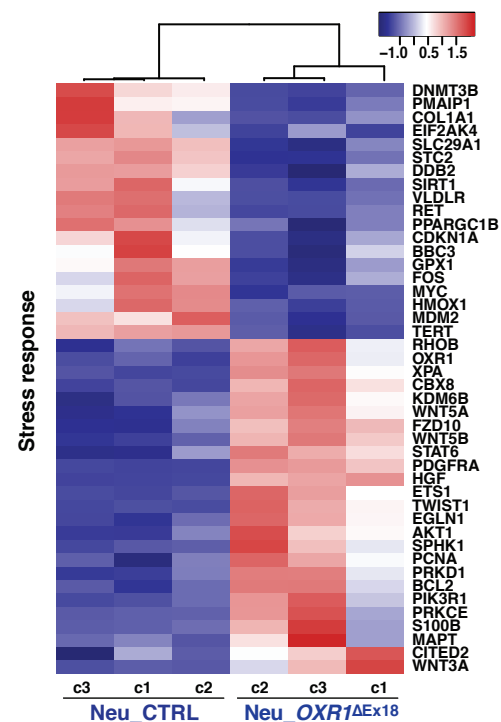


Figure.S3

t

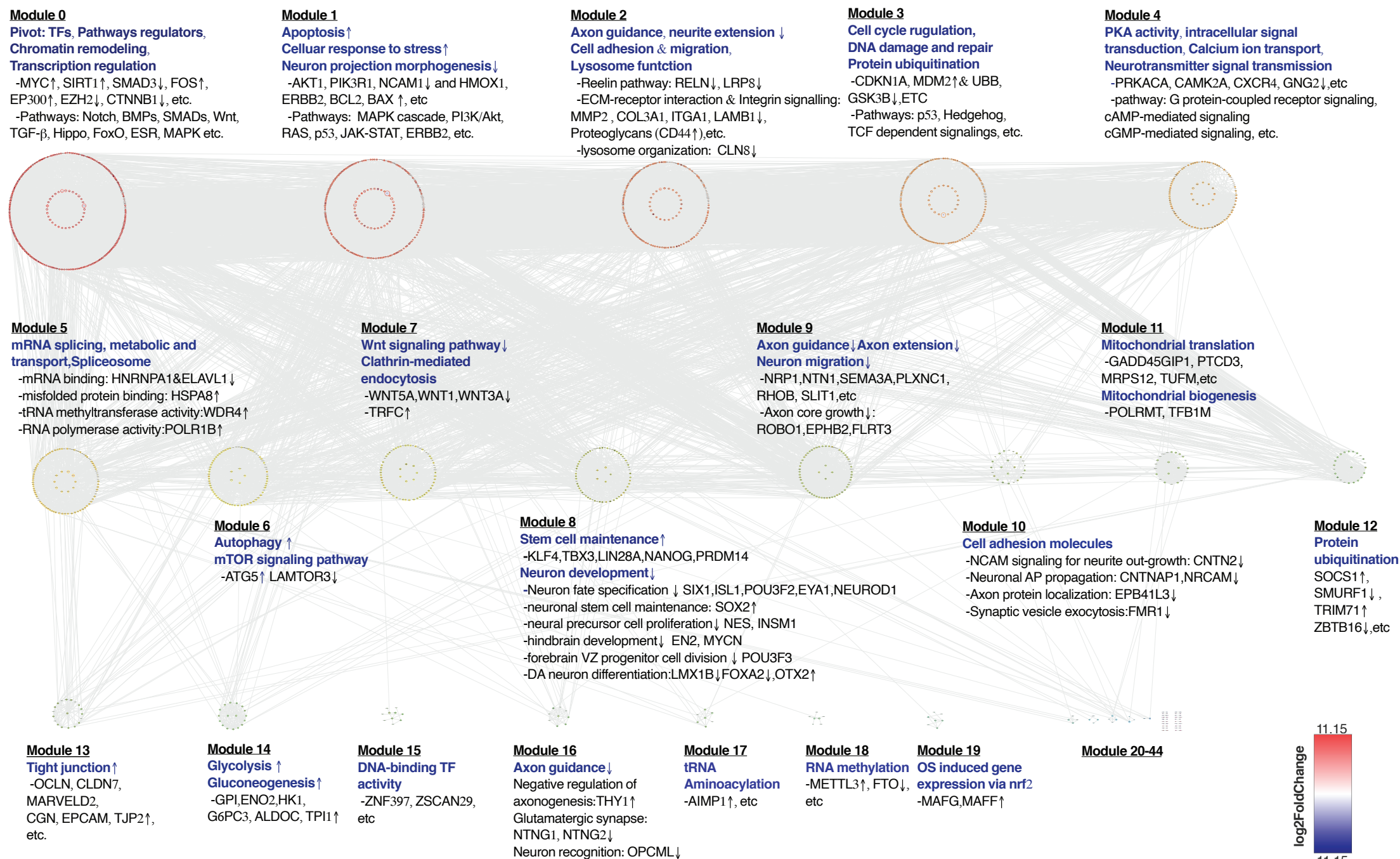


Figure S3. Generation and characterization of 2D neuronal differentiation models derived from induced pluripotent stem cells. **a**, Genotyping of generated iPS from healthy control clones (CTRL) and OXR1-deficient iPS clones ($OXR1^{\Delta Ex18}$). After total RNA and cDNA were prepared, PCR was performed using primer spanning exon 18 region. The shorter (185bp) PCR products were detected in all three $OXR1^{\Delta Ex18}$ iPSC clones. **b**, Immunoblot of OXR1 in CTRL and $OXR1^{\Delta Ex18}$ iPS showing complete lack of OXR1 protein in patient sample. **c**, Karyotype analysis of fibroblast cells and iPSC clones. Only qualified iPS clones were selected in further experiments. **d**, Immunofluorescence of pluripotent transcription factors OCT4, SOX2, NANOG, and cell surface marker SSEA4 in iPS. **e**, Gene expression of endogenously expressed pluripotency transcription factors in both iPS and fibroblasts from CTRL and $OXR1^{\Delta Ex18}$ by qPCR analysis. The gene expression levels were comparable with human embryonic stem (ES) cells. **f**, iPS 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 $OXR1^{\Delta Ex18}$. 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 $OXR1^{\Delta Ex18}$. The scale bars, 200 μ m. **k**, Representative images of TUNEL staining of apoptotic cells during neural differentiation (left) and quantification of TUNEL staining in control and $OXR1^{\Delta Ex18}$ neuronal cells (right). About 1,300 cells of each group from 8 different microscopic fields were counted. The scale bars, 200 μ m. **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**, The numbers of up- and down-regulated DEGs were listed using three different Log₂FC cut-offs, e.g., Log₂FC>|0.5|, Log₂FC>|1| or Log₂FC>|1.5|, respectively. **n**, Gene set enrichment analysis (GSEA) showing significant reduction in neuron fate commitment following neuronal differentiation in $OXR1^{\Delta Ex18}$ 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 $OXR1^{\Delta Ex18}$ and CTRL at neuron stage, identified by GSEA. The NES and FDR values were indicated next to each bar. **p**, Impaired hindbrain development in $OXR1^{\Delta Ex18}$ neurons. GSEA showing downregulated of hindbrain development in Neu_ $OXR1^{\Delta Ex18}$, as compared with Neu_CTRL. **q**, Heatmaps of leading-edge genes of hindbrain development by GSEA in the comparison of Neu_ $OXR1^{\Delta Ex18}$ 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_ $OXR1^{\Delta Ex18}$ 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.

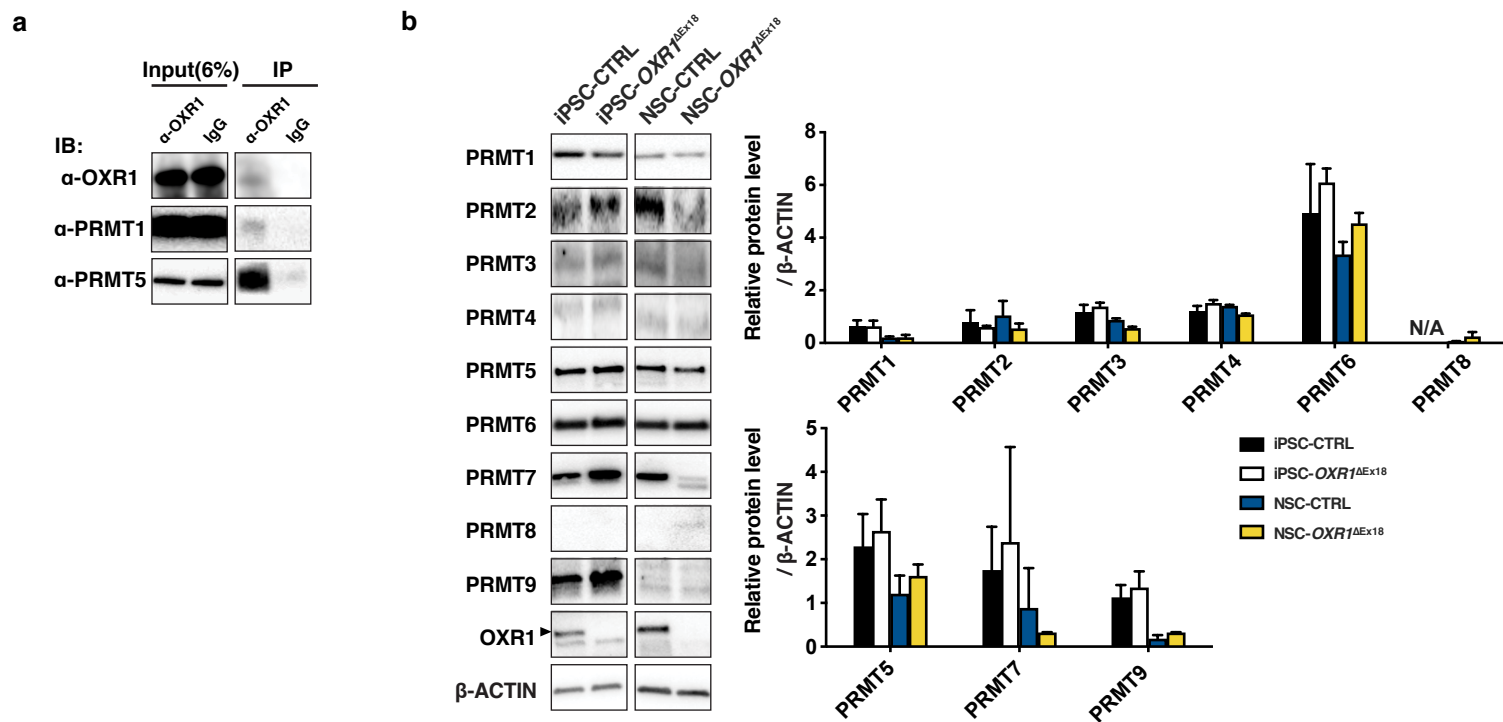


Figure S4. OXR1 interacts with PRMT1 and PRMT5. **a**, Immunoprecipitation of OXR1 in U2OS cells demonstrating that OXR1 bound to PRMT1 and PRMT5 respectively. 6% of extracted protein was loaded as input control. **b**, Western blot analysis of PRMTs in iPSC and NSC. Quantifications of type I (upper) and type II/III (down) of PRMTs. β -ACTIN was used as loading control. Results are shown as means+SD, *t*-test. iPSC, induced pluripotent stem cells; NSC, neural stem cells.

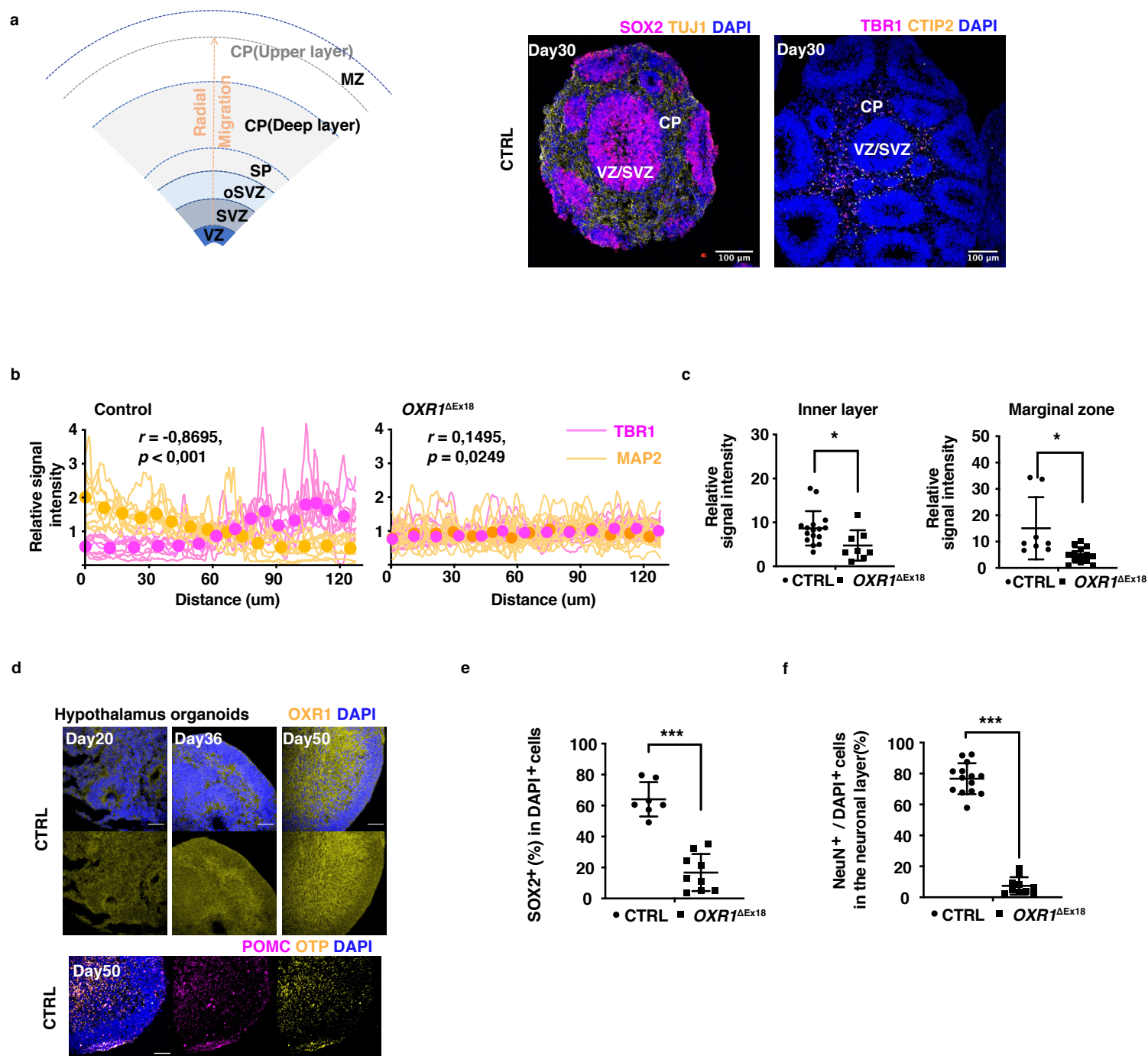


Figure S5. Generation and characterization of cerebral organoids and hypothalamus organoids derived from iPS. **a**, A schematic of cortical layer structure of cerebral organoids or cortical organoids at $\geq$ day 75 (left). The dotted arrow in orange indicating the radial migration of neural progenitor cells from VZ/SVZ. The SP like region is usually interspersed between SVZ and CP. Representative confocal images of cortical region at day 30 (right). The progenitor zone consists of VZ and SVZ, stained with SOX2. TBR1 and CITP2 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**, The quantification of Figure 5b, the distribution of TBR1⁺ and MAP2⁺ fluorescent signals in control and *OXR1*^{ΔEx18} organoids at day 75. The dot lines represent the average relative fluorescence intensity. The Spearman correlation analysis of TBR1⁺ and MAP2⁺ layers in control: $r = -0,8695$ [95% CI, $-0,8989$ to $-0,8323$], $p < 0,001$; in *OXR1*^{ΔEx18}: $r = 0,1495$ [95% CI, $0,01518$ to $0,2785$], $p = 0,0249$. **c**, The quantification of Figure 5c, the relative fluorescence intensity of REELIN at the marginal zone and inner layers of organoids (Student's *t*-test; *, $p < 0.05$). **d**, 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 day 50 (bottom). **e**, The quantification of Figure 5g, the percentage of SOX2⁺ in all DAPI⁺ cells of hippocampus organoids at day 50. Student's *t*-test; ***, $p < 0.001$. **f**, The quantification of Figure 5h, the percentage of NeuN⁺ neurons in the neuronal layers of hippocampus organoids at Day 50. Student's *t*-test; ***, $p < 0.001$.

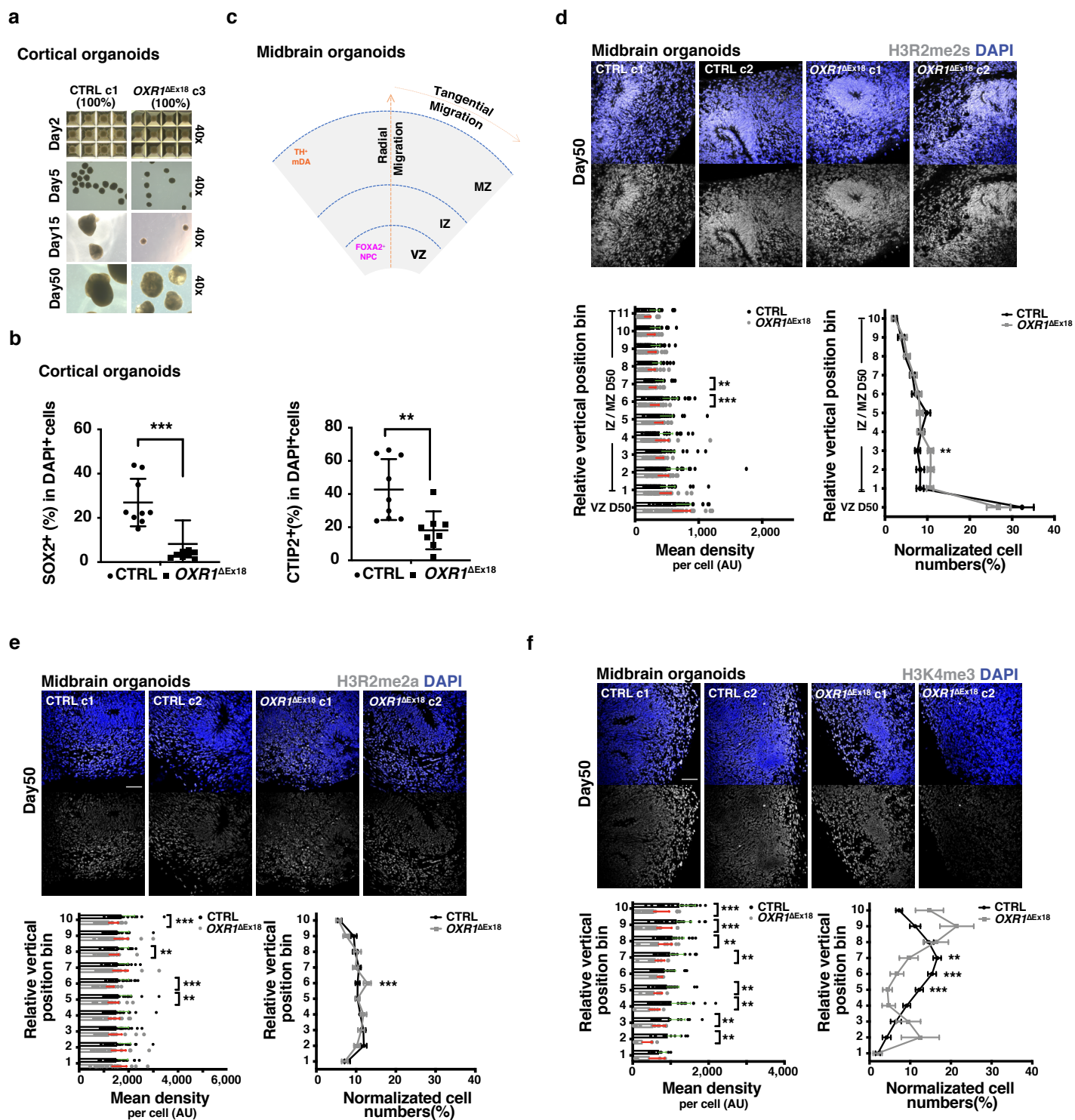


Figure S6. OXR1 impacts midbrain development by shaping histone arginine and lysine methylations. **a**, Representative bright-field images (40x) show the progression of cortical organoids, derived from healthy control (CTRL) and patient ($OXR1^{\Delta Ex18}$) iPSCs. The starting cell number of each clone is indicated (100% refer to 10,000 iPSC cells per microwell of Aggrewell-800 plate on day 0). **b**, The quantification of **Figure. 6b**, the percentage of SOX2⁺ and CTIP2⁺ cells in all DAPI⁺ cells of cortical organoids at Day50. Student's *t* test; ***p* < 0.01; ****p* < 0.001. **c**, 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. **d-f**, Representative images, and quantifications of the distribution of H3R2me2s⁺ (**d**), H3R2me2a⁺ (**e**), and H3K4me3⁺ (**f**) 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; Student's *t* test; ***p* < 0.01; ****p* < 0.001. In H3R2me2s⁺ (**d**), CTRL, *n* = 23; $OXR1^{\Delta Ex18}$, *n* = 33, from two independent batches; in H3R2me2a⁺ (**e**), CTRL, *n* = 31; $OXR1^{\Delta Ex18}$, *n* = 21, from two independent batches; in H3K4me3⁺ (**f**), CTRL, *n* = 31; $OXR1^{\Delta Ex18}$, *n* = 21, from two independent batches. VZ, ventricular zone; IZ, intermediate zone; MZ, mantle zone, NPC, neural progenitor cells.