Supplementary material

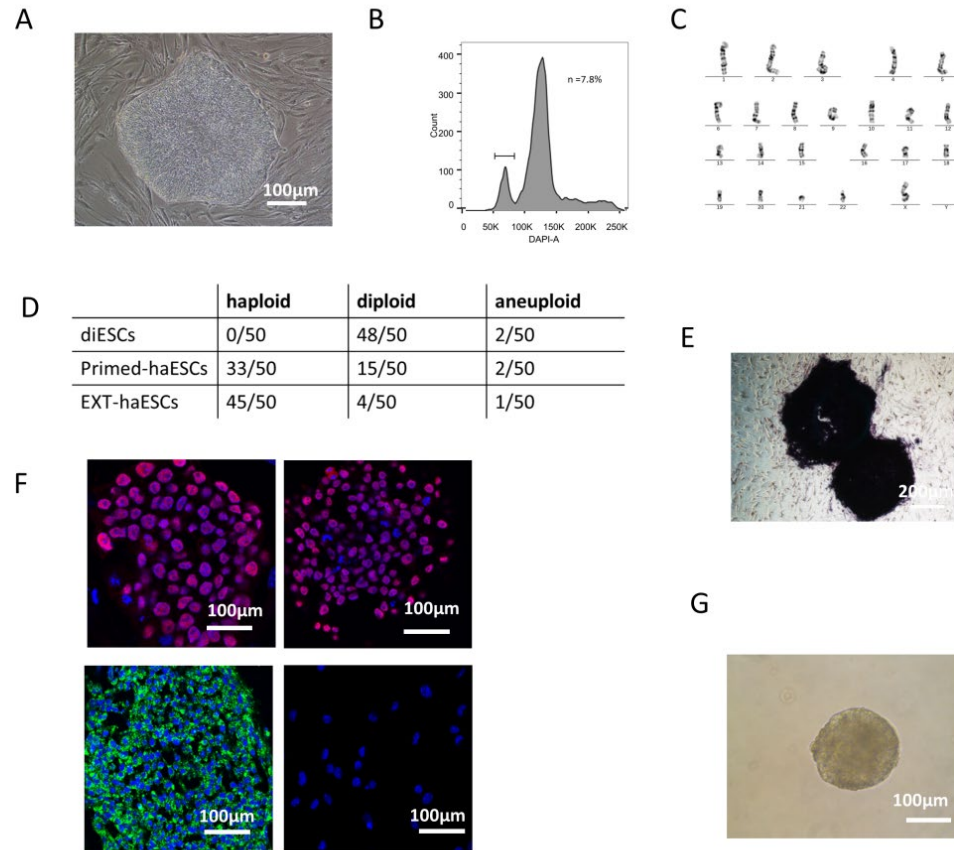|	haploid	diploid	aneuploid
diESCs	0/50	48/50	2/50
Primed-haESCs	33/50	15/50	2/50
EXT-haESCs	45/50	4/50	1/50

Supplementary Figure 1 Identification the pluripotency and haploidy of the human haploid embryonic stem cells. A: Morphology of traditional human haploid embryonic stem cells (haESCs). Scale bar, 100 μm; B: Fluorescence activated cell sorting analysis of DNA content of the human haESC line hPGES1 cultured in traditional medium after 6 wk without sorting; C: G-band analysis of the

hPGES1 cell line cultured in traditional medium with a haploid (23) chromosome set; D: Statistical analysis of chromosome numbers; E: Alkaline phosphatase staining of the human haESC line hPGES1 cultured in traditional medium. Scale bar, 200 μm; F: Immunofluorescence analysis of primate ESC markers in human haESCs cultured in traditional medium, including Oct4 (red), Nanog (red) and SSEA4 (green). DAPI (blue) was used for staining the nuclei. Mouse embryonic fibroblasts were used as a negative control. Scale bar, 100 μm; G: Morphology of embryoid bodies from haploid-enriched cells cultured in traditional medium. Scale bar, 100 μm. diESC: Diploid embryonic stem cell; EXT-haESC: Extended haploid embryonic stem cell; haESC: Haploid embryonic stem cell.
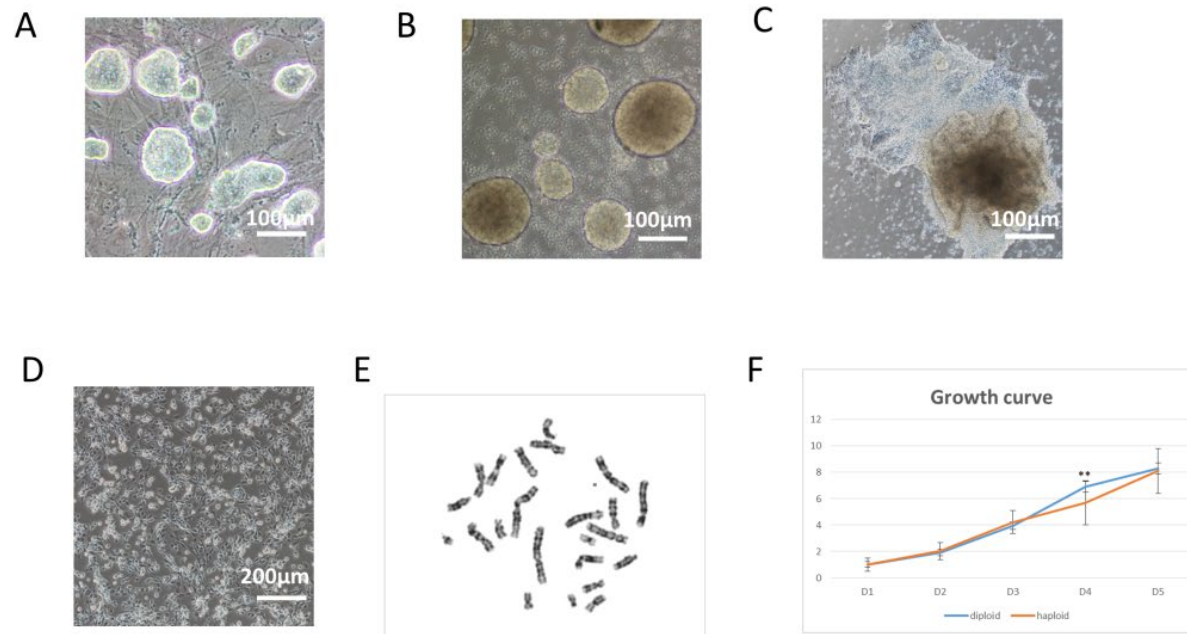
Supplementary Figure 2 Human haploid neural stem cells were derived from extended pluripotent haploid embryonic stem cells.
A: Well-formed human EhPGES1. Scale bar, 100 µm; B: Well-formed embryoid bodies from human EhPGES1. Scale bar, 100 µm; C: Morphology of rosettes that were manually selected and expanded in neural stem cell medium. Scale bar, 100 µm; D: Morphology of haploid neural stem cells (haNSCs) before sorting. Scale bar, 200 µm; E: Karyotype analysis diagram of haNSCs; F: Growth curve of haNSCs and diploid NSCs. *t*-test, **$P < 0.01$.
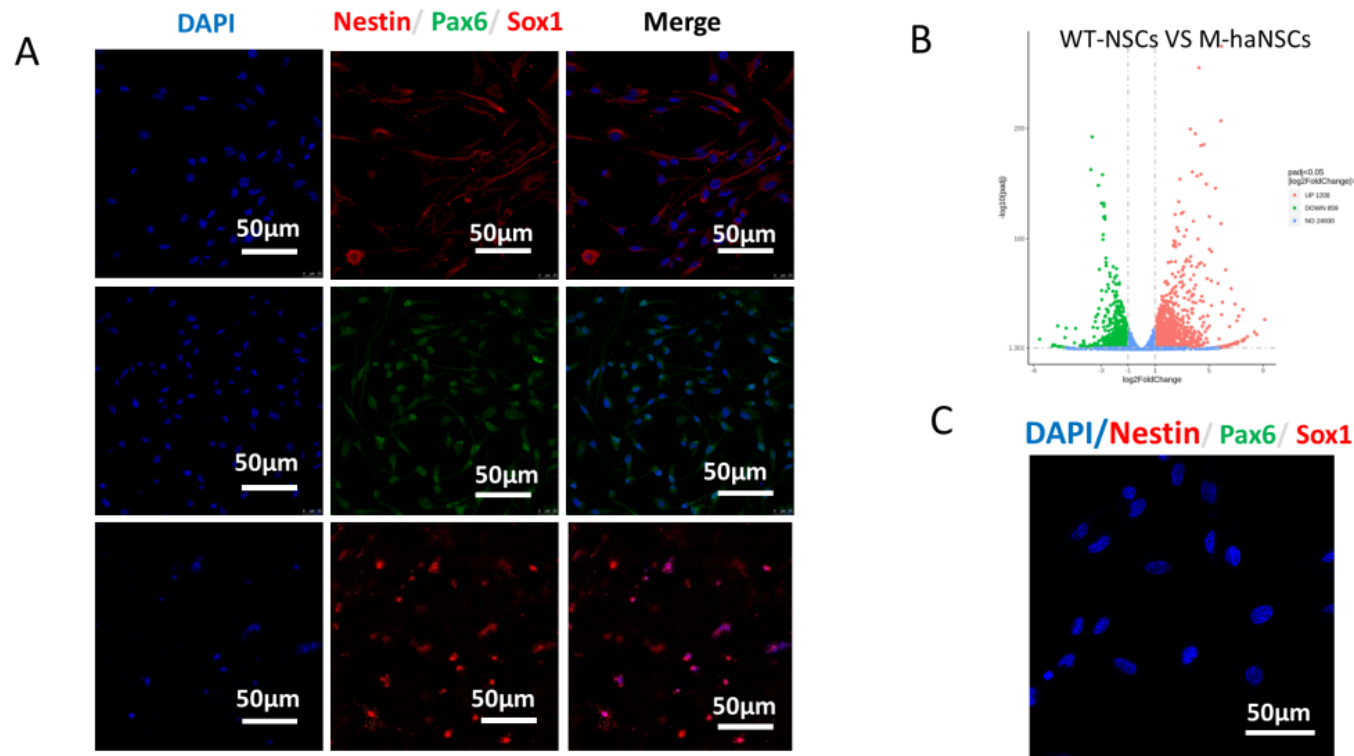
Supplementary Figure 3 Derived haploid neural stem cells were multipotent to neural subtypes *in vitro*. A: Immunostaining of neural stem cell (NSC)-specific markers in diploid embryonic stem cell-derived NSCs. Nestin (red), PAX6 (green) and SOX1 (red). DAPI (blue) was used for staining the nuclei. Scale bar, 50 μm; B: Volcano analysis showed that the transcriptome profile of monolayer culture haNSCs was highly similar to its diploid NSC counterparts; D: Mouse embryonic fibroblasts were used as the negative control. Scale bar, 50 μm. M-haNSC: Monolayer haploid neural stem cell; WT-NSC: Wildtype neural stem cell.

A

Medium	Cytokines	Morphology
Traditional	bFGF	flattened
Optimized	LIF, CHIR99021, (S)-(+)-Dimethin denemaleate, Minocycline hydrochloride, IWR-endo-1, Y-27632	dome-shaped

B

Traditional   Optimized

100μm   100μm
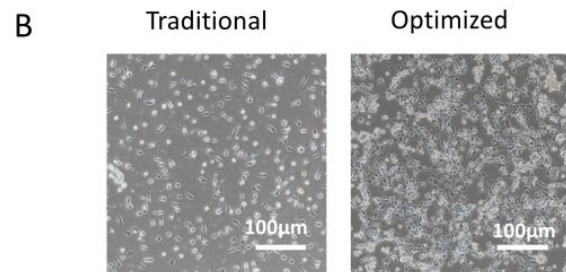
C

| Medium	haploid	diploid
Traditional	0	6
Optimized	2	2

Supplementary Figure 4 Better pluripotency yielded higher efficiency of neural differentiation in human haploid embryonic stem cells. A: Summary of contents and characteristics of human haploid embryonic stem cells in optimized and traditional medium; B: Morphology of sorted haploid neural stem cells (haNSCs) from the traditional group (left) and optimized group (right). Scale bar, 100 μm; C: Statistics of haNSCs obtained from haploid embryonic stem cells cultured in different systems (traditional and optimized). We generated two haNSC lines from the optimized groups, and no haNSC lines were obtained from the traditional groups. bFGF:

Basic fibroblast growth factor.

MAPK	Notch	smoothened	Rho protein	Wnt	BMP
CSF2RB	NOS3	MEGF8	CDH13	APC	MEGF8
ERBB3	SPEN	SEPTIN2	RASGRF1	ROR1	PCSK6
MAP3K4	EGFL7	RORA	RASGRF2	PPP2R1A	USP9X
ROR1	IFT74	SCUBE3	KALRN	PSMA1	LEF1
PDGFA			SHTN1	TBL1X	
PKHD1				UBC	
PPP2R1A				LGR5	
PSMA1				VGLL4	
PTPRJ				MAGI2	
RASGRF1				GPC6	
RASGRF2				RTF1	
SPTB				LEF1	
UBC				RNF220	
BCAR3				LGR6	
SPAG9				TBL1XR1	
TENM1				DAB2IP	
NOD1					
MINK1					
TLR9					
DAB2IP					
SH3RF3					
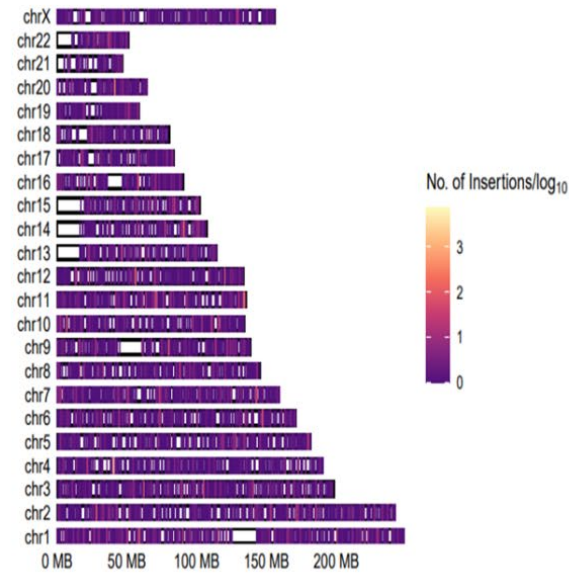
Supplementary Figure 5 Bioinformatics analysis of insertion sites in the derived haploid neural stem cells treated with manganese chloride. A: Summary of trapped genes mapped to critical pathways related to neurotoxicity; B: Hits across the whole genome.

Supplementary Table 1 Primers used in this work

Name	Sequence, 5′ to 3′
Gapdh F	AATCCCATCACCATCTTCCAGGAG
Gapdh R	AATCCCATCACCATCTTCCAGGAG
Sox1 F	CAACCAGGACCGGGTCAAACG
Sox1 R	GCCTCGGACATGACCTTCCACT
Nestin F	CTGCTACCCTTGAGACACCTG
Nestin R	GGGCTCTGATCTCTGCATCTAC
Pax6 F	GTACTGAATGACTCAACTGCTCGG
Pax6 R	CTTTAGAAGGAAGCGACACTCTGC
LEFT-L-1	CCTCGATATACAGACCGATAAAACA
LEFT-R-1	CAAGGCCTACTAGTATTATGCCCAGT
LEFT-L-2	CATGATTATCTTTAACGTACGTCACAAT
LEFT-R-2	GTACATGACCTTATGGGACTTTCCTAC
RIGHT-L-1	GGTCATAGGGCCGGGATTC
RIGHT-R-1	GACTGAGATGTCCTAAATGCACAGC
RIGHT-L-2	TCTCCTCCACGTCACCGC
RIGHT-R-2	GAGCAATATTTCAAGAATGCATGCGTC
SPLNK-GATC-TOP	GATCCCACTAGTGTCGACACCAGTCTCTAATTTTTTTTTTCAAAAAAA
SPLNK-BOT	CGAAGAGTAACCGTTGCTAGGAGAGACCGTGGCTGAATGAGACTGGTGTCGACACTAGTGG

F: Forward; R: Reverse.

Supplementary Table 2 Single haploid neural stem cell spheres were multipotent to neural subtypes *in vitro*

Cell types	Oligodendrocytes			Astrocytes			Neurons		
Repeats	1	2	3	1	2	3	1	2	3
Positive cells	33.3%	26.5%	19.3%	28.5%	23.8%	21.7%	9.8%	13.1%	11.6%

Each repeat was a single haploid neural stem cell sphere randomly selected for differentiation.

Supplementary Table 3 Comparison of maintenance of haploidy, differentiation capacity and survival rate of optimized and traditional human haploid embryonic stem cells

Group	DNA content after 6 wk without sorting, % haploid cells			EB			Area ratio of rosette			Surviving cells, %		
Repeats	1	2	3	1	2	3	1	2	3	1	2	3
Optimized	58.6	53.3	49.8	29	33	41	2.9	3.3	2.5	80.3	79.1	77.6
Traditional	23.4	33.6	19.8	18	22	17	1.0	1.0	1.0	44.5	51.3	55.6

All experiments were performed in triplicate. EB: Embryoid body.