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Supplemental information

Induced pluripotent stem cells and cerebral organoids from the critically endangered Sumatran rhinoceros

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Figure S1. CTG banding of SR-iPSCs confirms large block of heterochromatin at the terminal end of the long (q) arm of the X chromosome. Related to Figures 1E and 1F.

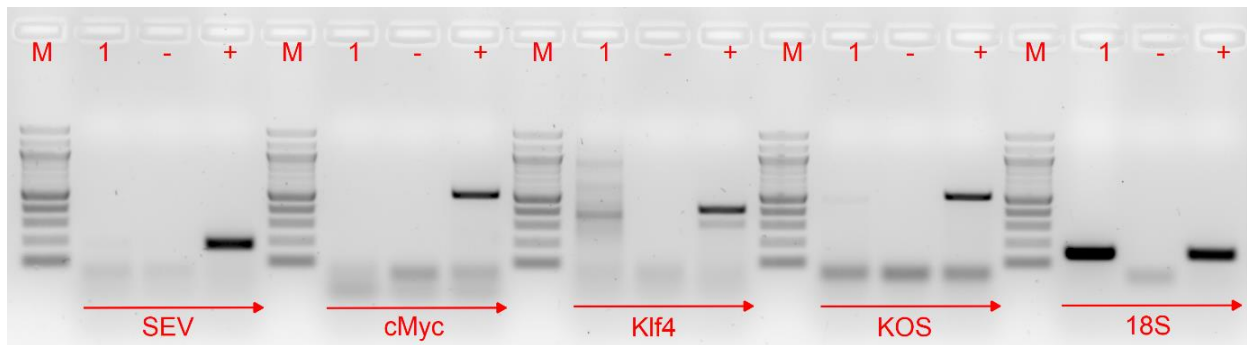


Figure S2. Absence of expression of exogenous reprogramming factors is demonstrated by reverse transcription PCR (RT-PCR). Related to Figure 1.

RNA isolated from SR-iPSCs (1) was reversely transcribed and amplified by PCR using primers targeting regions of the Sendai virus genome (SEV), and the reprogramming factors (cMyc, Klf4, KOS). Amplification of 18S RNA was included as loading control. Positive control (+) comprises a fibroblast sample collected at an early time point of reprogramming. Water was used as negative control (-). Unspecific amplification in the absence of Klf4 target RNA was also reported for human iPSCs. M: 100 bp ladder.

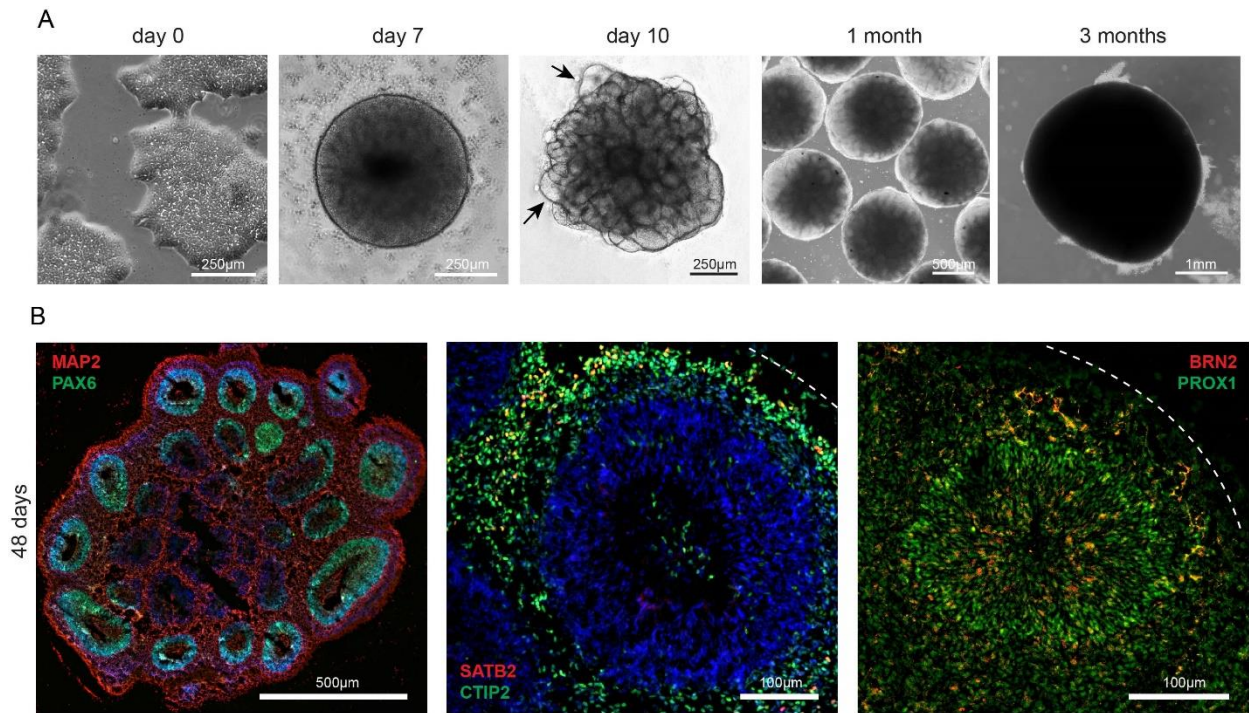


Figure S3. Generation of human cerebral organoids. Related to Figure 3.

A. Exemplary phase contrast images during organoid generation from human iPSCs at corresponding days of differentiation. Day 0: Feeder-free human iPSC colonies before dissociation; day 7: embryoid body showing a smooth and brightened surface; day 10: after addition of matrigel to the medium, organoids showed neuroepithelial bud outgrowth resembling neural tube-like structures (arrows), which are still visible after 1 month in culture. 3 months: mature organoid. Scale bars are defined within each image.

B. Immunostaining of 48 days old human brain organoids. Radially organized neural progenitors stain positive for PAX6, while neurons express MAP2, CTIP2, BRN2 and PROX1. Scale bars are defined within each image.

Table S1. List of antibodies. Related to STAR Methods.

Marker for	Antibody	Species	Company	Cat. #	Dilution
pluripotency	SOX2	rabbit	BioLegend	630802; RRID:AB_2195784	1:50
	OCT3/4	rabbit	Abcam	ab19857; RRID:AB_445175	1:100
	NANOG	rabbit	Thermo Fisher	PA1-097; RRID:AB_2539867	1:50
	SSEA3	rat	Thermo Fisher	MA1-020X; RRID:AB_2536686	1:200
	TRA-1-60	mouse	Cell Signaling	4746; RRID:AB_2119059	1:200
cardiomyocytes	ACTN2	mouse	Sigma-Aldrich	A7811; RRID:AB_476766	1:600
	TNNT2	rabbit	Abcam	ab45932; RRID:AB_956386	1:400
endoderm	GATA4	mouse	Santa Cruz	sc-25310; RRID:AB_627667	1:100
	GATA4	rabbit	Cell Signaling	36966; RRID:AB_2799108	1:400
	GATA6	goat	R and D Systems	AF1700; RRID:AB_2108901	1:100
	SOX17	goat	R and D Systems	AF1924; RRID:AB_355060	1:100
neural progenitor cells	NESTIN	mouse	Thermo Fisher	MA1-110; RRID:AB_2536821	1:50
	SOX1	goat	Thermo Fisher; ICC Kit	A24354	1:50
	SOX2	rabbit	BioLegend	630802; RRID:AB_2195784	1:100
	PAX6	rabbit	Thermo Fisher	42-6600; RRID:AB_2533534	1:200
	BRN2	mouse	Santa Cruz	sc-393324; RRID:AB_2737347	1:100
	FOXC1	mouse	Thermo Fisher	PA5-26794; RRID:AB_2544294	1:100
neurons	MAP2	guinea pig	Synaptic Systems	188 004; RRID:AB_2138181	1:500
	CTIP2	rat	Abcam	ab18465; RRID:AB_2064130	1:500
	SATB2	mouse	Santa Cruz	sc-81376; RRID:AB_1129287	1:100
astrocytes	GFAP	mouse	Thermo Fisher	A-21282; RRID:AB_2535827	1:500
2 nd Antibody	anti-goat Alexa Fluor 647	donkey	Thermo Fisher	A21447; RRID:AB_2535864	1:500
	anti-mouse Alexa Fluor 488	donkey	Thermo Fisher	A21202; RRID:AB_141607	1:500
	anti-rabbit Alexa Fluor 555	donkey	Thermo Fisher	A31572; RRID:AB_162543	1:500
	anti-rabbit Alexa Fluor 488	donkey	Thermo Fisher	A21206; RRID:AB_2535792	1:500
	anti-mouse Alexa Fluor 488	goat	Thermo Fisher	A21042; RRID:AB_2535711	1:500
	anti-rat Alexa Fluor 555	goat	Thermo Fisher	A21434; RRID:AB_2535855	1:500
	anti-guinea pig Alexa Fluor 647	goat	Thermo Fisher	A21450; RRID:AB_2735091	1:500