Supplementary Figures

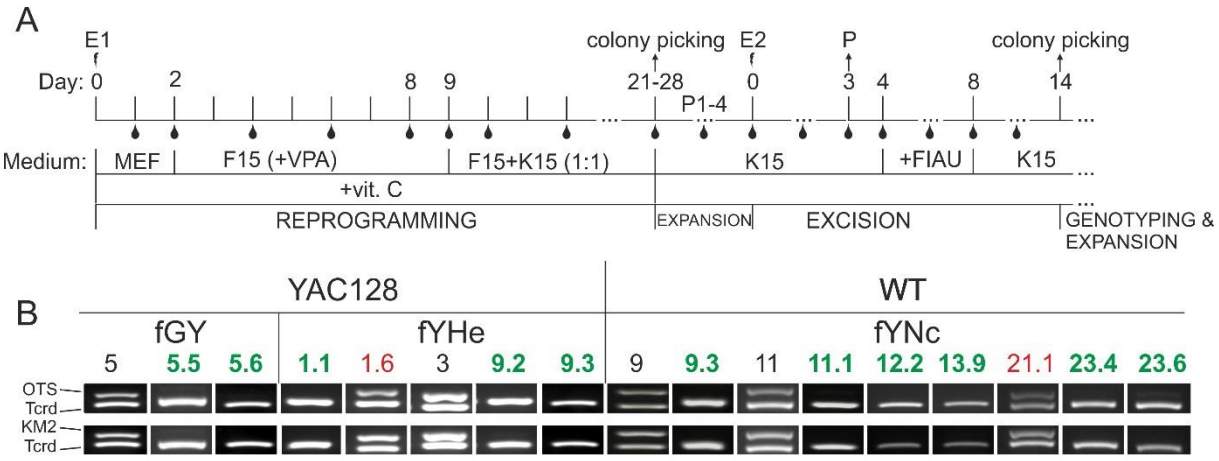

Fig. S1

Schematic illustration of the method used for iPSC generation using the piggyBAC system. (A) Reprograming of fibroblasts into iPSCs, harvesting iPSC colonies and the excision of reprogramming cassette. The black drops, P and E indicate medium changes, passage and electroporation, respectively. (B) The excision of the cassette was validated using multiplex PCR genotyping which lack of reprogramming cassette. Oct-to-Sox (OTS) was a PCR product for which primers were designed to bind to the linker between Oct3/4 and Sox2. Similarly, KM2 was a PCR product for which the primers bound to the linker between Klf4 and cMyc. The majority of the clones exhibited the excision of the cassette after selection using FIAU and are labeled with green numbers, whereas only a few of the clones still contained the cassette (red numbers). Pre-excision clones (positive control) are labeled with black numbers. Only the clones in which excision occurred (green labeled) were used in the studies described herein.

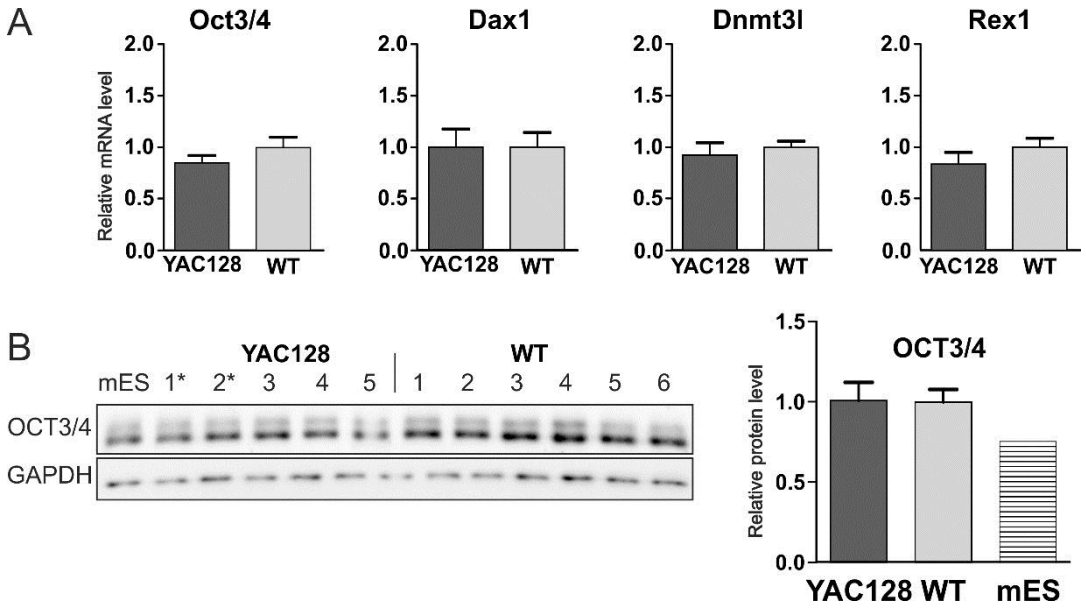

Fig. S2

Analysis of the expression of the pluripotency genes Oct3/4, Dax1, Dnmt3l and Rex1 using (A) qPCR and (B) western blotting demonstrated no differences between the YAC128 and WT lines. *YAC128/Oct-eGFP lines.

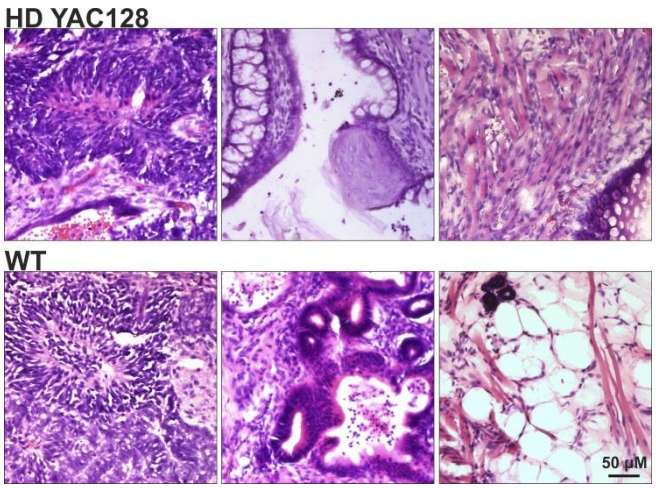

Fig. S3

Teratomas generated from HD-YAC128 and WT iPSCs contained cell types originating from the three germ layers: neuroepithelial cells (ectoderm); gut and respiratory epithelial cells, glands (endoderm); and muscle fibers, adipose tissue and cartilage (mesoderm).

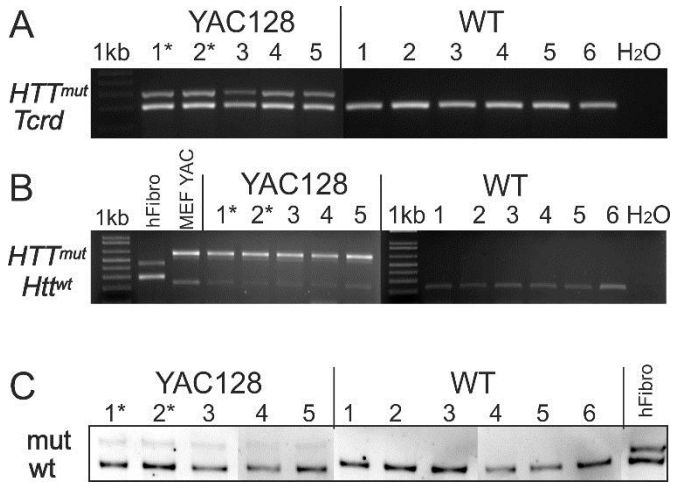

Fig. S4

Mutant huntingtin was expressed in YAC128 iPSCs (A) Genotyping for the presence of the mutant *HTT* gene in YAC128 and WT iPSCs. The multiplex touchdown-PCR reaction utilized endogenous *Tcrd* gene as the internal control (B) RT-PCR analysis of *HTT* mRNA expression. (C) Western blotting analysis of huntingtin expression in the HD YAC128 and WT iPSCs. (*) YAC128/Oct-eGFP lines; hFibro, human HD fibroblasts (GM04281, Coriell) containing 17 and 68 CAGs; MEF YAC, MEFs obtained from YAC128 mice.

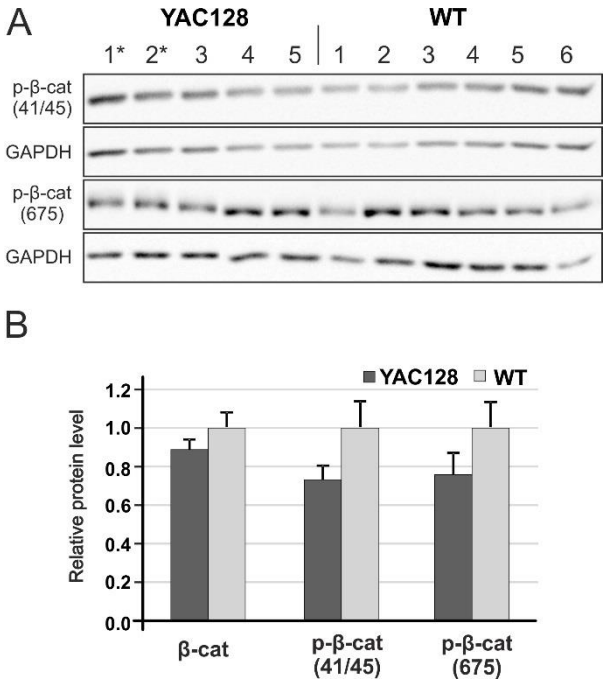

Fig. S5

The levels of p41/45-β-catenin and p675-β-catenin were similar in YAC128 iPSCs and WT iPSCs. (A) The levels of p41/45-β-catenin, p675-β-catenin and GAPDH were investigated in clonal lines of HD YAC128 (n=5) and WT iPSCs (n=6) using western blotting. (B) The quantification diagram for the levels of expression of p41/45-β-catenin and p675-β-catenin demonstrates similar degrees of activation in the HD YAC128 and WT iPSC lines. *YAC128/Oct-eGFP lines.

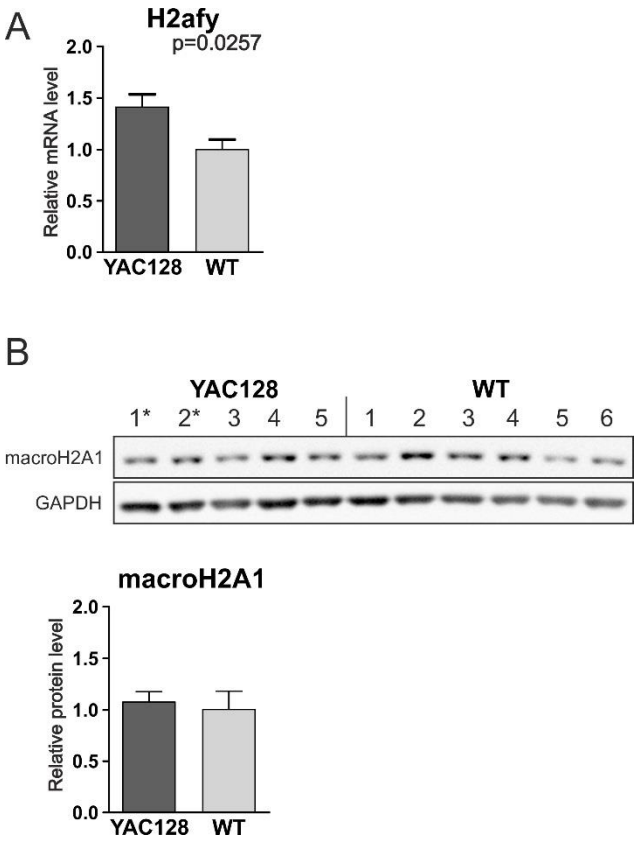

Fig. S6

The expression of the *H2afy* gene was increased (A) in the YAC128 iPSCs. The level of the resulting histone macroH2A1 (B) protein was similar in YAC128 and WT iPSCs. *YAC128/Oct-eGFP lines.

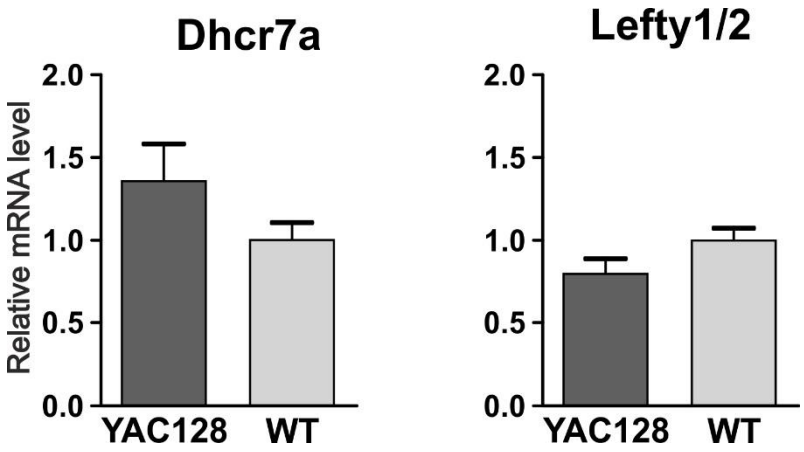

Fig. S7

The levels of mRNA expression of genes that play roles in lipid metabolism (*Dhcr7*), and signaling pathways (*Lefty1/2*) were similar in YAC128 and WT iPSCs, as revealed using real-time qPCR analysis.

SUPPLEMENTARY TABLE 1. List of primers used

Primer name		Sequence 5' -> 3'	Comment/Source
Genotyping			
pPB-OTS	F	TTCCCAACGAGAAGAGTATGAGGCTACA	transposon removal
	R	GCTCCGTCTCCATCATGTTATACATTGG	
pPB-KM2	F	AGGCGAGAAACCTTACCACTGTGACTG	transposon removal
	R	AGTCGAGGTCATAGTTCCTGTTGGTGAA	
NI26_27	F	CCTCTTATATATGGATGCTAATCTCATTC	YAC128 transgene
	R	AATACACAACACATGAGAGCATATAGAAC	
Tcrd	F	CAAATGTTGCTTGTCTGGTG	internal control
	R	GTCAGTCGAGTGCACAGTTT	
RT-PCR			
mCripto (Tdgf1)	F	ATGGACGCAACTGTGAACATGATGTTCGCA	(1)
	R	CTTTGAGGTCCTGGTCCATCACGTGACCAT	
mDax1 (Nr0b1)	F	TGCTGCGGTCCAGGCCATCAAGAG	(1)
	R	GGGCACTGTTCAGTTCAGCGGATC	
mDnmt3l	F	CCCTCTTCCTGTATGATGATGATGG	(2)
	R	CCTCTGCAGCAGTCCACTCCGTGAG	
mDppa3	F	GAGGACGCTTTGGATGATACAGACG	(2)
	R	CAACAAAGTGCGGACCCTTCTCTTG	
mEcat1 (Khdc3)	F	TGTGGGGCCCTGAAAGGCGAGCTGAGAT	(1)
	R	ATGGGCCGCCATACGACGACGCTCAACT	
mERas	F	ACTGCCCCTCATCAGACTGCTACT	(1)
	R	CACTGCCTTGTACTCGGGTAGCTG	
mEsrrb	F	AACCTGCCGATTTCCCCACCTGCTA	(2)
	R	GGCTCATCTGGTCCCCAAGTGTCAG	
mGapdh	F	AATGGTGAAGGTCGGTGTG	
	R	AAGATGGTGATGGGCTTCC	
emKlf4 endo	F	GGCGAGAAACCTTACCACTGT	(3)
	R	TACTGAACTCTCTCTCCTGGCA	
emcMyc endo	F	TCAAGCAGACGAGCACAAGC	(3)
	R	TACAGTCCCAAAGCCCCAGC	
emNanog endo	F	GTGCATATACTCTCTCCTTCCC	(3)
	R	AGCTACCCTCAAACTCCTGGT	
emOct3/4 endo (Pou5f1)	F	CCAACGAGAAGAGTATGAGGC	(3)
	R	GTGCTTTTAATCCCTCCTCAG	
mRex1 (Zfp42)	F	ACGAGTGGCAGTTTCTTCTTGGGA	(1)
	R	TATGACTCACTTCCAGGGGGCACT	
emSox2 endo	F	TCTGTGGTCAAGTCCGAGGC	(3)
	R	TTCTCCAGTTCGCAGTCCAG	
mUtf1	F	GGATGTCCCGGTGACTACGTCTG	(1)
	R	GGCGGATCTGGTTATCGAAGGGT	
mZfp296	F	CCATTAGGGGCCATCATCGCTTTC	(1)
	R	CACTGCTCACTGGAGGGGGCTTGC	
HD-dl	R	CACGGTCTTTCTTGGTAGCTG	human huntingtin mRNA (mismatch with mouse sequence) ; (4)
	F	CCCTGGAAAAGCTGATGAAG	

qPCR			
qmDax1 (Nr0b1)	R	ATCTGCTGGGTTCTCCACTG	(5)
	F	CTATCTGAAAGGGACCGTGC	
qmDnmt3	R	GCTTGCTCCTGCTTCTGACT	(5)
	F	GGTGTGGAGCAACATTCCAG	
qmOct4 (Pou5f1)	R	TCTTCTGCTTCAGCAGCTTG	(5)
	F	GTTGGAGAAGGTGGAACCAA	
qmRex1 (Zfp42)	R	TATGACTCACTTCCAGGGGG	(5)
	F	AGAAGAAAGCAGGATCGCCT	
Dhcr7	R	TGAGGTCACAGACGACCAAT	(5)
	F	ACAGGCCAGTCTGATGGAAG	
Gapdh	R	TTGATGGCAACAATCTCCAC	(5)
	F	CGTCCCGTAGACAAAATGGT	
Gpx1	R	CAATGTAAAATTGGGCTCGAA	(5)
	F	GTTTCCCGTGCAATCAGTTC	
GSK3β	R	GTGGTTACCTTGCTGCCATC	(5)
	F	GACCGAGAACCACCTCCTTT	
H2afy	R	TGCTCACTTCTCCCTGCTTC	
	F	GCCAAAAAGGCCAAGTCTCC	
Lefty1/2	R	TGCAGTAGACTGCTCAGGACC	(5)
	F	CATGAAGTCCCTGTGGCTTT	
Prdx1	R	TTGATGGTATCACTGCCAGG	(5)
	F	CCGCTCTGTGGATGAGATTA	
Sod1	R	TACTGATGGACGTGGAACCC	(5)
	F	GAACCATCCACTTCGAGCA	
Trp53	R	TCCGACTGTGACTCCTCCAT	(5)
	F	CTAGCATTCAGGCCCTCATC	

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3. Kaji, K. *et al.* Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature* **458,** 771–775 (2009).
4. Fiszer, A., Mykowska, A. & Krzyzosiak, W. J. Inhibition of mutant huntingtin expression by RNA duplex targeting expanded CAG repeats. *Nucleic Acids Res.* **39,** 5578–5585 (2011).
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SUPPLEMENTARY TABLE 2.

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