SUPPLEMENTARY INFORMATION

EXPERIMENTAL PROCEDURES

Cell culture

Cell culture techniques have been described previously[1]. C1 hiPSCs[2] and the hESC lines WIBR#3[3] were maintained on mitomycin C inactivated mouse embryonic fibroblast (MEF) feeder layers in hESC medium [DMEM/F12 (Invitrogen) supplemented with 15% fetal bovine serum (FBS) (Hyclone), 5% KnockOutTM Serum Replacement (Invitrogen), 1 mM glutamine (Invitrogen), 1% nonessential amino acids (Invitrogen), 0.1 mM β-mercaptoethanol (Sigma) and 4 ng/ml FGF2 (R&D systems)]. Cultures were passaged every 5 to 7 days either manually or enzymatically with collagenase type IV (Invitrogen; 1.5 mg/ml). In order to partially remove feeder cells prior to FACS analysis, OCT4-eGFP targeted hESCs were passaged using collagenase. Cells were plated onto matrigel coated plates and cultured for 24-48 hours in mTESRI medium prior FACS analysis.

Genome editing with TALENs in transformed cells

TALENs were designed and assembled as described[4] except that the RVD in the C-terminal "half repeat" was not fixed as NG. Tandem arrays of TALE repeats were assembled from smaller fragments containing shorter tandem arrays of TALE repeats by PCR as described (ref 10).[4] All TALENs used the +63 truncation point[4]. The complete sequence of all TALENs used in this work is provided in supplementary information. K562 cells were obtained from the American Type Culture Collection (ATCC CCL-243) and grown and transfected with TALENS as described previously [4] . Four hundred thousand cells were transfected (estimated transfection efficiency ~90%), genomic DNA was extracted without selecting for transfected cells with Epicentre QuickExtract solution. Approximately 8,000 genome equivalents were used as input for each

PCR. TALEN activity was assayed via Surveyor nuclease (Transgenomic) using the following primers: PPP1R12C, Cel-I F2 (5'-ccc ctt acc tct cta gtc tgt gc-3') and Cel-I R1 (5'-ctc agg ttc tgg gag agg gta g-3'); PITX3, GJC 289F (5'-tta cga gga aac gct gct gg-3') and GJC 290R (5'-cca agc cag cgc ata ttc tc-3') along with GJC 291F (5'-aaa gcc aaa cag cac gcc tc-3') and GJC 292R (5'-gtc tgt gtg tag ggc cta gt-3'); OCT4, Group1F (5'-agg cgg ctt gga gac ctc tca-3') and Group1R (5'-tag gag atg tga gag acc ctg aca agg-3') along with GJC 295F (5'-ctt tct gtc att cac ttg cag-3') and GJC 296R (5'-act gaa gga caa acc aag at-3'). We used the primers indicated at a final concentration of 1 micromolar each in 50 microliter reactions with Accuprime Taq HiFi (Invitrogen) in buffer II, and ran the PCR reactions on a BioRad Tetrad as follows: an initial denaturation step (95 degrees, 3 min); next, 35 cycles of a denaturation step of 30 sec at 95 degrees, annealing step of 30 sec at 60 degrees, and an extension step of 30 sec at 72 degrees; a final extension step of 7 min at 72 degrees.

Targeting of hESCs and hiPSCs using TALEN mediated homologous recombination

hESCs and hiPSCs were cultured in Rho Kinase (ROCK)-inhibitor (Calbiochem; Y-27632) 24 hours prior to electroporation. Cell were harvested using 0.25% trypsin/EDTA solution (Invitrogen) and 1×10^7 cells resuspended in phosphate buffered saline (PBS) were electroporated if not otherwise indicated with 40 µg of donor plasmids (previously described in[5] or designed and assembled by D.H. and H.W.) and 5 µg of each TALEN encoding plasmid (Gene Pulser Xcell System, Bio-Rad: 250 V, 500µ F, 0.4 cm cuvettes[5]). Cells were subsequently plated on MEF feeder layers (DR4 MEFs for puromycin selection) in hESC medium supplemented with ROCK-inhibitor for the first 24 hours. Individual colonies were picked and expanded after puromycin selection (0.5 µg/ml) 10 to 14 days after electroporation.

Immunocytochemistry

Cells were fixed in 4% paraformaldehyde in PBS and immunostained according to standard protocols using the following antibodies: SSEA4 (mouse monoclonal, Developmental Studies Hybridoma Bank); Tra-1-60 and Tra-1-81, (mouse monoclonal, Chemicon International); hSOX2 (goat polyclonal, R&D Systems); OCT-3/4 (mouse monoclonal, Santa Cruz Biotechnology); hNANOG (goat polyclonal R&D Systems) and appropriate Molecular Probes Alexa Fluor® dye conjugated secondary antibodies (Invitrogen).

FACS analysis

GFP-positive cells were measured using the BD LSR II flow cytometer and analyzed with FlowJo data analysis software.

Fibroblast differentiation of OCT4-eGFP hESCs

For EB induced differentiation, hESC colonies were harvested using 1.5 mg/ml collagenase type IV (Invitrogen), separated from the MEF feeder cells by gravity, gently triturated and cultured for 7 days in non-adherent suspension culture dishes (Corning) in DMEM supplemented with 15% fetal bovine serum. EBs were plated onto adherent tissue culture dishes and passaged according to primary fibroblast protocols using trypsin.

Removal of PGK-Puro cassette by transient Cre-recombinase expression

hESCs targeted in the PITX3 locus were cultured in Rho Kinase (ROCK)-inhibitor (Calbiochem; Y-27632) 24 hours prior to electroporation. Cell were harvested using 0.25% trypsin/EDTA solution (Invitrogen) and 1×10^7 cells resuspended in PBS were electroporated with pTurbo-Cre (40 µg; Genbank Accession Number AF334827) and pEGFP-N1 (10µg; Clontech) as described previously[5] (Gene Pulser Xcell System, Bio-Rad: 250 V, 500µF, 0.4 cm cuvettes). Cells were subsequently plated on MEF feeder layers (DR4 MEFs for puromycin selection) in hESC medium supplemented with ROCK-inhibitor. Cre-recombinase expressing cells were enriched by FACS sorting (FACS-Aria; BD-Biosciences) of a single cell suspension for EGFP expressing cells 60 hours after electroporation

followed by replating at a low density in ROCK-inhibitor containing hESC medium. Individual colonies were picked 10 to 14 days after electroporation.

Teratoma formation and analysis:

hESCs were collected by collagenase treatment (1.5mg/ml) and separated from feeder cells by subsequent washes with medium and sedimentation by gravity. hESCs aggregates were collected by centrifugation, resuspended in 250µl of phosphate buffered saline (PBS) and injected subcutaneously in the back of SCID mice (Taconic). Tumors generally developed within 4-8 weeks and animals were sacrificed before tumor size exceeded 1.5 cm in diameter. Teratomas were isolated after sacrificing the mice and fixed in formalin. After sectioning, teratomas were diagnosed based on hematoxylin and eosin staining. Immunostaining of paraffin section was performed with standard techniques using a rabbit polyclonal anti-GFP antibody (Abcam 290).

Experimental determination of genome-wide specificity of action by the TALENs

The actual binding site for TALENs directed against the PPP1R12C locus was determined by in vitro SELEX exactly as described[6]; the SELEX data are shown in Supplemental Figure 9. The top ranked off-target sites were identified as previously described[6] using the SELEX-derived base frequency matrices in Supplemental Table 3. Capital letters indicate either (i) a match to the SELEX consensus or (ii) a non-targeted position within the TALEN heterodimer site. The genome searches for candidate off-target sites allowed for TALEN site pairings with 11 - 26 bp between individual targets. For logistical reasons, in this specific experiment we used TALENs carrying conventional FokI domains, but since TALENS carrying enhanced high-fidelity obligate heterodimer FokI domains[7] directed against the PPP1R12C locus are robustly active both in transformed cells and hESCs/iPSCs (Table 1), we analyzed off-targets solely for TALEN heterodimers. Human ESCs were electroporated with TALEN expression constructs exactly as described for ZFNs[5], and genomic DNA was harvested 72

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hours later. Either 5µg of each TALEN and 40µg of a pEGFP-N1 expression plasmid or, as a control, 50µg of the pEGFP-N1 plasmid alone were electroporated. Illumina NextGen single-read 75 bp sequencing was used to interrogate the PPP1R12C on-target and top 20 SELEX-based off-target heterodimer sites for both control and TALEN-treated cells. Standard sequencing adapters were added via PCR in two steps using High-Fidelity Phusion Hot Start II Polymerase (NEB). In the first round, each site was amplified in a 20-cycle PCR using 200 ng genomic DNA and the primers listed in Supplemental Table 3, which also encoded the sequences 5'-CTACACTCTTTCCCTACACGACGCTCTTCCGATCT (For Seq) and 5'-CAAGCAGAAGACGGCATACGAGCTCTTCCGATCT (Rev Seq) at the 5' ends. In the second round, an additional 20 cycles were run using 1 ul of product from the first PCR round and the primers SOLEXA-OUT-F1 (5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACG) and SOLEXA-OUT-R1 (5'-CAAGCAGAAGACGGCATA) to generate complete Illumina sequencing amplicons. The resulting PCR products were then gel-purified on 2% TBE-Agarose gels using Qiaquick Gel Extraction (Qiagen) and subsequently re-purified on Qiaquick PCR Purification columns (Qiagen). The samples were normalized to 20 nM each and combined in equal volumes so that no more than 11 sites were sequenced in a single Illumina lane. Control and TALEN-treated samples were processed in separate sequencing lanes. Sequences were binned by locus and then aligned to the appropriate wild-type sequence. Insertions and deletions (Indels) that occurred within a 10 bp window centered between the TALEN binding sites and contained 5 of fewer mismatched bases (excluding the InDel) were considered potential NHEJ events. This tended to yield a background InDel frequency of 0.04%-0.05%. To further reduce the background InDel frequency, InDels in the TALEN treated samples were compared to InDels in the corresponding control. Any InDel that had a normalized frequency at least 10-fold higher in the TALEN treated samples than in the corresponding control was considered for further analysis and background corrected by subtracting the frequency (corrected for overall sample size) of the

same InDel in the control samples. The converse correction was also performed (i.e. InDels that were observed at least 10-fold more frequently in the control than in the corresponding TALEN treated sample was considered for further analysis and corrected by subtracting the frequency of the same InDel observed in the corresponding TALEN treated sample). This correction yields a background level of approximately 0.0002% (i.e. one event for every 500,000 sequences). For unknown reasons, the control sample for off-target 19 yielded sequences for the incorrect locus, and while the TALEN-treated sample for that off-target yielded sequences in which InDels occurred at frequencies comparable to those where background correction demonstrates no off-target activity, lack of data from a control sample prevents us from fully analyzing that sample. In summary this analysis showed that in PPP1R12C TALEN-edited cells unintended cleavage occurs only at low-frequency: 17 loci remained wild-type, one of these sites was disrupted 169-fold less often than the intended target, and a second site was disrupted 1,140-fold less frequently (Figure S10 and Table S3 and 4).

Supplementary discussion and off-target analysis

Targeting the Stop Codon of OCT4- a position with redundant TALEN binding sites

Nucleases are routinely designed to target unique sites in the genome. In some rare cases, however, this is impossible; for instance, in editing the OCT4 locus, we found that a significant stretch of the DNA surrounding its stop codon occurs at 4 other unlinked positions in the genome within known OCT4 pseudogenes. The nucleases that cut at the stop, therefore, have 4 perfect matches elsewhere in the human genome (coordinates listed as per hg19 on genome.ucsc.edu):

% match	chr	strand	start	end
100.00%	6	-	31132351	31132405
100.00%	12	-	8286340	8286394
100.00%	1	+	155404027	155404080
98.20%	8	+	128429164	128429218
100.00%	10	-	69769937	69769988

We genotyped all 4 of these other perfect target sites in 5 distinct single-cell-derived clones, all correctly edited at the OCT4 locus. The genotyping was done by PCR and Sanger sequencing, and was significantly complicated by the existence of a ~1.4 kb stretch of near-perfect homology between the sites ("ND" – no interpretable data obtained; "wt/?" – wild-type chromatids were obtained, but not at a number where a statistically significant argument could be made for wild-type homozygosity).

clone #	clone ID	chr 1	chr 8	chr 10	chr 12
1	7263	Δ16/?	N.D.	wt	Δ8/wt
2	7282	N.D.	wt	wt	N.D.
3	7111	wt/ Δ8	wt	wt	wt
4	7189	wt/?	Δ9/Δ5	wt	Δ3/Δ1
5	7187	wt/?	Δ8/wt	wt	Δ7/wt
6 (ctrl)	7464	wt	wt	wt	wt

One of the sites on chromosome 10 (chr 10) remained wild-type in all the clones. The site on chr 8 was wild-type in 2 clones, and carried small insertion or deletions (indels) in two others. For the site on chr 12 one clone remained wild-type, while 3 clones carried small indels. The site on chr 1 did not yield comprehensive data, but of note, clone #3 was wild-type for all off-target sites except an indel at one allele of that site. Since Southern blotting on these clones conclusively shows that the donor-specified insert was only transferred to the OCT4 locus, these data show that even in such rare circumstances (the occurrence of 4 perfect matches to the nuclease site), by relying on homology-directed repair it is possible to obtain cells that carry the insert at just one site, and with a minimal number of off-target mutations that do not affect cell phenotype in any measurable way. In the majority of cases, however, we design nucleases – both ZFNs and TALENs – to unique sites in the genome, and experimental measurement of nuclease action at off-target sites that inevitably carry multiple mismatches to the intended site has shown these to remain wild-

type, with very rare exceptions (e.g., Perez et al, Nature Biotechnology 2008; Hockemeyer et al Nature Biotechnology 2009).
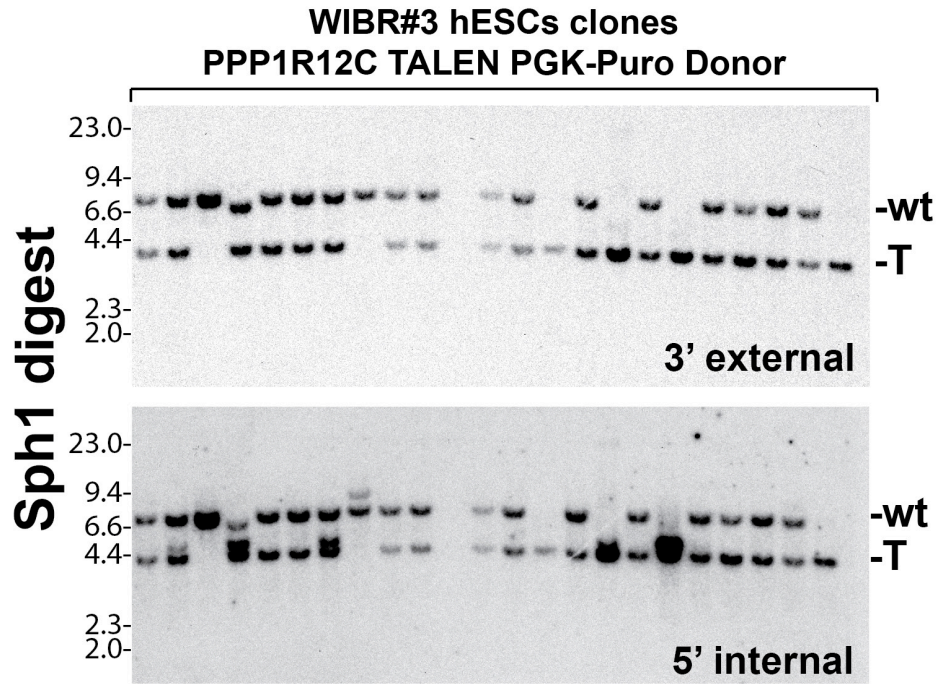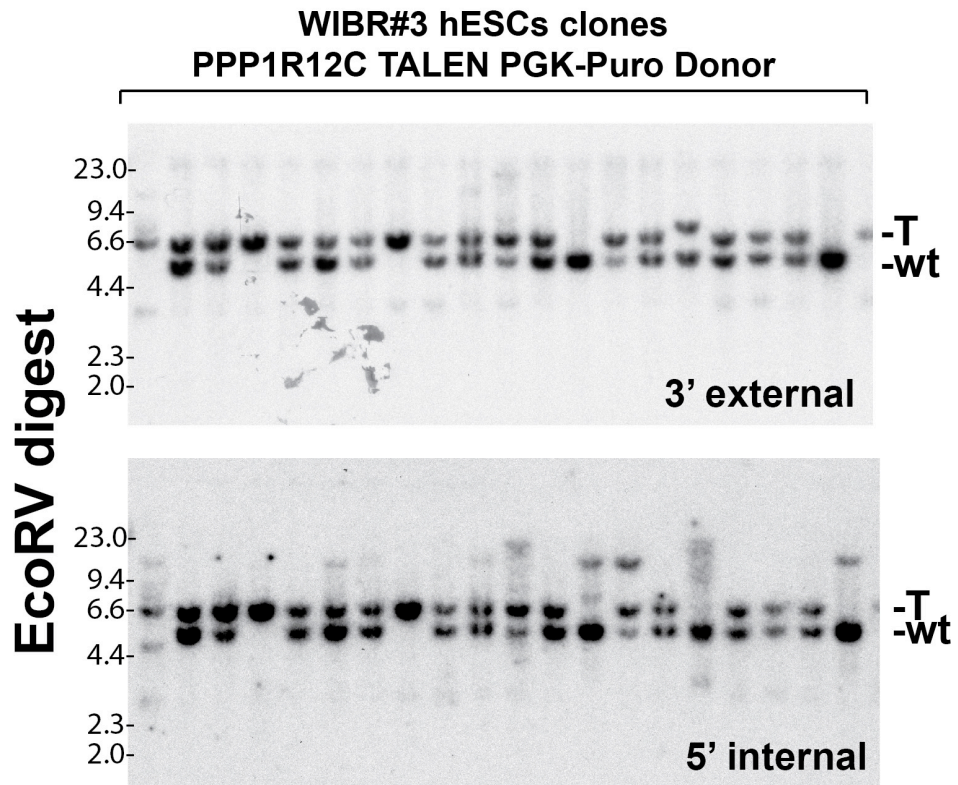
Supplemental Figure 1

A
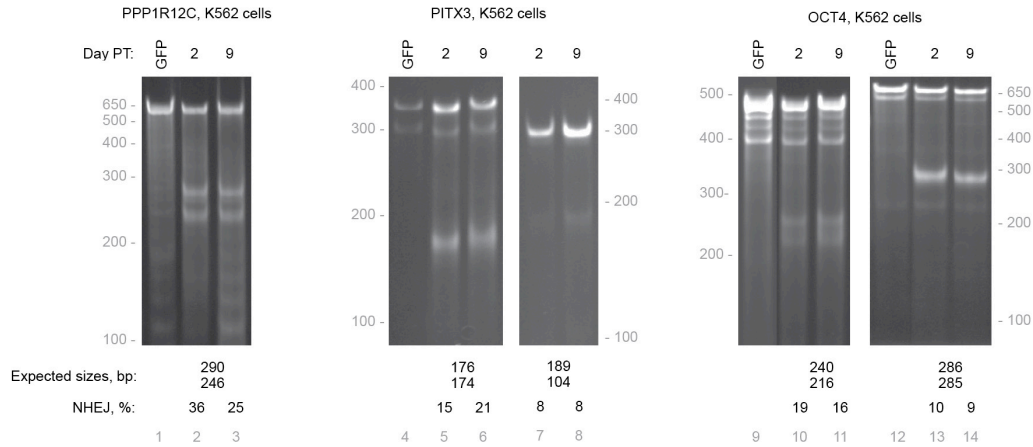
Assay of TALEN activity with the Surveyor nuclease. The AAVS1, PITX3, and OCT4 loci were PCR amplified and modification of the locus was measured using the Surveyor nuclease. The expected sizes of correctly digested fragments are shown below each panel. The degree of modification was quantified and is shown below each lane. GFP indicates GFP control transfection, 2 and 9 indicate days after transfection.

Supplemental Figure 2

A

WIBR#3 hESCs clones
PPP1R12C TALEN PGK-Puro Donor

B

WIBR#3 hESCs clones
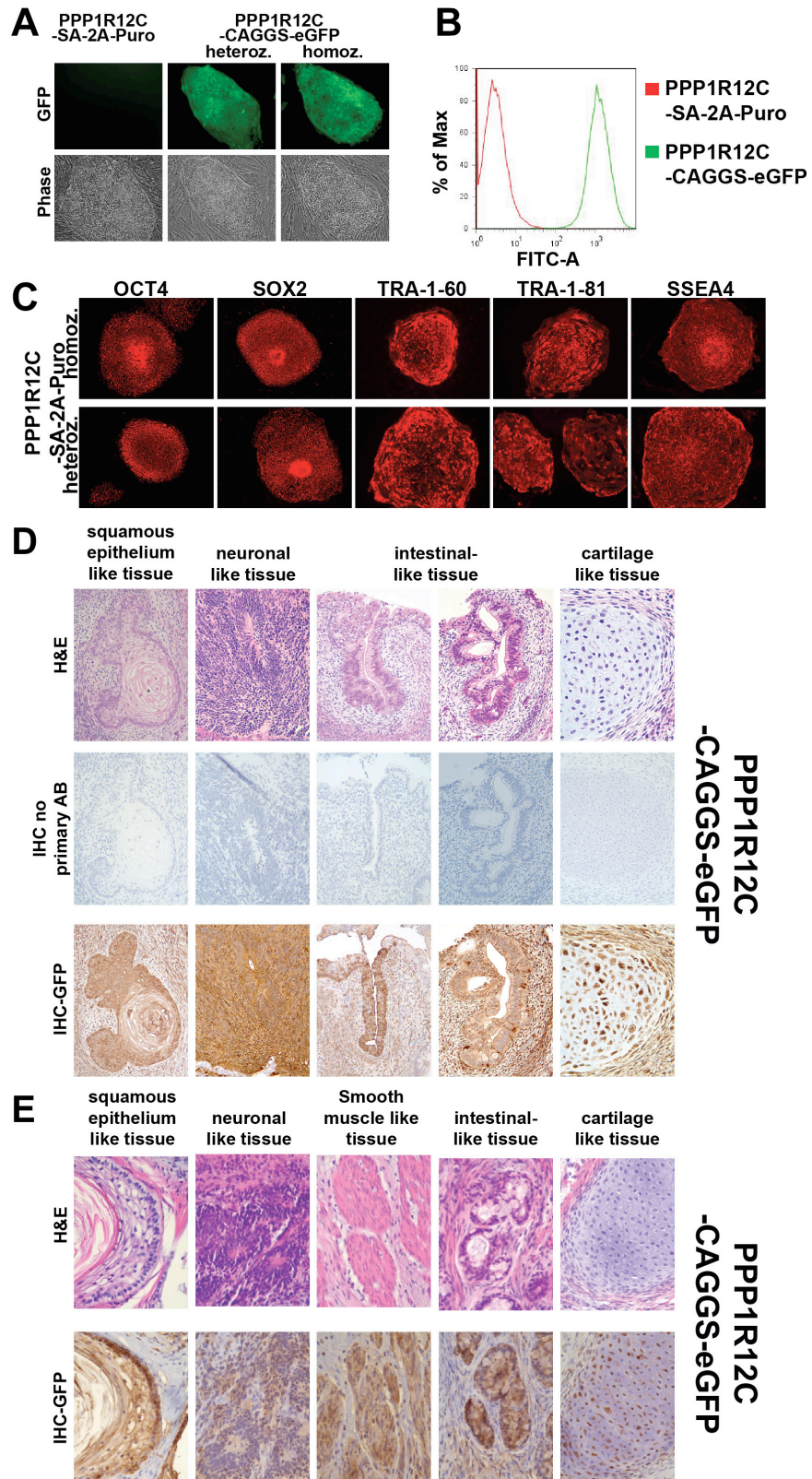**PPP1R12C TALEN PGK-Puro Donor**

Targeting PPP1R12C with a PGK-Puro cassette using TALENs

A. Southern blot analysis of WIBR#3 hESCs cells targeted using the AAVS1-PGK-Puro donor plasmid and the PPP1R12C TALENs. Genomic DNA was digested with SphI and hybridized with the ^{32}P-labeled external 3'-probe or with the internal 5'-probe. Fragment sizes are: PGK-Puro: 5'-probe: wt=6.5 kb, targeted=4.2 kb; 3'-probe: wt=6.5 kb, targeted=3.7 kb.

B. Southern blot analysis of WIBR#3 hESCs cells targeted using the PPP1R12C-PGK-Puro donor plasmid and the PPP1R12C TALENs. Genomic DNA was digested with EcoRV and hybridized with the ^{32}P-labeled external 3'-probe or with the internal 5'-probe. As previously described for ZFN targeting of the PPP1R12C locus, a fraction of clones, although targeted, carried additional integrations[5]. Since EcoRV does not cut in the donor vector, the detection of higher than expected molecular weight restriction fragments in some of the targeted clones suggests that the additional integration detected with the internal 5'-probe might represent aberrant or multiple integrations of the donor plasmids in the PPP1R12C (AAVS1) locus rather than an integration caused by off-target effects of the PPP1R12C TALENs. These clones were considered as not correctly targeted and not analyzed further. The majority of clones obtained were correctly targeted on one or both TALEN-targeted alleles, and lacked randomly integrated DNA.

Supplemental Figure 3

A

PPP1R12C -SA-2A-Puro

PPP1R12C -CAGGS-eGFP heteroz. homoz.

GFP

Phase

B

■ PPP1R12C -SA-2A-Puro

■ PPP1R12C -CAGGS-eGFP

C

| | OCT4 | SOX2 | TRA-1-60 | TRA-1-81 | SSEA4 |

PPP1R12C -SA-2A-Puro homoz.

PPP1R12C -SA-2A-Puro heteroz.

D

squamous epithelium like tissue | neuronal like tissue | intestinal-like tissue | cartilage like tissue

H&E

IHC no primary AB

IHC-GFP

PPP1R12C -CAGGS-eGFP

E

squamous epithelium like tissue | neuronal like tissue | Smooth muscle like tissue | intestinal-like tissue | cartilage like tissue

H&E

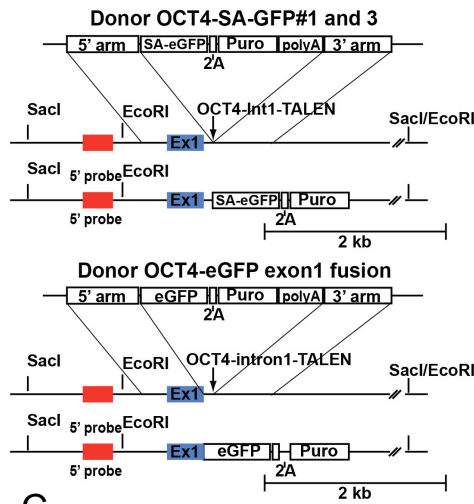IHC-GFP

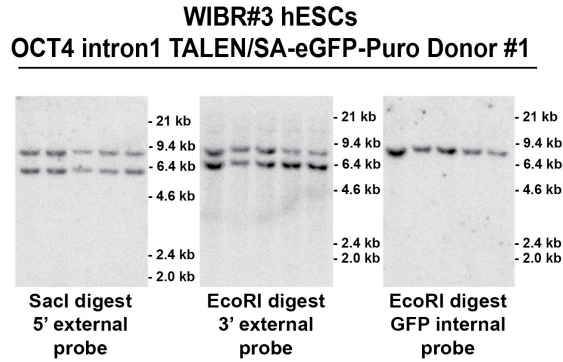PPP1R12C -CAGGS-eGFP

12

Targeting PPP1R12C using TALENs

A. Phase contrast picture and fluorescence imaging of eGFP in heterozygous or homozygous WIBR#3 hESC clones targeted with the PPP1R12C-CAGGS-eGFP donor as shown in (A) using the PPP1R12C TALENs.

B. FACS analysis of eGFP fluorescence in WIBR#3 hESCs targeted in the PPP1R12C locus with the SA-2A-Puro donor or the CAGGS-eGFP donor.

C. Immunofluorescence staining of WIBR#3 hESCs clones targeted with AAVS1 TALENs as shown in Figure 1A and B using the PPP1R12C-SA-2A-Puro donor plasmids. Cells were stained for the pluripotency markers OCT4, SOX2, Tra-1-60, Tra-1-81 and SSEA4.

D. Hematoxylin and eosin staining (H&E) of teratoma sections generated from WIBR#3 hESCs cells targeted with the PPP1R12C-CAGGS-eGFP donor plasmid and PPP1R12C **TALENs** (top row) and immunohistochemistry (IHC) staining for eGFP in neighboring sections (bottom row). As a control for staining specificity, neighboring sections were also stained omitting primary antibody incubation (middle row).

E. H&E of teratoma sections generated from WIBR#3 hESCs cells targeted with the PPP1R12C-CAGGS-eGFP donor plasmid and PPP1R12C **ZFN** (top row) and IHC staining for eGFP expression in neighboring sections (bottom row).
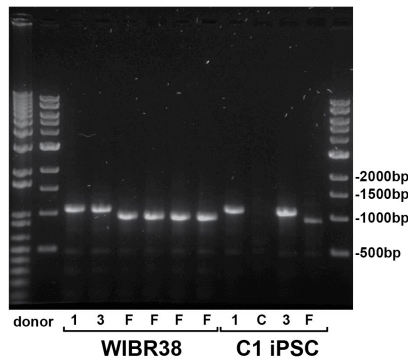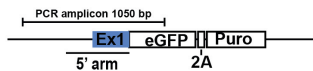
Supplemental Figure 4
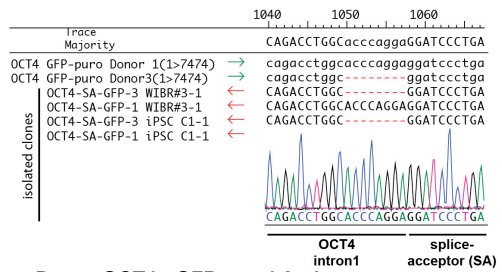
A

Donor OCT4-SA-GFP#1 and 3

Donor OCT4-eGFP exon1 fusion

B

WIBR#3 hESCs
OCT4 intron1 TALEN/SA-eGFP-Puro Donor #1

SacI digest
5' external
probe

EcoRI digest
3' external
probe

EcoRI digest
GFP internal
probe

C

Donor OCT4-SA-eGFP#1 and 3:

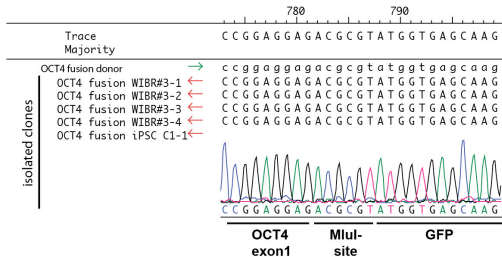**Donor OCT4-eGFP exon1 fusion (F):**

D

**Donor OCT4-SA-eGFP#1 and 3:**

Donor OCT4-eGFPexon1 fusion:

Targeting OCT4 to generate an OCT4-EXON1-GFP fusion protein

A. Schematic overview depicting the targeting strategy for the OCT4 locus using TALENs directed against the first intron of OCT4. We used three different donor plasmids to target the OCT4 locus. The first two donor plasmids (top schematic) (previously described as donor 1 and donor 3[5]) were designed to integrate a splice acceptor eGFP-2A-Puro cassette into the first intron of OCT4. The design of donor1 and 3 differed only in the positioning of the homology arms with respect to the TALEN cut. In donor 1, the 5' homology arm was comprised of homology sequences upstream of the 94 bp of the first OCT4 intron. In donor 3 this homology started at

position 102 bp. The third donor – named OCT4 EXON1-GFP fusion--(bottom panel) was engineered so that the (left) 5' homology arm starts with the last nucleotide of the first exon of OCT4. This targeting strategy requires that the DSB, generated by the TALENs, gets resected and single stranded DNA is generated to the start of the homology arm. Homology based DNA repair will result in a fusion of exon 1 to the reading frame of the eGFP-2A-puromycin cassette. Probes used for Southern blot analysis are shown as red boxes, exons of the OCT4 locus are shown as blue boxes and arrows indicate the genomic site cut of the OCT4 TALEN; SA-eGFP: splice acceptor eGFP sequence, 2A: self-cleaving peptide sequence, Puro: puromycin resistance gene, polyA: polyadenylation sequence.

B. Southern blot analysis of WIBR#3 hESCs targeted with the indicated OCT4-Intron1 TALENs using the indicated donor plasmids. Genomic DNA was digested either with SacI and hybridized with the external 5'-probe (left panel) or digested with EcoRI and hybridized with the external 3'-probe (middle panel) or internal eGFP probe (right panel). Shown are three independent single cell derived clones for each donor.

C. Genotyping PCR of hESCs targeted using the indicated donors and OCT4-Intron1 TALENs as described in (A) and found to be correctly targeted based on Southern blot analysis as shown in (B). The PCR strategy is shown below. Primers were positioned in genomic regions 5' (outside) of the homology arms and in eGFP. Expected fragment size: Donor 1:1180 bp (lane labeled with 1), donor 3: 1172 bp (lane labeled with 3), donor fusion: 1050 bp (lane labeled with F). Primer sequences were: FW: GAGCAGAAGGATTGCTTTGG; BW: AAGTCGTGCTGCTTCATGTG. Size markers are 1kb+ (NEB) first lane and 1kb (NEB) second and last lane.

D. Sequencing analysis and sequence alignment of PCR products shown in (C). Sequencing confirmed the correct integration of the transgenes at the position specified by the donor plasmid. Top panel: sequence analysis and alignment for donor 1 and 3. Bottom panel: sequence analysis and alignment for OCT4 fusion donor. MluI indicates an in-frame restriction site used during donor design. This data illustrates that the position of transgene insertion using a given TALEN can be modified using alternative donor designs.

Supplemental Figure 5
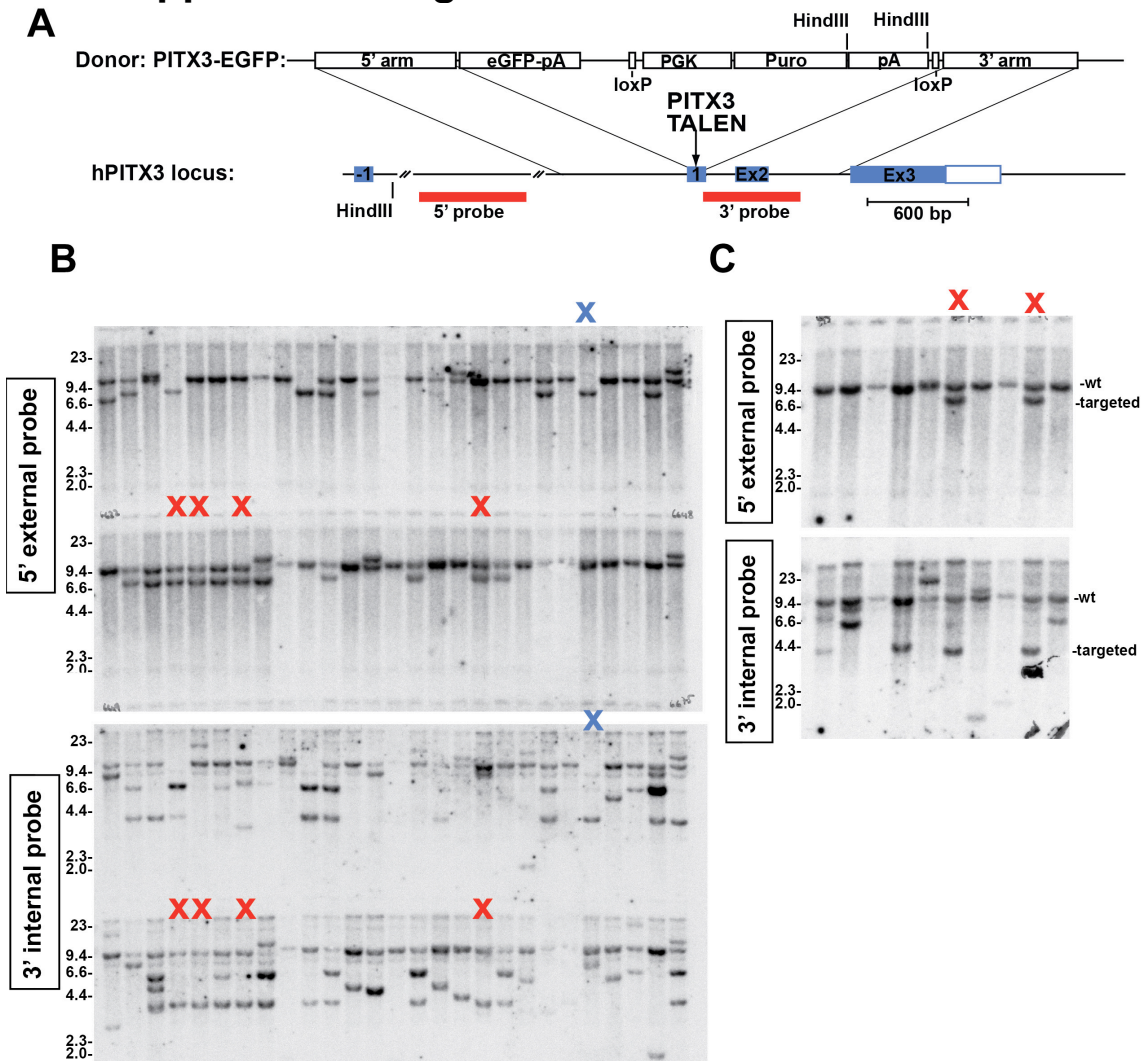
Targeting PITX3 using TALENs

A. Schematic overview depicting the targeting strategy for the PITX3 gene. Probes for Southern blot analysis are shown as red boxes, the first exons of the PITX3 locus are shown as blue boxes and arrows indicate the genomic site cut by the PITX3 TALENs. The donor plasmid used to target the PITX3 locus is shown above and contained 5' and 3' homologous sequences of approximately 800 bp flanking the predicted TALEN cut site. eGFP: enhanced green fluorescent protein, PGK: human phophoglycerol kinase promoter, Puro: puromycin resistance gene, loxP: loxP sites, pA: polyadenylation sequence. Two constructs (PITX3 donor FW and BW) that differed only in the orientation of this selection cassette with respect to the PITX3 gene were successfully used to target PITX3 (See also Table 1).

B. Southern blot analysis of WIBR#3 hESCs targeted with the PITX3 FW donor plasmid using the PITX3 TALENs. Genomic DNA was digested with HindIII and probed with ^{32}P-labeled external 5'-probe (top panel) or with the internal 3'-probe (bottom panel). Fragment sizes are: 5' probe: wt=8.8 kb, targeted=7.4 kb; 3'-probe: wt=8.8 kb, targeted=4.3 kb. Heterozygous targeted clones are indicated in red and the homozygous targeted clone is indicated in blue.

C. Southern blot analysis of the C1 iPSCs targeted with PITX3 FW donor plasmids using the PITX3 TALENs. Genomic DNA was digested with HindIII and probed with ^{32}P-labeled external 5' probe (top panel) or with the internal 3'-probe (bottom panel). Fragment sizes are: 5' probe: wt=8.8 kb, targeted=7.4 kb; 3'-probe: wt=8.8 kb, targeted=4.3 kb. Heterozygous targeted clones are indicated in red.

Supplemental Figure 6

OCT4-eGFP
PGK Puro

WIBR#3

OCT4-eGFP

PE-A

Mean FITC intensity: 15.7
% GFP+:0.77

FITC-A

clone1
Mean FITC intensity: 11.5
% GFP+:0.82

clone1-1
Mean FITC intensity: 30.3
% GFP+:44.6

OCT4-2A-eGFP
PGK Puro

OCT4-2A-eGFP

clone1
Mean FITC intensity: 47.1
% GFP+:49.8

clone1-1
Mean FITC intensity: 105.6
% GFP+:58.7

clone2
Mean FITC intensity: 32.9
% GFP+:45.9

clone2-1
Mean FITC intensity: 157.5
% GFP+:72.3

clone2-2
Mean FITC intensity: 154.6
% GFP+:71.6

OCT4-eGFP
-2A-Puro

clone1
Mean FITC intensity: 35.5
% GFP+:45.9

clone2
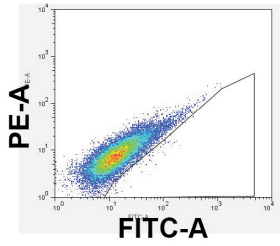Mean FITC intensity: 26.1
% GFP+:45.2

FACS analysis of OCT4-eGFP targeted cells

Graphs show FACS analysis of eGFP fluorescence (FITC-A, x axis) against PE-A auto-fluorescence (y-axis) in the indicated cell types. Left column shows wild-type WIBR#3 hESCs. Middle column shows one hESC clone targeted with OCT4-eGFP-PGK-Puro (top) and two clones targeted with OCT4-2A-eGFP-PGK-Puro (middle and bottom) before removal of the Puro selection cassette. eGFP fluorescence is low in these clones most likely due to the lack of an appropriate polyadenylation signal. The right column shows eGFP fluorescence for one clone after Cre-mediated removal of Puro (top), and for three clones expressing OCT4 and eGFP from a OCT4-2A-eGFP modified locus (middle). For all of these clones a significant increase in the percentage of eGFP positive cells (%GFP+) and the mean FITC intensity can be detected after eGFP is expressed using the natural OCT4 3' UTR. The bottom panels show the eGFP fluorescence of WIBR#3 clones targeted with the OCT4-eGFP-2A-Puro gene trap vector.
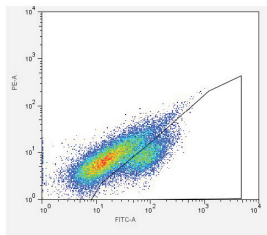
Supplemental Figure 7
WIBR#3

hESCs

OCT4-eGFP

hESCs   **fibroblast -like**
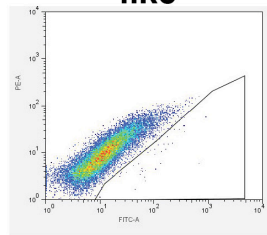
OCT4-2A-eGFP
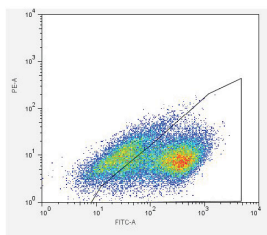
hESCs   **fibroblast -like**
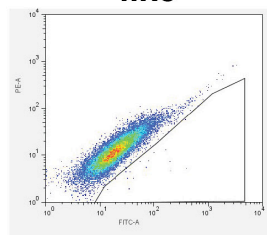
OCT4-eGFP -2A-Puro
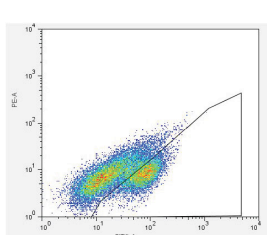
hESCs   **fibroblast -like**
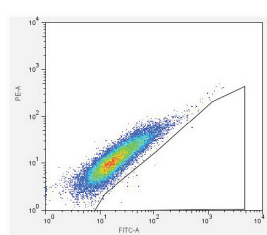
FACS analysis of OCT4-eGFP targeted cells after differentiation

Shown is FACS analysis of eGFP fluorescence (FITC-A, x axis) against PE-A auto-fluorescence (y-axis) in the indicated cell types. Top row shows unmodified wild-type hESCs. The graphs below show hESCs with the indicated modification at the OCT4-STOP codon. Shown on the left side are undifferentiated hESCs and on the right side the same cell line is shown after differentiation into fibroblast-like cells.

Supplemental Figure 8

A

Donor: PITX3-eGFP:

B

external probe

GFP-probe
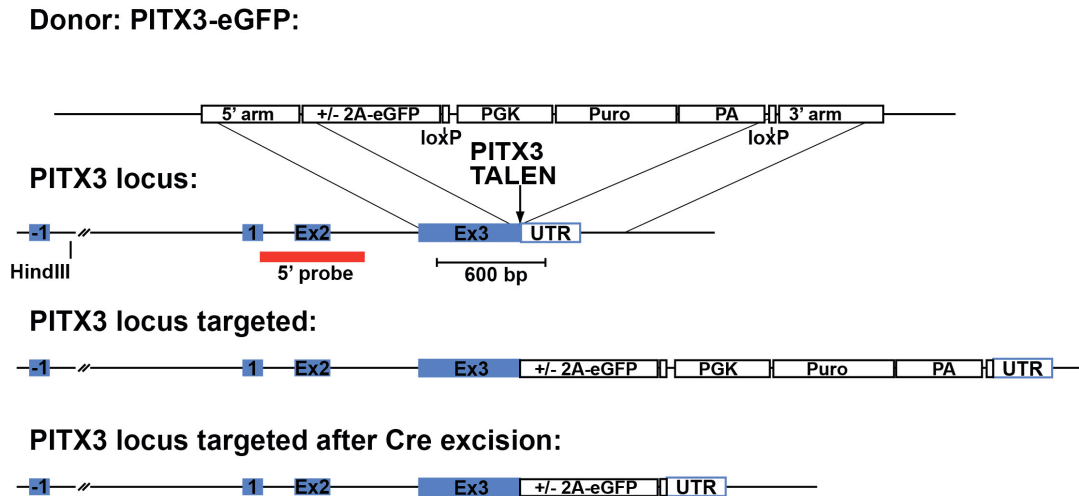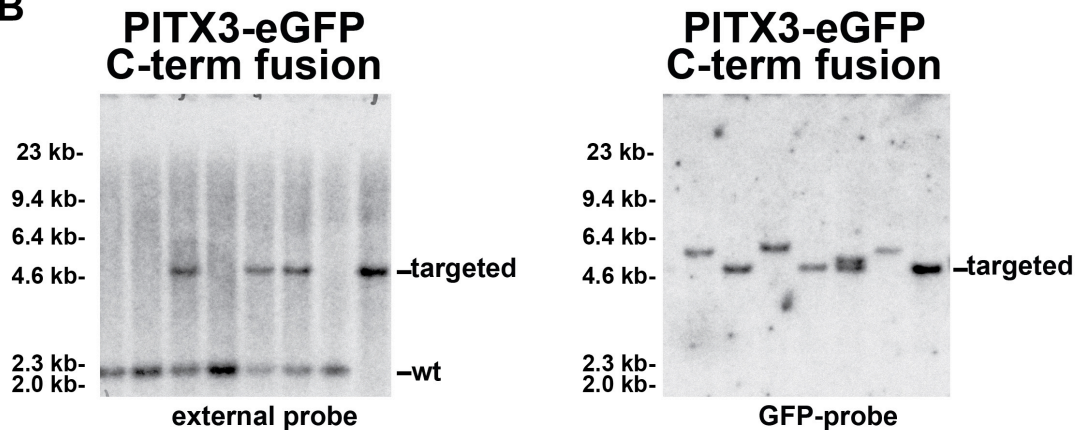
Using TALENs to generate a C-terminal fusion protein and eGFP-reporter line for PITX3

A. Schematic overview depicting the targeting strategy for the PITX3 locus using the PITX3-STOP TALENs. The external probe used for Southern blot analysis is indicated as a red box, exons of the PITX3 locus are shown as blue boxes and arrows indicate the genomic site cut by PITX3-STOP TALEN. Shown above is a schematic of the donor plasmid used to target the OCT4 locus (+/- 2A: indicates that two independent donor plasmids that only differ in this 2A-self cleaving peptide sequence were used), eGFP: enhanced green fluorescent protein, loxP-sites are indicated, PGK: human phophoglycerol kinase promotor, Puro: puromycin resistance gene, UTR: untranslated region of the PITX3 gene. Donor plasmids were constructed so that after the removal of the PGK-Puro cassette a PITX3-eGFP fusion protein or eGFP alone (from the 2A sequence) will be expressed using the natural 3' UTR of the PITX3 gene.

B. Southern blot analysis of WIBR#3 hESCs targeted in the PITX3 locus to generate a C-terminal PITX3-eGFP fusion protein. Genomic DNA was digested with NcoI and hybridized with the ^{32}P-labeled external 5'-probe (left panel) or with the internal GFP probe (right panel).

Supplemental Figure 9

A

PPP1R12C TALEN left

B

PPP1R12C TALEN right
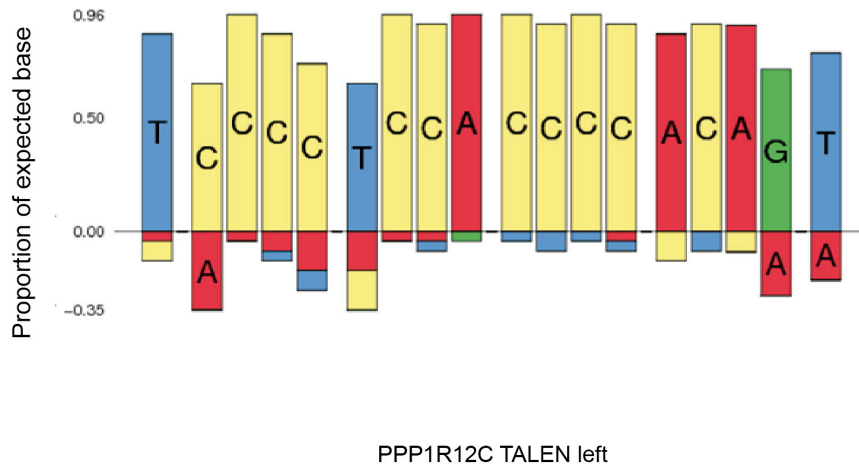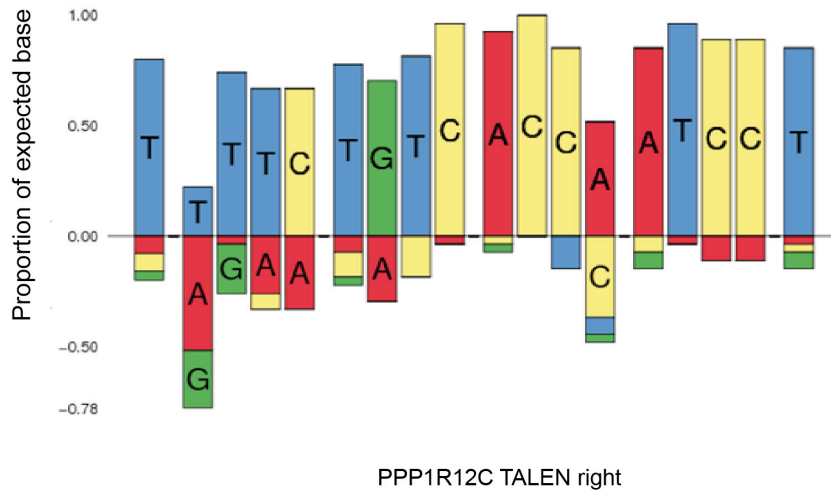
Graphical depiction of a SELEX-derived base frequency matrix for the PPP1R12C TALENs.

At each matrix position, the frequency of the predicted target base is shown above the x-axis, whereas the remaining base frequencies are shown below the x-axis.

Supplemental Figure 10

```
PPP1R12C deletions
locus   Number Size   Loc.    Sequence
P1R12C                        TATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCCCATCCTTAG
P1R12C  1991   -1     52      TATCTGTCCCCTCCACCCCACAGTGGGGCCACT:GGGACAGGATTGGTGACAGAAAA
P1R12C  1855   -1     51      TATCTGTCCCCTCCACCCCACAGTGGGGCCAC:AGGGACAGGATTGGTGACAGAAAA
P1R12C  1174   -7     51      TATCTGTCCCCTCCACCCCACAGTGGGGCCAC:::::::AGGATTGGTGACAGAAAAGCCCCA
P1R12C  843    -10    46      TATCTGTCCCCTCCACCCCACAGTGGG::::::::::ACAGGATTGGTGACAGAAAAGCCCCATCC
P1R12C  671    -5     44      TATCTGTCCCCTCCACCCCACAGTG:::::CTACTAGGGACAGGATTGGTGACAGAAAGC
P1R12C  622    -1     50      TATCTGTCCCCTCCACCCCACAGTGGGGCCA:TAGGGACAGGATTGGTGACAGAAAA
P1R12C  595    -2     51      TATCTGTCCCCTCCACCCCACAGTGGGGCCAC::GGGACAGGATTGGTGACAGAAAAG
P1R12C  571    -9     47      TATCTGTCCCCTCCACCCCACAGTGGGG:::::::::ACAGGATTGGTGACAGAAAAGCCCCATC
P1R12C  568    -9     43      TATCTGTCCCCTCCACCCCACAGT:::::::::AGGGACAGGATTGGTGACAGAAAAGCCCCATC
P1R12C  566    -17    53      TATCTGTCCCCTCCACCCCACAGTGGGGCCACTA:::::::::::::::::AGAAAAGCCCCATCCTTAG
P1R12C  565    -8     52      TATCTGTCCCCTCCACCCCACAGTGGGGCCACT::::::::GATTGGTGACAGAAAAGCCCCAT
P1R12C  564    -10    48      TATCTGTCCCCTCCACCCCACAGTGGGGC::::::::::AGGATTGGTGACAGAAAAGCCCCATCC
P1R12C  557    -9     41      TATCTGTCCCCTCCACCCCACA:::::::::CTAGGGACAGGATTGGTGACAGAAAAGCCCCATC
P1R12C  553    -9     50      TATCTGTCCCCTCCACCCCACAGTGGGGCCA:::::::::GGATTGGTGACAGAAAAGCCCCATC
P1R12C  551    -16    36      TATCTGTCCCCTCCACC::::::::::::::::AGGGACAGGATTGGTGACAGAAAAGCCCCATCCTTAG
P1R12C  549    -12    42      TATCTGTCCCCTCCACCCCACAG::::::::::::GGACAGGATTGGTGACAGAAAAGCCCCATCCTT
P1R12C  532    -18    32      TATCTGTCCCCTC:::::::::::::::::::CTAGGGACAGGATTGGTGACAGAAAAGCCCCATCCTTAG
P1R12C  519    -11    45      TATCTGTCCCCTCCACCCCACAGTGG:::::::::::ACAGGATTGGTGACAGAAAAGCCCCATCCT
P1R12C  457    -3     48      TATCTGTCCCCTCCACCCCACAGTGGGC:::TAGGGACAGGATTGGTGACAGAAAGC
P1R12C  430    -25    29      TATCTGTCCC:::::::::::::::::::::::::GGACAGGATTGGTGACAGAAAAGCCCCATCCTTAG
P1R12C  321    -23    33      TATCTGTCCCCTCC:::::::::::::::::::::::ACAGGATTGGTGACAGAAAAGCCCCATCCTTAG
P1R12C  290    -6     50      TATCTGTCCCCTCCACCCCACAGTGGGGCCA::::::ACAGGATTGGTGACAGAAAAGCCCC
P1R12C  261    -4     49      TATCTGTCCCCTCCACCCCACAGTGGGGCC::::GGGACAGGATTGGTGACAGAAAAGCC
P1R12C  57     -9     39      TATCTGTCCCCTCCACCCCA:::::::::CACTAGGGACAGGATTGGTGACAGAAAAGCCCCAT
P1R12C  11     -7     52      TATCTGTCCCCTCCACCCCACAGTGGGGCCACT:::::::GGATTGGTGACAGAAAAGCCCCA


PPP1R12C Insertions
locus   Number Size   Ins.    Sequence
P1R12C                        TATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGACAGAA
P1R12C  1694   +2     AC      TATCTGTCCCCTCCACCCCACAGTGGGGCCACACTAGGGACAGGATTGGTGACAGA
P1R12C  1558   +1     T       TATCTGTCCCCTCCACCCCACAGTGGGGCCACTTAGGGACAGGATTGGTGACAGA
P1R12C  1402   +4     ACTA    TATCTGTCCCCTCCACCCCACAGTGGGGCCACTAACTAGGGACAGGATTGGTGACA
P1R12C  1183   +1     A       TATCTGTCCCCTCCACCCCACAGTGGGGCCACTAAGGGACAGGATTGGTGACAGA
P1R12C  826    +3     CTA     TATCTGTCCCCTCCACCCCACAGTGGGGCCACTACTAGGGACAGGATTGGTGACAG
P1R12C  632    +3     TAA     TATCTGTCCCCTCCACCCCACAGTGGGGCCATAA::AGGGACAGGATTGGTGACAGAA
P1R12C  619    +4     GTGA    TATCTGTCCCCTCCACCCCACAGTGGGGCCAGTGACTAGGGACAGGATTGGTGACA
P1R12C  588    +2     TA      TATCTGTCCCCTCCACCCCACAGTGGGGCCACTATAGGGACAGGATTGGTGACAGA
P1R12C  573    +2     CT      TATCTGTCCCCTCCACCCCACAGTGGGGCCACTCTAGGGACAGGATTGGTGACAGA
P1R12C  553    +4     CTCC    TATCTGTCCCCTCCACCCCACAGTGGGGCCACCTCCTAGGGACAGGATTGGTGACA
P1R12C  481    +1     C       TATCTGTCCCCTCCACCCCACAGTGGGGCCACCTAGGGACAGGATTGGTGACAGAA
P1R12C  308    +3     GAC     TATCTGTCCCCTCCACCCCACAGTGGGGCCACGACTAGGGACAGGATTGGTGACAG
P1R12C  293    +4     CACT    TATCTGTCCCCTCCACCCCACAGTGGGGCCACTCACTAGGGACAGGATTGGTGACA
P1R12C  274    +4     TGCT    TATCTGTCCCCTCCACCCCACAGTGGGGCCACTTGCTAGGGACAGGATTGGTGACA
P1R12C  264    +5     TACTT   TATCTGTCCCCTCCACCCCACAGTGGGGCCACTTACTTAGGGACAGGATTGGTGAC
P1R12C  263    +4     CCAC    TATCTGTCCCCTCCACCCCACAGTGGGCCACCCACTAGGGACAGGATTGGTGACA
P1R12C  17     +4     CTTA    TATCTGTCCCCTCCACCCCACAGTGGGGCCACTACTTAGGGACAGGATTGGTGACA
P1R12C  11     +6     AAGCTT  TATCTGTCCCCTCCACCCCACAGTGGGGCAAGCTTCACTAGGGACAGGATTGGTGA
P1R12C  10     +2     CC      TATCTGTCCCCTCCACCCCACAGTGGGGCCACCCTAGGGACAGGATTGGTGACAGA


OT10 Deletions
locus   Number Size   Loc.    Sequence
OT10                          TGTTCTTCCACCCAGCCCTCAGTGCTCTGCTGAGGCAGGATGGGTGGCTGGAAAAATGGGGTCTTT
OT10    211    -5     45      TGTTCTTCCACCCAGCCCTCAGTGCT:::::GAGGCAGGATGGGTGGCTGGAAAAATGGGG
OT10    149    -3     48      TGTTCTTCCACCCAGCCCTCAGTGCTCTG:::AGGCAGGATGGGTGGCTGGAAAAATGG
OT10    3      -1     49      TGTTCTTCCACCCAGCCCTCAGTGCTCTGC:GAGGCAGGATGGGTGGCTGGAAAAAT
OT10    2      -1     45      TGTTCTTCCACCCAGCCCTCAGTGCT:TGCTGAGGCAGGATGGGTGGCTGGAAAAAT


OT14 Deletions
locus   Number Size   Loc.    Sequence
OT14                          AGTATTTTCTATAACTCATATTTTCACAAAACAATGTTTTTAGAAATTTTTGGGGTGG
OT14    51     -3     59      AGTATTTTCTATAACTCATATTTTCACAAAACAATGTTTTAA:::AAATTTTTGGGGTG
```

Off-target analysis of the PPP1R12C locus

Insertion and deletions (InDels) observed in loci with InDel frequencies above background. Only InDels that occurred at least 10 times are shown for PPP1R12C and only InDels that occurred at least two times are shown for off target site (OT) 10 and OT14. Inserted bases are shown in red and deleted bases are indicated by the appropriate number of colons. The first column shows the locus, the second column shows the number of occurrences of each InDel, the third column shows the size of the InDel, the fourth column shows either the position of the 5' edge of the deletion or the identity of the inserted bases, and the fifth column shows the aligned sequence read. Many of these sequences can be annotated in multiple ways- only one annotation is shown. The wild-type sequence of each locus is shown above the corresponding InDels and the spacer between the TALEN binding sites is underlined.

Supplemental Table 1:

PPP1R12C
```
        101077
gtCCCCTCCACCCCACAGTggggccactagggacaggattggtgacagaaag
cagggggaggtgggggtgtcaccccggtgatccctgTCCTAACCACTGTCTTTtc
                                            101079
```

PITX3
```
        101146
gtCAGACGCTGGCACTccgcacccccagctcccagagcacggctgcaagggccag
cagtctgcgaccgtgaggcgtgggggtcgagggtctcgTGCCGACGTTCCCGGtc
                                            101148
```

PITX3 (flanking stop codon)
```
        101236
gtACGCCGTGGAAAGGCCcgtatgagcggccccgcccgtagatcatccccgag
catgcggcacctttccgggcatactcgccggggcgggCATCTAGTAGGGGCtc
                                            101238
```

OCT4 (intron 1)
```
        101123
gtCACCTGCAGCTGCCCAGacctggcacccaggagaggagcaggcagggtcag
cagtggacgtcgacgggtctggaccgtgggtcctcTCCTCGTCCGTCCCAGtc
                                            101125
```

OCT4 (flanking stop codon)
```
        101138
ctCTGGGCTCTCCCATgcattcaaactgaggtgcctgcccttctaggaatggggggac
gagacccgagagggtacgtaagtttgactccacggacgGGAAGATCCTTACCCCCtg
                                            101142
```

Supplementary sequence information. **TALE amino acid sequences (RVDs underlined).**

```
PPP1R12C  101077
MVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPE
ATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPL
NLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASNGGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPAQVVAIANNNGGKQALETVQRLLPVLCQDH
```

```
GLTPEQVVAIASNGGGRPALESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLPHAPALI
KRTNRRIPERTSHRVA
```

PPP1R12C 101079
```
MVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPE
ATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPL
NLTPDQVVAIASNGGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASNGGGKQALETVQRLLPVLCQDHGLTPDQVVAIANNNGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
GLTPEQVVAIASNGGGRPALESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLPHAPALI
KRTNRRIPERTSHRVA
```

PITX3 101146
```
MVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPE
ATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPL
NLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH
GLTPDQVVAIANNNGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIANNNGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQDH
GLTPDQVVAIANNNGGKQALETVQRLLPVLCQDHGLTPDQVVAIANNNGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGRPALESIVAQLSRPDPAL
AALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVA
```

PITX3 101148
```
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ATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPL
NLTPDQVVAIANNNGGKQALETVQRLLPVLCQDHGLTPDQVVAIANNNGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
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GLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPAQVVAIANNNGGKQALETVQRLLPVLCQDH
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KRTNRRIPERTSHRVA
```

PITX3 101236
```
MVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPE
ATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPL
NLTPDQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPDQVVAIANNNGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIANNNGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIANNNGGKQALETVQRLLPVLCQDH
GLTPDQVVAIANNNGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH
GLTPAQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQDH
GLTPDQVVAIANNNGGKQALETVQRLLPVFCQAHGLTPDQVVAIANNNGGKQALETVQRLLPVLCQAH
```

```
GLTPAQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGRPALESIVAQLSRPDPAL
AALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVA
```

PITX3 101238

```
MVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPE
ATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPL
NLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIANNNGGKQALETVQRLLPVLCQAH
GLTPDQVVAIANNNGGKQALETVQRLLPVLCQAHGLTPAQVVAIANNNGGKQALETVQRLLPVLCQDH
GLTPDQVVAIANNNGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIANNNGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGRPALESIVAQLSRPDPAL
AALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVA
```

OCT4 intron1 101123

```
MVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPE
ATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPL
NLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
GLTADQVVAIASNGGGKQALETVQRLLPVLCQDHGLTPDQVVAIANNNGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQDH
GLTPDQVVAIANNNGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIANNNGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVFCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQDH
GLTPEQVVAIASNKGGRPALESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLPHAPALI
KRTNRRIPERTSHRVA
```

OCT4 intron1 101125

```
MVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPE
ATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPL
NLTPDQVVAIANNNGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAH
GLTPDQVVAIANNNGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAH
GLTPDQVVAIANNNGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASNGGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPAQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGRPALESIVAQLSRPDPAL
AALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVA
```

OCT4 Stop codon 101138

```
MVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPE
ATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPL
NLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAH
GLTPDQVVAIANNNGGKQALETVQRLLPVLCQAHGLTPAQVVAIANNNGGKQALETVQRLLPVLCQDH
GLTPDQVVAIANNNGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASNGGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVFCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
```

```
GLTPDQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGRPALESIVAQLSRPDPAL
AALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVA
```

OCT4 Stop codon 101142
```
MVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPE
ATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPL
NLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNIGGKQALETVQRFLPVLCQAH
GLTPDQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQDH
GLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQDH
GLTPDQVVAIANNNGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAH
GLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPAQVVAIANNNGGKQALETVQRLLPVLCQDH
GLTPEQVVAIASNKGGRPALESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLPHAPALI
KRTNRRIPERTSHRVA
```

Binding sites of TALENs and TALE amino acid sequences

Supplemental Table 2:

Target	TALEN A	TALEN B	Activity	
PPP1R12C intron 1	GGGTACTTTTATCTGT	CCCTAGTGGCCCCACT	17%	
	CCCCTCCACCCCACAGT	TTTCTGTCACCAATCCT	36%	x
	GGTTCTGGGTACTTTT	CCCTAGTGGCCCCACT	7%	
	GGGTACTTTTATCTGT	TTTCTGTCACCAATCCT	10%	
OCT4 intron 1	GGGGAAGTGGTCACCt	GCTCCTCTCCTGGGT	2%	
	CACCTGCAGCTGCCCAG	GACCCTGCCTGCTCCT	9%	x
	GACCCTGCCTGCT	GACCCTGCCTGCTCCT	4%	
	GACCCTGCCTGCT	GCAGCTGCCCAGACCT	8%	
PITX3 exon 1	TGCAGCCGTGCTCT	GTCGCTGTCAGACGCT	16%	
	TGCAGCCGTGCTCT	CAGACGCTGGCACT	8%	
	GGCCCTTGCAGCCGT	GTCGCTGTCAGACGCT	15%	
	GGCCCTTGCAGCCGT	CAGACGCTGGCACT	21%	x

TALENs assembled according to the cipher previously described[4] and recognizing the DNA stretches indicated were tested for genome editing activity at the endogenous locus (indicated) using Surveyor endonuclease exactly as previously described [6]. The gene disruption activity is indicated; the pairs used for gene addition are indicated with an "x" in the last column.[4]

Supplemental Table 3:

Site	Score	Chr	Location	Gene	Sequence	Site configuration TALEN-L / gap / TALEN-R	For Seq Primer	Rev Seq Primer
PPP1R12C	8.50E-06	19	55627106	PPP1R12C	CTtTTCTGTCACCAATCCTGTCCCTAGTGGCCCCACTGTGGGGTGGAGGGGAC	PPP1R12C-R / 15 / PPP1R12C-L	ctctggttctgggtacttttatctg	gtgtgtcaccagataaggaatctg
OTS 1	3.56E-11	1	206211317		TgGgTAaGTCACtCAaCCTCTCTGAATCATTTCAATTTACTCACCAtTGTGGGGTGGgGGGGAA	PPP1R12C-R / 26 / PPP1R12C-L	ccctgtattattttccttccc	agtacccaggagcagcaaga
OTS 2	2.75E-12	X	31479737	DMD	CTcTTCcaTCACtAATtCTGATGAACAGCTATCAAGCTATTCAtgGTaGGGTGGAGGGTAG	PPP1R12C-R / 23 / PPP1R12C-L	tttcattcattaactcttctcttcc	ccactcactcttcatattcaact
OTS 3	1.09E-12	1	24697472	C1orf201	TTCCCCaCCACaCCACAaTGCCCTGTCTCAAGaAactGTGACAGAATAA	PPP1R12C-L / 11 / PPP1R12C-R	ccttaaagctttgtacacaaattca	tgagccatcatgcccag
OTS 4	1.04E-12	18	60653602		GTATTCTGTCACtAtTCCTGGGACATTTCCTGAAGGACgaTGGGGcGtgGGGGgA	PPP1R12C-R / 17 / PPP1R12C-L	tccatccattgtcctttctc	gtcctccagtttcaaatttaa
OTS 5	9.91E-13	10	101836508	CPN1	CTCCCtcCCACCCCACctaCCATGAAGAATAGGATTGGgGgCAGgACtG	PPP1R12C-L / 11 / PPP1R12C-R	gctggcaggggttttcc	ttggcctgcttagatttgaa
OTS 6	7.02E-13	6	167286954		CccgTCaGTCACCCcTCCTCTTGGGAGTGGGGCACaGTGGGGTGGAGtGGgT	PPP1R12C-R / 14 / PPP1R12C-L	cctggccagactcatgct	atctctggatcgtgtgcatt
OTS 7	6.43E-13	3	124702671	HEG1	ATCCCtTCCACCtCACcaaATAGTCCTCGCTAGCAGAAGGgTTGGgtACAGAACAA	PPP1R12C-L / 18 / PPP1R12C-R	cctgatgttttacacaactctatcc	gtcattttactcggtcttgactg
OTS 8	5.57E-13	9	135721616	AK8	GTATcCaGaCACCCAcCCTGGGCCTTCTGCAATGGAtgTGTGGGGTGGAtGGGgC	PPP1R12C-R / 17 / PPP1R12C-L	catctgtgggcccctct	tcaaatgttttgaggttggaaa
OTS 9	5.48E-13	22	51139502	SHANK3	GctCCCcCCACCCCcCAaaAAAATCCATTTCCGGGACtGGAgTGGgGACAGAAaAG	PPP1R12C-L / 18 / PPP1R12C-R	cccctccctaacctttaagtc	tctgccagaggccagagt
OTS 10	3.93E-13	15	67305689		CTtCCacCCAgCCCtCAGTGCTCTGCTGAGGCAGGATGGGTGgCtGgAaAA	PPP1R12C-L / 13 / PPP1R12C-R	gaatggatgaatgagtgaatgt	tcctgagttctcggttctttg
OTS 11	3.78E-13	4	148942809	ARHGAP10	GTtCCCaCCACCCCACAacCTTCTTACATCTGAACAAtGAcaGaTGACAGtAaAT	PPP1R12C-L / 17 / PPP1R12C-R	cctggactattatccacagctg	ccagtggagtcatatttcctg
OTS 12	3.10E-13	3	70995950		ATgCCCcCCACCCCAgAGTATATTCATTAGGACTCTTTGAttATaaGaGACAGAAaAT	PPP1R12C-L / 20 / PPP1R12C-R	cgtggttgcctttgtgaaat	tgcctggattttcaattatgtg
OTS 13	2.87E-13	6	45952064	CLIC5	CaGTaCTGTCACCtgTgCTATCCCTGGACTCACCAtgtTGGGGTGGAaGGGAC	PPP1R12C-R / 15 / PPP1R12C-L	tgctgtcttcaggtccgc	agctgaagctcccaaagtca
OTS 14	2.73E-13	5	165831361		ATtTTCTaTaACtCATatTTTCACAAAACAATGTTTTTAGAAAttTtTGGGGTGGAGGGGgT	PPP1R12C-R / 16 / PPP1R12C-L	aaaaattctgtttctaagaagt	ttcatccatcctatgaaaactca
OTS 15	2.42E-13	12	120531852	CCDC64	GTGgTCaGcCACCCcaCCTTGAGGGCAGCACAGGCACCacGGGGTGGAGGGGAG	PPP1R12C-L / 16 / PPP1R12C-R	aatggcaccagaggggtct	cagccactgcaggcttct
OTS 16	2.42E-13	2	74063519	STAMBP	CTCCCCTCCACCCCgtgGTGCCCGCGCTGCCCTCAAGGtgGGGTGgCtGAcCAC	PPP1R12C-L / 16 / PPP1R12C-R	aatggcaccagaggggtct	agctgttgcagggctctc
OTS 17	2.22E-13	3	110893534		CTGTTCcaTCgCCAcTgCTGCTCAAACCACTGAGGttTGgGGGGTGGgGGGTgG	PPP1R12C-R / 16 / PPP1R12C-L	cctgtcgtctgcatgctc	gtgtgctgggtactgaaacg
OTS 18	2.10E-13	12	12838163	GPR19	CcATcCCcaTCACCCATCCTCACTCTTTAAATATGGTCTTTTTTTtttTtTttGGTGGgGGGGAG	PPP1R12C-R / 25 / PPP1R12C-L	catacctacctccctgtccc	ctgtggttttccccataagc
OTS 19	2.02E-13	19	6213603	MLLT1	AaGgaCTGTCcCtgcTCCTGCCCAGGAAGAGTCCAACTGTGGGGTGttGGGGgG	PPP1R12C-R / 16 / PPP1R12C-L	aggccacaccgggag	acaaggcggggaggctac
OTS 20	1.86E-13	9	141119479	FAM157B	AcACCCcCCACCCCcCAcaATTTGCTGTTAAAAGtGGAgTGtTGAtAGtATAG	PPP1R12C-L / 15 / PPP1R12C-R	ggaatattggttggtatggga	cacgaacagtggactctatttt

Location of SELEX-predicted PPP1R12C TALEN off-target sites and primer
sequences for off-target site analysis

Supplemental Table 4:

Locus	TALENs			Negative Control		
	total	InDels	%InDels	total	InDels	%InDels
PPP1R12C	2E+06	27409	1.8226	1464358	2	0.0001
OT1	2E+06	1	0.0001	1272038	2	0.0002
OT2	3E+06	4	0.0001	3228784	4	0.0001
OT3	5E+06	7	0.0002	3872171	8	0.0002
OT4	4E+06	3	0.0001	3948044	17	0.0004
OT5	3E+06	6	0.0002	2465847	3	0.0001
OT6	3E+06	6	0.0002	3092092	5	0.0002
OT7	2E+06	3	0.0001	2803754	4	0.0001
OT8	2E+06	0	0.0000	1424981	4	0.0003
OT9	2E+06	2	0.0001	1362994	3	0.0002
OT10	3E+06	366	0.0108	2871927	1	0.0000
OT11	3E+06	0	0.0000	4632791	17	0.0004
OT12	3E+06	3	0.0001	2963102	7	0.0002
OT13	3E+06	6	0.0002	4524613	6	0.0001
OT14	3E+06	53	0.0016	2597184	7	0.0003
OT15	3E+06	3	0.0001	3218486	5	0.0002
OT16	3E+06	7	0.0002	3020878	11	0.0004
OT17	2E+06	1	0.0001	1649244	0	0.0000
OT18	ND	ND	ND	ND	ND	ND
OT19	4E+06	3	0.0001	1862693	4	0.0002
OT20	4E+06	6	0.0002	3101727	7	0.0002

Summary of off-target analysis

The first column lists the predicted off-targets loci (OT) as described in Supplemental Table 3. The second column lists the total number of sequencing reads (total), the number of insertions and deletions (InDels) and their fraction to the total number of reads (in %) after electroporation with PPP1R12C TALENs. The third column shows the same values for eGFP control hESCs.

Additional References

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