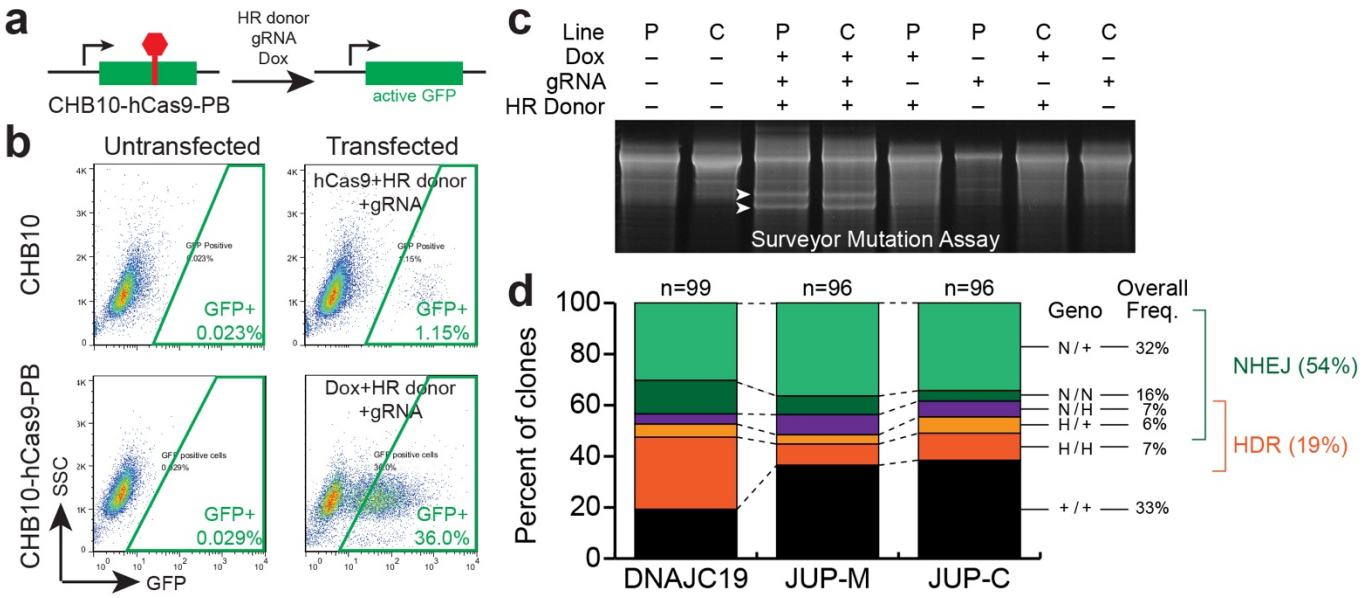
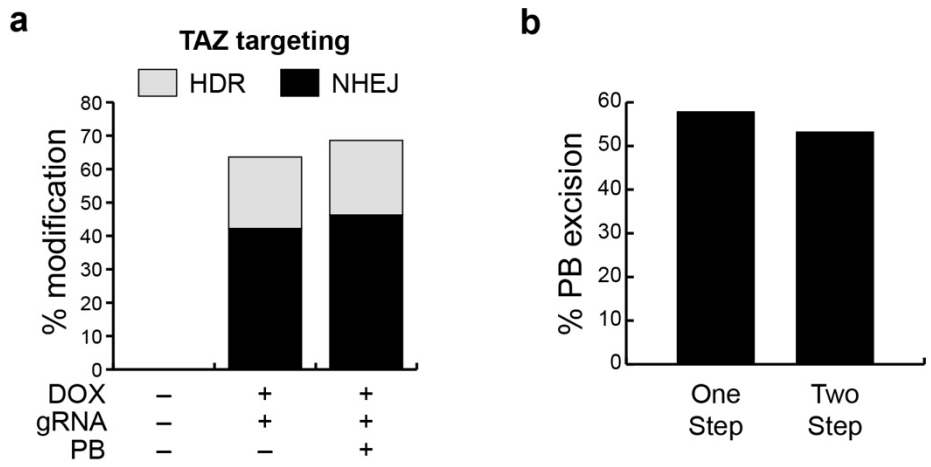


Supplementary Figure 1

Efficient genome editing in human pluripotent stem cells.

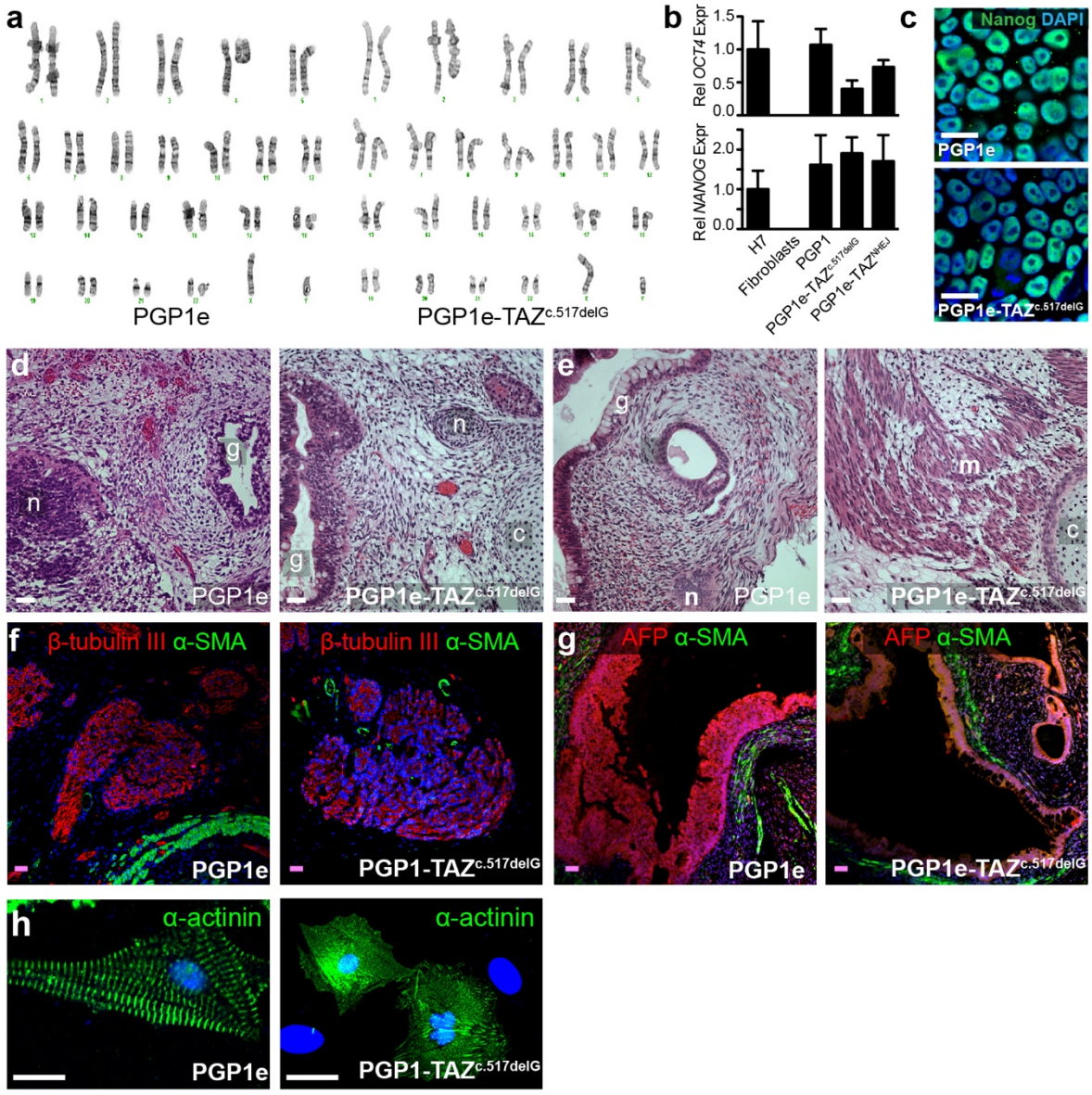
Our genome editing protocol was highly efficient in an additional pluripotent human cell line (CHB10) and at several different loci. **a-b**. We tested the efficiency of our protocol in a different pluripotent cell line, the human embryonic stem cell line CHB10, and a different locus, an integrated GFP reporter that is inactive due to a stop codon in the open reading frame (red symbol, panel a). Efficiency of HDR-mediated correction of the frameshift mutation was measured by FACS detection of GFP expression (panel b). **c**. Genome editing at the autosomal DNAJC19 locus of PGP1-hCas9-PB (P) or CHB10-hCas9-PB (C) pluripotent cell lines. Arrowheads indicate bands diagnostic of targeted genome modification. **d.** Genome editing efficiency at three autosomal sites, one in DNAJC19, and two within JUP (named JUP-M and JUP-C). Graph summarizes the results of Sanger sequencing of individual clones. Both homozygous and heterozygous mutations were efficiently recovered. Genotypes at each of the cell's two alleles is indicated as N, indel mutation from NHEJ; H, point mutation from HDR; +, wild-type. Overall frequency of each genotype class across the 3 experiments is summarized to the right.



Supplementary Figure 2

Efficient genome editing and piggyBac excision in a single step.

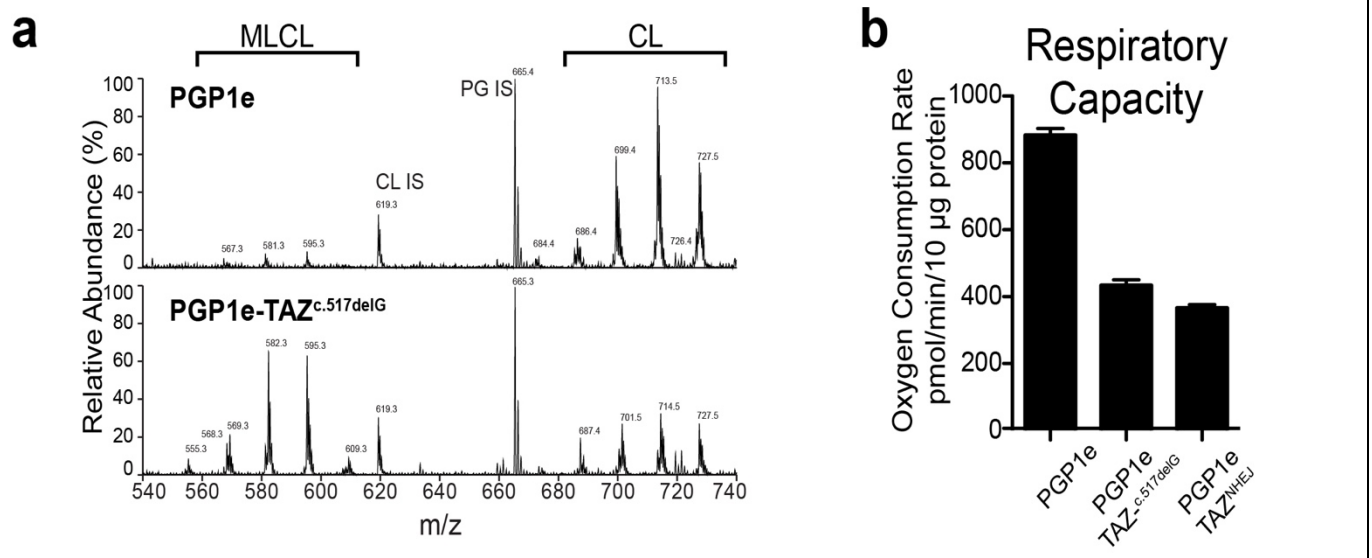
a. The co-transfection of the excision-only piggyBac mutant (PB) at the same as gRNA and HDR donor (”one-step” genome editing) did not substantially reduce the yield of genome-edited clones compared to sequential editing followed by excision (”two-step” genome editing). TAZ targeting frequencies were determined by next generation sequencing of genomic DNA from pooled cells. **b.** The frequency of transgene excision was not substantially different between one-step and two-step protocols. With either protocol, the majority of the recovered clones had successfully undergone excision of the piggyBac transgene, as determined by PCR genotyping of at least 79 independent clones per group over three separate experiments. These results show that including the excision-only piggyBac mutant into the transfection mix with gRNA and donor DNA permits efficient, single step genome editing and transgene excision.



Supplementary Figure 3

Efficient transposon removal by piggyBac transposase transient transfection yielded high quality iPSCs.

a. iPSC lines before after Cas9 genome editing had normal 46-XY karyotype. PGP1-hCas9-PB after transposon removal was designated PGP1e, and PGP1-hCas9-PB-TAZc.517delG was designated PGP1-TAZc.517delG. bar = 20 μm. **b-c.** Expression of pluripotency markers by control and mutant lines, as determined by qRTPCR (b) or immunostaining (c). **d-g.** Hematoxylin and eosin staining of teratomas indicated formation of structures from all three germ layers. n, neural. g, glandular. c, cartilagenous. m, musclar. white bar = 100 μm; pink bar = 200 μm. **h.** Cardiac differentiation of genome-edited, piggyBac excised iPSCs. bar = 20 μm.



Supplementary Figure 4

iPSCs with induced mutation at the TAZ locus recapitulate features of Barth Syndrome patients.

TAZ mutation causes mitochondrial dysfunction and cardiomyopathy by blocking maturation of cardiolipin, the major phospholipid of the inner mitochondrial membrane. (Wang, G., McCain, M. L., Yang, L., He, A., et al. Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. Nat Med 20, 616-623 (2014); Houtkooper, R. H., Turkenburg, M., Poll-The, B. T., Karall, D., et al. The enigmatic role of tafazzin in cardiolipin metabolism. Biochim Biophys Acta 1788, 2003-2014 (2009)). **a.** Cardiolipin phospholipid mass spectroscopy analysis control (PGP1e) and TAZ mutant (PGP1e-TAZc.517delG) iPSC-derived cardiomyocytes. As expected, PGP1e-TAZ$^{c.517delG}$ iPSC-derived cardiomyocytes (iPSC-CMs) showed abnormal cardiolipin maturation, a signature of Barth syndrome **b.** Normal CL content is required for optimal function of the mitochondrial electron transport chain (Pfeiffer, K., Gohil, V., Stuart, R. A., Hunte, C., et al. Cardiolipin stabilizes respiratory chain supercomplexes. J Biol Chem 278, 52873-52880 (2003)). Respiratory capacity, the rate of oxygen consumption in the presence of the mitochondrial uncoupler trifluorocarbonylcyanide phenylhydrazone (FCCP), is a measure of maximal electron transport chain activity. Oxygen consumption rate of control and mutant iPSC-CMs. Cells were treated with FCCP and analyzed using a Seahorses Biosciences Extracellular Flux Analyzer. Respiratory capacity, the rate of oxygen consumption in the presence of the mitochondrial uncoupler trifluorocarbonylcyanide phenylhydrazone (FCCP), is a measure of maximal electron transport chain activity. We confirmed that respiratory capacity was markedly impaired in iPSC-CMs containing introduced TAZ mutations.