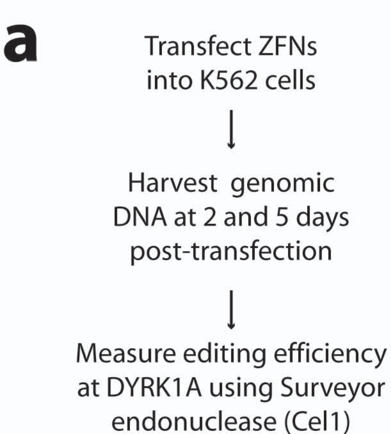
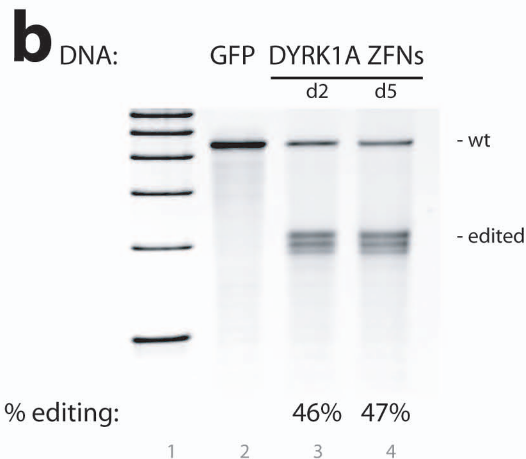
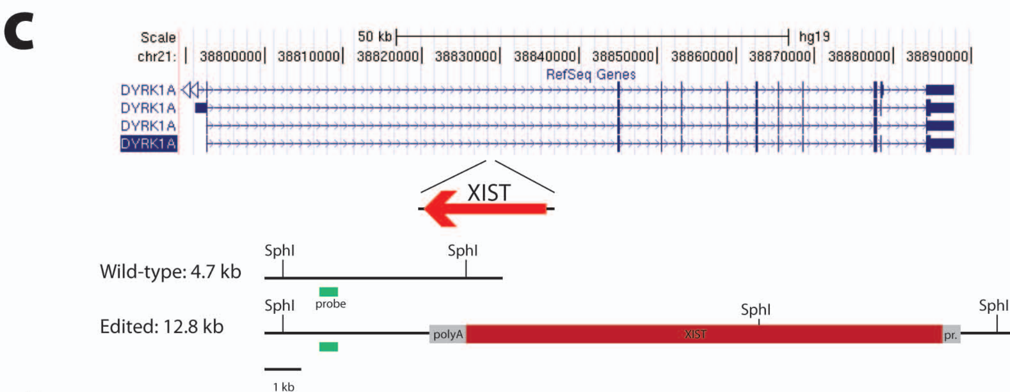
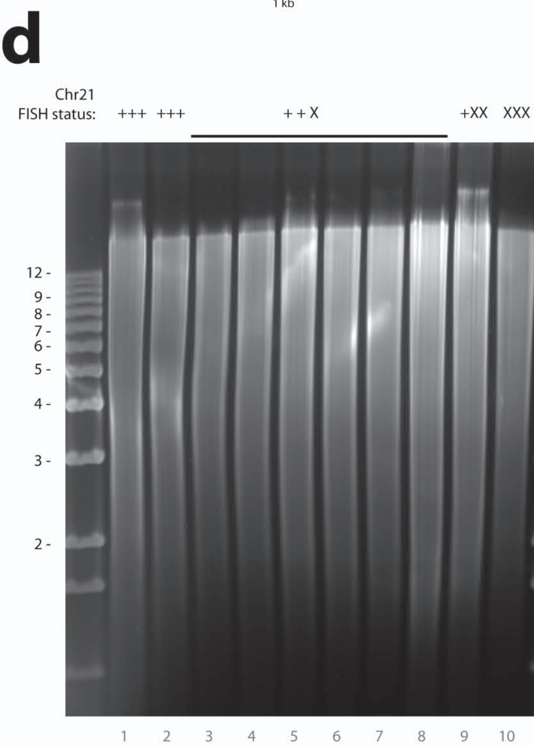
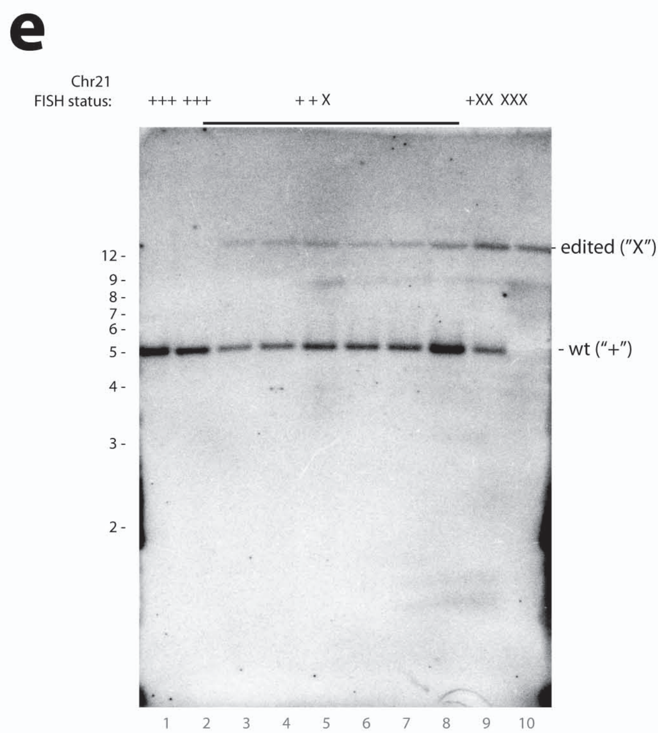


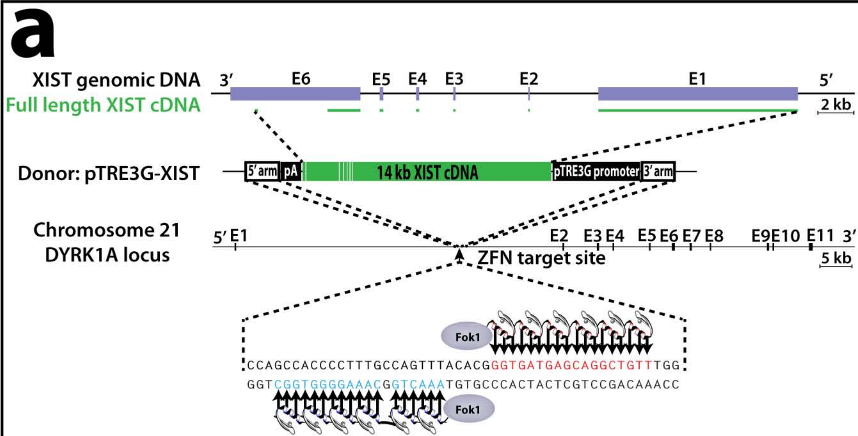



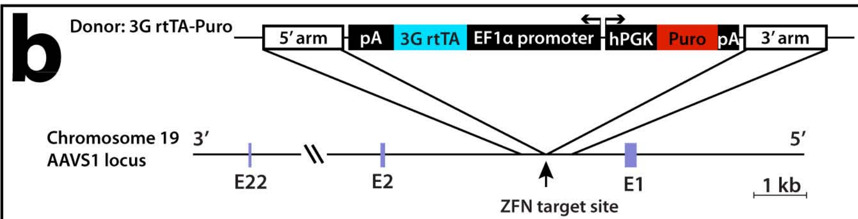



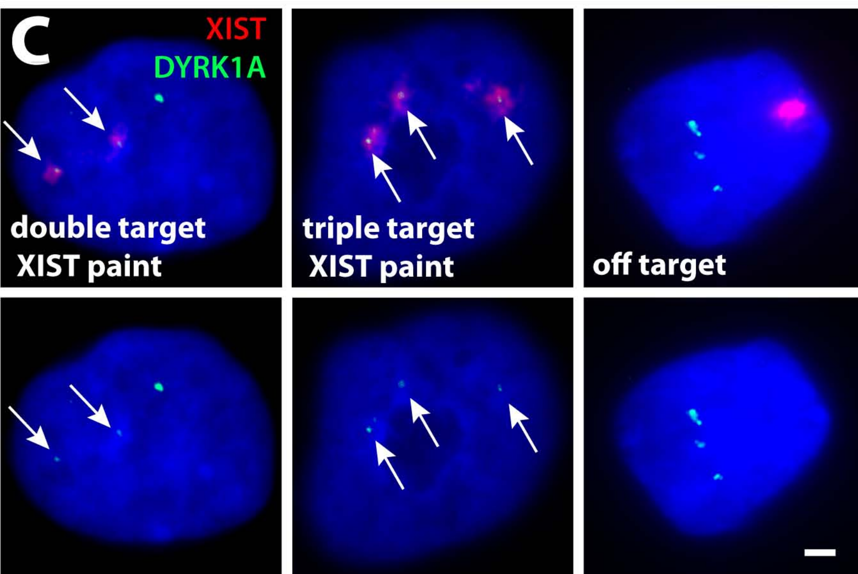



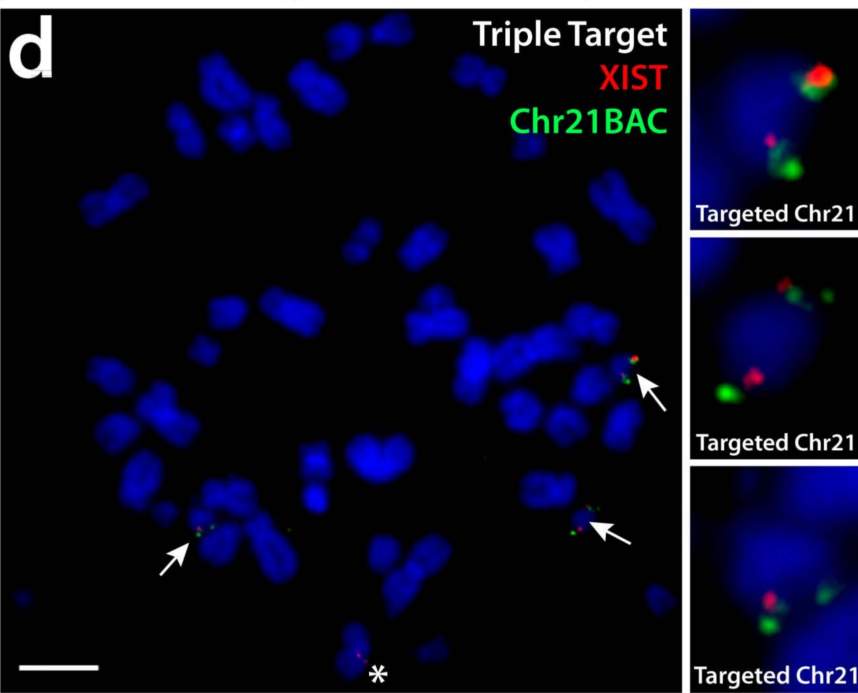

**Supplementary Figure 1. Engineered ZFN-driven editing of the endogenous *DYRK1A* locus in human tissue culture cells and in DS iPSCs.** **a.** Experimental outline of ZFN cleavage activity. **b.** Genome editing activity of the engineered ZFNs at the endogenous *DYRK1A* locus. Cells were transfected with an eGFP-expressing plasmid, or a plasmid expressing ZFNs engineered against *DYRK1A*. The position of bands deriving from wild-type and edited chromatids is shown to the right of the gel image. **c.** Schematic for southern blotting probes: Top is the intron-exon structure of *DYRK1A* with the position of the ZFN-targeted region. Bottom is the restriction digest and probing scheme for Southern-blotting. The wild-type allele is expected to produce a 4.7 kb band, while a correctly edited allele carrying the full-length *XIST* transgene is a 12.8 kb band. **d.** Genomic DNA isolated from single-cell-derived DS iPSCs clones and parental line was analyzed for southern blotting. Lanes 1 and 2 for parental line, lanes 3-8 for six single targeted clones, lane 9 for one double targeted clone, lane 10 for one triple targeted clone. The ladder in the left-most lane is annotated with molecular weights of the size markers to the left of the image. **e.** Results of Southern blotting. The parental line only shows a strong wild-type band (lanes 1 & 2), six single targeted clones show a strong wild-type band and weak edited band (lanes 3-8), one double targeted clone shows a weak wild-type band and strong edited band (lane 9), the triple targeted clone only shows a strong edited band (lane 10).

**Supplementary Figure 2. Efficient targeted addition of a 21 kb *XIST* transgene in HT1080 cells by ZFN technology. a.** Simple diagram of our first *XIST* Chr21 targeting transgene which carried a selectable marker. **b.** Metaphase DNA FISH in HT1080 cell. The *XIST* gene (red) overlaps one of two Chr21s (*DYRK1A* BAC probe, green). The box and enlargement indicate the targeted Chr21. The non-targeted Chr21 is marked by an arrow and the endogenous *XIST* gene on the single male X by an arrowhead. Scale: 5 µm.

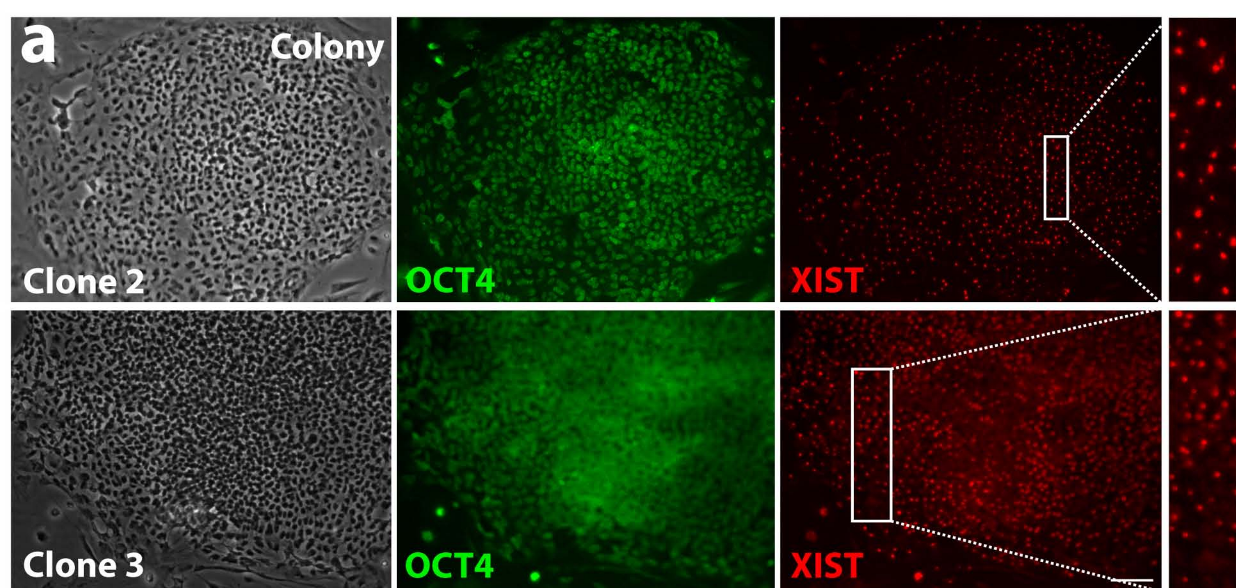


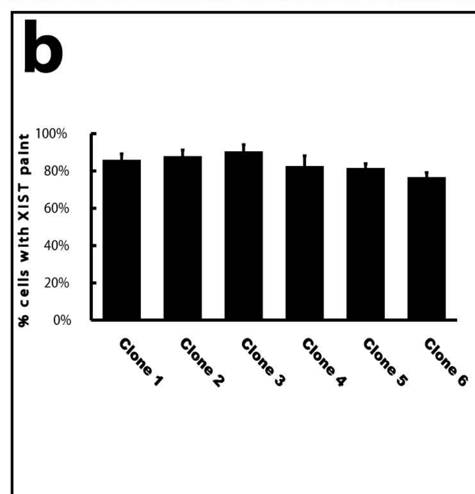



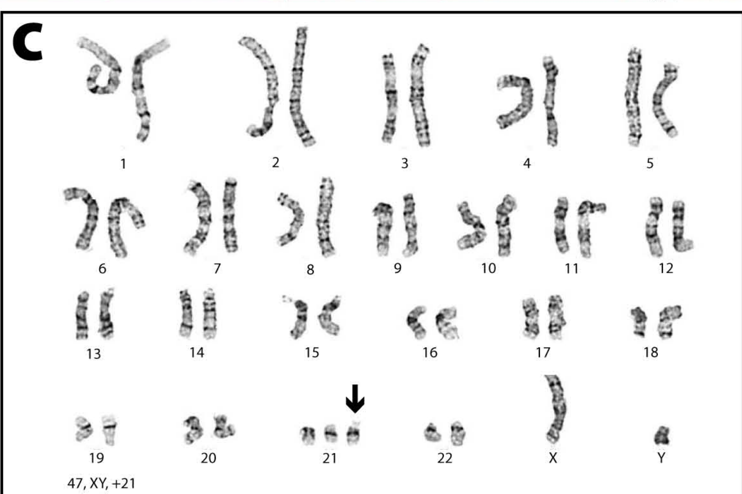



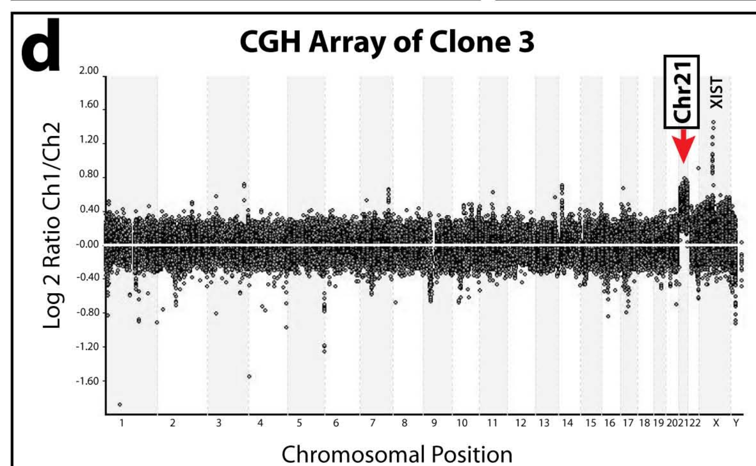



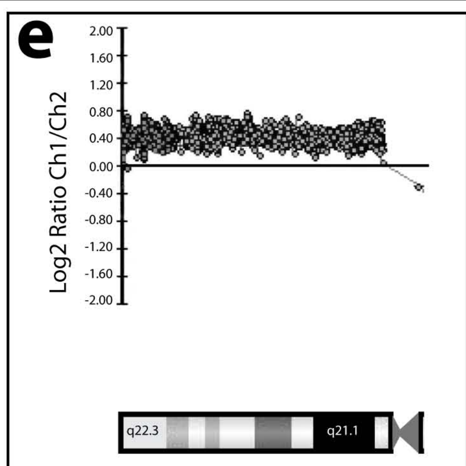

**Supplementary Figure 3. Accurate targeted addition of a 19 kb *XIST* transgene to trisomic Chr21 in Down syndrome iPSCs. a.** the 19kb *XIST* transgene construct contains dox-inducible pTRE3G promoter and two homologous arms that is targeted to *DYRK1A* locus on Chr21 by ZFNs. **b.** The rtTA-Puro construct contains a puromycin selection gene and rtTA cassette that is targeted to the *AAVS1* safe harbor locus on Chr19 by ZFNs. **c.** Interphase DNA/RNA FISH shows that the *XIST* transgene only targeted a single Chr21 (Figure 1c main text), however a low number of targeted colonies exhibit cells with two (left) or all three (middle) Chr21s targeted with the *XIST* transgene (arrows). Only a single off-target colony was found (right) after much searching. Scale: 2 µm. **d.** Metaphase DNA FISH for a triple targeted DS iPSC. All three Chr21s (*DYRK1A* BAC) overlap one *XIST* transgene (arrows). Each targeted Chr21 is enlarged in small panels (right). The single endogenous *XIST* gene is also seen on the male X-chromosome (asterisk). Scale: 5 µm.



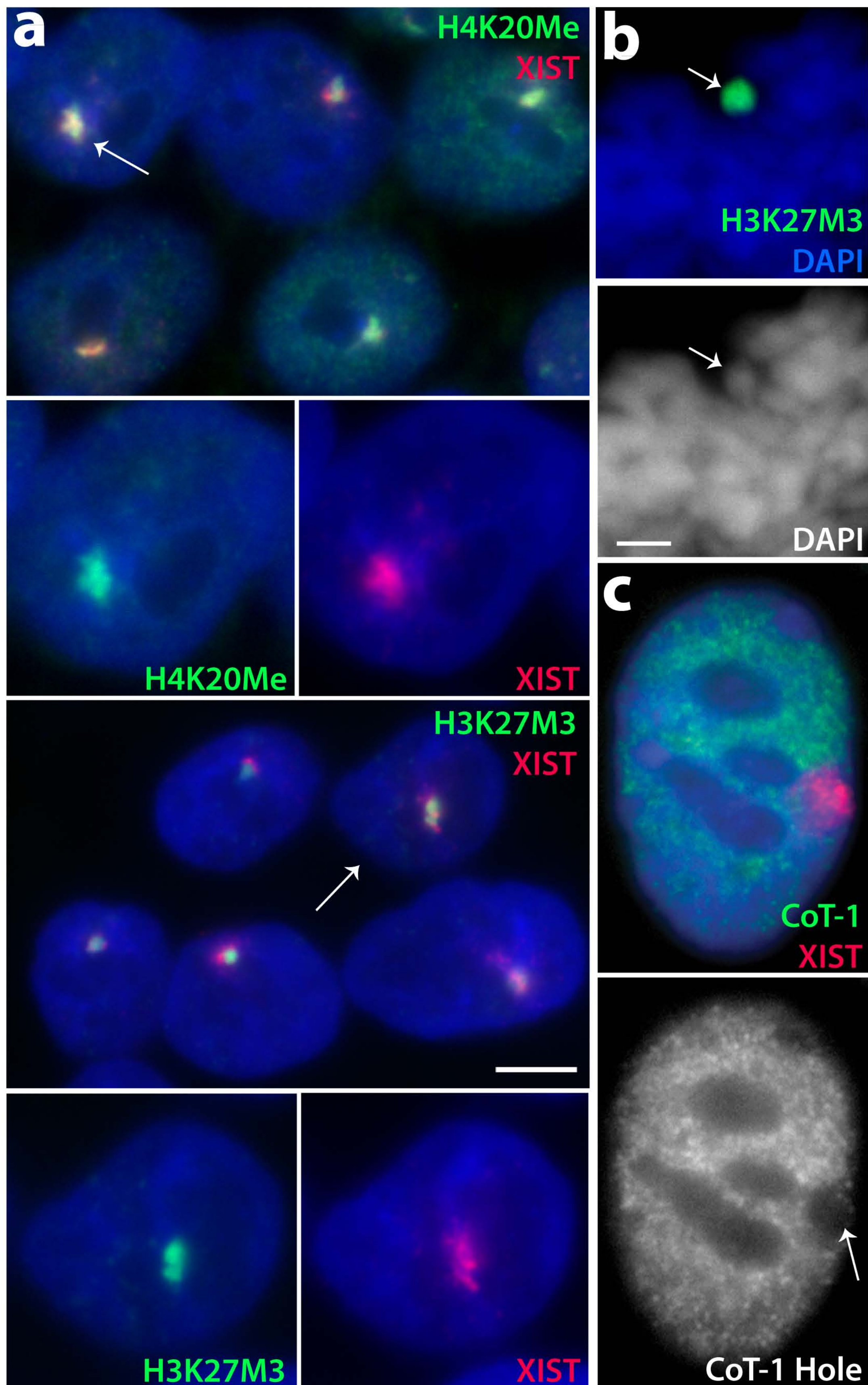



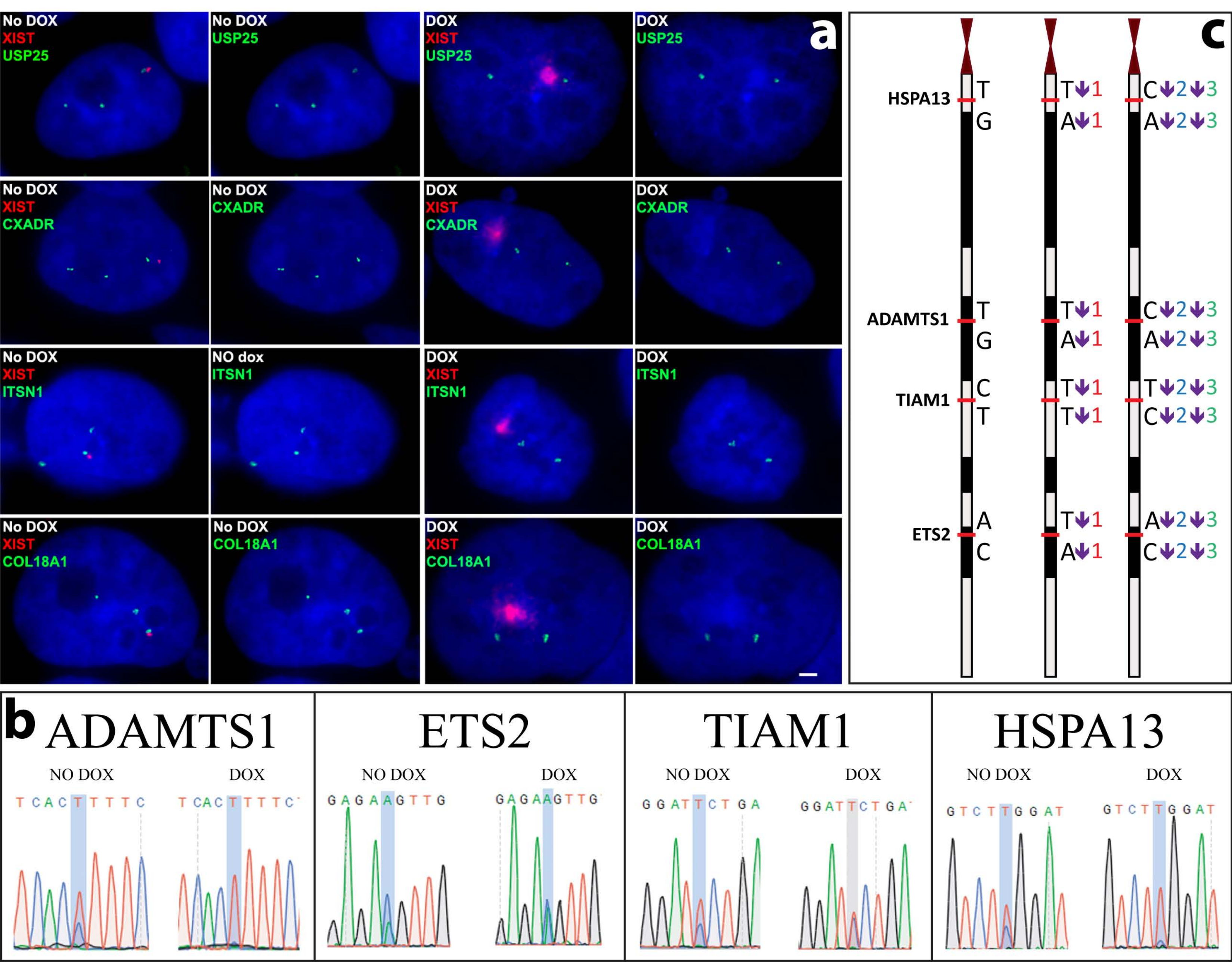



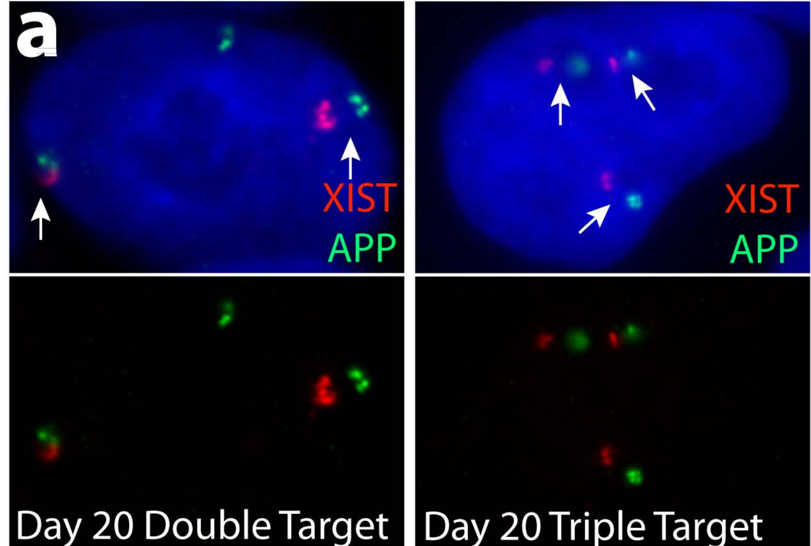

**Supplementary Figure 4. Genome edited iPSC clones show OCT4 expression, robust *XIST* RNA expression, and Karyotyping and CGH confirm full trisomy 21. a.** OCT4 immunostaining & *XIST* RNA FISH. All six clones isolated and studied show "pluripotent" colony morphology, OCT4 immunostaining, and highly consistent *XIST* expression in cells throughout the colonies. Panels at the right show enlargements of cells expressing *XIST* RNA. Scale 100 µm. **b.** Percentage of cells with *XIST* "paint" (a large, well-localized nuclear RNA territory) in six independent clones. Mean ± SE from 500 nuclei. **c.** A high resolution G-band karyotype was performed to further verify genome integrity of these subclones. Only Chr21 trisomy was observed, and karyotype is consistent with a male chromosome complement. **d.** Genomic Microarray analysis demonstrated a gain of one chromosome 21(red arrow) (and detected addition of the *XIST* transgene in these male cells). All other peaks are known human polymorphic variants and are not clinically significant. Note: Chr21 is increased 1.5 fold (from 2 to 3 chromosomes) while the *XIST* gene is increased 2 fold (from 1 to 2 copies). **e.** Close-up of Chr21 CGH shows full chromosome 21 trisomy with no deletions or duplications. This analysis was done on transgenic clone 3.

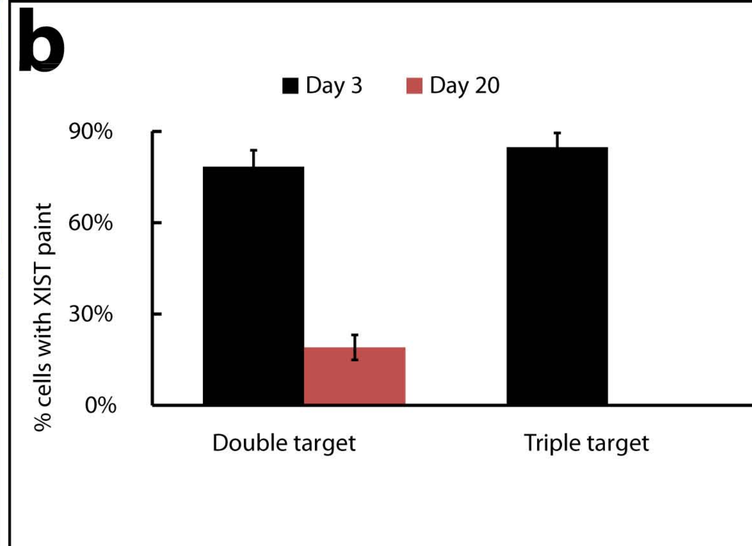



**Supplementary Figure 5. *XIST* RNA mediated recruitment of heterochromatic hallmarks to the targeted Chr21 in edited iPSCs. a.** *XIST* RNA recruits a number of heterochromatic epigenetic marks to the inactivating chromosome, for example H4K20me (top) and H3K27M3 (bottom). Channels are separated for each indicated cell (arrows) at bottom. Scale: 5 µm. **b.** H3K27me3 (green) coats the small Chr21 (arrows) in a mitotic cell. Scale: 2 µm. **c.** *XIST* RNA territory delineates hnRNA (Cot-1) “hole” (arrow), indicating Chr21 silencing.




**Supplementary Figure 6. *XIST* RNA induces allele-specific gene silencing on the targeted Chr21 in all DS iPS clones. a.** RNA FISH for four of the six Chr21-linked genes examined. RNA FISH for *APP* (Fig 3a) and *DYRK1A* (Fig 1c) is shown in main text. In all no-dox treated cells, *XIST* RNA (red) is not expressed and all three transcription foci (green) of four Chr21 genes are visible (left panels). In dox treated cells, *XIST* RNA is expressed (red), paints the inactivating Chr21, and silences the transcription focus of that allele (right panels). Scale: 2 μm. **b.** Allele-specific SNP analysis for four Chr21 genes. *ADAMTS1* goes from TTC to TT, *ETS2* from CCA to CA, *TIAM1* from TTC to TC, and *HSPA13* from TTC to TT. **c.** Alignment of eight Chr21 SNP alleles repressed in clones 1-3. Both clones 2 and 3 silence the far right chromosome and the center chromosome is silenced in Clone 1.




c

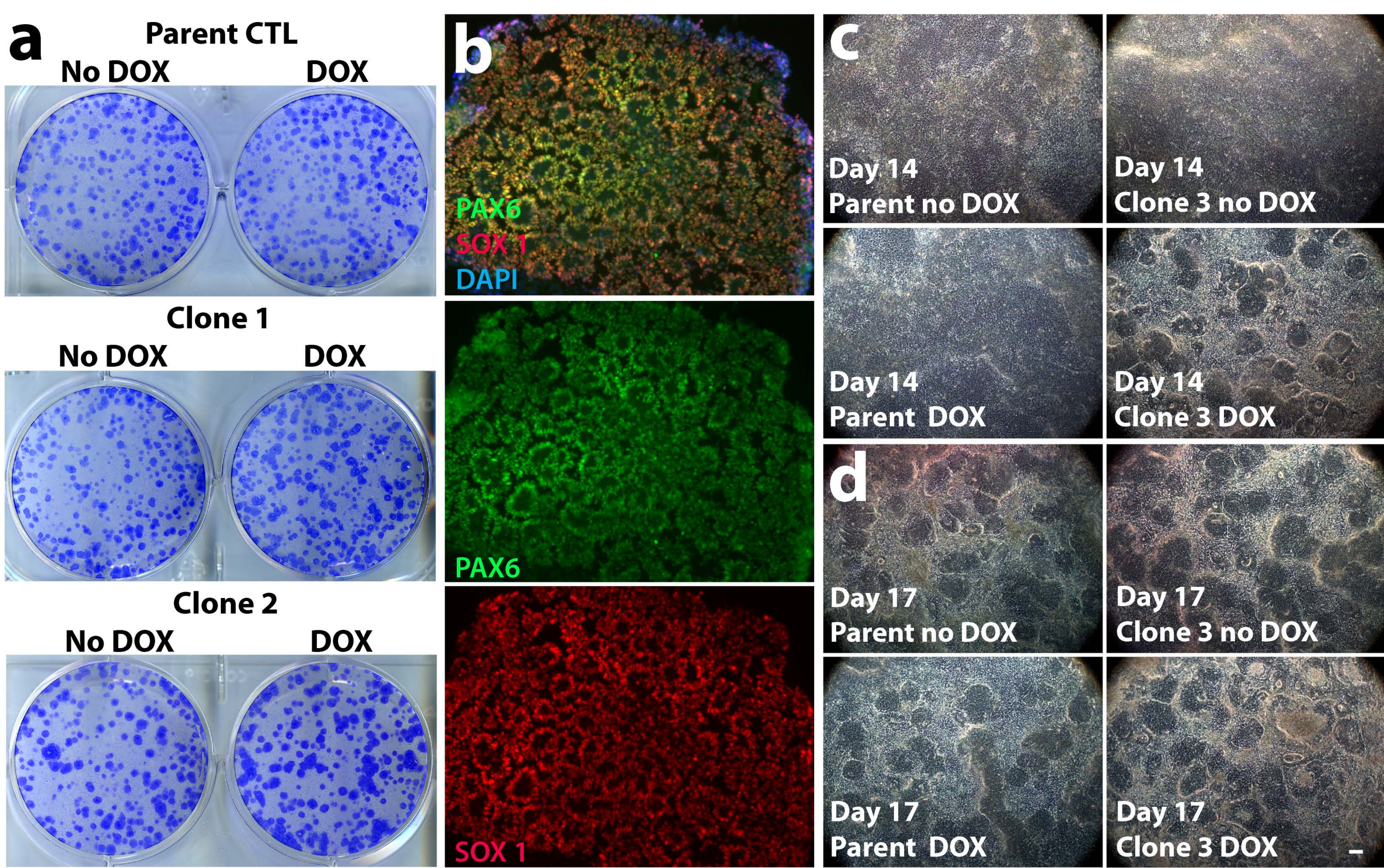
Relative expression level
DOX /no DOX

1.2
1
0.8
0.6
0.4
0.2
0

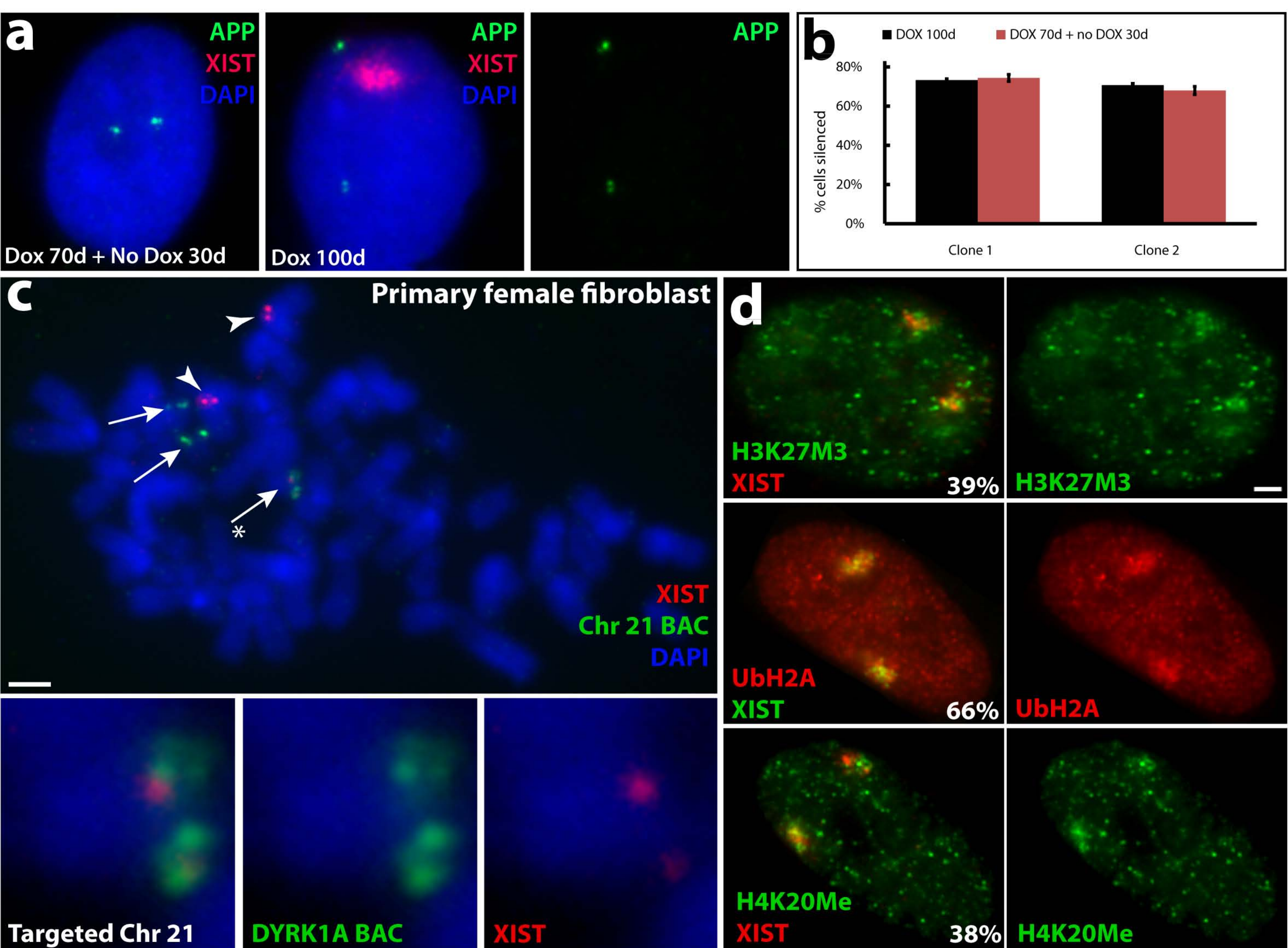
CXADR
COL6A1
PTTG1IP
ADAMTS1
BTG3
TIAM1
USP16
APP

no DOX
DOX

**Supplementary Figure 7. Selection against silencing in double targeted and triple targeted clones, and qRT-PCR validation for microarray silencing. a.** RNA FISH shows *XIST* expression is silenced during long-term culture (20 days) in double targeted (arrows, left) and triple targeted (arrows, right) iPSCs. The *APP* gene is not silenced by the low level *XIST* RNA expressed in these cells. **b.** Percentage of *XIST* paint on days 3 & 20 after dox induction. Although *XIST* RNA is robustly expressed in early time points (3 days) in the double and triple targeted clones, *XIST* becomes almost entirely silenced in later time points (20 days). Mean ± SE from 100 nuclei. **c.** qRT-PCR for eight Chr 21 linked genes for Clone 3. Mean ± SE from triplicate samples.




**Supplementary Figure 8. Correction of Chr21 trisomy improves cell proliferation and neural rosette formation. a.** Proliferation analysis by crystal violet staining for the parental line and clones 1 and 2. One week after dox induction, clones 1 & 2 show more tightly packed cells in colonies, and increased colony size and numbers compared to no-dox cells. This change was not seen in the parental line. **b.** PAX6 and SOX1 staining for differentiated neural progenitor cells. **c-d.** Neural rosette formation on days 14(c) and 17(d). The corrected trisomic iPSCs exhibit numerous neural rosettes by day 14, while the non-corrected trisomic iPSCs as well as the parental line takes longer (17days) to form neural rosettes. Scale: 100 μm.




**Supplementary Figure 9. Stable silencing in cortical neurons and targeted addition in primary fibroblasts. a.** RNA FISH in differentiated cortical neurons. The left cell shows the third *APP* transcript locus (green) remains silenced after the cell was treated with dox for 70 days and then dox was removed for 30 days, as similarly shown in the cell (middle and right) that was treated with dox for 100 days. This is consistent with other evidence that multi-layered chromatin modifications triggered by *XIST* maintain a largely irreversible silent state. **b.** Quantification of *APP* silencing by RNA FISH. *APP* silencing is stable upon withdrawal of *XIST* RNA. Mean ± SE from 100 nuclei. **c.** Metaphase DNA FISH in human DS primary fibroblasts. Long arrows indicate three Chr 21s, and asterisk indicates *XIST* targeted Chr21 that is enlarged at the bottom panel. Arrowheads indicate endogenous *XIST* genes on the two X-chromosomes in the female cell. Scale: 5 μm.**d.** Immunostaining for targeted primary fibroblasts indicates enrichment of H3K27me3, UbH2A, and H4K20me on both the targeted Chr21 and endogenous Xi in many cells (percentages shown on figures). Scale: 2 μm. This is consistent with evidence from our lab and the Wutz lab that chromosome silencing does not necessarily require the optimal pluripotent cell context. Future studies will be required to assess the ability of various somatic cell contexts to support chromosome silencing.