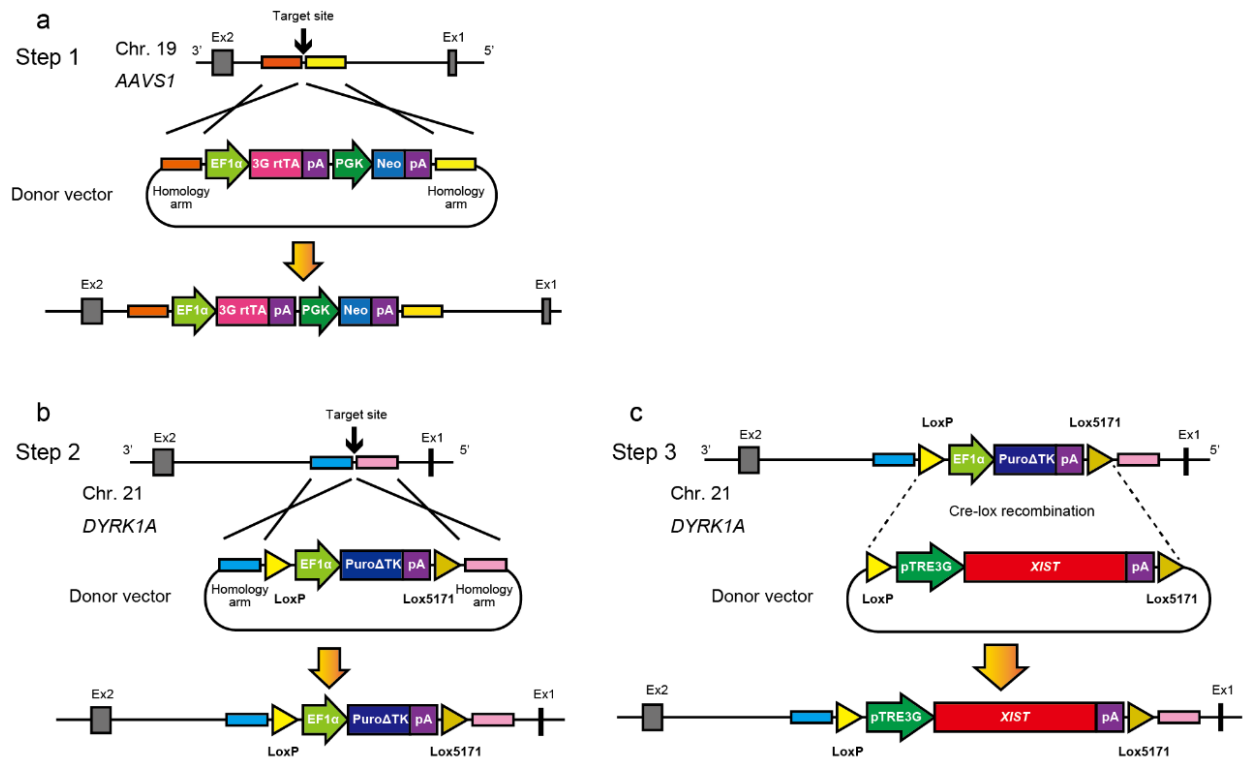


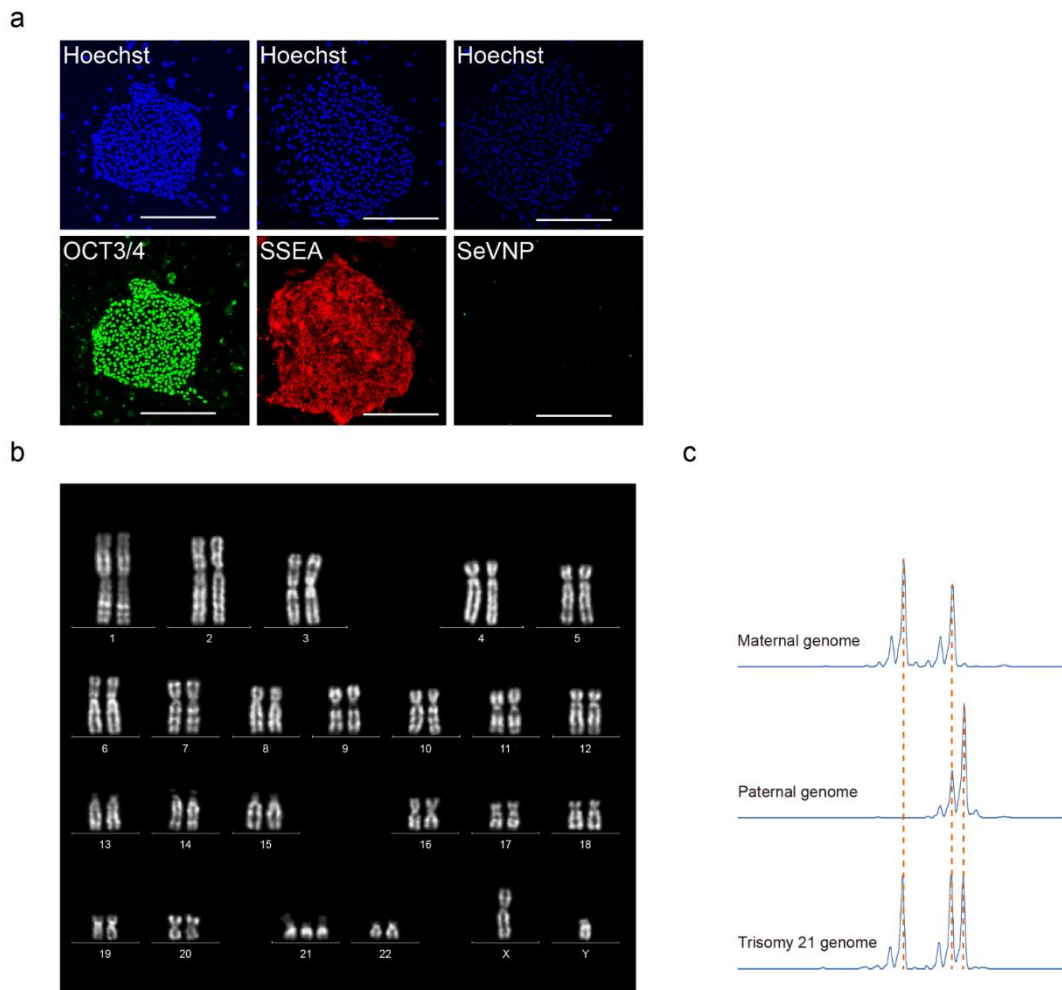
Supplementary Information

A human isogenic iPSC-derived cell line panel identifies major regulators of aberrant astrocyte proliferation in Down syndrome

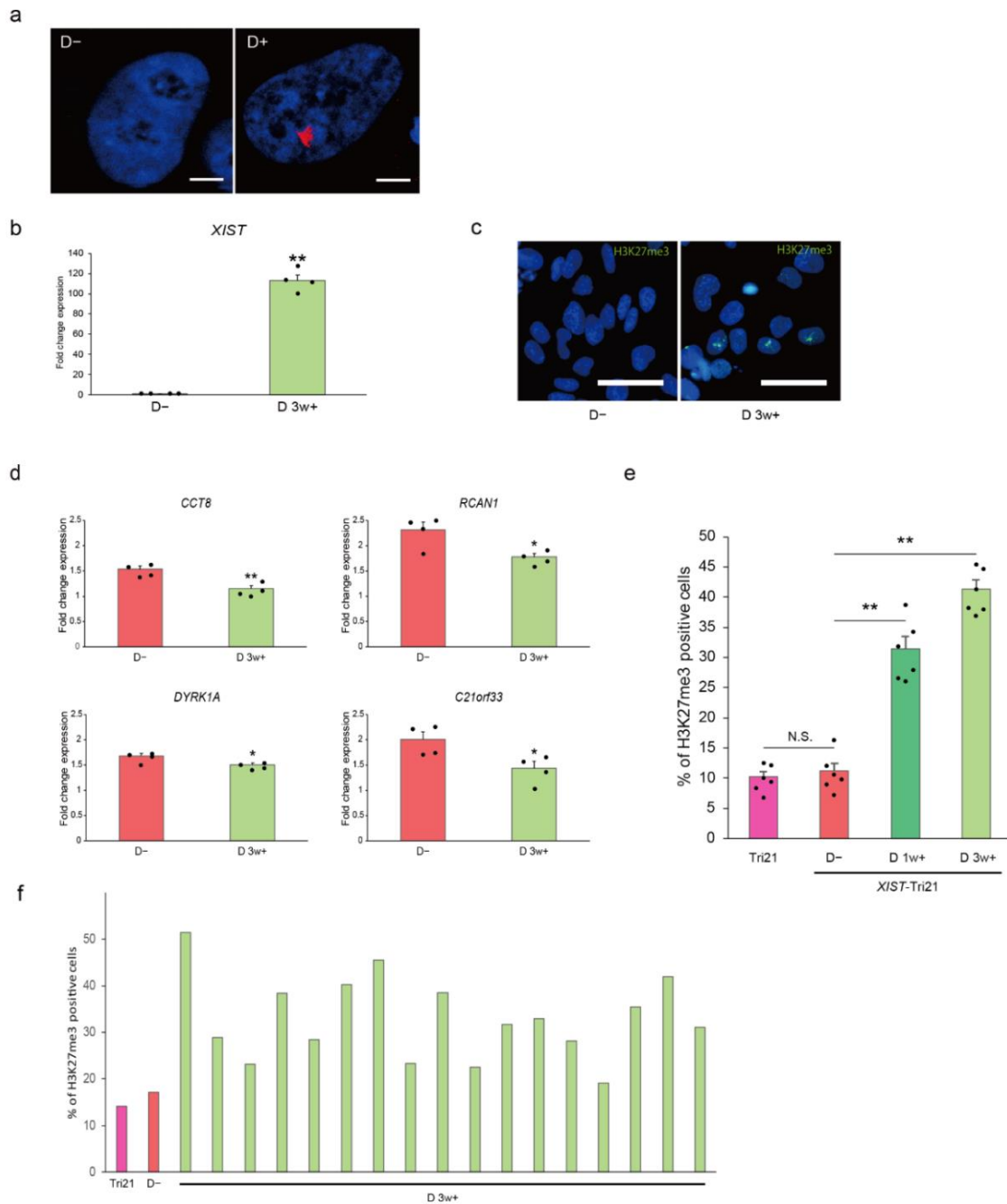
Keiji Kawatani¹, Toshihiko Nambara¹, Nobutoshi Nawa¹, Hidetaka Yoshimatsu¹,
Haruna Kusakabe¹, Katsuya Hirata^{1,2}, Akira Tanave³, Kenta Sumiyama³, Kimihiko
Banno^{1,4}, Hidetoshi Taniguchi¹, Hitomi Arahori¹, Keiichi Ozono¹, and Yasuji
Kitabatake^{1*}



Supplementary Figure 1 | Generation of an *XIST*-mediated, chromosome 21-silencing system. a) (Step 1) Schematic representation depicting the targeted insertion of rtTA construct into the *AAVS1* locus of chromosome 19 in Tri21 iPSC line using a ZFN. Top: *AAVS1* gene locus including exons (grey boxes), ZFN-target sites (black arrow), and homology arms (orange and yellow bars). Middle: donor vector including homology arms, a human EF1 α promoter (light-green arrow), the rtTA (pink box), poly(A) sequences (purple boxes), a human PGK promoter (green arrow), and the neomycin-resistance gene (Neo, blue box). Bottom: schematic of the rtTA-inserted *AAVS1* locus. **b) (Step 2)** Targeted insertion of the exchange cassette into the *DYRK1A* locus on chromosome 21. Top: *DYRK1A* gene locus including exons (grey boxes), CRISPR–Cas9-target sites (black arrow), and homology arms (blue and pink bars). Middle: donor vector including homology arms, the *loxP* sequence (yellow triangle), drug-resistance genes (Puro Δ TK, blue box), poly(A) sequences, and the *lox5171* site (dark yellow triangle). Bottom: schematic of the exchange cassette-inserted *DYRK1A* locus. **c) (Step 3)** Cre recombinase-mediated cassette exchange used to insert human *XIST* cDNA. Top: Exchange cassette-inserted *DYRK1A* locus in chromosome 21. Middle: donor vector including the *loxP* sequence, the tetracycline-response element (green arrow, pTRE3G), human *XIST* cDNA (red box), poly(A) sequences, and the *lox5171* sequence. Bottom: schematic of the *XIST*-inserted *DYRK1A* locus.

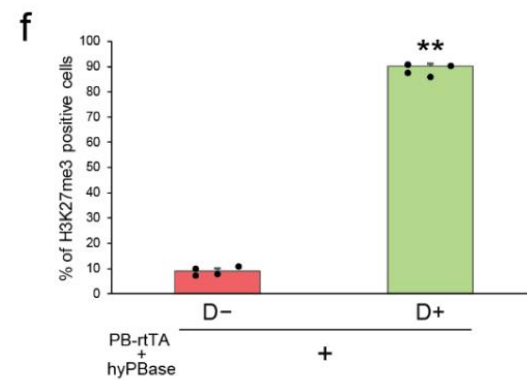
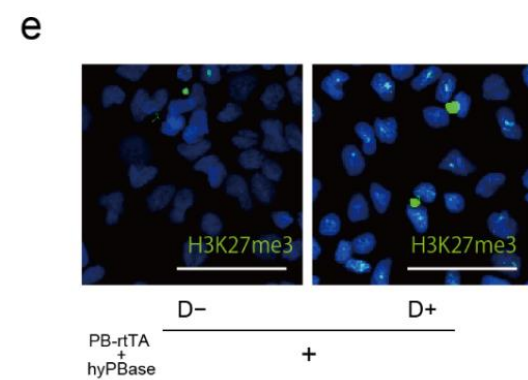
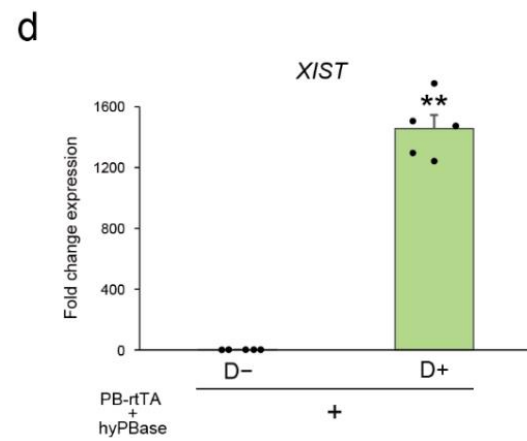
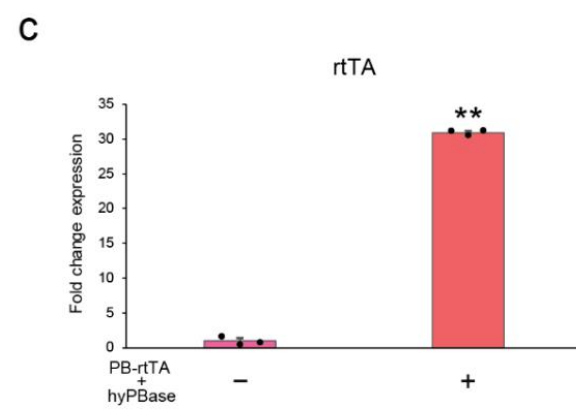
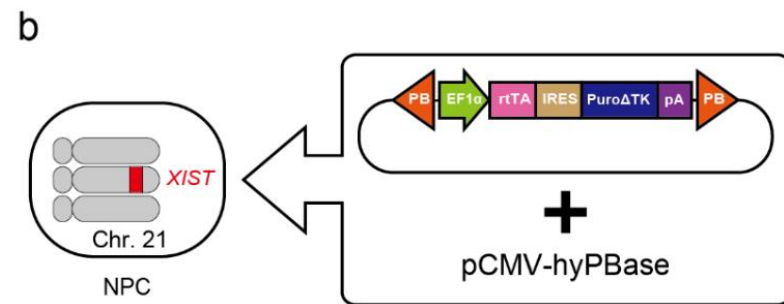
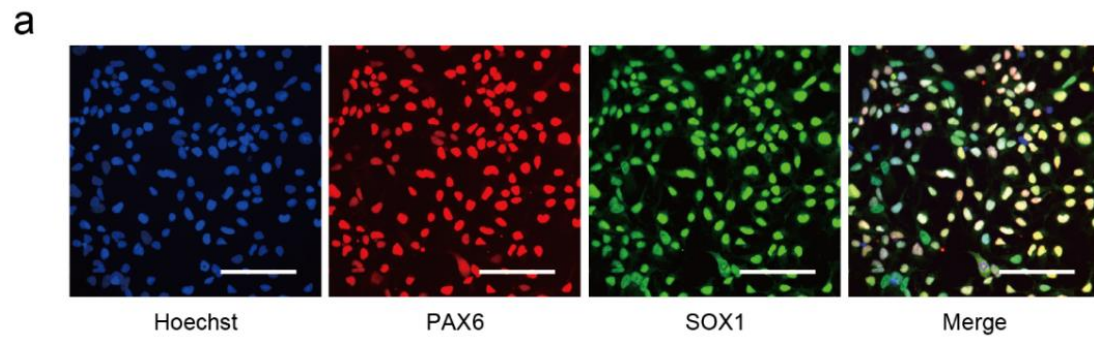


Supplementary Figure 2 | Analysis of an *XIST*-inserted trisomy 21 iPSC line. a) Immunocytochemical staining of the *XIST*-Tri21 iPSC line using specific antibodies against OCT3, OCT4, SSEA, and Sendai virus NP protein (SeVNP). Nuclei were stained with Hoechst 33342. Scale bars: 500 μ m. **b)** Karyotype analysis of the *XIST*-Tri21 iPSC line. **c)** STR analysis of the *XIST*-Tri21 iPSC line. The STR sequence of the *RUNX1* locus in chromosome 21 was compared between a patient with DS and his parents' genomes.

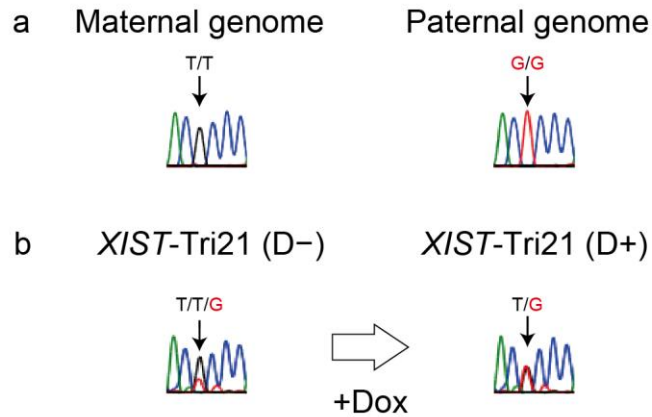


Supplementary Figure 3 | Dox treatment induces chromosome silencing in the *XIST*-Tri21 iPSC line. a) FISH analysis of *XIST* RNA in untreated (D-) *XIST*-Tri21 iPSCs and *XIST*-Tri21 iPSCs treated with Dox (D+) for 3 weeks. *XIST* RNA was labelled with a Dylight594-conjugated probe (red). Nuclei were stained with DAPI. Scale bars: 5 μ m. **b)** Relative expression levels of *XIST* in untreated (D-) and Dox-treated (D+) *XIST*-Tri21 iPSC lines (n = 4 experiments per cell line). Gene-expression levels were normalised to those of the D- cell lines. Error bars represent the SEM. **c)** Immunocytochemical staining of the *XIST*-Tri21 iPSC line using an anti-H3K27me3 antibody. Nuclei were stained with Hoechst 33342. Scale bars: 50 μ m. **d)** Relative expression levels of genes on chromosome 21 in untreated (D-) and Dox-treated (D+) *XIST*-Tri21 iPSC lines (n = 4 experiments per line). Gene-expression levels were normalised to

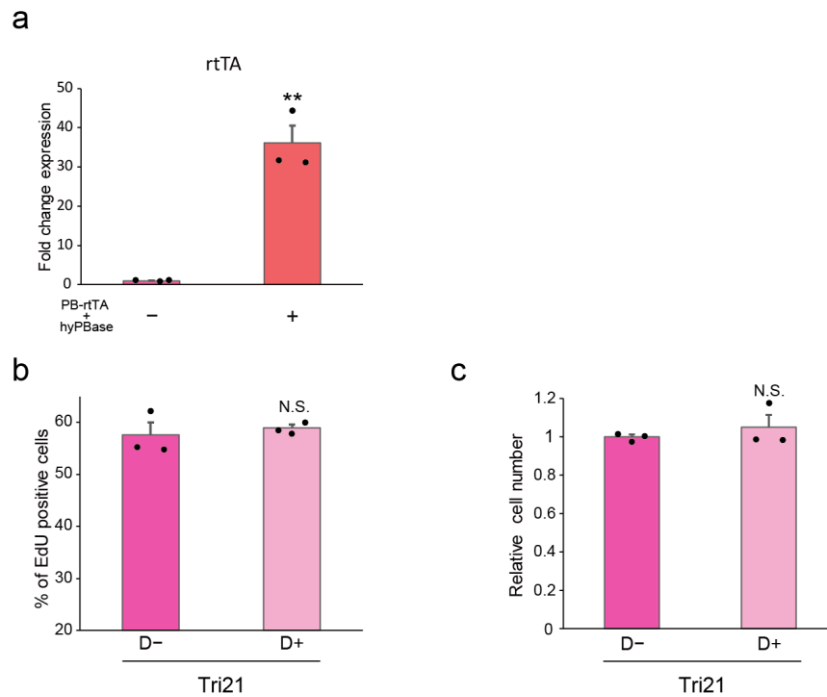
those of cDi21 lines ($n = 4$ experiments per cell line). Error bars represent the SEM. **e)** Percentage of H3K27me3-positive cells in Tri21 iPSCs, untreated (D-) *XIST*-Tri21 iPSCs, and *XIST*-Tri21 iPSCs, treated with Dox for 1 week (D 1w+) or 3 weeks (D 3w+). Less than 45% of *XIST*-Tri21 iPSCs were H3K27me3-positive even after 3 weeks of Dox administration ($n = 6$ experiments per cell line). Two *XIST*-Tri21 lines were used for each experiment (**b**, **d**, **e**). **f)** Percentage of H3K27me3-positive Tri21 iPSCs, untreated (D-) *XIST*-Tri21 iPSCs, and (D 3w+) *XIST*-Tri21 iPSCs after single-cell cloning. A relatively low efficiency of H3K27me3 induction was continuously observed in single-cell-derived clones of *XIST*-Tri21 iPSCs. Error bars represent the SEM. The data shown were analysed using Student's *t*-test (**d**, **e**) or Welch's two-sample *t*-test (**b**). * $P < 0.05$, ** $P < 0.01$; N.S., not significant ($P > 0.05$).



Supplementary Figure 4 | Additional transfection of rtTA into *XIST*-Tri21 NPCs. **a)** Immunocytochemical staining of *XIST*-Tri21 NPCs using PAX6- and SOX1-specific antibodies. Nuclei were stained with Hoechst 33342. Scale bars: 100 μm . **b)** Schematic depicting the additional transfection of an rtTA using a PB transposon vector and a hyperactive PB transposase into *XIST*-Tri21 NPCs. **c)** Relative rtTA-expression levels in *XIST*-Tri21 NPCs, with or without rtTA transfection (n = 3 experiments per cell line). rtTA expression from the gene cassette inserted into the *AAVS1* safe harbour locus was lost in NPCs, but restored by the transduction of PB-rtTA. Expression was normalised to that of untransfected cell lines. Error bars represent the SEM. **d)** Relative expression levels of *XIST* RNA in rtTA-transfected *XIST*-Tri21 NPCs. D+ cells were treated with Dox for 5 days. Expression levels were normalised to those of Dox-untreated lines (n = 5 experiments per cell line). **e)** Immunocytochemistry of rtTA-transfected *XIST*-Tri21 NPCs using an H3K27me3-specific antibody. D+ cells were treated with Dox for 5 days. Nuclei were stained with Hoechst 33342. Scale bars: 50 μm . **f)** Percentage of H3K27me3-positive cells in rtTA-transfected *XIST*-Tri21 NPCs. D+ cells were treated with Dox for 5 days (n = 4 experiments per cell line). Each of the data was obtained from three lines (**c**, **d**, **f**). Error bars represent the SEM. The data shown were analysed using Student's *t*-test (**c**, **f**) or Welch's two-sample *t*-test (**d**). * $P < 0.05$, ** $P < 0.01$.

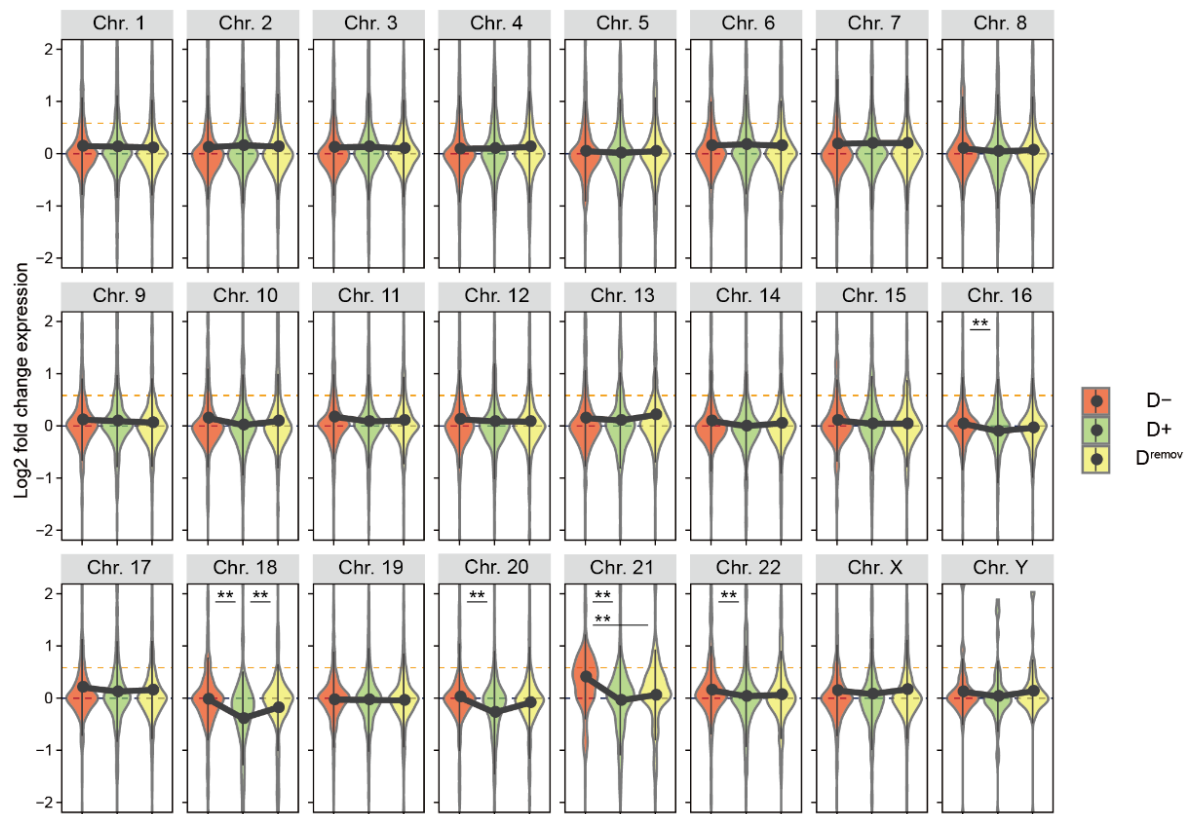


Supplementary Figure 5 | SNP analysis of mRNA extracted from *XIST*-Tri21 APCs, with or without Dox treatment, revealed the parental origin of the *XIST*-inserted chromosome 21. a) SNP analysis of the *ETS2* gene in chromosome 21 using the parents' genomic DNA showed T/T polymorphisms in the maternal genome and G/G polymorphisms in the paternal genome. **b)** SNP analysis of the *ETS2* gene using total cDNA (i.e., not genomic DNA) prepared from *XIST*-Tri21 APCs, with and without *XIST* expression. cDNA derived from *XIST*-Tri21 APCs without Dox treatment (D-) showed T/T/G polymorphisms, whereas cDNA derived from Dox-treated (chromosome-inactivated) *XIST*-Tri21 APCs lost a T allele, indicating that *XIST* cDNA was inserted into one of two maternal copies of chromosome 21 (hereafter the *XIST*-inserted maternal chromosome is referred to as M2).

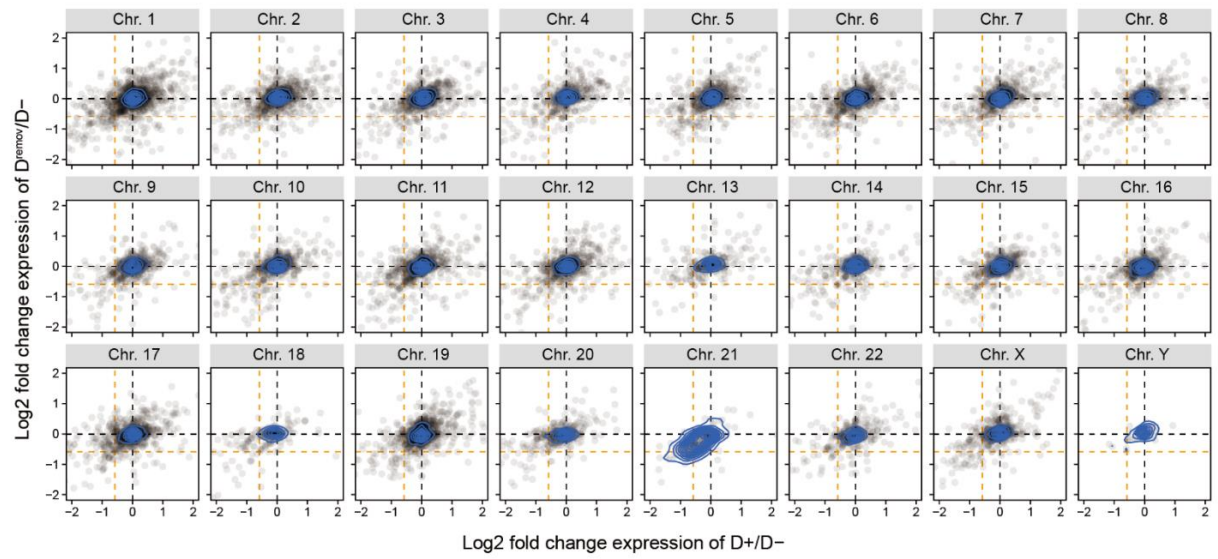


Supplementary Figure 6 | Dox administration does not effect the basal proliferative ability of Tri21 APCs. a)

Relative rtTA-expression levels in *XIST*-Tri21 APCs. The expression levels were normalised to those of untransfected APCs (n = 3 experiments per cell line). Data were obtained from three lines. **b)** Percentage of EdU-positive Tri21 APCs (i.e., not containing the *XIST* transgene; n = 3 experiments per cell line). Dox was administered to the D+ line for 6 weeks. **c)** Relative numbers of Tri21 APCs 1 day after seeding. The cell numbers were normalised to those of the D- APC line (n = 3 experiments per cell line). Error bars represent the SEM. The data shown were analysed by Student's *t*-test (**b, c**) or Welch's two-sample *t*-test (**a**). **P* < 0.05, ***P* < 0.01; N.S., not significant (*P* > 0.05).

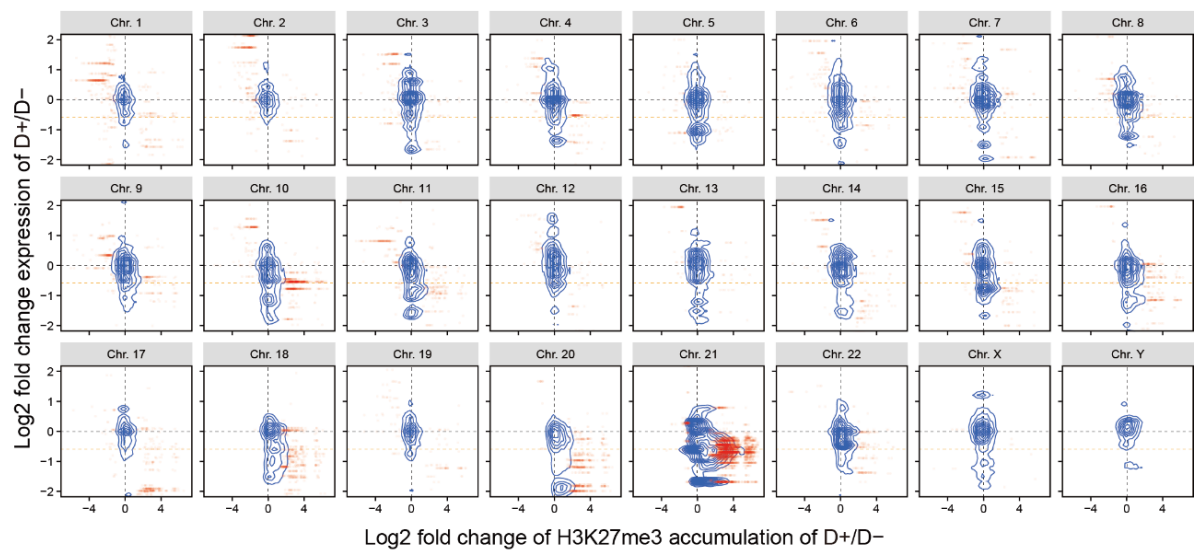


Supplementary Figure 7 | Transcriptional profiling of each chromosome in Dox-treated *XIST*-Tri21 APCs. Violin plots of relative log-transformed expression ratios for genes in each chromosome in *XIST*-Tri21 APC lines (n = 3 per cell line). D⁻ cell (red); D⁺ cell (green); D^{remov} cell lines (yellow). Gene-expression levels with positive read counts (as determined by RNA-seq analysis) were normalised to those of cDi21 lines. The upper orange dashed lines indicate a ratio of 1.5, whereas the lower black dashed lines indicate a ratio of 1.0. The plots show mean expression levels with error bars indicating the SD. The data shown were analysed by the Kruskal–Wallis test with Bonferroni’s correction. ** $P < 0.01$

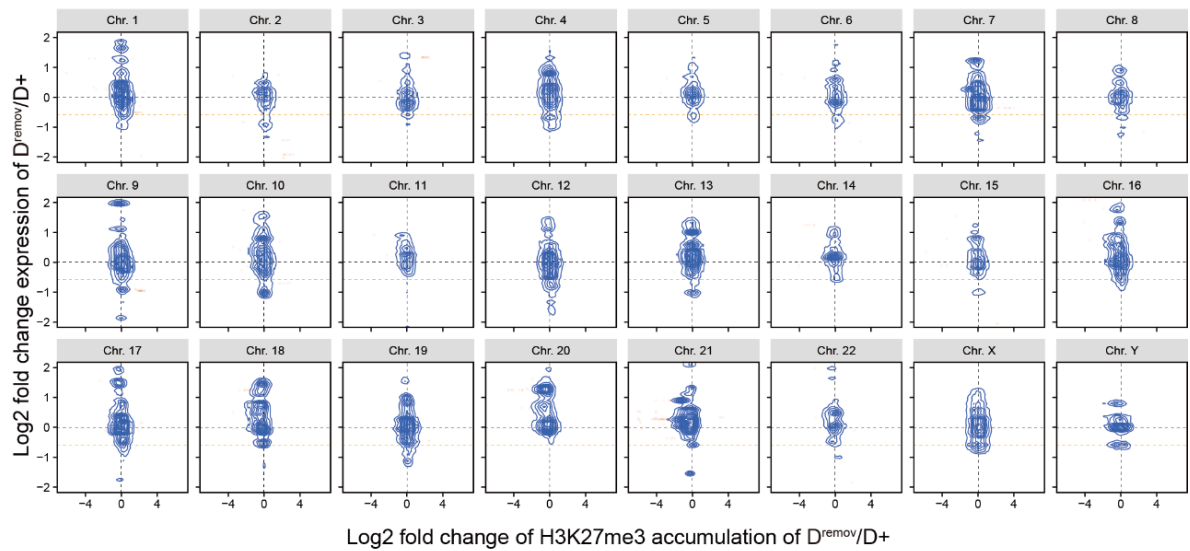


Supplementary Figure 8 | Transcriptional profiling showed sustained gene suppression in *XIST*-Tri21 APC lines after Dox removal. Log2-fold changes in the mean gene-expression ratios (Dox-treated lines: Dox-untreated lines, X-axis; D^{remov}: Dox-untreated lines, Y-axis). The grey data points represent the full set of 18,440 genes with positive read, as determined by RNA-seq, with the point densities for all sets represented by blue line contours. The orange dashed lines indicate a two-thirds decrease in the gene-expression levels for each cell line.

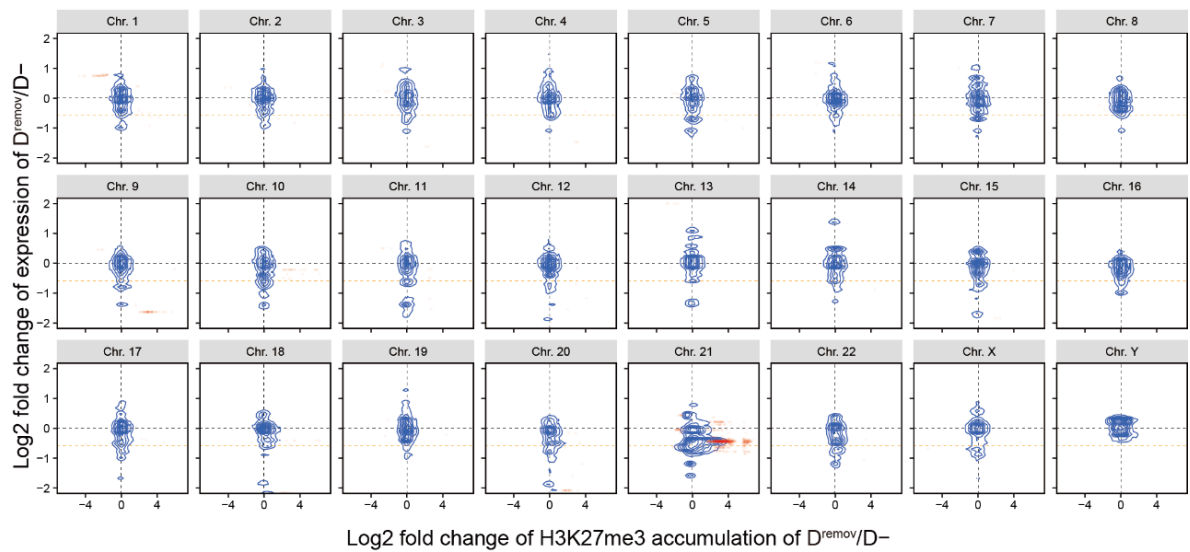
a D+ and D-



b D^{remov} and D+

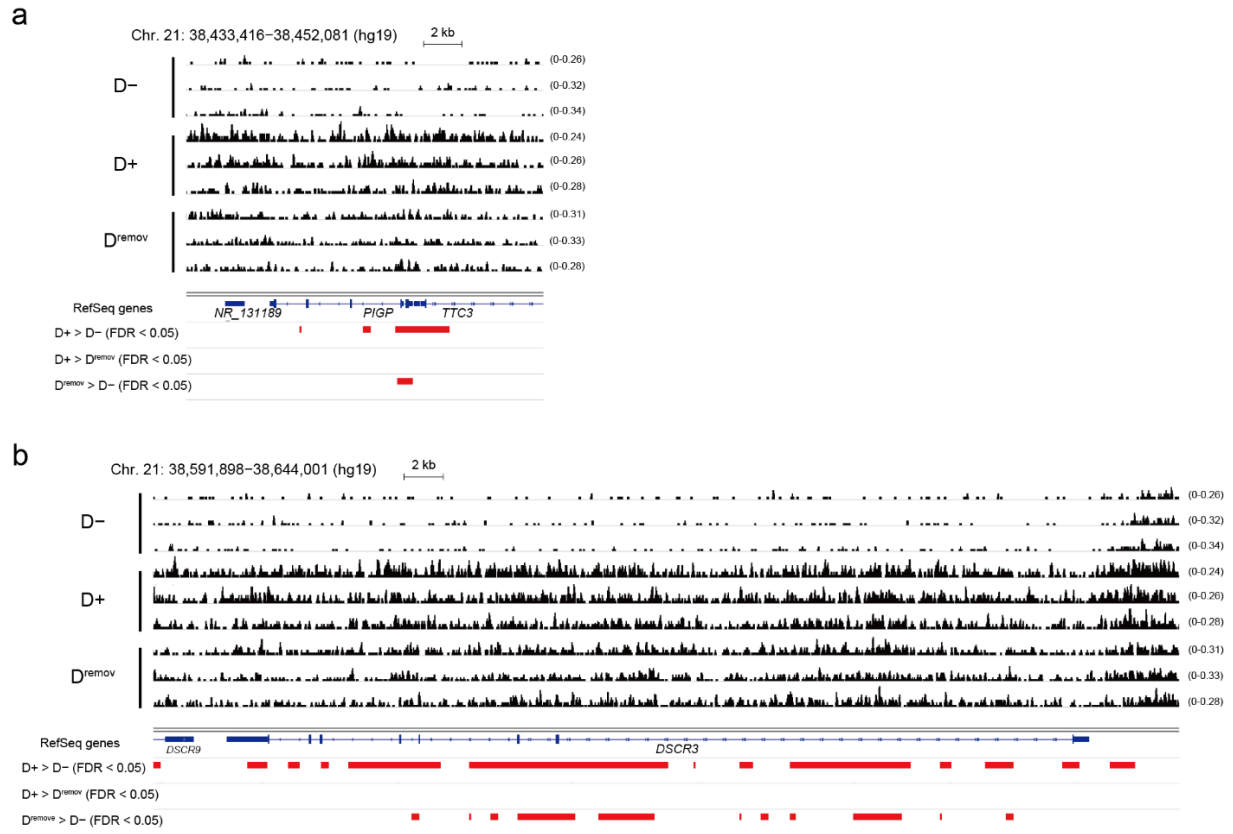


c D^{remov} and D-

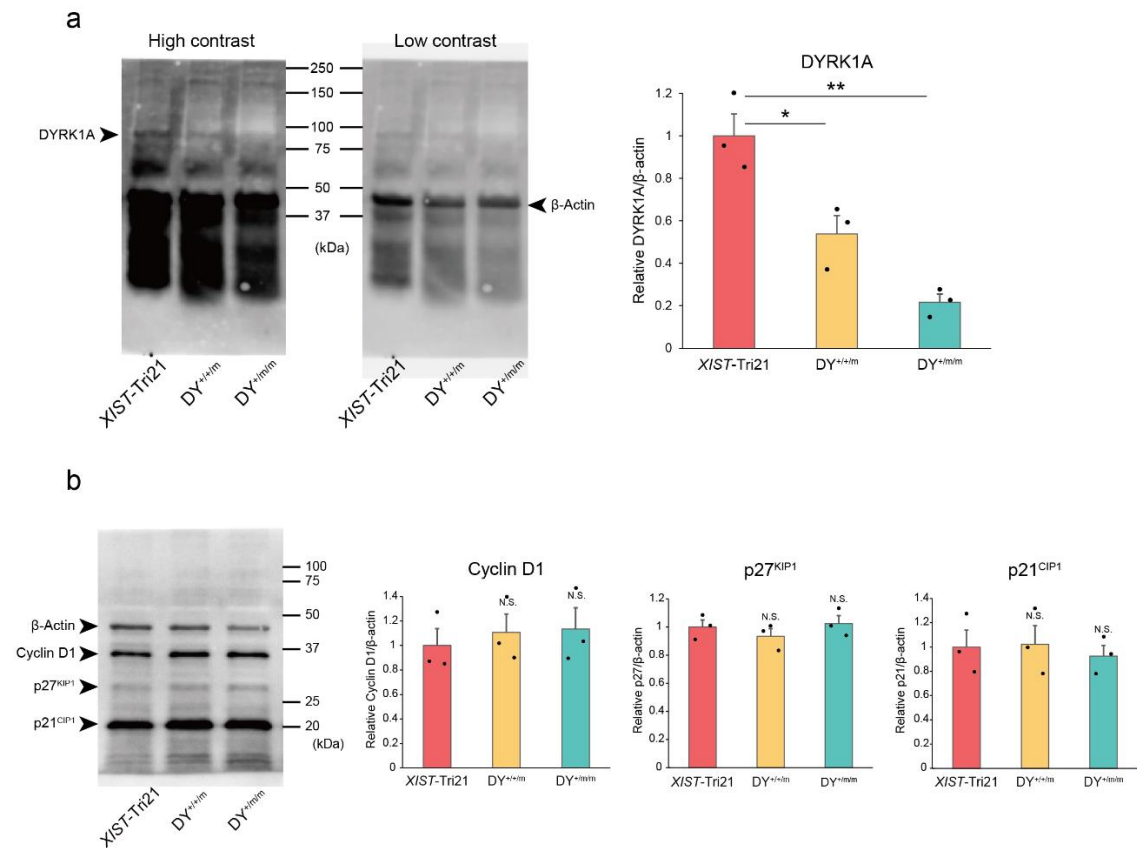


Supplementary Figure 9 | Integrative analysis of the RNA-seq and ChIP-seq data revealed a negative correlation between H3K27me3 enrichment and transcriptional silencing in D+ and D^{remov} *XIST*-Tri21 APC lines. a–c)

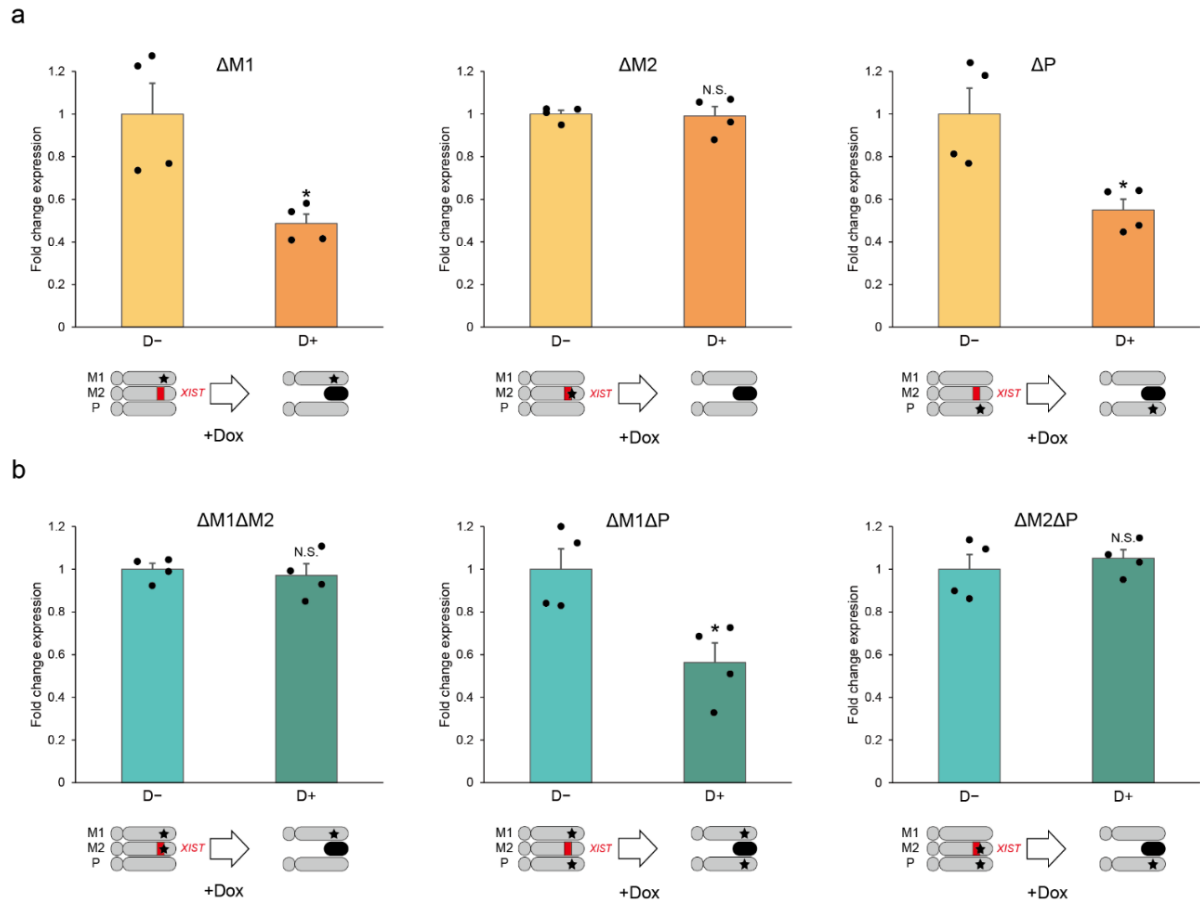
H3K27me3 accumulation within each gene body and 2 kb upstream of the transcription start site were compared between D+ and D– cell lines (a), D^{remov} and D+ cell lines (b), and D^{remov} and D– *XIST*-Tri21 APC lines (c). Genes with a significant difference in H3K27me3 accumulation between two lines are represented with red data points, with the density for all pair points shown by blue line contours. The orange dashed lines indicate a two-thirds decrease in the gene-expression level of each cell line. With the ChIP-seq data, the FDRs were calculated using the Benjamini–Hochberg method, and FDR < 0.05 was considered to reflect a statistically significant difference.



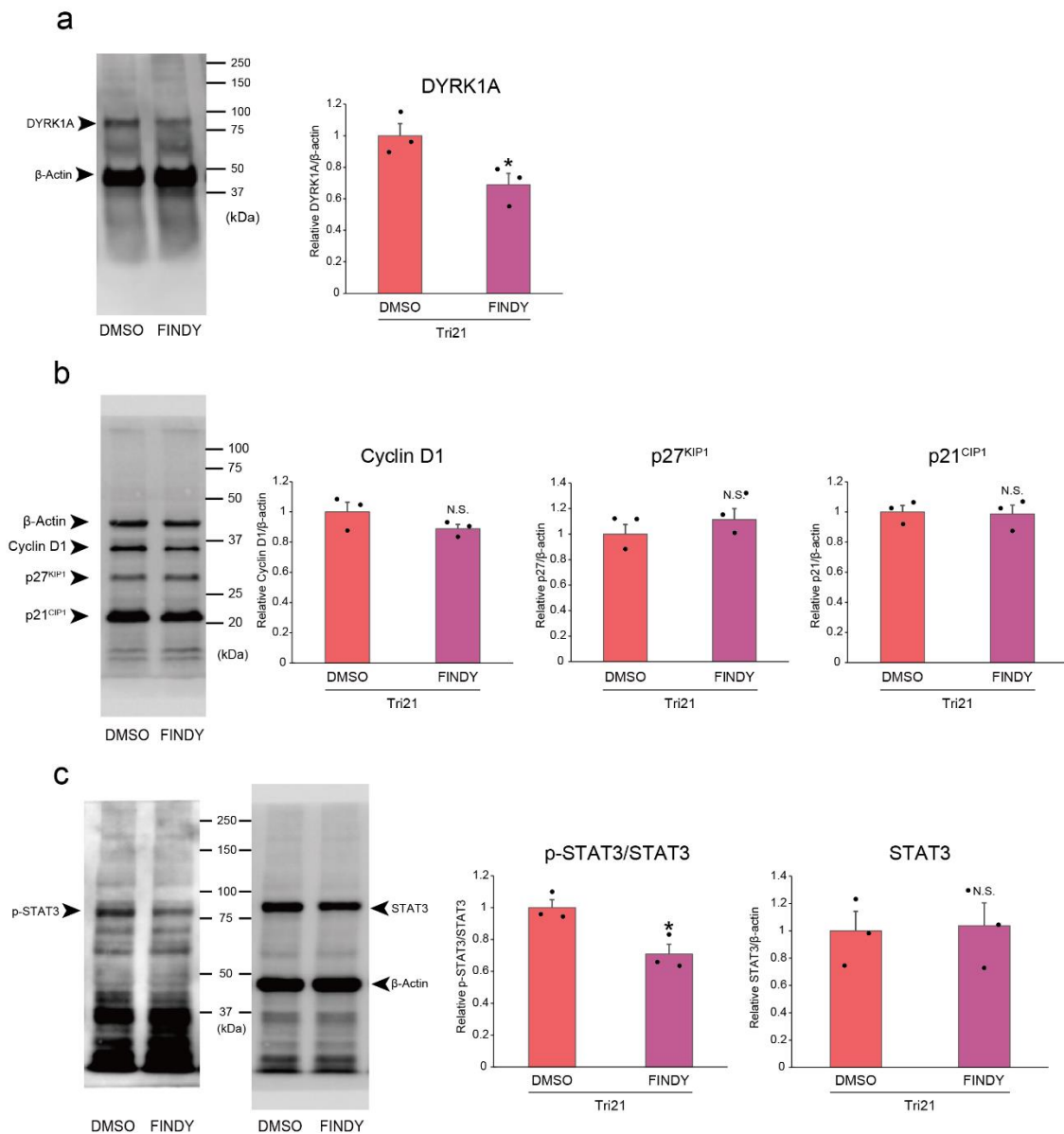
Supplementary Figure 10 | Map showing the distribution of H3K27me3 modifications in *PIGP* and *DSCR3* in *XIST-Tri21* APCs. **a, b) Integrative Genomics Viewer screenshot of H3K27me3 ChIP-seq track peaks for *PIGP* (**a**) and *DSCR3* (hg19) (**b**) in Dox-untreated cell lines, Dox-treated cell lines, and D^{remov} cell lines. The Y-axis shows the number of fragments per base pair per million reads. (Red bars) The distribution of the regions with significantly higher H3K27me3 accumulation in each comparison. The FDRs were calculated using the Benjamini–Hochberg method. FDR < 0.05 was considered to reflect a statistically significant difference.**



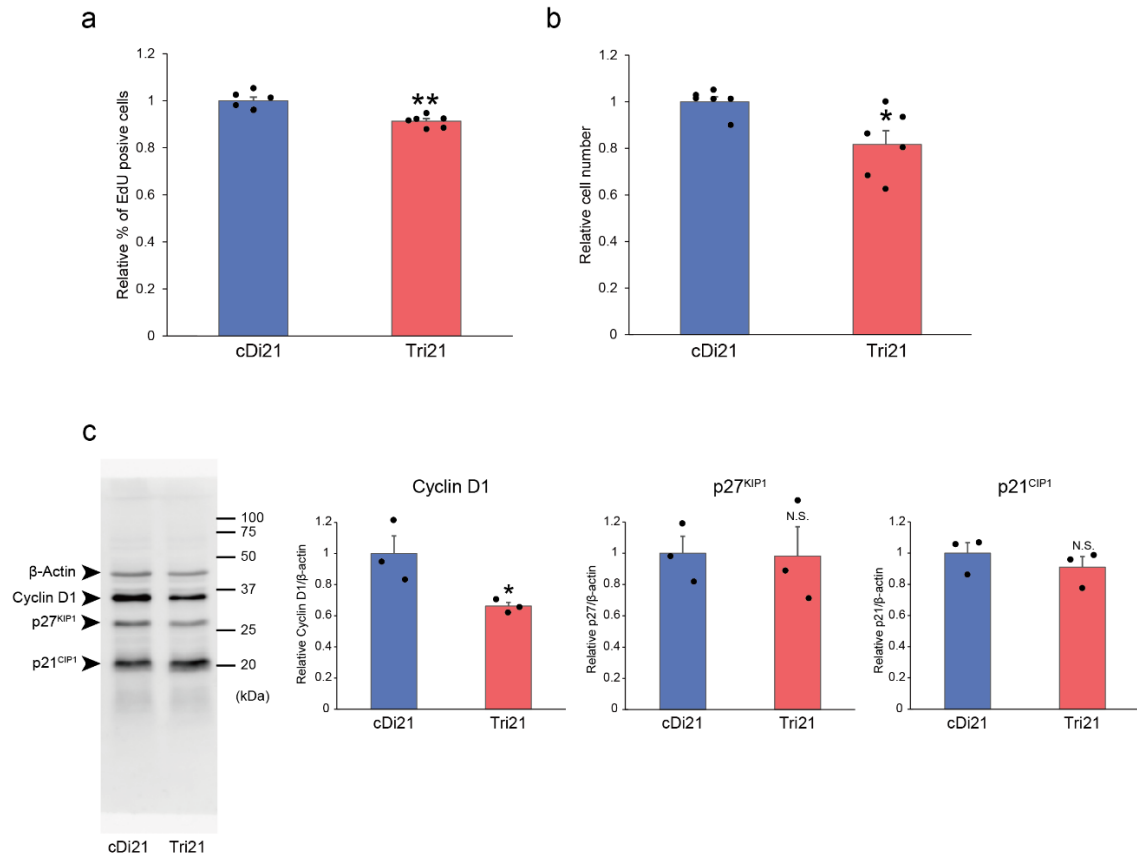
Supplementary Figure 11 | Targeted deletion of *DYRK1A* in APCs had little effect on cell cycle-related protein levels. a, b Immunoblot analysis of DYRK1A (**a**) and cell cycle-related proteins (Cyclin D1, p27^{KIP1}, p21^{CIP1}) (**b**) in *DYRK1A*-targeted *XIST*-Tri21 APCs. The high-contrast and low-contrast images were taken from the same membrane. β-Actin was used as a loading control and expression levels were normalised to that of the *XIST*-Tri21 line (n = 3 experiments per cell line). Each of the data was obtained from three lines. Error bars represent the SEM. Data were analysed by Student's *t*-test. **P* < 0.05, ***P* < 0.01; N.S., not significant (*P* > 0.05).



Supplementary Figure 12 | *DYRK1A* expression levels in $DY^{+/+/-}$ and $DY^{+/m/m}$ *XIST*-Tri21 APCs depend on the copy numbers of active *DYRK1A* genes. a, b) Relative *DYRK1A*-expression levels in $DY^{+/+/-}$ (a) and $DY^{+/m/m}$ (b) *XIST*-Tri21 APCs, with or without Dox treatment. Expression levels were normalised to that of Dox-untreated lines (n = 4 experiments per cell line). Error bars represent the SEM. The data shown were analysed by Student's *t*-test. * $P < 0.05$; N.S., not significant ($P > 0.05$). (Lower) Schematic depiction of the combination of targeted *DYRK1A* alleles and inactivated chromosome 21. The parental origin of chromosome 21 is indicated (M1, M2, or P). Red rectangle, *XIST* transgene; black stars, *DYRK1A*-target sites; black chromosomes, inactivated chromosome 21. Expected copy numbers of the active *DYRK1A* gene decreased from two to one in $\Delta M1$ - and ΔP - $DY^{+/+/-}$ APC lines, and from one to zero in $\Delta M1\Delta P$ - $DY^{+/m/m}$ APC lines after Dox treatment. In contrast, the expected copy numbers of the active *DYRK1A* gene remained unchanged as two in the $\Delta M2$ - $DY^{+/+/-}$ APC line, or as one in the $\Delta M1\Delta M2$ - and $\Delta M2\Delta P$ - $DY^{+/m/m}$ APC lines.



Supplementary Figure 13 | DYRK1A inhibitor decreases p-STAT3 levels in Tri21 APCs. a–c) Immunoblot analysis of DYRK1A (a), cell cycle-related proteins (Cyclin D1, p27^{KIP1}, p21^{CIP1}) (b), and p-STAT3 and STAT3 (c) in Tri21 APCs after 2-day treatment with FINDY (2.5 μ M). (c) The left blotted membrane was probed with an anti-p-STAT3 antibody. The right blotted membrane was stripped and re-probed with anti-STAT3 and anti- β -actin antibodies. β -Actin was used as a loading control and expression levels were normalised to that of Tri21 APCs without FINDY treatment (n = 3 experiments per condition). Error bars represent the SEM. Data were analysed by Student's *t*-test. * P < 0.05; N.S., not significant (P > 0.05).

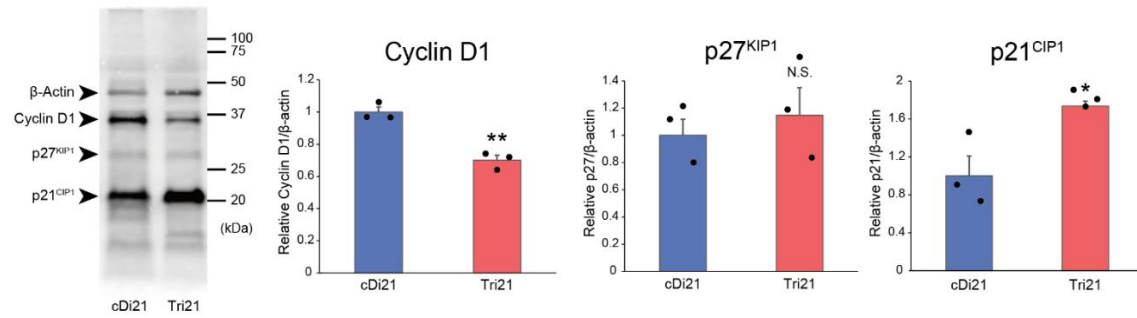


Supplementary Figure 14 | Tri21 NPCs exhibit impaired proliferation and decreased levels of Cyclin D1. a)

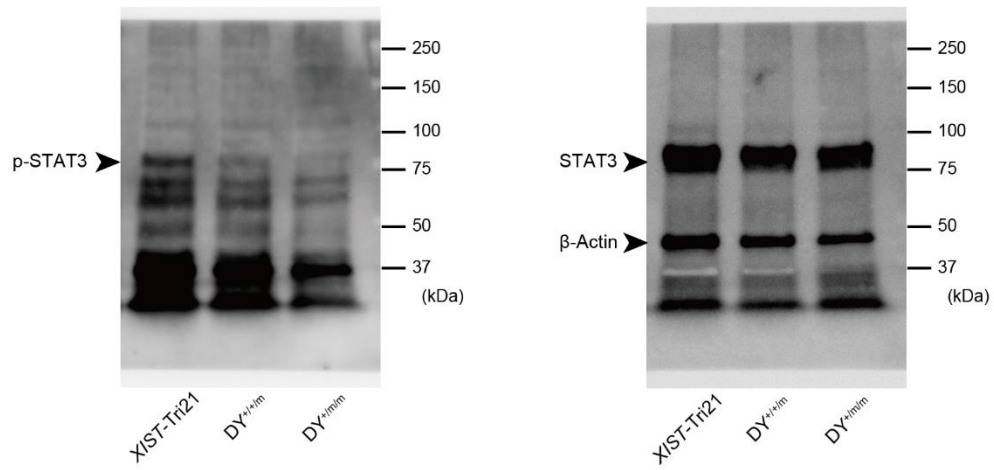
Percentage of EdU-positive NPCs, which was normalised to that of the cDi21 cell lines (n = 6 experiments per cell line).

b) Relative numbers of NPCs 1 day after seeding. Cell numbers were normalised to that of the cDi21 cell lines (n = 6 experiments per cell line).

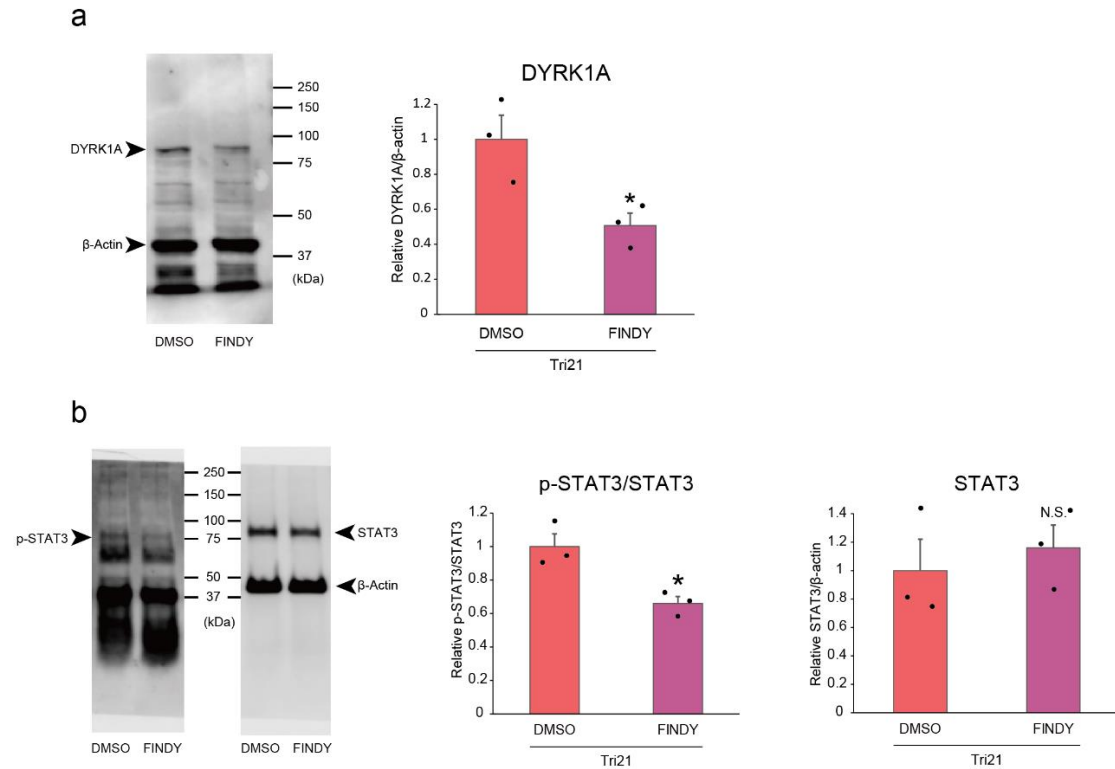
c) Immunoblot analysis of Cyclin D1, p27^{KIP1} and p21^{CIP1} in NPCs. β-Actin was used as a loading control and expression levels were normalised to that of the cDi21 cell line (n = 3 experiments per cell line). Error bars represent the SEM. Data were analysed by Student's *t*-test (**a**, **c**) or Welch's two-sample *t*-test (**b**). **P* < 0.05, ***P* < 0.01; N.S., not significant (*P* > 0.05).



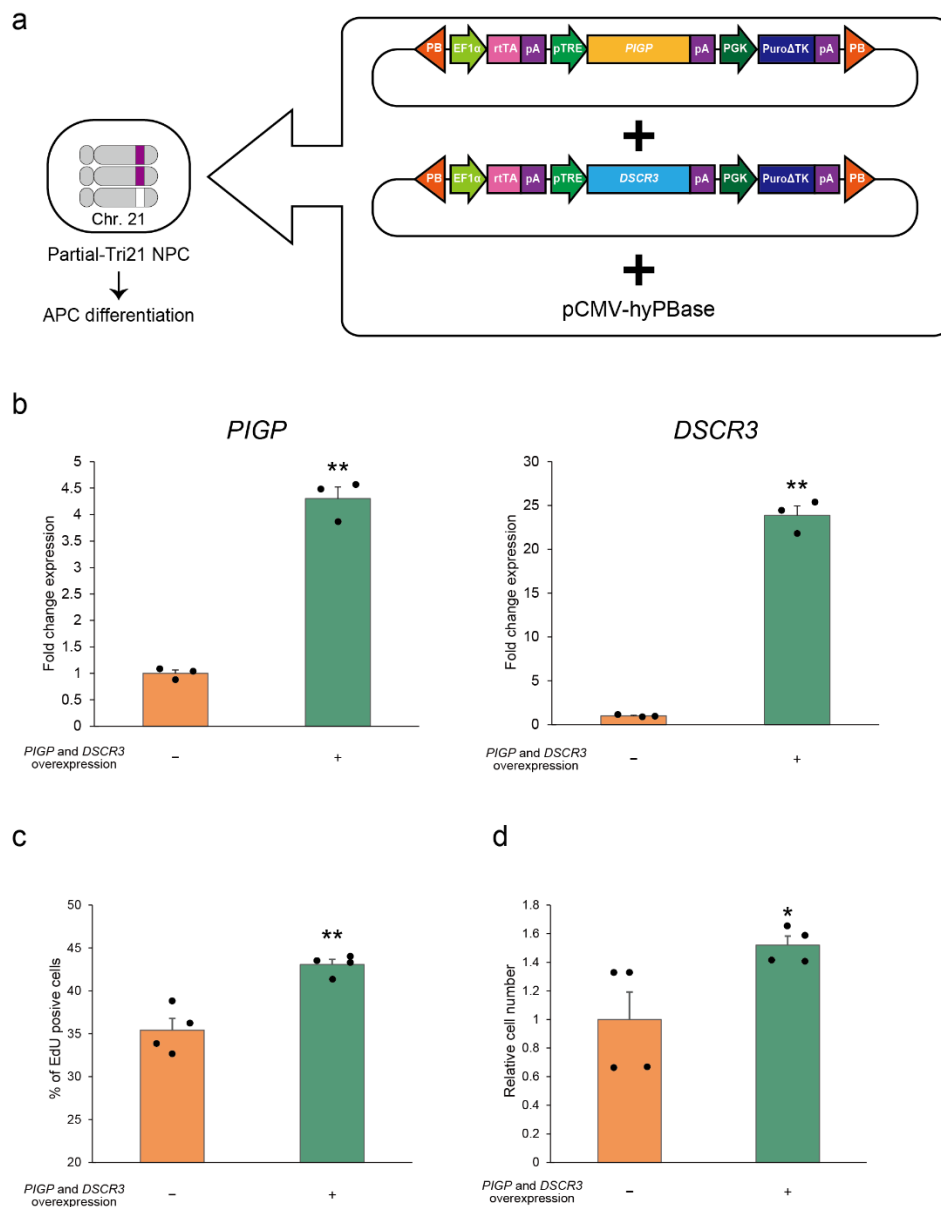
Supplementary Figure 15 | Changes in Cyclin D1 and p21 levels in Tri21 APCs. Immunoblot analysis of cell cycle-related proteins (Cyclin D1, p27^{KIP1}, p21^{CIP1}) in APCs. β -Actin was used as a loading control and expression levels were normalised to that of the cDi21 cell line (n = 3 experiments per cell line). Each of the data was obtained from two lines. Error bars represent the SEM. Data were analysed by Student's *t*-test. * P < 0.05, ** P < 0.01; N.S., not significant (P > 0.05).



Supplementary Figure 16 | Full immunoblots related to the data shown in Fig. 5g. Full immunoblot images for p-STAT3 and STAT3. β -Actin was detected as a loading control. The left blotted membrane was probed with an anti-p-STAT3 antibody. The right blotted membrane was stripped and re-probed with anti-STAT3 and anti- β -actin antibodies.

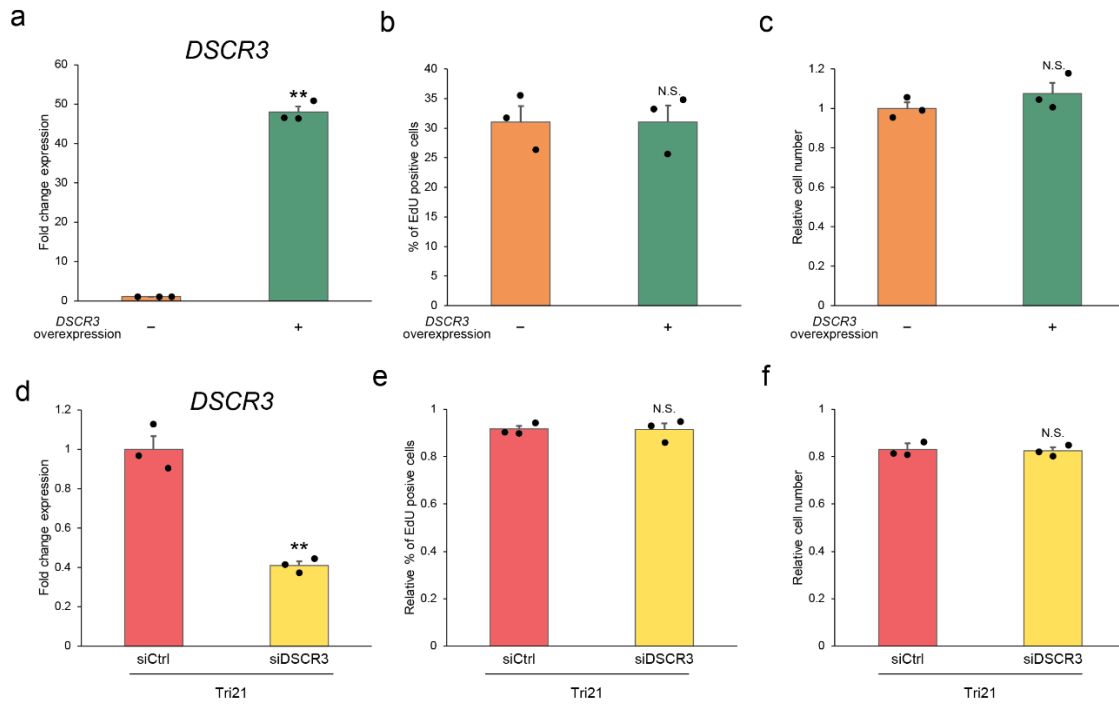


Supplementary Figure 17 | DYRK1A inhibitor decreases p-STAT3 levels in Tri21 NPCs. a, b) Immunoblot analysis of DYRK1A (**a**) and p-STAT3 and STAT3 (**b**) in Tri21 NPCs after 2-day treatment with FINDY (2.5 μ M). (**b**) The left blotted membrane was probed with an anti-p-STAT3 antibody. The right blotted membrane was stripped and re-probed with anti-STAT3 and anti- β -actin antibodies. β -Actin was used as a loading control and expression levels were normalised to that of Tri21 NPCs without FINDY treatment ($n = 3$ experiments per condition). Error bars represent the SEM. Data were analysed by Student's t -test. * $P < 0.05$; N.S., not significant ($P > 0.05$).



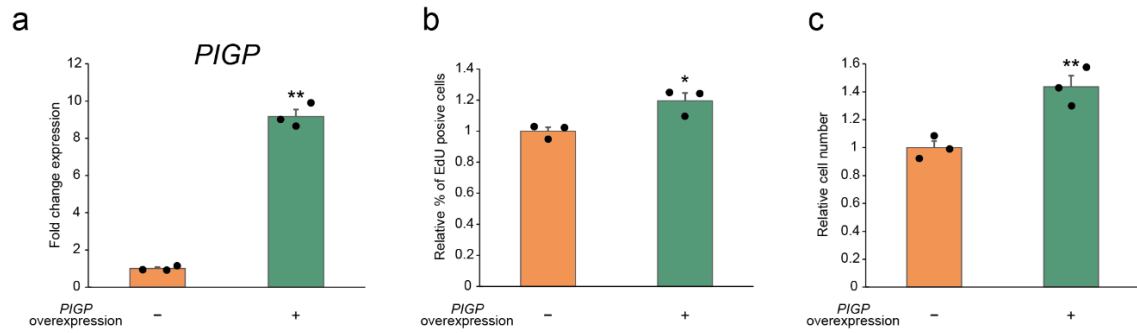
Supplementary Figure 18 | Co-overexpression of *PIGP* and *DSCR3* promotes Tri21 APC proliferation. a)

Schematic depicting the transfection of Dox-inducible *PIGP* and *DSCR3* transgenes using PB transposon vectors and a hyperactive PB transposase into Partial-Tri21 NPCs. Transduced NPCs were differentiated into APCs. **b)** Partial-Tri21 APCs stably expressing the *PIGP* and *DSCR3*-overexpression vectors. *PIGP* and *DSCR3* overexpression was induced by a 6-week Dox treatment. Expression levels were normalised to that of untreated APCs (n = 3 experiments per condition). **c, d)** Percentage of EdU-positive cells (**c**) and relative cell numbers 1 day after seeding (**d**) in *PIGP* and *DSCR3*-overexpressing Partial-Tri21 APCs (n = 4 experiments per condition). Cell numbers were normalised to that of untreated APCs. Error bars represent the SEM. Data were analysed by Student's *t*-test (**c, d**) or Welch's two-sample *t*-test (**b**). **P* < 0.05, ***P* < 0.01.



Supplementary Figure 19 | Neither overexpression nor knockdown of *DSCR3* affected Tri21 APC proliferation.

A Dox-inducible *DSCR3* transgene was transduced into Partial-Tri21 NPCs using a PB transposon vector and a hyperactive PB transposase. Transfected NPCs were differentiated into APCs, and *DSCR3* overexpression was induced by a 6-week treatment with Dox. **a)** Relative *DSCR3*-expression levels in overexpression vector-transfected Partial-Tri21 APCs. The expression levels were normalised to those of untreated APCs (n = 3 experiments per condition). **b,** **c)** Percentage of EdU-positive cells (**b**) and relative cell numbers 1 day after seeding (**c**) in *DSCR3*-overexpressing Partial-Tri21 APCs (n = 3 experiments per condition). The cell numbers were normalised to those of untreated APC lines. **d)** Relative *DSCR3*-expression levels in siRNA-treated Tri21 APCs. The expression levels were normalised to those in control siRNA (siCtrl)-treated Tri21-APCs (n = 3 experiments per condition). **e, f)** Percentage of EdU-positive cells (**e**) and relative cell numbers 1 day after seeding (**f**) in siRNA-treated Tri21 APCs (n = 3 experiments per condition). The percentage of EdU-positive cells and cell numbers were normalised to that of the Tri21 cell line without siRNA treatment. siDSCR3, *DSCR3* siRNA. Error bars represent the SEM. The data shown were analysed by Student's t-test (**b–f**) or Welch's two-sample t-test (**a**). * $P < 0.05$, ** $P < 0.01$; N.S., not significant ($P > 0.05$).



Supplementary Figure 20 | *PIGP* overexpression promotes Tri21 NPC proliferation. **a)** Partial-Tri21 NPCs stably expressing the *PIGP*-overexpression vector. *PIGP* overexpression was induced by a 5-day Dox treatment. Expression levels were normalised to that of untreated NPCs (n = 3 experiments per condition). **b, c)** Percentage of relative EdU-positive cells (**b**) and relative cell numbers 1 day after seeding (**c**) in *PIGP*-overexpressing Partial-Tri21 NPCs (n = 3 experiments per condition). The data were normalised to that of untreated NPCs. Error bars represent the SEM and the data were analysed by Student's *t*-test. **P* < 0.05, ***P* < 0.01.

Supplementary Table 1 | Details of isogenic cell lines generated in this study (related to Figure 1).

Cell	Line	The number of line	The number of passage
iPSC	Tri21	1 line	40 – 50
	<i>XIST</i> -Tri21	2 lines	
	cDi21 (M1 + P)	2 lines	
	cDi21 (M2 + P)	1 line	
	Partial-Tri21	1 line	
	DY ^{+/m} - <i>XIST</i> -Tri21	Each 1 line	
	DY ^{+/m/m} - <i>XIST</i> -Tri21	Each 1 line	
NPC, APC	Tri21	1 line	7 – 10 (NPC) 7 – 8 (APC)
	<i>XIST</i> -Tri21	3 lines	
	cDi21 (M1 + P)	2 lines	
	cDi21 (M2 + P)	1 line	
	Partial-Tri21	3 lines	
	DY ^{+/m} - <i>XIST</i> -Tri21	Each 1 line	
	DY ^{+/m/m} - <i>XIST</i> -Tri21	Each 1 line	
	PIGP overexpression	1 line	
	<i>DSCR3</i> overexpression	1 line	
	<i>PIGP</i> and <i>DSCR3</i> co-overexpression	1 line	

Tri21 iPSCs were derived from a male patient with DS, from which two *XIST*-Tri21 iPSC lines were generated. *XIST*-Tri21 NPCs were independently transfected using a *piggyBac* vector encoding rtTA to generate three lines. cDi21 iPSC lines consisted of three lines, of which two had a deleted maternal allele 2 of chromosome 21 (cDi21 M1 + P) and one had a deleted maternal allele 1 (cDi21 M2 + P). Three Partial-Tri21 NPC lines were separately differentiated with a line of Partial-Tri21 iPSCs. The passage numbers for each cell line are indicated in the table.

Supplementary Table 2 | *XIST*-mediated silencing does not affect astrocyte and APC markers.

Gene	D −	D+	D ^{remov}
GFAP	96.3	96.8	96.5
S100β	98.7	98.5	99.2
CD44	99.8	99.9	99.8
Vimentin	100.0	99.9	100.0

(%)

The average percentage of cells positive for GFAP, S100β, CD44, or vimentin in *XIST*-Tri21 APCs (n = 3 experiments per cell line). Each of the data was obtained from three lines. APCs of passage 8 were used for the analysis.

Supplementary Table 3 | PCA scores for genes on chromosome 21, based on the RNA-seq data.

Gene	Component 1	Component 2	Gene	Component 1	Component 2	Gene	Component 1	Component 2
SOD1	0.1272	0.0017	CHAF1B	0.0862	0.0504	JAM2	0.0428	0.1008
ITSN1	0.1253	0.0012	WDR4	0.0859	0.0244	SLC19A1	0.0427	0.0312
C21orf59	0.1231	0.0006	PTTG1IP	0.0857	0.0238	MX1	0.0417	0.1359
DONSON	0.1230	0.0049	PRMT2	0.0856	0.0513	MCM3AP-AS1	0.0414	0.0940
RRP1	0.1226	0.0032	GART	0.0846	0.1267	LINC00515	0.0393	0.0696
C21orf33	0.1201	0.0587	YBEY	0.0846	0.1090	KCNJ6	0.0393	0.0696
DYRK1A	0.1195	0.0035	C21orf91	0.0843	0.1172	LCA5L	0.0389	0.0121
DSCR3	0.1193	0.0265	TRAPPC10	0.0837	0.0108	C21orf62	0.0389	0.0096
HLCS	0.1188	0.0544	HSF2BP	0.0829	0.0029	NRIP1	0.0379	0.0499
PFKL	0.1163	0.0318	RSPH1	0.0825	0.0604	B3GALT5	0.0375	0.0926
N6AMT1	0.1160	0.0150	UBE2G2	0.0817	0.0247	LINC00162	0.0373	0.0287
U2AF1	0.1147	0.0135	COL18A1	0.0813	0.1031	GRIK1-AS2	0.0370	0.1087
BACH1	0.1144	0.0168	SH3BGR	0.0804	0.1101	ADARB1	0.0365	0.1455
MRPL39	0.1139	0.0499	WRB	0.0801	0.0691	RCAN1	0.0356	0.0998
ATP5O	0.1132	0.0480	LSS	0.0799	0.0712	LOC101927797	0.0354	0.1458
AGPAT3	0.1131	0.0146	C21orf58	0.0795	0.0741	LRRC3-AS1	0.0342	0.1065
CCT8	0.1126	0.0374	PCNT	0.0794	0.0772	RIPK4	0.0334	0.0893
URB1	0.1122	0.0309	SON	0.0793	0.1108	LINC00316	0.0319	0.0095
SCAF4	0.1120	0.0220	MX2	0.0789	0.0949	LRRC3	0.0314	0.1211
TMEM50B	0.1119	0.0427	IFNGR2	0.0771	0.0950	ERG	0.0298	0.0796
APP	0.1114	0.0522	DSCAM	0.0771	0.0267	SIK1	0.0294	0.0577
ATP5J	0.1110	0.0701	BACE2	0.0771	0.0198	ETS2	0.0285	0.0725
DNAJC28	0.1104	0.0351	PSMG1	0.0770	0.1219	GRIK1	0.0285	0.0725
CHODL	0.1080	0.0576	CBR3-AS1	0.0747	0.1290	RIPPLY3	0.0285	0.0725
MORC3	0.1067	0.0534	FAM207A	0.0729	0.0232	RUNX1-IT1	0.0235	0.1391
LOC642852	0.1059	0.0321	SLC37A1	0.0711	0.0480	PDE9A	0.0218	0.1087
PD XK	0.1058	0.0450	EVA1C	0.0701	0.0695	NCAM2	0.0211	0.0077
C21orf2	0.1058	0.0577	TFF2	0.0697	0.0982	LOC100129027	0.0209	0.1630
BTG3	0.1051	0.0127	AIRE	0.0693	0.0185	LOC100133286	0.0193	0.1345
PWP2	0.1046	0.0069	LINC00189	0.0693	0.0185	KCNE2	0.0189	0.1329
PIGP	0.1044	0.0314	TMPRSS15	0.0688	0.0761	DSCR9	0.0187	0.0942
SPATC1L	0.1041	0.0367	LOC100506403	0.0687	0.0112	LINC00158	0.0185	0.0702
USP16	0.1033	0.0508	LINC00114	0.0687	0.0112	KCNE1	0.0176	0.0609
BRWD1	0.1014	0.0468	ZNF295-AS1	0.0687	0.0112	COL6A2	0.0176	0.1059
IL10RB-AS1	0.1012	0.0211	LINC00313	0.0687	0.0112	CLIC6	0.0160	0.1100
CBR3	0.1004	0.0667	DIP2A-IT1	0.0687	0.0112	TFF3	0.0156	0.0483
LOC101928796	0.0997	0.0104	HUNK	0.0687	0.0112	PCBP3	0.0130	0.0710
PKNOX1	0.0984	0.0302	FTCD	0.0675	0.0777	LINC00649	0.0124	0.0922
SYNJ1	0.0982	0.0925	PAXBP1-AS1	0.0668	0.0637	CBS	0.0116	0.0428
USP25	0.0978	0.0398	RUNX1	0.0664	0.0679	ITGB2	0.0105	0.1184
DIP2A	0.0965	0.0806	MIS18A	0.0662	0.1012	COL6A1	0.0105	0.1586
NDUFV3	0.0959	0.0084	SLC5A3	0.0654	0.1170	LINC00479	0.0100	0.1398
TCP10L	0.0958	0.0215	MAP3K7CL	0.0654	0.0751	KRTAP20-2	0.0094	0.0813
SETD4	0.0951	0.0412	LINC00310	0.0640	0.0501	PCP4	0.0093	0.0128
CSTB	0.0947	0.0759	CYYR1	0.0631	0.0130	IGSF5	0.0089	0.0809
GABPA	0.0946	0.0924	ABCG1	0.0629	0.0775	ITGB2-AS1	0.0074	0.0455
S100B	0.0940	0.0379	RWDD2B	0.0607	0.0232	TMPRSS2	0.0060	0.1166
CRYZL1	0.0930	0.0818	C2CD2	0.0602	0.0106	TRPM2	0.0059	0.0483
LTN1	0.0925	0.0736	HSPA13	0.0586	0.0409	TPTE	0.0053	0.0455
TIAM1	0.0921	0.0849	PRDM15	0.0570	0.0676	LINC00160	0.0049	0.0380
IL10RB	0.0916	0.0827	SUMO3	0.0567	0.0123	TMPRSS3	0.0047	0.0799
TTC3	0.0910	0.1090	SSR4P1	0.0548	0.1445	MIR155HG	0.0045	0.0776
IFNAR2	0.0908	0.0953	DOPEY2	0.0544	0.1216	CBR1	0.0045	0.0760
RRP1B	0.0900	0.1140	ICOSLG	0.0540	0.0249	ANKRD30BP2	0.0045	0.0612
ADAMTS5	0.0897	0.0387	LINC00322	0.0521	0.1401	GRIK1-AS1	0.0036	0.0768
PAXBP1	0.0896	0.0933	ZBTB21	0.0511	0.1283	KCNJ15	0.0025	0.0231
IFNAR1	0.0895	0.0105	ADAMTS1	0.0505	0.1181	RBM11	0.0012	0.0418
HMGN1	0.0868	0.1171	SIM2	0.0501	0.0424	BAGE2	0.0003	0.0115
MCM3AP	0.0867	0.0088	POFUT2	0.0489	0.1077			
MRPS6	0.0863	0.1074	CXADR	0.0432	0.1145			

Absolute values from the PCA of 178 genes in chromosome 21 showing positive read counts, in at least one of the APC lines, based on RNA-seq data.

Supplementary Table 4. sgRNA sequences for CRISPR–Cas9.

Purpose	Target site	Sequence
<i>XIST</i> insertion	<i>DYRK1A</i> intron 1	AGTTTACACGGGTGATGAGC
<i>DYRK1A</i> targeting	<i>DYRK1A</i> intron 8	GATGGAAAACGGGTAAAATA

Supplementary Table 5. Primers for *DYRK1A* targeting.

Purpose	Forward primer (5' to 3')	Reverse primer (5' to 3')
RT-PCR (exon 8–10)	F1; TGACCAAGCACCAAAGCAAG	R2; CGGGTTCCTGGTGGTTTGT
5' Junctional PCR	F2; CACGCCACTGCCCTCTAGCTTGGGAGACAG	R3; GGCCGATGCAAAGTGCCGATAAACATAACG
3' Junctional PCR	F3; TTGACGGCAATTCGATGATGCAGCTTGGG	R2; CTGGTGGCAGCGTCCGCTAAAATACAAGGC
Outside PCR	F2; CACGCCACTGCCCTCTAGCTTGGGAGACAG	R2; CTGGTGGCAGCGTCCGCTAAAATACAAGGC
STR	GCACCCAACCCAGTTTTCAGAG (Fluor)	GCTGTCTTCCCACTATAACATAAAC

Supplementary Table 6. Primers for Quantitative RT-PCR.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
<i>ACTB</i>	TCAAGATCATTGCTCCTCCTGAG	ACATCTGCTGGAAGGTGGACA
<i>XIST</i>	AGCTCCTCGGACAGCTGTAA	GGACACATGCAGCGTGGA
<i>CCT8</i>	TTTTTCCTGATTCCGGCCATT	AACCATGCCATGCAATACTGA
<i>RCAN1</i>	GCTCCGCCAAATCCAGACAA	GCTGCGTGCAATTCATACTTTTC
<i>DYRK1A</i>	ACCACCTCCAGTACATCGTCAAG	CCGGGCTCTCCCACTGTT
<i>C21orf33</i>	GATGCGGAGTCTACGATGGG	CCAGGTCTGTGATTTTGCCAC
rtTA	CTGGGAGTTGAGCAGCCTAC	TCCAGCATCTCGATTGGCAG
<i>DSCR3</i>	CCTACACTGGAGACCACCAACTT	GATGAGGTGGTCAGGGTGAAG
<i>PIGP</i>	TGTTTGCGGATTAACATGATGAGTACCT	TCTGCTGTTGATTTTTTGCATAGTT
<i>HLCS</i>	AGGACAAAGGGCCCAACAG	CGCTGCCCAGATGGACTT

Supplementary Table 7. Primers for allele-specific SNP analysis.

Purpose	Forward primer (5' to 3')	Reverse primer (5' to 3')
PCR for allele-specific SNP analysis	GCATGAATGGCCAGATGCTG	CAATCCTGCCTTTCCTGGGT
Sanger sequencing	TTGTGGGTGACATTCTCTGG	-