Supplementary Material

Supplementary Table S1 Summary of cell lines with the tet-O HAC generated and analyzed in this study.

Supplementary Figure S1. Integrity of the tet-O HAC during MMCT.

Three cell lines containing the tetO HAC were analyzed by Southern blot. The original tet-O HAC generated in human HT1080 cells was represented as a AB2.2.18.21 cell line (Nakano et al., *Dev. Cell* 14:507-522). In chicken DT40 cells, this HAC was represented as a clone DT40/BHI 2-2, i.e. the HAC with approximately 20 copies of the loxP. The HAC-containing hamster cell line used for Southern analysis is CHOBHIG-11. DT40/BHI 2-2 HAC was transferred into hamster cells from chicken cells via MMCT. Genomic DNA was digested by SpeI, separated by CHEF and hybridized with a probe specific to the BAC vector sequence repeated 47 times in the HAC. SpeI is not present in alphoid satellite arrays. There is one SpeI recognition site in the vector. Three independently obtained DT40 clones, one CHO clone after MMCT and the original clone of tet-O HAC in HT1080 were compared.

Supplementary Figure S2. The copy number of the loxP cassette inserted into the tet-O HAC in different DT40 clones.

The number of the loxP cassette inserted into the tet-O HAC was determined by real-time PCR. The CENPH gene on chicken chromosome Z was used as an internal control. Primers hyg-679F and hyg-742R were used to amplify Hygromycin gene sequences in the loxP cassette.

Supplementary Figure S3. The tet-O HAC elimination assay in DT40 and CHO cells.

(**a**) The HAC elimination assay was performed by expression of the rtTA gene in DT40 and CHO cells. The rTA-containing plasmid was transfected into DT40BHI1-38 and DT40BHI1-2-2 clones containing the tetO-loxP-HAC. After 7 days of selection in the presence of doxycycline two randomly selected clones (designated as on-2 and on-3) were analyzed for each culture. FISH analysis with the digoxigenin-labeled hCOT-1 probe was used to evaluate loss of the tet-O HAC.

(**b**) The same assay was performed for three clones of CHO cells (designated as on-8,

on-15 and on-16) containing the tetO-EGFP-HAC. Additional experiments were performed with ganciclovir selection. Cells were cultured in the presence of doxycycline. Ganciclovir was added on day 7 and cells were cultured for 5 days prior to analysis.

Figure S1

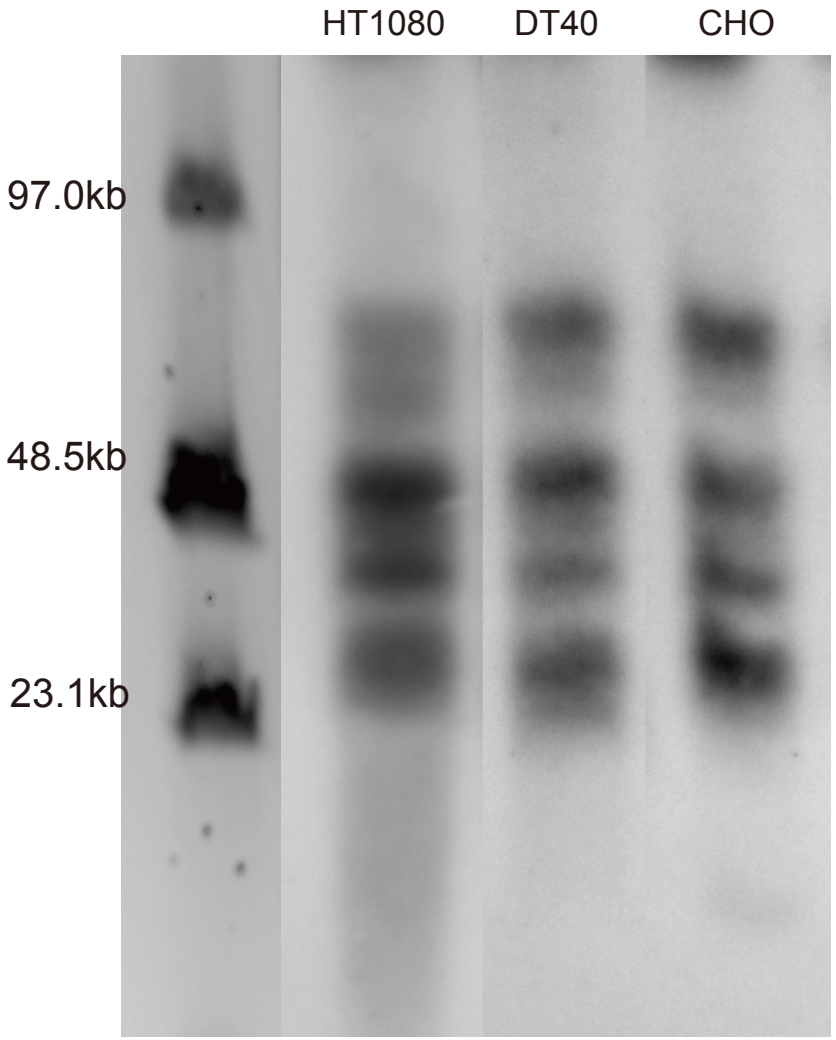
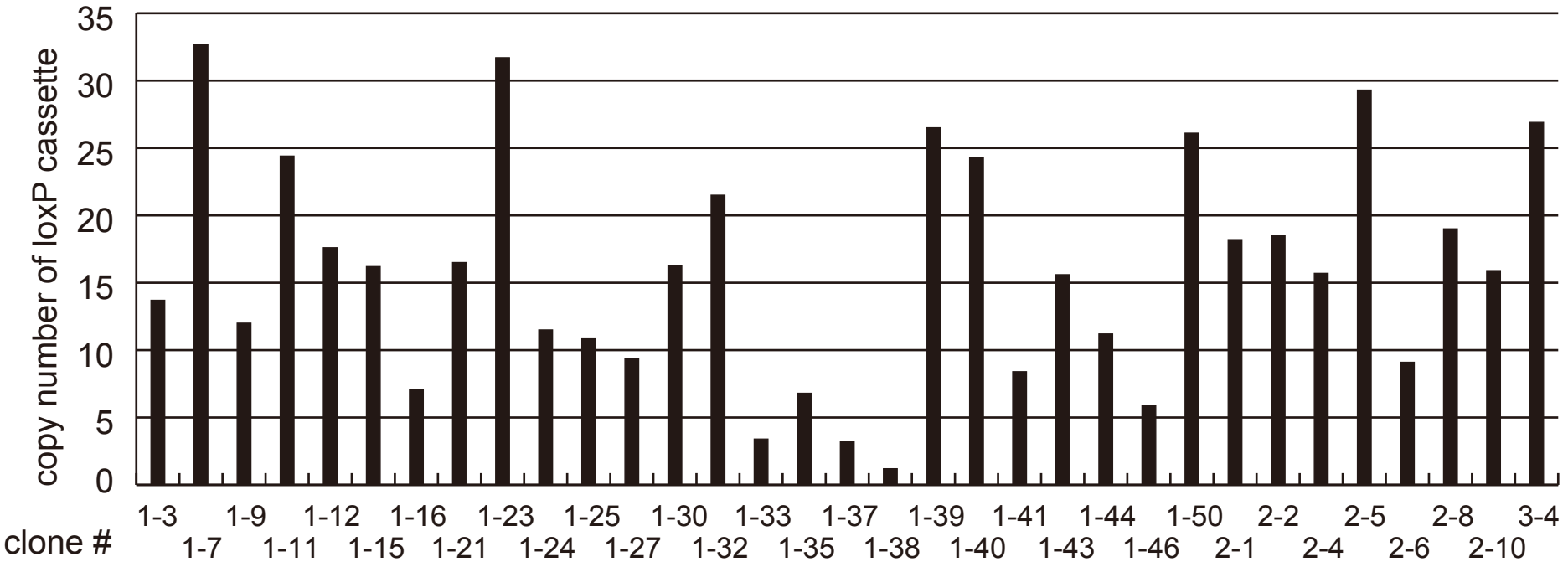


Figure S2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15
10
5
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clone #
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1-16
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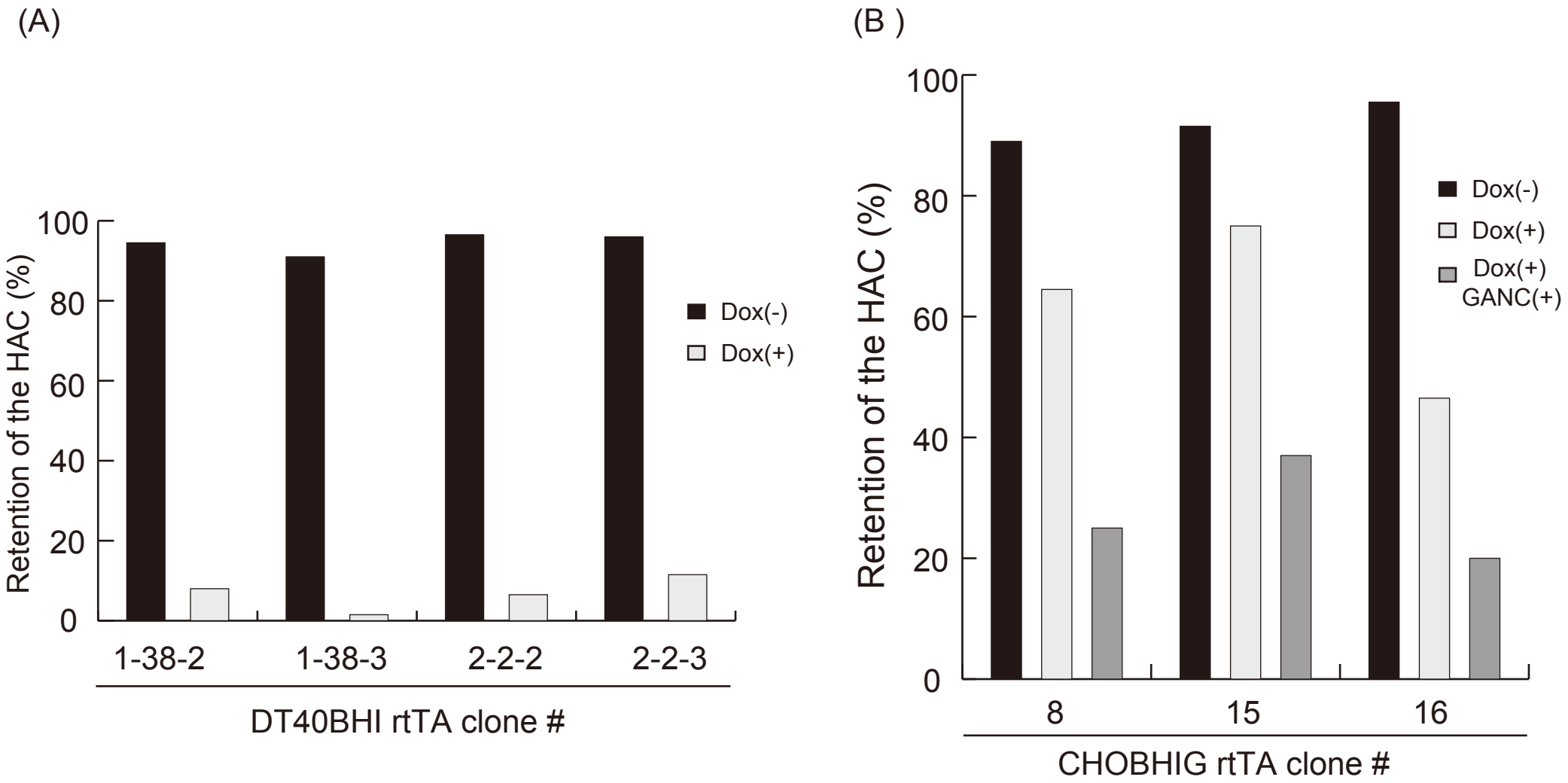
3-4

FIgure S3


(A)
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80
60
40
20
0
Retention of the HAC (%)
Dox(-)
Dox(+)
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1-38-3
2-2-2
2-2-3
DT40BHI rtTA clone #
(B)
100
80
60
40
20
0
Retention of the HAC (%)
Dox(-)
Dox(+)
Dox(+)
GANC(+)
8
15
16
CHOBHIG rtTA clone #

Supplementary Table S1 Summary of cell lines with the tet-O HAC generated and analyzed in this study

Source of cell line	Method used to get a clone	HAC-positive clones		Description of clones
		based on PCR	based on FISH	
DT40 cells containing tet-O HAC	F	3/3	3/3	transfer of HAC from hybrid cells to DT40
DT40 cells containing loxP-cassette in HAC	EP	50/62	6/6	insertion of loxP cassette into DT40
CHO cells containing tetO-loxP-HAC	F	24/41	2/9	transfer of tet-O-loxP-HAC into CHO
CHO cells containing tetO-EGFP-HAC	LP	10/10	1/1	insertion of EGFP into HAC into CHO
HT1080 cells containing tetO-EGFP-HAC	F	15/15	6/6	transfer of tet-O-EGFP-HAC into HT1080

CHO, Chinese hamster ovary; EP, electroporation; LP, lipofection; F, fusion by microcell-mediated chromosome transfer