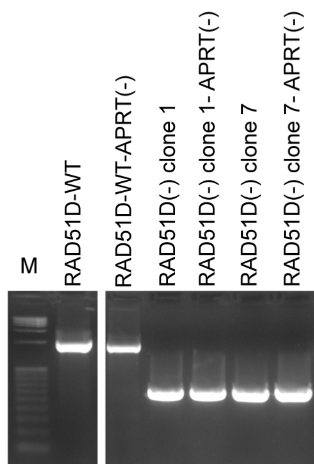
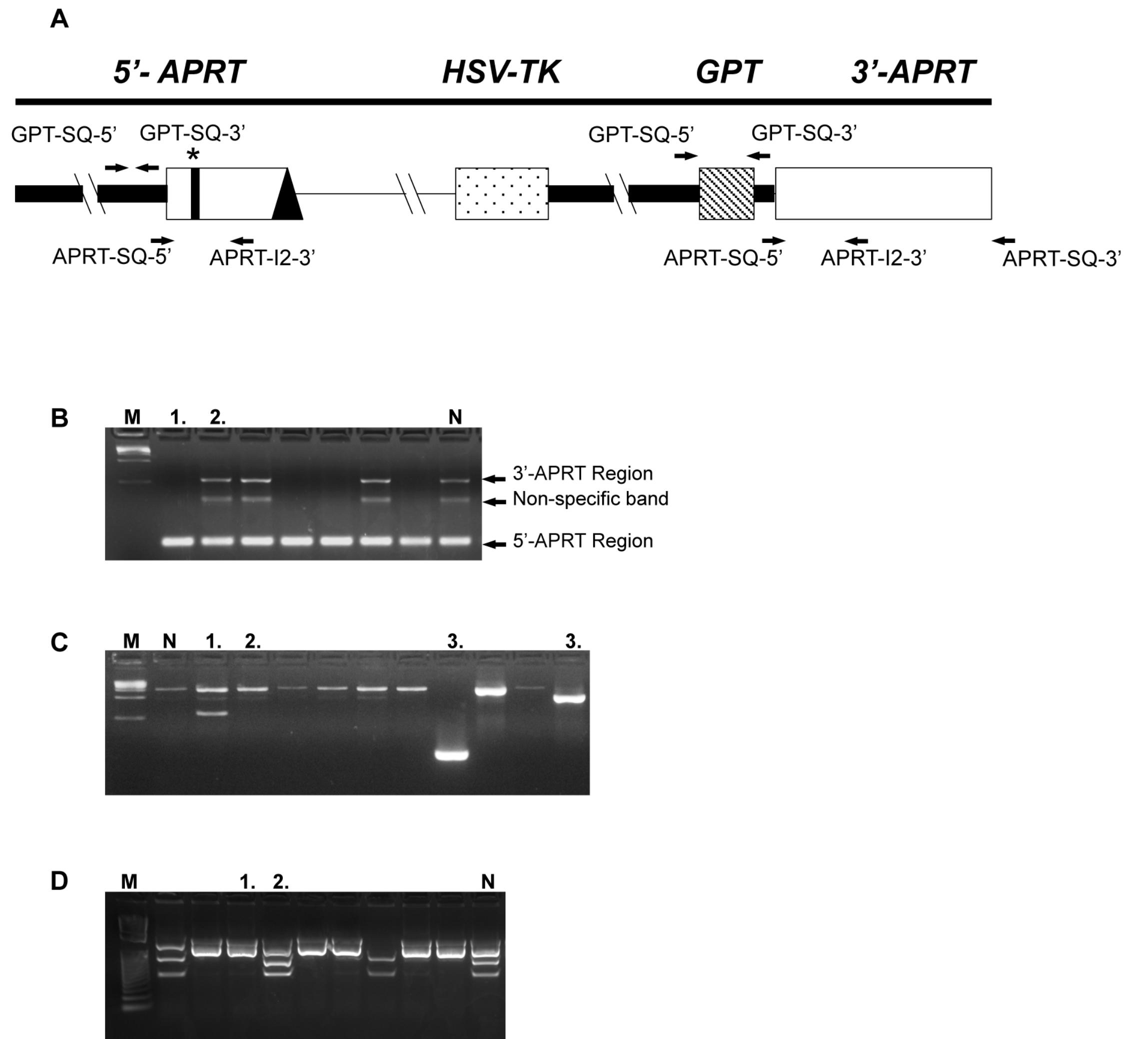
Supplementary Table 1. Sequences inserted into intron 2 of the 3’-*Aprt* gene, and PCR primer sequences.

Inserted triplex target and *I-SceI* sequence	TAGGGATAACAGGGTAATGGCGCGCCGAGGGGGAGGGGGTGGTGGGGGGGGAAGGAT ATCCCTATTGTCCCATTACCGCGCGGCTCCCCCTCCCCCACCACCCCCCCCTTCCTA ***I-SceI* target site** **Triplex-forming oligo target site**
PCR Primers	**Sequence (5’-3’)**
choRAD51D-5’	ATTTGCTGCCACATCTAAGGAA
choRAD51D-3’	CTGTCATTCCTCCACTGGAATC
GPT-SQ-5’	CACAGAGCAAAGGAACGTAAGG
GPT-SQ-3’	TTGAAAGCCTGGGGTTATTTCT
APRT-SQ-5’	CAATATCCAGCAAATGCGTTACT
APRT-SQ-3’	GCTACTCTCTCCATTTTGGTCCT
APRT-I2-3’	GACGAATTCTCTGGGGTTCTC



Supplementary Figure S1. PCR analysis to confirm *Rad51D* status in genetically modified CHO cell lines. PCR was performed using choRAD51D-5'/choRAD51D-3' primer pairs (Supplementary Table 1), which flank the LoxP sites in both *Rad51D* knock-in alleles. Here we show results from all three cell lines used in this study as well as a clone isolated from each after *I-SceI* treatment followed by 8-azaadinine selection. M is a mixture of 1-kb and 100-bp markers. The larger band indicates an intact *Rad51D* knock-in locus. The smaller band indicates Cre-mediated deletion of exon 4 of *Rad51D*.



Supplementary Figure S2. Representative PCR analysis of HR/mutation events in CHO cells. (**A**) Location of PCR primers from Supplementary Table 1 along the *Aprt* reporter locus. Note that GPT-SQ-5'/GPT-SQ-3' and APRT-SQ-5'/APRT-I2-3' primer pairs amplify two regions of the reporter locus resulting in amplicons from both *Aprt* repeats (**B**) GPT-SQ-5'/GPT-SQ-3' PCR analyses on representative clones following *I-SceI* DSB induction and selection with 8-azaadinine. M is a 1-kb marker, N is a non-treated/non-selected control, 1. represents a clone that has lost *gpt* sequence through LTGC or SSA, and 2. represents a clone that has maintained both

5’ and 3’ regions indicating an intact locus. (**C**) APRT-SQ-5’/APRT-SQ-3’ PCR analyses on representative clones following *I-SceI* DSB induction and selection with 8-azaadinine. M is a 1-kb marker, N is a non-treated/non-selected control, 1. multiple bands indicate that the sample consists of multiple clones (these samples were excluded from analysis), 2. represents a clone with a full-length 3’-*Aprt* region, and 3. represent clones with internal deletions of 3’-*Aprt* regions. (**D**) APRT-SQ-5’/APRT-I2-3’ PCR followed by *EcoRV* digest on representative spontaneous clones following selection with 8-azaadinine. M is a mixture of 1-kb and 100-bp markers, N is a non-treated/non-selected control, 1. represents a clone that has lost the *EcoRV* site in the 3’-*Aprt* gene due to gene conversion or SSA, and 2. represents a clone that has retained the *EcoRV* site in the 3’-*Aprt* gene resulting in digestion of the PCR amplicon. The 5’-*Aprt* amplicon remains intact. These clones were classified as “Other”.

Supplementary Table 2. Calculated frequency of recombination and mutation events obtained after spontaneous marker loss calculated by multiplying (frequency of marker loss) X (% of endpoint occurrence).

	APRT (-)		**GPT (-)**		**HSV-TK (-)**		**APRT (-)/HSV-TK (-)**	
	RAD51D-WT Frequency	RAD51D (-) Frequency	RAD51D-WT Frequency	RAD51D (-) Frequency	RAD51D-WT Frequency	RAD51D (-) Frequency	RAD51D-WT Frequency	RAD51D (-) Frequency
STGC	$6.4*10^{-6}$	$4.5*10^{-6}$	$4.7*10^{-7}$	ND	NA	NA	NA	NA
LTGC	$1.7*10^{-6}$	ND	$1.6*10^{-6}$	ND	NA	NA	NA	NA
SSA	$6.1*10^{-6}$	$8.6*10^{-5}$	$6.7*10^{-6}$	$6.7*10^{-5}$	$1.2*10^{-5}$	$6.3*10^{-5}$	$3.6*10^{-6}$	$6.4*10^{-5}$
Deletion	$1.2*10^{-6}$	$4.5*10^{-5}$	$1.6*10^{-6}$	$1.0*10^{-4}$	$1.3*10^{-5}$	$6.3*10^{-5}$	$7.7*10^{-8}$	$1.7*10^{-6}$
Other[a]	$5.7*10^{-6}$	$6.9*10^{-5}$	$5.2*10^{-6}$	ND	$1.5*10^{-5}$	$4.1*10^{-5}$	ND	ND
N	66	50	30	30	38	37	42	39

[a]Other indicates small mutations to coding sequence, such as point mutations, frameshifts, and small indels that do not change the overall structure of the region and are not readily discernable by Southern blot or PCR analysis.
NA-Not applicable.
ND-None Detected

Supplementary Table 3. Calculated frequency of recombination and mutation events obtained after *I-SceI* DSB-induced marker loss calculated by multiplying (frequency of marker loss) X (% of endpoint occurrence).

	APRT (-)		GPT (-)		HSV-TK (-)		APRT (-)/HSV-TK (-)	
	RAD51D-WT Frequency	RAD51D (-) Frequency	RAD51D-WT Frequency	RAD51D (-) Frequency	RAD51D-WT Frequency	RAD51D (-) Frequency	RAD51D-WT Frequency	RAD51D (-) Frequency
STGC	$2.7*10^{-4}$	$3.4*10^{-4}$	ND	ND	NA	NA	NA	NA
LTGC	$4.2*10^{-5}$	$3.8*10^{-5}$	$1.2*10^{-4}$	ND	NA	NA	NA	NA
SSA	$2.0*10^{-4}$	$1.5*10^{-3}$	$8.1*10^{-5}$	$1.2*10^{-3}$	$1.3*10^{-4}$	$1.1*10^{-3}$	$1.1*10^{-4}$	$9.9*10^{-4}$
Deletion	$1.7*10^{-4}$	$1.9*10^{-3}$	$2.9*10^{-5}$	$3.5*10^{-4}$	$1.8*10^{-5}$	$1.1*10^{-4}$	$1.9*10^{-5}$	$6.3*10^{-5}$
Other[a]	$1.3*10^{-5}$	$3.8*10^{-5}$	$3.5*10^{-5}$	ND	$1.8*10^{-5}$	ND	ND	ND
N	66	50	30	30	38	37	42	39

[a]Other indicates small mutations to coding sequence, such as point mutations, frameshifts, and small indels that do not change the overall structure of the region and are not readily discernable by Southern blot or PCR analysis.

NA-Not applicable.

ND-None Detected