Supplementary Data

A *cis*-Acting Element Downstream of the Mouse Mammary Tumor Virus Major Splice Donor Critical for RNA Elongation and Stability

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Table S1: List of primers and their description used in this study.

Primer Name	Primer Sequence Forward (F)/Reverse (R)	Target	Purpose/ Reference
OFM24	CATGTTTGTGATGGGTGTGA ACCA- (F)	GAPDH; multiple species	Mustafa et al., 2000 [1].
OFM25	GTTGCTGTAGCCGTATTCAT TGTC- (R)	GAPDH; multiple species	Mustafa et al., 2000 [1].
OFM112	CAT CAC AAG AGC GGA ACG GAC- (F)	MMTV 5' UTR upstream of SD1	Mertz et al., 2005 [2]. C3H230 (nt 1401-1419)
OFM113	CCT CTA AAT CAT CCC AAT CCT- (R)	MMTV Gag	Mertz et al., 2005 [2]. Gag 620 (nt 2127-2107)
OFM114	TCT ACC TAT TGG ATT GGT CTT ATT GG- (R)	MMTV 3' U3	Mertz et al., 2005 [2]. LTR408 (nt 8964-8938)
OFM115	ATC GCC TTT AAG AAG GAC GCC TTC T- (F)	MMTV Env	Mertz et al., 2005 [2]. Env7255 (nt 7231-7256)
OTR 580	TGA GCT GCG TGT GGC TCC- (F)	Spliced β-actin mRNA	β-actin S, Tan et al., 1995 [3]. A 247 bp product w/OTR 581.
OTR 581	GGC ATG GGG GAG GGC ATA CC- (R)	Spliced or unspliced β-actin mRNA	β-actin A, Tan et al., 1995 [3]. A common reverse primer for both spliced & unspliced actin.
OTR 582	CCA GTG GCT TCC CCA GTG- (F)	Unspliced β-actin mRNA	β-actin S-1, Tan et al., 1995 [3]. A 203 bp product w/OTR 581.
OTR 671	GTC CTA ATA TTC ACG TCT CGT GTG- (F)	MMTV R	Nt 1179-1202 of HYB MTV.
OTR 672	CTG TTC GGG CGC CAG CTG CCG CAG-(R)	MMTV PBS	Nt 1321-1298 of HYB MTV.
OTR 930	GCT TGT GTG TTG GAG GTC GCT GAG- (F)	CMV promoter	Nt 90-113 of pcDNA3. For sequencing.
OTR 552	CGA CTA GTG ATA TCG TTC CCC TGG TCC CAT AAG- (R)	MMTV *gag*	*Spe I* (nt 1885-1867 of HYB-MTV *gag*)

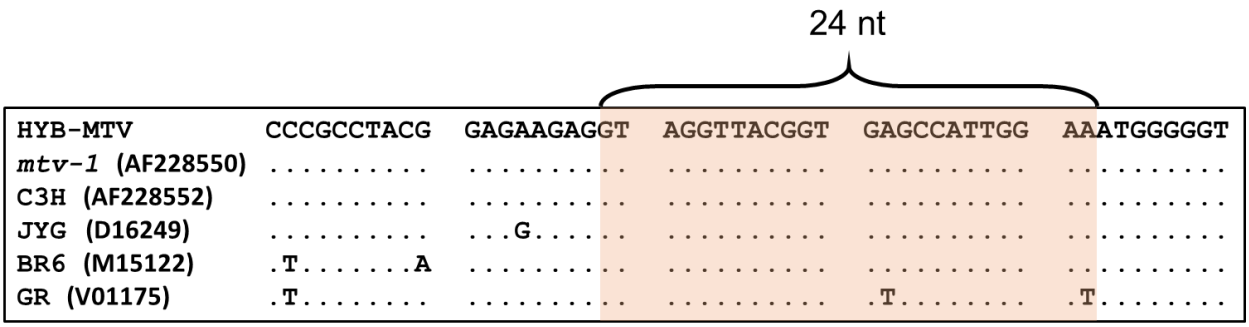

Figure S1: Sequence alignment of the 5' end of different MMTV strains. The shaded boxed region highlights the 24-nt region analyzed. Presence of a dot shows sequence identity, whereas differences are shown as capital letters.

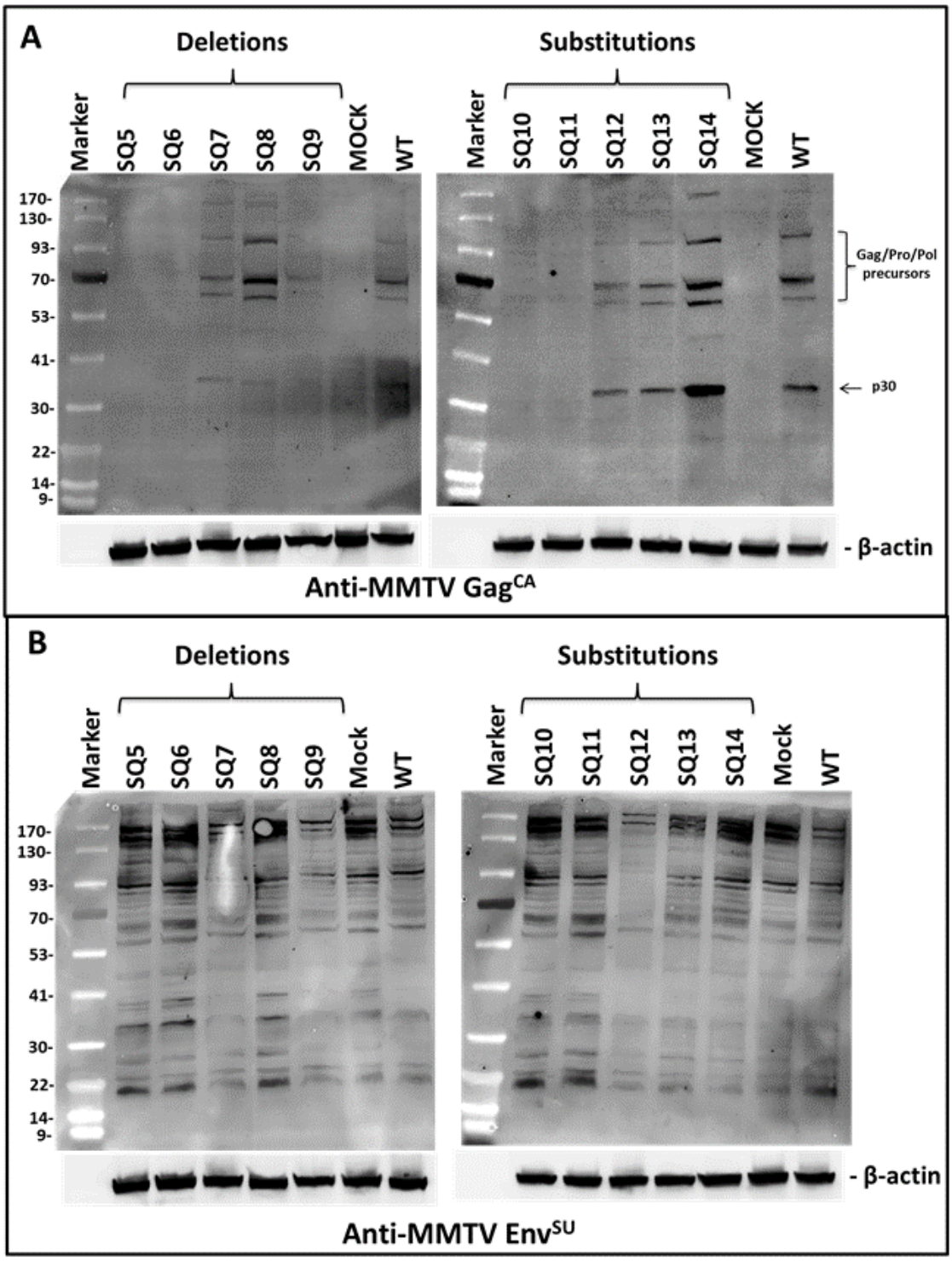

Figure S2. The 5' UTR mutants show a similar defect in cells of mouse origin for Gag/Pol protein expression. Western blot analysis of normal mouse mammary epithelial HC11 cells transfected with the wild type (WT) and mutant clones. **(A)** α- MMTV Gag^{CA} antibody and **(B)** α-MMTV Env^{SU} antibody along with human β-actin serving as a loading control in both gels. Mock, HC11 cells transfected with pcDNA3 plasmid alone.

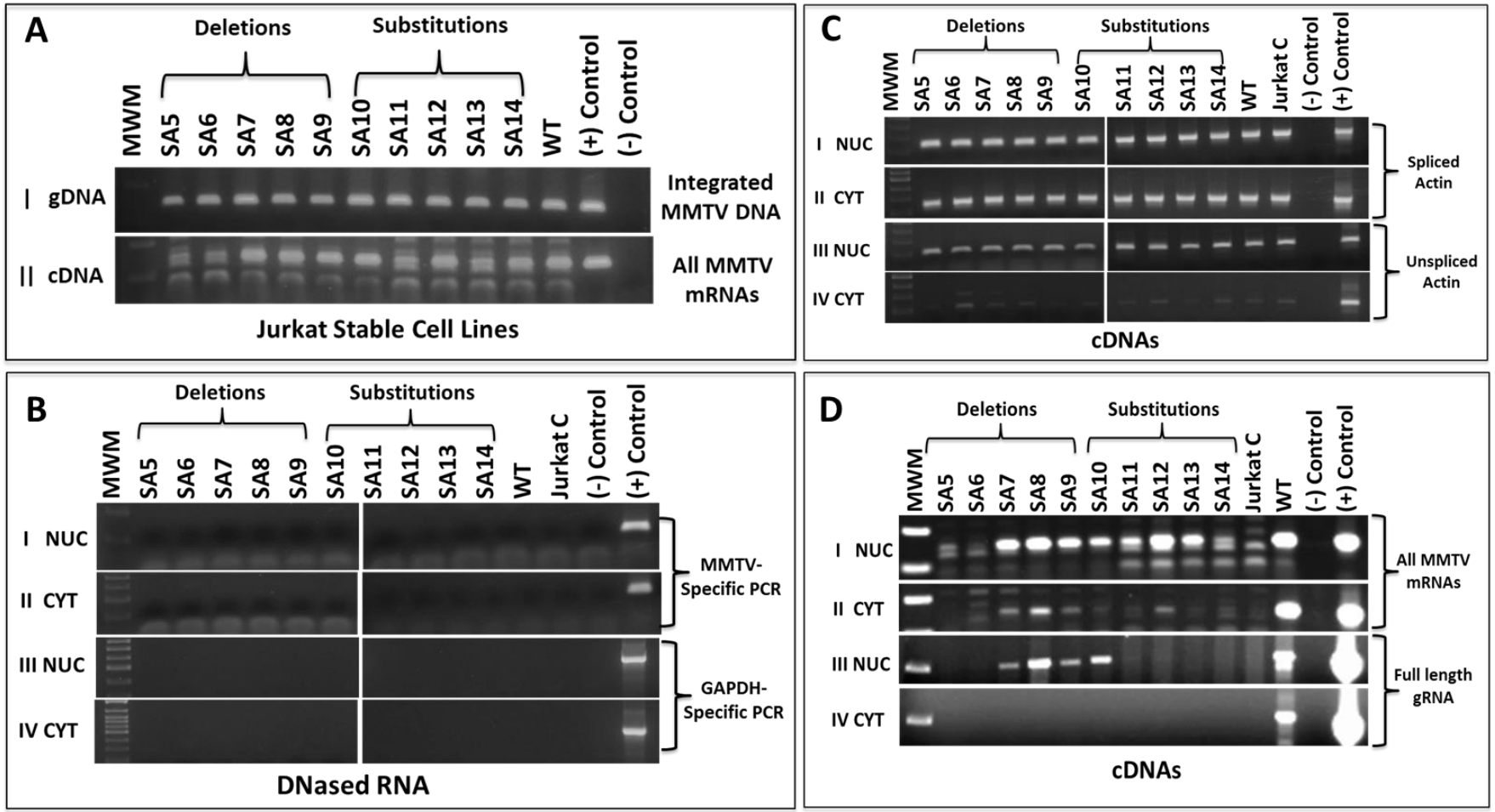

Figure S3. Characterization of Jurkat stable cell lines expressing the wild type and mutant proviruses containing the MMTV 5' LTR. (A) Analysis of DNA and RNA extracted from wild-type (WT) and mutant constructs stably transfected into Jurkat cells using PCR with primers OTR671/OTR672. Panel I: PCR amplification of MMTV-specific sequences from gDNA; Panel II: RT-PCR analysis of all MMTV-specific mRNAs from total RNA. **(B)** PCR analysis of NUC and CYT RNAs following DNase-treatment with MMTV-specific primers OTR671/OTR672 in panels I & II and GAPDH primers in panels III & IV. **(C)** RT-PCR analysis of NUC and CYT samples for spliced (panels I & II) and unspliced β-actin mRNAs (panels III & IV). **(D)** RT-PCR analysis of NUC and CYT samples for all MMTV mRNAs (OTR671/OTR672) in panels I & II, and full-length gRNAs (OFM112/OFM113) in panels III & IV. The gel images in this figure were spliced together from several independent gels due to the large number of samples.

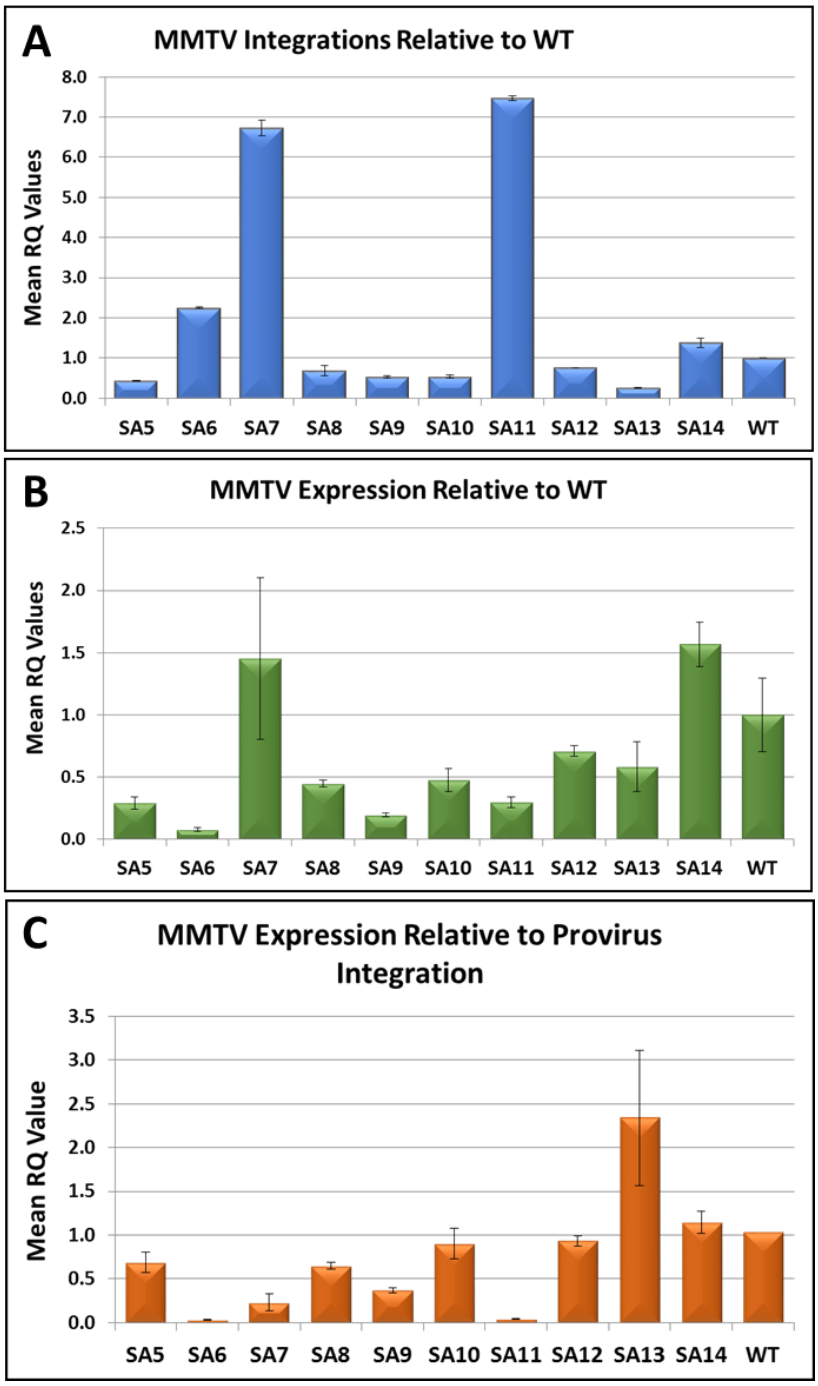

Figure S4: Quantitative PCR analysis of virus expression in Jurkat stable cell lines. Real time PCR was conducted on **(A)** 50 ng gDNA, and **(B)** cDNAs from the same Jurkat (JC) stable cell lines expressing the wild type (WT) and mutant (SA5-SA14) MMTV clones. The previously-published custom-made Taqman Assay 1 (detects HYB-MTV 5' U5 region) was used to quantitate MMTV gDNA and all MMTV transcripts from the LTR promoter in these samples [34, 35] **(C)** Normalization of the MMTV RNA expression relative to the integration level in the Jurkat stable cell lines.

References:

[1] Mustafa F, Lozano M, Dudley JP. C3H mouse mammary tumor virus superantigen function requires a splice donor site in the envelope gene. J Virol. 2000;74:9431-40.

[2] Mertz JA, Simper MS, Lozano MM, Payne SM, Dudley JP. Mouse mammary tumor virus encodes a self-regulatory RNA export protein and is a complex retrovirus. J Virol. 2005;79:14737-47.

[3] Tan W, Felber BK, Zolotukhin AS, Pavlakis GN, Schwartz S. Efficient expression of the human papillomavirus type 16 L1 protein in epithelial cells by using Rev and the Rev-responsive element of human immunodeficiency virus or the cis-acting transactivation element of simian retrovirus type 1. J Virol. 1995;69:5607-20.