Expanded View Figures

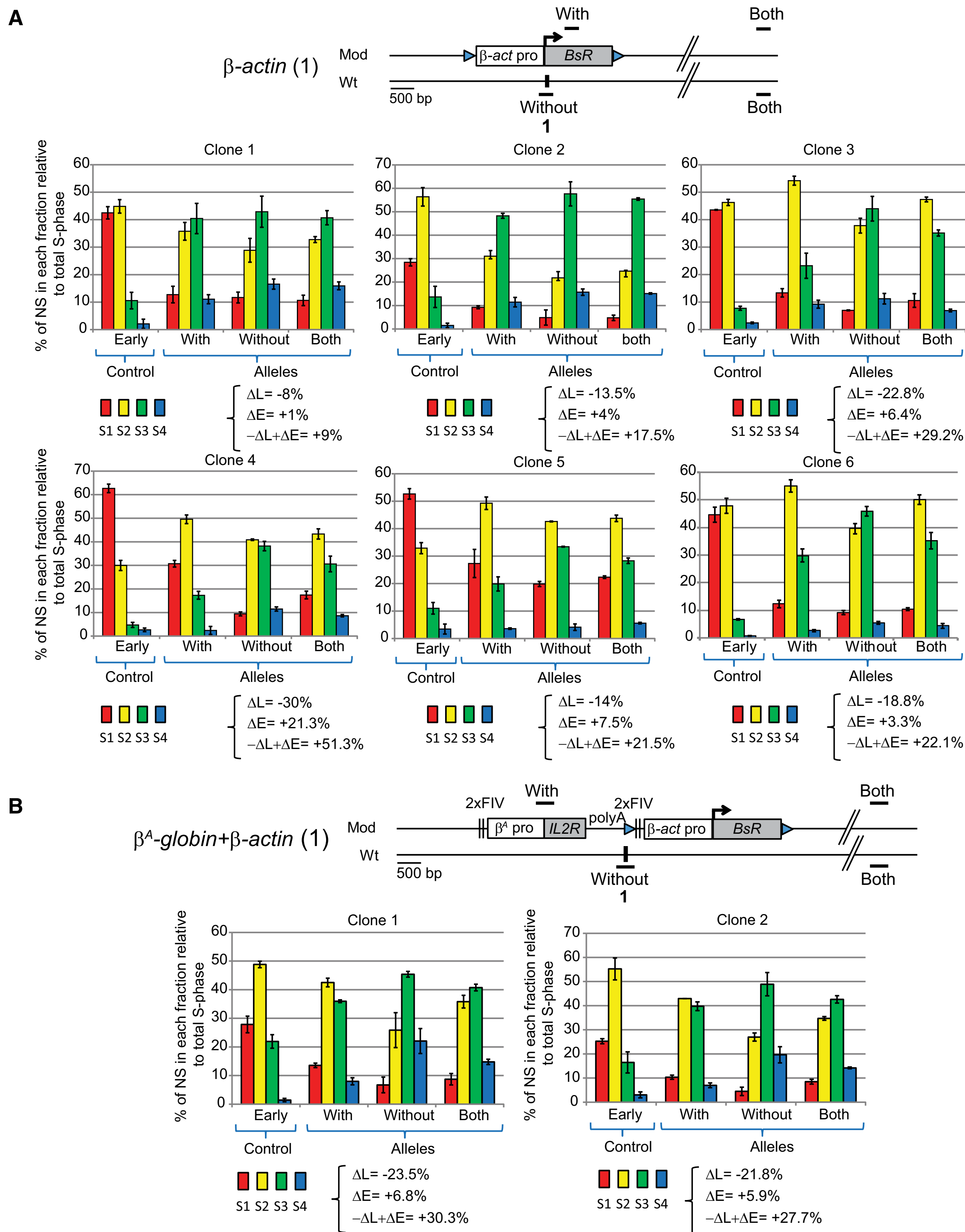

Figure EV1.

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◀ **Figure EV1. Additional RT assays for transgenes shifting the timing of replication.**

A, B RT profiles of each chromosomal allele are determined after targeted transgene integration using the allele-specific analysis of RT method by real-time PCR quantification described in Appendix Fig S1. Differences in $-\Delta L + \Delta E$ values calculated at the target site following transgene integration are indicated. Blue triangles represent reactive *loxP* sites. Error bars correspond to the standard deviation for qPCR duplicates. Analysis of six *β-actin* clonal cell lines (A) or two *β^A-globin + β-actin* clonal cell lines (B) described in Fig 1 is reported. Black vertical bars represent insertion sites.

Figure EV2. One advanced replicon inserted at site 1 or at site 3 directs a shift to earlier replication independently of the presence of a *GFP* reporter construct inserted at site 2. ▶

RT profiles of each chromosomal allele are determined after targeted transgene integration using the allele-specific analysis of RT method by real-time PCR quantification described in Appendix Fig S1. Differences in $-\Delta L + \Delta E$ values calculated at the target site following transgene integration are indicated.

A Analysis of one clonal cell line containing one *GFP* reporter construct composed of the *GFP* reporter gene under the control of the *β^A-globin* promoter (β^A pro) and linked to a 1.6 kb fragment of human chromosome 7 (h.K7) inserted at site 2.

B, C Analysis of clonal cell lines containing one *β^A-globin + β-actin* construct described in Fig 1 inserted at site 1 and one *GFP* reporter construct inserted at site 2 on the same chromosome (B) or on the other chromosome (C).

D, E Analysis of clonal cell lines containing one *β^A-globin+β-actin* construct inserted at site 3 and one *GFP* reporter construct inserted at site 2 on the same chromosome (D) or on the other chromosome (E).

Data information: Blue and black triangles represent reactive *loxP* sites and recombined inactive *loxP* sites, respectively. Black vertical bars represent insertion sites. Error bars correspond to the standard deviation for qPCR duplicates.

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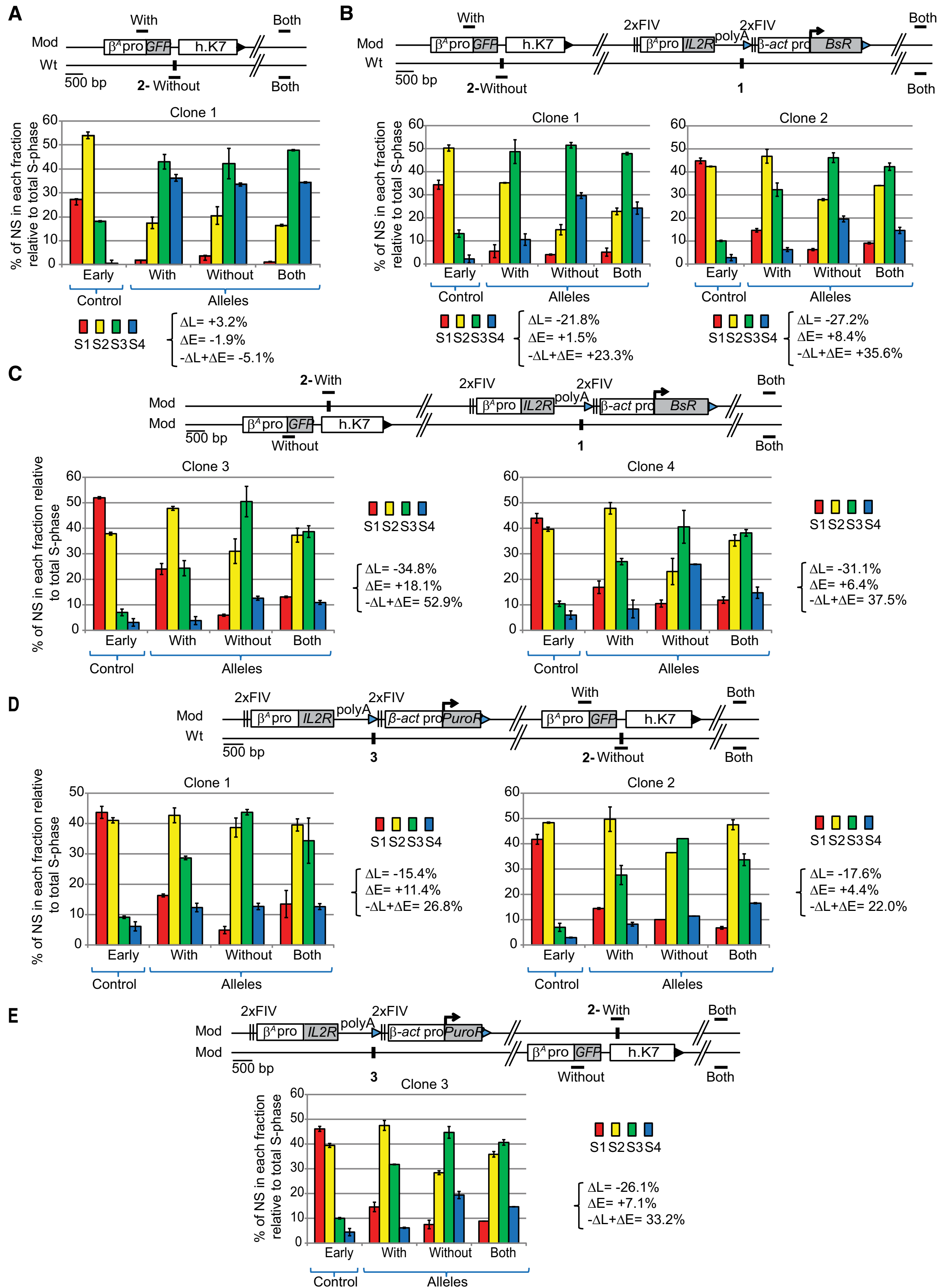

Figure EV2.

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Figure EV3. Two β^A-*globin* + β-*actin* constructs as well as two β-*actin* constructs inserted at sites 1 and 3 form an early-replicated domain.

A–C RT profiles of each chromosomal allele are determined after targeted transgene integration using the allele-specific analysis of RT method by real-time PCR quantification described in Appendix Fig S1. Differences in $-\Delta L + \Delta E$ values calculated at the target site following transgene integration are indicated. Error bars correspond to the standard deviation for qPCR duplicates. (A, B) Analysis of two 1 + 2 + 3 (A) and three 1 + 3 (B) clonal cell lines described in Fig 3A. (C) Analysis of five clonal cell lines containing two β-*actin* constructs inserted at sites 1 and 3 on the same chromosome described in Fig 3B. Blue and black triangles represent reactive *loxP* sites and recombined inactive *loxP* sites, respectively. Black vertical bars represent insertion sites. Error bars correspond to the standard deviation for qPCR duplicates.

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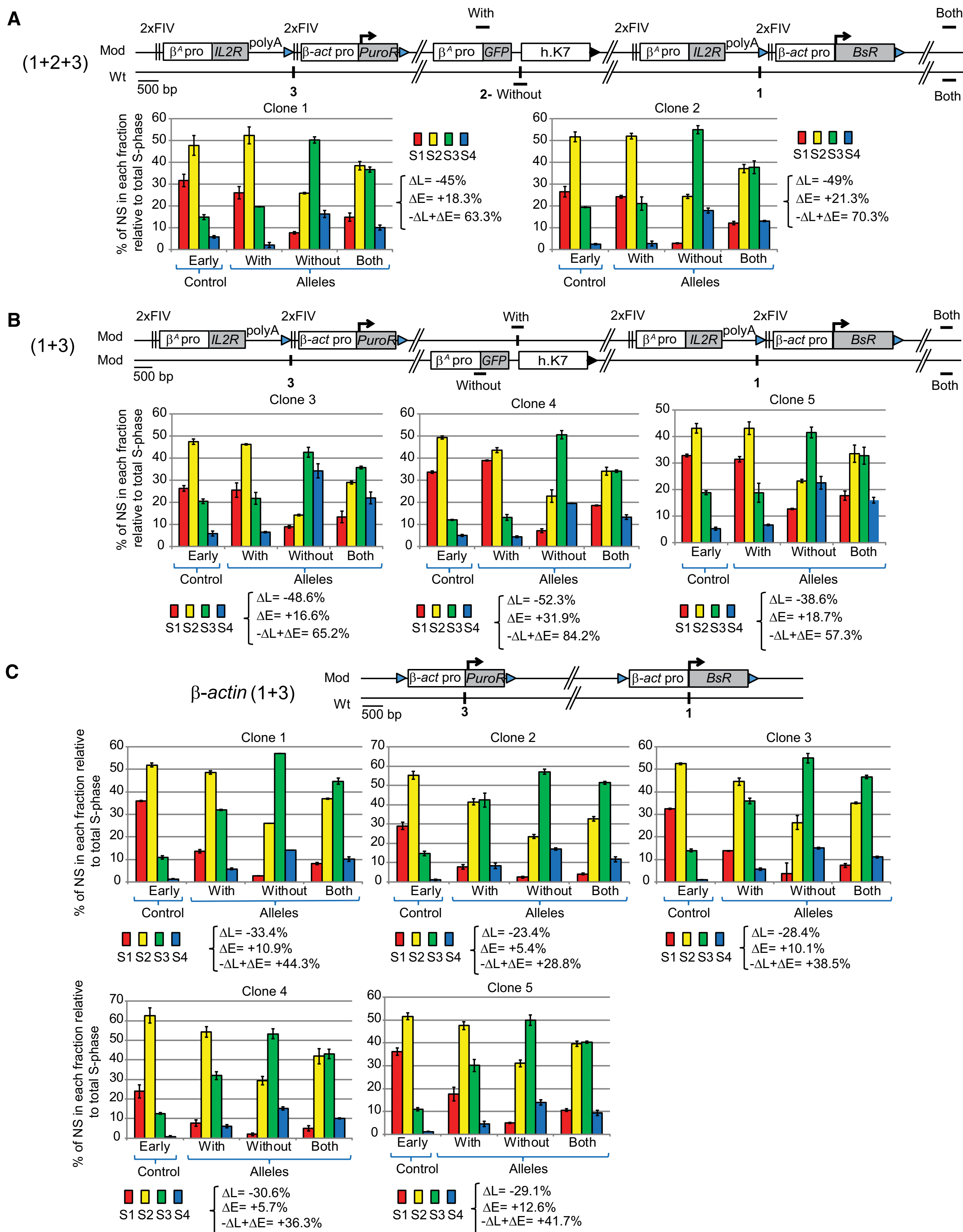

Figure EV3.

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Figure EV4. The open chromatin structure of the whole advanced replicon is imposed by the active *β-actin* promoter.

A Relative quantification, by real-time qPCR, of mRNA expression levels (RT-QPCR+) or background levels (RT-QPCR−), was performed in the β^A*-globin* and β^A*-globin + β-actin* clonal cell lines. For relative quantification, mRNA levels were normalized against *BU1A* mRNA levels arbitrarily set at 100 (first table). Crossing point (Cp) values for RT-QPCR+ and RT-QPCR− experiments are reported in the second table. NA corresponds to nonamplified samples, NS to nonspecific signals, and LD to the limit of detection.

B–D The β^A*-globin* (B), *β-actin* (C), and β^A*-globin + β-actin* (D) transgenes are shown on the left, with the positions of the amplicons used for quantification (thick black lines with names indicated above). The endogenous active *MED14* promoter and two genomic regions located within the condensed region upstream from the *β-globin* locus (cond1 and cond2) were analyzed as controls. Quantification, by real-time qPCR, of total chromatin (input) extracted from the β^A*-globin* (B), *β-actin* (C), and β^A*-globin + β-actin* (D) clonal cell lines after digestion with increasing concentrations of micrococcal nuclease (MNAse, 2.5 U, 10 U, 40 U, 160 U/ml). Error bars indicate the standard deviation for qPCR triplicates made on two independent clones.

E Immunoprecipitations of H3K9K27 acetylation (ac), H3K4 trimethylation (me3), or H3K4 dimethylation (me2) on formaldehyde-cross-linked chromatin extracted from the β^A*-globin* (green) and β^A*-globin+β-actin* (purple) clonal cell lines. The endogenous active *BU1A* promoter (*BU1A pro*) was analyzed as a control. Data are presented as enrichments of immunoprecipitated material relative to input DNA and normalized against *BU1A* enrichment. Error bars indicate the standard deviation for at least qPCR duplicates made on two independent clones.

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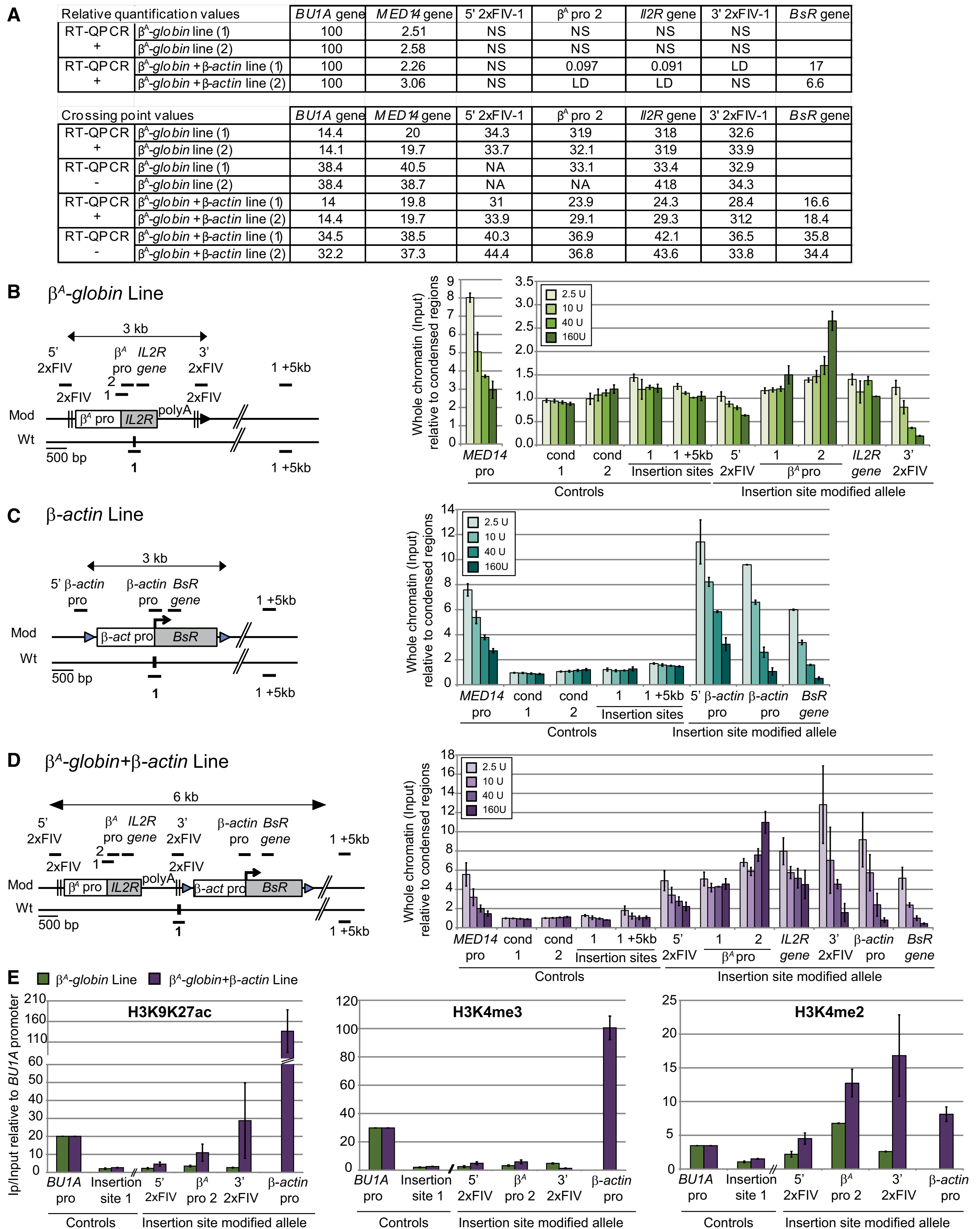

A

Relative quantification values		*BU1A* gene	*MED14* gene	5' 2xFIV-1	β^A pro 2	*Il2R* gene	3' 2xFIV-1	*BsR* gene
RT-QPCR +	β^A-*globin* line (1)	100	2.51	NS	NS	NS	NS	
	β^A-*globin* line (2)	100	2.58	NS	NS	NS	NS	
RT-QPCR +	β^A-*globin* + β-*actin* line (1)	100	2.26	NS	0.097	0.091	LD	17
	β^A-*globin* + β-*actin* line (2)	100	3.06	NS	LD	LD	NS	6.6

Crossing point values		*BU1A* gene	*MED14* gene	5' 2xFIV-1	β^A pro 2	*Il2R* gene	3' 2xFIV-1	*BsR* gene
RT-QPCR +	β^A-*globin* line (1)	14.4	20	34.3	31.9	31.8	32.6	
	β^A-*globin* line (2)	14.1	19.7	33.7	32.1	31.9	33.9	
RT-QPCR -	β^A-*globin* line (1)	38.4	40.5	NA	33.1	33.4	32.9	
	β^A-*globin* line (2)	38.4	38.7	NA	NA	41.8	34.3	
RT-QPCR +	β^A-*globin* + β-*actin* line (1)	14	19.8	31	23.9	24.3	28.4	16.6
	β^A-*globin* + β-*actin* line (2)	14.4	19.7	33.9	29.1	29.3	31.2	18.4
RT-QPCR -	β^A-*globin* + β-*actin* line (1)	34.5	38.5	40.3	36.9	42.1	36.5	35.8
	β^A-*globin* + β-*actin* line (2)	32.2	37.3	44.4	36.8	43.6	33.8	34.4

Figure EV4.

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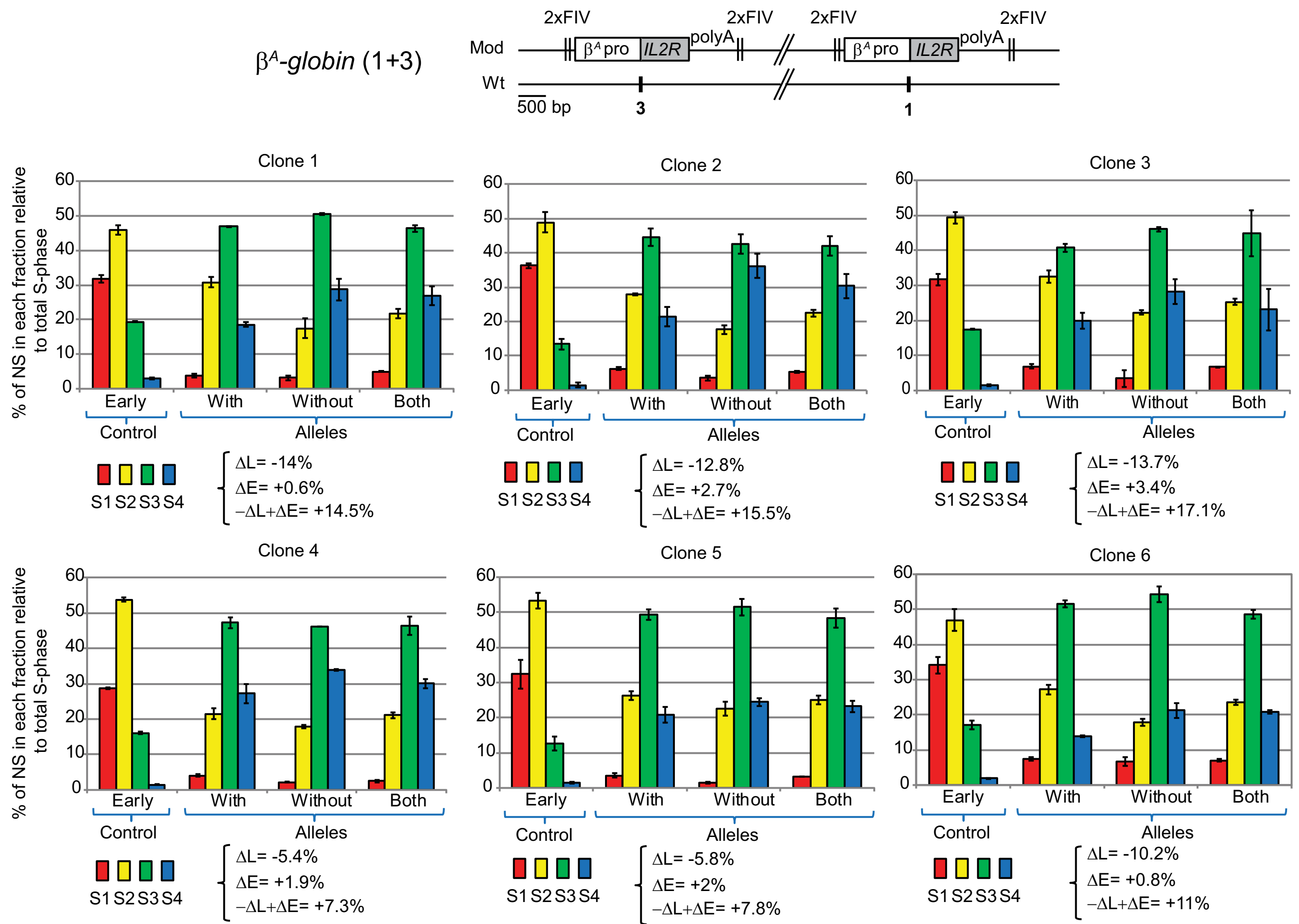

Figure EV5. Two β^A-*globin* constructs inserted at sites 1 and 3 are not sufficient to form an early-replicated domain.

RT profiles of each chromosomal allele are determined after targeted transgene integration using the allele-specific analysis of RT method by real-time PCR quantification described in Appendix Fig S1. Differences in $-\Delta L + \Delta E$ values calculated at the target site following transgene integration are indicated. Error bars correspond to the standard deviation for qPCR duplicates. Analysis of six β^A-*globin* (1 + 3) clonal cell lines described in Fig 4B.

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