Supporting Information

Han et al. 10.1073/pnas.1018726108

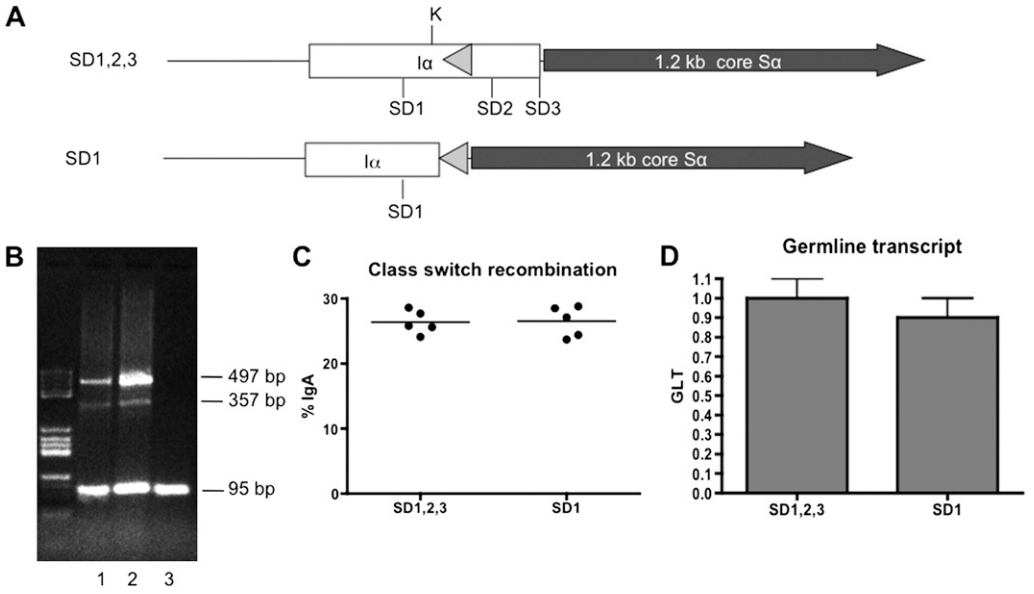

Fig. S1. Alternative splicing sites in Iα-Cα germ-line transcription (GLT). (*A*) Replacement Sα region with or without downstream splice donors SD2 and SD3. (*B*) RT-PCR of GLT from cells with (lane 1, unstimulated; lane 2, stimulated) and without (lane 3, stimulated) SD2 and SD3. (*C*) Class-switch assay of cells with or without SD2 and SD3. One of at least three independent experiments was shown. (*D*) The level of GLTs in stimulated cells. Error bars represent SEM of at least three independent experiments. Each experiment contains at least two independent clones from each construct.

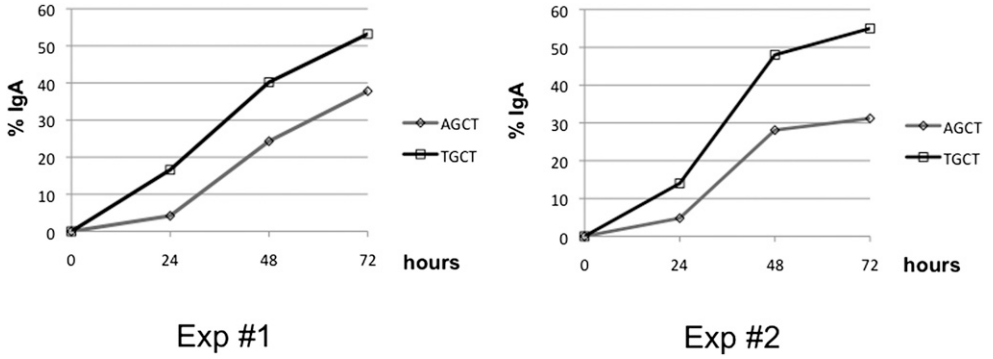

Fig. S2. Class-switch recombination (CSR) kinetics. CSR efficiency was determined at 24, 48, and 72 h after cytokine stimulation. Two independent experiments (experiments 1 and 2) were shown.

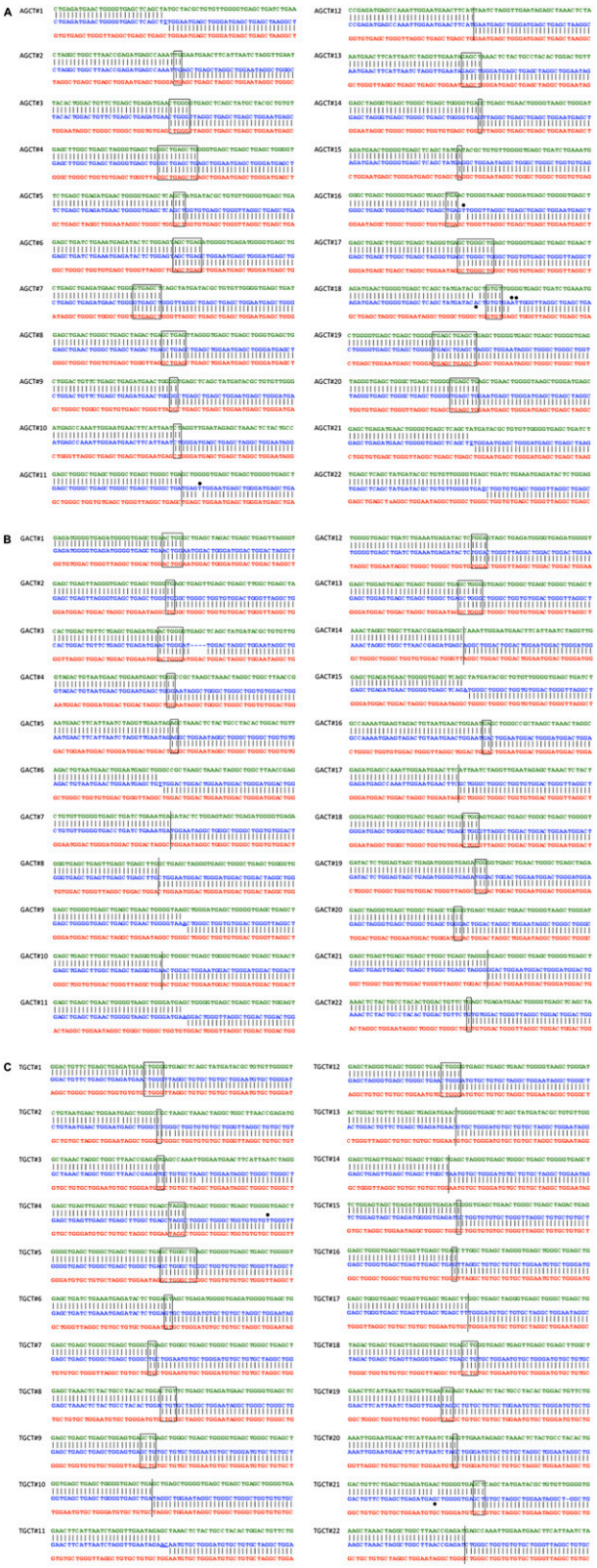

Fig. S3. Sμ-Sα junction sequences. (*A*–*C*). Alignment of switch junctions with germ-line sequences. Germ-line Sμ (green) and Sα (red) sequences are listed on the top and bottom, respectively, of each junction sequence (blue) in the middle. Microhomologies (boxes) are identified as the largest perfect matches to the germ-line sequences. Nucleotide additions are underlined. Long vertical lines indicate direct joins. Small vertical lines indicate identity between the junction and germ-line sequences. Mutations around the junction are indicated by dots.

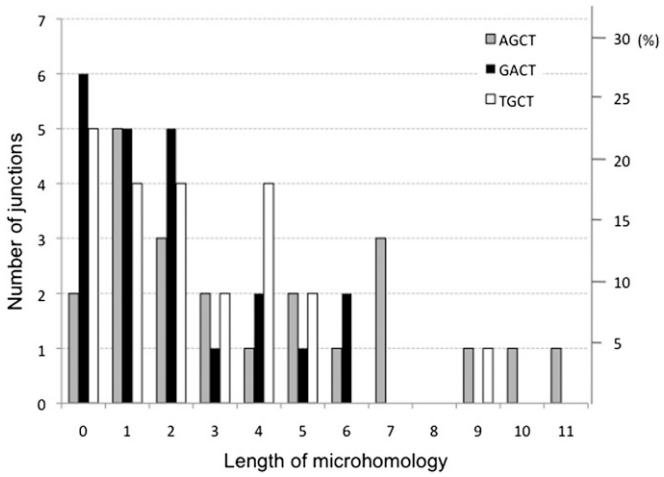

Fig. S4. Switch-junction microhomology. The number (left *y* axis) and percentage (right *y* axis) of junctions with different length of terminal microhomolgy were indicated.

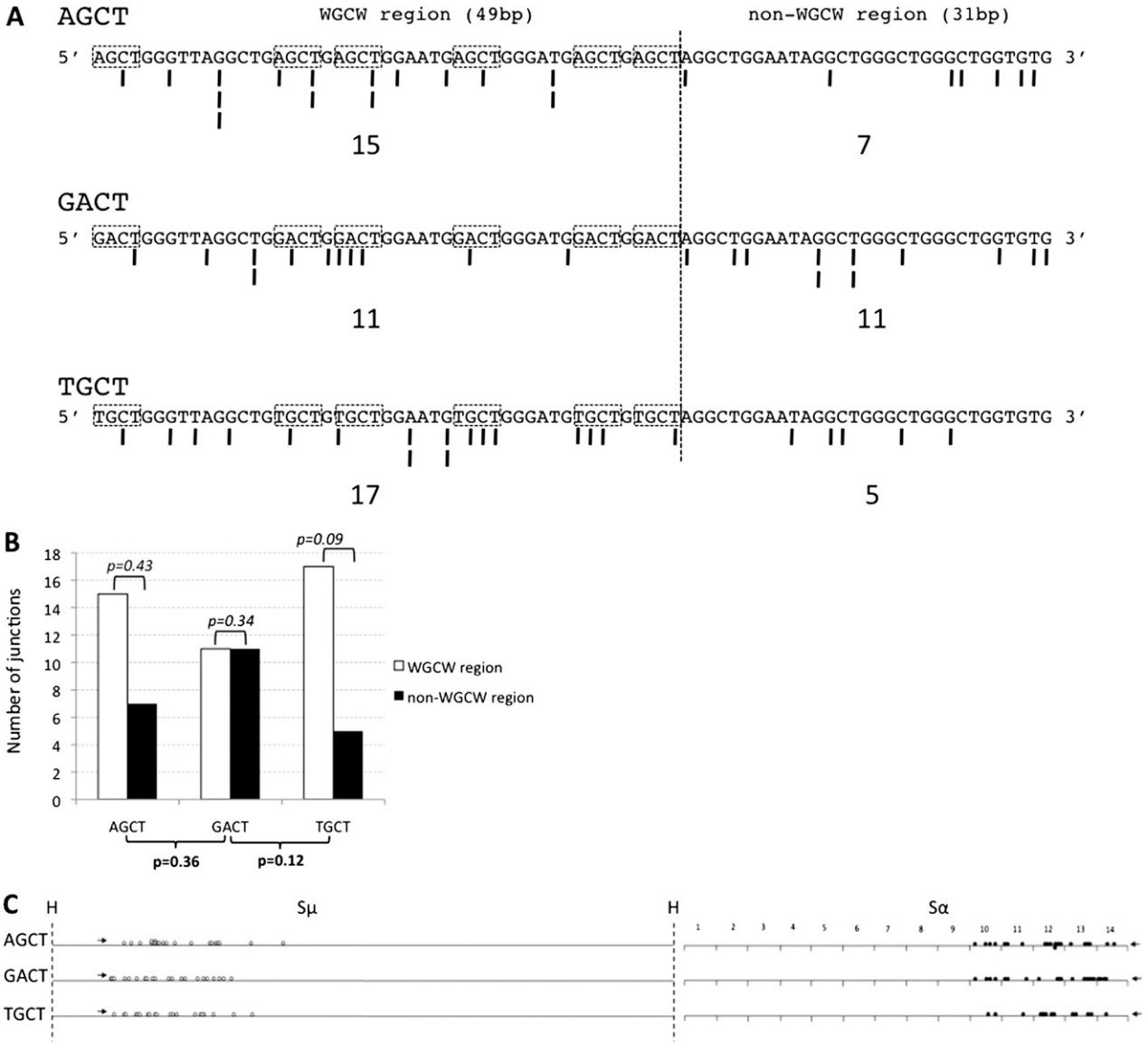

Fig. S5. Switch junction positions. (*A*) The 80-mer repeating unit was arbitrarily divided (vertical dotted line) into two regions: WGCW and non-WGCW. Each short vertical bar below the 80-mer repeat indicates the position of a junction, which is defined by the most upstream nucleotide matching the unrearranged Sα. The total number of junctions in each region is indicated. (*B*) Statistical analysis of switch junction distribution. *P* values in italic were from a χ^2 test to determine whether breaks are overrepresented within the WGCW region in each construct. *P* values in bold were from a Fisher's Exact test to determine whether break distribution between two different constructs is statistically significant. (*C*) Positions of junctions on the germ-line Sμ and Sα. Open and closed circles indicate junction positions for μ and α, respectively. Arrows indicate PCR primer sites. Repeats in each Sα mutant construct are numbered. H, HindIII site.

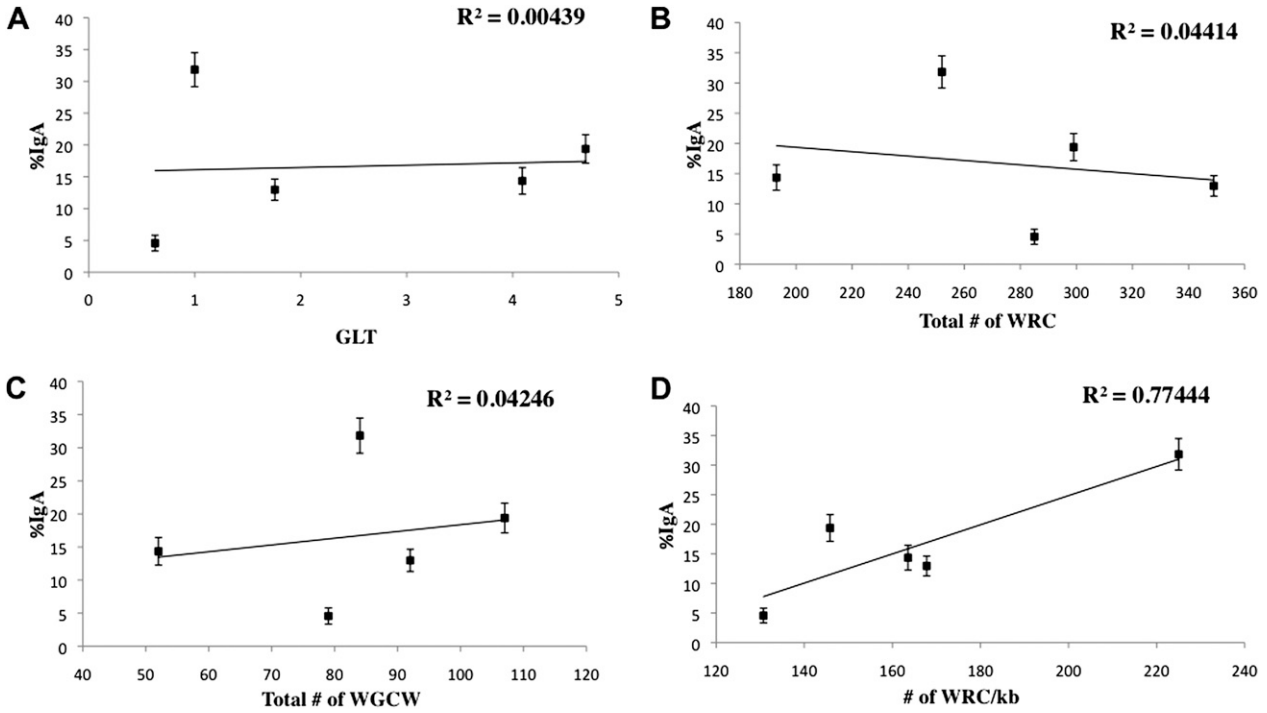

Fig. S6. CSR level versus various switch region motifs. CSR efficiency does not correlates with GLT (*A*), total number of WRC (*B*), or WGCW (*C*). CSR efficiency correlates weakly with WRC density (*D*). All symbols are the same as in Fig. 4.

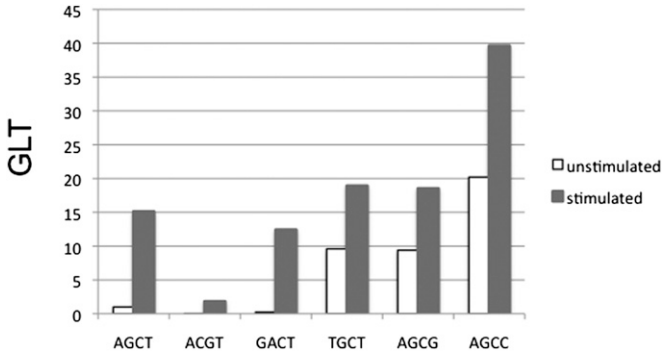

Fig. S7. GLT in stimulated and unstimulated cells. High level of GLT in unstimulated cells derived from TGCT, AGCG, and AGCC mutants.

Table S1. Oligonucleotides and primers

Oligonucleotide or primer	Sequence
Oligonucleotides used for the amplification of Iα-Cα GLT	
KY742	5′ CCTGGCTGTTCCCCTATGAA 3′
KY743	5′ GAGCTCGTGGGAGTGTCAGTG 3′
Oligonucleotides used in real-time PCR	
αCα-Taqman	5′ FAM-CTGCGAGAAATCCCACCATCTACCCA-3′ BHQ
IαCα-Fwd	5′ CCTATGAAGGACACTCAACAACATTG 3′
IαCα-Rev	5′ ACAGAGCTCGTGGGAGTGTCA 3′
Actβ-Taqman	5′FAM-ATCGTGGGCCGCCCTAGGCAC 3′BHQ
Actβ-Fwd	5′ ATGCTCCCCGGGCTGTA 3′
Actβ-Rev	5′ ATAGGAGTCCTTCTGACCCATTCC 3′
PCR primers used to amplify switch junction	
KY761	5′ AACTCTCCAGCCACAGTAATGACC 3′ (first round)
KY743	5′ GAGCTCGTGGGAGTGTCAGTG 3′ (first round)
KY762	5′ GCTTGAGCCAAAATGAAGTAGACT 3′ (second round)
KY812	5′ ATCGATGGATCCGATATCGTC 3′ (second round)