Supporting Information

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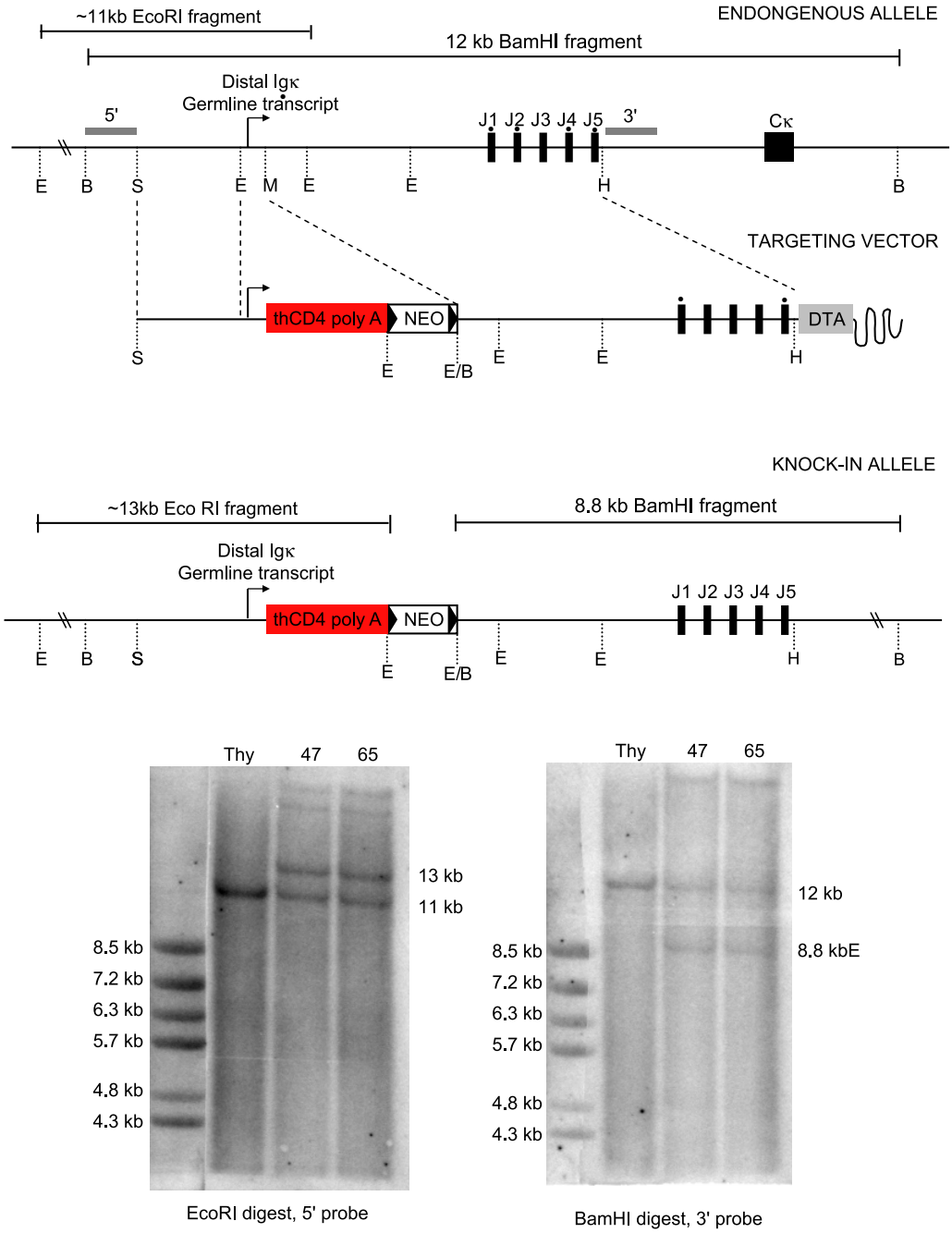

Fig. S1. Generation of dκGT-hCD4 and Cκ-iYFP knock-in mice. (*a*) Structure of the dκGT-hCD4 targeting construct and Southern blots showing the knock-in *Igκ* allele in ES cell clones. Position of restriction sites are shown (B, BamHI; E, EcoRI; H, HincII; M, MscI; S, SalI). 5′ and 3′ probes used for Southern blot are shown as gray lines. Southern blot of neomycin resistant ES cell clones using either the 5′ or 3′ probe on ES cell genomic DNA digested with the given restriction enzyme. Both ES cell clones were used for blastocyst injection. (*b*) Structure of the Cκ-iYFP targeting construct and Southern blots showing the knock-in *Igκ* allele in ES cell clones. Position of restriction sites are shown (Ba, BamHI; B, BglII; E, EcoRI; Bs, BsmBI; C, ClaI; N, NotI). 5′ and 3′ probes used for Southern blot are shown as gray lines. Southern blot of neomycin resistant ES cell clones using either the 5′ or 3′ probe on ES cell genomic DNA digested with the given restriction enzyme. Asterisks indicate the two ES cell clones chosen for blastocyst injection.

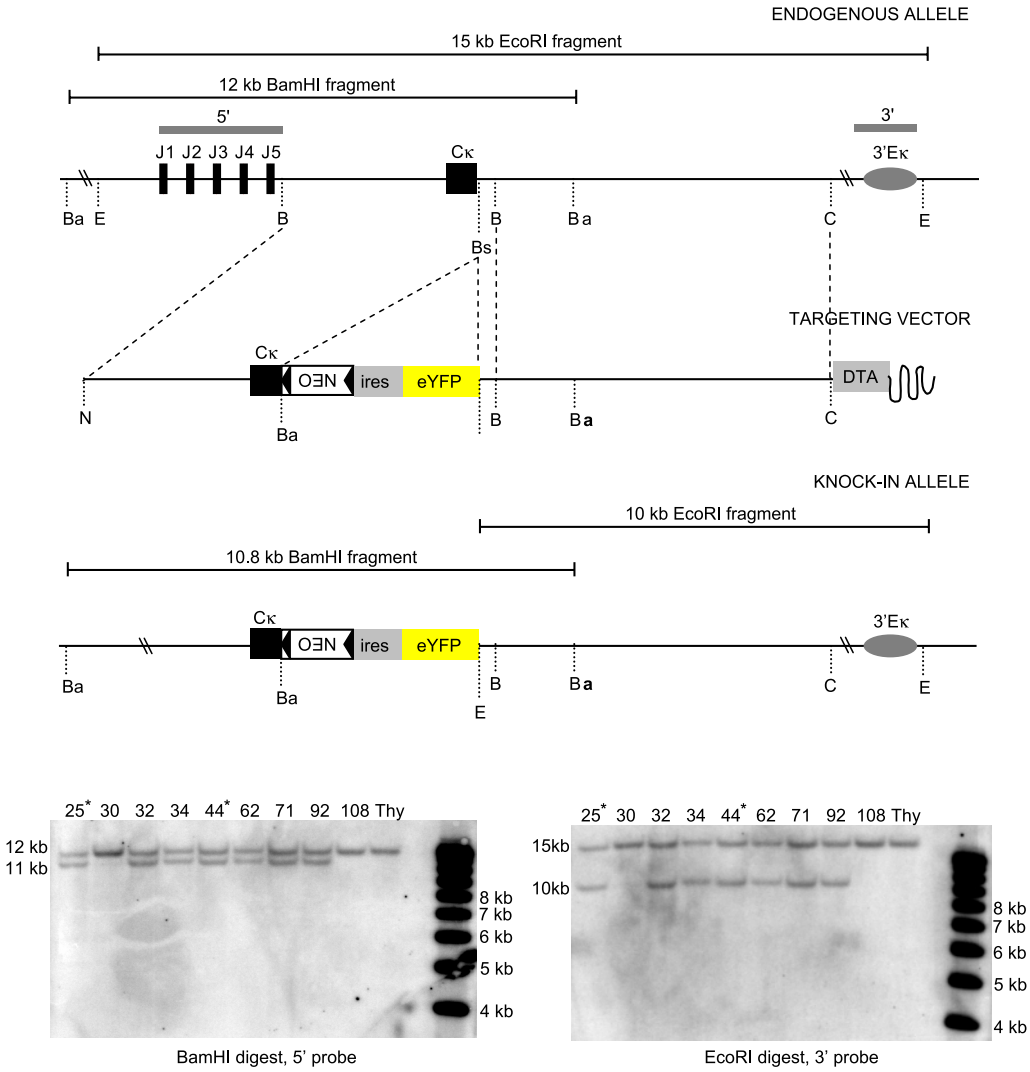

Fig. S1 continued.

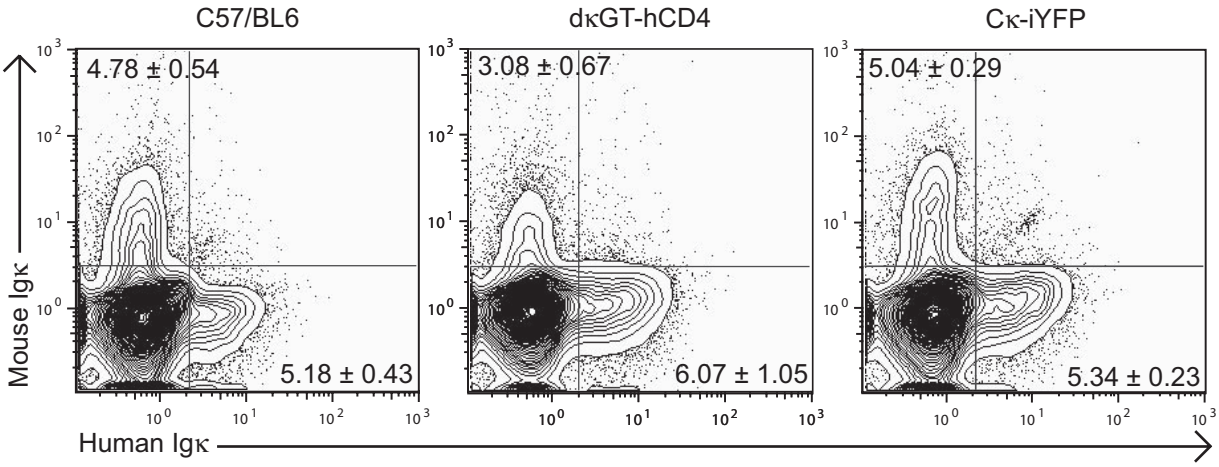

Fig. S2. Vκ-to-Jκ rearrangement of the dκGT-hCD4 knock-in allele is partially defective, while the rearrangement of the Cκ-iYFP knock-in allele is similar to the wild-type allele. Flow cytometric analysis of human or mouse Igκ surface expression in immature B cells in C57BL6/hCκ, dκGT-hCD4/hCκ, or Cκ-iYFP/hCκ compound heterozygous mice. Bone marrow was harvested from mice and labeled with appropriate antibodies to delineate immature B cells ($B220^{+}IgM^{+}IgD$-) and Igκ expression. Data are representative of 2 independent experiments analyzing 3–5 mice per experiment (± SD).

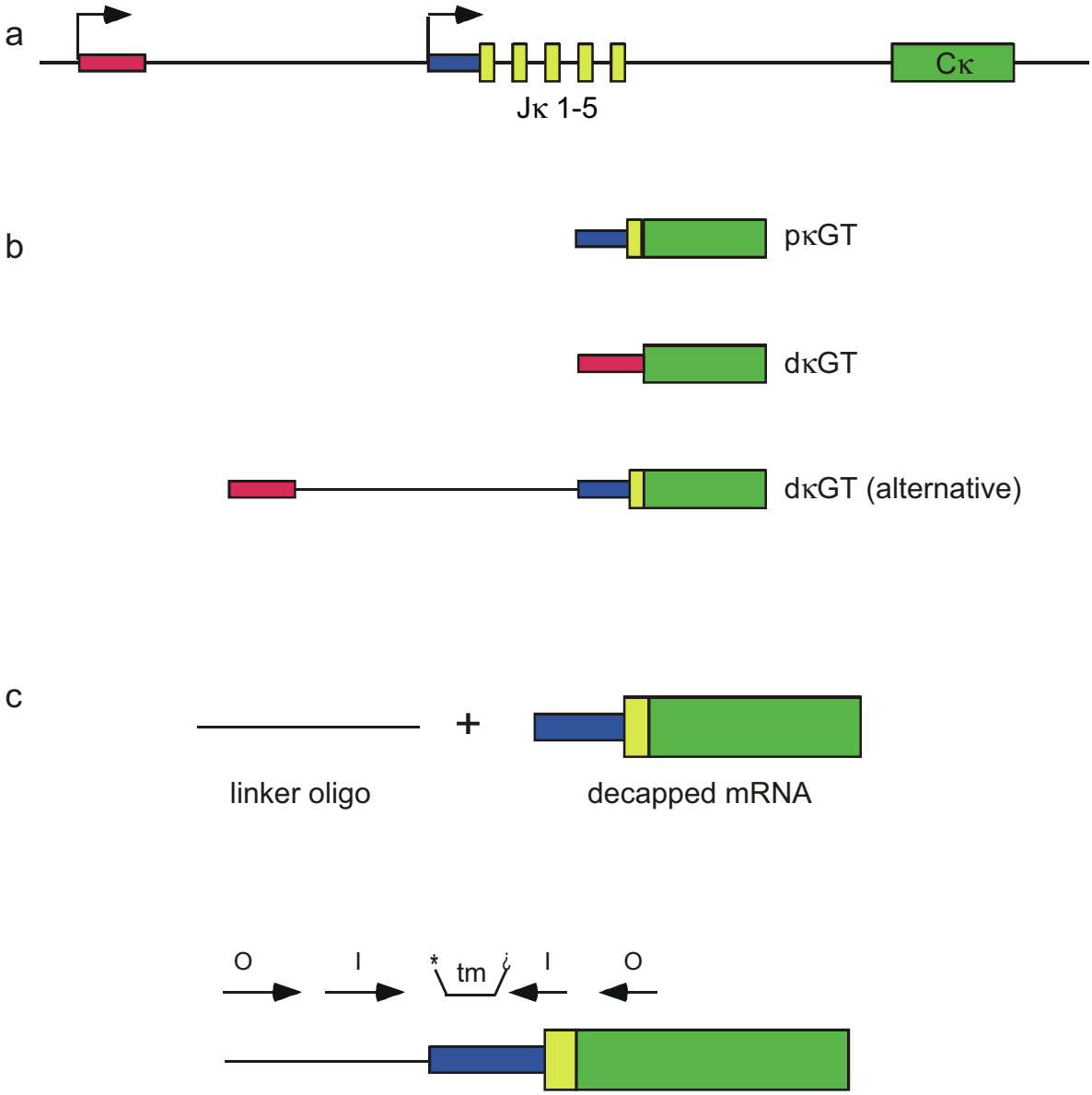

Fig. S3. Diagrams of (*a*) the germline κ locus, (*b*) the various processed germline κ transcripts, and (*c*) the RLM-RACE assay for the proximal promoter initiated germline transcript. The red and blue regions represent the first exons of the originally described distal and proximal germline κ transcripts; the yellow blocks represent the Jκ gene segments, and the green block represents the Cκ exon. The arrows represent the outer (O) and inner (I) nested PCR primers and tm denotes the position of the taqman probe. The diagrams are not drawn to scale.

Table S1. Sequences of primers used in these experiments

Primer name	Sequence 5′-3′
1	GACACATGGGGGAAGGCAGAGAGCTC
2	CCCTCTGAGGTTAGTAAACCCTGATC
3	GCCTTTCTTCAGGGACAAGTG
4	ATGCTCCTGACACATTCTTTGTCTG
5	GCACACTTAGCTCTCATTTCCCAC
6	CAGGGTGTTCAGAAGCAGAGAAGATG
7	GGATGCAGAGGCTGTCAGATTCCTTGCAGC
8	ATAAGCAGTCCTATGTGACATGCTTC
9	CAGCCAGACAGTGGAGTACTAC
Reverse Cκ primer	TGTTCAAGAAGCACACGACTGA
Cκ realtime PCR probe	FAM-TTCCCACCATCCAGTGAGCAGTTAACATC-TAMRA