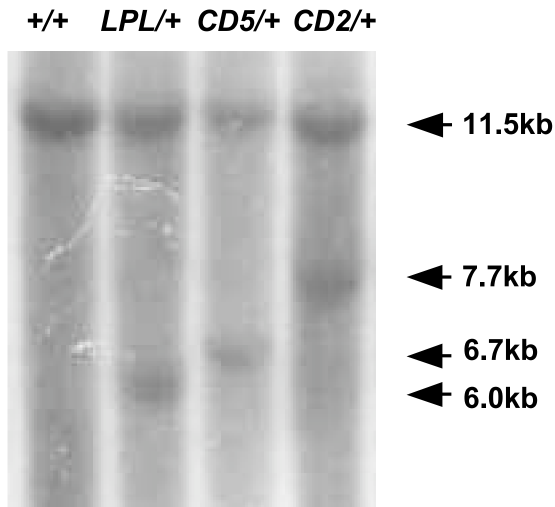
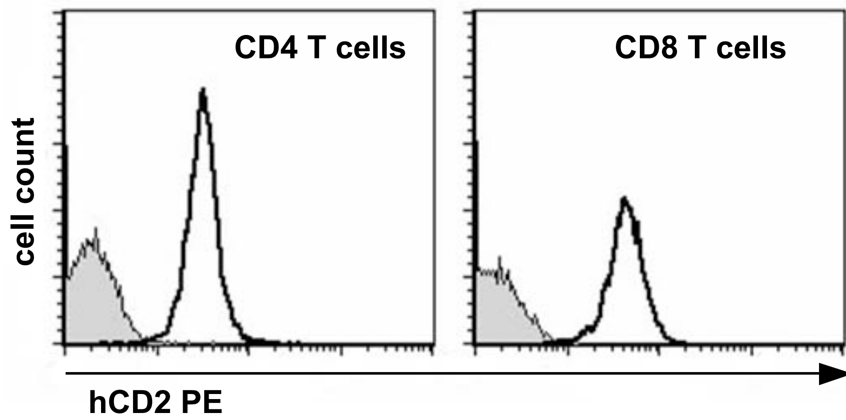


Fig. 4. Southern analysis of targeted alleles. All targeting constructs were built using genomic DNA spanning from the BglII site and the 3' HindIII site (Fig.1*a*). Selection markers and recombination sites were inserted at the NheI site in the middle. Pgk-DTA was attached outside of the targeting module to provide a negative selection. The targeting efficiency in ES cells is 12.1% (7/58) for the *CD2* allele, 12.5% (6/48) for the *CD5* allele, and 7.5% (3/40) for the *LPL* allele. Four mice with different genotypes were included in this Southern analysis. Tail DNA was digested with HindIII and hybridized with a probe derived from the genomic region external of the short homologous arm of the targeting construct (Fig.1*a*). HindIII digestion of the wild type, the *LPL* allele, the *CD5* allele, and the *CD2* allele produce a ~11.5-, ~6.0-, ~6.7-, and a ~7.7-kb fragment, respectively.

a



b

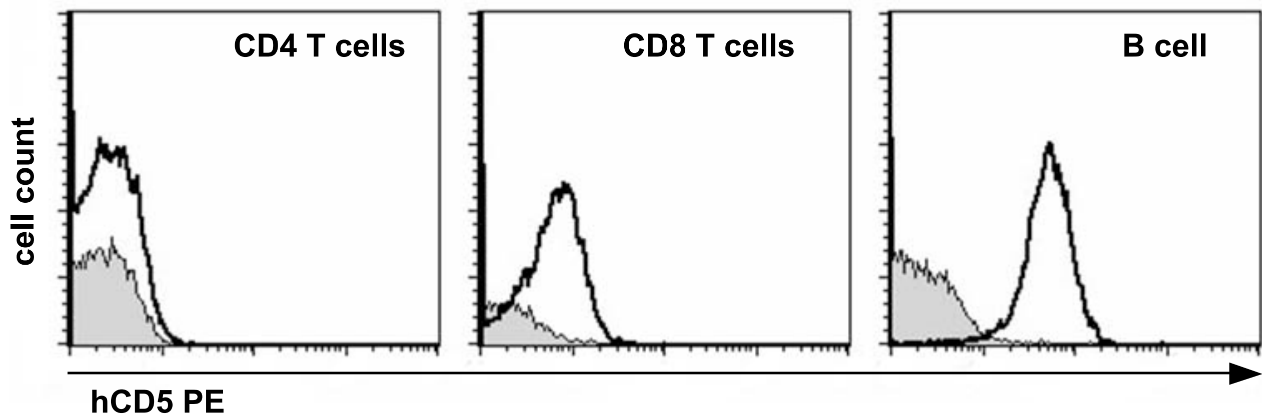


Fig. 5. (*a*) Expression of hCD2 in lymph node T cells. Gray area, from a wild-type mouse; dark line, from a *CD2/*+ mouse. (*b*) Expression of hCD5 in lymphocytes isolated from lymph nodes. Gray area, from a wild-type mouse; black line, from a *CD5/*+ mouse. For cell gating, B cell, $IgM^{+}B220^{+}$; CD4 T cells, $CD4^{+}CD8$; CD8 T cells, $CD4^{-}CD8^{+}$.

Table 1. Summary of PCR results shown in Fig. 2.

Genotype predicted	F1/R1	F2/R2	F3/R3	F4/R4	F5.1/R5	Mouse No.
CD2pCD2d/CD5pCD5d	+	+	+	+	-	2, 3, 9, 10, 12
CD2pCD2d/CD2pCD2d	+	+	-	-	-	1, 4
CD5pCD5d/CD5pCD5d	-	-	+	+	-	6, 7, 8
CD2pCD5d/CD2pCD2d	+	+	-	+	+	5
CD2pCD5d/CD5pCD5d	+	-	+	+	+	11
CD5pCD2d/CD2pCD2d	+	+	+	-	+	None
CD5pCD2d/CD5pCD5d	-	+	+	+	+	None

The first column shows seven possible genotypes as depicted in Fig.2*a*. The predicted PCR result with each primer pair is indicated in the middle columns. The outcome of PCR assay for each mouse included in Fig. 2*b* is listed in the last column. Five of seven predicted genotypes were observed in this group of progeny. The remaining genotypes were also observed in the expanded progeny test. Eight of 87 progeny from this type of cross were scored positive for recombination between the *CD2* and *CD5* alleles.

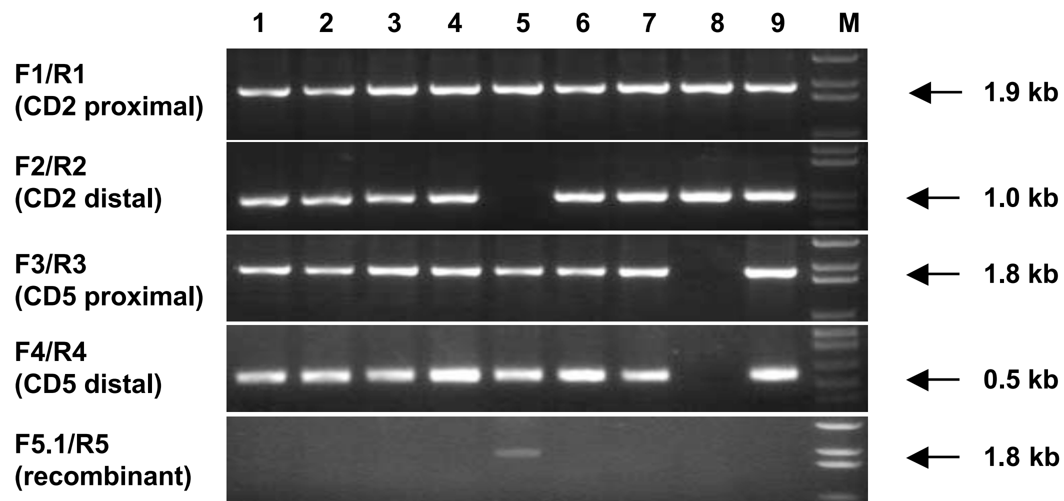


Fig. 6. PCR analysis of Flpe mediated germline recombination between the *CD2* and *CD5* alleles. Tail samples were from 9 progeny of a cross between *CD2/CD5;Actin-Flpe* and *CD2/CD5* mice. Positions of PCR primers are shown in Fig.1*a*. The size of each PCR product is indicated on the right. M: DNA size markers. Mouse number 5 contained a recombinant chromosome and a *CD5* allele, number 8 was homozygous for the *CD2* allele, and the rest were *CD2/CD5* heterozygous.

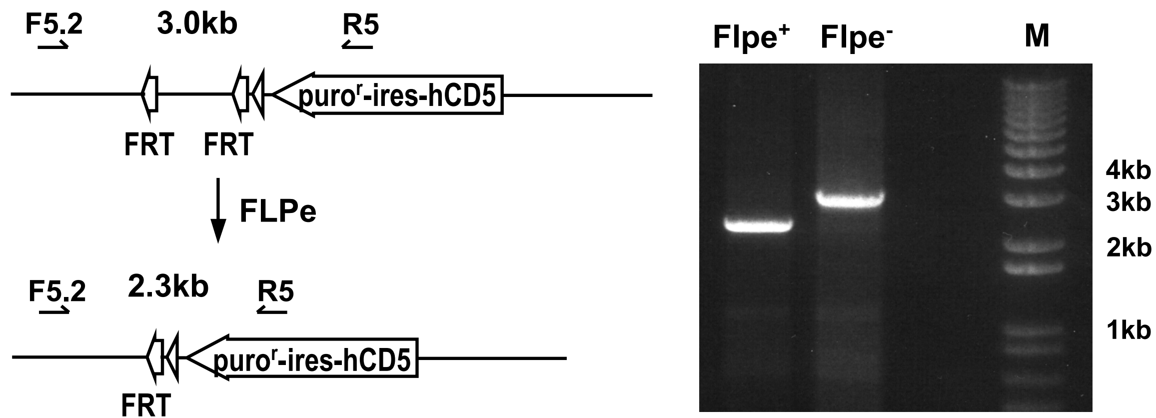


Fig. 7. Verification of the Flpe recombinase activity. Total thymocyte DNA was used in the PCR. Because of the 0.7-kb spacer between the FRT sites, primer pair F5.2/R5 amplifies a 3.0-kb fragment from the *CD2/CD5* mouse (Flpe^{-}) and a 2.3-kb fragment from *CD2/CD5; Actin-Flpe* mouse (Flpe^{+}). A 1-kb ladder (M) was loaded in the gel as size markers. Similar results were obtained with tail DNA (data not shown).