ELSEVIER

Journal of Immunological Methods 180 (1995) 273–280

JOURNAL OF IMMUNOLOGICAL METHODS

Short communication

Cassette vectors directing expression of T cell receptor genes in transgenic mice

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Received 31 August 1994; revised 9 November 1994; accepted 3 January 1995

Abstract

We describe a pair of cassette vectors that can be used to express rearranged T cell receptor genes in transgenic mice. Short DNA fragments containing rearranged Vα and Vβ segments are readily amplified from T cells and introduced between artificial cloning sites. Transgene-derived mRNAs are transcribed under the control of the natural TCRα and -β promoter/enhancer elements. Using this vector, we have obtained transgenic mouse lines which display transgene-encoded TCR α and β chains on a majority of T cells.

Keywords: T cell receptor; Transgenic mouse; Cassette vector

1. Introduction

In recent years, transgenic mouse strains carrying rearranged T cell receptor (TCR) genes have contributed greatly to our understanding of fundamental processes in T cell biology. Since expressed TCR chains largely prevent the further rearrangement of TCR loci, the transgene-encoded receptors are present on the majority of T cells (Uematsu et al., 1988), at least prior to selective influences which shape the repertoire. This provides one with the possibility of tracking the behavior and fate of T cells with defined specificities, which was previously possible only indirectly, by testing the functional capabilities of very minor populations. The central concept of positive selection in the thymus was thus given a firm experimental basis (e.g., Teh et al., 1988; Sha et al., 1988; Berg et al., 1989), and several studies uncovered the bewildering array of strategies with which the immune system achieves self-tolerance (Kisielow et al., 1988; Pircher et al., 1989; Ohashi et al., 1991; Schönrich et al., 1991). TCR transgenic mice have also been instrumental in analysing the behavior of autoimmune T cells

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SSDI 0022-1759(95)00002-X

aggressively directed against self-antigens (Ohashi et al., 1991; Katz et al., 1993; Goverman et al., 1993).

Construction of TCR transgenics still remains an unwieldy endeavour. In some instances, investigators have isolated large fragments of genomic

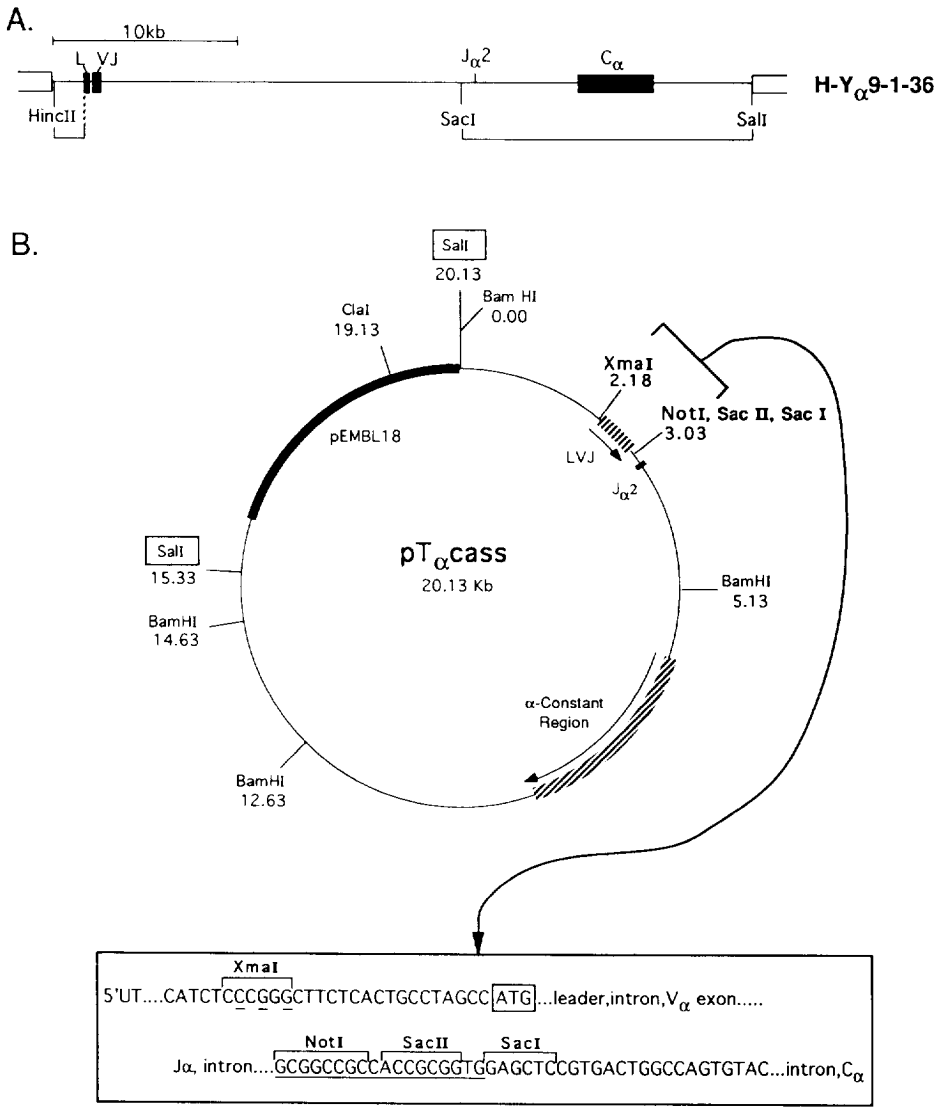

Fig. 1. Cassette plasmid for the expression of TCRα genes. *A*: origin of the fragments used in the vector construction, from plasmid H-Yα9-1-36 (Bluthmann et al., 1988). The fragments used to generate our construct are bracketed. The transcriptional promoter and 5′ untranslated sequences range from a *Hin*cII site to an artificial *Xma*I site introduced by site-directed mutagenesis 17 bp upstream of the ATG codon (stippled vertical line). The large *Sac*I-*Sal*I fragment provides the constant region and the enhancer elements. *B*: map of the pTαcass plasmid. The TCRα genetic elements are shown as a thin line, with the position of the exons broadly stippled. The prokaryotic plasmid sequences are shown as a thick line. The *Xma*I, and *Not*I, *Sac*II and *Sac*I between which the rearranged VDJ segments can be cloned are typed boldface, and the nucleotide sequence around these sites is shown at the bottom of the figure. The *Sal*I sites which can be used to separate the TCRα segments from the prokaryotic DNA are bracketed.

DNA from the TCR α and β loci of mature T cells; such fragments are cumbersome to manipulate, and it has never been quite clear exactly which flanking sequences are required for proper expression. In other instances, heterologous promoter fragments such as MHC class I (Pircher et al., 1989) or CD2 (Mamalaki et al., 1993) have been used to drive TCR constructs, or an IgH enhancer fragment has been added to boost expression levels (Berg et al., 1988). Such combinations are often not ideal, because they subject the TCR genes to an abnormal timing and regulation of expression.

With these considerations in mind, we thought it worthwhile to engineer TCR cassette vectors, into which short segments of rearranged α and β variable regions could easily be introduced. These short fragments can be quickly obtained by PCR

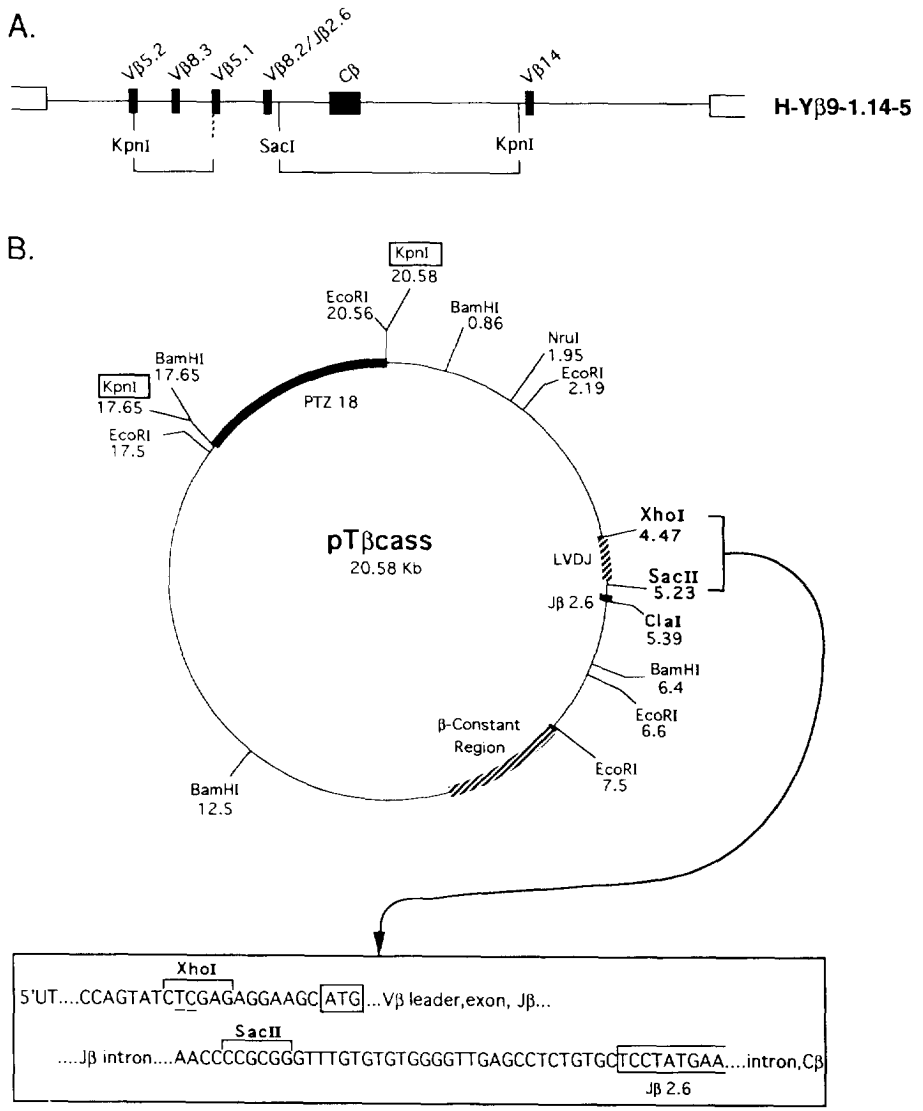

Fig. 2. Cassette plasmid for the expression of TCRβ genes. *A*: origin of the fragments used, taken from pH-Yβ9-1.14-5 (Uematsu et al., 1988). *B*: map of the pTβcass. Symbols as in Fig. 1.

from T cell cDNA or genomic DNA, and inserted into convenient cloning sites, bypassing the requirement for cumbersome cloning of large genomic segments of unknown structure. We also considered it preferable that transcription from these constructs be driven by the natural TCR regulatory elements.

2. Materials and methods

DNA manipulation and transgenic mice. Construction of the vectors employed standard techniques of recombinant DNA engineering. During the manipulation of such large recombinant plasmids, which are prone to spurious recombination events during cloning procedures, aberrant short plasmids are often obtained, even in RecA$^-$ strains. Colony screening thus employed several probes corresponding to different regions of the desired constructs; the desired recombinants are usually a rather small minority.

The transgenic mice were constructed by injection of linearised DNA fragments, devoid of prokaryotic sequences, into fertilised eggs of C57Bl/6/J × SJL/J F2 hybrids. Transgenic founders were identified by Southern blot analysis of tail DNA. Transmission of the transgenes to subsequent generations was also ascertained by Southern blot DNA hybridization, or by cytofluorimetric analysis of transgene expression on blood lymphocytes.

Proliferation assays and cytofluorimetry. Lymph node cells from transgenic mice or negative littermate controls were cultured in RPMI medium, supplemented with 10% fetal calf serum and 1 mM glutamine, with titrated doses of ConA or of specific antigenic peptides corresponding to the specificity of the transgene-encoded TCR (in the experiment shown in Fig. 5, peptide 41–61 of bovine RNAse; Peccoud et al., 1990). T cell activation was assessed by [^{3}H]thymidine incorporation in the cultures after 24–36 h. Three-color flow cytometry was performed as described (Katz et al., 1993); in the example shown in Fig. 4, staining employed anti-CD4 and anti-CD8 reagents (Caltag), and the mAb 44.22.1, specific for Vβ6 (Acha-Orbea et al., 1985).

3. Results and discussion

The DNA segments used for both the α and β chain constructs originated from fragments of rearranged DNA used to produce HY-specific TCR transgenic mice (Uematsu et al., 1988; Bluthmann et al., 1988). Since both the α and β transgenes are efficiently expressed in these mice, and do not require further enhancer motifs, we assumed that all essential promoter and enhancer elements could be found within these fragment. The origin of the segments we used and the map of the resulting cassette vectors are presented in Figs. 1 and 2. We will not detail here the several intermediate steps involved in the construction of the cassettes.

For the α chain (Fig. 1), we included essentially all of the 5′ flanking region upstream of the Vα region of the H-Y TCRα construct, from the *Hin*cII site down to an artificial *Xma*I site that was created 23 bp upstream of the ATG codon. This stretch of DNA should contain all required proximal promoter motifs, the transcriptional initiation site, and most of the 5′-untranslated region of the mRNA. For the Cα segment, we reasoned that all indispensable enhancer components must be present downstream of the 3′-most Jα segment (Jα2), since TCRα genes in which all sequences upstream of Jα2 are deleted by rearrangements involving Jα2 are nevertheless efficiently transcribed. We thus introduced the entire segment downstream of a convenient unique SacI site, found a few hundred bases upstream of Jα2 (Koop et al., 1992). This fragment contains the 3′ enhancer of the α locus (Winoto and Baltimore, 1989). During the assembly of this construct, we introduced unique *Not*I and *Sac*II sites next to the *Sac*I site. Rearranged VαJα segments can thus be introduced between the *Xma*I site on one side, and one of the *Not*I or *Sac*II, sites on the other (Fig. 1B).

For the β chain construct (Fig. 2), the upstream promoter region was that of Vβ5.1, which is the promoter actually used in the H-Y constructs (the leader exon of Vβ5.1 is spliced onto the coding exon of the rearranged Vβ8.2/Jβ2.6 segment after transcription; Uematsu et al., 1988). This region is present on a fragment extending

from a *Kpn*I site (itself in the Vβ5.2 coding region) to an artificial *Xho*I site engineered 13 bp upstream of the ATG codon of Vβ5.1 (Fig. 1B). As for the equivalent α chain segment, all necessary promoter, initiator, and 5′-untranslated sequence elements should be present in this fragment. The Cβ coding region and flanking regulatory sequences are provided on a segment which extends from a *Sac*II site upstream of the Jβ2.6 segment to a *Kpn*I site approximately 9 kb down from the Cβ exons (this was actually assembled from two portions: a short segment from the *Sac*II site to a *Sac*I site just downstream of the Jβ2.6 region, followed by the *Sac*I-*Kpn*I fragment shown in Fig. 2A). Rearranged VDJβ segments can thus be introduced between the *Xho*I site on one side, and the *Sac*II site on the other or alternatively the *Cla*I site, if the rearangement involves Jβ2.6 or if removal of the Jβ2.6 segment is thought preferable.

The rearranged regions are most conveniently obtained by PCR amplification, with primers which introduce the required unique restriction enzyme sites. Both genomic DNA and cDNA can be used as starting material for the amplification. However, we have used genomic DNA in our cases. First, this strategy automatically provides the splice donor and intron sequences to link to the J-C intron at the *Not*I or *Sac*II sites. Second, a short intron separates the leader and V region coding sequences; we do not know that this intron is truly required for expression, but have felt it safer to include it, since it could contain regulatory motifs for transcription or mRNA transport and splicing. Should cDNA be used, one would simply need to artificially add the beginning of the J-C intron down from the J segment, most conveniently by linking-PCR.

The cassette vectors also include pairs of unique restriction enzyme sites that can be used to excise the TCR genes free of prokaryotic plasmid DNA: *Sal*I sites in pTα cass, and *Kpn*I sites in pTβ cass. A few TCRβ variable regions actually contain a *Kpn*I site. In these instances, a partial *Kpn*I digestion is required.

We illustrate the use of these vectors in Fig. 3. These TCR segments derive from kLy11.10, an A^k-restricted, hen egg lysozyme-specific T cell hybridoma; the TCR is composed of Vα1Jα16 and Vβ11Jβ1.3. The nucleotide sequences up-

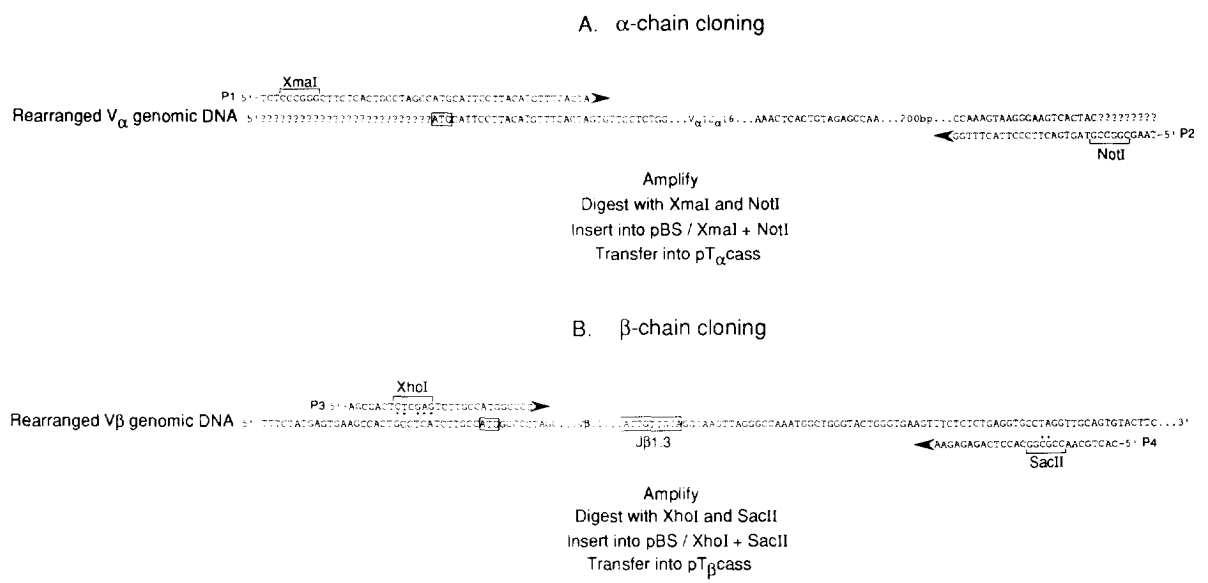

Fig. 3. A practical example of the use of the cassette vectors. The T cell hybridoma from which the V regions originate utilises the Vα1Jα16 and Vβ11Jβ1.3 combinations. The figure shows the known sequence of these elements, together with the PCR oligonucleotides used to create the desired fragments, flanked with the appropriate restriction enzyme sites. Dots between the genomic sequence and the primer sequences represent mismatches which generate the artificial sites.

stream of the ATG codon for this particular member of the Vα1 family are not known. We thus designed an upstream PCR primer (P1) which provides the equivalent sequence between the *Xma*I site and the ATG codon from the H-Yα, 9-1-36 Vα region, followed by the true Vα1 sequence from the ATG onwards. The opposite PCR primer (P2) contains 25 bp of sequence complementary to the intron downstream of Jα16, flanked by an additional *Not*I site. Following amplification from kLy11.10 genomic DNA and digestion with *Xma*I and *Not*I, the correct product was cloned into pBluescript, and verified by DNA sequencing. The fragment was subsequently transferred from pBluescript to pTCRαcass. We prefer to go through this intermediate step in pBluescript than to clone directly the PCR product in pTαcass, because sequencing to verify the faithfulness of the PCR products is easier in pBluescript than in the large final product. Furthermore, cloning into pTacass plasmid is not trivial, because of the large size of the plasmid which has a tendency to recombine into shorter derivatives. It is thus preferable to start from a significant amount of insert, checked and grown in pBluescript.

Similarly, the rearranged VDJβ segment was amplified with a primer (P3) in which a few mismatches introduced an *Xho*I site upstream of the ATG codon in Vβ11. The opposite primer was complementary to the sequence downtream of Jβ1.3, with two mismatches resulting in a SacII site. The amplified product was again cloned into pBluescript before transfer into pTβcass.

We have successfully used these cassettes in several instances. They were employed to transfect $58\alpha^{-}\beta^{-}$ cells (Gabert et al., 1987), yielding transfectants with the expected antigenic reactivity, in terms of antigenic peptide specificity as well as of sensitivity to MHC mutations (data not shown). The α chain of a diabetogenic TCR was expressed in transgenic mice in this fashion (Katz et al., 1993). The pTαcass and pTβcass vectors were also used to derive transgenic lines with dominant expression of TCRs conferring reactivity to the 46–61 peptide of hen egg lysozyme (clone kLy11.10), or the 41–61 peptide of bovine RNase (clone RNase28; V. Kouskoff, in preparation). High level expression of the β chain was detected in these cases with Vβ-specific reagents, as illustrated for lymph node cells in Fig. 4. Proper expression was also found as expected in thymic subpopulations, with the exception of abnormal overexpression in immature double negatives: this is an unavoidable corollary of rearranged TCR transgenes, whose expression commences at a stage where normal TCRβ genes are undergoing rearrangement. Frequently expression of the transgenic α chain could not be monitored directly for lack of specific monoclonal

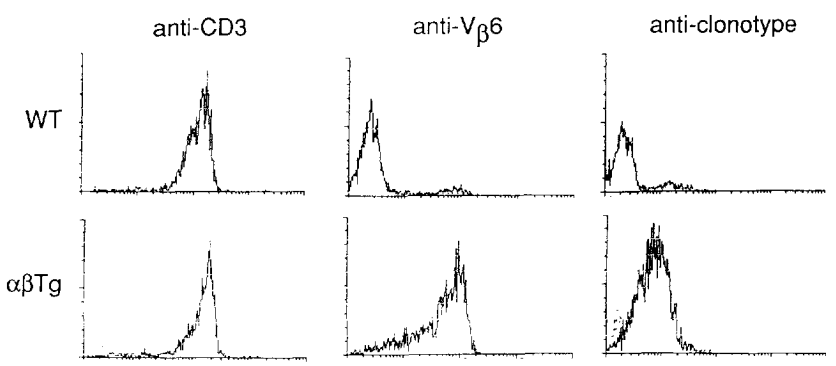

Fig. 4. Expression of cassette-encoded transgenes. CD4-positive lymph node cells were prepared from a TCRαβ transgenic mouse of the KRN line (an MHC class II-restricted receptor; VK, unpublished) and from a negative littermate. The cells were analysed by multiparameter cytofluorimetry after counterstaining with anti-CD3, anti-Vβ6 (which detects the transgene-encoded TCRβ chain), or with an anti-clonotypic reagent which primarily detects the particular αβ combination of the KRN receptor (note that the low staining intensity in this panel does not reflect a low expression of transgene-encoded TCRα chain, but rather a reagent of poor staining capacity).

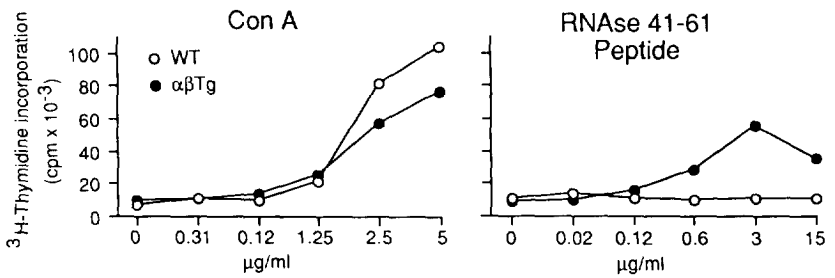

Fig. 5. Antigen recognition by transgene-encoded TCR chains. Primary stimulation of lymph node cells from a KRN mouse or a negative littermate in culture, with ConA or with the antigenic peptide RNAse 41–61. Cell proliferation, measured by incorporation of [^{3}H]thymidine, was evaluated after 24 h.

reagents. Yet its expression could be revealed with the use of clonotype-specific polyclonal antisera, as exemplified in Fig. 4. We could also readily detect reactivity to the antigenic peptide presented by the appropriate MHC molecules in large numbers of lymph node T cells, the extent and kinetics of the proliferative response being similar to those obtained with general mitogens (Fig. 5); this reactivity, as judged by the results of mixed transfection experiments, denotes expression of both α and β chains of the transgenic TCR.

We hope that this vector will prove useful to other investigators.

Acknowledgements

We are grateful to P. Gerber, J. Hergueux, C. Marfing, M. Duval and P. André for assistance in the generation and analysis of the mice, to Y. Uematsu, H. Bluthmann, M. Steinmetz and H. von Boehmer for the starting plasmids, to the staff of the CSAL/CNRS and to P. Michel, N. Zinck and S. Falcone for maintaining the mice. This work was supported by institutional funds from the INSERM, the CNRS, and the Centre Hospitalo-Universitaire Régional and by grants to DM and CB from the Association pour la Recherche sur le Cancer and the Juvenile Diabetes Foundation. VK and KS were supported by fellowships from the Ligue Nationale Française Contre le Cancer and the Cancer Research Institute, respectively.

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