The 5'HS4 insulator element is an efficient tool to analyse the transient expression of an Eμ-GFP vector in a transgenic mouse model.

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Dear Editor,

Several studies have highlighted the potential benefits of insulators on construct expression in transgenic animals (Houdebine et al., 2000; Giraldo et al., 2003; Soulier et al., 1999). As reviewed by Giraldo et al. (2003) in a previous issue of *Transgenic Research*, the 5'HS4 element, derived from the chicken β-globin locus (the first insulator identified in vertebrates) has been used with success to improve heterologous construct expression in transgenic animals. The intronic immunoglobulin heavy chain (IgH) enhancer Eμ, which consists of a core enhancer flanked by 5' and 3' matrix attachment regions (MAR), has been implicated in the control of IgH locus recombination and transcription (Khamlichi et al., 2000). Results of transfection of a recombination test gene have suggested that what is required for rearrangement seems not to be Eμ-mediated transcription *per se* but an open chromatin state (Engler et al., 1991). The repeated observations that these elements are not needed to maintain endogenous IgH expression in B cell lines suggested that continued expression of IgH genes in these cell lines does not require Eμ and MARs (Aguilera et al., 1985; Wabl and Burrows, 1984). Altogether these results thus suggest a transient expression of Eμ in early B cell development in order to generate an open chromatin state for actions of other functional elements (among which the various enhancers located in 3' of the IgH locus). The bright autofluorescence of the green fluorescent protein (GFP) in living cells provides a powerful strategy for investigating at the same time by flow cytometry cell lineage and differentiation stage (Guglielmi et al., 2003b). Unfortunately, previous results showed the lack of expression of an Eμ-GFP transgene in a transgenic mouse model (Guglielmi et al., 2003a); these results being related to intense transgene DNA methylation. We thus decided to flank a Eμ-GFP construct by the 5'HS4 insulator and to undertake the generation of transgenic mice harboring an insulated pV_H promoter-GFP reporter gene linked to the Eμ element (Figure 1A) in order to identify precisely the first B cell maturation stage showing expression of the Eμ-GFP transgene.

Transgenic mice were generated as previously reported (Guglielmi et al., 2003a, 2003b). Two different founders were generated with 5 and 10 copies of the transgene,

respectively; these two founders giving similar results. Femoral bone marrow cells were analysed using confocal microscopy (Figure 1B). For these experiments the MitoTracker Red was used as internal control in order to stain all cells in red. As shown in Figure 1B, GFP^+ cells were found in the bone marrow of transgenic mice. This result markedly differs from our previous study highlighting no GFP^+ cells in transgenic mice harbouring the construct without insulators (Guglielmi et al., 2003a). To examine the B specific expression of the GFP transgene, cells were labelled with appropriate antibodies and submitted to flow cytometry analysis (Figure 2). The differentiation of a bone marrow stem cell into a mature B cell can be subdivided into at least four steps that are characterised by Ig gene rearrangement and expression of surface markers. Briefly, $CD43^+CD117^+$ cells represent an uncommitted lymphohematopoietic progenitor population with the ability to generate B cells, myeloid cells and T cells. The pro-B stage is characterised by the progressive loss of CD117 and CD43 antigens and the acquisition of the B220 antigen by murine B cell precursors. The CD19 antigen expression begins at the late pro-B stage and, similarly to that found for the B220 antigen, is present during all the B cell maturation process. The CD25 antigen (the IL-2 receptor) is expressed only at the pre-B stage. Membrane IgM are expressed in immature B cells. Finally, immature B cells differentiate in the spleen into mature B cells expressing IgM and IgD. Flow cytometry analysis clearly indicated that GFP^+ B lymphocytes were detected in bone marrow of transgenic animals. All GFP^+ cells were $B220^+CD19^+$ (*i.e.* belonged to the B cell lineage). Results highlighted GFP^+ cells from pro-B cells ($B220^+CD19^+CD43^+CD117^+$) to pre-B cells ($B220^+CD19^+CD25^+IgM^-$). A few bone marrow GFP^+ cells were IgM^+ showing low transgene expression in immature B cells. No GFP^+ cells were found in thymus (*i.e.,* T cells) (data not shown).

The present study shows that a construct associating GFP with the Eμ element may confer, when insulated properly, an expression from pro-B cells to pre-B cells that is strictly confined to the B lineage. These results favour the hypothesis of a transient expression of Eμ in the B cell development in order to generate an open chromatin state for actions of other functionally elements such as the various enhancers located 3' of the IgH locus. This study confirms the great potential benefits of insulators, especially the chicken β-globin HS4 one,

for construct expression in transgenic mice (Houdebine et al., 2000; Giraldo et al., 2003).

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LEGENDS TO FIGURES

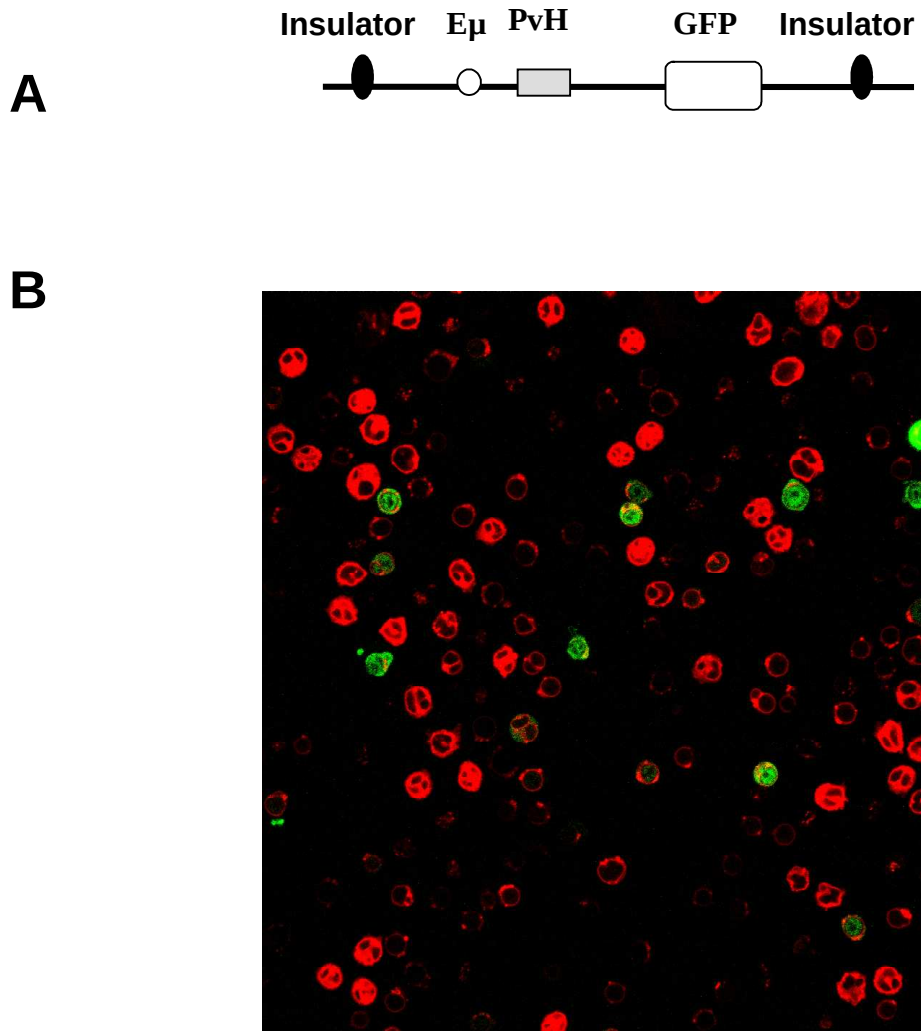

Figure 1: Presence of GFP^+ cells in insulated Eμ-GFP transgenic mice.

A: Schematization of the insulated Eμ-GFP transgene. The pV_H promoter was a 0.2-kB HindIII fragment derived from a rearranged murine V_H segment. The Eμ enhancer, a 0.9-Kb StuI fragment, was cloned upstream the pV_H promoter. The GFP coding sequence was a NotI-SalI fragment excised from the pEGFP-1 vector (Clontech Laboratories, Palo Alto, CA). The transgene was flanked by two copies of the core element of the chicken β-globin HS4 insulator (a 250-bp fragment containing the whole insulator activity). B: Detection of GFP^+ cells in femoral bone marrow of transgenic mice. Cells were incubated with 500 nM MitoTracker Red (Molecular Probes, Eugene, OR) for 40 min in order to stain all cells in red (MitoTracker Red is well known to stain mitochondria). Stained cells were then imaged by confocal scanning microscopy (LSM-510 Carl Zeiss, Jena, Germany) using an excitation at

488 and 594 nm for GFP and MitoTracker Red, respectively. Representative results from four transgenic mice derived from two different founders are shown. Results from mice with 10 copies of the transgene are shown.

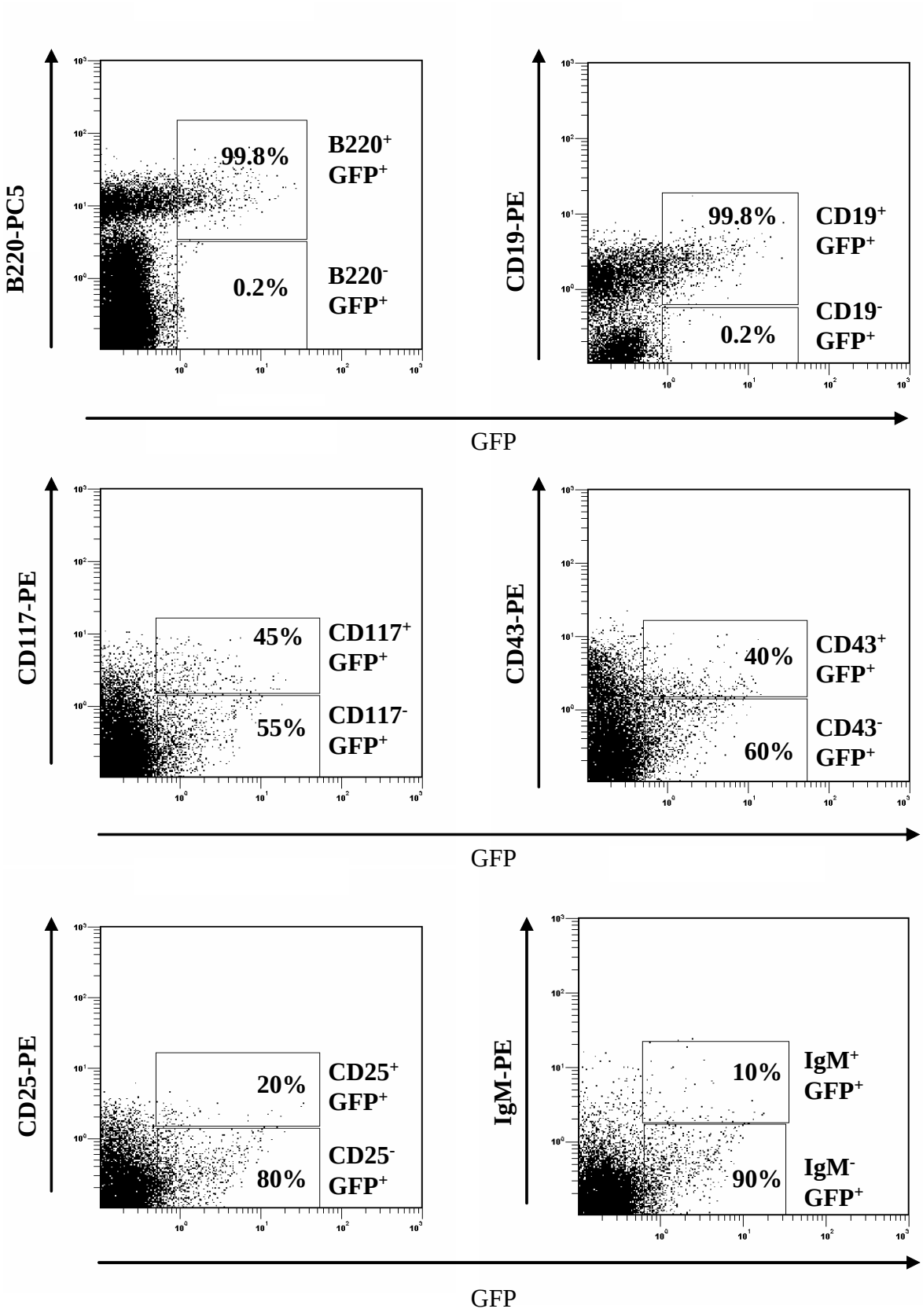

Figure 2: Characterisation of GFP$^+$ cells in bone marrow of insulated Eμ-GFP transgenic mice.

Single-cell suspensions from bone marrow were washed and stained ($5x10^5$ cells per assay) with various antibodies (Southern Biotechnologies, Birmingham, AL): anti-B220 conjugated with SpectralRed (PC5), anti-CD117 (c-kit), anti-CD19, anti-CD25 and anti-IgM conjugated with phycoerythrin (PE). The emitted fluorescence of GFP was measured at 530 nm (fluorescein-isothiocyanate (FITC) band pass filter). Cells were analysed on a Coulter XL apparatus (Beckman Coulter, Fullerton, CA). Representative results from six transgenic mice derived from two different founders are shown. Results from mice with 10 copies of the transgene are shown.