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Vieira *et al.* reply:

Jensen *et al.* analyzed the fetal livers of mice doubly deficient in IL-7 and the TSLP receptor (*Il7*$^{-/-}$*Tpte2*$^{-/-}$ mice) at embryonic day 17.5. They conclude, in contrast to our conclusions[1,2], that the cytokine TSLP is not important in IL-7-independent B cell lymphopoiesis. They base their conclusion on the apparent observation that the number of precursor B cells is identical in the fetal livers of *Il7*$^{-/-}$ and *Il7*$^{-/-}$*Tpte2*$^{-/-}$ mice at embryonic day 17.5. Additionally, they demonstrate that, as expected[3,4], single-knockout mice deficient only in the TSLP receptor show no obvious impairment in fetal liver lymphopoiesis at embryonic day 17.5.

Their analysis of pro–B cells and pre–B cells relies on gating for B220^{+} cells distinguished by the amount of CD43 expression (although this separation seems fairly arbitrary in their figure). This definition excludes the population of B220$^{\text{lo–neg}}$ B cell precursors described before[5] and is problematic for two reasons. First, CD19^{+}B220$^{\text{lo–neg}}$ precursors are found in fetal liver[5], are more highly represented in the fetal livers of *Il7*$^{-/-}$ mice than in controls by embryonic day 15 (**Fig. 1**) and give rise to B lymphocytes in response to TSLP at high frequency[5] (data not shown). Therefore, before any conclusions can be drawn from the data obtained using the mice analyzed by Jensen *et al.*, it is essential to know whether this population of CD19^{+} B220$^{\text{lo–neg}}$ pro–B cells is reduced when TSLP signaling is impaired. Second, no information is provided on the frequency at which the cells identified by Jensen *et al.* as B cell precursors were capable of generating B lymphocytes, either *in vitro* or *in vivo*. Direct comparison of such frequencies in *Il7*$^{-/-}$ and *Il7*$^{-/-}$*Tpte2*$^{-/-}$ mice with identical genetic backgrounds is necessary. More detailed analysis of the double-knockout mice, including data obtained at later time points, is therefore needed before it is known whether or not pro–B cells are much lower in number in such mice compared to *Il7*$^{-/-}$ mice. Given these issues, the conclusion of Jensen *et al.* is unsupported.

Furthermore, we are of the opinion that their conclusion that "Flt3L rather than TSLP is the key regulator of IL-7-independent B lymphopoiesis" would be firmly grounded only if fetal liver precursors were able to differentiate into B lymphocytes in the presence of Flt3L and in the absence of IL-7 and TSLP. However, pro–B cells do not differentiate into B cells at any detectable frequency when cultured with Flt3L alone (data not shown). Because Flt3 is not detected on pro–B cells, it seems likely to us that the small number of B lineage cells that are nevertheless generated in the combined absence of IL-7 and TSLP was due to the action of Flt3L on precursors before the pro–B cell stage. Nevertheless, fetal liver hematopoietic stem cells also do not form B cell colonies in response to Flt3L alone (data not shown).

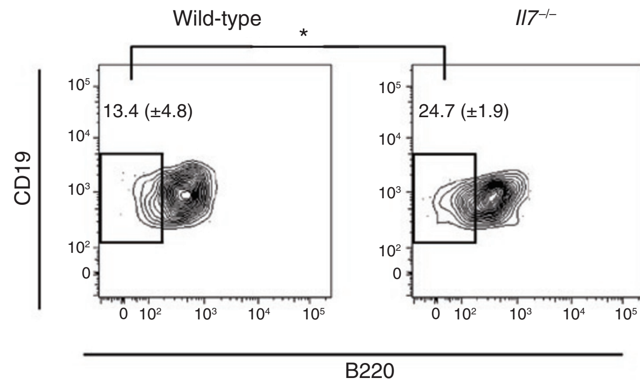

Figure 1 Expression of B220 by CD19^{+} fetal liver cells. Flow cytometry of fetal liver cells at embryonic day 15, stained with antibodies to CD19, CD43 and B220, showing coexpression of CD19 and B220 by gated CD19^{+}CD43^{+} cells. Numbers above outlined areas indicate percent B220$^{\text{lo–neg}}$ cells as a fraction of CD19^{+} cells (mean ± s.d.; five fetuses per group). *, $P = 0.0012$.

Whether or not the fetal livers of doubly deficient (*Il7*$^{-/-}$*Tpte2*$^{-/-}$) mice at embryonic day 17.5 have fewer pro–B cells than do singly deficient (*Il7*$^{-/-}$) mice, our work showed that when TSLP signaling is present (as in γc-deficient or IL-7-deficient mice), ten times more B lymphocytes are generated than when only Flt3L signals are available[1]. In addition, other published data[4] support our interpretation. That study found that the residual population of splenic B220$^{\text{hi}}$ B lymphocytes present in *Jak3*$^{-/-}$ mice (lacking IL-7 signaling) is absent when the TSLP receptor is additionally knocked out in these mice, whereas the population of bone marrow B220$^{\text{lo}}$ cells (comprising natural killer, dendritic and other non–B lineage cells[6]) is unaffected[4]. As Jak3 is involved in non-IL-7 signaling pathways, Jensen *et al.* could exclude the possibility of their involvement by simply counting the B cells in adult *Il7*$^{-/-}$*Tpte2*$^{-/-}$ mice and comparing that to the number of B cells in *Il7*$^{-/-}$ mice of identical genetic background. Notably, by embryonic day 17.5, there is a statistically significant reduction in the number of B lymphocytes in the fetal livers of *Il7*$^{-/-}$*Tpte2*$^{-/-}$ mice relative to that of *Il7*$^{-/-}$ mice.

We therefore see no reason at this point to modify our conclusion that TSLP is the main factor driving the IL-7-independent generation of B cells. Although our work has focused on the factors that drive the proliferation and differentiation of pro–B cells and pre–B cells, we have never proposed that TSLP, as a single factor, could generate B lymphocytes. Like Jensen *et al.*, we have analyzed mice doubly deficient in Flt3L and IL-7 and have found that Flt3L is indeed essential to the TSLP-driven generation of B cells (data not shown; *Flt3l*$^{-/-}$ mice from S.E. Jacobsen, Lund University). Other factors are certainly involved in and even essential for hematolymphopoiesis.

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COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

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