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1 *Trans*-silencing effect of the 3'RR immunoglobulin heavy chain enhancer on Igκ transcription
2 at the pro-B cell stage.

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21 By their impact on nuclear organisation, enhancers are master regulators of cell fate.^{1,2} The
22 immunoglobulin heavy chain (IgH) locus undergoes numerous changes (such as transcription,
23 accessibility, DNA breaks and mutations) throughout B-cell differentiation. Several of these
24 events are controlled by the IgH 3' regulatory region (3'RR). The 3'RR is the master control
25 element of mature B-cell IgH transcription,³ somatic hypermutation (SHM),^{4,5} conventional
26 class switch recombination (CSR),⁶⁻¹¹ and locus suicide recombination (LSR).¹² In contrast
27 the 3'RR was expected to be dispensable for V(D)J recombination.^{13,14} During B-cell
28 development, the heavy and light chain loci are poised for their VDJ and VJ rearrangements,
29 respectively. The IgH locus rearranges first with D-J joining at the pro-B-cell stages followed
30 by V-DJ joining at the pre-B-cell stage. The Igk locus is poised for VJ rearrangements at the
31 pre-B cell stage. A transient association (*trans*-mediated by Igk enhancer elements) between
32 IgH and Igk loci has been demonstrated at the pre-B cell stage.^{15,16} Recent, unexpected and
33 novel findings reported that the 3'RR acts as a *cis* transcriptional silencer of sense and
34 antisense germinal V, D and J transcription at the pro-B cell stage.^{17,18} In light of these
35 intriguing 3'RR features, we undertook the current study to determine if such a *trans* silencing
36 effect could also be found on Igk transcription.

37 Our research has been approved by our local ethics committee review board (Comité Régional
38 d'Ethique sur l'Expérimentation Animale du Limousin, Limoges, France) and carried out
39 according the European guidelines for animal experimentation. Pro-B cell experiments were
40 performed with RAG-deficient (RAG^{-/-}) and double 3'RR⁶-RAG-deficient (Δ 3'RR-RAG^{-/-})
41 mice developed in our animal facility. Femoral pro-B cells were recovered with the
42 EasySepTM mouse B-cell isolation Kit (STEMCELL Technologies, France). RNA was
43 extracted using Trisol (ThermoFisher Scientific) according to manufacturer's instructions.
44 Two pooled RNA samples (from four to six mice) were obtained for each genotype. RNA
45 libraries were obtained using TruSeq Stranded Total RNA with Ribo-Zero Gold (Illumina),

46 according to manufacturer's instructions. RNAseq experiments were done by the genomics
47 platform of Nice Sophia Antipolis as previously reported.^{7,8,18} Data were deposited in Gene
48 Expression Omnibus under the accession number GSE117449. Mature B-cells (CD43⁻
49 splenocytes) were obtained from four 129 *wt* mice (Charles Rivers Laboratories, France) and
50 four $\Delta 3'$ RR mice (in a 129 background) before and after 48h of *in vitro* stimulation (1×10^6
51 cells per ml in RPMI 1640 with 10% fetal calf serum) with 5 μ g/ml LPS. Two pooled RNA
52 samples (from two mice each) were obtained for each genotype. RNAseq experiments were
53 done as above and RNAseq data were deposited with the accession number GSE90760.

54 Femoral pro-B cells were isolated from RAG^{-/-} and $\Delta 3'$ RR-RAG^{-/-} mice to explore potential
55 transcriptional cross-talk between IgH and Ig κ loci in immature B-cells. A schematic
56 representation of these two loci is reported in Figure 1A. RNAseq experiments led to an
57 unexpected novel finding. Deletion of the IgH 3'RR enhancer markedly enhanced sense and
58 antisense transcription of the Ig κ locus in *trans* in pro-B cells (Figure 1B). This effect was not
59 found in mice deficient for the IgH E μ enhancer ($\Delta E\mu$ -RAG^{-/-} mice); the E μ enhancer (located
60 in blue in Figure 1A) being the major control element for IgH VDJ recombination (data not
61 shown).² As a positive control we found no such *trans* effect on the Ig λ locus. We next
62 examined if this effect could be detected in mature B-cells. Deletion of the 3'RR had no *trans*
63 silencer (nor activator) effect on Ig κ (and Ig λ) transcription in resting and LPS-stimulated
64 splenocytes (Figure 1C and 1D). These results are expected since a close association between
65 the Ig κ and IgH loci has not been reported in mature B cells.

66 The concept of a pro-B 3'RR *cis*-mediated transcriptional silencing activity was first reported
67 (using RT-QPCR) by Braikia and coll,¹⁷ and recently confirmed (using RNAseq analysis) by
68 us.¹⁸ The current study is the first report of a *trans* silencing effect of the 3'RR. This effect is
69 found at the Ig κ locus known to have a feedback inhibition effect on the establishment of
70 allelic exclusion of the IgH locus in pre-B cells. The present study reinforces the concept of a

71 mutual crosstalk through enhancer/silencer effects between IgH and Igk loci during immature
72 B-cell stages. It is possible that the *trans* silencer effect of the 3'RR on Igκ transcription
73 would use the same mechanism as that of its *cis* silencing effect on transcription of V, D and
74 J segments of the IgH locus. The 3'RR *trans* silencing on the Igκ locus would be of interest to
75 prevent its usage until the end of IgH D-J recombination. Clearly the resolution of how the
76 3'RR mediates *trans* transcriptional silencing on the Igκ locus is an exciting challenge to
77 meet.

78

79 **Author contributions:** HI, NG, AS, FB, OAM, JCM and YD designed and performed
80 experiments and wrote the manuscript. YD obtained financial grants.

81

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153

154 **Legend to Figure**

155 **Figure 1: Influence of the 3'RR enhancer on Igk transcription in pro-B cells.**

156 A: Schematic representation of the IgH, Igk and Ig λ loci (not to scale). B: Igk and Ig λ sense
157 (top) and antisense (bottom) transcription in pro-B cells of RAG^{-/-} and RAG^{-/-} Δ 3'RR mice. C:
158 Igk and Ig λ sense and antisense transcription in resting splenocytes of 129 *wt* mice and
159 Δ 3'RR mice. D: Igk and Ig λ sense and antisense transcription in LPS-stimulated splenocytes
160 of 129 *wt* mice and Δ 3'RR mice. Same mice as in C. E: Quantitative representation for C κ and
161 C λ transcription (in reads per million). Error bars show extreme values of 2 independent
162 experiments. Same samples as in C and D

