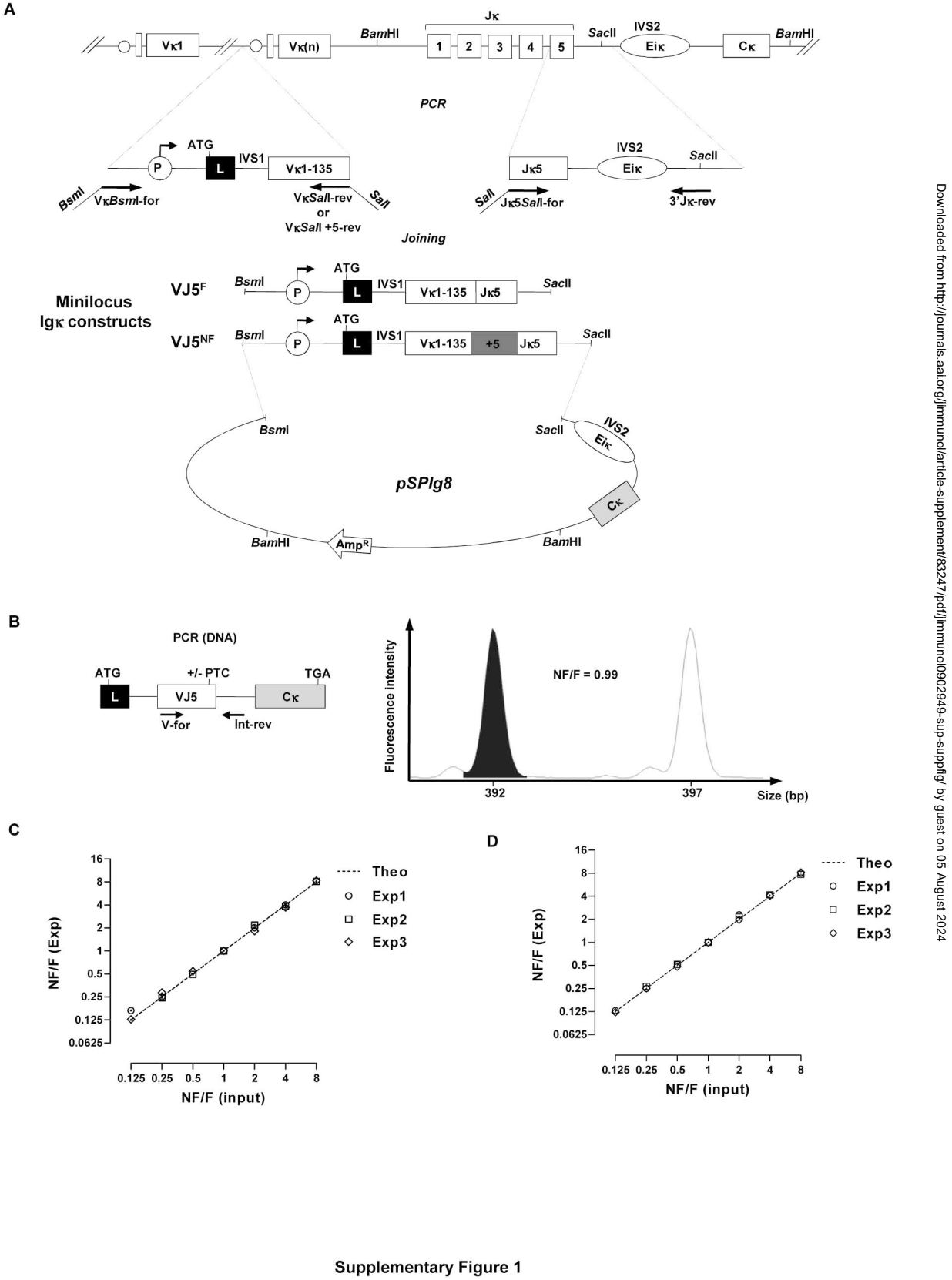
SUPPLEMENTARY INFORMATION

Supplementary Figure 1: Elaboration of functional and non-functional Igκ "mini-locus" constructs and quantification using capillary electrophoresis

A) In-frame and out-of-frame VJκ rearrangements were generated by joining artificially the Vκ1-135 and Jκ5 segments using a *Sal* I restriction site encoded by PCR primers. Amplification of Vκ1-135 segment was performed using the forward primer "Vκ*Bsm*I-for" (5'-AAGAAGAATGCTACAGAGTCAGAGTAAAGTCAA-3') and two different backward primers Vκ*Sal*I-rev (5'- TTGTCGACAGGAAAATGTGTACCTTGCCAGC-3') or Vκ*Sal*I+5-rev (5'-TTGTCGACCTGTGAGGAAAATGTGTACCTTGCCAGC-3'), generating in-frame junction ($VJ5^F$: functional) and out-of-frame junction ($VJ5^{NF}$: non functional) respectively. Amplification of Jκ5 segment was performed using Jκ5*Sal*I-for (5'-AAGTCGACTTCGGTGCTGGGACCAAGCTGGAG-3') and 3'Jκ-rev (5'-TGTTCTCTTCAGATTAGTG-3') primers. Both $VJ5^F$ and $VJ5^{NF}$ constructs were subsequently cloned into the pSPIg8 plasmid spanning 12.7kb of murine Igκ locus and, containing all the elements required for expression of Igκ light chains (enhancer Eiκ; Cκ exon; polyA) (35). B) In cell lines co-transfected with $VJ5^F$ and $VJ5^{NF}$ plasmids, PCR products were analysed by capillary electrophoresis in order to identify specifically both F (coloured) and NF constructs. To check whether equimolar ratios of both constructs were stably integrated in transfected cells, determination of F and NF peak areas (NF/F) was systematically performed on genomic DNA. Validation of this method was performed by mixing different amounts of $VJ5^F$ and $VJ5^{NF}$ constructs (NF/F_{input}), either from plasmid DNA (C) or cDNA (D), and by analysing the experimental NF/F ratios (NF/F_{exp}) after PCR (25 cycles).



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