

Supplementary Materials for

PreTCR and TCR $\gamma\delta$ Signal Initiation in Thymocyte Progenitors Does Not Require Domains Implicated in Receptor Oligomerization

Juliet Mahtani-Patching, Joana F. Neves, Dick John Pang, Kostadin V. Stoenchev, Ana M. Aguirre-Blanco, Bruno Silva-Santos, Daniel J. Pennington*

*To whom correspondence should be addressed. E-mail: d.pennington@qmul.ac.uk

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The PDF file includes:

Fig. S1. pT α chains that lack regions implicated in preTCR oligomerization initiate signaling that complements pT α deficiency.

Fig. S2. Signal initiation from TCR $\Delta I\gamma\Delta I\delta$ induces the expression of $\gamma\delta$ cell signature genes.

Fig. S3. A truncated TCR γ chain lacking both extracellular Ig-like domains pairs with pT α to initiate signaling.

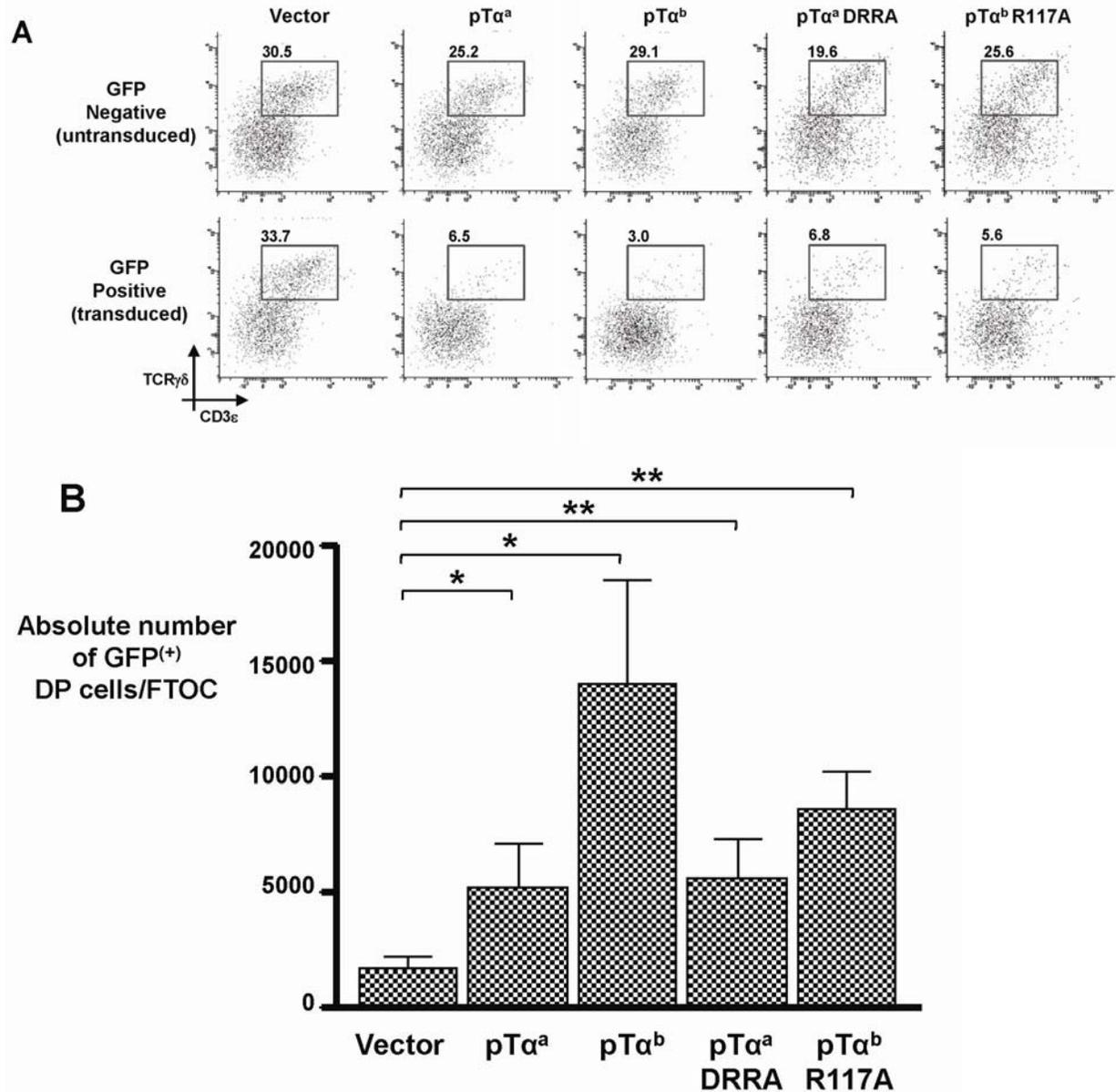


Fig. S1. pTα chains that lack regions implicated in preTCR oligomerization initiate signaling that complements pTα deficiency. **(A)** Representative flow cytometric analysis after 8-day FTOC of E14 pTα^{-/-} thymocytes transduced with GFP-expressing retroviral vector alone or with GFP-expressing vector encoding pTα^a, pTα^b, pTα^aDRRA, or pTα^bR117A. The top row shows untransduced (GFP⁻) cells, whereas the bottom row shows transduced (GFP⁺) cells. The percentages of gated cells are indicated. **(B)** Bar chart of the absolute numbers of GFP⁺ pTα-deficient DP thymocytes after 5 to 11 days of FTOC (n = 8 experiments) after retroviral transduction with vector alone or with vectors encoding pTα^a, pTα^b, pTα^aDRRA, or pTα^bR117A. DP, CD4⁺CD8⁺ cells. **, $P \leq 0.01$; *, $P \leq 0.05$.

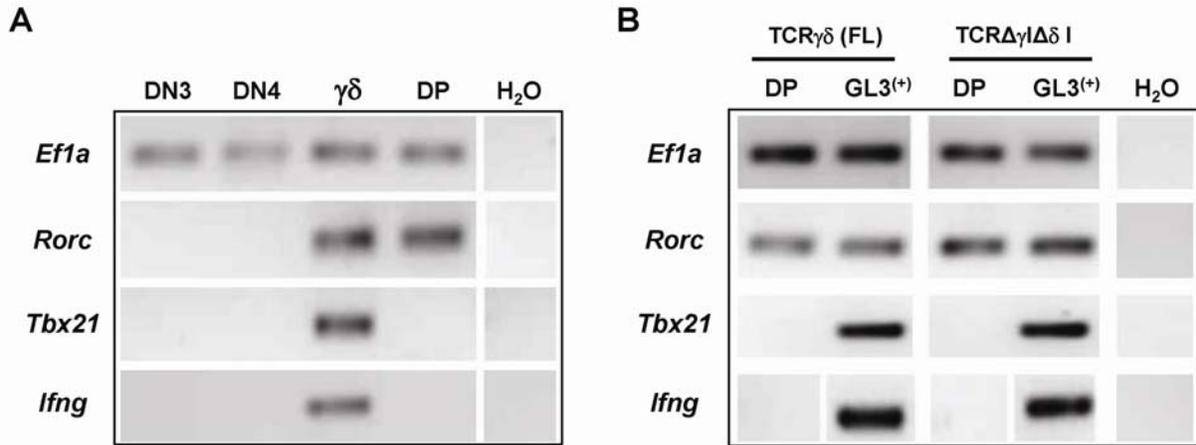


Fig. S2. Signal initiation from TCR $\Delta I\gamma\Delta I\delta$ induces the expression of $\gamma\delta$ cell signature genes. Multiplex PCR analysis of 200 cells for the expression of *Ef1a*, *Rorc* (which encodes ROR γ t), *Tbx21* (which encodes T-bet), and *Ifng* (which encodes IFN- γ) in sorted (A) DN3, DN4, $\gamma\delta$, and DP thymocytes from wild-type C57BL/6 mice, (B) CD4⁺CD8⁺ DP and GL3⁺ thymocytes generated from RAG-2^{-/-} E14 thymocytes transduced retroviruses encoding either full-length TCR $\gamma\delta$ (FL) or variable domain truncated TCR $\Delta I\gamma\Delta I\delta$ after 7 days in FTOC.

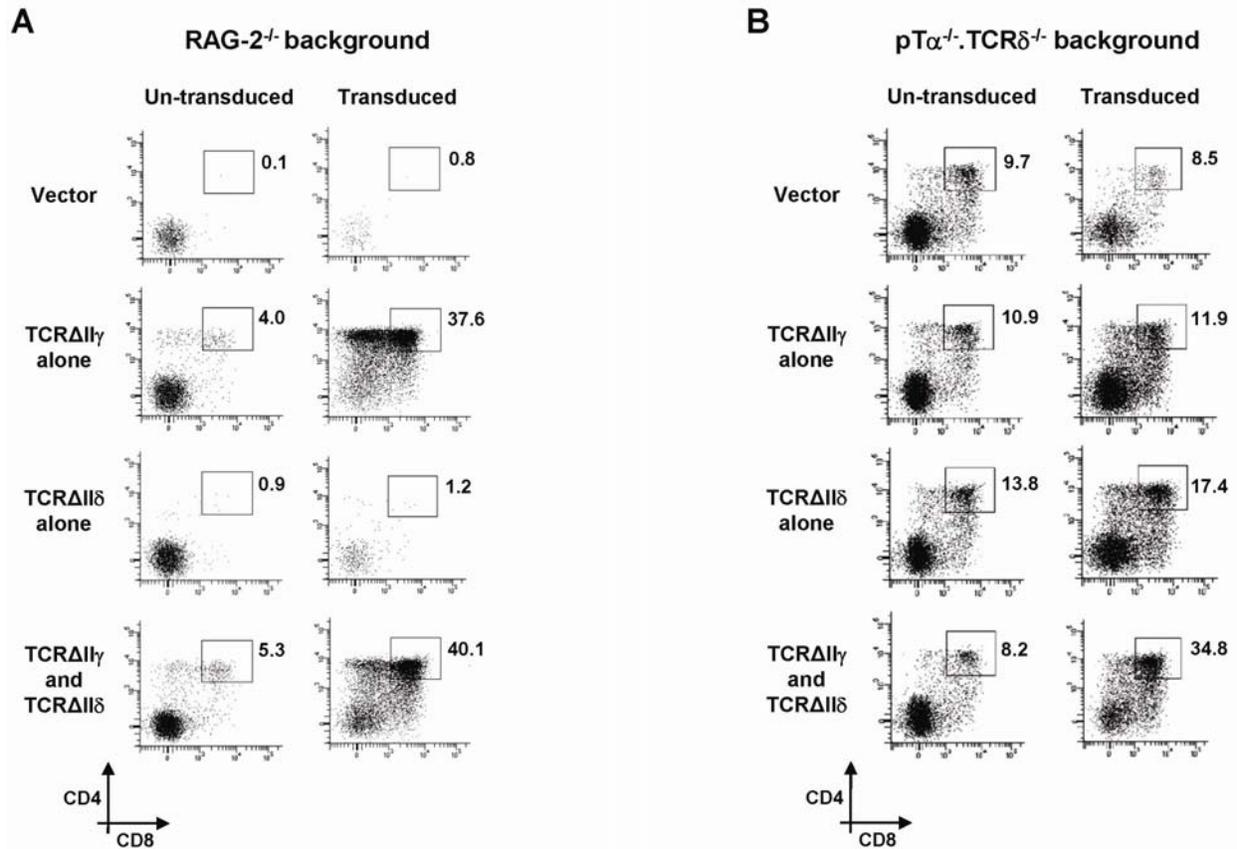


Fig. S3. A truncated TCR γ chain lacking both extracellular Ig-like domains pairs with pT α to initiate signaling. Representative flow cytometric analysis after 7 days of culture on OP9-DL1 cells of E14 (**A**) RAG-2^{-/-} or (**B**) pT α ^{-/-}.TCR δ ^{-/-} thymocytes transduced with GFP-expressing retroviral vector alone or with GFP-expressing retroviral vector encoding the TCRΔI γ or TCRΔI δ chains, as indicated. The percentages of gated cells are shown.