# European Journal of Immunology

## Supporting Information

## for

## DOI 10.1002/eji.201444442


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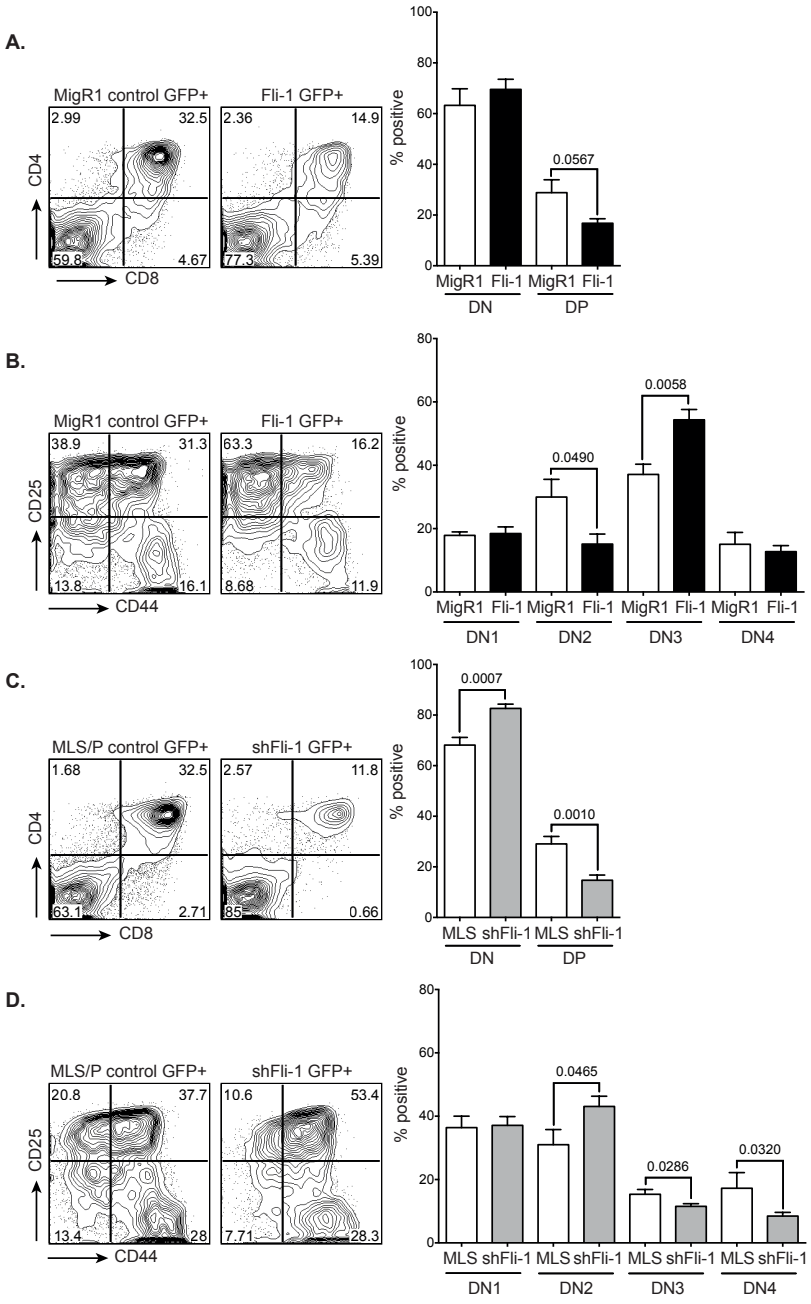
**Fli-1 regulates the DN2 to DN3 thymocyte transition and promotes $\gamma\delta$ T-cell commitment by enhancing TCR signal strength**

## Supporting Information

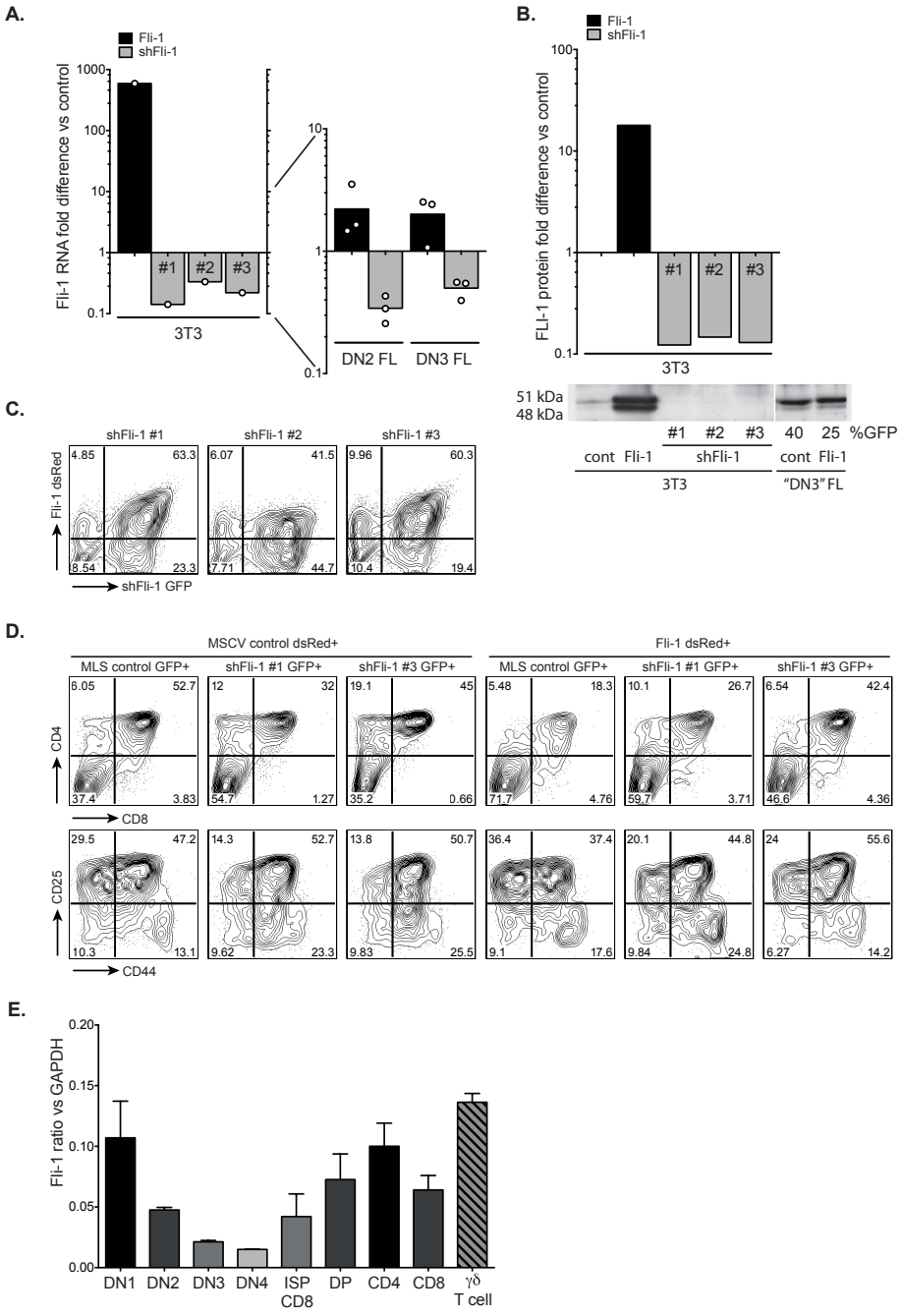
## Figure 1




Supporting Information Figure 1. Longer-term effects of Fli-1 overexpression and Fli-1 knockdown on in vitro T-cell development.

GFP$^+$ MigR1 control, Fli-1 and shFli-1 transduced FL cells were grown on OP9-DL1 cells for 12 days and analysed for expression of T-cell markers by flow cytometry. A. GFP$^+$-gated analysis of CD4 and CD8 expression (DP analysis) and B. Lineage negative-gated GFP$^+$ DN1-4 populations as determined by CD25 and CD44 expression (DN analysis) in control and Fli-1 overexpressing cells. Flow cytometry plots are representative of 5 independent experiments. Data in graphs are represented as mean + SEM of 5 independent experiments. C and D. DP and DN analysis in control and Fli-1 knockdown cells. Flow cytometry plots are representative of 9 independent experiments. Data in graphs are represented as mean + SEM of 9 independent experiments. *p* values as indicated. Unpaired two-tailed student's t-test.

## Figure 2




Supporting Information Figure 2. Levels and specificity of Fli-1 overexpression and Fli-1 knockdown and endogenous levels of Fli-1.

A. Fli-1 mRNA levels analysed by QRT-PCR in Fli-1 overexpressing and Fli-1 knockdown 3T3 cells 6 days after transduction and in sorted GFP$^+$ DN2 and DN3 thymocytes 4 days after transduction compared to control transduced cells (in thymocytes only shFli-1 #1 or #3 were used; 3 independent experiments). B. Fli-1 protein expression examined by Western blot in transduced 3T3 cells (quantification on top) and sorted DN3 cells transduced with MigR1 control or Fli-1 18 days after transduction (GFP percentages of both populations are indicated). C. Fli-1 dsRed overexpressing 3T3 cells co-transduced with three different Fli-1 knockdown constructs (shFli-1 #1 and 3 target the 3'UTR, #2 targets the Fli-1 ORF). D. MLS control and shFli-1 #1 and 3 transduced GFP$^+$ DN2 cells were sorted, transduced with MSCV dsRed control or Fli-1 dsRed and then analysed 12 days later for DP and DN progression (representative of > 3 experiments). E. Expression of Fli-1 mRNA in thymocyte subsets sorted from wild type thymi (mean + SEM of Fli-1 vs. GAPDH of 3 or 4 independent experiments).

## Materials and Methods

### Western Blot analysis

Cells were lysed in RIPA buffer (25 mM Tris-Cl [pH 7.6], 150 mM NaCl, 1% NP40, 1% sodium deoxycholate, and 0.1% SDS) with protease inhibitor (Complete Mini EDTA free protease inhibitor tablets, Roche Diagnostics, Castle Hill, NSW, Australia) and equal amounts (20μg) of total proteins per sample were separated via 10% SDS-PAGE and transferred to Immobilon-P (PVDF) transfer membrane (Millipore, North Ryde, NSW, Australia) for Western blotting. Proteins were detected using primary antibodies against FLI-1 (sc-356, Santa Cruz, CA USA) and beta-Actin (A5316, Sigma-Aldrich, Castle Hill, NSW, Australia) and secondary HRP conjugated antibodies followed by visualisation using ECL reagents (Santa Cruz, CA USA).