Supplementary Figure S1. Levels of TCR expressed on $IK^{-/-}$ and $IK^{+/+}$ H-Y and DO11.10 ζTg thymocyte subsets. A. Thymocytes were stained with anti-CD4-APC, anti-CD8α-PE and anti-TCRβ-FITC. Average mean fluorescence intensity (MFI) of anti-TCRβ-FITC staining in double negative (DN), double positive (DP) CD4 single positive (CD4 SP) and CD8 single positive (CD8 SP) subsets is shown. SEM and sample size (n) are also shown. N/A= not applicable. Statistical analyses were performed using paired student T tests and the results are reported in the text where applicable. B. A representative histogram of anti-TCRβ staining in DP sub-population in $IK^{-/-}$ DO11.10 ζ1Tg (IK-/-, black line) vs. $IK^{+/+}$ DO11.10 ζ1Tg (IK +/+, shaded) thymuses.

Supplementary Figure S2. CD8 SP cells that develop in the Ik-/- DO11.10 ζ1 Tg mice have upregulated surface expression of TCRβ**.** Thymocytes from $IK^{-/-}$ DO11.10 ζ1Tg mice were stained with anti-CD4-APC, anti-CD8α-PE and anti-TCRβ-FITC. A table showing the average percentage and absolute number ($x10^{-6}$) of CD8 SP cells as well as the average Mean Fluorescence Intensity (MFI) of anti-TCRβ-FITC staining in DP and CD8 SP thymocytes is shown (lower). SEM and sample size (n) are also shown. A representative histogram of anti-TCRβ staining in DP (shaded) and CD8 SP (black line) thymocytes is shown (upper).

Supplementary Figure S3. CD4 SP thymocytes that develop with reduced TCR signaling potential in the absence of Ikaros are exported to the periphery. Splenocytes from 3-4 wk old $IK^{+/+}$ and $IK^{-/-}$ DO11.10 ζTg mice were stained with anti-CD4-APC, anti-CD8α-PE and anti-TCRβ-FITC. A. Average percentage (left) and absolute number (right) of $CD4^{+}$

T cells present in the spleens of $IK^{+/+}$ (filled) and $IK^{-/-}$ (open) ζTg mice. Error bars represent the SEM. In order to eliminate background staining only TCRβ+ cells were included in the analyses of % and absolute # of CD4 SP T cells in the $IK^{+/+}$ and $Ik^{-/-}$ DO11.10 ζ0, ζ1 and ζ3 spleens. Such an analysis was not possible for $IK^{+/+}$ and $IK^{-/-}$ $\zeta^{-/-}$ spleens due to the lack of TCR surface expression. B. Representative flow cytometry plots of $IK^{+/+}$ (upper) and $IK^{-/-}$ (lower) splenocytes.

Supplementary Figure S4. Ikaros is not required for upregulation of CD40L by mature T cells in response to PMA and ionomycin activation. Thymocytes from $IK^{-/-}$ and $IK^{+/+}$ mice were activated with PMA and ionomycin followed by staining with anti-CD4-APC, anti-CD8α-FITC and anti-CD40L-PE. Representative flow cytometry plots (left) of post-activation thymocytes with CD4 SP and DP gates that were used to analyze upregulation of CD40L are shown. Representative histograms (right) of anti-CD40L-PE staining (black line) compared to isotype control (shaded) in post-activation whole thymus, CD4 SP and DP thymocytes.

Supplementary Figure S5. Some phosphorylation events triggered by TCR signaling are altered in the absence of Ikaros..

$IK^{-/-}$ and $IK^{+/+}$ thymocytes were incubated with anti-CD3ε monoclonal antibody (hamster IgG isotype). Goat anti-hamster IgG was then added to induce TCR cross-linking for the indicated amounts of time. 50 μg of protein extract prepared from these cells were separated by gel electrophoresis on 8% SDS-polyacrylamide gels, transferred to PVDF membrane and immunoblotted with anti-phosphotyrosine (for analysis of src family

kinase pathways)(A) and anti-phospo-Erk (for analysis of MAPK pathway)(B). Anti-HDAC and anti-Erk were used as loading controls.