**Supplementary Fig. 1.**  
(A) The data shows binding of purified DL4-IgG fusion protein to immature DN4 (CD25⁻CD44⁻) thymocytes taken immediately *ex vivo* from normal control mice, relative to a control stain for background binding of secondary antibodies.  
(B) Data show comparison of relative cell size, based on flow cytometric analysis of forward scatter, between region A and region B gated DN4 thymocytes taken immediately *ex vivo* from normal control mice. Data is representative of two independent experiments.

**Supplementary Fig. 2.**  
(A) The data shows flow cytometry for surface expression of CD71 in sorted DN3 (CD25⁺CD44⁻) wild type thymocytes co-cultured on OP9-DL1 monolayers for 24 hours, with regions A and B indicating cells expressing low and high levels of CD71 respectively.  
(B) The data shows cell size based on flow cytometric analysis of forward scatter of sorted DN3 (CD25⁺CD44⁻) wild type thymocytes co-cultured on OP9-DL1 monolayers for 24 hours that are expressing either low (region A-gated) or high (region B-gated) levels of CD71.  
(C) The data shows flow cytometry for surface expression of CD98 in sorted DN3 (CD25⁺CD44⁻) wild type thymocytes co-cultured on OP9-DL1 monolayers for 24 hours, with regions A and B indicating cells expressing low and high levels of CD98 respectively.  
(D) The data shows cell size based on flow cytometric analysis of forward scatter of sorted DN3 (CD25⁺CD44⁻) wild type thymocytes co-cultured on OP9-DL1 monolayers for 24 hours that are expressing either low (region A-gated) or high (region B-gated) levels of CD98.
Supplementary Fig. 3.
A diagram illustrating the generation of T-PDK1\textsuperscript{L155E/+} (PDK1\textsuperscript{L155E/fl\Deltaneo}Lck-Cre\textsuperscript{+}) mice is shown. PDK1\textsuperscript{L155E/fl\Deltaneo} mice, which contain the L155E mutation and an otherwise normal (fl\Deltaneo) allele, were bred with mice expressing the Cre recombinase under the control of the p56\textsuperscript{\textit{ck}} proximal promoter, resulting in a T cell specific deletion of PDK1 from one allele such that in these mice T cells express only the PDK1 L155E mutant. The black boxes represent exons, continuous lines represent introns and triangles represent \textit{loxP} sites. The position of the L155E knock-in mutation in the PIF domain of PDK1 is denoted by an asterix (*). PCR primers and their locations are indicated with arrows.

Supplementary Fig. 4.
These data show the PCR products of two PCR reactions using sorted DP cells from T-PDK1\textsuperscript{L155E/+} (PDK1\textsuperscript{L155E/fl\Deltaneo}Lck-Cre\textsuperscript{+}) mice and mature peripheral T cells from control mice T-PDK1\textsuperscript{+/fl\Deltaneo} (PDK1\textsuperscript{+/fl\Deltaneo}) and T-PDK1\textsuperscript{+/-} (PDK1\textsuperscript{+/fl\Deltaneo}Lck-Cre\textsuperscript{+/-}) in each reaction. Data on the left shows the products of PCR reaction using p99 and p100 primers where a single 200 bp band indicates the presence of a WT or PIF (L155E) allele and a 250 bp band indicates the presence of a fl\Deltaneo allele. Data on the right shows the PCR reaction products using p80 and p100 primers. Under these conditions a PCR product is generated only when successful deletion of the fl\Deltaneo allele has occurred in Lck-Cre positive animals.