



**Figure 2.** Gating strategy used to characterize thymocytes from C57BL/6J mice by flow cytometry using an 11-color panel. From all events, thymocytes were distinguished living by their FSC/SSC properties (A). Doublets were excluded twice (B and C), followed by exclusion of dead cells (D). From alive cells, two subpopulations were characterized. One strategy is based on CD4+ and CD8 expression and considered double negative (CD4-CD8-, DN), double positive (CD4+CD8+, DP), simple positive (CD4-CD8+ and CD4+CD8-, SP) the thymocytes represented in graph H. On CD4+ cells, regulatory T cells were distinguished as FoxP3+ (Precursor Tregs) (I) and CD25+FoxP3+ (Thymic T regs) (J). TOX-1+ cells on DP cells is shown (K). The other strategy characterized CD4+ and CD8+ thymocytes on CD45+CD3+ cells (E, F, G). From these gates, the expression of PD-1 on CD8 is shown in graph L.