



Figure 3. Gating strategy used to analyze thymic stromal cells from C57BL/6J mice by flow cytometry using a 6-color panel. From all events, thymus cells were distinguished by their FSC/SSC properties (A). Doublets were excluded twice (B and C), followed by exclusion of dead cells (D). Alive cells were initially divided into CD45+ and CD45- (E). Hematopoietic antigen presenting cells were gated in CD45+ cells expressing MHC class II (IA-IE+) (F). Within CD45-, cells were recognized as TECs based on the CD326 expression (CD326+) (G). TECs were further characterized over the basis of (H) MHC class II expression levels (IA-IE) as MHCIIlow and MHCIIhigh, (I) FoxN1 expression, and (J) were further subdivided into mTEC and cTEC based on the CD205 expression as CD205- (mTECs) and CD205+ (cTECs).