

# Human iPSC-based artificial thymus as a new platform for *in vitro* T cell generation

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## Abstract

*In vitro* production of T cells has the potential for wide-ranging clinical applications in various immunodeficiencies and cancer. However, efficient generation of self-MHC restricted naïve T cells bearing diverse TCR repertoires has remained challenging. iPSCs have the potential to differentiate into any cell type with patient-specific MHC and near-unlimited proliferation capacity. Therefore, we established a human iPSC-based induction system to derive thymic epithelial cells (iTECs) – the major stromal component of the thymus supporting the development of T cells and assuring their self-MHC restriction – to reconstitute thymic function *in vitro*. When cultured with CD4/CD8 double-positive (DP) thymocytes, these iTECs can efficiently generate both CD4 and CD8 single-positive (SP) thymocytes with a diverse TCR repertoire similar to that found in the pediatric thymus. Most of these SP thymocytes are TCRab<sup>+</sup> and show conventional naïve T cell phenotypes. Furthermore, iTECs recapitulate the remarkable stromal cell diversity crucial for thymic function, including cells reminiscent of mature cortical TECs (cTECs) and heterogeneous medullary TEC (mTEC) populations that, at the transcriptomic level, are nearly indistinguishable from their primary counterparts in the human thymus. Our system presents a new platform for the *in vitro* production of T cells with diverse TCR repertoires for potential off-the-shelf regenerative and cancer therapies as well as basic immunological studies.