

ZsGreen reporter expression is specific to the GATA3-expressing cells

Flow cytometric analysis of ZsGreen reporter expression among various lymphoid and myeloid

cell population.



ZsGreen insertion does not disrupt endogenous GATA3 expression during T cell

development

A. Flow cytometric analysis of GATA3 protein expression during CD4 and CD8 committed thymocytes population.

B. Flow cytometric comparison of GATA3 levels during various stages of CD4 and CD8 double negative thymocyte population.



-10 ³ 0 10³ 10⁴ NKp46

ZsGreen expression by ILC subsets correlates well with GATA3 protein levels

A. Gating strategy for identifying different ILC subsets from intestinal lamina propria based on the transcription factor staining.

B. Flow cytometry histogram represents the GATA3 protein expression among various ILCs by transcription factor staining.

C. Gating strategy for identifying different ILC subsets from intestinal lamina propria based on cell surface marker staining.

D. ZsGreen expression among various ILC subsets analyzed by flow cytometry.



Normal induction of ZsGreen expression by GATA3-deficient CD4 T cells cultured under

Th2 conditions

A. Schematic diagram for naïve CD4 T cells cultured under Th2 polarized conditions in the presence of 4-HT to delete the exon 4 of the *Gata3* gene.

B. Flow cytometry histogram showing GATA3 protein levels and ZsGreen reporter expression by cells harvested at the end of culture.



ZsGreen continues to be expressed by the GATA3-deficient "ILC2s"

A. Schematic diagram for ILC2 isolation from the Gata3^{fl/fl}CreERT2 mice and Gata3^{ZsG-}

^{fl/fl}CreERT2 mice followed by 4-HT treatment to delete the exon 4 of the *Gata3* gene.

B. Flow cytometry histogram showing GATA3 protein levels and ZsGreen reporter expression

from ILC2s with or without 4-HT treatment.



GATA3-deficient "ILC2s" undergo apoptosis and decline over time in vitro

A. Schematic diagram represents co-culture of ILC2s isolated from IL-25-primed $Gata3^{ZsG-}$ ^{fl/fl}CreERT2 mice (CD45.2) and WT mice (CD45.1) with and without 4-HT to delete the exon 4 of *Gata3* gene.

B. Gating strategy for distinguishing *Gata3*^{fl/fl}CreERT2 ILC2s (CD45.2) from WT ILC2s (CD45.1) based on the congenic marker.

C. Flow cytometry data show the relative abundance of ILC2s after Gata3 exon 4 deletion.

D. The relative abundance of congenically marked ILC2s from C was counted, and the percentages were plotted.

E. Gating strategy for analyzing the apoptosis marker annexin V levels on cultured ILC2s.

F. Flow cytometry data for apoptosis analysis with ILC2s cultured for two days with 4-HT.

G. The percent of cells undergoing apoptosis (annexin V+) from figure F was counted, and the values were plotted.



GATA3-deficient "Th2" cells reduce in number over time in vitro

A. Schematic diagram shows the naïve CD4 T cells isolation from *Gata3*^{fl/fl}CreERT2 mice (CD45.2) and WT mice (CD45.1), and co-culturing them in 1:1 ratio under Th2 polarizing conditions followed by exon 4 deletion by 4-HT treatment.

B. Gating strategy for identifying the *Gata3*^{fl/fl}CreERT2 (CD45.2) Th2 cells from WT (CD45.1)
Th2 cells.

C. The relative abundance of WT (CD45.1) and GATA3-deficient (CD45.2) Th2 cells were analyzed by flow cytometry.

D. Values from C were plotted.