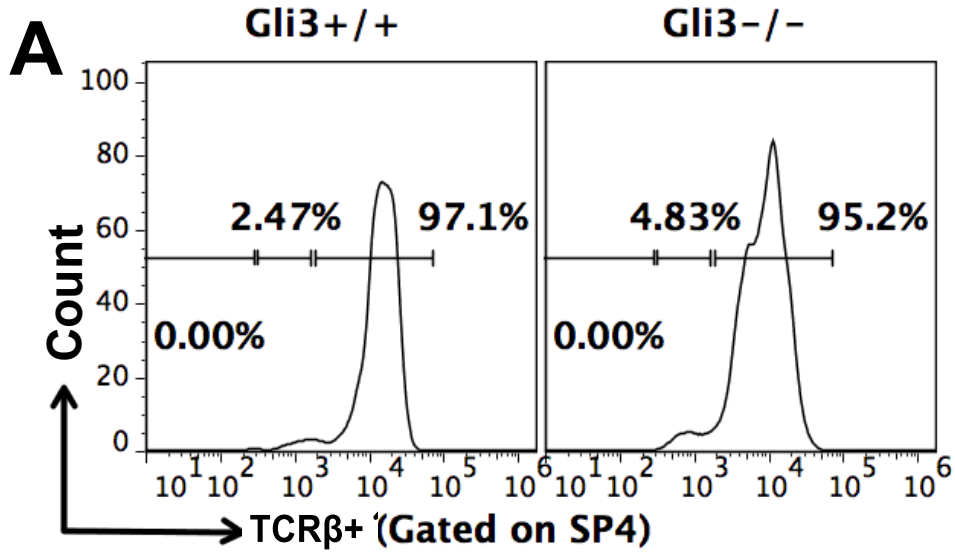
S1



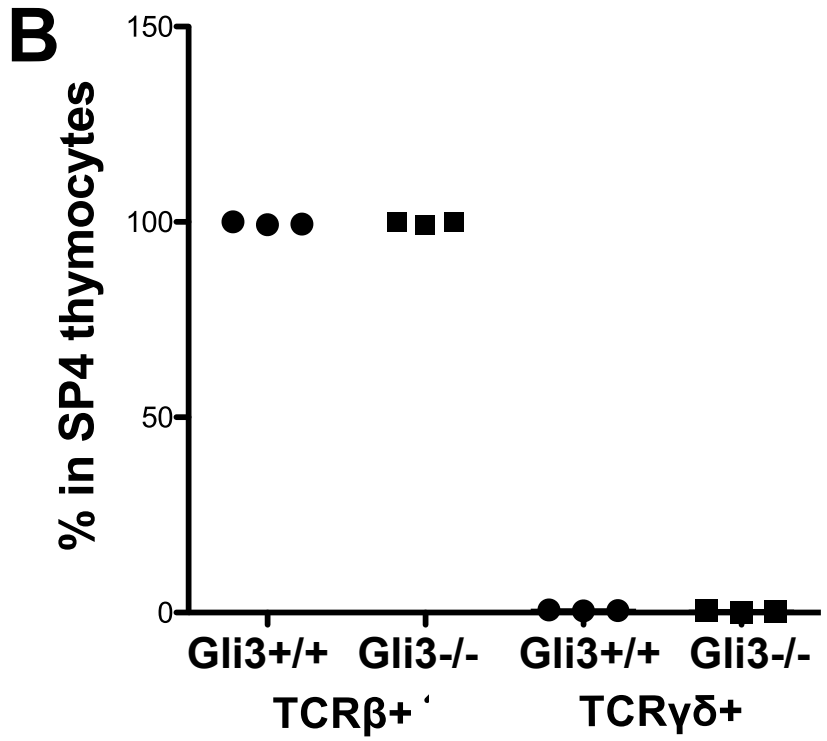


Figure S1: Expression of TCRβ and TCRγδ in WT and Gli3-deficient E17.5+4 Days FTOCs.

(A) Histograms showing the expression of TCRβ, gated on SP4 cells from Gli3+/+ and Gli3-/-. (B) Scatter plot showing the percentage of TCRβ+ and TCRγδ+ cells in the SP4 population from Gli3+/+ and Gli3-/- (n=3).

S2

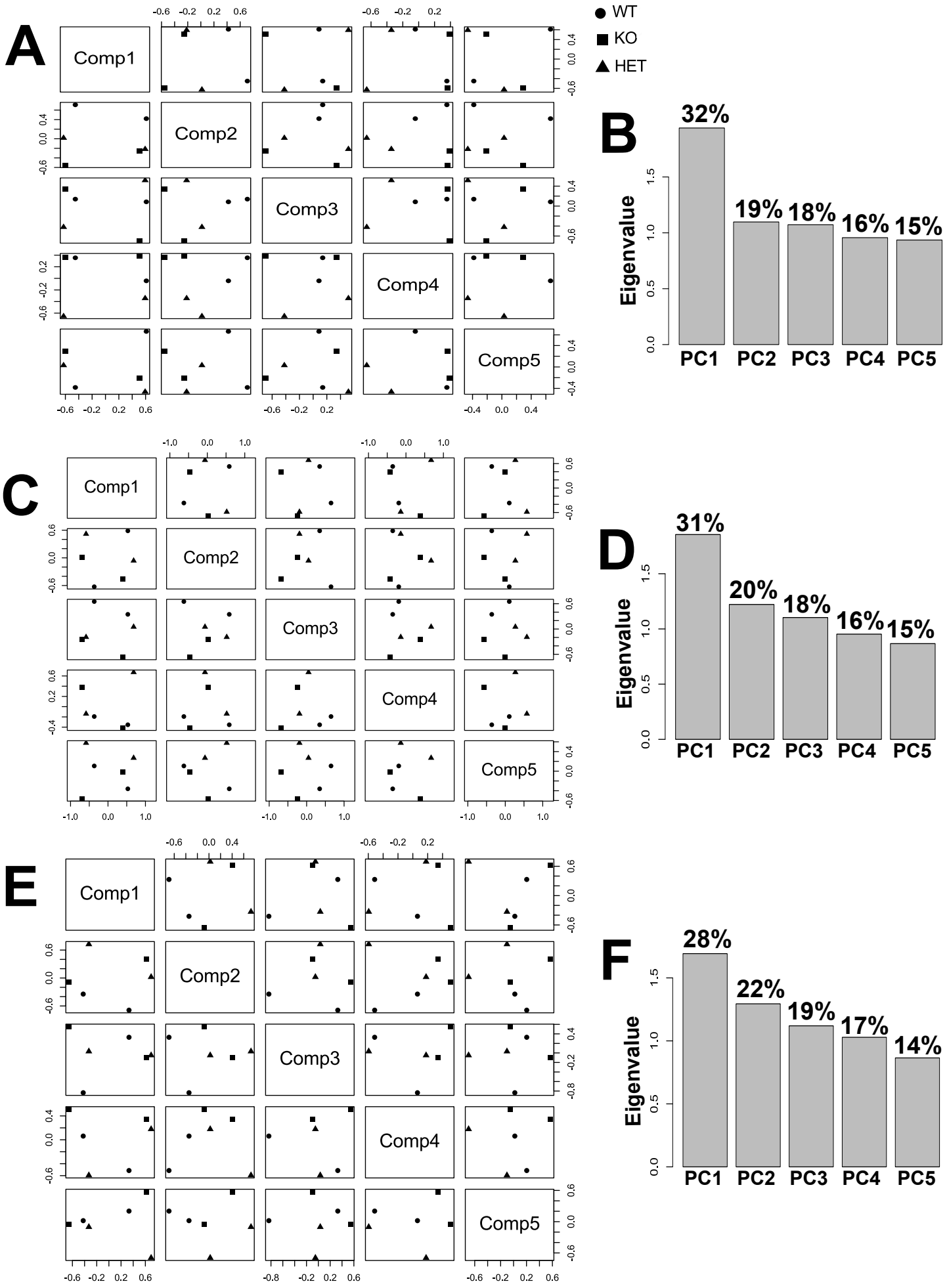


Figure S2: Principal Component Analyses of E18.5 WT and Gli3-mutant facs-sorted CD69-DP, CD69+DP and SP4 populations.

PCA of the CD69-DP (A), the CD69+DP (C) and SP4 (E) datasets. Eigenvalues and percentage variability associated with each principal component for CD69-DP (B), the CD69+DP (D) and SP4 (F).

Table S1: Summary of intersected DEG and PCA genes annotated in the heatmap (Figure 6) of known relevant function.

Gene Name	Function in Thymus	Reference
TCR repertoire selection Genes		
Egr1	Egr1 is rapidly upregulated after TCR stimulation and increased Egr1 expression increases thymic selection.	(Shao et al., 1997)
Egr2	Egr2-deficiency impairs positive selection of both CD4 and CD8 SP thymocytes. It also upregulates survival molecule Bcl-2 during positive selection allowing survival of positively selected thymocytes	(Lauritsen et al., 2008)
Nab1	The Nab family members interact with the Egr1/2 to regulate different function in thymocytes and T cells.	(Collins et al., 2008; Decker et al., 2003)
Nab2		
Itk	Itk is very importance for the efficient positive and negative selection of thymocytes.	(Andreotti et al., 2010)
Tox	TOX is important for the positive selection towards the CD4+ T cell lineage in the thymus	(Aliahmad et al., 2011)
Lef1	LEF-1 is important for the positive selection of CD4 SP thymocytes from the DP stage. Lef1 directly induce the master regulator of CD4 lineage commitment, ThPok.	(Steinke et al., 2014)
Pten	Loss of Pten leads to defects in negative selection	(Suzuki et al., 2001)
Socs1	Socs1 deficiency leads to impaired positive and negative selection in the thymus. Socs1 is important for CD4 T cell development.	(Catlett and Hedrick, 2005)
Id2	Id2 and Id3 allow CD8+ T cell development	(Jones-Mason et al., 2012)
Fas	Fas-FasL interaction mediates activation-induced cell death of mature postthymic T cells, allowing effective negative selection and elimination of autoimmune cells	(Kishimoto and Sprent, 1997)
CamK4	CaMK4 regulates the Ca2+-dependent gene transcription, and loss of CaMK4 in thymocytes impairs positive selection.	(Raman et al., 2001)
Cd6	Increase in CD6 expression allow DP thymocytes differentiate to a SPs.	(Singer et al., 2002)

Cd53	CD53 expression is correlated with positive selection. DP thymocytes expressing CD53 are undergoing selection or have just undergone selection.	(Puls et al., 2002)
TCR signal strength modulators		
Cd81	Loss of CD81 in thymocytes increases the TCR signal strength	(Cevik et al., 2012)
Cd5	High cell surface CD5 expression correlates with a stronger TCR signal and vice versa	(Azzam et al., 2001)
Nr4a1	The expression of the Nr4a family member is also correlated with increases in TCR signal strength.	(Moran et al., 2011; Nowyhed et al., 2015)
Nr4a3		
Hedgehog Signalling and Target Genes		
Ihh	Hedgehog protein expressed in DP cells	(Outram et al., 2009)
Bmp2	Hedgehog signalling target involved in T-cell development in thymus	(Hager-Theodorides et al., 2002; Outram et al., 2000)
Hoxa7	Homeobox (Hox) family members are targets of Hedgehog signalling and interact with various Hh family members to control developmental processes.	(Roberts et al., 1995)
H19	Hh signaling increases H19 expression	(Chan et al., 2014)
Kif13b	Kif13b promotes Shh signalling	(Schou et al., 2017)
Tgfb1	TGF-β-increases Shh signalling to induced fibrosis.	(Chung and Fu, 2013)
Itgav	Itgav is a target of Hedgehog signalling and can modulate Hedgehog signalling	(Kosinski et al., 2010; Zhou et al., 2013)

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Table S2: PCA Gene list with relevant PC scores and Intersected Gene list for each sorted thymocyte population.

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