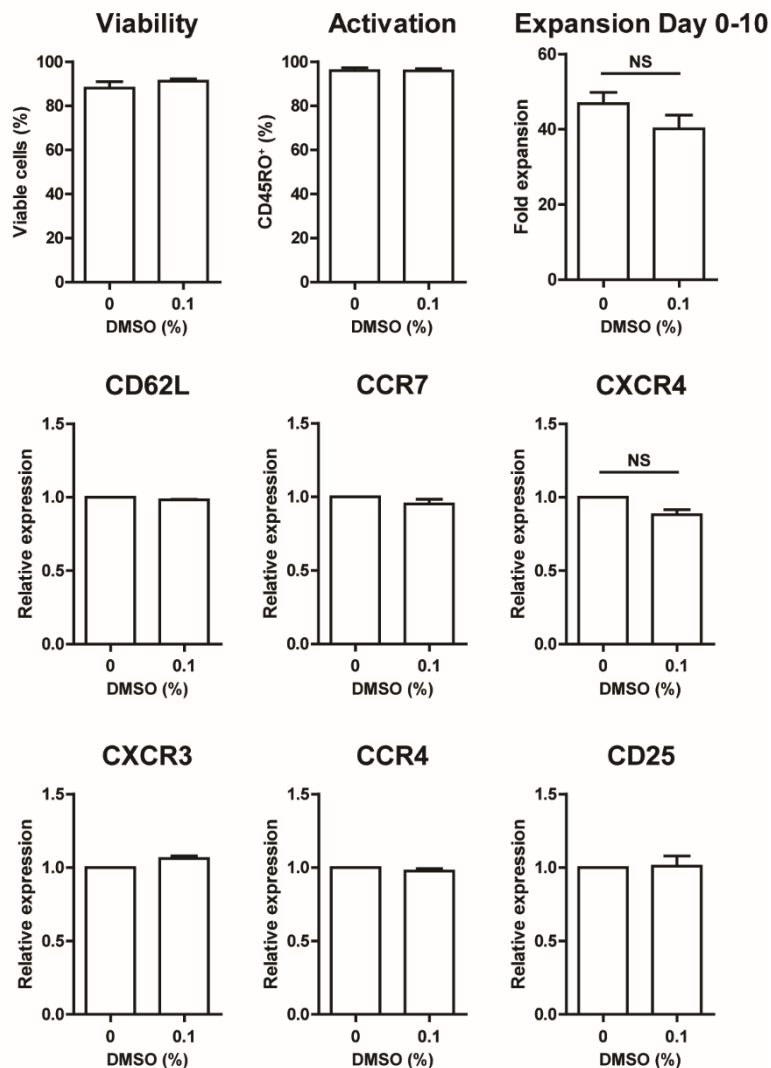


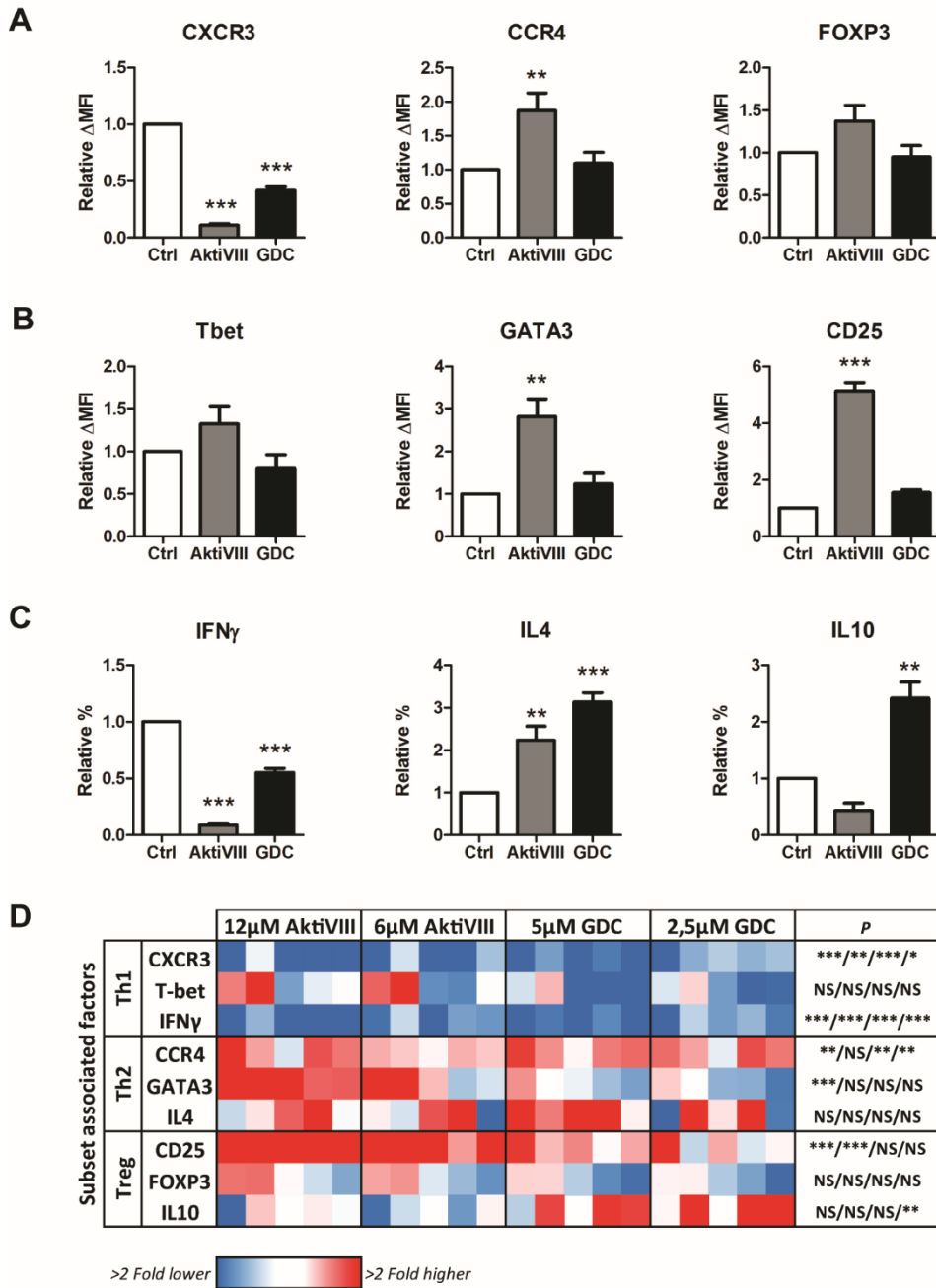
## Supplementary Figure 1



### Supplementary Figure 1 DMSO has no effect on viability, activation, expansion and differentiation of CD4<sup>+</sup> T cells

CD4<sup>+</sup> T<sub>N</sub> cells were stimulated with DCs in absence or presence of 0.1% DMSO and analyzed on day 10 for viability, activation, expansion, memory (CD62L, CCR7, CXCR4) and T cell subset-associated (CXCR3, CCR4, CD25) phenotype. Mean + SEM of 2 independent donors. Statistical analysis was performed using a two-tailed unpaired T test, no significant changes were found.

## Supplementary Figure 2

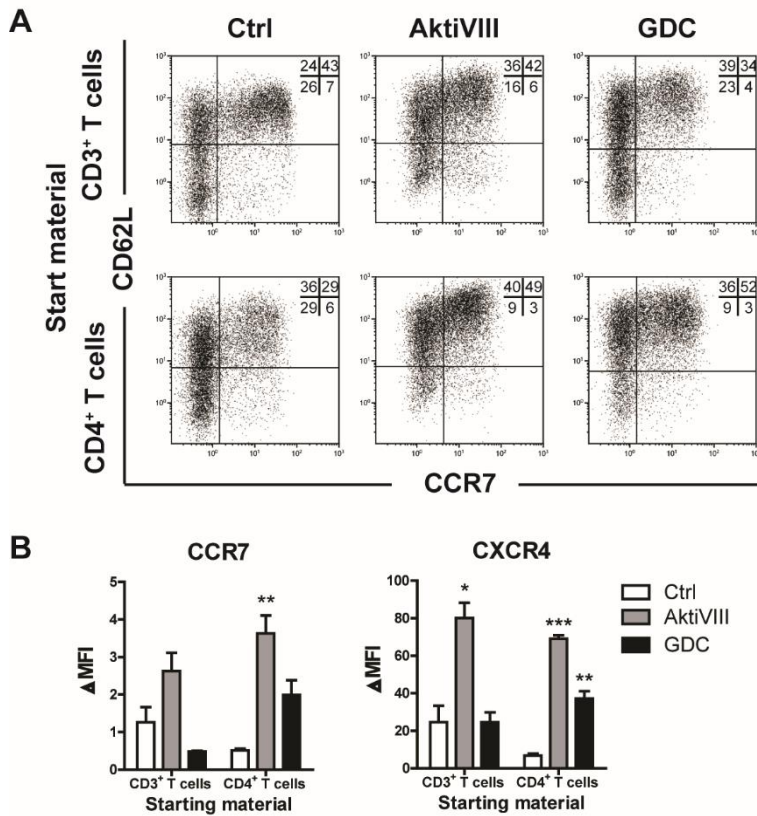


### Supplementary Figure 2 AKT-inhibition modulates CD4<sup>+</sup> T helper cell skewing

CD4<sup>+</sup> T<sub>N</sub> cells were expanded with allogeneic dendritic cells in presence of DMSO (Ctrl), AktiVIII or GDC for 10 days. (A-C) Relative (A) surface expression, (B) intracellular transcription factor expression and (C) cytokine production of CD3<sup>+</sup>CD4<sup>+</sup>CD45RO<sup>+</sup> T cells, after O/N restimulation with allogeneic dendritic cells in absence of AKT-inhibitors, corresponding to data shown in heat-map (Figure 2D, 18μM AktiVIII

and 10 $\mu$ M GDC). Mean+SEM shown for 4-5 independent donors. **(D)** Heatmap showing fold change compared to Ctrl condition of different T-helper cell associated parameters for 4-5 independent donors using 12 or 6 $\mu$ M AktiVIII and 5 or 2.5 $\mu$ M GDC. Grey = no data. Cytokine production was corrected for non-stimulated conditions. Statistical analysis was performed using Repeated Measures ANOVA followed by Bonferroni's Multiple Comparison Test of AKT-inhibited versus Ctrl T cells. \*\*\* $p$ <0.001, \*\* $p$ <0.01, \* $p$ <0.05.

### Supplementary Figure 3

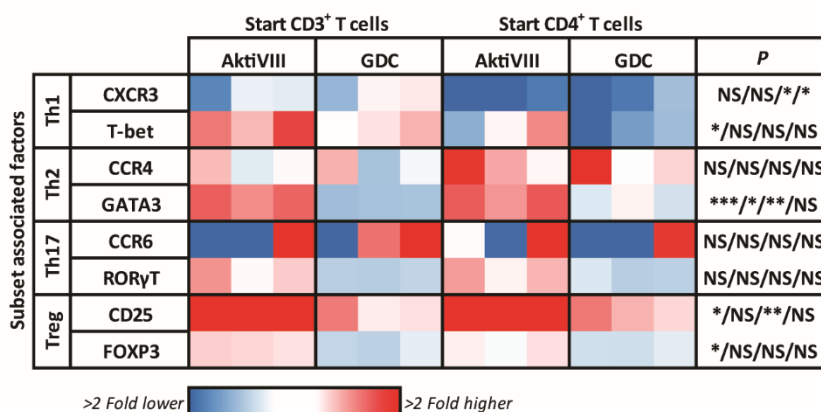


#### Supplementary Figure 3 Preserved memory differentiation of CD4<sup>+</sup> T cells by AKT-inhibition in CD3<sup>+</sup> T cell and CD4<sup>+</sup> T cell cultures

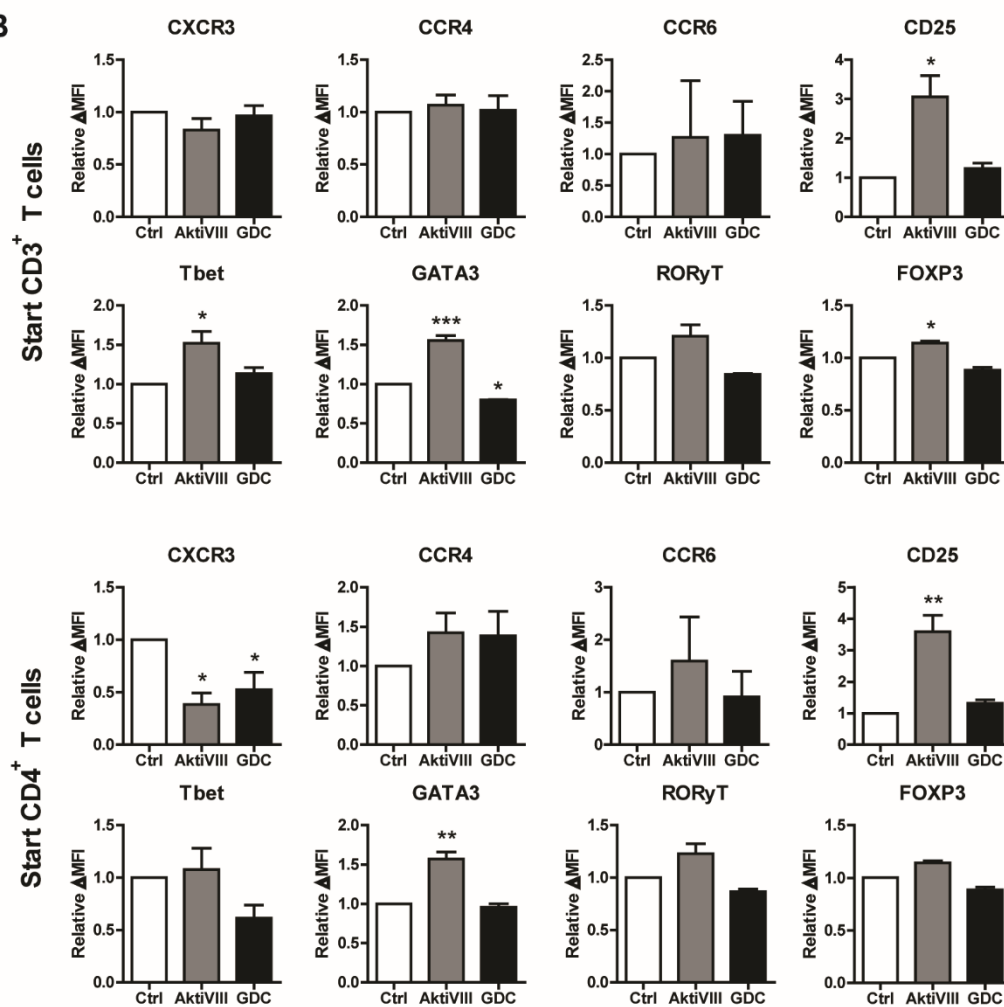
CD3<sup>+</sup> T cells or CD4<sup>+</sup> T cells were stimulated with allogeneic dendritic cells in presence of DMSO (Ctrl), AktiVIII (18μM) or GDC (10μM). CD4<sup>+</sup> T cell differentiation was analyzed on day 10. **(A)** Dotplots of CD62L and CCR7 expression gated on CD3<sup>+</sup>CD4<sup>+</sup>CD45RO<sup>+</sup> T cells of 1 representative donor. Gates were placed based on background staining, numbers represent percentage gated cells. **(B)** CCR7 and CXCR4 expression of 1 representative donor out of 3 donors, Mean+SD, n=2. Statistical analysis was performed using One-way ANOVA followed by Bonferroni's Multiple Comparison Test of AKT-inhibited versus Ctrl T cells within each starting population. \*\*\**p*<0.001, \*\**p*<0.01, \**p*<0.05.

## Supplementary Figure 4

**A**



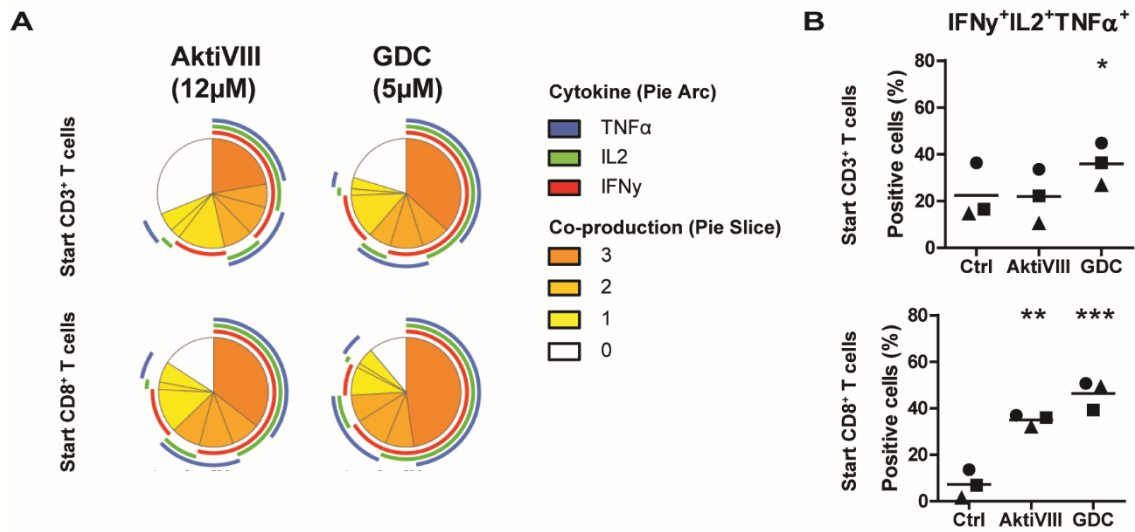
**B**



**Supplementary Figure 4 AKT-inhibition does not impede Th1 skewing of CD4<sup>+</sup> T cells in cultures with total CD3<sup>+</sup> T cells**

Total CD3<sup>+</sup> T cells or CD4<sup>+</sup> T cells were stimulated with allogeneic dendritic cells in presence of DMSO (Ctrl), AktiVIII (18μM) or GDC (10μM) for 10 days. **(A)** Heatmap and **(B)** statistical analysis showing fold change of expression of extracellular markers and transcription on AKT-inhibited compared to Ctrl conditions, gated on CD3<sup>+</sup>CD4<sup>+</sup>CD45RO<sup>+</sup> T cells within each starting material on day 10, mean+SEM of 3 independent donors. Statistical analysis was performed using Repeated Measures ANOVA followed by Bonferroni's Multiple Comparison Test of AKT-inhibited versus Ctrl T cells. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .

## Supplemental Figure 5



### Supplementary Figure 5 AKT-inhibition of allogeneic dendritic cell expanded CD8<sup>+</sup> T cells enhances polyfunctionality also at lower dosages

Total CD3<sup>+</sup> T cells or CD8<sup>+</sup> T cells were stimulated with allogeneic dendritic cells in presence of DMSO (Ctrl), AktiVIII (12  $\mu$ M) or GDC (5 $\mu$ M) for 10 days. Cells were restimulated in absence of AKT-inhibition for 7 days, and cytokine production was analyzed after O/N allogeneic dendritic cell stimulation. **(A)** Percentages IFN $\gamma$ -, IL2-, and TNF $\alpha$ -producing CD3<sup>+</sup>CD8<sup>+</sup> T cells, analyzed using SPICE software. The pie arcs depict the proportion of cells that produces a specific cytokine and pie slices the proportion of cells co-producing 0 to 3 different cytokines. **(B)** Percentages IFN $\gamma$ -, IL2-, TNF $\alpha$ -triple-producing CD3<sup>+</sup>CD8<sup>+</sup> T cells. Statistical analysis was performed using Repeated Measures ANOVA followed by Bonferroni's Multiple Comparison Test of AKT-inhibited versus Ctrl T cells within each starting population. \*\*\* $p$ <0.001, \*\* $p$ <0.01, \* $p$ <0.05.