# Human in vitro-induced regulatory T cells display Dlgh1 dependent and PKC-θ restrained suppressive activity

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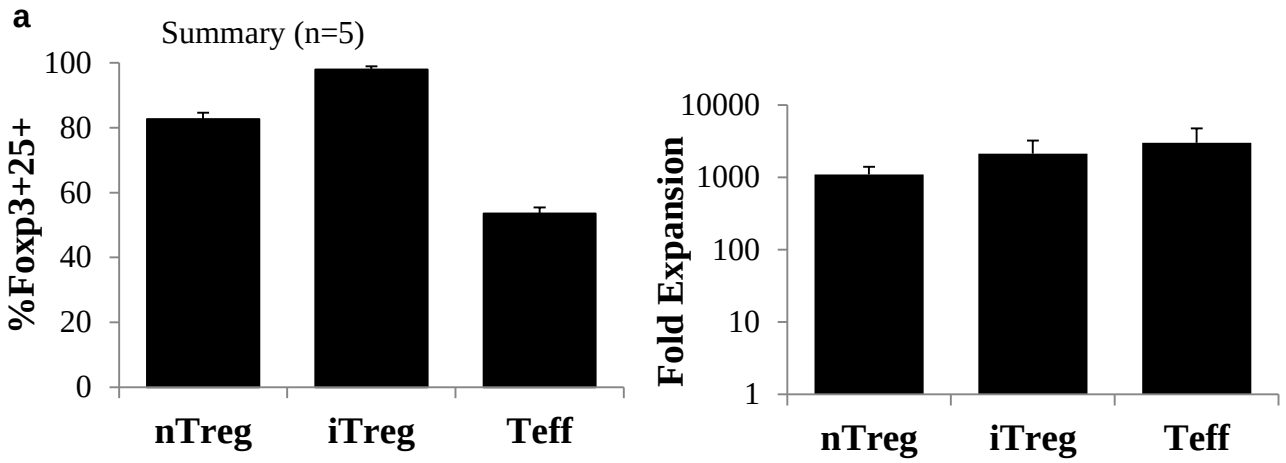
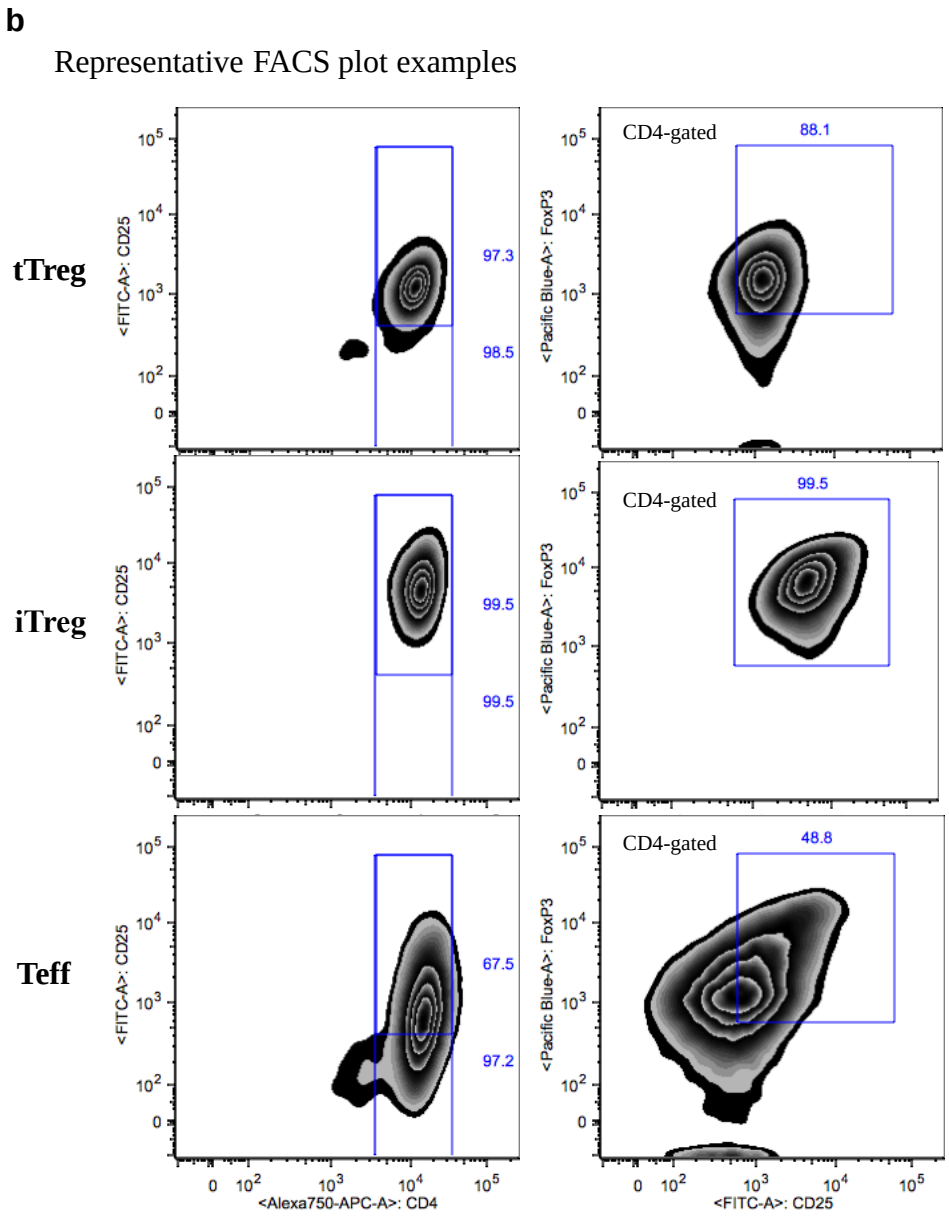
*Running title: Dlgh1 and PKC-θ oppositely controls in vitro-induced human Tregs*

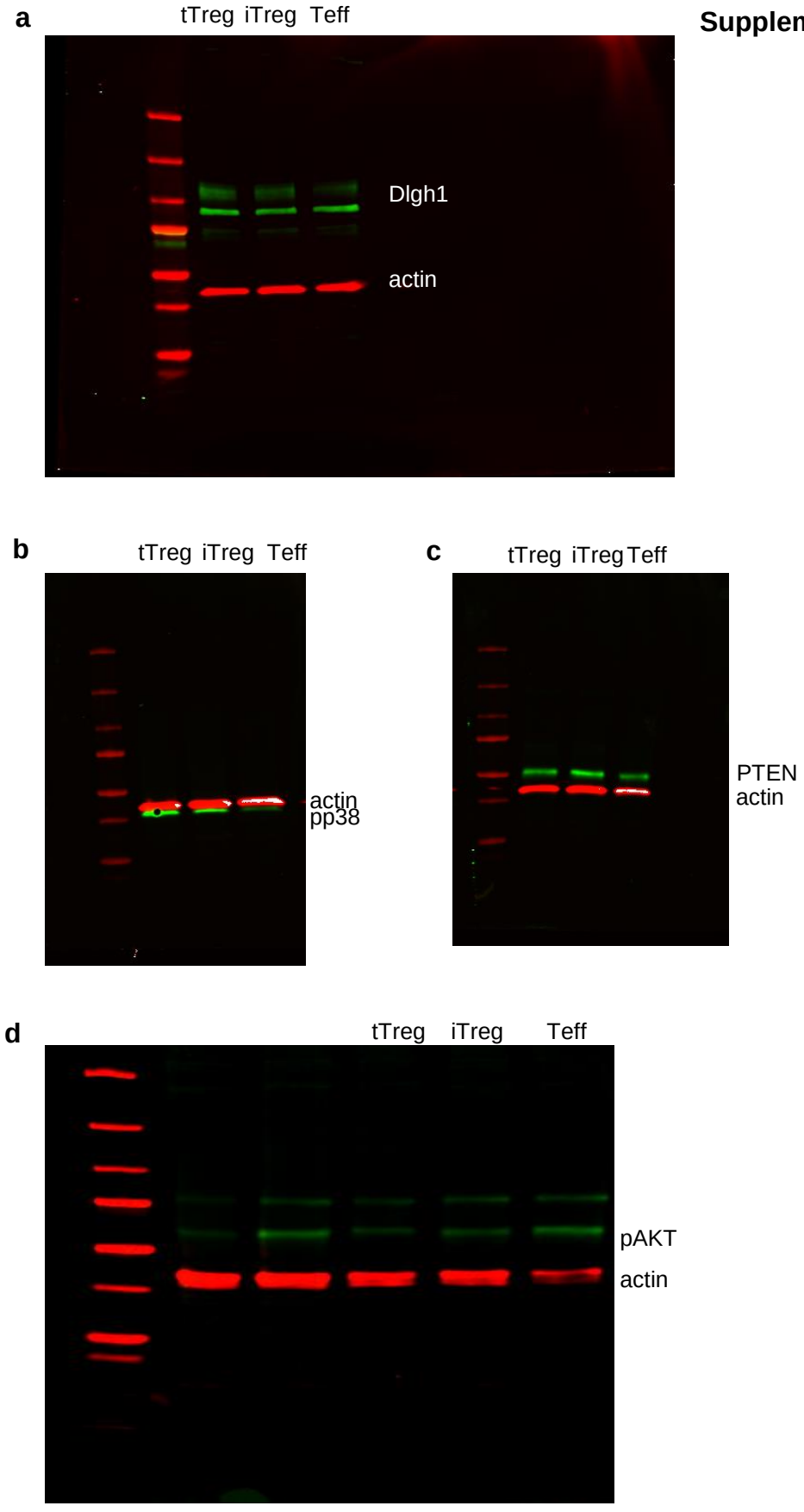
**Supplementary Figure 1**

Characteristics of expanded T cell subsets:

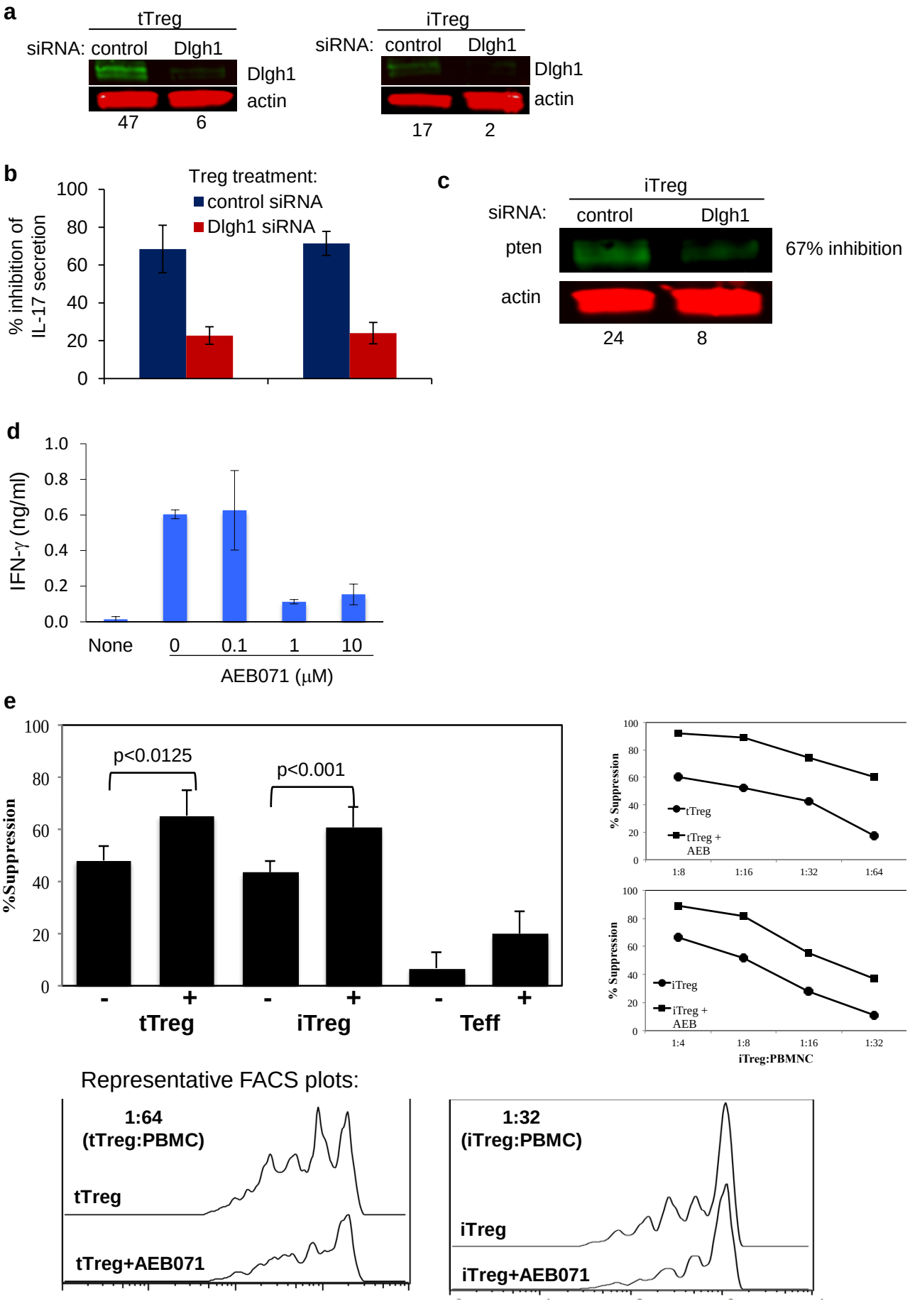


**Supplementary Figure 2**



**Supplementary Figure 3**



**Supplementary figure legend**

**Figure 1.** tTreg, iTreg, and Teff cells were purified from UCB and expanded in vitro. Summary of $CD25^{+}Foxp3^{+}$ purity and average fold expansion for the indicated cell types on day 14 of culture (a). Representative examples of CD4, CD25 and Foxp3 staining for the cultured cells are shown (b).

**Figure 2.** Full-length gels for Figure 3b-e.

**Figure 3.** Dlgh1 and PKC-θ oppositely regulates iTregs. Treg subsets, tTregs and iTregs were transfected with small interfering RNA (siRNA) targeting Dlgh1 or with control siRNA by AMAXA and plated in presence of IL-2 (300 IU/ml). After 48 hours Dlgh1 (a) and PTEN (c) expression was measured by Western blot analysis. SiRNA-transfected Tregs were mixed with $CD4^{+}$ $CD25^{-}$ T cells at 1:3 ratio and plated on immobilized anti-CD3 antibodies (5 μg/ml). The supernatants were analyzed for IL-17 secretion after 48 hours by ELISA (b). $CD4^{+}$ $CD25^{-}$ T cells were treated with indicated concentrations of PKC-θ inhibitor (AEB071) and plated on immobilized anti-CD3 mAb. The supernatants were analyzed for IFN-γ after 48hours by ELISA (d). In vitro expanded tTregs, iTregs and Teffs were treated ± AEB071 (10μM) for 30 minutes, washed, and then assayed for suppressive function using a proliferation assay in which cultured cells are incubated with CFSE-loaded allogeneic PBMC at doses from 1:4 to 1:64 (expanded cell:PBMC) and stimulated with anti-CD3 beads for 4 days. Since allo-PBMC are from different donors for each assay, overall suppression varies enough such that no single ratio of (expanded cell:PBMC) was useful for all experiments. Therefore, the ratio at which untreated Treg suppression was closest to 50% was chosen for analysis in each experiment (varied from 1:8 to 1:32). Data represent a summary of 4-8 independent experiments.