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Supporting Information

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Peripherally induced human regulatory T cells uncouple Kv1.3 activation from TCR-associated signaling

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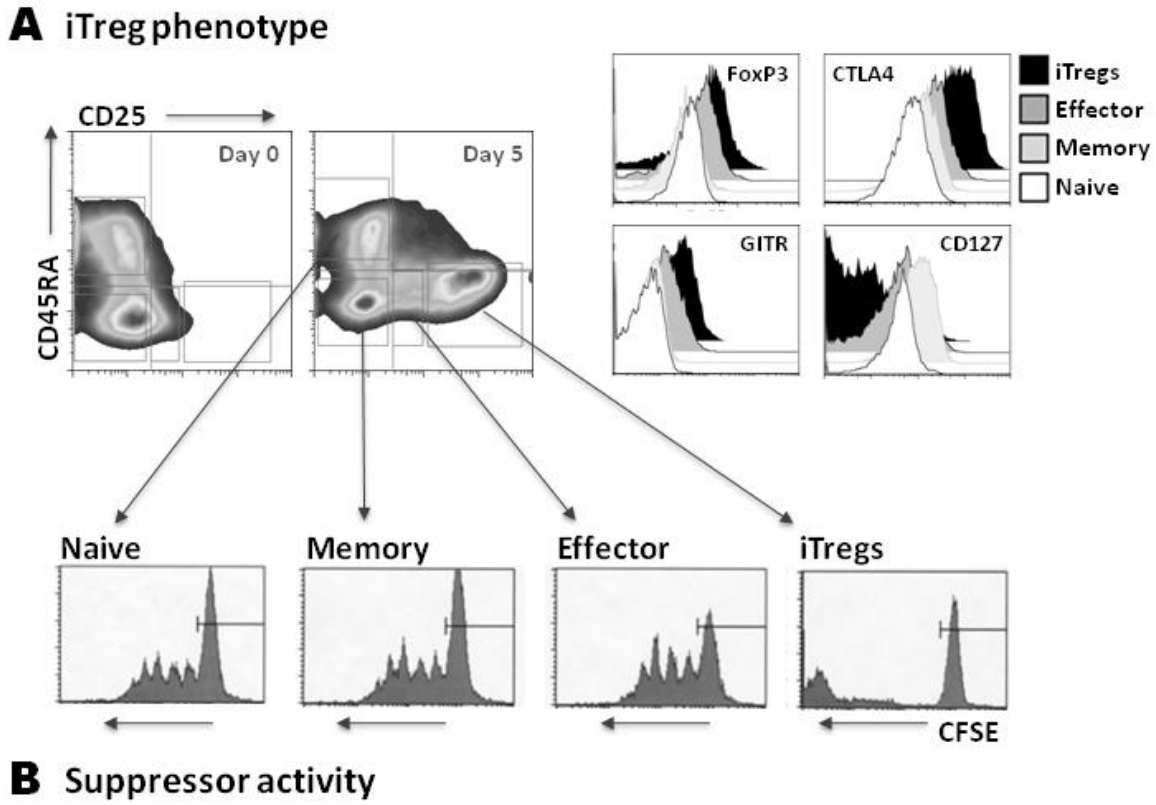
SUPPORTING INFORMATION

Supporting Figure A



Generation of iTregs. (A). Cell isolation, culture conditions, sorting. Human PBMC from fresh buffy coats of healthy anonymous blood donors obtained from the Kentucky Blood Center (Lexington, KY) were isolated by centrifugation over a Ficoll-Hypaque gradient. $CD4^+$ T cells were purified by negative selection (StemCell Technologies kit) and $CD25^-$ further selected (Miltenyi) following manufacturer instructions. $CD4^+CD25^-$ T cells were cultured at a

concentration of $2x10^6$/mL in RPMI, 10% FBS, 2mM L-Glutamine, 50μM 2-ME, IL-2 (5 ng/mL), coated anti-CD3 antibody (400 ng/mL) and TGFβ (2 ng/mL). Cells were maintained in culture for six days with a change of medium on the third day. Naïve ($CD45RA^+$ /$CD25^-$), memory ($CD45RA^-$ /$CD25^-$), effector ($CD45RA^-$ /$CD25^{low}$/$CD127^{high}$) and iTreg ($CD45RA^-$ /$CD25^{high}$/$CD127^{low}$) cells were sorted using a Cytomation MoFlo (Beckman Coulter) high speed sorter (University of Kentucky Cytometry Core Facility). The acquisition of Treg-like phenotype (high expression of CD25, FoxP3, GITR and CTLA4 and low expression or absence of CD45RA and CD127) is concomitant with cells displaying a strong capacity to suppress the proliferation of $CD4^+$ T cells. Between 15 and 35% of original $CD4^+CD25^-$ T cells reproducibly develop to iTreg phenotype. **(B). Suppression assay.** Purified $CD4^+CD25^-$ cells were labeled with CFSE (Molecular Probes) at a final concentration of 5μM. CFSE labeled cells (10^5 per well) were cultured with CD3-CD28 beads in the presence of sorted naïve, memory, effector T cells or iTregs ($3x10^4$ per well- ratio target CFSE: labeled cells: effector cells of 3:1-). After 5 days of co-culture, cells were harvested and the CFSE profile of the stained cells analyzed by flow cytometry. Cell divisions of target cells were analyzed by sequential dilution of CFSE fluorescence. The presence of iTreg cells completely abolished the proliferation $CD4^+$ T cells in response to CD3-CD28 beads.

Reagents. Antibodies for immunofluorescence assays were obtained from Miltenyi (CD45RA, CD127 and CD25), BD (FoxP3 and CTLA4) and eBioscience (GITR). The CD3 antibody (OKT3 clone) to culture and activate T cells was purchased from BioXCell, and CD3-CD28-coated beads used in suppressor assays to activate target cells were from Miltenyi (Inspector kit).