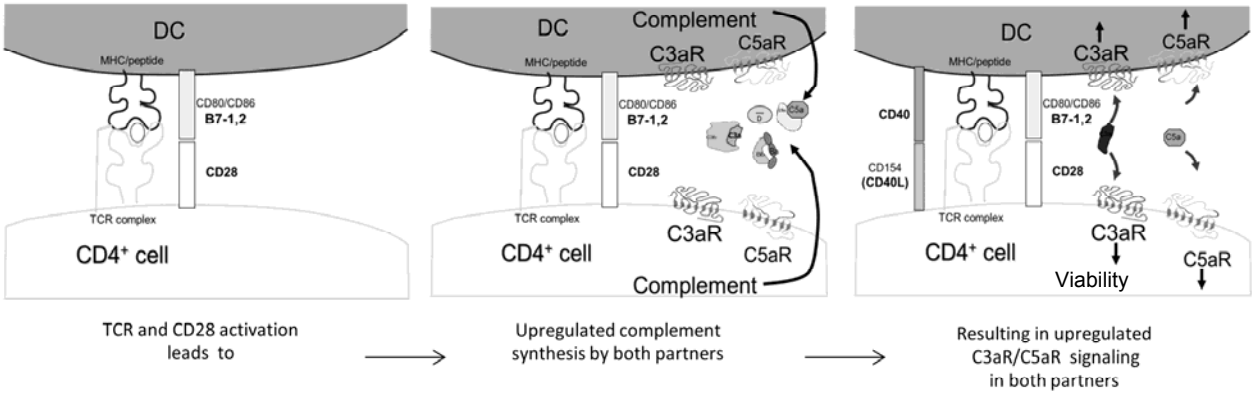
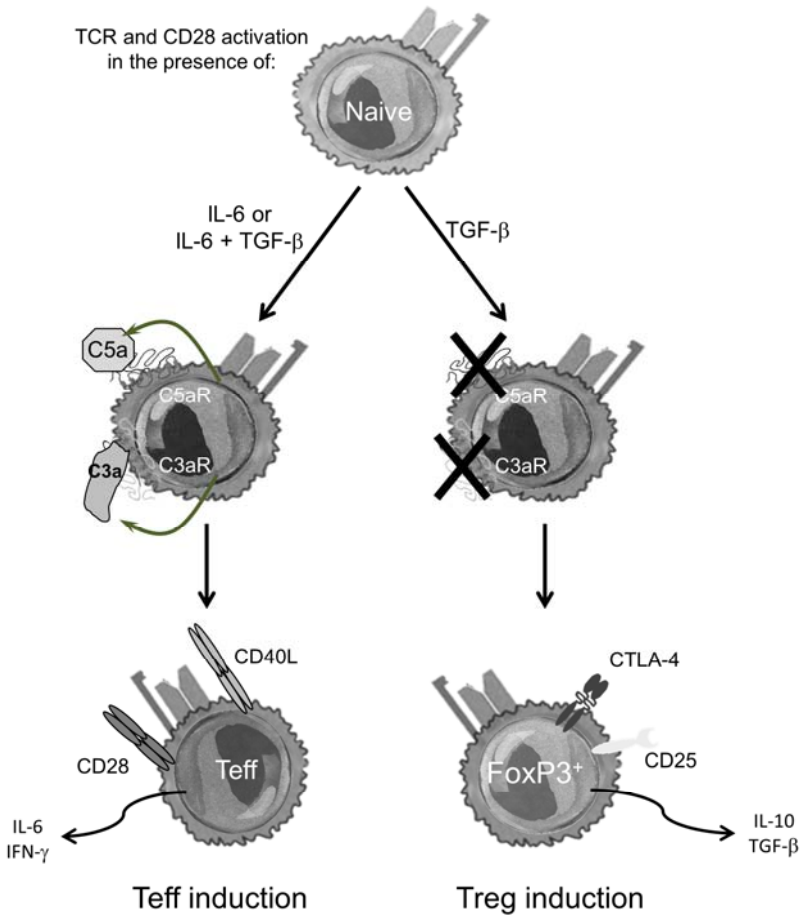
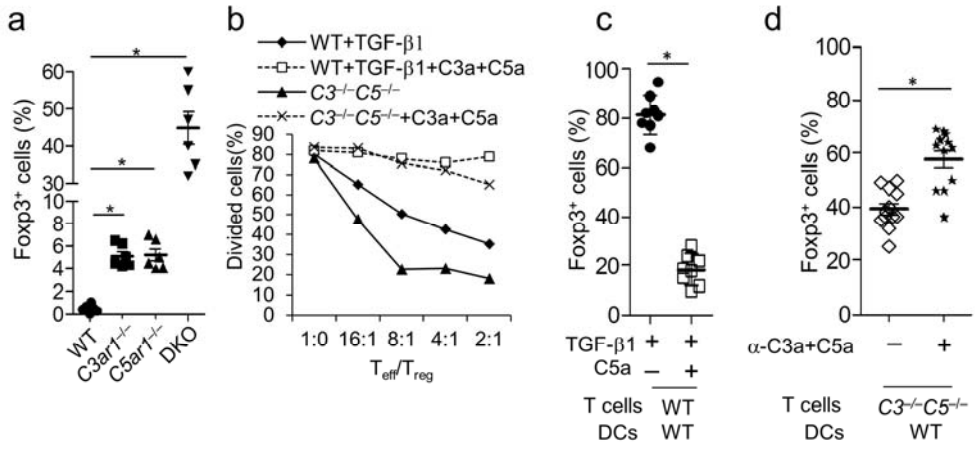
Supplemental Online Material



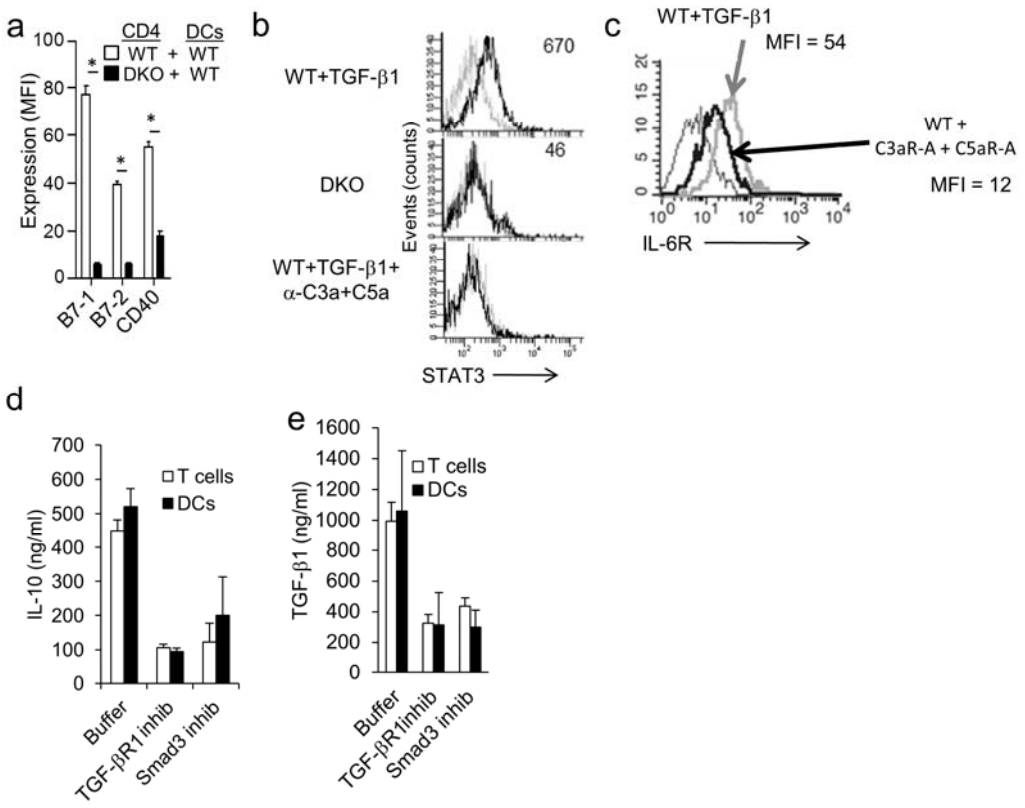
Supplementary Figure 1: **C3aR/C5aR signaling connected with T effector cell activation and viability.**



Supplementary Figure 2: The presence or absence of $CD4^+$ T cell C3aR/C5aR signaling biases between effector T cell and iT_{reg} polarization.

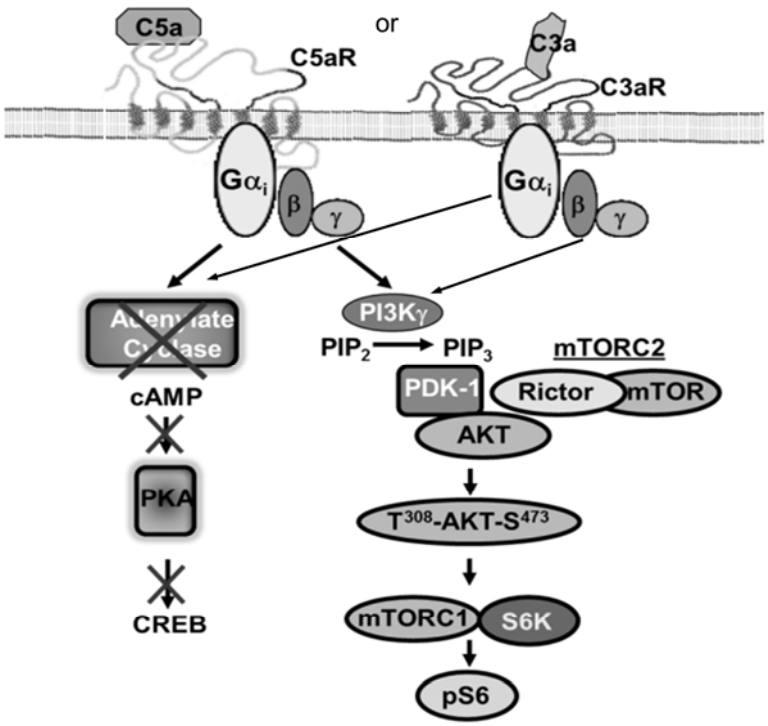


Supplementary Figure 3: Optimal iT_{reg} induction requires the dual absence of C3aR and C5aR signaling in $CD4^+$ cells and iT_{reg} induction is inhibited by C3a + C5a. **(a)** Sorted WT, *C3ar1*$^{-/-}$, *C5ar1*$^{-/-}$, or *C3ar1*$^{-/-}$*C5ar1*$^{-/-}$ $Foxp3^-$ $CD4^+$ T cells were incubated with WT DCs and $Foxp3^+$ $CD4^+$ T cells assayed for CD25 and Foxp3 expression (*P<0.001, n=7). **(b)** Sorted $Foxp3^+$ cells were incubated with 10^6 CellTracker Red™ labeled $Foxp3^-CD25^-CD4^+$ WT cells in differing T_{eff}/iT_{reg} ratios + anti-CD3 (5 μg/ml) and $2.5x10^5$ $CD11c^+$ DCs and relative suppressive capacity determined as in Fig 1. **(c)** Sorted WT $Foxp3^-$ $CD4^+$ T cells and WT DCs were incubated with rhTGF-β1 (5 ng/ml) ± C5a (100 ng/ml) after which percent $Foxp3^+CD25^+$ cells was determined. **(d)** Sorted *C3*$^{-/-}$*C5*$^{-/-}$ $CD25^-$ $CD4^+$ T cells were cultured for 3 days with WT DCs, and anti-CD3 ± anti-C3a + anti-C5a mAbs (10 μg/ml). The cells were assayed for Foxp3 expression by flow cytometry (*P<0.001; n=6).

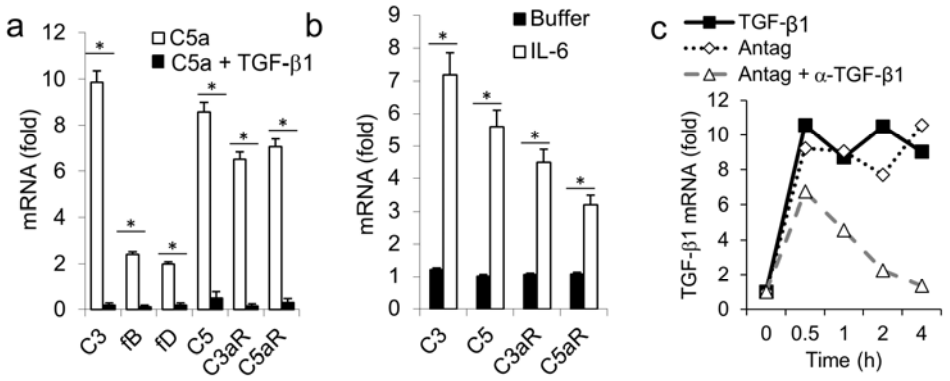


Supplementary Figure 4: Changes connected with greater suppressor activity of iT_{regs} induced by C3aR + C5aR antagonism than by exogenous TGF-β1. Dependence of TGF-β1 and IL-10 production by C3aR + C5aR antagonized iT_{reg} on auto-inductive TGF-β1 signaling. **(a)** Sorted WT or *C3ar1*$^{-/-}$*C5ar1*$^{-/-}$ Foxp3$^+$ iT_{regs} were induced in the presence of WT DCs after which the DCs were assayed for B7-1,2 and CD40 expression. Mean Fluorescence Intensity (MFI) (*$P < 0.01$; n=5). **(b)** iT_{regs} were induced from sorted WT Foxp3$^-$ T cells in the presence of anti-CD3, IL-2, DCs, and of TGF-β1 (5 ng/ml), or from sorted Foxp3$^-$ *C3ar1*$^{-/-}$*C5ar1*$^{-/-}$ cells in the absence of TGF-β1. Sorted Foxp3$^+$ cells prepared in the two ways were assayed for p-STAT3 by flow cytometry. Anti-C3a + anti-C5a mAbs were added to the Foxp3$^+$ cells prepared with TGF-β1 after which STAT3 phosphorylation was reassessed (representative plots of n=6). **(c)** WT Foxp3$^-$ CD4$^+$ T cells were incubated with anti-CD3, rhIL-2 (5 ng/ml) and CD11c$^+$ WT DCs in the presence of rhTGF-β1 (5 ng/ml) or C3aR-A + C5aR-A (10 nM). Foxp3$^+$ cells were assayed for IL-6R by flow cytometry (representative plots of n=6). **(d)** *C3ar1*$^{-/-}$*C5ar1*$^{-/-}$ Foxp3$^-$ cells were incubated for 3 days with anti-CD3, rhIL-2 (5

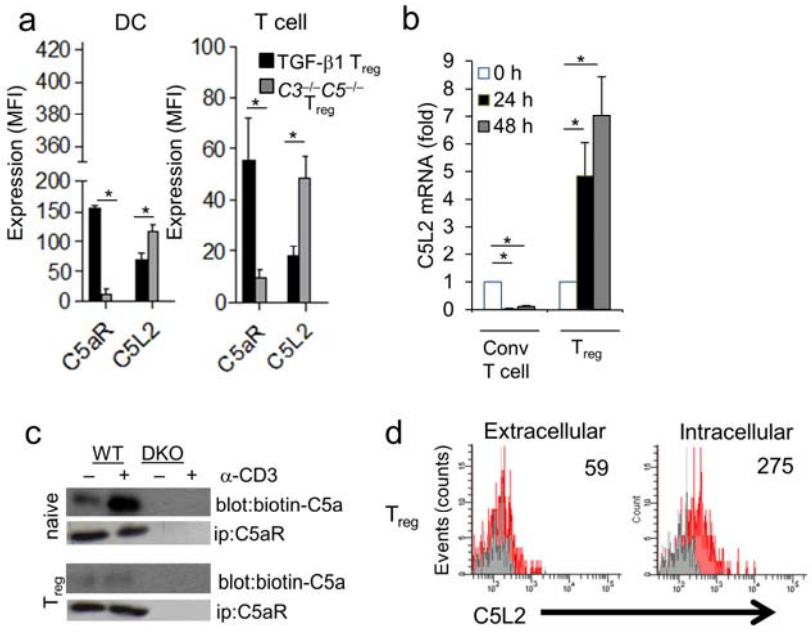
ng/ml) and CD11c^{+} WT DCs ± TGF-βR1 inhibitor (10 nM),or Smad3 inhibitor (5 μM). Sorted CD4^{+} T cells (unfractionated) and sorted DCs were cultured for 24 hr and the supernatants assayed IL-10 by ELISA (n=7). **(e)** Flow sorted CD4^{+} T cells (unfractionated) and DCs from the induction cultures containing TGF-βR1 inhibitor (10 nM), or Smad3 inhibitor (5 μM) in panel **(e)** were cultured for 24 hr, after which endogenously produced TGF-β1 protein was assessed by ELISA (n=7).



Supplementary Figure 5: Effects of downstream C3aR/C5aR GPCR signaling on CREB activation and PI-3Kγ-AKT-mTOR-p-S6 signaling.

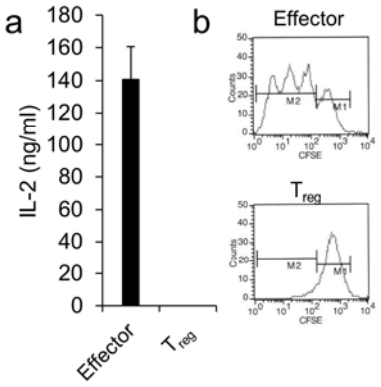


Supplementary Figure 6: Differential effects of TGF-β1 and IL-6 on local complement production by DCs. **(a)** Sorted WT Foxp3^{-} CD4^{+} T cells were activated for 1 hr with anti-CD3+CD28 beads plus C5a (100 ng/ml) or TGF-β1 (5 ng/ml), after which cells were assayed for C3, C5, C3aR, and C5aR mRNA expression by qPCR (*P<0.001, **P<0.02, n=5). **(b)** CD11c^{+} DCs were stimulated for 1 hr with IL-6 (5 ng/ml) and assayed for complement mRNA transcripts by qPCR (*P<0.001, n=5). **(c)** Sorted WT Foxp3^{-} cells were activated in the presence DCs for 0.5, 1, 2, 4 hr with TGF-β1 (5 ng/ml), C3aR-A+C5aR-A (10 nM, Antags), or C3aR-A + C5aR-A (10 nM) plus anti-TGF-β1 mAb (5 μg/ml). CD4^{+} T cells were then sorted and assayed for TGF-β1 mRNA levels by qPCR (values are presented as fold increase over time 0 after normalization to β-actin).



Supplementary Figure 7: C5L2 and C5aR expression in iT_{regs} induced by C3aR and C5aR antagonism vs exogenous TGF-β1, control for C5aR and C5L2 IPs using C3ar1$^{-/-}$ C5ar1$^{-/-}$ CD4$^+$ cells, and intracellular staining for C5L2. **(a)** Sorted Foxp3$^-$ CD4$^+$ T cells were incubated with WT DCs in the presence TGF-β1 (5 ng/ml) or with *C3$^{-/-}$C5$^{-/-}$*DCs in the absence of TGF-β1. DCs (left side) were assayed for C5aR and C5L2 expression by gating on CD11c$^+$ cells. Responder T cells (right side) were assayed for C5aR and C5L2 expression by gating on Foxp3$^-$ cells and on Foxp3$^+$ cells. (*P < 0.01; n=5). **(b)** Sorted Foxp3$^+$ iT_{regs} generated from WT Foxp3$^-$ CD4$^+$ T cells and WT DCs plus TGF-β1 (5 ng/ml), and sorted conventional Foxp3$^-$ CD4$^+$ WT cells (Conv T cell) were incubated with anti-CD3+CD28 Dynabeads and assayed at 0, 24, and 48 hr for C5L2 mRNA transcripts by qPCR. **(c)** Upper blots: Unactivated sorted conventional WT Foxp3$^-$CD4$^+$ T cells and unactivated *C3ar1$^{-/-}$C5ar1$^{-/-}$* Foxp3$^-$CD4$^+$ T cells were incubated for 30 min with anti-CD3, after which the cells were incubated for 5 min with biotin labeled C5a and chilled to 4^{o}C. Plasma membrane fractions were purified by ultracentrifugation and anti-C5aR IPs prepared. Following elution with alkali, the eluates from the anti-C5aR IPs were loaded on gels and electrophoresed proteins were immunoblotted for biotinylated-C5a with streptavidin-HRP. Lower blots: iT_{regs} prepared sorted Foxp3$^-$ WT CD4$^+$ T cells with anti-CD3, IL-2,

WT DCs and TGF-β1 (5 ng/ml) or from sorted Foxp3^{-} *C3ar1*$^{-/-}$*C5ar1*$^{-/-}$ CD4^{+} T cells in the absence of TGF-β1. The cells were incubated for 30 min with anti-CD3, after which they were incubated for 5 min with biotin labeled C5a, and immunoblots of anti-C5aR IPs analyzed as above. **(d)** WT Foxp3^{-}CD4^{+} T cells were incubated for 3 days with anti-CD3, rhIL-2 (5 ng/ml), and WT DCs in the presence of C3aR-A + C5aR-A (10 nM). Foxp3^{+} CD4^{+} T cells were assayed for extracellular C5L2 expression and permeabilized cells assayed for intracellular C5L2 expression (*$P < 0.01$ for MFI; n=5).



Supplementary Figure 8: iT_{regs} that arise from human $CD4^+$ cells in which C3aR and C5aR signaling are blocked are anergic. **(ab)** Flow sorted $CD45RA^+CD25^-CD4^+$ human T cells were incubated for 3 days with soluble anti-CD3, IL-2, and autologous DCs in the presence and absence of C3aR-A + C5aR-A after which cells were washed and sorted on CD25. After sorting, $CD25^-$ (Effectors) and $CD25^+$ (T_{regs}) were CFSE-labeled and incubated for 48 hr in complete RPMI 1640 + 10% FBS. The cells were then stimulated with PMA + Ionomycin for 3 days after which **(a)** culture supernatants were assayed for IL-2 by ELISA, and **(b)** proliferation was assessed by CFSE dilution.