## Supplemental Figure Legends

**Figure S1: Costimulatory blockade with anti-CD40L+anti-B71/2 mAbs markedly diminished complement and cytokine production during T cell APC interactions.** A) Following interaction of OT-II cells with DCs and ova for 18 hr with 3 μg/ml anti-CD40L mAbs or control IgG, mRNAs in flow separated cells were assayed for C3, C3aR, C5aR and IFNγ gene expression by qPCR. In parallel cultures, IFNγ was assessed by ELISPOT. No complement or cytokine upregulation occurred without T cells. Data are normalized to no ova. B) OT-II cells were incubated at 37°C for 18 hr with anti-CD40L+anti-B71/2 mAbs and 0.1 μM $ova_{323-339}$ and complement as well as cytokine mRNA assayed by qPCR. The results are normalized to no $ova_{323-339}$ with each DC. Identical studies to except that Mar T cells were stimulated with 3μg/ml anti-CD40L and 0.1 μM Dby peptide.

**Figure S2: C5aR-A and C3aR-A are specific blockers for their respective GPCRs.** A) Fura-2 loaded THP-1 cells were alternatively incubated with buffer (left) or C5aR-A (right) following which C5a (left) or C5a and then fMLP (right) was added and $Ca^{++}$ flux recorded. A similar experiment was done except the effect of the C3aR-A on fMLP induction of $Ca^{++}$ flux was measured. A small agonist effect of the C3aR•A is observed. The C5aR-A inhibited C5a induction but not fMLP induction of a $Ca^{++}$ flux even at 100-fold higher concentrations. Testing its specificity showed that it likewise did not inhibit fMLP induction of a $Ca^{++}$ flux. Neither the C5aR, the C3aR antagonist, nor the two antagonists together affected upregulation of the activation marker STAT1 or ionomycin

induced $Ca^{++}$ flux (data not shown). Titrations with anti-CD3 mAb showed that the antagonists also did not alter the $Ca^{++}$ flux which occurs rapidly following TCR stimulation (not shown). B) WT $CD4^+$ T cells preincubated with buffer, 10 ng/ml C5aR-A, or 10 ng/ml C3aR-A were incubated with 1 μg/ml anti-CD3 + 100 ng/ml C5a or anti-CD3 + 100 ng/ml C3a for 10 min and pAKT generation assessed by western blotting.

**Figure S3: .Locally produced C5a and C3a interacting with C5aR and C3aR function in an autocrine and paracrine fashion to upregulate complement production** OT-II T cells were incubated for 1 hr WT or *C5aR*$^{-/-}$*C3aR*$^{-/-}$ DCs plus $_{ova323-339}$, flow separated, and OT-II cells assayed for C3, C5aR and C3aR mRNA expression and DCs analyzed for C3 mRNA expression.

**Figure S4: The absence of C3a/C5a•C3aR/C5aR signaling blocks T cell generation of C3a and C5a.** WT, *C3aR*$^{-/-}$*C5aR*$^{-/-}$, or *C3*$^{-/-}$*C5*$^{-/-}$ T cells were incubated ± 3 mg/ml anti-CD3/28 for 18 hr, and concentrated culture supernatants were analyzed by western blotting for C3a or C5a production.

**Figure S5: The absence of C3a/C5a•C3aR/C5aR signaling blocks APC cytokine expression.** OT-II cells plus $ova_{323-339}$ and WT or *C5aR*$^{-/-}$*C3aR*$^{-/-}$ DCs were incubated for 1 hr, and flow separated partners were assayed for cytokine mRNA expression levels by qPCR (left). The DC•OT-II cell mixtures alternatively were cultured for 48 hr and supernatants assayed for IL-12 by ELISA (right).

**Figure S6. C5aR/C3aR ligation activates PI-3Kγ which in turn promotes AKT phosphorylation.** A) WT T cells were incubated with anti-CD3/CD28 for increasing times ± C5aR-A + C3aR-A (10 ng/ml each). Extracts then were western blotted with anti-phospho-Ser$^{473}$ AKT mAb (rep of 5 exps). B) WT or *C5aR*$^{-/-}$*C3aR*$^{-/-}$ T cells were incubated with anti-CD3/CD28 and extracts western blotted with anti-phospho-Ser$^{473}$ AKT mAb (rep of 5 exps).

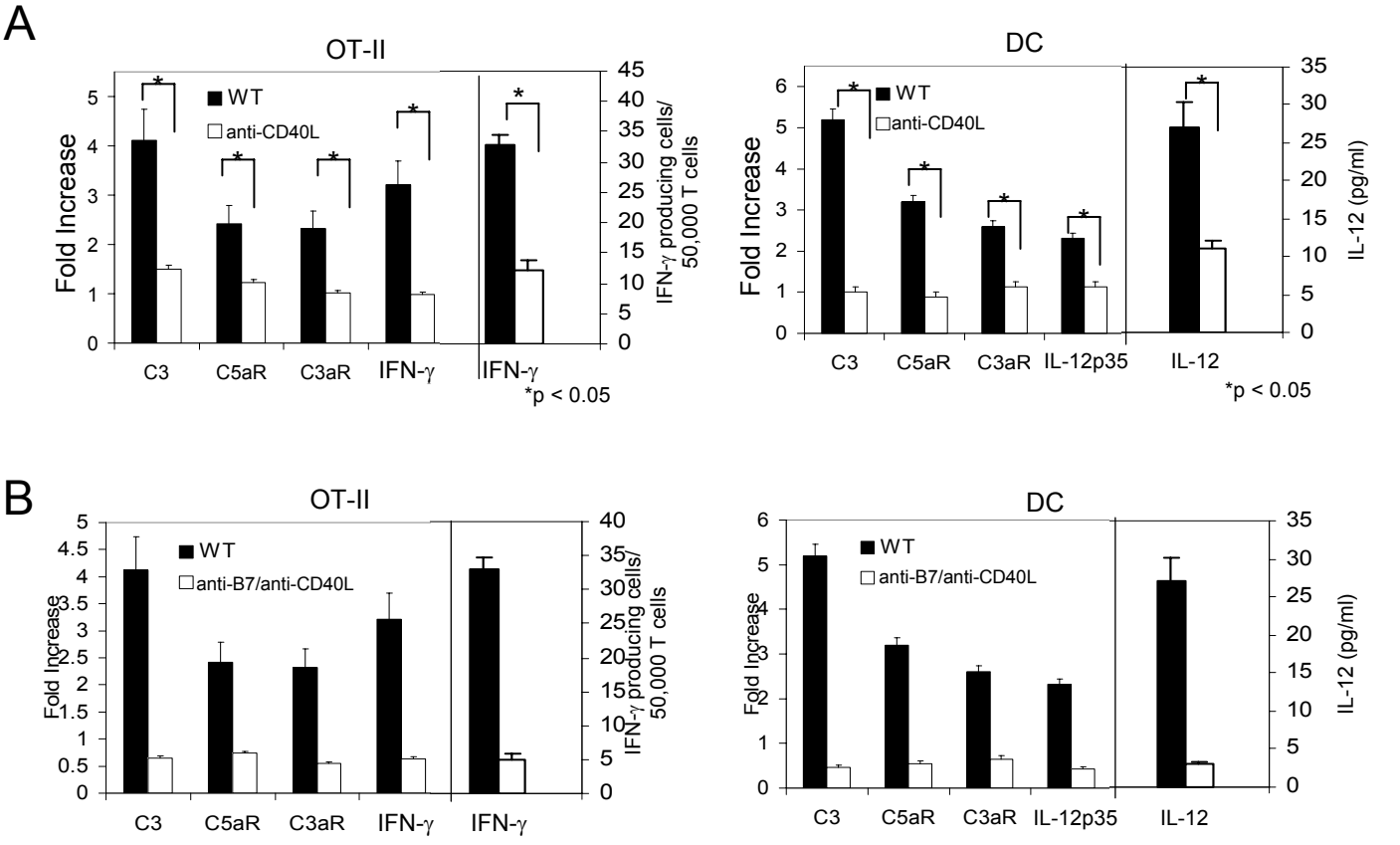
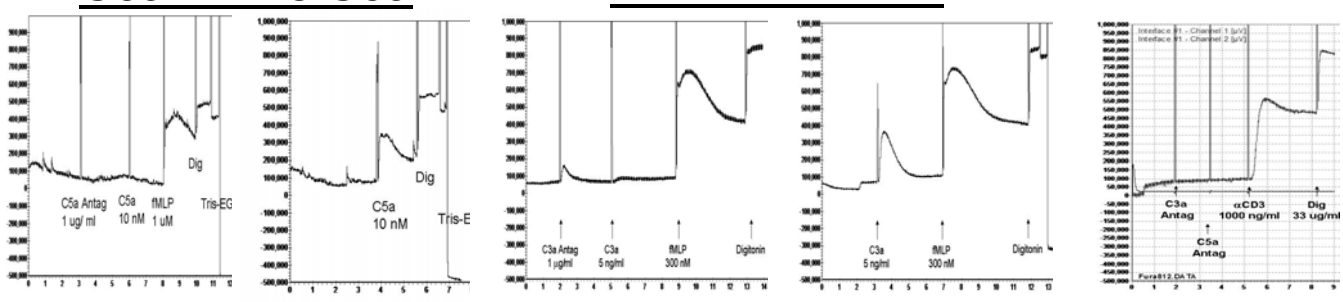

A
OT-II
WT
anti-CD40L
Fold Increase
IFN-γ producing cells/ 50,000 T cells
C3
C5aR
C3aR
IFN-γ
IFN-γ
*p < 0.05
DC
WT
anti-CD40L
Fold Increase
IL-12 (pg/ml)
C3
C5aR
C3aR
IL-12p35
IL-12
*p < 0.05
B
OT-II
WT
anti-B7/anti-CD40L
Fold Increase
IFN-γ producing cells/ 50,000 T cells
C3
C5aR
C3aR
IFN-γ
IFN-γ
DC
WT
anti-B7/anti-CD40L
Fold Increase
IL-12 (pg/ml)
C3
C5aR
C3aR
IL-12p35
IL-12

Figure S1

A


C5aR-A vs C5a
C3aR-A vs C3a
C5a Antag 1 ug/ ml
C5a 10 nM
fMLP 1 uM
Dig
Tris-EG
C5a 10 nM
Dig
Tris-E
C3a Antag 1 ug/ml
C3a 5 ng/ml
fMLP 300 nM
Digitonin
C3a 5 ng/ml
fMLP 300 nM
Digitonin
C3a Antag
αCD3 1000 ng/ml
Dig 33 ug/ml
C5a Antag
Time (min)

B

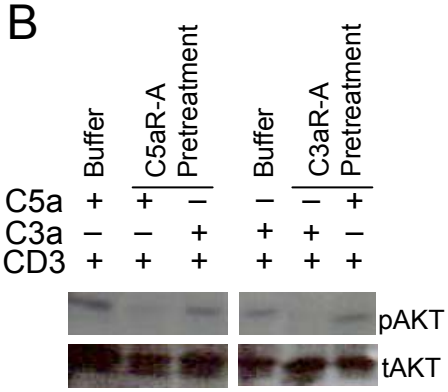

Buffer
C5aR-A Pretreatment
Buffer
C3aR-A Pretreatment
C5a + + – – –
C3a – – + + –
CD3 + + + + +
pAKT
tAKT

Figure S2

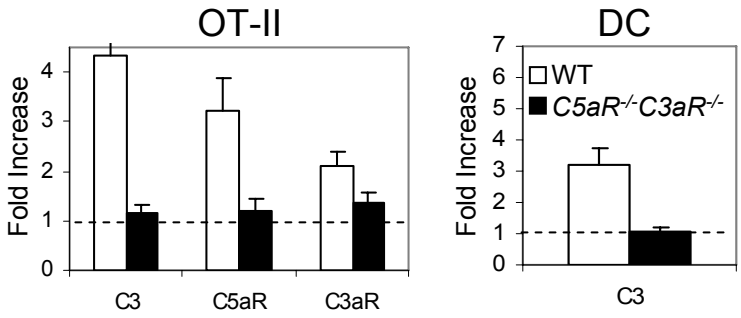

OT-II
Fold Increase
0
1
2
3
4
C3
C5aR
C3aR
DC
Fold Increase
0
1
2
3
4
5
6
7
WT
C5aR-/-C3aR-/-
C3

Figure S3

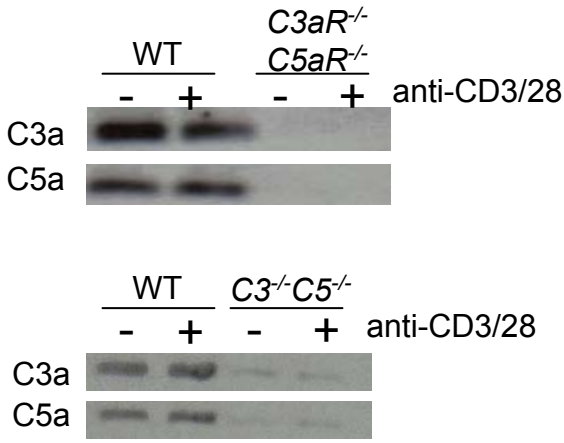



Figure S4

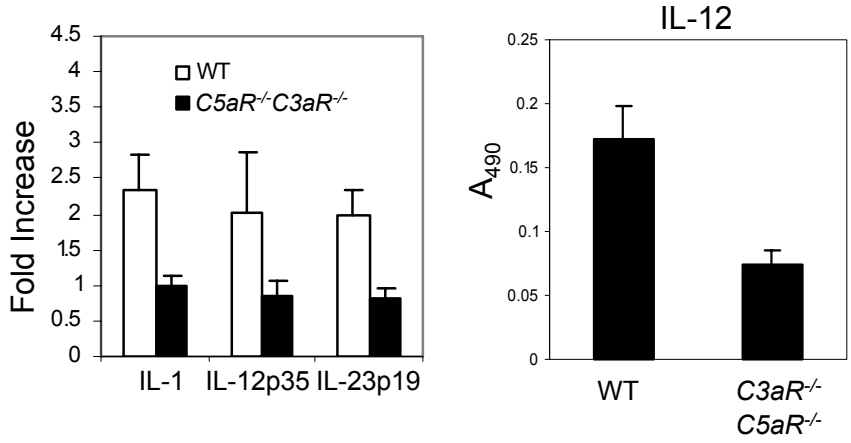

Fold Increase
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
WT
C5aR-/-C3aR-/-
IL-1
IL-12p35
IL-23p19
IL-12
A490
0.25
0.2
0.15
0.1
0.05
0
WT
C3aR-/-
C5aR-/-

Figure S5

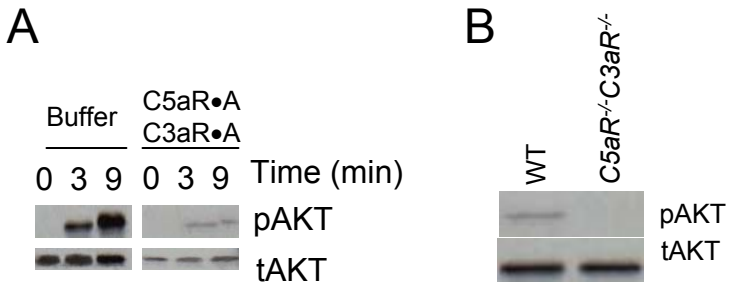

A
Buffer
C5aR•A
C3aR•A
0 3 9 0 3 9
Time (min)
pAKT
tAKT
B
WT
C5aR-/-C3aR-/-
pAKT
tAKT

Figure S6