Supplementary Information

The TSC-mTOR pathway regulates macrophage polarization

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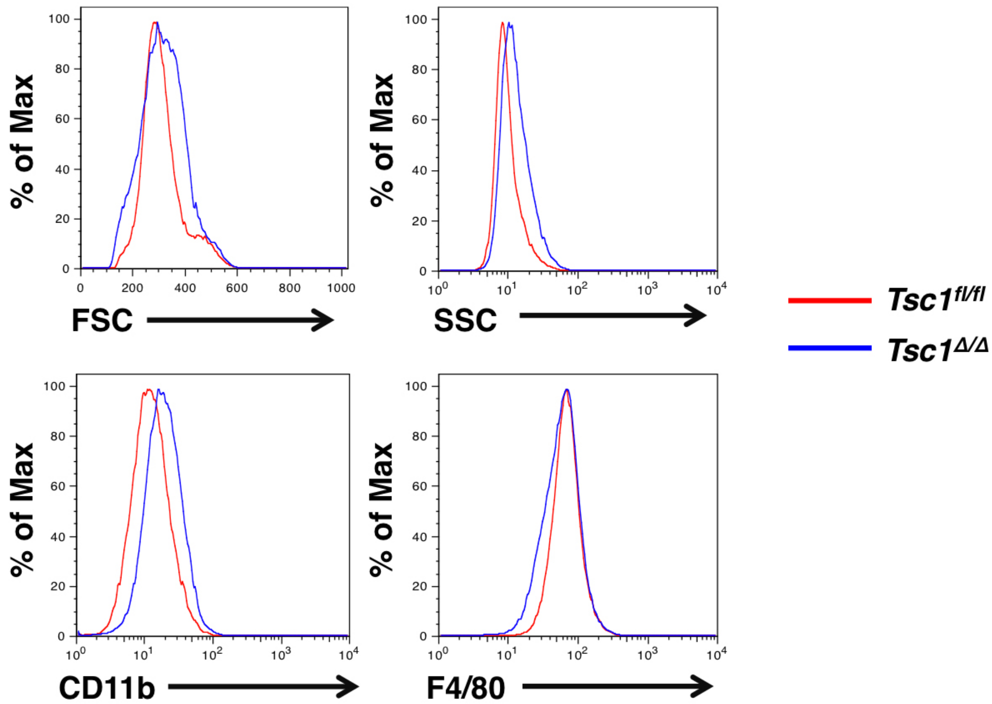
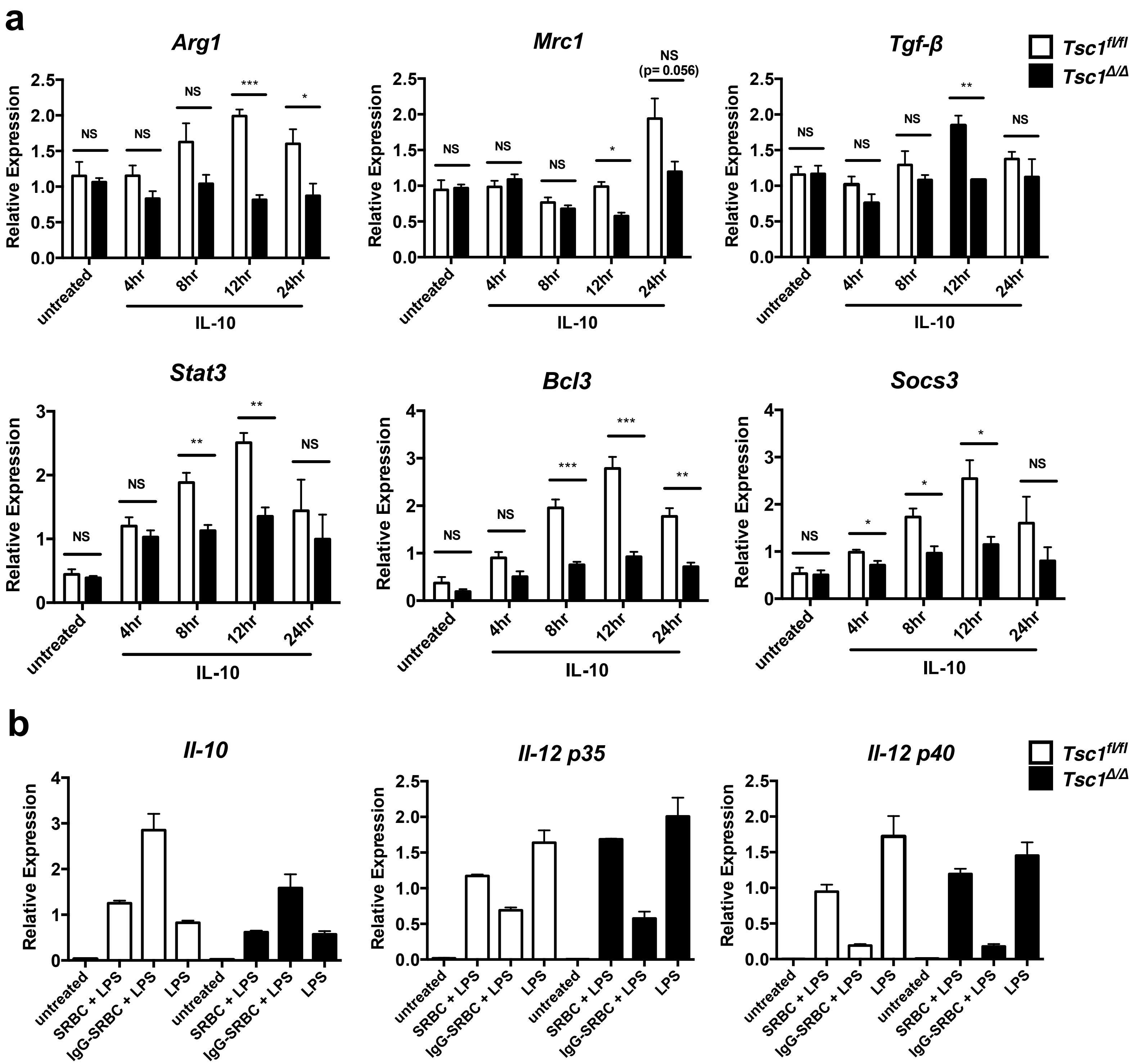
Supplementary Figures S1-S9
Supplementary Table S1



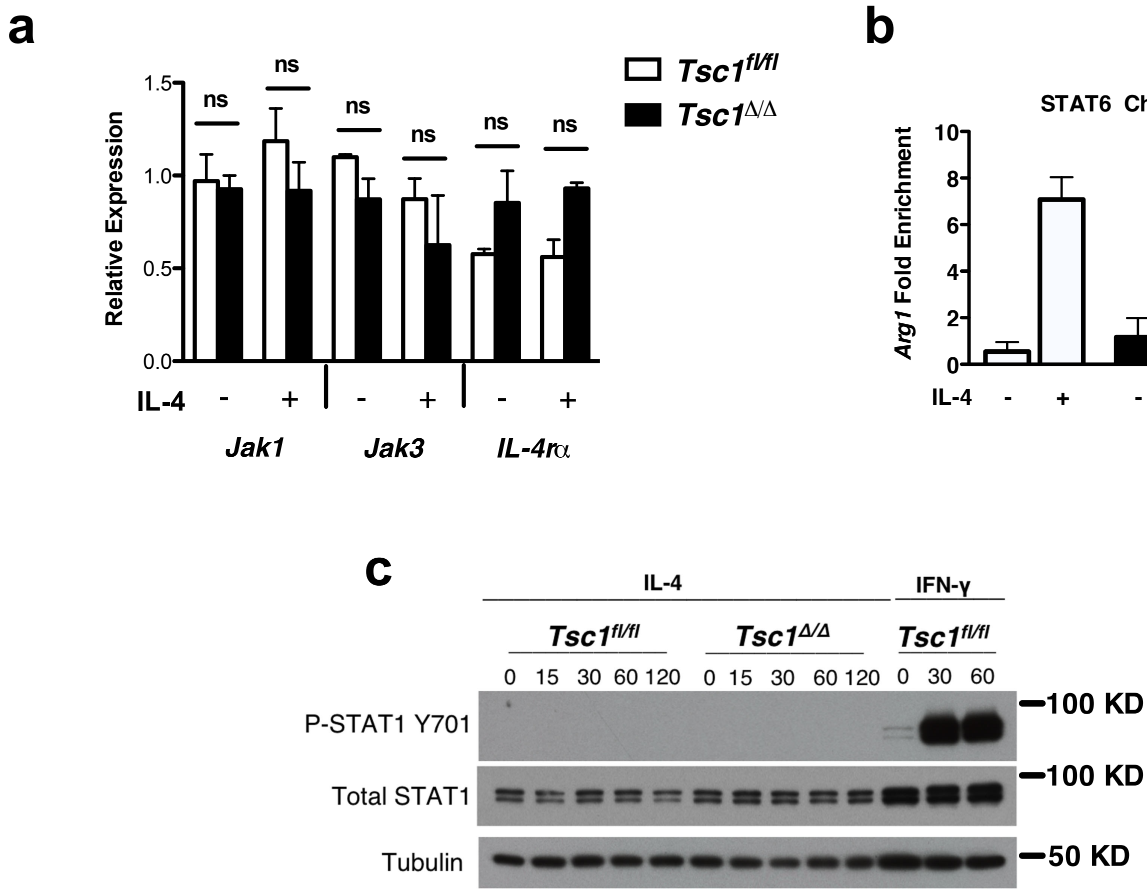
Supplementary Figure S1. Increased Cell Size and Granularity in *Tsc1*$^{\Delta/\Delta}$ BMDMs

Flow cytometry analysis of *Tsc1*$^{fl/fl}$ and *Tsc1*$^{\Delta/\Delta}$ BMDMs stained with macrophage markers CD11b and F4/80. Histogram plots are shown as % of maximum. Red= *Tsc1*$^{fl/fl}$ and Blue= *Tsc1*$^{\Delta/\Delta}$



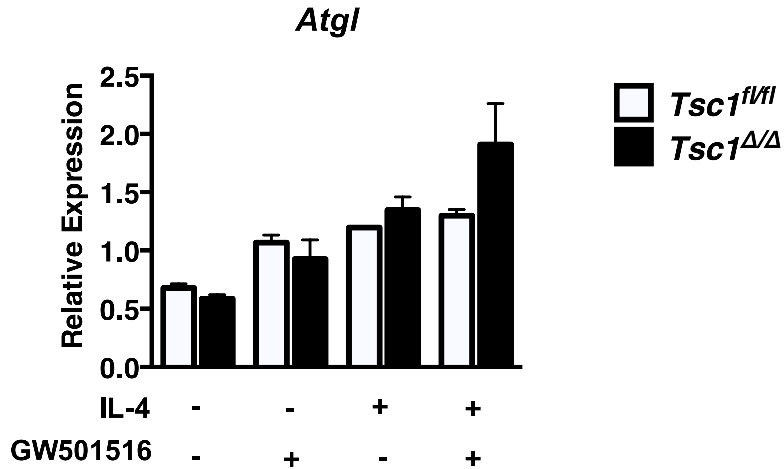
Supplementary Figure S2. Impaired M2c but not M2b Polarization in *Tsc1*$^{\Delta/\Delta}$ BMDMs

a. M2c gene expression in *Tsc1*$^{fl/fl}$ and *Tsc1*$^{\Delta/\Delta}$ BMDMs stimulated with 10ng/ml IL-10 for the indicated times (n=2 representative experiments). **b**. M2b gene expression of *Tsc1*$^{fl/fl}$ and *Tsc1*$^{\Delta/\Delta}$ BMDMs stimulated simultaneously with LPS and non-opsonized (SRBC) or opsonized SRBC (IgG- SRBC) for 6h (Data representative of 3 independent experiments). Graphs are shown as mean ± SEM, *p<0.05, **p<0.01, ***p<0.001 determined by Student's t-tests.



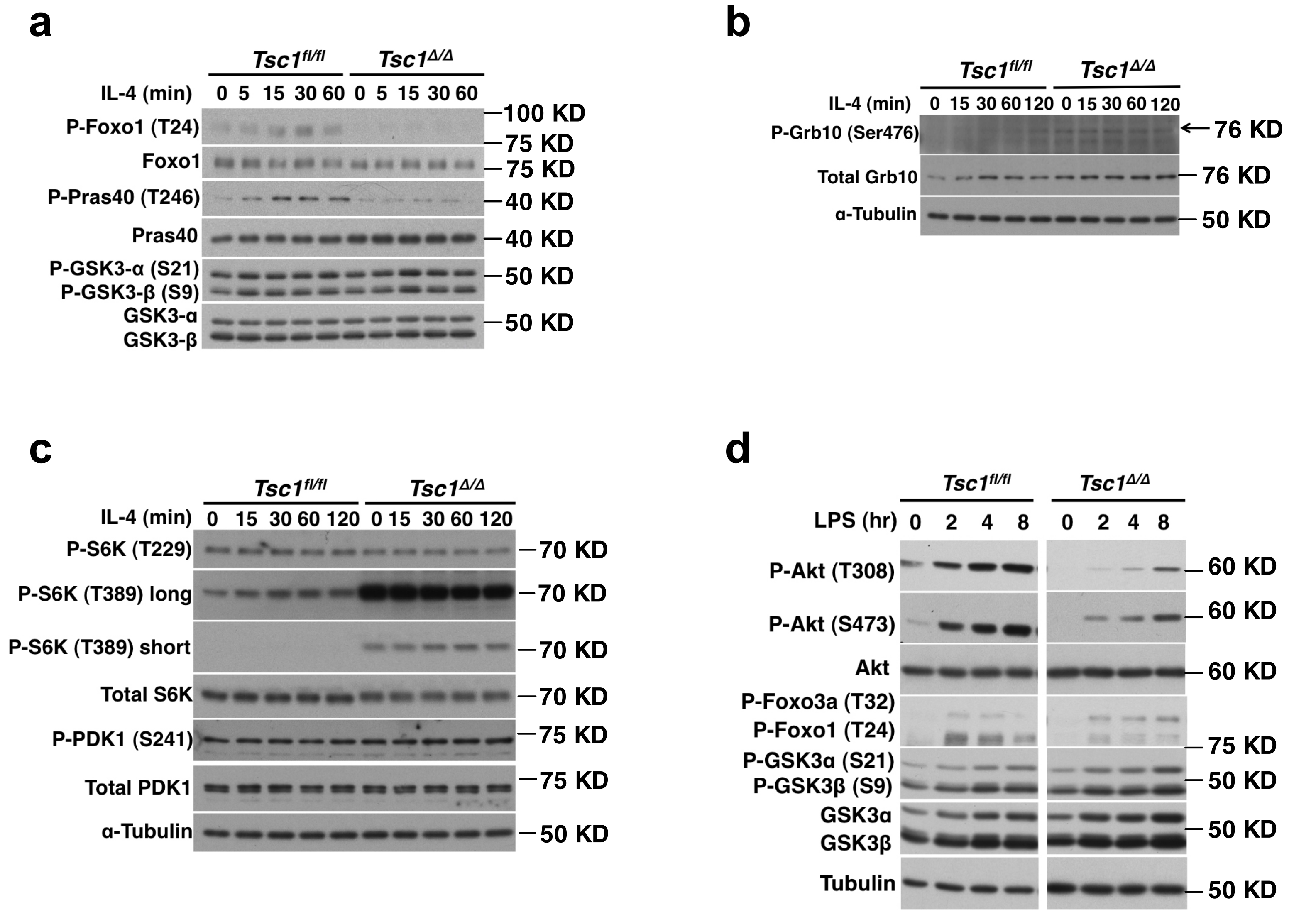
Supplementary Figure S3. The JAK-STAT6 Pathway is Intact in *Tsc1*$^{\Delta/\Delta}$ BMDMs

a. Gene expression of components of the IL-4R-JAK-STAT6 pathway (n=2 representative experiments). **b.** STAT6 chromatin immunoprecipitation. Data is shown as fold enrichment for the *Arg1* promoter and is representative of 3 independent experiments. **c.** IL-4 fails to activate STAT1 in BMDMs. Immunoblot analysis of STAT1 activation in lysates from *Tsc1*$^{fl/fl}$ and *Tsc1*$^{\Delta/\Delta}$ BMDMs stimulated with IL-4 or IFN (control) for the indicated time points. Graphs are shown as mean ± SEM.



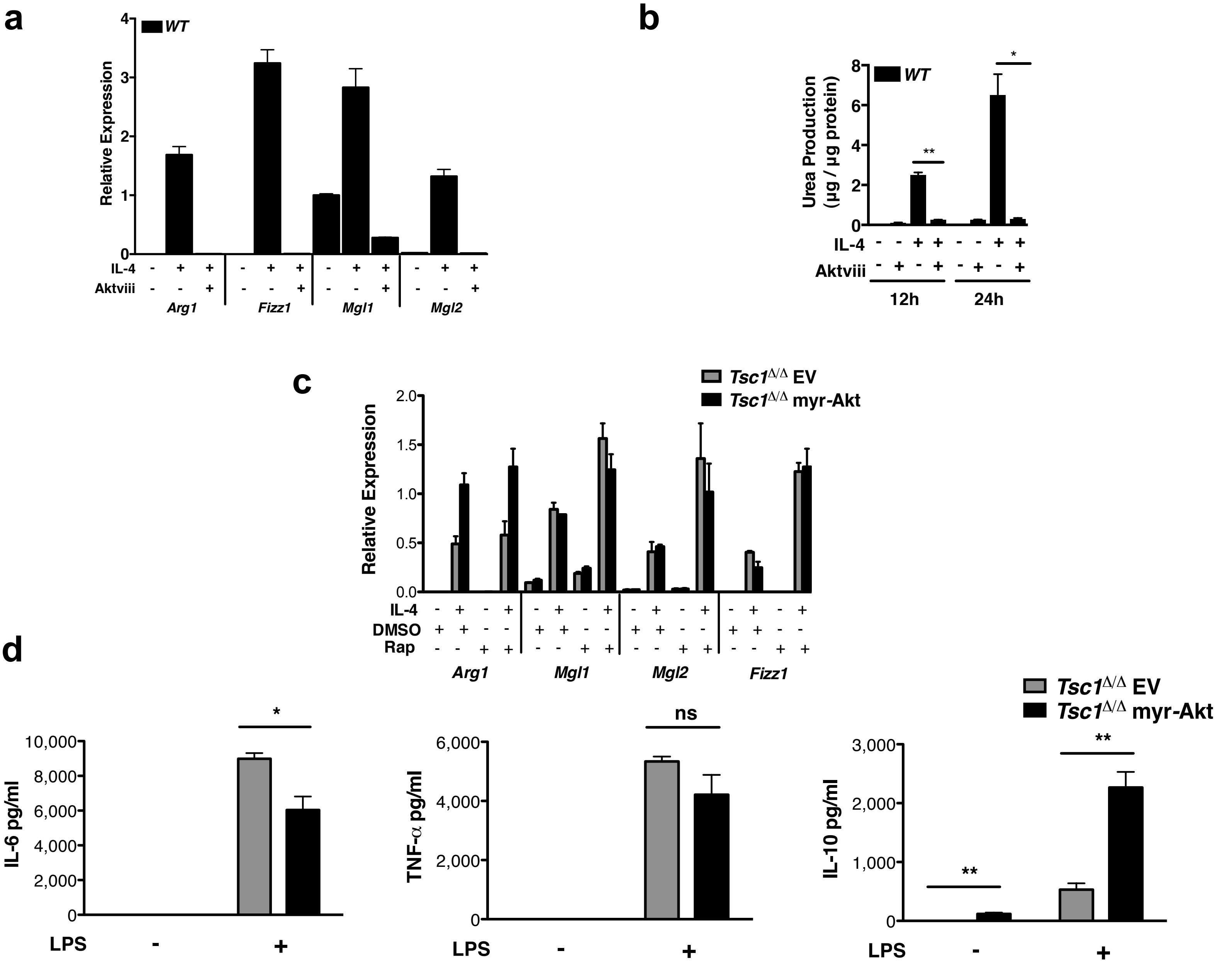
Supplementary Figure S4. PPARδ Activity is Normal in *Tsc1$^{\Delta/\Delta}$* BMDMs

Gene expression of *Atgl* in *Tsc1$^{fl/fl}$* and *Tsc1$^{\Delta/\Delta}$* BMDMs stimulated with IL-4 in the presence or absence of GW501516. DMSO vehicle was used as control. Data is shown as mean ± SEM (n=2 representative experiments).



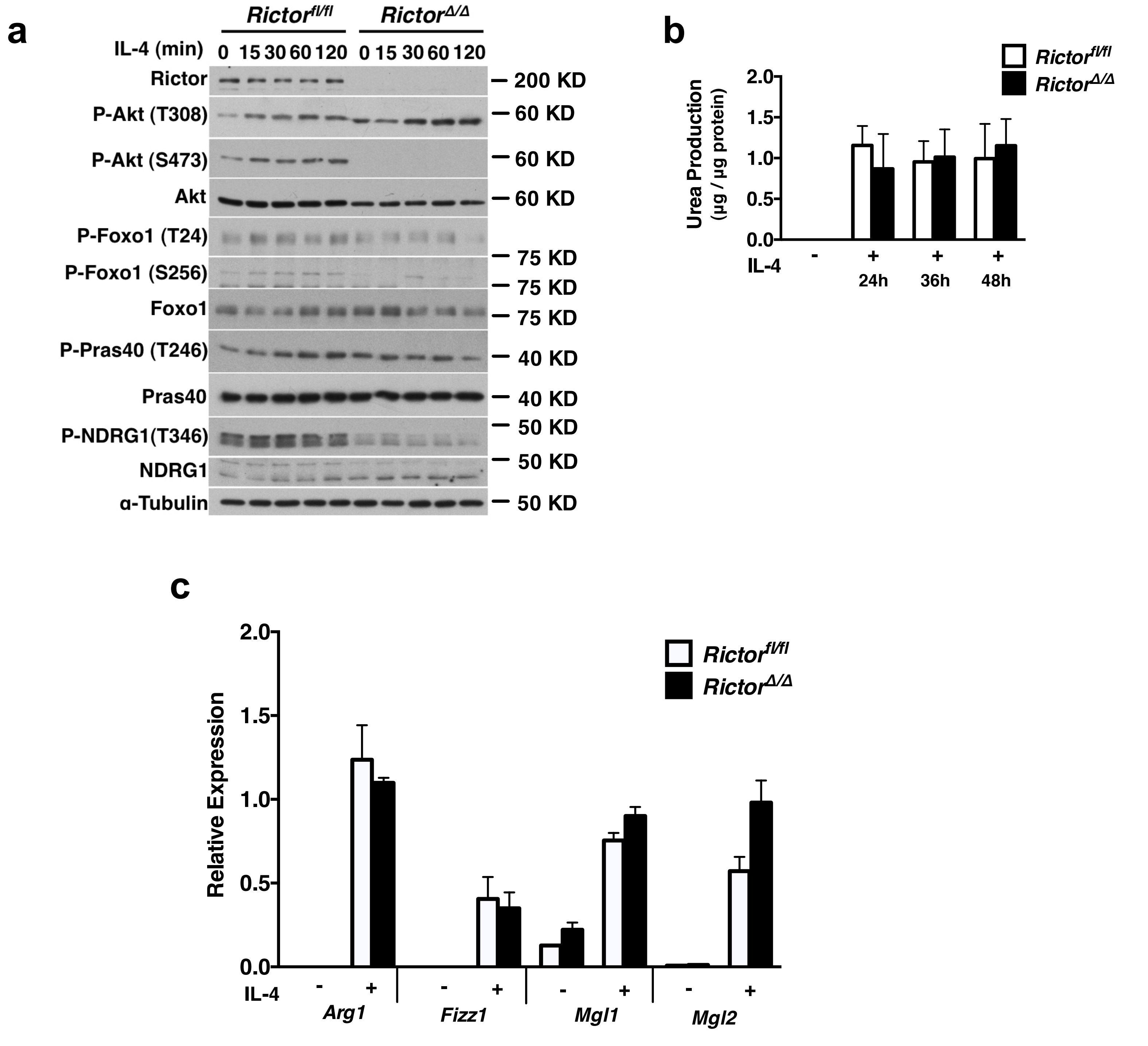
Supplementary Figure S5. IL-4 and LPS-Induced Akt Activation is Attenuated in *Tsc1*$^{\Delta/\Delta}$ BMDMs

a-c. Immunoblots of *Tsc1*$^{fl/fl}$ and *Tsc1*$^{\Delta/\Delta}$ BMDMs stimulated with IL-4 as indicated. **a.** Analysis of Akt targets. **b.** Elevated levels of total and phosphorylated Grb10 in *Tsc1*$^{\Delta/\Delta}$ BMDMs. **c.** Analysis of phosphorylated and total PDK1. Phosphorylation of PDK1 is not inducible, and levels of phosphorylated and total PDK1 are normal in *Tsc1*$^{\Delta/\Delta}$ BMDMs. **d**. Immunoblot analysis of Akt signaling in lysates from *Tsc1*$^{fl/fl}$ and *Tsc1*$^{\Delta/\Delta}$ BMDMs stimulated with LPS as indicated.



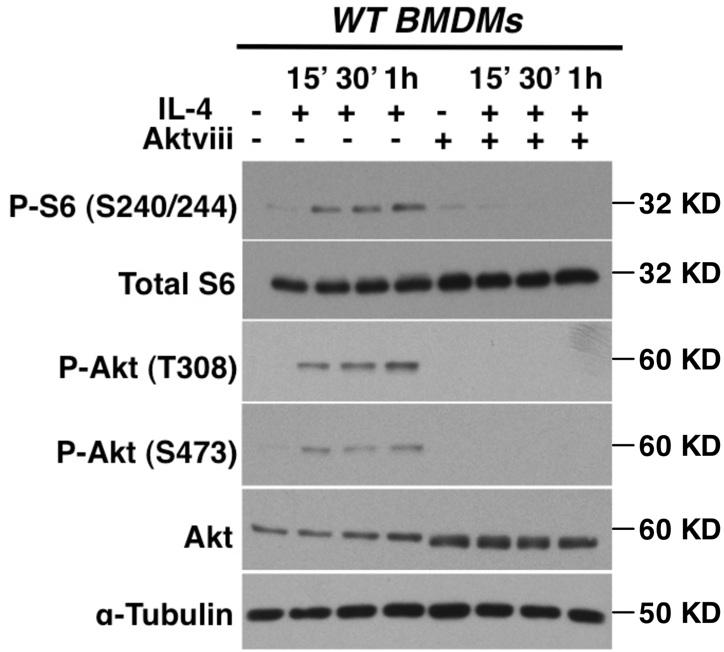
Supplementary Figure S6. Akt Signaling is Critical for Polarization in *Tsc1*$^{\Delta/\Delta}$ BMDMs

a-b. WT BMDMs were pretreated with Aktviii for 1h before IL-4 stimulation. **a.** M2 gene expression was measured after 24h of IL-4 stimulation (representative of 3 independent experiments). **b.** Urea production normalized to total protein (n=3). **c.** M2 gene expression in myr-Akt *Tsc1*$^{\Delta/\Delta}$ BMDMs and EV *Tsc1*$^{\Delta/\Delta}$ BMDMs pretreated with rapamycin or DMSO for 30min and stimulated with IL-4 for 12h (n=2 representative experiments). **d.** Cytokine concentration in supernatants from myr-Akt *Tsc1*$^{\Delta/\Delta}$ BMDMs and EV *Tsc1*$^{\Delta/\Delta}$ BMDMs treated with LPS for 6h (n=3). Graphs are shown as mean ± SEM, *p<0.05, **p<0.01 determined by Student's t-tests.



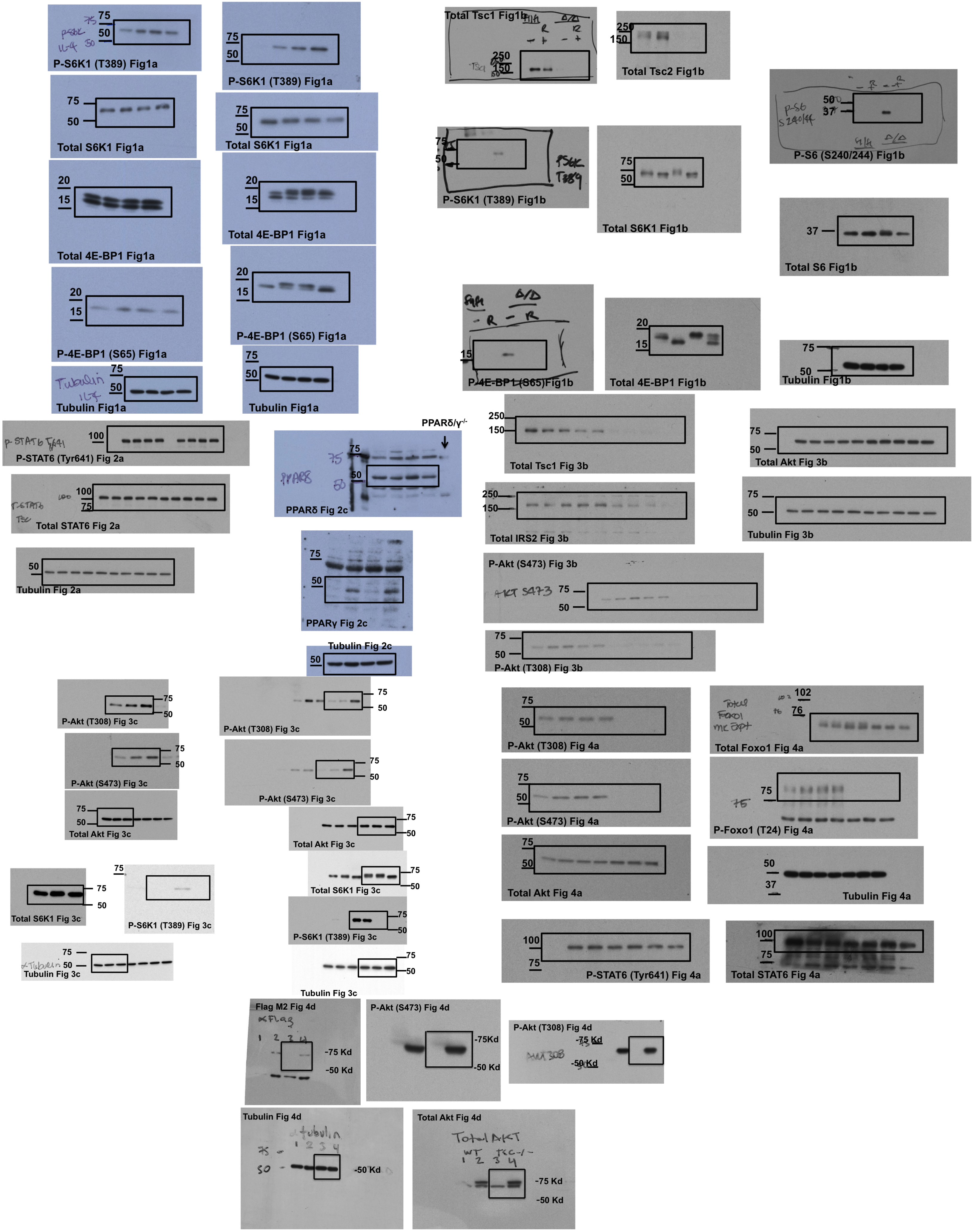
Supplementary Figure S7. Intact M2 Polarization in *Rictor*$^{\Delta/\Delta}$ BMDMs

a. Reduced phosphorylation of the Akt target Foxo1, but not Pras40 in *Rictor*$^{\Delta/\Delta}$ BMDMs. Immunoblot analysis of lysates from *Rictor*$^{fl/fl}$ and *Rictor*$^{\Delta/\Delta}$ BMDMs stimulated with IL-4 as indicated. **b.** Urea production normalized to total protein after stimulation with IL-4 as indicated (n=2 representative experiments). **c.** M2 gene expression analysis in *Rictor*$^{fl/fl}$ and *Rictor*$^{\Delta/\Delta}$ BMDMs stimulated with IL-4 for 24h (n=2 representative experiments). Graphs are shown as mean ± SEM.



Supplementary Figure S8. Akt Signaling is Critical for mTORC1 Activation in BMDMs

Immunoblot analysis of WT BMDMs pretreated with Aktviii for 1h before IL-4 stimulation for the indicated times. Reduced phosphorylation of S6 on S240/244 indicates attenuated mTORC1 activity in the presence of Aktviii.



Supplementary Figure S9. Full Blots for Main Text Figures

Supplementary Table S1. qPCR Primer Sequences.

Gene	Primer Sequence (5'-3')
Arg-1	F: ACC TGG CCT TTG TTG ATG TCC CTA R: AGA GAT GCT TCC AAC TGC CAG ACT
Mgl-1	F: TGC AAC AGC TGA GGA AGG ACT TGA R: AAC CAA TAG CAG CTG CCT TCA TGC
Mgl-2	F: GCA TGA AGG CAG CTG CTA TTG GTT R: TAG GCC CAT CCA GCT AAG CAC ATT
Fizz-1	F: TCC AGC TGA TGG TCC CAG TGA ATA R: ACA AGC ACA CCC AGT AGC AGT CAT
Ym1	F: AGA AGG GAG TTT CAA ACC T R: GTC TTG CTC ATG TGT GTA AGT GA
Mrc1	F: TGG GCT ACA GGA GAA CCC AAC TTT R: GCA GTG GCA TTG ATG CTG CTG TTA
Pgc1β	F: CTT CCG TTG GCC CAG ATA C R: CTG CTG GGC CTC TTT CAG TA
Fabp4	F: ATG AAA TCA CCG CAG ACG ACA GGA R: TGT GGT CGA CTT TCC ATC CCA CTT
Cd36	F: TCA TGC CAG TCG GAG ACA TGC TTA R: AAC TGT CTG TAC ACA GTG GTG CCT
Il-6	F: ACA AAG CCA GAG TCC TTC AGA GAG R: TTG GAT GGT CTT GGT CCT TAG CCA
Tnf-α	F: TCT CAG CCT CTT CTC ATT CCT GCT R: AGA ACT GAT GAG AGG GAG GCC ATT
Il-10	F: GCT CTT ACT GAC TGG CAT GAG R: CGC AGC TCT AGG AGC ATG TG
Tgfβ	F: GTG CGG CAG CTG TAC ATT GAC TTT R: TGT GTT GGT TGT AGA GGG CAA GGA
Stat3	F: TGT GAT GCC TCC TTG ATC GTG ACT R: CCA CAG GAT TGA TGC CCA AGC ATT
Bcl3	F: TCG CAA TTA TGA AGG GCT CAC TGC R: TGC ACC ATG TTC AGG CTG TTG TTC

Socs3	F: TAG ACT TCA CGG CTG CCA ACA TCT R: GGA GCT AGT CCC GAA GCG AAA TCT
Il-12 p35	F: TGC CCT CCT AAA CCA CCT CAG TTT R: TTT CTC TGG CCG TCT TCA CCA TGT
Il-12 p40	F: GGC TGG TGC AAA GAA ACA TGG ACT R: AGA GAC GCC ATT CCA CAT GTC ACT
Atgl	F: TGG AAC CAA AGG ACC TGA TGA CCA R: AGA TGC TAC CCG TCT GCT CTT TCA
Cd68	F: TTC TGC TGT GGA AAT GCA AG R: CAA TGA TGA GAG GCA GCA AG
Jak1	F: GAT AAG GAG TAC TAC ACA GTC AAG R: CAG ACA TCA GAG GCG ATA TAA A
Jak3	F: CTT GAT GAG CCA AGT ATC CTA C R: TGC TCC TAG ATA CAC AAA TTC C
Il4ra	F: AGA TGC AAG CCT TCA GAA TCT GGG R: CGT GGA TGC CCA AGA ACA CAC TAT
Ppar γ	F: TCA TGA CCA GGG AGT TCC TC R: CAG GTT GTC TTG GAT GTC CTC
Ppar δ	F: AGC ACA TCT ACA ACG CCT ACC TGA R: TCG ATG TCG TGG ATG ACA AAG GGT
Hprt	F: TTT CCC TGG TTA AGC AGT ACA GCC C R: TGG CCT GTA TCC AAC ACT TCG AGA