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Supplemental Information

Mitochondrial Integrity Regulated by Lipid Metabolism Is a Cell-Intrinsic Checkpoint for Treg Suppressive Function

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Figure S1 (Related to Figure 1). Measurement of FABP3, 4 and 5 in *in vitro* differentiated Th cell subsets

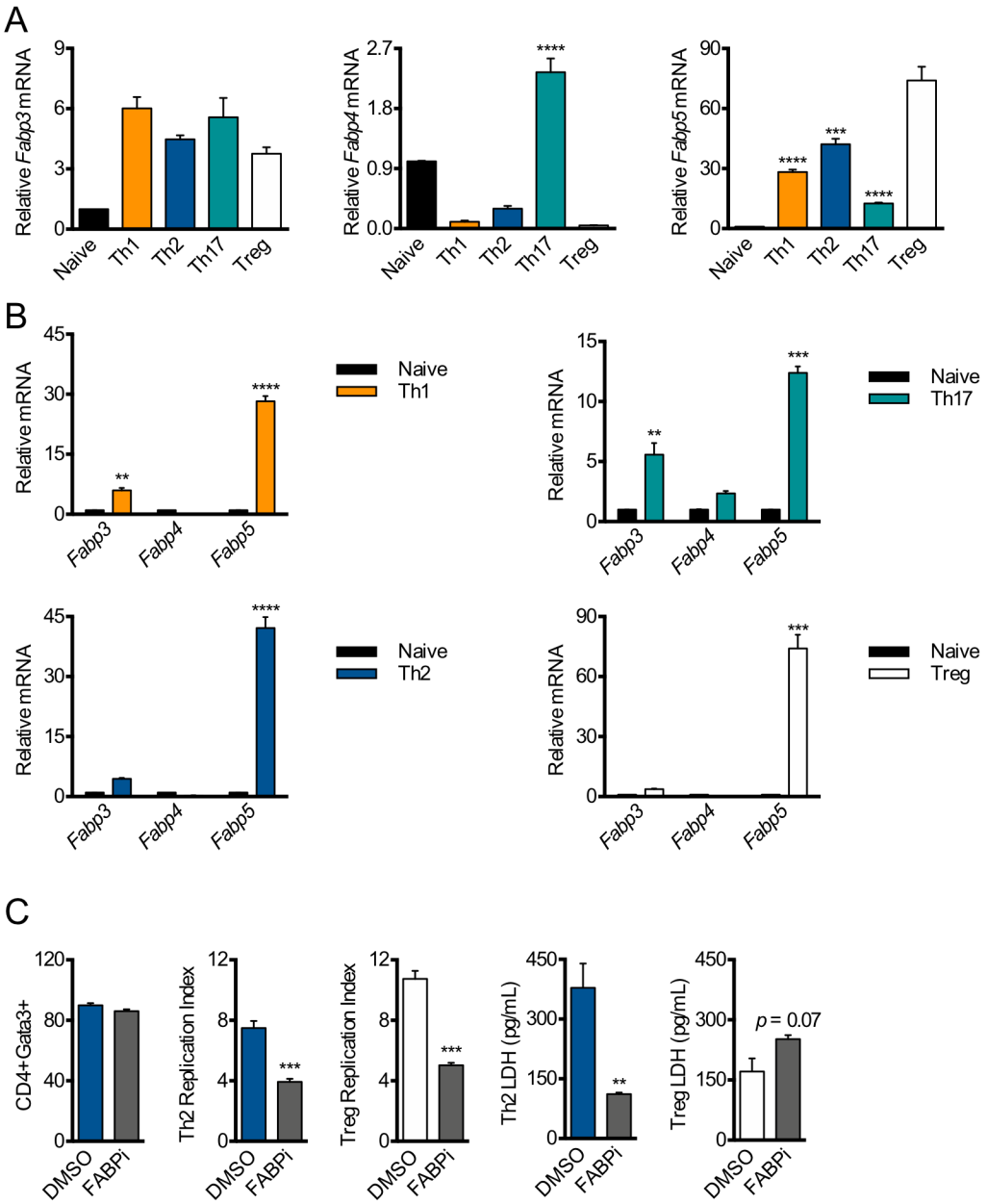

Figure S1 (Related to Figure 1). Measurement of FABP3, 4 and 5 in *in vitro* differentiated Th cell subsets. (**A**) Naïve CD4$^+$ T cells were cultured for four days under Th1, Th2, Th17 or Treg differentiation conditions. Mean relative expression (±SEM) of *Fabp3*, *Fabp4* or *Fabp5* mRNA in *in vitro* differentiated Th cell subsets compared to naïve CD4$^+$ T cells (n = 4). Results represent two independent experiments. (**B**) Mean relative expression (±SEM) of *Fabp3*, *Fabp4* or *Fabp5* mRNA in *in vitro* differentiated Th cell subsets, on a per subset basis, compared to naïve CD4$^+$ T cells (n = 4). Results represent two independent experiments. (**C**) Naïve CD4$^+$ T cells were cultured for three days under Th2, or Treg differentiation conditions in the presence of BMS309403 or vehicle control. Quantification of GATA3 expression, replication index (±SEM) and LDH from the supernatant of Th2 cells or Tregs cultured in the presence or absence of the FABP5 inhibitor BMS309403 (n = 4). Results represent three independent experiments. * P < 0.05, ** P <0.01, *** P <0.005, **** P < 0.001. P values were calculated using a two-way ANOVA with Bonferoni correction (**A**) or two-tailed, unpaired T test (**B**, **C)**. For clarity, only significant differences between Tregs and Th subsets are depicted in (**A**).

Figure S2 (Related to Figure 1). Bioenergetics of Tregs after BMS309403 treatment and of Tregs from *Fabp4/5* dKO mice.

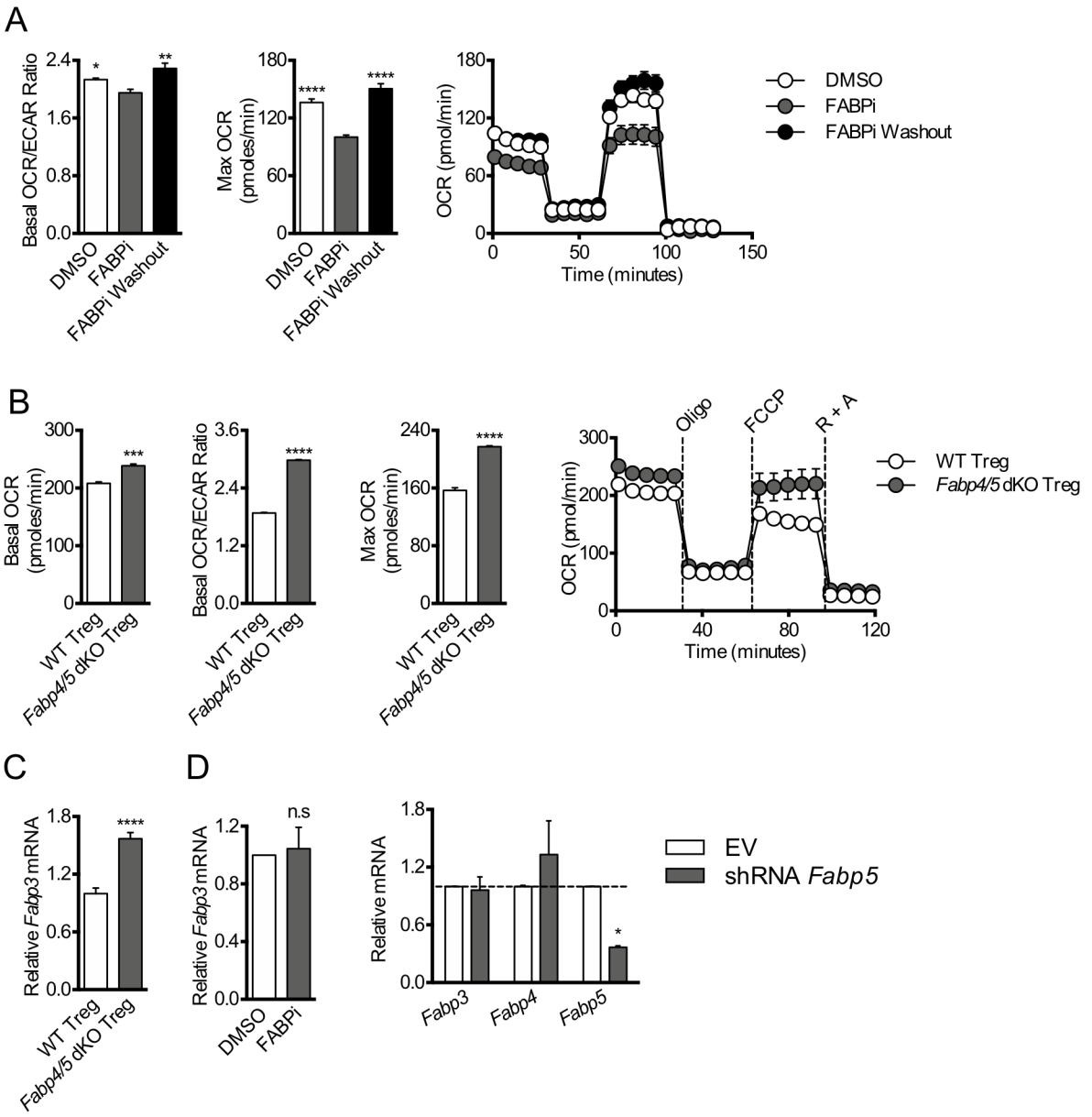

Figure S2 (Related to Figure 1). Bioenergetics of Tregs after BMS309403 treatment and of Tregs from *Fabp4/5* dKO mice. (**A**) Mean (±SEM) basal OCR, basal ECAR, OCR/ECAR ratio and maximal respiration (after FCCP) of *in vitro* differentiated mouse Tregs (*n* = 5) following overnight FABP5 inhibition or FABP5 inhibitor wash out. Results represent two independent experiments. (**B**) Mean (±SEM) basal OCR, OCR/ECAR ratio and maximal respiration of *in vitro* differentiated Tregs from WT or *Fabp4/5* dKO mice. OCR of *in vitro* differentiated Tregs at baseline and in response to oligomycin (Oligo), FCCP, and rotenone and antimycin A (R + A) (*n* = 3). Results represent two independent experiments. (**C**) Mean (±SEM) expression of *Fabp3* in WT or *Fabp4/5* dKO *in vitro* generated Tregs measured by qPCR (*n* = 3). Results represent two independent experiments. (**D**) Mean (±SEM) expression of *Fabp3* in Tregs following overnight BMS309403 treatment measured by qPCR (*n* = 3) and mean (±SEM) expression of *Fabp3*, *Fabp4* and *Fabp5* in Tregs following shRNA mediated knockdown of *Fabp5*. Results represent two independent experiments. Results represent two independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$. *P* values were calculated using a two-tailed, unpaired T test (**A**, **B**, **C, D)**.

Figure S3 (Related to Figures 1 and 2). IL-15 Tmem cells do not show metabolic defects following FABP5 inhibition.

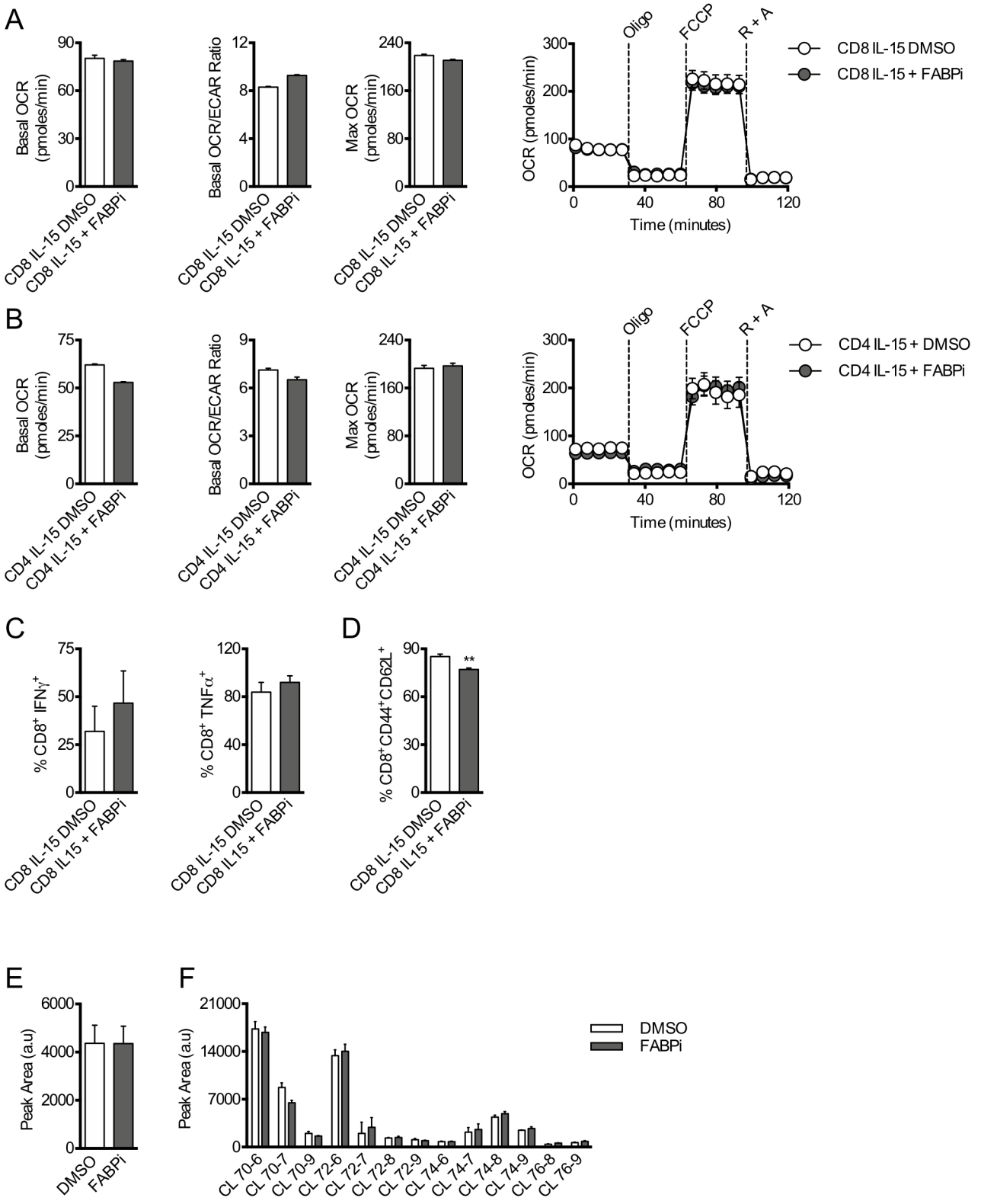

Figure S3 (Related to Figures 1 and 2). IL-15 Tmem cells do not show metabolic defects following FABP5 inhibition. Naïve $CD8^+$ or $CD4^+$ T cells were cultured with IL-2 for 3 days. Cells were then cultured in the presence of IL-15 from days 4-6 to generate *in vitro* Tmem cells, in the presence or absence of BMS309403. (**A**) Mean (±SEM) basal OCR, OCR/ECAR ratio and maximal respiration of *in vitro* differentiated $CD8^+$ Tmem cultured in the presence or absence of BMS309403 during IL-15 culture. OCR of *in vitro* differentiated $CD8^+$ Tmem cultured in the presence or absence of BMS309403 overnight at baseline and in response to oligomycin (Oligo), FCCP, and rotenone and antimycin A (R + A) (n = 4). Results represent three independent experiments. (**B**) Mean (±SEM) basal OCR, OCR/ECAR ratio and maximal respiration of *in vitro* differentiated $CD4^+$ Tmem cultured in the presence or absence of BMS309403 during IL-15 culture. OCR of *in vitro* differentiated $CD4^+$ Tmem cultured in the presence or absence of BMS309403 overnight at baseline and in response to oligomycin (Oligo), FCCP, and rotenone and antimycin A (R + A) (n = 4). Results represent three independent experiments. (**C**) Mean (±SEM) expression of IFNγ and TNFα by Tmem cells cultured as (**A**) (n = 4). Results represent two independent experiments. (**D**). Mean (±SEM) expression of $CD8^+CD44^+CD62L^+$ by Tmem cells cultured as (**A**). Results represent two independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$. P values were calculated using a two-tailed, unpaired T test (**A**, **B**, **C**, **D**). (**E**) Mean (±SEM) quantification of total cardiolipin content in IL-15 cells (n = 4). (**F**) Mean (±SEM) quantification of individual cardiolipin species in IL-15 cells (n = 4). Results represent one experiment, P values were calculated using a two-tailed, unpaired T test (**E**) or a two-way ANOVA with Bonferroni correction (**F**).

Figure S4 (Related to Figure 4). Opa1-deficient Tregs have metabolic defects, but no type I IFN signaling.

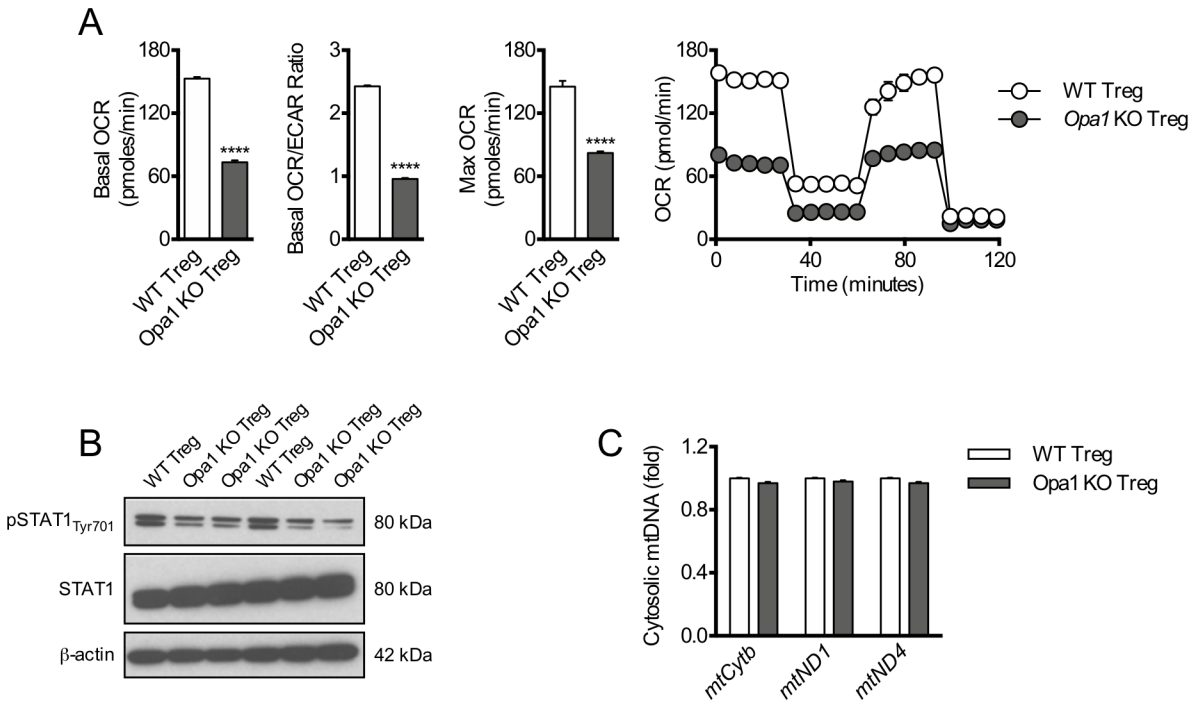

Figure S4 (Related to Figure 4). Opa1-deficient Tregs have metabolic defects, but no type I IFN signaling. Naïve CD4$^+$ T cells from WT or *Opa1*$^{-/-}$ mice were cultured for 4 days under Treg differentiation conditions before metabolic assessment. (**A**) Mean (±SEM) basal OCR, OCR/ECAR ratio and maximal respiration of *in vitro* differentiated Tregs from WT or *Opa1*$^{-/-}$ mice. OCR of *in vitro* differentiated Tregs from WT or *Opa1*$^{-/-}$ mice at baseline and in response to oligomycin (Oligo), FCCP, and rotenone and antimycin A (R + A) (n = 2-4). (**B**) Protein expression of pSTAT1$_{Tyr701}$ in *in vitro* generated Tregs from WT or *Opa1*$^{-/-}$ mice. Results represent two independent experiments. (**C**) Quantification of cytosolic mtDNA content of WT or *Opa1*$^{-/-}$ Tregs (n = 4). Results represent two independent experiments. **** $P < 0.001$. P values were calculated using a two-tailed, unpaired T test (**A**).

Figure S5 (Related to Figure 4). Type I interferon signaling induced by FABP5 inhibition is downstream of metabolic deficiencies.

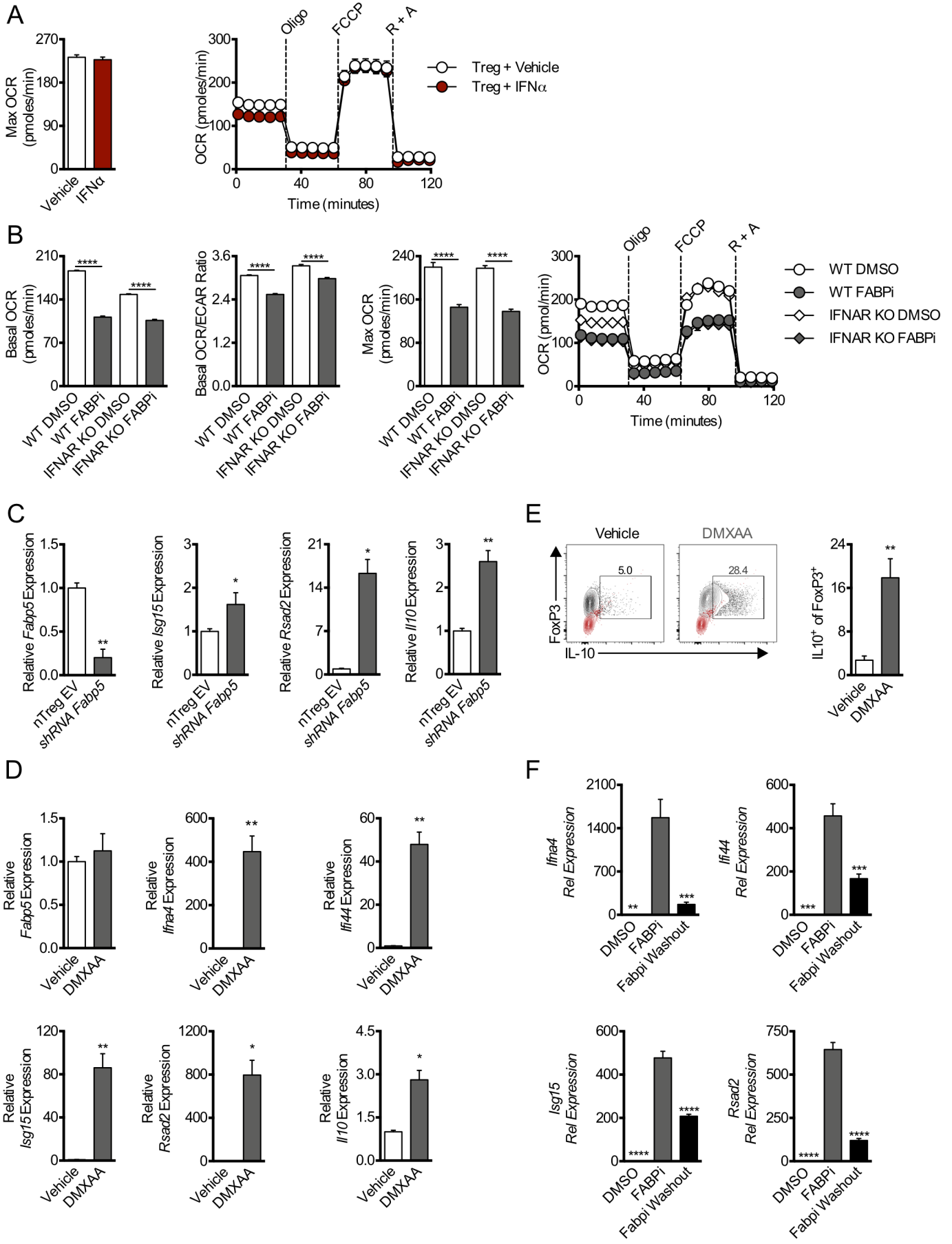

Figure S5 (Related to Figure 4). Type I interferon signaling induced by FABP5 inhibition is downstream of metabolic deficiencies. Naïve $CD4^+$ T cells were cultured for 3 days under Treg cell differentiation conditions before overnight IFNα treatment. (**A**) Mean (±SEM) maximal respiration (after FCCP) and OCR of in vitro differentiated murine Tregs cultured in the presence or absence of IFNα at baseline and in response to oligomycin (Oligo), FCCP, and rotenone and antimycin A (R + A) (n = 4). Results represent three independent experiments. (**B**) Mean (±SEM) basal OCR, OCR/ECAR ratio and maximal respiration (after FCCP) of *in vitro* differentiated murine Tregs from WT or IFNAR KO mice following acute FABP5 blockade at baseline and in response to oligomycin (Oligo), FCCP, and rotenone and antimycin A (R + A) (*n* = 4). Results represent three independent experiments. (**C**) Mean (±SEM) expression of *Fabp5* and type I IFN related genes in nTregs after shRNA against *Fabp5* measured by qPCR (*n* = 4). Results represent two independent experiments. (**D**) Mean (±SEM) expression of type I IFN related genes in Tregs after DMXAA treatment measured by qPCR (*n* = 4). Results represent two independent experiments. (**E**) Mean (±SEM) protein expression of IL-10 in Tregs following after overnight DMXAA treatment (*n* = 5). Results represent two independent experiments. Gating controls depicted in red. (**F**) Mean (±SEM) expression of type I IFN related genes in Tregs after BMS309403 treatment, or BMS309403 washout measured by qPCR (*n* = 4). Results represent two independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$. *P* values were calculated using a two-tailed, unpaired T test (**A**, **B**, **C**, **D, E**) or one-way ANOVA with Bonferroni correction (**F**).