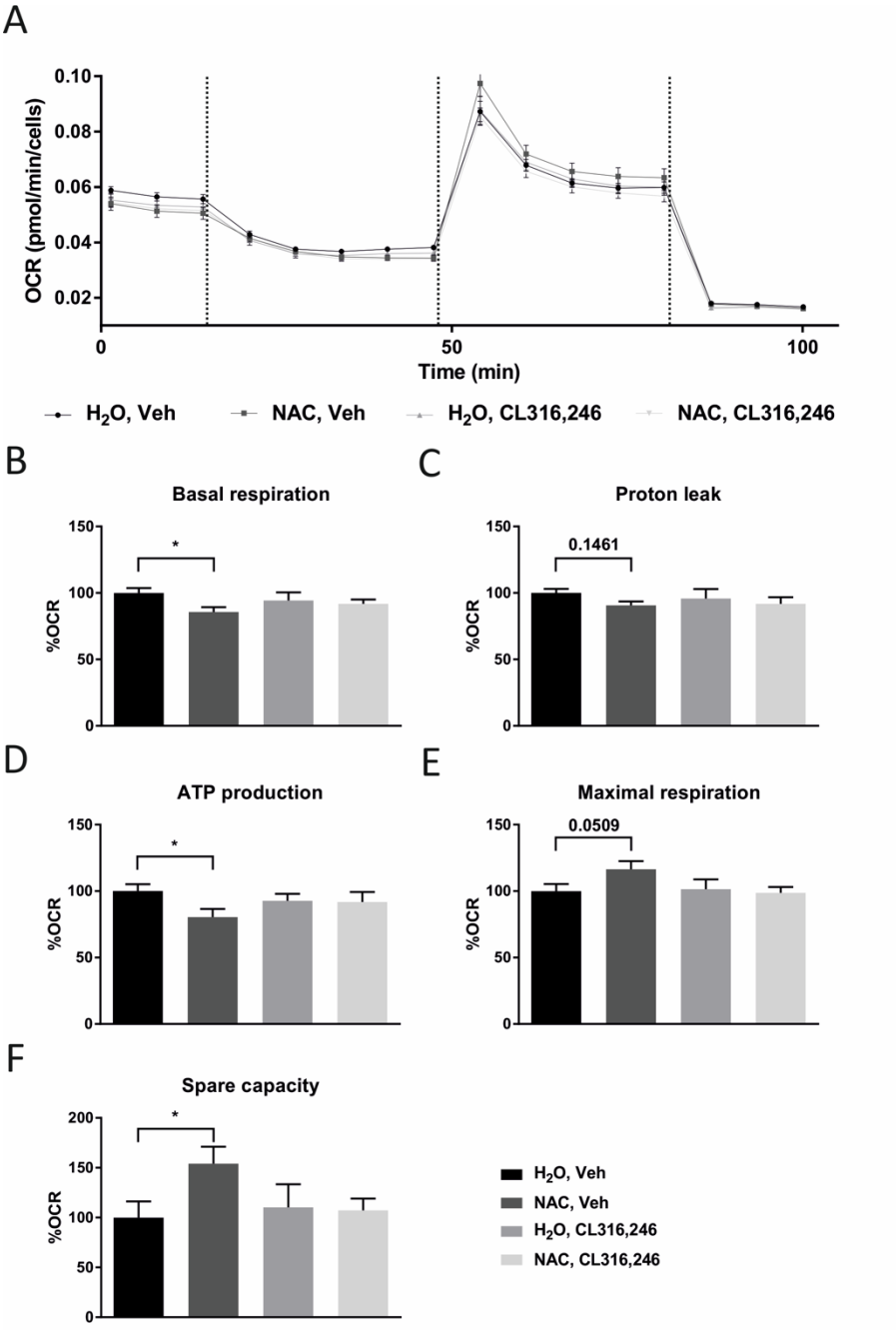
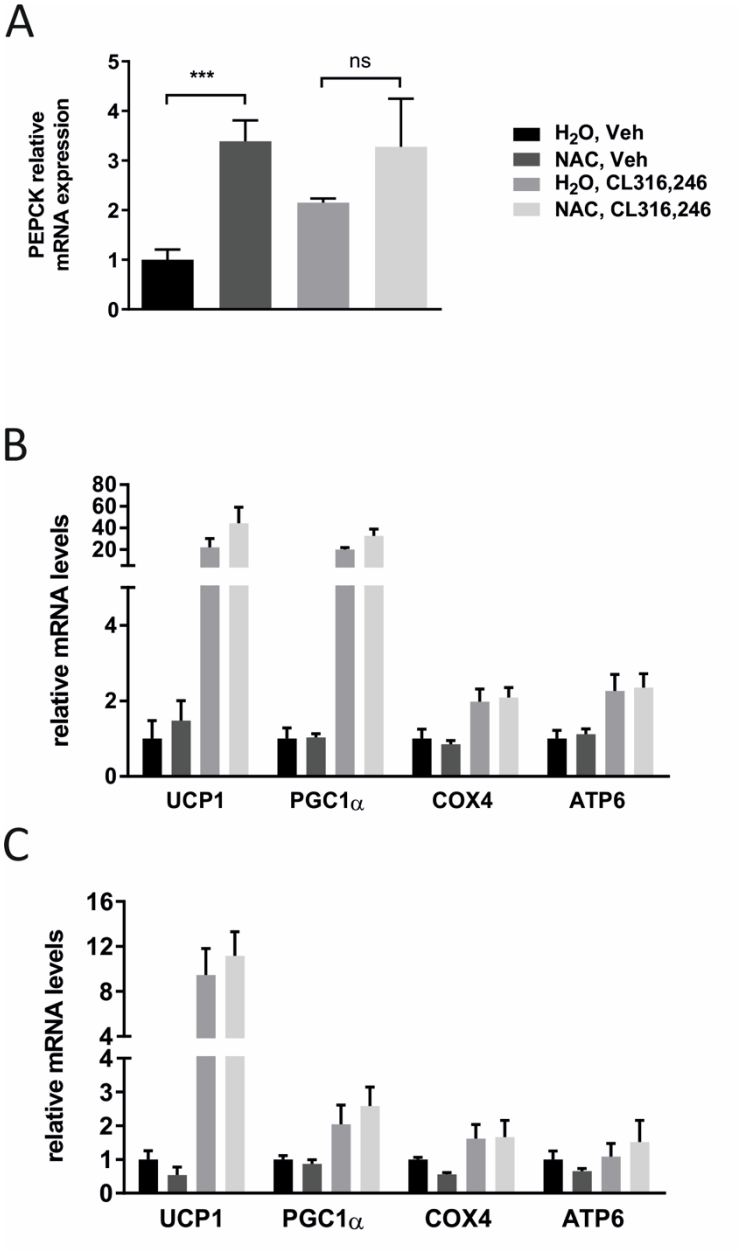
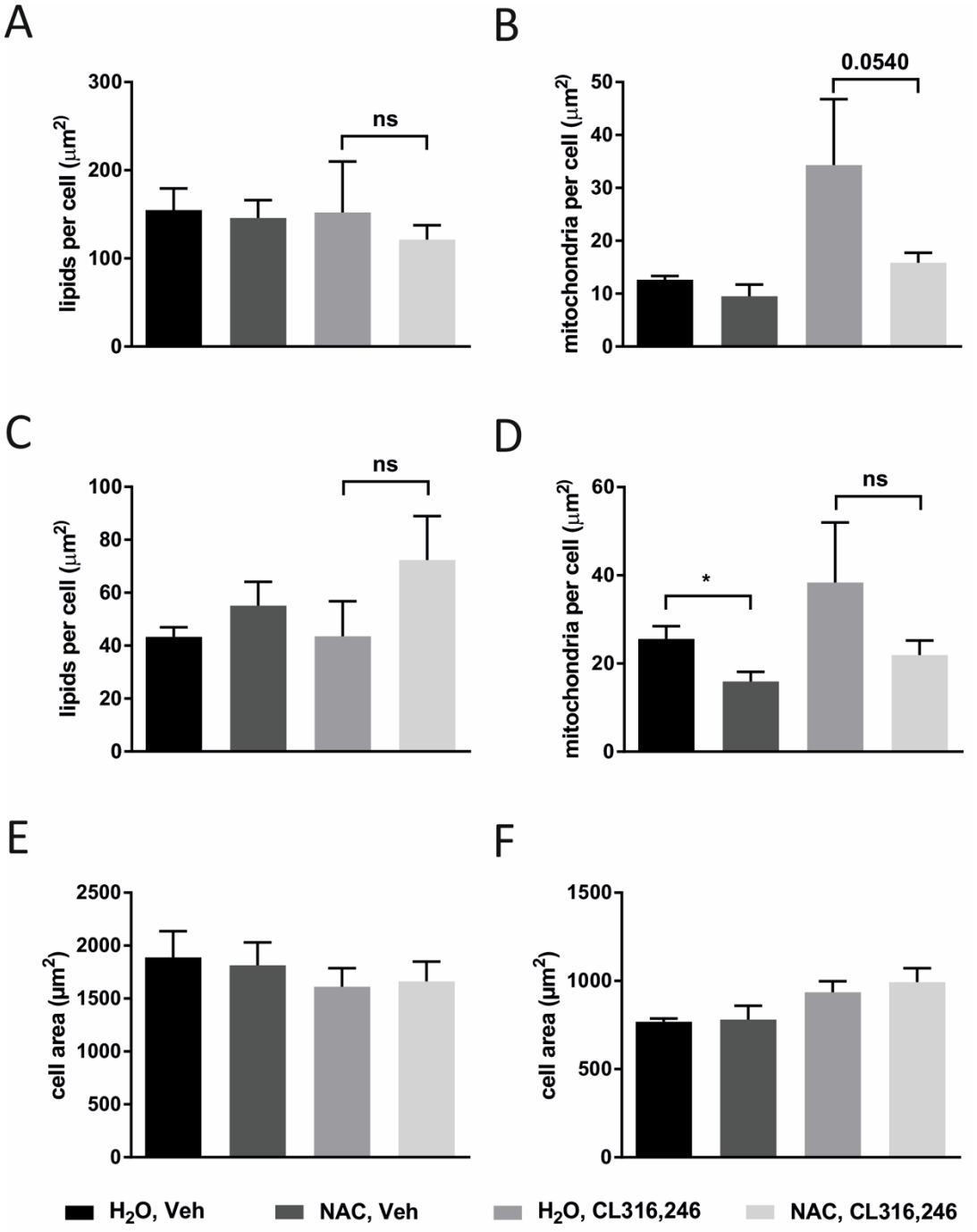
Supplemental Figures



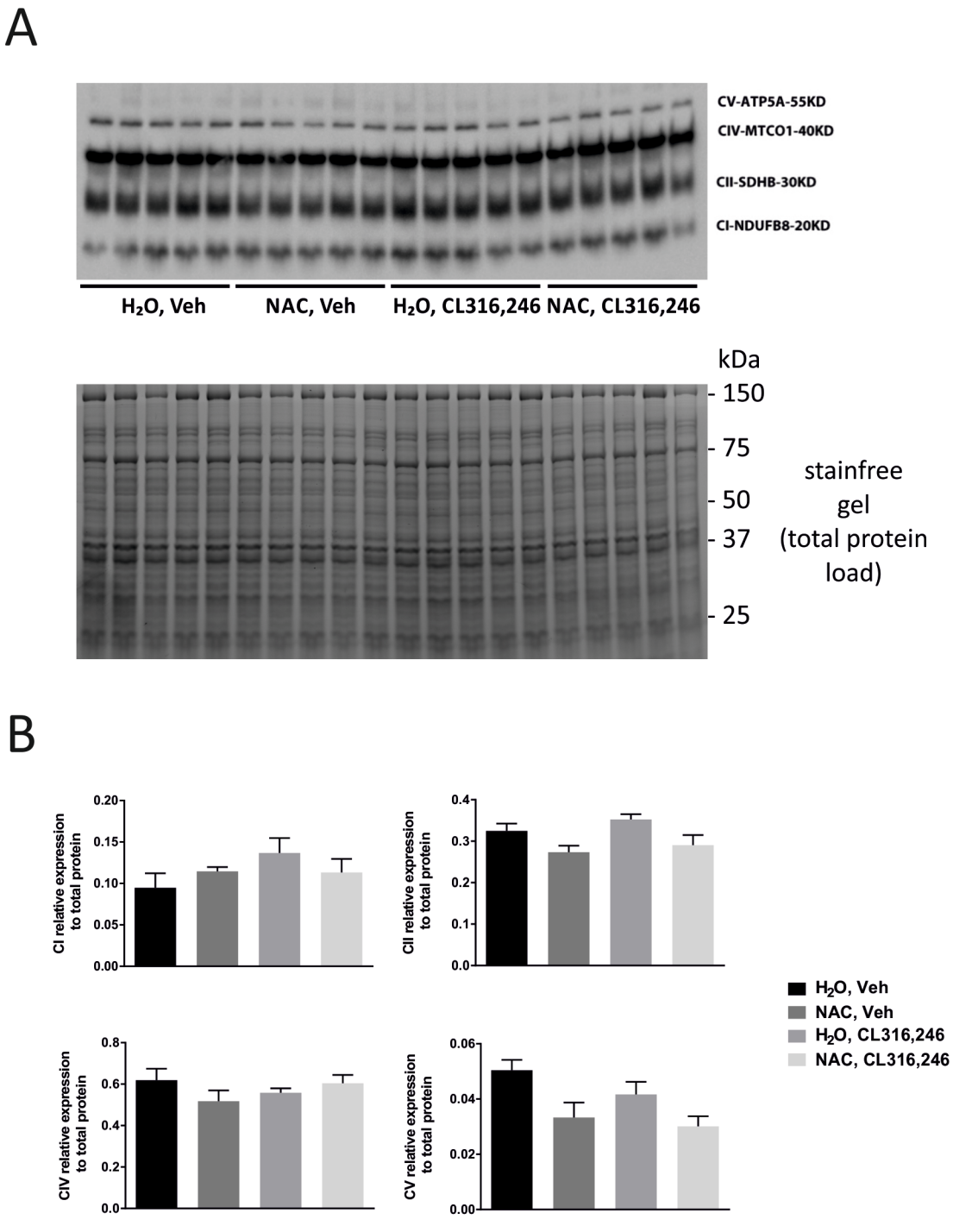
Supplemental Figure 1. NAC reduces oxygen consumption and modifies mitochondrial function in 3T3-L1 adipocytes. (A) Cultured adipocytes treated with 1mM NAC for 48h and 1µM CL316,246 for 24h were analyzed using the Seahorse technology; cells were sequentially treated with 1µM oligomycin, 0,6µM FCCP, and 0,5µM rotenone plus antimycin A to assess mitochondrial function. All metabolic parameters are calculated following Agilent's instructions and are shown relative to those in control cells. (B) Basal respiration, (C) Proton leak, (D) ATP production, (E) Maximal respiration and (F) Spare capacity (n=8/group, *p<0,05).



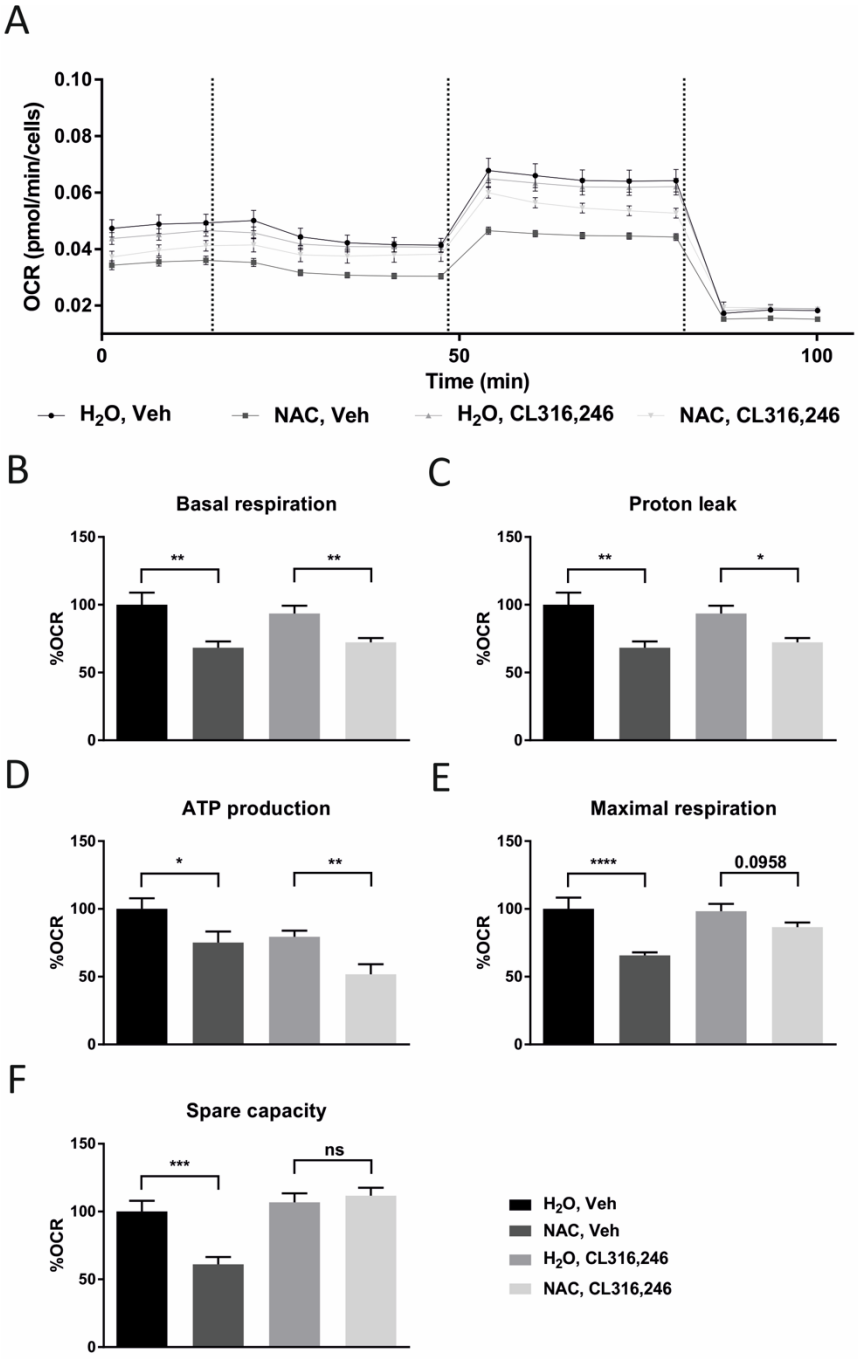
Supplemental Figure 2. NAC increases the PEPCK expression, but has no effect on the acute CL316,246-induced upregulation of browning markers in IWAT. (A) Relative PEPCK levels in IWAT 10 days after CL316,246 treatment (n=10/group, ***p<0.001). (B) Relative mRNA expression of UCP1, PGC1α, COX4, and ATP6 in IWAT, 3h post CL316,246 injection (n=10/group). (C) Relative mRNA expression of UCP1, PGC1α, COX4, and ATP6 in IWAT, 24h post CL316,246 injection (n=10/group).



Supplemental Figure 3. NAC pretreatment reduces mitochondrial activity in white and brown adipose tissue in response to β3-AR stimulation. (A) Quantification of lipid content and **(B)** mitochondrial density/activity per cell of fresh pieces of IWAT of C57/Bl6 mice pretreated for 1 week with either water or NAC 1g/l solution followed by 10 days injection with either vehicle or 1µg of CL316,246 per g of body weight (n=5/group, *p<0.05, **p<0.01). **(C)** Quantification of lipid content and **(D)** mitochondrial density/activity of fresh pieces of BAT. **(E)** Average cell area in IWAT and **(F)** BAT of the analyzed experiments.



Supplemental Figure 4. NAC treatment does not affect OXPHOS protein expression in BAT. (A) Immunoblot of BAT lysates labeled with Total OXPHOS Rodent WB Antibody Cocktail (Abcam). (B) Quantification of the different components separately (n=5/group).



Supplemental Figure 5. NAC reduces oxygen consumption and modifies mitochondrial function in primary brown adipocytes. (A) Primary brown cultured adipocytes treated with 1mM NAC for 48h and 1µM CL316,246 for 24h were analyzed using the Seahorse Technology; cells were sequentially treated with 1µM oligomycin, 0,6µM FCCP, and 0,5µM rotenone plus antimycin A to assess mitochondrial function. All metabolic parameters are calculated following Agilent's instructions and are shown relative to those in control cells. (B) Basal respiration, (C) Proton leak, (D) ATP production, (E) Maximal respiration and (F) Spare capacity (n=14/group, *p<0,05, **p<0,01, ***p<0,001, ****p<0,0001).