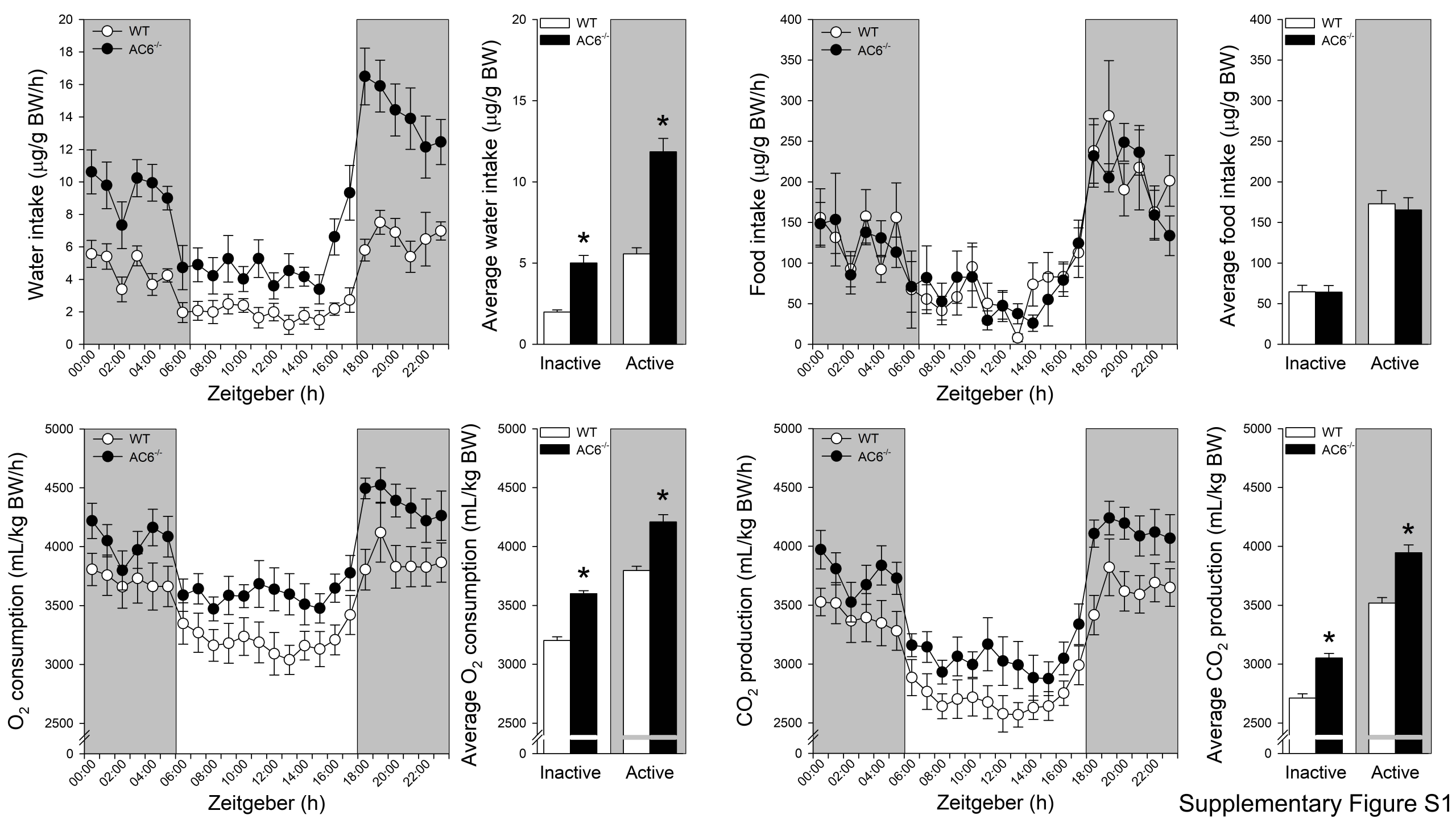
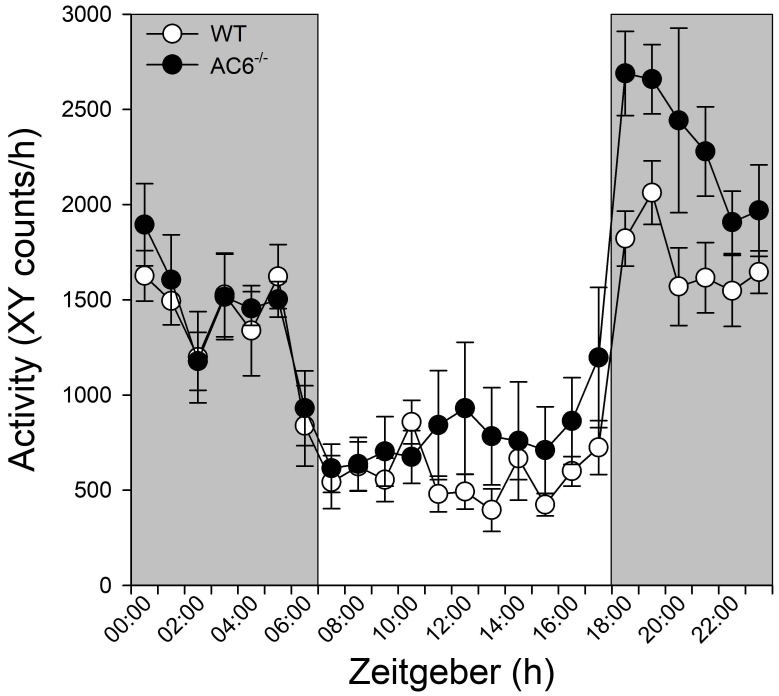
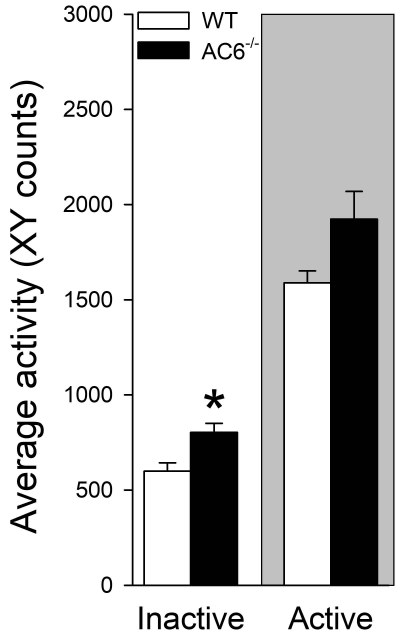
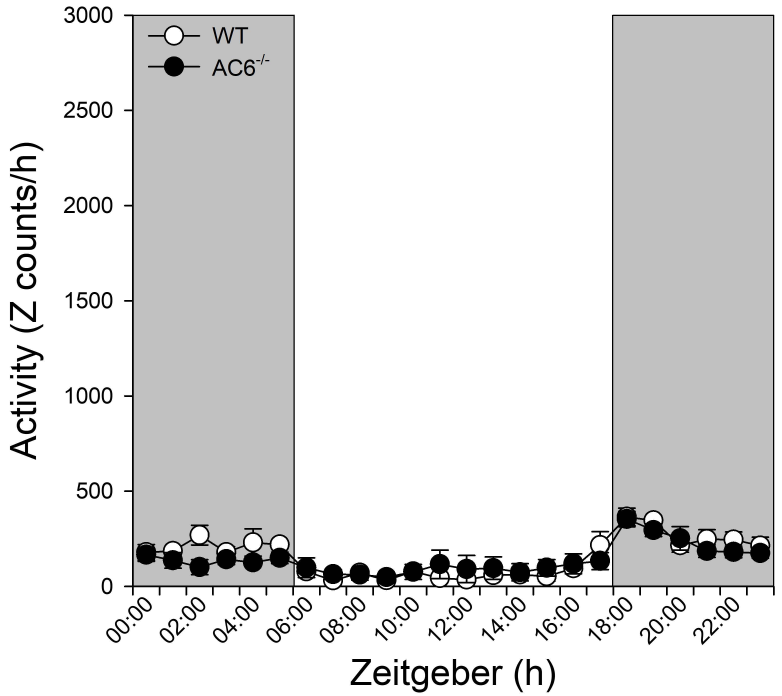
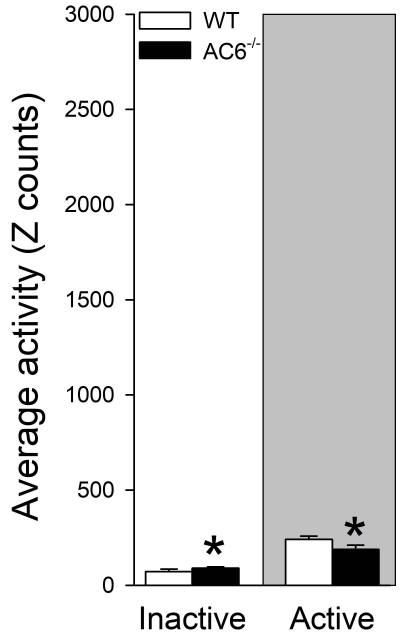


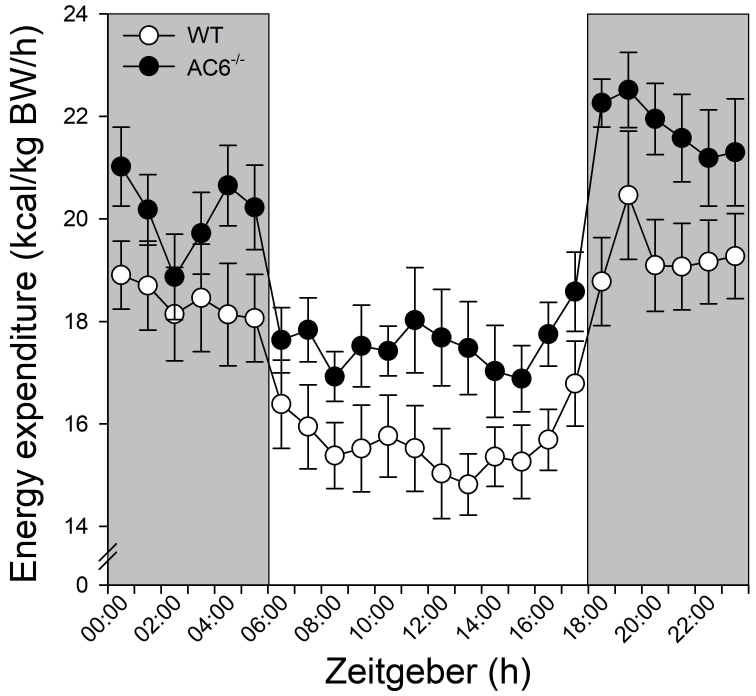
Supplementary Figure S1

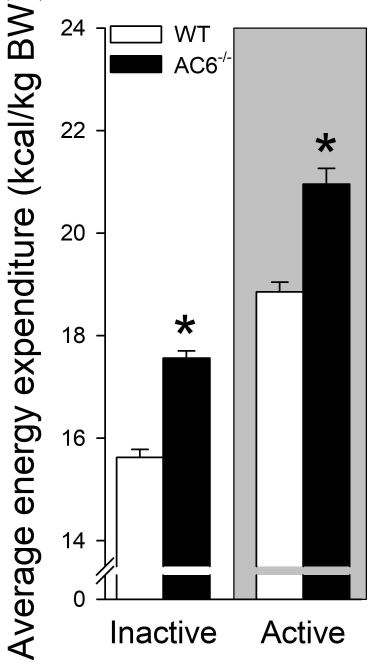




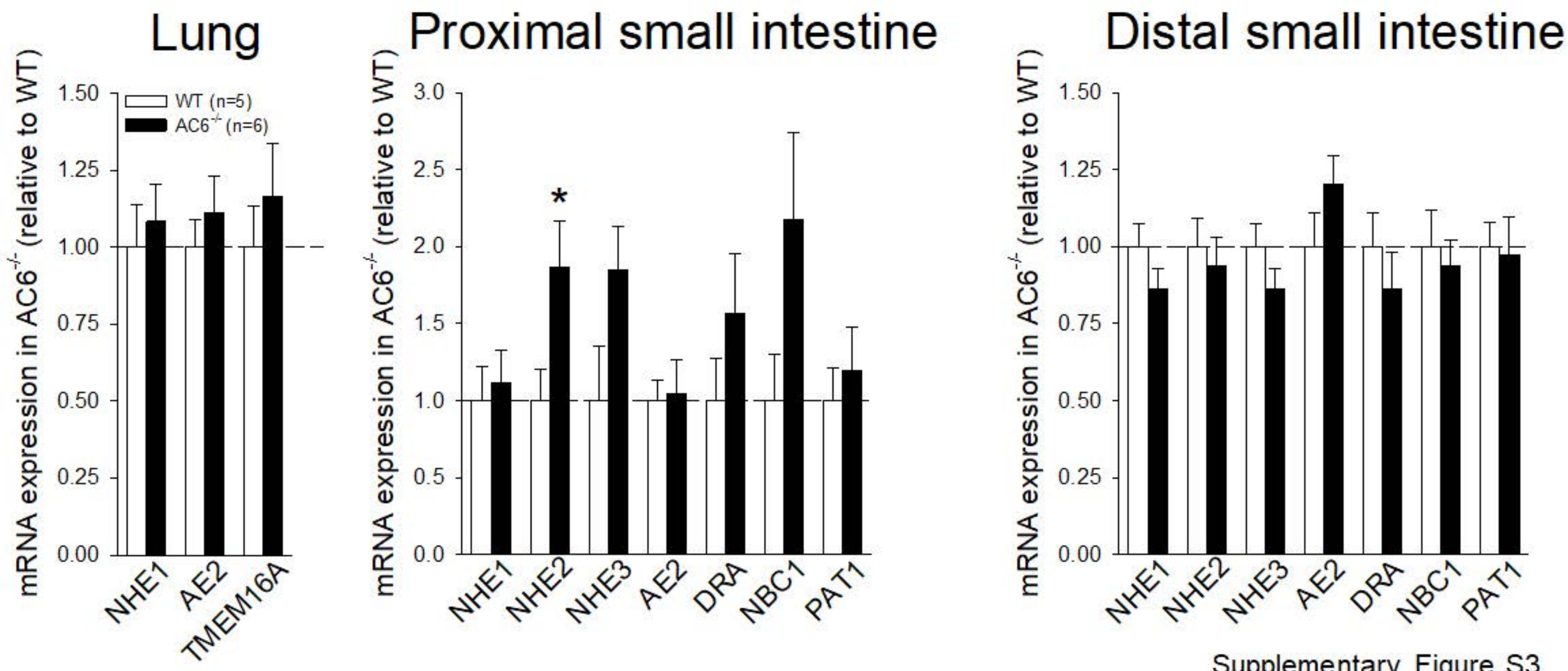






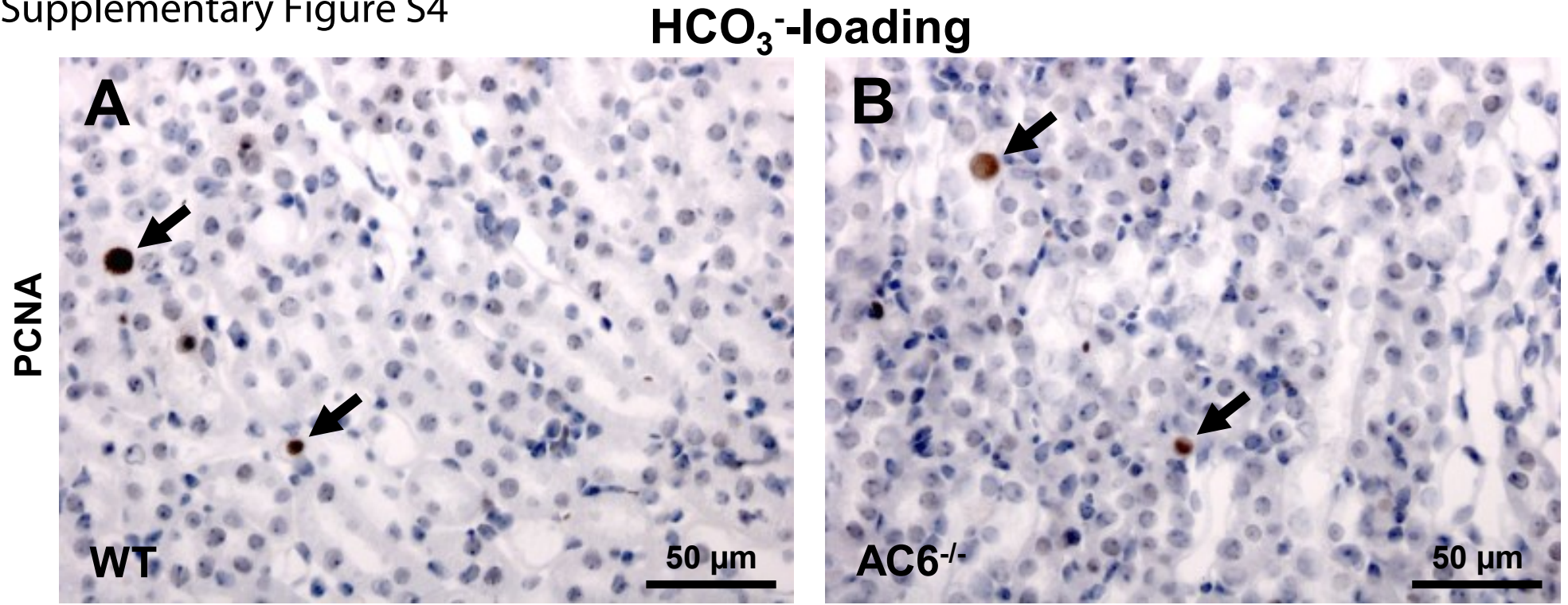


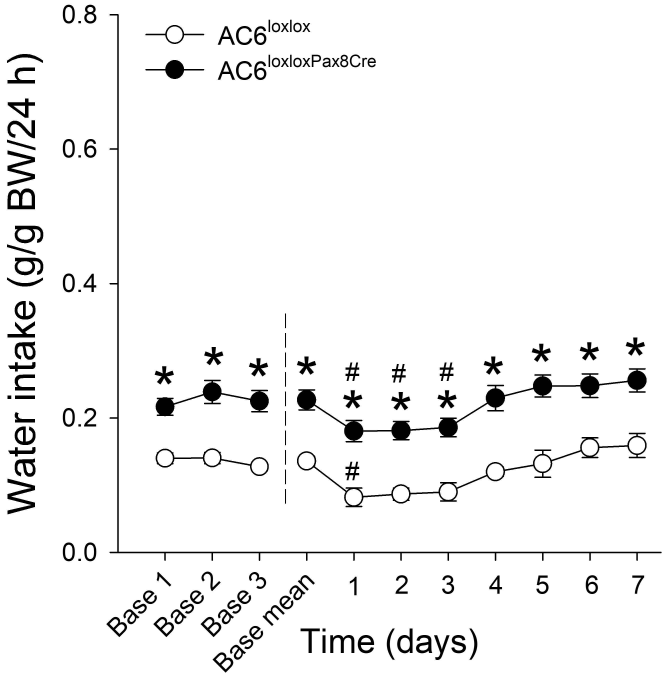
Supplementary Figure S2

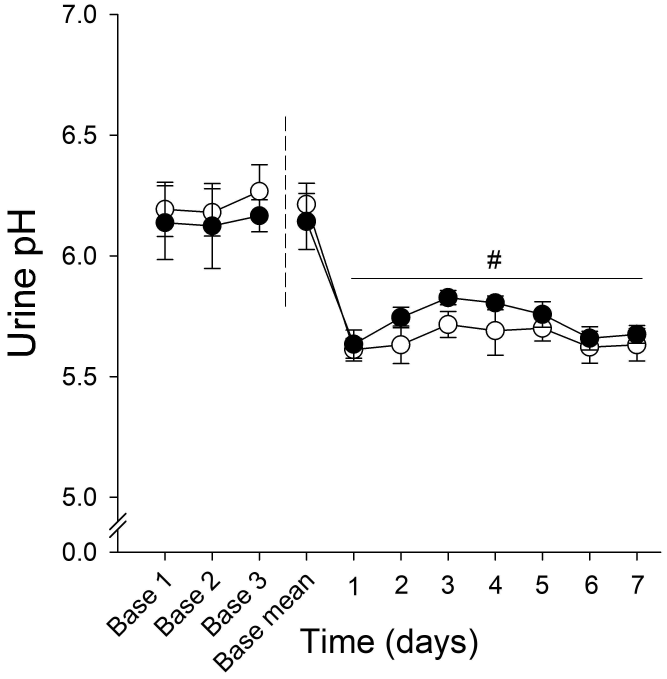


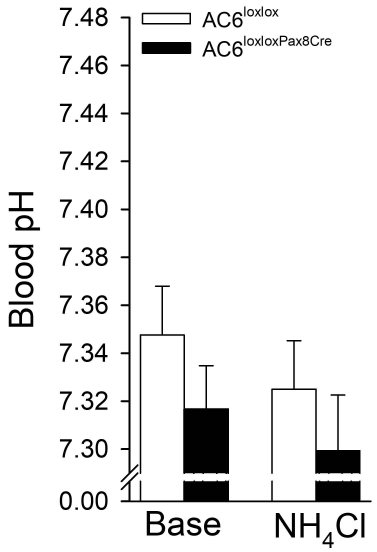
Supplementary Figure S3

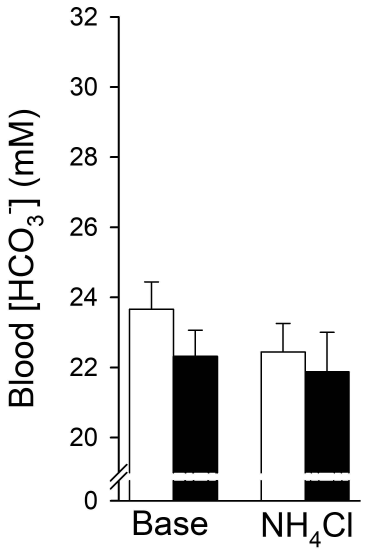
Supplementary Figure S4











Supplementary Figure S5

Supplementary Figure Legends

Supplementary Figure S1. $AC6^{-/-}$ mice have normal circadian rhythm but elevated O_2 consumption and CO_2 production. Consistent with our previously published data (1) $AC6^{-/-}$ mice show increased fluid intake. The experiments in PhenoMaster® metabolic cages identified a significantly higher water intake during their inactive and active period. No differences in food intake were observed between genotypes. Both, O_2 consumption and CO_2 production were significantly higher during the inactive and active period in $AC6^{-/-}$ versus WT mice. *n*=6/genotype, **P*<0.05 versus WT.

Supplementary Figure S2. $AC6^{-/-}$ mice have increased energy expenditure. Experiments in PhenoMaster® metabolic cages identified that activity in the XY-axis was significantly higher in $AC6^{-/-}$ during the inactive period. Activity in the Z-axis is only a fraction compared to the XY activity. Whereas 24-hour Z-axis activity was not different between genotypes, Z-activity in $AC6^{-/-}$ mice was elevated and reduced during the inactive and active period, respectively. $AC6^{-/-}$ mice have, independent of the active/inactive period, increased energy expenditure compared to WT mice. *n*=6/genotype, **P*<0.05 versus WT.

Supplementary Figure S3. mRNA expression of various transporters and channels in lung and proximal/distal small intestine of WT (*n*=5) and $AC6^{-/-}$ (*n*=6) mice. No differences between genotypes were observed in lung tissue mRNA expression. The proximal small intestine of $AC6^{-/-}$ mice had significantly higher expression of NHE2, and tendencies for higher NHE3 and NBC1 expression. No significant differences in transporter profiles were observed between genotypes in the distal small intestine. **P*<0.05 versus WT. Na^+-H^+ exchanger 1, NHE1; Na^+-H^+ exchanger 2, NHE2; Na^+-H^+ exchanger 3, NHE3, anion exchanger 2, AE2; down regulated in adenoma, DRA; Na^+-HCO_3^- cotransporter 1, NBC1; putative anion transporter 1, PAT1; Ca^{2+}-activated Cl^- channel, TMEM16A.

Supplementary Figure S4. $AC6^{-/-}$ mice do not present more PCNA-positive cells HCO_3^- after 8 days of HCO_3^- challenge. Representative light microscopy images of 2-µm kidney sections from $AC6^{-/-}$ and WT mice subjected to HCO_3^- challenge for 8 days. Immunolabeling was performed using an antibody targeting PCNA. (A and B) No difference was found between the numbers of cells labeled for PCNA in $AC6^{-/-}$ compared to WT mice. n=6/genotype. Arrows indicate PCNA labeled cells.

Supplementary Figure S5. No differences were observed between $AC6^{loxlox}$ and $AC6^{loxloxPax8Cre}$ mice in response to 7 days of NH_4Cl challenge. Because daily water intake is double in $AC6^{loxloxPax8Cre}$ mice, NH_4Cl was supplied to this genotype at a 50% lower concentration compared to $AC6^{loxlox}$ mice (0.14 M in $AC6^{loxloxPax8Cre}$ versus 0.28 M in

$AC6^{loxlox}$ mice). Fluid intake was slightly but significantly reduced on day 1 in $AC6^{loxlox}$ mice and on days 1-3 in $AC6^{loxloxPax8Cre}$ mice. Urinary pH was lowered to a similar extent during NH_4Cl challenge (n=7-8/genotype). Blood pH and HCO_3^- concentrations were not significantly different between genotypes and did not significantly change after 7 days NH_4Cl challenge (n=7-8). *P<0.05 versus $AC6^{loxlox}$, $^{\#}P$<0.05 baseline mean versus NH_4Cl challenge in both genotypes. Statistical comparisons on left and right side of dashed line in panel were performed separately using two-way repeated measurements ANOVAs. Values indicate mean ± S.E.M. Base, Baseline.

1. Rieg T, Tang T, Murray F, Schroth J, Insel PA, Fenton RA, et al. Adenylate cyclase 6 determines cAMP formation and aquaporin-2 phosphorylation and trafficking in inner medulla. J Am Soc Nephrol. 2010;21(12):2059-68.