Supplementary information and figures

Detailed methodological description of the molecular analyses

RNA isolation, cDNA, and qPCR

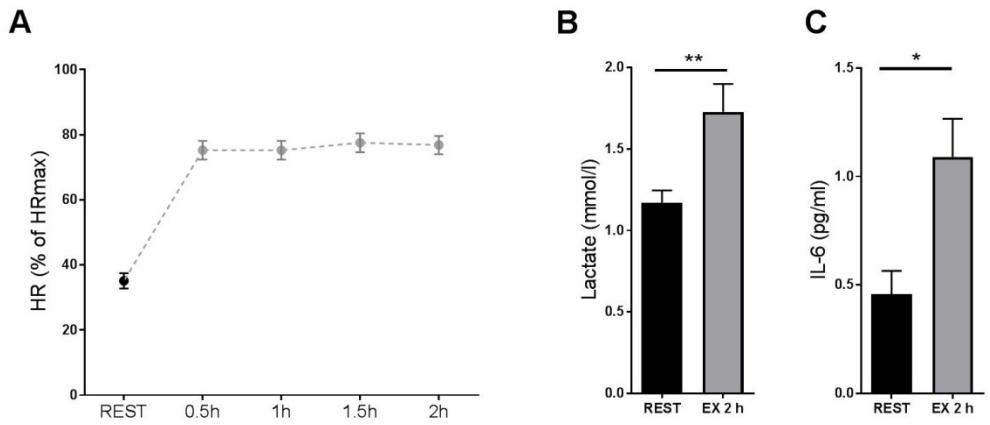
Total RNA was isolated by the Trizol method (Invitrogen Life Technologies), and RNA concentration was determined by a nanodrop spectrophotometer (Thermo Fischer Scientific). Reverse transcription reactions were performed using Reverse Transcriptase kit and random primers (Applied Biosystem) according to manufacturer's protocol. All amplifications were performed in triplets using SYBR green master mix (Applied Biosystem) and sequence specific primers. The relative expressions of the measured genes were determined after normalization to the endogenous control β-actin. All primer sequences can be specified upon request.

Western blotting

To investigate the regulation of YAP and TAZ in the cancer cells, they were stimulated for 15, 30, 60, and 120 min with media containing 10% resting/exercise conditioned serum from breast cancer patients +/- 10 µM propranolol or 10 µM EPI/NE/propranolol. Total protein from these cells and murine tumors were isolated as previously described (15). Protein concentration was measured by colorimetric protein assay (Bio-Rad). Loading buffer (Life Technologies) and 100 mM DTT was added and samples boiled for 3 min. Ten µg of the lysate was loaded on a TGX 4-15% Criterion gel (Bio-Rad) and run at 125V for 60 min. As molecular weight markers Precision blue protein dual color and Precision blue protein all blue (Bio-Rad) were used. Protein was transferred to a PVDF membrane using a TransBlot Turbo System at 25 V for 10 min (Bio-Rad). The membranes were blocked for 1 h with 1% FSG and incubated with the primary antibodies pYAP (Ser127), YAP, and TAZ (Cell Signaling) at concentrations 1:1000 overnight at 4°C. Membranes were subsequently washed and incubated with secondary IgG HRP conjugated antibody. Signal was detected using Supersignal West Femto Luminal/Enhancer Solution (Thermo Scientific) and exposed in a charge-

coupled device camera (Bio-Rad). For normalization all membranes were incubated in 0.5% Reactive Brown (Sigma Aldrich) for 10 min. Blots were analyzed and quantified using the software Image Lab 5.2 (Bio-Rad). Phos-tag YAP: Twenty ug of protein was loaded on phos-tag gels and run at 125 V for 2 hours. Gels were washed in Turbo transfer buffer (Bio-Rad) with 1 mM EDTA for 15 min and without EDTA for 15 min, before transfer and further development as described above.

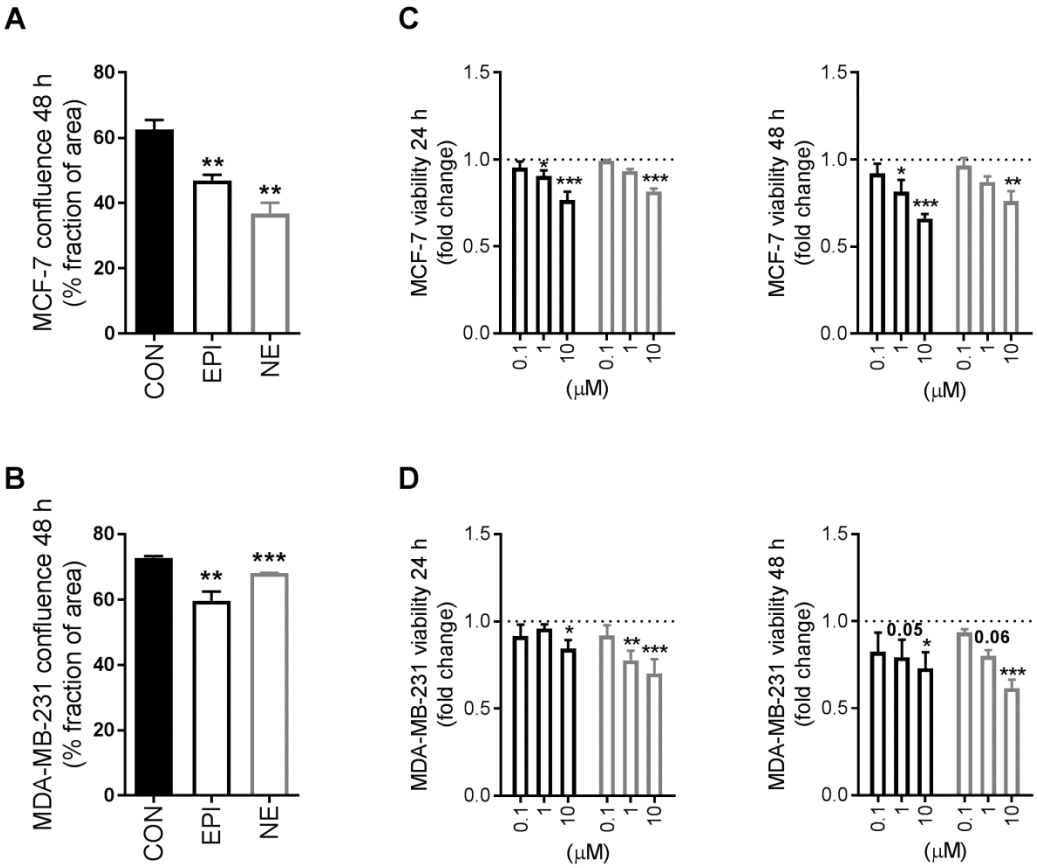
Supplementary figures



Supplementary figure S1

Heart rate and systemic responses to 2 hours of ergometer bicycling at 55% of VO_{2peak} in healthy women

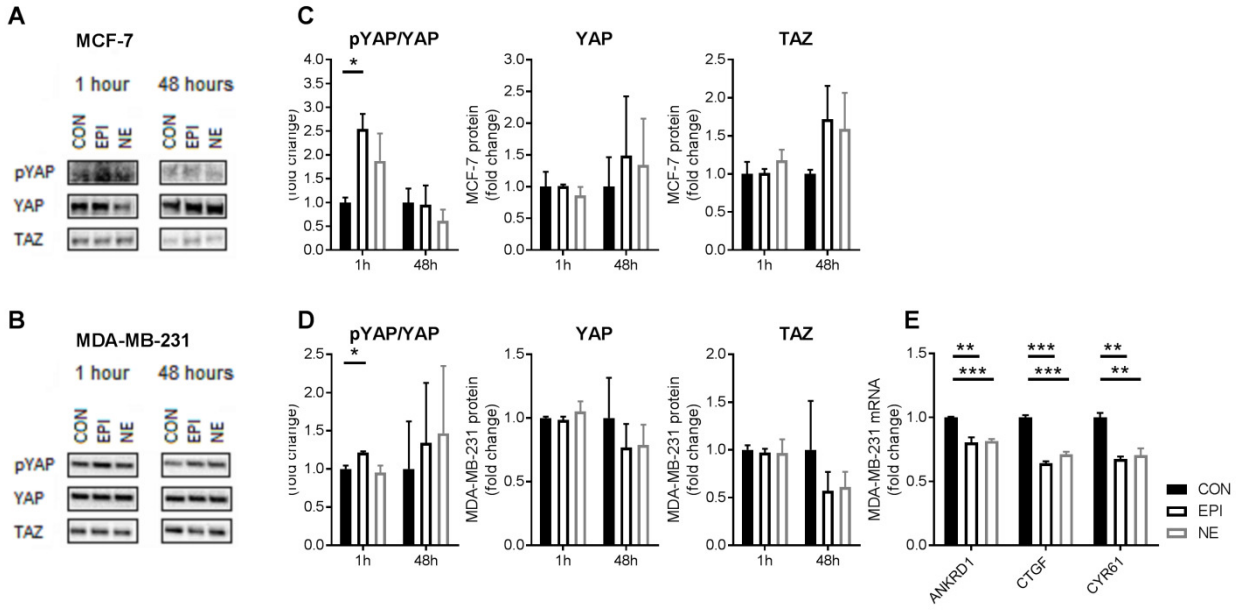
A) Percent of maximal heart rate (HR_{max}) during 2 hours of exercise. **B-C)** Serum levels of lactate and IL-6 at rest and after 2 hours of exercise. Data are presented as means ± SEM. Statistical significance was tested by paired t-tests. *p<0.05, **p<0.01.



Supplementary figure S2

Direct growth inhibitory effects of epinephrine and norepinephrine in vitro

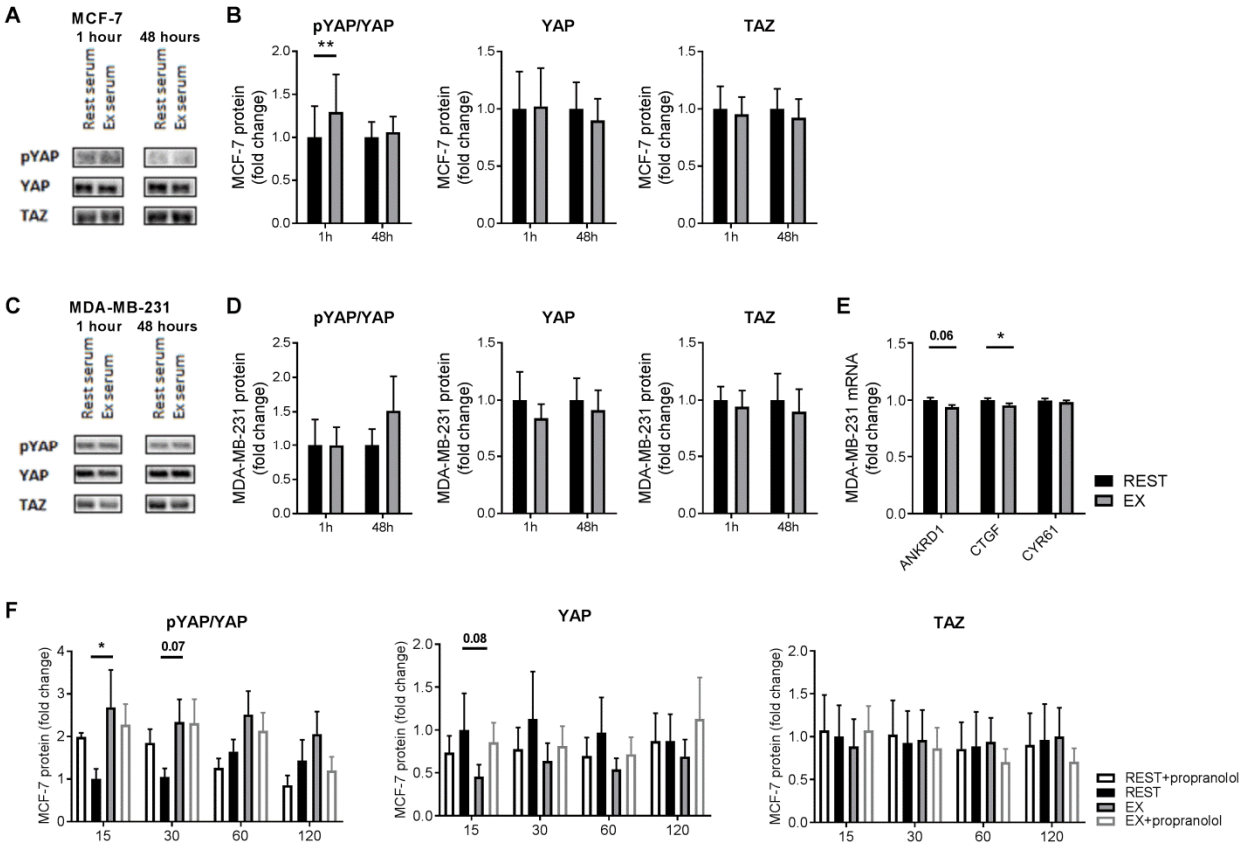
Confluence of **A)** MCF-7 and **B)** MDA-MB-231 cells stimulated with 10 µM epinephrine (EPI), 10 µM norepinephrine (NE), or vehicle (CON) for 48 hours measured as % fraction of area by ImageJ (n=3). Viability measured by MTT assay of **C)** MCF-7 and **D)** MDA-MB-231 cells after stimulation with epinephrine and norepinephrine at concentrations of 0, 0.1, 1, and 10 µM for 1-2 days (n=3). Results are shown as fold change from non-stimulated cells (dotted line). Data are presented as means ± SEM. Statistical significance of confluence was tested by an unpaired t-test and viability was tested by 2-way ANOVA with repeated measures. *p<0.05, **p<0.01, ***p<0.001.



Supplementary figure S3

Regulation of the Hippo-pathway in breast cancer cells by epinephrine and norepinephrine

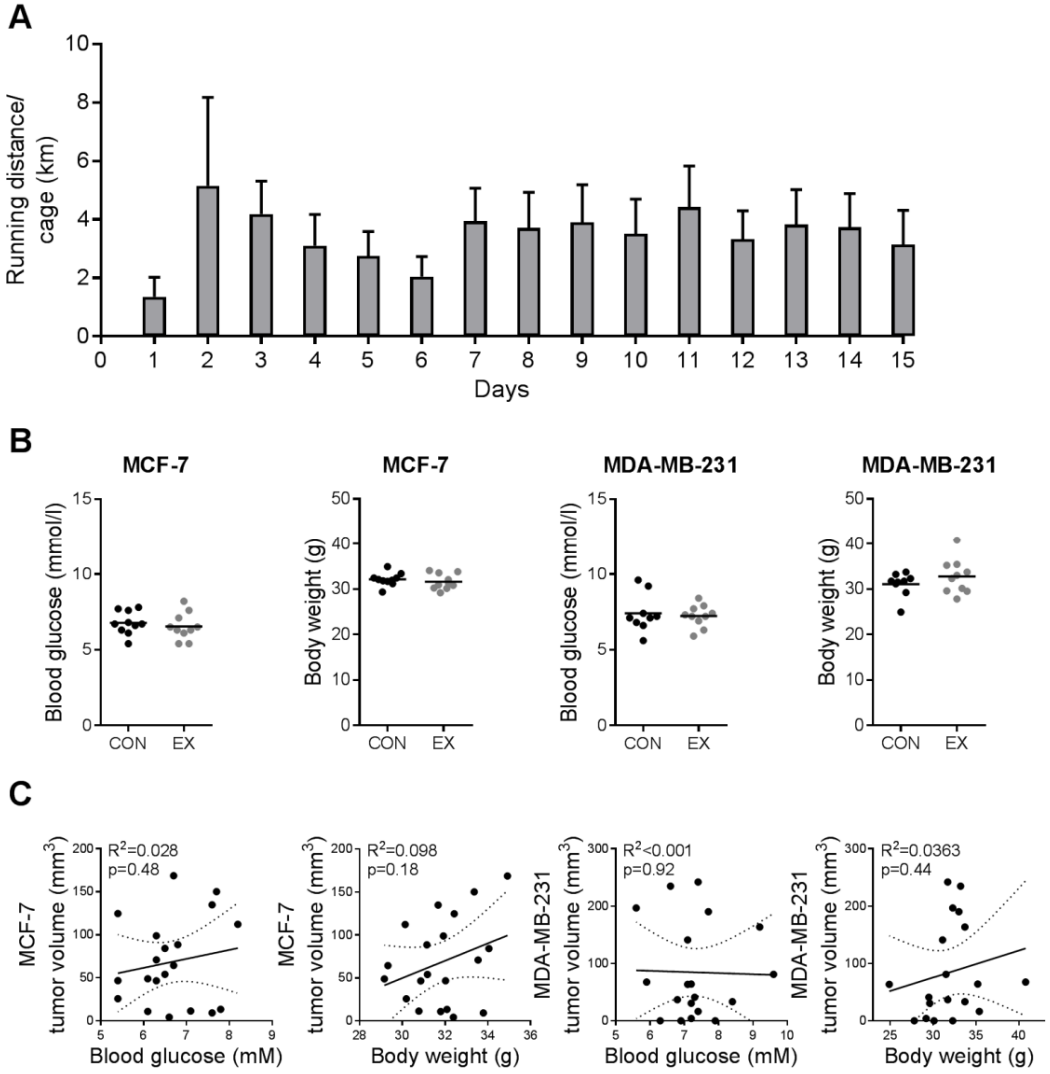
Representative immunoblots of pYAP (Ser127), YAP, and TAZ protein in **A)** MCF-7 and **B)** MDA-MB-231 cells after stimulation with 10 µM epinephrine (EPI) or 10 µM norepinephrine (NE) for 1 or 48 hours. Quantification of pYAP/YAP, YAP, and TAZ protein expression in **C)** MCF-7 and **D)** MDA-MB-231 cells after 1 hour (n=2) and 48 hours (n=3). Protein expression was normalized to total protein (reactive brown). **E)** mRNA expression of genes downstream of YAP/TAZ in MDA-MB-231 cells after 48 hours of incubation with 10 µM epinephrine (n=3) or norepinephrine (n=3). Gene expression was normalized to β-actin. Data are presented as means ± SEM. Statistical significance was tested by unpaired t-tests for protein and gene expressions. *p<0.05, **p<0.01, ***p<0.001.



Supplementary figure S4

Activation of the Hippo pathway in breast cancer cells by exercise-conditioned serum from breast cancer patients

Intracellular protein expression was determined by immunoblotting after *in vitro* incubations of the cancer cells with 10% REST or EX serum for 1 hour (n=6) or 48 hours (n=11-12). **A-D)** Representative immunoblots for MCF-7 and MDA-MB-231 and quantification of pYAP (Ser127)/YAP, YAP, and TAZ protein expression in MCF-7 and MDA-MB-231 cells related to total protein (reactive brown). **E)** mRNA expression of genes downstream of YAP/TAZ in MDA-MB-231 (n=20) cells after 48 hours of incubation with 10% REST/EX serum. All genes were related to β-actin. **F)** Quantification of protein expression of pYAP/YAP, YAP, and TAZ in MCF-7 cells (n=3) after stimulation with medium containing 10% serum from breast cancer patients at rest or after 2 hours of exercise (EX) +/- propranolol for 15-120 minutes. Data are depicted as mean ± SEM and statistical significance of protein expressions was tested by 2-way ANOVA with repeated measures, while mRNA levels were tested by paired t-tests . *p<0.05, **p<0.01



Supplementary figure S5

Effect of voluntary wheel running on physiological adaptations and tumor volume

A) Running distances were monitored daily after introduction of running wheels in the cages. Each cage contained two mice to avoid isolation stress. **B)** Blood glucose and body weight of the mice with MCF-7 and MDA-MB-231 tumors at the end of the study. **C)** Correlation analyses of MCF-7 and MDA-MB-231 tumor volumes to blood glucose and body weight of the mice. Data are presented as means ± SEM. Statistical significance was tested by unpaired t-tests for B, and linear correlation analyses for C.