CTRP13-Mediated Effects on Endothelial Cell Function and Their Potential Role in Obesity

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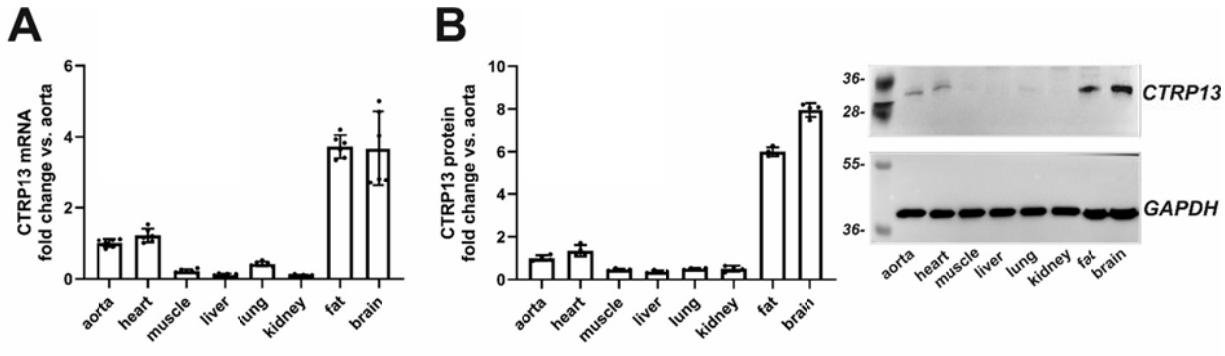
Supplement

Supplementary Table S1: Primer sequences

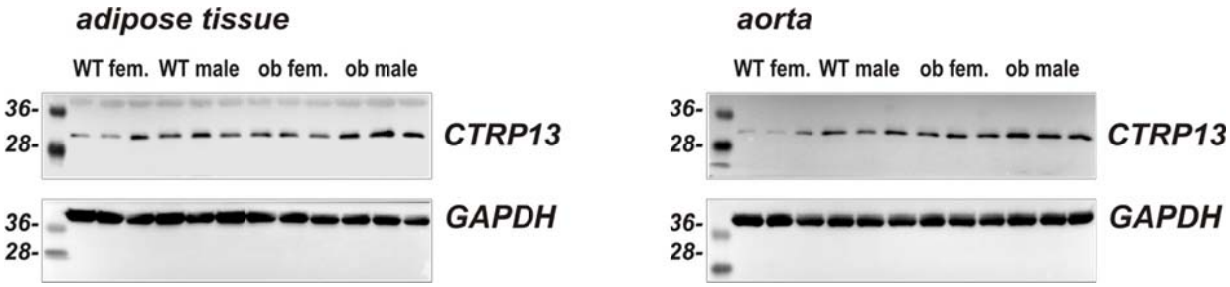
	GenBank accession #	Forward Primer	Reverse Primer
CTRP13 human	NM_001010908.2	GCA ATT GCC CAA GAT GCT GAT	TTT CCT CCATGG GCT TTC CC
PRKAA1 human	NM_006251.6	GCG CAG ACT CAG TTC CTG	TCT TCA CAG CTA CTT TAT GCC C
PRKAA2 human	NM_006252.4	AAG ATC GGA CAC TAC GTGC	ATT TTT CCA ACA ACA TCT AAA CTGC
TNF-alpha human	NM_000594.3	CCC ATG TTG TAG CAA ACC CTC	TAT CTC TCA GCT CCA CGC CA
ICAM-1 human	NM_000201.3	TGT GAC CAG CCC AAG TTG TT	TGG AGT CCA GTA CAC GGT GA
IL-8 human	NM_000584.4	GCT CTG TGT GAA GGT GCA GTT	ACC CAG TTT TCC TTG GGG TC
Col1A1 human	NM_000088.4	CCT GGT GAA TCT GGA CGT GA	ACC CAC GAT CAC CAC TCT TG
Col3A1 human	NM_000090.4	ACG GAA ACA CTG GTG GAC AG	GAA GCT CGG CTG GAG AGA AG
alpha-SMA human	NM_001613.4	GAG TCT GCT GGC ATC CAT GA	GTA CAT AGT GGT GCC CCC TG
TGF-beta1 human	NM_000660.7	CGT CTG CTG AGG CTC AAG TT	CTA AGG CGA AAG CCC TCA AT
GAPDH human	NM_002046.7	GTC ACC AGG GCT GCT TTT AAC	TTG ACG GTG CCA TGG AAT TTG
HPRT-1 human	NM_000194.3	TGA CCA GTC AAC AGG GGA CA	TGC CTG ACC AAG GAA AGC AAA
18S rRNA human	X03205.1	TGG AGC GAT TTG TCT GGT TA	ACG CCA CTT GTC CCT CTA AG
CTRP13 rat	NM_001109403	AGA GCC CGG ACC	GCG ATC TTG GGC ACC GTG

		ACC GGG	CT
18S rRNA rat	NR_046237	TGG AGC GAT TTG TCT GGT TA	ACG CCA CTT GTC CCT CTA AG
GAPDH rat	NM_017008.4	CAT CAC CAT CTT CCA GGA GGG	GGT TCA CAC CCA TGA CGA ACA
HPRT-1 rat	NM_012583.2	CGA GCC GAC CGG TTC TGT CAT G	GAG GGC CAC AAT GTG ATG GCC TC
CTRP13 mouse	NM_153155.3	GGC AAG TTC ACC TGC TCC AT	GAG CAA TTG CAC TAG CAC GC
GAPDH mouse	BC023196.2	CAT CAC CAT CTT CCA GGA GCG	CGT TTG GCT CCA CCC TTC AA
HPRT-1 mouse	NM_013556.2	GAT CAG TCA ACG GGG GAC AT	AGA GGT CCT TTT CAC CAG CAA
18S rRNA mouse	NR_003278.3	TGG AGC GAT TTG TCT GGT TA	ACG CCA CTT GTC CCT CTA AG

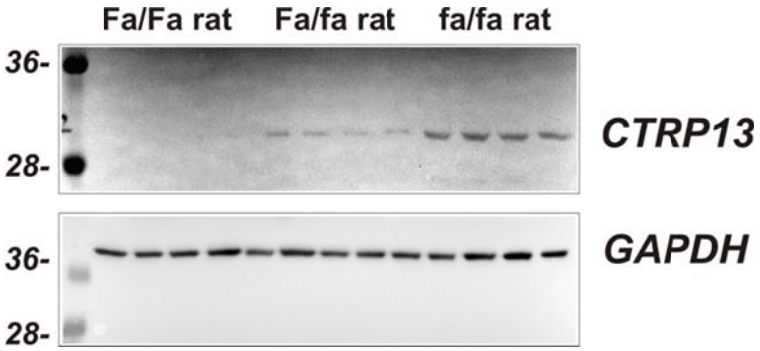
Supplementary Figures



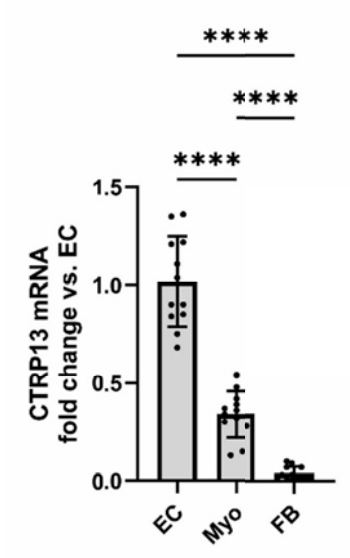
Supplementary Fig. S1: CTRP13 mRNA and protein expression in various mouse organs
(**A**) Results from qPCR analyses of CTRP13 mRNA expression in various organs of wild-type mice. (**B**) Quantification of CTRP13 protein expression in various mouse organs (left panel) and representative CTRP13 western blots (uncropped images with size markers as indicated) from these organs (right panel). GAPDH served as the loading control. All data represent mean ± SEM from 6 male animals.



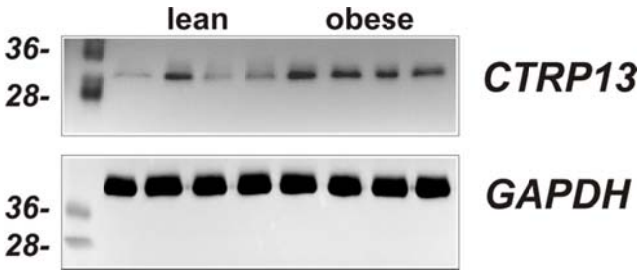
Supplementary Fig. S2: CTRP13 expression in adipose and aortic tissue of obese and lean mice
Representative CTRP13 western blots (uncropped images with size markers as indicated) from adipose tissue (left panel) and aortic tissue (right panel) of male and female wild-type or ob/ob mice. GAPDH served as loading control.



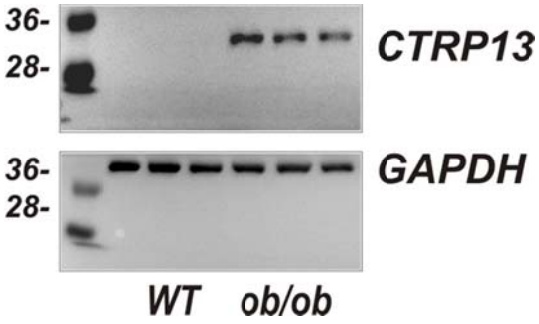
Supplementary Fig. S3: CTRP13 expression in the aorta of ZDF rats
Representative CTRP13 western blots (uncropped images with size markers as indicated) from aortic tissue of male obese ZDF (fa/fa) rats, lean heterozygous (Fa/fa) rats and wild-type (Fa/Fa) rats. GAPDH served as loading control.



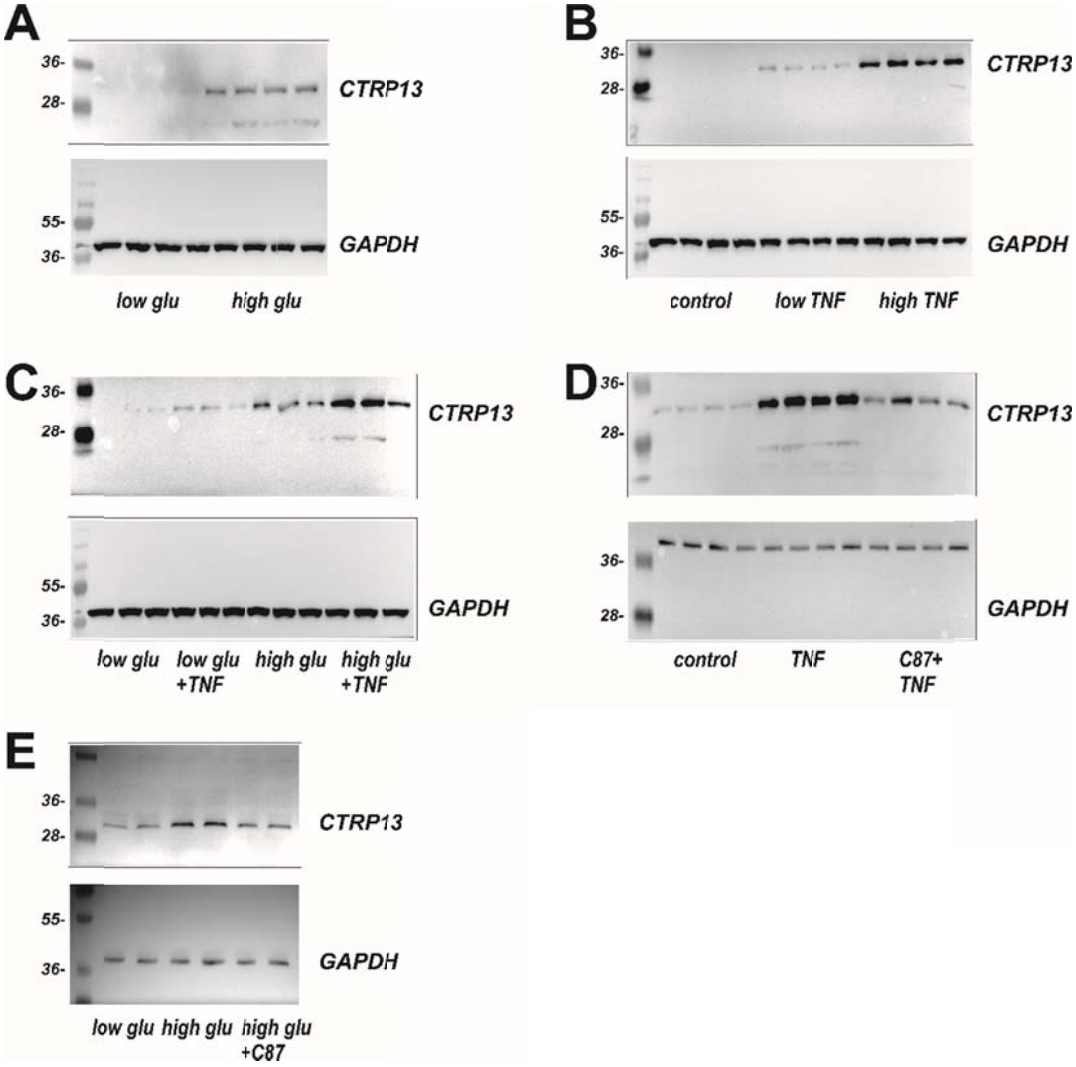
Supplementary Fig. S4: CTRP13 mRNA expression in rat cardiac endothelial cells, cardiomyocytes or cardiac fibroblasts
CTRP13 mRNA expression was analyzed in adult rat cardiac endothelial cells (EC), cardiomyocytes (Myo) or cardiac fibroblasts (FB) by qPCR. Results are presented relative to CTRP13 expression in ECs. Data are shown as mean±SEM, with n=12 per group. ****: $p<0.0001$.



Supplementary Fig. S5: CTRP13 expression in the mammary artery of obese and lean patients
Representative CTRP13 western blots (uncropped images with size markers as indicated) from mammary arteries of lean (BMI 18.5-25 kg/m^2) or obese (30-35 kg/m^2) patients. GAPDH served as loading control.

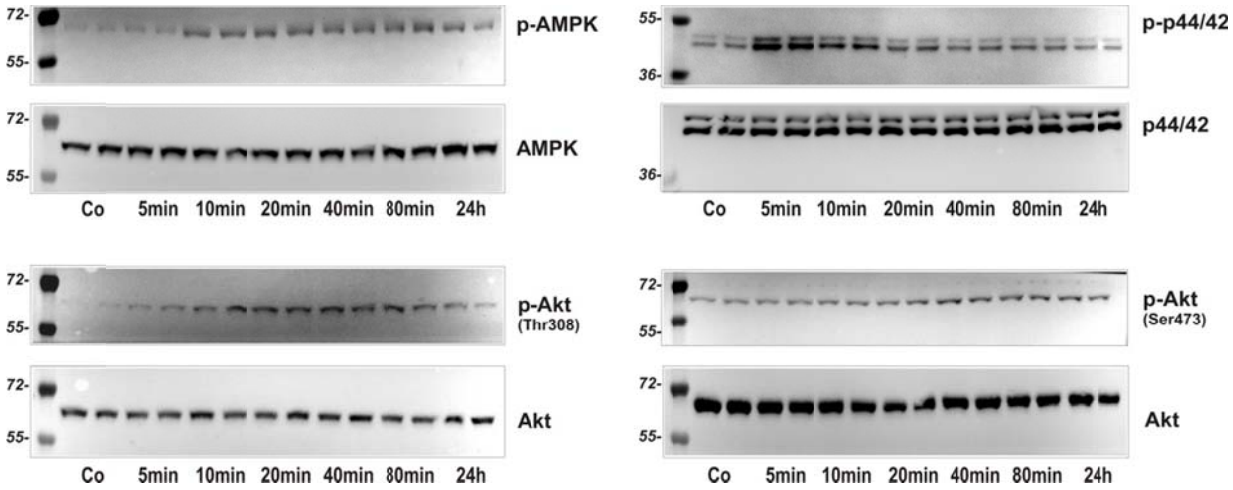


Supplementary Fig. S6: Induction of CTRP13 in HUVECs by mouse serum
Representative CTRP13 western blots (uncropped images with size markers as indicated) from cells grown in Opti-MEM™ supplemented with either 10% serum from wild-type (WT) or ob/ob mice for 48h. GAPDH served as loading control.



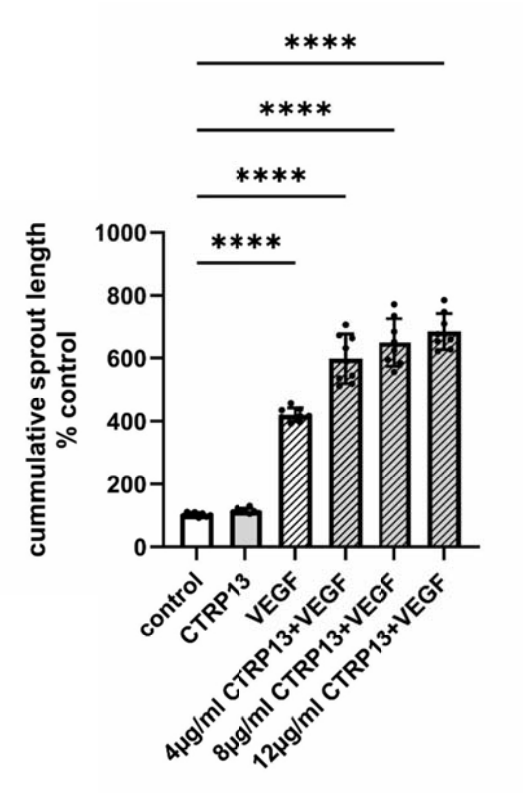
Supplementary Fig. S7: Impact of high glucose and TNF-alpha on CTRP13 protein expression in HUVECs

Representative CTRP13 western blots (uncropped images with size markers as indicated) from (**A**) HUVECs cultured under normal glucose conditions (5 mM D-glucose, low glu) or high glucose conditions (25 mM D-glucose, high glu) for 24 h. (**B**) HUVECs cultured under normal glucose conditions (5 mM D-glucose, low glu) or treated with 1 ng/ml or 10 ng/ml TNF-alpha for 24 h. (**C**) HUVECs cultured under normal glucose conditions (5 mM D-glucose, low glu) or high glucose conditions (25 mM D-glucose, high glu) and treated ± 1 ng/ml TNF-alpha for 24 h. (**D**) HUVECs cultured under normal glucose conditions (5 mM D-glucose, low glu), treated with ± 1 ng/ml TNF-alpha for 24 h, and preincubated with the TNF-alpha-specific small-molecule inhibitor C87 (2 μM) for 60 min as indicated. GAPDH served as loading control.



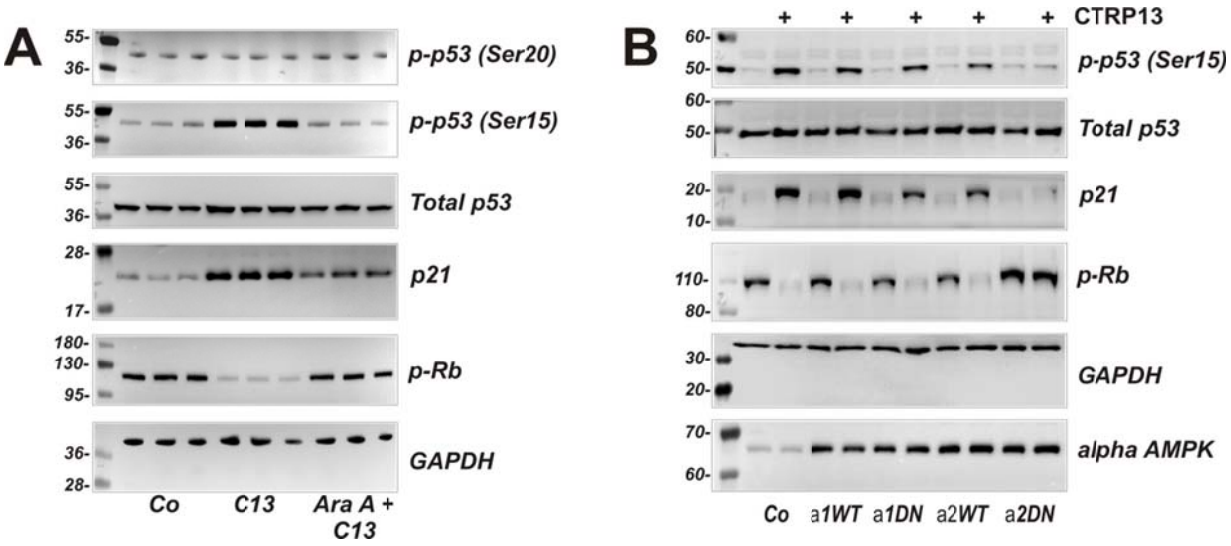
Supplementary Fig. S8: Time course of signaling pathway activation in response to CTRP13 in HUVECs

Representative western blots (uncropped images with size markers as indicated) for phosphorylation of AMPK (Thr172), p44/42 MAPK (Thr202/Tyr204) and Akt (Thr308 or Ser473) after treatment with CTRP13 (4 µg/ml) for the indicated time in Opti-MEM™ supplemented with 1% FBS. Total AMPK, total p44/42 MAPK or total Akt served as loading control.



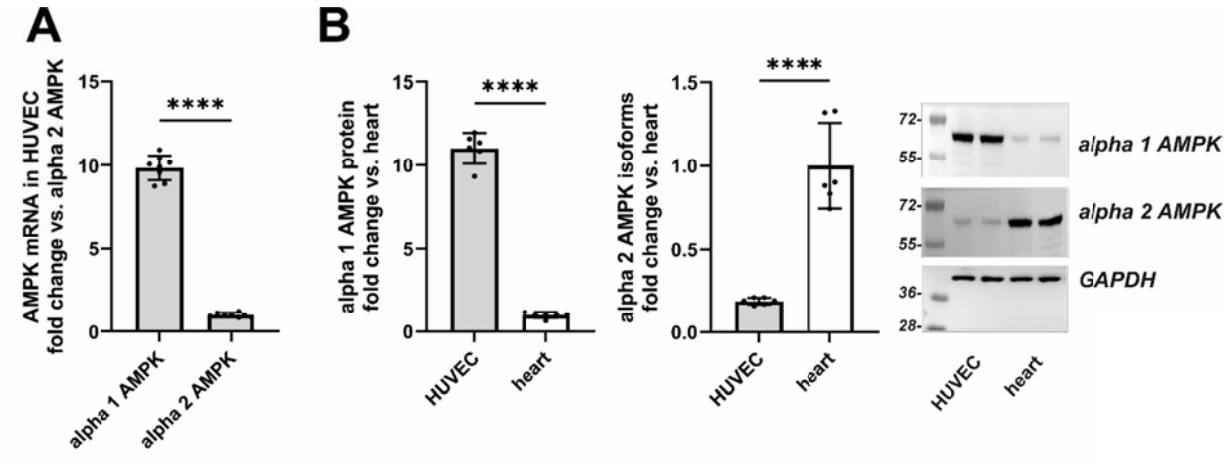
Supplementary Fig. S9: Sprouting assay

Cumulative sprout length. HUVECs were cultured as hanging drops to form spheroids. The spheroids were embedded into a collagen matrix and incubated with EGM containing 4 µg/ml CTRP13, 25 ng/ml VEGF or VEGF + CTRP13 at increasing concentration (4, 8, 12 µg/ml), and then incubated at 37°C overnight in a cell culture incubator. After 24 h, cell sprouts were photographed and cumulative sprout length was analyzed. Data are mean ± SEM from 4 independent experiments with 2 biological replicates each. ****$p < 0.0001$ vs. control.



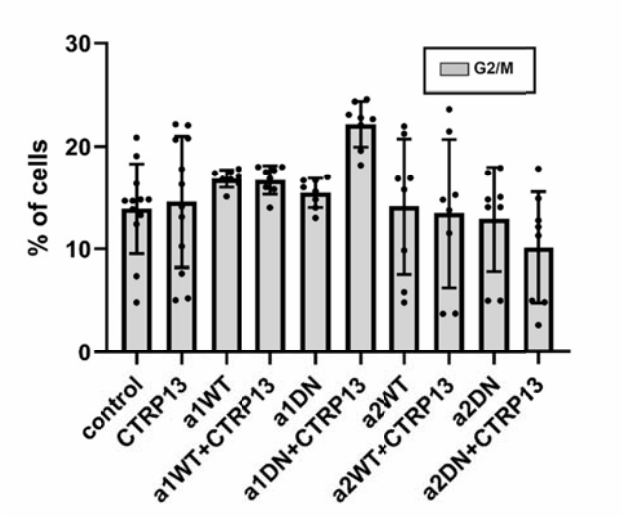
Supplementary Fig. S10: Impact of CTRP13 on cell cycle regulators and cell cycle progression

(**A**) Cells were either left untreated in Opti-MEM™ supplemented with 1% serum or treated with CTRP13 (4 µg/ml) for 24 h. Sixty minutes prior to CTRP13 treatment, HUVECs were incubated with the AMPK inhibitor AraA (500 µM) as indicated. Representative western blots (uncropped images with size markers as indicated) for phosphorylation of p53 (Ser15 or Ser20) or Rb and protein expression of p53 and p21. GAPDH served as loading control. (**B**) Adenoviral overexpression of wild-type (WT) or dominant negative (DN) alpha 1 and alpha 2 AMPK was performed in HUVECs. Forty-eight hours later cells were either left untreated in Opti-MEM™ supplemented with 1% serum or treated with CTRP13 (4 µg/ml) for 24 h. Western blots were performed as described in (A).



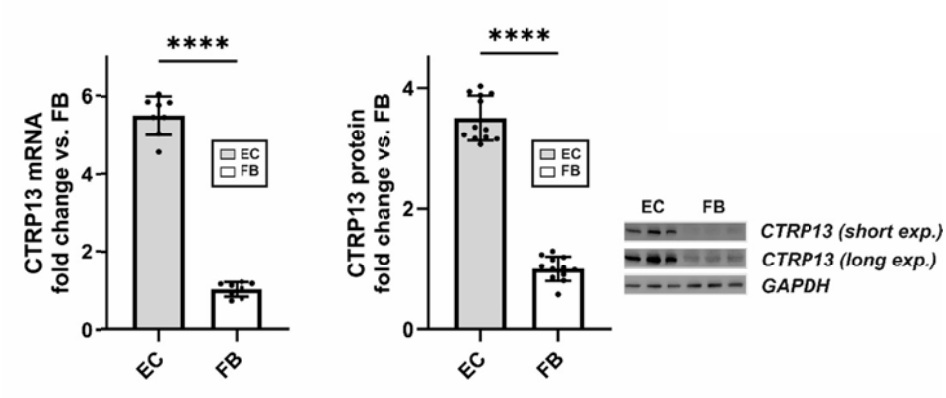
Supplementary Fig. S11: alpha AMPK isoform expression in HUVECs

(**A**) Alpha 1 and alpha 2 AMPK mRNA expression was analyzed in HUVECs by qPCR. (**B**) Quantification of CTRP13 protein expression in HUVECs (left panel). Results are presented in comparison to alpha 1 and alpha 2 AMPK expression in human cardiac tissue. Representative western blots (uncropped images with size markers as indicated) from HUVECs and human cardiac tissue (right panel). GAPDH served as loading control. Data are shown as mean±SEM, with n=6-8 per group. ***: $p<0.001$.



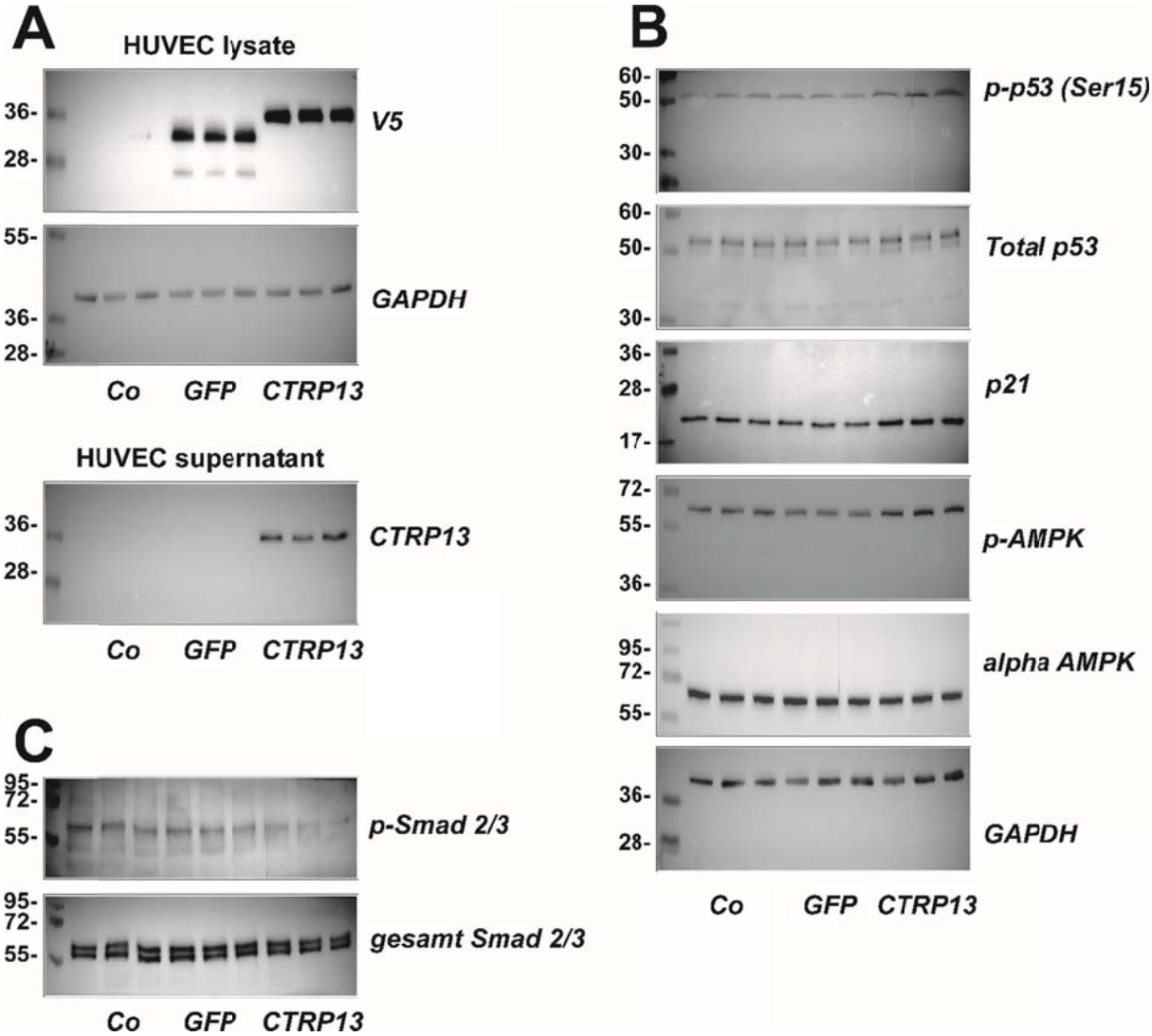
Supplementary Fig. S12: Role of alpha AMPK isoforms in mediating the CTRP13 effects on cell cycle progression (G2/M phase)

Adenoviral overexpression of wild-type (WT) or dominant negative (DN) alpha 1 and alpha 2 AMPK was performed in HUVECs. Forty-eight hours later cells were either left untreated in Opti-MEM™ supplemented with 1% serum or treated with CTRP13 (4 µg/ml) for 24 h. Results from FACS-based cell cycle analyses. Only the results regarding the impact of alpha AMPK isoforms in mediating the CTRP13 effects on the G2/M phase are depicted. Data are shown as mean ± SEM from 4 independent experiments with 2-3 biological replicates each.



Supplementary Fig. S13: CTRP13 expression in human endothelial cells and human fibroblasts

(**A**) CTRP13 mRNA expression was analyzed in HUVECs and human RA fibroblasts by qPCR. (**B**) Quantification of CTRP13 protein expression in HUVECs and human fibroblasts (left panel). Representative western blots (uncropped images with size markers as indicated) from HUVECs and human fibroblasts (right panel). GAPDH served as loading control. Data are shown as mean ± SEM, with n=8-12 per group. ****: $p<0.001$.



Supplementary Fig. S14: **Impact of endothelial CTRP13 on human fibroblasts.**
Adenoviral overexpression of CTRP13 or GFP in HUVECs was performed. Conditioned medium from HUVECs overexpressing either CTRP13 or GFP or HUVECs treated with conditioned medium from 293A cells without overexpression was utilized to culture human fibroblasts in 50% (vol/vol) HUVECs medium in DMEM for 24 h. (**A**) Overexpression of V5-tagged CTRP13 or GFP in HUVEC lysates (upper panel) or secretion of CTRP13 into cell culture medium (lower panel) was analyzed by Western blotting. GAPDH served a loading control. (**B**) Western blots were performed for phosphorylation of p53 (Ser15), total protein expression of p53, p21 and alpha-AMPK and AMPK phosphorylation (Thr172). GAPDH served as loading control. (**C**) Western blots were performed for phosphorylation of Smad2 (Ser465/467)/ Smad3 (Ser423/425).