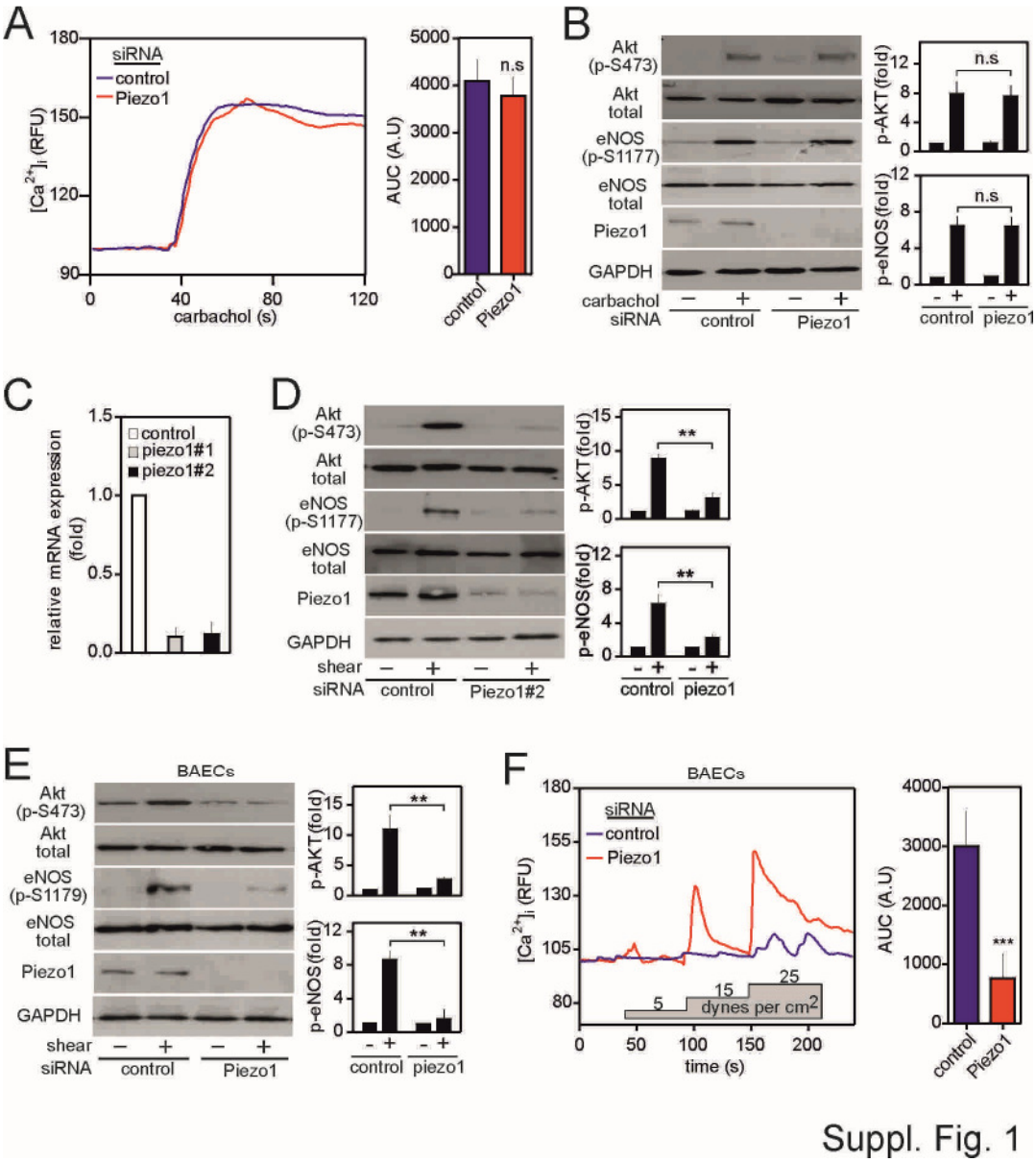
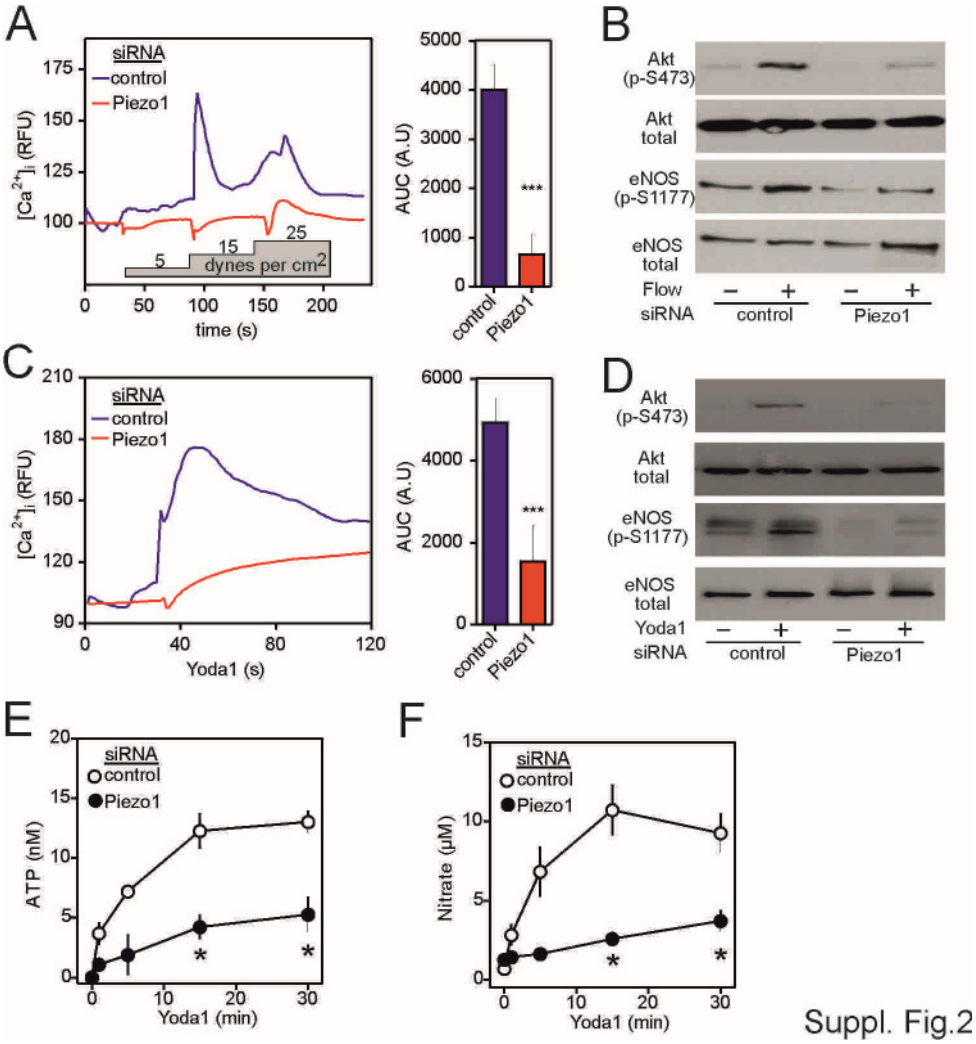
Supplementary Information (Ms Wang et al.)

Supplementary Data

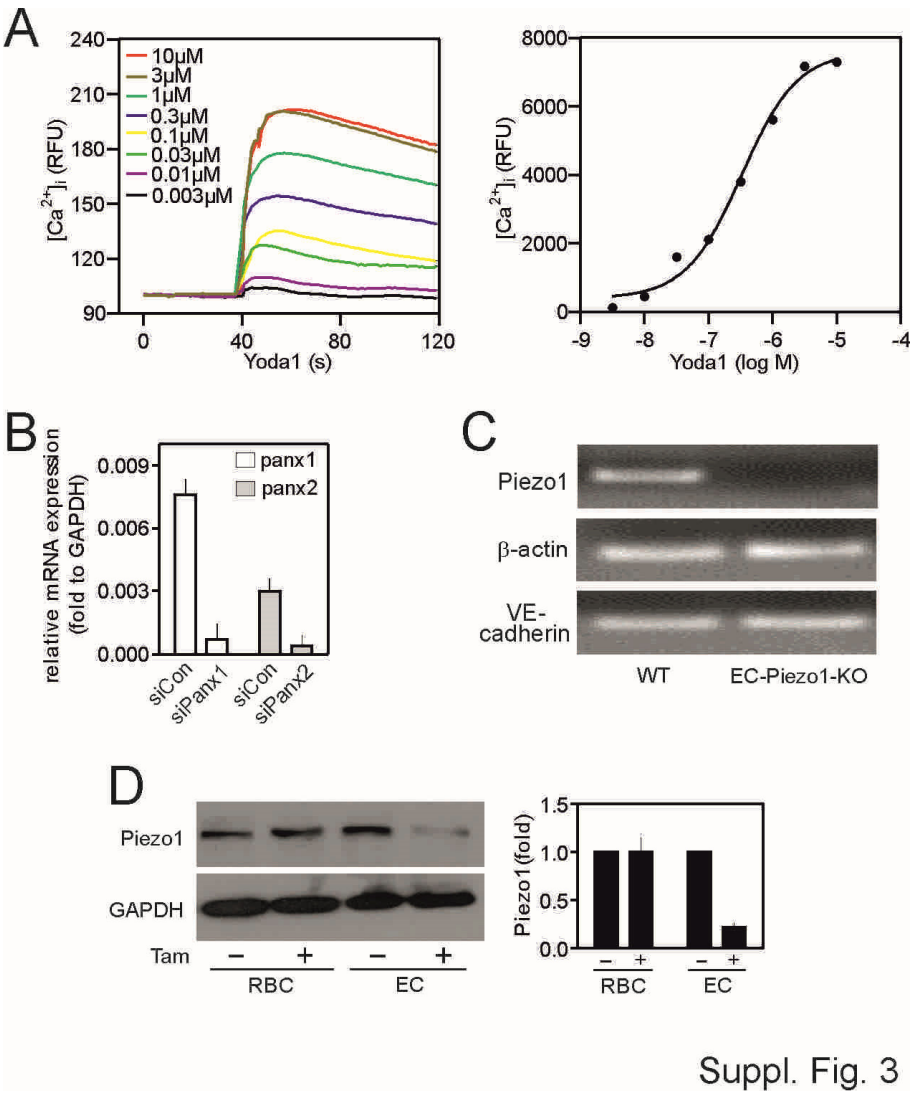


Supplementary Figure 1. Piezo1 mediates endothelial responses to fluid shear stress in HUAECs and BAECs in vitro. (**A-F**) HUAECs (A-D) or bovine aortic endothelial cells (BAECs; E,F) were transfected with scrambled (control) siRNA or siRNA directed against Piezo1 and were exposed to 10 µM carbachol (A,B) or fluid shear (15 dynes/cm^2 in D and 20 dynes/cm^2 in E) (D-F) for the indicated time (5 min in B,D,E). In A and F, experiments with Fluo-4-loaded HUAECs (A; n=12, control; n=15, Piezo1; 3 independent experiments) and BAECs (F; n=16, control; n=14,

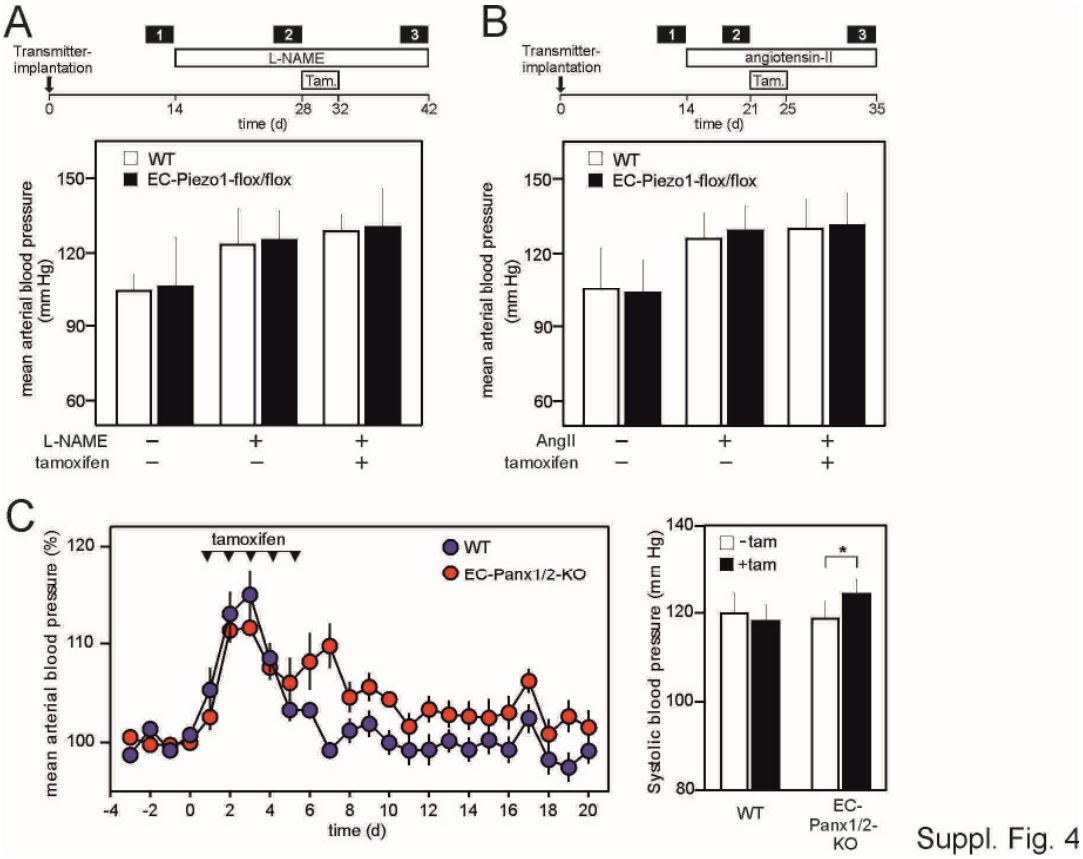
Piezo1) are shown. $[Ca^{2+}]_i$ was determined as fluorescence intensity (RFU, relative fluorescence units). Bar diagrams show areas under the curve (AUC). Shown are means -/+ s.e.m.; ***, P≤0.001 (two-tailed Student's *t* test). Phosphorylated Akt and eNOS was determined by Western blotting for phosphorylated Akt and eNOS as well as total Akt and eNOS. Knock-down of Piezo1 was verified by anti-Piezo1 immunoblotting (B,D,E) or RT-PCR (**C**). Bar diagrams show the densitometric evaluation. Shown are means -/+ s.e.m.; *, P≤0.05; **, P≤0.01; ***, P≤0.001 (two-way ANOVA and Bonferroni's *post hoc* test).



Supplementary Figure 2. Flow and Yoda1 induce endothelial responses via Piezo1 in HUVECs. HUVECs were transfected with scrambled (control) siRNA or siRNA directed against Piezo1. (**A, C**) Fluo-4-loaded HUVECs (n=10, control; n=12, Piezo1 knock-down) were exposed to the indicated shear forces (A) or to 1 µM Yoda1 (C), and $[Ca^{2+}]_i$ was determined as fluorescence intensity (RFU, relative fluorescence units). Bar diagrams show areas under the curve (AUC). Shown are means -/+ s.e.m.; ***, P≤0.001 (two-tailed Student's *t* test). (**B, D-F**) HUVECs were exposed to flow (12 dynes / cm^2) or 1 µM Yoda1 for the indicated time (5 min in B and D). B,D, Akt and eNOS activation was determined by Western blotting for phosphorylated Akt and eNOS as well as total Akt and eNOS (n=3). E,F, ATP concentration (E, n=3) and concentration of nitrate (F, n=6) in the cell medium. Shown are means -/+ s.e.m.; *, P≤0.05; **, P≤0.01 (two-way ANOVA and Bonferroni's *post hoc* test).



Supplementary Figure 3. (**A**) Fluo-4-loaded HUAECs (n=12-24) were exposed to the indicated concentrations of Yoda1, and $[Ca^{2+}]_i$ was determined as fluorescence intensity (RFU, relative fluorescence units). (**B**) Expression of pannexin1 and pannexin2 in HUAECs transfected with scrambled (control) siRNA or siRNA directed against Pannexin1 and Pannexin2 was determined by RT-PCR. (**C**) Expression of Piezo1 in isolated skeletal muscle endothelial cells from wild-type (WT) mice and tamoxifen-induced EC-Piezo1-KO animals was determined by RT-PCR. (**D**) Effect of tamoxifen treatment of Tie2-CreER^{T2};Piezo1$^{flox/flox}$ mice on Piezo1 protein levels in red blood cells (RBC) and skeletal muscle endothelial cells (EC). 10 days after tamoxifen treatment, red blood cells and skeletal muscle endothelial cells were isolated and lysates were analyzed by immunoblotting using anti-Piezo1 antibodies. Bar diagrams show the statistical analysis. Shown are means -/+ s.e.m.; ***, P≤0.001 (two-way ANOVA).



Supplementary Figure 4. (A,B) Effect of Tamoxifen-induced endothelial Piezo1 deficiency on blood pressure of L-NAME- (A) and angiotensin II- treated mice (B). 14 days after transmitter implantation, wild-type and non-induced EC-Piezo1-KO mice were treated with L-NAME (1 mg/ml in the drinking water for 28 days) or were implanted with an osmotic minipump releasing angiotensin-II (Alzet 2001, volume 200 µl, Ang-II-release: 2000 ng/kg/min for 21 days). After 14 days (L-NAME) or 7 days (angiotensin-II; see scheme), all mice were treated for 5 consecutive days with tamoxifen (Tam.) to induce endothelium-specific Piezo1 deficiency in the test group. The bar diagram shows mean arterial blood pressure on days 11-13 after transmitter implantation just before treatment with L-NAME or angiotensin II (L-NAME-/AngII-, tamoxifen-; period 1 in schemes), during the 3 days before tamoxifen treatment (L-NAME+/AngII+, tamoxifen-; period 2 of schemes) and during day 7-9 after the end of the tamoxifen treatment (L-NAME+/AngII+, tamoxifen+; period 3 of schemes). (C) Blood pressure in wild-type (n=6) and EC-Pannexin1/Pannexin2-KO mice (n=6) before, during and after induction. Average blood pressure 5 days before induction was set to 100%. Bar diagrams show systolic arterial blood pressure 4 days before tamoxifen treatment and in the 2nd week after induction. Shown are means -/+ s.e.m.; *, P≤0.05 (two-way ANOVA and Bonferroni's *post hoc* test (B)).