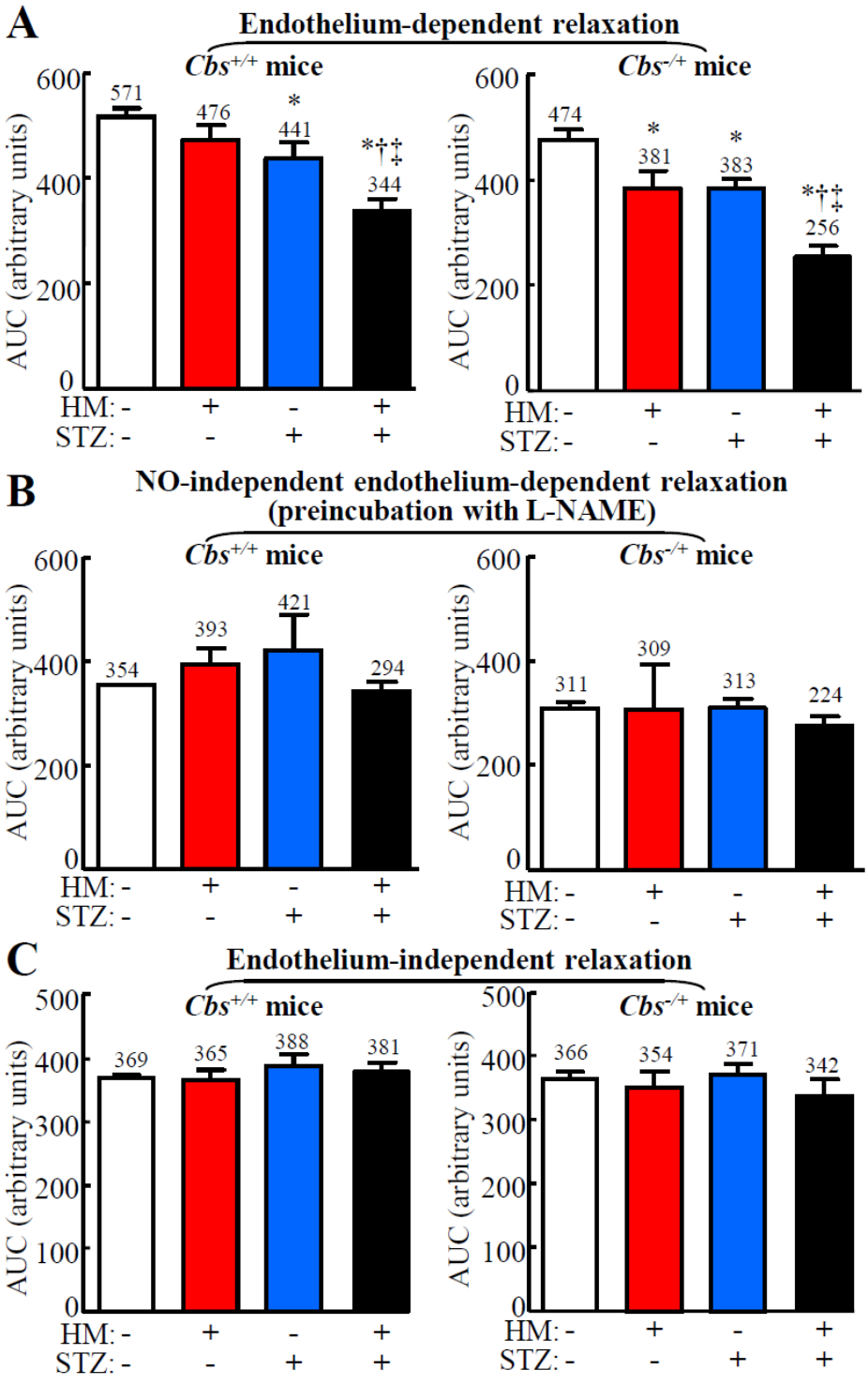
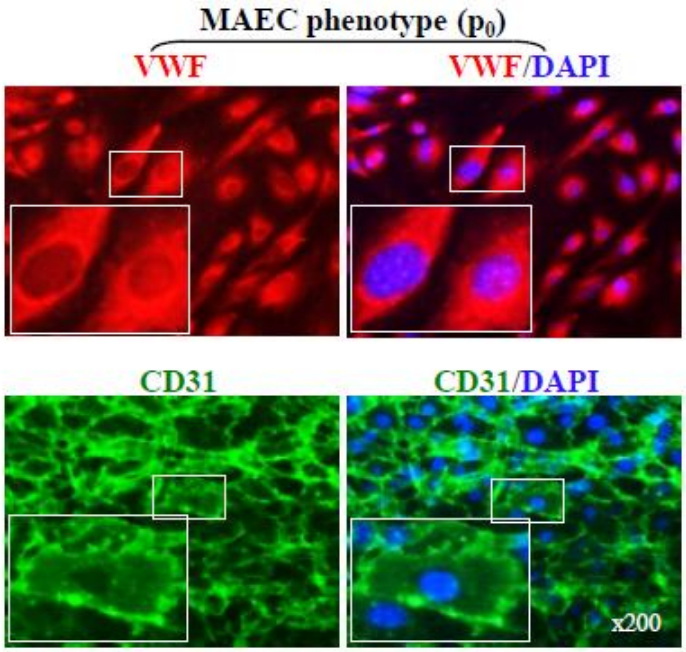
SUPPLEMENTARY DATA

Supplementary Figure 1. Hyperhomocysteinemia aggravated hyperglycemia-induced endothelial dysfunction in mouse thoracic aorta mainly via nitric oxide. A. Endothelium-dependent vascular relaxation to ACh. B. Endothelium-dependent vascular relaxation to ACh in the presence of LNAME (100 μmol/L, 30 min). C. Endothelium-independent vascular relaxation to SNP. Aortic rings were pre-contracted with phenylephrine (1 μmol/L) and examined for relaxation response to cumulative additions of ACh or SNP, and represented as the area under the curve (AUC). n=5-10, values are mean ± SEM. *p<0.05 vs vehicle-treated corresponding mice on CT diet; †p<0.05 vs vehicle-treated corresponding mice on HM diet; ‡p<0.05 vs STZ-treated corresponding mice on CT diet.



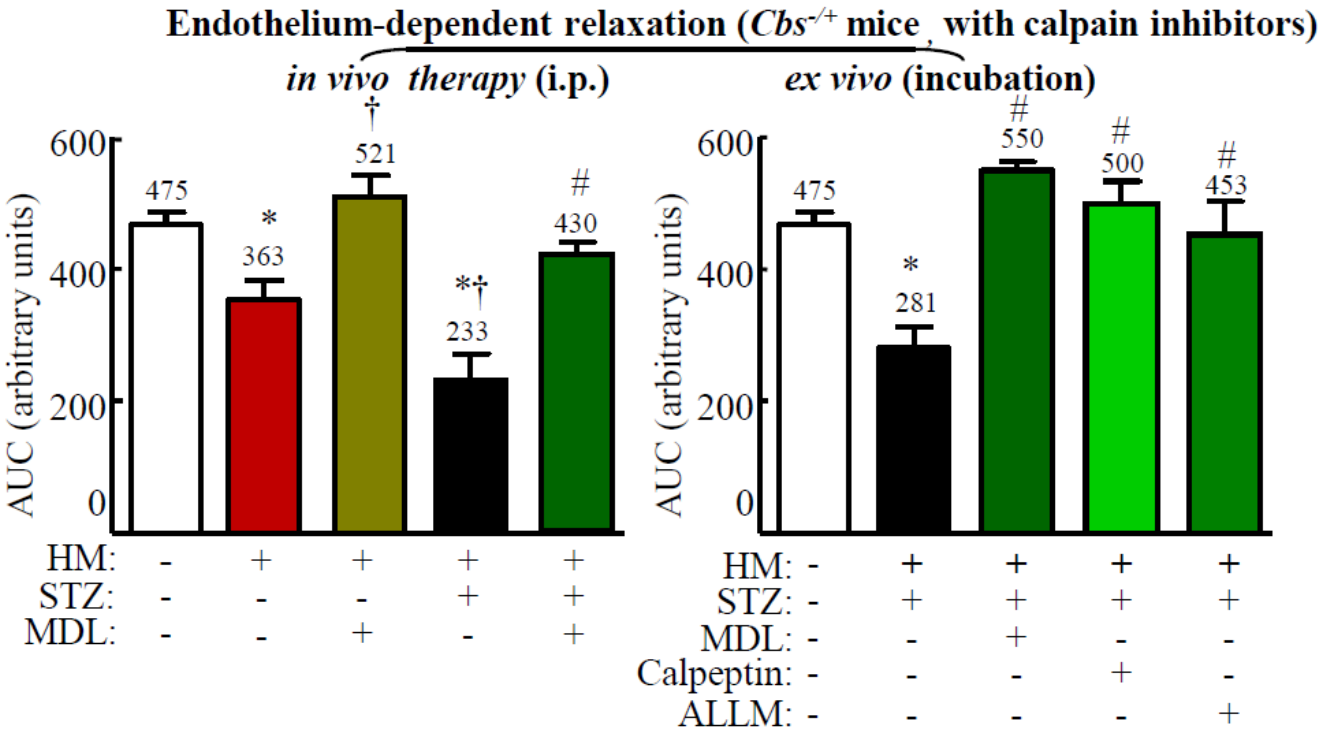
SUPPLEMENTARY DATA

Supplementary Figure 2. Identification of mouse aortic endothelial cells (MAECs). Cultured MAECs isolated from control *Cbs*+/- mice (p0) were identified by endothelial makers VWF (red, Von Willebrand factor, vWF, Santa Cruz, SC-2780, 1:200) and CD31 (green, CD31, BD Biosciences, BD553370, 1:100). Endothelial cells were stained with DAPI (blue, 4',6-Diamidino-2- Phenylindole).



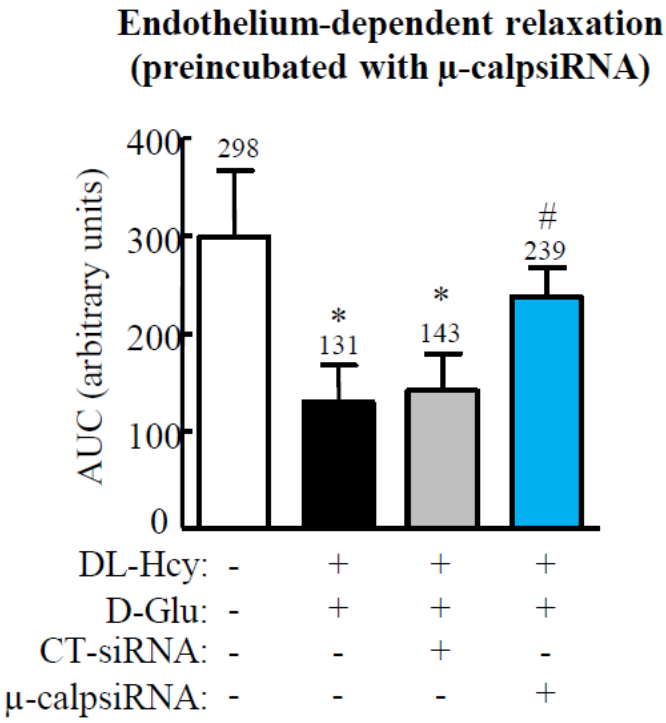
SUPPLEMENTARY DATA

Supplementary Figure 3. Activation of calpain mediated hyperhomocysteinemia/hyperglycemia-induced endothelial dysfunction in mouse aorta. Endothelium-dependent vascular relaxation to ACh in the aorta of *Cbs*-/+ and STZ-treated *Cbs*-/+ mice on HM diet pretreated with calpain inhibitors *in vivo* (left panel) or *ex vivo* (right panel). In *in vivo*, calpain inhibitor MDL was administrated by i.p. injection (2 mg/kg/day, 2 weeks) whereas in *ex vivo*, aortic rings were incubated with calpain inhibitors MDL, calpeptin and ALLM (20 μmol/L for all) for 1 hr. Aortic rings were pre-contracted with phenylephrine (1 μmol/L) and examined for relaxation response to accumulative concentrations of ACh, and represented as the AUC. n=3-10, values are mean ± SEM. *p<0.05 vs vehicle-treated *Cbs*-/+ mice on CT diet; †p<0.05 vs vehicle-treated *Cbs*-/+ mice on HM diet; ‡p<0.05 vs STZ-treated *Cbs*-/+ mice on CT diet; #p<0.05 vs STZ-treated *Cbs*-/+ mice on HM diet.



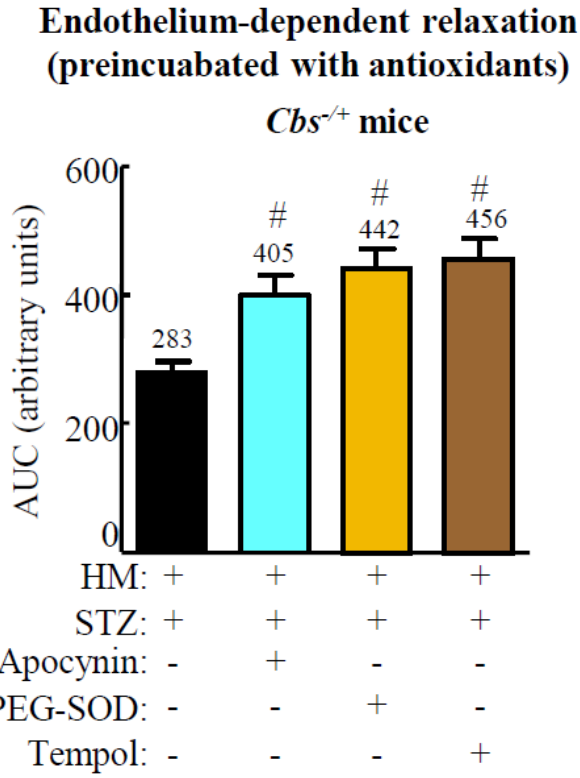
SUPPLEMENTARY DATA

Supplementary Figure 4. *μ-calpain* siRNA rescued hyperhomocysteinemia/hyperglycemia-induced ED. Endothelium-dependent vascular relaxation to ACh in control mouse aorta treated with or without DL-Hcy (500 μmol/L) and D-Glu (25 mmol/L) for 48 hrs in the presence and absence of μ- calpsiRNA *in vitro*. Aortic rings were pre-contracted with phenylephrine (1 μmol/L) and examined for relaxation response to cumulative additions of ACh and represented as the AUC. n=3-5, values are mean ± SEM. *p<0.05 vs control aorta; #p<0.05 vs aorta treated with DLHcy/ D-Glu and DL-Hcy/D-Glu + CTsiRNA. CT siRNA, control siRNA.



SUPPLEMENTARY DATA

Supplementary Figure 5. Oxidative stress regulated hyperhomocysteinemia/hyperglycemia-induced endothelial dysfunction. Endothelium-dependent vascular relaxation in aorta of STZ-treated *Cbs*-/+ mice on HM diet in the presence and absence of antioxidants PEG-SOD (150 U/mL), Tempol (1 mmol/L), or apocynin (10 μmol/L) for 1hr. Aortic rings were pre-contracted with phenylephrine (1 μmol/L) and examined for relaxation to cumulative additions of ACh and represented as the AUC. n=3-5, values are mean ± SEM. #p<0.05 vs STZ-treated *Cbs*-/+ mice on HM diet.



SUPPLEMENTARY DATA

Supplementary Figure 6. Endothelial nitric oxide synthase (eNOS) uncoupling was not regulated by hyperhomocysteinemia/hyperglycemia. A. eNOS dimer and monomer expression in HAECs. HAECs were treated with and without DL-Hcy (500 or 1000 μmol/L) and/or D-glucose (D-Glu, 25 mmol) for 24 or 48 hrs. B. Images of *in situ* ·O2 - production in HAECs. C. Quantifications of O2 - production. HAECs were treated with or without DL-Hcy (500 μmol/L) and D-Glu (25 mmol) for 24 hrs. n=3-5, values are mean ± SEM. *p<0.05 vs control; †p<0.05 vs DL-Hcytreated HAECs in corresponding time point; ‡p<0.05 vs D-Glu-treated HAECs in corresponding time point. DHE, dihydroethidium.