ONLINE APPENDIX

Activation of AMP-activated Protein Kinase Inhibits oxidized Low Density Lipoprotein-triggered Endoplasmic Reticulum (ER) Stress and Endothelium Dysfunction *in vivo*

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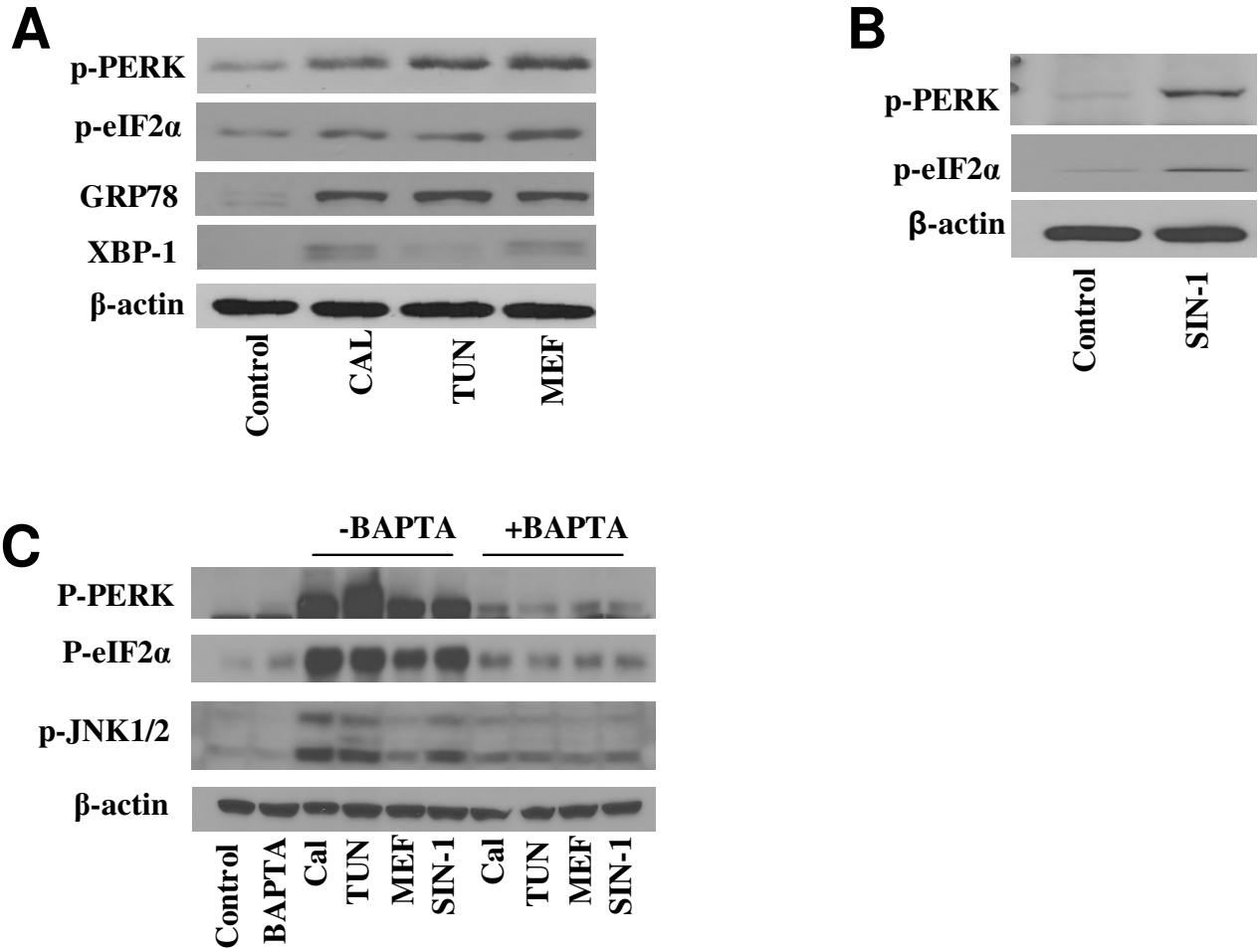
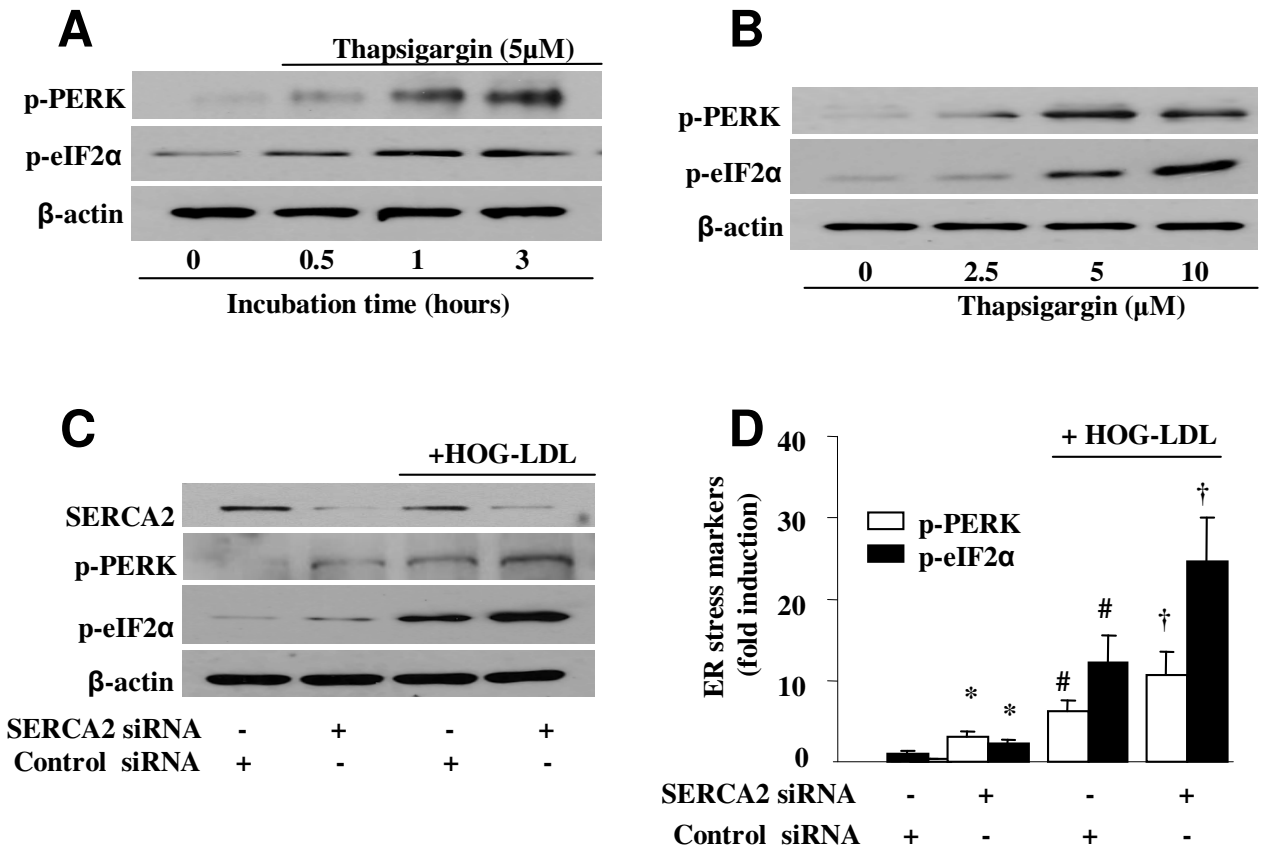
Supplemental Figure 1

Intracellular calcium mobilizers induce ER stress in a calcium dependent manner in BAEC. (A) ER stress was induced in BAEC cells with treatment of calcimycin (5 μM), tunicamycin (10μg/ml) or meflouquin (5 μM) for 30min respectively; **(B)** BAEC were treated by SIN-1 (1mM) for 30 min. (**C**) intracellular mobilizers caused ER stress were attenuated by pretreatment of BAEC with BAPTA-AM (10 μM); n≥ 3 for each experiment.



Supplemental Figure 2

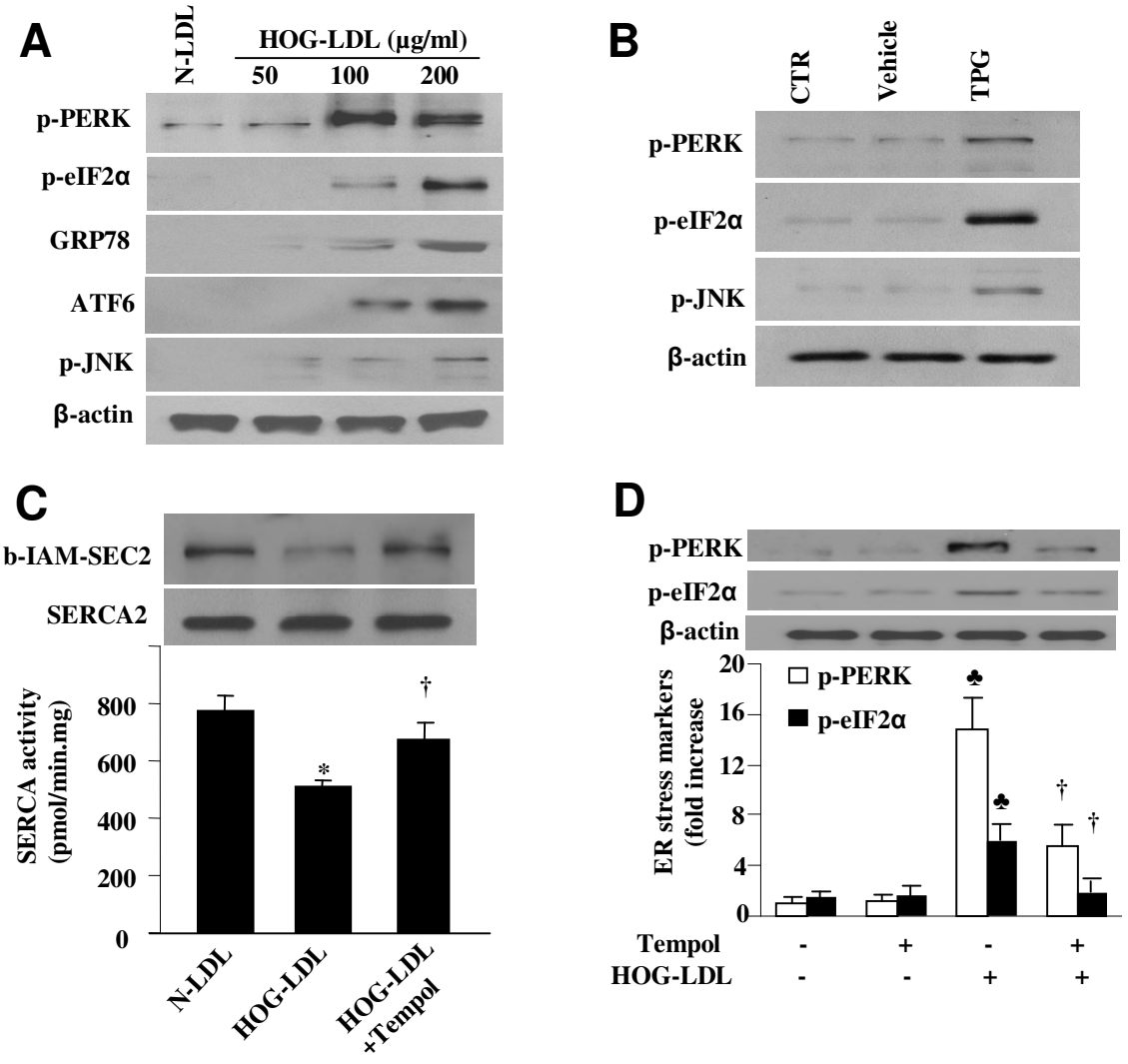
Decrease in SERCA activity/expression contributed to HOG-LDL induced ER stress. **(A & B)** Dose- and time- dependent effects of thapsigagin on ER stress in BAEC. The blot is a representative of at least three blots from three independent experiments; **(C & D)** Gene silencing of SERCA2 augmented HOG-LDL-triggered ER stress in HUVEC. n=6, *P<0.05, control siRNA *vs* SERCA siRNA; #P<0.05, control siRNA *vs.* control siRNA plus HOG-LDL; +P<0.05, SERCA-specific siRNA plus HOG-LDL *vs.* control siRNA plus HOG-LDL.



Supplemental Figure 3

Effects of Tempol on HOG-LDL-induced ER stress and SERCA oxidation in cultured human umbilical vein endothelial cells (HUVEC)

(A) HOG-LDL-induced ER stress in HUVEC. HUVEC were exposed to different concentrations of HOG-LDL (50-200 μg/ml) for 6 h. After the incubation, the cells were lysed and ER stress makers were detected with specific antibodies in western blots. The blot is a representative of three blots from three individual experiments; (**B**) ER stress in thapsgargin-treated HUVEC. HUVEC were exposed to thapsgargin (5μM) for 2 h. ER stress was monitored as described above. (**C**) Tempol ablates HOG-LDL-induced SERCA oxidation and SERCA activity. *p<0.05, HOG-LDL *vs.* N-LDL; †p<0.05, HOG-LDL + Tempol *vs.* HOG-LDL alone; n=3. (**D**) HOG-LDL induced ER stress can be prevented by Tempol supplement. ♣p<0.05, HOG-LDL *vs.* controls; †p<0.05, HOG-LDL + Tempol *vs.* HOG-LDL alone; n=3.



Supplemental Figure 4