**Supplementary information**

**CircDLPAG4/HECTD1 mediates ischemia/reperfusion injury in endothelial cells via ER stress**

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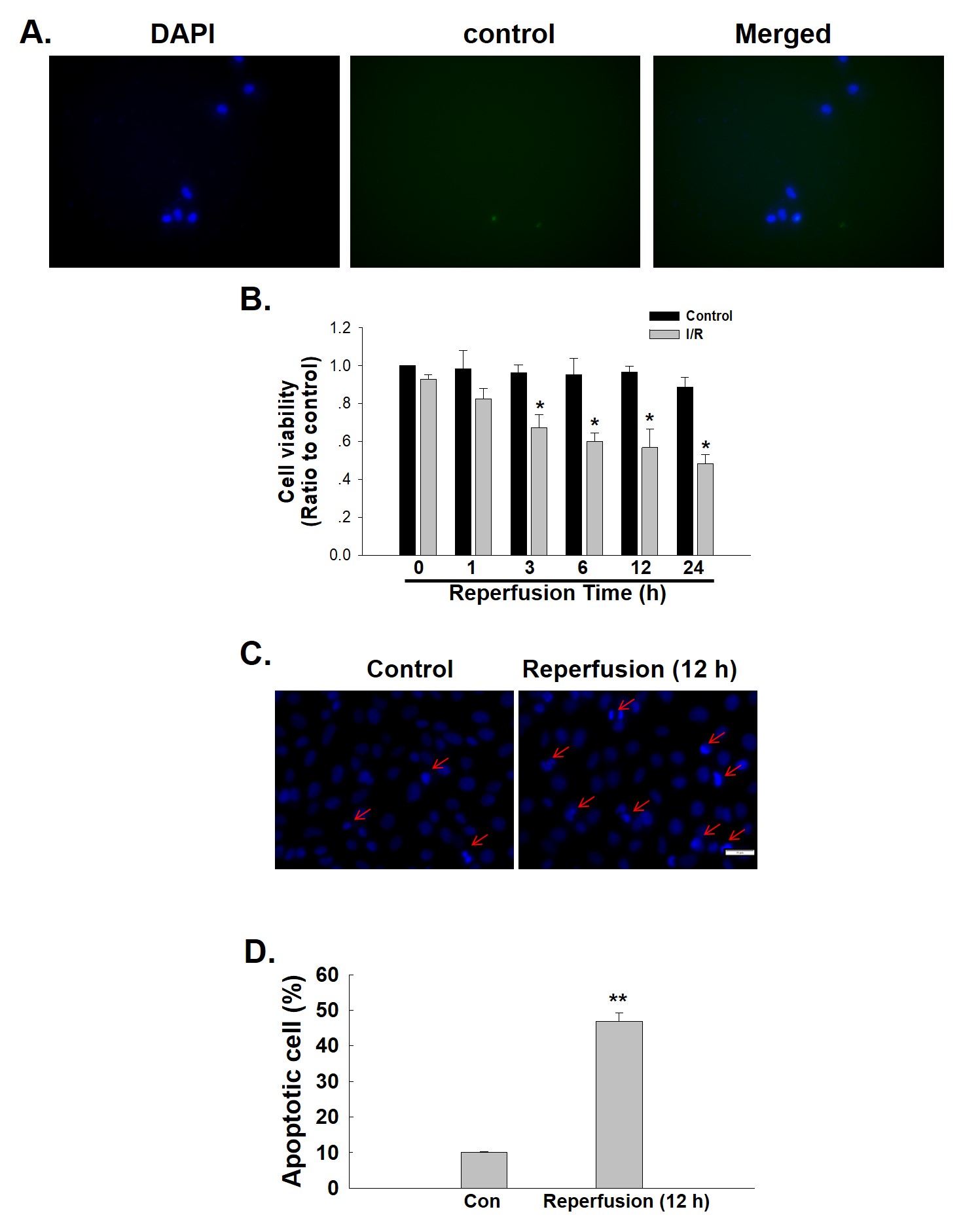
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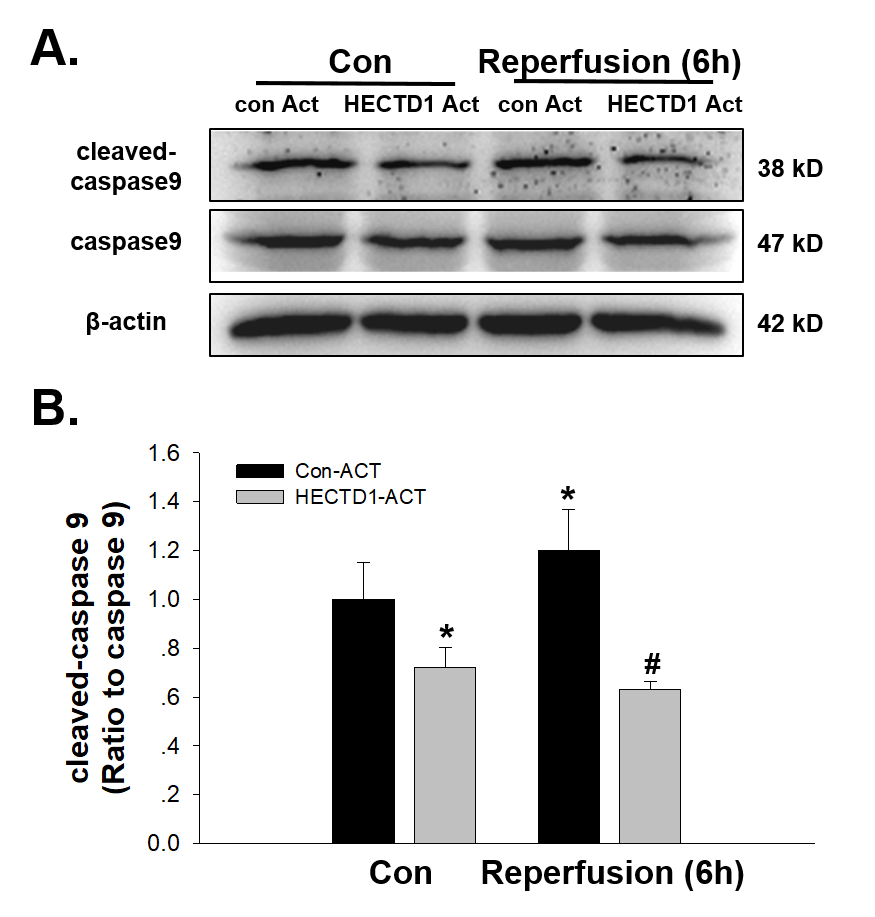
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**Supplementary Figure S1**

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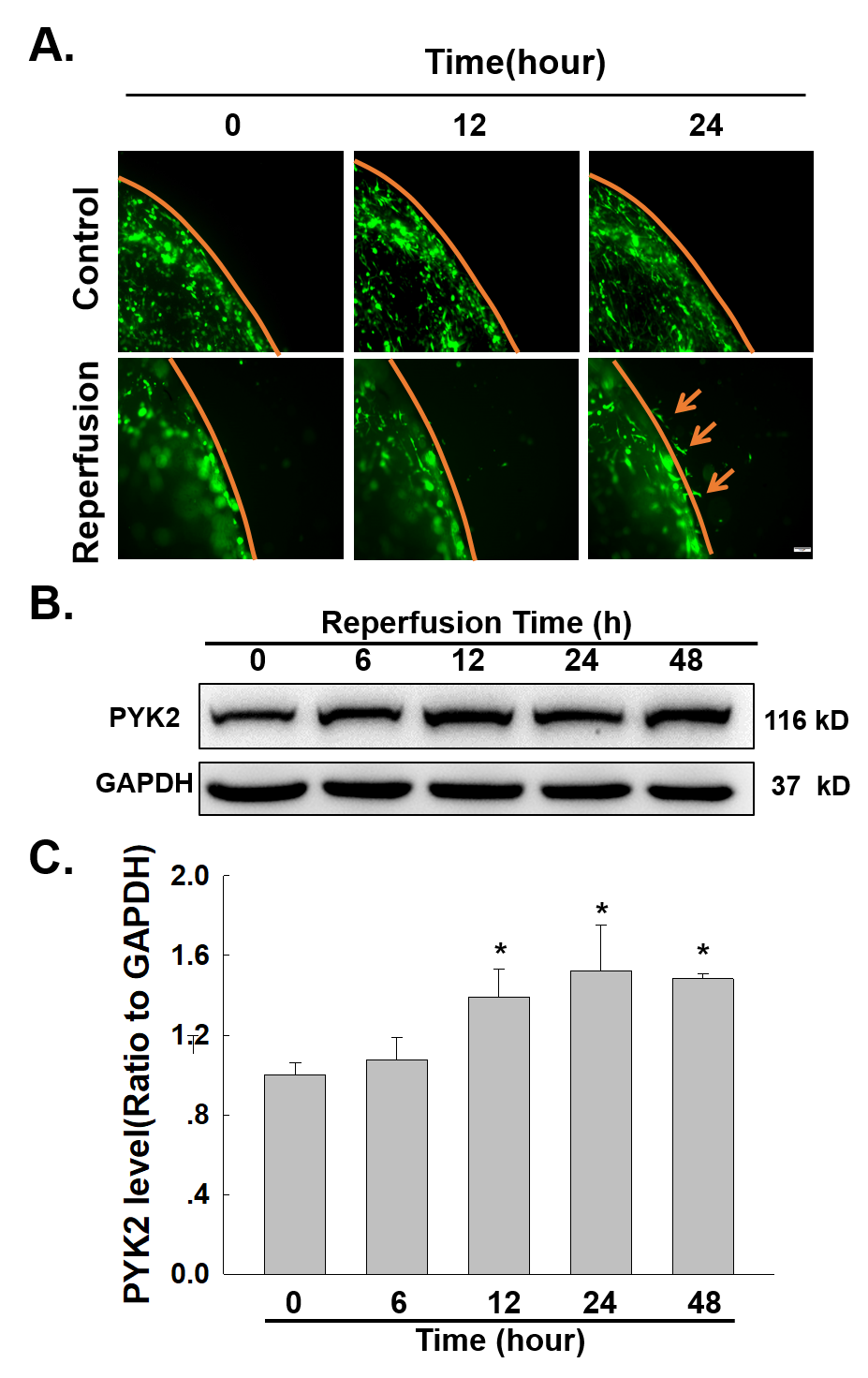
**Figure S1. Effect of I/R on cell viability in HUVECs.** (A) Representative images of immunocytochemistry without primary antibody as negative control to show the specificity of detection of target proteins. (B) CCK-8 assay results showing an I/R-induced decrease in HUVEC viability. \**P <* 0.05 vs*.* the 0 h group, n=5. (C) Representative images of Hoechst 33342 staining of HUVECs after I/R. (D) Hoechst 33342 staining demonstrating the apoptosis induced by I/R; n = 5; *\*\*P* < 0.01 vs. the control group.

**Supplementary Figure S2**



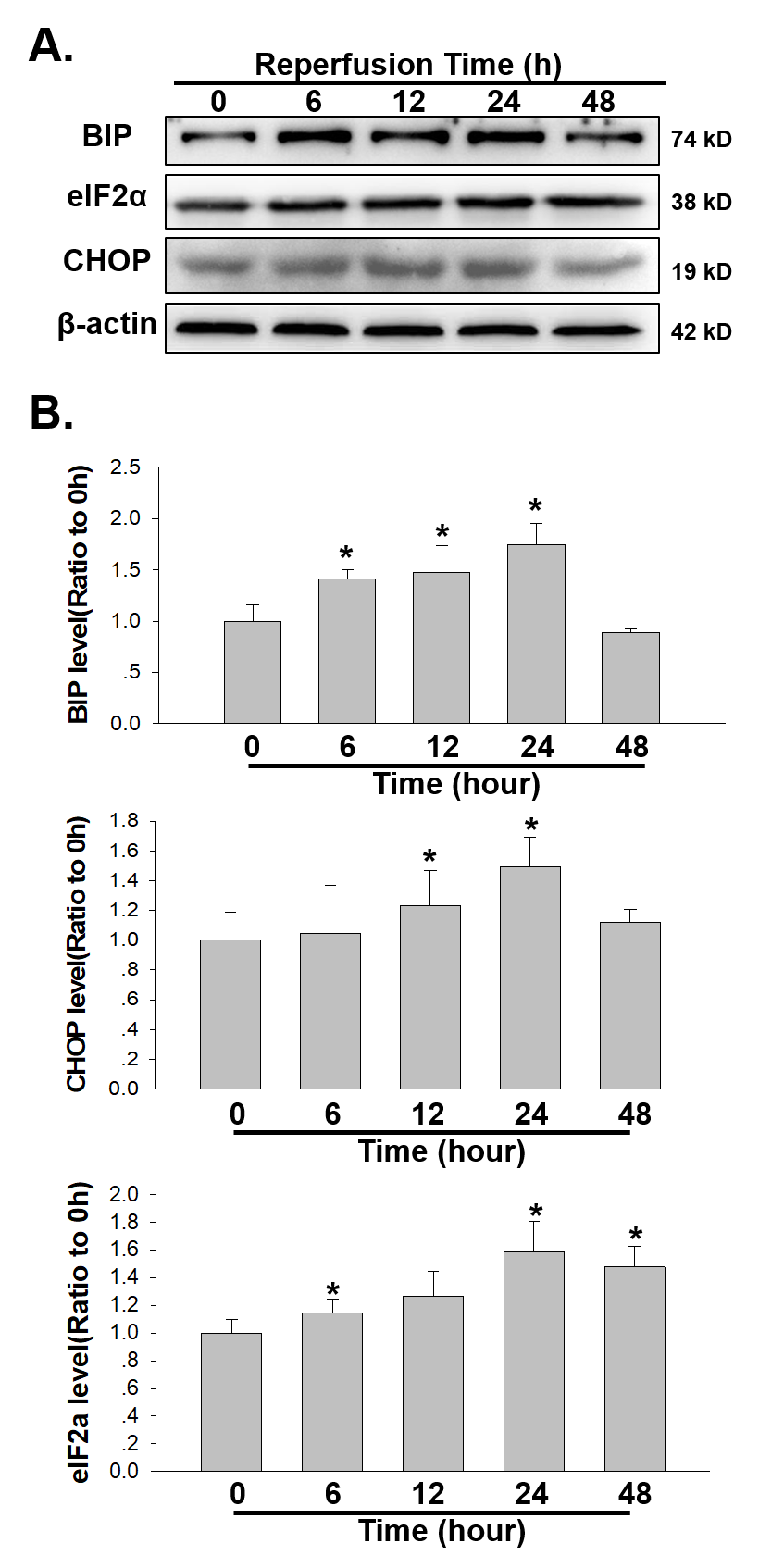
**Figure S2. Involvement of HECTD1 in I/R-induced apoptosis in HUVECs.** (A) Representative western blot depicting the effect of HECTD1 ACT on caspase-9 protein expression. (B) Densitometric analysis of caspase-9 protein levels from five independent experiments; \**P* < 0.05 vs. the control group; #*P* < 0.05 vs. the I/R group.

**Supplementary Figure S3**



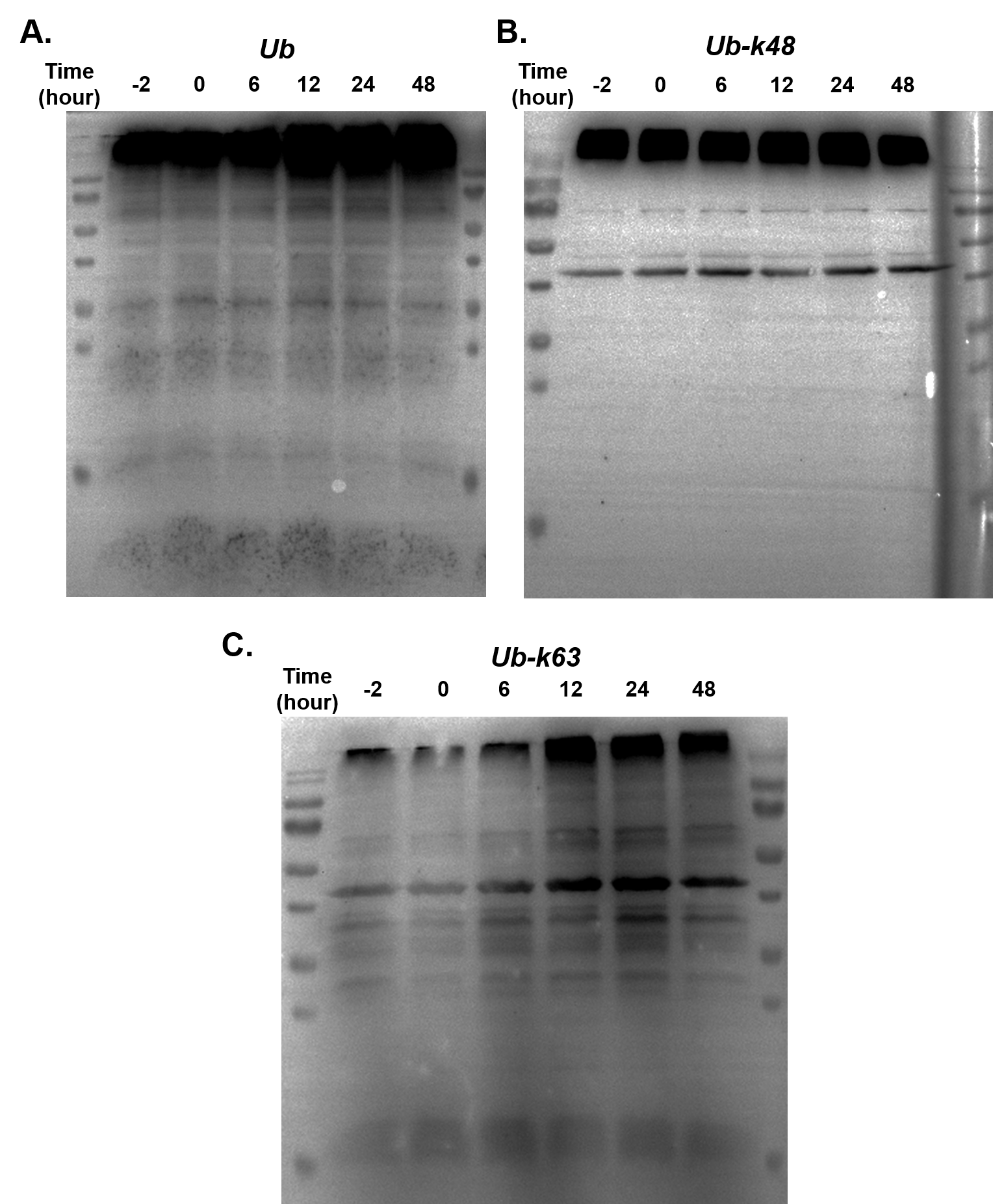
**Figure S3. Effect of I/R on cell migration in HUVECs.** (A) Representative images from a scratch assay showing the I/R-induced increase in cell migration. (B) Representative western blot showing that I/R induced the expression of PYK2. (C) Densitometric analysis of PYK2 expression from five experiments; \**P* < 0.05 vs. the 0 h group.

**Supplementary Figure S4**



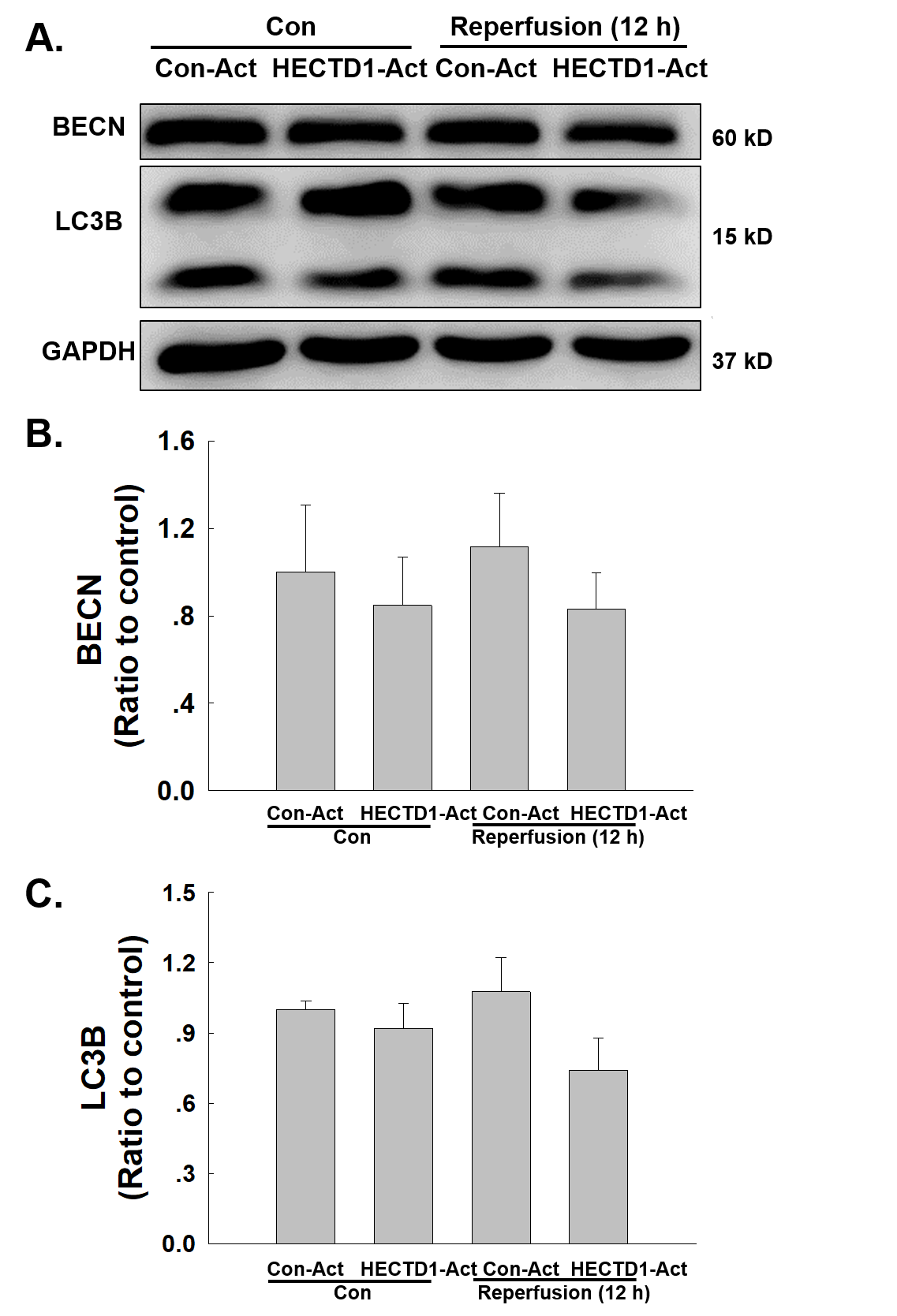
**Figure S4. Effect of I/R on ER stress in HUVECs.** (A) Representative western blot showing that I/R increased the expression of BIP, eIF2α and CHOP. (B) Densitometric analysis of BIP, eIF2α and CHOP protein levels from five independent experiments; \**P* < 0.05 vs. the control group.

**Supplementary Figure S5**



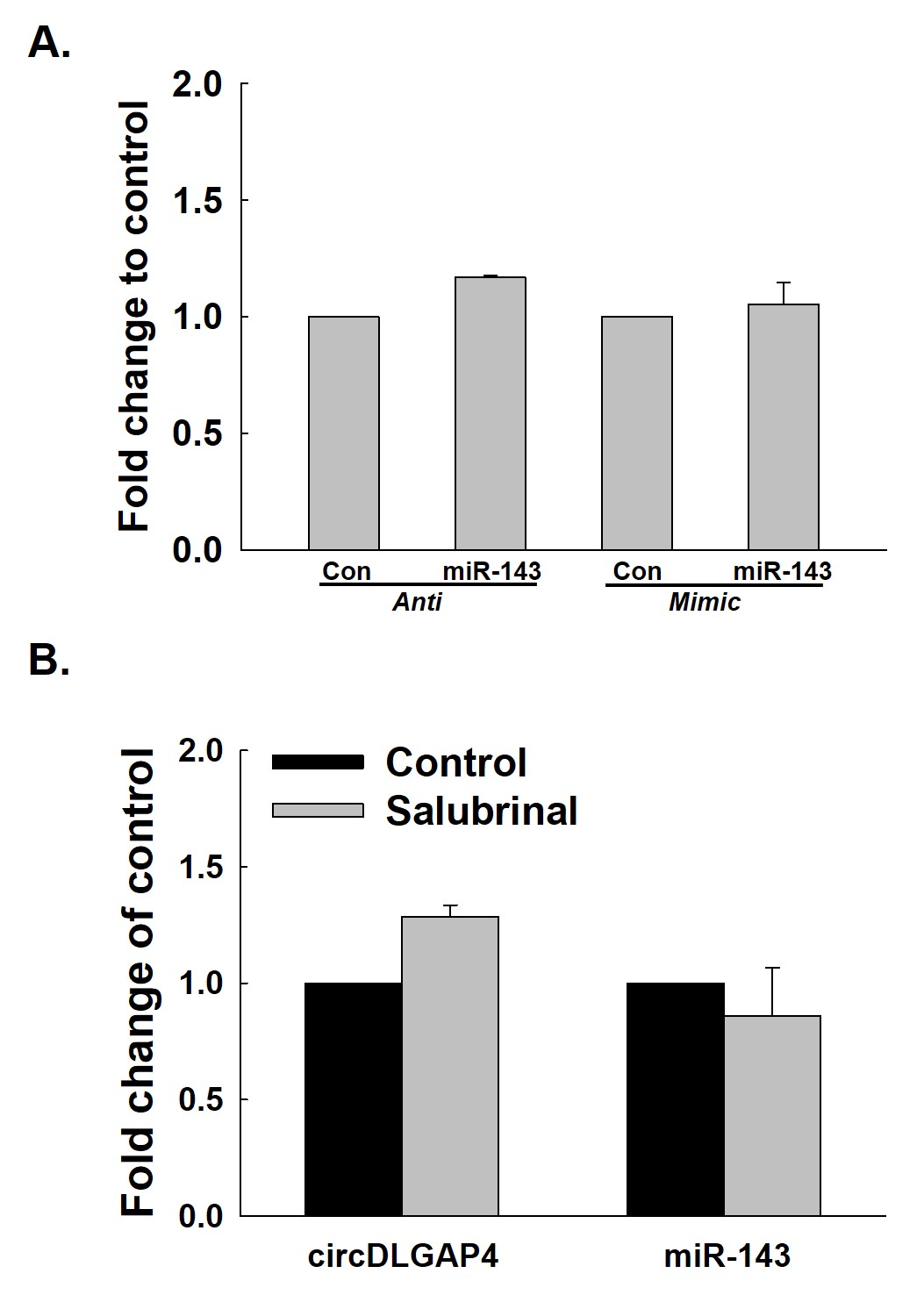
**Figure S5. Effect of I/R on ubiquitination in HUVECs.** (A) Representative western blot showing the effect of I/R on Ub. (B) Representative western blot showing the effect of I/R on Ub-k48. (C) Representative western blot showing the effect of I/R on Ub-k63.

**Supplementary Figure S6**



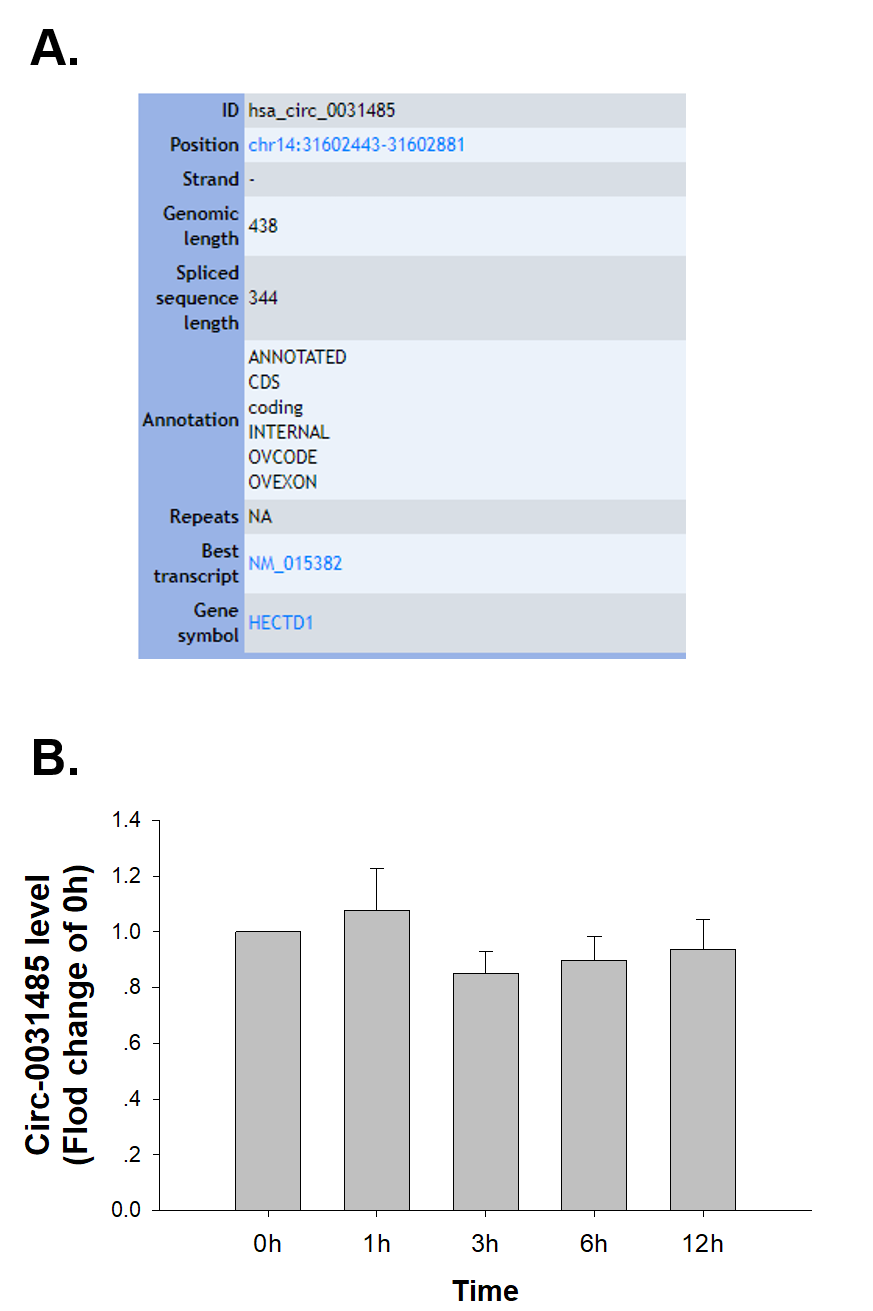
**Figure S6. Involvement of HECTD1 in I/R-induced autophagy in HUVECs.** (A) Representative western blot depicting the effect of HECTD1 ACT on BECN and LC3B protein expression. Densitometric analysis of BECN (B) and LC3B (C) protein levels from five independent experiments; \**P* < 0.05 vs. the control group, #*P* < 0.05 vs. the I/R group.

**Supplementary Figure S7**

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**Figure S7. Interaction of ER stress and circDLGAP4/miR143 pathway in HUVECs. (A)** As shown in the qRT-PCR analysis, regulation of miR-143 has no effect on circDLGAP4 expression (n=5). (B) As shown in the qRT-PCR analysis, salubrinal pretreatment regulation has no effect on circDLGAP4 and miR-143 expression (n=5).

**Supplementary Figure S8**



**Figure S8. Effect of I/R on circ0031485 in HUVECs.** (A) ID number and alias of circ0031485 from the circBase database. (B) As shown in the qRT-PCR analysis, I/R had no effect on circ0031485 expression (n=5).

**Supplementary Table S1  
Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)  
primers and FISH probes**

|  |  |  |
| --- | --- | --- |
| **mRNA qPCR primers** | | |
| **Gene** | **Forward (5′-3′)** | **Reverse (5′-3′)** |
| **GAPDH (human)** | **ACCATCTTCCAGGAGCGAGAT** | **GGGCAGAGATGATGACCCTTT** |
| **HECTD1 (human)** | **ACGGTTGTACGCAAGGTTGA** | **GGCGCTCTCTCATGATCTCC** |
| **Non-coding RNA qPCR primers** | | |
| **Name** | **Forward (5′-3′)** | **Reverse (5′-3′)** |
| **hsa\_circ\_0060180** | **CGAGACGGCTACTGGTTCCT** | **GCTTTGAAGTGGTGCGTGG** |
| **FISH probes** |  | |
| **Non-coding RNA** | **Sequence** | |
| **hsa\_circ\_0060180**  **(biotin-labeled FISH probe)** | **5′-AAAACTAGGCATGATGAACCTTCTTCAGAGAGGTT-3′** | |
| **miR-143 (DIG-labeled FISH probe)** | **5′-GAGCTACAGTGCTTCATCTCA-3′** | |

**The primers specific for the mature miR-143 and RNU6-6P/RNU6B snRNA were purchased from RiboBio.**