Molecules and Cells

SUPPLEMENTARY MATERIALS AND METHODS

Clinical samples

Between 2012-2014, patients were recruited for heart transplantation surgery at Asan Medical Center, Seoul, Korea. The Institutional Review Board (IRB) approved the study (approval No. 2012-0453). Written and informed consent forms were obtained from all the patients. The study was performed in accordance with the Declaration of Helsinki (2013). At least 1 year after the surgery, patients were diagnosed CAV based on luminal hyperplasia (>0.5 mm) demonstrated on intravascular ultrasound (IVUS) and coronary angiography (CAV group) or not (Control group). Both groups received standard postoperative care, and had no acute rejection or infection until assessment. The clinical information of the subjects is summarized in Supplementary Table S2.

MicroRNA microarray

The total RNA including miRNA was extracted from the biopsy samples from patients with CAV and healthy controls as previously described (Han et al., 2018). The RNAs were prepared and hybridized to a chip, the Agilent Human miRNA microarray, release 19.0, 8×60K (Agilent, USA). The miRNA data were aligned to miRBase (release 21; Agilent, UK) and analyzed by the Ebiogen Inc. service (Korea). The fold expression of miRNA was evaluated by the miRNA level of the CAV samples, which was normalized to that of the control samples.

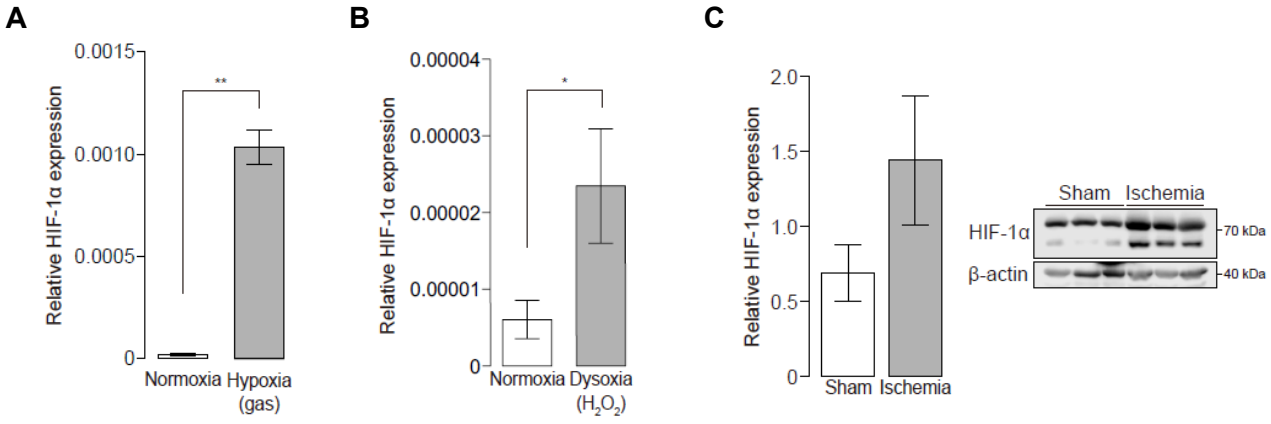
Supplementary Table S1. Primers used in this study

Name	Sequence (5′ to 3′)	Description
BCL2_F	TCGCCCTGTGGATGACTGA	For RT-qPCR
BCL2_R	CAGAGACAGCCAGGAGAAATCA	
HuP0_F	CCATTCTATCATCAACGGGTACAA	
HuP0_R	AGCAAGTGGGAAGGTGTAATCC	
HPRT_F	CCTGGCGTCGTGATTAGTG	
HPRT_R	CAGAGGGCTACAATGTGATGG	
HIF-1α_F	GGTTCCAGCAGACCCAGTTA	
HIF-1α_R	AGGCTCCTTGGATGAGCTTT	
BCL2_WT_F	TAGGCGATCGCTCGAAGTCAACATGCCTGCCCCAAA	For cloning to a psiCHECK-2 vector
BCL2_WT_R	AATTCCCGGGCTCGACCGTCTGCTCTTCAGATGGTGA	
BCL2_MUT_F	TATTTTCCGCAAAAGGGAAATATCATTTATTTTTTACA	
BCL2_MUT_R	CTTTTGCGGAAAATAGCTGATTCGACGTTTTG	

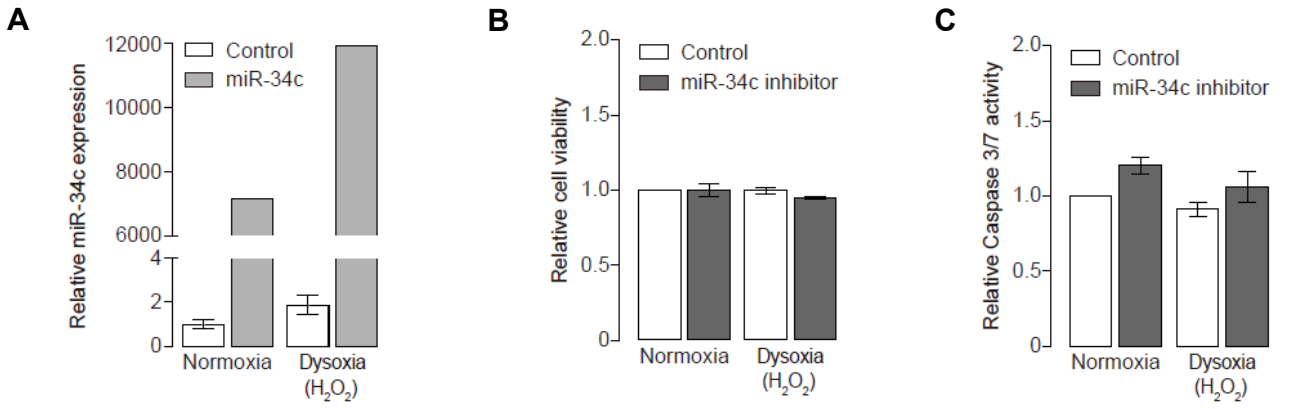
Supplementary Table S2. Patient demographics

	Control	CAV
Sex	M 3, F 2	M 5, F 1
Age (y)	58 ± 5.357	47 ± 7.944
ABO blood-type	A 1, B 2, O 2	A 2, B 2, O 2
Intimal thickness (1 y)	0.833 ± 0.214	1.207 ± 0.173
	No progression	All progression by +0.857 ± 0.129

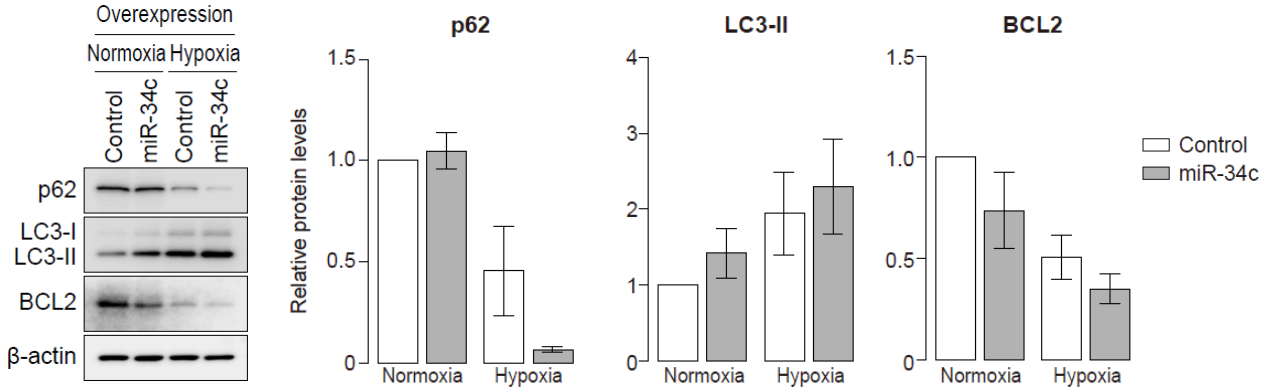
M, male; F, female.



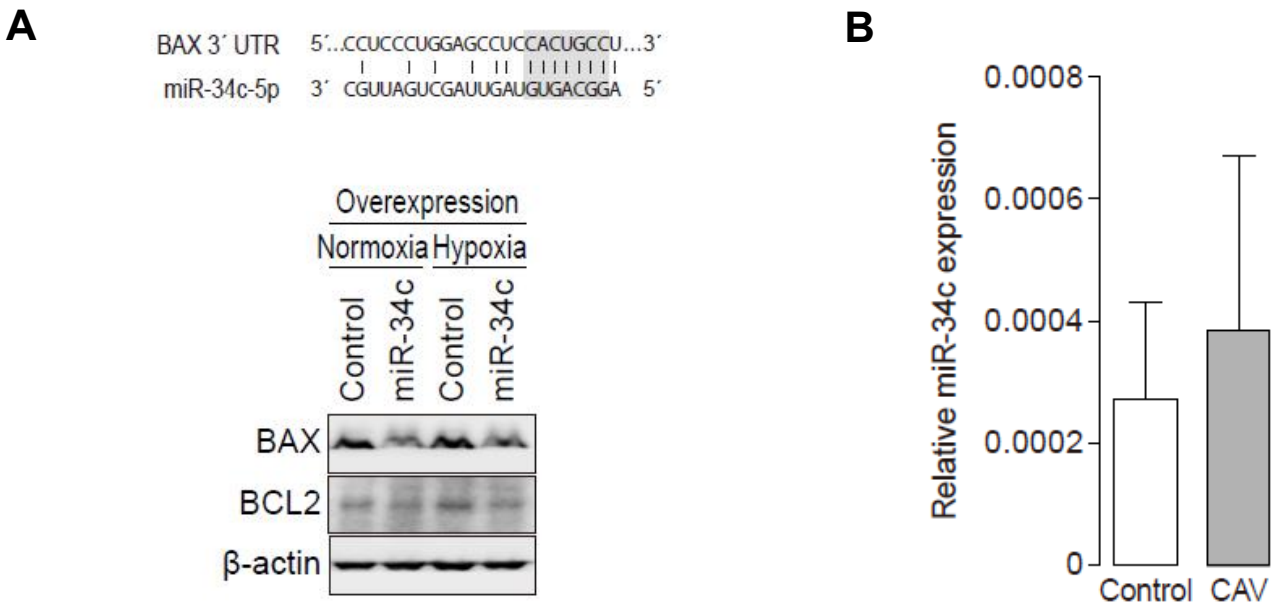
Supplementary Fig. S1. Validation of HIF-1α expression in both HUVEC and the ischemic mouse vessels. (A and B) Relative HIF-1α expressions of HUVECs under (A) hypoxia (1% O_2) and (B) dysoxia (700 nM of H_2O_2) were measured by a RT-qPCR. The mRNA levels of *HIF-1α* were normalized to both *HPRT* and *HuP0* used as internal controls (normoxia: n = 3, hypoxia and dysoxia: n = 8; mean ± SEM; $*P < 0.05$, $**P < 0.01$). (C) The upregulation of HIF-1α in the mouse vessels was used as an indicator of ischemia. The band intensities of HIF-1α were quantitated and normalized to those of β-actin (mean ± SEM; n = 3).



Supplementary Fig. S2. Validation of miR-34c in miR-34c-overexpressing HUVECs and evaluation of the caspase 3/7 activity and cell viability in miR-34c-inhibited HUVECs. (A) Relative miR-34c levels in the miR-34c-overexpressing HUVECs with or without H_2O_2 treatment. The graph shows the mean ± SEM (n = 3). (B and C) In the presence or absence of H_2O_2 in miR-34c-inhibited HUVECs, CCK8 cell viability assay (B) and caspase 3/7 assay (C) were performed. The inhibition of miR-34c was performed with mirVana (Thermo Fisher Scientific, USA), following the manufacturer's instructions. Each value was normalized to that of the control sample under conditions of normoxia (mean ± SEM; n = 3).



Supplementary Fig. S3. Repression of BCL2 and activation of autophagy in miR-34c-overexpressing HUVECs under hypoxia. Relative protein levels of BCL2 and autophagic markers (p62 and LC3-II) was analyzed and normalized to those of β-actin in miR-34c overexpressing HUVECs under hypoxia by gas-induction (1% O_2). The graph indicates the mean ± SEM (n = 3).



Supplementary Fig. S4. Downregulation of BAX in miR-34c-overexpressing HUVECs and validation of miR-34c expression in CAV patient samples. (A) A target site of miR-34c-5p in BAX 3′ UTR was bioinformatically predicted using the TargetScan prediction tool. The seed region is displayed in gray (upper panel). The BAX and BCL2 protein levels were analyzed in miR-34c-overexpressing HUVEC by western blotting. β-actin was used as a loading control (lower panel). Control, empty vector (mock) control; miR-34c, miR-34c-overexpressing. (B) Relative miR-34c expression in the human heart biopsy samples from the control or CAV groups (normalized to U6 snRNA). The graph shows the mean ± SEM (Control: n = 5, CAV: n = 6).