Endothelial β1 Integrin-Mediated Adaptation to Myocardial Ischemia

Carina Henning[1,*] Anna Branopolski[1,2,*] Paula Follert[1] Oksana Lewandowska[1] Aysel Ayhan[2] Marcel Benkhoff[2] Ulrich Flögel[3] Malte Kelm[2] Christian Heiss[2,4,5,*] Eckhard Lammert[1,6,7,*]

[1] Institute of Metabolic Physiology, Department of Biology, Heinrich-Heine-University, Düsseldorf, Germany
[2] Division of Cardiology, Pulmonology, and Vascular Medicine, Heinrich-Heine-University, Düsseldorf, Germany
[3] Institute for Molecular Cardiology, University Hospital Düsseldorf, Heinrich-Heine-University, Düsseldorf, Germany
[4] Department of Clinical and Experimental Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom
[5] Surrey and Sussex Healthcare NHS Trust, Redhill, Surrey, United Kingdom
[6] Institute for Vascular and Islet Cell Biology, German Diabetes Center (DDZ)—Leibniz Center for Diabetes Research, Düsseldorf, Germany
[7] German Center for Diabetes Research (DZD e.V.), Neuherberg, Germany

Address for correspondence Dr. rer. nat. Eckhard Lammert, Institute of Metabolic Physiology, Heinrich-Heine-University Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany (e-mail: lammert@hhu.de).

Dr. med. Christian Heiss, Department of Clinical and Experimental Medicine, Faculty of Health and Medical Sciences, University of Surrey, Stag Hill, Guildford U2 7XH, United Kingdom (e-mail: c.heiss@surrey.ac.uk).

Thromb Haemost 2021;121:741–754.

Abstract

Background Short episodes of myocardial ischemia can protect from myocardial infarction. However, the role of endothelial β1 integrin in these cardioprotective ischemic events is largely unknown.

Objective In this study we investigated whether endothelial β1 integrin is required for cardiac adaptation to ischemia and protection from myocardial infarction.

Methods Here we introduced transient and permanent left anterior descending artery (LAD) occlusions in mice. We inhibited β1 integrin by intravenous injection of function-blocking antibodies and tamoxifen-induced endothelial cell (EC)-specific deletion of *Itgb1*. Furthermore, human *ITGB1* was silenced in primary human coronary artery ECs using small interfering RNA. We analyzed the numbers of proliferating ECs and arterioles by immunohistochemistry, determined infarct size by magnetic resonance imaging (MRI) and triphenyl tetrazolium chloride staining, and analyzed cardiac function by MRI and echocardiography.

Results Transient LAD occlusions were found to increase EC proliferation and arteriole formation in the entire myocardium. These effects required β1 integrin on ECs, except for arteriole formation in the ischemic part of the myocardium. Furthermore, this integrin subunit was also relevant for basal and mechanically induced proliferation of human coronary artery ECs. Notably, β1 integrin was needed for cardioprotection induced by transient LAD occlusions, and the absence of endothelial β1 integrin resulted in impaired growth of blood vessels into the infarcted myocardium and reduced cardiac function after permanent LAD occlusion.

Conclusion We showed that endothelial β1 integrin is required for adaptation of the heart to cardiac ischemia and protection from myocardial infarction.

Keywords
- endothelial β1 integrin
- cardiac vascular growth
- myocardial infarction
- cardioprotection

* *These authors contributed equally to this work.*

received
October 22, 2020
accepted
October 28, 2020
published online
January 14, 2021

DOI https://doi.org/10.1055/s-0040-1721505.
ISSN 0340-6245.

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

Introduction

Coronary artery disease (CAD) is the leading cause of death around the globe.[1] Due to diminished blood supply of the heart, it can result in myocardial infarction (MI), heart failure, and death. Current prevention strategies aim to reduce risk factors that negatively affect the cardiovascular system, such as hyperglycemia or diabetes mellitus, hyperlipidemia, smoking, hypertension, physical inactivity, and unhealthy dietary patterns.[2,3] Notably, cardiac vascular growth was also observed as an important factor to protect from MI or to improve its outcome. Hereby, functional growth of coronary vessels creates additional and alternative routes to provide the ischemic myocardium with fresh blood, thus reducing MI size or preserving cardiac function after MI.[4–7]

Hypoxic and mechanical stimuli are thought to be involved in vascular growth.[8] Hypoxia activates hypoxia-inducible factor-1α to induce signals, such as vascular endothelial growth factor-A (VEGF-A) that preferentially triggers vascular growth in a paracrine manner.[9,10] In contrast, mechanical stimuli directly activate mechanosensitive proteins, including integrins, in cells of the blood vessels.[11–14] There, they stimulate vascular growth by cross-activating VEGF receptors,[10,15,16] endothelial cell (EC) reorientation,[17] and trigger the release of angiocrine signals needed for tissue growth and survival.[18]

The mechanosensitive β1 integrin is part of several integrin receptors, acts as an essential "bridge" between the basement membrane (BM) or extracellular matrix and cytoskeleton of ECs, and—along with some of its mechanosensitive α subunits—is required for embryonic and postnatal vascular development.[19–23] The β1 integrins are of particular interest, since they bind collagen type I, which is involved in vascular growth and left ventricular (LV) remodeling and dysfunction.[24] They also influence fibrosis and wound repair,[25] and to date the β1 integrin has been targeted among others in cardiomyocytes, myofibroblasts, and pericytes,[26–29] but not in the adult cardiac endothelium, to uncover its function in ischemia-induced protection of the heart from MI and preservation of cardiac function.

Here, we studied the hypothesis that endothelial β1 integrin is relevant for the ischemia-induced coronary vascular growth and cardiac adaptation to transient and chronic ischemia. Therefore, transient left anterior descending artery (LAD) occlusions were performed to mimic temporary ischemia observed in patients with CAD and discussed as a trigger for coronary growth.[4] Besides, short periods of ischemia induce ischemic preconditioning, which involves multiple different signaling pathways, including those activated by endothelial nitric oxide synthase (eNOS).[30–33] Additionally, a permanent LAD occlusion was performed to mimic the situation in patients with MI, but without receiving proper reperfusion.[34] To this end, both models were combined with pharmacologic and genetic manipulation of β1 integrin and analyses using different imaging techniques were performed. We first asked whether transient occlusions of the LAD induced EC proliferation and vascular growth only in the ischemic myocardium or also in the non-ischemic, right ventricular (RV) and septal myocardium. We then studied by infusion of β1 integrin function-blocking antibodies and induction of EC-specific genetic deletion of *Itgb1* whether β1 integrin on the endothelium is required for cardiac vascular growth induced by transient LAD occlusion and preservation of heart function. Further, we wished to find out whether β1 integrin and eNOS interact with each other and if β1 integrin is needed for basal and mechanically induced proliferation of human coronary artery ECs (HCAECs) as a human correlate of the experiments on the adult mouse heart. Finally, we studied whether endothelial β1 integrin had a general function to protect the heart and preserve cardiac function upon MI, independent of short episodes of ischemia.

Our study uncovers an important role of endothelial β1 integrin in cardiac adaptation to LV ischemia as well as preservation of cardiac function after an MI.

Methods

Mice

For wild-type studies with antibody treatment, 10- to 15-week-old male C57BL/6J mice from Janvier were used. β1 integrin blocking antibody (BD Bioscience, 555002) and control antibody (BD Bioscience, 553957) were intravenously injected (1 mg/mL, 100 µL) 1 day before each 15-minute ischemia and before and after permanent occlusion. For genetic deletion experiments, global *NOS3* (*eNOS* KO) mice[35] were used. For endothelial *Itgb1* deletion (*Itgb1*^{iECKO}), *Cdh5-Cre*^{ERT2}; homozygous *Itgb1-loxP* mice[36,37] were crossed with homozygous *Itgb1-loxP* mice. As controls for *eNOS* KO mice, the corresponding C57BL/6J mice from Jackson Laboratory were used. As controls for *Itgb1*^{iECKO} mice, *Cdh5-Cre*^{ERT2} mice were used, which received the same tamoxifen injections. Both mouse lines were treated with 100 µL tamoxifen (75 mg/kg bodyweight) for 5 consecutive days. All experiments were performed according to the German animal protection laws (Animal Ethics Committee of the Landesamt für Natur, Umwelt und Verbraucherschutz, North Rhine-Westphalia).

Myocardial Ischemia

For the induction of transient LAD occlusions, a closed-chest model was used, as previously described.[4] Briefly, in an initial operation an occluder was implanted for temporary occlusion of the LAD, therefore a 7–0 prolene suture (Ethicon, Johnson and Johnson) was placed around the proximal LAD, ends were threaded through a polyethylene tube, the thorax was closed, and the suture ends were placed into a subcutaneous pocket. For permanent LAD occlusion, mice were treated the same way but the LAD was closed with a 7–0 silk suture (Seraflex, Serag-Wiessner) and myocardial ischemia was induced directly. In general, 7 days after the initial operation, the first ischemia for transient LAD occlusions was induced. However, during the proliferation analysis in blocking AB-treated mice, and when RepI/R and MI were combined for magnetic resonance imaging (MRI), the first ischemia was induced 2 days after the initial operation, so as to shorten the experimental procedure. Mice were anesthetized in an inhalation anesthetic set with oxygen-enriched air (O_2 40%) and isoflurane (3% vol., Piramal

Healthcare). After anesthesia, the mice were placed in a supine position on a 37°C preheated surgery table, followed by respiration with oxygen-enriched air (O_2 40%) and isoflurane (2% vol., Piramal Healthcare). After skin disinfection, electrocardiogram (ECG) electrodes were fixed to the fore- and hindfeet and the skin was reopened to pull out the suture ends. Afterwards, 15-minute or 60-minute ischemia was induced by sticking the suture ends to two magnets and setting them under tension to ligate the LAD. Induction of ischemia was controlled in ECG by ST-segment elevation followed by reperfusion of the LAD. Suture ends were placed back into the subcutaneous pocket or cut off after the last ischemia. The 15-minute ischemia was repeated three times every other day. Additional 60-minute ischemia was followed only if the infarct size (IS) and cardiac function were quantified. All mice received an occluder implantation and afterward were randomized to sham or ischemia/reperfusion (I/R) procedure. Mice were intraperitoneally (IP) injected with ketamine (100 mg/kg bodyweight, Ketanest S., Pfizer Pharma GmbH) and xylazine (10 mg/kg bodyweight, Rompun TM, Bayer Healthcare) for anesthesia and treated with Temgesic (0.05–0.1 mg/kg bodyweight) for analgesia.

Cardiac Function

For analysis of IS and cardiac function, ^{1}H MRI measurements were performed at 400.13 MHz using a Bruker AVANCE III 9.4 T Wide Bore NMR spectrometer (Bruker) driven by ParaVision 5.1, as described previously.[38] In the case of late gadolinium enhancement (LGE) measurements, mice received an IP injection of 0.2 mmol Gd-DTPA per kg bodyweight immediately prior to the measurement. To calculate the LV ejection fraction (EF), defined as the difference between end-diastolic volume (EDV) and end-systolic volume (ESV) divided by EDV, the ventricular borders were selected manually by means of the ParaVision region-of-interest tool (Bruker). For quantification of IS, LGE-positive areas were selected manually and related to the LV volume. Transthoracic echocardiography was also used to analyze LV cardiac function, as previously described.[39] Therefore, measurements were performed with a MS-400 scan head (Vevo 2100, VisualSonics, FUJIFILM). EDV, ESV, and EF were calculated from long-axis B mode in the Vevo laboratory software 1.7.1 (VisualSonics). Only mice that survived until the end of the experiment were used for analysis.

Osmotic Pump Implantation

Osmotic pumps (Alzet, model 1002) were filled with 20 mg/mL 5-bromo-2′-deoxyuridine (BrdU, Sigma) in phosphate buffer saline (PBS) and were implanted IP during the first 15-minute ischemia. The pumps remained in the mice until heart isolation.

Pimonidazole Treatment

To determine hypoxic regions in the myocardium during ischemia, mice were treated with pimonidazole (Hypoxyprobe Green Kit, Hypoxyprobe, Inc., Burlington, Massachusetts, United States). More specifically, after anesthesia, pimonidazole (60 mg/kg bodyweight) dissolved in PBS was injected IP and the LAD was occluded as described before, without reperfusion. After ischemia induction, hearts were isolated, perfused with ice-cold PBS, deep-frozen in nitrogen, and embedded in Tissue-Tek O.C.T. (Sakura Finetek GmbH) for cryosectioning.

Immunohistochemistry

Heart isolation was performed 7 days after the last 15-minute ischemia, except for the proliferation analysis in AB-treated mice, which was 2 days after the last 15-minute ischemia. For proliferation analysis, sections were pretreated with 2 M HCl for 30 minutes at 37°C and neutralized with 0.1 M sodium tetraborate, followed by primary and secondary antibody incubation. The following primary antibodies were used in this study: rat anti-BrdU (Abcam, ab6326), goat anti-PECAM-1 (R&D Systems, AF3628), mouse anti-αSMA-Cy3 (Sigma, C6198), mouse anti-αSMA (Sigma, A5228), and rat anti-β1 integrin (Millipore, MAB1997), and incubated overnight at 4°C. Secondary antibodies conjugated with fluorophores AF488 (Invitrogen Molecular probes), Cy3 (Jackson ImmunoResearch), and Cy5 (Jackson ImmunoResearch) were incubated in the dark for 1 or 3 hours at room temperature. Cell nuclei were stained with DAPI (Sigma). For hypoxia analysis, sections were stained with fluorophore-coupled antibodies for HP-FITC-MAb (detects pimonidazole adducts) and mouse anti-αSMA-Cy3 (Sigma, C6198) overnight at 4°C. Cell nuclei were stained with DAPI (Sigma).

Hearts with considerable tissue damage and areas of cryosections containing part of the occlusion system were excluded from quantification.

Triphenyl Tetrazolium Chloride Staining

The triphenyl tetrazolium chloride (TTC) staining was performed to determine the IS and area at risk (AAR), after 60-minute I/R. After anesthesia, hearts were isolated, cannulated over the aorta and washed with NaCl solution to remove remaining blood clots. Afterwards, the LAD was permanently closed with a silk suture and the hearts were perfused with 1% Evans blue solution (in NaCl). Hearts were frozen at −20°C for approximately 1 hour, transversally cut, transferred into TTC solution containing 8 parts 0.1 M Na_2HPO_4, 2 parts 0.1 M NaH_2PO_4 (pH 7.4), and 10 mg/mL TTC and incubated at 37°C for 5 to 10 minutes under shaking. Images were taken with a stereomicroscope (Leica MZ6 with Leica KL 1500 LCD), and infarcted area and AAR in the LV were analyzed with the software DISKUS (Vers. 4.30.7, Carl H. Hilgers, DISKUS mikroskopische Diskussion).

Microfil Perfusion

To image the entire coronary vasculature, hearts were washed and retrograde-perfused via the thoracic aorta with Microfil compound (Flow Tech Inc.), previously described but modified.[40] For optical clearing, hearts were incubated in methyl salicylate (Sigma Aldrich) and afterward imaged under a stereomicroscope (Leica MZ6 with Leica KL 1500 LCD).

Primary Human Cell Culture, Transfection, and Mechanical Stretching

Primary HCAECs (male, 21 years old), purchased from PELO-Biotech, were cultured in a humidified atmosphere at 5% CO_2 and 37 °C. HCAECs were grown in a microvascular EC growth medium kit enhanced (PELOBiotech), plated on stretch chambers (STEX) or on 12-well plates, and used up to passage 6. To selectively knock down the expression of β1 integrin, HCAECs were transfected with one of the following *ITGB1*-siRNAs (β1-siRNA) (Invitrogen) via electroporation (4D-Nucleofector System, LONZA) and incubated for 48 hours:

β1-siRNA1: 5′-CCUAAGUCAGCAGUAGGAACAUUAU-3′
β1-siRNA2: 5′-UGCGAGUGUGGUGUCUGUAAGUGUA-3′
β1-siRNA3: 5′-GGGAGCCACAGACAUUUACAUUAAA-3′.

As control, a non-targeting small interfering RNA (siRNA; Invitrogen) with a similar GC content was used. Additionally, HCAECs were mechanically stretched for 30 minutes in the case of proliferation studies and for 15 minutes to analyze β1 integrin activation by means of a manual cell-stretching system (STREX, STB-10). Knockdown efficiencies were analyzed by quantitative real-time polymerase chain reaction (PCR) and Western blotting.

In Vitro Proliferation Analysis and β1 Integrin Activation

For analysis of in vitro proliferation, 10 µM BrdU (Sigma) was added to the transfected HCAECs and incubated for 2 hours. In the case of stretching experiments, BrdU was added at the beginning of the stretching. After pretreatment of fixed cells with 2 M HCl and subsequent neutralization with 0.1 M sodium tetraborate, immunofluorescence staining was performed using mouse anti-BrdU antibody (BD Bioscience, 555627) as primary and anti-mouse antibody conjugated with AF488 (Invitrogen Molecular probes) as secondary antibodies. For β1 integrin activation analysis, HCAECs were fixed in 4% PFA after mechanical stretching, and the immunostaining was performed using mouse anti-human activated β1 integrin antibody (Merck Millipore, MAB2079Z) as primary and anti-mouse antibody conjugated with Cy5 (Jackson ImmunoResearch) as secondary antibodies.

Microscopy and Analysis

All immunofluorescence stainings were imaged using a confocal laser scanning microscope (LSM 710 or LSM 880, Zeiss). Proliferating ECs and arterioles were counted manually and related to the myocardial area in mm^2. Proliferating HCAECs were quantified either by manual or a semiautomated counting method in FIJI (ImageJ, NIH). The myocardial area was determined applying the freehand selection tool in FIJI, area or intensity of PECAM-1, DAPI or β1 integrin in heart sections and in HCAECs after mechanical stretching was analyzed with FIJI as well.

Magnetic-Activated Cell Sorting of ECs

For collection of cardiac ECs from *Itgb1*^{iECKO} and control mice, hearts were isolated and dissociated with a gentleMACS dissociator (Miltenyi Biotec). All further steps were performed according to the customer protocol (Miltenyi Biotec; "Preparation of single-cell suspensions from mouse heart," "CD45 MicroBeads," and "CD31 MicroBeads"). EC pellets were used either for quantitative real-time PCR and therefore homogenized with peqGold TriFast (Peqlab) or lysed in RIPA buffer for Western blot analysis.

Quantitative Real-Time PCR

Total RNA from cultivated HCAECs was extracted with an RNeasy Mini kit (Qiagen), according to the manufacturer's instructions. Total RNA isolation from magnetic-activated cell sorting (MACS)-sorted murine ECs was performed by using the phenol/chloroform extraction. Quantitative real-time PCR was performed with Brilliant III Ultra-Fast SYBR Green QPCR Master Mix and the thermal cycler Stratagene Mx3000P (Agilent Technologies). All samples were analyzed in triplicates. The following primers were used:

human *ITGB1* forward: 5′-CATCTGCGAGTGTGGTGTCT-3′
human *ITGB1* reverse: 5′-GGGGTAATTTGTCCCGACTT-3′
human *RPLP0* forward: 5′-GCAGCATCTACAACCCTGAAG-3′
human *RPLP0* reverse: 5′-CACTGGCAACATTGCGGAC-3′
human *HPRT* forward: 5′-TGACACTGGCAAAACAATGCA-3′
human *HPRT* reverse: 5′-GGTCCTTTTCACCAGCAAGCT-3′
human *B2M* forward: 5′-TTTCATCCATCCGACATTGA-3′
human *B2M* reverse: 5′-CCTCCATGATGCTGCTTACA-3′
mouse *Itgb1* forward: 5′-AATGCCAAGTGGGACACGGG-3′
mouse *Itgb1* reverse: 5′-TGACTAAGATGCTGCTGCTGTGAGC-3′
mouse *Rplp0* forward: 5′-GATGCCCAGGGAAGACAG-3′
mouse *Rplp0* reverse: 5′-ACAATGAAGCATTTTGGATAATCA-3′
mouse *Hprt*: forward: 5′-CACAGGACTAGAACACCTGC-3′
mouse *Hprt* reverse: 5′-GCTGGTGAAAAGGACCTCT-3′
mouse *β2m* forward: 5′-GAGCCCAAGACCGTCTACTG-3′
mouse *β2m* reverse: 5′-GCTATTTCTTTCTGCGTGCAT-3′.

The efficiencies of β1 integrin knockout (in vivo) or knockdown (in vitro) were determined by means of the ΔΔ*CT* method, where *RPLP0*/*Rplp0*, *HPRT*/*Hprt*, and *B2M*/*β2m* were used as housekeeping genes for normalization.

Western Blotting

Cultivated human or MACS-sorted murine ECs were lysed in RIPA buffer (50 mM Tris/HCl, pH 7.4, 150 mM NaCl, 1% IGEPAL, 0.25% Na-deoxycholate, 1 mM EDTA) containing protease inhibitors (cOmplete Protease Inhibitor Cocktail Tablets, Roche) and phosphatase inhibitors (Phosphatase Inhibitor Cocktail, Roche). Total protein concentrations were determined by means of the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Equal amounts of protein were used for the SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). For protein separation, 4 to 15% SDS-gels (Bio-Rad) and Mini-PROTEAN Tetra Cell (Bio-Rad) were used. Western blotting was performed by using the Trans-Blot Turbo Transfer System (Bio-Rad) and rabbit anti-GAPDH (Abcam, ab9485), rat anti-β1 integrin (Millipore, MAB1997) for MACS-sorted murine ECs or goat anti-β1 integrin (Santa Cruz, sc-6622) for human ECs as primary antibodies and HRP-conjugated donkey anti-rabbit (Cell Signaling Technologies, 7074), donkey anti-rat (Jackson ImmunoResearch, 712-035-150) and donkey anti-goat (Jackson

ImmunoResearch, 705–035–147) as secondary antibodies. To determine the efficiencies of β1 integrin-knockout (in vivo) or knockdown (in vitro), a semiquantitative band density analysis was performed (FIJI, ImageJ, NIH), where GAPDH was used as a housekeeping protein for normalization.

Statistical Analysis

Statistical significance was determined by using Excel (Microsoft) or Prism (GraphPad Inc.). To compare two groups, an unpaired two-tailed Student's *t*-test was used, whereas for multiple comparisons a one-way analysis of variance with subsequent Dunnett's posthoc test was performed. To compare survival rates, in a Kaplan–Meier curve a log-rank (Mantel–Cox) test was used. *p*-Values less or equal to 0.05 are shown either in the figures or mentioned in the figure legend. All values are shown as means ± standard error of the mean.

Results

Transient Episodes of LV Cardiac Ischemia Lead to β1 Integrin-Dependent Cardiac EC Proliferation and Arteriole Formation in the Entire Myocardium

We used a closed-chest mouse model to introduce transient LAD occlusions, leading to repetitive episodes of ischemia followed by reperfusion (RepI/R) in the LV myocardium (►**Fig. 1**), similar to a previously published mouse model.[4] The procedure employed an occluder to block the blood flow through the LAD three times every other day for 15 minutes each (►**Fig. 1A–G**). The LAD occlusions resulted in a transient transmural ischemia. This was indicated by ST-segment elevation on ECG that resolved when the LAD was opened again (►**Fig. 1D**). Hypoxia staining revealed that only the LV ("ischemic myocardium"), but not the RV and septum ("non-ischemic myocardium"), turned hypoxic during the LAD occlusions (►**Supplementary Fig. S1A–D**, available in the online version and ►**Fig. 1E, F**). As a control (RepSham), an occluder was implanted around the LAD, and the surgery was performed three times as described above (►**Fig. 1A–D**), but without occluding the LAD.

First, we studied the proliferation of cardiac ECs in response to the repeated LAD occlusions and the role of β1 integrin in this proliferative response by intravenously injected β1 integrin blocking antibodies (β1-B-AB) versus isotype-matched control antibodies (ctrl-AB) into mice (►**Fig. 1H–O**). When mice were treated with ctrl-AB, RepI/R increased cardiac EC proliferation in both the ischemic (►**Fig. 1R**) and non-ischemic myocardia (►**Fig. 1H–K, P**). In contrast, β1-B-AB abolished the RepI/R-induced cardiac EC proliferation in both, the ischemic and non-ischemic myocardia (►**Fig. 1L–O, Q, S**).

Next, we counted the number of arterioles in the myocardium of the hearts undergoing either RepI/R or RepSham treatments (►**Fig. 2**). Notably, the arterioles increased in number, both in the ischemic and non-ischemic myocardia at the level of papillary muscles upon RepI/R (►**Fig. 2A–D, I, K**). In contrast, no increase in the number of arterioles was observed upon RepI/R when mice were treated with β1-B-AB (►**Fig. 2E–H, J, L**).

Transient Cardiac Ischemia Protects from Myocardial Infarction and Subsequent Cardiac Dysfunction via β1 Integrin

We then investigated whether the RepI/R treatment reduced the IS of an MI induced by an additional 60-minute ischemia (►**Fig. 3A**). Consistent with previous results,[4] transient cardiac ischemia reduced the IS as compared with the RepSham treatment, analyzed in MRI and TTC staining (►**Fig. 3B–E, J**; ►**Supplementary Fig. S2A–E**; and ►**Video 1**, available in the online version). RepI/R also improved cardiac function after MI, as determined by preserved LV EF (►**Fig. 3L**).

Video 1

Comparison of myocardial function upon RepI/R treatment and myocardial infarction (MI) in adult mice. MRI-based visualization of myocardial function in adult mice. Mice received either a ctrl-AB (*top-left* and *top-right panels)* or a β1-B-AB (*bottom-left* and *bottom-right panels*) before RepSham or RepI/R treatments followed by MI (60-minute ischemia) with reperfusion. MRI, magnetic resonance imaging. Online content including video sequences viewable at: https://www.thieme-connect.com/products/ejournals/html/10.1055/s-0040-1721505.

Next, we analyzed the role of β1 integrin function in myocardial protection from MI. When mice were treated with β1-B-AB before induction of an MI (►**Fig. 3A**), transient cardiac ischemia no longer reduced the IS to a significant extent and no longer protected the heart from infarct-related LV dysfunction (►**Fig. 3F–I, K, M**; ►**Supplementary Fig. S2F–J**; and ►**Video 1**, available in the online version).

Requirement of Endothelial β1 Integrin for Cardiac EC Proliferation and Arteriole Formation in the Myocardium

To investigate whether β1 integrin in the endothelium was required for RepI/R-induced cardiac EC proliferation, we selectively deleted the *Itgb1* gene in the ECs of adult mice (*Itgb1*^{iECKO}) using tamoxifen-inducible *Cdh5-Cre*^{ERT2} expression (►**Fig. 4**). These adult mice expressed *Cre* recombinase only in the endothelium, but not in myeloid cells,[36] and were compared with tamoxifen-injected *Cre* control mice (►**Fig. 4A**). An up to 70% knockdown efficiency of β1 integrin in ECs sorted from tamoxifen-treated mouse hearts was observed on both mRNA and protein levels (►**Supplementary Fig. S3**; for uncropped Western blots, see ►**Supplementary Fig. S6**, available in the online version). In the controls, transient ischemia induced a twofold increase in cardiac EC proliferation in the non-ischemic myocardium as well as a three- to fourfold increase in EC proliferation in the ischemic myocardium (►**Fig. 4B–E, J, L**). In contrast, tamoxifen-induced EC-selective gene depletion of β1 integrin strongly reduced the proliferative response of cardiac EC to the short periods of cardiac ischemia (►**Fig. 4F–I, K, M**).

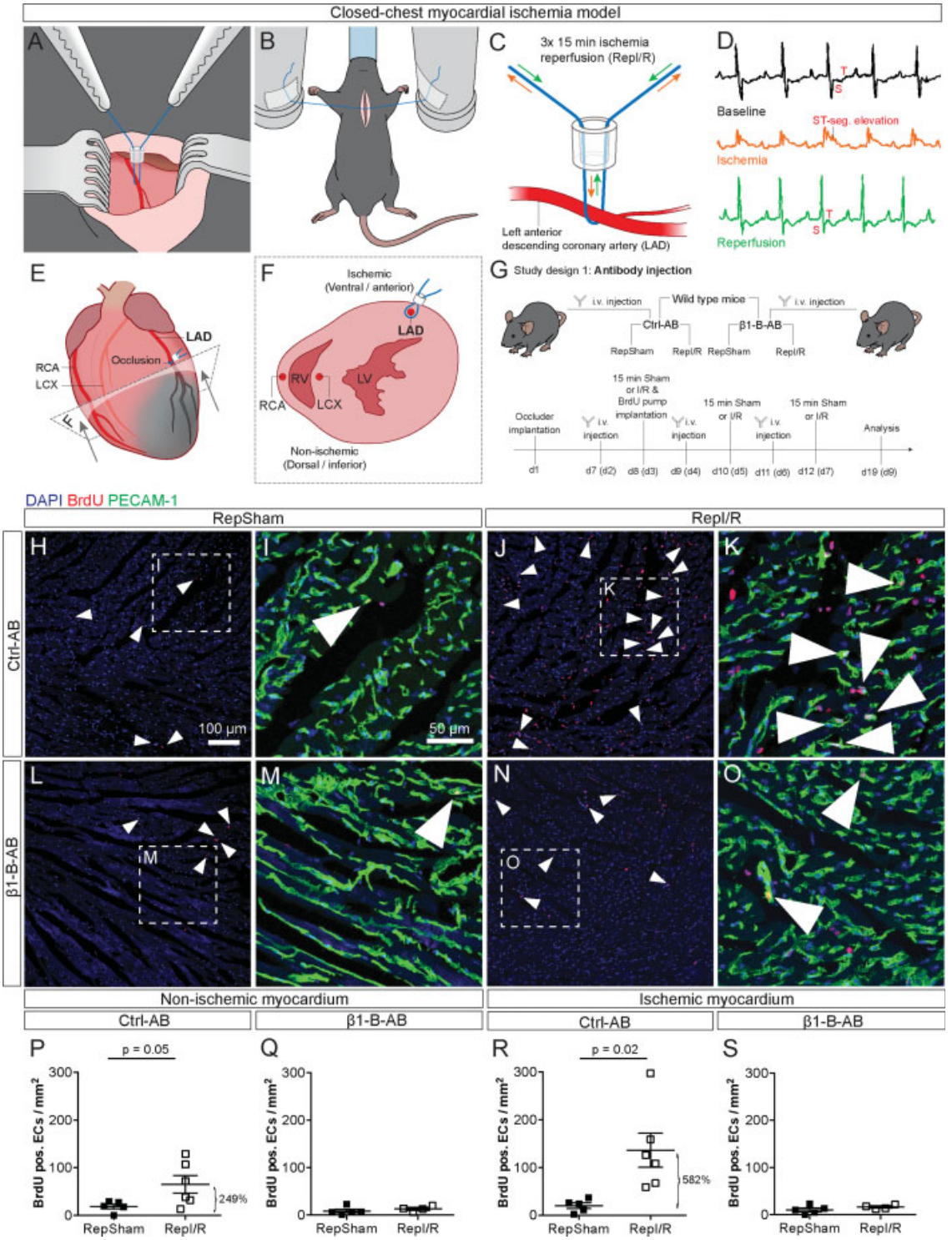

Fig. 1 Transient episodes of left ventricular (LV) cardiac ischemia require β1 integrin to induce cardiac EC proliferation in the entire myocardium. (**A–C**) Illustrations of (A) an implanted occluder at the LAD to introduce (**B, C**) 3× 15-minute myocardial ischemia treatment with reperfusion (RepI/R) in a closed-chest mouse model. (**D**) ECG recording before (baseline; *black line*), during (ischemia; *orange line*), and after the LAD occlusion (reperfusion; *green line*). (**E**) Illustration of an adult mouse heart, including the LAD with an occluder installed. The location of cross-section through the mouse heart (as illustrated in panel F and used for immunohistochemical analyses) is also indicated. (**F**) Schematic illustration of a cross-section through the ischemic and non-ischemic myocardium. (**G**) Study design to inhibit β1 integrin by antibody injection. A shortened experimental protocol is shown in brackets. (**H–O**) Representative immunofluorescence images of sections through the non-ischemic myocardium; mice received either (H–K) ctrl-AB or (L–O) β1-B-AB before each 15-minute ischemia or sham treatment. Sections were stained for BrdU (*red*), platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31; *green*), and DAPI (*blue*). (**P–S**) Quantification of BrdU-positive ECs per mm^2 in (P, Q) the non-ischemic and (R, S) the ischemic myocardium upon ctrl-AB or β1-B-AB treatment. Reported values are means ± SEM. Ctrl-AB with RepSham: $n = 5$; ctrl-AB with RepI/R: $n = 6$; β1-B-AB with RepSham: $n = 5$; β1-B-AB with RepI/R: $n = 4$. Statistical significance was determined using unpaired two-tailed Student's *t*-test. EC, endothelial cell; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; SEM, standard error of mean.

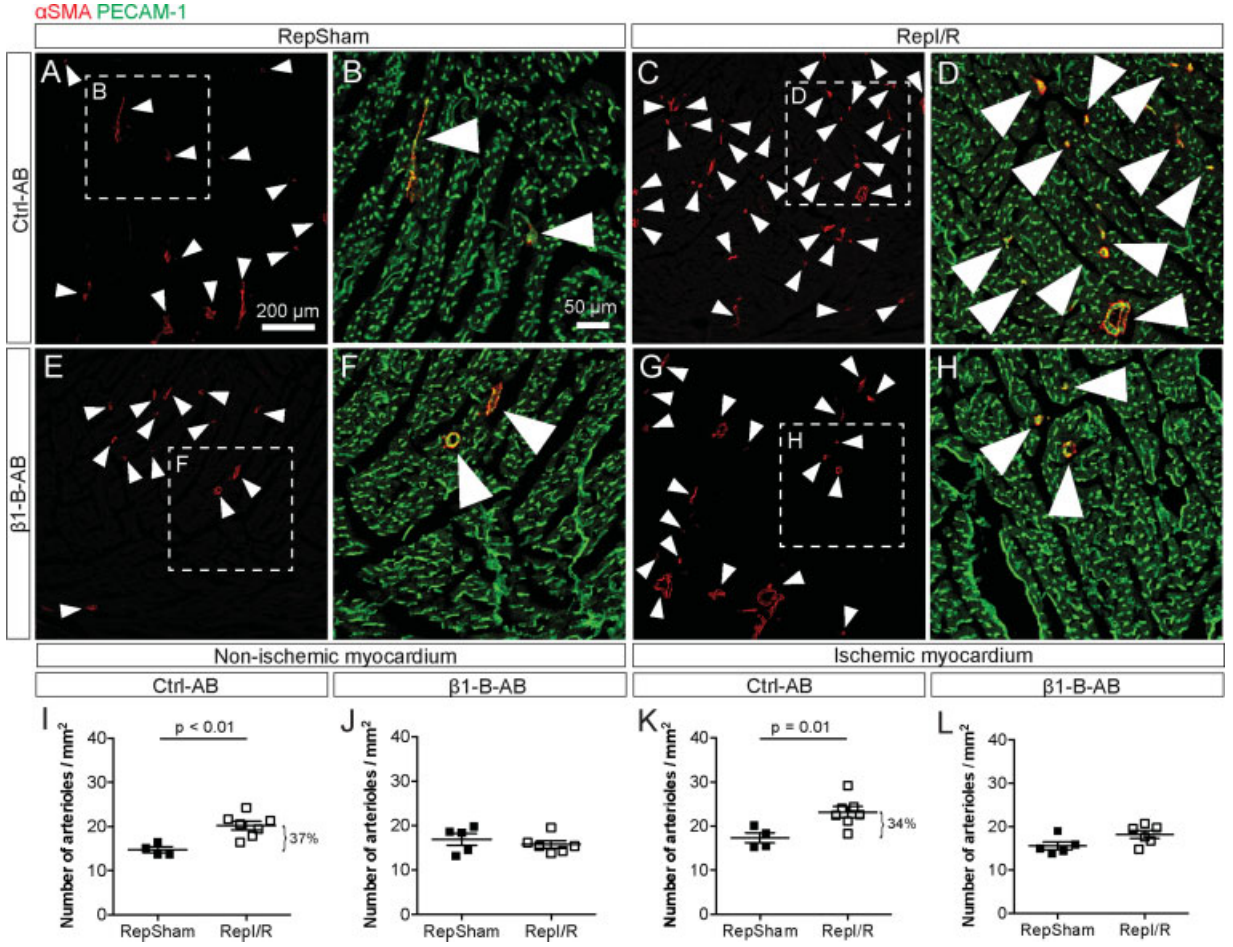

Fig. 2 Transient episodes of cardiac ischemia require β1 integrin to induce arteriole formation in the entire myocardium. (**A–H**) Representative immunofluorescence images of sections through the non-ischemic myocardium of adult mouse hearts; mice received either (A–D) ctrl-AB or (E–H) β1-B-AB before RepSham or RepI/R treatments. Sections were stained for α-smooth muscle actin (αSMA; *red*) and PECAM-1/CD31 (*green*). (**I–L**) Quantification of αSMA/PECAM-1-positive arterioles per mm^2 in (I, J) the non-ischemic and (K, L) the ischemic myocardium upon ctrl-AB or β1-B-AB treatment. Reported values are means ± SEM. Ctrl-AB with RepSham: $n = 4$; ctrl-AB with RepI/R: $n = 7$; β1-B-AB with RepSham: $n = 5$; β1-B-AB with RepI/R: $n = 6$. Statistical significance was determined using unpaired two-tailed Student's *t*-test. SEM, standard error of mean.

Next, we analyzed the number of arterioles within the ischemic and non-ischemic myocardia in the *Itgb1*^{iECKO} mice (►**Fig. 5**). Consistent with the previous experiments, more arterioles were found in both parts of the myocardium upon RepI/R (►**Fig. 5A–D, I, K**). In contrast to the situation in the ischemic myocardium (►**Fig. 5K, L**), endothelial β1 integrin was required for a statistically significant RepI/R-induced arteriole formation in the non-ischemic myocardium (►**Fig. 5E–H, I, J**).

Contribution of Endothelial β1 Integrin to Ischemia-Induced Cardioprotection

We next analyzed the role of endothelial β1 integrin in cardioprotection conveyed by transient cardiac ischemia. To this end, an MI was induced after RepI/R treatments (►**Fig. 5O**), and the hearts were analyzed over the following days for IS and LV EF. Notably, the IS in adult *Itgb1*^{iECKO} mice was found to be substantially larger compared with that of tamoxifen-induced controls (►**Fig. 5M-R**), and the LV EF was reduced on the first day after the MI (►**Fig. 5S**).

Growth of Blood Vessels into the Infarcted Myocardium and Maintenance of Cardiac Function upon MI Require Endothelial β1 Integrin

Next, we occluded the LAD permanently to induce a severe MI (without reperfusion), with the goal to study the role of endothelial β1 integrin in vascularization and function of the infarcted myocardium in the absence of RepI/R (►**Fig. 6**). Growth of blood vessels into the infarcted myocardium was analyzed by Microfil compound injection (►**Supplementary Fig. S1E-H**, available in the online version). Here, β1-B-AB was found to reduce vessel density in the infarcted area and to numerically reduce heart function (►**Fig. 6A–G**). Tamoxifen-induced EC-specific depletion of β1 integrin prevented the growth of blood vessels into the infarcted area (►**Fig. 6H–K**), and worsened heart function (►**Fig. 6L–N**). Notably, 3 of 8 mice (37.5%) without endothelial β1 integrin died in the first 4 days after the MI induction, while 2 of 10 control animals (20%) died only after 7 days (►**Fig. 6S**). Histology of the hearts revealed myocardial rupture due to thinning of the LV myocardium as the likely reason for cardiac death (►**Fig. 6O–R**, exemplified for *Itgb1*^{iECKO} mice).

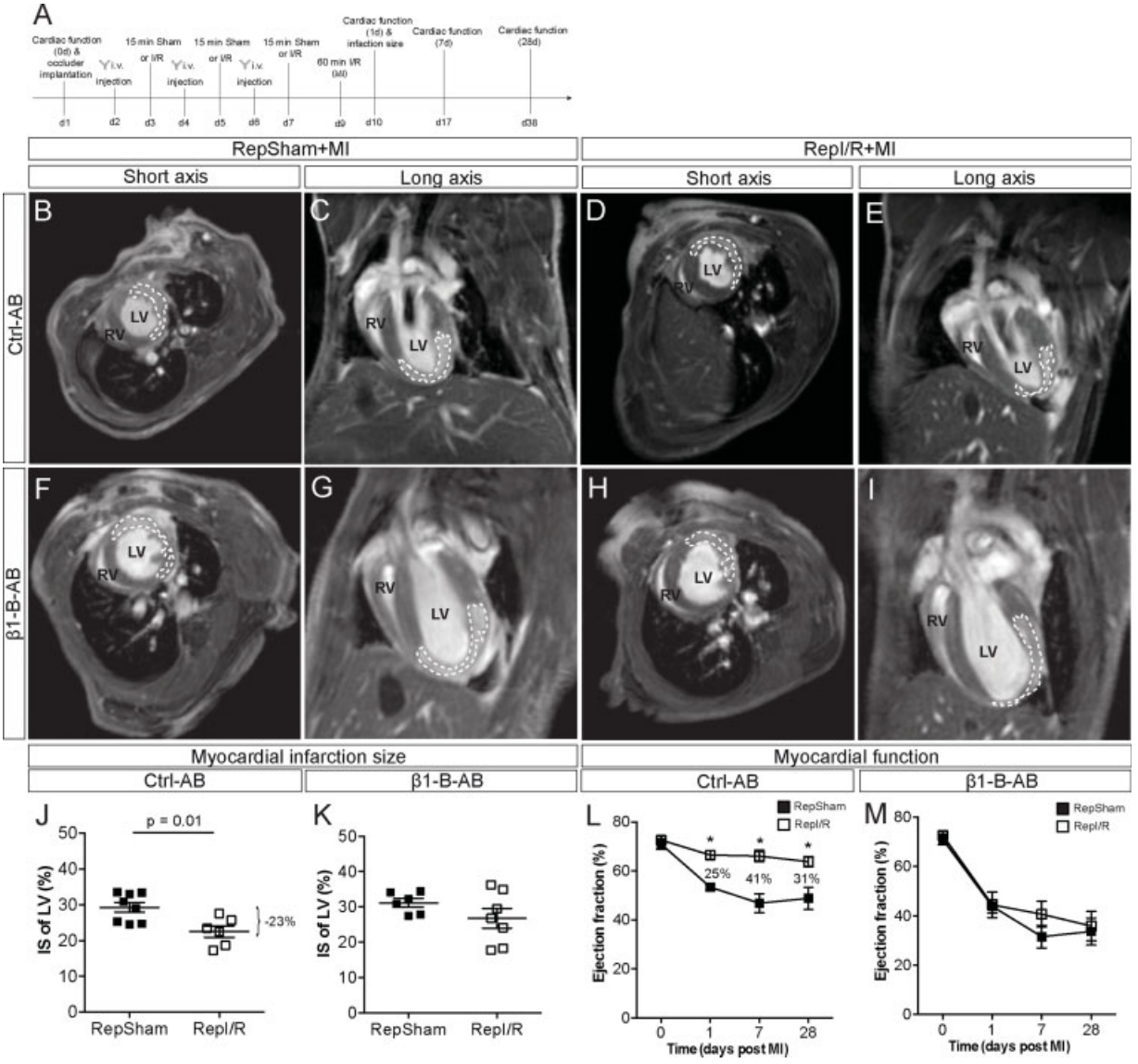

Fig. 3 Transient episodes of cardiac ischemia require β1 integrin to reduce infarct size (IS) and preserve LV heart function after myocardial infarction (MI). (**A**) Timeline of experimental procedure. (**B–I**) Representative cardiac MRI of mouse hearts after RepSham or RepI/R treatments and MI (60-minute ischemia) in short and long axis. IS or infarcted areas (as revealed by LGE) in the left myocardium are indicated by dashed lines. Mice received (B–E) ctrl-AB or (F–I) β1-B-AB before RepSham or RepI/R treatments followed by MI. (**J, K**) Quantification of the IS 1 day after MI with reperfusion in (J) ctrl-AB and (K) β1-B-AB treated mice. (**L, M**) Quantification of the LV ejection fraction at 0, 1, 7, and 28 days after MI with reperfusion in (L) ctrl-AB and (M) β1-B-AB treated mice. At day 0, wild-type mice without AB treatment were used as controls. Reported values are means ± SEM. Ctrl-AB with RepSham: $n = 8$; ctrl-AB with RepI/R: $n = 6$; β1-B-AB with RepSham: $n = 6$; β1-B-AB with RepI/R: $n = 7$. Statistical significance was determined using unpaired two-tailed Student's *t*-test; $^{*}p$-values: 1 d < 0.01; 7 d < 0.01; 28 d $= 0.01$. LGE, late gadolinium enhancement; LV, left ventricular; MRI, magnetic resonance imaging; SEM, standard error of mean.

Identification of eNOS-Regulated β1 Integrin Expression in Cardiac Endothelium

Previously others and we provided evidence for eNOS as a possible downstream effector of β1 integrin in vitro.[41–43] To analyze the relevance in vivo, we checked β1 integrin expression in *eNOS* KO mice. Here, the intensity of endothelial β1 integrin expression was found to be increased in *eNOS* KO mice when their myocardium was compared with that of control mice (►**Supplementary Fig. S4A–C**, available in the online version), whereas the EC area was not substantially different (►**Supplementary Fig. S4D**, available in the online version).

Requirement of β1 Integrin for Basal and Mechanically Induced Proliferation of Primary HCAECs

To investigate whether our in vivo results on endothelial β1 integrin in adult mouse hearts were (in principle) applicable to the human situation, *ITGB1*, the gene for human β1 integrin, was silenced in primary HCAECs. Using three different siRNAs, we obtained a more than 90% knockdown efficiency on the mRNA level, even when the *ITGB1* gene expression was normalized to three different housekeeping genes (►**Supplementary Fig. S5A–C**, available in the online version). The siRNAs also reduced the human β1 integrin protein to a substantial extent (►**Supplementary Fig. S5D, E**;

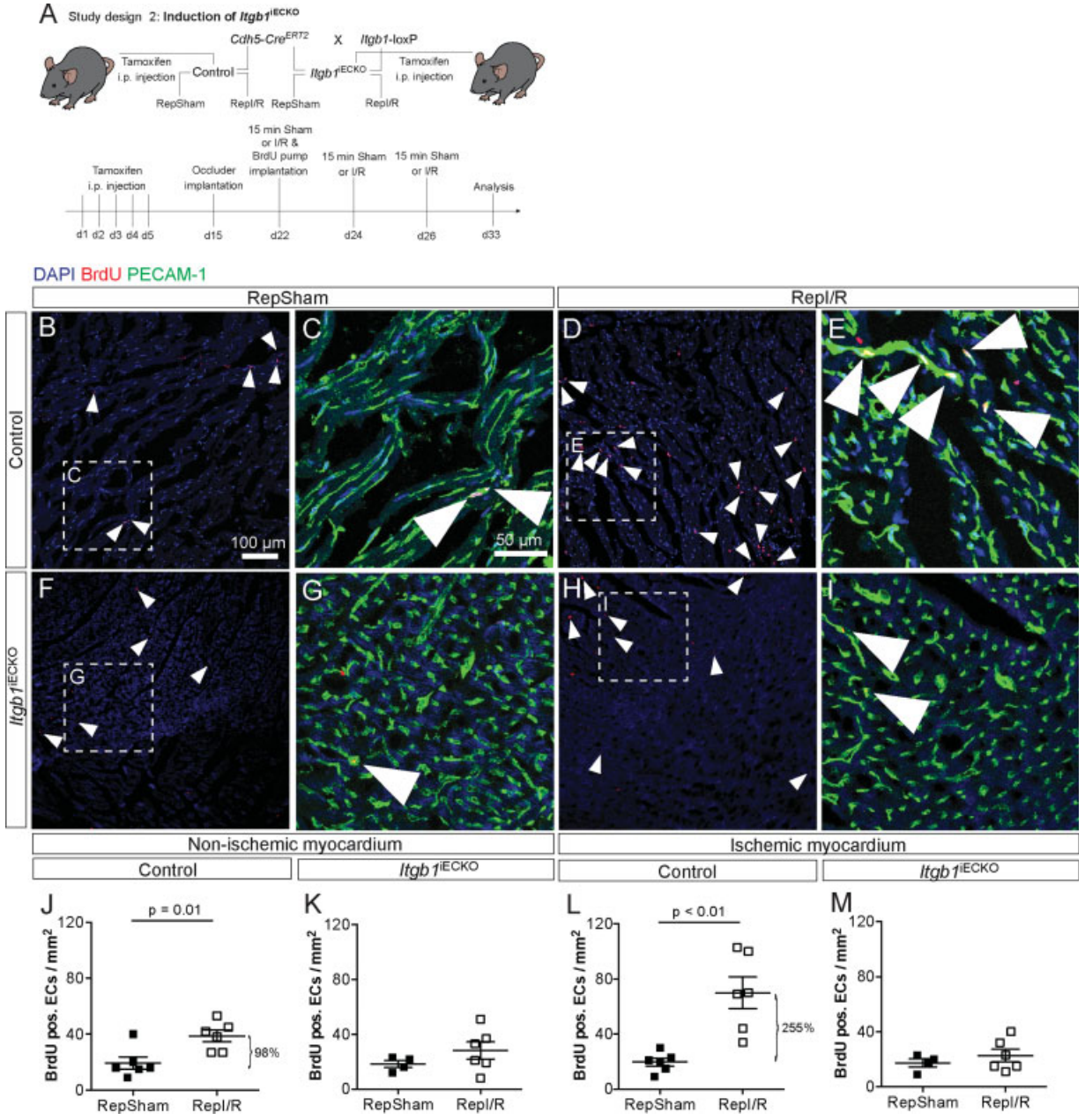

Fig. 4 Induction of cardiac EC proliferation by transient episodes of cardiac ischemia strictly requires endothelial *Itgb1* expression. (**A**) Study design to knock out the gene for β1 integrin (*Itgb1*) by tamoxifen treatment in ECs of mice. (**B–I**) Representative immunofluorescence images of sections through the non-ischemic myocardium of mouse hearts taken from tamoxifen-induced (B–E) *Cre* control mice (indicated as control) and (F–I) EC-specific β1 integrin KO (indicated as *Itgb1*^{iECKO}) mice upon RepSham or RepI/R treatments. Sections were stained for BrdU (*red*), PECAM-1/CD31 (*green*), and DAPI (*blue*). (**J–M**) Quantification of the BrdU-positive ECs per mm^2 (J, K) non-ischemic and (L, M) ischemic myocardium of control or *Itgb1*^{iECKO} mice. Reported values are means ± SEM. Control with RepSham: $n = 6$; control with RepI/R: $n = 6$; *Itgb1*^{iECKO} with RepSham: $n = 4$; *Itgb1*^{iECKO} with RepI/R: $n = 6$. Statistical significance was determined using unpaired two-tailed Student's *t*-test. EC, endothelial cell; SEM, standard error of mean.

for uncropped Western blots, see ►**Supplementary Fig. S6**, available in the online version). Notably, knockdown of *ITGB1* reduced the number of proliferating HCAECs by more than 20% (►**Supplementary Fig. S7A–E**, available in the online version), and even the total number of *ITGB1*-silenced HCAECs was numerically reduced compared with control-silenced cells within 2 days of cultivation (►**Supplementary Fig. S7F**, available in the online version). We also found that mechanical stretching (mimicking the effect of vasodilation on ECs[18]) activated β1 integrin in HCAECs (►**Supplementary Fig. S8A–E**, available in the online version) and that the integrin was strictly required for mechanically induced proliferation of HCAECs (►**Supplementary Fig. S8F–O**, available in the online version).

Discussion

The β1 integrin is part of the largest group of integrins and needed for assembly of BMs, cardiomyocyte function and differentiation, mechanosensing, EC reorientation, cross-activation of VEGF receptors, flow-mediated vasodilation, release of angiocrine signals, and other processes.[10,14–23,41,44,45] Studies could

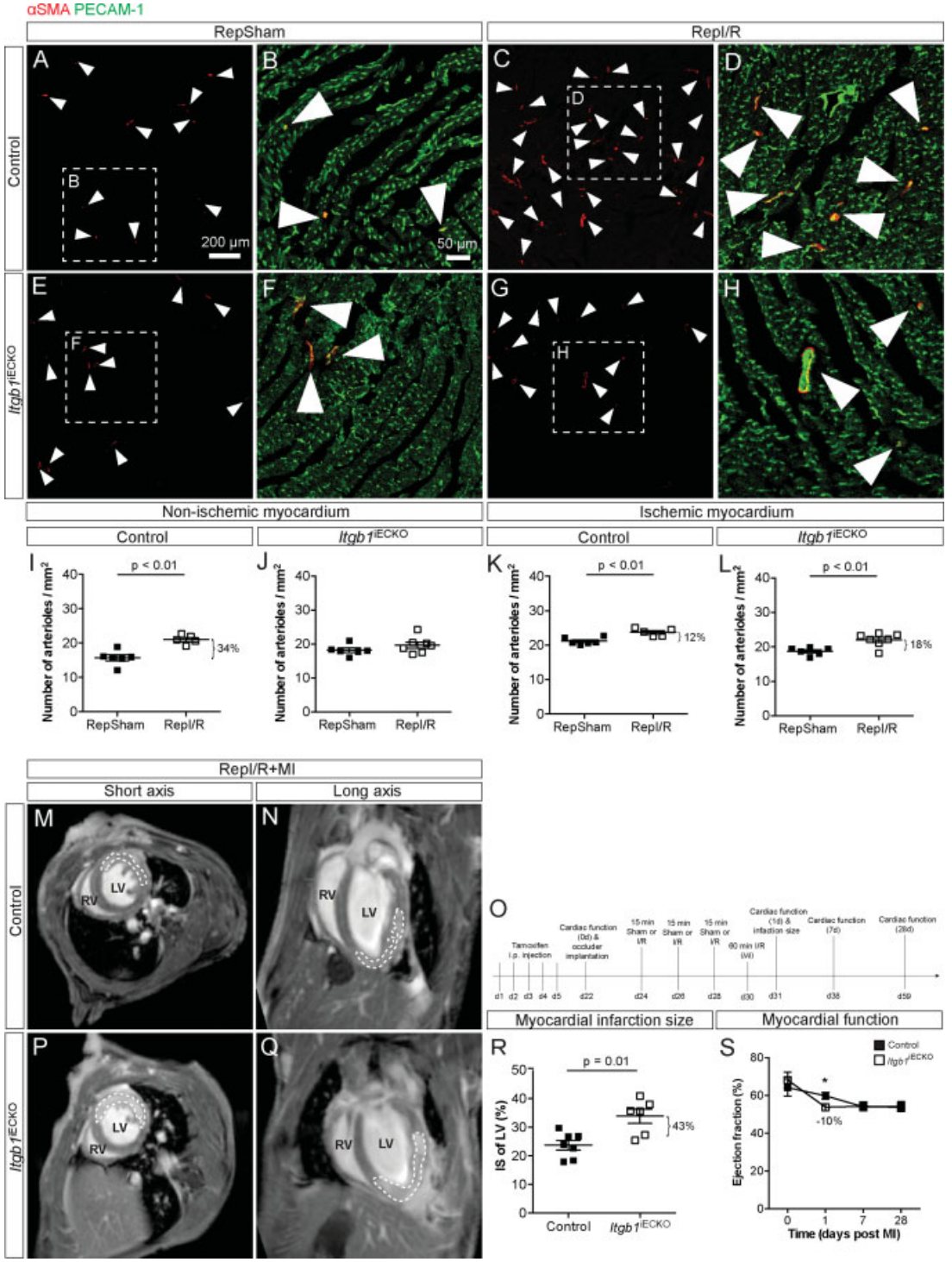

Fig. 5 Transient episodes of cardiac ischemia require endothelial *Itgb1* expression to induce arteriole formation in the non-ischemic myocardium and reduce infarct size (IS) after myocardial infarction (MI). (**A–H**) Representative immunofluorescence images through the non-ischemic myocardium of (A–D) control and (E–H) *Itgb1*^{iECKO} mice after RepSham or RepI/R treatments. Sections were stained for αSMA (*red*) and PECAM-1/CD31 (*green*). (**I–L**) Quantification of αSMA/PECAM-1-positive arterioles per mm^2 (I, J) non-ischemic and (K, L) ischemic myocardium of control or *Itgb1*^{iECKO} mice. Reported values are means ± SEM. Control with RepSham: $n = 6$; control with RepI/R: $n = 5$; *Itgb1*^{iECKO} with RepSham: $n = 6$; *Itgb1*^{iECKO} with RepI/R: $n = 7$. (**M, N, P, Q**) Representative MRI of (M, N) control and (P, Q) *Itgb1*^{iECKO} mouse hearts after RepI/R treatments and MI (60-minute ischemia) in short and long axis. IS or infarcted areas (as revealed by LGE) in the left myocardium are indicated by dashed lines. (**O**) Timeline of the experimental procedure. (**R**) Quantification of the IS 1 day after MI in control and *Itgb1*^{iECKO} mice undergoing RepI/R treatment. (**S**) Quantification of the LV ejection fraction at 0, 1, 7, and 28 days after MI with reperfusion in control versus *Itgb1*^{iECKO} mice. Reported values are means ± SEM. Control with RepI/R: $n = 7$; *Itgb1*^{iECKO} with RepI/R: $n = 6$. Statistical significance was determined using unpaired two-tailed Student's *t*-test. $^*p$-Values: 1 d = 0.04. LGE, late gadolinium enhancement; LV, left ventricular; MRI, magnetic resonance imaging; SEM, standard error of mean.

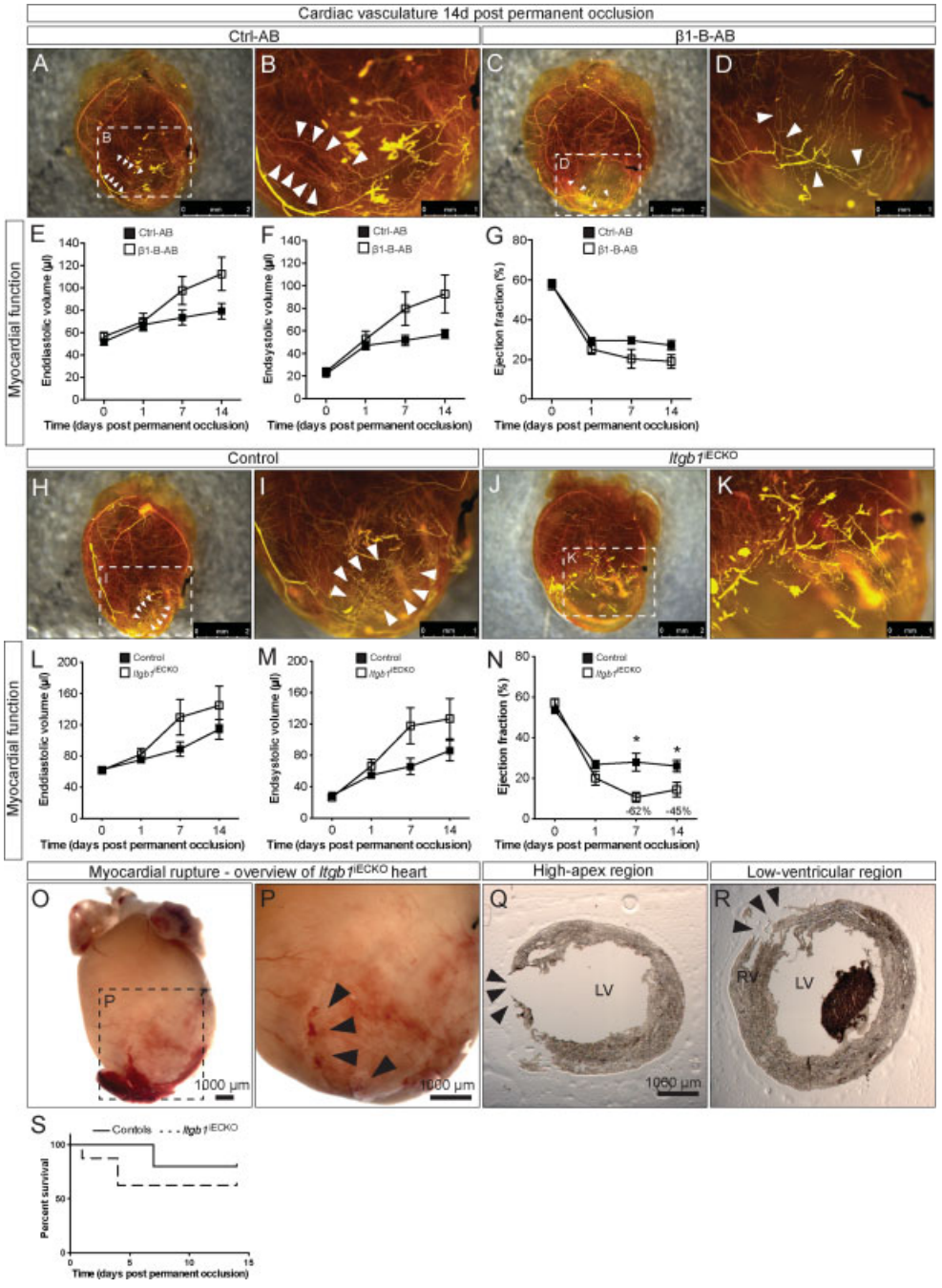

Fig. 6 Growth of blood vessels into the infarcted myocardium, LV function, and cardiac survival after permanent LAD occlusion require endothelial *Itgb1* expression. (**A–D, H–K**) Representative bright-field images of Microfil-perfused mouse hearts to visualize the coronary vasculature 14 days after permanent LAD occlusion. Mice were treated with (A, B) ctrl-AB and (C, D) β1-B-AB. Alternatively, tamoxifen-induced (H, I) control and (J, K) *Itgb1*^{iECKO} mice were used. (**E–G, L–N**) Quantification of LV myocardial function represented by (E, L) the end-diastolic volume, (F, M) the end-systolic volume, and (G, N) the LV ejection fraction at 0, 1, 7, and 14 days after LAD occlusion in wild-type mice treated with (E–G) ctrl-AB versus β1-B-AB or in (L–N) control versus *Itgb1*^{iECKO} mice. Reported values are means ± SEM. (E–G) Ctrl-AB: $n = 5$; β1-B-AB: $n = 5$; (L–N) control: $n = 7$; *Itgb1*^{iECKO} $n = 5$. Statistical significance was determined using unpaired two-tailed Student's *t*-test. $^{*}p$-Values: (N) 7 d = 0.01; 14 d = 0.03. (**O–R**) Representative bright-field images of an *Itgb1*^{iECKO} mouse heart with myocardial rupture as (O, P) overview or as (Q, R) transversal sections. Arrows indicate the rupture. In panels (Q) and (R), the LV and RV are labeled. (**S**) Quantification of survival in a Kaplan–Meier curve in control versus *Itgb1*^{iECKO} mice. Control 0 d: $n = 10$, 7 d: $n = 8$; *Itgb1*^{iECKO} 0 d: $n = 8$, 1 d: $n = 7$, and upon 4 d: $n = 5$. Of note, one *Itgb1*^{iECKO} mouse died after 0 d measurement of cardiac function, but before permanent LAD occlusion, and therefore was excluded from the Kaplan–Meier curve. Only mice which received a permanent LAD occlusion are shown. Statistical significance was determined using the log-rank (Mantel–Cox) test. LAD, left anterior descending artery; LV, left ventricular; SEM, standard error of mean.

show that a global heterozygous knockout of β1 integrin worsened the outcome after MI.[46] Furthermore, it was shown that cardiomyocyte-specific deletion of β1 integrin causes fibrosis, cardiac failure, issues in mechanotransductive response,[28,47] and bone mesenchymal stem cells overexpressing *Itgb1* revealed a protective role after MI for cardiomyocytes.[48] In addition, an EC-specific knockout of β1 integrin was used to study the role of this integrin in vascular leakage and endotoxemia in the mouse

heart.[49] However, the role of endothelial β1 integrin in cardioprotective ischemic events, ischemia-induced cardiac vascular growth, and preservation of LV cardiac function after MI has, to our knowledge, not yet been investigated.

Here, we provide evidence for an important role of endothelial β1 integrin in vascular growth and cardioprotection (see ►**Fig. 7**). We found that short episodes of myocardial ischemia are sufficient to induce proliferation of cardiac ECs as well as formation of arterioles in the ischemic and non-ischemic myocardia in a β1 integrin-dependent manner (see ►**Fig. 7**). In contrast to EC proliferation, arteriole formation depends on endothelial β1 integrin in the non-ischemic, but not in the ischemic part of the adult mouse heart. This is particularly striking given that RepI/R treatment more strongly induces cardiac EC proliferation in the ischemic myocardium compared with the non-ischemic one. These regional differences in the adult heart might be due to additional responses taking place when the myocardium encounters hypoxia and subsequent inflammatory events.[50,51] For example, whereas arteriole formation in the non-ischemic myocardium is likely to predominantly depend on mechanical stimulation (e.g., via increased shear stress/mechanical stretch) and thus β1 integrin as a mechanosensory protein on ECs, hypoxia-induced responses also take place in the ischemic myocardium. The latter responses might trigger arteriole formation independent of local EC proliferation and β1 integrin on ECs.

One possible scenario might be the recruitment of smooth muscle cells (SMCs) by migration, causing an arterialization of capillaries,[52] which could be independent of EC proliferation. Further, it is noteworthy that Red-Horse and colleagues have recently shown that collaterals are also formed via "artery reassembly" in the neonatal heart,[53] and that Adams and colleagues have shown that ECs from sprouting capillaries (that develop in hypoxic areas) can relocate to participate in

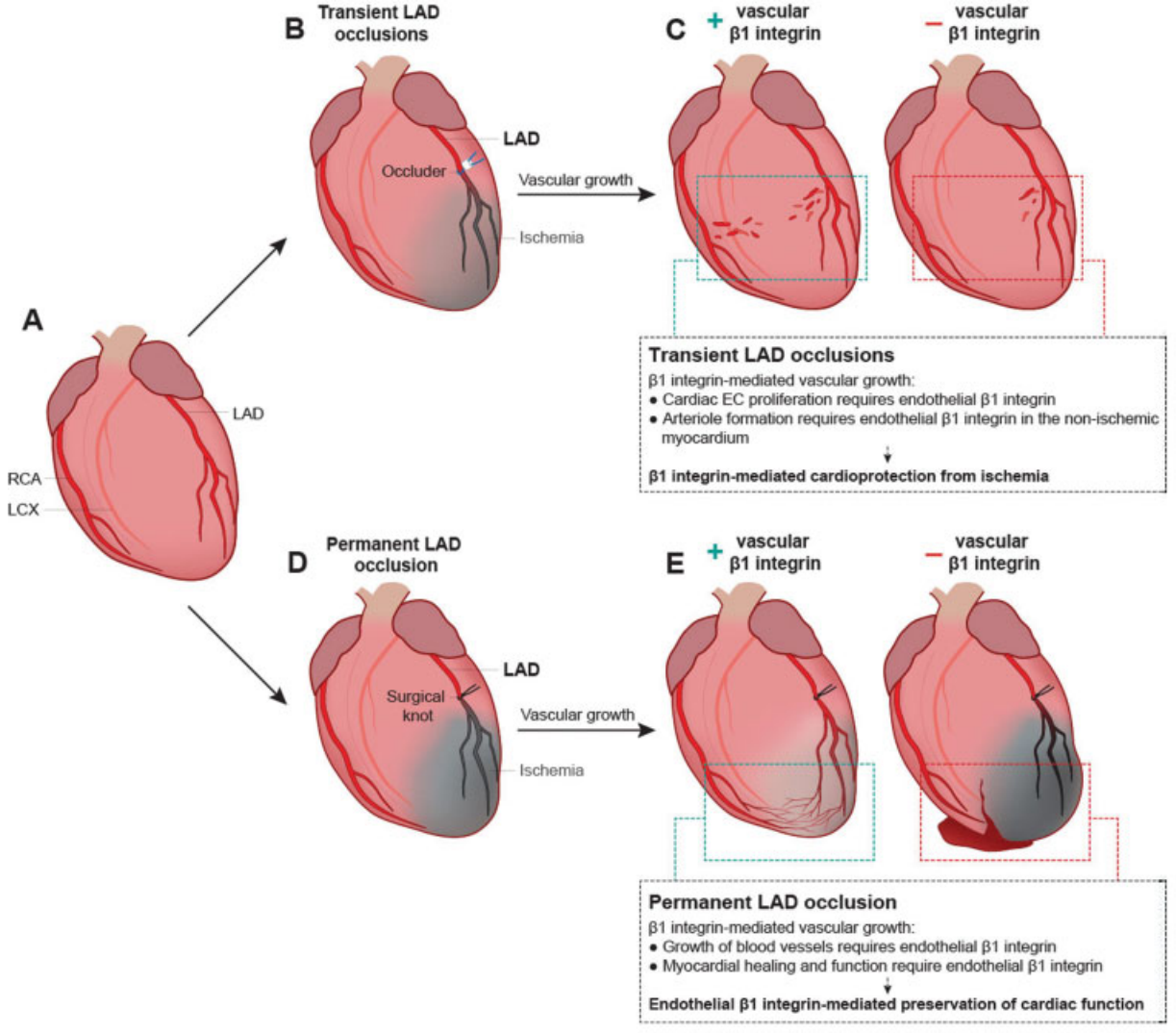

Fig. 7 Visual Summary: Endothelial β1 integrin is required for myocardial ischemia-induced vascular growth, growth of blood vessels into the infarcted myocardium and preservation of cardiac function. (**A**) Adult mouse heart, with a coronary vasculature similar to the human situation, received (**B**) transient LAD occlusions, mimicking temporary ischemia observed in patients with CAD. (**C**) These transient occlusions trigger β1 integrin-dependent vascular growth and cardioprotection. (**D**) Permanent LAD occlusion, mimicking the situation in patients with MI, not receiving proper reperfusion. (**E**) Endothelial β1 integrin is required for growth of blood vessels into the infarcted myocardium and preservation of cardiac function.

the formation of arteries,[54] thus representing possible scenarios by which arterioles might form in the ischemic myocardium without increased cardiac EC proliferation.

The here-presented data show that pharmacological inhibition of β1 integrin abolished RepI/R-induced cardioprotection, shown in MRI measurements and TTC staining. Since a simultaneous decrease in cardiac function is observed in the RepSham and the RepI/R group, β1 integrin seems to have a general role in cardioprotection that extends beyond its role in RepI/R-induced cardioprotection.

To validate this point, the role of β1 integrin was also analyzed in a chronic ischemia model without RepI/R. In this model, we could generate a stronger ischemia, which induced growth of blood vessels into the infarcted myocardium, comparable to a previous study.[40] Notably, our experimental results revealed that endothelial β1 integrin was required for proper vascular growth and maintenance of cardiac function independent of RepI/R. Further, we showed in the mouse heart that endothelial β1 integrin is regulated by eNOS (as indicated by increased β1 integrin expression in *eNOS*-deficient mice). Since eNOS contributes to ischemic preconditioning,[30–33] our findings also point to a possible role of β1 integrin in this relevant phenomenon.

Multiple different cell types, including SMCs, cardiomyocytes, myeloid cells (such as monocytes and macrophages), and endothelial progenitor cells, were previously reported to be involved in cardiac vascular growth.[4,40,55,56] Thus, our findings are remarkable in that β1 integrin on ECs, meaning a single-cell surface receptor on a single-cell type, is needed to induce vascular growth and cardioprotection. In summary, we uncover a key role of endothelial β1 integrin in cardiac vascular adaptation to ischemia, function, and protection from MI.

What is known about this topic?

- Short episodes of myocardial ischemia can protect the heart from myocardial infarction (MI).
- To date, β1 integrin has been targeted among others in cardiomyocytes, myofibroblasts, and pericytes, but not in endothelial cells (ECs) to uncover its endothelial role in myocardial ischemia.
- A role of endothelial β1 integrin has been identified in vascular leakage and endotoxemia of the mouse heart.

What does this paper add?

- Endothelial β1 integrin is shown to be required for cardiac EC proliferation during short episodes of left ventricular (LV) ischemia in mice in vivo and for proliferation of human coronary artery ECs in vitro.
- Endothelial β1 integrin is required for arteriole formation induced by short episodes of LV ischemia in the non-ischemic myocardium.
- Endothelial β1 integrin has a general role in vascular growth into the infarct region and preservation of cardiac function after chronic myocardial infarction.

Funding
This project was supported and funded by the Deutsche Forschungsgemeinschaft (DFG) through the SFB 1116 ("Master Switches in Cardiac Ischemia"), the SFB 974 ("Communication and Systems Relevance during Liver Damage and Regeneration"), the IRTG 1902 ("Intra- and Interorgan Communication of the Cardiovascular System") and the Federal Ministry of Health, the Ministry of Culture and Science of North Rhine-Westphalia, and the German Center for Diabetes Research (DZD e.V.).

Conflict of Interest
None declared.

Acknowledgments
We especially thank J. Fischer for his help and our colleagues at SFB 1116 for their collaborative efforts. We also thank D. Eberhard for critical and helpful suggestions to the manuscript, Y. Koh for schematic illustration of the visual summary and closed-chest myocardial ischemia model, S. Becher for her great advice with regard to myocardial ischemia experiments, S. Jakob, B. Bartosinska, and A. Köster for technical support, and A. Leinweber, M. Schröter, and S. Tittelbach for mouse care.

References

1 GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017;390(10106):1151–1210
2 Benjamin EJ, Virani SS, Callaway CW, et al;American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2018 update: a report from the American Heart Association. Circulation 2018;137(12):e67–e492
3 Cannon B. Cardiovascular disease: biochemistry to behaviour. Nature 2013;493(7434):S2–S3
4 Lavine KJ, Kovacs A, Weinheimer C, Mann DL. Repetitive myocardial ischemia promotes coronary growth in the adult mammalian heart. J Am Heart Assoc 2013;2(05):e000343
5 Gloekler S, Seiler C. Cardiology patient page. Natural bypasses can save lives. Circulation 2007;116(11):e340–e341
6 Seiler C, Stoller M, Pitt B, Meier P. The human coronary collateral circulation: development and clinical importance. Eur Heart J 2013;34(34):2674–2682
7 Jamaiyar A, Juguilon C, Dong F, et al. Cardioprotection during ischemia by coronary collateral growth. Am J Physiol Heart Circ Physiol 2019;316(01):H1–H9
8 Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. Nat Med 2000;6(04):389–395
9 Cochain C, Channon KM, Silvestre JS. Angiogenesis in the infarcted myocardium. Antioxid Redox Signal 2013;18(09):1100–1113
10 Simons M, Gordon E, Claesson-Welsh L. Mechanisms and regulation of endothelial VEGF receptor signalling. Nat Rev Mol Cell Biol 2016;17(10):611–625
11 Ingber D. Integrins as mechanochemical transducers. Curr Opin Cell Biol 1991;3(05):841–848
12 Planas-Paz L, Strilić B, Goedecke A, Breier G, Fässler R, Lammert E. Mechanoinduction of lymph vessel expansion. EMBO J 2012;31(04):788–804
13 Ross TD, Coon BG, Yun S, et al. Integrins in mechanotransduction. Curr Opin Cell Biol 2013;25(05):613–618

14 Sun Z, Costell M, Fässler R. Integrin activation by talin, kindlin and mechanical forces. Nat Cell Biol 2019;21(01):25–31
15 Somanath PR, Ciocea A, Byzova TV. Integrin and growth factor receptor alliance in angiogenesis. Cell Biochem Biophys 2009;53 (02):53–64
16 Tugues S, Honjo S, König C, et al. Tetraspanin CD63 promotes vascular endothelial growth factor receptor 2-β1 integrin complex formation, thereby regulating activation and downstream signaling in endothelial cells in vitro and in vivo. J Biol Chem 2013; 288(26):19060–19071
17 Thodeti CK, Matthews B, Ravi A, et al. TRPV4 channels mediate cyclic strain-induced endothelial cell reorientation through integrin-to-integrin signaling. Circ Res 2009;104(09):1123–1130
18 Lorenz L, Axnick J, Buschmann T, et al. Mechanosensing by β1 integrin induces angiocrine signals for liver growth and survival. Nature 2018;562(7725):128–132
19 Lei L, Liu D, Huang Y, et al. Endothelial expression of beta1 integrin is required for embryonic vascular patterning and postnatal vascular remodeling. Mol Cell Biol 2008;28(02):794–802
20 Tanjore H, Zeisberg EM, Gerami-Naini B, Kalluri R. Beta1 integrin expression on endothelial cells is required for angiogenesis but not for vasculogenesis. Dev Dyn 2008;237(01):75–82
21 Carlson TR, Hu H, Braren R, Kim YH, Wang RA. Cell-autonomous requirement for beta1 integrin in endothelial cell adhesion, migration and survival during angiogenesis in mice. Development 2008;135(12):2193–2202
22 Zovein AC, Luque A, Turlo KA, et al. Beta1 integrin establishes endothelial cell polarity and arteriolar lumen formation via a Par3-dependent mechanism. Dev Cell 2010;18(01):39–51
23 Yamamoto H, Ehling M, Kato K, et al. Integrin β1 controls VE-cadherin localization and blood vessel stability. Nat Commun 2015;6:6429
24 Lindsey ML, Iyer RP, Zamilpa R, et al. A novel collagen matricryptin reduces left ventricular dilation post-myocardial infarction by promoting scar formation and angiogenesis. J Am Coll Cardiol 2015;66(12):1364–1374
25 Martin K, Pritchett J, Llewellyn J, et al. PAK proteins and YAP-1 signalling downstream of integrin beta-1 in myofibroblasts promote liver fibrosis. Nat Commun 2016;7:12502
26 Abraham S, Kogata N, Fässler R, Adams RH. Integrin beta1 subunit controls mural cell adhesion, spreading, and blood vessel wall stability. Circ Res 2008;102(05):562–570
27 Mishina H, Watanabe K, Tamaru S, et al. Lack of phospholipase A2 receptor increases susceptibility to cardiac rupture after myocardial infarction. Circ Res 2014;114(03):493–504
28 Li R, Wu Y, Manso AM, et al. β1 integrin gene excision in the adult murine cardiac myocyte causes defective mechanical and signaling responses. Am J Pathol 2012;180(03):952–962
29 Okada H, Lai NC, Kawaraguchi Y, et al. Integrins protect cardiomyocytes from ischemia/reperfusion injury. J Clin Invest 2013; 123(10):4294–4308
30 Bolli R. Preconditioning: a paradigm shift in the biology of myocardial ischemia. Am J Physiol Heart Circ Physiol 2007;292 (01):H19–H27
31 Bolli R, Li QH, Tang XL, et al. The late phase of preconditioning and its natural clinical application–gene therapy. Heart Fail Rev 2007; 12(3–4):189–199
32 Dawn B, Bolli R. Role of nitric oxide in myocardial preconditioning. Ann N Y Acad Sci 2002;962:18–41
33 Heusch G. Molecular basis of cardioprotection: signal transduction in ischemic pre-, post-, and remote conditioning. Circ Res 2015;116(04):674–699
34 Lindsey ML, Bolli R, Canty JM Jr, et al. Guidelines for experimental models of myocardial ischemia and infarction. Am J Physiol Heart Circ Physiol 2018;314(04):H812–H838
35 Shesely EG, Maeda N, Kim HS, et al. Elevated blood pressures in mice lacking endothelial nitric oxide synthase. Proc Natl Acad Sci U S A 1996;93(23):13176–13181
36 Benedito R, Roca C, Sörensen I, et al. The notch ligands Dll4 and Jagged1 have opposing effects on angiogenesis. Cell 2009;137 (06):1124–1135
37 Potocnik AJ, Brakebusch C, Fässler R. Fetal and adult hematopoietic stem cells require beta1 integrin function for colonizing fetal liver, spleen, and bone marrow. Immunity 2000;12(06):653–663
38 Bönner F, Jacoby C, Temme S, et al. Multifunctional MR monitoring of the healing process after myocardial infarction. Basic Res Cardiol 2014;109(05):430
39 Erkens R, Kramer CM, Lückstädt W, et al. Left ventricular diastolic dysfunction in Nrf2 knock out mice is associated with cardiac hypertrophy, decreased expression of SERCA2a, and preserved endothelial function. Free Radic Biol Med 2015;89:906–917
40 Zhang H, Faber JE. De-novo collateral formation following acute myocardial infarction: dependence on $CCR2^{+}$ bone marrow cells. J Mol Cell Cardiol 2015;87:4–16
41 Henning C, Branopolski A, Schuler D, et al. Requirement of β1 integrin for endothelium-dependent vasodilation and collateral formation in hindlimb ischemia. Sci Rep 2019;9(01):16931
42 Yang B, Rizzo V. Shear stress activates eNOS at the endothelial apical surface through $\beta 1$ containing integrins and caveolae. Cell Mol Bioeng 2013;6(03):346–354
43 Xanthis I, Souilhol C, Serbanovic-Canic J, et al. β1 integrin is a sensor of blood flow direction. J Cell Sci 2019;132(11):jcs229542
44 Israeli-Rosenberg S, Manso AM, Okada H, Ross RS. Integrins and integrin-associated proteins in the cardiac myocyte. Circ Res 2014;114(03):572–586
45 Hodgkinson CP, Gomez JA, Payne AJ, et al. Abi3bp regulates cardiac progenitor cell proliferation and differentiation. Circ Res 2014;115(12):1007–1016
46 Krishnamurthy P, Subramanian V, Singh M, Singh K. Deficiency of beta1 integrins results in increased myocardial dysfunction after myocardial infarction. Heart 2006;92(09):1309–1315
47 Shai SY, Harpf AE, Babbitt CJ, et al. Cardiac myocyte-specific excision of the beta1 integrin gene results in myocardial fibrosis and cardiac failure. Circ Res 2002;90(04):458–464
48 Li L, Guan Q, Dai S, Wei W, Zhang Y. Integrin β1 increases stem cell survival and cardiac function after myocardial infarction. Front Pharmacol 2017;8:135
49 Hakanpaa L, Kiss EA, Jacquemet G, et al. Targeting β1-integrin inhibits vascular leakage in endotoxemia. Proc Natl Acad Sci U S A 2018;115(28):E6467–E6476
50 van der Vorst EPC, Weber C. Novel features of monocytes and macrophages in cardiovascular biology and disease. Arterioscler Thromb Vasc Biol 2019;39(02):e30–e37
51 Zernecke A, Bernhagen J, Weber C. Macrophage migration inhibitory factor in cardiovascular disease. Circulation 2008;117(12):1594–1602
52 Mac Gabhann F, Peirce SM. Collateral capillary arterialization following arteriolar ligation in murine skeletal muscle. Microcirculation 2010;17(05):333–347
53 Das S, Goldstone AB, Wang H, et al. A unique collateral artery development program promotes neonatal heart regeneration. Cell 2019;176(05):1128.e18–1142.e18
54 Pitulescu ME, Schmidt I, Giaimo BD, et al. Dll4 and Notch signalling couples sprouting angiogenesis and artery formation. Nat Cell Biol 2017;19(08):915–927
55 la Sala A, Pontecorvo L, Agresta A, Rosano G, Stabile E. Regulation of collateral blood vessel development by the innate and adaptive immune system. Trends Mol Med 2012;18(08):494–501
56 Yue Y, Wang C, Benedict C, et al. Interleukin-10 deficiency alters endothelial progenitor cell-derived exosome reparative effect on myocardial repair via integrin-linked kinase enrichment. Circ Res 2020;126(03):315–329