### SUPPLEMENTAL METHODS

#### **Construction of recombinant lentiviral vectors**

Lentiviral vector carrying the murin Apelin precursor gene, 234-bp cDNA, (Lenti-Apelin) was constructed. The cDNA was inserted into the ptrip-DU3-CMV-MCS vector plasmid derived from the pTRIP-DU3-EF1a-EGFP.<sup>1</sup> Lentiviral vector particles for Lenti-Apelin and Lenti-GFP were produced in parallel using the tri-transfection procedure with the plasmids pLvPack and pLvVSVg (Sigma-Aldrich), into HEK-293FT cells. Viral supernatants were harvested 48 hours after transfection, passed through 0.45-µm filters (Millipore) and concentrated over 100-fold through a single step ultracentrifugation at 24000rpm at 4°C for 1h30. The viral pellet was resuspended in Dulbecco's Phosphate Buffered Salin with MgCl2 and CaCl2. Viral stocks were stored at -80°C until use. Functional viral titers were assessed on HT1080 cells following serial dilutions and scored for GFP expression by flow cytometry.

#### Generation of Myocardial infarction mice and gene transfer

Experiments were carried out in adult male C57BL/6J mice. Ten- to eleven-week-old mice were anesthetized with ketamine-xylazine (125 mg/kg-5mg/kg) followed by isoflurane (3%), and were endotracheally intubated. After thoracotomy, the left anterior descending coronary artery was ligated through a left sided thoracotomy and lentiviral vectors were injected at the border between the ischemic area and the non-ischemic area of the myocardium (2-2.5 x 10<sup>6</sup> TU). Sham-operated mice undergoes thoracotomy without coronary artery ligation. After the surgical procedure, a subcutaneous injection of buprenorphine (Buprecar, 1.5 mg/kg) was performed.

## Echocardiography

Transthoracic echocardiography was performed to evaluate the left ventricular systolic function in the sham-operated and operated groups using the VEVO 2100 system (VisualSonics, Toronto, ON, Canada). As echocardiography is a noninvasive test, animals were followed after 1, 3 and 6 weeks post-surgery. Animals were anesthetized with inhaled isoflurane (2%) vaporized in medical O<sub>2</sub>. The thoracic hair was removed with a depilatory agent and an ultrasound gel was placed to form an interface between the chest and the transducer. The mice were then placed on a heating pad and their rectal temperature was maintained between 36 and 37 °C during the examination. The paws were connected to an ECG. Echocardiographic measurements were obtained from the parasternal long axis view from B-mode images and from the parasternal short axis view from M- and B-mode images at three different levels. Simpson measurements of the left ventricle included the following: heart volume in systole and diastole, stroke volume, cardiac output, fractional shortening and ejection fraction.

## Lymphography

Mice were anesthetized with a subcutaneous injection of ketamine (15 mg/kg), xylazine (1 mg/kg). FITC-dextran (Sigma) was injected into the footpad of control or APL-KO mice. After 5 min, the skin was analyzed under the microscope (Leica Dmi8).

#### Co-culture of lymphatic endothelial cells (LECs) with H9C2 (myoblast)

Co-culture: H9C2 control cells (myoblast) or H9C2 transduced by the lentivector apelin were cultures on transwell inserts with LECs on bottom chamber during 24 hours before incubation in hypoxic or normoxic conditions.

### Immunoblotting assay

LECs samples were prepared in RIPA lysis buffer and protein determination were performed with BCA assay (Interchim). Western blot analysis was quantified with Image J.

### S1P dosage

Briefly lipids from around 2ml of supernatant were extracted according to modified acidic (HCl 2M) Bligh and Dyer in the presence of Internal standard Sphingosine-1-phosphate (d20:1) (5

ng). The extract was washed and methylated with TMS diazomethane in hexane (50  $\mu$ L) at room temperature for 10 min. Addition of acetic acid (6  $\mu$ L) quenched the methylation. The organic phase is concentrated and then dissolved in 20  $\mu$ L of MeOH and 5ml was injected on a HPLC system (Agilent 1290 Infinity) coupled to Agilent 6460 triple quadrupole MS (Agilent Technologies) equipped with electrospray ionization source in positive mode with the source parameters as follows : source temperature was set at 300°C, nebulizer gas (nitrogen) flow rate was 10 L/min, sheath gas temperature was 300°C, sheath gas (nitrogen) flow rate was 12 L/min and the spray voltage was adjusted to +4000 V. The analytical column was an Acquity UPLC BEH-C8 (100 x 2,1 mm, 1,7  $\mu$ m) (Waters) maintained at 35 °C. The mobile phases consisted of Water, FA (99.9:0.1;v/v/v) (A) and ACN, FA (99.9:0.1, v/v) (B). The gradient was as follows: 50% B at 0 min, 60% B at 2 min, 60% B at 3 min, 100% B at 4 min, 100% B at 8,5 min and 50% B at 9 min. The flow rate was 0.3 mL/min. Quantification were done in Selected Reaction Monitoring detection mode (SRM) with the transition 409-> 264 m/z for sphingosine-1-Phosphate and 437-> 293m/z for the internal standard using a collision energy of 15eV, with calibration curves.

# SUPPLEMENTAL FIGURES LEGENDS



**Figure S1: cardiac lymphatic vasculature expressed NRP2, VEGFR3 and podoplanin**. (A) Visualization of lymphatic vasculature by LYVE-1 detection on adult heart paraffin cross-section. Arrows indicate the presence of lymphatic vessels. (B) Quantification of lymphatic vessels density in the heart. AV: atrioventricular. (C) LYVE-1 and CD45 immunostaining in the

myocardium. Arrows indicated the presence of lymphatic capillaries LYVE-1+CD45-. (D) Larger collecting lymphatic vessels expressed NRP2, VEGFR3 and podoplanin. These vessels are located at the sub-epicardial surface of the heart, close to the blood vasculature and in the interventricular septum. Scale bars: 1mm (A), 20µm (D), 50µm (C)



Figure S2: Lymphatic remodeling after chronic cardiac ischemia

(A) Images of heart cross-section stained for LYVE-1 showing the remodeling of lymphatic vasculature post-MI at 2 weeks and at 6 weeks post-MI. Arrows show growing lymphatic vessels at the infarct border zone. (B) Immunostaining of collagen I, NRP2 and CD31 in sham and 6 weeks after myocardial infarction. Note the disrupted and disordered architecture of collagen fibers in ischemic hearts compared to the regular and well-organized structures surrounding the cardiomyocytes in sham mice. (C) Growing lymphatic vessels in the ischemic zone expressed VEGFR3. (D) Developing lymphatic vessels expressed podoplanin suggesting the migration of lymphatic vessels. Scale bar: 1mm (A), 100µm for confocal images (B, C, D).



**Figure S3: Dilation of lymphatic vasculature in apelin-KO mice.** (A) Analysis of dermal lymphatic vasculature in apelin-KO versus control mice. Note the hyperplasic vessels in apelin-KO mice. (B) Quantitative evaluation of the number of branch points in ear dermal skin. (C) Quantification of the number of lymphatic vessels per fields in dermal back skin. (D) Quantification of the diameter of lymphatic vessels in adult dermal skin of apelin-KO mice. (n= 5-6 per group, values are means ±SD; \**P*<.001). (E) Visualization of altered lymphatic drainage

in adult skin of apelin-KO (arrows) compared to control. Scale bar: 100 $\mu$ m (A) except ear skin 200 $\mu$ m.



**Figure S4: Lymphangiogenesis in lymph node of apelin-KO mice**. Representative images of inguinal and mesenteric lymph nodes with LYVE-1 (green) and CD31 (red) antibodies and DAPI staining showing an important lymphangiogenic process in lymph nodes of apelin-KO mice compared to control. Scale bar: 500µm



**Figure S5: Lymphatic vasculature in apelin-KO mice post-MI**. (A) Detection of lymphatic vessels by LYVE-1 immunostaining (green) and the lectin wheat agglutinin germ (WGA, red) on cryo-sections 4 days post-MI. Note the highly dilated and hyperplasic lymphatic vessels post-MI. (B) Area of lymphatic vessels in apelin-KO and control mice post-MI.



Figure S6: Analysis of heart function in presence or absence of the lentivector apelin. Echocardiography analysis in sham, 6 weeks post-MI with or without the overexpression of the lentivector apelin. Values are mean  $\pm$ SD, n=6-10, \*\**P*<0.001, \**P*<0.02.



Figure S7: Apelin is expressed in lymphatic endothelial cells under hypoxic conditions *in vitro*. (A) Expression of lymphangiogenic factors under hypoxic stress *in vitro* in LECs compared to normoxia. (n=3 independent samples per group, \*P<.05). (B) Time-course of the induction of apelin expression in LECs under hypoxic stress (n=3 independent samples per

group, \**P*<.05). (C) Apelin immunostaining in LECs cultured under hypoxia or normoxia condition. We observed an increase of apelin peptide close to the membrane of LECs under hypoxia. (D) Quantification of apelin expression from images shown in F (n=3 different experiments). (E) VE-cadherin and apelin immunostaining in LECs in co-culture with myoblast H9C2 or H9C2 constitutively expressing apelin under normoxic or hypoxic stress. VE-cadherin is maintained at cell-cell junctions in presence of H9C2-apelin under hypoxic stress

# SUPPLEMENTAL TABLE

	Sham	MI 2 weeks	MI 6 weeks
LV end diastolic volume (µI)	81±16.1	180.5±19.7	148.9±22.2
LV end systolic volume (µI)	38.8±13.9	141.9±19.9	122.3±28.6
Ejection fraction (%)	53.7±8	22.9±3.1	22.4±7.4
Fraction shortening (%)	28±6.4	3.4±0.9	10.7±3.6

Supplemental table 1: Echocardiography analysis in sham, 2 weeks and 6 weeks post-MI. Values are mean  $\pm$  SEM (n=4-6).

# REFERENCES

1. Sirven A, Ravet E, Charneau P, Zennou V, Coulombel L, Guetard D, Pflumio F and Dubart-Kupperschmitt A. Enhanced transgene expression in cord blood CD34(+)derived hematopoietic cells, including developing T cells and NOD/SCID mouse repopulating cells, following transduction with modified trip lentiviral vectors. *Mol Ther.* 2001;3:438-48.