# CD73 positive adipose derived mesenchymal stem cells enhance cardiac repair with experimental myocardial infarction by promoting angiogenesis

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# CD73 positive adipose derived mesenchymal stem cells enhance cardiac repair with experimental myocardial infarction by promoting angiogenesis

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**Abstract:**

**Background:** Cardiovascular disease is the leading cause of death in developed and developing countries. The lack of effective regenerative therapies in the treatment of ischemia - related diseases requires new therapies to improve clinical outcomes. Thus, MSCs have become a focus in stem cell treatment of myocardial injury. At present, most studies use mixed MSCs in vivo and in vitro. A promising therapeutic strategy for myocardial injury should be using the dominant subgroup with essential biological characteristics. The aim of this study was to utilize the dominant $CD73^+$subgroup of adipose derived mesenchymal stem cells (ADMSCs) for the therapy of myocardial infarction (MI).

**Methods:** Adult mix gender SD rats, with a body weight of 230±18g, were randomly divided into sham operation group (SHAM), MI group (MI), mixed ADMSCs transplantation group (MI+ADMSCs), $CD73^+$ADMSCs transplantation group (MI+$CD73^+$ADMSCs) and $CD73^-$ADMSCs transplantation group (MI+$CD73^-$ADMSCs). $CD73^+$ADMSCs were isolated using flow cytometry and then

cultured. Overexpression and inhibition of CD73 gene of ADMSCs using lentiviral vectors. Differential genes analysis of $CD73^+$ADMSCs vs. $CD73^-$ADMSCs were based on GO analysis. The effect of CD73 on the secretion of cytokines was measured by ELISA. Myocardial infarction model and cell transplantation model were replicated. Detection of cardiac function of rats by color doppler ultrasound after operation. The expression of VEGF and factor VIII and neovascularization were detected by immunohistochemistry and Western Blotting.

**Results:** We demonstrated that, compared to mixed ADMSCs and $CD73^-$ADMSCs, $CD73^+$ADMSCs were more effective in the promotion function of cardiac recovery in a rat model of MI. $CD73^+$ subset promoted vascular regeneration in myocardial injured regions. We also showed that expression of CD73 promoted secretion of VEGF, HIF-1α and HGF factors in ADMSCs. $CD73^+$ADMSCs displayed significantly different transcription profile compared to $CD73^-$ADMSCs, in particular, concerning VEGF pathways.

**Conclusions:** Overall, $CD73^+$ADMSCs were the dominant subgroup and the presence of the surface marker CD73 can be used as a MSCs cell quality control for treatment of myocardial injury by angiogenesis.

**Key words:** CD73, VEGF, Adipose derived mesenchymal stem cells, Angiogenesis, Myocardial infarction, Rats.

## Background

Multiple clinical trials have been performed in patients with chronic ischemic heart failure and acute MI using a variety of cell types, including mesenchymal stem cells (MSCs), bone marrow stem cells and cardiac resident stem cells [1]. These therapies utilizating stem cell to regenerate lost cardiac muscle are clinically attractive. The cardioprotective effects observed with cell therapy involving MSCs, pluripotent stem cells derived from mesoderm, may be in large part secondary to secreted paracrine factors that enhance endogenous reparative pathways rather than the generation of new cardiac tissue [2, 3]. The results from clinical and preclinical studies have been variable and the effect of MSCs transplantation have been shown to be limited, with

generally modest benefits in human trials and disappointingly low levels of cell persistence and cardiomyocyte differentiation [4,5].

MSCs are a heterogeneous and can be divided into many subgroups with different biological characteristics [6, 7]. Morphologically, MSCs can be divided into three subgroups: a small fast self-renewing cell subgroup, a fibroblast-like subgroup, and a large slow-growing square or flat cell subgroup [8]. Functionally, $CD56^{+}$MSCs are proliferative, more chondrogenic and less adipogenic than $CD56^{-}$MSCs [9]. Tseng reported that $CD105^{+}$-ADMSCs have the potential to differentiate into chondrocytes [10]. At present, bulk MSCs are used in most in vivo and in vitro studies, and the heterogeneity of unsorted MSCs compromises comparability of cell therapy studies [11, 12].

MSCs exhibit a variety of immunophenotypes. CD44, CD73, CD29, CD90 and CD105 are the most common markers of MSCs, which also lack cell surface antigen CD45 [13]. As reported by Florian et al, the expression patterns of CD73, CD105, CD90 varied in different morphological subsets [8]. All three markers were stably expressed in the rapid self-renewal cells whereas most flat cells lack one or more hMSC surface markers, predominantly CD73 [8]. Also, there were significant differences in CD73 expression in MSCs from different sources [14]. We have found that $CD73^{+}$MSCs are the dominant subgroup with regard to potential for myocardial differentiation [15].

CD73, ecto-5'-nucleotidase, is a glycoprotein anchored on the cell membrane by glycosyl-phosphoryl inositol. It is widely expressed on the cell membrane and is implicated in signal transduction [16, 17]. Previous studies have shown that CD73 has the ability to promote cell proliferation and that $CD45^{-}CD14^{-}CD73^{+}$cell subsets have stronger clone forming ability [18]. CD73 can promote tumor angiogenesis, release cytokines under ischemic conditions and has the ability to make tumor cells escape immune recognition [19, 20]. CD73 plays an important role in myocardial protection during myocardial ischemic preconditioning [21], and the expression of CD73 is upregulated in immune cells after MI and is linked to myocardial repair and improvement of cardiac function [22]. In conclusion, CD73 may be a sensitive marker

for screening subsets of MSCs most suitable for treatment of myocardial injury.

Adipose derived mesenchymal stem cells (ADMSCs) have many similarities with MSCs from alternative sources [23]. Among all transplantable seed cells, ADMSCs have the advantages of easy sampling, multi-directional differentiation potential, with few ethical problems, rapid proliferation in vitro and low immunogenicity [2]. Therefore, ADMSCs have become a primary choice for stem cell treatment of MI. Most studies utilize unsorted ADMSCs. Although CD73 has been used as an important criterion for the identification of ADMSCs for many years, its role in ADMSCs is still unclear [24, 25]. The role of CD73 in the myocardium differentiation and secretion of cytokines from ADMSCs also needs to be clarified.

In the present study, $CD73^+$ and $CD73^-$ subsets of ADMSCs were separated by flow cytometry and evaluated their morphology and genomics. Using lentivirus and APCP (inhibitor of CD73) to alter the expression or activity of CD73, then to observe the changes in cytokines secretion of ADMSCs in vitro. We also evaluated the effect of $CD73^+$ADMSCs transplantation on repair of myocardial infarction in rats. The main objective of the study was to evaluate the utility of the dominant subset of ADMSCs for improved myocardial infarction treatment, and also to provide theoretical and experimental basis for clinical application of $CD73^+$ cells.

## Methods

### Experimental animals and groups

We used adult mix gender SD rats with a body weight of 230±18g. The rats were randomly divided into sham operation group (SHAM), MI group (MI), mixed ADMSCs transplantation group (MI+ADMSCs), $CD73^+$ADMSCs transplantation group (MI+$CD73^+$ADMSCs) and $CD73^-$ADMSCs transplantation group (MI+$CD73^-$ADMSCs). The above animals are provided by the animal center of Xinxiang Medical University. All experiments comply with the Ethics Committee of Xinxiang Medical University (Ethical review approval number: XYLL-2015023).

### Culture, sorting and detection of ADMSCs

$CD73^+$ADMSCs were isolated using flow cytometry and then cultured. P3-P5 cells were used for all subsequent experiments.

Upon completion of experiments, we obtained groin adipose tissue from sacrificed animals with subsequent isolation and culture of ADMSCs as described [23]. When cells reached 80% confluence, they were passaged using1:2 splits and low sugar DMEM (Dulbecco's modified Eagle's medium); containing 10% FBS (Fetal bovine serum) (Hyclone, USA). The morphology of cells was observed by inverted microscope. At passage 3 cells were collected and suspended in PBS at concentration $2\times10^6$ / ml. Immunofluorescence and flow cytometry analysis were conducted in 1ml cell suspensions using monoclonal antibodies against CD29, CD44 and CD73 (1:500, BD Bioscience). With CD73 as the standard, flow cytometry (FACS Aria II, BD Bioscience) was used to sort out two subgroups of $CD73^+$ADMSCs and $CD73^-$ADMSCs. Subpopulations were further sub-cultured and the cells from fourth to sixth passages were used for the follow-up experiments. Quantitative real-time PCR and Western blotting were used to detect the expression of CD73 in two subgroups. Gene chip was used to detect differential gene expression with consequent GO analysis (Jikai Biotechnology Co. China). The primer sequences were as follows. CD73, 269bp, Forward primer: GGTTGTGGGGATTGTTGGATA, Reverse primer: GCACTTCTTTGGAAGGTGGAT.GAPDH, 231bp,
Forward primer: TGGTGAAGGTCGGTGTGAAC,
Reverse primer: GCTCCTGGAAGATGGTGATGG.

**Overexpression and inhibition of CD73 gene of ADMSCs using lentiviral vectors**

CD73 gene over-expression and CD73 gene SiRNA lentivirus were constructed by Jikai Biotechnology Co. The cells in logarithmic growth phase (amount $5\times10^4$) were seeded in a 6-well tissue culture plate in complete culture medium in incubator supplied with 5% CO2 at 37℃. Upon achieving 30%-40% confluence, appropriate amount of medium, virus, Eni.S and Polybrene were added according to the MOI (Multiple of infection) value of cell, best infective condition and experimental groups. After 48h-72h infection time, lentivirus reporter gene GFP expression were detected.

**The effect of CD73 on the secretion of cytokines**

All experimental groups (ADMSCs, $CD73^-$ADMSCs, $CD73^+$ADMSCs and $CD73^+$ADMSCs+APCP) were cultured in complete medium. Upon reaching 80 - 90% confluence, cells were cultured in serum-free medium. APCP (80 μmol/L; alpha, beta—methyleneadenosine-5'- diphosphate), was used to inhibit the activity of CD73 in $CD73^+$ADMSCs+ APCP group. After 24 hours, the concentration of cytokines in the conditioned medium was measured by ELISA. The selection principles for cytokines were as follows: the cytokines secreted by MSCs [3] and the cytokines closely related to myocardial repair [4] including vascular endothelial growth factor (VEGF), hypoxia inducible factor-1α (HIF-1α), basic fibroblast growth factor (bFGF), and hepatocyte growth factor (HGF). Cytokine detection kit was purchased from Wuhan Huamei biotech Co., Ltd.

**Myocardial infarction model and cell transplantation**

After trans-nasal anesthesia with aether, the rats were immobilized in supine position and anesthetized by intraperitoneal injection with 1% pentobarbital sodium (40mg/kg). After skin preparation and disinfection, a median tracheotomy and intubation were performed. The chest was opened by the third or fourth rib space on the left side of the sternum and the heart was exposed. At the lower 1-2mm of the junction between left atrial ear and the pulmonary artery cone, left anterior descending coronary artery (LAD) was ligated by 5/0 line suture needle. Then the thorax was closed and the rats were injected 1.6 million units of penicillin into abdominal cavity. After resumption of spontaneous breathing, tracheal intubation was removed and the animal was monitored.

ADMSCs were incubated 4h with DAPI (4',6-diamidino-2-phenylindole) at a concentration of 10μg/ml before transplantation. After the cells were washed by PBS for 2 times, the cells were trypsinized, centrifuged and resuspended. $1.2 \times 10^6$ cells were injected into the central epicardium of the infarcted areas in each group.

**Detection of cardiac function of rats by color doppler ultrasound after operation**

PHILPS iE33 ultrasound apparatus and S12-4 heart probe were used to evaluate the cardiac function at 1st week, 2nd week, 3rd week and 4th week after MI. 1% pentobarbital sodium (40mg/kg) was injected intraperitoneally before the analysis. The evaluated indicators of ultrasonic diagnosis were as follows: left ventricular ejection fraction (LVEF)，left ventricular end-systolic volume (LVESV)，left ventricular end-diastolic volume (LVEDV).

**The expression of VEGF and factor VIII and neovascularization**

The tissue blocks including infarct area, infarcted edge area and peripheral tissue around the infarct border were harvested and sliced continuously with the thickness of 8μm. VEGF and factor VIII were detected by immunohistochemistry. The primary antibodies were polyclonal antibody against VIII factor and monoclonal antibody against VEGF (1:200, Beijing BOOSEN Biotechnology Co., Ltd.). The secondary antibody was CY3. 10 visual fields were randomly selected from each slide and analyzed by motic images advanced software.

Western Blotting was used to detect VEGF and VIII factor in 120mg myocardium from the edge of the left ventricular infarction. The images were scanned by the gel imaging system. The ratio between the absorbance of target bands and the absorbance of β-tubulin indicated the difference of the content of target proteins in each group.

**Data analysis**

Statistical analyses were performed using the SPSS version 23.0 statistical software. The data were presented as means ± Standard deviation (SD). Each experiment was performed at least 3 times independently. The difference between groups was analyzed using one-way ANOVA and Tukey's t test. $P< 0.05$ were considered to be statistically significant. Immunocytochemical staining positive products were quantitatively analyzed by HMIAS-2000 high definition medical image and text analysis system. The intensity of the positive reaction product was expressed by the integral optical density of the image processing system.

## Results

### Morphological characteristics and immunophenotyping of ADMSCs

After the primary cells were cultured for 30min, a large number of suspended circular cells were visible under the inverted microscope. After 2-3 days, the growth medium was replaced for the first time and ADMSCs assumed short rod or round shapes. After 7-10 days ADMSCs reached up to 80% confluence. At passages 3 to 6 the cells were mostly spindle shaped and polygonal. The majority of passage 3 ADMSCs expressed CD29 and CD44 (81.3% and 99.1%, respectively) while the expression of CD73 was observed in only 15.7% of cells and CD45 was virtually undetectable (Fig.1).

### Characterization of CD73+ and CD73- subgroups

$CD73^+$ADMSCs exhibited mainly spindle and rod like shapes whereas polygonal large cells were largely CD73 negative (Fig. 2A). The expression of CD73 in $CD73^+$ADMSCs and $CD73^-$ADMSCs was difference that was statistically significant ($P<0.05$) (Fig. 2B and 2C). These subtypes also displayed significant differences in gene expression patterns. Based on GO analysis, the differences concerned 10 signaling pathways, including VEGF pathway, which are implicated in angiogenesis of ADMSCs (Fig. 3).

### Effect of CD73 on cytokine secretion of ADMSCs in vitro

CD73 positive ADMSCs released more VEGF, HIF-1 α and HGF than CD73 negative counterparts ($P<0.01$) while secretion of bFGF remained similar ($P>0.05$). Inhibition of CD73 activity of $CD73^+$cells by APCP resulted in reduction of secreted VEGF ($P<0.01$), HIF-1α ($P<0.05$) and HGF ($P<0.01$) with no effect on bFGF ($P>0.05$). The expression of HIF-1α and HGF in CD73-ADMSCs was lower than that in unsorted ADMSCs. (Fig. 4A) When CD73 expression was upregulated in CD73 negative subsets, VEGF secretion increased ($P<0.05$), and VEGF secretion decreased with the down-regulation of CD73 in the positive subset ($P<0.01$). (Fig. 4B)

These results indicate that CD73 promotes secretion of VEGF, HIF-1 α and HGF factors.

**Results of cardiac function test**

Compared to the sham group, the LVEF and the LVEDV significantly decreased ($P$<0.01) and the LVESV significantly increased at 1-4 weeks after MI ($P$<0.01). Compared to MI group, however, the LVEF and LVEDV in MI rats from cell transplantation groups were significantly increased ($P$<0.01). Importantly, the LVEDV did not significantly increase at 1st and 2nd weeks post-MI in $CD73^-$ cells transplantation group ($P$>0.05). The LVESV significantly decreased at 2nd and 4th weeks in all three cell transplantation groups ($P$<0.01). Compared to ADMSCs and $CD73^-$ADMSCs groups, $CD73^+$ADMSCs transplanted rats exhibited increase of LVEF (1st, 2nd and 4th weeks) and LVEDV (4th week) ($P$<0.05).The LVESV of $CD73^+$ADMSCs group decreased compared with ADMSCs group on 2nd weekend ($P$<0.05).These results indicate that $CD73^+$ADMSCs, ADMSCs and $CD73^-$ADMSCs can significantly improve cardiac function of rats with myocardial infarction, but that $CD73^+$ADMSCs transplantation appears to be more effective (Fig. 5A).

**Protein expression of cytokine in myocardial infarction region**

The analysis of cytokines content in myocardium was carried out at the end of 4th week after MI using Western blotting. Although there was no difference in the content of VIII factor and VEGF between sham operated and MI groups, the myocardium of all transplanted animals exhibited higher expression of these cytokines ($P$<0.05) (Fig. 5B).

**Effect of transplantation on angiogenesis**

Immunohistochemical staining was used to detect the expression of VIII factor and VEGF. By the end of the 4th week post MI, the neovascularization density in all transplantation groups was significantly higher than in untreated MI ($P$<0.01). However, the extent of increase in $CD73^+$ADMSC animals significantly exceeded

increases observed in other transplantation groups ($P< 0.05$) (Fig. 5C and 5D). These results indicate that injection of $CD73^+$ADMSC is more effective in promoting myocardial angiogenesis.

**Discussion**

Unlike most similar studies, the present study utilized $CD73^+$ and $CD73^-$ subsets of mice' ADMSCs for transplantation. We demonstrated that presence of CD73 promotes secretion of VEGF, HIF-1α and HGF factors by ADMSCsin vitro. $CD73^+$ADMSCs promoted vascular regeneration in vivo, which could be closely related to regulate the microenvironment of myocardial infarction area. We also have shown that $CD73^+$ADMSCs are more effective than bulk ADMSCs in the promotion of cardiac function recovery in the rat model of myocardial infarction.

CD73 is a glycoprotein that functions as exogeneous-5'-nucleotidase and participates in signal transduction. CD73 hydrolyzes 5'-adenosine monophosphate (AMP) into adenosine and phosphoric acid and then interacts with adenosine receptors on the cell surface to regulate many biological effects [26]. CD73 also has non-hydrolase function, which is also a signal and adhesion molecule that regulate cell-extracellular matrix interaction [27]. However, our understanding of the role of CD73 in the biology of MSCs is rather limited.

Routinely, most laboratories isolate bulk MSC populations and enrich mesenchymal stromal cells by plastic adherence followed by expansion [28]. In this study, $CD73^+$ADMSCs and $CD73^-$ADMSCs were separated by flow cytometry. The $CD73^+$cells had either spindle or rod shape, while $CD73^-$cells mostly large and polygonal, in line with observations in Florian Haasters study [8]. Small differences in the transcriptome reported between MSCs from different sources do have a noticeable impact on the behavior of the cells [9]. Gene chip analysis of this study revealed a number of differentially expressed genes in the subgroups, $CD73^+$ADMSCs displayed significantly different transcription profile compared to $CD73^-$ADMSCs, in particular, concerning VEGF pathways.

MSCs promote tissue repair by secreting soluble cytokines, promoting vascular

regeneration, blocking apoptosis, suppressing inflammation, and stimulating the regeneration of host cells [29]. Our study showed that expression of CD73 stimulates secretion of VEGF, HIF-1α and HGF. The effects of $CD73^+$ ADMSCs in promoting myocardial angiogenesis were superior to those of unsorted ADMSCs and $CD73^-$ADMSCs. Our data suggests that evaluation based on presence of the surface marker CD73 can be used as a cell quality control.

Inhibition of CD73 with anti-CD73 antibodies or APCP reduced tumor angiogenesis in mice [30]. The mechenism is probably related to CD73's interaction with adenosine receptor A2B [31]. This interaction will stimulates vascular smooth muscle cells, endothelial cells, immune cells and tumor cells to secrete VEGF. Our results showed CD73 was beneficial to angiogenesis in infarcted regions by upregulating secretion of VEGF in ADMSCs. Overexpression of HIF-1α in MSCs increases exsome secretion under normoxic conditions [32]. Several molecules are linked to the secretion of exosomes including Rab proteins, proteins of the SNARE complex and ceramides [33], and HIF-1α expression has been associated with the expression of Rab22A, which is implicated in the formation of microvesicles [34].

These cytokines not only promote vascular regeneration, but also protect myocardium in hypoxia [35]. Our results indicate that $CD73^+$ADMSCs, ADMSCs and $CD73^-$ADMSCs can significantly improve cardiac function of rats with myocardial infarction, but that $CD73^+$ADMSCs transplantation appears to be more effective. Exogenously expressed VEGF promotes myocardial repair at least in part through SDF-1α/CXCR4 (troma-derived factor-1α/chemokine receptor-4) diated recruitment of cardiac stem cell (CSC) [36]. MSCs over-expressing SDF-1 stimulate effective angiogenesis resulting in prevention of progressive heart dysfunction after myocardial infarction [37]. In vivo, MSC-stimulated SDF-1α expression in infarcted hearts resulted in massive mobilization and homing of bone marrow stem cells and CSC [37].

High expression of HGF also has obvious cardioprotective effect, inhibits cardiocyte apoptosis, promotes vascular regeneration, and plays an important role in the process of anti-ventricular remodeling and repair [38, 39]. Gallo et al reported that

the HGF/Met axis also plays an important role in regulating self-renewal and myocardial regeneration through the enhancement of cardiac progenitor cells [40].

The analysis of the effects of MSC transplantation in the treatment of myocardial injury was mainly focused on cytokine secretion and the differentiation ability of transplanted cells [41]. Clearly, transplantation of ADMSCs triggers multiple mechanisms that collectively improve heart function. Cytokines secreted from MSCs exert protective effects by salvaging injured neighboring cells through regulation of apoptosis, inflammation, fibrosis and angiogenesis [42]. In recent years, studies have shown that MSCs may be involved in regulation of immune responses [43]. CD73 and CD39 can be regarded as "immune checkpoint mediator" and can regulate function of various types of immune cells [17]. Our study showed that expression of CD73 stimulated production of HGF in vitro. In addition to well characterized effects of HGF on epithelial cells, endothelial cells and haemopoietic progenitor cells, it also has been shown to regulate chemotaxis of T cells into heart tissue [44]. Promoting angiogenesis and anti-inflammation can interact, and VEGF expression is higher at low doses of TNF-α [45].

It is essential for myocardial repairing about cell transplantation that $CD73^{+}$ADMSCs regulate myocardial microenvironment especially by promoting angiogenesis.

## Conclusion

In the context of possible clinical application, flow cytometry based characterization of MSC subsets will require development of appropriate standards. Since unsorted bulk MSCs may contain unwanted cells. Our data suggests that evaluation based on presence of the surface marker CD73 can be used as a cell quality control for the treatment of myocardial injury (Fig. 6).

## Abbreviations

ADMSCs: adipose derived mesenchymal stem cells; MI: myocardial infarction; MSCs: mesenchymal stem cells; APCP: alpha, beta—methyleneadenosine-5'-

diphosphate; DMEM: Dulbecco's modified Eagle's medium; ; FBS: Fetal bovine serum; MOI: multiple of infection; VEGF: vascular endothelial growth factor; HIF-1α: hypoxia inducible factor-1α; bFGF: basic fibroblast growth factor; HGF: hepatocyte growth factor; DAPI: 4',6-diamidino-2-phenylindole; LAD: left anterior descending coronary artery; LVEF: left ventricular ejection fraction; LVESV: left ventricular end-systolic volume; LVEDV: left ventricular end-diastolic volume; AMP: 5'-adenosine monophosphate; ECM: Extracellular matrix; CSC: cardiac stem cell; FBS: Fetal bovine serum; SDF-1α/CXCR4: troma-derived factor-1α/chemokine receptor-4.

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**Authors' contributions**

Qiong Li, Huifang Hou: conception, design and manuscript writing. Qiong Li, Zhikun Guo: administrative support and financial support. Huifang Hou, Jianhui Gao: conception and manuscript finalization. Meng Li, Xia Yu, Hongbo Zuo: collection of data. Min Zhang: data analysis and interpretation, final approval of manuscript.

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## Availability of data and materials

Not applicable.

## Ethics approval and consent to participate

The experimental protocol was approved by the Ethics Committee of Xinxiang Medical University (Ethical review approval number: XYLL-2015023), and all procedures were carried out according to the Guidelines for the Regulation of Animal Experiments.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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**Figure 1. The morphology and phenotypical characterization of Adipose derived mensenchymal stem cells (ADMSCs).**

(A) Morphology of ADMSCs at different passages.P1: passage 1, P2: passage 2, P3: passage 3; Scale bar: 20μm. (B) Phenotypical characterizations of ADMSCs using multicolor immunofluorescence. Immunofluorescence was performed for surface markers CD29, CD44, CD73 and CD45. DAPI working solution was used to label nucleus. Scale bars: 10μm. (C) Phenotypical characterizations of ADMSCs using flowcytometer.

**Figure 2. Adipose derivedmensenchymal stem cells(ADMSCs) can be divided to $CD73^+$ADMSCs and $CD73^-$ADMSCs.**

(A) Morphology of $CD73^+$- and $CD73^-$-ADMSCs in culture was detected by HE staining. Blue arrows spindle cells, Red arrows showed big polygonal cells. Scale bars: 20μm. (B) Detection of CD73mRNA in $CD73^+$ADMSCs and $CD73^-$ADMSCs by Real-time quantitative PCR. Results are presented as mean ± SEM. $^*P<0.05$, vs. $CD73^-$ADMSCs. (C) The expression of CD73 in $CD73^+$ADMSCs and $CD73^-$ADMSCs by Western blotting.

**Figure 3. Differential genes analysis of $CD73^+$ADMSCs vs. $CD73^-$ADMSCs.**

(A) Fire maps of differential genes. (B) Go analysis for differentially expressed genes by KEGG.

**Figure 4. Cytokine secretion of ADMSCs by ELISA *in vitro*.**

(A) Secretion of cytokines in unsorted ADMSCs, $CD73^+$ADMSCs, $CD73^-$ADMSCs，and $CD73^+$ADMSCs+APCP. $^*P<0.05$, $^{**}P<0.01$, vs. ADMSCs; $^{\#}P<0.05$, $^{\#\#}P<0.01$, vs $CD73^-$ADMSCs; $^{\$}P<0.05$, $^{\$\$}P<0.01$, vs $CD73^+$ADMSCs; (B) Secretion of cytokines of sorted ADMSCs with Changing CD73 expression. $^{**}P<0.01$, vs. $CD73^+$ADMSCs; $^{\#}P<0.05$, vs $CD73^-$ADMSCs.

One-way ANOVA, followed by post Tukey's test for multiple comparisons. Data are presented as mean ± SEM. $CD73^+$ADMSCs: CD73 positive ADMSCs; $CD73^-$ADMSCs: CD73 negative ADMSCs; APCP: inhibitor of CD73, alpha, beta—methyleneadenosine-5'- diphosphate. $CD73^+$ADMSCs+siRNA: CD73 interference lentivirals transfected $CD73^+$ADMSCs; $CD73^-$ADMSCs+OE: CD73 overexpression of lentivirus transfected $CD73^-$ADMSCs.

**Figure 5. Improvement of cardiac function, neovascularization after CD73$^{+}$ADMSCs, CD73$^{-}$ADMSCs and ADMSCs were transplanted to infracted heart.**

(A) Cardiac hemodynamic monitoring. LVEF: Left ventricular ejection fraction; LVESV(ml): left ventricular end-systolic volume; LVEDV(ml): left ventricular end-diastolic volume.

(B), (C) and (D) VIII factor and VEGF expression in myocardial infarction area after transplantation of ADMSCs 4 weeks post MI by IHC and Western blotting.

$^{*}P$<0.05, $^{**}P$<0.01, *vs.* SHAM; $^{\#}P$<0.05, $^{\#\#}P$<0.01, *vs.* MI; $^{\$}P$<0.05, $^{\$\$}P$<0.01, *vs.* MI+ADMSCs; $^{+}P$<0.05, $^{++}P$<0.01, *vs.* CD73$^{-}$ADMSCs, n=5~6. One-way ANOVA, then followed by post Tukey's test for multiple comparisons. Data are presented as mean ± SEM.

SHAM: Sham operation group; MI: myocardial infarction group; MI+ADMSCs: the group that ADMSCs were translated into myocardial infarction; CD73$^{+}$ADMSCs: the group that CD73 positive ADMSCs were translated into myocardial infarction; CD73$^{-}$ADMSCs: the group that CD73 negative ADMSCs were translated into myocardial infarction; IOD: Integrated optical density; VEGF: vascular endothelial growth factor.

**Fig.6 The diagram of mechanisms responsible for improved cardiac repair after myocardial transplantation of CD73 positive ADMSCs.**

# Figures

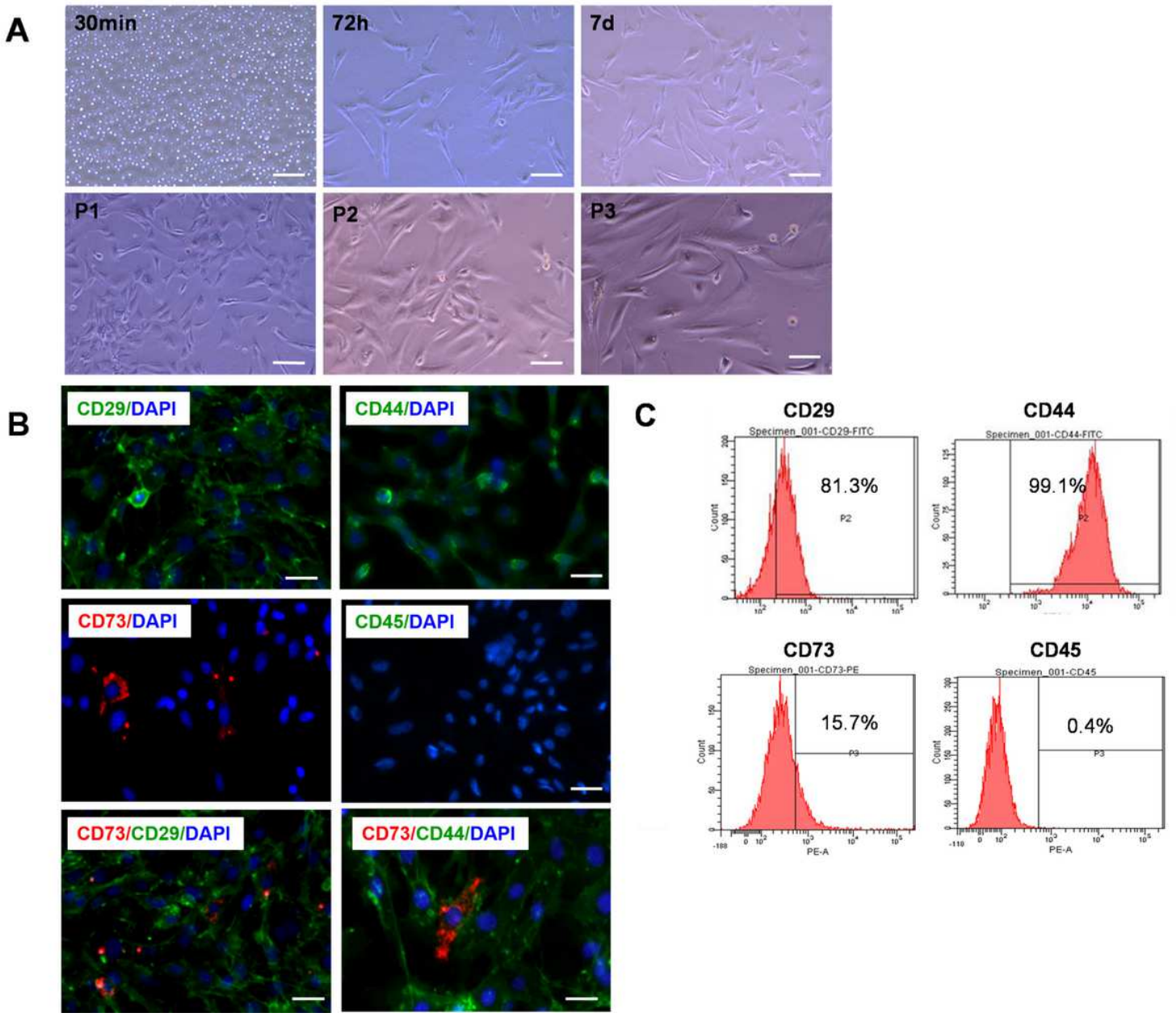
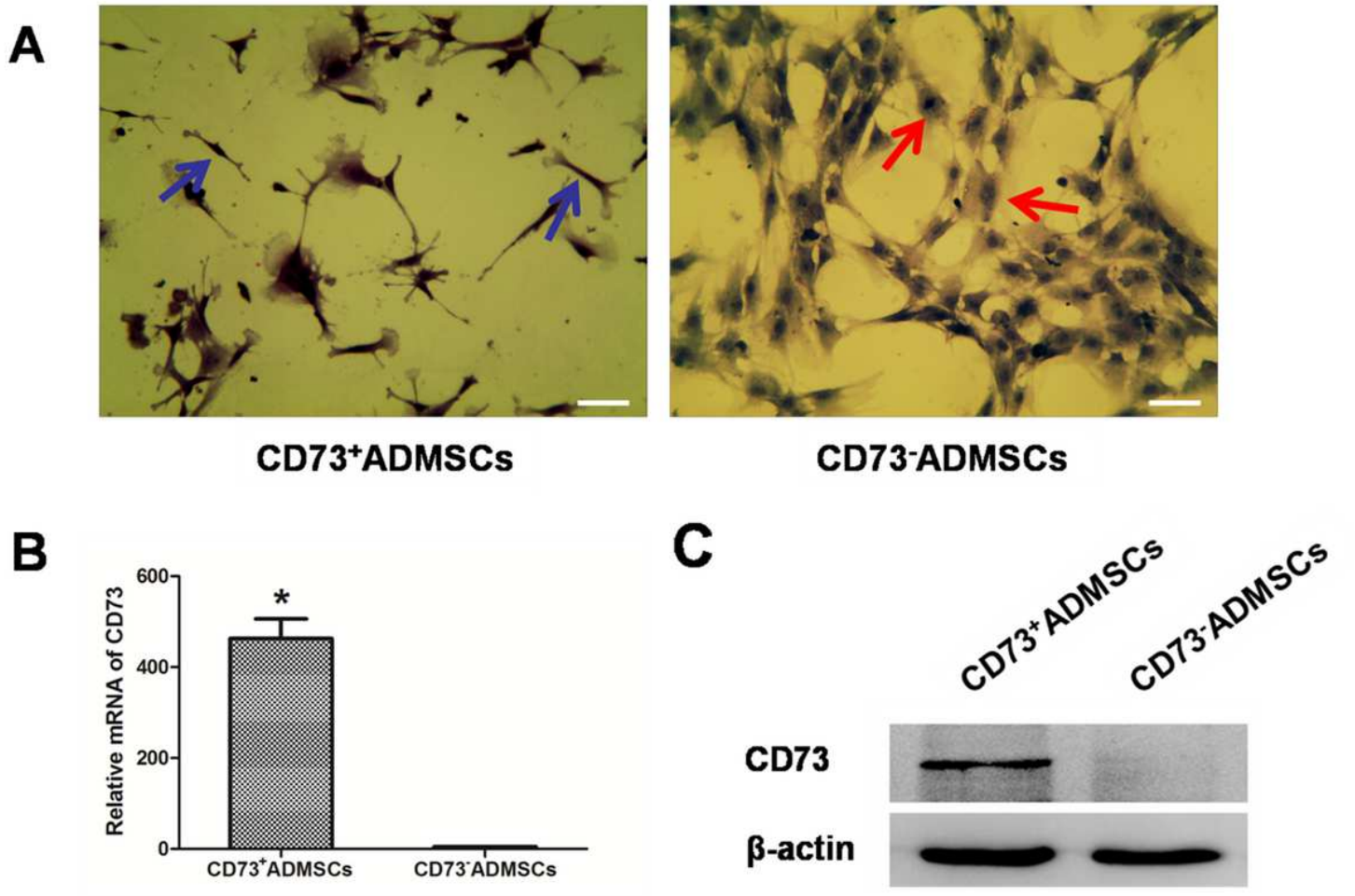


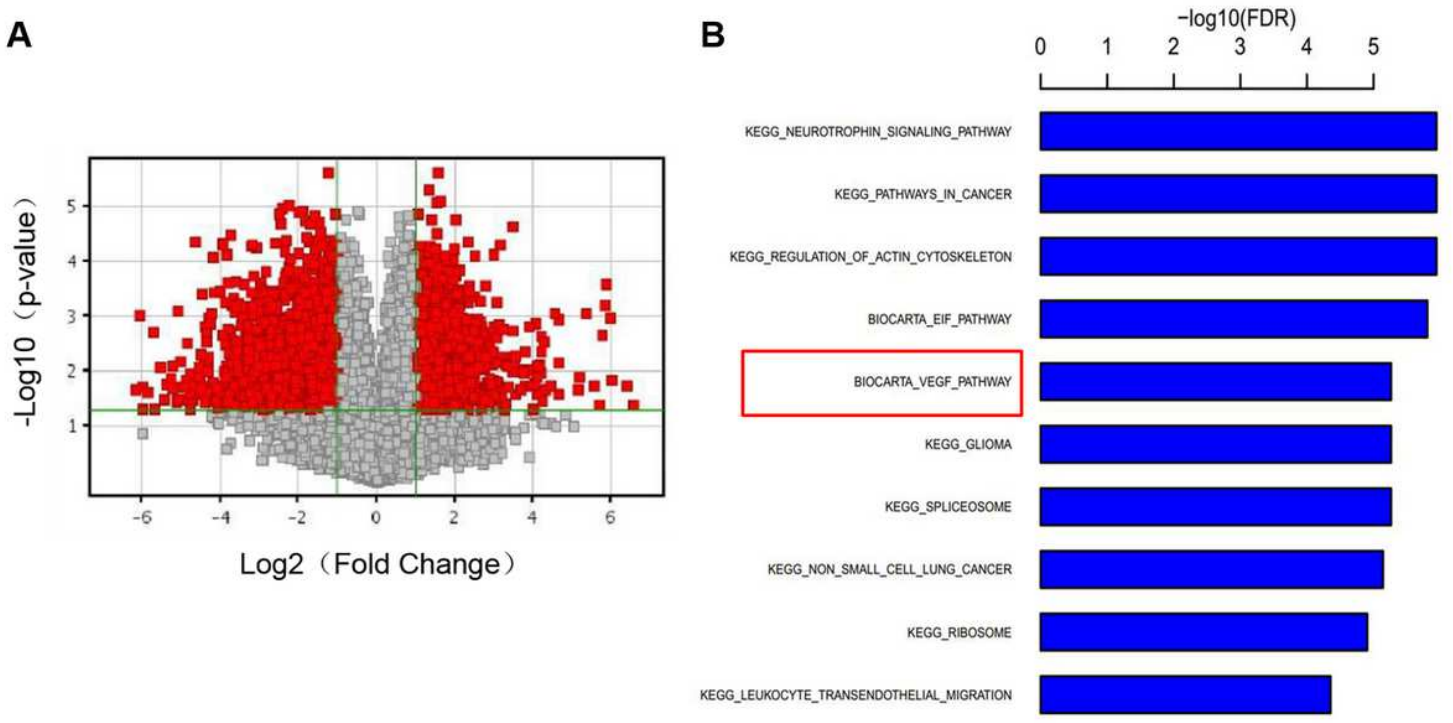

**Figure 1**

The morphology and phenotypical characterization of Adipose derived mensenchymal stem cells (ADMSCs). (A) Morphology of ADMSCs at different passages.P1: passage 1, P2: passage 2, P3: passage 3; Scale bar: 20μm. (B) Phenotypical characterizations of ADMSCs using multicolor immunofluorescence. Immunofluorescence was performed for surface markers CD29, CD44, CD73 and CD45. DAPI working solution was used to label nucleus. Scale bars: 10μm. (C) Phenotypical characterizations of ADMSCs using flowcytometer.



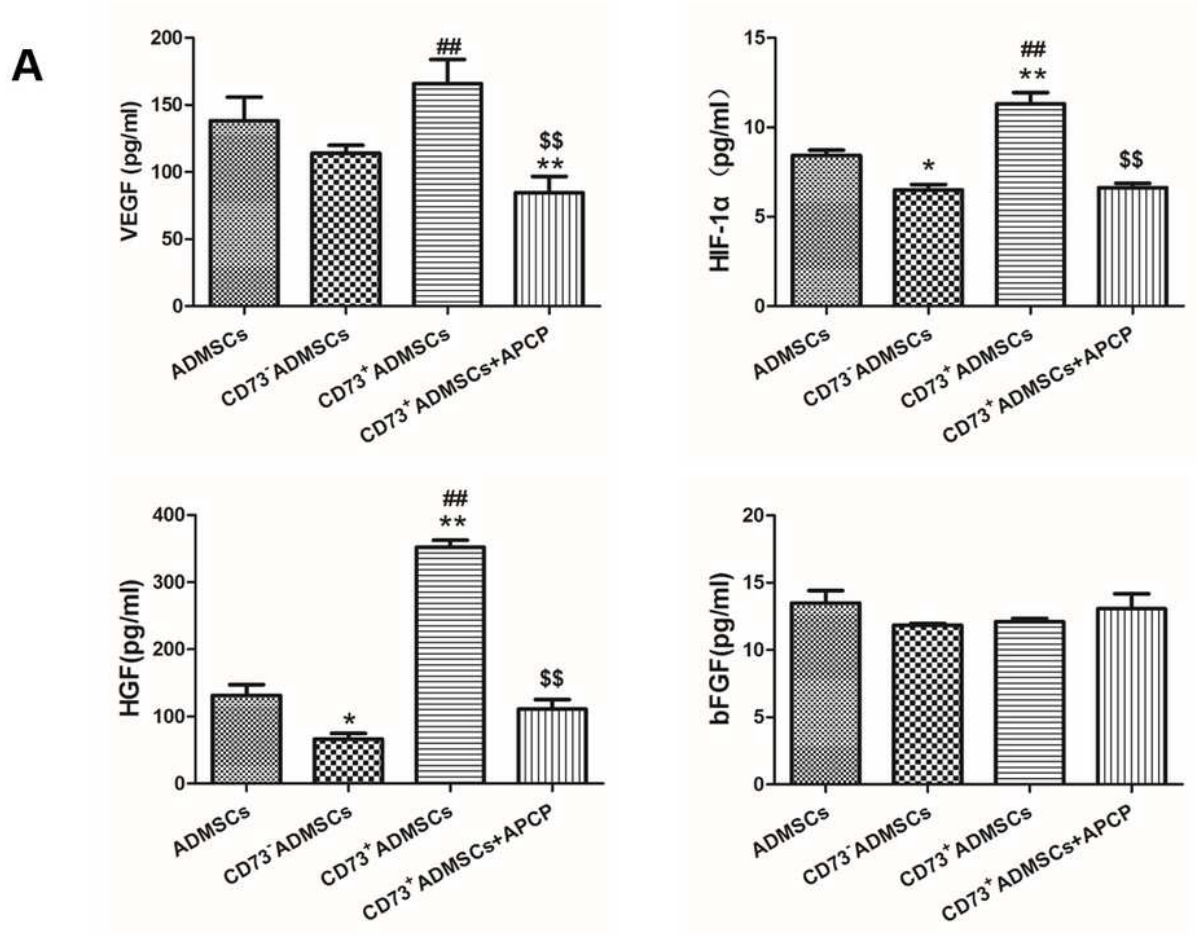

## Figure 2

Adipose derivedmensenchymal stem cells(ADMSCs) can be divided to CD73+ADMSCs and CD73-ADMSCs. (A) Morphology of CD73 +- and CD73–ADMSCs in culture was detected by HE staining. Blue arrows spindle cells, Red arrows showed big polygonal cells. Scale bars: 20μm. (B) Detection of CD73mRNA in CD73+ADMSCs and CD73-ADMSCs by Real-time quantitative PCR. Results are presented as mean ± SEM.*P<0.05, vs. CD73-ADMSCs. (C) The expression of CD73 in CD73+ADMSCs and CD73-ADMSCs by Western blotting.




## Figure 3

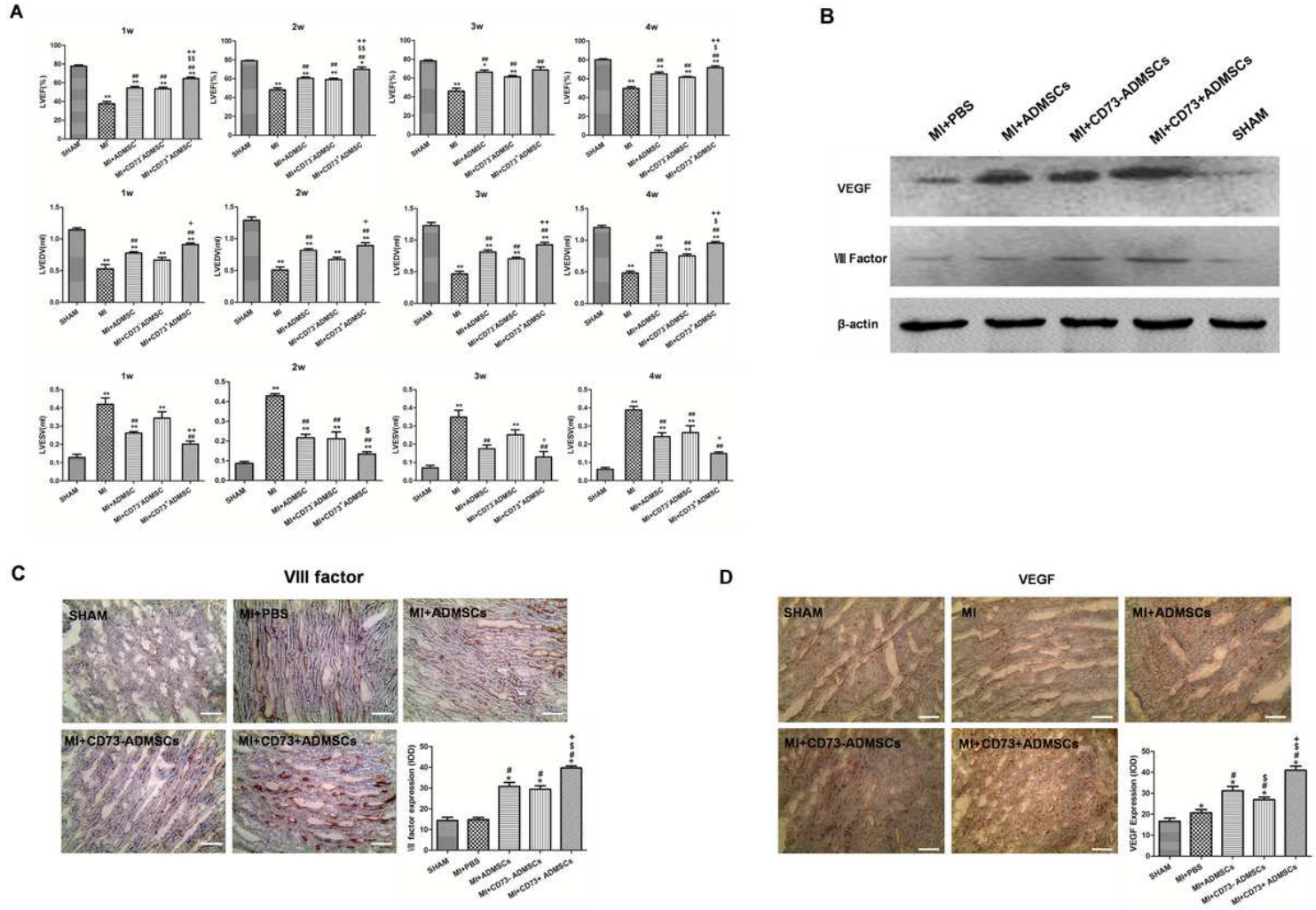
Differential genes analysis of CD73+ADMSCs vs. CD73-ADMSCs. (A) Fire maps of differential genes. (B) Go analysis for differentially expressed genes by KEGG.




## Figure 4

Cytokine secretion of ADMSCs by ELISA in vitro. (A) Secretion of cytokines in unsorted ADMSCs, CD73+ADMSCs, CD73-ADMSCs □ and CD73+ADMSCs+APCP. *P<0.05, ** P<0.01, vs. ADMSCs; □ P<0.05, ##P<0.01, vs CD73-ADMSCs; $P<0.05, $$P<0.01, vs CD73+ADMSCs; (B) Secretion of cytokines of sorted ADMSCs with Changing CD73 expression. ** P<0.01, vs. CD73+ADMSCs; □ P<0.05, vs CD73-ADMSCs. One-way ANOVA, followed by post Tukey's test for multiple comparisons. Data are presented as mean ±

SEM. CD73 +ADMSCs: CD73 positive ADMSCs; CD73-ADMSCs: CD73 negative ADMSCs; APCP: inhibitor of CD73, alpha, beta—methyleneadenosine-5'- diphosphate. CD73+ADMSCs+siRNA: CD73 interference lentivirals transfected CD73+ADMSCs; CD73-ADMSCs+OE: CD73 overexpression of lentivirus transfected CD73-ADMSCs.




## Figure 5

Improvement of cardiac function, neovascularization after CD73+ADMSCs, CD73-ADMSCs and ADMSCs were transplanted to infracted heart. (A) Cardiac hemodynamic monitoring. LVEF: Left ventricular ejection fraction; LVESV(ml): left ventricular end-systolic volume; LVEDV(ml): left ventricular end-diastolic volume. (B) , (C) and (D) VIII factor and VEGF expression in myocardial infarction area after transplantation of ADMSCs 4 weeks post MI by IHC and Western blotting. *P<0.05, **P<0.01, vs. SHAM; #P<0.05, ##P<0.01, vs. MI; $ P<0.05, $$P<0.01, vs. MI+ADMSCs; +P<0.05, ++P<0.01, vs. CD73-ADMSCs, n=5~6. One-way ANOVA, then followed by post Tukey's test for multiple comparisons. Data are presented as mean ± SEM. SHAM: Sham operation group; MI: myocardial infarction group; MI+ADMSCs: the group that ADMSCs were translated into myocardial infarction; CD73+ADMSCs: the group that CD73 positive ADMSCs were translated into myocardial infarction; CD73-ADMSCs: the group that CD73 negative ADMSCs were translated into myocardial infarction; IOD: Integrated optical density; VEGF: vascular endothelial growth factor.

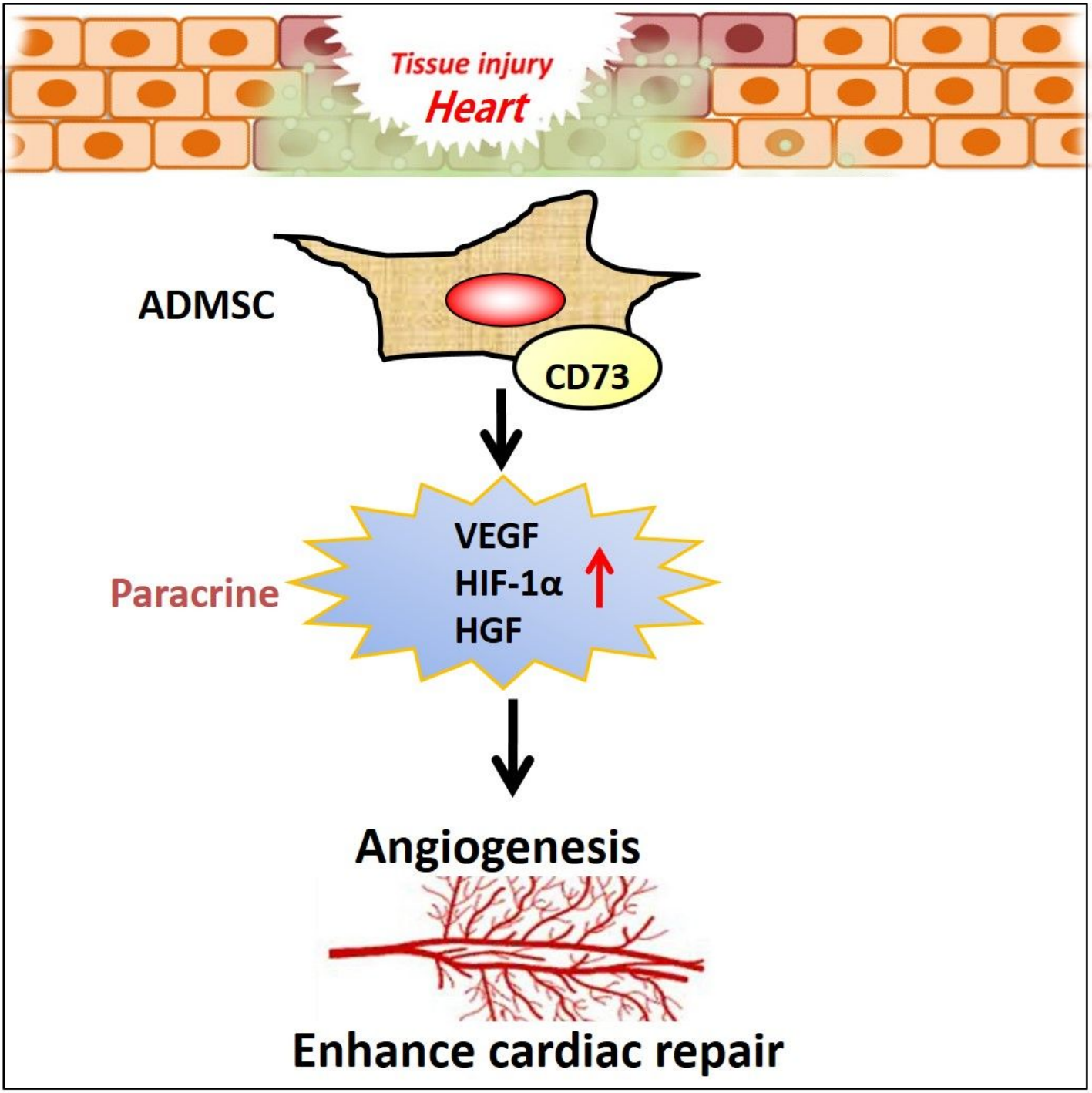



**Figure 6**

The diagram of mechanisms responsible for improved cardiac repair after myocardial transplantation of CD73 positive ADMSCs.

## Supplementary Files

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- Ethicalreviewreport.pdf