SUPPLEMENTAL MATERIAL

MATERIALS AND METHODS

Echocardiography

Mice were anesthetized with inhalation of 1-2% isoflurane. A comprehensive echocardiography study was performed using a Vevo 2100 system (VisualSonics, Canada). Representative M-mode images were acquired and analyzed to evaluate cardiac function.

Immunoprecipitation

The cultured cardiac fibroblasts incubated under hypoxic conditions for 24 hours were lysed in NP40 lysis buffer (50 mM Tris, 150 mM NaCl, 1% Nonidet P-40, 5 mM EDTA, complete protease inhibitor cocktail, 10 mM NaF, 1 mM sodium orthovanadate, 25 mM β-glycerophosphate). The supernatant was collected after centrifuging at 12,000 rpm and then immunoprecipitated overnight at 4°C with the anti-Stat3 antibody (Cell Signaling Technology) or IgG (Santa Cruz). Protein A/G beads (Santa Cruz) were added into cell lysates for an additional incubation of 2 h. Beads then were centrifuged, and protein complex was denatured and resolved by SDS-PAGE gel. The gel was stained with coomassie brilliant blue solution and subjected to mass spectrometry sequencing and data analysis 1.

Mass spectrometry

Protein slices in fresh SDS-PAGE gel were excised and plated into a 96-well microtitre plate. Protein slices were destained with 200 μl of 50 mM NH₄HCO₃ and 50% acetonitrile and then dried with 200μl of acetonitrile. The dried pieces of gels were incubated in ice-cold digestion solution (trypsin 12.5 ng/μl, 20 mM NH₄HCO₃) for 20 minutes and then transferred into a 37°C incubator for digestion overnight. Peptides in the supernatant were collected using extract solution (5% formic acid in 50% acetonitrile) and dried under the protection of N₂.

The dried peptides were dissolved in solvent A (5% acetonitrile, 0.1% formic acid)
and analyzed on TripleTOF 5600 systems (AB SCIEX, USA). Briefly, peptides were separated on reverse-phase column (ZORBAX 300SB-C18 column, 5μm, 300 Å, 0.1×15 mm; Micromass) using an Eksigent 1D PLUS system (AB SCIEX) at an analytical flow rate of 300 nL/min. Survey scans were acquired from 400 to 1500 with up to 15 precursors selected for MS/MS and dynamic exclusion for 20 seconds.

For protein identification, MS/MS data were searched using MASCOT version 2.3.02 (Matrix Science, United Kingdom) against the mouse subset of the UniProt sequence databases. Only peptides with significant scores greater than “identity score” were counted as identified.

REFERENCE

Online Figure I. The interaction of Stat3 with Smad3 in cultured cardiac fibroblasts infected with EphrinB2 overexpression lentiviruses was detected via co-immunoprecipitation.
Online Figure I. Original mass spectrogram of Stat3-pulled down Smad3 in cultured cardiac fibroblasts under hypoxic conditions.
**Online Table I.** Echocardiographic analysis of MI mice with injection of lentiviruses.

<table>
<thead>
<tr>
<th>Group</th>
<th>Scramble sham</th>
<th>EphrinB2 shRNA sham</th>
<th>Scramble MI</th>
<th>EphrinB2 shRNA MI</th>
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<td>FS (%)</td>
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</tbody>
</table>

Values are mean ± SEM;

* P<0.05 vs Scramble MI.