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Supplementary Materials for

The long noncoding RNA *Wisper* controls cardiac fibrosis and remodeling

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The PDF file includes:

Table S4. Echocardiographic parameters measured 7 and 28 days after MI in mice injected with GapmeRs 2 days and 9 days after MI.
Table S5. Characteristics of patients suffering from AOS and developing cardiac fibrosis.
Table S6. List of the primers used in this study.

Other Supplementary Material for this manuscript includes the following:
(available at
www.sciencetranslationalmedicine.org/cgi/content/full/9/395/eaai9118/DC1)

Table S7. Individual-level data and exact *P* values (Excel format).

Material and Methods

Primary cell culture and transfection

Neonatal mouse CM and fibroblast isolation - Neonatal C57B6 mice were sacrificed within the first 24h after birth and beating hearts were collected. Atria and great vessels were carefully dissected away and placed on ice in ADS buffer (H_2O, NaCl 116 mM, HEPES 20 mM, NaH_2PO_4 1 mM, KCl 5.4 mM, $MgSO_4$ 0.8 mM, glucose 5.5 mM). The hearts were minced using a sterile sharp razorblade, and placed in a 1.5 ml-tubes (5-6 hearts per tube) containing 1 ml of PIB digestion buffer (ADS buffer; 0.05mg/ml collagenase type II (Worthington); 1mg/ml pancreatin; Sigma). Tissues were incubated at 37°C with shaking for 15 min. Supernatants were then collected in tubes containing complete medium (DMEM 75%, M199 25% ml, penicillin/streptavidin 1x, L-glutamine 1x, horse serum 10%, fetal cow serum 5%; Gibco). PIB buffer was added to undigested tissue fragments and digestion was repeated twice. Cells were collected by centrifugation at 800 rpm for 10 min at room temperature (RT). Pellet was then resuspended in an adequate volume of complete medium (2 ml each 5 hearts). Cells were then plated in 10 cm dishes for 45 min at 37°C, 10% CO_2 (pre-plating 1); after this step the non-myocytes adhere and the CMs remain in suspension. Supernatant was transferred to a new 10 cm dish and pre-plating step was repeated (pre-plating 2). After the second pre-plating the supernatant was collected in a new tube, CMs were counted and seeded on gelatin coated 3.5 cm plates ($3x10^5$ cells per dish). The non-myocytes fraction was cultured in fibroblast medium (DMEM, fetal cow serum 10%, penicillin/streptavidin 1x) and seeded into 10 cm dishes. Cells were split using trypsin to minimize CMs contamination. Confluence was maintained below the 85% and medium was changed every second day.

Adult mouse cardiac fibroblast isolation- 12-week old C57/BL6 were injected with 100 U of heparin (Liquerin) 30 min before being sacrificed to avoid blood coagulation in the heart. Beating hearts were removed and washed in cold PBS. Then, hearts were canulated through the aorta and the vessel was tied using a surgical rope. Using a 1 ml syringe, HBSS solution (hanks balanced salt solution, GIBCO) was pumped in the heart through the aorta in order to wash out the blood. In the same way, 1 ml of digesting solution (HBSS; 2 mg/ml Collagenase type II (Worthington); 0.05 mg/ml Protease XIV; Sigma)) was pumped in the heart followed by 5 min of digestion at 37°C. This step was performed 3 times each heart. After the third digestion, the

atria and big vessels were removed and the remaining ventricles were minced and resuspended in complete medium (DMEM, penicillin/streptavidin 1x, fetal cow serum 10%). The solution was pipetted up and down 10-20 times to reach a single cell suspension and the supernatant was collected avoiding undigested pieces. Cells were collected by centrifugation at 800 rpm for 10 min at RT. The pellet was resuspended in adequate volume of complete medium and cells were plated in 10 cm dishes overnight (pre-plating 1). After this step, the non-myocytes adhere and the CMs remain in suspension. Supernatant was transferred to a new 10 cm dish and pre-plating step repeated for another 24 h (pre-plating 2). Fresh complete medium was added to the pre-plating dishes to culture the non-myocytes cells and the supernatant was discarded. Cells were split to maintain a confluence not over the 85% and medium was changed every two days.

Mouse lung and tail fibroblast isolation- The lungs and the tail tips were collected from 12 weeks old C57/BL6 and washed in HBSS (Gibco). They were both minced using a sterile and sharp razorblade, and placed in a 2 ml tubes (2 lungs per tube or 5 tails per tube) containing 1 ml digestion buffer (HBSS + 10 mg/ml Collagenase type II, Worthington). Cells were incubated at 37°C with shaking for 40 min. After digestion fibroblast medium (DMEM, Fetal Cow Serum 10%, Penicillin/streptavidin 1x) was added and cells were collected by centrifugation at 800 rpm for 10 min at RT. The supernatant was discarded. The cellular pellet was resuspended in proper volume of fibroblast medium and then filtered into a 70um strainer to remove undigested pieces. Cells were counted and seeded in 10 cm dishes. Cells were split to maintain a confluence not over 85% and medium was changed every two days.

Human fibroblasts- Adult human dermal fibroblasts were purchased (Gibco). Primary cell cultures of human CFs were obtained by mechanical tissue enzymatic digestion with collagenases (Roche) and explant outgrowth from atrial samples obtained as discarded surgical tissue. Cultures of primary CFs were shown to express specific fibroblasts markers (i.e. vimentin) and to respond to TGF-β (1 ng/mL) stimulation. Cells were plated at a density of 5000 cells/cm^2 and maintained in a fibroblast medium (low glucose DMEM (Gibco) with fetal bovine serum (10%; Sigma), fibroblast growth factor-2 (FGF-2; 5.8 µg/µL; Peprotech) glutamine (2.5 mM; Gibco) penicillin/streptomycin (1%; Gibco), Fungizone (0.1%; Gibco) and HEPES (1.5%; Lonza). Medium was changed every 48 hours. Once cells reached a 60-70% confluence cells were starved in medium without fetal bovine serum (and FGF-2 0.285 µg/µL) for 2, 4, 8, 14, 24 or 40 hours. Subsequently, cells were collected for further analysis. Human dermal fibroblasts

were used in passages between 3 and 6 and human CFs in passages 2 to 4. Between 6 and 10 independent samples were analyzed for each condition.

GapmeR transfection in mouse primary cell- Adult CFs, neonatal CFs and lung fibroblasts ($3.5x10^5$ cells per 3.5 cm dish; passage 2) and CMs ($1x10^6$ cells per 3.5 cm dish) were cultured overnight. The next day, medium was changed two hours before transfection. Then, a final concentration of 10 nM (if not differently specified in the text) of LNA lncRNA GapmeRs (GapmeR-*Wisper* or GapmeR-negative control A or GapmeR-Wisp2) (Exiqon) was transfected on cells using Xtreme gene HP DNA transfection reagent (Promega). After 48 hours, medium was changed and cells were collected for future experiments. Total RNA was obtained using miRNeasy kit (Qiagen) and the knock down confirmed by qRT-PCR.

GapmeR transfection in human CFs- Transfection was performed in 25 cm^2 flasks in cells at 80% confluence. A final concentration of 25 nM of LNA lncRNA GapmeRs (GapmeR-*Wisper* or GapmeR-negative control A; Exiqon) was transfected on cells using Xtreme gene HP DNA transfection reagent (Promega) in complete medium. After 24 hours, cells were kept in normal medium or exposed to serum starvation for another 24 hours, and subsequently were collected for further studies. Three independent samples were analyzed for each condition.

Animal experiments – Tissue collection and preparation

Mice were sacrificed by deep anesthesia followed by cervical dislocation, and organs were collected. Hearts were dissected from sham and MI mice at different time points after artery ligation. Atria and big vessels were eliminated. The remaining ventricles were rinsed in diethylpyrocarbonate (DEPC)-treated PBS to minimize residual blood contamination. Furthermore, the ventricles were divided in 3 parts: top section (from the top to the ligature), central section (from the ligature to the center of the infarcted area) and the tip of the heart (from the center of the infarcted area to the bottom). The heart tip (50% viable muscle, 50% infarcted zone) was used to collect total RNA. The central section was fixed in formalin and embedded in paraffin. The top section was snap frozen in liquid nitrogen and stored at -80’C for future analysis.

RNA isolation, cDNA preparation and quantitative PCR analysis

Total RNA from tissues and cultured cells isolated from mice was extracted using miRNeasy kit (Qiagen) according to the manufacturer's instructions and quantified with Nanodrop instrument. The quality control was performed with a Agilent 2100 bioanalyzer (Agilent Technologies). Two steps cDNA synthesis was performed with SuperScript II (Invitrogen), and quantification was carried out using QuantStudio 6/7 (Thermofisher). Gene expression was normalized to *Gapdh* and quantified using the ΔΔCt method.

Total RNA from human cells was obtained using the Maxwell 16 LEV simplyRNA Purification Kit (Promega) according to manufacturer's instructions, and was quantified with a Nanodrop instrument. Reverse transcription was performed using the high capacity cDNA reverse transcription kit (Applied Biosystems). Real-time PCR for collagen-related genes was performed with a StepOne Plus Real-time PCR system according to the manufacturer's recommendations (Applied Biosystems) using specific TaqMan fluorescent probes. Data were normalized to ribosomal 18S expression. *WISPER* and *WISP2* expression were analyzed with a 7900HT Fast Real-Time PCR system (Applied Biosystems) using the power SYBR green PCR master mix (Applied Biosystems) and specific primers. Gene expression was normalized to *GAPDH*. Primer sequences and fluorescent-labeled TaqMan probes used in mouse and human samples are listed in Table S6.

Western blotting

Proteins were extracted from neonatal CFs transfected with GapmeR-*Wisper* (10 nM) or GapmeR-Scrambled (10 nM) using lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 0.25 % DOC, 1% Nonidet P-40, 1% Triton X-100 containing protease and phosphatase inhibitors (Roche)). Proteins were resuspended in SDS-PAGE buffer, separated on acrylamide gels and transferred to PVDF membranes (BioRad). Membranes were incubated with mouse monoclonal anti-α-SMA primary antibodies (Sigma, 1:2000 dilution), and then with horseradish-conjugated secondary antibodies (Amersham Bioscience, 1:2000 dilution). α-SMA abundance was quantified by densitometry and normalized to the total amount of proteins.

Thymidine incorporation assay

Adult CFs and lung fibroblasts were transfected with 10 nM of GapmeR-*Wisper* or GapmeR-Scrambled for 24h. $4x10^3$ cells/well were seeded in 96 round bottom-well plates in quadruplicate. 1 µCi of [H^3]-Thymidine was added in each well at 0h, 24h and 48h after cell seeding, and incubated for 18 h at 37°C. Cells were then harvested and transferred into a filter plate. Radioactivity was measured using a scintillation beta-counter (TopCount, Canberra Packard). Measurements were performed in quadruplicate for each condition in 5 independent experiments and normalized to 0 h values.

Wound closure assay

Adult CFs and lung fibroblasts were transfected with 10 nM of GapmeR-*Wisper* or GapmeR-Scrambled for 48h. $3.5x10^5$ cells were seeded in 3.5-cm well and let them attached overnight. The scratch test was performed using a sterile 10 µl filter tip. Medium was replaced twice to remove floating cells. Three pictures for each condition were taken at 20x magnification every 24 hours. The size of the wound has been measured using ImageJ. Measurements were performed in triplicate for each condition in at least 3 independent experiments and normalized to 0 h values (100%).

Annexin V-positive cells quantification

Adult CFs and lung fibroblasts were transfected with 10 nM of GapmeR-*Wisper* or GapmeR-Scrambled. Transfected cells were collected after 48h and resuspended in FACS medium. Cells were stained using the FITC Annexin V Apoptosis detection Kit (BD Pharmingen) according to the manufacturer's instructions. Annexin V and PI signals were quantified with a Galios FACS apparatus within 30 min after staining. Results were analyzed with FlowJo.

***Wisper in situ* hybridization**

Infarcted (14 days post-MI) and sham-operated hearts were fixed for 48h in 10% neutral buffered formalin and sections of 4 µm were obtained. Sections were de-waxed, dehydrated, air-dried briefly and incubated in 1.3 mg/ml Pepsin/0.1mM HCl solution for 5 min at 37°C. After a brief wash in DEPC MQ, sections were incubated overnight at 42.5°C in 1x hybridization buffer

(ENZO Life sciences,) with either 100 nM *Wisper* DIG labeled probe (Exiqon) or a scrambled control probe. The following day the sections were washed once in 5xSSC and twice in 0.2xSSC at hybridization temperature. Subsequently, sections were blocked in DIG blocking buffer from the DIG Wash and Block Buffer Set (Roche) for 20 min followed by a 45 min-incubation in 1:500 anti-DIG-AP, Fab fragments (Roche). Sections were washed 3x5 min with TBS and incubated 3 min in DIG detection buffer at RT. Alkaline phosphatase signal was detected by using NBT/BCIP tablets (Roche) for 4 hours at 30°C in darkness. Finally, sections were counterstained with fast red (Sigma) briefly washed in MQ, quickly dehydrated through an ethanol gradient and mounted with entellan (EMS). Pictures were taken using a Nikon Stereomicroscope SMZ 25 at different magnifications.

BrdU labeling and detection

Mice were supplied with a solution of 10 mg/ml 5-Bromo-2-deoxyuridine (BrdU; Sigma) supplemented with 1% glucose directly in the drinking water for 7 days. The drinking solution was changed every second day. For BrdU detection, frozen heart tissue sections were fixed in 2% paraformaldehyde (PFA) in PBS for 20 min at room temperature. DNA was fragmented by incubation in 2N HCl at 37°C for 20 min and neutralization in 0.1M Borate buffer, pH 8.5. BrdU detection was performed using rat anti-BrdU antibody (Abcam; 1:100) and Alexa-Fluor 594 conjugated anti-rat antibody (Molecular Probes; 1:250). For BrdU-laminin co-immunostaining, tissue sections were first subjected to immunofluorescence staining to detect protein antigens. Tissue sections were post-fixed with 2% PFA for 10 min at room temperature, and then processed for BrdU detection.

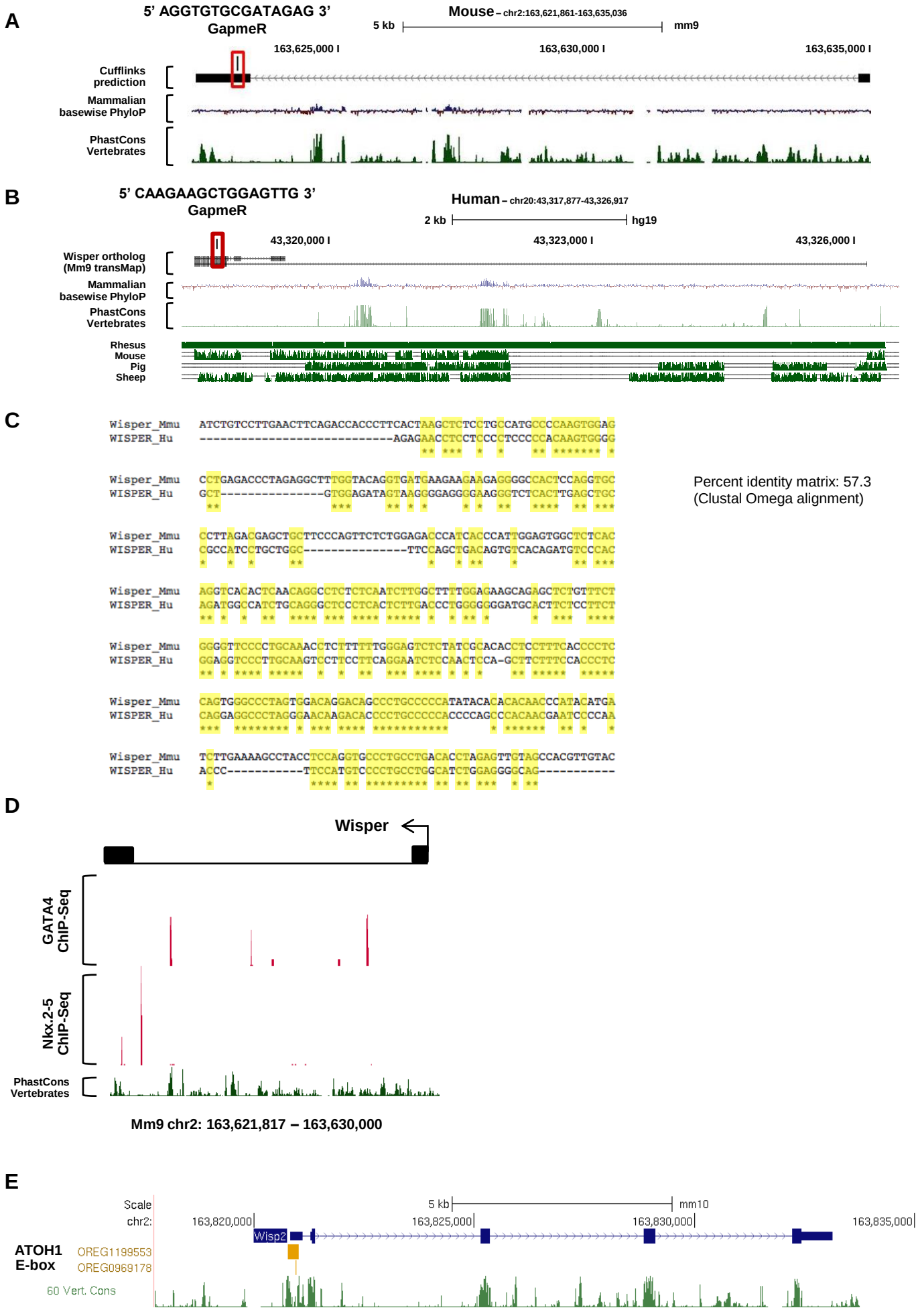

A
5' AGGTGTGCGATAGAG 3'
GapmeR
Mouse – chr2:163,621,861-163,635,036
5 kb
mm9
163,625,000
163,630,000
163,635,000
Cufflinks prediction
Mammalian basewise PhyloP
PhastCons Vertebrates
B
5' CAAGAAGCTGGAGTTG 3'
GapmeR
Human – chr20:43,317,877-43,326,917
2 kb
hg19
43,320,000
43,323,000
43,326,000
Wisper ortholog (Mm9 transMap)
Mammalian basewise PhyloP
PhastCons Vertebrates
Rhesus
Mouse
Pig
Sheep
C
Wisper_Mmu ATCTGTCCTTGAACTTCAGACCACCCTTCACTAAGCTCTCCTGCCATGCCCCAAGTGGAG
WISPER_Hu ----------AGAGAACCTCCTCCCCTCCCCCACAAGTGGGG
Wisper_Mmu CCTGAGACCCTAGAGGCTTTGGTACAGGTGATGAAGAAGAAGAGGGGCCACTCCAGGTGC
WISPER_Hu GCT----------GTGGAGATAGTAAGGGGAGGGAAGGGTCTCACTTGAGCTGC
Wisper_Mmu CCTTAGACGAGCTGCTTCCCAGTTCTCTGGAGACCCATCACCCATTGGAGTGGCTCTCAC
WISPER_Hu CGCCATCCTGCTGGC----------TTCCAGCTGACAGTGTCACAGATGTCCCAC
Wisper_Mmu AGGTCACACTCAACAGGCCTCTCTCAATCTTGGCTTTTGGAGAAGCAGAGCTCTGTTTCT
WISPER_Hu AGATGGCCATCTGCAGGGCTCCCTCACTCTTGACCCTGGGGGGGGATGCACTTCTCCTTCT
Wisper_Mmu GGGGTTCCCCTGCAACACCTCTTTTTTGGGAGTCTCTATCGCACACCTCCTTTCACCCCTC
WISPER_Hu GGAGGTCCCTTGCAAGTCCTTCCTTCAGGAATCTCCAACTCCA-GCTTCTTTCCACCCCTC
Wisper_Mmu CAGTGGGCCCTAGTGGACAGGACAGCCCTGCCCCCATATACACACACAACCCATACATGA
WISPER_Hu CAGGAGGCCCTAGGGAACAAGACACCCCTGCCCCCACCCCAGCCCACAACGAATCCCCAA
Wisper_Mmu TCTTGAAAAGCCTACCTCCAGGTGCCCTGCCTGACACCTAGAGTTGTAGCCACGTTGTAC
WISPER_Hu ACCC----------TTCCATGTCCCCTGCCTGGCATCTGGAGGGGCAG----------
Percent identity matrix: 57.3
(Clustal Omega alignment)
D
Wisper
GATA4 ChIP-Seq
Nkx.2-5 ChIP-Seq
PhastCons Vertebrates
Mm9 chr2: 163,621,817 – 163,630,000
E
Scale
5 kb
mm10
chr2:
163,820,000
163,825,000
163,830,000
163,835,000
Wisp2
ATOH1 E-box
OREG1199553
OREG0969178
60 Vert. Cons

Figure S1. Conservation between the mouse and human *Wisper* transcripts. (A and B) UCSC genome browser views of *Wisper* showing the region targeted by GapmeRs and its sequence in the mouse (A) and human genome (B). (C) Pairwise alignment of the murine and human sequence of *Wisper* by Clustal Omega. (D) UCSC Screenshot of GATA4 and NKX2-5 ChIP-Seq data in the adult murine heart at the *Wisper* locus. (E) UCSC Screenshot of the *Wisp2* locus showing ATOH1 binding sites and E-box in the promoter.

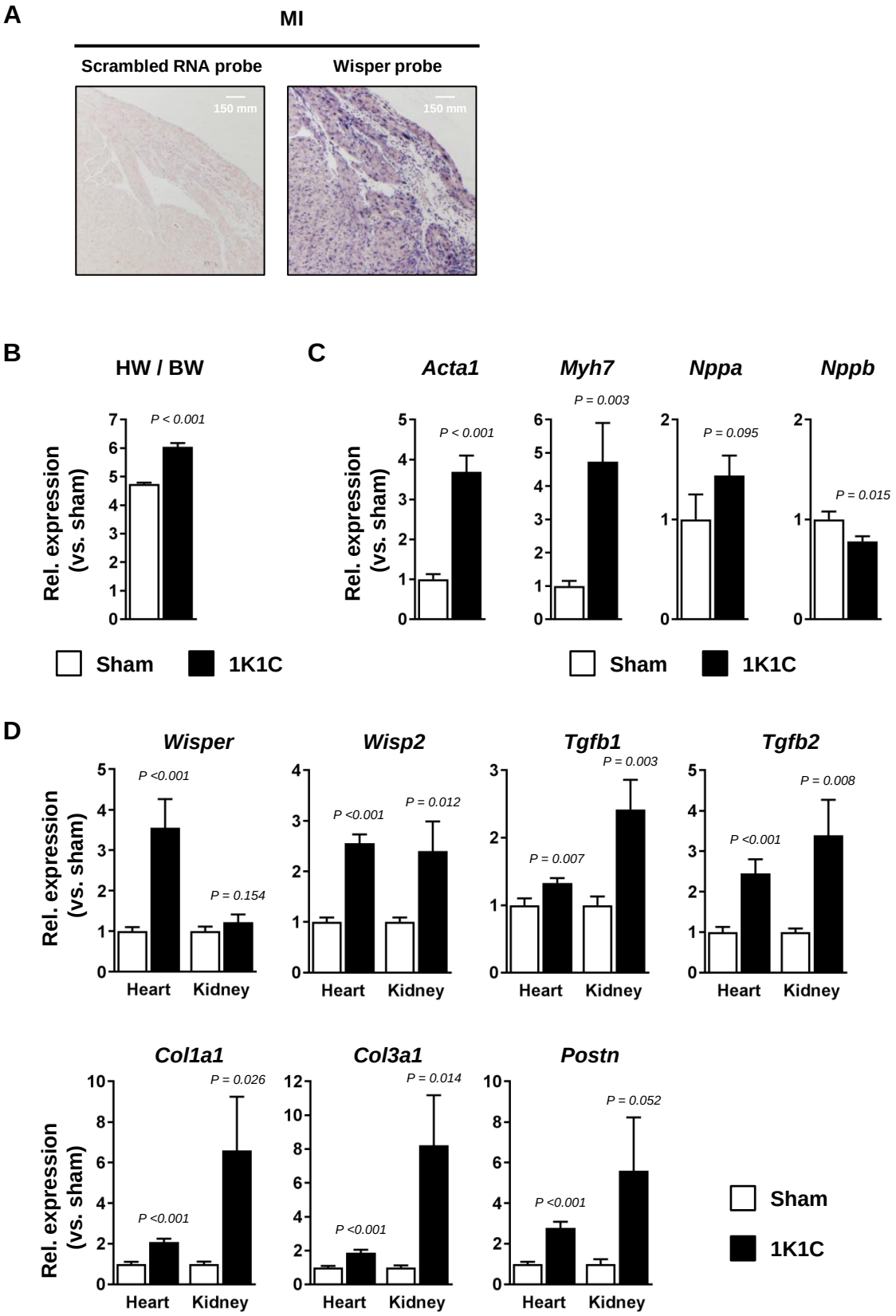

A
MI
Scrambled RNA probe
Wisper probe
150 mm
150 mm
B
HW / BW
Rel. expression (vs. sham)
P < 0.001
Sham
1K1C
C
Acta1
Myh7
Nppa
Nppb
Rel. expression (vs. sham)
P < 0.001
P = 0.003
P = 0.095
P = 0.015
Sham
1K1C
D
Wisper
Wisp2
Tgfb1
Tgfb2
Rel. expression (vs. sham)
P <0.001
P = 0.154
P <0.001
P = 0.012
P = 0.007
P = 0.003
P <0.001
P = 0.008
Heart
Kidney
Col1a1
Col3a1
Postn
P <0.001
P = 0.026
P <0.001
P = 0.014
P <0.001
P = 0.052
Sham
1K1C

Figure S2. *Wisper* is expressed in the stressed fibrotic heart but not in the stressed fibrotic kidney. (A) In situ hybridization on infarcted heart sections: Left panel: scrambled RNA negative control; Right panel: *Wisper* probe (B) Cardiac hypertrophy in mice subjected to the one-kidney one-clip (1K1C) model of renovascular hypertension. 1K1C mice: n=8; Sham-operated mice: n = 7. Heart weight (HW) to tibial length (TL) ratio. Bars represent mean ± SEM. *P values* determined by Student's t test. (C) Cardiac stress marker expression measured by qRT-PCR in sham and 1K1C mice 14 days after surgery. Bars represent means normalized to sham ± SEM. *P values* determined by Student's t test. (D) *Wisper* and fibrosis-associated gene expression measured by qRT-PCR in sham and 1K1C mice. Bars represent means normalized to sham ± SEM. *P values* determined by two-way ANOVA (Fisher's test).

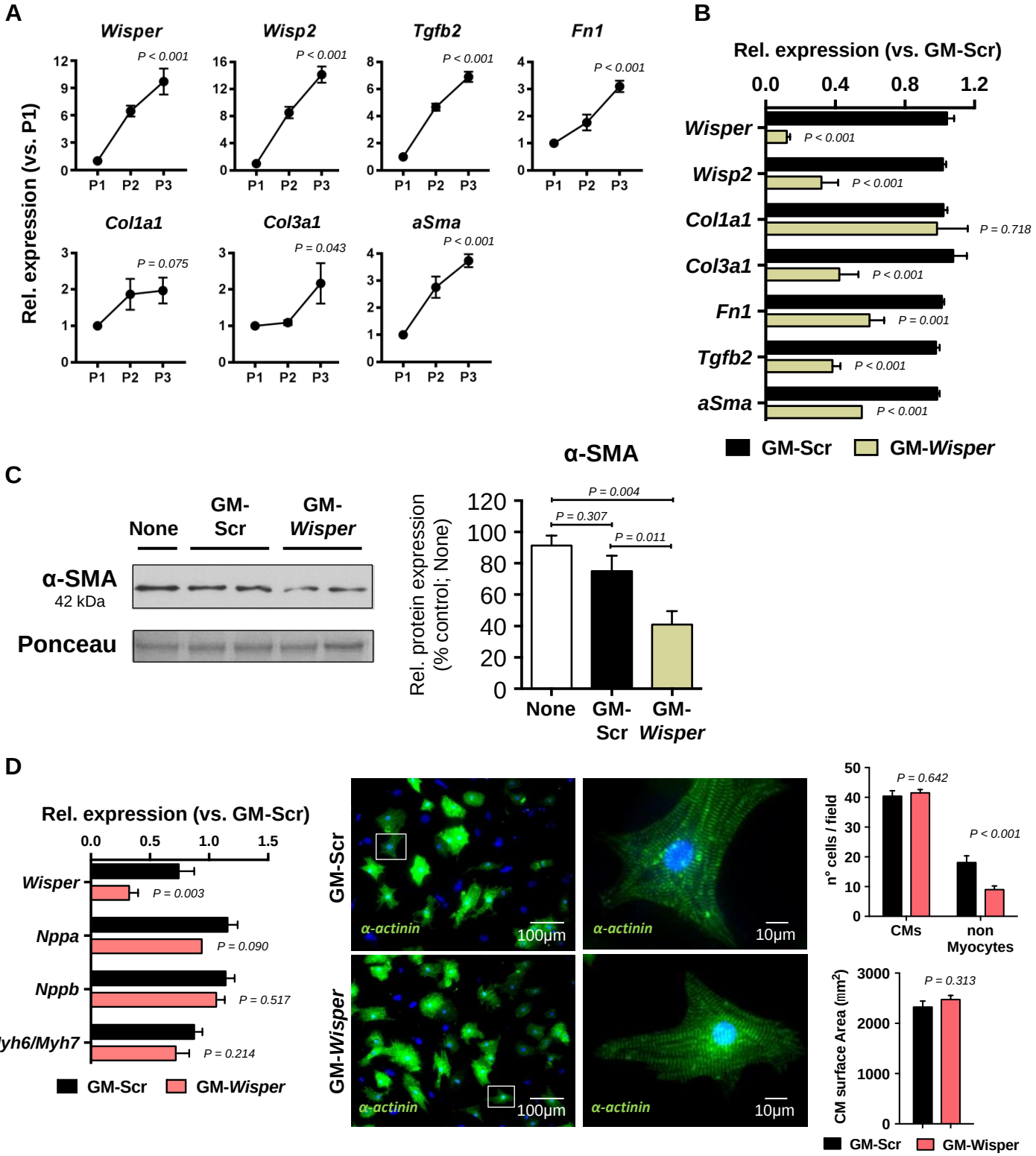
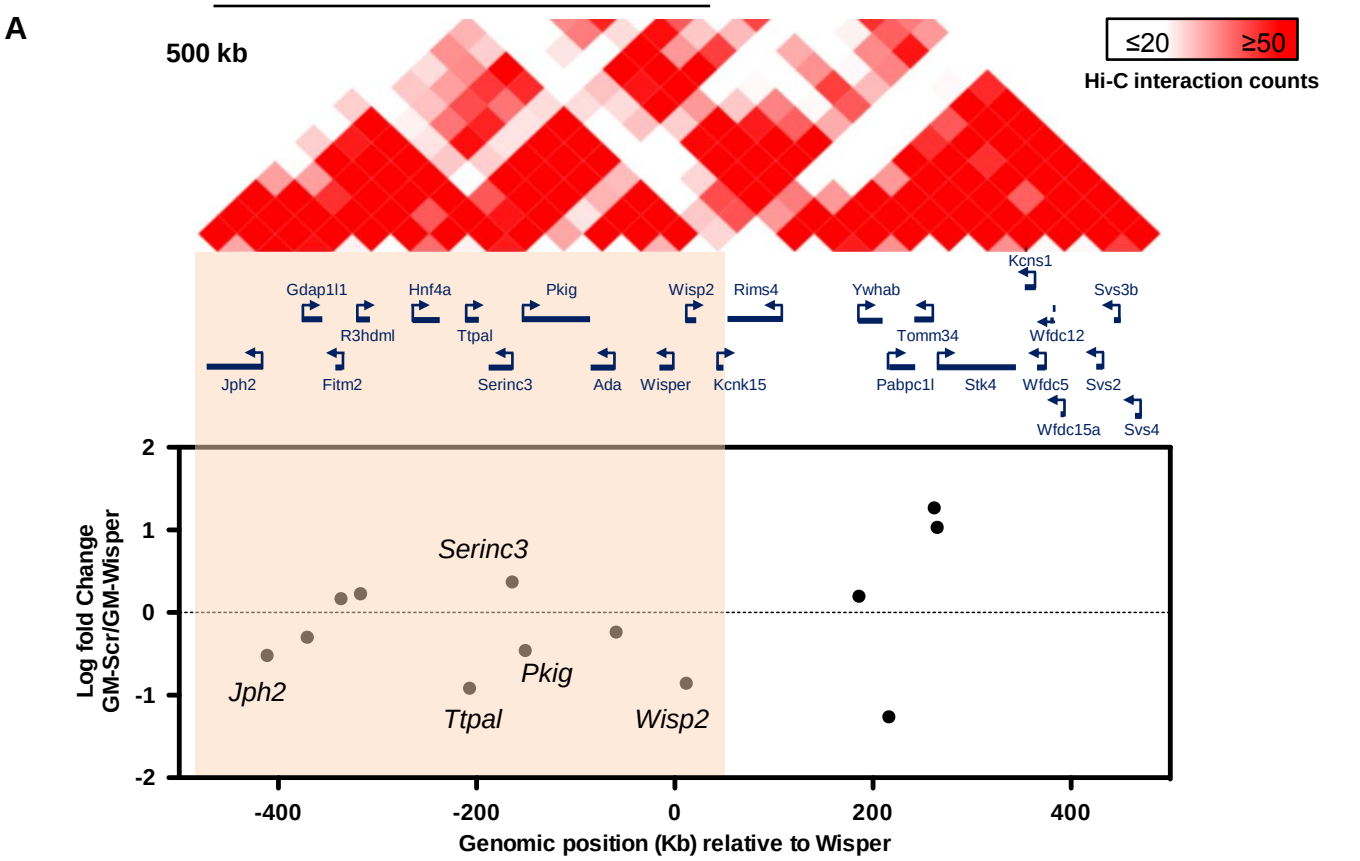

A
Wisper
Wisp2
Tgfb2
Fn1
Col1a1
Col3a1
aSma
Rel. expression (vs. P1)
P < 0.001
P = 0.075
P = 0.043
P1 P2 P3
B
Rel. expression (vs. GM-Scr)
0.0 0.4 0.8 1.2
P = 0.718
P = 0.001
GM-Scr
GM-Wisper
C
None
GM-Scr
GM-Wisper
α-SMA
42 kDa
Ponceau
α-SMA
Rel. protein expression (% control; None)
P = 0.004
P = 0.307
P = 0.011
D
Rel. expression (vs. GM-Scr)
0.0 0.5 1.0 1.5
Wisper
Nppa
Nppb
Myh6/Myh7
P = 0.003
P = 0.090
P = 0.517
P = 0.214
GM-Scr
GM-Wisper
α-actinin
100μm
10μm
n° cells / field
P = 0.642
P < 0.001
CMs
non Myocytes
CM surface Area (mm²)
P = 0.313

Figure S3. Effects of *Wisper* knockdown in neonatal CFs and CMs.

(A) Gene expression measured by qRT-PCR in neonatal cardiac fibroblasts during differentiation into myofibroblasts. (B) PCG expression measured by qRT-PCR in neonatal cardiac fibroblasts after GapmeR-mediated depletion of *Wisper* (10nM; 48h) (n = 4). Bars represent means normalized to GM-Scr ± SEM. *P values* determined by two-way ANOVA (Fisher's test). (C) α-SMA protein quantification by Western blotting in neonatal cardiac fibroblasts. Cells were kept untreated (None) or transfected with scrambled GapmeRs (GM-Scr) or GapmeRs targeting *Wisper* (GM-*Wisper*). Bars represent mean normalized to protein detected by Ponceau staining ± SEM ($n \geq 5$). Graph shows mean normalized to untreated cells. *P values* determined by by one-way ANOVA. (D) Left panel: *Wisper* and stress marker expression measured by qRT-PCR upon GapmeR-induced *Wisper* depletion (10nM, 48h; n = 3); Central panel: Immunohistochemistry of GapmeR-treated cardiomyocytes (α-actinin: green; DAPI: blue). Right panel: Quantification of α-actinin-positive cardiomyocytes and α-actinin-negative non-myocytes following GM-*Wisper* transfection. Cardiomyocyte surface area calculated from immunofluorescence images after transfection with GM-Scr or GM-*Wisper* (n = 5). Bars represent means normalized to GM-Scr. *P values* determined by two-way ANOVA (Fisher's test).


A
500 kb
≤20
≥50
Hi-C interaction counts
Gdap1l1
R3hdml
Hnf4a
Ttpal
Pkig
Wisp2
Rims4
Ywhab
Kcns1
Svs3b
Tomm34
Wfdc12
Jph2
Fitm2
Serinc3
Ada
Wisper
Kcnk15
Pabpc1l
Stk4
Wfdc5
Svs2
Wfdc15a
Svs4
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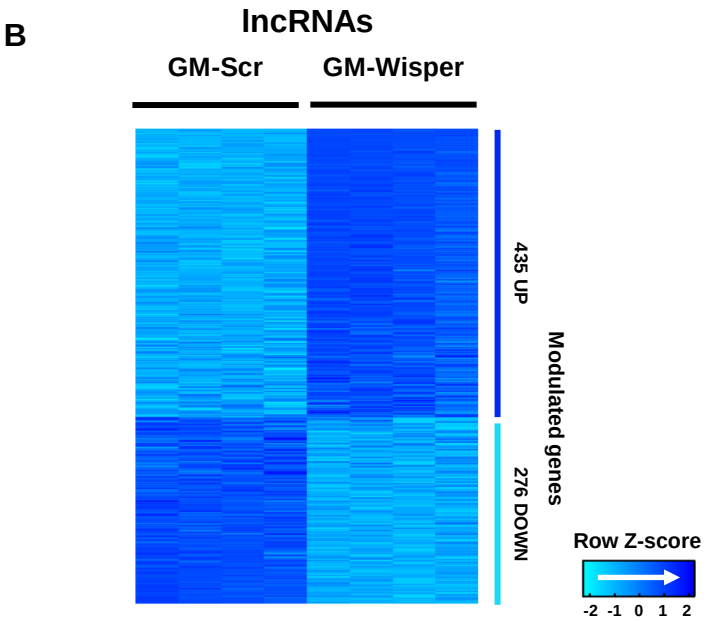
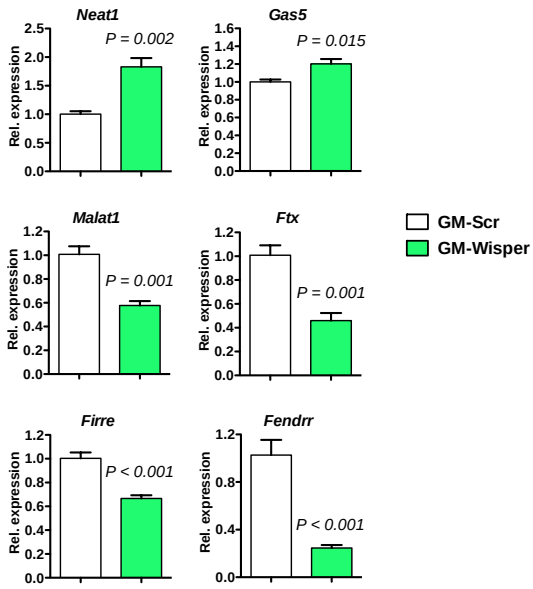
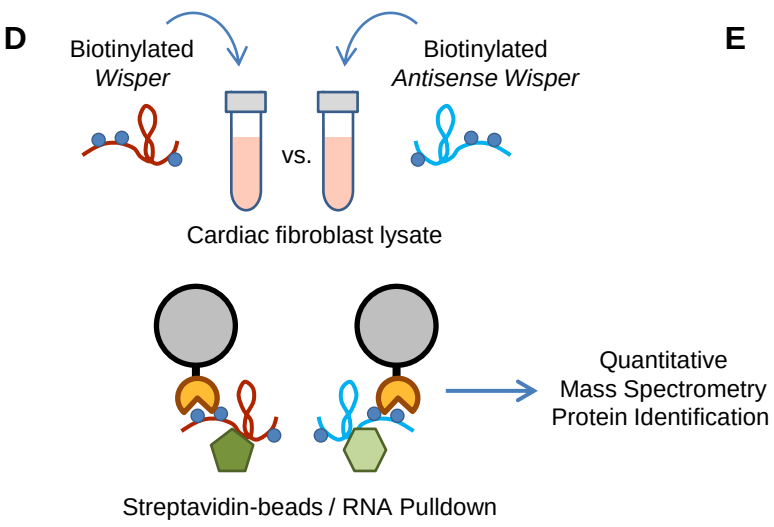
Serinc3
Jph2
Ttpal
Pkig
Wisp2
Genomic position (Kb) relative to Wisper


B
lncRNAs
GM-Scr
GM-Wisper
435 UP
276 DOWN
Modulated genes
Row Z-score


C
Neat1
P = 0.002
Gas5
P = 0.015
Malat1
P = 0.001
Ftx
P = 0.001
Firre
P < 0.001
Fendrr
P < 0.001
Rel. expression
GM-Scr
GM-Wisper


D
Biotinylated Wisper
Biotinylated Antisense Wisper
vs.
Cardiac fibroblast lysate
Quantitative Mass Spectrometry Protein Identification
Streptavidin-beads / RNA Pulldown

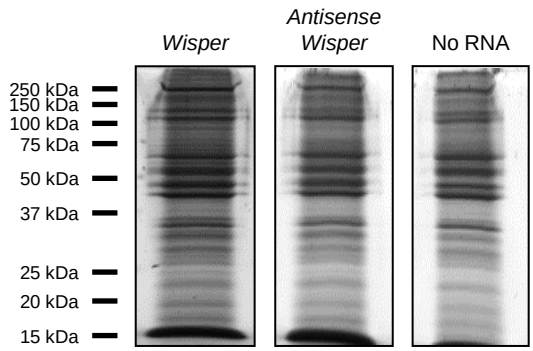

E
Wisper
Antisense Wisper
No RNA
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa

Figure S4. TAD centered on the murine *Wisper* locus; long noncoding transcriptome after Wisper knockdown in fibroblasts; *Wisper* pulldown assay.

(A) Upper panel: Hi-C interaction density heatmap of a genomic region; Lower panel: Intra-TAD transcriptional changes in gene expression upon *Wisper* depletion in adult cardiac fibroblasts. RNA sequencing data; significantly modulated genes are indicated (n = 4). (B) Hierarchical clustering of lncRNAs differentially expressed in adult CFs following transfection with GM-*Wisper* or GM-Scr assessed by RNA-Seq (fold change >2 and *adjusted P<0.05*). (C) Expression of specific lncRNAs assessed by RNA-Seq in adult cardiac fibroblasts after transfection with GM-Wisper or GM-Scr. (D) Schematic of the RNA pulldown assay. (E) Pulldown fractions, reduced by alkylation and digested by trypsin; Coomassie staining.

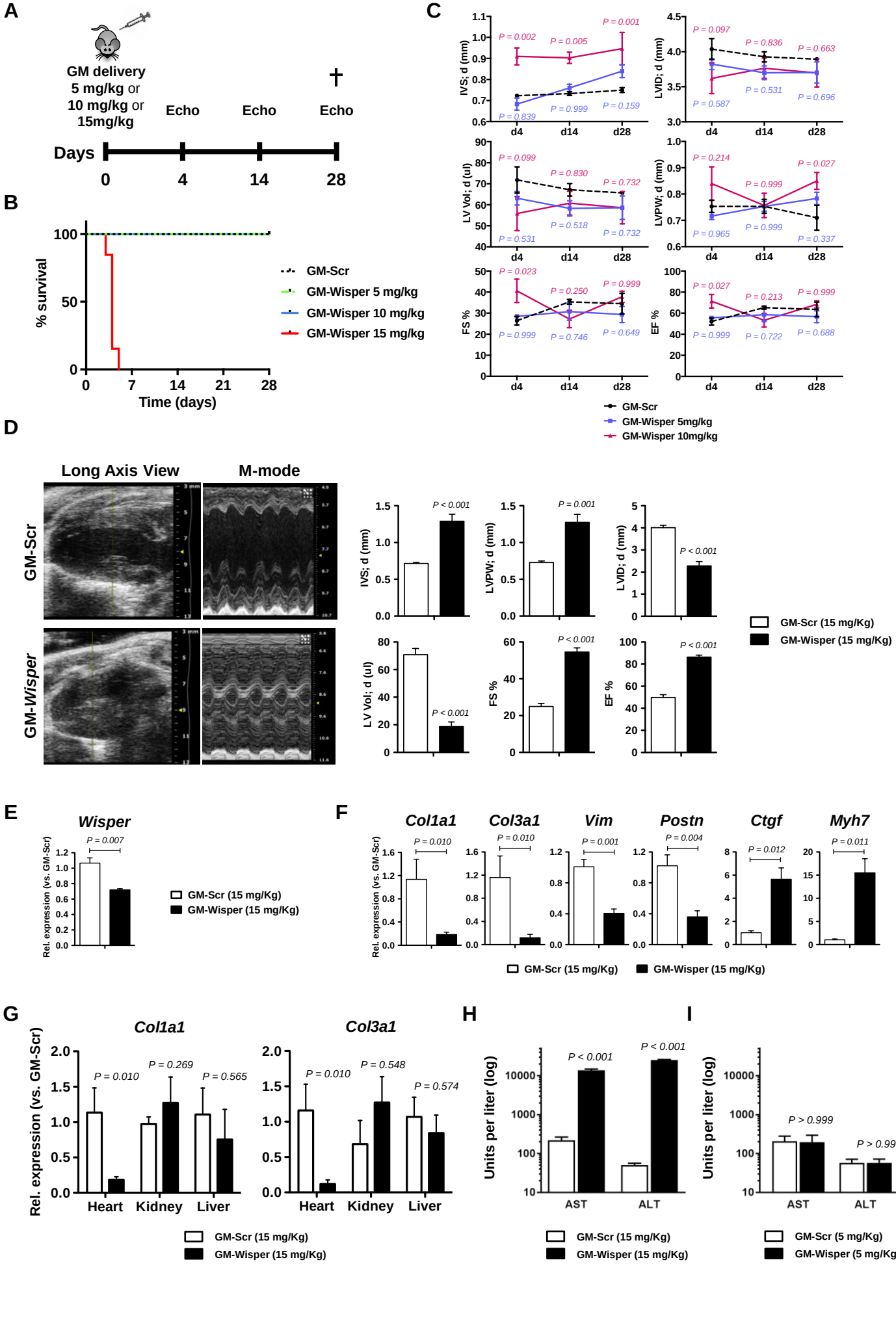

A
GM delivery
5 mg/kg or
10 mg/kg or
15mg/kg
Echo
Echo
Echo
Days
0
4
14
28
B
% survival
Time (days)
GM-Scr
GM-Wisper 5 mg/kg
GM-Wisper 10 mg/kg
GM-Wisper 15 mg/kg
C
IVS; d (mm)
LVID; d (mm)
LV Vol; d (ul)
LVPW; d (mm)
FS %
EF %
d4
d14
d28
GM-Scr
GM-Wisper 5mg/kg
GM-Wisper 10mg/kg
D
Long Axis View
M-mode
GM-Scr
GM-Wisper
GM-Scr (15 mg/Kg)
GM-Wisper (15 mg/Kg)
E
Wisper
Rel. expression (vs. GM-Scr)
P = 0.007
F
Col1a1
Col3a1
Vim
Postn
Ctgf
Myh7
P = 0.010
P = 0.001
P = 0.004
P = 0.012
P = 0.011
G
Col1a1
Col3a1
P = 0.010
P = 0.269
P = 0.565
P = 0.548
P = 0.574
Heart
Kidney
Liver
H
Units per liter (log)
AST
ALT
I
P > 0.999
GM-Scr (5 mg/Kg)
GM-Wisper (5 mg/Kg)

Figure S5. Dose-response analysis after injection of GapmeRs targeting *Wisper* in vivo. (A) Overview of the experimental setup using different doses of GapmeRs targeting *Wisper* (GM-Wisper) or scrambled GapmeRs (GM-Scr). (B) Animal survival following GapmeR injection. (C). Echocardiographic parameters following injection of different doses of GM-Wisper or GM-Scr. Graphs show means ± SEM (n = 3). *P values* calculated by two-way ANOVA (Bonferroni's test). (D) Echocardiographic parameters 4 days after injection of GapmeR (15 mg/kg). Long axis view of control and GM-Wisper-injected hearts. Bars represent mean ± SEM (n = 5). *P values* vs. GM-Scr determined by Student's *t* test. (E) *Wisper* expression in adult cardiac fibroblasts isolated 2 days after GapmeR injection (15 mg/kg). Bars represent mean ± SEM (n = 3). *P values* vs. GM-Scr determined by Student's *t* test. (F) Expression of fibrotic/stress markers measured by qRT-PCR in the heart of mice injected with GapmeRs (15 mg/kg). Bars represent mean ± SEM (n = 4). *P values* vs. GM-Scr determined by Student's *t* test. (G) Collagen expression measured in different tissues after GapmeR injection (15 mg/kg). Bars represent mean ± SEM ($n \geq 3$). *P values* vs. GM-Scr determined by two-way ANOVA test. (H and I) Levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) measured in the plasma of mice injected with 15 mg/kg (H) or 5 mg/kg GapmeRs (I) GM-Scr: white; GM-*Wisper*: black). Bars represent mean ± SEM (GM-Scr, n = 3; GM-*Wisper*, n = 4). *P values* vs. GM-Scr determined by two-way ANOVA test (Bonferroni's test).

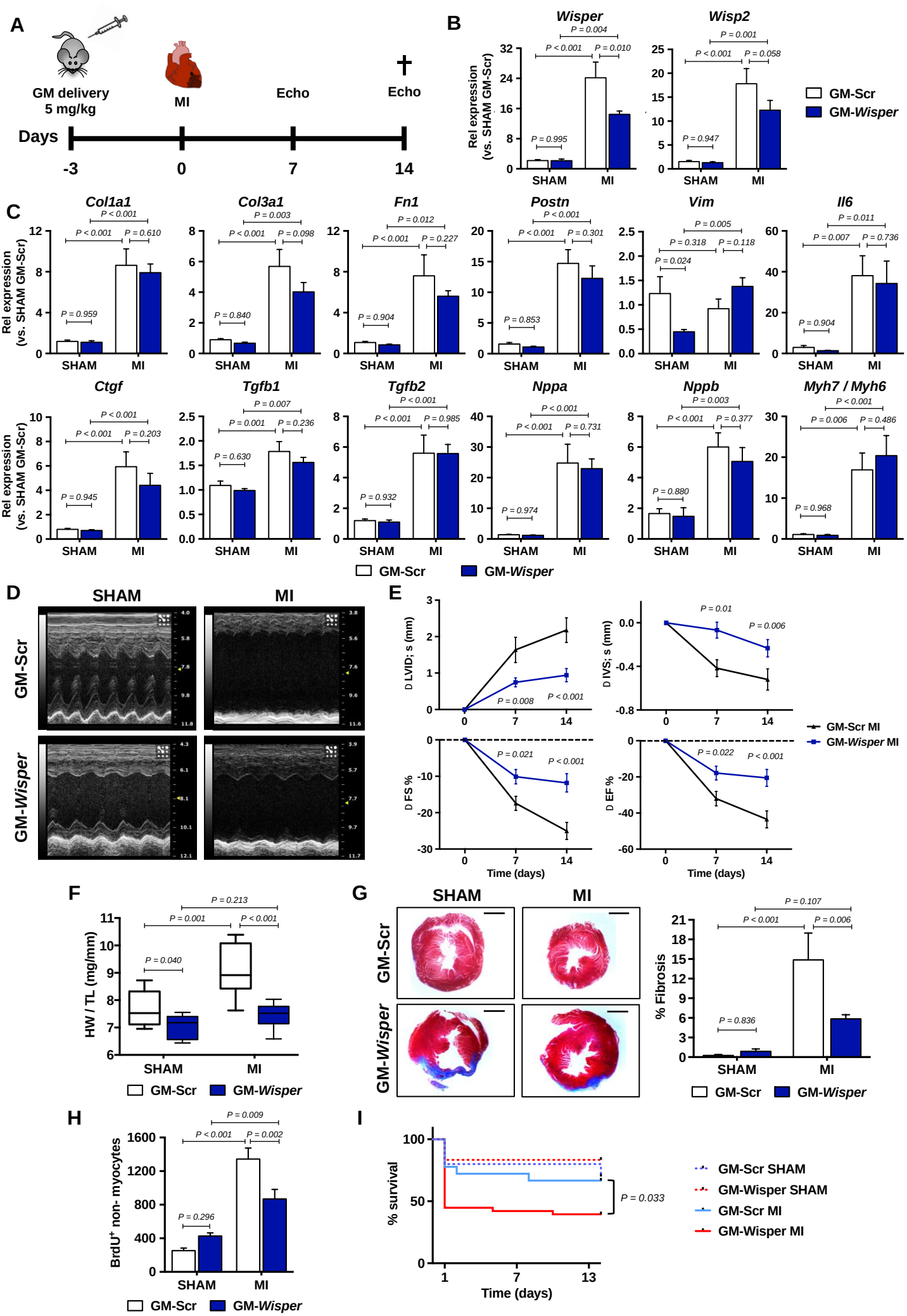
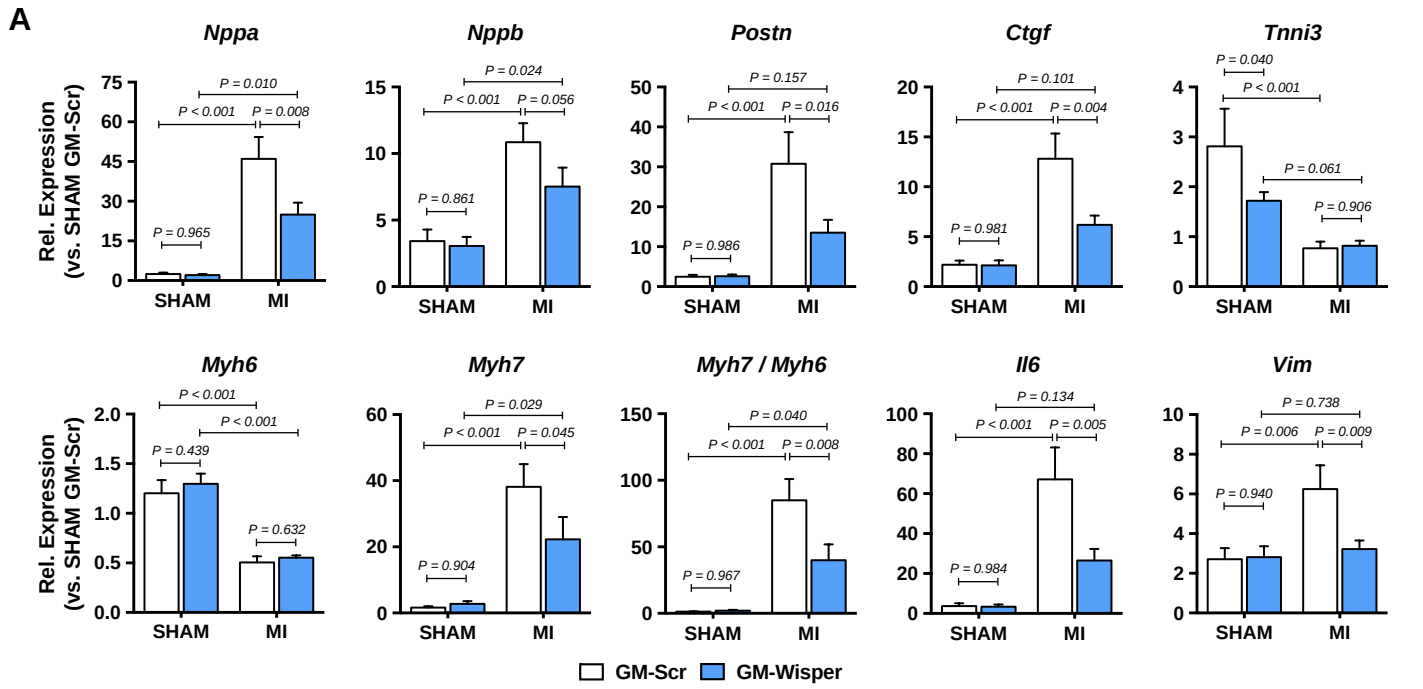
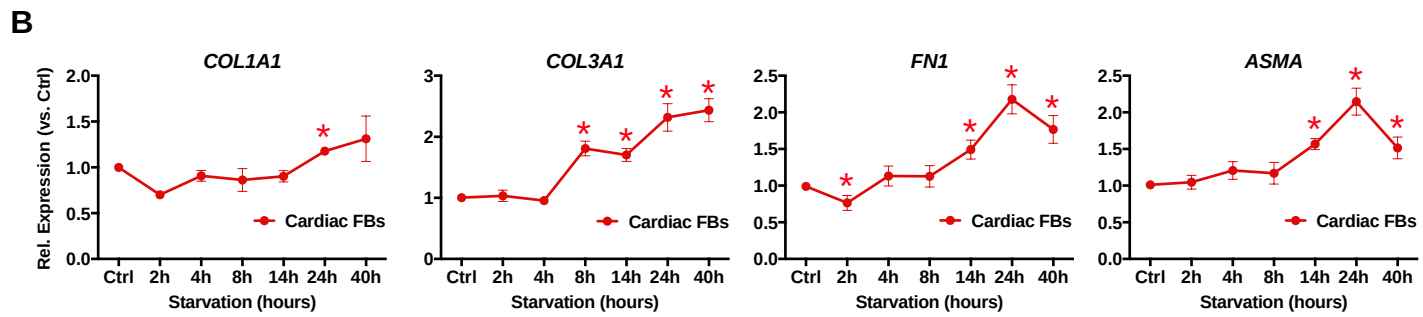

A
GM delivery
5 mg/kg
MI
Echo
Echo
Days
-3
0
7
14
B
Wisper
Wisp2
Rel expression
(vs. SHAM GM-Scr)
P = 0.004
P < 0.001
P = 0.010
P = 0.995
P = 0.001
P < 0.001
P = 0.058
P = 0.947
SHAM
MI
GM-Scr
GM-Wisper
C
Col1a1
Col3a1
Fn1
Postn
Vim
Il6
Ctgf
Tgfb1
Tgfb2
Nppa
Nppb
Myh7 / Myh6
Rel expression
(vs. SHAM GM-Scr)
P < 0.001
P = 0.610
P = 0.959
P = 0.003
P = 0.098
P = 0.840
P = 0.012
P = 0.227
P = 0.904
P = 0.301
P = 0.853
P = 0.005
P = 0.318
P = 0.118
P = 0.024
P = 0.011
P = 0.007
P = 0.736
P = 0.904
P = 0.203
P = 0.945
P = 0.007
P = 0.236
P = 0.630
P = 0.985
P = 0.932
P = 0.731
P = 0.974
P = 0.377
P = 0.880
P = 0.486
P = 0.968
SHAM
MI
GM-Scr
GM-Wisper
D
SHAM
MI
GM-Scr
GM-Wisper
E
D LVID; s (mm)
D IVS; s (mm)
D FS %
D EF %
P = 0.01
P = 0.006
P = 0.008
P < 0.001
P = 0.021
P = 0.022
Time (days)
GM-Scr MI
GM-Wisper MI
F
HW / TL (mg/mm)
P = 0.213
P = 0.001
P < 0.001
P = 0.040
SHAM
MI
GM-Scr
GM-Wisper
G
SHAM
MI
GM-Scr
GM-Wisper
% Fibrosis
P = 0.107
P < 0.001
P = 0.006
P = 0.836
SHAM
MI
GM-Scr
GM-Wisper
H
BrdU+ non- myocytes
P = 0.009
P < 0.001
P = 0.002
P = 0.296
SHAM
MI
GM-Scr
GM-Wisper
I
% survival
P = 0.033
Time (days)
GM-Scr SHAM
GM-Wisper SHAM
GM-Scr MI
GM-Wisper MI

Figure S6. Preventative *Wisper* depletion inhibits cardiac fibrosis and improves function. (A) Overview of the experimental setup for the preventative inhibition of *Wisper*. GapmeR targeting *Wisper* (GM-*Wisper*) or scrambled GapmeRs (GM-Scr) were injected 3 days before surgery at a dose of 5 mg/kg. Two weeks thereafter, mice were sacrificed and tissues collected. (B and C) *Wisper* expression (B) and fibrotic/stress PCG expression (C) were measured by qRT-PCR in sham-operated and MI mice. GM-Scr: white; GM-*Wisper*: blue (sham n = 6; MI n = 8) (D) M mode images of the left ventricle of control and GM-Wisper-injected hearts. (E) Echocardiographic assessment of cardiac dimension (LVID; IVS) and function (FS%; EF%). Graphs show mean values normalized to the average value of the sham-operated group ± SEM. (sham n = 6; MI n = 8) (F) Heart weight (HW) to tibial length (TL) ratio following GapmeR injection ($n \geq 6$). (G) Quantification of fibrosis by Masson's trichrome staining on transversal ventricle sections. Fibrosis was measured using ImageJ (n = 6). (H) Quantification of BrdU-positive non-myocyte cells after GapmeR injection in sham-operated and MI mice. Mice received BrdU 3 days before GapmeR injection and were analyzed 2 weeks after MI (n = 6). (I) Survival after preventative GapmeR injection. *P values* calculated by Log-rank (Mantel-Cox) test vs. the GM-Scr MI group. In (B), (C) and (G) bars represent means normalized to GM-Scr sham ± SEM. In (B), (C), (E), (F), (G) and (H) *P values* were calculated by two-way ANOVA test.


A
Nppa
Nppb
Postn
Ctgf
Tnni3
Myh6
Myh7
Myh7 / Myh6
Il6
Vim
Rel. Expression (vs. SHAM GM-Scr)
SHAM
MI
GM-Scr
GM-Wisper


B
COL1A1
COL3A1
FN1
ASMA
Rel. Expression (vs. Ctrl)
Cardiac FBs
Ctrl
2h
4h
8h
14h
24h
40h
Starvation (hours)

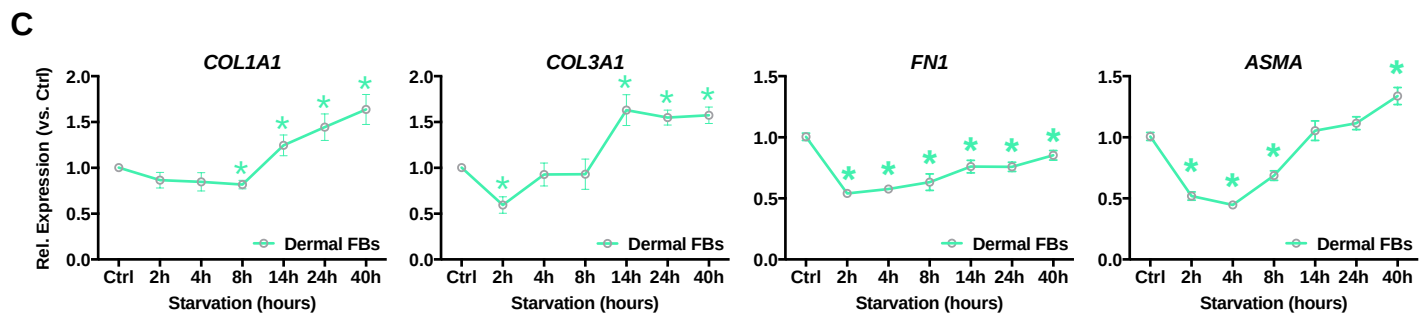

C
COL1A1
COL3A1
FN1
ASMA
Rel. Expression (vs. Ctrl)
Dermal FBs
Ctrl
2h
4h
8h
14h
24h
40h
Starvation (hours)

Figure S7. Expression of cardiac markers of stress after therapeutic depletion of *Wisper* in the infarcted heart; expression of myofibroblast genes in differentiating cardiac and dermal human fibroblasts. (A) RNA expression level of fibrotic/stress cardiac markers measured by qRT-PCR following injection of GapmeRs targeting Wisper (GM-Wisper, light blue) or scrambled GapmeRs (GM-Scr, white) in sham-operated and MI mice. Bars represent means normalized to GM-Scr Sham ± SEM. *P values* were calculated by two-way ANOVA (Fisher's test). (B and C) Time course of *COL1A1, COL3A1, FN1* and *aSMA* expression after induction of differentiation by serum starvation in human cardiac (B) and dermal fibroblasts (C). Graphs show means normalized to control ± SEM (n = 6 to 14). *P values* vs. control determined by one-way ANOVA. * $P < 0.05$.

Table S1. Echocardiographic parameters measured 1, 7, 14, and 28 days after MI.

	1d post-MI					7d post-MI				
	Sham (n=8)		MI (n=7)		Sham vs. MI	Sham (n=7)		MI (n=6)		Sham vs. MI
	Mean	St. Dev.	Mean	St. Dev.	*P* value	Mean	St. Dev.	Mean	St. Dev.	*P* value
Body weight (g)	22.743	1.858	22.746	1.340	0.997035	26.136	2.074	23.662	2.428	0.0728147
Heart rate	515.595	33.521	497.674	44.375	0.389669	503.325	48.790	472.209	44.553	0.2582581
IVS;d (mm)	0.690	0.023	0.670	0.062	0.405440	0.719	0.025	0.542	0.222	0.0582405
IVS;s (mm)	1.062	0.055	0.854	0.145	0.002338	1.050	0.107	0.624	0.281	0.0033832
LVID;d (mm)	3.596	0.261	3.963	0.384	0.047210	3.948	0.315	5.435	1.409	0.0194847
LVID;s (mm)	2.380	0.359	3.301	0.607	0.003016	2.882	0.408	4.555	1.149	0.0048123
LVPW;d (mm)	0.669	0.018	0.667	0.126	0.957602	0.684	0.030	0.674	0.239	0.9130412
LVPW;s (mm)	1.016	0.117	0.885	0.199	0.136539	0.957	0.076	0.821	0.217	0.1477382
LVID Trace (CO) (ml/min)	16.923	2.229	11.618	3.595	0.004015	17.641	2.135	11.229	3.050	0.0009818
LVID Trace (SV) (ul)	33.036	4.126	23.789	7.454	0.009697	36.457	5.790	23.438	6.085	0.0022759
%FS	34.106	5.977	17.238	8.188	0.000497	27.356	5.588	8.732	4.875	0.0000548
%EF	63.516	8.330	35.687	15.130	0.000601	53.362	8.604	18.944	10.072	0.0000360
delta FS%	n.m.	n.m.	-16.868	8.188	0.000497	n.m.	n.m.	-18.624	4.875	0.0000548
delta EF%	n.m.	n.m.	-27.830	15.130	0.000601	n.m.	n.m.	-34.417	10.072	0.0000360
LV Mass (mg)	79.770	9.788	90.712	15.384	0.119376	97.692	11.324	142.142	67.594	0.1125914
LV Vol;d (ul)	54.970	9.292	69.292	15.217	0.043631	70.591	11.801	152.439	88.564	0.0329385
LV Vol;s (ul)	20.295	6.771	46.491	19.503	0.003378	33.585	9.774	129.475	85.078	0.0124903
LA MI trace (mm)	0.000	0.000	5.549	2.094	4.266E-06	0.000	0.000	7.025	1.474	6.431E-08
SA MI trace (mm)	0.000	0.000	3.621	1.331	3.221E-06	0.000	0.000	5.851	1.073	1.591E-08
LV diastole area LA (mm2)	17.880	1.643	22.849	2.737	8.132E-04	22.390	1.831	32.374	12.810	6.464E-02
LV systole area LA (mm2)	11.467	2.432	18.960	3.748	4.494E-04	15.584	2.247	28.318	12.468	2.174E-02
LV diastole area SA (mm2)	8.176	1.829	11.096	3.275	4.901E-02	11.121	1.705	23.541	12.332	2.240E-02
LV systole area SA (mm2)	3.772	1.908	8.151	3.528	9.334E-03	6.555	1.432	20.173	11.313	8.778E-03
% area shortening LA	36.349	9.605	17.708	7.323	1.089E-03	30.530	6.606	13.797	6.109	6.390E-04
% area shortening SA	55.513	14.044	29.019	14.858	3.566E-03	41.386	6.790	15.431	7.732	4.745E-05
LV mass/BW echo	3.511	0.368	3.973	0.501	6.103E-02	3.733	0.287	6.250	3.619	9.176E-02

	14d post-MI					28d post-MI				
	Sham (n=7)		MI (n=8)		Sham vs. MI	Sham (n=6)		MI (n=10)		Sham vs. MI
	Mean	St. Dev.	Mean	St. Dev.	*P* value	Mean	St. Dev.	Mean	St. Dev.	*P* value
Body weight (g)	28.131	1.262	28.488	1.462	0.6247692	28.182	28.182	28.918	1.986	0.4322619
Heart rate	539.247	36.690	516.350	91.303	0.5466856	579.005	579.005	531.402	43.156	0.0619227
IVS;d (mm)	0.761	0.087	0.550	0.223	0.0349765	0.821	0.821	0.493	0.112	0.0001187
IVS;s (mm)	1.127	0.143	0.590	0.276	0.0004796	1.276	1.276	0.572	0.190	4.590E-06
LVID;d (mm)	3.805	0.153	5.147	0.740	0.0004233	3.761	3.761	5.654	0.678	1.373E-05
LVID;s (mm)	2.480	0.250	4.669	0.953	0.0000546	2.236	2.236	5.200	0.795	8.177E-07
LVPW;d (mm)	0.758	0.073	0.574	0.329	0.1725612	0.747	0.747	0.684	0.333	0.657863
LVPW;s (mm)	1.208	0.092	0.726	0.468	0.0191913	1.217	1.217	0.878	0.432	0.091369
LVID Trace (CO) (ml/min)	22.247	2.935	12.709	5.822	0.0017940	24.503	24.503	13.956	5.536	0.002321
LVID Trace (SV) (ul)	40.261	5.624	25.976	12.946	0.0183477	43.668	43.668	27.762	11.813	0.010867
%FS	34.874	5.177	9.851	6.566	0.0000019	40.876	40.876	8.292	4.229	4.513E-08
%EF	64.472	6.787	20.986	13.355	0.0000031	71.605	71.605	17.922	8.692	2.388E-08
delta FS%	n.m.	n.m.	-25.022	6.566	0.0000019	n.m.	n.m.	-32.584	4.229	4.513E-08
delta EF%	n.m.	n.m.	-43.486	13.355	0.0000031	n.m.	n.m.	-53.684	8.692	2.388E-08
LV Mass (mg)	101.447	14.863	110.968	29.555	0.4556725	101.666	101.666	140.861	36.660	0.023029
LV Vol;d (ul)	62.340	5.938	129.795	44.157	0.0015362	60.843	60.843	159.988	44.528	0.000109
LV Vol;s (ul)	22.280	5.579	106.225	51.287	0.0008826	17.840	17.840	133.449	47.820	4.708E-05
LA MI trace (mm)	0.000	0.000	8.209	2.193	2.131E-07	0.000	0.000	9.751	1.791	2.877E-09
SA MI trace (mm)	0.000	0.000	4.693	3.066	1.429E-03	0.000	0.000	7.240	2.249	1.903E-06
LV diastole area LA (mm2)	23.364	2.443	32.889	5.216	7.031E-04	22.433	2.245	37.881	5.414	1.220E-05
LV systole area LA (mm2)	16.292	3.380	28.243	5.720	3.309E-04	12.623	3.409	33.223	5.208	5.958E-07
LV diastole area SA (mm2)	10.074	0.825	18.695	7.457	9.690E-03	10.175	2.120	24.106	6.017	9.219E-05
LV systole area SA (mm2)	3.609	0.775	15.810	7.562	9.839E-04	3.374	1.078	20.771	5.954	6.304E-06
% area shortening LA	30.651	9.572	14.569	5.412	1.306E-03	43.964	11.863	12.302	4.206	1.810E-06
% area shortening SA	64.268	6.042	17.862	10.971	1.969E-07	67.314	5.571	14.595	4.384	5.243E-12
LV mass/BW echo	3.612	0.538	3.889	0.971	5.146E-01	3.620	0.393	4.938	1.507	5.708E-02

n.m. = not measurable

Table S2. **Echocardiographic parameters measured 4, 14, and 28 in untouched mice injected with 5, 10, or 15 mg/kg of GM-Scrambled (control) or GM-*Wisper* (Wisper).**

	day 4				
Gapmer against	Control	Wisper	Wisper		
concentration	15 mg/kg	5 mg/kg	10 mg/kg	Ctrl / Wisper 5mg/kg	Ctrl / Wisper 10mg/kg
n =	n=3	n=3	n=3	p value	
Body weight (g)	28.097	25.287	26.097	0.1236	0.2005
Heart rate (Beats/min)	516.874	432.807	458.521	0.2223	0.4654
IVS;d (mm)	0.720	0.680	0.914	0.2328	0.0083
IVS;s (mm)	1.162	1.059	1.450	0.3532	0.2439
LVID;d (mm)	4.036	3.826	3.620	0.2814	0.1915
LVID;s (mm)	2.969	2.736	2.160	0.1578	0.0635
LVPW;d (mm)	0.752	0.715	0.838	0.2428	0.2757
LVPW;s (mm)	0.974	0.957	1.263	0.8463	0.2009
% FS	26.431	28.489	40.616	0.3834	0.0730
%EF	52.091	55.613	71.410	0.3509	0.0545
LV Mass (mg)	106.971	91.905	108.271	0.1403	0.9076
LV Vol;d (µl)	71.813	63.086	55.849	0.2800	0.1939
LV Vol;s (µl)	34.384	27.992	16.533	0.1502	0.0536

	day 14				
Gapmer against	Control	Wisper	Wisper		
concentration	15 mg/kg	5 mg/kg	10 mg/kg	Ctrl / Wisper 5mg/kg	Ctrl / Wisper 10mg/kg
n =	n=3	n=3	n=3	p value	
Body weight (g)	28.937	26.310	25.767	0.1165	0.0765
Heart rate (Beats/min)	570.370	505.002	469.117	0.3395	0.0274
IVS;d (mm)	0.734	0.761	0.905	0.2451	0.0053
IVS;s (mm)	1.236	1.096	1.254	0.1415	0.9206
LVID;d (mm)	3.926	3.700	3.762	0.1393	0.3696
LVID;s (mm)	2.536	2.570	2.748	0.8814	0.4436
LVPW;d (mm)	0.752	0.756	0.754	0.9021	0.9728
LVPW;s (mm)	1.219	1.046	1.042	0.1786	0.2570
% FS	35.365	30.734	27.215	0.3374	0.1299
%EF	65.291	58.650	53.263	0.3594	0.1419
LV Mass (mg)	101.650	96.684	110.851	0.3877	0.1690
LV Vol;d (µl)	67.127	58.276	60.792	0.1313	0.3770
LV Vol;s (µl)	23.230	24.520	29.027	0.8136	0.4153

	day 28				
Gapmer against	Control	Wisper	Wisper		
concentration	15 mg/kg	5 mg/kg	10 mg/kg	Ctrl / Wisper 5mg/kg	Ctrl / Wisper 10mg/kg
n =	n=3	n=3	n=3	p value	
Body weight (g)	30.060	27.393	28.447	0.1405	0.3453
Heart rate (Beats/min)	548.942	532.581	548.942	0.0891	1.0000
IVS;d (mm)	0.749	0.840	0.947	0.0458	0.0337
IVS;s (mm)	1.237	1.245	1.384	0.9299	0.2329
LVID;d (mm)	3.892	3.704	3.697	0.2782	0.3837
LVID;s (mm)	2.547	2.624	2.308	0.8024	0.4383
LVPW;d (mm)	0.712	0.783	0.849	0.2475	0.0780
LVPW;s (mm)	1.135	1.043	1.204	0.6457	0.7340
% FS	34.529	29.395	37.761	0.4551	0.5936
%EF	63.575	56.688	68.383	0.4843	0.5663
LV Mass (mg)	100.092	105.254	119.919	0.6485	0.4324
LV Vol;d (µl)	65.634	58.593	58.592	0.2817	0.4138
LV Vol;s (µl)	23.821	25.849	18.876	0.7758	0.4599

Table S3. **Echocardiographic parameters measured 7 and 14 days after MI in mice injected with GapmeRs 3 days before MI.**

	Gapmer injected PRE MI - Echo 7 days post-MI							
	(n=8) CTRL sham	(n=8) WISPER sham	(n=8) CTRL mi	(n=8) WISPER mi	CTRL sham / WISPER sham	CTRL sham / CTRL mi	WISPER sham / WISPER mi	CTRL mi / WISPER mi
	Average				p value			
Body weight (g)	27.449	27.566	27.403	26.633	0.88927	0.91670	0.24134	0.32804
Heart rate	491.863	464.644	465.295	482.723	0.10584	0.38772	0.45453	0.65074
IVS;d (mm)	0.767	0.735	0.625	0.764	0.43587	0.07859	0.67218	0.15433
IVS;s (mm)	1.120	1.008	0.683	0.941	0.10777	0.00031	0.41082	0.02824
LVID;d (mm)	3.992	3.926	5.012	4.292	0.64335	0.00387	0.00567	0.02871
LVID;s (mm)	2.906	2.850	4.551	3.597	0.67579	0.00049	0.00039	0.02121
LVPW;d (mm)	0.734	0.713	0.601	0.777	0.66626	0.22839	0.10985	0.10543
LVPW;s (mm)	1.050	0.981	0.756	0.987	0.35265	0.02654	0.92179	0.06904
Cardiac Output (ml/min)	18.859	17.442	11.009	13.608	0.67049	0.00302	0.08332	0.20967
Stroke volume (µl)	38.583	36.678	24.154	28.770	0.71521	0.00160	0.06978	0.25299
%FS	27.292	27.483	9.854	16.286	0.81948	0.00001	0.00091	0.03584
%EF	53.285	53.668	21.248	34.069	0.81099	0.00002	0.00094	0.03498
LV Mass (mg)	106.831	99.125	119.258	125.232	0.46499	0.19519	0.00459	0.60099
LV Vol;d (ul)	70.061	67.312	122.801	83.017	0.64407	0.00671	0.00608	0.03211
LV Vol;s (ul)	33.006	31.402	100.587	55.072	0.66378	0.00213	0.00053	0.02611
LA MI trace (mm)	0.000	0.000	8.805	5.521	0.00000	0.00000	0.00000	0.00935
LA LV lenght (mm)	18.089	17.668	19.588	19.109	0.71454	0.02712	0.01924	0.48950
SA MI trace (mm)	0.000	0.000	4.864	3.607	0.00000	0.00133	0.00001	0.36288
SA LV lenght (mm)	11.923	12.251	15.741	13.671	0.42011	0.00341	0.00170	0.07667
LV diastole area LA (mm2)	25.048	23.979	32.868	28.196	0.62910	0.00315	0.00467	0.06080
LV systole area LA (mm2)	17.292	17.381	28.071	22.329	0.84085	0.00100	0.00013	0.04222
LV diastole area SA (mm2)	11.208	11.746	19.767	14.389	0.47824	0.00570	0.00276	0.05989
LV systole area SA (mm2)	5.365	7.123	16.862	11.793	0.07439	0.00103	0.00023	0.09030
Fractional Area Change LA (%)	31.242	27.100	15.351	20.645	0.34135	0.00074	0.07775	0.14931
Fractional Area Change SA (%)	51.815	39.673	17.059	18.212	0.09316	0.00008	0.00092	0.82199
% MI length LA	0.000	0.000	44.574	28.828	0.00000	0.00000	0.00000	0.00635
% MI length SA	0.000	0.000	28.525	26.268	0.00000	0.00037	0.00001	0.75902
LV mass/BW	3.887	3.594	4.360	4.695	0.37038	0.18040	0.00037	0.41920

	Gapmer injected PRE MI - Echo 14 days post-MI							
	(n=8) CTRL sham	(n=8) WISPER sham	(n=8) CTRL mi	(n=8) WISPER mi	CTRL sham / WISPER sham	CTRL sham / CTRL mi	WISPER sham / WISPER mi	CTRL mi / WISPER mi
					p value			
Body weight (g)	28.131	28.676	28.488	27.620	0.31082	0.49145	0.09819	0.19640
Heart rate	539.247	506.129	516.350	515.824	0.24945	0.42070	0.78162	0.98872
IVS;d (mm)	0.761	0.723	0.550	0.620	0.41391	0.02983	0.08597	0.47046
IVS;s (mm)	1.127	0.999	0.590	0.766	0.12437	0.00031	0.02347	0.18413
LVID;d (mm)	3.805	4.073	5.147	4.562	0.01260	0.00017	0.00315	0.05831
LVID;s (mm)	2.480	2.933	4.669	3.875	0.03446	0.00002	0.00187	0.05630
LVPW;d (mm)	0.758	0.705	0.574	0.662	0.12860	0.14797	0.53525	0.51941
LVPW;s (mm)	1.208	1.007	0.726	0.859	0.01459	0.01569	0.20443	0.49983
Cardiac Output (ml/min)	22.247	20.608	12.709	14.637	0.58444	0.00084	0.10802	0.50446
Stroke volume (µl)	40.261	40.576	25.976	28.962	0.90023	0.01269	0.04682	0.63342
%FS	34.874	28.265	9.851	15.301	0.10817	0.00000	0.00602	0.13259
%EF	64.472	54.085	20.986	31.829	0.07883	0.00000	0.00450	0.12511
LV Mass (mg)	101.447	102.050	110.968	110.544	0.75739	0.36029	0.39572	0.97565
LV Vol;d (ul)	62.340	73.454	129.795	96.061	0.01267	0.00073	0.00287	0.05967
LV Vol;s (ul)	22.280	34.431	106.225	66.427	0.02739	0.00042	0.00134	0.05844
LA MI trace (mm)	0.000	0.000	8.209	6.282	0.00000	0.00000	0.00000	0.05500
LA LV lenght (mm)	17.501	17.258	20.142	18.623	0.52427	0.00146	0.02245	0.03023
SA MI trace (mm)	0.000	0.000	4.693	3.156	0.00000	0.00069	0.00000	0.20409
SA LV lenght (mm)	11.277	12.266	15.268	14.079	0.01603	0.00320	0.00178	0.32613
LV diastole area LA (mm2)	23.364	23.364	32.889	27.690	0.83288	0.00046	0.01933	0.03329
LV systole area LA (mm2)	16.292	15.562	28.243	22.143	0.69639	0.00013	0.00203	0.02784
LV diastole area SA (mm2)	10.074	11.835	18.695	15.219	0.02196	0.00564	0.00271	0.22225
LV systole area SA (mm2)	3.609	6.771	15.810	11.022	0.00743	0.00044	0.01499	0.12270
Fractional Area Change LA (%)	30.651	33.639	14.569	20.418	0.67850	0.00054	0.00153	0.09861
Fractional Area Change SA (%)	64.268	44.057	17.862	28.748	0.00693	0.00000	0.07641	0.10606
% MI length LA	0.000	0.000	40.391	33.747	0.00000	0.00000	0.00000	0.11402
% MI length SA	0.000	0.000	29.031	86.231	0.00000	0.00004	0.19686	0.38532
LV mass/BW	3.612	3.559	3.889	3.988	0.95453	0.42791	0.20003	0.82857

Table S4. Echocardiographic parameters measured 7 and 28 days after MI in mice injected with GapmeRs 2 days and 9 days after MI.

	7 days post-MI							
	(n=6) CTRL sham	(n=6) WISPER sham	(n=9) CTRL mi	(n=9) WISPER mi	CTRL sham / WISPER sham	CTRL sham / CTRL mi	WISPER sham / WISPER mi	CTRL mi / WISPER mi
	Average				p value			
Body weight (g)	26.40	24.15	26.06	24.25	0.05158325905	0.72787141487	0.93653680871	0.10533498193
Heart rate	523.05	410.33	526.24	434.42	0.00730263132	0.92144053565	0.47303877758	0.00683884500
IVS;d (mm)	0.72	0.74	0.51	0.70	0.36934055604	0.00443014917	0.44230809558	0.01237719223
IVS;s (mm)	1.04	1.03	0.53	0.86	0.79589172890	0.00000291509	0.20352796377	0.00788565174
LVID;d (mm)	3.92	3.65	5.31	4.52	0.06429074273	0.00008349795	0.00260574458	0.00776593311
LVID;s (mm)	2.68	2.57	4.87	3.87	0.51431281574	0.00000370856	0.00329455959	0.01232343019
LVPW;d (mm)	0.68	0.71	0.79	0.74	0.14721796457	0.45677431532	0.70302661890	0.67344967386
LVPW;s (mm)	1.06	1.06	0.96	0.93	0.99844489881	0.59543189407	0.22736158300	0.83502750777
Cardiac Output (ml/min)	20.37	12.85	12.59	11.05	0.01961169760	0.01969611734	0.29836555438	0.47028791592
Stroke volume (µl)	37.87	31.82	24.61	25.86	0.08711722668	0.00960931146	0.07259114988	0.74513056305
%FS	31.64	29.74	8.45	15.41	0.53836105335	0.00000078755	0.00539831678	0.07003248976
%EF	59.69	57.57	18.39	31.41	0.63554297792	0.00000041233	0.00401301085	0.06026633875
LV Mass (mg)	99.60	88.29	144.45	126.48	0.09953549207	0.02649296004	0.00100166286	0.26868570963
LV Vol;d (ul)	67.20	56.63	138.00	95.07	0.05816154139	0.00032981263	0.00333170501	0.00841455620
LV Vol;s (ul)	27.09	24.38	113.97	68.88	0.48830456170	0.00006797125	0.00416442802	0.01152369920
LA MI trace (mm)	0.00	0.00	9.05	6.49	0.00000000000	0.00000002969	0.00000269122	0.01280551395
LA LV lenght (mm)	18.51	18.08	22.56	20.95	0.49932568824	0.00000498961	0.00076123536	0.01136277439
SA MI trace (mm)	0.00	0.00	6.77	3.87	0.00000000000	0.00000124170	0.00002415105	0.00253690524
SA LV lenght (mm)	11.49	11.19	16.47	13.96	0.48306058908	0.00002172457	0.00165855903	0.00629952614
LV diastole area LA (mm2)	23.03	21.77	35.22	29.11	0.32862174328	0.00001487783	0.00185090928	0.00661563384
LV systole area LA (mm2)	15.32	14.83	30.77	24.38	0.56440465352	0.00000170768	0.00075143158	0.01218595518
LV diastole area SA (mm2)	10.44	9.97	21.27	15.47	0.54094746602	0.00019148233	0.00327742977	0.01202636252
LV systole area SA (mm2)	4.73	5.21	18.18	12.54	0.57911034229	0.00000494016	0.00052183779	0.00820747320
Fractional Area Change LA (%)	33.43	31.51	12.81	16.77	0.54954444618	0.00000014166	0.00361263696	0.22573326543
Fractional Area Change SA (%)	54.82	48.50	14.27	19.90	0.31218279331	0.00000033812	0.00006835578	0.14330127851
% MI length LA	0.00	0.00	40.01	30.75	0.00000000000	0.00000000817	0.00000105760	0.02755230716
% MI length SA	0.00	0.00	40.77	27.18	0.00000000000	0.00000003964	0.00000464756	0.00450167575
LV mass/BW	3.79	3.66	5.57	5.16	0.62001597394	0.02447207499	0.00002176357	0.51109177567

	28 days post-MI							
	(n=6) CTRL sham	(n=6) WISPER sham	(n=9) CTRL mi	(n=9) WISPER mi	CTRL sham / WISPER sham	CTRL sham / CTRL mi	WISPER sham / WISPER mi	CTRL mi / WISPER mi
					p value			
Body weight (g)	28.18	27.79	28.45	28.96	0.71120661037	0.71408855279	0.38181800297	0.61003852878
Heart rate	579.00	545.59	530.92	534.35	0.25741392365	0.07496517727	0.71339524469	0.89565629413
IVS;d (mm)	0.82	0.72	0.50	0.59	0.10098326585	0.00026642392	0.22695101414	0.34672174764
IVS;s (mm)	1.28	1.09	0.57	0.72	0.04344714196	0.00001119649	0.02722120191	0.26097433112
LVID;d (mm)	3.76	3.82	5.64	4.94	0.64369367973	0.00003684243	0.00007533994	0.02620473360
LVID;s (mm)	2.24	2.40	5.16	4.22	0.45252511164	0.00000267777	0.00002314680	0.01655785688
LVPW;d (mm)	0.75	0.68	0.72	0.78	0.00731042189	0.85002670169	0.39654804018	0.67151773233
LVPW;s (mm)	1.22	1.10	0.92	0.96	0.16484502016	0.14123062591	0.43705308680	0.83731600553
Cardiac Output (ml/min)	24.50	23.93	14.33	19.72	0.82794981294	0.00446234391	0.07944137033	0.04438193768
Stroke volume (µl)	43.67	43.07	28.74	35.10	0.86014444350	0.01903144555	0.05837549396	0.22407072717
%FS	40.88	37.25	8.76	14.95	0.38063333567	0.00000016682	0.00000275060	0.01627921519
%EF	71.61	67.60	18.89	31.25	0.45276597512	0.00000008019	0.00000574455	0.01529745513
LV Mass (mg)	101.67	90.86	145.37	136.41	0.04834833487	0.01215271966	0.00333511698	0.57269185356
LV Vol;d (ul)	60.84	62.87	159.42	116.57	0.67259684371	0.00024596243	0.00034436125	0.03038084117
LV Vol;s (ul)	17.84	20.71	131.63	82.17	0.53810594758	0.00011774115	0.00044998251	0.02370673977
LA MI trace (mm)	0.00	0.00	9.73	7.47	0.00000000000	0.00000001419	0.00000013793	0.01880556204
LA LV lenght (mm)	18.74	17.80	23.07	21.90	0.05439210787	0.00001125316	0.00000021351	0.04561962872
SA MI trace (mm)	0.00	0.00	7.32	4.16	0.00000000000	0.00000471223	0.00003381162	0.00456251201
SA LV lenght (mm)	11.38	11.51	17.51	15.33	0.83914869485	0.00004573384	0.00000330270	0.01856568492
LV diastole area LA (mm2)	22.43	21.57	37.55	32.40	0.45525033954	0.00003275187	0.00001111751	0.03437460217
LV systole area LA (mm2)	12.62	13.67	33.23	27.37	0.49805428585	0.00000192223	0.00000597092	0.02431864057
LV diastole area SA (mm2)	10.17	10.41	23.87	17.82	0.82042161524	0.00021986621	0.00014660728	0.02119013549
LV systole area SA (mm2)	3.37	3.97	20.57	13.81	0.32318172328	0.00001812596	0.00003564711	0.01395682533
Fractional Area Change LA (%)	43.96	36.58	11.57	15.73	0.18477653285	0.00000309884	0.00003740301	0.15363351387
Fractional Area Change SA (%)	67.31	61.80	14.66	23.29	0.17311822254	0.00000000004	0.00000198501	0.03149509332
% MI length LA	0.00	0.00	42.10	33.98	0.00000000000	0.00000000358	0.00000005048	0.03246553200
% MI length SA	0.00	0.00	41.10	26.91	0.00000000000	0.00000002339	0.00002730382	0.00552641172
LV mass/BW	3.62	3.28	5.15	4.71	0.12896926191	0.02518469736	0.00307840966	0.44706670379

Table S5. Characteristics of patient suffering from AOS and developing cardiac fibrosis.

	Fibrosis Group	
	Non Severe	**Severe**
n	11	15
CVF (Avg.)	7.40	26.38
Age (Avg.)	71	71.47
Gender	10 Females; 1 Males	8 Females; 7 Males
Hypertension	8 Yes; 3 No	11 Yes; 4 No
Atrial fibrillation	2 Yes; 9 No	8 Yes; 7 No
BMI (Avg.)	28.95	28.61
SBP (Avg.) (mm Hg)	121.82	118.53
DBP (Avg.) (mm Hg)	71.09	67.67
HR (Avg.) (beats/min)	72.09	77.47
LVMI (Avg.)	125.57	155.15
LVH	5 Yes; 6 No	9 Yes; 6 No
RWT (Avg.)	0.62	0.62
AVAi (Avg.)	0.32	0.39
LVEDD (Avg.)	4.14	4.66
LVESD (Avg.)	2.29	3.11
LVEF (Avg.)	75.44	60.72
DT (Avg.)	290.91	269.13
IVRT (Avg.)	84.00	119.13

BMI, body mass index
SBP, systolic blood pressure
DBP, diastolic blood pressure
HR, heart rate
LVMI, left ventricular mass index (g/m2)
LVH, LV hypertrophy
RWT, relative wall thickness
AVAi, aortic valve area index
LVEDD, LV end-diastolic diameter
LVESD, LV end-systolic diameter
LVEF, left ventricular ejection fraction
DT, deceleration time
IVRT, isovolumic relaxation time

Table S6. List of the primers used in this study.

PRIMER LIST			
Species	**Gene Name**	**Forward primer (5'—3')**	**Reverse primer (5'—3')**
Mouse	Wisper	CTGCTTCTCCAAAAGCCAAG	TAGACGAGCTGCTTCCCAGT
Mouse	Wisp2	GTACCTGGATGGGGAGACCT	TCCTGGCACCTGTATTCTCC
Mouse	XLOC_006255	CAGATCTCTGCCCTCTGGAA	ATTGGGATGACTGGCTGTGT
Mouse	XLOC_015277	TGGGACAGCAGAGCTAAGGT	AGATTCCAGCACGCACTTCT
Mouse	XLOC_015960	GTGATGGAGGCTGAGCTAGG	CAGGAGGAATGCCAAAAGAA
Mouse	XLOC_022878	CTCAGGAGACTGGGAAGC	AACCACTGGCTTTTGACA
Mouse	XLOC_025989	GTACAGTCAGGGCCTCAAGC	CCAGGAAGGAATTTGCAGAC
Mouse	Gapdh	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
Mouse	Tubg1	ATGCCGAGAGAAATCATCACCC	CCGTGGCGAACTCTTCTACG
Mouse	Neat1	TGGCCCCTTTTGTTCATTAGC	TGGAAGGCCATTGTTTCAGG
Mouse	Xist	GGAAAGCCCCAAGTAAAAGG	CCAGGAACCATTCTTGCCTA
Human	WISPER	CCATCTGTGGGACATCTGTG	TGGGGGCTGTGGAGATAGTA
Human	WISP2	ACAGCTGCCGGAACATAAAG	GGTACGCACCTTTGAGAGGA
Species	**Gene Name**	**Taqman probe reference**	**Supplier**
Mouse	Ctgf	Mm_01192931_g1	Applied biosystem
Mouse	Col1a1	Mm_00801666_g1	Applied biosystem
Mouse	Col3a1	Mm_00802331_m1	Applied biosystem
Mouse	Fn1	Mm_00692666_m1	Applied biosystem
Mouse	Vim	Mm_01333430_m1	Applied biosystem
Mouse	Postn	Mm_00450111_m1	Applied biosystem
Mouse	a-SMA	Mm_00725412_s1	Applied biosystem
Mouse	Bcl2	Mm_00477631_m1	Applied biosystem
Mouse	Bax	Mm_00432051_m1	Applied biosystem
Mouse	Myh6	Mm_00440354_m1	Applied biosystem
Mouse	Myh7	Mm_00600555_m1	Applied biosystem
Mouse	Nppa	Mm_01255747_g1	Applied biosystem
Mouse	Nppb	Mm_00435304_g1	Applied biosystem
Mouse	Il6	Mm_00446190_m1	Applied biosystem
Mouse	Tgfb1	Mm_01178820_m1	Applied biosystem
Mouse	Tgfb2	Mm_00436955_m1	Applied biosystem
Mouse	Tnni3	Mm_00437164_m1	Applied biosystem
Mouse	Gapdh	Mm99999915_g1	Applied biosystem
Human	COL1A1	Hs.PT.58.15517795	IDT
Human	COL3A1	Hs.PT.58.40254063	IDT
Human	FN1	Hs.PT.58.21141138	IDT
Human	ASMA	Hs.PT.56a.20825574	IDT
Human	18S ribos. RNA	Hs.PT.39a.222148556.g	IDT
Human	CTGF	Hs00140014m1	Applied biosystem
Human	TGFB1	Hs00998133m1	Applied biosystem

GapmeRs Sequences			
Species	**Gene Name**	**Sequence (5'—3')**	**Supplier**
both	Negative CTRL A	AACACGTCTATACGC	Exiqon
mouse	Mm_Wisper	AGGTGTGCGATAGAG	Exiqon
mouse	Mm_Wisp2	CGCAAGTGCCAAGAGT	Exiqon
human	Hu_WISPER	CAAGAAGCTGGAGTTG	Exiqon