Supplemental Material:

Supplemental Materials and Methods:

Mouse echocardiography. The Vevo2100 imaging system (VisualSonics, Toronto, Canada) was used with 22-55 MHz linear transducer probe (MS550D) was employed for 2-D b-mode and m-mode analysis. Heart rate was maintained at 400-500 bpm via isoflurane anesthesia. The mitral valve leaflet was visualized and its function was assessed at long axis b-mode view by placing the transducer on the left lateral chest wall. End-systolic and end-diastolic LV dimensions and wall thicknesses were measured according to the American Society of Echocardiography guidelines as applied to mice (Henry, 1980). LV wall thickness was measured at the level of intraventricular septum and the posterior wall. LV volume was calculated from Simoson's method of disks and ejection fraction determined from the formula (LV end-diastolic-end-systolic volume)/(LV end-diastolic volume). Offline image analyses were performed using dedicated VisualSonics Vevo2100 1.2.0 software.

Cell culture and WISP treatment of isolated adult rat cells (Myocytes and Fibroblasts). Each isolated cells was cultured in a cell type specific media. Adult rat myocytes were cultured in MEM, minimal essential media (Gibco, Catalog 11095-080) supplemented with butanedione monoxime and insulin-transferrin-selenium. Rat fibroblasts were cultured in DMEM (Gibco, Catalog 11965-092) supplemented with 10% fetal bovine serum (FBS). Rat coronary endothelial cells were cultured in M199 (Gibco, Catalog 11150-059) supplemented with 20% FBS, 250U/ml penicillin, 0.626μg/ml amphotericin, and 250μg/ml streptomycin. Media was changed every 24 hours and all experiments were performed within 72 hours of isolation. Rat myocytes and

fibroblasts were treated with phosphate buffered saline (PBS) or 5ng/ml, 10ng/ml or 20ng/ml of recombinant mouse Wisp-1 (R&D Systems ®, Catalog 1680-WS-050).

Table 1. ***Antibody -Based on Application (Use)***

(Species Reactivity) Antibody ***(H-Human, M-Mouse, R-Rat)***	***USE***	***Company***	***Catalog, Clone, Lot***
(H)Wisp-1, goat polyclonal	IFC/ICC WB	Abcam	Ab ab65943
(M) α-Actinin, monoclonal	IFC/ICC WB	Sigma Aldrich	A7732
(R/M)Wisp-1 (V-19), goat polyclonal	IFC/ICC WB	Santa Cruz Biotech.	sc-8866
(H/M/R)Isolectin GS-IB4-Alexafluor 568	IFC/ICC	Life Technologies	121412
(H/M/R)IntegrinαV (H-75), rabbit polyclonal	IFC/ICC	Santa Cruz Biotech.	sc-10719
(H/M/R)Integrinß5 (H-96) rabbit polyclonal	IFC/ICC	Santa Cruz Biotech.	sc-14010
(H/M/R)PECAM-1 (M-20), goat polyclonal	IFC/ICC WB	Santa Cruz Biotech.	sc-1506
(H/M/R)ATG 5-12 Complex-Alexa Fluor 647, mouse monoclonal IgG2b	IFC/ICC	R&D Systems	IC5294T-100 Clone #603813, Lot:AEYL01170
(H/M)ATG 7, mouse monoclonal IgG1	IFC/ICC WB	R&D Systems	IC6608R-100 Clone #683906, Lot:AEYK0117021
(H/M/R)LC3B, polyclonal rabbit	IFC/ICC WB	Cell Signaling	#2775S
Alexafluor-488 anti-mouse	IFC/ICC	Thermo-Fisher	A-11001
Alexafluor-546 donkey-anti-mouse	IFC/ICC	Thermo-Fisher	A10036
Alexafluor-568 donkey anti-goat,	IFC/ICC	Thermo-Fisher	
(H/M/R)HDAC1 (C-19), goat polyclonal	ChIP	Santa Cruz Biotech.	sc-6298X
(H/M/R)HDAC2 (H-54), rabbit polyclonal	ChIP	Santa Cruz Biotech.	sc-7899X
(H/M/R)HDAC3 (H-99), rabbit polyclonal	ChIP	Santa Cruz Biotech.	sc-11417X
(H/M/R) anti-p300 (H-C20), rabbit polyclonal	ChIP	Santa Cruz Biotech.	sc-585
CBP (A-22) rabbit polyclonal	ChIP	Santa Cruz Biotech.	sc-369
ß-Catenin (E-5) mouse monoclonal	ChIP	Santa Cruz Biotech.	sc-7963
TCF-4(D-4) mouse monoclonal	ChIP	Santa Cruz Biotech.	sc-166699
Normal IgG rabbit Normal IgG goat	ChIP ChIP	Santa Cruz Biotech.	sc-2027 sc-2028

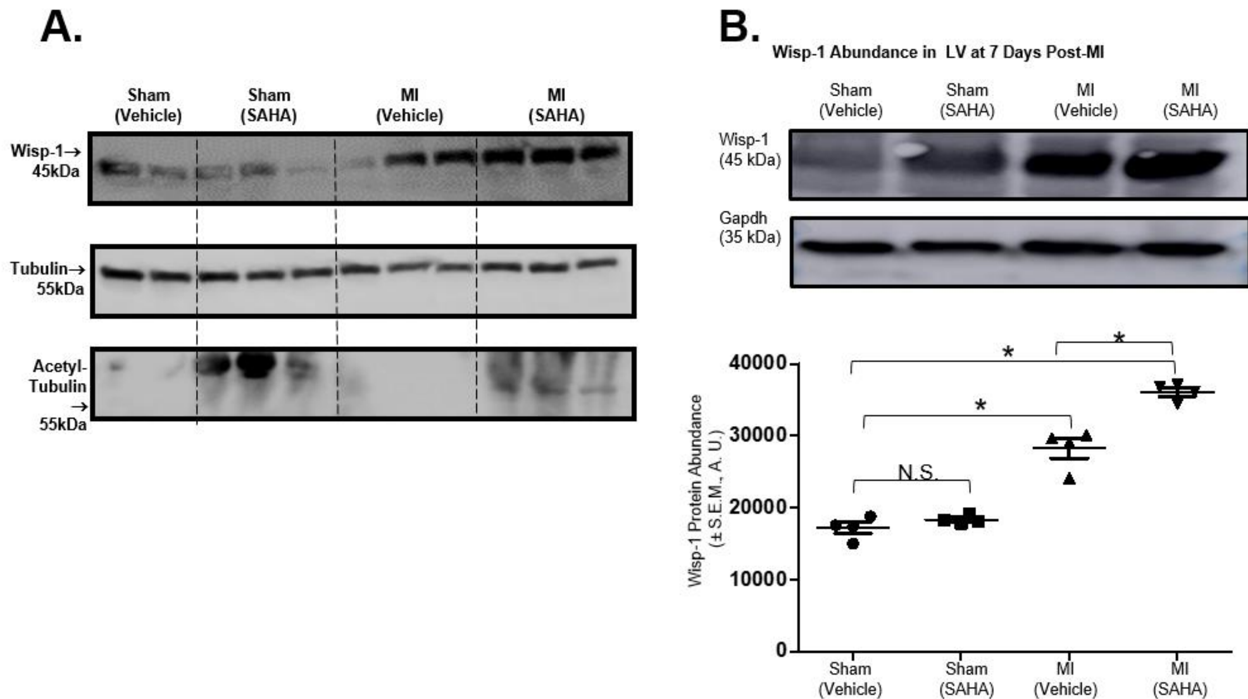
Normal IgG mouse	ChIP	Santa Cruz Biotech.	sc-2025
(H/M) anti-BAD aa 1-21, rabbit polyclonal	WB	R&D Systems	AF819
(H/M/R) anti-Phospho-BAD (pSer 136), rabbit polyclonal	WB	Thermo-Scientific	PA5-35538
(H/M/R) anti-Histone H3-(FL-136), rabbit polyclonal	WB	Santa Cruz Biotech.	sc-10809
(H/M/R) anti-Rac-1 (C-14), rabbit polyclonal	WB	Santa Cruz Biotech.	sc-217
(H/M/R) anti-RhoA (119), rabbit polyclonal	WB	Santa Cruz Biotech.	sc-179
(H/M/R) anti-VEGF (A-20), rabbit polyclonal	WB	Santa Cruz Biotech.	sc-152
(H/M/R) α-Tubulin (TU-02) mouse monoclonal	WB	Santa Cruz Biotech.	sc-8035
(H/M/R) Acetyl.- α-Tubulin (6-11B-1) mouse monoclonal	WB	Santa Cruz Biotech.	sc-23950
(H/M/R) GAPDH (FL-335) rabbit polyclonal	WB	Santa Cruz Biotech.	sc-25778
2°Anti-goat-IgG-HRP	WB	Santa Cruz Biotech.	sc-2741
2°Anti-rabbit-IgG-HRP	WB	Santa Cruz Biotech.	sc-2030
2°Anti-mouse-IgG-HRP	WB	Santa Cruz Biotech.	sc-2005

RNAi Sequences

Table 2. Comparison of RNAi mediated approaches to knockdown *Wisp-1* in vitro

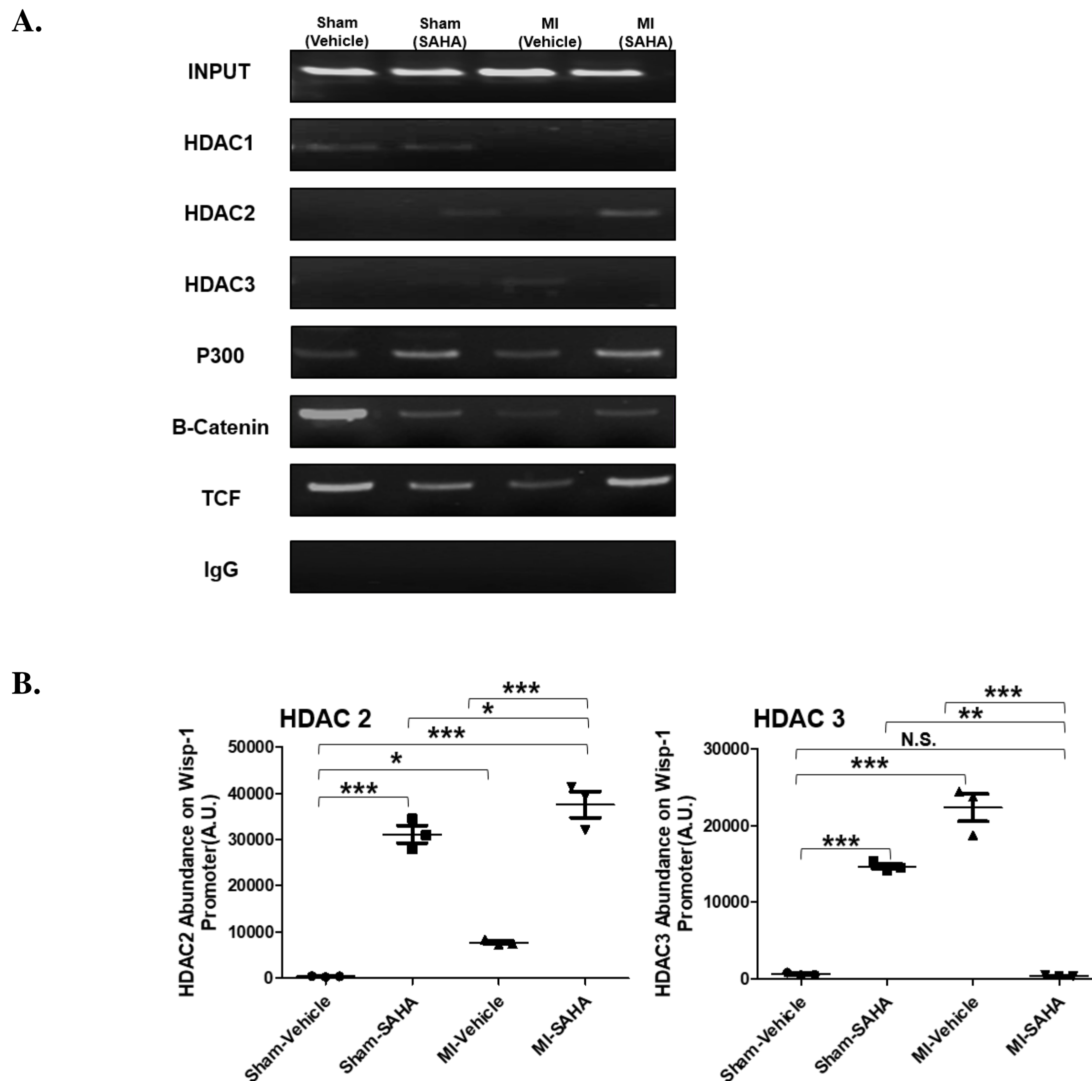
shRNA (Santa Cruz Biotechnology)	siRNA (Ambion, Life Technologies)
shRNA (Santa Cruz Biotechnology) Catalog no. sc-39335-V (Pooled A, B and C targets): Lot# I1909 sc-39335-VA: Hairpin sequence: GATCCGGACATCCATACACTCATTTTCAAGAGAA ATGAGTGTATGGATGTCCTTTTT Corresponding siRNA sequences (sc-39335A): • Sense: 5'GGACAUCCAUACACUCAUUtt3' • Antisense: 5'AAUGAGUGUAUGGAUGUCCtt'3	siRNA (Ambion, Life Technologies) Catalog no. AM16704, ID:137312 Lot# AS028HLJ Sense sequence: 5'CGCUCCUAUCAACCCAAGUtt 3' antisense sequence: 5'ACUUGGGUUGAUAGGAGCGtg3'
sc-39335-VB: Hairpin sequence: GATCCGGAATCCCAATGACATCTTTTCAAGAGAA AGATGTCATTGGGATTCCTTTTT Corresponding siRNA sequences (sc-39335B): • Sense:5'GGAAUCCCAAUGACAUCUUtt3' • Antisense:5' AAGAUGUCAUUGGGAUUCCtt	
sc-39335-VC: Hairpin sequence: GATCCCAACTAGGCAGGCACAAATTCAAGAGAA TTTGTGCCTGCCTAGTTGTTTTT Corresponding siRNA sequences (sc-39335C): • Sense: 5'CAACUAGGCAGGCACAAAUtt3' • Antisense: 5'AUUUGUGCCUGCCUAGUUGtt3'	

Supplemental Figure 1. Wisp-1 in LV at 7 days post-MI.



Supplemental Figure 1. **(A)** Representative image of Western blot of Wisp-1 and tubulin acetylation in left ventricle (LV)**.** 10-12 week old male CD1 mice were subjected to either Sham (control) or ligation of the coronary artery surgery and received daily I.P. injections of DMSO (vehicle-control) or the HDAC inhibitor SAHA (25mg/kg) for 7 days post-MI. (**B**) Representative Western blot and densitometry graph analysis of Wisp-1 protein from whole LV homogenates of post-MI myocardium or sham control tissue. GAPDH (loading control). N=4 per group. Results depicted as mean ±S.E.M., * $p \leq 0.05$, Not significant. P-values obtained by 1-Way ANOVA with Bonferroni's post test.).

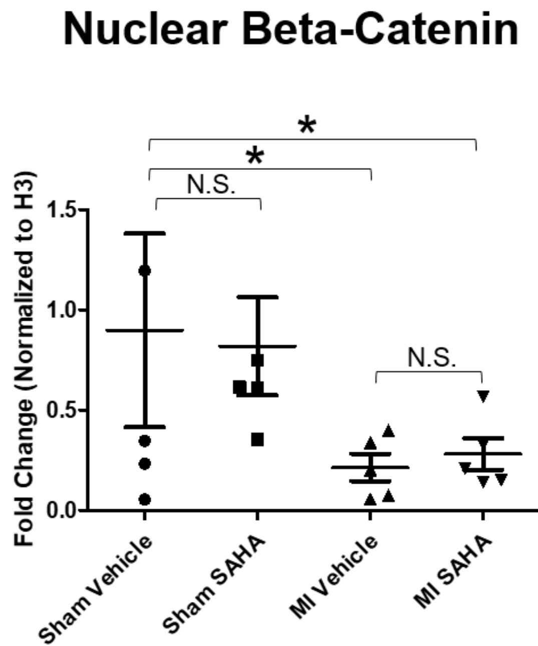
Supplemental Figure 2. Representative Image of ChIP Assay (*Wisp-1* promoter) and PCR Product Quantitation.



Supplemental Figure 2. 10-12 week old male CD1 mice were subjected to either Sham (control) or ligation of the coronary artery surgery and received daily I.P. injections of DMSO (vehicle-control) or the HDAC inhibitor SAHA (25mg/kg) for 7 days post-MI **(A)** Representative image of gels from ChIP analyses. Images were taken using the Image Quant LAS GE imaging system and taken at 1/4th second exposure. **(B)** Experiments were repeated 4 times and gel band intensities

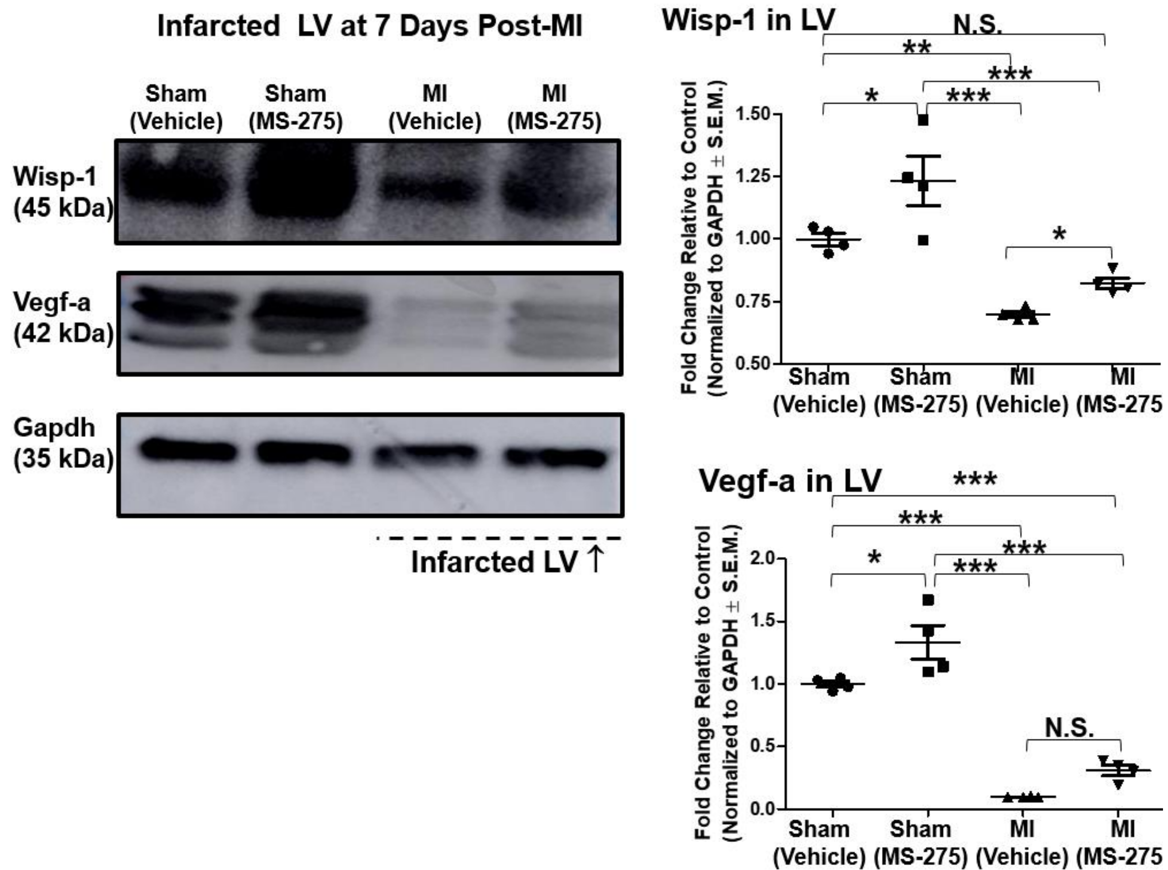
were quantified using ImageJ-Fuji software. ChIP analyses of left ventricle homogenates for HDAC1, HDAC2, HDAC3, P300, ß-catenin, TCF and IgG (isotype control) on the proximal promoter of *Wisp-1*. Data are from 4 mice per group and was repeated 3 times. Results depicted as mean ±S.E.M., * $p \leq 0.05$, ** $p \leq 0.01$., *** $p \leq 0.001$, N.S., not significant. P-values obtained by 1-Way ANOVA with Tukey's post test.

Supplemental Figure 3. HDAC inhibition does not impact subcellular localization of ß-catenin.



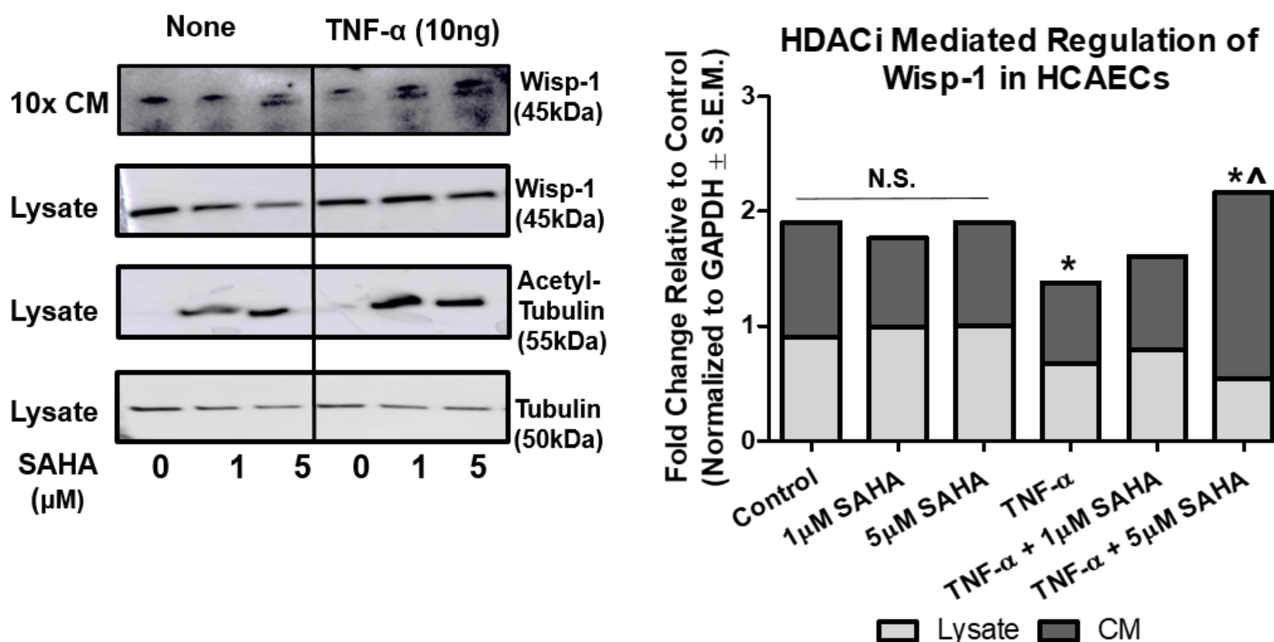
Supplemental Figure 3. Tissue samples from sham or MI operated mice hearts were removed, finely minced, and homogenized by poltroon. NE-PER Nuclear and Cytoplasmic extraction Reagents (Pierce biotechnology, Rockford, IL) were used to separate nuclear and cytosolic components of the cardiac lysates. Protein expression was then analyzed via Western blot. Graph is represented as +/- S.E.M, of 3 experimental replicates. * P-value ≤0.05. N.S., not significant. One-way ANOVA.

Supplemental Figure 4. MS-275 mediated increase of Wisp-1 in LV at 7 days post-MI.



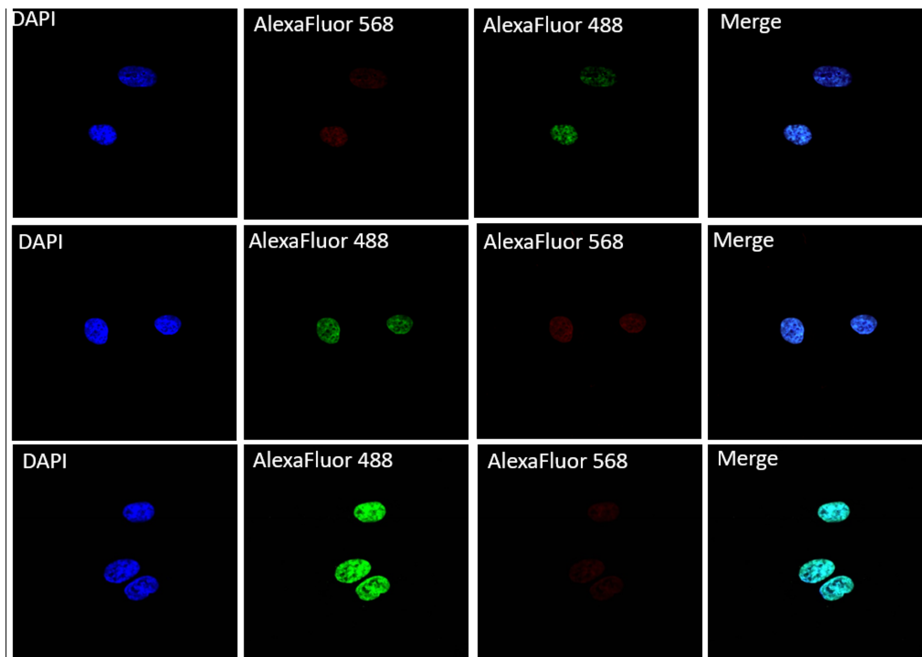
Supplemental Figure 4. 10-12 week old male CD1 mice were subjected to either Sham (control) or ligation of the coronary artery surgery and received daily I.P. injections of DMSO (vehicle-control) or the class I HDAC inhibitor MS-275 (5mg/kg) for 7 days post-MI. Western blot analysis of Wisp-1 and Vegf-a protein from infarcted LV homogenates of post-MI myocardium or sham control tissue with (Gapdh loading control). Results depicted as mean ±S.E.M., n=4/group *p≤0.05, **p≤0.01., ***p≤0.001, N.S., not significant. P-values obtained by 1-Way ANOVA with Bonferroni's post test.

Supplemental Figure 5. HDAC inhibition promotes production of WISP-1 in endothelial cells.



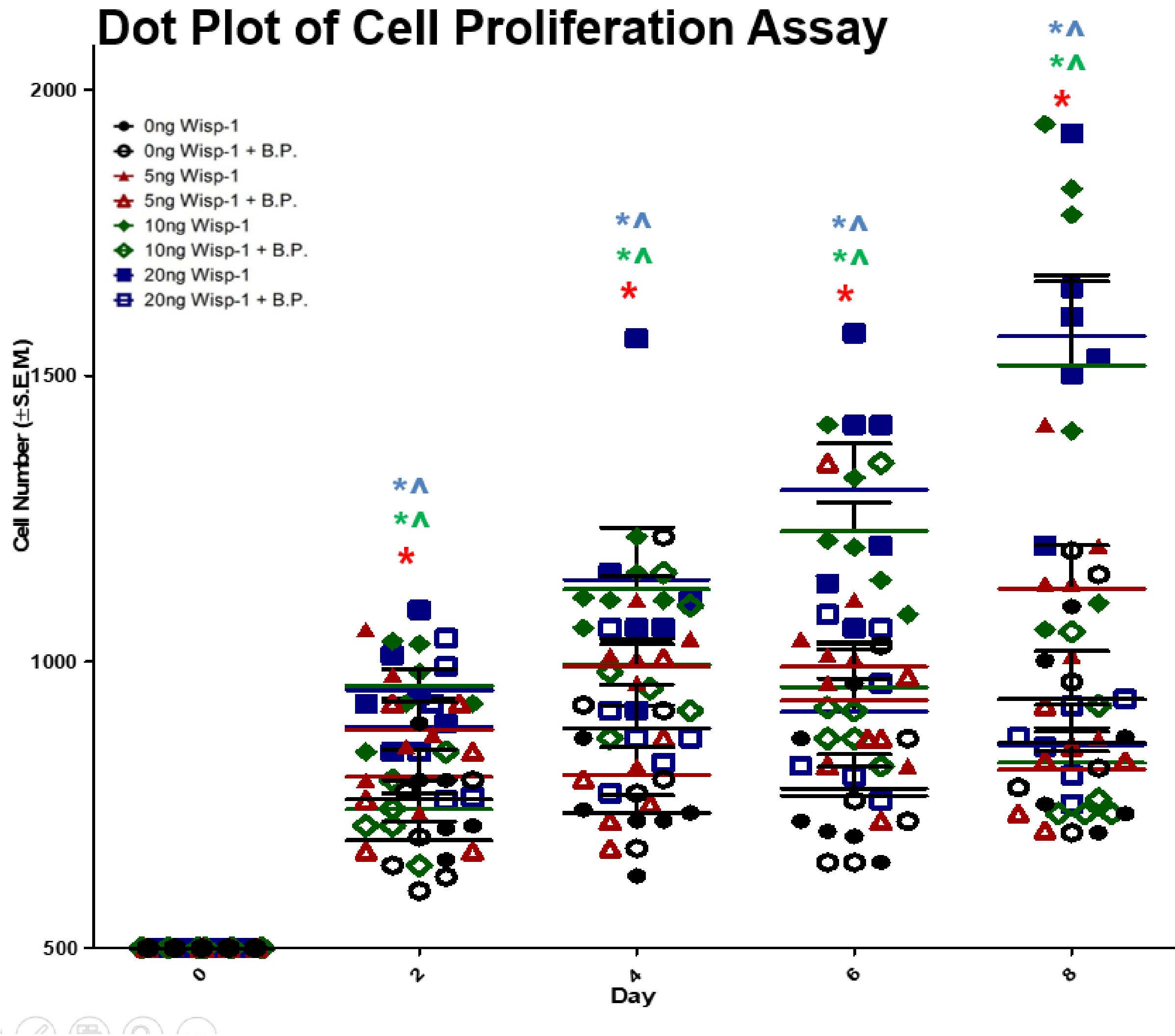
Supplemental Figure 5. Human coronary artery cells (HCAECs) were treated with either vehicle (DMSO, 0.01%), TNF-α (10ng/ml), HDAC inhibitor, SAHA (1, or 5μM) or both TNF-α and SAHA. (A) Expression and secretion of WISP-1 in conditioned media (C.M) was measured by western blot analysis using antibodies against Wisp-1, tubulin and acetylated-tubulin. Image is representative of the replicated experiments. Graph shows quantitated densitometry differences normalized to loading control (Tubulin) ±S.E.M. of arbitrary units. * $p \leq 0.05$ relative to control, ^ $p \leq 0.05$ relative to TNF-α, n=3. N.S., not significant. All p-values were obtained by 1-way ANOVA.

Supplemental Figure 6. Assessment of secondary antibody auto-fluorescence.



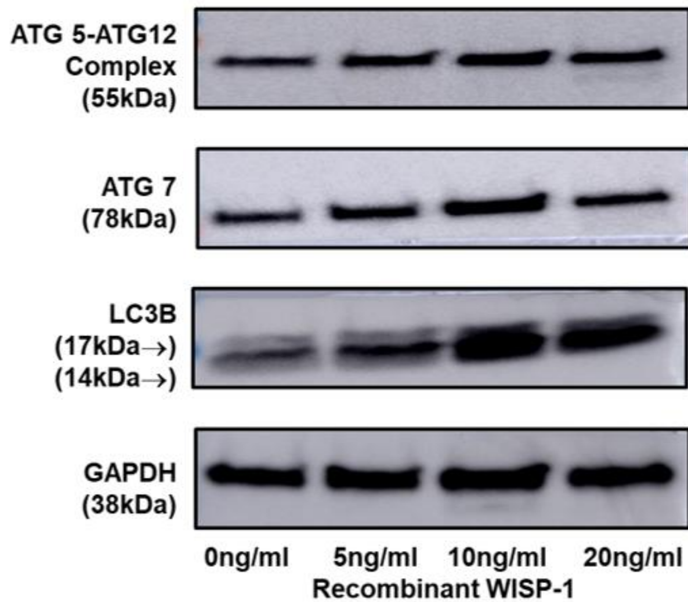
Supplemental Figure 6. Negative control of immunocytochemical analyses (no 1° antibody added, only DAPI) on isolated human coronary artery endothelial cells. 2° Antibodies AlexFluor 568 and 488).

Supplemental Figure 7. Dot Plot analysis of Cell proliferation



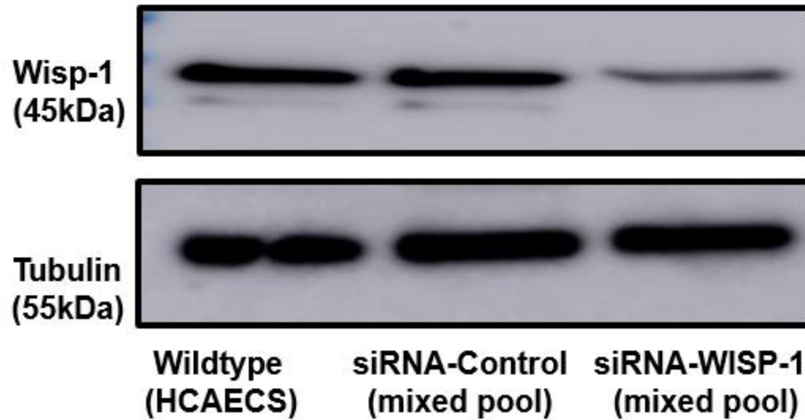
Supplemental Figure 7. 250 cells were seeded in a multi-well plate in triplicate in complete media. Cells were treated with either PBS (0ng/ml), 0ng/ml, 5ng/ml, 10ng/ml or 20ng/ml recombinant Wisp-1 every 48 hours for 8 days. Cells were detached and counted on day 2, 4, 6, and 8 post seeding.). Assay was performed in triplicate experimental groups and are representative of 3 independent experiments. Results depicted as mean ±S.E.M.,* $p \leq 0.05$ relative to control, ^ $p \leq 0.05$ relative to 5ng/ml, # relative to 10ng/ml ^ $p \leq 0.05$ relative to 5ng/ml and # $p \leq 0.05$ relative to 10ng/ml. P-values obtained by 1-way ANOVA with Bonferroni's post test.

Supplemental Figure 8. Western blots of WISP-1 regulated autophagy signaling in human coronary artery endothelial cells respectively.



Supplemental Figure 8. Equal numbers of HCAECs were seeded overnight. The following day, cells were treated with 0ng/ml, 5ng/ml, 10ng/ml or 20ng/ml recombinant WISP-1 (rec.WISP-1) for 72 hours then assessed for autophagy markers, ATG 5-ATG 12 complex, ATG-7 and LC3BII (17 and 14 kDa) and GAPDH (loading control). Image is representative of 4 repeated experiments.

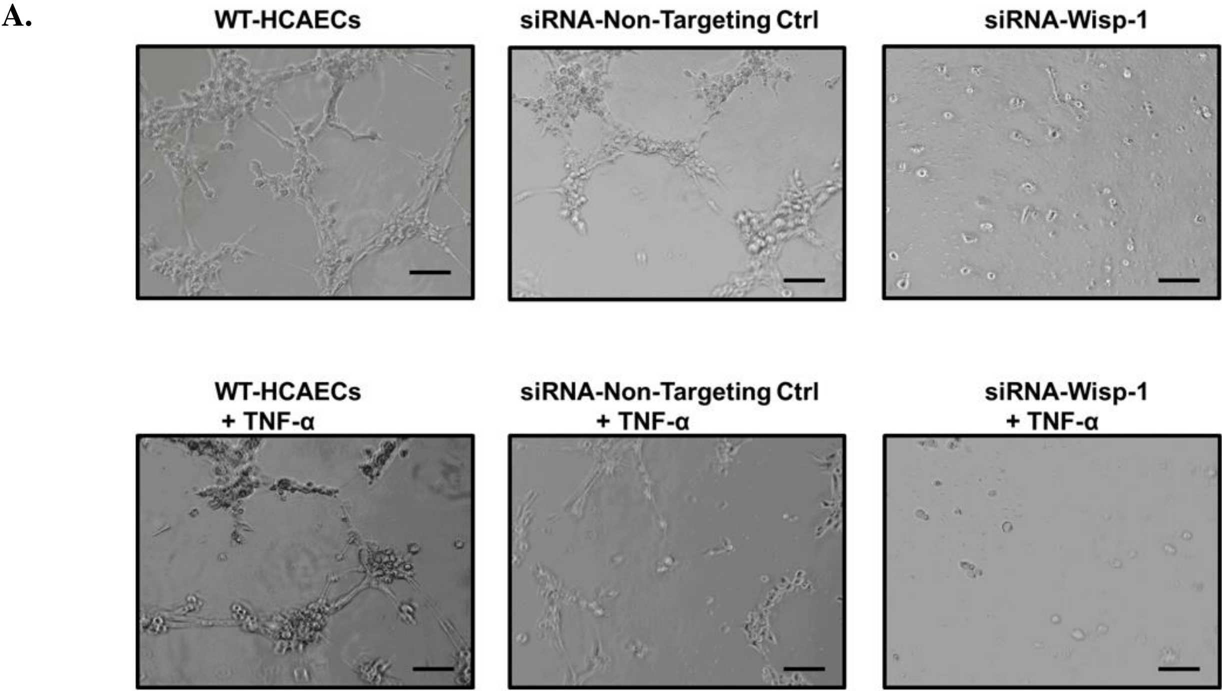
Supplemental Figure 9. WISP-1 siRNA mediated knockdown in isolated human coronary artery endothelial cells.



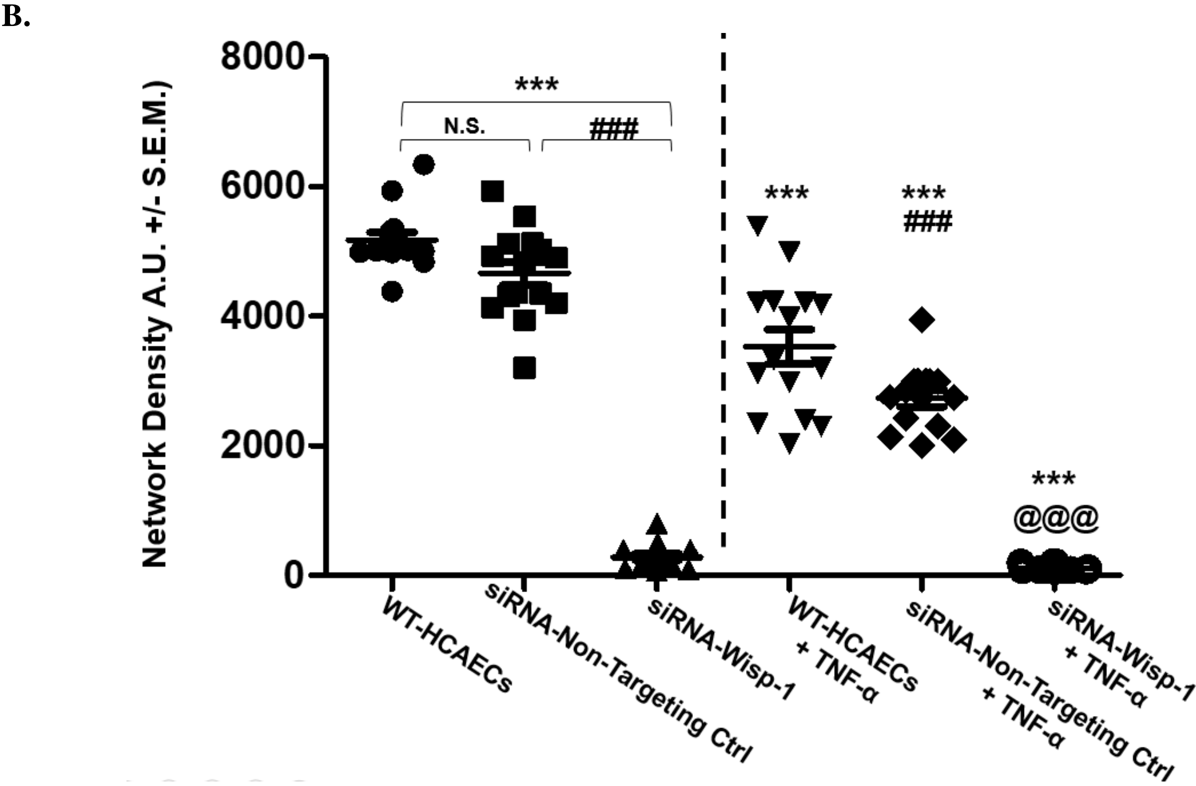
Supplemental Figure 9. *siRNA Transfection.* Human Wisp-1 siRNA was purchased from Life Technologies (Carlsbad, CA ((Catalog no. AM16704, ID:137312, Lot# AS028HLJ)). The scrambled siRNA was purchased as a control siRNA (Catalog no. AM4611, Lot#ASO26WKF). HCAECs were cultured in six-well plates and transfected with siRNAs (Wisp-1 siRNA and control siRNA; 20 nM) using Lipofectamine 2000 (Life Technologies Catalog no.11668019) Transfection Reagent (Life Technologies) according to the manufacturer's instruction followed by 48 hours of incubation in DMEM supplemented with 10%FBS at 37°C in a CO2 incubator. Wisp-1 knock down was validated via Western Blot.

Supplemental Figure 10. Impact of siRNA mediated knockdown of WISP-1 knockdown on network branching densities.

A.



B.



Supplemental Figure 10. HCAECs were transfected with either control, non-targeting/scrambled siRNA, or WISP-1 siRNA constructs for transient knockdown. 72 hours after transfection, 30,000 HCAECs/well (48-well plate) were seeded, in triplicate, on growth factor reduced Matrigel^{TM}, (Corning, Tewksbury, MA (Catalog No. 356231)). (**A**) Density of network branching determined by software analyses of images (5/well) after 8 hours. **(B)** Graphs show branching density determined by software analyses. Experiments were performed in triplicate with an n=15 per group (average density of 5 photographs per group). Results depicted as mean ±S.E.M. of arbitrary units (A.U.) ***p≤0.001 relative to WT, ### p≤0.001 relative to siRNA Non-targeting control, @@@ p≤0.001 relative to siRNA Non-targeting control with TNF-α treatment, N.S., not significant. P-values obtained by 1-Way ANOVA with Bonferroni's post test.