

SUPPLEMENTAL MATERIAL

Detailed Methods

Myocardial infarction: MI was generated via permanent coronary ligation as previously described.¹ C57BL/6J male mice (8-10 weeks old) were anesthetized using 65 mg/kg pentobarbital. Mice were ventilated using a standard rodent ventilator. An incision was performed between the fourth and the fifth intercostals space. After the pericardium was removed a descending coronary artery was permanently ligated with a silk suture. Sham-operated animals underwent an identical surgical operation lacking the occlusion of the coronary artery. To prevent any post-operative discomfort animals received buprenorphine (0.03-0.06 mg/kg). Immediately after myocardial infarction and injection of r-Wnt3a protein osmotic minipumps (Alzet, 1007D) were implanted subcutaneously in the back of each mouse. Each pump was loaded with BrdU dissolved in NaHCO₃ (150 mmol/L) buffer at a concentration of 40 mg/ml. BrdU pumps remained in place for a period of 7 days.

Heart fixation: Mice were anesthetized by intra-peritoneal injection of pentobarbital (65 mg/ml) followed by intra-peritoneal administration of 100 IU/ml anticoagulant heparin. Hearts were rapidly excised and washed briefly in Krebs buffer (NaCl 137 mmol/L, KCl 4.0 mmol/L, CaCl₂ 1.8 mmol/L, KH₂PO₄ 1.2 mmol/L, MgSO₄ 1.2 mmol/L, NaHCO₃ 24.9 mmol/L, and dextrose 11.2 mmol/L (pH 7.4)). A short cannula was connected to the aortic root to initiate retrograde perfusion by the Langendorff apparatus. A thin cannula was pierced through the apex of the left ventricle (LV) to vent the besian drainage. A ventricular balloon was inserted into the LV through the mitral valve via an incision in the left atrium. The balloon was connected to a pressure transducer (Statham P23Db, Gould) for recording the LV pressure. Hearts were paced (Grass Instruments) with platinum wires placed on the epicardial surface of the right ventricle. Coronary perfusion pressure was held constant during the duration of the experiment at 80 mm Hg. The balloon was inflated with saline to adjust the end-diastolic pressure (EDP) to 5 mm Hg. When the end-diastolic pressure reached 5 mm Hg, hearts were arrested in diastole with KCl and were perfused with 10% buffered formalin solution at a hydrostatic pressure of 40-50 mm Hg for 15 minutes. Hearts remained overnight in 10% buffered formalin solution at 4°C. Subsequently, the balloon and the excess formalin were removed and the hearts were weighed. Hearts were imbedded in paraffin, cut in three parts (apex, middle, base) and analyzed by immuno-histochemistry.

Echocardiography: Echocardiograms were obtained at baseline (1 day prior coronary occlusion) and 7 days following MI, with a Vevo 2100 Imaging digital ultrasound system (VisualSonics). Echocardiography was performed using an 18-38 MHz linear-array transducer. Prior to echocardiography the chest was shaved and the animals were placed on a heated platform in the supine position. Echocardiographic analysis was performed in conscious mice at a physiological heart rate (>500 beats/minute). Data acquisition was initiated with the parasternal cardiac long axis view and subsequently with a short axis view, at the level of mid-papillary muscles. Echocardiographic measurements were performed from M-mode images. All data were acquired and analyzed in a blinded fashion.

Immuno-histochemistry. Formalin fixed tissue slides were deparaffinized via incubation at 65°C for 30 minutes and re-hydrated through sequential three minute incubations in xylene, ethanol (96%, 90%, 80%) and water. Antigen retrieval was performed by microwave heating in citrate buffer (0.1 M, pH 6.0) for 10 minutes. Slides were incubated with primary antibodies against BrdU (Roche) and α -sarcomeric-actin (Sigma) for 1 hour at 37°C, followed by incubation with appropriate secondary antibodies at the same conditions. Slides were subsequently washed and mounted with DAPI-containing medium (Vector Vectashield). Infarct area and border zone were scanned for the presence of BrdU positive

cardiomyocytes (CM) through examination of 10-20 mosaic-pictures of each section obtained by epi-fluorescence microscopy (Zeiss, Axiovert 200M). Each mosaic picture was composed of 9-12 high power (63X) images. The ratio of BrdU⁺ CM over total nucleated CM was calculated for every image. Confocal imaging was performed with a LSM 700 system (Zeiss). Image acquisition and analysis were performed in a blinded fashion. Myocyte cross-sectional area was assessed by staining the cardiac tissue with Alexa Fluor 555 conjugated wheat germ agglutinin (WGA) (Invitrogen). Apoptotic cell death was determined by Tunel staining (Roche) according to the instructions of the manufacturer.

RT-PCR gene array. RNA samples from expanded CSP cells treated with Vehicle or Wnt3a-CM (a), CSP cells infected with Mock or IGFBP3-o/e (b), CSP cells infected with sh-Scramble or sh-IGFBP3 (c) and freshly isolated CSP cells harvested from sham and post-MI (1, 3 and 7 days after injury) (d) were used for cDNA synthesis (RT²-First strand kit, SABiosciences). RT-PCR gene-arrays (SABiosciences) focused on cell-cycle regulation (a, b and c), signal transduction pathways (b) and Wnt signaling pathway (d) were performed according to the instructions of the manufacturer using a MyiQ cycler (Bio-Rad). Data were acquired from 3 independent experiments each with three replications and analysis was performed as follows: ΔCt values of all examined genes were obtained by subtracting the mean threshold cycle (Ct) value of 5 housekeeping genes (Gusb, Hprt1, Hsp90ab1, Gapdh, Actb) from the Ct value of each gene. Subsequently, $\Delta\Delta Ct$ values for all genes were calculated as follows: $\Delta\Delta Ct = \Delta Ct_{\text{experimental}} - \Delta Ct_{\text{control}}$. Fold differences were calculated based on the formula: $Fold\ difference = 2^{-\Delta\Delta Ct}$.

Production of Wnt3a conditioned medium (CM). Vehicle-CM (Vehicle) and Wnt3a-CM were produced from L-M (TK-) cells and L-M (TK-)-Wnt3a cells according the instructions of American Type Culture Collection (ATCC).

Cell expansion and lentiviral infection. CSP cells were isolated as previously described² and cultured in expansion medium [α -MEM (Lonza) with 20% FBS (Hyclone), 2 mmoles/L, L-Glutamine and 1% penicillin/streptomycin] at a density of 10-15 cells/mm. CSP cells of passages 3-5 were infected with lentivirus and 48 hours later were used in proliferation assays. Lentivirus with sh-Scramble, sh-IGFBP3, empty vector (Mock), IGFBP3-o/e, IGFBP3^{mIGF} or pBARL (TCF-controlled luciferase reporter) and pSL9 (Ef1-a-controlled Renilla luciferase control)³, were used as described in the manuscript.

Proliferative capacity assay. In vitro proliferation capacity of CSP cells was determined by cell counting, cell cycle markers including BrdU and phospho-Histone H3 (p-H3), and DNA content measurements through propidium iodide (PI) staining. Briefly, CSP cells were cultured at an initial density of 3.5-7 cells/mm² in our established α -MEM based expansion medium with 20% FBS with various concentrations of Vehicle, Wnt3a-CM, BIO ((2'Z,3'E)-6-Bromoindirubin-3'-oxime), IGF-1 (Peprotech), recombinant Wnt3a protein or recombinant SFRP2 (R&D). Culture medium was replaced every 72 hours and following 6 days in culture, cell number was determined using hemacytometer and flow cytometer (C6 Accuri cytometer). For immuno-cytochemistry detection, CSP cells were cultured on coverslips in expansion medium for five days. For BrdU incorporation assays, at day 5 CSP cells were pulsed with 10 μ M BrdU for 1 hour and BrdU was detected using a labeling kit (Roche) according to the instructions of the manufacturer. p-H3 (Abcam) was detected by immunocytochemical staining as previously described.⁴ CSP cells were visualized using Zeiss epi-fluorescent microscopy. For DNA content measurements, CSP cells were fixed using 70% ethanol following 5 days culture and were stained with PI (PBS, 0.1% Triton-X 100, 2 μ g/ml PI, 400 ng/ml RNase-A) for one hour at 37°C under light-free conditions. CSP cells were analyzed by FACS and data were processed by ModFit Lt (Verity Software House).

IGFBP-3 over-expression: IGFBP-3 cDNA was excised by XbaI/XmaI enzymatic digestion from vector pSport-6 (Open Biosystems). The cDNA-fragment was blunted and ligated into lentiviral expression vector LSLV-83 (kindly provided by Dr. Paul Allen) downstream of the constitutively active promoter EF1a. Infected cells were selected with neomycin (1mg/ml) for a period of 7-10 days.

IGFBP-3 shRNA. pLKO.1-puro lentiviral vectors encoding Scramble or IGFBP3 (TRCN0000112128) shRNAs were obtained from Open Biosystems. CSP cells were infected with lentiviruses and selected using puromycin treatment (4µg/ml) over a period of 7-10 days.

IGFBP-3 mutagenesis. Lentiviral plasmid containing WT-IGFBP3 cDNA was utilized as a template for site-directed mutagenesis (quick-change mutagenesis kit, Stratagene), according to manufacturer's instructions. In brief, complementary primers encoding the desired mutations were annealed to the template LSLV-83-IGFBP3 plasmid and Pfu polymerase was used to replicate the template, incorporating the mutated primers. The template plasmid was digested with DpnI, prior to transformation. The utilized primers are as follows:

IGFBP3^{mIGF}-sense:CCCGCTGAGGGCGGGGGGAATGGCCGCGGG
IGFBP3^{mIGF}-antisense:CCCGCGGCCATTCCCCCGCCCTCAGCGGG.
All mutated plasmids were sequence verified.

Cell viability assay: Annexin-V staining (Abcam) was used to determine cell death, according to the instructions of the manufacturer, using a flow cytometer (C6 Accuri cytometer).

Actinomyosin-D treatment: CSP cells were treated with 5µg/ml Actinomyosin-D for a period of 30 minutes, prior to application of Vehicle or Wnt3a-CM (25%) for a period of 24 hours.

Western blot: Immunoblot was performed as previously described.⁵ Primary antibodies against IGFBP3 (Santa Cruz) or GAPDH (R&D) followed by appropriate secondary antibodies (Alexa-Fluor 680, Molecular probes) were applied. All incubations were performed for two hours at room temperature under constant movement. Antibody binding was detected using the Odyssey infrared system (LI-COR).

Online Tables

Online Table I: Cell cycle-focused RT-PCR gene array in CSP cells treated with Vehicle or Wnt3a-CM.

Cell cycle RT-PCR array							
Description	Symbol	8 hours (Δ Ct)		Fold change	48 hours (Δ Ct)		Fold Change
		Vehicle	Wnt3a-CM		Vehicle	Wnt3a-CM	
Integrin beta 1 (fibronectin receptor beta)	Itgb1	-1.87	-2.28	1.33	-0.14	-1.36	2.33*
Cyclin-dependent kinase inhibitor 2A	Cdkn2a	5.09	4.08	2.00*	2.52	1.49	2.04*
Cyclin-dependent kinase inhibitor 1A (P21)	Cdkn1a	0.17	0.34	-1.12	1.60	1.23	1.30
Peripheral myelin protein 22	Pmp22	3.11	2.99	1.08	1.94	1.63	1.24
Cyclin D1	Ccnd1	3.37	3.28	1.06	2.49	2.23	1.20
Transformed mouse 3T3 cell double minute 2	Mdm2	2.61	2.64	-1.01	1.65	1.39	1.20
DNA-damage inducible transcript 3	Ddit3	4.47	4.26	1.15	4.82	4.61	1.16
Protein phosphatase 2 (formerly 2A), regulatory subunit B", alpha	Ppp2r3a	9.88	9.90	-1.01	10.09	9.92	1.12
Adenylate kinase 1	Ak1	1.85	1.67	1.13	2.82	2.68	1.10
Protein phosphatase 1D magnesium-dependent, delta isoform	Ppm1d	4.91	5.06	-1.10	4.24	4.74	-1.41*
Structural maintenance of chromosomes 1A	Smc1a	4.70	4.62	1.05	3.99	4.48	-1.41*
Nucleophosmin/nucleoplasmin 2	Npm2	9.83	9.90	-1.04	9.46	9.98	-1.43*
Cell division cycle 25 homolog A (S. pombe)	Cdc25a	3.36	3.55	-1.14	4.36	4.88	-1.44*
Transcription factor Dp 1	Tfdp1	1.33	1.38	-1.03	1.04	1.60	-1.48*
Stromal antigen 1	Stag1	5.04	5.51	-1.38	4.78	5.39	-1.52*
Telomeric repeat binding factor 1	Terf1	6.22	6.54	-1.24	4.71	5.34	-1.54*
Cyclin B1	Ccnb1	4.54	4.46	1.05	3.17	3.80	-1.55*
Proliferating cell nuclear antigen	Pcna	1.71	2.03	-1.25*	0.92	1.57	-1.57*
Protein phosphatase 3, catalytic subunit, alpha isoform	Ppp3ca	4.67	5.07	-1.31	4.08	4.77	-1.62*

RAD21 homolog (S. pombe)	Rad21	4.71	5.01	-1.22	1.95	2.65	-1.62*
Cyclin-dependent kinase 4	Cdk4	2.13	2.47	-1.26	1.20	1.91	-1.63*
Inhibin alpha	Inha	8.30	8.48	-1.13	7.77	8.47	-1.63*
Mdm2, transformed 3T3 cell double minute p53 binding protein	Mtbp	8.32	8.67	-1.27	5.13	5.84	-1.63*
Minichromosome maintenance deficient 3 (S. cerevisiae)	Mcm3	2.95	3.10	-1.11	2.86	3.57	-1.64*
MutS homolog 2 (E. coli)	Msh2	4.23	4.23	-1.00	3.80	4.54	-1.66*
Checkpoint kinase 1 homolog (S. pombe)	Chek1	5.53	6.15	-1.53	5.48	6.22	-1.67*
RAD9 homolog (S. pombe)	Rad9	5.62	5.90	-1.21	5.19	5.97	-1.72*
NIMA (never in mitosis gene a)-related expressed kinase 2	Nek2	N/A	N/A	N/A	4.28	5.07	-1.73*
RAD51 homolog (S. cerevisiae)	Rad51	6.23	6.86	-1.55	4.99	5.81	-1.77*
MAD2 mitotic arrest deficient-like 1 (yeast)	Mad211	3.91	4.21	-1.2	3.28	4.12	-1.79*
Transformation related protein 53	Trp53	2.98	3.15	-1.12	1.83	2.68	-1.81*
Retinoblastoma-like 1 (p107)	Rbl1	5.51	5.96	-1.37	5.69	6.57	-1.85*
Myeloblastosis oncogene	Myb	N/A	N/A	N/A	12.28	13.25	-1.96*
Breast cancer 2	Brca2	6.72	8.67*	-3.88*	6.67	7.70	-2.04*
WEE 1 homolog 1 (S. pombe)	Wee1	5.16	5.53	-1.29	5.24	6.27	-2.04*
Breast cancer 1	Brca1	6.20	6.73	-1.44	5.58	6.62	-2.06*
Cyclin F	Ccnf	4.40	4.32	1.06	3.71	4.76	-2.06*
Minichromosome maintenance deficient 2 mitotin (S. cerevisiae)	Mcm2	4.23	4.47	-1.18	2.75	3.81	-2.07*
Antigen identified by monoclonal antibody Ki67	Mki67	5.02	6.59	-2.96*	2.94	4.07	-2.19*
Transformation related protein 63	Trp63	10.02	9.90	1.08	10.58	13.40	-7.09*
Calcium/calmodulin-dependent protein kinase II alpha	Camk2a	N/A	N/A	N/A	9.14	9.14	-1.00
Polycystic kidney disease 1 homolog	Pkd1	4.06	3.80	1.20	3.74	3.76	-1.01
Notch gene homolog 2 (Drosophila)	Notch2	6.57	6.80	-1.17	5.23	5.29	-1.04
Microtubule-actin crosslinking factor 1	Macf1	2.53	2.61	-1.05	3.01	3.12	-1.07

Amyloid beta (A4) precursor protein-binding, family B, member 1	Apbb1	9.45	8.78	1.59	8.76	8.87	-1.08
Cyclin C	Ccnc	6.36	6.59	-1.17	7.30	7.44	-1.10
Tumor susceptibility gene 101	Tsg101	6.80	6.51	1.22	7.03	7.17	-1.10
Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	Nfatc1	2.99	3.02	-1.01	4.46	4.63	-1.12
RAD17 homolog (S. pombe)	Rad17	5.93	5.90	1.01	4.81	4.99	-1.13
SMT3 suppressor of mif two 3 homolog 1 (yeast)	Sumo1	2.24	2.37	-1.09	2.10	2.28	-1.13
TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor	Taf10	2.37	2.36	1.00	1.31	1.53	-1.16
G protein-coupled receptor 132	Gpr132	N/A	N/A	N/A	12.38	12.60	-1.17
Sestrin 2	Sesn2	7.65	7.39	1.19	5.57	5.80	-1.17
RAN, member RAS oncogene family	Ran	-1.57	-1.44	-1.09	-1.58	-1.33	-1.19
CDK5 regulatory subunit associated protein 1	Cdk5rap1	7.71	7.14	1.48	7.76	8.03	-1.2
C-abl oncogene 1, receptor tyrosine kinase	Abl1	N/A	N/A	N/A	9.49	9.76	-1.21
E2F transcription factor 4	E2f4	2.73	2.93	-1.15	3.96	4.25	-1.22
Pescadillo homolog 1, containing BRCT domain (zebrafish)	Pes1	3.67	3.86	-1.13	2.20	2.50	-1.23
DnaJ (Hsp40) homolog, subfamily C, member 2	Dnajc2	3.39	3.47	-1.05	3.22	3.54	-1.25
Dystonin	Dst	4.79	4.75	1.02	3.12	3.44	-1.25
Protamine 1	Prm1	N/A	N/A	N/A	13.23	13.57	-1.26
Retinoblastoma-like 2	Rbl2	6.52	6.78	-1.19	5.31	5.64	-1.26
Growth arrest and DNA-damage-inducible 45 alpha	Gadd45a	6.55	5.96	1.50*	5.06	5.42	-1.28
Cyclin-dependent kinase 2	Cdk2	4.61	5.06	-1.36	3.62	4.01	-1.31
Caspase 3	Casp3	4.88	5.05	-1.12	4.79	5.20	-1.32
E2F transcription factor 3	E2f3	6.44	6.56	-1.08	5.85	6.30	-1.36
Src homology 2 domain-containing transforming protein C1	Shc1	2.93	2.88	1.03	3.85	4.30	-1.36
Stratifin	Sfn	7.34	6.98	1.28	8.39	8.85	-1.37
Cyclin E1	Ccne1	6.99	6.98	1.00	5.01	5.47	-1.38

Proteasome (prosome, macropain) assembly chaperone 2	Psmg2	4.83	5.23	-1.32	3.07	3.56	-1.4
E2F transcription factor 1	E2f1	6.53	6.71	-1.12	5.48	5.98	-1.41
CDC28 protein kinase 1b	Cks1b	2.16	2.09	1.04	3.01	3.56	-1.46
Cyclin-dependent kinase inhibitor 1B	Cdkn1b	5.93	5.92	1.00	3.79	4.36	-1.48
S-phase kinase-associated protein 2 (p45)	Skp2	5.60	6.07	-1.37	5.42	6.00	-1.49
Ataxia telangiectasia mutated homolog (human)	Atm	5.42	6.23	-1.75	5.56	6.14	-1.50
Meiotic recombination 11 homolog A (<i>S. cerevisiae</i>)	Mre11a	5.78	5.71	1.04	5.10	5.68	-1.50
Hus1 homolog (<i>S. pombe</i>)	Hus1	5.41	5.58	-1.11	6.29	6.94	-1.57
Calcium/calmodulin-dependent protein kinase II, beta	Camk2b	10.02	9.90	1.08	11.89	12.55	-1.58
Cyclin A2	Ccna2	3.89	4.09	-1.15	3.55	4.25	-1.62
Cyclin B2	Ccnb2	6.37	6.39	-1.01	4.10	4.91	-1.74
Minichromosome maintenance deficient 4 homolog (<i>S. cerevisiae</i>)	Mcm4	2.34	2.54	-1.14	2.32	3.18	-1.82
E2F transcription factor 2	E2f2	N/A	N/A	N/A	11.09	12.32	-2.35
Cyclin A1	Ccna1	N/A	N/A	N/A	N/A	N/A	N/A
Schlafen 1	Slfn1	N/A	N/A	N/A	N/A	N/A	N/A

Online Table II: Pathway finder focused RT-PCR gene array in CSP cells treated with Vehicle or Wnt3a-CM.

Pathway finder RT-PCR array					
Description	Gene #	Gene Symbol	Δ Ct		Fold change
			Vehicle	Wnt3a-CM	
Insulin-like growth factor binding protein 3	31	Igfbp3	10.01	5.08	43.44*
Wingless-related MMTV integration site 2	57	Wnt2	10.1	6.55	13.94*
Fas (TNF receptor superfamily member 6)	20	Fas	8.08	5.15	10.14*
Transcription factor 7, T-cell specific	49	Tcf7	8.47	5.55	9.81*
Cyclin-dependent kinase inhibitor 2A (p16)	15	Cdkn2a	3.18	0.19	9.11*
Lymphoid enhancer binding factor 1	37	Lef1	8.02	4.97	8.52*
Prostate transmembrane protein, androgen induced 1	52	Tmepai	7.18	4.49	6.88*
Etoposide induced 2.4 mRNA	19	Ei24	4.21	3.36	1.92*
Heat shock protein 1	29	Hspb1	0.26	-0.63	1.92*
Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	16	Cdkn2b	5.94	2.46	12.33
Intercellular adhesion molecule 1	30	Icam1	9.85	6.24	12.17
Fatty acid synthase	21	Fasn	9.41	7.32	9.91
FBJ osteosarcoma oncogene	23	Fos	9.12	7.07	9.83
Bone morphogenetic protein 4	8	Bmp4	8.79	7.06	9.73
Prostaglandin-endoperoxide synthase 2	46	Ptgs2	6.54	4.43	9.42
Chemokine (C-C motif) ligand 2	10	Ccl2	6.9	6.87	8.74
Baculoviral IAP repeat-containing 5	7	Birc5	6.33	7.85	8.61
Myelocytomatosis oncogene	39	Myc	6.21	6.01	6.43
Baculoviral IAP repeat-containing 2	5	Birc2	7.92	6.38	6.28
B-cell leukemia/lymphoma 2	3	Bcl2	7.31	6.31	6.09
WNT1 inducible signaling pathway protein 1	56	Wisp1	4.96	2.87	5.90
Nuclear receptor interacting protein 1	42	Nrip1	7.93	6.37	4.71
Transformed mouse 3T3 cell double minute 2	38	Mdm2	4.5	2.82	3.45
Retinol binding protein 1, cellular	47	Rbp1	2.34	1.5	3.41
Telomerase reverse transcriptase	50	Tert	8.16	6.66	3.34
Cyclin-dependent kinase inhibitor 1A (P21)	13	Cdkn1a	0.88	-0.23	3.08
Baculoviral IAP repeat-containing 3	6	Birc3	6.56	5.47	2.96
Breast cancer 1	9	Brca1	6.82	7.74	2.85
Patched homolog 1	45	Ptch1	6.35	4.98	2.84
Homeo box A1	27	Hoxa1	7.48	6.32	2.77
Jun oncogene	36	Jun	5.65	4.6	2.76
TRAF family member-associated Nf-kappa B activator	48	Tank	5.97	5.66	2.75
Peroxisome proliferator activated receptor gamma	44	Pparg	6.8	6.29	2.41

Inhibitor of kappaB kinase beta	33	Ikbbk	6.73	5.97	2.36
Transferrin receptor	51	Tfrc	5.58	5.44	2.34
Cyclin-dependent kinase 2	12	Cdk2	5.53	5.79	2.05
Early growth response 1	18	Egr1	5.37	6.19	1.88
Hexokinase 2	26	Hk2	4.76	4.2	1.55
Bcl2-associated X protein	2	Bax	1.16	0.58	1.53
Heat shock factor 1	28	Hsf1	4.37	3.84	1.52
Ngfi-A binding protein 2	40	Nab2	5.53	5.36	1.49
Vascular cell adhesion molecule 1	54	Vcam1	5.27	5.67	1.49
Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	41	Nfkbia	4.97	4.48	1.44
Growth arrest and DNA-damage-inducible 45 alpha	24	Gadd45a	4.69	4.36	1.43
Insulin-like growth factor binding protein 4	32	Igfbp4	2.75	2.27	1.43
Interferon regulatory factor 1	35	Irf1	5.86	5.4	1.43
Cyclin-dependent kinase inhibitor 1B	14	Cdkn1b	6.77	7.02	1.33
Bcl2-like 1	4	Bcl2l1	5.24	5.45	1.23
Glycogen synthase 1, muscle	25	Gys1	4.86	4.65	1.20
Vascular endothelial growth factor A	55	Vegfa	4.66	4.51	1.12
Ornithine decarboxylase, structural 1	43	Odc1	2.11	2.14	1.05
Activating transcription factor 2	1	Atf2	3.55	4.46	1.04
Transformation related protein 53	53	Trp53	2.48	2.48	1.02
Cyclin D1	11	Ccnd1	2.9	3.21	1.02
Interleukin 4 receptor, alpha	34	Il4ra	6.09	6.67	0.73
Fibronectin 1	22	Fn1	-1.26	-0.41	0.63
CCAAT/enhancer binding protein (C/EBP), beta	17	Cebpb	1.68	2.55	0.60
Bone morphogenetic protein 2	-	Bmp2	N/A	N/A	N/A
Chemokine (C-C motif) ligand 20	-	Ccl20	N/A	N/A	N/A
CD5 antigen	-	Cd5	N/A	N/A	N/A
Cadherin 1	-	Cdh1	N/A	N/A	N/A
Colony stimulating factor 2 (granulocyte-macrophage)	-	Csf2	N/A	N/A	N/A
Chemokine (C-X-C motif) ligand 1	-	Cxcl1	N/A	N/A	N/A
Chemokine (C-X-C motif) ligand 9	-	Cxcl9	N/A	N/A	N/A
Cytochrome P450, family 19, subfamily a, polypeptide 1	-	Cyp19a1	N/A	N/A	N/A
Engrailed 1	-	En1	N/A	N/A	N/A
Fas ligand (TNF superfamily, member 6)	-	Fasl	N/A	N/A	N/A
Fibroblast growth factor 4	-	Fgf4	N/A	N/A	N/A
Forkhead box A2	-	Foxa2	N/A	N/A	N/A
Gene regulated by estrogen in breast cancer protein	-	Greb1	N/A	N/A	N/A
Hedgehog-interacting protein	-	Hhip	N/A	N/A	N/A

Interleukin 1 alpha	-	Il1a	N/A	N/A	N/A
Interleukin 2	-	Il2	N/A	N/A	N/A
Interleukin 2 receptor, alpha chain	-	Il2ra	N/A	N/A	N/A
Leptin	-	Lep	N/A	N/A	N/A
Lymphotoxin A	-	Lta	N/A	N/A	N/A
Matrix metalloproteinase 10	-	Mmp10	N/A	N/A	N/A
Matrix metalloproteinase 7	-	Mmp7	N/A	N/A	N/A
NLR family, apoptosis inhibitory protein 1	-	Naip1	N/A	N/A	N/A
Nitric oxide synthase 2, inducible	-	Nos2	N/A	N/A	N/A
Selectin, endothelial cell	-	Sele	N/A	N/A	N/A
Selectin, platelet	-	Selp	N/A	N/A	N/A
Tumor necrosis factor	-	Tnf	N/A	N/A	N/A
Wingless-related MMTV integration site 1	-	Wnt1	N/A	N/A	N/A

Online Table III: Cell cycle-focused RT-PCR gene-arrays in CSP cells treated with Vehicle or Wnt3a-CM (48hours) and CSP cells infected with Mock or IGFBP3 o/e lentiviruses.

Cell cycle RT-PCR array						
Symbol	Wnt3a-CM treatment (48 hours)			IGFBP3 o/e		
	AVG Δ Ct		Fold change	AVG Δ Ct		Fold change
	Vehicle	Wnt3a-CM	Wnt3a/Vehicle	Mock	IGFBP3 o/e	IGFBP3-o/e / Mock
Itgb1	-0.14	-1.36	2.33	-1.32	-1.63	1.24
Cdkn2a	2.52	1.49	2.04	1.14	0.49	1.56
Cdkn1a	1.6	1.23	1.3	0.57	-0.01	1.49
Pmp22	1.94	1.63	1.24	0.83	0.39	1.35
Ccnd1	2.49	2.23	1.20	0.95	0.7	1.19
Mdm2	1.65	1.39	1.20	0.88	0.76	1.09
Ddit3	4.82	4.61	1.16	2.9	2.75	1.11
Ak1	2.82	2.68	1.10	1.21	0.79	1.33
Notch2	5.23	5.29	-1.04	4.23	4.67	-1.36
Macf1	3.01	3.12	-1.07	2.22	2.27	-1.04
Rad17	4.81	4.99	-1.13	4.1	4.36	-1.2
Taf10	1.31	1.53	-1.16	0.57	0.85	-1.22
Sesn2	5.57	5.8	-1.17	4.25	4.27	-1.01
Ran	-1.58	-1.33	-1.19	-1.45	-1.06	-1.31
Cdk5rap1	7.76	8.03	-1.20	6.79	6.84	-1.04
E2f4	3.96	4.25	-1.22	3.14	3.41	-1.21
Pes1	2.2	2.5	-1.23	2.01	2.29	-1.22
Dnajc2	3.22	3.54	-1.25	2.74	3.09	-1.28
Rbl2	5.31	5.64	-1.26	4.55	5.42	-1.83
Cdk2	3.62	4.01	-1.31	3.83	4	-1.12
E2f3	5.85	6.3	-1.36	5.3	5.33	-1.02
Shc1	3.85	4.3	-1.36	2.63	2.76	-1.10
Sfn	8.39	8.85	-1.37	7.06	7.1	-1.03
Ccne1	5.01	5.47	-1.38	5.11	5.24	-1.09
Psmg2	3.07	3.56	-1.40	3.13	3.24	-1.08
E2f1	5.48	5.98	-1.41	4.57	4.76	-1.14
Ppm1d	4.24	4.74	-1.41	3.76	4.13	-1.29
Smc1a	3.99	4.48	-1.41	3.31	3.79	-1.40
Npm2	9.46	9.98	-1.43	8.65	8.75	-1.07
Cdc25a	4.36	4.88	-1.44	3.68	3.99	-1.25

Cks1b	3.01	3.56	-1.46	2.58	3.07	-1.41
Cdkn1b	3.79	4.36	-1.48	2.5	3.11	-1.52
Tfdp1	1.04	1.6	-1.48	1.08	1.42	-1.27
Skp2	5.42	6	-1.49	6.31	6.77	-1.37
Atm	5.56	6.14	-1.50	4.83	5.25	-1.34
Mre11a	5.1	5.68	-1.50	5.87	5.95	-1.06
Stag1	4.78	5.39	-1.52	3.86	4.37	-1.43
Terf1	4.71	5.34	-1.54	4.7	5.29	-1.51
Ccnb1	3.17	3.8	-1.55	3.11	4.38	-2.42
Hus1	6.29	6.94	-1.57	6.08	6.76	-1.60
Pcna	0.92	1.57	-1.57	1.33	2.25	-1.89
Ccna2	3.55	4.25	-1.62	2.79	4.22	-2.69
Ppp3ca	4.08	4.77	-1.62	2.86	3.16	-1.23
Rad21	1.95	2.65	-1.62	1.4	2.05	-1.57
Cdk4	1.2	1.91	-1.63	0.63	0.87	-1.18
Inha	7.77	8.47	-1.63	6.97	7.05	-1.06
Mtbp	5.13	5.84	-1.63	4.99	5.51	-1.43
Mcm3	2.86	3.57	-1.64	3.13	3.98	-1.80
Msh2	3.8	4.54	-1.66	3.71	4.12	-1.33
Chek1	5.48	6.22	-1.67	5.8	6.76	-1.95
Rad9	5.19	5.97	-1.72	4.73	5.23	-1.41
Nek2	4.28	5.07	-1.73	4.87	6.22	-2.55
Ccnb2	4.1	4.91	-1.74	4.1	5.13	-2.04
Rad51	4.99	5.81	-1.77	5.45	6.73	-2.42
Mad2l1	3.28	4.12	-1.79	3.27	4.4	-2.19
Trp53	1.83	2.68	-1.81	1.85	2.64	-1.72
Mcm4	2.32	3.18	-1.82	2.77	3.39	-1.54
Rbl1	5.69	6.57	-1.85	5.21	6.09	-1.85
Brca2	6.67	7.7	-2.04	5.97	6.64	-1.59
Wee1	5.24	6.27	-2.04	5.58	5.94	-1.28
Brca1	5.58	6.62	-2.06	5.7	7.24	-2.91
Ccnf	3.71	4.76	-2.06	3.47	4.78	-2.49
Mcm2	2.75	3.81	-2.07	3.15	4.42	-2.42
Mki67	2.94	4.07	-2.19	1.75	3.49	-3.34
E2f2	11.09	12.32	-2.35	11.35	11.75	-1.32
Trp63	10.58	13.4	-7.09	11.7	11.79	-1.06
Ppp2r3a	10.09	9.92	1.12	8.83	9.05	-1.17
Camk2a	9.14	9.14	-1.00	8.09	6.95	2.2
Pkd1	3.74	3.76	-1.01	2.73	2.43	1.23
Apbb1	8.76	8.87	-1.08	6.27	6.22	1.04
Ccnc	7.3	7.44	-1.10	5.99	5.82	1.13

Tsg101	7.03	7.17	-1.10	6.54	6.52	1.02
Nfatc1	4.46	4.63	-1.12	2.93	2.91	1.01
Sumo1	2.1	2.28	-1.13	1.76	1.61	1.11
Gpr132	12.38	12.6	-1.17	11.95	11.79	1.12
Abl1	9.49	9.76	-1.21	8.13	7.72	1.33
Dst	3.12	3.44	-1.25	2.2	2.07	1.09
Prm1	13.23	13.57	-1.26	12.07	11.79	1.21
Gadd45a	5.06	5.42	-1.28	4.41	3.51	1.86
Casp3	4.79	5.2	-1.32	3.98	3.86	1.09
Ccna1	13.27	13.75	-1.39	12.07	11.79	1.21
Sfn1	13.27	13.75	-1.39	12.07	11.79	1.21
Camk2b	11.89	12.55	-1.58	10.26	9.78	1.40
Myb	12.28	13.25	-1.96	11.85	11.79	1.04

Online Table IV: Cell cycle-focused RT-PCR gene arrays in CSP cells infected with sh-Scramble and sh-IGFBP3 lentiviruses and treated with Vehicle or Wnt3a-CM (5 days).

Cell cycle RT-PCR array							
Description	Symbol	Sh-Scramble			Sh-IGFBP3		
		(Δ Ct)		Fold change	(Δ Ct)		Fold change
		Vehicle	Wnt3a -CM		Vehicle	Wnt3a -CM	
Minichromosome maintenance deficient 3 (<i>S. cerevisiae</i>)	Mcm3	4.79	1.60	-9.18	1.58	2.00	1.33
Cyclin B1	Ccnb1	6.55	3.65	-7.51	1.93	3.32	2.61
Proliferating cell nuclear antigen	Pcna	4.66	1.78	-7.40	1.52	1.97	1.36
Cyclin-dependent kinase 2	Cdk2	7.43	4.56	-7.35	4.20	4.64	1.35
Cyclin A2	Ccna2	5.21	2.69	-5.77	1.12	2.21	2.12
Cyclin B2	Ccnb2	8.64	6.26	-5.23	4.61	5.02	1.33
Breast cancer 1	Brca1	7.76	5.59	-4.53	4.59	5.71	2.17
Minichromosome maintenance deficient 4 homolog (<i>S. cerevisiae</i>)	Mcm4	4.64	2.49	-4.46	2.06	2.25	1.14
MAD2 mitotic arrest deficient-like 1 (yeast)	Mad2l1	5.72	3.60	-4.37	3.05	3.12	1.05
E2F transcription factor 3	E2f3	7.48	5.55	-3.83	5.21	5.96	1.68
Antigen identified by monoclonal antibody Ki 67	Mki67	3.21	1.36	-3.63	1.25	1.50	1.19
Meiotic recombination 11 homolog A (<i>S. cerevisiae</i>)	Mre11a	8.64	6.82	-3.55	5.53	6.98	2.72
Breast cancer 2	Brca2	7.38	5.58	-3.50	3.52	4.55	2.04
Minichromosome maintenance deficient 2 mitotin (<i>S. cerevisiae</i>)	Mcm2	4.85	3.17	-3.22	2.49	2.89	1.32
RAN, member RAS oncogene family	Ran	0.93	-0.63	-2.96	-1.10	-0.94	1.11
Cyclin-dependent kinase inhibitor 1B	Cdkn1b	5.02	3.48	-2.92	3.11	3.24	1.09
Checkpoint kinase 1 homolog (<i>S. pombe</i>)	Chek1	6.89	5.43	-2.77	5.45	5.60	1.11
CDC28 protein kinase 1b	Cks1b	4.51	3.05	-2.77	2.65	2.78	1.09
Retinoblastoma-like 1 (p107)	Rbl1	6.76	5.32	-2.73	5.50	5.60	1.07
S-phase kinase-associated protein 2 (p45)	Skp2	8.54	7.10	-2.73	6.01	7.48	2.76
WEE 1 homolog 1 (<i>S. pombe</i>)	Wee1	7.44	6.04	-2.65	5.08	6.36	2.42
Caspase 3	Casp3	6.79	5.50	-2.46	4.30	5.99	3.22
SMT3 suppressor of mif two 3 homolog 1 (yeast)	Sumo1	4.33	3.06	-2.43	3.35	3.37	1.01
Cyclin F	Ccnf	4.41	3.17	-2.38	2.59	2.63	1.03

Mdm2, transformed 3T3 cell double minute p53 binding protein	Mtbp	5.70	4.50	-2.31	3.53	4.37	1.79
Retinoblastoma-like 2	Rbl2	8.63	7.45	-2.28	6.41	7.28	1.82
MutS homolog 2 (E. coli)	Msh2	4.31	3.16	-2.23	2.30	2.65	1.27
Pescadillo homolog 1, containing BRCT domain (zebrafish)	Pes1	1.99	0.86	-2.20	1.12	0.47	-1.57
Telomeric repeat binding factor 1	Terf1	5.49	4.38	-2.17	4.98	5.02	1.03
Ataxia telangiectasia mutated homolog (human)	Atm	5.78	4.70	-2.13	3.43	4.60	2.24
Tumor susceptibility gene 101	Tsg101	8.42	7.38	-2.07	5.42	6.43	2.01
C-abl oncogene 1, receptor tyrosine kinase	Abl1	7.41	6.60	-1.76	7.40	7.44	1.03
RAD51 homolog (S. cerevisiae)	Rad51	8.64	7.95	-1.62	7.17	7.68	1.42
Protein phosphatase 1D magnesium-dependent, delta isoform	Ppm1d	4.03	3.37	-1.59	2.86	2.93	1.05
RAD21 homolog (S. pombe)	Rad21	2.14	1.56	-1.50	1.58	1.21	-1.30
Transformation related protein 53	Trp53	1.88	1.34	-1.46	1.27	0.98	-1.23
Src homology 2 domain-containing transforming protein C1	Shc1	3.87	3.42	-1.37	2.41	2.92	1.42
NIMA (never in mitosis gene a) related expressed kinase 2	Nek2	4.80	4.36	-1.36	3.33	3.34	1.00
Cyclin C	Ccnc	8.64	8.25	-1.32	7.27	7.13	-1.11
Nucleophosmin/nucleoplasm in 2	Npm2	8.64	8.27	-1.30	8.47	8.03	-1.36
Protein phosphatase 3, catalytic subunit, alpha isoform	Ppp3ca	4.40	4.04	-1.29	2.79	2.66	-1.10
Structural maintenance of chromosomes 1A	Smc1a	4.22	3.91	-1.25	3.66	3.30	-1.29
Transcription factor Dp 1	Tfdp1	2.08	1.77	-1.25	1.04	1.15	1.08
Stromal antigen 1	Stag1	4.92	4.64	-1.22	4.49	4.20	-1.23
Cyclin E1	Ccne1	4.33	4.07	-1.20	3.28	4.89	3.04
E2F transcription factor 4	E2f4	2.94	2.70	-1.19	2.15	2.35	1.15
E2F transcription factor 1	E2f1	5.18	4.95	-1.18	3.82	4.28	1.37
RAD17 homolog (S. pombe)	Rad17	4.83	4.60	-1.18	4.48	4.40	-1.06
Adenylate kinase 1	Ak1	2.70	2.49	-1.16	1.27	2.16	1.85
RAD9 homolog (S. pombe)	Rad9	4.49	4.33	-1.12	4.37	3.99	-1.31
Hus1 homolog (S. pombe)	Hus1	5.87	5.73	-1.11	4.81	5.23	1.33
Growth arrest and DNA damage-inducible 45 alpha	Gadd45a	4.31	4.19	-1.09	3.45	4.07	1.53
Transformed mouse 3T3 cell double minute 2	Mdm2	2.92	2.85	-1.06	1.47	1.94	1.38
Proteasome (prosome, macropain) assembly chaperone 2	Psmg2	3.41	3.38	-1.03	2.73	3.04	1.24

Integrin beta 1 (fibronectin receptor beta)	Itgb1	-4.12	-1.26	7.22	-2.38	-1.51	1.82
Polycystic kidney disease 1 homolog	Pkd1	1.33	2.92	2.99	3.10	2.99	-1.08
DNA-damage inducible transcript 3	Ddit3	1.64	3.22	2.97	3.24	2.55	-1.62
Dystonin	Dst	0.26	1.84	2.97	1.46	0.73	-1.66
Sestrin 2	Sesn2	2.81	4.30	2.79	3.78	4.01	1.17
Microtubule-actin crosslinking factor 1	Macf1	0.44	1.75	2.47	1.36	1.32	-1.03
Cyclin D1	Ccnd1	1.46	2.70	2.35	1.66	2.58	1.89
Cyclin-dependent kinase inhibitor 1A (P21)	Cdkn1a	-0.79	0.45	2.35	0.07	0.24	1.12
Cyclin-dependent kinase inhibitor 2A	Cdkn2a	0.62	1.81	2.27	1.02	1.57	1.46
Calcium/calmodulin-dependent protein kinase II alpha	Camk2a	5.28	6.23	1.92	5.49	7.52	4.07
CDK5 regulatory subunit associated protein 1	Cdk5rap1	5.73	6.49	1.68	5.84	6.16	1.24
Stratifin	Sfn	6.24	6.99	1.67	6.26	7.06	1.74
Notch gene homolog 2 (Drosophila)	Notch2	3.58	4.27	1.60	3.73	3.74	1.00
Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	Nfatc1	3.94	4.45	1.42	3.85	3.41	-1.36
DnaJ (Hsp40) homolog, subfamily C, member 2	Dnajc2	2.04	2.44	1.31	1.89	1.77	-1.09
Amyloid beta (A4) precursor protein-binding, family B, member 1	Apbb1	5.56	5.93	1.29	5.68	5.79	1.08
Cyclin-dependent kinase 4	Cdk4	0.74	1.03	1.22	0.86	0.80	-1.05
Peripheral myelin protein 22	Pmp22	1.53	1.79	1.19	2.13	2.22	1.06
Inhibin alpha	Inha	6.58	6.81	1.17	7.22	6.23	-1.99
TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor	Taf10	0.11	0.22	1.07	0.10	-0.58	-1.61
Cell division cycle 25 homolog A (S. pombe)	Cdc25a	3.27	3.37	1.07	3.31	3.45	1.10
Calcium/calmodulin-dependent protein kinase II, beta	Camk2b	N/A	N/A	N/A	N/A	N/A	N/A
Cyclin A1	Ccna1	N/A	N/A	N/A	N/A	N/A	N/A
E2F transcription factor 2	E2f2	N/A	N/A	N/A	N/A	N/A	N/A
G protein-coupled receptor 132	Gpr132	N/A	N/A	N/A	N/A	N/A	N/A
Myeloblastosis oncogene	Myb	N/A	N/A	N/A	N/A	N/A	N/A
Protein phosphatase 2 (formerly 2A), regulatory subunit B", alpha	Ppp2r3a	N/A	N/A	N/A	N/A	N/A	N/A
Protamine 1	Prm1	N/A	N/A	N/A	N/A	N/A	N/A

Schlafen 1	Slfn1	N/A	N/A	N/A	N/A	N/A	N/A
Transformation related protein 63	Trp63	N/A	N/A	N/A	N/A	N/A	N/A

Online Table V: Wnt signaling focused RT-PCR gene array in freshly isolated CSP cells from sham and 1 day post-MI mice.

Wnt signaling RT-PCR array				
Description	Symbol	Day 1 post-MI (ΔCt)		
		Sham	post-MI	Fold change
Casein kinase 1, delta	Csnk1d	2.59	3.22	-1.55*
Jun oncogene	Jun	-2.42	-2.38	-1.02
F-box and WD-40 domain protein 11	Fbxw11	5.69	5.72	-1.02
Secreted frizzled-related protein 4	Sfrp4	5.67	5.72	-1.03
Amino-terminal enhancer of split	Aes	3.42	3.48	-1.04
Cyclin D3	Ccnd3	2.18	2.24	-1.04
Transducin-like enhancer of split 1, homolog of Drosophila E(spl)	Tle1	4.56	4.69	-1.09
Casein kinase 1, alpha 1	Csnk1a1	2.04	2.18	-1.10
Frizzled homolog 1 (Drosophila)	Fzd1	2.86	3.01	-1.10
Low density lipoprotein receptor-related protein 6	Lrp6	2.76	2.92	-1.11
Protein phosphatase 2a, regulatory subunit A (PR 65)	Ppp2r1a	1.53	1.71	-1.12
Casein kinase 2, alpha 1 polypeptide	Csnk2a1	2.48	2.64	-1.12
Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	Ppp2ca	1.39	1.58	-1.14
Dishevelled 2, dsh homolog (Drosophila)	Dvl2	4.31	4.52	-1.15
Kringle containing transmembrane protein 1	Kremen1	2.43	2.67	-1.17
C-terminal binding protein 1	Ctbp1	1.62	1.90	-1.21
Wingless-related MMTV integration site 4	Wnt4	5.44	5.72	-1.21
Secreted frizzled-related protein 2	Sfrp2	2.07	2.37	-1.22
Ras homolog gene family, member U	Rhou	4.83	5.14	-1.24
Secreted frizzled-related protein 1	Sfrp1	3.66	4.00	-1.26
Fos-like antigen 1	Fosl1	2.72	3.08	-1.27
Dishevelled, dsh homolog 1 (Drosophila)	Dvl1	5.01	5.37	-1.28
Catenin (cadherin associated protein), beta 1	Ctnnb1	0.37	0.78	-1.33
SUMO/sentrin specific peptidase 2	Senp2	5.31	5.72	-1.33
Low density lipoprotein receptor-related protein 5	Lrp5	2.51	2.97	-1.37
Frizzled homolog 7 (Drosophila)	Fzd7	3.80	4.26	-1.37

Frizzled homolog 2 (Drosophila)	Fzd2	5.67	5.20	1.38
Transcription factor 3	Tcf3	4.69	5.17	-1.39
Frizzled homolog 4 (Drosophila)	Fzd4	3.50	3.98	-1.39
C-terminal binding protein 2	Ctbp2	1.93	2.41	-1.39
Protein phosphatase 2d, regulatory subunit B (B56)	Ppp2r5d	3.18	3.70	-1.43
Axin 1	Axin1	3.51	4.13	-1.53
Catenin beta interacting protein 1	Ctnnbip1	4.56	5.22	-1.57
WNT1 inducible signaling pathway protein 1	Wisp1	5.02	5.72	-1.63
Frizzled homolog 5 (Drosophila)	Fzd5	4.04	4.78	-1.67
E1A binding protein p300	Ep300	4.61	5.37	-1.68
Cyclin D1	Ccnd1	4.52	5.27	-1.68
Frizzled homolog 6 (Drosophila)	Fzd6	4.48	5.28	-1.73
Naked cuticle 1 homolog (Drosophila)	Nkd1	3.89	4.87	-1.98
Adenomatous polyposis coli	Apc	4.03	5.24	-2.30
Solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1	Slc9a3r1	5.53	5.53	1.00
Myelocytomatosis oncogene	Myc	2.90	2.89	1.00
F-box and WD-40 domain protein 2	Fbxw2	3.22	3.20	1.01
B-cell CLL/lymphoma 9	Bcl9	5.76	5.72	1.02
Lymphoid enhancer binding factor 1	Lef1	5.76	5.72	1.02
Frizzled homolog 8 (Drosophila)	Fzd8	5.77	5.72	1.03
Cyclin D2	Ccnd2	2.72	2.65	1.05
SRY-box containing gene 17	Sox17	3.75	3.66	1.06
Wingless-type MMTV integration site 9A	Wnt9a	5.81	5.72	1.06
Pygopus 1	Pygo1	5.69	5.59	1.07
Wingless-related MMTV integration site 11	Wnt11	4.33	4.23	1.07
Dishevelled associated activator of morphogenesis 1	Daam1	3.57	3.45	1.08
Wingless related MMTV integration site 2b	Wnt2b	5.15	5.00	1.10
F-box and WD-40 domain protein 4	Fbxw4	5.87	5.72	1.10
Frizzled homolog 3 (Drosophila)	Fzd3	5.89	5.72	1.12
DIX domain containing 1	Dixdc1	5.61	5.40	1.15
Glycogen synthase kinase 3 beta	Gsk3b	5.97	5.72	1.18
Beta-transducin repeat containing protein	Btrc	6.03	5.72	1.23
Frizzled-related protein	Frzb	2.15	1.81	1.26
Porcupine homolog (Drosophila)	Porcn	6.03	5.64	1.30
Wingless-related MMTV integration site 5B	Wnt5b	5.56	5.16	1.32

Wingless-related MMTV integration site 5A	Wnt5a	6.15	5.72	1.34
Transcription factor 7, T-cell specific	Tcf7	5.86	5.21	1.57
Follicle stimulating hormone beta	Fshb	6.17	5.51	1.58
Wingless-related MMTV integration site 16	Wnt16	6.17	5.44	1.65
Wnt inhibitory factor 1	Wif1	4.42	3.08	2.52
Dickkopf homolog 1 (<i>Xenopus laevis</i>)	Dkk1	N/A	N/A	N/A
Fibroblast growth factor 4	Fgf4	N/A	N/A	N/A
Forkhead box N1	Foxn1	N/A	N/A	N/A
Frequently rearranged in advanced T-cell lymphomas	Frat1	N/A	N/A	N/A
Nemo like kinase	Nlk	N/A	N/A	N/A
Paired-like homeodomain transcription factor 2	Pitx2	N/A	N/A	N/A
Brachyury	T	N/A	N/A	N/A
Transducin-like enhancer of split 2, homolog of <i>Drosophila</i> E(spl)	Tle2	N/A	N/A	N/A
Wingless-related MMTV integration site 1	Wnt1	N/A	N/A	N/A
Wingless related MMTV integration site 10a	Wnt10a	N/A	N/A	N/A
Wingless-related MMTV integration site 2	Wnt2	N/A	N/A	N/A
Wingless-related MMTV integration site 3	Wnt3	N/A	N/A	N/A
Wingless-related MMTV integration site 3A	Wnt3a	N/A	N/A	N/A
Wingless-related MMTV integration site 6	Wnt6	N/A	N/A	N/A
Wingless-related MMTV integration site 7A	Wnt7a	N/A	N/A	N/A
Wingless-related MMTV integration site 7B	Wnt7b	N/A	N/A	N/A
Wingless-related MMTV integration site 8A	Wnt8a	N/A	N/A	N/A
Wingless related MMTV integration site 8b	Wnt8b	N/A	N/A	N/A

Online Table VI: Wnt signaling focused RT-PCR gene array in freshly isolated CSP cells from sham and 3 days post-MI mice.

Wnt signaling RT-PCR array				
Description	Symbol	Day 3 post-MI (ΔCt)		
		Sham	post-MI	Fold change
Frizzled homolog 5 (Drosophila)	Fzd5	4.03	5.03	-1.99*
Adenomatosis polyposis coli	Apc	4.03	5.07	-2.07*
Dishevelled 2, dsh homolog (Drosophila)	Dvl2	4.30	5.47	-2.25*
Amino-terminal enhancer of split	Aes	3.42	4.75	-2.52*
Transcription factor 3	Tcf3	4.68	6.09	-2.65*
E1A binding protein p300	Ep300	4.61	6.02	-2.66*
Secreted frizzled-related protein 4	Sfrp4	5.66	7.29	-3.00*
Frizzled homolog 6 (Drosophila)	Fzd6	4.48	6.44	-3.89*
Ras homolog gene family, member U	Rhou	4.83	4.84	-1.00
Frizzled-related protein	Frzb	2.15	2.18	-1.01
Frizzled homolog 1 (Drosophila)	Fzd1	2.86	2.91	-1.03
Casein kinase 1, alpha 1	Csnk1a1	2.04	2.23	-1.13
Wingless-related MMTV integration site 11	Wnt11	4.33	4.52	-1.14
Transducin-like enhancer of split 1, homolog of Drosophila E(spl)	Tle1	4.56	4.79	-1.16
Myelocytomatosis oncogene	Myc	2.90	3.16	-1.19
Secreted frizzled-related protein 1	Sfrp1	3.66	3.96	-1.22
Cyclin D1	Ccnd1	4.52	4.85	-1.25
Protein phosphatase 2a, regulatory subunit A (PR 65)	Ppp2r1a	1.53	1.91	-1.29
F-box and WD-40 domain protein 2	Fbxw2	3.22	3.61	-1.30
Wnt inhibitory factor 1	Wif1	4.42	4.85	-1.34
SUMO/sentrin specific peptidase 2	Senp2	5.31	5.74	-1.34
Fos-like antigen 1	Fosl1	2.72	3.19	-1.38
C-terminal binding protein 1	Ctbp1	1.62	2.10	-1.39
Low density lipoprotein receptor-related protein 6	Lrp6	2.76	3.26	-1.40
Dishevelled associated activator of morphogenesis 1	Daam1	3.57	4.12	-1.45
Transcription factor 7, T-cell specific	Tcf7	5.86	6.42	-1.47
F-box and WD-40 domain protein 11	Fbxw11	5.69	6.32	-1.54
Axin 1	Axin1	3.51	4.15	-1.55
Catenin (cadherin associated protein), beta 1	Ctnnb1	0.37	1.03	-1.57
Kringle containing transmembrane protein 1	Kremen1	2.43	3.13	-1.61

Frizzled homolog 2 (Drosophila)	Fzd2	5.67	6.36	-1.61
Casein kinase 1, delta	Csnk1d	2.59	3.31	-1.63
Solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1	Slc9a3r1	5.53	6.25	-1.64
C-terminal binding protein 2	Ctbp2	1.93	2.72	-1.72
Glycogen synthase kinase 3 beta	Gsk3b	5.97	6.76	-1.73
Wingless related MMTV integration site 2b	Wnt2b	5.15	5.94	-1.73
Cyclin D3	Ccnd3	2.18	2.99	-1.74
Wingless-related MMTV integration site 4	Wnt4	5.44	6.25	-1.75
Dishevelled, dsh homolog 1 (Drosophila)	Dvl1	5.01	5.82	-1.75
Casein kinase 2, alpha 1 polypeptide	Csnk2a1	2.48	3.30	-1.76
Low density lipoprotein receptor-related protein 5	Lrp5	2.51	3.34	-1.77
Wingless-related MMTV integration site 5A	Wnt5a	6.15	7.00	-1.80
Cyclin D2	Ccnd2	2.72	3.59	-1.82
Frizzled homolog 3 (Drosophila)	Fzd3	5.89	6.83	-1.91
Jun oncogene	Jun	-2.42	-1.47	-1.92
Porcupine homolog (Drosophila)	Porcn	6.03	7.00	-1.96
Frizzled homolog 8 (Drosophila)	Fzd8	5.77	6.76	-1.97
Frizzled homolog 7 (Drosophila)	Fzd7	3.80	4.85	-2.06
F-box and WD-40 domain protein 4	Fbxw4	5.87	6.95	-2.11
Wingless-type MMTV integration site 9A	Wnt9a	5.81	6.90	-2.11
Catenin beta interacting protein 1	Ctnnbip1	4.56	5.70	-2.20
Wingless-related MMTV integration site 5B	Wnt5b	5.56	6.71	-2.21
B-cell CLL/lymphoma 9	Bcl9	5.76	6.94	-2.25
Frizzled homolog 4 (Drosophila)	Fzd4	3.50	4.69	-2.27
Naked cuticle 1 homolog (Drosophila)	Nkd1	3.89	5.07	-2.27
DIX domain containing 1	Dixdc1	5.61	6.81	-2.28
Beta-transducin repeat containing protein	Btrc	6.03	7.23	-2.29
Lymphoid enhancer binding factor 1	Lef1	5.76	7.15	-2.61
Pygopus 1	Pygo1	5.69	7.16	-2.76
SRY-box containing gene 17	Sox17	3.75	5.44	-3.23
Secreted frizzled-related protein 2	Sfrp2	2.07	1.57	1.41
WNT1 inducible signaling pathway protein 1	Wisp1	5.02	4.71	1.23
Protein phosphatase 2d, regulatory subunit B (B56)	Ppp2r5d	3.18	3.03	1.11
Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	Ppp2ca	1.39	1.27	1.08
Dickkopf homolog 1 (Xenopus laevis)	Dkk1	N/A	N/A	N/A
Fibroblast growth factor 4	Fgf4	N/A	N/A	N/A
Forkhead box N1	Foxn1	N/A	N/A	N/A

Frequently rearranged in advanced T-cell lymphomas	Frat1	N/A	N/A	N/A
Follicle stimulating hormone beta	Fshb	N/A	N/A	N/A
Nemo like kinase	Nlk	N/A	N/A	N/A
Paired-like homeodomain transcription factor 2	Pitx2	N/A	N/A	N/A
Brachyury	T	N/A	N/A	N/A
Transducin-like enhancer of split 2, homolog of Drosophila E(spl)	Tle2	N/A	N/A	N/A
Wingless-related MMTV integration site 1	Wnt1	N/A	N/A	N/A
Wingless related MMTV integration site 10a	Wnt10a	N/A	N/A	N/A
Wingless-related MMTV integration site 16	Wnt16	N/A	N/A	N/A
Wingless-related MMTV integration site 2	Wnt2	N/A	N/A	N/A
Wingless-related MMTV integration site 3	Wnt3	N/A	N/A	N/A
Wingless-related MMTV integration site 3A	Wnt3a	N/A	N/A	N/A
Wingless-related MMTV integration site 6	Wnt6	N/A	N/A	N/A
Wingless-related MMTV integration site 7A	Wnt7a	N/A	N/A	N/A
Wingless-related MMTV integration site 7B	Wnt7b	N/A	N/A	N/A
Wingless-related MMTV integration site 8A	Wnt8a	N/A	N/A	N/A
Wingless related MMTV integration site 8b	Wnt8b	N/A	N/A	N/A

Online Table VII: Wnt signaling focused RT-PCR gene array in freshly isolated CSP cells from sham and 7 days post-MI mice.

Wnt signaling RT-PCR array				
Description	Symbol	Day 7 post-MI (ΔCt)		
		Sham	post-MI	Fold change
Catenin (cadherin associated protein), beta 1	Ctnnb1	0.09	0.47	-1.30*
C-terminal binding protein 1	Ctbp1	1.73	2.36	-1.54*
Wnt inhibitory factor 1	Wif1	3.50	4.34	-1.78*
Casein kinase 1, alpha 1	Csnk1a1	1.99	2.87	-1.83*
Cyclin D2	Ccnd2	2.13	3.58	-2.74*
Amino-terminal enhancer of split	Aes	2.77	4.74	-3.91*
Protein phosphatase 2a, regulatory subunit A (PR 65)	Ppp2r1a	1.79	1.80	-1.00
Frizzled homolog 1 (Drosophila)	Fzd1	3.11	3.13	-1.01
Pygopus 1	Pygo1	5.13	5.17	-1.02
Transcription factor 3	Tcf3	4.80	4.84	-1.02
Casein kinase 2, alpha 1 polypeptide	Csnk2a1	2.83	2.88	-1.03
Solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1	Slc9a3r1	4.94	5.02	-1.05
Frizzled-related protein	Frzb	2.12	2.23	-1.07
Frizzled homolog 5 (Drosophila)	Fzd5	4.11	4.22	-1.07
Wingless-related MMTV integration site 11	Wnt11	4.53	4.66	-1.09
Dishevelled 2, dsh homolog (Drosophila)	Dvl2	4.16	4.31	-1.10
Frizzled homolog 3 (Drosophila)	Fzd3	5.09	5.34	-1.18
Myelocytomatosis oncogene	Myc	3.08	3.32	-1.18
Frizzled homolog 8 (Drosophila)	Fzd8	5.01	5.27	-1.19
Frizzled homolog 4 (Drosophila)	Fzd4	3.14	3.41	-1.2
Secreted frizzled-related protein 1	Sfrp1	3.78	4.06	-1.21
Secreted frizzled-related protein 4	Sfrp4	5.04	5.34	-1.22
Protein phosphatase 2d, regulatory subunit B (B56)	Ppp2r5d	3.04	3.37	-1.24
C-terminal binding protein 2	Ctbp2	1.86	2.25	-1.31
Casein kinase 1, delta	Csnk1d	2.82	3.26	-1.35
Low density lipoprotein receptor-related protein 6	Lrp6	2.57	3.01	-1.35
B-cell CLL/lymphoma 9	Bcl9	4.88	5.34	-1.36
Naked cuticle 1 homolog (Drosophila)	Nkd1	3.72	4.16	-1.36
F-box and WD-40 domain protein 2	Fbxw2	2.66	3.12	-1.37
E1A binding protein p300	Ep300	4.21	4.69	-1.38
SRY-box containing gene 17	Sox17	2.71	3.19	-1.39
Transcription factor 7, T-cell specific	Tcf7	4.37	4.85	-1.39
Kringle containing transmembrane protein 1	Kremen1	2.47	2.96	-1.40

Low density lipoprotein receptor-related protein 5	Lrp5	2.10	2.63	-1.44
Porcupine homolog (Drosophila)	Porcn	4.79	5.34	-1.45
Frizzled homolog 7 (Drosophila)	Fzd7	3.93	4.49	-1.47
Wingless-related MMTV integration site 5A	Wnt5a	4.72	5.29	-1.48
Jun oncogene	Jun	-2.87	-2.22	-1.57
SUMO/sentrin specific peptidase 2	Senp2	4.24	4.93	-1.6
Ras homolog gene family, member U	Rhou	4.11	4.80	-1.61
Frizzled homolog 2 (Drosophila)	Fzd2	4.63	5.34	-1.62
Catenin beta interacting protein 1	Ctnnbip1	3.96	4.74	-1.71
Cyclin D3	Ccnd3	1.33	2.12	-1.72
Wingless related MMTV integration site 2b	Wnt2b	4.05	4.85	-1.73
Adenomatosis polyposis coli	Apc	3.79	4.64	-1.80
Fos-like antigen 1	Fosl1	3.24	4.17	-1.90
Cyclin D1	Ccnd1	3.46	4.41	-1.92
Axin 1	Axin1	3.45	4.47	-2.01
Dishevelled associated activator of morphogenesis 1	Daam1	3.15	4.20	-2.06
Dishevelled, dsh homolog 1 (Drosophila)	Dvl1	3.91	5.06	-2.21
Secreted frizzled-related protein 2	Sfrp2	3.07	1.66	2.66*
Transducin-like enhancer of split 1, homolog of Drosophila E(spl)	Tle1	5.04	4.21	1.77
Wingless-related MMTV integration site 5B	Wnt5b	4.70	4.44	1.19
Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	Ppp2ca	1.42	1.28	1.09
WNT1 inducible signaling pathway protein 1	Wisp1	5.13	5.01	1.09
Wingless-related MMTV integration site 4	Wnt4	5.13	5.00	1.09
Frizzled homolog 6 (Drosophila)	Fzd6	3.97	3.96	1.00
Glycogen synthase kinase 3 beta	Gsk3b	5.13	5.12	1.00
Beta-transducin repeat containing protein	Btrc	N/A	N/A	N/A
DIX domain containing 1	Dixdc1	N/A	N/A	N/A
Dickkopf homolog 1 (Xenopus laevis)	Dkk1	N/A	N/A	N/A
F-box and WD-40 domain protein 11	Fbxw11	N/A	N/A	N/A
F-box and WD-40 domain protein 4	Fbxw4	N/A	N/A	N/A
Fibroblast growth factor 4	Fgf4	N/A	N/A	N/A
Forkhead box N1	Foxn1	N/A	N/A	N/A
Frequently rearranged in advanced T-cell lymphomas	Frat1	N/A	N/A	N/A
Follicle stimulating hormone beta	Fshb	N/A	N/A	N/A
Lymphoid enhancer binding factor 1	Lef1	N/A	N/A	N/A
Nemo like kinase	Nlk	N/A	N/A	N/A
Paired-like homeodomain transcription factor 2	Pitx2	N/A	N/A	N/A
Brachyury	T	N/A	N/A	N/A

Transducin-like enhancer of split 2, homolog of Drosophila E(spl)	Tle2	N/A	N/A	N/A
Wingless-related MMTV integration site 1	Wnt1	N/A	N/A	N/A
Wingless related MMTV integration site 10a	Wnt10a	N/A	N/A	N/A
Wingless-related MMTV integration site 16	Wnt16	N/A	N/A	N/A
Wingless-related MMTV integration site 2	Wnt2	N/A	N/A	N/A
Wingless-related MMTV integration site 3	Wnt3	N/A	N/A	N/A
Wingless-related MMTV integration site 3A	Wnt3a	N/A	N/A	N/A
Wingless-related MMTV integration site 6	Wnt6	N/A	N/A	N/A
Wingless-related MMTV integration site 7A	Wnt7a	N/A	N/A	N/A
Wingless-related MMTV integration site 7B	Wnt7b	N/A	N/A	N/A
Wingless-related MMTV integration site 8A	Wnt8a	N/A	N/A	N/A
Wingless related MMTV integration site 8b	Wnt8b	N/A	N/A	N/A
Wingless-type MMTV integration site 9A	Wnt9a	N/A	N/A	N/A

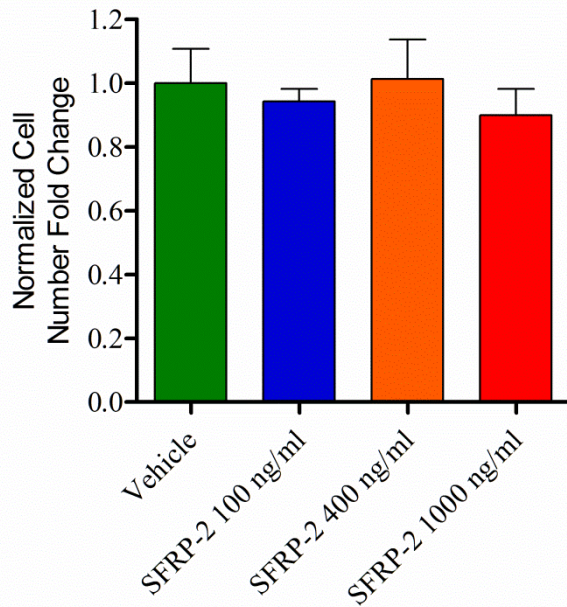
Online Table VIII: Order of Wnt signaling relates genes on the RT-PCR array 96-well plate corresponding to Figure 4A-C. MGDC (Mouse Genomic DNA Contamination), RTC (Reverse Transcription Control) and PPC (Polymerase PCR Control) represent internal array controls and are not depicted in **Figure 4A-C**.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Aes	Apc	Axin1	Bcl9	Btrc	Ctnnb1 p1	Ccnd1	Ccnd2	Ccnd3	Csnk1 a1	Csnk1 d	Csnk2 a1
B	Ctbp 1	Ctbp2	Ctnnb1	Daam 1	Dixdc1	Dkk1	Dvl1	Dvl2	Ep300	Fbxw1 1	Fbxw2	Fbxw4
C	Fgf4	Fosl1	Foxn1	Frat1	Frzb	Fshb	Fzd1	Fzd2	Fzd3	Fzd4	Fzd5	Fzd6
D	Fzd7	Fzd8	Gsk3b	Jun	Kremen1	Lef1	Lrp5	Lrp6	Myc	Nkd1	NIK	Pitx2
E	Porcn	Ppp2ca	Ppp2r1 a	Ppp2r5d	Pygo1	Rhou	Senp2	Sfrp1	Sfrp2	Sfrp4	Slc9a3 r1	Sox17
F	T	Tcf7l1	Tcf7	Tle1	Tle2	Wif1	Wisp1	Wnt1	Wnt10 a	Wnt11	Wnt16	Wnt2
G	Wnt2b	Wnt3	Wnt3a	Wnt4	Wnt5a	Wnt5b	Wnt6	Wnt7a	Wnt7b	Wnt8a	Wnt8b	Wnt9a
H	Gusb	Hprt	Hsp90ab1	Gapdh	Actb	MGDC	RTC	RTC	RTC	PPC	PPC	PPC

Supplemental References

1. Mouquet F, Pfister O, Jain M, Oikonomopoulos A, Ngoy S, Summer R, Fine A, Liao R. Restoration of cardiac progenitor cells after myocardial infarction by self-proliferation and selective homing of bone marrow-derived stem cells. *Circ Res*. 2005;97(11):1090-1092.
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4. Pfister O, Oikonomopoulos A, Sereti KI, Sohn RL, Cullen D, Fine GC, Mouquet F, Westerman K, Liao R. Role of the ATP-binding cassette transporter *Abcg2* in the phenotype and function of cardiac side population cells. *Circ Res*. 2008;103(8):825-835.
5. Shi J, Guan J, Jiang B, Brenner DA, Del Monte F, Ward JE, Connors LH, Sawyer DB, Semigran MJ, Macgillivray TE, Seldin DC, Falk R, Liao R. Amyloidogenic light chains induce cardiomyocyte contractile dysfunction and apoptosis via a non-canonical p38alpha MAPK pathway. *Proc Natl Acad Sci U S A*. 2010;107(9):4188-4193.

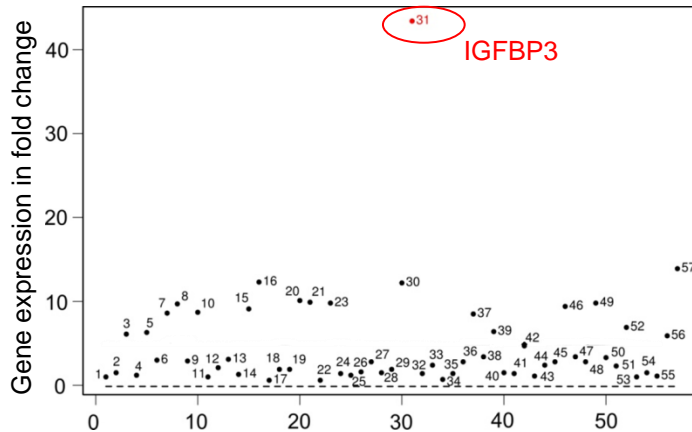
Online Figure I



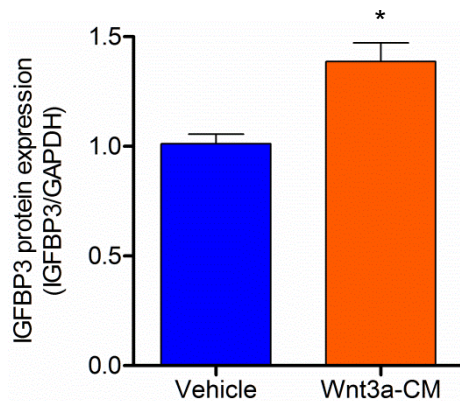
Online Figure I: Administration of SFRP2 does not alter CSP cell proliferation capacity in vitro. Treatment of CSP cells with increasing concentrations of SFRP2 protein had no effect on CSP cell numbers compared to Vehicle treated cells (n=3). Data are mean \pm s.e.m. Cell number in fold change is normalized to Vehicle group.

Online Figure II

A

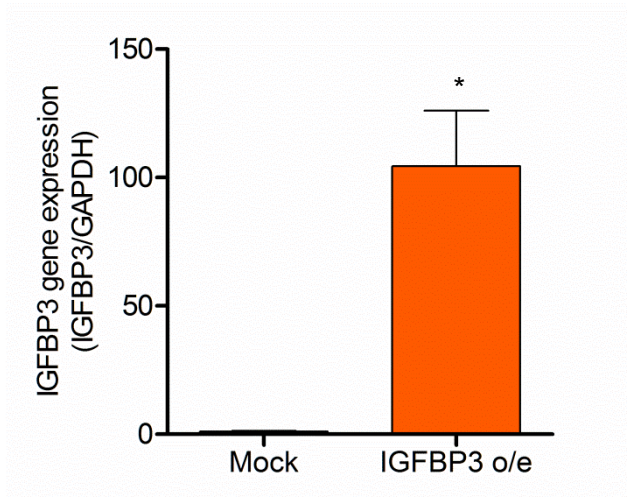


B



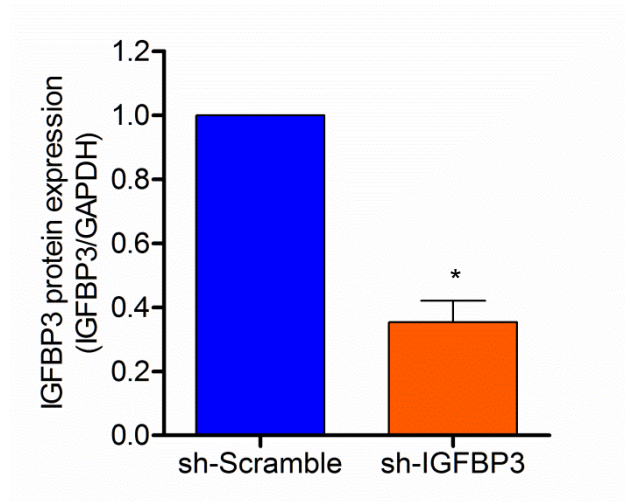
Online Figure II: Activation of Wnt signaling pathway up-regulates IGFBP3 expression in CSP cells. (A) Scatter plot analysis of pathway finder RT-PCR gene array in CSP cells following treatment (6 days) with Vehicle or Wnt3a-CM. IGFBP3 (red dot) was profoundly up-regulated among various genes related to different signaling pathways (n=3). The gene corresponding to each number can be found in Online Table II (second column). **(B)** Quantification of Western blot analysis of IGFBP3 protein expression in CSP cells following treatment with Wnt3a-CM (48 hours) (n=3). Data are mean \pm s.e.m. * P \leq 0.05.

Online Figure III



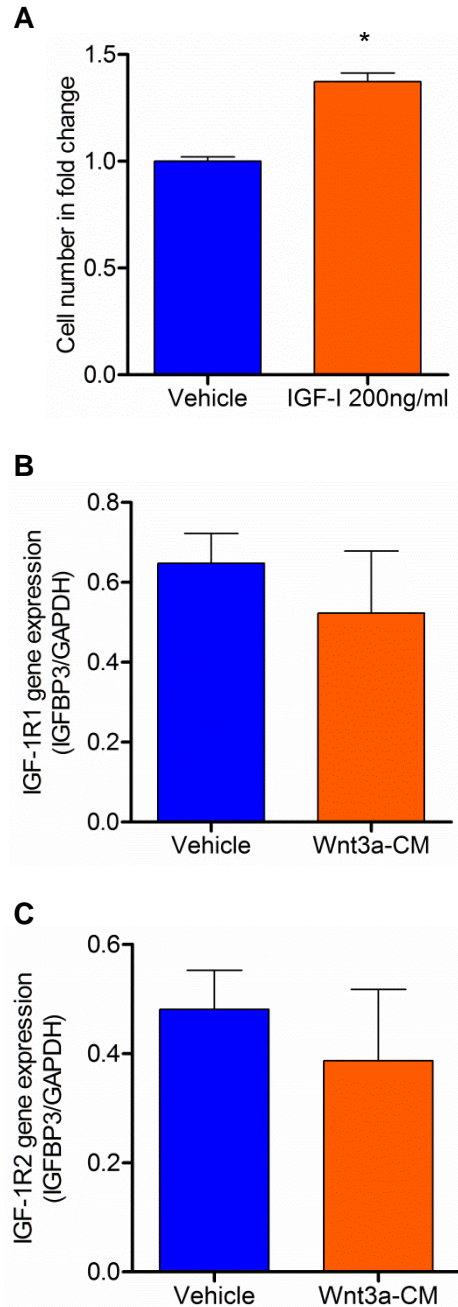
Online Figure III: Lentivirus-mediated over-expression of IGFBP3. RT-PCR mRNA expression analysis of IGFBP3 in CSP cells infected with Mock or IGFBP3-over-expressing lentivirus (IGFBP3 o/e) (n=3). Data are mean \pm s.e.m. * $P \leq 0.05$.

Online Figure IV



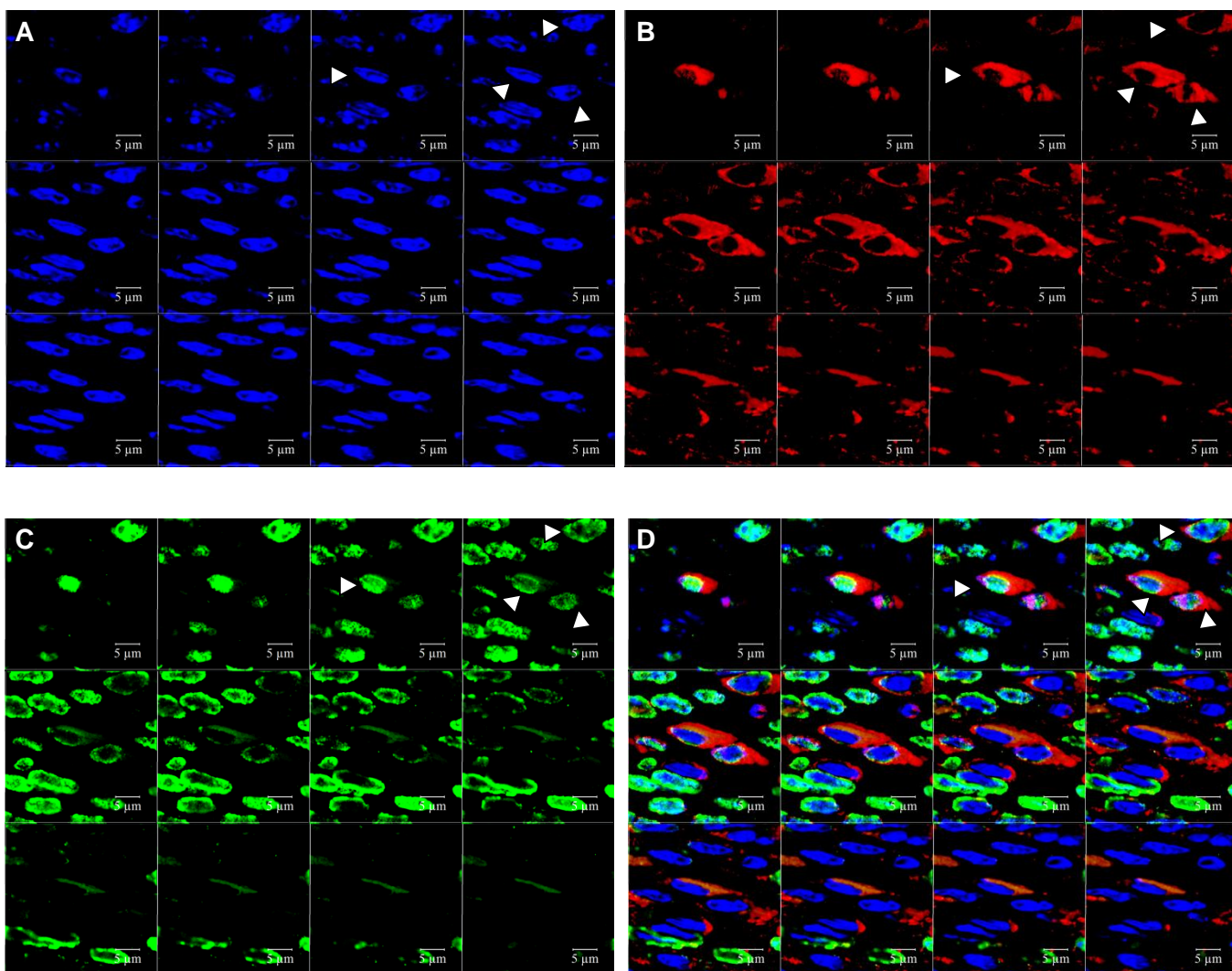
Online Figure IV: shRNA mediated down-regulation of IGFBP3. Quantification of Western blot analysis of IGFBP3 protein expression in sh-Scramble and sh-IGFBP3 CSP cells (n=3). Data are mean \pm s.e.m. * $P \leq 0.05$.

Online Figure V



Online Figure V: IGF-1 increases CSP proliferation capacity. (A) Treatment of CSP cells with 200ng/ml of IGF-1 protein resulted in increased proliferation capacity compared to Vehicle treated cells (n=3). RT-PCR mRNA expression analysis of (B) IGF-1R1 and (C) IGF-1R2 following Vehicle and Wnt3a treatment (n=3). Data are mean \pm s.e.m. * $P \leq 0.05$. 33

Online Figure VI



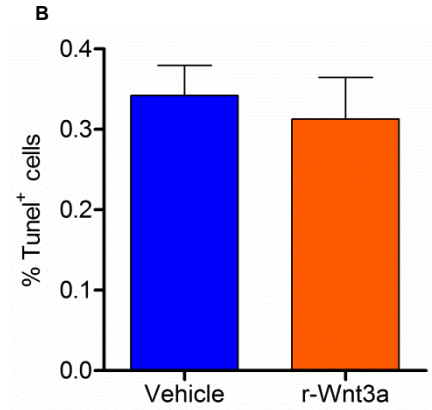
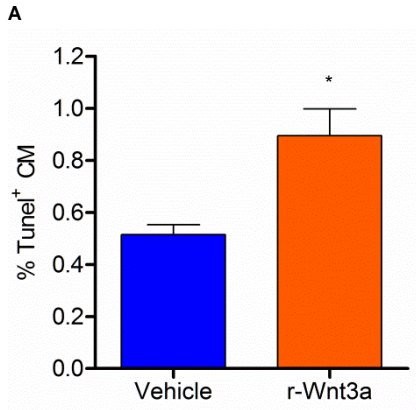
Alexa488 (BrdU)

Alexa-555 (α -s-Actin)

DAPI (Nuclei)

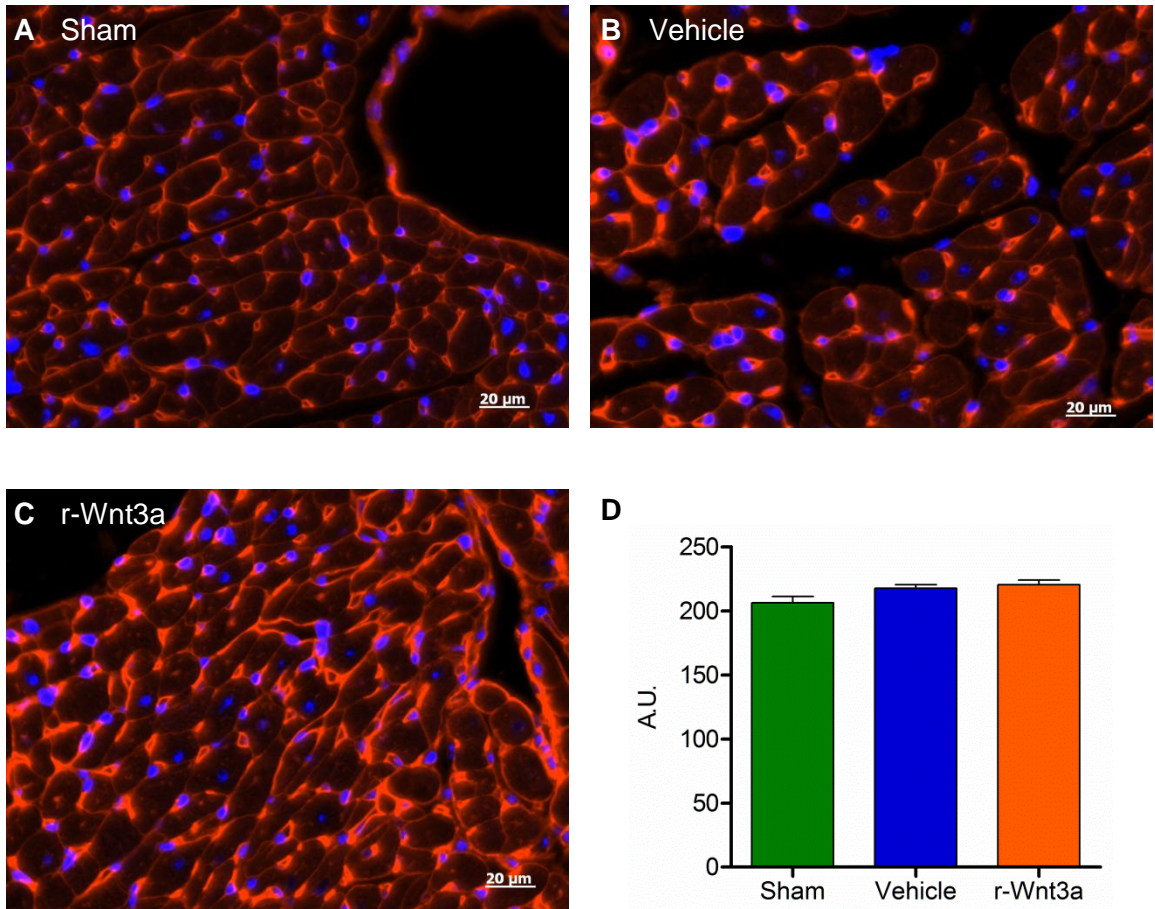
Online Figure VI: BrdU⁺ cardiomyocytes in infarct/border zone area of r-Wnt3a-injected heart. (A-D) Each panel shows a series of 12 representative confocal z-stack immuno-fluorescent images of BrdU⁺ cardiomyocytes in high magnification (x200). White arrowheads indicate the position of four BrdU⁺ cardiomyocytes. Myocardial sections were immuno-labeled with (A) DAPI (blue), (B) α -sarcomeric-actin (red), (C) BrdU (green). (D) Merged picture. (Scale bars, 5 μ m).

Online Figure VII



Online Figure VII: Administration of r-Wnt3a increases cardiomyocyte cell death post-MI. Injection of r-Wnt3a resulted in **(A)** increased TUNEL⁺ cardiomyocytes in the infarct/border zone area one week post-MI compared to Vehicle injected hearts. **(B)** r-Wnt3a had no effect on total cell death (Vehicle n=4, r-Wnt3a n=5).

Online Figure VIII



Online Figure VIII: Administration of r-Wnt3a does not alter cardiomyocyte cross-sectional area post-MI. Representative examples of WGA staining in (A) Sham, (B) Vehicle and (C) r-Wnt3a injected hearts one week post-MI. (D) Quantification of cardiomyocyte cross-sectional area revealed no significant differences between the groups (n=4). Sections were immuno-labeled with WGA (red) and DAPI (blue). A.U. arbitrary units. (Scale bars 20 μm).