### Supplementary Figure S1A



### Supplementary Figure S1B



### Supplementary Figure S1C





### Supplementary Figure S1D

MI



# Supplementary Figure S2









### Supplmentary Figure S3

### Cell cycle

ΒZ	IA		
		Trp53	transformation related protein 53
		Pdgfra	platelet derived growth factor receptor, alpha polypeptide
		Ednra	endothelin receptor type A
		Inhba	inhibin beta-A
		Myh9	myosin, heavy polipeptide 9, non-muscle
		ll31ra	interleukin 31 receptor A
		Acvr1b	activin A receptor, type 1B
		Pik3cd	phosphatidylinositol 3-kinase catalytic delta polypeptide
		Morf4I1	mortality factor 4 like 1
		Ovol2	ovo-like 2 (Drosophila)
		Nkx2-3	NK2 transcription factor related, locus 3 (Drosophila)
		Mycn	v-myc myelocytomatosis viral related oncogene
		Scg2	secretogranin II
		Pax3	paired box gene 3
		Fgf2	fibroblast growth factor 2
		Cfl1	cofilin 1, non-muscle
	_	Prkg2	protein kinase, cGMP-dependent, type II
		Atp7a	ATPase, Cu++ transporting, alpha polypeptide
		Hif1a	hypoxia inducible factor 1, alpha subunit
		Cat1	chromatin licensing and DNA replication factor 1
		Tes	testis derived transcript
		Radyb	RAD9 nomolog B (S. cerevisiae)
		STUUD	S100 protein, beta polypeptide, neural
		Gasz	growth arrest specific 2
		Cases1e	colony sumulating factor i (macrophage)
		Dalith	Calcium channel, voltage-dependent, P/Q type, alpha TA sub
		Dthib	B-cell leukenila/lymphoma TTB
		Fuilli Impdh1	inosine 5' phosphate debudrogenase 1
			histocompatibility 2 class II locus DMa
		Libr2	ubiquitin protein ligase E3 component p-recognin 2
			ubiquitin protein igase Lo component n-recognin z

### Tissue and cell development



	paired box gene 3 achaete-scute complex homolog-like 2 (Drosophila) activin A recentor type 1B
	EGF-like-domain, multiple 6
	phospholamban
	ovo-like 2 (Drosophila)
	lymphoid enhancer binding factor 1
	fibroblast growth factor 2
8	a disintegrin and metallopeptidase domain 18
	endothelin receptor type A
	myosin, heavy polypeptide 9, non-muscle
	ectodysplasin-A
	RUN and FYVE domain containing 3
	hypoxia inducible factor 1, alpha subunit
	zinc finger protein 39
	drebrin 1
	delta-like 3 (Drosophila)
	roundabout homolog 3 (Drosophila)

#### Gene expression

Trp53	transformation related protein 53
1 2864	longevity assurance homolog $A$ (S. cerevisiae)
Irf3	interferon regulatory factor 3
Bbx	hobby sox homolog (Drosonhila)
Pax3	paired box gene 3
Ascl2	achaete-scute complex homolog-like 2 (Drosophila)
Mrpl27	mitochondrial ribosomal protein L27
Eif4a1	eukarvotic translation initiation factor 4A1
Tle4	transducin-like enhancer of split 4, homolog of Drosophila E(spl
Nkx2-3	NK2 transcription factor related, locus 3 (Drosophila)
Taf1a1	TATA box binding protein (Tbp)-associated factor, RNA pol.I, A
Per3	period homolog 3 (Drosophila)
Sorbs3	sorbin and SH3 domain containing 3
Nfe2I3	nuclear factor, erythroid derived 2, like 3
Morf4I1	mortality factor 4 like 1
Nr0b2	nuclear receptor subfamily 0, group B, member 2
Zfp93	zinc finger protein 93
Mycn	v-myc myelocytomatosis viral related oncogene
 TIx2	T-cell leukemia, homeobox 2
 Ovol2	ovo-like 2 (Drosophila)
 Lef1	lymphoid enhancer binding factor 1
 HIC2	nypermethylated in cancer 2
 PSID1	PC4 and SFRS1 interacting protein 1
Tet	thyrotroph embryonic factor
Серро	CCAAT/ennancer binding protein (C/EBP), beta
Ppici	Wiekott Aldrich averdrome like (human)
 VVdSI	vacualar protein sorting 25 (vesst)
Noc2l	nucleolar complex associated 2 homolog (S. cerevisiae)
Fah	fumarylacetoacetate hydrolase
Strm	striamin
Fus	fusion, derived from t(12:16) malignant liposarcoma (human)
Max	Max protein
Aplp1	amyloid beta (A4) precursor-like protein 1
Bcl11b	B-cell leukemia/lymphoma 11B
Jarid1a	jumonji, AT rich interactive domain 1A (Rbp2 like)
Pum1	pumilio 1 (Drosophila)
Mrps9	mitochondrial ribosomal protein S9
Phox2a	paired-like homeobox 2a
Hif1a	hypoxia inducible factor 1, alpha subunit
 Pum2	pumilio 2 (Drosophila)
 Gfm2	G elongation factor, mitochondrial 2
 Rreb1	ras responsive element binding protein 1
 Ube3a	ubiquitin protein ligase E3A
wrps24	mitochondriai ribosomai protein S24
Zipsa Mfkhio	zine iniger protein 39 nuclear factor kappa light polynon
Free8	nuclear racion kappa light polypep
Vdr	vitamin D recentor
vui	



# Supplementary Figure S4

# Supplementary Table S1

Accession No.	Gene	Forward Primer	Reverse primer
NM_010849	Мус	AGCTGAAGCGCAGCTTTTT	GGCCTTTTCGTTGTTTTCCA
NM_020510	Fzd2	TCCGCATCCGCACCAT	CCATGAGCCTCTCCAGCTTCT
NM_010928	Notch2	AAATGAACCAAAGGTGTTCAGTGTT	CATTCAACGCGCTGGTTAAA
U43691	Notch4	TGTGGGCGAATTGGGTGTA	GCAGTAGATAGCAGAGGCTCCTTT
NM_009519	Wnt11	TTCCAGGCTGCTCCAAGAA	ATTCCAGAAAGCCGGTCTTTC
NM_010851	Myd88	TGGGCTACATGAGAGCCTACCT	ACAGTGCCCCCAGATTTTCC
NM_008416	JunB	CAGCTCAAGCAGAAGGTCATGA	GGGCAAGGGAGGCTCTCA
NM_010588	Jagged-1	CTTTCACCCTCATCGTGGA	TCAGCAGCTCCTCATCTGG

Gene/miR	Assay ID	
TBX5	Mm00803521_m1	
Tie-2	Mm00443242_m1;	
sm-MHC	Mm00443013_m1	
GAPDH	Mm99999915_g1	
Hes1	Mm01342805_m1	
Hey1	Mm00468865_m1	
Jagged-1	Mm00496902_m1	
Notch1	Mm00435245_m1	
miR-16	000391	
hsa-mir-208b	002290	
hsa-mir-301a	000528	
hsa-mir-483-5p	002338	
mmu-mir-675-5p	001940	
hsa-mir-711	001646	
mmu-mir-882	002610	
mmu-mir-204	000508	

# Supplementary Table S6

# PUTATIVE TARGETS\_MiROnTop

miR-208b	miR-301a
ADAM22	CSF1
ADAMDEC1	DDX49
ATP8B2	ERCC8
CHIC1	GNG12
EDNRA	GOLT1B
EMB	HIF1A
EPN3	IMPDH1
GUCA2B	MBP
HIC2	PLAT
PDLIM5	PTHLH
SLA	RANBP17
SLC1A2	SCD1
SLC22A4	SFRS2
SLC4A8	SLC12A2
TLE4	SNTB2
TRP53	TES
YWHAZ	UBE2G2
	VPS39

#### SUPPLEMENTARY FIGURE LEGENDS

**Supplementary Figure S1. Gene expression analysis in control and infarcted hearts**. Hierarchical clustering of differentially expressed genes shown in Figures 1 and 3 in the main text.

**Supplementary Figure S2.** Real time PCR validation of gene chip microarray expression analysis in the BZ and in the IA in the absence (A) and in the presence of HMGB1 treatment (B). Data were normalized to GAPDH, a housekeeping gene and represent means $\pm$ SE; (n=4, p<0.05).

**Supplementary Figure S3.** Clustered expression pattern of genes in HMGB1-treated hearts, within the indicated functional groups. Differentially expressed genes were categorized on the basis of known functions. Each row represents the expression of a single gene and columns 1 and 2 correspond to a sample pool of 3 BZ and IA of HMGB1-treated hearts. Expression levels are represented by a color tag, with red representing the highest levels and green the lowest levels of expression.

**Supplementary Figure S4.** Real time PCR validation of miR-208b and miR-301a expression in the indicated region of the heart after MI and HMGB1 treatment. Data were normalized to miR-16 expression and represent means±SE; (n=3, \*p<0.01 vs SO; \*\*p<0.05 vs BZ Ctrl; †p<0.001 vs BZ).

Supplementary Table S1. mRNA and miRNA probe list used in validation studies.

**Supplementary Table S2.** mRNA array results in the border zone and in the infarcted area of untreated and HMGB1-treated hearts.

Supplementary Table S3. IPA of expressed genes in untreated hearts.

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Supplementary Table S4. IPA of expressed genes in HMGB1-treated hearts.

**Supplementary Table S5.** List of modulated miRNA in the BZ and IA of untreated and HMGB1treated hearts.

Supplementary Table S6. List of putative miR208b and miR301a targets.

#### SUPPLEMENTARY MATERIALS AND METHODS

#### miRNA and mRNA isolation and quantification.

Quality of RNA was checked using the Agilent 2100 Bioanalyzer and nanodrop 1000. mRNAs and miRNA levels were analyzed using the SYBR-GREEN qPCR method (5 ng/assay, Qiagen) and the TaqMan quantitative real-time PCR (qPCR) method (1 ng/assay), respectively. Then both mRNA and miRNA were quantified with ABI Prism 7000 SDS (Applied Biosystems). Relative expression was calculated using the comparative Ct method (2–[delta][delta]Ct)<sup>1</sup>.

Validation was performed using mRNA primers reported in Supplementary Table S1. The expression of cardiogenic markers of Notch target genes was assessed using primers from (Applied Biosystems) (Supplementary Table S1). Individual mature miRNAs were measured using TaqMan MicroRNA single assays (Applied Biosystems) and primer codes were reported in Supplementary Table S1.

#### mRNA array.

For hybridization experiments, 10 µg of total RNA was used to synthesize double-stranded cDNA (Affymetrix, Santa Clara, CA,USA). After purification double stranded cDNA was used to produce Cr-3-5 labeled cRNA. Microarray analysis was performed in triplicate using the Mouse Genome 430A 2 array (Affymetrix) containing 14.000 genes, according to Affymetrix Expression Analysis technical manual. The results were analyzed using customized script which utilizes Bioconductor packages (<u>www.bioconductor.org</u>) based on the R language (www.r-project.org), for quality control analysis, data normalization, hierarchical cluster and identification of differentially expressed transcripts. The mRNA expression level of a transcript is directly related to the signal intensity and can be calculated for each probe set with different methods. Our R-script provides utilization of differentially expressed transcripts. Scanned images were first inspected for quality control (QC) using a variety of built-in QC tools package. QC consisted of visual examination of probe array images, scatter plots from replicates, RNA degradation plots and

*MAplots* was for quality control analysis. Specifically, the *gcrma* package was used for chip normalization and background correction. The genefilter package was used to separate genes with high variance according to the interquartile range method (IQR). *samr*-package, significance analysis of microarrays (SAM)<sup>2</sup>, was used for the detection of significantly expressed genes between two groups and to control the false discovery rate (FDR). Briefly, SAM calculates a score for each gene on the basis of the change in expression relative to the standard deviation of all measurements by compute t-statistic for each gene and then performs a set of permutations to determine the false discovery rate by shuffling the class labels (1000 permutations in our analysis). The settings for this analysis were as follows: two-class response. Once the program reported the list of ranked genes, the "delta value" was adjusted to a stringent false discovery rate (FDR%). Function and Pathway Analysis of the modulated genes was performed using Ingenuity Pathways Knowledge Base (version 8.8, Ingenuity Systems) as reference set and assuming direct and indirect relationships. A Fisher's exact test p-value< 0.05 was deemed as statistically significant.

#### miRNA array.

Two-color hybridization was performed with total RNA extracted for mRNA studies, using miRCURY LNA microRNA Arrays (v.10.0, EXIQON). The obtained data were analyzed using the Limma package from the Bioconductor Project, subjecting the arrays to locally weighted scatter plot smoothing (Loess). miRNAs with spots showing less than 1.5 times median signal intensity were not considered for subsequent analysis. Modulated miRNAs were validated by qPCR (Applied Biosystems). Bioinformatic prediction of miRNA target genes was performed using miRonTop<sup>3</sup>. mRNAs displaying reciprocal modulation to miR-208b in BZ and to miR-301a in the IA were analyzed using MiROnTop algorithm, looking for direct binding predictions.

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#### SUPPLEMENTARY REFERENCES

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