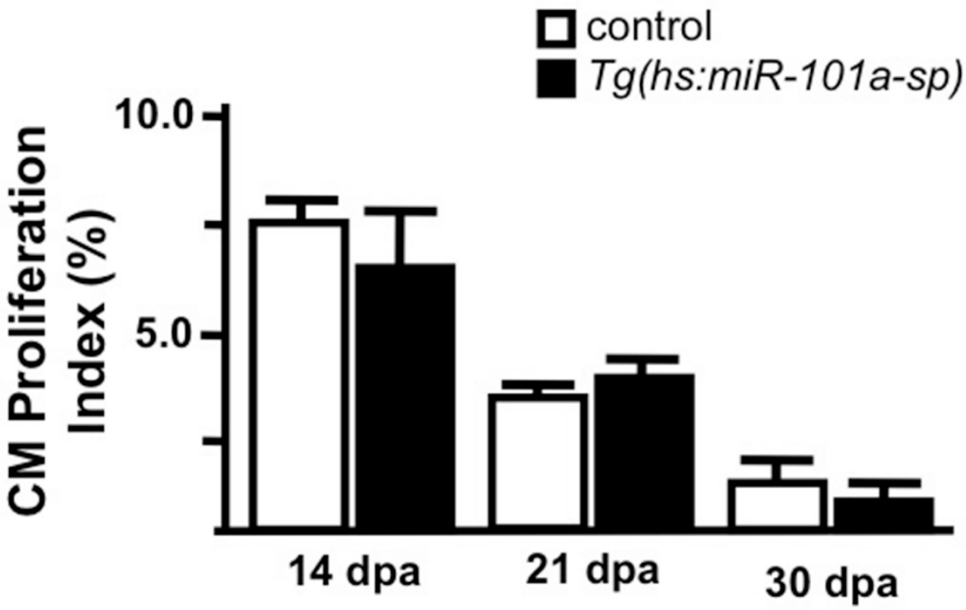

Fig. S1. CM proliferation indices in *Tg(hs:miR-101a-sp)* hearts at later stages of heart regeneration. Control or *Tg(hs:miR-101a-sp)* animals were injured, subjected to daily heat-treatment and processed to identify proliferating CMs. CM proliferation indices were determined by representing Mef+PCNA+ cells as a percentage of total Mef2+ cells. CM proliferation indices in *Tg(hs:miR-101a-sp)* hearts were comparable to control hearts at later stages of regeneration. (n=6-8; error bars represent SEM).

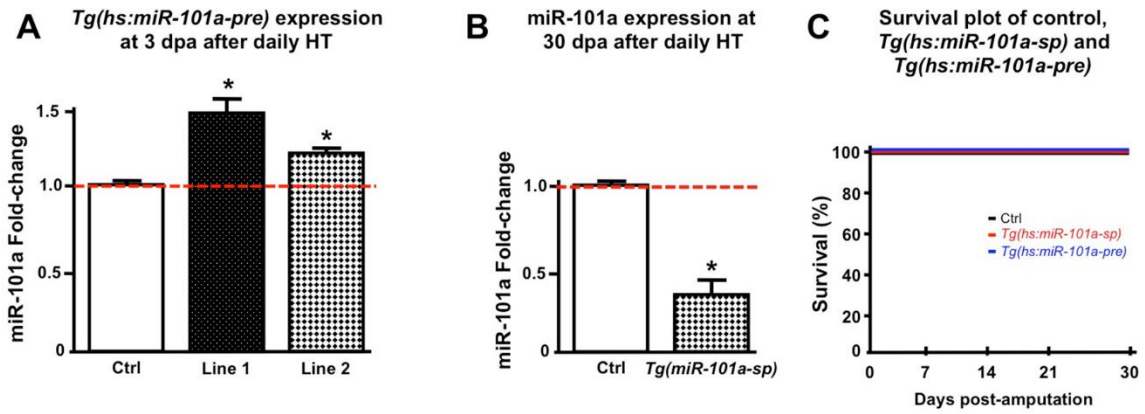

Fig. S2. Transgenic modulation of miR-101a *in vivo* alters miR-101a expression. **(A)** Heat-activation of miR-101a overexpression strains, *Tg(hs:miR-101a-pre),* led to an increase in miR-101a expression at 3 dpa in two independent strains, as revealed by qPCR. **(B)** Long-term daily heat-treatment of *Tg(hs:miR-101a-sp)* animals significantly suppressed miR-101a expression by ~65% in injured hearts at 30 dpa. **(C)** Daily heat-treatment of control, *Tg(hs:miR-101a-sp)* and *Tg(hs:miR-101a-pre)* animals did not significantly affect mortality. (n=8-10; *=Student's t-test p-value<0.05; error bars represent SEM).

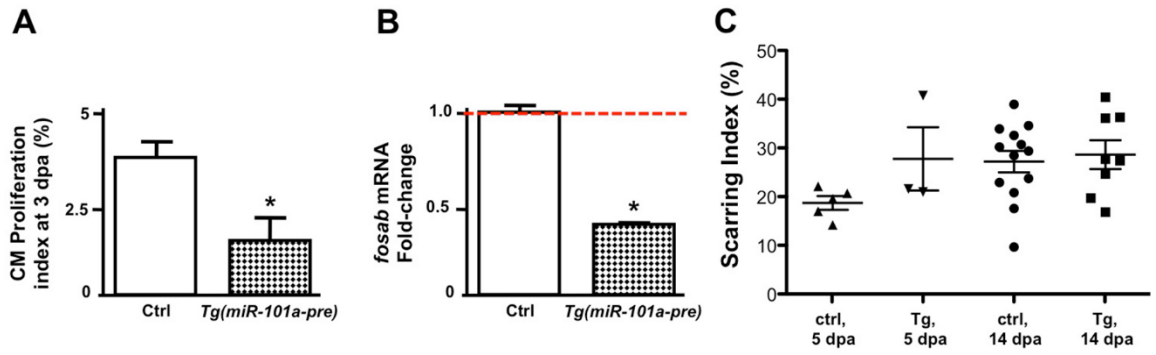

Fig. S3. Overexpression of miR-101a with *Tg(hs:miR-101a-pre)* strain inhibits CM proliferation. Control and *Tg(hs:miR-101a-pre)* hearts were resected, subjected to daily HT and extracted at 3 dpa for histology. Hearts were cryosectioned at 10μm, stained to detect Mef2 and PCNA and CM proliferation indices were determined. **(A)** When compared to control, overexpression of miR-101a reduced CM proliferation indices from 4.1% to 2.2%. **(B)** *fosab* expression in *Tg(hs:miR-101a-pre)* hearts was reduced by 55% when compared to heat-treated control hearts. **(C)** Sustained overexpression of miR-101a did not alter scar tissue formation in injured hearts. Scarring indices between in *Tg(hs:miR-101a-pre)* hearts at 5 and 14 dpa were not significantly different in comparison to controls. (*=Student's t-test p-value<0.05; error bars represent SEM).

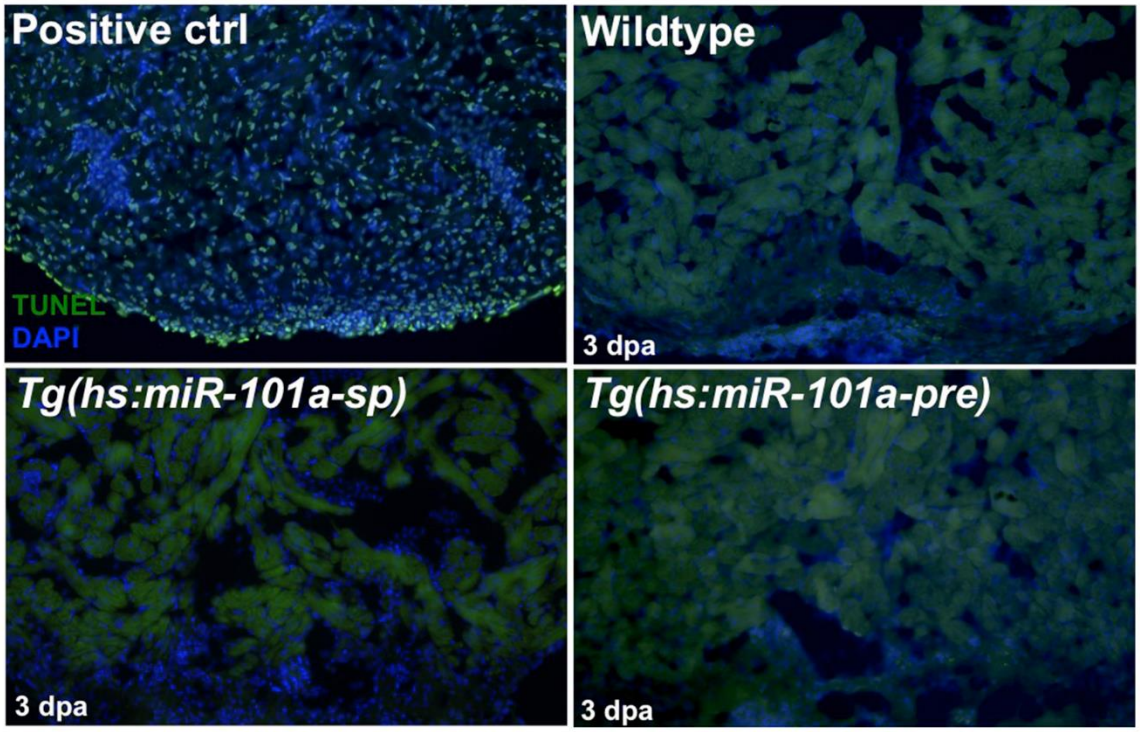

Fig. S4. Activation of *Tg(hs:miR-101a-sp)* and *Tg(hs:miR-101a-pre)* did not induce ectopic-programmed cell death. Control, *Tg(hs:miR-101a-sp)* and *Tg(hs:miR-101a-pre)* hearts were injured, heat-treated and extracted at 3 dpa for analysis of programmed cell death by TUNEL staining. We observed no differences in TUNEL+ cells between control and transgenic animals. An uninjured wildtype heart treated with DNaseI served as a positive control.

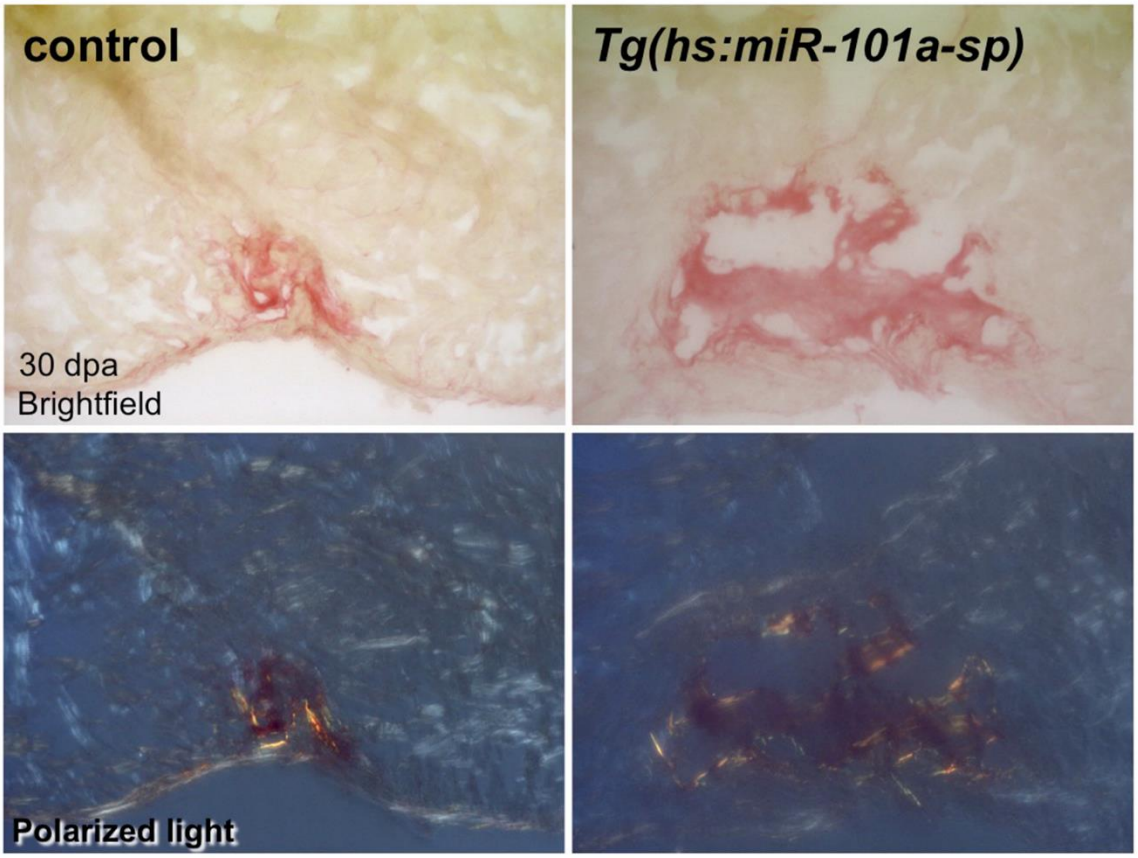

Fig. S5. Sustained depletion of miR-101a activity did not alter scar tissue composition. Control and *Tg(hs:miR-101a-sp)* hearts were resected, subjected to daily heat-treatment and processed for Sirius Red staining. Collagen composition in both control and *Tg(hs:miR-101a-sp)* hearts is primarily thick fibers (collagen type I, orange).

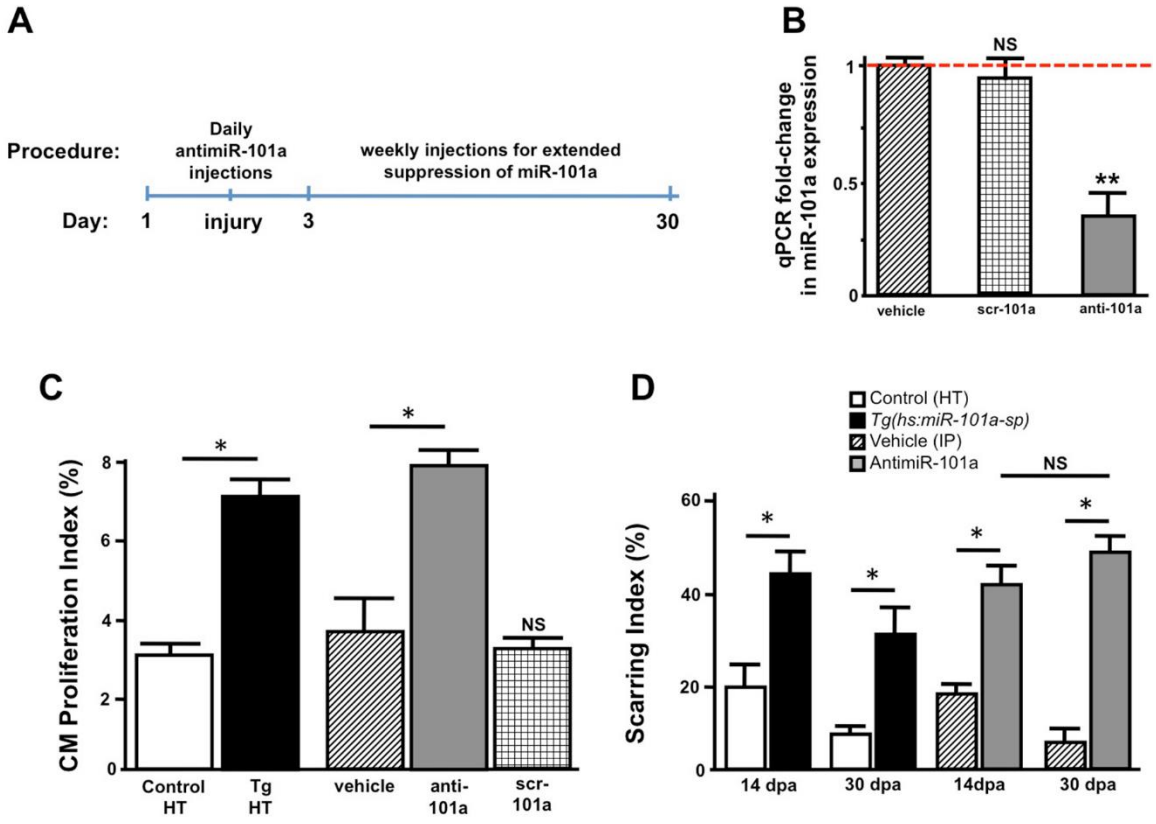

Fig. S6. LNA anti-miR-101a treatment recapitulates *Tg(hs:miR-101a-sp)* cardiac phenotypes. **(A)** Experimental schematic of LNA antimiR-101a treatment. (**B)** qPCR studies to detect miR-101a expression levels in hearts injected with vehicle, scrambled-101a and antimiR-101a oligonucleotides reveal a significant decrease in miR-101a expression in antimiR-101a treatment. No significant differences were observed between vehicle and scrambled-101a treatments. **(C)** Injured vehicle, scrambled-101a and antimiR-101a 3 dpa hearts were collected and processed to detect proliferating CMs. Relative to controls (vehicle and scrambled-101a), treatment with antimiR-101a increased CM proliferation indices comparably to *Tg(hs:miR-101a-sp)* indices. (**D)** Vehicle and antimiR-101a treated hearts were collected at 14 and 30 dpa, stained with AFOG and quantified for collagen and fibrin deposition within the total injury area. Sustained depletion of miR-101a by antimiR-101a led to significant scar tissue retention. (*= Student's t-test $p<0.001$; IP=intraperitoneal; HT=heat-treatment; error bars represent SEM).

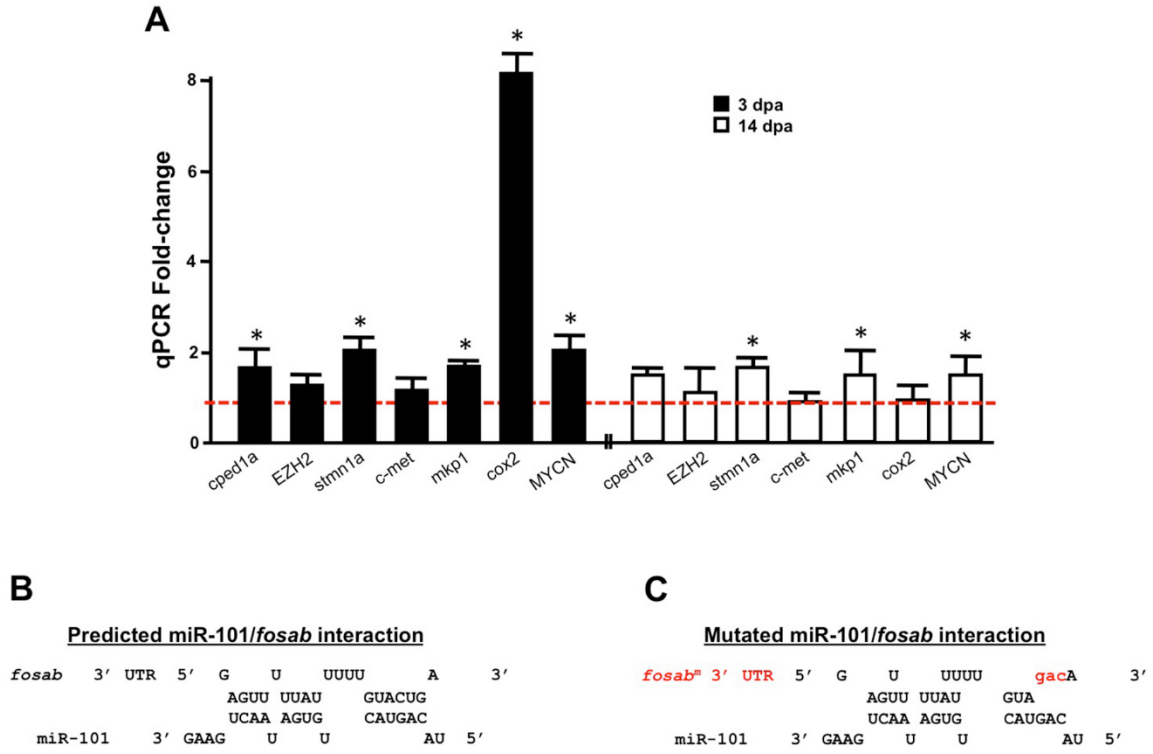

Fig. S7. miR-101a controls expression of multiple target genes. **(A)** Real-time qPCR studies of candidate miR-101 target genes at 3 and 14 dpa in *Tg(hs:miR-101a-sp)* hearts. Expression levels were normalized to *β-actin* and represented as fold-change over normalized gene expression levels in control hearts. **(B)** Alignment of the predicted base pair association between miR-101a and *fosab* -3'-UTR. **(C)** Mutation of 3-nucleotides in the *fosab*-3'-UTR is shown in red. (error bars represent SEM).

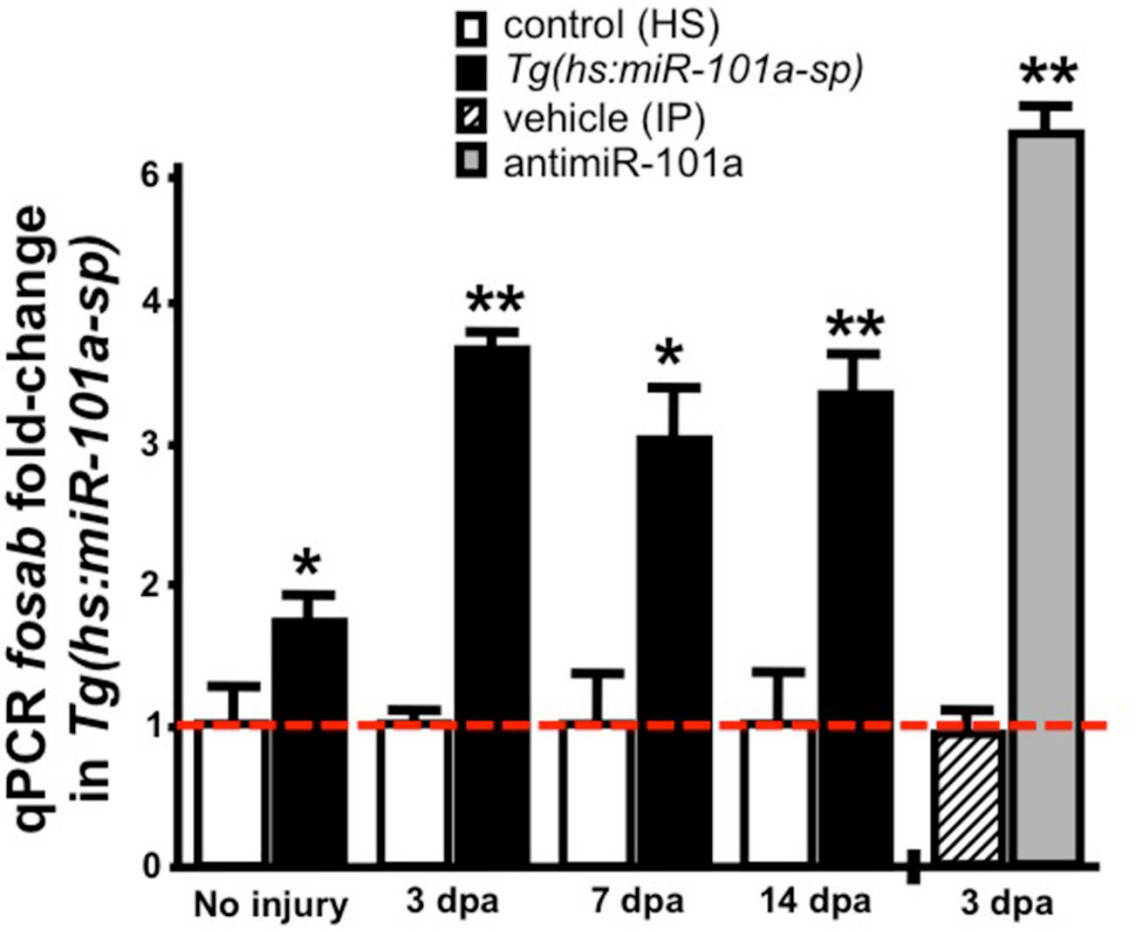

Fig. S8. Modulation of miR-101a *in vivo* alters *fosab* expression. *fosab* mRNA levels are significantly elevated in ventricles under conditions of miR-101a depletion with *Tg(hs:miR-101a-sp)* and antimiR-101a treatment when compared to controls. (n=8-10; *, **=Student's t-test p-value<0.05 and 0.001; error bars represent SEM).

Table S1. Candidate miR-101a target genes.

Gene Name	GenBank Accession	Description	Primer sequences for qPCR
MYCN	BX005358	v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog	FP: 5'-ACAGCAGCACTATAACT-3' RP: 5'-GAGTGAGGAAGCTAGAC-3'
STMN1A	C R925717	stathmin1a	FP: 5'-CTTTCCTTGGTGGAGAT-3' RP: 5'-GCCATAATTGCTGTCG-3'
CPEB1A	AL929134	cytoplasmic polyadenylation element binding protein 1a	FP: 5'-CAGACAAGCACAAGTATC-3' RP: 5'-TGACCGACAGTAGTATTTA-3'
C-MET	DP000237	MET proto-oncogene, receptor tyrosine kinase	FP: 5'-GAACTCCTTCGACATTAC-3' RP: 5'-TGTTCAGGAGGATGTAG-3'
MKP-1	CR381700	dual specificity phosphatase 1	FP: 5'-CTAGAGATGGGAGAAGTG-3' RP: 5'-CATGTGTTACAGGAGGA-3'
PTGS2A	CU570979	prostaglandin-endoperoxide synthase 2a	FP: 5'-AACTCTATCGTCACCAC-3' RP: 5'-CCTGTCATCTCCTCAAA-3'
FOSAB	AL929435	murine osteosarcoma viral oncogene homolog Ab	FP: 5'-GCTCCATCTCAGTCCCAGAG-3' RP: 5'-AGAGTGGGCTCCAGATCAGA-3'
EZH2	BX005392	enhancer of zeste 2 polycomb repressive complex 2 subunit	FP: 5'-GTGGAAACCAAAGCCACTGT-3' RP: 5'-CCAACACCACATGAAAGTGC-3'

Table S2. PCR primers and their application.

Gene Name	Application	Primer sequences
FOSAB	PCR amplification of 3'-UTR for EGFP sensor	FP: 5'-CTCGAGATGCTATGGAAGTGACTCTAAAGCAAT-3' RP: 5'-TCTAGAATGCAAACAATTCGCAAGTTCACATT T-3'

	assay	
miR101a	*Tg(hs:miR-101a-pre)* strain construct	FP: 5'-GTAAAGTCTAAGTTGTCGTCC-3' RP: 5'-CCCCACACAATTTCTTATT-3'
miR-101a	RNA duplex for EGFP senor assay	FP: 5'-rUrArCrArGrUrArCrUrGrUrGrArUrArArCrUrGrArArGTT-3' RP:5'-rCrUrUrCrArGrUrUrArUrCrArCrArGrUrArCrUrGrArATT-3'

Table S3. Predicted miR-101a binding site in candidate target genes.

Gene Name	Predicted miR-101a binding site highlighted
FOSAB	GATTCCACTAAAGACTATTAGTATTTATATCTGTGACTTGATTTCGAATGGCATTGCAG GGCTATGGAAGTGACTCTAAAGCAATGGATATTGCTTCCTATATTTATCTGAACCATAC ATGCCTGCCACATGTAGCAAGATAAGCATGGATCAACTGATTTTATTTGCATTAATGAT CTGGTTAGTGTTGAGTTAACCTAATATAGTTATTTTTCTGAGTTTACCGGTGCTAGATTT GTTGTATTTAGTGTGTTCTGAGCAATAGAGAACTATCATGTTATTTTTTTTCGGCCGTTT AACGGTTGATTTCTGAACGTGTGAATGTTGTTTACTCAAATGTGTGCTGCCTAACCATT GCATTAAATGGTCTGACGTCCAAGTATGAAGTTTTCTGTGAAAACGTAGTCTCTCTTTT AATTTATGGAGTTTTTATTTTTGTACTGAAATGTGAACTTGCGAATTGTTTGCAAAAAAC AAACAAAAAAAAACTAGACACAATAAATATTAAATGTATAAACTA
PTGS2A	AAGGTCTCAAAATCTTCAAAATCCCAGATACTTGATATGGTTTTGTAATTTATTTATTTT TTATATATATTTATTTGAATAATTTATTGCAGTAGTGTATTTATTGATTGTTTTTTTTTTT GTATTGTGATCGATGTGCAACTTTGTTTCATAAATTCATGTGAGGTATTCGACTAATTGG ACAAACACATTTCAAATGCCAATCAACTGTTGCAAATGTTTATTTCATAAATAAAAACA AATGATATTTTGCAAAAATAAAAAAAAAAAAAAAA
MKP-1	ATGGTCATCATGGAAGTGCCCACTATCGATTCGGCCTCACTTCGGGATATGTTGGAGGG AGACGACCCGGATTGTTTGGTTTTGGACTGTCGCTCCTTCTTTTCTTTCAGCGTATCTCA CATTTCGGGCTCCAGTAATGTGCGCTTCAGTACTATCGTGCGCCGGAGGGCCAGAGGG GGGCTTGGGCTTGAGCACATTGTGCCCAACGAGGACACCAGGAACAGGCTTCTGTCCG GGGAATACCAGAGTGTCGTTTTCCTGGATGACCACAGTCTAGAGATGGGAGAAGTGAA GAAAGACGGGACTTTAATGCTCGCTGTGAACGCGTTGTGCCGCAAACAATGTGGAGCA AGTGTATACCTTCTTAAAGGTGGATTTGACACATTTTCCGCTGAGTTTCCTGAAAAGTG TACAAAGACCGTCCCCCCACAAGGCTTGAGTTTACCTCTGAGCTCCAACTGCCATTCAA ACACCGCTGATTCCTCCTGTAACACATGTACAACCCCTCTGTATGATCAGGGTGGCCCT GTAGAAATCCTGCCTTTCCTATATTTGGGCAGTGCTTATCACGCTTCCAGAAAAGACAT GCTGGACATGTTGGGGATCACCGCTCTTATTAACGTCTCTTCCAACTGTCCCAACCACTT TGAAGACCACTACCAGTACAAAAGCATTCCAGTTGAAGACAACCACAAGGCTAACATC AGTTCCTGGTTCAACGAGGCCATCGAATTTATTGATTCTGTACGGAATAAAGGTGGACG TGTCTTTGTTCACTGCCAAGCGGGAATCTCGCGCTCTGCCACCATCTGTCTGGCTTATCT GATGCGCACCAACCGCGTCAAACTGGAAGAGGCCTTCGAATTCGTTAAACAGCGACGC AGCATCATCTCGCCCAACTTCAGCTTCATGGGCCAGCTTTTACAATTCGAGTCGCAAGT TCTGGCCTCCTCTACGTGTTCCTCAGAAGCAGGAAGCCCCGCCATTGGCAAGAACAGCA CTGTGTTCAACTTCCCCGTCCACACAGCGGCCAGTCCTCTTTCCTTCCTGCAGAGTCCCA TCACTACCTCACCCTCCTGCTGA

MYCN	ATGCCAGCTAAAACCATGAGCTCAGATCTGGAGTTTGACTCTTTGCAGCCGTGTTTCTA CCCGGACGAGGATGACTTCTACTTCTGCAGACCAGACGCCGCACCGCCTGGTGAGGAC ATCTGGAAGAAATTCGAGCTGCTGCCCACTCCTCCTCTGTCCCCGAGCCGGGCAGCGCT TCCAGGGGACCCGGGGGAGCTGGGCGCGGTGGCTGGGGATTGCTCGCTGATGGGCTTT GGATTAACCGACCCGTTGGACTGGGCTTCCGAGCTTCTGCTTTTACCGGGAGACGACAT TTGGGGGGCGTCGGACGGGGACCTCTTTGGCTCCGTTTTGGATACTACGGACAATTCCA TCATCATTCAGGACTGCATGTGGAGCGGCTTCTCGGCGCGAGAGAAACTGGAGCGGGT TGTGAATGAGAAACTCGGCAAAGTCGTTCCTACTCCAACCTGTACTGAAGCCGGTAAA GACACGACAGTCAAAGCGCCCGAAGTGAGCCACTCTATACCGGAGTGTGTGGACCCTA CCGTGGTTTTCCCTTATCCAGTCAACAAAAGAAACGGGAGCAGCAGCAGCCAAAGTGT GACACAACTGAGGAGCCAACAATTGCCTCAAGTCAGTGCAGGCGAGACTCCGAGCGAC TCTGATGATGATGATGAGGATGATGAAGATGAGGATGATGAGGAAGATGATGAAGAA GAGGAAGAAGATGAAGAAGAGGAAGAGATTGATGTCGTCACGGTGGAGAAGAGGCGT TCCATCACCAGCAGGACAACCAGCACTGGACTGTCTGCTGTCTCCAACTCTCAGGCAGG AGGGCGACTGGGCTCAGGGGTGAGCAAAGCTCCGCAGGAACTCATTTTAAAGAGGACA GCAGCAGCCTCCATCCACCAGCAGCAACATAACTACGCAGCCCCATCCCCTTACTCCGA ACAGCAAGACGTTCCCAGCGCTCCTCCAAGCAAGAAACTCAGAATCGACAGCAGCACT ATAACTCTACGCACTGGCAGGAACCAGAGCTCTTCTCCAAACTCCCCTACCAACAGCGT ACCAAGTCAGCGCTTGAGGAAGAGCGACTCCAGCAGCCCCAGATGCTCCGACTCGGAG GACAGCGAACGCAGACGCAACCACAACATCTTGGAGCGTCAGCGGCGCAATGACCTGC GGTCTAGCTTCCTCACTCTGCGGGATCAGGTGCCTGAGCTCGCACACAACGACAAGGC AGCAAAGGTGGTCATCCTAAAGAAGGCCACTGATTACGTCAGCTCCCTGGAGGCTCAG GAGTTCCGACTCCAGCAGGAGAAAGACAGATTGCAAGCCAAACGACAACAGCTCCTCC GCAGACTTGAGCAGGCCAGGACTCGCTAA
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CPEB1A	ATGGATTTCCGCAGGGGAAAGAAGAATTGCTGTTATCCCGCTTATGGAGAATGGTTAG CTCACAGGGCCTTTGGGCCCTTCATAGGTGCCAAACAACACACCAATTGGAAACACAC ACATGATGGTTCAGGCTCTTCCACAGTTAGCAGTATGCTGTTGGCTCCAGAGCAGCAGG ATCATCCGGCCGCTGTGCCCAGCGATGAAGAGCTCAGTCTGGGCCTGCTATCGCTCGCT CTCCCCTGCTGGTATCAGGAGCTCTGGAGCCGCCCTGAAGCCCAGCGGGATGTCCAGA CTCCATTGAATCTCTTGGGTTCATCACGAGGTGATGGTGATTGGGTTCAGCATCAGGCT CTCCAGGCCCATGAGTCTGCAGGAGGACGAGATTCACTGACCCCCACACTGGAGACTC ACTCTCTGCTACACTCCGGCTCCAGCAGCCCTGTCAACTCCGAGAACAGCGCCGTCTCT TCAGGGTCAGAGCACCTGGCGTCTCTCAGAGTTTCTCCTCCGTTGCCGCTGTTGCTCTCA GGACTGCGTGTGGAGGACCTGAAATTAGGCTCAGATGTTGGTTTGGATCAGAATCCGCT GAACACCTTCATGAACGCCTCCAGCGGCCGGGCTGAGGGCTCCGTGTTCAGCCGCTGGT CCACAGGTCCCGTCTGGCCAGGCTGGGACACTTTGGGCTTGAATAAACCCTCATTCTGC ATCGAGAGAGAGGCGAAGCTTCATAAACAAGCTGCAGCCGTGAATGAAGCCTCATACA GCTGGGAGGGAAGTCTTCCTCCGCGTCACTACAAAACCCCAGTGTACTCGTGTAAAGTG TTTCTGGGAGGAGTGCCGTGGGACATCACAGAGGCGAGTCTGCAGAGCACATTTAGTG TATTTGGCCCATTGAAAGTGGAGTGGCCTGGTAAAGATGGCAAACATCCGCGATGTCCT CCTCAAGGTTATGTCTATCTACTGTTTGACTGGGAGAAGTCTGTGAAGTCTCTCCTGCA GGCATGTACTCAACACCGCCTGCAGGCCGACGACTATCTCCAGTTTTACTACAAACTCT CCAGCAGGAGGATCCACAGCAAAGACGTGCAGGTCATCCCCTGGGTGATTTCAGACAG TAATTTTATCCGCTGTCCCTCTCAGCGCCTCTGCCGCAATAAGACTGTGTTTGTGGGCGC TCTGCACGGGATGCTGAACGCTGAGGGTCTGGCTCACATCATGGATGAGCTCTTTGGGG GTGTCATGTACGCCGGCATCGACACAGACAAGCACAAGTATCCTATAGGTTCAGGCAG AGTGACGTTCAGGAGCCAGAGGAGCTACTTGAAAGCTGTCACTGCAGCATTTGTGCAG

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