

# MicroRNA-146a, a therapeutic target and biomarker for peripartum cardiomyopathy

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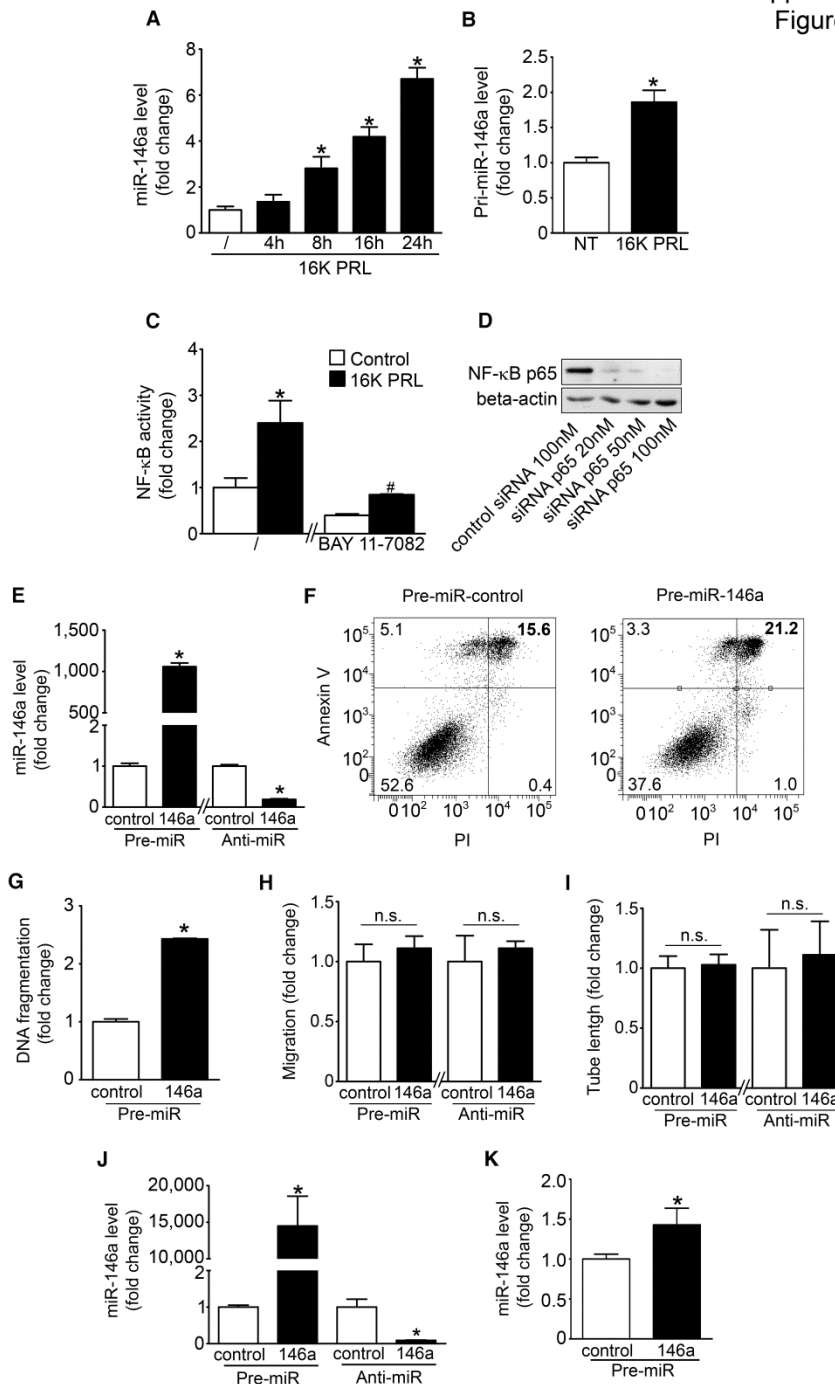
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## **Additional Footnotes:**

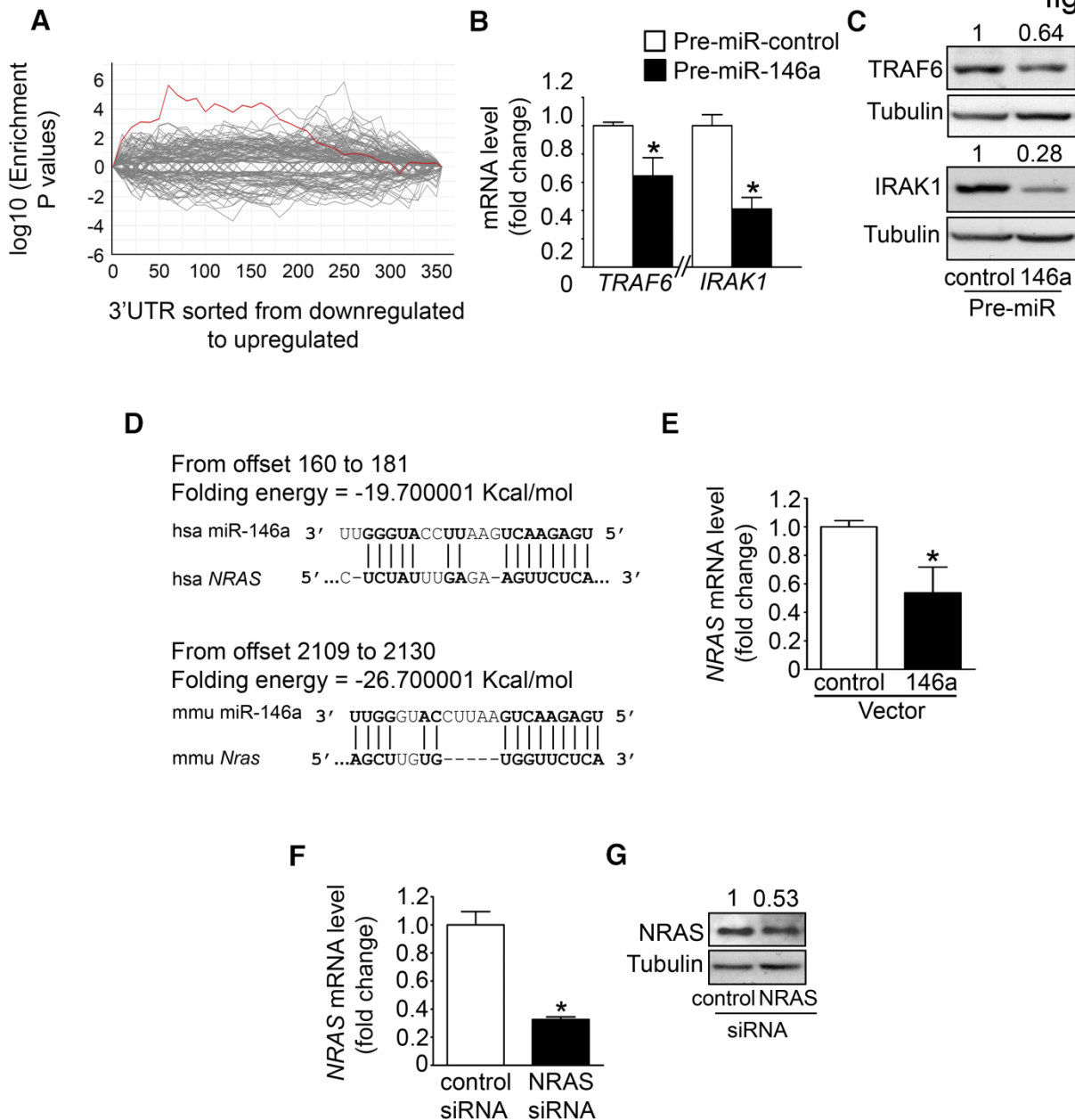
\*Equally contributing first authors

#Equally contributing last authors

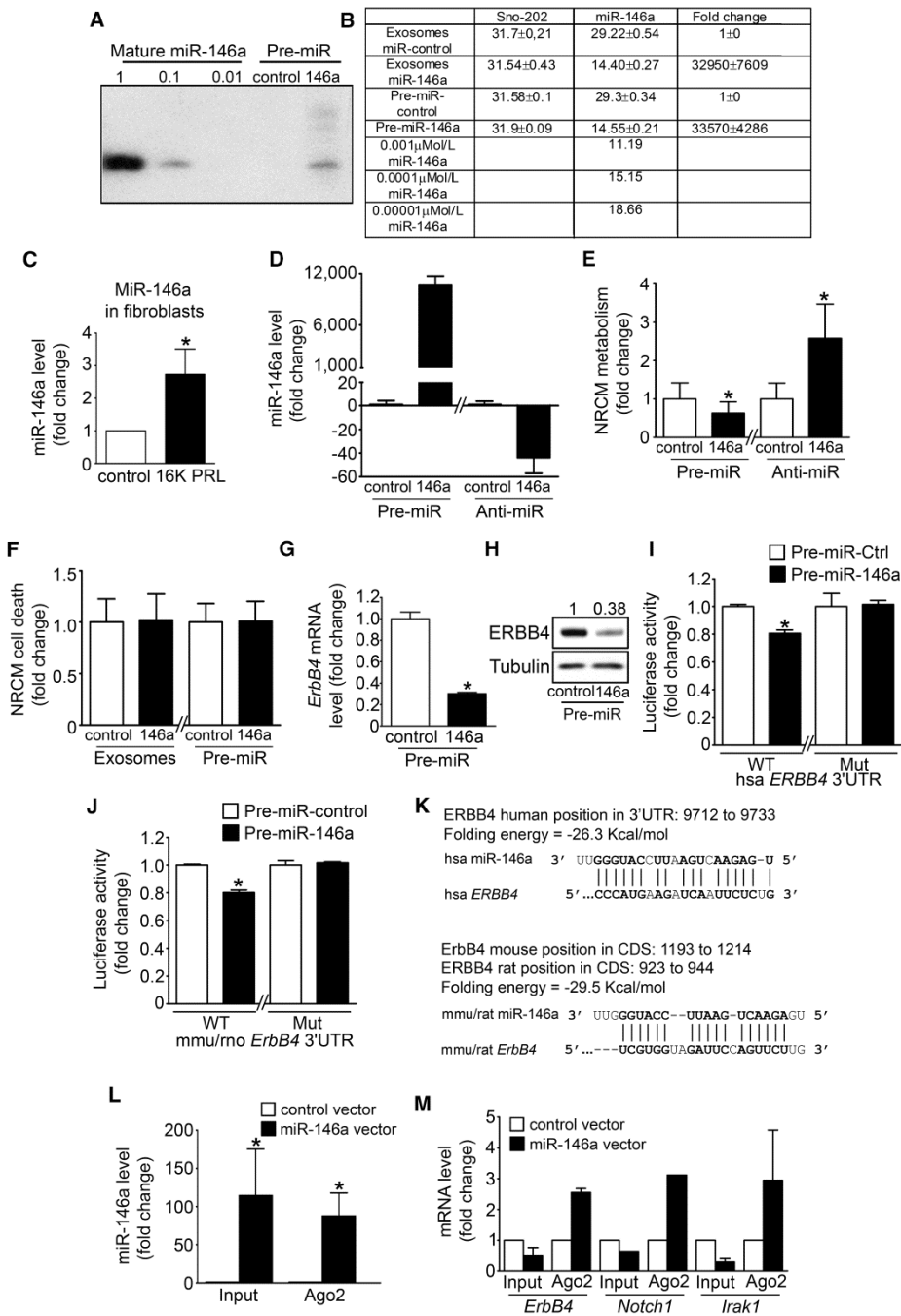
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**Figure S1:** (A) Bar graph depicting expression level of miR-146a in HUVEC treated with 16K PRL for the indicated times (50 nM). (B) Expression level of pri-miR-146a in HUVEC treated with and without (NT= non-treated) 16K PRL (50 nM, 24 h). (C) Bar graph summarizing NF-κB activity analyzed by ELISA in HUVEC treated with BAY 1170-82 (10 μM) and 16K PRL (50 nM) for 1 h. (D) Western blot depicting p65 protein levels in HUVEC transfected with p65 NF-κB siRNA or a control siRNA for 72 h (20 nM). (E) Bar graph summarizing miR-146a expression level in HUVEC transfected with pre-miR-ctrl or pre-miR-146a and anti-miR-control or anti-miR-146a. (F) Flow cytometry analysis for apoptosis by annexin-V-PI staining and (G) DNA fragmentation analysis 72 h after transfection of HUVEC with pre-miR-control or pre-miR-146a. (H-I) HUVEC transfected with pre-miR-control or pre-miR-146a and anti-miR-control or anti-miR-146a were assessed for migration (scratch wound assay) (H) and (I) tube formation (tubulogenesis assay) after 16 h. (J) MiR-146a level in aortic rings transfected with pre-miR-146a and pre-miR-control or anti-miR-146a and anti-miR-control. (K) Quantification of miR-146a level in eyes of laser induced choroidal neovascularization at day-7 after transfection with pre-miR-control and pre-miR-146a injected intravitreally ( $n \geq 8$  eyes/condition; 4 lesions/eyes). All data are means  $\pm$  SD ( $n \geq 3$ ). \*  $P < 0.05$  vs. corresponding control. #  $P < 0.05$  vs. control treated with 16K PRL.

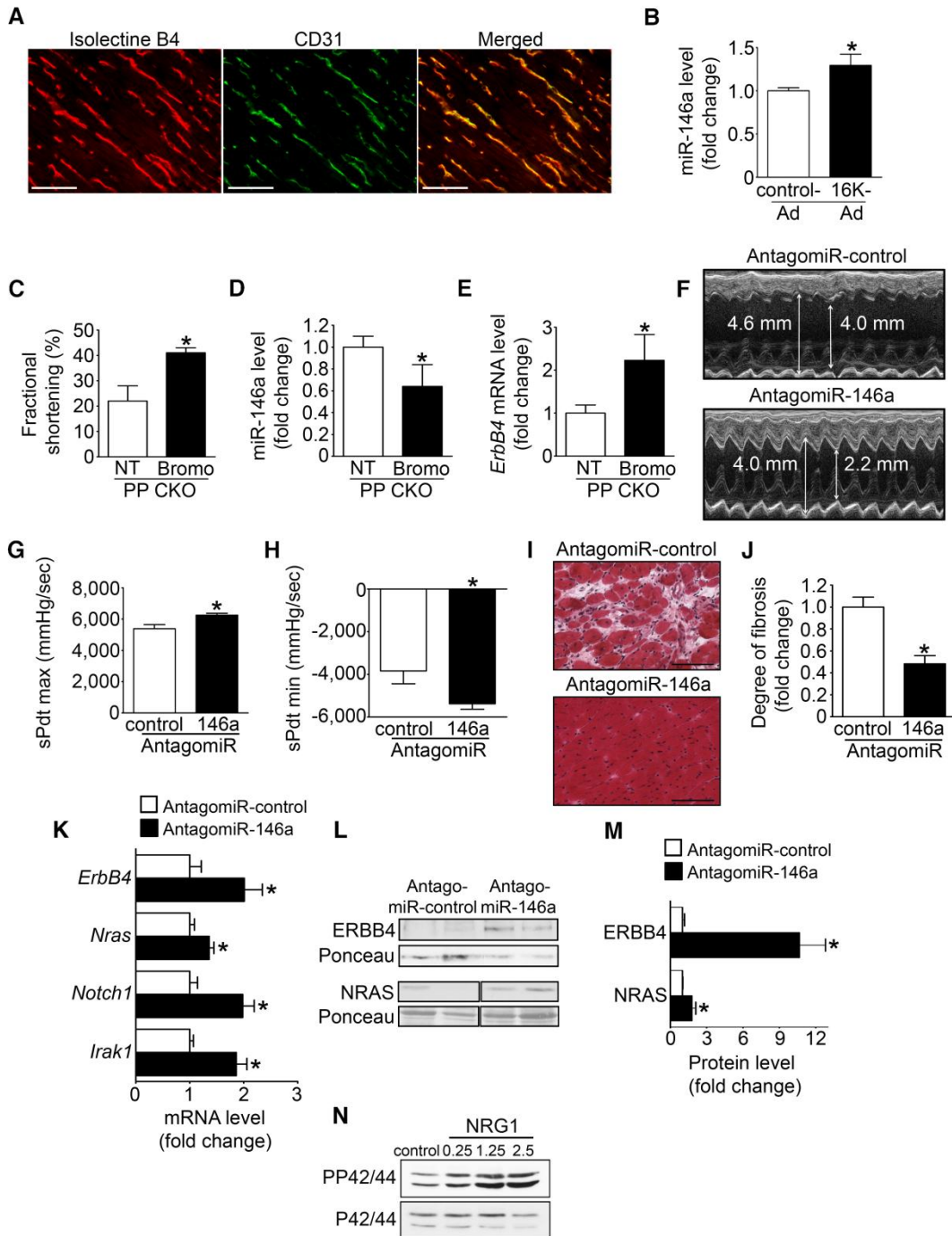


**Figure S2:** (A) Sylamer analysis: landscape plot for a pre-miR-146 overexpression mRNA analysis. Lines represent log of enrichment/depletion  $P$ -value for miRNA seeds in 3'UTRs, calculated in incremental parts of the submitted ranked gene list. Red line shows the profile for word that is complementary to seed region of miR-146a. The y-axis shows the hypergeometric significance for each word at each leading bin. Positive values indicate enrichment and negative values, depletion.  $x$ -axis: from left to right 3'UTR sorted from most to least down regulated (decreasing fold change). (B) Bar graph depicting *TRAF6* and *IRAK1* mRNA levels in HUVEC transfected with pre-miR-control or pre-miR-146a. (C) Western blot showing protein levels in HUVEC transfected with pre-miR-control or pre-miR-146a and anti-miR-control or anti-miR-146a (50 nM, 72 h) for *TRAF6* and *IRAK1*. (D) Focus on the pairing sequences of *NRAS* 3'UTR and miR-146a in human and mouse. (E) MiR-146a expression level in rat heart endothelial cell (RheA) infected with the lentiviral vector encoding pre-miR-146a or control. (F) Bar graph summarizing *NRAS* mRNA and (G) Western blot depicting *NRAS* protein levels in HUVEC transfected with *NRAS* siRNA or a control siRNA (20 nM) for 48 and 72 h, respectively. All data are means  $\pm$  SD ( $n \geq 3$ ). \*  $P < 0.05$  vs. corresponding control.



**Figure S3:** (A) Northern blot depicting miR-146a level. MiR-146a probes at different concentrations as indicated in columns 1, 2 and 3 and miR-146a expression levels in NRCM transfected with pre-miR-control or pre-miR-146a. (B) Ct values of miR-146a detection and the internal control sno-202 by qRT-PCR in NRCM exposed to control and miR-146a exosomes or transfected with pre-miR-146a and pre-miR-control and miR-146a expression corresponding of different quantities of miR-146a probes relative to figure S3A. (C) Quantification by qRT-PCR of miR-146a level in primary cardiac rat fibroblasts incubated with 16K-PRL (50 nM, 48 h). (D) MiR-146a level in NRCM transfected with pre-miR-control or pre-miR-146a and anti-miR-control or anti-miR-146a (100 nM, 72 h). (E) MTS assay in NRCM transfected with pre-miR-control or pre-miR-146a and anti-miR-control or anti-miR-146a (100 nM, 72 h). (F) NRCM cell death after exposure to control or miR-146a exosomes and transfected with pre-miR-146a or pre-miR-control. (G) *ErbB4* mRNA (qRT-PCR) and (H) protein levels (Western blot) in NRCM transfected with pre-miR-146a and pre-miR-control. (I-J) Luciferase activity from *ERBB4* 3'UTR WT reporter plasmid and the mutated *ERBB4* 3'UTR from human (I) and mouse (J) cotransfected into HEK293T cells with pre-miR-146a or pre-miR-control. (K) Focus on the pairing sequences of *ERBB4* 3'UTR and miR-146a in human and mouse.

(L-M) In put (levels of miR-146a and *ErbB4* mRNA in total RNA prior IP) and RIP assays on immunoprecipitated Ago2 complexes from NRCM infected with the lentiviral vector encoding pre-miR-146a or control. Quantification by qRT-PCR of miR-146a (L) and mRNA levels (M) in immunoprecipitated Ago2 complexes. All data are means  $\pm$  SD ( $n \geq 3$ ), \*  $P < 0.05$  vs. corresponding control.



**Figure S4:** (A) Fluorescence microscopy showing colocalization of Isolectin B4 (red) and CD31 (green) staining in blood vessels in LV sections of postpartum (after 2 pregnancies, PP) CKO mice, scale bar: 25  $\mu$ m. (B) MiR-146a expression level in LVs from WT mice injected with adenovirus expressing 16K-PRL (16K-Ad) or adenovirus control (control-Ad). (C) Fractional shortening, (D) miR-146a and (E) *ErbB4* mRNA levels in PP CKO mice treated with or without bromocriptine. (F) Representative M-mode pictures of PP CKO mice treated with antagomiR-control and antagomiR-miR-146a, arrows indicate LV diameter in systole and diastole. (G, H) Catheter measurements of sPdt max (G) and sPdt min (H) in PP CKO mice treated with antagomiR-control and antagomiR-miR-146a. (I) H&E staining of LV sections, scale bar: 100  $\mu$ m (J) and quantification of fibrosis in LVs of PP CKO mice treated with antagomiR-control and antagomiR-miR-146a. (K) *ErbB4*, *Nras*, *Notch1* and *Irak1* mRNA (qRT-PCR) and (L) ERBB4 and NRAS protein (Western blot), (M) quantified in bar graph in LVs of PP CKO mice treated with antagomiR-control and antagomiR-miR-146a. (N) MAPK phosphorylation (Western blot) in WT mice injected with recombinant NRG1 or NaCl (control). All data are means  $\pm$ SEM,  $n=3-7$ , \* $P<0.05$  vs. corresponding control.