

Ultrasound and Microbubble-targeted Delivery of a microRNA Inhibitor to the Heart Suppresses Cardiac Hypertrophy and Preserves Cardiac Function

Jonathan A. Kopechek, Ph.D.,^{1,2} Charles F. McTiernan, Ph.D.,¹ Xucai Chen, Ph.D.,¹ Jianhui Zhu, Ph.D.,¹ Maureen Mburu, M.D.,¹ Rafey Feroze, B.Sc.,¹ Daniel A. Whitehurst, B.Sc.,¹ Linda Lavery, B.Sc.,¹ Jissy Cyriac, M.Sc.,¹ Flordeliza S. Villanueva, M.D.^{1*}

¹Center for Ultrasound Molecular Imaging and Therapeutics, Heart and Vascular Institute, Pittsburgh Heart, Lung, Blood and Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, PA, USA

²Dept. of Bioengineering, University of Louisville, Louisville, KY, USA

ONLINE SUPPLEMENTAL MATERIAL

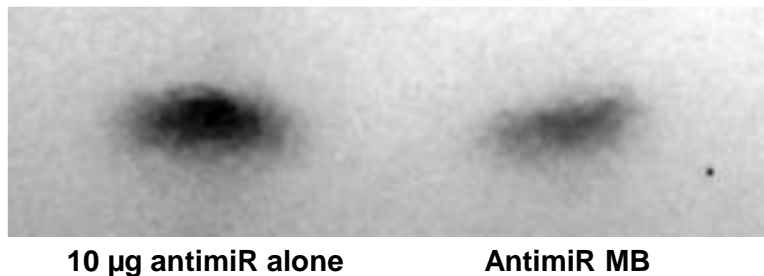


Figure S1: AntimiR loading on microbubbles. Methylene blue electrophoresis indicating that 40% of the antimiR added to the formulation could be loaded on to the microbubbles.

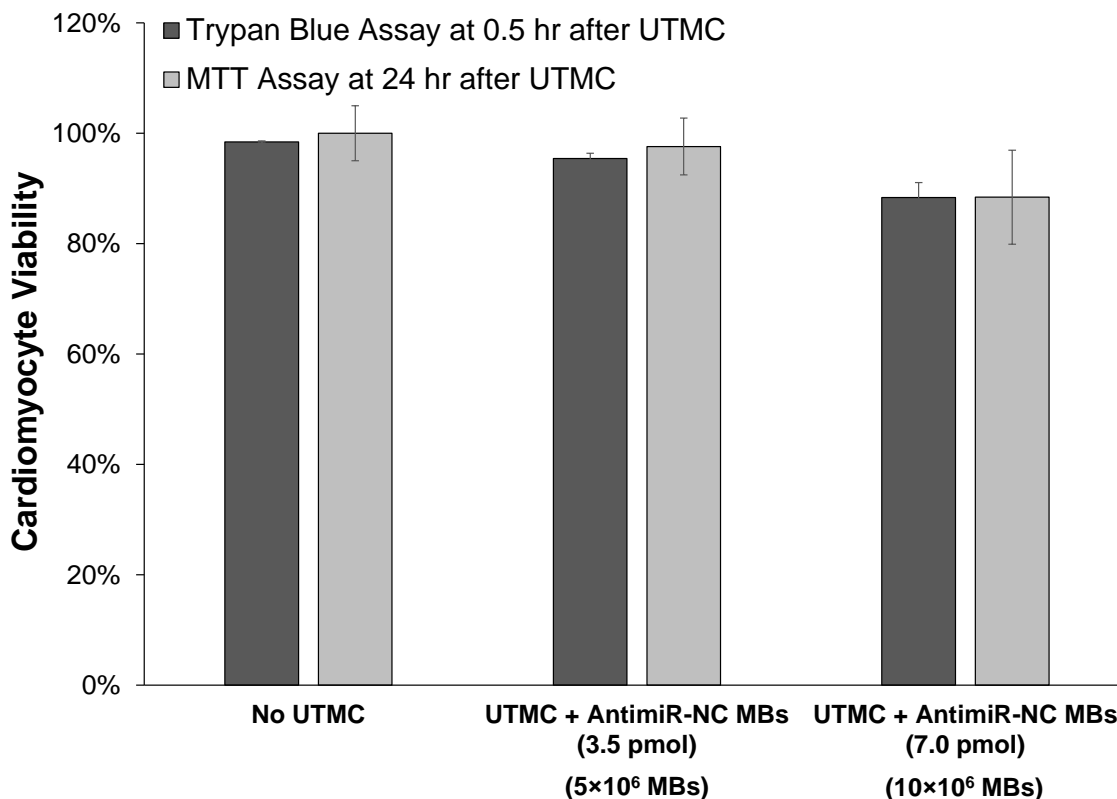


Figure S2: Viability after ultrasound treatment. Cardiomyocyte viability following UTMC was not significantly affected by UTMC treatment ($n=4/\text{group}$ for Trypan Blue Assay, $n=7-10/\text{group}$ for MTT Assay, $p=\text{NS}$).

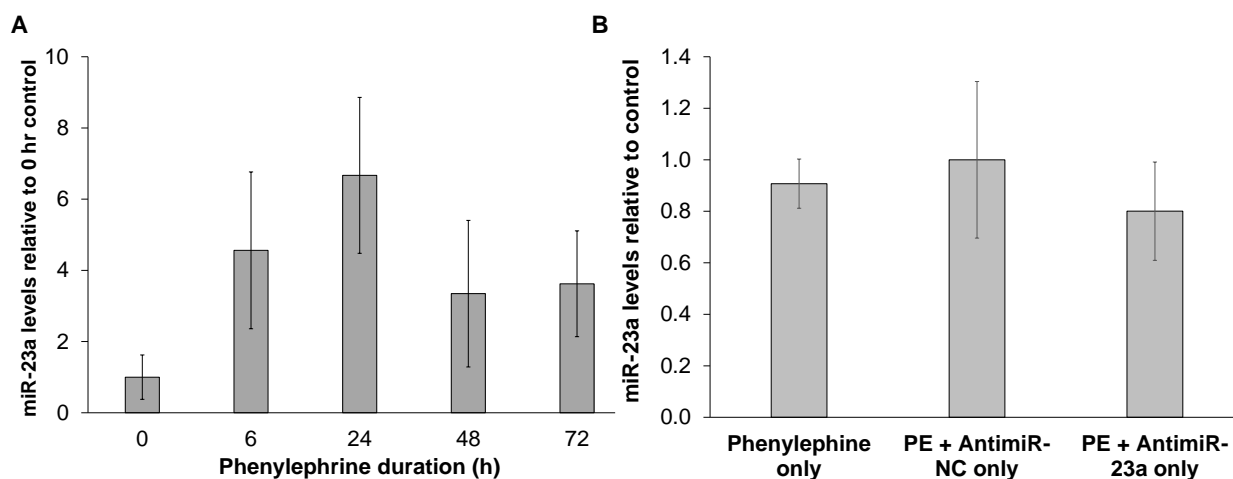


Figure S3: miR-23a levels in cardiomyocytes. (A) RT-PCR showing strong trend of increased levels of miR-23a in cardiomyocytes after phenylephrine exposure ($n=3/\text{group}$, $p=0.13$). (B) Compared to control groups, no significant difference in miR-23a levels was detected in cardiomyocytes treated with antimiR-23a or antimiR-NC alone (no microbubbles or ultrasound) following 24 h of phenylephrine exposure ($n=3-6/\text{group}$). PE = phenylephrine.

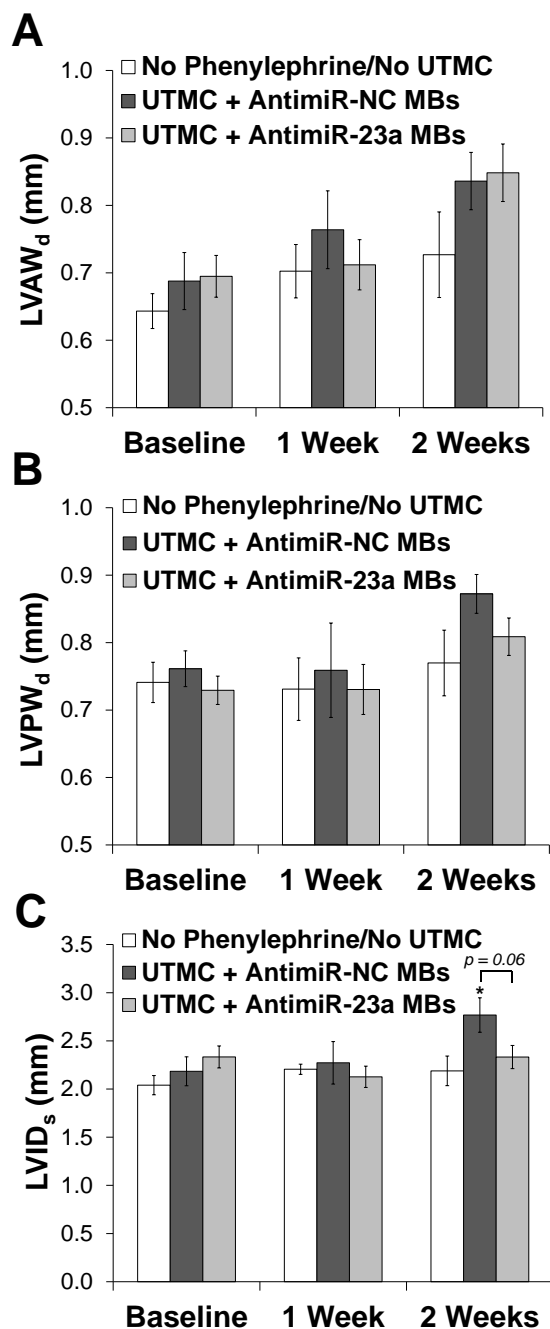


Figure S4: Diastolic wall thickness and systolic internal diameter. Echocardiographic analysis of mouse hearts following UTMC targeted delivery of antimiR-23a compared to UTMC delivery of antimiR-NC, or no phenylephrine. (A) Diastolic LV anterior wall thickness, (B) diastolic posterior wall thickness, and (C) systolic LV internal diameter ($n=5-9$ animals/group); UTMC + AntimiR-NC vs UTMC + AntimiR-23a microbubble groups, $p=0.06$; UTMC + AntimiR-NC microbubble group at 2 weeks vs baseline, $*p=0.03$.

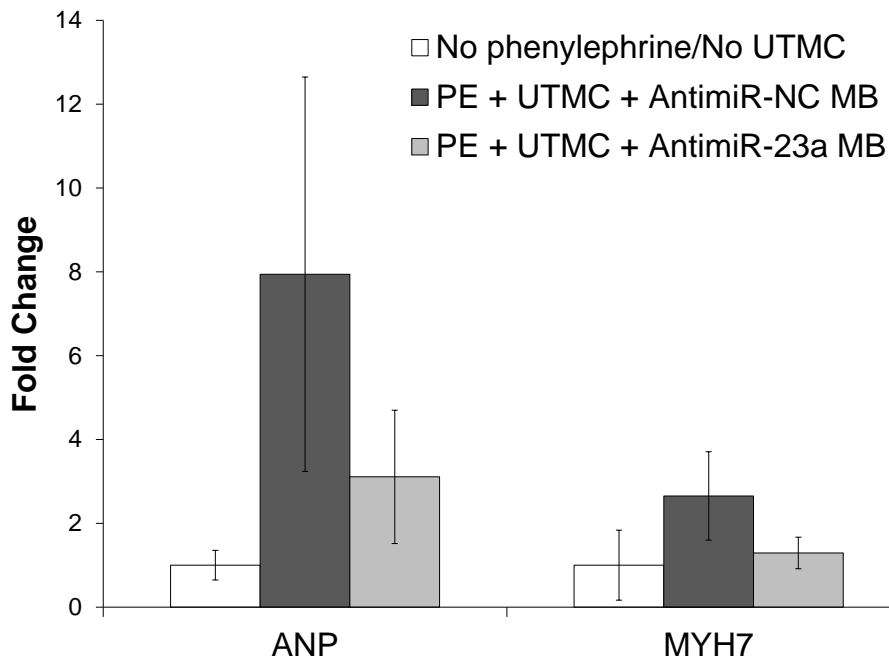


Figure S5: Expression of hypertrophy markers in mouse hearts. Myocardial expression levels of hypertrophy markers measured by RT-PCR ($n=5-9$ animals/group).

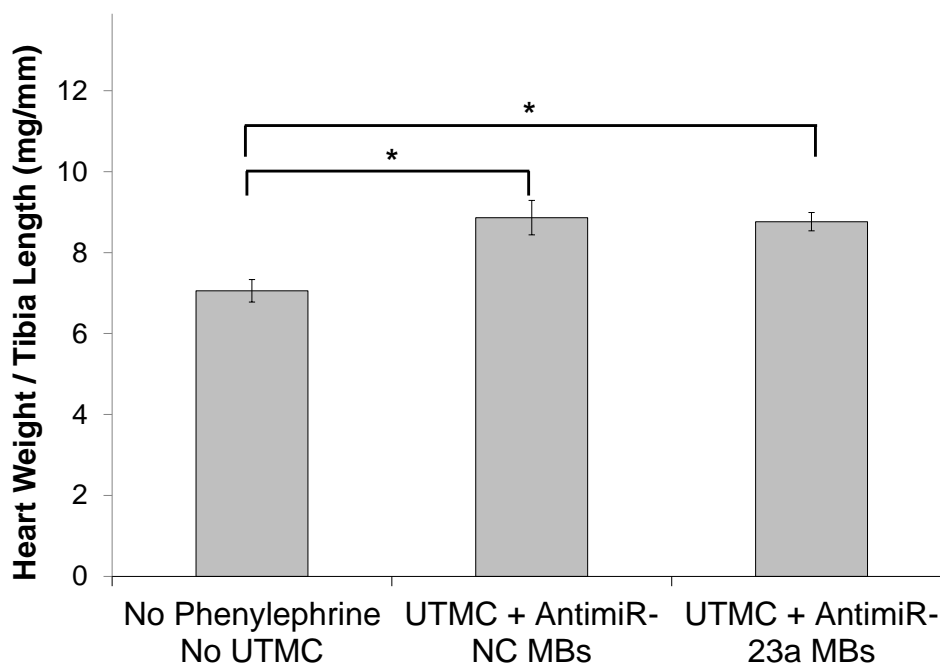


Figure S6: Heart weights. Normalized total heart weight measured immediately after sacrifice at 2 weeks ($n=5-9$ animals/group, $*p < 0.05$).