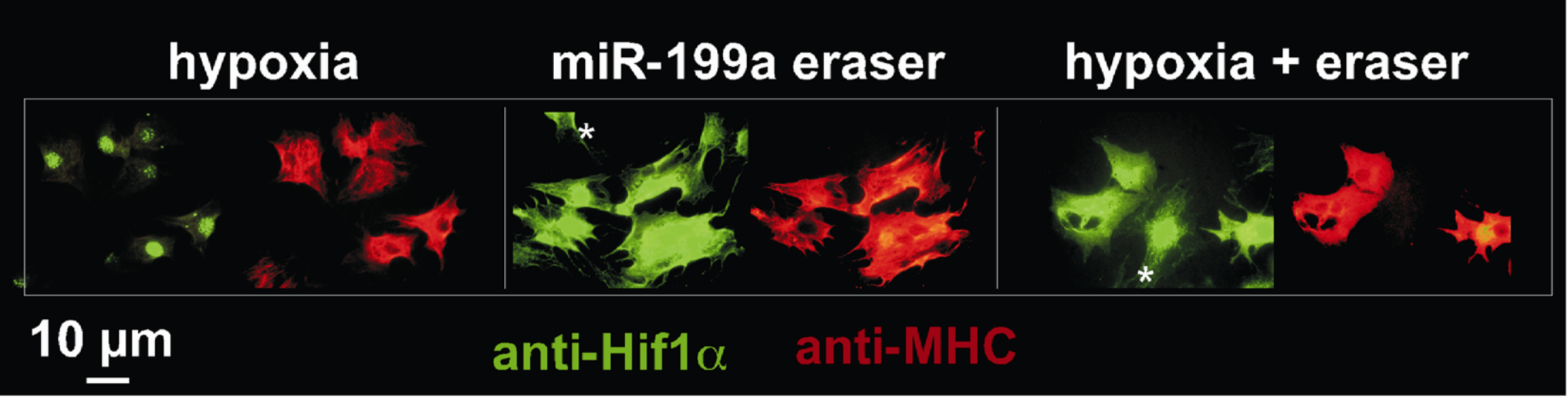
Supplement Material

Constructs cloned into recombinant adenovirus - The stem-loop precursor of mmu-miR-199a-1 was synthesized and cloned into pDC316 shuttle vector. For a negative control, a nonsense sequence was used in place of miR-199a, as previously described [1]. The miR-199a-eraser is a tandem repeat of the anti-sense of mature miR-199a sequence, as previously characterized by Sayed et al [1]. Human Hif-1α (NM_001530.2) cDNA was purchased from Origene and subcloned into pDC316 shuttle vector. A mutant (Hif1$\alpha\Delta$199a) was constructed by excising nt 2761-2921 that encompass the miR-199a target sequence. Hairpin-forming oligonucleotides encompassing nt 2465-2485 of rat HIF1A (NM_024359) or nt 2211-2231 of mouse Sirt1 (NM_019812.1), were used for gene silencing. The Sirt1-expressing adenovirus was kindly provided by Dr. Junichi Sadoshima.

Antibodies used - anti-Procaspase 12, anti-Caspase 9, anti-Caspase 6, and anti-GAPDH (Chemicon, MA); anti-cleaved Caspase 3 (Cell Signaling Technologies, MA), anti-BNip1 (B. D. Biosciences, CA), anti-Hif-1alpha (Novus Biologicals, CO), anti-p53 (Genscript, NJ), anti-H2B (Upstate biotechnology, MA), anti-actin (Santa Cruz), anti-cytochrome c (Santa Cruz Biotechnologies, CA), anti-iNOS (Ana Spec, CA), anti-Sir-2α (Upstate biotechnology, MA), anti-pHD2 (Novus Biologicals, CO), and anti-myosin-heavy chain (MHC) (Hybridoma Bank, University of Iowa, IO).

References

1. Sayed D, Rane S, Lypowy J, He M, Chen IY, Vashistha H, Yan L, Malhotra A, Vatner D, Abdellatif M. MicroRNA-21 Targets Sprouty2 and Promotes Cellular Outgrowths. *Mol Biol Cell.* 2008;18:3272-3282.



MiR-199a eraser induces upregulation of Hif-1α in myosin heavy chain positive or negative cells, in primary neonatal myocyte cultures. Myocytes were exposed to hypoxia for 24 h, miR-199a eraser for 24 h, or hypoxia+eraser, as indicated above each panel. Cells were then co-stained with anti-Hif-1α and anti-myosin heavy chain (MHC, red) (n=2). The anti-Hif-1α used in this experiment is a rabbit polyclonal versus the mouse monoclonal used in Fig.3a. *Marks non-myocytes that are negative for MHC staining. The data prove that miR-199a is intrinsic in both myocytes and non-myocytes and targets Hif-1α.

ONLINE FIGURE I