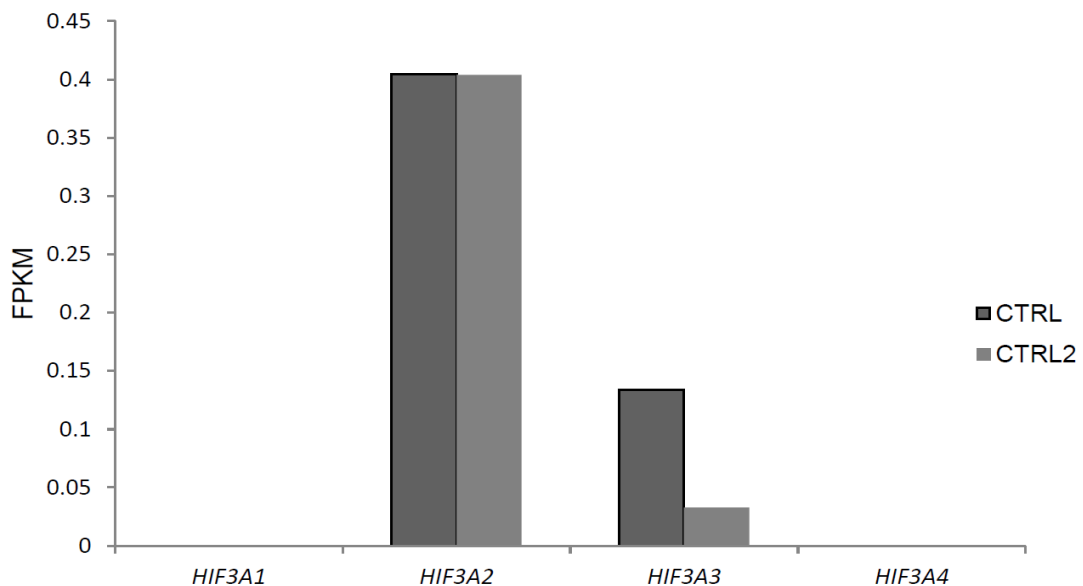


SUPPLEMENTARY INFORMATION

miR-429 regulates the transition between Hypoxia-Inducible Factor (HIF)1A and HIF3A expression in human endothelial cells

Anna Janaszak-Jasiecka, Sylwia Bartoszewska, Kinga Kochan, Arkadiusz Piotrowski;
Leszek Kalinowski; Wojciech Kamysz, Renata J. Ochocka, Rafał Bartoszewski, and James
F. Collawn

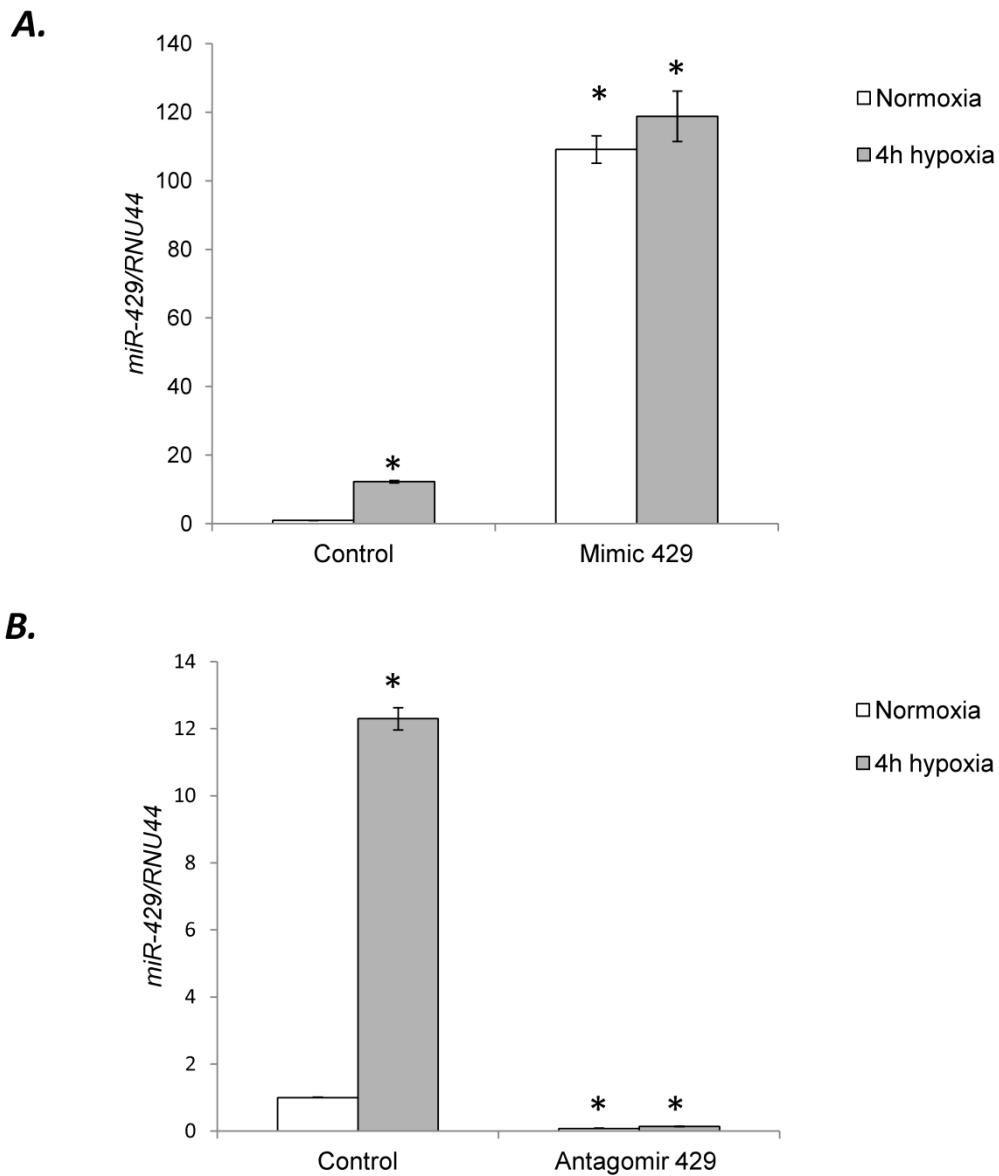
Janaszak & Bartoszewska et al, *Supplemental Figure 1*



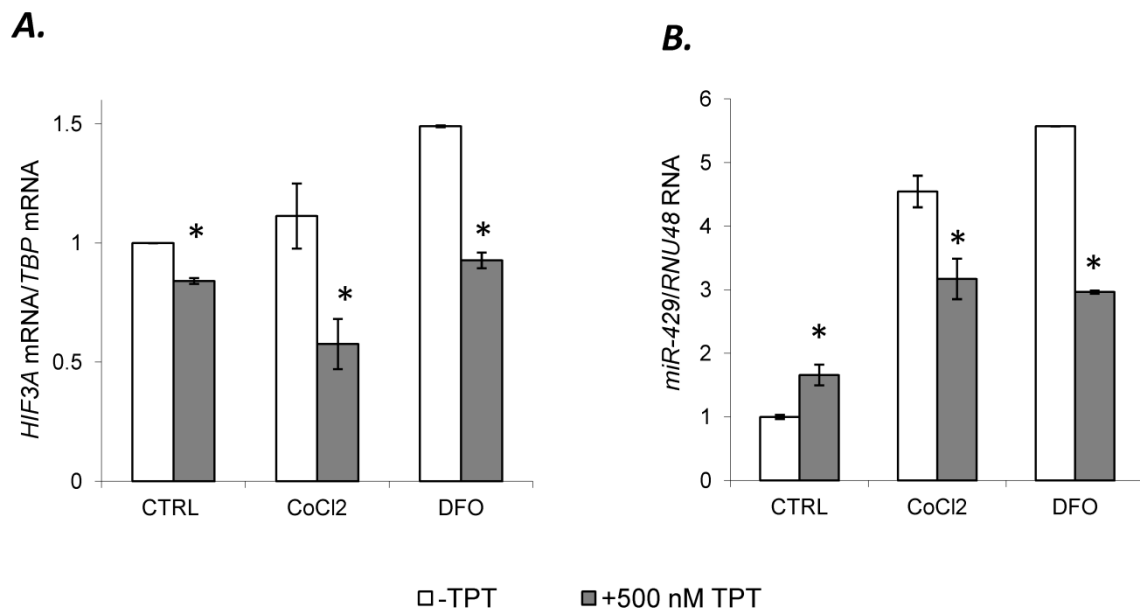
Supplemental Figure 1. The Next Generation Sequencing (NGS) analysis of *HIF3A* isoforms relative expression (Fragments Per Kilobase of transcript per Million mapped reads). In HUVECs, expression of only 2 isoforms was observed in all analyzed samples (two HUVEC control samples, CTRL and CTRL2). Neither the *HIF3A1* (NM_152794.3) nor *HIF3A4* (NM_152796.2) transcript variants were expressed in the samples.

	<i>DDIT4</i>	<i>HIF1A</i>	<i>miR-429</i>
<i>HIF3A</i>	C.C.= 0.426 p =0.03 n=24	C.C.= -0.796 p =3.3 e-7 n=24	C.C.= -0.546 p =0.006 n=24
<i>DDIT4</i>		C.C.= -0.272 p =0.198 n=24	C.C.= -0.051 p =0.811 n=24
<i>HIF1A</i>			C.C.= 0.893 p =4.1 e-9 n=24

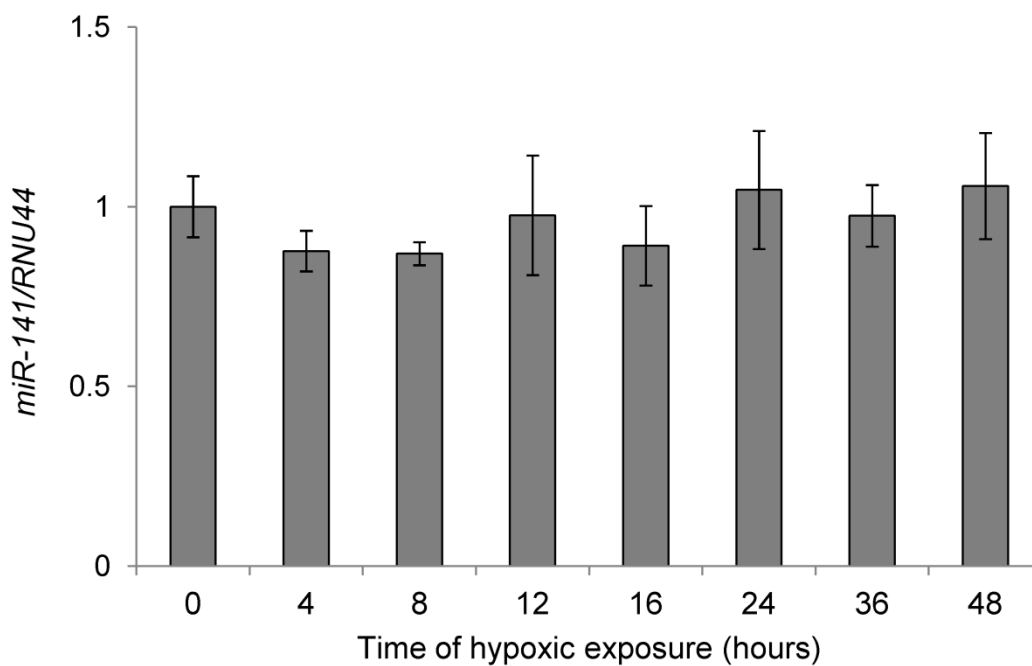
Supplemental Figure 2. Correlation of expression profiles of miR-429, *HIF1A*, *HIF3A2* and *DDIT4*. The qRT-PCR results of hypoxia-induced changes in mRNA and miRNA levels were subjected to Pearson product-moment correlation tests. The pair(s) of variables (mRNA-mRNA and mRNA-miR-429) with positive correlation coefficients (C.C.) and P values below 0.050 increased together (dark grey). For the pairs with negative correlation coefficients and P values below 0.050, one variable decreased while the other increased (light grey). For pairs with P values greater than 0.050 (white), there was no significant relationship between the two variables (n = number of tested values). Pearson product-moment correlation tests were performed with SigmaPlot software.



Supplemental Figure 3. Transfections of HUVECs with miR-429 mimic (**A**) and AntagomiR (**B**) were efficient both in normoxia and during hypoxia. miRNA levels were monitored in qRT-PCR experiments. The miRNA levels results from 2 independent experiments (n=8) are plotted normalized to RNU44 levels and expressed as a fold change over the control transfection (with c-67). Significant changes ($p < 0.05$) are marked with an "*".



Supplemental Figure 4. HIF-1 induces HIF3A (A) and miR-429 (B) expression during treatment with hypoxia mimetics. HUVECs were treated with hypoxia mimetics (100 μ M desferoxamine (DFO) for 6h or 200 μ M CoCl₂ for 12h) and with or without (grey and white, respectively) a HIF-1 functional inhibitor (topotecan (500 μ M TPT). mRNA and miRNA levels were monitored in qRT-PCR experiments. The miRNA levels results from 2 independent experiments (n=8) are plotted normalized to RNU44 levels and expressed as a fold change over the untreated control. The mRNA levels results from 2 independent experiments (n=12) are normalized to TBP mRNA levels and expressed as a fold-change over the untreated control. Significant changes (p<0.05) are marked with an "**"



Supplemental Figure 5. Hypoxia does not affect expression profile of miR-141 in HUVECs. The miRNA levels were monitored in quantitative real-time PCR experiments. The results from 2 independent experiments (n = 8) are plotted normalized to *RNU44* and expressed as a fold change over the normoxic control (*p < 0.05).