APPENDIX 1: QUANTITATIVE REAL-TIME RT-PCR MICRORNA EXPRESSION DATA IN INDIVIDUAL RATS

A1. CANDIDATE MICRORNA EXPRESSION IN INDIVIDUAL RATS

Time-course analysis of miR-30e, miR-338 and miR-210 expression was performed at days 3, 5 and 6, in individual rats. Between 3 and 11 rats were analysed for each treatment group, depending on the quality of the total RNA used for cDNA synthesis. Any animal from which the RNA yield was poor or the RNA was partially degraded was excluded from further analysis.

miR-30e was chosen as it was predicted to target FIH, miR-338 was chosen as it was predicted to target HIF-α and HIF-β, and miR-210 was chosen as it is known to be upregulated by HIF-α in response to relative hypoxia. Note that miR-338 expression was investigated in lieu of miR-338-3p as described in section 5.4.

Data were transformed where necessary prior to statistical analysis using two-way analysis of variance (ANOVA) to ensure data were normally distributed. Two-way ANOVA was used to compare the effects of strain (F344 and SD together), treatment (room air and cyclic hyperoxia together) and the interaction between strain and treatment (strain*treatment) on miRNA expression. The significance (alpha) level was set at 0.05.

A1.1 miR-30e expression

A1.1.a miR-30e expression at day 3

At day 3, expression of miR-30e was not significantly affected by strain ($F_{(1,37)}$=2.311, p=0.138) or treatment ($F_{(1,37)}$=0.229, p=0.636) in response to hyperoxia. miR-30e was downregulated 1.8 fold in F344 rats in response to hyperoxia in F344 cyclic hyperoxia-exposed rats (mean=0.187; 95% CI: 0.144-0.242) compared to F344 room air-exposed rats (mean=0.372; 95% CI: 0.231-0.600) as shown in Figure A1.1. On the other hand, miR-30e was upregulated 2.1 fold in cyclic hyperoxia-exposed SD rats (mean=0.478; 95% CI: 0.333-0.687) compared to SD room air-exposed rats (mean=0.224; 95% CI: 0.141-0.354). The interaction between strain*treatment was also statistically significant ($F_{(1,37)}$=15.125, p<0.001), suggesting there is a strain-dependent difference in miR-30e expression in response to hyperoxia at day 3.

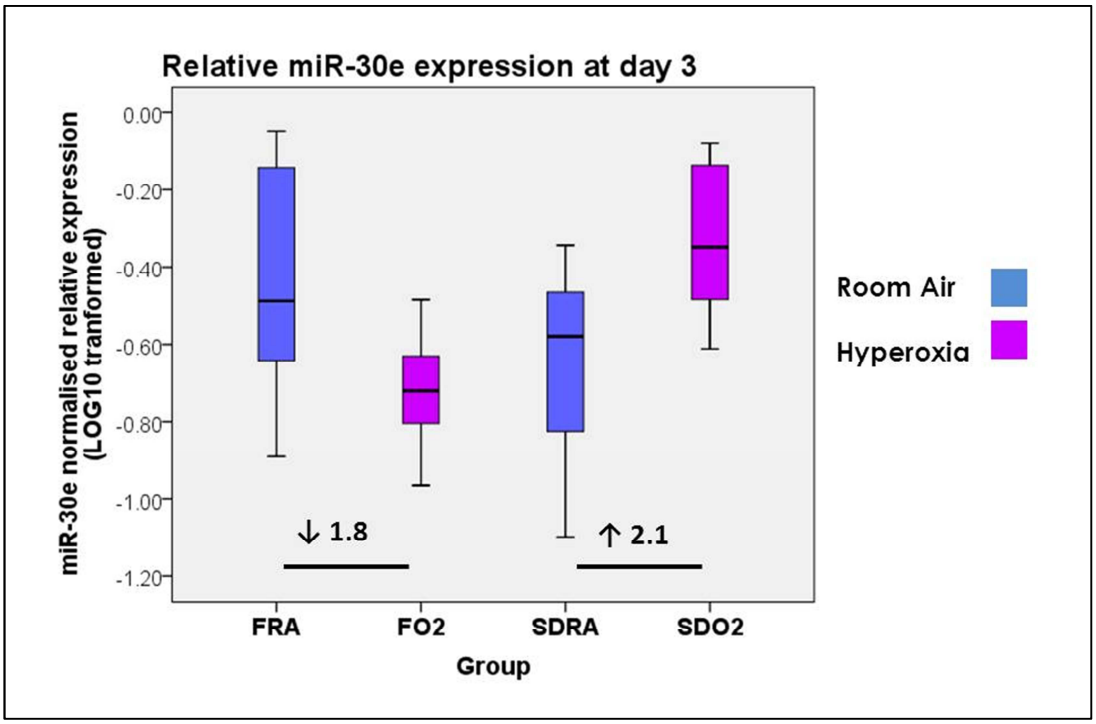

Figure A1.1 Retinal miR-30e expression in F344 and SD rats at day 3 in response to hyperoxia. Quantification was performed on individual rats. FRA= F344 room-air exposed, n = 10; FO_2= F344 cyclic hyperoxia-exposed, n = 9; SDRA= SD room air-exposed, n = 9; SDO_2= SD cyclic hyperoxia-exposed, n = 9. Data were log10 transformed prior to statistical analysis by two-way ANOVA. Expression levels were normalised to the small RNA reference gene miR-16. Numbers and arrows indicate direction of fold changes in hyperoxia.

A1.1.b miR-30e expression at day 5

Expression of miR-30e in response to hyperoxia at day 5 was significantly affected by strain ($F_{(1,34)}$=8.600, p<0.01) but not by treatment ($F_{(1,34)}$=0.0001, p=0.992). miR-30e was downregulated 1.7 fold in F344 rats in response to hyperoxia in F344 cyclic hyperoxia-exposed rats (mean=0.176; 95% CI: 0.102-0.249) compared to F344 room air-exposed rats (mean=0.293; 95% CI: 0.210-0.377; Figure A1.2). miR-30e was upregulated 1.4 fold in cyclic hyperoxia-exposed SD rats (mean=0.411; 95% CI: 0.294-0.528) compared to SD room air-

exposed rats (mean=0.293; 95% CI: 0.195-0.391). The interaction between strain*treatment was also statistically significant ($F_{(1,34)}$=8.634, p<0.01), suggesting there may be a strain-dependent difference in miR-30e expression in response to hyperoxia at day 5.

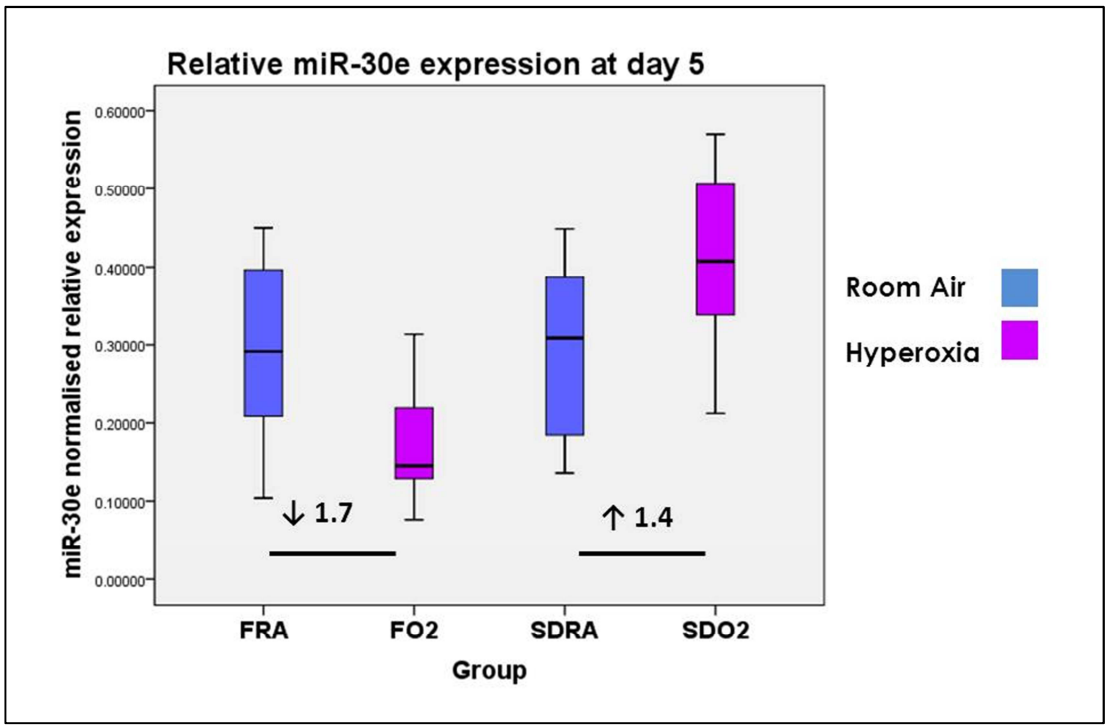

Figure A1.2 Retinal miR-30e expression in F344 and SD rats at day 5 in response to hyperoxia. Quantification was performed on individual rats. FRA= F344 room-air exposed, n = 11; FO2= F344 cyclic hyperoxia-exposed, n = 7; SDRA= SD room air-exposed, n = 9; SDO2= SD cyclic hyperoxia-exposed, n = 9. One room air-exposed F344 rat, one room air-exposed SD rat and two cyclic hyperoxia-exposed SD rats were considered outliers and were excluded from the data prior to statistical analysis, resulting in data from 10 F344 room air-exposed, 8 SD room air-exposed rats and 7 SD cyclic hyperoxia-exposed rats being analysed. Expression levels were normalised to the small RNA reference gene miR-16. Numbers and arrows indicate direction of fold changes in hyperoxia.

A1.1.c miR-30e expression at day 6

In response to relative hypoxia at day 6, miR-30e was not significantly affected by strain ($F_{(1,28)}$=0.836, p=0.370), or treatment ($F_{(1,28)}$=3.309, p=0.082), but was significantly affected by the interaction between strain and treatment ($F_{(1,28)}$=8.162, p<0.01). miR-30e was relatively unchanged in F344 rats in response to hyperoxia in F344 cyclic hyperoxia-exposed rats (mean=0.762; 95% CI: 0.658-0.883) compared to F344 room air-exposed rats (mean=0.866; 95% CI: 0.663-1.132; Figure A1.3). miR-30e was upregulated 1.8 fold in cyclic hyperoxia-exposed SD rats (mean=1.215; 95% CI: 1.004-1.470) compared to SD room air-exposed rats (mean=0.681; 95% CI: 0.538-0.863). Note that Levene's Test of Equality of Error Variances was significant, suggesting that the variance across all groups was not equal. Therefore the significance (alpha) level was accordingly adjusted to 0.01.

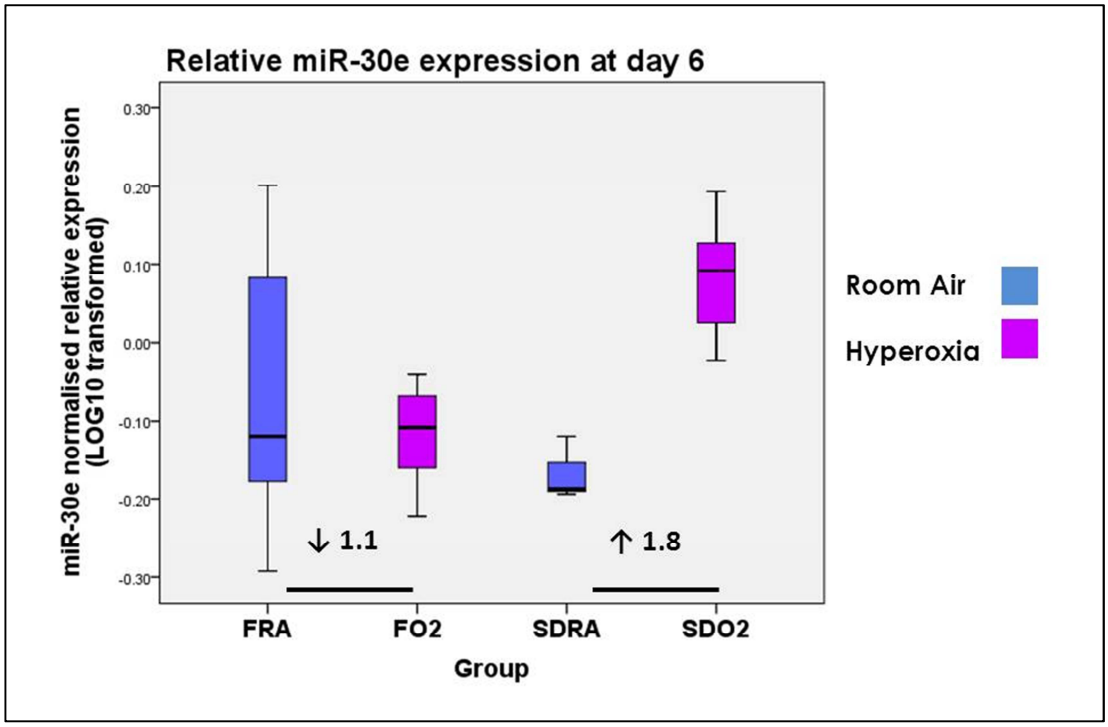

Figure A1.3 Retinal miR-30e expression in F344 and SD rats at day 6 in response to relative hypoxia. Quantification was performed on individual rats. FRA= F344 room-air exposed, n = 11; FO_2= F344 cyclic hyperoxia-exposed, n = 7; SDRA= SD room air-exposed, n = 4; SDO_2= SD cyclic hyperoxia-exposed, n = 7. One room air-exposed and one cyclic hyperoxia-exposed SD rat were considered an outlier and were excluded from the data prior to statistical analysis, resulting in data from 3 SD room air-exposed, and 6 SD cyclic hyperoxia-exposed rats being analysed. Expression levels were normalised to the small RNA reference gene miR-16. Numbers and arrows indicate direction of fold changes in hyperoxia.

A1.2 miR-338 expression

A1.2.a miR-338 expression at day 3

At day 3, miR-338 expression was significantly affected by strain ($F_{(1,33)}$=4.824, $p<0.05$), but not by treatment with cyclic hyperoxia ($F_{(1,33)}$=1.244, p=0.274) or the interaction between strain and treatment ($F_{(1,33)}$=0.047, p=0.829; Figure A1.4). miR-338 was relatively unchanged (1.1 fold downregulation) with exposure to hyperoxia in F344 rats (mean=0.0008;

95% CI: 0.0005-0.0012) compared to room air –exposed rats (mean= 0.0007; 95% CI: 0.005-0.0010). Expression of miR-338 in SD rats was upregulated 1.3 fold in cyclic hyperoxia–exposed rats (mean=0.0006; 95% CI: 0.0005-0.0007) compared to room air control rats (mean=0.0005; 95% CI: 0.0002-0.0007).

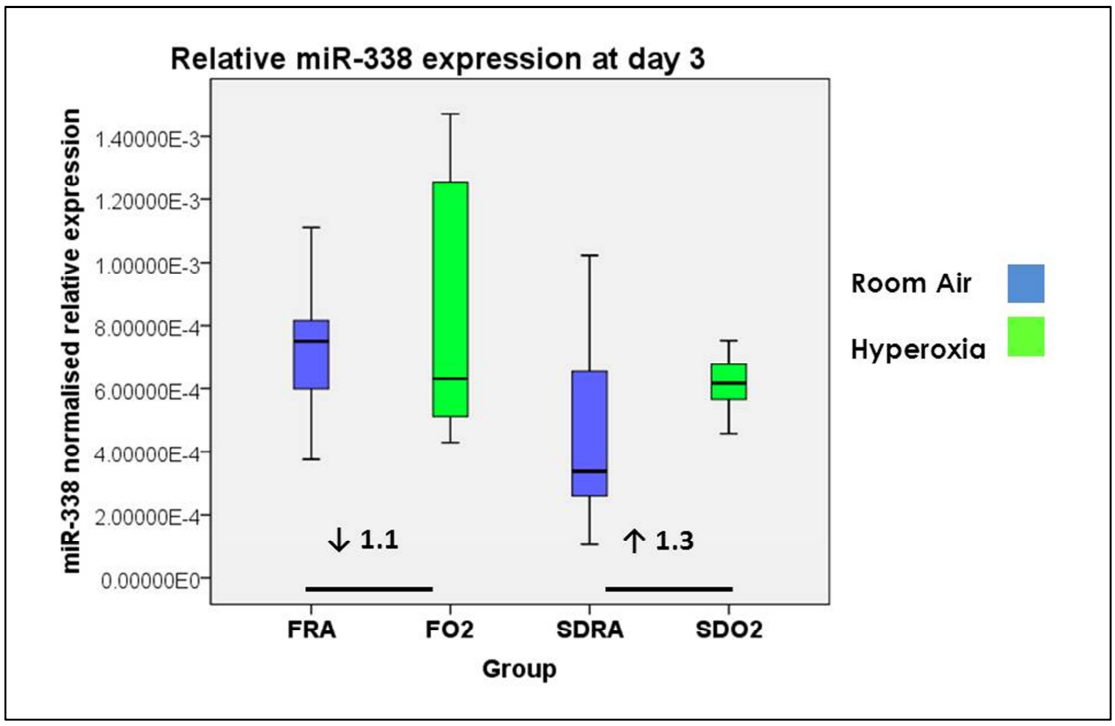

Figure A1.4 Retinal miR-338 expression in F344 and SD rats at day 3 in response to hyperoxia. Quantification was performed on individual rats. FRA= F344 room-air exposed, n = 10; FO_2= F344 cyclic hyperoxia-exposed, n = 9; SDRA= SD room air-exposed, n = 9; SDO_2= SD cyclic hyperoxia-exposed, n = 9. Four room air-exposed F344 rats were considered outliers and were excluded from the data prior to statistical analysis, resulting in data from 6 room air-exposed F344 rats being analysed. Expression levels were normalised to the small RNA reference gene miR-16. Numbers and arrows indicate direction of fold changes in hyperoxia.

A1.2.b miR-338 expression at day 5

miR-338 expression was not significantly affected by strain ($F_{(1,28)}$=0.280, p=0.601) or the interaction between strain and treatment ($F_{(1,28)}$=1.326, p=0.259) but it was significantly affected by treatment ($F_{(1,28)}$=6.071, p<0.05) at day 5 in response to hyperoxia. miR-338 was downregulated 1.3 fold with exposure to hyperoxia in F344 rats (mean=0.0015; 95% CI: 0.0008-0.0022) compared to room air–exposed rats (mean=0.0019; 95% CI: 0.0012-0.0026; Figure A1.5). Expression of miR-338 was also downregulated (2.1 fold) in response to hyperoxia in SD rats (mean=0.0012; 95% CI: 0.0008-0.0016) compared to room air-exposed rats (mean=0.0025; 95% CI: 0.0008-0.0042).

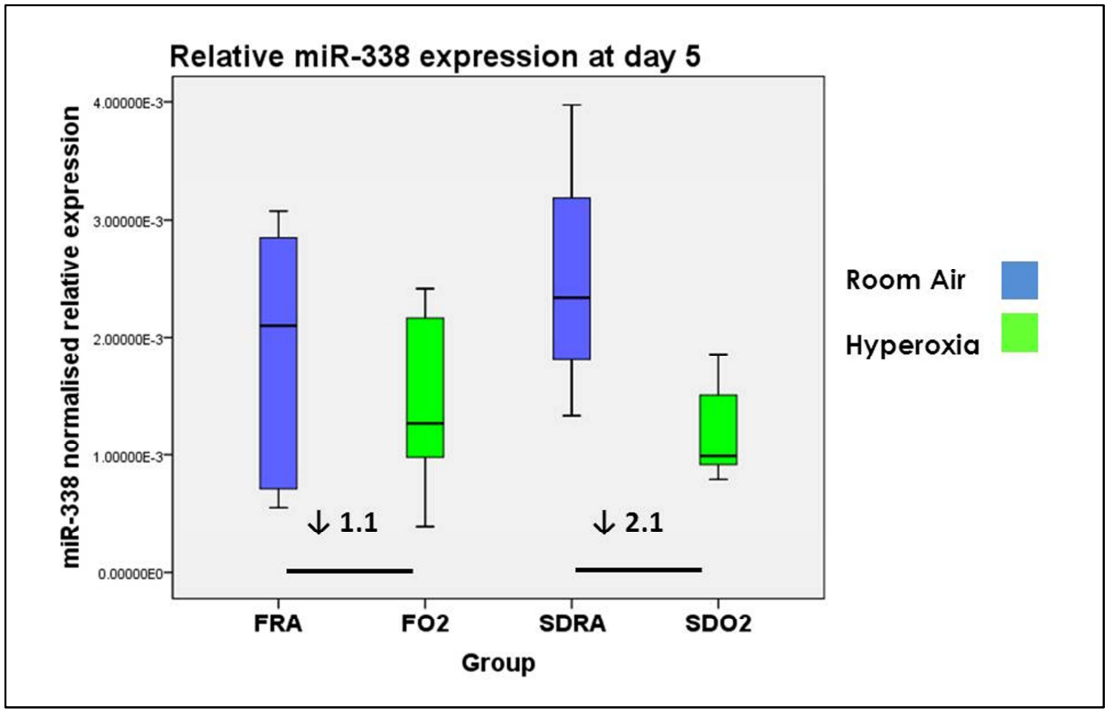

Figure A1.5 Retinal miR-338 expression in F344 and SD rats at day 5 in response to hyperoxia. Quantification was performed on individual rats. FRA= F344 room-air exposed, n = 11; FO_2= F344 cyclic hyperoxia-exposed, n = 7; SDRA= SD room air-exposed, n = 9; SDO_2= SD cyclic hyperoxia-exposed, n = 9. One room air-exposed F344, 5 room air-exposed SD rats and 2 cyclic hyperoxia-exposed SD rats were considered outliers and were excluded from the data prior to statistical analysis, resulting in data from 10 F344, 4 SD room air-exposed rats and 7 cyclic hyperoxia-exposed SD rats being analysed. Expression levels were normalised to the small RNA reference gene miR-16. Numbers and arrows indicate direction of fold changes in hyperoxia.

A1.2.c miR-338 expression at day 6

miR-338 expression was significantly affected by strain ($F_{(1,26)}$=6.337, $p<0.05$) and treatment ($F_{(1,26)}$=5.444, $p<0.05$) at day 6 in response to relative hypoxia. The interaction between strain and treatment was also statistically significant ($F_{(1,26)}$=15.034, $p<0.001$) with strain-dependent differential expression of miR-338 seen. miR-338 was relatively unchanged with exposure to hyperoxia in

F344 rats (mean=0.007; 95% CI: 0.005-0.011) compared to room air–exposed rats (mean=0.009; 95% CI: 0.007-0.013; Figure A1.6). miR-338 was upregulated 3.1 fold in response to hyperoxia in SD rats (mean=0.023; 95% CI: 0.021-0.025) compared to room air-exposed rats (mean=0.007; 95% CI: 0.004-0.015).

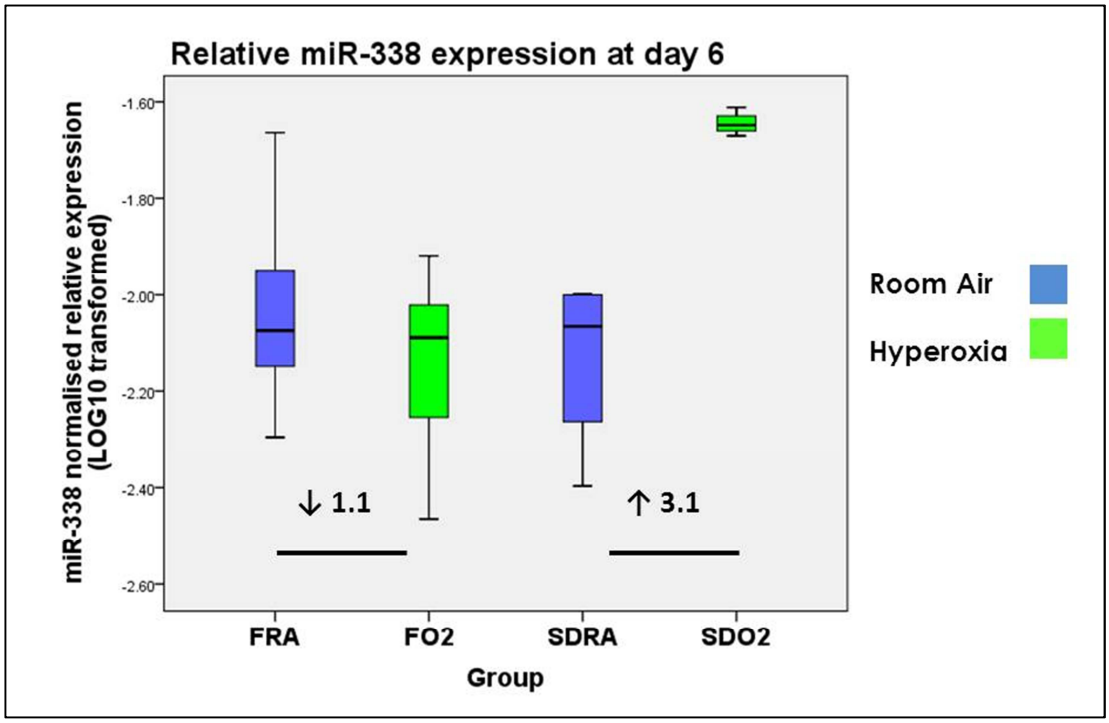

Figure A1.6 Retinal miR-338 expression in F344 and SD rats at day 6 in response to relative hypoxia. Quantification was performed on individual rats. FRA= F344 room-air exposed, n = 11; FO_2= F344 cyclic hyperoxia-exposed, n = 7; SDRA= SD room air-exposed, n = 4; SDO_2= SD cyclic hyperoxia-exposed, n = 7. Data were log10 transformed prior to statistical analysis by two-way ANOVA. Three cyclic hyperoxia-exposed SD rats were considered outliers and were excluded from the data prior to statistical analysis, resulting in data from 4 cyclic hyperoxia-exposed SD rats being analysed. Expression levels were normalised to the small RNA reference gene miR-16. Numbers and arrows indicate direction of fold changes in hyperoxia.

A1.3 miR-210 expression

A1.3.a miR-210 expression at day 3

miR-210 expression was significantly affected by strain ($F_{(1,37)}$=4.735, $p<0.05$), treatment ($F_{(1,37)}$=62.990, $p<0.001$) and the interaction between strain and treatment ($F_{(1,37)}$=4.975, $p<0.05$) in response to hyperoxia at day 3. miR-210 was downregulated 1.3 fold in cyclic hyperoxia-exposed F344 rats (mean=0.354; 95% CI: 0.286-0.437) compared to room air-exposed F344 rats (mean=0.598; 95% CI: 0.490-0.730; Figure A1.7). In SD rats, miR-210 was downregulated in response to hyperoxia with a 2.5 fold change observed in cyclic hyperoxia-exposed SD rats (mean=0.352; 95% CI: 0.279-0.444) compared to room air-exposed SD rats (mean=0.897; 95% CI: 0.734-1.096).

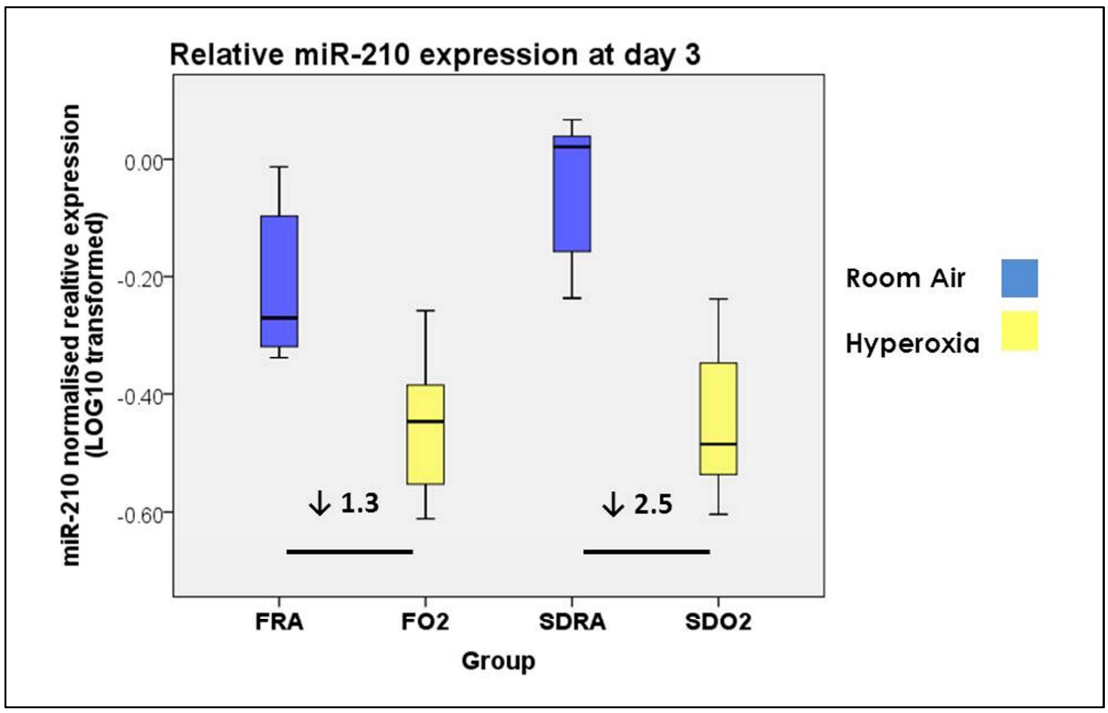

Figure A1.7 Retinal miR-210 expression in F344 and SD rats at day 3 in response to hyperoxia. Quantification was performed on individual rats. FRA= F344 room-air exposed, n = 10; FO_2= F344 cyclic hyperoxia-exposed, n = 9; SDRA= SD room air-exposed, n = 9; SDO_2= SD cyclic hyperoxia-exposed, n = 9. Data were log10 transformed prior to statistical analysis by two-way ANOVA. Expression levels were normalised to the small RNA reference gene miR-16. Numbers and arrows indicate direction of fold changes in hyperoxia.

A1.3.b miR-210 expression at day 5

At day 5, miR-210 expression was significantly affected by strain ($F_{(1,34)}$=13.569, p<0.001) and treatment ($F_{(1,34)}$=63.116, p<0.001) but was not significantly affected by the interaction between strain and treatment ($F_{(1,34)}$=5.416, p=0.0269) in response to hyperoxia. Note that Levene's Test of Equality of Error Variances was significant, suggesting that the variance across all groups was not equal. Therefore the significance (alpha) level was accordingly adjusted to 0.01.

In F344 cyclic hyperoxia-exposed rats, miR-210 was downregulated 1.6 fold (mean=0.439; 95% CI: 0.331-0.582) compared to room air-exposed rats (mean=0.703; 95% CI: 0.605-0.816, Figure A1.8). Again a greater down regulation of miR-210 was observed in the SD cyclic hyperoxia-exposed rats (2.4 fold, mean=0.492; 95% CI: 0.457-0.530) compared to SD room air-exposed rats (mean=1.164; 95% CI: 0.919-1.475).

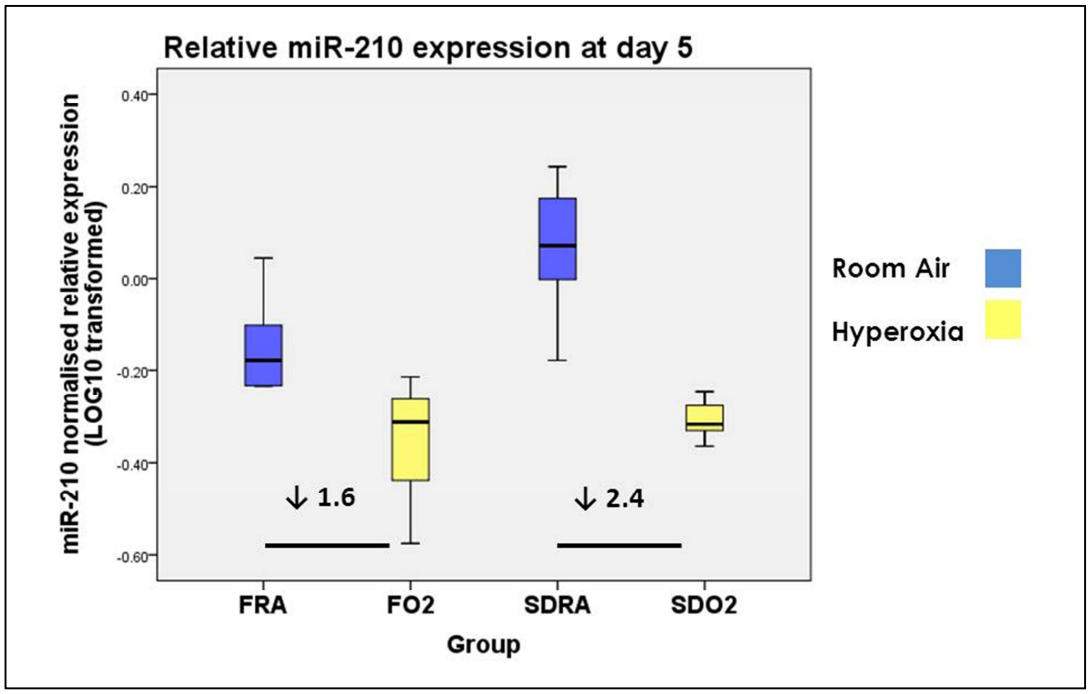

Figure A1.8 Retinal miR-210 expression in F344 and SD rats at day 5 in response to hyperoxia. Quantification was performed on individual rats. FRA= F344 room-air exposed, n = 11; FO_2= F344 cyclic hyperoxia-exposed, n = 7; SDRA= SD room air-exposed, n = 9; SDO_2= SD cyclic hyperoxia-exposed, n = 9. One F344 room air-exposed and one cyclic hyperoxia-exposed SD rat were considered outliers and were excluded from the data prior to statistical analysis, resulting in data from 10 F344 room air-exposed and 8 cyclic hyperoxia-exposed SD rats being analysed. Data were log10 transformed prior to statistical analysis by two-way ANOVA. Expression levels were normalised to the small RNA reference gene miR-16. Numbers and arrows indicate direction of fold changes in hyperoxia.

A1.3.c miR-210 expression at day 6

In response to relative hypoxia at day 6, miR-210 expression was significantly affected by strain ($F_{(1,28)}$=9.359, p<0.01), treatment ($F_{(1,28)}$=6.533, p<0.05), and the interaction between strain and treatment ($F_{(1,28)}$=29.477, p<0.001). A strain-dependent difference in miR-210 expression was observed with miR-210 being downregulated 1.5 fold (mean=0.041; 95% CI: 0.029-0.054) in F344 cyclic hyperoxia-exposed rats compared to room air-exposed rats (mean=0.062; 95% CI: 0.050-0.074; Figure A1.9), whereas there was a 2.3 fold upregulation of miR-210 expression in SD cyclic hyperoxia-exposed rats (mean=0.101; 95% CI: 0.079-0.124) compared to SD room-air exposed rats (mean=0.045; 95% CI: 0.017-0.073).

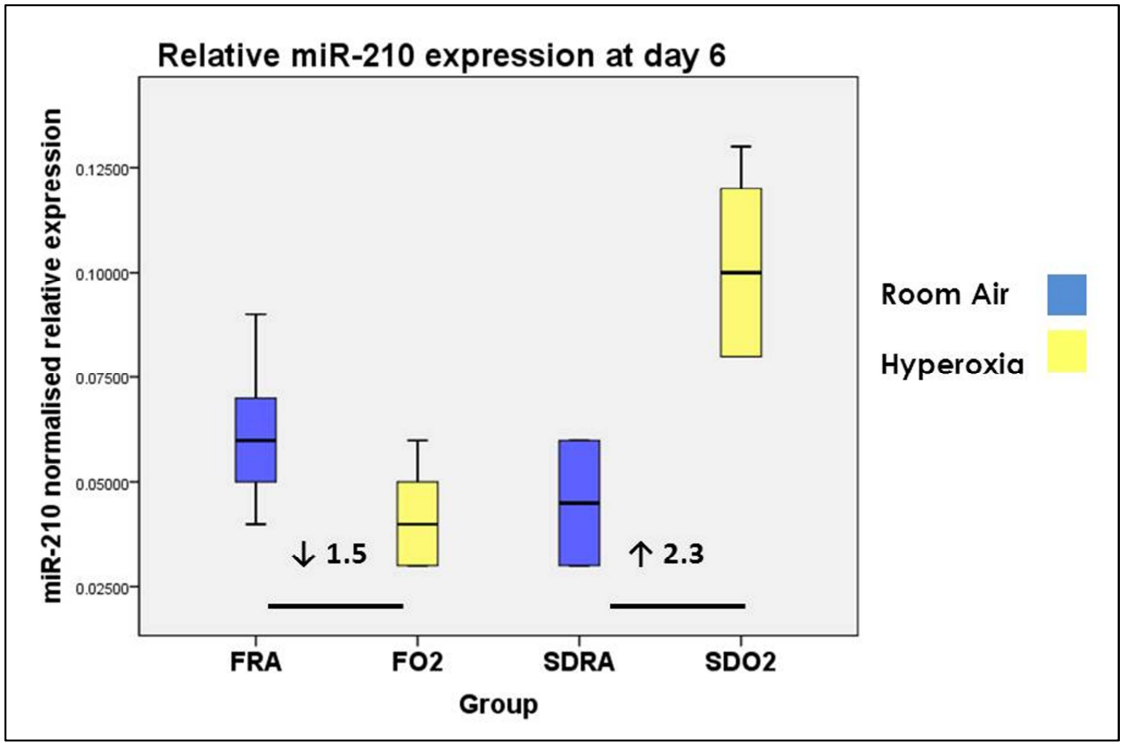

Figure A1.9 Retinal miR-210 expression in F344 and SD rats at day 6 in response to relative hypoxia. Quantification was performed on individual rats. FRA= F344 room-air exposed, n = 11; FO_2= F344 cyclic hyperoxia-exposed, n = 7; SDRA= SD room air-exposed, n = 4; SDO_2= SD cyclic hyperoxia-exposed, n = 7. Expression levels were normalised to the small RNA reference gene miR-16. Numbers and arrows indicate direction of fold changes in hyperoxia.