## Supplemental Material

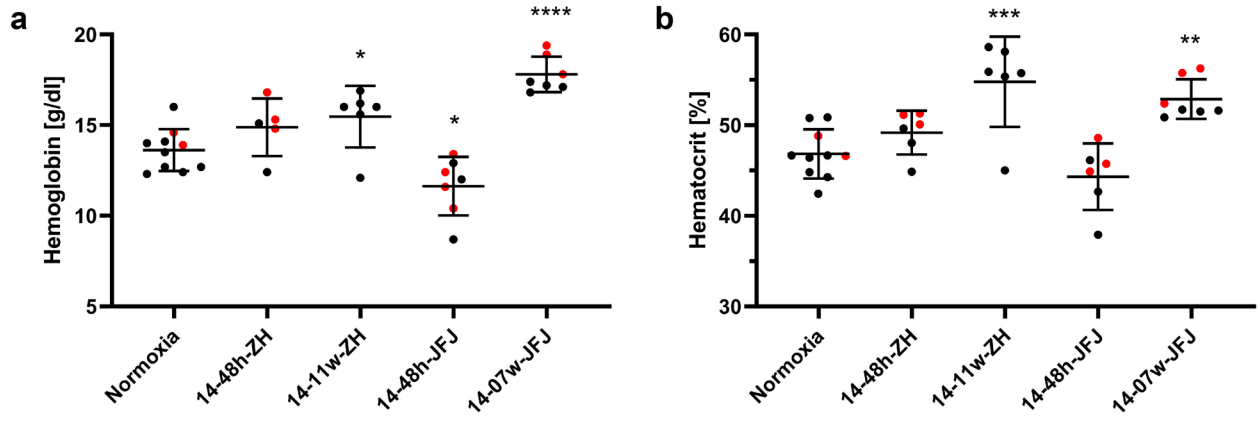


**Figure S1| Hemoglobin and hematocrit levels in mice exposed to different hypoxic conditions.**

**a)** Hemoglobin values in [g/dL]. **b)** Hematocrit in [%]. Shown are means ± SD, n≥5. Female samples are indicated in red. Significance tested with one-way ANOVA with Holm-Sidak's multiple comparison to normoxic group. *p<0.05; **p<0.005; ***p<0.0005 ****p<0.0001. Groups represent exposure to 14% $O_2$ for short (48 h) and long term (07-11 weeks) in normobaric (ZH) and hypobaric (JFJ) hypoxia.

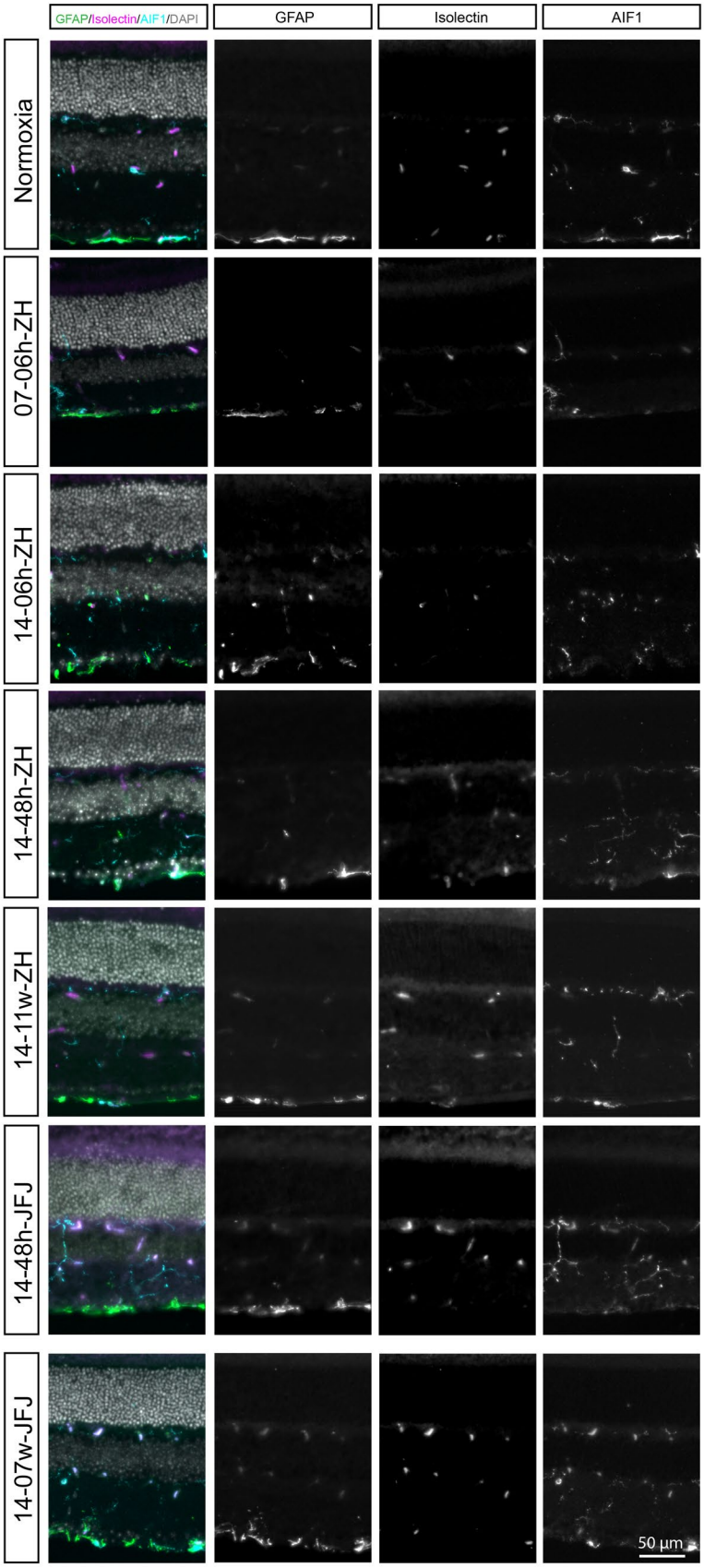


**Figure S2| Immunofluorescence of markers for gliosis, blood vessels and, microglia/macrophages.**

Immunostaining of glial fibrillary acidic protein (GFAP in green), isolectin as vascular stain (magenta), and allograft inflammatory factor 1 (AIF1 alias IBA1, blue) for microglia in indicated experimental groups. Cell nuclei were counterstained with DAPI (grey). Scale bar as indicated.

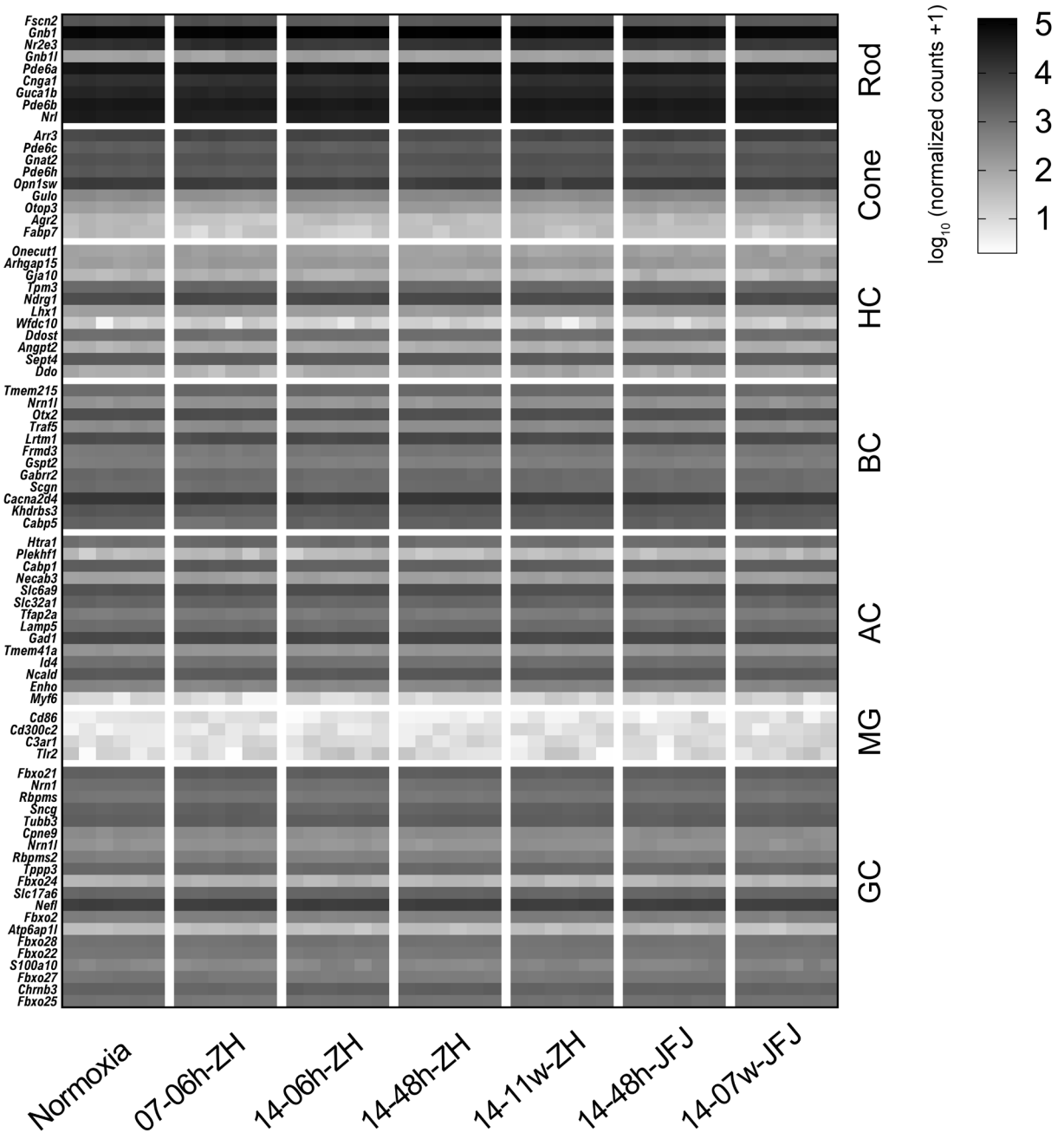


**Figure S3| Heatmap of cell type-specific genes.**

Plotted are $\log_{10}$(normalized counts+1). HC: horizontal cells; BC: bipolar cells; AC: amacrine cells; MG: microglia; GC: ganglion cells.

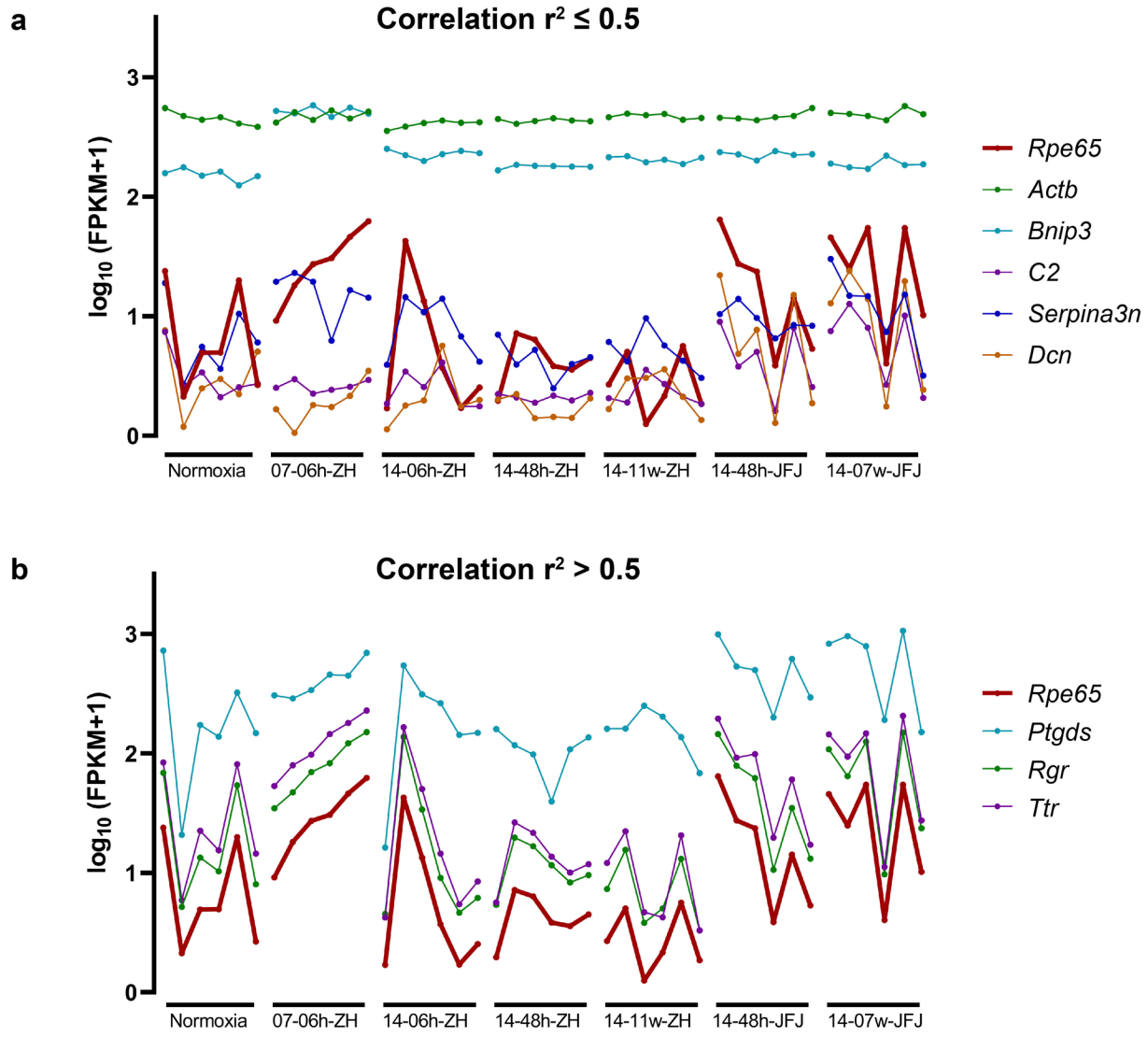


**Figure S4| Correlation to *Rpe65* expression as an indicator of contamination with RPE transcripts.**

**a)** Example genes which displayed a correlation coefficient $r^2 \leq 0.5$ to *Rpe65*. These genes are unlikely to result from an RPE contamination. Actin-beta (*Actb*) is displayed as a housekeeping gene, BCL2 Interacting Protein 3 (*Bnip3*) is exemplarily shown as a hypoxia regulated gene. **b)** Example genes which displayed a correlation coefficient higher than 0.5 to *Rpe65* expression, indicating that some of the signals were attributable to RPE contamination. The genes that had an $r^2 \geq 0.5$ were excluded from further analysis.

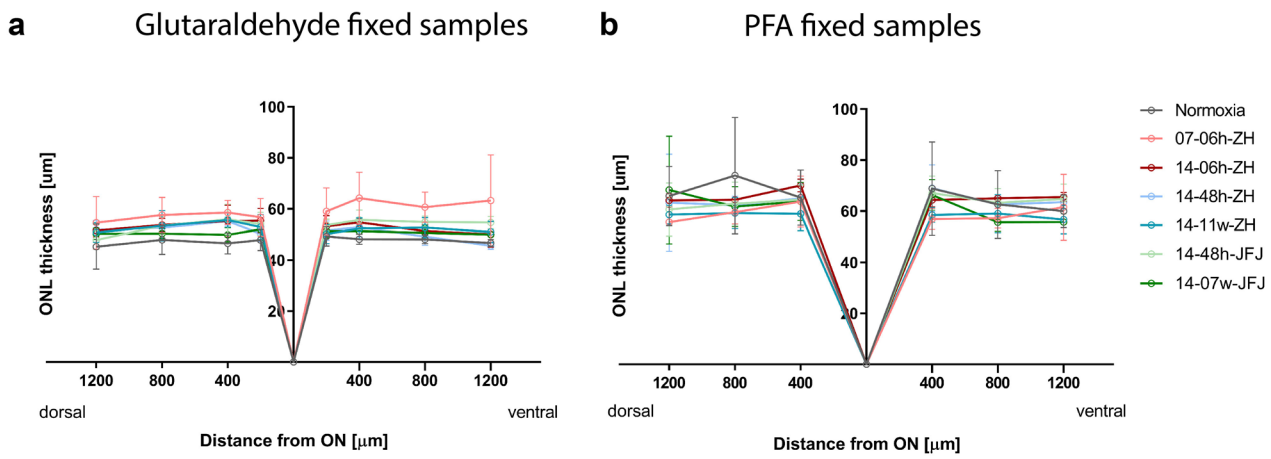


**Figure S5 |Thickness of the outer nuclear layer under hypoxic conditions.**

Spiderograms of outer nuclear layer (ONL) thickness measured at indicated points dorsal and ventral to the optic nerve head (ON) in different hypoxic conditions after **a)** glutaraldehyde fixation of samples for morphological evaluation and **b)** 4% PFA fixation of eyes serving for immunofluorescence analysis. ONL: outer nuclear layer; ON: optic nerve head; PFA: paraformaldehyde. Shown are means ± SD of n=6 per group.

**Table S1** | Hemoglobin and Hematocrit values

| Condition | Hemoglobin [g/dL] mean ± SD | Hematocrit [%] mean ± SD |
|---|---|---|
| Normoxia | 13.62 ± 1.15 | 46.83 ± 2.71 |
| 14-48h-ZH | 14.88 ± 1.58 | 49.17 ± 2.41 |
| 14-48h-JFJ | 11.63 ± 1.61 * | 44.32 ± 3.67*** |
| 14-11w-ZH | 15.47 ± 1.7 * | 54.79 ± 4.98 |
| 14-07w-JFJ | 17.8 ± 0.98 **** | 52.87 ± 2.19 ** |

Hypoxic groups were compared to normoxic controls. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

**Table S2** | Correlation between RNA-Seq and qPCR gene expression in the retina

| Gene name | FC RNA-Seq over Normoxia | | | | | |
|---|---|---|---|---|---|---|
| | 07-06h-ZH | 14-06h-ZH | 14-48h-ZH | 14-11w-ZH | 14-48h-JFJ | 14-07w-JFJ |
| *Adm* | 5.56 | 1.65 | 1.35 | 1.23 | 1.55 | 1.30 |
| *Bnip3* | 3.50 | 1.57 | 1.22 | 1.33 | 1.45 | 1.21 |
| *Egln1* | 6.16 | 2.17 | 1.49 | 1.35 | 1.62 | 1.50 |
| *Slc2a1* | 2.01 | 1.52 | 1.23 | 1.09 | 1.42 | 1.33 |
| *Pdk1* | 2.91 | 1.85 | 1.32 | 1.20 | 1.48 | 1.30 |
| *Vegfa* | 1.83 | 1.00 | 0.95 | 1.09 | 1.21 | 1.06 |

| Gene name | FC qPCR over Normoxia | | | | | |
|---|---|---|---|---|---|---|
| | 07-06h-ZH | 14-06h-ZH | 14-48h-ZH | 14-11w-ZH | 14-48h-JFJ | 14-07w-JFJ |
| *Adm* | 23.22 | 3.26 | 2.38 | 1.29 | 5.27 | 3.59 |
| *Bnip3* | 2.20 | 1.00 | 0.62 | 1.20 | 0.81 | 0.82 |
| *Egln1* | 3.60 | 1.33 | 0.76 | 1.18 | 0.89 | 0.87 |
| *Slc2a1* | 0.95 | 0.84 | 0.55 | 0.96 | 0.78 | 0.82 |
| *Pdk1* | 1.49 | 0.80 | 0.48 | 0.95 | 0.83 | 0.71 |
| *Vegfa* | 1.13 | 0.76 | 0.63 | 0.94 | 0.83 | 0.69 |

Shown are fold changes (FC) over normoxic controls for RNAseq and qPCR data for each experimental condition. N=6.

**Table S3** | Excluded gene list

| Group | Genes excluded from analysis |
|---|---|
| Crystallins | *Cryab; Cryba1; Cryba2; Cryba4; Crybb1; Crybb2; Crybb3; Crybg1; Crybg2; Crybg3; Cryga; Crygb; Crygc; Crygd; Cryge; Crygf; Crygn; Crygs; Cryl1; Crym; Cryz; Cryaa* |
| Hemoglobins | *Hba-a1; Hba-a2; Hba-x; Hbb-bh1; Hbb-bh2; Hbb-bs; Hbb-bt; Hbb-y* |
| RPE-specific | *Dct; Lgsn; Lrat; Mlana; Pmel; Rpe65; Slc45a2; Tyrp1* |

**Table S4** | Primers used for qPCR

| Gene | forward Primer (5'- 3') | reverse Primer (5'- 3') |
|---|---|---|
| ***Hif1a*** | TCATCAGTTGCCACTTCCCCA | CCGTCATCTGTTAGCACCATC |
| ***Epas1*** | GGAGCTCAAAAGGTGTCAGG | CAGGTAAGGCTCGAACGATG |
| ***Adm*** | TCCTGGTTTCTCGGCTTCTC | ATTCTGTGGCGATGCTCTGA |
| ***Slc2a1*** | CAGTGTATCCTGTTGCCCTTCTG | GCCGACCCTCTTCTTTCATCTC |
| ***Hk2*** | CCTGGTTTCAAAGCGGTCGG | TACTGGTCAACCTTCTGCACTTG |
| ***Col1a1*** | TGTTCAGCTTTGTGGACCTC | TCAAGCATACCTCGGGTTTC |
| ***Pdk1*** | GTTGAAACGTCCCGTGCT | AGTCTCTCGACGGATTCTGT |
| ***Angptl2*** | TCGCTGGTGAAGAGTCCAAC | GACCACATGCGTCAAACCAC |
| ***Vegfa*** | ACTTGTGTTGGGAGGAGGATGTC | AATGGGTTTGTCGTGTTTCTGG |
| ***Bnip3*** | CCTGTCGCAGTTGGGTTC | GAAGTGCAGTTCTACCCAGGAG |
| ***Egln1*** | GCAGCATGGACGACCTGAT | CAACGTGACGGACATAGCCT |

**Supplementary Dataset 1** (Supplementary Dataset 1.xlsx)

Differentially expressed genes (FDR ≤ 0.05) in each group over normoxia. A detailed list of genes shown in Fig. 1a. Given are the official gene name, ensembl number (Identifier), gene description, the $log_2$FC over normoxic controls, FDR and p-Value. Individual tabs show values of each DE gene over normoxia for each hypoxic condition: Tab1: 07-06h-ZH; Tab2: 14-06h-ZH; Tab3: 14-48h-ZH; Tab4; 14-11w-ZH; Tab5: 14-48h-JFJ; Tab6: 14-07w-JFJ. FC: fold change; FDR: false discovery rate.

**Supplementary Dataset 2** (Supplementary Dataset 2.xlsx)

List of 442 differentially expressed genes (FDR ≤ 0.05; $log_2$FC ≥ ± 1 or $log_2$FC ≤ - 1) exclusively in the 07-06h-ZH group versus normoxia. Given are the official gene name, ensembl number (Identifier), gene description, the $log_2$FC over normoxic controls, FDR and p-value. FC: fold change; FDR: false discovery rate.

**Supplementary Dataset 3** (Supplementary Dataset 3.xlsx)

Genes in the top 4 sets identified in the gene set enrichment analysis (GSEA) of 07-06h-ZH compared to normoxia with MSigDB hallmark datasets. Listed are genes contributing to the core enrichment (defined as genes most contributing to the enrichment results) of each set. Given are the rank according to enrichment, official gene name, ensembl number (Identifier), gene description, the $log_2$FC over normoxic controls, FDR, and p-value. Tab1: Hallmark hypoxia; Tab2: Hallmark glycolysis; Tab3: Hallmark TNFa signaling; Tab4: Hallmark coagulation. FC: fold change; FDR: false discovery rate.

**Supplementary Dataset 4** (Supplementary Dataset 4.xlsx)

List of differentially expressed genes (FDR ≤ 0.05; $log_2FC \geq + 1$ or $log_2FC \leq 1$) in hypobaric versus normobaric 14% hypoxia. Given are the official gene name, ensembl number (Identifier), gene description, the $log_2FC$ over normoxic controls, FDR and p-value. Tab1: 14-48h-JFJ over 14-48h-ZH; Tab2: 14-07w-JFJ over 14-11w-ZH. FC: fold change; FDR: false discovery rate.

**Supplementary Dataset 5** (Supplementary Dataset 5.xlsx)

Overview of RNA-Seq datasets comparing hypoxic groups to normoxia. Unfiltered RNA-Seq datasets used for the analysis in this manuscript. The reference genome used (GRC38.p5; https://www.ncbi.nlm.nih.gov/assembly/GCF_000001635.25/) contains 25,700 genes. Given are official gene name, ensembl number (Identifier), gene description, the $log_2FC$ over normoxic controls, FDR, and p-value for each gene. Individual tabs show values of each gene over normoxia for each hypoxic condition: Tab1: 07-06h-ZH; Tab2: 14-06h-ZH; Tab3: 14-48h-ZH; Tab4; 14-11w-ZH; Tab5: 14-48h-JFJ; Tab6: 14-07w-JFJ. FC: fold change; FDR: false discovery rate.