**Supplemental File**

## METHODS

**Animals**. All procedures with animals in this study were performed according to approved protocols under the supervision of the Institutional Animal Care and Use Committee of the University of Colorado Anschutz Medical Campus (Aurora, CO). All mice were C57bl6/J background. The experiments in mice began when they were 12 weeks old. Sprague Dawley (SD) male rats weighing 210-245 grams were used for rat experiments.

***Transgenic mice and genotyping***.

*Hif1α* floxed mice (1), *Hif-2α* floxed mice (2) were constructed wherein exon 2 (coding DNA binding domain) of the Hif1α or Hif2α gene was floxed with LoxP sites. *UbcCreERT*$^{+}$ mice express a fusion protein consisting of cre recombinase and the human estrogen receptor binding domain under control of a ubiquitously active Ubiquitin-C gene promoter, in which Cre recombinase is activated upon tamoxifen exposure. Crossing of Hif1α$^{fl/fl}$ or Hif2α$^{fl/fl}$ mice with UbcCreERT$^{+}$ mice generated *Hif1α*$^{fl/fl}$*;UbcCreERT*$^{+}$mice, *Hif2α*$^{fl/fl}$*;UbcCreERT*$^{+}$ mice and *Hif2α*$^{fl/WT}$*;UbcCreERT*$^{+}$ mice, in which tamoxifen treatment completely or partially deleted *Hif1α* or *Hif2α* gene in all cell types in adult mice (See Suppl Fig 1A-C for breeding information and Suppl Table 1 for Hifa reduction efficiency). Endothelial cell–specific knockout of *Hif2α* was generated by crossing *Hif2α*$^{fl/fl}$ mice to mice expressing cre recombinase under the control of the VE-cadherin promoter (from the Jackson laboratory, kindly provided by Dr. Eltzschig's laboratory, University of Colorado, Denver, CO, USA) (See Suppl Fig 1D for breeding

information)(3). In all cases, mouse genotypes were determined using real time PCR of tail genomic DNAs by a commercial vendor (Transnetyx, Cordova, TN, USA). Age-matched (about 12 wk old), male and female mice with the desired genotypes were used for experimentation.

***Tamoxifen treatment in mice.*** To delete Hif1α or Hif2α gene in adult mice, *UbcCreERT*$^{+}$ or *UbcCreERT*$^{-}$ (as control) mice were injected with 100 µl of tamoxifen solution (in peanut oil at 10mg/ml) i. p. once daily over five consecutive days. Mice were then allowed to rest for one week before being exposed to hypobaric hypoxia or normoxia.

***Antisense oligonucleotide treatment in mice***. *Hif1α* antisense oligonucleotide (henceforth *Hif1α*-ASO; 75mg/kg; 298745-11) or *Hif2α-ASO* (50mg/kg; 589983-5) or non-specific ASO (control-ASO; 549148-18) was injected intraperitoneally twice per week, beginning one week before the hypoxia exposure and continued during the hypoxia exposure. ASOs were provided by Ionis Pharmaceuticals, Inc. (Carlsbad, CA, USA). See Suppl Table for HIF1α and HIF2α mRNA reduction rate.

***Rats treated with HIF2 inhibitor PT2567***. Sprague Dawley (SD) male rats weighing 210-245 grams (Charles River Laboratories) were housed in chambers under normoxia or hypoxic (high altitude ~ 18,000 feet) conditions for four days or four weeks. Rats were dosed with vehicle methylcellulose (0.5%)/Tween-80 (0.5%) or PT2567 (300 mg/kg/day) (4), beginning the day they were placed in chambers. PT2567 was provided by Peloton Therapeutics, Inc.

***Exposure to chronic hypoxia.*** Mice or rats were housed for indicated times (5 weeks for mice, 4 days or 4 weeks for rats) in chambers equipped for continuous hypobaric

hypoxia (0.5 atm, equivalent to 5,486m/18,000 feet altitude and corresponding to $FiO_2$ 0.1). Chambers were vented to room atmosphere weekly for cage maintenance (twice weekly in case of ASO administration). Control mice were housed in identical chambers at sea level atmosphere (1.0 atm, 0m altitude, $FiO_2$ 0.21).

**Measurement of right ventricular systolic pressure in mice and mean pulmonary arterial pressure in rats**. RV pressure measurements were performed essentially as previously described (5). Briefly, mice were anesthetized with isoflurane. The RV was directly catheterized with a fluid-filled catheter interfaced with a pressure transducer connected to a dedicated computer. Trains of successive pressure waveforms were analyzed and averaged over three seconds to determine RV systolic pressure (RVSP). MPAP measurements were performed as described (6). Additional measurements were performed in ASO-experiments utilizing a 1.4 French Pressure Volume Conductance System SPR-839 (Millar Instruments, Houston, TX) inserted into the RV via a surgical transdiaphragmatic approach.

**Tissue preparation, hematocrit measurement, Fulton index and weight ratios**. Animals were deeply anesthetized. Blood samples were taken by left ventricular puncture and the hematocrit was measured using glass capillaries and a suitable centrifuge (IEC, model MB micro-capillary centrifuge). Lung vasculature was flushed with PBS through the RV after incision of the left atrium. For immunohistochemical analysis, left lungs were inflated with agarose in situ and fixed in paraformaldehyde (4%) after excision. Left lungs were then paraffin embedded and cut into 5 µm sections. Right lung lobes were excised, snap frozen in liquid nitrogen and stored at -80°C for RNA extraction to conduct pulmonary gene expression analysis. To determine right

ventricular hypertrophy, the atria were removed from the heart. Subsequently, the heart was dissected into sections of RV free wall and LV free wall including the septum (LV+S) or LV only. Heart tissue was patted dry, weights were taken and the Fulton index (Fulton index = $Weight_{RV}/Weight_{LV+S}$) or $Weight_{RV}/Weight_{LV}$ and the ratio of $weight_{RV}$/bodyweight were calculated. After taking weights, heart tissue was snap frozen in liquid nitrogen and stored at -80°C for RNA extraction to conduct right ventricle gene expression analysis.

**Immunohistochemistry of lung tissue**. Vascular remodeling was quantified by immunohistochemical staining with specific antibody and hematoxylin counterstaining as previously described (5). Alpha-smooth muscle actin antibody (αSMA) (Abcam ab 66133 rabbit polyclonal antibody; dilution 1:400) was used to assess vessel muscularization. Using a Nikon microscope, twelve fields (1$\mu m^2$ each) per lung were inspected and muscularized vessels (αSMA+) were counted. Anti-ED1 antibody, anti-Ki67 antibody, or anti-Tenascin C antibody was used to assess monocyte/macrophage accumulation, cell proliferation, or vessel remodeling respectively.

**Cell culture and treatments**. Normal human pulmonary artery endothelial cells and smooth muscle cells were provided by Pulmonary Hypertensive Breakthrough Initiative. Human ECs were cultured in endothelial cell basal medium plus supplements (Cat#: CC-3202, Lonza Walkersville, MD USA). Human SMCs were cultured in smooth muscle cell basal medium plus supplements (Cat#: CC-3182, Lonza Walkersville, MD USA). Human pulmonary artery Fibs are from control donors undergoing lobectomy or pneumonectomy at Papworth Hospital, Cambridge, UK and were cultured in fibroblast growth medium (DMEM) (15-018-CV, Corning, VA USA) supplemented with fetal bovine

serum (FBS) (10%, Cat#: 100-106, Gemini, CA USA) and Penicillin-Streptomycin (P/S) (30-002-CI, Corning, VA USA), MEM Nonessential Amino Acids (25-025-CI, Corning, VA USA) and L-Glutamine (4mM, 25-005-CI, Corning, VA USA). When hypoxia treatment was needed, cells were placed in normoxia (Nx, 21% O2) or hypoxia (Hx, 1.5% O2) for 16 h for HIF target gene studies, cultured under regular 10% FBS medium. To assess the role of HIF1 or HIF2 in the increased expression of HIF target genes in hypoxic EC cells, ECs were treated with HIF2 inhibitor PT2567 at concentration of 0.1, 1. or 10 μM or targeted with siRNA against Hif2*α* or Hif1*α* mRNA.

**Gene expression analysis***.* After tissue disruption and homogenization using a bead-mill system, RNA from organs such as lung and heart, or cells was isolated using RNeasy column kits (Qiagen). Synthesis of cDNA was performed using a commercially available kit according to the manufacturer's instructions (iScript Advanced cDNA Synthesis Kit, Bio-Rad, Hercules, CA, USA). Quantification of messenger RNA levels was performed by reverse transcription quantitative PCR (RT–qPCR) using iQ Universal SYBR Green Supermix (Bio-Rad) and CFX384 Real Time System (Bio-rad). All primer sets for RT–qPCR were validated for their specificity and amplification efficiency (85% to 110%) using melt curve analysis, RT–qPCR product sequencing and standard dilution analysis. Primer sequences are listed in Suppl. Table 6. The qPCR results were analyzed using the ΔΔCT method using 18S ribosomal RNA and beta actin messenger RNA as reference genes and presented in relative to samples from controls. For Fig. 3A) and Suppl. Fig. 8), commercially TaqMan probes and reagents were used (Applied Biosystems, Grand Island, NY), according to the manufacturer's instructions. At least

three independent experiments were performed for all in vitro results presented in this paper.

**Statistics**. In general, data is reported as mean +/- SEM. Statistical differences were evaluated by 2-way ANOVA test as well as an unpaired, two-tailed t-test. Figure legends specified the statistical analysis used for the data in each panel. Star (*) is used to indicate the difference between hypoxia versus normoxia in the same genotype (or treatment) group. # is used to show the differences between genotypes or treatments under hypoxic condition while ^ is used to mark the differences between genotypes or treatments under normoxic condition. Survival curves were compared by log-rank test. $P<0.05$ (* or # or ^) was considered statistically significant, other levels of statistical significance are reported as $p<0.01$ (** or ## or ^^) and $p<0.001$ (*** or ### or ^^^). Group size (n) is reported in the corresponding figure legends. Data was logged using Excel (Microsoft Corp.), graphing and calculations were performed using Graphpad Prism (GraphPad Software Inc.) and figures were designed using Powerpoint (Microsoft Corp.).

## References


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**Supplementary Figure 1. Breeding schemes to generate the transgenic mice used in the study. A)** Breeding scheme to generate *Hif1α$^{fl/fl}$;UBC-creER$^{+/-}$ and Hif1α$^{fl/fl}$;UBC-creER$^{-/-}$* mice. **B)** Breeding scheme to generate *Hif2α$^{fl/fl}$;UBC-creER$^{+/-}$ and Hif2α$^{fl/fl}$;UBC-creER$^{-/-}$* mice. **C)** Breeding scheme to generate *Hif2α$^{fl/wt}$;UBC-creER$^{+/-}$ and Hif2α$^{fl/wt}$;UBC-creER$^{-/-}$* mice **D.**) Breeding scheme to generate *Hif2α$^{fl/fl}$;Ve-cadherin-cre$^{+/-}$* and *Hif2α$^{fl/fl}$;Ve-cadherin-cre$^{-/-}$* mice.

**Supplementary Figure 2. Hif2 ASO reduces cardiac function of mice under hypoxia.** Mice were treated Control or HIF2a ASO as described in Fig. 3. At the end of the experiment, cardiac expression of Hif2a mRNA was determined by qRT-PCR (A). Levels of catecholamines in blood plasma as determined by HPLC (B). C-E: Parameters of cardiac function, Millar catheter measurements. Statistical significance determined by t-test (B) or by 2-way ANOVA (other panels).

**Supplementary Figure 3**. **HIF2 inhibitor PT2567 significantly normalizes the levels of some mis-expressed genes in lungs of rats exposed to hypoxia for 4 weeks**. A-C: levels of HIF target genes and PH related genes in the lungs from rats under normoxia or hypoxia, treated with control reagent or HIF2 inhibitor PT2567 (N=6 for each group). **A**) Classical HIF target genes; **B**) Genes involved in inflammation; **C**) Genes involved in signaling and proliferation. Statistical significance determined by t-test.

**Supplementary Figure 4**. **HIF inhibitor PT2567 significantly normalizes the levels of some mis-expressed genes in lungs of the rats exposed to hypoxia for four days**. A-C: levels of HIF target genes and PH related genes in the lungs of rats under normoxia or hypoxia, treated with control or HIF2 inhibitor PT2567 (N=6 for each group). **A**) Classical HIF target genes. **B**) Genes involved in inflammation. **C**) Genes involved in signaling and proliferation. Statistical significance determined by t-test.

**Supplementary Figure 5. Hif2α, not Hif1α siRNA significantly prevents gene expression changes observed in normal pulmonary artery endothelial cells in response to acute hypoxia.** To determine if HIF2 or HIF1 activity is responsible for hypoxia-mediated gene expression changes in EC, normal human pulmonary artery EC cells (N=3) were transfected with control or siRNAs targeting Hif1α or Hif2α mRNAs. Post-transfection of 16 hours, cells were exposed to normoxia or hypoxia (1.5% O2) for additional 16 hours, and then cells were collected for RNA preparation and qRT-PCR. **A**) Hif1α and Hif2α mRNA levels, to monitor the knockdown efficiency; **B**) Select classical HIF target genes; **C**) Genes involved in inflammation that are significantly induced by hypoxia in EC (Fig 6B); **D**) Genes involved in signaling and proliferation that are significantly altered by hypoxia in EC (Fig 6C). Statistical significance determined by t-test.

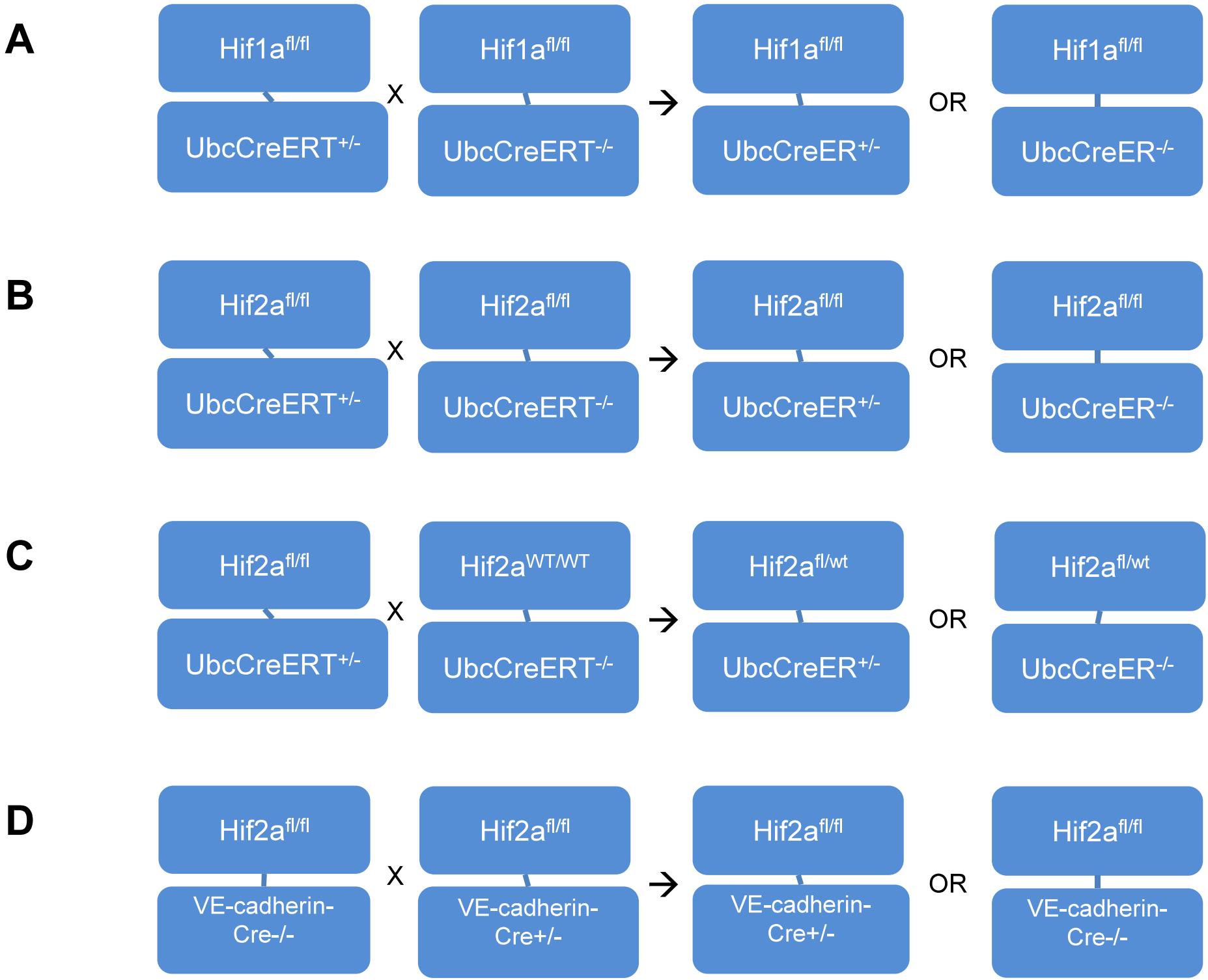
**Supplementary Figure 6. Signals generated from normal pulmonary vascular EC under hypoxia, in a HIF2 activity dependent manner, significantly activate normal pulmonary vascular SMCs.** Conditional medium prepared from normal pulmonary vascular ECs (N=3), cultured under normoxia or hypoxia (1.5% O2 for 24 hours), in the presence of DMSO or HIF2 inhibitor PT2567 (1 μM), were added to culture

medium of normal pulmonary vascular SMCs (N=3) that were cultured under normaxia. After 24 hours, SMC cells were collected for RNAs that were used to examine the mRNA levels of the indicated genes involved in cell proliferation (CCNE1 and CCNE2), pro-inflammation (CCL2) and anti-apoptosis (BCL2, BCL2L1 and BIRC5). Statistical significance determined by t-test.

**Supplementary Figure 7**. **Endothelial deletion of Hif2α markedly attenuates some pathologic gene expression in the lungs of chronically (5 weeks) hypoxic mice. A-C**: levels of HIF target genes and PH related genes in the lungs of normoxic or hypoxic mice, with or without EC deletion of Hif2α gene. **A**) Classical HIF target genes. **B**) Genes involved in inflammation. **C**) Genes involved in signaling and proliferation. Statistical significance determined by t-test.

**Supplementary Figure 8**. **Endothelial deletion of Hif2α markedly attenuates pathologic gene expression in the RVs of chronically hypoxic mice.** In the hypertrophic heart, cardiac myocytes display an altered gene signature, such as isoform switching from α-myosin heavy chain (α-MHC, Myh6) to β-MHC (Myh7) and re-expression of skeletal muscle α-actin (Acta1). **A**) Increased expression of Acta1 was attenuated in hypoxic EC-Hif2α KO mice. **B**) Myh6 expression was reduced in hypoxia-exposed mice, but was not reversed in hypoxic EC-Hif2α KO mice. **C**) Increased expression of Myh7 was attenuated in hypoxic EC-Hif2α KO mice. Statistical significance determined by t-test.

Supplemental Figure 1



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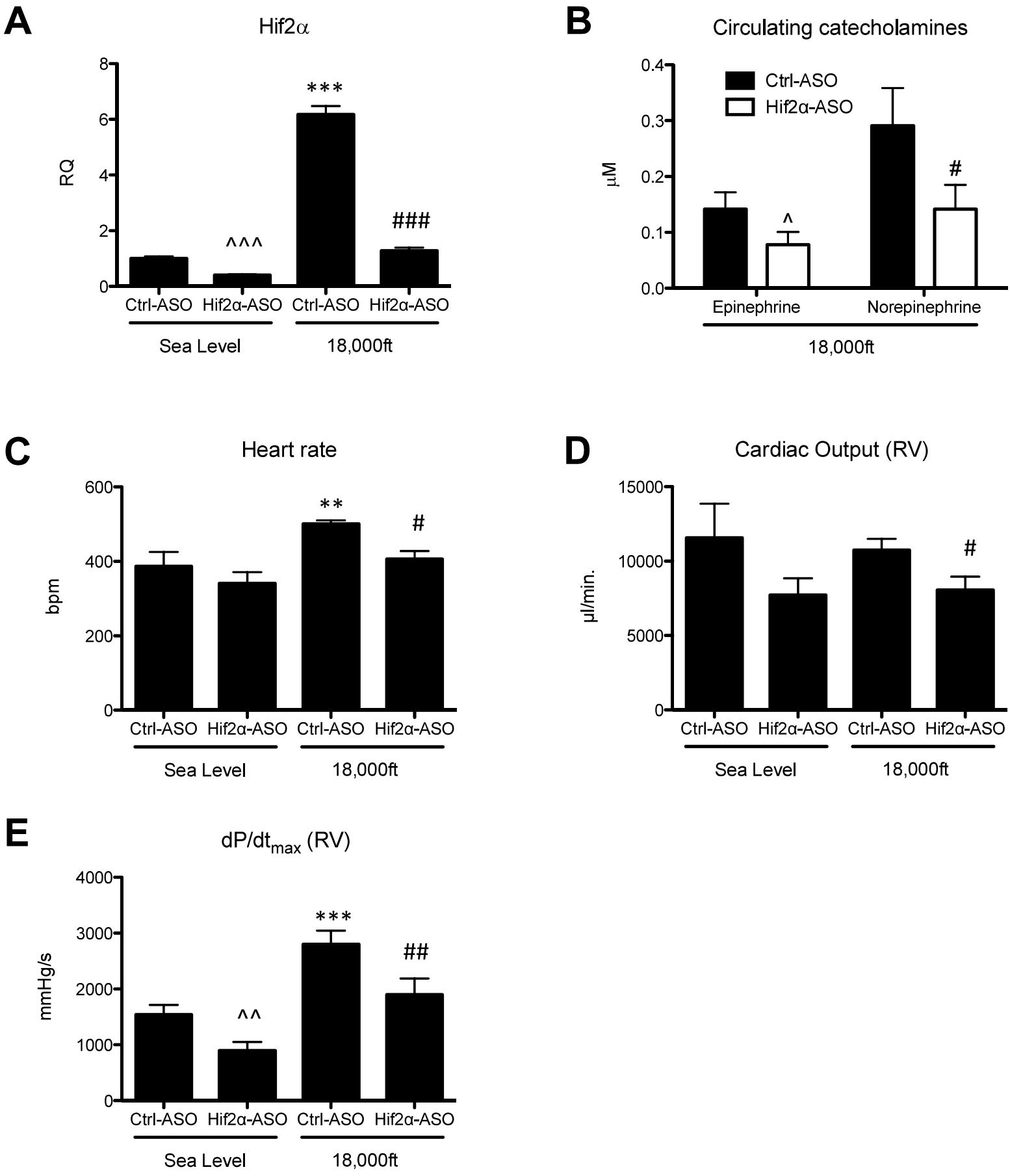

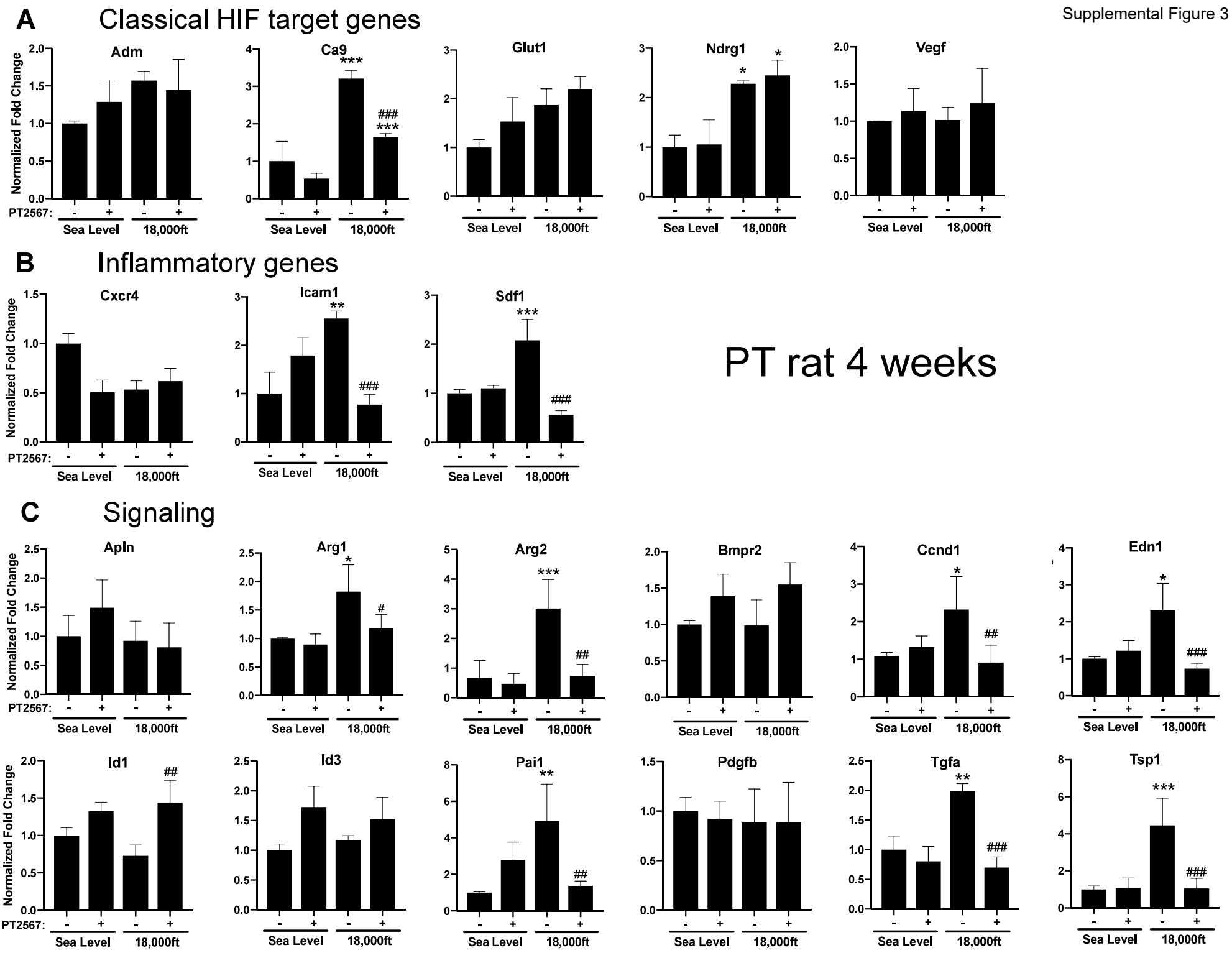
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Glut1
Ndrg1
Vegf
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Inflammatory genes
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Sdf1
PT rat 4 weeks
C
Signaling
Apln
Arg1
Arg2
Bmpr2
Ccnd1
Edn1
Id1
Id3
Pai1
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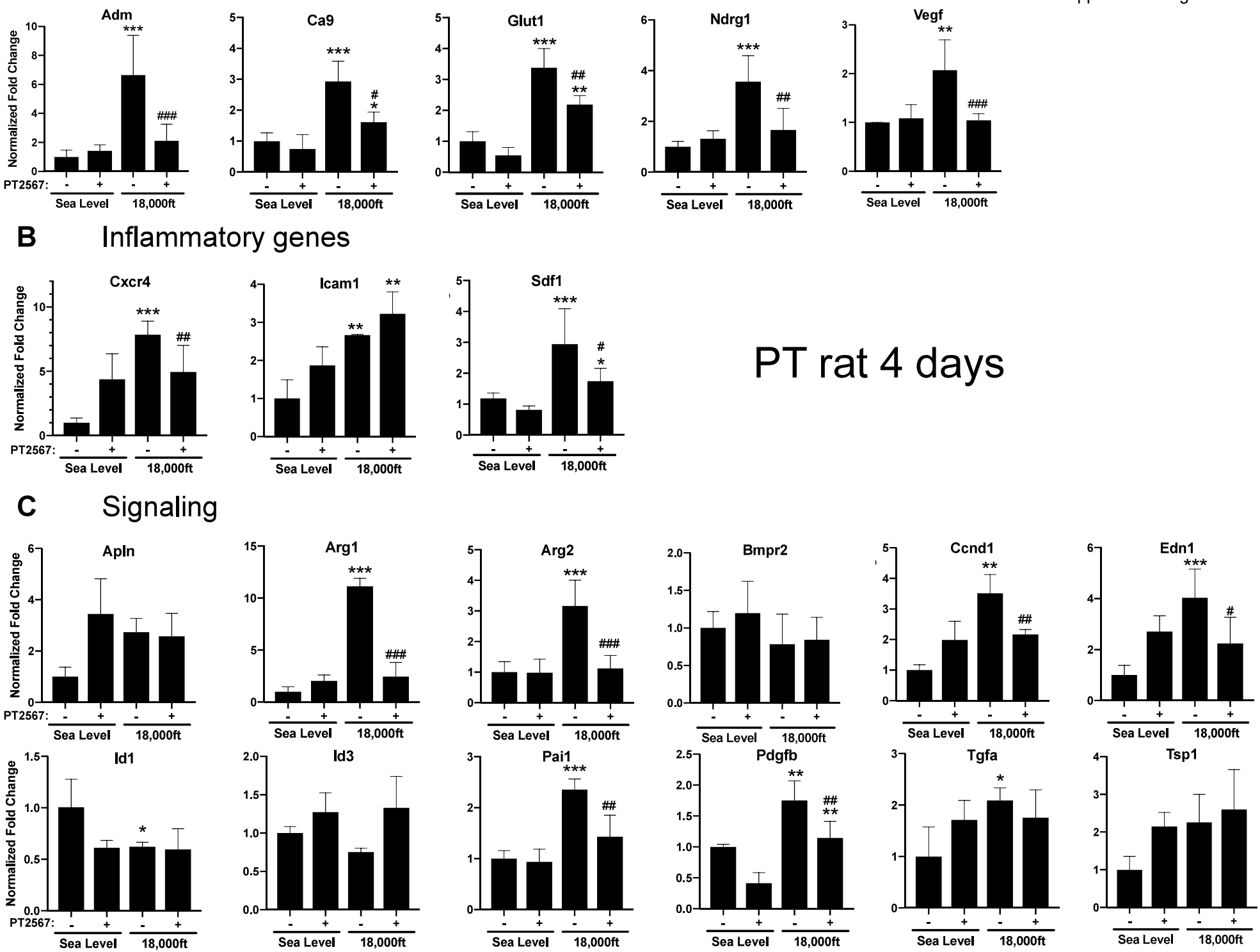
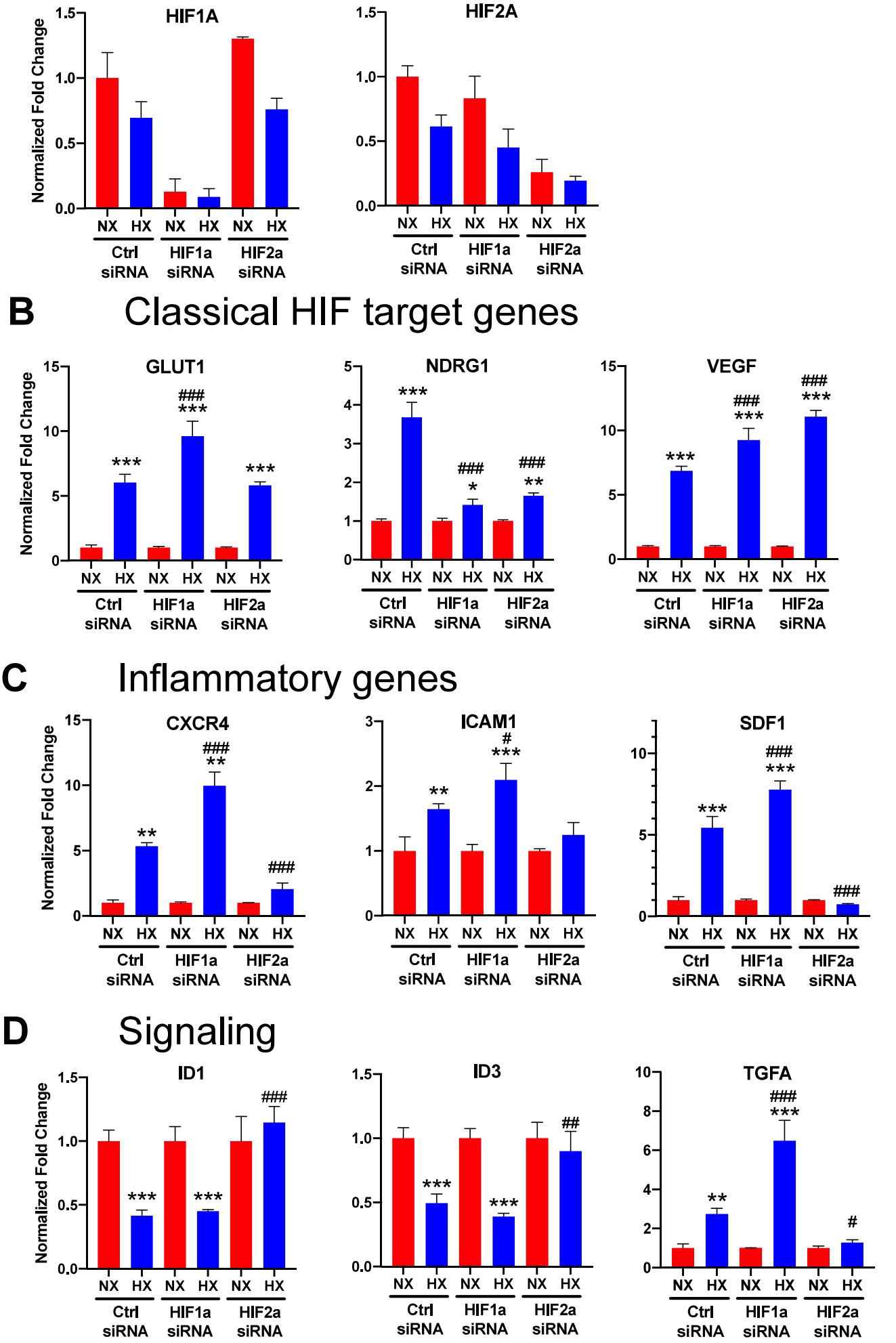
Tsp1
Normalized Fold Change
PT2567:
Sea Level
18,000ft

Supplemental Figure 4

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VEGF
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Inflammatory genes
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SDF1
D
Signaling
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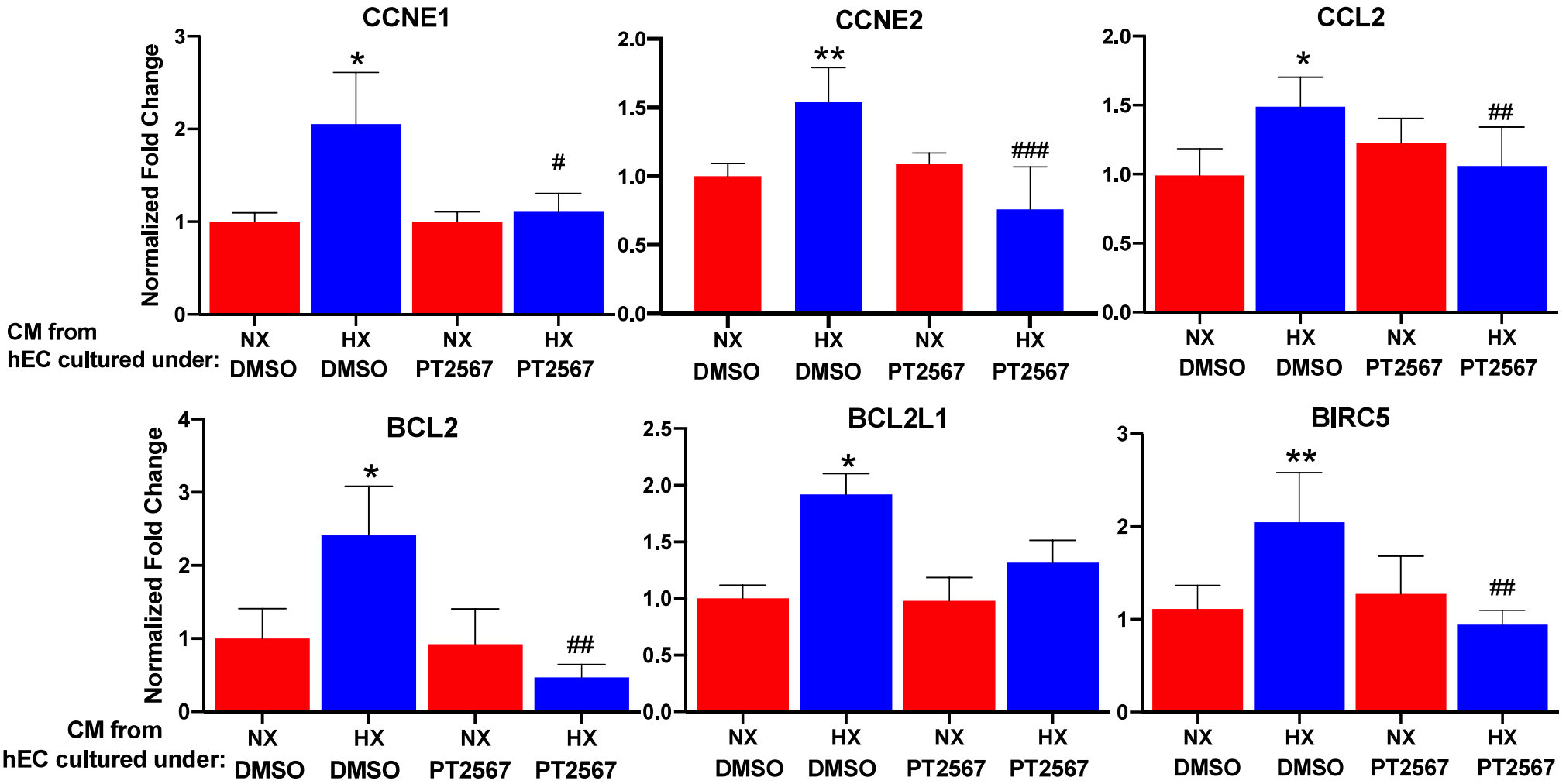
ID3
TGFA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CCNE2
CCL2
BCL2
BCL2L1
BIRC5
Normalized Fold Change
CM from
hEC cultured under:
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HX DMSO
NX PT2567
HX PT2567
*
**
#
##
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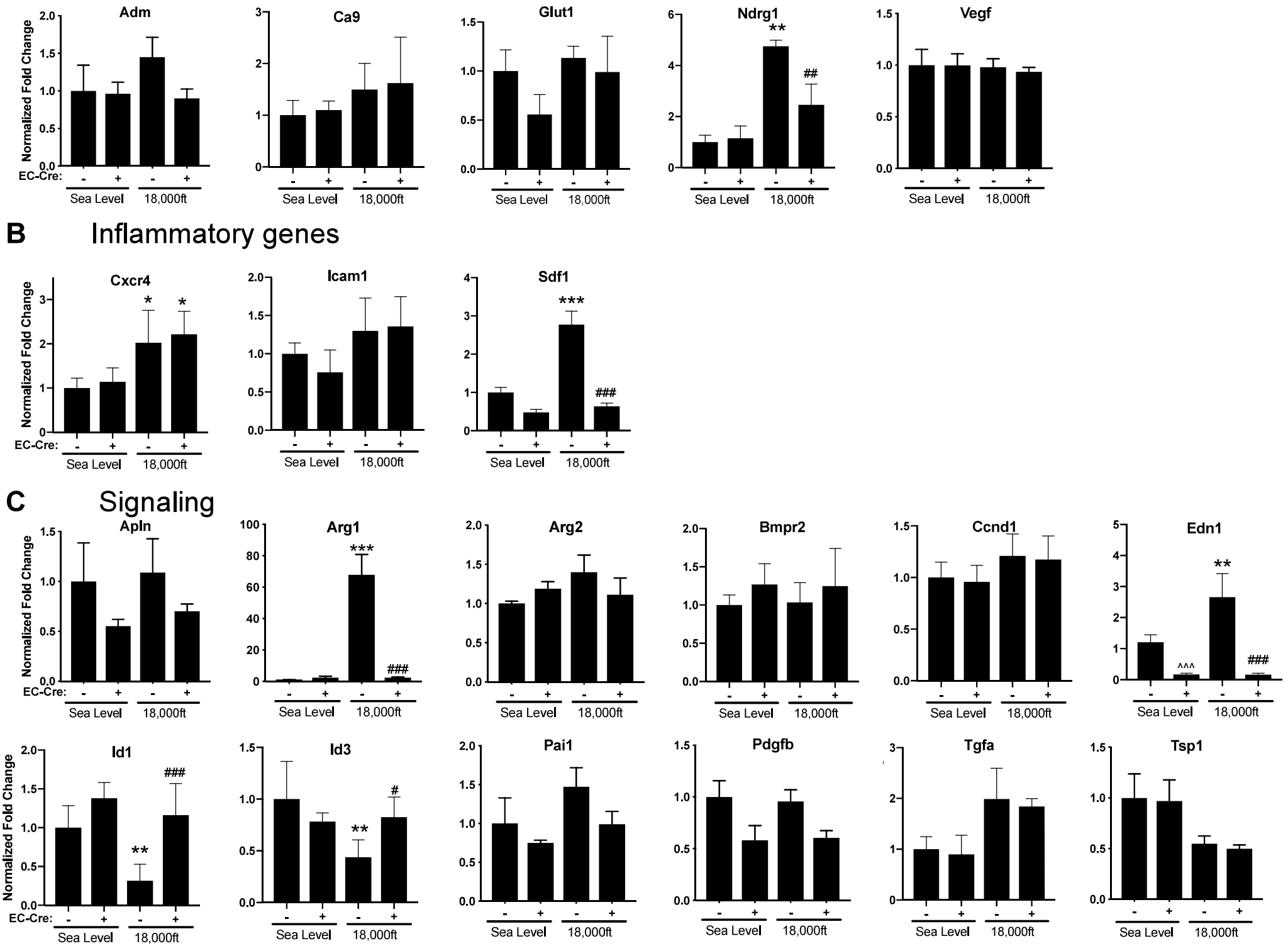
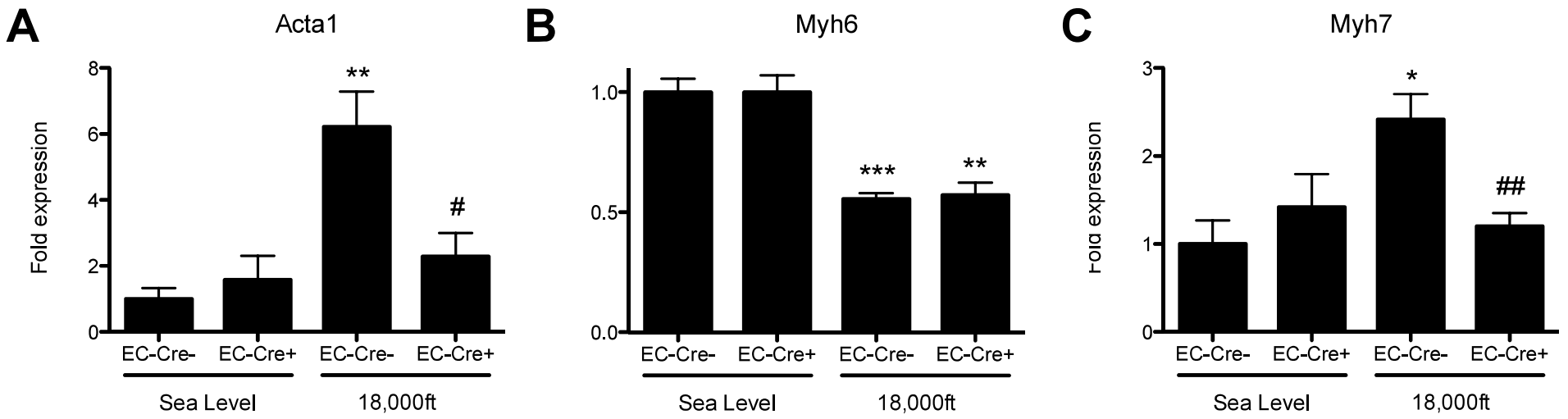
Supplemental Figure 7



Supplemental Figure 8



| Mice | Reduction of mRNA |
|---|---|
| $Hif1\alpha^{fl/fl}$;$UbcCreERT^{+}$ | Hif1α: -80% ± 5% |
| $Hif2\alpha^{fl/fl}$;$UbcCreERT^{+}$ | Hif2α: -71% ± 6% |
| $Hif2\alpha^{fl/WT}$;$UbcCreERT^{+}$ | Hif2α: -36% ± 4% |
| Hif1α-ASO | Hif1α: -60% ± 5% (RV) |
| Hif2α-ASO | Hif2α: -72% ± 4% |

**Supplementary Table 1. Hif1a and HIF2a mRNA reduction in floxed and ASO treated mice.** Rate of reduction in Hifa mRNA after hypoxia exposure (experimental setup: Fig. 1A and Fig. 3C)) in cre-positive mice and in ASO-treated animals, compared to the appropriate controls (cre-negative mice or Ctrl-ASO). Similar results were obtained for normoxic animals (data not shown). Expression was studied in lung tissue, with the exception of Hif1α ASO, for which RV tissue was used (no lung tissue available). There was no reduction of Hif1α, when Hif2α was targeted and vice versa.

| | Sea Level | | 18,000ft | |
|---|---|---|---|---|
| | **Hif1α$^{fl/fl}$; *UbcCre-*** | **Hif1α$^{fl/fl}$; *UbcCre+*** | **Hif1α$^{fl/fl}$; *UbcCre-*** | **Hif1α$^{fl/fl}$; *UbcCre+*** |
| **Weigth (g)** | 29.3 | 27.3 | 24.2 (**) | 22.0 (**)(^) |
| **Females / Males (n)** | 6/3 | 9/2 | 3/7 | 5/4 |
| **Heart Rate (bpm)** | 366 | 340 | 448 (***) | 435 (*) |
| | | | | |

**Supplementary Table 2**. Parameters were taken after exposure to either sea level or high altitude at the end of the experiment. *, ** and ***: $p<0.05$, $p<0.01$ and $p<0.001$ for sea level vs. high altitude within the same genotype. ^$p<0.1$ for cre-negative vs. Cre-positive within the same condition. T-test was used.

| | Sea Level | | 18,000ft | |
|---|---|---|---|---|
| | **Hif2α$^{fl/WT}$; *UbcCre-*** | **Hif2α$^{fl/WT}$; *UbcCre+*** | **Hif2α$^{fl/WT}$; *UbcCre-*** | **Hif2α$^{fl/WT}$; *UbcCre+*** |
| **Weight (g)** | 25,0 | 26,3 | 23,5 | 23,1 |
| **Females / Males (n)** | 5/2 | 1/3 | 3/7 | 5/7 |
| **Heart Rate (bpm)** | 397,1 | 390,0 | 390,0 | 360,0 |
| | | | | |

**Supplementary Table 3**. Parameters were taken after exposure to either sea level or high altitude at the end of the experiment.

| | Sea Level | | 18,000ft | |
|---|---|---|---|---|
| | ***Ctrl-ASO*** | ***Hif2a-ASO*** | ***Ctrl-ASO*** | ***Hif2a-ASO*** |
| **Weight (g)** | 27,6 | 26,2 | 23,3 (**) | 22,4 (***)(^) |
| **Females / Males (n)** | 0/8 | 0/8 | 0/8 | 0/7 |
| **Heart Rate (bpm)** | 386,7 | 340,5 | 500,5 (**) | 406,4 (##) |
| | | | | |

**Supplementary Table 4**. Parameters were taken after exposure to either sea level or high altitude at the end of the experiment. **, ***: $p<0.01$ and $p<0.001$ for sea level vs. high altitude within the same ASO-treatment group. ^, ##: $p<0.1$ and $p<0.01$ for Ctrl-ASO vs. Hif2a-ASO within the same condition. T-test was used.

| | Sea Level | | 18,000ft | |
|---|---|---|---|---|
| | **Hif2α$^{fl/fl}$; *EC-Cre-*** | **Hif2α$^{fl/fl}$; *EC-Cre+*** | **Hif2α$^{fl/fl}$; *EC-Cre-*** | **Hif2α$^{fl/fl}$; *EC-Cre+*** |
| **Weight (g)** | 23,8 | 25,7 | 22,4 | 21,3 |
| **Females / Males (n)** | 5/4 | 4/4 | 4/3 | 6/1 |

**Supplementary Table 5**. Parameters were taken after exposure to either sea level or high altitude at the end of the experiment.

| Gene | | Mouse | Rat | Human |
|---|---|---|---|---|
| Acta1 | fwd | AATGCTTCTAGGCGACTCG | | |
| | rev | CCTGCAACCACAGCACGATT | | |
| Adm | fwd | GACAAAGACAAGGACGGCATG | GGTATCGGAGCATCGCTACAG | GAG TGG AAG TGC TCC CCA CTT |
| | rev | GCCTTGAGGGCTGATCTTGTT | GAACGGCGAGCGAACCCAAT | CTT GTA CCA TGG GCG CCT A |
| Apln | fwd | GACCGAGTTGCAGCATGAATC | TGCCACTGATGCTGCCTCCA | ATGAATCTGCGGCTCTGCGT |
| | rev | TCAAGGAGAGCCAGAGCAGC | CTTCACCAGGTAGCGCATGTT | GCGGCATCAGGGACCCTCC |
| Arg1 | fwd | GCTTGCGAGACGTAGACCCT | CAGAGGAGGTGACTCGTACTGTG | ACTCGAACAGTGAACACAGCAGT |
| | rev | TGCCAATCCCCAGCTTGTCT | ACAAGACAAGGTCAACGCCA | CAATAGGCTTGTGATTACCCTCC |
| Arg2 | fwd | GAAGAGGCCAAGGCAACAGC | AGGCTGCACTCACTCGAGGT | AATCCTCAGTTGGCCACCTC |
| | rev | TGTCCTCCTTCTCTTGTCTGACC | ATGATTGGCAGGGAGGCGAT | CAATCACATCTACTGCCAGGTTAGC |
| Bcl2 | | | | TCCACCAAGAAAGCAGGAAAC |
| | | | | TGGACAGGATAGCAGCACAGGA |
| Bcl2L1 | | | | CAG GAG AAC GGC GGC TGG GA |
| | | | | TCG GCT GCT GCA TTG TTC CCA |
| Birc5 | | | | GTGGCTGCTTCTCTCTCTCT |
| | | | | GCCTTCTTCCTCCCTCACTT |

**Supplementary Table 6**. Primer sequences used for qRT-PCRs.

| Gene | | Mouse | Rat | Human |
|---|---|---|---|---|
| Bmpr2 | fwd | AGAAGAGCACAGAGGCCCAAT | GTC TAC ACA GTG TGA GCA TGA TGG | TGCTATGCAGAATGAACGCAAC |
| | rev | CTGTATACTGCTGCCATCCAGG | CTC CAG CAG CTT CAG GTT ATC CAA | AAGGACCAATTTTTGGCACACG |
| Ca9 | fwd | TGAAGGGTCTCTGACCACACC | CTGCCTCTCTGCTGGTGACA | GGT GTC ATC TGG ACT GTG TTT |
| | rev | ACACAGTCCAGATGACCCCC | AGGAGGAAGGCGATGCTGGT | GGT GTC AGA GAG GGT GTG GAG |
| Ccl2 | fwd | | | CATGAAAGTCTCTGCCGCCCT |
| | rev | | | TGAGCGAGCCCTTGGGGAAT |
| Ccna1 | fwd | | CTTCAGTACTTAAGGCGGCAA | |
| | rev | | GGCCAAATTCTCAGTCCTGATG | |
| Ccnd1 | fwd | CGT GGC CTC TAA GATGAA GGA | ATGGTGGCTGCTGGGAGTGT | CGT GGC CTC TAA GATGAA GGA |
| | rev | GTA GAT GCA CAG CTT CTC GGC | TAGCAGGAGAGGAAGTTGTTGG | GTA GAT GCA CAG CTT CTC GGC |
| Ccne1 | fwd | | | GGACACCATGAAGGAGGACG |
| | rev | | | TTCCTGGAGCGAGCCGAGAA |
| Ccne2 | fwd | | | GTAGCTGGTCTGGCGAGGTT |
| | rev | | | ACGGCTACTTCGTCTTGACATTC |
| Cd68 | fwd | CACAGGCAGCACAGTGGACA | CTTTGGATTCAAACAGGACCG | |
| | rev | CTTGGAGCTCTCGAAGAGATGA | GGTAGACTGTACTGTGGCTCTGATG | |

**Supplementary Table 6 (continued)**. Primer sequences used for RT-PCRs.

| Gene | | Mouse | Rat | Human |
|---|---|---|---|---|
| Cxcr4 | fwd | CCCTCGCCTTCTTCCACTG | TGTTCCAGTTCCAGCACATC | ATCTGTGACCGCTTCTACCCC |
| | rev | CGAGGAAGGCATAGAGGATGG | CCTTGGAGTGTGACAGCTTG | AAACTGGAACACAACCACCCA |
| Edn1 | fwd | GCACCGGAGCTGAGAATGGA | CGAGCTGAGAAGGAAGTGCAGAG | AAAGAGTGTGTCTACTTCTGCCACC |
| | rev | ACAGGAACAGCGCTTGGACC | AAGGAGGAGCAGGAGCAACG | CAACGTGCTCGGGAGTGTTG |
| Epo | fwd | TTGGAAAAGAATGGAGGTGGA | | |
| | rev | GACAGGCCTTGCCAAACTTC | | |
| Glut1 | fwd | GGA GAG GTG TCA CCT ACA GC | GATGATGCGGGAGAAGAAGG | TGTGGGCCTTTTCGTTAACC |
| | rev | AAG GAT GCC AAC GAC GAT TC | AGCACCACGGCGATGAGGAT | ATCATCAGCATTGAATTCCGC |
| Hif1a | fwd | TGCAGCAAGATCTCGGCGAA | | TTT TAC CAT GCC CCA GAT TCA |
| | rev | AGTGGCAACTGATGAGCAAGC | | AGT GCT TCC ATC GGA AGG ACT |
| Hif2a (Epas1) | fwd | GACGGAGGTCTTCTATGAGTTGG | | TACAAGGAGCCCCTGCTGTC |
| | rev | CGCATGATGGAGGCTTTGTC | | TGCTGGATTGGTTCACACATG |
| Icam1 | fwd | GAGGAGATACTGAGCCGCCA | CTTCCGACTAGGGTCCTGAA | CTGTATACTGCTGCCATCCAGG |
| | rev | GCTAGCCAGCACCGTGAATG | CTTCAGAGGCAGGAAACAGG | CTGGCTTCGTCAGAATCACGTT |
| Id1 | fwd | GGTGGAGATCCTGCAGCATGTA | TTC TGC TCT ACG ACA TGA ACG GCT | GCACCCTCAACGGCGAGATCAGCG |
| | rev | CCGACTTCAGACTCCGAGTTCA | ACA TGC TGC AGT ATC TCC ACC TTG | CAGCGACACAAGATGCGATCGTCC |

**Supplementary Table 6 (continued)**. Primer sequences used for qRT-PCRs.

| Gene | | Mouse | Rat | Human |
|---|---|---|---|---|
| Id3 | fwd | GGAACGTAGCCTGGCCATT | CTG TCG GAA CGT AGC CTA GCC ATT | CAG CGC GTC ATC GAC TAC ATT CTC |
| | rev | TCCAAGAGGCTAAGAGGCTCC | GCG AGT AGC AGT GGT TCA TGT CGT | CCA TCA GGG GGT CCA GGG GCT GGC |
| Myh6 | fwd | GGGCAGCTCATGCGCATTGA | | |
| | rev | TGCGCTCTTCAGCAGCGGTT | | |
| Myh7 | fwd | CAGCAGTTGGATGAGCGACT | | |
| | rev | GATGCGTGCCTGAAGCTCCT | | |
| Ndrg1 | fwd | ATGCCTGGAACCCACACTGT | GTGGAGGTGGTGCACACGTA | GAA GCA AGC ATC TCC GCA TC |
| | rev | AACTACCAGCCAGAGCAGGGC | TTGTAGGCGCTGATGAACAGG | CAA CCG GCC ACT GGT TAA TG |
| Pai1 | fwd | TTTGGACCCAGTTCCCACC | TTGTGAAGGAGGAACGCTGC | |
| | rev | TGAAAGTGTTGTGCCCTCCAC | ATTGGCTCTTGTTGGCTGTCG | |
| Pdgfb | fwd | AATGCTGAGCGACCACTCCA | CAGCAGAGCCTGCCGTAATC | GGCCGAGTTGGACCTGAACA |
| | rev | TACGGAGTCTCTGTGCAGCAGG | GAACACCTCTGTACGCGTCTTG | TCCAGCTCGCCTCCAGAGTG |
| Pgk1 | fwd | GGAGAGTCCAGAGCGACCCT | | |
| | rev | GCAACTTTAGCGCCTCCCA | | |
| Sdf1 | fwd | ATCGGTGGCTGCGAGCTGAA | TGTGCATTGACCCGAAATTA | AGA TTG TAG CCC GGC TGA AG |
| | rev | ACATGCCTGGGATGCTGCGT | TCCTCAGGGGTCTACTGGAA | TCG GGT CAA TGC ACA CTT GT |

**Supplementary Table 6 (continued)**. Primer sequences used for qRT-PCRs.

| Gene | | Mouse | Rat | Human |
|---|---|---|---|---|
| Tgfa | fwd | GGGAAGGAAATGTGTTCCCTG | CAACAAGTGCCCAGATTCCC | GAAGGGAAGAACCGCTTGCT |
| | rev | GGCTCAATGGGTCAGAGCAT | GGCTTCTCTTCCTGCACCAA | TGGGCTCTTCAGACCACTGTT |
| Tnc | fwd | | TGCTGAAGGTTGTGGCTGTG | |
| | rev | | ATTCAGGCTCAGAGCAGTTGG | |
| Tsp1 | fwd | CAATTTTCAGGGGGTGCTGC | ACATCTGCTCTGTCACCTGTGG | CCTGTGATGATGACGATGA |
| | rev | CCGTTCACCACGTTGTTGTC | GGGTTGTTGCAGAGTCGGCT | CTGATCTGGGTTGTGGTTGTA |
| Vegf | fwd | TGACAAGCCAAGCGCGTGAG | GGGATCTTTCATCGGACCAGT | TGT GCC CAC TGA GGA GTC C |
| | rev | CTCCTCTTCCTTCATGTCAG | CGGTGTCTGTCTGTCTGTCCGT | GGT TTG ATC CGC ATA ATC TGC |
| | | | | |
| **House keeping:** | | | | |
| 18S | fwd | GAA TTC CCA GTA AGT GCG GG (for mouse, rat and human) | | |
| | rev | GGG CAG GGA CTT AAT CAA CG (for mouse, rat and human) | | |
| Actb | fwd | GCCCTGAGGCTCTTTTCCAG | CCAGCCATGTACGTAGCCATCCAG | GCCCTGAGGCACTCTTCCA |
| | rev | TGCCACAGGATTCCATACCC | TCACAATGCCAGTGGTACGACCAG | ATGCCACAGGACTCCATGC |

**Supplementary Table 6**. **Forward and Reverse sequences of qPCR primers used in the manuscript, listed in mouse, rat, and human genes**. The amplicon sizes are typically 50-80 mer and without significant secondary structures. All primer sets were validated by melt curve analysis, cloning and sequencing of qPCR products, and standard dilution assay to ensure specific PCR products and to have amplification efficiencies between 90% and 110%. *Hif1α* floxed mice and *Hif-2α* floxed mice were constructed wherein exon 2 (coding DNA binding domain) of the Hif1α or Hif2α gene was floxed with LoxP sites. To assess HIF1a and HIF2a deletion in HIF flox mice, qPCR primers for mouse HIF1a and HIF2a mRNAs are located in the exon 2 of HIF1α or HIF2α gene.

| | Gene | Cell type | Normoxia | Hypoxia |
|---|---|---|---|---|
| Hypoxic induction of classical HIF target genes In normal pulmonary artery vascular cells | ADM | EC | 100%±9.7 | 250.7%±24.3*** |
| | | Fib | 100%±0.6 | 221.3%±41.2*** |
| | | SMC | 100%±22.3 | 93.7%±6.8 |
| | CA9 | EC | 100%±10.1 | 1328.6%±274.4* |
| | | Fib | 100%±18.6 | 19164.2%±3575.7** |
| | | SMC | 100%±26.7 | 235.1%±26.7 |
| | GLUT1 | EC | 100%±27 | 301.3%±33*** |
| | | Fib | 100%±1.6 | 477.0%±8.7*** |
| | | SMC | 100%±20.2 | 461.7±140.2*** |
| | NDRG1 | EC | 100%±8.6 | 301.8%±15.6*** |
| | | Fib | 100%±8.7 | 333.5%±30.3*** |
| | | SMC | 100%±9.6 | 312.6%±13.6*** |
| | VEGF | EC | 100%±8.2 | 193.5%±6.9*** |
| | | Fib | 100%±5.3 | 208.5%±27.1*** |
| | | SMC | 100%±7.2 | 209.6%±30*** |
| Hypoxic induction Of inflammatory genes in normal pulmonary artery vascular cells | CXCR4 | EC | 100%4.8 | 250.0%±13.2*** |
| | | Fib | 100%±2.9 | 46.3%±23.9 |
| | | SMC | 100%±3.3 | 195.6%±18.2*** |
| | SDF1 | EC | 100%±13.9 | 321.5%±13.9*** |
| | | Fib | 100%±0.61 | 123.2%±53.0 |
| | | SMC | 100%±3.3 | 109.4%±32.42 |
| | ICAM1 | EC | 100%±23.6 | 307.4%±18.8*** |
| | | Fib | 100%±3.7 | 138.6%±4.4 |
| | | SMC | 100%±41.6 | 162.7%±18.8 |

**Supplementary Table 7**. Gene expression in pulmonary artery cells by qRT-PCR, as in Fig. 6. Statistical significance as determined by t-test.

| | Gene | Cell type | Normoxia | Hypoxia |
|---|---|---|---|---|
| Hypoxic induction Of signaling factors in normal pulmonary artery vascular cells | APLN | EC | 100%±12.0 | 208.3%±57.7*** |
| | | Fib | 100%±52.7 | 595.8%±64.5*** |
| | | SMC | 100%±4.3 | 530.%±16.4*** |
| | ARG1 | EC | 100%±30.8 | 89.4%±15.4 |
| | | Fib | 100%±21.3 | 89.8%±32.3 |
| | | SMC | 100%±39.8 | 82.7%±4.9 |
| | ARG2 | EC | 100%±22.4 | 97.9%±11.2 |
| | | Fib | 100%±15.5 | 79.0%±10.1 |
| | | SMC | 100%±8.7 | 87.2%±27.3 |
| | BMPR2 | EC | 100%±16.5 | 94.7%±17.1 |
| | | Fib | 100%±25.1 | 127.8%±15.4 |
| | | SMC | 100%±27.0 | 76.6%±15.0 |
| | CCND1 | EC | 100%±5.1 | 103%±6.1 |
| | | Fib | 100%±9.6 | 90%±5.8 |
| | | SMC | 100%±30.8 | 110%±32.7 |
| | EDN1 | EC | 100%±13.1 | 118.0%±22.7 |
| | | Fib | 100%±38.3 | 366.5%±17.4*** |
| | | SMC | 100%±25.0 | 144.7%±8.9*** |
| | ID1 | EC | 100%±14.7 | 30.0%±4.3*** |
| | | Fib | 100%±12.3 | 83.6%±12.3 |
| | | SMC | 100%±14.4 | 96%±7.1 |
| | ID3 | EC | 100%±4.6 | 54.8%±12.0*** |
| | | Fib | 100%±4.3 | 96.4%±6.0 |
| | | SMC | 100%±10.0 | 91.2%±1.2 |

**Supplementary Table 7, continued.**

| | Gene | Cell type | Normoxia | Hypoxia |
|---|---|---|---|---|
| Hypoxic induction Of signaling factors in normal pulmonary artery vascular cells (continued) | PDGFB | EC | 100%±25.7 | 161.2%±23.3* |
| | | Fib | 100%±33.5 | 120.1%±12.0 |
| | | SMC | 100%±43.2 | 257.3%±39.6** |
| | TGFA | EC | 100%±17.8 | 775.3%±127.1*** |
| | | Fib | 100%±23.0 | 80%±15.0 |
| | | SMC | 100%±60.0 | 346.9%±16.9*** |
| | TSP1 | EC | 100%±20.1 | 104.0%±4.6 |
| | | Fib | 100%±8.1 | 141.7%±28.1 |
| | | SMC | 100%±30.0 | 159.9%±62.5* |

**Supplementary Table 7, continued.**

| | Gene | | Normoxia | Hypoxia | Δ |
|---|---|---|---|---|---|
| Hypoxic induction of classical HIF target genes in EC | GLUT1 | DMSO | 100.0%±1.2 | 721.26%±75.1*** | |
| | | PT2567 0.1µM | 103.0%±13.3 | 555.5%±20.8*** | 23% # |
| | | PT2567 1µM | 88.9%±14.62 | 346.8%±21.2** | 36.5% ### |
| | | PT2567 10µM | 116.7%±19.6 | 242.6%±84.4* | 51.9% ### |
| | NDRG1 | DMSO | 100.0%±12.6 | 603.3%±39.7*** | |
| | | PT2567 0.1µM | 94.5%±21.7 | 578.3%±23.9*** | 4,10% |
| | | PT2567 1µM | 96.2%±13.2 | 400.0%±57.8*** | 33.7% ## |
| | | PT2567 10µM | 99.4%±8.1 | 522.4%±7.0*** | 13.4% # |
| | VEGF | DMSO | 100.0%±30.9 | 516.0%±87.8*** | |
| | | PT2567 0.1µM | 97.1%±20.6 | 384.4%±50.4*** | 25.5% # |
| | | PT2567 1µM | 114.8%±18.0 | 271.0%±54.0** | 47.5% ### |
| | | PT2567 10µM | 92.6%±20.9 | 276.0%±33.6** | 46.5% ### |
| Hypoxic induction of inflammatory genes in EC | CXCR4 | DMSO | 100.0%±15.0 | 350.0%±31.9** | |
| | | PT2567 0.1µM | 127.9%±26.3 | 319.3%±73.3** | 8,77% |
| | | PT2567 1µM | 79.9%±1.01 | 85.9%±11.05 | 75.5% ## |
| | | PT2567 10µM | 110.5%±18.0 | 98.2%±26.7 | 71.9% ## |
| | ICAM1 | DMSO | 100.0%±33.1 | 450.8%±41.8** | |
| | | PT2567 0.1µM | 109.5%±60.0 | 105.6%±51.1 | 76.6% ## |
| | | PT2567 1µM | 116.9%±35.4 | 115.9%±10.2 | 74.3% ## |
| | | PT2567 10µM | 122.8%±15.5 | 143.2%±19.2 | 68.2% ## |
| | SDF1 | DMSO | 100.0%±23.6 | 395.8%±18.3** | |
| | | PT2567 0.1µM | 184.6%±10.3 | 424.6%±1.02** | 107,30% |
| | | PT2567 1µM | 59.0%±31.3 | 45.5%±133.3 | 88.5% ### |
| | | PT2567 10µM | 99.1%±28.4 | 115.5%±4.74 | 70.8% ### |

**Supplementary Table 8**. Gene expression in EC by qRT-PCR, as in Fig. 7. Δ (inhibitory rate) refers how effective of HIF2 inhibitor PT2567 in reducing the induction of the gene by the hypoxia. Statistical significance as determined by t-test.

| | Gene | | Normoxia | Hypoxia | Δ |
|---|---|---|---|---|---|
| Hypoxic induction of signaling factors in EC | ID1 | DMSO | 100.0%±25.0 | 30.1%±4.3** | |
| | | PT2567 0.1µM | 107.5%±24.3 | 48.5%±10.1* | 161,00% |
| | | PT2567 1µM | 134.5%±35.4 | 63.0%±3.3*** | 209.3% ## |
| | | PT2567 10µM | 82.9%±0.53 | 69.4%±8.2 | 230.6% ## |
| | ID3 | DMSO | 100.0%±4.6 | 57.8%±10.7** | |
| | | PT2567 0.1µM | 77.7%±31.2 | 63.7%±20.2 | 110,20% |
| | | PT2567 1µM | 89.4%±25.5 | 74.2%±12.6 | 128,40% |
| | | PT2567 10µM | 71.9%±10.05 | 77.3%±5.95 | 124,40% |
| | TGFA | DMSO | 100.0%±46.4 | 384.7%±64.9*** | |
| | | PT2567 0.1µM | 139.2%±22.04 | 180.7%±5.8*** | 53.02% ### |
| | | PT2567 1µM | 147.6%±78.6 | 134.7%±3.1 | 65% ### |
| | | PT2567 10µM | 185.5%±158.8 | 140.4%±10.5 | 63.5% ### |

**Supplementary Table 8, continued.**