Table S1. **Sequence of human and mouse primers used for RT-qPCR measurements.**

Ca9, carbonic anhydrase IX; Ndrg1, N-myc downstream regulated gene 1; L28, ribosomal protein L28; Hif1a, hypoxia inducible factor 1, α subunit; Hif2a, hypoxia inducible factor 2, α subunit; Bnip3, BCL2/adenovirus E1B interacting protein 1; Glut1, glucose transporter, member 1; Pdk1, pyruvate dehydrogenase kinase, isoenzyme 1; Phd1-3, prolyl-4-hydroxylase domain 1-3; Sod1, superoxide dismutase 1; Sod2, superoxide dismutase 2; Glrx, glutaredoxin; Ednrb, endothelin receptor type B; Mmp3, matrix metallopeptidase 3; Svct1, sodium-dependent vitamin C transporter 1; Svct2, sodium-dependent vitamin C transporter 2; Epo, erythropoietin; S12, Ribosomal protein S12.

	Species	Accession No.	Forward primer	Reverse primer
CA9	human	NM_001216	5′- gggtgtcatctggactgtgtt -3′	5′- cttctgtgctgccttctcatc -3′
NDRG1	human	NM_006096	5′- atgtacccctccatggatca -3′	5′- tgtggaccacttccacgtta -3′
L28	human	NM_000991	5′- gcaattccttccgctacaac -3′	5′- tgttcttgcggatcatgtgt -3′
Hif1a	mouse	NM_010431	5′- ggttccagcagacccagtta -3′	5′- aggctccttggatgagcttt -3′
Hif2a	mouse	NM_010137	5′- taaagcggcagctggagtat -3′	5′- actgggaggcatagcactgt -3′
Bnip3	mouse	NM_009760	5′- gctcccagacaccacaagat -3′	5′- tgagagtagctgtgcgcttc -3′
Ca9	mouse	NM_139305	5′- gctgtcccatttggaagaaa -3′	5′- ggaaggaagcctcaatcgtt -3′
Glut1	mouse	NM_011400	5′- tctctgtcggcctctttgtt -3′	5′- gcagaagggcaacaggatac -3′
Pdk1	mouse	NM_172665	5′- ggcggctttgtgatttgtat -3′	5′- acctgaatcgggggataaac -3′
Phd1	mouse	NM_053208	5′- ttgcctgggtagaaggtcac -3′	5′- gctcgatgttggctaccact -3′
Phd2	mouse	NM_053207	5′- agccatggttgcttgttacc -3′	5′- ctcgctcatctgcatcaaaa -3′
Phd3	mouse	NM_028133	5′- caacttcctcctgtccctca -3′	5′- ggctggacttcatgtggatt -3′
Ndrg1	mouse	NM_010884	5′- tcaagatggcagactgtgga -3′	5′- gttgggggtgatgttgagac -3′
Sod1	mouse	NM_011434	5′- ccagtgcaggacctcatttt -3′	5′- cacctttgcccaagtcatct -3′
Sod2	mouse	NM_013671	5′- ggccaagggagatgttacaa -3′	5′- gaaccttggactcccaca -3′
Glrx	mouse	NM_053108	5′- aacaacaccagtgcgattca -3′	5′- atctgcttcagccgagtcat -3′
Ednrb	mouse	NM_007904	5′- cagtcttctgcctggtcctc -3′	5′- ggactgcttttcctcaaacg -3′
Mmp3	mouse	NM_010809	5′- ctatacgagggcacgaggag -3′	5′- ccacccttgagtcaacacct -3′
Svct1	mouse	NM_011397	5′- tctttggcctcacactaccc -3′	5′- tccttttaccatgcccatc -3′
Svct2	mouse	NM_018824	5′- tgccaggaagggtgtacttc -3′	5′- ccggtaccaaatatgccatc -3′
Epo	mouse	NM_007942	5′- ggccatagaagtttggcaag -3′	5′- cctctcccgtgtacagcttc -3′
S12	mouse	NM_011295	5'- gaagctgccaaagccttaga -3'	5'- aactgcaaccaaccaccttc -3'

Table S2. Blood parameters of *Gulo*$^{-/-}$ mice. All mice received ascorbate free provender for 5 weeks. Control animals received vitamin C via their drinking water. Heparinized whole blood samples were collected from mice kept at ambient oxygen tension or in a hypoxic environment (8% oxygen for 24 hours) by cardiac puncture. The values represent mean values ± SD of at least three animals per group as indicated.

	20% oxygen [Fi O_2]		**8% oxygen [Fi O_2]**	
	+Asc (n=3)	**-Asc (n=4)**	**+Asc (n=4)**	**-Asc (n=5)**
Hemoglobin(g/dl)	12.57±0.6807	13.43±0.3304	12.33±2.022	12.20±1.030
Hematocrit (%)	40.23±1.528	42.88±0.3862	40.68±6.247	39.72±3.492
Erythrocytes (10^6/µl)	8.777±0.5724	9.250±0.09019	8.625±1.275	8.496±0.6687
Retikulocytes (10^3/µl)	260.3±42.34	227.0±21.46	295.3±106.4	273.2±53.18

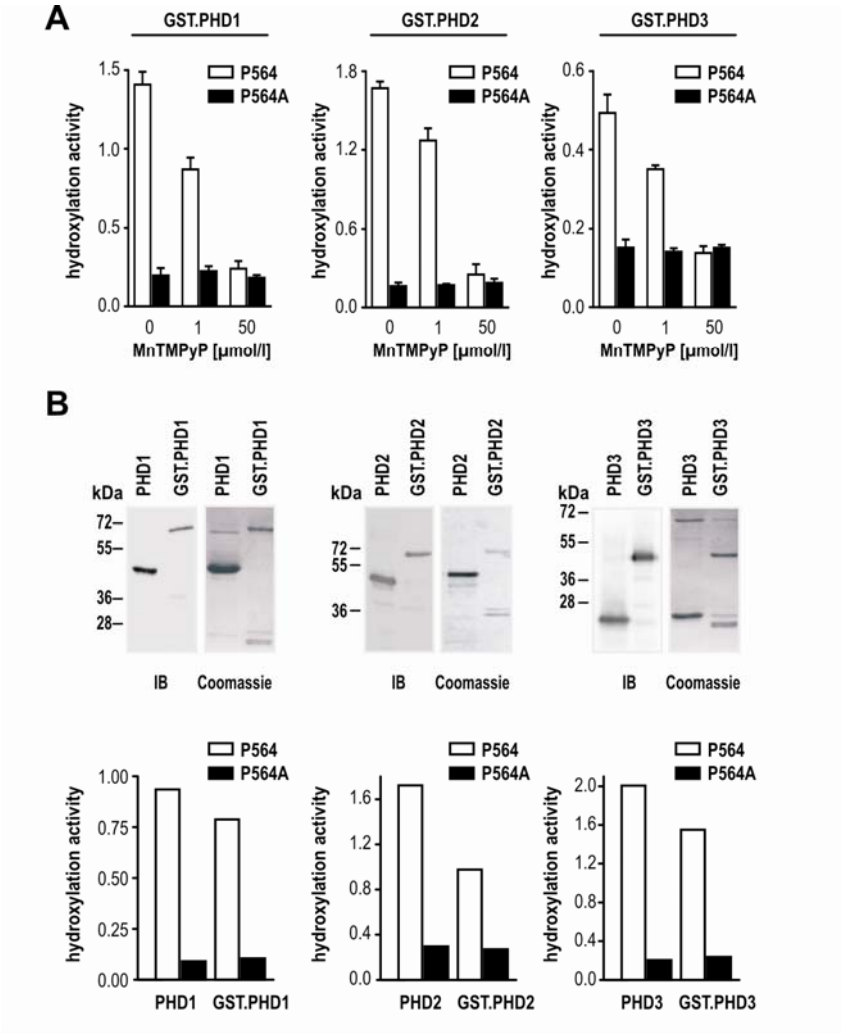

Figure S1. Inhibition of PHDs by MnTMPyP and *in vitro* hydroxylation activity of untagged PHD1-3. (A) Inhibition of PHD1-3 hydroxylation activity by Mn(III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride (MnTMPyP) measured by an *in vitro* hydroxylation assay. The presence of 50 µM MnTMPyP completely abolished hydroxylase activity of all three PHDs. Shown are mean values ± SEM of an experiment performed in triplicates. (B) Comparison of the purity (upper panels. IB, immunoblotting; Coomassie, protein staining by Coomassie blue) and hydroxylation activity (lower panels) of GST-tagged and untagged PHD1-3 enzyme preparations. Representative experiments performed in duplicates are shown. A Gateway-Technology compatible expression vector for GST fusion proteins bearing the PreScission protease cleavage site was generated by introducing a Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro peptide into pDEST20 (Invitrogen) by site-directed mutagenesis. The C201S mutation

was introduced into wild-type PHD2 by site-directed mutagenesis. PHD1, PHD2 and PHD3 expression vectors were generated by homologous recombination with respective Entry vectors (Invitrogen). For expression of recombinant proteins, Sf9 cells were infected with baculovirus stock and cultured in Grace's insect medium at 27ºC in a humidified incubator for 96-110 hours. Cells were collected by centrifugation and lysed in ice-cold 0.1% NP-40, 10 mM Tris-HCl pH 7.5, 100 mM NaCl, 100 mM glycine and 10 mM DTT. Cleared lysates were incubated with PBS-equilibrated GSH-sepharose beads (GE Healthcare) for 2 hours at 4°C with gentle agitation. For cleavage, beads were washed twice with PBS and equilibrated twice with PreScission cleavage buffer (50 mM Tris-Cl pH 7.0, 150 mM NaCl). PreScission protease (GE Healthcare) was added to the protein-bound glutathione-sepharose beads (80 U in 960 ml cleavage buffer per 1 ml of bead volume) and incubated for 5 hours at 4ºC.

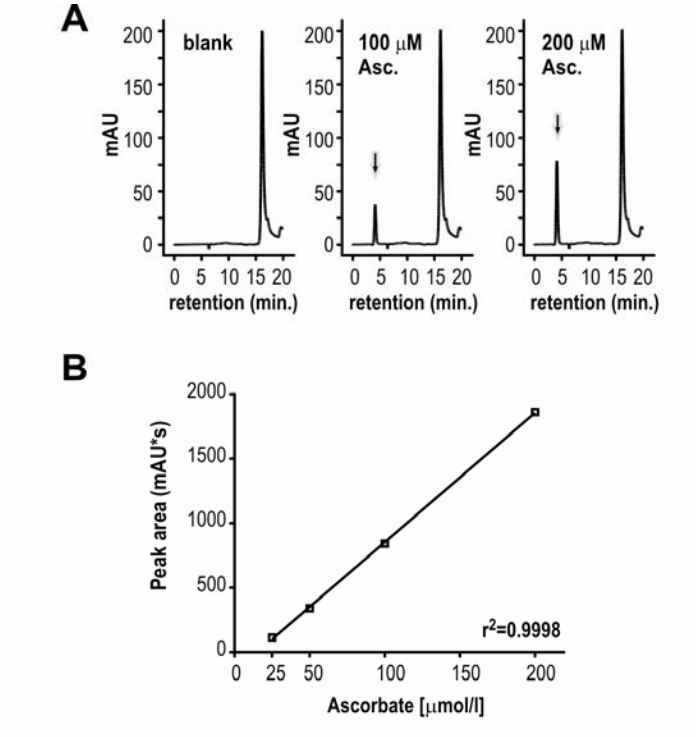

Figure S2. HPLC determination of ascorbate concentrations. (A) Exemplary chromatograms derived from samples of blank (60 mM phosphoric acid, left), or spiked standard solutions containing 100 µM (middle) and 200 µM (right) ascorbate, respectively. Probes were loaded on a Nucleosil C18 column and eluted applying an acetonitrile gradient (0-60%). Arrows indicate the position of the peaks specific for ascorbate. (B) Standard curve as obtained after plotting the peak areas of ascorbate elutions (given in arbitrary units; AU) against known ascorbate concentrations in spiked samples. Regression analysis was performed using Prism 4.0 GraphPad software.

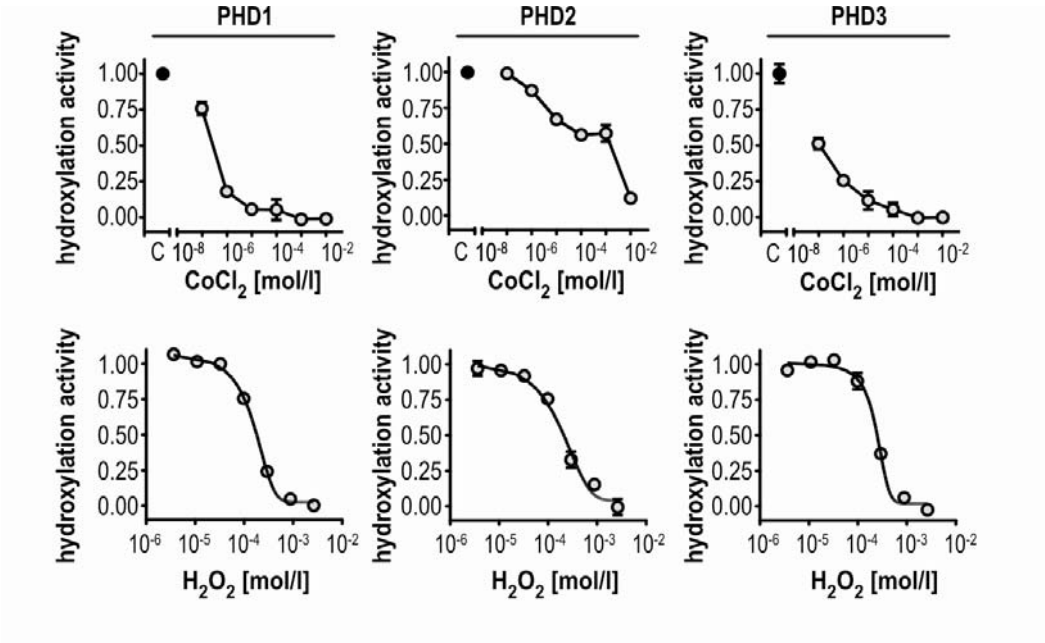

Figure S3. $CoCl_2$ and H_2O_2 inhibit the *in vitro* hydroxylation activity of PHD1-3.

Dose-dependent inhibition of PHD activity by $CoCl_2$ (upper panel) and H_2O_2 (lower panel). Shown are mean values ± SEM of a representative experiment performed in triplicates.