Online Supplemental Material

Cell Counts

Differential murine peripheral blood cell counts were determined by Coulter counter (Leeds Veterinary School, UK)

Murine neutrophil glucose and ATP assays

For glucose analysis, neutrophils were cultured for 2 hours at $1x10^6$/ml in pre-equilibrated (normoxia vs hypoxia) serum free media, recovered, washed and lysed in pre-equilibrated buffer (40mM HEPES, 150mM KCL, 0.05% P-40). [Glucose] in cell lysates was determined by colorimetric assay using a glucose HK assay kit, as per manufacturer's instructions. For ATP analysis neutrophils were cultured at 0.2x106/ml and at each time point directly lysed ($0.2x10^5$) in 50µl tumor lysis buffer (Ammonium meta-vanadate, Hepes, Triton X). Lysates were subsequently incubated with Luciferin-luciferase reagent (Innovative Diagnostik Systeme) and samples read by luminometer (MPLX, Berthold Diagnostic Systems) against an ATP standard curve, with light output directly proportional to [ATP].

Apoptosis assays

Flow cytometric analysis (FACSCalibur™, Becton Dickinson) of PE-conjugated Annexin V (BD Pharmingen™) and To-Pro®-3 (Molecular Probes) staining was performed as previously described (28). Caspase activity was measured by detection of cleavage of a fluorescent caspase substrate FITC-VAD (Molecular Probes, Invitrogen) as previously described (Marriott et al., 2004).

Marriott HM, Ali F, Read RC, Mitchell TJ, Whyte MK, Dockrell DH. Nitric oxide levels regulate macrophage commitment to apoptosis or necrosis during pneumococcal infection. *FASEB J.* 2004;18:1126-1128.

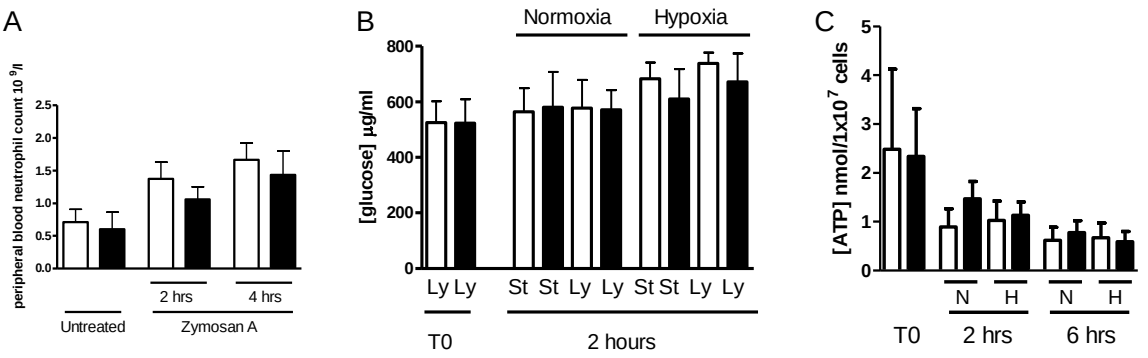
Supplementary Table 1 Transcripts showing 2 fold or greater decrease in abundance in hypoxic PHD3-/- neutrophils compared to WT controls.

Log FC	PPLR	Gene Title
-4.002544296	0.00049	EGL nine homolog 3 (C. elegans)
-3.660739691	1.00E-06	EGL nine homolog 3 (C. elegans)
-2.562627179	0.0007	inactive X specific transcripts
-2.395124508	0.011826	inactive X specific transcripts
-2.054470585	1.93E-05	Spi-C transcription factor (Spi-1/PU.1 related)
-1.730112135	0.000955	guanylate binding protein 6
-1.723701728	0.000405	interleukin 18 receptor 1
-1.603144173	0.003028	galactosidase, alpha
-1.597289988	0.009287	BTB and CNC homology 1
-1.574272782	0.000128	fatty acid binding protein 4, adipocyte
-1.492353593	0.000351	G-protein signalling modulator 1 (AGS3-like, C. elegans)
-1.440153231	0.001066	solute carrier family 30 (zinc transporter), member 1
-1.395755974	0.000254	Interleukin 1 receptor accessory protein-like 2
-1.377239746	0.001827	serum response factor
-1.373146135	0.000631	cytoplasmic polyadenylation element binding protein 2
-1.36531521	0.002668	jumonji, AT rich interactive domain 1A (Rbp2 like)
-1.330614168	0.005278	Aminolevulinic acid synthase 1
-1.321529759	0.004646	DnaJ (Hsp40) homolog, subfamily C, member 2
-1.3169723	0.01365	lymphocyte antigen 9
-1.294465871	0.000366	Spi-C transcription factor (Spi-1/PU.1 related)
-1.277480127	0.000952	NACHT, leucine rich repeat and PYD containing 4C
-1.275345305	0.015897	DEAD (Asp-Glu-Ala-Asp) box polypeptide 10
-1.246186455	0.002429	MAM domain containing 1
-1.229611668	0.010458	solute carrier family 25, member 44
-1.226613832	0.003546	breast cancer 1

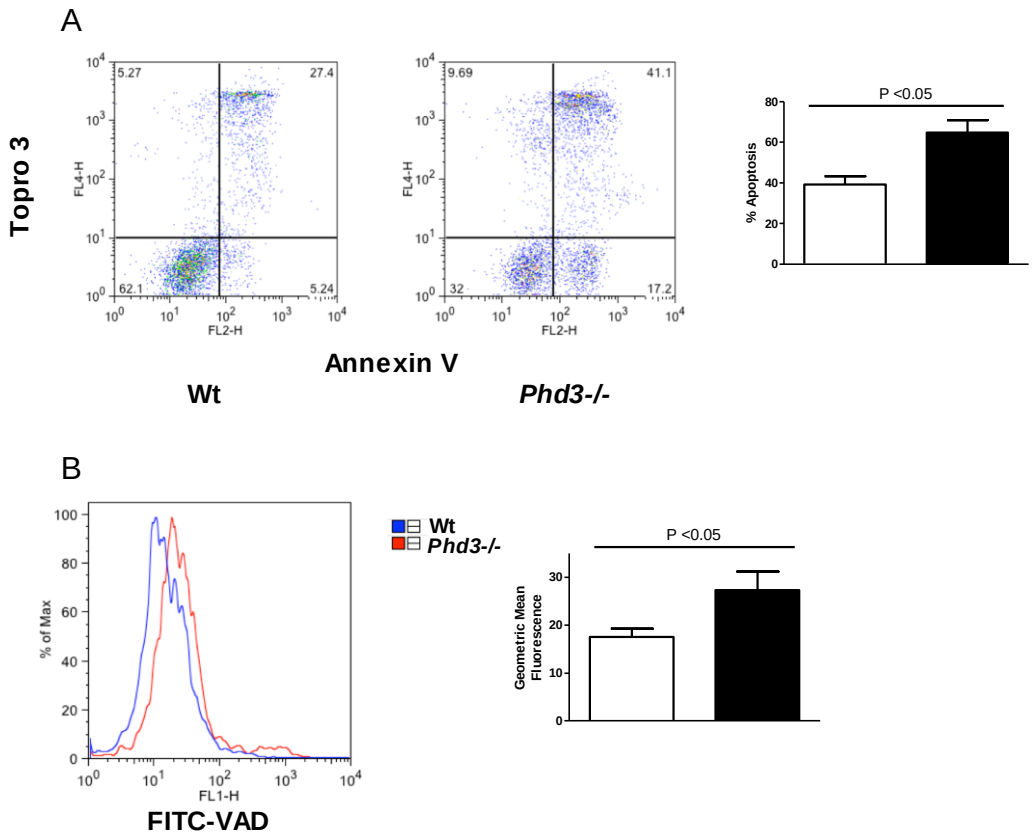
-1.225736305	0.003292	patatin-like phospholipase domain containing 1
-1.212334951	0.004345	hyaluronan mediated motility receptor (RHAMM)
-1.201089967	0.010207	kinesin family member 1B
-1.178638216	0.002367	anti-Mullerian hormone type 2 receptor
-1.169951585	0.003071	WD repeat domain 75
-1.166363855	0.000688	solute carrier family 20, member 1
-1.164771366	0.001533	oligonucleotide/oligosaccharide-binding fold containing 2A
-1.161822411	0.036751	ribonuclease, RNase A family, 6
-1.161507124	0.009264	Myocyte enhancer factor 2A
-1.105808437	0.000711	jumonji, AT rich interactive domain 1A (Rbp2 like)
-1.101601052	0.001205	wingless-related MMTV integration site 5A
-1.097561822	0.05912	G protein-coupled receptor 107
-1.086726232	0.044066	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2
-1.085498546	0.005698	transmembrane protein 170
-1.074945718	0.014539	histone cluster 2, H3c1
-1.072501734	0.013116	coiled-coil domain containing 122
-1.066100576	0.020376	DPH3 homolog (KTI11, S. cerevisiae)
-1.062907017	0.004907	glutaminyl-tRNA synthase (glutamine-hydrolyzing)-like 1
-1.056764651	0.002531	lipoma HMGIC fusion partner-like 2
-1.049399075	0.065697	Ras interacting protein 1
-1.04573322	0.004191	splicing factor 3a, subunit 3
-1.04547089	0.022613	F-box protein 34
-1.044333421	0.018758	tubulointerstitial nephritis antigen-like
-1.043751385	0.002386	zeta-chain (TCR) associated protein kinase
-1.025538336	0.017964	Thioredoxin-like 2
-1.016423647	0.030642	inhibitor of DNA binding 2
-1.012245469	0.001591	inner membrane protein, mitochondrial
-1.007133936	0.040876	RGM domain family, member A

-1.005076582	0.016875	frizzled homolog 3 (Drosophila)
-1.004819081	0.020861	interleukin 1 alpha

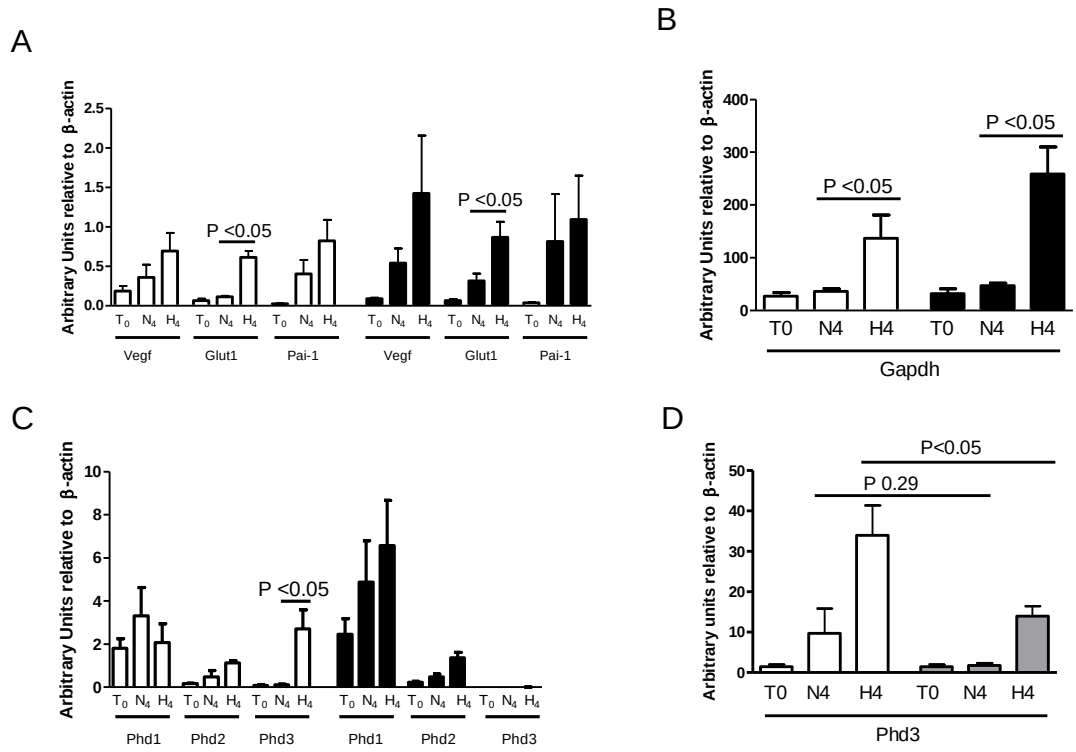
Gene array. cDNA from wildtype and PHD3-/- neutrophils aged for 4 hours in hypoxia was run on Affymetrix whole mouse gene array chips in n=3 independent experiments. Individual arrays were normalised and log fold change in transcript abundance determined for PHD3-/- compared to wild-type cells by panther analysis. For each gene PPLR values determine the probability of the estimated fold change being close to the determined values. Genes showing a greater than 2 fold decrease are listed in order of magnitude of response. Geo accession number GSE26023.



Supplemental Figure 1. Normal neutrophil numbers and metabolism in *Phd3* null mice. (A) Peripheral blood counts. Peripheral blood samples were obtained by terminal IVC bleeds from wild type (open bars) and *Phd3-/-* animals (filled bars) 2 and 4 hrs following the installation of intra-peritoneal zymosan and in aged matched untreated animals. Differential cell counts were performed by Coulter counter (n=5). (B) Intra- and extra-cellular [glucose] measurement. Murine peripheral blood PMN isolated from wild type (open bars) and *Phd3* null (filled bars) animals were cultured for 2 hours in normoxia and hypoxia and [glucose] determined in whole cell lysates (ly) and supernatants (St) by colorimetric assay (n=7). (C) ATP analysis. Murine peripheral blood PMN isolated from wild type (open bars) and *Phd3-/-* (filled bars) animals were cultured for 2 or 6 hours in normoxia (N) and hypoxia (H), cells lysed and [ATP] determined against a standard curve by luminometry (n=3).



Supplemental Figure 2. Hypoxia delays apoptosis of wildtype but not *Phd3-/-* neutrophils. Murine peripheral blood PMN were isolated from WT (open bar) and *Phd3-/-* (filled bar) animals. PMN were cultured in normoxia or hypoxia for 6 h. (A) Representative dot plots are shown for Annexin V and ToPro3 staining for wild-type and *Phd3-/-* neutrophils cultured in hypoxia. Mean data for WT (open bars) and PHD3-/- (filled bars) cells (n=4). (B) Representative histograms are shown for wild-type (blue line) vs *Phd3-/-* neutrophils (red line) FITC VAD fluorescence (n=4).



Supplemental Figure 3. Preservation of Hif transcriptional activity and Phd isoform expression in *Phd3-/-* neutrophils. (A) Hif targets. cDNA from freshly isolated murine peripheral blood PMN and PMN aged for 4 hours in either normoxia (N) or hypoxia (H) was analysed by TaqMan for *Vegf, Glut1* and *Pai-1* transcript abundance relative to β-actin in both WT (open bars) and *Phd3-/-* (filled bars) cells (n=5). (B) Expression of Gapdh transcript was similarly evaluated (n=5). (C) PHD enzymes. cDNA from freshly isolated murine peripheral blood PMN and PMN aged for 4 hours in either normoxia (N) or hypoxia(H) was analysed by TaqMan for *Phd1, Phd2* and *Phd3* transcript abundance relative to *β-actin* in both WT (open bars) and *Phd3-/-* (filled bars)cells (n=5). (D) *Phd3* and *Hif-1α-/-*. cDNA from freshly isolated murine peripheral blood PMN and PMN aged for 4 hours in either normoxia (N) or hypoxia (H) was analysed by TaqMan for *Phd3* transcript abundance relative to *β-actin* in both WT (open bars) and *Hif-1α-/-* (filled bars) cells (n=4).