SUPPLEMENT SECTION

METHODS

Malek, *et al*. study comparison. SAM result data from the rat study by Malek, *et al*. [1] was retrieved from the TIGR Programs for Genomic Applications (PGA) website (http://pga.tigr.org/). Nonblank GenBank IDs representing the significantly differentially-expressed genes between normoxia and hypoxia conditions for male Dahl SS rats were extracted and translated into a set of representative Entrez Gene identifiers using the DAVID tool (http://niaid.abcc.ncifcrf.gov) [2]. Using a similar process, significantly differentially-expressed probe sets derived using SAM were translated into representative Entrez Gene identifiers for two sets of the present study: normoxia versus hypoxia and normoxia versus hypoxia/SU5416. The common distinct Entrez Gene identifiers between two experimental sets of the present study and the normoxia versus hypoxia set from Malek, *et al.* was then derived using the comparison tool of the Whitehead Institute (http://jura.wi.mit.edu/bioc/tools/compare.html) and these numbers are displayed in **Supplemental Table 4 and 5**. The probability of finding common genes between these group is calculated using the hypergeometric distribution (assuming arrays contain > 15,000 genes in rat).

Geraci, *et al*. study comparison. The list of significantly differentially-expressed genes in *H. sapiens* for PH was taken from Table 3 and Supplement Table 1 of Geraci, *et al.*'s paper [3] and were manually translated from gene names to

Entrez Gene identifiers. The GeneCards database (http://www.genecards.org/index.shtml) was used to retrieve orthologuous rat gene symbols and IDs. Genes from the normoxia versus hypoxia and normoxia versus hypoxia/SU5416 analyses sets were similarly translated as above using DAVID [2] into Entrez Gene identifiers, selecting only those associated with the species *rattus norvegicus*, *rattus rattus*, and *rattus sp*. The resulting lists of Entrez gene identifiers of the study by Geraci, *et al.* were compared to those or our study using the comparison tool of the Whitehead Institute as above.

Girgis, *et al*. study comparison. Similar to the previous study comparisons, data from a previous study examining the effects of simvastatin on a rat model of hypoxia [4] was compared with the results of this study. All identifiers were converted to Entrez Gene identifiers using DAVID [2], using only results falling into the “rattus norvegicus” and “rattus sp.” species. Specifically, three comparisons were conducted: first, the hypoxia versus hypoxia/simvastatin pair was compared to the hypoxia/SU6516 versus hypoxia/SU6516/sorafenib. Additionally, the normoxia versus hypoxic condition was compared to our normoxia versus hypoxia and normoxia versus hypoxia/SU5416 datasets.

Gharib, *et al*. study comparison. Differentially-expressed mice probes derived by Gharib, *et al.* [5] were downloaded from http://physiolgenomics.physiology.org/cgi/content/full/00265.2004/DC1. Results are presented as 5,141 probe ids (from the NIA 15K mouse cDNA chip [6, 7]

representing 1,752 distinct mice genes divided in 9 clusters) and translated the probe IDs into gene symbols using the annotation file of NIA 15K chip found at http://lgsun.grc.nia.nih.gov/cDNA/15k.html. A Mouse to rat ortholog translation table "HMD_Rat2.rpt" was downloaded from ftp://ftp.informatics.jax.org/pub/reports/. Two sets of genes were retrieved from the differential expressed genes spanning 35 days of experiments by examining their 7 expression patterns: normoxia versus hypoxia group, and hypoxia versus re-oxygenation group. The first set, normoxia versus hypoxia, (1,133 genes with 738 of them having rat orthologs) was selected from clusters 2,4,5,6 and 7. These clusters show significant expression change (beyond the 1^{st} and 3^{rd} quartile of expression value for each cluster) between day 1 (normoxia stage) and day 21 (hypoxia stage). This hypoxia-driven set was used to compare to two of our sets including normoxia versus hypoxia and normoxia versus hypoxia/SU5416. The second set, hypoxia versus re-oxygenation, (405 genes with 266 having rat orthologs), was selected from clusters 2 and 7 and compared to our hypoxia/SU5416 versus hypoxia/SU5416/sorafenib. These clusters show significant expression change (beyond the 1^{st} and 3^{rd} quartile of expression value for each cluster) between day 21 (hypoxia stage) and day 35 (re-oxygenation stage). In addition, the set was filtered to include only the specific mouse genes for which the average normalized expression value between the two periods changed by more than 0.1 on a log scale.

RESULTS

SUPPLEMENT TABLE 4. Number of biological processes significantly overrepresented in differentially-expressed genes derived from three comparison sets.

GO Functional Category	Count of distinct GO terms under the broad GO categories		
	N vs H	N vs H-SU	H-Su vs H-SU-Sor
Development (GO:0032502)	69	73	12
Immune System (GO:0006952, GO:0002376)	19	24	5
Muscle Contraction or Development (GO:0006937, GO:0007517)	2	5	3
Cell Metabolism (GO:0044237, GO:0008152)	90	65	18
Cell Differentiation (GO:0030154)	36	39	6
Cell Proliferation (GO:0008283)	7	8	3

SUPPLEMENT TABLE 5. 57 common hypoxia-driven distinct genes across the differentially-expressed set between normoxia and hypoxia from the Malek, *et al.* study and the differentially-expressed set between normoxia versus hypoxia of our study.

Entrez Gene ID	Gene Symbol
24440	Hbb
24772	Cxcl12
24791	Sparc
24875	Vipr1
24914	Lox
25054	Ntrk2
25330	Lipe
25339	Npr3
25532	Rab4a
25644	Bmp6
25655	Gja4
29147	Jag2
29393	Col1a1
54292	Rgs12
56765	Plvap

58948	Dlgh3
60357	Prom1
60423	Slc28a2
64155	Scn7a
65155	Alas1
79252	Adamts1
81640	Amd1
81660	Gatm
83834	Nrn1
84407	Cdh11
84575	Fads1
85332	Prkcdbp
89784	Idi1
113900	Nupr1
116501	Slc9a3r2
245956	Scn3b
245963	Egfl7
246138	Ly6b
246327	Prim1
289083	RGD1308584_predicted
290905	Col4a1
293186	Xlkd1_predicted
295490	Emcn
298006	Ccl21b
299357	RGD1359202
306628	Col4a2_predicted
307861	Terf2ip
308393	RGD1560435_predicted
308508	Uble1b
309804	Cdc2l6_predicted
310811	Palmd
311071	Zfhx1b
311209	Tp53i11_predicted
313722	Spsb1_predicted
315259	Prickle1
315655	Rdx
315970	LOC315970
360551	RGD1563179_predicted
360914	Plac8_predicted
361303	Lims2
432392	Fut8
641523	LOC641523

SUPPLEMENT TABLE 6. 35 common distinct genes across the differentially-expressed set between normoxia and hypoxia from the Malek, *et al.* study and the differentially-expressed set between normoxia versus hypoxia-SU5416 of our study.

Entrez Gene ID	Gene Symbol
24626	Pde4b
24772	Cxcl12
24914	Lox
25043	Eln
25054	Ntrk2
25532	Rab4a
25644	Bmp6
25661	Fn1
29147	Jag2
29266	Mcpt2
29393	Col1a1
29436	Tfpi
54294	Rgs5
60423	Slc28a2
65204	Cnn1
79252	Adamts1
81640	Amd1
83834	Nrn1
84050	Enpp2
84348	Cmkor1
84407	Cdh11
85251	Col18a1
89784	Idi1
113900	Nupr1
116501	Slc9a3r2
192262	C1s
245963	Egfl7
246327	Prim1
293186	Xlkd1_predicted
293823	RGD1311350
294335	Susd2_predicted
298006	Ccl21b
313722	Spsb1_predicted
360785	Ap1s1_predicted
361303	Lims2

SUPPLEMENT TABLE 7. Overlap of Genbank IDs differentially-expressed between normoxia versus hypoxia from Girgis, *et al.* study and our hypoxia-driven gene set resulted in 20 common genes.

Entrez Gene ID	Gene Name
29517	SERUM/GLUCOCORTICOID REGULATED KINASE
24617	SERINE (OR CYSTEINE) PROTEINASE INHIBITOR, CLADE E, MEMBER 1
64511	FARNESYLTRANSFERASE, CAAX BOX, BETA
24654	PHOSPHOLIPASE C, BETA 1
29602	PROSTAGLANDIN F2 RECEPTOR NEGATIVE REGULATOR
81640	S-ADENOSYLMETHIONINE DECARBOXYLASE 1
140868	FATTY ACID BINDING PROTEIN 5, EPIDERMAL
83823	REGULATOR OF G-PROTEIN SIGNALING 19 INTERACTING PROTEIN 1
24791	SECRETED ACIDIC CYSTEINE RICH GLYCOPROTEIN
83834	NEURITIN
293186	EXTRA CELLULAR LINK DOMAIN-CONTAINING 1 (PREDICTED)
24825	TRANSFERRIN
25339	NATRIURETIC PEPTIDE RECEPTOR 3
293701	ESTROGEN RELATED RECEPTOR, ALPHA
63997	SOLUTE CARRIER FAMILY 29 (NUCLEOSIDE TRANSPORTERS), MEMBER 1
140914	OXIDIZED LOW DENSITY LIPOPROTEIN (LECTIN-LIKE) RECEPTOR 1
25741	PHOSPHOFRUCTOKINASE, LIVER, B-TYPE
246138	LYMPHOCYTE ANTIGEN 6 COMPLEX, LOCUS B
245963	EGF-LIKE DOMAIN 7
117183	RESPONSE GENE TO COMPLEMENT 32

SUPPLEMENT TABLE 8. Overlap of Genbank IDs differentially-expressed between normoxia versus hypoxia from Girgis, *et al.* study and our hypoxia/SU5416-driven gene set resulted in 17 common genes.

Entrez Gene ID	Gene Name
24617	SERINE (OR CYSTEINE) PROTEINASE INHIBITOR, CLADE E, MEMBER 1
64511	FARNESYLTRANSFERASE, CAAX BOX, BETA
81640	S-ADENOSYLMETHIONINE DECARBOXYLASE 1
24373	FOLLISTATIN
140868	FATTY ACID BINDING PROTEIN 5, EPIDERMAL
25427	CYTOCHROME P450, SUBFAMILY 51
24654	PHOSPHOLIPASE C, BETA 1
83834	NEURITIN
293186	EXTRA CELLULAR LINK DOMAIN-CONTAINING 1 (PREDICTED)
24825	TRANSFERRIN
64369	PHOSPHATIDIC ACID PHOSPHATASE 2A
65029	AMILORIDE BINDING PROTEIN 1 (AMINE OXIDASE, COPPER-CONTAINING)
63997	SOLUTE CARRIER FAMILY 29 (NUCLEOSIDE TRANSPORTERS), MEMBER 1
140914	OXIDIZED LOW DENSITY LIPOPROTEIN (LECTIN-LIKE) RECEPTOR 1
24626	PHOSPHODIESTERASE 4B, CAMP SPECIFIC
245963	EGF-LIKE DOMAIN 7
117183	RESPONSE GENE TO COMPLEMENT 32

SUPPLEMENT TABLE 9. 47 common hypoxia-driven genes between the differentially-expressed rat ortholog set (from mice) in the Gharib, *et al*. study and differentially-expressed rat genes from our study with a similar time period (3 weeks).

Rat genes in our study	Mouse genes in Gharib *et al.*	Cluster Id	Clone ID	Gene Name
Plat	Plat	4	H3080H11	plasminogen activator, tissue
Akap2	Akap2	6	H3090C12	A kinase (PRKA) anchor protein 2
Fads1	Fads1	4	H3031E12	fatty acid desaturase 1
Akt1	Akt1	4	H3020C06	thymoma viral proto-oncogene 1
RGD1564876 _ predicted	Slc35e3	5	H3093E08	solute carrier family 35, member E3
Kit	Kit	6	H3136A01	kit oncogene
Nr3c1	Nr3c1	6	H3147F05	nuclear receptor subfamily 3, group C, member 1
Por	Por	7	H3090A06	P450 (cytochrome) oxidoreductase
Kif23_ predicted	Kif23	4	H3068A08	kinesin family member 23
Ctdspl_ predicted	Ctdspl	5	H3061F04	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like
Rgs12	Rgs12	6	H3155E02	regulator of G-protein signaling 12
Rod1	Rod1	6	H3001B02	ROD1 regulator of differentiation 1 (S. pombe)
Fkbp5	Fkbp5	2	H3138G12	FK506 binding protein 5
Tef	Tef	6	H3028E11	thyrotroph embryonic factor
Aldh3b1	Aldh3b1	4	H3149A06	aldehyde dehydrogenase 3 family, member B1
Fntb	Fntb	5	H3001H04	farnesyltransferase, CAAX box, beta
Sfpq	Sfpq	2	H3066C06	splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)
Tekt2	Tekt2	5	H3084B10	tektin 2
Atp1b1	Atp1b1	5	H3005E10	ATPase, Na+/K+ transporting, beta 1 polypeptide
Procr	Procr	4	H3022E10	protein C receptor, endothelial
Eno1	Eno1	4	H3027E08	enolase 1, alpha non-neuron
Cldn7	Cldn7	5	H3084E04	claudin 7
Adcy3	Adcy3	5	H3113D06	adenylate cyclase 3
RGD1307736	2410014A08 Rik	4	H3119B11	RIKEN cDNA 2410014A08 gene
LOC501069	Golga4	7	H3037A05	golgi autoantigen, golgin subfamily a, 4
Tcf3_ predicted	Tcf3	5	H3004D03	transcription factor 3
Slc28a2	Slc28a2	4	H3014C12	solute carrier family 28 (sodium-coupled nucleoside transporter), member 2
Pdlim7	Pdlim7	6	H3082E06	PDZ and LIM domain 7
Camk2g	Camk2g	5	H3093E05	calcium/calmodulin -dependent protein kinase II gamma

Lmcd1_ predicted	Lmcd1	4	H3134B01	LIM and cysteine-rich domains 1
Mt1a	Mt1	4	H3020C02	metallothionein 1
Coro1b	Coro1b	4	H3018F07	coronin, actin binding protein 1B
Crebbp	Crebbp	6	H3075G02	CREB binding protein
Phactr1	Phactr1	5	H3018G12	phosphatase and actin regulator 1
Atrx	Atrx	6	H3067F06	alpha thalassemia/mental retardation syndrome X-linked homolog (human)
Adipor2	Adipor2	7	H3137B07	adiponectin receptor 2
Aplp2	Aplp2	7	H3154H04	amyloid beta (A4) precursor-like protein 2
Lamc1	Lamc1	4	H3044A05	laminin, gamma 1
Agtrap	Agtrap	5	H3027C07	angiotensin II, type I receptor-associated protein
Dcxr	Dcxr	7	H3098H02	dicarbonyl L-xylulose reductase
Ucp2	Ucp2	4	H3136E12	uncoupling protein 2, mitochondrial
RGD1564237 _ predicted	Gpihbp1	2	H3153H06	GPI-anchored HDL-binding protein 1
Bgn	Bgn	4	H3127D03	Biglycan
Zadh1	Zadh1	6	H3010E06	zinc binding alcohol dehydrogenase, domain containing 1
Col4a1	Col4a1	4	H3112C01	procollagen, type IV, alpha 1
Stk4_ predicted	Stk4	7	H3080D05	serine/threonine kinase 4
MGC105830	Rab1b	6	H3025A10	RAB1B, member RAS oncogene family

SUPPLEMENT TABLE 10. 26 shared genes between the differential expressed genes between normoxia and hypoxia/SU5416 from our study to the orthologous rat genes found in the Gharib, *et al*. dataset between normoxia (day 1) versus hypoxia (21 days) in mice.

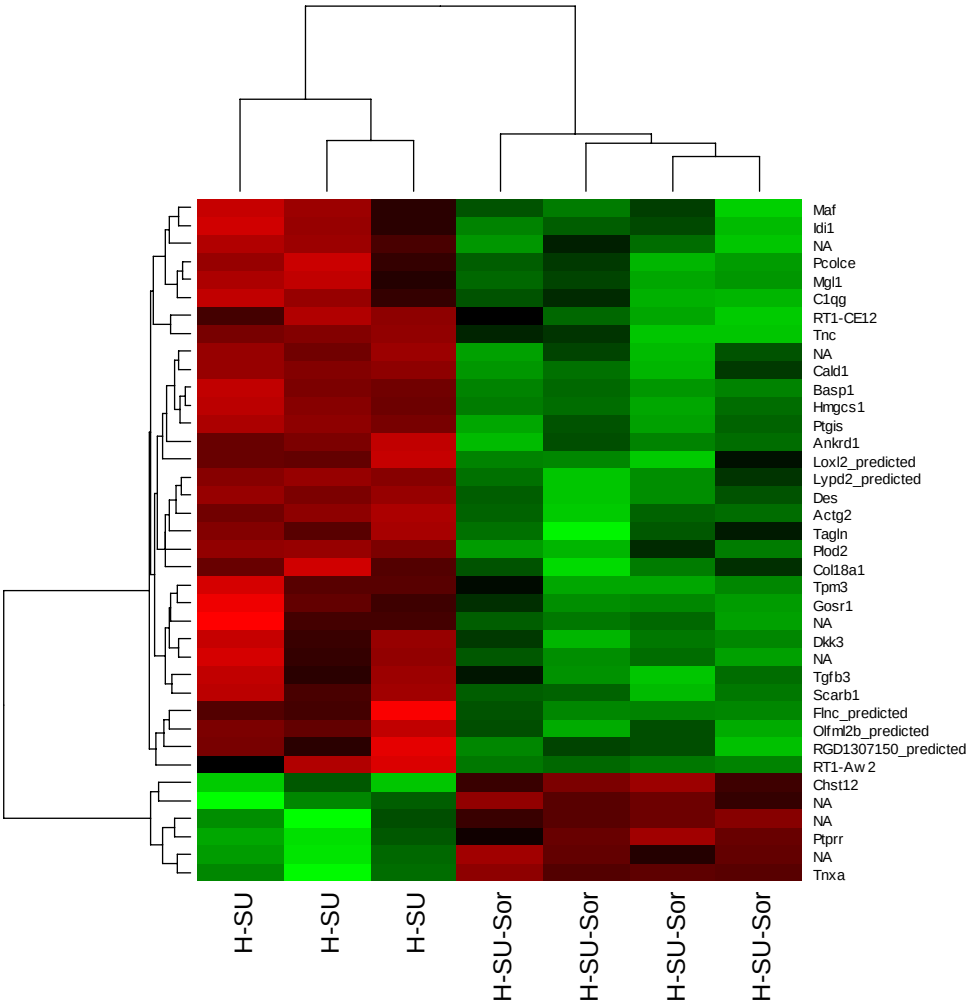
Rat genes in our study	Mouse genes in Gharib, *et al.*	Cluster ID	Clone ID	Gene Name
Rrbp1_ predicted	Rrbp1	4	H3009F11	ribosome binding protein 1
C1qa	C1qa	4	H3139F06	complement component 1, q subcomponent, alpha polypeptide
Fn1	Fn1	4	H3116A10	fibronectin 1
Kit	Kit	6	H3136A01	kit oncogene
Nr3c1	Nr3c1	6	H3147F05	nuclear receptor subfamily 3, group C, member 1
Por	Por	7	H3090A06	P450 (cytochrome) oxidoreductase
Kif23_ predicted	Kif23	4	H3068A08	kinesin family member 23
Carhsp1	Carhsp1	4	H3112B05	calcium regulated heat stable protein 1
Vldlr	Vldlr	5	H3096H12	very low density lipoprotein receptor
Fntb	Fntb	5	H3001H04	farnesyltransferase, CAAX box, beta
Sfpq	Sfpq	2	H3066C06	splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)
Eno1	Eno1	4	H3027E08	enolase 1, alpha non-neuron
Actr2	Actr2	6	H3002C02	ARP2 actin-related protein 2 homolog (yeast)
RGD1307736	2410014A08 Rik	4	H3119B11	RIKEN cDNA 2410014A08 gene
LOC501069	Golga4	7	H3037A05	golgi autoantigen, golgin subfamily a, 4
Ugt1a1	Ugt1a1	2	H3155C10	UDP glycosyltransferase 1 family, polypeptide A5
Slc28a2	Slc28a2	4	H3014C12	solute carrier family 28 (sodium-coupled nucleoside transporter), member 2
Pdlim7	Pdlim7	6	H3082E06	PDZ and LIM domain 7
Mt1a	Mt1	4	H3020C02	metallothionein 1
Adipor2	Adipor2	7	H3137B07	adiponectin receptor 2
Cnn1	Cnn1	4	H3053E04	calponin 1
Car8	Car8	6	H3115C01	carbonic anhydrase 8
C1r	C1r	7	H3136D05	complement component 1, r subcomponent
Ugt1a2	Ugt1a2	2	H3155C10	UDP glycosyltransferase 1 family, polypeptide A5
Bgn	Bgn	4	H3127D03	biglycan
MGC105830	Rab1b	6	H3025A10	RAB1B, member RAS oncogene family

SUPPLEMENT TABLE 11. Search for differentially-expressed genes common to the two models of PH of our current study and previous PH studies.

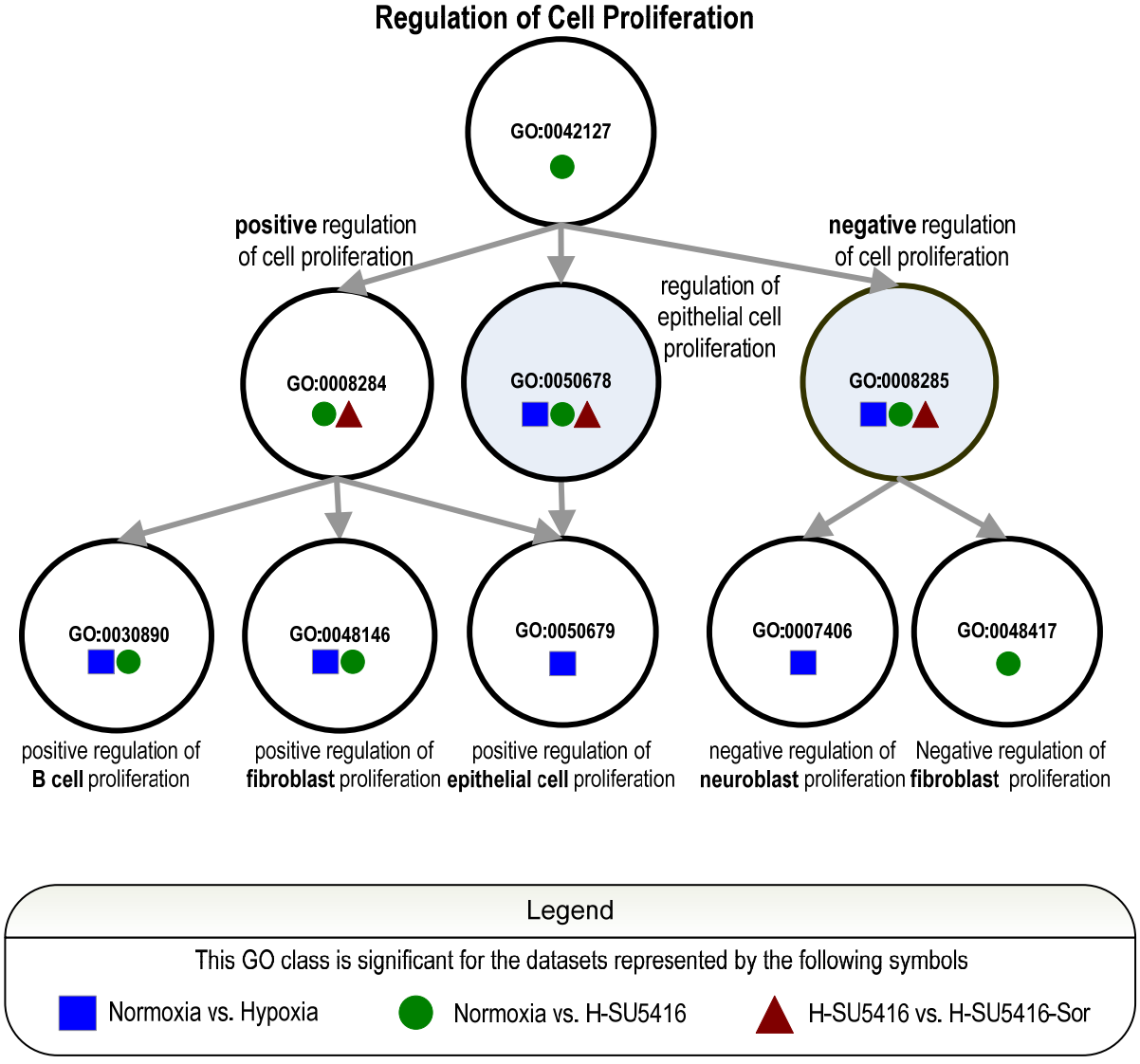
Previous Study Conditions	# Genes in common (probability)		
	Normoxia vs Hypoxia	Normoxia vs H-SU	Total Unique Genes
Malek , *et al.* Normoxia vs Hypoxia	57 ($p<10^{-11}$)	35 ($p<10^{-10}$)	72
Geraci, *et al.* Normoxia vs PH	4 (NS)	1(NS)	5
Girgis, *et al.* Normoxia vs Hypoxia	20 (p=0.026)	17 ($p<10^{-5}$)	25
Gharib, *et al.* Normoxia vs Hypoxia	47 (p=0.016)	26 (p=0.021)	58

NS= not statistically significant. (See Supplement Methods for description of the calculation of the probability).

SUPPLEMENT FIGURE 1: Original R-derived Heatmap of 38 significant genes. This is the unmodified heatmap where the NA values for gene names have not been referenced to their underlying annotation and not replaced with appropriate descriptions.



SUPPLEMENT FIGURE 2. GO terms of "Cell proliferation" significantly overrepresented by differentially-expressed genes across three comparison sets. The network tree illustrates the relationship of GO terms via a GO hierarchy (used to construct the comparisons in Figure 8) under a single overarching functional category, *Cell proliferation*. The majority of biological processes that compromise *Cell proliferation* include the regulation of proliferation of B lymphycytes, fibroblasts, neuroblasts, and epithelial cells.



REFERENCES

1. Malek, R.L., et al., *Physiogenomic resources for rat models of heart, lung and blood disorders.* Nat Genet, 2006. **38**(2): p. 234-9.
2. Dennis, G., Jr., et al., *DAVID: Database for Annotation, Visualization, and Integrated Discovery.* Genome Biol, 2003. **4**(5): p. P3.
3. Geraci, M.W., et al., *Gene expression patterns in the lungs of patients with primary pulmonary hypertension: a gene microarray analysis.* Circ Res, 2001. **88**(6): p. 555-62.
4. Girgis, R.E., et al., *Differential gene expression in chronic hypoxic pulmonary hypertension: effect of simvastatin treatment.* Chest, 2005. **128**(6 Suppl): p. 579S.
5. Gharib, S.A., et al., *Global gene annotation analysis and transcriptional profiling identify key biological modules in hypoxic pulmonary hypertension.* Physiol Genomics, 2005. **22**(1): p. 14-23.
6. Kargul, G.J., et al., *Verification and initial annotation of the NIA mouse 15K cDNA clone set.* Nat Genet, 2001. **28**(1): p. 17-8.
7. Tanaka, T.S., et al., *Genome-wide expression profiling of mid-gestation placenta and embryo using a 15,000 mouse developmental cDNA microarray.* Proc Natl Acad Sci U S A, 2000. **97**(16): p. 9127-32.