

Bioprospecting for hypoxia tolerance: applied genomics of the blind subterranean mole rat (Spalax)

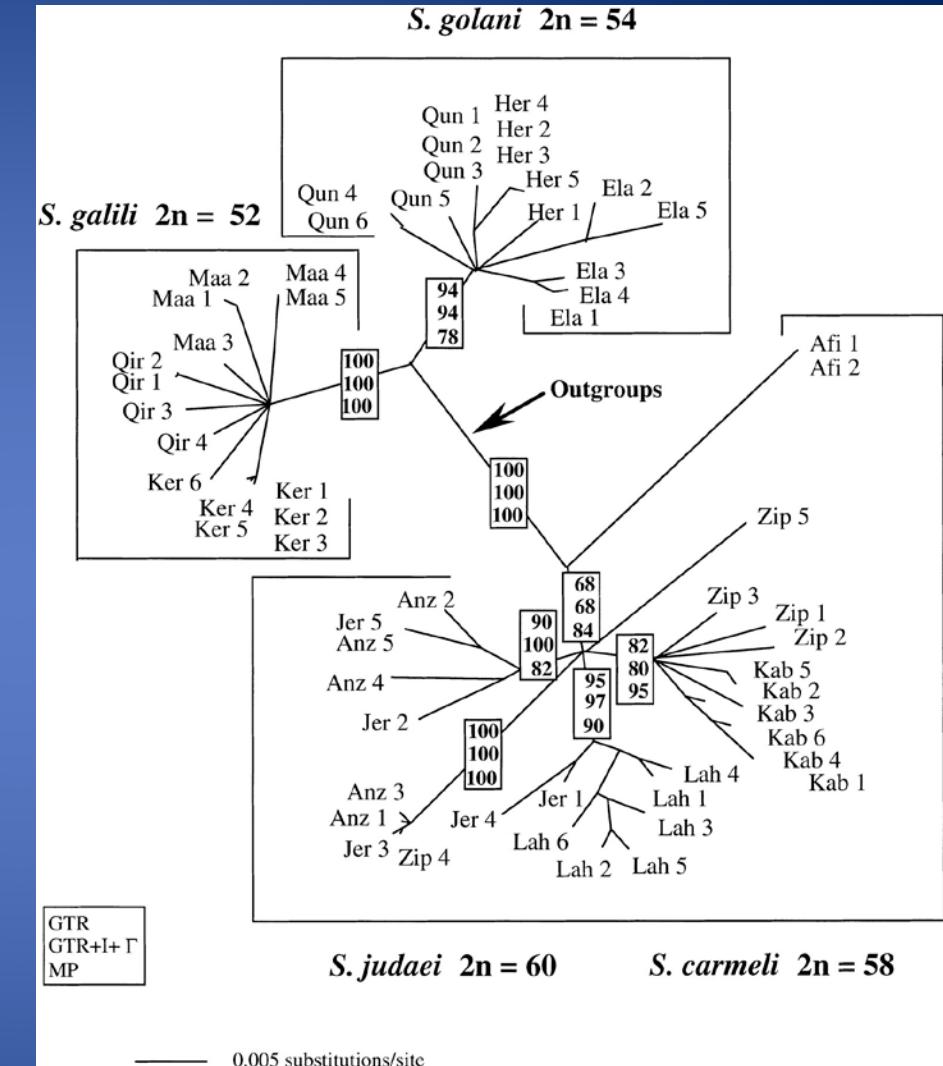
Assaf Malik, Alvaro Hernandez, Avraham Korol,
Aaron Avivi, Mark Band



Blind Subterranean Mole Rat *Spalax ehrenburgi*



Rainfall



Reyes, A. et al. Mol Biol Evol 2003 20:622-632;

Oxygen levels in natural environment vs. in lab

Atmospheric Oxygen (21%)

Measurements in burrows 7.2% O₂ and 6.1% CO₂

Lowest O₂ levels in lab:

2.8% O₂ *S. galili* (Mediterranean)

14 hours

3.7% O₂ *S. judaei* (Arid)

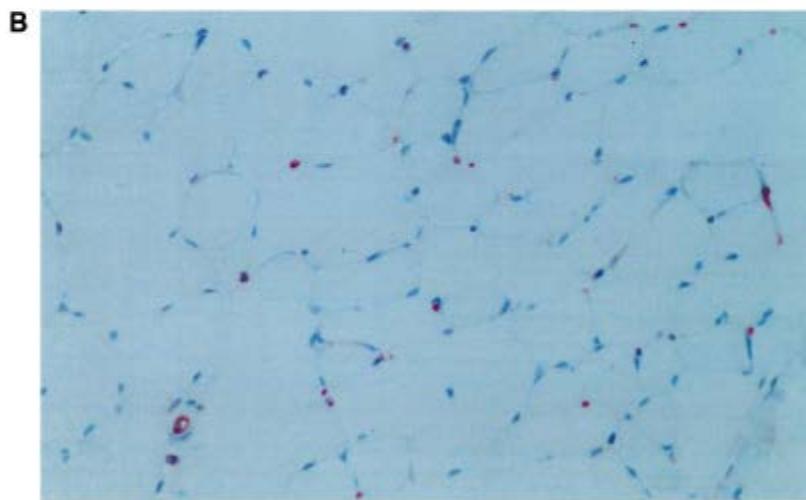
3 hours

3.0% O₂ *Rattus*

Normoxic O₂ levels (21%)

Vascular endothelial cells (red)
in *Spalax* (A), rat (B) muscle.

A. Avni et al./FEBS Letters 452 (1999)

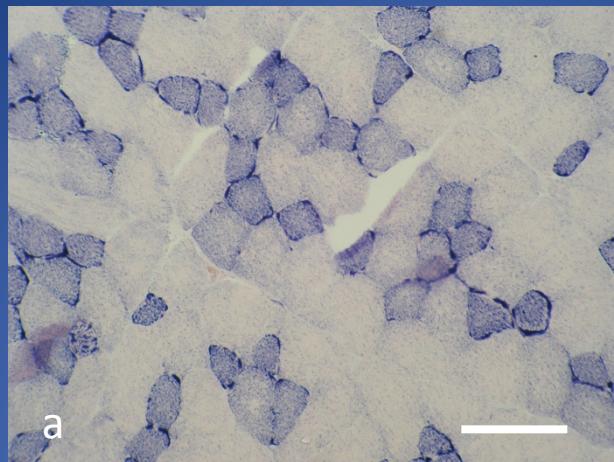


Vegf
VegfR
Heperanase
Hif1a

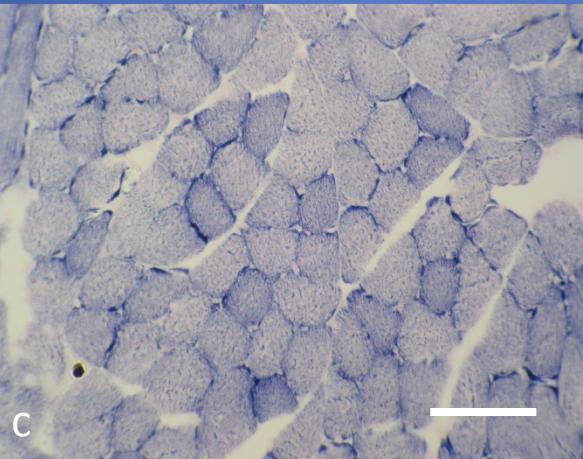
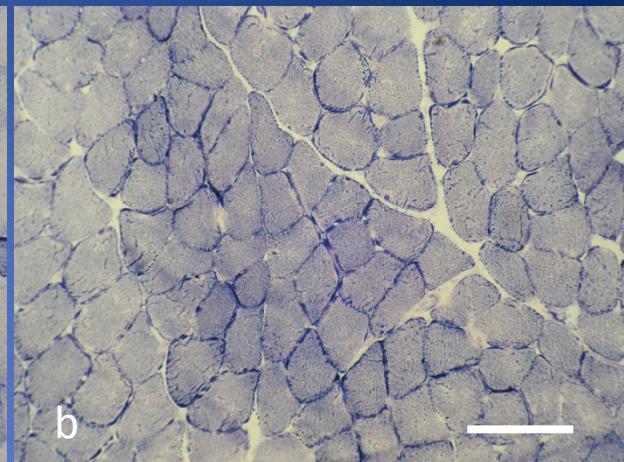
Type I oxidative
Type II oxidative
Type II glycolytic

Rat- normal dist.
Spalax- Type I oxidative

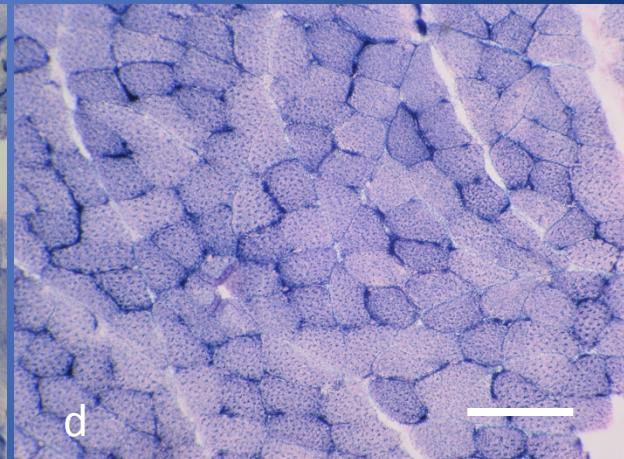
Rat neck



Spalax neck



Spalax gastrocnemius



Spalax quadriceps

Succinate Dehydrogenase (SDH) activities in (a) rat neck (b) Spalax neck and (c, d)
Spalax leg muscles

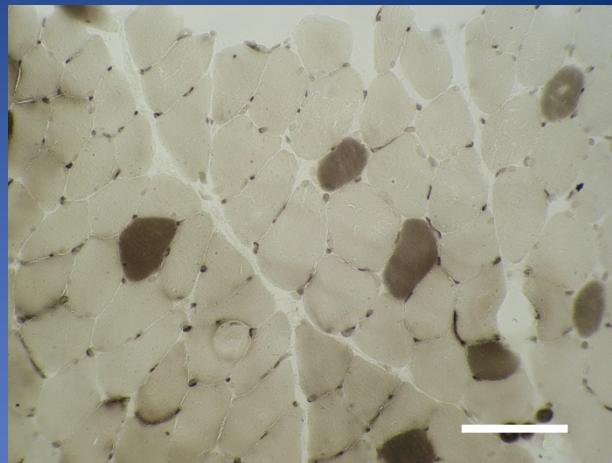
Type I dark
stain



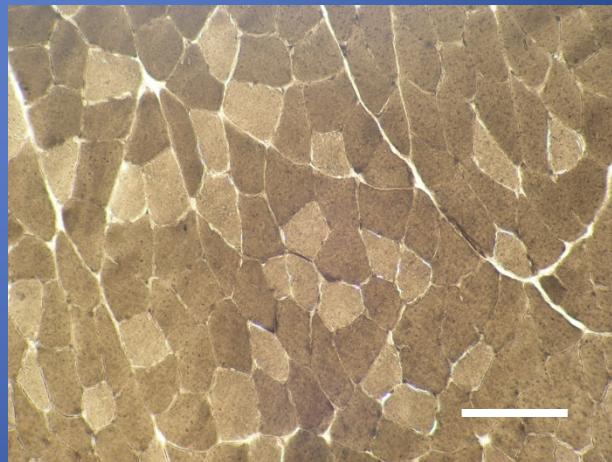
Rat neck ATP-ase pH4.2



Spalax neck ATPase pH4.6



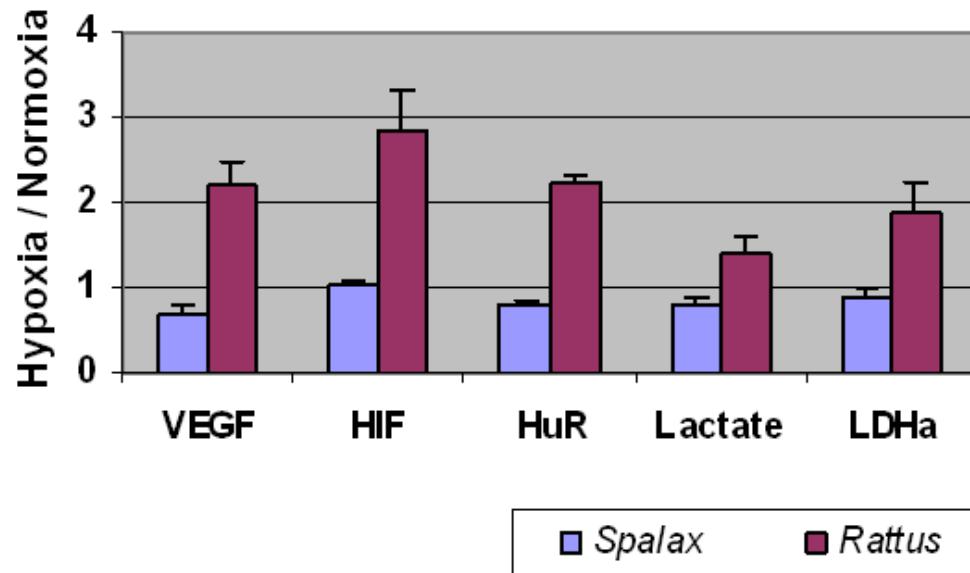
Spalax neck ATP-ase pH4.2



Type I light
stain

Spalax neck ATPase pH10.2

in vivo Muscle Gene Expression



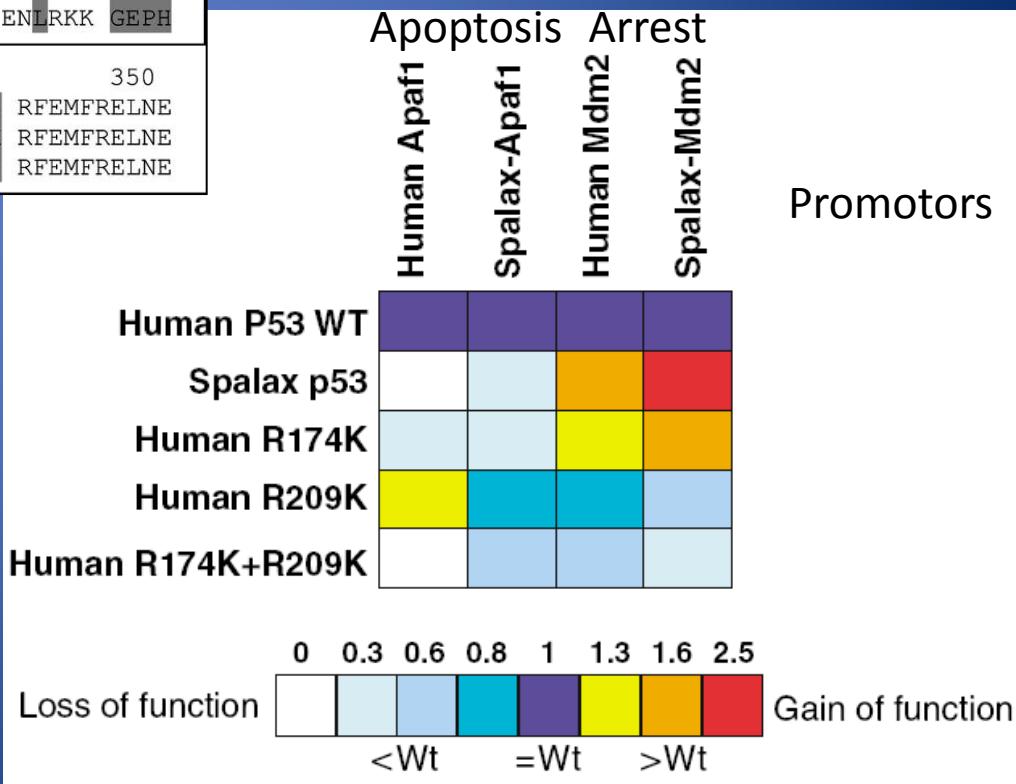
Exposure of *Rattus* and *Spalax* to a hypoxic environment revealed that in *Rattus* and in those *Spalax* tissues where the blood vessel concentration was similar to *Rattus* tissues, *VEGF* as well as *HIF-1 α* and *HuR* increased to a similar degree. However, in *Spalax* muscle there was no increase in the expression of these genes while under the same environmental conditions.

Spalax p53

	151	hs.174	200
sP53	VD STPPPGR VRAMAIYKKs	QHMTEVV K RC PHHERCSDSD	GLAPPQHLIR
mP53	VS ATPPAGSR VRAMAIYKKs	QHMTEVV R RC PHHERCSDGD	GLAPPQHLIR
hP53	VD STPPPGR VRAMAIYKQS	QHMTEVV R RC PHHERCSDSD	GLAPPQHLIR
	201	hs.209	250
sP53	VE GNLRAEYL D D K HTRFRHSV VVPYEPPEVG SDCTTIHYNY MCNSSCMGGM		
mP53	VE GNLYPEYL E D R QTFRFRHSV VVPYEPPEAG SEYTTIHYKY MCNSSCMGGM		
hP53	VE GNLRVEYL D D R NTFRFRHSV VVPYEPPEVG SDCTTIHYNY MCNSSCMGGM		
	251		300
sP53	NRRPILTIIIT LEDSSGNLLG RNSFEVRVCA CPGRDRRTEE ENFRKK G ELC		
mP53	NRRPILTIIIT LEDSSGNLLG RDSFEVRVCA CPGRDRRTEE ENFRKK E VLC		
hP53	NRRPILTIIIT LEDSSGNLLG RNSFEVRVCA CPGRDRRTEE ENLRKK G EPH		
	301		350
sP53	PELP PGSTKR ALPTGTSSSP QPKKKPLDGE YFTL K IRGR E RFEMFRELNE		
mP53	PELP PGSAKR ALPTCTSASP PQKKKKPLDGE YFTL K IRGR K RFEMFRELNE		
hP53	HELP PGSTKR ALPNNTSSSP QPKKKPLDGE YFTL Q IRGR E RFEMFRELNE		

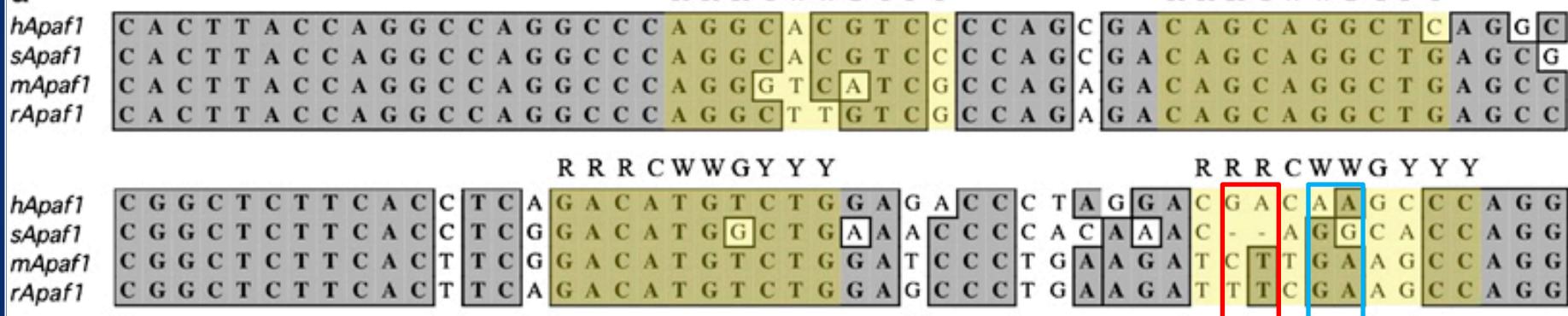
Relative transactivation of human and *Spalax Apaf1* and *Mdm2* promotores

Spalax specific amino acid changes within the p53 DNA binding domain mimic common mutations, arginine->lysine R174K and R209K, observed in tumors.

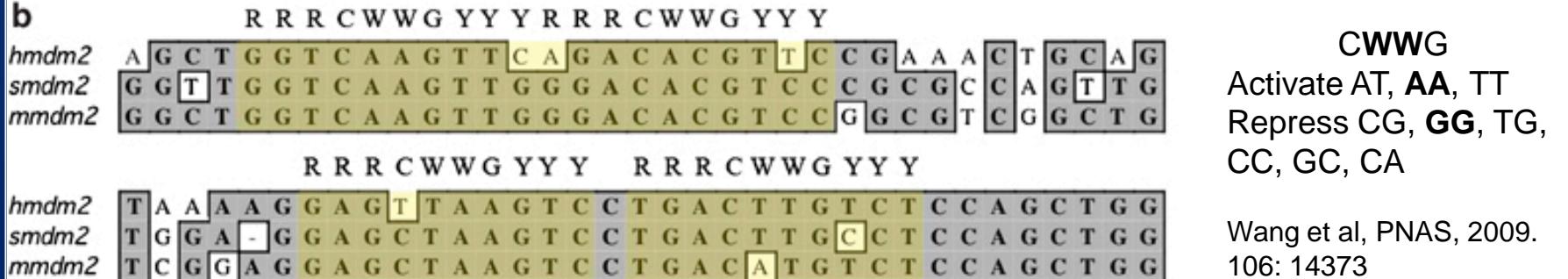


Nucleotide sequence alignment of human, *Spalax* and mouse *Apaf1* promoter regions and *Mdm2* introns containing *p53* response elements.

a



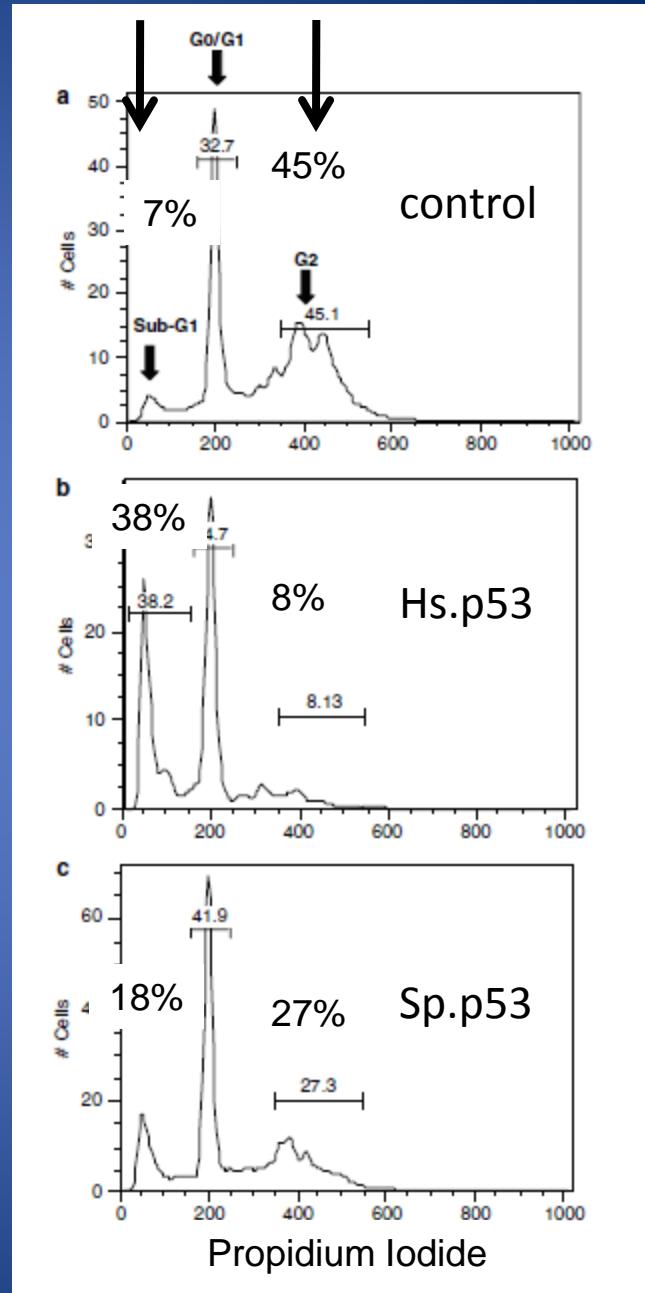
b



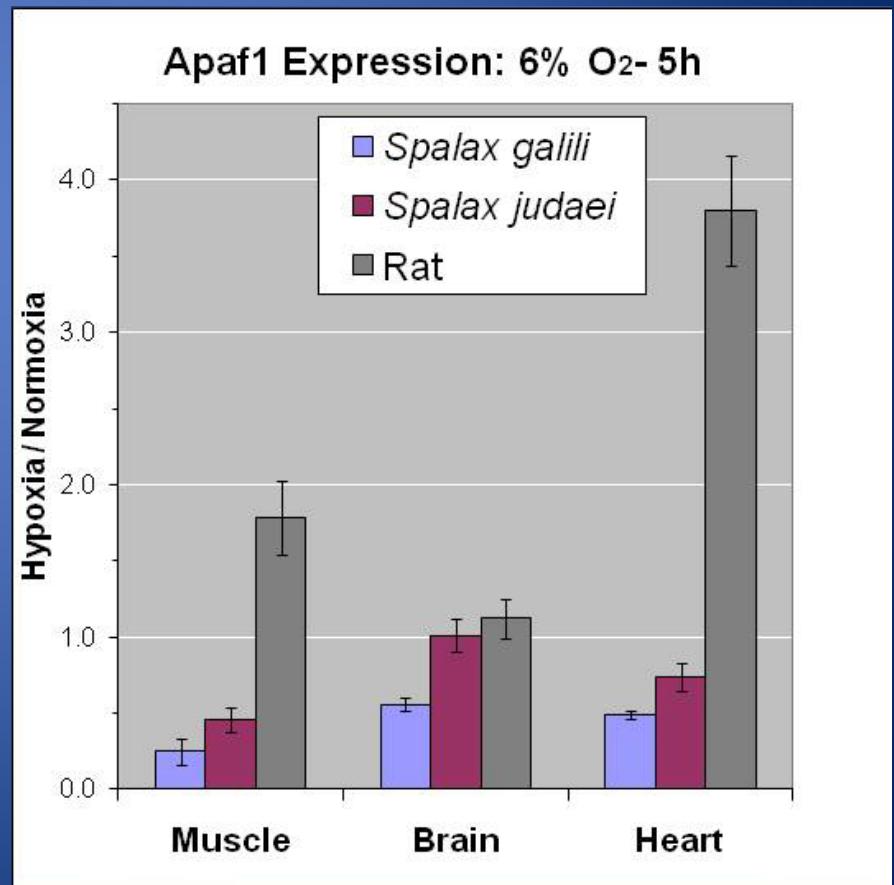
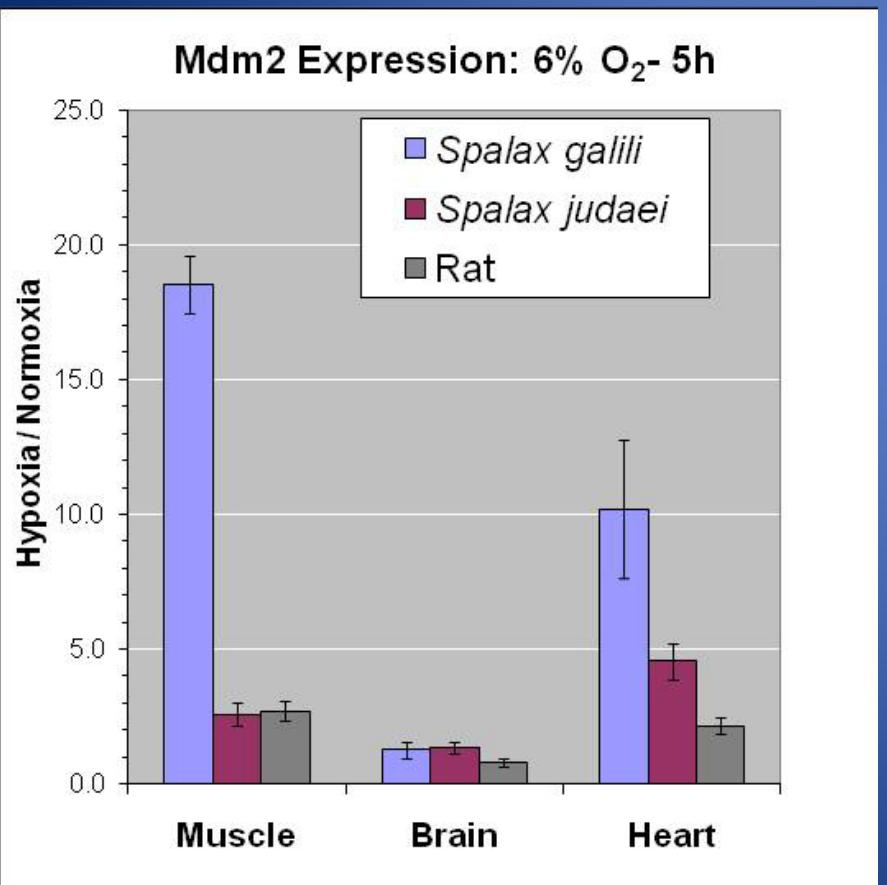
FACS analysis of transfected cells

***Spalax* p53 induces lower rates of apoptosis as compared to human WT p53**

Apoptosis Cell Division



Mdm2 and *Apaf1* transcript expression in *Spalax* and Rat tissues



DMBA-TPA treatment.



Top Left: Mice 1 week necrosis

Top Right: Mice 7 weeks developing tumors. 12 weeks, metastases in spleen, liver and kidney (not shown)

Bottom Left: *Spalax* 1 week necrosis

Bottom Center: *Spalax* 7 weeks, healing

Bottom Left: *Spalax* 9 weeks, completely healed



Conclusions:

Extreme hypoxic conditions

Evolved p53 variant analogous to cancer progression

Favors growth arrest versus apoptosis

Lys174 - critical for apoptosis inactivation

New animal model for cancer?

Transcriptome Sequencing of the Blind Subterranean Mole Rat, *Spalax galili*: Utility and Potential for the Discovery of Novel Evolutionary Patterns

Assaf Malik, Abraham Korol, Sariel Hübner, Alvaro G. Hernandez, Jyothi Thimmapuram, Shahjahan Ali, Fabian Glaser, Arnon Paz, Aaron Avivi, Mark Band

- **454 transcriptome sequencing**
- **Normalized normoxic (21% O₂) and hypoxic (3%, 10% O₂) cDNA libraries**
- **Muscle and Brain**

Discovery:

- **Splice variants, SNPs, novel transcribed regions, differential expression.**

Sequencing Stats:

	<u># reads</u>	<u>Avg. Length</u>
Sanger	7336	636 bp
FLX	846,747	218 bp
Titanium	2,358,558	351 bp

Total Assembly 48,206,674 base

Avg. Isotig Size = 1597 51,000 isotigs
N50 IsotigSize = 2491 12,000 genes

Largest Isotig 65905 (Titin)

Novel inserts-example Sec23a

Function: ER-Golgi protein trafficking

Regulation: p53 SRY ATF6

Sec23a (isotig20294)

S	tgtcctaattctcaactggcagggtctccagctggactgg//cagtgttggatcaaagaggatacatggtaatgggtgac
M	tgtcctaacccttactg-----//-----gaggatacatggtaatgggtgac
R	tgccttaacccttactg-----//-----gaggatacatggtaatgggtgac
H	tgtcccaacccttactg-----//-----gaggatacatggtaatgggtgat

S	..C..P..N..L..T..G..R..V..S..Q..L..D..W//.Q..C..W..D..Q..R..G..Y..M..V..M..G..D
M	..C..P..N..L..T.-----//-----G..G..Y..M..V..M..G..D
R	..C..P..N..L..T.-----//-----G..G..Y..M..V..M..G..D
H	..C..P..N..L..T.-----//-----G..G..Y..M..V..M..G..D

Comparison of sequence with mouse, rat, human shows splice variant with unique insert, confirmed by RT-PCR and resequencing of 360 base across the region in Spalax and Rat.

96 bp insert in coding sequence parallel to 7129 bp human intron.

In humans F382L amino acid substitution in SEC23A causes skeletal deformations.

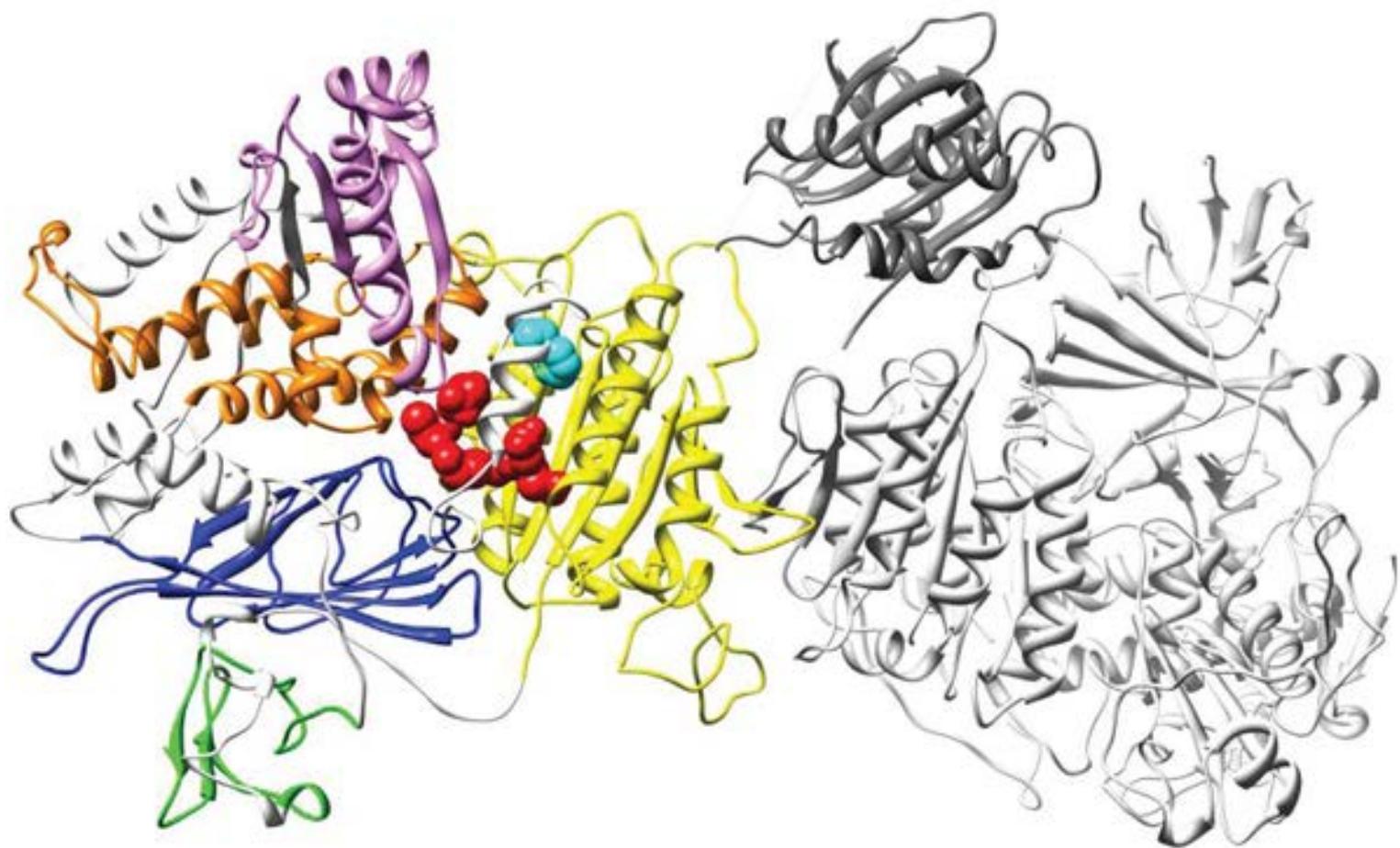


Figure 4. A model of the *S. galil* sec23a/sec24 hetero-dimer, color-coded by functional domains.

Differential Expression- Muscle

Pooled 3% and 10% vs. Normoxic

Isotig name	Normoxic	Hypoxic	FDR	Gene name
isotig03675	144	1926	3.1E-183	lipin 1
isotig22428	159	1847	7.0E-159	xin actin-binding repeat containing 1
isotig16056	31	693	1.8E-85	ERBB receptor feedback inhibitor 1
isotig03993	29	554	1.2E-63	G protein-coupled receptor 157
isotig05949	185	1273	8.5E-63	glutamate-ammonia ligase (glutamine synthetase)
isotig32854	19	391	2.6E-46	OTU domain containing 1
isotig05974	42	518	5.4E-46	syndecan 4
contig57880	3872	11750	6.1E-43	titin
isotig23386	5	273	5.2E-42	similar to hypothetical protein MGC6835
isotig01962	37	454	4.0E-40	ankyrin repeat domain 1 (cardiac muscle)
isotig01944	167	952	1.7E-34	splicing factor, arginine/serine-rich 3
isotig33484	37	417	1.9E-34	leucine rich repeat containing 30
isotig08700	28	356	3.4E-32	FK506 binding protein 5
isotig19475	10	249	1.0E-31	forkhead box O1
isotig18032	16	282	5.4E-31	activating transcription factor 3
isotig06808	23	321	6.4E-31	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
contig11513	12	250	1.1E-29	elongation factor RNA polymerase II 2
isotig14054	40	394	5.1E-29	regulator of calcineurin 1
isotig00986	81	567	1.9E-28	ubiquitin interaction motif containing 1
isotig13244	59	464	5.6E-27	nuclear receptor interacting protein 1
contig35926	22	286	3.1E-26	stomatin; ABO-family member 5
isotig41385	39	368	5.3E-26	jumonji domain containing 1C
isotig11426	30	323	1.1E-25	bestrophin 3

Agilent Spalax earray
8 x 15K (13200 unique genes)

Muscle and Brain

21% O₂ (Normoxia)

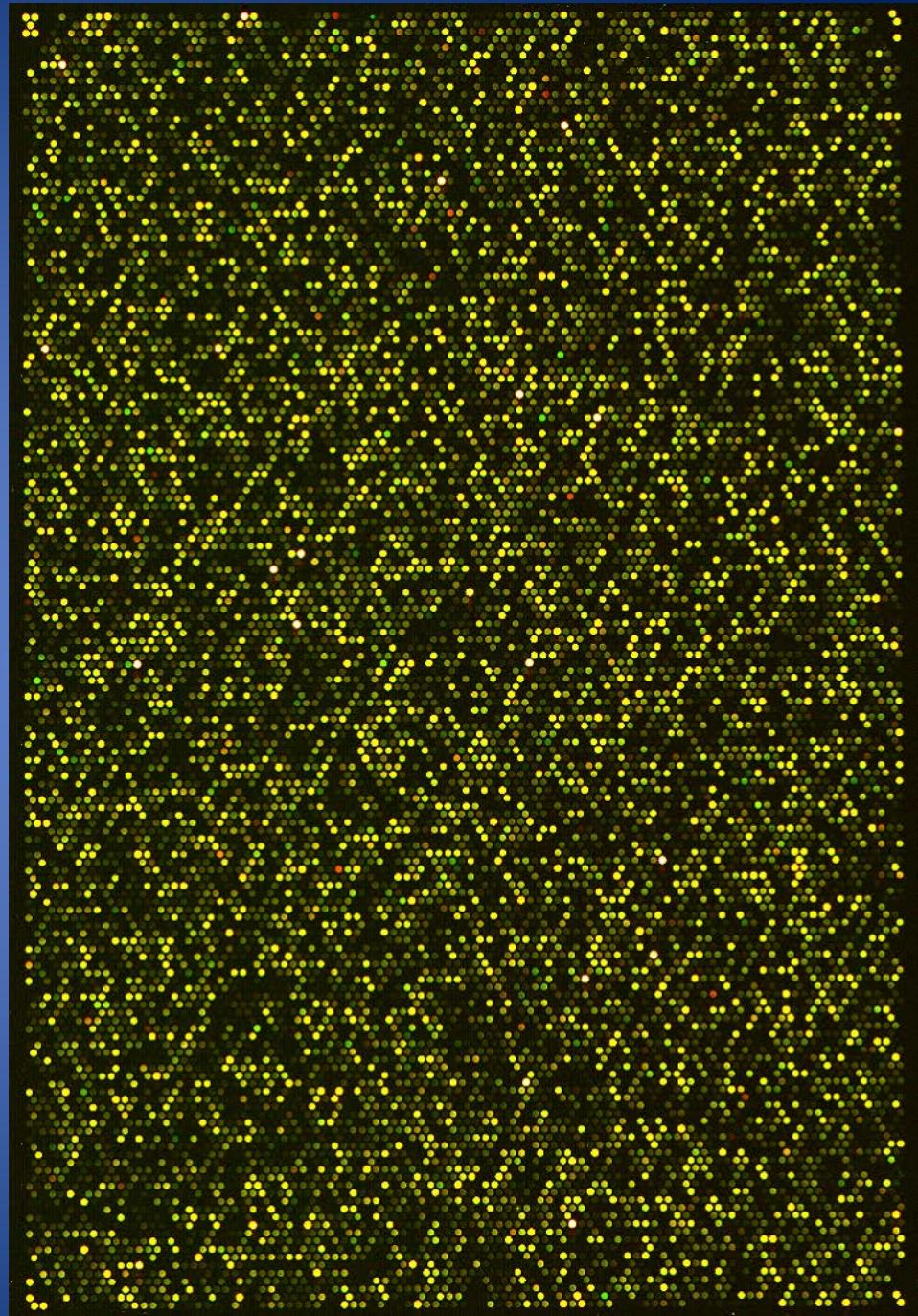
3% O₂, 5 hours

6% O₂, 8 hours

10% O₂, 44 hours

N=4 for each Tissue x Treatment

1660 response to hypoxia (FDR 0.05)



Conclusions:

Model for hypoxia tolerance

Cancer resistance

Full genome sequencing- identification of regulatory regions

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