Macromolecole della risposta alla pressione parziale di ossigeno (3)

In mammals, O2 sensing leads to **chronic (days …weeks)** adaptation

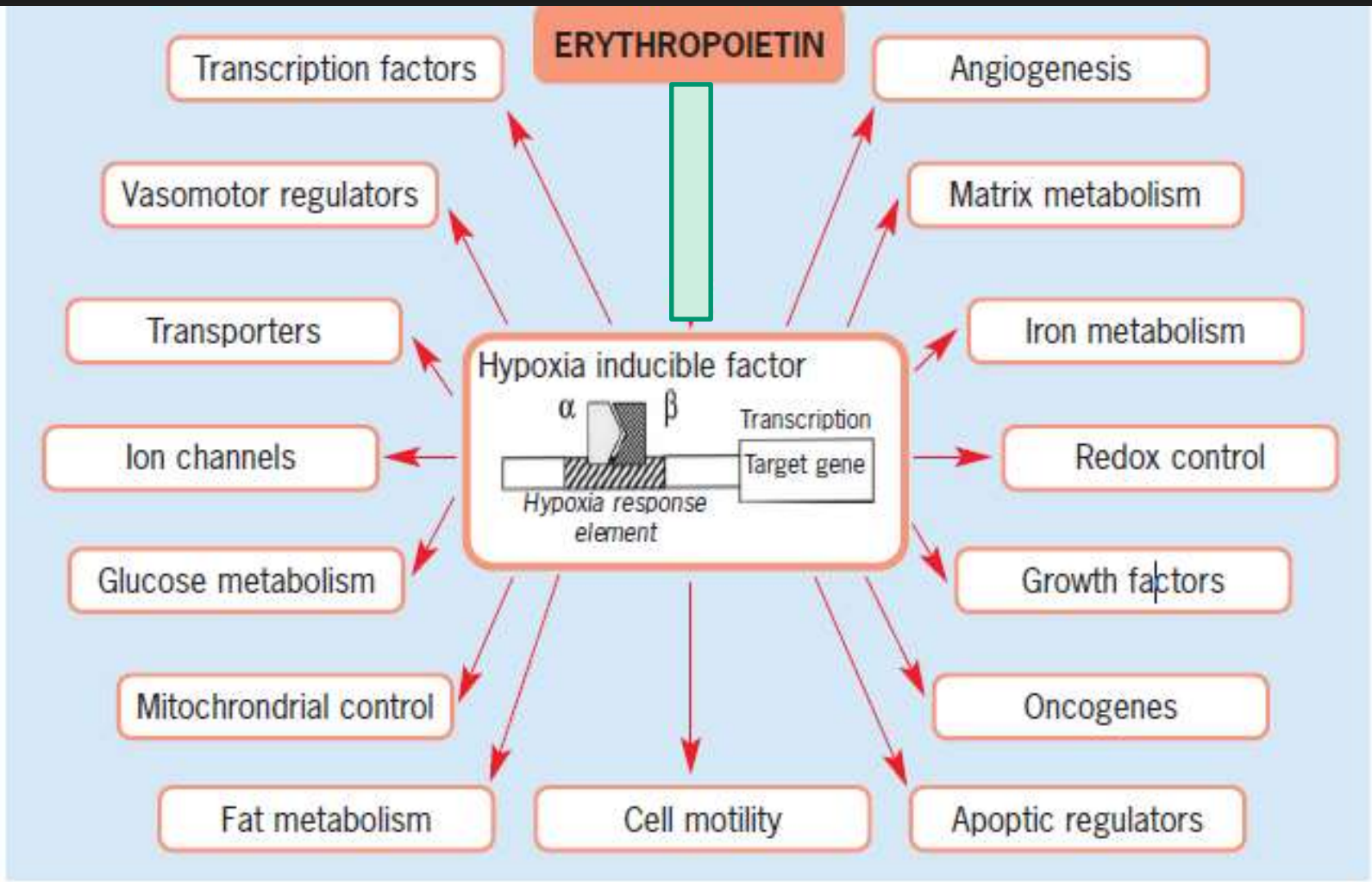
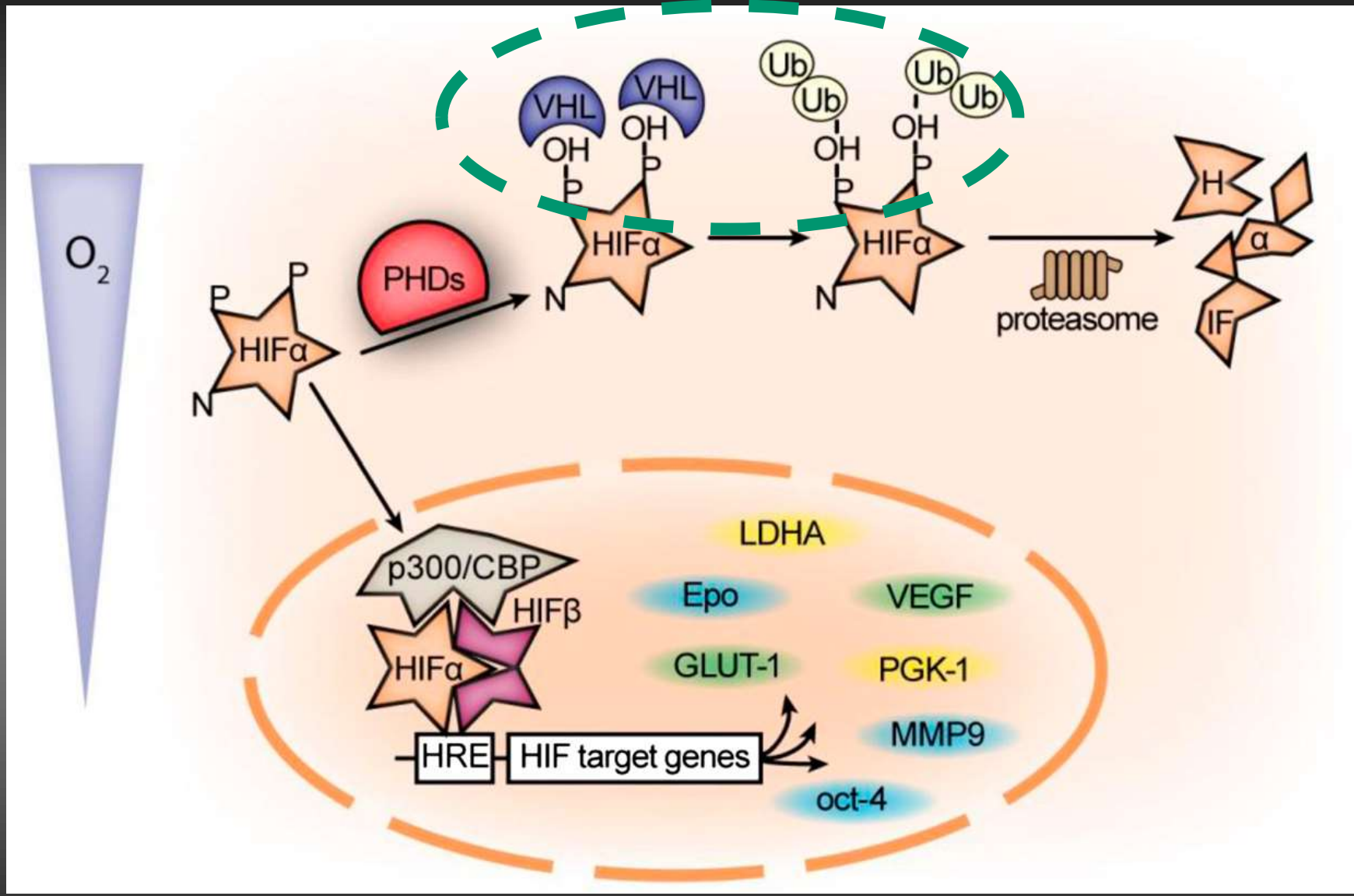
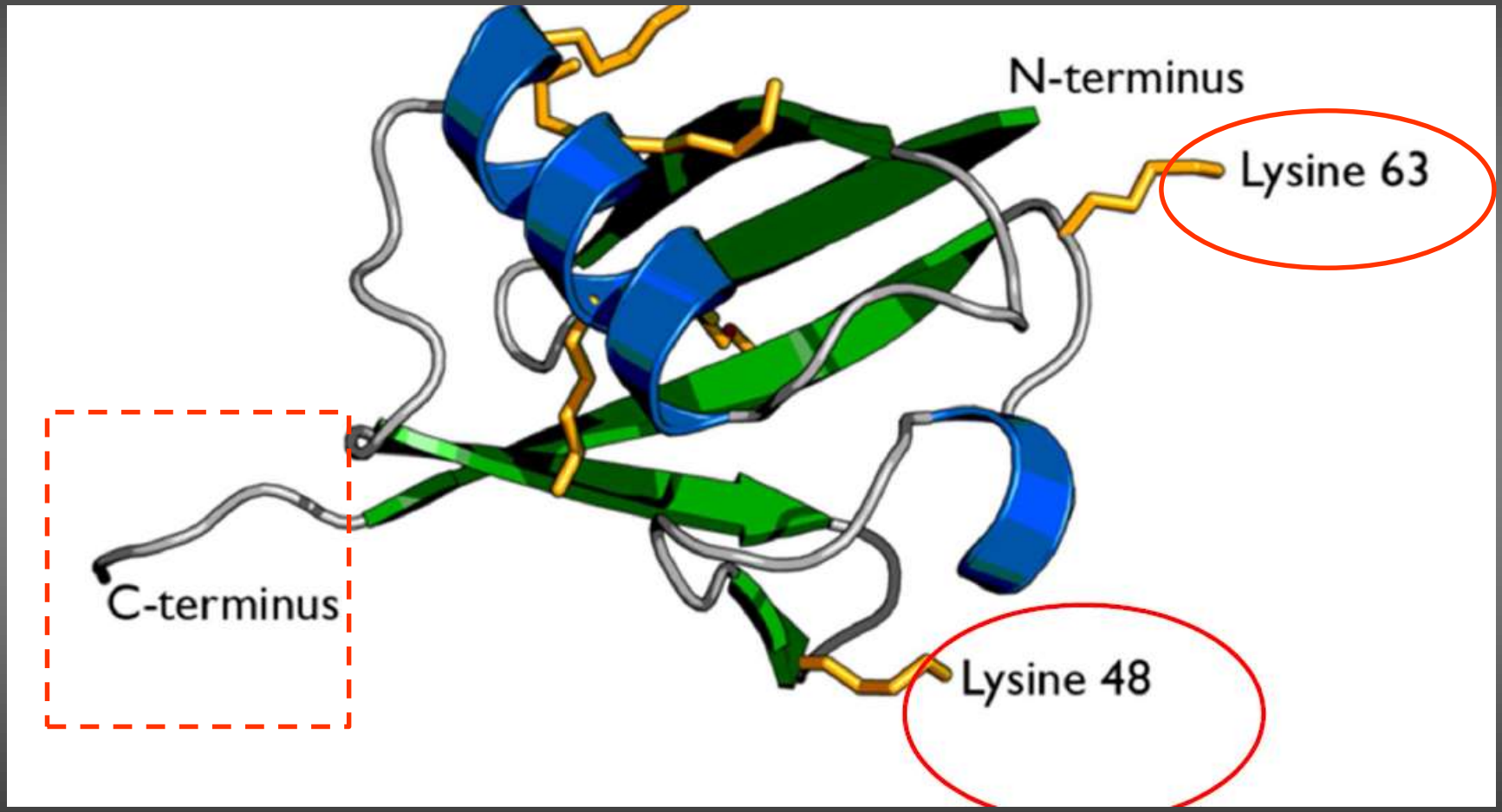


Fig 1. The hypoxia-inducible factor (HIF) transcriptional cascade directly regulates genes with key functions in a broad range of processes. The complex binds in a sequence-specific manner to control elements in DNA, termed hypoxia-response elements, at target gene loci.

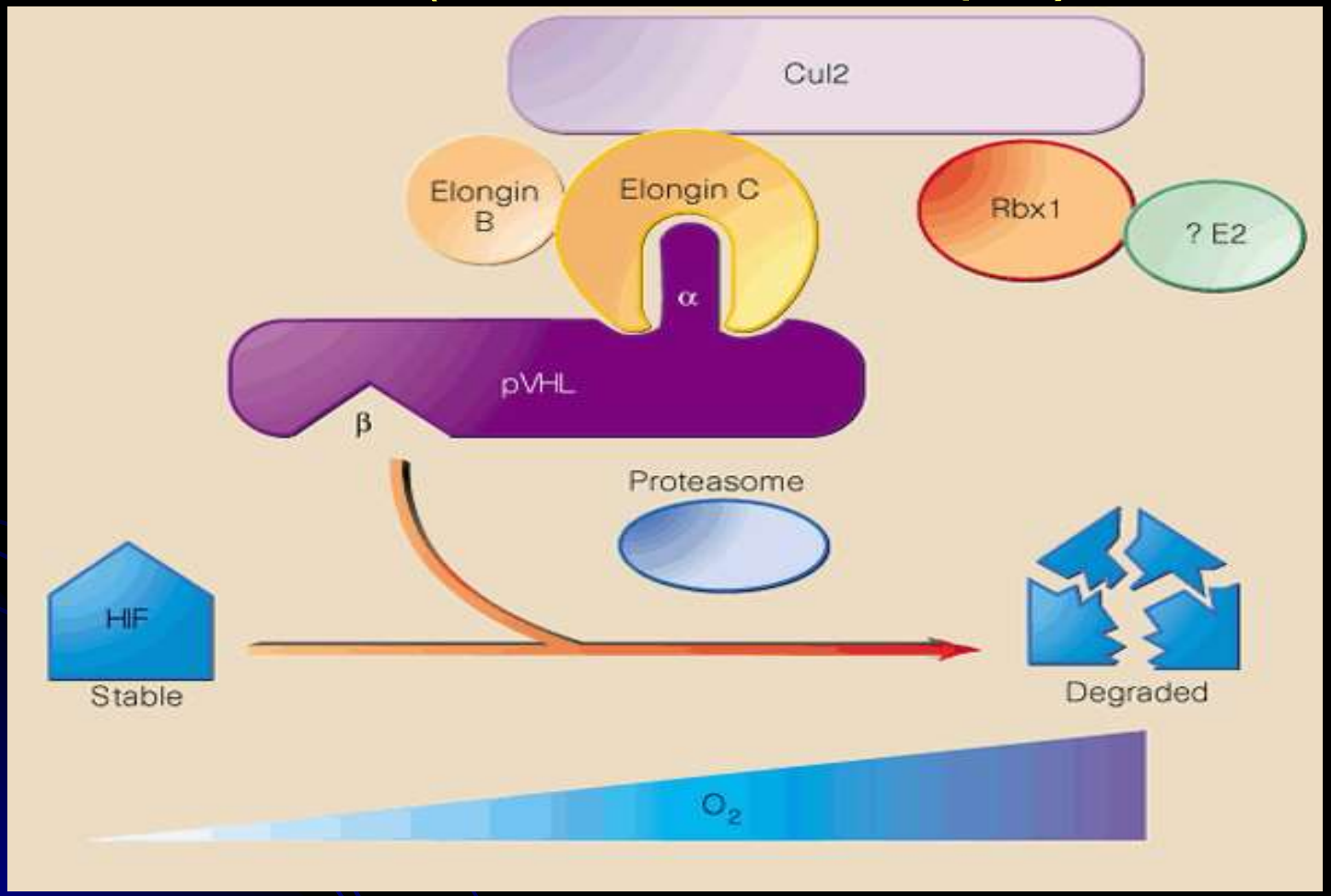

O2
VHL
VHL
OH
OH
P
P
HIFα
N
Ub
Ub
Ub
Ub
OH
OH
P
P
HIFα
N
H
α
IF
proteasome
PHDs
P
P
HIFα
N
p300/CBP
HIFβ
HIFα
HRE
HIF target genes
LDHA
Epo
VEGF
GLUT-1
PGK-1
MMP9
oct-4

Quesiti

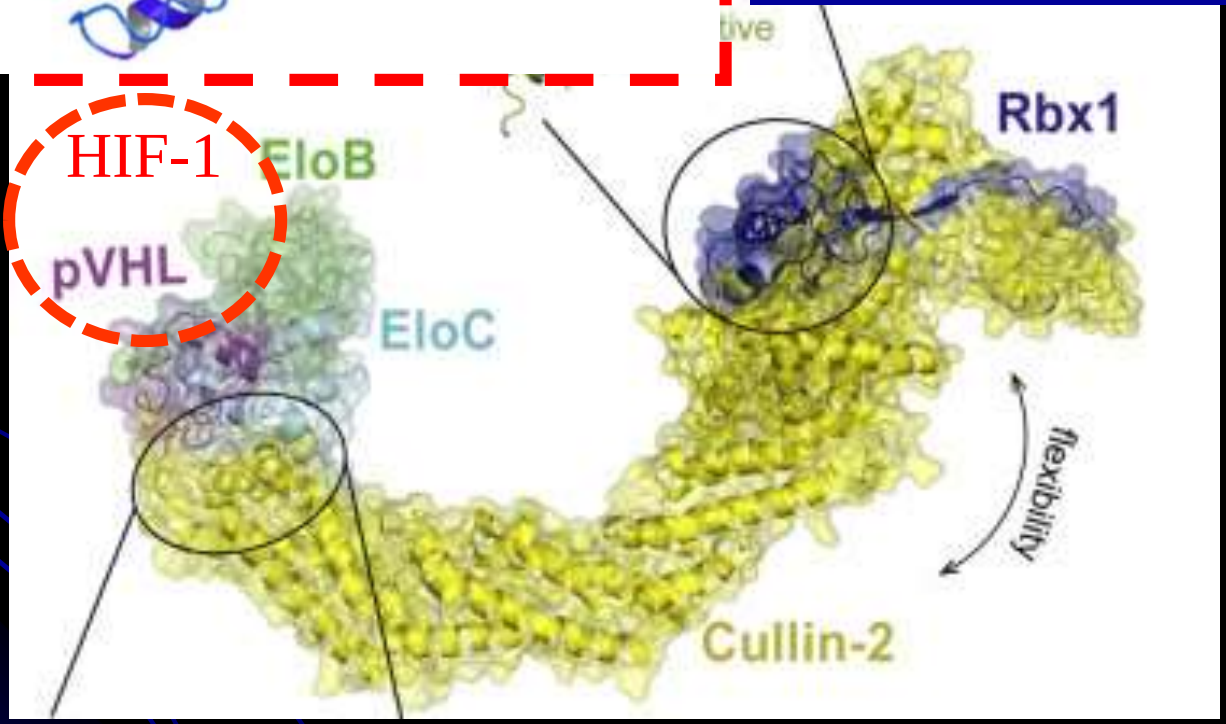
UBIQUITINA



VHL Function: the difference in kd for hydroxylated versus non-hydroxylated CODD is ~1,000-fold (33 nM versus 34 μM)

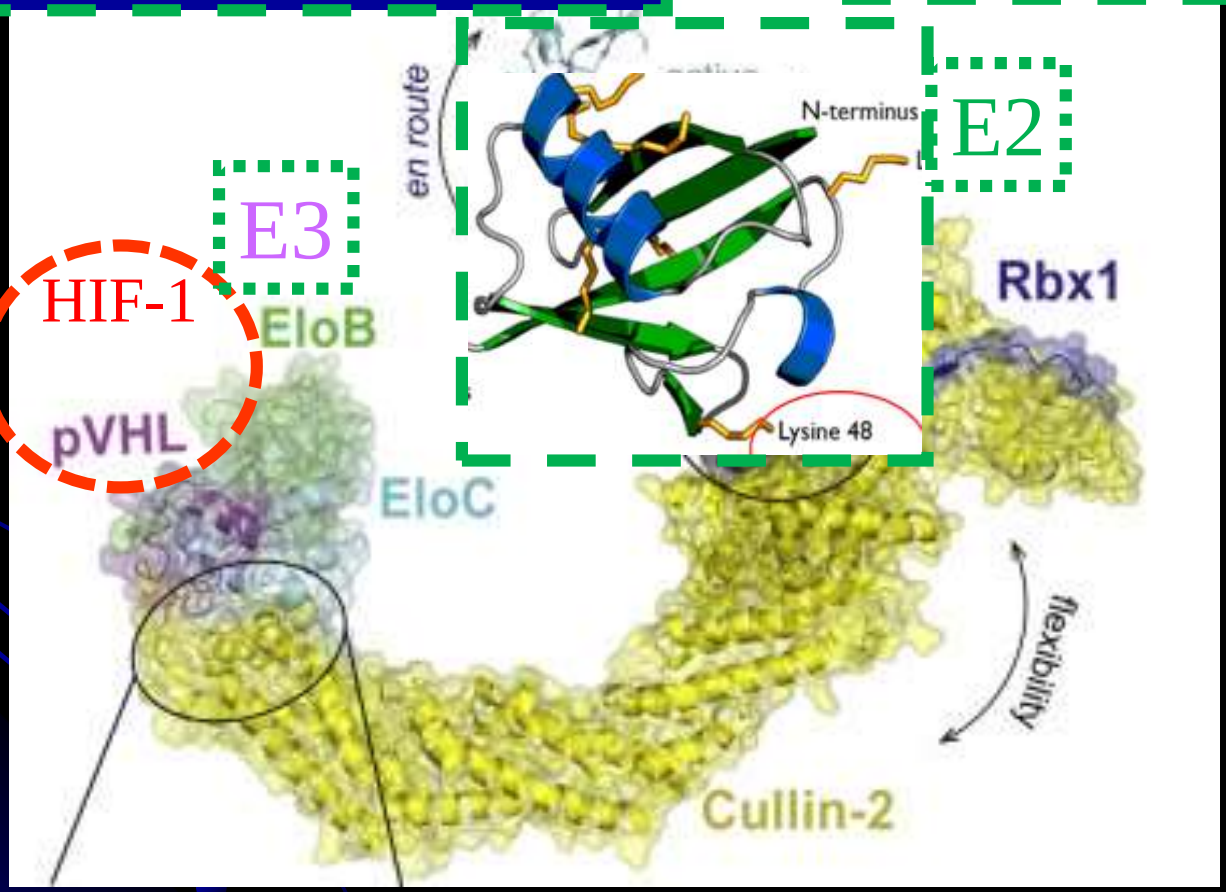


Cul2 recruits pVHL at its N-terminal region through an adaptor subunit constituted by a dimeric complex formed by Elongin B (EloB) and Elongin C (EloC)

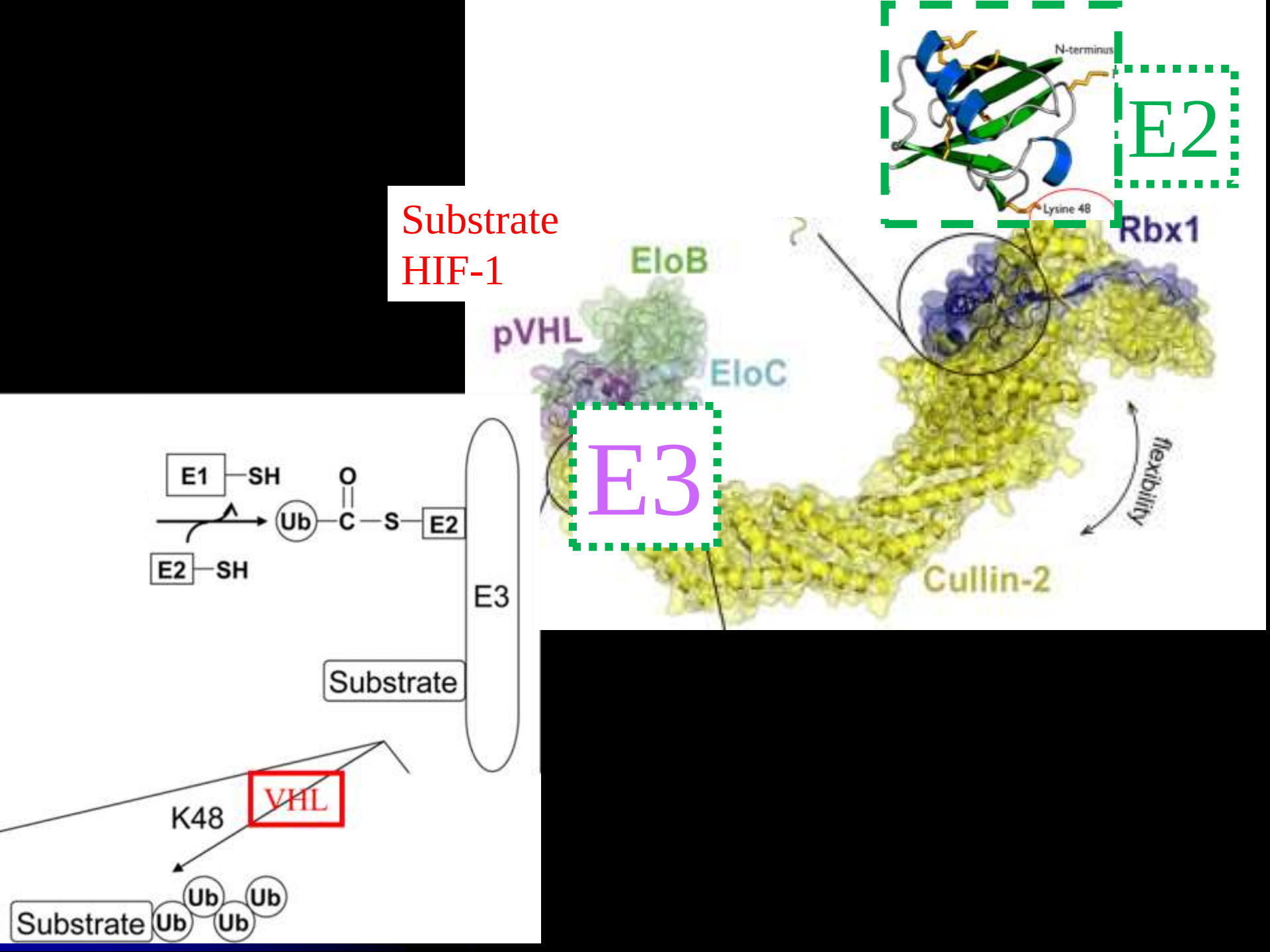


Cullin RING E3 ubiquitin ligases catalyze the transfer of ubiquitin from the E2-conjugating enzyme to the target substrate

the E2 enzyme loaded with ubiquitin is recruited by the RING finger protein Rbx1

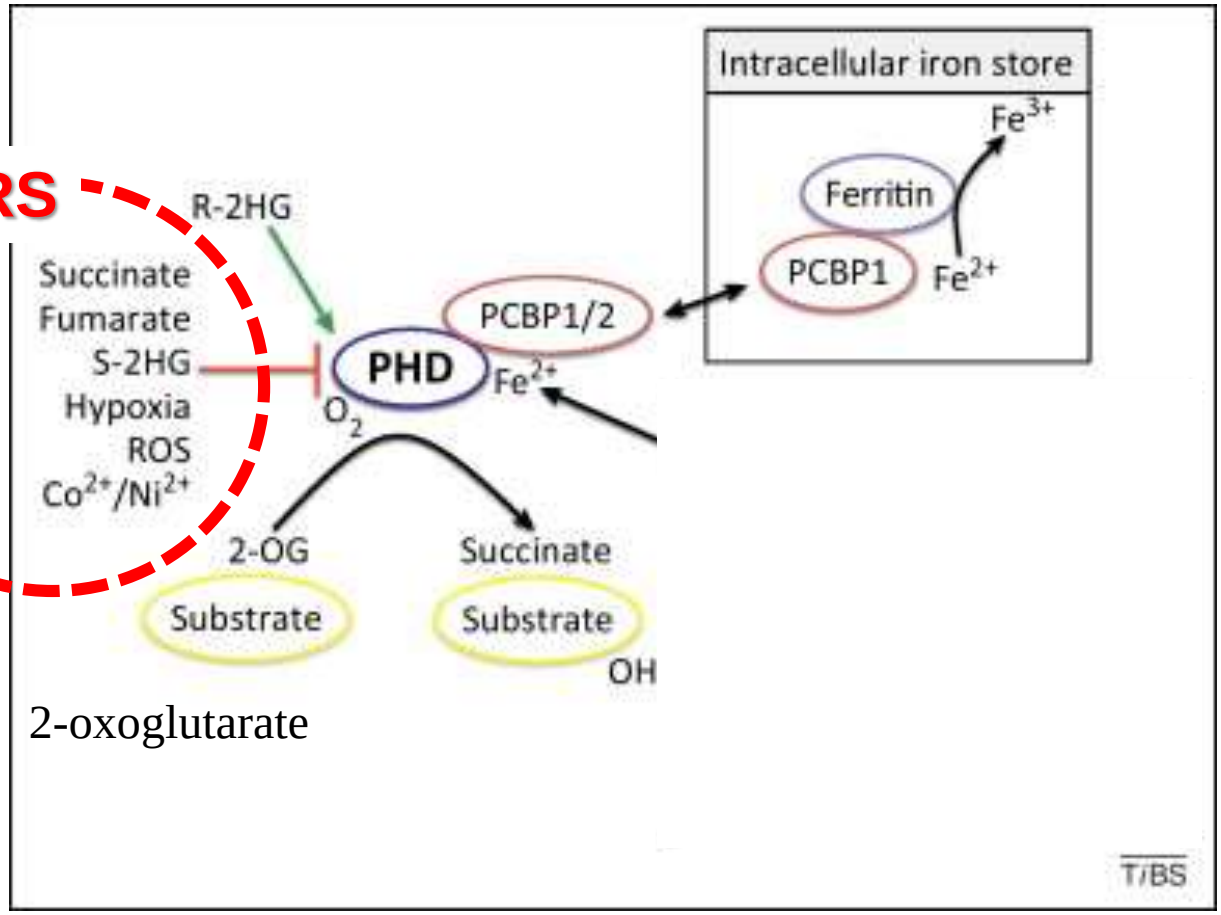


Substrate
HIF-1



Quesiti

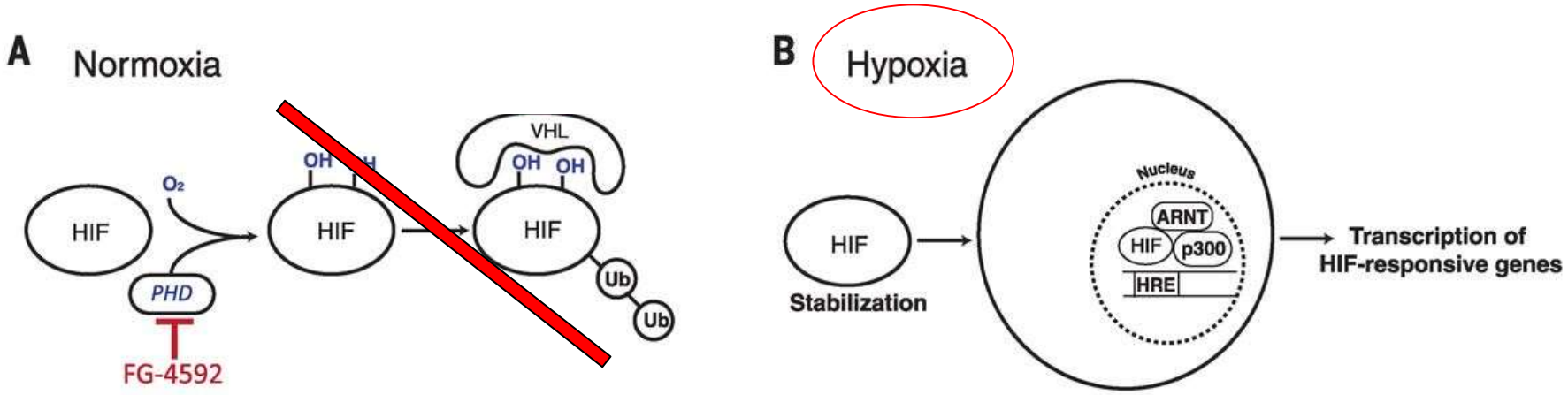
Regulation of prolyl hydroxylase domain (PHD) enzyme activity



g **Emerging novel functions of the oxygen-sensing prolyl hydroxylase domain enzymes** Brian W. Won

INIBITORI FARMACOLOGICI DI PHD

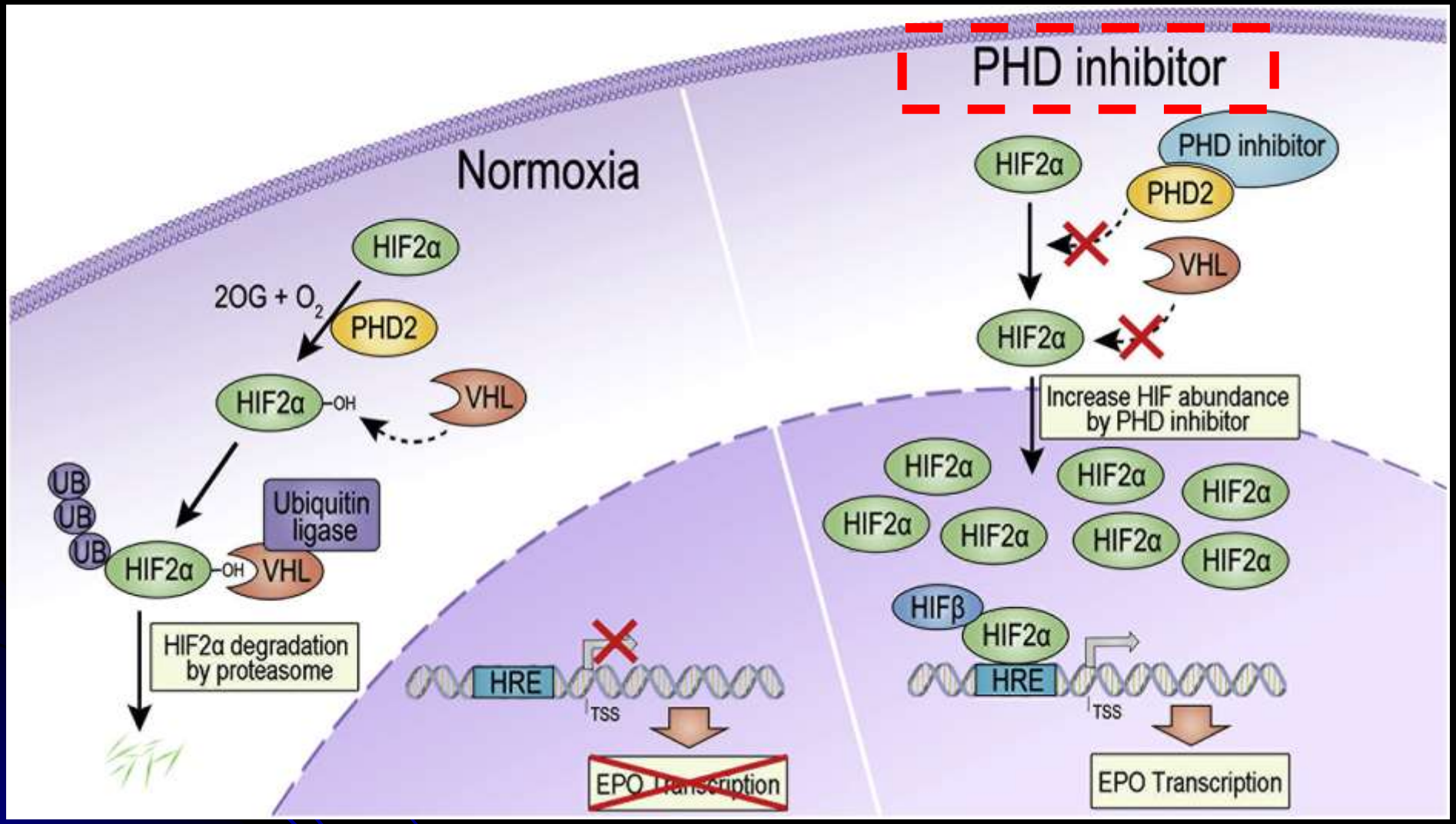
activation of the HIF response by PHD inhibition



FG-4592 acts as competitive antagonists of 2-oxoglutarate, a cofactor that accepts one oxygen from molecular dioxygen to become succinate as the second oxygen forms trans-4-hydroxyproline

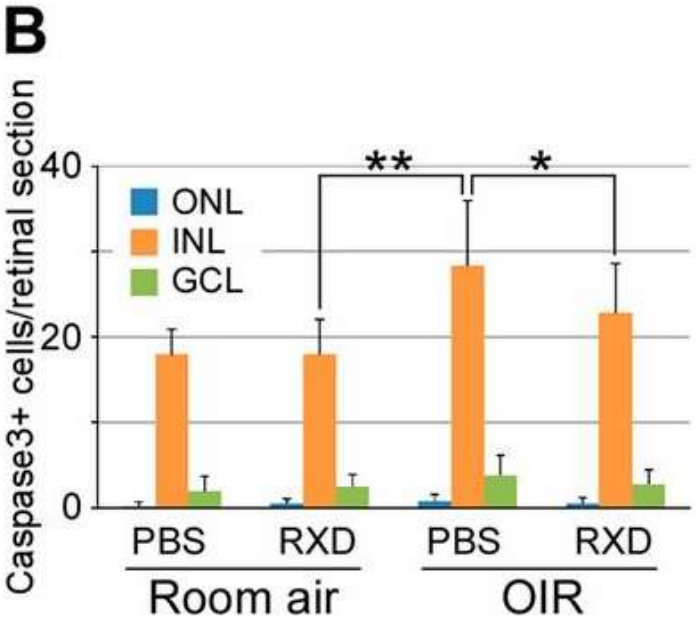
Published by AAAS

Isha H. Jain et al. Science 2016;352:54-61


PHD inhibitor
Normoxia
HIF2α
2OG + O2
PHD2
HIF2α -OH
VHL
UB
UB
UB
Ubiquitin ligase
HIF2α -OH VHL
HIF2α degradation by proteasome
HRE
TSS
EPO Transcription
PHD inhibitor
HIF2α
PHD2
VHL
HIF2α
Increase HIF abundance by PHD inhibitor
HIF2α
HIF2α
HIF2α
HIF2α
HIF2α
HIF2α
HIF2α
HIFβ
HIF2α
HRE
TSS
EPO Transcription

Effect of Roxadustat on neural retina apoptosis.

outer nuclear layer (ONL),
inner nuclear layer (INL)
ganglion cell layer (GCL)

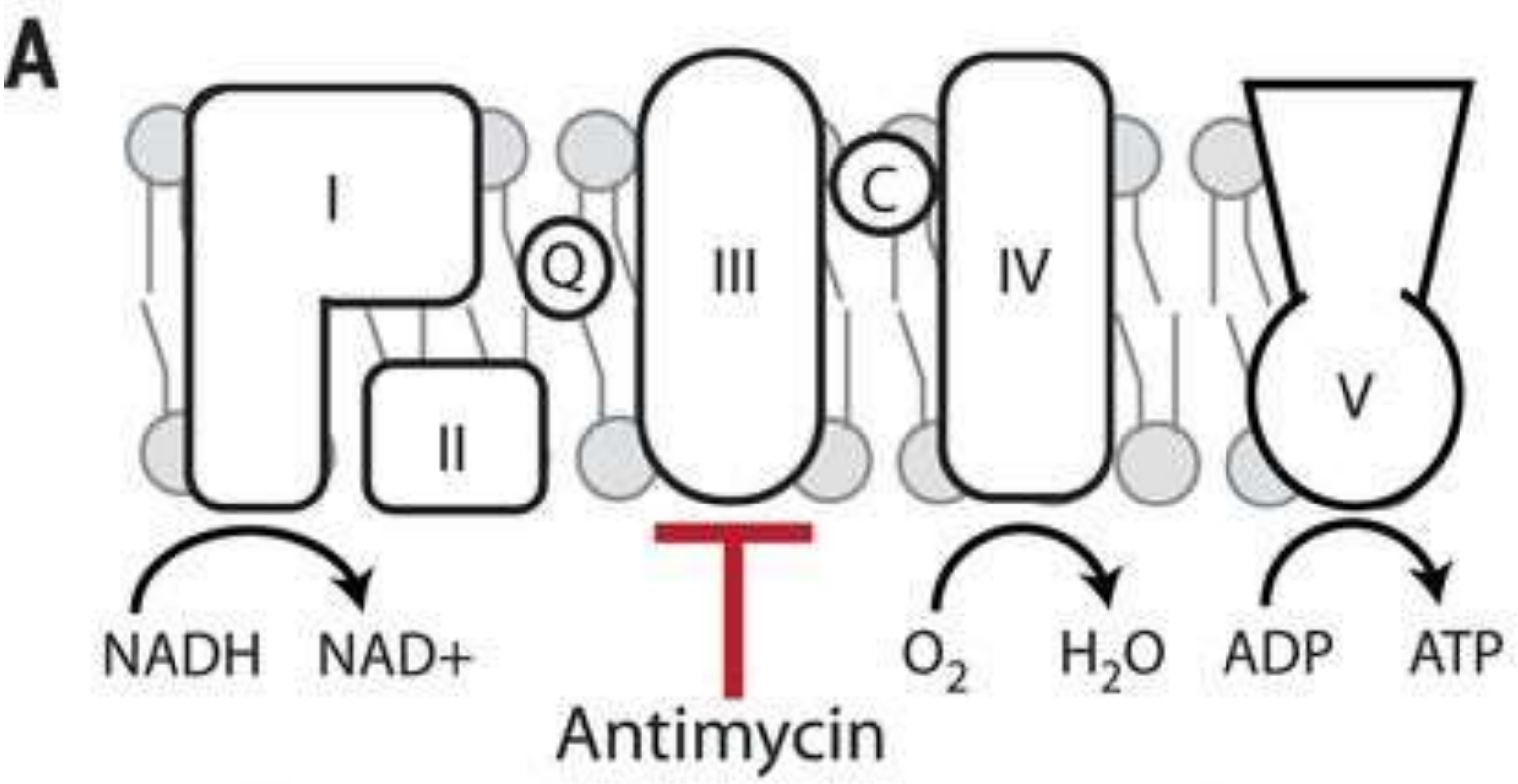


RXD?
+EPO
-Apoptosis

Quantification of active caspase 3-positive cells demonstrates statistically significant reduction in apoptosis in the inner nuclear layer of animals treated with Roxadustat (RXD) FG-4592

George Hoppe et al. PNAS 2016;113:E2516-E2525

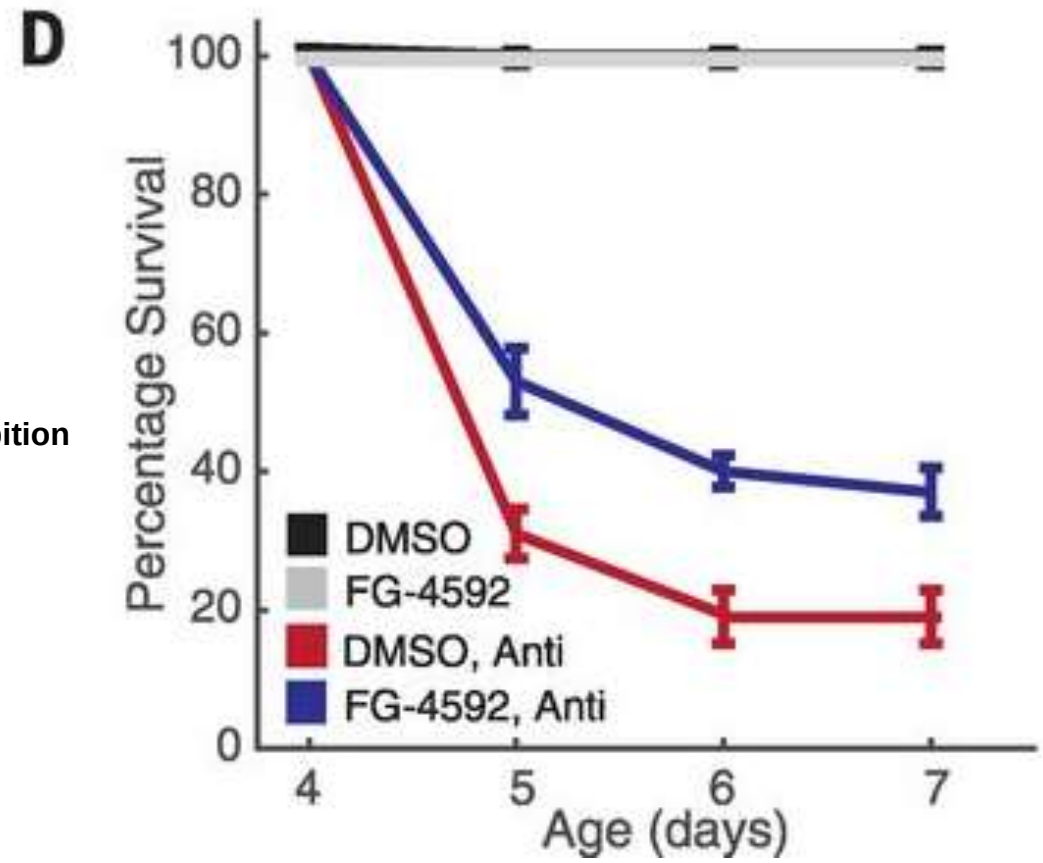
Respiratoty Chain inhibition



Isha H. Jain et al. Science 2016;352:54-61

FG-4592 treatment activates the HIF response in zebrafish embryos and alleviates death caused by Respiratoty Chain inhibition.

Isha H. Jain et al. Science 2016;352:54-61



Antimycin=Anti

Respiratoty Chain inhibition

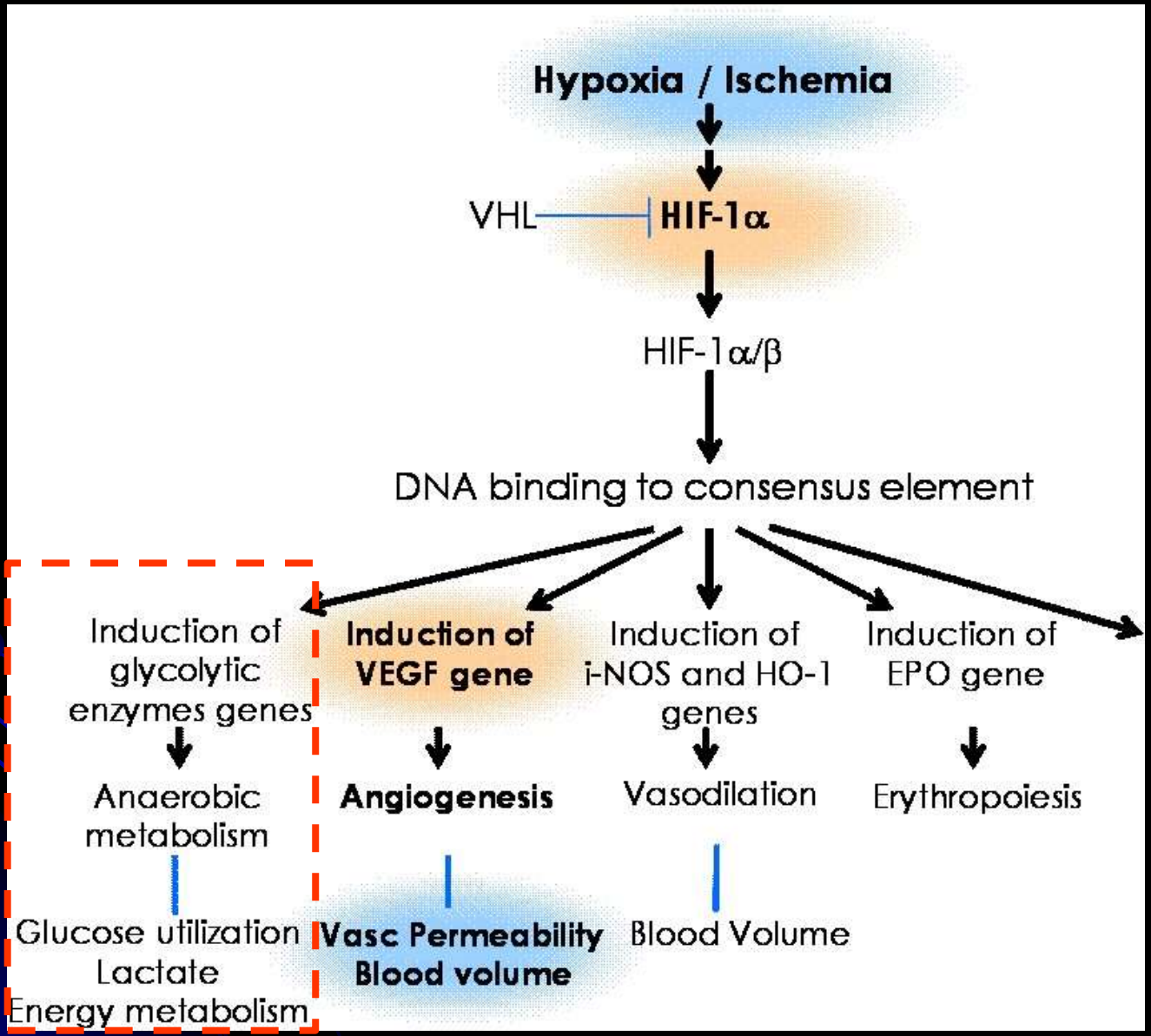
RC inhibition by 2.5 nM antimycin in 4 days post fertilization (dpf) embryos results in significant death within the first 24 hours of treatment

Coexposure of antimycin with FG-4592 (2.5 µM) doubles embryo survival, whereas FG-4592 alone has no impact.

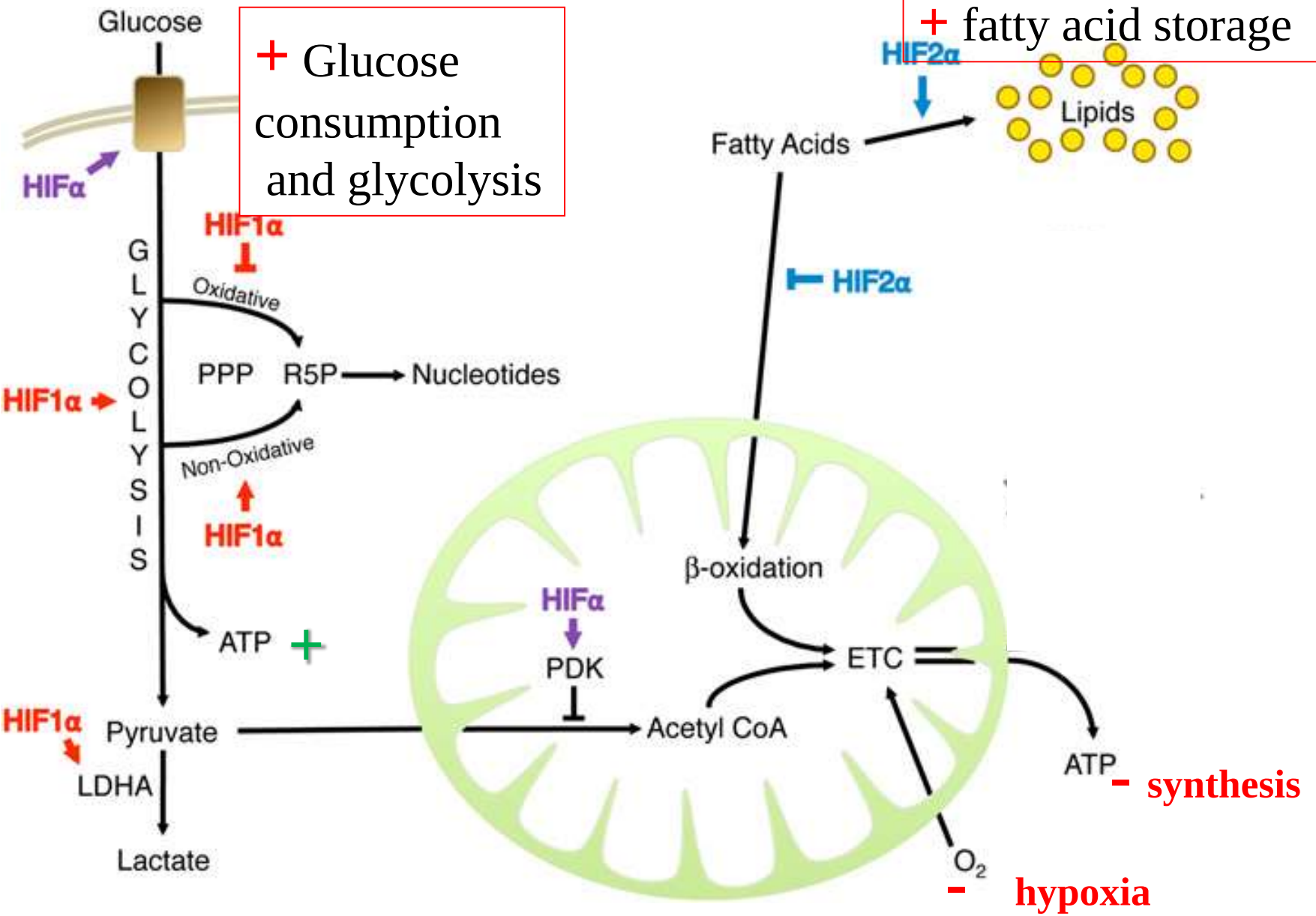
Exposure to FG-4592 rescues antimycin-induced zebrafish embryonic death.

HIF Metabolismo e Mitocondrio

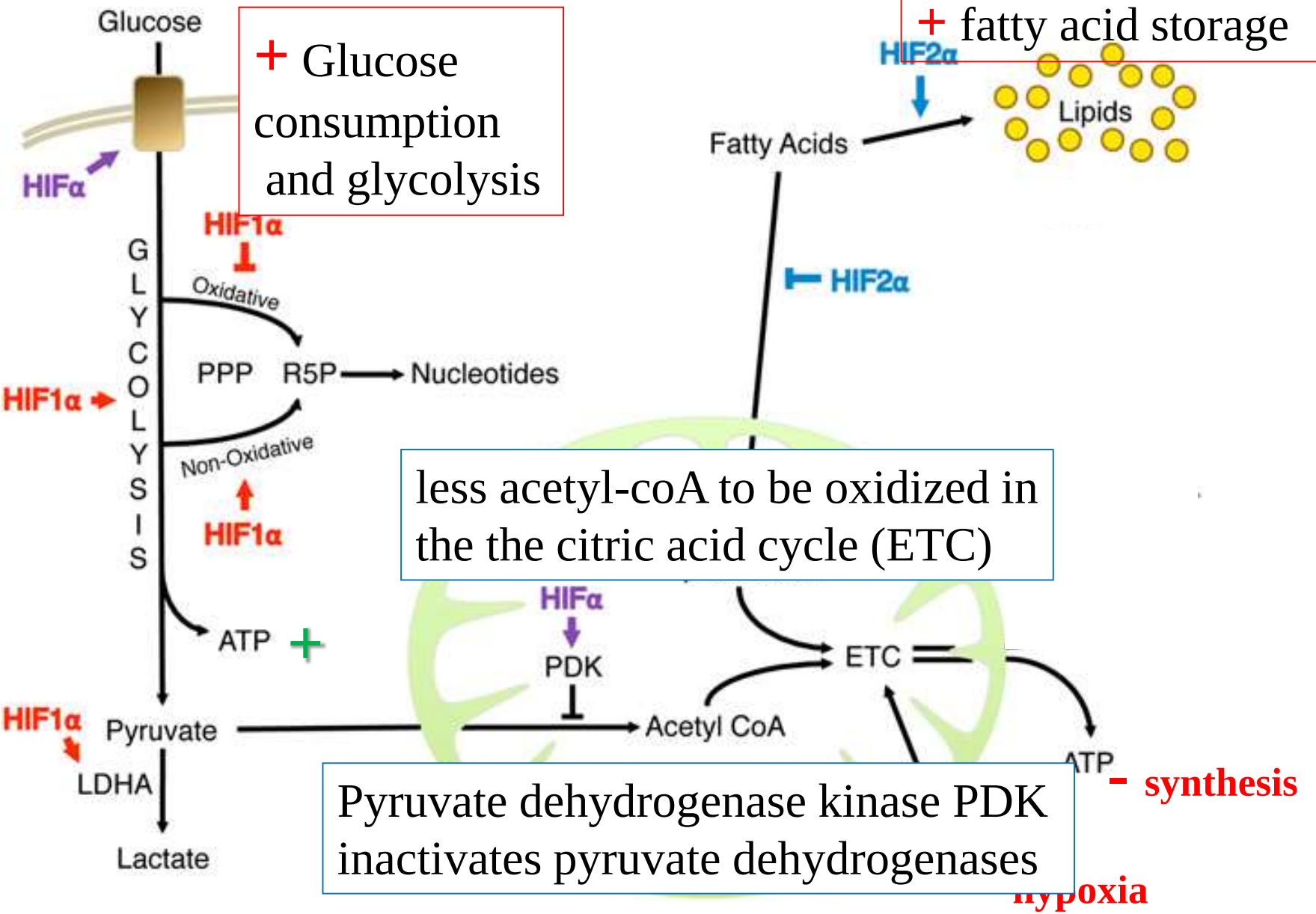
HIF transcription factor effects



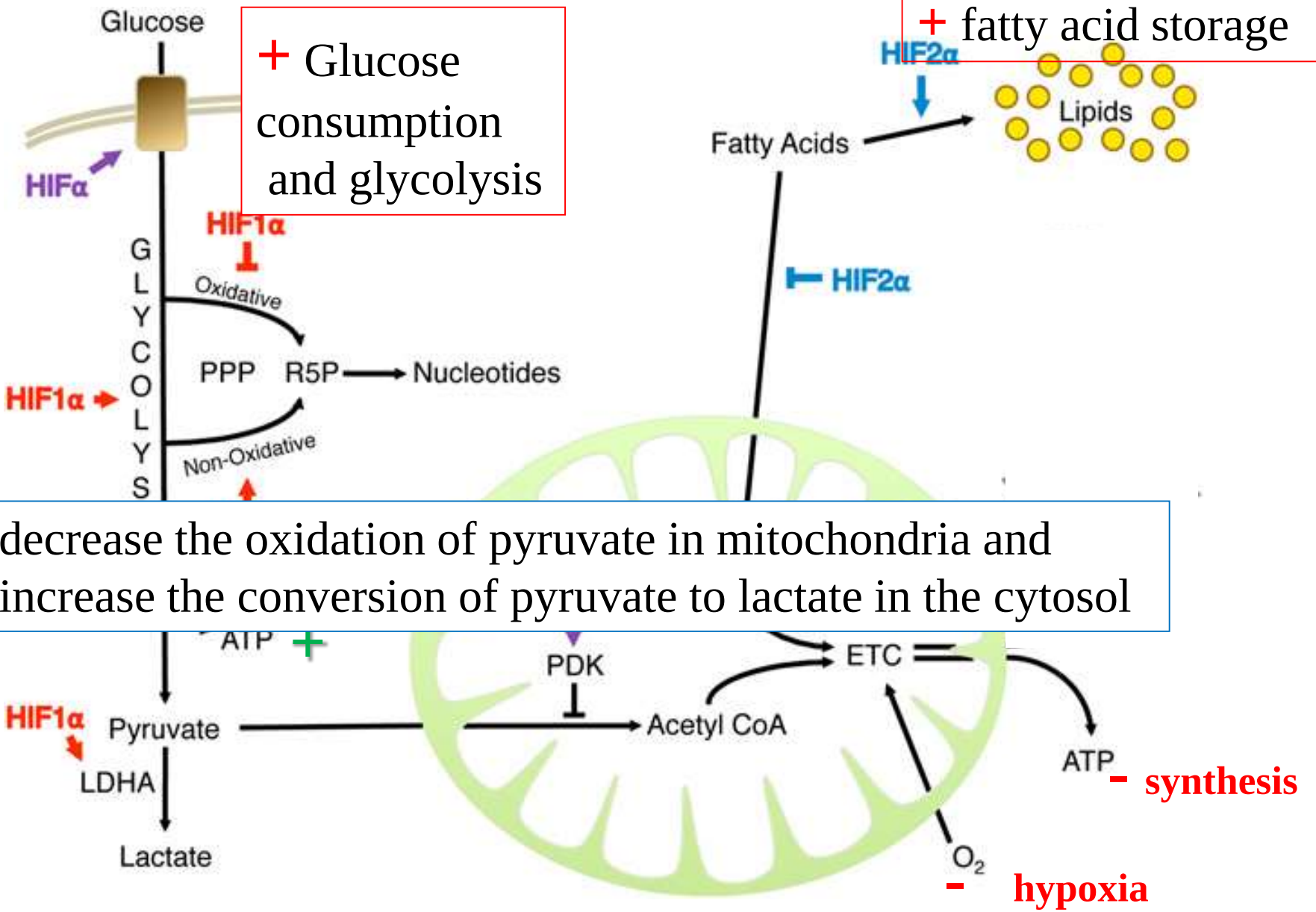
HIFa Control of Cell Metabolism



HIFa Control of Cell Metabolism



HIFa Control of Cell Metabolism



How to model Mitochondrial disease

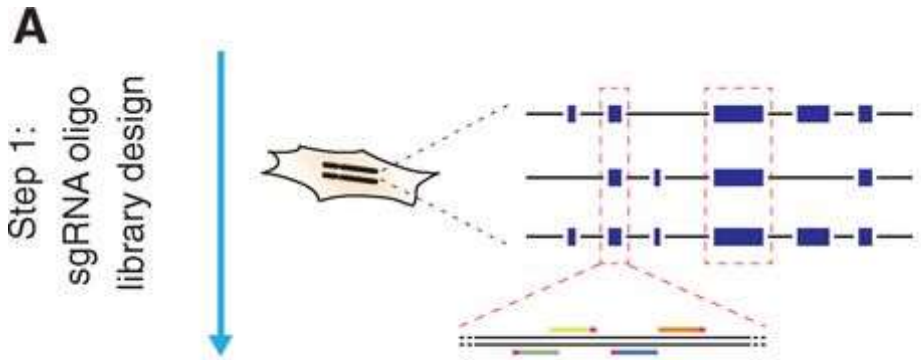
Delivery of Cas9 and sgRNA provides efficient depletion of target genes

synthetic single-guide RNA (sgRNA) targeted to specific coding regions of genes

programming the CRISPR (clustered regularly interspaced short palindromic repeats)–associated nuclease Cas9 to modify specific genomic loci

Ophir Shalem et al. Science 2014;343:84-87

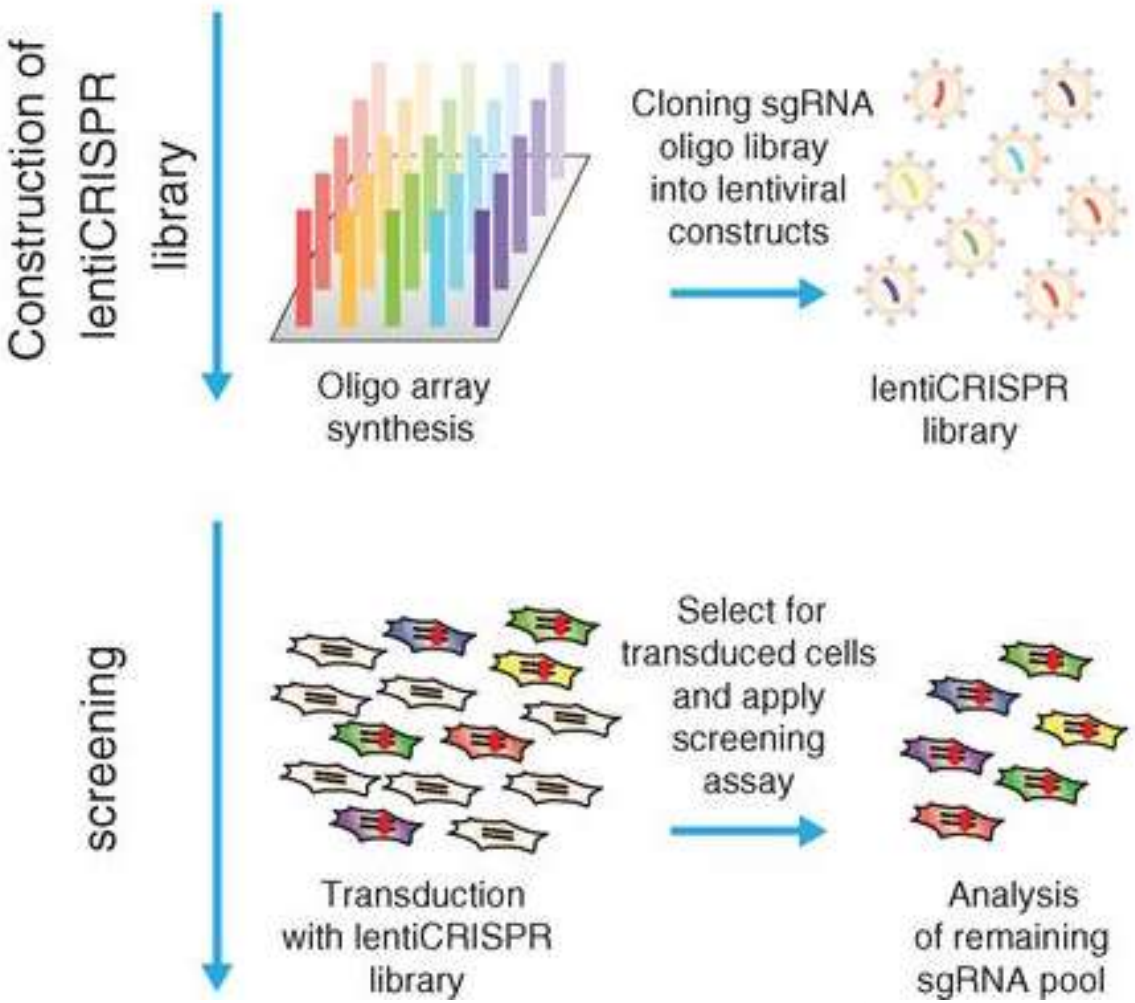
Design of sgRNA library for genome-scale knockout of coding sequences in human cells



CRISPR library sgRNAs targeting exons of 18,080 genes in the human genome with an average coverage of 3 to 4 sgRNAs per gene

Ophir Shalem et al. Science 2014;343:84-87

genome-scale screening

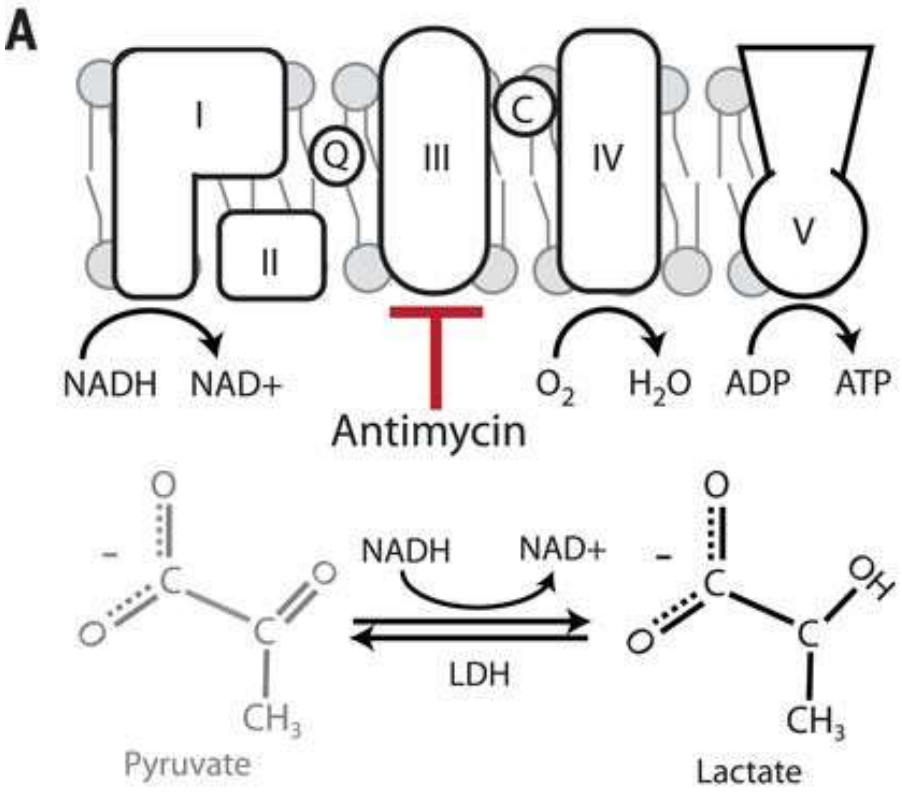


negative selection screening

PCR amplification «sgRNAs» HiSeQ

Ophir Shalem et al. Science 2014;343:84-87

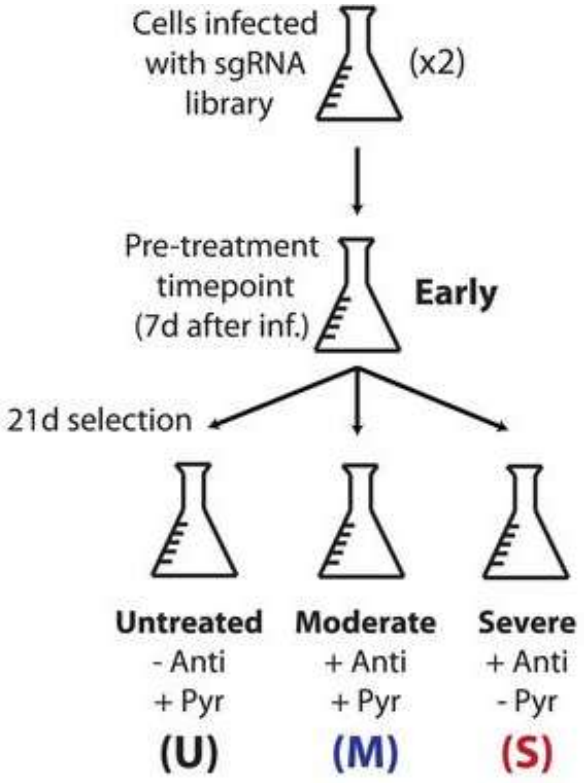
Genome-scale Cas9-mediated knockout screen during states of mitochondrial dysfunction.



Mitochondrial disease was modeled with the addition of the complex III inhibitor antimycin (moderate disease)
addition of antimycin and **removal of pyruvate (severe disease S).**

Science
AAAS

Genome-scale Cas9-mediated knockout screen during states of mitochondrial dysfunction.



cells were infected with the genome-scale Cas9-mediated knockout library

3 conditions U untreated, M moderate disease, S severe disease

Samples were taken at a **pretreatment time point and after 3 weeks of selection**

Science AAAS

Isha H. Jain et al. Science 2016;352:54-61

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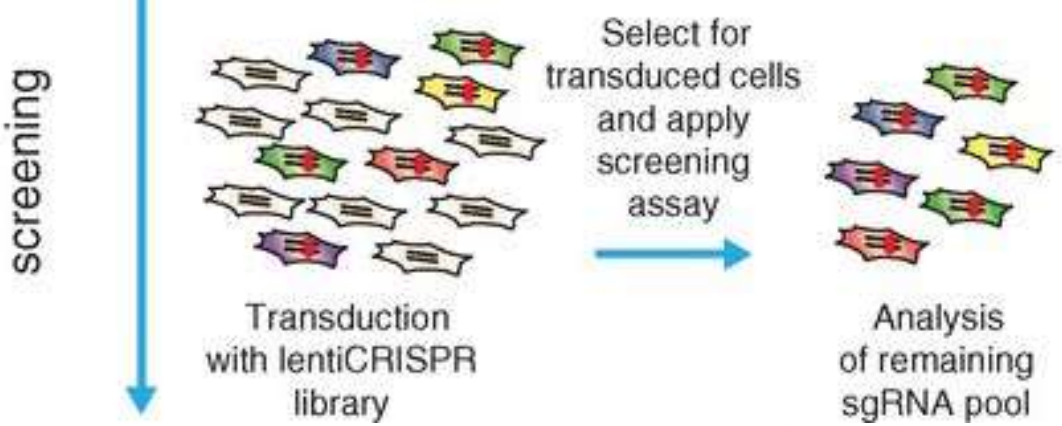
genome-scale screening

enrichment of sgRNAs

pretreatment conditions **U** relative to Severe disease S



negative selection screening

PCR amplification «sgRNAs» HiSeQ

Ophir Shalem et al. Science 2014;343:84-87

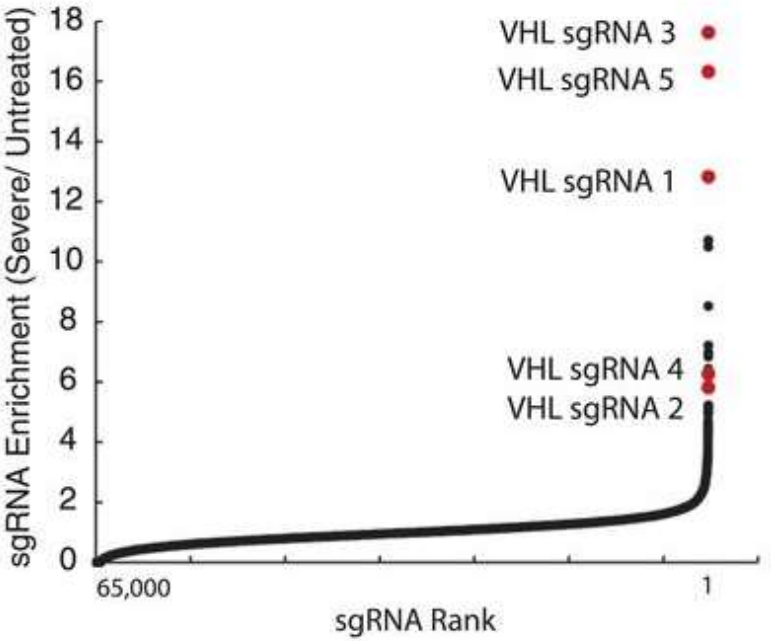
Genome-scale Cas9-mediated knockout screen identifies VHL inhibition as protective during states of mitochondrial dysfunction.

enrichment of sgRNAs in severe disease S relative to pretreatment conditions **U**

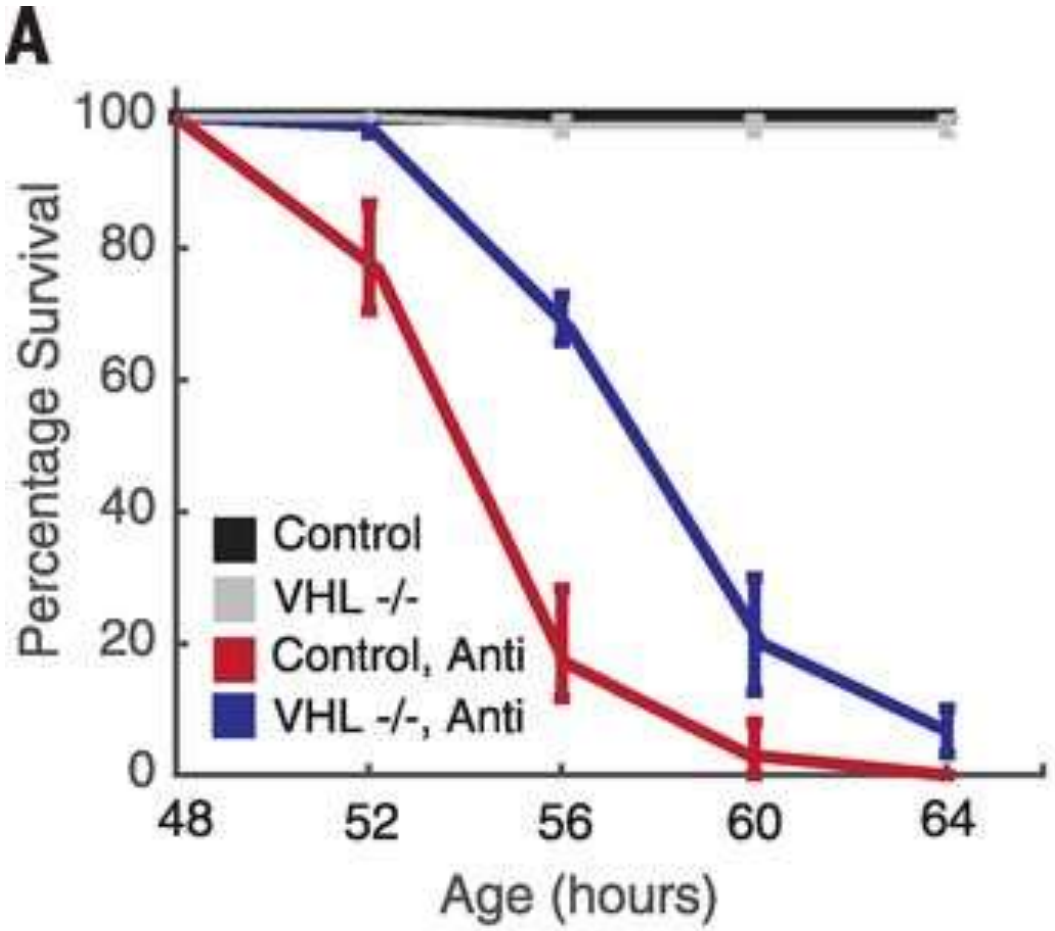
D

Gene	sgRNA Ranks	Rank
VHL	1, 2, 3, 12, 14	1
RGS20	13, 145, 2266, 8296, 27675, 29239	2
SIN3A	32, 242	3
ESPNL	168, 199, 8244, 8519, 12532, 58512	4
EXOC3L4	47, 267, 6259, 7589	5
DOCK7	177, 299, 4796, 10550, 18350, 23644	6
NDUFS6	8, 403, 2876, 7677	7
CLSTN1	7, 412, 11644, 46491	8
CD101	139, 372, 14840, 30593, 57365, 61388	9
TRIO	277, 342, 1831, 23700, 37855	10

E



vhl KO activates the HIF response in zebrafish embryos and alleviates death caused by RC inhibition.



Anti = Respiratory Chain inhibition

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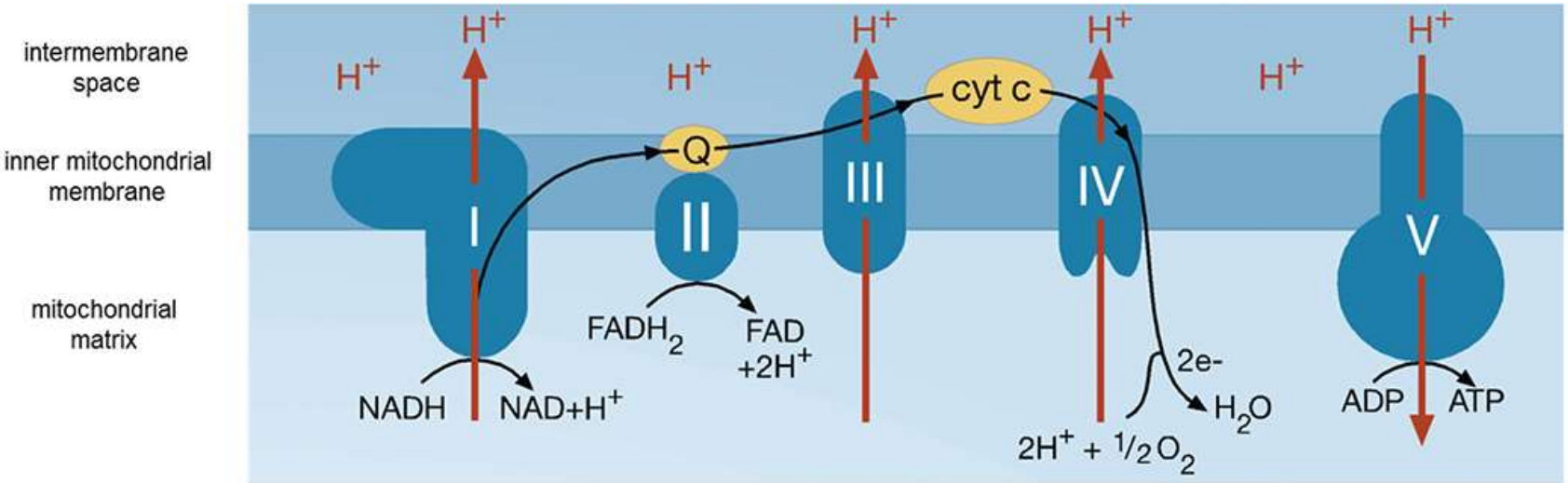
Isha H. Jain et al. Science 2016;352:54-61

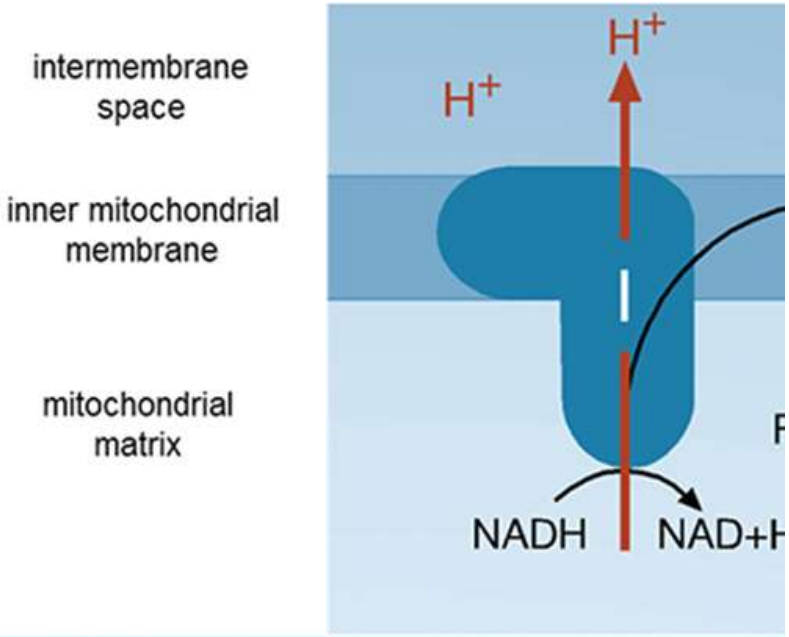
Mutations in mitochondrial disease



OXPHOS Component	Complex I	Complex II	Complex III	Complex IV	Complex V
mtDNA structural subunit genes	***MTND1***[120] ***MTND2***[121] ***MTND3***[122] ***MTND4***[123] ***MTND4L***[124] ***MTND5***[125] ***MTND6***[126]	-	***MTCYB***[127]	***MTCO1***[128] ***MTCO2***[129] ***MTCO3***[130]	***MTATP6***[131] ***MTATP8***[132]
Nuclear structural subunit genes	***NDUFS1***[133] ***NDUFS2***[134] ***NDUFS3***[135] ***NDUFS4***[136] *NDUFS5* ***NDUFS6***[137] ***NDUFS7***[138] ***NDUFS8***[139] ***NDUFA1***[140] ***NDUFA2***[141] *NDUFA3 NDUFA5 NDUFA6 NDUFA7 NDUFA8* ***NDUFA9***[142] ***NDUFA10***[143] ***NDUFA11***[21] ***NDUFA12***[144] ***NDUFA13***[145] *NDUFAB1* ***NDUFV1***[146] ***NDUFV2***[147] ***NDUFV3*** *NDUFB1 NDUFB2* ***NDUFB3***[148] *NDUFB4 NDUFB5 NDUFB6 NDUFB7 NDUFB8* ***NDUFB9***[149] *NDUFB10* ***NDUFB11***[150] *NDUFC1 NDUFC2*	***SDHA***[25] ***SDHB***[151] *SDHC* ***SDHD***[152]	***UQCRB***[153] *UQCRC1* ***CYC1***[156] ***UQCRC2***[154] *UQCRFS1 UQCRH* ***UQCRQ***[155] *UQCR10 UQCR11*	***COX4***[157] *COX5A COX5B* ***COX6A***[57] ***COX6B***[158] *COX6C COX7A* ***COX7B***[159] *COX7C* ***COX8***[160]	***ATP5A1***[76] *ATP5B ATP5C1 ATP5D* ***ATP5E***[161] *ATP5F1 ATP5G1 ATP5G2 ATP5G3 ATP5H ATP5I ATP5O ATP5J ATP5J2 ATP5L ATP5L2*
Assembly factor and ancillary protein genes	***NDUFAF1***[162] ***NDUFAF2***[163] ***NDUFAF3***[164] ***NDUFAF4***[165] ***NDUFAF5***[166] ***NDUFAF6***[167] *NDUFAF7* ***FOXRED1***[168] ***ACAD9***[30] *ECSIT* ***NUBPL***[168] ***TMEM126B***[28, 37] *TIMMDC1 C17orf89*	***SDHAF1***[41] *SDHAF2 SDHAF3 SDHAF4*	***BCS1L***[49] ***LYRM7***[169] *UQCC1* ***UQCC2***[170] ***UQCC3***[171] ***TTC19***[172] *PTCD2*	*COA1* ***COA3***[173] *COA4* ***COA5***[174] ***COA6***[175] *COA7* ***COX10***[176] *COX11* ***COX14***[177] ***COX15***[178] *COX16 COX17 COX18 COX19* ***COX20***[179] ***SCO1***[180] ***SCO2***[181] ***SURF1***[182] *PET117* ***LRPPRC***[183] ***PET100***[184] ***CEP89***[185] ***TACO1***[186] *OXA1L* ***APOPT1***[187] ***NDUFA4***[53] ***FASTKD2***[188]	*ATPAF1* ***ATPAF2***[189] ***TMEM70***[58]

bold= genes with mutations

The Journal of Pathology2 NOV 2016 DOI: 10.1002/path.4801



OXPHOS Component	Complex I
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Nuclear structural subunit genes	***NDUFS1***[133] ***NDUFS2***[134] ***NDUFS3***[135] ***NDUFS4***[136] *NDUFS5* ***NDUFS6***[137] ***NDUFS7***[138] ***NDUFS8***[139] ***NDUFA1***[140] ***NDUFA2***[141] *NDUFA3 NDUFA5 NDUFA6 NDUFA7 NDUFA8* ***NDUFA9***[142] ***NDUFA10***[143] ***NDUFA11***[21] ***NDUFA12***[144] ***NDUFA13***[145] *NDUFAB1* ***NDUFV1***[146] ***NDUFV2***[147] ***NDUFV3*** *NDUFB1 NDUFB2* ***NDUFB3***[148] *NDUFB4 NDUFB5 NDUFB6 NDUFB7 NDUFB8* ***NDUFB9***[149] *NDUFB10* ***NDUFB11***[150] *NDUFC1 NDUFC2*

Mouse model of Leigh syndrome Ndufs4 -/- (KO) NADH:ubiquinone oxidoreductase subunit S4

Science AAAS

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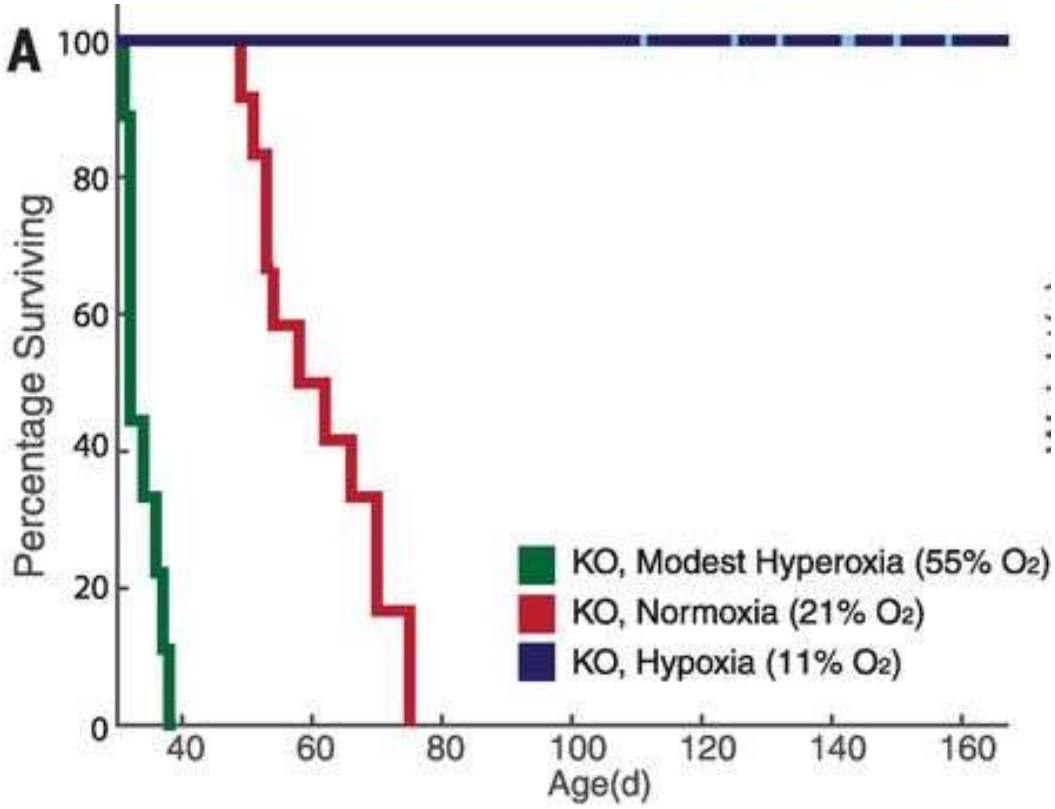
Isha H. Jain et al. Science 2016;352:54-61

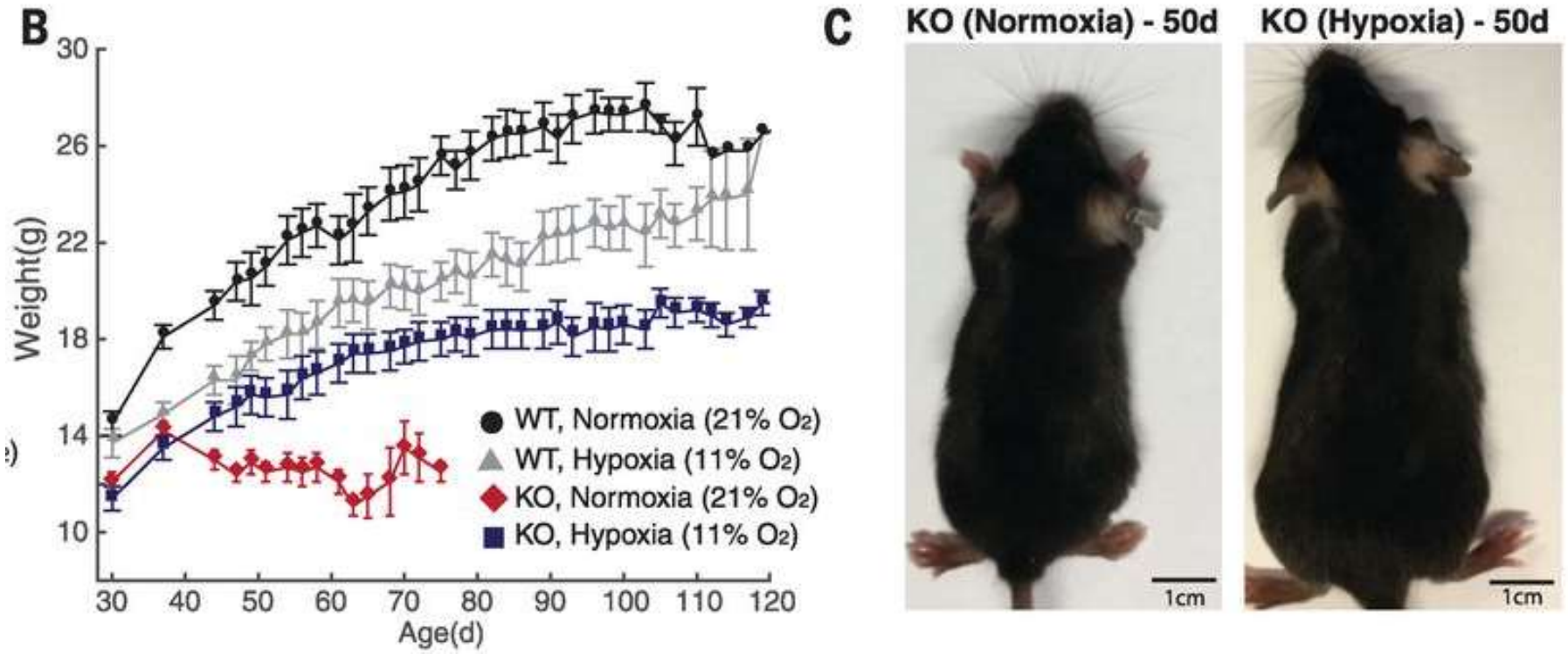
Chronic hypoxia extends life span and alleviates disease in a mouse model of Leigh syndrome (KO) .



chronic hyperoxia exacerbates disease

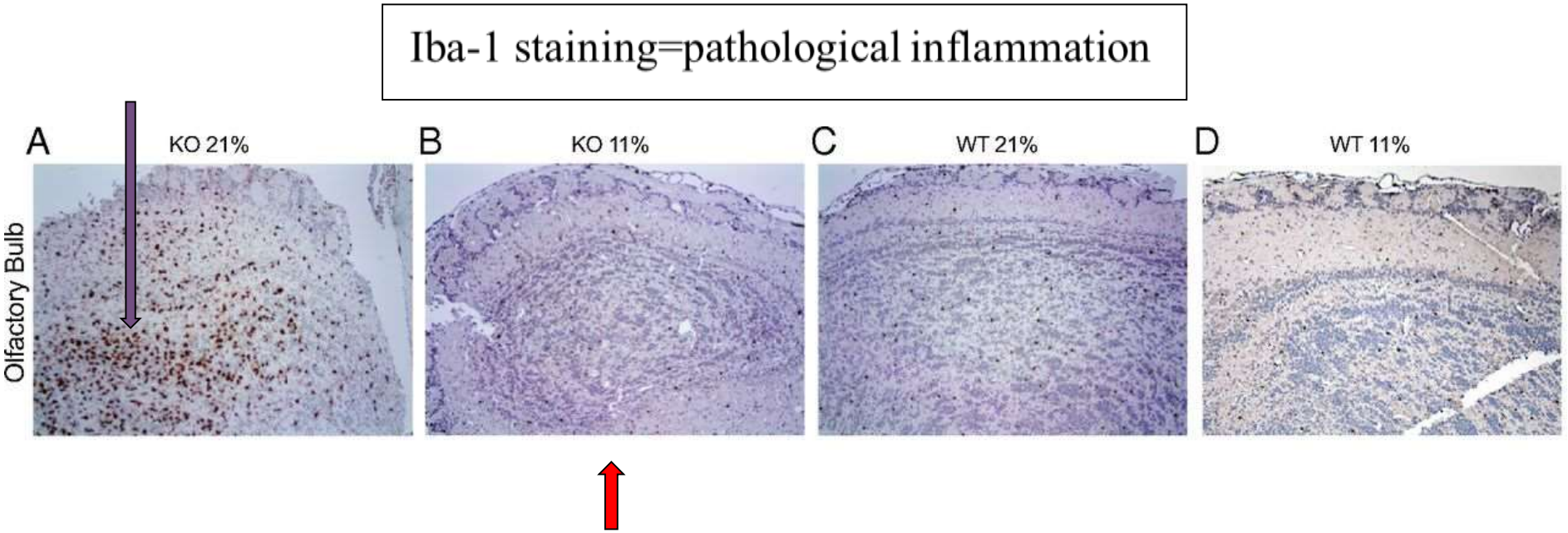
Isha H. Jain et al. Science 2016;352:54-61

Fig. 5 Chronic hypoxia extends life span and alleviates disease in a mouse model of Leigh syndrome (KO) whereas chronic hyperoxia exacerbates disease.



Isha H. Jain et al. Science 2016;352:54-61

Breathing 11% O2 in late-stage neurological disease reverses pathological inflammation in the brains of Ndufs4 KO mice.



KO mice breathing 21% O2 up to 55 d and then breathing 11% O2 to 160 d.

Iba-1 is up-regulated in microglia following nerve injury,
central nervous system ischemia, and several other brain diseases

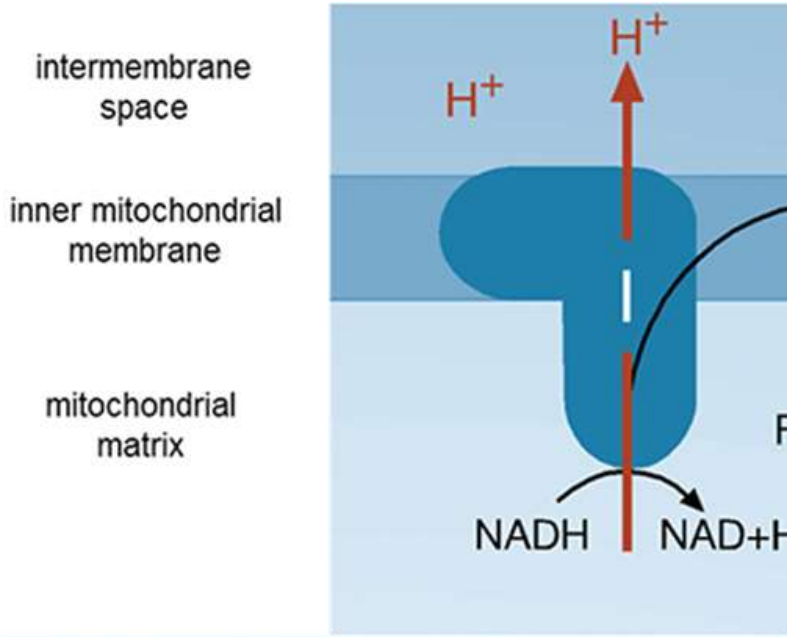
Michele Ferrari et al. PNAS 2017;114:E4241-E4250

Genome-scale Cas9-mediated knockout screen and states of mitochondrial dysfunction.

enrichment of sgRNAs in severe disease **S** relative to pretreatment conditions **U**

D

Gene	sgRNA Ranks	Rank
VHL	1, 2, 3, 12, 14	1
RGS20	13, 145, 2266, 8296, 27675, 29239	2
SIN3A	32, 242	3
ESPNL	168, 199, 8244, 8519, 12532, 58512	4
EXOC3L4	47, 267, 6259, 7589	5
DOCK7	177, 299, 4796, 10550, 18350, 23644	6
NDUFS6	8, 403, 2876, 7677	7
CLSTN1	7, 412, 11644, 46491	8
CD101	139, 372, 14840, 30593, 57365, 61388	9
TRIO	277, 342, 1831, 23700, 37855	10



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Nuclear structural subunit genes	*NDUFS1*[133] *NDUFS2*[134] *NDUFS3*[135] *NDUFS4*[136] *NDUFS5* *NDUFS6*[137] *NDUFS7*[138] *NDUFS8*[139] *NDUFA1*[140] *NDUFA2*[141] *NDUFA3* *NDUFA5*

Ndufs6 NADH:ubiquinone oxidoreductase subunit S6

Genome-scale Cas9-mediated knockout screen and states of mitochondrial dysfunction.

enrichment of sgRNAs in severe disease **S** relative to pretreatment conditions **U**

D

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NDUFS6	8, 403, 2876, 7677	7
CLSTN1	7, 412, 11644, 46491	8
CD101	139, 372, 14840, 30593, 57365, 61388	9
TRIO	277, 342, 1831, 23700, 37855	10

Sin3A= global transcription regulators
platform for chromatin-modifying activities

Within 24 h of reoxygenation, the hypoxia-induced transcription returned to basal levels
the nucleosome structure was reassembled in the hypoxia-inducible form

Nucleosome reassembly required the transcriptional coregulator SIN3A