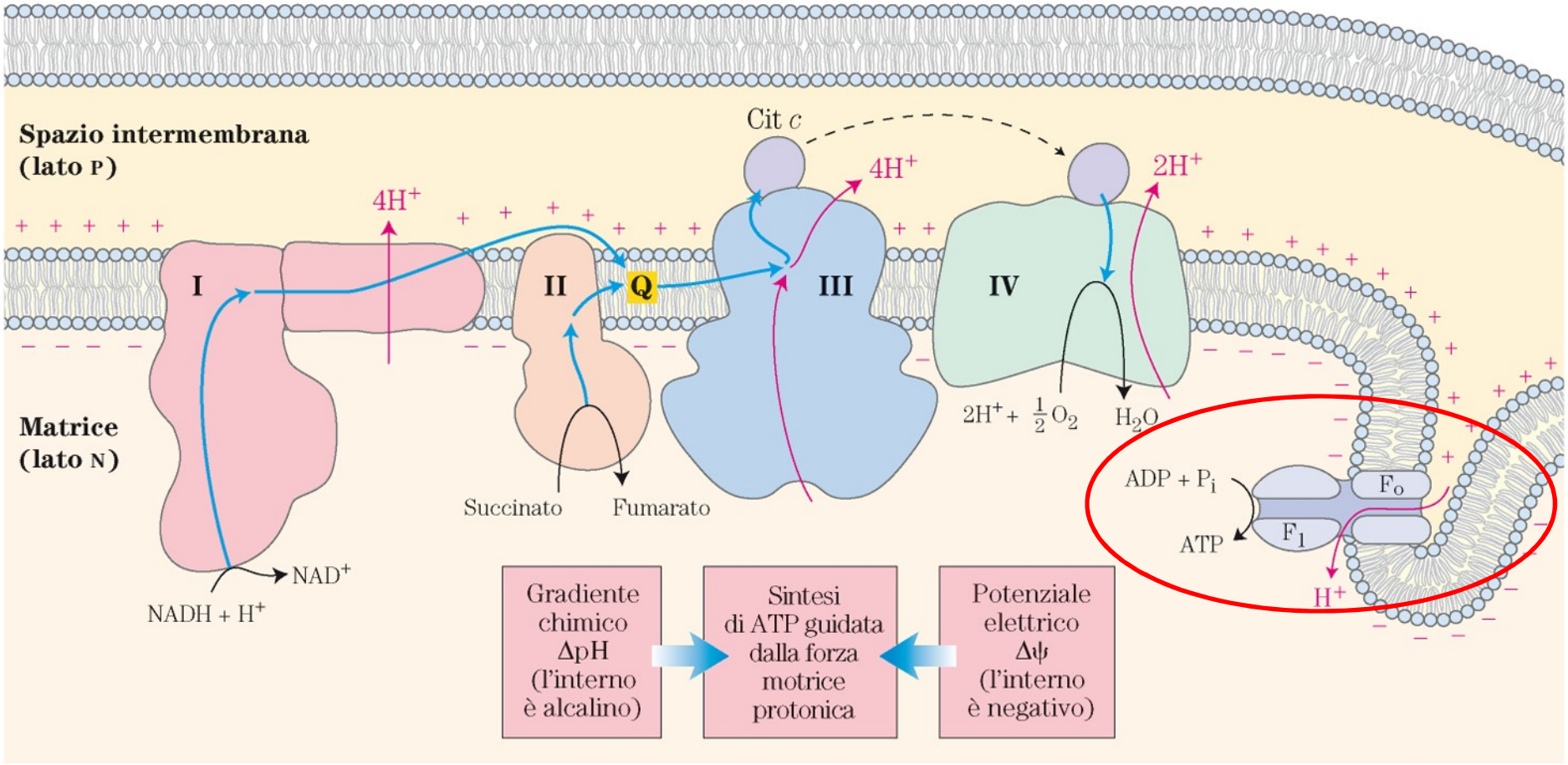
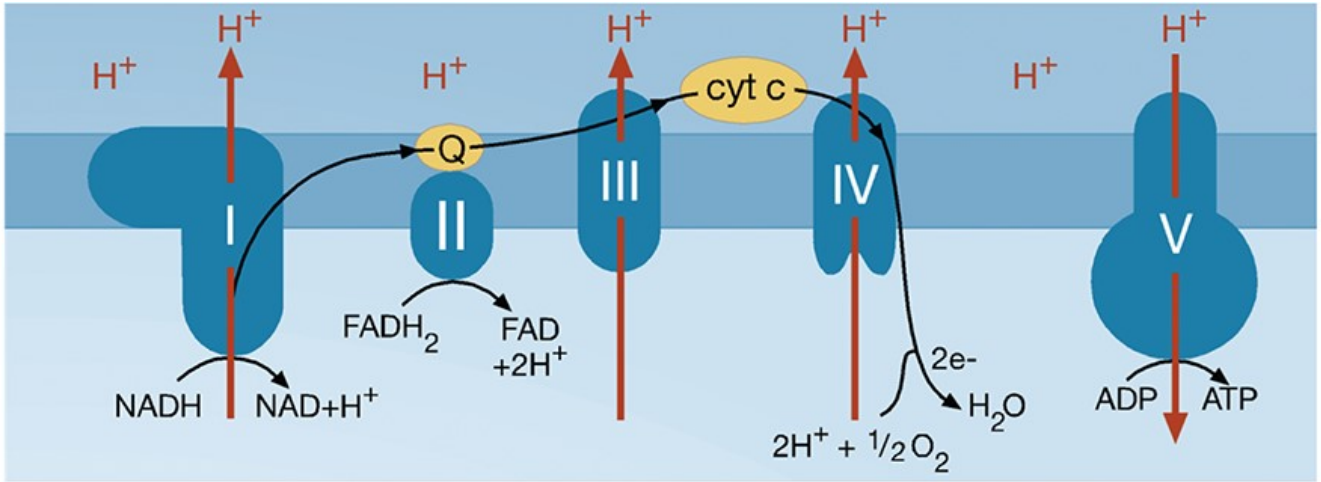
La fosforilazione ossidativa nel mitocondrio



Mutations in mitochondrial disease

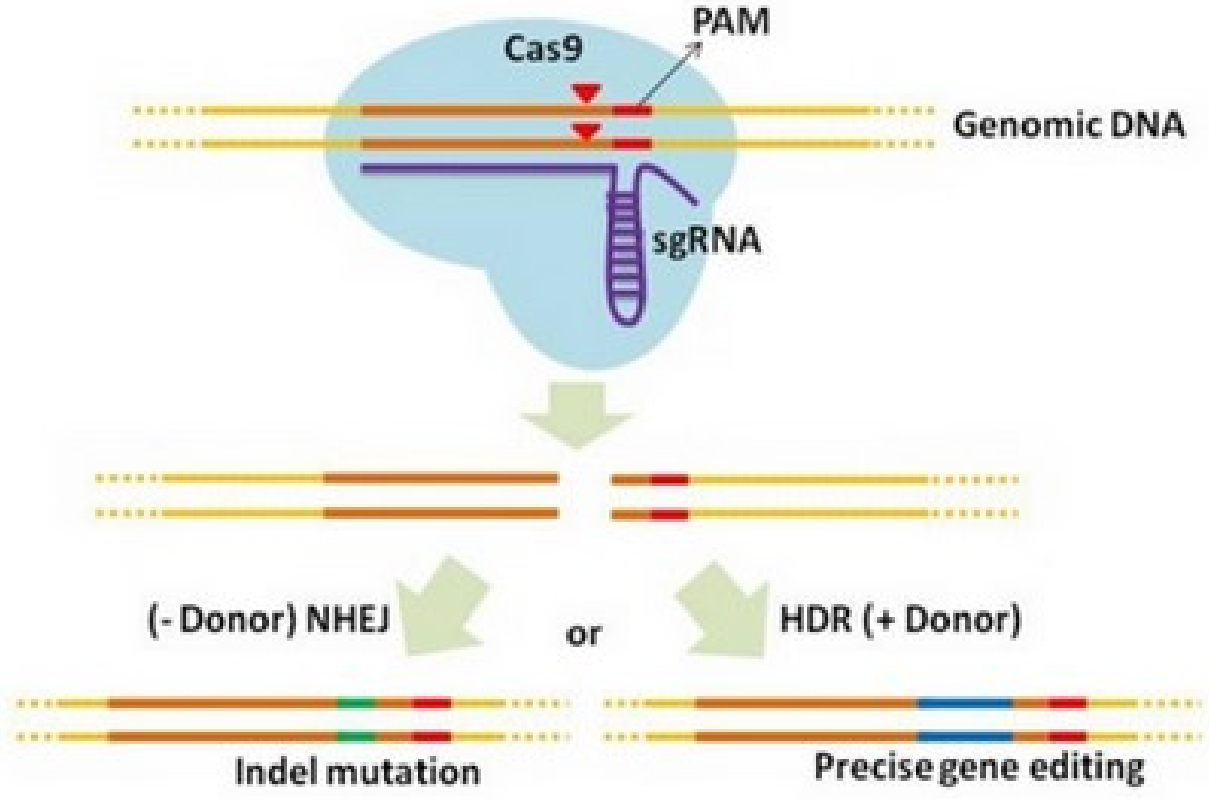


OXPHOS Component	Complex I	Complex II	Complex III	Complex IV	Complex V
mtDNA structural subunit genes	***MTND1***[120] ***MTND2***[121] ***MTND3***[122] ***MTND4***[123] ***MTND4L***[124] ***MTND5***[125] ***MTND6***[126]	-	***MTCYB***[127]	***MTCO1***[128] ***MTCO2***[129] ***MTCO3***[130]	***MTATP6***[131] ***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 Pathology
2 NOV 2016 DOI: 10.1002/path.4809
http://onlinelibrary.wiley.com/doi/10.1002/path.4809/full#path4809-fig-0001

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



Doudna et al., 2014

Lentiviral delivery of Cas9 and sgRNA provides efficient depletion of target genes

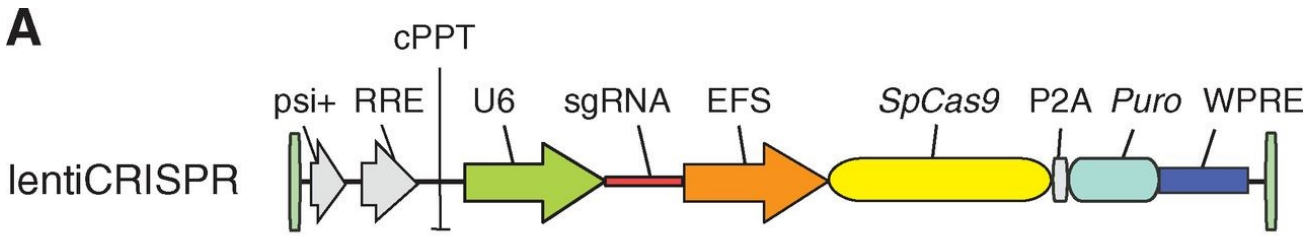
synthetic single-guide RNA (sgRNA) targeted to specific coding regions of genes

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

Lentiviral expression vector for Cas9 and sgRNA (lentiCRISPR)

ability to simultaneously deliver Cas9 and sgRNA through a single vector enables application to any cell type of interest

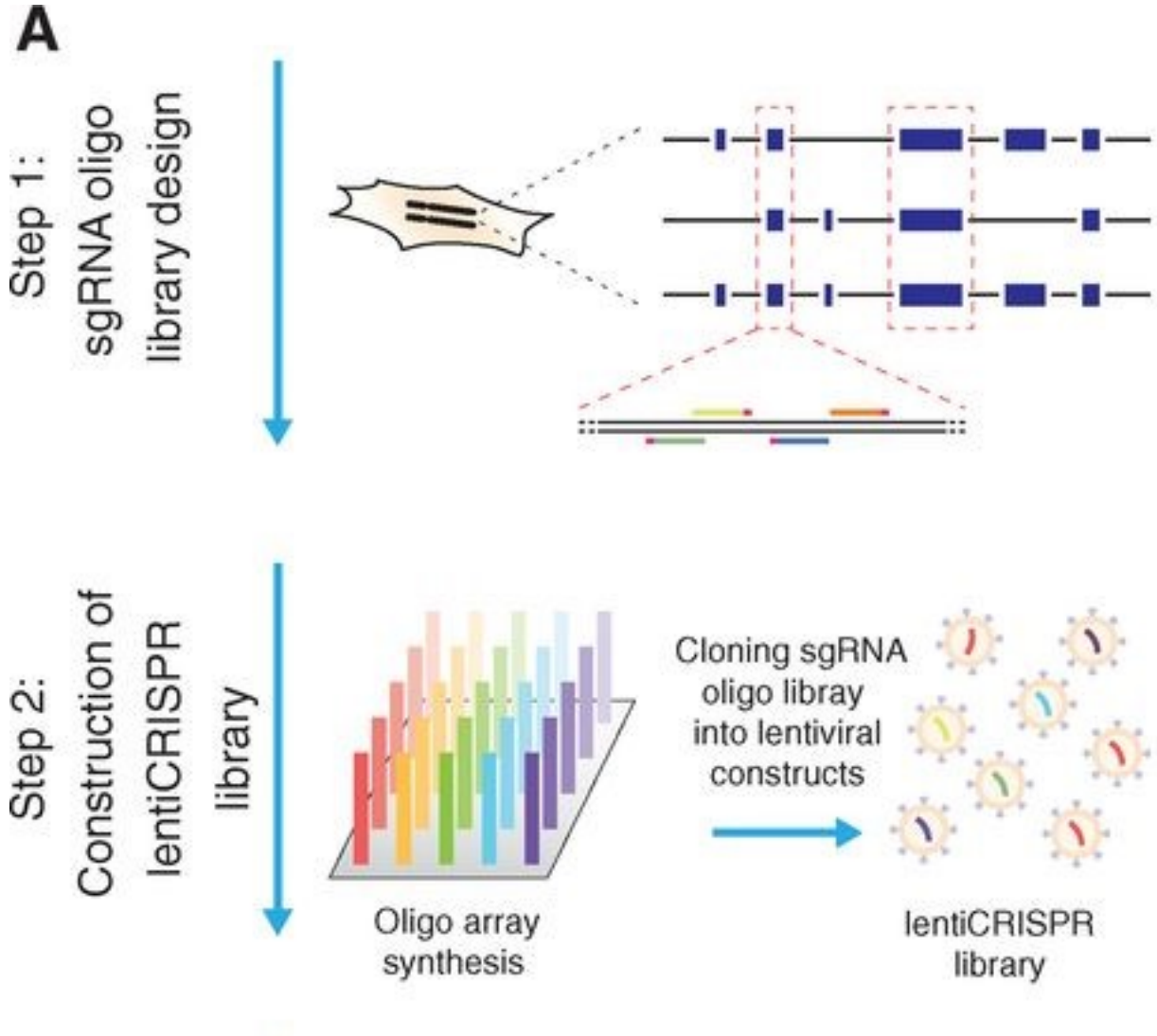


synthetic single-guide RNA (sgRNA)
targeted to specific coding regions of genes

puro, puromycin selection marker; psi+, psi packaging signal; RRE, rev response element; cPPT, central polypurine tract; EFS, elongation factor-1α short promoter; P2A, 2A self-cleaving peptide; WPRE, posttranscriptional regulatory element

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

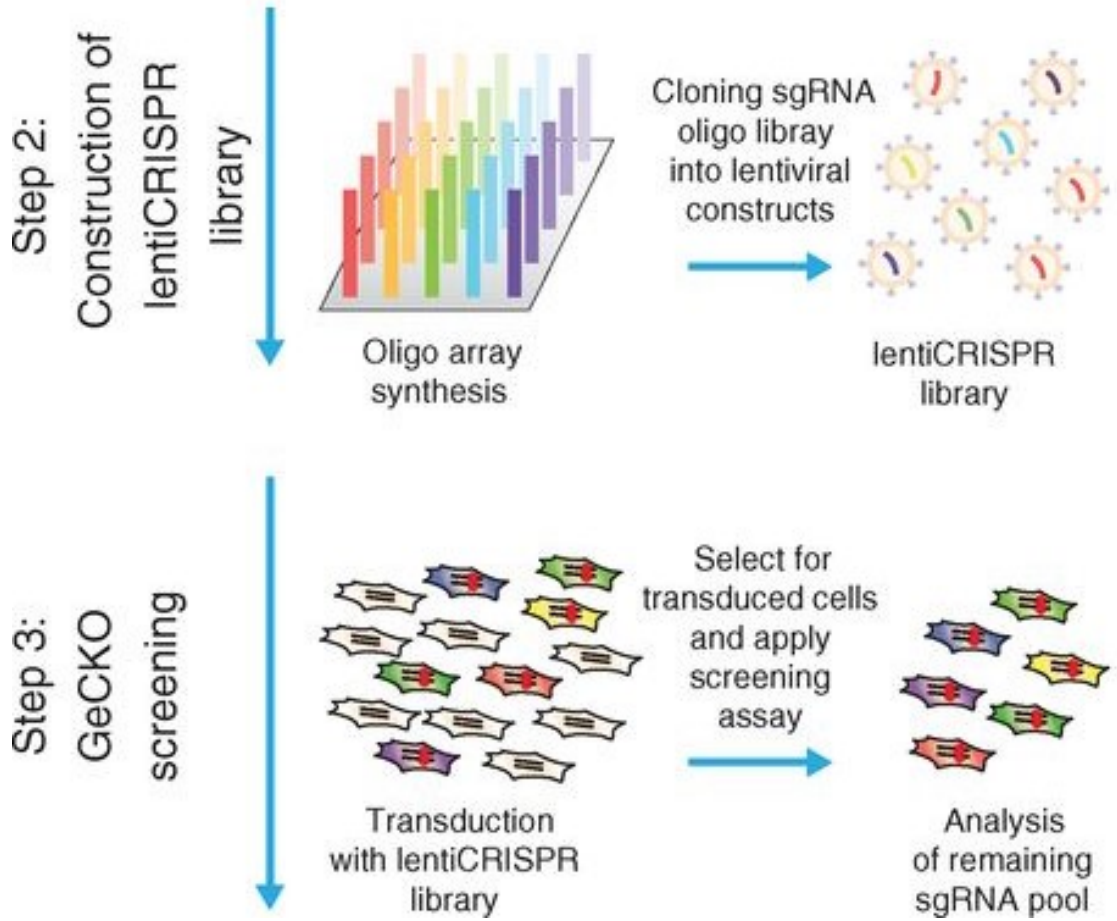
GeCKO library design for genome-scale negative selection screening
Design of sgRNA library for genome-scale knockout of coding sequences in human cells



pooled lentiCRISPR 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

GeCKO library design for genome-scale negative selection screening
Design of sgRNA library for genome-scale knockout of coding sequences in human cells



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

PCR amplification «sgRNAs» HiSeQ

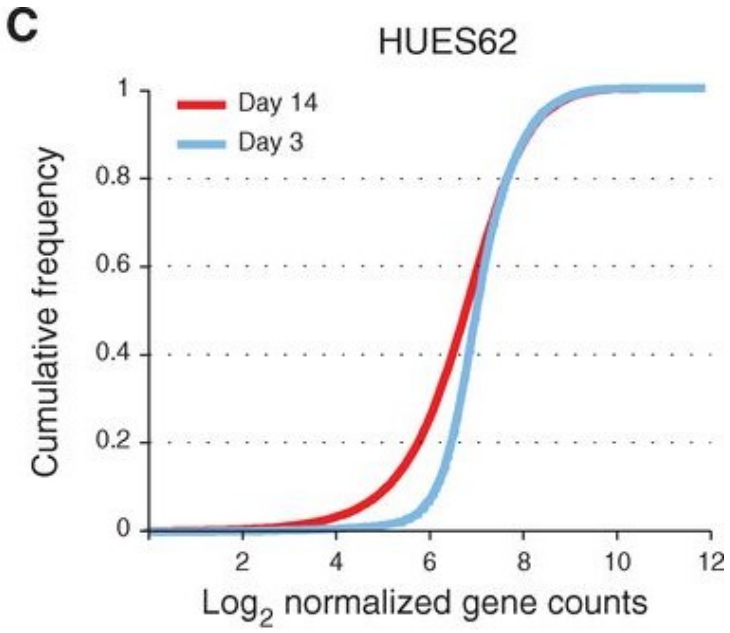
Ophir Shalem et al. Science 2014;343:84-87

GeCKO library design and application for genome-scale negative selection screening

human embryonic stem cells

Shift in the 14-day curve represents the depletion in a subset of sgRNAs

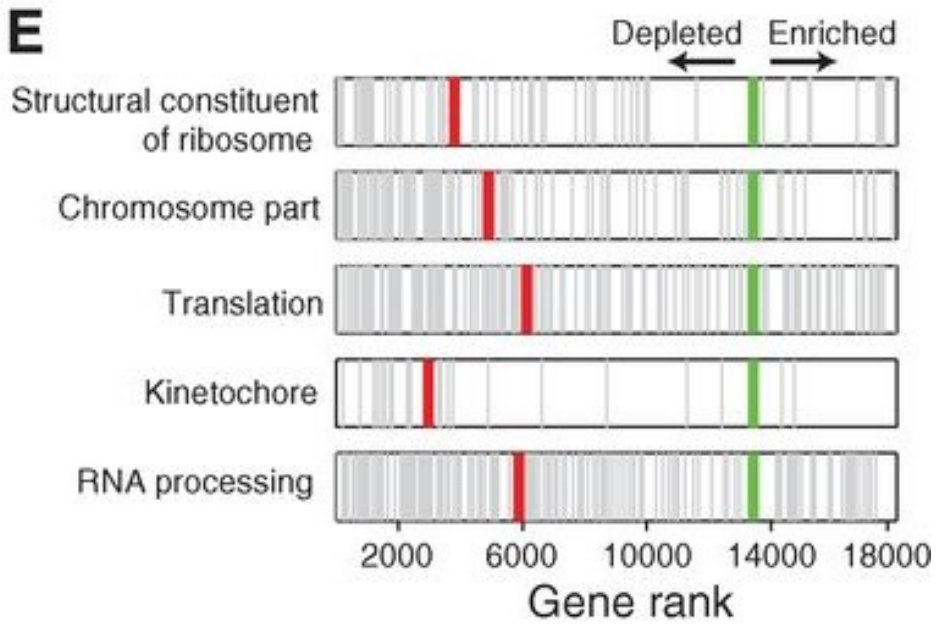
human embryonic stem cells



Published by AAAS **Ophir Shalem et al. Science 2014;343:84-87**

GeCKO library design and application for genome-scale negative selection screening

The five most significantly depleted gene sets

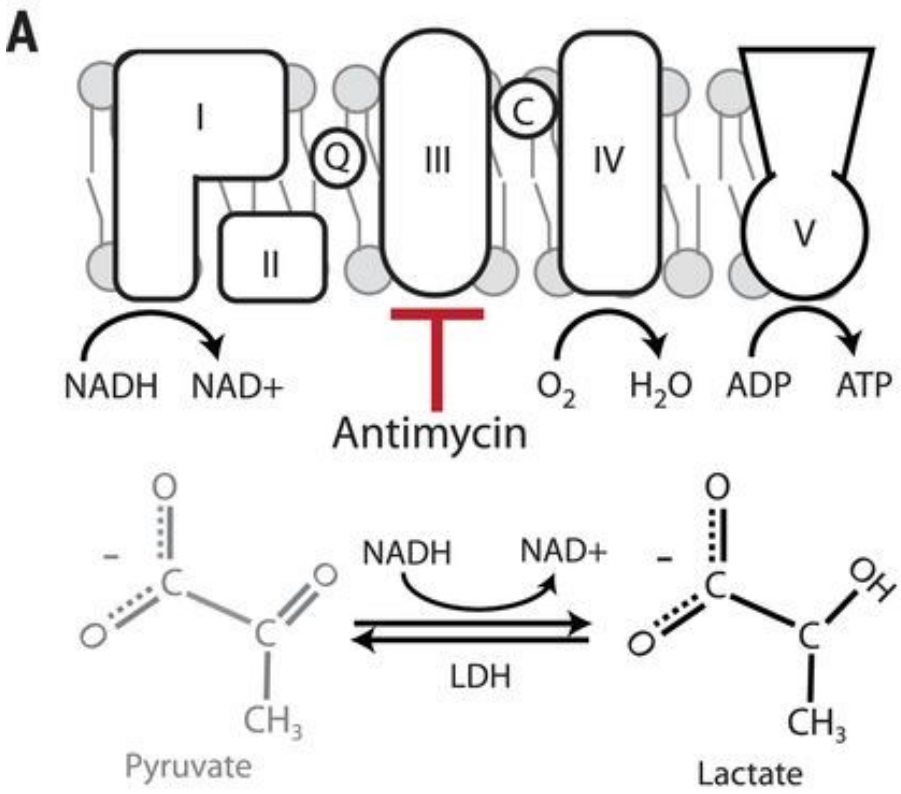


18,080 genes in the human genome with an average coverage of 3 to 4 sgRNAs per gene

Published by AAAS **Ophir Shalem et al. Science 2014;343:84-87**

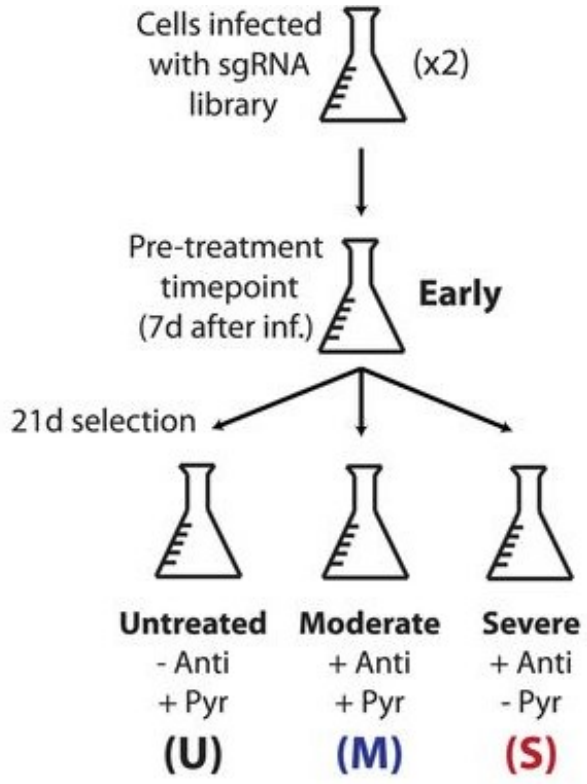
How to model Mitochondrial disease

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).

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

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

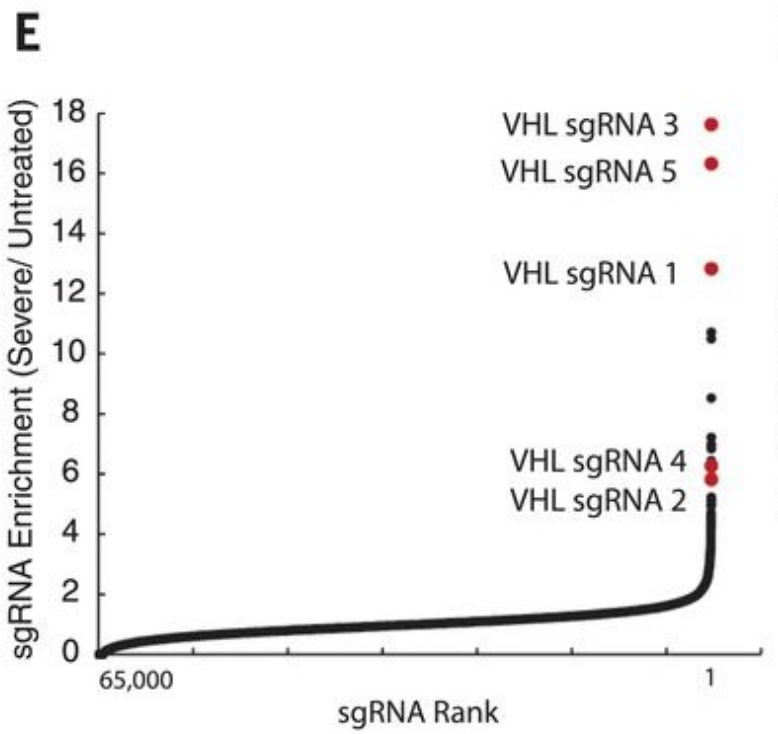
Genome-scale Cas9-mediated knockout screen identifies VHL inhibition as protective during states of mitochondrial dysfunction.

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

D

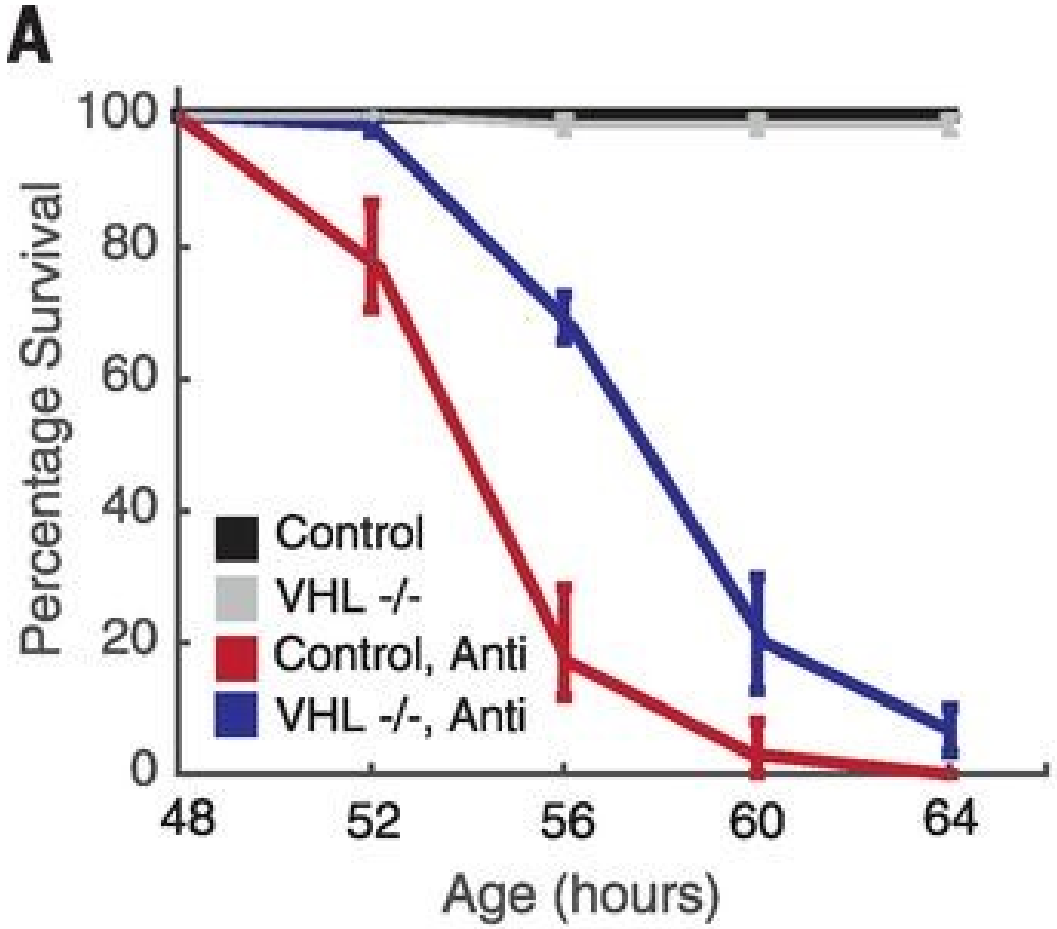
Gene	sgRNA Ranks	Rank
VHL	1, 2, 3, 12, 14	1
RGS20	13, 145, 2266, 8296, 27675, 29239	2
SIN3A	32, 242	3
ESPNL	168, 199, 8244, 8519, 12532, 58512	4
EXOC3L4	47, 267, 6259, 7589	5
DOCK7	177, 299, 4796, 10550, 18350, 23644	6
NDUFS6	8, 403, 2876, 7677	7
CLSTN1	7, 412, 11644, 46491	8
CD101	139, 372, 14840, 30593,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

E



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

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



Anti = Respiratory Chain inhibition

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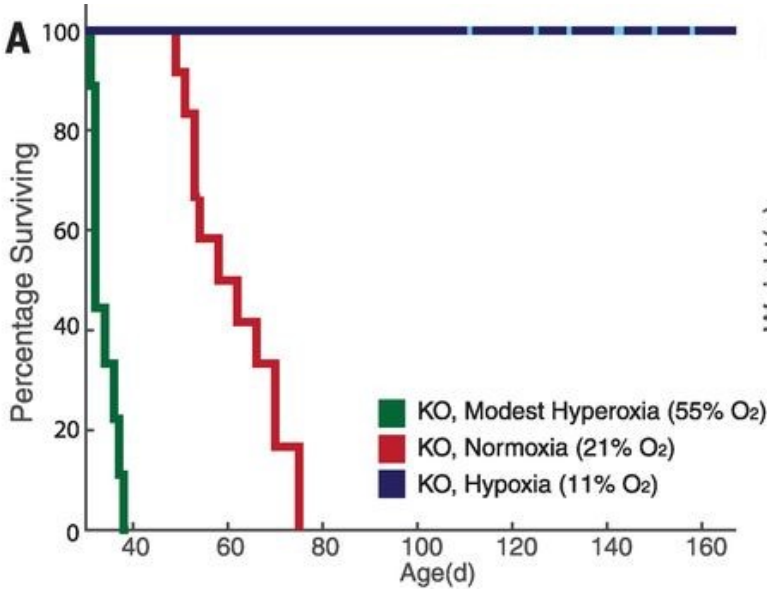
Chronic hypoxia extends life span and alleviates disease in a mouse model of Leigh syndrome (KO) whereas chronic hyperoxia exacerbates disease.



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

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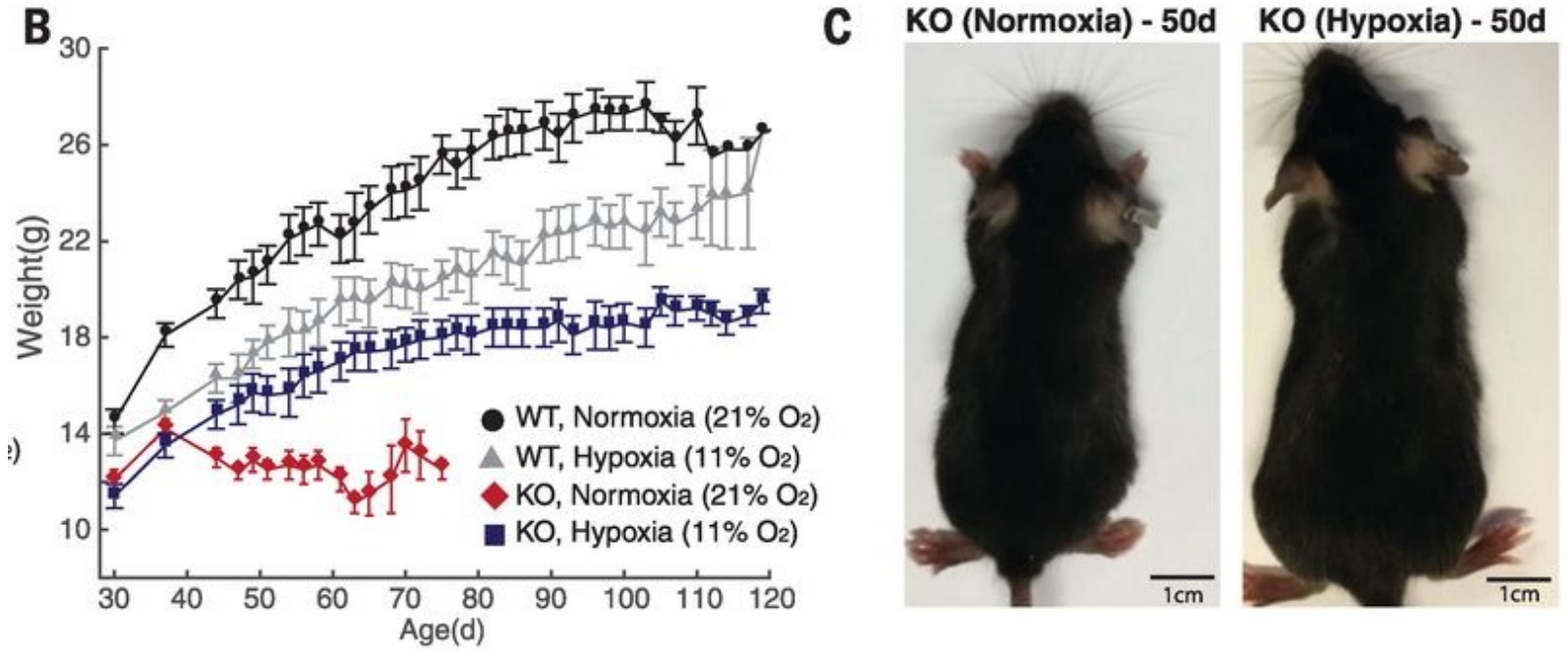
Fig. 5 Chronic hypoxia extends life span and alleviates disease in a mouse model of Leigh syndrome (KO) whereas chronic hyperoxia exacerbates disease.



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

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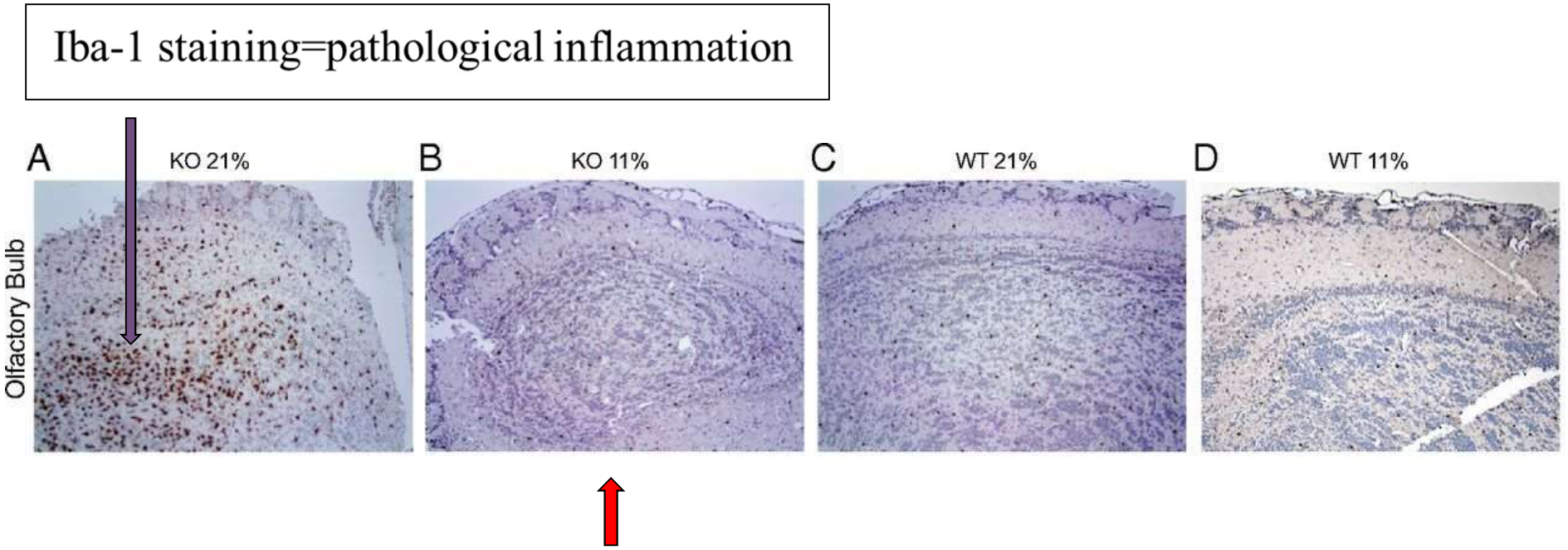
Breathing 11% O2 in late-stage neurological disease reverses pathological inflammation in the brains of Ndufs4 KO mice.

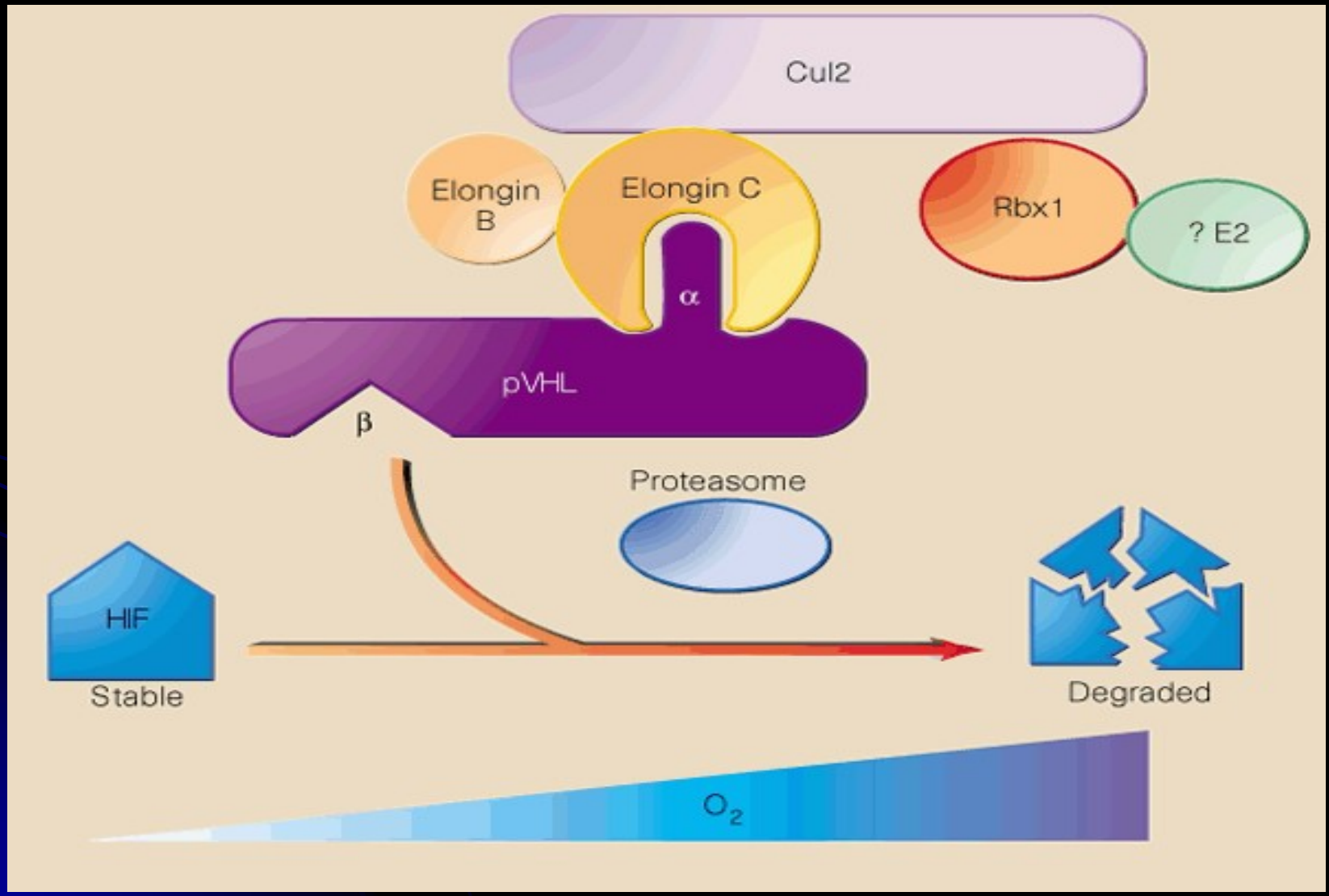


KO mice breathing 21% O2 up to 55 d and then breathing 11% O2 to 160 d.

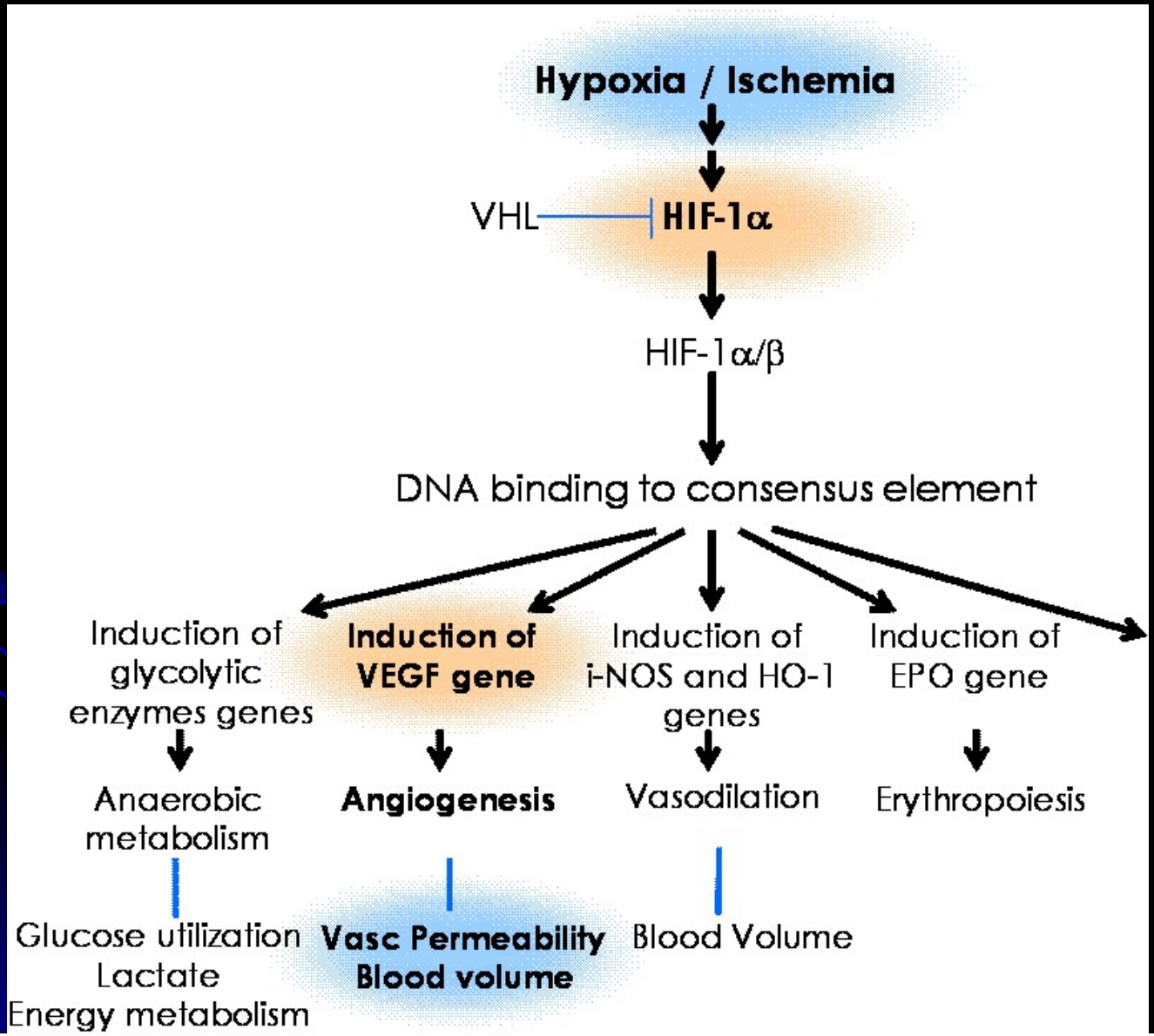
The Function of VHL and pVHL Protein

- Helps to regulate and destroy the alpha subunit of hypoxia-inducible factor or HIF-1
- HIF-1 is a transcription factor that has a myriad of target genes
- Products are involved in angiogenesis, erythropoiesis, energy metabolism, glucose transport

Normal VHL Function



HIF transcription factor



von Hippel-Lindau (VHL)

Patients with a germline mutation in von Hippel-Lindau (VHL) develop renal cell cancers and hypervascular tumors of the brain, adrenal glands, and pancreas as well as erythrocytosis

These phenotypes are driven by aberrant expression of HIF2α, which induces expression of genes involved in cell proliferation, angiogenesis, and red blood cell production

Inactivation of vhl in zebrafish (vhl–/–) led to constitutive activation of HIF2α