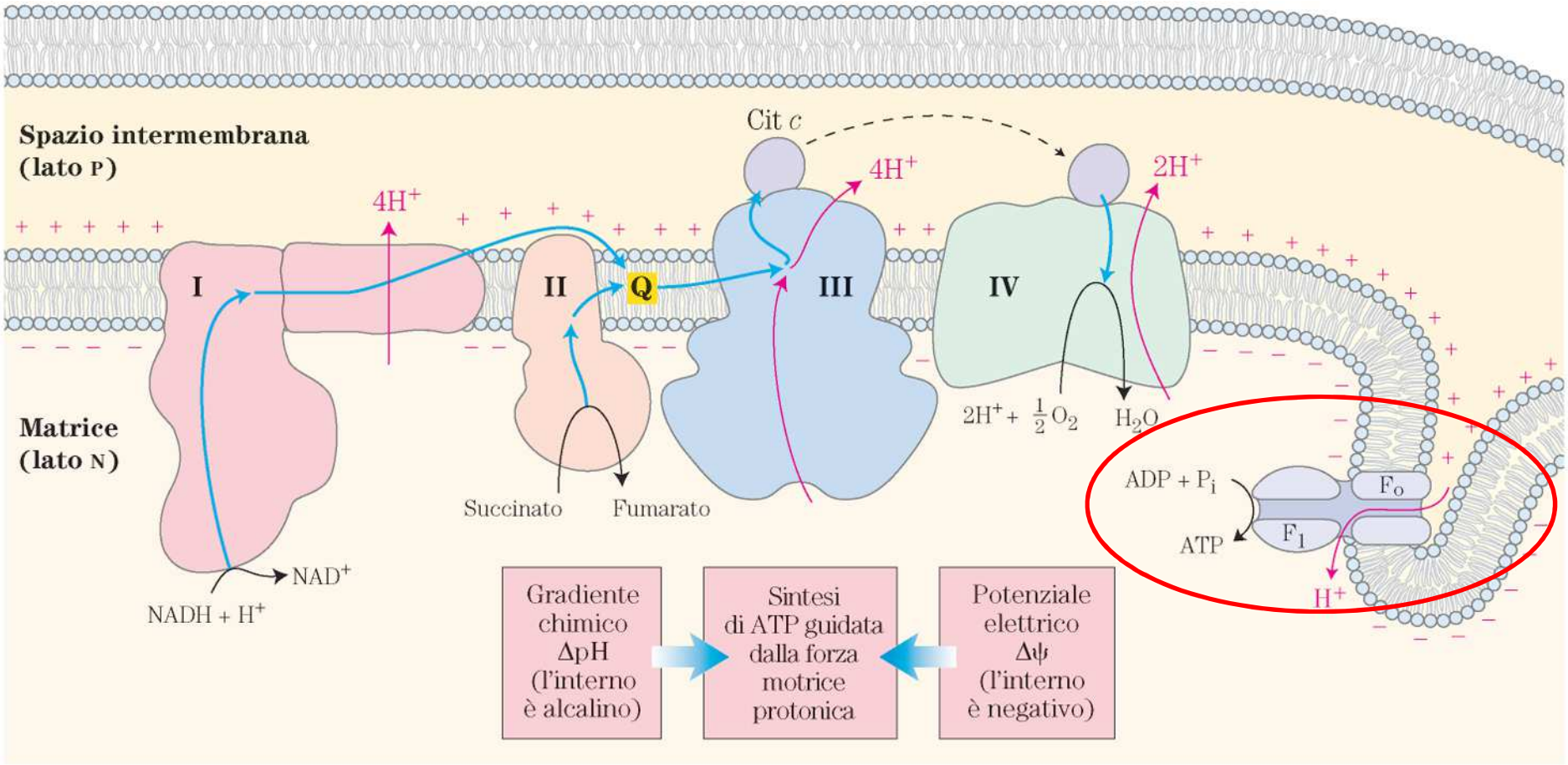
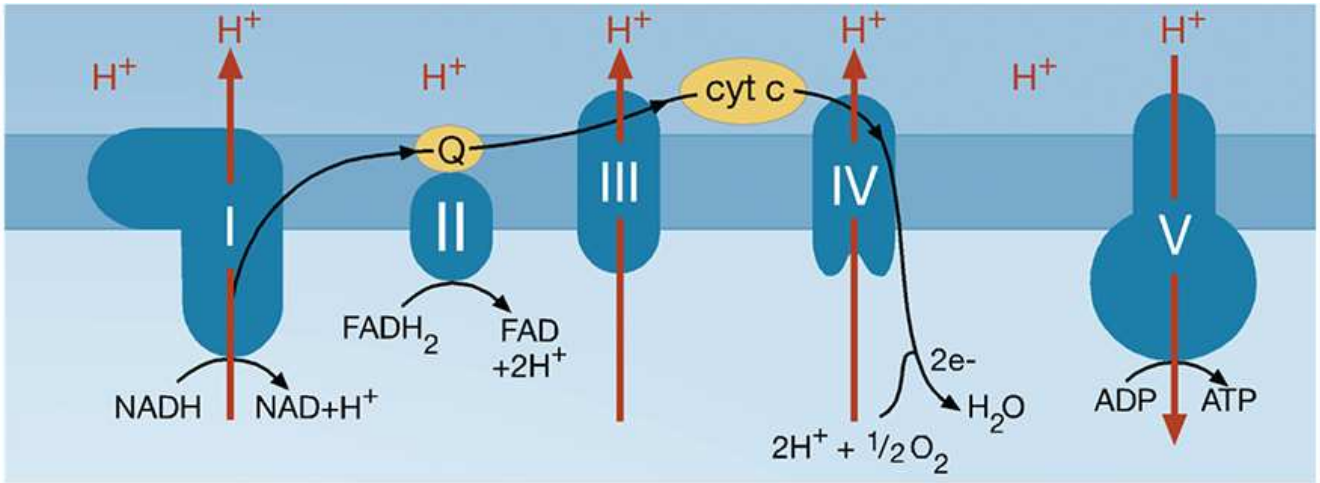
VHL inhibition as protective during states of mitochondrial dysfunction

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

La fosforilazione ossidativa nel mitocondrio



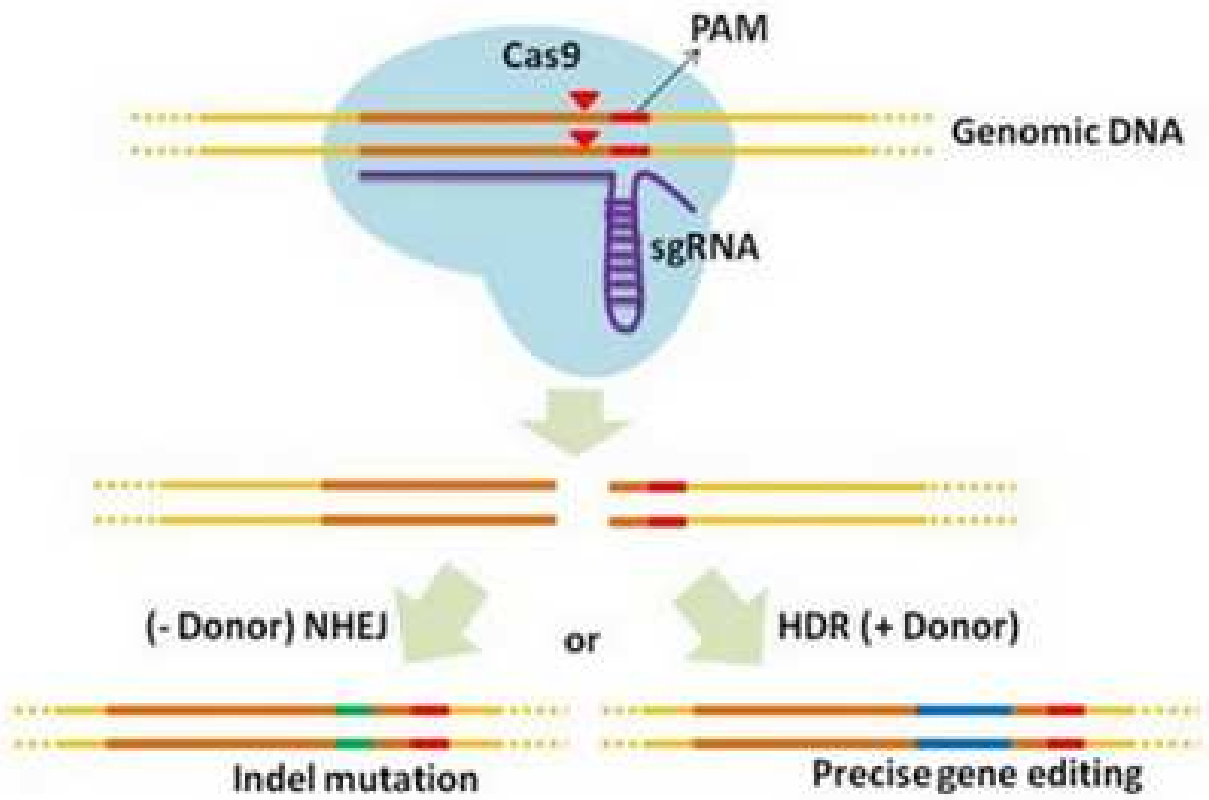
Mutations in mitochondrial disease



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]

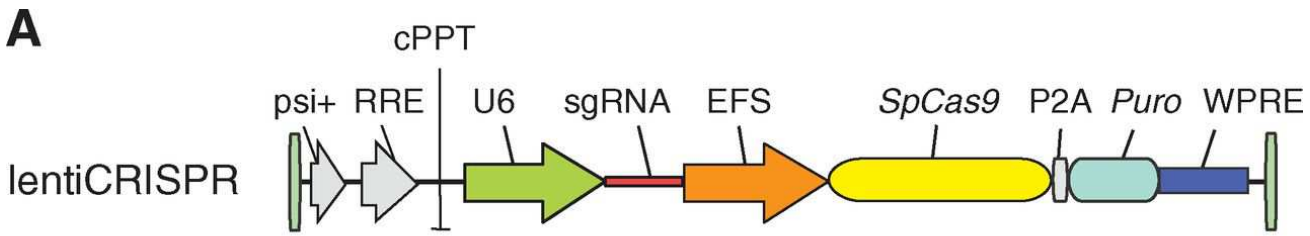
The Journal of Pathology
2 NOV 2016 DOI: 10.1002/path.4809
http://onlinelibrary.wiley.com/doi/10.1002/path.4809/full#path4809-fig-0001

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



Doudna et al., 2014

Fig. 1 Lentiviral delivery of Cas9 and sgRNA provides efficient depletion of target genes.(A) Lentiviral expression vector for Cas9 and sgRNA (lentiCRISPR). 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.



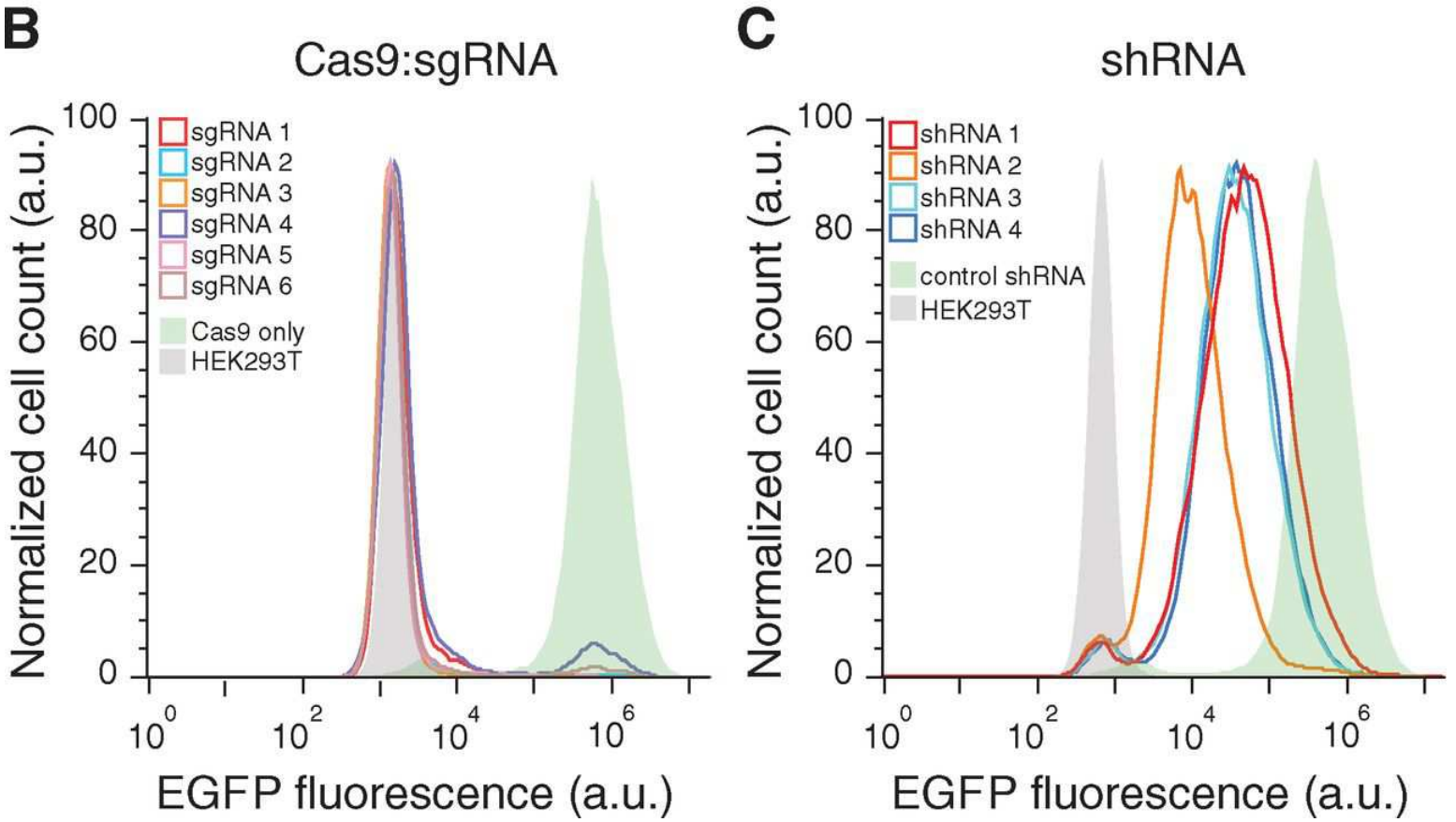
synthetic single-guide RNA (sgRNA) (10), which when targeted to coding regions of genes

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

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

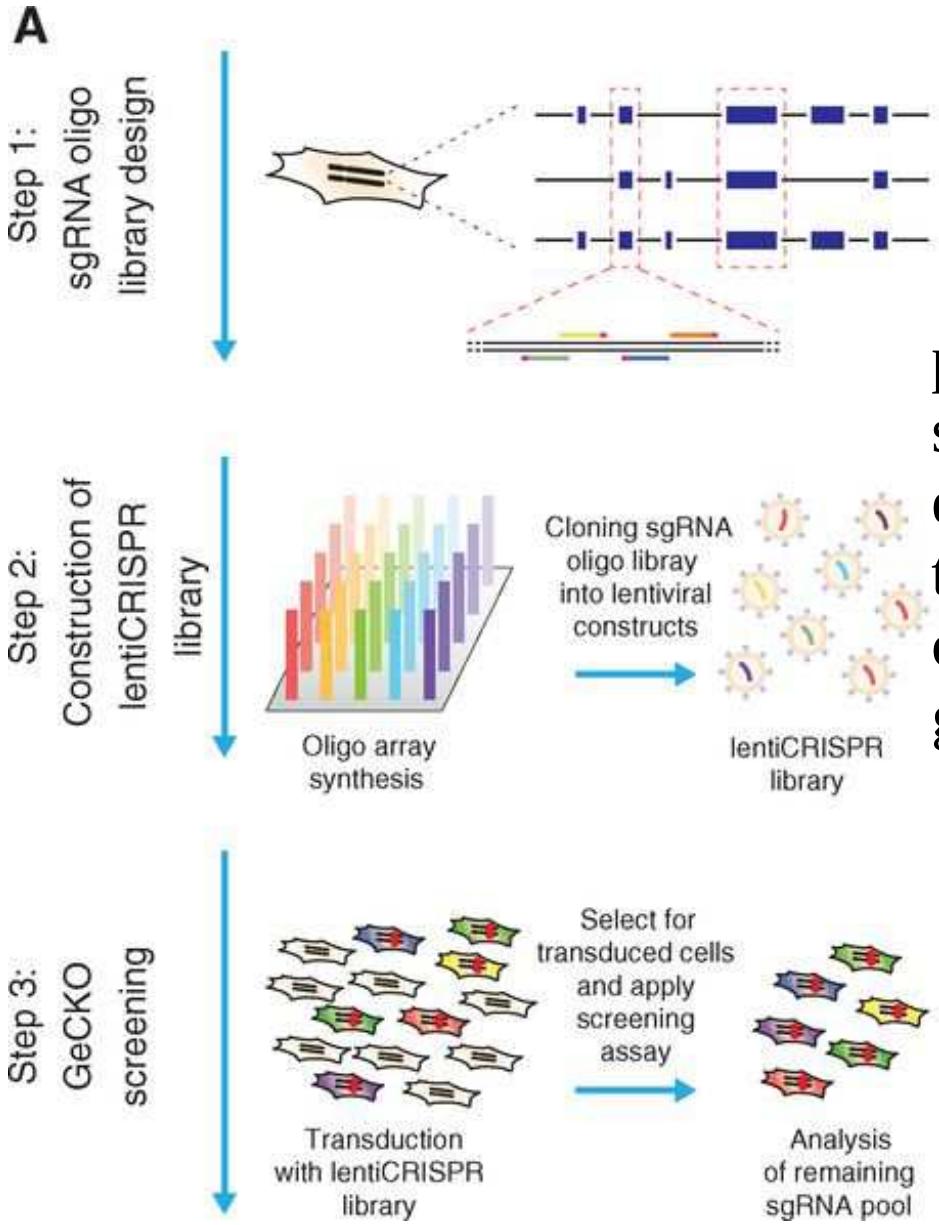
Fig. 1 Lentiviral delivery of Cas9 and sgRNA provides efficient depletion of target genes.(A) Lentiviral expression vector for Cas9 and sgRNA (lentiCRISPR). puro, puromycin selection marker; psi+, psi packaging signal; RRE, rev response element; cPPT, central polypurine tract; EFS, elongation factor-1α short promoter; P2A, 2A self-cleaving peptide; WPRE, posttranscriptional regulatory element.



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

sgRNAs targeting enhanced green fluorescent protein (EGFP) in a human embryonic kidney (HEK) 293T cell line containing a single

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 5′ constitutive exons (Fig. 2A) of 18,080 genes in the human genome with an average coverage of 3 to 4 sgRNAs per gene

PCR amplification «sgRNAs»
HiSeQ

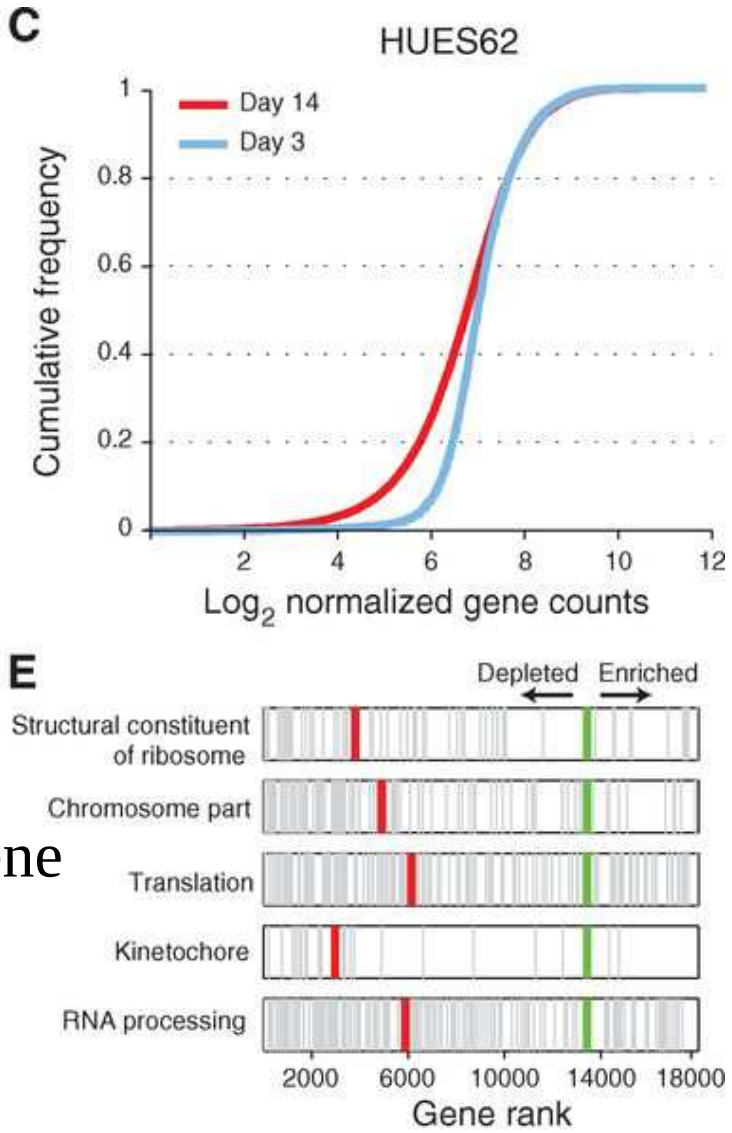
Ophir Shalem et al. Science 2014;343:84-87

Fig. 2 GeCKO library design and application for genome-scale negative selection screening.(A) Design of sgRNA library for genome-scale knockout of coding sequences in human cells (see supplementary text).

human embryonic stem cells

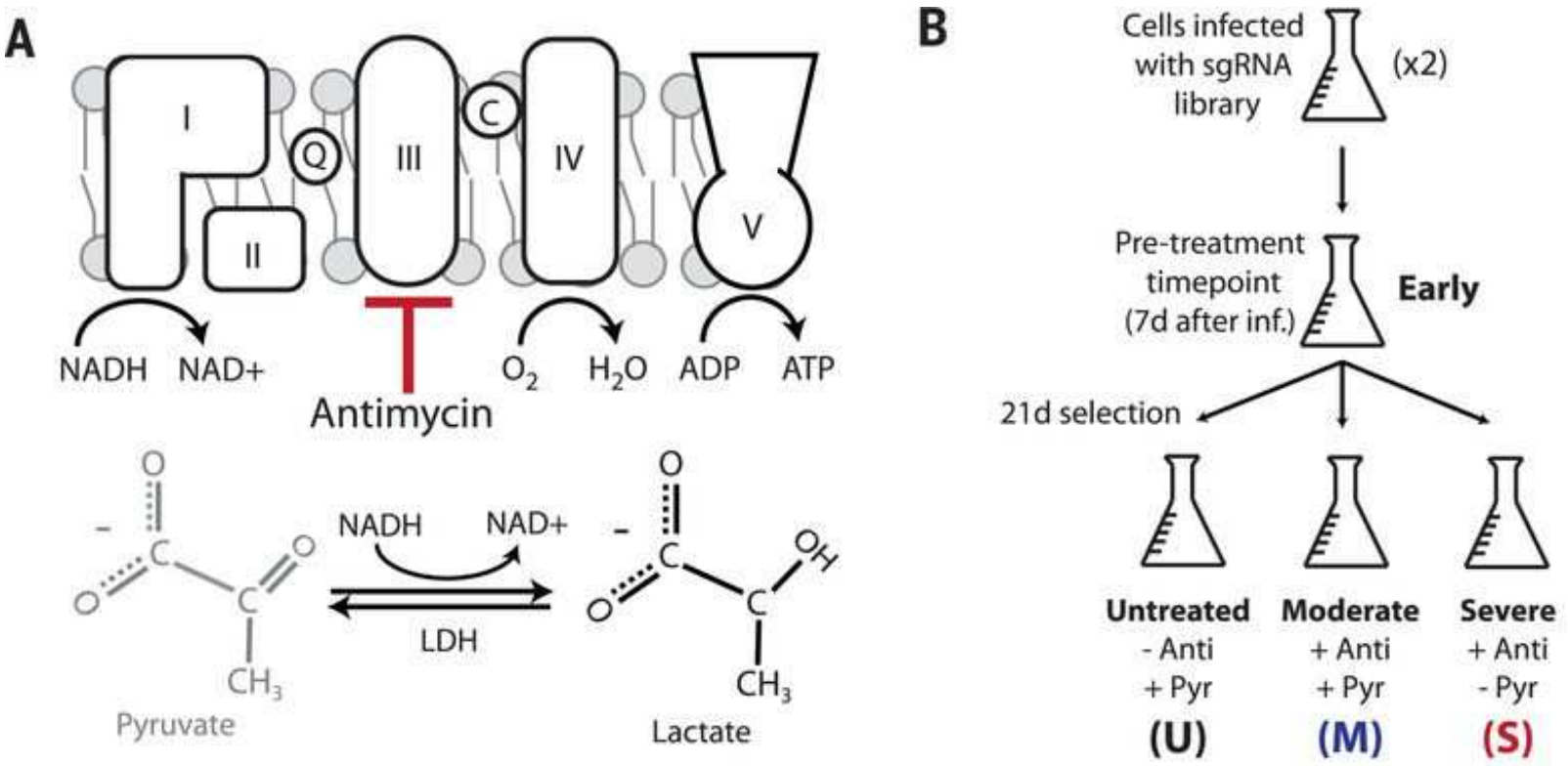
Shift in the 14-day curve represents the depletion in a subset of sgRNAs

The five most significantly depleted gene sets



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

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



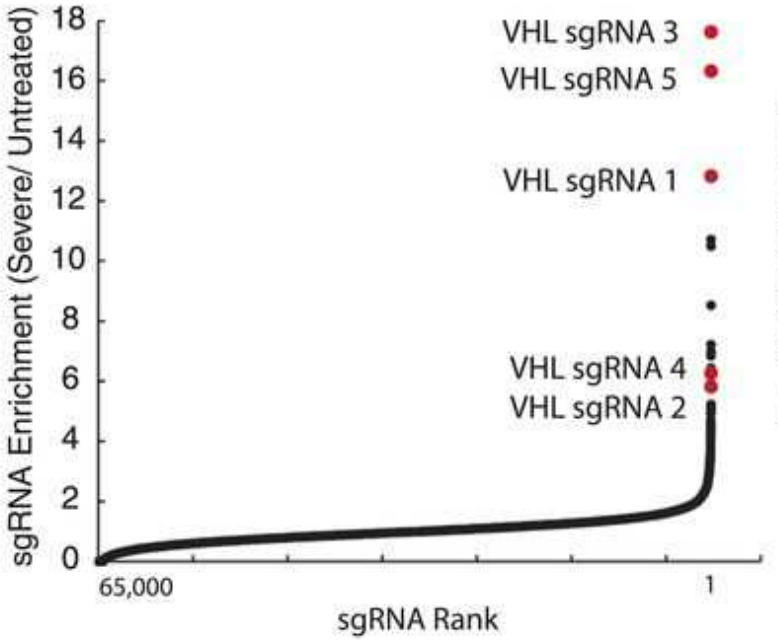
Isha H. Jain et al. Science 2016;352:54-61

Fig. 1 Genome-scale Cas9-mediated knockout screen identifies VHL inhibition as protective during states of mitochondrial dysfunction.

D

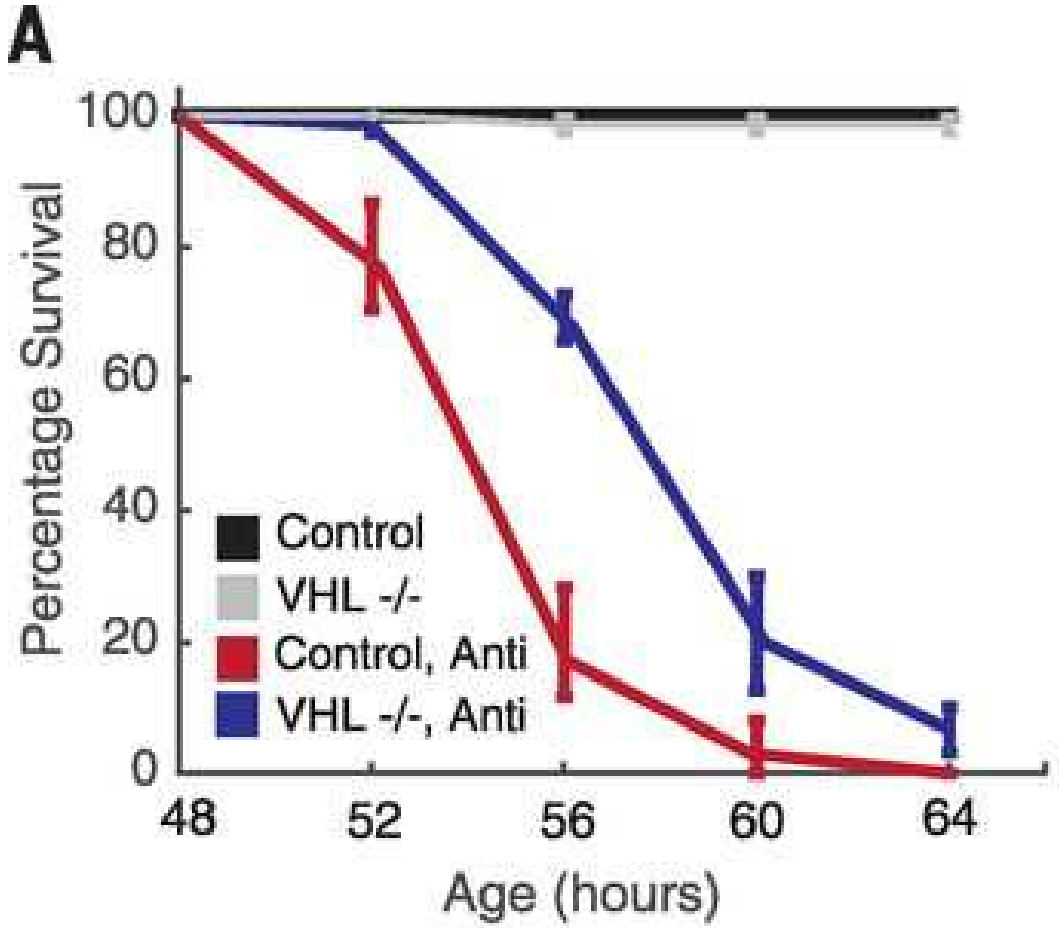
Gene	sgRNA Ranks	Rank
VHL	1, 2, 3, 12, 14	1
RGS20	13, 145, 2266, 8296, 27675, 29239	2
SIN3A	32, 242	3
ESPNL	168, 199, 8244, 8519, 12532, 58512	4
EXOC3L4	47, 267, 6259, 7589	5
DOCK7	177, 299, 4796, 10550, 18350, 23644	6
NDUFS6	8, 403, 2876, 7677	7
CLSTN1	7, 412, 11644, 46491	8
CD101	139, 372, 14840, 30593,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

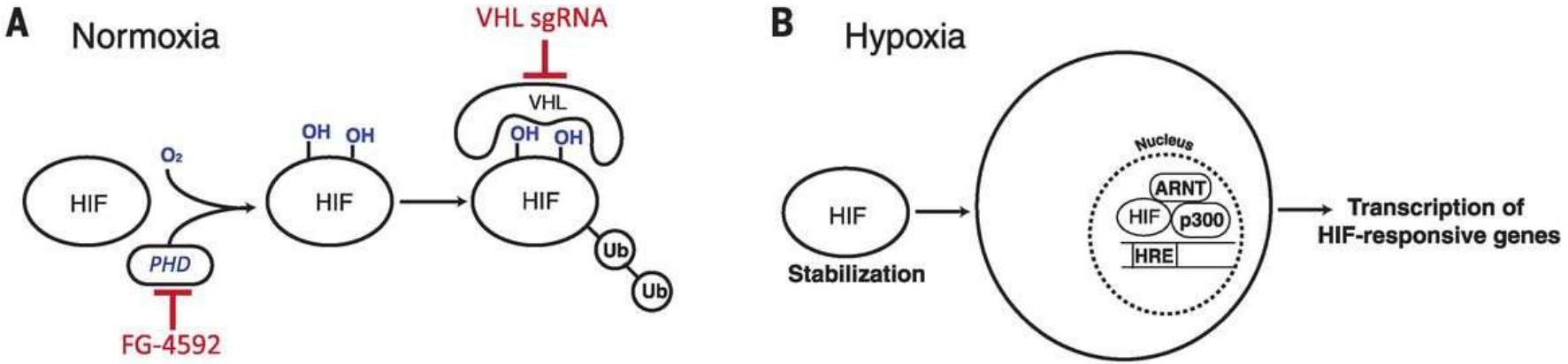


Anti = Respiratory Chain inhibition

Published by AAAS

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

Fig. 2 Genetic or small-molecule activation of the HIF response is protective against multiple forms of RC inhibition, in multiple cell types.



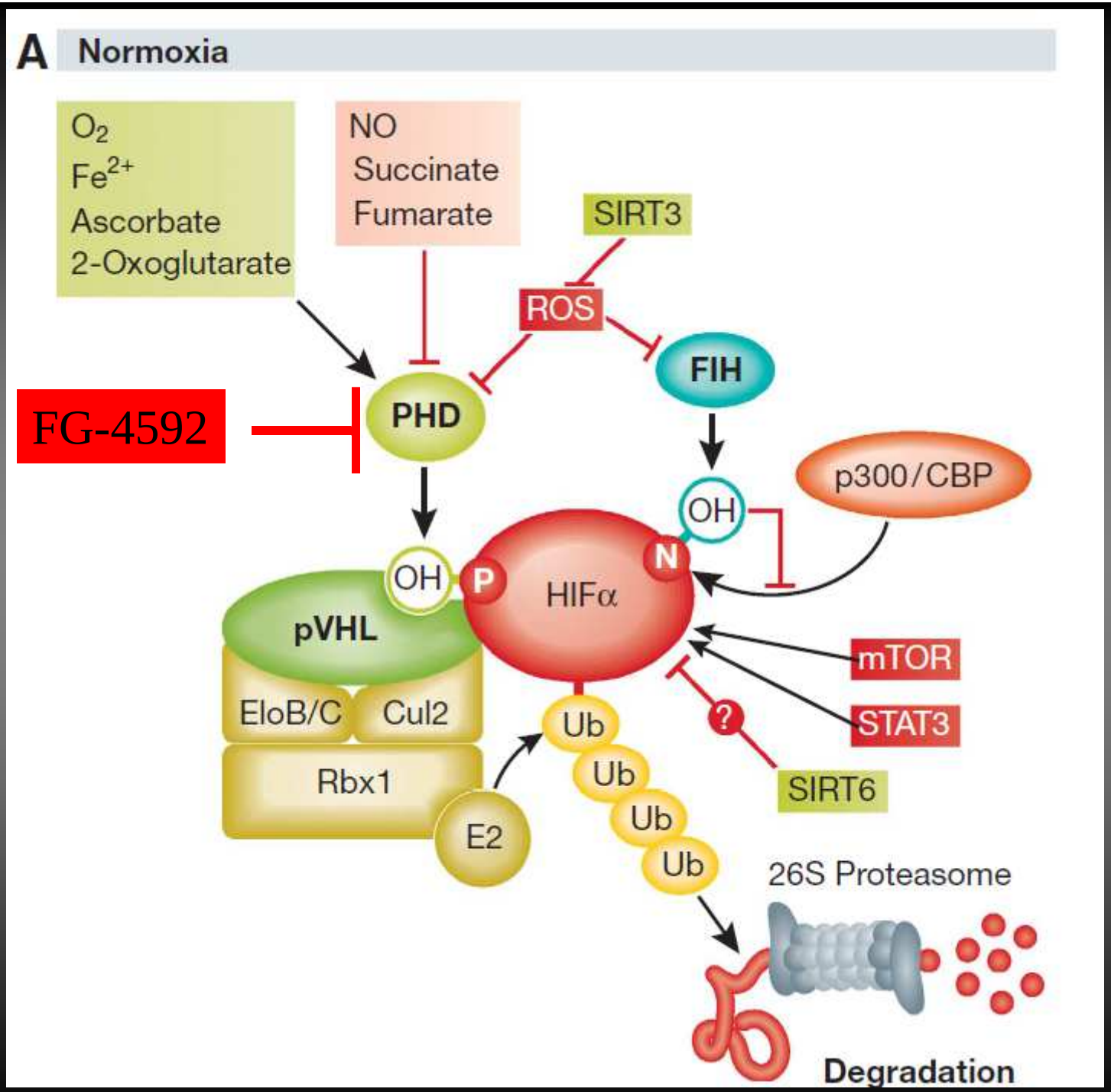
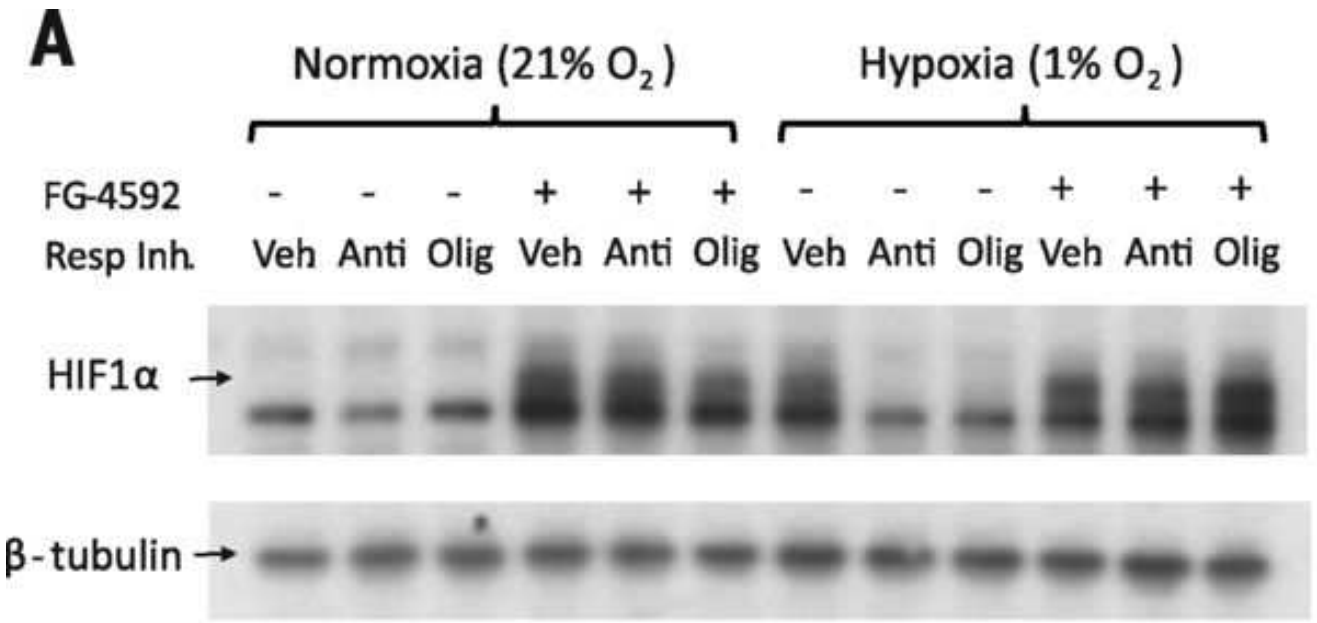

A Normoxia
O2
Fe2+
Ascorbate
2-Oxoglutarate
NO
Succinate
Fumarate
SIRT3
ROS
FIH
FG-4592
PHD
p300/CBP
OH
OH
P
N
HIFα
pVHL
EloB/C
Cul2
Rbx1
E2
Ub
Ub
Ub
Ub
mTOR
STAT3
?
SIRT6
26S Proteasome
Degradation

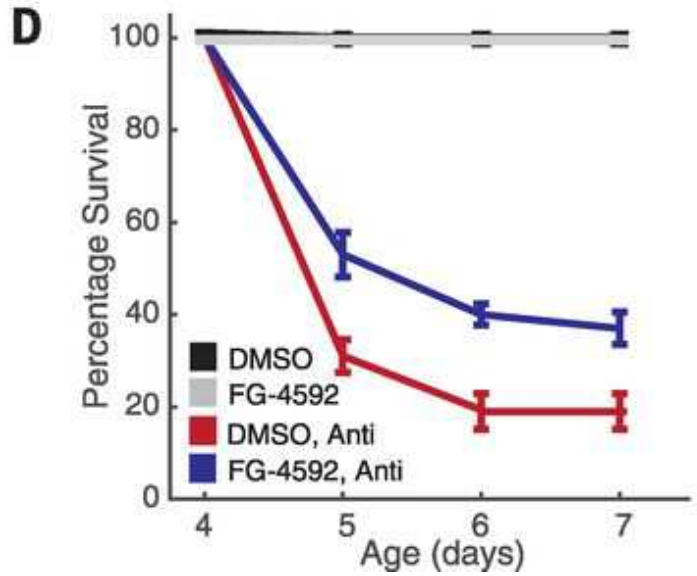
Fig. 3 FG-4592 causes normoxic stabilization of HIF1α and rewires energy metabolism.



HIF1α Immunoblot
± Respiratory chain RC inhibition with antimycin or oligomycin
± FG-4592 under normoxia (21% O2) or hypoxia (1% O2)
RC inhibition prevents HIF1α stabilization during hypoxia
FG-4592 administration overcomes this paradox and stabilizes HIF1α even during normoxia.

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.



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

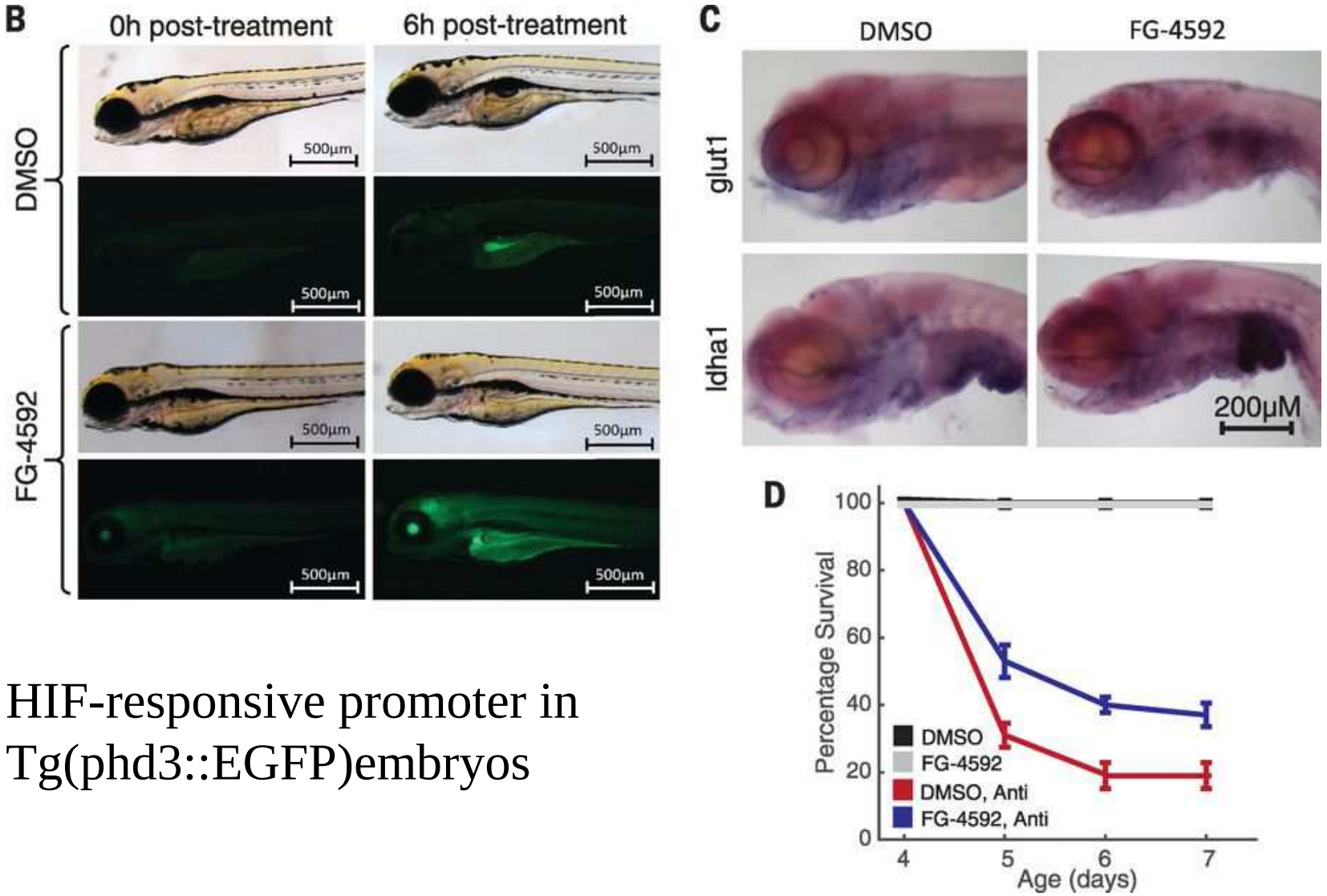
RC inhibition by 2.5 nM antimycin in 4 days post fertilization (dpf) embryos results in significant death within the first 24 hours of treatment.

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

Published by AAAS

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.

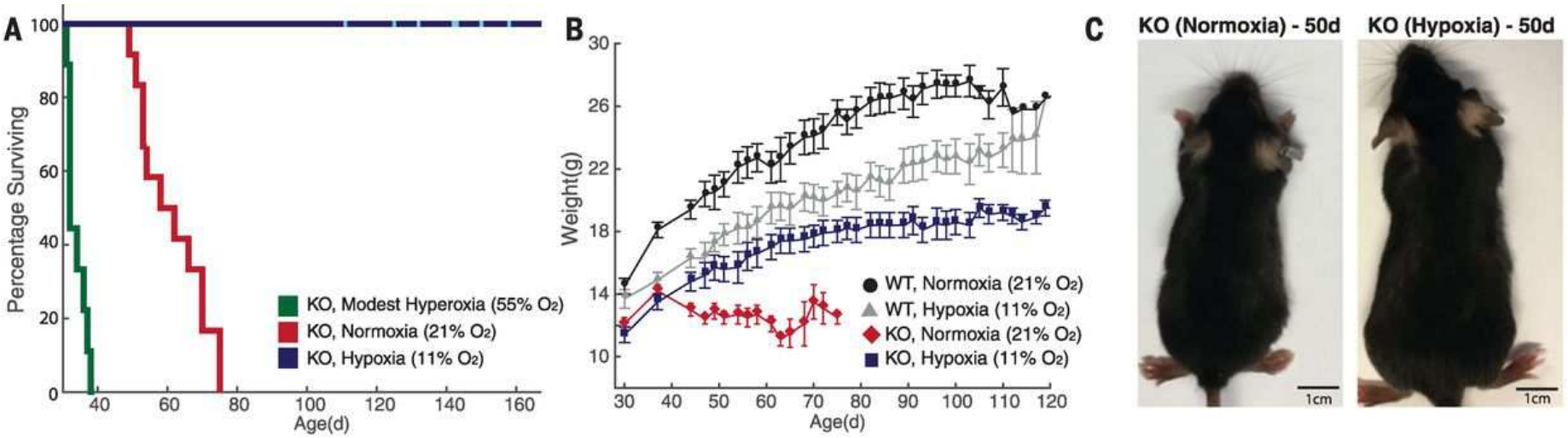


HIF-responsive promoter in Tg(phd3::EGFP)embryos

Published by AAAS

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