JOURNAL CLUB

Tabriz University of Medical Sciences

PARKIN TARGETS HIF-1A FOR UBIQUITINATION AND DEGRADATION TO INHIBIT BREAST TUMOR PROGRESSION

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Parkin targets HIF-1α for ubiquitination and degradation to inhibit breast tumor progression

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Mutations in E3 ubiquitin ligase Parkin have been linked to familial Parkinson's disease. Accumulating evidence suggests that Parkin is a tumor suppressor, but the underlying mechanism is poorly understood. Here we show that Parkin is an E3 ubiquitin ligase for hypoxia-inducible factor 1α (HIF-1α). Parkin interacts with HIF-1α and promotes HIF-1α degradation through ubiquitination, which in turn inhibits metastasis of breast cancer cells. Parkin downregulation in breast cancer cells promotes metastasis, which can be inhibited by targeting HIF-1α with RNA interference or the small-molecule inhibitor YC-1. We further identify lysine 477 (K477) of HIF-1α as a major ubiquitination site for Parkin. K477R HIF-1α mutation and specific cancer-associated Parkin mutations largely abolish the functions of Parkin to ubiquitinate HIF-1α and inhibit cancer metastasis. Importantly, Parkin expression is inversely correlated with HIF-1α expression and metastasis in breast cancer. Our results reveal an important mechanism for Parkin in tumor suppression and HIF-1α regulation.

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RESONE

- Parkin protein is one of the most important proteins involved in in vivo metabolism.
- One of the important functions of this protein is to suppress tumors.
- Breast cancer is one of the most common cancers among women. According to the World Health Organization, in 2020, about 2.3 million people were diagnosed with this cancer and 685,000 people died worldwide.
- The treatment of this cancer is one of the main concerns for scientists. In this article, the effect of Parkin on preventing the progression of cancer, as well as the path it takes, has been studied.

INTRODUCTION

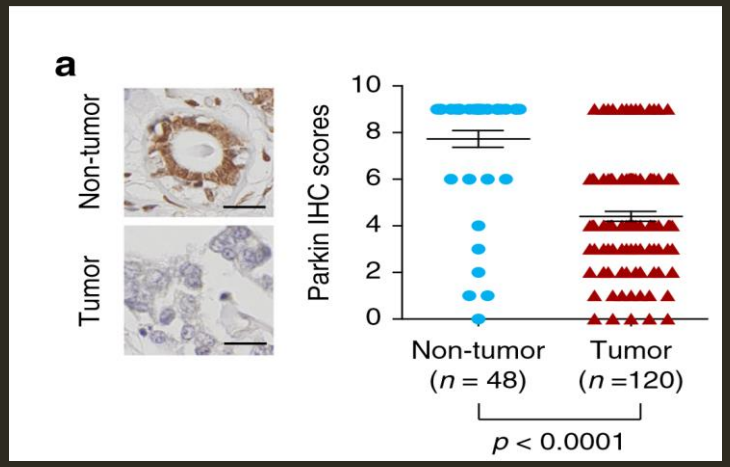
- The familial form of Parkinson's disease is caused by a mutation in the parkin protein
- Parkin acts as a E3 ubiquitin ligase
- evidence suggests that Parkin is a tumor suppressor
- Loss of heterozygosity and copy number loss of Parkin have been observed in human cancers, including breast cancer
- Parkin was also reported to interact with Cdc20/ Cdh1 to mediate ubiquitination and degradation of key mitotic regulators to maintain genomic stability

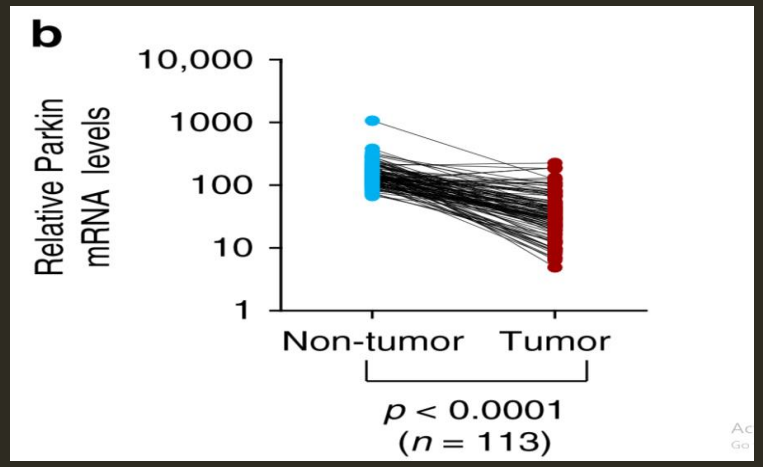
HIF-1

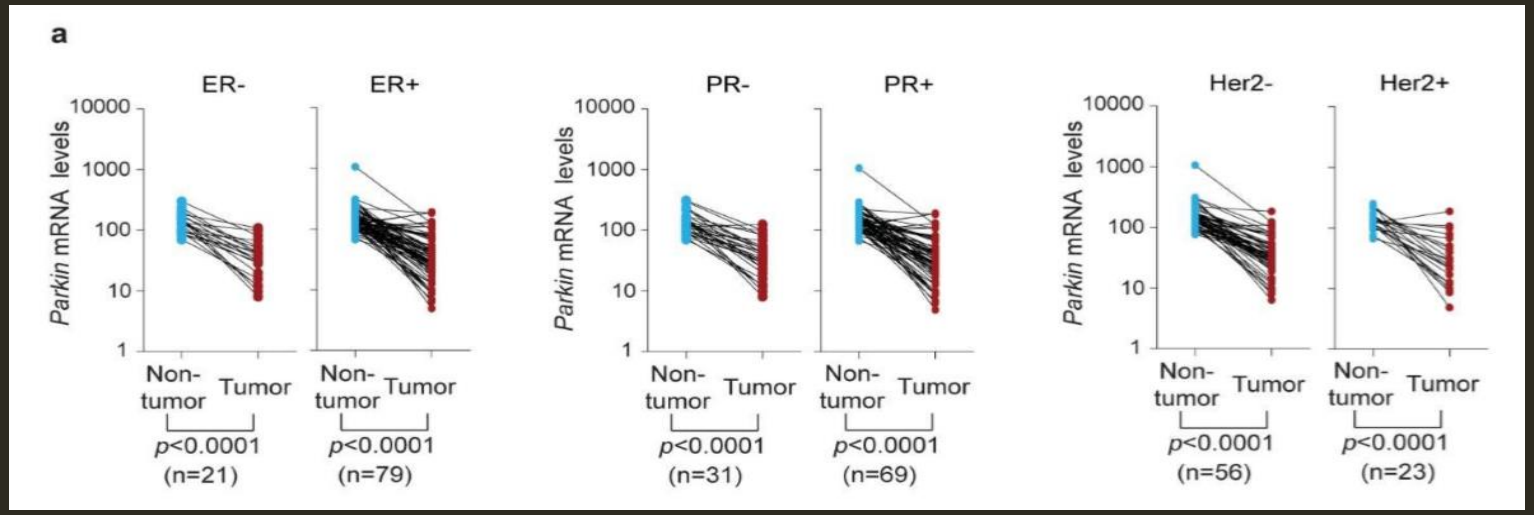
- HIF-1 is a heterodimeric transcription factor composed of an α and β subunit.
- HIF-1 binds to the hypoxia response element (HRE) to regulate gene expression

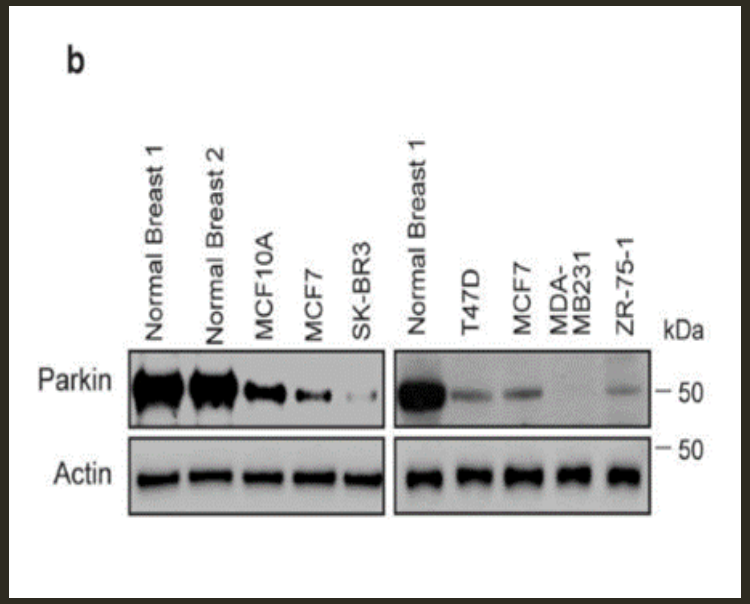
- HIF-1α is also regulated by VHL-independent mechanisms in cells.
- A recent study listed HIF-1α as a potential ubiquitination substrate of Parkin

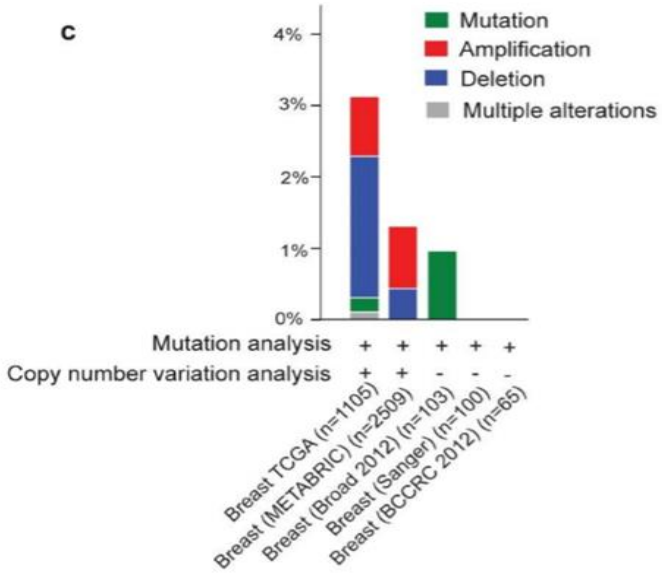
HIF-1A IS A PARKIN-INTERACTING PROTEIN

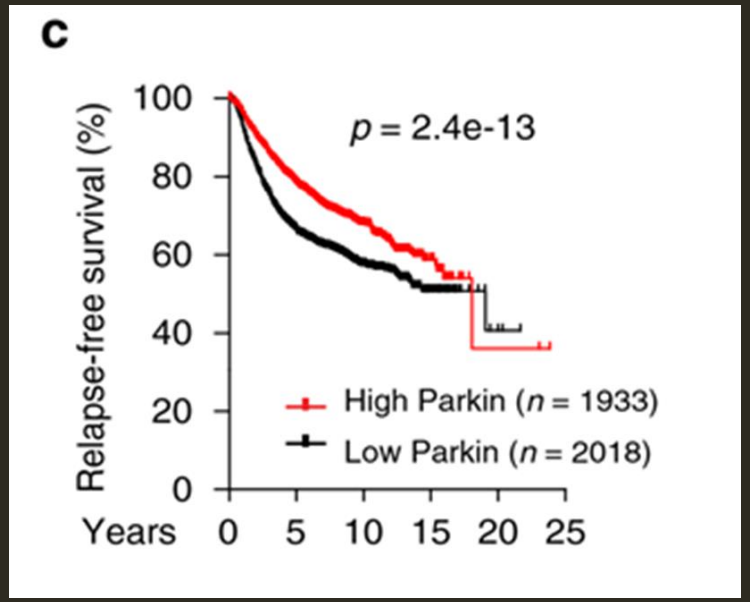




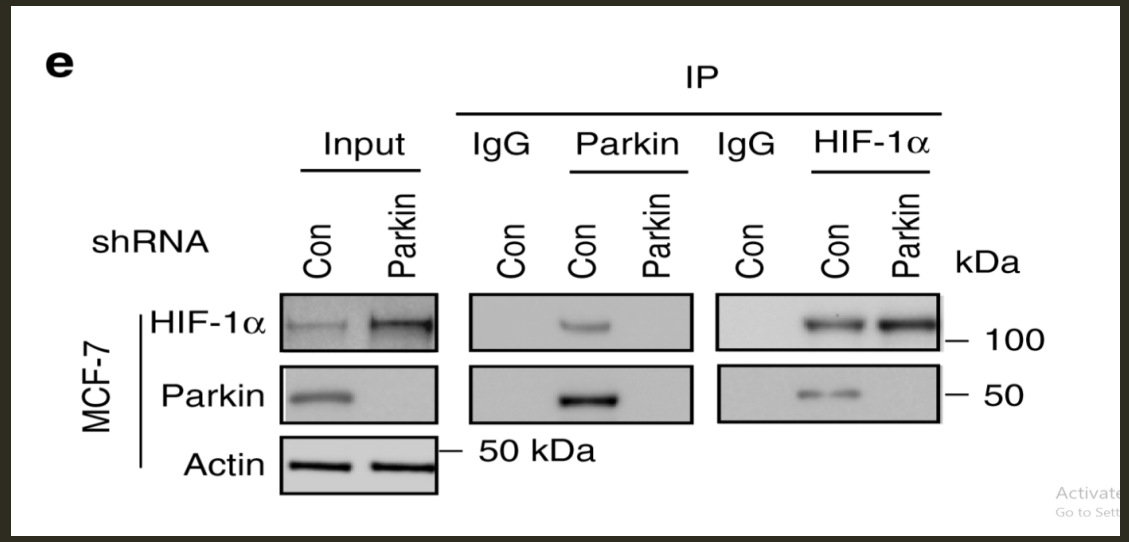


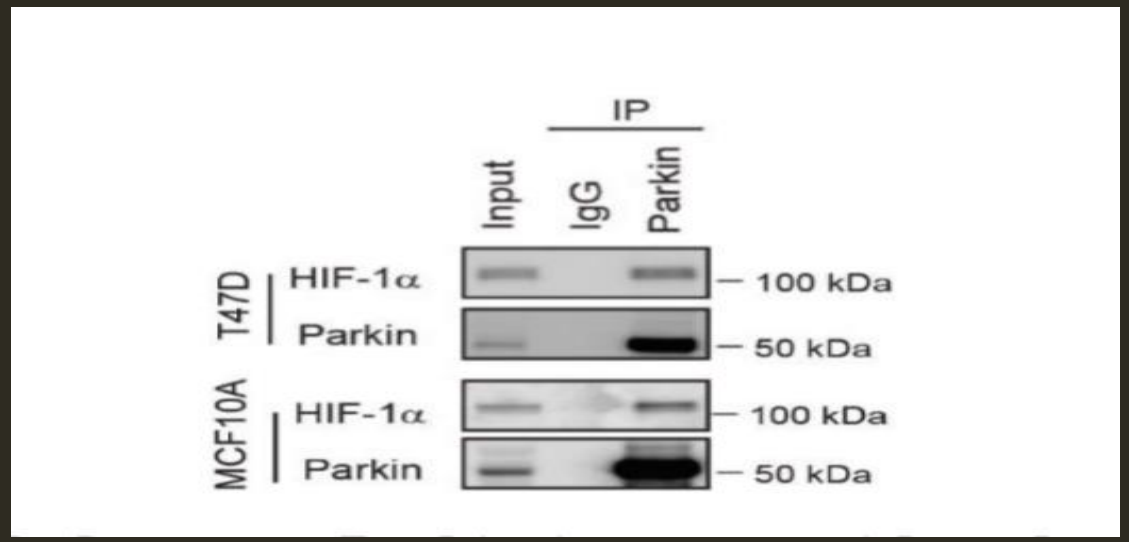


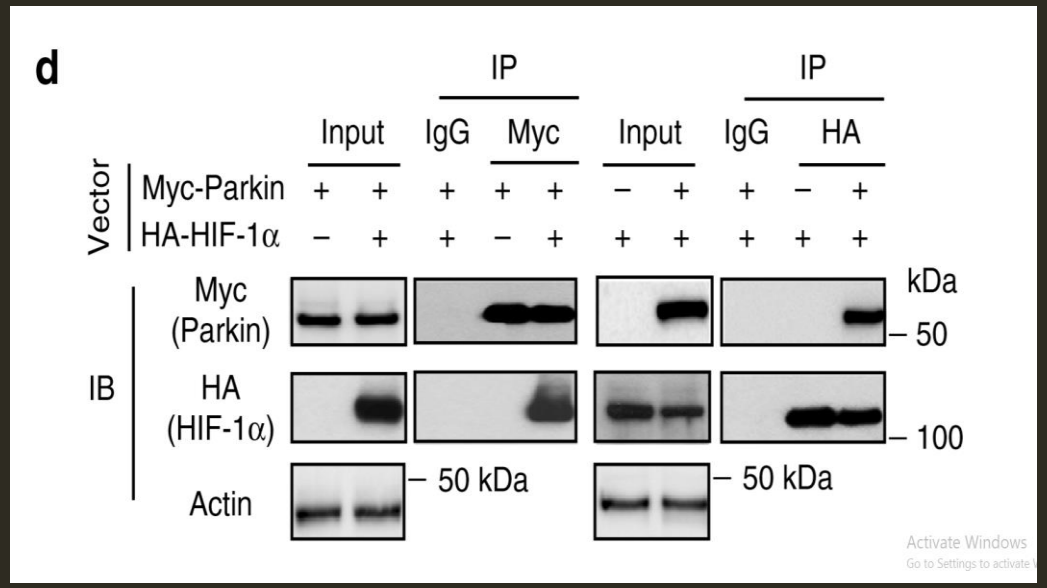


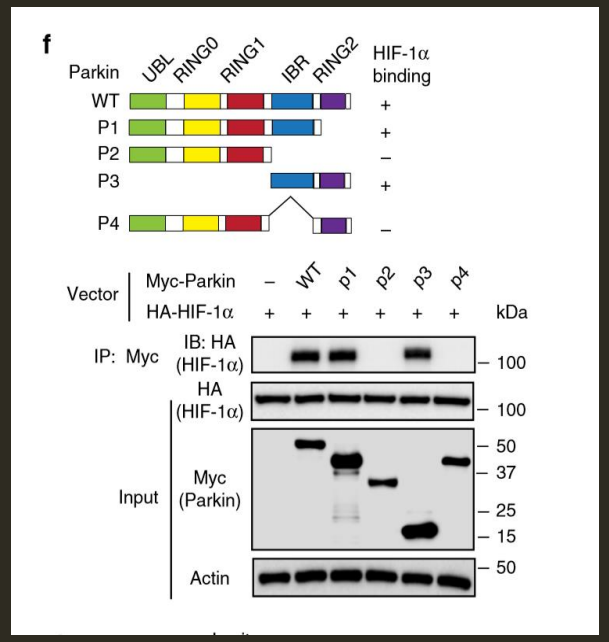


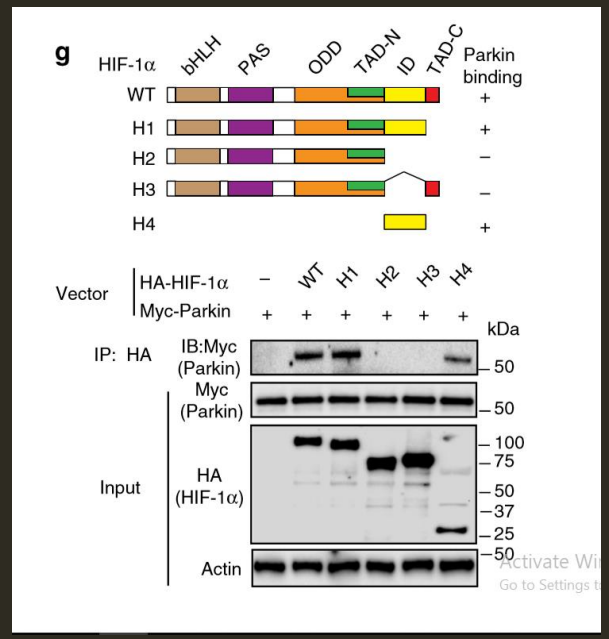
Potential interacting proteins for Parkin	Number of peptides	
	Control	Parkin
Laminin	0	17
HSP70	0	15
Rpn10	0	5
UBL4A	0	5
HIF-1α	**0**	**4**
OSGIN1	0	4
TOMM40	0	4
ABCE1	0	4
14-3-3	0	3
ATAD1	0	2
Tubulin	0	2

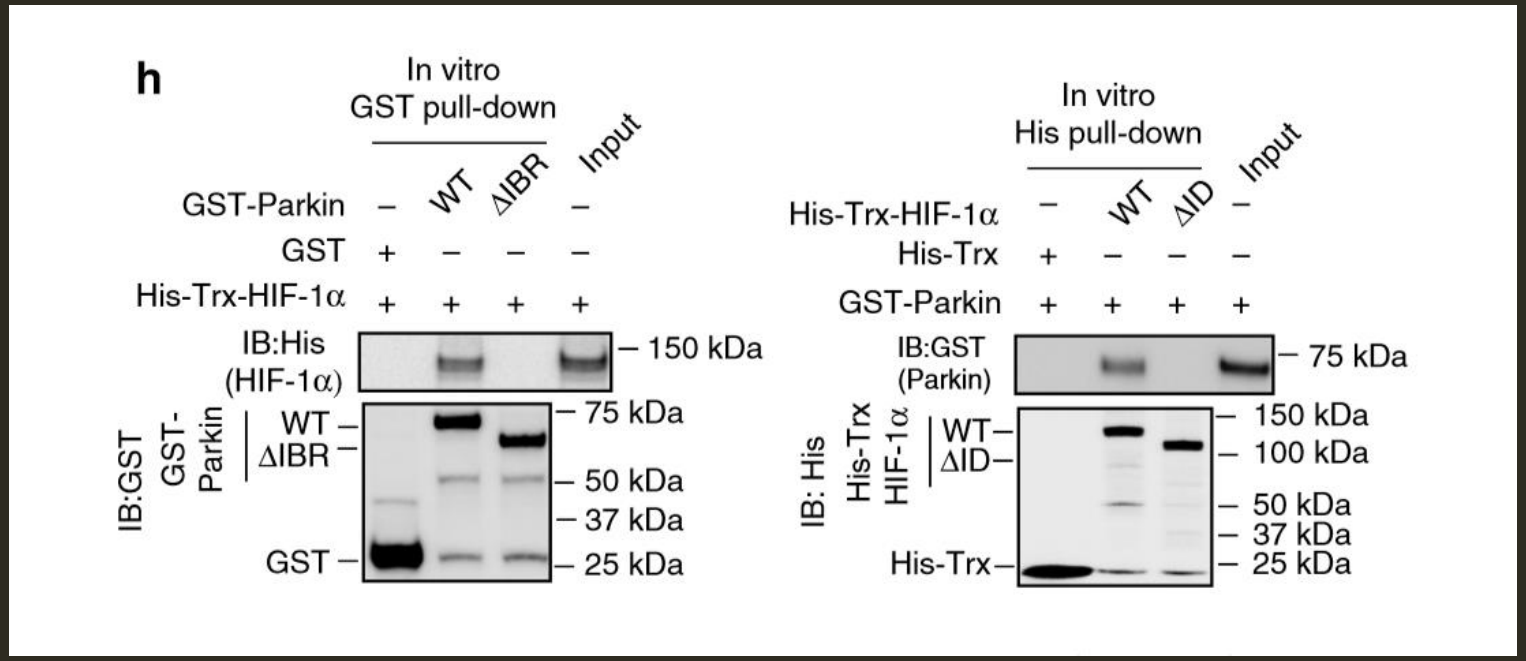

e
IP
Input
IgG
Parkin
IgG
HIF-1α
shRNA
Con
Parkin
Con
Con
Parkin
Con
Con
Parkin
kDa
MCF-7
HIF-1α
Parkin
Actin
100
50
50 kDa


IP
Input
IgG
Parkin
T47D
HIF-1α
Parkin
100 kDa
50 kDa
MCF10A
HIF-1α
Parkin
100 kDa
50 kDa

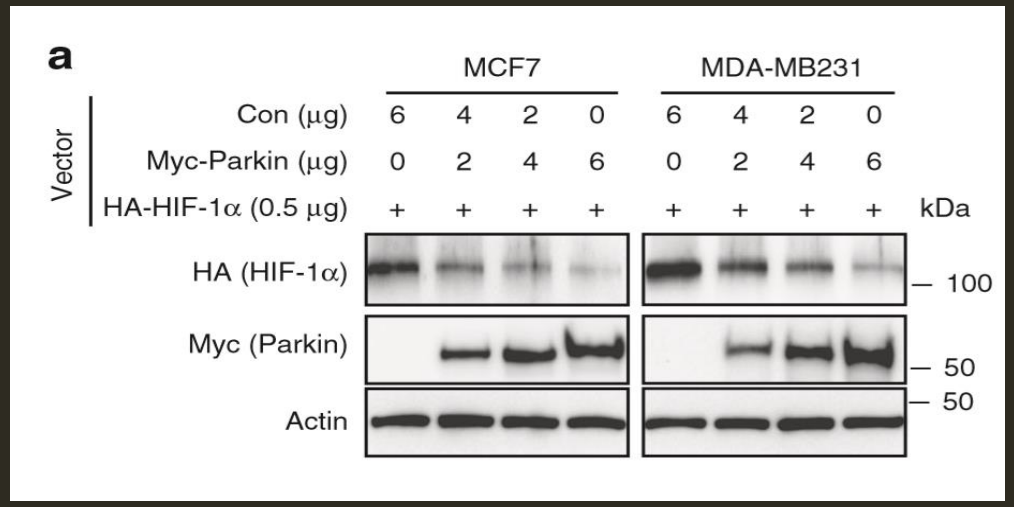

d
IP
IP
Input
IgG
Myc
Input
IgG
HA
Vector
Myc-Parkin
HA-HIF-1α
kDa
IB
Myc
(Parkin)
HA
(HIF-1α)
Actin
50
100
50 kDa
50 kDa

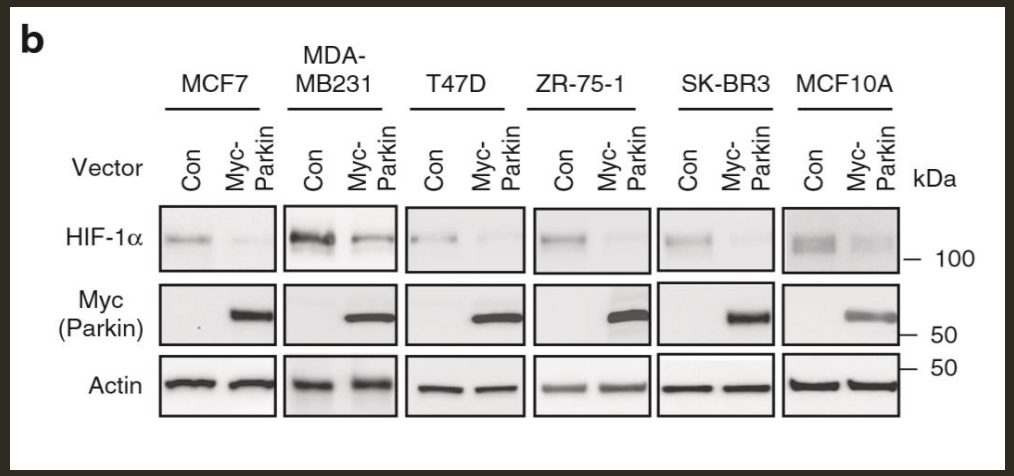

f
Parkin
UBL
RING0
RING1
IBR
RING2
HIF-1α binding
WT
P1
P2
P3
P4
Vector
Myc-Parkin
HA-HIF-1α
WT
p1
p2
p3
p4
kDa
IP: Myc
IB: HA (HIF-1α)
HA (HIF-1α)
Input
Myc (Parkin)
Actin
100
100
50
37
25
15
50

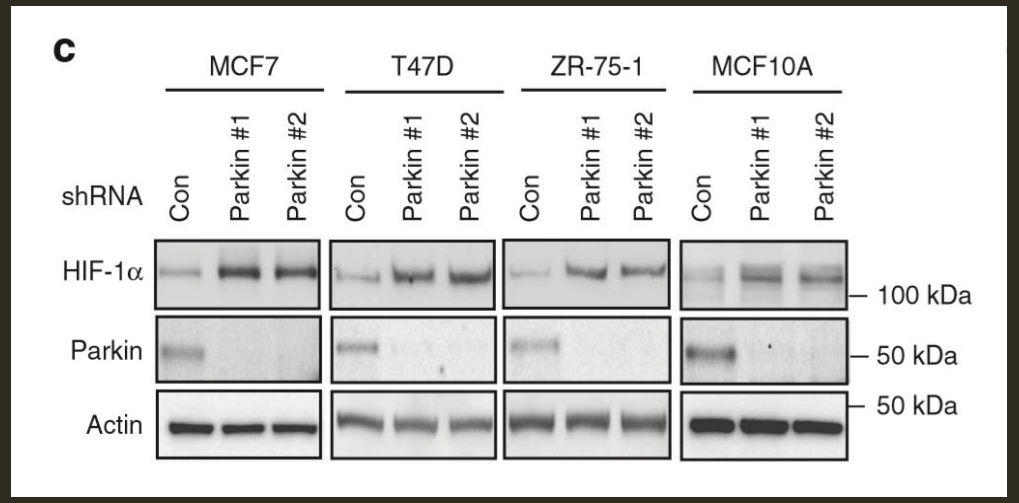

g
HIF-1α
bHLH
PAS
ODD
TAD-N
ID
TAD-C
Parkin binding
WT
H1
H2
H3
H4
Vector
HA-HIF-1α
Myc-Parkin
WT
H1
H2
H3
H4
kDa
IP: HA
IB:Myc (Parkin)
Myc (Parkin)
Input
HA (HIF-1α)
Actin
50
50
100
75
50
37
25
50

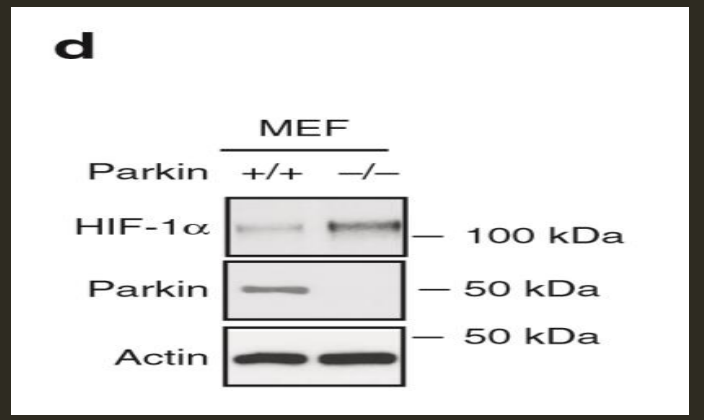

h
In vitro GST pull-down
GST-Parkin
GST
His-Trx-HIF-1α
WT
ΔIBR
Input
IB:His (HIF-1α)
150 kDa
IB:GST GST-Parkin
WT
ΔIBR
GST
75 kDa
50 kDa
37 kDa
25 kDa
In vitro His pull-down
His-Trx-HIF-1α
His-Trx
GST-Parkin
WT
ΔID
Input
IB:GST (Parkin)
75 kDa
IB: His His-Trx HIF-1α
WT
ΔID
His-Trx
150 kDa
100 kDa
50 kDa
37 kDa
25 kDa

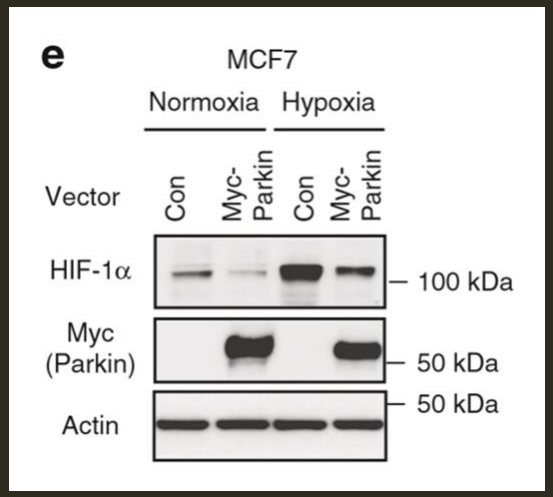
PARKIN DOWNREGULATES HIF-1A PROTEIN LEVELS IN CELLS

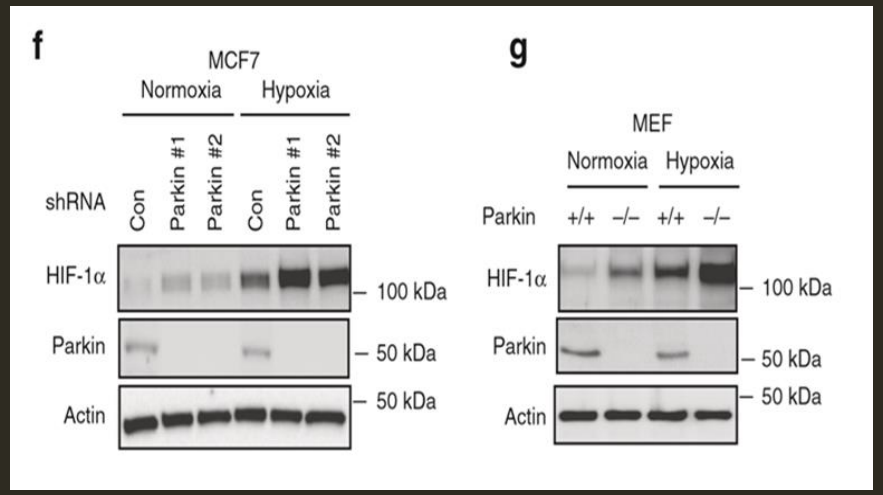


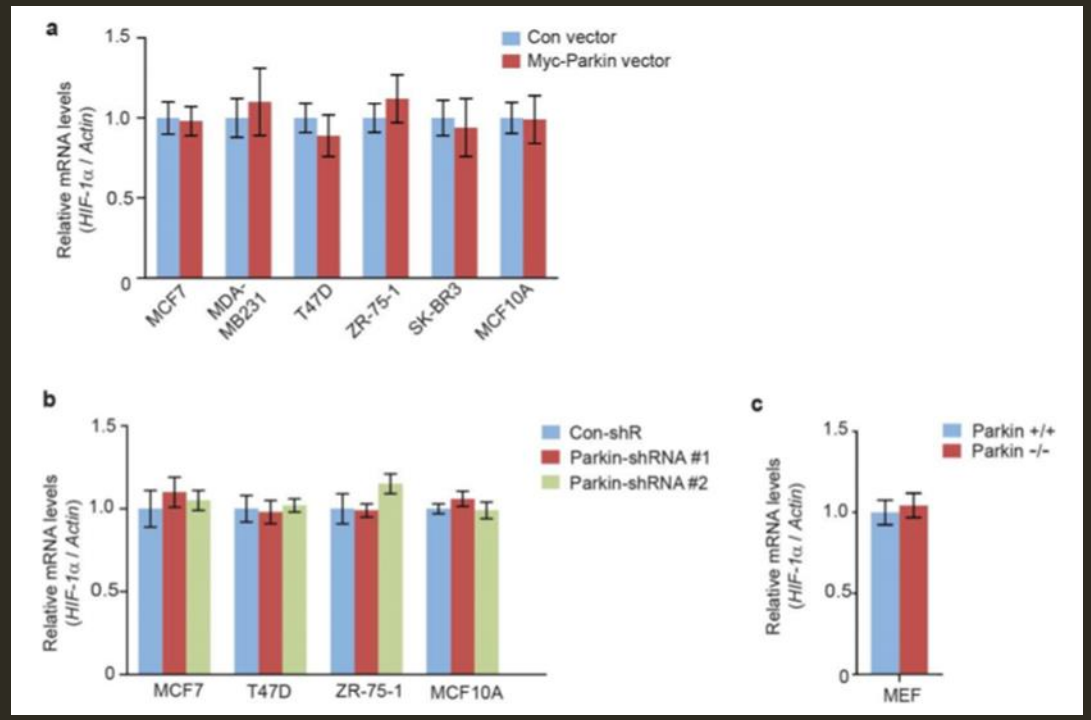


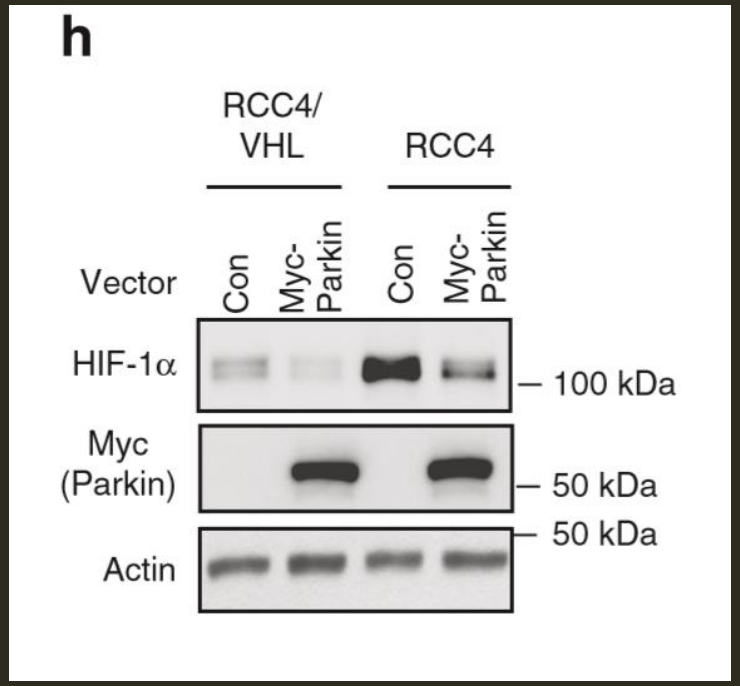


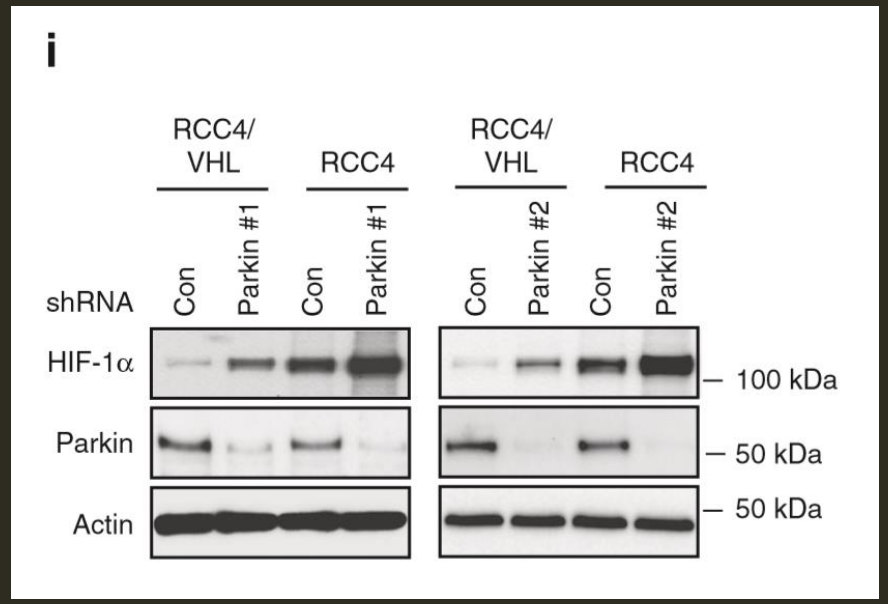


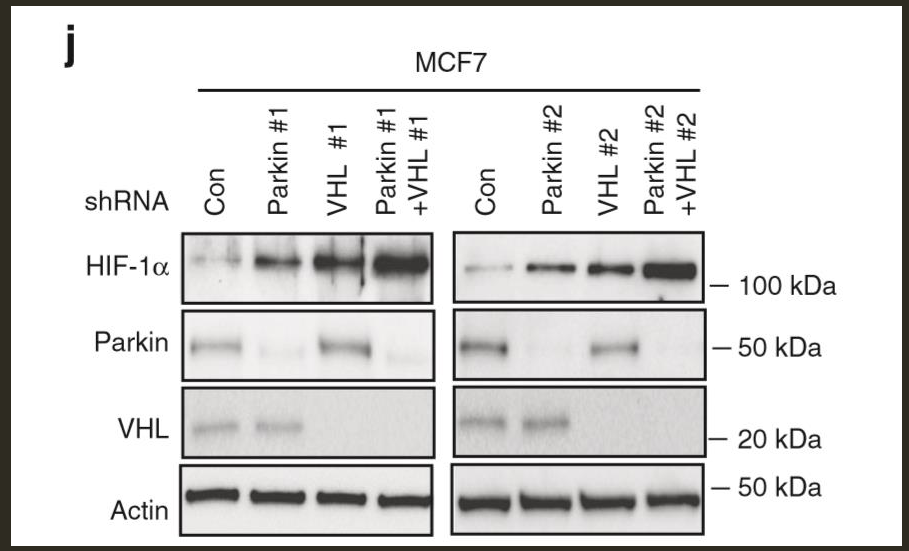

e
MCF7
Normoxia
Hypoxia
Vector
Con
Myc-Parkin
Con
Myc-Parkin
HIF-1α
100 kDa
Myc (Parkin)
50 kDa
Actin
50 kDa


f
MCF7
Normoxia
Hypoxia
shRNA
Con
Parkin #1
Parkin #2
Con
Parkin #1
Parkin #2
HIF-1α
100 kDa
Parkin
50 kDa
Actin
50 kDa
g
MEF
Normoxia
Hypoxia
Parkin
+/+
–/–
+/+
–/–
HIF-1α
100 kDa
Parkin
50 kDa
Actin
50 kDa

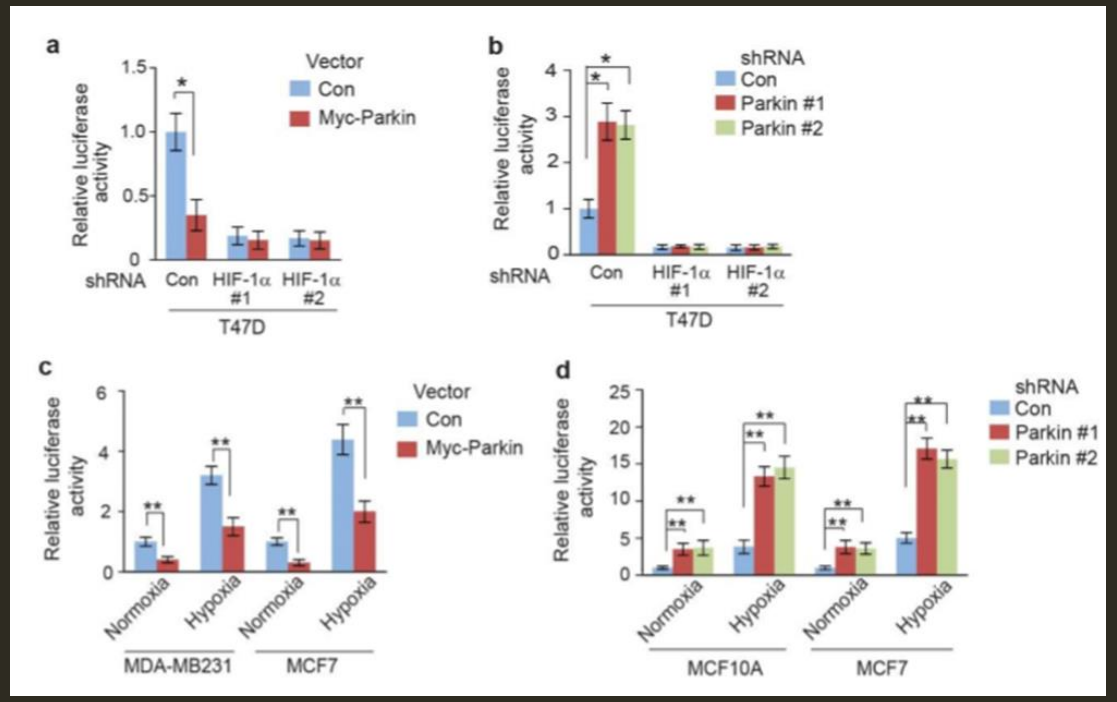

a
Con vector
Myc-Parkin vector
Relative mRNA levels (HIF-1α / Actin)
1.5
1.0
0.5
0
MCF7
MDA-MB231
T47D
ZR-75-1
SK-BR3
MCF10A
b
Con-shR
Parkin-shRNA #1
Parkin-shRNA #2
Relative mRNA levels (HIF-1α / Actin)
MCF7
T47D
ZR-75-1
MCF10A
c
Parkin +/+
Parkin -/-
Relative mRNA levels (HIF-1α / Actin)
MEF

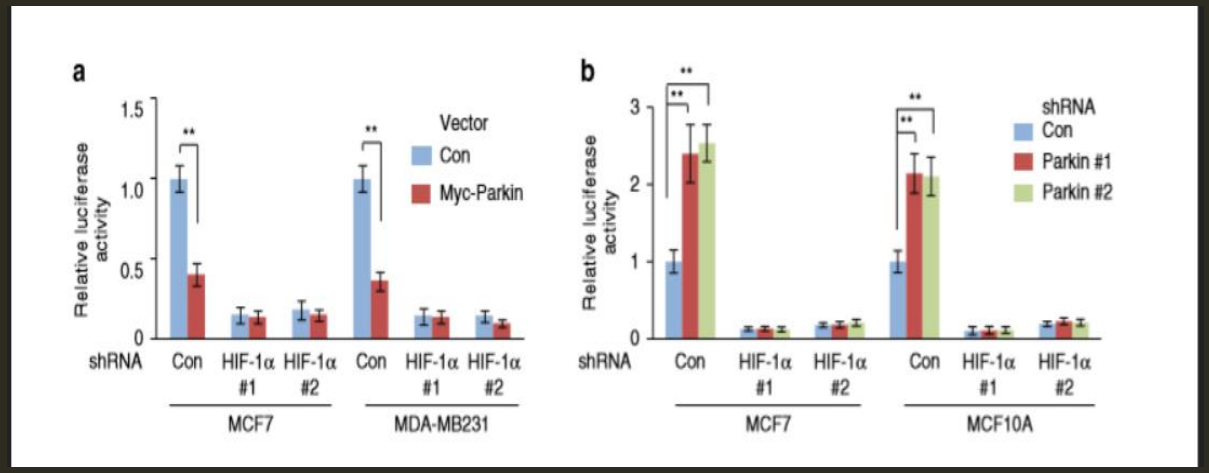

h
RCC4/
VHL
RCC4
Vector
Con
Myc-
Parkin
Con
Myc-
Parkin
HIF-1α
100 kDa
Myc
(Parkin)
50 kDa
50 kDa
Actin

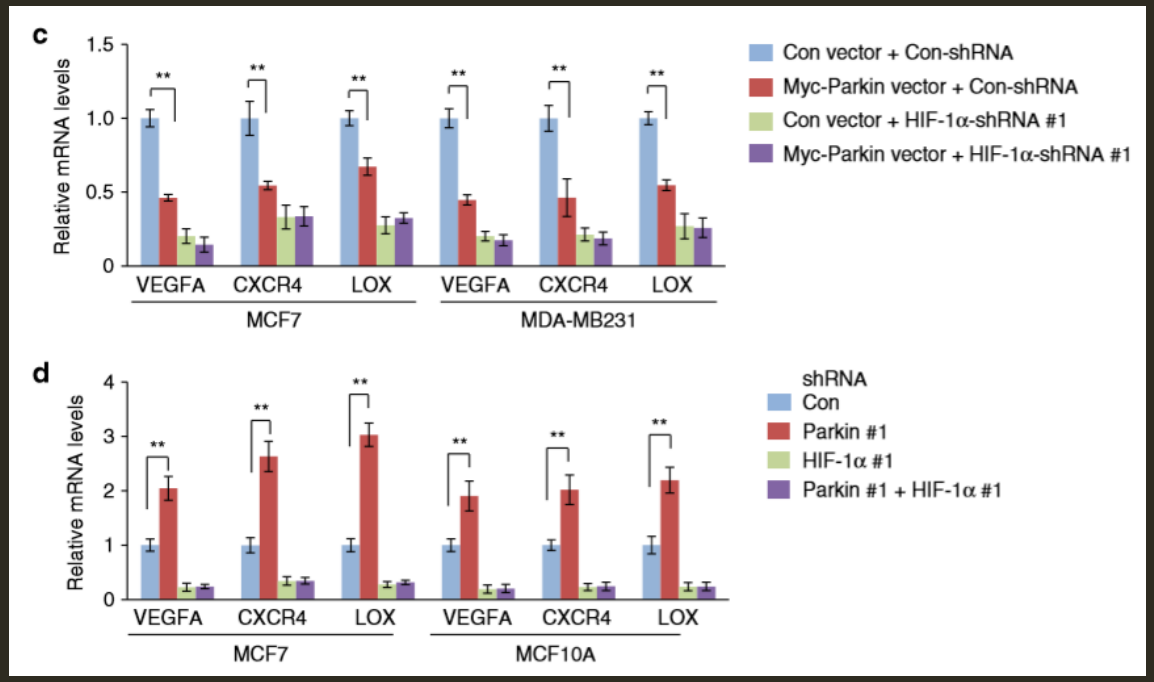

i
RCC4/
VHL
RCC4
RCC4/
VHL
RCC4
shRNA
Con
Parkin #1
Con
Parkin #1
Con
Parkin #2
Con
Parkin #2
HIF-1α
100 kDa
Parkin
50 kDa
Actin
50 kDa

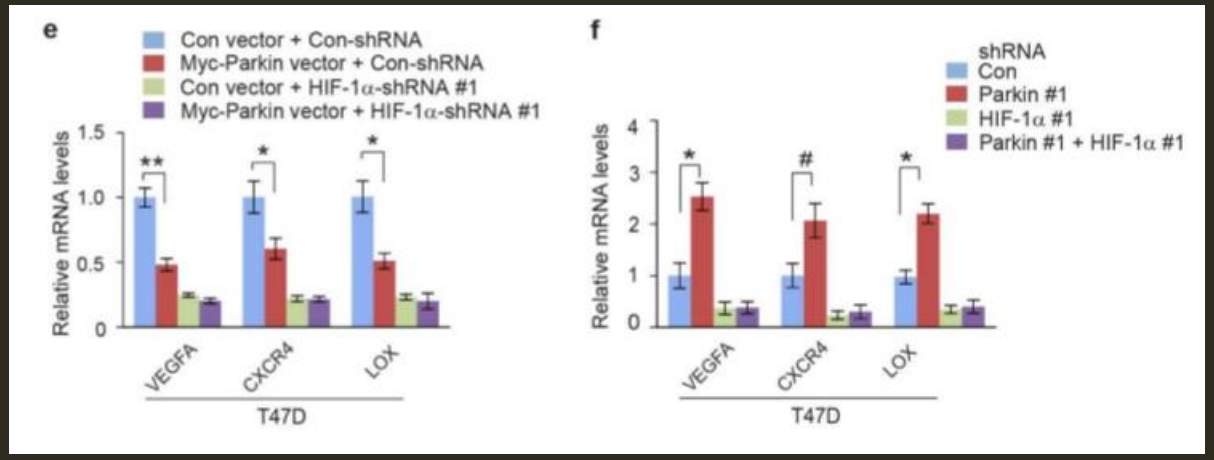

j
MCF7
shRNA
Con
Parkin #1
VHL #1
Parkin #1
+VHL #1
Con
Parkin #2
VHL #2
Parkin #2
+VHL #2
HIF-1α
100 kDa
Parkin
50 kDa
VHL
20 kDa
Actin
50 kDa

PARKIN NEGATIVELY REGULATES HIF-1-ATRANSCRIPTIONAL ACTIVITY

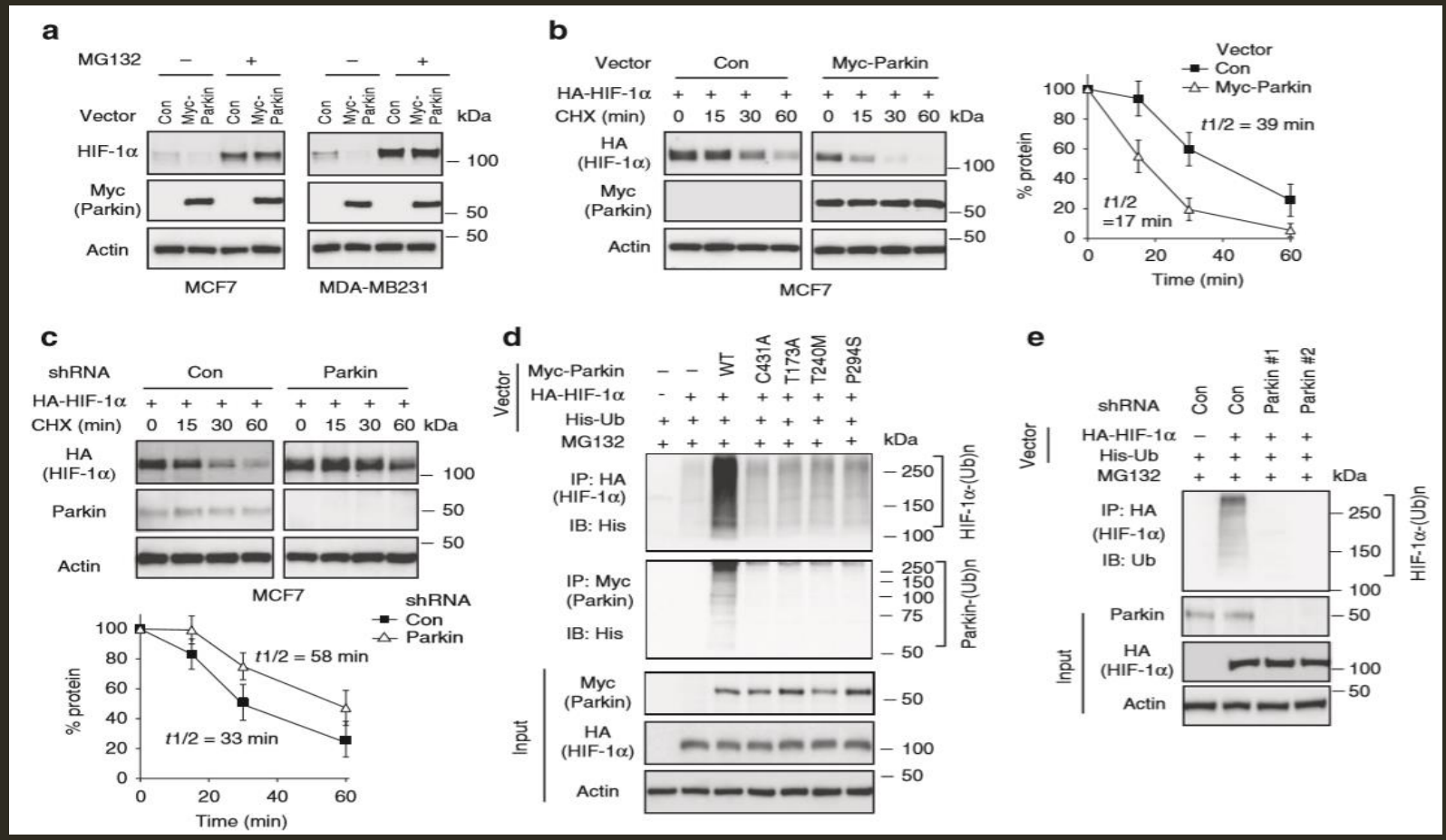


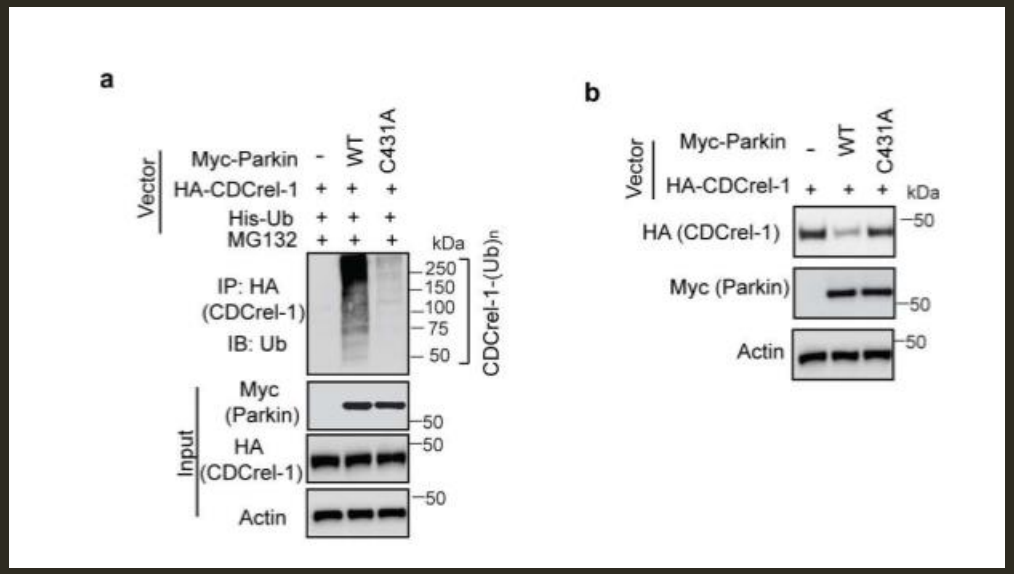


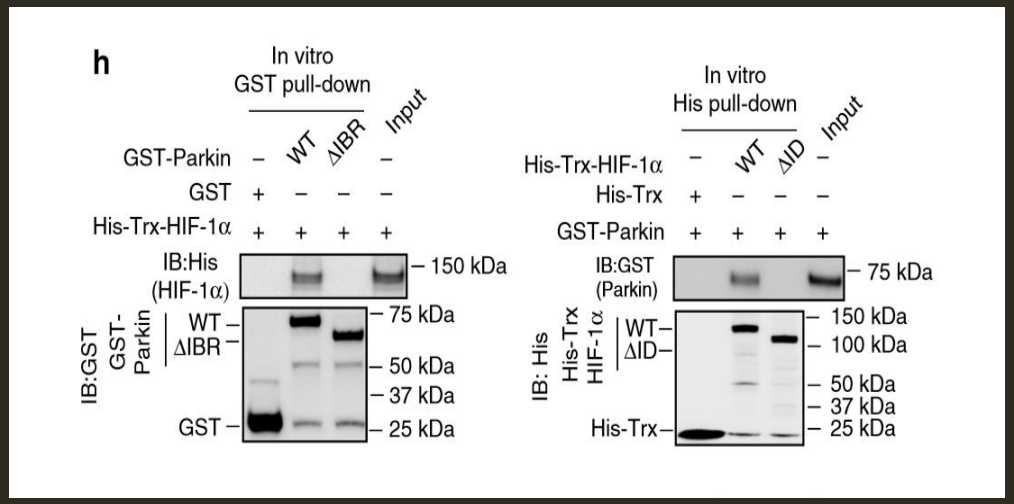

c
Relative mRNA levels
1.5
1.0
0.5
0
**
VEGFA
CXCR4
LOX
MCF7
MDA-MB231
Con vector + Con-shRNA
Myc-Parkin vector + Con-shRNA
Con vector + HIF-1α-shRNA #1
Myc-Parkin vector + HIF-1α-shRNA #1
d
Relative mRNA levels
4
3
2
1
0
MCF7
MCF10A
shRNA
Con
Parkin #1
HIF-1α #1
Parkin #1 + HIF-1α #1

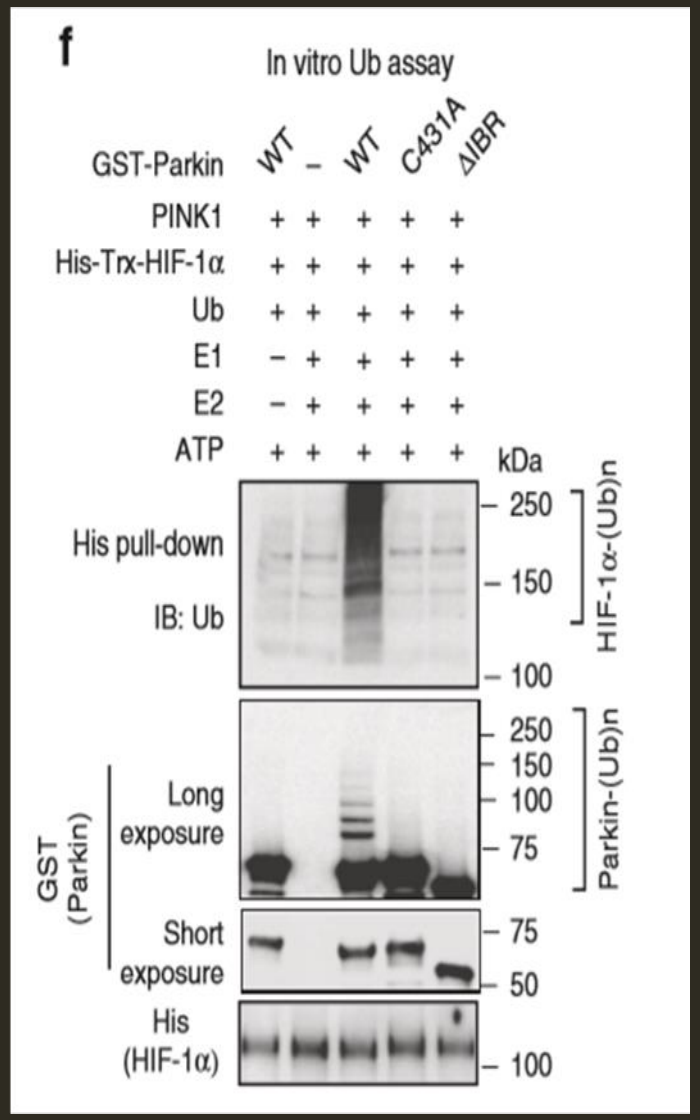

e
Con vector + Con-shRNA
Myc-Parkin vector + Con-shRNA
Con vector + HIF-1α-shRNA #1
Myc-Parkin vector + HIF-1α-shRNA #1
Relative mRNA levels
1.5
1.0
0.5
0
**
*
*
VEGFA
CXCR4
LOX
T47D
f
shRNA
Con
Parkin #1
HIF-1α #1
Parkin #1 + HIF-1α #1
Relative mRNA levels
4
3
2
1
0
*
#
*
VEGFA
CXCR4
LOX
T47D

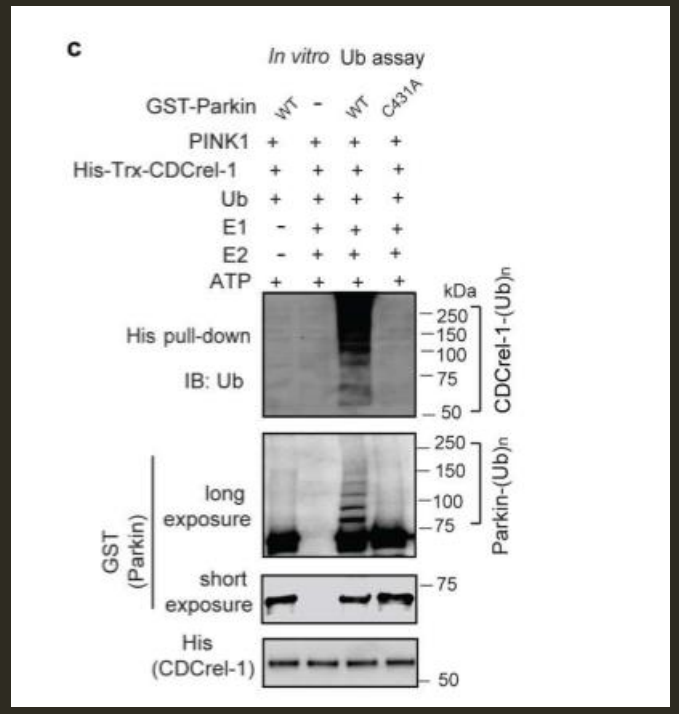
PARKIN PROMOTES UBIQUITINATION AND DEGRADATION OF HIF-1A

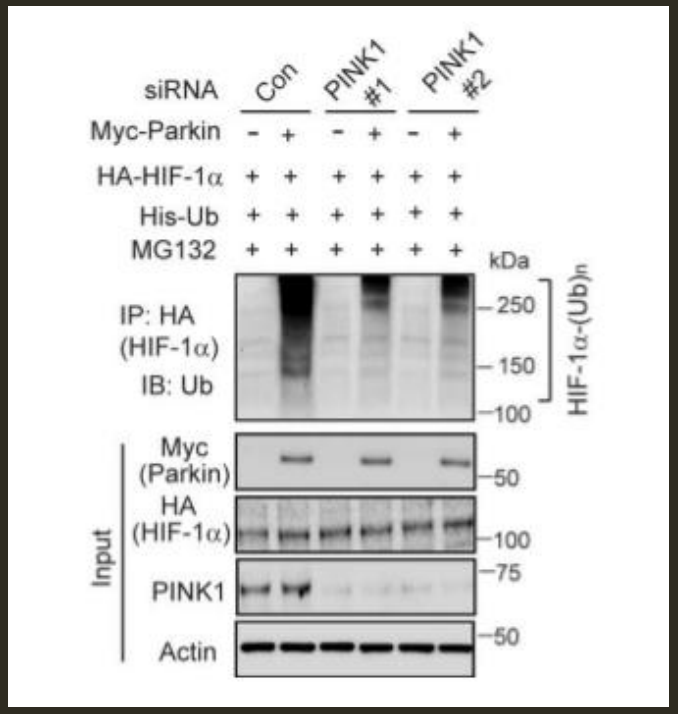


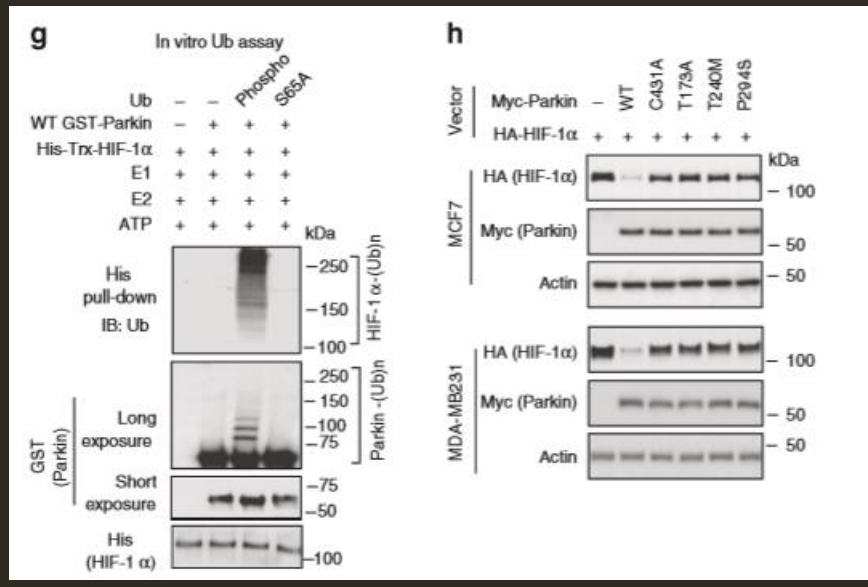

a
Vector
Myc-Parkin
HA-CDCrel-1
His-Ub
MG132
WT
C431A
kDa
IP: HA (CDCrel-1)
IB: Ub
250
150
100
75
50
CDCrel-1-(Ub)n
Input
Myc (Parkin)
HA (CDCrel-1)
Actin
b
Vector
Myc-Parkin
HA-CDCrel-1
WT
C431A
kDa
HA (CDCrel-1)
Myc (Parkin)
Actin
50


h
In vitro GST pull-down
GST-Parkin
GST
His-Trx-HIF-1α
WT
ΔIBR
Input
IB:His (HIF-1α)
150 kDa
IB:GST GST-Parkin
75 kDa
50 kDa
37 kDa
25 kDa
In vitro His pull-down
His-Trx-HIF-1α
His-Trx
GST-Parkin
WT
ΔID
Input
IB:GST (Parkin)
75 kDa
IB: His His-Trx HIF-1α
150 kDa
100 kDa
50 kDa
37 kDa
25 kDa
His-Trx

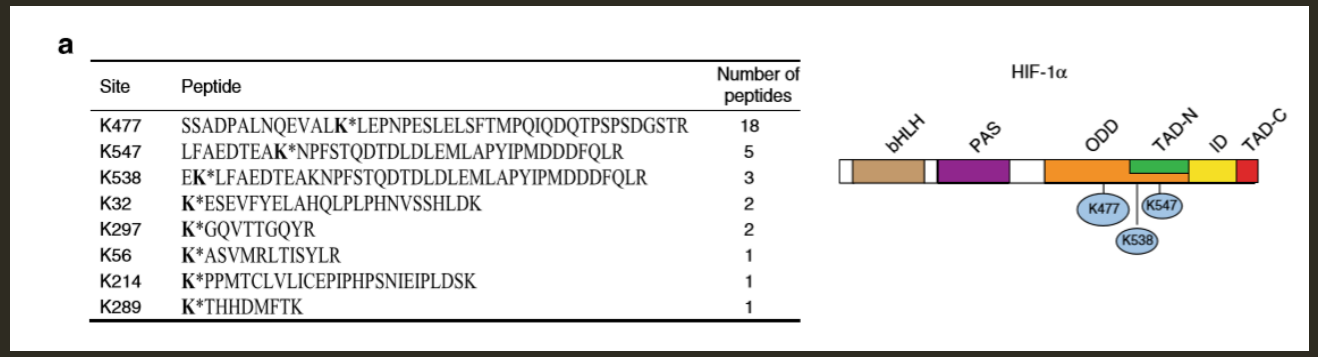

f
In vitro Ub assay
GST-Parkin
WT
–
WT
C431A
ΔIBR
PINK1
His-Trx-HIF-1α
Ub
E1
E2
ATP
kDa
His pull-down
IB: Ub
250
150
100
HIF-1α-(Ub)n
GST (Parkin)
Long exposure
Short exposure
75
50
Parkin-(Ub)n
His (HIF-1α)

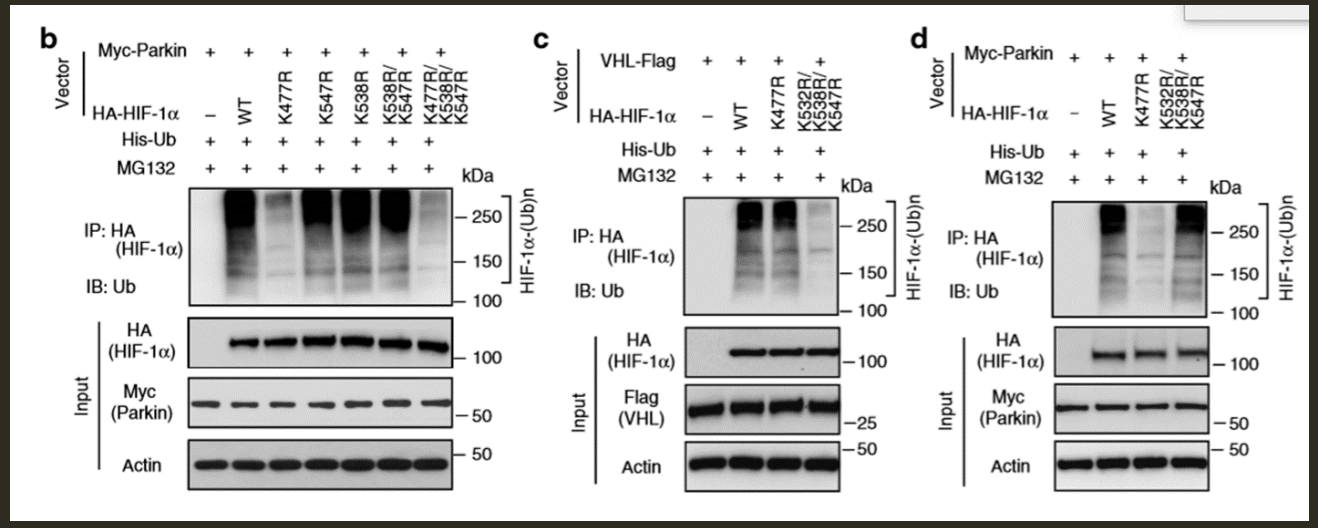

c
In vitro Ub assay
GST-Parkin
WT
-
WT
C431A
PINK1
His-Trx-CDCrel-1
Ub
E1
E2
ATP
kDa
His pull-down
IB: Ub
250
150
100
75
50
CDCrel-1-(Ub)n
GST (Parkin)
long exposure
short exposure
Parkin-(Ub)n
His (CDCrel-1)

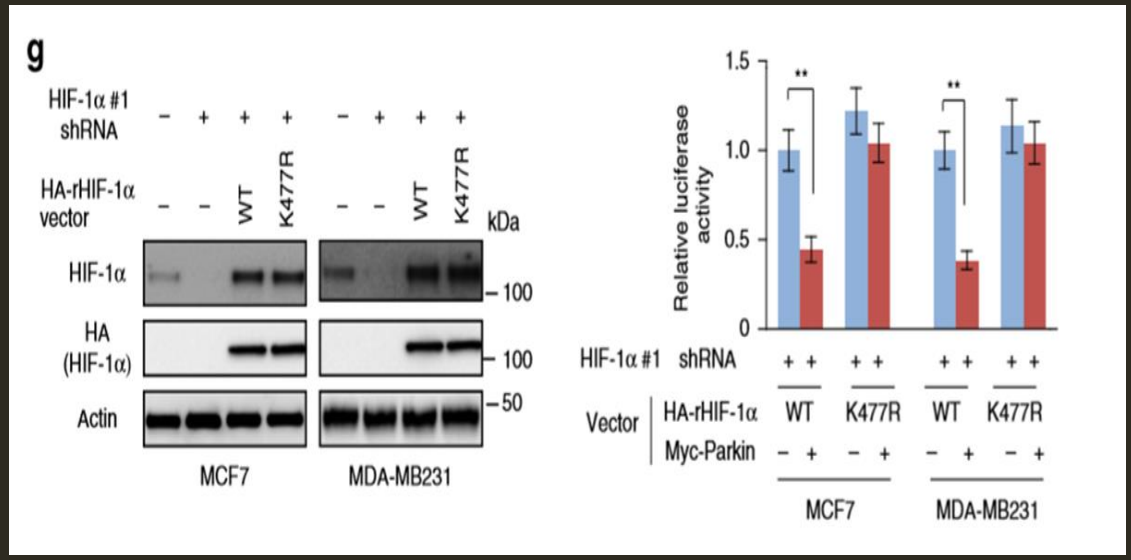

siRNA
Con
PINK1 #1
PINK1 #2
Myc-Parkin
HA-HIF-1α
His-Ub
MG132
kDa
IP: HA (HIF-1α)
IB: Ub
250
150
100
HIF-1α-(Ub)n
Input
Myc (Parkin)
HA (HIF-1α)
PINK1
Actin
50
100
75

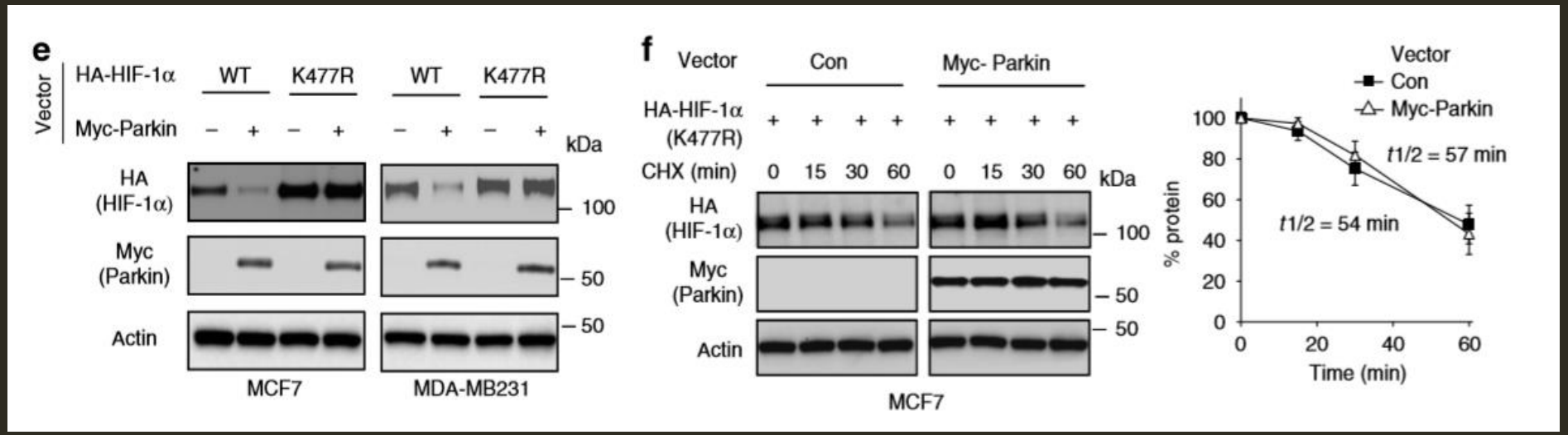

g
In vitro Ub assay
Ub
WT GST-Parkin
His-Trx-HIF-1α
E1
E2
ATP
Phospho S65A
kDa
His pull-down
IB: Ub
250
150
100
HIF-1 α-(Ub)n
GST (Parkin)
Long exposure
Short exposure
Parkin -(Ub)n
75
50
His (HIF-1 α)
h
Vector
Myc-Parkin
WT
C431A
T173A
T240M
P294S
HA-HIF-1α
MCF7
HA (HIF-1α)
Myc (Parkin)
Actin
MDA-MB231

UBIQUITINATION OF HIF-1A AT LYSINE 477 BY PARKIN.

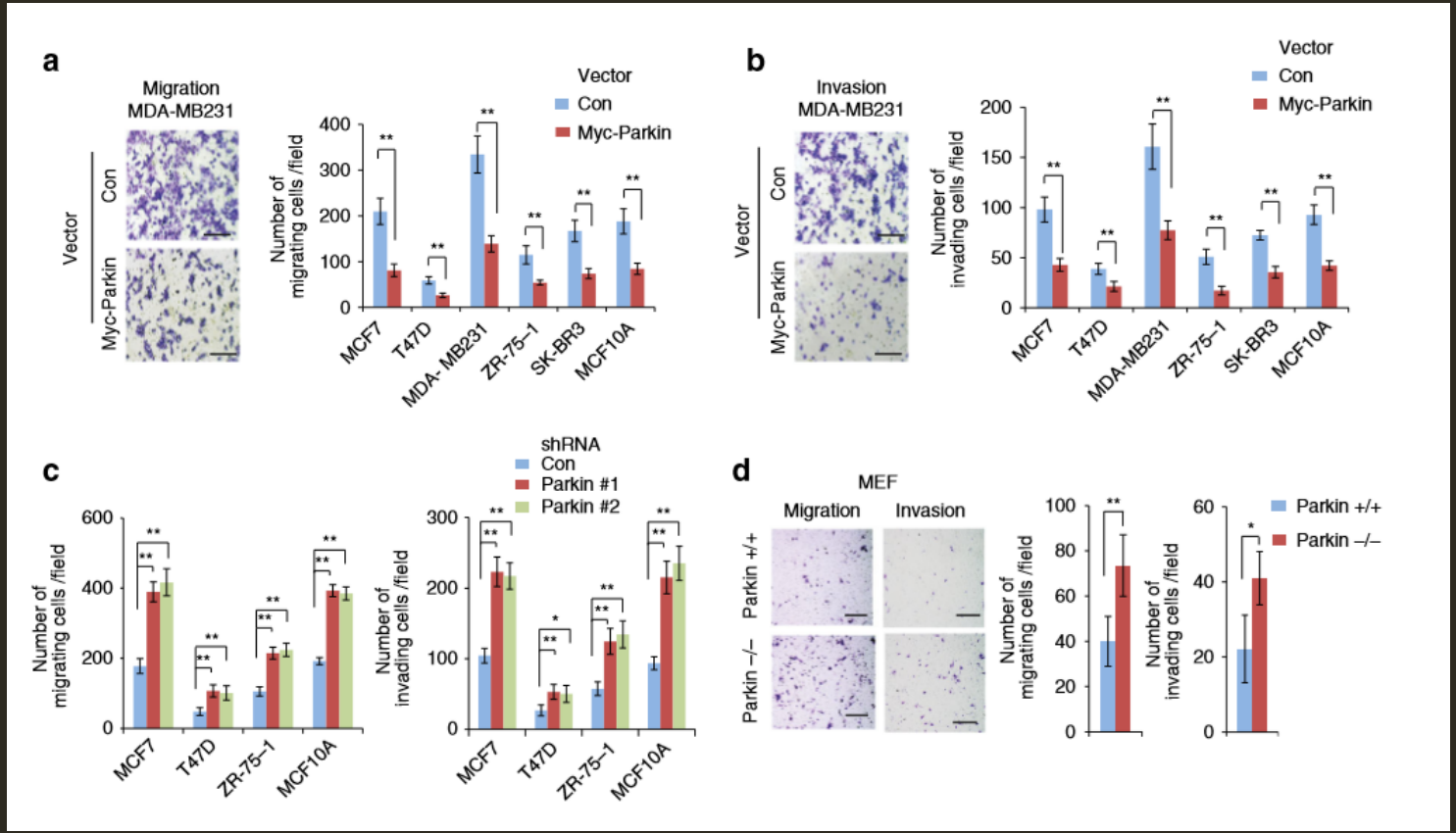


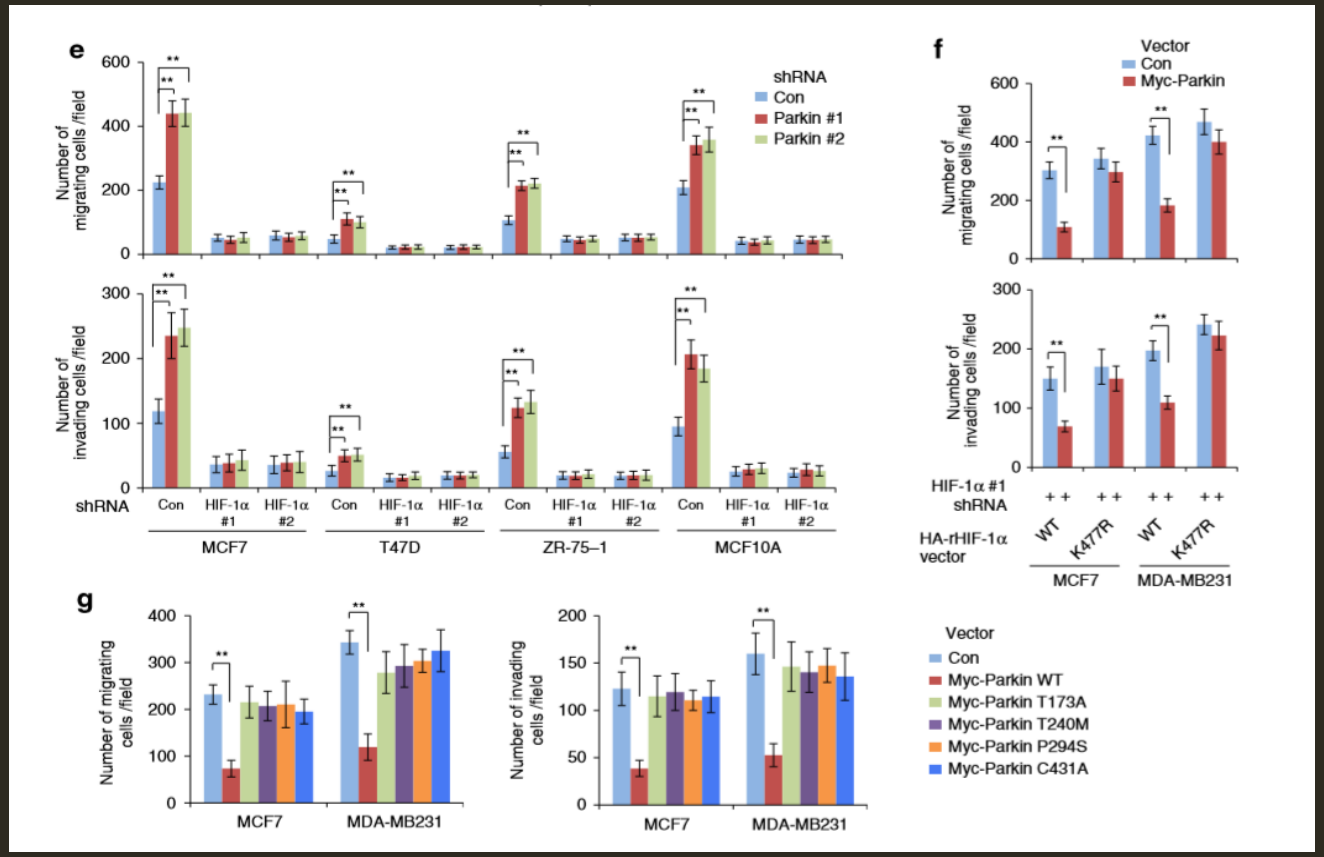


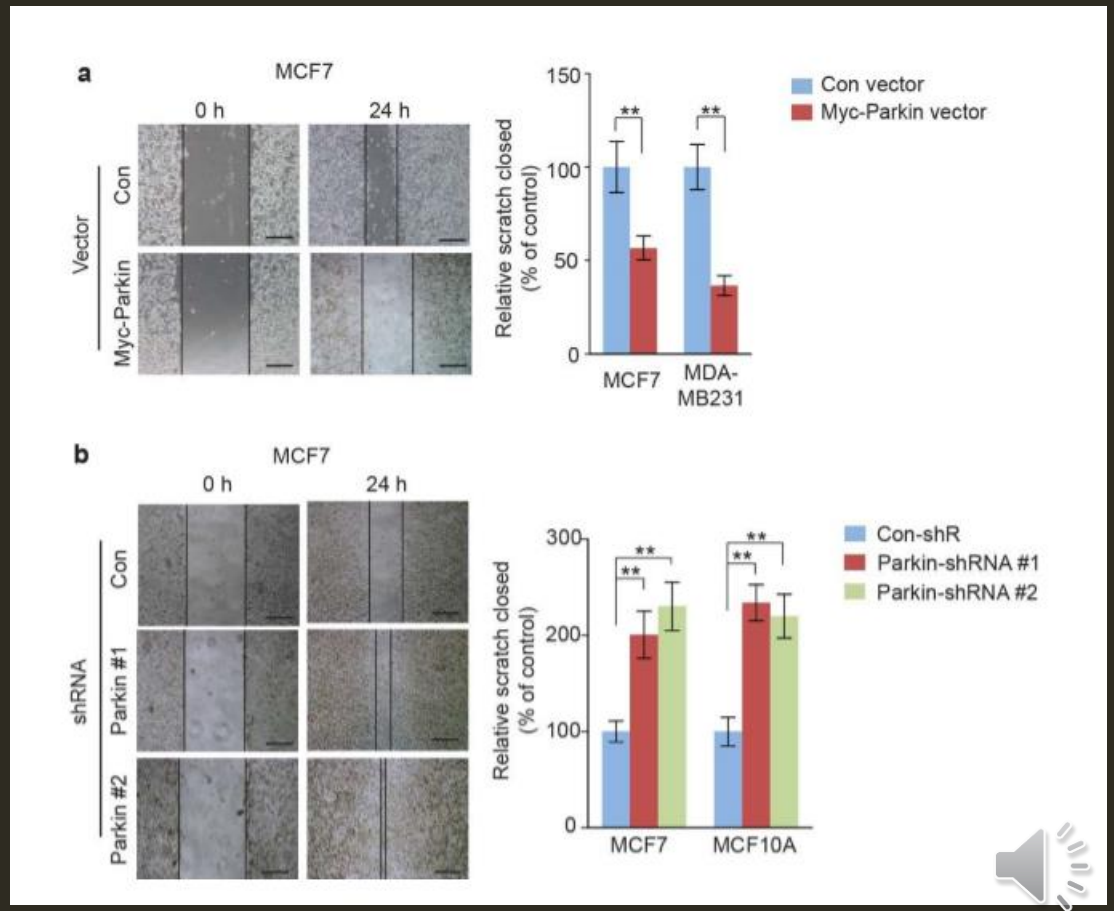

g
HIF-1α #1 shRNA
HA-rHIF-1α vector
WT
K477R
kDa
HIF-1α
HA (HIF-1α)
Actin
100
100
50
MCF7
MDA-MB231
Relative luciferase activity
1.5
1.0
0.5
0
**
**
HIF-1α #1 shRNA
Vector
HA-rHIF-1α
Myc-Parkin
WT
K477R
WT
K477R
MCF7
MDA-MB231


e
Vector
HA-HIF-1α
Myc-Parkin
WT
K477R
WT
K477R
kDa
HA (HIF-1α)
Myc (Parkin)
Actin
100
50
50
MCF7
MDA-MB231
f
Vector
Con
Myc- Parkin
HA-HIF-1α (K477R)
CHX (min)
0 15 30 60 0 15 30 60
kDa
HA (HIF-1α)
Myc (Parkin)
Actin
100
50
50
MCF7
Vector
Con
Myc-Parkin
t1/2 = 57 min
t1/2 = 54 min
% protein
100
80
60
40
20
0
0
20
40
60
Time (min)

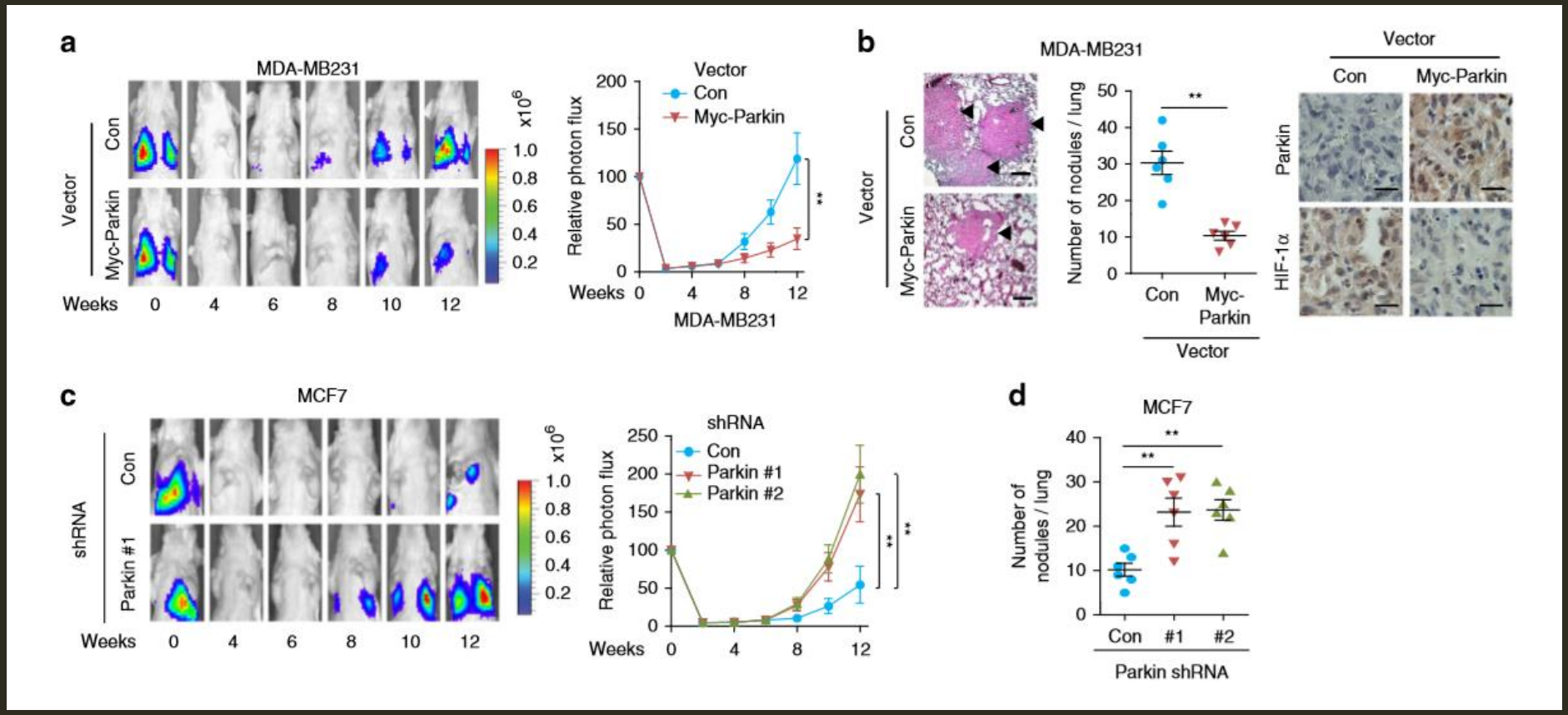
PARKIN INHIBITS CELL MIGRATION AND INVASION THROUGH HIF-1A

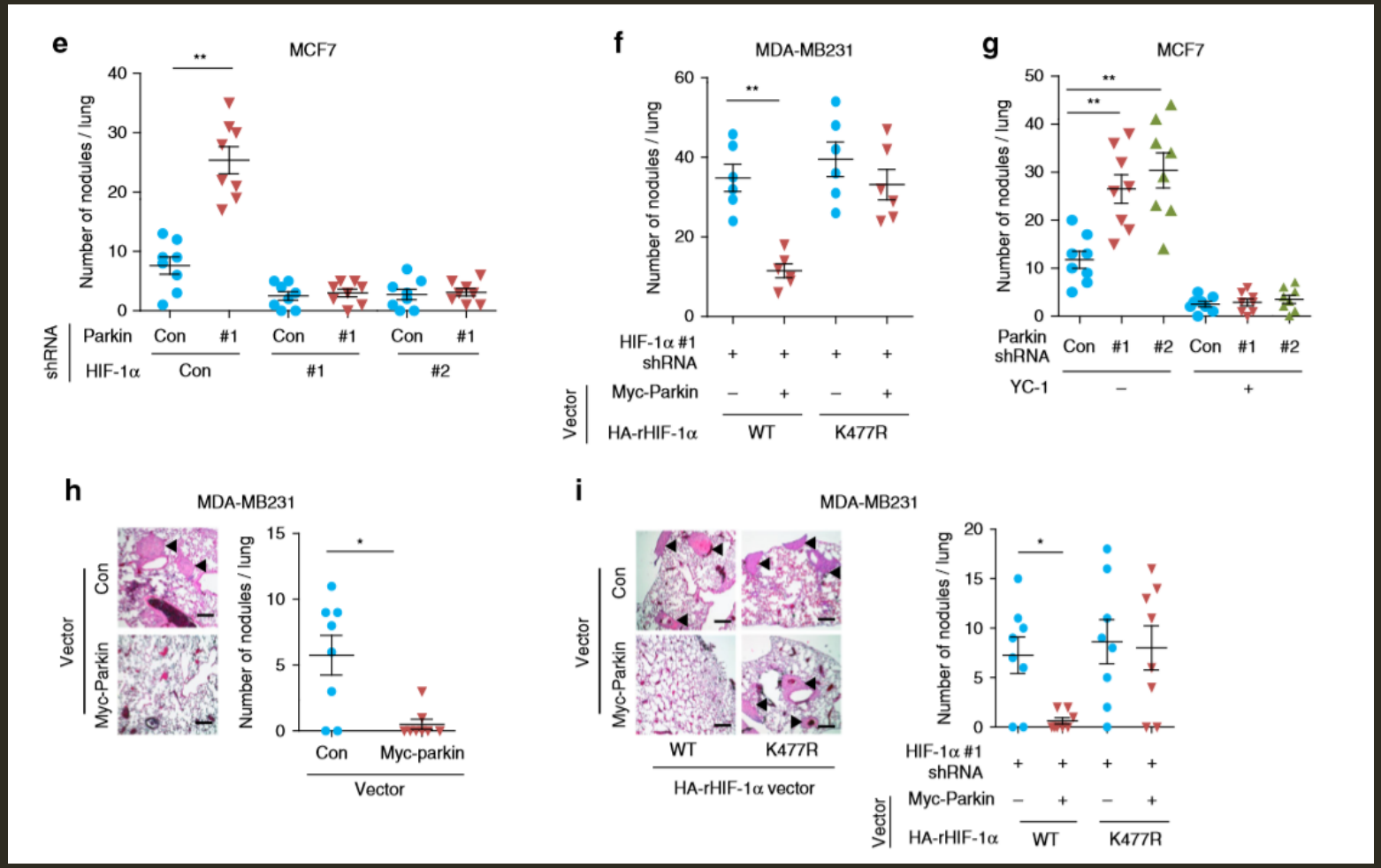



e
Number of migrating cells /field
Number of invading cells /field
shRNA
Con
Parkin #1
Parkin #2
HIF-1α #1
HIF-1α #2
MCF7
T47D
ZR-75–1
MCF10A
f
Vector
Con
Myc-Parkin
HIF-1α #1 shRNA
HA-rHIF-1α vector
WT
K477R
MCF7
MDA-MB231
g
Number of migrating cells /field
Number of invading cells /field
Vector
Con
Myc-Parkin WT
Myc-Parkin T173A
Myc-Parkin T240M
Myc-Parkin P294S
Myc-Parkin C431A

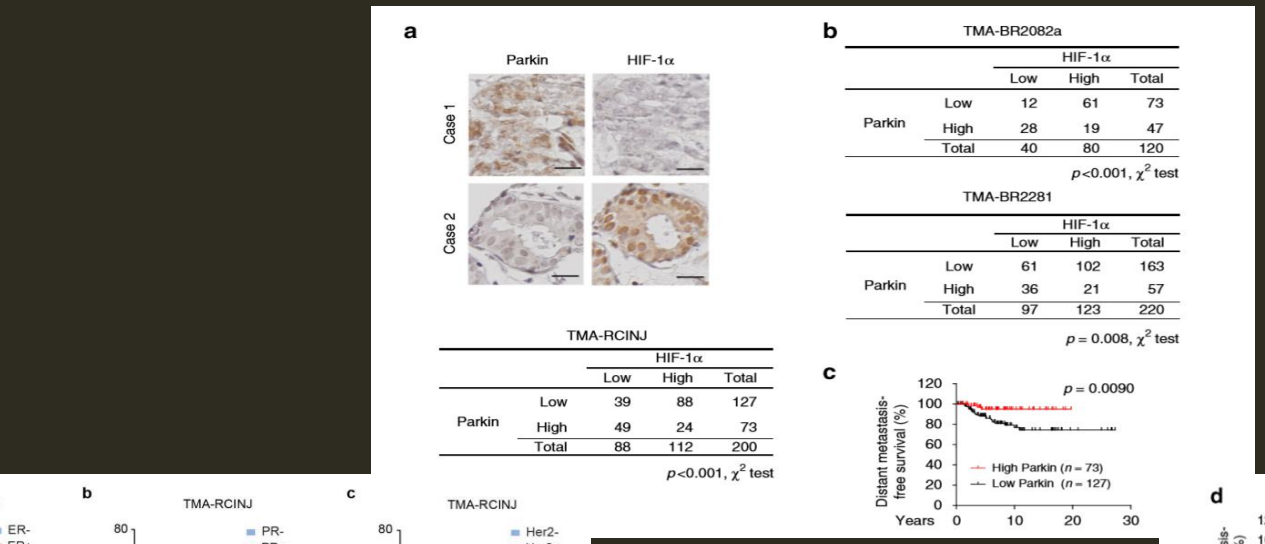

a
MCF7
0 h
24 h
Vector
Con
Myc-Parkin
Relative scratch closed (% of control)
Con vector
Myc-Parkin vector
MCF7
MDA-MB231
b
MCF7
0 h
24 h
shRNA
Con
Parkin #1
Parkin #2
Relative scratch closed (% of control)
Con-shR
Parkin-shRNA #1
Parkin-shRNA #2
MCF7
MCF10A

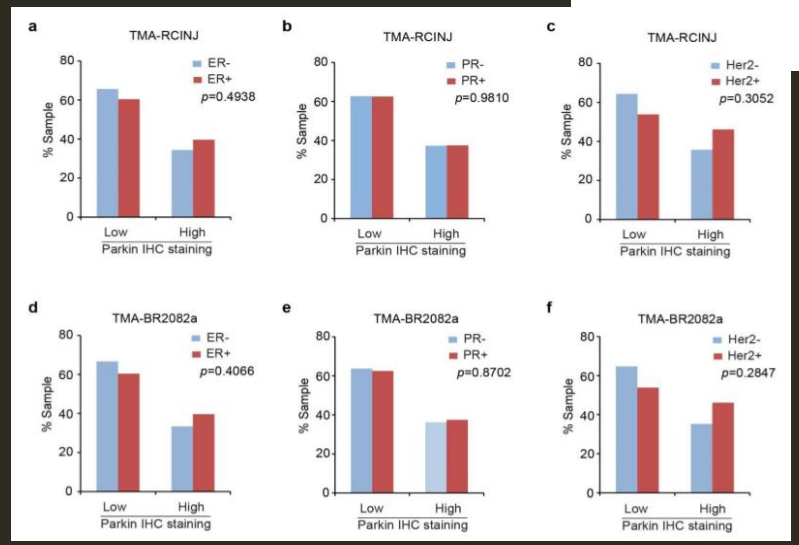
PARKIN INHIBITS CANCER METASTASIS THROUGH HIF-1A REGULATION.

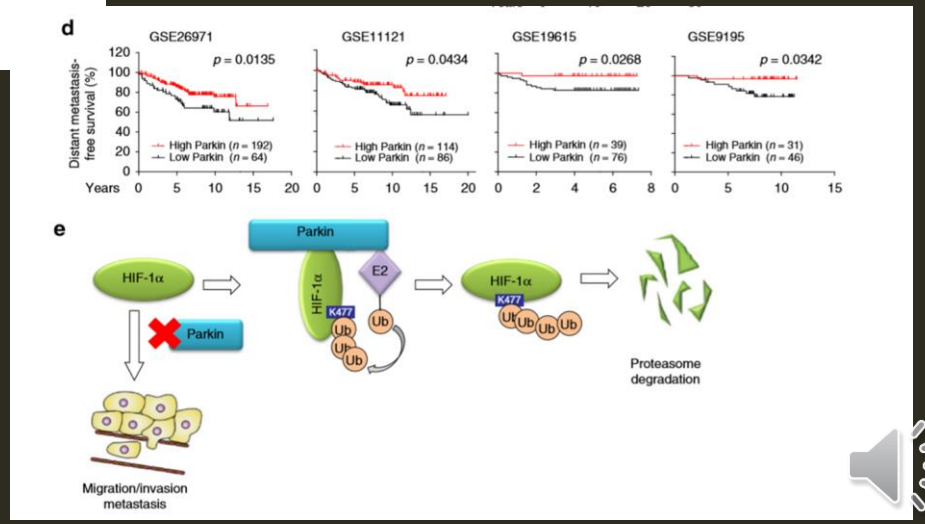



e
MCF7
Number of nodules / lung
shRNA
Parkin
HIF-1α
Con
#1
#2
f
MDA-MB231
HIF-1α #1 shRNA
Myc-Parkin
HA-rHIF-1α
Vector
WT
K477R
g
MCF7
Parkin shRNA
YC-1
h
MDA-MB231
Vector
Con
Myc-Parkin
Myc-parkin
i
MDA-MB231
HA-rHIF-1α vector

PARKIN EXPRESSION CORRELATES WITH BREAST CANCER METASTASIS.







Thank you for attention