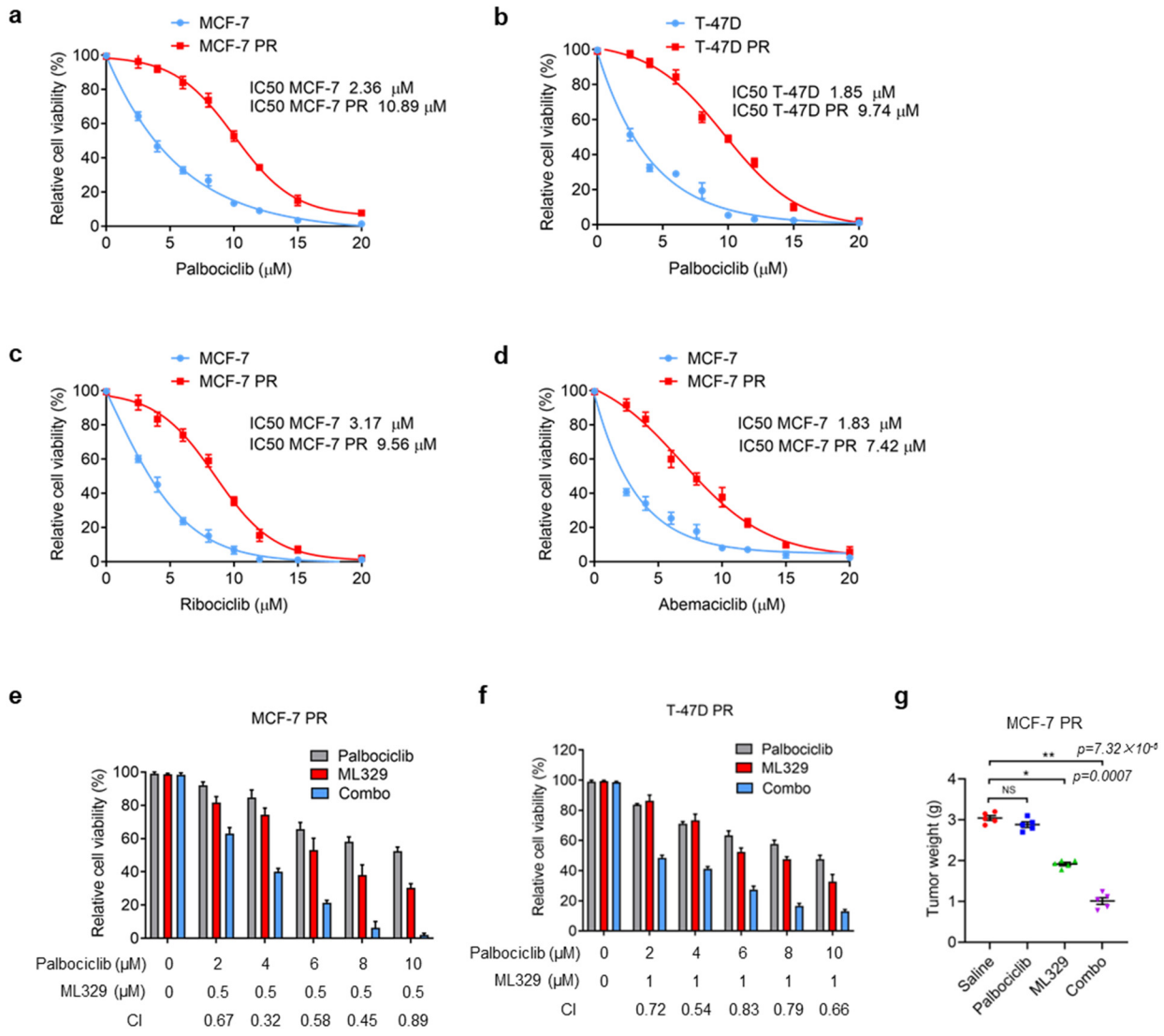
# *O*-GlcNAcylation of MITF regulates its activity and CDK4/6 inhibitor resistance in breast cancer

## Supplementary Information

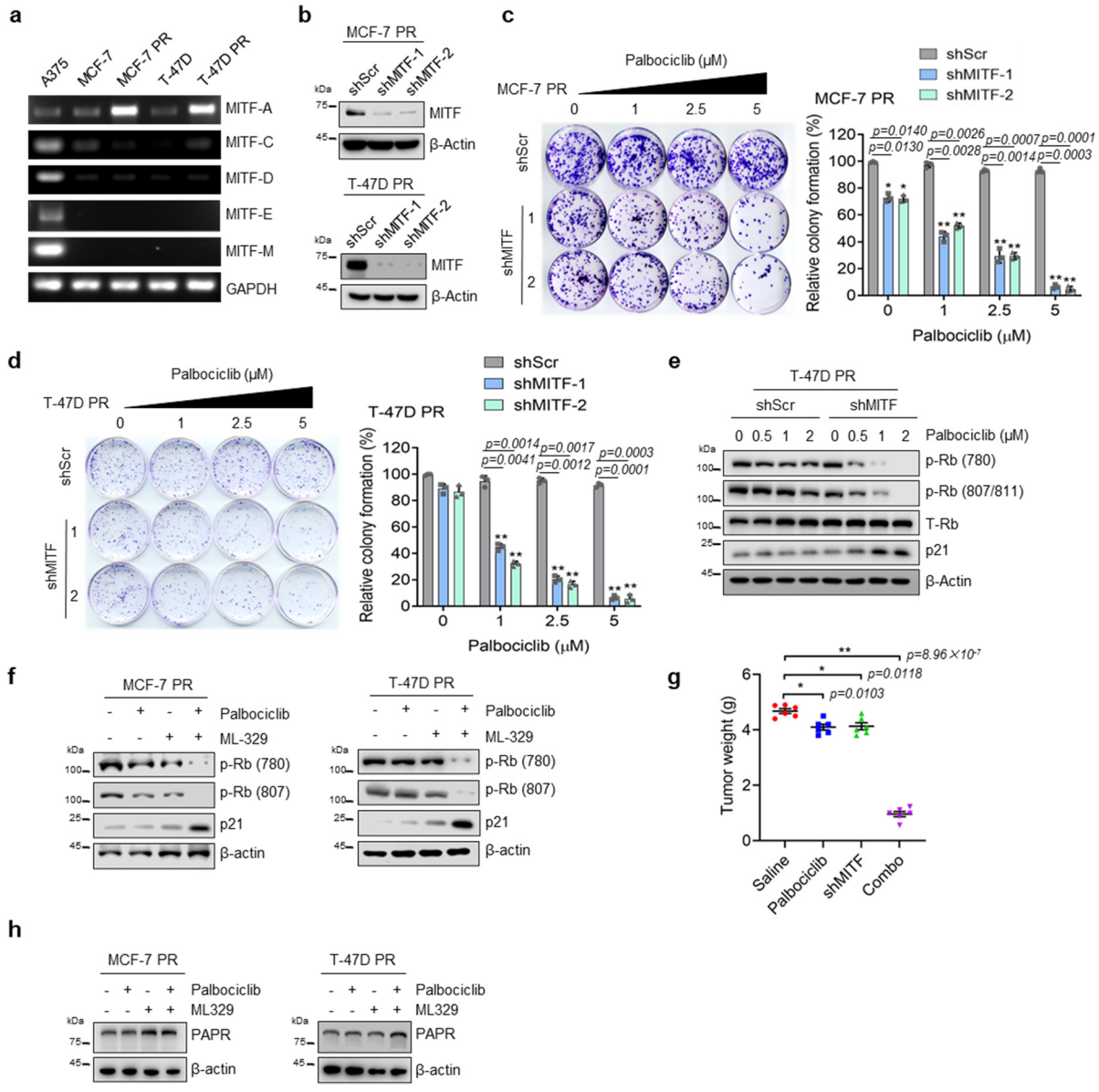
**This file includes:**




**Supplementary Figure 1. qHTS identifies MITF inhibitor ML329 that overcomes palbociclib resistance**

(**a-b**) Viability of MCF-7 and MCF-7 PR cells (**a**), T-47D and T-47D PR cells (**b**) treated with increasing concentrations of palbociclib for 3 days (n = 3 independent experiments). The $IC_{50}$ was indicated. (**c-d**) Viability of MCF-7 PR cells treated with increasing concentrations of ribociclib (**c**) and abemaciclib (**d**) for 3 days (n = 3 independent experiments). The $IC_{50}$ was indicated. (**e-f**) The synergistic effects of ML329 and palbociclib on MCF-7 PR cells (**e**) and T-47D PR cells (**f**) (n = 3 independent experiments). CI values are presented below the bars. (**g**) Tumor weight of MCF-7 PR xenografts was assessed after treatments with the following compounds: saline, palbociclib (25mg/kg), ML329 (10mg/kg), and a combination of palbociclib with ML329 for 2

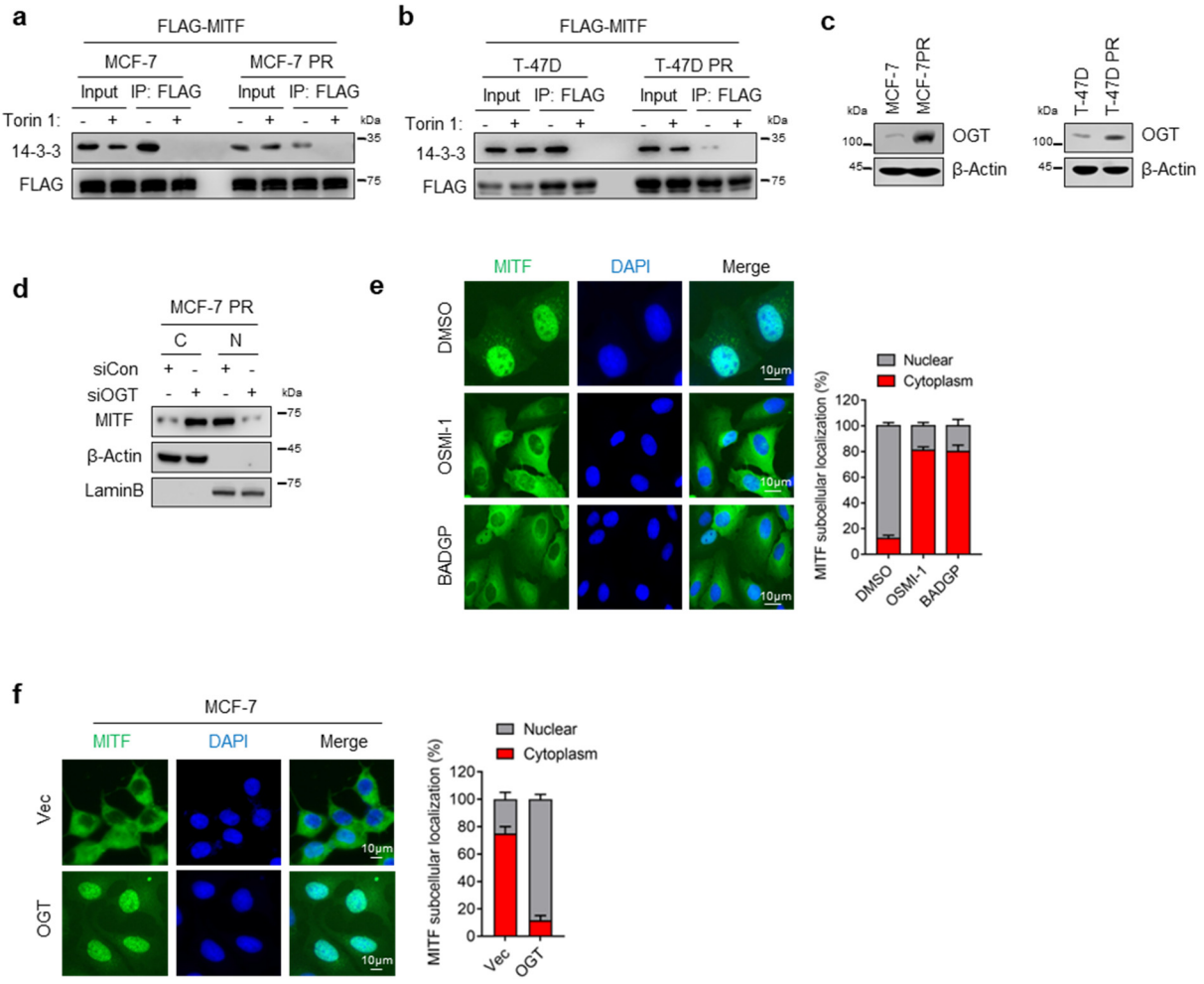
weeks. n = 6 mice/group. **, $p \leq 0.01$, *, $p \leq 0.05$. All error bars are expressed as mean ± SEM. Two-tailed Student's t-tests were employed for statistical evaluation. Source data are provided as a Source Data file.




**Supplementary Figure 2. Inhibition of MITF overcomes palbociclib resistance by activating the senescence pathway in breast cancer cells**

(**a**) RT–PCR analysis to examine the expression levels of MITF isoforms in cells as indicated. Note, MITF-M was detected in A375 melanoma cells but not in breast cancer cells. (**b**) MCF-7 PR and T-47D PR cells treated as indicated were collected and then followed by immunoblotting for the indicated proteins. (n = 3 independent experiments). (**c-d**) Representative images of colony

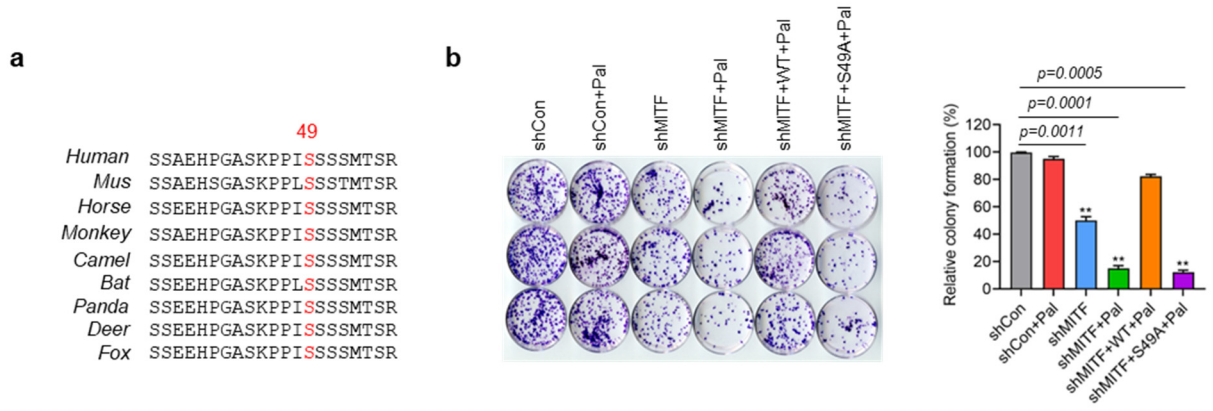
formation and quantification results in MCF-7 PR cells (**c**) and T-47D PR cells (**d**) with indicated treatments (n = 3 independent experiments). (**e**) T-47D PR cells treated as indicated were collected and then followed by immunoblotting for the indicated proteins. (n = 3 independent experiments). (**f**) MCF-7 PR and T-47D PR cells treated as indicated were collected and then followed by immunoblotting for the indicated proteins. (n = 3 independent experiments). (**g**) Tumor weight of MCF-7 PR xenograft treated with saline, palbociclib (25mg/kg), ML329(5mg/kg), or a combination of both for 3 weeks. n = 6 mice/group. (**h**) MCF-7 PR and T-47D PR cells treated as indicated were collected and then immunoblotted for indicated proteins. (n = 3 independent experiments). **, $p \leq 0.01$, *, $p \leq 0.05$. All error bars are expressed as mean ± SEM. Two-tailed Student's t-tests were employed for statistical evaluation. Source data are provided as a Source Data file.




**Supplementary Figure 3. OGT interacts with MITF and promotes its nuclear translocation**

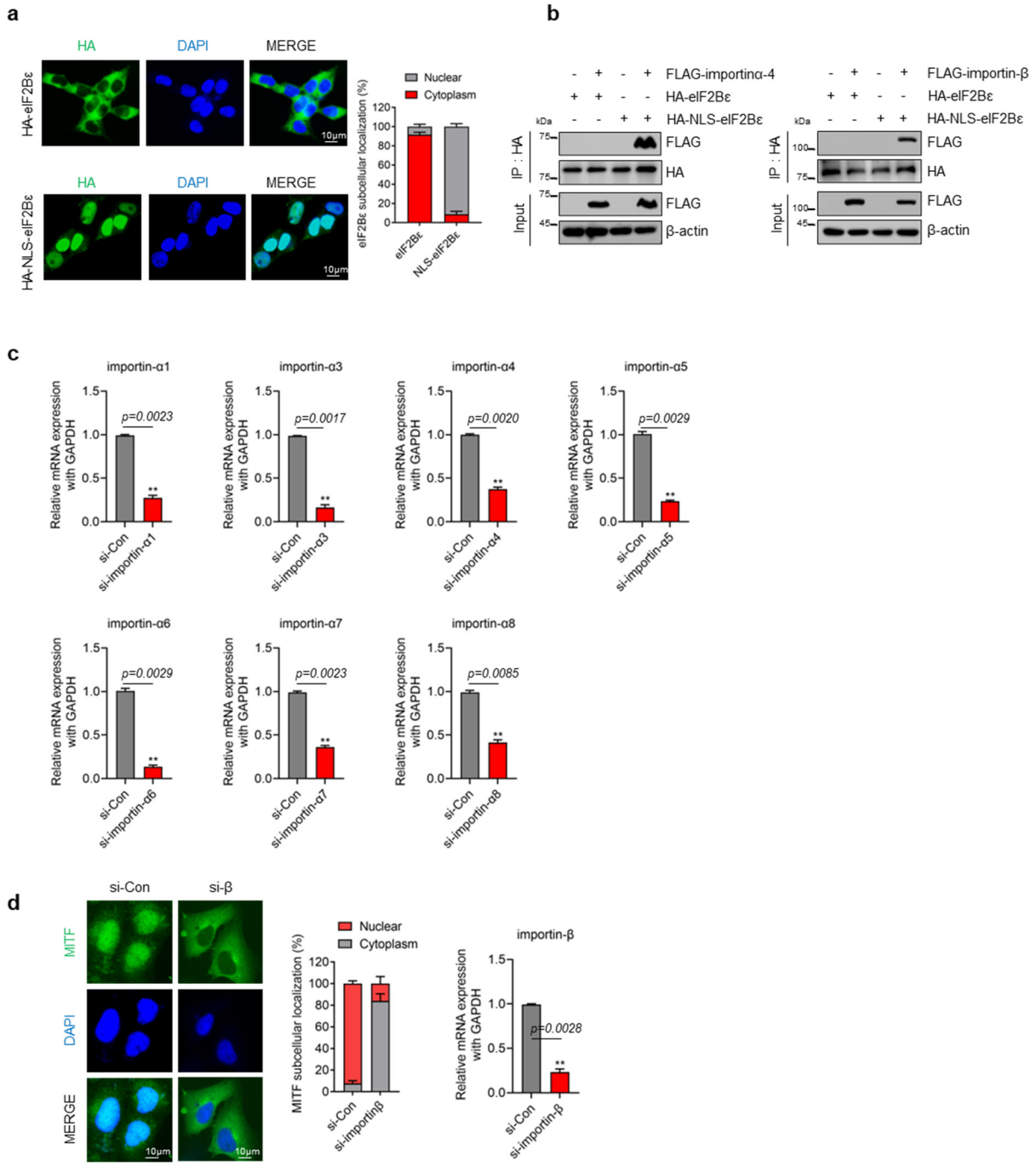
(**a**) MCF-7 and MCF-7 PR cells were transfected with the indicated plasmids for 48 hr before being harvested. FLAG-IPs were immunoblotted for the indicated proteins. (n = 3 independent experiments). (**b**) T-47D and T-47D PR cells were transfected with the indicated plasmids for 48 hr before being harvested. FLAG-IPs were immunoblotted for the indicated proteins. (n = 3 independent experiments). (**c**) MCF-7 & MCF-7 PR cells and T-47D & T-47D PR cells were collected and followed by immunoblotting for the indicated proteins. (n = 3 independent experiments). (**d**) MCF-7 PR cells were collected after indicated treatments, and then cytoplasmic and nuclear fractions were separated and cell lysates were subjected to immunoblotting for

indicated proteins. (n = 3 independent experiments). (**e**) MCF-7 PR cells were treated as indicated, followed by immunostaining to examine the localization of MITF (n = 3 independent experiments). The scale bar represents 10 μm. Right panel, quantification results. (**f**) MCF-7 cells were treated as indicated, and then followed by immunostaining to examine the localization of MITF (n = 3 independent experiments). The scale bar represents 10 μm. Right panel, quantification results. All error bars are expressed as mean ± SEM. Two-tailed Student's t-tests were employed for statistical evaluation. Source data are provided as a Source Data file.




**Supplementary Figure 4. O-GlcNAcylation of MITF at S49 is required for its nuclear accumulation and resistance**

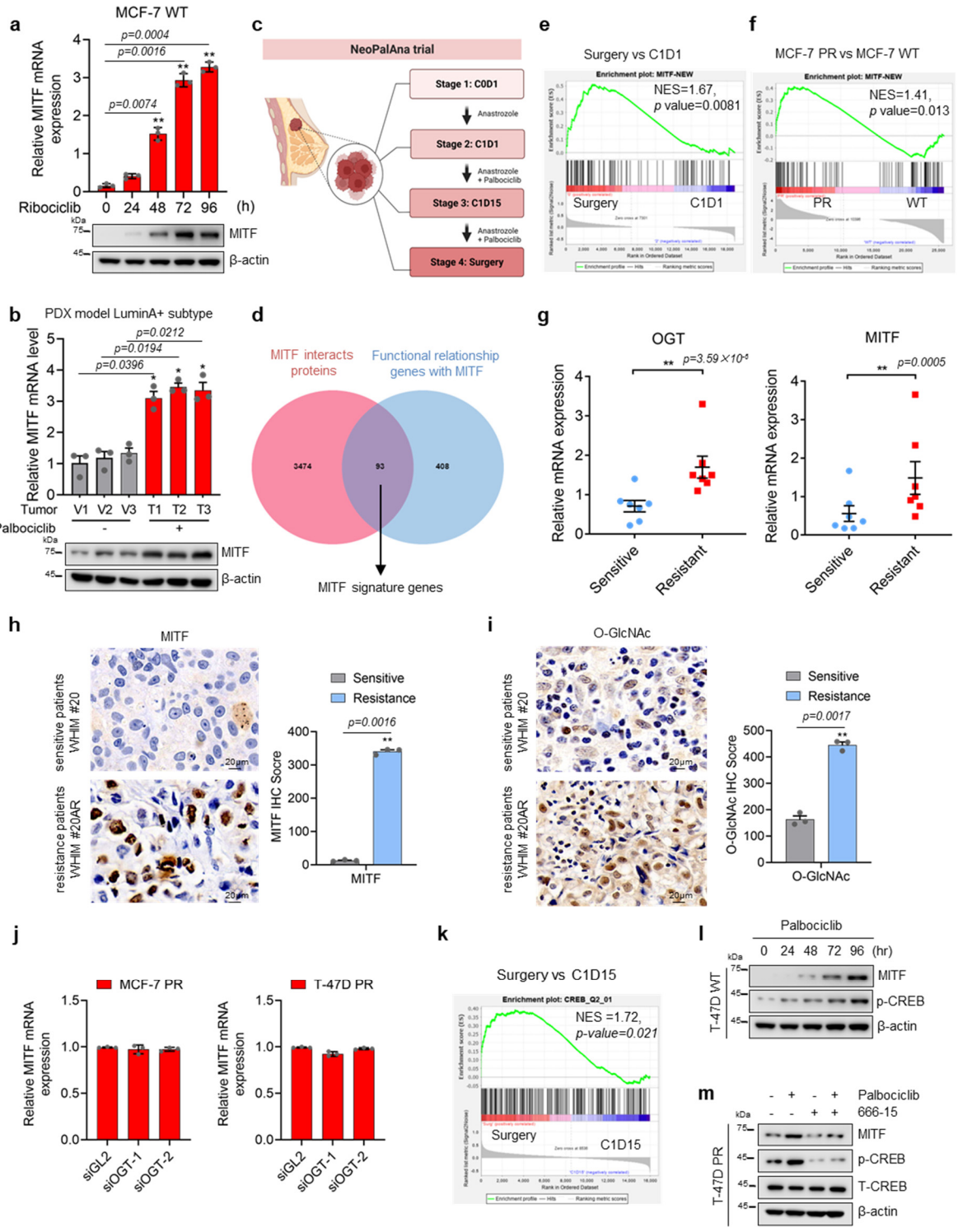
(**a**) MITF S49 site is conserved across various species. (**b**) Left panel, representative images of colony formation in MCF-7 PR cells with indicated treatments (n = 3 independent experiments). Right panel, quantification results. **, p≤0.01. All error bars are expressed as mean ± SEM. Two-tailed Student's t-tests were employed for statistical evaluation. Source data are provided as a Source Data file.




**Supplementary Figure 5. O-GlcNAcylation within the nuclear localization signal (NLS) promotes the interaction of MITF with importin α/β and nuclear translocation**

(**a**) pcDNA3.1-HA-eIF2Bε and pcDNA3.1-HA-NLS-eIF2Bε plasmids were transfected into MCF-7 PR cells as indicated, and the eIF2Bε cellular localization was examined by immunostaining (n

= 3 independent experiments). The scale bar represents 10 μm. (**b**) MCF-7 PR cells were transfected with indicated plasmids for 48 hr before being harvested for co-IP. HA-IPs were then immunoblotted for indicated proteins. (n = 3 independent experiments). (**c**) MCF-7 PR cells were collected after the indicated treatment and subjected to qPCR to examine the expression of indicated genes (n = 3 independent experiments). (**d**) MCF-7 PR cells were transfected with the indicated siRNAs and then followed by immunostaining to detect MITF cellular localization (n = 3 independent experiments). The scale bar represents 10 μm. Right panel, quantification results are shown on the left. **, $p \leq 0.01$. All error bars are expressed as mean ± SEM. Two-tailed Student's t-tests were employed for statistical evaluation. Source data are provided as a Source Data file.


a
MCF-7 WT
Relative MITF mRNA expression
p=0.0004
p=0.0016
p=0.0074
Ribociclib 0 24 48 72 96 (h)
MITF
β-actin
b
PDX model LuminA+ subtype
Relative MITF mRNA level
p=0.0212
p=0.0194
p=0.0396
Tumor V1 V2 V3 T1 T2 T3
Palbociclib - +
MITF
β-actin
c
NeoPalAna trial
Stage 1: C0D1
Anastrozole
Stage 2: C1D1
Anastrozole + Palbociclib
Stage 3: C1D15
Anastrozole + Palbociclib
Stage 4: Surgery
d
MITF interacts proteins
Functional relationship genes with MITF
3474
93
408
MITF signature genes
e
Surgery vs C1D1
Enrichment plot: MITF-NEW
NES=1.67,
p value=0.0081
Surgery
C1D1
f
MCF-7 PR vs MCF-7 WT
Enrichment plot: MITF-NEW
NES=1.41,
p value=0.013
PR
WT
g
OGT
p=3.59×10-5
Relative mRNA expression
Sensitive
Resistant
MITF
p=0.0005
Relative mRNA expression
Sensitive
Resistant
h
MITF
sensitive patients WH-IM #20
resistance patients WH-IM #20AR
20μm
Sensitive
Resistance
p=0.0016
MITF IHC Socre
MITF
i
O-GlcNAc
sensitive patients WH-IM #20
resistance patients WH-IM #20AR
20μm
Sensitive
Resistance
p=0.0017
O-GlcNAc IHC Socre
O-GlcNAc
j
MCF-7 PR
Relative MITF mRNA expression
siGL2
siOGT-1
siOGT-2
T-47D PR
Relative MITF mRNA expression
siGL2
siOGT-1
siOGT-2
k
Surgery vs C1D15
Enrichment plot: CREB_Q2_01
NES =1.72,
p-value=0.021
Surgery
C1D15
l
Palbociclib
0 24 48 72 96 (hr)
T-47D WT
MITF
p-CREB
β-actin
m
Palbociclib
666-15
T-47D PR
MITF
p-CREB
T-CREB
β-actin

**Supplementary Figure 6. MITF is activated in response to palbociclib and elevated in tumors from palbociclib-resistant breast cancer patients**

(**a**) MCF-7 cells were collected after indicated treatments and then subjected to qPCR or immunoblotting to examine the expression of indicated genes (n = 3 independent experiments) and proteins. (**b**) PDX model LuminA+ tumors from mice were collected after indicated treatments and then subjected to qPCR and immunoblotting to examine the expression of indicated genes (n = 3 independent experiments) and proteins. (**c**) Schematic of the stages of the NeoPalAna trial. (**d**) Venn diagram to show the overlapping of proteins that interact with MITF (by mass-spec analysis) and genes that are functionally associated with MITF (by PahtwayNet analysis). A total of 93 genes were identified and these genes were defined as MITF signature geneset. (**e-f**) GSEA profiling to show enrichment of the MITF signature geneset in the Surgery group vs. C1D1 group (**e**), and MCF-7 PR cells vs. MCF-7 cells (**f**). (**g**) Palbociclib-resistant and sensitive breast cancer PDX cells were collected and followed by qPCR to examine the expression of indicated genes (n = 3 independent experiments). (**h-i**) Representative images of IHC staining and quantification results for MITF (**h**) and O-GlcNAc (**i**) in PDX lines WHIM 20AR and WHIM 20 (n = 3 independent experiments). The scale bar represents 20 μm. (**j**) MCF-7 PR and T-47D PR cells were collected after treatments as indicated and then subjected to qPCR to examine the expression of genes as indicated (n = 3 independent experiments). (**k**) GSEA profiling to show enrichment of the CREB geneset in the Surgery group vs. C1D15 group. (**l-m**) T-47D WT cells (**l**) and T-47D PR cells (**m**) were collected after indicated treatments and then followed by immunoblotting for the indicated proteins. (n = 3 independent experiments). **, $p \leq 0.01$. All error bars are expressed as mean ± SEM. Two-tailed Student's t-tests were employed for statistical evaluation. Source data are provided as a Source Data file.