# Ubiquitination and Regulation of AURKA Identifies a Hypoxia-Independent E3 Ligase Activity of VHL

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*Running Title:* Hypoxia-independent ubiquitination of AURKA by VHL

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## SUPPLEMENTARY FIGURE LEGENDS

### Supplementary Figure 1. VHL regulates AURKA protein levels

**(a)** Lysates from A-498 and isogenic $VHL_L$ ($VHL_{24}$) and $VHL_S$ ($VHL_{19}$) expressing stable cell lines cultured under confluent and serum starved (for 48h) conditions probed with the indicated antibodies. **(b)** Densitometric quantitation of the ratio of AURKA to GAPDH expression from A-498, A-498 $VHL_L$ and $VHL_S$ cells, averaged from 6 independent experiments. *$p<0.05$. Error bars denote standard error of the mean (SEM). **(c)** Lysates from hTERT RPE-1 cells overexpressing varying doses of $VHL_{24}$ (μg), as denoted, probed for the indicated antibodies.

### Supplementary Figure 2. VHL interacts with AURKA

hTERT RPE-1 cells expressing EGFP-AURKA and HA-$VHL_{30}$ used in co-immunoprecipitation assays to show binding between HA-$VHL_{30}$ and EGFP-AURKA. Pulldown efficiencies are indicated for each immunoprecipitation.

### Supplementary Figure 3. VHL R167Q mutant fails to ubiquitinate and degrade AURKA

**(a)** *In vivo* ubiquitination assay using lysates from 786-0 and isogenic 786-0 $VHL_{24}$ (WT), and R167Q cell lines grown to confluence and serum starved for 48 hours in the absence of MG132. Ubiquitinated AURKA probed using anti-ubiquitin P4D1 antibody. Arrowhead indicates the expected molecular weight of mono-ubiquitinated AURKA. Input lysates probed with AURKA, VHL and tubulin. **(b)** Graphical representation of

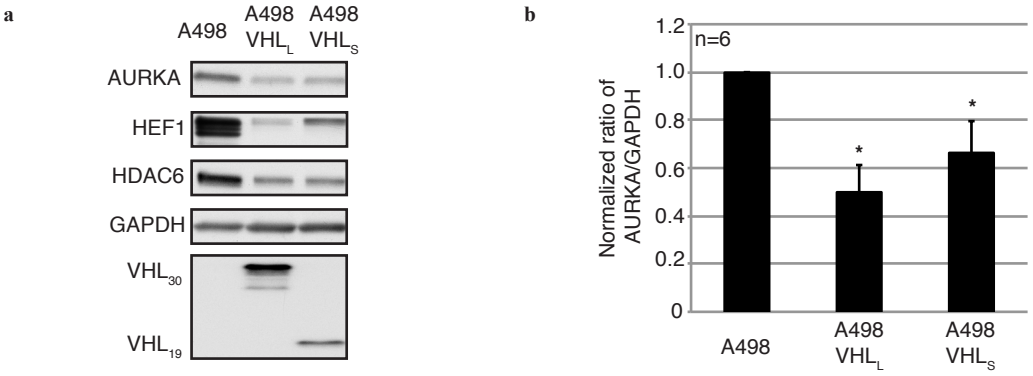
densitometric quantitation (measured from 50-200kD) from the representative experiment shown in (**a**) denoting a ratio of ubiquitinated AURKA (probed using the P4D1 anti-ubiquitin antibody) to the pull down efficiency (using an anti-AURKA antibody). Black bar (parental 786-0), gray bar (WT), and black bar with white dots (R167Q).

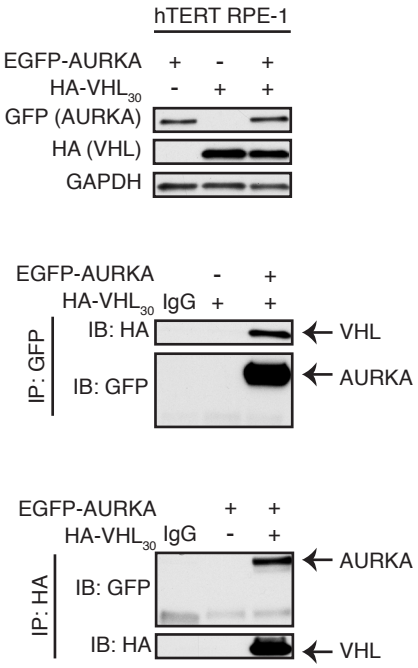
**Supplementary Figure 4. VHL regulates AURKA protein half-life and abundance**

*In vivo* ubiquitination assay performed using hTERT RPE-1 cells overexpressing EGFP-AURKA and HA-Ub with and without overexpressed $VHL_{24}$ in the absence of MG132. Cells were grown to confluence and harvested following serum withdrawal at the indicated time points. Ubiquitinated AURKA immunoprecipitated using an anti-HA antibody and probed with an anti-AURKA antibody. Input lysates immunoblotted for the indicated antibodies.

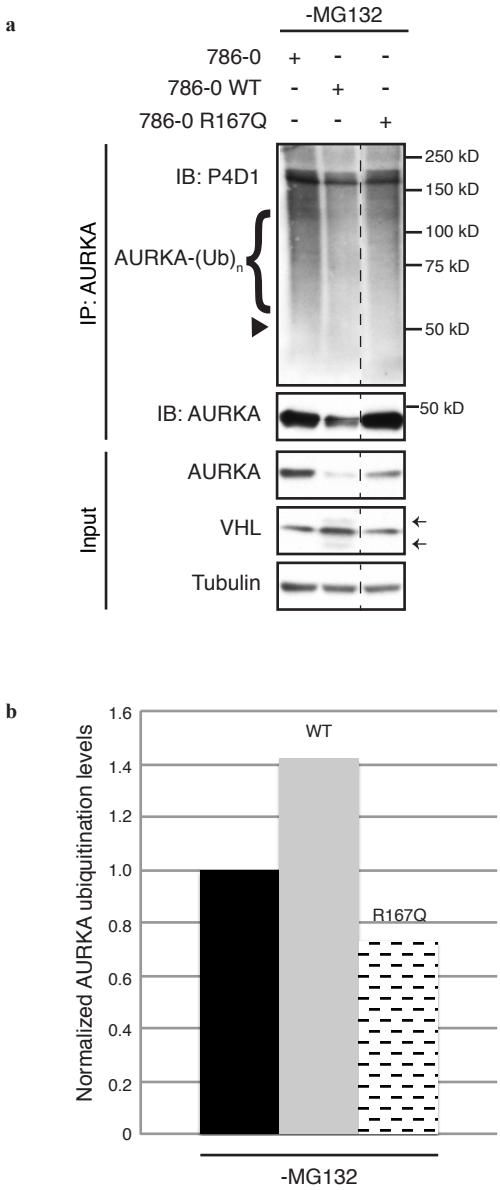
**Supplementary Figure 5. Rescue of ciliogenesis in VHL-deficient cells with alisertib and rocilinostat**

**(a)** Graph showing fold change in VHL mRNA transcript levels. **(b-c)** Lysates from siC and siVHL transfected cells treated with vehicle (DMSO), (**b**) rocilinostat (ACY1215) or (**c**) alisertib (MLN8237) probed for the indicated antibodies.

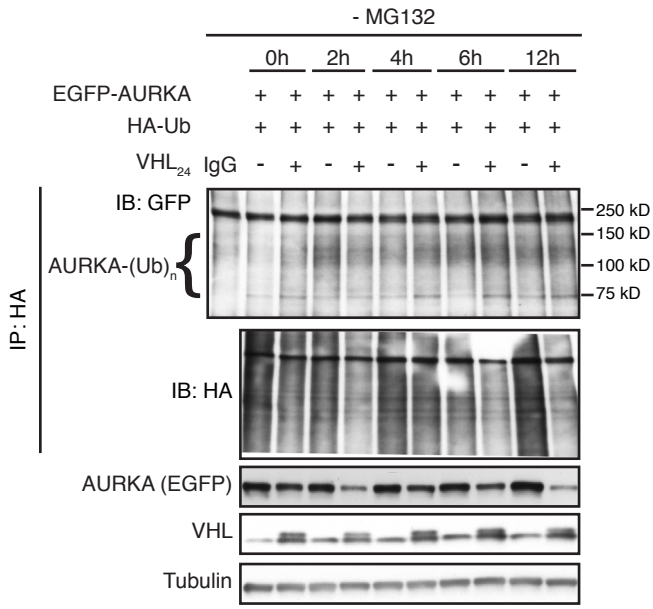

a
A498
A498 VHL_L
A498 VHL_S
AURKA
HEF1
HDAC6
GAPDH
VHL30
VHL19
b
n=6
Normalized ratio of AURKA/GAPDH
1.2
1.0
0.8
0.6
0.4
0.2
0
*
*
A498
A498 VHL_L
A498 VHL_S
c
hTERT RPE-1
VHL24 (μg)
-
3
4
5
6
AURKA
VHL
GAPDH



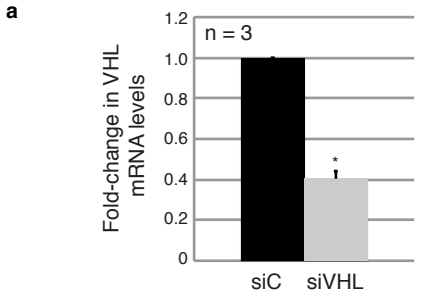

Supplementary Figure 2

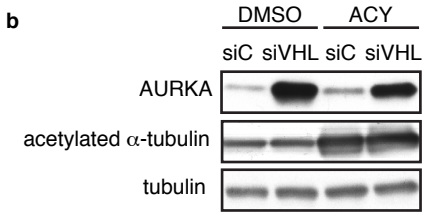


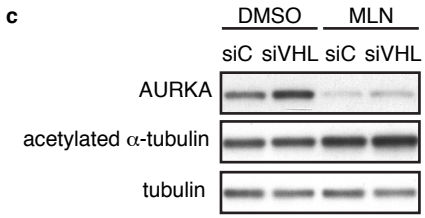

Supplementary Figure 3




Supplementary Figure 4


a
n = 3
Fold-change in VHL mRNA levels
1.2
1.0
0.8
0.6
0.4
0.2
0
*
siC
siVHL


b
DMSO
ACY
siC siVHL siC siVHL
AURKA
acetylated α-tubulin
tubulin


c
DMSO
MLN
siC siVHL siC siVHL
AURKA
acetylated α-tubulin
tubulin