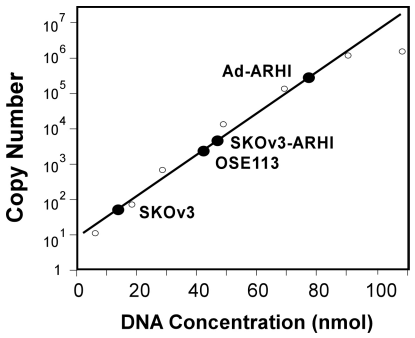
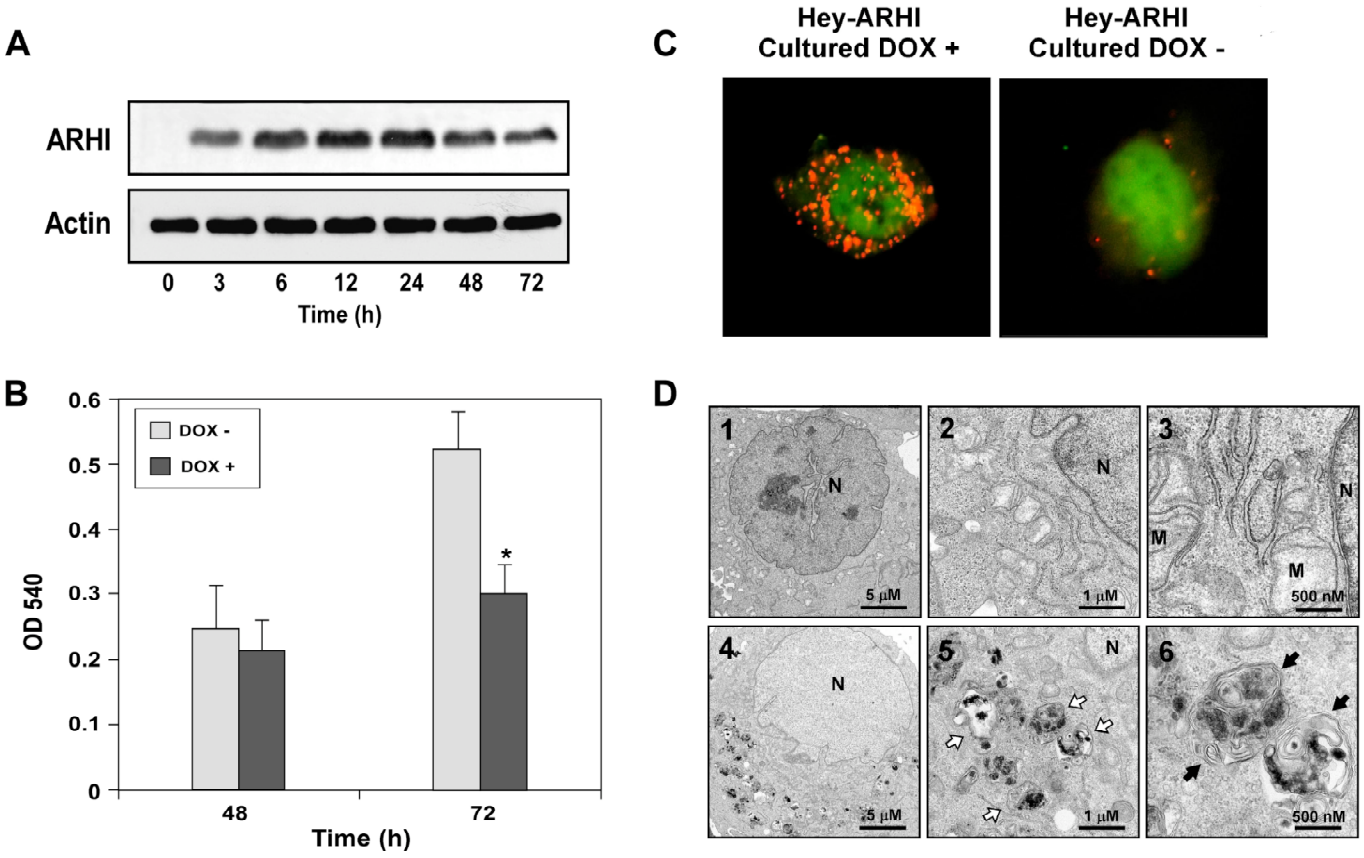
SUPPLEMENTARY FIGURES

Supplementary Fig. S1. Comparison of ARHI expression in OSE113, parental SKOv3, SKOv3-ARHI, or adenovirus-infected SKOv3 (Ad-ARHI) cells.



Supplementary Fig. S2. Characterization of ARHI-inducible ovarian cancer cell line Hey-ARHI. (A) Kinetics of induction of ARHI. Lysates from Hey-ARHI cells cultured with or without DOX were western blotted for ARHI and actin (as loading control). (B) Cell proliferation of inducible Hey-ARHI ovarian cancer cells in the presence or absence of DOX. (C) Cultured inducible Hey-ARHI cells were treated with or without DOX to induce ARHI expression. Cells were then stained with acridine orange and examined by fluorescence microscopy. Large orange punctate spots were considered to be acidic vesicular organelles, a marker for autophagosomes. (D) Electron microscope images of uninduced (panels 1-3) and induced (panels 4-6) Hey-ARHI cells. Open arrowhead indicate autophagosome vacuoles and solid arrowheads indicate typical double-membrane of autophagosome. No autophagosomes were observed in uninduced cells.

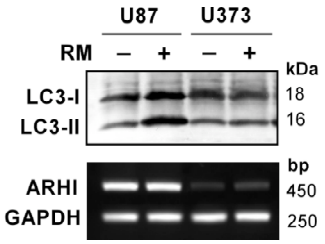


Supplementary Fig. S3. (A) OSE106 cells were transfected with GFP-LC3 plasmid alone or co-transfected with siARHI or siControl. Transfected cells were treated with rapamycin and examined for autophagy 24 hrs later. Green vacuoles from two independent experiments were counted. (B) ARHI is necessary for rapamycin-induced autophagy in glioma cells. *Left panel:* Conversion of LC3-I to LC3-II and ARHI mRNA levels were examined in rapamycin-sensitive and pro-autophagic (U87) or rapamycin-insensitive (U373) glioma cells by Western blotting or semiquantitative RT-PCR analysis. *Right panel:* U87 cells were transfected with siARHI or siControl in the presence or absence of rapamycin. Conversion of LC3-I to LC3-II was examined by Western blotting. (C) ARHI is associated with LC3 in the pelleted fraction of the lysed cells. SKOv3-ARHI and -NTD cells with or without DOX were transfected with pGFP-LC3 and harvested at the indicated times. Cell lysates and pellets were analyzed by Western blotting with anti-GFP or anti-ARHI antibody. (D) ARHI is associated with LC3 in live cells. SKOv3-ARHI and -NTD cells were treated as in (C) and then incubated with BASED for *in vivo* cross-linking. Whole cell lysates (WCL) were immunoprecipitated with anti-ARHI and Western blotted to detect cross-linked ARHI-LC3 complexes. The WCL used for immunoprecipitation was included as input controls.

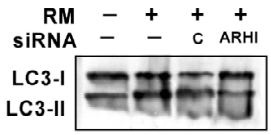
A

Total vesicles / 100 cells			
siRNA	–	Control	ARHI
Rapamycin -	97	89	23
Rapamycin +	100	78	30

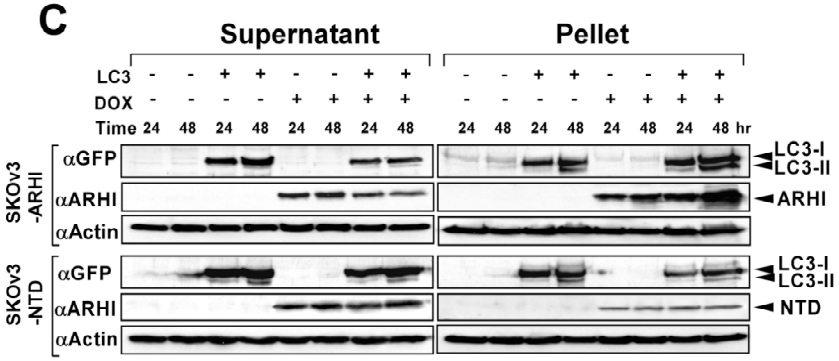
B i



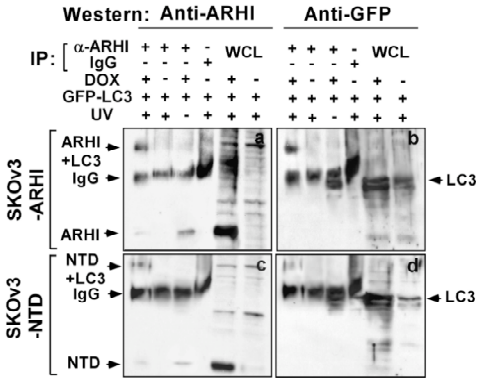
ii



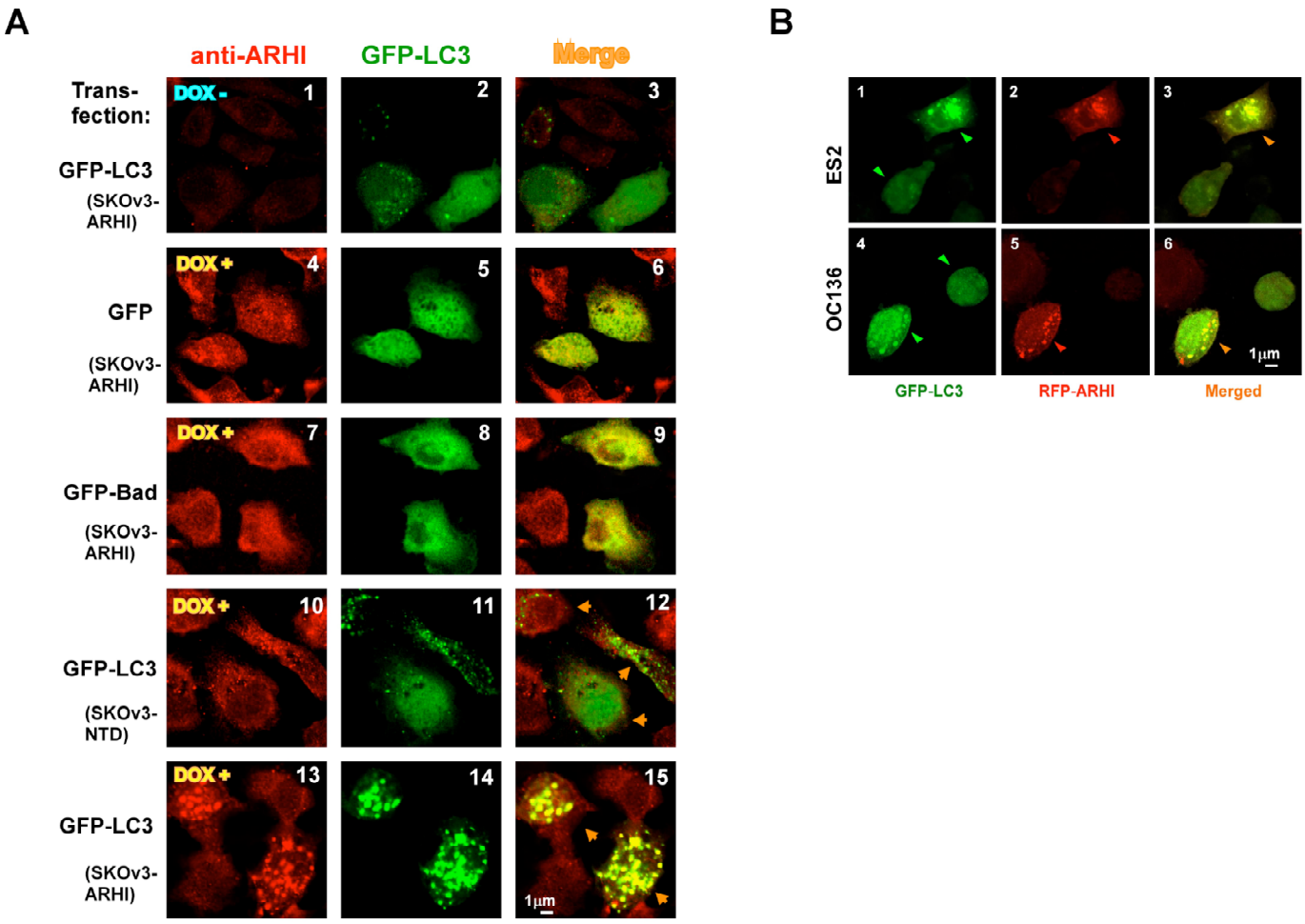
C



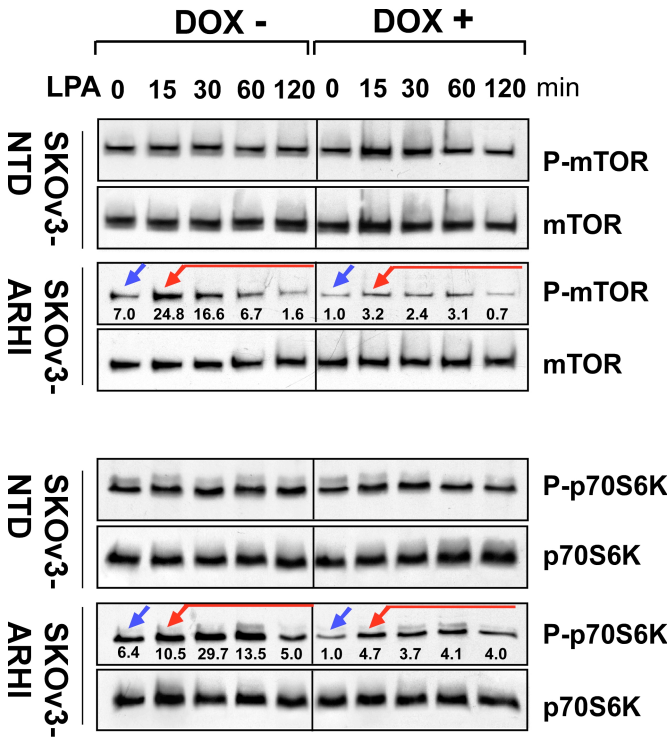
D



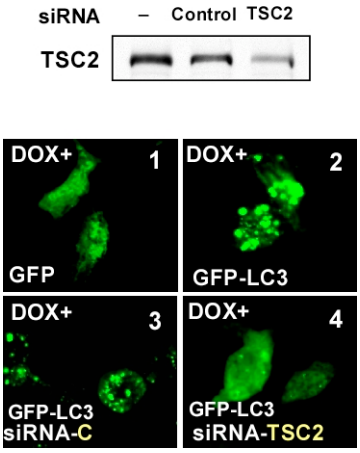
Supplementary Fig. S4. (A) ARHI, but not NTD, co-localized with LC3. SKOv3-ARHI or –NTD cells were transfected with pGFP-LC3 to detect distribution of LC3 in cells before (panels 1-3) and after (panels 4-15) ARHI or NTD expression. pGFP vector (panels 4-6) and pGFP-Bad (panels 7-9) plasmids were transfected as controls. Arrowheads indicate transfected cells exhibiting both ARHI and LC3 signals (panels 10-15). Data shown are representative of at least 3 independent experiments. (B) ARHI co-localizes with MAP-LC3 in autophagosomes. ES2 and OC316 ovarian cancer cells were co-transfected with GFP-LC3 and RFP-ARHI plasmids and examined 24 hr later by fluorescence microscopy. LC3- and ARHI-transfected cells are marked by green (panels 1 and 4) and red (panels 2 and 5) arrowheads, respectively. Co-localization of LC3 and ARHI in transfected cells is indicated by the orange arrows (panels 3 and 6).


A
anti-ARHI
GFP-LC3
Merge
Trans-fection:
GFP-LC3 (SKOv3-ARHI)
GFP (SKOv3-ARHI)
GFP-Bad (SKOv3-ARHI)
GFP-LC3 (SKOv3-NTD)
GFP-LC3 (SKOv3-ARHI)
DOX -
DOX +
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
1μm
B
ES2
OC136
GFP-LC3
RFP-ARHI
Merged
1μm

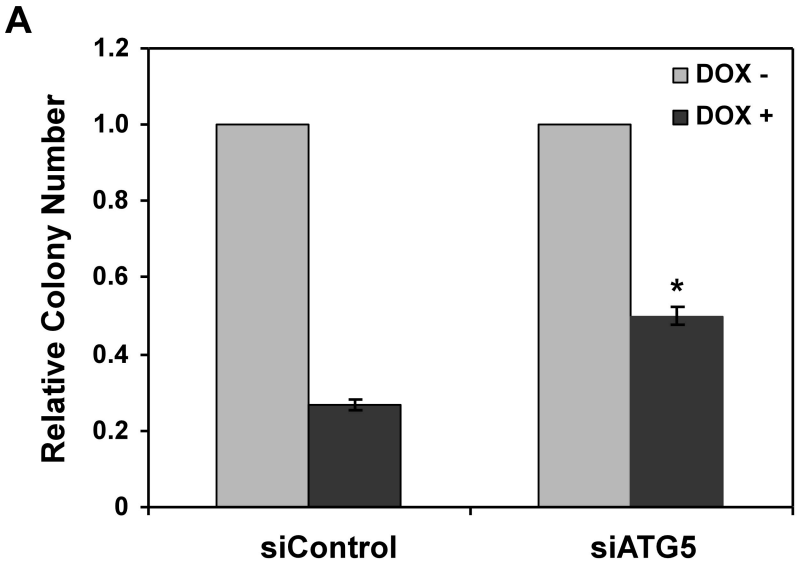
Supplementary Fig. S5. Reduced phosphorylation of PI3K signaling proteins in ARHI-induced SKOv3-ARHI cells. Phosphorylation levels of mTOR and p70S6K were measured by Western blotting before (blue arrow) and after stimulation (red arrow) with 10 nM of lysophosphatidic acid (LPA) in the presence or absence of DOX. The numbers under the protein bands are fold changes, where the basal levels in DOX-treated SKOv3-ARHI cells were assigned as 1.0.

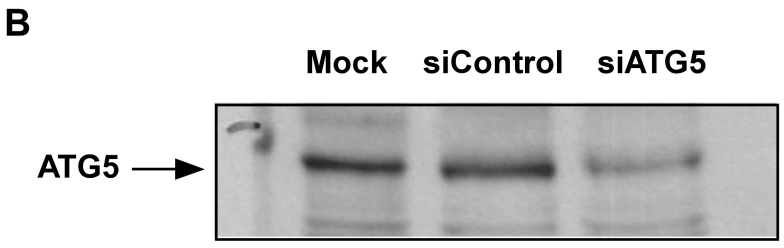


Supplementary Fig. S6. *Upper panel*: TSC2- or control siRNA was transfected into SKOv3-ARHI cells for 48 hrs. Cell lysates were analyzed by Western blotting using anti-TSC2 antibody. *Lower panel*: SKOv3-ARHI cells were transfected with TSC2 siRNA for 24 hrs followed by co-transfection of TSC2 siRNA and pGFP or pGFP-LC3 along with DOX induction for 24 hrs. Green vacuoles were viewed and counted under a confocal microscope and presented in **Fig. 3D**.

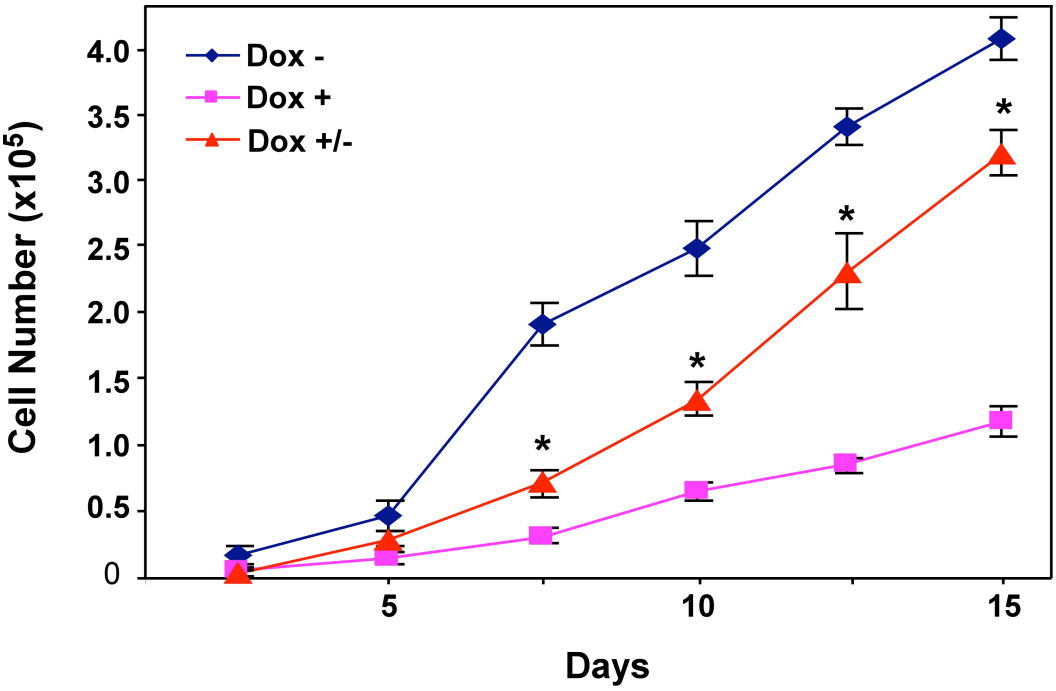


Supplementary Fig. S7. Inhibition of autophagy rescues cells from autophagic death. SKOv3-ARHI cells were transfected with siATG5 for two consecutive days to maximize ATG5 knockdown. Cells were then trypsinized, counted, and seeded in 6-well plates in triplicates and treated with or without DOX for 13 days. (A) Cell colonies were stained with Coomassie blue and counted. Colony numbers in the DOX-treated samples are normalized to those of untreated samples, which is given a value of 1.0. *P<0.05, compared with siControl samples. (B) Knockdown of ATG5 by siATG5 as demonstrated by western blotting.

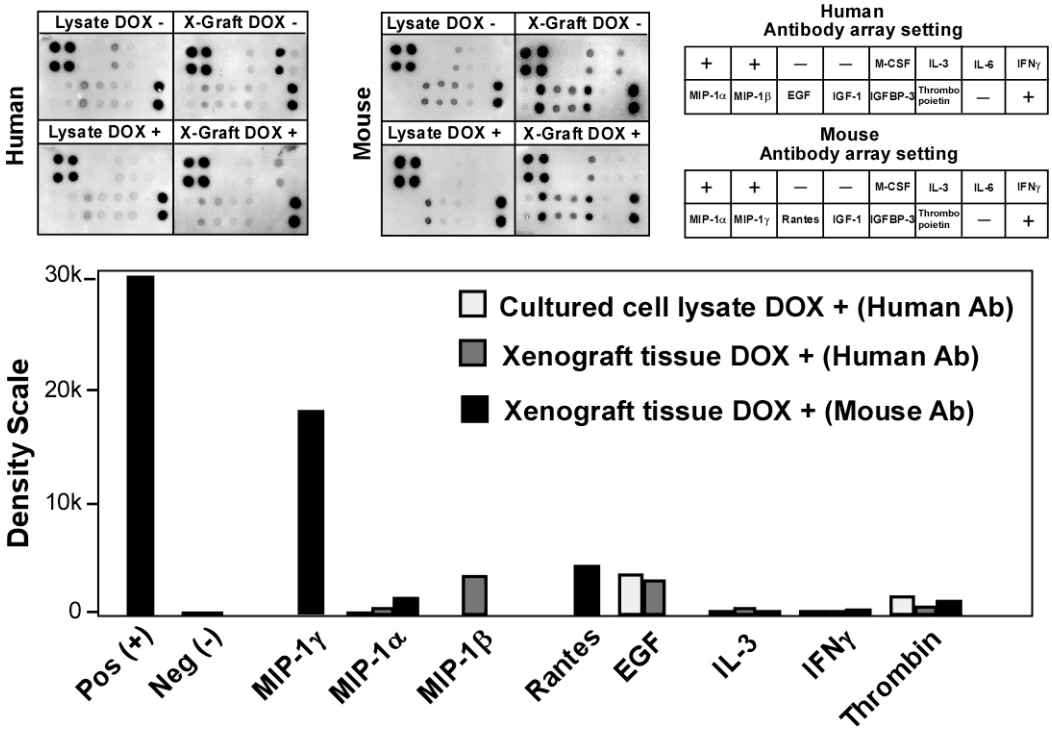




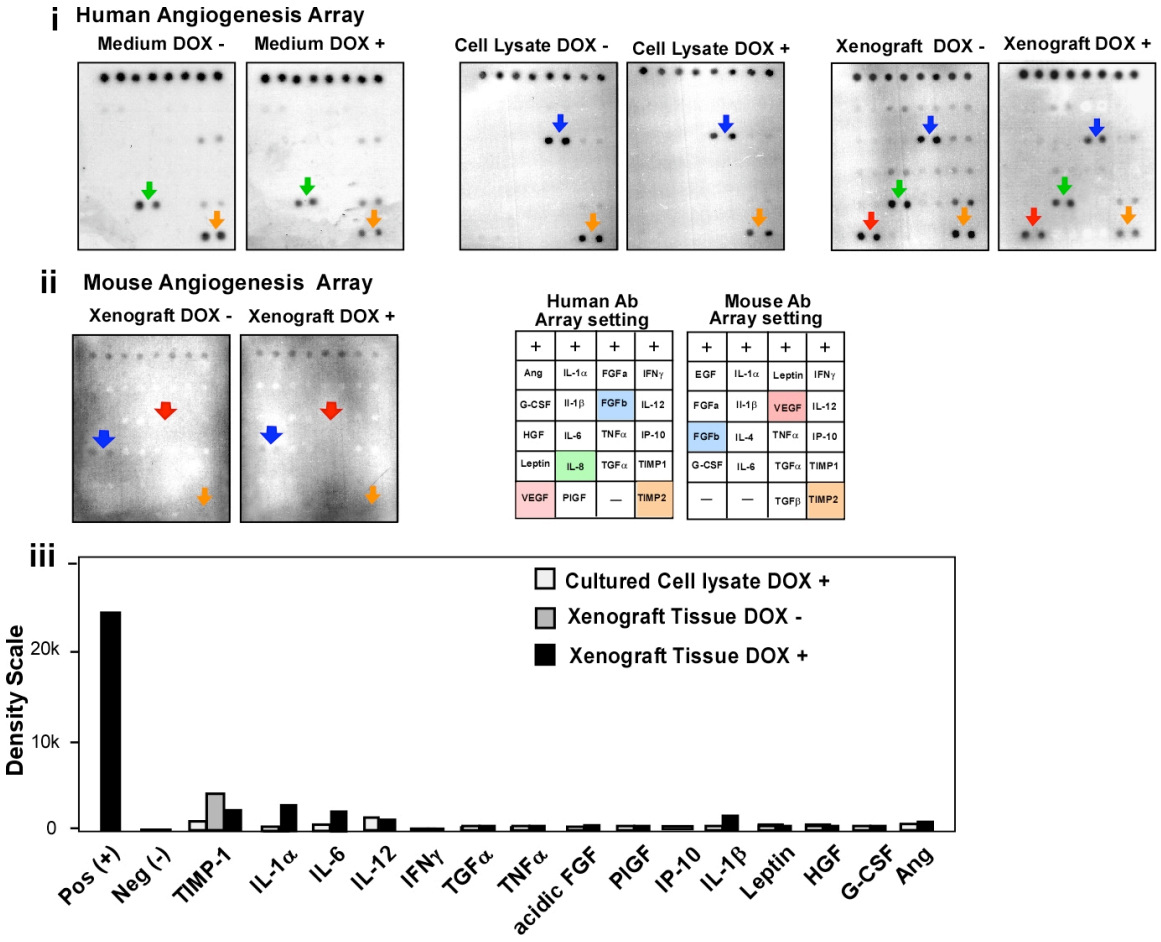
Supplementary Fig. S8. SKOv3-ARHI cells resume exponential growth after DOX withdrawal. SKOv3-ARHI cells were seeded in triplicates in 24-well plates and cultured without DOX (Dox -), with DOX (Dox +), or with DOX for 3 days followed by DOX withdrawal (Dox +/-). Cells were counted at indicated times after seeding. *P<0.01, compared with DOX+treated samples.



Supplementary Fig. S9. Most of detected growth factors are of host origin. Microarray analysis of cytokines, growth factors, and inflammatory factors from cultured cells or xenograft tissues were performed with antibodies specific for human or mouse antigens. Each antibody was spotted in duplicates and two sets of positive and negative controls were spotted at the upper-right corner of each membrane. * P<0.05, compared with *in vitro* samples.

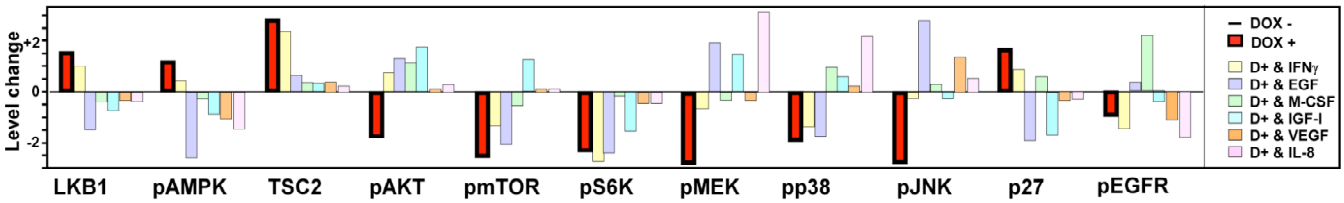


Supplementary Fig. S10. Detection of angiogenic factors in cultured cells and xenografts. Antibodies specific for human (panel i) or mouse (panel ii) angiogenic factors or inflammatory proteins were used in the angiogenic microarray analysis. Colored arrowheads indicate significant signals. Densitometry scanning of antibody spots was represented as arbitrary density units and shown in panel iii. Arrowheads indicate corresponding spots in the microarray membranes. *P<0.05, compared with *in vitro* samples.

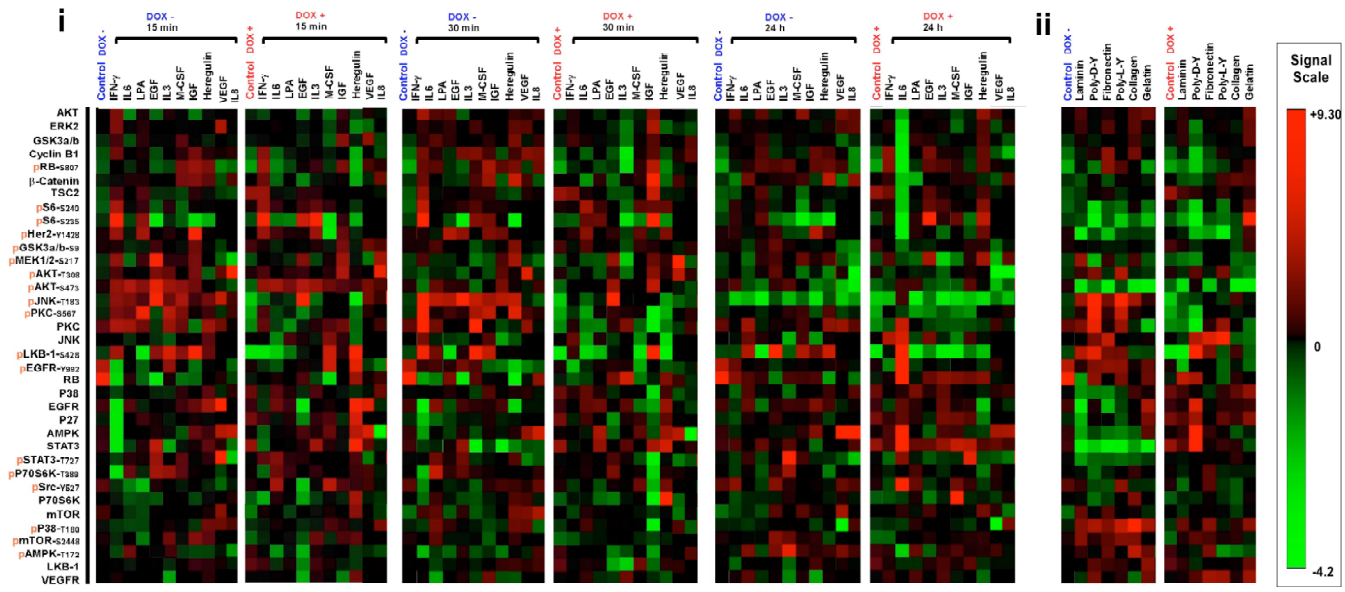


Supplementary Fig. S11. (A) ARHI induces autophagy through regulation of PI3K/AKT/mTOR and AMPK/TSC1/2 signaling pathways and growth factors/cell matrix proteins mitigate ARHI's autophagy-promoting activity. SKOv3-ARHI cells were cultured with or without DOX 24 hrs. ARHI-expressing cells were the treated with indicated growth factors or cytokines for 15 min before they were harvested and cell lysates prepared for reverse-phase protein microarray analysis. Fold changes in the levels of selected protein expression or phosphorylation after ARHI induction and addition of growth factors are shown. (B) SKOv3-ARHI or –NTD cells were cultured with or without DOX. Growth factors or cell matrix were added in the cultured condition. Cells were harvested at indicated times and cell lysates prepared for reverse-phase protein microarray analysis. (C) Phosphorylated AKT, mTOR and p70S6K, and total TSC2 expression were measured by Western blotting before and at different times after stimulation by VEGF (10 ng/mL) and IL-8 (10 ng/mL).

A


Level change
+2
0
-2
DOX -
DOX +
D+ & IFNγ
D+ & EGF
D+ & M-CSF
D+ & IGF-I
D+ & VEGF
D+ & IL-8
LKB1
pAMPK
TSC2
pAKT
pmTOR
pS6K
pMEK
pp38
pJNK
p27
pEGFR

B


i
ii
Signal Scale
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0
-4.2
AKT
ERK2
GSK3a/b
Cyclin B1
pRB-S807
β-Catenin
TSC2
pS6-S240
pS6-S235
pHer2-Y1248
pGSK3a/b-S9
pMEK1/2-S217
pAKT-T308
pAKT-S473
pJNK-T183
pPKC-S567
PKC
JNK
pLKB-1-S428
pEGFR-Y992
RB
P38
EGFR
P27
AMPK
STAT3
pSTAT3-T727
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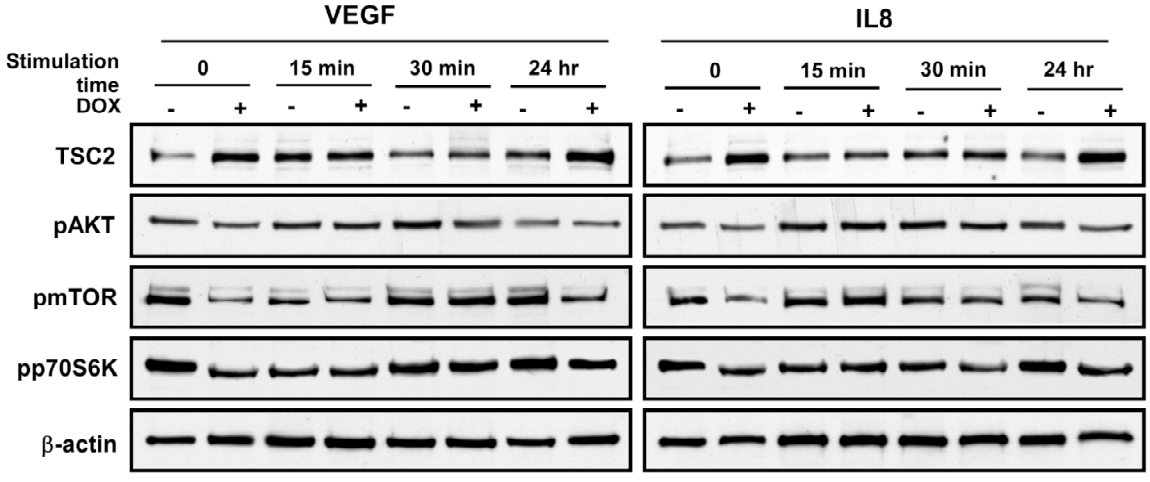
LKB-1
VEGFR

C


VEGF
IL8
Stimulation time
DOX
0
15 min
30 min
24 hr
TSC2
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pmTOR
pp70S6K
β-actin