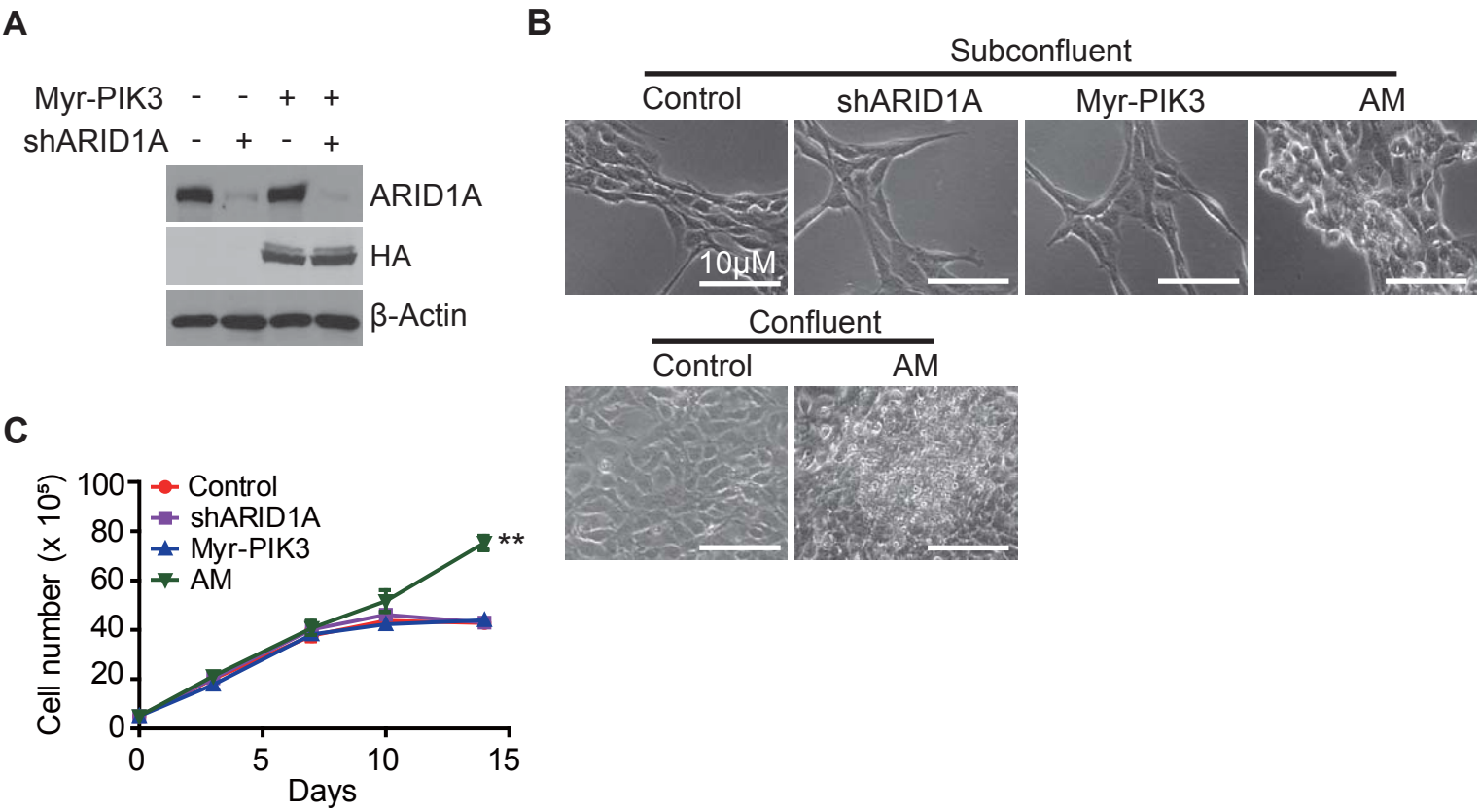

A
Myr-PIK3 - - + +
shARID1A - + - +
ARID1A
HA
β-Actin
B
Subconfluent
Control
shARID1A
Myr-PIK3
AM
10μM
Confluent
Control
AM
C
Cell number (x 10⁵)
100
80
60
40
20
0
Control
shARID1A
Myr-PIK3
AM
**
0
5
10
15
Days

Figure S2. Kim et al.

A

Gene Ontology Term	%	p-Value
Cytokine activity	19.64286	7.38E-10
Growth factor activity	10.71419	2.03E-04
Chemokine activity	5.357143	0.01057
Chemokine receptor binding	5.357143	0.011933
Interleukin-1 receptor binding	3.571429	0.052898

B

KEGG Pathway	%	p-Value
Cytokine-cytokine receptor interaction	16.07143	1.25E-04
NOD-like receptor signaling pathway	8.928571	6.29E-04
Graft-versus-host disease	7.142857	0.001616

C

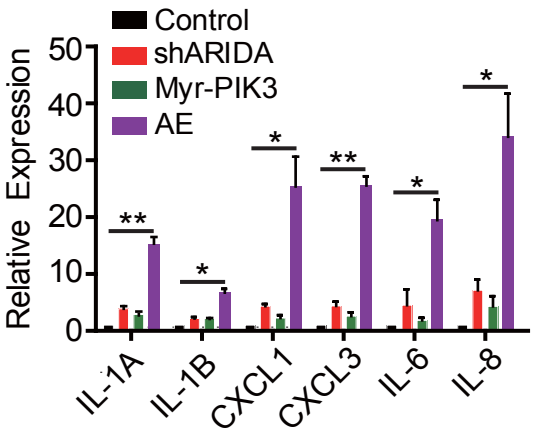


Figure S3. Kim et al.

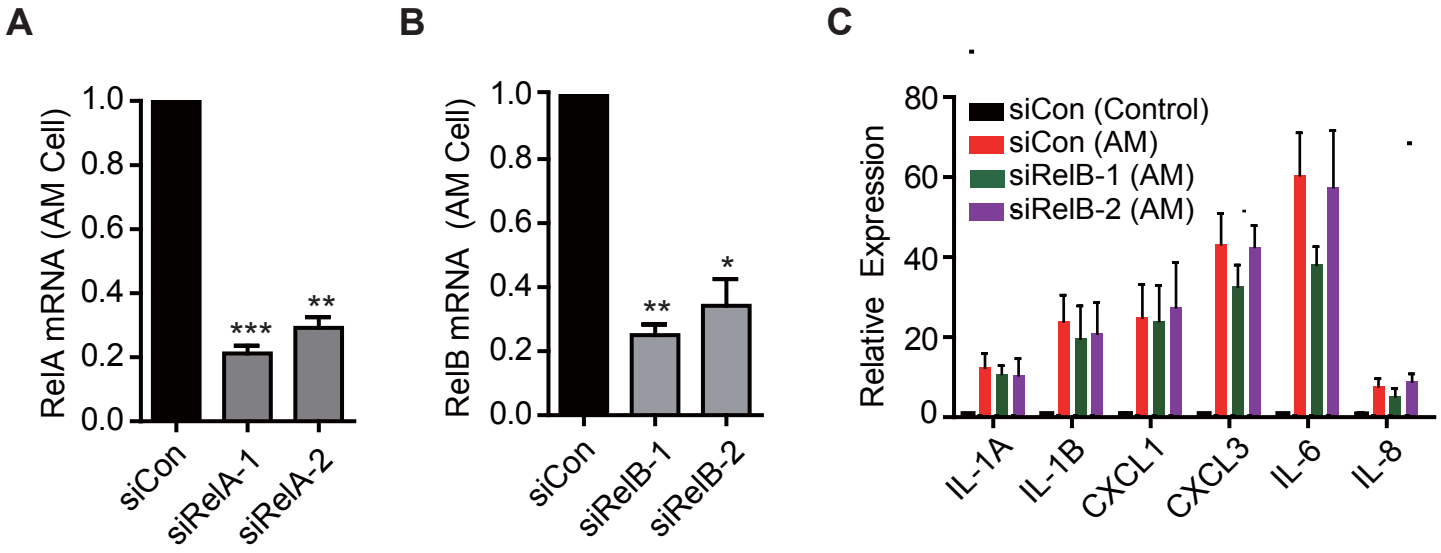
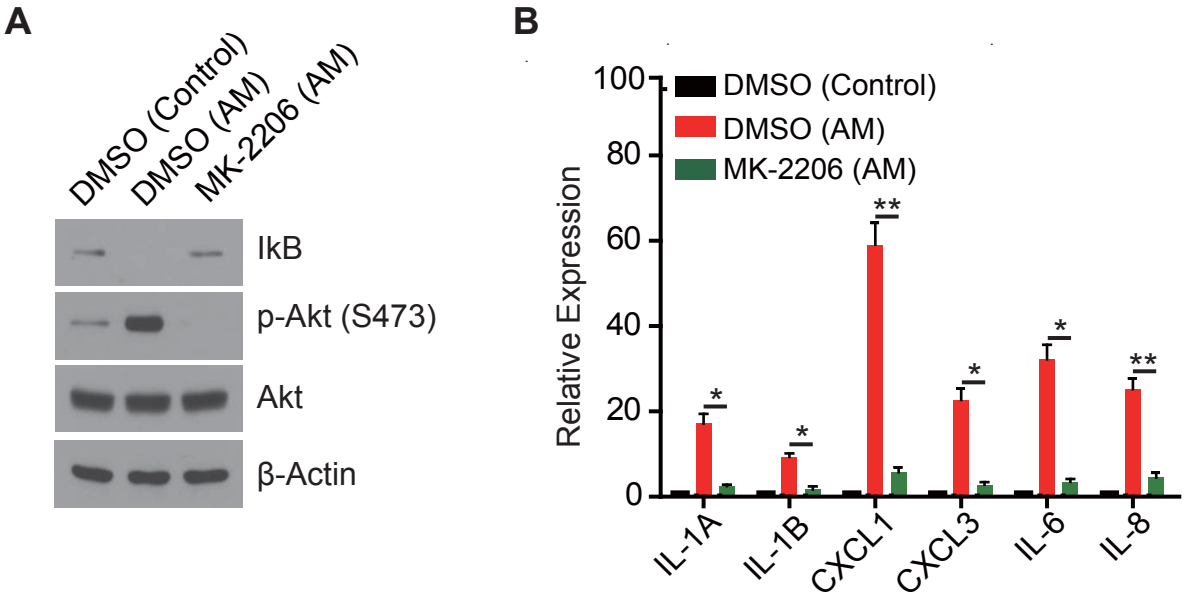
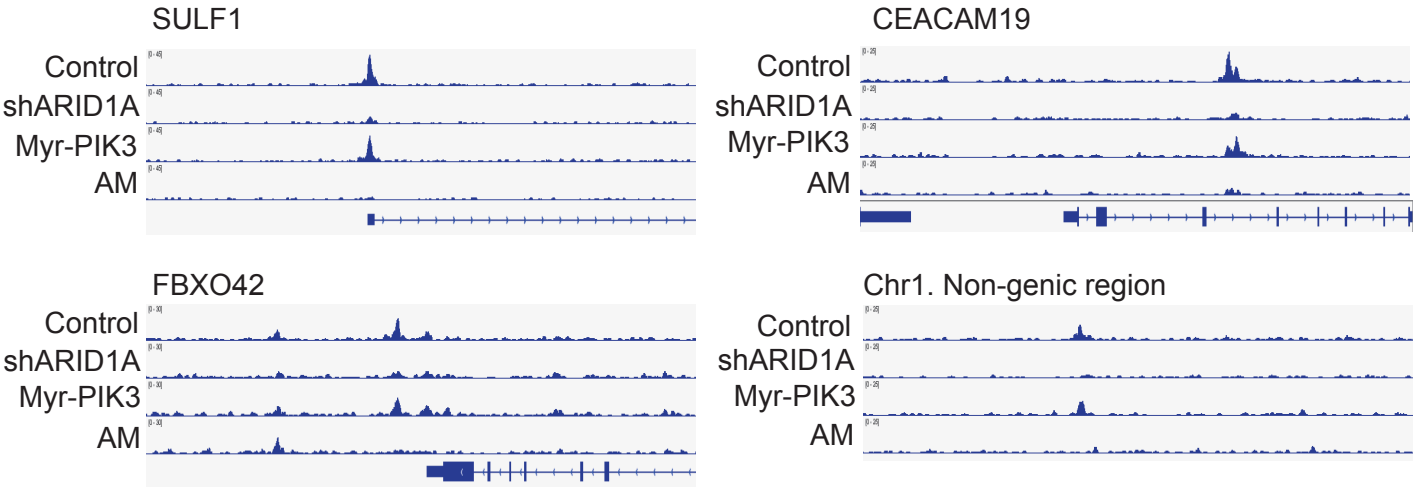


Figure S4. Kim et al.



A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Myr-PIK3
AM
CEACAM19
Control
shARID1A
Myr-PIK3
AM
FBXO42
Control
shARID1A
Myr-PIK3
AM
Chr1. Non-genic region
Control
shARID1A
Myr-PIK3
AM

B

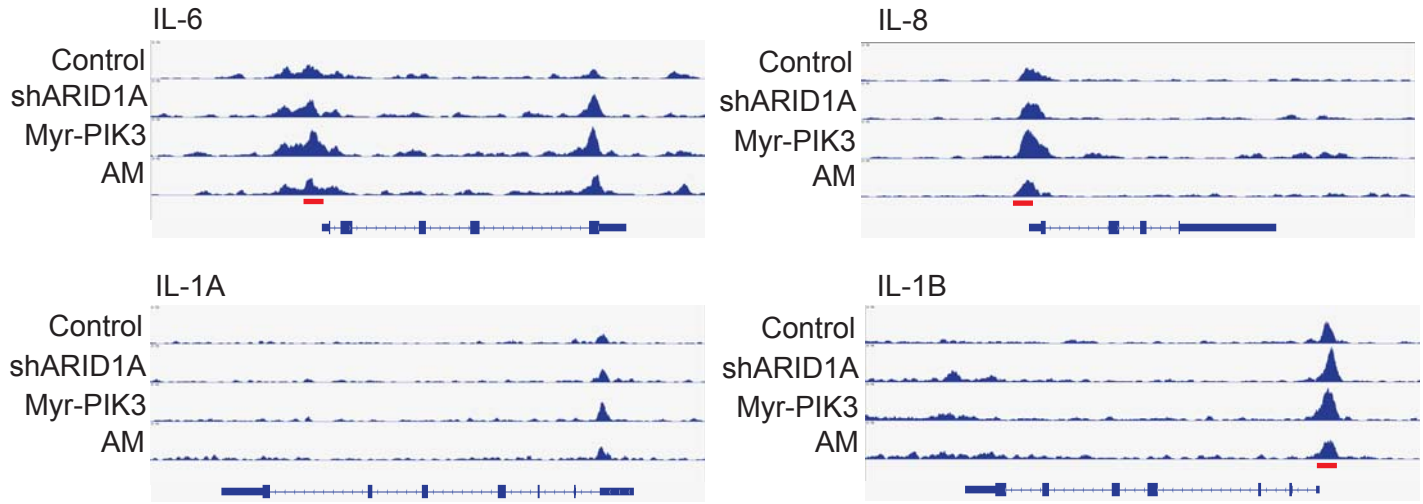

IL-6
Control
shARID1A
Myr-PIK3
AM
IL-8
Control
shARID1A
Myr-PIK3
AM
IL-1A
Control
shARID1A
Myr-PIK3
AM
IL-1B
Control
shARID1A
Myr-PIK3
AM

Figure S6. Kim et al.

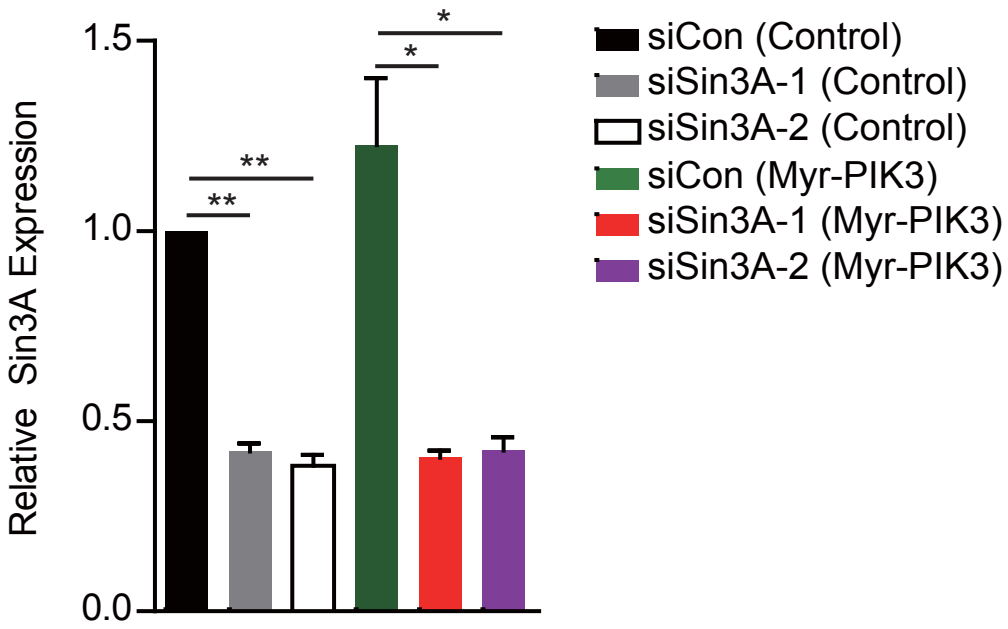
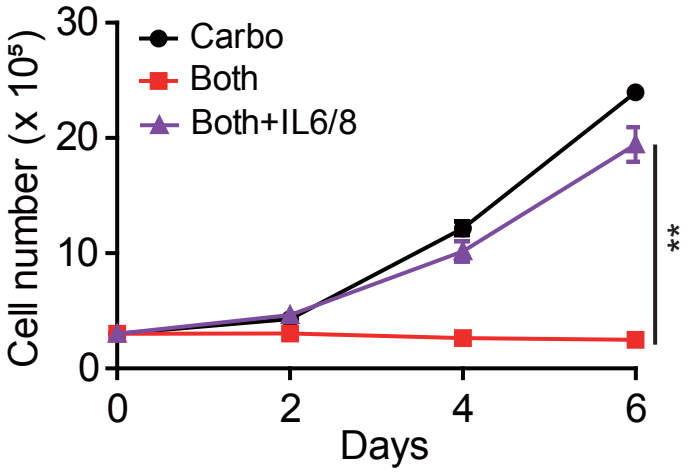


Figure S7. Kim et al.



Supplemental Figure Legends

Figure S1. ARID1A depletion and PIK3CA activation transform T29 normal human ovarian epithelial cells - related to Figure 1.

(A) Establishment of double mutant cells in T29 cell line. T29 cells were transduced with retroviruses expressing ARID1A shRNA and the indicated PIK3CA mutants. Western blot analyses confirm successful expression of the indicated proteins.

(B) Phase contrast images of established cell lines under sub-confluent and confluent conditions. Scale bar, 50μm.

(C) Growth curve of the established T29 cell lines. $5x10^5$ of the indicated cells were plated on 6-well plates, and counted every two days (n=3). Error bars, S.E.M. Statistical analysis comparing AM and control cells is done. Paired student's t-test (two-tailed). **, *p-value* < 0.01.

Figure S2. Cytokine genes are abberantly up-regulated in ARID1A/PIK3CA mutant cells - related to Figure 2

(A) Gene-ontology analysis of the genes presented in Figure 2A.

(B) KEGG pathway analysis of the genes presented in Figure 2A.

(C) Confirmation of the RNA-Seq results of the T80(AE) cells by qRT-PCR. Expression level of selected cytokines genes were confirmed by qRT-PCR and normalized by β-actin transcripts (n=3). Error bars, S.E.M. Paired student's t-test (two-tailed). *, *p-value* < 0.05; **, *p-value* < 0.01.

Figure S3. RelB NF-kB transcription factor is dispensable for cytokine gene expression in the ARID1A/PIK3CA mutant cells - related to Figure 3

(A) Confirmation of RelA knock-down by qRT-PCR (n=3). Error bars, S.E.M.

(B) Confirmation of RelB knock-down by qRT-PCR (n=3). Error bars, S.E.M.

(C) RelB knockdown does not alter cytokine gene expression. Cells were transfected as indicated and the expression level of selected cytokines genes were analyzed by qRT-PCR three days after transfection, normalized by β-actin transcripts (n=3). Error bars, S.E.M.

All statistical analyses were performed by paired student's t-test (two-tailed). *, *p-value* < 0.05; **, *p-value* < 0.01; ***, *p-value* < 0.001.

Figure S4. An AKT inhibitor abolishes RelA activation and cytokine gene induction - related to Figure 4

(A) DMSO or MK-2206 (10 μM), an AKT-specific inhibitor, was applied to the indicated cells for three days before the cells were harvested for Western blotting using the indicated antibodies.

(B) Expression of the selected cytokine genes in cells from panel A were analyzed by qRT-PCR and normalized by β-actin transcripts (n=3). Error bars, S.E.M. Paired student's t-test (two-tailed). *, *p-value* < 0.05; **, *p-value* < 0.01.

Figure S5. ARID1A depletion does not alter chromatin accessibility of cytokine genes

(A) Genome browser views of DNase-seq results of selected cytokine genes in the various T80 cells.

(B) Genome browser views of DNase-seq results of selected loci exemplify the effect of ARID1A depletion. Red bars indicate RelA binding elements.

Figure S6. Confirmation of Sin3A knock-down - related to Figure 5

Confirmation of Sin3A knock-down by qRT-PCR in the control and Myr-PIK3CA expressing cells (n=3). Error bars, S.E.M. Paired student's t-test (two-tailed). *, *p-value* < 0.05; **, *p-value* < 0.01.

Figure S7. Cytokines are responsible for carboplatin resistance of T80 (AM) cells - related to Figure 7

$3x10^5$ T80 (AM) cells were plated on 6-well plates. One day after seeding, cells were treated with the indicated Carboplatin/IKK-IIV with or without recombinant IL6 and IL8 (both 10 μg/μl). Cell number was counted every two days after seeding (n=3). Error bars, S.E.M. Paired student's t-test (two-tailed). **, *p-value* < 0.01.

Table S1. List of genes up-regulated in ARID1A/PI3KCA mutant cells - related to Figure 2.

List of genes in Figure 2A. Fold changes of each genes (AM cell/Control cell) are indicated. Secreted cytokines or growth factors are indicated in Red.

Gene name	AM/Con (T80)	AM/Con (T29)	Gene name	AM/Con (T80)	AM/Con (T29)
SNORA39	Control no exp.	Control no exp.	BMF	7.41	3.84
SNORA66	Control no exp.	Control no exp.	GPR68	7.41	11.04
IL-24	558.16	12.61	HIST1H2BK	6.82	5.60
EHF	529.20	25.11	HIST1H3E	6.64	Control no exp.
MMP3	106.29	127.75	HIST1H1C	6.48	3.53
PTGS2	71.33	39.94	PAK3	5.88	18.36
CLDN1	49.99	5.73	HIST2H4B	5.86	2.60
CXCL1	41.64	34.24	ITGA2	5.74	7.03
NPTX1	35.99	23.96	HERC5	5.12	6.67
IL8	34.95	22.47	PNPLA3	4.81	5.41
KYNU	31.79	77.46	HIST2H4A	4.69	3.93
CSF2	30.66	38.80	HIST2H2AA4	4.67	4.63
IL4I1	28.76	10.52	HTR7	4.56	6.49
MYEOV	27.52	16.47	STC1	4.55	10.09
FEZ1	19.91	13.51	IER3	4.18	3.24
MMP1	19.60	73.50	TNFRSF11B	4.18	4.00
PTPRN2	19.85	Control no exp.	HIST1H3D	3.99	17.23
IL-1A	18.50	23.80	HIST2H2AA3	3.84	4.39
VNN1	17.29	4.08	EML1	3.56	95.13
BIRC3	16.52	6.35	NR1D1	3.55	2.17
TRPA1	14.27	62.27	MAGEC2	3.55	10.06
AREG	14.10	2.15	KRT15	3.46	2.41
PLAU	13.58	50.71	HLA-B	3.45	2.10
CYP1A1	12.74	4.85	MAP3K5	3.16	16.86
IL-6	10.38	5.93	MMD	2.96	3.61
CXCL3	10.38	11.81	FLJ27352	2.59	2.37
AGPAT9	9.10	3.14	HIST1H2BG	2.59	4.20
IL-1B	8.74	28.51	BFSP1	2.54	5.68
GDF15	8.71	3.62	RGS17	2.33	5.67
LCE1F	8.17	11.36			

Table S2. List of primer sequences for RT-qPCR and ChIP-qPCR used in this study

RT-qPCR oligos	Sequence
β-actin F	CAT GTA CGT TGC TAT CCA GGC
β-actin R	CTC CTT AAT GTC ACG CAC GAT
IL-1A F	AGA TGC CTG AGA TAC CCA AAA CC
IL-1A R	CCA AGC ACA CCC AGT AGT CT
IL-1B F	ATG ATG GCT TAT TAC AGT GGC AA
IL-1B R	GTC GGA GAT TCG TAG CTG GA
IL-6 F	ACT CAC CTC TTC AGA ACG AAT TG
IL-6 R	CCA TCT TTG GAA GGT TCA GGT TG
IL-8 F	ACT GAG AGT GAT TGA GAG TGG AC
IL-8 R	AAC CCT CTG CAC CCA GTT TTC
CXCL1 F	AAC CGA AGT CAT AGC CAC AC
CXCL1 R	GTT GGA TTT GTC ACT GTT CAG C
CXCL3 F	CGC CCA AAC CGA AGT CAT AG
CXCL3 R	GCT CCC CTT GTT CAG TAT CTT TT
RelA F	ATG TGG AGA TCA TTG AGC AGC
RelA R	CCT GGT CCT GTG TAG CCA TT
RelB F	CCA TTG AGC GGA AGA TTC AAC T
RelB R	CTG CTG GTC CCG ATA TGA GG
Sin3A F	GGT GGA GGA TGC GCT ATC TTA
Sin3A R	GGG TGT CGA TGC TCT GAG ATT T
ChIP-qPCR oligos	**Sequence**
IL-1B RelA binding element F	ATC TCA TGG AAC GCC ATT TC
IL-1B RelA binding element R	GGG AGG AGG ATG TCG TGT TA
IL-6 RelA binding element F	TGC AGG TTT TGG TGT GGA TA
IL-6 RelA binding element R	GCT GAC AAG GAT GTG AAG CA
IL-8 RelA binding element F	CTG GGC TTT TCC ACA TGT CT
IL-8 RelA binding element R	TCC TGC TTG TCT CCA CAA AA
CXCL1 RelA binding element F	CTC GGG ATC GAT CTG GAA CT
CXCL1 RelA binding element R	GTG AGA GGA GCG GAA GAG C
IL-6 amplicon 1 F	CGA TAT AGC CGA GCT GGA AG
IL-6 amplicon 1 R	TTC CTG GCG CAT AGT AAT CC
IL-6 amplicon 2 F	AAA TGC CCA ACA GAG GTC AC
IL-6 amplicon 2 R	AAA CCA GAC CCT TGC ACA AC
IL-8 amplicon 1 F	AGC TCC AGT GCA CCA CTT TC
IL-8 amplicon 1 R	TGA GGA ATA GGA GGG CTT CA
IL-8 amplicon 2 F	CAT CAG TTG CAA ATC GTG GA
IL-8 amplicon 2 R	GAA GCT TGT GTG CTC TGC TG

Supplemental Experimental Procedures

Cell lines and reagents

T80 and T29 cell lines were kind gifts from Dr. Jin-Sung Liu, and were cultured in 1:1 DMBC 105 (Sigma) and Medium 199 (Invitrogen), supplemented with 2mM L-Glutamine, 50ng/ml human EGF, Penicillin/Streptomycin and 10% Fetal bovine serum. 293T, TOV-21G, ES-2 and OV-90 cell lines were purchased from ATCC, and cultured by supplier's instruction. Ruxotinilib, IKK-IIV, Trichostatin A, Carboplatin and MK-2206 were purchased from Selleckchem and dissolved in DMSO (Sigma). Drugs were used with indicated concentrations and DMSO was used in 0.1% (v/v). Neutralizing antibody against IL6 (MAB206), recombinant IL-6 (206-IL) and IL-8 (208-IL) were purchased from R&D systems. Zeocin was purchased from Invitrogen.

Constructs

pBABE zeocin vector, pBABE puro HA-PIK3CA E545K and pBABE puro HA-Myr-PIK3CA were obtained from Addgene. IKK2-CA construct was obtained from Addgene and subcloned into pMSCV puro vector. IkB-SR construct was obtained from Addgene and subcloned into pBABE zeocin vector. Scrambled (5'-CCTAAGGTTAAGTCGCCCTCG-3') and ARID1A targeting (5'- GCCTGATCTATCTGGTTCAAT-3') shRNAs were cloned to pLKO.1 neo vector.

Virus generation and transduction

For retroviral vectors (pBABE and pMSCV), 293T cells were transfected with 5:5:2 ratio of pBABE/pMSCV : Gag-pol : Vsv-G using FuGene HD (Promega). For lentiviral vectors, 293T cells were transfected with pLKO.1, pMD2.G and psPAX2 following Addgene's guideline using Fugene HD. One day after transfection, cell media were changed and virus containing supernatants were collected every 24 hours for two days. Target cells were infected with virus containing supernatant supplemented with 6 μg/μl polybrene. One day after infection, selection was initiated using 1 μg/ml puromycin or 400 μg/ml G418 (Sigma).

siRNA transfection

Control (4390843) and selected siRNAs against RelA (4427038-s11914 and s11916), RelB (4427037-s11917 and s11918) and Sin3A (4427037-s24799 and s24800) were purchased from Thermofisher Scientific. For transfection, RNAi max (Life technology) was used according to manufacturer's instruction. Cells were analyzed three days after transfection.

Soft agar assay

A total of 1 ml of 0.5% bottom agar in T80 growth medium was solidified in 6-well plates. 3 x 10^4 cells were added to 0.4% top agar in T80 growth medium and layered onto bottom agar. A total of 2 ml medium was added on the top to prevent drying and was replenished every 3 days. After three weeks, colonies were stained with 0.1% crystal violet followed by washing with distilled water. Stained colonies were then examined under a dissecting microscope.

Western blotting

Cells were lysed in lysis buffer (50mM Tris-Cl, pH 7.5, 150mM NaCl, 1mM $MgCl_2$ and 1% Triton X-100) for 20-30 minutes on ice. After clearing the lysates by centrifugation, protein concentrations were measured by Bradford method, and boiled with Laemilli buffer. Denatured proteins were separated by SDS-PAGE and then transferred to nitrocellulose membrane. Membranes were blocked with 5% skim milk and treated with primary antibodies diluted in 5% skim milk or Bovine Serum Albumin overnight at 4°C. Membranes were developed using ECL reagent (Amersham). The primary antibodies used for Western blotting are as follows; β-actin (1:10000, Santa Cruz, AC-15), IkB (1:1000, Santa Cruz, C-21), phospho-IkB (1:1000, Cell Signaling, #9246), RelA (1:1000, Santa Cruz, C-20), RelB (1:1000, Santa Cruz, C-19) Lamin B (1:500, Santa Cruz, M-20), α-tubulin (1:000, Calbiochem), ARID1A (1:1000, Cell Signaling, #12354) and HA (1:1000, Cell Signaling, #3724).

Reverse Transcription and qPCR

Cellular RNAs were isolated using Trizol reagent (Ambion) following manufacture's instruction. 1μg of RNA was reverse transcribed using oligo dT primer and Impro-II RT (Promega). Synthesized cDNAs were diluted three-fold with water. Target genes were amplified using SYBR green master mix (AB applied bioscience). C_t values of target genes were normalized using that of β-actin. The primer sequences used are listed in Supplementary Table S2.

Immunofluorescence

Cells were washed once with PBS and fixed with 4% Paraformaldehyde at room temperature for 15 minutes. Cells were permeabilized by incubating with 0.3% Triton X-100 diluted in PBS at room temperature for 15 minutes. After blocking with 1% bovine serum albumin, antibody against RelA (1:100, Santa Cruz, C-20) diluted in blocking buffer was treated at 4°C overnight. On the next day, samples were washed three times with PBS (10 minutes each) and then incubated with an anti-rabbit Alexa-594 secondary antibody (1:1000, Invitrogen) at room temperature for an hour. After washing three times with PBS, samples were stained with DAPI diluted in PBS (30 nM, Thermofisher) for 5 minutes. Solution was then replace with PBS and examined under a fluorescence microscope.

Chromatin Immunoprecipitation

Cells were fixed with 1% formaldehyde in phosphate buffered saline (PBS) for 10 minutes at room temperature, followed by quenching with 125 mM Glycine for 5 minutes. Cell pellets were resuspended in hypotonic buffer (20 mM HEPES, pH 7.9, 2 mM KCl and 1 mM DTT) and then incubated on ice for 15 minutes. Cells were passed through a 27G syringe ten times to lyse cell membrane and centrifuged two minutes to pellet nuclei. Nuclear pellets were resuspended in 100-300 μl SDS buffer [50 mM Tris-Cl (pH 7.5), 10 mM EDTA and 1% SDS] and transferred to TPX tube (Diagenode). Samples were sonicated using bio-ruptor for 50 cycles with 30 sec on and 30 sec off in high power. Samples were then centrifuged for 20 minutes at 13,000 rpm, and supernatants were transferred to a new tube. Samples were then diluted 10 times with dilution buffer [20 mM Tris-Cl (pH7.5), 167 mM NaCl, 1.2 mM EDTA, 1.1% Triton X-100 and 0.1% SDS], and DNA concentration was measured. 5-10 μg chromatin were incubated with 2-3 μg

antibody. After overnight incubation at 4°C, samples were further incubated with 15 µl protein A dynabeads (Thermofisher) for 1 hour. Beads were then washed three times with washing buffer [50 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100 and 0.1% SDS] for three times and once with high salt buffer (washing buffer with 500 mM NaCl). Chromatin was then eluted from beads by incubating the beads with elution buffer (100 mM sodium carbonate, 400 mM NaCl, and 1% SDS) at 68°C overnight with constant shaking. Eluted samples were sequentially treated with RNase for 1 hour and Proteinase K for two hours. DNA was precipitated with 2 volumes of Ethanol and 300 mM sodium acetate. Glycogen was added to aid the precipitation of the DNA. DNA pellets were washed once with 70% Ethanol and resuspended in 50 µl autoclaved water. With the exception of ChIP assay of H4 and Acetylated-H4, 10% of input chromatin and isolated DNA were used for normalization. 1µl sample was used for target region amplification. Antibodies used for ChIP assay include RelA (Santa Cruz, C-20), H4 (Abcam, ab31830), Acetylated-H4 (Millipore, 06-866) and HDAC1 (Abcam, ab7028). The sequences of the ChIP primers are listed in supplementary Table S2.