Expanded View Figures

A

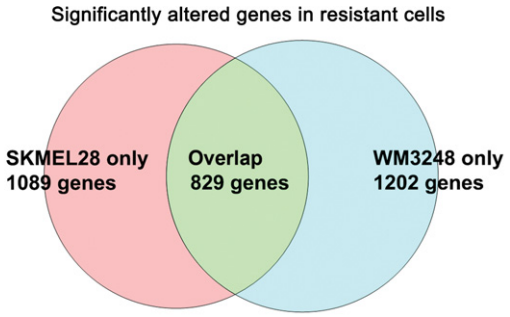

Figure EV1. Comparison of expression profiles between PLX4032-resistant and parental cells reveals significant alterations of multiple genes encoding actin cytoskeleton regulators.

A Venn diagram showing the number of significantly altered genes in a microarray analysis comparing parental and resistant cells of SKMEL28 and WM3248.

B A list showing significantly enriched gene ontology terms (Benjamini–Hochberg FDR < 0.05) related to the actin cytoskeleton on functional annotation charts which analyze 829 significantly altered genes.

B

GO Category	GO Term	Overlap Gene Count	*P*-value	Benjamini
GOTERM_BP_FAT	GO:0030036~actin cytoskeleton organization	22	0.00112	0.03189
GOTERM_CC_FAT	GO:0001725~stress fiber	7	5.18E-04	0.0164
GOTERM_CC_FAT	GO:0032432~actin filament bundle	7	8.21E-04	0.02108
GOTERM_MF_FAT	GO:0003779~actin binding	31	1.12E-04	0.03035

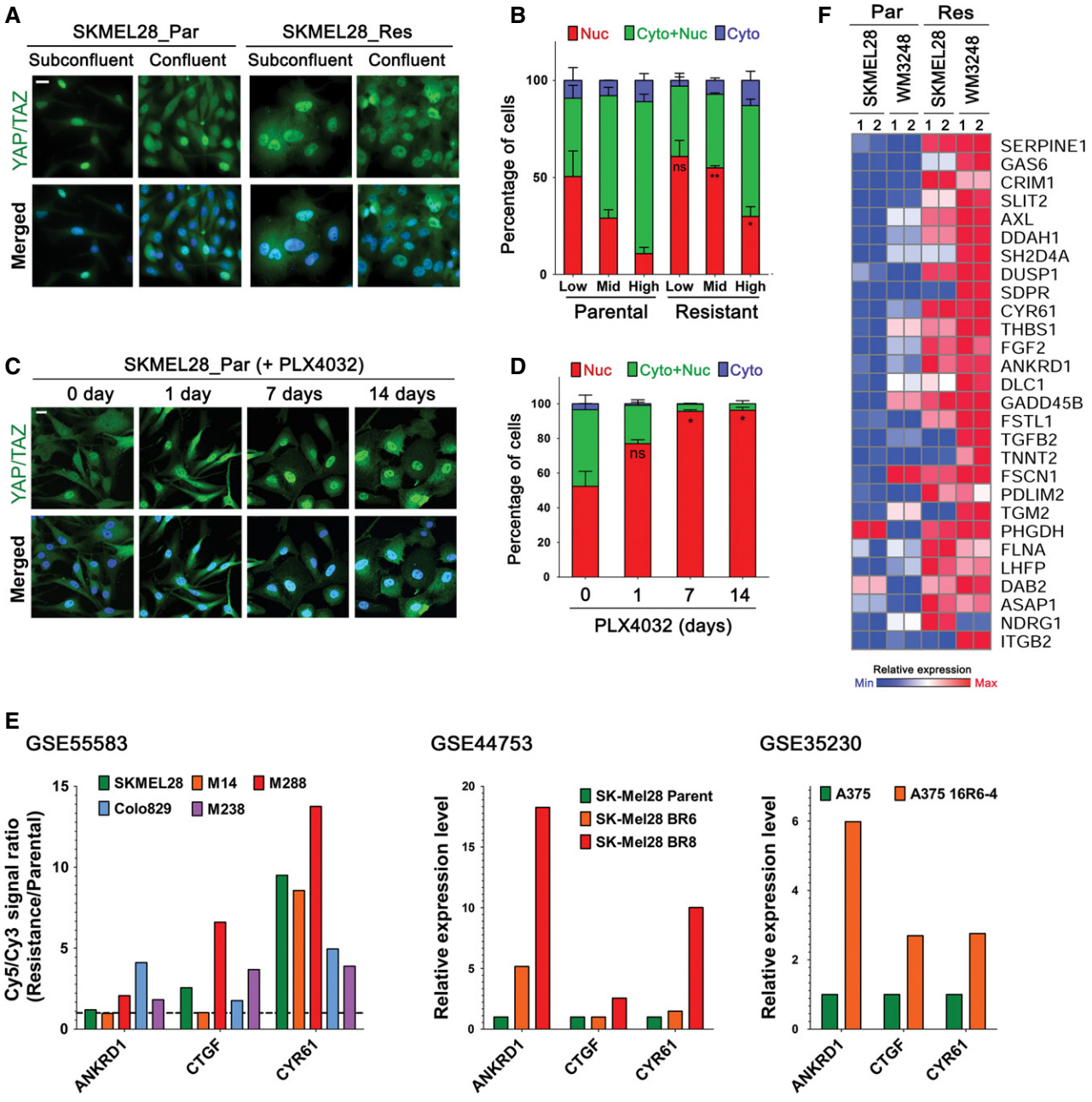

Figure EV2. Resistant melanoma cells exhibit both YAP/TAZ nuclear localization and YAP signature gene upregulation.

A Immunofluorescence micrographs showing YAP/TAZ localization in parental and resistant SKMEL28 cells in subconfluent and confluent cultures. Cells were labeled with anti-YAP/TAZ antibody.

B Quantification of the experiment presented in (A). Cells were seeded at low, middle, and high confluency. Proportion of nuclear YAP/TAZ in resistant cells was compared with that in parental cells of the same confluency.

C Immunofluorescence micrographs showing YAP/TAZ localization. Parental SKMEL28 cells were treated with PLX4032 (2 μM) for the indicated times before labeling with anti-YAP/TAZ antibody.

D Quantification of the experiment presented in (C).

E Comparison of YAP/TAZ target gene expressions (ANKRD1, CTGF, and CYR61) between parental and BRAF inhibitor-resistant melanoma cells in published gene expression datasets (GSE55583, GSE44753, and GSE35230 from GEO database).

F A heatmap showing upregulated YAP signature genes in resistant cells identified by GSEA analysis (the leading edge subset) comparing expression profiles of resistant and parental cells.

Data information: All data are mean and SEM [two biologic replicates (D) or three biologic replicates (B)], and *P*-values were determined by *t*-test (*$P < 0.05$ and **$P < 0.01$). The nuclei were stained with DAPI (blue), and scale bars represent 20 μm.

Figure EV3. Validation of the specificity of YAP/TAZ knockdown, and comparison of YAP/TAZ knockdown sensitivity between parental and resistant melanoma cells.

A PLX4032 dose–response curves of parental and resistant cells after YAP/TAZ knockdown. Cells were transfected with either control or YAP/TAZ siRNA set 2 (siYT#2) for 72 h, and then treated with PLX4032 at the indicated concentrations for additional 72 h. Relative cell viability, compared to DMSO control, was measured by CCK8 assay. Sigmoidal dose–response curves were fitted to data, and significance of the difference in IC_{50} values was determined by extra sum-of-squares *F* test.

B Time-course analyses of cell viability after YAP/TAZ knockdown. Cells were transfected with the indicated siRNAs for 48 h, and then treated with PLX4032 (2 μM) for the indicated times. Relative cell viability, compared to initial cell viability, was measured by CCK8 assay. Significance of the difference between control and YAP/TAZ depletion for parental or resistant cells were determined by *t*-test.

C Quantification of BrdU assay of SKMEL28 and WM3248 cells transfected with either control siRNA or siYT#2. Cells were treated as in Fig 4C.

D Cell viability analyses of SKMEL28 and WM3248 cells transfected with variable doses of YAP/TAZ siRNAs and control siRNA. Cells were transfected with siRNAs for 144 h and cell viability, compared with that of control siRNA-transfected cells, was measured by CCK8 assay.

E Immunoblotting for the indicated proteins 48 h after transfection of siRNAs followed by 24-h PLX4032 or DMSO treatment.

F Cell viability analyses of resistant SKMEL28 and WM3248 cells transfected with siRNAs and siRNA-resistant flag-YAP-5SA retrovirus. Cells were transfected with retrovirus for 24 h, and then transfected with the indicated siRNAs (forward transfection) for 72 h (SKMEL28) or 144 h (WM3248). Relative cell viability, compared with that of mock and control siRNA-transfected cells, was measured by CCK8 assay.

G Immunoblotting of the indicated proteins in transfected HEK293T. HEK293T cells were transfected with siRNA-resistant Flag-YAP5SA retrovirus for 24 h, and then transfected with the indicated siRNAs for 72 h (reverse transfection).

Data information: All data are mean and SEM (three biologic replicates). In all graphs except (A), *P*-values were determined by *t*-test (*$P < 0.05$ and **$P < 0.01$).
Source data are available online for this figure.

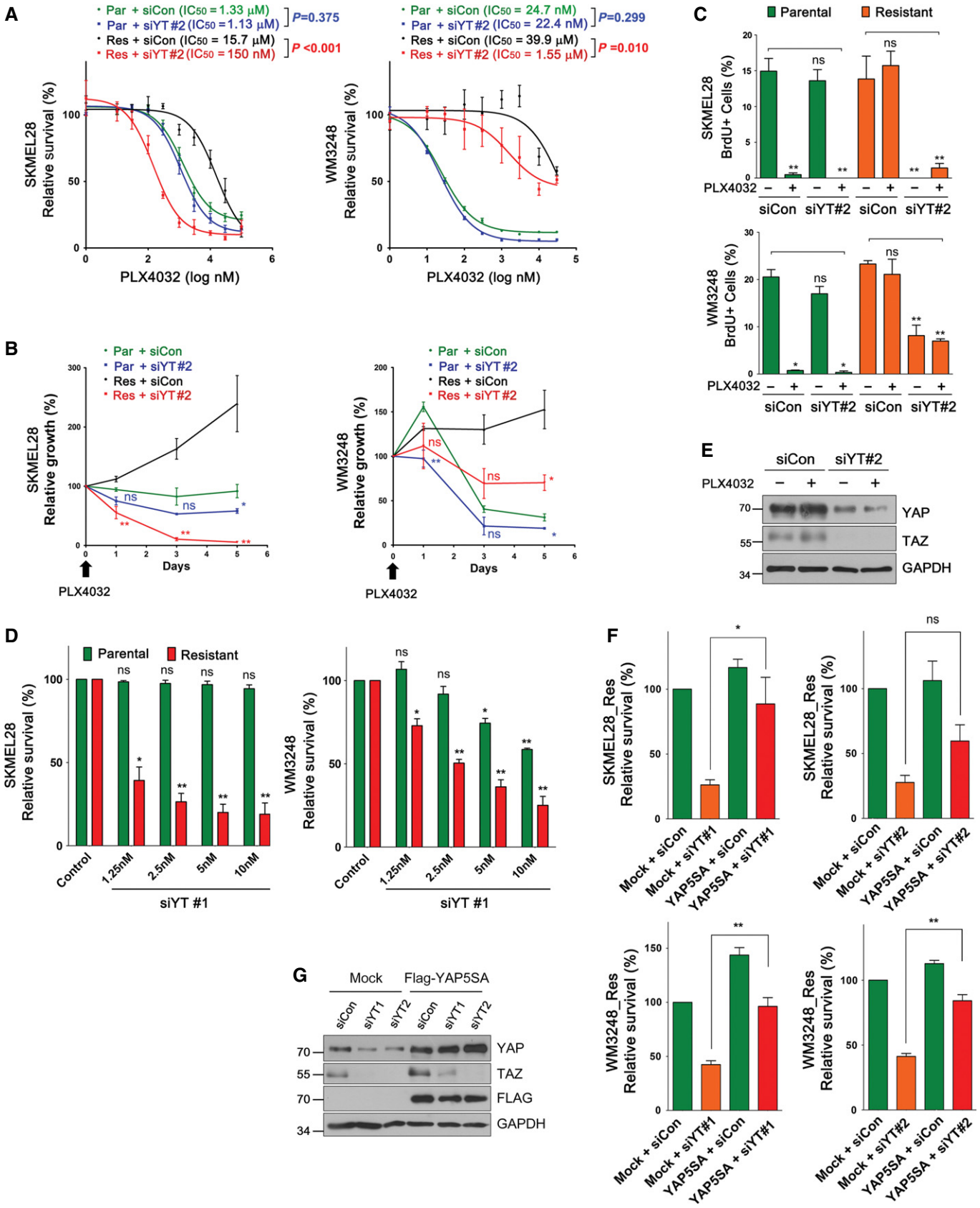
A
Par + siCon (IC50 = 1.33 μM)
Par + siYT#2 (IC50 = 1.13 μM)
Res + siCon (IC50 = 15.7 μM)
Res + siYT#2 (IC50 = 150 nM)
P=0.375
P <0.001
SKMEL28 Relative survival (%)
PLX4032 (log nM)
Par + siCon (IC50 = 24.7 nM)
Par + siYT#2 (IC50 = 22.4 nM)
Res + siCon (IC50 = 39.9 μM)
Res + siYT#2 (IC50 = 1.55 μM)
P=0.299
P =0.010
WM3248 Relative survival (%)
PLX4032 (log nM)
B
Par + siCon
Par + siYT#2
Res + siCon
Res + siYT#2
SKMEL28 Relative growth (%)
Days
PLX4032
WM3248 Relative growth (%)
Days
PLX4032
C
Parental
Resistant
SKMEL28 BrdU+ Cells (%)
PLX4032
siCon siYT#2 siCon siYT#2
WM3248 BrdU+ Cells (%)
PLX4032
siCon siYT#2 siCon siYT#2
D
Parental
Resistant
SKMEL28 Relative survival (%)
Control 1.25nM 2.5nM 5nM 10nM
siYT #1
WM3248 Relative survival (%)
Control 1.25nM 2.5nM 5nM 10nM
siYT #1
E
siCon siYT#2
PLX4032
YAP
TAZ
GAPDH
F
SKMEL28_Res Relative survival (%)
Mock + siCon
Mock + siYT#1
YAP5SA + siCon
YAP5SA + siYT#1
SKMEL28_Res Relative survival (%)
Mock + siCon
Mock + siYT#2
YAP5SA + siCon
YAP5SA + siYT#2
WM3248_Res Relative survival (%)
Mock + siCon
Mock + siYT#1
YAP5SA + siCon
YAP5SA + siYT#1
WM3248_Res Relative survival (%)
Mock + siCon
Mock + siYT#2
YAP5SA + siCon
YAP5SA + siYT#2
G
Mock
Flag-YAP5SA
siCon siYT1 siYT2 siCon siYT1 siYT2
YAP
TAZ
FLAG
GAPDH

Figure EV3.

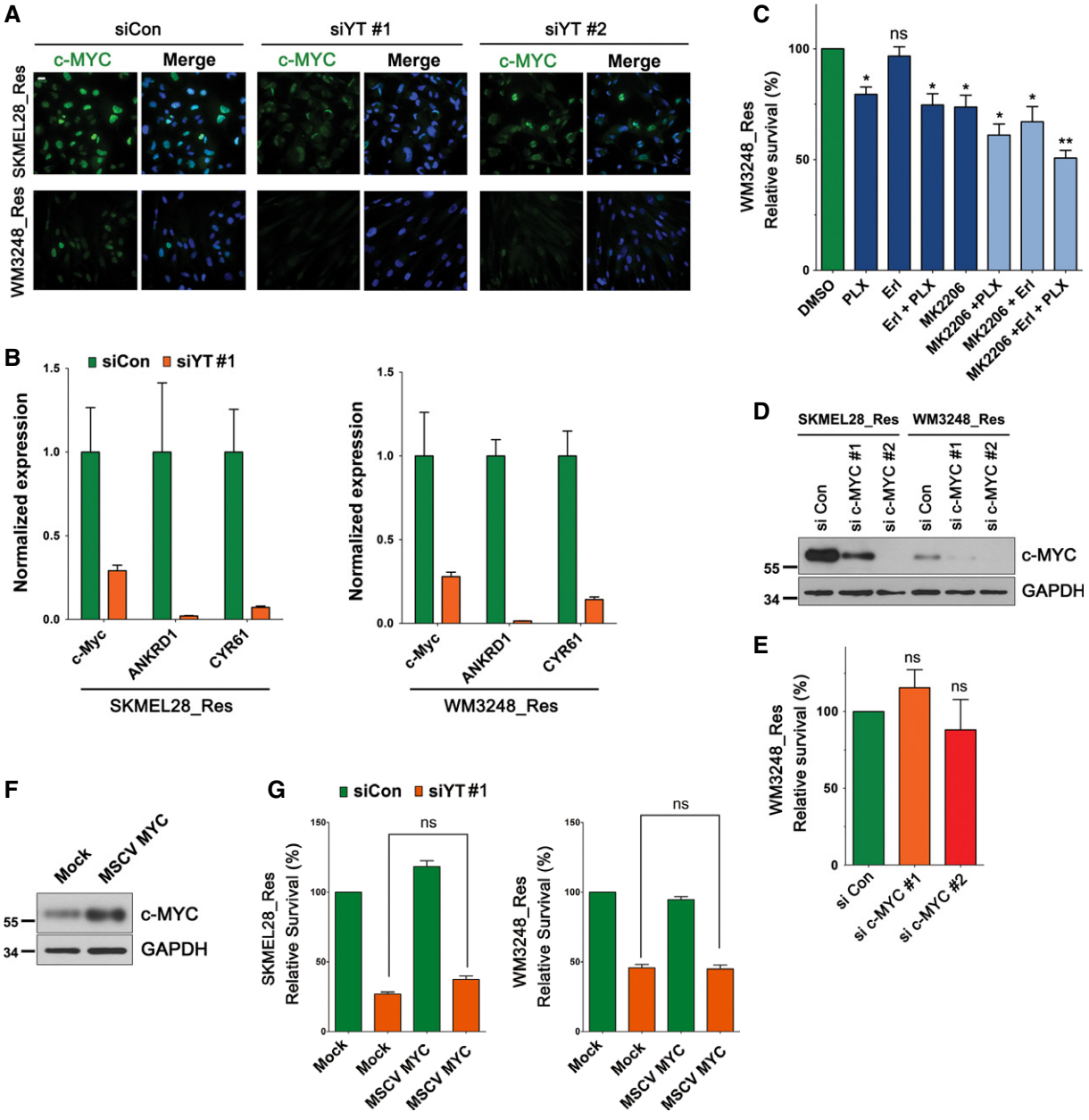

Figure EV4. YAP/TAZ knockdown downregulates c-MYC expression in resistant cells, and AKT inhibition suppresses the viability of resistant WM3248 cells.

A Immunofluorescence micrographs showing c-MYC in resistant SKMEL28 and WM3248 cells 72 h after transfection of control siRNA or YAP/TAZ siRNAs. Cells were labeled with anti-c-MYC antibody.

B qRT–PCR analyses of the expression of c-MYC and YAP/TAZ target genes. Resistant SKMEL28 and WM3248 cells were transfected with the indicated siRNAs for 72 h.

C Cell viability analyses of resistant WM3248 cells treated with PLX4032 (PLX, 2 μM), Erlotinib (Erl, 2.5 μM), MK-2206 (2.5 μM) or their combinations. Relative cell viability, compared with DMSO control, was analyzed by CCK8 assay after drug treatment for 72 h.

D Immunoblotting for c-MYC proteins 72 h after transfection of the indicated siRNAs.

E Cell viability analyses of resistant WM3248 cells. Cells were transfected with the indicated siRNAs for 5 days. Relative cell viability, compared with that of control siRNA-transfected cells, was measured by CCK8 assay.

F Immunoblotting for c-MYC proteins 48 h after retrovirus transfection of MSCV mock or MSCV-c-MYC.

G Cell viability analyses of resistant SKMEL28 and WM3248 cells. Cells were transfected with the indicated retrovirus for 24 h, and then transfected with the indicated siRNAs for additional 72 h (forward transfection). Relative cell viability, compared with that of mock- and control siRNA-transfected cells, was measured by CCK8 assay.

Data information: All data are mean and SEM [two biological replicates (G) or three biological replicates (B, C, and E)], and *P*-values were determined by *t*-test (*$P < 0.05$ and **$P < 0.01$). The nuclei were stained with DAPI (blue), and scale bars represent 20 μm.
Source data are available online for this figure.

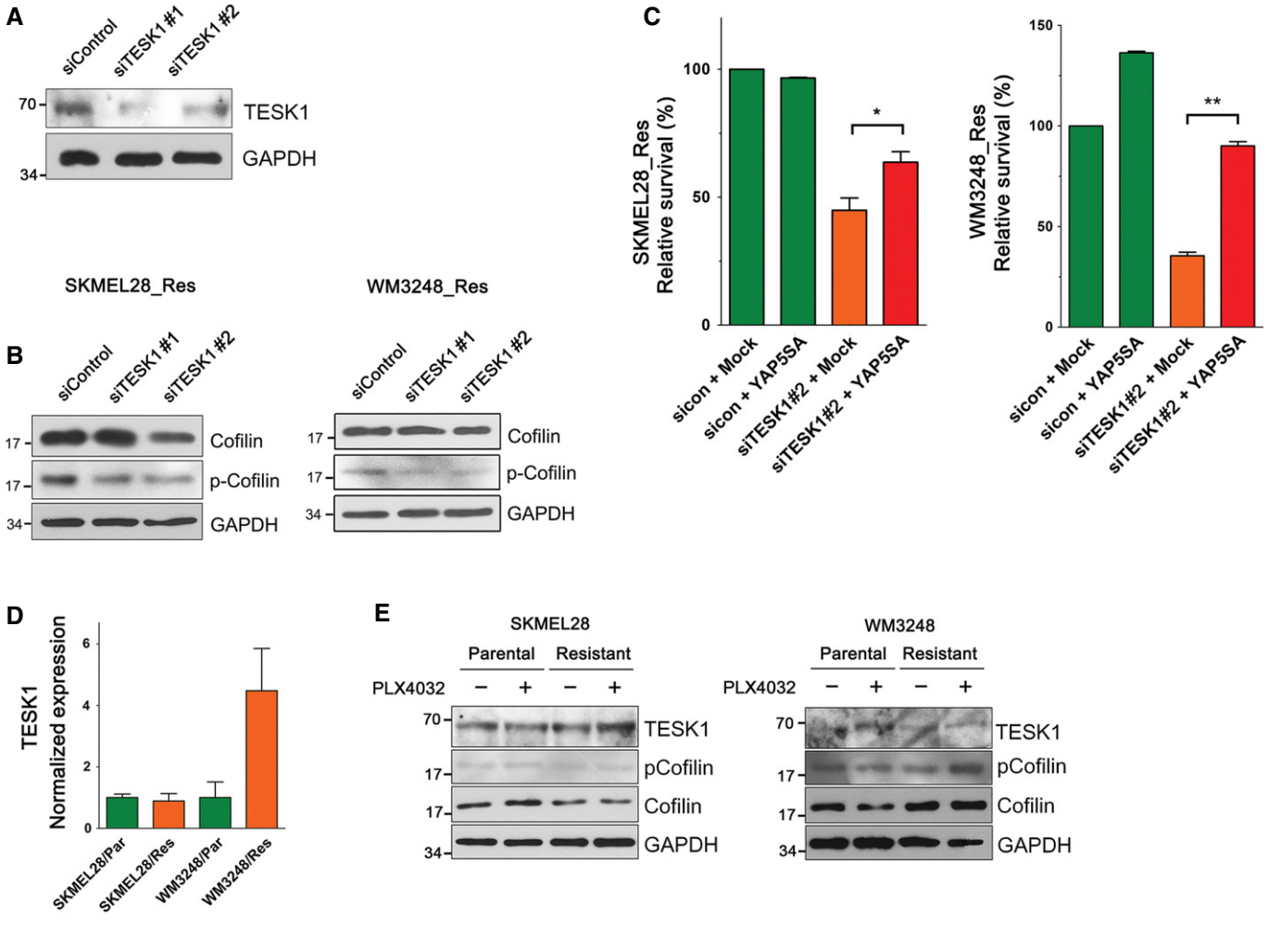

Figure EV5. TESK1 knockdown decreased phospho-Cofilin levels, and TESK1 knockdown-mediated cell viability loss is rescued by YAP-5SA.

A Immunoblotting of the indicated proteins in SKMEL28 cells transfected with either control siRNA or TESK1 siRNAs for 60 h.

B Immunoblotting with anti-Cofilin and anti-phospho-Cofilin antibodies. Lysates of resistant SKMEL28 and WM3248 cells were collected after transfection with the indicated siRNAs for 72 h (SKMEL28) or 48 h (WM3248).

C Cell viability analyses of resistant SKMEL28 and WM3248 cells. Cells were transfected with mock or YAP-5SA retrovirus for 24 h, and then transfected with the indicated siRNAs for additional 144 h (forward transfection). Relative cell viability, compared with that of mock- and control siRNA-transfected cells, was measured by CCK8 assay.

D TESK1 expression levels in parental and resistant cells measured by qRT–PCR.

E Immunoblotting of the indicated proteins in parental and resistant cells. Cells were plated and incubated without drugs for 24 h, and then treated with PLX4032 (2 μM) or DMSO for 24 h.

F A cBioPortal oncomap for genetic alterations of TESK1 in human melanoma tumor samples in TCGA database.

Data information: All data are mean and SEM (three biologic replicates), and *P*-values were determined by *t*-test (*$P < 0.05$ and **$P < 0.01$).
Source data are available online for this figure.