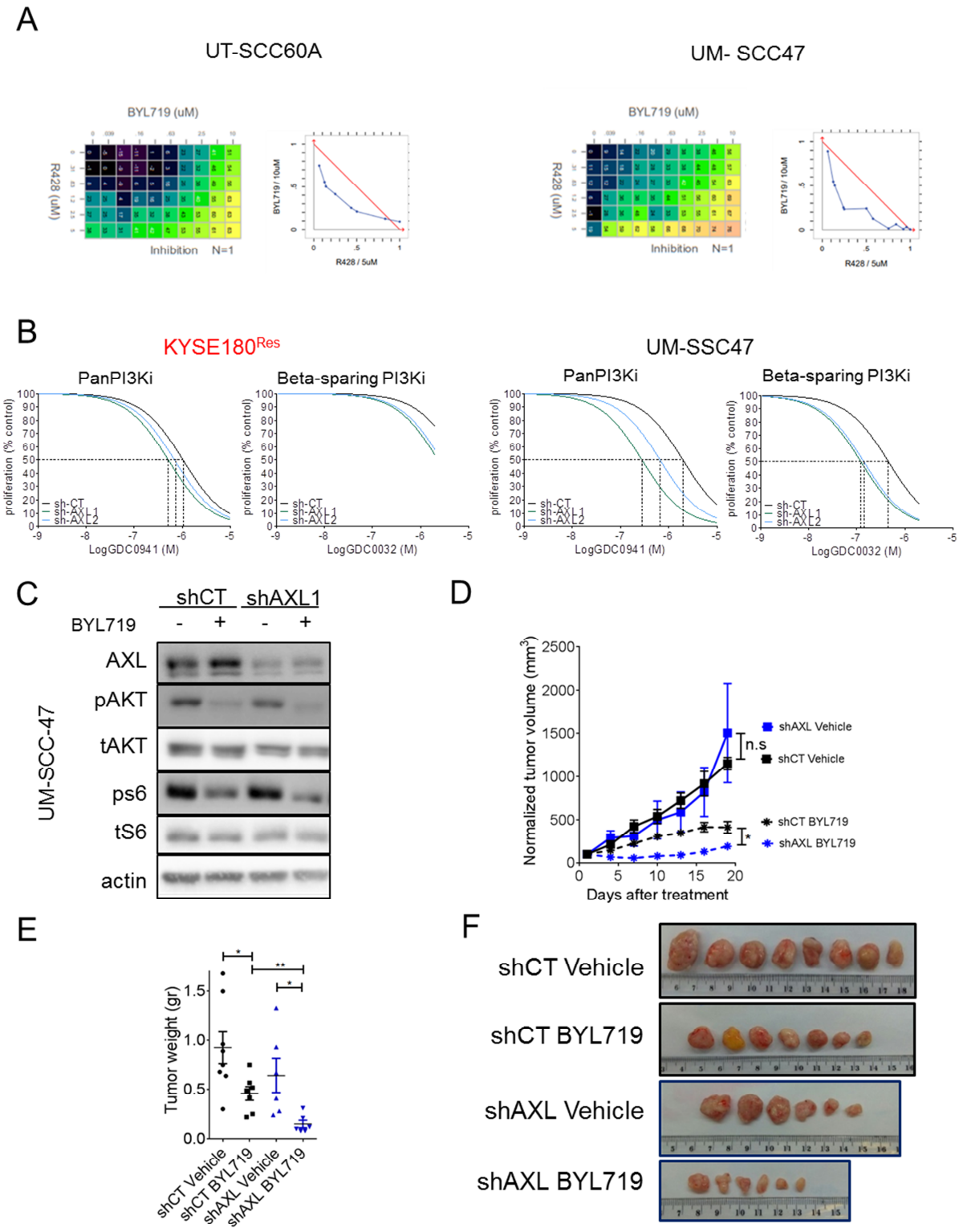
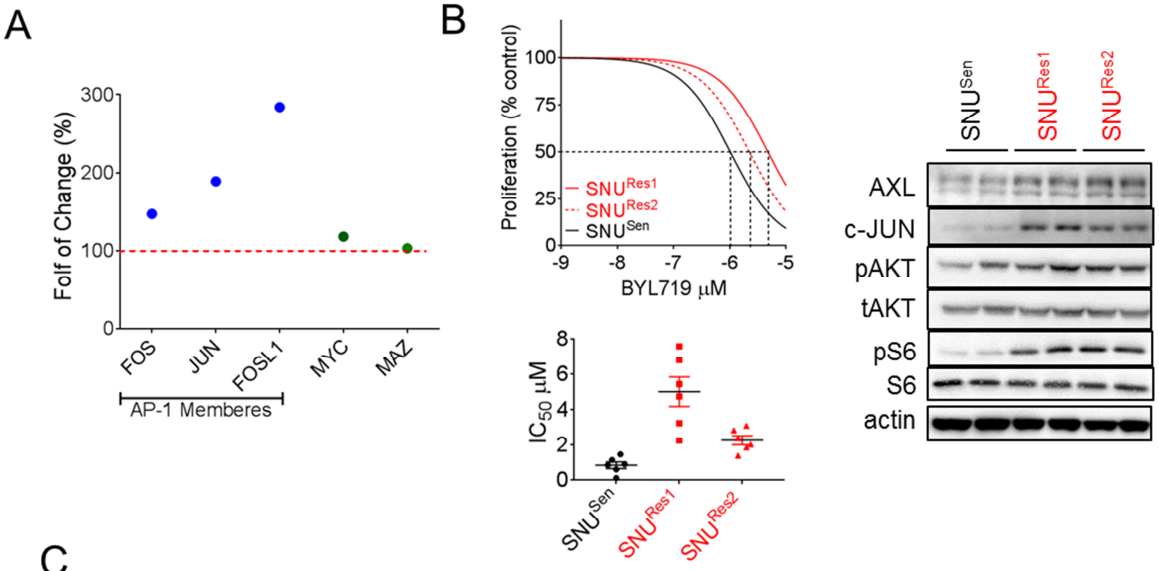
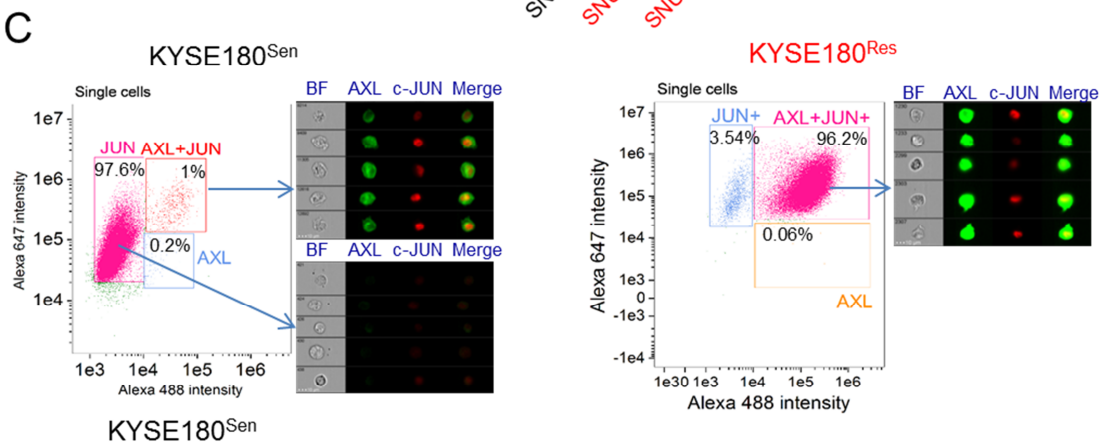
Supplementary Figures:

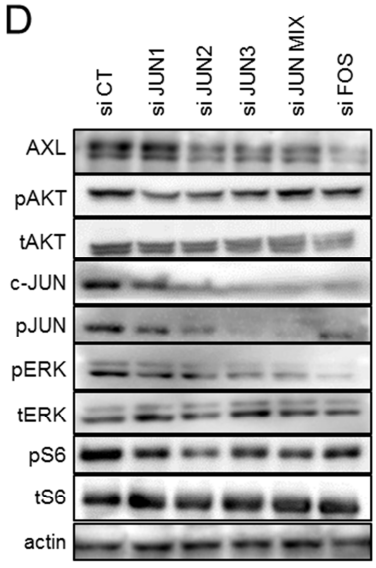


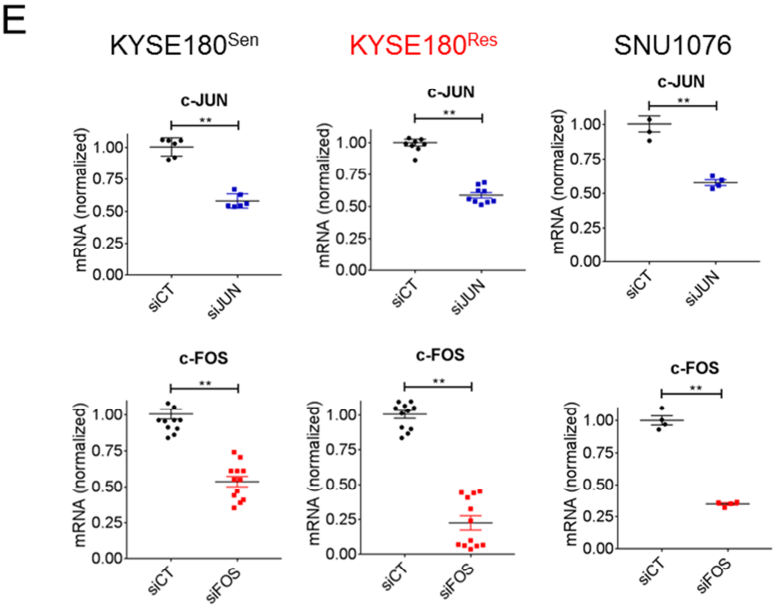
Supplementary Figure 1

Supplementary Figure 1: AXL knockdown sensitizes HNSCC and ESCC cells to BYL719 in vitro and in vivo. **A.** Viability was assessed in cell lines treated with escalating doses of BYL719 and R428 for 4 days. Synergy test for the interaction between BYL719 and R428. The synergy test was analyzed using Chalice software (Horizon), and a synergy score was extracted. **B.** Viability was assessed in cell lines treated with escalating doses of GDC0941 and GDC0032 for 4 days. IC:50 analysis of GDC0941 and GDC0032 values in HPV^{Neg} and HPV^{Pos} HNSCC and ESCC cells following infection with shRNAs to silence AXL expression (shAXL1 and shAXL2), or a non-targeting control shRNA (shCT). **C.** WB analysis showing the activation of the AKT and mTOR (p-S6) pathways in shCT and shAXL1 UM-SCC47 (HPV^{Pos}) tumor cells after BYL719 treatment (2 μM, 24 hours). **D.** Tumor growth of shCT or shAXL1 UM-SCC47 CDXs in mice (tumors n=6-8) treated daily with BYL719 (25 mg/kg). **E.** Averaged tumor weights of the tumors presented in D at the end of the experiment. **F.** Images of the tumors presented in D. One-way ANOVA p values are shown *p< 0.05; **p< 0.01.



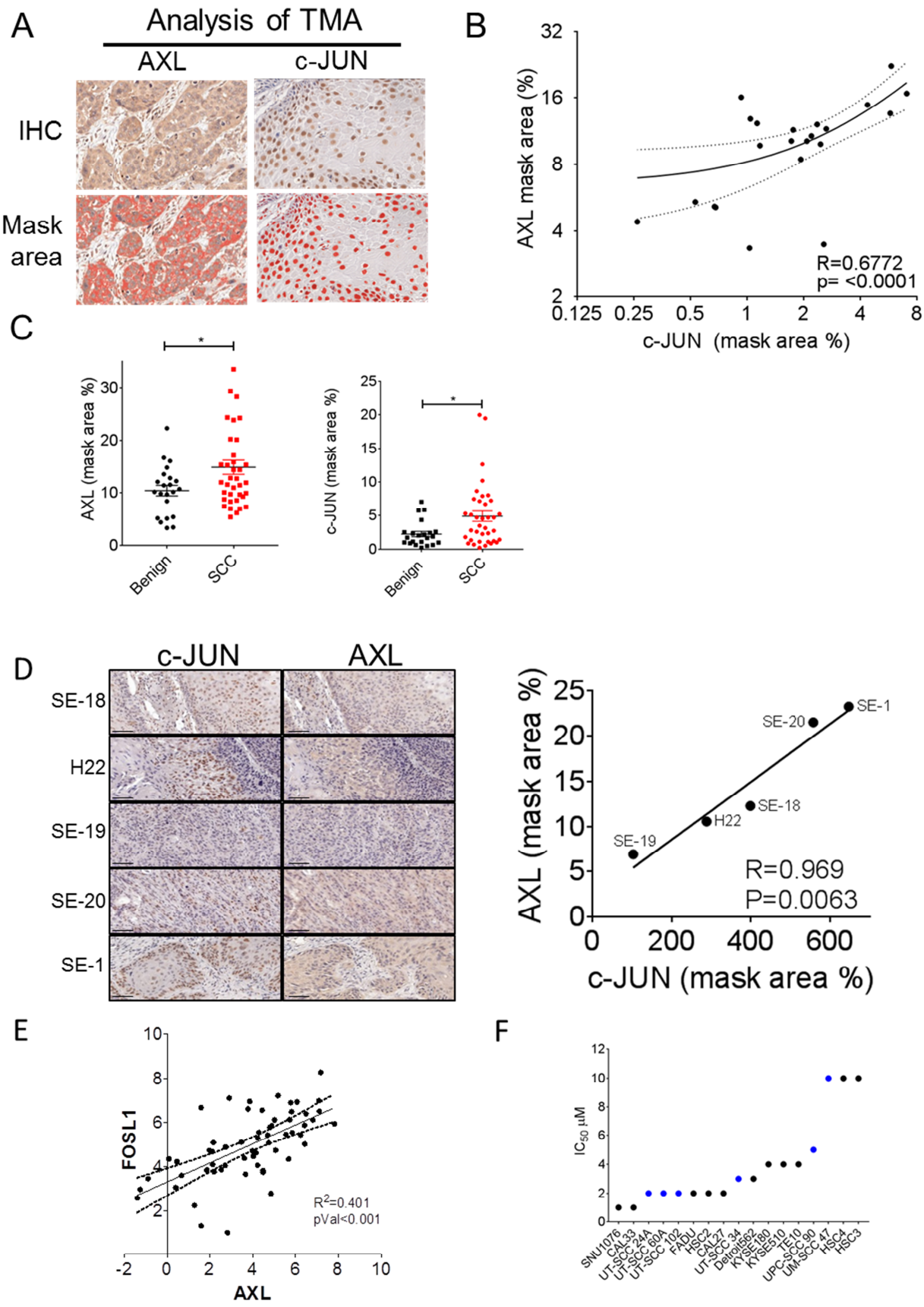






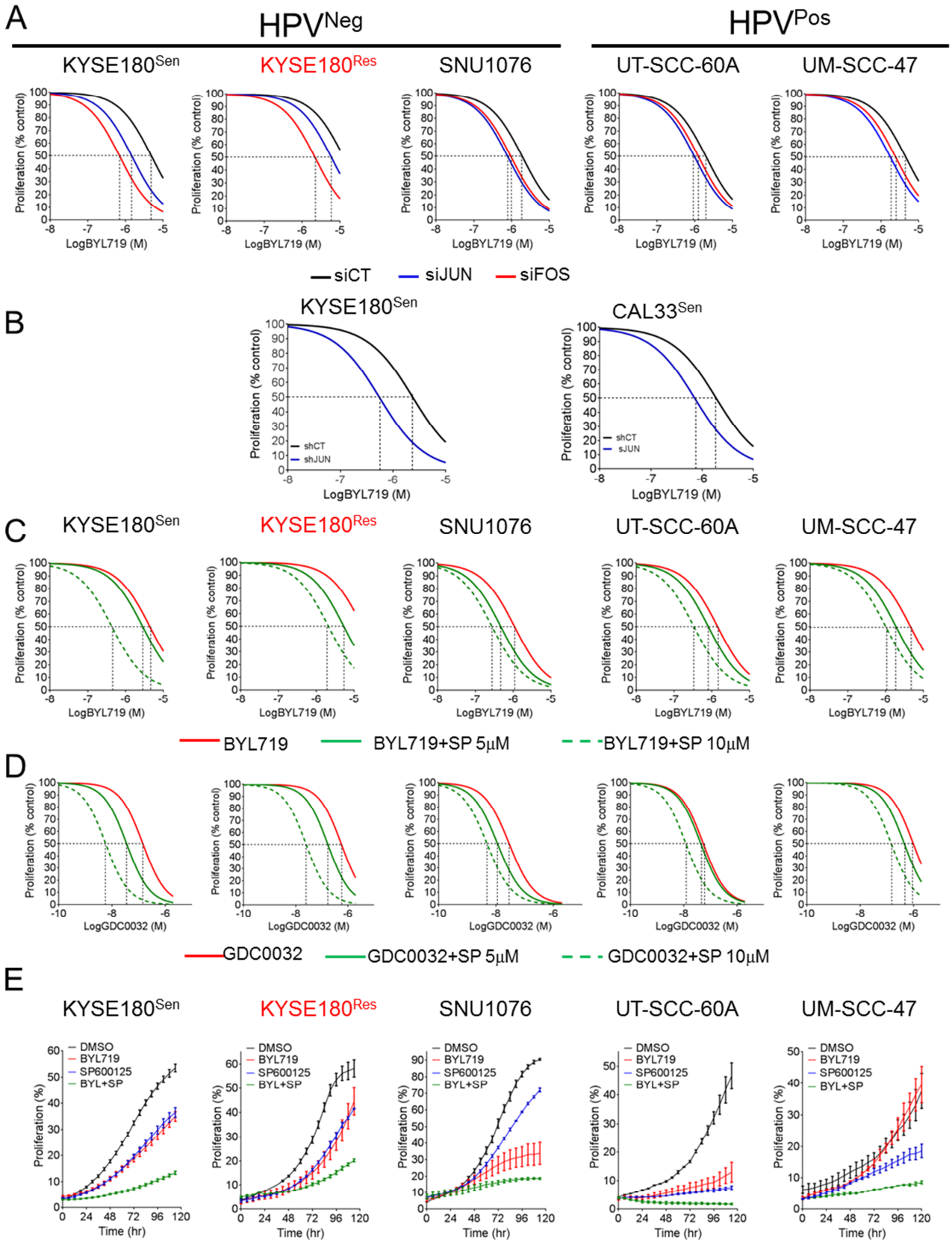
Supplementary Figure 2

Supplementary Figure 2: The AP-1 transcriptional complex regulates AXL expression in HNSCC and ESCC. **A.** RNA sequencing data showing the levels of AP-1, MYC, MAZ in BYL719-resistant cells compared to the corresponding sensitive cells. **B.** Viability was assessed in cell lines treated with escalating doses of BYL719 for 4 days. Left - IC:50 values of SNU1076- sensitive and BYL719-acquired resistance cells (n=6). Right - WB analysis showing AXL, c-JUN, AKT and mTOR (pS6) levels. **C.** Imaging flow cytometry (ImageStream) analysis of the AXL and c-JUN positive cell populations in $KYSE180^{Sen}$ and $KYSE180^{Res}$ cells. Cells were labeled with DAPI for nuclear staining (blue), AXL (green) and c-JUN (red) antibodies. **D.** WB analysis of $KYSE180^{Sen}$ cells after transfection with siRNAs: siCT- a non-targeting sequence; siJUN1,2,3 are 3 different sequences for the targeting of c-JUN; siJUN mix is the combination of all siJUN sequences; siFOS for the targeting of c-FOS. **E.** qPCR analysis showing the relative mRNA levels of c-JUN and c-FOS in $KYSE180^{Sen}$, $KYSE180^{Res}$, and SNU1076 cells after transfection with siJUN or siFOS (n>3). The mRNA levels were normalized to GAPDH levels and presented as a percentage of the siCT cells. Unpaired 2-sided t test p values are shown **p< 0.01.

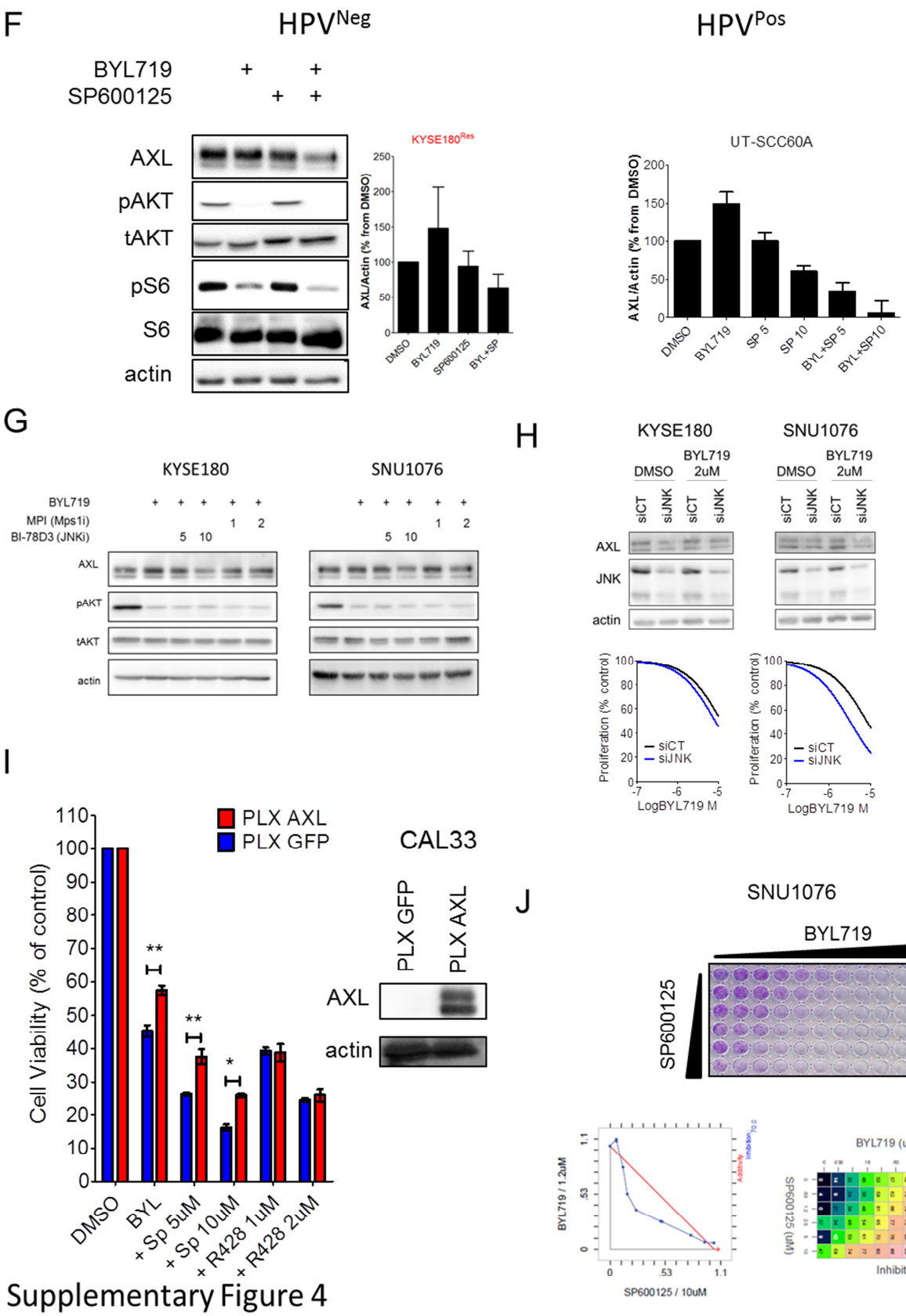


Supplementary Figure 3

Supplementary Figure 3: AXL and c-JUN levels are correlated in clinical samples of HNSCC tumors and in cell lines. **A**. A representative image for AXL and c-JUN analysis following IHC staining of a tumor sample from an HNSCC patient. The image analysis software HistoQuant^{TM} was used for the analysis of stained area, by adjusting the software to identify the brown stained area. The mask area identified by the software is shown in red in the lower panel. **B.** Correlation between AXL and c-JUN expression in benign tumors of the tissue microarray (TMA), calculated using HistoQuant^{TM} analysis software. **C.** Comparison of AXL and c-JUN expression in benign vs. SCC tissues of the TMA of HNSCC patients (n>10). **D.** IHC staining of AXL and c-JUN in HNSCC PDXs. The size of the scale bar represents 50μm. The expression levels are calculated as in A, and a correlation between AXL and c-JUN is presented. **E.** Correlation between AXL and FOSL1 expression in all esophagus and head and neck cell lines. **F.** Viability was assessed in cell lines treated with escalating doses of BYL719 for 4 days. BYL719 IC:50 in HPV^{Neg} (Black) and HPV^{Pos} (Blue) HNSCC and ESCC cell lines. Unpaired 2-sided t test p values are shown **p< 0.05.

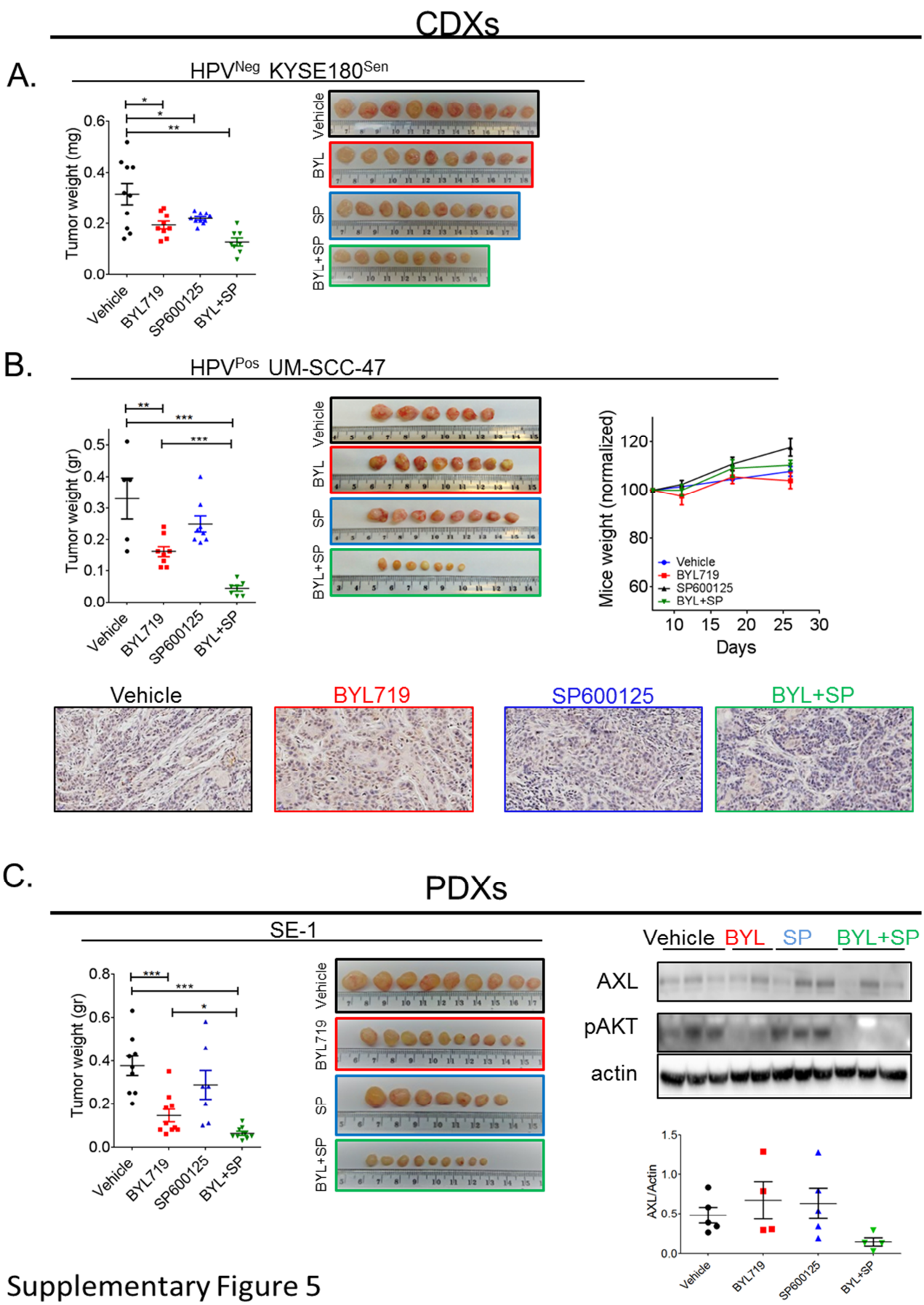


Supplementary Figure 4



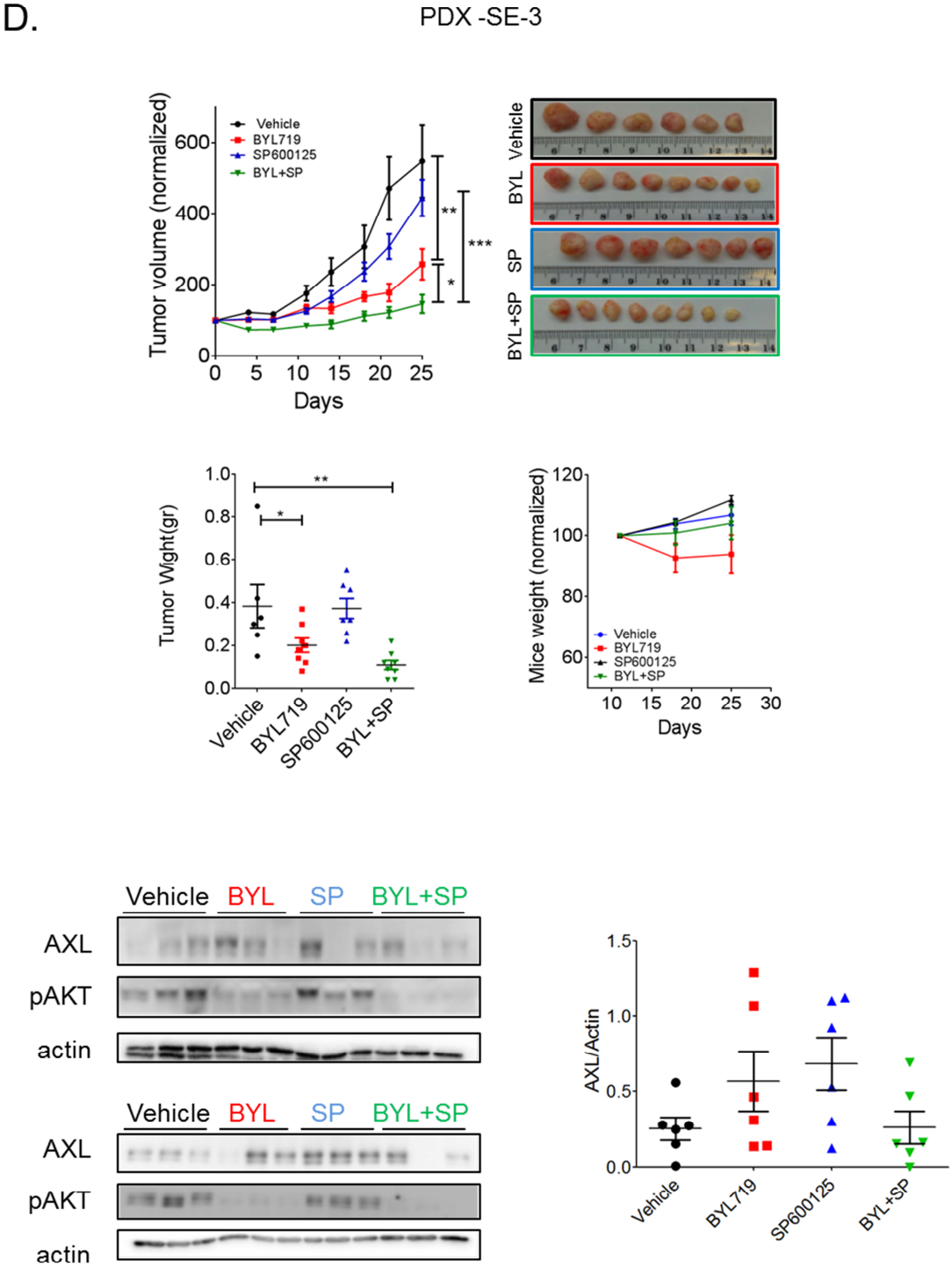
Supplementary Figure 4

Supplementary Figure 4: Silencing of c-JUN and c-FOS or blocking c-JUN N-terminal kinase (JNK) sensitize HNSCC and ESCC cells to BYL719 in vitro. **A.** Viability was assessed in cell lines treated with escalating doses of BYL719 for 4 days. Analysis of BYL719 IC:50 values in HNSCC and ESCC cells after transfection with siRNAs to silence c-JUN and c-FOS expression. **B.** Viability was assessed in cell lines treated with escalating doses of BYL719 and SP600125 for 4 days. Analysis of BYL719 IC:50 values in HNSCC and ESCC cells after infection with shRNAs to silence c-JUN expression **C.** Viability was assessed in cell lines treated with escalating doses of BYL719 for 4 days. Analysis of BYL719 IC:50 values following JNK inhibition with SP600125 (5 and 10μM) in HNSCC and ESCC cells. **D.** Viability was assessed in cell lines treated with escalating doses of BYL719 for 4 days. Analysis of GDC0032 IC:50 values following JNK inhibition with SP600125 (5 and 10μM) in HNSCC and ESCC cells. **E.** Cell proliferation assay showing the growth kinetics of the indicated cells following treatments with BYL719 (2 μM), SP600125 (10 μM) or the BYL719–SP600125 combination. **F.** Right - WB analysis showing AXL level, and AKT/mTOR pathway activation in KYSE180^{Res} cells treated with BYL719 (2 μM), SP600125 (10 μM) and the combination therapy for 24 hours. Left Quantification of WB was assessed by Photoshop. **G.** WB analysis showing AXL level, and AKT pathway activation in KYSE180 and SNU1076 cell lines treated with BYL719 (2 □M), in combination with JNK inhibitor BI-78D3 or Msp1 inhibitor MPI for 24 hours. H. Upper panel - WB analysis showing AXL levels and JNK levels after transfection with siRNA for the silencing of JNK, in presence of BYL719 (2 □M), Lower panel – Tumor cells viability was assessed in cell lines treated with escalating doses of BYL719 for 4 days. I. Left- Tumor cell viability was assessed in CAL33 cell line following infection with PLX-AXL vector for AXL over expression and PLX-GFP for control, upon treatment with BYL719 (1uM) , SP600125 (5 or 10 uM) and R428 (1 or 2 uM). Right- WB analysis showing AXL overexpression following infection with PLX-AXL and PLX-GFP. **J.** Synergy test for the interaction between BYL719 and SP600125. A representative image of a plate fixed and stained with crystal violet is presented on the right, and the analysis of growth inhibition (in percentage) is presented in the middle panel. The synergy test was analyzed using the Chalice software (Horizon), and a synergy score was extracted (presented on the left).



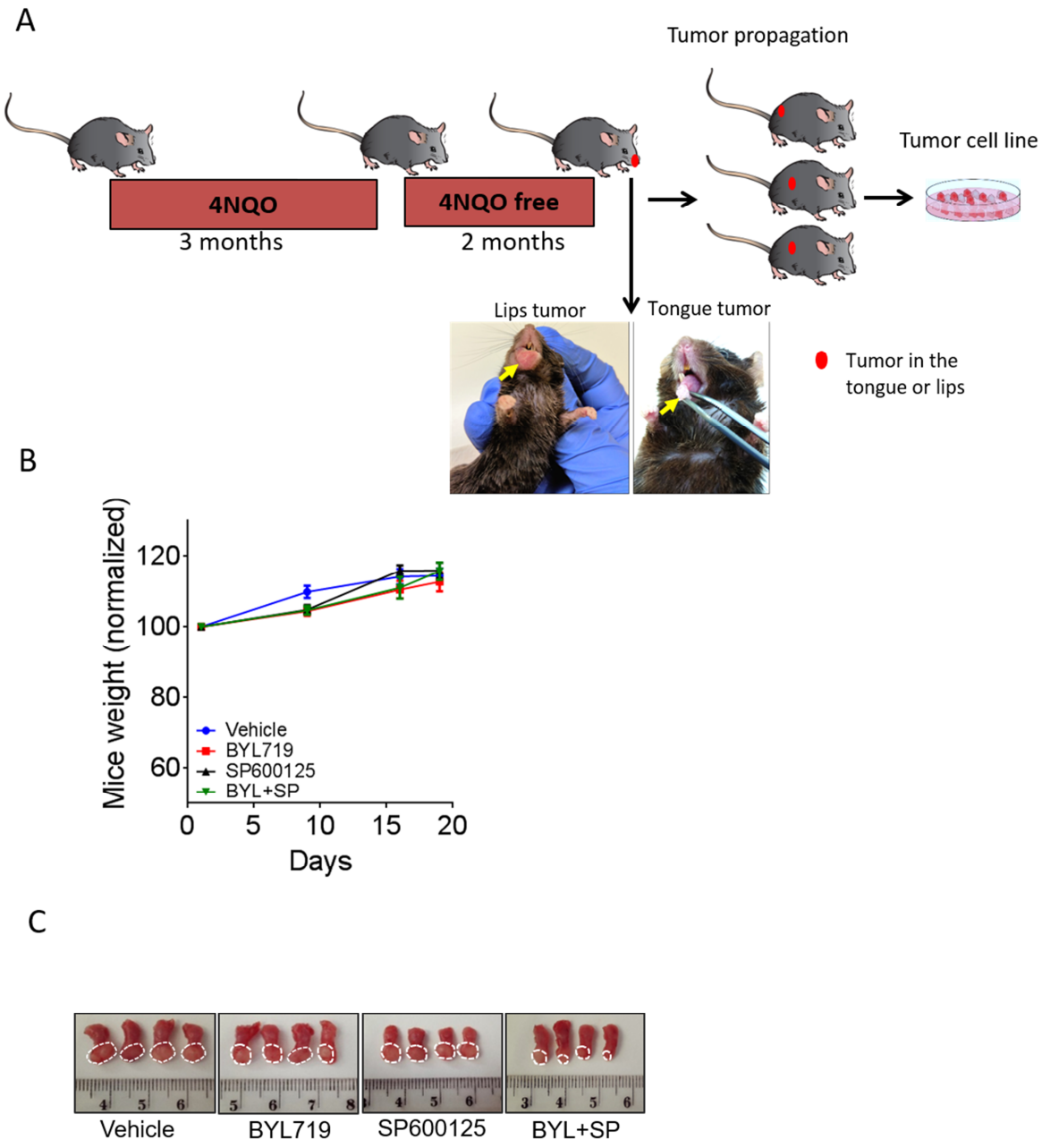
Supplementary Figure 5

D.



Supplementary Figure 5

Supplementary Figure 5: SP600125 enhances BYL719 efficacy in vivo in CDX and PDX models. **A**. Tumor weights and images of KYSE180^{Sen} CDXs (tumors n=8-10) treated with BYL719 (25 mg/kg); SP600125 (15 mg/kg) or the BYL719–SP600125 combination. **B**. Upper- Tumor weights, images and mouse weights of UM-SCC47 CDXs (tumors n=6-8) treated as in A. Lower- IHC staining for AXL **C.** Left - Tumor weights and images of HNSCC PDX SE-1 (tumors n=7-10) treated as in A. Right - WB analysis showing AXL level, and pAKT in PDX-SE1 treated with BYL719, SP600125, and the combination therapy. Quantification of WB was assessed by Photoshop (n=4-5) **D.** Tumor growth, tumor images, tumor weights (tumors n=6-8), and mice weights of HNSCC PDX SE-3 treated as in A. WB analysis showing AXL level, and pAKT in PDX-SE1 treated with BYL719, SP600125, and the combination therapy. Quantification of WB was assessed by Photoshop. One-way ANOVA p values are shown *p< 0.05; **p< 0.01; ***p< 0.001.



Supplementary Figure 6: SP600125 increases BYL719 efficacy in vivo in syngeneic head and neck cancer models. **A.** Upper panel - pictures of mice after 4NQO exposure. Lower panel - Establishment of HNSCC mouse model: 4NQO was added to the drinking water of C57B6/c mice for 3 months, and oral cavity tumors developed after about 2 months. Tumors were then used for the generation of cell lines from the lip and tongue tumors. **B**. Weights of C57BL/6 mice bearing a syngeneic model of a 4NQO-induced lip tumor, treated with BYL719 (25 mg/kg), SP600125 (15 mg/kg) or the BYL719–SP600125 combination. **C.** Images of mouse tongues from an orthotopic model of a tongue SCC cell line, treated as described in B.

Supplementary material and methods tables:

Table 1. Xlsx file – Gene expression and signatures.

Table 2. Xlsx file – AXL and c-JUN expression in TMA.

Table 3:

Cell Lines:

Cell line	Source	Medium	Medium Cat. Number
CAL27	ATCC	DMEM	01-052-1A
CAL33	DSMZ	DMEM	01-052-1A
Detroit 562	ATCC	EMEM	01-025-1A
FaDu	ATCC	EMEM	01-025-1A
HSC-2	HSRRB	EMEM	01-025-1A
HSC-3	HSRRB	EMEM	01-025-1A
HSC-4	HSRRB	EMEM	01-025-1A
SNU-1076	KCLB	RPMI 1640	01-100-1A
KYSE-180	DSMZ	RPMI 1640	01-100-1A
KYSE-510	DSMZ	RPMI 1640	01-100-1A
TE-10	RIKEN	RPMI 1640	01-100-1A
HEK293	ATCC	DMEM	01-052-1A

Table 4:

Antibodies:

Primary antibody	Company	Cat #	Working dilution
WB primary antibody			
Axl (C89E7) rabbit mAb	CST	8661	1-1000
c-Jun (60-A8) rabbit mAb	CST	9165	1-1000
Phospho-c-Jun (Ser73) (D47G9) rabbit mAb	CST	3270	1-1000
MITF (D5G7V) rabbit mAb	CST	12590	1-1000
SP1 rabbit antibody (PEP 2)	Santa Cruz Biotechnology	Sc-59	1-1000
SAPK/JNK Antibody	CST	9252	1-1000
Phospho-SAPK (Thr183/Try185) (81E11) rabbit mAb	CST	4668	1-1000
Phospho-Akt (Ser473) (D9E) XP® rabbit mAb	CST	4060S	1-1000
Akt (pan) (11E7) rabbit mAb	CST	4685S	1-1000
p-S6 ribosomal protein (S240/244) rabbit mAb	CST	5364L	1-1000
S6 ribosomal protein (5G10) rabbit mAb	CST	2217L	1-1000
Actin	MP Biomedicals	691001	1-1000
IHC/IHF primary antibody			
AXL (clone 7E10), mouse	Abnova	MAB10498	1-500
c-JUN (60-A8) rabbit mAb	CST	9165	1-400
P-S6 ribosomal protein (S240/244) rabbit mAb	CST	5364L	1-2000
Ki-67 (proliferation marker) antibody, rabbit	Bioss Antibodies	bs-2130R	1-200

CST = Cell Signaling Technology, Inc.

Table 5:

PIK3CA and PTEN status

Cell line	PIK3CA	PTEN
KYSE180	amplification	-
SNU1076	mutation	-
CAL33	mutation	-
KYSE510	mutation	-
TE10	amplification	-
FADU	amplification	-
HSC2	mutation	-
HSC4	-	mutation
Detroit 562	mutation	-
CAL27	-	-
HSC3	-	-
UT-SCC24	NA	NA
UT-SCC34	NA	NA
UM-SCC47	-	-
UT-SCC60A	NA	NA
UPC-SCC90	mutation	amplification
UT-SCC102	NA	NA