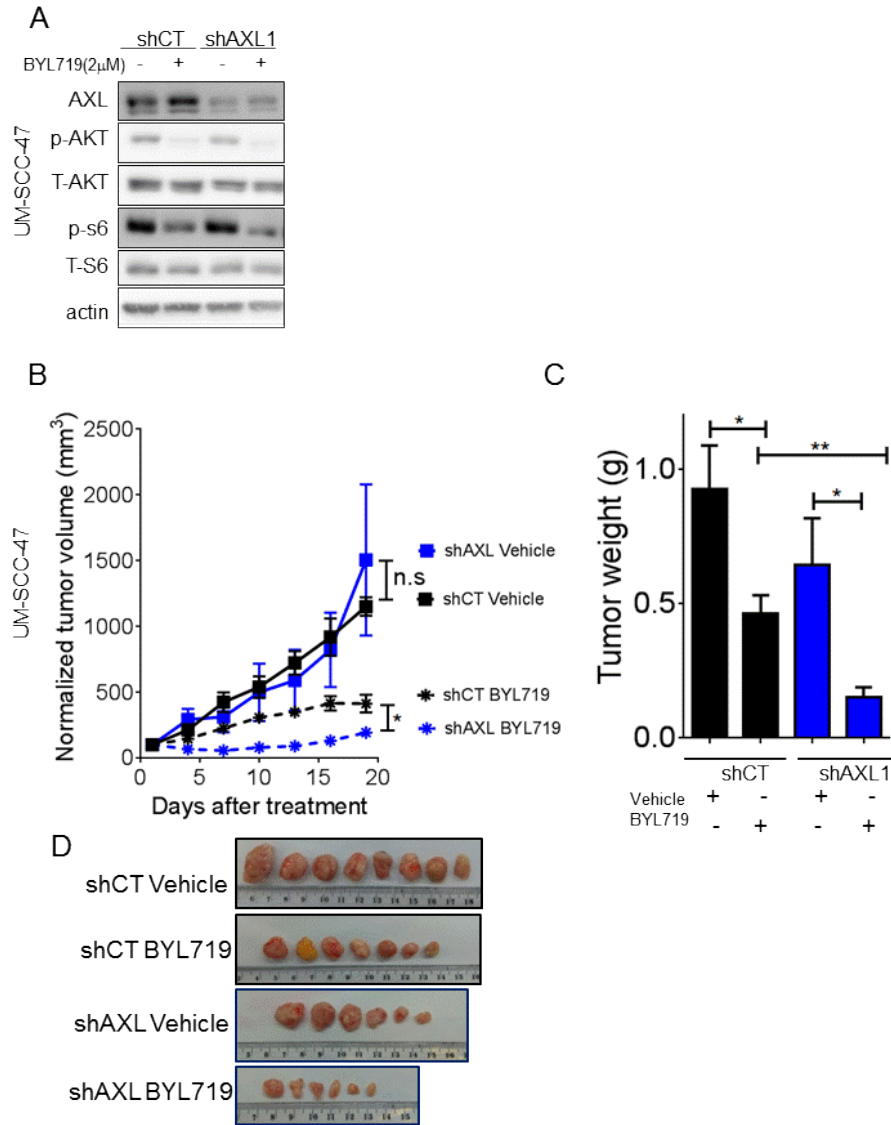


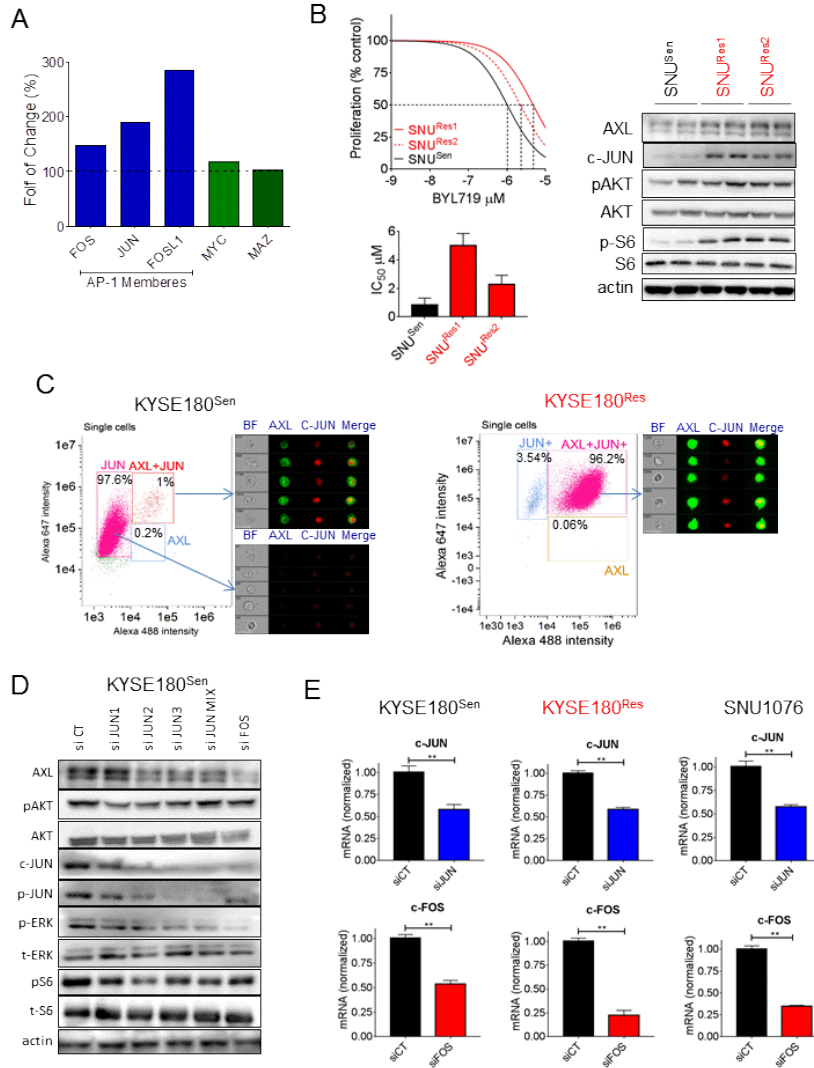
Supplementary Figures:



Supplementary Figure 1

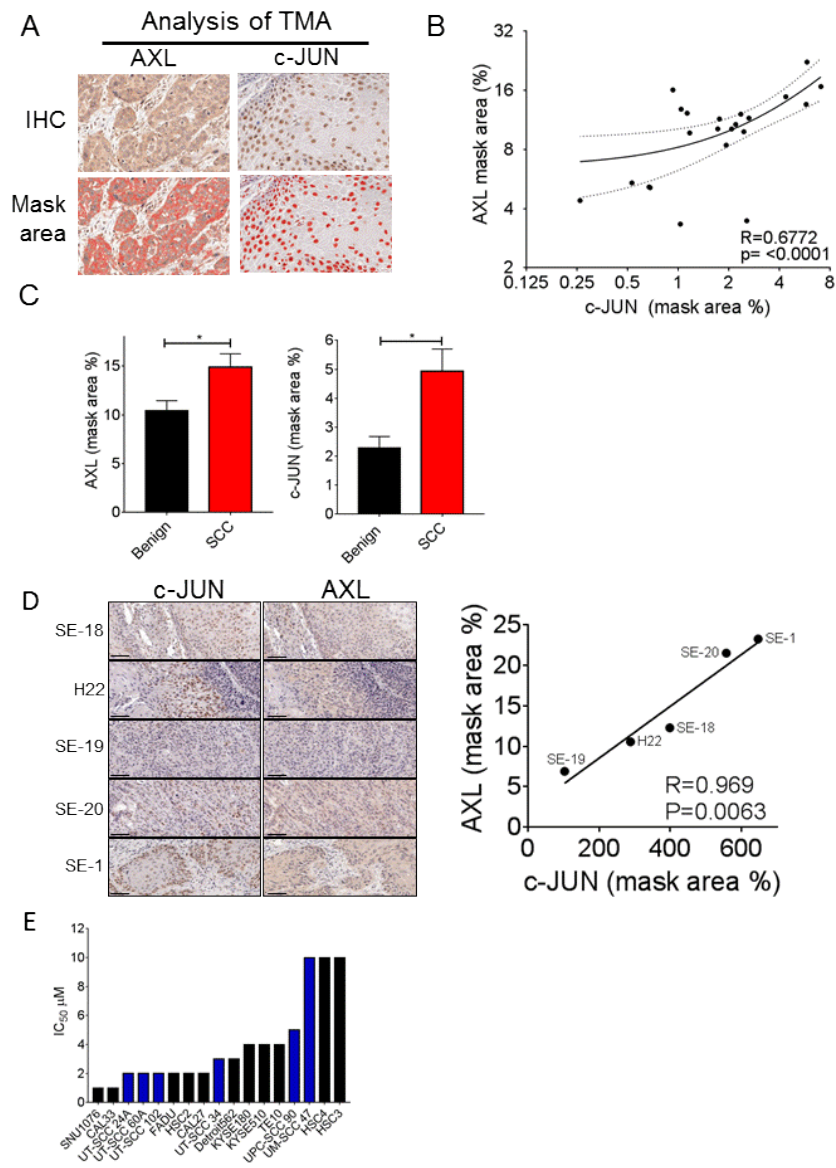
Supplementary Figure 1: AXL knockdown sensitizes HNSCC and ESCC cells to BYL719 in vitro and in vivo.

A. 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 μ M, 24 hours). **B.** Tumor growth of shCT or shAXL1 UM-SCC47 CDXs treated daily with BYL719 (25 mg/kg). **C.** Averaged tumor weights of the tumors presented in B at the end of the experiment. **D.** Images of the tumors presented in C.



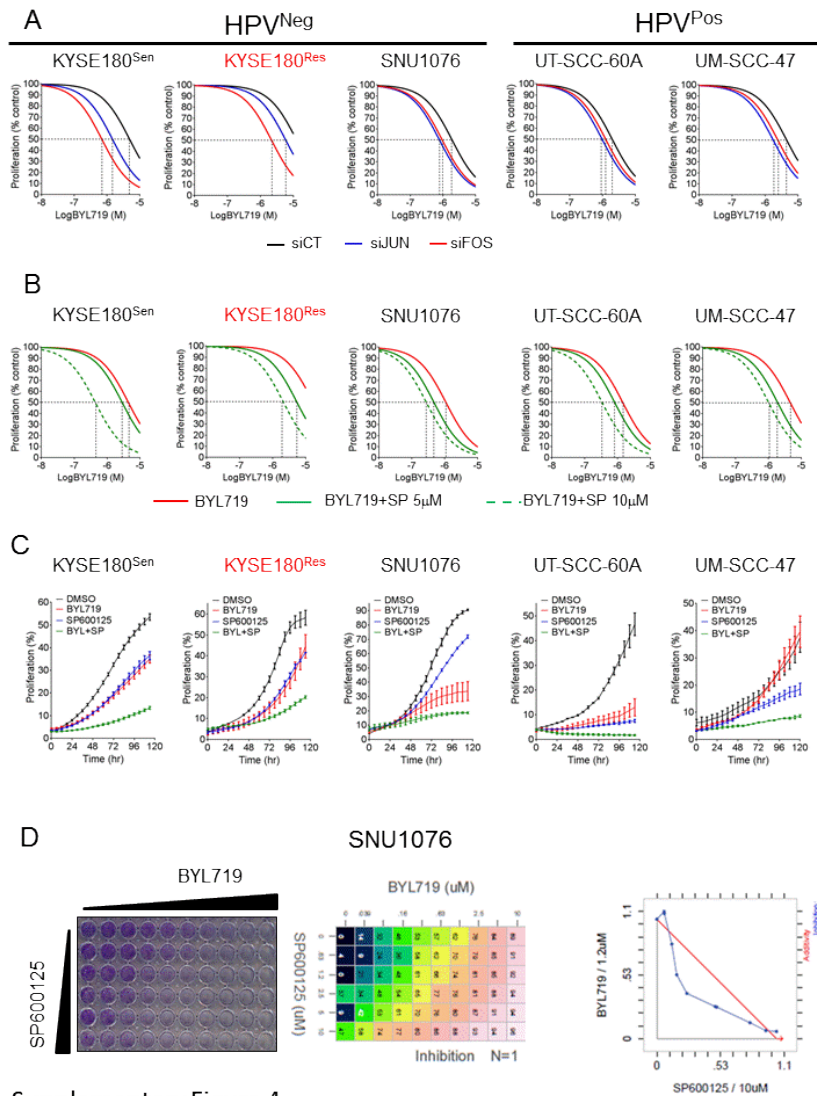
Supplementary Figure 2

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.** Left - IC₅₀ values of SNU1076- sensitive and BYL719-acquired resistance cells. 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. The mRNA levels were normalized to GAPDH levels and presented as a percentage of the siCT cells. **P= 0.01.



Supplementary Figure 3

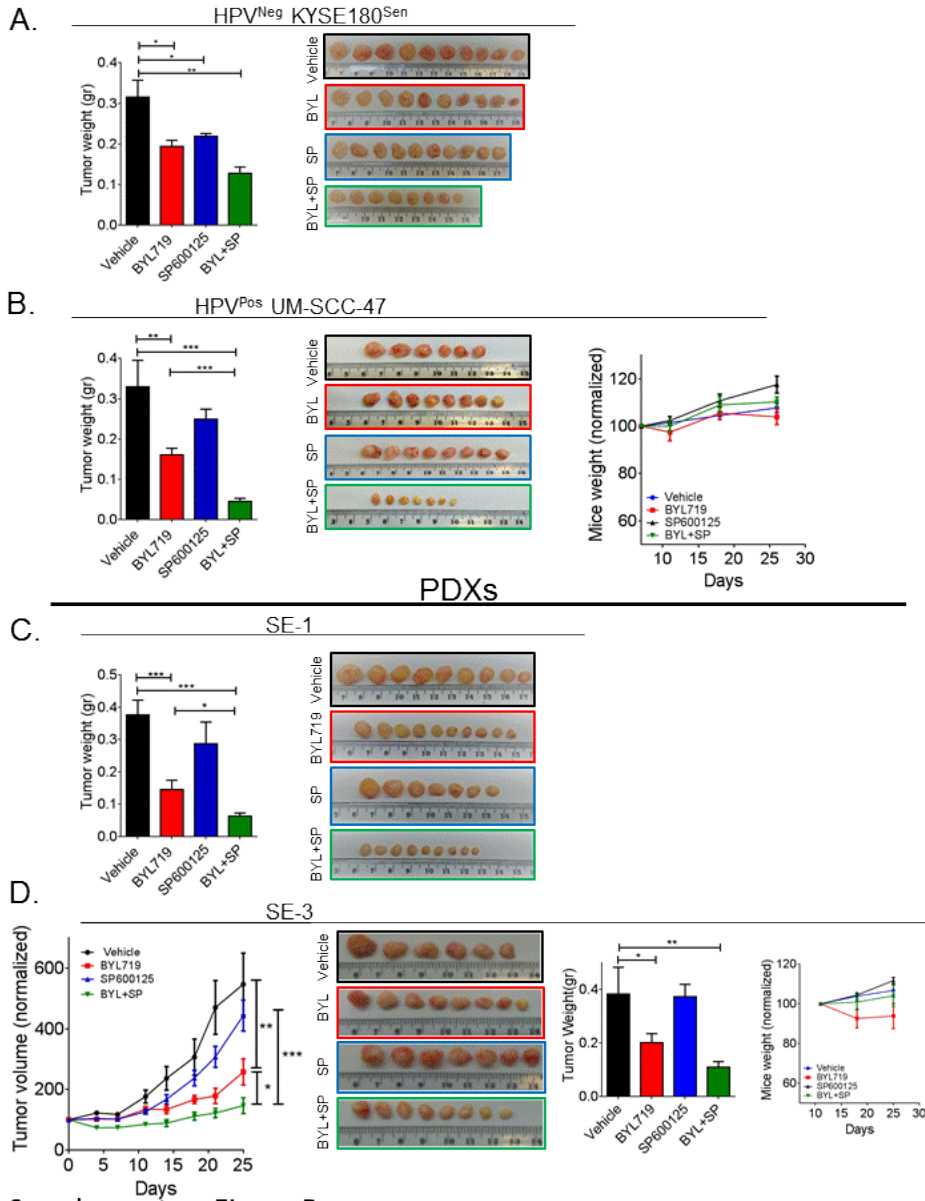
Supplementary Figure 3: AXL and c-JUN levels are correlated in clinical samples of HNSCC tumors and in cell lines. **A.** A Representative image for AXL and c-JUN analysis following IHC staining of a tumor sample from an HNSCC patient. The image analysis software HistoQuant™ was used for the analysis 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™ analysis software. **C.** Comparison of AXL and c-JUN expression in benign vs. SCC tissues of the TMA of HNSCC patients. **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.** BYL719 IC:50 in HNSCC and ESCC cell lines. *P = 0.05.



Supplementary Figure 4

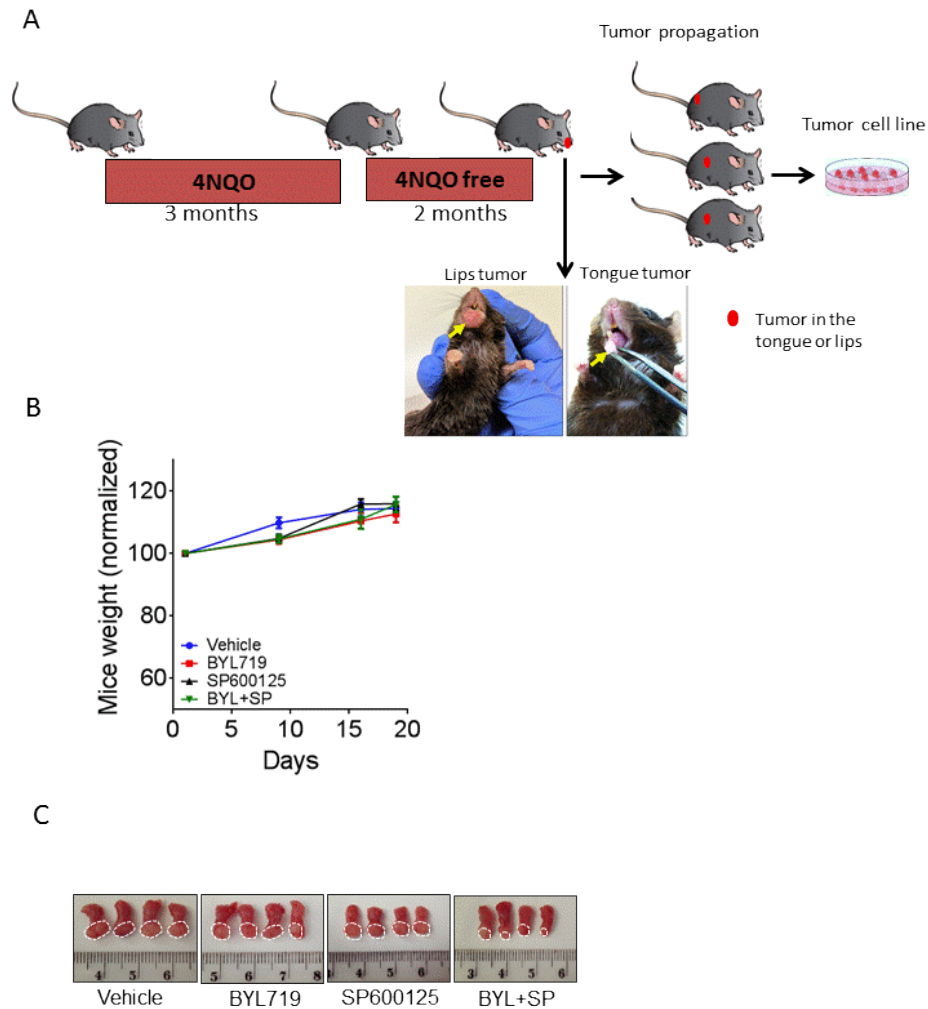
Supplementary Figure 4: Silencing of *c-JUN* and *c-FOS* or blocking *c-JUN* N-terminal kinase (*JNK*) sensitize HNSCC and ESCC cells to BYL719 in vitro. **A.** Analysis of BYL719 IC:50 values in HNSCC and ESCC cells after transfection with siRNAs to silence *c-JUN* and *c-FOS* expression. **B.** Analysis of BYL719 IC:50 values following *JNK* inhibition with SP600125 (5 and 10 μ M) in HNSCC and ESCC cells. **C.** Cell proliferation assay showing the growth kinetics of the indicated cells following treatments with BYL719 (2 μ M), SP600125 (10 μ M) or the BYL719–SP600125 combination. **D.** 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). *P = 0.05; **P= 0.01; ***P = 0.001;

CDXs



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 treated with BYL719 (25 mg/kg; *P = 0.05; **P= 0.01; ***P = 0.001. *P = 0.05; **P= 0.01; ***P = 0.001), SP600125 (15 mg/kg) or the BYL719–SP600125 combination. **B.** Tumor weights, images and mouse weights of UTSCC47 CDXs treated as in A. **C.** Tumor weights and images of HNSCC PDX SE-1 treated as in A. **D.** Tumor growth, tumor images, tumor weights, and mouse weights of HNSCC PDX SE-3 treated as in A.



Supplementary Figure 6

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:

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

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.