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Supplemental Information

A Novel Aurora-A Inhibitor (MLN8237) Synergistically Enhances the Antitumor Activity of Sorafenib in Hepatocellular Carcinoma

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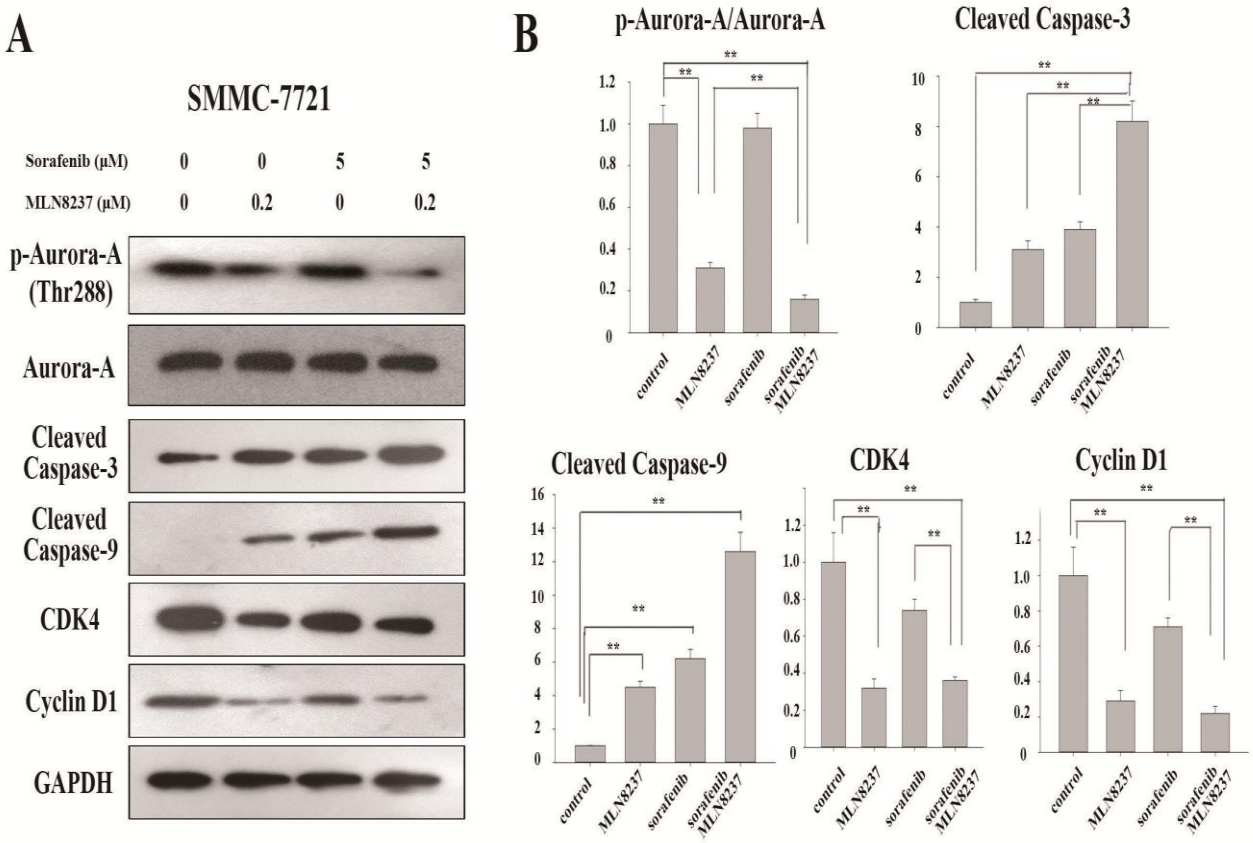

Figure S1. (A) and (B) SMMC-7721 cells were treated with sorafenib, MLN8237, or both for 48 h. Western blotting was then performed to monitor the expression of p-Aurora, cleaved caspase-3, cleaved caspase-9, CDK4, and cyclin D1. GAPDH was used as an internal control.

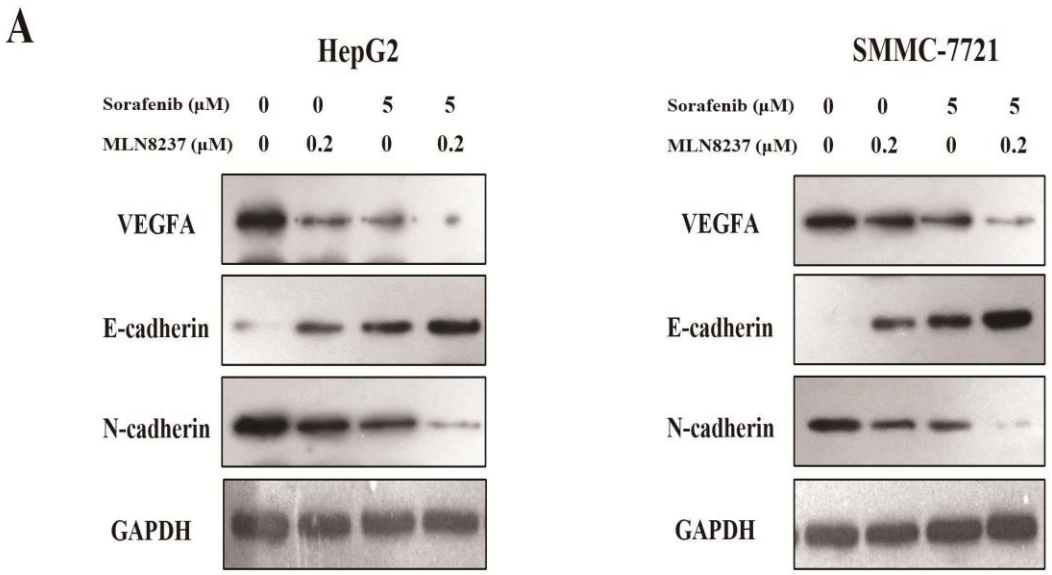

Figure S2. (A) HepG2 and SMMC-7721 cells were treated with sorafenib, MLN8237, or both for 48 h. Western blotting was then performed to monitor the expression of VEGFA, E-cadherin, N-cadherin. GAPDH was used as an internal control.

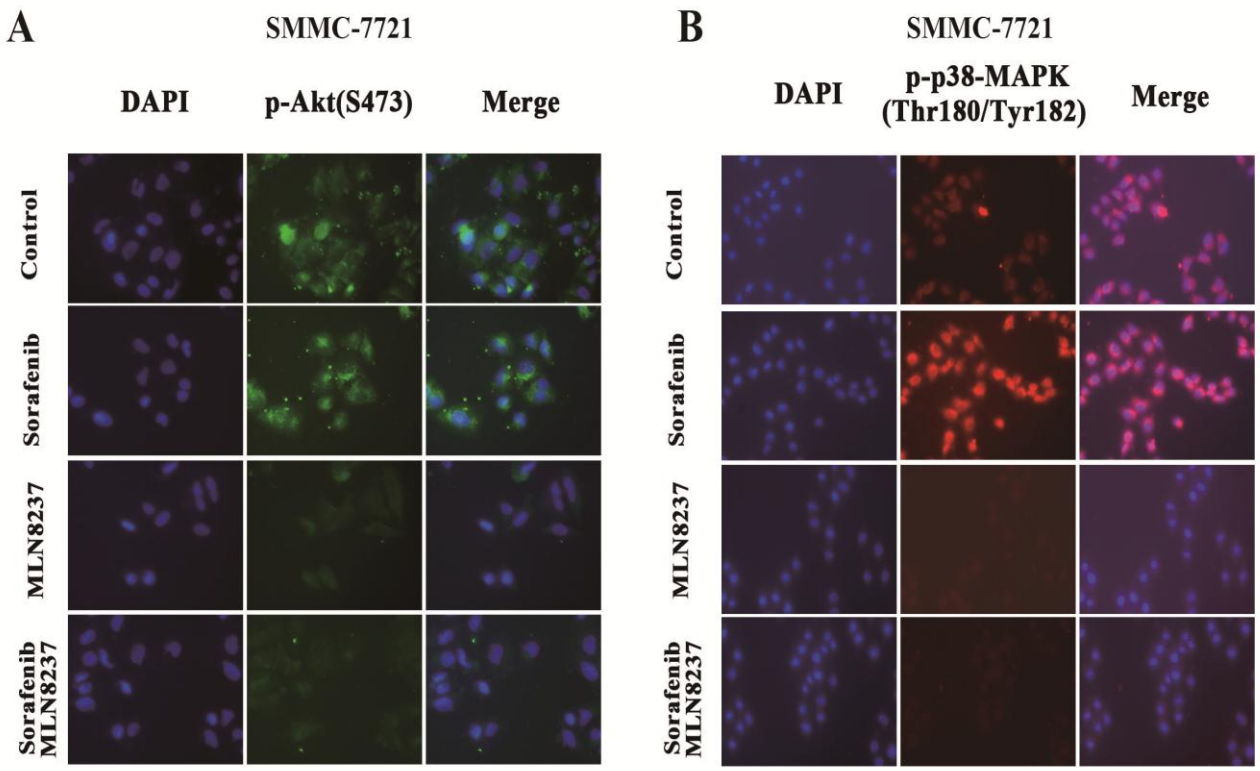

Figure S3. (A) and (B) SMMC-7721 cells treated with sorafenib, MLN8237, or both for 48 h. Immunofluorescence to determine the expression of p-Akt and p-p38 MAPK in SMMC-7721 cell.

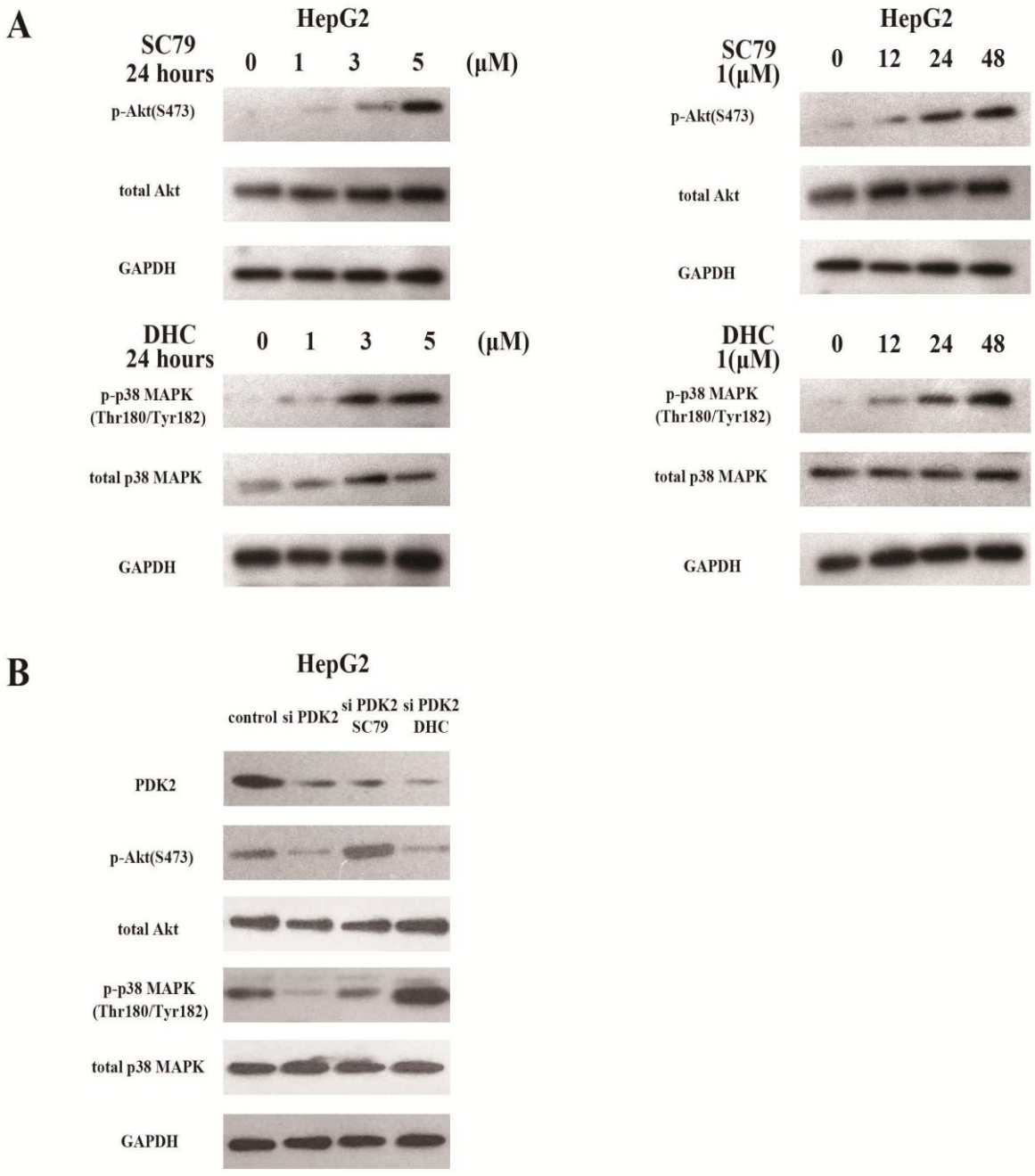

Figure S4. (A) HepG2 cells treated with different concentrations and different times of SC79, DHC. Western blotting was then performed to monitor the expression of p-Akt, total Akt, p-p38 MAPK, and total p38 MAPK. GAPDH was used as an internal control. (B) HepG2 cells treated with si PDK2 alone or combination with SC79/DHC. Western blotting was then performed to monitor the expression of PDK2,p-Akt, total Akt, p-p38 MAPK, and total p38 MAPK. GAPDH was used as an internal control.

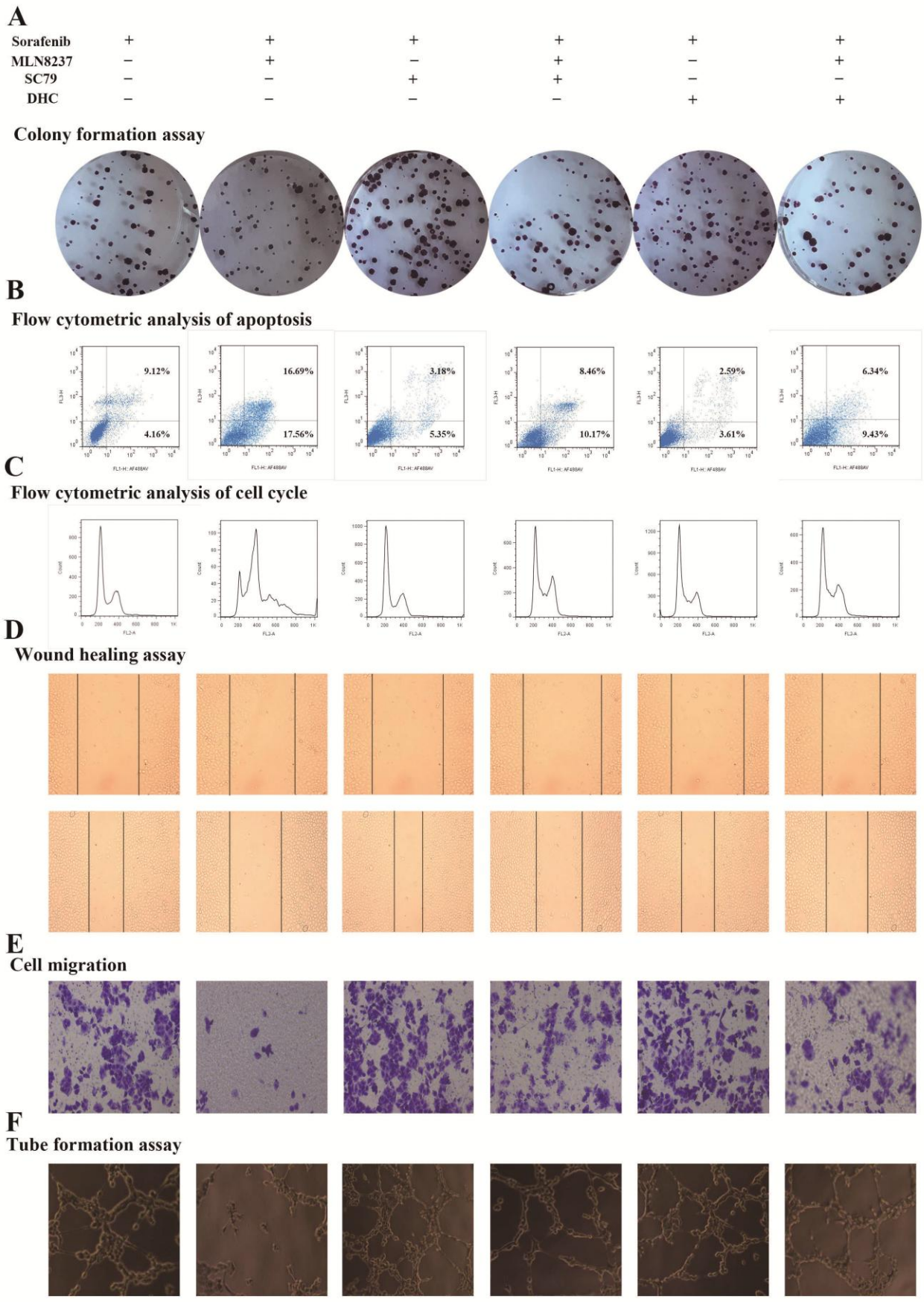

Figure S5. The original data for Figure 5 C-H.

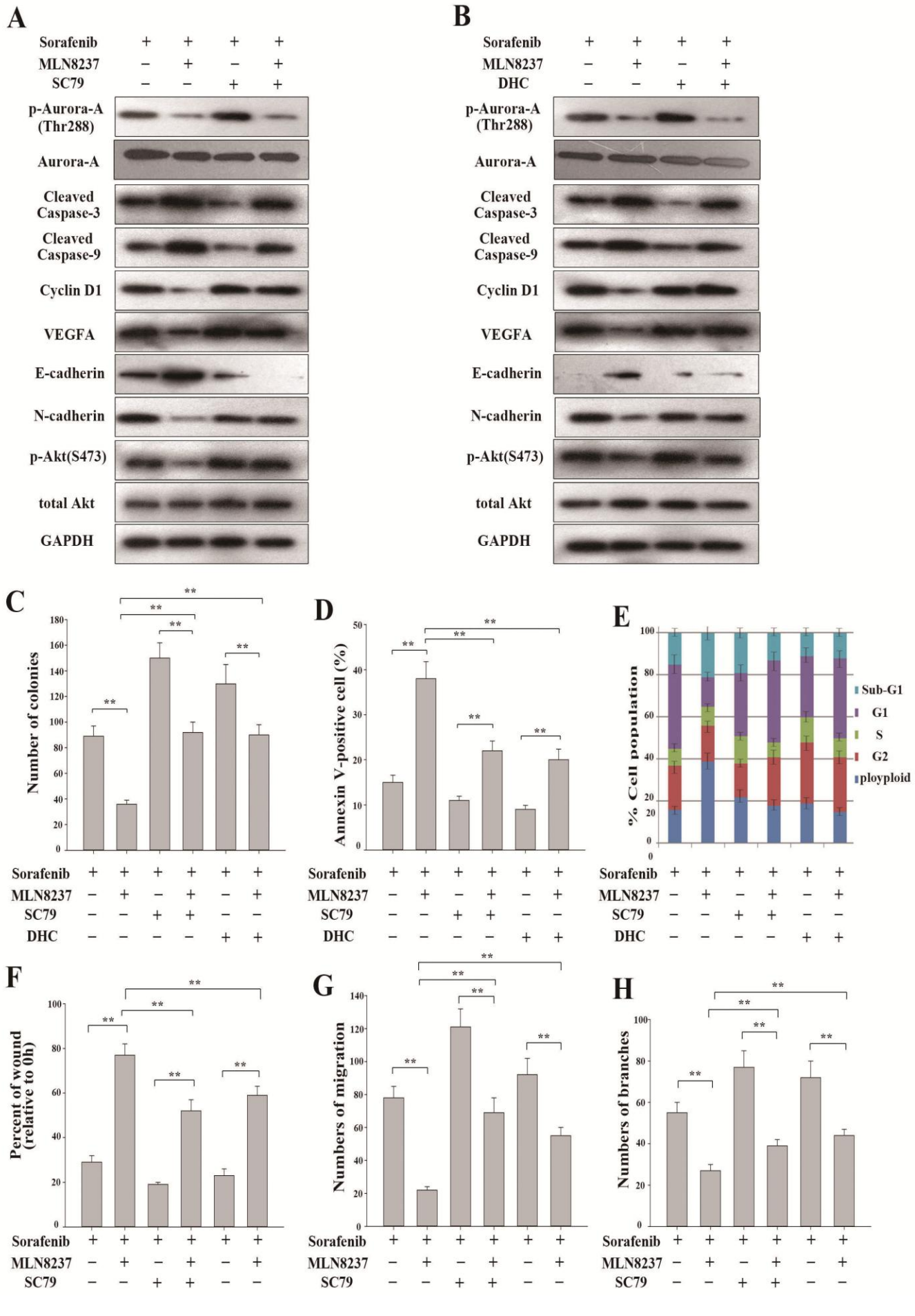
A
Sorafenib + + + +
MLN8237 − + − +
SC79 − − + +
p-Aurora-A (Thr288)
Aurora-A
Cleaved Caspase-3
Cleaved Caspase-9
Cyclin D1
VEGFA
E-cadherin
N-cadherin
p-Akt(S473)
total Akt
GAPDH
B
Sorafenib + + + +
MLN8237 − + − +
DHC − − + +
p-Aurora-A (Thr288)
Aurora-A
Cleaved Caspase-3
Cleaved Caspase-9
Cyclin D1
VEGFA
E-cadherin
N-cadherin
p-Akt(S473)
total Akt
GAPDH
C
Number of colonies
Sorafenib + + + + + +
MLN8237 − + − + − +
SC79 − − + + − −
DHC − − − − + +
D
Annexin V-positive cell (%)
Sorafenib + + + + + +
MLN8237 − + − + − +
SC79 − − + + − −
DHC − − − − + +
E
% Cell population
Sub-G1
G1
S
G2
ploypoid
Sorafenib + + + + + +
MLN8237 − + − + − +
SC79 − − + + − −
DHC − − − − + +
F
Percent of wound (relative to 0h)
Sorafenib + + + + + +
MLN8237 − + − + − +
SC79 − − + + − −
G
Numbers of migration
Sorafenib + + + + + +
MLN8237 − + − + − +
SC79 − − + + − −
H
Numbers of branches
Sorafenib + + + + + +
MLN8237 − + − + − +
SC79 − − + + − −

Figure S6. (A) SMMC-7721 cells were treated with sorafenib alone or with the combination of sorafenib and MLN8237 in the presence or absence of the p-Akt activator SC79. Western blotting was then performed to analyse and monitor the expression of p-Aurora, cleaved caspase-3, cleaved caspase-9, CDK4, cyclin D1, VEGFA, E-cadherin, N-cadherin, p-Akt, and total Akt in the cells. GAPDH was used as an internal control. (B) SMMC-7721 cells were treated with sorafenib alone or with the combination of sorafenib and MLN8237 in the presence or absence of the p-p38 MAPK activator DHC. Western blotting was then performed to analyze and monitor the expression of p-Aurora, cleaved caspase-3, cleaved caspase-9, CDK4, cyclin D1, VEGFA, E-cadherin, N-cadherin, p-p38 MAPK, and total p38 MAPK. GAPDH was used as an internal control. (C) SMMC-7721 cells were treated with sorafenib alone or with the combination of sorafenib and MLN8237 in the presence or absence of the p-Akt activator SC79 and the p-p38 MAPK activator DHC. A clonogenic assay was then conducted to examine the proliferative capability of the cells. (D) Apoptosis monitored via flow cytometry. (E) Cell cycle monitored via flow cytometry. (F) Examination of the invasive and metastatic capabilities of the cells via a scratch wound healing assay. (G) Examination of the invasive and metastatic capabilities of the cells via a transwell assay. (H) Examination of the angiogenic ability of HUVECs via a tube formation assay.