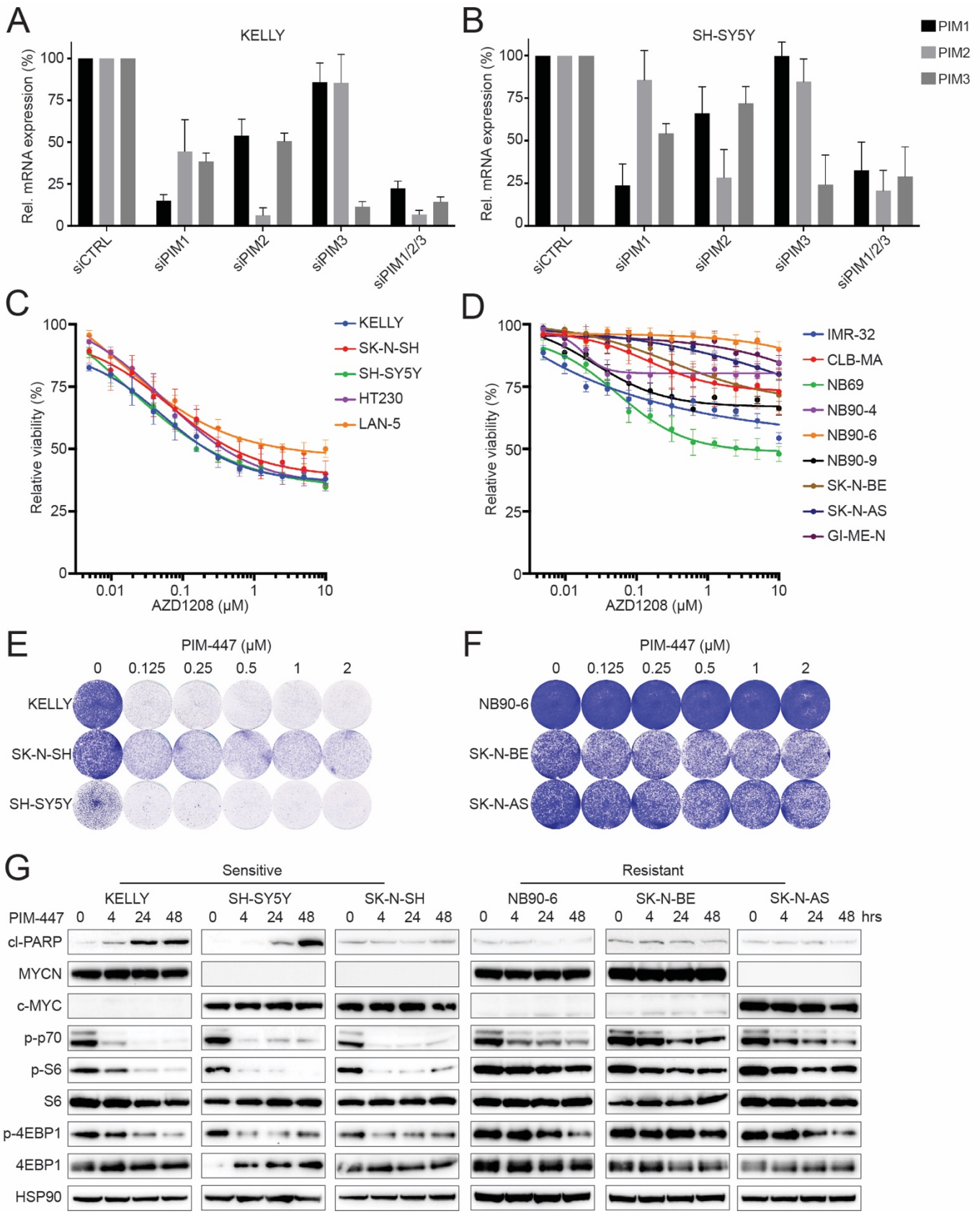
A
KELLY
Rel. mRNA expression (%)
100
75
50
25
0
siCTRL
siPIM1
siPIM2
siPIM3
siPIM1/2/3
B
SH-SY5Y
PIM1
PIM2
PIM3
C
Relative viability (%)
AZD1208 (μM)
0.01
0.1
1
10
KELLY
SK-N-SH
SH-SY5Y
HT230
LAN-5
D
IMR-32
CLB-MA
NB69
NB90-4
NB90-6
NB90-9
SK-N-BE
SK-N-AS
GI-ME-N
E
PIM-447 (μM)
0
0.125
0.25
0.5
1
2
KELLY
SK-N-SH
SH-SY5Y
F
NB90-6
SK-N-BE
SK-N-AS
G
Sensitive
Resistant
KELLY
SH-SY5Y
SK-N-SH
NB90-6
SK-N-BE
SK-N-AS
PIM-447
0
4
24
48
hrs
cl-PARP
MYCN
c-MYC
p-p70
p-S6
S6
p-4EBP1
4EBP1
HSP90

Supplemental figure 2. PIM kinases are a potential therapeutic target in neuroblastoma

(A-B) Knockdown levels of *PIM1*, *-2*, and *-3*. KELLY and SH-SY5Y cell lines were transfected with scrambled siRNA (siCTRL) or siRNAs targeting *PIM1*, *-2*, or *-3*, cultured for 2 days, followed by RNA isolation and qRT (n=3).

(C-D) Neuroblastoma cell lines demonstrate differential sensitivity to AZD1208 in a short-term assay. Cells were plated in 384-well plates, treated for 5 days with increasing concentrations of AZD1208, and viability was measured using CellTiter-Blue (n=3).

(E-F) Neuroblastoma cell lines demonstrate differential sensitivity to PIM-447 in a colony formation assay. Cells were plated in 6-well plates and treated with increasing concentrations of AZD1208. Drug-containing medium was refreshed every 3 days. Cells were fixed, stained, and scanned when untreated wells reached confluency (n=3).

(G) PIM-447-resistant cell lines demonstrate sustained mTOR signaling upon treatment. Cells were treated with 2 μM PIM-447 for 0, 24, and 48 hours followed by western blot analysis (n=3).