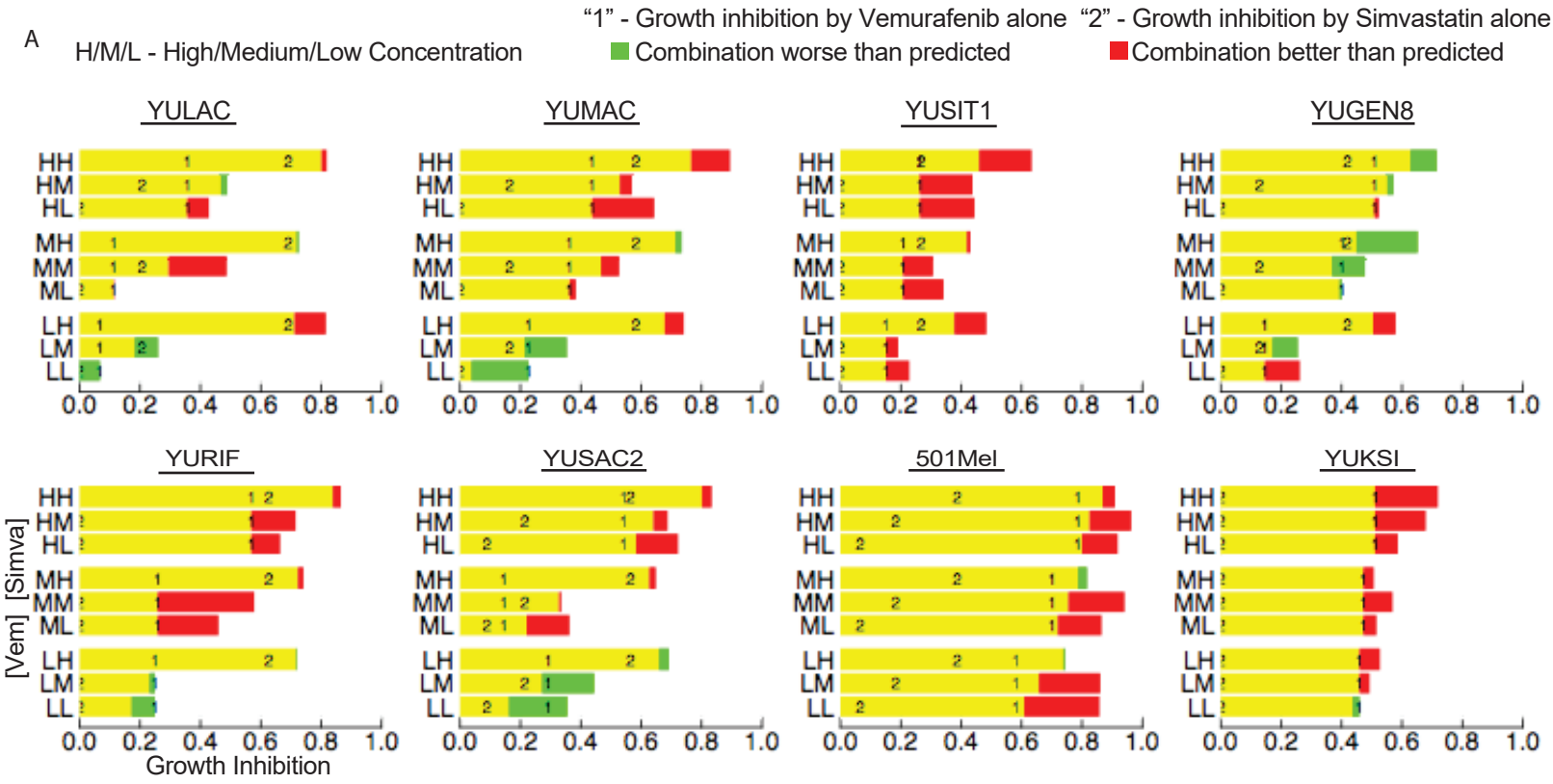
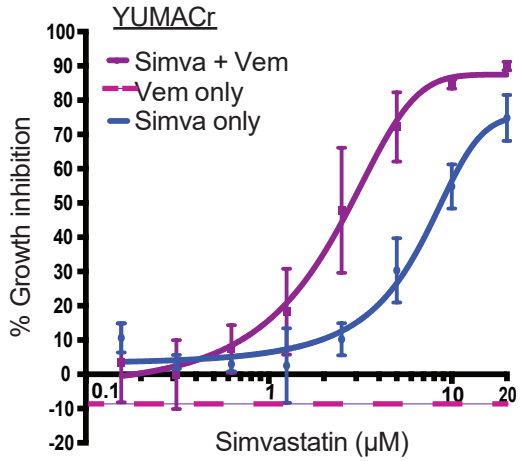
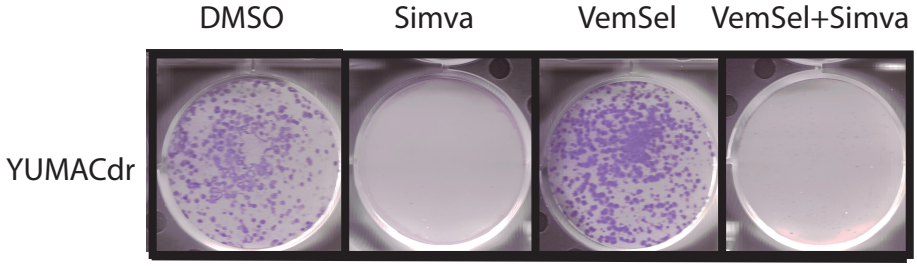
A


H/M/L - High/Medium/Low Concentration
"1" - Growth inhibition by Vemurafenib alone
"2" - Growth inhibition by Simvastatin alone
Combination worse than predicted
Combination better than predicted
YULAC
YUMAC
YUSIT1
YUGEN8
YURIF
YUSAC2
501Mel
YUKSI
[Vem] [Simva]
HH
HM
HL
MH
MM
ML
LH
LM
LL
0.0 0.2 0.4 0.6 0.8 1.0
Growth Inhibition

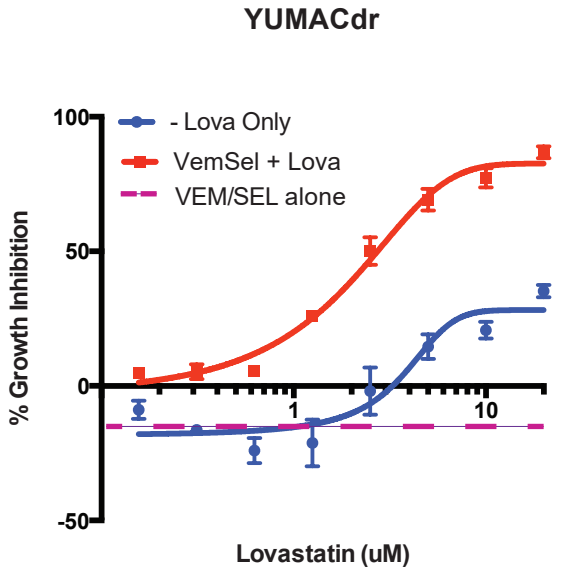
B


YUMACr
Simva + Vem
Vem only
Simva only
% Growth inhibition
100
90
80
70
60
50
40
30
20
10
0
-10
-20
0.1
1
10
20
Simvastatin (μM)

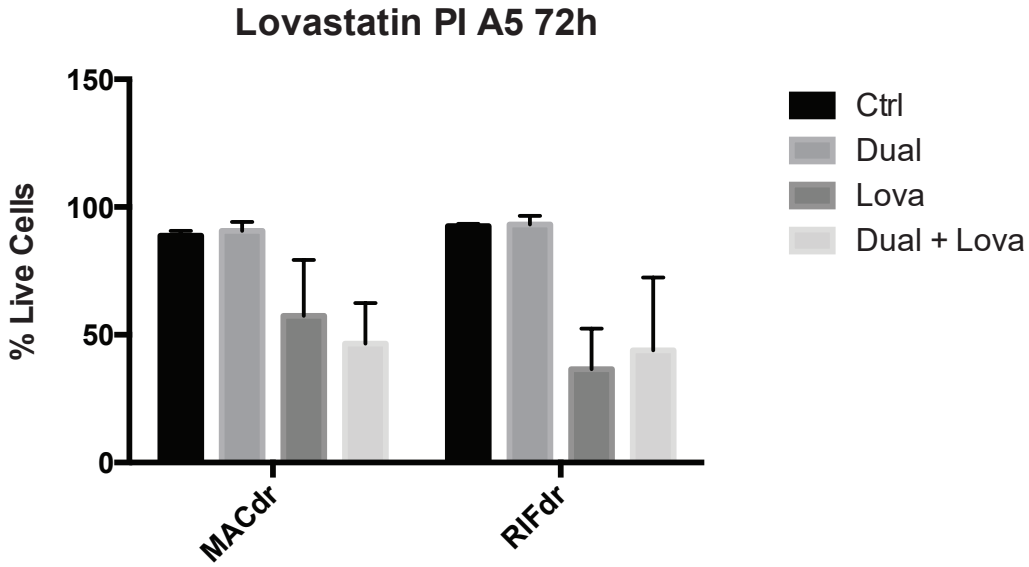
C


DMSO
Simva
VemSel
VemSel+Simva
YUMACdr

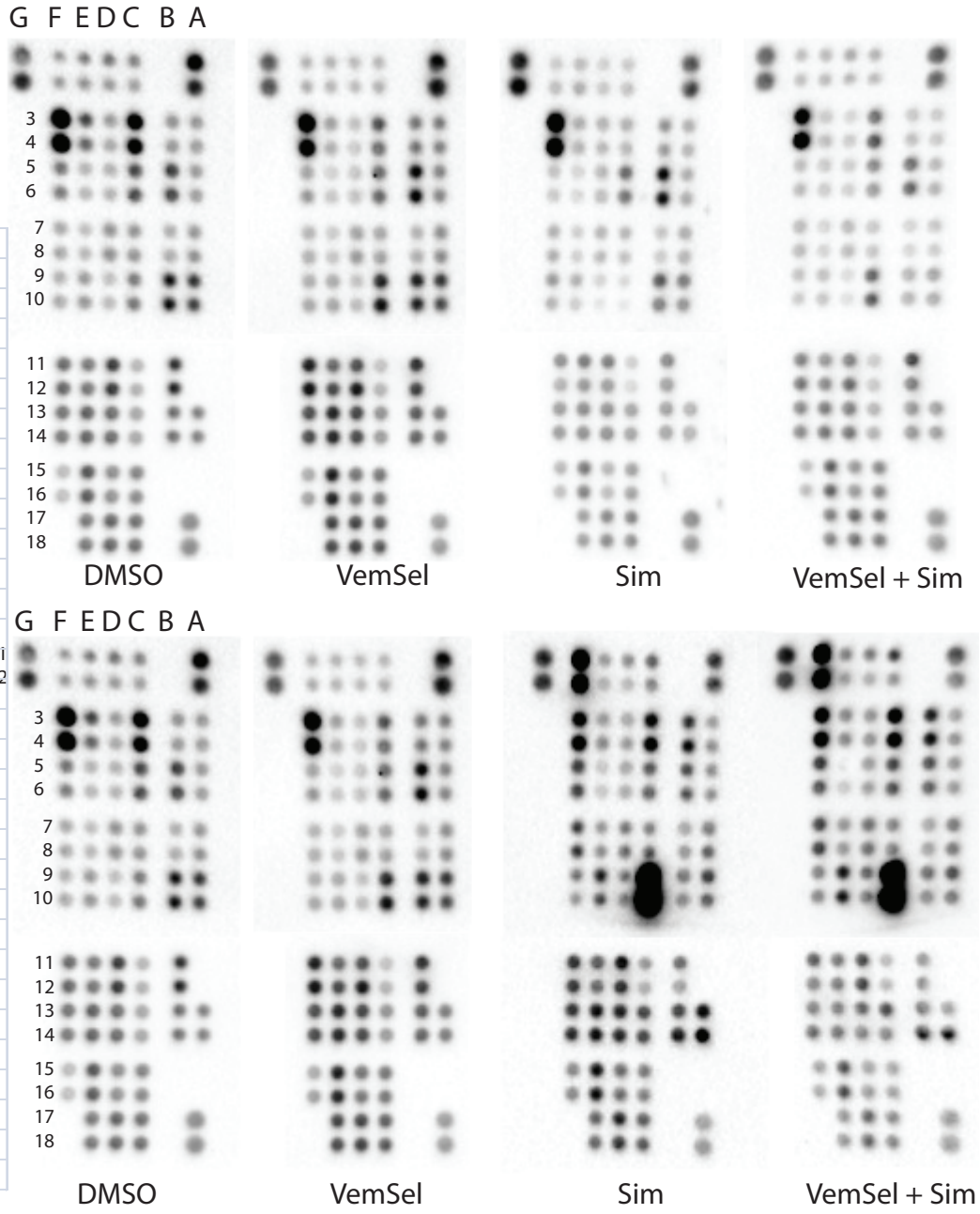
A


YUMACdr
- Lova Only
VemSel + Lova
VEM/SEL alone
% Growth Inhibition
100
50
0
-50
1
10
Lovastatin (uM)

B


Lovastatin PI A5 72h
% Live Cells
150
100
50
0
MACdr
RIFdr
Ctrl
Dual
Lova
Dual + Lova

Coordinate	Target	phospho-site
A1, A2	Reference Spot	-----
A3, A4	p38α	T180/Y182
A5, A6	ERK1/2	T202/Y204, T185/Y187
A7, A8	JNK 1/2/3	T183/Y185, T221/Y223
A9, A10	GSK-3α/β	S21/S9
B3, B4	EGFR	Y1086
B5, B6	MSK1/2	S376/S360
B7, B8	AMPKα1	T183/Y185, T221/Y223
B9, B10	AKT 1/2/3	S473
C1, C2	TOR	S2448
C3, C4	CREB	S133
C5, C6	HSP27	S78/S82
C7, C8	AMPKα2	T172
C9, C10	β-catenin	-----
D1, D2	Src	Y419
D3, D4	Lyn	Y397
D5, D6	Lck	Y394
D7, D8	STAT2	Y689
D9, D10	STAT5a	Y694
E1, E2	Fyn	Y420
E3, E4	Yes	Y426
E5, E6	Fgr	Y412
E7, E8	STAT6	Y641
E9, E10	STAT5b	Y699
F1, F2	Hck	Y411
F3, F4	Chk-2	T68
F5, F6	FAK	Y397
F7, F8	PDGF Rβ	Y751
F9, F10	STAT5a/b	Y694/Y699
G1, G2	Reference Spot	-----
G3, G4	PRAS40	T246
G9, G10	PBS (Negative Control)	-----

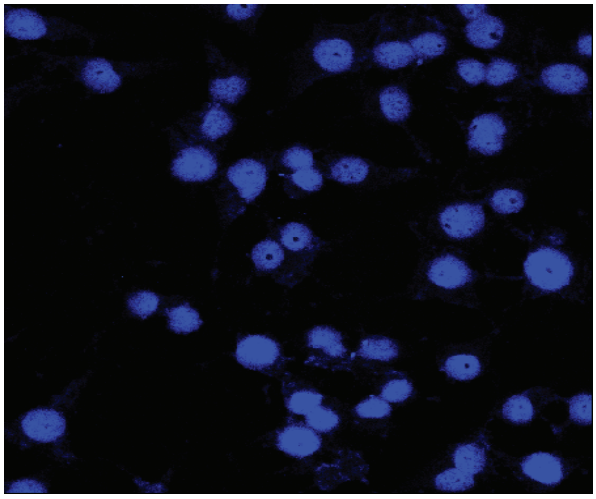


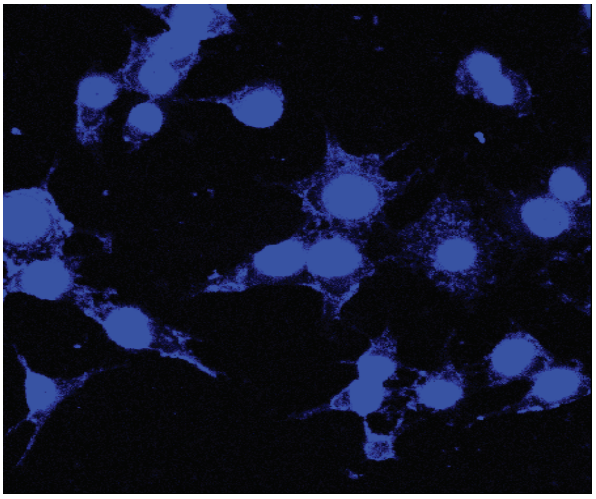
YUMACdr

VemSel

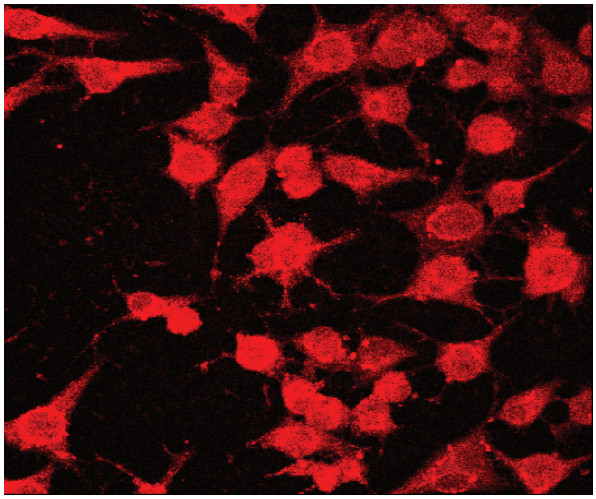
VemSel + Simvastatin

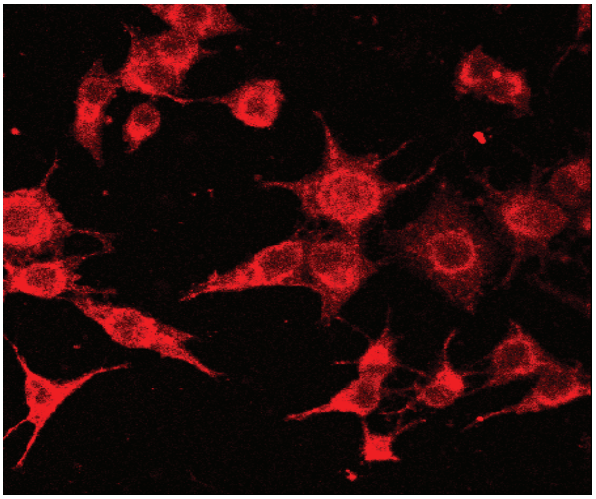
DAPI



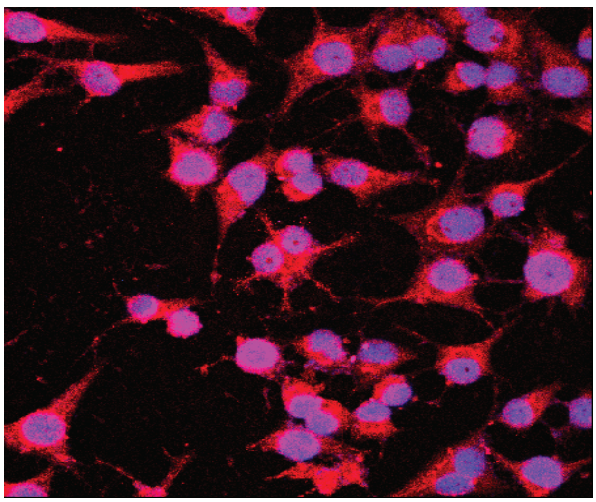


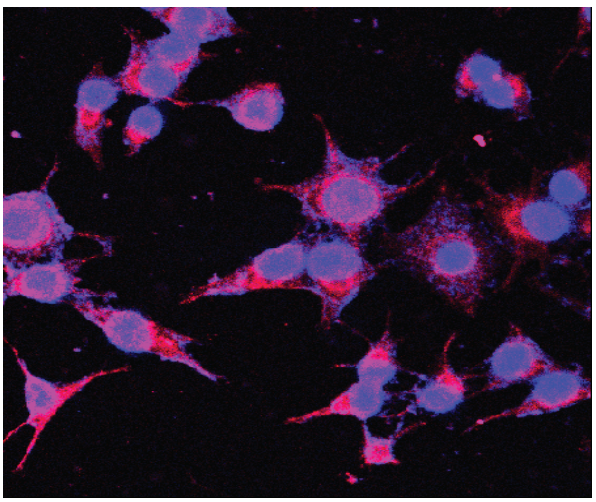
YAP





Merge



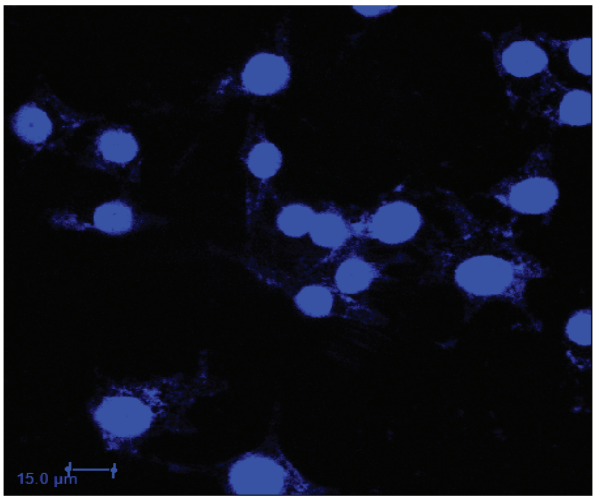


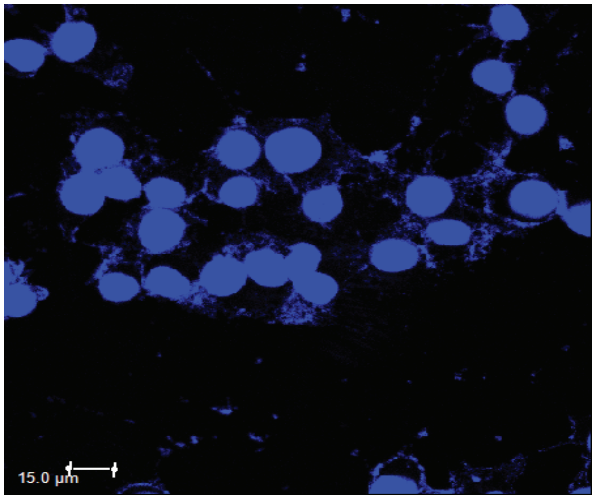
YUMACdr

VemSel

VemSel + Simvastatin

DAPI





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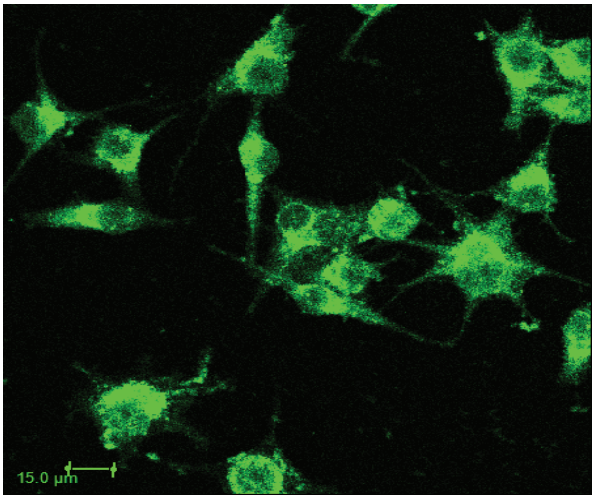
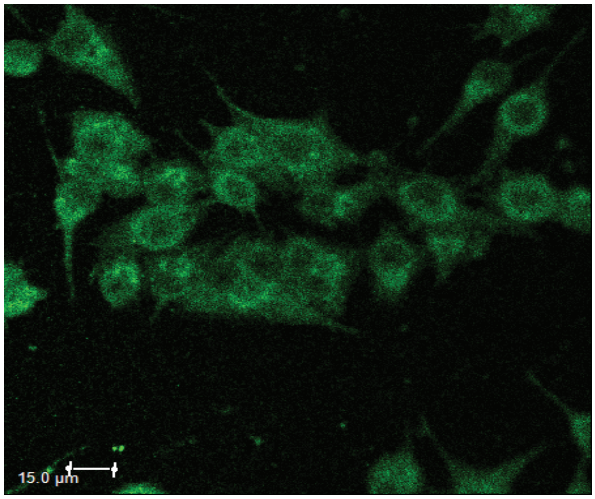
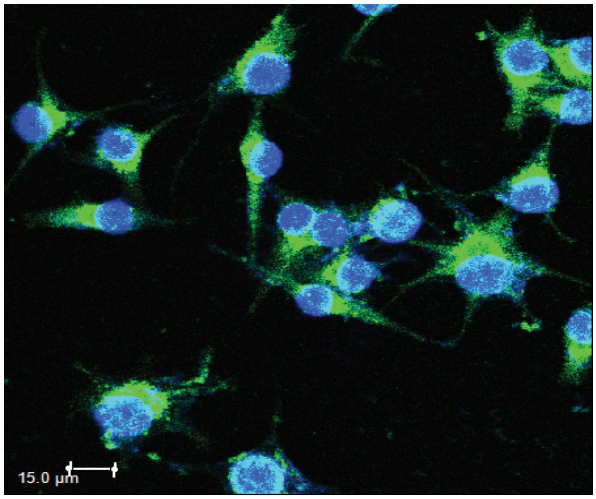
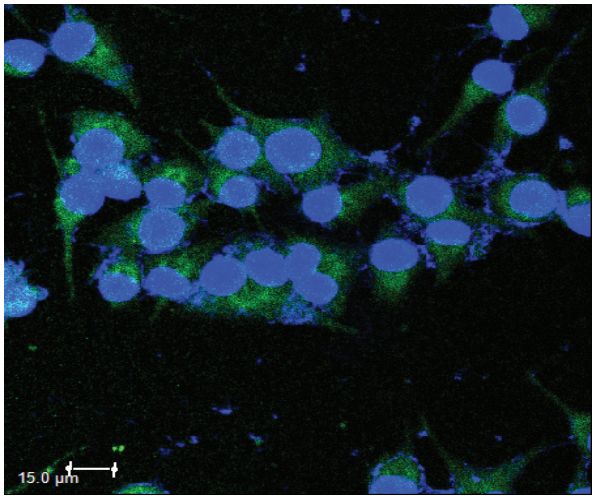
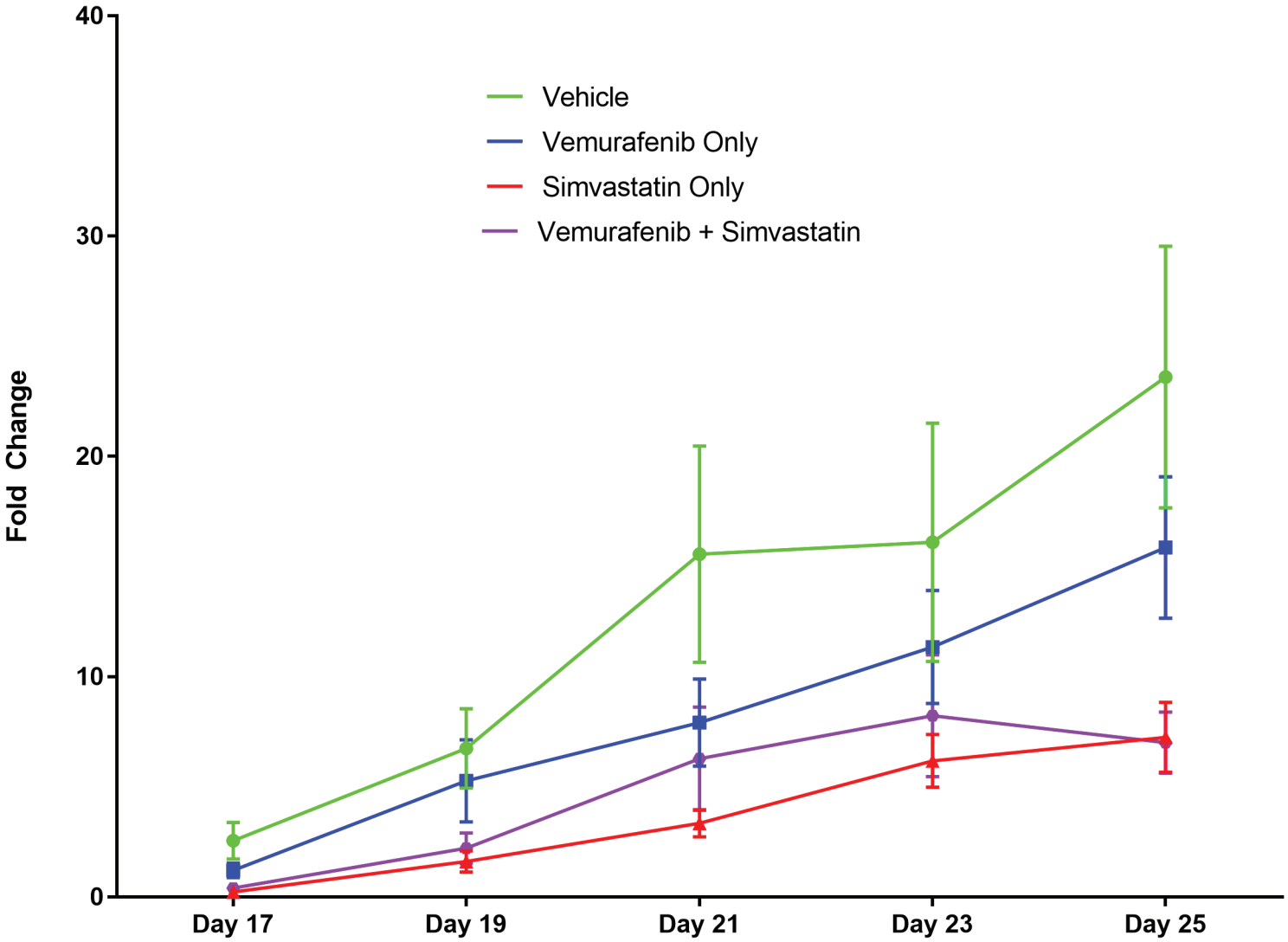




Merge






40
30
20
10
0
Fold Change
Vehicle
Vemurafenib Only
Simvastatin Only
Vemurafenib + Simvastatin
Day 17
Day 19
Day 21
Day 23
Day 25

Supplementary Figure 1: High-throughput drug screening identifies statins as showing significant synergy with MAPK inhibitors. (A). In a panel of 8 *BRAF*-mutant melanomas screened in a 72 hour assay, we observed synergistic growth inhibition in at least 3 vemurafenib/simvastatin drug conditions in 100% of lines. (B). Simvastatin was found to be greatly potentiated in combination with vemurafenib in lines with acquired resistance to vemurafenib. Assays were performed in triplicate. Error bars represent SEM, n=3. (C). 5µmol/L simvastatin significantly reduced colony formation as both mono- and combination therapy over 14 day assay. n=3.

Supplementary Figure 2: Lovastatin recapitulates growth inhibitor activity of simvastatin. (A,B). Treatment with 5µmol/L lovastatin showed significant growth inhibition and apoptosis induction in 72 hour dose-response assay and 72 hour propodium iodide/annexin V flow cytometry. Error bars represent SEM, n=3.

Supplementary Figure 3: Major effects of statins are restricted to a limited number of pathways. Human phospho-kinase antibody array profiling of lysates from resistant lines YUMACdr and YURIFdr treated with or without 5 µmol/L vemurafenib plus 150nmol/L selumetinib and with or without 5µmol/L simvastatin for 24 hours showing significant downregulation of AKT activation.

Supplementary Figure 4: Treatment with statins delocalizes the transcription factor YAP away from the nucleus. Treatment of resistant YUMACdr cells with 5 µmol/L vemurafenib plus 150nmol/L selumetinib with or without 5µmol/L simvastatin shows significant delocalization of the transcription factor YAP away from the nucleus with HMG-CoA reductase blockade.

Supplementary Figure 5: Treatment with statins delocalizes NRAS away from its site of greatest activity at the plasma membrane. Treatment of resistant YUMACdr cells with 5 µmol/L vemurafenib plus 150nmol/L selumetinib with or without 5µmol/L simvastatin shows significant relocalization of NRAS from the plasma membrane to the cytoplasm, as well as overall downregulation with statin treatment.

Supplementary Figure 6: Mevalonate pathway activity plays a significant role in melanoma activity *in vivo* (A). Four cohorts of five mice each were grafted with YUMACr vemurafenib-resistant human melanoma cells. Treatment was begun once the first mice showed tumors of at least $1mm^3$. Mice receiving vemurafenib were fed vemurafenib-containing chow, while mice receiving simvastatin received 30mg/kg simvastatin injection in DMSO every other day after an initial loading phase of 4 daily doses. The experiment was concluded when the first mouse reached tumor size endpoint of $1cm^3$. Tumor volumes were measured every 2 days after treatment began.