

Supplemental Figure 2

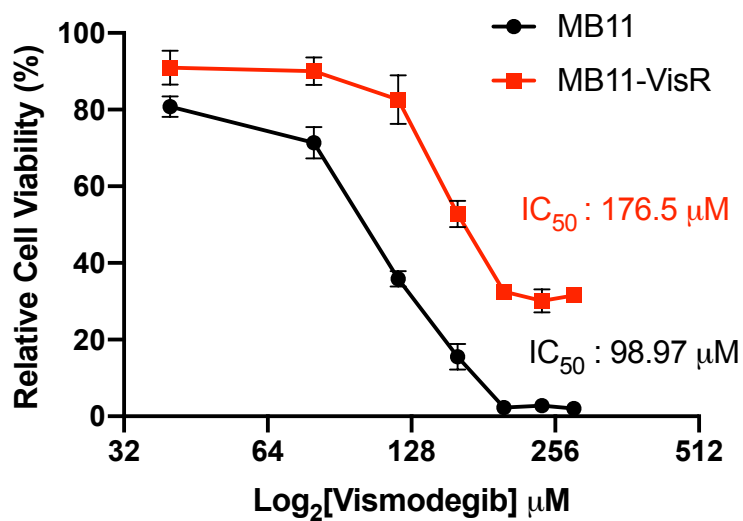
A

MB11			
Vismodegib (μM)	Metformin (mM)	Killing Effect	Combination index (CI)
40	10	0.79	0.78232
60	10	0.892	0.60672
80	10	0.926	0.59870
100	10	0.936	0.66080
Chou-Talalay quantitative definition for additive effect (CI = 1), synergism (CI < 1), and antagonism (CI > 1) in drug combination			

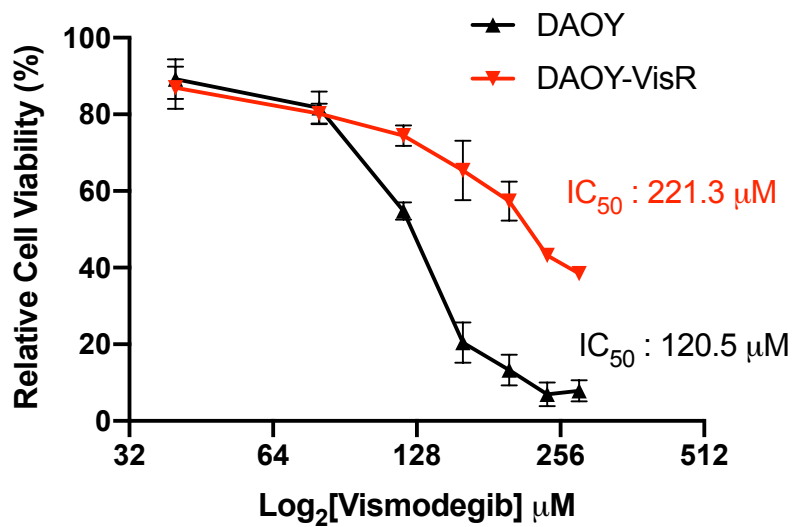
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DAOY			
Vismodegib (μM)	Metformin (mM)	Killing Effect	Combination index (CI)
40	10	0.662	0.98673
60	10	0.784	0.83380
80	10	0.809	0.88266
100	10	0.888	0.74332
Chou-Talalay quantitative definition for additive effect (CI = 1), synergism (CI < 1), and antagonism (CI > 1) in drug combination.			

A



B



A

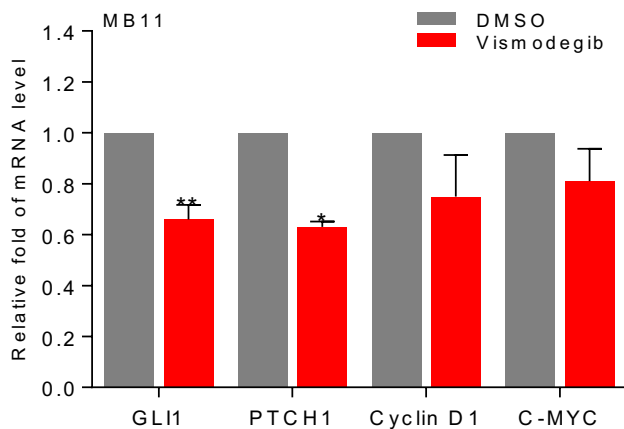
MB11-VisR			
Vismodegib (μM)	Metformin (mM)	Killing Effect	Combination index (CI)
40	10	0.32	1.86983
60	10	0.415	1.41692
80	10	0.469	1.29105
100	10	0.575	1.00714
120	10	0.536	1.24318
160	10	0.756	0.72770
200	10	0.828	0.64337
240	10	0.91	0.47581
Chou-Talalay offers quantitative definition for additive effect (CI = 1), synergism (CI < 1), and antagonism (CI > 1) in drug combinations			

B

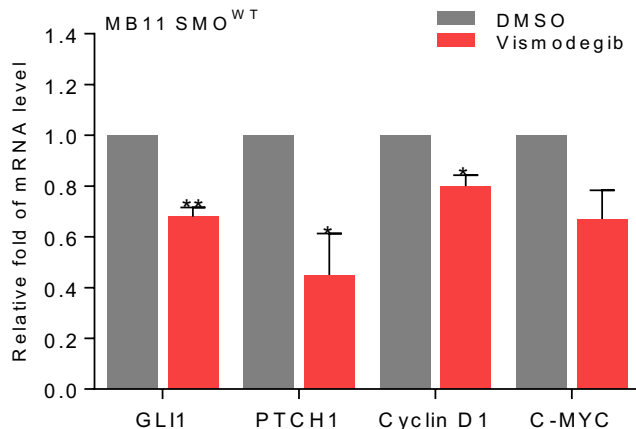
DAOY-VisR			
Vismodegib (μM)	Metformin (mM)	Killing Effect	Combination index (CI)
40	10	0.322	1.76747
60	10	0.275	2.31826
80	10	0.418	1.48716
100	10	0.516	1.15830
120	10	0.739	0.55717
160	10	0.91	0.22257
200	10	0.906	0.25887
240	10	0.91	0.27526
Chou-Talalay offers quantitative definition for additive effect (CI = 1), synergism (CI < 1), and antagonism (CI > 1) in drug combinations			

Supplemental Figure 5

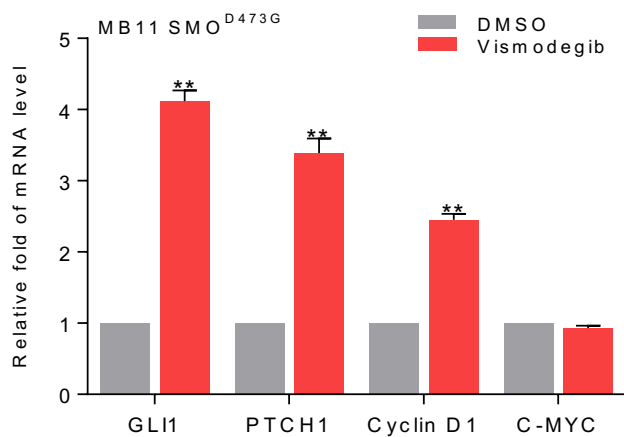
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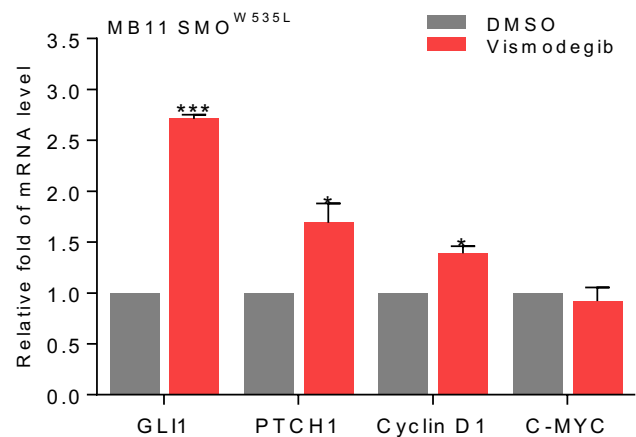
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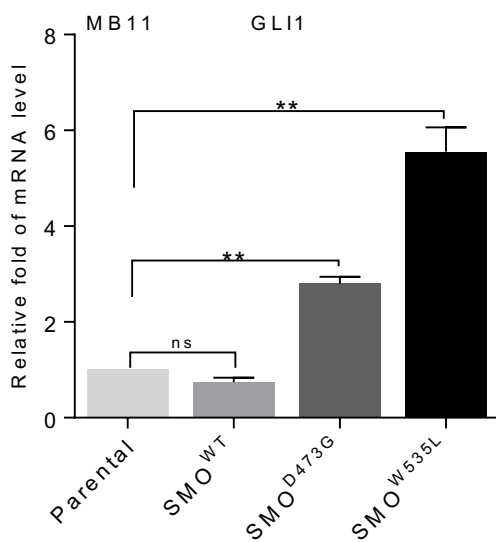
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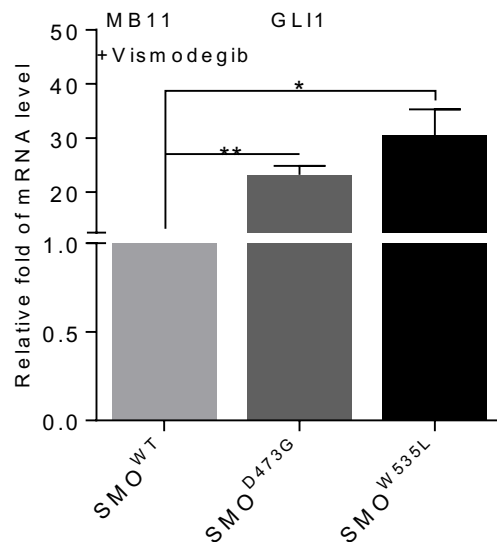
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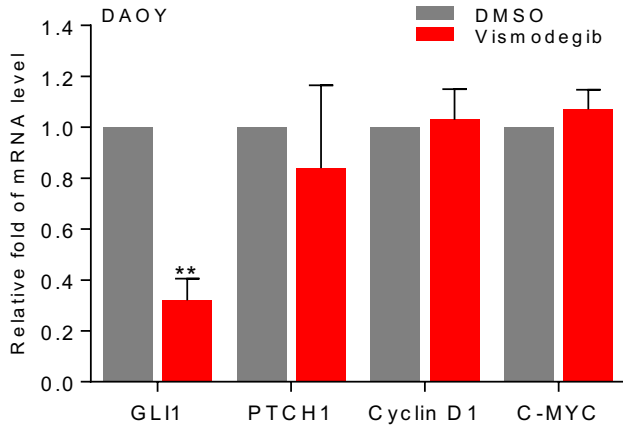
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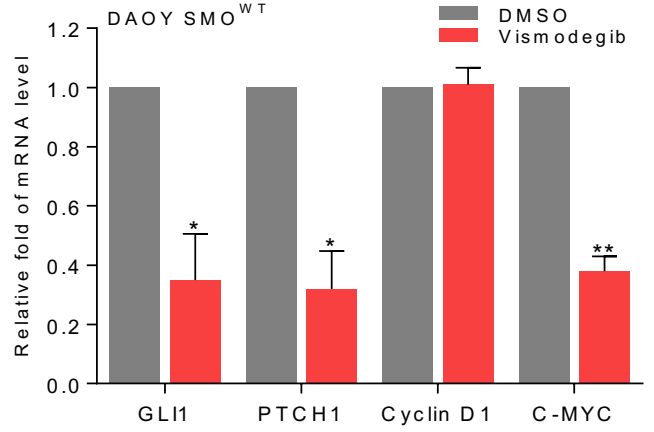
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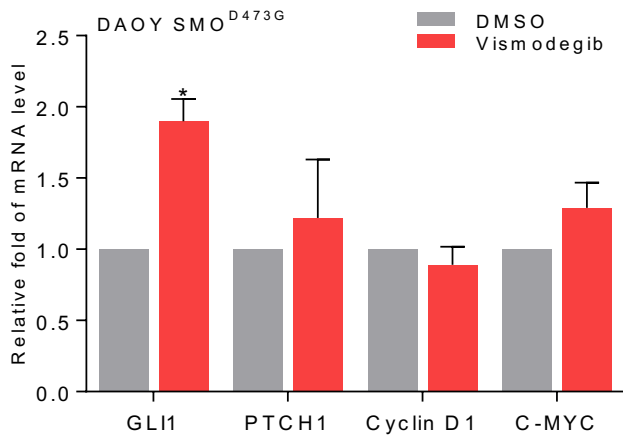
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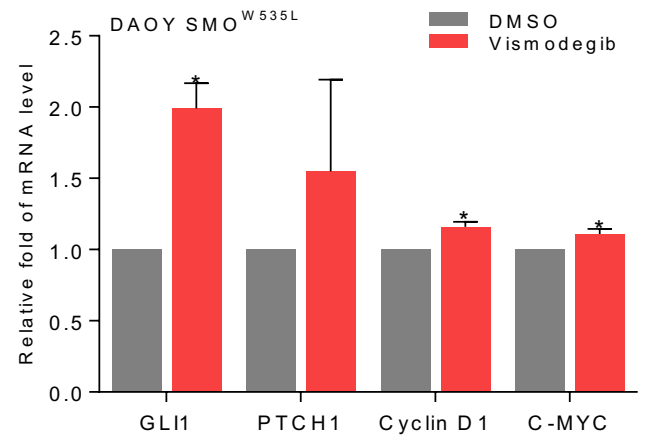
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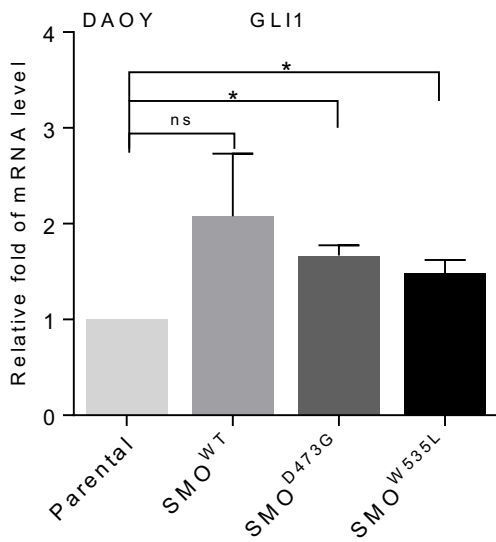
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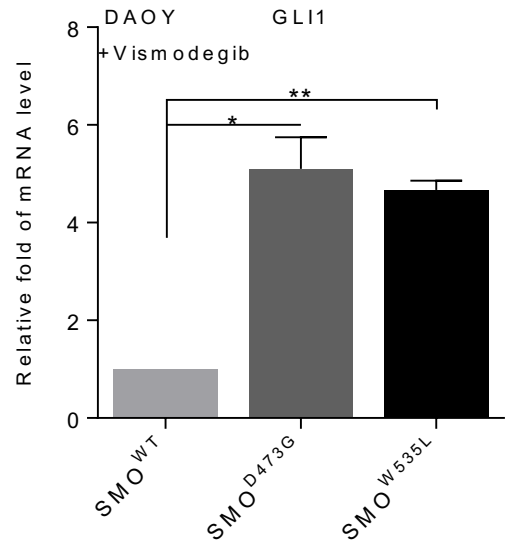
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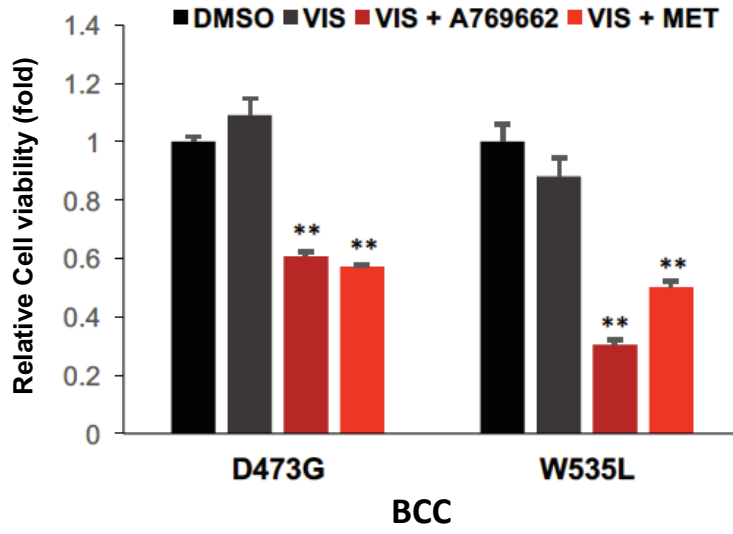


E



F





SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Combination of AMPK agonist and SMO inhibitor has better growth inhibitory effect on MB cell growth and colony formation. Human MB cell line, **(A)** DAOY and mouse MB cell line **(B)** Med1 were treated with DMSO, Vismodegib (10 μ M), A769662 (20 μ M), Vis and A769662 (10 μ M+20 μ M) and subjected to MTT cell growth assay for three consecutive days. **(C)** Med1 cells were treated with DMSO, Vismodegib (10 μ M), Metformin (5 mM), Vis and Met (10 μ M+5 mM) and subjected to soft agar colony formation assay for 2 weeks. The experimental points were in triplicate and repeated three times (***) $p < 0.01$).

Supplemental Figure 2. Metformin synergizes with Vismodegib on MB cells. As showed in Figure 1F (MB11) and 1H (DAOY), MB11 and DAOY cells were treated with Vismodegib (from 40 to 100 μ M), Metformin (10 mM) and Vismodegib plus Metformin for 48 hours and subjected to cell growth assay. The synergism of Metformin and Vismodegib was calculated by Chou-Talalay quantitative definition as showed in **(A)** and **(B)**. Combination index (CI) < 1 means synergism in drug combination.

Supplemental Figure 3. MB11-VisR and DAOY-VisR cell lines were resistant to Vismodegib.

(A) MB11-VisR and **(B)** DAOY-VisR cell lines were treated with Vismodegib from 40 to 280 μ M for 48 hours and subjected to MTT cell growth assay. The experimental points were in triplicate and repeated three times (***) $p < 0.01$).

Supplemental Figure 4. Metformin synergizes with vismodegib on MB-VisR cells.

As showed in Figure 4B (MB11-VisR) and 4D (DAOY-VisR), MB-VisR cells were treated with DMSO, Vismodegib (From 40 to 240 μ M), Metformin (10 mM), Vismodegib (From 40 to 240 μ M) and Metformin (10 mM) for 48 hours and subjected to cell growth assay. The synergism of Metformin and Vismodegib was calculated by Chou-Talalay quantitative definition as showed in **(A) and (B)**. Combination index (CI) <1 means synergism in drug combination.

Supplemental Figure 5. MB11-SMO^{D473G} and SMO^{W535L} MB stable cells are resistant to Vismodegib. **(A)** MB11, **(B)** MB11-SMO^{WT}, **(C)** MB11-SMO^{D473G} and **(D)** MB11-SMO^{W535L} cell lines were treated with Vismodegib (10 μ M) for 24 hours and mRNA was collected, and the amount of HH/GLI1 downstream targets, *GLI1*, *PTCH1*, *Cyclin D1* and *C-MYC* mRNA was analyzed by qRT-PCR with *GAPDH* mRNA as the internal control. The bars indicate mRNA level relative to that of DMSO-treated cells. **(E)** In MB11 Parental, MB11-SMO^{WT}, MB11-SMO^{D473G} and MB11-SMO^{W535L} cell lines, *GLI1* mRNA was analyzed by qRT-PCR with *GAPDH* mRNA as the internal control. The bars indicate mRNA level relative to that of MB11 Parental cell line. **(F)** MB11-SMO^{WT}, MB11-SMO^{D473G} and MB11-SMO^{W535L} cell lines were treated with Vismodegib (10 μ M) for 24 hours and mRNA was collected. *GLI1* mRNA was analyzed by qRT-PCR with *GAPDH* mRNA as the internal control. The bars indicate mRNA level relative to that of Vismodegib treated MB11-SMO^{WT} cell line. All the experimental points were in triplicate and independently repeated three times (“*” p<0.05, “**” p<0.01, “***” p<0.001; “ns” non-significant).

Supplemental Figure 6. DAOY-SMO^{D473G} and SMO^{W535L} MB stable cells are resistant to Vismodegib. (A) DAOY, (B) DAOY-SMO^{WT}, (C) DAOY-SMO^{D473G} and (D) DAOY-SMO^{W535L} cell lines were treated with Vismodegib (10 μ M) for 24 hours and mRNA was collected, and the amount of HH/GLI1 downstream targets, *GLI1*, *PTCH1*, *Cyclin D1* and *C-MYC* mRNA was analyzed by qRT-PCR with *GAPDH* mRNA as the internal control. The bars indicate mRNA level relative to that of DMSO-treated cells. (E) In DAOY Parental, DAOY-SMO^{WT}, DAOY-SMO^{D473G} and DAOY-SMO^{W535L} cell lines, *GLI1* mRNA was analyzed by qRT-PCR with *GAPDH* mRNA as the internal control. The bars indicate mRNA level relative to that of DAOY Parental cell line. (F) DAOY-SMO^{WT}, DAOY-SMO^{D473G} and DAOY-SMO^{W535L} cell lines were treated with Vismodegib (10 μ M) for 24 hours and mRNA was collected. *GLI1* mRNA was analyzed by qRT-PCR with *GAPDH* mRNA as the internal control. The bars indicate mRNA level relative to that of Vismodegib treated DAOY-SMO^{WT} cell line. All the experimental points were in triplicate and independently repeated three times (“*” $p < 0.05$, “**” $p < 0.01$; “ns” non-significant).

Supplemental Figure 7. Combination of Vismodegib and AMPK agonist exerts best killing effect on SMO mutant variant BCC. SMO^{D473G} or SMO^{W535L} BCC cells were seeded into 96-well plates and allowed to grow for 24 hours, after which medium was replaced with either 100nM Vismodegib (VIS), 0.75 mM A769662, 100 μ M Metformin or a combination of Vismodegib and A769662 or Vismodegib and Metformin at the indicated concentrations. Cells were cultured in drug treatment for 48 hours after which MTT assay

was performed. All the experimental points were in triplicate and independently repeated three times (***) $p < 0.01$).