Supplemental materials and methods

Western blot analysis

Cell lysis and immunoblotting were performed as previously described [32]. Proteins were detected via incubation with HRP-conjugated secondary antibodies and ECL Western Blotting detection reagents (Cat # RPN1206, GE Healthcare) or SuperSignal West Dura Extended Duration Substrate (Cat # 34076, Thermo Fisher Scientific). Primary antibodies used were as follows: pERK (#4370), pPKD (s744/748) (#2054), RASGRP3 (#3334), pFAK(Y397) (#8556), PARP (#9542), FAK (#3285) from Cell Signaling Technology (Danvers, MA, USA); p-p90RSK (#ab32413), pRasGRP3 (#ab124823) all from ABCAM (Burlingame, CA, USA); ERK2 (#SC-154), GNA15 (# sc-393878), GNAS (#sc-135914) all from Santa Cruz (Santa Cruz, CA, USA); β-actin (#A1978) from Sigma; GNA14(#H00009630-B02P) from Abnova (Taipei City, Taiwan). SuperSignal West Dura Extended Duration Substrate was used to detect pPKD expression. ECL Western Blotting detection reagents were utilized to detect all other proteins.

IP1 assay

Accumulation of IP1 was measured using the IP-One Gq kit (Cat# 62IPAPEB) from Cisbio Inc (Bedford, MA) according to the manufacture's instruction with slight modification. This IP1 assay is a competitive homogenous time resolved fluorescence (HTRF) immunoassay, in which native IP1 produced by cells competes with d2-labeled IP1 (acceptor) for binding to anti-IP1-Cryptate (donor). Briefly, 293FT transfected cells or melanoma cells were trypsinized, counted, and seeded into tissue culture grade flat white 384-well plates, with triplicate per sample. Cell numbers per well were adjusted to yield IP1 amounts in the linear range of the assay kits. The same cell numbers were then used to treat with or without drug in the presence of stimulation buffer containing LiCl to prevent IP1 degradation for 2 hours at 37^{o}C, unless indicated differently in experimental figures. IP1 d2 reagent and IP1 Tb Cryptate antibody was added and incubated for another one hour at room temperature. Plates were read in a Spark 20M plate reader. The plate was first subjected to excitation wavelength at 320nm and then the fluorescence was measured at 615 nm and 665 nm, simultaneously. The ratio of the

acceptor and donor emission signals was calculated for each individual well based on the following formula: Ratio=signal at 665nm / signal at 615nm x10^4. The IP1 concentrations were interpolated from a standard curve generated with control reagents provided with the kits and graphed using Prism 6.0 (GraphPad, La Jolla, CA, USA).

cAMP assay

Accumulation of cAMP was measured using the competitive immunoassay-based CAMP-GS Dynamic Kit (Cat# 62AM4PEB) from Cisbio Inc (Bedford, MA) by following the supplier's instructions with modifications. This assay also uses a HTRF technology similar to IP1 assay. Briefly, 293FT cells were transfected with various plasmids for 24 hours, trypsinized, and counted. 20,000 cells were seeded into 384-well plates with triplicate per sample and incubated with stimulation buffer in the presence or absence of YM drug at 37^{o}C for 2 hours. Then cells were added with d2-labeled cAMP (red acceptor) and monoclonal cAMP Europium Cryptate-labeled antibody (Europium donor) and incubated at room temperature for 1 hour. The plates were read and data were analyzed as described for the IP1 assay.

Cell proliferation assay

For drug IC_{50} analysis: 1000-3000 cells were plated in triplicate into 96 well tissue culture plates per well and, the following day, were treated with or without the indicated concentrations of reagents for 4 days. Cell viability was analyzed by using CyQUANT NF cell proliferation assay (Life technologies Corporation, Eugene, Oregon) according to the manufacturer's instruction. Plates were read in a SpectraMax M2 (Molecular Devices, Sunnyvale, CA). IC_{50} values were calculated using Prism 6 software. For short term proliferation assays (less than one week), cells were plated in triplicate into 6-well plates at the same number of cells and cultured for the indicated times. Cells were collected by trypsinization and counted in a TC20 automated cell counter (Bio-Rad, Hercules, CA). For long term proliferation assays, cells were plated at 6-well plates and then treated with or without reagents. After 10 to 14 days, cells were stained with Crystal Violet.

Cell Fractionation

Subcellular fractionation was performed using the Subcellular Protein Fractionation Kit of Thermo Scientific (Waltham, MA, USA) following the manufacturer's instructions. Fractions from cytosolic, membrane and nuclear parts were subjected to western blotting with the indicated antibodies.

Supplemental figure legends

Supplemental Fig. S1: YM-254890 is a GNAQ/11-specific inhibitor

(A) Oncogenic signaling output of GNA14, GNA15 and GNAS. 293 FT cells were transfected for 24 hours and subjected to IP1 assay (GNA14, GNA15) (top panel) and cAMP assay (GNAS) (top panel) as well as western blot (bottom panel).

(B) The effect of YM on MAPK signaling downstream of Gαq. 293FT cells transfected were treated with DMSO (D), 100nM YM (YM), 1μM LXS196(LXS) and 100nM Trametinib (TRA) for 24hrs. LXS196 but not YM-254890 blocks the effect of TPA (200nM) on pERK, pp90RSK and pPKD. A representative of western blot of three independent experiments is shown.

Supplemental Fig. S2 PKC inhibitor AHT956 inhibited FAK phosphorylation in a dose dependent manner in UM cells.

MP41 cells were treated AHT956 at indicated dosages for 24 hours and subjected to western blot.

Supplemental Fig. S3 GNAQ/11 mutant UM cells display high intrinsic PLC β activity compared to CM cells carrying BRAF/NRAS mutations

(A) IP1 levels increased with higher cell numbers for UM cells but not for CM cells. The indicated numbers of UM (OMM1.3 and UPMD1) or CM cells (MUM2C and MM415) were incubated for 2 hours in the presence of lithium chloride (LiCl) to inhibit the degradation of newly generated IP1 followed by quantification of IP1.

(B) IP1 levels increased with times for UM cells but not for CM cells. 10,000 of UM (92-1, OMM1.3, UPMD1 and MP41) or CM cells (MUM2C and MM415) were incubated for indicated times.

Supplemental Fig. S4 Combined inhibition of PKC & MEK but not FAK & MEK or FAK & PKC synergistically reduces cell viability in UM cells

Left panel: Drug dose matrix data of OMM1.3 cell line (left panel). The numbers in the individual cells indicate the percentage of viability of cells treated for 96 h with the corresponding compound combination relative to vehicle control-treated cells. The data

were visualized over matrix using a color scale. Synergy was calculated using Bliss model with Combenefit software and indicates strong synergy for the LXS196 and Trametinib combination (middle and right panel).

Supplemental Fig. S5 Loewe model indicates strong synergy for the LXS196 and Trametinib combination but not LXS196/VS-4718 or VS-4718/Trametinib combination in both MP41 and OMM1.3 cells

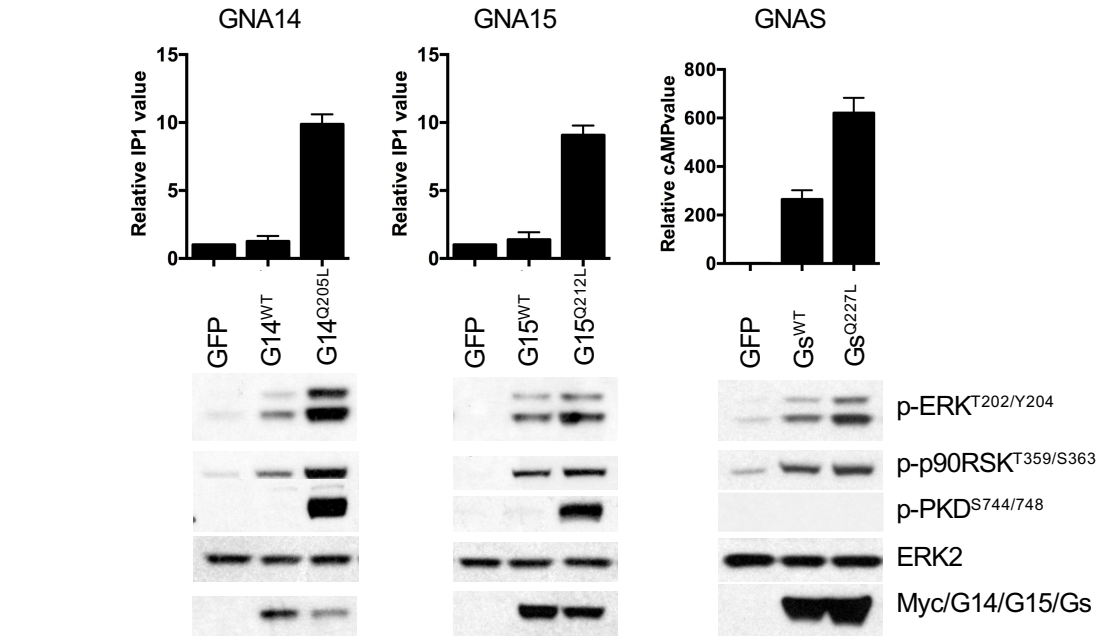
Supplemental Fig. S6: YM-254890 selectively suppresses Gαq signaling in UM, irrespective of the genetic background

(A) YM-254890 reduces IP1 production in melanoma cell lines with Gαq pathway mutations but not with other mutations. 10^4 Cells were treated with indicated concentration of YM for 2 hours and then subjected to IP1 measurement.

(B) In GNAQ or GNA11 mutant melanoma cell lines YM-254890 blocked both RasGRP3/MAPK and FAK signaling in a concentration-dependent manner. Cells were treated with increasing concentrations of YM-254890 for 24 h and probed. As noted, RasGRP3 expression was undetectable in CM cells with BRAF mutation.

Supplemental Fig. S1

A



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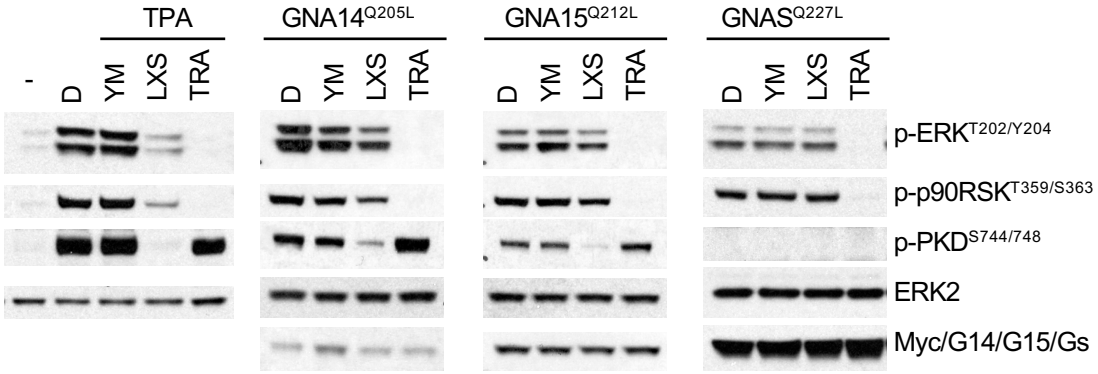

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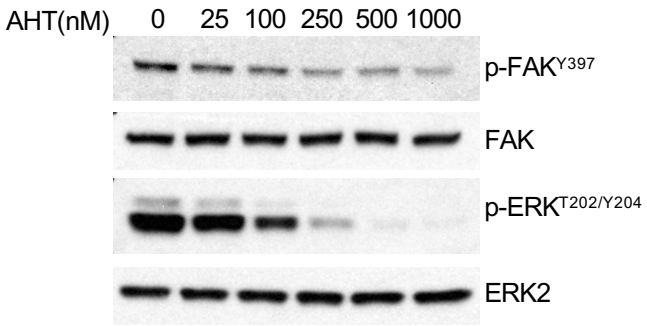
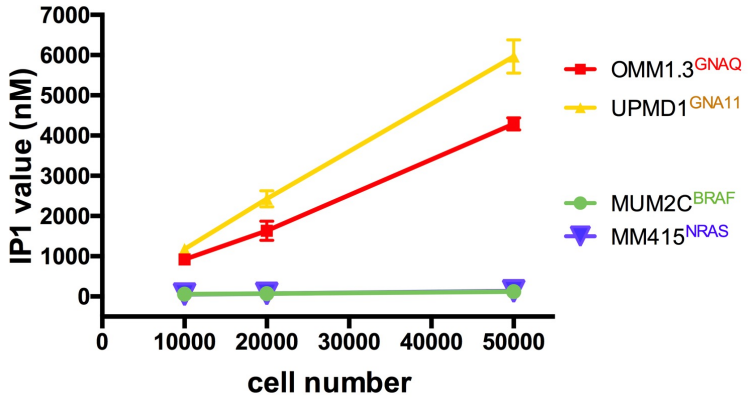


Supplemental Fig. S3

A



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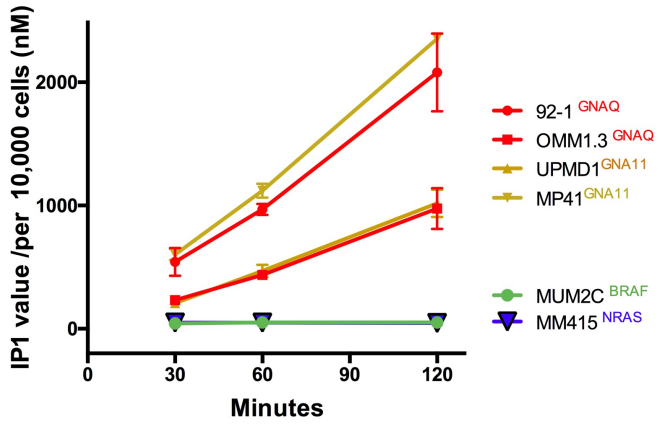

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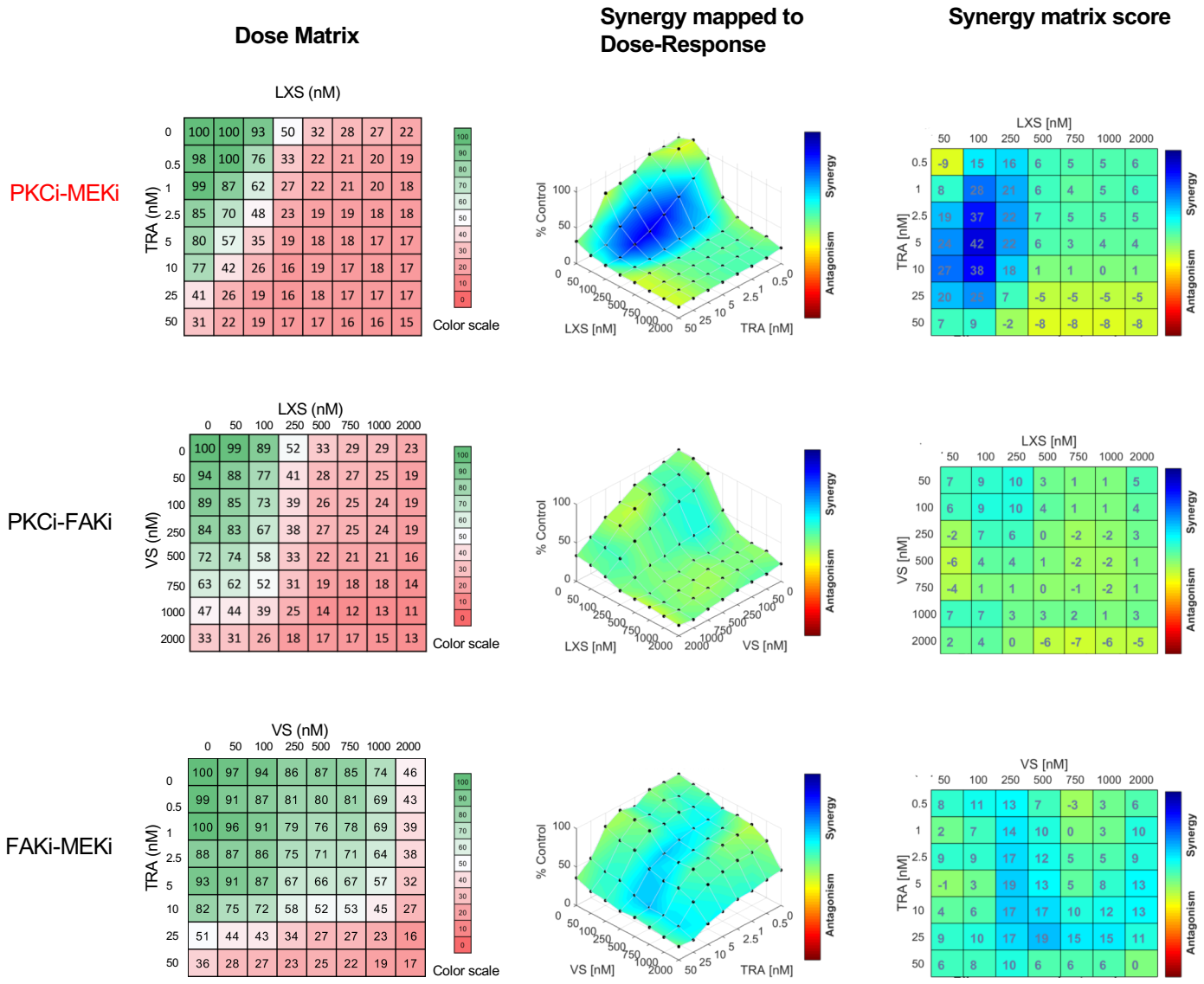

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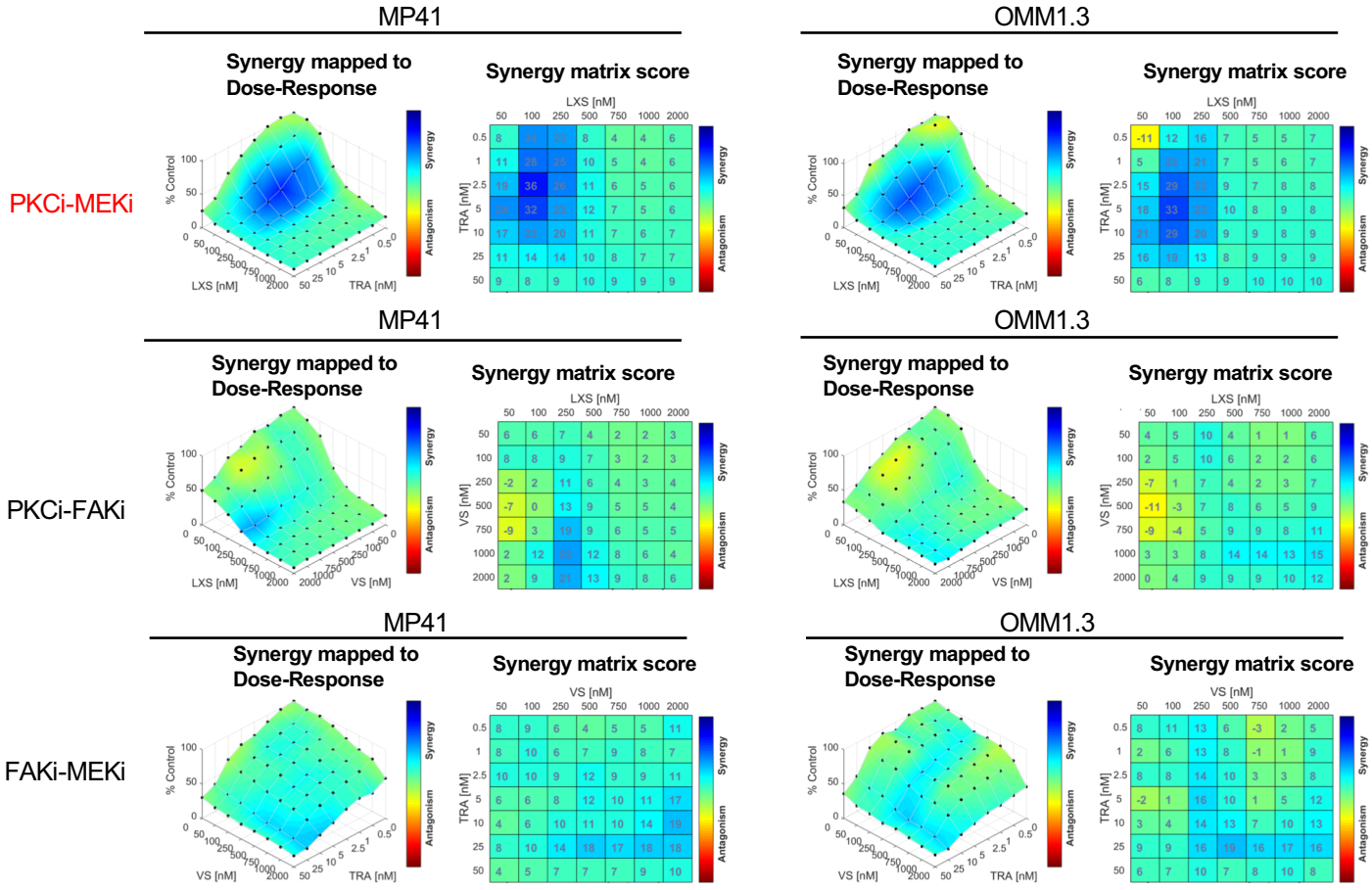

Supplemental Fig. S6

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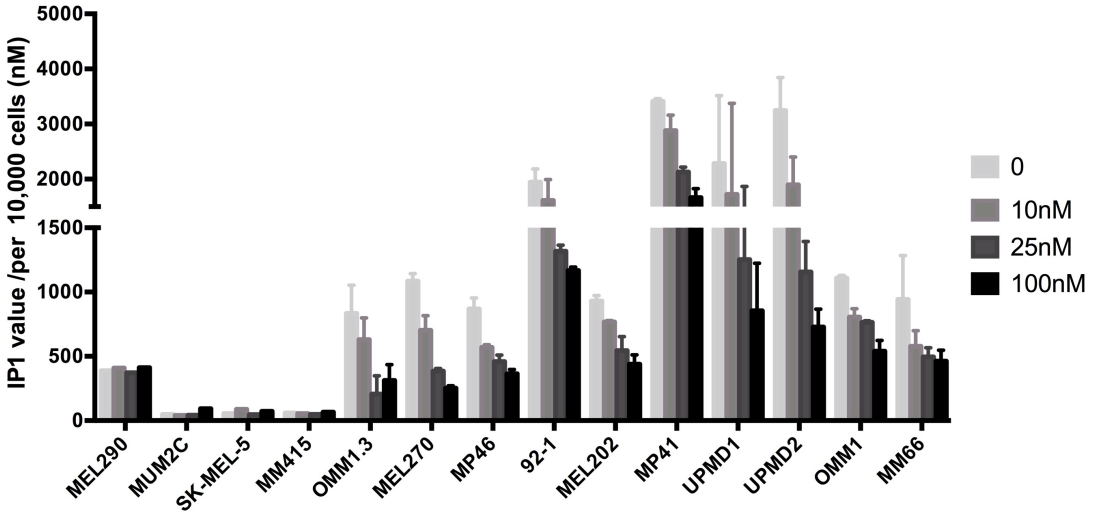

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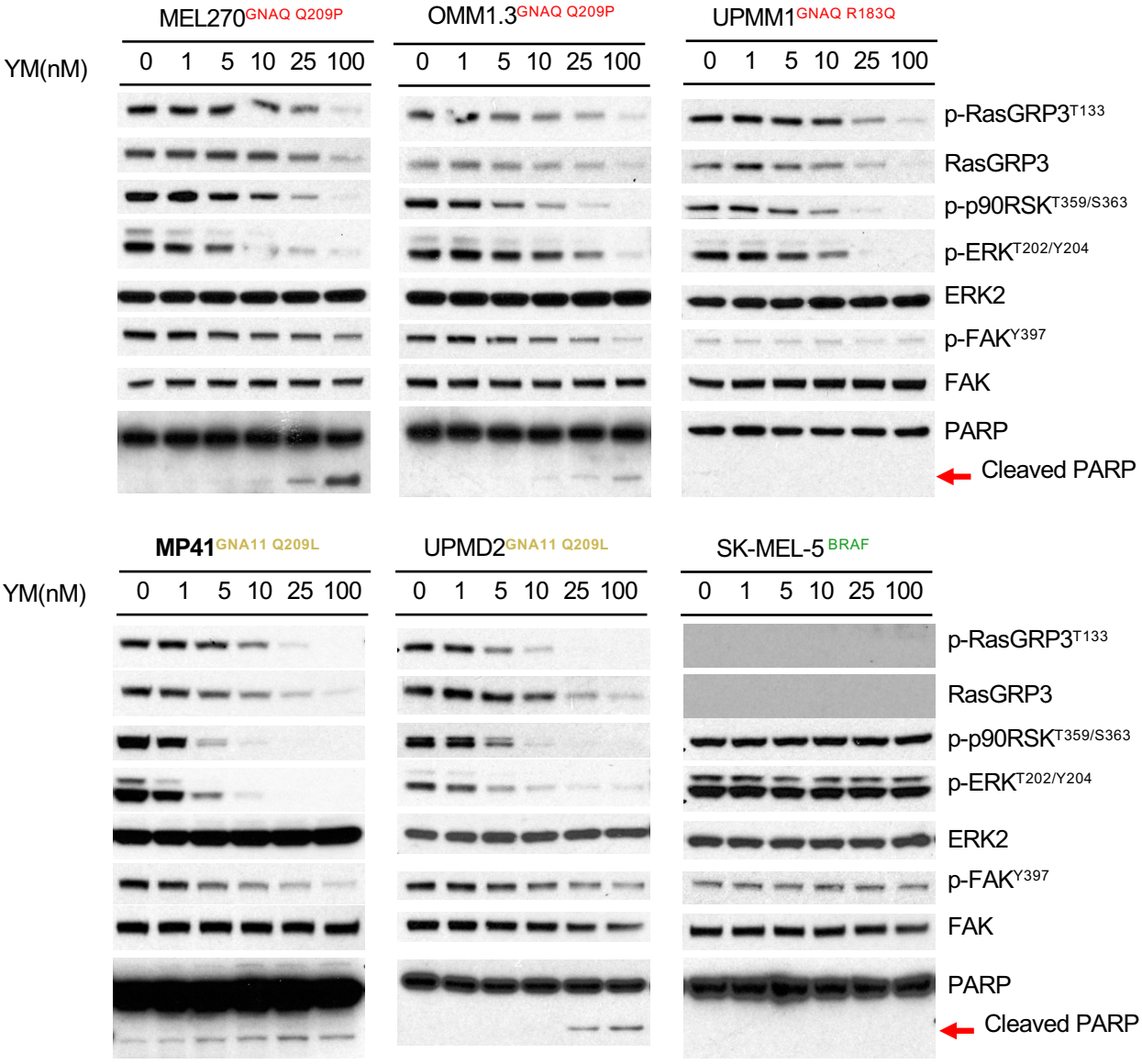

Supplemental Table 1 Genetic status of melanoma cell lines

Cell line	origin	Primary /Metstatic	GNAQ mutation	GNA11 mutation	SF3B1 mutaion	EIF1AX mutation	BAP1 expression	BRAF	NRAS
OMM1.3	UM	liver metastasis	Q209P						
MEL202	UM	primary	Q209L		R625G				
92-1	UM	primary	Q209L			G6D			
MEL270	UM	primary	Q209P						
MP46	UM	primary	Q209L				No		
UPMM1	UM	primary	R183Q						
MP41	UM	primary		Q209L					
UPMD1	UM	primary		Q209L					
UPMD2	UM	primary		Q209L					
MM66	UM	liver metastasis		Q209L					
OMM1	UM	subcutaneous metastasis		Q209L					
MEL290	?	primary							
MUM2C	CM	primary						V600E	
UACC257	CM	primary						V600E	
SK-MEL-5	CM	primary						V600E	
MM415	CM	lynph node							Q61L
MM485	CM	lynph node							Q61R