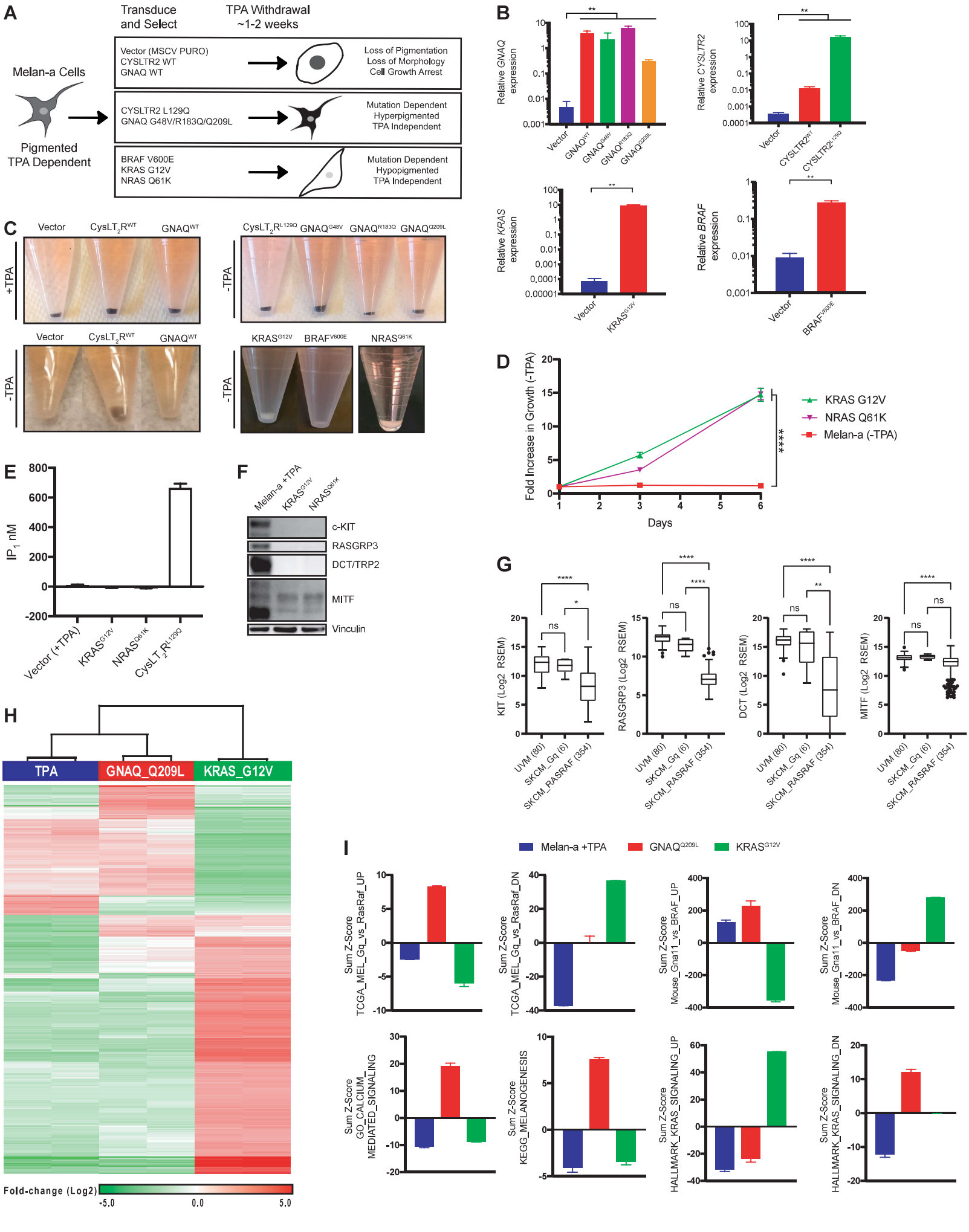
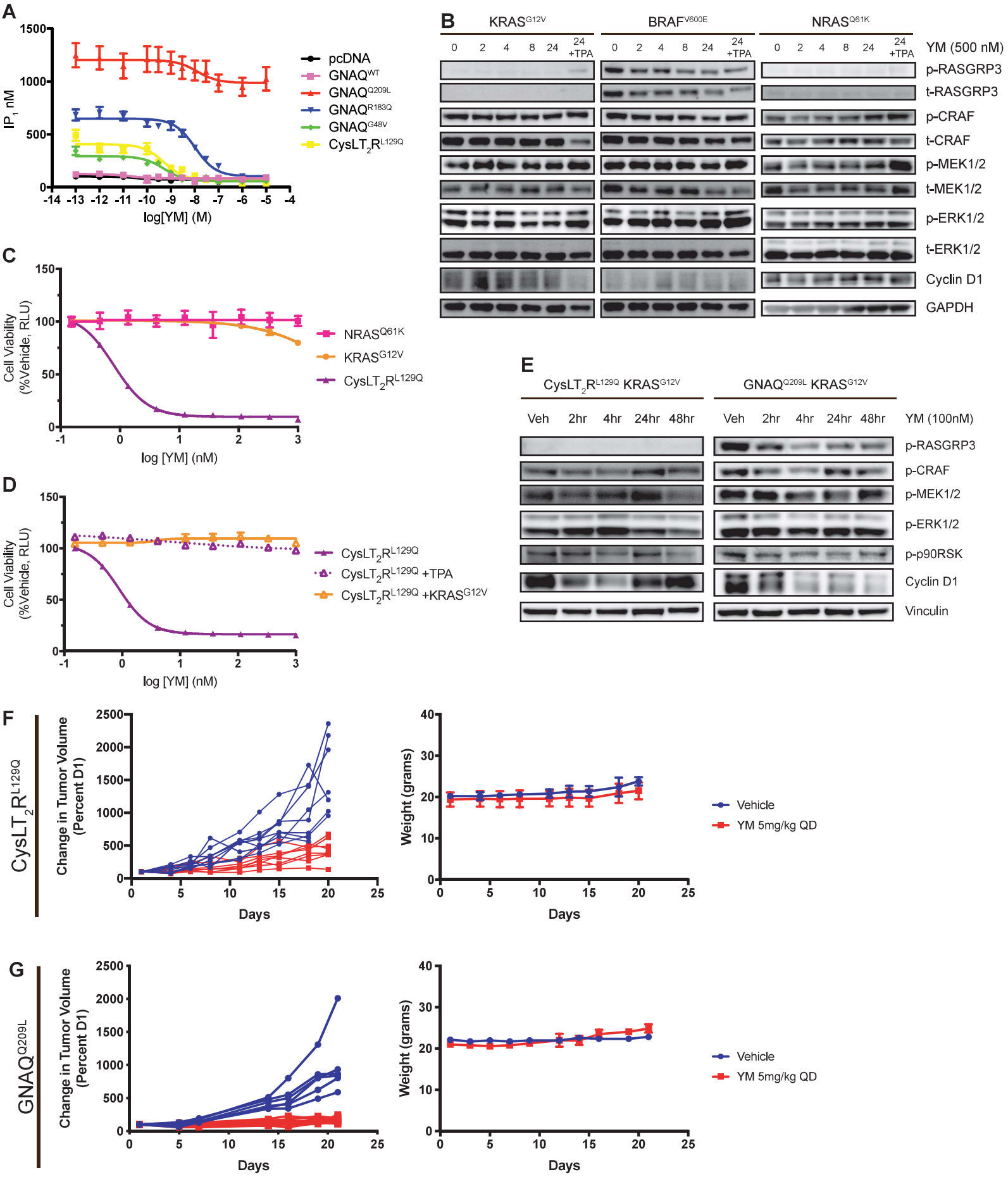
Supplementary Figure 1

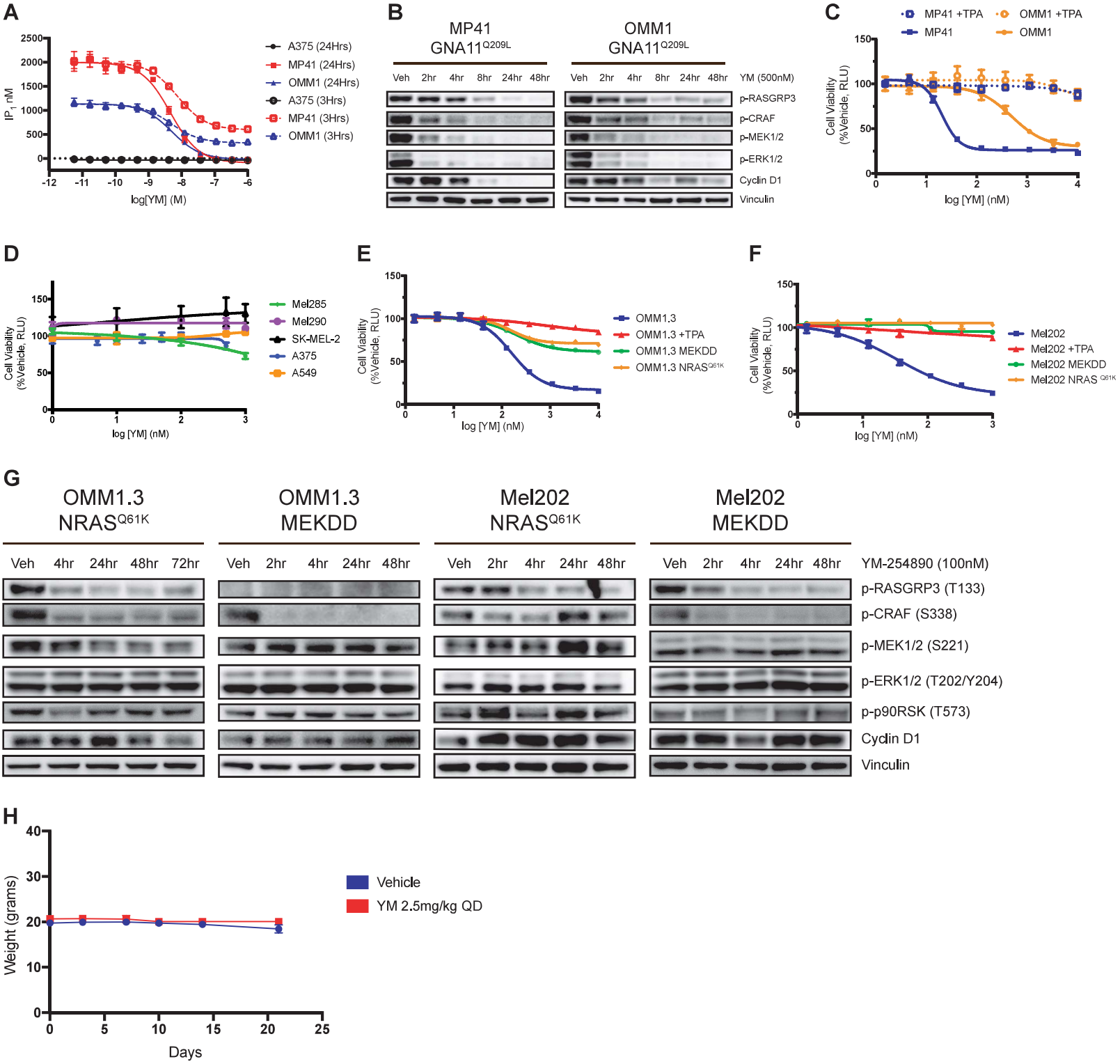


Supplementary Figure 1. **(A)** Workflow diagram of generating mutation dependent melan-a cells. Cells were transduced with vector control (MSCV PURO), human cDNAs with WT or activating mutations found in UVM patients (CYSLTR2 and GNAQ), or downstream MAPK activating mutations ($KRAS^{G12V}$, $BRAF^{V600E}$, or $NRAS^{Q61K}$). TPA was then withdrawn from the media to drive mutation dependence. **(B)** Expression of *GNAQ*, *CYSLTR2*, *KRAS*, and *BRAF* in melan-a cells transduced with cDNAs. All samples were compared to expression of RPL27 (mean ± SD, n = 3). **(C)** Cells pellets in melan-a cells after TPA withdrawal for 2 weeks to show pigmentation. **(D)** Growth assay for $NRAS^{Q61K}$ melan-a cells compared to parental and $KRAS^{G12V}$ melan-a cells without TPA for six days. Growth was assayed by Celltiter-Glo 2.0 at D1, D3, and D6. Fold increase in luminescence is shown relative to D1 cell number (mean ± SEM, n = 3). **(E)** Basal level of IP_1 accumulation in $NRAS^{Q61K}$ melan-a cells compared to other melan-a cells. **(F)** Western blot of melanocyte lineage markers (MITF, TRP2/DCT, c-KIT, and RASGRP3) upon TPA withdrawal for 2 weeks in $NRAS^{Q61K}$ melan-a cells. **(G)** Tukey box plot of expression of KIT, RASGRP3, DCT, and MITF from TCGA datasets in UVM and $G\alpha_q$-mutated SKCM compared to RAS/RAF mutated SKCM. **(H)** Unsupervised hierarchical clustering of RNA-seq data from melan-a cells (+TPA), $GNAQ^{Q209L}$, and $KRAS^{G12V}$ melan-a cells (-TPA) (duplicates shown). **(I)** Sum Z-scores of 8 gene sets highlighting the differences between groups from (H) (n = 2). Custom genes sets (first row) include genes up or downregulated in UVM and $G\alpha_q$-mutated SKCM versus RAS/RAF mutant SKCM from TCGA (TCGA_MEL_Gq_vs_RasRaf) as well as genes up or downregulated in our $GNA11^{Q209L}$ $Bap1^{-/-}$ versus $BRAF^{V600E}$ $Bap1^{-/-}$ GEMM (Mouse_Gna11_vs_Braf). For all cases $P > .05$; ns, $P < 0.05$; *, $P < 0.005$; **, $P < 0.0005$; ***, $P < 0.0001$, ****.


A
IP1 nM
log[YM] (M)
pcDNA
GNAQWT
GNAQQ209L
GNAQR183Q
GNAQG48V
CysLT2RL129Q
B
KRASG12V
BRAFV600E
NRASQ61K
0 2 4 8 24 24 +TPA
YM (500 nM)
p-RASGRP3
t-RASGRP3
p-CRAF
t-CRAF
p-MEK1/2
t-MEK1/2
p-ERK1/2
t-ERK1/2
Cyclin D1
GAPDH
C
Cell Viability (%Vehicle, RLU)
log [YM] (nM)
NRASQ61K
KRASG12V
CysLT2RL129Q
D
Cell Viability (%Vehicle, RLU)
log [YM] (nM)
CysLT2RL129Q
CysLT2RL129Q +TPA
CysLT2RL129Q +KRASG12V
E
CysLT2RL129Q KRASG12V
GNAQQ209L KRASG12V
Veh 2hr 4hr 24hr 48hr
YM (100nM)
p-RASGRP3
p-CRAF
p-MEK1/2
p-ERK1/2
p-p90RSK
Cyclin D1
Vinculin
F
CysLT2RL129Q
Change in Tumor Volume (Percent D1)
Days
Weight (grams)
Days
Vehicle
YM 5mg/kg QD
G
GNAQQ209L
Change in Tumor Volume (Percent D1)
Days
Weight (grams)
Days
Vehicle
YM 5mg/kg QD

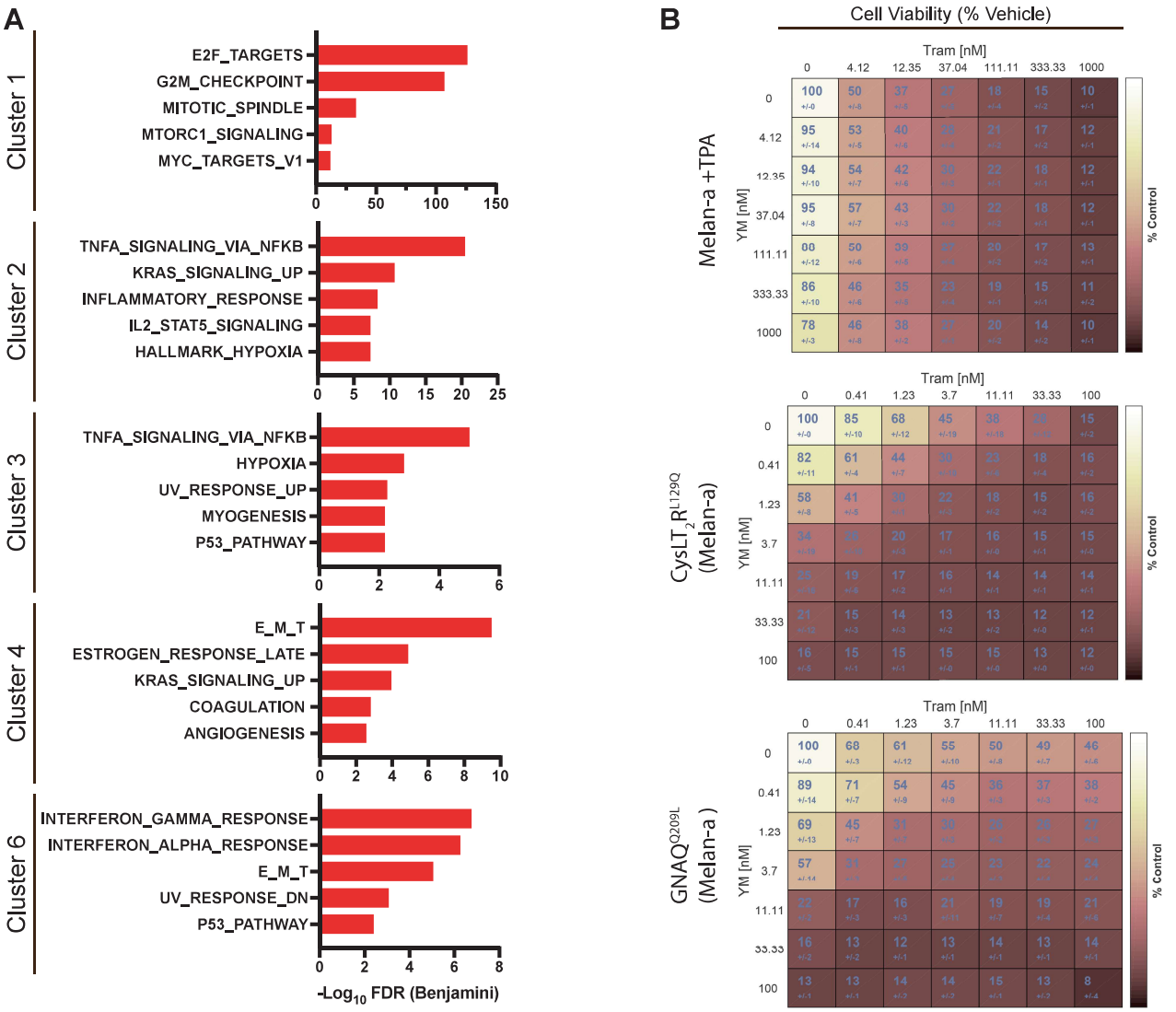
Supplementary Figure 2. **(A)** IP_1 accumulation in HEK-293T cells transfected with constructs at 11ng/well. Cells were treated with YM at increasing dose for 24 hours. Data are expressed as IP_1 concentration (nM) (mean ± SEM, n = 3). **(B)** Western blot analysis of $KRAS^{G12V}$, $BRAF^{V600E}$, and $NRAS^{Q61K}$ melan-a cells. Samples were treated with 500nM YM for 0, 2, 4, 8, and 24 hours with the last lane being treatment plus TPA add back. **(C)** Viability curves for $NRAS^{Q61K}$ melan-a cells treated with YM for 5 days assayed by Celltiter-glo 2.0 (mean ± SEM, n = 3). **(D)** Viability curves for $CysLT_2R^{L129Q}$ melan-a cells supplemented with TPA or expressing oncogenic $KRAS^{G12V}$ treated with YM for 5 days assayed by Celltiter-glo 2.0. Data are expressed as the percentage RLU relative to that observed with vehicle (mean ± SEM, n = 3). **(E)** Western blot analysis of $CysLT_2R^{L129Q}$ and $GNAQ^{Q209L}$ melan-a cells expressing oncogenic $KRAS^{G12V}$ treated with 100nM YM for 0, 2, 4, 24, and 48 hours. **(F)** Percent tumor volume of $CysLT_2R^{L129Q}$ and **(G)** $GNAQ^{Q209L}$ melan-a allografts as individual flanks treated with vehicle or YM for 20 or 21 days, respectively. Mouse weight throughout the course of experiment is plotted (right) (mean ± SEM, n = 3-5).

Supplementary Figure 3



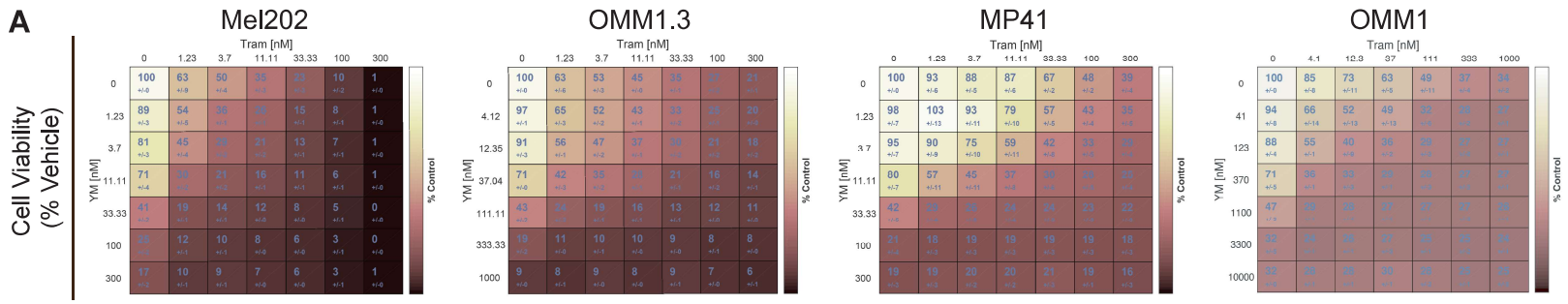
Supplementary Figure 3. (A) IP_1 accumulation assay in MP41 and OMM1 cells (*GNA11*^{Q209L}) compared to A375 cells. Cells were treated with increasing concentrations of YM for 3 hours (dotted lines) and 24 hours (solid lines). Data are expressed as IP_1 concentration (mean ± SEM, n = 3). **(B)** Western blot analysis of indicated proteins in MP41 and OMM1 cells treated with 500nM YM at 0, 2, 4, 24, and 48 hours. **(C)** Viability Curves for MP41 and OMM1 cells treated with YM in the presence or absence of TPA for 5 days. **(D)** Viability curves for non-Gq mutant UVM cell lines Mel285 and Mel290, cutaneous melanomas A375 (BRAF^{V600E} from Figure 4C for reference) and SK-MEL-2 (NRAS^{Q61R}), and lung adenocarcinoma cells A549 (KRAS^{G12S}) treated with YM for 5 days. **(E)** Viability curves for OMM1.3 and **(F)** Mel202 cells treated with YM for 5 days in the presence or absence of TPA or expressing indicated MAPK activating mutations. All viability curves were assayed by Celltiter-glo 2.0 and expressed as the percentage RLU relative to that observed with vehicle (mean ± SEM, n = 3). **(G)** Western blot analysis of indicated proteins in OMM1.3 or Mel202 cells expressing NRAS^{Q61K} or MEKDD treated with 100nM YM at 0, 2, 4, 24, and 48 hours. **(H)** Weight for OMM1.3 xenografted mice from Fig. 3D (mean ± SEM, n = 5).

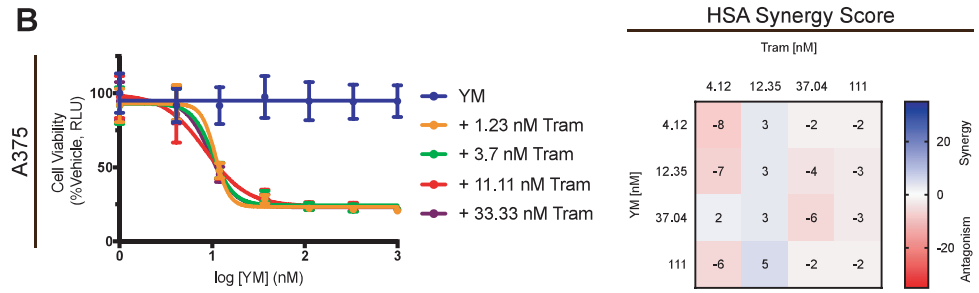
Supplementary Figure 4

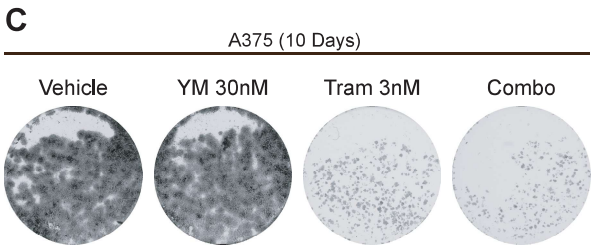


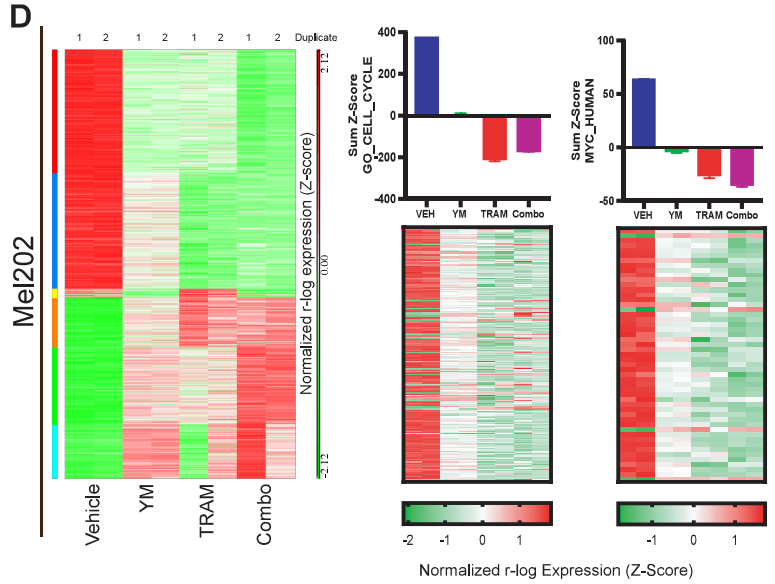
Supplementary Figure 4. (A) Top five HALLMARK gene set enrichments for each cluster of Fig. 4A, cluster 5 yielded no significantly enriched gene sets. Epithelial_Mesenchymal_Transition is abbreviated with E_M_T. **(B)** Cell viability diagrams for melan-a cell synergy analysis (mean ± SEM, n = 3).

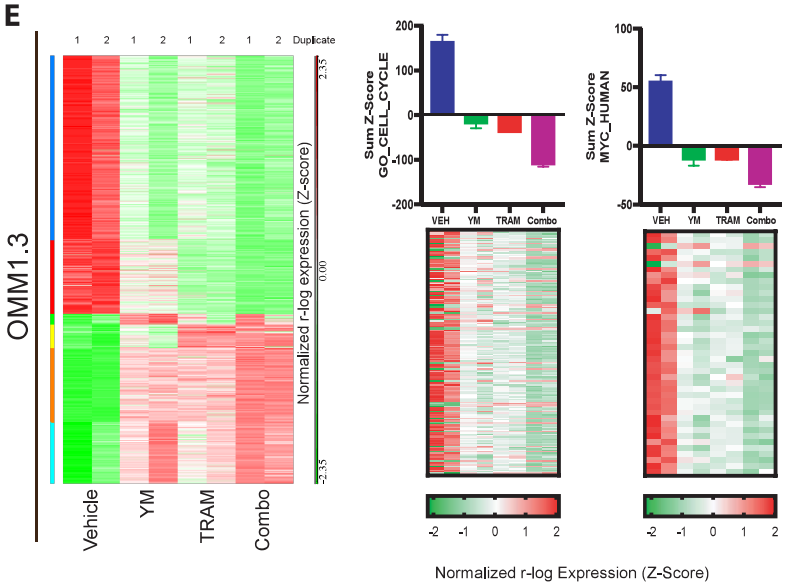
Supplementary Figure 5

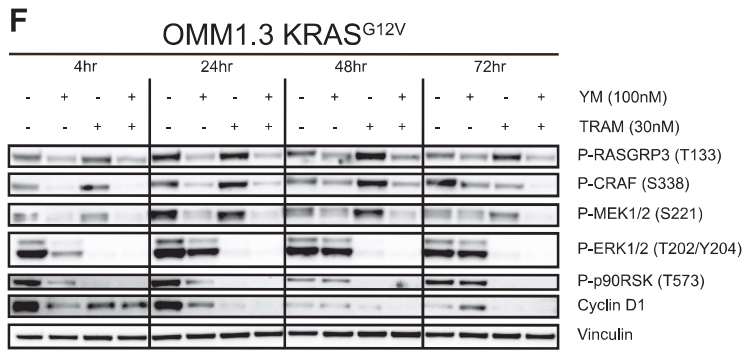






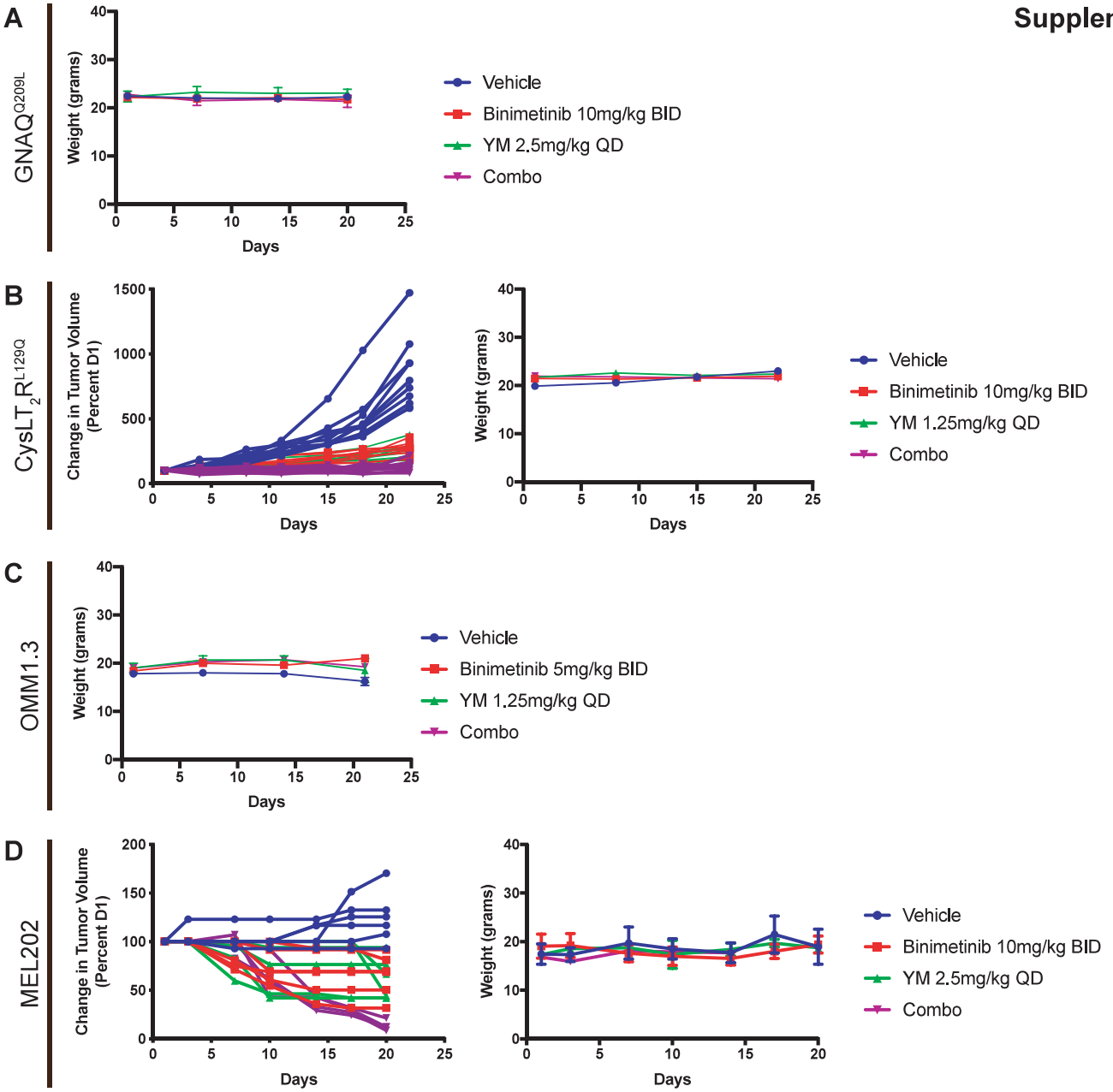


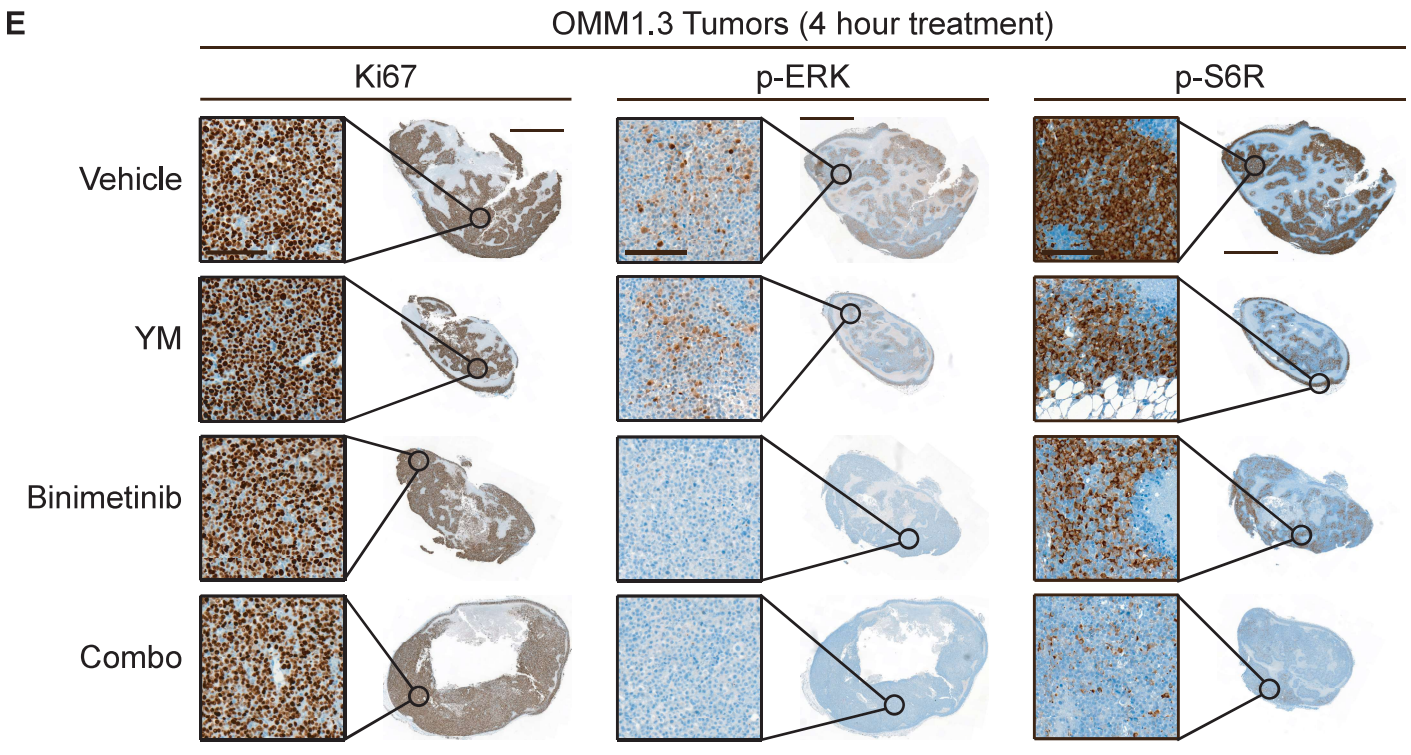




Supplementary Figure 5. (A) Cell viability diagrams for human UVM cell synergy analysis (mean ± SEM, n = 3). **(B)** Viability curves for A375 cells treated with increasing doses of YM in combination with four doses of trametinib (left) and corresponding HSA synergy diagrams (right). (mean ± SEM, n = 3). **(C)** Growth assays for A375 cells in the presence of vehicle, YM, trametinib, or Combo. **(D)** Mel202 cells treated with vehicle, YM (30nM), trametinib (10nM), or Combo for 24 hours, and **(E)** OMM1.3 cells treated with vehicle, YM (100nM), trametinib (30nM), or Combo for 24 hours (left) (duplicates shown). Data was r-log transformed, Z-scored, and then k-means clustered. Sum Z scores (top) shown for cell cycle (middle) and MYC (right) with heatmaps (bottom) showing expression of individual signature genes (mean ± SEM, n = 2). **(F)** Western blot analysis of indicated proteins for OMM1.3 cells expressing $KRAS^{G12V}$ (~50% population expression). Cells were treated with vehicle, YM, trametinib, or Combo for 4, 24, 48, and 72 hours.

Supplementary Figure 6





Supplementary Figure 6. (A) Weight of mice throughout experiment from Fig. 6A (mean ± SEM, n = 3-5). **(B)** Percent tumor volume of $CysLT_2R^{L129Q}$ melan-a allografts as individual flanks treated with vehicle, binimetinib, YM, or Combo for 22 days. Mouse weight throughout the course of experiment is plotted (right) (mean ± SEM, n = 5). **(C)** Weight for OMM1.3 xenografted mice from Fig. 6C (mean ± SEM, n = 2-5). **(D)** Percent tumor volume of Mel202 xenografts as individual flanks treated with vehicle, binimetinib, YM, or Combo for 20 days. Mouse weight throughout the course of experiment is plotted (right) (mean ± SEM, n = 2-3). **(E)** Immunohistochemistry of OMM1.3 tumors from Fig. 6D for Ki67, p-ERK, and p-S6R. Scale bar, 50μm for insert (left) and 2mm for whole tumor (right).