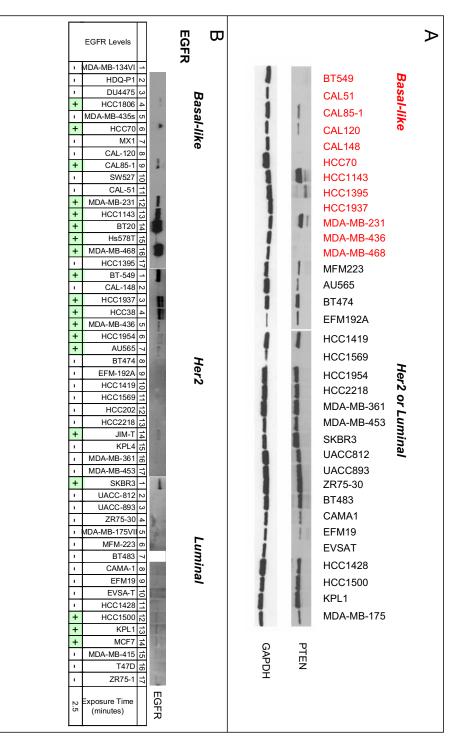
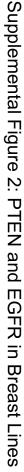
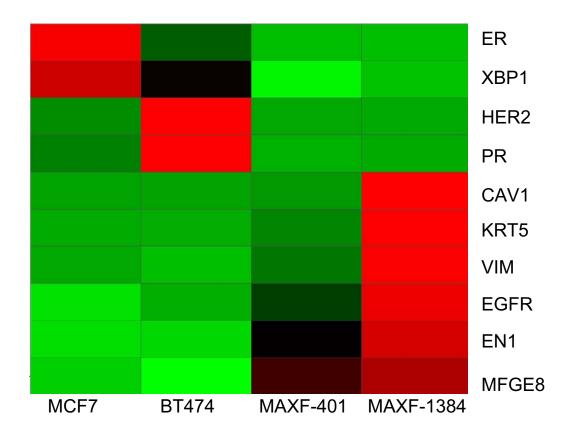


Supplemental Figure 1: In vitro viability data

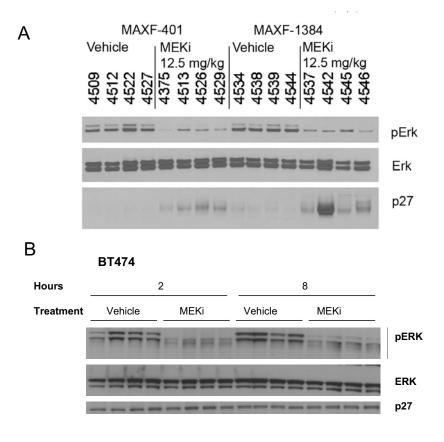




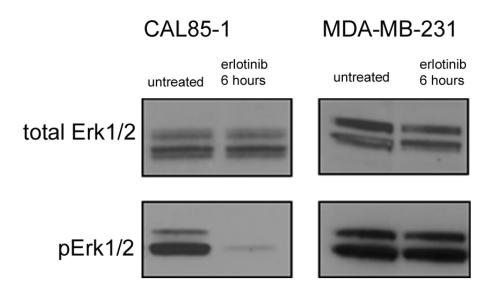
Supplemental figure 3: Molecular Classification of Breast Tumor Models

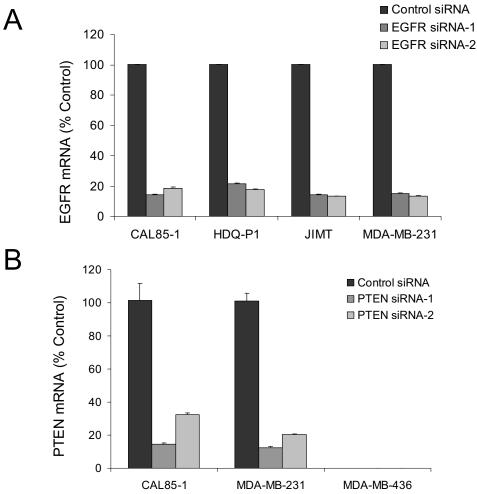


Supplemental figure 4: Pharmacodynamic response to MEK Inhibition in Xenograft Tumors



Supplemental Figure 5: $1\mu M$ erlotinib treatment downregulates pErk in CAL85-1 but not MDA-MB-231 KRAS mutant cells



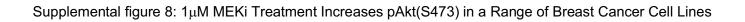


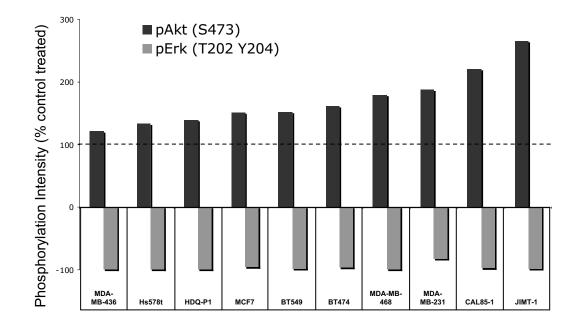
Supplemental figure 6: Quantitation of EGFR and PTEN siRNA Knockdown

Supplemental figure 7: PTEN siRNA Reduces G1 Arrest in Response to MEK Inhibition

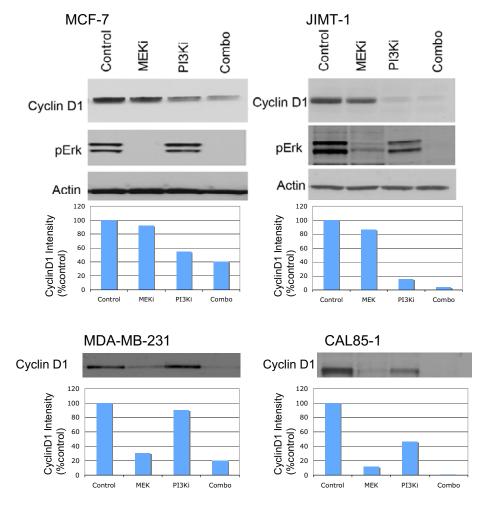
MDA-MB-231

ΜΕΚί: 0 μΜ MEKi: 0 μM MEKi: 1 μM MEKi: 1 μM Control siRNA PTEN siRNA-2 Control siRNA PTEN siRNA-2 1500 2500 1500 G1: 40.0% 2000 G1: 39.9% G1: 73.9 % G1: 56.9 % S 10.2% S: 9.8% S: 5.5% S: 3.6% 2000 1500 1000 1500 · 생종 장 북 G2/M: 34.6% 1000 G2/M: 37.4% G2/M: 15.3% G2/M: 23.8% # Celk # Cells 왕 이 1000 북 37.4 23.8 1000 8.11 9.66 500 7.86 500 I۲ 39.9 73.9 56. 500 500 ٥ 0 0-0 -0 200 800 1000 0 200 400 600 FL2-H: FL2-H 800 1000 400 600 FL2-H: FL2-H 200 1000 800 1000 800 D 200 400 600 FL2-H: FL2-H ۵ 400 600 FL2-H: FL2-H

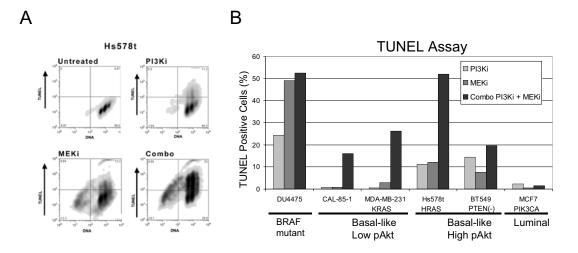




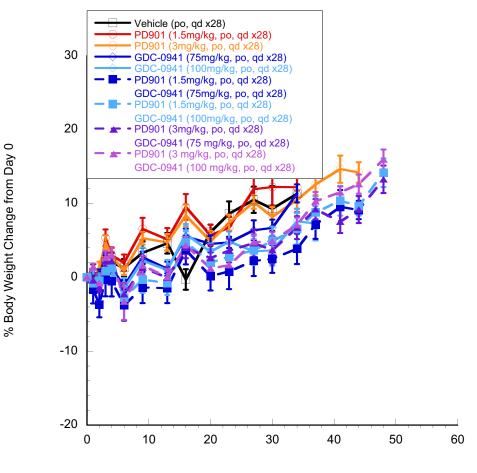
Supplemental figure 9: Cyclin D1 downregulation in response to MEKi, PI3Ki or combination treatment in cell lines that show in vitro synergy (MDA-MB-231 and CAL85-1 data from Figure 5A) or lack of synergy (MCF-7 and JIMT-1). Graphs show quantitation of immunoblot bands using NIH Image J software.



Supplemental figure 10: FACS assay for TUNEL apoptosis marker shows synergistic increases in apoptotic response in basal-like non-BRAF mutant cell lines but not a luminal cell line. Panel A shows raw FACS data from Hs578t cells and panel B quantitation of data for multiple cell lines. Molecular subtypes and key genetic alterations are indicated under the graph.



Supplemental figure 11: A MEK and PI3 kinase inhibitor combination regimen is well tolerated in mice harboring MX-1 basal-like PTEN null tumors



Time (Days)

Hoeflich et al Supplemental Materials and Methods:

Cell lines

Breast cancer cell lines AU565, BT-20, BT-474, BT-549, BT-483, CAMA-1, DU4475, HCC1143, HCC1395, HCC1419, HCC1428, HCC1500, HCC1569, HCC1937, HCC1954, HCC2218, HCC38, HCC70, Hs578T, KPL-1, KPL-4, MCF-7, MDA-MB-134-VI, MDA-MB-175-VII, MDA-MB-231, MDA-MB-361, MDA-MB-415, MDA-MB-436, MDA-MB-453, MDA-MB-468, SK-BR-3, SW-527, T-47D, UACC-812, UACC-893, ZR-75-1 and ZR-75-30 were obtained from American Type Culture Collection (ATCC, Manassas, VA). The cell lines CAL-120, CAL-148, CAL-51, CAL-85-1, EFM-19, HDQ-P1, HCC1806, HCC202, EFM-192A, EVSA-T, JIMT-1, and MFM-223 were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany). MX-1 was obtained from the Piedmont Research Center (Wilmington, NC), and BT474M1 is a subclone of BT474 that was obtained from California Pacific Medical Center. All cell lines were maintained in RPMI 1640 or DMEM supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO), non-essential amino acids and 2 mmol/L L-glutamine. The integrity of the cell line panel and confirmation of unique identity all cell lines is of great importance to the conclusions of the study, so we have taken several steps to ensure this integrity. First, all cell lines were newly acquired from the above vendors and characterized and archived at low passage number. As described previously we have profiled the cell lines on Affymetrix 100K mapping SNP arrays (17). SNPs for which no genotype was called in any cell line were excluded. The percentage agreement in genotype at all other SNPs was calculated for each pair of cell lines. The percent agreement values fell clearly into two groups, one around 70% and

one around 99%. Two pairs of cell lines (KPL-1 and MCF-7, AU565 and SKBR3) had percent agreement values above 95% and thus were considered to be of common origin. Otherwise the cell lines used in this study were genetically distinct based on this analysis. In addition, our independent gene expression analyses and classification into molecular subtype for all of the cell lines in the study were compared with the classification described by Neve et al (26) and we found near perfect agreement between the cell lines that overlap between the studies, thus supporting the molecular subtype classification we describe. The one exception is the HCC1500 cell line, which is luminal in our analyses of cells derived from two independent vials ordered from ATCC but is described by Neve et al as basal-like.

High Content Assays for Cell Proliferation

Cells were plated at 5-10K per well (depending on cell line growth properties) in PackardView 96 well plates and allowed to adhere overnight incubating at 37°C. The following day the cells were treated with the MEK inhibitor and allowed to incubate for 72 hours at 37°C. BrdU labeling reagent (Cat.# B9285, Sigma) was then added to the cells at a final concentration of 200nM for an additional 5 hours, and then plates were fixed and processed according using the manufacturer's standard protocol. Cells were counterstained with Hoechst-33258 to allow identification of nuclei and the percentage of cells positive for BrdU immunofluorescence was then quantitated for at least 1000 cells per well using Cellomics Target Activation software (www.cellomics.com).

Chou and Talalay combination index experiments

For in vitro combination studies CAL85-1, Hs578t, MCF7 and JIMT-1 cells were plated out in 384 well format and compounds were added in a fixed dose ratio ranging from 1/16 to 8X the EC₅₀ of each drug, both alone and in combination. For the MEK inhibitor the range of concentrations spanned 0.04uM to 20µM and for the PI3 Kinase inhibitor the range of concentrations spanned 0.025µM to 13µM.

Protein analyses

For in vitro pharmacodynamic studies, BT474, HDQ-P1, CAL85-1 and MDA-MB-231 cells were plated in 12 well plates and allowed to grow for until cells reached 60-80% confluence. Cells were dosed with 0, 0.1, or 1.0 μ M MEK inhibitor and duplicate plates were made for each timepoint. Cells were incubated in the compound for 6 or 24 hours, then washed with cold PBS and processed for Western blotting using standard protocols, as described in the Supplemental Experimental Methods. CAL85-1 cells were cultured in media containing 10 ng/ml EGF to assess pAKT levels since basal levels of pAKT are undetectable in this cell line when cultured in 10% FBS. For analysis of protein expression in tumor xenografts, lysate from tumor samples was collected by adding DNase Lysis Buffer to frozen tissue and pulverized using the TissueLyser (Qiagen, Valencia, CA) as described by the manufacturer.

Primary blotting antibodies used were p27(C-19) (Santa Cruz Biotechnology cat # sc-528), Cyclin D1(DCS-6) (Santa Cruz Biotechnology cat# SC-20044), ERK #9102, pERK (Thr202/Tyr204) (Cell Signaling Technology, cat #9101), Total AKT and pAKT (S473) (Cell Signaling Technology, cat #9272 and cat #9271). Secondary blotting antibodies used were polyclonal Goat anti-mouse IgG HRP and Polyclonal Goat anti-rabbit IgG HRP (both from Dako, Glostrup, Denmark Cat.# P0161 and P0448, or Cell Signaling Technology Cat. #7076 and #7074).

Quantitative analysis of protein expression using reverse phase protein arrays was performed at Theranostics Health (Rockville, Md) as described previously (Boyd et al., 2008) and in the Supplemental Experimental Procedures.

siRNA Experiments

Transfection efficiency of siRNA was evaluated by qRT-PCR. Optimal siRNA duplex and lipid concentrations were determined for each cell-line. For the adherent cell lines CAL85-1, HDQ-P1, MDA-MB-231 or MDA-MB-436, cells were plated at 6000 cells per well in a 96 well plate with 0.125uL of Lipofectamine RNAiMAX (Cat.#13778-150 Invitrogen, Carlsbad, CA) and 50nM of siRNA per well. Cells were incubated for 3 days in siRNA then the MEK inhibitor was added for 24 hours, followed by addition of CellTiter Glo or processing for Cell Cycle Profiling. For quantitation of siRNA knockdown by PTEN or EGFR duplexes, RNA was collected and isolated using the QIAGEN TurboCapture mRNA Kit (Cat.#72251). cDNA was made using the High Capacity cDNA Revese Transcription Kit (Cat.#4368813) from ABI and qPCR was done using assays on demand from ABI. EGFR(cat.#Hs00193306 m1) and PTEN (cat.#Hs02621230 s1) primer/probe sets were normalized to the average of the housekeeping genes PPIA (cat.#Hs99999904 ml) and UBC (cat.#Hs00824723 m1) and then again to the corresponding NTC siRNA for

each cell line using the $\Delta\Delta$ CT method.

Gene Expression Microarray Analyses

For supervised analysis of breast cancer cell lines, gene expression data were filtered to remove probe sets that showed little variation across the cell lines. Briefly, probes that did not show at least a five fold variation across the samples (max/min>10) and an absolute intensity difference of at least 250 (max-min >250) were excluded from hierarchical clustering analysis. Cell lines were binned into sensitive and resistant classes based on an EC₅₀ cutoff of 1 μ M and the Cyber-T algorithm was implemented to identify genes differentially expressed between the classes. Data preprocessing prior to clustering analysis involved log transforming and median centering gene expression values, after which average linkage clustering was carried out using Spotfire software (www.spotfire.com).

Identification of Activated RAS and MEK signatures

Stocks of recombinant adenoviruses expressing GFP, HRAS (G12V), and MEK1 (S217E S221E) transgenes (henceforth referred to as gain of function constructs, gf) as well as null control vectors, were purchased and propagated in HEK 293 cells according to vendor supplied protocol (Cell BioLabs, San Diego, CA). Viral vectors were isolated and functionally titered using assay kits (Cell BioLabs, San Diego, CA). Optimal multiplicity of infection (MOI) was determined for MCF10A cells by GFP transfection. Cells were then optimally transfected with MEK1, HRAS, and null control vectors. Cells were lysed 24 hours post-transfection with collection of total RNA and protein via a kit (Qiagen, Valencia, CA). Expression upon transfection for MEK1(gf) and HRas(gf) was confirmed by western blot

(data not shown). Isolated RNA was reversed transcribed to cDNA and then run on Human Genome U133P 2.0 Array chips (Affymetrix, Santa Clara, CA). At least five independent replicates were profiled for each expression construct. Differentially expressed genes between cells infected with control vector and MEK(gf) or RAS(gf) vectors were identified by via the Cyber-T algorithm (Baldi and Long, 2001). We developed pathway activation signatures using a variation of the strategy employed by Bild et al (Bild et al., 2006). The positive training data comprised five HRAS and six MEK1 samples, and the negative training data comprised eighteen control samples. The use of test set data when defining metagenes has been shown to improve predictor performance, but has been controversial (Coombes et al., 2007; Potti and Nevins, 2007). We devised an alternative approach to ensure the use of metagenes that would generalize beyond the training set without using test set data. This approach used a large corpus of unrelated microarray data which reflects real and diverse patterns of gene expression. Specifically, microarray data for 9,833 normal tissue samples with HGU133 Plus 2.0 expression data were collected from Gene Logic, Genentech and the Gene Expression Omnibus (GEO) and expression values for each probe were centered about their median. The singular value decomposition of this collection of normal tissue expression data was calculated and the resulting eigenarray matrix was used as a basis for transformation of the training data. The transformed features of the training data were than ordered according to their difference across training set classes by the Rank Product procedure (Breitling et al., 2004). L2 penalized logistic regression models were trained using iteratively re-weighted ridge regression (Park and Hastie, 2008) on the reduced singular value decomposition (West, 2003) of the top N features. The top N features which minimized cross validation error were used to train

the final model. Predicted pathway activation levels derived from the model for each cell line are shown in Supplemental table 1.

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Supplementa	напис	1. 100	100 20105		SCHSILIVE	vo reolotant	CCH IIIICS

Suppleme	ental tac		J genes in	sensitive vs resistant cell lines
ProbeID	UNQ	UNQ_Short_Name	HUGO Symbol	SRCNAME
227919_at	UNQ28863	YHRL28863	NA	
205428_s_at	UNQ9956	CALB2	CALB2 CD274	calbindin 2, 29kDa (calretinin)
227458_at	UNQ6713 UNQ16971	PDL1/B7-H1 OTUD1	OTUD1	CD274 molecule
226140_s_at 201042_at	UNQ7380	TGM2	TGM2	OTU domain containing 1 transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)
235911_at	NA	NA	NA	NA
205032_at	UNQ1682	ITGA2	ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
226757_at	UNQ21425	IFIT2	IFIT2	interferon-induced protein with tetratricopeptide repeats 2
223961_s_at	UNQ5695	CISH	CISH	cytokine inducible SH2-containing protein
227475_at	UNQ24348	FOXQ1	FOXQ1	forkhead box Q1
215543_s_at	UNQ10018	LARGE	LARGE	like-glycosyltransferase
204363_at	UNQ11	TF	F3	coagulation factor III (thromboplastin, tissue factor)
209343_at	UNQ20640	EFHD1	EFHD1	EF-hand domain family, member D1
208250_s_at	UNQ5963	DMBT1	NA	NA
214434_at	UNQ14471	HSPA12A	HSPA12A	heat shock 70kDa protein 12A
203638_s_at	UNQ942	FGFR2 TRIO	FGFR2	fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrou triate (unational domain (DTDPE intercention)
209011_at	UNQ1870 UNQ3146	PLCB4	TRIO PLCB4	triple functional domain (PTPRF interacting) phospholipase C, beta 4
203896_s_at 209270_at	UNQ1262	LAMB3	LAMB3	prospholipase C, beta 4 Iaminin, beta 3
227314_at	UNQ1682	ITGA2	ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
1555742_at	NA	NA	NA	
204508_s_at	UNQ13558	CA12	CA12	carbonic anhydrase XII
201566_x_at	UNQ3216	ID2	ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
229450_at	UNQ13624	IFIT3	IFIT3	interferon-induced protein with tetratricopeptide repeats 3
213524_s_at	UNQ4406	G0S2	G0S2	G0/G1switch 2
204039_at	UNQ8046	mc/EPB	NA	NA
203963_at	UNQ13558	CA12	CA12	carbonic anhydrase XII
225847_at	UNQ11715	AADACL1	AADACL1	arylacetamide deacetylase-like 1
218086_at	UNQ7114	NPDC1	NPDC1	neural proliferation, differentiation and control, 1
201565_s_at	UNQ3216	ID2	ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
212148_at	UNQ4995	PBX1	PBX1	pre-B-cell leukemia homeobox 1
214164_x_at	UNQ13558	CA12 IFIT2	CA12	carbonic anhydrase XII Isterferen indused erstele with tetratricementide reports 2
217502_at	UNQ21425		IFIT2	interferon-induced protein with tetratricopeptide repeats 2
204747_at	UNQ13624 NA	IFIT3 NA	IFIT3 NA	interferon-induced protein with tetratricopeptide repeats 3 NA
201095_at 200672_x_at	NA UNQ7408	NA SPTBN1	NA SPTBN1	NA spectrin, beta, non-erythrocytic 1
200672_x_at 218806_s_at	UNQ1408 UNQ14258	VAV3	VAV3	spectrin, beta, non-erythrocytic 1 vav 3 oncogene
218796_at	UNQ20949	C20orf42	C20orf42	chromosome 20 open reading frame 42
235457_at	UNQ12517	MAML2	NA	NA NA
201995_at	UNQ5295	EXT1	EXT1	exostoses (multiple) 1
210457_x_at	UNQ5772	HMGA1	HMGA1	high mobility group AT-hook 1
223112_s_at	UNQ14082	NDUFB10	NDUFB10	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10, 22kDa
226333_at	UNQ923	IL6R	IL6R	interleukin 6 receptor
60474_at	UNQ20949	C20orf42	C20orf42	chromosome 20 open reading frame 42
206074_s_at	UNQ5772	HMGA1	HMGA1	high mobility group AT-hook 1
218856_at	UNQ437	TNFRSF21	TNFRSF21	tumor necrosis factor receptor superfamily, member 21
224909_s_at	UNQ16674	EAPS16674	NA	NA
209369_at	UNQ14083	ANXA3	ANXA3	annexin A3
227342_s_at	UNQ25939	MYEOV	MYEOV	myeloma overexpressed gene (in a subset of t(11;14) positive multiple myelomas)
215867_x_at	UNQ13558	CA12	CA12	carbonic anhydrase XII
212770_at	UNQ4601	TLE4	TLE3	transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)
226487_at	UNQ20292 UNQ437	FLJ14721 TNFRSF21	C12orf34 TNFRSF21	chromosome 12 open reading frame 34 tumor necrosis factor receptor superfamily. member 21
214581_x_at 204268 at	UNQ437 UNQ4926	S100A2	S100A2	studior necrosis factor receptor supernamily, member 21
230183_at	UNQ5295	EXT1	EXT1	exostoses (multiple) 1
210692_s_at	UNQ3295	SLC43A3	SLC43A3	solute carrier family 43, member 3
1553530_a_at	UNQ1687	ITGB1	ITGB1	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
235106_at	UNQ34598	DHX37	NA	анаран, ана - (лананалан такран) ана раураран, анара са се спорта се су паста - у паста - у паста - у паста - у NA
213113_s_at	UNQ32943	SLC43A3	SLC43A3	solute carrier family 43, member 3
217997_at	UNQ7723	PHLDA1	PHLDA1	pleckstrin homology-like domain, family A, member 1
201889_at	UNQ4407	FAM3C	FAM3C	family with sequence similarity 3, member C
213198_at	UNQ917	ACVR1B	ACVR1B	activin A receptor, type IB
219433_at	UNQ16823	BCOR	BCOR	BCL6 co-repressor
227606_s_at	UNQ16509	DNMD16509	STAMBPL1	STAM binding protein-like 1
209946_at	UNQ417	VEGFC	VEGFC	vascular endothelial growth factor C
224925_at	UNQ16674	EAPS16674	NA	NA
212531_at	UNQ1721	LCN2	LCN2	lipocalin 2 (oncogene 24p3)
1553858_at	UNQ11983	ZBTB3	ZBTB3	zinc finger and BTB domain containing 3
212151_at	UNQ4995	PBX1 NA	PBX1 NA	pre-B-cell leukemia homeobox 1
231035_s_at 212071_s_at	NA UNQ7408	NA SPTBN1	NA SPTBN1	IVA enactrin hala non-anthrostic 1
212071_s_at 228340_at	UNQ7408 UNQ4601	SPIBN1 TLE4	SPIBN1 TLE3	spectrin, beta, non-erythrocytic 1 transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)
227272_at	UNQ26769	LOC374614	NA	NA
218000_s_at	UNQ7723	PHLDA1	PHLDA1	pleckstrin homology-like domain, family A, member 1
225688_s_at	UNQ20005	PLCXD2	PHLDB2	pleckstrin homology-like domain, family B, member 2
		OASL	OASL	2'-5'-oligoadenylate synthetase-like
205660_at	UNQ17129	OAGE		
205660_at 223194_s_at	UNQ17129 UNQ11816	C6orf85	C6orf85	chromosome 6 open reading frame 85
			C6orf85 CISH	chromosome 6 open reading frame 85 cytokine inducible SH2-containing protein
223194_s_at 221223_x_at 223082_at	UNQ11816 UNQ5695 UNQ16865	C6orf85 CISH SH3KBP1	C6orf85 CISH SH3KBP1	cytokine inducible SH2-containing protein SH3-domain kinase binding protein 1
223194_s_at 221223_x_at 223082_at 1553678_a_at	UNQ11816 UNQ5695 UNQ16865 UNQ1687	C6orf85 CISH SH3KBP1 ITGB1	C6orf85 CISH SH3KBP1 ITGB1	cytokine inducible SH2-containing protein SH3-domain kinase binding protein 1 Inliegin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
223194_s_at 221223_x_at 223082_at 1553678_a_at 208228_s_at	UNQ11816 UNQ5695 UNQ16865 UNQ1687 UNQ942	C6orf85 CISH SH3KBP1 ITGB1 FGFR2	C6orf85 CISH SH3KBP1 ITGB1 FGFR2	cytokine inducible SH2-containing protein SH3-domain kinase binding protein 1 inlegrin, beta 1 (libronctin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndro
223194_s_at 221223_x_at 223082_at 1553678_a_at 208228_s_at 223601_at	UNQ11816 UNQ5695 UNQ16865 UNQ1687 UNQ942 UNQ942 UNQ20862	C6orl85 CISH SH3KBP1 ITGB1 FGFR2 OLFM2	C6orf85 CISH SH3KBP1 ITGB1 FGFR2 OLFM2	cytokine inducible SH2-containing protein SH3-domain hisses binding protein 1 inlegim, beta 1 (libronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) libroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndro offactomedin 2
223194_s_at 221223_x_at 223082_at 1553678_a_at 208228_s_at 223601_at 209530_at	UNQ11816 UNQ5695 UNQ16865 UNQ1687 UNQ942 UNQ20862 UNQ2071	C6orf85 CISH SH3KBP1 ITGB1 FGFR2 OLFM2 CACNB3	C6orf85 CISH SH3KBP1 ITGB1 FGFR2 OLFM2 CACNB3	cytokine inducible SH2-containing protein SH3-domair kinase binding protein 1 integrin, beta 1 (libronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrou olfactomedin 2 calcium channel, voltage-dependent, beta 3 subunit
223194.s_at 221223_x_at 223082_at 1553678_a_at 208228_s_at 223801_at 209530_at 219014_at	UNQ11816 UNQ5695 UNQ16865 UNQ1687 UNQ942 UNQ20862 UNQ2071 UNQ4513	C6orf85 CISH SH3KBP1 ITGB1 FGFR2 OLFM2 CACNB3 PLAC8	C6orf85 CISH SH3KBP1 ITGB1 FGFR2 OLFM2 CACNB3 PLAC8	cytokine inducible SH2-containing protein SH3-domain kinase binding protein 1 mingrin, beta 1 (Bronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) fibrobiast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndroi olfactomedin 2 calcium channel, voltage-dependent, beta 3 subunit placenta-specific 8
223194.s_at 221223_x_at 223082_at 1553678_a_at 20828_s_at 223601_at 209530_at 219014_at 205490_x_at	UNQ11816 UNQ5695 UNQ16865 UNQ1687 UNQ942 UNQ20862 UNQ2071 UNQ4513 NA	C6ort85 CISH SH3KBP1 ITGB1 FGFR2 OLFM2 CACNB3 PLAC8 NA	C6orf85 CISH SH3KBP1 ITGB1 FGFR2 OLFM2 CACNB3 PLAC8 NA	cytokine inducible SH2-containing protein SH3-domain kinase binding protein 1 Inlegin, beta 1 (Ibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) Ifbroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndro offactomedin 2 calcium channel, voltage-dependent, beta 3 subunit placenta-specific 8 NA
223194 s. at 221223 x. at 223082. at 1553678. a. at 208228 s. at 208530. at 209530. at 209530. at 205490 x. at 211599 x. at	UNQ11816 UNQ5695 UNQ16865 UNQ1687 UNQ42 UNQ20862 UNQ2071 UNQ4513 NA UNQ42	C6orl85 CISH SH3KBP1 TGB1 FGFR2 OLFM2 CACNB3 PLAC8 NA cMet	C6orf85 CISH SH3KBP1 ITGB1 FGFR2 OLFM2 CACNB3 PLAC8 NA MET	cytokine inducible SH2-containing protein SH3-domain kinase binding protein 1 integrin, beta 1 (libronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrou ollactomedin 2 calcium channel, voltage-dependent, beta 3 subunit placente-specific 8 NA met proto-oncogene (hepatocyte growth factor receptor)
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223194 s. at 221223 x. at 223082 at 1553678 a. at 208228 s. at 209530. at 209530. at 219014. at 205490 x. at 211599 x. at 201508. at 213368 x. at 227556. at	UNQ11816 UNQ5695 UNQ16865 UNQ16867 UNQ942 UNQ20862 UNQ2071 UNQ4513 NA UNQ42 UNQ45187 UNQ1567	Céord85 CISH SHAGBP1 TITGB1 FGFR2 CACNB3 PLAC8 PLAC8 GPRC5A PPFIA3 CBX4 CBX4 CBX4	C6orl85 CISH SH3KBP1 TGB1 FGFR2 OLFM2 CACNB3 PLAC8 NA MET GPRC5A PPFIA3 CBX4	cytokine inducible SH2-containing protein SH3-domain knase binding protein 1 integrin, beta 1 (libronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndroi allactomedin 2 calcium channel, voltage-dependent, beta 3 subunit placente-specific 8 NA met proto-oncogene (hepatocyte growth factor receptor) G protein-coupled receptor, family C, group 5, member A protein hyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 3 chromobox Ahmolog 4 (Pc class Honolog, Dresophia)
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223194 s. at 221222 x. at 223082, at 1553678, a, at 206226 s. at 20830, at 219014, at 205430, x. at 211909 x. at 205490 x. at 203106, at 21388, x. at 20358, at 20368, s. at 227558, at 209826, s. at 223484, at	UNQ11816 UNQ5695 UNQ16865 UNQ16867 UNQ20862 UNQ20862 UNQ2071 UNQ4513 NA UNQ4513 UNQ42 UNQ14562 UNQ14562 UNQ10567 UNQ1665 UNQ16558	Coords CisH SHXBP1 ITGB1 FCFR2 CACNB3 PLAC8 NA CACNB3 PLAC8 NA CACNB3 PLAC8 CACNB3 PLAC8 CACNB3 CACN	C60r85 CISH SH3KBP1 ITGB1 FGFR2 OLFM2 CACNB3 PLAC8 NA MET GPRC5A GPRC5A PPFIA3 CBX4 OSBPL3 CISr48	cytokine inducible SH2-containing protein SH3-domain knase binding protein 1 integrin, beta 1 (libronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndroi allactomedin 2 calcium channel, voltage-dependent, beta 3 subunit placente-specific 8 NA met proto-oncogene (hepatocyte growth factor receptor) G protein-coupled receptor, family C, group 5, member A protein hyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 3 chromobox Ahmolog 4 (Pc class Honolog, Dresophia)
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223194 g, at 221223 x, at 221223 x, at 223082, at 1555075 g, at 223082, at 223081, at 20540, at 20540, x at 20540, x at 20540, x at 20340, at 213068, x at 213368, x at 223484, at 1554097, a, at 217999, a, at	UNQ11816 UNQ16865 UNQ1687 UNQ1687 UNQ942 UNQ2071 UNQ4513 NA UNQ4513 UNQ14562 UNQ14562 UNQ14562 UNQ14562 NA UNQ14553 NA UNQ14552 NA	Coords5 CISH SHXSBP1 JTGB1 FGFR2 OLFM2 CACNB3 PLAC8 NA dMet GPRC5A PPFIA3 CBM4 GPRC5A PPFIA3 CBM4 CBM4 CBM4 CBM51 NA PLDA1	Coort85 CISH SH3KBP1 JITGB1 FCFR2 OLFM2 CACNB3 PLAC8 NA MET GPRC5A PPFIA3 CB3M GSRPL3 CI5ort48 NA NA	cytokine inducible SH2-containing protein SH3-domain kinase binding protein 1 Intigrin, beita 1 (Itbronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) Itbroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndro oflactomedin 2 calcium channel, voltage-dependent, beta 3 subunit placente-specific 8 NA M Sprotein-coupled receptor, family C, group 5, member A protein-focupie phosphatiase, receptor type, 1 polypeptide (PTPRF), interacting protein (liprin), alpha 3 chromobox homolog 4 (Pc class homolog, Crosophila) oxysterol binding protein-like 3 chromosome 15 open reading frame 48 NA pleckstrin homology-like domain, family A, member 1
223194 g. at 221223 x. at 221223 x. at 223082; at 1555378 g. at 208228 g. at 20820 g. at 20820 g. at 20830, at 20830, at 20308, at 213904, at 203408, x. at 203108, at 21398 x. at 20368, x. at 20368, s. at 209826, g. at 217999, g. at 217999, g. at 217943, g. at	UNQ11816 UNQ16865 UNQ1687 UNQ1687 UNQ2682 UNQ2082 UNQ2071 UNQ4513 NA UNQ42 UNQ1587 UNQ1267 UNQ1455 UNQ10567 UNQ14055 UNQ14055 UNQ1723 UNQ7723 UNQ19	Céord85 CISH SHAGBP1 JITGB1 FGFR2 CACNB3 PLAC8 CACNB3 PLAC8 CACNB3 PLAC8 CACNB3 PLAC8 CACNB3	Coord85 CISH SH3KBP1 TIGB1 FGFR2 CACNB3 PGFR2 CACNB3 PLAC8 GPRC5A GPRC5A GPRC5A CASPL3 CISord48 NA PHLDA1 GFFP3	cytokine inducible SH2-containing protein SH3-domain hinase binding protein 1 Integrin, beta 1 (libronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) Interoblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndro offactomedn 2 calcium channel, votlage-dependent, beta 3 subunit placents-specific 8 NA Met proto-oncegane (hepatocyte growth factor receptor) G protein-coupled receptor, family C, group 5, member A protein tyrosine phosphatase, receptor type, 1 polypeptide (PTPRF), interacting protein (liprin), alpha 3 chromobox homolog 4 (Pc class homolog, Drosophila) exysterol binding protein-like 3 chromosome 15 open reading frame 48 NA pleckstrin homology-like domain, family A, member 1 insulin-like growth factor binding protein 3
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Supplemental table 2: GSEA analysis

p-value	Direction			Source
1.86E-48			Breast cancer_ER_POS Genes whose expression is consistently positively correlated with estrogen recep	
2.54E-45			ras-induced senescent IMR90 fibroblast genes Gene expression changes in ras-induced senescent IMR90	
1.22E-30	U	314	BREAST CANCER STROMA GENES genes expressed by stroma of GeneLogic human breast cancer samples	Gene logic corporation, Gaithersburg, Md
6.39E-14	U		BAF57_BT549_UP Up-regulated following stable re-expression of BAF57 in Bt549 breast cancer cells that lack fur	
4.44E-12			HCC_SURVIVAL_GOOD_VS_POOR_DN Genes highly expressed in hepatocellular carcinoma with poor survival.	
2.53E-09			SERUM_FIBROBLAST_CELLCYCLE Cell-cycle dependent genes regulated following exposure to serum in a vari	
2.55E-09			AGUIRRE_PANCREAS_CHR12 Genes on chromosome 1 with copy-number-driven expression in pancreatic ader	
5.83E-09	U	136	CHANG_SERUM_RESPONSE_UP CSR (Serum Response) signature for activated genes (Stanford)	Broad/MIT MSigDB
8.45E-09	U			Broad/MIT MSigDB
1.68E-08	U	80	CORDERO_KRAS_KD_VS_CONTROL_UP Genes upregulated in kras knockdown vs control in a human cell line	Broad/MIT MSigDB

Supplemental table 3: Top 50 genes induced by MEK and RAS

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22855 9.83 0 0 1.242 UN02146 LRP8 LRP8 Iow density ispontene receptor - related protein 8, apolicapceten 8, apolicapcet			qvalue				Description
218856_01 9.526 0 1.466 UNQ347_TITERSP21TIFERSP21 Unron accises factor receptor superfamily, member 21 21827_01 11.55 0 0.75 UNQ2148(ETG3_BTG BTG family, member 21 21827_01 11.55 0 0.3048 UNQ2343 GALTH1_4 CAPNAIGLANTH Caphan 14UDP-Ascapt-alpha-D_glatectaaming-transferase 14 (GalNAC-T14) 21827_01 11.51 0 0.091 UNQ357 MTFFD1_HTFD2 melytenetister ceptor superfamily, member 21 22830_01 11.51 0 0.091 UNQ357 MTFFD1_HTFD2 melytenetister/superfamily, member 24 22830_01 11.51 0 0.998 UNQ3748 RAFB RAFB 22830_01 11.52 0 1.562 UNQ1775 BACR_B RAFB RAFB 22840_01 1.862 0 1.952 UNQ1775 BACR_B RAFB RAFB RAFB 22840_01 1.862 0 1.952 UNQ1775 BACR_B RAFB							
213132. 11.135 0 0.75 UN02194(ETG3 BTG3 BTG family, member 3 213127.1 0.566 0 3.044 UN02345 (ALTH1 4 CMH14GLNT14 202019.1 9.343 0 0.311 UN0368 EPH44 EPH44 22523.1 11.133 0 0.321 UN0368 EPH44 EPH44 EPH respire 7 AA 22523.1 11.133 0 0.327 WIN156 MTFD1 MTHPD1 methylenstetrahydrolatic dehydrogenase (NADP-4 dependent) 1-like 22523.2 11.133 0 1.297 WIN156 SERTMTFD1 MTHPD1 methylenstetrahydrolatic dehydrogenase (NADP-4 dependent) 1-like 22523.2 11.04 0 1.867 WIN156 SERTMTFD FTH101 methylenstetrahydrolatic dehydrogenase (NADP-4 dependent) 1-like 22523.2 11.04 0 1.867 WIN156 SERTMTFD FTH101 methylenstetrahydrolatic dehydrogenase (NADP-4 dependent) 1-like 22695.2 -11.504 0 1.367 WIN175 RAGE RAGE ECL-associated ahanogene 2 200052.4 -11.504 0 1.22 WIN1466 CDTL CTL derticless homolog (Drosophia) 200852.4 9.858 0 2.268 WIN250 KTER RTTR krmain 16 (Goano- epidemolyfe pamolysinatark setatodema)							
10:566 0 3.046 UN02434 GALT14 CAPN4 (GALNA-T14 captan 14(UD-Astacta-anip-color_patactasamic-polypeption Naccolygalactosaminytransferate 14 (GalNA-T14) 202303, 0:541 0 0.381 UN02356 MTHFD1 MTHFD1 methytenetatarydorolate dehydrogenase (NADP- dependent) 1, metherytentarydorolate cyclohydrolate, cyclohy							
202010 3.343 0							
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230356_at 13.106 0 1.643 UNO1034/Sax1 NKX1-2 NK1 formedbox2 21844_at 9.993 0 1.702 UNO2078PLEX2 PLEK2 plexkstrin 2 20425_s 13.816 0 1.253 UNQ14985 PMAIP1 photo-1/2-myristate-13-acetate-induced protein 1 217272_s -9.096 0 -2.241 UNQ12083 ERPINB1 SERPINB1 SERPINB1 sprin peptidase inhibitor, clade B (ovalburnin), member 13 204386_at 12.945 0 -2.241 UNQ12083 ERPINB1 SERPINB1 SERPINB1 KIA1244 204786_at 9.311 0 2.027 UNQ2058 MYB KY8 v-myb myeloblastosis viral oncogene homolog (avian) 22514_at 16.406 0 1.957 UNQ19471 RKSEF RASEF RASEF 21807_at -9.498 0 -1.617 UNQ2116FTPSINP1 TP53INP1 tumor protein p53 inducible nuclear protein 1	204734_at	-9.46	0	0	-2.46 UNQ7983 KRT19	KRT15 KRT19	keratin 15 keratin 19
21864_art 9.993 0 0 1.702 UNQ20788 PLEK2 PLEK2 pleckstrin 2 204285_s 13.816 0 0 1.253 UNQ14986 PMAIP1 PMAIP1 photbol-12-myristate-13-acetate-induced protein 1 204285_s 13.816 0 0 -2.241 UNQ12082 SERTINBIS SERTINBIS serpin peptidase inhibitor, clade B (ovalbumin), member 13 231856_at 12.945 0 0 4.543 UNQ12147 SPCR121 KIAA1244 KIAA1244 204798_at 9.311 0 0 2.027 UNQ2058 MVB MVB v-mymoleblastics viral oncogene homolog (avian) 235144_at 16.405 0 1.957 UNQ11647 FS3INPI TPS3INPI tumor protein p53 inducble nuclear protein 1 225912_at -9.298 0 -1.617 UNQ21166 TPS3INPI tumor protein p53 inducble nuclear protein 1 21807_at -9.498 0 0 -1.617 UNQ21166 TPS3INPI yaanine nucleotide exchange factor	213906_at	16.874	0	0	1.787 UNQ5673 MYBL1	MYBL1	v-myb myeloblastosis viral oncogene homolog (avian)-like 1
204285_s 13.816 0 0 1.253 UNC1498EPMAIP1 photbol-12-mystate-13-acetater-induced protein 1 217272_s 0.006 0 -2.241 UNC120825EPRINB1 SERPINB1S serpin peptidase inhibitor, clade B (ovalburnin), member 13 231856_at 12.945 0 4.543 UNC12147SPQR1214/KIAA1244 KIAA1244 204798_at 9.311 0 2.027 UNC2056 MYB MYB v-myb myeloblastosis viral oncogene homolog (avian) 23184_at 16.406 0 1.957 UNC1947/TRASEF RASEF RASEF 21807_at -9.498 0 -1.617 UNC21146TPS3INP1 TP53INP1 tumor protein p53 inducible nuclear protein 1	230356_at	13.106	0	0	1.643 UNQ10354 Sax1	NKX1-2	NK1 homeobox 2
217272_s -9.096 0 -2.241 UNO12083 SERPINB1 SERPINB13 serpin peptidase inhibitor, clade B (ovalibumin), member 13 231856_at 12.945 0 0 4.543 UNO12147 SPQR1214/KIAA1244 KIAA1244 Q47789_at 9.311 0 2.027 UNO2058 MVR MYB v-myb wyeloblastosis viral oncogene homolog (avian) 225412_at 16.406 0 1.957 UNO1947/ RASEF RASEF RAS and EF-hand domain containing 225912_at -9.259 0 0 -1.617 UNO21166 TPS3INP1 tumor protein p53 inducible nuclear protein 1 21807_at -9.498 0 0 -1.25 UNO14526 VAV3 VAV3 va/a guarine nucleotide exchange factor	218644_at	9.993	0	0	1.702 UNQ20788 PLEK2	PLEK2	pleckstrin 2
231856_art 12.945 0 4.543 UNO12147 SPORT214 KIAA1244 KIAA1244 204798_at 9.311 0 2.027 UNQ2058 MYB vmyb myeloblastosis viral oncogene homolog (avian) 225914_at 16.406 0 1.957 UNQ1947 IRASEF RASEF RASEF 225912_at -9.259 0 -1.617 UNQ2146T P53INP1 TP53INP1 TP53INP1 tumor protein p53 inducible nuclear protein 1 21807_at -9.498 0 -1.152 UNQ2146E VAV3 Vav3 vav3 vav3 guarine nucleotide exchange factor	204285_s_	13.816	0	0	1.253 UNQ14985 PMAIP1	PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1
204798_at 9.311 0 0 2.027 UNQ2058 MYB MYB v-myb myeloblastosis viral oncogene homolog (avian) 23514_at 16.406 0 1.957 UNQ19471 RASEF RASEF RAS and EF-hand domain containing 225912_at -9.259 0 0 -1.617 UNQ2166 TP53INP1 Tbrotein p53 inducible nuclear protein 1 18007_at -9.498 0 0 -1.125 UNQ14254 VAV3 VAV3 vav3 guanine nucleotide exchange factor	217272_s_	-9.096	0	0	-2.241 UNQ12083 SERPINB	1 SERPINB13	serpin peptidase inhibitor, clade B (ovalbumin), member 13
Z35144 at 225912, at 92.59 0 0 1.957 UNC19471RASEF RASE and EF-hand domain containing 225912, at -9.259 0 0 -1.617 UNC2116617P53INP1 tumor protein p53 inducible nuclear protein 1 21807, at -9.498 0 -1.125 UNC1425EVAV3 VAV3 vav3 guarnie nucleotide exchange factor	231856_at	12.945	0	0	4.543 UNQ12147 SPQR121	4 KIAA1244	KIAA1244
Z35144 at 225912, at 92.59 0 0 1.957 UNC19471RASEF RASE and EF-hand domain containing 225912, at -9.259 0 0 -1.617 UNC2116617P53INP1 tumor protein p53 inducible nuclear protein 1 21807, at -9.498 0 -1.125 UNC1425EVAV3 VAV3 vav3 guarnie nucleotide exchange factor	204798_at	9.311	0	0			v-myb myeloblastosis viral oncogene homolog (avian)
225912_at -9.259 0 0 -1.617 UNQ21166TP53INP1 TP53INP1 tumor protein p53 inducible nuclear protein 1 218807_at -9.498 0 0 -1.125 UNQ14258 VAV3 VAV3 vav 3 guanine nucleotide exchange factor	235144_at	16.406	0	0		RASEF	
	225912_at	-9.259	0	0	-1.617 UNQ21166 TP53INP1	TP53INP1	
202095_s 10.425 0 0 1.59 UNQ6162 BIRC5 BIRC5 baculoviral IAP repeat-containing 5 (survivin)	218807_at	-9.498	0	0	-1.125 UNQ14258 VAV3	VAV3	vav 3 guanine nucleotide exchange factor
	202095_s_	10.425	0	0	1.59 UNQ6162 BIRC5	BIRC5	baculoviral IAP repeat-containing 5 (survivin)

A	4: Kas pathway predictors
Cell Line	mcf10a_RAS MEK predictor
MDA-MB-134VI	1.561522258
HDQ-P1	3.005412519
DU4475	5.088272929
MT-3	0.094212097
HCC1806	6.057952552
MDA-MB-435S	7.897471489
HCC70	5.209717563
HCC1954	7.271376772
CAL-120	7.825616103
MX1	1.526650039
CAL-85-1	5.877978641
SW527	3.000730351
CAL-51	4.83582616
MDA-MB-231	7.812897994
HCC1143	9.828949712
BT-20	-0.715458478
MDA-MB-175-VII	-5.916575386
HS 578T	-0.493018017
MDA-MB-468	8.422287698
HCC1395	-1.454714578
MFM-223	1.755087639
AU565	-0.684587495
BT-474	-0.432212789
BT-483	-3.779623789
BT-549	3.470857318
CAL-148	-4.634639056
CAMA-1	1.063519427
EFM-19	-0.931087696
EFM-192A	0.989251324
EVSA-T	-2.805201836
HCC1187	2.760688293
HCC1419	-5.284668636
HCC1428	-0.996595596
HCC1500	-0.632811063
HCC1569	3.282208361
HCC1937	4.819642478
HCC2218	-10.28517067
HCC38	2.66314843
JIM-T	3.152998211
KPL-1	-6.409216711
KPL4	-2.411538379
MCF7	-5.795687238
MDA-MB-361	-2.048195804
MDA-MB-415	0.793425341
MDA-MB-436	3.041703724
MDA-MB-453	-1.91388501
SK-BR-3	-4.747140587
T-47D	-1.580969544
UACC-812	-3.741796407
UACC-893	-2.44463976
ZR-75-1	-1.924760377
ZR-75-30	-2.646637775
LIN-10-00	-2.0+0001110