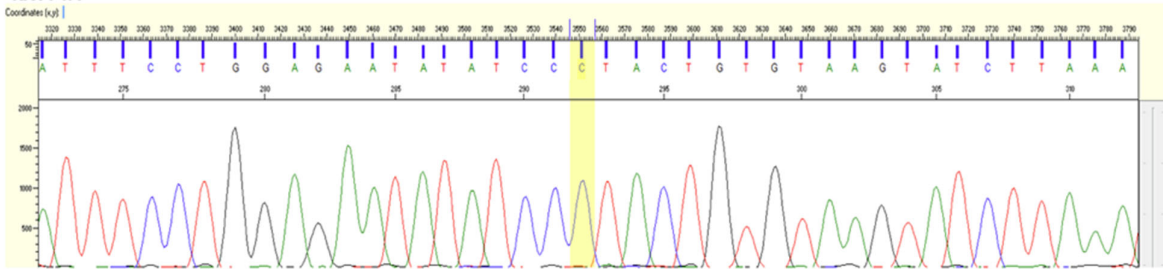
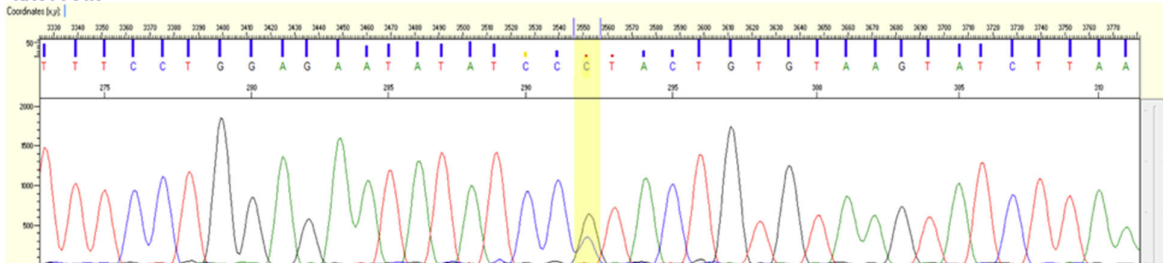


# **RAC1 WT**



# **RAC1 P34R**



**A**

PDX.008.CL\_NCBJ\_NG\_007873.3\_201

CTCTTCATAATGCTTGCTCTGATAGGAAAATGAGATCTACTGTTTTCTTTACTTACTACCTCAGATATATTTCTTCATGAAGACCTCACAGTAAAAATAGGT  
GATTTTGGTCTAGCTACAGTGAAAATCTCGATGGAGTGGTCCCATCAGTTTGAACAGTTGTCTGGATCCATTTGTGGATGGTAAGAATTGAGGCT

A T V K

>PDX.008.CL\_NC12\_CONTIG\_198\_p1 59467 pairs of NGS reads, 74.52%

CTCTTCATAATGCTTGCTCTGATAGGAAAATGAGATCTACTGTTTTCTTTACTTACTacacctcagatatattcttcataagacctcacagtaaaataggtgattttgtctagcag  
tgaattctcgatggagtggtccCATCAGTTTGAACAGTTGTCTGGATCCATTTGTGGATGGTAAGAATTGAGGCT

V N

>PDX.008.CL\_NC12\_CONTIG\_201\_p2 20325 pairs of NGS reads, 25.47%

CTCTTCATAATGCTTGCTCTGATAGGAAAATGAGATCTACTGTTTTCTTTACTTACTacacctcagatatattcttcataagacctcacagtaaaataggtgattttgtctagcag  
acagtgaattctcgatggagtggtTCCCATCAGTTTGAACAGTTGTCTGGATCCATTTGTGGATGGTAAGAATTGAGGCT

T V K

KTC\_NCBJ\_NG\_007873.3\_201

CTCTTCATAATGCTTGCTCTGATAGGAAAATGAGATCTACTGTTTTCTTTACTTACTACCTCAGATATATTTCTTCATGAAGACCTCACAGTAAAAATAGGT  
GATTTTGGTCTAGCTACAGTGAAAATCTCGATGGAGTGGTCCCATCAGTTTGAACAGTTGTCTGGATCCATTTGTGGATGGTAAGAATTGAGGCT

A T V K

>KTC\_NC11\_CONTIG\_201\_p1 40686 pairs of NGS reads, 51.74%

CTCTTCATAATGCTTGCTCTGATAGGAAAATGAGATCTACTGTTTTCTTTACTTACTacacctcagatatattcttcataagacctcacagtaaaataggtgattttgtctagcag  
acagtgaattctcgatggagtggtTCCCATCAGTTTGAACAGTTGTCTGGATCCATTTGTGGATGGTAAGAATTGAGGCT

T V K

>KTC\_NC11\_CONTIG\_201\_p2 37941 pairs of NGS reads, 48.25%

CTCTTCATAATGCTTGCTCTGATAGGAAAATGAGATCTACTGTTTTCTTTACTTACTacacctcagatatattcttcataagacctcacagtaaaataggtgattttgtctagcag  
acagtgaattctcgatggagtggtTCCCATCAGTTTGAACAGTTGTCTGGATCCATTTGTGGATGGTAAGAATTGAGGCT

T E K

= normal sequence ATVK

= V600E mutation (ATEK)

= deletion of Threonine (T) and K601N mutation (AVN). Comes from deletion of TAC, which produces GCA instead of GCT (still an Alanine) but deletes ACA (Threonine), plus point mutation at K601

**B**

**Figure S1. A:** Sanger sequencing of RAC1 exon 2, after PCR amplification with primers framing codon 34. **B:** NGS analysis of BRAF exon 15 (MGH CCIB DNA Core)



Cytogenetics and Cell Authentication core

CCAC database is a comprehensive databases of short tandem repeat (STR) profiles. The database includes profiles from 20 public database/ publications and the cell lines developed by MD Anderson research labs. The database contains over 4650 unique human cancer cell line STR profiles, one of the largest cancer cell line STR online search database in the world

For questions please contact Xuesong Li (713)-792-6839, Xli@mdanderson.org

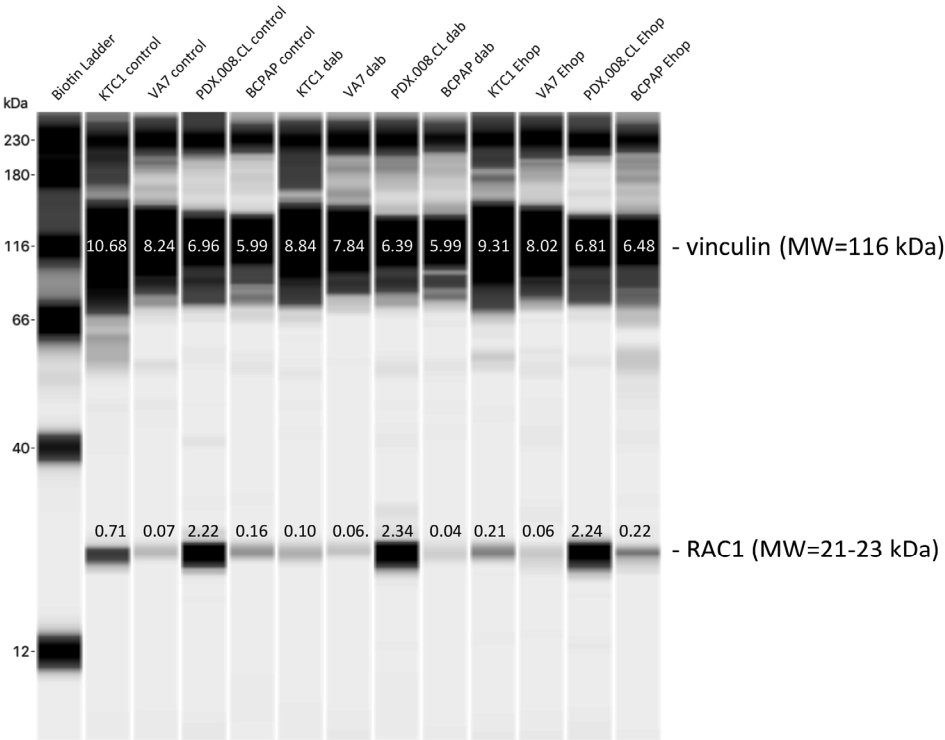
Annual cell line authentication is required by  
UTMDACC INSTITUTIONAL POLICY # ACA1044

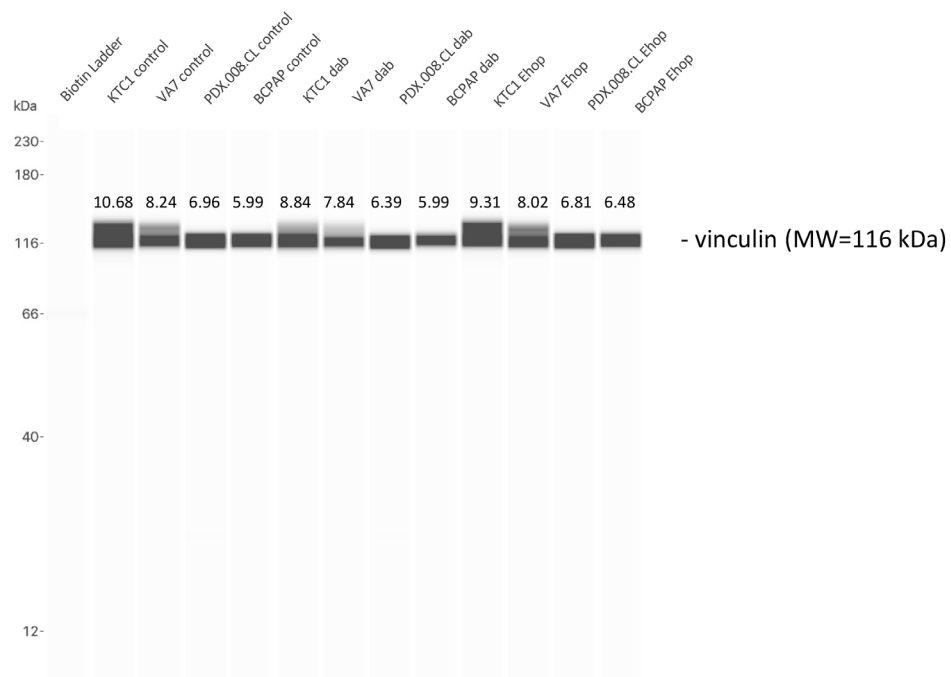
SET 518	2/25/2020
Expiration date: 2/25/2021	

Source	Sample_Name	AMEL	CSF1PO	D13S317	D16S539	D18S51	D21S11	D3S1358	D5S818	D7S820	D8S1179	FGA	TH01	TPOX	vWA	Comments
Hofmann Marie Claude	KTC1	X,Y	10,12	11,12	12	12,13	29	14,15	11,12	11	11,14	23,26	9	11	14,17	
CCCLC customer database -SET 262	KTC1	X,Y	10,12	11,12	12	12,13	29	14,15	11,12	11	11,14	23,26	9	11	14,17	MATCH

Source	Sample_Name	AMEL	CSF1PO	D13S317	D16S539	D18S51	D21S11	D3S1358	D5S818	D7S820	D8S1179	FGA	TH01	TPOX	vWA	Comments
Hofmann Marie Claude	FNA001	X,Y	11,12	11,12	11	12,17	28	14,17	10,12	10,12	12,13	23,25	7,9,1	8	16	Profile Unique No match found in the database

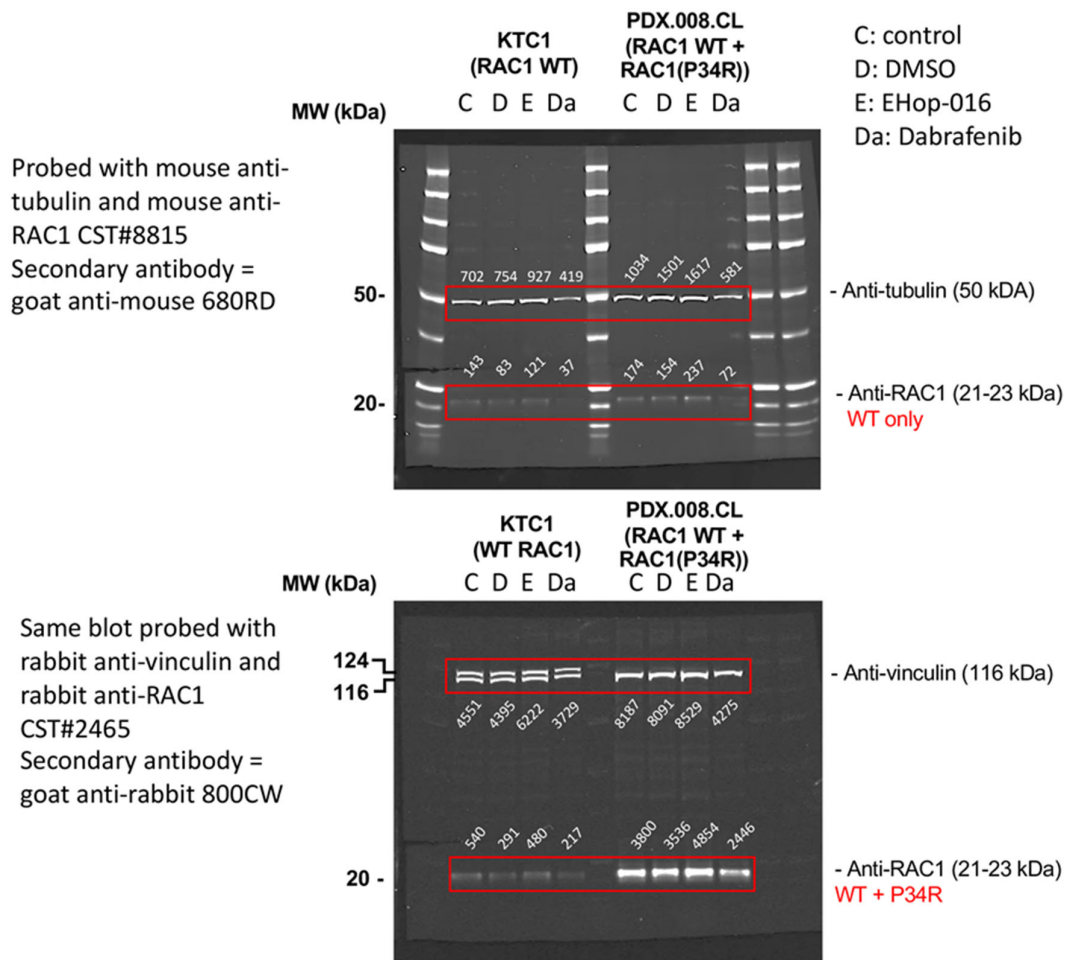
Figure S2. STR analysis of KTC1 and PDX.008.CL cells. FNA001 was the original name given to PDX.008.CL cells.



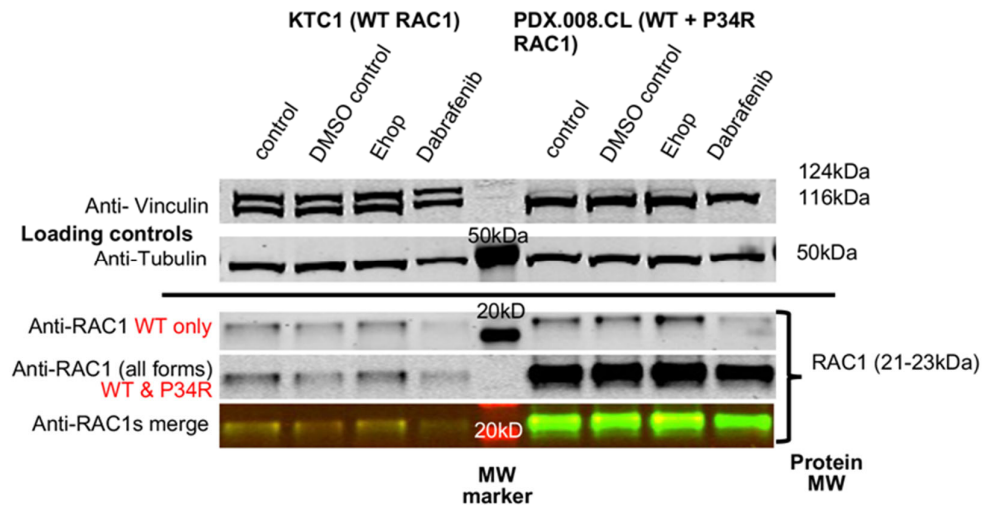


B

**Figure S3. A:** Wes digital capillary protein analysis of simultaneous total RAC1 and Vinculin expression in different papillary thyroid cancer cell lines treated with a BRAF V600E inhibitor (dabrafenib 0.1  $\mu$ M) or RAC1 inhibitor (Ehop-016 0.1 $\mu$ M). Vinculin was used as loading control. **B:** Same capillary set, but brightness-adjusted for Vinculin. VA7 = KTC1 cell line with an acquired KRAS<sup>G12D</sup> mutation.



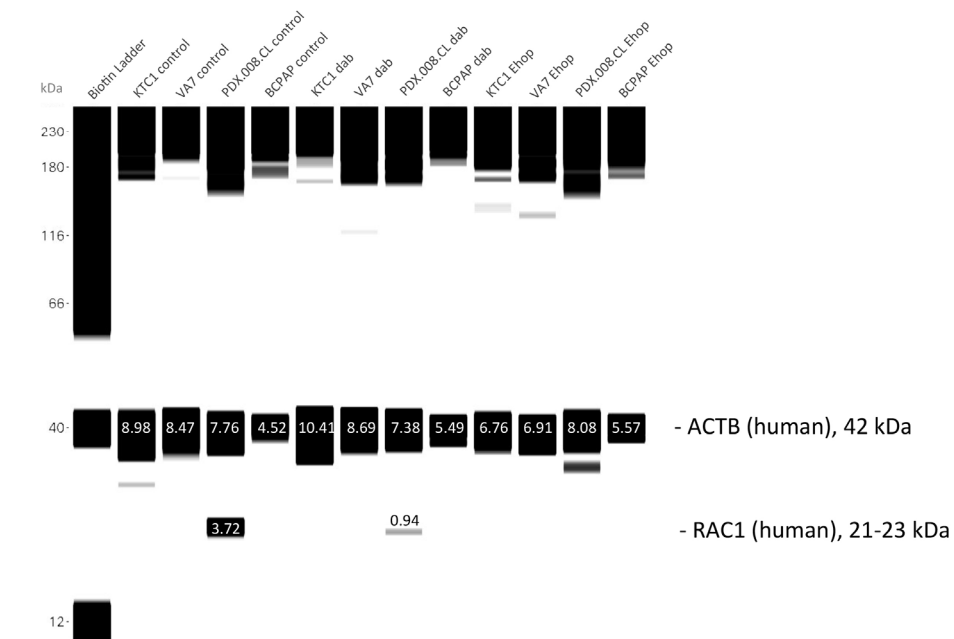
A



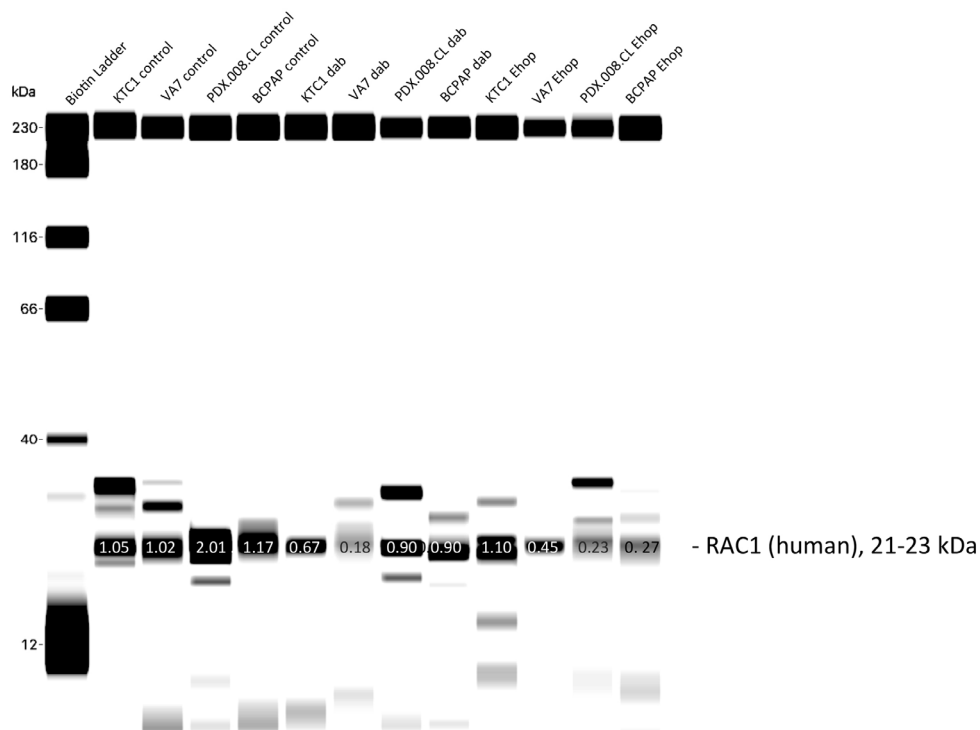
B

**Figure S4. A:** Conventional Western blot probing for RAC1, with vinculin or tubulin as simultaneous loading controls (LICOR fluorescence). KTC1 and PDX.008.CL were treated with and without the Ehop-016 RAC1 inhibitor or dabrafenib.

By subtracting non-P34R-detecting anti-RAC1 staining (detects only WT) from total anti-RAC1 staining (detects both WT and P34R forms), the relative amount of P34R mutant RAC1 can be calculated. **B:** Same blot, cut in strips and showing merged fluorescence for RAC1.

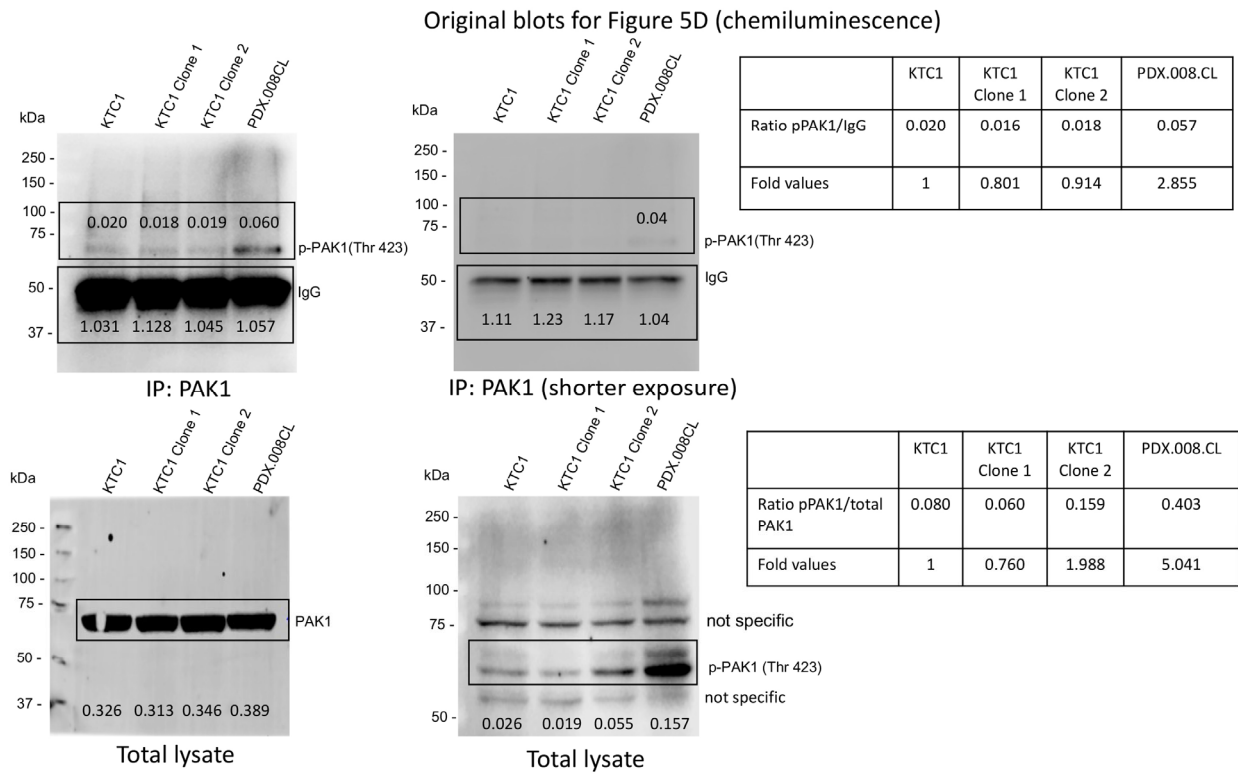


A

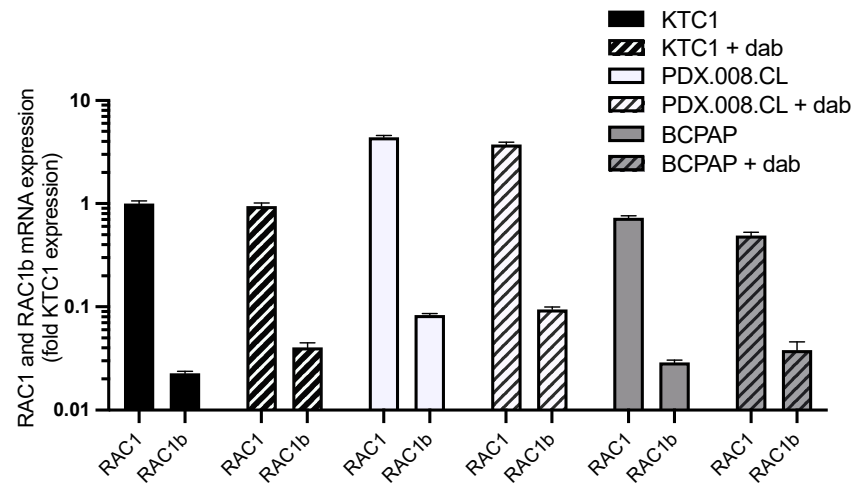


B

**Figure S5. A:** Wes digital capillary protein analysis of RAC1 and ACTB. ACTB is used as a loading control. **B:** Same samples after immunoprecipitation with a GST-PAK1-PBD fusion protein that strongly binds GTP-bound RAC1, then probed for total RAC1. VA7 = KTC1 cell line with an acquired KRAS<sup>G12D</sup> mutation.



**Figure S6.** Western blots of samples after immunoprecipitation with an anti-PAK1 antibody, then probing with a p-PAK (Thyr 423) antibody. PAK1 and pPAK1 in total lysates are also shown.



**Figure S7.** Expression of *RAC1b* mRNA in comparison to *RAC1* mRNA in two PTC cell lines and PDX.008.CL. *RAC1b* expression corresponds to 2% of total *RAC1* expression.

**Table S1.** CM50 Gene Panel.

Gene /HGNC	Genomic Location	Strand	Accession Number	Exons (Codons) Tested	Gene Name
ABL1	chr9:133589268-133763062	+	NM_005157	4 (232-260), 5 (275-279), 6 (314-360), 7 (380-412)	ABL proto-oncogene 1, non-receptor tyrosine kinase
AKT1	chr14:105235687-105262080	-	NM_005163	3 (16-52), 6 (154-183)	AKT serine/threonine kinase 1
ALK	chr2:29415640-30144477	-	NM_004304	23 (1172-1204), 25 (1270-1279)	anaplastic lymphoma receptor tyrosine kinase
APC	chr5:112043202-112181936	+	NM_000038	16 (860-891), 16 (1089-1125), 16 (1284-1326), 16 (1342-1384), 16 (1426-1471), 16 (1483-1524), 16 (1543-1582)	APC, WNT signaling pathway regulator
ATM	chr11:108093559-108239826	+	NM_000051	8 (326-355), 9 (407-412), 12 (601-626), 17 (834-865), 26 (1292-1325), 34 (1674-1707), 35 (1726-1757), 36 (1790-1815), 39 (1926-1946), 50 (2436-2454), 54 (2650-2667), 55 (2682-2711), 56 (2718-2736), 59 (2865-2891), 61 (2933-2950), 63 (2996-3026), 63 (3041-3057)	ATM serine/threonine kinase
BRAF	chr7:140433813-140624564	-	NM_004333	11 (439-473), 15 (581-611)	B-Raf proto-oncogene, serine/threonine kinase
CDH1	chr16:68771195-68869444	+	NM_004360	3 (65-96), 8 (337-374), 9 (380-408)	cadherin 1
CDKN2A	chr9:21967751-21994490	-	NM_000077	2 (51-90), 2 (98-140)	cyclin dependent kinase inhibitor 2A
CSF1R	chr5:149432854-149492935	-	NM_005211	7 (297-319), 22 (953-973)	colony stimulating factor 1 receptor
CTNNB1	chr3:41240942-41281939	+	NM_001904	3 (9-48)	catenin beta 1
EGFR	chr7:55086725-55275031	+	NM_005228	3 (96-123), 7 (279-297), 15 (575-601), 18 (695-726), 19-20 (729-796), 20 (807-823), 21 (855-875)	epidermal growth factor receptor
ERBB2	chr17:37844393-37884915	+	NM_004448	19-20 (752-797), 21 (839-882)	erb-b2 receptor tyrosine kinase 2
ERBB4	chr2:212240442-213403352	-	NM_005235	3 (136-141), 4 (167-186), 6 (225-247), 7 (254-290), 8 (295-323), 9 (333-367), 15 (580-623), 23 (919-948)	erb-b2 receptor tyrosine kinase 4
EZH2	chr7:148504464-148581441	-	NM_004456	16 (625-649)	enhancer of zeste 2 polycomb repressive complex 2 subunit
FBXW7	chr4:153242410-153456172	-	NM_033632	5 (264-287), 8 (378-403), 9 (434-473), 10 (478-509), 11 (567-594)	F-box and WD repeat domain containing 7
FGFR1	chr8:38268656-38326352	-	NM_015850	4 (120-148), 7 (247-273)	fibroblast growth factor receptor 1
FGFR2	chr10:123237844-123357972	-	NM_000141	7 (250-275), 7 (296-313), 9 (936-399), 12 (546-558)	fibroblast growth factor receptor 2

FGFR3	chr4:1795039-1810599	+	NM_000142	7 (247-277), 9 (367-402), 14 (631-653), 16 (690-719), 18 (771-807)	fibroblast growth factor receptor 3
FLT3	chr13:28874483-29069265	-	NM_004119	11 (437-466), 14 (570-610), 16 (663-685), 20 (828-847)	fms related tyrosine kinase 3
GNA11	chr19:3094408-3121454	+	NM_002067	5 (202-219)	G protein subunit alpha 11
GNAQ	chr9:80335191-80646219	-	NM_002072	5 (206-245)	G protein subunit alpha q
GNAS	chr20:57414795-57486250	+	NM_000516	8-9 (196-240)	GNAS complex locus
HNF1A	chr12:121416549-121440314	+	NM_000545	3 (192-221), 4 (253-282)	HNF1 homeobox A
HRAS	chr11:532242-535550	-	NM_005343	2 (5-35), 3 (42-82)	HRas proto-oncogene, GTPase
IDH1	chr2:209100953-209119806	-	NM_005896	4 (101-135)	isocitrate dehydrogenase (NADP(+)) 1, cytosolic
IDH2	chr15:90627212-90645708	-	NM_002168	4 (133-177)	isocitrate dehydrogenase (NADP(+)) 2, mitochondrial
JAK2	chr9:4985245-5128183	+	NM_004972	14 (603-622)	Janus kinase 2
JAK3	chr19:17935593-17958841	-	NM_000215	4 (128-140), 13 (568-580), 16 (709-733)	Janus kinase 3
KDR	chr4:55944426-55991762	-	NM_002253	6-7 (244-291), 11 (471-480), 19 (872-894), 21 (961-988), 26 (1135-1156), 27 (1192-1221), 30 (1283-1310), 30 (1324-1357)	kinase insert domain receptor
KIT	chr4:55524095-55606881	+	NM_000222	2 (23-58), 9 (494-514), 10-11 (525-587), 13 (627-661), 14 (664-684), 15 (714-724), 17 (802-828), 18 (832-858)	KIT proto-oncogene receptor tyrosine kinase
KRAS	chr12:25358180-25403854	-	NM_004985	2-3 (5-66), 4 (114-150)	KRAS proto-oncogene, GTPase
MET	chr7:116312459-116438440	+	NM_001127500	2 (159-188), 1 (339-378), 11 (816-856), 14 (981-1012), 16 (1105-1132), 19 (1246-1274)	MET proto-oncogene, receptor tyrosine kinase
MLH1	chr3:37034841-37092337	+	NM_000249	12 (373-415)	mutL homolog 1
MPL	chr1:43803475-43820135	+	NM_005373	10 (501-522)	MPL proto-oncogene, thrombopoietin receptor
NOTCH1	chr9:139388896-139440238	-	NM_017617	26 (1566-1602), 27 (1673-1680), 34 (2436-2476)	notch receptor 1
NPM1	chr5:170814708-170837888	+	NM_002520	11 (283-295)	nucleophosmin (nucleolar phosphoprotein B23, numatrin)
NRAS	chr1:115247085-115259515	-	NM_002524	2 (3-31), 3 (43-69), 4 (124-150)	neuroblastoma RAS viral oncogene homolog
PDGFRA	chr4:55095264-55164412	+	NM_006206	12 (552-583), 14 (644-668), 15 (671-709), 18 (819-854)	platelet derived growth factor receptor alpha
PIK3CA	chr3:178866311-178952497	+	NM_006218	2 (54-90), 2 (106-118), 5 (316-351), 7-8 (390-422), 8 (449-468),	phosphatidylinositol-4,5-bisphosphate 3-



				10 (522-549), 14 (677-720), 19 (898-924), 21 (1017-1051), 21 (1065-1069)	kinase catalytic subunit alpha
PTEN	chr10:89623195-89728532	+	NM_000314	1 (1-25), 3 (55-70), 5 (99-135), 6 (165-184), 7 (212-215), 7 (231-267), 8 (282-300), 8 (312-342)	phosphatase and tensin homolog
PTPN11	chr12:112856536-112947717	+	NM_002834	3 (46-82), 13 (485-527)	protein tyrosine phosphatase, non-receptor type 11
RB1	chr13:48877883-49056026	+	NM_000321	4 (130-159), 6 (196-203), 10 (314-345), 11 (350-366), 14 (452-463), 17-18 (547-582), 20 (655-691), 21 (703-724), 22 (743-770)	RB transcriptional corepressor 1
RET	chr10:43572517-43625797	+	NM_020975	10-11 (608-654), 13 (762-786), 15-16 (875-924)	ret proto-oncogene
SMAD4	chr18:48556583-48611411	+	NM_005359	3 (98-136), 4 (142-146), 5 (165-202), 6 (242-263), 8 (307-319), 9 (326-365), 10 (384-424), 1 (443-474), 12 (494-532)	SMAD family member 4
SMARCB1	chr22:24129150-24176705	+	NM_003073	2 (35-72), 4-5 (144-206), 9 (373-386)	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1
SMO	chr7:128828713-128853385	+	NM_005631	3 (186-228), 5 (307-331), 6 (391-419), 9 (511-542), 11 (608-646)	smoothened, frizzled class receptor
SRC	chr20:35973088-36033821	+	NM_005417	14 (499-533)	SRC proto-oncogene, non-receptor tyrosine kinase
STK11	chr19:1205798-1228434	+	NM_000455	1 (22-64), 4 (155-181), 4-5 (191-207), 6 (253-285), 8 (317-361)	serine/threonine kinase 11
TP53	chr17:7571720-7590868	-	NM_000546	2 (1-20), 4 (68-113), 5 (126-138), 5-6 (149-223), 7 (225-258), 8 (263-307), 10 (332-367)	tumor protein p53
VHL	chr3:10183319-10195354	+	NM_000551	1 (78-108), 2 (114-150), 3 (155-174)	von Hippel-Lindau tumor suppressor

Table S2. T200.1 Gene Panel.

Gene	Genomic_Location	Strand	Accession Number (mRNA)	Name
ABL1	chr9:133589268-133763062	+	NM_005157	ABL proto-oncogene 1, non-receptor tyrosine kinase
ABL2	chr1:179068462-179198819	-	NM_007314	ABL proto-oncogene 2, non-receptor tyrosine kinase
ACVR1B	chr12:52345451-52390863	+	NM_004302	activin A receptor type 1B
ACVR2A	chr2:148602570-148688393	+	NM_001278579	activin A receptor type 2A
AJUBA	chr14:23440410-23451848	-	NM_198086	ajuba LIM protein
AKT1	chr14:105235687-105262080	-	NM_005163	AKT serine/threonine kinase 1
AKT2	chr19:40736224-40791302	-	NM_005163	AKT serine/threonine kinase 2
AKT3	chr1:243651535-244006886	-	NM_005465	AKT serine/threonine kinase 3
AKTIP	chr16:53525192-53537170	-	NM_001012398	AKT interacting protein
ALK	chr2:29415640-30144477	-	NM_004304	anaplastic lymphoma receptor tyrosine kinase
APC	chr5:112043202-112181936	+	NM_000038	APC, WNT signaling pathway regulator
AR	chrX:66763874-66950461	+	NM_000044	androgen receptor
ARAF	chrX:47420499-47431320	+	NM_001654	A-Raf proto-oncogene, serine/threonine kinase
ARID1A	chr1:27022522-27108601	+	NM_006015	AT-rich interaction domain 1A
ARID1B	chr6:157099064-157531913	+	NM_017519	AT-rich interaction domain 1B
ARID2	chr12:46123620-46301819	+	NM_152641	AT-rich interaction domain 2
ASXL1	chr20:30946147-31027122	+	NM_015338	additional sex combs like 1, transcriptional regulator
ATM	chr11:108093559-108239826	+	NM_000051	ATM serine/threonine kinase
ATR	chr3:142168077-142297668	-	NM_001184	ATR serine/threonine kinase
ATRX	chrX:76760356-77041719	-	NM_000489	ATRX, chromatin remodeler
AURKA	chr20:54944445-54967351	-	NM_198433	aurora kinase A
AURKB	chr17:8108049-8113883	-	NM_004217	aurora kinase B
AXIN1	chr16:337440-402676	-	NM_003502	axin 1
AXIN2	chr17:63524683-63557740	-	NM_004655	axin 2
AXL	chr19:41725108-41767671	+	NM_021913	AXL receptor tyrosine kinase
B2M	chr15:45003685-45010357	+	NM_004048	beta-2-microglobulin
BAP1	chr3:52435025-52444009	-	NM_004656	BRCA1 associated protein 1
BCL11A	chr2:60678302-60780633	-	NM_022893	B-cell CLL/lymphoma 11A

BCL2	chr18:60790579-60986613	-	NM_000633	BCL2, apoptosis regulator
BCOR	chrX:39910499-40036582	-	NM_017745	BCL6 corepressor
BIRC2	chr11:102217966-102249401	+	NM_001166	baculoviral IAP repeat containing 2
BRAF	chr7:140433813-140624564	-	NM_004333	B-Raf proto-oncogene, serine/threonine kinase
BRCA1	chr17:41196312-41277468	-	NM_007294	BRCA1, DNA repair associated
BRCA2	chr13:32889617-32973809	+	NM_000059	BRCA2, DNA repair associated
BTK	chrX:100604435-100641212	-	NM_000061	Bruton tyrosine kinase
CARD11	chr7:2945710-3083509	-	NM_032415	caspase recruitment domain family member 11
CASP8	chr2:202098166-202152434	+	NM_001228	caspase 8
CBL	chr11:119076990-119178859	+	NM_005188	Cbl proto-oncogene
CCND1	chr11:69455873-69469242	+	NM_053056	cyclin D1
CCND2	chr12:4382902-4414522	+	NM_001759	cyclin D2
CCND3	chr6:41902671-42016610	-	NM_001136017	cyclin D3
CCNE1	chr19:30302901-30315215	+	NM_001238	cyclin E1
CD274	chr9:5450503-5470567	+	NM_014143	CD274 molecule
CD79A	chr19:42381190-42385439	+	NM_001783	CD79a molecule
CD79B	chr17:62006098-62009704	-	NM_000626	CD79b molecule
CDC27	chr17:45195311-45266665	-	NM_001114091	cell division cycle 27
CDC73	chr1:193091088-193223942	+	NM_024529	cell division cycle 73
CDH1	chr16:68771195-68869444	+	NM_004360	cadherin 1
CDK12	chr17:37617739-37690800	+	NM_016507	cyclin dependent kinase 12
CDK4	chr12:58141510-58146230	-	NM_000075	cyclin dependent kinase 4
CDK6	chr7:92234235-92465941	-	NM_001259	cyclin dependent kinase 6
CDKN1B	chr12:12870302-12875305	+	NM_004064	cyclin dependent kinase inhibitor 1B
CDKN2A	chr9:21967751-21994490	-	NM_000077	cyclin dependent kinase inhibitor 2A
CDKN2C	chr1:51434367-51440309	+	NM_001262	cyclin dependent kinase inhibitor 2C
CEBPA	chr19:33790840-33793430	-	NM_004364	CCAAT/enhancer binding protein alpha
CHEK1	chr11:125495031-125546150	+	NM_001274	checkpoint kinase 1
CHEK2	chr22:29083731-29137822	-	NM_007194	checkpoint kinase 2
CIC	chr19:42788817-42799949	+	NM_015125	capicua transcriptional repressor
COL2A1	chr12:48366748-48398285	-	NM_001844	collagen type II alpha 1 chain
CREBBP	chr16:3775056-3930121	-	NM_004380	CREB binding protein
CSF1R	chr5:149432854-149492935	-	NM_005211	colony stimulating factor 1 receptor
CTCF	chr16:67596310-67673088	+	NM_006565	CCCTC-binding factor
CTLA4	chr2:204732511-204738683	+	NM_005214	cytotoxic T-lymphocyte associated protein 4
CTNNB1	chr3:41240942-41281939	+	NM_001904	catenin beta 1

CYLD	chr16:50775961-50835846	+	NM_015247	CYLD lysine 63 deubiquitinase
CYP2C19	chr10:96522463-96612671	+	NM_000769	cytochrome P450 family 2 subfamily C member 19
DAXX	chr6:33286335-33290793	-	NM_001350	death-domain associated protein
DDR2	chr1:162602228-162750247	+	NM_006182	discoidin domain receptor tyrosine kinase 2
DDX3X	chrX:41192651-41209524	+	NM_001356	DEAD-box helicase 3, X-linked
DICER1	chr14:95552565-95623759	-	NM_177438	dicer 1, ribonuclease III
DNMT3A	chr2:25455830-25565459	-	NM_175629	DNA methyltransferase 3 alpha
EGFR	chr7:55086725-55275031	+	NM_005228	epidermal growth factor receptor
ELF3	chr1:201979690-201986315	+	NM_004433	E74 like ETS transcription factor 3
EP300	chr22:41488614-41576081	+	NM_001429	E1A binding protein p300
EPCAM	chr2:47596287-47614167	+	NM_002354	epithelial cell adhesion molecule
EPHA2	chr1:16450832-16482582	-	NM_004431	EPH receptor A2
EPHA3	chr3:89156674-89531284	+	NM_005233	EPH receptor A3
EPHA5	chr4:66185281-66535653	-	NM_004439	EPH receptor A5
ERBB2	chr17:37844393-37884915	+	NM_004448	erb-b2 receptor tyrosine kinase 2
ERBB3	chr12:56473809-56497291	+	NM_001982	erb-b2 receptor tyrosine kinase 3
ERBB4	chr2:212240442-213403352	-	NM_005235	erb-b2 receptor tyrosine kinase 4
ERCC2	chr19:45854649-45873845	-	NM_000400	ERCC excision repair 2, TFIIH core complex helicase subunit
ERCC3	chr2:128014866-128051752	-	NM_000122	ERCC excision repair 3, TFIIH core complex helicase subunit
ERCC4	chr16:14014014-14046205	+	NM_005236	ERCC excision repair 4, endonuclease catalytic subunit
ERCC5	chr13:103498191-103528351	+	NM_000123	ERCC excision repair 5, endonuclease
ESR1	chr6:152011631-152424408	+	NM_000125	estrogen receptor 1
ETV1	chr7:13930856-14031050	-	NM_004956	ETS variant 1
EZH2	chr7:148504464-148581441	-	NM_004456	enhancer of zeste 2 polycomb repressive complex 2 subunit
FADD	chr11:70049269-70053508	+	NM_003824	Fas associated via death domain
FAM123B	chrX:63404997-63425624	-	NM_139285	APC membrane recruitment protein 1 (AMER1, WTX)
FANCA	chr16:89803959-89883065	-	NM_000135	Fanconi anemia complementation group A

FANCD2	chr3:10068113-10143614	+	NM_033084	Fanconi anemia complementation group D2
FBXW7	chr4:153242410-153456172	-	NM_033632	F-box and WD repeat domain containing 7
FGFR1	chr8:38268656-38326352	-	NM_015850	fibroblast growth factor receptor 1
FGFR2	chr10:123237844-123357972	-	NM_000141	fibroblast growth factor receptor 2
FGFR3	chr4:1795039-1810599	+	NM_000142	fibroblast growth factor receptor 3
FGFR4	chr5:176513921-176525126	+	NM_002011	fibroblast growth factor receptor 4
FH	chr1:241660857-241683085	-	NM_136850	fumarate hydratase
FLT1	chr13:28874483-29069265	-	NM_002019	fms related tyrosine kinase 1, vascular endothelial growth factor receptor 1
FLT3	chr13:28577411-28674729	-	NM_004119	fms related tyrosine kinase 3, CD135
FLT4	chr5:180028506-180076624	-	NM_182925	fms related tyrosine kinase 4, vascular endothelial growth factor receptor 3
FOXA1	chr14:38059191-38064489	-	NM_004496	forkhead box A1
FOXL2	chr3:138663066-138665982	-	NM_023067	forkhead box L2
FTO	chr16:53737875-54148379	+	NM_001080432	fat mass and obesity associated
GABRA6	chr5:161112658-161129598	+	NM_000811	gamma-aminobutyric acid type A receptor alpha6 subunit
GATA1	chrX:48644982-48652717	+	NM_002049	GATA binding protein 1
GATA2	chr3:128198265-128212030	-	NM_032638	GATA binding protein 2
GATA3	chr10:8096667-8117164	+	NM_002051	GATA binding protein 3
GNA11	chr19:3094408-3121454	+	NM_002067	G protein subunit alpha 11
GNAQ	chr9:80335191-80646219	-	NM_002072	G protein subunit alpha q
GNAS	chr20:57414795-57486250	+	NM_000516	GNAS complex locus
GSK3B	chr3:119540802-119813264	-	NM_002093	glycogen synthase kinase 3 beta
H3F3A	chr1:226250408-226259703	+	NM_002107	H3 histone, family 3A
H3F3B	chr17:73772515-73775860	-	NM_005324	H3 histone, family 3B (H3.3B)
HIST1H3B	chr6:26031817-26032288	-	NM_003537	histone cluster 1, H3b
HLA-A	chr6:29910247-29913661	+	NM_002116	major histocompatibility complex, class I, A
HNF1A	chr12:121416549-121440314	+	NM_000545	HNF1 homeobox A
HRAS	chr11:532242-535550	-	NM_005343	HRas proto-oncogene, GTPase
HSP90AB1	chr6:44214849-44221614	+	NM_007355	heat shock protein 90 alpha family class B member 1

IDH1	chr2:209100953-209119806	-	NM_005896	isocitrate dehydrogenase (NADP(+)) 1, cytosolic
IDH2	chr15:90627212-90645708	-	NM_002168	isocitrate dehydrogenase (NADP(+)) 2, mitochondrial
IGF1R	chr15:99192761-99507759	+	NM_000875	insulin like growth factor 1 receptor
IL7R	chr5:35856991-35876923	+	NM_002185	interleukin 7 receptor
JAK1	chr1:65298906-65432187	-	NM_002227	Janus kinase 1
JAK2	chr9:4985245-5128183	+	NM_004972	Janus kinase 2
JAK3	chr19:17935593-17958841	-	NM_000215	Janus kinase 3
KDM5C	chrX:53220503-53254604	-	NM_004187	lysine demethylase 5C
KDM6A	chrX:44732423-44971845	+	NM_001291415	lysine demethylase 6A
KDR	chr4:55944426-55991762	-	NM_002253	kinase insert domain receptor
KEAP1	chr19:10596796-10614054	-	NM_203500	kelch like ECH associated protein 1
KIT	chr4:55524095-55606881	+	NM_000222	KIT proto-oncogene receptor tyrosine kinase
KRAS	chr12:25358180-25403854	-	NM_004985	KRAS proto-oncogene, GTPase
LRP1B	chr2:140988996-142889270	-	NM_018557	LDL receptor related protein 1B
MAP2K1	chr15:66679211-66783882	+	NM_002755	mitogen-activated protein kinase kinase 1 (MEK1)
MAP2K2	chr19:4090320-4124126	-	NM_030662	mitogen-activated protein kinase kinase 2 (MEK2)
MAP2K4	chr17:11924135-12047051	+	NM_003010	mitogen-activated protein kinase kinase 4 (MEK4)
MAP3K1	chr5:56110900-56191978	+	NM_005921	mitogen-activated protein kinase kinase kinase 1 (MEKK1)
MAP3K13	chr3:185000729-185206882	+	NM_004721	mitogen-activated protein kinase kinase kinase 13 (MEKK13)
MAP3K4	chr6:161412822-161538417	+	NM_005922	mitogen-activated protein kinase kinase kinase 4 (MEKK4)
MAPK1	chr22:22113947-22221970	-	NM_002745	mitogen-activated protein kinase 1 (ERK)
MCL1	chr1:150547027-150552214	-	NM_021960	BCL2 family apoptosis regulator
MDM2	chr12:69201971-69239212	+	NM_002392	MDM2 proto-oncogene
MED12	chrX:70338406-70362304	+	NM_005120	mediator complex subunit 12
MEN1	chr11:64570986-64578766	-	NM_000244	menin 1
MET	chr7:116312459-116438440	+	NM_001127500	MET proto-oncogene, receptor tyrosine kinase
MITF	chr3:69788586-70017488	+	NM_198159	melanogenesis associated transcription factor
MLH1	chr3:37034841-37092337	+	NM_000249	mutL homolog 1
MLL	chr11:118307205-118397539	+	NM_001197104	KMT2A, histone 3- lysine methyltransferase 2A

MLL2	chr12:49412758-49449107	-	NM_003482	KMT2D, histone 3- lysine methyltransferas 2De, mixed lineage leukemia protein-2
MLL3	chr7:151832010-152133090	-	NM_170606	KMT2C, histone 3-lysine methyltransferase 2C
MPL	chr1:43803475-43820135	+	NM_005373	MPL proto-oncogene, thrombopoietin receptor
MSH2	chr2:47630263-47710360	+	NM_000251	mutS homolog 2
MSH6	chr2:48010221-48034092	+	NM_000179	mutS homolog 6
MST1	chr3:49721380-49726196	-	NM_020998	macrophage stimulating 1
MST1R	chr3:49924436-49941306	-	NM_002447	macrophage stimulating 1 receptor
MTOR	chr1:11166588-11322608	-	NM_004958	mechanistic target of ra- pamycin
MUTYH	chr1:45794914-45806142	-	NM_012222	mutY DNA glycosylase
MYD88	chr3:38179969-38184512	+	NM_001172567	myeloid differentiation primary response 88
NBN	chr8:90945564-90996899	-	NM_002485	nibrin
NCOR1	chr17:15933408-16118874	-	NM_006311	nuclear receptor core- pressor 1
NF1	chr17:29421945-29704695	+	NM_001042492	neurofibromin 1
NF2	chr22:29999545-30094589	+	NM_000268	neurofibromin 2 (merlin)
NFE2L2	chr2:178095031-178129859	-	NM_006164	nuclear factor, erythroid 2 like 2
NKX2-1	chr14:36985604-36989430	-	NM_001079668	NK2 homeobox 1
NOTCH1	chr9:139388896-139440238	-	NM_017617	notch receptor 1
NOTCH2	chr1:120454176-120612317	-	NM_024408	notch receptor 2
NOTCH3	chr19:15270444-15311792	-	NM_000435	notch receptor 3
NOTCH4	chr6:32162620-32191844	-	NM_004557	notch receptor 4
NPM1	chr5:170814708-170837888	+	NM_002520	nucleophosmin (nucleolar phosphoprotein B23, nu- matrin)
NRAS	chr1:115247085-115259515	-	NM_002524	neuroblastoma RAS viral oncogene homolog
NSD1	chr5:176560080-176727214	+	NM_172349	nuclear receptor binding SET domain protein 1
NTRK1	chr1:156785542-156851642	+	NM_002529	neurotrophic receptor ty- rosine kinase 1
NTRK3	chr15:88419988-88799962	-	NM_001012338	neurotrophic receptor ty- rosine kinase 3
PALB2	chr16:23614483-23652678	-	NM_024675	partner and localizer of BRCA2
PAX5	chr9:36838531-37034476	-	NM_016734	paired box 5
PBRM1	chr3:52579368-52719866	-	NM_018313	polybromo 1
PDCD1	chr2:242792033-242801058	-	NM_005018	programmed cell death 1
PDGFRA	chr4:55095264-55164412	+	NM_006206	platelet derived growth factor receptor alpha
PDGFRB	chr5:149493402-149535422	-	NM_002609	platelet derived growth factor receptor beta
PHF6	chrX:133507342- 133562822	+	NM_032458	PHD finger protein 6

PIK3CA	chr3:178866311-178952497	+	NM_006218	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PIK3CG	chr7:106505924-106547592	+	NM_002649	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma
PIK3R1	chr5:67511584-67597649	+	NM_181523	phosphoinositide-3-kinase regulatory subunit 1
PLCG1	chr20:39766161-39804357	+	NM_002660	phospholipase C gamma 1
PMS2	chr7:6012870-6048737	-	NM_000535	PMS1 homolog 2, mismatch repair system component
POLE	chr12:133200348-133263945	-	NM_006231	polymerase (DNA) epsilon, catalytic subunit
PPM1D	chr17:58677544-58743640	+	NM_003620	protein phosphatase, Mg2+/Mn2+ dependent 1D
PPP1R3A	chr7:113516882-113559082	-	NM_002711	protein phosphatase 1 regulatory subunit 3A
PPP2R1A	chr19:52693055-52729678	+	NM_014225	protein phosphatase 2 scaffold subunit Aalpha
PRDM1	chr6:106534195-106557814	+	NM_001198	PR domain 1
PREX2	chr8:68864603-69143897	+	NM_024870	phosphatidylinositol-3,4,5-trisphosphate dependent Rac exchange factor 2
PRG4	chr1:186265405-186283688	+	NM_005807	proteoglycan 4
PTCH1	chr9:98205264-98279247	-	NM_001083602	patched 1
PTEN	chr10:89623195-89728532	+	NM_000314	phosphatase and tensin homolog
PTK2	chr8:141668481-142011412	-	NM_153831	protein tyrosine kinase 2
PTPN11	chr12:112856536-112947717	+	NM_002834	protein tyrosine phosphatase, non-receptor type 11
PTPRB	chr12:70910632-71031220	-	NM_001109754	protein tyrosine phosphatase, receptor type B
RAC1	chr7:6414126-6443598	+	NM_006908	ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)
RAD51	chr15:40987327-41024356	+	NM_002875	RAD51 recombinase
RAD51C	chr17:56769963-56811692	+	NM_058216	RAD51 paralog C
RAF1	chr3:12625100-12705700	-	NM_001354689	Raf-1 proto-oncogene, serine/threonine kinase
RARA	chr17:38465423-38513895	+	NM_000964	retinoic acid receptor alpha
RB1	chr13:48877883-49056026	+	NM_000321	RB transcriptional corepressor 1
RET	chr10:43572517-43625797	+	NM_020975	ret proto-oncogene
RICTOR	chr5:38938022-39074501	-	NM_152756	RPTOR independent companion of MTOR complex 2
RNF43	chr17:56431038-56494931	-	NM_017763	ring finger protein 43



ROS1	chr6:117609530-117747018	-	NM_002944	ROS proto-oncogene 1, receptor tyrosine kinase
RPS6KB1	chr17:57970443-58027786	+	NM_003161	ribosomal protein S6 kinase B1
RPTOR	chr17:78518625-78940173	+	NM_020761	regulatory associated protein of MTOR complex 1
RUNX1	chr21:36160098-36421595	-	NM_001754	runt related transcription factor 1
RUNX1T1	chr8:92967195-93115454	-	NM_004349	RUNX1 translocation partner 1
SDHB	chr1:17345225-17380665	-	NM_003000	succinate dehydrogenase complex iron sulfur subunit B
SDHC	chr1:161284166-161334535	+	NM_003001	succinate dehydrogenase complex subunit C
SDHD	chr11:111957571-111966518	+	NM_003002	succinate dehydrogenase complex subunit D
SETBP1	chr18:42260138-42648475	+	NM_015559	SET binding protein 1
SETD2	chr3:47057898-47205467	-	NM_014159	SET domain containing 2
SF3B1	chr2:198256698-198299771	-	NM_012433	splicing factor 3b subunit 1
SMAD2	chr18:45359466-45457515	-	NM_005901	SMAD family member 2
SMAD3	chr15:67358195-67487533	+	NM_005902	SMAD family member 3
SMAD4	chr18:48556583-48611411	+	NM_005359	SMAD family member 4
SMARCA2	chr9:2015342-2193623	+	NM_003070	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2
SMARCA4	chr19:11071598-11172958	+	NM_001128849	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4
SMARCB1	chr22:24129150-24176705	+	NM_003073	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1
SMARCD1	chr12:50478983-50494494	+	NM_003076	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 1
SMC1A	chrX:53401070-53449618	-	NM_006306	structural maintenance of chromosomes 1A
SMC3	chr10:112327449-112364392	+	NM_005445	structural maintenance of chromosomes 3
SMO	chr7:128828713-128853385	+	NM_005631	smoothened, frizzled class receptor
SOCS1	chr16:11348274-11350039	-	NM_003745	suppressor of cytokine signaling 1

SOS1	chr2:39208690-39347604	-	NM_005633	SOS Ras/Rac guanine nucleotide exchange factor 1
SOX9	chr17:70117161-70122560	+	NM_000346	SRY-box 9
SPEN	chr1:16174359-16266950	+	NM_015001	spen family transcriptional repressor
SPOP	chr17:47676246-47755525	-	NM_001007226	speckle type BTB/POZ protein
SRC	chr20:35973088-36033821	+	NM_005417	SRC proto-oncogene, non-receptor tyrosine kinase
SRSF2	chr17:74730197-74733493	-	NM_003016	serine and arginine rich splicing factor 2
STAG2	chrX:123094475-123236505	+	NM_001042749	stromal antigen 2
STAT3	chr17:40465343-40540513	-	NM_139276	signal transducer and activator of transcription 3
STK11	chr19:1205798-1228434	+	NM_000455	serine/threonine kinase 11
STK19	chr6:31938952-31949223	+	NM_004197	serine/threonine kinase 19
SUFU	chr10:104263719-104393214	+	NM_016169	SUFU negative regulator of hedgehog signaling
SYK	chr9:93564012-93660842	+	NM_003177	spleen associated tyrosine kinase
TBC1D4	chr13:75858809-76056250	-	NM_014832	TBC1 domain family member 4
TBX3	chr12:115108059-115121969	-	NM_005996	T-box 3
TERT	chr5:1253287-1295162	-	NM_198253	telomerase reverse transcriptase
TET2	chr4:106067032-106200960	+	NM_001127208	tet methylcytosine dioxygenase 2
TGFB1	chr19:41836812-41859831	-	NM_000660	transforming growth factor beta 1
TGFBR1	chr9:101867412-101916473	+	NM_004612	transforming growth factor beta receptor 1
TGFBR2	chr3:30647994-30735633	+	NM_001024847	transforming growth factor beta receptor 2
TNF	chr6:31543350-31546112	+	NM_000594	tumor necrosis factor
TNFAIP3	chr6:138188581-138204449	+	NM_001270508	TNF alpha induced protein 3
TOP1	chr20:39657462-39753126	+	NM_003286	topoisomerase (DNA) I
TOP2A	chr17:38544773-38574202	-	NM_001067	topoisomerase (DNA) II alpha
TP53	chr17:7571720-7590868	-	NM_000546	tumor protein p53
TSC1	chr9:135766735-135820020	-	NM_000368	tuberous sclerosis 1
TSC2	chr16:2097990-2138713	+	NM_000548	tuberous sclerosis 2
TSHR	chr14:81421869-81612646	+	NM_000369	thyroid stimulating hormone receptor
U2AF1	chr21:44513066-44527688	-	NM_006758	U2 small nuclear RNA auxiliary factor 1
VEGFA	chr6:43737946-43754223	+	NM_001025366	vascular endothelial growth factor A
VHL	chr3:10183319-10195354	+	NM_000551	von Hippel-Lindau tumor suppressor

WHSC1L1	chr8:38132561-38239790	-	NM_023034	Wolf-Hirschhorn syn- drome candidate 1-like 1
WT1	chr11:32409322-32457081	-	NM_000378	Wilms tumor 1
XPO1	chr2:61705069-61765418	-	NM_003400	exportin 1
ZRSR2	chrX:15808574-15841382	+	NM_005089	zinc finger CCCH-type, RNA binding motif and serine/arginine rich 2

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**Table S3.** Primary and secondary antibodies.

<b>Primary Antibody</b>	<b>Host/ Isotype</b>	<b>Dilution/ Concentration</b>	<b>Source</b>	<b>Catalog Number</b>
anti-RAC1 <sup>a</sup>	Rabbit polyclonal IgG	1:200	Cell Signaling Technology	2465
anti-RAC1 <sup>b</sup>	Mouse monoclonal IgG	1:500	Cell Signaling Technology	8815
anti-ERK1/2	Rabbit polyclonal IgG	1:1000	Cell Signaling Technology	4695
anti-pERK1/2 (Thr202/Tyr204)	Rabbit polyclonal IgG	1:1000	Cell Signaling Technology	4370
anti-AKT	Rabbit polyclonal IgG	1:1000	Cell Signaling Technology	4691
anti-pAKT (Thr308)	Rabbit polyclonal IgG	1:2000	Cell Signaling Technology	4056
anti-PAK1	Rabbit polyclonal IgG	1:1000	Cell Signaling Technology	2602
anti-pPAK1 (Thr423)	Rabbit polyclonal IgG	1:1000	Cell Signaling Technology	2601
anti-TUBA4A	Mouse monoclonal IgG	1:500	Sigma-Aldrich	T9026
anti-VCL	Rabbit monoclonal IgG	1:500	Cell Signaling Technology	13901
<b>Secondary Antibody</b>	<b>Host/ Isotype</b>	<b>Dilution/ Concentration</b>	<b>Source</b>	<b>Catalog Number</b>
Mouse IgG	Goat	1:5000/WB	LI-COR	926-68070 (680RD)
Rabbit IgG	Goat	1:5000/WB	LI-COR	926-32211 (800CW)
Rat IgG	Goat	1:5000/WB	LI-COR	925-68076 (800RD)
Mouse IgG	Goat	1:5000/WB	LI-COR	925-32210 (800CW)

<sup>a</sup>Recognizes both RAC1 wild-type and RAC1 (P34R), <sup>b</sup>Recognizes only RAC1 wild-type.

**Table S4.** TaqMan and IDT probes/primers used for RT-qPCR and copy number assays.

<b>RT-qPCR:</b>	
<b>Gene</b>	<b>Probe ID</b>
<i>TWIST1</i>	Hs04989912_s1
<i>MET</i>	Hs01565584_m1
<i>BRAF</i>	Hs00269944_m1
<i>EGFR</i>	Hs01076090_m1
<i>RAC1</i>	Hs00251654_m1
<i>GAPDH</i>	Hs02758991_g1
<i>RPLP0</i>	Hs00420895_gH
<b>Copy number assays:</b>	
<b>Gene</b>	<b>Probe ID</b>
	<b>TaqMan</b>
<i>RAC1</i>	Hs04938353_cn
<i>TWIST1</i>	Hs02813890_cn
<i>EGFR</i>	Hs07526740_cn
<i>MET</i>	Hs01432482_cn
<i>BRAF</i>	Hs04949885_cn
<i>RPPH1</i> (RNase P, CNV reference assay)	Cat. # 4403326

**Table S5.** TaqMan and IDT probes/primers used for RT-qPCR and copy number assays.

<b>RT-qPCR:</b>	
<b>Gene</b>	<b>Probe ID</b>
<i>TWIST1</i>	Hs04989912_s1
<i>MET</i>	Hs01565584_m1
<i>BRAF</i>	Hs00269944_m1
<i>EGFR</i>	Hs01076090_m1
<i>RAC1</i>	Hs00251654_m1
<i>GAPDH</i>	Hs02758991_g1
<i>RPLP0</i>	Hs00420895_gH
<b>Copy number assays:</b>	
<b>Gene</b>	<b>Probe ID</b>
	<b>TaqMan</b>
<i>RAC1</i>	Hs04938353_cn
<i>TWIST1</i>	Hs02813890_cn
<i>EGFR</i>	Hs07526740_cn
<i>MET</i>	Hs01432482_cn
<i>BRAF</i>	Hs04949885_cn
<i>RPPH1</i> (RNase P, CNV reference assay)	Cat. # 4403326