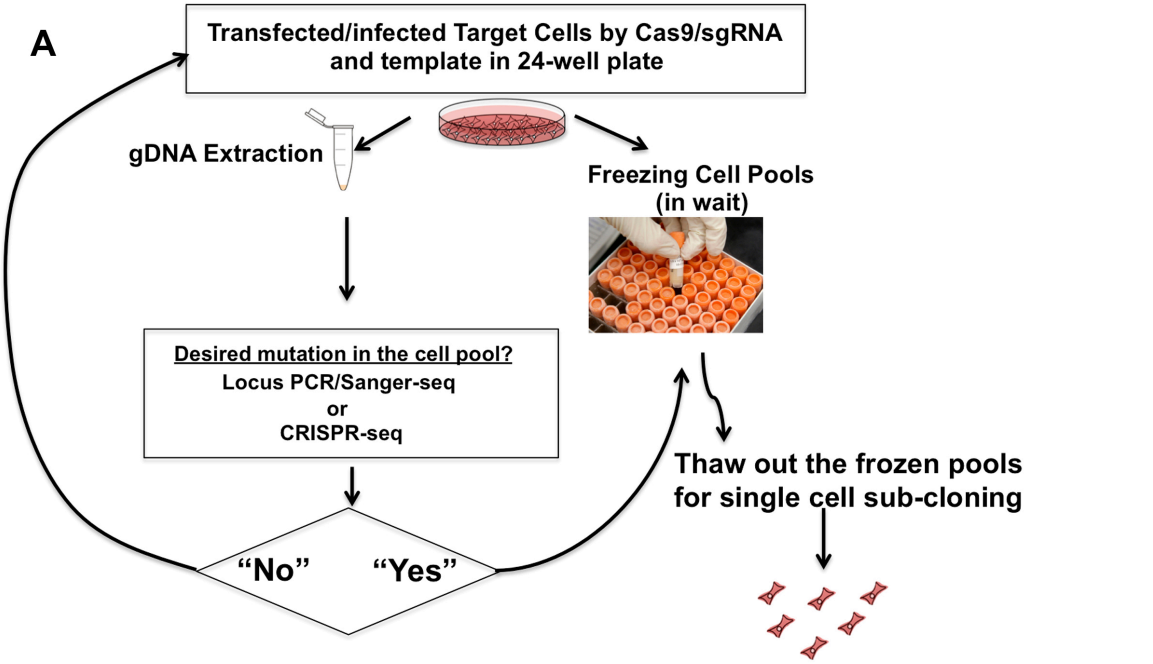
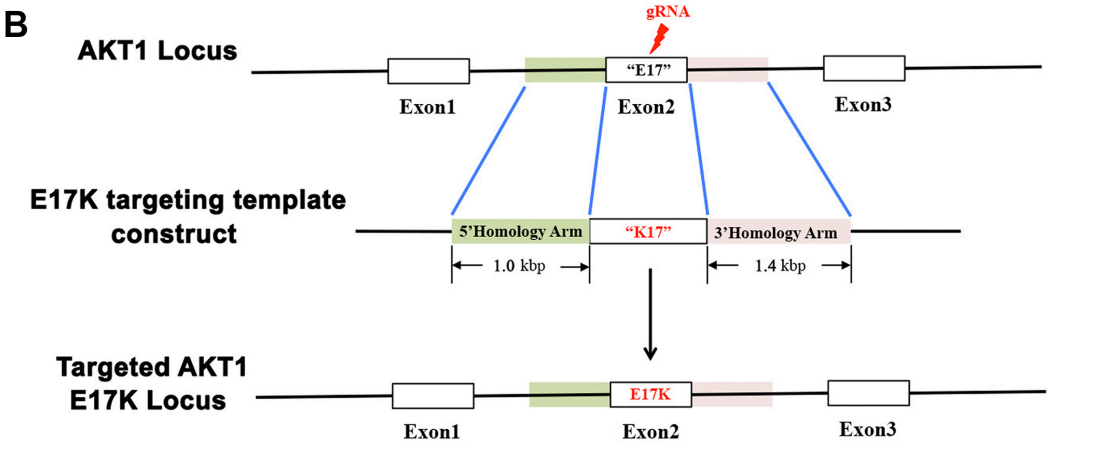
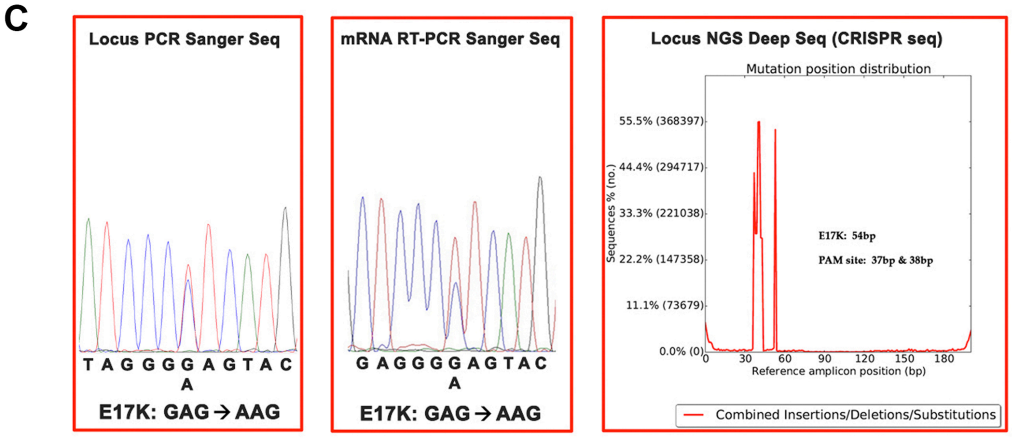
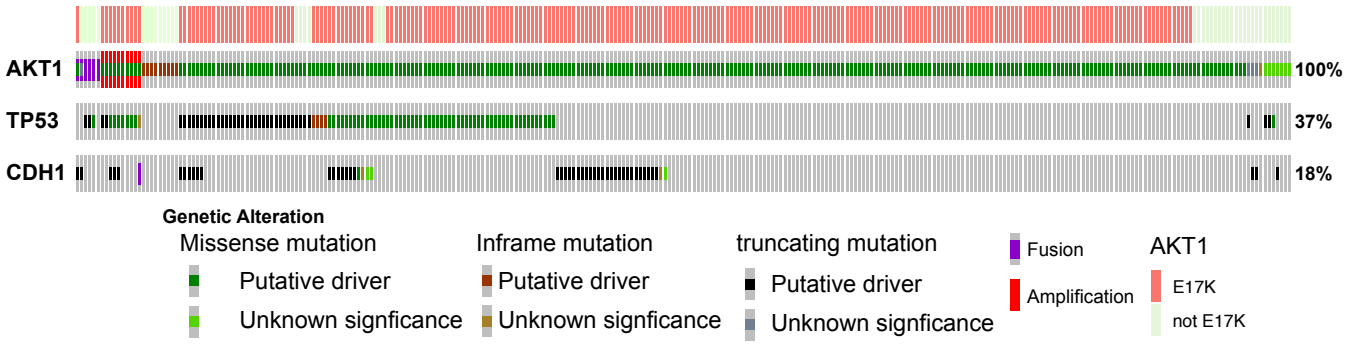

A
Transfected/infected Target Cells by Cas9/sgRNA and template in 24-well plate
gDNA Extraction
Freezing Cell Pools (in wait)
Desired mutation in the cell pool?
Locus PCR/Sanger-seq
or
CRISPR-seq
"No"
"Yes"
Thaw out the frozen pools for single cell sub-cloning
Validation of KI:
DNA, mRNA RT-PCR sequencing, Western blots

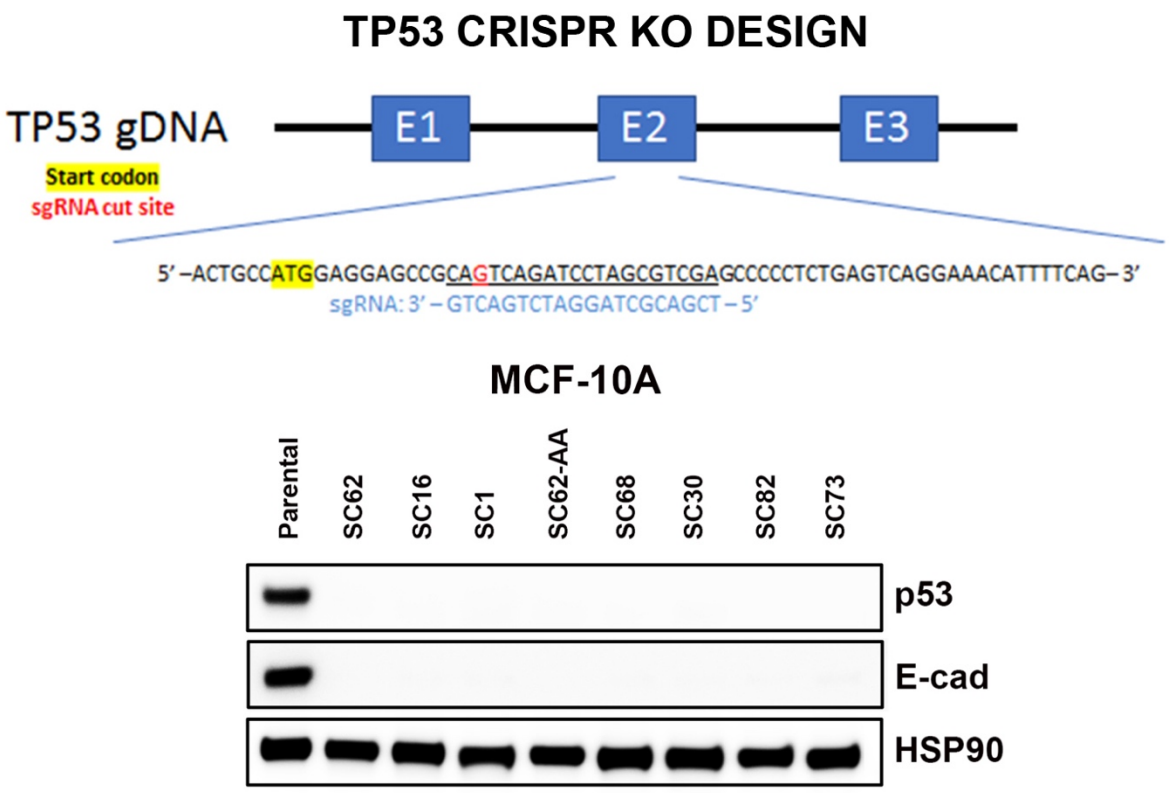

B
AKT1 Locus
gRNA
"E17"
Exon1
Exon2
Exon3
E17K targeting template construct
5'Homology Arm
"K17"
3'Homology Arm
1.0 kbp
1.4 kbp
Targeted AKT1 E17K Locus
E17K
Exon1
Exon2
Exon3


C
Locus PCR Sanger Seq
T A G G G G A G T A C A
E17K: GAG → AAG
mRNA RT-PCR Sanger Seq
G A G G G G A G T A C A
E17K: GAG → AAG
Locus NGS Deep Seq (CRISPR seq)
Mutation position distribution
55.5% (368397)
44.4% (294717)
33.3% (221038)
22.2% (147358)
11.1% (73679)
0.0% (0)
Sequences % (no.)
E17K: 54bp
PAM site: 37bp & 38bp
0
30
60
90
120
150
180
Reference amplicon position (bp)
Combined Insertions/Deletions/Substitutions

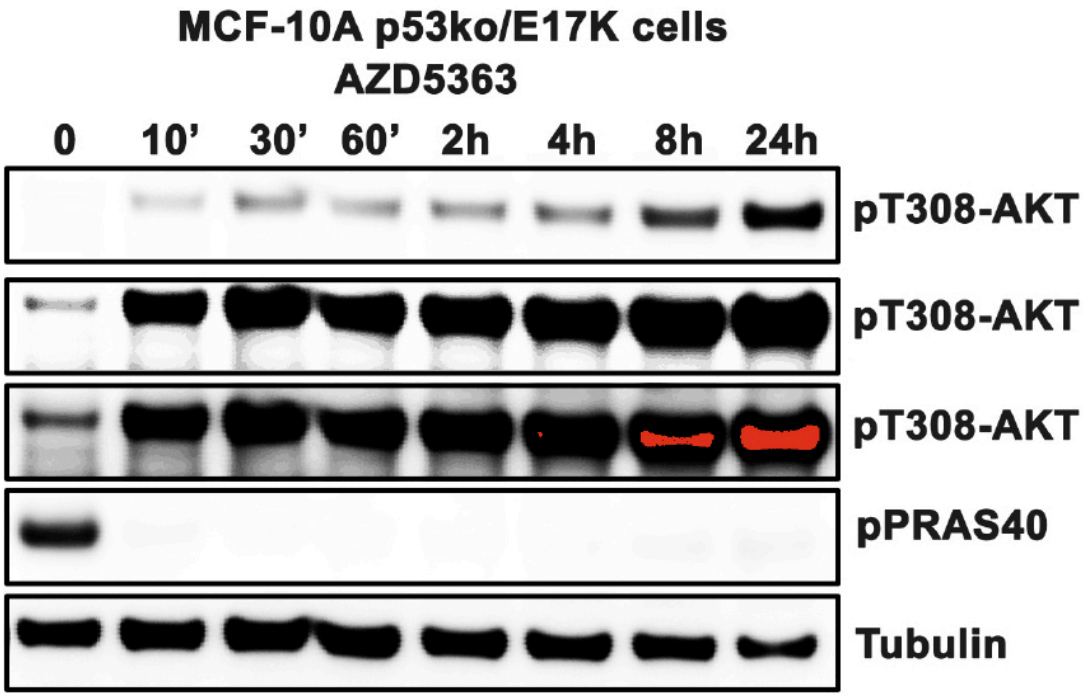
Supplementary Fig 1. Generation of MCF-10A isogenic cells via *TP53* knockout and AKT1 E17K knock-in. A. CRISPR Knock-in Survey: MCF-10A cells were infected with Cas9/sgRNA and AKT1 E17K template lentiviruses. A portion of the cell pool was used to extract genomic DNA (gDNA) and the rest was frozen. gDNA was tested for the desired AKT1 E17K mutation by locus PCR/Sanger or CRISPR-seq. If the AKT1 E17K mutation was present, the cell pool was thawed and sorted for single clones. B. Schematic of AKT1 E17K CRISPR knock-in (KI) strategy. MCF-10A cells were simultaneously infected with lentivirus containing a 2.4kbp template with homologous arms around the AKT1 E17 site with the K17 mutation and a CRISPR/Cas9 sgRNA targeting 12bp upstream of E17. C. Validation of the presence of a heterozygous AKT1 E17K mutation. Left and middle panels: Sanger Sequencing results of the genomic locus PCR product and the AKT1 mRNA RT-PCR product. Right panel: Next generation sequencing (NGS) result of the 200bp genomic locus PCR product. The point mutations at 37bp and 38bp are the PAM site mutations. The point mutation at 54bp is G → A (E17K).



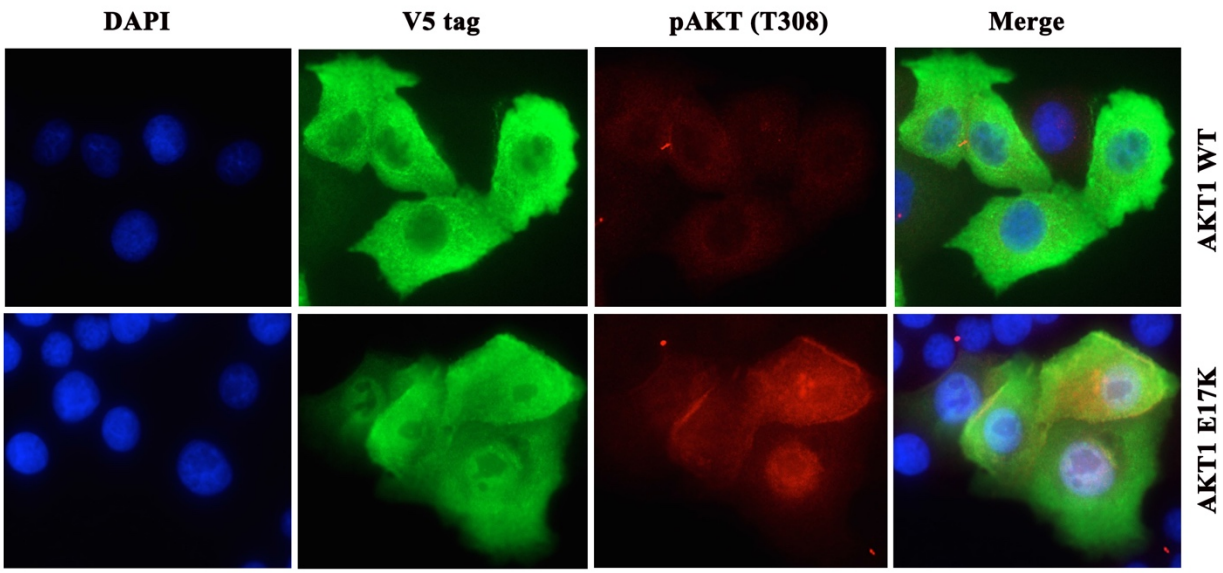
Supplementary Fig 2. Frequent co-occurrence of *AKT1*, *TP53* and *CDH1* mutations in breast cancers. Tumors were sequenced prospectively with results reported to patients and their physicians as part of the MSK-IMPACT tumor profiling initiative. Only tumors with AKT1 mutations were included in the OncoPrint (294 breast cancers had AKT1 mutations of a total of 5635 breast cancers sequenced. 249 tumors harbored AKT1 E17K mutations).



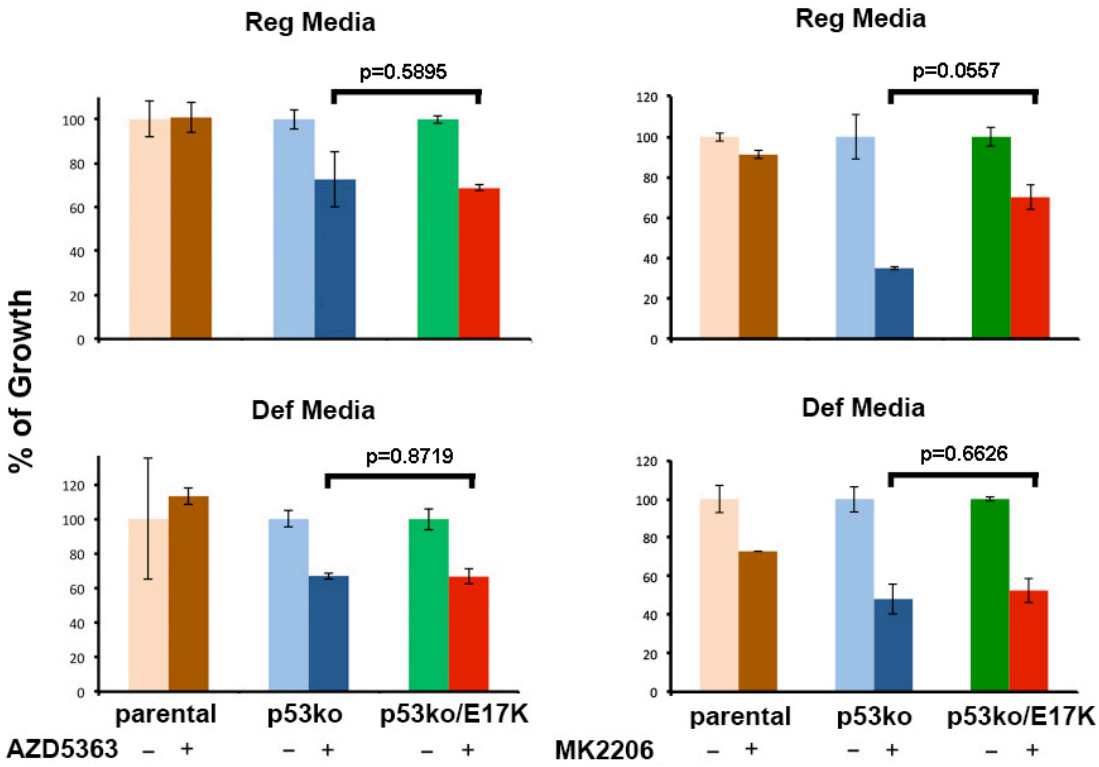
Supplementary Fig 3. *TP53* CRISPR knockout in MCF-10A cells. Lentiviral CRISPR Cas9/sgRNAs targeting the *TP53* gene were used to infect MCF-10A cells (one sgRNA sequence is shown). Single cell clones were screened for p53 knockout and E-cadherin expression via Western blot analysis.



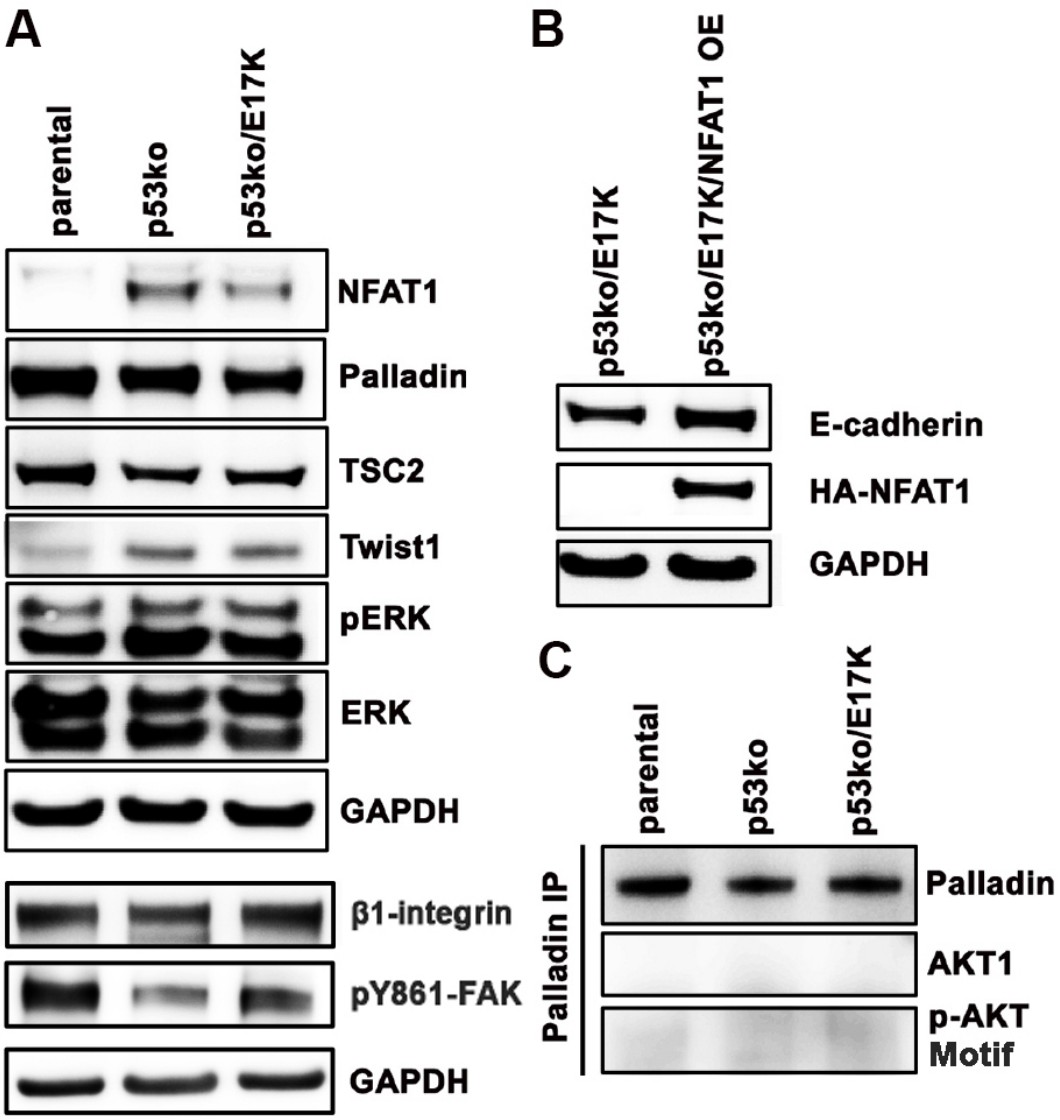
Supplementary Fig 4. Phosphorylation of the AKT1 effector PRAS40 was AKT-dependent in MCF-10A p53ko/E17K cells. Cells were grown in growth factor deficient media and then treated with 3μM AZD5363 for the indicated time periods. Expression of pT308-AKT is shown using three exposures from shortest to longest.



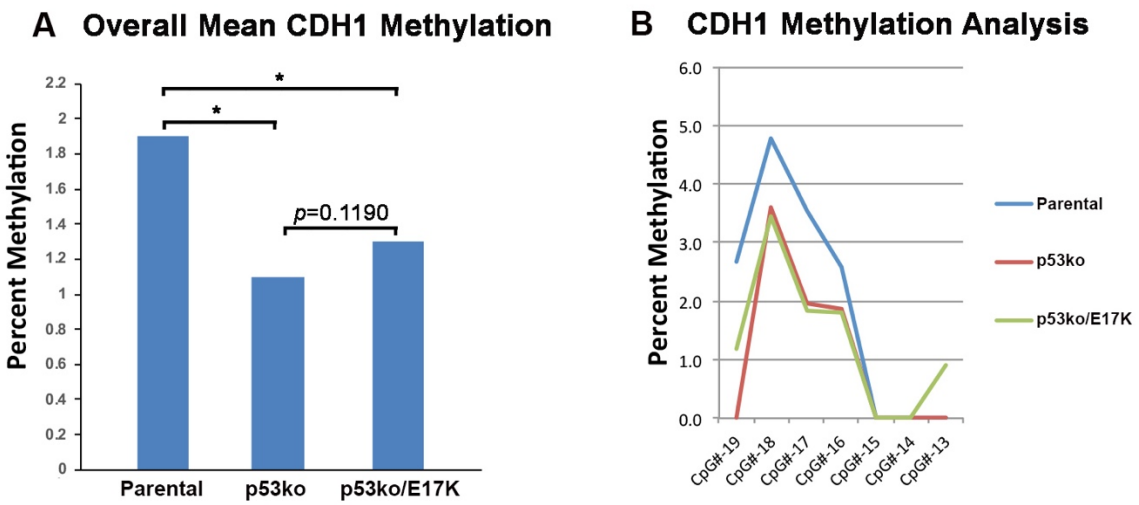
Supplementary Fig 5. Immunofluorescence using a pT308 specific AKT1 antibody of MCF-10A cells following lentiviral infection with V5 tagged (V5 tag) wildtype (WT) or E17K mutant AKT1.



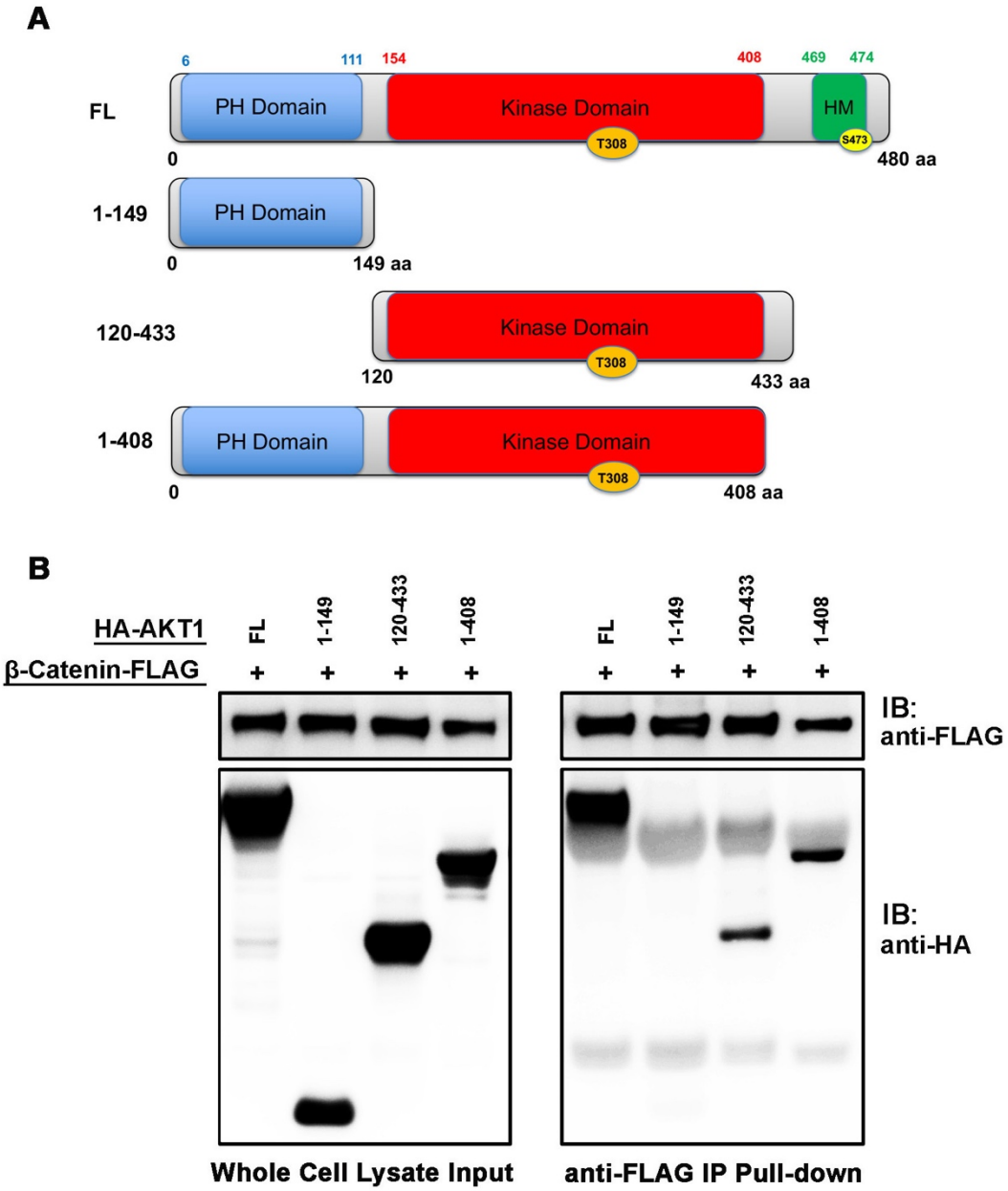
Supplementary Fig 6. Knock-in of the AKT1 E17K mutation did not enhance sensitivity to the pan-AKT kinase inhibitor AZD5363 or the allosteric pan-AKT inhibitor MK2206 in 2D culture conditions. MCF-10A parental, p53ko and p53ko/E17K cells were grown in regular (Reg) or growth factor deficient (Def) media with or without addition of AZD5363 (3 μM) or MK2206 (2.5 μM) for 7 days. Cell counts were determined by Vi-CELL. Percent (%) growth for drug treated cells was calculated by: 100 x (mean of treated cell number)/(mean of untreated, control cell number). Error bars represent mean ± SEM. Percentages inhibition were calculated for p53ko and p53ko/E17K cells under each treatment condition, which were analyzed by Student's *t* test and *P* values were determined.



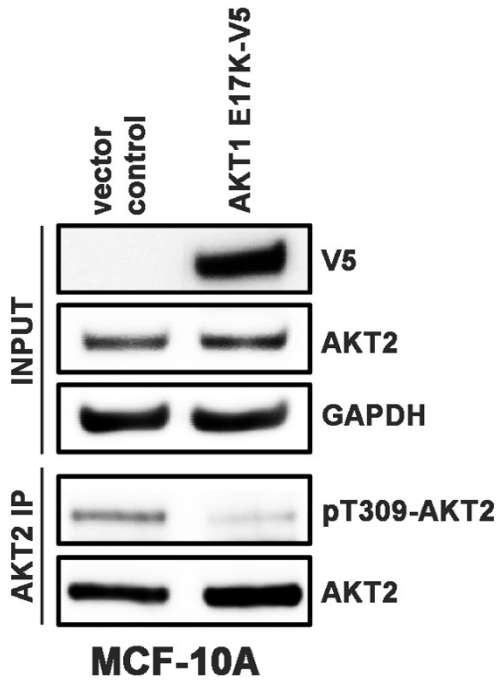
Supplementary Fig 7. Survey of previously postulated mechanisms for AKT1-mediated inhibition of cell migration in breast cancer cells. A. Whole cell lysates were subjected to Western blot analysis with the indicated antibodies. B. p53ko/E17K cells were transfected with WT NFAT1 and lysed after two days. Whole cell lysates were then subjected to Western blot analysis. C. Cells were lysed and immunoprecipitated with anti-palladin antibody, and immunoprecipitates were then subjected to Western blot analysis.



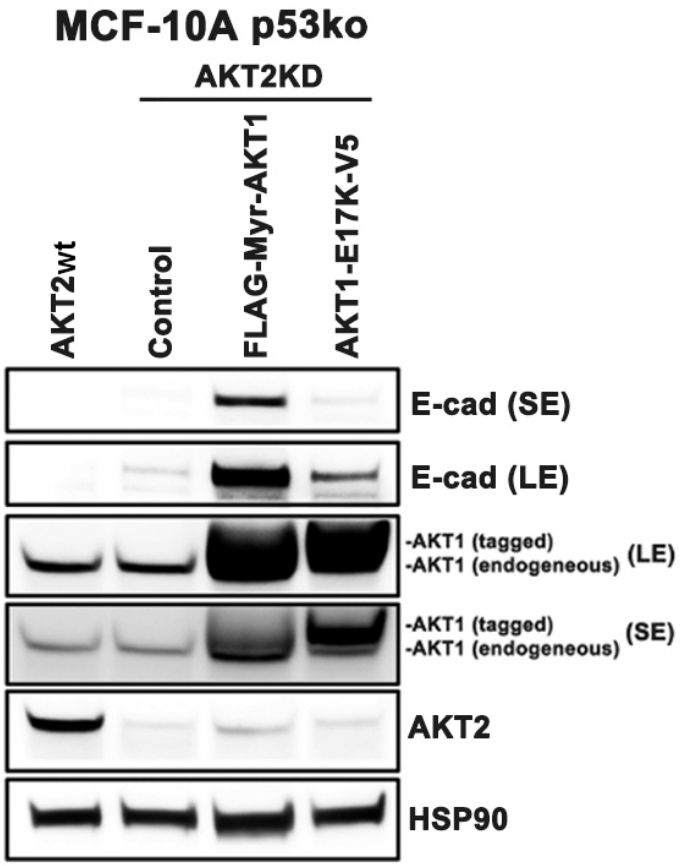
Supplementary Fig 8. *CDH1* promoter methylation status in the MCF-10A isogenic cells. MCF-10A genomic DNA was extracted from cell pellets using QuickExtract DNA Extraction Solution per the manufacturer's instructions (Epicentre). The DNA samples were then sent to EpigenDx (Hopkinton, MA) where they underwent bisulfite modification and Pyrosequencing surrounding the *CDH1* promoter region (assay ADS907). Results were reported as percent methylation for seven CpG islands. A. Overall mean methylation in each cell line at 7 different CpG islands within the *CDH1* gene promoter. *P* values were determined by Student's *t* test. **P*<0.05. B. Percent methylation at each CpG island.



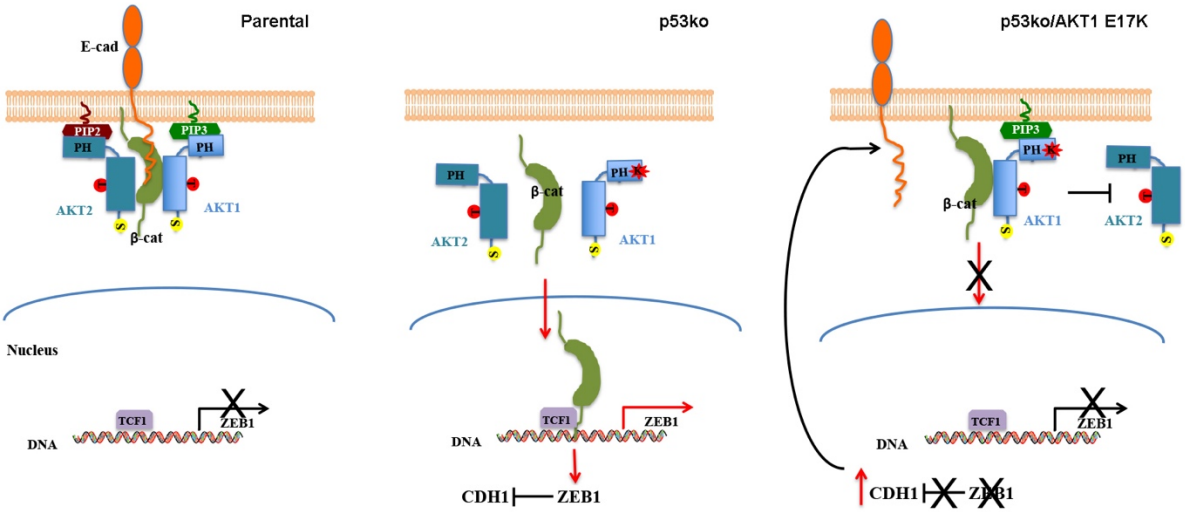
Supplementary Fig 9. β-catenin interacts with the kinase domain of AKT1. A. Schematic representation of different truncated fragments of AKT1 used for coimmunoprecipitation (Co-IP). PH: pleckstrin homology domain. HM: hydrophobic motif. B. Co-IP assays in 293T cells transfected with DNA constructs of the indicated proteins and immunoblotted with FLAG and HA antibodies. IB: immunoblotting.



Supplementary Fig 10. Expression of AKT1 E17K decreased phosphorylation of AKT2 in MCF-10A cells. Western blots of input cell lysates and immunoprecipitates from MCF-10A cells lentiviral-infected by AKT1-E17K-V5 using the indicated antibodies.



Supplementary Fig 11. AKT2 knockdown induces E-cadherin expression in MCF-10A p53ko cells. Western blots of lysates from MCF-10A p53ko cells (AKT2 wt) and those in which AKT2 was knocked down using CRISPR/Cas9 (AKT2KD), with or without co-lentiviral infection of FLAG tagged Myr-AKT1 or V5 tagged AKT1 E17K. SE: shorter exposure. LE: longer exposure.



Supplementary Fig 12. Model of the mechanism of paradoxical inhibition of cell migration by activated AKT1. In parental MCF-10A cells, β-catenin is localized to the cell membrane and thus unable to transactivate the *ZEB1* promoter. In p53 null cells, AKT2 is activated and β-catenin is translocated into the cell nucleus, where it can function as a transcriptional activator for *ZEB1*. ZEB1 then inhibits the expression of *CDH1*, leading to epithelial mesenchymal transition (EMT) and increased cell migration and invasion. Expression of the AKT1 E17K mutation in a p53 null background results in localization of AKT1 to the cell membrane where it sequesters β-catenin leading to decreased ZEB1 mRNA and protein expression. This decrease in ZEB1 relieves the transcriptional repression on *CDH1* resulting in increased E-cadherin expression on the cell membrane and a paradoxical reduction in cell migration and invasion.

Supplementary Table 1. List of oligonucleotide sequences

Name	Sequence
AKT1 E17K Template-F	TGAATTCACCCCTCAGATGAAGCAGTTG
AKT1 E17K Template-R	TGAATTCCTCCCCTTATACGTGCAGGC
AKT1E17K-PAM mutagen-F	CACCACTCGCACGTCTGTAGGGAAGTACATCAAGACCTGGCG
AKT1E17K-PAM mutagen-R	CGCCAGGTCTTGATGTACTTCCCTACAGACGTGCGAGTGGTG
AKT1 E17K-1 sgRNA	GTACTCCCCTACAGACGTGC
AKT1-ATG sgRNA	CTCGGGCACCATGAGCGACG
AKT2 sgRNA	GCGGCCTCTTACCACGCTTG
TP53-1 sgRNA	CAGTCAGATCCTAGCGTCGA
TP53-3 sgRNA	GAAACTGTGAGTGGATCCAT
ZEB1 sgRNA	TGACTGTGAAGGTGTACCAG
PTEN sgRNA	AAACAAAAGGAGATATCAAG

Supplementary Table 2. List of antibodies

Antibody	Vendor	Cat#
AKT1	CST	2938
AKT/pT308	CST	2965, 13038
p-AKT (RXXS*/T*) motif	CST	9614
AKT2	CST	2964, 5239
β-catenin	CST	8480
β-catenin/pS552	CST	5651
β1-Integrin	CST	9699
DDK	ORIGENE	TA50011
E-cadherin	BD Transduction Lab	610181
FAK/pY861	Abcam	4804
FLAG	Sigma Aldrich	F9291, F3165
GAPDH	Millipore Chemicon	MAB374
HA	CST	3724
HSP90	CST	4877
N-cadherin	CST	13116
NFAT1	CST	5861
p53	SCBT	sc-126
palladin	Proteintech	10853-1-AP
PRAS40-pT246	CST	2997
PTEN	CST	9559
Slug	CST	9585
Snail	CST	3879
TSC2	CST	3635
tubulin	Sigma Aldrich	T9026
Twist	Abcam	50887
V5	SCBT	81594
ZEB1	CST	3396

Supplementary Table 3. List of plasmids

Plasmid Name	Vendor	Cat#
LentiCRISPR v2	Addgene	52961
LV-GFP	Addgene	25999
Retro WT NFAT1	Addgene	11100
myr-FLAG-AKT1-pcw107 lentiV	Addgene	64606
1227 pcDNA3 Myr HA Akt2	Addgene	9016
1036 pcDNA3 Myr HA Akt1	Addgene	9008
pCDNA3-HA-Akt1	Addgene	73408
pCDNA3-HA-Akt1-aa1-149	Addgene	73410
pCDNA3-HA-Akt1-aa120-433	Addgene	73411
pCDNA3-HA-Akt1-aa1-408	Addgene	73412
human beta-catenin pcDNA3	Addgene	16828
Lenti-C-Myc-DDK-IRES-Neo-PIK3CA E545K	OriGene	RC400384