Supplementary Figure 1. Genomic DNA and cDNA sequencing verification of additional gene-targeted MCF-7 derivatives. Sequences of PIK3CA exon 9 from Het-2 and Het-3, in which one mutant copy of PIK3CA has been corrected back to wild type (and one mutant allele remains) are shown. Below, sequences from AKT1 exon 3 from two additional AKT1 E17K knock-in clones are shown.
Supplementary Figure 2. PI3K pathway signaling in low serum conditions. Cells were grown in medium containing 0.5% CD-FBS and equal amounts of protein lysates were loaded on SDS-PAGE gels and blotted with the antibodies shown. All three gene targeted Het clones with a single mutant PIK3CA E545K allele and all three AKT1 E17K cell lines are shown, along with parental MCF-7 and MCF-7$^{PIK3CAWT}$ cells. All clones with the same genotype show similar signaling output, and the pattern of signal activation that is dependent on mutant PIK3CA or AKT1 is identical to the pattern seen in 5% FBS medium conditions (Figure 2).
Supplementary Figure 3. Estrogenic stimulation of growth. Cells of the indicated genotypes were grown in media with 0.5% CD-FBS, with or without estrogen supplementation (1nM β-estradiol). Cells were counted on day 4. Relative cell number in estrogen compared to no estrogen is shown. The graph depicts the average of two experiments, with standard deviations.
Supplementary Figure 4. GDC-0941 and MK-2206 treatment of MCF-7 cells and three gene-targeted derivatives with a single mutant copy of PIK3CA (Het 1-3). Cells were treated with increasing concentrations of the indicated drugs. Viable cell number was determined with a luminescence assay. Experiments were performed in triplicate and repeated three times. Averages and standard errors of all three experiments are shown.
Supplementary Figure 5. MK-2206 treatment of isogenic MCF-10A cells. Cells were grown in medium containing EGF and treated with increasing concentrations of MK-2206. Viable cell number was determined with a luminescence assay. Experiments were performed in triplicate and repeated three times. Averages and standard deviations of all three experiments are shown. 10A KI control cells are MCF-10A cells with a gene targeting event at the AKT1 locus that preserved the wild type AKT1 sequence.
Supplementary Figure 6. Perifosine treatment of MCF-7 and cells with wild type PIK3CA and AKT1, mutant PIK3CA, or mutant AKT1 genes. Cells were grown in the media described in Methods and treated with increasing concentrations of perifosine. Viable cell number was determined with a luminescence assay. Experiments were performed in triplicate and repeated three times. Averages and standard deviations of all three experiments are shown.