Figure S1. Alignment of human and canine PIK3CA. Canine PIK3CA (GenBank accession no. BCZ50593.1) was Sanger-sequenced and aligned with that of human PIK3CA (NP_006209.2) using Genetyx software. Conserved residues are denoted (*). The mutation hot-spot (1047His) in human PIK3CA is boxed. PIK3CA, p110 α phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

Human Canine	1:MPPRPSSGELWGIHLMPPRILVECLLPNGMIVTLECLREATLITIKHELFKEARKYPLHQ 1:MPPRPSSGELWGIHLMPPRILVECLLPNGMIVTLECLREATLITIKHELFKEARKYPLHQ ************************************	60 60
Human Canine	61:LLQDESSYIFVSVTQEAEREEFFDETRRLCDLRLFQPFLKVIEPVGNREEKILNREIGFA 61:LLQDESSYIFVSVTQEAEREEFFDETRRLCDLRLFQPFLKVIEPVGNREEKILNREIGFA ************************************	120 120
Human Canine	121:IGMPVCEFDMVKDPEVQDFRRNILNVCKEAVDLRDLNSPHSRAMYVYPPNVESSPELPKH 121:IGMPVCEFDMVKDPEVQDFRRNILNVCKEAVDLRDLNSPHSRAMYVYPPNVESSPELPKH ************************************	180 180
Human Canine	181:IYNKLDKGQIIVVIWVIVSPNNDKQKYTLKINHDCVPEQVIAEAIRKKTRSMLLSSEQLK 181:IYNKLDKGQIIVVIWVIVSPNNDKQKYTLKINHDCVPEQVIAEAIRKKTRSMLLSSEQLK ************************************	240 240
Human Canine	241:LCVLEYQGKYILKVCGCDEYFLEKYPLSQYKYIRSCIMLGRMPNLMLMAKESLYSQLPMD 241:LCVLEYQGKYILKVCGCDEYFLEKYPLSQYKYIRSCIMLGRMPNLMLMAKESLYSQLPMD ************************************	300 300
Human Canine	301:CFTMPSYSRRISTATPYMNGETSTKSLWVINSALRIKILCATYVNVNIRDIDKIYVRTGI 301:CFTMPSYSRRISTATPYMNGETSTKSLWVINSALRIKILCATYVNVNIRDIDKIYVRTGI ************************************	360 360
Human Canine	361:YHGGEPLCDNVNTQRVPCSNPRWNEWLNYDIYIPDLPRAARLCLSICSVKGRKGAKEEHC 361:YHGGEPLCDNVNTQRVPCSNPRWNEWLNYDIYIPDLPRAARLCLSICSVKGRKGAKEEHC ***********************************	420 420
Human Canine	421:PLAWGNINLFDYTDTLVSGKMALNLWPVPHGLEDLLNPIGVTGSNPNKETPCLELEFDWF 421:PLAWGNINLFDYTDTLVSGKMALNLWPVPHGLEDLLNPIGVTGSNPNKETPCLELEFDWF ***********************************	480 480
Human Canine	481:SSVVKFPDMSVIEEHANWSVSREAGFSYSHAGLSNRLARDNELRENDKEQLKAISTRDPL 481:SSVVKFPDMSVIEEHANWSVSREAGFSYSHAGLSNRLARDNELRENDKEQLRAICTRDPL ************************************	540 540
Human Canine	541:SEITEQEKDFLWSHRHYCVTIPEILPKLLLSVKWNSRDEVAQMYCLVKDWPPIKPEQAME 541:SEITEQEKDFLWSHRHYCVTIPEILPKLLLSVKWNSRDEVAQMYCLVKDWPPIKPEQAME ************************************	600 600
Human Canine	601:LLDCNYPDPMVRGFAVRCLEKYLTDDKLSQYLIQLVQVLKYEQYLDNLLVRFLLKKALTN 601:LLDCNYPDPMVRGFAVRCLEKYLTDDKLSQYLIQLVQVLKYEQYLDNLLVRFLLKKALTN ************************************	660 660
Human Canine	661:QRIGHFFFWHLKSEMHNKTVSQRFGLLLESYCRACGMYLKHLNRQVEAMEKLINLTDILK 661:QRIGHFFFWHLKSEMHNKTVSQRFGLLLESYCRACGMYLKHLNRQVEAMEKLINLTDILK ************************************	720 720
Human Canine	721:QEKKDETQKVQMKFLVEQMRRPDFMDALQGFLSPLNPAHQLGNLRLEECRIMSSAKRPLW 721:QEKKDETQKVQMKFLVEQMRRPDFMDALQGFLSPLNPAHQLGNLRLEECRIMSSAKRPLW ************************************	780 780
Human Canine	781:LNWENPDIMSELLFQNNEIIFKNGDDLRQDMLTLQIIRIMENIWQNQGLDLRMLPYGCLS 781:LNWENPDIMSELLFQNNEIIFKNGDDLRQDMLTLQIIRIMENIWQNQGLDLRMLPYGCLS ************************************	840 840
Human Canine	841:IGDCVGLIEVVRNSHTIMQIQCKGGLKGALQFNSHTLHQWLKDKNKGEIYDAAIDLFTRS 841:IGDCVGLIEVVRNSHTIMQIQCKGGLKGALQFNSHTLHQWLKDKNKGEIYDAAIDLFTRS ************************************	900 900
Human Canine	901:CAGYCVATFILGIGDRHNSNIMVKDDGQLFHIDFGHFLDHKKKKFGYKRERVPFVLTQDF 901:CAGYCVATFILGIGDRHNSNIMVKDDGQLFHIDFGHFLDHKKKKFGYKRERVPFVLTQDF ************************************	960 960
Human Canine	961:LIVISKGAQECTKTREFERFQEMCYKAYLAIRQHANLFINLFSMMLGSGMPELQSFDDIA 961:LIVISKGAQECTKTREFERFQEMCYKAYLAIRQHANLFINLFSMMLGSGMPELQSFDDIA ***********************************	1020 1020
Human Canine	1021:YIRKTLALDKTEQEALEYFMKQMNDA <mark>H</mark> HGGWTTKMDWIFHTIKQHALN 1068 1021:YIRKTLALDKTEQEALEYFMKQMNDAHHGGWTTKMDWIFHTIKQHALN 1068	

Figure S2. Mutation sensitivities of anti-cancer drug and phosphorylation profiles of canine mammary gland tumor cell lines. (A) Electropherograms of Sanger sequencing of PIK3CA of 3 canine mammary gland tumor cell lines. (B) PIK3CA WT or heterozygous/homozygous mutant cells were treated with increasing concentrations of alpelisib (0, 1, 2, 5, 10, 20, 50 and 100 μ M) for 24 h. (C) Cells were treated with doxorubicin (0, 0.1, 0.5, 1, 5, 10 and 50 μ M) for 24 h. The IC₅₀ values and cell viability using an MTT assay were determined by measuring the absorbance at 560 nm in a microplate reader. The values are shown from four independent experiments as the mean \pm SD. (D) Detection of Akt phosphorylation in canine mammary gland tumor cell lines with 10 μ M alpelisib treatment using western blot analysis with 100 μ M EGF stimulations. PIK3CA, p110 α phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; WT, wild-type; M, lanes of loaded protein molecular weight marker.

