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Supplementary Informations

Inhibition of AKT suppresses the initiation and progression of *BRCA1*-associated mammary tumors

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Supplementary Table 1. Quantification of mammary gland structure in *Brcal*- and *Akt1*-mutant mice.

	Length (mm)	Branch (N)	N
<i>Brcal</i> ^{co/co}	252±25	338±55	11
<i>Brcal</i> ^{co/co} <i>Akt1</i> ^{-/-}	71±29**	81±42**	11
<i>Brcal</i> ^{co/co} <i>MMTV-Cre</i>	233±24	315±52	10
<i>Brcal</i> ^{co/co} <i>MMTV-Cre</i> <i>Akt1</i> ^{-/-}	86±21**	98±26**	19

¹Lengths and branching numbers of ducts between the lymph node and distal end were measured in mammary glands of 2-month-old mice, using the Branch software (ver. 1.1).

²The numbers represent mean±SE. Significant differences are indicated by asterisks; ***P*<0.01.

Baek *et al.***Supplementary Table 2. List of MK-2206 response-associated genes**

Gene symbol	Name	GO molecular function/ biological process	Chr #	Entrez Gene ID	Accession No.	PCR primers for comparative RT-qPCR
<i>Apln</i>	apelin	apelin receptor binding	X	30878	NM_013912.4	Forward: 5'-CACTGATGTTGCCTCCAGAT-3' Reverse: 5'-GTTCTGGGCTTCACCAGGTA-3'
<i>Tmc5</i>	transmembrane channel-like gene family 5	ion transport	7	74424	NM_001105252.1	Forward: 5'-AAAAAGGTTCTGGGTTGCT-3' Reverse: 5'-TGGAAGGAAGACATGATGCT-3'
<i>Pipox</i>	pipecolic acid oxidase	L-pipecolate oxidase activity	11	19193	NM_008952.2	Forward: 5'-CCCAATCAGCCTCCCTAGTC-3' Reverse: 5'-TGCTGTTCTGTGATGGTGAA-3'
<i>Slc4a2</i>	solute carrier family 4 (anion exchanger), member 2	enzyme binding	5	20535	NM_001253892.1	Forward: 5'-AGCCAGGACGAAGCTATGGA-3' Reverse: 5'-GTGGATAGCGGATGATGGAT-3'
<i>Plxna1</i>	plexin A1	semaphorin receptor activity	6	18844	NM_008881.2	Forward: 5'-TGGGACCCTCCTAACAGTCA-3' Reverse: 5'-TTCCCTCTCAATGCCTCCAT-3'
<i>Srgap3</i>	SLIT-ROBO Rho GTPase activating protein 3	Rac GTPase binding	6	259302	NM_080448.4	Forward: 5'-GCCAGTTACGCTGTGCAGAT-3' Reverse: 5'-GCTCTGCTCTTGGGACATTT-3'
<i>Iqsec1</i>	IQ motif and Sec7 domain 1	lipid binding	6	232227	NM_001134383.1	Forward: 5'-CATTAGCCAGCGGTACTGT-3' Reverse: 5'-AAGGCCAGGATGAAGATGGT-3'

Supplementary Table 3. List of enriched GO terms

GO ID	Description	Gene count	False discovery rate	Proteins
GO.0007165	signal transduction	18	1.86E-06	Agtr1a, Apln, Aplnr, Farp2, Fyn, Nrp1, Pak2, Pak3, Plxn1, Rac1, Rnd1, Rras, Sema3a, Sema3e, Sema3f, Srgap3, Trem2, Tyrobp
GO.0016358	dendrite development	6	2.11E-06	Fyn, Nrp1, Pak2, Pak3, Rac1, Sema3a
GO.0044700	single organism signaling	18	2.11E-06	Agtr1a, Apln, Aplnr, Farp2, Fyn, Nrp1, Pak2, Pak3, Plxn1, Rac1, Rnd1, Rras, Sema3a, Sema3e, Sema3f, Srgap3, Trem2, Tyrobp
GO.0007154	cell communication	18	3.14E-06	Agtr1a, Apln, Aplnr, Farp2, Fyn, Nrp1, Pak2, Pak3, Plxn1, Rac1, Rnd1, Rras, Sema3a, Sema3e, Sema3f, Srgap3, Trem2, Tyrobp
GO.0035290	trunk segmentation	3	6.93E-06	Nrp1, Sema3a, Sema3f
GO.0048532	anatomical structure arrangement	4	6.93E-06	Nrp1, Rac1, Sema3a, Sema3f
GO.0060666	dichotomous subdivision of terminal units involved in salivary gland branching	3	1.2E-05	Nrp1, Plxn1, Sema3a
GO.0007264	small GTPase mediated signal transduction	7	2.59E-05	Agtr1a, Farp2, Pak2, Pak3, Rac1, Rnd1, Rras
GO.0051271	negative regulation of cellular component movement	6	4.23E-05	Nrp1, Rras, Sema3a, Sema3f, Sema6d, Srgap3
GO.0061549	sympathetic ganglion development	3	6.35E-05	Nrp1, Sema3a, Sema3f
GO.0040013	negative regulation of locomotion	6	7e-05	Nrp1, Rras, Sema3a, Sema3f, Sema6d, Srgap3
GO.0022603	regulation of anatomical structure morphogenesis	9	7.78E-05	Agtr1a, Fyn, Nrp1, Pak3, Rac1, Rras, Sema3a, Sema3e, Sema3f
GO.0051716	cellular response to stimulus	17	0.000122	Apln, Aplnr, Farp2, Fyn, Nrp1, Pak2, Pak3, Plxn1, Rac1, Rnd1, Rras, Sema3a, Sema3e, Sema3f, Srgap3, Trem2, Tyrobp
GO.0040012	regulation of locomotion	8	0.000128	Nrp1, Plxn1, Rac1, Rras, Sema3a, Sema3f, Sema6d, Srgap3
GO.0051270	regulation of cellular component movement	8	0.000129	Nrp1, Plxn1, Rac1, Rras, Sema3a, Sema3f, Sema6d, Srgap3
GO.0016477	cell migration	8	0.000143	Agtr1a, Fyn, Nrp1, Pak2, Pak3, Rac1, Sema3a, Sema3f
GO.0022604	regulation of cell morphogenesis	7	0.000143	Fyn, Nrp1, Pak3, Rac1, Sema3a, Sema3e, Sema3f
GO.0006544	glycine metabolic process	3	0.000164	Dmgdh, Gldc, Shmt1
GO.0007350	blastoderm segmentation	3	0.000164	Nrp1, Sema3a, Sema3f
GO.0032879	regulation of localization	12	0.000199	Agtr1a, Apln, Fyn, Nrp1, Plxn1, Rac1, Rras, Sema3a, Sema3f, Sema6d, Srgap3, Trem2
GO.0048731	system development	15	0.000219	Agtr1a, Aplnr, Farp2, Fyn, Nrp1, Pak2, Plxn1, Rac1, Rnd1, Rras, Sema3a, Sema3e, Sema3f, Sema6d, Trem2
GO.0021783	preganglionic parasympathetic fiber development	3	0.000235	Nrp1, Sema3a, Sema3f
GO.0051674	localization of cell	8	0.000257	Agtr1a, Fyn, Nrp1, Pak2, Pak3, Rac1, Sema3a, Sema3f
GO.0042327	positive regulation of phosphorylation	8	0.000293	Agtr1a, Apln, Fyn, Nrp1, Pak2, Pak3, Rac1, Trem2
GO.0060445	branching involved in salivary gland morphogenesis	3	0.000306	Nrp1, Plxn1, Sema3a
GO.0048667	cell morphogenesis involved in neuron differentiation	6	0.000314	Fyn, Nrp1, Pak3, Rac1, Sema3a, Sema3f
GO.0007166	cell surface receptor signaling pathway	10	0.00035	Farp2, Fyn, Nrp1, Plxn1, Rac1, Sema3a, Sema3e, Sema3f, Trem2, Tyrobp
GO.0048486	parasympathetic nervous system development	3	0.00035	Nrp1, Sema3a, Sema3f
GO.0090066	regulation of anatomical structure size	6	0.000416	Agtr1a, Nrp1, Pak3, Rac1, Sema3a, Sema3f
GO.0030036	actin cytoskeleton organization	6	0.000464	Farp2, Iqsec1, Pak2, Pak3, Rac1, Rnd1
GO.2000026	regulation of multicellular organismal development	10	0.000636	Agtr1a, Fyn, Nrp1, Pak3, Rac1, Rras, Sema3a, Sema3e, Sema3f, Tyrobp
GO.0042325	regulation of phosphorylation	9	0.000665	Agtr1a, Apln, Fyn, Nrp1, Pak2, Pak3, Rac1, Rras, Trem2
GO.0021828	gonadotrophin-releasing hormone neuronal migration to the hypothalamus	2	0.000774	Nrp1, Sema3a
GO.1903375	facioacoustic ganglion development	2	0.000774	Nrp1, Sema3a
GO.0007275	multicellular organismal development	15	0.000838	Agtr1a, Aplnr, Farp2, Fyn, Nrp1, Pak2, Plxn1, Rac1, Rnd1, Rras, Sema3a, Sema3e, Sema3f, Sema6d, Trem2
GO.0007435	salivary gland morphogenesis	3	0.00137	Nrp1, Plxn1, Sema3a
GO.0021886	hypothalamus gonadotrophin-releasing hormone neuron differentiation	2	0.00137	Nrp1, Sema3a
GO.0021888	hypothalamus gonadotrophin-releasing hormone neuron development	2	0.00137	Nrp1, Sema3a
GO.0021884	forebrain neuron development	3	0.00144	Nrp1, Rac1, Sema3a
GO.0031344	regulation of cell projection organization	6	0.00144	Fyn, Nrp1, Pak3, Rac1, Sema3a, Sema3f
GO.0001934	positive regulation of protein phosphorylation	7	0.00169	Agtr1a, Fyn, Nrp1, Pak2, Pak3, Rac1, Trem2
GO.0032535	regulation of cellular component size	5	0.00171	Nrp1, Pak3, Rac1, Sema3a, Sema3f
GO.0007431	salivary gland development	3	0.00179	Nrp1, Plxn1, Sema3a
GO.0010769	regulation of cell morphogenesis involved in differentiation	5	0.00179	Nrp1, Pak3, Rac1, Sema3a, Sema3f
GO.0048513	organ development	12	0.00183	Agtr1a, Aplnr, Farp2, Fyn, Nrp1, Plxn1, Rac1, Rras, Sema3a, Sema3f, Sema6d, Trem2
GO.0061551	trigeminal ganglion development	2	0.00184	Nrp1, Sema3a
GO.0060284	regulation of cell development	7	0.00199	Fyn, Nrp1, Pak3, Rac1, Sema3a, Sema3f, Tyrobp
GO.0001932	regulation of protein phosphorylation	8	0.00222	Agtr1a, Fyn, Nrp1, Pak2, Pak3, Rac1, Rras, Trem2
GO.0007266	Rho protein signal transduction	3	0.00229	Agtr1a, Pak2, Pak3

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GO ID	Description	Gene count	False discovery rate	Proteins
GO.0048813	dendrite morphogenesis	3	0.0025	Fyn, Rac1, Sem3a
GO.0050731	positive regulation of peptidyl-tyrosine phosphorylation	4	0.0025	Fyn, Nrp1, Pak2, Trem2
GO.0050920	regulation of chemotaxis	4	0.00261	Nrp1, Rac1, Sem3a, Sem3f
GO.0030334	regulation of cell migration	6	0.00335	Plxn1, Rac1, Rras, Sem3a, Sem6d, Srgap3
GO.0035272	exocrine system development	3	0.00345	Nrp1, Plxn1, Sem3a
GO.0014032	neural crest cell development	3	0.00372	Nrp1, Sem3a, Sem3f
GO.0050896	response to stimulus	16	0.00385	Apln, Aplnr, Farp2, Nrp1, Pak2, Pak3, Plxn1, Rac1, Rnd1, Rras, Sem3a, Sem3e, Sem3f, Srgap3, Trem2, Tyrobp
GO.0009653	anatomical structure morphogenesis	10	0.00398	Aplnr, Fyn, Nrp1, Pak3, Plxn1, Rac1, Rras, Sem3a, Sem3e, Sem3f
GO.0007265	Ras protein signal transduction	4	0.00417	Agtr1a, Farp2, Pak2, Pak3
GO.0030336	negative regulation of cell migration	4	0.00448	Rras, Sem3a, Sem6d, Srgap3
GO.0030154	cell differentiation	12	0.00455	Farp2, Fyn, Nrp1, Pak2, Rac1, Rnd1, Rras, Sem3a, Sem3e, Sem3f, Sem6d, Trem2
GO.0006935	chemotaxis	5	0.00526	Agtr1a, Nrp1, Rac1, Sem3a, Sem3f
GO.0061138	morphogenesis of a branching epithelium	4	0.00553	Nrp1, Plxn1, Sem3a, Sem3e
GO.0009071	serine family amino acid catabolic process	2	0.00653	Gldc, Shmt1
GO.0044763	single-organism cellular process	20	0.00676	Agtr1a, Apln, Aplnr, Dmgdh, Farp2, Fyn, Gldc, Iqsec1, Nrp1, Pak2, Plxn1, Rac1, Sem3a, Sem3e, Sem3f, Sem6d, Slc4a2, Srgap3, Trem2, Tyrobp
GO.1901606	alpha-amino acid catabolic process	3	0.00698	Gldc, Pipox, Shmt1
GO.0051239	regulation of multicellular organismal process	10	0.00789	Apln, Fyn, Nrp1, Pak3, Rac1, Rras, Sem3a, Sem3e, Sem3f, Tyrobp
GO.0048519	negative regulation of biological process	13	0.00817	Agtr1a, Apln, Fyn, Nrp1, Pak2, Rac1, Rnd1, Rras, Sem3a, Sem3e, Sem3f, Sem6d, Srgap3
GO.0010720	positive regulation of cell development	5	0.00941	Fyn, Nrp1, Pak3, Rac1, Sem3a
GO.0044767	single-organism developmental process	14	0.00976	Aplnr, Farp2, Fyn, Nrp1, Pak2, Plxn1, Rac1, Rnd1, Rras, Sem3a, Sem3e, Sem3f, Sem6d, Trem2
GO.0051094	positive regulation of developmental process	7	0.0125	Agtr1a, Fyn, Nrp1, Pak3, Rac1, Rras, Sem3a
GO.0046653	tetrahydrofolate metabolic process	2	0.0139	Pipox, Shmt1
GO.0060996	dendritic spine development	2	0.0139	Pak2, Pak3
GO.0065008	regulation of biological quality	10	0.0162	Apln, Aplnr, Fyn, Nrp1, Pak3, Rac1, Sem3a, Sem3e, Sem3f, Slc4a2
GO.0031346	positive regulation of cell projection organization	4	0.0167	Fyn, Nrp1, Pak3, Rac1
GO.0050919	negative chemotaxis	2	0.0168	Sem3a, Sem3f
GO.1902531	regulation of intracellular signal transduction	7	0.0187	Agtr1a, Fyn, Nrp1, Pak2, Pak3, Rras, Trem2
GO.0044707	single-multicellular organism process	14	0.0188	Apln, Farp2, Fyn, Nrp1, Pak2, Plxn1, Rac1, Rnd1, Rras, Sem3a, Sem3e, Sem3f, Sem6d, Trem2
GO.0060384	innervation	2	0.0218	Nrp1, Sem3a
GO.0021854	hypothalamus development	2	0.0233	Nrp1, Sem3a
GO.0043408	regulation of MAPK cascade	5	0.0241	Nrp1, Pak2, Pak3, Rras, Trem2
GO.0023056	positive regulation of signaling	7	0.0258	Agtr1a, Apln, Fyn, Nrp1, Pak2, Pak3, Trem2
GO.0008361	regulation of cell size	3	0.0287	Nrp1, Sem3a, Sem3f
GO.0051241	negative regulation of multicellular organismal process	6	0.0291	Apln, Nrp1, Rac1, Sem3a, Sem3e, Sem3f
GO.0048583	regulation of response to stimulus	10	0.0319	Agtr1a, Fyn, Nrp1, Pak2, Pak3, Rac1, Rras, Sem3a, Sem3f, Trem2
GO.0051272	positive regulation of cellular component movement	4	0.0332	Nrp1, Rac1, Sem3a, Sem6d
GO.0040017	positive regulation of locomotion	4	0.0357	Nrp1, Rac1, Sem3a, Sem6d
GO.0010770	positive regulation of cell morphogenesis involved in differentiation	3	0.0367	Nrp1, Pak3, Rac1
GO.0014910	regulation of smooth muscle cell migration	2	0.039	Plxn1, Sem6d
GO.0010647	positive regulation of cell communication	7	0.0407	Agtr1a, Apln, Fyn, Nrp1, Pak2, Pak3, Trem2
GO.0009069	serine family amino acid metabolic process	2	0.0455	Dmgdh, Gldc
GO.0018108	peptidyl-tyrosine phosphorylation	3	0.0456	Agtr1a, Fyn, Nrp1
GO.0021543	pallium development	3	0.0463	Nrp1, Rac1, Sem3a
GO.2001236	regulation of extrinsic apoptotic signaling pathway	3	0.0483	Fyn, Nrp1, Pak2

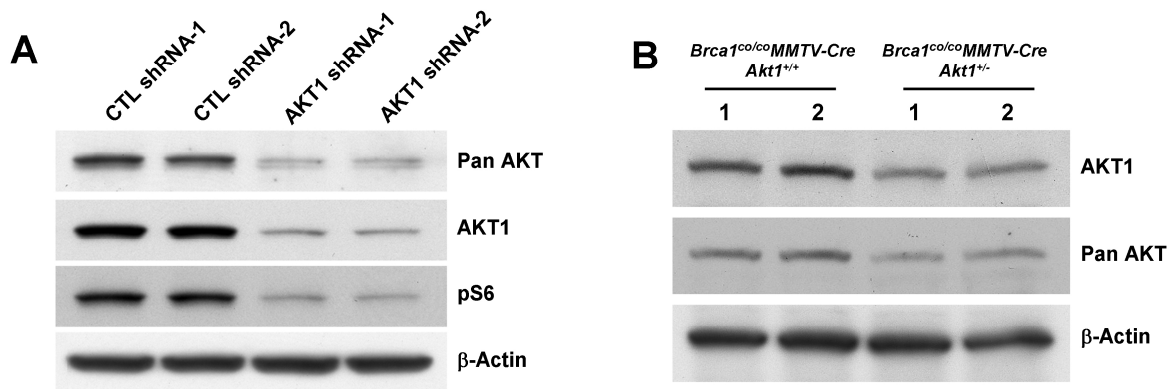
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Supplementary Table 4. KEGG pathway analysis of 7 genes associated with tumor volumes (FDR<0.05).

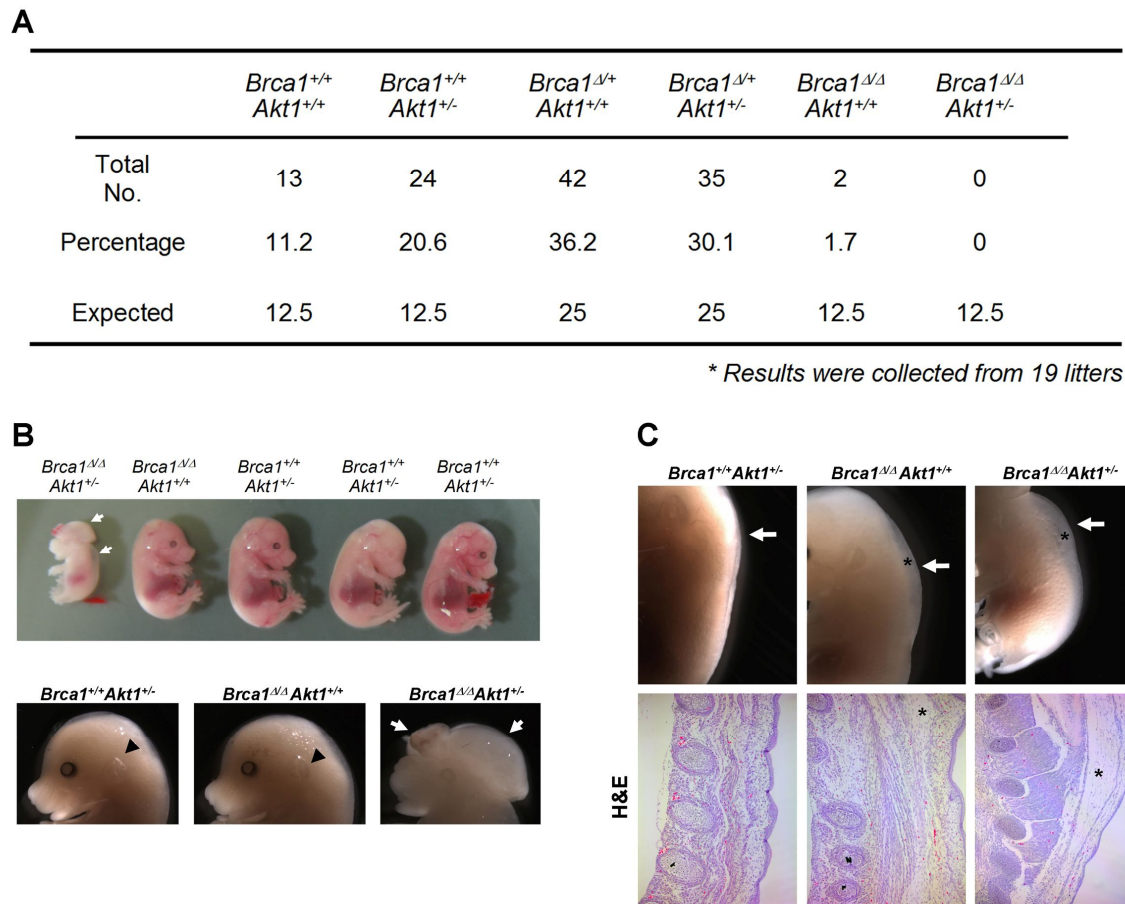
Pathway ID	Pathway	Proteins	Count	FDR
260	Glycine, serine and threonine metabolism	Dmgdh, Gldc, Pipox, Shmt1, Shmt2	5	0.000
460	Cyanoamino acid metabolism	Shmt1, Shmt2	2	0.001
5211	Renal cell carcinoma	Pak2, Pak3, Rac1	3	0.003
4510	Focal adhesion	Fyn, Pak2, Pak3, Rac1	4	0.004
4520	Adherens junction	Farp2, Fyn, Rac1	3	0.004
4810	Regulation of actin cytoskeleton	Pak2, Pak3, Rac1, Rras	4	0.004
4014	Ras signaling pathway	Pak2, Pak3, Rac1, Rras	4	0.004
670	One carbon pool by folate	Shmt1, Shmt2	2	0.007
1200	Carbon metabolism	Gldc, Shmt1, Shmt2	3	0.007
4660	T cell receptor signaling pathway	Fyn, Pak2, Pak3	3	0.007
4650	Natural killer cell mediated cytotoxicity	Fyn, Rac1, Tyrobp	3	0.007
630	Glyoxylate and dicarboxylate metabolism	Shmt1, Shmt2	2	0.008
4015	Rap1 signaling pathway	Farp2, Rac1, Rras	3	0.036

Supplementary Table 5. Summary of PCR primers for mouse genotyping

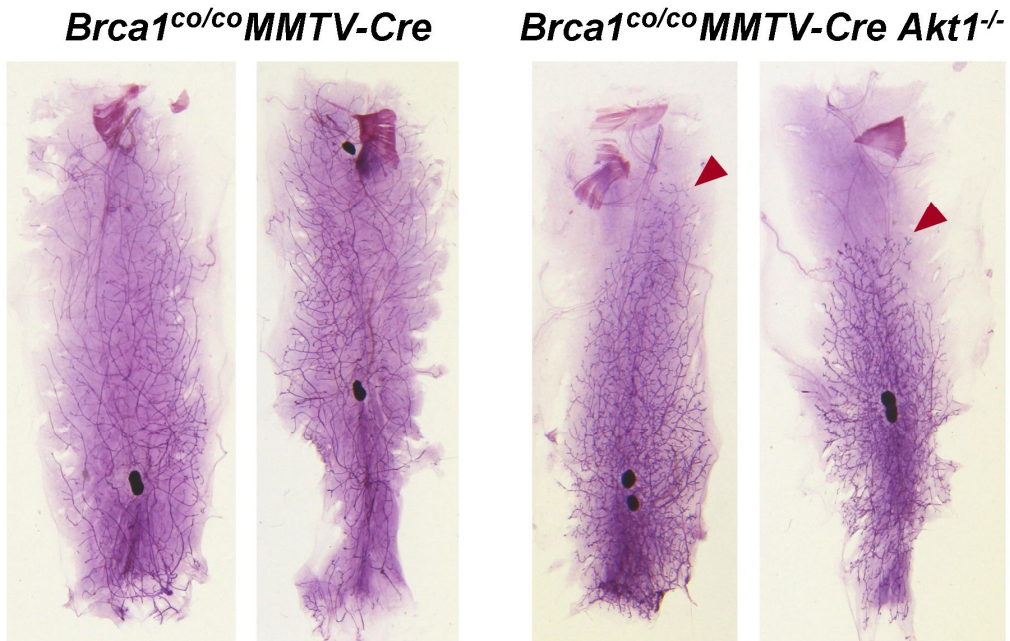
Gene Allele	PCR primers	Size of PCR product
<i>Brcal-co</i> (conditional)	Forward: 5'– CTG GGT AGT TTG TAA GCA TGC –3' Reverse: 5'– CAA TAA ACT GCT GGT CTC AGG –3'	WT: 470 bp co: 530 bp
<i>Brcal-Δ11-WT</i>	Forward: 5'– CTG GGT AGT TTG TAA GCA TGC –3' Reverse: 5'– CAA TAA ACT GCT GGT CTC AGG –3'	450 bp
<i>Brcal-Δ11-KO</i>	Forward: 5'– CTG GGT AGT TTG TAA GCA TGC –3' Reverse: 5'– CTG CGA GCA GTC TTC AGA AAG –3'	600 bp
<i>MMTV-cre</i>	Forward: 5'– CTG ATC TGA GCT CTG AGT G –3' Reverse: 5'– CAT CAC TCG TTG CAT CGA CC –3'	250 bp
<i>Akt1-WT</i>	Forward: 5'– GCT CCA TAA GCA CAC CTT CAG G –3' Reverse: 5'– AGC TCT TCT TCC ACC TGT CTC –3'	259 bp
<i>Akt1-KO</i>	Forward: 5'– GCT CCA TAA GCA CAC CTT CAG G –3' Reverse: 5'– GTG GAT GTG GAA TGT GTG CGA G –3'	143 bp



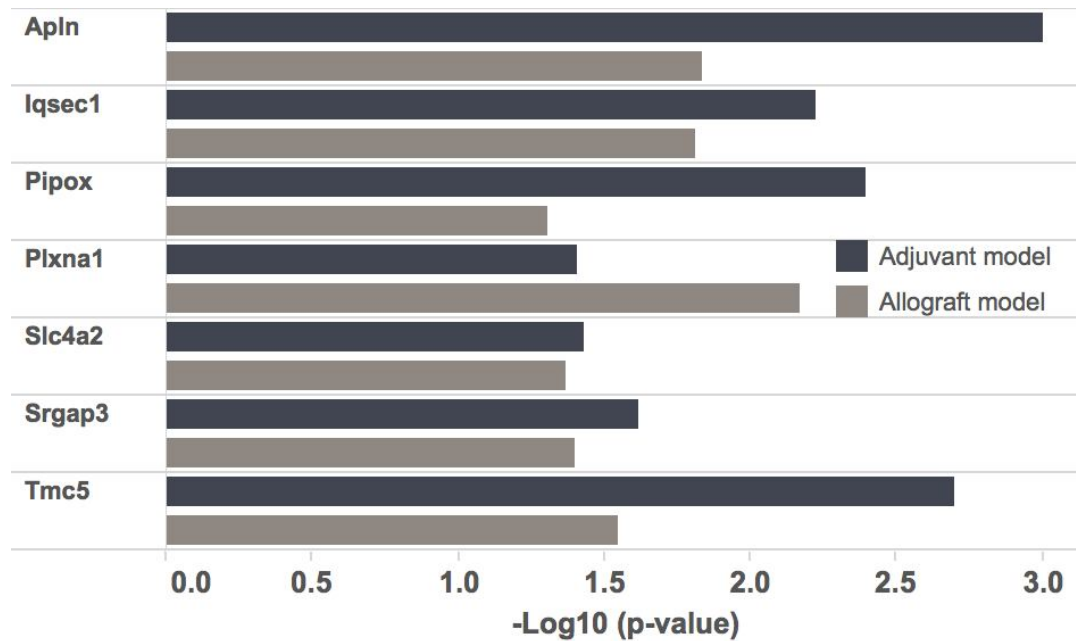
Supplementary Fig. 1. Measurements of AKT1 in MCF7 and *Brca1/Akt1*-mutant mice. (A) AKT1 is the major AKT isoform in the mammary epithelial cell-derived cell line, MCF7. MCF7 cells were transfected with control or *AKT1*-shRNAs, and the expression patterns of the indicated proteins were analyzed by Western blotting. Suppression of AKT1 expression readily reduced pan AKT expression and S6 phosphorylation. (B) AKT1 and pan AKT expression patterns in small intestines from *Brca1^{co/co}MMTV-Cre Akt1^{+/+}* and *Brca1^{co/co}MMTV-Cre Akt1^{+/-}* mice are shown. Protein levels of the AKT1 and pan AKT were analyzed by Western blotting. β -actin was used as the loading control.



Supplementary Fig. 2. Developmental defects observed in *Brca1^{Δ11/Δ11}Akt1^{+/-}* embryos. (A) Genotypes of offsprings derived from crosses between *Brca1^{+/Δ11}Akt1^{+/+}* (female) and *Brca1^{+/Δ11}Akt1^{+/-}* mice (male). From this cross, we expected to acquire the embryos with 6 following genotypes (numbers in the parentheses were expected ratio of the cross); *Brca1^{+/+}Akt1^{+/+}* (12.5%), *Brca1^{+/+}Akt1^{+/-}* (12.5%), *Brca1^{+/Δ11}Akt1^{+/+}* (25%), *Brca1^{+/Δ11}Akt1^{+/-}* (25%), *Brca1^{Δ11/Δ11}Akt1^{+/+}* (12.5%), *Brca1^{Δ11/Δ11}Akt1^{+/-}* (12.5%). (B) Embryos (14.5 dpc) of the indicated genotypes are shown. The white arrows point to exencephaly and lack of neural tube closure in *Brca1^{Δ11/Δ11}Akt1^{+/-}* embryos. Bottom panels were magnification of head area of embryos. Ears (black arrow heads) were identified in *Brca1^{+/+}Akt1^{+/-}* and *Brca1^{Δ11/Δ11}Akt1^{+/+}* embryos but not in *Brca1^{Δ11/Δ11}Akt1^{+/-}*. (C) Comparisons of dorsal body cavities and spinal cords of *Brca1^{+/+}Akt1^{+/-}*, *Brca1^{Δ11/Δ11}Akt1^{+/+}*, and *Brca1^{Δ11/Δ11}Akt1^{+/-}* embryos. *Brca1^{Δ11/Δ11}Akt1^{+/-}* mutant embryos exhibited lymphedemas (asterisk, accumulations of tissue fluid,) caused from the absent or malformed lymph nodes and/or lymphatic channels while *Brca1^{Δ11/Δ11}* mutant embryos also showed accumulations of tissue fluid but extent is milder than *Brca1^{Δ11/Δ11}Akt1^{+/-}* mutant embryos



Supplementary Fig. 3. Whole-mount stainings of mammary glands from 6-month-old *Brca1^{co/co}MMTV-Cre* and *Brca1^{co/co}MMTV-Cre Akt1^{-/-}* mice. The distal end tips of the mammary glands cover the entire fat pad area in *Brca1^{co/co}MMTV-Cre* mice, whereas the ends of the ducts (arrows) do not extend to the end of the fat pad in *Brca1^{co/co}MMTV-Cre Akt1^{-/-}* mice.

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Supplementary Fig. 4. Validation of differentially expressed genes with MK-2206 responsiveness. Identified 7 genes were upregulated in the MK-2206-sensitive samples of both models, compared to the MK-2206-insensitive samples ($P < 0.05$). All the genes were validated using RT-qPCR with primers appeared in supplementary table 3 (fold change > 1.5).