SUPPLEMENTARY INFORMATION

Title: Tumor Suppressor BRCA1 epigenetically controls oncogenic miRNA-155

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Supplementary Table 1 **Expression analysis of R1699Q embryoid bodies**

Column #	Probeset ID	Gene Title	Gene Symbol
Down-regulated			
33597	1449289_a_at	beta-2 microglobulin	B2m
36731	1452428_a_at	beta-2 microglobulin	B2m
39742	1455439_a_at	lectin, galactose binding, soluble 1	Lgals1
44651	1460351_at	S100 calcium binding protein A11 (calgizzarin)	S100a11
6821	1422507_at	cystatin B	Cstb
40559	1456256_at	eukaryotic translation initiation factor 5	Eif5
6820	1422506_a_at	cystatin B	Cstb
7743	1423429_at	reproductive homeobox 5	Rhox5
378	1416039_x_at	cysteine rich protein 61	Cyr61
11440	1427126_at	heat shock protein 1B	Hspa1b
3912	1419573_a_at	lectin, galactose binding, soluble 1	Lgals1
11441	1427127_x_at	heat shock protein 1B	Hspa1b
32541	1448233_at	prion protein	Prnp
12397	1428083_at	RIKEN cDNA 2310043N10 gene	2310043N10Ril
22447	1438133_a_at	cysteine rich protein 61	Cyr61
8372	1424058_at	proline-rich coiled-coil 1	Prrc1
7262	1422948_s_at	histone cluster 1, H4h /// histone cluster 1, H4c /// h	Hist1h4a /// His
30331	1446017_at	Transcribed locus	---
3648	1419309_at	podoplanin	Pdpn
24568	1440254_at	hypothetical protein LOC100041277 /// hypothetica	LOC100041277
3430	1419091_a_at	annexin A2	Anxa2
238	1415899_at	Jun-B oncogene	Junb
2910	1418571_at	tumor necrosis factor receptor superfamily, memb	Tnfrsf12a
43825	1459522_s_at	glycogenin	Gyg
33295	1448987_at	acyl-Coenzyme A dehydrogenase, long-chain	Acadl
34232	1449929_at	dynein light chain Tctex-type 3	Dynlt3
44614	1460314_s_at	histone cluster 2, H3c1 /// histone cluster 2, H2aa1	Hist1h3a /// His
32626	1448318_at	adipose differentiation related protein	Adfp
44157	1459854_s_at	dynein light chain Tctex-type 3	Dynlt3
2911	1418572_x_at	tumor necrosis factor receptor superfamily, memb	Tnfrsf12a
36621	1452318_a_at	heat shock protein 1B	Hspa1b
18817	1434503_s_at	lysosomal-associated membrane protein 2	Lamp2
12408	1428094_at	lysosomal-associated membrane protein 2	Lamp2
1101	1416762_at	S100 calcium binding protein A10 (calpactin)	S100a10
21485	1437171_x_at	gelsolin	Gsn
38321	1454018_at	tousled-like kinase 2 (Arabidopsis)	Tlk2
1748	1417409_at	Jun oncogene	Jun
32647	1448339_at	transmembrane protein 30A	Tmem30a
12017	1427703_at	platelet-activating factor acetylhydrolase, isoform	LOC100046589
23286	1438972_x_at	RIKEN cDNA 2810410L24 gene	2810410L24Ril
Up-regulated			
34929	1450626_at	mannosidase, beta A, lysosomal	Manba
40644	1456341_a_at	Kruppel-like factor 9 /// RIKEN cDNA 2310051E17 g	2310051E17Ril

12602	1428288_at	RIKEN cDNA 2310051E17 gene	2310051E17Ril
35156	1450853_at	transducin-like enhancer of split 4, homolog of Dro	Tle4
491	1416152_a_at	splicing factor, arginine/serine-rich 3 (SRp20)	Sfrs3
34385	1450082_s_at	ets variant gene 5	Etv5
19231	1434917_at	cordon-bleu	Cobl
12366	1428052_a_at	zinc finger, MYM domain containing 1	Zmym1
33716	1449408_at	junction adhesion molecule 2	Jam2
36761	1452458_s_at	peptidylprolyl isomerase (cyclophilin) like 5	Ppil5
12603	1428289_at	Kruppel-like factor 9 /// RIKEN cDNA 2310051E17 g	2310051E17Ril
12456	1428142_at	ets variant gene 5	Etv5
2099	1417760_at	nuclear receptor subfamily 0, group B, member 1	Nr0b1
5312	1420998_at	ets variant gene 5	Etv5
18103	1433789_at	small nucleolar RNA host gene (non-protein codin	Snhg3
33065	1448757_at	promyelocytic leukemia	Pml
4412	1420085_at	fibroblast growth factor 4	Fgf4
33423	1449115_at	metal response element binding transcription fact	Mtf2
36643	1452340_at	RIKEN cDNA 6820424L24 gene	6820424L24Ril
21240	1436926_at	estrogen related receptor, beta	Esrrb
20097	1435783_at	RIKEN cDNA B230112C05 gene	B230112C05Ri
490	1416151_at	splicing factor, arginine/serine-rich 3 (SRp20)	Sfrs3
4413	1420086_x_at	fibroblast growth factor 4	Fgf4
7383	1423069_at	activity-dependent neuroprotective protein	Adnp
2773	1418434_at	makorin, ring finger protein, 1	Mkrn1
12533	1428219_at	RING1 and YY1 binding protein /// predicted gene,	EG628746 /// R
11408	1427094_at	polymerase (DNA directed), epsilon 2 (p59 subunit	Pole2
41001	1456698_s_at	heterogeneous nuclear ribonucleoprotein D-like	Hnrpdl
4882	1420568_at	stimulated by retinoic acid gene 8	Stra8
24054	1439740_s_at	uridine-cytidine kinase 2	Uck2
33198	1448890_at	Kruppel-like factor 2 (lung)	Klf2
6600	1422286_a_at	TG interacting factor 1	Tgif1
39296	1454993_a_at	splicing factor, arginine/serine-rich 3 (SRp20)	Sfrs3
36340	1452037_at	mannoside acetylglucosaminyltransferase 2	Mgat2
32792	1448484_at	S-adenosylmethionine decarboxylase 1	Amd1
4420	1420093_s_at	heterogeneous nuclear ribonucleoprotein D-like	Hnrpdl
36352	1452049_at	ribosomal protein L7-like 1	Rpl7l1
8072	1423758_at	GTPase activating protein (SH3 domain) binding p	G3bp2
17884	1433570_s_at	MAK10 homolog, amino-acid N-acetyltransferase s	Mak10
12508	1428194_at	ubiquitin specific peptidase 9, X chromosome	Usp9x
19071	1434757_at	core-binding factor, runt domain, alpha subunit 2,	Cbfa2t2
19315	1435001_at	phospholipase A2, activating protein	Plaa
2840	1418501_a_at	oxidation resistance 1	Oxr1
39603	1455300_at	RIKEN cDNA E130014J05 gene	E130014J05Ril
14034	1429720_at	MAK10 homolog, amino-acid N-acetyltransferase s	Mak10
890	1416551_at	ATPase, Ca++ transporting, cardiac muscle, slow t	Atp2a2
33418	1449110_at	ras homolog gene family, member B	Rhob
7011	1422697_s_at	jumonji, AT rich interactive domain 2	Jarid2
1743	1417404_at	ELOVL family member 6, elongation of long chain	Elovl6
17828	1433514_at	ethanolamine kinase 1	Etnk1
13039	1428725_at	protein inhibitor of activated STAT 2	Pias2

13760	1429446_at	serologically defined colon cancer antigen 1	Sdccag1
8830	1424516_at	RIKEN cDNA B230354K17 gene	B230354K17Ri
516	1416177_at	RNA binding motif protein, X chromosome retroge	Rbmxrt
21424	1437110_at	RIKEN cDNA 2810474O19 gene	2810474O19Ri
35588	1451285_at	fusion, derived from t(12;16) malignant liposarcom	Fus
11456	1427142_s_at	jumonji, AT rich interactive domain 1B (Rbp2 like)	Jarid1b
24695	1440381_at	RIKEN cDNA 2410085M17 gene	2410085M17Ri
34341	1450038_s_at	ubiquitin specific peptidase 9, X chromosome	Usp9x
38920	1454617_at	arrestin domain containing 3	Arrdc3
20072	1435758_at	similar to Beta-1,4-galactosyltransferase 6 (Beta-1,	LOC675709
5848	1421534_at	deafness, autosomal dominant 5 homolog (human)	Dfna5h
36	1415697_at	GTPase activating protein (SH3 domain) binding p	G3bp2
19811	1435497_at	RIKEN cDNA 5730590G19 gene /// hypothetical pro	5730590G19Ri
1361	1417022_at	solute carrier family 7 (cationic amino acid transpo	Slc7a3
40522	1456219_at	similar to OPR	LOC10004598
13159	1428845_at	BCL2-associated transcription factor 1	Bclaf1
11214	1426900_at	jumonji domain containing 1C	Jmjd1c
35017	1450714_at	antizyme inhibitor 1	Azin1
19078	1434764_at	A kinase (PRKA) anchor protein 11	Akap11
19019	1434705_at	C-terminal binding protein 2	Ctbp2
13697	1429383_at	casein kinase 1, gamma 3 /// similar to casein kinas	Csnk1g3 /// LO
2409	1418070_at	chromodomain protein, Y chromosome-like	Cdyl
35013	1450710_at	jumonji, AT rich interactive domain 2	Jarid2
12507	1428193_at	ubiquitin specific peptidase 9, X chromosome	Usp9x
5236	1420922_at	ubiquitin specific peptidase 9, X chromosome	Usp9x
11036	1426722_at	solute carrier family 38, member 2	Slc38a2
10852	1426538_a_at	transformation related protein 53	Trp53
11485	1427171_at	rearranged L-myc fusion sequence	Rlf
39025	1454722_at	phosphatase and tensin homolog	Pten
12538	1428224_at	heterogeneous nuclear ribonucleoprotein D-like	Hnrpdl
18913	1434599_a_at	tight junction protein 2	Tjp2
501	1416162_at	RAD21 homolog (S. pombe)	Rad21
18217	1433903_at	expressed sequence AU021838	AU021838
14948	1430634_a_at	phosphofructokinase, platelet	Pfkp
8347	1424033_at	splicing factor, arginine/serine-rich 7	Sfrs7
19508	1435194_at	heat shock protein 4	Hspa4
25996	1441682_s_at	exportin, tRNA (nuclear export receptor for tRNAs)	Xpot
19081	1434767_at	expressed sequence C79407	C79407
18626	1434312_at	ADP-ribosylation factor 6	Arf6
44138	1459835_s_at	DnaJ (Hsp40) homolog, subfamily A, member 1	Dnaja1
35288	1450985_a_at	tight junction protein 2	Tjp2
34356	1450053_at	Kinesin family member 2A	Kif2a
34355	1450052_at	kinesin family member 2A	Kif2a
39655	1455352_at	expressed sequence AU023006	AU023006
8829	1424515_at	RIKEN cDNA B230354K17 gene	B230354K17Ri
11562	1427248_at	Wolf-Hirschhorn syndrome candidate 2 (human)	Whsc2
13086	1428772_at	exportin, tRNA (nuclear export receptor for tRNAs)	Xpot
12053	1427739_a_at	transformation related protein 53	Trp53
13197	1428883_at	transmembrane protein 57	Tmem57

7738	1423424_at	zinc finger protein of the cerebellum 3	Zic3
9037	1424723_s_at	cleavage stimulation factor, 3' pre-RNA, subunit 3	Cstf3
39476	1455173_at	G1 to S phase transition 1	Gspt1
1267	1416928_at	RNA binding motif protein 12	Rbm12
39077	1454774_at	zinc finger protein 445	Zfp445
34993	1450690_at	RAN binding protein 2	Ranbp2
8667	1424353_at	leucine-rich PPR-motif containing	Lrpprc
36540	1452237_at	HIV-1 Rev binding protein	Hrb
12263	1427949_at	zinc finger protein 294	Zfp294
13902	1429588_at	RIKEN cDNA 2810474O19 gene	2810474O19Ri
42677	1458374_at	expressed sequence C79407	C79407
13762	1429448_s_at	CXXC finger 6	Cxxc6
18050	1433736_at	host cell factor C1	Hcfc1
8061	1423747_a_at	pyruvate dehydrogenase kinase, isoenzyme 1	Pdk1
18995	1434681_at	RIKEN cDNA 4932441K18 gene	4932441K18Ri
18616	1434302_at	Ras association (RalGDS/AF-6) and pleckstrin hon	Raph1
3793	1419454_x_at	protein inhibitor of activated STAT 2	Pias2
21381	1437067_at	putative homeodomain transcription factor 2	Phtf2
33017	1448709_at	AT rich interactive domain 1A (Swi1 like) /// similar	Arid1a /// LOC
18108	1433794_at	senataxin	Setx
40406	1456103_at	promyelocytic leukemia	Pml
40308	1456005_a_at	BCL2-like 11 (apoptosis facilitator)	Bcl2l11
2987	1418648_at	EGL nine homolog 3 (C. elegans)	Egln3
34340	1450037_at	ubiquitin specific peptidase 9, X chromosome	Usp9x
6578	1422264_s_at	Kruppel-like factor 9 /// RIKEN cDNA 2310051E17 g	2310051E17Ri
20284	1435970_at	nemo like kinase /// similar to nemo-like kinase	LOC10004446
18351	1434037_s_at	p300/CBP-associated factor	Pcaf
32538	1448230_at	ubiquitin specific peptidase 10	Usp10
18914	1434600_at	tight junction protein 2	Tjp2
20882	1436568_at	junction adhesion molecule 2	Jam2
13609	1429295_s_at	thyroid hormone receptor interactor 13	Trip13
567	1416228_at	protein (peptidyl-prolyl cis/trans isomerase) NIMA-	Pin1
9300	1424986_s_at	F-box and WD-40 domain protein 7, archipelago ho	Fbxw7
19092	1434778_at	Transcribed locus	---
869	1416530_a_at	purine-nucleoside phosphorylase /// similar to puri	LOC10004556
28704	1444390_at	PR domain containing 14	Prdm14
44480	1460179_at	DnaJ (Hsp40) homolog, subfamily A, member 1	Dnaja1
11457	1427143_at	jumonji, AT rich interactive domain 1B (Rbp2 like)	Jarid1b
22057	1437743_at	AE binding protein 2	Aebp2
44978	1460678_at	kelch domain containing 2	Klhdc2
7028	1422714_at	ubiquitin-conjugating enzyme E2l /// predicted gen	EG546265 /// U
17993	1433679_at	far upstream element (FUSE) binding protein 3	Fubp3
36512	1452209_at	plakophilin 4	Pkp4
12845	1428531_at	integrator complex subunit 7	Ints7
33393	1449085_at	PHD finger protein 10	Phf10
1306	1416967_at	SRY-box containing gene 2	Sox2
111	1415772_at	nucleolin	Ncl
40267	1455964_at	Cdc2-related kinase, arginine/serine-rich	Crkrs
11648	1427334_s_at	RIKEN cDNA 2810474O19 gene	2810474O19Ri

38969	1454666_at	similar to BKLF	LOC10004685
6166	1421852_at	potassium channel, subfamily K, member 5	Kcnk5
33285	1448977_at	transcription factor AP-2, gamma	Tcfap2c
259	1415920_at	cleavage stimulation factor, 3' pre-RNA subunit 2,	Cstf2t
31938	1447624_s_at	storkhead box 2	Stox2
14048	1429734_at	RIKEN cDNA 4632434I11 gene	4632434I11Rik
35359	1451056_at	proteasome (prosome, macropain) 26S subunit, nc	Psmd7
13312	1428998_at	PHD finger protein 3	Phf3
19079	1434765_at	E1A binding protein p300	Ep300
47	1415708_at	taurine upregulated gene 1	Tug1
36741	1452438_s_at	TAF4A RNA polymerase II, TATA box binding prote	LOC10004693
23051	1438737_at	zinc finger protein of the cerebellum 3	Zic3
21141	1436827_at	gene model 944, (NCBI)	Gm944
2979	1418640_at	sirtuin 1 ((silent mating type information regulatior	Sirt1
7012	1422698_s_at	jumonji, AT rich interactive domain 2	Jarid2
11067	1426753_at	PHD finger protein 17	Phf17
35762	1451459_at	AT hook containing transcription factor 1	Ahctf1
8099	1423785_at	EGL nine homolog 1 (C. elegans)	Egln1
7428	1423114_at	ubiquitin-conjugating enzyme E2D 3 (UBC4/5 hom	Ube2d3
32568	1448260_at	ubiquitin carboxy-terminal hydrolase L1	Uchl1
39066	1454763_at	ankyrin repeat domain 17	Ankrd17
20329	1436015_s_at	serine/threonine kinase 4	Stk4
18610	1434296_at	cDNA sequence BC049349	BC049349
40420	1456117_at	ribosomal RNA processing 1 homolog B (S. cerevi	Rrp1b
9172	1424858_at	L-2-hydroxyglutarate dehydrogenase	L2hgdh
4254	1419927_s_at	RAB interacting factor	Rabif
44629	1460329_at	similar to Beta-1,4-galactosyltransferase 6 (Beta-1,	LOC675709
2246	1417907_at	ubiquitin-conjugating enzyme E2L 3	Ube2l3
17983	1433669_at	A kinase (PRKA) anchor protein 8	Akap8
40814	1456511_x_at	ES cell-expressed Ras	Eras
39799	1455496_at	phosphoribosylformylglycinamidine synthase (FG	Pfas
39134	1454831_at	forkhead box N2	Foxn2
19658	1435344_at	RIKEN cDNA 1110029I05 gene /// hypothetical prote	1110029I05Rik
12721	1428407_at	heterogeneous nuclear ribonucleoprotein A0	Hnrpa0
36579	1452276_at	SWI/SNF-related, matrix-associated actin-depende	Smarcad1
2410	1418071_s_at	chromodomain protein, Y chromosome-like	Cdyl
2853	1418514_at	metal response element binding transcription fact	Mtf2
18171	1433857_at	FAT tumor suppressor homolog 1 (Drosophila)	Fat1
22603	1438289_a_at	SMT3 suppressor of mif two 3 homolog 1 (yeast)	Sumo1
10761	1426447_at	nucleoporin 35	Nup35
33188	1448880_at	ubiquitin-conjugating enzyme E2L 3	Ube2l3
19334	1435020_at	serologically defined colon cancer antigen 1	Sdccag1
6372	1422058_at	nodal	Nodal
1961	1417622_at	solute carrier family 12, member 2 /// similar to sol	LOC10004723
32548	1448240_at	membrane-bound transcription factor peptidase, s	Mbtps1
18826	1434512_x_at	splicing factor, arginine/serine-rich 3 (SRp20) /// pr	EG632248 /// S
10967	1426653_at	minichromosome maintenance deficient 3 (S. cere	LOC10004567
2280	1417941_at	N-acetylneuraminic acid phosphatase	Nanp
35380	1451077_at	ribosomal protein L5 /// similar to ribosomal protei	EG665407 /// E

13657	1429343_at	RNA binding motif protein, X-linked-like 2 /// simila	LOC10004643
6770	1422456_at	N-ethylmaleimide sensitive fusion protein	Nsf
39259	1454956_at	ribosomal protein S6 kinase, polypeptide 1	Rps6kb1
21236	1436922_at	peptidylprolyl isomerase (cyclophilin) like 5	Ppil5
19167	1434853_x_at	makorin, ring finger protein, 1	Mkrn1
19788	1435474_at	TAF5 RNA polymerase II, TATA box binding protein	Taf5
40918	1456615_a_at	bromodomain PHD finger transcription factor	Bptf
2620	1418281_at	RAD51 homolog (S. cerevisiae)	Rad51
12948	1428634_at	TWIST neighbor	Twistnb
19683	1435369_at	FAST kinase domains 5	Fastkd5
422	1416083_at	zinc finger, AN1-type domain 5 /// similar to zinc fin	LOC100047896
8225	1423911_at	protein phosphatase 2, regulatory subunit B (B56),	LOC10004639
3333	1418994_at	RIKEN cDNA 2410116G06 gene	2410116G06Ril
39122	1454819_at	---	---
1494	1417155_at	v-myc myelocytomatosis viral related oncogene, n	Mycn
18280	1433966_x_at	asparagine synthetase	Asns
19281	1434967_at	zinc finger, SWIM domain containing 6	Zswim6
37136	1452833_at	Rap guanine nucleotide exchange factor (GEF) 2	Rapgef2
35200	1450897_at	Rho GTPase activating protein 5	Arhgap5
32985	1448677_at	COX4 neighbor	Cox4nb
6371	1422057_at	nodal	Nodal
18531	1434217_at	RIKEN cDNA C330019G07 gene	C330019G07Ri
4724	1420410_at	nuclear receptor subfamily 5, group A, member 2	Nr5a2
19256	1434942_at	ESF1, nucleolar pre-rRNA processing protein, hom	Esf1
18353	1434039_at	amyloid beta precursor protein (cytoplasmic tail) b	Appbp2
12683	1428369_s_at	Rho GTPase activating protein 21	Arhgap21
3754	1419415_a_at	retinoic acid receptor, gamma	Rarg
20706	1436392_s_at	transcription factor AP-2, gamma	Tcfap2c
17841	1433527_at	iron responsive element binding protein 2	Ireb2
22592	1438278_a_at	cDNA sequence BC003993	BC003993
10665	1426351_at	heat shock protein 1 (chaperonin)	Hspd1
40118	1455815_a_at	tyrosine 3-monooxygenase/tryptophan 5-monooxy	Ywhab
21609	1437295_at	protein kinase N2	Pkn2
10942	1426628_at	transmembrane protein 34	Tmem34
20092	1435778_at	ankyrin repeat domain 11	Ankrd11
5637	1421323_a_at	GTPase activating protein (SH3 domain) binding pı	G3bp2
2322	1417983_a_at	ubiquitin-conjugating enzyme E2 variant 2	Ube2v2
1094	1416755_at	DnaJ (Hsp40) homolog, subfamily B, member 1	Dnajb1
8187	1423873_at	LSM1 homolog, U6 small nuclear RNA associated (	Lsm1
8543	1424229_at	dual-specificity tyrosine-(Y)-phosphorylation regul	Dyrk3
39251	1454948_at	ubiquitin specific peptidase 7	Usp7
17835	1433521_at	ankyrin repeat domain 13c	Ankrd13c
5266	1420952_at	Son cell proliferation protein	Son
33525	1449217_at	caspase 8 associated protein 2	Casp8ap2
18996	1434682_at	zinc finger protein 770	Zfp770
8802	1424488_a_at	pyrophosphatase (inorganic) 2 /// similar to Pyroph	LOC100048145
6926	1422612_at	hexokinase 2 /// hypothetical protein LOC10004341	Hk2 /// LOC100
11398	1427084_a_at	mitogen-activated protein kinase kinase kinase kin	Map4k5
18148	1433834_at	membrane-associated ring finger (C3HC4) 6	6-Mar

32452	1448144_at	heterogeneous nuclear ribonucleoprotein A/B	Hnrpab
26722	1442408_at	sulfatase 2	Sulf2
6045	1421731_a_at	flap structure specific endonuclease 1	Fen1
12785	1428471_at	sorbin and SH3 domain containing 1	Sorbs1
35620	1451317_at	YTH domain family 2	Ythdf2
1384	1417045_at	BH3 interacting domain death agonist	Bid
18835	1434521_at	regulatory factor X domain containing 2 homolog (	LOC10004861
38960	1454657_s_at	MAK10 homolog, amino-acid N-acetyltransferase s	Mak10
38419	1454116_a_at	MTERF domain containing 1	Mterfd1
18352	1434038_at	DnaJ (Hsp40) homolog, subfamily C, member 13	Dnajc13
13120	1428806_at	casein kinase 1, gamma 1	Csnk1g1
40824	1456521_at	nuclear receptor subfamily 5, group A, member 2	Nr5a2
1742	1417403_at	ELOVL family member 6, elongation of long chain	Elovl6
17851	1433537_at	RIKEN cDNA 4833408C14 gene	4833408C14Ri
35161	1450858_a_at	ubiquitin-conjugating enzyme E2D 3 (UBC4/5 hom	Ube2d3
11194	1426880_at	enhancer trap locus 4	Etl4
18193	1433879_a_at	RIKEN cDNA C130032J12 gene	C130032J12Ri
6798	1422484_at	cytochrome c, somatic	Cycs
459	1416120_at	ribonucleotide reductase M2	Rrm2
21662	1437348_at	F-box protein 28	Fbxo28
408	1416069_at	phosphofructokinase, platelet	Pfkp
2241	1417902_at	solute carrier family 19 (thiamine transporter), mem	Slc19a2
33900	1449592_at	transcription factor 15	Tcf15
23134	1438820_at	ring finger protein 17	Rnf17
19314	1435000_at	G1 to S phase transition 1	Gspt1
19554	1435240_at	bromodomain adjacent to zinc finger domain, 2B	Baz2b
772	1416433_at	replication protein A2	Rpa2
11096	1426782_at	G protein-coupled receptor 125	Gpr125
19384	1435070_at	AE binding protein 2	Aebp2
39117	1454814_s_at	expressed sequence AU021838	AU021838
1254	1416915_at	mutS homolog 6 (E. coli)	Msh6
19454	1435140_at	insulin degrading enzyme	Ide
35589	1451286_s_at	fusion, derived from t(12;16) malignant liposarcom	Fus
6359	1422045_a_at	protein tyrosine phosphatase, non-receptor type 1	Ptpn12
35944	1451641_at	debranching enzyme homolog 1 (S. cerevisiae)	Dbr1
37017	1452714_at	tetratricopeptide repeat, ankyrin repeat and coiled-	Tanc1
1505	1417166_at	PC4 and SFRS1 interacting protein 1	Psip1
19697	1435383_x_at	necdin	Ndn
23379	1439065_x_at	Predicted gene, OTTMUSG00000010173	OTTMUSG000
35754	1451451_at	grancalcin	Gca
8215	1423901_at	thyroid hormone receptor interactor 12	Trip12
35383	1451080_at	ubiquitin specific peptdiase 1	Usp1
264	1415925_a_at	nucleoporin 62	Nup62
3360	1419021_at	mcf.2 transforming sequence	Mcf2
21054	1436740_at	similar to p47 protein	LOC10004156
23071	1438757_at	RIKEN cDNA C130069I09 gene /// similar to crooke	C130069I09Rik
627	1416288_at	DnaJ (Hsp40) homolog, subfamily A, member 1	Dnaja1
33019	1448711_at	minichromosome maintenance deficient 3 (S. cere	Mcm3ap
11899	1427585_at	DNA cytosine methyltransferase mRNA	---

7589	1423275_at	integrator complex subunit 6	Ints6
8195	1423881_at	SAPS domain family, member 3	Saps3
22406	1438092_x_at	H2A histone family, member Z	H2afz
44902	1460602_at	deleted in liver cancer 1	Dlc1
18534	1434220_at	nucleoporin 98	Nup98
11012	1426698_a_at	heterogeneous nuclear ribonucleoprotein M	Hnrpm
2109	1417770_s_at	proteasome (prosome, macropain) 26S subunit, AT	Psmc6
472	1416133_at	RIKEN cDNA C920006C10 gene	C920006C10Ri
18875	1434561_at	additional sex combs like 1 (Drosophila)	Asxl1
40893	1456590_x_at	aldo-keto reductase family 1, member B3 (aldose r	Akr1b3
32435	1448127_at	ribonucleotide reductase M1	Rrm1
12777	1428463_a_at	protein phosphatase 2, regulatory subunit B (B56),	Ppp2r5e
16356	1432042_a_at	smu-1 suppressor of mec-8 and unc-52 homolog ((	Smu1
8327	1424013_at	eukaryotic translation termination factor 1	Etf1
36483	1452180_at	PHD finger protein 17	Phf17
17910	1433596_at	DnaJ (Hsp40) homolog, subfamily C, member 6	Dnajc6
19255	1434941_s_at	ESF1, nucleolar pre-rRNA processing protein, hom	Esf1
36589	1452286_at	SLAIN motif family, member 2	Slain2
21623	1437309_a_at	replication protein A1	Rpa1
15820	1431506_s_at	peptidyl prolyl isomerase H /// similar to peptidyl p	EG665989 /// L
12547	1428233_at	cleavage and polyadenylation specific factor 6	Cpsf6
25029	1440715_s_at	DNA segment, Chr 11, ERATO Doi 497, expressed	D11Ertd497e
10291	1425977_a_at	STE20-like kinase (yeast)	Slk
12642	1428328_at	nucleoporin 50	Nup50
381	1416042_s_at	nuclear autoantigenic sperm protein (histone-bind	LOC10004397
2453	1418114_at	recombination signal binding protein for immunog	Rbpj
7612	1423298_at	adducin 3 (gamma)	Add3
37566	1453263_at	MAK10 homolog, amino-acid N-acetyltransferase s	Mak10
8062	1423748_at	pyruvate dehydrogenase kinase, isoenzyme 1	Pdk1
39507	1455204_at	phosphatidylinositol transfer protein, cytoplasmic	Pitpnc1
2361	1418022_at	NMDA receptor-regulated gene 1	Narg1

Supplementary Table 2. Summary of miRNA array analysis

	Probeset ID	Systematic Name	Fold Change (WT vs. MI)	Fold Change (WT vs. RQ)
Up-regulated	3674	miR-155	1.52	**3.18**
	8598		1.31	**2.92**
	4476		1.34	**2.66**
	14869	miR-652	-1.09	1.61
	2246		-1.15	1.57
	836		-1.17	1.54
Down-regulated	8889	miR-148	-1.19	-1.69
	18032		-1.17	-1.66
	6907		-1.19	-1.64
	1739	miR-152	-1.14	-1.62
	4328		1.10	-1.66
	10016		1.02	-1.57
	6331		1.08	-1.56
	4115	miR-744	-1.03	-1.64
	9932		-1.01	-1.61
	11089		-1.00	-1.60

Supplementary Table 3. Altered signaling pathways in R1699Q embryoid body

Up-regulated Pathway	-Log (P-value)
Hypoxia Signaling in the Cardiovascular System	5.90E+00
Protein Ubiquitination Pathway	5.60E+00
Wnt/b-catenin Signaling	5.35E+00
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	2.83E+00
Estrogen Receptor Signaling	2.68E+00
PI3K/AKT Signaling	2.68E+00
Tight Junction Signaling	2.63E+00
p53 Signaling	2.43E+00
Down-regulated Pathway	**-Log (P-value)**
IGF-1 Signaling	2.20E+00

Supplementary Table 4. Tumors analyzed from the *Brca1*$^{ko/+}$;*Trp53*$^{ko/+}$;*Tg*^{R1699Q} and *Brca1*$^{ko/+}$;*Trp53*$^{ko/+}$;*Tg*^{M1652I} mice

Tg	Number/Gen	Tumor	Age (months)
M1652I	28 Male	Liver	20
	29 Male	Liver	23
	30 Male	Liver	20
	169 Female	Liver	14
R1699Q	93 Male	Liver	18
	163 Male	Skin	14
	435 Male	GI*	15
	489 Male	GI	15
	491 Male	Skin	15
	515 Female	Mammary	14
	519 Male	GI	16
	578 Male	Liver	13

* GI : Gastrointestinal tract

Supplementary Table 5. Primers used in this study

Primers for BAC Mutagenesis	Sequences
M1652I M1 (Hit Forward))	CTGCTGGGTATAATGCAATGGAAGAAAGTGTGAGCAGGGAGAAGCCAGAATTGAC AGCTTCAACAGAAAGGGTCAACAAAGGATCCTAGAATTCCTCGAG
M1652I M2 (Hit Backward)	TCTTTCCAGAATGTTGTTAAGTCTTAGTCATTAGGGAGATACATATGGATACACTCA CAAATTCTTCTGGGGTCAGGCCACTCGAGGAATTCTAGGATCC
M1652I M3 (Fix Forward)	CTGCTGGGTATAATGCAATGGAAGAAAGTGTGAGCAGGGAGAAGCCAGAATTGAC AGCTTCAACAGAAAGGGTCAACAAAAGAATGTCCATCGTGGTGTC
M1652I M4 (Fix Backward)	TCTTTCCAGAATGTTGTTAAGTCTTAGTCATTAGGGAGATACATATGGATACACTCA CAAATTCTTCTGGGGTCAGGCCAGACACCACGATGGACATTCT
R1699Q M1 (Hit Forward)	GCTTCTTAGGACAGCACTTCCTGATTTTGTTTTCAACTTCTAATCCTTTGAGTGTTTT TCATTCTGCAGATGCTGAGTTTGGATCCTAGAATTCCTCGAG
R1699Q M2 (Hit Backward)	AAGGGAGGAGGGGAGAAATAGTATTATACTTACAGAAATAGCTAACTACCCATTTT CCTCCCGCAATTCCTAGAAAATATCTCGAGGAATTCTAGGATCC
R1699Q M3 (Fix Forward)	GCTTCTTAGGACAGCACTTCCTGATTTTGTTTTCAACTTCTAATCCTTTGAGTGTTTT TCATTCTGCAGATGCTGAGTTTGTGTGTGAACAGACACTGAA
R1699Q M4 (Fix Backward)	AAGGGAGGAGGGGAGAAATAGTATTATACTTACAGAAATAGCTAACTACCCATTTT CCTCCCGCAATTCCTAGAAAATATTTCAGTGTCTGTTCACACAC
ChIP primers	**Sequences**
mBIC Brca1-1 ChIP	AAG TTT GCA ACT TCC CCT GC
	TCG TGA CTC ATA ACC GAC CAG
mBIC Brca1-2 ChIP	GAG CTT CTG TGC CTG TTT GC
	CTA ATA GAT CGG GTC CAT TCC
hBIC BRCA1 ChIP1	CTG TAG GTT CCA AGA ACA GG
	TCC GCT ATC CGC TCC CTT CC
hBIC BRCA1 ChIP2	GAC CAG AGA TTG CGC TGG AT
	AAG AAA GGC AAC CGC TCG GC
hEsrrg ChIP	GTT CTG ATG GCC ATT CAT GG
	CAC ATT GAT TCC AGC TGT TCG
hStat5a ChIP	GAA AAG CCC TAG CCG TCG AG
	ACA GAG ATG GTG AGG AGT GG
hCyclinB1 ChIP	CTG ATT TTC CCA TGA GAG GC
	GAT TGT CCA GTT TCC CAA GG
Genotyping primers	**sequences**
Brca1 Conditional	TAT CAC CAC TGA ATC TCT ACC G
	GAC CTC AAA CTC TGA GAT CCA C
	TAT TCT TAC TTC GTG GCA CAT C
	TCC ATA GCA TCT CCT TCT AAA C
K14 Cre	TTT TCA AGG CAA TCA GGG TA
	CAT CAC TCG TTG CAT CGA CC
Brca1 knockout	GGA CGG CAG ATA AAT CCA TTT CTT CC
	GGT ACA AAG CCA GTG TGG GTT ACA TG

	GGA ATG TTT CCA CCC AAT GTC GAG C
	CATC AGA GCC GATT GTC TGT TG
Trp53	CGT GAT ATT GCT GAA GAC CTT GGC
	CCT CAA TAA GCT ATT CTG CCA GCT G
	CTG TCT TCC AGA TAC TCG GGA TAC
Brca1 R1699Q / M1652I BAC TG	ATG GAG GAA CCC ACA TAG GC
	TAT GGG ATA GAG GTG AGA TCC
Real-time PCR primers	**sequences**
Oct4	CCG TGT GAG GTG GAG TCT GGA G
	GCG ATG TGA GTG ATC TGC TGT AGG
Nanog	GAA ATC CCT TCC CTC GCC ATC
	CTC AGT AGC AGA CCC TTG TAA GC
B-T	TAC ACA CCA CTG ACG CAC ACG
	GAG GCT ATG AGG AGG CTT TG
mBrca1	CAG GTC TAT TGT TGT GAG CC
	TCT GTA CCA GGT AGG CAT CC
hBRCA1 (LOH)	AAC CAC AGT CGG GAA ACA AG
	TAA CTG TCT GTA CAG GCT TG
Gadd45a	TAG CTG AGC TGC TGC TAC TG
	GTT CCG GGA GAT TAA TCA CG
CyclinB1	GAG AAG CTT TCT CCT GAA CC
	TTT GGT CTA ACT GAC TGC TC
pri-miR155	CCT CAT GAA ACC AGC TCA TCT G
	CAG GTA GGA GTC AGT CAG AG
Actin	TCC TCC TGA GCG CAA GTA CT
	ACG CGT TCA ATC CAA AAC AG
Lamb1	CTA TCC AAC TGG ATT TGG AAG C
	TCC GTT GAG GGT TCA ATG TCG
mmu-miR-155	Mm_miR-155_1 miScript Primer Assay (UUAAUGCUAAUUGUGAUAGGGGU)
Hs miR-155	Hs_miR-155_1 miScript Primer Assay
	(UUAAUGCUAAUCGUGAUAGGGGU)
RNU6B_2	miScript PCR control (Cat no.218380)
RNU5A_1	miScript PCR control (Cat no. MS00013993)
Northern / Southern primers	**sequences**
mmu-miR-155 probe	CCC CTA TCA CAA TTA GCA TTA A
Hs-miR-155 probe	CCC CTA TCA CGA TTA GCA TTA A
Hs U6 Probe	AAC CGT ATG CGT GTT GTC AGG
mmu U6 probe	GCT AAT CTT CTC TGT ATC GT
mBRCA1 Probe (LOH)	AAC TGC TAA AGC GTC TCC AC
	CAA CAT AAA CGC TAA CCA ACC

Others	**sequences**
miR-155 Reporter construct	CTA GCC CCT ATC ACA ATT AGC ATT AA
	AGC TTT AAT GCT AAT TGT GAT AGG GG
R1699Q Site-Directed Mutagenesis	GCT GAG TTT GTG TGT GAA CAG ACA CTG AAA TAT TTT CTA GG
BRCA1 ShRNA	Target set for NM_007294 (12 Clones, Open Biosystem Cat. No.RHS4529-NM_007294)
Promoter mutagenesis	
BIC Br1-2 Mut1	5'- ACC TCG AGT AGA TGG TAC AAA CCC TAA TAG ATC GGG TCC ATT CCT GAA AGC TGA AAC AAA CAA ACA AAC AAA ACA ACA AAA CAA CAA AAA AGT GAC CTA CGC TCCTAG GAG ATT TCT GAG AAA AAA AAA AGC AAA AAA CAA AAC AAA GCC GCC GCC GCC GCC GCC GAA AAA CCG CCG AGA CAA CTC AAG TGC CTC AGG -3
BIC Br1-2 Mut2F	CGT AGG TCA CTT TTT TCG GCT TTT CGG CTT TTG TTT GTT TGT TTG
BIC Br1-2 Mut2R	CAA ACA AAC AAA CAA AAG CCG AAA AGC CGA AAA AAG TGA CCT ACG

1 Cable, P. L. *et al*. Novel consensus DNA-binding sequence for BRCA1 protein complexes. *Mol Carcinog* **38**, 85-96 (2003).

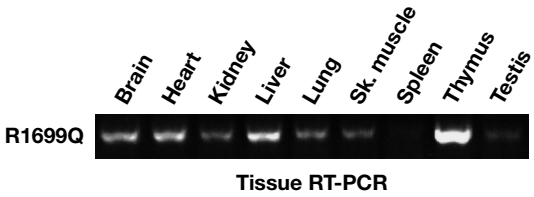
Supplementary Table 6. Specific mutations in BRCA1 in human breast tumors

Tumor Sample No.	Tumor ID	Specific Mutations
1	143	R1751X
2	628	E402X
3	696	IVS 4-1G>C
4	764	185 delAG
5	967	E879X
6	1097	S1383X
7	1118	S157X
8	8798	187delAG
9	8799	L752X
10	8801	5385insC
11	8804	C61G (300T>G)
12	8805	del exon 1
13	8806	C61G (300T>G)
14	8808	187delAG

SUPPLEMENTARY FIGURES

Supplementary Figure 1. R1699Q BAC transgenic expression does not rescue the embryonic lethality of *Brca1*$^{ko/ko}$ mice. **a**. Representative expression profile of R1699Q RNA in multiple tissues from a R1699Q BAC transgenic mouse. **b**. Summary of the number of newborns from *Brca1*$^{+/ko}$; *Tg*^{R1699Q} intercrosses. **c**. Representative picture of embryos dissected on E7.5. Genotypes of embryos are indicated at the top (scale bar=100μM).

a

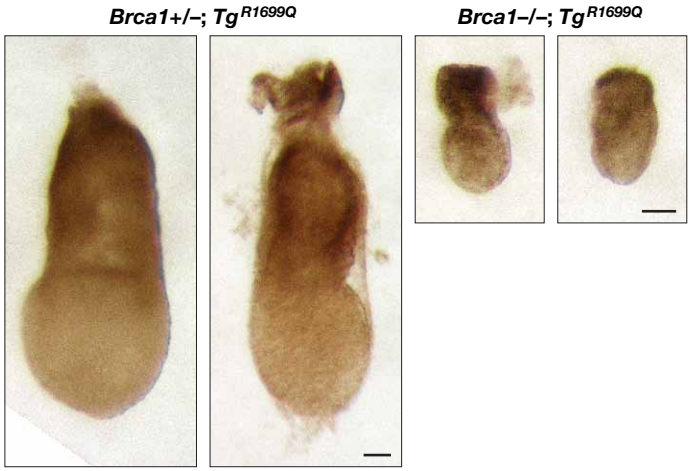


Tissue RT-PCR

b

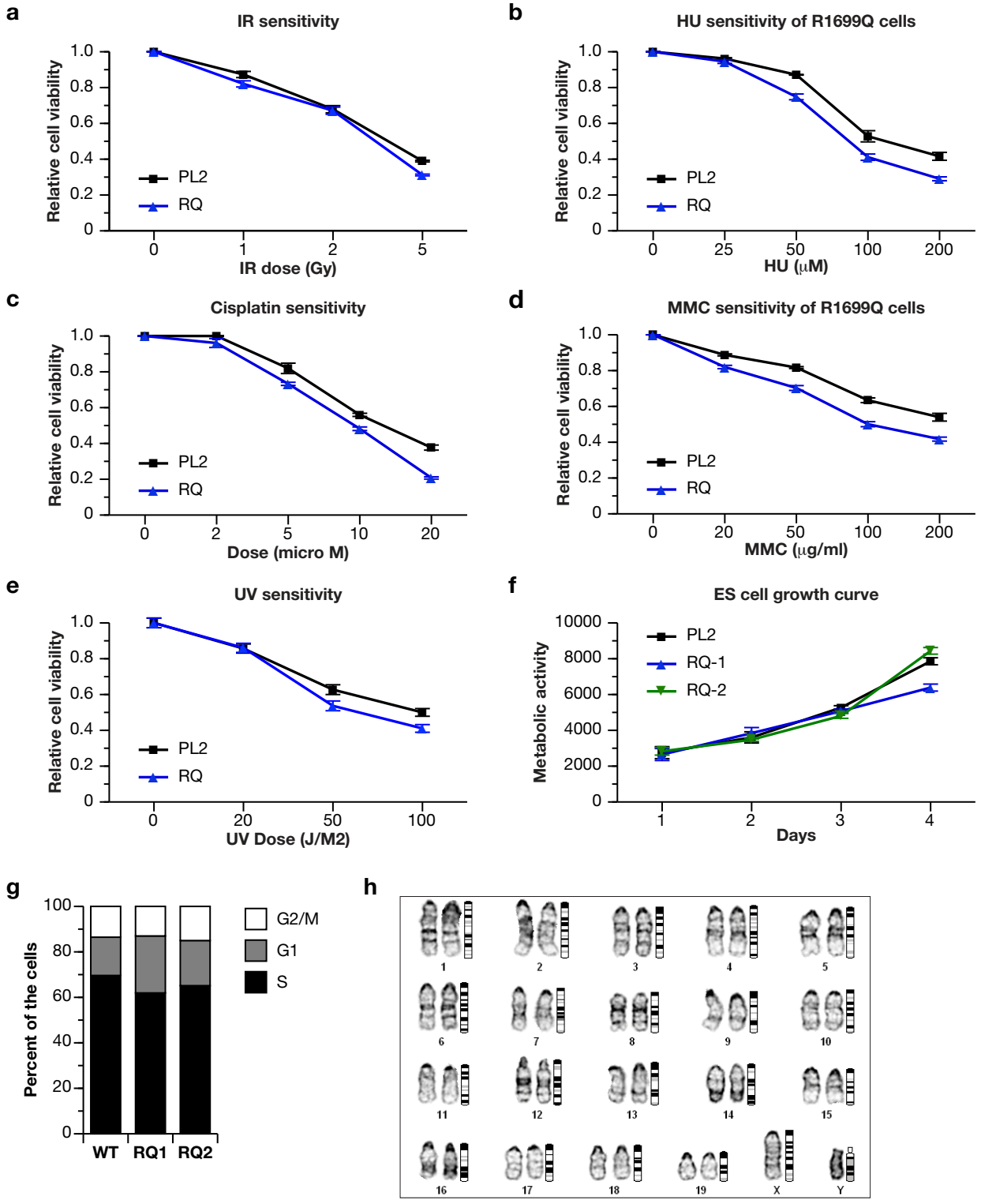
	Brca1		
R1699Q	**+/+**	**+/–**	**–/–**
Non-TG	**27**	**62**	**0**
TG	**57**	**125**	**0**

c



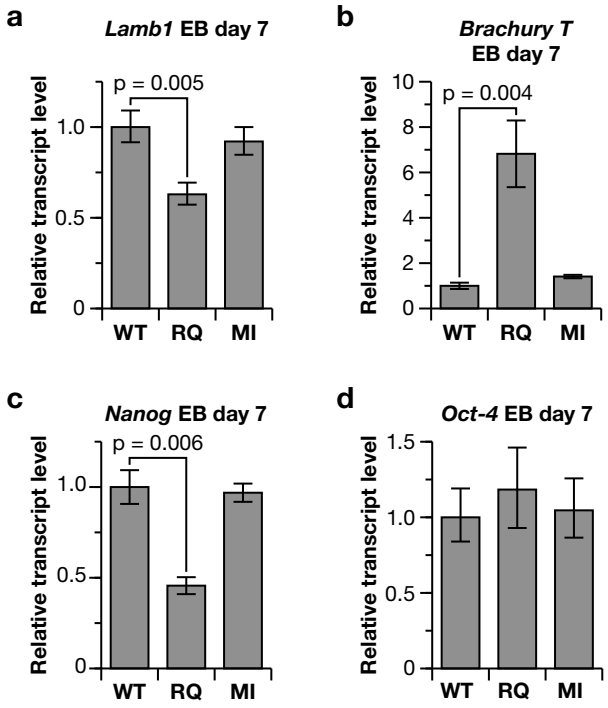
Chang – Sup Figure 1

Supplementary Figure 2. R1699Q ES cells do not show hypersensitivity to DNA damaging agents (**a-e**), and show normal cell growth (**f, g**) and karyotype (**h**). PL2F8a expressing wild type BRCA1 (PL2) and R1699Q ES cells were treated with Cisplatin, MMC, HU, UV and IR with indicated doses. After 48 hours, the metabolic activity of the cells was measured by alamar blue assay. Cell growth was tested in 96-well plates without feeder cells. Cell cycle was analyzed by PI-BrdU staining (**g**). For karyotyping, ES cells in 6-well plates were treated with colcemid and chromosomes at metaphase were examined. One representative picture is shown out of two independent clones analyzed.



Chang – Sup Figure 2

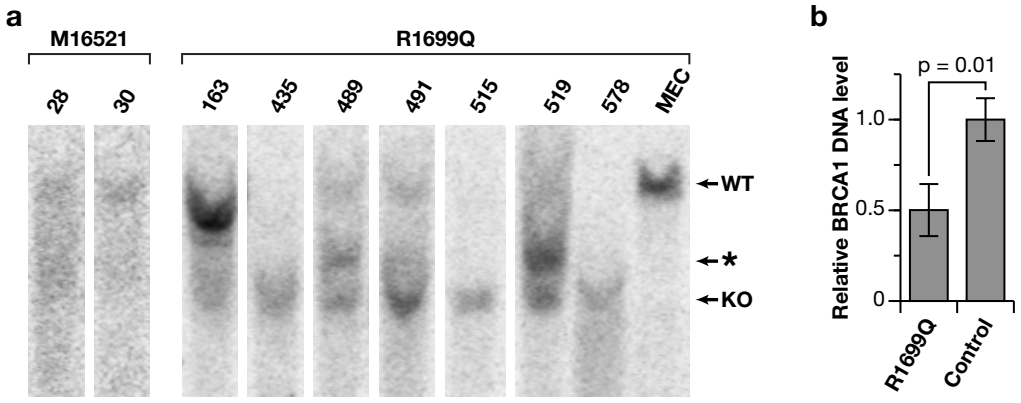
Supplementary Figure 3. Differential expressions of *Lamb1* (**a**), *Brachury T* (**b**), *Nanog* (**c**) and *Oct-4* (**d**) in R1699Q EBs. Wild type (WT), R1699Q (RQ), M1652I (MI) ES cells were cultured in suspension to generate EBs. On day 7, cells were harvested and transcript level of each gene was measured by real-time PCR.



Chang – Sup Figure 3

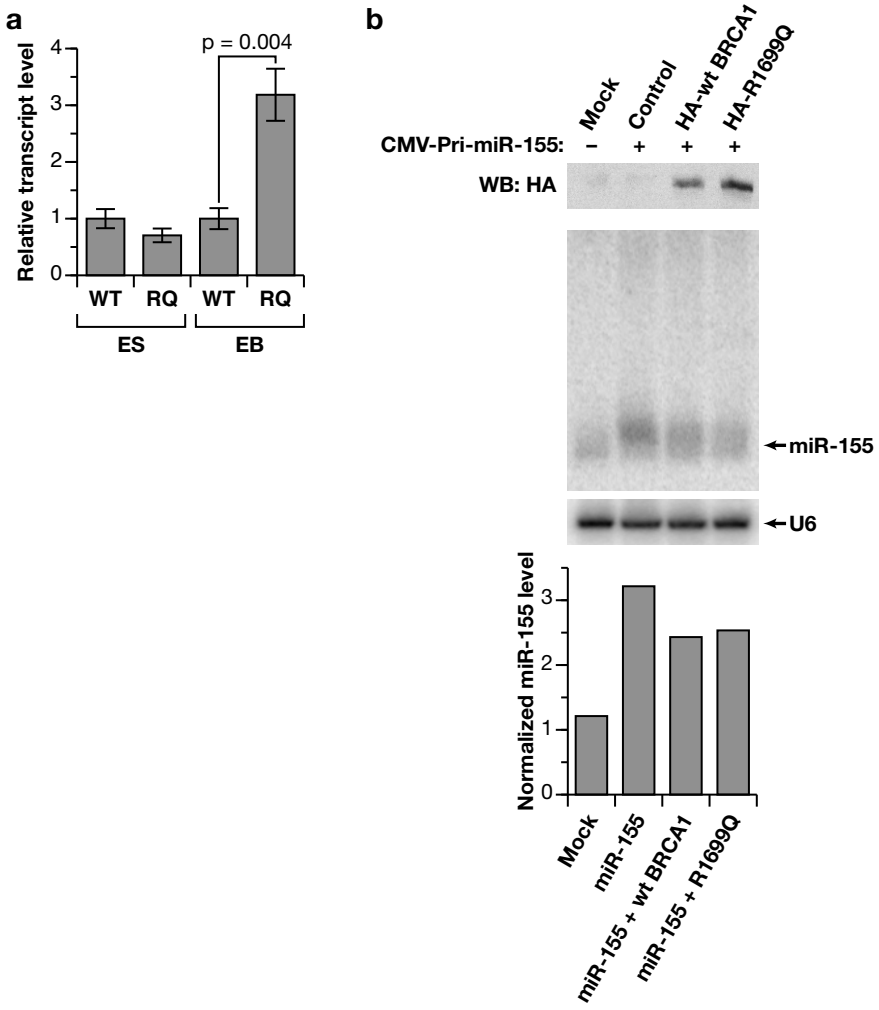
Supplementary Figure 4. LOH analysis of tumors with R1699Q mutation

a. Southern hybridization of *Brca1* shows LOH of *Brca1* in 6 out of 7 tumors from *Brca1*$^{ko/+}$;*Trp53*$^{ko/+}$;*Tg*^{R1699Q} mouse. Note that the tumor 163 with no LOH had low level of miR-155 in the Fig 3a (asterisk : signal from partial digested DNA). b. Real time PCR quantitation of *BRCA1* gene in the FFPE human tumor with R1699Q mutation. Control is a normal breast tissue. *CYCLIN B1* was used as normalizer.



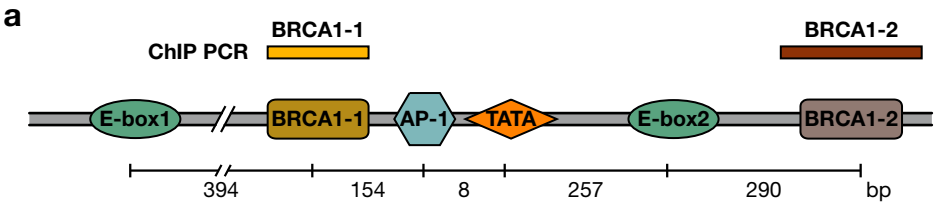
Chang – Sup Figure 4

Supplementary Figure 5. BRCA1 R1699Q mutation specifically increases miR-155 transcription. **a**. Real-time PCR quantitation of pri-miR-155 in WT and R1699Q ES cells and EBs on day 7. **b**. The effect of BRCA1 on the processing of miR-155 was tested in BRCA1 deficient MDA-MB 436 cells. A construct expressing pri-miR-155 (CMV-pri-miR-155) was co-transfected with HA-tagged WT or R1699Q BRCA1 expression plasmids. Top panel : Western blot of BRCA1, two middle panels : Northern blot of miR-155 and U6 snRNA. Bottom panel: Quantitation of the matured form of miR-155, normalized by U6 snRNA.



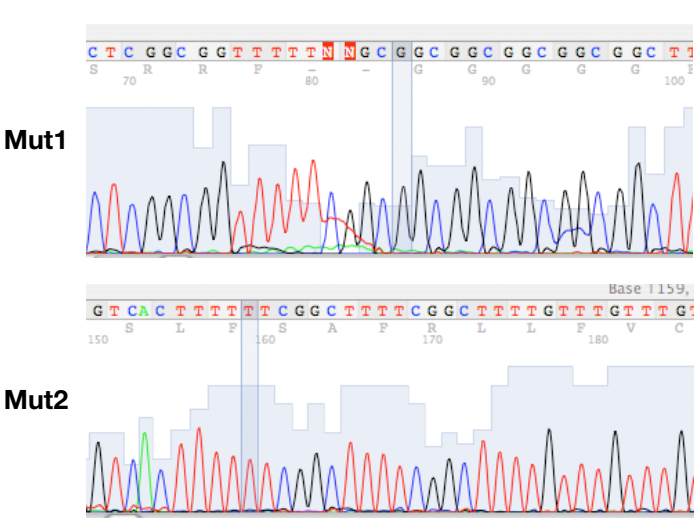
Chang – Sup Figure 5

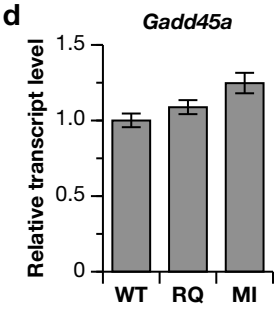
Supplementary Figure 6. BRCA1 binding on miR-155 promoter. **a**. Schematic diagram showing the two putative BRCA1 binding regions in the miR-155 promoter. **b**. Sequence of the BRCA1-2 region showing two clusters of putative BRCA1 binding sites (open box : first cluster, shaded box : second cluster). Out of 8 base pair of proposed consensus sequence (T/G-T/G-N-T-GTTG [1]), the last four stringent bases were colored as red (in the first cluster) or blue (in the second cluster). **c**. Sequencing results showing the generation of mutants (Mut1: for the first cluster, Mut2 : for the second cluster and Double : mutant of both clusters (not shown)) in BRCA1-2 region. **d** and **e**. Real-time PCR quantitation of know transcriptional targets of BRCA1 in embryoid bodies generated from *Brca1*$^{ko/ko}$ ES cells rescued by WT, R1699Q (RQ) and M1652I (MI) BRCA1: *Gadd45a* (d) and *Cyclin B1* (e) in EB cells.

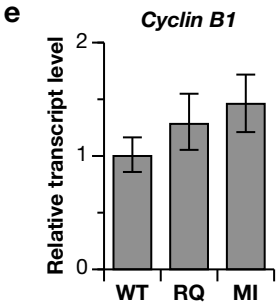


b Mouse miR-155 promoter (BRCA1-2)

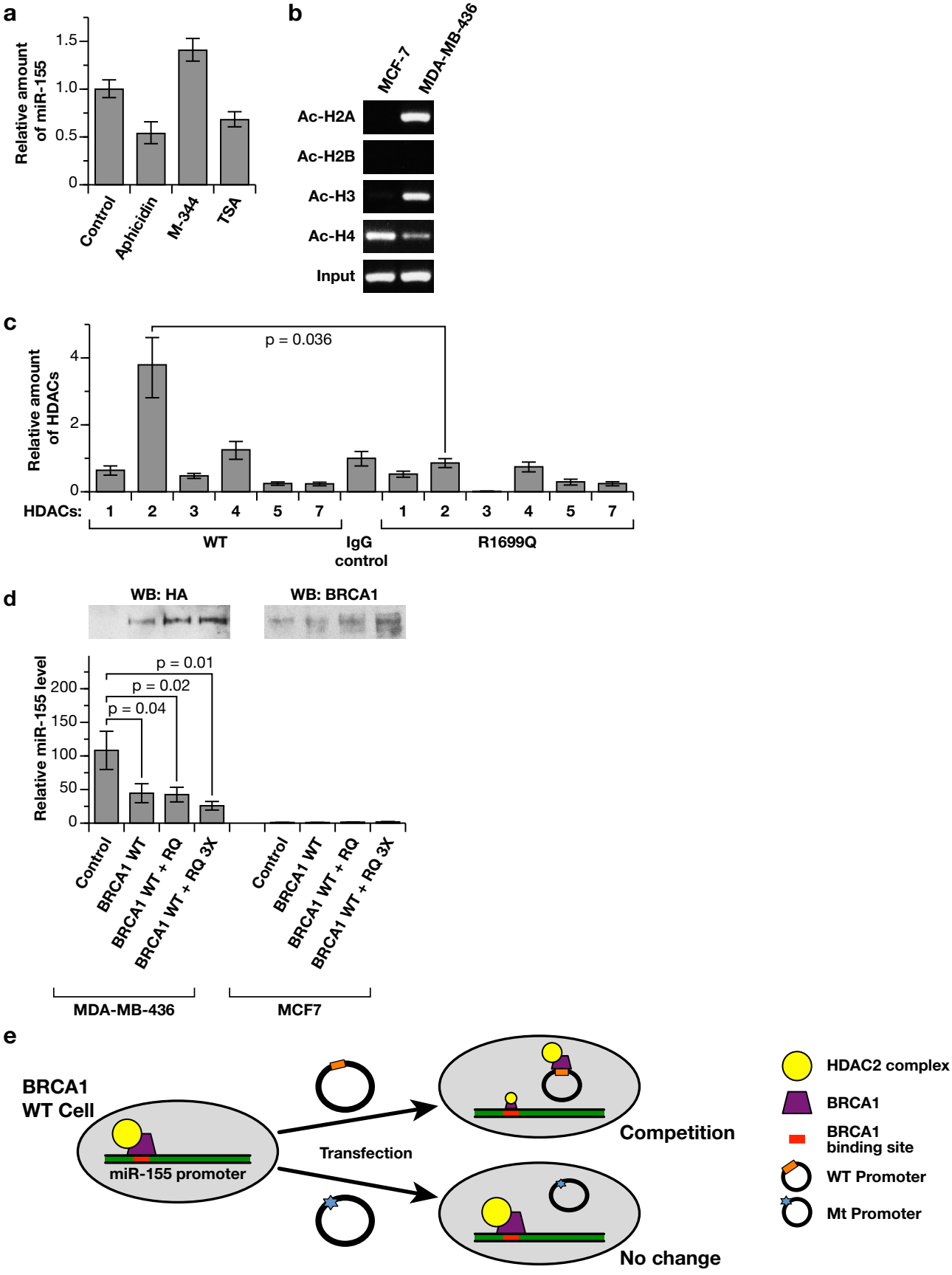
GGGACCGGCTTCAGTTCATAAATAATCCAAACTAATCTAACCAAGTTAGGTTCCCGTGGTTTTTAACGGA
AGAATGAAGCCTGGCTAACCCTTGAGCTTCTGTGCCTGTTTGCTCCCTTCGTGCCAAGGTGAGATTTTTT
TCCTGAGGCACTTGAGTTGTCTGTTGGGTTTTTGTTGTTGTTGTTGTTGTTGTTTGTTTTGTTTTTTGCT
TTTTTTTTTCTCAGAAATCTCCTAGGAGCGTAGGTCACTTTTTTGTTGTTTTGTTGTTTTGTTTGTTTGT
TTGTTTCAGCTTTCAGGAATGGACCCGATCTATTACCATCTAAATCAG





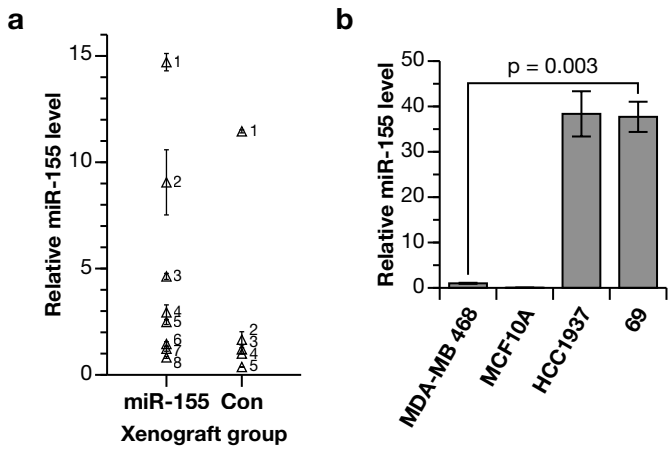


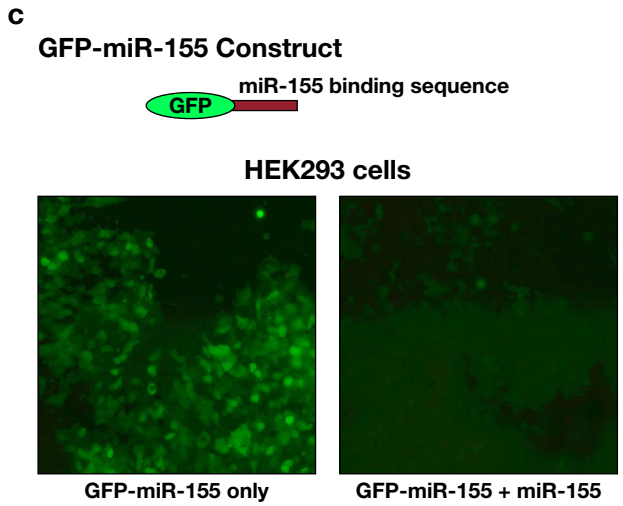
Supplementary Figure 7. BRCA1 epigenetically controls the miR-155 promoter. **a. E**ffect of different HDAC inhibitors on miR-155 level in BRCA1-deficient HCC1937 cells. **b.** ChIP analysis of BRCA1-positive MCF7 and BRCA1-deficient MDA-MB-436 cells. Antibodies specific to acetylated histones H2A, H2B, H3 and H4 were used to test differential histone acetylation on the miR-155 promoter. **c.** ChIP analysis to test association of HDAC1- HDAC5 and HDAC7 on the miR-155 promoter in WT or R1699Q EBs on day 7. Rabbit IgG was used as negative control. **d**. Increasing amount HA-R1699Q expression plasmid was transfected into MDA-MB-436 cells or MCF7 cells along with HA-wt BRCA1. The miR-155 level from the transfected cells is shown. Note that basal level of miR-155 (Con; vector only) in MDA-MB-436 cells is much higher than that in MCF7 cells. **e**. Schematic diagram showing the experimental design of Fig 4i, Note that the possible competition between mouse and human miR-155 promoters for the BRCA1-HDAC2 complex, only when WT but not the mutant mouse miR-155 promoter is transfected into human cell line.

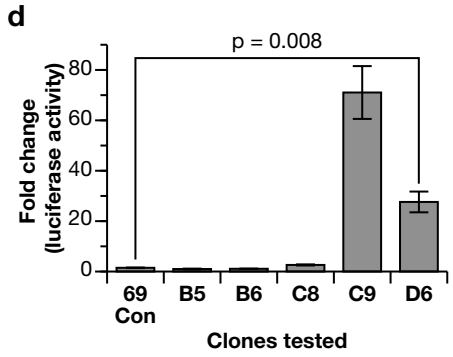


Chang – Sup Figure 7

Supplementary Figure 8. *In vivo* effect of miR-155 on tumor growth. **a** Real time PCR quantitation of miR-155 from the tumors shown in Fig 5a. **b**. Real time PCR quantitation of miR-155 in mouse Brca1 deficient tumor cell line 69. Note that the miR-155 level is comparably high to human BRCA1 deficient HCC1973 cell **c**. Validation of antagomiR (Sponge). GFP-miR-155 construct was transfected with control or miR-155 expression plasmid (CMV-Pri-miR-155) and the efficient reduction of GFP signal was checked under the fluorescence microscope. **d**. Screening of clones with stable miR-155 knockdown. miR-155 luciferase reporter was transfected into 96 candidate clones and two clones (C9 and D6) showed high luciferase activity compared to parental or other clonal cells.



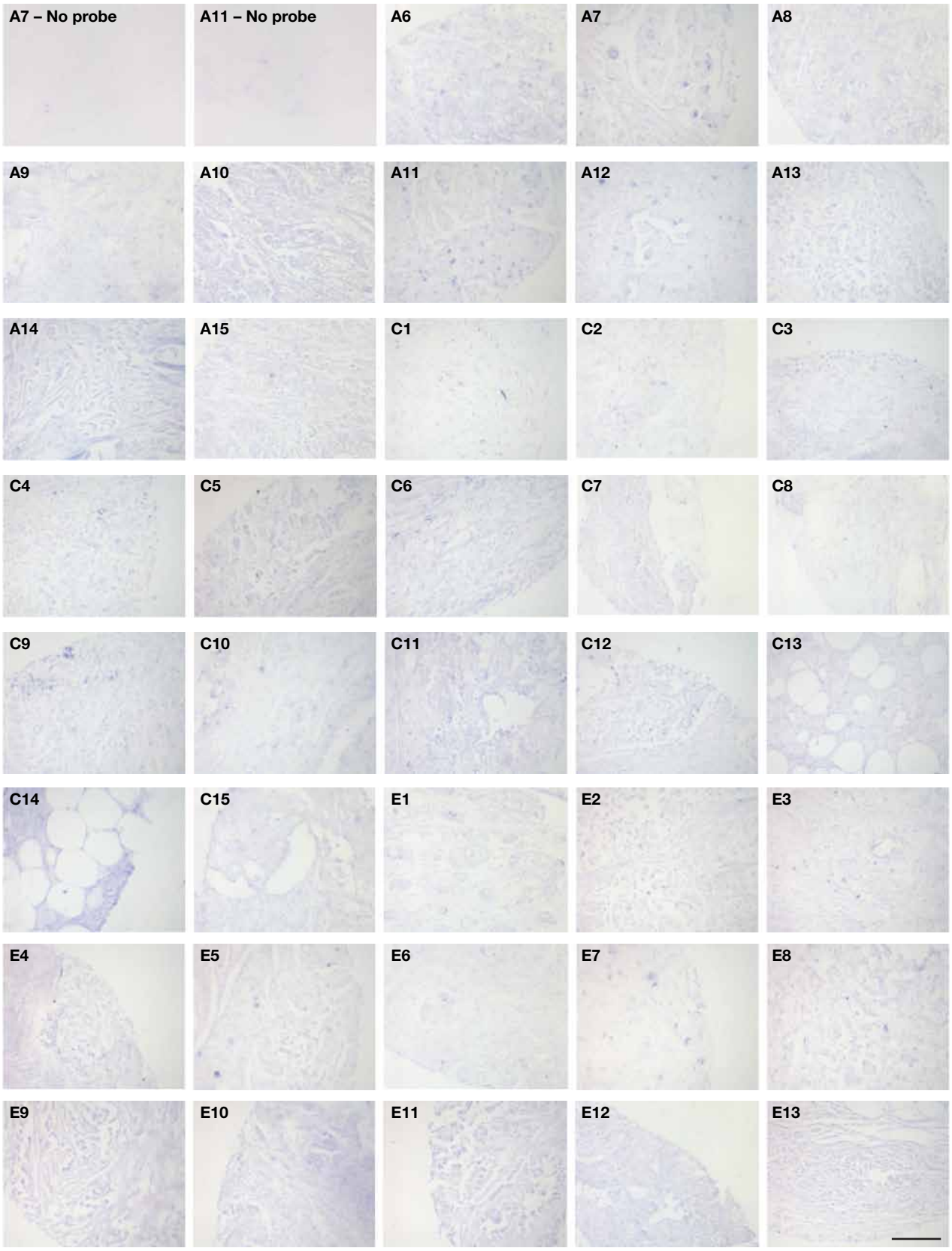




Chang – Sup Figure 8

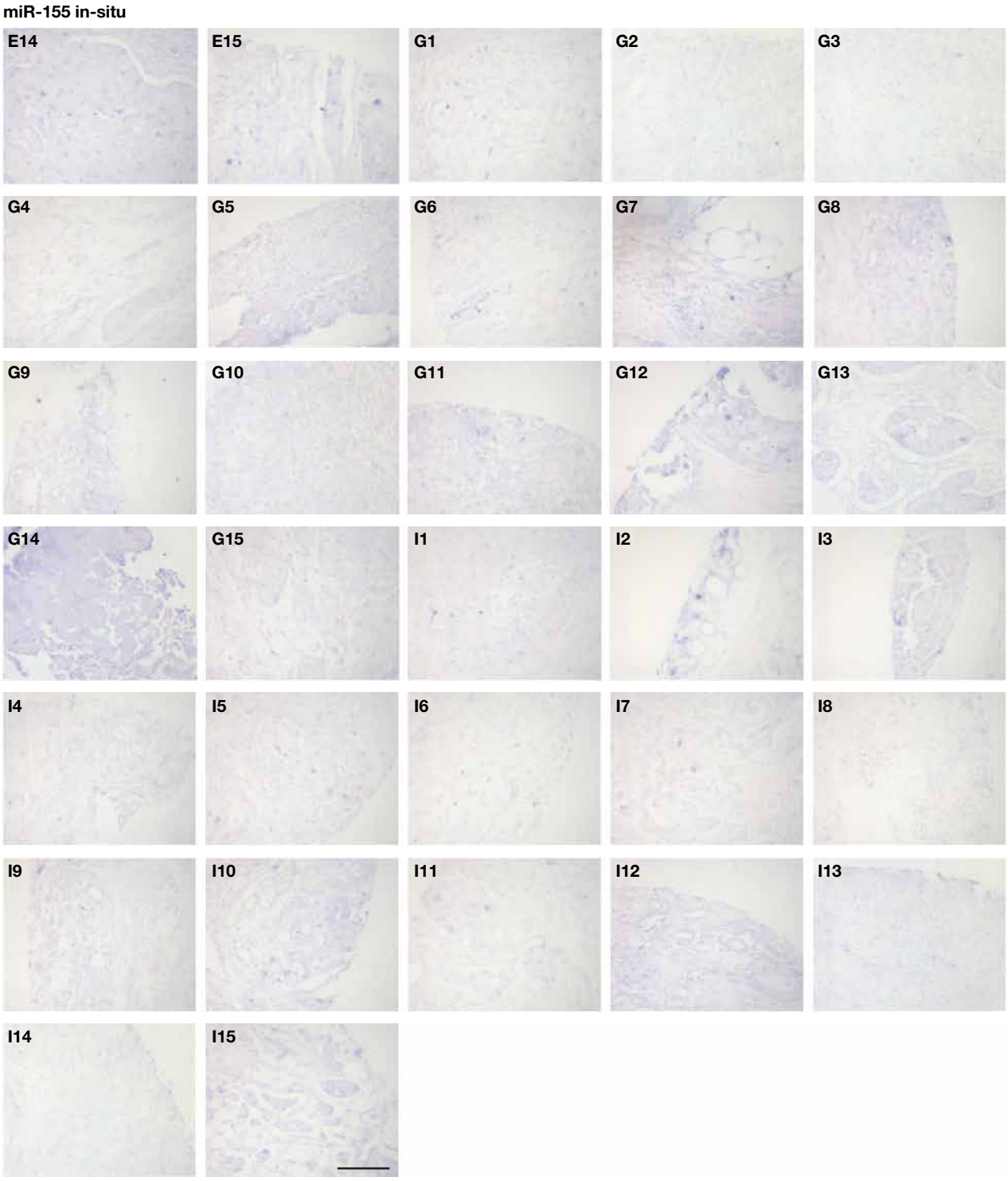
Supplementary Figure 9. Human breast tumor tissue array analysis **a**. BRCA1 IHC of 70 tumor tissues with IgG control. **b**. miR-155 *in-situ* hybridization of 70 tumor tissues and two representative pictures of no probe controls.

a

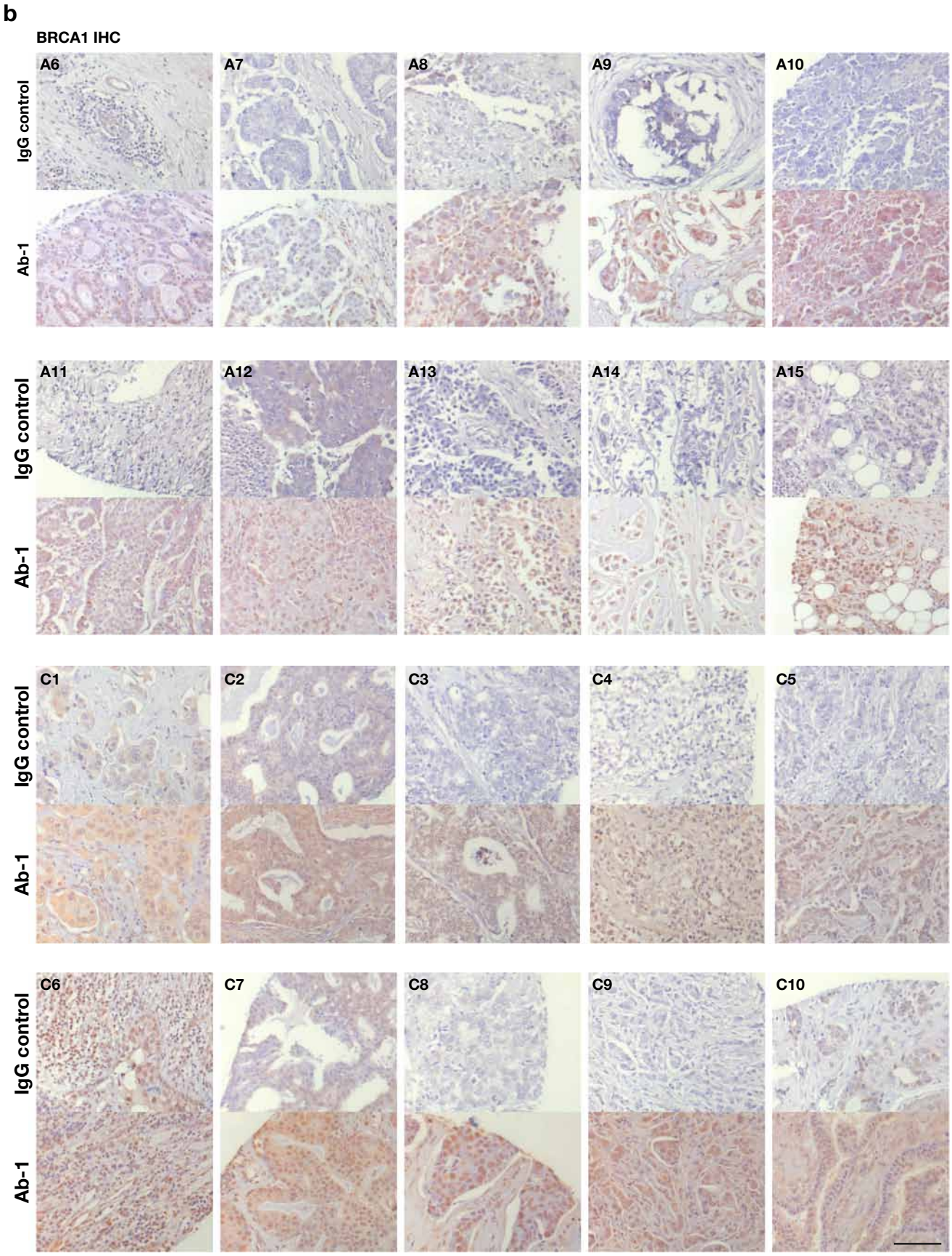


Chang – Sup Figure 9a

a (continued)

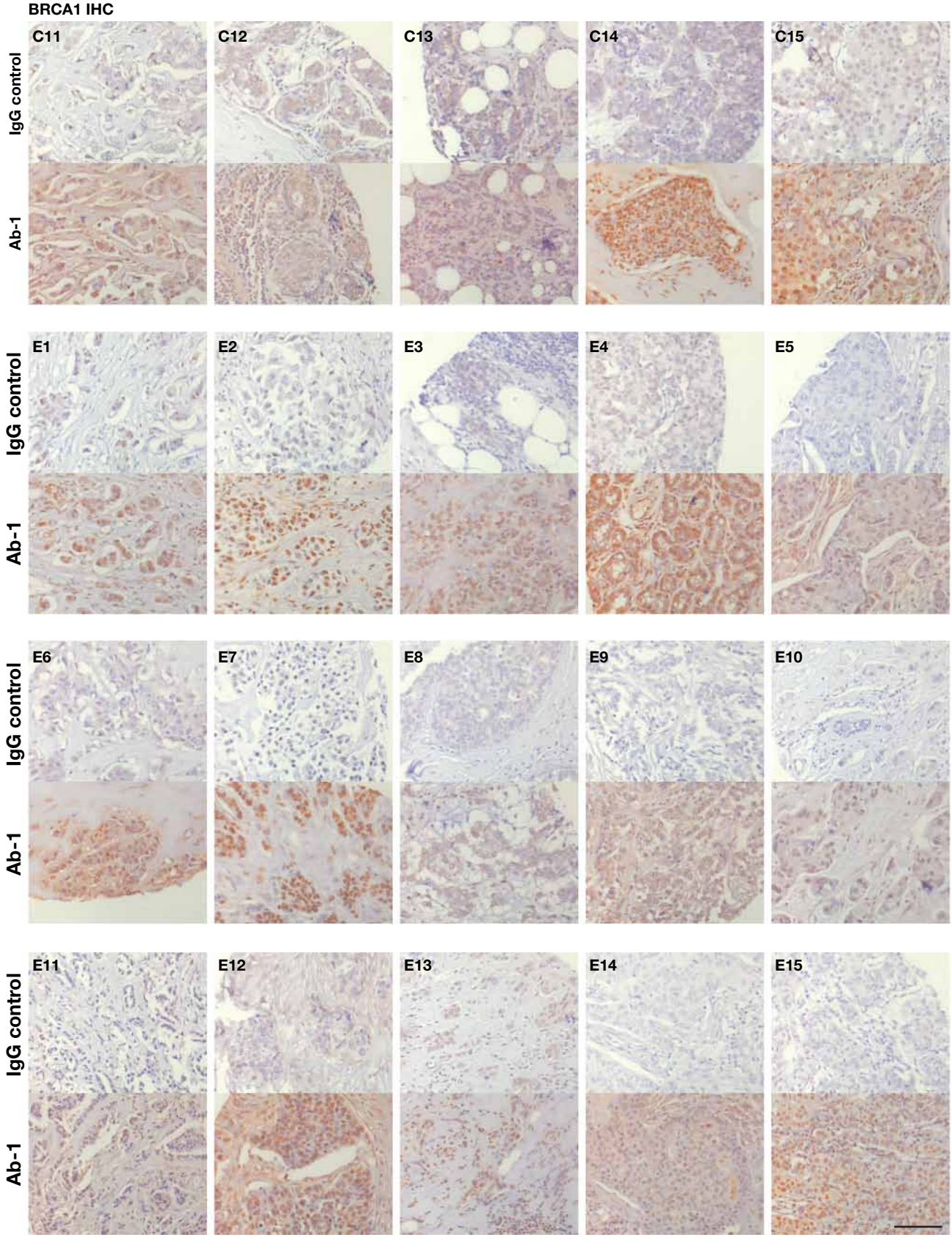


Chang – Sup Figure 9a



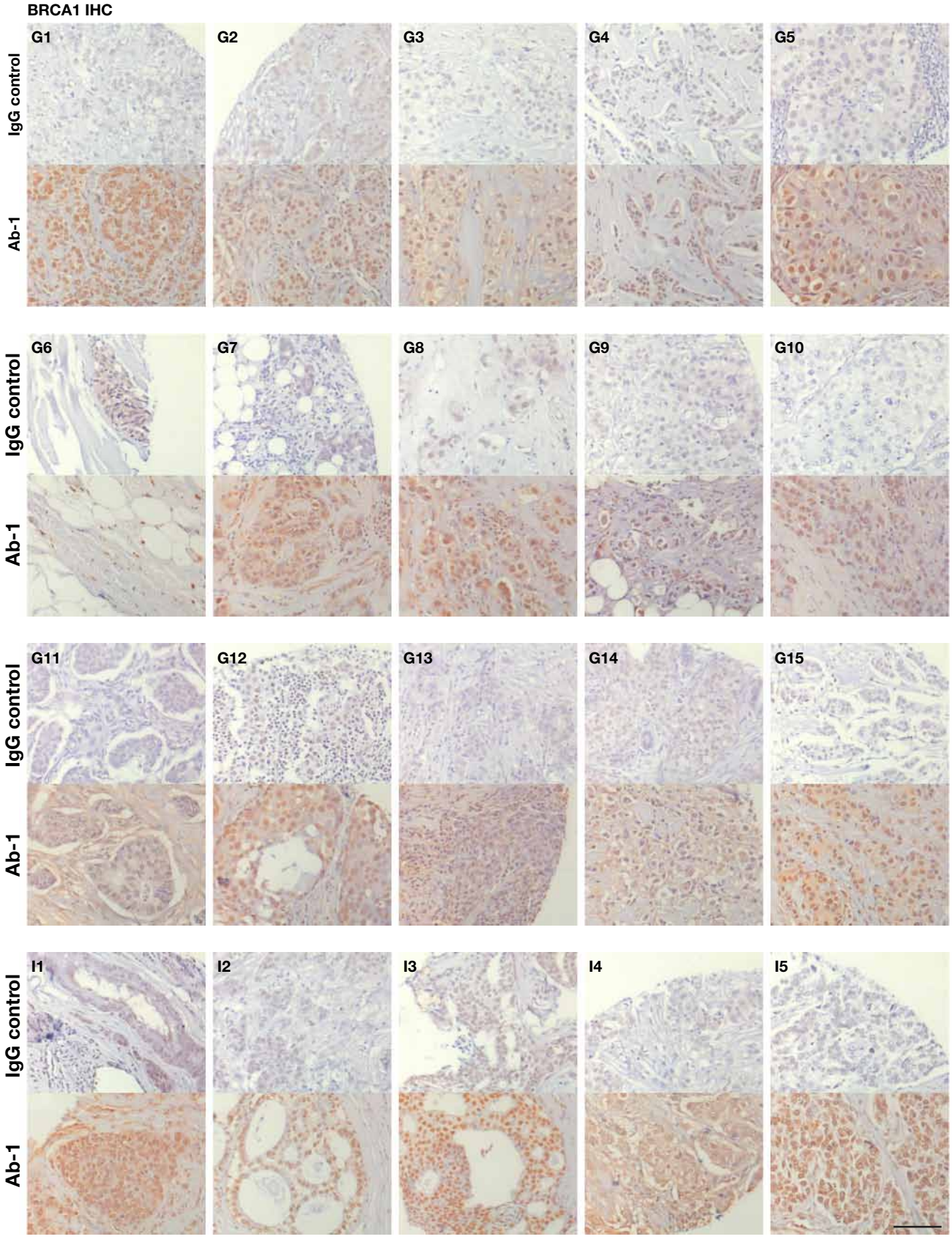
Chang – Sup Figure 9b

b (continued)



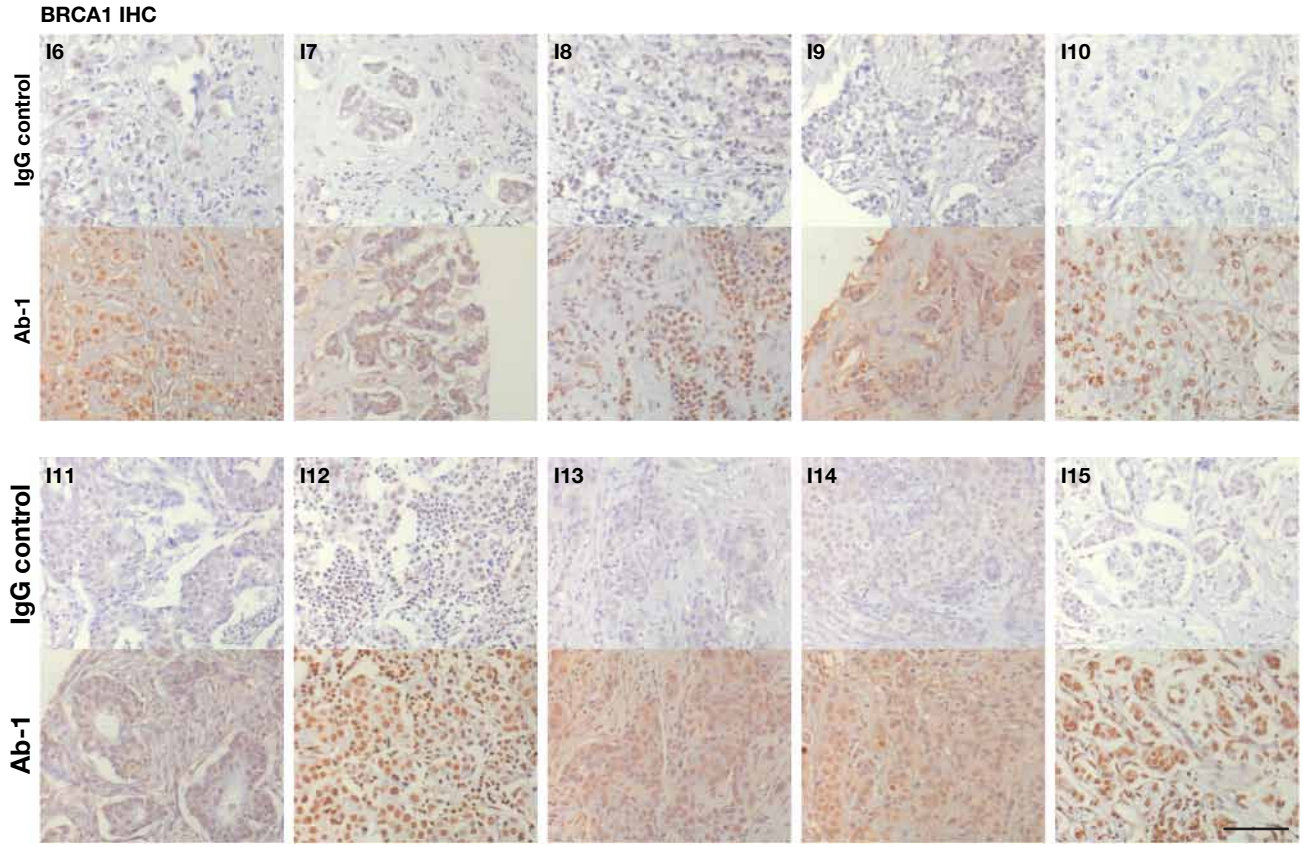
Chang – Sup Figure 9b

b (continued)



Chang – Sup Figure 9b

b (continued)



Chang – Sup Figure 9b

SUPPLEMENTARY METHODS

Cell culture and transfection

ES cells were cultured on mitotically inactivated feeder cells as described earlier[1]. The Human breast cancer cell lines MCF7, HCC1937, MDA-MB-436 (obtained from American Type Culture Collection (ATCC), Rockville, MD), HEK 293 and MDA-MB-468 (generous gift from Dr. Esta Sterneck, NCI) were cultured in DMEM supplemented with 10% FBS and antibiotics. MCF10A cells were cultured in F-12/DMEM (1:1) with supplements as described (ATCC, CRL-10317). Mouse BRCA1 deficient cell line (#69) was cultured in DMEM supplemented with 10% FBS and antibiotics. For transfection of MDA-MB 436 cells, 15μl of Lipofectamine 2000 (Invitrogen) was mixed with 5μg of DNA per one 100mm culture dish with 5ml of Opti-MEM (Invitrogen). For the reporter assay in HCC1937 or MDA-MB-468 cells, the cells in 24 well plates were transfected with 20ng-100ng of DNA mixed with 0.1-0.5 μl of lipofectamine 2000. For stable knockdown of BRCA1 in HEK293 cells, 12 clones of BRCA1 shRNA set (see the supplementary Table 6) was transfected and the cells were selected under 1mg/ml G-418 for 1 week. The G-418 positive clones were further expanded to 6 wells and analyzed for BRCA1 level.

DNA constructs

A bacterial artificial chromosome clone (RPCI 11-812-5O) containing the full-length human *BRCA1* was used to generate R1699Q using recombineering-based "Hit and Fix" method as described previously[2]. M1652I variant was described previously[1]. Sequence of oligonucleotides used to generate the mutations in the BAC is described in

Supplementary Table 6. The reporter plasmid of miR-155 promoter was constructed by cloning 1.5 kb genomic fragment of mouse *BIC* prompter containing the TATA box into pGL3 Enhancer plasmid (Promega). The first mutation on the BIC promoter (Mut1) was generated by designing a 200mer oligonucleotide with 9 TGGT > GCCG change for putative BRCA1 binding sites. The second mutation (Mut2) was generated by QuickChange II site directed mutagenesis kit (Stratagene). The double mutant was generated by introducing Mut2 in Mut1 reporter construct. miRNA-155 luciferase reporter was constructed by cloning the oligonucleotide of the complementary sequence for matured miR-155 into pMIR-REPORT luciferase reporter plasmid (Ambion). The GFP sponge of miR-155 was generated by using the same oligonucleotide for luciferase reporter, cloned into pEGFP-C1 (Clontech). The HA tagged BRCA1 expression plasmid (BRCA1-HA) is a generous gift from Dr. David M. Livingston. The R1699Q mutation (c.5095G>A) was introduced in BRCA1-HA using QuickChange II site directed mutagenesis kit (Stratagene). For the inducible miR-155, two complementary oligonucleotides with the sequence of the miR-155 precursor were cloned into pSUPERIOR.retro.puro plasmid (OligoEngine) into the *Bgl*II and *Hin*dIII sites. pcDNA/TR6 vector (Invitrogen) was used to express Tet repressor. The pCS2+BIC134-283 (Designated as CMV-pri-miR-155, generous gift from Dr. David L. Turner) was used for the over-expression of miR-155. All DNA constructs were confirmed by sequencing.

Embryoid body analysis

To generate embryoid bodies, ES cells were trypsinized and the feeder cells were removed by incubating the cells on gelatinized plate for one hour. The ES cells in supernatant were counted and diluted to $5X10^4$ cells/ml. The cell suspension was cultured in Petri dish, in DMEM-10 media. The media was changed on day 3 and later it was changed every other day. After 14 days, the embryoid bodies were collected.
For histology, the embryoid bodies were fixed in 10% formalin and embedded in paraffin and sectioned. The sections were stained with H&E. To analyze cell death, DeadEnd TUNEL staining kit (Promega) was used as per the manufacture's protocol. For miRNA *in situ* hybridization in embryoid body, DIG labeled LNA-miR-155 probe (Exiqon) was used, followed by the colorimetric detection using REMBRANDT® In Situ Hybridization and Detection kit (Panpath).

Western blots and co-immunoprecipitation (co-IP) analysis

For western blot, cells were lysed on ice in RIPA buffer with protease inhibitor tablet (Roche) for 20minutes. The cleared lysate was added with 2X protein sample buffer and used for western. For co-IP, freshly isolated mammary epithelial cell (10^7 cells) was lysed in modified RIPA buffer (150 mM NaCl, 10 mM Tris, pH 7.2, 0.1% SDS, 0.5% Triton X-100, 0.5% Deoxycholate, 5 mM EDTA) with protease inhibitors. The human specific BRCA1 antibody or rabbit monoclonal HDAC2 antibody was used for IP. The co-immunoprecipitated BRCA1 and HDAC2 were detected by E1 (for BRCA1) or mouse monoclonal HDAC2 antibody.

LOH analysis of the tumors

To test the LOH of the tumors from the *Brca1*$^{ko/+}$;*Trp53*$^{ko/+}$;*Tg*^{R1699Q} mice, Southern hybridization was performed using freshly isolated tumor DNA digested with PstI. The probe for *Brca1* detection was generated by PCR (See Supplementary Table5 for sequence) and labeled with Prime-It random primer labeling kit (Stratagene). To measure the copy number of BRCA1 in the R1699Q human tumor, two of 10mM sections of tumor and normal sample were dissolved in paraffin dissolver (Clontech) and the genomic DNA was isolated by FFPE genomic DNA isolation kit (Qiagen). The copy number of *BRCA1* in the genomic DNA was quantified by real-time PCR using *CCNB1* as a control.

Teratoma analysis

The R1699Q and WT ES cells were harvested and washed in PBS. Cells were counted and diluted to a concentration of $5X10^7$ cell/ml in PBS. 100 μl of the cell suspension was injected subcutaneously into athymic nude mouse (C3H/HeNCr-nu). The growth of teratoma was measured after one week, and then measured every other day. Tumor volume (in mm^3) was calculated as a product of 2 x length x width. Mice were maintained under limited access conditions at the National Cancer Institute (Frederick) and animal care was provided according to the procedures outlined in the Guide for the Care and Use of Laboratory Animals, under an approved Animal Care and Use Committee (ACUC) protocol.

Inducible miR-155 expression in ES cells

For inducible expression of the miR-155, the pSUPERIOR-puro-miR-155 (see DNA constructs), along with pcDNA/TR6 and pmiRep-miR-155, were co-electroporated into wild-type ES cells and selected with puromycin (300 μg/ml) for 5 days. The puromycin resistant colonies were picked and expanded in a 96-well plate. After splitting the cells into three 96 well plates, one plate was treated with tetracycline to test miR-155 induction. By measuring the ratio of miR-155 reporter luciferase activity from induced and uninduced, the ES clone with Tet induced miR-155 expression was identified and used for further analysis. The induction of miR-155 was confirmed by miRNA Northern hybridization as well as real time PCR (Fig 2d).

Tumor tissue array analysis

For human breast tumor analysis, Breast Tumor Tissue Array (BioChain Institute, Inc.) containing 70 breast tumor tissues was used. To access the BRCA1 level in these tumor samples, two tissue array slides were deparaffinized, hydrated and boiled in citrate buffer for antigen exposure. After blocking, the slides were probed with anti-BRCA1 antibody (Ab-1, Calbiochem) or normal mouse IgG (Oncogene), respectively. The BRCA1 signal was detected by Elite ABC HRP detection kit (Vectastain). For the detection of miR-155, DIG labeled LNA-miR-155 probe (Exiqon) was used, following the manufacturer's protocol. The miR-155 expression was visualized by anti-DIG-AP conjugate antibody and NBT/BCIP substrate (included in RAMBRANDT RISH kit used above). One set of tissue microarray was stained with H&E. The stained tissue array was analyzed under the Axioplan2 upright microscope (Zeiss).

Generation and analysis of the R1699Q BAC transgenic mice

BAC DNA with R1699Q mutation was prepared by Qiagen Maxiprep kit. The purified DNA was diluted in TE buffer to 10ng/ml concentration and used for the microinjection. Mice were genotyped by Southern analysis as described previously[1]. The BAC transgenic founder mice were mated with mice carrying a null allele of *Brca1* (*Brca1*$^{ko/+}$) to obtain BAC transgenic mice on *Brca1* heterozygous background (*Brca1*$^{ko/+}$;*Tg*^{R1699Q}). The expression of R1699Q *BRCA1* was examined in several tissues by RT-PCR. To test the embryo development of *Brca1*$^{ko/ko}$;*Tg*^{R1699Q} transgenic mice, a pair of R1699Q transgenic mouse with Brca1 heterozygous background (*Brca1*$^{ko/+}$;*Tg*^{R1699Q}) were intercrossed. Embryos at days 7.5 of gestation were dissected under the microscope (LEICA MZ8) and photographed. Embryos were genotyped by PCR using primers as listed in Supplementary Table 6. To generate the *cis*-*Brca1*$^{ko/+}$;*Trp53*$^{ko/+}$;*Tg*^{R1699Q} mouse, *Brca1*$^{ko/+}$;*Trp53*$^{ko/+}$ mice were crossed with *BRCA1;Tg*^{R1699Q} mouse and the co-segregation of *Brca1* and *Trp53* was screened by genotyping PCR. *cis*-*Brca1*$^{ko/+}$;*Trp53*$^{ko/+}$;*Tg*^{M1652I} mouse was generated by the same method.

Mouse Mammary Epithelial Cell (MEC) isolation and culture

To isolate mammary epithelial cells, the protocol from Stem Cell Technology was used (http://www.stemcell.com/en/Products/All-Products/EpiCultB-Mouse-Medium-Kit.aspx). Briefly, freshly dissected mammary gland was digested in digestion media (Epicult-B medium supplemented with 5% FBS and Collagenase/Hyaluronidase) for 8 hrs at 37ºC. The dissociated tissue was centrifuged and red blood cells were removed by treating with 0.8% NH_4Cl with 0.1 mM EDTA. The collected organelles were further digested in

trypsin-EDTA, followed by DNase I and dispase treatment to make a single cell suspension. The resulting cells were plated in 150mm gelatinized culture dish with Epicult-B medium with 5% FBS. One the next day the media was changed to serum free Epicult-B medium supplemented with EGF and FGF and cultured up to near confluency.

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SUPPLEMENTARY DISCUSSION

Breast cancer risk of R1699Q variant of BRCA1

In the past 10 years, many different studies have been undertaken to characterize the R1699Q variant of BRCA1 but its precise effect remains unclear. It was shown to be defective in phospho-specific binding to BACH1[1]. However, structural and biophysical studies suggest that R1699Q is not significantly different from the wild-type BRCA1[2]. This was also supported by the trypsin sensitivity assay, which showed R1699Q behaves similarly to wild type[3]. Yet, another study suggests the R1699Q variant destabilizes BRCA1 based on a similar assay[4], making the interpretation difficult. The transcriptional activation assays have also given inconsistent results, depending on the cell line used[3-6]. A study using functional and multifactorial likelihood approaches concluded that R1699Q is associated with low to moderate risk of developing the disease compared to other clearly deleterious variant[4], while another study classified the same variant as deleterious based on cancer family history[7]. The latter study also reported one family in which R1699Q did not segregate with the disease. In summary, the breast cancer risk in R1699Q mutation carriers is likely to be higher compared to the general population, but its precise risk is not known. Future large-scale epidemiological studies may provide a better assessment of the precise risk of this variant.

In this study, we have characterized the R1699Q variant using our mouse ES cell-based assay to examine its effect on BRCA1 function. We found the R1699Q variant to result in a 10-fold reduction in ES cell survival compared to cells expressing the wild-type BRCA1. This suggested that R1699Q is deleterious. This conclusion was further

supported by our *in vivo* studies showing that R1699Q fails to rescue the embryonic lethality of *Brca1-null* mice. Our previous work has demonstrated that most BRCA1 variants that result in ES cell lethality or show reduced cell survival are high-risk variants[8]. These variants also show defect in DNA repair function or cell cycle regulation. Surprisingly, the R1699Q variant had no effect on any of these functions. Instead, we uncovered a defect in ES cell differentiation, which in part was caused by the up-regulation of a miRNA, miR-155.

Epigenetic regulation of miR-155 by BRCA1

In this study, we have focused on understanding mechanistically how BRCA1 controls miRNA-155. The up-regulation of miRNA-155 in many cancers has been reported[9-12]. Several transcription factors that can activate the miR-155 promoter have been identified including AP-1, NF-kB, SMAD4, FOXP3 and HOXA9[13-15]. However, to date there has been no insight into how miRNA-155 may be regulated epigenetically. Our study not only demonstrates that miR-155 is epigenetically regulated, but also uncovers the role of BRCA1 in this control. We found marked increase in acetylation of histones H2A and H3 on the miR-155 promoter in R1699Q mutant cells. The histone acetylation and deacetylation is regulated by the various HAT/HDAC complexes and is important for chromatin organization. The association of BRCA1 with HDAC complex has been described previously[16]. It is also reported that BRCA1-mediated repression of ER-a promoter can be reversed by HDAC inhibitor, trichostatin A[17]. As we have detected an increase in acetylation of histones H2A and H3 on the miR-155 promoter in R1699Q ES cells as well as BRCA1-deficient tumor cell lines, we predicted that the interaction of

mutant BRCA1 with the HDAC complex is reduced or disrupted. Indeed, the ChIP experiment revealed an increase in binding of HDAC2 to the miR-155 promoter in the presence of wild type BRCA1 (Figure 4E). Based on the ChIP and coimmunoprecipitate results, we conclude that R1699Q BRCA1 is defective in the interaction with HDAC complex.

The mutational analysis of miR-155 promoter indicated that the putative BRCA1 binding site is critical for the epigenetic repression. Our observation that there is no or marginal association between BRCA1 and the three promoters with putative BRCA1 binding sites (*ESSRG, CCNB1* and *STAT5A*) suggests the association of BRCA1 with the miR-155 promoter is specific. Also, it suggests that the putative BRCA1 binding sites[18] may not necessarily be a good indicator of actual association with BRCA1. A genome-wide ChIP analysis for BRCA1 may provide a better understanding of the predictive value of these putative binding sites and the role of BRCA1 in epigenetic regulation of other promoters.

Does R1699Q have any dominant negative effect?

Because R1699Q BRCA1 fails to bind to HDAC2 but can associate with the miR-155 promoter, we tested the possibility that it may have a dominant negative effect. Although we did not find any *in vitro* evidence to support this, we cannot completely rule out this possibility. Lack of dominant negative effect was also supported by the *in vivo* observation that $Brca1^{ko/+};Tg^{R1699Q}$ mice did not show any overt phenotype and $Brca1^{cko/ko};Tg^{R1699Q}$ EB cells did not show miR-155 up-regulation (data not shown).

Also, tumors from *Brca1*$^{ko/+}$;*Trp53*$^{ko/+}$;*Tg*^{R1699Q} mice that showed high miR-155 had lost the WT allele of *Brca1* (Fig. 3a,b and Supplementary Figure 4).

Different levels of miR-155 expression in BRCA1-deficient cells

Interestingly, we found a 3-4 fold increase in miR-155 level in MECs from *Brca1*$^{cko/cko}$; *K14 Cre* mice and HEK 293 cells with BRCA1 knockdown, whereas the miR-155 levels in the tumors or tumor cell lines were much higher (50-150 fold) than the controls. This difference suggests the effect of additional regulatory signals or factors that may be involved in the transcriptional regulation of miR-155 promoter in addition to the BRCA1-mediated epigenetic control. And, these signals may depend upon the physiological or topological state of the cell. Such differences are also visible in the cells of the R1699Q ES cell derived embryoid bodies that are genotypically identical (Figure 2b). Human tumors samples also exhibit a similar variation in their pattern of miR-155 expression (Figure 5d). We also observed substantial differences in the increase of miR-155 expression between human and mouse tumors (3-6 fold compared to 50-180 fold). We attribute this to the difference in the quality of samples used for RNA extraction. For mouse tumors we used freshly-frozen samples whereas for human tumors we extracted RNA from 5-15 years old archived FFPE sections.

miR-155 as a biomarker

This study also shows a correlation between BRCA1 deficiency and miR-155 up-regulation in BRCA1-deficient tumors. However, because multiple transcription factors regulate the miR-155 promoter, it can be activated by other signals, unrelated to BRCA1.

Indeed, we observed high levels of miR-155 in one of the four tumors from Her2/Neu transgenic mice as well as in 10 cases of human breast tumors that were BRCA1 positive. Therefore, miR-155 alone may not be sufficient to determine the functional status of BRCA1. However, miR-155 may be part of a metagene signature that may be useful to determine the functional status of BRCA1. Future studies will be focused on evaluating the use of miR-155 expression to determine the BRCA1 status.

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