Supplementary Figures and Figure legends



Figure S1 Related to Figure 1

Figure S1. (A) TCGA database available from cBioportal for SIRT4 expression in human breast cancers. **(B)** Copy number compared to gene expression for SIRT4 mRNA versus copy number in human breast cancers using the cBioportal database. **(C)** Putative 3D structures of SIRT4 protein generated from the cBioportal database. **(D)**

Gene expression of SIRT4 in human breast cancer compared to normal tissues from 3 Gene Expression Omnibus (GEO) data sets (GSE2034, 25307, 7390).



Figure S2 Related to Figure 1

Figure S2. (A) Immunoblotting of SIRT4 in breast tumors compared to normal tissues (n=6). (B) Representative IHC staining images of SIRT4 in breast tumors compared to normal tissues; Scale bars, 100 μ m (B) and 20 μ m in the insets (B).



Figure S3. Representative H&E staining (A) and IHC staining images of Ki67 (B) for sections of mammary glands isolated from SIRT4^{WT} and SIRT4^{-/-} mice; Scale bars, 100 μ m (A) and 20 μ m in the insets (A), 100 μ m (B).

Figure S4 Related to Figure 2



Figure S4. (A) mRNA expression of SIRT4 in tumors isolated from MMTV-neu and MMTV-neu; SIRT4^{-/-} mice. **(B)** Quantification of Fig.2E. **(C)** Distribution of Linneg mouse mammary cells according to their expression of CD24 and CD49f were analyzed on MMTV-neu and MMTV-neu; SIRT4^{-/-} mice (left) and its quantification (right). **(D)** Representative H&E staining images for sections of lung metastasis isolated from mice mentioned above; Scale bars, 200 μm (D).

Figure S5 Related to Figure 3

<u>SIRT4^{-/-} SIF</u>		
	RP2 RP2 retinitis nigmentosa 2 (X-linked recessive)	
	FTSJ1 FTSJ1 FtsJ homolog 1 (E, coli)	
	TEE3 TEE3 transcription factor binding to IGHM enhancer 3	
	ATP6AP2 ATP6AP2 ATPase. H+ transporting, lysosomal accessory prote	in 2
	GPKOW GPKOW G patch domain and KOW motifs	
	PLP2 PLP2 proteolipid protein 2 (colonic epithelium-enriched)
	SMC1A SMC1A structural maintenance of chromosomes 1A	
	HUWE1 HUWE1 HECT, UBA and WWE domain containing 1	
	CFP CFP complement factor properdin	
	HDAC6 HDAC6 histone deacetylase 6	
	<u>APEX2 APEX2 APEX nuclease (apurinic/apyrimidinic endonuclease)</u>	2
	UBOLN2 UBOLN2 ubiquilin 2	
	PHF8 PHF8 PHD finger protein 8	
	WDR45 WDR45 WD repeat domain 45	
	AKAF V-rat murine sarcoma 3611 viral oncogene homolog	
	DDX3X DDX3X DEAD (ASP-G(u-A(a-ASP) DOX polypeptide 3, X-(inked	
	SIGEAC STRUCTURE STRUCTURE STRUCTURE AND A STRUCTURE STR	r) mombor A2
	SLC35A2 SLC35A2 SOLUTE CARRIER TAMILY 35 (UDP-galactose transporte	r), member Az
	Thest 70591 70591 and for protoin 91	DLOG B (yeast)
	Cover and the second se	
	NDP NDP Norria (japan (psaudal))	
	PORP1 PORP1 polyalutamine binding protein 1	
	PAGE1 PAGE1 P antique family member 1 (prostate associated)	
	RRM3 RRM3 RNA binding motif (RNP1, RRM) protein 3	
	SX5 SX5 Synovial sarcoma, X breaknoint 5	
	CCDC22 CCDC22 coiled-coil domain containing 22	
	TSR2 TSR2 TSR2, 205 rRNA accumulation, homolog (S. cerevisia	e)
	TSPYL2 TSPYL2 TSPY-like 2	
	EBP EBP emopamil binding protein (sterol isomerase)	
	PHF16 PHF16 PHD finger protěin 16	
	ZNF41 ZNF41 zinc finger protein 41	
	ZNF157 ZNF157 zinc finger protein 157	
	PRAF2 PRAF2 PRA1 domáin family, member 2	
	INE1 INE1 inactivation escape 1	
	PIM2 PIM2 pim-2 oncogene	
	TMEM47 TMEM47 transmembrane protein 47	
	RRAGB RRAGB Ras-related GTP binding B	
	USP11 USP11 ubiquitin specific peptidase 11	
	IRU IRU trophinin	
	MAUA MAUA monoamine oxidase A	mambar 7
	SECSAY SECSAY Solute carrier family 9 (Solum/nydrogen exchanger	(MACUK fomilu)
	CATA1 CATA1 CATA binding protein 1 (alobin transcription facto	r 1)
	CACNAIE CACNAIE calcium channel voltage_dependent alpha 15 subun	i+
	MADE MADE monoamine ovidase B	
	PEKER1 PEKER1 6-phosphofructo-2-kinase/fructose-2 6-hiphosphatas	e 1
	MAGED2 MAGED2 melanoma antigen family D. 2	
	PAGE4 PAGE4 P antigen family, member 4 (prostate associated)	
	RBM10 RBM10 RNA binding motif protein 10	
	GAGE3 GAGE3 G antigen 3	

Figure S5. Top 50 of the genes displayed in Fig.3A.



Figure S6. Immunoblotting (A) and mRNA expression (B) of SIRT4 among breast cancer cell lines.



Figure S7. **(A-D)** Transfection efficiency of MDA-MB-231 (A, C) and MCF-7 cells (B, D) transfected with control vector, SIRT4, and/or sh-SIRT4 measured by GFP (A, B) or mRNA expression (C, D). **(E-H)** In vitro MTT (E, F) and sphere formation assay (G, H) with transformed MDA-MB-231 (E, G) and MCF-7 cells (F, H) described above. **(I, J)** Representative ventral view images of bioluminescence from mice (n=3) with injections of MDA-MB-231 cells described above (I) and its quantification (J).



Figure S8 Related to Figure 3

Figure S8. (A, B) Migration assay (upper) and its quantification (bottom) of transformed MDA-MB-231 (A) and MCF-7 cells (B) described in Supplementary Figure 8. (C, D) Immunoblotting of transformed MDA-MB-231 (C) and MCF-7 cells (D) described above. (E) Representative ventral view images of bioluminescence from mice (n=3) with injections of MDA-MB-231 cells described above; Scale bars, 100 μ m (A) and 100 μ m (B).

Figure S9 Related to Figure 4



Figure S9. Immunoblotting (A, C) and mRNA (B, D) expression of SIRT4 and SIRT1 in transformed MDA-MB-468 (A, B) and MCF-7 cells (C, D) described in Fig.3G.



Figure S10. **(A, B)** Representative IHC staining images of SIRT4 (A) and SIRT1 (B) in mammary tissues from 36 breast cancer patients. (B) Quantification of (A, B). **(C)** Negative association between SIRT4 and SIRT1 protein expression in breast tumor tissues.



Figure S11 Related to Figure 5

Figure S11. **(A)** Immunoblotting of acetyl-histone H4 at lys16 (H4K16ac), acetylhistone H3 at lys9 (H3K9ac), Oct4, SOX2, and Nanog in MDA-MB-468 (left panel) as well as MCF-7 cells (right panel) transfected with control (NC) or shSIRT4 vector. **(B-D)** transformed MCF-7 (B), MDA-MB-231 (C), and BT579 cells (D) as described in Fig.3 were chromatin immunoprecipitated for IgG and H4K16ac. Pull-down at the putative H4K16ac binding sites was assessed by qRT- PCR and calculated as percent of IgG input. Error bars are SEM for 3 technical replicates.



Figure S12. (A, B, C) Immunoblotting (B) and mRNA expression (C) of BRCA1 in MDA-MB-468, MCF7 and BT579 cells describe in Fig5. (D) Reverse relationship between SIRT4 and BRCA1 generated from 3 databases (GSE1456, 2034, and 4922).
(E) Representative IHC staining images of SIRT4 and BRCA1 in tumor sections isolated from human mammary tissues; Scale bars, 100 μm (D).



Figure S13 Related to Figure 6

Figure S13. (A, B) Immunofluorescence images of MDA-MB-468 cells described in Fig.6A stained with antibodies against H4K16ac/DAPI (A) and SOX2/DAPI (B). (C) Representative IHC staining images (left) and its quantification (right) of Ki67 in MDA-MB-468 cells described above; Scale bars, 20 μ m (A) and 20 μ m (B).



Figure S14 Related to Figure 7

Figure S14. (A-D) Measurement of Glutamine uptake, NH4+ production, Glucose uptake, and lactate production (from left to right panel) in MDA-MB-468 (A), MCF-7 (B), MDA-MB-231 (C), and BT579 (D) cells.



Figure S15. (A) Immunoblotting of indicated proteins isolated from MDA-MB-468 cells with or without BPTES (10 μ M) treatment. (**B**, **C**, **D**) Quantification of Hoechst SP assay (B), sphere formation efficiency (C), and CD44+/CD24- subpopulations (D) in MDA-MB-468 cells with or without BPTES treatment.

Figure S15 Related to Figure 7



Full unedited gel for western blots figures

















MDA-MB-468











