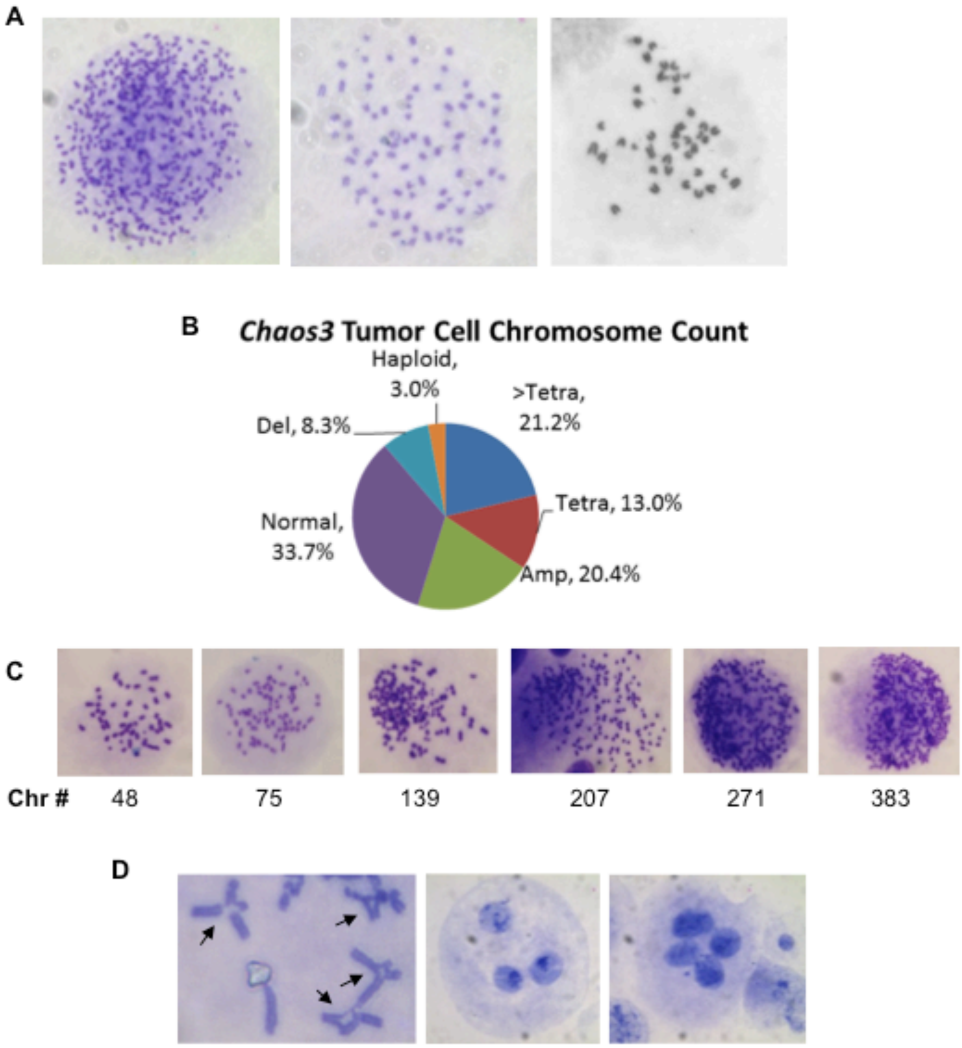

Figure S1 *Chaos3* tumors demonstrate high levels of GIN and aneuploidy. (A) Metaphase spreads from cells of 3 *Chaos3* mammary tumors. Note aneuploidy in left and middle spreads compared to the normal 40 chromosomes (left to right: 414, 83, 40). (B) Examination of 16 *Chaos3* tumors reveal a normal chromosome count in an average of only 1/3 of the cells (>Tetra= Beyond Tetraploidy; Tetra=Tetraploid; Amp=Amplification; Del=Deletion). (C) Metaphase spreads from one *Chaos3* mammary tumor (16864a). Chromosome count is indicated beneath the images. Note the extreme variation of aneuploidy found within a single tumor. (D) Additional abnormal features displayed by tumor cells, including: cruciform structures (left) and abnormal multi-nucleated cells (middle and right).

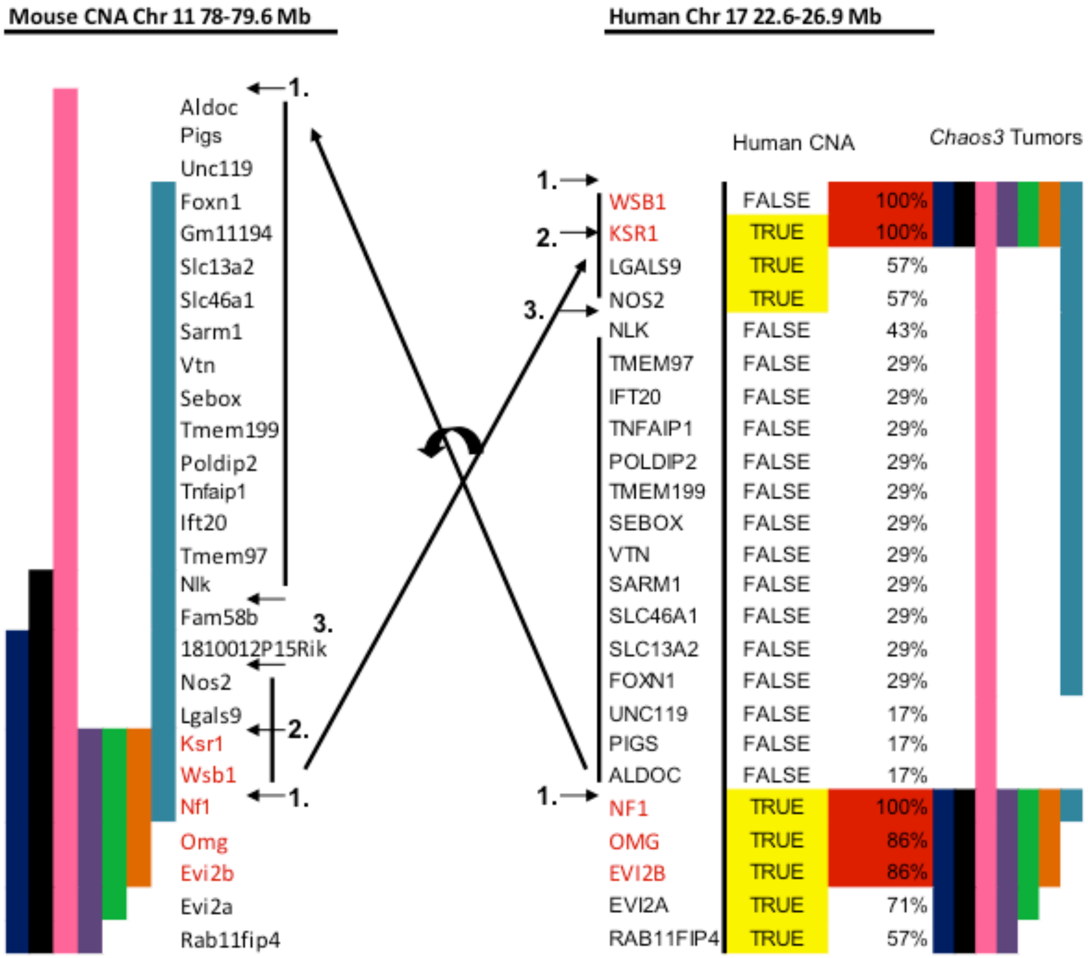

Figure S2 Genomic sequence around *Nf1* is prone to CNA and contains a genomic rearrangement. Colored vertical bars represent the deleted region in 7 *Chaos3* mammary tumors as detected by aCGH, and the percentages reflect how many of these tumors contain CNA for a given mouse gene. Gene names in red denote the *Chaos3* critical region. Mouse and human genomic orientations of the *Nf1* region are depicted. TRUE/FALSE indicates TCGA Level 4 (limited dataset) analysis of a subset of invasive breast carcinomas for segmental CNAs; it is possible that the intervals between *NOS2* and *NF1* are actually part of more inclusive deletion events. Numbers in bold with small arrows indicate positions of interest: **1.** Proximal to *Nf1*, a breakpoint of chromosomal inversion between human and mouse occurred between and including *Wsb1* to *Aldoc*. This is a site of both human and mouse tumor CNA, and the human CNA begins with *NF1*. **2.** The mouse critical CNA begins at *Ksr1*, which has flipped orientation in humans and starts/forms a second smaller CNA, with the caveats mentioned above. **3.** The mouse genome has an insertion between *Nlk* and *Nos2*, where human statistically-declared CNAs end.

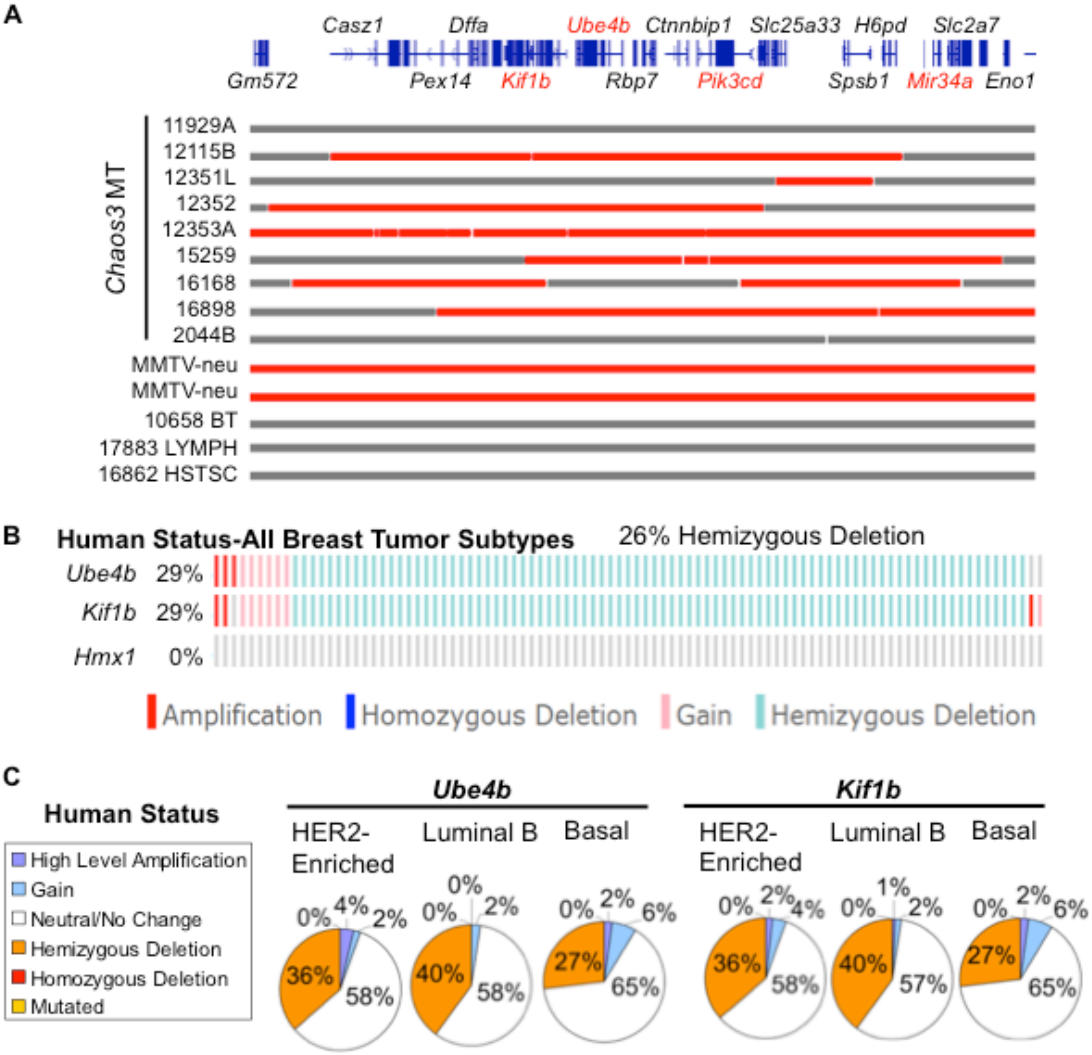

Figure S3 *Ube4b* and *Kif1b*, deleted in over half of *Chaos3* mammary tumors, show frequent deletion in human breast tumors. (A) Recurrent Chr 4 deletions specific to mammary tumors (MT). Horizontal bars represent tumors examined by aCGH. Red portions of bars indicate deleted regions in *Chaos3* and MMTV-neu MTs. Cancer-related genes are in red. Note that *Chaos3* non-MTs do not demonstrate this deletion. BT=bone tumor; LYMPH = Lymphoma; HSTSC=histiocytic sarcoma. (B) **"**Oncoprints" of *Ube4b* and *Kif1b* alterations in 320 human breast tumors (TCGA data) generated by the cBio portal (see Methods). Rows contain bars representing individual tumors, and samples are aligned for visualization of alterations within the same tumor across multiple genes. *Hmx1* does not have a known role in cancer. (C) Percentage of *Ube4b* and *Kif1b* CNAs in 55 Her2-Enriched, 125 Luminal B, and 93 Basal human breast tumors (TCGA). Note that ~40% of HER2-Enriched and Luminal B tumors have hemizygous deletion of *Ube4b* and *Kif1b*, as do 27% of Basal-type breast tumors.

Files S1-S4

Supporting Data

Available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.142802/-/DC1.

Table S1 Validated Gene Mutations in *Chaos3* Mammary Tumors

Sample	Name	Mutation	Effect	Description	Function
15259	Myo1g	G/A	Splice Site	Myosin-Ig	Precursor of minor histocompatibility antigen HA-2
2042	Acsl6	G/T	E>D	Long-chain-fatty-acid--CoA ligase 6	Catalyze formation of acyl-CoA from fatty acids, ATP, and CoA.
2042	Tdrd6	T/C	H>R	Tudor domain-containing protein 6	Required for spermiogenesis, chromatoid body architecture, and regulation of miRNA expression.
2042	Ttn	C/T	D>N	Titin (Connectin)	Cardiac and skeletal muscle protein. Disease Associations: Familial Cardiomyopathy, Tibial muscular dystrophy
2044b	Ttn	C/G	V>L	Titin (Connectin)	

Note: The mutations were validated by Sanger sequencing of PCR products.

Table S2 *Chaos3* **-specific and Mammary Tumor-specific Recurrent Deletions Overlapping Human Breast Cancer CNAs.**

					Tumors								
Mouse CNA	**Gene**	**Hum Chr**	**Human CNA**	***Chaos3* CNA**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Mmu Chr 4	SLC2A7	1	FALSE	33%		x			x				
148.4-149.5 Mb	SLC2A5	1	FALSE	50%		x			x	x			
	GPR157	1	FALSE	50%		x			x	x			
	MIR34A	1	FALSE	50%		x			x	x			
	H6PD	1	FALSE	57%		x			x	x	x		
	SPSB1	1	FALSE	86%		x	x		x	x	x	x	
	SLC25A33	1	FALSE	86%		x	x		x	x	x	x	
	TMEM201	1	FALSE	86%		x	x		x	x	x	x	
	PIK3CD	1	FALSE	86%		x		x	x	x	x	x	
	CLSTN1	1	FALSE	71%		x		x	x		x	x	
	CTNNBIP1	1	FALSE	71%		x		x	x		x	x	
	LZIC	1	FALSE	71%		x		x	x		x	x	
	NMNAT1	1	FALSE	71%		x		x	x		x	x	
	RBP7	1	FALSE	71%		x		x	x		x	x	
	UBE4B	1	TRUE	71%		x		x	x		x	x	
	KIF1B	1	TRUE	71%		x		x	x		x	x	
	PGD	1	TRUE	33%		x		x				x	
	APITD1	1	TRUE	33%		x		x				x	
	CORT	1	TRUE	33%		x		x				x	
	DFFA	1	TRUE	33%		x		x				x	
	PEX14	1	TRUE	33%		x		x				x	
	CASZ1	1	TRUE	33%		x		x					
	TARDBP	1	FALSE	17%		x							
	MASP2	1	FALSE	17%		x							
	SRM	1	FALSE	17%		x							
Mmu Chr 5	CLIP1	12	FALSE	43%	x	x					x		
122-125 Mb	ZCCHC8	12	FALSE	43%	x	x					x		
	RSRC2	12	FALSE	43%	x	x					x		
	KNTC1	12	FALSE	71%	x	x		x			x		x
	GPR81	12	FALSE	71%	x	x		x			x		x
	DENR	12	FALSE	71%	x	x		x			x		x
	CCDC62	12	FALSE	71%	x	x		x			x		x
	HIP1R	12	FALSE	71%	x	x		x			x		x
	VPS37B	12	FALSE	86%	x	x	x	x			x		x
	ABCB9	12	FALSE	86%	x	x	x	x			x		x
	OGFOD2	12	FALSE	86%	x	x	x	x			x		x
	ARL6IP4	12	FALSE	86%	x	x	x	x			x		x
	PITPNM2	12	FALSE	86%	x	x	x	x			x		x
	MPHOSPH9	12	FALSE	86%	x	x	x	x			x		x
	CDK2AP1	12	FALSE	86%	x	x	x	x			x		x
	SBNO1	12	FALSE	86%	x	x	x	x			x		x

	SETD8	12	FALSE	86%	x	x	x	x			x		x
	RILPL2	12	FALSE	86%	x	x	x	x			x		x
	SNRNP35	12	FALSE	100%	x	x	x	x			x	x	x
	RILPL1	12	FALSE	100%	x	x	x	x			x	x	x
	TMED2	12	FALSE	100%	x	x	x	x			x	x	x
	DDX55	12	FALSE	100%	x	x	x	x			x	x	x
	EIF2B1	12	FALSE	100%	x	x	x	x			x	x	x
	GTF2H3	12	FALSE	100%	x	x	x	x			x	x	x
	TCTN2	12	FALSE	100%	x	x	x	x			x	x	x
	ATP6V0A2	12	FALSE	43%						x		x	x
	CCDC92	12	FALSE	29%								x	x
	Zfp664	12	FALSE	14%								x	
	Fam101a	12	TRUE	14%								x	
	Ncor2	12	TRUE	14%								x	
	Scarb1	12	FALSE	14%								x	
Mmu Chr 11	WSB1	17	FALSE	100%	x	x	x	*	x	*	x	x	x*
78-79.6 Mb	KSR1	17	TRUE	100%	x	x	x	*	x	*	x	x	x*
	LGALS9	17	TRUE	57%		x		x*		x*		x	x*
	NOS2	17	TRUE	57%		x		x*		*		x	
	NLK	17	FALSE	43%		x				x*		x	
	TMEM97	17	FALSE	29%		x				*		x	
	IFT20	17	FALSE	29%		x				*		x	
	TNFAIP1	17	FALSE	29%		x				x*		x	
	POLDIP2	17	FALSE	29%		x						x	
	TMEM199	17	FALSE	29%		x						x	
	SEBOX	17	FALSE	29%		x						x	
	VTN	17	FALSE	29%		x						x	
	SARM1	17	FALSE	29%		x						x	
	SLC46A1	17	FALSE	29%		x						x	
	SLC13A2	17	FALSE	29%		x						x	
	FOXN1	17	FALSE	29%		x						x	
	UNC119	17	FALSE	17%		x							
	PIGS	17	FALSE	17%		x							
	ALDOC	17	FALSE	17%		x							
	NF1	17	TRUE	100%	x	x	x	x*	x	x*	x	x	x
	OMG	17	TRUE	86%	x	x	x	x*	x		x		x
	EVI2B	17	TRUE	86%	x	x	x	*	x		x		x
	EVI2A	17	TRUE	71%	x	x	x	*	x		x		
	RAB11FIP4	17	TRUE	57%	x	x	x	x*	x				

Legend. x= deleted as determined by aCGH analysis. Some qPCR genotyping from the Chr 11 interval was added, and deleted probes are indicated as x*. Presumed deleted probes are indicated by “*”. Tumor Codes: A: 2044B; B: 12353A; C: 12351L; D: 12352; E: 15259; F: 16168; G: 12115B; H: 16898; I: 11929A. Mmu = *Mus musculus*. Some of the deletions extend further than indicated. The True (deleted) and False (not deleted) calls for human gene deletions are from TCGA level 4 data (see Methods) and refer to whether that locus is deleted at levels statistically above background. Human genes in red are potentially cancer-relevant if deleted. Red shaded regions are the “critical regions” of a deletion set. Note that the Mmu Chr 11 deletion cluster is organized in the human genome order, which is inverted and has an insertion. Thus, the critical region is actually contiguous. The “Chaos3 CNA” column refers to the % of Chaos3 mammary tumors analyzed by aCGH that contained deletions of that particular locus. ND=no data.

Table S3 ***Chaos3*** **Mammary Tumor Non-specific Recurrent Deletions Overlapping Human Breast Cancer CNAs.**

Mouse Region	Human Gene	Hs Chr	Human CNA	*Chaos3* CNA	A	B	C	D	E	F	G
Mmu Chr 4	AIM1L	1	FALSE	43%		x	x			x	
132.4-133.5 Mb	LIN28	1	FALSE	71%		x	x	x		x	x
	DHDDS	1	FALSE	71%		x	x	x		x	x
	HMGN2	1	FALSE	71%		x	x	x		x	x
	RPS6KA1	1	FALSE	86%		x	x	x	x	x	x
	ARID1A	1	FALSE	100%	x	x	x	x	x	x	x
	PIGV	1	FALSE	100%	x	x	x	x	x	x	x
	ZDHHC18	1	TRUE	86%	x	x	x		x	x	x
	SFN	1	TRUE	86%	x	x	x		x	x	x
	GPN2	1	TRUE	86%	x	x	x		x	x	x
	GPATCH3	1	TRUE	86%	x	x	x		x	x	x
	NR0B2	1	TRUE	86%	x	x	x		x	x	x
	NUDC	1	TRUE	86%	x	x	x		x	x	x
	TRNP1	1	TRUE	86%	x	x	x		x	x	x
	FAM46B	1	FALSE	86%	x	x	x		x	x	x
	SLC9A1	1	FALSE	71%	x		x		x	x	x
	WDTC1	1	FALSE	43%	x				x		x
	TMEM222	1	FALSE	43%	x				x		x
	SYTL1	1	FALSE	43%	x				x		x
	MAP3K6	1	FALSE	43%	x				x		x
	CD164L2	1	FALSE	43%	x				x		x
	GPR3	1	FALSE	43%	x				x		x
Mmu Chr 10	PPAP2C	19	TRUE	33%				x	x		
79.4-80.2 Mb	MIER2	19	TRUE	33%				x	x		
	THEG	19	TRUE	33%				x	x		
	C2CD4C	19	TRUE	33%				x	x		
	SHC2	19	TRUE	33%				x	x		
	ODF3L2	19	TRUE	33%				x	x		
	MADCAM1	19	TRUE	33%				x	x		
	CDC34	19	TRUE	33%				x	x		
	GZMM	19	TRUE	33%				x	x		
	BSG	19	TRUE	33%				x	x		
	HCN2	19	TRUE	33%				x	x		
	POLRMT	19	TRUE	33%				x	x		
	FGF22	19	TRUE	33%				x	x		
	RNF126	19	TRUE	33%				x	x		
	FSTL3	19	TRUE	33%				x	x		
	PRSSL1	19	TRUE	33%				x	x		
	PALM	19	TRUE	33%				x	x		
	PTBP1	19	TRUE	33%				x	x		
	PRTN3	19	TRUE	33%				x	x		
	ELANE	19	TRUE	33%				x	x		

CFD	19	TRUE	33%		x	x		
MED16	19	TRUE	33%		x	x		
KISS1R	19	TRUE	50%		x	x		x
ARID3A	19	TRUE	50%		x	x		x
WDR18	19	TRUE	50%		x	x		x
GRIN3B	19	TRUE	50%		x	x		x
CNN2	19	TRUE	50%		x	x		x
ABCA7	19	TRUE	50%		x	x		x
HMHA1	19	TRUE	50%		x	x		x
POLR2E	19	TRUE	50%		x	x		x
GPX4	19	TRUE	50%		x	x		x
SBNO2	19	TRUE	50%		x	x		x
STK11	19	TRUE	50%		x	x		x
ATP5D	19	TRUE	50%		x	x		x
MIDN	19	TRUE	50%		x	x		x
CIRBP	19	TRUE	33%		x	x		
EFNA2	19	TRUE	33%		x	x		
MUM1	19	TRUE	33%		x	x		
NDUFS7	19	TRUE	33%		x	x		
GAMT	19	TRUE	33%		x	x		
DAZAP1	19	TRUE	33%		x	x		
RPS15	19	TRUE	33%		x	x		
APC2	19	TRUE	33%		x	x		
PCSK4	19	TRUE	33%		x	x		
REEP6	19	TRUE	33%		x	x		
ADAMTSL5	19	TRUE	33%		x	x		
MEX3D	19	TRUE	50%		x	x	x	
MBD3	19	TRUE	50%		x	x	x	
TCF3	19	TRUE	50%		x	x	x	
ONECUT3	19	TRUE	67%	x	x	x	x	
ATP8B3	19	TRUE	67%	x	x	x	x	
REXO1	19	TRUE	67%	x	x	x	x	
KLF16	19	TRUE	67%	x	x	x	x	
SCAMP4	19	TRUE	67%	x	x	x	x	
ADAT3	19	TRUE	67%	x	x	x	x	
CSNK1G2	19	TRUE	67%	x	x	x	x	
BTBD2	19	TRUE	67%	x	x	x	x	
MKNK2	19	TRUE	67%	x	x	x	x	
MOBKL2A	19	TRUE	67%	x	x	x	x	
AP3D1	19	TRUE	67%	x	x	x	x	
DOT1L	19	TRUE	67%	x	x	x	x	
PLEKHJ1	19	TRUE	67%	x	x	x	x	
SF3A2	19	TRUE	67%	x	x	x	x	
AMH	19	TRUE	67%	x	x	x	x	
JSRP1	19	TRUE	67%	x	x	x	x	
OAZ1	19	TRUE	67%	x	x	x	x	

	LINGO3	19	TRUE	67%	x			x	x	x	
	LSM7	19	TRUE	33%				x	x		
	TMPRSS9	19	FALSE	33%				x	x		
	TIMM13	19	FALSE	33%				x	x		
	LMNB2	19	FALSE	33%				x	x		
	GADD45B	19	FALSE	17%				x			

Legend. x= deleted. Tumor Codes: A: 15259; B: 12353A; C: 12115B; D: 11929A; E: 16898; F: 2044B; G: 16892. Mmu = *Mus musculus*. Hs = *Homo sapiens*. Some of the deletions extend further than indicated. The True (deleted) and False (not deleted) calls for human gene deletions are from TCGA level 4 data (see Methods) and refer to whether that locus is deleted at levels statistically above background. Human genes in red are potentially cancer-relevant. Red shaded regions are the "critical regions" of a deletion set. The "Chaos3 CNA" column refers to the % of Chaos3 mammary tumors analyzed by aCGH that contained deletions of that particular locus.

Table S4 qPCR analysis of *Nf1* locus in tumors

Geno & Type	Tumor #	*Nf1* 5'	*Nf1* 3' (*Omg*)
Chaos3 MT	15259	17.4	52.9
Chaos3 MT	12351 L	35.5	12.0
Chaos3 MT	12353A	25.5	16.7
Chaos3 MT	12352	12.7	0.7
Chaos3 MT	2044B CL	63.7	48.0
Chaos3 MT	11929A	16.7	16.3
Chaos3 MT	16168	9.2	ND
Chaos3 MT	16898	9.6	75.1
Chaos3 MT	12115B	31.2	38.7
Chaos3 MT	2042 CL	78.9	92.3
Chaos3 MT	919 CL	51.8	0.1
Chaos3 MT	21040	0.1	0.1
Chaos3 MT	21253	0.3	0.2
Chaos3 MT	20317	29.5	48.7
Chaos3 MT	19957	32.7	5.4
Chaos3 MT	19958	11.2	12.4
Chaos3 MT	19959	14.4	72.4
Chaos3 MT	20783	7.0	10.4
Chaos3 MT	20164	20.6	22.8
Chaos3 MT	20888	27.3	26.0
Chaos3 MT	20892	6.4	34.9
Chaos3 MT	20893	7.4	24.5
Chaos3 MT	20138	41.1	10.8
Chaos3 MT	21039	68.4	66.7
Chaos3 MT	21809	62.0	60.0
Chaos3 MT	20894	85.1	67.1
Chaos3 MT	20889	12.0	18.4
Chaos3 MT	21333	40.8	71.4
Chaos3 MT	20626	36.7	22.6
Chaos3 MT	20318	14.5	24.9
Chaos3 MT	20890	13.6	38.9
Chaos3 MT	20891	2.5	1.4
Chaos3 MT	21123	34.7	39.0
Chaos3 MT	19660	44.8	48.3
Chaos3 MT	20459	53.7	69.8
Chaos3 MT	21597	28.6	5.3
Chaos3 MT	22182	62.6	78.2
Chaos3 MT	21416	63.8	56.8
Chaos3 MT	22236	31.6	8.0
Chaos3 MT	22418	24.9	24.2
Chaos3 MT	22180	23.9	49.0
Chaos3 MT	22235	8.1	8.2
Chaos3 MT	22166	21.7	21.0
Chaos3 MT	22168	8.0	7.6
Chaos3 MT	22238	3.4	3.4

Chaos3 MT	21417	3.3	14.8
Chaos3 MT	21419	19.2	50.6
Chaos3 MT	21255	7.0	6.1
Chaos3 MT	21124	8.5	29.1
Chaos3 MT	21810	26.3	27.0
Chaos3 MT	21811	47.3	51.7
Chaos3 MT	21254	9.5	7.9
Chaos3 MT	21041	24.4	21.2
Chaos3 MT	22420	40.5	17.1
Chaos3 MT	22476	75.5	70.7
Chaos3 MT	22414	3.3	17.1
Chaos3 MT	22416	3.5	14.6
Chaos3 MT	22417	7.1	19.4
Chaos3 MT	23116	11.2	10.8
Chaos3 MT	22418	115.0	110.3
Chaos3 non-MT	19160	198.2	207.5
Chaos3 non-MT	10658	87.1	ND
Chaos3 non-MT	16862	98.5	101.2
Chaos3 non-MT	17883	97.0	88.0
PyVT		96.3	105.1
MMTV-neu1		108.6	93.2
MMTV-neu2		103.8	104.4
Chaos3 +/+ MT		107.3	109.4

Legend. qPCR values are presented as the percentage vs C3H DNA. *Nf1* probes were at the 5' and 3' ends of the gene. The 3’ probe actually corresponds to the *Omg* gene that lies within an *Nf1* intron near the 3' end. Note that copy number differences between the *Nf1* 5' and 3' are observed in some tumors, indicating a breakpoint within *Nf1*. Deletion calls were made as follows: Heterozygous = 15-80%; Homozygous = <15%. If either *Nf1* or *Omg* were <15%, the tumor sample was called as homozygous deleted because full *Nf1* transcripts cannot be made. MT = mammary tumor.