Supporting Information

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SI Text

Supplemental Methods

Karyotypic Analysis. The cultures were harvested and G banded by use of standard protocols. Clonal chromosomal abnormalities identified by G banding were described according to the International Committee on Standard Genetic Nomenclature for MICE (2005). For karyotype analysis using G-banding, 40–85 metaphases from exponentially growing cells were examined using a computer-assisted program (Applied Imaging). Chromosome numbers for each metaphase was counted and frequency of occurrence in percent was calculated.

Spectral Karyotyping (SKY). The mixture of mouse chromosome paints was obtained from Applied Spectral Imaging (ASI). Hybridization and detection were carried out according to the manufacturer's protocol. Chromosomes were counterstained with DAPI. For each case, a minimum of 10 metaphase cells was analyzed by SKY (tumor 305, 311, 1979, and 1980). Images were acquired with a SD200 Spectra cube (ASI) mounted on a Zeiss Axioplan II microscope using a custom designed optical filter (SKY-1) (Chroma Technology), and analyzed using SKY View 1.2 software (ASI).

Comparative Genomic Hybridization (CGH). High-molecular-weight DNA was extracted from tumor tissue (305, 311) and normal placenta by standard methods and subjected to CGH according to the previously published method with some modifications [Kallioniemi A, *et al.* (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 258:818–8211]. The metaphase preparations were captured and processed by use of QUIPS software (Applied Imaging).

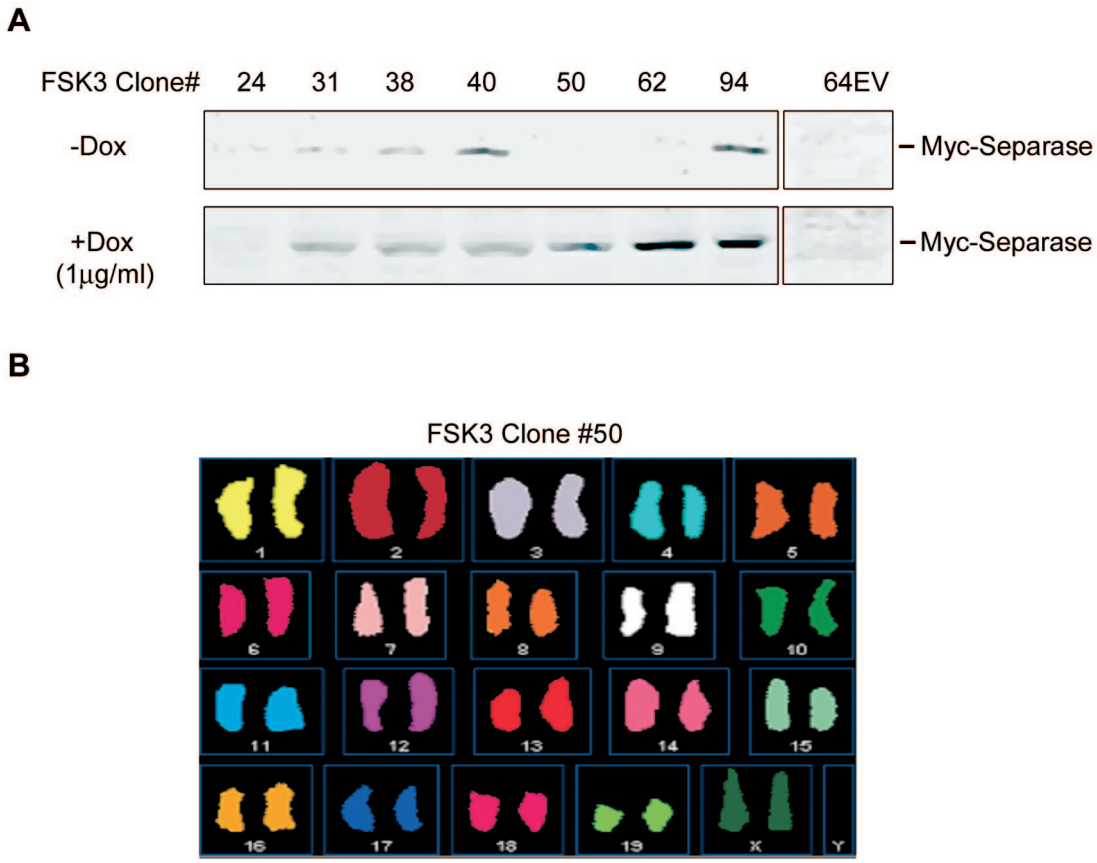

Fig. S1. (*A*) Western blot analysis of mSeparase protein in Tet-inducible FSK3 mouse mammary epithelial cell clones. Conditional expression of myc-epitope tagged mSeparase protein was examined in the presence (+Dox) and absence (-Dox) of 1 μg/ml of doxycycline. Clone #64EV is an empty vector clone used as a negative control. Myc-mSeparase protein was detected using 9E10 monoclonal antibody. (*B*) SKY analysis of FSK3 Clone #50 at passage 14 before Separase induction ($n = 10$), showing a representative classified colored karyotype with normal diploid chromosome.

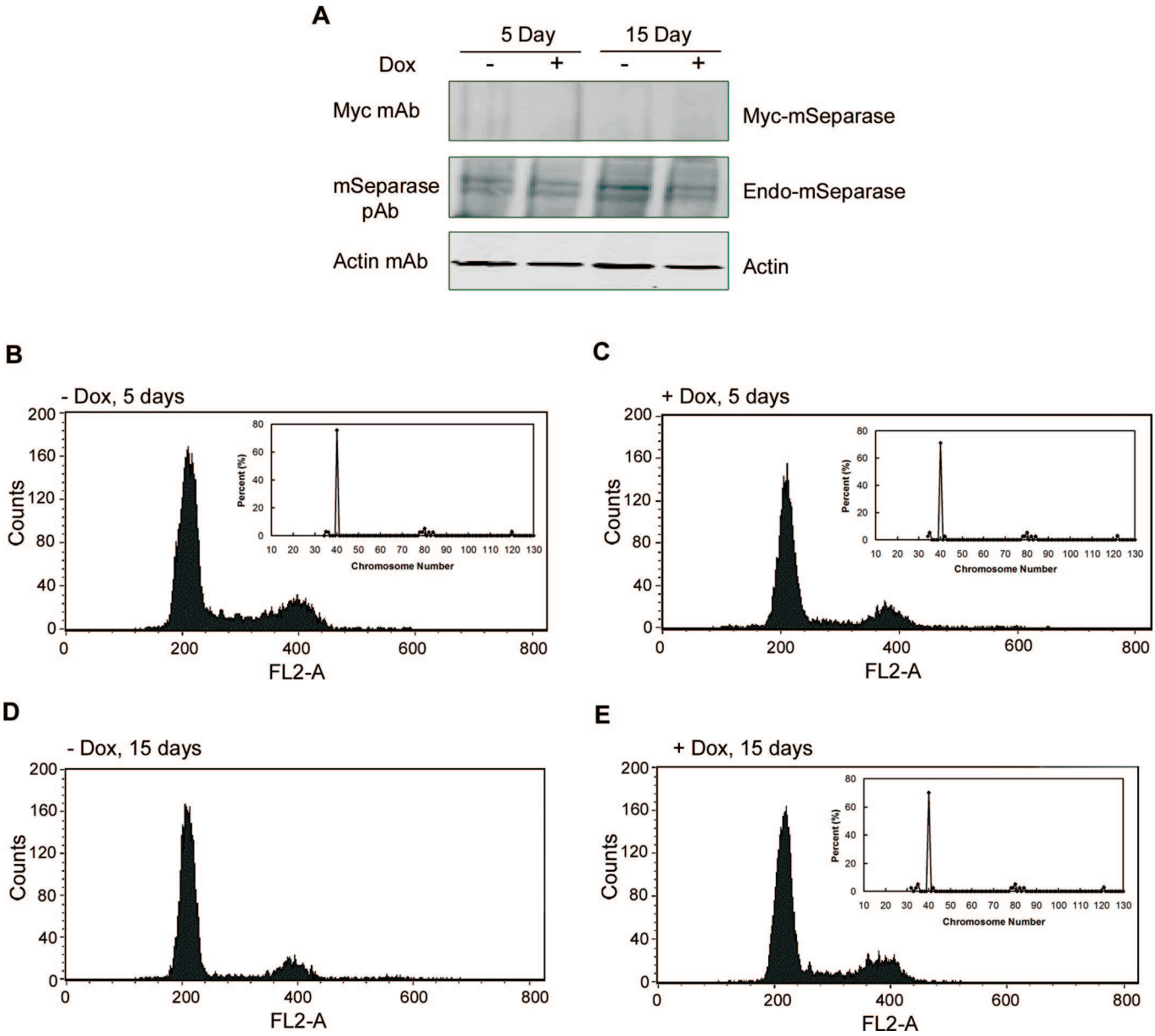

Fig. S2. Control experiment showing no aneuploidy in doxycycline-induced FSK3 Tet-on cells containing Empty Vector (Clone#64EV). Cells were assayed at 5 and 15 days following doxycycline (1 μg/ml) treatment. (*A*). Separase protein was assayed using Western blot. (*B-E*). FACS analysis of the propidium iodide (PI) stained nuclei obtained from cells treated in the presence or absence of doxycycline for a period 5 and 15 days. *Insets* show distribution of chromosome number except for *D*, where data are not available.

Table S1. Oncogenic activity of Separase *in vivo*

Clone no.	Induced	Not induced	Tumor appearance (week)	Tumor ID	Modal* chromosome number with range
50	5/6	0/6	3–4	305	42 (39–83)
62	5/6	0/6	3–4	311	41 (37–81)
64EV (control)	0/6	0/6	-		40 (40–80)

Formation of mammary tumors within 3–4 weeks of transplant. Separase was induced for a period of 3 weeks by feeding the mice with 1 mg of doxycycline per liter of drinking water.
*Most common chromosome number.

Table S2. Pathological characteristics of the breast tissue samples used in Fig. 5

	Diagnosis				Diagnosis		
Sample	Primary	Secondary	Type	Sample	Primary	Secondary	Type
1N			N-Normal	11	Infiltrating ductal carcinoma	Carcinoma	T-tumor
1T	Infiltrating ductal carcinoma I	Carcinoma	T-tumor	12	signet ring		T-tumor
2N			N-Normal	13	n/a	Carcinoma	T-tumor
2T	Infiltrating ductal carcinoma	Carcinoma	T-tumor	14	n/a	Carcinoma	T-tumor
3N			N-Normal	15	adeno	Carcinoma	T-tumor
3T	Infiltrating ductal carcinoma	Carcinoma	T-tumor	16	n/a	Carcinoma	T-tumor
4N			N-Normal	17	Infiltrating ductal carcinoma	Carcinoma	T-tumor
4T	Infiltrating ductal carcinoma	Carcinoma	T-tumor	18	Infiltrating ductal carcinoma	Carcinoma	T-tumor
5N			N-Normal	19	Infiltrating ductal carcinoma	Carcinoma	T-tumor
5T	Infiltrating ductal carcinoma	Carcinoma	T-tumor	20	Infiltrating ductal carcinoma	Carcinoma	T-tumor
6N			N-Normal	21	Infiltrating ductal carcinoma	Carcinoma	T-tumor
6T		Carcinoma	T-tumor	22	n/a	carcinoma	T-tumor
7N			N-Normal	23	n/a	sarcoma	T-tumor
7T		Carcinoma	T-tumor	24	Infiltrating ductal carcinoma	Carcinoma	T-tumor
8N			N-Normal	25	sarcomatoid		T-tumor
8T		Carcinoma	T-tumor	26*	Infiltrating ductal carcinoma	Carcinoma	T-tumor
9N			N-Normal	27*	Infiltrating ductal carcinoma	Ductal carcinoma in situ	T-tumor
9T		Carcinoma	T-tumor	28*	Infiltrating ductal carcinoma	Carcinoma	T-tumor
10N			N-Normal	29*	Infiltrating ductal carcinoma	Carcinoma	T-tumor
10T		Carcinoma	T-tumor	30*	Infiltrating ductal carcinoma	Carcinoma	T-tumor

*Samples obtained from the Lester and Sue Smith Breast Center at Baylor College of Medicine. The rest of the samples are from the M. D. Anderson Cancer Center tissue repository (provided by Adel K. El-Naggar).