Supplemental Table S1. Rate of FAM175A/Abraxas mutation and copy number loss in various tumors

tissue	*mutation_rate*	*sample_examined*	*copy_loss_rate*	*sample_examined*
breast	N.D.	967	30.7%	782
central nervous system	N.D.	571	5.7%	140
endometrium	2.5%	281	10.2%	246
haematopoietic	0.1%	1031	0.5%	192
kidney	0.1%	660	12.7%	300
large intestine	1.3%	600	28.0%	486
liver	0.3%	312	N/A	N/A
lung	0.6%	869	43.3%	476
ovary	N.D.	502	59.5%	462
pancreas	N.D.	366	12.5%	32

Supplemental Table S2. Summary of Fam175A/Abraxas mutations in various tumors

See separate attached file.

Supplemental Experimental Procedures

Generation of *Abraxas* null mice

A gene-targeting construct was made with a 4 kb homology region 5' to *exon 5* of the *Abraxas* genomic sequence placed upstream of the first *loxP* site and 2.4 kb homology region 3' to exon 5 placed downstream of the second *loxP* site. The DNA sequence between the two *loxP* sites includes two-FRT sites flanked *Neo* gene cassette and a fragment including *exon 5* and adjacent intron sequences. The targeting vector was electroporated into 129/C56BL6 G4 ES cells by the Mouse Genetic Engineering Core Facility of MD Anderson Cancer Center. Positive recombinants were identified and verified by Southern blot analysis for proper targeting (Figure S1). The 5' probe was amplified by primers BW774 (5'-CAAGCAATAAGGTGGTGACTGG-3') and BW775 (5'-TTCCCTTCCTGGTAACTGAT-3') and 3' probe was amplified by primers BW766 (5'-AGCGTCTTAAGCCTCGGATGC-3') and BW767 (5'-AACTGAAAATAACGCTGAGGATGC-3'). With assistance of the Mouse Genetic Engineering Core Facility, three independent ES cells clones heterozygous for the *Abraxas* targeted allele were injected into C56BL6 blastocysts and transferred to foster mothers. Chimeric progenies were then backcrossed to C57BL/6 mice to identify germline transmission and generation of $Abraxas^{Neo/+}$ heterozygous mice. Breeding $Abraxas^{Neo/+}$ mice with Zona pellucid glycoprotein 3 promter (*Zp3*)-*Cre* transgenic mice, which express Cre recombinase under the control of the *Zp3* promter in the growing oocyte (Lewandoski et al., 1997), resulted in deletion of *exon 5* in the recombined allele, generating $Abraxas^{+/\Delta}$ animals. $Abraxas^{+/-}$ mice were intercrossed to produce $Abraxas^{-/-}$ mice.

Mouse tail genotyping was performed using mouse genotyping kit (KAPA Biosystems) and primers Abraxas (F1) 5'-TGAGTATGGGCTGCTGTAGAC-3'; Abraxas (F2) 5'-GACATGAGCCTGTTCCAATCC-3'; Abraxas (R1) 5'-TGAGATAACCTCACCATGTAGCC-3'; Cre (F1) 5'-CCTGGAAAATGCTTCTGTCCG-3'; Cre (R1) 5'-CAGGGTGTTATAAGCAATCCC-3'. PCR conditions are 95°C for 2 min, repeat cycle 95°C 15 sec, 56°C 15 sec, 72°C 15 sec for 30 times, and extend at 72° for 2 min.

All experiments with mice followed protocols approved by the MD Anderson Cancer Center Institutional Animal Care and Use Committee, protocol 110812132, and conformed to the guidelines of the United States Animal Welfare Act and the National Institutes of Health.

RT-PCR and Quantitative RT-PCR

RNA was isolated by standard protocol using Trizol (Invitrogen). cDNA was synthesized with the SuperScriptIII kit (Invitrogen). Real-time quantitative RT-PCR was performed on an ABI7900 (Applied Biosystems) with a standard two-step PCR protocol (1x 95^{o}c-10 min, 40x 95^{o}c-15 sec and 60^{o}c-1 min). Transcripts were amplified using SYBR Green PCR Master Mix (Applied Biosystems) The following primers were used: Primer 1 (exon 2 forward): 5' GCATTACTGATTCACAGATGGAC-3'; Primer 2 (exon5 forward): 5' ACACATCTTTCAAGCCCTGAG; Primer 3 (exon6 reverse): 5' TGATCAGACATTCCCAGATTGGT-3'; GAPDH (Fwd) 5' AGGTCGGTGTGAACGGATTTG-3', GAPDH (Rev) 5' TGTAGACCATGTAGTTGAGGTCA-3'.

Comet Assay

Trypsinized 2 x 10^5 cells were pelleted and suspended in 1ml ice-cold 1 x PBS buffer followed by standard protocol. Fifty ml of cell suspension was mixed with 100 ml of 0.75% pre-warmed low melting-point agarose and dropped slowly on a fully frosted slide pre-coated with 0.75% agarose. After solidifying, the slides were submerged in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 10% DMSO; 4°C) for one hour at 4°C followed by unwinding DNA in electrophoresis buffer (0.3N NaOH and 1mM EDTA, pH 13.1; 4°C) for 30min at 4°C. Subsequently, electrophoresis was performed at 2 V cm^{-1} for 10min. After electrophoresis, slides were dehydrated in 70% alcohol for 30min and air-dried overnight. Cells were then rehydrated in 1 x PBS and stained with SybrGold in the dark. Nuclear DNA on slides was visualized under a fluorescent microscope (Leica DM4000 B) and images were captured at 400 x magnifications using Leica DFC 300 FX color camera. To measure levels of DNA damage, 50 cells of each sample were analyzed for tail moment (a.u.) by the CometScore software (TriTek Corp.). Quantitative results of tail moment were graphed by Prism 5 software.

Supplemental Reference

Lewandoski, M., Wassarman, K.M., and Martin, G.R. (1997). Zp3-cre, a transgenic mouse line for the activation or inactivation of loxP-flanked target genes specifically in the female germ line. Curr Biol *7*, 148-151.