Supporting Information

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SI Materials and Methods

The following quantitative RT-PCR primers were used:

mDlc1: 5′-CCACTGATATCCCGGAAAGA-3′ and 5′-AAGCTGTGCCACCTCAGTCT-3′
mFgl1: 5′-GGAGGGGGATGGACTGTAAT-3′ and 5′-GCCAGTATTCGCCATTGTTT-3′
mVps37a: 5′-TGCAAAGGCAACATGAACTC-3′ and 5′-CGATTCTTCCTCAGCTTCGT-3′
mArhgef10: 5′-GAGATGCCGACCAGCGATG-3′ and 5′-TCGTTGTAAACCGTCTCGATG-3′
mTnfrsf10b: 5′-CGGGCAGATCACTACACCC-3′ and 5′-TGTTACTGGAACAAAGACAGCC-3′
mFbxo25: 5′-AAGGTGTGACCCCTGTAGC-3′ and 5′-CCTCTTTTTGGCTGCGTATTCA-3′
mScara5: 5′-CATGGATTTCACAATGATTCGCC-3′ and 5′-TCCCCGTCCTTCTTGTCCC-3′
mBnip3l: 5′-ATGTCTCACTTAGTCGAGCCG-3′ and 5′-CTCATGCTGTGCATCCAGGA-3′
mTrim35: 5′-TTCCGGGCCAAGTGTAAGAAC-3′ and 5′-CCAAGTCGTTTGCACCTCA-3′
mGapdh: 5′-GGTGAAGGTCGGTGTGAACG-3′ and 5′-CTCGCTCCTGGAAGATGGTG-3′
mActin: 5′-CCACCGATCCACACAGAGTA-3′ and 5′-GGCTCCTAGCACCATGAAGA-3′

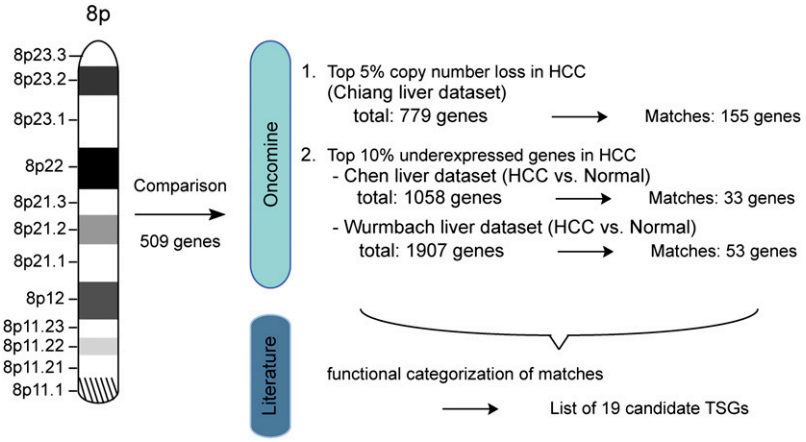

Fig. S1. Selection criteria for chromosome 8p candidate tumor–suppressor genes (TSGs). A complete list of genes and chromosome locations is provided in Table S1.

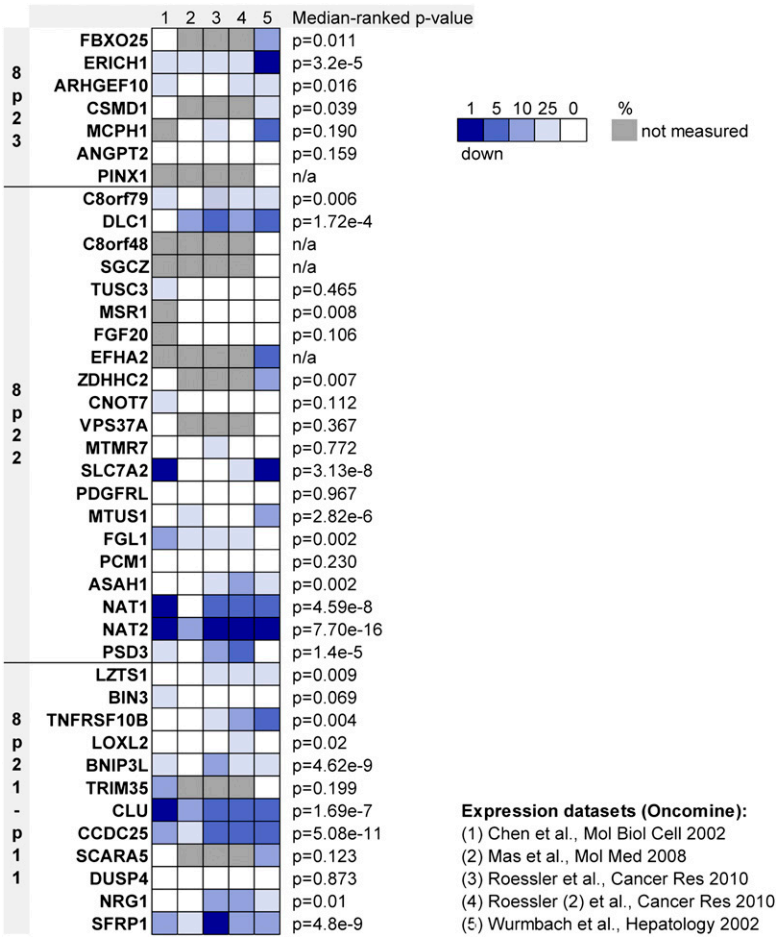

Fig. S2. Gene expression of 8p genes selected for RNAi screening across multiple datasets comparing hepatocellular carcinoma (HCC) to normal liver. The Oncomine database (oncomine.org) was used to analyze gene expression across multiple datasets comparing HCC to normal (1–4). *P* value is the median-ranked *P* value across the different datasets.

1. Chen X, et al. (2002) Gene expression patterns in human liver cancers. *Mol Biol Cell* 13:1929–1939.
2. Mas VR, et al. (2009) Genes involved in viral carcinogenesis and tumor initiation in hepatitis C virus-induced hepatocellular carcinoma. *Mol Med* 15:85–94.
3. Roessler S, et al. (2010) A unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients. *Cancer Res* 70:10202–10212.
4. Wurmbach E, et al. (2007) Genome-wide molecular profiles of HCV-induced dysplasia and hepatocellular carcinoma. *Hepatology* 45:938–947.

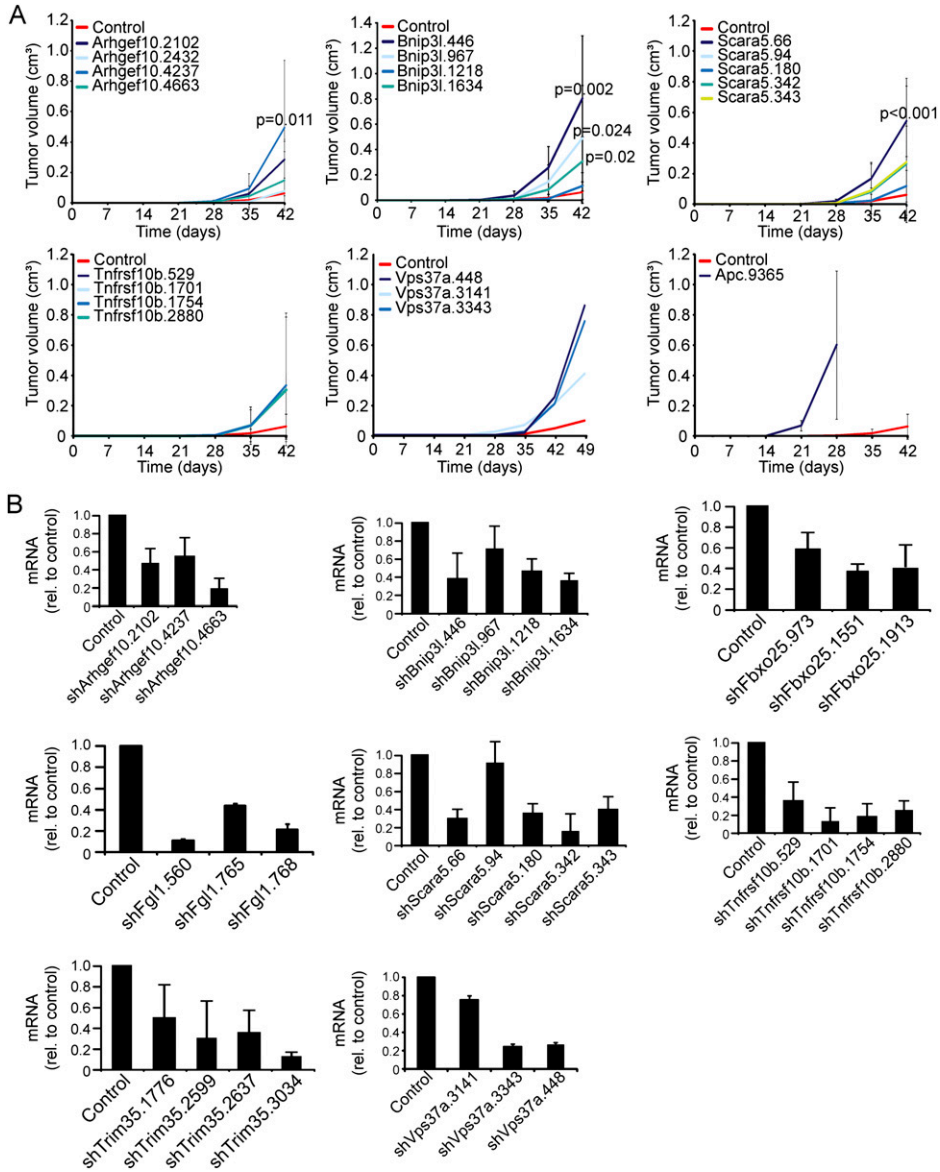

Fig. S3. Functional validation of individual shRNAs targeting chromosome 8p genes. (*A*) Average tumor volumes of s.c. injected *p53*$^{-/-}$*;Myc* immortalized liver cells infected with indicated individual shRNAs used in the pooled screening (Fig. 2 *A* and *B*). Error bars denote SD (n = 8). The Student t test comparing normalized samples at day 42 relative to control was used to calculate P values. Apc was used as positive control. (*B*) Quantitative RT-PCR of *p53*$^{-/-}$*;Myc* immortalized liver cells infected with indicated individual shRNAs used for injections in *A*. Error bars denote SD.

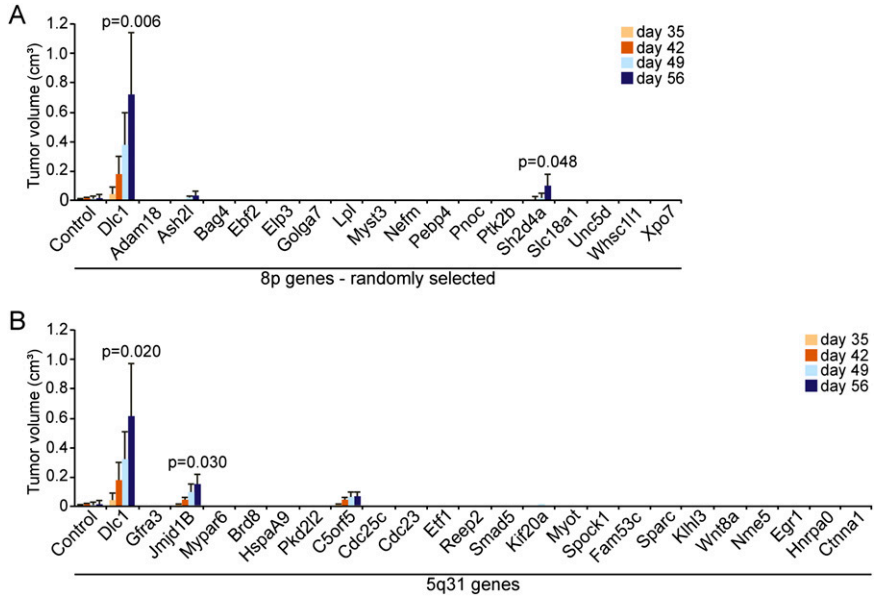

Fig. S4. Control in vivo RNAi screen for randomly selected 8p genes and 5q31 genes. (*A-B*) Average tumor volumes of s.c. injected *p53*$^{-/-}$*;Myc* immortalized liver cells infected with shRNA pools targeting indicated 8p genes or 5q31 genes, respectively. Error bars denote SD (n = 4). The Student t test comparing normalized samples at day 56 relative to control was used for statistical calulations.

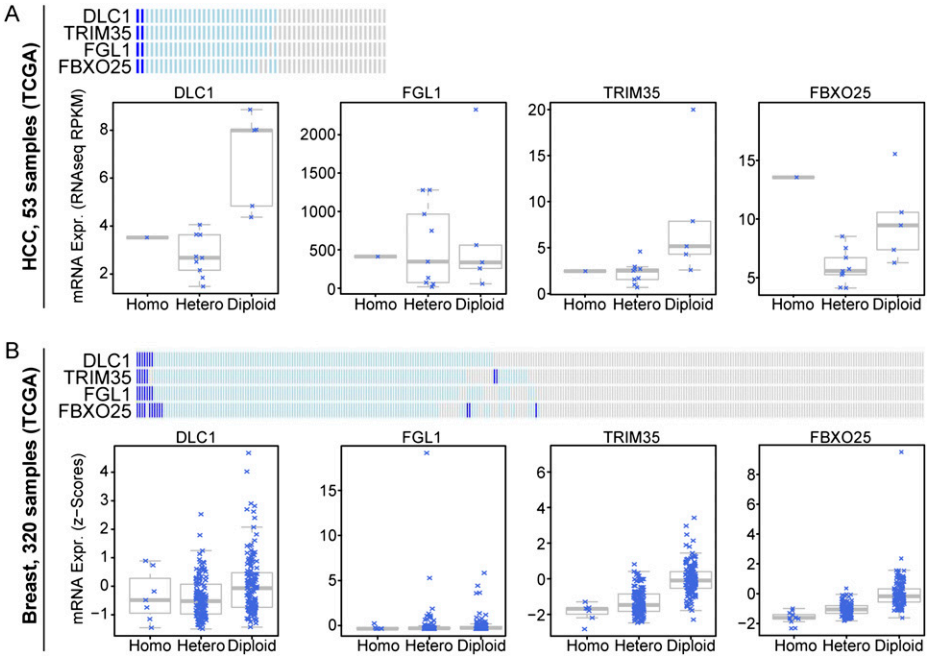

Fig. S5. Comparison of copy number loss to gene expression. (*A* and *B*, *Upper*) Putative copy number events based on the gene expression compared with copy number (GISTIC) algorithm for *DLC1*, *TRIM35*, *FGL1*, and *FBXO25* in each individual sample, with dark blue indicating homozygous loss and light blue indicating heterozygous loss. Of note, "homozygous" samples also can be tetraploid tumors with only a single remaining 8p arm. (*A* and *B*, *Lower*) GISTIC algorithm for the indicated genes for HCC (*A*) and invasive breast cancer (*B*). Data analysis is based on available TCGA data processed by the MSKCC cBio Core (www.cbioportal.org).

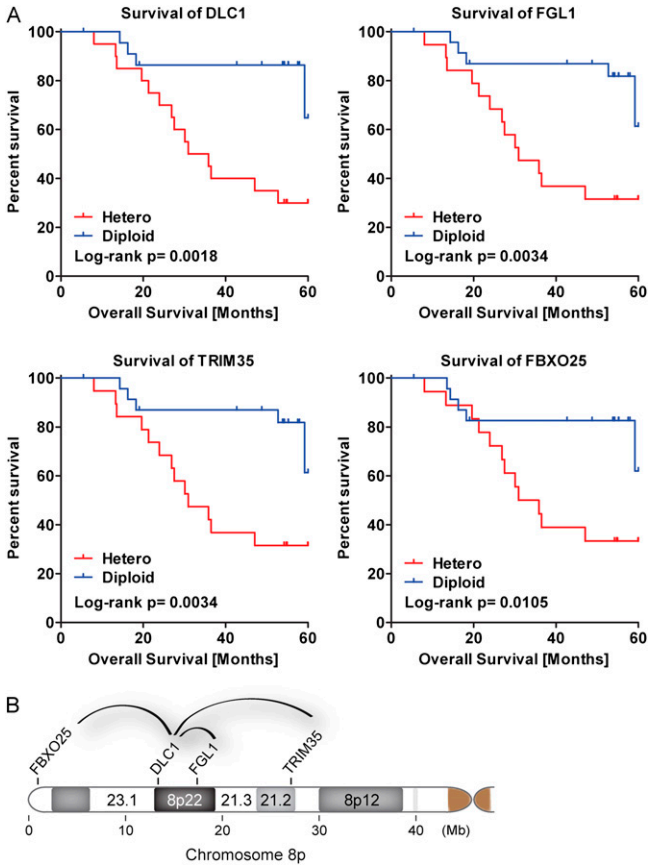

Fig. S6. Survival association of copy number loss of *DLC1*, *TRIM35*, *FGL1*, and *FBXO25* in HCC patients. (*A*) Survival curves of HCC patients comparing copy number (diploid vs. heterozygous loss) for the indicated individual chromosome 8p TSGs. Statistical tests were performed as described previously (1). (*B*) Schematic illustration of the chromosome position of the validated HCC TSGs showing cooperativity with *DLC1*.

1. Roessler S, et al. (2010) A unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients. *Cancer Res* 70:10202–10212.

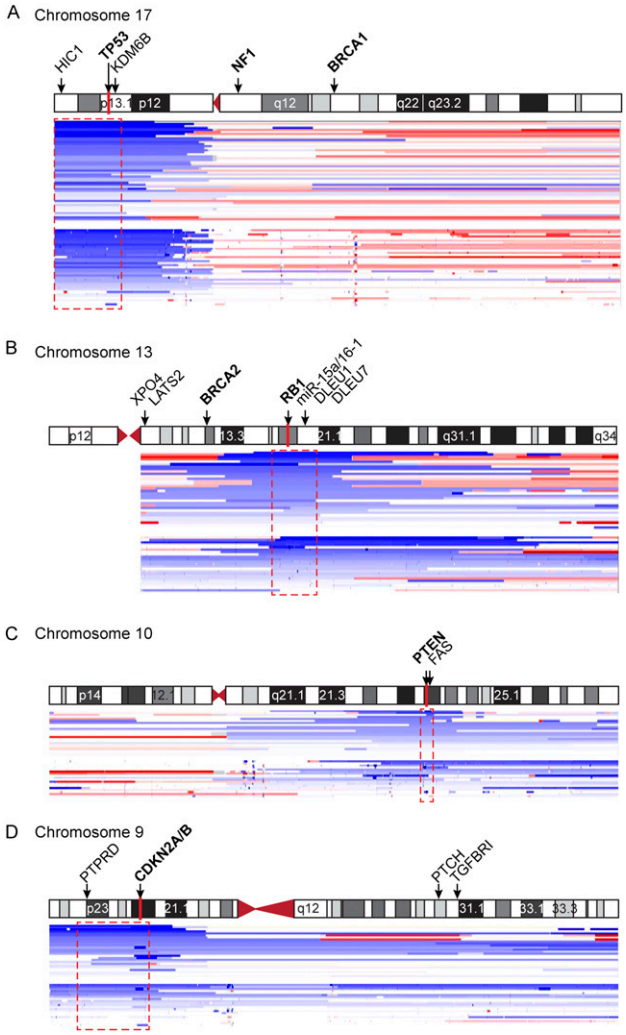

Fig. S7. Deletions at established TSG loci often include multiple candidate TSGs. Schematic diagrams of human chromosomes 17 (*A*), 13 (*B*), 10 (*C*), and 9 (*D*), with deletions indicated in blue and amplifications indicated in red from individual HCCs (based on 140 samples) as in Fig. 1*A*. The location of the most well-established TSG on each chromosome (*TP53*, *RB1*, *PTEN*, and *CDKN2A*/*B*) is indicated as a red line on the chromosome, with other established or candidate TSGs labeled in black. The red box highlights the most commonly deleted region.

Table S1. Gene name, chromosome location, and deletion frequency of selected 8p candidate TSGs outside 8p22

	Gene symbol	Cytoband	Description	Start no.	Stop no.	Deletion frequency, %
8p23	*FBXO25*	8p23.3	F-box protein 25	356808	419876	44.02
	ERICH1	8p23.3	Glutamate-rich 1	614200	681226	43.54
	ARHGEF10	8p23	Rho guanine nucleotide exchange factor 10	1772149	1906807	41.15
	CSMD1	8p23.2	CUB and Sushi multiple domains 1	2792875	4852328	41.63
	MCPH1	8	Microcephalin 1	6264121	6506026	41.63
	ANGPT2	8p23.1	Angiopoietin 2	6357172	6420784	41.63
	PINX1	8p23	PIN2-interacting protein 1	10622884	10697299	42.11
8p21-p11	*LZTS1*	8p22	Leucine zipper, putative tumor suppressor 1	20103676	20112803	41.63
	BIN3	8	Bridging integrator 3	22477931	22526661	44.02
	TNFRSF10B	8p22-p21	Tumor necrosis factor receptor superfamily, member 10b	22877646	22926700	47.37
	LOXL2	8p21.3-p21.2	Lysyl oxidase-like 2	23154410	23261722	46.41
	BNIP3L	8p21	BCL2/adenovirus E1B 19kDa interacting protein 3-like	26240523	26270644	44.02
	TRIM35	8	Tripartite motif-containing 35	27142404	27168834	49.28
	CLU	8p21-p12	Clusterin	27454451	27472327	47.85
	CCDC25	8	Coiled-coil domain containing 25	27590833	27630170	46.41
	SCARA5	8	Scavenger receptor class A, member 5 (putative)	27727736	27850198	46.41
	DUSP4	8p12-p11	Dual specificity phosphatase 4	29193611	29208185	44.02
	NRG1	8	Neuregulin 1	31497268	32622073	36.36
	SFRP1	8p12-p11.1	Secreted frizzled-related protein 1	41119478	41166980	32.06

Selection criteria are shown in Fig. S1. *DOK2*, a recently reported 8p lung TSG (1), is not strongly underexpressed in HCC and thus is not included in the list of candidate TSGs (Fig. S1).

1. Berger AH, et al. (2010) Identification of DOK genes as lung tumor suppressors. *Nat Genet* 42:216–223.

Table S2. Overview of the results from the pooled screening for 8p23 and 8p21-11 TSGs listed as fold increase compared with the experimental control

	Gene	Fold increase compared with control*	SEM (n = 6)	P value (t test)	Penetrance (no. of tumors/ no. of injection sites)
8p23	*Fbox25*	25.15	±6.00	0.220	6/6
	Erich1	2.63	±1.91	0.420	2/6
	Arhgef10	28.9	±5.10	0.047	5/6
	Csmd1	7.36	±3.03	0.110	3/6
	Mcph1	1.08	±0.55	0.890	2/6
	Angpt2	10.61	±3.72	0.210	6/6
	Pinx1	8.0	±2.80	0.120	4/6
8p21-p11	*Lzts1*	3.89	±1.99	0.220	2/6
	Bin3	9.90	±3.38	0.042	3/6
	Tnfrsf10b	11.6	±3.30	0.140	4/6
	Loxl2	13.0	±4.90	0.160	2/6
	Bnip3l	14.02	±3.10	0.036	6/6
	Trim35	9.83	±2.23	0.013	6/6
	Clu	0.82	±0.76	0.150	1/6
	Ccdc25	1.28	±1.04	0.720	6/6
	Scasra5	8.3	±2.40	0.043	4/6
	Dusp4	0.4	±0.70	0.360	1/6
	Nrg1	2.4	±1.30	0.230	4/6
	Sfrp1	0.77	±1.24	0.840	1/6

*Fold increase was calculated to the corresponding experimental shControl on day 49 for *Bnip3l* and on day 42 for *Trim35*.

Table S3. Somatic mutations of 8p TSGs reported in databases or the literature

Gene	COSMIC*	HGMD†	Literature
ARHGEF10	Colon: 1/33; S28L (missense)		
BNIP3L	0/181		Ovarian cancer, 1/40 [Lai et al. (1)]
DLC1	Kidney: 1/101; R347* (nonsense)Lung: 1/12; K237N, R1294C (missense)Pancreas: 1/2; R1425Q (missense)		HCC, ovarian, colorectal, and prostate [Wilson et al. (2); Park et al. (3); Liao et al. (4)]
FBXO25	Ovarian: 1/1; A347D (missense)		
FGL1	0/44		
TNFRSF10B	0/605	Squamous cell carcinoma, head and neck (insertion) [Pai et al. (5)]	Colorectal, truncating mutation [Macartney-Coxson et al. (6)]; nonsmall cell lung cancer [Lee et al. (7)]; metastatic breast cancer [Shin et al. (8)]
TRIM35	0/180		
SCARA5	Melanoma: 1/1; E270K (missense)		
VPS37A	0/44		

*www.sanger.ac.uk/genetics/CGP/cosmic/.
†www.hgmd.cf.ac.uk/ac/index.php.

1. Lai J, et al. (2003) Analysis of the candidate 8p21 tumour suppressor, BNIP3L, in breast and ovarian cancer. *Br J Cancer* 88:270–276.
2. Wilson PJ, et al. (2000) Sequence variants of DLC1 in colorectal and ovarian tumours. *Hum Mutat* 15:156–165.
3. Park SW, et al. (2003) DNA variants of DLC-1, a candidate tumor suppressor gene in human hepatocellular carcinoma. *Int J Oncol* 23:133–137.
4. Liao YC, et al. (2008) Mutations in the focal adhesion targeting region of deleted in liver cancer-1 attenuate their expression and function. *Cancer Res* 68:7718–7722.
5. Pai SI, et al. (1998) Rare loss-of-function mutation of a death receptor gene in head and neck cancer. *Cancer Res* 58:3513–3518.
6. Macartney-Coxson DP, et al. (2008) Metastatic susceptibility locus, an 8p hot-spot for tumour progression disrupted in colorectal liver metastases: 13 candidate genes examined at the DNA, mRNA and protein level. *BMC Cancer* 8:187.
7. Lee SH, et al. (1999) Alterations of the DR5/TRAIL receptor 2 gene in non-small cell lung cancers. *Cancer Res* 59:5683–5686.
8. Shin MS, et al. (2001) Mutations of tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) and receptor 2 (TRAIL-R2) genes in metastatic breast cancers. *Cancer Res* 61:4942–4946.