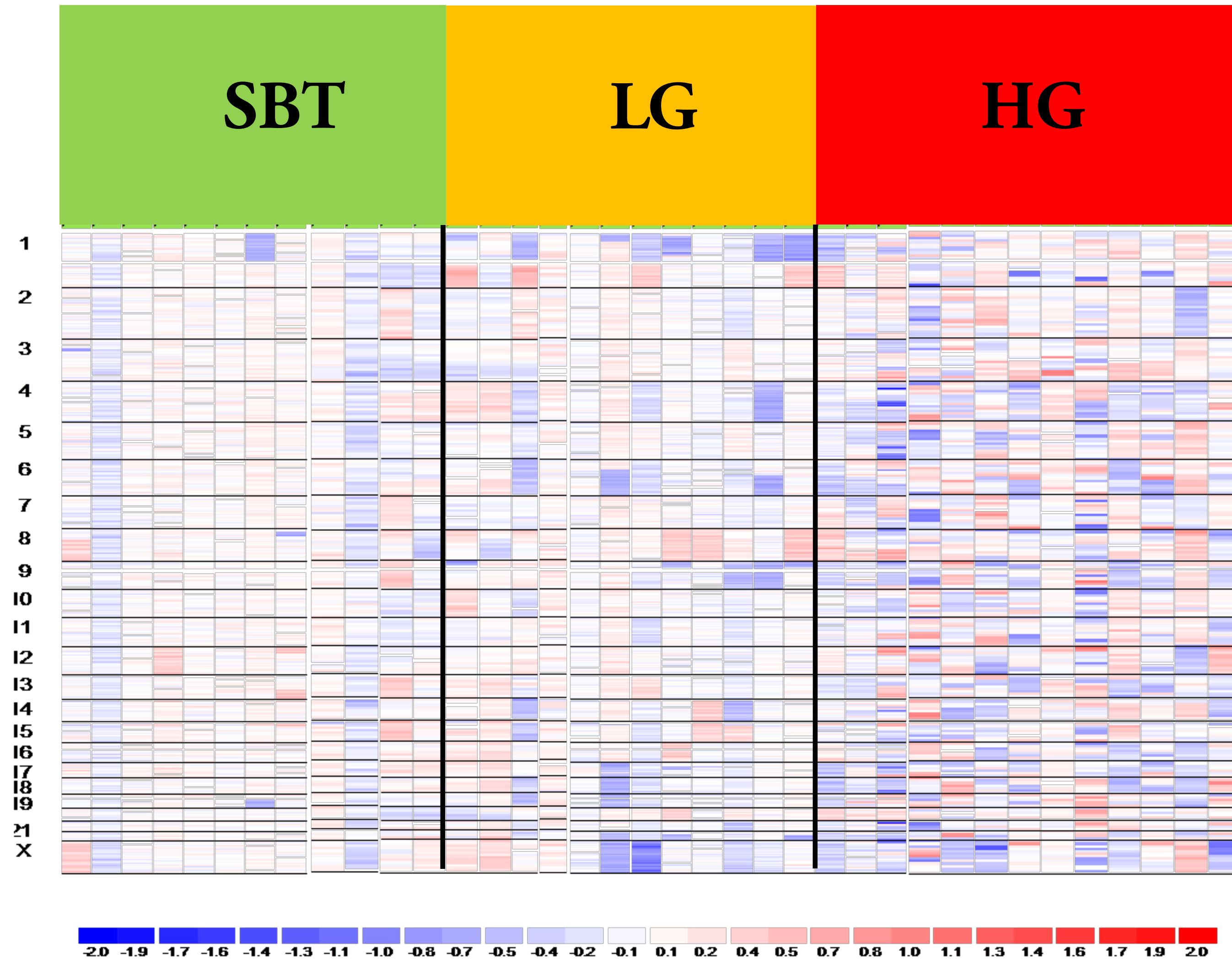
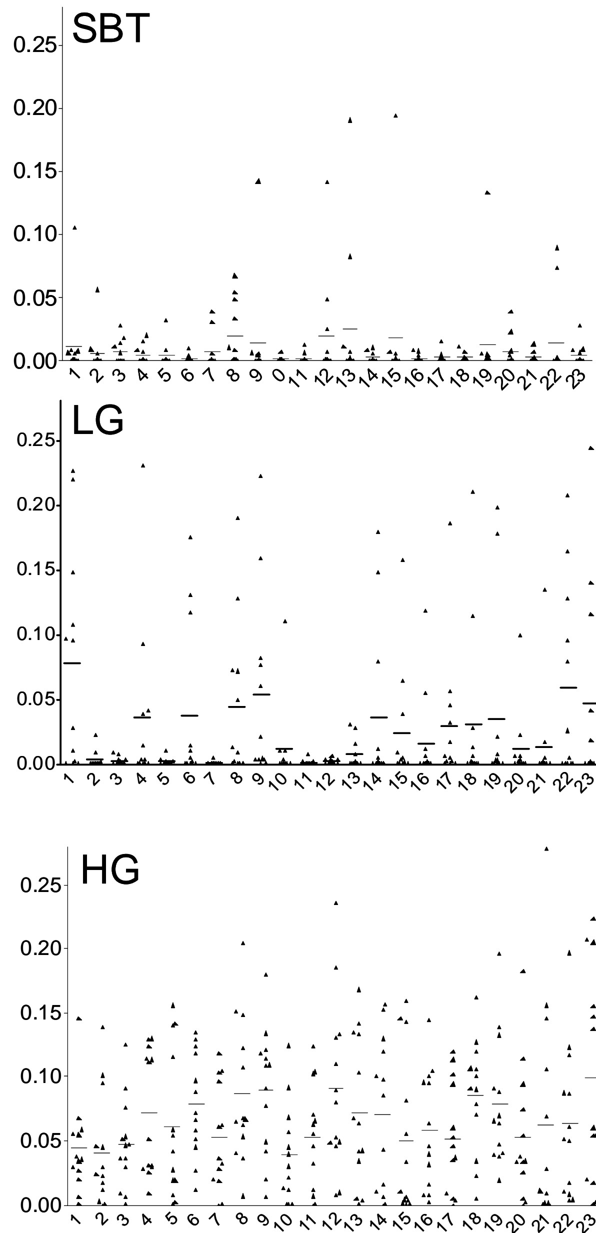
Supplementary Fig 1



Supplementary Figure 1. Genome-wide distribution of DNA copy number changes in ovarian serous tumors. DNA copy number changes are represented as pseudo-color gradients corresponding to the copy number increase (red boxes) and decrease (blue boxes) as compared to pooled normal samples. Each column represents an individual tumor sample. SBT: serous borderline tumor, LG: low-grade serous tumor, HG: high-grade serous tumor. As compared to SBT and LG tumors, HG tumors demonstrate diffuse and discrete DNA copy number gain (red boxes) and loss (blue boxes) in many chromosomes. Chromosome numbers are shown in the left column.



Supplementary Figure 2. Chromosome instability index for each chromosome. CIN index of each chromosome is plotted for each tumor type. The index is generally low in all the chromosomes of SBT while the index is generally high and dispersed in HG tumors. Several chromosomes in LG tumors (ch2, 3, 5, 7, 10, 11 and 12) exhibit a very low CIN index.

Supplementary Table 1. Serous tumor samples for SNP array analysis

Dx	Primary or Recurrent	TP53 status	KRAS/BRAF
SBT1	NA	wt	wt
SBT2	NA	wt	1799 T>A Val600Glu ; wt KRAS
SBT3	NA	wt	1799 T>A Val600Glu ; wt KRAS
SBT4	NA	wt	35 G>A Gly12Asp; wtBRAF
SBT5	NA	wt	1799 T>A Val600Glu ; wt KRAS
SBT 6	NA	wt	35 G>T Gly12Asp; wtBRAF
SBT7	NA	wt	35 G>T Gly12Asp; wtBRAF
SBT 8	NA	wt	35 G>A Gly12Asp; wtBRAF
SBT9	NA	wt	1799 T>A Val600Glu ; wt KRAS
SBT 10	NA	wt	35 G>A Gly12Asp; wtBRAF
SBT11	NA	wt	wt
SBT12	NA	wt	wt
LG1	R	wt	wt
LG2	P	wt	wt
LG3	P	wt	35 G>A Gly12Asp; wtBRAF
LG4	P	wt	35 G>A Gly12Asp; wtBRAF
LG5	P	wt	wt
LG6	P	wt	wt
LG7	R	wt	1799 T>A Val600Glu ; wt KRAS
LG8	P	wt	wt
LG9	P	wt	wt
LG10	R	wt	1799 T>A Val600Glu ; wt KRAS
LG11	P	wt	wt
LG12	R	wt	wt
HG1	R	715A>T, Asn239Tyr	35 G>A Gly12Asp; wtBRAF
HG2	R	wt	wt
HG3	P	641 A>G, His214Arg	wt
HG4	R	524 G>A, Arg175His	wt
HG5	P	wt	wt
HG6	R	747 G>T, Arg249Ser	wt
HG7	P	Exon 6, 5' intron 1G>T	wt
HG8	R	721 T>C, Ser241Pro	wt
HG9	P	452C>G, Pro151Arg	wt
HG10	P	726 1bp del, Cys242Stop	wt
HG11	P	Exon 6, 5' intron 1G>T	wt
HG12	P	168-169 2bp del, frameshift	wt
HG13	P	743 G>A, Arg248Gln	wt

Supplementary Table 2. Key regions of sub-chromosomal amplification in LG and HG tumors

Cytoband	Location (Mb)	Candidate gene	No. of Amplifications	Inferred copy number	microRNA
LG Tumors					
1q25.2	Chr1:174.541-174.547		3	3.10	
1q31.2	Chr1:188.094-188.217		3	3.08	

Cytoband	Location (Mb)	Candidate gene	No. of Amplifications	Inferred copy number	microRNA
HG Tumors					
1q44	Chr1:241.197-241.365	FAM152A, FAM36A, HNRNPU	3	3.11	
3q26.2	Chr3:170.61-170.718	MDS1	3	3.02	
3q26.2	Chr3:172.541-172.586	TNIK	3	3.23	
3q26.31	Chr3:173.559-173.608	FNDC3B	3	3.08	
3q26.33	Chr3:183.720-183.774		3	3.35	
3q27.1	Chr3:184.374-184.422	MCF2L2	3	3.19	
3q27.1	Chr3:185.685-185.710		3	3.22	
3q27.2	Chr3:186.013-186.114	VPS8	3	3.44	
3q27.2	Chr3:186.324-186.382	C3orf70	3	3.21	
3q27.2	Chr3:186.625-186.814	MAP3K13, TMEM41A, LIPH, SENP2	3	3.33	
3q27.2	Chr3:186.980-187.116	IGF2BP2	3	3.33	
3q27.2	Chr3:187.313-187.373	DGKG	3	3.11	
5p15.33	Chr5:0.461-0.697	AHRR, LOC116349, EXOC3, SLC9A3, CEP72	4	3.13	
5p15.33	Chr5:1.209-1.289	SLC6A19, SLC6A18	3	3.20	
5p15.33	Chr5:1.496-1.523	SLCA3,LPCAT1	3	3.14	
5p15.33	Chr5:3.436-3.710	IRX1	3	3.55	
5p15.32	Chr5:5.236-5.248	ADAMTS16	3	3.27	
5p15.32	Chr5:5.471-5.489	KIAA0947	3	3.20	
5p15.31	Chr5:6.625-6.675	NSUN2	3	3.19	
5p15.31	Chr5:7.305-7.573	ADCY2,	3	3.13	
5p15.31	Chr5:9.229-9.283	SEMA5A	3	3.11	
5p15.2	Chr5:13.663-13.757	DNAH5	3	3.46	
5p15.2	Chr5:14.832-14.868	ANKH	3	3.38	
5p15.1	Chr5:15.601-15.788	FBXL7	3	3.68	
5p15.1	Chr5:16.121-16.222	MARCH11	3	3.54	
5p15.1	Chr5:16.619-16.708	FAM134B	3	3.61	
5p15.1	Chr5:16.838-16.967	MYO10	3	3.44	

5p15.1	Chr5:16.289-17.253	ZNF622, FAM134B, MYO10	3	3.43	
7q36.3	Chr7:158.218-158.240	WDR60	3	3.34	
8q22.2	Chr8:99.304-99.323	NPAL2	3	3.24	
8q24.11	Chr8:118.897-118.948	EXT1	3	3.05	
11q22.1	Chr11:99.317-99.357	CNTN5	3	3.18	
12p13.33	Chr12:2.156-2.206	CACNA1C	3	3.12	
12p13.33	Chr12:2.743-2.848	FKBP4, ITFG2, NRIP2, FOXM1	3	3.17	
12p13.32	Chr12:4.589-4.638	DYRK4, AKAP3, NDUFA9	3	3.29	
12p13.31	Chr12:6.469-6.513	MRPL51, NCAPD2	3	3.19	U85
12p13.31	Chr12:6.714-6.775	MLF2, PTMS, LAG3, CD4	3	3.31	
12p13.31	Chr12:7.161-7.427	RBP5, CLSTN3, PEX5,ACSM4, CD163L1	3	3.35	
12p13.31	Chr12:7.676-8.081	APOBEC1, DPPA3, CLEC4C, GDF3, NANOG,	4	3.42	
12p13.31	Chr12:9.707-9.790	CLEC2D, CLECL1	4	3.24	
12p13.31-13.2	Chr12:10.013-10.197	CLEC12A,, CLEC1B,	4	3.26	
12p13.2	Chr12:11.625-12.757	ETV6, BCL2L14, LRP6,	4	3.26	
12p13.2-13.1	Chr12:12.778-13.591	APOLD1, DOX47,	4	3.49	
12p13.1-12.3	Chr12:13.675-17.794	ART4, MGP, ERP27,	3	3.66	
12p12.3-12.2	Chr12:18.043-20.126	PIK3C2G, PLCZ1,	3	3.81	
12p12.2	Chr12:20.497-20.660	PDE3A	4	3.63	
12p12.2-12.1	Chr12:20.662-22.764	KIAA0528, ETNK1	3	3.67	
12p12.1	Chr12:23.370-24.083	SOX5	3	3.43	
12p12.1	Chr12:24.872-24.888	BCAT1	3	3.46	
12p12.1-11.23	Chr12:24.992-27.297	KRAS, IFLTD1, RASSF8,	3	3.49	
12p11.23-11.22	Chr12:27.364-28.915	PTHLH, CCDC91	3	3.46	
12p11.22-11.21	Chr12:29.314-32.637	ERGIC2, OVCH1	3	3.32	
12q24.23	Chr12:116.653-116.659	KSR2	3	3.07	
15q11.2	Chr15:19.969-20.045		4	3.04	
17q25.3	Chr17:74.741-74.808	HRNBP3	3	3.36	
18q11.2	Chr18:22.874-23.027	CHST9	3	3.30	
19p13.12	Chr19:15.114-15.135	Notch3	3	3.05	
19q13.12	Chr19:41.709-41.778	ZNF260, ZNF529	3	3.13	
21q21.1	Chr21:20.368-20.490		3	3.24	
22q11.21	Chr22:19.378-19.442	PIK4CA	3	3.39	

Xp22.2	ChrX:11.325-11.769	ARHGAP6	3	3.24
Xp22.2	ChrX:12.249-12.307	FRMPD4	3	3.35
Xp22.11	ChrX:22.295-22.730		3	3.12
Xp11.1-q11.2	ChrX:57.734-62.515	ZXDA, SPIN4	3	3.14

Note: Chromosome location is based on genome assembly from May 2004, hg17

Supplementary Table 3. Key regions of sub-chromosomal deletion in LG and HG tumors

Cytoband	Location (Mb)	Candidate gene	[1]No. of Deletions	[2]Inferred copy number	[3]Measured copy number	microRNA
LG Tumors						
9p21.3	Chr9:21.945-22.000	CDKN2A, CDKN2B	2/4	0.59*	0.09	
22q11.23	Chr22:24.044-24.240	LRP5L	1/3	0.28	0.03	
HG Tumors						
2q22.1	Chr2:141.719-141.827	LRP1B	1/2	0.41	[4]NP	
4p15.1-14	Chr4:33.9934-38.2422	CENTD1	1/5	0.27	0.03	
4q28.2	Chr4:130.513-130.662		1/2	0.34	NP	
4q34.3	Chr4:182.433-182.752		1/6	0.36	NP	
7p15.3	Chr7:24.6407-24.736	OSBPL3	1/2	0.32	0.28	
7q31.1	Chr7:111.021-111.566	DOCK4	1/1	0.15	0.02	
7q32.1	Chr7:127.194-127.306	SND1	1/1	0.23	NP	
8p23.3-23.1	Chr8:0.189-8.336	CSMD1	1/5	0.20	0.03	
9p24.2	Chr9:2.869-3.024		1/3	0.29	NP	
9p21.3	Chr9: 21.489-24.413	CDKN2A, CDKN2B,	1/5	0.22	0.03	*mir-31*
9p21.2-21.1	Chr9:27.792,-28.577	LINGO2	1/5	0.25	0.13	
9p21.1	Chr9:30.2558-30.4361		1/3	0.45	NP	
10p12.2-12.1	Chr10:24.596-24.644	KIAA1217	1/0	0.24	NP	
13q14.2	Chr13:47.59-48.095	RB1	1/5	0.26	0.01	
14q21.3-22.1	Chr14:49.859-51.382	CDKL1	1/2	0.23	0.03	
17p13.3	Chr17:0.0068-0.139	RPH3AL	2/1	0.41	0.22	
17q23.2	Chr17:51.518-51.519		1/1	0.43	NP	
21q11.2-21.1	Chr21:14.369-15.823	LIP1	1/1	0.24	NP	
21q21.1	Chr21:15.823-17.532	USP25, C21orf34	1/2	0.36	0.02	*miR-99a, let-7c, miR-125b-2*

Note: Chromosome location is based on human genome assembly from May 2004, hg17.

[1] No. of homozygous deletion/No. of hemizygous deletion

[2] Copy number based on the dCHIP analysis

[3] Copy number determined by quantitative real-time PCR

[4] NP: not performed

Supplementary Table 4. Nucleotide sequences of PCR primers used to amplify cDNA of miR-34a target genes.

Candidate Target Gene	Forward primer	Reverse primer
Rad51AP1	*TCGTCATTATCCTCACTCTCACA	CTTCTGGAAGGCAGTGATGG
MDC1	AATGGCTGTGTAGCCAGGAC	CTTCATGTTGACTCCACCCC
CHEK1	TCATCCATTTCTAACAAATTCACTT	TGGGCTATCAATGGAAGAAAA
CDKN2C	CAAATCGGGATTAGCACCTC	ACGTCAATGCACAAAATGGA
BIRC3	GTCAAATGTTGAAAAAGTGCCA	GGGAAGAGGAGAGAGAAAGAGC
EMP1	GAGTTCTGAAGGGTCCCAGC	TGCGGTCACATACTTCCAGA
BCL2	GAGAAATCAAACAGAGGCCG	CTGAGTACCTGAACCGGCA
CCND1	GGCGGATTGGAAATGAACTT	TCCTCTCCAAAATGCCAGAG
CDK6	TGTCTGTTCGTGACACTGTGC	ATGCCGCTCTCCACCAT
E2F1	GGCCAGGTACTGATGGTCA	GACCCTGACCTGCTGCTCT
E2F3	CTAGCTCCAGCCTTCGCTTT	AGCCTCCTCTACACCACGC

*nucleotide sequence from 5’ to 3’

Supplementary Table 5. Nucleotide sequences of PCR primers used to amplify the specific genomic loci.

Cytoband	Candidate Gene	Forward primer	Reverse primer
1p36.23	miR-34a	*GCGAAAGTTTGCAAAGAAGG	GGAATCCTTTCTCCCCAGAG
4p15.1-14	CENTD1	GCACTGCCCTTTTCTCCTTTT	AAGCATAGCAGCACCCATTTT
7p15.3	OSBPL3	ACTCAGCTCCCAAGACAGGA	CTTTTTCTCAGGGTCCACCA
7q31.1	DOCK4	TTTGGCATTTCAACTGAGTCC	ATTCCATCGGCAAAGAACAGA
8p23.3-23.1	CSMD1	CGGCCATGAGAAGAAATGAT	ATGGGATGAAGGCAACAGAG
9p21.3	CDKN2A	GAAACCCGAAGAACAATGGA	GAATTCCCATCTGCCGTCTA
9p21.2-21.1	LINGO2	GAATGCTCCTGGTTCCACAT	CTGTCACAGAAGGCGATTGA
13q14.2	RB1	TCCATTGCCCACAGGATACTC	AGCCGACTAACACGCAAGAAG
14q21.3-22.1	CDKL1	GCCCCTATGTCTCATGGAAGA	TCCACTTTGATGCTGATGCAC
17p13.3	RPH3AL	GAGAAGGTGTGGAGCTGAGG	GGCCTGTAAAGTTTGGGTCA
21q21.1	USP25	ATACTGTGGGTTTGGCACGAT	CTTCCTCCGTTATGTGCCTTG
22q11.23	LRP5L	CACACAGCTAGGCCATCAGA	CTTGGCCTCAACCTGCTTAG

*Nucleotide sequence from 5’ to 3’

Supplementary Material and Methods

Quantitative real-time PCR

gDNA copy number of the candidate genes was validated by quantitative real-time PCR using an iCycler (Bio-Rad, Hercules, CA) with SYBR green dye (Molecular Probes, Eugene, OR). Averages in the threshold cycle number (Ct) of triplicate measurements were obtained. The results were expressed as the difference between the Ct of the gene of interest and the Ct of a *Line-1* gene for which gDNA copy number is relatively constant among tumor tissues[1]. cDNA copy number was measured using the same procedure except the relative copy number of each candidate gene was normalized to the copy number of *APP*, a gene which mRNA expression is constant among samples[2].The primer sequences are listed in supplementary Table 4 and 5.

Statistic analysis

The differences in parameters were determined by unpaired *t* test for data presented in Fig 1B and Fig 6, and p value was determined by two-tailed analysis. Paired *t* test was performed to determine the significance of difference in the CIN index between matched normal and tumor samples (Fig 1C). The significant level (alpha) was set at 0.05. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Transfection and functional study of miR-34a

Synthetic miR-34a (mimic) and control miRNA were purchased from ABI (Applied Biosystems, Foster City, CA) and transfected into a LG cell line, MPSC-1, using Lipofectamine 2000 (Invitrogen). The level of miR-34a was determined by the TaqMan microRNA assay kit (Applied Biosystems). Following transfection, cells were seeded into 96-well plates and the viable cell number was measured by the Celltiter Blue reagent (Promega, Madison, WI) using a microplate reader. Data were expressed as mean ± 1 standard deviation from five replicates in each experimental group. Apoptotic cells were detected by staining with Annexin V-FITC and cell death was detected by nuclear propidium iodide staining using a kit purchased from BioVision

(Mountain View, CA). The apoptotic cells were defined by Annexin V-positive, propidium iodide-negative cells and the percentage of apoptotic cells was determined by counting approximately 300 cells from each well. The data were expressed as mean ± 1 standard deviation from triplicates.

[1]Wang TL, Maierhofer C, Speicher MR, et al. Digital karyotyping. Proc Natl Acad Sci U S A 2002;99:16156-61.

[2]Buckhaults P, Zhang Z, Chen YC, et al. Identifying tumor origin using a gene expression-based classification map. Cancer Res 2003;63:4144-9.