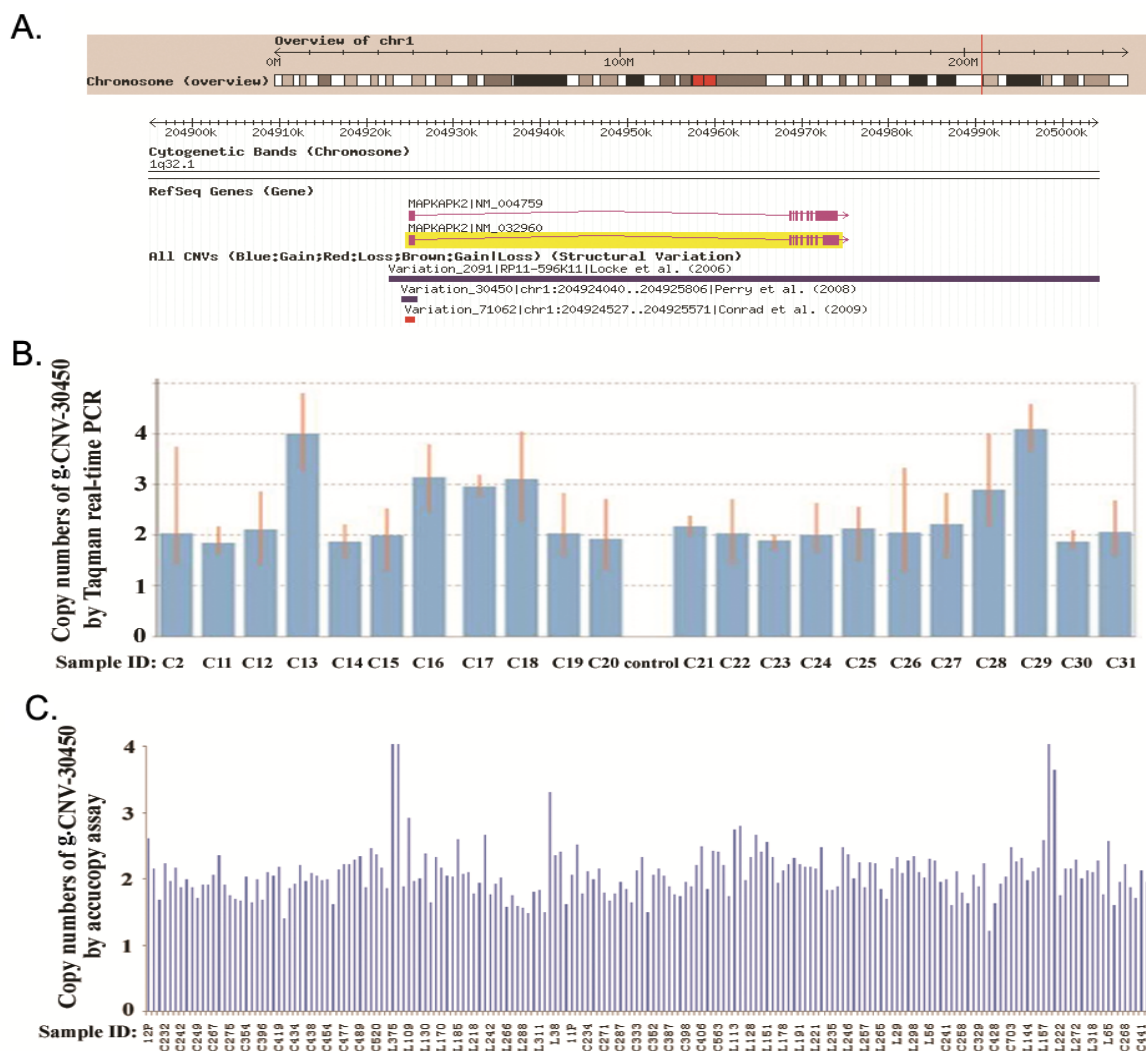# Supplemental Data

A Functional Copy-Number Variation in *MAPKAPK2*

Predicts Risk and Survival of Lung Cancer

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Figure S1. The Chromosomal Location of *MAPKAPK2* with CNVs Information and Genotyping of g.CNV-30450

(**A**) Schematic of *MAPKAPK2* in Chr.1q32 and the three identified CNVs.

(B) g.CNV-30450 genotyping by the Taqman real-time PCR: in 10μl reaction systems, triplicate 10-ng DNA samples were mixed with TaqMan PCR Master Mix (Applied Biosystems, Foster City, CA, USA), the control RNase P probe (VIC labeled, Applied Biosystems) and the experimental probe (FAM labeled, cat# Hs01173160 Applied Biosystems), then the mixture were run on an ABI 7900HT fast real-time PCR System (Applied Biosystems) and the genotypes were automatically determined by software CopyCaller 1.0 (Applied BioSystems). The mixture without genomic DNA was defined as zero copy number and 3 samples with two copies of g.CNV-30450 locus found by Affymetrix Genome-Wide Human SNP Array 6.0 were used as standard sample. Columns, mean from three independent experiments; bars, SD.

(C) g.CNV-30450 genotyping by the Accucopy assay with a patented Multiplex AccuCopy[TM] Kit (Genesky Bio-Tech Co., Ltd., Shanghai, China).

A.

B.
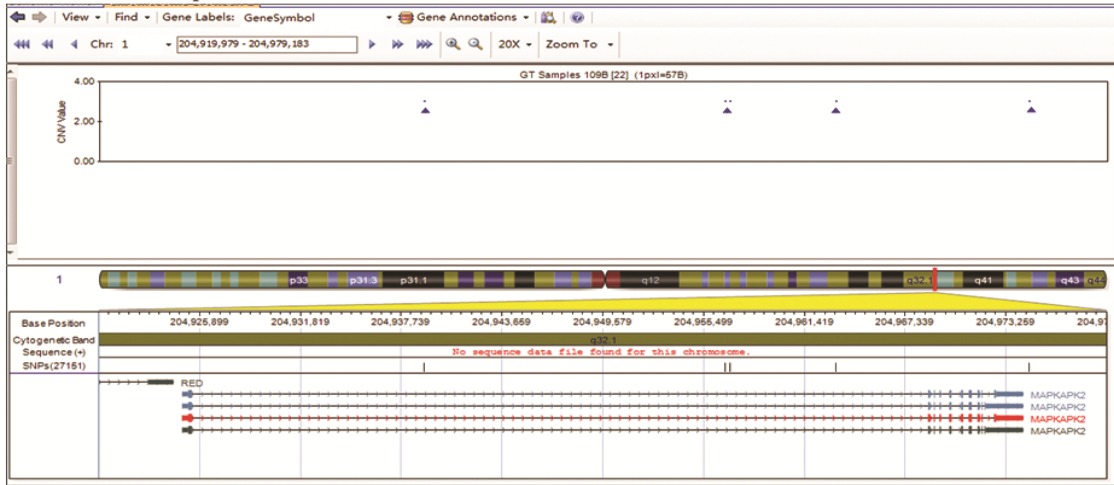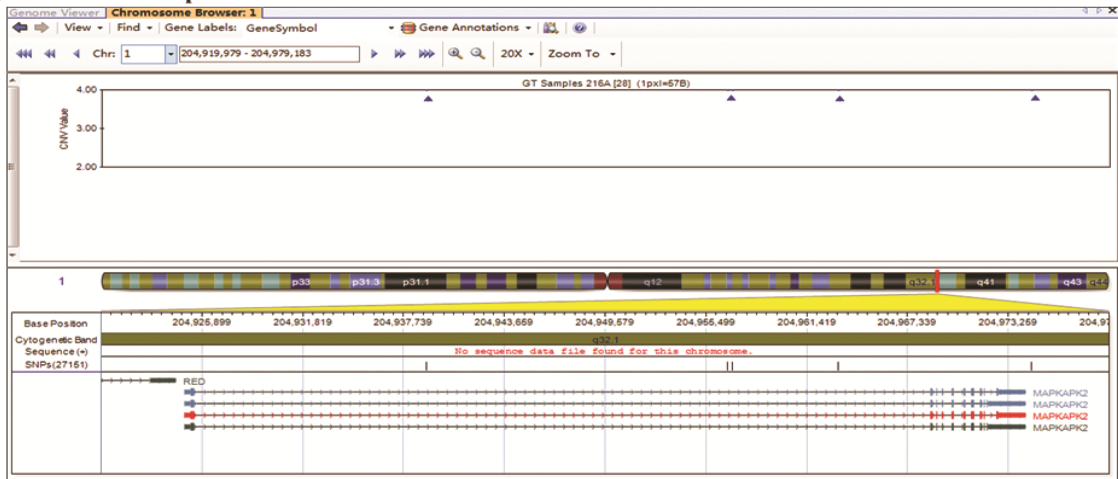
Figure S2. *MAPKAPK2* **g.CNV-30450 Calculation with Affymetrix Genome-wide Human**

SNP Array 6.0

(**A**) 3-copy genotype.

(**B**) 4- copy genotype.
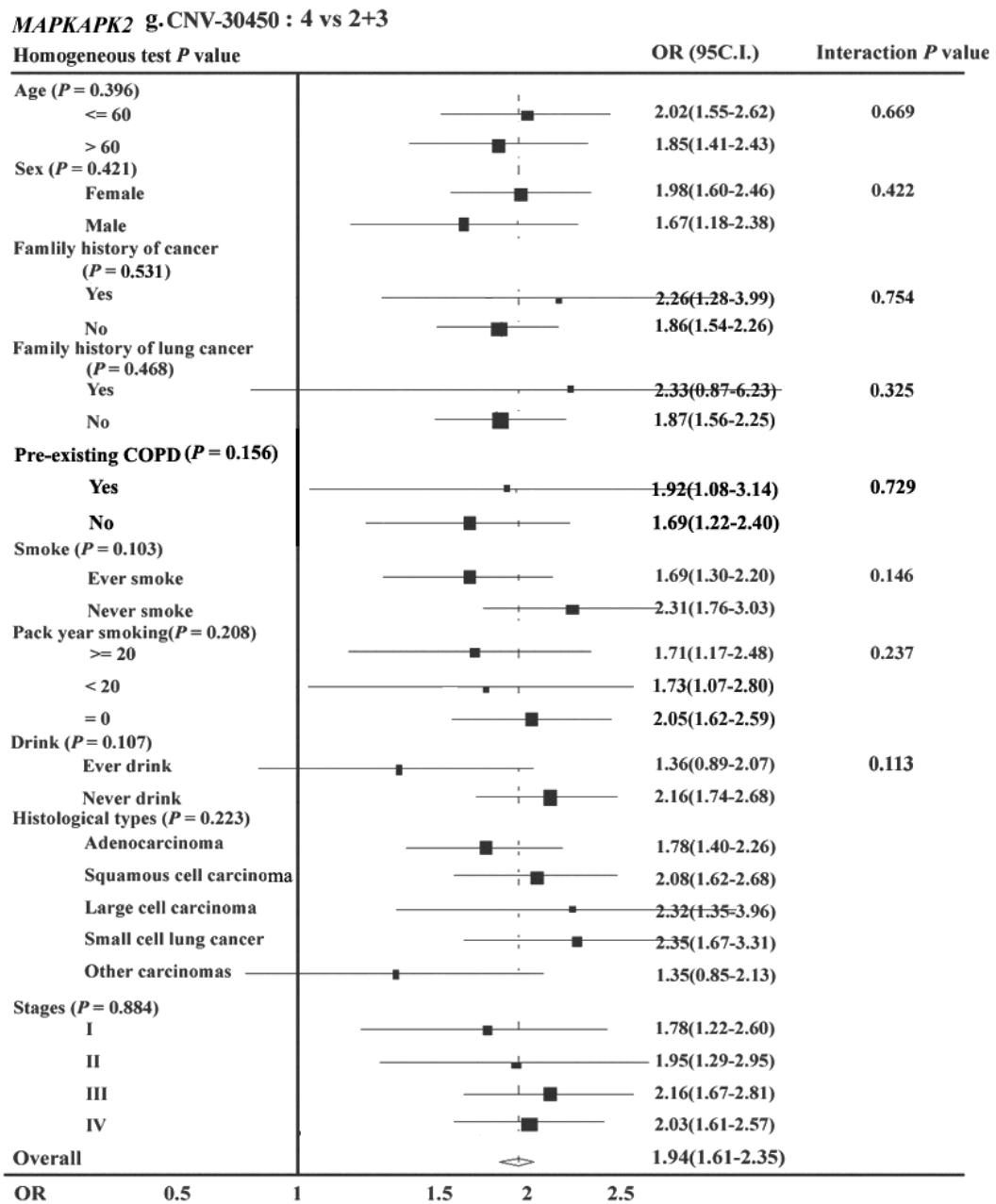
Figure S3. Stratification Analysis of the g.CNV-30450 Genotypes and Lung Cancer Risk

P value for the homogeneity test in each stratum was tested by Breslow-Day Test. A multiplicative interaction model was applied for the interaction analysis.

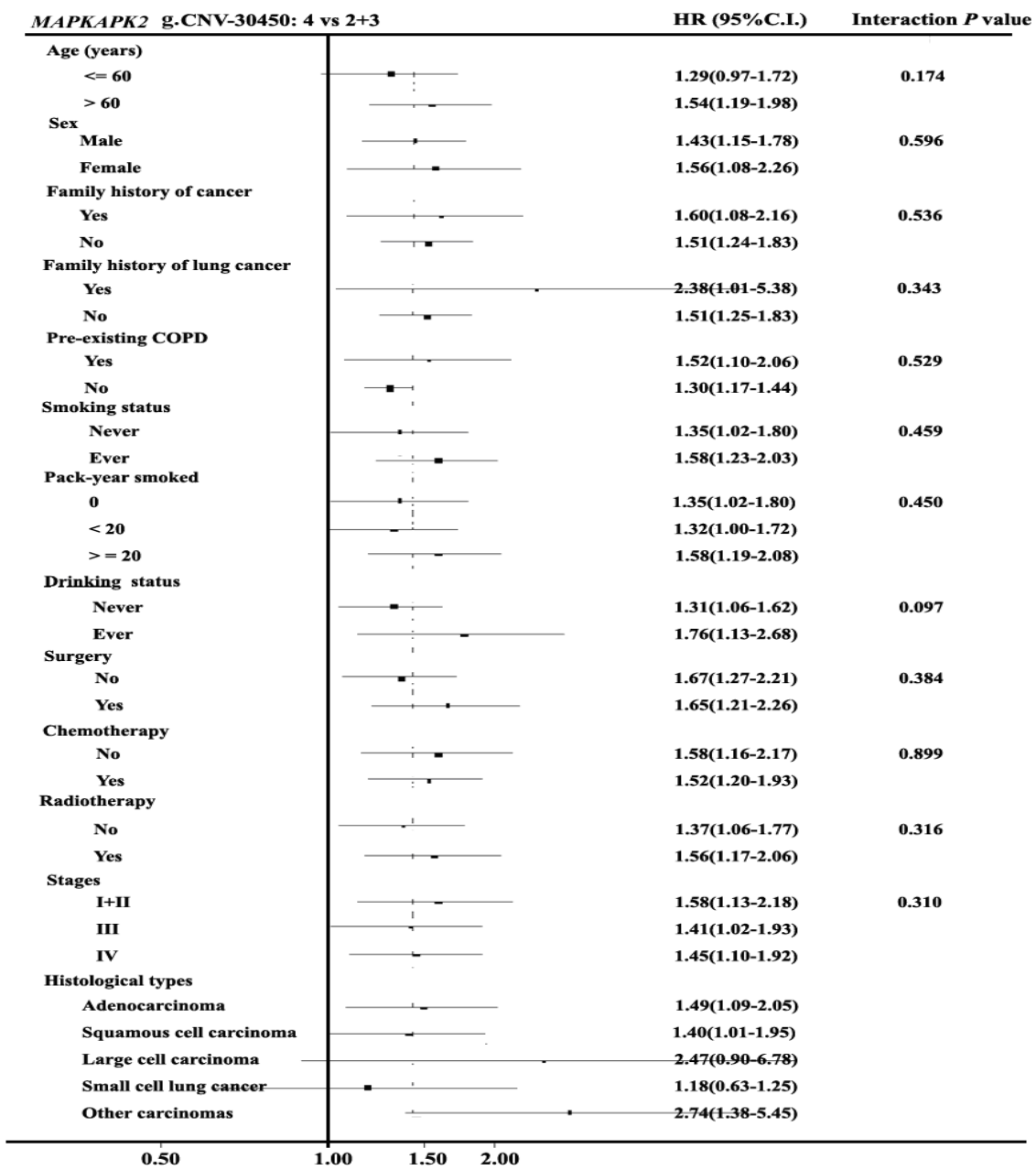| MAPKAPK2 g.CNV-30450: 4 vs 2+3	HR (95%C.I.)	Interaction P value
Age (years)		
<= 60	1.29(0.97-1.72)	0.174
> 60	1.54(1.19-1.98)	
Sex		
Male	1.43(1.15-1.78)	0.596
Female	1.56(1.08-2.26)	
Family history of cancer		
Yes	1.60(1.08-2.16)	0.536
No	1.51(1.24-1.83)	
Family history of lung cancer		
Yes	2.38(1.01-5.38)	0.343
No	1.51(1.25-1.83)	
Pre-existing COPD		
Yes	1.52(1.10-2.06)	0.529
No	1.30(1.17-1.44)	
Smoking status		
Never	1.35(1.02-1.80)	0.459
Ever	1.58(1.23-2.03)	
Pack-year smoked		
0	1.35(1.02-1.80)	0.450
< 20	1.32(1.00-1.72)	
>= 20	1.58(1.19-2.08)	
Drinking status		
Never	1.31(1.06-1.62)	0.097
Ever	1.76(1.13-2.68)	
Surgery		
No	1.67(1.27-2.21)	0.384
Yes	1.65(1.21-2.26)	
Chemotherapy		
No	1.58(1.16-2.17)	0.899
Yes	1.52(1.20-1.93)	
Radiotherapy		
No	1.37(1.06-1.77)	0.316
Yes	1.56(1.17-2.06)	
Stages		
I+II	1.58(1.13-2.18)	0.310
III	1.41(1.02-1.93)	
IV	1.45(1.10-1.92)	
Histological types		
Adenocarcinoma	1.49(1.09-2.05)	
Squamous cell carcinoma	1.40(1.01-1.95)	
Large cell carcinoma	2.47(0.90-6.78)	
Small cell lung cancer	1.18(0.63-1.25)	
Other carcinomas	2.74(1.38-5.45)	

0.50   1.00   1.50   2.00

Figure S4. Stratification Analysis of the g.CNV-30450 Genotypes and Lung Cancer Survival

A multiplicative interaction model was supplied for the interaction analysis.
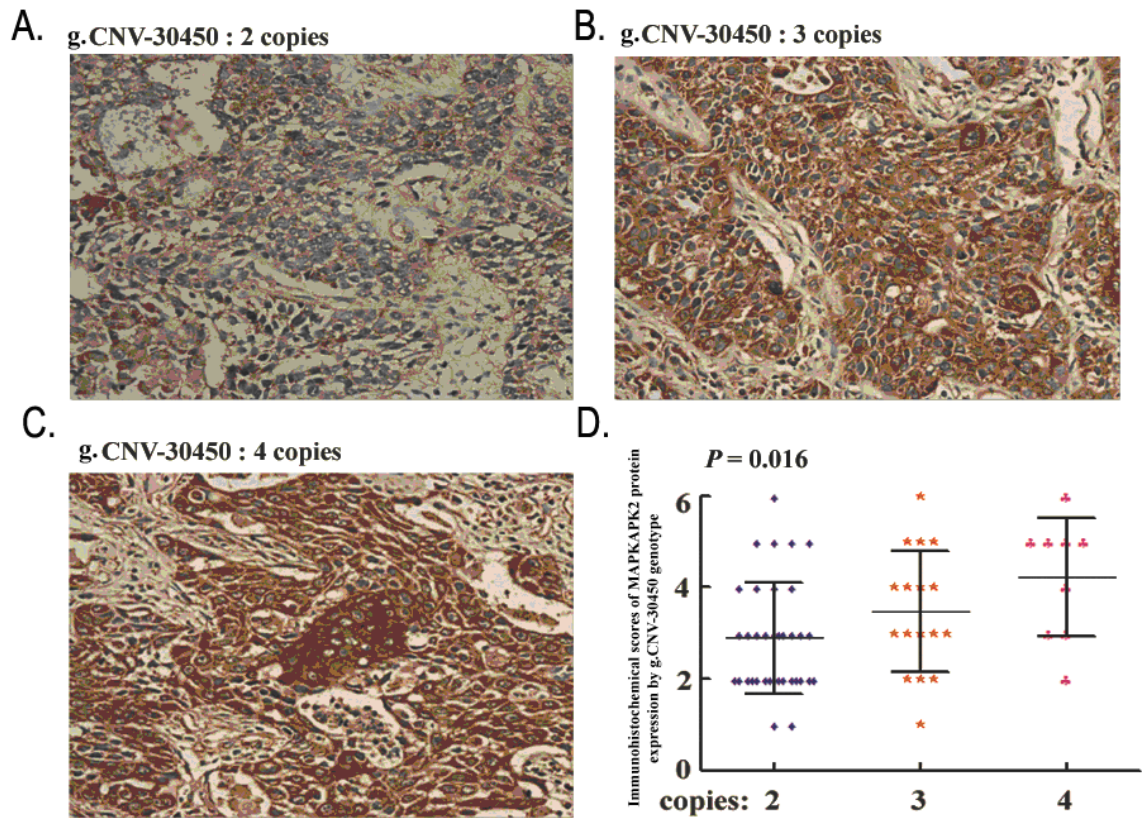
Figure S5. MAPKAPK2 Protein Expressions in Lung Cancer Tissues In Situ by Immunohistochemistry

The MAPKAPK2 cytoplasmic expression was scored to the sum of the intensity and percentage and defined as follows: 0-2, negative or weak; 3-4, moderate; and 5-6, strong by using the Image Pro Plus software. **A,** 2-copy genotype. **B,** 3-copy genotype. **C,** 4-copy genotype. **D,** MAPKAPK2 expression in situ by g.CNV-30450 genotypes. Columns, mean from three independent experiments; bars, SD. The copy number of g.CNV-30450 was CNV in blood DNA. The 4 copies of g.CNV-30450 had significantly higher MAPKAPK2 expressions (both intensity and positive percentage) than that of 2 or 3 copies as ANOVA test shown.
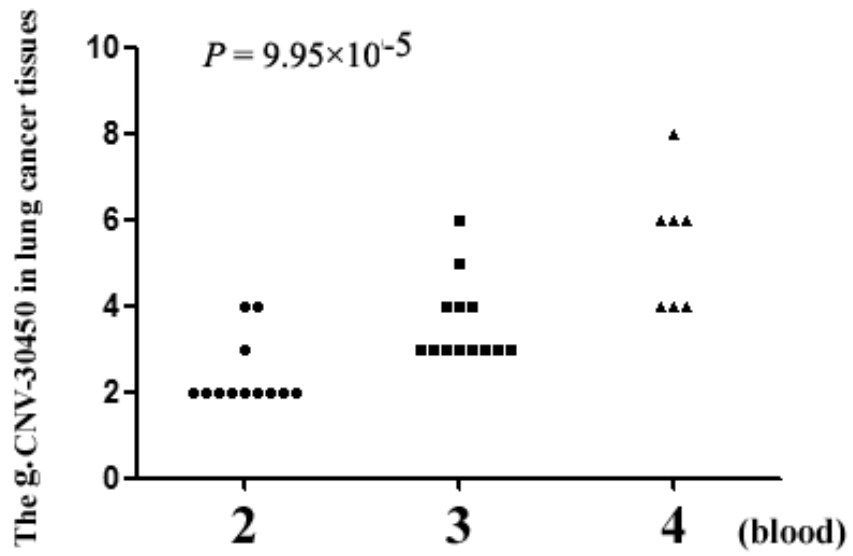
Figure S6. The Relationship between g.CNV-30450 in Peripheral-Blood and Tumor Tissues

The 4 copies genotype might cause a more copy number variation in somatic genome.

Table S1. Frequency Distributions of Selected Variables in Individuals with Lung Cancer and Cancer-free Controls

Variables	Discovery set (Southern Chinese)			Validation set I (Eastern Chinese)			Validation set II (Southern Chinese)		
	Case (n=1056) n (%)	Control (n=1056) n (%)	P^a	Case (n=503) n (%)	Control (n=623) n (%)	P^a	Case (n=773) n (%)	Control (n=778) n (%)	P^a
Age (years)									
≤ 60	536(50.8)	534(50.6)	0.931	273(54.3)	343(55.1)	0.793	438(56.7)	447(57.5)	0.752
> 60	520(49.2)	522(49.4)		230(45.7)	280(44.9)		335(43.3)	331(42.5)	
Sex									
Male	746(70.6)	746(70.6)	1.000	345(66.6)	433(70.4)	0.496	545(70.5)	547(70.3)	0.933
Female	310(29.4)	310(29.4)		158(31.4)	184(29.6)		228(29.5)	231(29.7)	
Family history of cancer									
Yes	104(9.9)	103(9.8)	0.942	25(5.0)	44(7.1)	0.046	32(4.1)	47(6.1)	0.088
No	952(90.1)	953(90.2)		478(95.0)	579(92.9)		741(95.9)	730(93.9)	
Family history of lung cancer									
Yes	42(4.0)	30(2.8)	0.150	10(2.0)	13(2.1)	0.907	27(3.5)	14(1.8)	0.038
No	1014(96.0)	1026(97.2)		493(98.0)	610(97.9)		746(96.5)	763(98.2)	
Pre-existing COPD									
Yes	148(14.0)	113(10.7)	0.021	69(13.7)	56(9.0)	0.012	67(8.7)	43(5.5)	0.016
No	908(86.0)	943(89.3)		434(86.3)	567(91.0)		706(91.3)	735(94.5)	
Smoking status									
Current	394(37.3)	366(34.6)	0.028	118(23.5)	168(26.6)	1.79×10^{-7}	187(24.2)	219(28.1)	4.99×10^{-14}
Former	207(19.3)	176(16.8)		105(20.9)	57(9.2)		197(25.5)	80(10.3)	
Never	455(43.1)	514(48.7)		280(55.6)	400(64.2)		389(50.3)	479(61.6)	
Pack-years smoked									
≥20	459(43.5)	314(29.7)	9.40×10^{-12}	165(32.8)	165(26.5)	0.011	291(37.7)	218(28.0)	3.34×10^{-5}
<20	142(13.4)	228(21.6)		58(11.5)	58(9.3)		93(12.0)	81(10.4)	

			P^a			P^a			P^a
0	455(43.1)	514(48.7)		280(55.7)	400(64.2)		389(50.3)	479(61.6)	
Drinking status									
Current	165(15.6)	186(17.6)	0.042	36(7.2)	75(12.0)	0.017	80(10.4)	106(13.6)	0.062
Former	64(6.1)	41(3.9)		28(5.6)	40(6.4)		75(9.7)	59(7.6)	
Never	827(78.3)	829(78.5)		439(87.2)	508(81.6)		618(79.9)	613(78.8)	
Histological types									
Adenocarcinoma	384(36.4)			231(45.9)			330(42.7)		
Squamous cell carcinoma	369(34.9)			158(31.4)			238(30.8)		
Large cell carcinoma	43(4.1)			23(4.6)			35(4.5)		
Small cell lung cancer	128(12.1)			65(12.9)			92(11.9)		
Other carcinomas [b]	132(12.5)			26(5.2)			78(10.1)		
Stages									
I	154(14.6)			46(9.2)			98(12.7)		
II	94(8.9)			53(10.5)			83(10.8)		
III	333(31.5)			157(31.2)			214(27.7)		
IV	475(45.0)			247(49.1)			378(48.8)		

[a] P values for a χ^2 test.
[b] Mixed-cell or undifferentiated carcinoma.

Table S2. Analysis of the Effects of Lung Cancer-Affected Individuals' Characteristics and Clinical Features on Lung Cancer Survival

Variables	Discovery set (Southern Chinese)		Validation set I (Eastern Chinese)		Validation set II (Southern Chinese)		MST (months)	Log-rank P value	Cox model Crude HR (95%CI)	Cox model Adjust [a] HR (95%CI)
	Case n (%)	No. of death	Case n (%)	No. of death	Case n (%)	No. of death				
Total	510	413	296	203	331	208				
Age (years)								2.89×10^{-5}		
≤ 60	229 (44.9)	190	151 (51.0)	92	174 (52.6)	94	16		1.00 (ref.)	1.00 (ref.)
> 60	281 (50.1)	223	145 (49.0)	111	157 (47.4)	114	10		**1.33 (1.16-1.53)**	**1.24 (1.07-1.44)**
Sex								**0.044**		
Male	366 (71.8)	301	200 (67.6)	138	229 (69.2)	144	13		1.00 (ref.)	1.00 (ref.)
Female	144 (28.2)	112	96 (32.4)	65	102 (30.8)	64	15		**0.86 (0.74-1.00)**	0.91 (0.75-1.08)
Family history of cancer										
No	461 (90.4)	372	285 (96.3)	197	318 (96.1)	202	13	0.194	1.00 (ref.)	1.00 (ref.)
Yes	49 (9.6)	41	11 (3.7)	6	13 (3.9)	6	19		0.84 (0.63-1.10)	0.81 (0.61-1.06)
Family history of lung cancer										
No	489 (95.9)	397	292 (98.6)	201	322 (97.3)	203	13	0.108	1.00 (ref.)	1.00 (ref.)
Yes	21 (4.1)	16	4 (1.4)	2	9 (2.7)	5	17		0.94 (0.84-1.04)	0.89 (0.66-1.21)
Pre-existing COPD										
No	72(14.1)	59	252(85.1)	165	300(90.6)	183	14	**0.002**	1.00 (ref.)	1.00 (ref.)
Yes	438(85.9)	354	44(14.9)	38	31(9.4)	25	10		**1.34 (1.11-1.64)**	1.15 (0.93-1.41)
Smoking status								1.07×10^{-5}		
Never	211 (41.4)	167	165 (55.7)	106	181 (54.7)	107	16		1.00 (ref.)	1.00 (ref.)
Ever	299 (58.6)	246	131 (42.3)	97	150 (45.3)	101	11		**1.35 (1.18-1.55)**	**1.19 (1.01-1.41)**
Pack-years smoked								1.29×10^{-6}		
0	221 (43.3)	167	166 (56.1)	107	181 (54.7)	107	16		1.00 (ref.)	1.00 (ref.)
<20	59 (11.6)	43	24 (8.1)	17	26 (7.8)	18	13		1.11 (0.87-1.42)	1.06 (0.81-1.39)

								P	HR (95% CI)	HR (95% CI)[a]
≥20	240 (47.1)	203	106 (35.8)	79	124 (34.5)	83	11		**1.18 (1.10-1.28)**	**1.09 (1.00-1.20)**
Drinking status								0.212		
Never	401 (78.6)	322	262 (88.5)	178	288 (87.0)	181	14		1.00 (ref.)	1.00 (ref.)
Ever	109 (21.4)	91	34 (11.5)	25	43 (13.0)	27	12		1.12 (0.93-1.34)	1.08 (0.89-1.32)
Surgery								2.58×10^{-15}		
No	334 (65.5)	289	162 (54.7)	133	191 (57.7)	138	12		1.00 (ref.)	1.00 (ref.)
Yes	176 (34.5)	124	134 (45.3)	70	140 (42.3)	70	18		**0.56(0.49-0.65)**	**0.57(0.49-0.66)**
Chemotherapy								**0.001**		
No	203 (39.8)	162	85 (28.7)	60	91 (27.5)	61	9		1.00 (ref.)	1.00 (ref.)
Yes	307 (60.2)	251	211 (71.3)	143	240 (72.5)	147	15		**0.78(0.68-0.91)**	**0.77(0.66-0.90)**
Radiotherapy								**0.001**		
No	274 (53.7)	218	118 (39.9)	82	153 (46.2)	86	10		1.00 (ref.)	1.00 (ref.)
Yes	236 (46.3)	195	178 (60.1)	121	178 (53.8)	122	15		**0.80(0.69-0.91)**	**0.86(0.75-0.99)**
Stages								6.58×10^{-10}		
I+ II	96 (18.8)	56	58 (19.6)	33	75 (22.7)	29	24		1.00 (ref.)	1.00 (ref.)
III	169 (33.1)	145	94 (31.8)	63	96 (49.0)	67	12		**1.85 (1.48-2.32)**	**1.76 (1.40-2.21)**
IV	245 (48.1)	212	144 (48.6)	107	160 (28.3)	112	11		**1.35 (1.22-1.51)**	**1.38 (1.23-1.53)**
Histological types								0.588		
Adenocarcinoma	179 (35.1)	144	132 (44.6)	89	148 (44.7)	91	13		1.00 (ref.)	1.00 (ref.)
Squamous cell carcinoma	164 (32.2)	133	95 (32.1)	65	104 (31.4)	66	15		1.01 (0.86-1.19)	0.92 (0.78-1.11)
Large cell carcinoma	24 (4.7)	17	17 (5.7)	12	19 (5.8)	12	11		0.99 (0.84-1.17)	0.99 (0.82-1.18)
Small cell lung cancer	62 (12.1)	52	39 (13.2)	30	42 (12.7)	31	13		1.05 (0.97-1.13)	1.04 (0.97-1.13)
Other [b]	81 (15.9)	67	13 (4.4)	7	18 (5.4)	8	15		0.97 (0.91-1.05)	0.96 (0.90-1.25)

Abbrevations: MST, median survival time; HR, hazard ratio;

[a] Adjusted in a multivariate Cox regression model that included age, sex, pre-existing COPD, smoking status, stages, surgery, chemotherapy, and radiotherapy status.

[b] Mixed-cell or undifferentiated carcinoma.

Table S3. Multivariate Cox Regression Analysis of Surrounding Factors and Lung Cancer Survival

Factors	HR (95%CI) [a]	COX mode *P* value
age	**1.25 (1.08-1.45)**	**0.003**
sex	0.92 (0.77-1.11)	0.390
Pre-existing COPD	1.12 (0.91-1.38)	0.288
Smoking status	**1.20 (1.02-1.43)**	**0.032**
Surgery	**0.57 (0.49-0.67)**	**2.38×10^{-12}**
Chemotherapy	**0.78 (0.67-0.90)**	**0.001**
Radiotherapy	**0.85 (0.74-0.99)**	**0.031**
Stages	**1.26 (1.17-1.37)**	**1.97×10^{-9}**
MAPKAPK2 g.CNV-30450 [a]	**1.49 (1.23-1.79)**	**3.63×10^{-5}**

[a] g.CNV-30450: 4 copies vs. 2/3 copies.

Table S4. The Primers Used for SYBR-Green Real-Time PCR

Gene	Primer
MAPKAPK2	5'-CAGCAGTTCCCGCAGTTC-3' (forward)
	5'-CGAATTTCTCCTGGGTCCTC-3' (reverse)
β-actin	5'-GGCGGCACCACCATGTACCCT-3' (forward)
	5'-AGGGGCCGGACTCGTCATACT-3' (reverse)