

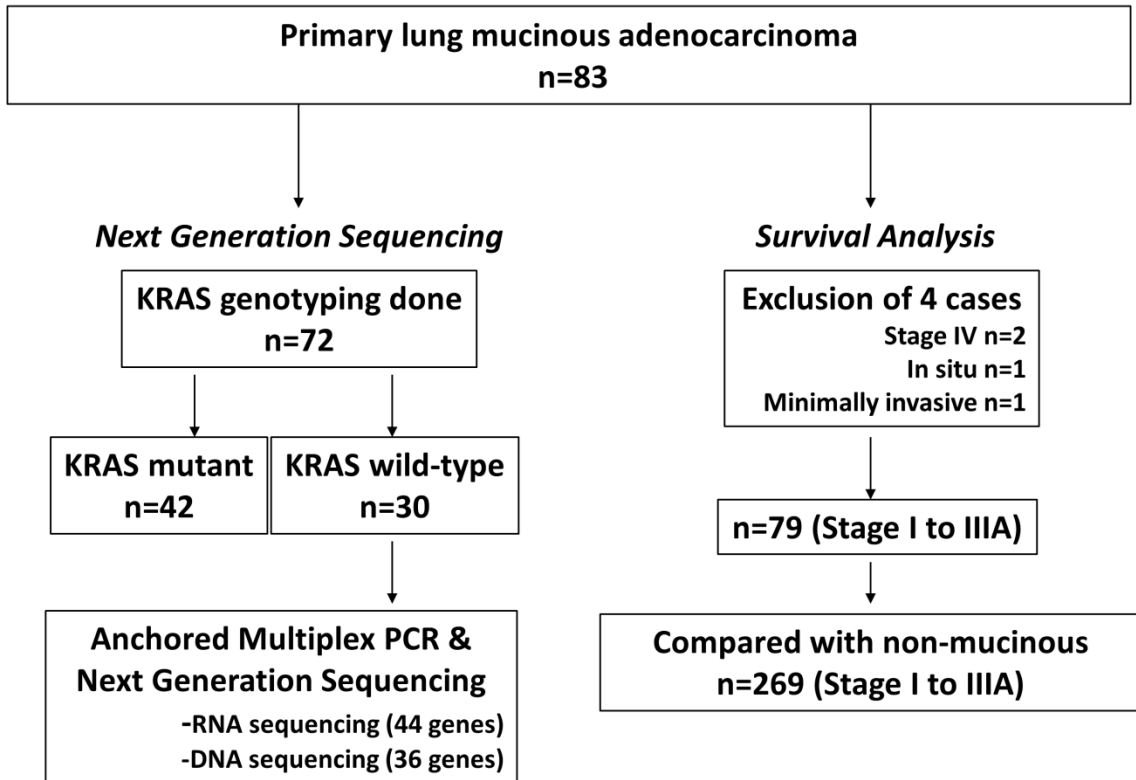
Supplementary Figures and Tables

Title: Unique genetic and survival characteristics of invasive mucinous adenocarcinoma of the lung

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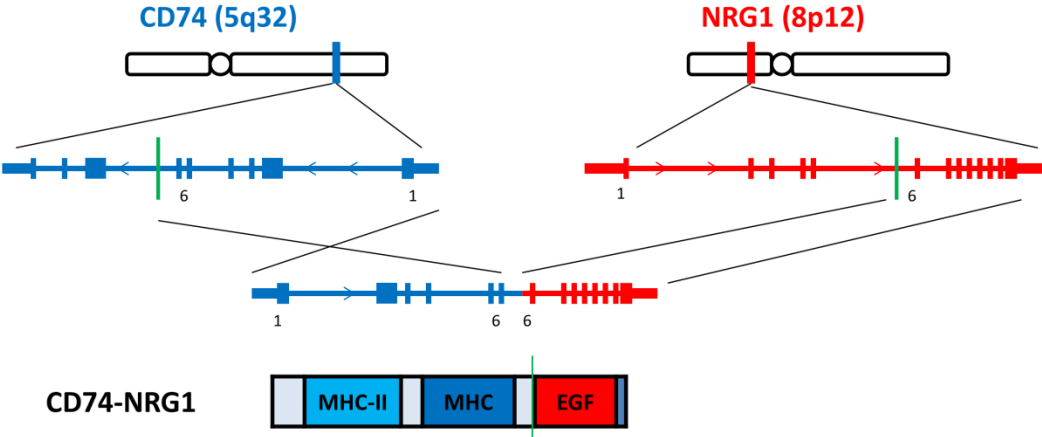
Supplementary Data	Title or caption
Supplementary Figure 1	Workflow diagram of cases enrolled in this study
Supplementary Figure 2	Schematic depiction of gene fusion involving <i>CD74</i> and <i>NRG1</i>
Supplementary Figure 3	Schematic depiction of gene fusion involving <i>VAMP2</i> and <i>NRG1</i>
Supplementary Figure 4	Schematic depiction of gene fusions involving <i>TRIM4</i> and <i>BRAF</i> , and <i>TPM3</i> and <i>NTRK1</i> with predicted mechanisms for each fusion
Supplementary Figure 5	Sequencing read pileup in the Integrative Genomics Viewer shows <i>TP53</i> mutations H179D (A) and C238Y (B)
Supplementary Figure 6	Representative images of p53 immunohistochemistry from <i>TP53</i> wild-type cases
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Supplementary Table 5	Comparison of mutational status between MGH-USA and Yonsei-Korea cohort
Supplementary Table 6	Case list of mucinous adenocarcinomas found in TCGA data
Supplementary Table 7	Univariate and multivariate analyses of contributing factors to a mutation burden

Supplementary Figure 1. Workflow diagram of cases enrolled in this study. Of 83 total mucinous adenocarcinomas 72 cases were examined for *KRAS* mutations, 30 cases were *KRAS* negative. For these cases, anchored multiplex PCR and next generation sequencing were performed. For survival analysis, a total of 79 mucinous cases were compared with a total of 269 non-mucinous adenocarcinomas.



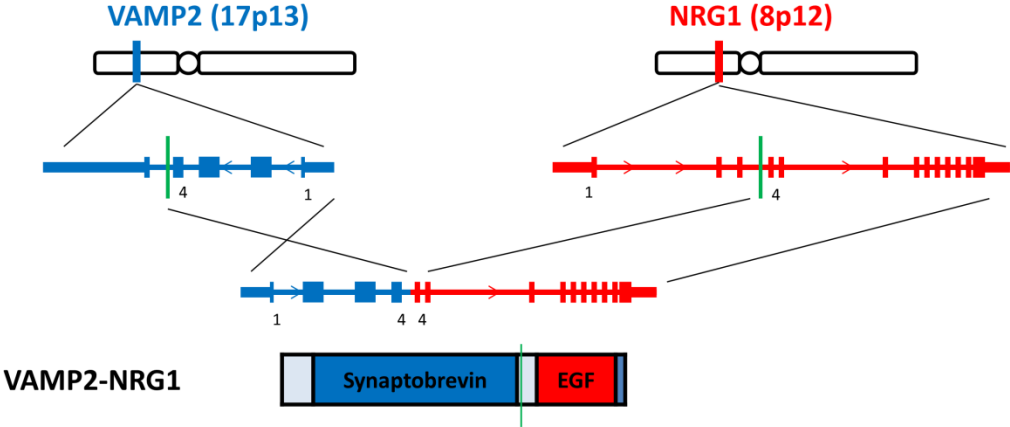
Supplementary Figure 2. Schematic depiction of gene fusion involving *CD74* and *NRG1*.

CD74-NRG1 fusion



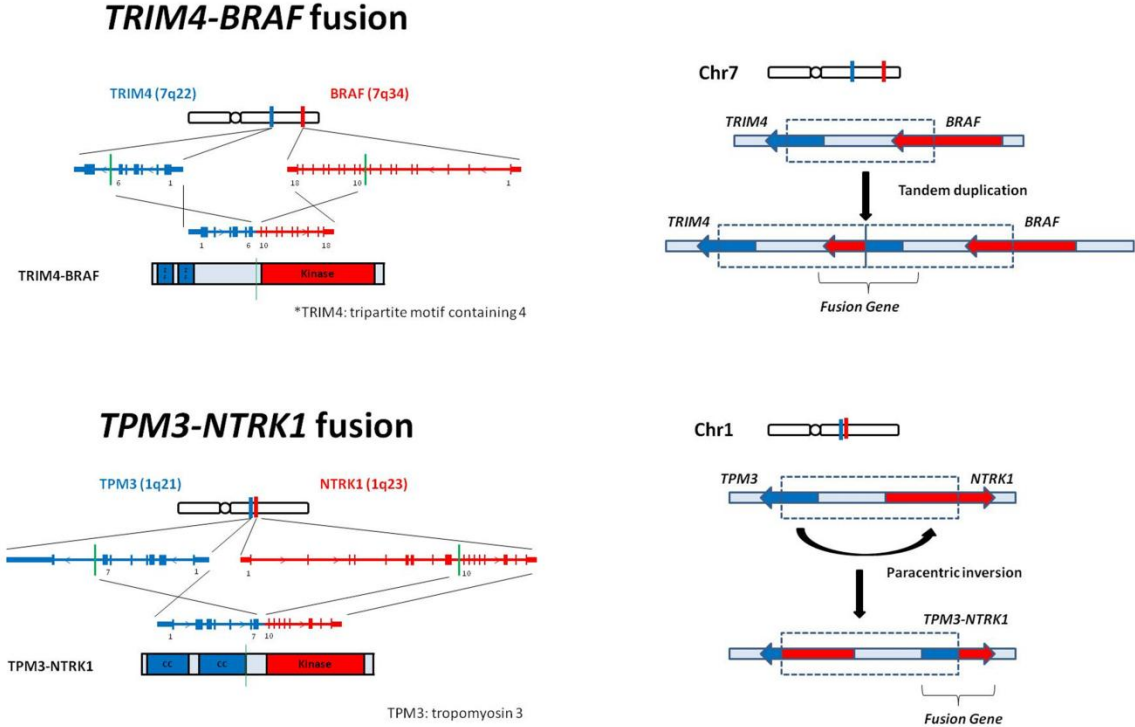
Supplementary Figure 3. Schematic depiction of gene fusion involving *VAMP2* and *NRG1*.

VAMP2-NRG1 fusion

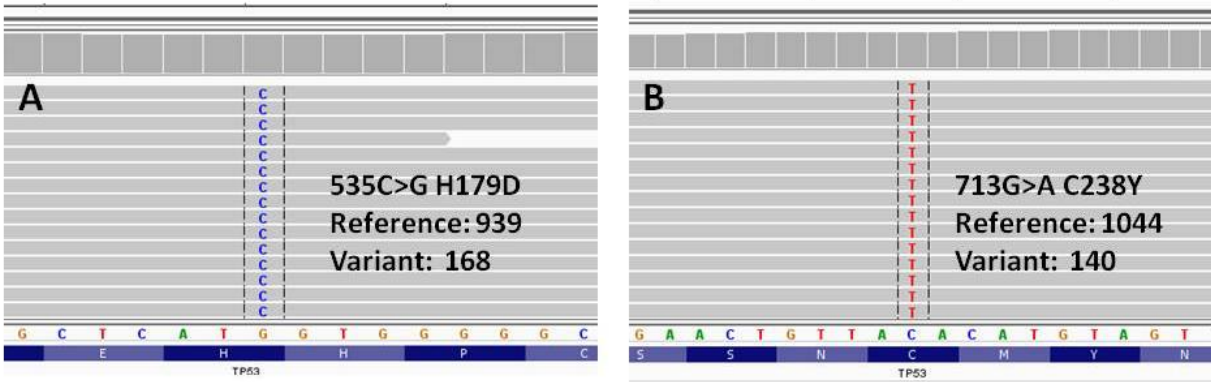


VAMP2: vesicle-associated membrane protein 2

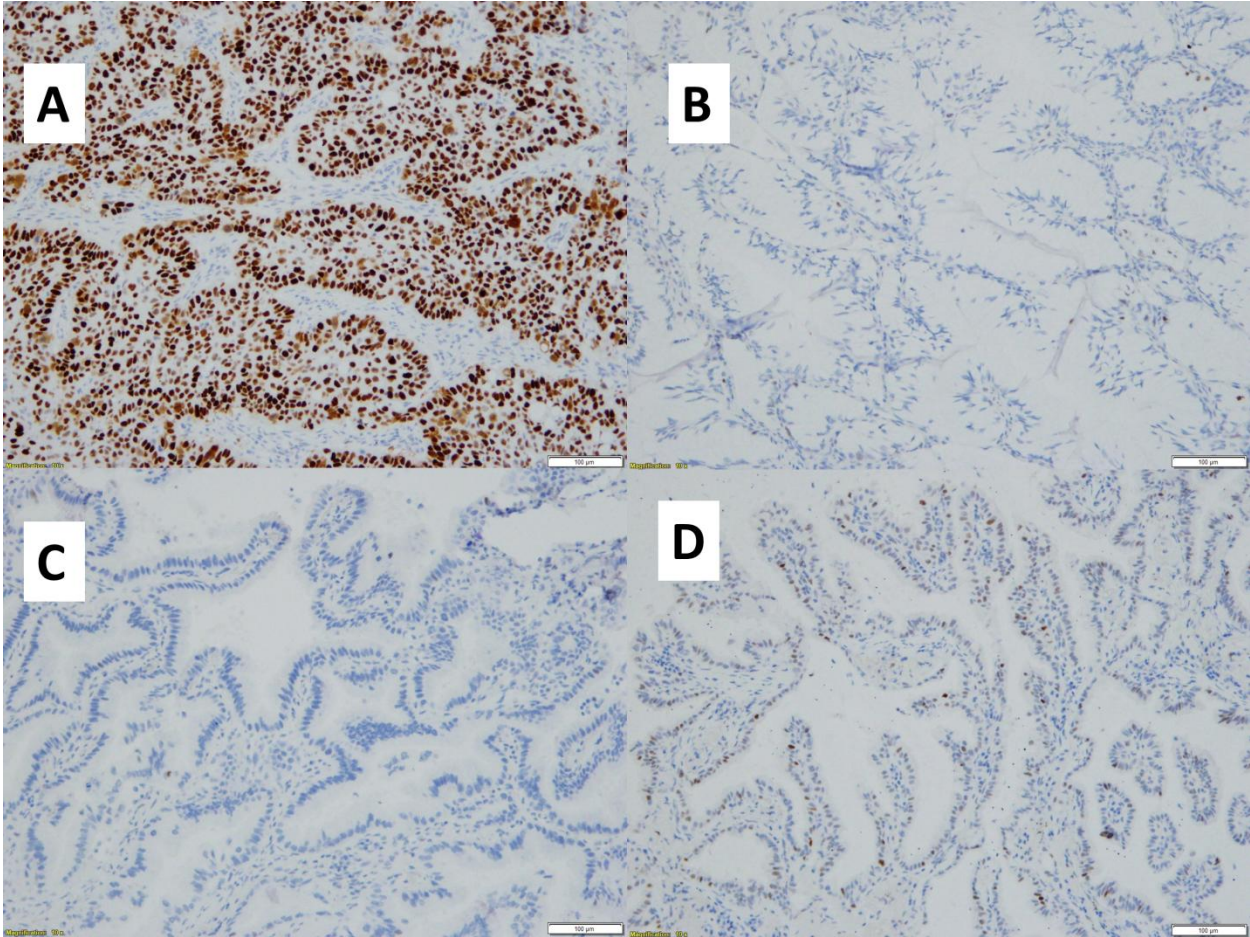
Supplementary Figure 4. Schematic depiction of gene fusions involving *TRIM4* and *BRAF*, and *TPM3* and *NTRK1* with predicted mechanisms for each fusion.



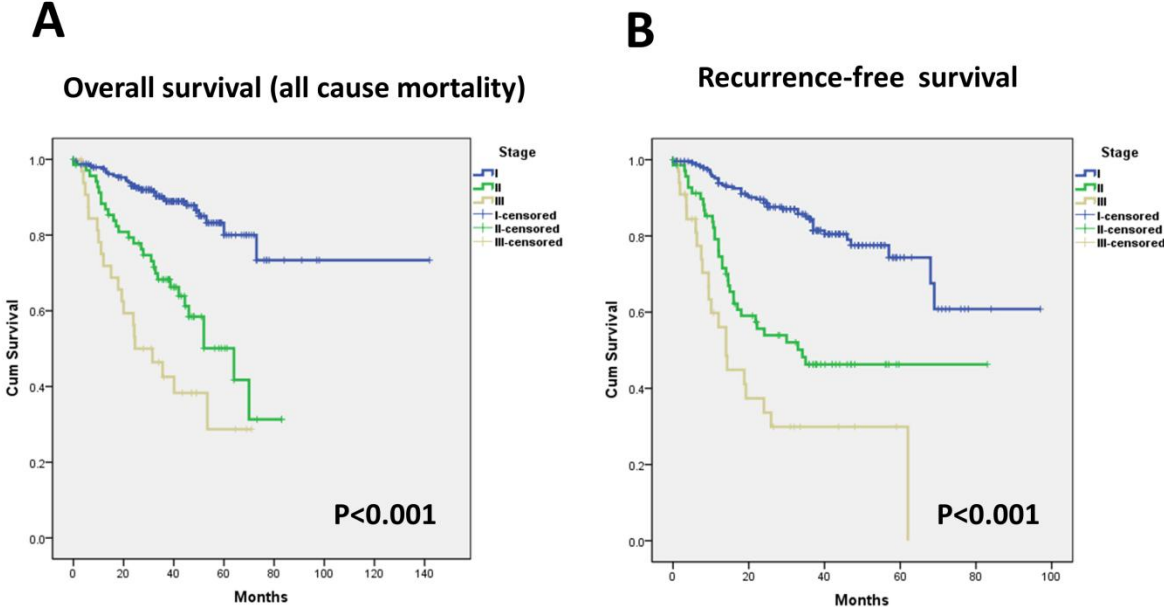
Supplementary Figure 5. Sequencing read pileup in the Integrative Genomics Viewer shows *TP53* mutations H179D (A) and C238Y (B).



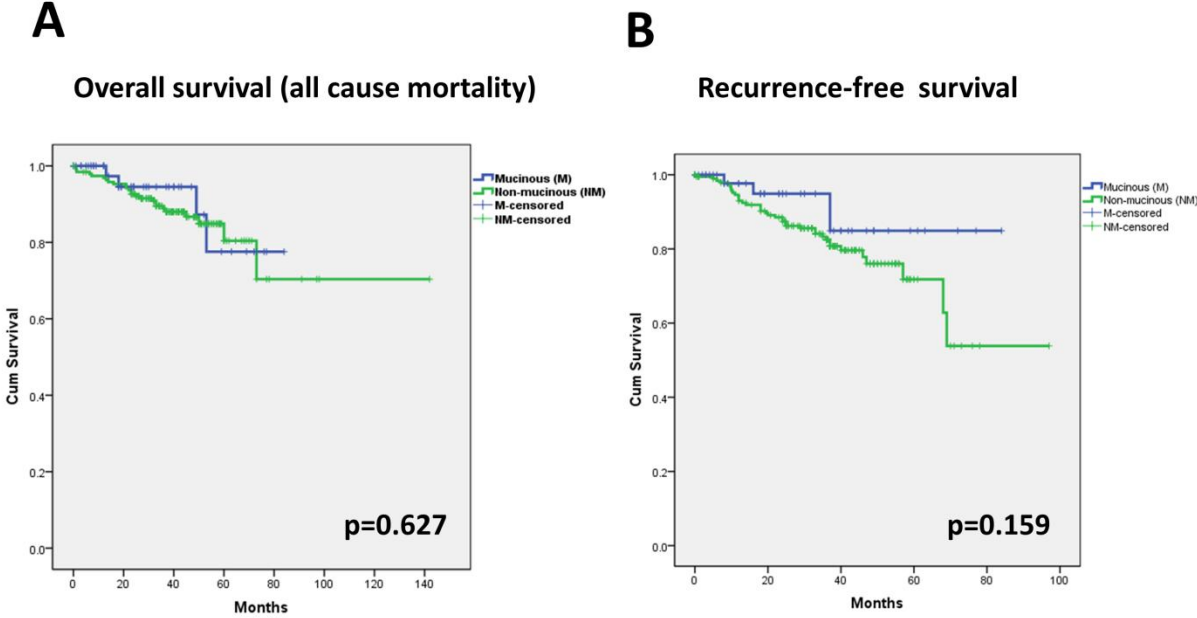
Supplementary Figure 6. Representative images of p53 immunohistochemistry from *TP53* wild-type cases. Control known-mutant case shows diffuse and strong staining (A). *TP53* wild-type cases show weak and focal (<50%) staining (B, C, and D).



Supplementary Figure 7. Survival curves of all patients for (A) overall and (B) recurrence-free survival according to stage.



Supplementary Figure 8. Survival curves comparing mucinous and non-mucinous cases for (A) overall and (B) recurrence-free survival in stage I only.



Supplementary Table 1. Gene lists and exons covered for each panel used for anchored multiplex PCR.

Gene Fusion Panel	Exons covered	Mutation Panel	Exons covered
ADCK4	1, 2, 4, 5, 6, 9, 10, 12, 13, 14, 15	AKT1	3
AKT3	1, 2, 3	ALK	22, 23, 25
ALK	1, 19, 20, 21, 22, 29	APC	16
AXL	14, 15	BRAF	11, 15
B2M	2	CDH1	1 ~ 16
BRAF	1, 8, 9, 10, 11, 17	CDKN2A	1, 2, 3
BRD4	1, 10, 11, 12	CTNNB1	3
CCDC6	1 ~ 8	DDR2	12, 13, 14, 15, 16, 17, 18
CD74	1 ~ 8	EGFR	7, 15, 18, 19, 20, 21
CHTOP	2, 3, 4, 5, 6	ERBB2	20
CTBP1	6	FBXW7	1 ~ 11
EGFR	7 ~ 9, 14 ~ 18, 23 ~ 28	FGFR2	7, 9, 12, 14
ERBB2	2, 3, 4	FGFR3	7, 8, 9, 14, 16
ERBB4	17, 18, 20	FOXL2	1
EWSR1	1, 3 ~ 8, 12, 13	GNA11	5
FGFR1	1, 7 ~ 13, 15 ~ 18	GNAQ	4, 5
FGFR2	3, 4, 17	GNAS	6, 7, 8, 9
FGFR3	3, 7 ~ 12, 15 ~ 18	IDH1	3, 4
GAPDH	6	IDH2	4
INSR	13 ~ 18, 21, 22	KIT	8, 9, 11, 17
INSRR	13 ~ 18, 21, 22	KRAS	2, 3, 4, 5
JAK1	1 ~ 25	MAP2K1	2, 3
JAK2	1, 6, 9, 11, 12, 16, 17, 19, 24	MET	19
MAST1	2, 8, 19, 20, 26	NOTCH1	25, 26, 34
MAST2	1, 5, 29	NRAS	2, 3, 4, 5
MET	11 ~ 16, 20, 21	PDGFRA	18
MUSK	8, 9, 11, 12, 13, 14	PIK3CA	2, 5, 10, 21
NFIB	1, 7, 8, 9	PIK3R1	1 ~ 10
NOTCH1	2, 27, 28, 34	PTEN	1 ~ 9
NOTCH2	1, 27, 33	RET	11, 16
NRG1	2, 3, 4, 6	ROS1	38
NTRK1	1, 8 ~ 17	SMAD4	2 ~ 12
NTRK2	9, 10, 11, 13 ~ 20	SMO	9
NTRK3	1, 11 ~ 16, 18, 19	STK11	1 ~ 9
NUMBL	3, 4, 5, 6, 7, 9, 10	TP53	1 ~ 11
PDGFB	1, 2, 6	VHL	1, 2, 3
PDGFRA	1, 9, 10, 11, 13, 14, 20 ~ 23		
PPARG	3 ~ 8		
PRKACA	2, 3, 4		
RAF1	1, 17		
RET	1, 7, 8, 9, 10, 11, 12, 13, 19		
RHOA	1 ~ 5		
ROS1	1, 31 ~ 37, 43		
TMPRSS2	1 ~ 5		

Supplementary Table 2. Clinical features according to the two institutions

Histology		MGH-USA (n=98)	Yonsei-Korea (n=254)	p-value
Mean age (range)		63.9 (36-87)	62.0 (34-90)	0.146
Gender				
	Male	37 (37.8)	129 (50.8)	0.028
	Female	61 (62.2)	125 (49.2)	
Smoking status				
	Never	19 (19.4)	157 (61.8)	<0.001
	Ever	79 (80.6)	97 (38.2)	
Stage				
	I	62 (63.3)	185 (72.8)	0.218
	II	26 (26.5)	44 (17.3)	
	III	9 (9.2)	24 (9.4)	
	IV	1 (1.0)	1 (0.4)	

Supplementary Table 3. Sensitivity according to sequencing methods reported in the literatures

Method	Sanger sequencing	SNaPSHOT	Next generation sequencing
Sensitivity	25% ¹	approximately 5% ²	~0.02% ³

References:

1. Pao W, Ladanyi M. Epidermal growth factor receptor mutation testing in lung cancer: searching for the ideal method. *Clin Cancer Res* 2007;13:4954-4955.
2. Dias-Santagata D, Akhavanfard S, David SS, et al. Rapid targeted mutational analysis of human tumours: a clinical platform to guide personalized cancer medicine. *EMBO Mol Med* 2010;2:146-158.
3. Newman AM, Bratman SV, To J, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med* 2014;20:548-554.

Supplementary Table 4. Detailed information of three discrepant cases for *KRAS* mutation

Sample	No 1.	No 2.	No 3.
The previous sequencing method	Sanger sequencing	Sanger sequencing	SNaPSHOT
DNA extraction method	Previously extracted by DNeasy DNA isolation kits (Qiagen, Valencia, CA). Reextracted by Agencourt FormaPure Kit (Beckman Coulter, Indianapolis, IN).	Previously extracted by DNeasy DNA isolation kits (Qiagen, Valencia, CA). Reextracted by Agencourt FormaPure Kit (Beckman Coulter, Indianapolis, IN).	Same (by Agencourt FormaPure Kit (Beckman Coulter, Indianapolis, IN))
The allelic frequency of <i>KRAS</i> mutation in NGS	9.16%	6.33%	5.67%
The estimated tumor cellularity	55%	50%	30%

Supplementary Table 5. Comparison of mutational status between MGH-USA and Yonsei-Korea cohort

Factors	MGH-USA (n=31)	Yonsei-Korea (n=41)	P value
<i>KRAS</i> mutation	21 (67.7)	24 (58.5)	0.424
No <i>KRAS</i> mutation	10 (32.3)	17 (41.5)	
Fusion-positive	2 (6.5)	7 (17.1)	0.177
Fusion-negative	29 (93.5)	34 (82.9)	

Supplementary Table 6. Case list of mucinous adenocarcinomas found in TCGA data.

CASE ID	Age	Gender	Drivers	TP53	Smoking
TCGA-80-5608	[Not Available]	FEMALE	KRAS G12A	No	Current smoker
TCGA-05-4403	76	MALE	KRAS G12C	No	Current reformed smoker for > 15 years
TCGA-50-5936	58	MALE	KRAS G12C	No	[Not Available]
TCGA-55-6983	81	MALE	KRAS G12C	No	Current reformed smoker for < or = 15 years
TCGA-55-7728	64	FEMALE	KRAS G12V	No	Current reformed smoker for < or = 15 years
TCGA-05-4433	82	MALE	KRAS G12V	No	Current reformed smoker for > 15 years
TCGA-44-6146	64	MALE	KRAS G12V	No	Current reformed smoker for > 15 years
TCGA-97-7552	70	MALE	unknown	No	Current reformed smoker for > 15 years
TCGA-78-7163	60	MALE	ALK fusion	No	Current reformed smoker for < or = 15 years
TCGA-78-7540	66	FEMALE	KRAS G12V	R273C	Lifelong Non-smoker
TCGA-75-7030	[Not Available]	MALE	KRAS G12D	No	Lifelong Non-smoker
TCGA-55-6543	60	FEMALE	RET fusion	No	Current reformed smoker for < or = 15 years

Supplementary Table 7. Univariate and multivariate analyses of contributing factors to a mutation burden

Analysis	Contributing factor	p value
Univariate	Mucinous histology	0.0099
Univariate	<i>TP53</i> status	<0.0001
Univariate	Smoking status	<0.0001
After adjustment for <i>TP53</i> status	Mucinous histology	0.0858*
After adjustment for smoking status	Mucinous histology	0.0112**
Multivariate	Mucinous histology	0.0725
Multivariate	<i>TP53</i> status	<0.0001
Multivariate	Smoking status	0.0003

*This indicates that mucinous histology depends on *TP53* status for a mutation burden.

**This indicates that mucinous histology does not depend on smoking status a mutation burden.