

## **Supporting Information**

# **Isolation and Retrieval of Extracellular Vesicles for Liquid Biopsy of Malignant Ground-Glass Opacity**

Wenjun Mao<sup>1,†</sup>, Yi Wen<sup>2,†</sup>, Haozhi Lei<sup>3,†</sup>, Rongguo Lu<sup>1</sup>, Shengfei Wang<sup>1</sup>, Yuheng Wang<sup>3</sup>, Ruo Chen<sup>1</sup>, Yuanyuan Gu<sup>3</sup>, Lin Zhu<sup>3</sup>, Komal K. Abhange<sup>2</sup>, Zachary J. Quinn<sup>2</sup>, Yundi Chen<sup>2</sup>, Fei Xue<sup>2</sup>, Mingfeng Zheng<sup>1,\*</sup>, and Yuan Wan<sup>2,\*</sup>

<sup>1</sup>Department of Cardiothoracic Surgery, Wuxi People's Hospital Affiliated to Nanjing Medical University, Wuxi, Jiangsu 214023, China

<sup>2</sup>The Pq Laboratory of Micro/Nano BiomeDx, Department of Biomedical Engineering, Binghamton University-SUNY, Binghamton, NY 13902, USA

<sup>3</sup>PerMed Biomedicine Institute, Shanghai, 201203, China

† Equal contribution

\* Tel: +1 607-777-5477, Fax: +1 607-777-5780, Email: ywan@binghamton.edu; Tel: +86-13003305673, Email: zhengmfmedical@126.com

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**Table S1.** Sequence quality control and coverage

Patient	Target	All reads	>=Q30 (%)	GC (%)	Mapped reads	Mapped reads (%)	Read and mate paired	Read and mate paired (%)	Target Reads	Target Reads in all reads ( % )	[Target] Coverage (>=30x) (%)	Average depth (rmdup)
1	Tissue DNA	47863558	95.70%	50.00%	47719260	99.70%	47713558	99.69%	42316077	88.41%	99.61%	1495.64
	EV DNA	221389333	95.21%	48.16%	220938318	99.80%	220905400	99.78%	181475531	81.97%	99.80%	170.68
2	Tissue DNA	44516976	95.52%	49.15%	44345894	99.62%	44339876	99.60%	34680343	77.90%	99.45%	1222.34
	EV DNA	126929054	95.76%	45.02%	126670453	99.80%	126649556	99.78%	102009669	80.37%	99.66%	87.67
3	Tissue DNA	47454052	95.54%	51.22%	47328573	99.74%	47323100	99.72%	41967067	88.44%	99.43%	2054.63
	EV DNA	174646298	95.98%	43.72%	174281599	99.79%	174256156	99.78%	138721993	79.43%	99.71%	487.01
4	Tissue DNA	39493041	94.91%	51.38%	39232000	99.34%	39224864	99.32%	33661921	85.24%	99.13%	1426.18
	EV DNA	206415270	96.15%	43.80%	206156134	99.87%	206124836	99.86%	172986051	83.80%	99.77%	153.74
5	Tissue DNA	52140416	95.25%	51.72%	52071842	99.87%	52070740	99.87%	44099146	84.58%	99.34%	1092.52
	EV DNA	195568684	96.39%	47.50%	195438340	99.93%	195433230	99.93%	173843195	88.89%	99.73%	525.58
6	Tissue DNA	50910449	96.05%	49.02%	50764291	99.71%	50763400	99.71%	42628280	83.73%	99.55%	1055.58
	EV DNA	198000889	96.30%	47.24%	197859730	99.93%	197854064	99.93%	177272106	89.53%	99.81%	399.19
7	Tissue DNA	83657054	95.76%	49.49%	83365582	99.65%	83362354	99.65%	73415622	87.76%	99.71%	1036.62
	EV DNA	119593616	95.43%	41.43%	119016121	99.52%	119003034	99.51%	34361388	28.73%	79.04%	137.45
8	Tissue DNA	67941910	94.90%	51.60%	67389421	99.19%	67381554	99.18%	59534929	87.63%	99.43%	1710.69
	EV DNA	332663780	95.54%	48.08%	332187536	99.86%	332145580	99.84%	272902275	82.04%	99.77%	123.74

**Table S2.** Comparison key mutations in lung adenocarcinoma between 3 smokers and 5 never smokers

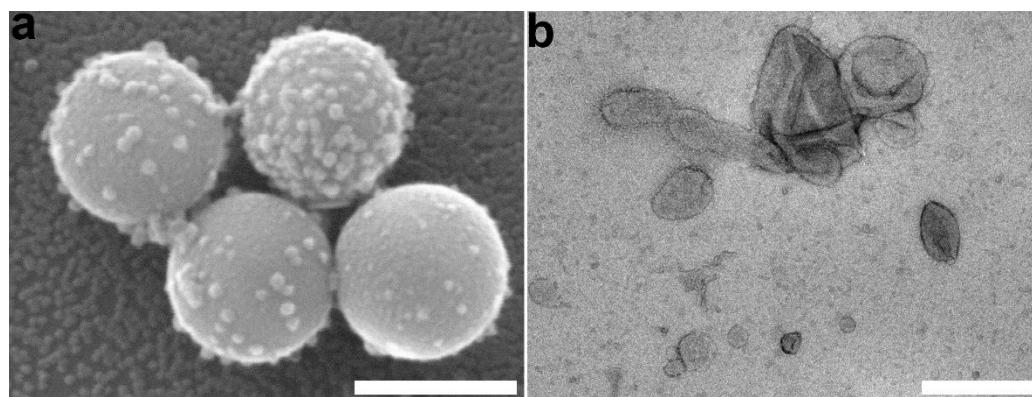
Mean ( <b>KRAS, STK11, TP53</b> ) Mutations in EV-DNA	6.0/Patient (18/3 Patients)	4.4/Patient (22/5 Patients)
Mean ( <b>KRAS, STK11, TP53</b> ) Mutations in Tissue-DNA	2.3/Patient (7/3 Patients)	1.2/Patient (6/5 Patients)
Mean ( <b>KRAS</b> ) Mutaton Number in EV-DNA	0/Patient (0/3 Patients)	0.4/Patient (2/5 Patients)
Mean ( <b>KRAS</b> ) Mutation Number in Tissue-DNA	1/Patient (3/3 Patients)	0.4/Patient (2/5 Patients)
Mean ( <b>STK11</b> ) Mutation Number in EV-DNA	2.3/Patient (7/3 Patients)	0.6/Patient (3/5 Patients)
Mean ( <b>STK11</b> ) Mutation Number in Tissue-DNA	0.3/Patient (1/3 Patients)	0/Patient (0/5 Patients)
Mean ( <b>TP53</b> ) Mutation Number in EV-DNA	3.7/Patient (11/3 Patients)	3.4/Patient (17/5 Patients)
Mean ( <b>TP53</b> ) Mutation Number in Tissue-DNA	1/Patient (3/3 Patients)	0.8/Patient (4/5 Patients)
Mean ( <b>EGFR</b> ) Mutation Number in EV-DNA	2.0/Patient (6/3 Patients)	2.0/Patient (10/5 Patients)
Mean ( <b>EGFR</b> ) Mutation Number in Tissue-DNA	0/Patient (0/3 Patietns)	1.0/Patient (5/5 Patients)
Mean ( <b>KRAS, STK11, TP53</b> ) Fraction in EV Samples	55.6% (5/9 Samples)	53.3% (8/15 Samples)
Mean ( <b>KRAS, STK11, TP53</b> ) Fraction in Tissue Samples	44.4% (4/9 Samples)	33.3% (5/15 Samples)
Mean ( <b>KRAS</b> ) Fraction in EV Samples	0 (0/3 Samples)	40% (2/5 Samples)
Mean ( <b>KRAS</b> ) Fraction in Tissue Samples	33.3% (1/3 Samples)	40% (2/5 Samples)
Mean ( <b>STK11</b> ) Fraction in EV Samples	66.7% (2/3 Samples)	40% (2/5 Samples)
Mean ( <b>STK11</b> ) Fraction in Tissue Samples	33.3% (1/3 Samples)	0 (0/5 Samples)
Mean ( <b>TP53</b> ) Fraction in EV Samples	100% (3/3 Samples)	80% (4/5 Samples)
Mean ( <b>TP53</b> ) Fraction in Tissue Samples	66.7% (2/3 Samples)	60% (3/5 Samples)
Mean ( <b>EGFR</b> ) Fraction in EV Samples	100% (3/3 Samples)	80% (4/5 Samples)
Mean ( <b>EGFR</b> ) Fraction in Tissue Samples	0 (0/3 Samples)	40% (2/5 Samples)

**Table S3.** Mutation counts in respective EV DNA and tissue DNA

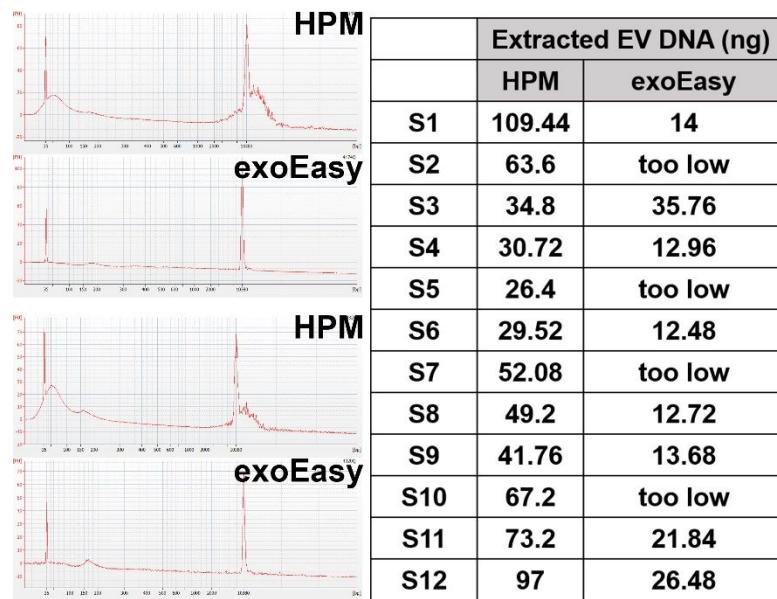
	Never Smoker		Never Smoker		Never Smoker		Smoker		Smoker		Never Smoker		Smoker		Never Smoker		
	P1 EV DNA	P1 Tissue DNA	P2 EV DNA	P2 Tissue DNA	P3 EV DNA	P3 Tissue DNA	P4 EV DNA	P4 Tissue DNA	P5 EV DNA	P5 Tissue DNA	P6 EV DNA	P6 Tissue DNA	P7 EV DNA	P7 Tissue DNA	P8 EV DNA	P8 Tissue DNA	
<b>EGFR</b>	5			1	4	2		3		1				2		2	1
<b>BRAF</b>				1		1			3		1		1	1		1	
<b>KRAS</b>		1	1			1		3							1		
<b>PIK3CA</b>		1	1		1	2											1
<b>TP53</b>	3	1	7	2	5		9	1	1	2			1		2	1	
<b>NF1</b>	2			5	2	5	1	6	3		1		1		1		1
<b>STK11</b>						2		6			1			1		1	
<b>CDKN2A</b>						1		1	1				1				

**Table S4.** Detected DNA mutations involved in LUAD evolution

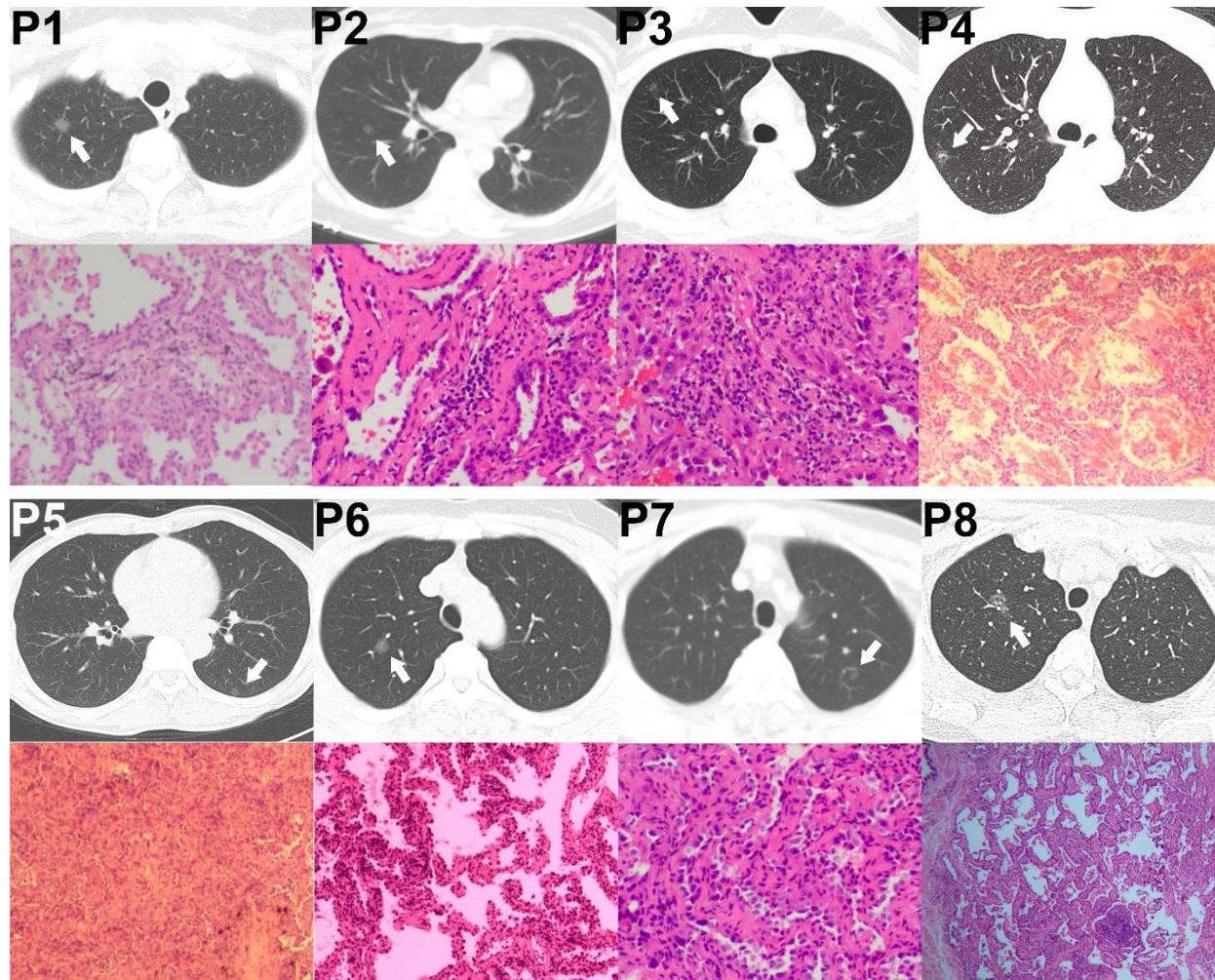
		P1		P2		P3		P4		P5		P6		P7		P8		
		EV	Tissue															
Initiating Drivers	ALK			1				2								1		
	BRAF			1		1			3		1		1		1		1	
	EGFR	5		1	4	2			3		1			2		2	1	
	ERBB2							1	1									
	KRAS		1	1			1		3						1			
	PRDM1																	
	TSC2	3	3	2		1		2	1			1	1		4			
	PHOX2B															1		
	FBXW7					1			1		1	1		1				
	CHEK2		2	4		2	2	1	2		2			2		2		
	APC	1	1	2	1	3	2	3	7						1	3		
	BCOR	1																
	TP53	3	1	7	2	5		9	1	1	2			1		2	1	
	FAT1																	
Clonal/Subclonal Drivers	ATM	5	3	4	2	7	2	4	9			1				1	3	
	ARID1B			2		2		1				1	1					
	KDM5C			1				2										
	ATRX	1	1			1												
	RB1							1	2						1	2		
	SMARCA4	1													1			
	NRAS	1		1									2					
	NOTCH2																	
	WRN																	
	RASA1			1	1		1	1										
	CREBBP			1	1	3		1	2	1					4	1		
	FUBP1																	
	MGA																	
	NF1	2		5	2	5	1	6	3		1		1		1		1	
	PIK3CA		1	1		1	2										1	
	KMT2D	4	1	2		9	2	4	1	1	1	1			3	2	1	
	CTNNB1				1			1					1					
	STK11					2		6			1			1		1		
Late Drivers	KMT2C																	
	SMAD4			2				1	1									
	NOTCH1					3		1					1					
	FLT4			1				1										
	EP300					3									1		1	
	CIC					1		1	1									



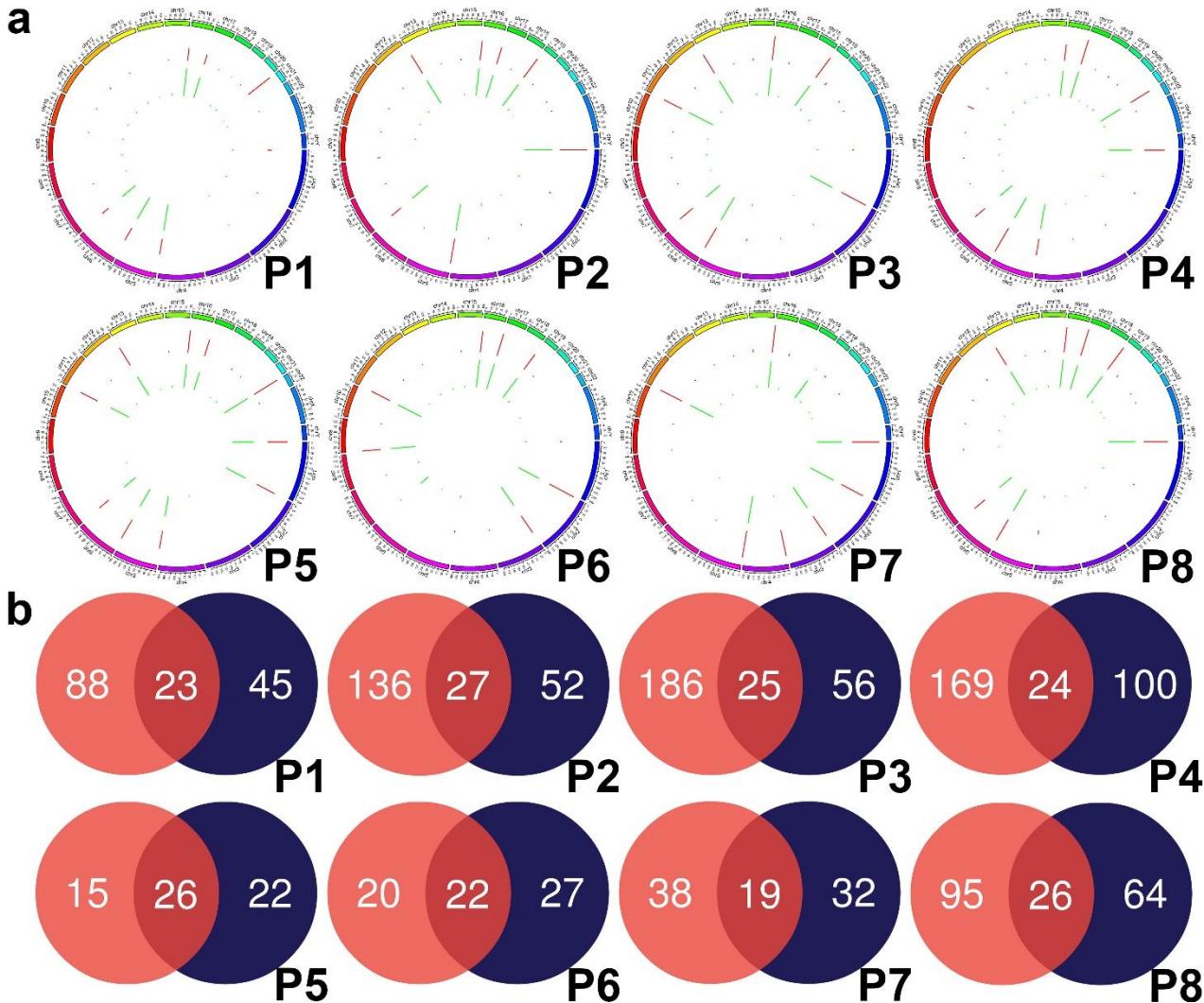
**Figure S1.** Morphological characterization of isolated and retrieved EVs. a, SEM image showing the EVs are enriched on the surface of HPMs (scale bar is 1  $\mu$ m). b, TEM image showing the retrieved EVs (scale bar is 200 nm).



**Figure S2.** Measurement of extracted EV-DNA from EVs isolated by HPM and commercialized exoEasy Maxi Kit (Qiagen), respectively. DNA analysis using Bioanalyzer 2100 shows EV-DNA in HPM group contains DNA fragments longer than 10 kbp, whereas EV-DNA in exoEasy group only contains short DNA fragments. The total mass of EV-DNA in HPM groups was much higher than that of exoEasy group ( $t$ -test,  $p<0.0005$ ).



**Figure S3.** LDCT images of patients with GGO and paired postoperative immunohistochemical staining of resected tumor tissues. The location of GGO in each LDCT image is indicated with a white arrow. The resected tumor tissues were routinely stained with H&E for an examination of the tissue architecture. In few cases, immunostaining was performed to confirm the findings. P1: adenocarcinoma in situ. P2: adenocarcinoma in situ with local microinvasion, immunostaining shows TTF-1 (+), CK7 (+), NapsinA (+), SP-B (+), Ki-67 (2% +), EGFR (+), ALK (-), CgA (-), CK5/6 (-), and P63 (+). P3: minimal invasive adenocarcinoma, immunostaining shows TTF-1 (+), CK7 (+), NapsinA (+), SP-B (+), Ki-67 (5% +), EGFR (+), ALK (+), CgA (-), CK5/6 (-), and P63 (-). P4: adenocarcinoma in situ with local microinvasion. P5: adenocarcinoma in situ with local microinvasion. P6: adenocarcinoma in situ with local microinvasion. P7: adenocarcinoma in situ with local microinvasion, immunostaining shows TTF-1 (+), CK7 (+), NapsinA (+), SP-B (+), Ki-67 (2% +), EGFR (+), ALK (-), CgA (-), CK5/6 (-), and P63 (-). P8: adenocarcinoma in situ.



**Figure S4.** Mutation analysis of EV-DNA and paired tissue-DNA. a, Circular plot illustrating gene mutations in tissue-DNA (innermost ring) and EV-DNA (second inner-most ring). b, Venn diagram of the overlapped mutations between EV-DNA and tissue-DNA.