

Supplementary Information

BCAP31, a cancer/testis antigen-like protein, can act as a probe for non-small-cell lung cancer metastasis

Author:

Jing Wang⁺¹, Dongbo Jiang⁺¹, Zichao Li⁺¹, Shuya Yang⁺¹, Jiayi Zhou¹, Guanwen Zhang¹, Zixin Zhang¹, Yuanjie Sun¹, Zhipei Zhang², Xiaofei Li², Liang Tao¹, Jingqi Shi¹, Yuchen Lu¹, Lianhe Zheng³, Chaojun Song^{*1}, Kun Yang^{*1}

+Co-first authors. These authors contributed equally to this work.

*Corresponding author

Affiliations:

1 Department of Immunology; 2 Department of Thoracic Surgery, Tangdu Hospital; 3 Department of Orthopedics, Tangdu Hospital; the Fourth Military Medical University, No.169, Changle W. Rd., Xi'an 710032, China

List of supplementary material:

1 Supplementary Figure S1. BCAP31 mRNA expression had no effect on NSCLC prognosis.

2 Supplementary Figure S2. B cell receptor-associated protein 31 (BCAP31) did not significantly affect proliferation, cell cycle and apoptosis in three NSCLC cell types.

3 Supplementary Table S1. Seven transcript expression microarray datasets extracted from the Gene Expression Omnibus (GEO).

4 Supplementary Table S2. GSEA reports of BCAP31-associated molecules, biological processes and pathways (sheets 1–4) and label free MS of analysis for mechanism exploration demonstrated the involvement of BCAP31 in primitive functions (sheets 5–6).

5 Supplementary Video S1- S23. The HoloMonitor M4 recorded cell states by real-time monitoring. The videos from S1-S23 were respectively for A549, AN, AS, AN', AO, ALN, ALS, ALN', ALO, PLA-801D, PN, PS, PN', PO, PLN, PLS, PLN', PLO, GLC-82, GN, GS, GN', GO.

Legends:

Supplementary Figure S1.

BCAP31 mRNA expression had no effect on NSCLC prognosis.

A meta-analysis of the relationship between BCAP31 and patients, and the overall survival curve. Both showed that BCAP31 was not related to NSCLC patient survival at the mRNA level.

Supplementary Figure S2.

BCAP31 did not significantly affect proliferation, cell cycle, and apoptosis in three NSCLC cell types.

(A) GSEA database analysis of the BCAP31 relationship with cell cycle and apoptosis.

38 **(B)** Flow cytometry images of cell cycle at 24 h, 48 h, and 72 h after BCAP31 expression was
39 changed using transient transfection. Data were analyzed using a *t* test in each group, and the
40 results were not statistically significant.

41 **(C)** Flow cytometry images of cell apoptosis at 24 h, 48 h, and 72 h after BCAP31 expression
42 was knocked down using transient transfection. Data were analyzed using a *t* test in each group,
43 and the results were not statistically significant.

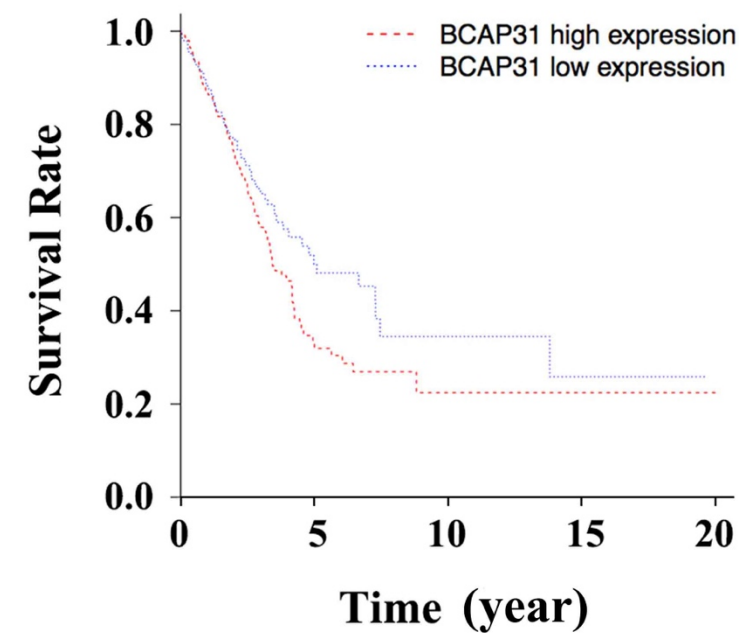
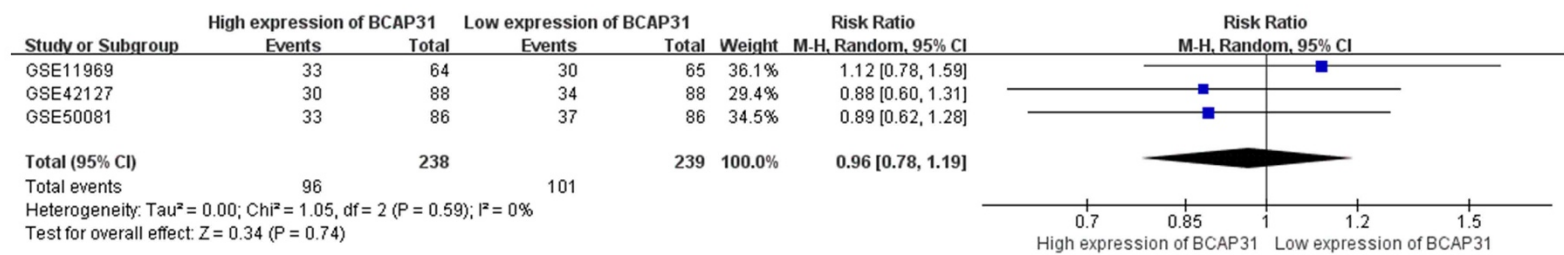
44 **(D)** After transient transfections, clone formation assays of three NSCLC cell types were
45 performed, using 100, 200, and 400 cells seeded in each well of a 6-well plate. Means were
46 calculated and analyzed using a *t* test in each group, and the results were not statistically
47 significant.

48 **(E)** Cell proliferation (non-transfected and transiently transfected cells) was recorded using an
49 xCELLigence RTCA DP instrument and showed a similar trend as the clone formation assays.

50 **(F)** In a HoloMonitor M4 irregularity recording of NSCLC cells, which represents cell activity,
51 each cell strain had an stable level of irregularity showing a good condition.

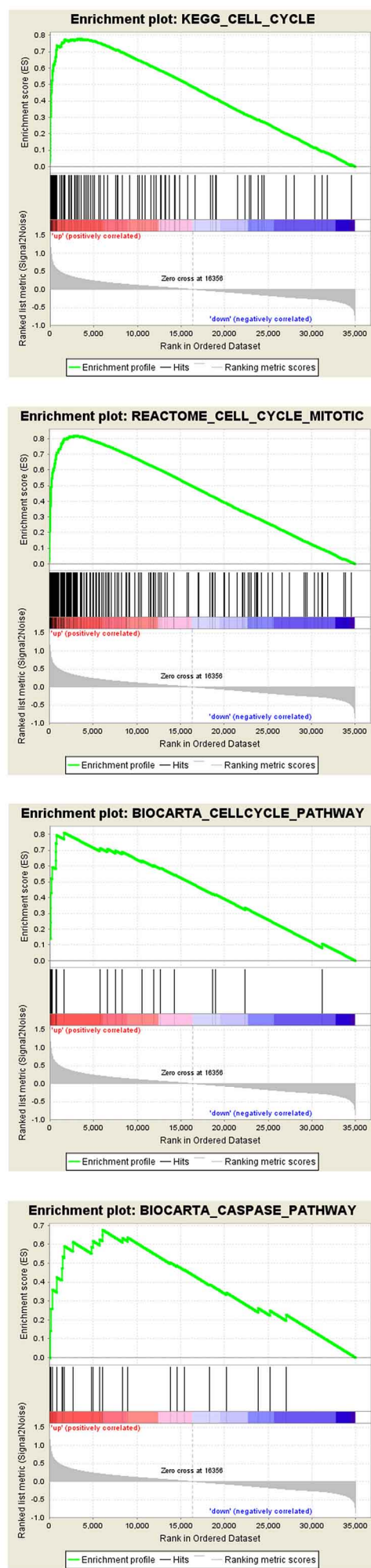
52 **(G)** In a HoloMonitor M4 optical thickness recording of NSCLC cells, the results were
53 irregular in different cell lines.

54 AN, PN, GN: control groups of decreased BCAP31 expression with transient transfection in
55 three cell lines; AS, PS, GS: experimental groups of decreased BCAP31 expression with
56 transient transfection in three cell lines; AN', PN', GN': control groups of increased BCAP31
57 expression with transient transfection in three cell lines; AO, PO, GO: experimental groups of
58 increased BCAP31 expression with transient transfection in three cell lines. All experiments
59 were repeated at least three times.

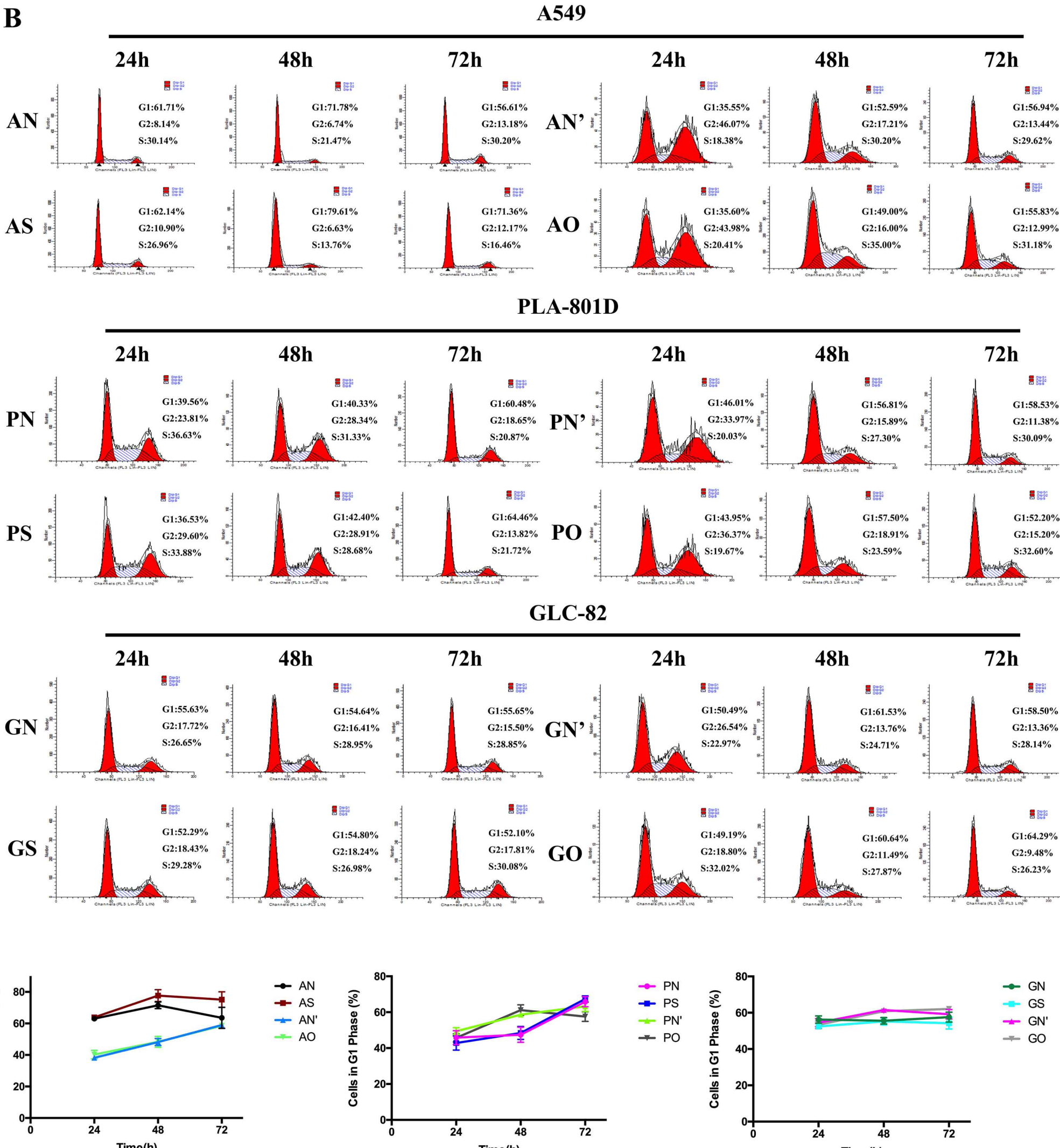


Supplementary Figure S1

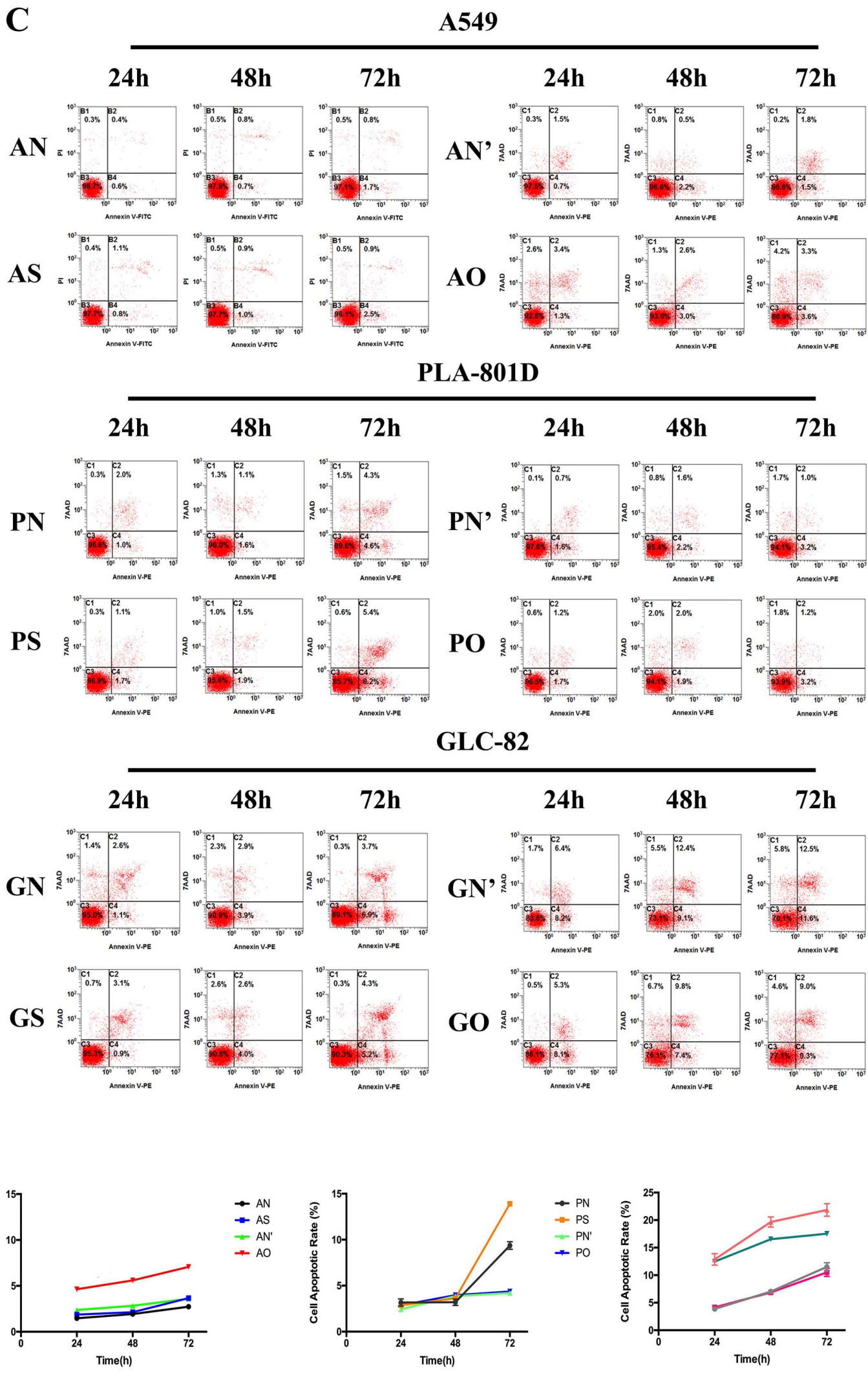
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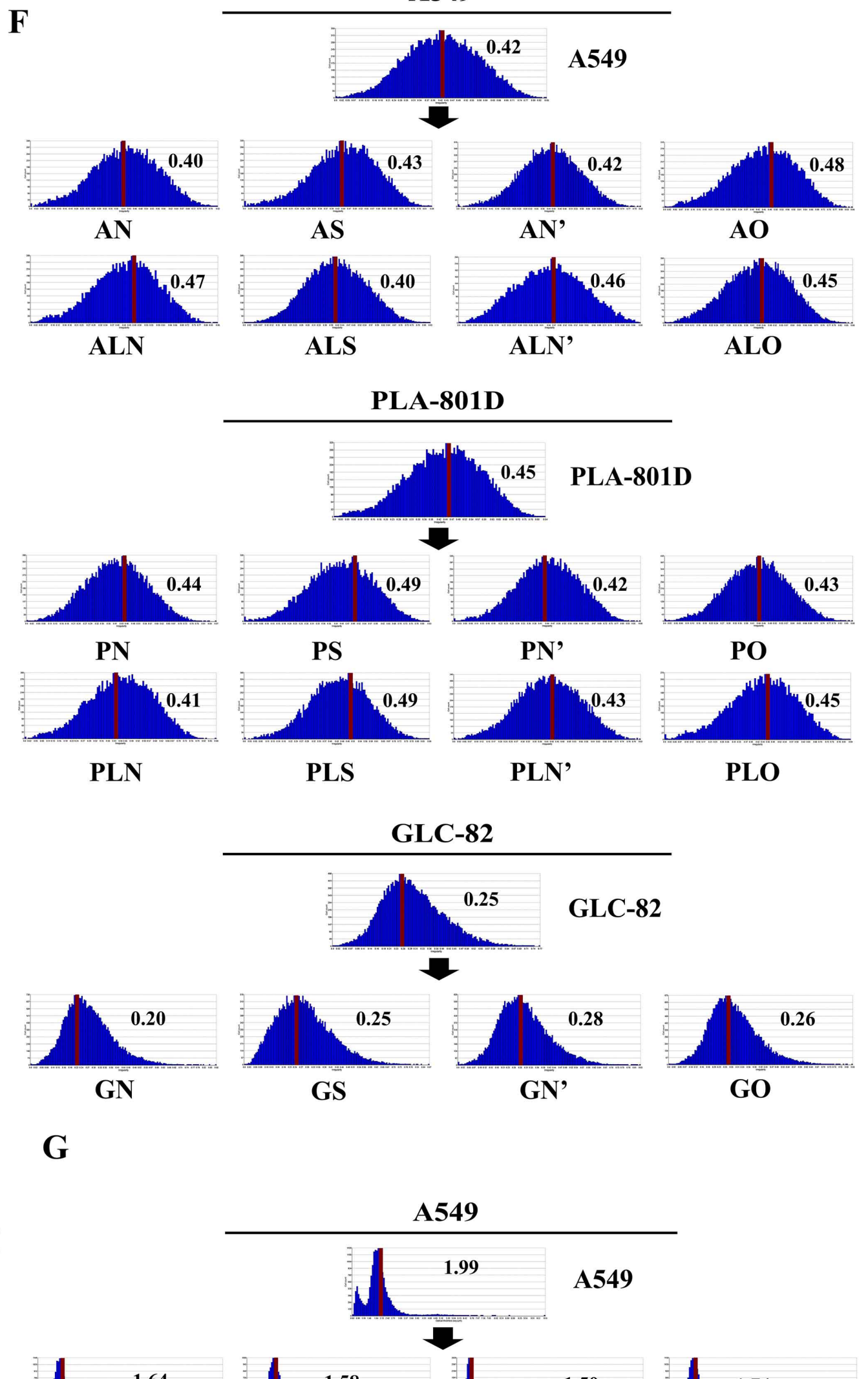
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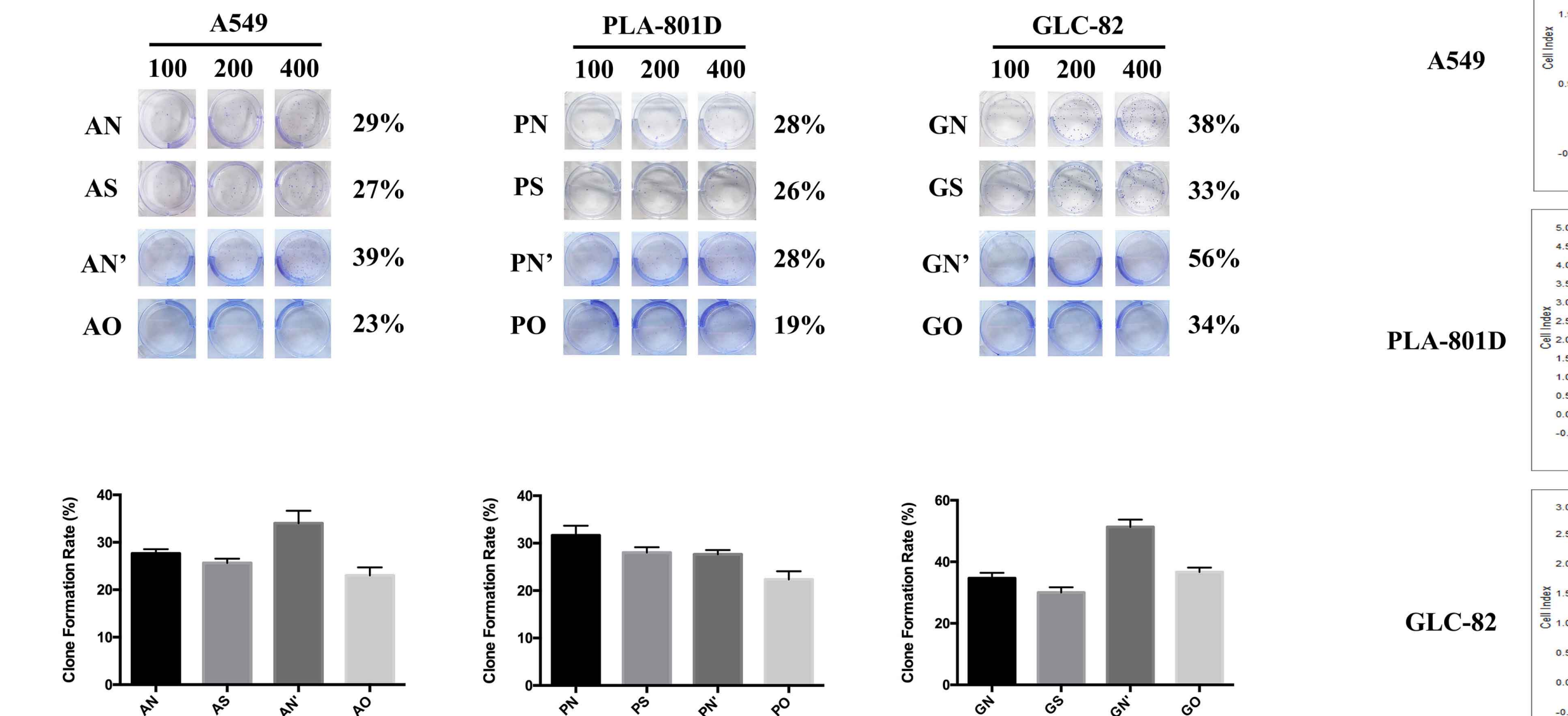
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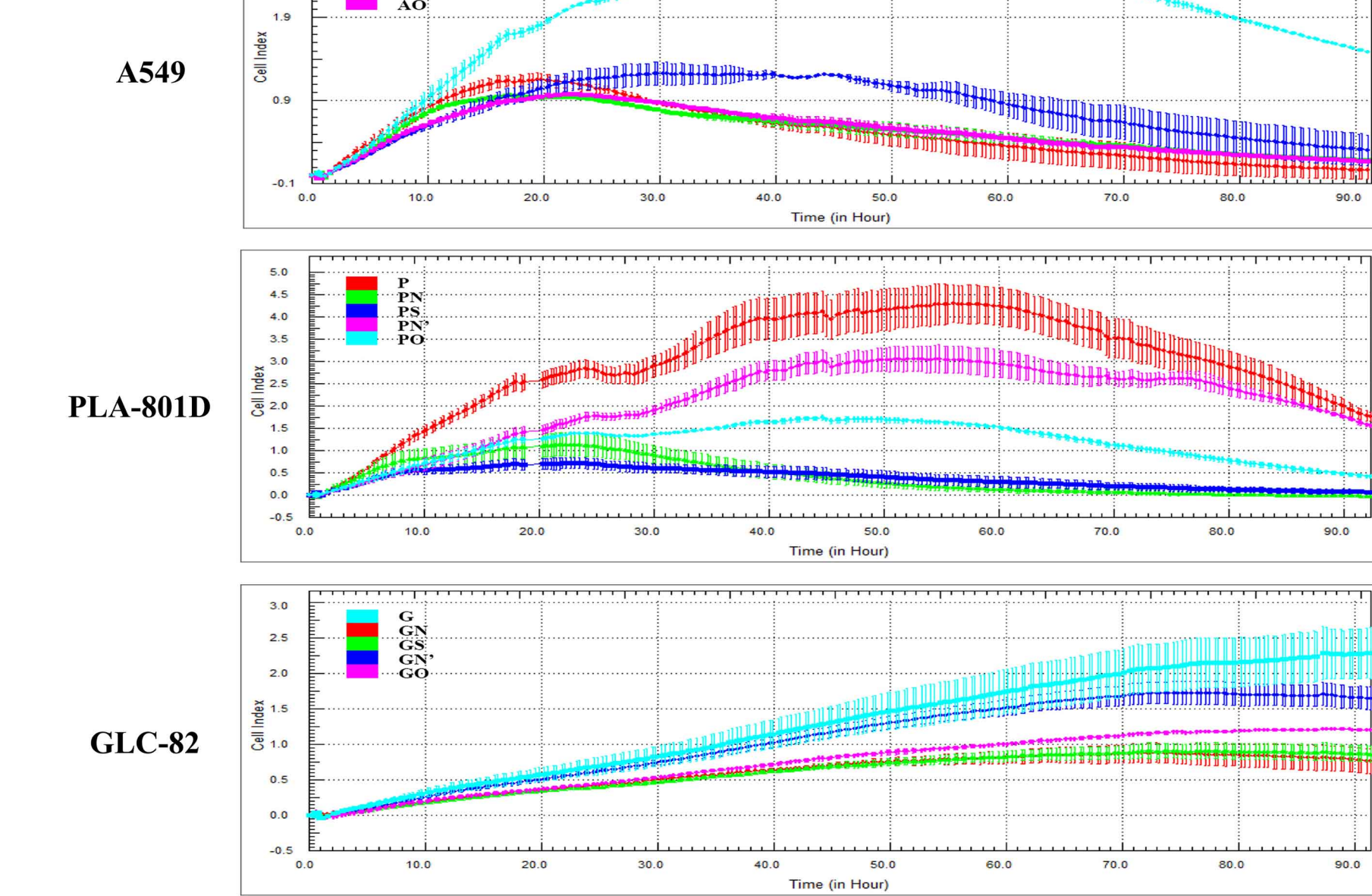
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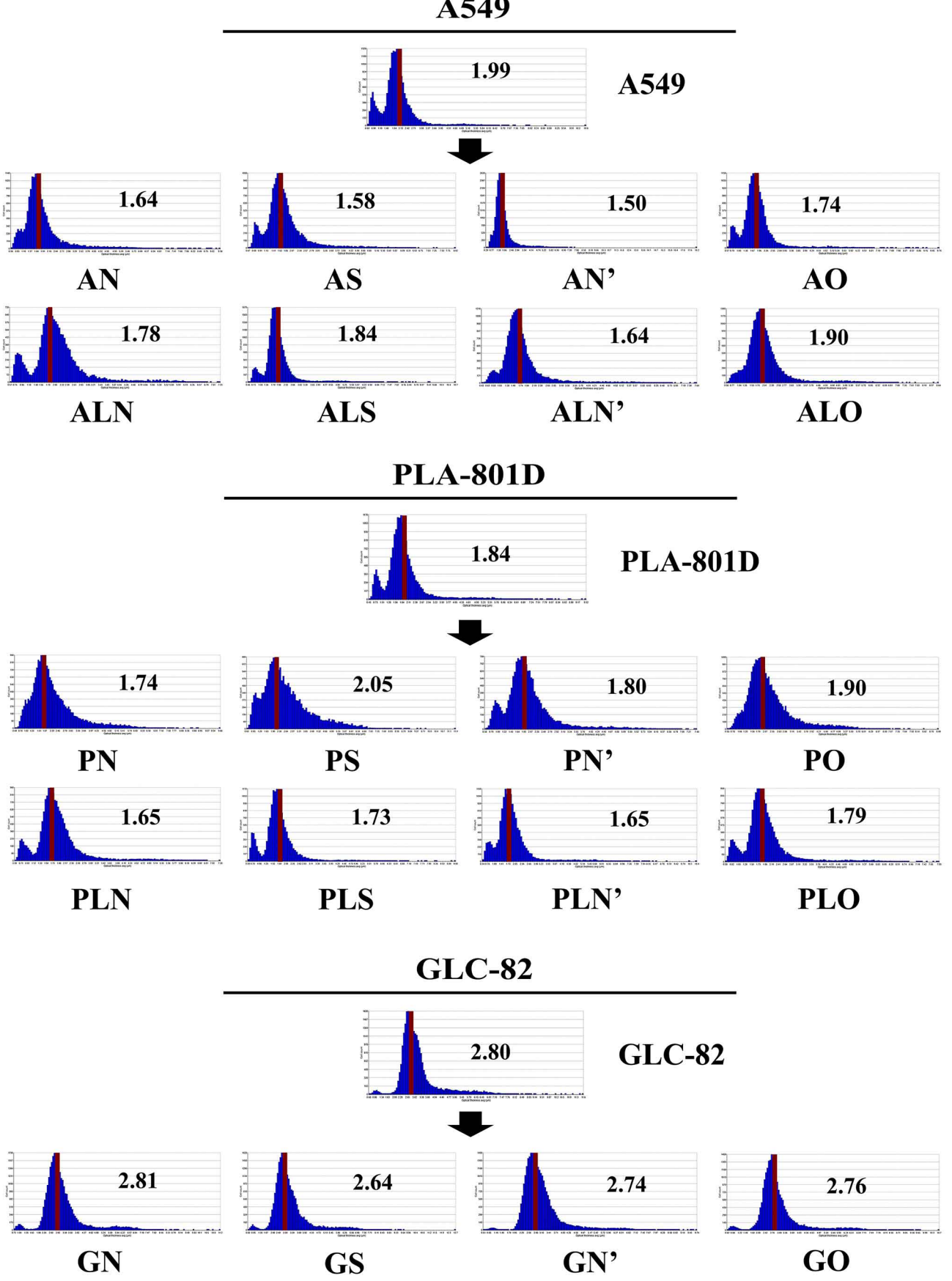
D



E



G



Supplementary Figure S2

Supplementary Table S1. Clinical Relevance of BCAP31 from Gene Expression Omnibus																													
		GSE11969 (n=129)			GSE13255 (n=137)			GSE14814 (n=133)			GSE33532 (n=80)			GSE42127 (n=176)			GSE50081 (n=172)			GSE103512 (n=55)			Total						
BCAP31		High	Low	P	High	Low	P	High	Low	P	High	Low	P	High	Low	P	High	Low	P	High	Low	P	High	Low	P				
Relapse	Y	36	32	.4246													23	26	.6123				59	58	.8696				
	N	28	33															63		60				91		93			
Status	Alive	31	35	.5389										58	54	.5308	53	49	.5347				142	139	.6697				
	Dead	33	30											30	34			33		37				96		101			
Gender	F	21	24	.6243	31	37	.3470	21	21	.9530	12	4	.0253	43	40	.6505	23	37		5	14	.0141	156	177	.1758				
	M	43	41			37		32			45	46			28		36		45	48			63	49			22	14	
Age	<60	24	26	.7707	17	23	.2834	17	27	.2520	5	11	.0935				17	8	.0515	10	10	.9188	90	105	.3497				
	>60	40	39			51		46			39	40			35	29		69		78			17	18			251	250	
Smoking	Y	21	25	.5030	62	66	.2904																83	91	.3883				
	N	43	40			6		3																49		43			
Histology	AD	35	55	.0006							19	21	.6015	62	71	.1144	62	67	.3786	11	19	.0435	189	233	.0009				
	SQ	27	8								7	9			26		17			24	19			16		9		100	62
	Mix	2	2									14		10			0	0			0		0			0	0		16
K-Ras	WT	61	58	.1965																			61	58	.1965				
Status	MuT	3	7																					3		7			
P53	WT	30	46	.0058																			30	46	.0058				
Status	MuT	34	19																					34		19			
EGFR	WT	50	46	.3384																			50	46	.3384				
Status	MuT	14	19																					14		19			
COPD	Y				30	33	.6632																30	33	.6632				
	N				38	36																		38		36			
Y: Yes; N: No; F: Female; M: Male; AD: Adenocarcinoma; SQ: Squamous cell carcinoma; WT: Wild type; MuT: Mutant type; EGFR: Epidermal Growth Factor Receptor; COPD: Chronic Obstructive Pulmonary Disease																													