In Situ Mapping of Activated PDGFRβ Defines a Prognostic Discrepancy Between Histological Subtypes of NSCLC.

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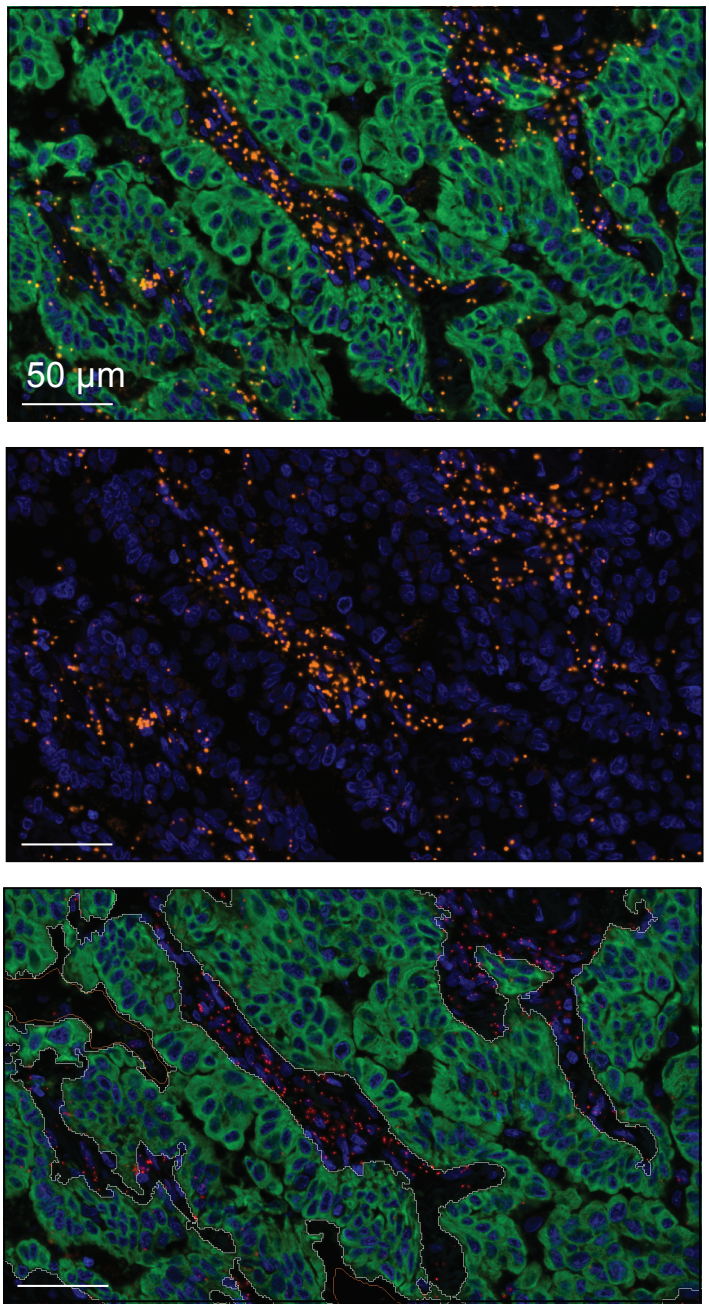
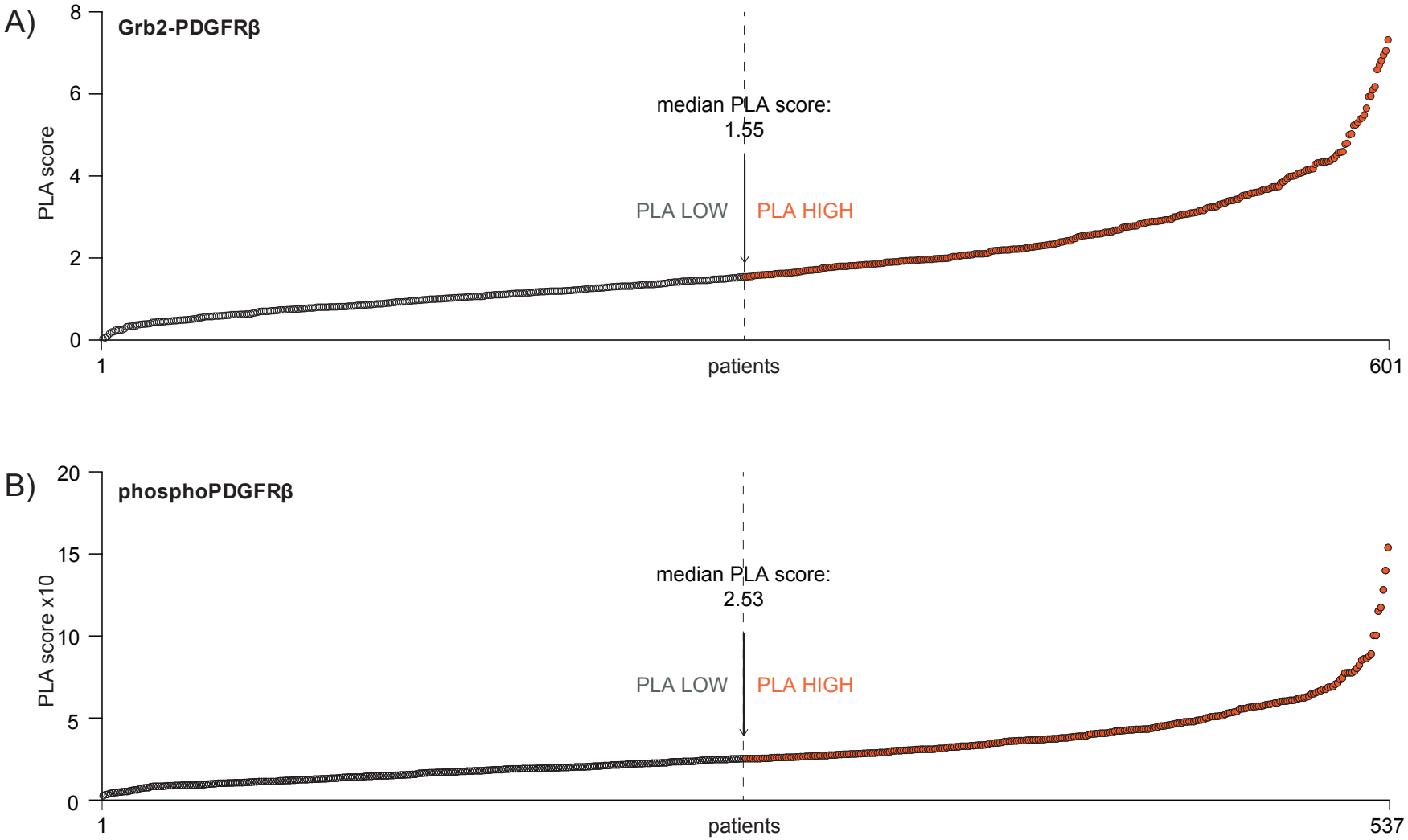
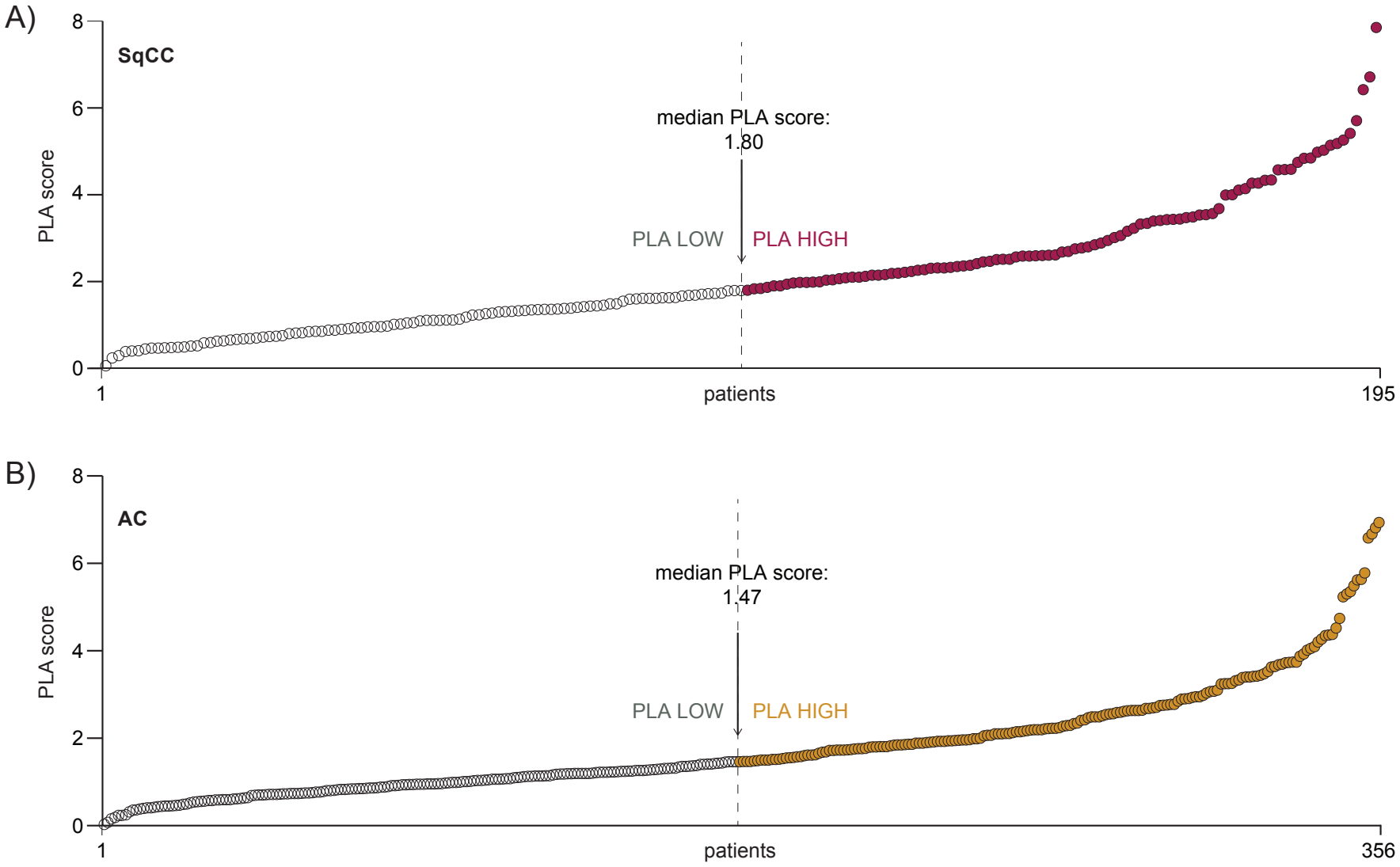

50 µm

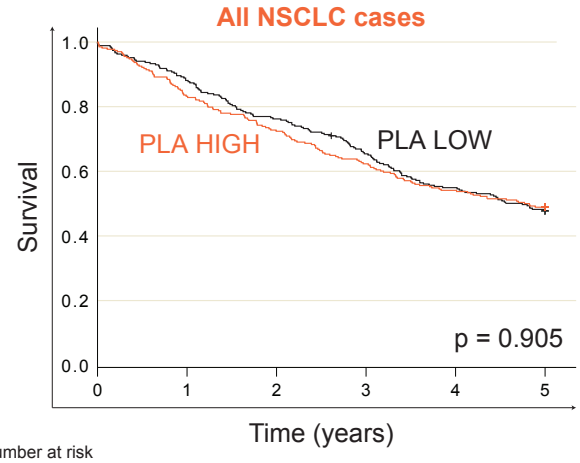
Supplementary Figure 1. Representative immunofluorescence staining and digital annotation of Grb2-PDGFRβ proximity ligation assay (PLA) signals in relation to cytokeratin (CK) expression. The top panel shows the triplex immunofluorescence image with CK staining (green), Grb2-PDGFRβ PLA signals (orange), and nuclei counterstained with DAPI (blue). The middle panel displays the same field of view with the CK channel omitted, highlighting the distribution of PLA signals across both tumor (CK-positive) and stromal regions. The bottom panel illustrates the digitally generated annotation masks used for image analysis (orange contours), with tumor regions delineated by white contours and PLA signals identified specifically within the stromal compartment indicated by white pixel dots.


A)
Grb2-PDGFRβ
PLA score
8
6
4
2
0
median PLA score:
1.55
PLA LOW
PLA HIGH
1
patients
601
B)
phosphoPDGFRβ
PLA score x10
20
15
10
5
0
median PLA score:
2.53
PLA LOW
PLA HIGH
1
patients
537

Supplementary Figure 2. Frequency distributions of PLA activation scores in the NSCLC patient cohort. A) Distribution of the Grb2–PDGFRβ activation score among the 601 patients with successful staining and analysis. The arrow indicates the median score (1.55 dots per stromal cell). **B)** Distribution of the phosphoPDGFRβ activation score (displayed ×10) among the 537 patients with successful staining and analysis. The arrow indicates the median score (2.53 dots per stromal cell).

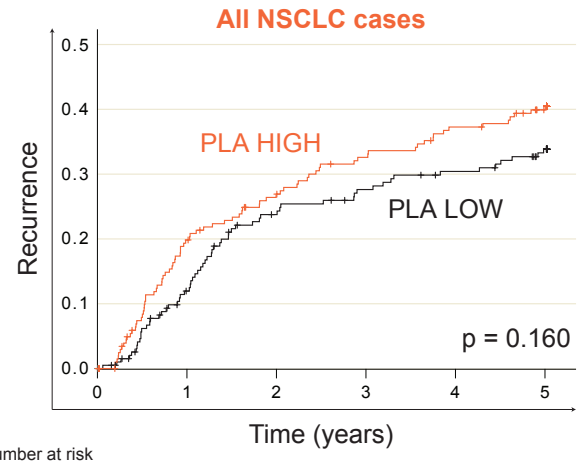

A)
SqCC
PLA score
8
6
4
2
0
median PLA score:
1.80
PLA LOW
PLA HIGH
1
patients
195
B)
AC
PLA score
8
6
4
2
0
median PLA score:
1.47
PLA LOW
PLA HIGH
1
patients
356

Supplementary Figure 3. Frequency distributions of Grb2–PDGFRβ PLA activation scores across the two most common NSCLC histological subtypes separately. A) Distribution of the Grb2–PDGFRβ activation score among the 195 squamous cell carcinoma (SqCC) patients with successful staining and analysis. The arrow indicates the median score (1.80 dots per stromal cell). **B)** Distribution of the Grb2–PDGFRβ activation score among the 356 adenocarcinoma (AC) patients with successful staining and analysis. The arrow indicates the median score (1.47 dots per stromal cell).



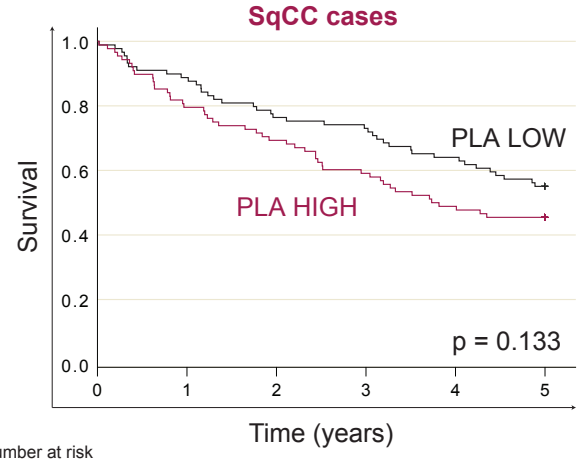
Number at risk

PLA LOW	269	237	205	175	147	128
PLA HIGH	268	223	194	167	145	131



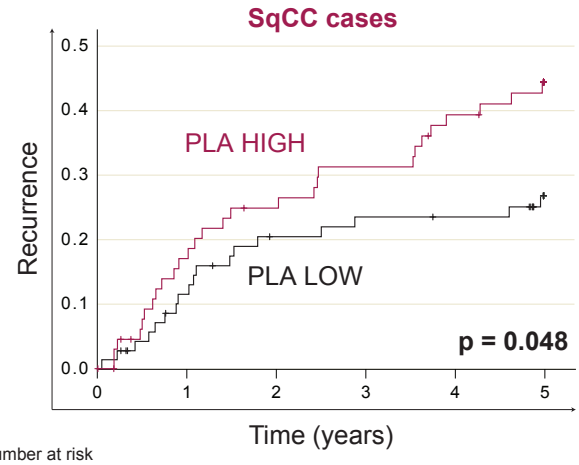
Number at risk

PLA LOW	199	165	139	130	123	112
PLA HIGH	209	161	142	130	120	107



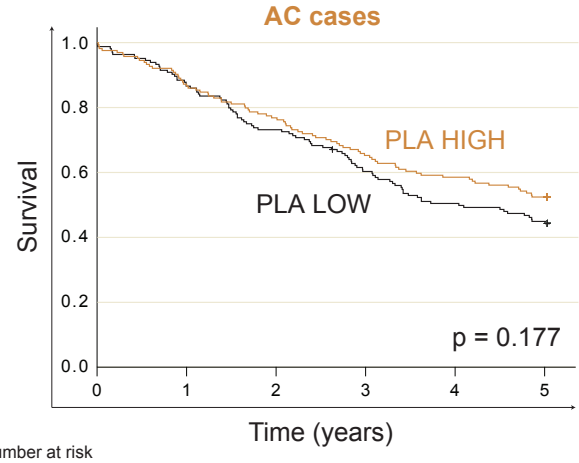
Number at risk

PLA LOW	89	79	68	65	57	49
PLA HIGH	88	70	61	52	43	40



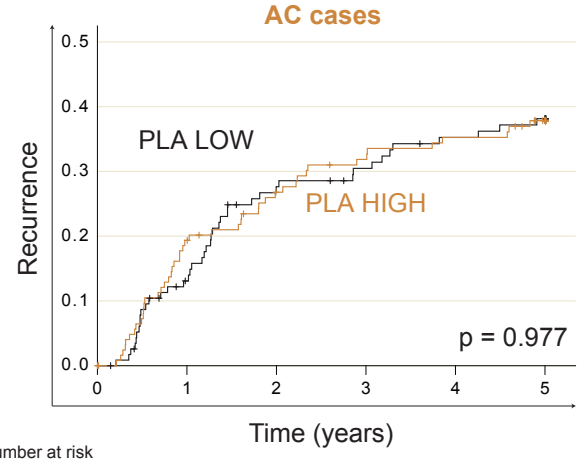
Number at risk

PLA LOW	72	60	52	50	49	42
PLA HIGH	68	53	47	43	37	33



Number at risk

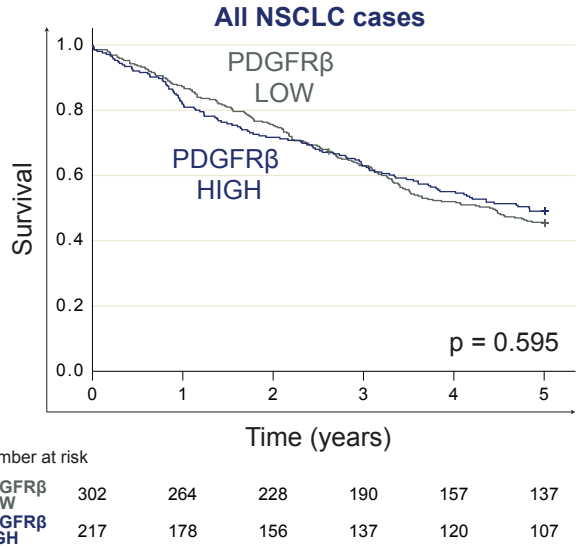
PLA LOW	164	142	120	98	82	72
PLA HIGH	164	142	125	107	96	86



Number at risk

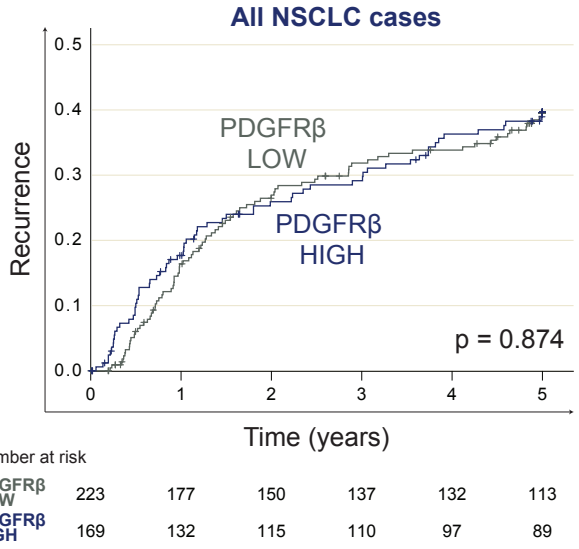
PLA LOW	117	96	79	73	67	64
PLA HIGH	127	100	87	80	76	68

Supplementary Figure 4. PhosphoPDGFRβ PLA activation score in relation to survival and recurrence. (Left column) Kaplan Meier curves demonstrating overall survival (truncated at 5 years) in all NSCLC patients (top), squamous cell carcinoma (SqCC) patients (middle), and adenocarcinoma (AC) patients (bottom). (Right column) Cumulative hazard plots for recurrence within 5 years of diagnosis for the same patient groups. Statistics are based on Log-rank tests. Low and high groups were defined using the median phosphoPDGFRβ score as a cut-point in each respective patient group separately.



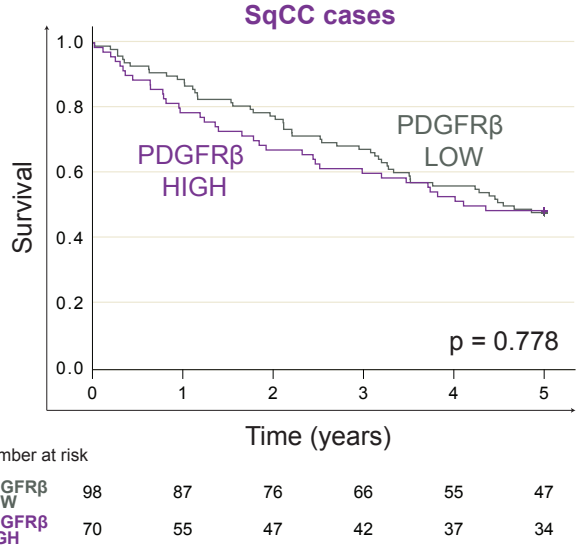
Number at risk

PDGFRβ LOW	302	264	228	190	157	137
PDGFRβ HIGH	217	178	156	137	120	107



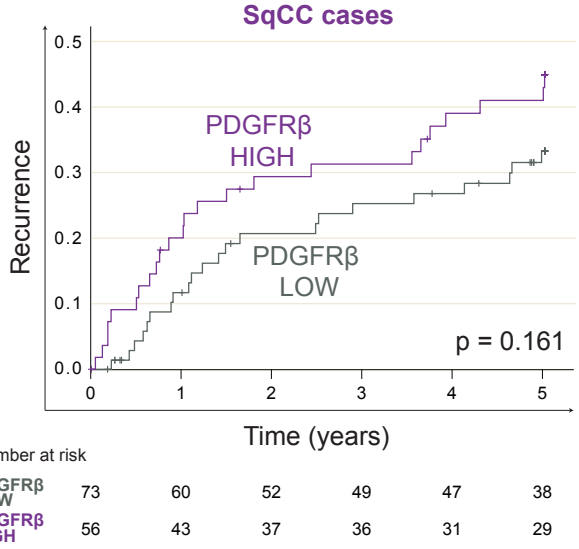
Number at risk

PDGFRβ LOW	223	177	150	137	132	113
PDGFRβ HIGH	169	132	115	110	97	89



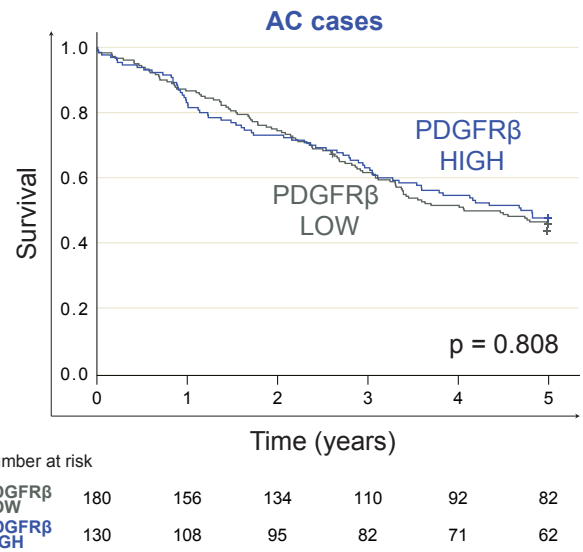
Number at risk

PDGFRβ LOW	98	87	76	66	55	47
PDGFRβ HIGH	70	55	47	42	37	34



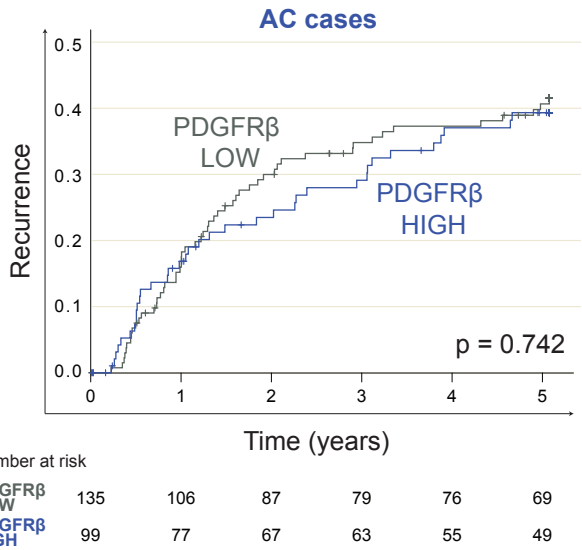
Number at risk

PDGFRβ LOW	73	60	52	49	47	38
PDGFRβ HIGH	56	43	37	36	31	29



Number at risk

PDGFRβ LOW	180	156	134	110	92	82
PDGFRβ HIGH	130	108	95	82	71	62



Number at risk

PDGFRβ LOW	135	106	87	79	76	69
PDGFRβ HIGH	99	77	67	63	55	49

Supplementary Figure 5. PDGFRβ protein expression level in relation to survival and recurrence. (Left column) Kaplan Meier curves demonstrating overall survival (truncated at 5 years) in all NSCLC patients (top), squamous cell carcinoma (SqCC) patients (middle), and adenocarcinoma (AC) patients (bottom). (Right column) Cumulative hazard plots for recurrence within 5 years of diagnosis in the same patient groups. Statistics are based on Log-rank tests. Statistics are based on Log-rank tests. Low and high groups were defined using the median PDGFRβ protein expression level as cut-point, which was value=7 for all groups, based on semiquantitative intensity scoring (see Materials and Methods for annotation strategy).

Supplementary Table 1. Comparison of the distribution of clinicopathological characteristics between the phosphoPDGFRβ PLA LOW and PLA HIGH groups. P-values are based on Fisher's Exact tests for comparisons between 2 variables, and Fisher-Freeman-Halton Exact tests for comparisons between >2 variables. All tests were 2-sided.

	n (Valid %)								
	All NSCLC patients n = 537			**SqCC patients only n = 177**			**AC patients only n = 328**		
Variable	**PLA LOW** n = 269 (50.1)	**PLA HIGH** n = 268 (49.9)	**p-value**	**PLA LOW** n = 89 (50.3)	**PLA HIGH** n = 88 (49.7)	**p-value**	**PLA LOW** n = 164 (50.0)	**PLA HIGH** n = 164 (50.0)	**p-value**
Histology:									
SqCC	82 (30.5)	95 (35.4)	0.451	-	-	-	-	-	-
AC	171 (63.6)	157 (58.6)		-	-		-	-	
Other	16 (5.9)	16 (6.0)		-	-		-	-	
Stage 8th edition:									
I	144 (53.5)	126 (47.0)	0.369	43 (48.3)	34 (38.6)	0.427	91 (55.5)	87 (53.0)	0.842
II	67 (24.9)	78 (29.1)		26 (29.2)	31 (35.2)		36 (22.0)	40 (24.4)	
III	53 (19.7)	61 (22.8)		20 (22.5)	23 (26.1)		32 (19.5)	34 (20.7)	
IV	5 (1.9)	3 (1.1)		0 (0.0)	0 (0.0)		5 (3.0)	3 (1.8)	
Age at diagnosis:									
<70 years	158 (58.7)	159 (59.3)	0.930	51 (57.3)	49 (55.7)	0.880	100 (61.0)	96 (58.5)	0.736
≥70 years	111 (41.3)	109 (40.7)		38 (42.7)	39 (44.3)		64 (39.0)	68 (41.5)	
Biological sex:									
Male	128 (47.6)	138 (51.5)	0.389	51 (57.3)	61 (69.3)	0.119	67 (40.9)	73 (44.5)	0.577
Female	141 (52.4)	130 (48.5)		38 (42.7)	27 (30.7)		97 (59.1)	91 (55.5)	
Smoking history:									
Current/former smoker	242 (90.0)	237 (88.4)	0.677	86 (96.6)	83 (94.3)	0.496	144 (87.8)	141 (86.0)	0.744
Never smoker	27 (10.0)	31 (11.6)		3 (3.4)	5 (5.7)		20 (12.2)	23 (14.0)	
Performance status ECOG:									
0	158 (58.7)	156 (58.2)	0.660	45 (50.6)	45 (51.1)	0.576	101 (61.6)	106 (64.6)	0.454
1	97 (36.1)	102 (38.1)		41 (46.1)	37 (42.0)		54 (32.9)	53 (32.3)	
2-4	14 (5.2)	10 (3.7)		3 (3.4)	6 (6.8)		9 (5.5)	5 (3.0)	
KRAS [a]**:**									
WT	197 (73.5)	187 (69.8)	0.388	87 (97.8)	82 (93.2)	0.168	110 (61.3)	94 (57.3)	0.500
Mutated	71 (26.5)	81 (30.2)		2 (2.2)	6 (6.8)		63 (38.7)	70 (42.7)	
EGFR [a]**:**									
WT	238 (88.8)	240 (89.6)	0.890	88 (98.9)	88 (100.0)	1.000	136 (83.4)	136 (82.9)	1.000
Mutated	30 (11.2)	28 (10.4)		1 (1.1)	0 (0.0)		27 (16.6)	28 (17.1)	
TP53 [b]**:**									
WT	68 (39.8)	67 (39.6)	1.000	10 (17.2)	7 (13.2)	0.607	53 (51.0)	59 (55.1)	0.582
Mutated	103 (60.2)	102 (60.4)		48 (82.8)	46 (86.8)		51 (49.0)	48 (44.9)	

SqCC = Squamous cell carcinoma. AC = Adenocarcinoma. ECOG = Eastern Cooperative Oncology Group. *KRAS* = Kirsten rat sarcoma virus oncogene. *EGFR* = Epidermal growth factor receptor. *TP53* = Cellular Tumor Antigen p53. WT = Wildtype (genotype).
[a] = data missing for 1 patient. [b] = data missing for 197 patients.

Supplementary Table 2. Comparison of 5-year recurrence events between the Grb2-PDGFRβ PLA LOW and HIGH groups in squamous cell carcinoma (SqCC) patients. P-values are based on 2-sided Fisher's Exact tests.

n (Valid %)

	SqCC patients only n = 195			SqCC patients only n = 177		
	As via Grb2-PDGFRβ assay			As via phosphoPDGFRβ assay		
Variable	**PLA LOW** n = 98 (50.3)	**PLA HIGH** n = 97 (49.7)	**p-value**	**PLA LOW** n = 89 (50.3)	**PLA HIGH** n = 88 (49.7)	**p-value**
Recurrence at 5 years [a]:						
None	57 (77.0)	45 (57.0)	**0.010**	56 (75.7)	40 (58.8)	**0.048**
Yes	17 (23.0)	34 (43.0)		18 (24.3)	28 (41.2)	
Recurrence organ [b] (valid n=50/46):						
Brain	1 (6.3)	7 (20.6)	0.409	3 (16.7)	4 (14.3)	1.000
All other	15 (93.8)	27 (79.4)		15 (83.3)	24 (85.7)	
Recurrence site/organ [b] (valid n=50/46):						
Lung	10 (62.5)	11 (32.4)	0.066	8 (44.4)	12 (42.9)	1.000
All other	6 (37.5)	23 (67.6)		10 (55.6)	16 (57.1)	

SqCC = Squamous cell carcinoma. Sites/organs in which recurrences were reported: brain, lung, bones, liver, skin, mediastinum, adrenal glands, pleura, other.

[a] = data missing for 42 patients in the Grb2-PDGFRβ assay/data missing for 37 patients in the pPDGFRβ assay.

[b] = data is based on solely patients with recurrence – recurrence site/organ specified (brain or lung) can have additional recurrences.

Supplementary Table 3. Spearman's rank correlation coefficients between the Grb2-PDGFRβ PLA (continuous values) and other stromal factors previously assessed via multiplexed immunofluorescence or immunohistochemistry(37). P-values are two-sided and were adjusted for multiple testing using the Benjamini-Hochberg procedure for each patient set (all NSCLC patients, SqCC patients, AC patients) individually.

	n (Valid %)								
Variable	**All NSCLC patients**	**p-value**	**Adjusted p-value**	**SqCC patients only**	**p-value**	**Adjusted p-value**	**AC patients only**	**p-value**	**Adjusted p-value**
Immune infiltration									
B cell	0.03	0.619	0.689	-0.10	0.360	0.565	0.15	0.068	0.146
CD4-effector	0.10	0.119	0.257	-0.01	0.923	0.923	0.16	**0.044**	0.140
CD4-Treg	0.13	**0.043**	0.133	0.10	0.368	0.565	0.16	0.049	0.140
CD8-effector	0 06	0.352	0.460	-0.11	0.303	0.565	0.18	**0.026**	0.140
CD8-Treg	0.13	**0.047**	0.133	0.05	0.631	0.767	0.17	**0.037**	0.140
CD163	0.08	0.192	0.363	0.12	0.303	0.565	0.11	0.186	0.316
iDC	0.08	0.247	0.370	-0.05	0.632	0.767	0.13	0.132	0.249
mDC	-0.03	0.648	0.689	-0.09	0.399	0.565	0.05	0.524	0.631
pDC	-0.20	**0.002**	**0.013**	-0.27	**0.015**	0.082	-0.17	**0.047**	0.140
M1 macrophage	-0.04	0.532	0.646	-0.13	0.232	0.565	-0.05	0.557	0.631
M2 macrophage	0.07	0.254	0.370	0.04	0.732	0.777	0.11	0.205	0.317
NK cell	0.07	0.261	0.370	0.15	0.187	0.565	0.04	0.640	0.680
NKT cell	-0.02	0.746	0.746	-0.05	0.685	0.777	-0.02	0.825	0.825
Stroma ratio									
Stroma area (CK negative)	0.12	**0.003**	**0.018**	0.11	0.119	0.508	0.13	**0.014**	0.140
Mesenchymal markers									
LRRC15	0.22	**<0.001**	**0.003**	0.33	**0.001**	**0.011**	0.14	0.069	0.146
FAP	0.12	**0.040**	0.133	0.28	**0.004**	**0.034**	0.05	0.484	0.631
CD31	-0.09	0.121	0.257	-0.12	0.241	0.565	-0.06	0.449	0.631

SqCC = Squamous cell carcinoma. AC = Adenocarcinoma.