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Supplemental information

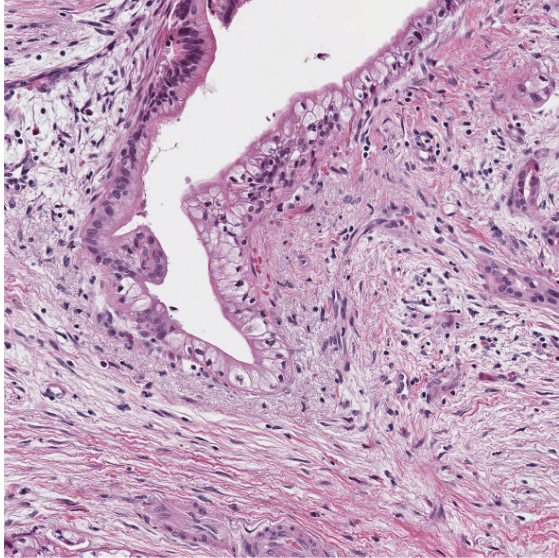
**Development of an artificial intelligence-derived
histologic signature associated with adjuvant
gemcitabine treatment outcomes in pancreatic cancer**

Vivek Nimgaonkar, Viswesh Krishna, Vrishab Krishna, Ekin Tiu, Anirudh Joshi, Damir Vrabac, Hriday Bhambhani, Katelyn Smith, Julia S. Johansen, Shalini Makawita, Benjamin Musher, Arnav Mehta, Andrew Hendifar, Zev Wainberg, Davendra Sohal, Christos Fountzilas, Aatur Singhi, Pranav Rajpurkar, and Eric A. Collisson

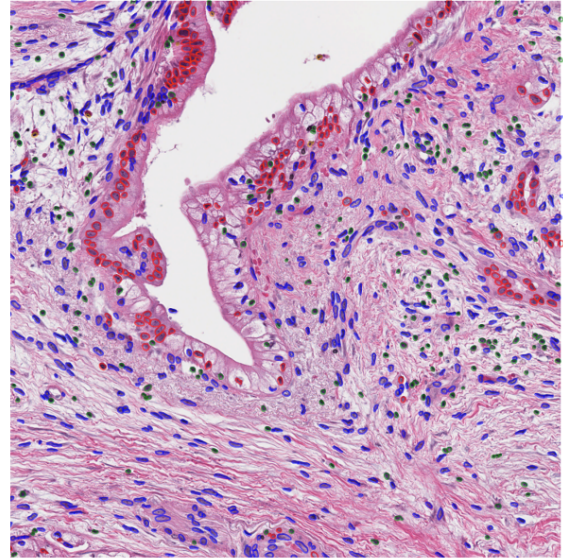
Supplementary Information

Supplementary Figure 1

A



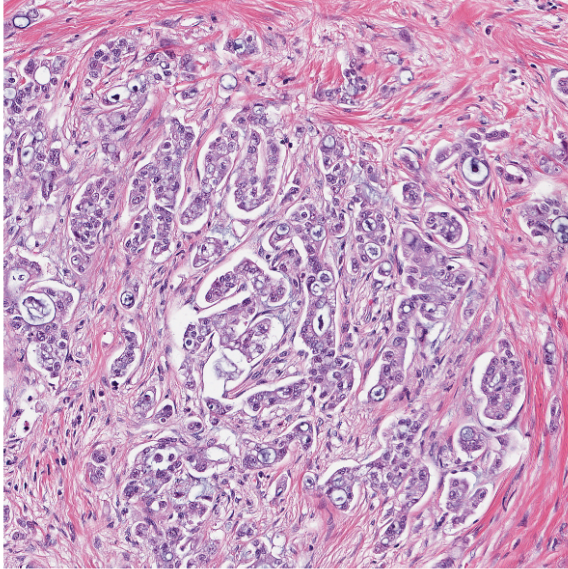
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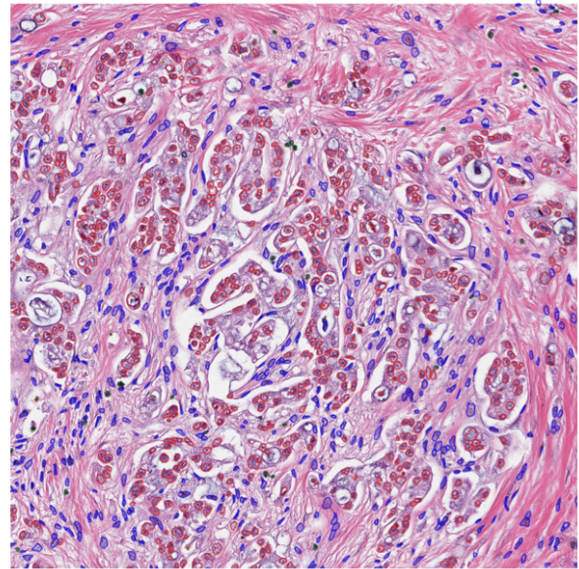
Supplementary Figure 1: Example of nuclear segmentation in the TCGA Cohort. Related to Figure 2. A) A digitally scanned image of a patient sample from the TCGA cohort without nuclear segmentation. B) The digitally scanned image of the patient sample from the TCGA cohort displayed in panel A with nuclear segmentation: red labels are neoplastic cells, blue labels are connective tissue cells, green labels are inflammatory cells, orange labels are non-neoplastic cells, and yellow labels are necrotic cells.

Supplementary Figure 2

A



B

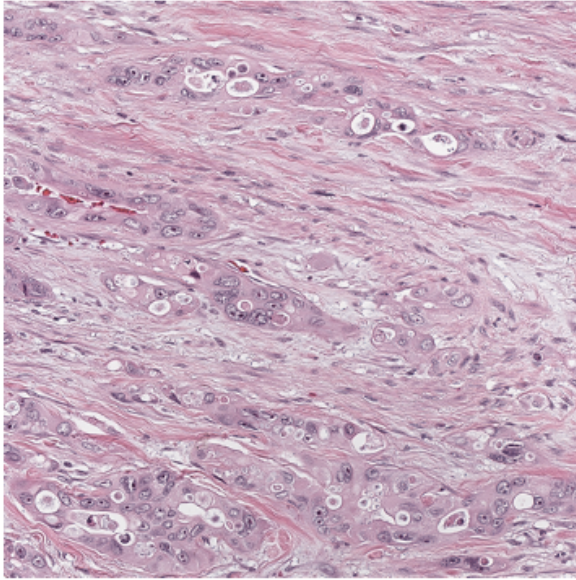


Supplementary Figure 2: Example of nuclear segmentation in the Copenhagen Cohort. Related to Figure 2.

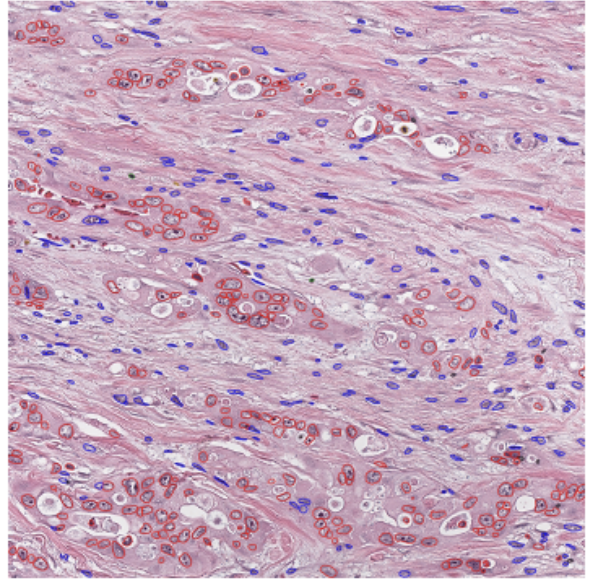
A) A digitally scanned image of a patient sample from the Copenhagen cohort without nuclear segmentation. B) The digitally scanned image of the patient sample from the Copenhagen cohort displayed in panel A with nuclear segmentation: red labels are neoplastic cells, blue labels are connective tissue cells, green labels are inflammatory cells, orange labels are non-neoplastic cells, and yellow labels are necrotic cells.

Supplementary Figure 3

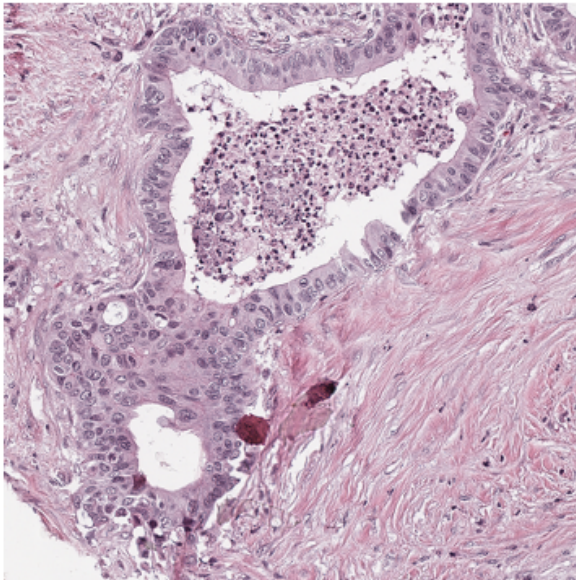
A



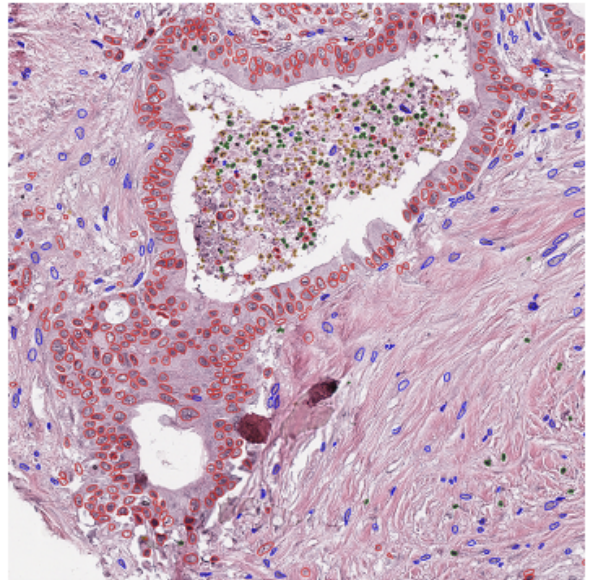
B



C



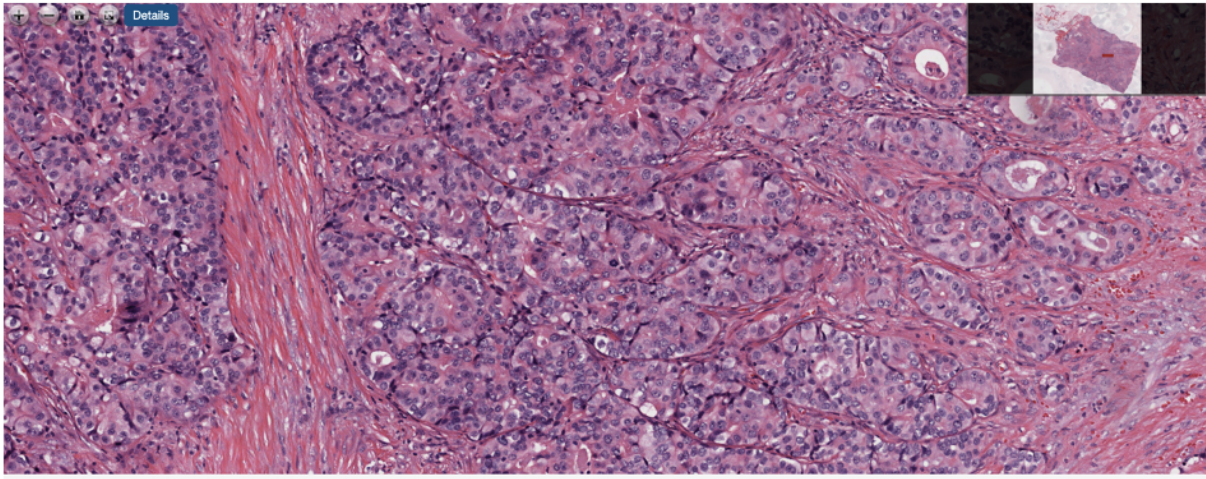
D



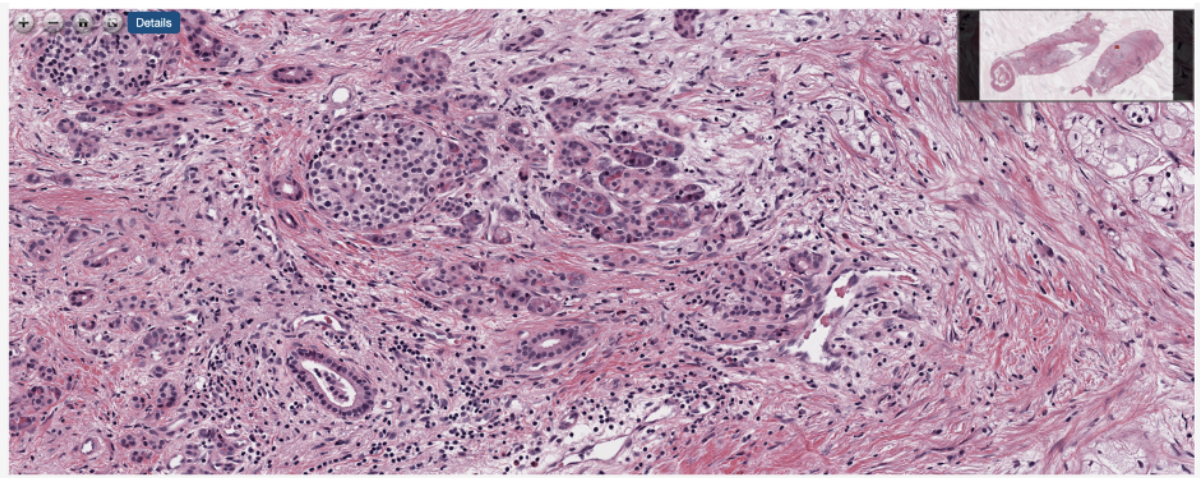
Supplementary Figure 3: Example of nuclear segmentation in the UPMC Cohort. Related to Figure 2. A) A digitally scanned image of a patient sample from the UPMC cohort without nuclear segmentation. This patient did not receive neoadjuvant chemotherapy prior to resection. B) The digitally scanned image of the patient sample from the UPMC cohort displayed in panel A with nuclear segmentation: red labels are neoplastic cells, blue labels are connective tissue cells, green labels are inflammatory cells, orange labels are non-neoplastic cells, and yellow labels are necrotic cells. C) A digitally scanned image of a patient sample from the UPMC cohort without nuclear segmentation. This patient did receive neoadjuvant chemotherapy prior to resection. D) The digitally scanned image of the patient sample from the UPMC cohort displayed in panel C with nuclear segmentation: red labels are neoplastic cells, blue labels are connective tissue cells, green labels are inflammatory cells, orange labels are non-neoplastic cells, and yellow labels are necrotic cells.

Supplementary Figure 4

A



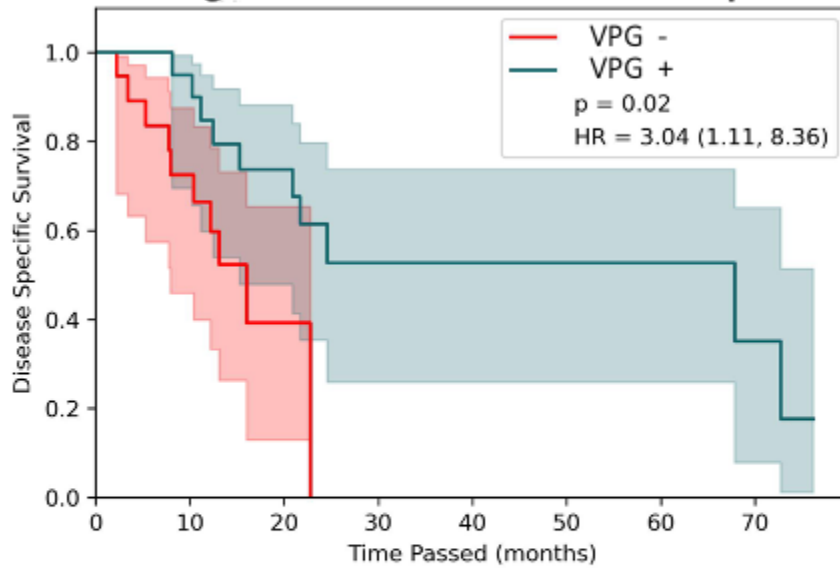
B



Supplementary Figure 4: Examples of histologic images for VPG+ and VPG- slides. Related to Figure 2. A) A digitally scanned image of a VPG+ slide from the TCGA cohort. The feature contributing to the VPG signature describes variation in nuclear morphology and demonstrates significant variation visually, as compared to: B) A digitally scanned image of a VPG- slide from the TCGA cohort. Both slides correspond to patients with tumor grade of G3.

Supplementary Figure 5

**DSS Stratified by the Histologic Signature
among Test Set Patients with RNASeq Data**

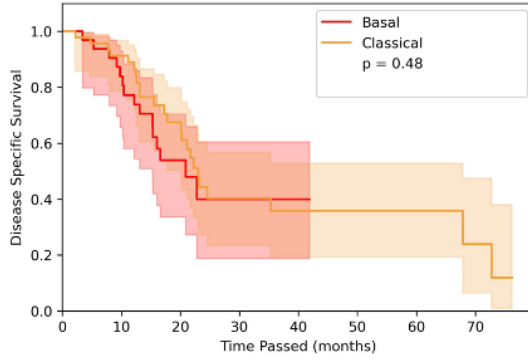


VPG -								
At risk	19	12	2	0	0	0	0	0
Censored	0	2	8	9	9	9	9	9
Events	0	5	9	10	10	10	10	10
VPG +								
At risk	20	19	12	6	5	4	3	2
Censored	0	0	3	6	7	8	9	9
Events	0	1	5	8	8	8	8	9

Supplementary Figure 5: The histologic signature stratifies patients by outcome following adjuvant gemcitabine among a sub-population of the TCGA test cohort with RNA Seq data available. Related to Figure 3. Kaplan meier curve describing DSS among patients in a sub-population of the TCGA cohort test set with RNASeq data available (n=39, the same population of patients discussed in Figure 3A-C).

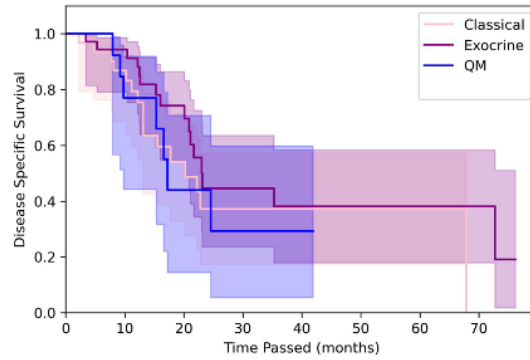
Supplementary Figure 6

A DSS Stratified by the Moffitt clusters in the entire TCGA cohort with RNASeq data available



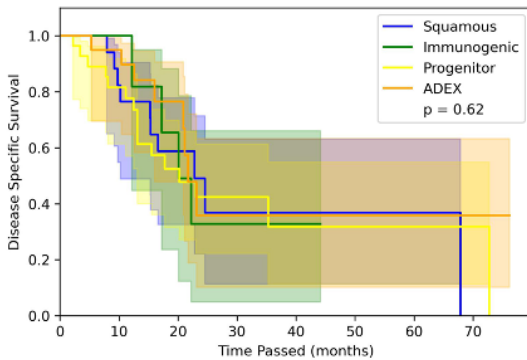
Basal							
At risk	32	25	9	3	1	0	0
Censored	0	2	10	14	16	17	17
Events	0	5	13	15	15	15	15
Classical							
At risk	47	40	21	9	7	5	4
Censored	0	3	13	17	18	20	21
Events	0	4	13	21	22	22	23

B DSS Stratified by the Collisson clusters in the entire TCGA cohort with RNASeq data available



Classical							
At risk	31	24	10	3	2	2	2
Censored	0	3	9	13	14	14	14
Events	0	4	12	15	15	15	15
Exocrine							
At risk	35	31	16	7	5	3	2
Censored	0	2	11	14	15	17	18
Events	0	2	8	14	15	15	15
QM							
At risk	13	10	4	2	1	0	0
Censored	0	0	3	4	5	6	6
Events	0	3	6	7	7	7	7

C DSS Stratified by the Bailey clusters in the entire TCGA cohort with RNASeq data available

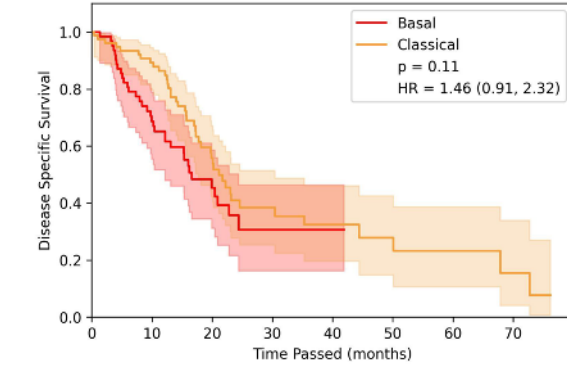


Squamous							
At risk	17	14	7	3	2	1	1
Censored	0	0	3	5	6	7	7
Events	0	3	7	9	9	9	10
Immunogenic							
At risk	14	12	4	1	1	0	0
Censored	0	2	7	8	8	9	9
Events	0	0	3	5	5	5	5
Progenitor							
At risk	28	21	11	5	3	2	2
Censored	0	2	5	9	10	11	11
Events	0	5	12	14	15	15	15
ADEX							
At risk	20	18	8	3	2	2	1
Censored	0	1	8	9	10	10	11
Events	0	1	4	8	8	8	8

Supplementary Figure 6: RNASeq clusters do not stratify patients by DSS following adjuvant gemcitabine across the entire gemcitabine-treated TCGA dataset. Related to Figure 3. A-C) Kaplan meier curves describing DSS among all patients in the TCGA cohort with RNASeq data available (n=79) when stratified by A) Moffitt clusters, B) Collisson clusters, and C) Bailey clusters.

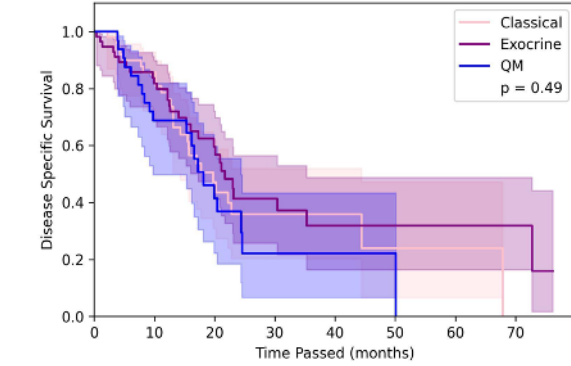
Supplementary Figure 7

A DSS stratified by Moffitt cohorts among all TCGA patients (including those without adjuvant gemcitabine treatment)



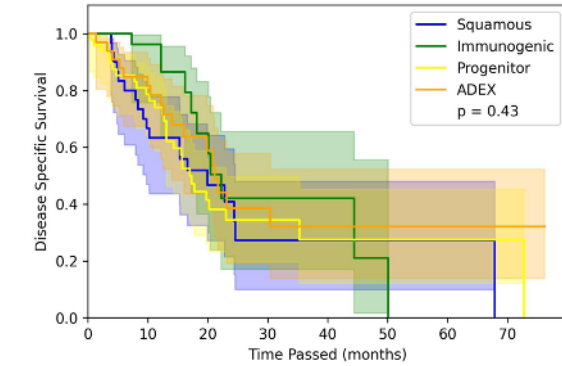
Basal								
At risk	63	39	15	6	1	0	0	0
Censored	0	5	18	23	28	29	29	29
Events	0	19	30	34	34	34	34	34
Classical								
At risk	80	62	29	13	10	6	4	2
Censored	1	11	24	32	33	36	37	38
Events	0	8	28	36	38	39	40	41

B DSS stratified by Collisson cohorts among all TCGA patients (including those without adjuvant gemcitabine treatment)



Classical								
At risk	52	37	14	6	4	2	2	0
Censored	0	8	16	21	23	24	24	25
Events	0	7	22	25	25	26	26	27
Exocrine								
At risk	58	42	21	10	5	3	2	2
Censored	1	7	18	23	26	28	29	29
Events	0	10	20	26	28	28	28	28
QM								
At risk	33	22	9	3	2	1	0	0
Censored	0	1	8	11	12	13	13	13
Events	0	10	16	19	19	19	20	20

C DSS stratified by Bailey cohorts among all TCGA patients (including those without adjuvant gemcitabine treatment)



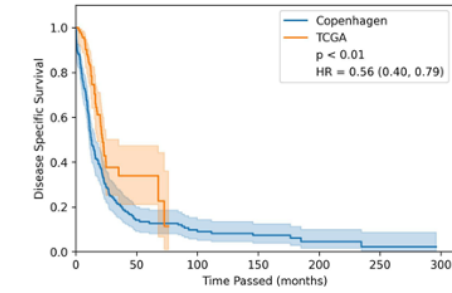
Squamous								
At risk	30	20	9	4	2	1	1	0
Censored	0	0	6	8	10	11	11	11
Events	0	10	15	18	18	18	18	19
Immunogenic								
At risk	28	22	9	3	3	1	0	0
Censored	0	5	13	16	16	17	17	17
Events	0	1	6	9	9	10	11	11
Progenitor								
At risk	50	34	14	6	4	2	2	1
Censored	0	6	11	17	18	20	20	21
Events	0	10	25	27	28	28	28	28
ADEX								
At risk	35	25	12	6	2	2	1	1
Censored	1	5	12	14	17	17	18	18
Events	0	6	12	16	17	17	17	17

Supplementary Figure 7: RNASeq clusters do not stratify patients by DSS across the entire TCGA dataset regardless of adjuvant treatment (n=143). Related to Figure 3. A-C) Kaplan meier curves describing DSS among all patients in the TCGA cohort with RNASeq data available regardless of adjuvant treatment received (n=143) when stratified by A) Moffitt clusters, B) Collisson clusters, and C) Bailey clusters.

Supplementary Figure 8

A

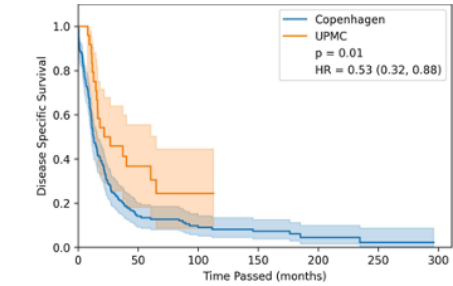
DSS among the adjuvant treated TCGA Cohort and untreated Copenhagen Cohort



Copenhagen							
At risk	161	21	12	8	3	1	0
Censored	0	2	4	6	9	10	11
Events	0	138	145	147	149	150	150
TCGA							
At risk	93	5	0	0	0	0	0
Censored	0	44	47	47	47	47	47
Events	0	44	46	46	46	46	46

B

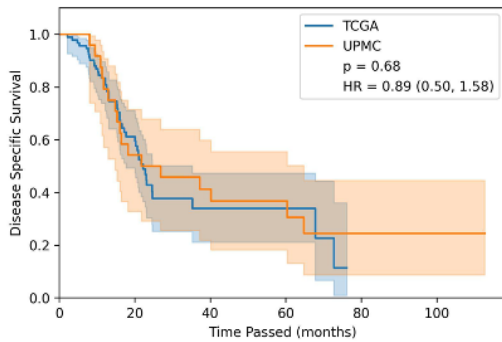
DSS among the adjuvant treated UPMC Cohort and untreated Copenhagen Cohort



Copenhagen							
At risk	161	21	12	8	3	1	0
Censored	0	2	4	6	9	10	11
Events	0	138	145	147	149	150	150
UPMC							
At risk	24	6	4	0	0	0	0
Censored	0	3	3	7	7	7	7
Events	0	15	17	17	17	17	17

C

DSS among the adjuvant treated TCGA and UPMC Cohorts

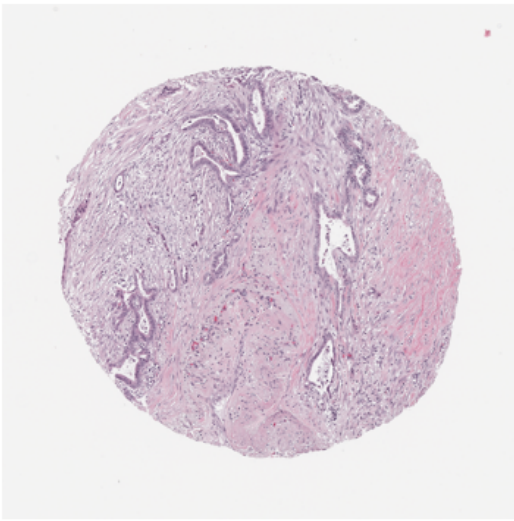


TCGA						
At risk	93	35	8	4	0	0
Censored	0	27	41	45	47	47
Events	0	31	44	44	46	46
UPMC						
At risk	24	13	9	6	4	4
Censored	0	0	1	3	3	3
Events	0	11	14	15	17	17

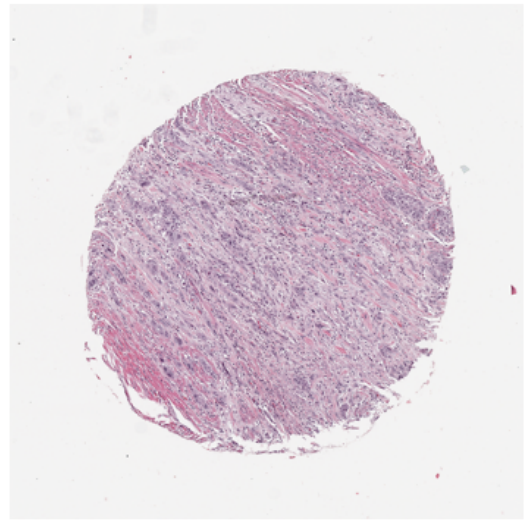
Supplementary Figure 8: Adjuvant gemcitabine-treated cohorts have different DSS from an untreated cohort. Related to Figure 4. A) Kaplan meier curves describing DSS among all TCGA cohort patients (n=93) and all Copenhagen cohort patients (n=161). The p-value for the log-rank test is <0.01. B) Kaplan meier curves describing DSS among all UPMC cohort patients (n=24) and all Copenhagen cohort patients (n=161). The p-value for the log-rank test is 0.01. C) Kaplan meier curves describing DSS among all TCGA cohort patients (n=161) and all UPMC cohort patients (n=24). The p-value for the log-rank test is 0.68.

Supplementary Figure 9

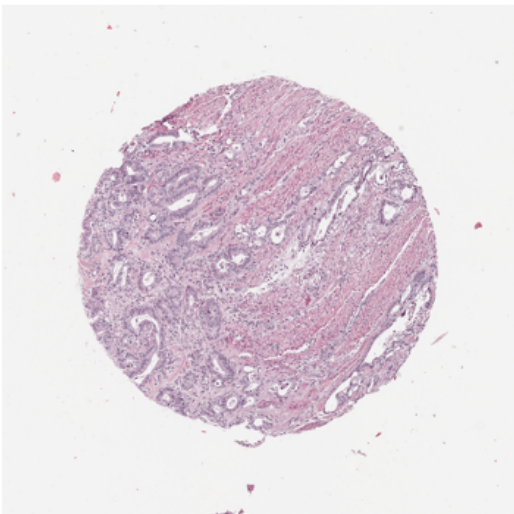
A



B



C



Supplementary Figure 9: Examples of images of microarray specimens from the UPMC cohort (external validation set). Related to Figure 4. A, B, C) Three representative examples of scanned images of tissue microarray samples included in the external validation set (UPMC Cohort).

Supplementary Table 1

	Training	Test	
n	46	47	
Age, Median (IQR)	65 (56, 74.8)	65 (60, 71)	p=0.65
Gender (%)			p=0.26
Female	23 (50)	17 (36)	
Male	23 (50)	30 (64)	
Tumor Grade (%)			p=0.09
G1	2 (4)	10 (21)	
G2	30 (65)	22 (47)	
G3	13 (28)	14 (30)	
G4	1 (2)	1 (2)	
Adjuvant Regimen Received (%)			p=0.98
Gemcitabine alone	43 (93)	45 (96)	
Gemcitabine in combination another agent	3 (7)	2 (4)	
Length of Adjuvant Therapy (%)			p=0.26
<3 months	19 (41)	27 (57)	
3-6 months	10 (22)	9 (19)	
> 6 months	17 (37)	11 (23)	

Supplementary Table 1: Clinical characteristics of the training and test sets from the TCGA. Related to Figure 2. Table describing clinical characteristics among patients in the TCGA training and test sets. Patients were randomly divided between the two groups. P-values correspond to chi-squared tests run with the exception of the variable age, for which a Wilcoxon Rank Sum Test was run.

Supplementary Table 2

	Signature +	Signature -	
n	23	24	
Age, Median (IQR)	67 (59, 71)	65 (62, 71)	p=0.72
Gender (%)			p=0.62
Female	7 (30)	10 (42)	
Male	14 (70)	16 (58)	
Tumor Grade (%)			p=0.60
G1	4 (17)	6 (25)	
G2	12 (52)	10 (42)	
G3	6 (26)	8 (33)	
G4	1 (4)	0 (0)	
Adjuvant Regimen Received (%)			p=1
Gemcitabine alone	22 (96)	23 (96)	
Gemcitabine in combination another agent	1 (4)	1 (4)	
Length of Adjuvant Therapy (%)			p=0.40
<3 months	11 (48)	16 (67)	
3-6 months	7 (30)	4 (17)	
> 6 months	5 (21)	4 (17)	

Supplementary Table 2: Clinical characteristics of the internal test set from the TCGA. Related to Figure 2. Table describing clinical characteristics among patients in the TCGA test set who were signature + vs. signature -. P-values correspond to chi-squared tests run with the exception of the variable age, for which a Wilcoxon Rank Sum Test was run.

Supplementary Table 3

	Signature +	Signature -	
n	29	17	
Age, Median (IQR)	60 (55, 71)	66 (54, 71)	p=0.59
Gender (%)			p=0.47
Female	13 (45)	5 (29)	
Male	16 (55)	12 (71)	
ECOG (%)			p=0.37
0	6 (21)	1 (6)	
1	4 (14)	2 (12)	
Not available	19 (66)	14 (82)	
Tumor Grade (%)			p=0.05
G1	3 (10)	0 (0)	
G2	23 (79)	10 (59)	
G2-3	0 (0)	2 (12)	
G3	3 (10)	5 (29)	
Neoadjuvant Therapy Received			p=0.67
None	15 (52)	9 (53)	
5-FU Backbone	6 (21)	5 (29)	
Gemcitabine Backbone	8 (28)	3 (18)	
Adjuvant Regimen Received (%)			p=0.45
Gemcitabine alone	21 (72)	11 (65)	

	Gemcitabine in combination with another agent	6 (21)	6 (35)	
	Gemcitabine in combination with radiation	2 (7)	0 (0)	
Length of Adjuvant Therapy (%)				p=0.96
	<3 months	6 (21)	4 (24)	
	3-6 months	19 (66)	10 (59)	
	> 6 months	3 (10)	2 (12)	
	Date not available	1 (3)	1 (6)	

Supplementary Table 3: Clinical characteristics of the external test set from UPMC. Related to Figure 4.

Table describing clinical characteristics among patients in the UPMC cohort who were signature + vs. signature -. P-values correspond to chi-squared tests run with the exception of the variable age, for which a Wilcoxon Rank Sum Test was run.

Supplementary Table 4

	Signature +	Signature -	
n	74	87	
Age, Median (IQR)	62 (53, 69)	63 (57, 69)	p=0.54
Gender (%)			p=0.86
Female	36 (49)	40 (46)	
Male	38 (51)	47 (54)	
Tumor Grade (%)			p=0.09
G0	0 (0)	1 (1)	
G1	27 (36)	19 (22)	
G2	15 (20)	24 (28)	
G3	32 (43)	39 (45)	
G4	0 (0)	4 (5)	

Supplementary Table 4: Clinical characteristics of the external test set from Copenhagen. Related to Figure 4. Table describing clinical characteristics among patients in the Copenhagen cohort who were signature + vs. signature -. P-values correspond to chi-squared tests run with the exception of the variable age, for which a Wilcoxon Rank Sum Test was run.