Supplementary Methods

Immunohistochemistry

Primary antibodies S100 (Dako Omnis GA50461-2, 1:2), cytokeratin AE1/AE3 (EMD Millipore, IHCR2025-6, 1:6), and myelin basic protein (MBP, Cell Signaling #78896, 1:1200) were incubated for 2h at room temperature. Mouse (X0931) or rabbit IgG (X0936, both Dako) were used as negative controls (2 µg/mL). Antigen retrieval was performed with citrate buffer pH 6.0. Epitope blocking was with 1% bovine serum albumin in 1x phosphate buffered saline (1h, room temperature). Secondary antibody reactions were with streptavidin-biotin complex, including biotinylated goat anti-rabbit IgG (#GR602H), biotinylated goat anti-mouse IgG (#GM601H), streptavidin HRP Label (#HP604H), and 3,3'-Diaminobenzidine (DAB) chromogen (#DB801R, all from Biocare Medical). Slides were counterstained with Mayer's hematoxylin.

Cell culture and qPCR

50B11 cells were cultured in Neurobasal medium (Gibco, #21103-049) supplemented with 10% FBS (Gibco, #10082-147), B27 supplement (18 µl/ml, Gibco, #17504-044), 20% glucose (10 µl/ml, Sigma, #G-7520), 200 mM L-Glutamine (2.49 µl/ml, ATCC, #30-2214), and 1% Penicillin/Streptomycin (Gibco, #15140-122). S16 cells were cultured in DMEM (ATCC, #30-2002), supplemented with 10% FBS and 5% Penicillin/Streptomycin. ATCC cells were genotyped at the vendor.

UM-SCC-29 (from T. Carey, University of Michigan, genotyped at our laboratory) was used to generate cancer conditioned medium as follows: cells were plated in a 100 mm dish at 1.2 million cells per dish in DMEM (Gibco, #11965-092) supplemented with 10% FBS and 1% Penicillin/Streptomycin for 24h. Cells were washed once in either blank DMEM (#30-2002) or blank Neurobasal medium (Gibco, #21103049) and incubated with 5 mL of blank medium for 16h to generate conditioned medium. 50B11 and S16 cells were plated at 0.7 million cells per dish in their corresponding medium described above. After 24h, cells were washed once and incubated in blank medium for 16h. UM-SCC-29 conditioned medium was collected and centrifuged at 10,000 rpm for 10 minutes at 4ºC, and applied to 50B11 and

S16 cells for 6, 10, 24, and 48h. For 50B11 cells, supplements B27, glucose and L-glutamine were added to the conditioned medium. Cells were harvested in QIAzol (QIAGEN, #79306).

Dorsal root ganglia (DRG) co-culture experiments were performed as follows. UM-SCC-29 cells were plated in a 24-well plate using DMEM complete medium, 4 wells per time point, at the designated numbers: $6x10^4$, $5x10^4$, and $4 x10^4$ for 24, 48, and 72h, respectively. Medium was also added to control blank wells. One day after plating, 48 DRGs from one 9 week-old female rat (Charles River Sprague Dawley rat, # 001) were extracted and cultured for 1h in complete DMEM supplemented with 2% Penicillin/Streptomycin. All wells containing UM-SCC-29 or blank were then washed once with blank DMEM; DMEM supplemented with 0.5% FBS was subsequently added to all wells. Cell culture transwell inserts with pore size of 0.4 µm (Falcon, # 353095) were added to the wells and filled with 300 µl of blank DMEM. DRGs were then transferred to the inserts (2 DRGs per insert). Each group received 2 cervical, 4 thoracic and 2 lumbar DRGs. After 24, 48 and 72h, 8 DRGs from each group were pooled, lyzed in Qiazol (QIAGEN, #79306) and homogenized using a rotor (Omni International, # TH01) and QiaShredder columns (Qiagen, # 79654).

RNA was extracted with the miRNA extraction kit (QIAGEN, #217004) and converted to cDNA. qPCR was performed using SybrGreen Mastermix (Applied Biosystems, #4309155). Primers are listed in Supplementary Table S2.

Tissue analysis for myelin basic protein

Sections from the same 8 samples used to perform the spatial transctiptomic assessment were stained for MBP as previously described in the immunohistochemistry section. Parameters to classify nerves into close to tumor or far from tumor were also the same used in the GeoMx analysis. Using digital images from tissue slides and the Halo platform, MBP staining intensity and proportion were quantified. Intensity was scored as zero for no stain, 1 for weak, 2 for moderate, and 3 for strong inensity stain. Proportion was scored as zero for no nerve fibers stained, 1 for one to 25%, 2 for 26 to 50%, and 3 for over 50% of nerve fibers presenting stain. A combined score was obtained by multiplying intensity versus proportion.
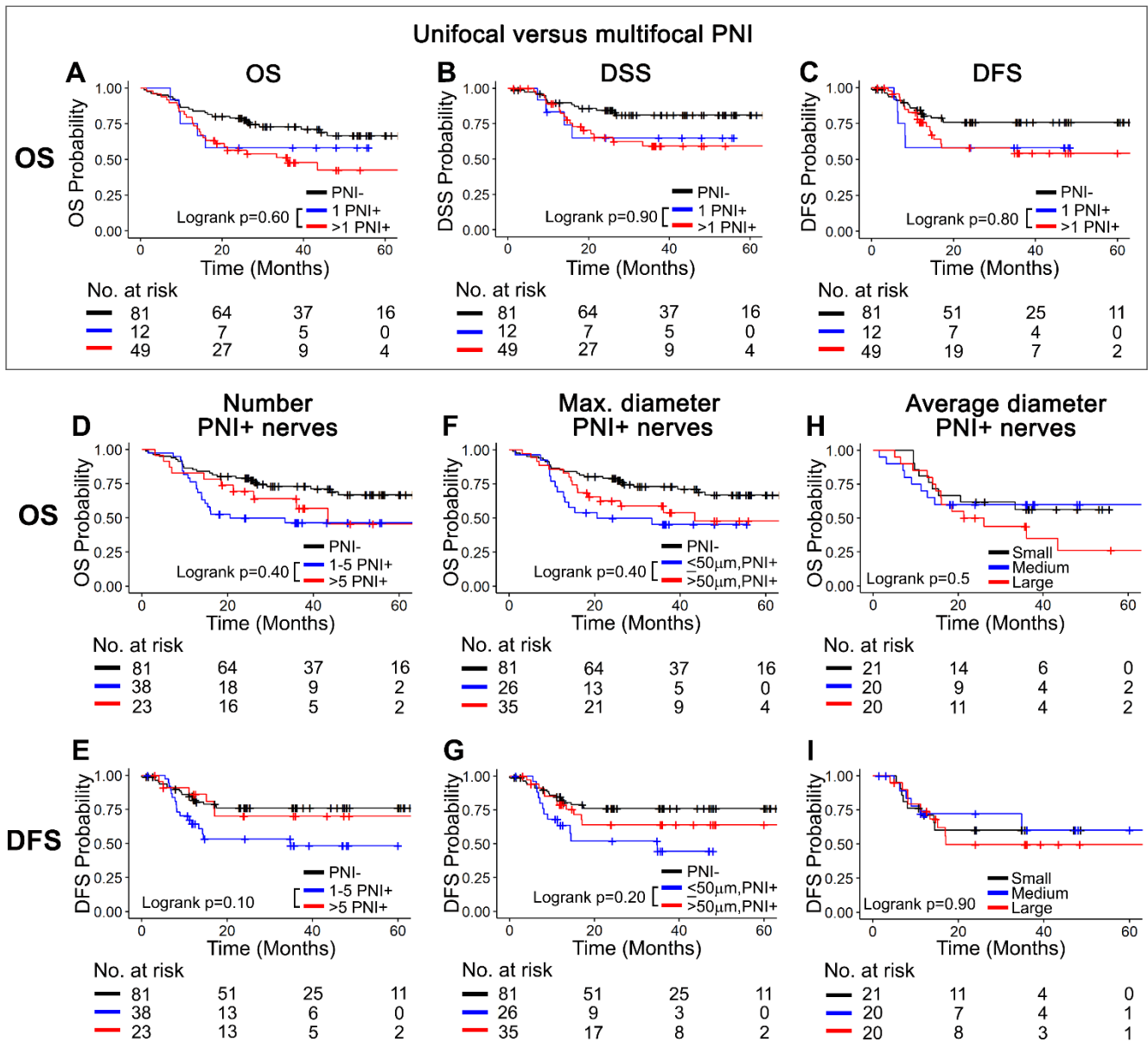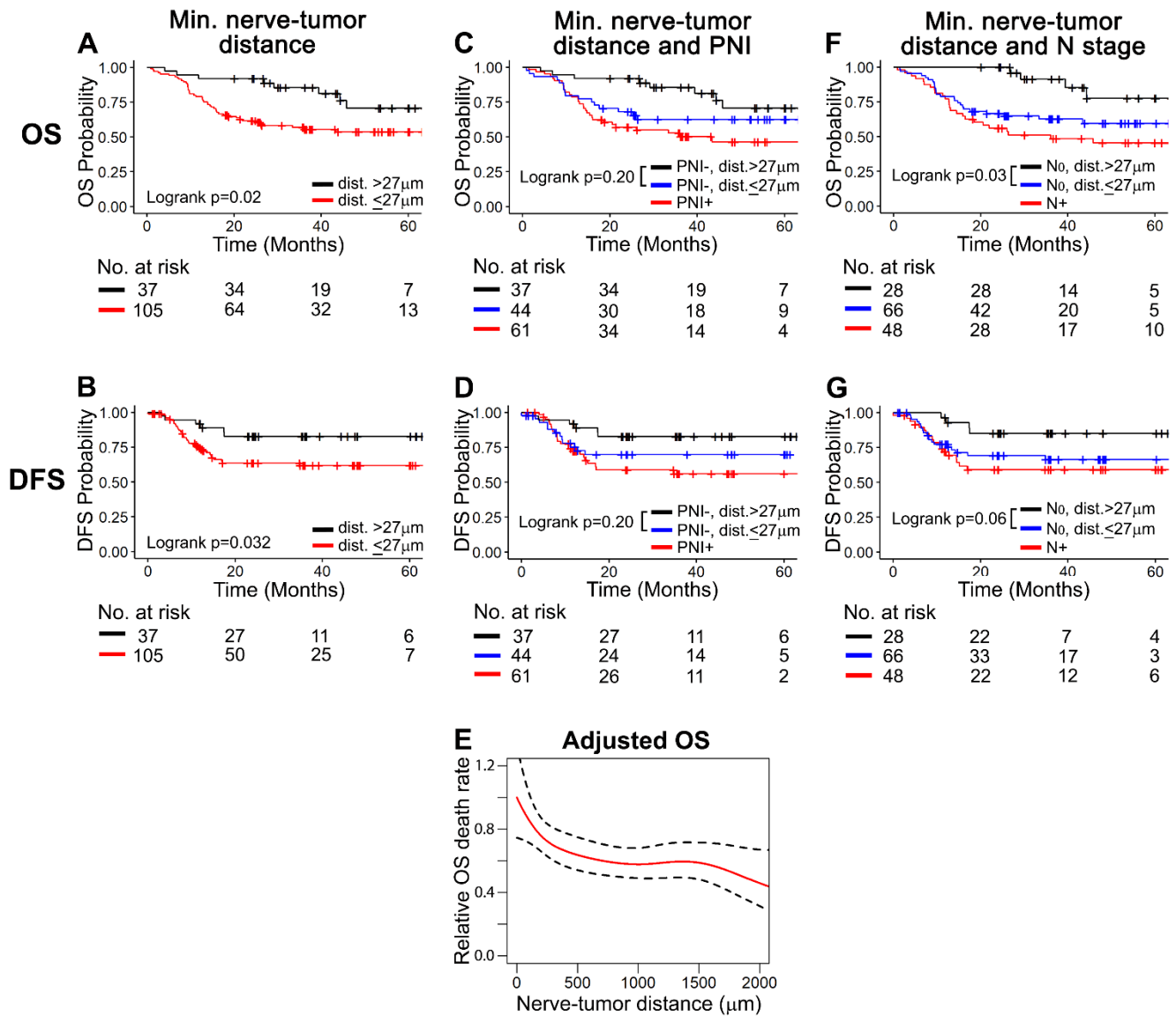
Supplementary Figures
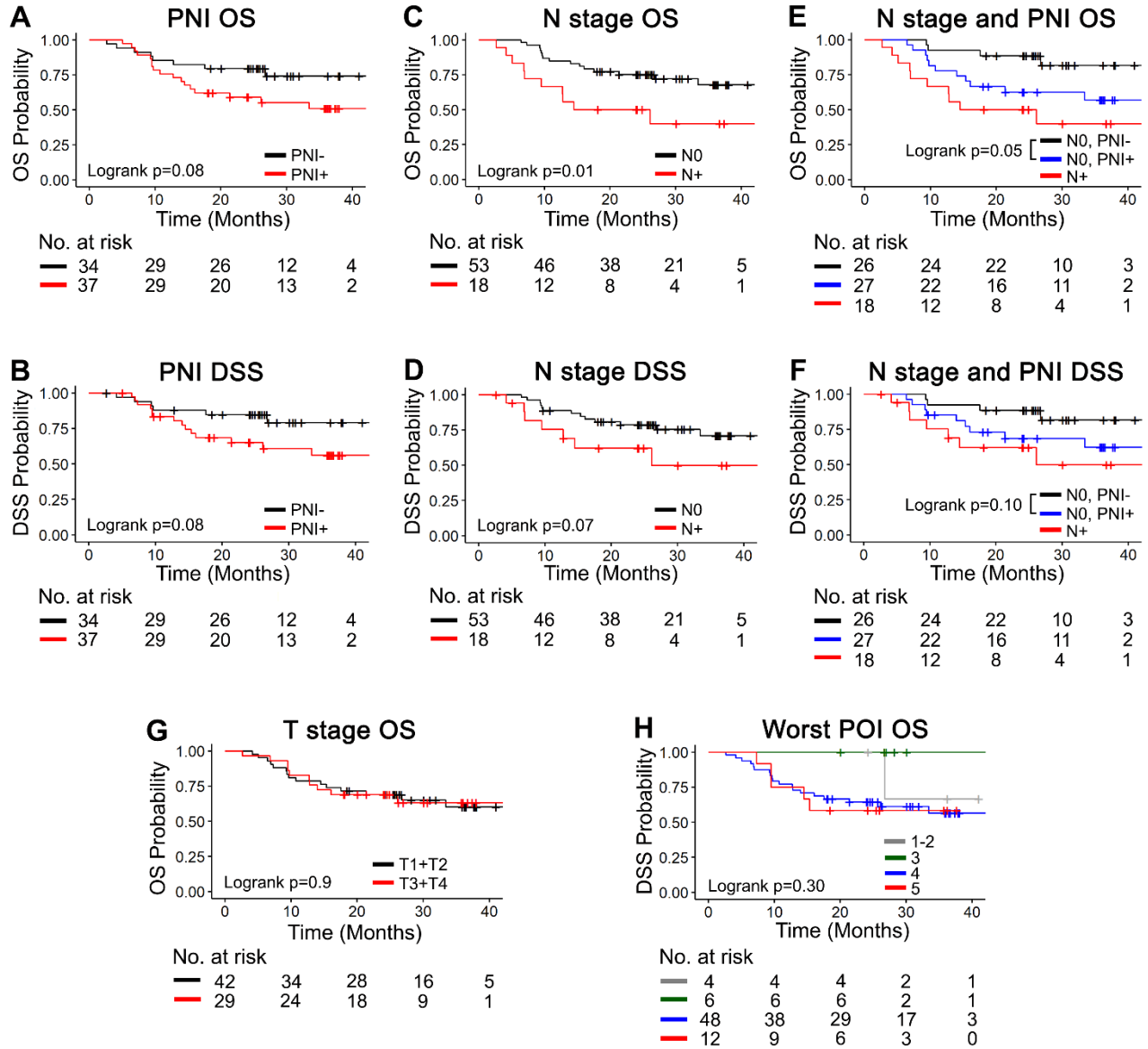
Supplementary Figure 1: Kaplan-Meier plots for OS and DSS related to PNI and other tumor characteristics for cohort 2 patients (n=71). **A** and **B,** PNI status. **C** and **D,** Lymph node metastasis clinical status. **E** and **F,** Node-negative patients stratified by PNI status. **G,** Tumor stage with patients stratified as T1+T2 and T3+T4. **H,** Worst pattern of invasion (POI). The number of patients at risk for each group at each time point is shown below the plots. PNI was assessed using H&E + IHC.
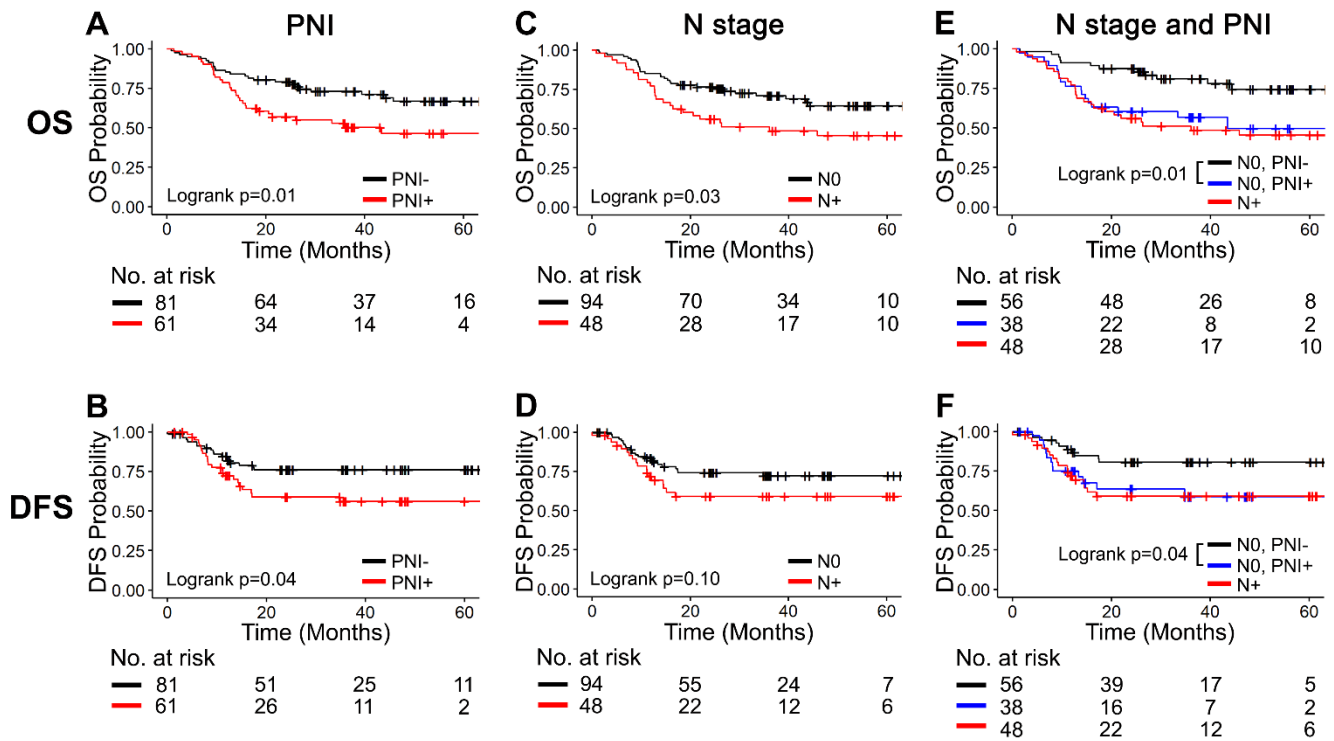
Supplementary Figure 2: Kaplan-Meier plots for OS and DFS related to PNI and N stage (n=142 patients). **A** and **B,** PNI status. **C** and **D,** Lymph node metastasis clinical status. **E** and **F,** Node-negative patients stratified by PNI status. The number of patients at risk for each group at each time point is shown below the plots. PNI was assessed using H&E + IHC.

Supplementary Figure 3: Kaplan-Meier plots for OS, DSS, and DFS related to PNI-positive nerve characteristics (n=142 patients). **A, B,** and **C,** PNI status as unifocal (one PNI-positive nerve per patient) versus multifocal (more than one PNI-positive nerve per patient). **D** and **E,** Number of PNI-positive nerves per patient with a cut-off of five PNI-positive nerves. **F** and **G,** Maximum nerve diameter of PNI-positive nerves per patient; a cutoff of 50 μm was calculated using regression tree analysis. **H** and **I,** Average diameter of PNI-positive nerves per patient, stratifying patients into small (diameter < 29.2 μm), medium (29.2 - 47.5 μm) and large (> 47.5 μm) nerve diameter. The number of patients at risk for each group at each time point is shown below the plots. PNI was assessed using H&E + IHC.

Supplementary Figure 4: Nerve tumor distance and survival (n=142 patients). **A** and **B,** Kaplan-Meier plots for OS and DFS stratifying patients based on minimum nerve-tumor distance of 27 μm. **C** and **D,** Kaplan-Meier plots for OS and DFS with PNI-negative patients stratified by minimum nerve-tumor distance of 27 μm. **E,** Adjusted Cox Additive modeling of nerve-level data weighted by number of nerves per patient and adjusted for age, ACE comorbidities, and tumor differentiation. Data is plotted as relative OS death rate as a function of nerve-tumor distance. **F** and **G,** Kaplan-Meier plots for OS and DFS with node-negative patients stratified by minimum nerve-tumor distance of 27 μm. The number of patients at risk for each group at each time point is shown below the plots. PNI was assessed using H&E + IHC.

A AJCC Stage re-classification

Stage (AJCC 7th Edition)	Stage (AJCC 8th Edition)					
	NA	I	II	III	IV	Total
I	1	11	8	0	1	21
II	1	7	13	10	9	40
III	0	2	1	5	10	18
IV	0	0	2	11	50	63
Total	2	20	24	26	70	142

B AJCC 8th Edition stage frequency

T	Frequency	Percent
1	24	16.90
2	43	30.28
3	37	26.05
4	36	25.35
Missing	2	1.41

N	Frequency	Percent
0	71	50.00
1	8	5.63
2a	11	7.75
2b	12	8.45
2c	2	1.41
3b	25	17.60
Missing	13	9.15

Stage	Frequency	Percent
I	20	14.08
II	24	16.90
III	26	18.31
IV	70	49.29
Missing	2	1.41

C AJCC 8th edition update from Figure 2C: Univariate Cox modeling for DSS

Stage AJCC 8th Edition	Hazard Ratio [CI]	P value
I	0.000 *	0.984
II	0.497 [0.204, 1.209]	0.123
III	**0.214 [0.065, 0.709]**	**0.012**
IV	ref.	

* Hazard ratio and confidence interval not estimable
ref. indicates the reference variable
Values in bold indicate significance at the 0.05 level

**D** AJCC 8th edition update from Figure 3C: Adjusted Weighted Cox modeling of all nerves

Variable	Hazard Ratio	P value
Log(Nerve-tumor distance)	**0.90**	**0.02**
AJCC 8th Edition Stage IV vs lower	**3.79**	**0.0004**
Well differentiated vs others	0.53	0.09
Comorbidities	1.23	0.22

Values in bold indicate significance at the 0.05 level

E

Adjusted DSS

F AJCC 8th edition update from Figure 4C: Adjusted Weighted Cox modeling of nerves in the tumor bulk

| Variable	Hazard Ratio	P value
Log(Nerve Diameter)	**2.63**	**0.01**
AJCC 8th Edition Stage IV vs lower	**3.02**	**0.003**
Well differentiated vs others	**0.47**	**0.04**
Comorbidities	1.32	0.15

Values in bold indicate significance at the 0.05 level

G

Adjusted DSS
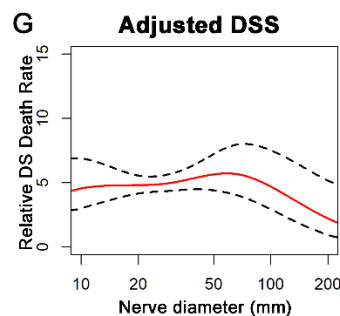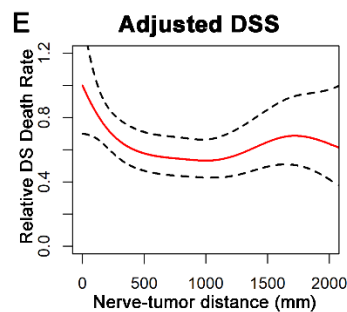
Supplementary Figure 5: Re-staging of 142 patients according to AJCC 8th edition. **A,** Comparison between 7th and 8th edition AJCC staging; 2 patients with non-available information were excluded from all subsequent analyses using AJCC 8th edition. **B,** Updated pathologic T, N and stage frequencies from 142 patients. **C,** Univariate Cox modeling using AJCC 8th edition and with DSS as outcome. **D,** Adjusted Weighted Cox modeling of all nerves in 140 samples. **E,** Adjusted Cox Additive modeling of nerve-level data weighted by number of nerves per patient and adjusted for AJCC 8th edition stage and differentiation status. Data are plotted as relative DSS death rate as a function of nerve-tumor distance. **F,** Adjusted Weighted Cox modeling of all nerves in 140 samples. **G,** Adjusted Cox Additive modeling of nerve-level data weighted by number of nerves per patient and adjusted for AJCC 8th edition stage and differentiation status. Data is plotted as relative DSS death rate as a function of nerve diameter.
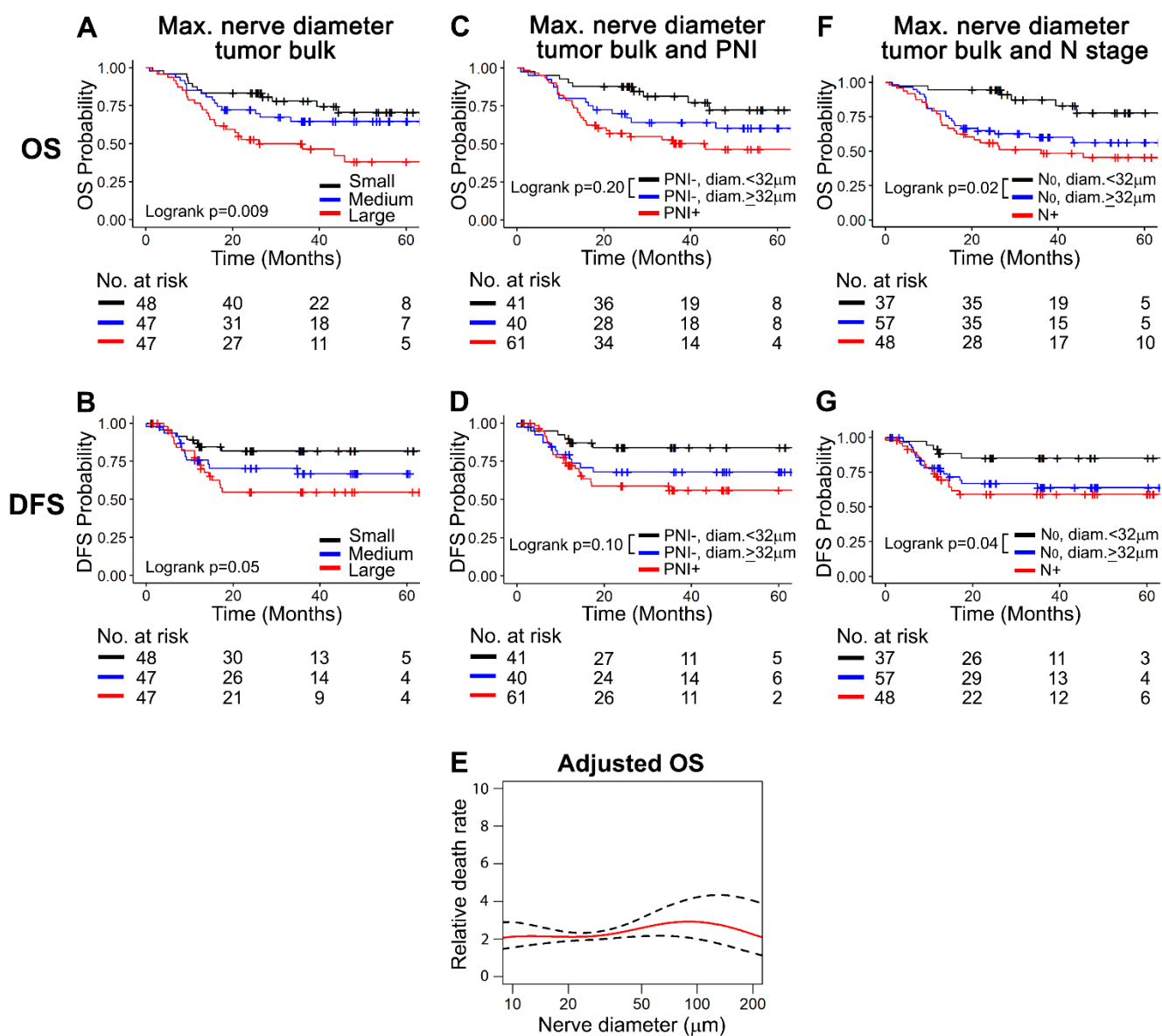
Supplementary Figure 6: Association of nerve diameter in tumor bulk with survival (n=142 patients). **A** and **B,** Kaplan-Meier plots for OS and DFS stratifying patients based on maximum nerve diameter in tumor bulk per patient. Patients are split into tertiles of nerve diameter in tumor bulk. **C** and **D,** Kaplan-Meier plots for OS and DFS with PNI-negative patients stratified by a maximum nerve diameter in the tumor bulk of 32 μm. **E,** Adjusted Cox Additive modeling of nerve-level data weighted by the number of nerves per patient and adjusted for age, ACE comorbidities and tumor differentiation status. Data are plotted as relative OS death rates as a function of nerve diameter in tumor bulk. **F** and **G,** Kaplan-Meier plots for OS and DFS with node-negative patients stratified by maximum nerve diameter in tumor bulk of 32 μm. The number of patients at risk for each group at each time point is shown below the plots. PNI is assessed using H&E + IHC.
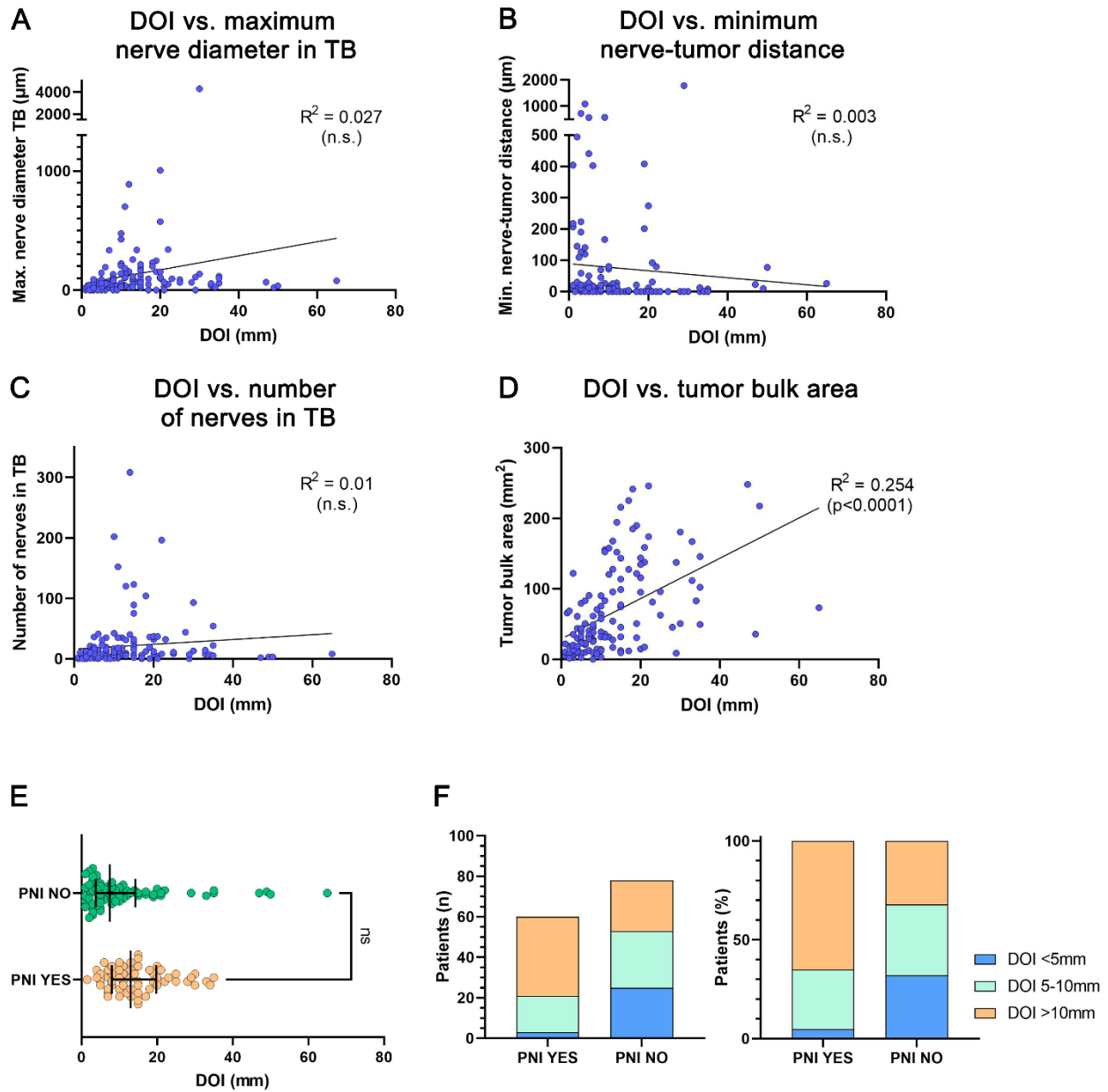
Supplementary Figure 7: Correlation between depth of invasion (DOI) and nerve-related parameters. **A, B,** and **C,** Correlation between DOI and nerve-related characteristics: maximum nerve diameter in tumor bulk, minimum nerve-tumor distance and number of nerves in tumor bulk. **D,** Correlation between DOI and tumor bulk area. Pearson's correlation with significance level at $p<0.05$ was used for **A, B, C** and **D**. **E,** DOI of PNI-positive and PNI-negative patients; no significant difference between groups (t test). **F,** PNI-positive and PNI-negative patients' frequencies in three thresholds of DOI; left graph shows number of patients and right graph shows percentage of patients in each group. All data is from 138 patients with available DOI information in pathology reports. PNI is assessed using H&E + IHC.
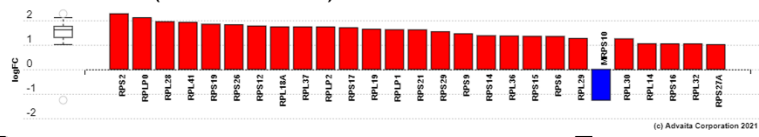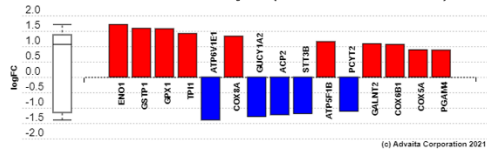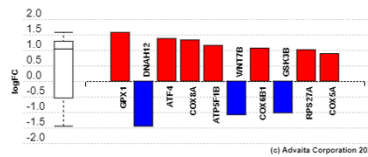
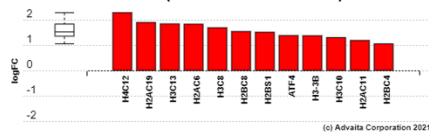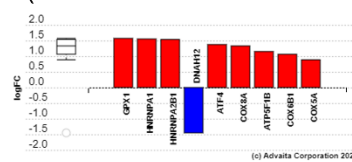**Supplementary Figure 8:** iPathway guide analysis of 159 DEGs in NC vs NF. **A** through **F,** Top 6 pathways ordered by gene representation. Genes in red are upregulated and genes in blue are downregulated in NC. Pathways Ribosome **(A)**, Alcoholism **(C),** and Systemic Lupus Erythematosus **(D)** were significant at FDR <0.05. **G,** Top 10 gene ontology (GO) terms for molecular function. **H,** Top 10 GO terms for biological process. **I,** Top 10 GO terms for cellular component. All GO terms in **G**, **H**, and **I** are significant at FDR<0.05.

Supplementary Tables

Table S1: Descriptive statistics for OS, DSS, and DFS

	Cohort 1 (n=71)[¶]	Cohort 2 (n=71)	Combined (N=142)
Overall Survival (OS)			
Number of events, N (%)	30 (42.2)	25 (35.2)	55 (38.7)
3-year OS probability	0.66 (0.56, 0.78)	0.60 (0.48, 0.75)	0.64 (0.56, 0.73)
Median follow-up time (months)	56.2 (53.9, 61.6)	30.1 (25.7, 35.7)	48.0 (38.0, 53.7)
Disease Free Survival (DFS)			
Number of events, N (%)	18 (25.3)	23 (32.4)	41 (29.6)
3-year DFS probability	0.72 (0.62, 0.84)	0.62 (0.50, 0.77)	0.69 (0.60, 0.77)
Median follow-up time (months)	47.5 (44.3, 48.1)	24.1 (24.0, 34.9)	35.6 (24.7, 39.3)
Disease Specific Survival (DSS)			
Number of events, N (%)	15 (19.7)	20 (28.2)	35 (24.6)
3-year DSS probability	0.77 (0.67, 0.88)	0.66 (0.55, 0.81)	0.73 (0.65, 0.81)
Median follow-up time (months)	57.0 (48.6, 56.3)	27.0 (24.9, 31.0)	39.5 (36.2, 48.0)

[¶] Schmitd et al., 2018

The median survival probability for OS, DFS, and DSS is not estimable

Table S2: Primer sequences

Gene	Species	Forward	Reverse	Product size (bp)
Cfl1	Rat	AGGAGATTCTGGTAGGAGATGT	GGTCTCATAGGTTGCGTCATAG	116
Marcks	Rat	TGGGTCTTTCCCACTTTAACC	CCCATTTCAGTTGAGTGCTACTA	90
Fabp5	Rat	AGAAGTGGGAAGGGAAAGAAAG	GTACCTTCTCATAGACCCGAGTA	111
Hnrnpa1	Rat	ACTCTGAAGCCATCTTGGTAAA	CCACCCAAGCAACCATAAATAAA	103
Gstp1	Rat	GAGACCTCACCCTTTACCAATC	CTCCTTCTGGTCTTTCCCATAAA	83
Cox5a	Rat	GAGGGCATGTAGACGGTTAAA	CCTTATGAGGTCCTGCTTTGT	83
Pdcd2	Rat	GCATCGGGTACACAGAAGAA	GAGGTAGTGAGGCATAAGGAAC	104
Anxa2	Rat	GGCAGAGGATGGTTCTGTTATT	GACATCGGTTCCTTTCCTCTTC	94
Mbp	Rat	CCTGTCCCTCAGCAGATTTAG	TCCCTTGTGAGCCGATTTATAG	97

bp – base pairs

Table S3: Relationship between PNI (assessed by H&E + IHC) and other variables (Unadjusted logistic regression of PNI status)

Predictor	OR, [95% CI]
Age	1.01 [0.98, 1.03]
Tumor Stage (AJCC 7th Edition)	
I	ref.
II	3.14 [0.89, 11.04]
III	**5.31 [1.27, 22.24]**
IV	**3.86 [1.17, 12.78]**
T Stage	
1	ref.
2	2.64 [0.85, 8.20]
3	**5.14 [1.29, 20.52]**
4	**3.32 [1.07, 10.34]**
N Stage	
N_0	ref.
N+	1.36 [0.67, 2.73]
Differentiation	
Poor	0.26 [0.07, 1.01]
Moderate	ref.
Well	0.62 [0.31, 1.24]
Worst POI [Φ]	
1-2	ref.
3	2.08 [0.62, 6.94]
4-5	**2.81 [1.09, 7.26]**

[Φ] Worst pattern of invasion according to Brandwein-Gensler et al., 2005.

Values in bold indicate significance at the 0.05 level.

ref. indicates the reference variable

Table S4: Unadjusted Cox modeling of patient characteristics (n=142).

Parameter	Unit or group	OS HR [95% CI]	p	DFS HR [95% CI]	p	DSS HR [95% CI]	p
Age	1 year	1.01 [0, 1.03]	0.541	0.99 [0.97, 1.02]	0.530	0.98 [0.96, 1.01]	0.176
ACE Comorbidities	None	ref.		ref.		ref.	
	Mild	1.83 [0.83, 4.06]	0.135	1.16 [0.53, 2.57]	0.710	0.98 [0.42, 2.31]	0.963
	Moderate	**2.51 [1.00, 6.26]**	**0.049**	1.21 [0.45, 3.26]	0.702	1.45 [0.53, 4.01]	0.472
	Severe	**4.77 [1.87, 12.18]**	**0.001**	2.22 [0.79, 6.23]	0.132	2.15 [0.70, 6.60]	0.179
T stage	1 or 2	ref.		ref.		ref.	
	3 or 4	1.57 [0.92, 2.68]	0.097	1.26 [0.68, 2.33]	0.463	1.45 [0.74, 2.81]	0.277
N stage	N_0	ref.		ref.		ref.	
	N-positive	**1.81 [1.06, 3.08]**	**0.029**	1.66 [0.90, 3.08]	0.106	**2.40 [1.24, 4.66]**	**0.009**
AJCC Stage*	I	**0.29 [0.10, 0.82]**	**0.02**	0.46 [0.16, 1.34]	0.15	0.34 [0.10, 1.14]	0.08
	II	**0.50 [0.26, 0.97]**	**0.04**	0.55 [0.25, 1.17]	0.12	0.45 [0.19, 1.05]	0.07
	III	0.53 [0.22,1.26]	0.15	0.52 [0.18, 1.49]	0.22	0.58 [0.20, 1.70]	0.32
	IV	ref.		ref.		ref.	
PNI	No	ref.		ref.		ref.	
	Yes	**1.93 [1.13, 3.29]**	**0.016**	**1.92 [1.04, 3.56]**	**0.039**	**2.33 [1.18, 4.58]**	**0.014**
Expanded N stage	N_0, PNI-negative	ref.		ref.		ref.	
	N_0, PNI-positive	**2.45 [1.19, 5.06]**	**0.015**	**2.36[1.03, 5.38]**	**0.042**	**3.36 [1.24, 9.09]**	**0.017**
	N-positive	**2.72 [1.39, 5.33]**	**0.004**	**2.47 [1.14, 5.35]**	**0.022**	**4.41 [1.75, 11.12]**	**0.002**
Differentiation	Moderate	ref.		ref.		ref.	
	Poor	0.85 [0.35, 2.07]	0.726	1.08 [0.44, 2.66]	0.872	1.39 [0.55, 3.53]	0.487
	Well	0.70 [0.40, 1.23]	0.217	**0.47 [0.24, 0.93]**	**0.030**	0.60 [0.28, 1.25]	0.170
Worst POI $^\Phi$	1 or 2	ref.		ref.		ref.	
	3	1.01 [0.39, 2.66]	0.979	0.35 [0.07, 1.63]	0.180	0.20 [0.02, 1.59]	0.127
	4 or 5	1.47 [0.73, 2.97]	0.279	1.44 [0.66, 3.15]	0.354	1.38 [0.60, 3.18]	0.447
log(Minimum nerve-tumor distance)		**0.67 [0.49, 0.92]**	**0.013**	**0.66 [0.46, 0.95]**	**0.026**	**0.56 [0.36, 0.85]**	**0.007**
log(Average nerve-tumor distance)		**0.45 [0.29, 0.68]**	**0.000**	**0.48 [0.30, 0.77]**	**0.002**	**0.41 [0.25, 0.66]**	**0.000**
log(Maximum nerve diameter in tumor bulk)		**1.48 [1.05, 2.09]**	**0.025**	1.47 [0.99, 2.17]	0.053	**1.74 [1.11, 2.73]**	**0.016**
log(Average nerve diameter in tumor bulk)		**1.75 [1.06, 2.90]**	**0.028**	1.76 [0.99, 3.12]	0.053	**2.22 [1.11, 4.44]**	**0.025**

*American Joint Committee on Cancer (AJCC) TNM staging system 7th Edition.

$^\Phi$ Worst pattern of invasion according to Brandwein-Gensler et al., 2005.

PNI was assessed using H&E + IHC

ref. indicates the reference variable

Table S5: Characteristics of 9137 nerves measured

Nerve-related characteristic		Missing, N (%)
Nerve-Level PNI	N (%)*	
No	8505 (93.1)	225 (2.4)
Yes	407 (4.5)	
Nerve location	N (%)*	
Tumor margin	6156 (67%)	6 (<1)
Tumor bulk	2975 (33%)	
Nerve diameter range (µm)	9-4708	
Nerve diameter median (µm)	25	
Nerve-tumor distance range (µm)	0-2089	
Nerve-tumor distance median (µm)	549	
Number of PNI-positive nerves per patient (range)	0-36	

* Note: Percent includes NA values.
PNI was assessed using H&E + IHC

Table S6: Nerve-related characteristics of the sample

Nerve and sample characteristics	Cohort 1 (n=71)[¶Φ]	Cohort 2 (n=71)	Combined (n=142)
	N (%)* or Mean[†]	N (%)* or Mean[†]	N (%)* or Mean[†]
Tumor bulk area (mm^2)	71.79	58.96	65.37
Margin Area (mm^2)	31.50	47.31	39.41
Missing	9 (12.7)	0 (0)	9 (6.3)
Total area analyzed (bulk + margin, mm^2)	103.29	106.27	104.78
Number of nerves in total area	40.6	88.1	64.4
Number of nerves in tumor bulk	16.8	24.9	20.9
Number of nerves in tumor margin	23.8	63.2	43.5
Average nerve diameter (µm)	41.1	33.8	37.4
Maximum nerve diameter (µm)	333.3	185.0	258.6
Average nerve area (µm^2)	14,305.7	7,835.0	11,047.4
Maximum nerve area (µm^2)	317,163	188,318.3	252,283.9
Average nerve-tumor distance (µm)	618.6	841.6	730.1
Minimum nerve-tumor distance (µm)	77.3	72.8	75.1

[¶] Schmitd et al., 2018.
[Φ] Cohort 1 has one patient with no nerves to evaluate.
[†] The mean values presented are the mean of the number, average, minimum and maximum values across patients.
* Percent includes NA values.

Table S7: Adjusted Cox modeling of nerve-level data and survival

Adjusted Weighted Cox modeling*	OS		DFS		DSS	
Model 1: All Nerves (n=9114[a])	HR [95% CI]	p-value	HR [95% CI]	p-value	HR [95% CI]	p-value
Log(Nerve-tumor Distance)	**0.88 (0.81, 0.95)**	**0.000**	0.92 (0.84, 1.00)	0.05	**0.87 (0.79, 0.96)**	**0.005**
AJCC Stage IV vs. lower [Φ]	**2.03 (1.17, 3.51)**	**0.01**	1.83 (0.98, 3.43)	0.06	**2.07 (1.05, 4.05)**	**0.03**
Well differentiated vs. others	0.70 (0.41, 1.20)	0.19	**0.42 (0.22, 0.82)**	**0.01**	0.50 (0.25, 1.02)	0.06
Comorbidities	**1.59 (1.21, 2.08)**	**0.000**	1.22 (0.87, 1.73)	0.25	1.30 (0.87, 1.94)	0.19
Age	1.01 (0.98, 1.04)	0.49				
Model 2: Nerves within Tumor Bulk (n=3000[b])	HR [95% CI]	p-value	HR [95% CI]	p-value	HR [95% CI]	p-value
Log(Nerve Diameter)	**2.16 (1.13, 4.12)**	**0.02**	**2.06 (1.07, 3.94)**	**0.03**	**2.50 (1.24, 5.06)**	**0.01**
AJCC Stage IV vs. lower [Φ]	1.47 (0.75, 2.88)	0.26	1.62 (0.72, 3.67)	0.24	1.80 (0.76, 4.23)	0.18
Well differentiated vs. others	0.72 (0.40, 1.27)	0.48	**0.40 (0.17, 0.96)**	**0.04**	0.48 (0.19, 1.20)	0.12
Comorbidities	**1.63 (1.20, 2.23)**	**0.002**	1.36 (0.92, 2.01)	0.13	1.41 (0.93, 2.15)	0.11
Age	1.01 (0.98, 1.04)	0.59				

* Weighted by the number of nerves within each subject.
[a] From 9137 nerves assessed in 142 patients (23 missing values).
[Φ] American Joint Committee on Cancer TNM staging system 7th Edition.
[b] 25 subjects with no nerves in tumor bulk were assigned 1 nerve with a diameter of zero μm.

Table S8: Adjusted Cox modeling of nerve diameter in the tumor bulk

Parameter	Unit or group	OS HR [95% CI]	p	DFS HR [95% CI]	p	DSS HR [95% CI]	p
Maximum nerve diameter in tumor bulk	Small	ref.		ref.		ref.	
	Medium	1.78 [0.82, 3.84]	0.142	**3.33 [1.28, 8.67]**	**0.014**	**5.13 [1.63, 16.17]**	**0.005**
	Large	**2.55 [1.23, 5.31]**	**0.012**	**3.07 [1.2, 7.86]**	**0.019**	**4.58 [1.45, 14.52]**	**0.010**
PNI and Maximum nerve diameter in tumor bulk	PNI-negative, nerve diameter <32μm	ref.		ref.		ref.	
	PNI-negative, nerve diameter ≥32μm	1.54 [0.67, 3.51]	0.309	2.29 [0.83, 6.29]	0.109	**3.73 [1.01, 13.8]**	**0.049**
	PNI-positive	**2.74 [1.27, 5.9]**	**0.010**	**3.47 [1.32, 9.16]**	**0.012**	**6.96 [1.96, 24.76]**	**0.003**

DFS and DSS are adjusted for AJCC stage and comorbidities. OS is adjusted for AJCC stage, comorbidities, and age.
PNI was assessed using H&E + IHC
ref. indicates the reference variable

Table S9: differentially expressed genes in NC vs NF

Top 50 upregulated in NC*			Top 50 downregulated in NC*		
Gene	Log2(FC)	Adjusted p-value	Gene	Log2(FC)	Adjusted p-value
KRT5	3.15	0.0842	MBP	-2.41	0.0828
KRT6A	2.79	0.0003	TCAP	-1.83	0.0008
TMSB10	2.51	0.0822	ACTA1	-1.80	0.0278
H4C12	2.28	0.0043	REC114	-1.78	0.0392
RPS2	2.27	0.0013	MME	-1.75	0.0144
RPLP0	2.11	0.0043	LCN12	-1.71	0.0122
DSP	2.08	0.0357	CEP164	-1.68	0.0710
H1-5	2.01	0.0230	A1BG	-1.62	0.0009
CFL1	2.01	0.0775	GDF7	-1.53	0.0822
COL4A1	2.00	0.0074	KLHL20	-1.52	0.0822
RPL28	1.95	0.0842	ELL	-1.50	0.0088
RPL41	1.92	0.0003	PDCD2	-1.50	0.0036
S100A11	1.92	0.0003	MYOZ1	-1.47	0.0003
H2AC19	1.89	0.0081	FBXL2	-1.46	0.0671
RPS19	1.85	0.0026	TRANK1	-1.46	0.0606
H3C13	1.84	0.0183	SLC16A2	-1.46	0.0117
CD44	1.82	0.0318	DNAH12	-1.45	0.0866
RPS26	1.82	0.0632	RNF212	-1.44	0.0309
H2AC6	1.82	0.0822	TTBK2	-1.42	0.0165
FABP5	1.82	0.0042	MCRS1	-1.40	0.0318
MYL6	1.81	0.0236	ATP6V1E1	-1.40	0.0435
COL3A1	1.78	0.0331	CES4A	-1.38	0.0208
RPS12	1.77	0.0309	KLK7	-1.35	0.0463
RPL18A	1.74	0.0643	OR2H2	-1.35	0.0405
RPL37	1.74	0.0030	ATG5	-1.34	0.0643
RPLP2	1.74	0.0429	EPPK1	-1.33	0.0720
NPM1	1.71	0.0059	ZBTB40	-1.32	0.0318
ENO1	1.71	0.0175	R3HCC1L	-1.32	0.0842
RPS17	1.70	0.0043	TAF1B	-1.31	0.0671
PTMA	1.69	0.0589	KRBOX4	-1.31	0.0657
H3C8	1.68	0.0055	GUCY1A2	-1.29	0.0885
ITGAV	1.66	0.0309	UBAC1	-1.28	0.0649
RPL19	1.64	0.0043	TGIF2	-1.28	0.0429
EMILIN1	1.64	0.0435	CWF19L2	-1.27	0.0828
RPLP1	1.62	0.0643	OTUD4	-1.26	0.0504
RPS21	1.62	0.0529	MRPS10	-1.26	0.0671
GSTP1	1.58	0.0117	HEPHL1	-1.25	0.0450
GPX1	1.57	0.0135	PLPP7	-1.23	0.0806
TAGLN2	1.57	0.0089	ACP2	-1.23	0.0510
HNRNPA1	1.55	0.0589	RASSF3	-1.22	0.0656
RPS29	1.54	0.0806	STT3B	-1.19	0.0822
HNRNPA2	1.54	0.0734	ICOS	-1.18	0.0842
H2BC8	1.53	0.0124	ZNF33B	-1.17	0.0376
HNRNPH1	1.53	0.0572	ZNF577	-1.16	0.0292
H2BS1	1.50	0.0178	ANAPC4	-1.15	0.0882
STAT2	1.47	0.0713	CASR	-1.15	0.0849
RPS9	1.46	0.0043	GRHL3	-1.14	0.0708
EIF4A1	1.44	0.0036	ITGB6	-1.14	0.0572
HDLBP	1.42	0.0610	PCYT2	-1.11	0.0572
TPI1	1.42	0.0987	MRPL58	-1.09	0.0888

* Ordered by log2(Fold Change)