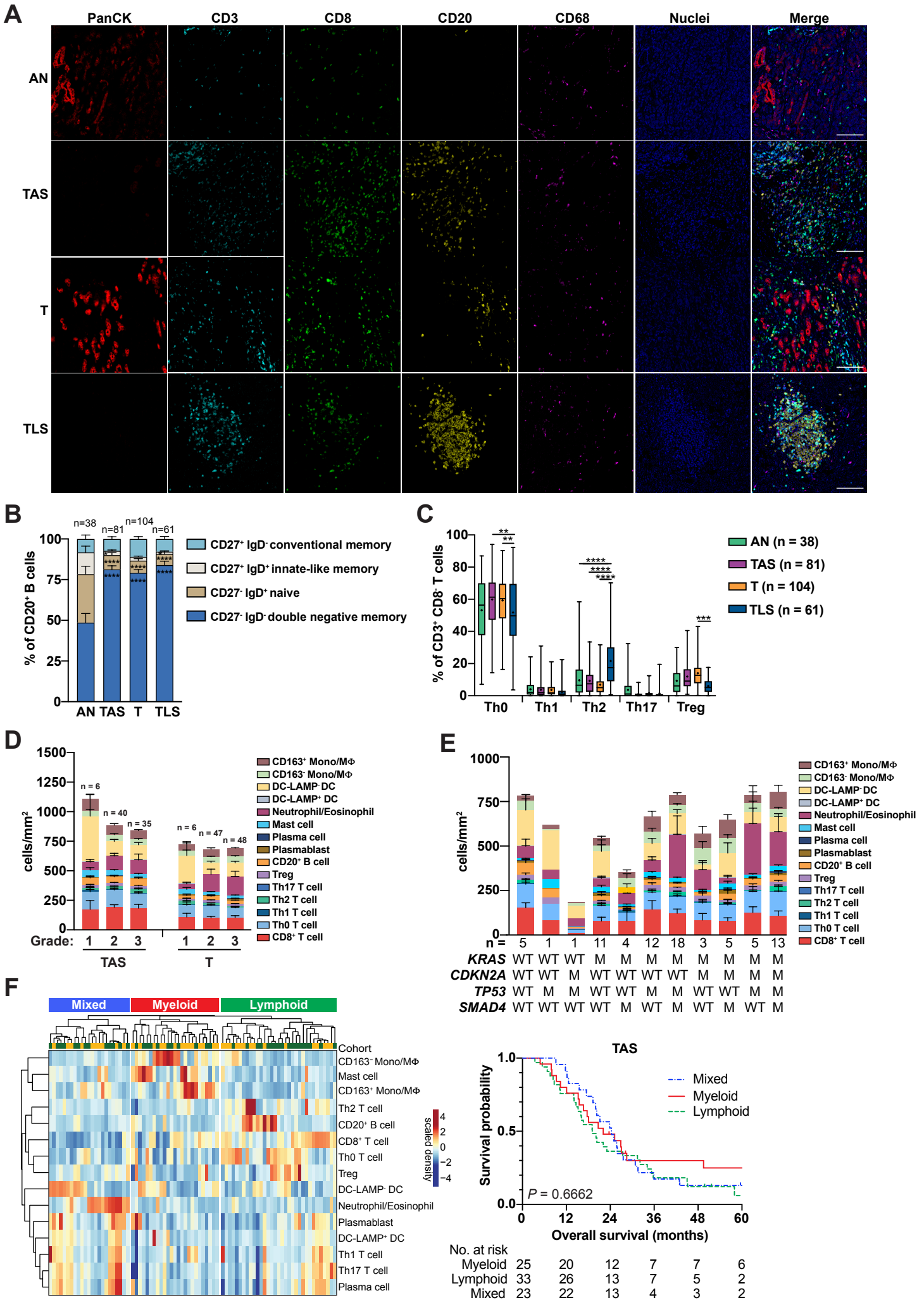
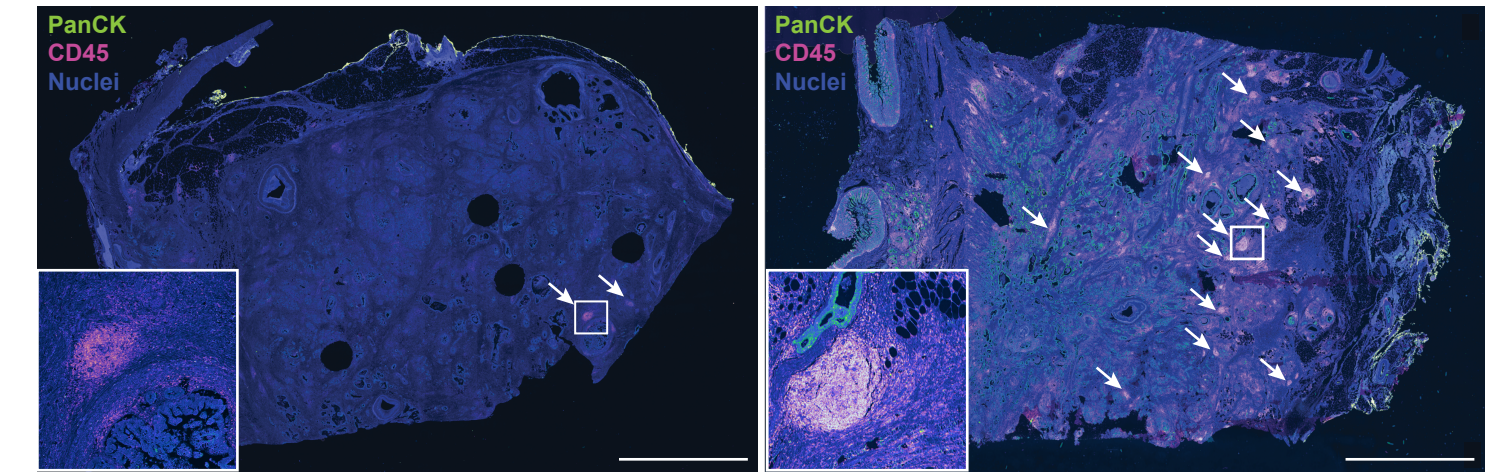
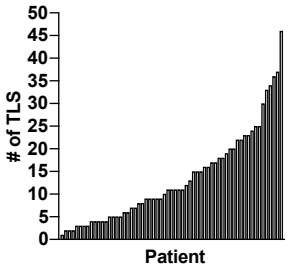
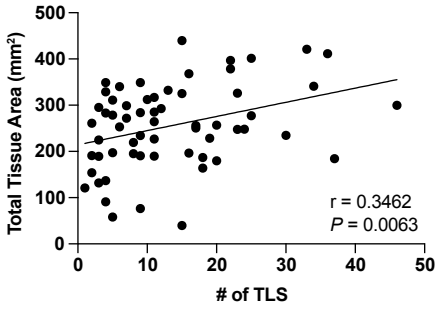
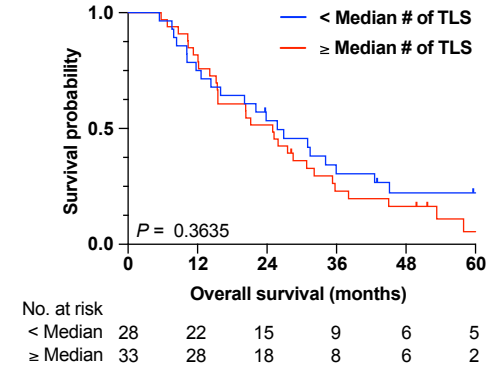
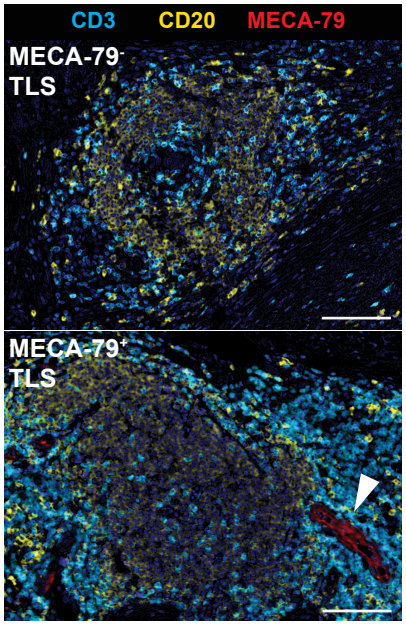

A
PanCK
CD3
CD8
CD20
CD68
Nuclei
Merge
AN
TAS
T
TLS
B
% of CD20+ B cells
CD27+ IgD- conventional memory
CD27+ IgD+ innate-like memory
CD27- IgD+ naive
CD27- IgD- double negative memory
AN TAS T TLS
n=38
n=81
n=104
n=61
C
% of CD3+ CD8- T cells
AN (n = 38)
TAS (n = 81)
T (n = 104)
TLS (n = 61)
Th0
Th1
Th2
Th17
Treg
D
cells/mm2
CD163+ Mono/MΦ
CD163- Mono/MΦ
DC-LAMP- DC
DC-LAMP+ DC
Neutrophil/Eosinophil
Mast cell
Plasma cell
Plasmablast
CD20+ B cell
Treg
Th17 T cell
Th2 T cell
Th1 T cell
Th0 T cell
CD8+ T cell
Grade: 1 2 3 1 2 3
TAS
T
E
cells/mm2
n = 5 1 1 11 4 12 18 3 5 5 13
KRAS WT WT WT M M M M M M M M
CDKN2A WT WT M WT WT WT WT M M M M
TP53 WT M M WT WT M M WT WT M M
SMAD4 WT WT WT WT M WT M WT M WT M
F
Mixed
Myeloid
Lymphoid
Cohort
CD163- Mono/MΦ
Mast cell
CD163+ Mono/MΦ
Th2 T cell
CD20+ B cell
CD8+ T cell
Th0 T cell
Treg
DC-LAMP- DC
Neutrophil/Eosinophil
Plasmablast
DC-LAMP+ DC
Th1 T cell
Th17 T cell
Plasma cell
scaled density
TAS
Survival probability
P = 0.6662
Overall survival (months)
No. at risk
Myeloid 25 20 12 7 7 6
Lymphoid 33 26 13 7 5 2
Mixed 23 22 13 4 3 2

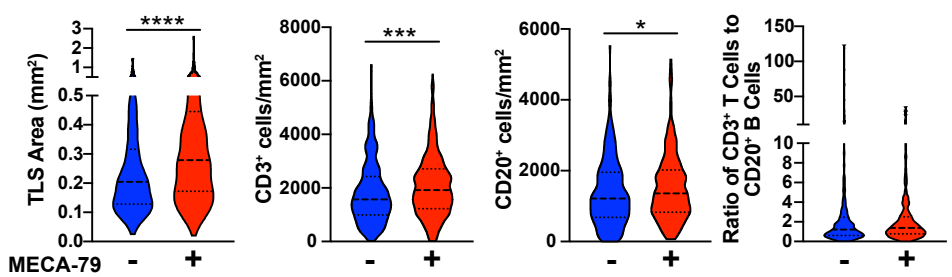

G
PanCK
CD45
Nuclei
PanCK
CD45
Nuclei

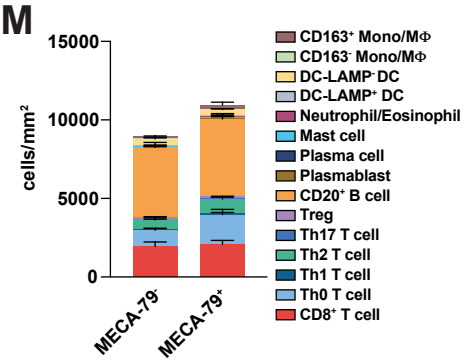

H
# of TLS
Patient

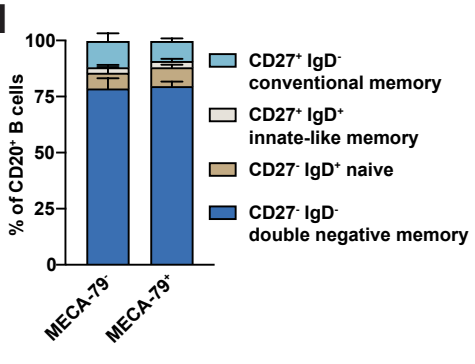

I
Total Tissue Area (mm²)
# of TLS
r = 0.3462
P = 0.0063

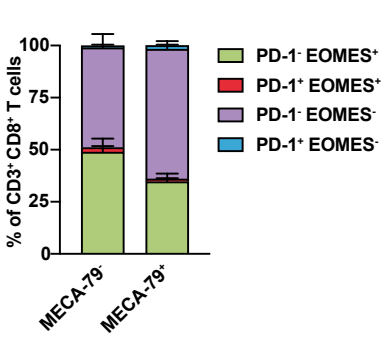

J
< Median # of TLS
≥ Median # of TLS
Survival probability
P = 0.3635
Overall survival (months)
No. at risk
< Median 28 22 15 9 6 5
≥ Median 33 28 18 8 6 2

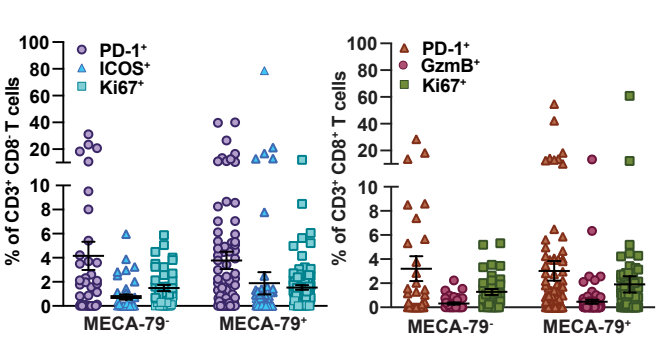

K
CD3
CD20
MECA-79
MECA-79⁻ TLS
MECA-79⁺ TLS


L
TLS Area (mm²)
CD3⁺ cells/mm²
CD20⁺ cells/mm²
Ratio of CD3⁺ T Cells to CD20⁺ B Cells
MECA-79

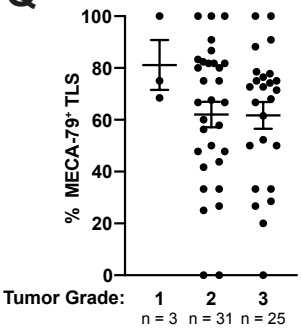

M
cells/mm²
CD163⁺ Mono/MΦ
CD163⁻ Mono/MΦ
DC-LAMP⁻ DC
DC-LAMP⁺ DC
Neutrophil/Eosinophil
Mast cell
Plasma cell
Plasmablast
CD20⁺ B cell
Treg
Th17 T cell
Th2 T cell
Th1 T cell
Th0 T cell
CD8⁺ T cell
MECA-79⁻
MECA-79⁺


N
% of CD20⁺ B cells
CD27⁺ IgD⁻ conventional memory
CD27⁺ IgD⁺ innate-like memory
CD27⁻ IgD⁺ naive
CD27⁻ IgD⁻ double negative memory
MECA-79⁻
MECA-79⁺

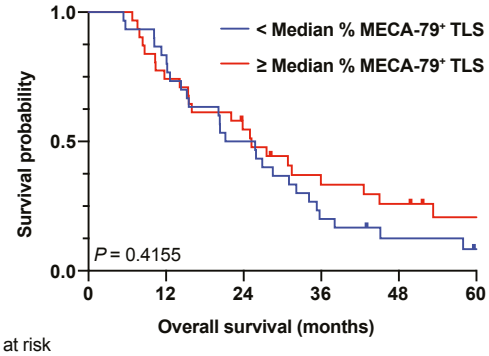

O
% of CD3⁺ CD8⁺ T cells
PD-1⁻ EOMES⁺
PD-1⁺ EOMES⁺
PD-1⁻ EOMES⁻
PD-1⁺ EOMES⁻
MECA-79⁻
MECA-79⁺


P
% of CD3⁺ CD8⁺ T cells
PD-1⁺
ICOS⁺
Ki67⁺
PD-1⁺
GzmB⁺
Ki67⁺
MECA-79⁻
MECA-79⁺

Q



R



No. at risk						
< Median	30	26	16	7	4	2
≥ Median	31	24	17	10	8	5

S

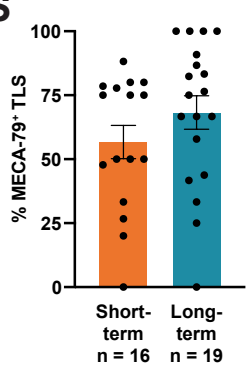


Figure S4, related to Figure 4. Leukocyte composition based on tumor grade, molecular status, and histopathologic region

(A) Single-channel pseudocolored images corresponding to composite images shown in Figure 4C. Scale bars, 100 μm. **(B-C)** B and T cell phenotyping in indicated histopathological regions of treatment-naïve tissue specimens, reflected as % of total $CD20^+$ B cells (panel B) and % of total presumed $CD4^+$ T cells identified from $CD3^+CD8^-$ cell populations (panel C). 'n' indicates number of patients in each category. Statistical differences in population frequencies across region types were evaluated by mixed model repeated measures ANOVA with Tukey-Kramer post-hoc correction. **(D)** Leukocyte composition of 'TAS' and 'T' from treatment-naïve PDAC based on histologic grade of primary tumors from Cohorts 1 and 2. Grade 1 = well-differentiated; Grade 2 = moderately differentiated; Grade 3 = poorly differentiated. 'n' indicates number of patient specimens in each category. **(E)** Leukocyte composition of treatment-naïve PDACs segregated by combined mutational profiles of *KRAS*, *CDKN2A*, *TP53*, and *SMAD4* in the subset of treatment-naïve specimens for which molecular status of all four genes was identified. (WT) indicates wild-type/intact gene; (M) indicates mutant/altered/lost gene. Number of unique cases in each group is reflected in top row. (B-E) Data are represented as mean ± SEM. **(F)** Unsupervised hierarchical clustering (left) of TAS regions from treatment naïve PDACs in Cohorts 1 and 2 (n = 81 total; columns) showing leukocyte subtypes (rows) and corresponding Kaplan-Meier curves comparing overall survival (right) for each cluster. *P*-value determined by log-rank test. **(G)** Pseudocolored images showing $PanCK^+$ (green) and $CD45^+$ (pink) cells in representative PDAC specimens with low abundance of TLS (left; 2 TLS total) and high abundance of TLS (right; 36 TLS total). Arrows indicate examples of TLS in each specimen, with higher magnification images of TLS shown in boxed insets. Scale bars, 5 mm. **(H)** Range of

TLS quantity across the 61 treatment-naïve tissue specimens in which TLS were identified. **(I)** Spearman correlation of total area of surgical resection specimen and number of TLS (n = 61 patients). **(J)** Kaplan-Meier curves displaying OS based on quantity of TLS among treatment-naïve patients in which at least one TLS was identified. *P*-value determined by log-rank test. **(K)** Representative pseudocolored images of TLS lacking HEVs (MECA-79$^-$) or containing HEVs (MECA-79$^+$, arrowhead). Scale bars, 100 μm. **(L)** Analysis of indicated parameters of individual TLS within treatment-naïve PDAC specimens (MECA-79$^-$ TLS, n = 268; MECA-79$^+$ TLS, n = 519). Comparisons were made using Mann-Whitney U test. **(M-P)** Profiling of leukocyte composition and functionality of a subset of MECA-79$^-$ and MECA-79$^+$ TLS quantitively analyzed with the full lymphoid, myeloid and functional mIHC antibody panels (n = 39 and 92 TLS, respectively, from a total of 61 patient specimens). **(M)** Lymphoid and myeloid composition, **(N)** frequency of indicated B cell subsets, **(O)** frequency of CD8$^+$ T cells exhibiting expression of PD-1 and/or EOMES, and **(P)** frequency of CD3$^+$CD8$^-$ cells (left) and CD3$^+$CD8$^+$ T cells (right) within TLS that express the indicated functional biomarkers. Statistical differences evaluated by Kruskal-Wallis tests with Dunn's correction. **(Q)** Treatment-naïve PDAC specimens with the indicated proportion of MECA-79$^+$ TLS out of total TLS, arranged by histologic tumor grade. Statistical comparisons made using Kruskal-Wallis test with Dunn's correction. **(R)** Kaplan-Meier curves displaying OS based on proportion of MECA-79$^+$ TLS out of total TLS identified per patient (n = 61). *P*-value determined by log-rank test. **(S)** Proportion of MECA-79$^+$ TLS in treatment-naïve short-term versus long-term survivors who had at least one TLS present. Statistical differences evaluated by two-tailed, unpaired Mann-Whitney U test. All data presented as mean ± SEM.