Gene signature of circulating platelet-bound neutrophils is associated with poor prognosis in cancer patients

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Table S6. NPAs-associated neutrophil signature as opposed to the remaining neutrophil clusters and all the other leukocytes.

List of 16 genes of the NPA signature generated based on the public scRNAseq data from NSCLC patients' whole blood leukocytes - GSE127465.

16_gene_NPA_signature
RTN3
RAB3D
IL10RB
GPR82
SPAG9
B4GALT5
RBPJ
IDH2
TMUB2
ZNF397
NXPE3
TRIM41
HELLPAR
CCNI
TSC22D4
YY1

Figure S1. Identification of a cluster of neutrophils expressing platelet genes in public scRNAseq data from healthy donors' peripheral blood leukocytes.

*(**A-B**)* Two dimensional-UMAP representation of re-clustered NSCLC patients' whole blood leukocytes from scRNAseq data (GSE145230). *(**A**)* Major immune cell types were labeled using Clustifyr and SingleR annotation tools from pre-selected immune cell signatures. *(**B**)* Each of the 22 clusters defined by the analysis were labeled based on the type of immune cell. The 6 clusters of neutrophils were annotated as follow: Neu 0, Neu1, Neu 6, Neu 15, Neu 17 and Neu 18. Dark arrows show Neu 17 neutrophil cluster of interest displaying high expression of platelet genes. *(**C**)* Violin plot representing MCP counter abundance score per cell of the "in house 11 genes" neutrophil signature (Table S2) across all major blood immune cell types. *(**D**)* Violin plot representing ssGSEA enrichment score per cell of the Raghavachari platelet signature (Table S2) across all major blood immune cell types. *(**E**)* Violin plots representing the $\log_2$ gene expression (count per million, CPM) of neutrophil-specific genes (*CXCR2*, *CSF3R* and *FCGR3B*) (Table S2) across all clusters of blood immune cells. *(**F**)* Violin plots representing the $\log_2$ gene expression (CPM) of platelet-specific genes (*PF4*, *PPBP* and *NRGN*) (Table S3) across clusters of blood immune cells.

In *(**C**), (**E**)* and *(**F**)* P values were calculated with Wilcoxon test, taking Neu 5 cluster as the population of reference for each pairwise comparison with other clusters. In *(**D**)* P values were calculated with Wilcoxon test, taking Platelet cluster as the population of reference for each pairwise comparison with other clusters. P values were adjusted with Bonferroni test. Adjusted p-values (Adj P) were displayed on graphs, **** Adj P ≤ 0.0001, *** Adj P ≤ 0.001, ** Adj P ≤ 0.01.

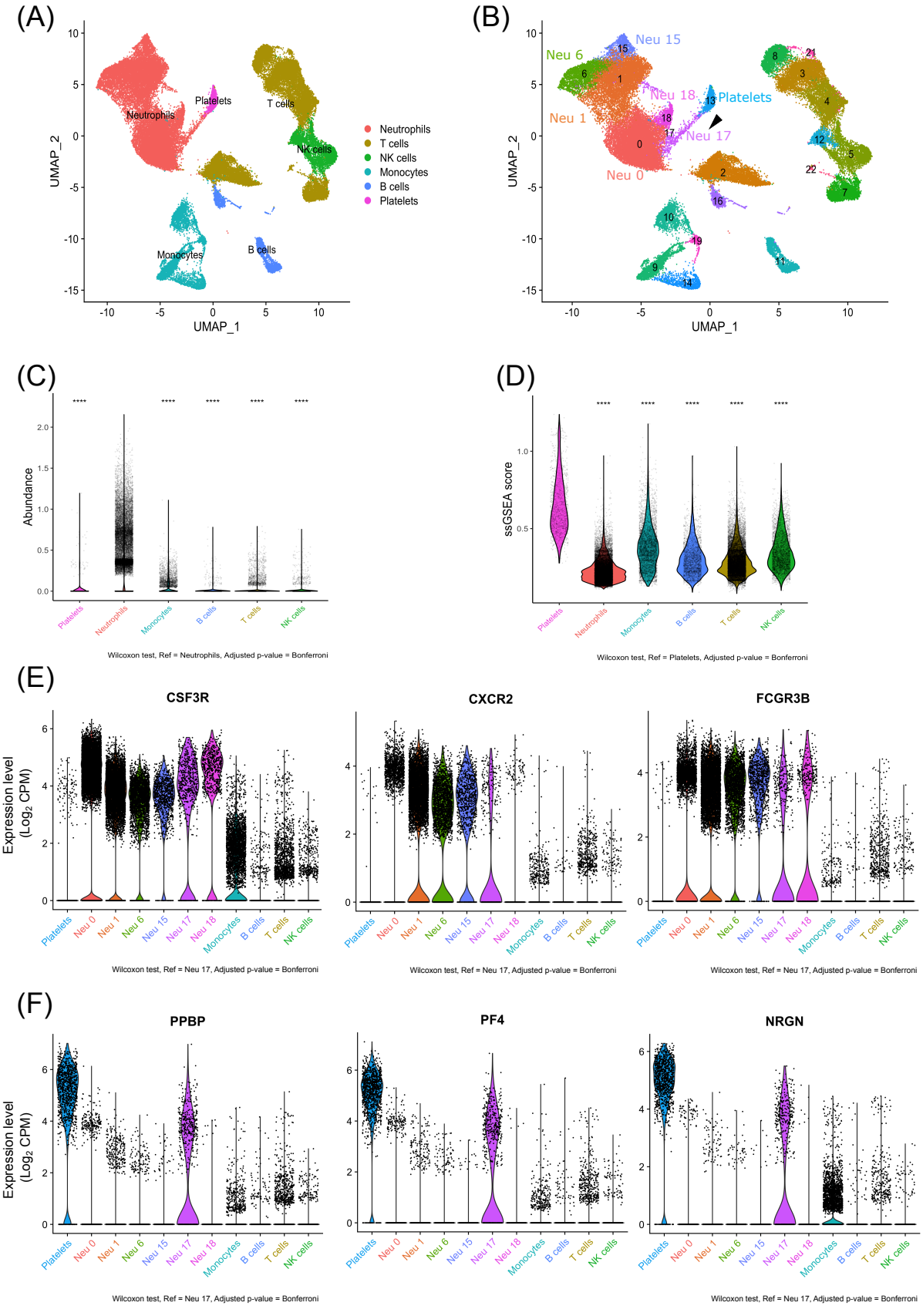
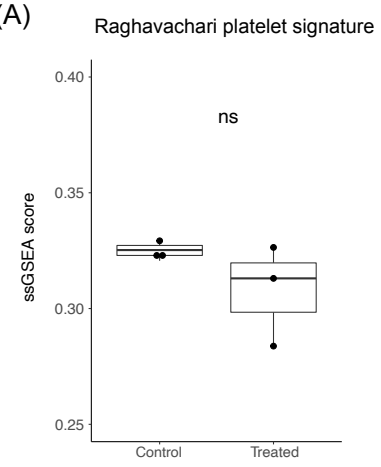

(A)
UMAP_2
UMAP_1
Neutrophils
Platelets
T cells
NK cells
Monocytes
B cells
(B)
Neu 15
Neu 6
Neu 18
Platelets
Neu 1
Neu 17
Neu 0
(C)
Abundance
Wilcoxon test, Ref = Neutrophils, Adjusted p-value = Bonferroni
(D)
ssGSEA score
Wilcoxon test, Ref = Platelets, Adjusted p-value = Bonferroni
(E)
CSF3R
CXCR2
FCGR3B
Expression level (Log2 CPM)
Wilcoxon test, Ref = Neu 17, Adjusted p-value = Bonferroni
(F)
PPBP
PF4
NRGN
Expression level (Log2 CPM)
Wilcoxon test, Ref = Neu 17, Adjusted p-value = Bonferroni

Figure S2. Enrichment analysis of platelet signatures in stimulated versus unstimulated neutrophils.

*(**A**)* Box plot representing ssGSEA enrichment scores of two independent platelet signatures Raghavachari platelet signature (Table S2) in GM-CSF-treated neutrophils ("treated") versus untreated neutrophils ("Control"). Blood from 3 HDs were used for each group (GSE15139). Differential enrichment of platelet signature between "treated" and "control" groups was assessed by the Wilcoxon rank-sum (Mann–Whitney) statistical test (p-value = 0.4). *(**B**)* Box plot representing ssGSEA enrichment scores of the Raghavachari platelet signature (Table S2) in neutrophils treated either with plasma from septic patients ("Septic plasma", n = 35) or from HDs ("Uninfected plasma", n=19) (GSE49757). Differential enrichment of platelet signature between "Septic plasma" and "Uninfected plasma" groups was assessed by the Wilcoxon rank-sum (Mann–Whitney) statistical test (p-value = 0.05202).

(A)



(B)

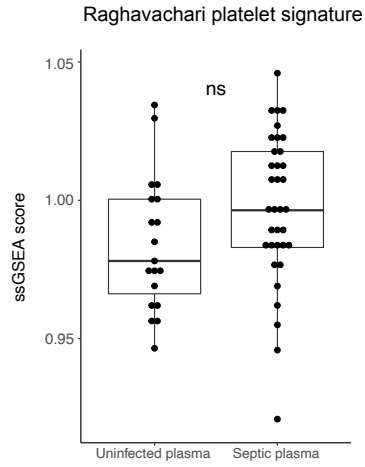


Figure S3. Two dimensional-UMAP representation of each neutrophil cluster.

Two dimensional-UMAP representation of individual neutrophil clusters (from NSCLC patients' whole blood leukocytes from scRNAseq data (GSE127465)) whose projection was based on UMAP parameters used to discriminate the major subsets of leukocytes.

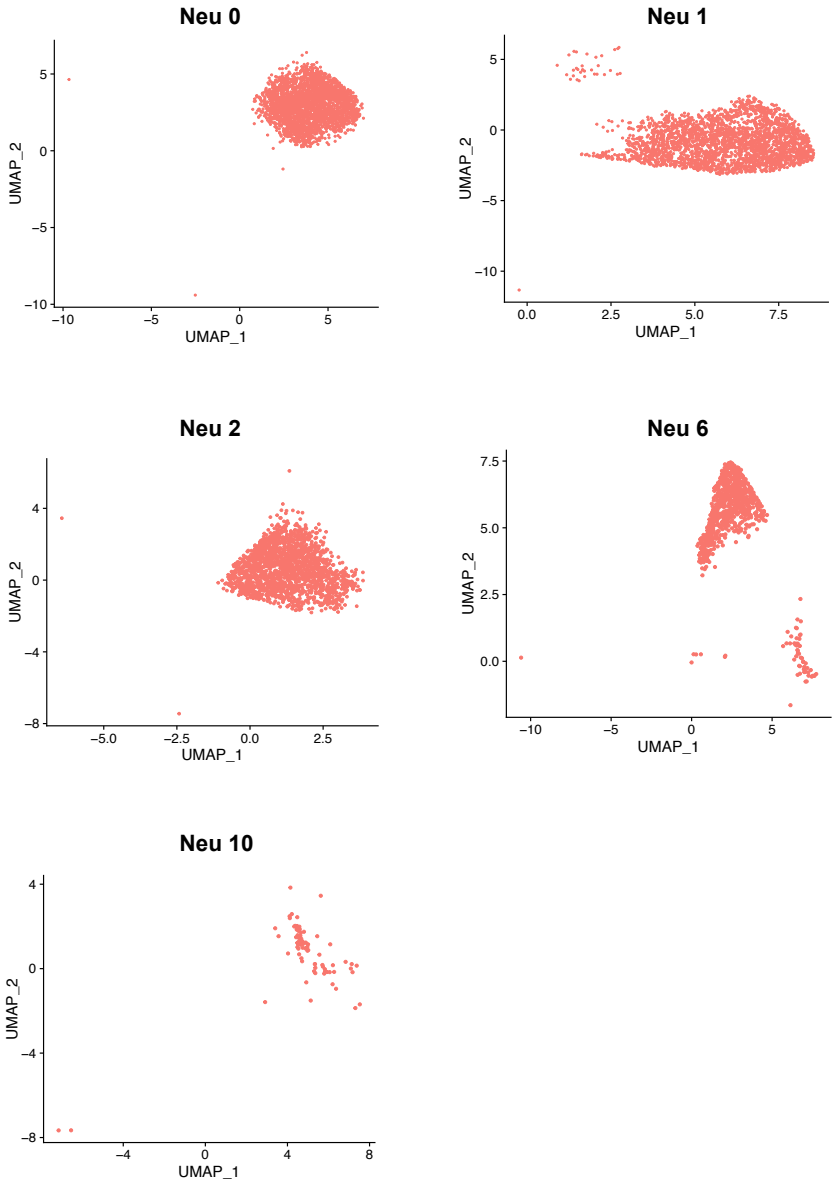


Figure S4. Two dimensional-UMAP representation of NSCLC patients' tumor scRNAseq data.

Expression of each of the 16 genes of the NPA signature (Table S5) plotted on the two dimensional-UMAP representation of NSCLC patients' stromal and patient-specific tumors from scRNAseq data (GSE127465)).

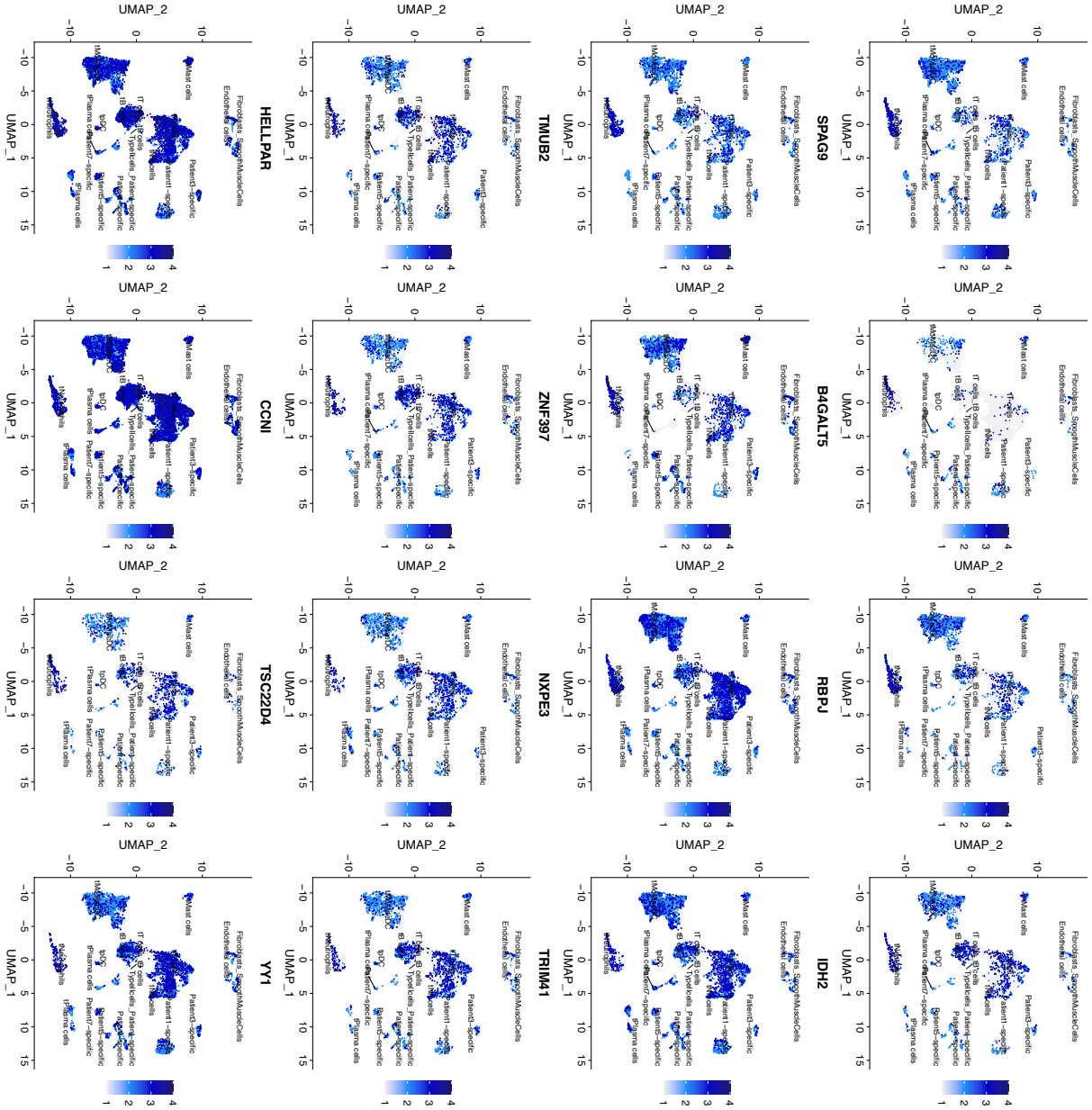
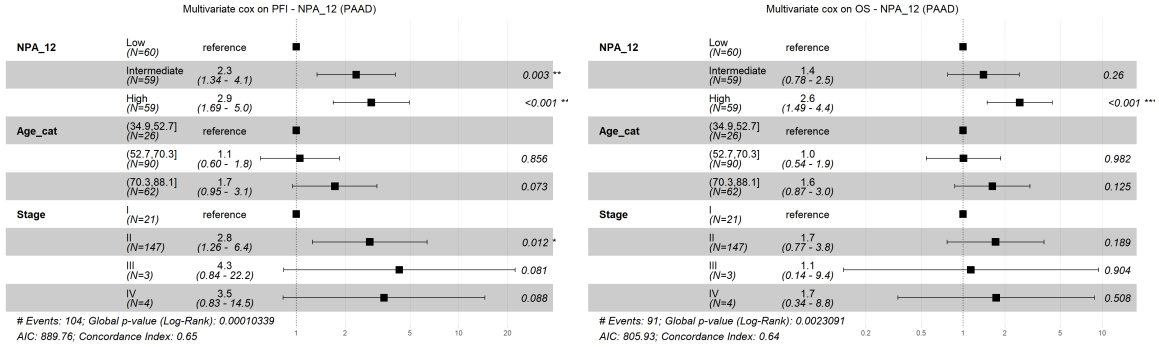


Figure S5. Multivariate analysis (Forest plot) of the prognostic impact of the 12-genes NPAs-associated neutrophil signature.

Multivariate analysis taking into account age and stage as cofounding factors. Cohort of PAAD ***(A)*** and LIHC ***(B)*** cancer patients were cut at tercile values based on mean expression of "NPA_12" signature. For both cohorts, progression free-interval (panel on the left) and overall survival (panel on the right) were assessed.

(A)



(B)

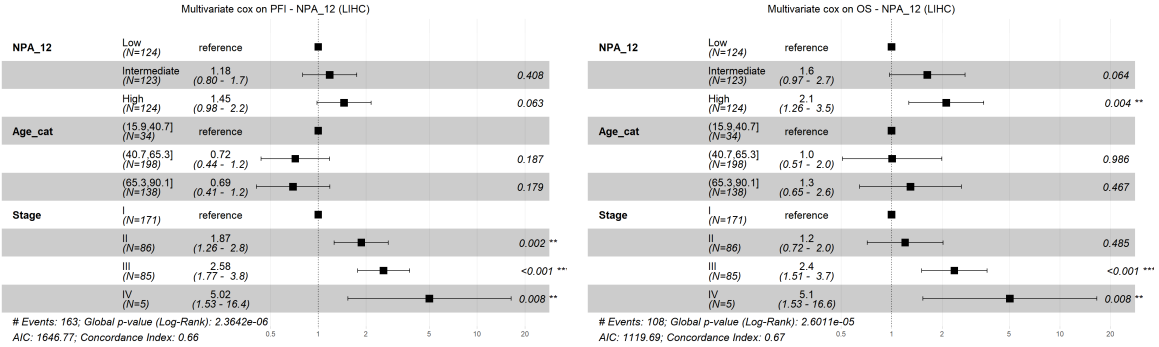


Figure S6. Detailed gating strategy of multiple filtering steps to identify NPAs.

Gating strategy to identify NPAs from whole blood samples acquired with ImageStream®X (ISX) imaging flow cytometer. Scatter plot, histograms and fluorescence images were generated by IDEAS image analysis software (Amnis Corporation, Seattle, WA).