SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURES

Figure Supplementary 1

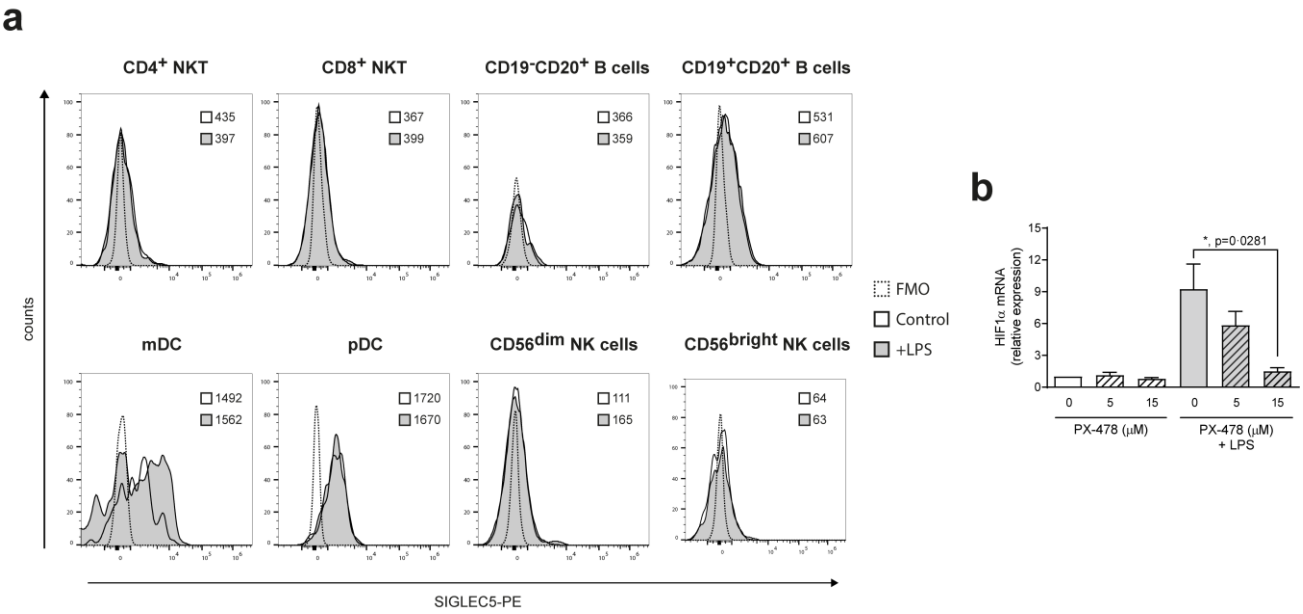


Figure S1. SIGLEC5 expression after LPS stimulation in some immune cells and PX-478 inhibits HIF1α expression in human monocytes. (a) SIGLEC5 expression on the cell surface of a wide panel of blood circulating immune populations stimulated (grey filled line) or not (black clear line) with lipopolysaccharide (LPS), including B lymphocytes, Natural Killer (NK) cells, and myeloid and plasmacytoid dendritic cells (DC). **(b)** Relative expression (mRNA) by RT-qPCR of HIF1α on $CD14^+$ cells from HV, pre-treated or not with different concentrations of a specific inhibitor of HIF1α (PX-478) for 3 hours, and then challenged (grey filled columns) or not (black clear columns) with LPS (10 ng/mL) for 16 hours is shown (n=7). Data shown as mean ± SEM. Statistical analysis was performed using paired t-test (**p<0·01).

Suplementary Figure 2

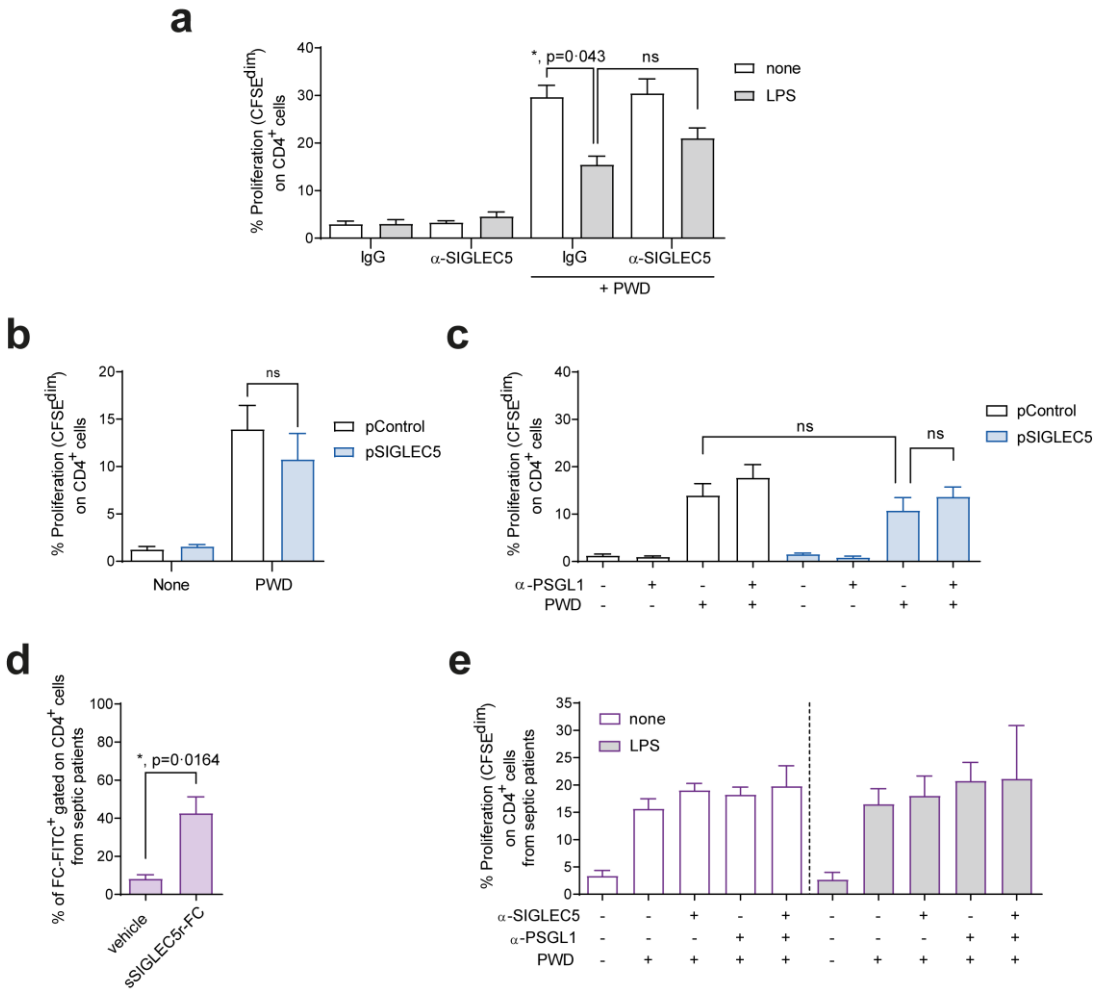
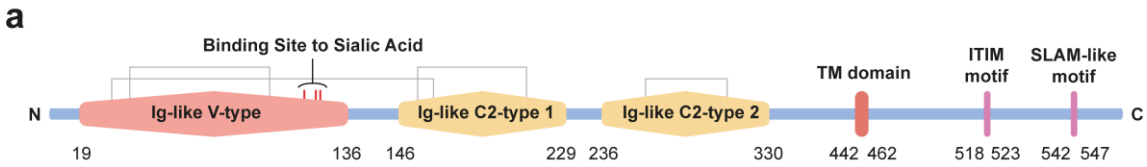


Figure S2. Effect of the SIGLEC5/PSGL1 axis in CD4$^+$ T cell proliferation. (a) Proliferation levels (CFSE^{dim}) of CD4$^+$ cells from HVs, stimulated or not with PWD and co-cultured for 5 days with autologous monocytes pre-challenged (grey filled columns) or not (black clear columns) with 10 ng/mL of LPS for 16 hours. In some indicated conditions, a blocking antibody against SIGLEC5 (α-SIGLEC5) or an unspecific IgG was added (n=3, left panel). **(b)** Proliferation levels (CFSE^{dim}) of CD8$^+$ cells from HVs, stimulated or not with PWD and co-cultured for 5 days with autologous monocytes pre-nucleofected (blue filled column) or not (black clear column) with an expression vector of SIGLEC5 (pSIGLEC5) (n=4). **(c)** Proliferation levels (CFSE^{dim}) of CD4$^+$ cells from HVs in presence or not of PWD and co-cultured for 5 days with autologous monocytes pre-nucleofected with an expression vector of SIGLEC5 (pSIGLEC5, blue filled columns) or an empty vector (pControl, black clear columns) (n=6). In some conditions, a blocking antibody against PSGL1 (α-PSGL1) was added. **(d)** Quantification of the binding of sSIGLEC5r-FC to CD4$^+$ cells from septic patients (n=4). The binding was revealed by an antibody (α-FC-FITC). **(e)** Proliferation levels (CFSE^{dim}) of CD4$^+$ cells from septic patients in presence or not of PWD and co-cultured for 5 days with autologous monocytes pre-challenged (grey filled columns) or not (black clear columns) with 10 ng/mL of LPS for 16 hours. In some conditions, the α-PSGL1 and α-SIGLEC5 antibodies were added (n=6). Data shown as mean ± SEM. Statistical analysis was performed using paired t-test (*p<0·05; **p<0·01).

Suplementary Figure 3



b

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Siglec5      PVYELQVQKSVTV---QEGLCVLVPCSFSYPWRSWYS----SPPLYVYWFRDGEIPYYAE    53
VISTA        ----FKVATPYSLYVCPEGQNVTLTCRLLGPV-----DKGHDVTFYKTWYRSS----RGE    47
PD-L1        ----FTVTVPKDLYVVEYGSNMTIECKFPVEK-----QLD-LAALIVYWEMED----KNI    46
PD-L2        ----FTVTVPKELYIIEHGSNVTLECNFDTGS-----HVN-LGAITASLQ----------    40
ICOSL        -------DTQEKEVRAMVGSDVELSCACPEGS-----RFD-LNDVYVYWQTSE----SKT    43
CD80         ---VIHVTK------EVKEV-ATLSCGHNV-------SVEELAQTRIYWQKEKKMVLT--    41
CD86         ------------------NETADLPCQFANSQ-----NQS-LSELVVFWQDQE----NLV    32
B7-H3        ----LEVQVPEDPVVALVGTDATLCGSFSPEP-----GFS-LAQLNLIWQLTD----TKQ    46
B7-H4        ----HSITVTTVASAGNIGEDGILSCTFEP-------DIK-LSDIVIQWLKEG----VLG    44
CTLA-4       -----HVAQPAVVLASSRGI-ASFVCEYASPGKATEVRVTVLRQADS--QVTEVCAAT--    50
ILDR2        ----LQVTVPDKKKVAMLFQPTVLRCHFSTSS-----HQPA----VVQWKFKSYCQDRMG    47
                                      . *

Siglec5      VVA-----------------------------------TNNPDRRVKPETQGRFRL-LGD    77
VISTA        VQT-----CSERRPIRNLTFQDLHLH-H----------GGHQAANTSHDLAQRHGLESAS    91
PD-L1        IQF--------------------VH-----------GEEDLKVQHSSYRQRARLLKDQ    73
PD-L2        ------------------------------------KVENDTSPHRERATLLEEQ    59
ICOSL        VVT--------------------YHIP----------QNSSLENVDSRYRNRALMSPAG    72
CD80         MM-----------------------------------SGDMNIWPEYK---NR-TIF    59
CD86         LNE--------------------VY-L----------GKEKFDSVHSKYMGRTSFDSD-    59
B7-H3        --L--------------------VH-----------SFAEGQDQGSAYANRTALFPDL    71
B7-H4        LVH--------------------EFKE----------GKDELSEQDEMFRGRTAVFADQ    73
CTLA-4       YM-----------------------------------MGNELTFLDDS---IC-TGT    68
ILDR2        ESLGMSSTRAQSLSKRNLEWDPYLDCLDSRRTVRVVASKQGSTVTLGDFYRGRE-----I   102

Siglec5      VQKKNCSLSIGDARMEDTGSYFFRVERGRDVKYSYQQNKLN--------       118
VISTA        DHHGNFSITMRNLTLLDSGLYCCLVVEIRHHHSEHRVHGAMELQV----       136
PD-L1        LSLGNAALQITDVKLQDAGVYRCMISYGGA-DYKR-----IT-------       109
PD-L2        LPLGKASFHIPQVQVRDEGQYQCIIIYGVAWDYKY-----LTLK-----       98
ICOSL        MLRGDFSLRLFNVTPQDEQKFHCLVLSQSL-GFQE----VLSVE-----       111
CD80         DITNNLSIVILALRPSDEGTYECVVLKYEKDAFK--REHLAEVT-----       101
CD86         ----SWTLRLHNLQIKDKGLYQCIIHHKKPTGMIR----IHQMNS-ELS       99
B7-H3        LAQGNASLRLQRVRVADEGSFTCFVSIRDF-GSAA-----VSLQVAQVA       114
B7-H4        VIVGNASLRLKNVQLTDAGTYKCYIITSKGKGNAN-----LEYK-----       112
CTLA-4       SSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYY----------------       102
ILDR2        TIVHDADLQIGKLMWGDSGLYYCIITTPDDLEGKNE--DSVE-------       142
                 .  : :      * : :   :
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c

	% Identity	% Similarity
VISTA	15·4	33·6
PD-L1	25·2	38·2
PD-L2	15·1	27·7
ICOSL	18·2	26·6
CD80	16·3	30·4
CD86	16·5	32·2
B7-H3	19	31
B7-H4	15·8	33·8
CTLA-4	14·1	22·5
ILDR2	18·8	30·5

Figure S3. Human SIGLEC5 exhibits Ig-like-V-type regions and high similarity to other immune checkpoints. (a) Schematic representation of human SIGLEC5 protein primary structure. Black and grey boxes represent the main domains,

interconnecting lines represent disulphide bridges, red ticks on the Ig-like-V-type domain represent the amino acids responsible for the sialic acid binding. The transmembrane domain (TM) is also displayed. **(b)** Multiple sequence alignment of the Ig-like-V-type domains of SIGLEC5 and other proteins postulated and confirmed as immune checkpoints. DxGxYxC motifs are highlighted in blue and the amino acids responsible for the sialic acid binding are highlighted in red. Canonical and non-canonical cysteine implicated on the inter-disulphide bridge are represented in green and yellow, respectively. **(c)** Percentages of identity and similarity between the Ig-like-V-type domains of SIGLEC5 and other proteins postulated and/or confirmed as immune checkpoints.

Suplementary Figure 4

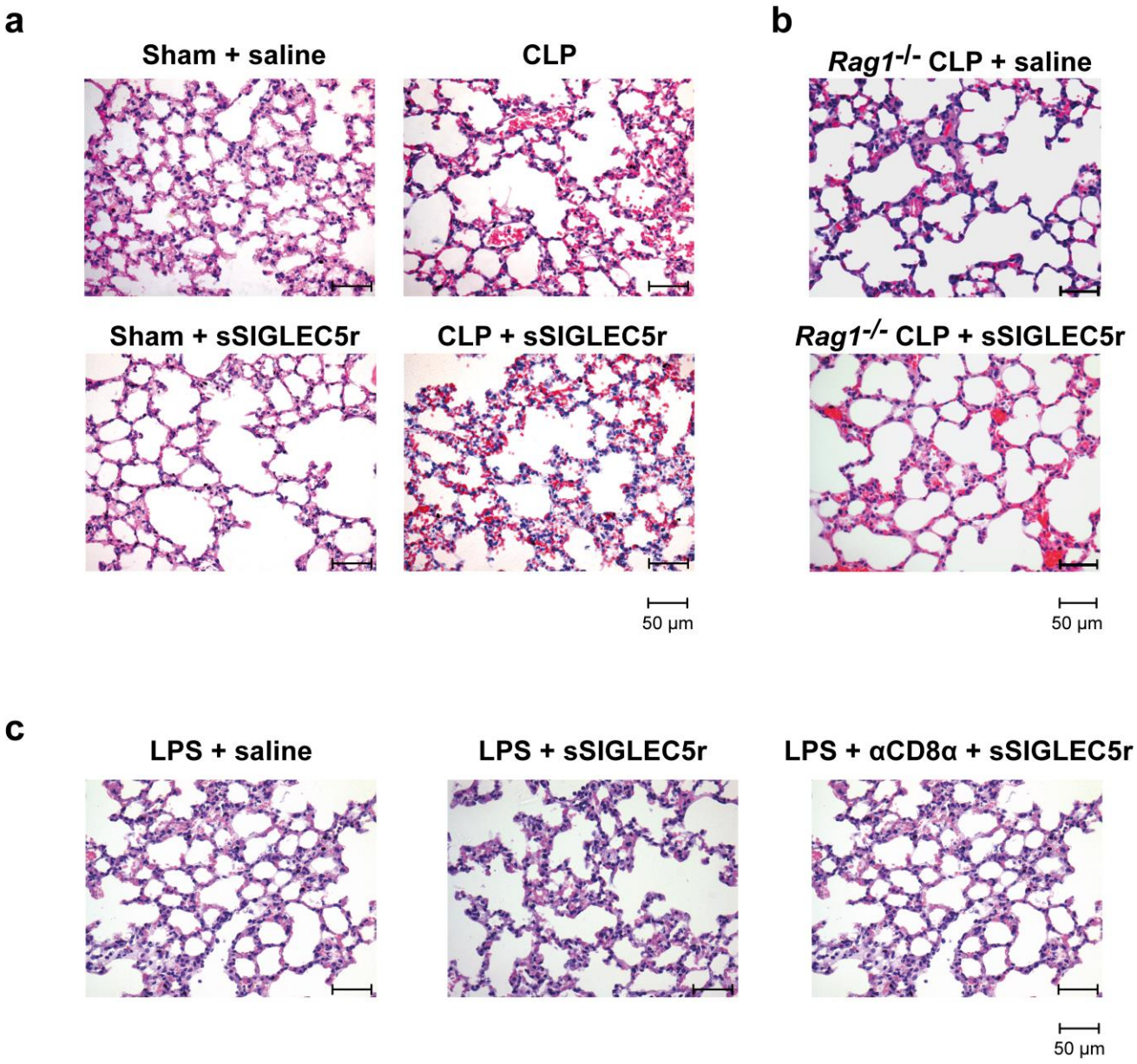

Figure S4. SIGLEC5 induced a acute lung injury in endotoxemia in vivo mice models in a CD8α-dependent manner. **(a)** A set of representative images of Haematoxylin/Eosin stained lung sections from CLP-mice and Sham-mice in presence or not of sSIGLEC5r at 400x magnification are shown; scale bar 50 µm. **b)** A set of representative images of Haematoxylin/Eosin stained lung sections from *Rag1*$^{-/-}$ CLP-mice in presence or not of sSIGLEC5r at 400x magnification are shown; scale bar 50 µm. **(c)** A set of representative images of Haematoxylin/Eosin stained lung sections from LPS-mice depleted or not with an anti-mouse CD8α in presence or not of sSIGLEC5r at 400x magnification are shown; scale bar 50 µm.

Suplementary Figure 5

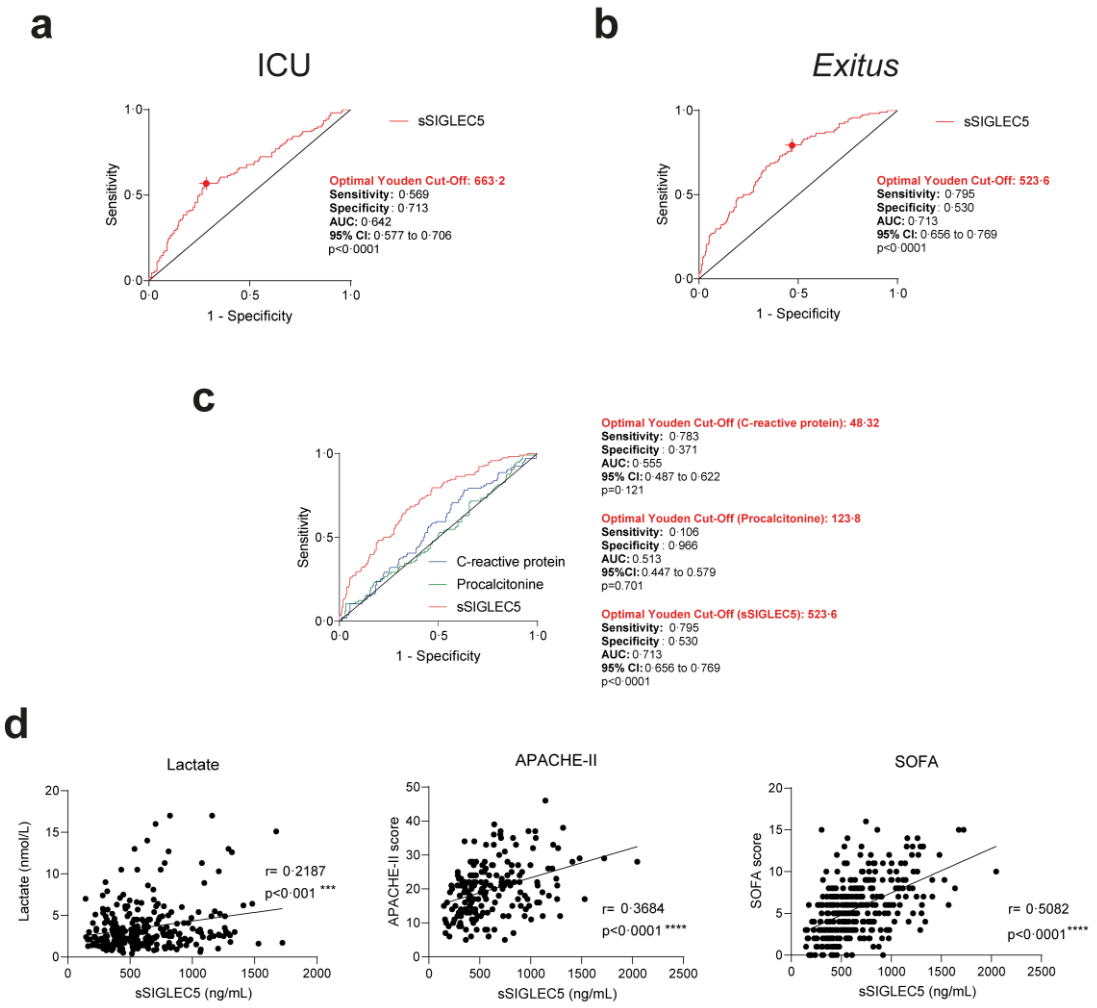

Figure S5. Soluble SIGLEC5 levels on plasma act as a better predictor of *exitus* than the admission to ICU in septic patients, C-reactive protein and procalcitonine and correlates with clinical prognosis indicators. (a) Receiver-operating-characteristic (ROC) curve describing the predictive performance of plasmatic sSIGLEC5 concentration at sepsis diagnosis (red line; area under the curve [AUC] of 0·642 [95% CI, 0·577 to 0·706], p<0·0001), to identify which patients of the cohort (n=346) would go to the ICU at the time of admission. **(b)** Receiver-operating-characteristic (ROC) curve describing the predictive performance of plasmatic sSIGLEC5 concentration at sepsis diagnosis (red line; area under the curve [AUC] of 0·713 [95% CI, 0·656 to 0·769], p<0·0001), to identify which patients of the cohort (n=346) would be dead within 60 days after diagnosis. **(c)** Receiver-operating-characteristic (ROC) curves describing the predictive performance of plasmatic sSIGLEC5, C-reactive protein and procalcitonine concentrations (red line as sSIGLEC5 with area under the curve [AUC] of 0·713 [95% CI, 0·656 to 0·769], p<0·0001; green line as procalcitonine with area under the curve [AUC] of 0·513 [95% CI, 0·447 to 0·579], p=0·701; and blue line as C-reactive protein with area under the curve [AUC] of 0·555 [95% CI, 0·487 to 0·622], p=0·121), to identify which patients of the cohort (n=346) would be dead within 60 days after diagnoses. **(d)** Correlations of sSIGLEC5 levels in septic patients with Lactate, APACHE-II and SOFA scores. (**a-c**) ROC analysis by Clopper-Pearson (****p<0·0001). (**d**) Spearman Pearson to quantitative correlations (***p<0·001; ****p<0·0001).

SUPPLEMENTARY TABLES

Table S1. Baseline characteristics of SIRS and septic patients included in the study separated by sex.

Characteristic*	All Female Patients (n=186)	All Male Patients (n=240)	Female Sepsis (n=82)	Male Sepsis (n=106)	Female Septic Shock (n=69)	Male Septic Shock (n=89)	Female SIRS** (n=35)	Male SIRS** (n=45)
Age - yr	67·5±17·5	67·2±15·1	68·2±18·6	66·4±16·1	68·3±16·6	69·3±13·9	64·4±16·8	65·3±15·0
APACHE II Score	17·6± 8·1	18·3± 7·1	16·1±6·3	18·7±6·1	22·4±9·4	22·0±7·9	12·9±3·8	13·6±4·9
SOFA Score	4·7±3·3	5·8±3·2	3·1±2·3	4·7±2·4	7·3±3·1	7·6±3·6	3·5±2·0	4·4±2·2
Lactate, nmol/L	3·2±3·7	3·3±2·8	2·8±2·3	3·1±2·5	4·5±4·3	4·5±4·3	1·8±0·7	1·5±0·8
CRP, mg/L	81·1±104·3	66·4±105·5	76·6±91·6	77·3±114·2	86·9±118·6	56·8±97·0	-	-
PCT, ng/mL	27·9±53·3	21·5±48·6	15·9±50·0	23·9±52·7	29·6±47·6	33·7±54·3	-	-

*Data are presented as mean±SD, or number (%).

**Non-infectious SIRS.

† P values were calculated by ANOVA test.

APACHE II: Acute Physiology and Chronic Health Evaluation II; CRP: C-reactive protein; INR, International Normalized Ratio; PCT: Procalcitonin; SIRS, Systemic Inflammatory Response Syndrome; SOFA: Sequential Organ Failure Assessment.

Table S2. Description of the patients with Aneurysm or Stroke, and Healthy Volunteers.

Characteristic*	Aneurysm (N=11)	Stroke (N=16)	Healthy Volunteers (N=100)
Age (years)	62±6·88	70±13·61	50±11·98
Male sex – n (%)	5(38·46)	10(58·82)	54(54)
APACHE II	11·08±7·68	-	-
CRP, mg/L	18·19±30·70	9·56±11·36	-
INR	1·02±0·06	1·08±0·26	-

* Data are presented as mean±SD

APACHE II: Acute Physiology and Chronic Health Evaluation II; CRP: C-reactive protein; INR: international normalized ratio.

Table S3. List of fluorochrome-conjugated antibodies to the SIGLEC5 characterization in the main circulating immune populations

Marker	Fluorochrome	Source	Clone	Reference (RRID)
CD3	BV510	Biolegend	OKT3	317332 (AB_2561943)
CD4	cFluor-YG584	Cytek Biosciences	SK3	R7-20041 (AB_2885083)
CD8	BUV805	BD	SK1	612889 (AB_2833078)
CD11c	BUV661	BD	B-Ly6	612967 (AB_2870241)
CD14	Spark Blue™ 550	Biolegend	63D3	367148 (AB_2832724)
CD16	BUV496	BD	3G8	612944 (AB_2870224)
CD19	Spark NIR™ 685	Biolegend	HIB19	302270 (AB_2832581)
CD20	Pacific Orange	ThermoFisher Scientific	2H7	MHCD2030 (AB_10375578)
CD24	PE/Dazzle™ 594	Biolegend	ML5	311134 (AB_2566349)
CD38	APC-Fire™ 810	Biolegend	HIT2	303550 (AB_2860784)
CD45	PerCP	Biolegend	2D1	368506 (AB_2566358)
CD56	BUV737	BD	NCAM 16.2	612766 (AB_2813880)
CD123	Super Bright 436	ThermoFisher Scientific	6H6	62-1239-42 (AB_2662727)
HLA-DR	APC/Fire™ 750	Biolegend	L243	307658 (AB_2572101)

Table S4. Sequence of primers used for Hypoxia Response Elements (HRE) amplification by PCR.

HRE sites	Forward primers (5'-3')	Reverse primers (5'-3')	Amplicon (bp)
HRE1	AGAAGGGGAACTTGGGCATC	TCAGTATCTTCACCTGCGGC	99
HRE2	CTGGGTCTCTGGCTTCACTC	CCAAGTTCCCCTTCTGTGCG	497
HRE3	GGTGAGTGAGAGCTGTGGAC	TCCCTGACAACTTGCCTTCC	398
HRE4	TCATGTCTCCAGAGGAGGCT	ATCCCTCCTGTGGTCTGGTT	236

106 **Table S5. Sequence of primers used in RT-pPCR.**

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Gene	Forward primers (5'-3')	Reverse primers (5'-3')
β-ACTIN	GTGGGGCGCCCCAGGCACCA	CTCCTTAATGTCACGCACGATTTC
HIF1α	TTCCAGTTACGTTCCTTCGATCA	TTTGAGGACTTGCGCTTTCA
SIGLEC5	CAAGGGAGATCGAACCTCGG	TGCGGGCTTTCACTATTAAAAAGA

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