Immunoprofiling reveals novel mast cell receptors and the continuous nature of human lung mast cell heterogeneity

Short title: Immunoprofiling of human lung mast cells

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Abstract

Background: Immunohistochemical analysis of granule-associated proteases has revealed that human lung mast cells constitute a heterogeneous population of cells, with distinct subpopulations identified. However, a systematic and comprehensive analysis of cell-surface markers to study human lung mast cell heterogeneity has yet to be performed.

Methods: Human lung mast cells were obtained from lung lobectomies, and the expression of 332 cell-surface markers was analyzed using flow cytometry and the LEGENDScreen^{TM} kit. Markers that exhibited high variance were selected for additional analyses to reveal whether they were correlated and whether discrete mast cell subpopulations were discernable.

Results: We identified the expression of 102 surface markers on human lung mast cells. Several markers showed high continuous variation in expression within the mast cell population. Six of these markers were correlated: SUSD2, CD49a, CD326, CD34, CD66 and HLA-DR. The expression of these markers was also correlated with the size and granularity of mast cells. However, no marker produced an expression profile consistent with a bi- or multimodal distribution.

Conclusions: LEGENDScreen analysis identified more than 100 cell-surface markers on mast cells, including 23 that, to the best of our knowledge, have not been previously described on human mast cells. Several of the newly described markers are known to be involved in sensing the microenvironment, and their identification can shed new light on mast cell functions. The exhaustive expression profiling of the 332 surface markers failed to detect distinct mast cell subpopulations. Instead, we demonstrate the continuous nature of human lung mast cell heterogeneity.

Keywords: Human lung mast cells, heterogeneity, SUSD2

Introduction

Heterogeneity among mast cells has been known for a long time and was first attributed to differential expression of proteoglycans in rodent mast cells, which gave them distinct staining patterns [1]. This led to the division of rodent mast cells into connective tissue mast cells and mucosal mast cells. In humans, mast cell heterogeneity has been based on the expression of mast cell proteases, i.e., cells expressing tryptase only (MC_T) and those expressing both tryptase and chymase (MC_{TC}) as well as carboxypeptidase A [2,3]. These subtypes have been defined using immunohistochemistry, a method that produced binary results, that is, the absence or presence of expression. The MC_{TC} subtype is more predominant in connective tissues such as the skin, while the MC_T subset is more prevalent in mucosal surfaces such as the airways and gastrointestinal tract [4].

Mast cells are found in the human lungs in all different compartments, i.e., under the epithelium, in smooth muscle bundles, around pulmonary vessels, in the parenchyma and in close proximity to sensory nerves [5]. Human lung mast cells (HLMCs) have several important functions in health and diseases, such as host defense, induction of acute inflammatory responses, vascular regulation, bronchoconstriction and tissue remodeling [6-9]. The heterogeneity of HLMCs was first described to be related to differences in cell size and functionality, i.e., the response to secretagogues [10,11]. Later, it was described that the MC_T subtype is the predominant subtype in the lungs, except around pulmonary vessels, where the MC_T and MC_{TC} subtypes are found in equal numbers [2]. However, the heterogeneity among HLMCs goes beyond size and protease expression, as demonstrated by the differential expression of certain mast cell-related markers (FcεRI, IL-9R, 5-LO, LTC_4S, etc.) among the MC_T and MC_{TC} populations in different lung compartments [12].

Mast cell heterogeneity has primarily been studied in a binary manner using immunohistochemistry, describing the absence or presence of expression. Here, we used a quantitative flow cytometry-based approach to study HLMC heterogeneity, profiling the expression of 332 markers. None of these markers distinctly divided the mast cells studied into subpopulations. However, several markers showed a high degree of variation within the mast cell population with a nonclustered gradient expression pattern. Six of these markers correlated with each other, revealing the continuous nature of HLMC heterogeneity rather than separation into distinct subpopulations.

Materials and Methods

Ethical approval

The local ethics committee approved the collection of lung tissue from patients undergoing lobectomy, and all patients provided informed consent (Regionala Etikprövningsnämnden Stockholm, 2010/181-31/2).

Cell preparation

Single-cell suspensions were obtained from macroscopically healthy human lung tissue as previously described [13]. Briefly, human lung tissue was cut into small pieces and enzymatically digested for 45 min with DNase I and collagenase. Thereafter, the tissue was mechanically disrupted by plunging through a syringe, the cells were washed, and debris was removed by 30% Percoll centrifugation. After preparation, the cells were stained and analyzed by flow cytometry.

Flow cytometry

The following antibodies were used for surface staining: anti-CD45-V500 (Clone HI30, BD Biosciences, San Jose, CA, USA), anti-CD14-APC-Cy7 (clone M5E2, BioLegend, San Diego, CA, USA), anti-CD117-APC (clone 104D2, BD Biosciences), anti-FcεRI-FITC (clone CRA1, Miltenyi Biotec, Bergisch Gladbach, Germany), anti-FcεRI-PE (clone CRA1, BioLegend), anti-SUSD2-PE (clone W3D5, BioLegend), anti-CD63- PE/Cy7 (clone H5C6, BD Biosciences), anti-CD49a-BV786 (clone SR84, BD Biosciences), anti-CD66a/c/e-A488 (clone ASL-32, BioLegend), anti-CD326-BV650 (clone 9C4, BioLegend), anti-CD34-BV421 (clone 581, BD Biosciences), anti-HLA-DR-PE/Cy5 (clone L243, BioLegend), and anti-CD344-PE/Vio770 (clone CH3A4A7, Miltenyi Biotec). When using a LEGENDScreen™ human cell screening kit, which contains 342 antibodies conjugated to PE (Cat. 70001, BioLegend) that are detailed in Supplementary Table S1, cells were first stained with anti-CD45, anti-CD117, anti-CD14 and anti-FcεRI antibodies; thereafter, they were stained with the kit reagents according to the manufacturer's instructions. Mast cells were gated as $CD45^{+}CD14^{low}CD117^{high}$ cells (Figure 1). For intracellular staining, cells were fixed with 4% paraformaldehyde (PFA) and permeabilized using 0.1% saponin in PBS with 0.01 M HEPES (PBS-S buffer). Nonspecific binding was blocked using blocking buffer (PBS-S with 5% dry milk and 2% fetal calf serum (FCS). The cells were thereafter stained with anti-tryptase

antibodies (clone G3, Millipore, Burlington, MA, USA) conjugated in-house with an Alexa Fluor 647 monoclonal antibody labeling kit (Thermo Fisher Scientific, Waltham, MA, USA) or anti-CPA3 antibodies (clone CA5, a kind gift from Andrew Walls, Southampton, UK) conjugated in-house with an Alexa Fluor™ 488 antibody labeling kit (Thermo Fisher Scientific). The cells were analyzed using a BD FACSCanto (BD, Franklin Lakes, NJ, USA) or BD LSRFortessa, and FlowJo software version 10 (FlowJo LLC, Ashland, OR, USA) was used for flow cytometry data analysis.

Statistical analysis

Statistical analyses were performed with GraphPad Prism software version 7.0b or the Python environment (3.7) with the following packages: statsmodels (0.10.1), seaborn (0.9.0), scipy (1.4.1), pandas (1.1.0), numpy (1.18.1), and matplotlib (3.1.3). The specific methods used are detailed in the figure legends. * $p < 0.05$; ** $p < 0.01$; *** $p<0.001$; **** $p<0.0001$.

Results

Immunoprofiling of HLMCs

The expression of cell-surface antigens was thoroughly investigated by flow cytometry using a LEGENDScreen^{TM} kit containing 342 antibodies, including 10 isotype controls. HLMCs were gated as $CD45^{+}CD14^{low}CD117^{high}$ cells (Figure 1A), and the gated HLMC population expressed high levels of tryptase, confirming the identity of the gated cells (Figure 1B). The expression of some relevant mast cell markers included in the LEGENDScreen kit is highlighted in Figure 1C, showing the percentage of positive cells, the median fluorescence intensity (MFI) and the significance of expression within the HLMC population. Many of the highly expressed markers on HLMCs, such as β2-microglobulin (B2M), CD44, and CD9, are broadly expressed (Figure 1C). To determine which of the markers are most relevant for HLMCs, we compared the expression to that of $CD45^{+}CD14^{+}CD117^{-}FSC^{int}SSC^{low}$ cells (Figure 1A). Well-known monocyte markers such as CD11b, CD11c, CD31, CD141, CXCR1 and HLA-DR showed higher expression on $CD14^{+}$ cells, whereas classic mast cell markers such as CD117, FcεRI, CD203c, Siglec-8, and TSLPR showed higher expression on HLMCs. The markers with the most significant differences between the HLMCs and CD14+ cells included CD9, CD59, CD274 and CD226 (Figure 1D). CD9 is a broadly expressed tetraspanin with a wide variety of functions; in mast cells, it is abundantly expressed and has been implicated in chemotaxis and activation [14]. CD59 can prevent complement-induced cytolytic cell death by preventing assembly of the complement membrane attack complex and

has also been implicated in T cell activation [15]. CD274 is also known as programmed death ligand-1 (PD-L1) and can cause blockade of T cell activation [16]. CD226 has received increasing interest in recent years and can play a role in many immunological processes [17], including enhancement of FcεRI-mediated activation in mast cells [18]. HLMCs significantly expressed (the MFI compared to that of the fluorescence minus one (FMO) control) 102 out of the 332 markers included in the LEGENDScreen kit (Figure 2). To the best of our knowledge, surface expression of 23 of these proteins on (nonneoplastic) human mast cells has not been described before (Table 1).

Heterogeneous expression of the high-affinity IgE receptor FcεRI

The LEGENDScreen analysis failed to produce significant staining of the high-affinity IgE receptor FcεRI (Figure 2). However, the use of the same antibody clone in the backbone staining panel likely explains this observation. To investigate this further, we separately studied the expression of FcεRI on mast cells from additional donors. The expression of CD117 and FcεRI on mast cells from four donors is shown in Figure 3A. Approximately half of the donors were ~100% positive for the marker (Figure 3B). However, even in the 100% positive individuals, the level of expression, i.e., the MFI, varied considerably (Figure 3C).

Heterogeneous expression of cell-surface markers with a continuous distribution

None of the markers clearly and consistently divided HLMCs into subpopulations (data not shown). However, several markers showed considerable continuous expression variation within the population, which was quantified by calculating the robust coefficient of variation (CV) (Table 2). The two antibodies with the highest CV recognized the same antigen, SUSD2, a marker identified on mesenchymal and pluripotent stem cells with functional domains inherent to adhesion molecules [19,20]. Costaining of the seven markers with the highest CV revealed that six of these markers were correlated (SUSD2, CD49a, CD326, CD34, CD66 and HLA-DR), while CD344 was not correlated with any of the other markers (Figure 4, FMO controls in Supplementary Figure S1). Furthermore, to investigate whether these markers correlated with either of the classic mast cell subtypes, MC_T or MC_{TC}, costaining with an anti-CPA3 antibody was performed, but no correlations were observed (Figure 4G, FMO control in Supplementary Figure S1). In addition, these markers did not show costaining with any of the other markers that were included for gating purposes in the LEGENDScreen analysis, including CD45, CD14, CD117 and FcεRI (data not shown).

Furthermore, cells with high expression of SUSD2 showed higher FSC and SSC, indicating that they were larger and had a higher inner complexity, i.e., contained more granules (Figure 4 H-J). SUSD2 has been linked to proliferation in cancer cells [21], which is why we investigated the proliferation status of cells with the proliferation marker Ki-67. However, in agreement with the fact that mast cells are long-lived cells with low turnover [22], no staining was observed (Supplementary Figure S2).

Discussion

Although attempts have been made to map cell-surface antigens on HLMCs [23-28], extensive mapping including the heterogeneity of cell-surface antigen expression has not been carried out. In this study, we identified significant expression of 102 markers on the HLMC surface, of which, to the best of our knowledge, 23 are novel mast cell markers (Table 1). Several of these markers, including SSEA-5, SUSD2, W4A5, CD243, CD111, CD131 and CD164, are described as markers expressed on stem cells. The expression of stem cell markers on mast cells is in accordance with results from the FANTOM5 consortium, in which mast cells exhibited similarities with stem cells [29]. In some cases, our results are in disagreement with previously published data; for example, CD4, CD10, CD36 and CD74 were previously shown to not be expressed by HLMCs [25,27]. This discrepancy might be explained by differences in the procedures, as in contrast to published data, we did not purify or culture the studied mast cells prior to analysis [24,26-28]. Culturing mast cells has been shown to alter their phenotype and expression of cell-surface receptors [29,30].

In an immunohistological study by Andersson et al., the expression of the receptor FcεRI on HLMCs differed among different compartments in the lungs, with mast cells present in the parenchyma being negative for FcεRI [12]. In our study, there was no clear-cut division between the negative and positive FcεRI population but rather a continuous spectrum of different levels of expression, and approximately 50% of the included patients expressed FcεRI on virtually all of their mast cells (Figure 3A). These discrepancies could be due to the different detection limits of the two different techniques used, immunohistochemistry and flow cytometry. We measured expression in a quantitative manner using flow cytometry, thus finding that there is a spectrum of different expression levels, while in the immunohistological study by Andersson et al., the cells were classified into FcεRI-positive/negative populations in a binary manner depending on the detection limit of the technique. We also observed a large

variation in expression among individuals (Figure 3), and in line with our results, this has previously been shown to be true for human skin mast cells [31]. The reasons for this variation could be manifold, as the surface expression of FcεRI can be regulated in many different ways. FcεRI is, for example, upregulated by IL-4 and stabilized on the cell surface by the binding of IgE antibodies[32,] and recently, it was described that IL-33 downregulates the expression of FcεRI [33,34], indicating that the state of inflammation in the tissue can influence FcεRI expression.

HLMCs have been shown to be heterogeneous; classically, they have been studied using immunohistochemistry in a binary manner, and they have been divided into the MC_T and MC_{TC} subtypes based on whether the mast cell proteases chymase and CPA3 are detectable [6]. How this heterogeneity is reflected by the heterogeneous expression of cell-surface markers has scarcely been investigated. We investigated the heterogeneity of cell-surface markers in a quantitative manner using flow cytometry and did not find any markers that distinctly and consistently divided the studied mast cells into subpopulations with a bi- or multimodal distribution (data not shown). We did, however, find several markers with considerable continuous variation in expression within the mast cell populations (Table 2), and costaining revealed that six of these markers, SUSD2, CD49a, CD326, CD34, CD66 and HLA-DR, were correlated (Figure 3). To investigate whether these markers are correlated with the classic mast cell subpopulations MC_T and MC_{TC}, we costained for SUSD2 and CPA3, but no correlation was detected, ruling out the possibility that these markers are extracellular markers of the classic mast cell subtypes (Figure 4G). CD344 did not correlate with the MC_T or MC_{TC} profile either (data not shown). Recently, Dwyer et al. identified a population of $CD38^{low}$ nasal polyp MCs that expressed chymase (MC_{TC} type), while $CD38^{high}$ MCs were a heterogenous pool of cells containing both chymase negative and positive cells[35]. The HLMCs in the present investigation also show high variation in CD38 expression (Table 2). However, CD38 did not distinctly separate the HLMCs into subpopulations. CD88 has also been reported to be a cell-surface marker that distinguishes the MC_{TC} subtype from the MC_T subtype [36]. However, in our hands, we did not detect any expression of CD88 on HLMCs (Figure 2); thus, we were unable to find an extracellular marker that distinguishes the classic mast cell subsets.

Considering that one of our six correlated heterogeneity markers, CD34, is expressed on circulating mast cell progenitors [37], we speculated that these markers could identify cells in different stages of maturation. However, if that was the case, one would expect cells with high expression of CD34 to be small and contain few granules similar to mast cell progenitors [37]. In contrast, the cells with high expression of SUSD2 (and by correlation also high in CD34) had relatively high FSC and SSC values (Figure 4H-J), suggesting that they were relatively large and granular and therefore unlikely to be immature mast cells. SUSD2 is a marker for pluripotent [20] and mesenchymal [19] stem cells, but it is also expressed in certain cancers, in which it has been linked to proliferation [21]; thus, one could imagine that cells with high SUSD2 expression are proliferating. However, we could not detect any staining for the proliferation marker Ki67 in HLMCs (Supplementary Figure S2). We also observed varying expression of HLA-DR, an MHC class II receptor that presents antigens to $CD4^+$ T cells, suggesting that cells with high expression of heterogeneity markers could be able to present antigens and activate $CD4^+$ T cells. There were initially conflicting results from murine experiments regarding whether mast cells are able to present antigens and activate T cells via MHC II (reviewed in [38]). However, human mast cells in close proximity to T cells in the tonsils express HLA-DR and CD80, indicating that they can present antigens to $CD4^+$ T cells [39]. Additionally, *In vitro*-derived human mast cells generated from CD34+ progenitors and *ex vivo* human skin mast cells have been shown to express MHC II and costimulatory ligands when stimulated with IFN-γ and activate T cells in an antigen-dependent manner [39,40]. In this context, it is worth noting that costimulatory ligands for T cell activation, including CD80, CD86, CTLA-4 (CD152), OX40L (CD252), Tim-1, Tim-4, 41BB-L (CD137L), ICOS-L (CD275), CD70, CD40, LIGHT (CD258) and CD112, were not detected on HLMCs, while CD48, CD58, CD155 and HVEM (CD270) were expressed (Figure 2). The coinhibitory ligands PD-L1 (CD274) and PD-L2 (B7-DC, CD273) were also expressed, while galectin-9 was not detected (Figure 2) [41]. Thus, HLMCs express receptors/ligands that endow them with the possibilities to interact with and regulate T cells and adaptive immunity [42,43].

In summary, we found the expression of 102 cell-surface antigens on HLMCs, and several of these antigens had high continuous variability in expression within the mast cell population. The expression of six of these markers correlated to each other (SUSD2, CD49a, CD326, CD34, CD66 and HLA-DR) and the size and granularity of the cells. Further studies are needed to determine how these cells differ functionally. To the contrary of the dogma of distinct mast cell subtypes, we demonstrate the continuous nature of HLMC heterogeneity.

Autor Contributions

ER, JSD and GN, conceived and designed the studies. ER, JSD and AR designed and performed the experiments. ER, DZHB and JSD analyzed the data. JS, ACO, MAA, MA and SED provided samples. ER, GN and JSD wrote the manuscript draft. All authors reviewed, critically revised, and approved the final manuscript.

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Table 1. Novel antigens identified on human lung mast cells

Marker (clone)	Description
CD36	Receptor binding a broad range of lipids
CD45RO	Isoform of CD45
CD66a/c/e	Adhesion molecules
CD74	Involved in MHC class II antigen processing and a receptor for macrophage migration inhibitory factor
CD111	Adhesion molecule
CD115	Receptor for M-CSF and IL-34
CD131	Common β subunit of the IL-3, IL-5 and GM-CSF receptors
CD143	Metallopeptidase
CD148	Tyrosine phosphatase involved in signal transduction
CD164	Sialomucin involved in cell adhesion and proliferation
CD166	Glycoprotein involved in cell adhesion and migration
CD205	Endocytic receptor involved in antigen uptake and processing
CD243	Involved in transportation of molecules across cell membranes
CD270	Receptor for TNFSF14, BTLA, LTA and CD160
CD277	Regulate T cell responses
CD317	Blocks the release of certain viruses from infected cells
CD344 (Frizzled-4)	Receptor for Wnt proteins and norrin
CLEC12A/CD371	C-type lectin-like receptor with an immunoreceptor tyrosine-based inhibitory motif (ITIM) domain
Integrin α9β1	Integrin mediating cell adhesion and migration
SUSD2 (W3D5, W5C5)	Potentially involved in cell adhesion as this transmembrane protein contains functional domains associated with adhesion molecules
(W4A5)	Antigen has yet to be described
Siglec-9	Lectin that binds sialic acid and has ITIM domains
SSEA-5	A glycan

Table 2. The 10 markers from the LEGENDScreen analysis with the highest robust coefficient of variation (robust CV)

Marker	Robust CV
SUSD2 (W5C5)	264
SUSD2 (W3D5)	246
CD344	172
CD49a	160
CD326	155
CD66a/c/e	153
CD34	134
HLA-DR	133
SSEA-5	131
CD63	124
CD38	123

Figure Legends

Figure 1. **Gating strategy and LEGENDScreen results**

Single-cell suspensions of human lung tissue were stained with anti-CD45, anti-CD117, anti-CD14 and anti-FcεRI antibodies and then stained with the LEGENDScreen human cell Screening kit. (A) Representative gating strategies for human lung mast cells and $CD14^+$ cells are shown. (B) Intracellular tryptase-stained human lung mast cells compared to isotype-stained mast cells and tryptase-stained $CD45^+CD117^-$ cells. (C) Scatter plot of the p-values, MFI and percent positive of each marker on human lung mast cells. The y-axis represents -log10 FDR-adjusted p-values from 2-sided individual t-tests (marker versus FMO controls), and the blue line represents the confidence cutoff of -log10 (0.05). The x-axis represents normalized ln(MFI) values (plate-matched FMO control subtracted from the marker), and the size of circles represents the percentage of positive cells with the positive gate set according to the FMO. Some mast cell markers are highlighted in blue. (D) Comparison of marker expression between mast cells and $CD14^+$ cells. Volcano plot showing log2-fold change in mast cells divided by $CD14^+$ cells (normalized MFI values with the plate-matched FMO subtracted) against -log10 p-values (independent 2-sided t-test) of mast cells against $CD14^+$ cells. Markers are annotated only if abs(log2fc) => 2 and p-value < 0.05. n=3.

Figure 2. **Expression of cell-surface antigens on human lung mast cells.**
Single-cell suspensions of human lungs were stained with anti-CD45, anti-CD117, anti-CD14 and anti-FcεRI antibodies and then stained with the LEGENDScreen human cell screening kit. Mast cells were gated as $CD45^{+}CD14^{low}CD117^{high}$ cells. Shown are the percent positive (%) for each marker and the MFI that was normalized to the plate-matched FMO control and log10 transformed. The significance of the MFI of the marker compared to that of the FMO control is shown (one-way ANOVA with Dunnett's multiple comparisons test); * $p < 0.05$, ** $p < 0.01$, *** $p<0.001$, **** $p<0.0001$. n=3.

Figure 3. FcεRI expression on human lung mast cells.
Examples of CD117/FcεRI expression on HLMCs gated as $CD45^{+}CD14^{low}CD117^{high}$ cells from four donors (A). Quantification of the percent positive for FcεRI (B) and the MFI of FcεRI normalized to that of the matched FMO control (C). n=9.

Figure 4. Correlations between markers with high CV, size and granularity.
HLMCs gated as $CD45^{+}CD14^{low}CD117^{high}$ cells were costained for SUSD2 (A-H), CD66a/c/e (A), CD49a (B), HLA-DR (C), CD34 (D), CD326 (E), CD344 (F), or CPA3 (G). Representative plots form 4 donors are shown. Pearson correlations to SUSD2 of the fluorescent intensity data in each donor was calculated using Graphpad prism and the average r value of four donors is shown. All correlations had a $p>0.0001$. SUSD2low, SUSD2intermediate and SUSD2high cells were gated, and the FSC (H) and SSC (I) values of the SUSD2low (dotted line) and SUSD2high (filled gray) populations are shown. Quantification of FSC and SSC is shown in (J), mean ± SEM, n=5. Two-way ANOVA with Bonferroni's multiple comparisons test was performed. * $p < 0.05$; ** $p < 0.01$; *** $p<0.001$; **** $p<0.0001$.

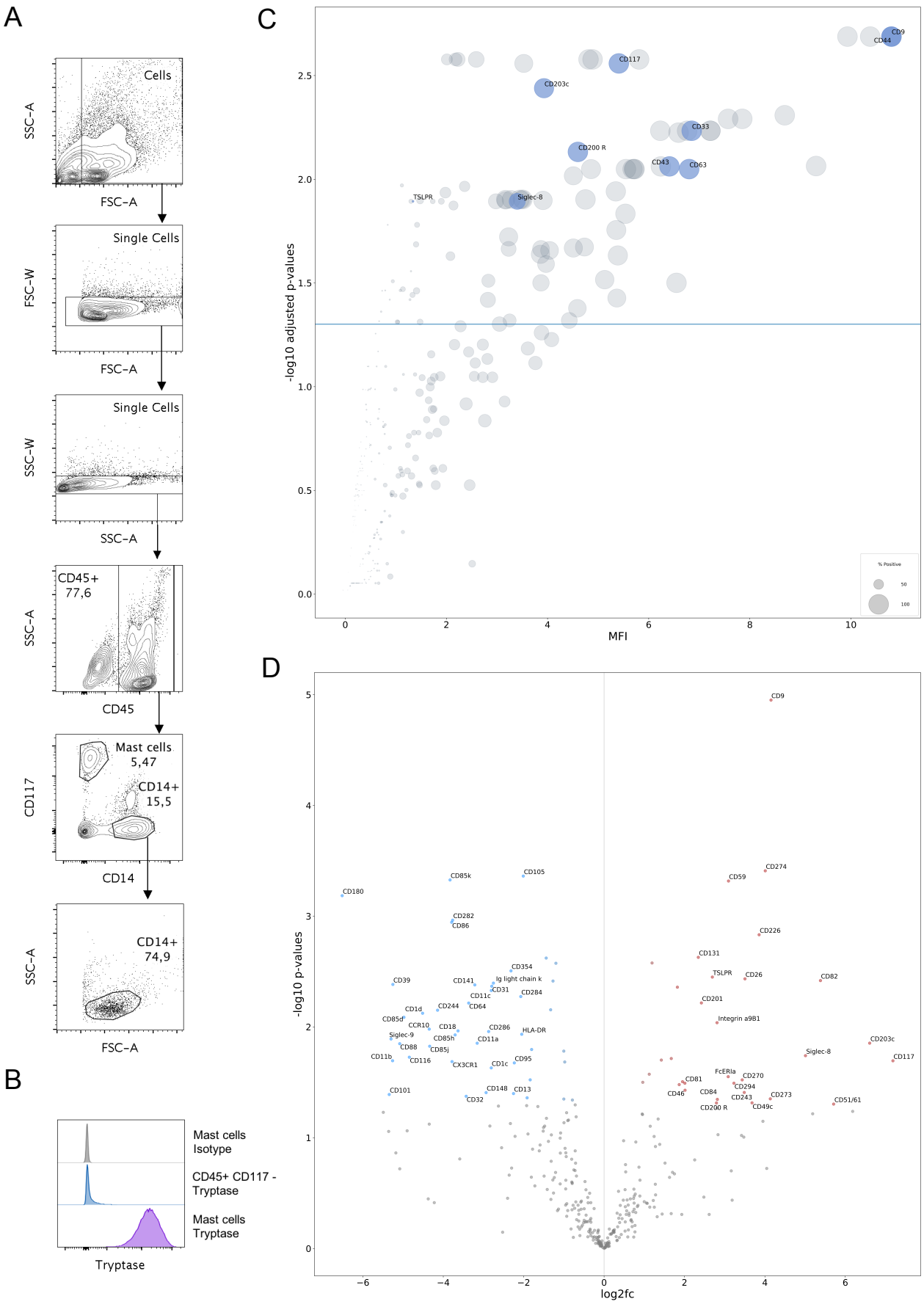
A
Cells
SSC-A
FSC-A
Single Cells
FSC-W
FSC-A
Single Cells
SSC-W
SSC-A
CD45+
77,6
SSC-A
CD45
Mast cells
5,47
CD14+
15,5
CD117
CD14
CD14+
74,9
SSC-A
FSC-A
B
Mast cells
Isotype
CD45+ CD117 -
Tryptase
Mast cells
Tryptase
Tryptase
C
-log10 adjusted p-values
MFI
CD9
CD44
CD117
CD203c
CD33
CD200 R
CD43
CD63
TSLPR
Siglec-8
% Positive
50
100
D
-log10 p-values
log2fc
CD9
CD180
CD85k
CD105
CD282
CD86
CD274
CD59
CD226
CD131
CD354
CD39
CD141
Ig light chain k
CD31
CD284
CD11c
CD244
CD64
CD1d
CD85d
CCR10
CD18
CD286
HLA-DR
Siglec-9
CD85h
CD11a
CD88
CD85j
CD11b
CD116
CX3CR1
CD1c
CD95
CD101
CD148
CD13
CD32
TSLPR
CD26
CD82
CD201
Integrin a9B1
CD203c
Siglec-8
CD117
FcERIa
CD270
CD81
CD294
CD46
CD84
CD243
CD273
CD200 R
CD49c
CD51/61

Figure 1

Marker	%	MFI	
CD1a	6,38	0,63	
CD1b	8,40	0,73	
CD1c	4,80	0,58	
CD1d	7,66	0,71	
CD2	52,23	2,44	
CD3	25,25	1,13	
CD4	75,90	3,23	**
CD5	8,56	0,63	
CD6	11,69	0,71	
CD7	22,17	0,93	
CD8a	16,26	0,92	
CD9	100,00	10,80	****
CD10	52,60	2,57	*
CD11a	72,90	4,08	****
CD11b	51,05	2,55	*
CD11b act	31,11	1,39	
CD11c	84,93	3,87	****
CD13	97,10	5,36	****
CD14	26,34	1,24	
CD15	2,94	0,46	
CD16	34,88	1,66	
CD18	94,13	5,13	****
CD19	2,01	0,30	
CD20	5,40	0,35	
CD21	10,04	0,58	
CD22	98,63	5,35	****
CD23	1,54	0,29	
CD24	21,46	1,09	
CD25	8,43	0,49	
CD26	92,17	4,51	****
CD27	3,42	0,46	
CD28	30,95	1,23	
CD29	100,00	7,22	****
CD30	35,11	1,74	
CD31	74,40	3,88	****
CD32	56,11	2,81	*
CD33	100,00	6,85	****
CD34	57,53	2,28	
CD35	22,32	1,11	
CD36	67,67	3,61	***
CD38	68,20	3,76	***
CD39	41,10	1,70	
CD40	33,20	1,61	
CD41	57,52	2,72	*
CD42b	2,32	0,35	
CD43	99,60	6,41	****
CD44	100,00	10,80	****
CD45	92,47	4,51	****
CD45RA	85,67	3,97	****
CD45RB	40,33	1,76	
CD45RO	89,70	4,59	****
CD46	100,00	7,85	****
CD47	100,00	8,69	****
CD48	99,37	5,55	****
CD49a	47,47	1,88	
CD49c	98,53	5,68	****
CD49d	97,37	4,86	****
CD49e	91,77	4,73	****
CD49f	53,73	2,16	
CD50	80,70	4,43	****
CD51	99,70	5,71	****
CD51/61	99,83	5,72	****
CD52	91,67	5,37	****
CD53	98,03	5,39	****
CD54	98,93	5,54	****
CD55	99,87	6,23	****
CD56	2,56	0,44	
CD57	3,28	0,40	
CD58	100,00	6,76	****
CD59	100,00	9,93	****
CD61	90,30	4,04	****
CD62E	4,82	0,57	
CD62L	6,08	0,48	
CD62P	35,24	1,77	
CD63	100,00	6,80	****
CD64	54,73	2,91	**
CD66a/c/e	66,50	2,83	*
CD66b	3,77	0,47	
CD69	99,43	6,24	****
CD70	4,99	0,56	
CD71	66,10	3,25	**
CD73	41,83	1,68	
CD74	54,54	2,72	*
CD79b	24,96	1,05	
CD80	18,30	0,99	
CD81	100,00	7,57	****

Marker	%	MFI	
CD82	100,00	10,38	****
CD83	55,90	3,15	**
CD84	100,00	7,22	****
CD85a	12,05	0,90	
CD85d	9,81	0,78	
CD85g	4,97	0,66	
CD85h	13,45	0,92	
CD85j	33,97	1,75	
CD85k	26,49	1,26	
CD86	9,28	0,65	
CD87	11,71	0,79	
CD88	8,53	0,71	
CD89	8,44	0,50	
CD90	16,83	0,94	
CD93	5,11	0,46	
CD94	1,93	0,25	
CD95	27,90	1,40	*
CD96	2,70	0,29	
CD97	99,87	4,89	****
CD99	100,00	5,81	****
CD100	31,75	1,52	*
CD101	2,79	0,27	
CD102	26,33	1,33	
CD103	1,01	0,16	
CD104	6,23	0,49	
CD105	5,09	0,65	
CD106	2,36	0,31	
CD107a	80,00	2,59	****
CD108	6,18	0,62	
CD109	2,14	0,20	
CD111	29,92	1,48	*
CD112	13,46	1,09	
CD114	4,93	0,52	
CD115	40,34	1,93	***
CD116	6,29	0,41	
CD117	100,00	5,41	****
CD119	55,00	1,98	***
CD122	1,42	0,21	
CD123	7,51	0,67	
CD124	5,40	0,73	
CD126	6,01	0,84	
CD127	2,18	0,21	
CD129	4,30	0,46	
CD131	65,37	2,18	****
CD132	17,18	1,12	
CD134	3,92	0,30	
CD135	1,95	0,28	
CD137	3,85	0,35	
CD137L	12,18	0,80	
CD138	18,23	0,88	
CD140a	1,79	0,33	
CD140b	5,49	0,59	
CD141	25,27	1,14	
CD146	32,83	1,41	*
CD144	2,80	0,32	
CD146	23,07	1,03	
CD148	56,17	2,01	***
CD150	2,34	0,18	
CD152	20,44	0,79	
CD154	4,58	0,21	
CD155	26,27	1,42	*
CD156c	93,80	3,91	****
CD158a/h	1,53	0,16	
CD158b	4,61	0,50	
CD158d	33,85	1,31	
CD158e1	2,05	0,15	
CD158f	2,00	0,23	
CD161	1,83	0,26	
CD162	91,83	3,53	****
CD163	18,49	0,99	
CD164	99,60	4,81	****
CD165	1,92	0,40	
CD166	87,97	3,17	****
CD167a	12,64	0,76	
CD169	2,42	0,44	
CD170	71,10	2,23	****
CD172a	74,23	3,05	****
CD172b	11,26	0,59	
CD172g	5,21	0,60	
CD178	32,82	1,14	
CD179a	9,19	0,67	
CD179b	2,16	0,19	
CD180	1,22	0,23	
CD181	5,65	0,42	
CD182	1,38	0,21	
CD183	6,53	0,43	

Marker	%	MFI	
CD184	3,89	0,41	
CD193	2,37	0,24	
CD195	4,45	0,45	
CD196	4,33	0,25	
CD197	3,23	0,42	
CD200	5,80	0,67	
CD200 R	99,70	4,60	****
CD201	35,50	1,48	*
CD202b	1,66	0,24	
CD203c	98,13	3,93	****
CD205	38,72	1,47	*
CD206	5,01	0,51	
CD207	1,85	0,19	
CD209	0,65	0,22	
CD210	3,21	0,38	
CD213a2	3,22	0,34	
CD215	18,85	1,04	
CD218a	3,54	0,44	
CD220	2,38	0,49	
CD221	7,94	0,63	
CD226	88,03	3,50	****
CD229	1,12	0,21	
CD231	7,13	0,64	
CD235ab	4,09	0,10	
CD243	77,07	2,82	**
CD244	1,88	0,34	
CD245	18,05	1,21	
CD252	2,56	0,25	
CD253	1,56	0,25	
CD254	1,48	0,32	
CD255	1,64	0,21	
CD257	6,68	0,67	
CD258	2,97	0,45	
CD261	2,33	0,16	
CD262	4,68	0,50	
CD263	2,49	0,28	
CD266	2,06	0,40	
CD267	2,19	0,25	
CD268	0,76	0,09	
CD270	99,73	4,75	****
CD271	1,80	0,42	
CD273	62,63	2,39	*
CD274	93,07	3,17	***
CD275	17,42	1,14	
CD276	93,13	3,86	****
CD277	91,00	3,28	***
CD278	1,98	0,18	
CD279	1,33	0,10	
CD282	1,35	0,19	
CD284	2,18	0,34	
CD286	1,72	0,23	
CD290	1,16	0,15	
CD294	24,30	1,45	
CD298	100,00	6,59	****
CD300e	27,83	0,89	
CD300F	96,23	3,51	****
CD301	4,04	0,57	
CD303	2,30	0,26	
CD304	29,57	1,46	
CD307	1,66	0,12	
CD307d	1,69	0,23	
CD314	3,33	0,41	
CD317	95,40	3,45	****
CD318	12,77	0,88	
CD319	4,97	0,55	
CD324	3,42	0,52	
CD325	1,76	0,16	
CD326	44,97	1,82	
CD328	64,57	2,76	**
CD344	1,04	-0,04	
CD335	5,22	0,38	
CD336	1,17	0,13	
CD337	2,20	0,17	
CD338	1,19	0,21	
CD340	1,18	0,22	
CD344	55,30	2,46	*
CD351	1,69	0,15	
CD352	11,95	0,70	
CD354	14,52	0,83	
CD355	1,69	0,13	
CD357	4,21	0,66	
CD360	1,64	0,19	
B2M	100,00	9,31	****
BTLA	2,11	0,30	
C3AR	49,57	1,96	
C5L2	4,57	0,59	

Marker	%	MFI	
CCR10	29,73	1,47	
CLEC12A	92,43	3,23	***
CLEC9A	1,90	0,28	
CX3CR1	0,89	0,28	
CXCR7	1,96	0,26	
OPRD	1,52	0,17	
DLL1	2,97	0,12	
DLL4	0,53	-0,05	
DR3	13,98	0,66	
EGFR	3,64	0,47	
erbB3	1,13	0,05	
FcεRIα	41,23	1,72	
FcRL6	1,77	0,09	
Galectin-9	4,48	0,15	
GARP	3,17	0,22	
HLA-A,B,C	99,40	6,55	****
HLA-A2	34,66	2,51	*
HLA-DQ	46,10	1,79	
HLA-DR	83,63	3,87	****
HLA-E	37,53	1,70	
HLA-G	8,80	0,60	
IFNGR2	1,93	0,25	
Ig light chain κ	25,93	1,36	
Ig light chain λ	35,50	1,65	
IgD	1,46	0,14	
IgM	1,15	0,23	
IL-28RA	1,73	0,22	
Integrin α9β1	76,67	2,98	****
Integrin β5	7,02	0,88	*
Integrin β7	11,48	1,15	***
Jagged 2	5,55	0,75	
LAP	0,74	0,32	
LT-B R	8,37	1,30	****
Mac- 2	2,64	0,27	
MAIR- II	11,33	1,28	****
MICA/MICB	1,20	0,26	
W3D5	45,03	2,08	****
W5C5	46,47	2,14	****
W7C6	1,78	0,48	
W4A5	53,27	2,36	****
MSCA-1	0,88	0,32	
NKp80	0,38	0,33	
Notch 1	1,78	0,67	
Notch 2	2,06	0,76	
Notch 3	1,14	0,48	
Notch 4	1,24	0,29	
NPC (57D2)	2,85	0,41	
Podoplanin	7,20	0,78	
Pre- BCR	2,31	0,37	
PSMA	5,65	0,40	
Siglec-10	24,81	1,73	****
Siglec-8	81,73	3,40	****
Siglec-9	17,99	1,38	****
SSEA-1	0,58	0,19	
SSEA-3	0,93	0,33	
SSEA-4	1,29	0,35	
SSEA-5	22,17	1,30	****
TCR γ/σ	2,91	0,49	
TCR Vβ13,2	0,79	0,19	
TCR Vβ23	2,10	0,31	
TCR Vβ8	0,95	0,12	
TCR Vβ9	0,49	0,28	
TCR Vσ2	0,52	0,12	
TCR Vγ9	1,18	0,21	
TCR Vα24-Jα18	6,25	0,79	
TCR Vα7,2	0,80	0,17	
TCR a/B	1,87	0,48	
Tim-1	1,05	0,26	
Tim-3	30,37	1,90	****
Tim-4	0,94	0,28	
TLT-2	0,46	0,17	
TRA-1-60-R	0,59	0,10	
TRA-1-81	0,64	0,12	
TSLPR	9,87	1,34	****
Ms IgG1 κ	1,04	0,24	
Ms IgG2a κ	4,22	0,75	
Ms IgG2b	0,93	0,27	
Ms IgG3 κ	1,54	0,34	
Ms IgM κ	0,43	0,06	
Rat IgG1	0,98	0,32	
Rat IgG2a	1,56	0,31	
Rat IgG2b	0,62	0,18	
Rat IgM κ	0,27	0,15	
AH IgG	1,37	0,31	

Figure 2

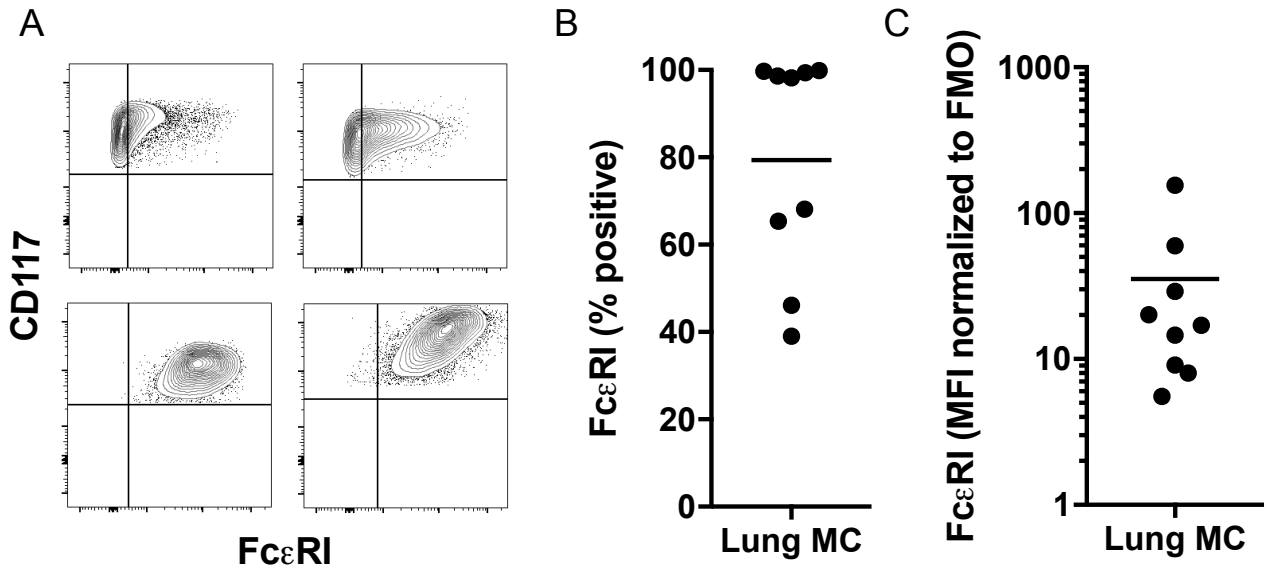
A
CD117
FcεRI
B
FcεRI (% positive)
100
80
60
40
20
0
Lung MC
C
FcεRI (MFI normalized to FMO)
1000
100
10
1
Lung MC

Figure 3

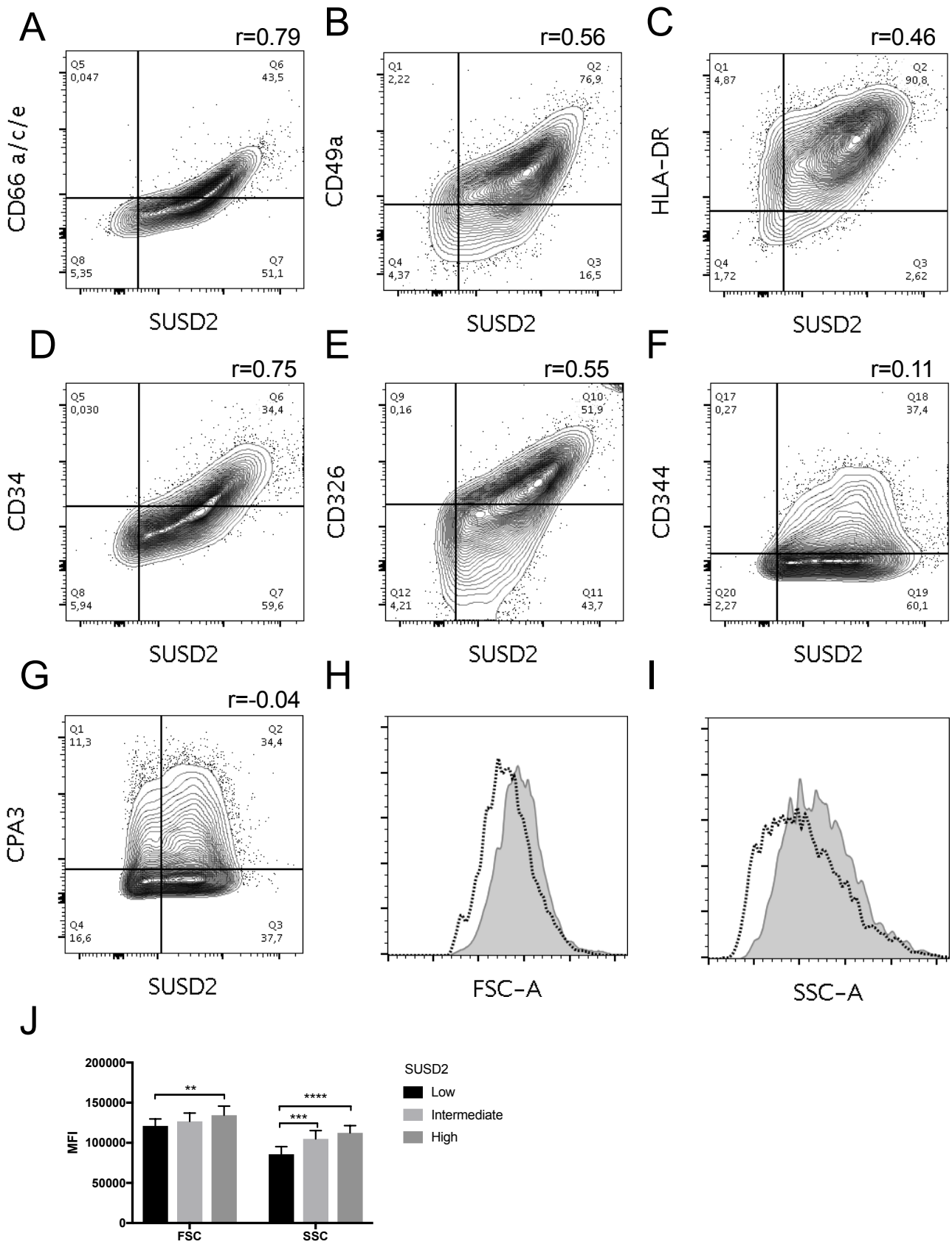
A
r=0.79
Q5 0,047
Q6 43,5
Q8 5,35
Q7 51,1
CD66 a/c/e
SUSD2
B
r=0.56
Q1 2,22
Q2 76,9
Q4 4,37
Q3 16,5
CD49a
SUSD2
C
r=0.46
Q1 4,87
Q2 90,8
Q4 1,72
Q3 2,62
HLA-DR
SUSD2
D
r=0.75
Q5 0,030
Q6 34,4
Q8 5,94
Q7 59,6
CD34
SUSD2
E
r=0.55
Q9 0,16
Q10 51,9
Q12 4,21
Q11 43,7
CD326
SUSD2
F
r=0.11
Q17 0,27
Q18 37,4
Q20 2,27
Q19 60,1
CD344
SUSD2
G
r=-0.04
Q1 11,3
Q2 34,4
Q4 16,6
Q3 37,7
CPA3
SUSD2
H
FSC-A
I
SSC-A
J
MFI
200000
150000
100000
50000
0
**
****
***
FSC
SSC
SUSD2
Low
Intermediate
High

Figure 4