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Mast cell surfaceome characterization reveals CD98hc is critical for optimal cell function

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Abstract

Background: Mast cells are involved in many distinct pathological conditions, suggesting that they recognize and respond to various stimuli and thus require a rich repertoire of cell surface proteins. However, mast cell surface proteomes have not been comprehensively characterized.

Objective: We aimed to further characterize the mast cell surface proteome to obtain a better understanding of how mast cells function in health and disease.

Methods: We enriched for glycosylated surface proteins expressed in mouse bone marrow-derived cultured mast cells (BMCMCs) and identified them using mass spectrometry analysis. The presence of novel surface proteins in mast cells was validated by qPCR and flow cytometry analysis in BMCMCs and peritoneal mast cells (PMCs). We developed a clustered regularly

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interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) gene editing approach to disrupt genes of interest in BMCMCs.

Results: The glycoprotein enrichment approach resulted in the identification of 1270 proteins in BMCMCs, 378 of which were localized to the plasma membrane. The most common protein classes among plasma membrane proteins are small GTPases, receptors and transporters. One such cell surface protein is CD98 heavy chain (CD98hc) encoded by the *Slc3a2* gene. *Slc3a2* gene disruption resulted in a significant reduction in CD98hc expression, adhesion, and proliferation.

Conclusions: Our study indicates that glycoprotein enrichment coupled with mass spectrometry can be used to identify novel surface molecules in mast cells. Moreover, we found that CD98hc plays an important role in mast cell function.

Capsule summary

Glycoprotein enrichment and mass spectrometry enabled a comprehensive characterization of the mast cell surface proteome. CD98hc expression is required for optimal mast cell function.

Keywords

Mast cells; glycoprotein enrichment; mass spectrometry; CRISPR/Cas9; surfaceome; CD98hc

INTRODUCTION

Mast cells are hematopoietic progenitor-derived, granule-containing immune cells that are widely distributed in tissues that interact with the external environment, such as the skin and mucosal tissues¹. It is well-known that mast cells are significantly involved in IgE-mediated allergic reactions². Moreover, recent clinical studies with therapies that can reduce mast cell numbers in severe asthma highlight the relevance of mast cells in human disease outcomes³. Recent studies using mouse modeling of mast cell or mast cell protease deficiency by our group and others indicate that mast cells and their granule proteases have pleiotropic regulatory roles in immunological responses and diseases, exemplified by bacterial and parasite infections, sepsis, auto-immune disease, and cancer⁴⁻⁶. Overall, these studies suggest that in addition to IgE-dependent mechanisms, mast cell function can be influenced by IgE-independent mechanisms involving cytokines, peptides, toll-like receptor ligands, or components of the complement system. The mast cell surface protein repertoire that transduces these stimuli is essential for the concerted biological function of mast cells in the complex signaling environments generated during homeostasis and disease. However, the mast cell surface proteome or surfaceome has yet not been comprehensively characterized.

Historically, antibody-based technologies have been utilized to characterize the cell surface proteomes of immune cells, including mast cells^{7, 8}. However, antibody-based technologies such as flow or mass cytometry are limited in their multiplexing capabilities and by the availability of high-quality antibodies. For example, approximately 370 antigens are targeted by monoclonal antibodies to cluster of differentiation (CD) molecules⁹, representing only a fraction of the estimated 2,900 proteins in the surface exposed proteome¹⁰. Mass spectrometry-based technologies offer the advantage of multiplexed and unbiased detection of proteins independent of existing antibody availability. Since most cell surface proteins

are glycosylated, the identification of most components of the cell surface proteome can be accomplished by the enrichment of glycoproteins exposed to the extracellular space. Glycoprotein enrichment in combination with mass spectrometry has been used to characterize the surfaceome of a relatively large number of tumor^{11–13} and human primary cells including macrophages^{14,15} and CD4⁺ T cells¹⁶. Therefore, we decided to use this approach to investigate the surface proteome profile of mast cells. Mass spectrometry analysis of BMCMC glycoprotein enriched samples resulted in the identification of 1,270 proteins. Plasma membrane proteins including GTPases, receptors and transporters were overrepresented in the glycoprotein enriched samples. Among the novel pool of surface proteins, we found that CD98hc was highly expressed in mast cells. Therefore, we investigated CD98hc's contribution to mast cell function and observed that CD98hc deletion by a CRISPR/Cas9 approach significantly impaired mast cell ability to degranulate, proliferate and adhere to fibronectin.

METHODS

Mass spectrometry (MS) sample preparation

For glycoprotein enrichment, BMCMCs were treated with 10mM sodium periodate (NaIO₄) (Bio-Rad, Hercules, CA) in PBS for 1h to oxidize hydroxyl groups on surface glycoproteins. Cells were lysed in lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 0.002% sodium azide, 1% octyl-b-D-1-thioglucopyranoside) supplemented with a cocktail of serine and cysteine protease inhibitors (catalog number 11836170001, Sigma Aldrich, St. Louis, MO) and passed through a 27.5 gauge needle 15 times. Lysates were incubated overnight at room temperature with hydrazide beads (catalog number 153-6047, Bio-Rad) in coupling buffer (100 mM sodium acetate, 150 mM NaCl in H₂O, pH 5.5). Bead-bound proteins were denatured with 8 M urea in 100 mM NH₄HCO₃. Immobilized denatured proteins were reduced with 50 mM dithiothreitol (DTT) for 1 h at 37°C, alkylated with 65 mM iodoacetamide (IAA) at room temperature in the dark for 30 min, and digested with trypsin (1:100, w/w) at 37 °C overnight. The digested tryptic peptides were recovered and stored at –80 °C until analysis. For whole cell proteomics, BMCMCs were treated with the same lysis, denaturation, reduction, alkylation, and digestion steps described for enriched glycoproteins.

Preparation of CRISPR/Cas9 reagents

CRISPR RNAs (crRNA) targeting *Slc3a2* were identified using the CCTop design tool¹⁷ and the COSMID CRISPR design tool¹⁸ and commercially synthesized by Integrated DNA Technologies (IDT, Coralville, IA). The ribonucleoprotein (RNP) crRNA sequence targeting exon 2 in mouse cells was GGGTGCTCAAGTACTCCAGA.

Mock edited cells were generated with Alt-R® CRISPR-Cas9 Negative Control crRNA #1 (catalog 1072544, Integrated DNA Technologies, Inc., Coralville, IA).

Gene editing

Slc3a2 disruption was accomplished using the Neon transfection system (catalog number MPK1025, Thermo Fisher Scientific). crRNA targeting *Slc3a2* and *trans*-activating RNA

(IDT) were complexed at a 1:1 ratio, as per the manufacturer's instructions. crRNA: *trans*-activating RNA complexes were mixed with Cas9 nuclease (IDT) at 1.2:1 M ratio (120 pmol RNA complex: 100 pmol Cas9 per reaction). Cells were washed with PBS and resuspended in Neon Buffer T at a final cell density of 4×10^7 cells/ml. Then, 100 pmol of complexed RNP per 4×10^6 cells was delivered to the cells by Neon electroporation (1450 V, 3 10 ms pulses). Cells were then transferred into pre-warmed cell culture medium with IL-3 (10 ng/ml) and 10% FBS. Cells were maintained in media after editing at a density of $\sim 5 \times 10^5$ cells/ml. Seven days after gene editing, CD98hc⁻ cells accounted for 85% of the total gene-edited BMCMCs.

Image analysis of gene edited cells

Cells were transferred onto glass slides using cytopsin and stained with May-Grunwald Giemsa. Photographs were taken using a Leica DM4000 B microscope with a Leica DFC310 FX camera (Leica, Allendale, NJ). LAS V4.5 software was used for capturing the images (Leica). The images were analyzed using the ImageJ software (NIH, Bethesda, MD). The largest diameter of cells was quantified using the measure function. The metachromatic area of each cell was estimated as the integrated density of the photos converted into binary images.

Statistical analyses

Statistical tests were performed using GraphPad Prism version 8.4.3 (GraphPad Software, Inc., La Jolla, CA). Data were analyzed for statistical significance using the Mann Whitney *U*-test. $P < 0.05$ was considered statistically significant.

Detailed methods used in this study are included in the Methods section in this article's Online Repository (available at www.jacionline.org), covering the following: C57BL/6 mice used, generation of BMCMCs, mass spectrometry analysis of cell surface proteins, single-cell sorting and qPCRs, flow cytometry, confocal microscopy, β -hexosaminidase release assay, PGD₂ and cys-LT release assays, cytokine and chemokine release assays, cell counting and proliferation assay, adhesion assay, analysis of ERK phosphorylation, and colocalization immunofluorescence for cutaneous mastocytosis.

RESULTS

Mast cell surface proteome profiling

To profile the cell surface proteome of mast cells, we used well-established approaches to enrich for glycoproteins^{15, 16, 19}, which are disproportionately located on the plasma membrane²⁰. Briefly, glycoproteins were oxidized prior to cell lysis and captured from the lysate by hydrazide beads. Following several washes in denaturing buffer, peptides digested from hydrazide beads were subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) for protein identification (Fig. E1A). Analysis of the protein expression profiles generated by this method demonstrated close agreement between replicates as well as readily discernable differences between BMCMC whole cell lysate and BMCMC glycoprotein enriched samples (Fig. E1B). Of the 1,750 proteins identified in BMCMC samples, 748 (~43%) were common between whole cell and enriched samples, 480 (~27%)

were present in whole cell lysates only, and 522 (~30%) were present in glycoprotein enriched samples only. The glycoprotein enrichment process substantially increased the proportion of transmembrane domain-containing proteins in the samples relative to whole cell lysate samples from 11% to 26% (Fig. 1A). Additionally, analysis of annotated subcellular localization listed on UniProt and GeneCards confirmed that plasma membrane proteins were over-represented in glycoprotein enriched samples relative to whole cell lysates from the same cells (Fig. 1A, 30% and 24%, respectively). This proportion of plasma membrane proteins is similar to that previously reported with the use of glycoprotein enrichment and mass spectrometry^{15, 19}. All further analysis in the study utilized only the glycoprotein-enriched samples. The most highly represented plasma membrane-annotated proteins are listed in Table 1 (see full list in Table E1). Importantly, our approach detected surface molecules known to be expressed in mast cells in the enriched samples, validating its usefulness for identifying mast cell surface molecules. These expected surface molecules include the beta and gamma subunits of the high affinity receptor for IgE (FcεRIβ and FcεRIγ, respectively), the receptor for stem cell factor (c-Kit, also known as CD117), the type 1 receptor for transforming growth factor-beta (Tgfr1), the receptor for IL-33 (Il1rl1, also known as St2) and the common beta subunit of the receptor for granulocyte-macrophage colony stimulating factor (GM-CSF), IL-3, and IL-5 (Csf2rb). We also observed numerous mast cell-associated leukocyte CD surface molecules among the plasma membrane proteins (Table E1). These CD molecules reported to be expressed during mast cell development including CD13 (Anpep), CD34, and CD117 (c-Kit)²¹ as well as those involved in mast cell activation including CD31 (Pecam)²², CD45 (Ptpre)²³, CD48^{24, 25}, CD63 (Lamp3)²⁶, and CD107a (Lamp1)²⁷.

Gene ontology analysis of the plasma membrane-localized proteins by PANTHER^{28, 29} revealed that these proteins are involved in a wide variety of biological processes (Fig. 1B). The most annotated processes included cellular processes, biological regulation, response to stimulus, metabolic processes, cellular compartment organization, signaling, developmental process, and multicellular organismal process (Fig. 1B), reflecting the functional diversity of proteins at the mast cell plasma membrane. Additional PANTHER analysis of the protein classes represented in our dataset revealed that the most common protein classes in our dataset were small GTPases, receptors, and transporters (Fig. 1B, PANTHER protein class data) which are protein classes normally enriched at the plasma membrane in a variety of cell types. In these classes, we examined the most highly represented proteins in our dataset. The three most highly represented small GTPases were Rab proteins—Rab7a, Rab14, and Rab5c— which have all been reported to be involved in the endocytic pathway³⁰ and to associate with macrophage phagosomes^{31, 32}. In RBL-2H3 cells, a basophilic leukemia cell line, Rab7a and Rab14 are involved in exocytosis³³, while Rab5c regulates secretory granule size through the fusion of secretory granules³⁴. Aside from the known mast cell receptors addressed above, the three most highly expressed receptors were Ly6a (Sca-1), plasminogen receptor (Plgrkt), and Spn (CD43). Ly6a is a murine bone marrow hematopoietic stem cell marker³⁵ that has been found on some mast cell progenitor cells in mice as well as BMCMCs³⁶ but not in granulocyte-myelocyte progenitors (GMPs), which are also thought to give rise to mast cells³⁷. Plgrkt has not yet been characterized in mast cells but is reportedly involved in murine macrophage migration³⁸. Spn is a sialoglycoprotein that

is a negative regulator of mast cell adhesion likely involved in mast cell migration³⁹. The highly expressed small GTPases and receptors reflect the potential relevance of these surface proteins in mast cell degranulation and ability to migrate; however, the most highly expressed transporters seen in our dataset do not currently have reported roles in mast cells despite their known contribution to cell function in other immune cells. These transporters include Atp1b3 (CD298), a sodium/potassium ATPase which has been reported to be involved in pain sensitivity in mice⁴⁰ and human T cell activation^{41, 42}; Clic1, a chloride ion channel that is important for macrophage function⁴³, and CD98hc, the heavy chain of an amino acid transport heterodimer⁴⁴ and integrin co-receptor⁴⁵ implicated in B and T cell activation^{46, 47, 48}. Overall, our data indicate that we were able to use glycoprotein enrichment and mass spectrometry to define the cell surface proteome of BMCMCs in a comprehensive manner that includes both known mast cell surface proteins as well as surface proteins of unknown mast cell function.

Identification and validation of novel surface proteins expressed in mast cells

The comprehensive nature of our glycoprotein enrichment technique allowed for the identification of mast cell surface proteins whose role in mast cell biology is not yet understood. The analysis of mast cell surface proteins by database searches (Pubmed and Google Scholar) revealed that approximately half of the mast cell plasma membrane proteins identified (223/378) have not previously been characterized in mast cells. The most highly represented novel mast cell surface proteins are listed in Table 2 (see full list in Table E2). All the novel mast cells surface genes were validated by independent verification of their expression in tissue mast cells using the Immunological Genome Project (ImmGen, <http://www.immgen.org/databrowser/>) database (Fig. E2). Gene ontology analysis of this set of surface proteins revealed that the uncharacterized surface proteins are involved in similar biological processes as the whole mast cell plasma membrane proteome (Fig. E3). Protein class analysis gave more dissimilar results as the most represented protein classes in this set of proteins are transporters, membrane traffic proteins, and scaffold/adaptor proteins (Fig. E3). Highly represented proteins in these classes include the transporters Atp1b3, Clic1, and CD98hc discussed above as well as Lman2 (VIP36), a lectin whose shedding stimulates macrophage phagocytosis⁴⁹; and Lamtor1, a scaffold protein for the mechanistic target of rapamycin (mTOR) complex that is involved in regulating the innate immune response of macrophages⁵⁰. To broadly verify the results of our proteomics analysis, a group of novel surface proteins was selected based on their wide range of estimated expression levels, and their involvement in a variety of biological processes reflecting the variety of our dataset (Table E3). Messenger RNA for all the selected novel surface proteins was detected in BMCMCs as well as PMCs, and both mouse mast cell types showed a similar pattern of expression among the genes measured (Fig. 2). Thus, our approach of the characterization of the mast cell's surfaceome by glycoprotein enrichment and mass spectrometry led to the identification of a novel pool of surface proteins to investigate in the context of mast cell biology.

CD98hc is highly expressed in resting mast cells

The highest mRNA expression in the set of genes encoding for novel surface proteins was seen for *Slc3a2* (Fig. 2). *Slc3a2* encodes the heavy chain of CD98 (CD98hc), which is the

only ubiquitously expressed heavy subunit of the heteromeric amino acid transporter CD98. CD98hc, carries the complex to the plasma membrane, whereas any of the six subunits of the L-type amino acid transporters (LATs) from the SLC7 family (LAT1, LAT2, xCT, y⁺LAT1, y⁺LAT2, and Asc-1) confers substrate specificity and constitutes the catalytic subunit of the transporter⁵¹.

In addition to CD98hc, mRNA for all the SLC7 subunits was detected in BMCMCs as well as PMCs (Fig. E4). Both mouse mast cell types showed a similar pattern of expression among the genes measured except for Asc-1 that is expressed at higher levels in BMCMCs than PMCs (Fig. E4).

The increased proportion of transporters in the set of uncharacterized mast cell surface proteins (Fig. E3) suggests that transporters may play an important and understudied role in mast cells. Furthermore, examination of the RNAseq IMMGEN database⁵², revealed that *Slc3a2* appears to be highly expressed in PMCs, heart mast cells and splenic basophils when compared to other immune cells (Fig. E2 and E5A). This observation was confirmed by flow cytometry on selected immune cells (Fig. E5B). We also confirmed that the CD98hc protein is strongly expressed in BMCMCs, though at lower levels than those observed in PMCs, by flow cytometry (Figs. 3A and 3B). Interestingly, confocal microscopy showed intracellular localization of CD98hc along with the cell surface staining observed by flow cytometry (Fig. 3C). It has been shown that CD98hc protein expression levels are upregulated in activated B and T cells⁴⁶⁻⁴⁸. On the contrary, *Slc3a2* mRNA and CD98hc protein expression levels were not significantly increased in mast cells upon activation by IgE-dependent and IgE-independent stimuli (Figs. E6A and E6B).

CD98hc deletion impairs the release of preformed mediators

Based on the findings that transporters are the most common type of uncharacterized mast cell surface proteins and the robust surface expression of CD98hc on resting mast cells as compared to other immune cells, we decided to examine CD98hc's contribution to mast cell function.

Since CD98hc deficiency in mice is embryonically lethal⁵³, we electroporated CRISPR-Cas9 ribonucleoprotein complexes into BMCMCs to delete CD98hc expression. Guide RNAs targeting exon 2 successfully disrupted the *Slc3a2* gene as evidenced by T7 Endonuclease I assay (Fig. 4A) and confirmed using DNA sequencing and Inference of CRISPR Edits (ICE) analysis (~85% indel percentage). We generated a large population of BMCMCs that do not express CD98hc (85-95%) as measured by flow cytometry 7 days after gene editing (Fig. 4B). In contrast, CD98hc-deleted BMCMCs express similar levels of FcεRIα and c-Kit as mock-edited cells (Fig. E7). Moreover, BMCMC viability was not affected by CD98hc deletion at 7-8 days after gene editing (98.9% and 99.8% for mock edited and *Slc3a2*-disrupted BMCMCs, respectively). However, morphological characterization of CD98hc-deleted BMCMCs showed that these cells were smaller (Figs. 4C and 4D) and had a smaller metachromatic area per cell (Figs. 4C and 4E) than cells expressing CD98hc.

To assess whether CD98hc plays a role in regulating mast cell activation and/or degranulation, we measured β -hexosaminidase release upon IgE-dependent activation of mock edited and *Slc3a2*-disrupted BMCMCs. Stimulation of mock edited BMCMCs resulted in significant β -hexosaminidase release when compared with cells incubated in medium alone (Fig. 4F). In contrast, CD98hc-deleted BMCMCs (*Slc3a2* RNP) showed a significant reduction in their ability to release β -hexosaminidase upon IgE-dependent activation. A similar reduction in β -hexosaminidase release was observed for CD98hc-deleted BMCMCs stimulated with calcium ionophore (A23187) (Fig. 4F). Based on this evidence, we hypothesized that CD98hc may influence the mast cell degranulation process, granule formation and/or storage of preformed mediators like β -hexosaminidase. Accordingly, we assessed whether CD98hc has an impact on the degranulation process itself by assessing the expression of the lysosomal-associated membrane protein 1 (LAMP1 or CD107a) which indicates granule mobilization towards the plasma membrane upon mast cell stimulation. As shown in Fig. E8, CD98hc-deleted BMCMCs exhibited a slight but not significant reduction in the percentage of cells that are positive for LAMP1 upon activation with either calcium ionophore (A23187) or IgE-dependent stimuli when compared with mock-edited cells. Overall, these data suggest that the reduced β -hexosaminidase release from CD98hc-deleted cells may be caused by defects in granule formation rather than a defect in the degranulation process itself.

Contrary to what was observed for β -hexosaminidase release, CD98hc deletion in BMCMCs did not impact the production and release of the arachidonic acid metabolites prostaglandin (PG)_{D2} and cys-leukotrienes (LTs) after IgE-dependent activation or stimulation with A23187 (Figs. E9A and E9B). Similarly, CD98hc deletion had no effect on the release of pro-inflammatory mediators such as tumor necrosis factor (TNF), IL-6 and monocyte chemoattractant protein-1 (MCP-1/CCL2) (Figs. E9C–E).

Taken together, this data indicates that CD98hc deletion impaired mast cell degranulation but did not affect the mast cell's ability to produce and release arachidonic acid metabolites and newly formed pro-inflammatory mediators.

CD98hc contributes to mast cell adhesion to fibronectin and cell proliferation

CD98hc can also be expressed without LATs at the plasma membrane⁴⁴, and it can have functions that are independent from its amino acid transporter role⁵⁴. More specifically, CD98hc behaves as a co-receptor of β -integrins and amplifies their downstream signaling^{55, 56} promoting cell adhesion and proliferation^{46, 55}. Therefore, we first examined whether CD98hc deletion influences the mast cell's ability to adhere to fibronectin (FN), a ligand for alpha (5) and beta (1) integrin^{57, 58}. For this purpose, we first ruled out that CD98hc deletion does not impact the expression of alpha 5 and beta 1 integrins (Fig. E10).

CD98hc-deleted BMCMCs exhibited a significant reduction in their ability to adhere to FN when compared with mock edited BMCMCs (Fig. 5A). Moreover, we observed that either SCF or IgE/antigen, stimuli known to promote mast cell adhesion to FN⁵⁹, significantly increased the adhesion of mock edited cells and CD98hc-deleted cells to FN (Fig. 5A). However, CD98hc-deleted BMCMCs exhibited a significant reduction in their ability to

adhere to FN after stimulation with either SCF or IgE/antigen when compared with mock edited cells (Fig. 5A).

Next, we examined the effects of CD98hc deletion on cell proliferation. Cell counting after plating equal numbers of cells and allowing them to grow for 5d showed that CD98hc-deleted cells were unable to significantly expand upon stimulation with SCF (Fig. 5B). To confirm whether the reduced cell expansion in CD98hc-deleted cells is due to impaired cell proliferation, we labeled the cells with carboxyfluorescein succinimidyl ester (CFSE) and analyzed the dilution of this fluorescent marker in subsequent generations of cells by flow cytometry. In BMCMC culture media, most cells stayed in the undiluted CFSE peak representing undivided cells (Fig. 5C) which is reflected in the proliferation index ranging from 1.08-1.30 (Fig. 5D). However, mock edited cells proliferated slightly more than CD98hc-deleted cells after 5d of culture (proliferation index of 1.18-1.36) (Figs. 5C and 5D). The addition of SCF to the culture media significantly stimulated proliferation in mock edited cells after 5d in culture as shown by a reduction in CFSE staining (Fig. 5C) and a significant increase in the proliferation index (1.63-1.96) when compared with cells incubated in media alone (Fig. 5D). In contrast, most of the CD98hc-deleted BMCMCs did not divide upon SCF stimulation as they remained in the undiluted CFSE peak and their proliferation index was significantly lower than that observed in mock edited cells (Fig. 5D, proliferation index 1.32-1.48). Overall, these data indicate that CD98hc plays a significant role in mast cell proliferation.

CD98hc is highly expressed in mast cells from patients with cutaneous mastocytosis

It has been reported that *SLC3A2* is differentially expressed by bone marrow mast cells (BMMCs) from patients with aggressive systemic mastocytosis versus both normal reactive BMMCs and BMMCs from patients with mild indolent systemic mastocytosis⁶⁰. Therefore, we decided to examine CD98hc expression in skin biopsies obtained from patients with cutaneous mastocytosis. We found that mast cells from patients with cutaneous mastocytosis overexpress CD98hc when compared with mast cells from patients with urticaria as a disease control (Fig. 6).

DISCUSSION

The ability of mast cells to respond to various IgE-independent stimuli as well as their well-documented plasticity shaped by their microenvironment prompted us to hypothesize that a thorough characterization of the mast cell surface proteome could identify novel targets for the modulation of mast cell response in normal conditions and disease. Accordingly, our current study had two main goals: to develop a technique that provides a comprehensive characterization of the mast cell surface proteome, and to investigate the contribution of novel surface proteins to mast cell function by using CRISPR/Cas9 as a rapid and efficient method for the disruption of genes of interest.

The development of low input proteomics methods has allowed for the proteome profiling of resident tissue cells⁶¹. Proteome analysis of mast cells has been recently reported for primary human connective tissue mast cells from skin and fat⁶² and PMCs^{61, 62}. These studies identified approximately 130-170 unique cell surface proteins in mast cells when

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mast cell proteome was compared with the proteome of macrophages⁵⁸ and other immune cells⁶¹. These findings suggest that a relatively small number of surface proteins can be detected by mass spectrometry analysis of the whole mast cell proteome. Enrichment for glycoproteins, which are disproportionately located on the plasma membrane²⁰, followed by mass spectrometry analysis has been successfully used as a comprehensive approach to characterize the cell surface proteome, or surfaceome, of immune cells including macrophages,^{15, 63} B cells, and T cells⁶⁴. Based on these studies, we decided to use glycoprotein enrichment coupled with mass spectrometry to characterize the mast cell surface proteome. BMCMCs were used in our studies because these cells can be generated in the large numbers that are required to target plasma membrane proteins via glycoprotein enrichment. We recognize we should be careful about extrapolating findings from our study on BMCMCs to tissue mast cells. Comparative transcriptome analysis of BMCMCs and tissue mast cells revealed that these two populations show many similarities but also many differences⁶⁵. Accordingly, we validated novel mast cell surface proteins in BMCMCs by independent verification of their expression in tissue mast cells using the Immunological Genome Project (ImmGen, <http://www.immgen.org/databrowser/>) database (Fig. E2). Moreover, we confirmed that PMCs express mRNA (Fig. 2) for a selected group of novel surface proteins with a wide range of estimated expression levels per our mass spectrometry data (Table E3).

Based on our analysis of annotated subcellular localization listed on UniProt and Genecards, we identified 378 plasma membrane proteins among the enriched glycoproteins, including GTPases, receptors, and transporters (Fig. 1B). The use of glycoprotein enrichment in combination with mass spectrometry typically leads to the identification of an average of 280 proteins, including 70 CD proteins, per cell type¹⁰. Based on this evidence we think that our approach has provided a comprehensive profiling of the mast cell surface proteome.

Despite the enrichment of plasma membrane proteins, we were not able to detect the presence of surface molecules known to be expressed by mast cells like the alpha subunit of the high affinity receptor for IgE (FcεRIα). Instead, we detected the FcεRIβ and FcεRIγ subunits (Supplemental Table E1). These observations are consistent with previous mass spectrometry analysis of mouse^{61, 62} and human mast cells⁶⁶. We were also not able to detect surface innate immune sensors in BMCMCs such as toll-like receptor (TLR)2, TLR3, TLR4 and TLR7. Similar observations were recently reported in mass spectrometry studies of PMCs^{61, 62}. Regardless of these observations, there is extensive literature supporting mast cell expression of these innate immune sensors that allow mast cells to respond to pathogens and contribute to host response to infection via secretion of cytokines, chemokines, and lipid mediators^{5, 67}. It is well-known that plasma membrane proteins are under-represented in proteomic studies due to their low abundance and their hydrophobic nature⁶⁸. Based on this evidence, we conclude that lack of expression of a surface protein in mast cells as determined by mass spectrometry is not definitive proof that the surface protein is not expressed in mast cells, and further assessment of its expression by alternative approaches is recommended.

Our analysis and subsequent validation of the plasma membrane proteins previously uncharacterized in mast cells revealed that CD98hc is highly expressed in both BMCMCs

and PMCs (Figs. 2 and 3). Moreover, according to publicly available datasets (Fig. E3A) and our own flow cytometry data (Fig. E3B), mast cells express the highest levels of CD98hc amongst immune cells. Interestingly, resting mast cells express high CD98hc levels and mast cell activation does not increase CD98hc expression further (Figs. E6A and E6B). In contrast, other immune cells such as lymphocytes only express higher CD98hc levels upon activation, an event that seems to be required for clonal expansion of these cells^{46, 69}. Overall, these observations suggest that CD98hc significantly contributes to mast cell function at steady state. To investigate the contribution of CD98hc to mast cell function, we developed a CRISPR/Cas9-based method to rapidly and efficiently delete CD98hc in BMCMCs (Figs. 4A and 4B). CD98hc-deleted BMCMCs were smaller in size (Figs. 4C and 4D), exhibited reduced metachromatic staining (Fig. 4C and 4E), and showed a significant reduction in β -hexosaminidase release when compared with mock edited cells (Fig. 4F). Moreover, we determined that CD98hc deletion does not impact the degranulation process itself as LAMP1 staining upon cell activation demonstrated that *Slc3a2* disruption did not significantly affect mobilization of mast cell granules to the membrane (Fig. E8). These observations suggest that CD98hc may contribute to mast cell granule formation and hence to preformed mediator storage.

Our findings seem to resemble those reported for other members of the solute carrier family which contribute to mast cell granule formation. For example, it has been shown that *Slc15a4*-deficient BMCMCs exhibit anomalous granule formation possibly due to the role of *Slc15a4* in the transport of amino acids required for the synthesis of mast cell pre-formed mediators⁷⁰.

Our studies were expanded to examine CD98hc's contribution to cell functions that are not associated with its transport activity but with its capability to bind to cytoplasmic tails of integrin- β chains, thus mediating signals that control cell adhesion and growth⁷¹. Both adhesion and proliferation upon stimulation were significantly impacted by CD98hc deletion (Fig. 5). Overall, our studies point to a critical role for CD98hc in mast cell degranulation, adhesion, and proliferation.

It has been shown that CD98-deficiency selectively impairs sustained activation of Erk1/2 after BCR ligation, an event that promotes B cell proliferation⁴⁶. Since Erk1/2 activation is also downstream of both Fc ϵ RI and c-Kit signalling⁷², we explored whether this pathway may integrate our findings on CD98hc contribution to mast cell degranulation, proliferation and adhesion. As shown in Fig. E11, analysis of Erk1/2 phosphorylation indicates that this signaling pathway is not altered in CD98hc-deleted BMCMCs. At this point, we think that multiple mechanisms are involved in how CD98hc influences mast cell function. This hypothesis is based on reports that showed that CD98hc facilitates amino acid transport using multiple partners with various amino acid specificities⁵¹, and that CD98hc has integrin-related activities that are independent from its function as an amino acid transporter^{46, 54-56}. Studies are in progress in our laboratory to determine the mechanism (s) by which CD98hc is involved in mast cell biology.

Can CD98hc contribute to the outcome of mast cell-associated disorders in humans? To gain insight into this question, we found that CD98hc is highly expressed in mast cells

from patients with cutaneous mastocytosis (Fig. 6). Mast cell CD98hc overexpression in mastocytosis is consistent with the increased demand of amino acids by rapidly proliferating cells⁷³. This result supports exploring the possibility of targeting CD98hc to treat mastocytosis and other mast cell-associated disorders in a similar manner as an approach currently under investigation for the treatment of acute myeloid leukemia^{74, 75}.

Conclusions

Overall, our studies indicate that glycoprotein enrichment in combination with mass spectrometry can be used to provide a comprehensive characterization of the mast cell surface proteome and hence improve our understanding of how mast cell function is influenced by signals generated by the environment in health and disease. Finally, we identified CD98hc as a surface protein that plays an important role in mast cell degranulation, adhesion and proliferation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

ATP	Adenosine triphosphate
BMCMCs	bone marrow derived-cultured mast cells
BMMCs	bone marrow mast cells
Cas9	CRISPR associated protein 9
CD	cluster of differentiation
CD98hc	CD98 heavy chain
CFSE	carboxyfluorescein succinimidyl ester
CRISPR	clustered regularly interspaced short palindromic repeats
Csf2rb	common beta subunit of the receptor for granulocyte-macrophage colony stimulating factor GM-CSF/IL-3/IL-5
FcεRIβ	beta subunit of the high affinity receptor for IgE
FcεRIγ	gamma subunit of the high affinity receptor for IgE

FN	fibronectin
GM-CSF	granulocyte-macrophage colony stimulating factor
GTP	guanosine triphosphate
Ig	immunoglobulin
IL	interleukin
ImmGen	Immunological Genome Project
LAMP1	lysosomal-associated membrane protein 1
LATs	L-type amino acid transporters
LC-MS/MS	liquid chromatography-mass spectrometry-mass spectrometry
LTs	leukotrienes
MCP-1:	monocyte chemoattractant protein-1
mTOR	mechanistic target of rapamycin
Plgrkt	plasminogen receptor
PMCs	peritoneal mast cells
PG	prostaglandin
RNP	ribonucleoprotein
SCF	stem cell factor
Tgfbβ1	type 1 receptor for transforming growth factor-beta
TLR	toll-like receptor
TNF	tumor necrosis factor

REFERENCES

1. Dudeck A, Koberle M, Goldmann O, Meyer N, Dudeck J, Lemmens S, et al. Mast cells as protectors of health. *J Allergy Clin Immunol* 2019; 144:S4–S18. [PubMed: 30468774]
2. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012; 18:693–704. [PubMed: 22561833]
3. Cahill KN, Katz HR, Cui J, Lai J, Kazani S, Crosby-Thompson A, et al. KIT Inhibition by Imatinib in Patients with Severe Refractory Asthma. *N Engl J Med* 2017; 376:1911–20. [PubMed: 28514613]
4. Rivellesse F, Nerviani A, Rossi FW, Marone G, Matucci-Cerinic M, de Paulis A, et al. Mast cells in rheumatoid arthritis: friends or foes? *Autoimmun Rev* 2017; 16:557–63. [PubMed: 28411167]
5. Piliponsky AM, Romani L. The contribution of mast cells to bacterial and fungal infection immunity. *Immunol Rev* 2018; 282:188–97. [PubMed: 29431211]
6. Varricchi G, de Paulis A, Marone G, Galli SJ. Future Needs in Mast Cell Biology. *Int J Mol Sci* 2019; 20.

7. Katz HR, LeBlanc PA, Russell SW. Two classes of mouse mast cells delineated by monoclonal antibodies. *Proc Natl Acad Sci U S A* 1983; 80:5916–8. [PubMed: 6577460]
8. Yu YR, O’Koren EG, Hotten DF, Kan MJ, Kopin D, Nelson ER, et al. A Protocol for the Comprehensive Flow Cytometric Analysis of Immune Cells in Normal and Inflamed Murine Non-Lymphoid Tissues. *PLoS One* 2016; 11:e0150606. [PubMed: 26938654]
9. Clark G, Stockinger H, Balderas R, van Zelm MC, Zola H, Hart D, et al. Nomenclature of CD molecules from the Tenth Human Leucocyte Differentiation Antigen Workshop. *Clin Transl Immunology* 2016; 5:e57. [PubMed: 26900471]
10. Bausch-Fluck D, Goldmann U, Muller S, van Oostrum M, Muller M, Schubert OT, et al. The in silico human surfaceome. *Proc Natl Acad Sci U S A* 2018; 115:E10988–E97. [PubMed: 30373828]
11. Bausch-Fluck D, Hofmann A, Bock T, Frei AP, Cerciello F, Jacobs A, et al. A mass spectrometric-derived cell surface protein atlas. *PLoS One* 2015; 10:e0121314. [PubMed: 25894527]
12. Hofmann A, Thiesler T, Gerrits B, Behnke S, Sobotzki N, Omasits U, et al. Surfaceome of classical Hodgkin and non-Hodgkin lymphoma. *Proteomics Clin Appl* 2015; 9:661–70. [PubMed: 26076441]
13. Fonseca AL, da Silva VL, da Fonseca MM, Meira IT, da Silva TE, Kroll JE, et al. Bioinformatics Analysis of the Human Surfaceome Reveals New Targets for a Variety of Tumor Types. *Int J Genomics* 2016; 2016:8346198. [PubMed: 28097125]
14. Raposo RA, Thomas B, Ridlova G, James W. Proteomic-based identification of CD4-interacting proteins in human primary macrophages. *PLoS One* 2011; 6:e18690. [PubMed: 21533244]
15. Kalxdorf M, Gade S, Eberl HC, Bantscheff M. Monitoring Cell-surface N-Glycoproteome Dynamics by Quantitative Proteomics Reveals Mechanistic Insights into Macrophage Differentiation. *Mol Cell Proteomics* 2017; 16:770–85. [PubMed: 28336715]
16. Graessel A, Hauck SM, von Toerne C, Kloppmann E, Goldberg T, Koppensteiner H, et al. A Combined Omics Approach to Generate the Surface Atlas of Human Naive CD4+ T Cells during Early T-Cell Receptor Activation. *Mol Cell Proteomics* 2015; 14:2085–102. [PubMed: 25991687]
17. Stemmer M, Thumberger T, Del Sol Keyer M, Wittbrodt J, Mateo JL. CCTop: An Intuitive, Flexible and Reliable CRISPR/Cas9 Target Prediction Tool. *PLoS One* 2015; 10:e0124633. [PubMed: 25909470]
18. Cradick TJ, Qiu P, Lee CM, Fine EJ, Bao G. COSMID: A Web-based Tool for Identifying and Validating CRISPR/Cas Off-target Sites. *Mol Ther Nucleic Acids* 2014; 3:e214. [PubMed: 25462530]
19. Song E, Zhu R, Hammoud ZT, Mechref Y. LC-MS/MS quantitation of esophagus disease blood serum glycoproteins by enrichment with hydrazide chemistry and lectin affinity chromatography. *J Proteome Res* 2014; 13:4808–20. [PubMed: 25134008]
20. In: nd, Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, et al., editors. *Essentials of Glycobiology*. Cold Spring Harbor (NY); 2009.
21. Jamur MC, Grodzki AC, Berenstein EH, Hamawy MM, Siraganian RP, Oliver C. Identification and characterization of undifferentiated mast cells in mouse bone marrow. *Blood* 2005; 105:4282–9. [PubMed: 15718418]
22. Wong MX, Roberts D, Bartley PA, Jackson DE. Absence of platelet endothelial cell adhesion molecule-1 (CD31) leads to increased severity of local and systemic IgE-mediated anaphylaxis and modulation of mast cell activation. *J Immunol* 2002; 168:6455–62. [PubMed: 12055265]
23. Grochoway G, Hermiston ML, Kuhny M, Weiss A, Huber M. Requirement for CD45 in fine-tuning mast cell responses mediated by different ligand-receptor systems. *Cell Signal* 2009; 21:1277–86. [PubMed: 19332117]
24. Malaviya R, Gao Z, Thankavel K, van der Merwe PA, Abraham SN. The mast cell tumor necrosis factor alpha response to FimH-expressing *Escherichia coli* is mediated by the glycosylphosphatidylinositol-anchored molecule CD48. *Proc Natl Acad Sci U S A* 1999; 96:8110–5. [PubMed: 10393956]
25. Munoz S, Hernandez-Pando R, Abraham SN, Enciso JA. Mast cell activation by *Mycobacterium tuberculosis*: mediator release and role of CD48. *J Immunol* 2003; 170:5590–6. [PubMed: 12759438]

26. Kraft S, Jouvin MH, Kulkarni N, Kissing S, Morgan ES, Dvorak AM, et al. The tetraspanin CD63 is required for efficient IgE-mediated mast cell degranulation and anaphylaxis. *J Immunol* 2013; 191:2871–8. [PubMed: 23945142]
27. Lyons DO, Plewes MR, Pullen NA. Soluble transforming growth factor beta-1 enhances murine mast cell release of Interleukin 6 in IgE-independent and Interleukin 13 in IgE-dependent settings in vitro. *PLoS One* 2018; 13:e0207704. [PubMed: 30444930]
28. Mi H, Muruganujan A, Ebert D, Huang X, Thomas PD. PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. *Nucleic Acids Res* 2019; 47:D419–D26. [PubMed: 30407594]
29. Mi H, Muruganujan A, Huang X, Ebert D, Mills C, Guo X, et al. Protocol Update for large-scale genome and gene function analysis with the PANTHER classification system (v.14.0). *Nat Protoc* 2019; 14:703–21. [PubMed: 30804569]
30. Wandinger-Ness A, Zerial M. Rab proteins and the compartmentalization of the endosomal system. *Cold Spring Harb Perspect Biol* 2014; 6:a022616. [PubMed: 25341920]
31. Garin J, Diez R, Kieffer S, Dermine JF, Duclos S, Gagnon E, et al. The phagosome proteome: insight into phagosome functions. *J Cell Biol* 2001; 152:165–80. [PubMed: 11149929]
32. Li Q, Jagannath C, Rao PK, Singh CR, Lostumbo G. Analysis of phagosomal proteomes: from latex-bead to bacterial phagosomes. *Proteomics* 2010; 10:4098–116. [PubMed: 21080496]
33. Azouz NP, Matsui T, Fukuda M, Sagi-Eisenberg R. Decoding the regulation of mast cell exocytosis by networks of Rab GTPases. *J Immunol* 2012; 189:2169–80. [PubMed: 22826321]
34. Azouz NP, Zur N, Efergan A, Ohbayashi N, Fukuda M, Amihai D, et al. Rab5 is a novel regulator of mast cell secretory granules: impact on size, cargo, and exocytosis. *J Immunol* 2014; 192:4043–53. [PubMed: 24696234]
35. Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. *Science* 1988; 241:58–62. [PubMed: 2898810]
36. Chen CC, Grimbaldeston MA, Tsai M, Weissman IL, Galli SJ. Identification of mast cell progenitors in adult mice. *Proc Natl Acad Sci U S A* 2005; 102:11408–13. [PubMed: 16006518]
37. Arinobu Y, Iwasaki H, Gurish MF, Mizuno S, Shigematsu H, Ozawa H, et al. Developmental checkpoints of the basophil/mast cell lineages in adult murine hematopoiesis. *Proc Natl Acad Sci U S A* 2005; 102:18105–10. [PubMed: 16330751]
38. Lighvani S, Baik N, Diggs JE, Khaldoyanidi S, Parmer RJ, Miles LA. Regulation of macrophage migration by a novel plasminogen receptor Plg-R KT. *Blood* 2011; 118:5622–30. [PubMed: 21940822]
39. Drew E, Merzaban JS, Seo W, Ziltener HJ, McNagny KM. CD34 and CD43 inhibit mast cell adhesion and are required for optimal mast cell reconstitution. *Immunity* 2005; 22:43–57. [PubMed: 15664158]
40. LaCroix-Fralish ML, Mo G, Smith SB, Sotocinal SG, Ritchie J, Austin JS, et al. The beta3 subunit of the Na⁺,K⁺-ATPase mediates variable nociceptive sensitivity in the formalin test. *Pain* 2009; 144:294–302. [PubMed: 19464798]
41. Chiampanichayakul S, Szekeres A, Khunkaewla P, Moonsom S, Leksa V, Drbal K, et al. Engagement of Na,K-ATPase beta3 subunit by a specific mAb suppresses T and B lymphocyte activation. *Int Immunol* 2002; 14:1407–14. [PubMed: 12456588]
42. Chruwkamlow N, Pata S, Mahasongkram K, Laopajon W, Kasinrerak W, Chiampanichayakul S. beta3 subunit of Na,K ATPase regulates T cell activation with no involvement of Na,K ATPase activity. *Immunobiology* 2015; 220:634–40. [PubMed: 25678464]
43. Jiang L, Salao K, Li H, Rybicka JM, Yates RM, Luo XW, et al. Intracellular chloride channel protein CLIC1 regulates macrophage function through modulation of phagosomal acidification. *J Cell Sci* 2012; 125:5479–88. [PubMed: 22956539]
44. Mastroberardino L, Spindler B, Pfeiffer R, Skelly PJ, Loffing J, Shoemaker CB, et al. Amino-acid transport by heterodimers of 4F2hc/CD98 and members of a permease family. *Nature* 1998; 395:288–91. [PubMed: 9751058]
45. Fenczik CA, Sethi T, Ramos JW, Hughes PE, Ginsberg MH. Complementation of dominant suppression implicates CD98 in integrin activation. *Nature* 1997; 390:81–5. [PubMed: 9363894]

46. Cantor J, Browne CD, Ruppert R, Feral CC, Fassler R, Rickert RC, et al. CD98hc facilitates B cell proliferation and adaptive humoral immunity. *Nat Immunol* 2009; 10:412–9. [PubMed: 19270713]
47. Cantor J, Slepak M, Ege N, Chang JT, Ginsberg MH. Loss of T cell CD98 H chain specifically ablates T cell clonal expansion and protects from autoimmunity. *J Immunol* 2011; 187:851–60. [PubMed: 21670318]
48. Cantor JM, Ginsberg MH. CD98 at the crossroads of adaptive immunity and cancer. *J Cell Sci* 2012; 125:1373–82. [PubMed: 22499670]
49. Shirakabe K, Hattori S, Seiki M, Koyasu S, Okada Y. VIP36 protein is a target of ectodomain shedding and regulates phagocytosis in macrophage Raw 264.7 cells. *J Biol Chem* 2011; 286:43154–63. [PubMed: 22016386]
50. Kimura T, Nada S, Takegahara N, Okuno T, Nojima S, Kang S, et al. Polarization of M2 macrophages requires Lamtor1 that integrates cytokine and amino-acid signals. *Nat Commun* 2016; 7:13130. [PubMed: 27731330]
51. Wagner CA, Lang F, Broer S. Function and structure of heterodimeric amino acid transporters. *Am J Physiol Cell Physiol* 2001; 281:C1077–93. [PubMed: 11546643]
52. Heng TS, Painter MW, Immunological Genome Project C. The Immunological Genome Project: networks of gene expression in immune cells. *Nat Immunol* 2008; 9:1091–4. [PubMed: 18800157]
53. Tsumura H, Suzuki N, Saito H, Kawano M, Otake S, Kozuka Y, et al. The targeted disruption of the CD98 gene results in embryonic lethality. *Biochem Biophys Res Commun* 2003; 308:847–51. [PubMed: 12927796]
54. Fenczik CA, Zent R, Dellos M, Calderwood DA, Satriano J, Kelly C, et al. Distinct domains of CD98hc regulate integrins and amino acid transport. *J Biol Chem* 2001; 276:8746–52. [PubMed: 11121428]
55. Feral CC, Nishiya N, Fenczik CA, Stuhlmann H, Slepak M, Ginsberg MH. CD98hc (SLC3A2) mediates integrin signaling. *Proc Natl Acad Sci U S A* 2005; 102:355–60. [PubMed: 15625115]
56. Cai S, Bulus N, Fonseca-Siesser PM, Chen D, Hanks SK, Pozzi A, et al. CD98 modulates integrin beta1 function in polarized epithelial cells. *J Cell Sci* 2005; 118:889–99. [PubMed: 15713750]
57. Pytela R, Pierschbacher MD, Ruoslahti E. Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. *Cell* 1985; 40:191–8. [PubMed: 3155652]
58. Zhang Z, Morla AO, Vuori K, Bauer JS, Juliano RL, Ruoslahti E. The alpha v beta 1 integrin functions as a fibronectin receptor but does not support fibronectin matrix assembly and cell migration on fibronectin. *J Cell Biol* 1993; 122:235–42. [PubMed: 8314844]
59. Dasty J, Metcalfe DD. Stem cell factor induces mast cell adhesion to fibronectin. *J Immunol* 1994; 152:213–9. [PubMed: 7504710]
60. Teodosio C, Garcia-Montero AC, Jara-Acevedo M, Sanchez-Munoz L, Pedreira CE, Alvarez-Twose I, et al. Gene expression profile of highly purified bone marrow mast cells in systemic mastocytosis. *J Allergy Clin Immunol* 2013; 131:1213–24, 24 e1–4. [PubMed: 23403045]
61. Myers SA, Rhoads A, Cocco AR, Peckner R, Haber AL, Schweitzer LD, et al. Streamlined Protocol for Deep Proteomic Profiling of FAC-sorted Cells and Its Application to Freshly Isolated Murine Immune Cells. *Mol Cell Proteomics* 2019; 18:995–1009. [PubMed: 30792265]
62. Plum T, Wang X, Rettel M, Krijgsveld J, Feyerabend TB, Rodewald HR. Human Mast Cell Proteome Reveals Unique Lineage, Putative Functions, and Structural Basis for Cell Ablation. *Immunity* 2020; 52:404–16 e5. [PubMed: 32049054]
63. Zarif JC, Yang W, Hernandez JR, Zhang H, Pienta KJ. The Identification of Macrophage-enriched Glycoproteins Using Glycoproteomics. *Mol Cell Proteomics* 2017; 16:1029–37. [PubMed: 28348171]
64. Haverland NA, Waas M, Ntai I, Keppel T, Gundry RL, Kelleher NL. Cell Surface Proteomics of N-Linked Glycoproteins for Typing of Human Lymphocytes. *Proteomics* 2017; 17. [PubMed: 29275045]
65. Akula S, Paivandy A, Fu Z, Thorpe M, Pejler G, Hellman L. How Relevant Are Bone Marrow-Derived Mast Cells (BMMCs) as Models for Tissue Mast Cells? A Comparative Transcriptome Analysis of BMMCs and Peritoneal Mast Cells. *Cells* 2020; 9.

66. Gschwandtner M, Paulitschke V, Mildner M, Brunner PM, Hacker S, Eisenwort G, et al. Proteome analysis identifies L1CAM/CD171 and DPP4/CD26 as novel markers of human skin mast cells. *Allergy* 2017; 72:85–97. [PubMed: 27091730]
67. Sandig H, Bulfone-Paus S. TLR signaling in mast cells: common and unique features. *Front Immunol* 2012; 3:185. [PubMed: 22783258]
68. Elschenbroich S, Kim Y, Medin JA, Kislinger T. Isolation of cell surface proteins for mass spectrometry-based proteomics. *Expert Rev Proteomics* 2010; 7:141–54. [PubMed: 20121483]
69. Kurihara T, Arimochi H, Bhuyan ZA, Ishifune C, Tsumura H, Ito M, et al. CD98 Heavy Chain Is a Potent Positive Regulator of CD4+ T Cell Proliferation and Interferon-gamma Production In Vivo. *PLoS One* 2015; 10:e0139692. [PubMed: 26444422]
70. Kobayashi T, Tsutsui H, Shimabukuro-Demoto S, Yoshida-Sugitani R, Karyu H, Furuyama-Tanaka K, et al. Lysosome biogenesis regulated by the amino-acid transporter SLC15A4 is critical for functional integrity of mast cells. *Int Immunol* 2017; 29:551–66. [PubMed: 29155995]
71. Prager GW, Feral CC, Kim C, Flan J, Ginsberg MFI. CD98hc (SLC3A2) interaction with the integrin beta subunit cytoplasmic domain mediates adhesive signaling. *J Biol Chem* 2007; 282:24477–84. [PubMed: 17597067]
72. Gilfillan AM, Rivera J. The tyrosine kinase network regulating mast cell activation. *Immunol Rev* 2009; 228:149–69. [PubMed: 19290926]
73. Cano-Crespo S, Chillaron J, Junza A, Fernandez-Miranda G, Garcia J, Polte C, et al. CD98hc (SLC3A2) sustains amino acid and nucleotide availability for cell cycle progression. *Sci Rep* 2019; 9:14065. [PubMed: 31575908]
74. Glaser R, Zhang HY, Yao KT, Zhu HC, Wang FX, Li GY, et al. Two epithelial tumor cell lines (HNE-1 and HONE-1) latently infected with Epstein-Barr virus that were derived from nasopharyngeal carcinomas. *Proc Natl Acad Sci U S A* 1989; 86:9524–8. [PubMed: 2556716]
75. Ablack JN, Ortiz J, Bajaj J, Trinh K, Lagarrigue F, Cantor JM, et al. MARCH Proteins Mediate Responses to Antitumor Antibodies. *J Immunol* 2020; 205:2883–92. [PubMed: 33077644]

Clinical implications

Characterization of the mast cell surface proteome can provide a better understanding of mast cell biology in the complex signaling environment that influences mast cell function in health and disease.

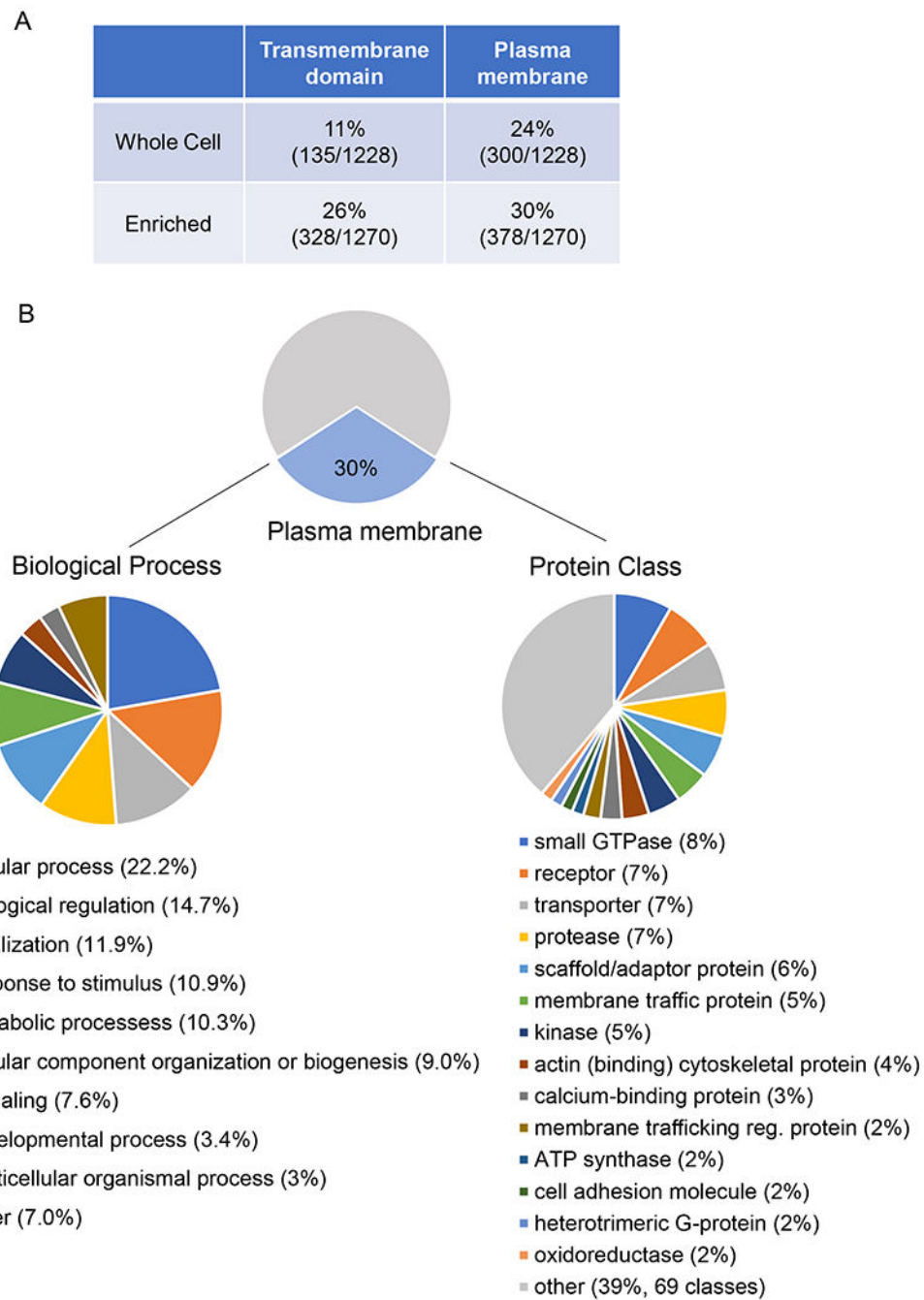


Figure 1. Characterization of mast cell surface proteome.

(A) Level of enrichment of number of transmembrane domain-containing proteins and cell surface proteins in glycoprotein-enriched samples compared to whole cell lysates. (B) Description of BMC MC cell surface proteome gene ontology (GO terms) by biological process (left) and PANTER protein classes (right).

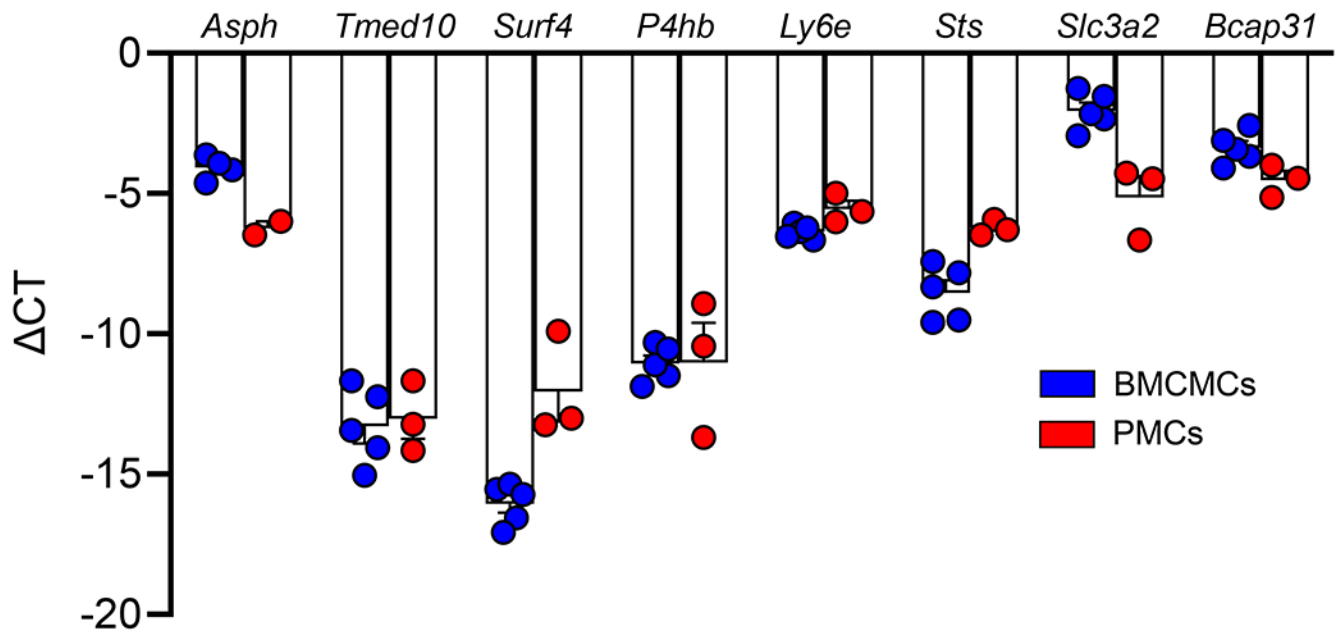


Figure 2. Validation of plasma membrane proteins identified but not previously characterized in mast cells.

Expression of selected cell surface proteins in BMCMCs and PMCs by qPCR. Messenger RNA expression levels for the indicated transcripts are expressed as Δ CT values normalized against Gapdh as the reference transcript. Data are shown as mean + SEM of the average for duplicate specimens from 3-5 independent experiments, circles show values from individual experiments.

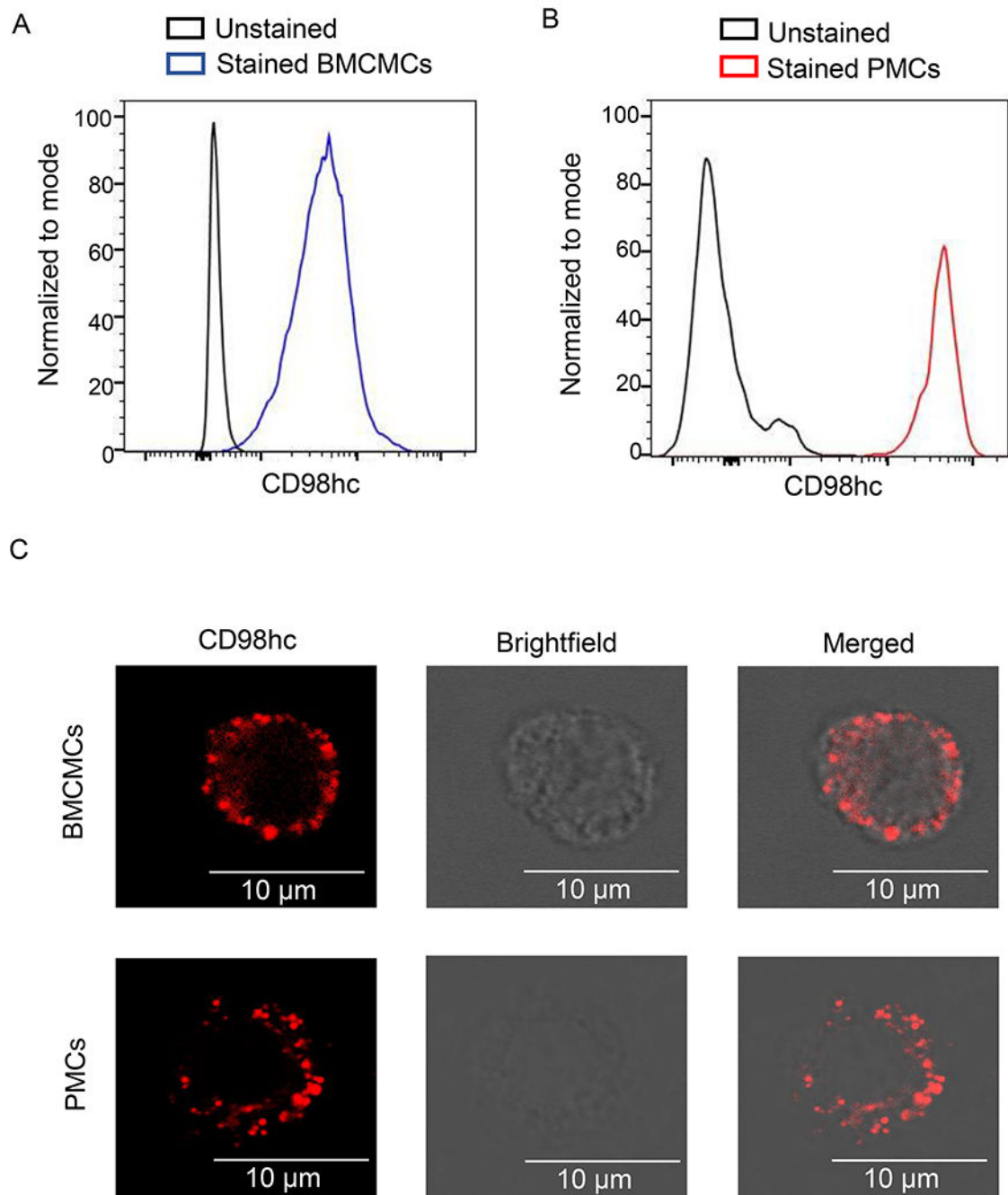


Figure 3. Mast cells express CD98hc on the cell surface.

(A, B) Representative flow cytometry analysis of CD98hc expression in BMCs (A) and mast cell population in the peritoneal cavity (B). Cells in B were immunostained for CD98hc in addition to c-Kit and FcεRIα to identify mast cells in the peritoneal cavity. (C, D) Representative confocal images of BMCs (C) and PMCs (D) labeled first with rabbit anti-mouse CD98hc antibody and then with Alexa Fluor 488-conjugated donkey anti-rabbit antibody. Confocal microscopy images show staining for CD98hc (red in left panel), cell surface (brightfield microscopy in middle panel) and a merge of CD98hc staining and cell

surface (right panel). Scale bar equals 10um. Flow cytometry data in **A** and **B**, and confocal images in **C** and **D** are representative of 3 independent experiments.

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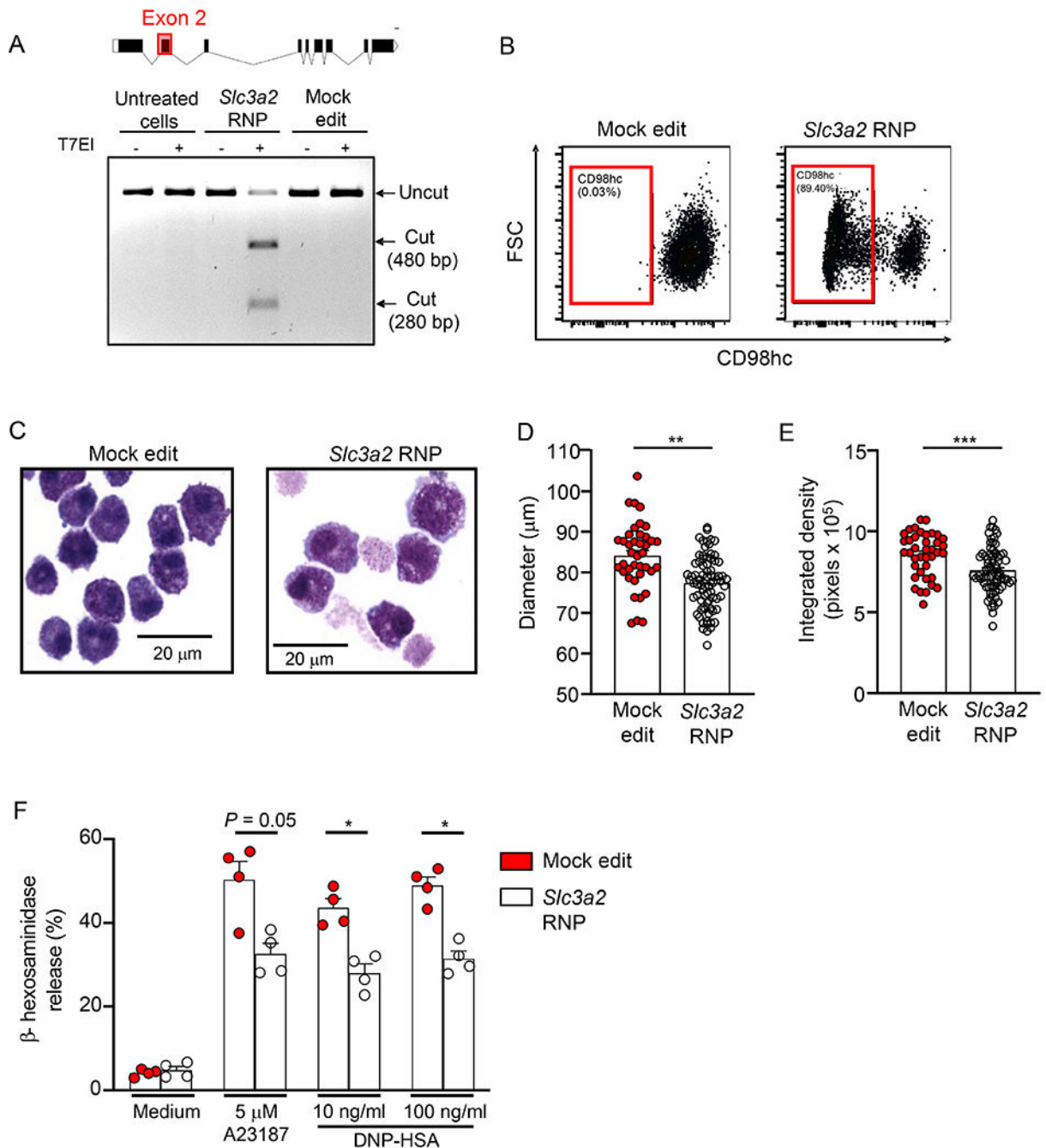


Figure 4. *Slc3a2* disruption in BMCMCs partially abrogates mast cell degranulation.

(A) *Slc3a2* coding exons and representative T7 Endonuclease I (T7EI) assay showing ribonucleoprotein (RNP) cleavage. (B) Representative flow cytometry analysis of CD98hc expression and (C) cytopins stained with May-Grunwald-Giemsa of mock- and *Slc3a2*-edited BMCMCs. (D, E) Diameter (D) and metachromatic area (E) of mock- and *Slc3a2*-edited BMCMCs. (F) β -hexosaminidase release by mock- and *Slc3a2*-edited BMCMCs upon activation. In F mock- and *Slc3a2*-edited BMCMCs were sensitized with IgE mAb to DNP (2 μ g/ml) overnight and then were challenged with either DNP-HSA (10 or 100

ng/ml) or calcium ionophore (A23187, 5 μ M) for 1 h. Data in **A-C** are representative of 3 independent experiments. Data in **D** and **E** are shown as mean + SEM from 2 independent experiments, circles show values from one individual cell. Data in **F** are shown as mean + SEM of the average of duplicate specimens from 4 independent experiments, circles show values from individual experiments. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

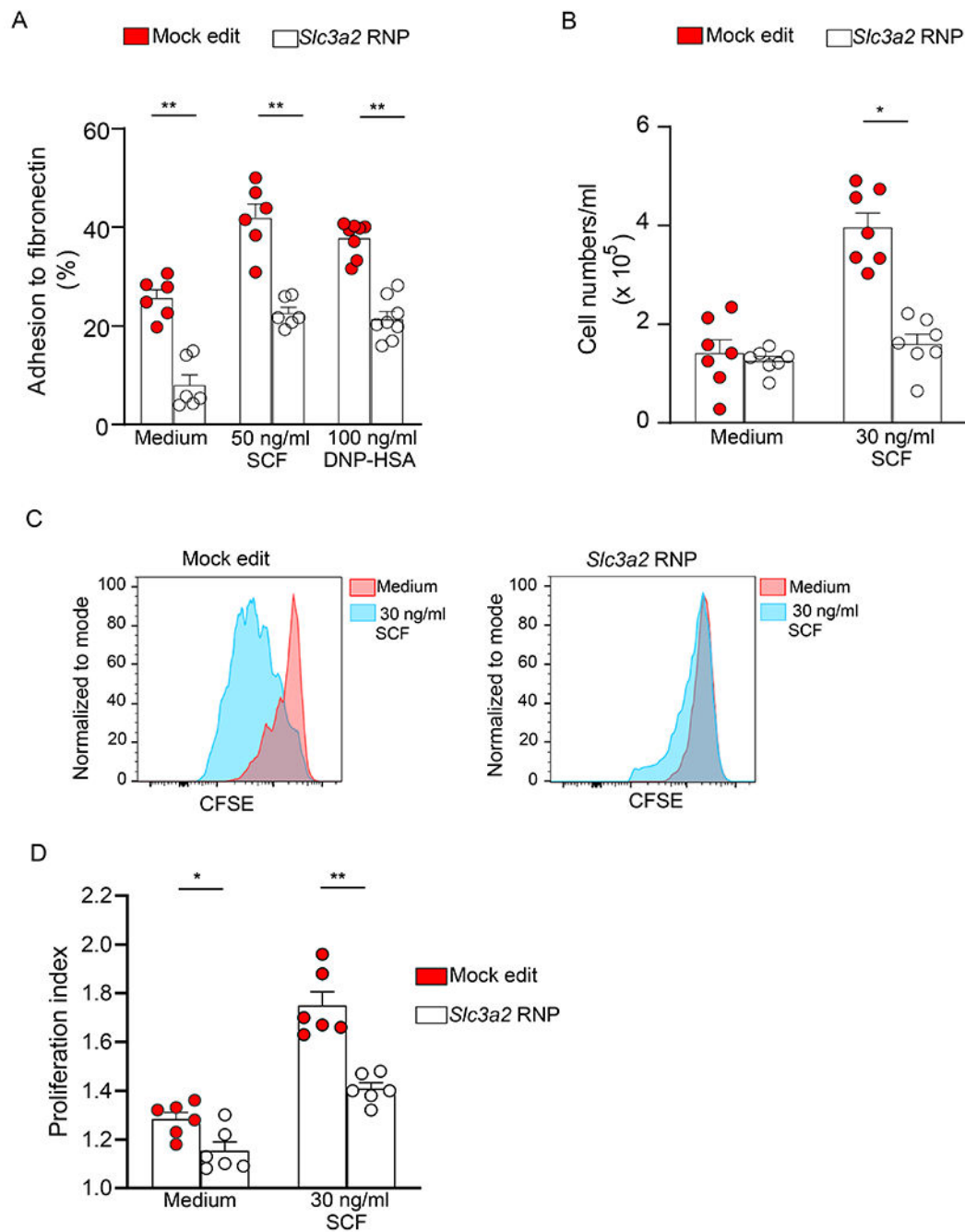


Figure 5. *Slc3a2* disruption in BMCMCs inhibits mast cell adhesion and proliferation.

(A) Mock- and *Slc3a2*-edited BMCMC adhesion to 96-well plates coated with fibronectin (50 μ g/ml) after the indicated treatment. Mock- and *Slc3a2*-edited BMCMCs were sensitized with IgE mAb to DNP (2 μ g/ml) overnight, loaded with calcein-AM (3 μ g/ml) and then challenged with DNP-HSA (100 ng/ml) or maintained in media supplemented with SCF (50 ng/ml). Percentage of fluorescent adherent cells relative to total number of adherent and non-adherent fluorescent cells was determined at 1 h after stimulation. (B-D) Numbers (B), representative flow cytometry analysis of CFSE dilution (C) and proliferation index (D)

at 5 d after incubation of mock- and *Slc3a2*-edited BMCMCs in media alone or in media supplemented with SCF (30 ng/ml). Data in **A**, **B** and **D** are shown as mean + SEM of the average of duplicate specimens from 4-6 independent experiments, circles show values from individual experiments. Flow cytometry data in **C** are representative of 3 independent experiments. * $P < 0.05$, ** $P < 0.005$.

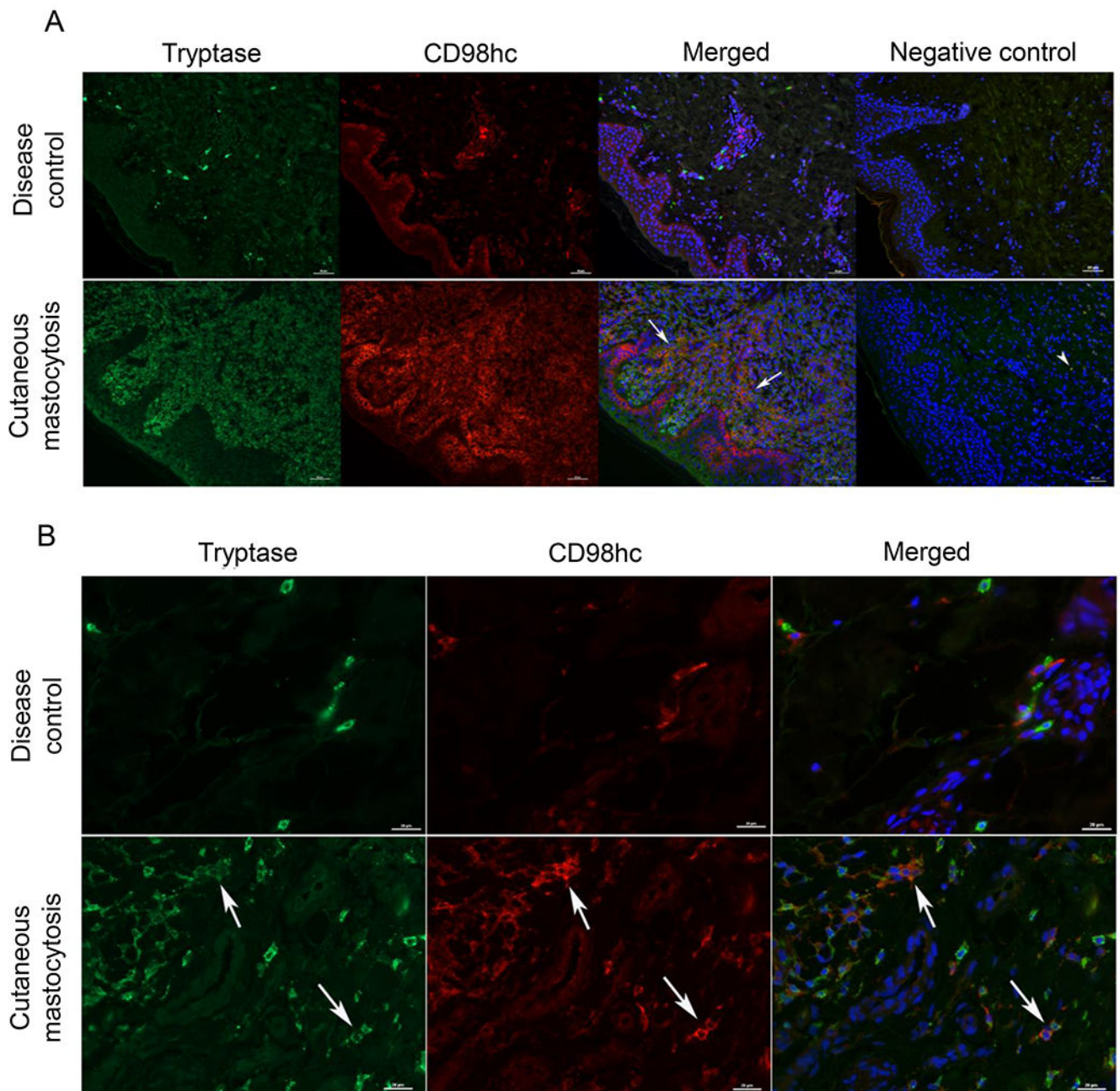


Figure 6. CD98hc is highly expressed in mast cells from patients with cutaneous mastocytosis. (A, B) Detection of CD98hc in mast cells located in skin biopsies from a disease control (chronic urticaria) and a newborn with congenital mastocytosis. Immunofluorescence was performed with anti-CD98hc (red fluorescence) and anti-tryptase mAb (green fluorescence) antibodies for mast cells located in skin. Nuclei were counterstained with DAPI (blue fluorescence). Mast cells expressing both CD98hc and tryptase are indicated with white arrows (yellow fluorescence). Negative controls were performed with secondary antibodies only (far right panel; arrowheads point to nonspecific signal). Images at 20x (A) and 40x

(B) are representative of 4 separate disease controls and 3 separate patients with cutaneous mastocytosis.

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Table 1.
Top 30 represented mast cell plasma membrane annotated proteins.

Plasma membrane proteins in glycoprotein enriched BMCMC lysates (top 30 proteins identified sorted by normalized spectral abundance factor).

Gene Name	UniProtKB Entry Name	Protein Name	Average number of peptides	Normalized peptide abundance
Atp5a1	ATPA	ATP synthase subunit alpha, mitochondrial	647.5	1.609362744
Pdia3	PDIA3	Protein disulfide-isomerase A3	300	0.817938914
Tmed10	TMEDA	Transmembrane emp24 domain-containing protein 10	96.5	0.604409682
Scin	ADSV	Adseverin	300.5	0.578482641
Arf3	ARF3	ADP-ribosylation factor 3	61	0.46384661
Tagln2	TAGL2	Transgelin-2	61.5	0.426144919
Rab7a	RAB7A	Ras-related protein Rab-7a	63	0.419253803
Rab14	RAB14	Ras-related protein Rab-14	58.5	0.375086815
Arf5	ARF5	ADP-ribosylation factor 5	45	0.343771887
P4hb	PDIA1	Protein disulfide-isomerase (PDI)	124.5	0.337299144
Rab5c	RAB5C	Ras-related protein Rab-5C	53	0.335955405
Eef1a1	EF1A1	Elongation factor 1-alpha 1	111.5	0.332567166
Phb	PHB	Prohibitin	63.5	0.32199888
Anpep	AMPN	Aminopeptidase N	222.5	0.317256399
Canx	CALX	Calnexin	133.5	0.310751474
Myh9	MYH9	Myosin-9	416	0.29230598
Vdac1	VDAC1	Voltage-dependent anion-selective channel protein 1	61.5	0.285514099
Hsp90ab1	HS90B	Heat shock protein HSP 90-beta	125.5	0.23793405
Atp1b3	AT1B3	Sodium/potassium-transporting ATPase subunit beta-3	48	0.237285781
Pdia6	PDIA6	Protein disulfide-isomerase A6	75	0.235160627
Ifitm1	IFM1	Interferon-induced transmembrane protein 1	17.5	0.227438107
Pdia4	PDIA4	Protein disulfide-isomerase A4	105	0.22702886
Slc25a11	M2OM	Mitochondrial 2-oxoglutarate/malate carrier protein	51.5	0.226485846
Gnai2	GNAI2	Guanine nucleotide-binding protein G(i) subunit alpha-2	58.5	0.226278247
Stoml2	STML2	Stomatin-like protein 2, mitochondrial	58	0.22612262
Gng12	GBG12	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-12	11	0.210576234
Anxa5	ANXA5	Annexin A5	48.5	0.209973681
Rhog	RHOG	Rho-related GTP-binding protein RhoG	28.5	0.205855353
Rac2	RAC2	Ras-related C3 botulinum toxin substrate 2	27.5	0.197982925
Pgam1	PGAM1	Phosphoglycerate mutase 1	36	0.195256676

Table 2.
Top 30 unique mast cell plasma membrane annotated proteins.

Plasma membrane proteins in glycoprotein enriched BMCMC lysates that have not been previously characterized in mast cells (top 30 proteins identified sorted by normalized spectral abundance factor).

Gene Name	UniProt ID	Protein Name	Average number of peptides	Normalized peptide abundance	Expression in MC in Immgen	Reported in High Throughput Studies
Tmed10	TMEDA	Transmembrane emp24 domain-containing protein 10	131.5	0.604	M/H	Y
Scin	ADSV	Adseverin (Scinderin)	300.5	0.578	N-M*	Y
Tagln2	TAGL2	Transgelin-2	61.5	0.426	H	N
Arf5	ARF5	ADP-ribosylation factor 5	45.0	0.344	M	N
P4hb	PDIA1	Protein disulfide-isomerase	124.5	0.337	X	Y
Canx	CALX	Calnexin	133.5	0.311	H	N
Myh9	MYH9	Myosin-9	416.0	0.292	M	Y
Vdac1	VDAC1	Voltage-dependent anion-selective channel protein 1	61.5	0.286	M	N
Atp1b3	AT1B3	Sodium/potassium-transporting ATPase subunit beta-3	48.0	0.237	H	N
Pdia6	PDIA6	Protein disulfide-isomerase A6	75.0	0.235	H	Y
Ifitm1	IFM1	Interferon-induced transmembrane protein 1	17.5	0.227	VL-M*	Y
Pdia4	PDIA4	Protein disulfide-isomerase A4	105.0	0.227	M-H	Y
Slc25a11	M2OM	Mitochondrial 2-oxoglutarate/malate carrier protein	51.5	0.226	H	N
Stoml2	STML2	Stomatin-like protein 2	58.0	0.226	M	N
Gng12	GBG12	Guanine nucleotide-binding protein	11.0	0.211	M	Y
Ppia	PPIA	Peptidyl-prolyl cis-trans isomerase A	24.0	0.201	M-H	Y
Pgam1	PGAM1	Phosphoglycerate mutase 1	36.0	0.195	M	N
Itm2c	ITM2C	Integral membrane protein 2C	37.0	0.189	H-VH	N
Hsp90aa1	HS90A	Heat shock protein HSP 90-alpha	101.0	0.189	M-H*	N
Clc1	CLIC1	Chloride intracellular channel protein 1	32.0	0.183	H	N
Ddost	OST48	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit	58.5	0.183	H	N
Itm2b	ITM2B	Integral membrane protein 2B	32.5	0.169	VH	Y
Arf4	ARF4	ADP-ribosylation factor 4	21.0	0.161	H	N
Plgrkt	PLRKT	Plasminogen receptor (KT)	16.5	0.155	H	N
Slc3a2	4F2	4F2 cell-surface antigen heavy chain (CD antigen CD98)	58.0	0.152	H	Y
Got2	AATM	Aspartate aminotransferase, mitochondrial	45.5	0.146	M	N
Gnb2l1	RACK1	Receptor of activated protein C kinase 1	33.5	0.145	H	N

Gene Name	UniProt ID	Protein Name	Average number of peptides	Normalized peptide abundance	Expression in MC in Immgen	Reported in High Throughput Studies
Nme2	NDKB	Nucleoside diphosphate kinase B	15.5	0.142	L	N
Mlec	MLEC	Malectin	29.5	0.139	M*-H	Y
Lman2	LMAN2	Vesicular integral-membrane protein VIP36	35.5	0.137	H	N

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