

Received Date : 07-Jan-2016

Revised Date : 01-Apr-2016

Accepted Date : 15-Apr-2016

Article type : Original Article: Experimental Allergy and Immunology

Proteome-analysis identifies L1CAM / CD171 and DPP4 / CD26 as novel markers of human skin mast cells

Maria Gschwandtner^{*1}, Verena Paulitschke^{*2}, Michael Mildner¹, Patrick M. Brunner², Stefan Hacker³, Gregor Eisenwort^{4,5}, Wolfgang R. Sperr^{4,5}, Peter Valent^{4,5}, Christopher Gerner^{6,7}, Erwin Tschachler¹

¹ Research Division of Biology and Pathobiology of the Skin, Department of Dermatology, Medical University of Vienna

² Department of Dermatology, Medical University of Vienna

³ Department of Plastic and Reconstructive Surgery, Medical University Vienna, Vienna, Austria

⁴ Department of Internal Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria

⁵ Ludwig Boltzmann Cluster Oncology, Medical University of Vienna, Vienna, Austria.

⁶ Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna

⁷ Institute of Cancer Research, Comprehensive Cancer Center, Medical University of Vienna

***Both authors contributed equally**

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.12919

This article is protected by copyright. All rights reserved.

Author for correspondence:

Erwin Tschachler, MD

Medical University of Vienna

Department of Dermatology

Research Division of Biology and Pathobiology of the Skin

Währinger Gürtel 18-20

1090 Vienna, Austria

Tel: +43-1-40400-77870 Fax: +43-1-4081287

Email: erwin.tschachler@meduniwien.ac.at

Short title

L1CAM/CD171 and DPP4/CD26 as novel human skin mast cell markers

Keywords (5)

Bone marrow

Mastocytosis

Proteomics

Psoriasis

Skin homeostasis

Abbreviations

DPP4: dipeptidylpeptidase 4

GO: gene ontology

HUVEC: human umbilical vein endothelial cells

L1CAM: neural cell adhesion molecule L1

PBMC: peripheral blood mononuclear cells

RLE: relative log expression

Abstract

Background:

The function of skin mast cells has been well documented in IgE-mediated allergic reactions whereas other mast cell functions are poorly defined. The present study aimed at identifying novel mast cell proteins by proteome analysis of primary human skin mast cells.

Methods:

The proteome of skin mast cells was compared to other cell types and analyzed using bioinformatics. The expression and function of two proteins hitherto not described in skin mast cells was investigated in isolated mast cells as well as in mast cells *in situ*.

Results:

Within the mast cell proteome we identified 49 highly expressed proteins previously not described in mast cells; 21 of these proteins were selectively expressed in mast cells. Two proteins, the neural cell adhesion molecule L1 and dipeptidylpeptidase 4, were further studied. L1 was found to be highly expressed in mast cells in normal, psoriasis and mastocytosis skin. Dipeptidylpeptidase 4 was expressed in mast cells in normal, psoriasis and mastocytosis skin as well as in bone marrow mast cells in patients with systemic mastocytosis. In normal skin, mast cells were identified as a major source of dipeptidylpeptidase 4 and we also found that skin mast cells and fibroblasts secrete an active form of this enzyme.

Conclusions:

In a systematic proteomics approach we identified two novel mast cell proteins potentially relevant to skin homeostasis, neural cell adhesion molecule L1 and dipeptidylpeptidase 4.

Introduction

Mast cells are derived from bone marrow and circulating precursor cells and mature once these cells reach their target tissues. Tissue mast cells are predominantly located in close proximity to body surfaces such as the skin, the airways and gastrointestinal tract(1).

Mast cells can be activated by immunological signals including crosslinking of Fcε-receptors by antigen/IgE complexes, complement proteins and toll-like receptor ligands as well as by non-immunologic stimuli among them peptides found in venoms, calcium ionophore and neuropeptides(2). Once activated, mast cells release various preformed and *de novo* synthesized mediators(3). The best studied functions of mast cells are their involvement in IgE-dependent allergic reactions(4) and the clonal mast cell expansion disorders systemic and cutaneous mastocytosis(1, 5, 6). Apart from this mast cells have been implicated in the pathogenesis of various diseases ranging from atherosclerosis(7) and thrombosis(8) to obesity, diabetes(9) and cancer(10).

Proteomics is a powerful method to identify the protein signatures of tissues and cells and helps to obtain important clues as to their function(11). So far the protein expression profile of primary mast cells was mainly investigated by antibody surface staining experiments(12, 13), but mast cells have not been well characterized by proteomics yet. Proteome analysis of LAD-2, a stem cell factor dependent and IgE-FcεRI crosslinking sensitive human mast cell line(14) has focused on the identification of IgE-mediated secretion of proteins, proteins subjected to membrane relocation processes and proteins involved in exocytosis(15). In human mast cells differentiated from CD34⁺ cells(16) and in murine bone marrow-derived mast cells(17) phosphoproteomic analysis has been used to identify the signaling events occurring after mast cell activation. The proteome of murine mucosal mast cell

analogues has been analyzed in response to cell activation(18, 19) and the proteome profile of murine mast cell derived exosomes has been delineated(20). Whereas the available data from murine mast cells provide important information about the function of these cells, the possibility of species differences in mast cell function remains. Also the widely used mast cell lines HMC-1 and LAD-2 have been shown to differ in several aspects from native mast cells(21). To the best of our knowledge a proteome analysis of primary human tissue mast cells has not been reported to date.

In the present study we isolated and enriched mast cells from healthy human skin for proteome analysis. Combining this approach with bioinformatics analyses we identified a number of proteins previously unknown to be expressed in mast cells.

Materials and Methods

Ethics statement

Experiments involving human material were done according to the Declaration of Helsinki principles after approval by the ethics committee of the Medical University of Vienna and written informed consent of the participants (vote number 1149/2011: isolation and culture of cells from and analysis of normal human skin biopsies; vote number 217/2010: analysis of biopsies from skin pathologies; vote number 1184/2014: isolation and investigation of bone marrow mast cells).

Isolation and culture of human skin mast cells, fibroblasts, keratinocytes and melanocytes

Tissue samples including skin and subcutaneous fat (100 to 300 cm²) were obtained from patients undergoing routinely performed body contouring surgeries and were used for isolation of mast cells, fibroblasts and keratinocytes. On clinical inspection and in histology the skin was inconspicuous. Subcutaneous tissue and reticular dermis were removed and the remaining split thickness skin was cut into 0.5 cm² pieces and placed over night at 4°C in 2.4 U/ml dispase II (Roche, Vienna, Austria). After separation of the epidermis, dermis was digested in collagenase I (Gibco,

Vienna, Austria) at 37°C for 2 hours. CD117⁺ mast cells were isolated using magnetic beads (MACS System, Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. To increase purity of recovered cells magnetic isolation was repeated with CD117⁺ cells from the first isolation round. CD117⁺ mast cells were then seeded in DMEM (Gibco) supplemented with 10% FCS, Penicillin/Streptomycin (both Biochrom, Berlin, Germany) and 100 ng/ml recombinant human stem cell factor (Peprotech, Rocky Hill, NY, USA). After isolation of mast cells, CD117⁻ adherent cells (=fibroblasts) were cultured in DMEM (Gibco) supplemented with 10% FCS and Penicillin/Streptomycin (both Biochrom). Keratinocyte single cell suspensions were prepared from dispase-separated epidermis after digestion with trypsin (Lonza, Basel, Switzerland) and cultured in DermaLife medium (Lifeline cell technology, Frederick, MD, USA). Primary human melanocytes were purchased from Lonza and cultured in melanocyte growth medium-4 (Lonza).

Preparation of proteins and proteome analysis

Mast cells (10⁶) were used the day after isolation. Proteome analysis was performed as described previously(22); briefly proteins were isolated from different cellular fractions, separated using SDS-PAGE, silver-stained gel lanes were sliced and digested separately with trypsin. Peptides were separated using nanoflow Ultra-High Performance Liquid Chromatography (UHPLC, Dionex Ultimate 3000) with a 50cm x 75µm 2µm particle size Pepmap column (Dionex) and a flow rate of 300nl/min, using solvent A (97.8% H₂O, 2% acetonitrile, 0.2% formic acid) and a 8% to 40% gradient of solvent B (80% acetonitrile, 19.8% H₂O, 0.2% formic acid) within 240 min. Peptides were analysed in positive ionisation mode at a resolution of 70000 (at m/z=200) and fragmented using higher-energy collisional dissociation in a QEXACTIVE orbitrap mass spectrometer (Thermo Fisher Scientific, Austria) in a data-dependent mode (150ms collection time, fragmentation at 30eV, resolution 17500, top 10 method). Data was analysed using ProteomeDiscoverer 1.4 (Thermo Fisher Scientific) equipped with Mascot 2.4 (Matrix Science, UK) and filtered allowing only peptides with a false discovery rate less than 0.01 (validation based on q-value). Protein identification was achieved searching against the SwissProt Database

(version 012013 with 20,264 entries) allowing a mass tolerance of 5ppm for MS spectra and 20ppm for MS/MS spectra as well as a maximum of 2 missed cleavages.

Proteome data analysis

The amounts of proteins overlapping with other cell types analysed previously(11) and specifically expressed proteins were assessed with Microsoft Excel. DAVID bioinformatics tool (DAVID Bioinformatics Resources 6.7; National Institute of Allergy and Infectious Diseases)(23, 24) was used for enrichment analysis. Enrichment scores for annotation clusters, fold-enrichment values for individual GO (gene ontology)-terms as well as Fisher exact p-values and false discovery rates using Benjamini-Hochberg coefficients adjusting for multiple comparisons were calculated using DAVID.

Immunofluorescence staining

Immunofluorescence staining of acetone fixed cytopins and 5 µm cryosections or formalin-fixed paraffin-embedded sections from normal, psoriasis and mastocytosis skin was done as described(25). The antibodies used are listed in Supplementary table 1. Images were recorded using the AX70 microscope with the imaging software MetaMorph from Olympus (Hamburg, GER). The relative DPP4/CD26 staining intensity on tryptase positive and tryptase negative cells in the skin was assessed by visual inspection and rated on a scale of 0-5 (0: no staining; 5: highly intense staining). DPP4/CD26 expression on isolated mast cells was analyzed with the help of ImageJ (National Institutes of Health, Bethesda, MD, USA); cells were classified into three groups: (i) no staining and (ii) weak or (iii) strong staining if the signal was not or was visible after reduction of brightness by a value of 100, respectively.

Toluidine blue staining

Toluidine blue working solution in 1% NaCl pH2.5 was prepared by 1:10 dilution of the stock solution (1% toluidine blue (Sigma-Aldrich) in 70% ethanol). Cytospins were hydrated in distilled water and stained for 3 min, washed in distilled water, dehydrated with two quick changes of 95% and 100% ethanol and cleared in xylene before mounting of the slides with Eukitt® (Sigma-Aldrich).

Quantitative real-time PCR

RNA was isolated with RNeasy 96 kit (Qiagen), cDNA synthesis was performed with iScript cDNA Synthesis Kit (Bio-Rad) and real-time PCR was carried out with LightCycler480 SYBR Green I Master (Roche) according to the instructions using the following primer pairs: DPP4/CD26-for 5'-tggtcaccagtgggtcataa-3' and DPP4/CD26-rev 5'-aaaaagtgccgtttggagac-3', L1CAM-for 5'-gtgagtgccatcatcctcct-3' and L1CAM-rev 5'-ctgtactcgccgaaggtctc-3', b2m-for 5'-gatgagtatgcctgccgtgtg-3' and b2m-rev 5'-caatccaaatgcggcatct-3'. The relative expression of the target genes was calculated by comparing with the housekeeping gene b2m using a formula described previously(26).

Protein microarray analyses

Cell free supernatants and cell lysates in 1% NP-40 buffer of mast cells (4×10^5 per well) that had been cultured for 24 hours were analyzed using Proteome Profiler™ Human Angiogenesis Antibody Array (R&D Systems) according to the manufacturer's instructions.

DPP4/CD26 activity assay

Mast cells ($1-2 \times 10^5$ cells per well, freshly isolated), fibroblasts (80% confluence) and keratinocytes (60% confluence) were cultured in flat bottom 96-well plates; supernatants and 1% NP-40 lysates were taken after 48h. After washing with PBS 100 μ l 0.1M Tris (pH 8.0) was added to the living cells, and supernatants and lysates

were diluted with 0.1M Tris to reach a total volume of 100 μ l. For some experiments the DPP4/CD26 inhibitor vildagliptin was added to the reaction mixture at 1 μ M. 100 μ l of the substrate 1 mM Gly-Pro-pNitroanilide (Sigma-Aldrich) were added to the respective wells and the plate was incubated at 37°C for 120 min. Absorbance was measured at 405 nm, background signals from culture media or lysis buffer were subtracted and the amount DPP4/CD26 activity was calculated from a standard curve of recombinant DPP4/CD26 ranging from 0.25 ng/ml to 8 ng/ml (Abcam).

Flow cytometry

Isolated human skin mast cells and bone marrow aspirates from patients diagnosed with mast cell leukemia and different systemic mastocytosis variants(27-29) were stained with the antibodies listed in Supplementary Table 1 and analyzed by flow cytometry. Human skin mast cells were activated two days after isolation; for 1h or 24h with 50 μ M compound 48/80 (Sigma-Aldrich, Vienna, Austria) or with 10 μ g/ml anti-IgE (Beckman Coulter, Vienna Austria) after overnight loading with 10 μ g/ml human IgE (Merck, Darmstadt, Germany).

Statistical analysis

Statistical analysis was performed using Graphpad Prism version 5 (GraphPad Software Inc.). Dot graphs depict the measurement values of individual donors and the median of all experiments. The following statistical tests were performed; Figure 5f: Mann-Whitney test and Figure 6c: Wilcoxon matched-pairs signed rank test; A p-value below 0.05 was regarded as significant and depicted with * or ^s, a p-value below 0.01 was depicted with ^{##}.

Results

Isolation and characterization of mast cells from human skin

Mast cells were enriched from dermal cell suspensions using anti-CD117 magnetic beads. The yield of mast cells correlated with the sample size and ranged from 0.63

to 3.75 million cells (Supplementary table 2). The purity of mast cells in most of these preparations was over 95% as assessed by immunostaining for tryptase (Figure 1a) and toluidine blue staining (Figure 1c).

The proteome of human skin mast cells

Three independent mast cell preparations (donors 1, 2 and 5 in Supplementary table 2) were used for proteome analysis and in total 3,553 different proteins were identified in mast cells (Supplementary file 1). Comparing our data with a previously published study we found that all proteins described for the LAD-2 mast cell line(15) were also present in the proteome of skin mast cells. In addition we identified 35 proteins not described in LAD-2 cells but also belonging to the pathways of exocytosis and redox regulation studied by Gage et al. Most of these proteins have not been described in mast cells previously (Supplementary table 3).

Next we compared the skin mast cell proteome with data obtained for dermal fibroblasts, endothelial cells (HUVEC) and peripheral blood mononuclear cells (PBMC) obtained with the same technology(11) (Supplementary file 1). 2,808 proteins were identified in all four cell types present in the dermis; 391, 209 and 686 were only expressed in fibroblasts, HUVEC and PBMC, respectively and 97 proteins were exclusively identified in mast cells (Figure 2a, Supplementary table 4, 5a-b). The majority of these proteins was also identified in the transcriptome of *ex vivo* cultured human skin mast cells(30); abundant proteins (peptide count ≥ 2) were identified with a mean relative log expression (RLE) of 392 and non-abundant proteins (peptide count =1) with a mean RLE of 50 (Supplementary table 5a-b).

As expected PAI-1 was not present in the mast cell proteome(31) (Figure 2b), but was readily detectable in other cell types. Known key mast cell proteins such as tryptase alpha/beta-1, chymase and CD117 were highly abundant specifically in mast cells. Twenty-one additional proteins were identified to be selectively present in the mast cell proteome, i.e. with a peptide count >5 and at least a 2-fold increased peptide count as compared to other cell types (Figure 2b). These proteins were also identified in the transcriptomic study(30) (Supplementary table 6), but for the majority information about their function in mast cells is scarce (Supplementary table 7).

Furthermore, 28 proteins, which have been well characterized in other cells and tissues, showed up with high peptide counts in the mast cell proteome and transcriptome (Figure 2c, Supplementary table 6). In a separate analysis we compared the proteome of mast cells to keratinocytes, the major cell type found in the epidermis. Eight proteins were uniquely expressed in mast cells and keratinocytes, but not in other dermal cells (Supplementary tables 8-9). The proteins previously classified as selectively expressed in mast cells (Figure 2b) were also underrepresented in keratinocytes (Supplementary table 10).

Comparison to the data on human proteins available in the DAVID bioinformatics database identified functional clusters enriched in the skin mast cell proteome, among them exocytosis related processes, protein transport and redox homeostasis as well as single GO-terms, e.g. mast cell degranulation and aminopeptidase activity (Figure 3).

Identification of L1CAM/CD171 as a novel marker of mast cells in the skin

The neural adhesion molecule L1 (L1CAM/CD171) was identified by proteomics as one of the markers selectively expressed in our mast cell preparations but not in the other local cells in the dermis (Figure 2b). Strong expression of L1CAM/CD171 by mast cells was confirmed at the mRNA level by real-time PCR. By contrast, melanocytes, keratinocytes and fibroblasts showed mRNA expression at much lower levels (Figure 4a). The vast majority of isolated skin mast cells strongly expressed L1CAM/CD171 as assessed by immunofluorescence analysis (Figure 4b) and co-localization studies showed that mast cells express L1CAM/CD171 in normal skin as well as in psoriatic lesions and cutaneous mastocytosis (Figure 4c). Besides in mast cells anti-L1CAM/CD171 reactivity was found in the basal epidermal layer of some normal skin samples confined to c-kit/CD117 positive melanocytes (Supplementary figure 1a). Analysis of dermal sheet preparations showed L1CAM/CD171 and FcERI double positive mast cells in close proximity to vessels and nerves (Supplementary Figure 1b).

Skin mast cells express and secrete active DPP4/CD26

DPP4/CD26 was identified as the enzyme with highest peptide counts of the enriched GO-term aminopeptidase activity (Figure 3). The high expression level of DPP4/CD26 detected by proteome analysis of mast cells and fibroblasts (Figure 2c) was confirmed by real-time PCR (Figure 5a). As detected by immunofluorescence staining isolated skin mast cells expressed DPP4/CD26 at varying levels ranging from strongly positive cells to cells with no detectable expression (Figure 5b-d). Similarly, mast cells in normal skin showed variable DPP4/CD26 staining intensity. Despite the heterogeneous expression pattern, tryptase positive mast cells were the major cell type showing reactivity with DPP4/CD26 antibodies in normal skin (Figure 5e-f). Occasionally low levels of expression were detected also in tryptase negative cells. In contrast, in lesional psoriatic skin DPP4/CD26 expression was significantly increased on tryptase negative dermal cells (Figure 5f, Supplementary figure 2) as has been described previously(32). Of note, we regularly found DPP4/CD26 staining in the dermis in areas in which no nuclei were detectable. Most likely this reflects an extracellular accumulation of the enzyme (Supplementary figure 3).

Analyses of mast cell leukemia and mastocytosis also showed variable DPP4/CD26 expression patterns on mast cells in the bone marrow (Figure 5g, Supplementary figure 4). The difference in DPP4/CD26 expression did not correlate with age, tryptase levels or white blood cell count and the presence of mediator symptoms, urticaria pigmentosa-like skin lesions or the *KIT* mutation D816V (Supplementary figure 5).

Using proteome profiler arrays DPP4/CD26 was detected in both lysates and supernatants of skin mast cells (Figure 6a). Cleavage of the substrate Gly-Pro-pNitroanilide corresponding to the enzymatic activity of DPP4/CD26 was observed in both cell lysates and supernatants of mast cells and fibroblasts but not at all or at the threshold of detection in keratinocytes (Figure 6b); specificity of the reaction was confirmed by blocking the reaction with the selective DPP4/CD26 inhibitor vildagliptin(33) (Figure 6c). DPP4/CD26 expression, secretion and activity were neither induced by compound 48/80- nor by IgE-mediated mast cell activation (Supplementary figure 6). Moreover, screening publicly available transcriptome data we found no evidence for the regulation of DPP4/CD26 expression in stimulated

Accepted Article
mast cells (data not shown). In the published dermal mast cell transcriptome study(30) no difference in stimulated mast cells was observed; however DPP4/CD26 mRNA expression was reduced upon cell expansion (Supplementary table 6). The widely used mast cell lines LAD-2, HMC-1.1 and HMC-1.2 did not express DPP4/CD26 and could not be induced to do so by stimulation with different cytokines (data not shown).

Discussion

Our study complements the available proteomics analyses of mast cell lines and *in vitro* generated mast cells(15, 16) by defining the proteome signature of primary human skin mast cells. Comparing our data to the proteome of the LAD-2 cell line, we confirmed that all proteins previously described for the cell line are also present in primary mast cells(15). However, in addition we identified 35 proteins that have not been described in mast cell so far. The detection of these additional proteins is very likely due to the use of a more advanced technology in our study as compared to previous studies, since we detected the corresponding mRNAs in both LAD-2 and primary mast cells (data not shown).

Based on detailed analysis of the proteomes of dermal cells – mast cells, fibroblasts, endothelial cells and PBMC – we identified 97 proteins exclusively and 21 proteins selectively expressed in mast cells. The majority of these proteins has also been identified in a transcriptomic study of human dermal mast cells(30) (Supplementary table 5a-b and 7); vice versa by proteomics we were able to identify 5 out of the 8 proteins presented as novel genes with mast cell-restricted expression pattern in the transcriptome study. One of those, von Willebrand factor A domain-containing protein 5A, was in our study found to be enriched in mast cells, but not exclusively expressed. Possible functions of the other four genes/proteins - mas-related G-protein coupled receptor member X2, regulator of G-protein signaling 13 (RGS13), germinal center associated signaling and motility-like protein (C1orf150) and sialic acid-binding Ig-like lectin 6 (Siglec 6) - have been briefly described in the mentioned study(30). However, for most of the other proteins which we have newly identified to be exclusively or selectively expressed in mast cells only putative functions have been described (see Supplementary table 7). Two novel mast cell

proteins were selected for further analysis: (I) L1CAM/CD171 known so far primarily for its association with cells of the nervous system(34) was found highly expressed selectively in mast cells but not in the other cells and (II) DPP4/CD26 which is known to be expressed in various cell types but also was among the most abundant mast cell proteins.

Immunoreactivity of mast cells for L1CAM/CD171 has previously been observed in peripheral nerve sheath tumors and neurofibromas(35). Here we demonstrate that L1CAM/CD171 is consistently found in mast cells in normal skin, psoriatic lesions and skin mastocytosis. This observation is rather surprising, since L1CAM/CD171 is a cell adhesion molecule known primarily for its role in the nervous system including myelination, neuronal migration, nerve regeneration as well as axon extension and guidance(34). In the dermis Schwann cells of sensory nerves are the only other cells which are regularly positive for L1CAM/CD171(36). Mutations in the L1CAM/CD171 gene result in a variety of pathologies ranging from mental retardation and hydrocephalus to spastic paraplegia(37). Recently, the role of L1CAM/CD171 in human disease has been extended by studies showing L1CAM/CD171 over-expression in different human cancers and its association with aggressive phenotype, metastasis and poor prognosis provoked by L1CAM/CD171-induced increase in cell survival, trans-endothelial migration and angiogenesis(38). In leucocytes L1CAM/CD171 has been shown to be of importance in steady state cell adhesion and migration(39, 40). It is therefore conceivable that L1CAM/CD171 together with P-selectin glycoprotein ligand-1, $\alpha\text{v}\beta 3$ -integrin and platelet/endothelial cell adhesion molecule-1 expression(41) might be part of the adhesion molecule-integrin network guiding mast cell migration from the bone marrow to their target tissue. On skin mast cells L1CAM/CD171 may also contribute to localizing mast cells in close proximity to nerves and vessels, e.g. via homophilic binding to another L1CAM/CD171 molecule on Schwann cells(36) or heterophilic binding to endothelial integrins(41). The confirmation of these hypotheses and detailed elaboration of the functional relevance of L1CAM/CD171 on mast cells in different tissues will be subject of future studies.

The second protein we analyzed in more detail is the aminopeptidase DPP4/CD26. This enzyme has broad substrate specificity and cleaves neurotransmitters, angiogenic factors and chemokines, e.g. GM-CSF, CCL3 (MIP-1 α), CCL11 (eotaxin), CCL22 (MDC), CXCL9 (MIG) and CXCL12 (SDF-1)(42-44). Cleavage by DPP4/CD26 can result in altered activity and changes in receptor binding affinity or receptor specificity; for example, LR78 β , a MIP-1 α isoform, obtains upon cleavage by DPP4/CD26 in addition to its strong binding to CCR5 also high affinity to CCR1(42) and DPP4/CD26 mediated inactivation of CXCL12 reduces the skin homing migratory response of T cells(45). In this regard, DPP4/CD26 has been also described as novel leukemic stem cell marker in chronic myeloid leukemia initiating the spread of stem cells by disturbance of the niche homing CXCL12-CXCR4 chemokine network(43). Furthermore, DPP4/CD26 is also involved in matrix remodeling and fibrogenic activity(46, 47). In inflammatory skin diseases, e.g. psoriasis and atopic dermatitis increased DPP4/CD26 expression has been observed(32, 48) and DPP4/CD26 inhibitors have been suggested as potential drugs for the modulation of immune functions(44).

It has been reported previously that DPP4/CD26 is expressed on the surface of human blood basophils, but not on isolated human lung and gastrointestinal mast cells(49-51). In the present study, we were able to identify DPP4/CD26 on bone marrow mast cells in patients with mastocytosis as well as on human skin mast cells. Compared to the skin, weak anti-DPP4 reactivity was detected only in a small proportion of mast cells in healthy lung tissue (data not shown). This marginal expression of DPP4/CD26 might not have been detected in previous studies due to differences in the used antibodies and labeling techniques or as a consequence of the heterogeneous DPP4/CD26 expression patterns described below. When we studied the presence of DPP4/CD26 in the skin we were confronted with variable results: As has been described before DPP4/CD26 expression was elevated in psoriasis skin(32), but primarily in fibroblasts. In contrast, in normal skin it was predominantly mast cells which exhibited anti-DPP4 reactivity. In general DPP4/CD26 expression was donor dependent with varying numbers of weak and strong DPP4/CD26 positive mast cells in the skin. Similarly, we observed in mast cell leukemia and systemic mastocytosis elevated DPP4/CD26 levels only in a minority of samples. The finding that the expression levels of DPP4/CD26 in both enriched

Accepted Article
mast cell preparations and by mast cells *in situ* are variable together with previous studies showing heterogeneous DPP4/CD26 expression in other cell types such as epidermal keratinocytes and T cells(31, 44) suggests that DPP4/CD26 expression is dependent on the activation state of the cells. However, so far we could not modulate DPP4/CD26 expression by primary mast cells and mast cell lines; neither upon IgE-dependent nor in response to IgE-independent activation. It is therefore conceivable that as an alternative explanation the expression levels of DPP4/CD26 might be determined by factors inherent to the respective individuals.

By immunofluorescence microscopy deposits of anti-DPP4 reactive material not associated with cells were found focally in both normal as well as in psoriatic skin. In this context it is interesting that we found strong DPP4/CD26 activity not only in mast cells lysates but also in cell free supernatant of both mast cells and fibroblasts. Release of DPP4/CD26 from mast cells into the intercellular spaces could result in an increase of the area of action by allowing DPP4-mediated modifications of substrates also in the tissue surrounding resident mast cells.

In summary proteomics has helped us to identify new proteins regularly produced by skin mast cells. Our finding that mast cells express the neural adhesion molecule L1CAM/CD171 and express and release the aminopeptidase DPP4/CD26 adds new fragments to complete the puzzle of the elusive functions of mast cells in physiological and pathological conditions.

Acknowledgements

The research reported in this manuscript was funded by grants from the Austrian Science Fund: T545-B19 to Maria Gschwandtner, SFB F4704-B20 to Peter Valent and a research grant from CE.R.I.E.S, Neuilly, France to Erwin Tschachler. Images at the laser scanning microscope were taken in cooperation with the Core Facility Imaging of the Medical University of Vienna.

Author contributions

MG, VP, MM, PV, CG and ET were involved in conception and design of the study. PMB, SH, GE, WRS, PV and ET were involved in obtaining skin and bone marrow samples. MG, VP, MM and GE performed the experiments. All authors were involved in the analysis and interpretation of the data. MG and ET drafted the article and all other authors provided critical review of the manuscript.

The authors declare no conflict of interest.

Figure legends

Figure 1:

Purification of mast cells from human skin.

Tryptase staining of CD117⁺ mast cells (a) and CD117⁻ cell fraction (b); Toluidine blue staining of CD117⁺ mast cells (c) and CD117⁻ cell fraction (d). One representative staining of ten is shown.

Figure 2:

Comparative profiling and identification of the unique human skin mast cell proteome signature.

(a) Venn diagram depicting the number of overlapping and selectively expressed proteins in mast cells, fibroblasts, endothelial cells (HUVEC) and peripheral blood mononuclear cells (PBMC). Peptide counts of selected proteins are depicted for mast cell enriched proteins (b, light grey highlighted: known mast cell absent protein; dark grey highlighted: known mast cell specific proteins) and for highly expressed but not mast cell specific proteins (c).

Figure 3:

Functional annotation cluster of the human skin mast cell proteome.

DAVID enriched functional clusters and characteristic examples of their GO-terms as well as not clustered enriched GO-terms are depicted. The fold-enrichment factor, protein count for each GO-term, p-value and false discovery rate are listed next to the graph. The group enrichment score indicated in bold numbers ranks the clusters according to their biological significance based on consistent low p-values of their GO-term members.

Figure 4:

L1CAM/CD171 is a marker for mast cells in the skin

(a) L1CAM/CD171 mRNA expression in the brain (as positive control), mast cells, fibroblasts (FB), melanocytes and keratinocytes (KC) was analyzed by quantitative real-time PCR in duplicate measurements. (b) Isolated skin mast cells stained on cytopins showed strong L1CAM/CD171 expression; one donor out of four analyzed is shown. (c) Representative immunofluorescence stainings of normal (n=8), psoriasis (n=8) and mastocytosis (n=4) skin showed L1CAM/CD171 expressing FcERI positive mast cells. Bar = 50 μ m;

Figure 5:

DPP4/CD26 is variably expressed on mast cells in skin and bone marrow

(a) DPP4/CD26 mRNA expression in mast cells (MC), fibroblasts (FB), keratinocytes (KC) and epidermis was analyzed in duplicate measurements by quantitative real-time PCR; relative expression levels of two to eleven donors are depicted. (b) Isolated skin mast cells stained on cytopins showed variable DPP4/CD26 expression levels; one donor out of ten is shown. (c) Flow cytometry of isolated skin mast cells confirmed the presence of cell populations with varying DPP4/CD26 staining intensity. (d) The percentage of isolated skin mast cells showing no, weak or strong DPP4/CD26 expression varied between different donors. (e) Representative immunofluorescence staining of normal skin showing DPP4/CD26 and tryptase

positive mast cells; bar = 50µm; (f) DPP4 staining intensity varied *in situ* in the skin; each dot represents one donor. Mast cells were the principal producers of DPP4/CD26 in normal skin; in contrast in lesional psoriasis skin tryptase negative fibroblasts showed elevated DPP4/CD26 expression; *p<0.05 comparing DPP4/CD26 expression on tryptase positive and negative cells; ##p<0.01 and §p<0.05 comparing tryptase negative cells from lesional psoriasis to normal and non-lesional psoriasis skin, respectively; (g) Flow cytometric analysis of DPP4/CD26 expression on mast cells in bone marrow aspirates from patients with mastocytosis in the skin (MIS), indolent systemic mastocytosis (ISM), systemic mastocytosis with an associated clonal hematologic non-mast cell lineage disease (SM-AHNMD), aggressive systemic mastocytosis (ASM) and mast cell leukemia (MCL) showed variable expression levels; MFI=mean fluorescence intensity.

Figure 6:

Isolated human skin mast cells express and secrete active DPP4/CD26.

(a) Proteome profiler array shows DPP4/CD26 protein expression in the cell lysate and supernatant of skin mast cells; one experiment out of three is shown. (b) Active DPP4/CD26 was demonstrated in living mast cells (MC) and fibroblasts (FB), and supernatants and cell lysates of these cells. (c) Substrate cleavage by DPP4/CD26 was absent after pre-incubation with the DPP4/CD26 inhibitor vildagliptin (1 µM); *p<0.05;

References

1. Theoharides TC, Valent P, Akin C. Mast Cells, Mastocytosis, and Related Disorders. *N Engl J Med* 2015;**373**(2):163-172.
2. Reber LL, Frossard N. Targeting mast cells in inflammatory diseases. *Pharmacol Ther* 2014;**142**(3):416-435.
3. Moon TC, Befus AD, Kulka M. Mast cell mediators: their differential release and the secretory pathways involved. *Front Immunol* 2014;**5**:569.
4. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012;**18**(5):693-704.

5. Hartmann K, Escribano L, Grattan C, Brockow K, Carter MC, Alvarez-Twose I, et al. Cutaneous manifestations in patients with mastocytosis: Consensus report of the European Competence Network on Mastocytosis; the American Academy of Allergy, Asthma & Immunology; and the European Academy of Allergology and Clinical Immunology. *J Allergy Clin Immunol* 2015.
6. Otsuka A, Kabashima K. Mast cells and basophils in cutaneous immune responses. *Allergy* 2015;**70**(2):131-140.
7. Shi GP, Bot I, Kovanen PT. Mast cells in human and experimental cardiometabolic diseases. *Nat Rev Cardiol* 2015.
8. Valent P, Baghestanian M, Bankl HC, Sillaber C, Sperr WR, Wojta J, et al. New aspects in thrombosis research: possible role of mast cells as profibrinolytic and antithrombotic cells. *Thromb Haemost* 2002;**87**(5):786-790.
9. Liu J, Divoux A, Sun J, Zhang J, Clément K, Glickman JN, et al. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nat Med* 2009;**15**(8):940-945.
10. Ribatti D. Mast cells as therapeutic target in cancer. *Eur J Pharmacol* 2015.
11. Slany A, Paulitschke V, Haudek-Prinz V, Meshcheryakova A, Gerner C. Determination of cell type-specific proteome signatures of primary human leukocytes, endothelial cells, keratinocytes, hepatocytes, fibroblasts and melanocytes by comparative proteome profiling. *Electrophoresis* 2014;**35**(10):1428-1438.
12. Ghannadan M, Baghestanian M, Wimazal F, Eisenmenger M, Latal D, Kargül G, et al. Phenotypic characterization of human skin mast cells by combined staining with toluidine blue and CD antibodies. *J Invest Dermatol* 1998;**111**(4):689-695.
13. Valent P, Schernthaner GH, Sperr WR, Fritsch G, Agis H, Willheim M, et al. Variable expression of activation-linked surface antigens on human mast cells in health and disease. *Immunol Rev* 2001;**179**:74-81.
14. Kirshenbaum AS, Akin C, Wu Y, Rottem M, Goff JP, Beaven MA, et al. Characterization of novel stem cell factor responsive human mast cell lines LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia; activation following aggregation of FcepsilonRI or FcgammaRI. *Leuk Res* 2003;**27**(8):677-682.
15. Gage MC, Keen JN, Buxton AT, Bedi MK, Findlay JB. Proteomic analysis of IgE-mediated secretion by LAD2 mast cells. *J Proteome Res* 2009;**8**(8):4116-4125.
16. Yamaoka K, Okayama Y, Kaminuma O, Katayama K, Mori A, Tatsumi H, et al. Proteomic Approach to FcepsilonRI aggregation-initiated signal transduction cascade in human mast cells. *Int Arch Allergy Immunol* 2009;**149 Suppl 1**:73-76.
17. Cao L, Yu K, Banh C, Nguyen V, Ritz A, Raphael BJ, et al. Quantitative time-resolved phosphoproteomic analysis of mast cell signaling. *J Immunol* 2007;**179**(9):5864-5876.

18. Ryu SY, Hong GU, Kim DY, Ro JY. Enolase 1 and calreticulin regulate the differentiation and function of mouse mast cells. *Cell Signal* 2012;**24**(1):60-70.
19. Pemberton AD, Brown JK, Wright SH, Knight PA, Miller HR. The proteome of mouse mucosal mast cell homologues: the role of transforming growth factor beta1. *Proteomics* 2006;**6**(2):623-631.
20. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007;**9**(6):654-659.
21. Guhl S, Babina M, Neou A, Zuberbier T, Artuc M. Mast cell lines HMC-1 and LAD2 in comparison with mature human skin mast cells--drastically reduced levels of tryptase and chymase in mast cell lines. *Exp Dermatol* 2010;**19**(9):845-847.
22. Paulitschke V, Berger W, Paulitschke P, Hofstätter E, Knapp B, Dingelmaier-Hovorka R, et al. Vemurafenib resistance signature by proteome analysis offers new strategies and rational therapeutic concepts. *Mol Cancer Ther* 2015;**14**(3):757-768.
23. Huang dW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 2009;**37**(1):1-13.
24. Huang dW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 2009;**4**(1):44-57.
25. Gschwandtner M, Mildner M, Mlitz V, Gruber F, Eckhart L, Werfel T, et al. Histamine suppresses epidermal keratinocyte differentiation and impairs skin barrier function in a human skin model. *Allergy* 2013;**68**(1):37-47.
26. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001;**29**(9):e45.
27. Valent P, Sperr WR, Schwartz LB, Horny HP. Diagnosis and classification of mast cell proliferative disorders: delineation from immunologic diseases and non-mast cell hematopoietic neoplasms. *J Allergy Clin Immunol* 2004;**114**(1):3-11; quiz 12.
28. Valent P, Akin C, Escribano L, Födinger M, Hartmann K, Brockow K, et al. Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria. *Eur J Clin Invest* 2007;**37**(6):435-453.
29. Valent P, Escribano L, Broesby-Olsen S, Hartmann K, Grattan C, Brockow K, et al. Proposed diagnostic algorithm for patients with suspected mastocytosis: a proposal of the European Competence Network on Mastocytosis. *Allergy* 2014;**69**(10):1267-1274.
30. Motakis E, Guhl S, Ishizu Y, Itoh M, Kawaji H, de Hoon M, et al. Redefinition of the human mast cell transcriptome by deep-CAGE sequencing. *Blood* 2014;**123**(17):e58-67.
31. Sillaber C, Baghestanian M, Bevec D, Willheim M, Agis H, Kapiotis S, et al. The mast cell as site of tissue-type plasminogen activator expression and fibrinolysis. *J Immunol* 1999;**162**(2):1032-1041.

32. van Lingen RG, van de Kerkhof PC, Seyger MM, de Jong EM, van Rens DW, Poll MK, et al. CD26/dipeptidyl-peptidase IV in psoriatic skin: upregulation and topographical changes. *Br J Dermatol* 2008;**158**(6):1264-1272.
33. Kirby M, Yu DM, O'Connor S, Gorrell MD. Inhibitor selectivity in the clinical application of dipeptidyl peptidase-4 inhibition. *Clin Sci (Lond)* 2010;**118**(1):31-41.
34. Chen L, Zhou S. "CRASH"ing with the worm: insights into L1CAM functions and mechanisms. *Dev Dyn* 2010;**239**(5):1490-1501.
35. Blessmann M, Gröbe A, Quaas A, Kaifi JT, Mistakidis G, Bernreuther C, et al. Adhesion molecule L1 is down-regulated in malignant peripheral nerve sheath tumors versus benign neurofibromatosis type 1-associated tumors. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012;**113**(2):239-244.
36. Reinisch CM, Tschachler E. The dimensions and characteristics of the subepidermal nerve plexus in human skin--terminal Schwann cells constitute a substantial cell population within the superficial dermis. *J Dermatol Sci* 2012;**65**(3):162-169.
37. Fransen E, Van Camp G, Vits L, Willems PJ. L1-associated diseases: clinical geneticists divide, molecular geneticists unite. *Hum Mol Genet* 1997;**6**(10):1625-1632.
38. Kiefel H, Bondong S, Hazin J, Ridinger J, Schirmer U, Riedle S, et al. L1CAM: a major driver for tumor cell invasion and motility. *Cell Adh Migr* 2012;**6**(4):374-384.
39. Maddaluno L, Verbrugge SE, Martinoli C, Matteoli G, Chiavelli A, Zeng Y, et al. The adhesion molecule L1 regulates transendothelial migration and trafficking of dendritic cells. *J Exp Med* 2009;**206**(3):623-635.
40. Ebeling O, Duczmal A, Aigner S, Geiger C, Schöllhammer S, Kemshead JT, et al. L1 adhesion molecule on human lymphocytes and monocytes: expression and involvement in binding to alpha v beta 3 integrin. *Eur J Immunol* 1996;**26**(10):2508-2516.
41. Dudeck A, Leist M, Rubant S, Zimmermann A, Dudeck J, Boehncke WH, et al. Immature mast cells exhibit rolling and adhesion to endothelial cells and subsequent diapedesis triggered by E- and P-selectin, VCAM-1 and PECAM-1. *Exp Dermatol* 2010;**19**(5):424-434.
42. Proost P, Menten P, Struyf S, Schutyser E, De Meester I, Van Damme J. Cleavage by CD26/dipeptidyl peptidase IV converts the chemokine LD78beta into a most efficient monocyte attractant and CCR1 agonist. *Blood* 2000;**96**(5):1674-1680.
43. Herrmann H, Sadovnik I, Cerny-Reiterer S, Rülcke T, Stefanzi G, Willmann M, et al. Dipeptidylpeptidase IV (CD26) defines leukemic stem cells (LSC) in chronic myeloid leukemia. *Blood* 2014;**123**(25):3951-3962.
44. Lambeir AM, Durinx C, Scharpé S, De Meester I. Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. *Crit Rev Clin Lab Sci* 2003;**40**(3):209-294.

45. Narducci MG, Scala E, Bresin A, Caprini E, Picchio MC, Remotti D, et al. Skin homing of Sézary cells involves SDF-1-CXCR4 signaling and down-regulation of CD26/dipeptidylpeptidase IV. *Blood* 2006;**107**(3):1108-1115.
46. Lessard J, Pelletier M, Biertho L, Biron S, Marceau S, Hould FS, et al. Characterization of dedifferentiating human mature adipocytes from the visceral and subcutaneous fat compartments: fibroblast-activation protein alpha and dipeptidyl peptidase 4 as major components of matrix remodeling. *PLoS One* 2015;**10**(3):e0122065.
47. Rinkevich Y, Walmsley GG, Hu MS, Maan ZN, Newman AM, Drukker M, et al. Skin fibrosis. Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. *Science* 2015;**348**(6232):aaa2151.
48. Tasic T, Bäumer W, Schmiedl A, Schwichtenhövel F, Pabst R, Raap U, et al. Dipeptidyl peptidase IV (DPP4) deficiency increases Th1-driven allergic contact dermatitis. *Clin Exp Allergy* 2011;**41**(8):1098-1107.
49. Krauth MT, Majlesi Y, Florian S, Bohm A, Hauswirth AW, Ghannadan M, et al. Cell surface membrane antigen phenotype of human gastrointestinal mast cells. *Int Arch Allergy Immunol* 2005;**138**(2):111-120.
50. Valent P, Majdic O, Maurer D, Bodger M, Muhm M, Bettelheim P. Further characterization of surface membrane structures expressed on human basophils and mast cells. *Int Arch Allergy Appl Immunol* 1990;**91**(2):198-203.
51. Füreder W, Agis H, Sperr WR, Lechner K, Valent P. The surface membrane antigen phenotype of human blood basophils. *Allergy* 1994;**49**(10):861-865.

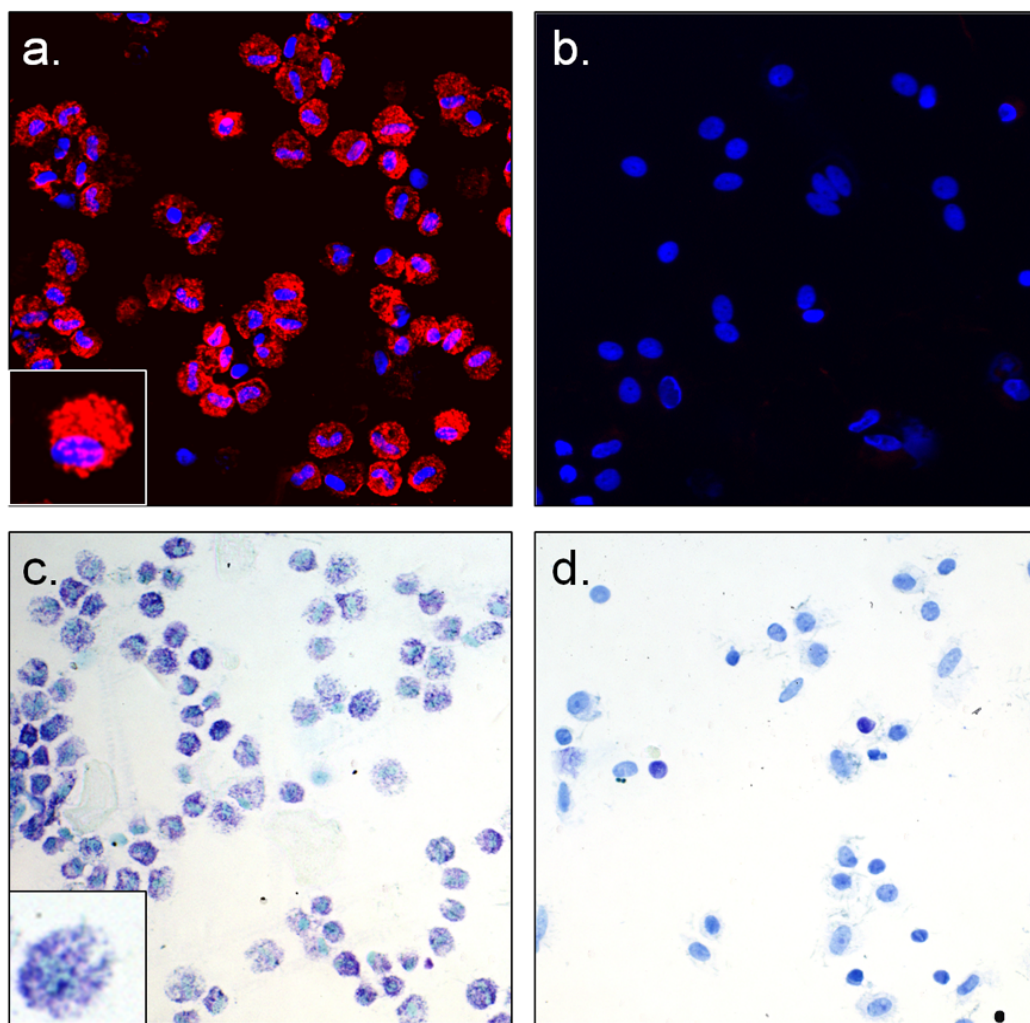


Figure 1

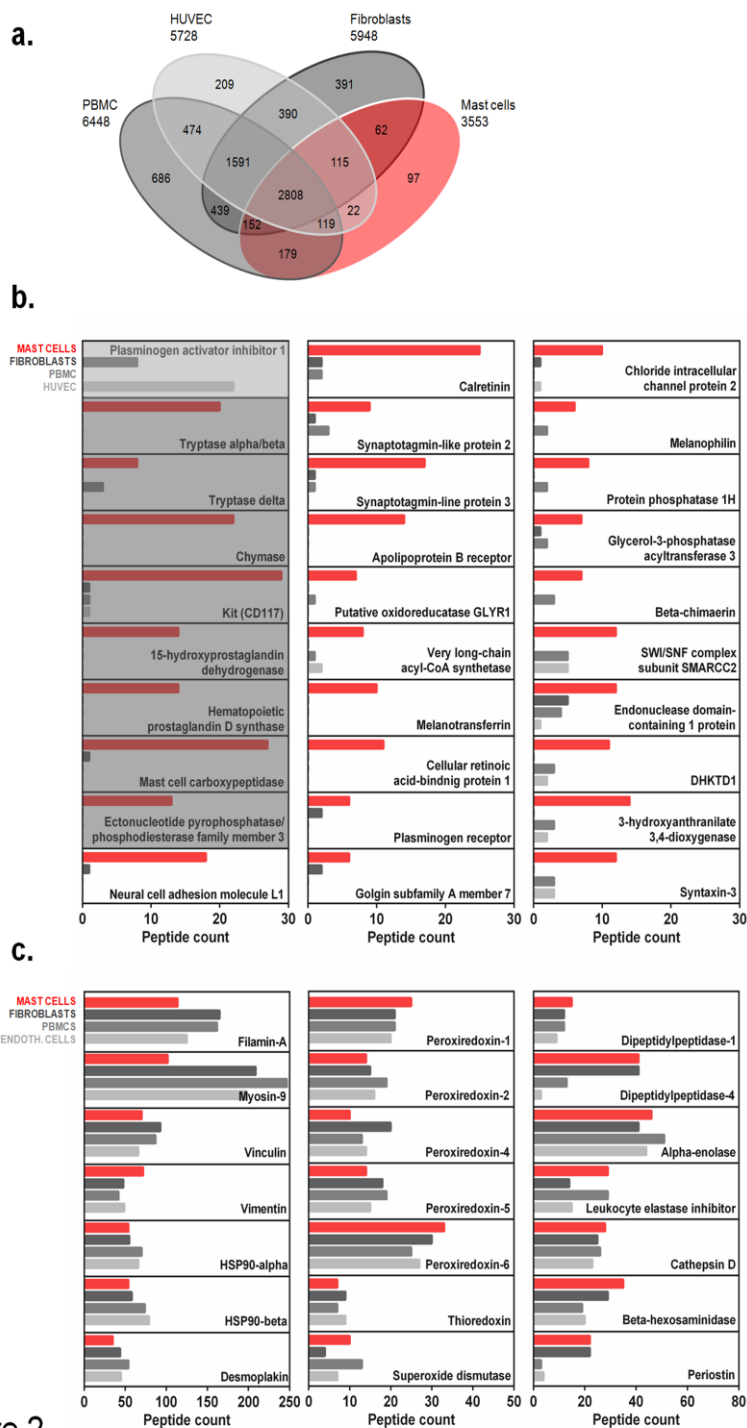


Figure 2

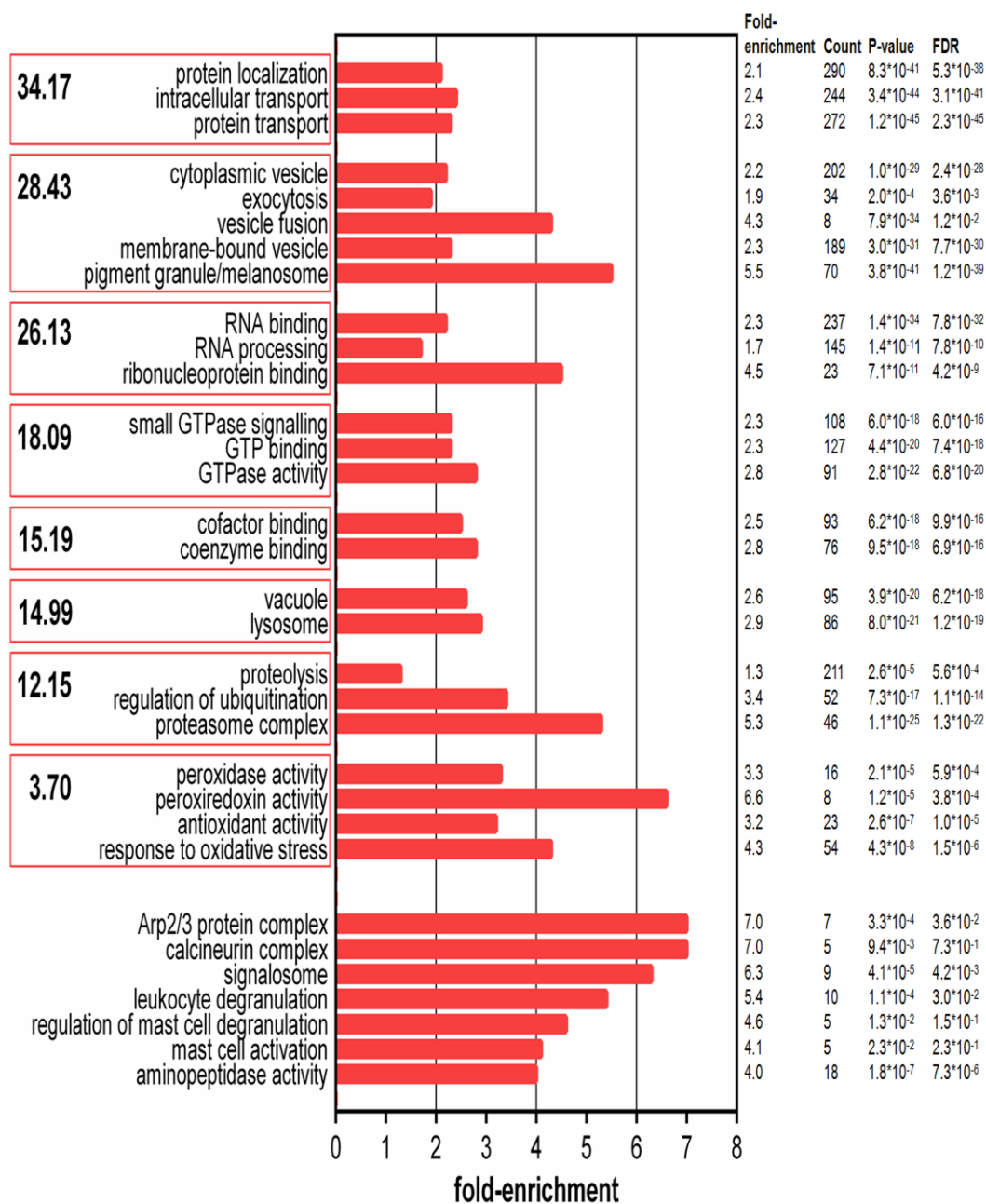


Figure 3

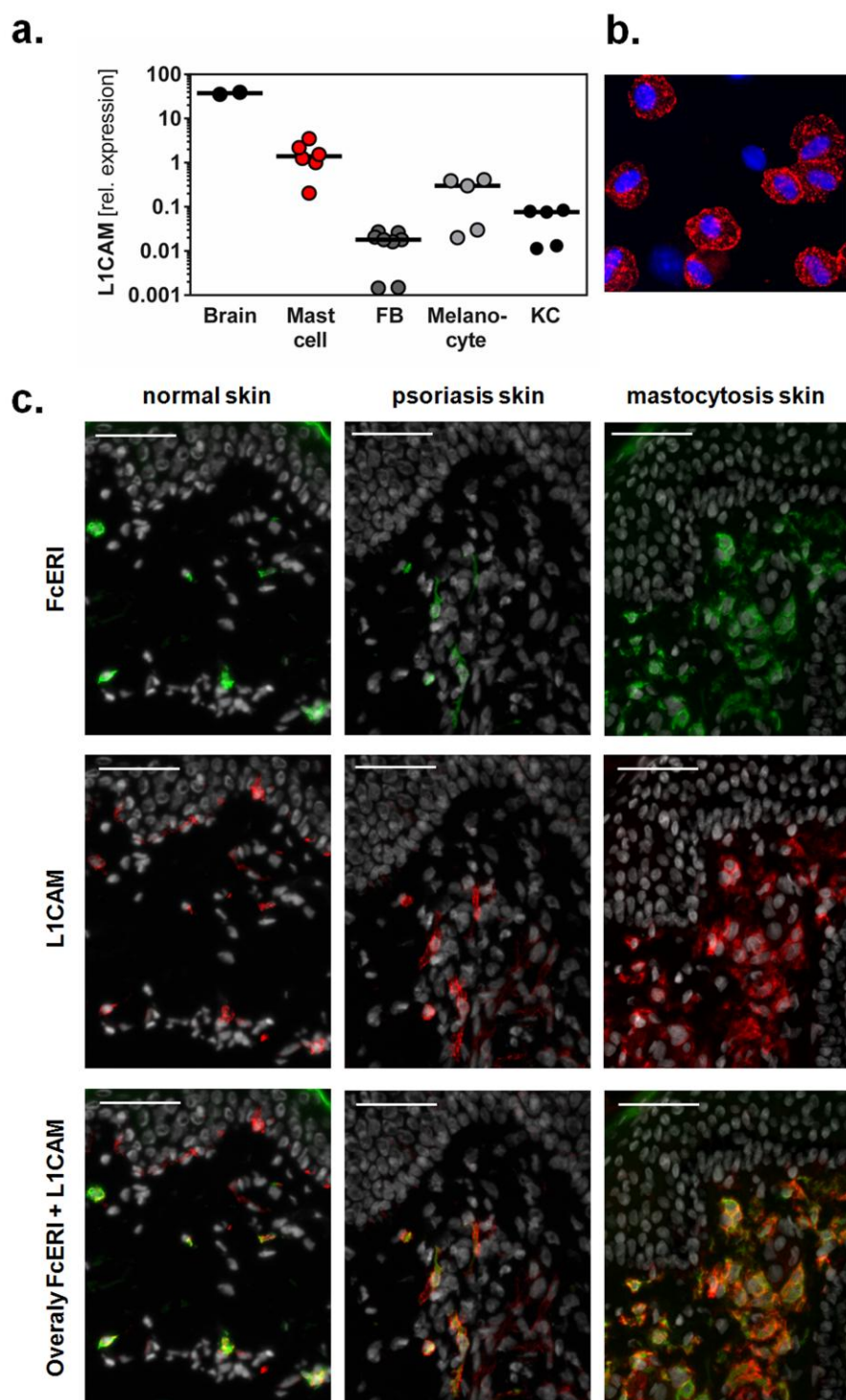


Figure 4

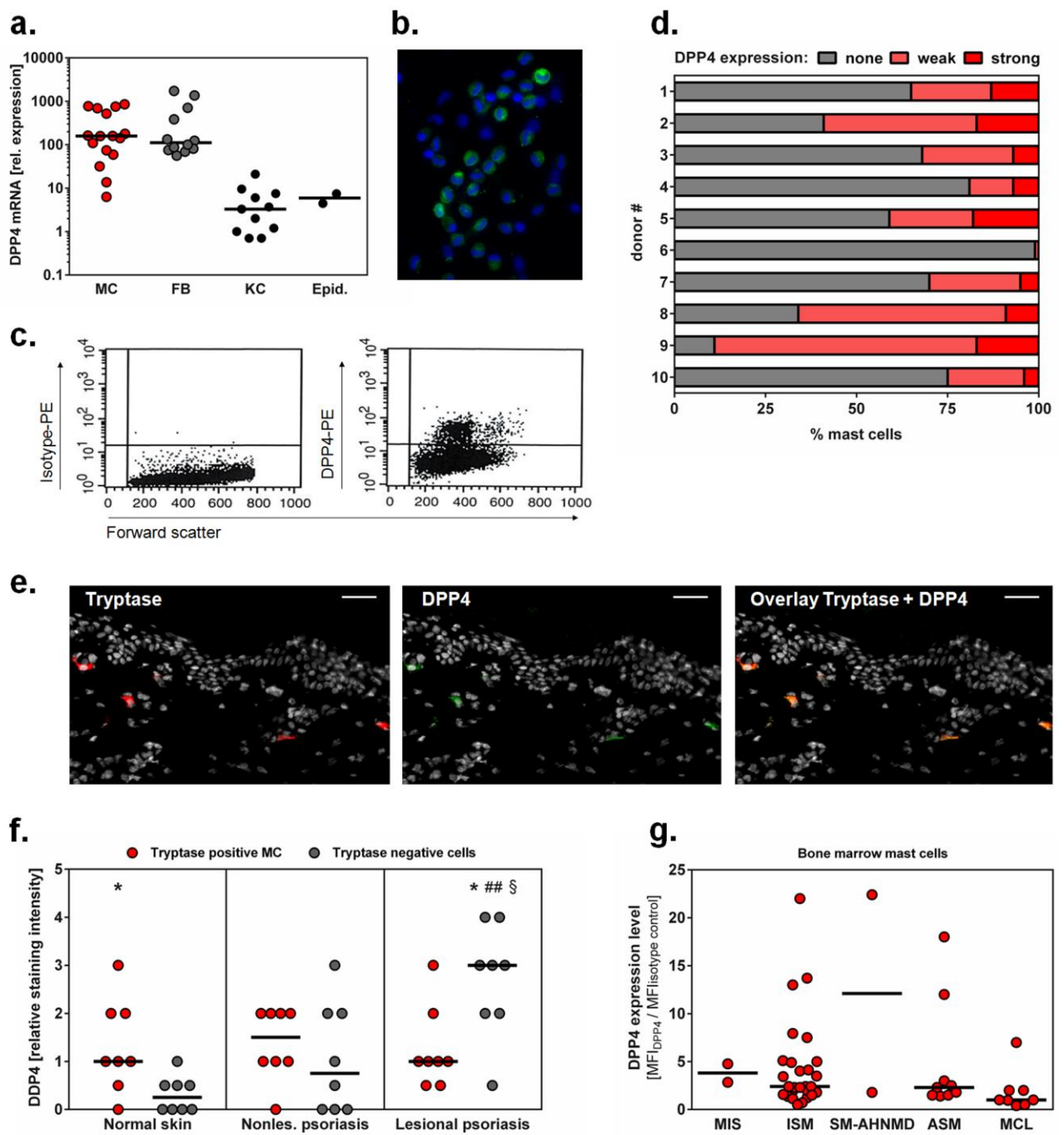


Figure 5

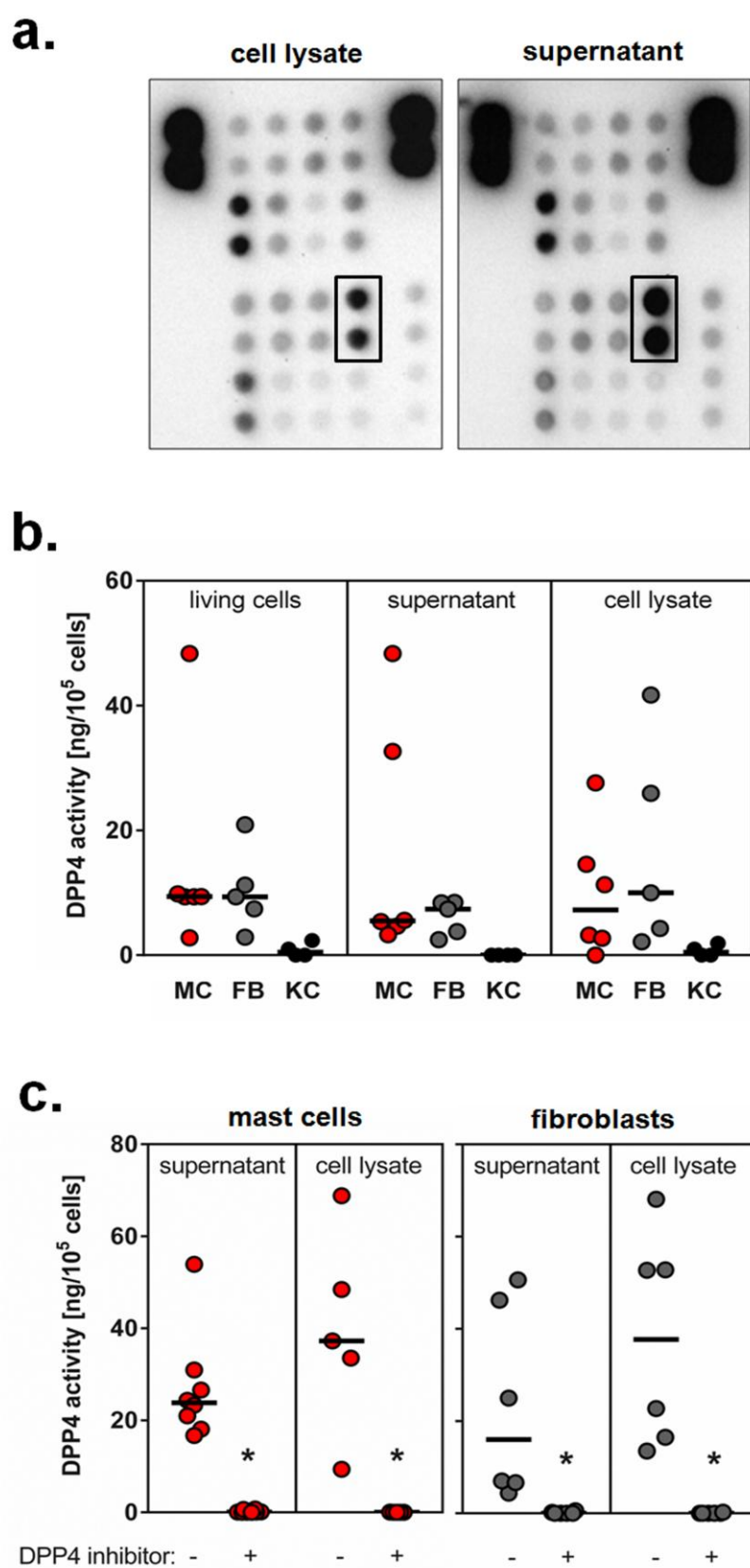


Figure 6

Supplementary table 1:

Suppliers and dilutions of the used antibodies (I-P: immunogen-purified; IF-staining: immuno-fluorescence-staining)

Antigen-Conjugation	Ig subclass	Clone	Company	IF-staining	Flow cytometry
CD26/DPP4	Mouse IgG2b	202-36	Abcam	5 µg/ml	-
CD26/DPP4-PE	Mouse IgG1	M-A261	BD Biosciences	-	2.5 µl per test
CD34-Pacific Blue	Mouse IgG1	581	BioLegend	-	0.15 µl per test
CD45-APC/Cy7	Mouse IgG1	HI30	BioLegend	-	0.5 µl per test
CD117-PE/Cy7	Mouse IgG1	104D2	eBioscience	-	0.3 µl per test
CD203c-PE	Mouse IgG1	97A6	Beckman Coulter	-	10 µl per test
FcERI-Alexa488	Mouse IgG2b	9E1	Serotec	1:20	-
FVIII	Rabbit IgG	Cat# A0082	DAKO	7 µg/ml	-
L1CAM	Mouse IgG1	UJ127	Neomarkers	2 µg/ml	-
Tryptase	Mouse IgG1	AA1	Abcam	1 µg/ml	-
mIgG1-Alexa546	Goat IgG		LifeTechnologies	4 µg/ml	-
mIgG2b-Alexa488	Goat IgG		LifeTechnologies	4 µg/ml	-
Rabbit-Alexa488	Goat IgG		LifeTechnologies	4 µg/ml	-
Rabbit-Alexa647	Goat IgG		LifeTechnologies	4 µg/ml	-

Supplementary table 2:

Donor characteristics, cell counts and purity of ten different experiments are summarized.

¹ absolute number of isolated CD117⁺ mast cells; ² percentage of CD117⁺ mast cells in the dermal cell suspension before magnetic separation; ³ purity of tryptase positive cells in %

Donor	Age	Sex	# ¹ CD117 ⁺	% ² CD117 ⁺	% Purity ³
1	37	f	1.60*10 ⁶	5.00	95.73
2	56	f	3.75*10 ⁶	2.08	98.46
3	65	f	1.64*10 ⁶	6.67	98.81
4	59	f	3.04*10 ⁶	5.99	98.53
5	36	m	2.50*10 ⁶	5.32	98.09
6	30	m	1.11*10 ⁶	5.84	99.20
7	65	f	0.63*10 ⁶	6.86	99.60
8	46	f	0.65*10 ⁶	8.22	97.06
9	23	f	0.86*10 ⁶	4.78	96.77
10	57	f	1.15*10 ⁶	5.00	97.59

Supplementary table 3:

Names and Uniprot accession numbers for the proteins identified in Lad-2 (Gage et al., J Proteome Res 2009; 8(8):4116-4125) and dermal mast cells and 35 new proteins identified only in skin mast cells. Some of the proteins in the latter group have been linked to mast cells previously as indicated by the references.

Function	Proteins identified in LAD2 and skin MC		Additional proteins identified in skin MC	
EXOCYTOSIS	Rab1b	Q9H0U4	14-3-3 protein zeta/delta	P63104
	Rab11b	Q15907	Calcineurin B homologous protein 1	Q99653
	Rab27b	O00194	Linker for activation of T-cell family member 2	Q9GZY6
	Gamma-enolase	P09104	P2X purinoceptor 1 ¹	P51575
	Ganglioside GM2 activator	P17900	Phosphatidylinositol 4-kinase type 2-alpha ²	Q9BTU6
			Protein unc-13 homolog D ^{3,4}	Q70J99
			Ras-related protein Rab-3D	O95716
			Ras-related protein Rab-27A	P51159
			Sec1 family domain-containing protein 1	Q8WVM8
			SNARE-associated protein Snapin	O95295
			Synaptobrevin homolog YKT6	O15498
			Syntaxin-binding protein 2	Q15833
			Syntaxin-binding protein 3	O00186
			Transmembrane emp24 domain-containing protein 10	P49755
			Tyrosine-protein kinase Lyn ⁵	P07948
			Vesicle-associated membrane protein 3, 7	Q15836
			Vesicle-associated membrane protein 7 ⁶	P51809
REDOX	NADH-ubiquinone oxidoreduct.	P28331	Chopper chaperone for superoxide dismutase	O14618
REGULATION	Glutathione peroxidase 1	P07203	Catalase	P04040
	Peroxioredoxin 2	P32119	Glutaredoxin-1	P35754
	Protein disulfide-isomerase A3	P30101	Glutathione peroxidase	P36969
			Heme oxygenase 1	P09601
			Mpv17	P39210
			Peroxioredoxin 1	Q06830,
			Peroxioredoxin 4	Q13162
			Peroxioredoxin 5	P30044
			Peroxioredoxin 6	P30041
			Protein disulfide-isomerase	P07237
			Protein DJ1 / PARK7 ⁷	Q99497
			Superoxide dismutase	P00441
			Superoxide dismutase, mit.	P04179
			Thioredoxin	P10599
			Thioredoxin-dependent peroxide reductase, mit.	P30048
			Thioredoxin-like protein 1	O43396
			Thioredoxin-related transmembrane protein 1	Q9H3N1

¹ Wareham K, Vial C, Wykes RC, Bradding P, Seward EP. Functional evidence for the expression of P2X1, P2X4 and P2X7 receptors in human lung mast cells. Br J Pharmacol 2009; 157:1215-24.

² Naveen B, Shankar BS, Subrahmanyam G. FcεRI cross-linking activates a type II phosphatidylinositol 4-kinase in RBL 2H3 cells. Mol Immunol 2005; 42:1541-9.

³ Singh RK, Mizuno K, Wasmeier C, Wavre-Shapton ST, Recchi C, Catz SD, et al. Distinct and opposing roles for Rab27a/Mlph/MyoVa and Rab27b/Munc13-4 in mast cell secretion. FEBS J 2013; 280:892-903.

⁴ Higashio H, Nishimura N, Ishizaki H, Miyoshi J, Orita S, Sakane A, et al. Doc2 alpha and Munc13-4 regulate Ca(2+) - dependent secretory lysosome exocytosis in mast cells. J Immunol 2008; 180:4774-84.

⁵ Ainsua-Enrich E, Alvarez-Errice D, Gilfillan AM, Picado C, Savos C, Rivera J, et al. The adaptor 3BP3 is required for early and late events in FcεRI signaling in human mast cells. J Immunol 2012; 189(6):2727-34.

⁶ Sander LE, Frank SP, Bolat S, Blank U, Galli T, Bigalke H, et al. Vesicle associated membrane protein (VAMP)-7 and VAMP-8, but not VAMP-2 or VAMP-3, are required for activation-induced degranulation of mature human mast cells. Eur J Immunol 2008; 38:855-63.

⁷ Kim dK, Kim HS, Kim AR, Kim JH, Kim B, Noh G, et al. DJ-1 regulates mast cell activation and IgE-mediated allergic responses. J Allergy Clin Immunol 2013; 131:1653-62.

Supplementary table 4:

Table stating the numbers depicted in Figure 2a (Venn diagram of four cell types analyzed by proteomics): number of proteins expressed selectively in only one cell type, number of proteins expressed overlapping in two cell types (and not expressed in the other two cell types), number of proteins expressed overlapping in three cell types and the proteins expressed in all four cell types. M: mast cell, F: fibroblast, H: HUVEC, P: PBMC;

# of proteins				
selectively expressed in one cell type				
M				97
	F			391
		H		209
			P	686
overlapping in two cell types				
M	F			62
M		H		22
M			P	179
	F	H		390
	F		P	439
		H	P	474
overlapping in three cell types				
M	F	H		115
M	F		P	152
M		H	P	119
	F	H	P	1591
expressed in all cell types				
M	F	H	P	2808

Supplementary table 5a and 5b:

Suppl. Table 5a: list of abundant proteins (peptide count ≥ 2) and Suppl. Table 5b: list of non-abundant proteins (peptide count =1) selectively expressed in mast cells, but not in fibroblasts, endothelial cells and peripheral blood mononuclear cells. * indicates that this protein was found in keratinocytes, for further information see Supplementary table 9. Mean RLE (relative log expression) counts observed in a transcriptomic study in ex-vivo cultured, 4-5 weeks expanded (Exp.) and expanded and stimulated (Exp. Stim.) mast cells (Motakis et al., Blood 2014; 123(17):e58-67) are given in the last three columns (- indicates that protein was not measured in the transcriptome study).

Supplementary table 5a:

Protein name	Uniprot Acc. #	# peptides	RLE Ex-vivo	RLE Exp.	RLE Exp. Stim.
Tryptase alpha/beta-1	Q15661	27	84	30	9
Chymase	P23946	22	1669	654	443
15-hydroxyprostaglandin dehydrogenase	P15428	14	1569	9950	7604
Apolipoprotein B receptor	Q0VD83	14	67	107	95
Hematopoietic prostaglandin D synthase	O60760	14	1230	3583	3090
CD203c	O14638	13	346	838	1805
*Cellular retinoic acid-binding protein 1	P29762	11	2	0	1
Melanotransferrin	P08582	10	123	24	12
*CD82 antigen	P27701	5	646	724	658
Linker for activation of T-cells family member 2	Q9GZY6	5	306	328	186
Tubulin polymerization-promoting protein	O94811	5	112	1	0
Bcl10-interacting CARD protein (C9orf89)	Q96LW7	4	48	52	22
Germinal center-assoc. sign. and motility-like protein (C1orf150)	Q5JQS6	4	1038	1141	3728
Leukemia inhibitory factor	P15018	4	226	277	646
Enoyl-CoA hydratase domain-containing protein 3, mit.	Q96DC8	3	12	8	7
Fibromodulin	Q06828	3	5	1	13
H/ACA ribonucleoprotein complex subunit 3	Q9NPE3	3	37	128	120
Insulin-like growth factor-binding protein 2	P18065	3	16	1	1
KH domain-cont., RNA-bind., signal transd.-assoc. protein 1	Q07666	3	252	221	128
Mas-related G-protein coupled receptor member X2	Q96LB1	3	983	86	56
Membrane primary amine oxidase	Q16853	3	-	-	-
Nicotinamide mononucleotide adenylyltransferase 1	Q9HAN9	3	11	17	16
Sialic acid-binding Ig-like lectin 6	O43699	3	761	1715	984
1-acyl-sn-glycerol-3-phosphate acyltransferase epsilon	Q9NUQ2	2	97	139	157
AP-1 complex subunit sigma-3	Q96PC3	2	398	27	33
Apolipoprotein A	P08519	2	-	-	-
Arylsulfatase D	P51689	2	49	46	22
B box and SPRY domain-containing protein OS	Q5W0U4	2	49	10	9
Beta-galactosidase-1-like protein	Q6UWU2	2	4	4	2
CDC42 small effector protein 2	Q9NRR3	2	174	116	79
Complement C1q tumor necrosis factor-related protein 3	Q9BXJ4	2	-	-	-
Hepatocyte growth factor activator	Q04756	2	-	-	-
High affinity immunoglobulin epsilon receptor subunit beta	Q01362	2	1390	2040	1292
Mitochondrial import receptor subunit TOM6 homolog	Q96B49	2	118	253	259
Protein DEK	P35659	2	104	103	135
Regulator of G-protein signaling 13	O14921	2	979	580	911
Sialic acid-binding Ig-like lectin 8	Q9NYZ4	2	31	39	29
Uncharacterized protein C1orf186	Q6ZWK4	2	-	-	-

Supplementary table 5b:

Protein name	Uniprot Acc.	# peptides	RLE Ex-vivo	RLE Exp	RLE Exp. Stim.
40S ribosomal protein S29	P62273	1	74	103	101
*Activity-dependent neuroprotector homeobox protein	Q9H2P0	1	81	67	45
ADAMTS-like protein 4	Q6UY14	1	4	2	3
ADP-ribosylation factor-like protein 4C	P56559	1	340	43	13
AP-4 complex subunit sigma-1	Q9Y587	1	4	5	7
ATP synthase subunit epsilon, mitochondrial	P56381	1	139	541	466
Cathepsin F	Q9UBX1	1	38	23	5
Cell adhesion molecule 1	Q9BY67	1	4	79	156
Cell surface glycoprotein CD200 receptor 1	Q8TD46	1	144	191	101
Choline/ethanolaminephosphotransferase 1	Q9Y6K0	1	30	48	42
Cholinephosphotransferase 1	Q8WUD6	1	76	47	22
Collagen alpha-1(XIV) chain	Q05707	1	3	0	0
Complement component C8 beta chain	P07358	1	-	-	-
Complement factor D	P00746	1	19	0	0
Cysteine-rich and transmembr. domain-cont. protein 1 (C5orf32)	Q9H1C7	1	118	159	121
Cytochrome c oxidase subunit 7A1, mitochondrial	P24310	1	18	9	3
Dolichol-phosphate mannosyltransferase subunit 3	Q9P2X0	1	24	42	24
ER membrane protein complex subunit 8 (C16orf42)	O43402	1	16	18	6
GDNF family receptor alpha-2	O00451	1	-	-	-
GPN-loop GTPase 3	Q9UHW5	1	21	29	17
Group IID secretory phospholipase A2	Q9UNK4	1	-	-	-
Guanine nucleotide-binding protein subunit gamma-10	P50151	1	16	29	20
Guanine nucleotide-binding protein subunit gamma-5	P63218	1	95	241	178
*H/ACA ribonucleoprotein complex subunit 4	O60832	1	66	57	73
Hermansky-Pudlak syndrome 4 protein	Q9NQG7	1	66	43	19
HLA class II histocompatibility antigen, DQ alpha 1 chain	P01909	1	3	0	0
Latent-transforming growth factor beta-binding protein 4	Q8N2S1	1	89	33	95
Leptin receptor overlapping transcript-like 1	O95214	1	119	196	142
Leukotriene C4 synthase OS	Q16873	1	232	2262	630
Microfibrillar-associated protein 5	Q13361	1	-	-	-
*MIP18 family protein FAM96A	Q9H5X1	1	40	102	72
*Mitochondrial inner membrane organizing system protein 1	Q5TGZ0	1	61	90	55
MRG-binding protein (C20orf20)	Q9NV56	1	10	14	6
NADH dehydrogenase 1a subcomplex subunit 3	O95167	1	41	122	75
NTF2-related export protein 1	Q9UKK6	1	27	22	19
NTF2-related export protein 2	Q9NPJ8	1	7	13	8
Nucleoporin-like protein 2	O15504	1	25	63	53
Osteomodulin	Q99983	1	-	-	-
Plasma serine protease inhibitor	P05154	1	-	-	-
Plexin domain-containing protein 2	Q6UX71	1	21	3	10
PQ-loop repeat-containing protein 3	Q8N755	1	28	39	19
Presenilin-2	P49810	1	9	23	8
Protein C19orf12	Q9NSK7	1	8	10	8
Protein FAM200A	Q8TCP9	1	10	14	15
Protein FAM84B	Q96KN1	1	19	0	1
Proteoglycan 3	Q9Y2Y8	1	-	-	-
Proton-coupled amino acid transporter 1	Q7Z2H8	1	35	19	13
Proton-coupled folate transporter	Q96NT5	1	7	3	3
Round spermatid basic protein 1	Q5VWQ0	1	52	23	12
SAGA-associated factor 29 homolog	Q96ES7	1	40	38	20
Selenoprotein W	P63302	1	110	145	87
Sex hormone-binding globulin	P04278	1	0	1	0
*Short coiled-coil protein OS	Q9UIL1	1	27	73	63
Signal-transducing adaptor protein 1	Q9ULZ2	1	35	224	163
Transmembrane protein 141	Q96I45	1	29	34	17
*Transmembrane protein 41B	Q5BJD5	1	26	46	42
Uncharacterized protein C6orf136	Q5SQH8	1	24	44	31
UPF0466 protein C22orf32, mitochondrial	Q9H4I9	1	32	41	27
Zinc finger and BTB domain-containing protein 6	Q15916	1	16	35	41

Supplementary table 6:

Expression levels (peptide count) of the proteins depicted in Figure 2b and 2c compared to the mean RLE (relative log expression) counts observed in a transcriptomic study in ex-vivo cultured, 4-5 weeks expanded (Exp.) and expanded and stimulated (Exp. Stim.) mast cells (Motakis et al., Blood 2014; 123(17):e58-67).

Protein name	Uniprot Acc. #	# peptides	RLE Ex- vivo	RLE Exp.	RLE Exp. Stim.
Tryptase alpha/beta-1	Q15661	20	84	30	9
Tryptase delta	Q9BZJ3	8	632	960	495
Chymase	P23946	22	1669	654	443
Kit / CD117 / SCF-receptor	P10721	29	3394	3492	1853
Prostaglandin D synthase	O60760	14	1569	9950	7604
HPGD	P15428	14	1230	3583	3090
MC-CPA (CPA3)	P15088	27	4632	7435	5866
Calretinin	P22676	13	346	838	1805
L1CAM	P32004	18	257	27	44
CD203c	O14638	25	341	526	366
SYTL-2	Q9HCH5	9	248	113	85
SYTL-3	Q4VX76	17	498	183	106
APOBR	Q0VD83	14	67	107	95
Put. Oxido-reductase GLYR1	Q49A26	7	67	98	72
SLC27A2	O14975	8	151	151	146
Melanotransferrin	P08582	10	123	24	12
CRABP1	P29762	11	2	0	1
Plasminogen receptor	Q9HBL7	6	69	45	26
Golgin subfamily A member 7	Q7Z5G4	6	60	84	54
CLIC2	O15247	10	45	12	24
Melanophilin	Q9BV36	6	359	358	157
Protein phosphatase 1H	Q9ULR3	8	208	123	40
AGPAT9	Q53EU6	7	115	138	272
Beta-chimaerin	P52757	7	235	113	85
SMARCC2	Q8TAQ2	12	59	45	39
ENDOD1	O94919	12	97	133	209
DHTKD1	Q96HY7	11	12	50	13
HAAO	P46952	14	26	21	4
Syntaxin-3	Q13277	12	238	247	73
Filamin-A	P21333	114	154	268	439
Myosin-9	P35579	102	215	369	209
Vinculin	P18206	70	205	114	147
Vimentin	P08670	72	8915	4212	2048
HSP90-alpha	P07900	54	2321	847	1819
HSP90-beta	P08238	54	1294	1083	2150
Desmoplakin	P15924	35	12,6	0	0
Peroxiredoxin-1	Q06830	25	419	788	1351
Peroxiredoxin-2	P32119	14	71	118	71
Peroxiredoxin-4	Q13162	10	19	63	77
Peroxiredoxin-5	P30044	14	29	87	64
Peroxiredoxin-6	P30041	33	469	913	477
Thioredoxin	P10599	7	410	537	809
Superoxide dismutase	P00441	10	116	236	143
Dipeptidylpeptidase-1	P53634	15	164	383	283
Dipeptidylpeptidase-4	P27487	41	33	4	4
Alpha-enolase	P06733	46	201	642	785
Leukocyte elastase inhibitor	P30740	29	1228	3858	3905
Cathepsin D	P07339	28	924	2820	759
Beta-hexosaminidase subunit beta	P07686	35	78	97	54
Periostin	Q15063	22	3	2	1

Supplementary table 7:

Function, biological relevance and literature entries (# of publications found in Pubmed for mast cells + respective protein / # of publications for the respective protein alone) for the mast cell specific proteins depicted in Figure 2b. The most important references are included at the bottom of the table.

Protein	Uniprot-ID	Function, biological relevance	Literature
Tryptase alpha/beta-1	Q15661	Major neutral protease; released upon degranulation/activation; vasoactive, proinflammatory and chemotactic action; used for diagnosis of mast cell disorders	3292 / 3971
Tryptase delta	Q9BZJ3	Neutral protease; (patho-)physiological function not determined	5 / 13
Chymase	P23946	Tissue damage, pain, angiotensin II synthesis	1737 / 2453
Kit / CD117 / SCF-receptor	P10721	Tyrosine protein kinase; cell surface receptor for Kitl/SCF; regulation of cell development, survival and proliferation; stem cell maintenance; melanocyte development	1315 / 8873 1680 / 11546
Prostaglandin D synthase	O60760	Catalyzes the conversion of PGH2 to PGD2; glutathione-peroxidase activity	12 / 45
HPGD	P15428	= 15-hydroxyprostaglandin dehydrogenase; Inactivation of prostaglandin	2 / 585
MC-CPA (CPA3)	P15088	Processing of substance P and bradykinin; angiotensin II synthesis	213 / 2013
Calretinin	P22676	Neuron-specific calcium-binding protein; expressed in mast cell lesions (urticaria pigmentosa, mastocytoma) ¹	6 / 3215
L1CAM	P32004	= neural cell adhesion molecule L1; neuron-neuron adhesion; development of nervous system; outgrowth of neurons; ²	1 / 357
CD203c	O14638	= Ectonucleotide pyrophosphatase/phosphodiesterase family member 3; expressed on basophils and mast cells; induced upon activation; diagnosis of allergy; ^{3,4}	34 / 176
SYTL-2	Q9HCH5	Acts as rab27a effector protein; cytotoxic granule exocytosis in lymphocytes	1 / 46
SYTL-3	Q4VX76	May act as rab effector protein; vesicle trafficking	0 / 22
APOBR	Q0VD83	Binds and internalizes triglyceride-rich lipoproteins	0 / 27
Put. Oxido-reductase GLYR1	Q49A26	Regulates p38 MAP kinase activity	0 / 7
SLC27A2	O14975	= very long-chain acyl-CoA synthase; probably involved in bile acid metabolism; activates long- and branched-chain fatty acids	0 / 21
Melanotransferrin	P08582	Involved in cellular iron uptake	0 / 100
CRABP1	P29762	= cellular retinoic acid-binding protein 1; fatty-acid binding protein	0 / 106
Plasminogen receptor	Q9HBL7	Neuroendocrine prohormone processing; regulation of inflammatory response, MMP expression and catecholamine release	0 / 10
Golgin subfamily A member 7	Q7Z5G4	May be involved in protein transport from Golgi to membrane; found in extracellular vesicular exosome	0 / 6
CLIC2	O15247	= chloride intracellular channel protein 2; involved in the formation of chloride channels	0 / 27
Melanophilin	Q9BV36	Rab effector protein involved in melanosome transport	1 / 81
Protein phosphatase 1H	Q9ULR3	Dephosphorylates CDKN1B; removes signal for proteasomal degradation	0 / 9
AGPAT9	Q53EU6	= glycerol-3-phosphatase acyltransferase 3; glycerolipid synthesis	0 / 8
Beta-chimaerin	P52757	GTPase-activating protein for p21-rac	0 / 37
SMARCC2	Q8TAQ2	Involved in chromatin remodeling (neural progenitor specific)	0 / 28
ENDOD1	O94919	May act as DNase or RNase; extracellular vesicular exosome	0 / 4
DHTKD1	Q96HY7	= probable 2-oxoglutarate dehydrogenase E1 component	0 / 8
HAAO	P46952	= 3-hydroxyanthranilate 3,4-dioxygenase; cofactor biosynthesis	0 / 7
Syntaxin-3	Q13277	Vesicular transport; mast cell exocytosis and degranulation ^{5,6}	31 / 2274

¹ Mangini J, Silverman JF, Dabbs DJ, Tung MY, Silverman AR. Diagnostic Value of Calretinin in Mast Cell Lesions of the Skin. *Int J Surg Pathol* 2000; 8:119-22.

² Blessmann M, Gröbe A, Quaas A, Kaifi JT, Mistakidis G, Bernreuther C, et al. Adhesion molecule L1 is down-regulated in malignant peripheral nerve sheath tumors versus benign neurofibromatosis type 1-associated tumors. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012; 113:239-44.

³ Hauswirth AW, Escobedo L, Prados A, Nuñez R, Mirkina I, Kneidinger M, et al. CD203c is overexpressed on neoplastic mast cells in systemic mastocytosis and is upregulated upon IgE receptor cross-linking. *Int J Immunopathol Pharmacol* 2008; 21:797-806.

⁴ Bühring HJ, Seiffert M, Giesert C, Marxer A, Kanz L, Valent P, et al. The basophil activation marker defined by antibody 97A6 is identical to the ectonucleotide pyrophosphatase/phosphodiesterase 3. *Blood* 2001; 97:3303-5.

⁵ Frank SP, Thon KP, Bischoff SC, Lorentz A. SNAP-23 and syntaxin-3 are required for chemokine release by mature human mast cells. *Mol Immunol* 2011; 49:353-8.

⁶ Brochetta C, Suzuki R, Vita F, Soranzo MR, Claver J, Madjene LC, et al. Munc18-2 and syntaxin 3 control distinct essential steps in mast cell degranulation. *J Immunol* 2014; 192:41-51.

Supplementary table 8:

Number of proteins expressed selectively in keratinocytes (K) and the numbers of proteins expressed overlapping in two cell types (but not expressed in the other cell types): K and mast cells (M), K and fibroblasts (F), K and HUVEC (H) and K and PBMC (P); In total 5271 proteins were identified in keratinocytes.

# of proteins			
selectively expressed in K			
K			144
overlapping in K and M, F, H or P			
K	M		8
K	F		107
K	H		71
K	P		294

Supplementary table 9:

List of the 8 proteins expressed in mast cells (MC) and keratinocytes (KC), but not expressed in fibroblasts, HUVEC and PBMC.

Protein name	Uniprot Acc. #	Peptide # in MC	Peptide # in KC
Cellular retinoic acid-binding protein 1	P29762	11	1
CD82 antigen	P27701	5	2
Activity-dependent neuroprotector homeobox protein	Q9H2P0	1	2
H/ACA ribonucleoprotein complex subunit 4	O60832	1	1
MIP18 family protein FAM96A	Q9H5X1	1	1
Mitochondrial inner membrane organizing system protein 1	Q5TGZ0	1	1
Shorted coiled-coil protein	Q9UIL1	1	1
Transmembrane protein 41B	Q5BJD5	1	2

Supplementary table 10:

Expression levels (peptide count) of the proteins selectively expressed or highly expressed in mast cells (depicted in Figure 2b and 2c) compared to the expression levels in keratinocytes.

Protein name	Uniprot Acc. #	Peptide # in MC	Peptide # in KC
Tryptase alpha/beta-1	Q15661	20	0
Tryptase delta	Q9BZJ3	8	0
Chymase	P23946	22	0
Kit / CD117 / SCF-receptor	P10721	29	2
Prostaglandin D synthase	O60760	14	0
HPGD	P15428	14	0
MC-CPA (CPA3)	P15088	27	0
Calretinin	P22676	13	2
L1CAM	P32004	18	3
CD203c	O14638	25	0
SYTL-2	Q9HCH5	9	0
SYTL-3	Q4VX76	17	0
APOBR	Q0VD83	14	0
Put. Oxido-reductase GLYR1	Q49A26	7	3
SLC27A2	O14975	8	3
Melanotransferrin	P08582	10	0
CRABP1	P29762	11	1
Plasminogen receptor	Q9HBL7	6	0
Golgin subfamily A member 7	Q7Z5G4	6	2
CLIC2	O15247	10	0
Melanophilin	Q9BV36	6	0
Protein phosphatase 1H	Q9ULR3	8	0
AGPAT9	Q53EU6	7	0
Beta-chimaerin	P52757	7	0
SMARCC2	Q8TAQ2	12	0
ENDOD1	O94919	12	4
DHTKD1	Q96HY7	11	5
HAAO	P46952	14	0
Syntaxin-3	Q13277	12	0
Filamin-A	P21333	114	115
Myosin-9	P35579	102	164
Vinculin	P18206	70	57
Vimentin	P08670	72	24
HSP90-alpha	P07900	54	55
HSP90-beta	P08238	54	52
Desmoplakin	P15924	35	154
Peroxiredoxin-1	Q06830	25	19
Peroxiredoxin-2	P32119	14	10
Peroxiredoxin-4	Q13162	10	10
Peroxiredoxin-5	P30044	14	12
Peroxiredoxin-6	P30041	33	13
Thioredoxin	P10599	7	4
Superoxide dismutase	P00441	10	5
Dipeptidylpeptidase-1	P53634	15	8
Dipeptidylpeptidase-4	P27487	41	1
Alpha-enolase	P06733	46	29
Leukocyte elastase inhibitor	P30740	29	11
Cathepsin D	P07339	28	13
Beta-hexosaminidase subunit beta	P07686	35	8
Periostin	Q15063	22	3

Supplementary Figure 1:

(a) Immunofluorescence stainings of normal skin showed that some c-kit/CD117 positive melanocytes in the basal epidermal layer express L1CAM (blue arrows). One representative experiment out of three is shown.

(b) Within dermal sheets FcERI and L1CAM double positive mast cells can be found in close proximity to vessels (FVIII positive) and nerves (L1CAM/CD171 positive). One representative experiment out of three is shown.

Split thickness skin (normal skin) was cut into 0.5 cm² pieces and floated on 3.8% ammoniumthiocyanate solution in PBS for 30 min at 37°C. Subsequently, the epidermis was separated from the underlying dermis and dermal sheets were washed in PBS followed by incubation in 10% sucrose in PBS overnight. Sheets were washed in PBS and immunofluorescence staining was done as follows: sheets were incubated in 10% goat serum and stained with FcERI, L1CAM/CD171 and FVIII (Supplementary table 1) over night at 4°C. After washing dermal sheets were incubated with secondary antibodies for 2h at RT and mounted in Fluoprep (Biomerieux) after additional intensive washing. Sheets were analyzed on a laser confocal microscope (LSM 700, Karl Zeiss): 1µm slices of 30-50 µm thick stacks were acquired and projected in one picture.

Supplementary Figure 2:

Representative immunofluorescence stainings of lesional psoriasis skin (n=8) and mastocytosis skin (n=4) showed variable DPP4/CD26 in the skin. Bar = 50 µm;

Supplementary Figure 3:

Immunofluorescence stainings of normal skin and lesional psoriasis skin showed deposits of DPP4/CD26 in areas without nuclei probably associated with structural components (yellow circles). Representative experiments are depicted. Bar = 50 µm.

Supplementary Figure 4:

(a) Mast cells in the bone marrow of patients with mastocytosis or mast cell leukemia were gated as depicted in the dot blots. (b-c) The histograms show representative stainings of two patients with aggressive systemic mastocytosis; one donor (b) with DPP4/CD26-negative mast cells and one donor (c) with DPP4 positive mast cells. Black-lined non-filled histogram: isotype control; red-lined filled histogram: DPP4/CD26 staining.

Supplementary Figure 5:

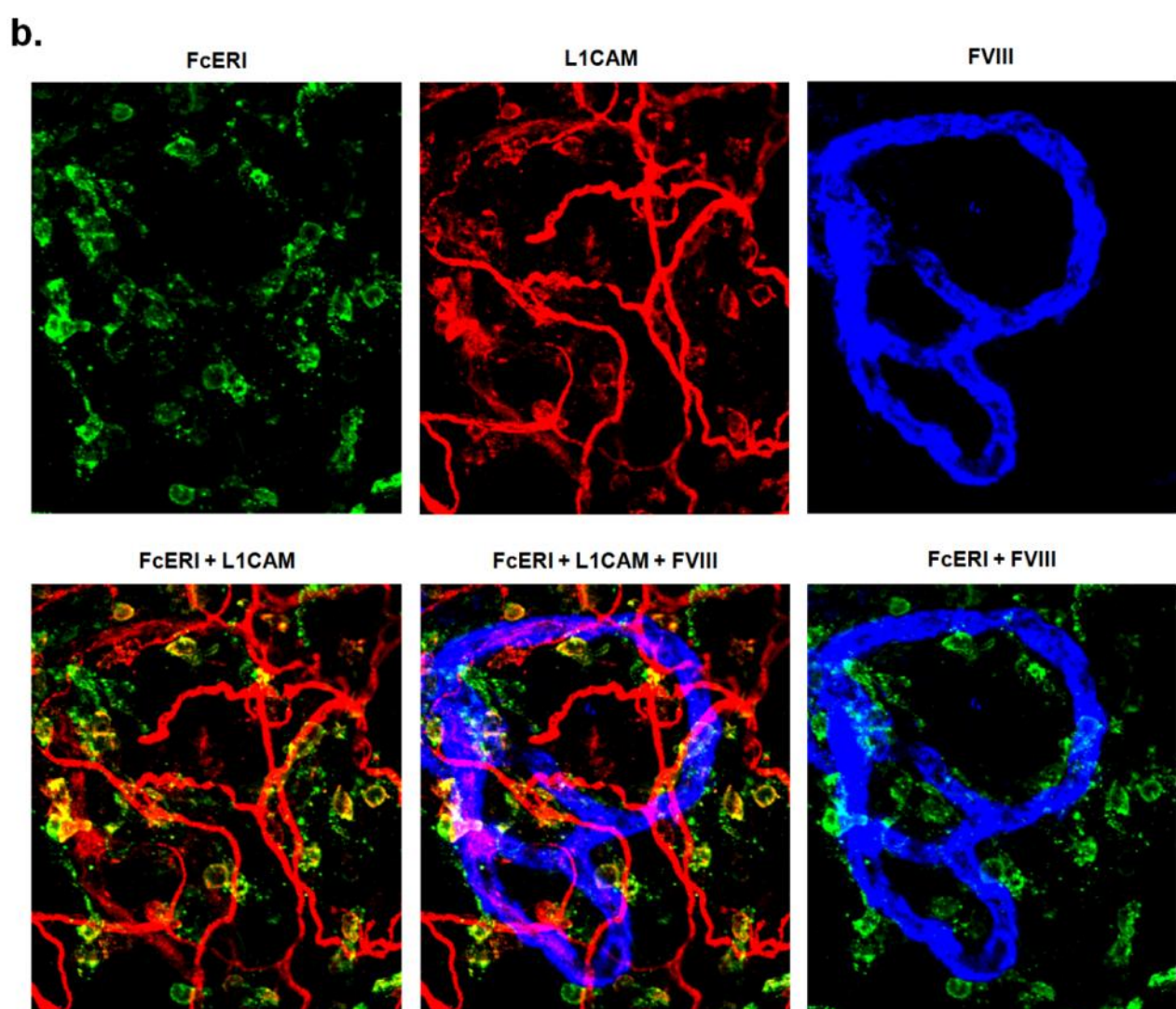
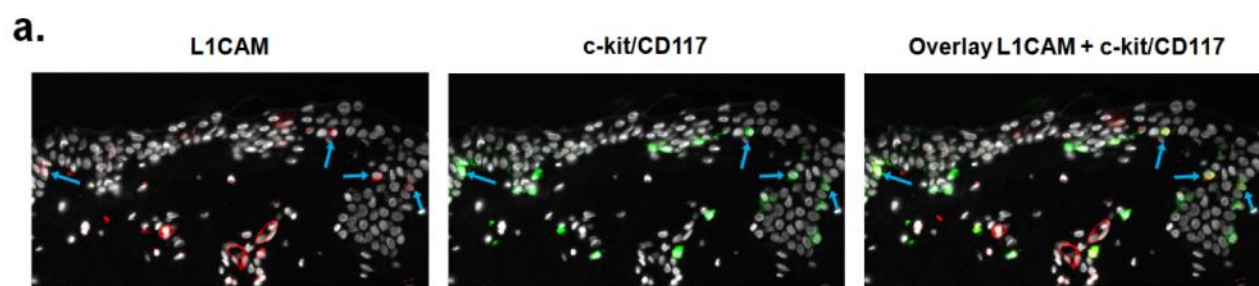
(a) The characteristics of the patients included in the study of DPP4/CD26 in mastocytosis and mast cell leukemia derived mast cells are summarized. (b) DPP4/CD26 expression levels on mast cells did not correlate with age, tryptase level and white blood cell count; correlation coefficients calculated with Pearson correlation test. (c) No significant differences in DPP4/CD26 expression levels on mast cells were observed when grouping patients according to the presence or absence of mediator-related symptoms, urticaria-pigmentosa (UP)-like skin lesions, *KIT* mutation D816V or according to gender as calculated by Mann-Whitney test.

Supplementary Figure 6:

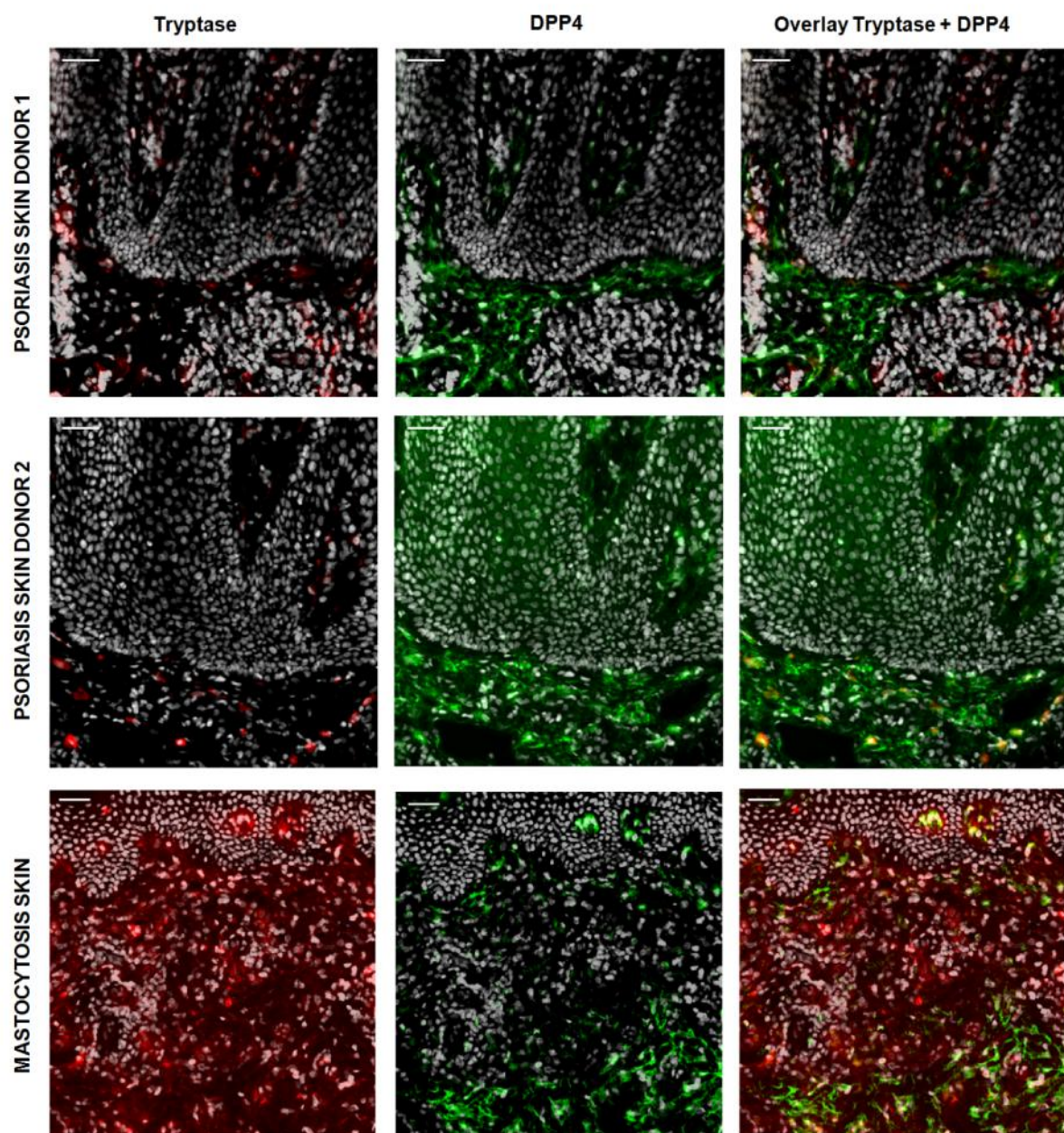
DPP4/CD26 expression as evaluated by flow cytometry (n=2) and (b) DPP4/CD26 activity (n=4 for supernatants; n=2 for cell lysate) was not changed in skin mast cells upon activation with compound 48/80 (50 μ M) or IgE and anti-IgE (each 10 μ g/ml); CD203c as positive control was induced upon stimulation (a).

Supplementary file 1:

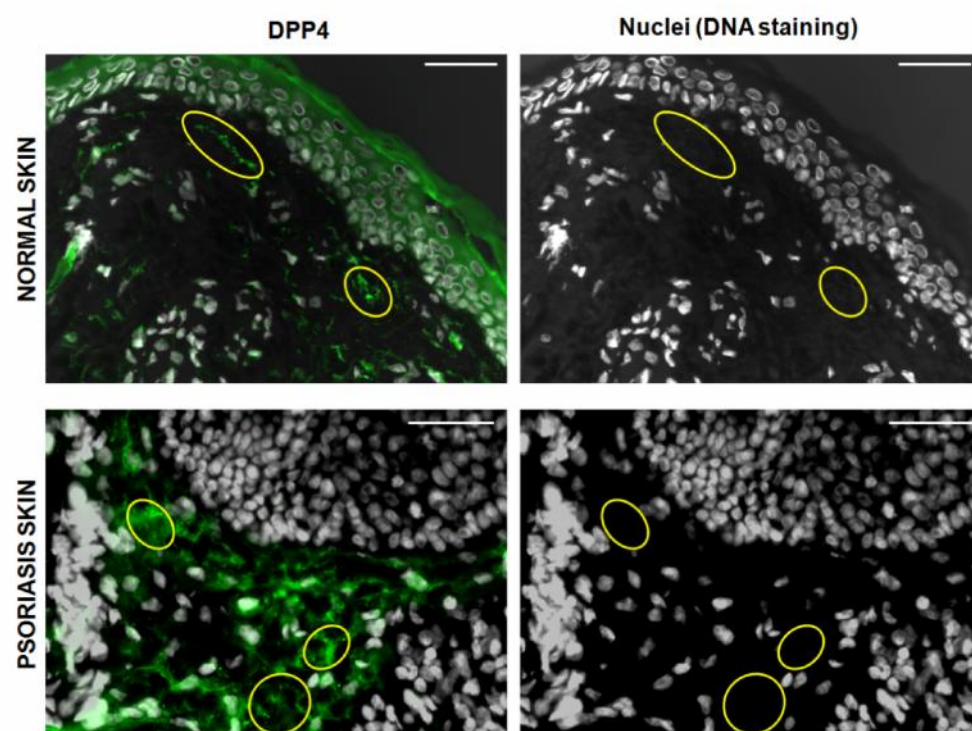
Excel list including Uniprot accession number, name, coverage, score and peptide count of the proteins identified in primary human skin mast cells, primary human dermal fibroblasts, human peripheral blood mononuclear cells and human umbilical vein endothelial cells.



Supplementary Figure 1

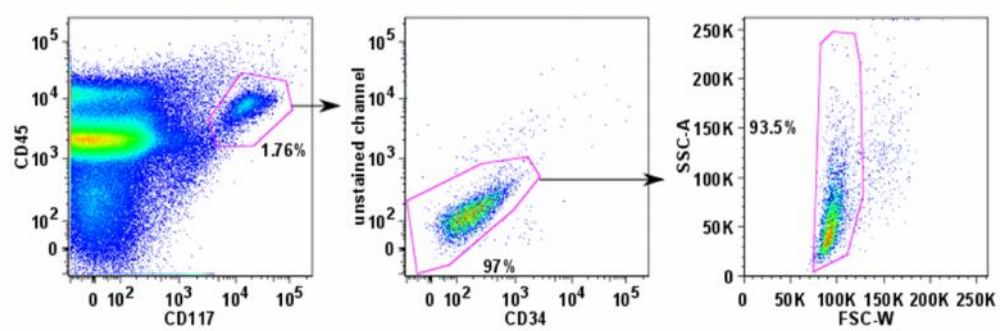


Supplementary Figure 2

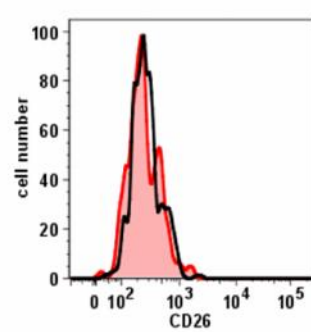


Supplementary Figure 3

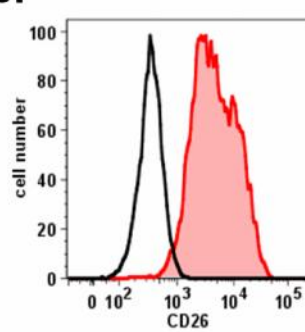
a.



b.



c.

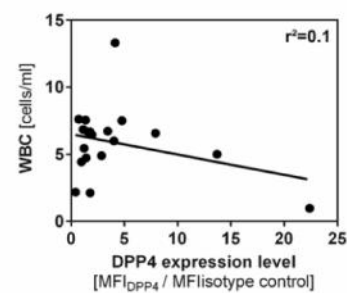
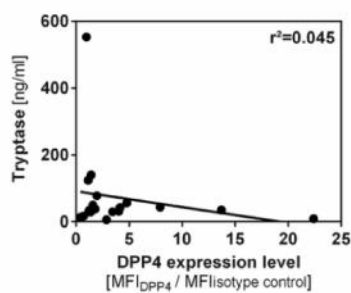
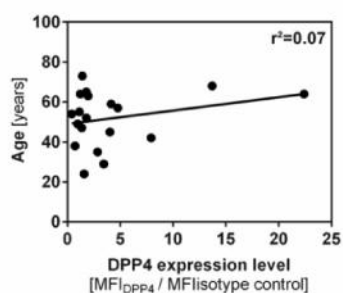


Supplementary Figure 4

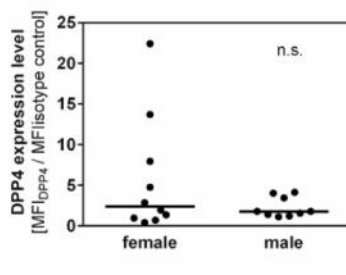
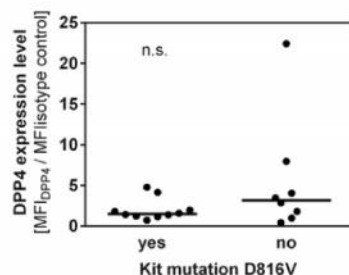
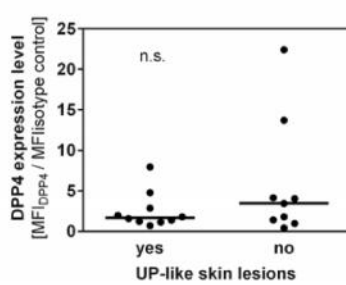
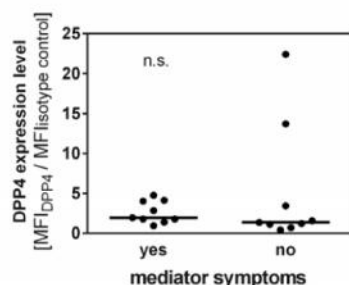
a.

Diagnosis (WHO)	Age	Sex	c-kit mutation D 816 V	serum tryptase [unit]	WBC [G/L]	Hb [g/dL]	PLT [g/L]	LDH [U/L]	Calcium [mmol/L]	spleen (palp)	hepato-megaly	LAP (palp or >2cm)	UP	Mediator symptoms	CD26 [corr. MFI]
MIS	35	f	no	5.8	4.89	11.8	243	136	2.31	no	no	no	yes	yes	2.85
MIS	57	f	yes	56	7.5	14.3	201	155	2.33	yes	no	no	yes	yes	4.77
ISM	45	m	no	31.1	6	16	202	162	2.21	no	no	no	no	yes	4.02
ISM	63	f	yes	77.7	6.46	12.7	288	169	2.4	no	no	no	yes	yes	1.95
ISM	64	m	yes	33.5	5.45	13.9	151	180	2.33	no	no	no	yes	no	1.22
ISM	59	m	yes	42	13.31	15.7	211	195	2.37	no	no	no	no	yes	4.13
ISM	68	f	n.d.	35	5	14.1	267	272	2.5	no	no	no	no	no	13.7
ISM	55	m	yes	124	6.84	13.8	223	117	2.42	no	no	no	yes	no	1.13
ISM	47	f	yes	28.8	7.55	14.6	286	149	2.34	no	no	no	yes	no	1.36
ISM	42	f	no	43.5	6.57	13	228	121	2.31	no	no	no	yes		7.93
ISM	29	m	no	29.7	6.72	14.4	340	171	2.54	no	no	no	no	no	3.44
ISM	24	m	yes	50.2	6.68	15.8	230	118	2.46	no	no	no	yes	no	1.57
ISM	38	f	yes	16.8	7.62	14	200	145	2.33	no	no	no	yes	no	0.71
ISM	52	m	yes	38.2	6.67	14.6	285	157	2.35	no	no	yes	yes	yes	1.79
ASM	73	m	yes	140	4.73	9.1	103	206	2.06	yes	no	yes	no	yes	1.41
MCL	54	f	no	13.4	2.17	10.6	32	173	2.51	no	no	no	no	no	0.41
MCL	49	f	no	553	4.43	10.6	190	105	2.17	no	no	no	no	yes	0.96
SM-AHNMD	64	f	no	8.9	0.98	11	48	112	2.29	no	no	no	no	no	22.4
SM-AHNMD	65	m	no	37.8	2.12	7.5	18	141	2.21	no	no	no	no	yes	1.79

b.

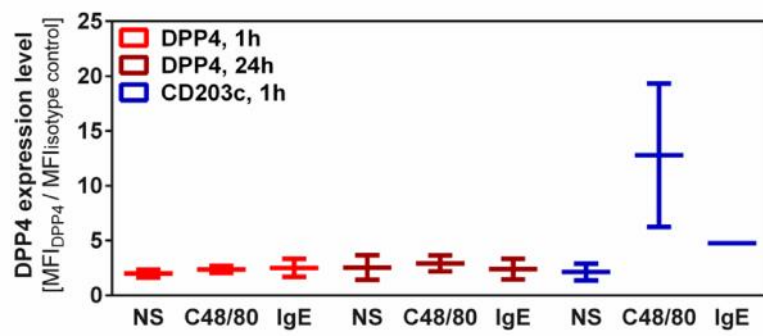


c.

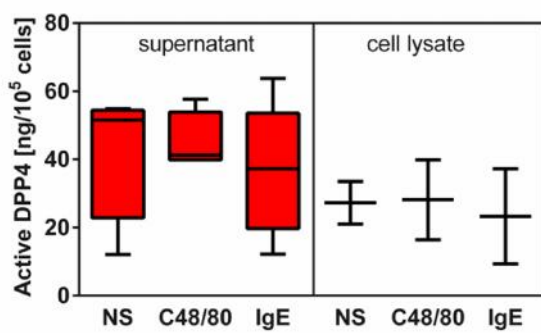


Supplementary Figure 5

a.



b.



Supplementary Figure 6