SUPPLEMENTARY MATERIALS

Supplementary Figures

Fig. S1. SLP76 is lost in mast cell lysates prepared with a panel of mild non-denaturing detergents. BMMC were solubilized in lysis buffers containing laurylmaltoside (LM), octylglycoside (OG), IGEPAL, Brij35 or TX100, supplemented with standard protease inhibitor cocktail (Roche). Equal amounts of cell lysates were electrophoresed and Western blotted with anti-SLP76, anti-LAT1, anti-PAG or anti-actin antibodies.

Fig. S2. Phenanthroline protects SLP76 from BMMC proteases in LM lysis buffer pH 8.0. (A, B) BMMC were solubilized in LM lysis buffer pH 7.4 (A) or pH 8.0 (B) supplemented with specific metalloprotease inhibitors or in SDS lysis buffer. Equal amounts of cell lysates were electrophoresed and Western blotted with anti-SLP76 antibodies. (C) BMMC were solubilized in LM lysis buffer pH 7.4 or pH 8.0 supplemented with Roche cocktail inhibitors or not. Equal amounts of cell lysates were electrophoresed and Western blotted with anti-SLP76 antibodies.

Supplementary Tables

Table S1: Genes expressed in BMMC. Known BMMC-specific genes are shown, along with their detection confidence level.

Table S2: Proteases expressed in BMMC. All the proteases retrieved on the AB1700 DNA-microarray platform are shown, along with their associated detection p-value. Protease families are also specified.
Table S3: Metal-binding proteases expressed in BMMC. Zn2+-dependent proteases are highlighted.

Table S4: Mast cells protease inhibitors cocktail.

Table S5: SLP76 interactome in resting and activated BMMC. Tables 1 and 2 have been completed with p-value of student’s t-tests and iBAQ/MS intensity from all samples.

Table S6: AP-MS data from some known SLP76 partners in T-cells. Student’s t-tests results (p-value and ratio) are gathered with raw iBAQ data.

Table S7: Proteins identification and quantification data. Number of total peptides, unique peptides, sequence coverage, MS intensity and iBAQ value in each sample are provided for each protein identified in the whole dataset.

Table S8: Peptides identification and quantification data. Sequence, best score, uniqueness of all peptides identified are provided with MS intensity in each sample.

Supplementary Materials and Methods:

DNA-microarray data analysis: The transcriptome of three biological replicates of resting BMMC cells were analyzed using AB1700 DNA-microarrays. An expression confidence level was computed for the genes covered by the platform, using the following fields from the hybridization output files (raw data):

- The S/N field is a computed test statistics of each probe's estimated signal-to-noise ratio. Thus, a detection confidence level can be computed according to a convenient probability distribution. As suggested by the manufacturer (cf. AB1700 platform
documentation downloaded by http://www.appliedbiosystems.com), we derived p-values directly from the Standard Normal probability distribution.

- The **Flag field** indicates, for each probe, the possible occurrence of quality issues. Values lower than or equal to 100 correspond to good quality probes. Before applying the statistical test, we set to zero all the S/N values corresponding to associated Flag greater than 100. In this way, all the poor quality measurements obtained a weak detection confidence level (i.e. p-value = 0.5).

Finally, in order to avoid multiple gene values (as we have three sample replicates, and as several probes may correspond to the same gene for a given replicate), we only kept the highest p-value obtained for each gene across all the corresponding probes in the dataset. We considered the genes showing a p-value lower than an arbitrarily chosen threshold (i.e. p-value < 0.1) as *expressed*. We tested this procedure by verifying the computed detection confidence level of known specific BMMC genes (Table S2). All these genes were declared expressed by our test.

Then, we estimated the number of protease-coding genes expressed in BMMC. From the MEROPS database (http://merops.sanger.ac.uk), we downloaded a list of 685 reported *Mus musculus* proteases genes annotated with their main family names (Serine, Metallo, Aspartic and Cysteine proteases). 539 out of the 685 annotated protease-coding genes were found to be represented in the DNA-microarray platform. Among them, 400 genes were declared expressed by the statistical test with a confidence level greater than 90% (Figure 3A). Table S2 reports the results for all the 539 protease-coding genes.

All data analyses were carried out using R programming language (http://www.r-project.org).