Supplementary Figure 1  Intensity dependent quantification variation and statistically defined substrate ratio cut-off. (A) MA-plot for reporter ion intensity ratios from an iTRAQ-TAILS experiment of secretomes from Mmp2-/- fibroblasts mixed at a 1:1 ratio without protease incubation. Black circles represent A-value dependent standard deviations calculated by averaging of 100 random sample means (n = 19) and a sliding window with size 1.5 and increment 0.1. Error bars are standard deviations of the mean of the 100 random sample means. Dashed line represents a curve fitted to those values by non-linear curve fitting. This curve was used to calculate weighting factors for averaging of ratios for peptides identified by multiple spectra. (B) Distribution of abundance ratios (GluC/control) of spectra assigned to GluC generated neo N-termini (n = 157). Solid line represents the probability density with a maximum at $2^{5.11} \approx 35$. The mean is an estimate for the dynamic range of iTRAQ quantification. (C) ROC curve analysis of substrate classifier performance. A peptide abundance ratio (GluC/control) of 10 for cleavage events provides maximum sensitivity (93%) at minimum false discovery rate (15%). Dashed line represents the same analysis for a classifier calculated from weighted averaged ratios if peptides were identified by multiple spectra.
Supplementary Figure 2  *Mmp2/-* fibroblast secretomes are MMP-2/MMP-9 naïve proteomes. *Mmp2/-* fibroblasts secretomes were incubated for 18 h at 37 °C with APMA-activated MMP-2, MMP-9, 100 μM APMA, or buffer alone, as described in Experimental Procedures. The gelatinolytic activity in secretome samples, active MMP-2 and MMP-9, pro-MMP-2 and pro-MMP-9, or secretomes without any treatment was monitored by gelatin zymography (9% SDS-PAGE gel with 1 mg/ml gelatin). Arrows indicate pro and active forms of MMP-2 and MMP-9.* Indicate autolytic products of MMP-2 and MMP-9. MMP-2 loses its hemopexin C domain due to autocatalysis (Δ hemopexin domain MMP-2). Panels to the left and right show lanes from silver-stained SDS-PAGE gels of the proMMP-2 and the proMMP-9 preparations respectively prior to APMA activation demonstrating the purity of each enzyme.
Supplementary Figure 3  Distribution of reporter ion intensity ratios for spectra assigned to original protein N-termini and derivation of quantification normalization factors. (A) Spectra (n = 388) assigned to original protein N-termini were extracted from a list of peptides with iProphet probability >0.95 that was compiled from Mascot and X! Tandem results on replicate 1 of the 4-plex-iTRAQ-TAILS experiment. Probability density functions (PDF) for all three ratios (MMP2(115)/ctrl(114), MMP9(116)/ctrl(114), APMA(117)/ctrl(114)) are plotted and maxima indicated by dashed lines. To correct for outliers data below the 10 and above the 90 percentile were removed, and normalization factors were calculated from the deviation of PDF-maxima from the expected ratio of 1.0. (B) Same analysis as in (A) but for data derived from replicate 2 (n = 362). The mean maximum of the bimodal distribution for MMP9(116)/ctrl(114) was determined by adjusting a normal distribution (dashed red line). Normalization factors were applied to ratios from individual spectra prior to intensity weighted averaging for peptides with multiple identifications.
Supplementary Figure 4  Thrombospondin 2 is processed by MMP-2 and MMP-9. MMP-2 (A) or MMP-9 (B) (0, 0.2, 0.4, 2.0, 4.0, or 10 pmoles) were incubated with thrombospondin 2 (TSP2, 20 pmoles). Products (indicated by arrows) were analyzed by: 10% SDS-PAGE with silver staining (top panel), or Western blotting: 9% SDS-PAGE α-N-terminal domain antibody (middle panel); 7.5% SDS-PAGE α-C-terminal domain antibody (lower panel). (C) Processing of TSP2 by MMP-2 could be prevented by including the MMP inhibitor BB94 in the in vitro incubation (this also prevents autoproteolysis of MMP-2). Bands resulting from MMPs are represented by arrows with asterisks.
**Supplementary Figure 5** MMP-9 processes cystatin C.

Cystatin C (75 pmoles) was incubated alone, or with MMP-9 (7.5 pmoles) for 18 h at 37 °C. The samples were mixed with sinapic acid on a MALDI plate and subjected to MALDI-TOF-MS (Voyager). The predicted m/z for cystatin C is between 13,300 and 13,400. Cystatin C alone (upper panel) gave peaks at m/z of 13,352 and 12,564. After incubation of MMP-9, the 13,352 m/z peak was no longer present suggesting that cystatin C had been cleaved by MMP-9. The peak at 12,564 remained and may represent a contaminant in the preparation.