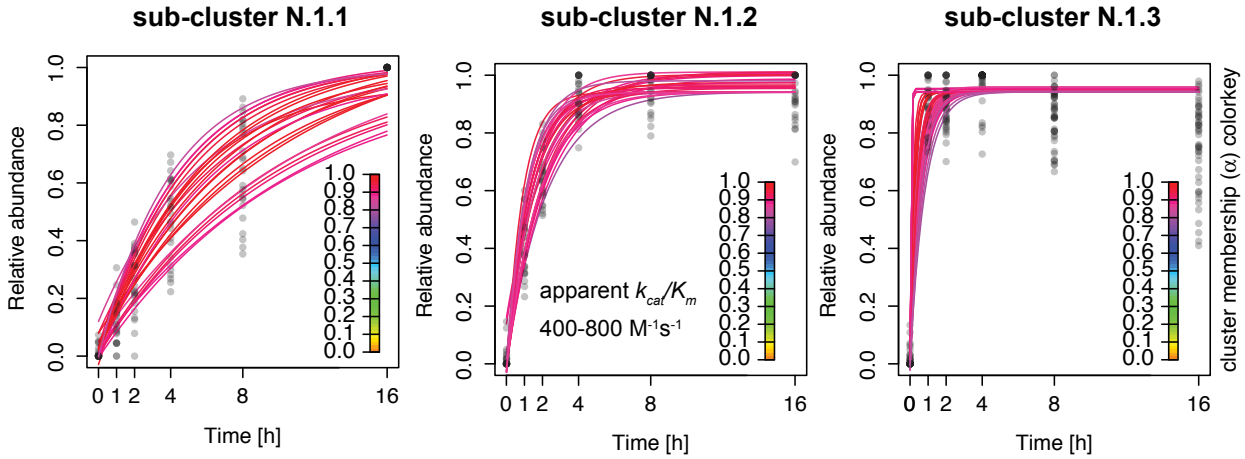

Figure S1 Determination of catalytic efficiencies for GluC cleavages monitored by 8plex-iTRAQ-TAILS. Data from sub-clusters in Figure 2A were fitted to the well-established pseudo-first-order kinetic equation $A/A_o = 1 - e^{-(k_{cat}/K_m * E_0 * t)}$ with A/A_0: relative abundance and E_0: enzyme concentration. Cleavages in sub-cluster N.1.2 were considered in a measurable range, and apparent k_{cat}/K_m values were determined using the equation $k_{cat}/K_m = \ln 2/E_0 * t_{1/2}$, where $t_{1/2}$ is the time point at which the relative abundance of a neo-N-terminal peptide reaches half of the maximum abundance.

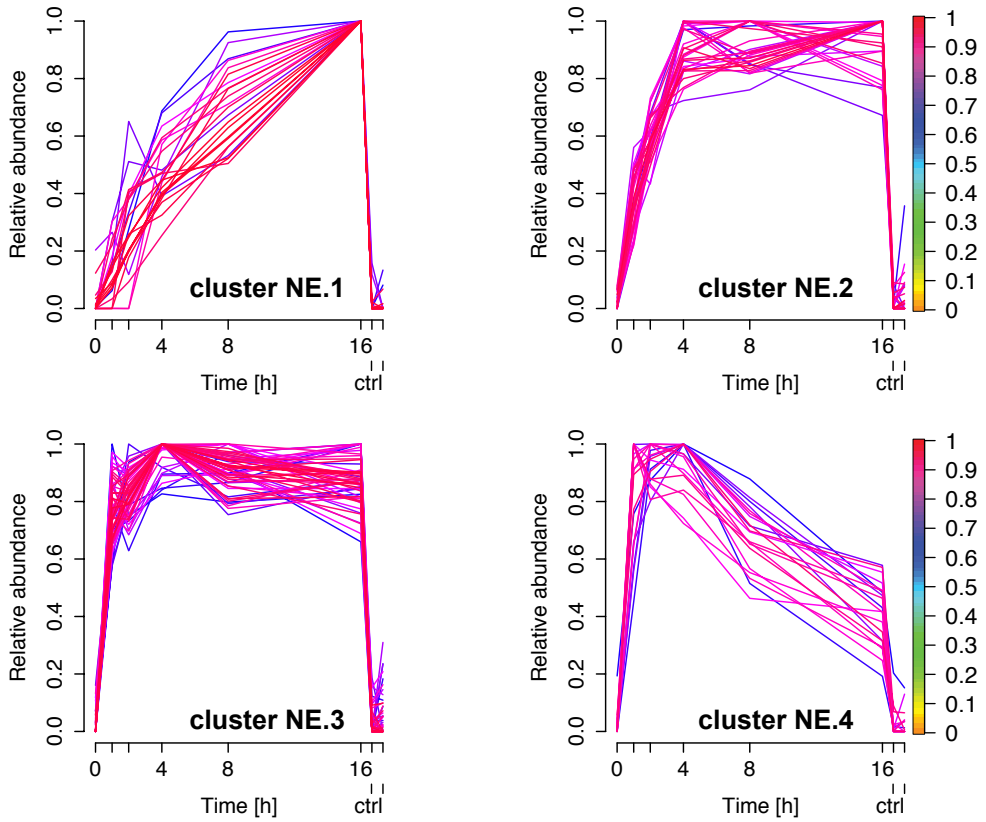

Figure S2 Fuzzy c means clustering of GluC-generated neo-N termini harboring a single E or D in their sequence. Peptides in clusters NE.1 to NE.3 are either not or to a low extend affected by secondary cleavage, while neo-N termini in cluster NE.4 decrease in abundance upon rapid increase at 2 to 4 hours of incubation. Colorkey indicates membership value α. For sub-dataset see Supplementary Table S6. ctrl:12 h and 16 h controls.

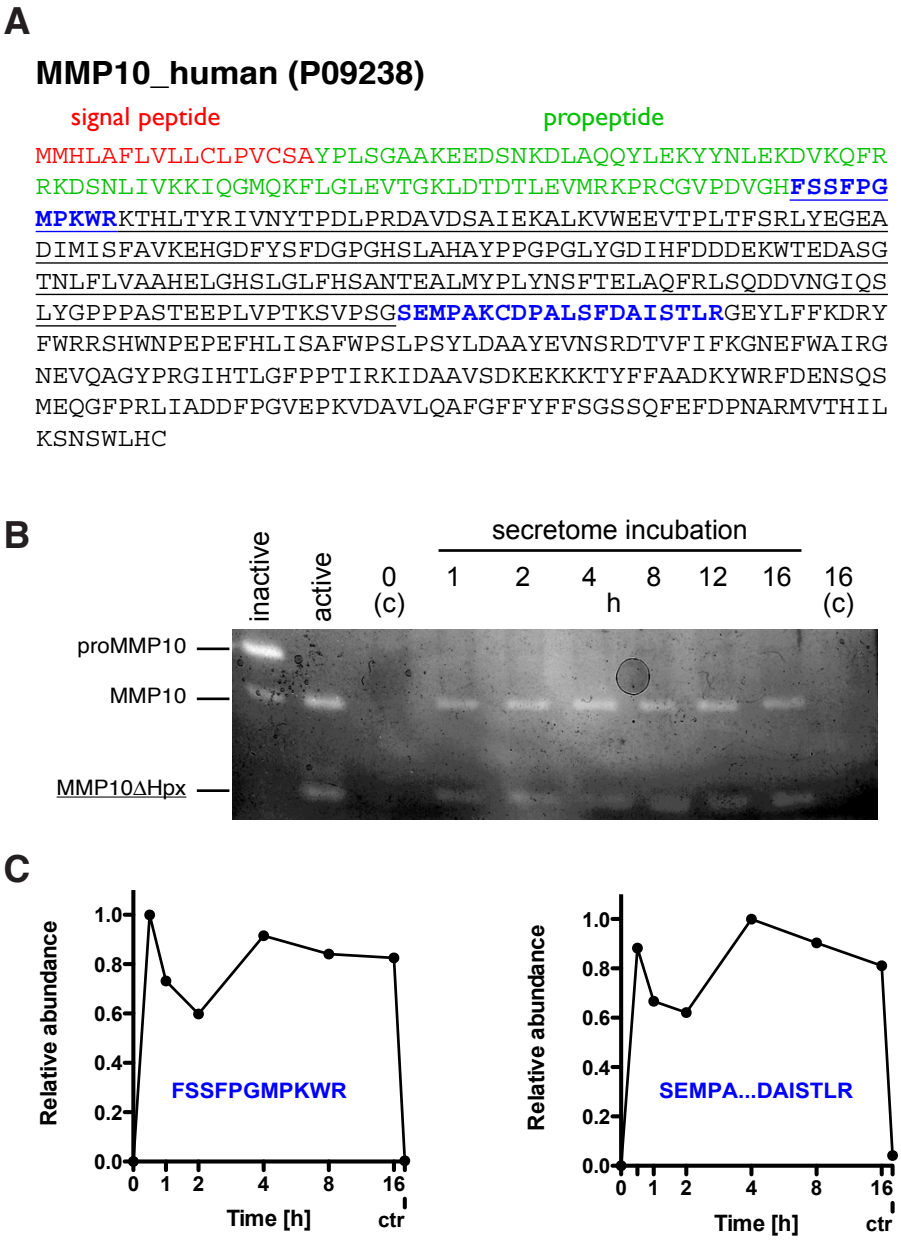

Figure S3 Activation and stability of recombinant human MMP10. (**A**) Amino acid sequence of human proMMP10. N-terminal peptides identified by 8plex-iTRAQ-TAILS are indicated in blue. The sequence of an additional autolytic fragment (MMP10ΔHpx) generated upon activation is underlined. Identified neo-N termini resulting from removal of the propeptide and partial autolysis perfectly match with a previous report from Nakamura et al. (1). (**B**) Casein zymography of recombinant human MMP10 before and after auto-activation and samples used for 8plex-iTRAQ-TAILS analysis. Bands correspond to molecular weights of proMMP10, MMP10 and MMP10ΔHpx. Both fragments generated upon auto-activation are stable over the entire time of incubation. (**C**) Kinetic abundance profiles of MMP10 N termini identified by 8plex-iTRAQ-TAILS. Time-dependent differences in abundance for both peptides are within the range of non-cleavage events (Figure 4C) validating the results from zymography. ctrl: 16 h control.

1. Nakamura, H., Fujii, Y., Ohuchi, E., Yamamoto, E., and Okada, Y. (1998) Activation of the precursor of human stromelysin 2 and its interactions with other matrix metalloproteinases. *Eur J Biochem* 253, 67-75

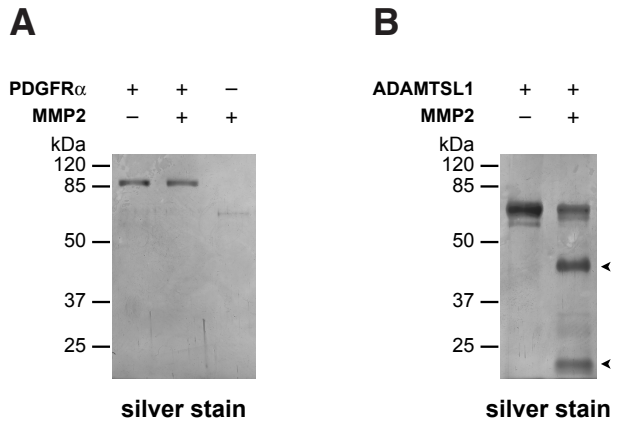

Figure S4 Processing of PDGFRα and ADAMTSL1 by MMP2. (**A**) Recombinant human PDGFRα (residues 24-524) was incubated with active human MMP2 at 37°C for 16 hours. Proteins were analyzed by SDS-PAGE and silver staining. Lack of processing by MMP2 indicates cleavage by a third activated protease other than MMP10 and MMP2 in MMP10-treated fibroblast supernatants. (**B**) Recombinant human ADAMTSL1 (isoform 1; residues 1-525) was incubated with active human MMP2, and cleavage was monitored by SDS-PAGE and silver staining. Arrows indicate cleavage fragments with equal molecular weights as generated by incubation with MMP10 (Figure 6B), suggesting concomitant activity of both proteases towards ADAMTSL1.