Supplemental Information

Supplemental Figures

Figure S1 (related to Figure 1)

LCMS of biotin-LVSR-AOMK

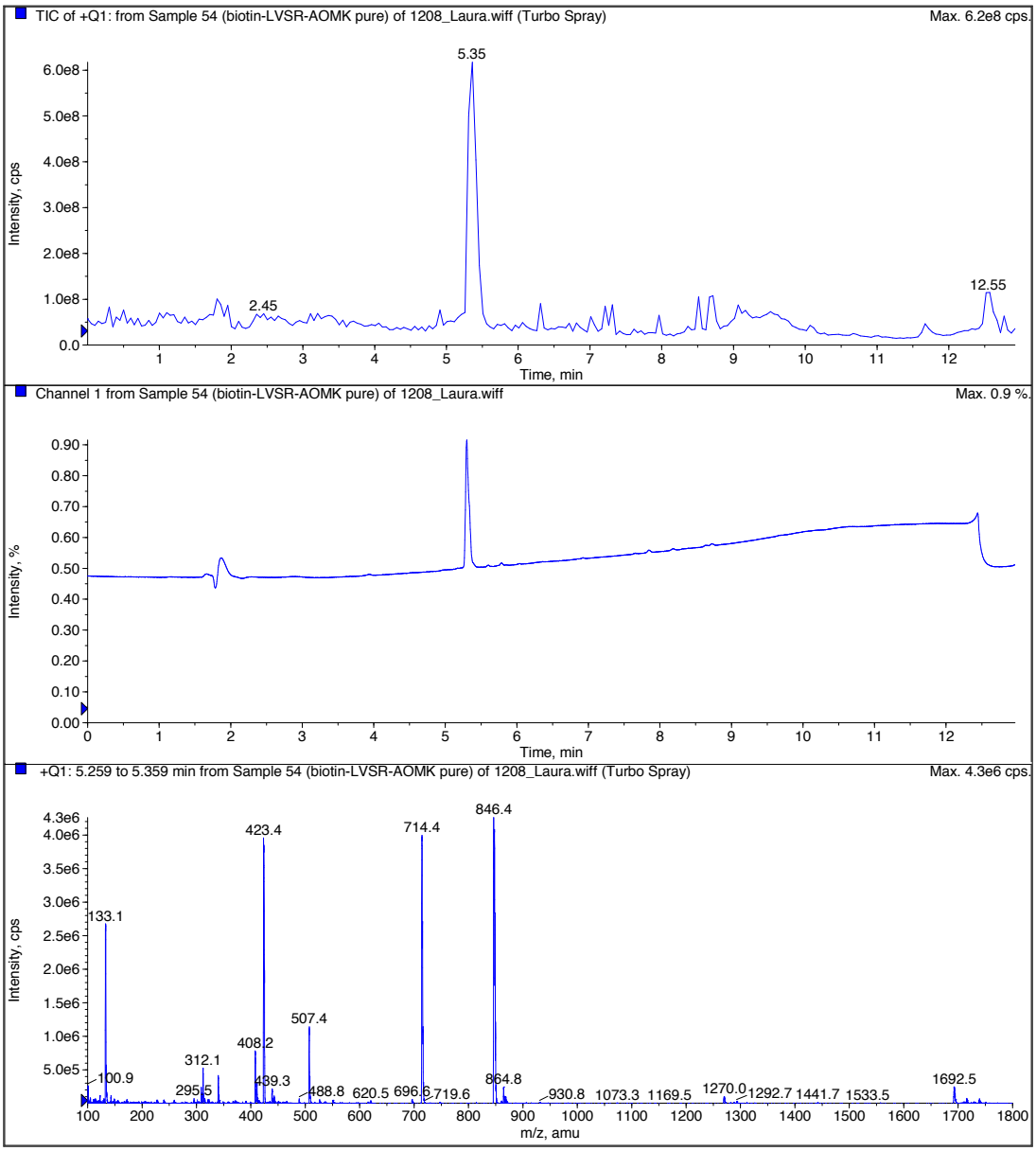


Figure S2 (related to Figure 1)

LCMS of Cy5-LVSR-AOMK

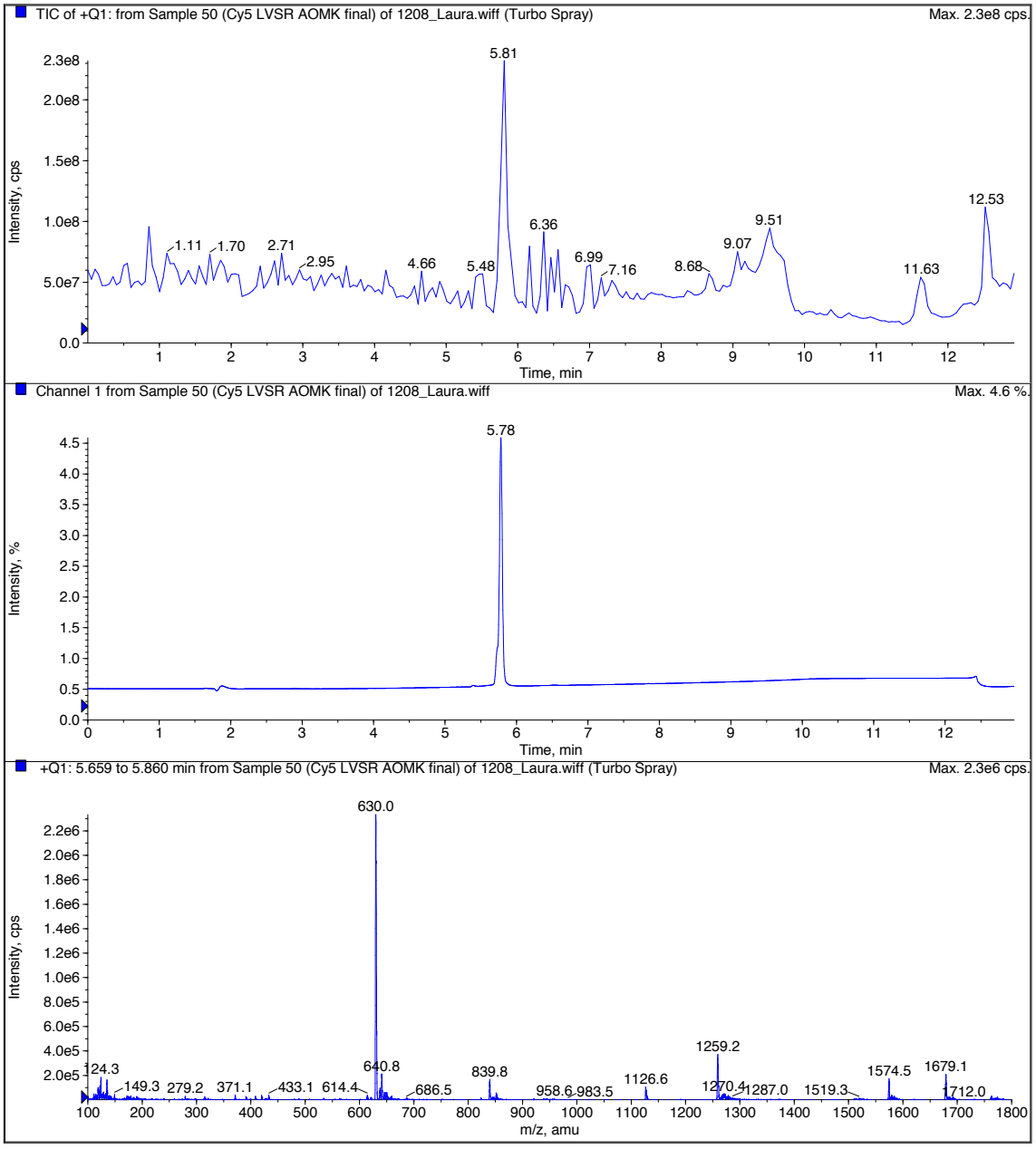
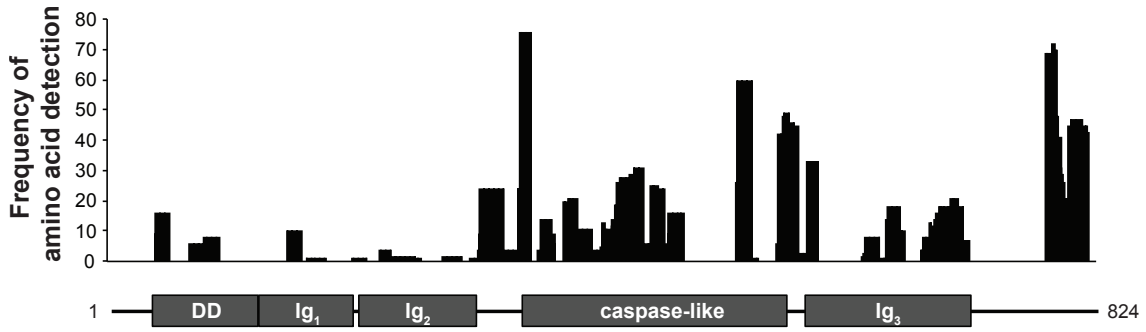
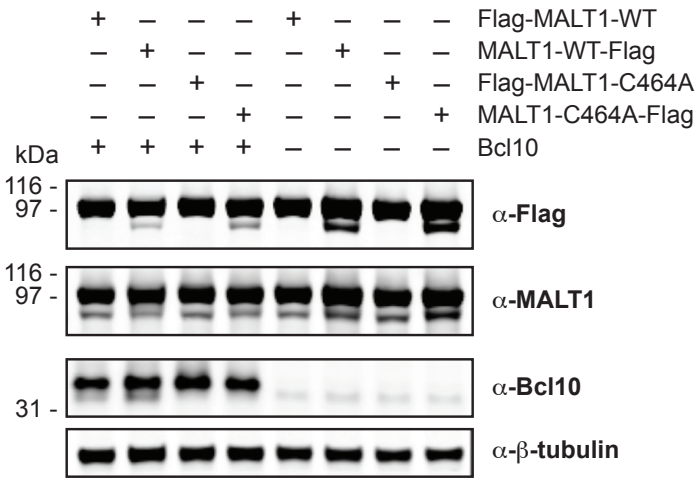


Figure S3 (related to Figure 2)



The 60 kDa species contains mostly the C-terminal fragment of MALT1 including the caspase-like domain and the Ig_3 domain. After mass spectrometry analysis, the frequency with which the different peptides were detected was determined and the amino acids covered by these peptides were calculated. The frequency with which each amino acid was observed in the different peptides was graphed versus the position in full-length MALT1 to determine which part of the protein made up the 60 kDa species. For example, amino acid 41 was discovered in 2 different peptides (encompassing either amino acids 40-52 or 41-52) with a total occurrence of 16. The frequency for this amino acid was thus graphed as 16. DD, death domain; Ig, immunoglobulin-like domain.

Figure S4 (related to Figure 3)



MALT1 gets cleaved upon overexpression. MALT1-WT or C464A containing an N- or C-terminal Flag-tag, respectively, were overexpressed in the presence or absence of Bcl10 in HEK-293A cells. Cell lysis was followed by SDS-PAGE and Western blot analysis with the indicated antibodies. Note that when Bcl10 is expressed in combination with MALT1-WT (lanes 1 and 2) a smaller Bcl10 cleavage product below the main species is visible. The size difference between overexpressed (lanes 1-4) and endogenous (lanes 5-8) Bcl10 is explained by the Flag-tag on the Bcl10 construct.

Supplemental Experimental Procedures

Synthesis of Cy5-LVSR-AOMK and biotin-LVSR-AOMK

All reagents were purchased from commercial suppliers and used without further purification. All solvents were HPLC grade. Reactions were analyzed by LC-MS using an API 150EX single-quadropole mass spectrometer (Applied Biosystems). Reverse HPLC was conducted with an AKTA explorer 100 (Amersham Pharmacia Biotech) using a C18 column.

The method for the synthesis of Cy5-LVSR-AOMK and biotin-LVSR-AOMK was adapted from a previously described literature procedure (Edgington et al., 2012; Edgington et al., 2013). Boc-Leu-Val-Ser(OtBu)-Arg(Pbf)-OH was prepared using standard solid phase peptide synthesis on 2-chlorotrityl resin. This acid was converted to a chloromethyl ketone (CMK) using the previously described method. Isobutylchloroformate (25 µL, 0.19 mmol, 1.1 equiv.) was added dropwise to a solution of Boc-Leu-Val-Ser(OtBu)-Arg(Pbf)-OH (150 mg, 0.17 mmol) and N-methylmorpholine (23 µL, 0.21 mmol, 1.2 equiv.) in dry THF (1 mL) at -78°C, and the reaction mixture was stirred for 1 h under argon atmosphere. Diazomethane was prepared from diazald (0.21 g, 0.93 mmol, 5.4 equiv.) and added dropwise to the reaction mixture at 0°C. After 30 min, the reaction was allowed to warm to room temperature, and the reaction mixture was stirred for 3 h. A 1:1 solution of hydrochloric acid and acetic acid (500 µL) was then added dropwise while stirring at 0°C. The reaction mixture was diluted with EtOAc, and the organic phase was washed with water, saturated $NaHCO_3$, and brine. The organic layer was dried with $MgSO_4$, filtered, and concentrated using a rotary evaporator, followed by HPLC purification. The resulting CMK was a white powder (10.1 mg, 0.011 mmol, 6.7%). Boc-Leu-Val-Ser(OtBu)-Arg(Pbf)-CMK (10.1 mg, 0.011 mmol) was then dissolved in 1 mL dry DMF and 2,6-dimethylbenzoic acid (2.5 mg, 0.017 mmol, 1.5

equiv.) was added along with KF (12.7 mg, 0.22 mmol, 20 equiv.). The reaction mixture was stirred overnight under argon atmosphere at room temperature. The DMF was removed *in vacuo* and 25% TFA in DCM was added for 45 min to remove the protecting groups followed by concentration of the reaction mixture under reduced pressure. The deprotected intermediate was then purified by HPLC to yield a white powder (2.7 mg, 0.004 mmol, 39%), which was divided into two aliquots. To one aliquot, Cy5-OSu was coupled by published methods (Edgington et al., 2012; Edgington et al., 2013) followed by HPLC purification to yield the compound Cy5-LVSR-AOMK (LE40) (1.7 mg, 0.001 mmol, 63%), a blue powder. To the other aliquot, Biotin-OSu was added according to the same method and purified by HPLC to yield the compound biotin-LVSR-AOMK (LE42) (1 mg, 0.001 mmol, 54%), a white powder.

Mass spectrometry analysis

Mass spectrometry analysis of proteins extracted from SDS-PAGE gels has been described elsewhere (Sacchetti et al., 2013). The analysis was performed by the proteomics core facility at Sanford-Burnham Medical Research Institute. Briefly, the coomassie-stained protein bands were excised from the gel and subjected to in-gel trypsin digestion using mass spectrometry grade trypsin (Promega). The digested samples were analyzed by 1D-LC/MS/MS using an LTQ linear ion trap mass spectrometer (Thermo Scientific). MS/MS spectra were searched against the Human IPI database using Sorcerer Enterprise with SEQUEST (SageN) software. The minimum trans-proteomic pipeline (TPP) probability score for proteins to 0.95 was set to assure a very low error rate (much less than FDR 1%) with reasonably good sensitivity.

Supplemental References

Edgington, L.E., van Raam, B.J., Verdoes, M., Wierschem, C., Salvesen, G.S., and Bogyo, M. (2012). An optimized activity-based probe for the study of caspase-6 activation. Chem Biol *19*, 340-352.

Edgington, L.E., Verdoes, M., Ortega, A., Withana, N.P., Lee, J., Syed, S., Bachmann, M.H., Blum, G., and Bogyo, M. (2013). Functional imaging of legumain in cancer using a new quenched activity-based probe. J Am Chem Soc *135*, 174-182.

Sacchetti, C., Motamedchaboki, K., Magrini, A., Palmieri, G., Mattei, M., Bernardini, S., Rosato, N., Bottini, N., and Bottini, M. (2013). Surface polyethylene glycol conformation influences the protein corona of polyethylene glycol-modified single-walled carbon nanotubes: potential implications on biological performance. ACS nano *7*, 1974-1989.