Cysteine cathepsins shed cell adhesion proteins and receptors from the surface of cancer cells

Authors: Barbara Sobotič[1,2], Matej Vizovišek[1,2], Robert Vidmar[1,2], Petra Van Damme[,3,4], Vasilena Gocheva[5], Johanna A. Joyce[5], Kris Gevaert[3,4], Vito Turk[1,2,6], Boris Turk[1,6,7,8*], Marko Fonović[1,6*]

Affiliations:

[1] Department of Biochemistry, Molecular and Structural Biology, Jozef Stefan Institute, Jamova cesta 39, SI-1000 Ljubljana, Slovenia

[2] International Postgraduate School Jozef Stefan, Jamova 39, SI-1000 Ljubljana, Slovenia

[3] Department of Biochemistry, Ghent University, B-9000 Ghent, Belgium

[4] Department of Medical Protein Research, VIB, B-9000 Ghent, Belgium

[5] Cancer Biology and Genetics Program, Memorial Sloan Kettering Cancer Center,

New York, NY 10065, USA

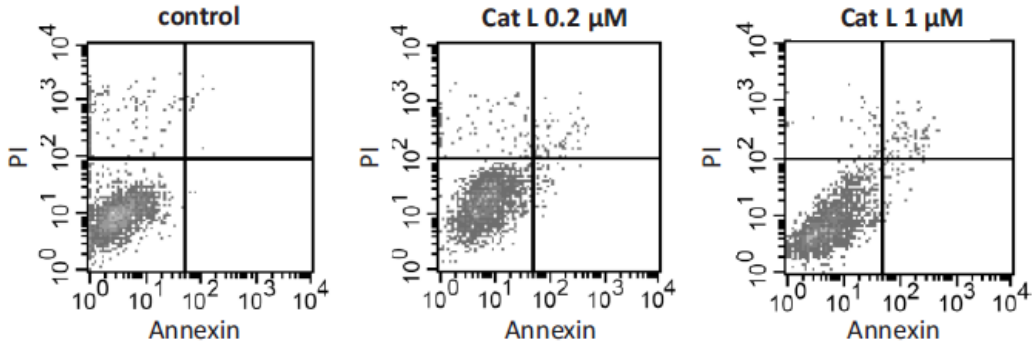
[6] Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins, Jamova cesta 39, SI-1000 Ljubljana, Slovenia

[7] Center of Excellence NIN, Ljubljana, Slovenia

[8] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

SUPPLEMENTARY FIGURES

Supplemental Figure S1



Live cells (PI and annexin negative)	95%	Live cells (PI and annexin negative)	94%	Live cells (PI and annexin negative)	95%

Supplemental Figure S1. Viability of MDA-MB-231 cancer cell line after cathepsin treatment.

Intact cells were treated for one hour with cathepsin L (1 µM, 0.2 µM) in PBS (pH 6.0, 0.5 mM DTT) or inhibited cathepsin L (with 20 µM E-64) as a negative control. Live cells after the treatment are shown as PI and Annexin negative cells. No significant difference in viability was observed between the control and the cathepsin treated cells.

Supplemental Figure S2

activated leukocyte cell adhesion molecule (ALCAM) ***(isoform 1)***

MESKGASSCRLLFCLLISATVFRPGLGWYTVNSAYGDTIIIPCRLDVPQNLMFGKWKYEKPDGSPVFIAF
RSSTKKSVQYDDVPEYKDRLNLSENYTLSISNARISDEKRFVCMLVTEDNVFEAPTIVKVFKQPSKPEIV
SKALFLETEQLKKLGDCISEDSYPDGNITWYRNGKVLHPLEGAVVIIFKKEMDPVTQLYTMTSTLEYKT
TKADIQMPFTCSVTYYGPSGQKTIHSEQAVFDIYYPTEQVTIQVLPPKNAIKEGDNITLKCLGNGNPPPEE
FLFYLPGQPEGIRSSNTYTLTDVRRNATGDYKCSLIDKKSMIASTAITVHYLDLSLNPSGEVTRQIGDALP
VSCTISASRNATVVWMKDNIRLRSSPSFSSLHYQDAGNYVCETALQEVEGLKKRESLTLIVEGKPQIKM
TKKTDPSGLSKTIICHVEGFPKPAIQWTITGSGSVINQTEESPYINGRYYSKIIISPEENVTLTCTAENQLER
TVNSLNVSAISIPEHDEADEISDENREKVNDQAKL***IVGIVVGLLLAALVAGVVYWL***YMKKSKTASKHVN
KDLGNMEENKKLEENNHKTEA

alkaline phosphodiesterase 1

MERDGCAGGGSRGGEGGRAPREGPAGNGRDRGRSHAAEAPGDPQAAASLLAPMDVGEEPLEKAARA
RTAKDPNTYK***VLSLVLSVCVLTTILGCIFGL***KPSCAKEVKSCKGRCFERTFGNCRCDAACVELGNCCLD
YQETCIEPEHIWTCNKFRCGEKRLTRSLCACSDDCKDKGDCCINYSSVCQGEKSWVEEPCESINEPQCPA
GFETPPTLLFSLDGFRAEYLHTWGGLLPVISKLKKCGTYTKNMRPVYPTKTFPNHYSIVTGLYPESHGIID
NKMYDPKMNASFSLKSKEKFNPEWYKGEPIWVTAKYQGLKSGTFFWPGSDVEINGIFPDIYKMYNGSV
PFEERILAVLQWLQLPKDERPHFYTLYLEEPDSSGHSYGPVSSEVIKALQRVDGMVGMLMDGLKELNL
HRCLNLILISDHGMEQGSCKKYIYLNKYLGDVKNIKVIYGPAARLRPSDVPDKYYSFNYEGIARNLSCRE
PNQHFKPYLKHFLPKRLHFAKSDRIEPLTFYLDPQWQLALNPSERKYCGSGFHGSDNVFSNMQALFVG
YGPGFKHGIEADTFENIEVYNLMCDLLNLTPAPNNGTHGSLNHLLKNPVYTPKHPKEVHPLVQCPFTRN
PRDNLGCSCNPSILPIEDFQTQFNLTVAEEKIIKHETLPYGRPRVLQKENTICLLSQHQFMSGYSQDILMPL
WTSYTVDRNDSFSTEDFSNCLYQDFRIPLSPVHKCSFYKNNTKVSYGFLSPPQLNKNSSGIYSEALLTTNI
VPMYQSFQVIWRYFHDTLLRKYAEERNGVNVVSGPVFDFDYDGRCDSLENLRQKRRVIRNQEILIPTHF
FIVLTSCKDTSQTPLHCENLDTLAFILPHRTDNSESCVHGKHDSSWVEELLMLHRARITDVEHITGLSFY
QQRKEPVSDILKLKTHLPTFSQED

CD44 antigen (CD44) ***(isoform 1)***

QIDLNITCRFAGVFHVEKNGRYSISRTEAADLCKAFNSTLPTMAQMEKALSIGFETCRYGFIEGHVVIPRI
HPNSICAANNTGVYILTSNTSQYDTYCFNASAPPEEDCTSVTDLPNAFDGPITITIVNRDGTRYVQKGEY
RTNPEDIYPSNPTDDDVSSGSSSERSSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTLMSTSATATET
ATKRQETWDWFSWLFLPSESKNHLHTTTQMAGTSSNTISAGWEPNEENEDERDRHLSFSGSGIDDDEDF
ISSTISTTPRAFDHTKQNQDWTQWNPSHSNPEVLLQTTTRMTDVDRNGTTAYEGNWNPEAHPPLIHHEH
HEEEETPHSTSTIQATPSSTTEETATQKEQWFGNRWHEGYRQTPKEDSHSTTGTAAASAHTSHPMQGRT
TPSPEDSSWTDFFNPISHPMGRGHQAGRRMDMDSSHSITLQPTANPNTGLVEDLDRTGPLSMTTQQSNS
QSFSTSHEGLEEDKDHPTTSTLTSSNRNDVTGGRRDPNHSEGSTTLLEGYTSHYPHTKESRTFIPVTSAKT
GSFGVTAVTVGDSNSNVNRSLSGDQDTFHPSGGSHTTHGSESDGHSHGSQEGGANTTSGPIRTPQIPE***W***
LIILASLLALALILAVCIAVNSRRRCGQKKKLVINSGNGAVEDRKPSGLNGEASKSQEMVHLVNKESSET
PDQFMTADETRNLQNVDMKIGV

cell surface glycoprotein MUC 18

VPGEAEQPAPELVEVEVGSTALLKCGLSQSQGNLSHVDWFSVHKEKRTLIFRVRQGQGQSEPGEYEQR
LSLQDRGATLALTQVTPQDERIFLCQGKRPRSQEYRIQLRVYKAPEEPNIQVNPLGIPVNSKEPEEVATC
VGRNGYPIPQVIWYKNGRPLKEEKNRVHIQSSQTVESSGLYTLQSILKAQLVKEDKDAQFYCELNYRLP
SGNHMKESREVTVPVFYPTEKVWLEVEPVGMLKEGDRVEIRCLADGNPPPHFSISKQNPSTREAEEETT
NDNGVLVLEPARKEHSGRYECQGLDLDTMISLLSEPQELLVNYVSDVRVSPAAPERQEGSSLTLTCEAE
SSQDLEFQWLREETGQVLERGPVLQLHDLKREAGGGYRCVASVPSIPGLNRTQLVNVAIFGPPWMAFK
ERKVWVVKENMVLNLSCEASGHPRPTISWNVNGTASEQDQDPQRVLSTLNVLVTPELLETGVECTASND
LGKNTSILFLELVNLTTLTPDSNTTTGLSTSTASPHTRANSTSTERKLPEPESRG***VVIVAVIVCILVLAVLG***
AVLYFLYKKGKLPCRRSGKQEITLPPSRKSELVVEVKSDKLPEEMGLLQGSSGDKRAPGDQGEKYIDLR
H

decay-accelerating factor CD55 ***(isoform 2)***

MTVARPSVPAALPLLGELPRLLLLVLLCLPAVWGDCGLPPDVPNAQPALEGRTSFPEDTVITYKCEESFV
KIPGEKDSVICLKGSQWSDIEEFCNRSCEVPTRLNSASLKQPYITQNYFPVGTVVEYECRPGYRREPSLSP
KLTCLQNLKWSTAVEFCKKKSCPNPGEIRNGQIDVPGGILFGATISFSCNTGYKLFGSTSSFCLISGSSVQ
WSDPLPECREIYCPAPPQIDNGIIQGERDHYGYRQSVTYACNKGFTMIGEHSIYCTVNNDEGEWSGPPPE
CRGKSLTSKVPPTVQKPTTVNVPTTEVSPTSQKTTTKTTTPNAQATRSTPVSRTTKHFHETTPNKGSGTT
SGTTRLLSGHTCFTLTGLLGTLVTMGLLT

epidermal growth factor receptor

LEEKKVCQGTSNKLTQLGTFEDHFLSLQRMFNNCEVVLGNLEITYVQRNYDLSFLKTIQEVAGYVLIAL
NTVERIPLENLQIIRGNMYYENSYALAVLSNYDANKTGLKELPMRNLQEILHGAVRFSNNPALCNVESI
QWRDIVSSDFLSNMSMDFQNHLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICAQQCSGRCRGKSPSD
CCHNQCAAGCTGPRESDCLVCRKFRDEATCKDTCPPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPR
NYVVTDHGSCVRACGADSYEMEEDGVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFKNCTSIS
GDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSL
AVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHA
LCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPD
NCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIP
S***IATGMVGALLLLLVVALGIGLFM***RRRHIVRKRTLRRLLQERELVEPLTPSGEAPNQALLRILKETEFKKI
KVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREATSPKANKEILDEAYVMASVDNPHVCRLLGICLTS
TVQLITQLMPFGCLLDYVREHKDNIGSQYLLNWCVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQHV
KITDFGLAKLLGAEEKEYHAEGGKVPIKWMALESILHRIYTHQSDVWSYGVTVWELMTFGSKPYDGIP
ASEISSILEKGERLPQPPICTIDVYMIMVKCWMIDADSRPKFRELIIEFSKMARDPQRYLVIQGDERMHLP
SPTDSNFYRALMDEEDMDDVVDADEYLIPQQGFFSSPSTSRTPLLSSLSATSNNSTVACIDRNGLQSCPIK
EDSFLQRYSSDPTGALTEDSIDDTFLPVPEYINQSVPKRPAGSVQNPVYHNQPLNPAPSRDPHYQDPHST
AVGNPEYLNTVQPTCVNSTFDSPAHWAQKGSHQISLDNPDYQQDFFPKEAKPNGIFKGSTAENAEYLR
VAPQSSEFIGA

ephrin type A receptor 2

QGKEVVLLDFAAAGGELGWLTHPYGKGWDLMQNIMNDMPIYMYSVCNVMSGDQDNWLRTNWVYR
GEAERIFIELKFTVRDCNSFPGGASSCKETFNLYYAESDLDYGTNFQKRLFTKIDTIAPDEITVSSDFEARH
VKLNVEERSVGPLTRKGFYLAFQDIGACVALLSVRVYYKKCPELLQGLAHFPETIAGSDAPSLATVAGT
CVDHAVVPPGGEEPRMHCAVDGEWLVPIGQCLCQAGYEKVEDACQACSPGFFKFEASESPCLECPEHT
LPSPEGATSCECEEGFFRAPQDPASMPCTRPPSAPHYLTAVGMGAKVELRWTPPQDSGGREDIVYSVTC
EQCWPESGECGPCEASVRYSEPPHGLTRTSVTVSDLEPHMNYTFTVEARNGVSGLVTSRSFRTASVSIN
QTEPPKVRLEGRSTTSLSVSWSIPPPQQSRVWKYEVTYRKKGDSNSYNVRRTEGFSVTLDDLAPDTTYL
VQVQALTQEGQGAGSKVHEFQTLSPEGSGN***LAVIGGVAVGVVLLLVLAGVGFFI***HRRRKNQRARQSPE
DVYFSKSEQLKPLKTYVDPHTYEDPNQAVLKFTTEIHPSCVTRQKVIGAGEFGEVYKGMLKTSSGKKEV
PVAIKTLKAGYTEKQRVDFLGEAGIMGQFSHHNIIRLEGVISKYKPMMIITEYMENGALDKFLREKDGEF
SVLQLVGMLRGIAAGMKYLANMNYVHRDLAARNILVNSNLVCKVSDFGLSRVLEDDPEATYTTSGGK
IPIRWTAPEAISYRKFTSASDVWSFGIVMWEVMTYGERPYWELSNHEVMKAINDGFRLPTPMDCPSAIY
QLMMQCWQQERARRPKFADIVSILDKLIRAPDSLKTLADFDPRVSIRLPSTSGSEGVPFRTVSEWLESIK
MQQYTEHFMAAGYTAIEKVVQMTNDDIKRIGVRLPGHQKRIAYSLLGLKDQVNTVGIPI

L1 cell adhesion molecule (L1CAM) ***(isoform 1)***

IQIPEEYEGHHVMEPPVITEQSPRRLVVFPTDDISLKCEASGKPEVQFRWTRDGVHFKPKEELGVTVYQS
PHSGSFTITGNNSNFAQRFQGIYRCFASNKLGTAMSHEIRLMAEGAPKWPKETVKPVEVEEGESVVLPC
NPPPSAEPLRIYWMNSKILHIKQDERVTMGQNGNLYFANVLTSDNHSDYICHAHFPGTRTIIQKEPIDLR
VKATNSMIDRKPRLLFPTNSSSHLVALQGQPLVLECIAEGFPTPTIKWLRPSGPMPADRVTYQNHNKTL
QLLKVGEEDDGEYRCLAENSLGSARHAYYVTVEAAPYWLHKPQSHLYGPGETARLDCQVQGRPQPEV
TWRINGIPVEELAKDQKYRIQRGALILSNVQPSDTMVTQCEARNRHGLLLANAYIYVVQLPAKILTADN
QTYMAVQGSTAYLLCKAFGAPVPSVQWLDEDGTTVLQDERFFPYANGTLGIRDLQANDTGRYFCLAA
NDQNNVTIMANLKVKDATQITQGPRSTIEKKGSRVTFTCQASFDPSLQPSITWRGDGRDLQELGDSDKY
FIEDGRLVIHSLDYSDQGNYSCVASTELDVVESRAQLLVVGSPGPVPRLVLSDLHLLTQSQVRVSWSPA

EDHNAPIEKYDIEFEDKEMAPEKWYSLGKVPGNQTSTTLKLSPYVHYTFRVTAINKYGPGEPSPVSETV
VTPEAAPEKNPVDVKGEGNETTNMVITWKPLRWMDWNAPQVQYRVQWRPQGTRGPWQEQIVSDPFL
VVSNTSTFVPYEIKVQAVNSQGKGPEPQVTIGYSGEDYPQAIPELEGIEILNSSAVLVKWRPVDLAQVKG
HLRGYNVTYWREGSQRKHSKRHIHKDHVVVPANTTSVILSGLRPYSSYHLEVQAFNGRGSGPASEFTFS
TPEGVPGHPEALHLECQSNTSLLLRWQPPLSHNGVLTGYVLSYHPLDEGGKGQLSFNLRDPELRTHNLT
DLSPHLRYRFQLQATTKEGPGEAIVREGGTMALSGISDF***GNISATAGENYSVVSWVPKEGQC***NFRFHILF
KALGEEKGGASLSPQYVSYNQSSYTQWDLQPDTDYEIHLFKERMFRHQMAVKTNGTGRVRLPPAGFA
TE

nectin-like protein 5

WPPPGTGDVVVQAPTQVPGFLGDSVTLPCYLQVPNMEVTHVSQLTWARHGESGSMAVFHQTQGPSYS
ESKRLEFVAARLGAELRNASLRMFGLRVEDEGNYTCLFVTFPQGSRSVDIWLRVLAKPQNTAEVQKVQ
LTGEPVPMARCVSTGGRPPAQITWHSDLGGMPNTSQVPGFLSGTVTVTSLWILVPSSQVDGKNVTCKV
EHESFEKPQLLTVNLTVYYPPEVSISGYDNNWYLGQNEATLTCDARSNPEPTGYNWSTTMGPLPPFAVA
QGAQLLIRPVDKPINTTLICNVTNALGARQAELTVQVKEGPPSEHSGISRN***AIIFLVLGILVFLILLG***
IGIYFYWSKCSREVLWHCHLCPSSTEHASASANGHVSYSAVSRENSSSQDPQTEGTR

neuropilin 1

FRNDKCGDTIKIESPGYLTSPGYPHSYHPSEKCEWLIQAPDPYQRIMINFNPHFDLEDRDCKYDYVEVFD
GENENGHFRGKFCGKIAPPPVVSSGPFLFIKFVSDYETHGAGFSIRYEIFKRGPECSQNYTTPSGVIKSPGF
PEKYPNSLECTYIVFVPKMSEIILEFESFDLEPDSNPPGGMFCRYDRLEIWDGFPDVGPHIGRYCGQKTPG
RIRSSSGILSMVFYTDSAIAKEGFSANYSVLQSSVSEDFKCMEALGMESGEIHSDQITASSQYSTNWSAER
SRLNYPENGWTPGEDSYREWIQVDLGLLRFVTAVGTQGAISKETKKKYYVKTYKIDVSSNGEDWITIKE
GNKPVLFQGNTNPTDVVVAVFPKPLITRFVRIKPATWETGISMRFEVYGCKITDYPCSGMLGMVSGLIS
DSQITSSNQGDRNWMPENIRLVTSRSGWALPPAPHSYINEWLQIDLGEEKIVRGIIIQGGKHRENKVFMR
KFKIGYSNNGSDWKMIMDDSKRKAKSFEGNNNYDTPELRTFPALSTRFIRIYPERATHGGLGLRMELLG
CEVEAPTAGPTTPNGNLVDECDDDQANCHSGTGDDFQLTGGTTVLATEKPTVIDSTIQSEFPTYGFNCEF
GWGSHKTFCHWEHDNHVQLKWSVLTSKTGPIQDHTGDGNFIYSQADENQKGKVARLVSPVVYSQNSA
HCMTFWYHMSGSHVGTLRVKLRYQKPEEYDQLVWMAIGHQGDHWKEGRVLLHKSLKLYQVIFEGEI
GKGNLGGIAVDDISINNHISQEDCAKPADLDKKNPEIKIDETGSTPGYEGEGEGDKNISRKPGNVLKTLD
P***ILITIIAMSALGVLLGAVCGVVL***YCACWHNGMSERNLSALENYNFELVDGVKLKKDKLNTQSTYSEA

plexin A1

EAGLPRAGGGSQPPFRTFSASDWGLTHLVVHEQTGEVYVGAVNRIYKLSGNLTLLRAHVTGPVEDNEK
CYPPPSVQSCPHGLGSTDNVNKLLLLDYAANRLLACGSASQGICQFLRLDDLFKLGEPHHRKEHYLSSV
QEAGSMAGVLIAGPPGQGQAKLFVGTPIDGKSEYFPTLSSRRLMANEEDADMFGFVYQDEFVSSQLKIP
SDTLSKFPAFDIYYVYSFRSEQFVYYLTLQLDTQLTSPDAAGEHFFTSKIVRLCVDDPKFYSYVEFPIGCE
QAGVEYRLVQDAYLSRPGRALAHQLGLAEDEDVLFTVFAQGQKNRVKPPKESALCLFTLRAIKEKIKE
RIQSCYRGEGKLSLPWLLNKELGCINSPLQIDDDFCGQDFNQPLGGTVTIEGTPLFVDKDDGLTAVAAY
DYRGRTVVFAGTRSGRIRKILVDLSNPGGRPALAYESVVAQEGSPILRDLVLSPNHQYLYAMTEKQVTR
VPVESCVQYTSCELCLGSRDPHCGWCVLHSICSRRDACERADEPQRFAADLLQCVQLTVQPRNVSVTM
SQVPLVLQAWNVPDLSAGVNCSFEDFTESESVLEDGRIHCRSPSAREVAPITRGQGDQRVVKLYLKSKE
TGKKFASVDFVFYNCSVHQSCLSCVNGSFPCHWCKYRHVCTHNVADCAFLEGRVNVSEDCPQILPSTQI
YVPVGVVKPITLAARNLPQPQSGQRGYECLFHIPGSPARVTALRFNSSSLQCQNSSYSYEGNDVSDLPVN
LSVVWNGNFVIDNPQNIQAHLYKCPALRESCGLCLKADPRFECGWCVAERRCSLRHHCAADTPASWM
HARHGSSRCTDPKILKLSPETGPRQGGTRLTITGENLGLRFEDVRLGVRVGKVLCSPVESEYISAEQIVCE
IGDASSVRAHDALVEVCVRDCSPHYRALSPKRFTFVTPTFYRVSPSRGPLSGGTWIGIEGSHLNAGSDVA
VSVGGRPCSFSWRNSREIRCLTPPGQSPGSAPIIININRAQLTNPEVKYNYTEDPTILRIDPEWSINSGGTLL
TVTGTNLATVREPRIRAKYGGIERENGCLVYNDTTMVCRAPSVANPVRSPPELGERPDELGFVMDNVR
SLLVLNSTSFLYYPDPVLEPLSPTGLLELKPSSPLILKGRNLLPPAPGNSRLNYTVLIGSTPCTLTVSETQLL
CEAPNLTGQHKVTVRAGGFEFSPGTLQVYSDSLLTLPLYAKDIPNYKSWVERYYADIAKMPAISDQDM
SAYLAEQSRLHLSQFNSMSALHEIYSYITKYKDEILAALEKDEQARRQRLRSKLEQVVDTMALSS***AIVGI***
GGGGGLLLLVIVAVLIAYKRKSRDADRTLKRLQLQMDNLESRVALECKEAFAELQTDIHELTNDLDGA
GIPFLDYRTYAMRVLFPGIEDHPVLKEMEVQANVEKSLTLFGQLLTKKHFLLTFIRTLEAQRSFSMRDRG
NVASLIMTALQGEMEYATGVLKQLLSDLIEKNLESKNHPKLLLRRTESVAEKMLTNWFTFLLYKFLKE
CAGEPLFMLYCAIKQQMEKGPIDAITGEARYSLSEDKLIRQQIDYKTLTLNCVNPENENAPEVPVKGLD

CDTVTQAKEKLLDAAYKGVPYSQRPKAADMDLEWRQGRMARIILQDEDVTTKIDNDWKRLNTLAHY
QVTDGSSVALVPKQTSAYNISNSSTFTKSLSRYESMLRTASSPDSLRSRTPMITPDLESGTKLWHLVKNH
DHLDQREGDRGSKMVSEIYLTRLLATKGTLQKFVDDLFETIFSTAHRGSALPLAIKYMFDFLDEQADKH
QIHDADVRHTWKSNCLPLRFWVNVIKNPQFVFDIHKNSITDACLSVVAQTFMDSCSTSEHKLGKDSPSN
KLLYAKDIPNYKSWVERYYADIAKMPAISDQDMSAYLAEQSRLHLSQFNSMSALHEIYSYITKYKDEIL
AALEKDEQARRQRLRSKLEQVVDTMALSS

plexin B2

LRPRKLDFFRSEKELNHLAVDEASGVVYLGAVNALYQLDAKLQLEQQVATGPALDNKKCTPPIEASQC
HEAEMTDNVNQLLLLDPPRKRLVECGSLFKGICALRALSNISLRLFYEDGSGEKSFVASNDEGVATVGL
VSSTGPGGDRVLFVGKGNGPHDNGIIVSTRLLDRTDSREAFEAYTDHATYKAGYLSTNTQQFVAAFED
GPYVFFVFNQQDKHPARNRTLLARMCREDPNYYSYLEMDLQCRDPDIHAAAFGTCLAASVAAPGSGR
VLYAVFSRDSRSSGGPGAGLCLFPLDKVHAKMEANRNACYTGTREARDIFYKPFHGDIQCGGHAPGSS
KSFPCGSEHLPYPLGSRDGLRGTAVLQRGGLNLTAVTVAAENNHTVAFLGTSDGRILKVYLTPDGTSSE
YDSILVEINKRVKRDLVLSGDLGSLYAMTQDKVFRLPVQECLSYPTCTQCRDSQDPYCGWCVVEGRCT
RKAECPRAEEASHWLWSRSKSCVAVTSAQPQNMSRRAQGEVQLTVSPLPALSEEDELLCLFGESPPHPA
RVEGEAVICNSPSSIPVTPPGQDHVAVTIQLLLRRGNIFLTSYQYPFYDCRQAMSLEENLPCISCVSNRWT
CQWDLRYHECREASPNPEDGIVRAHMEDSCPQFLGPSPLVIPMNHETDVNFQGKNLDTVKGSSLHVGS
DLLKFMEPVTMQESGTFAFRTPKLSHDANETLPLHLYVKSYGKNIDSKLHVTLYNCSFGRSDCSLCRAA
NPDYRCAWCGGQSRCVYEALCNTTSECPPPVITRIQPETGPLGGGIRITILGSNLGVQAGDIQRISVAGRN
CSFQPERYSVSTRIVCVIEAAETPFTGGVEVDVFGKLGRSPPNVQFTFQQPKPLSVEPQQGPQAGGTTLTI
HGTHLDTGSQEDVRVTLNGVPCKVTKFGAQLQCVTGPQATRGQMLLEVSYGGSPVPNPGIFFTYRENP
VLRAFEPLRSFASGGRSINVTGQGFSLIQRFAMVVIAEPLQSWQPPREAESLQPMTVVGTDYVFHNDTK
VVFLSPAVPEEPEAYNLTVLIEMDGHRALLRTEAGAFEYVPDPTFENFTGGVKKQVNKLIHARGTNLNK
AMTLQEAEAFVGAERCTMKTLTETDLYCEPPEVQPPPKRRQKRDTTHNLPEFIVKFGSREWVLGRVEY
DTRVSDVP***LSLILPLVIVPMVVVIAVSVY***CYWRKSQQAEREYEKIKSQLEGLEESVRDRCKKEFTDLMIE
MEDQTNDVHEAGIPVLDYKTYTDRVFFLPSKDGDKDVMITGKLDIPEPRRPVVEQALYQFSNLLNSKSF
LINFIHTLENQREFSARAKVYFASLLTVALHGKLEYYTDIMHTLFLELLEQYVVAKNPKLMLRRSETVV
ERMLSNWMSICLYQYLKDSAGEPLYKLFKAIKHQVEKGPVDAVQKKAKYTLNDTGLLGDDVEYAPLT
VSVIVQDEGVDAIPVKVLNCDTISQVKEKIIDQVYR

transferrin receptor protein 1

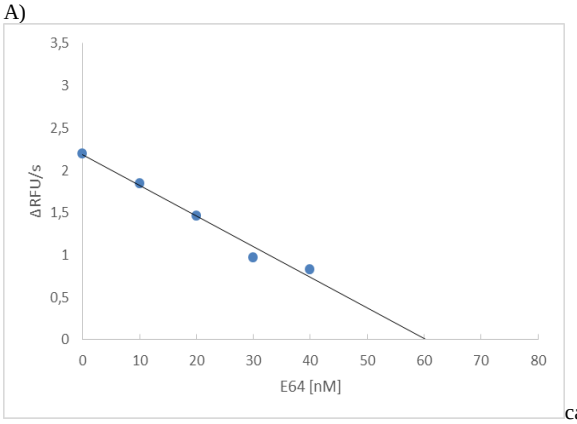
MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNSHVEMKLAVDEEENADNNTKANVTKPKRCSGSIC
YGTIAVIVFFLIGFMIGYLGYCKGVEPKTECERLAGTESPVREEPGEDFPAARRLYWDDLKRKLSEKLDS
TDFTGTIKLLNENSYVPREAGSQKDENLALYVENQFREFKLSKVWRDQHFVKIQVKDSAQNSVIIVDKN
GRLVYLVENPGGYVAYSKAATVTGKLVHANFGTKKDFEDLYTPVNGSIVIVRAGKITFAEKVANAESL
NAIGVLIYMDQTKFPIVNAELSFFGHAHLGTGDPYTPGFPSFNHTQFPPSRSSGLPNIPVQTISRAAAEKLF
GNMEGDCPSDWKTDSTCRMVTSESKNVKLTVSNVLKEIKILNIFGVIKGFVEPDHYVVVGAQRDAWGP
GAAKSGVGTALLLKLAQMFSDMVLKDGFQPSRSIIFASWSAGDFGSVGATEWLEGYLSSLHLKAFTYIN
LDKAVLGTSNFKVSASPLLYTLIEKTMQNVKHPVTGQFLYQDSNWASKVEKLTLDNAAFPFLAYSGIPA
VSFCFCEDTDYPYLGTTMDTYKELIERIPELNKVARAAAEVAGQFVIKLTHDVELNLDYERYNSQLLSF
VRDLNQYRADIKEMGLSLQWLYSARGDFFRATSRLTTDFGNAEKTDRFVMKKLNDRVMRVEYHFLSP
YVSPKESPFRHVFWGSGSHTLPALLENLKLRKQNNGAFNETLFRNQLALATWTIQGAANALSGDVWDI
DNEF

Supplemental Figure S2. Amino acid coverage of identified substrate ectodomains

The amino acid sequences of all identified shed extracellular domains are shown. Extracellular domains are marked in black, while cytosolic regions are red. Transmembrane regions are underlined and in italics. Peptides which were identified by mass spectrometry are shaded. For each substrate, multiple peptides were identified and they were all located exclusively in the substrate ectodomain, whereas none of the identified peptides were located in transmembrane or cytosolic domains. In the case of CD55, which is a GPI anchored protein, the aminoacid containing the GPI anchor is marked in red.

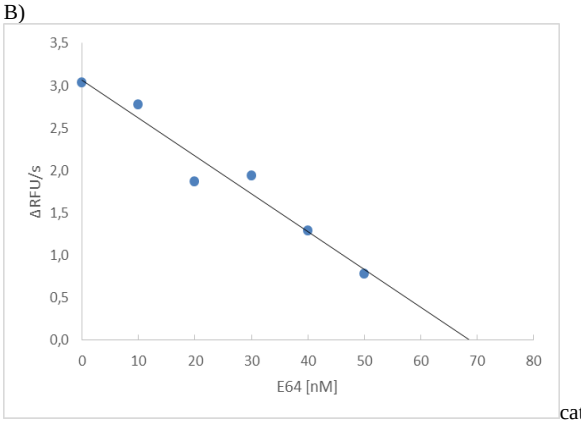
Supplemental Figure S3

A)



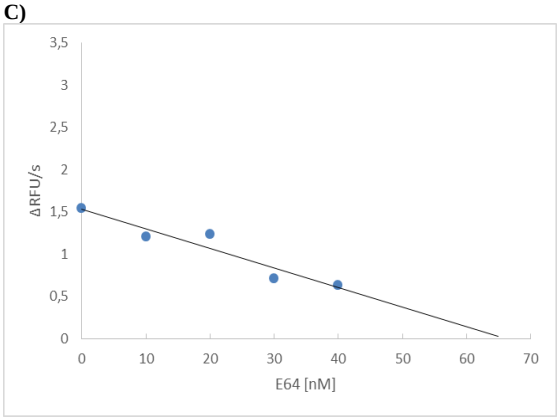
cathepsin concentration: 61 nM

B)



cathepsin concentration: 68 nM

C)

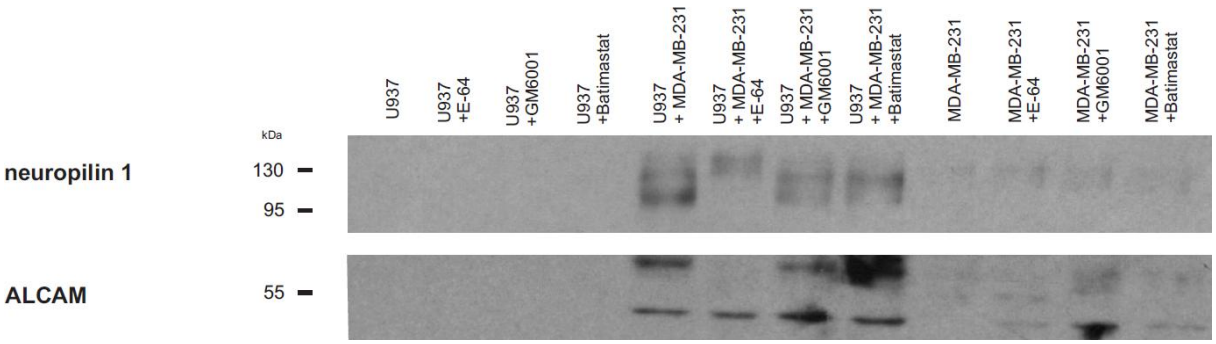


cathepsin concentration: 66 nM

Supplemental Figure S3. Active site titration of cathepsins in the macrophage secretome

Active site titration was used to determine the concentration of active cathepsins in the macrophage secretome. Secretome samples were incubated in the presence of increasing concentrations of E-64 (0-50 nM) and residual cathepsin activity was measured using the fluorogenic substrate Z-FR-AMC. Cathepsin concentrations were determined using linear regression analysis. Results of three biological replicates are shown (A, B and C) and the three determined cathepsin concentrations are listed. The average cathepsin concentration, calculated from the three experiments was 65 ± 4 nM.

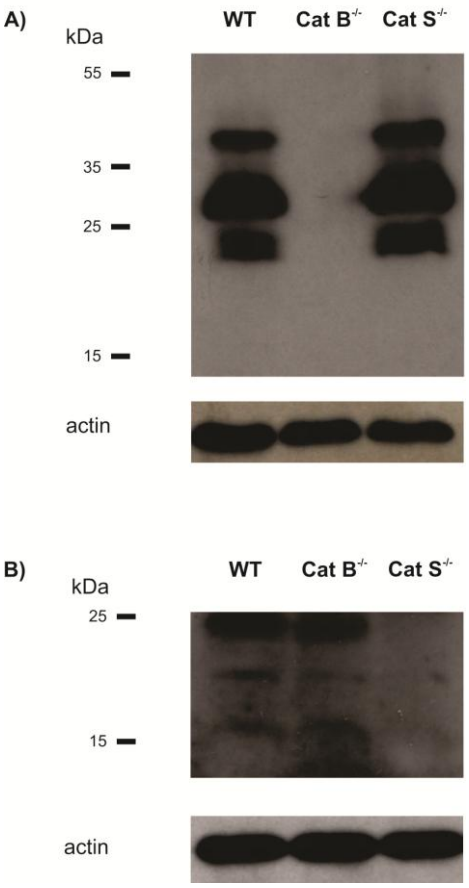
Supplemental Figure S4



Supplemental Figure S4. Metalloprotease inhibitors GM6001 and batimastat do not prevent substrate shedding in the co-culture of U937 cells differentiated into macrophages and MDA-MB-231 cells as cancer cells.

Immunoblot analysis of shedding of ALCAM and neuropilin 1 in the culture media. U937 cells were differentiated into macrophages and co-cultured with MDA-MB-231 cells. No shedding was detected in individual cell lines or in inhibitor-treated cells. Only the use of cysteine inhibitor E-64 abolished substrate shedding in co-culture while the broad-spectrum metalloprotease inhibitors GM6001 and batimastat had no effect.

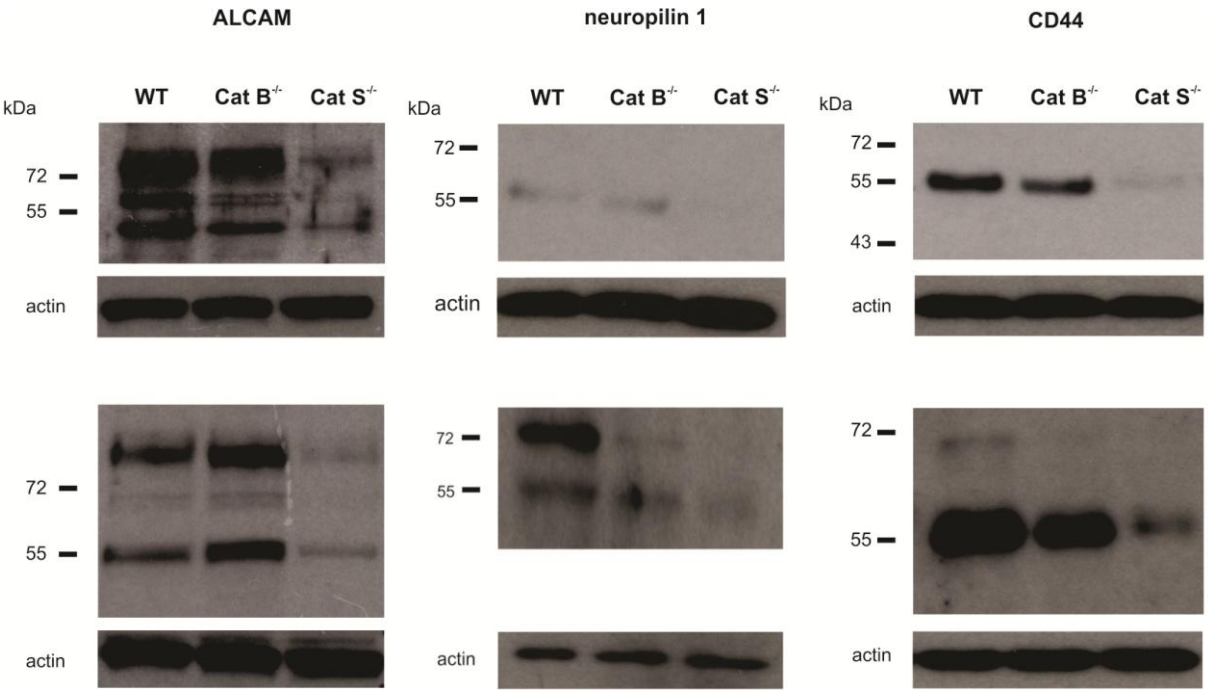
Supplemental Figure S5



Supplemental Figure S5. Detection of cathepsins B and S in RIP1-Tag2 tumors.

Immunological detection of cathepsin B (A) and cathepsin S (B) in tumor extracts was used to confirm their genetic ablation in catB-/- and catS-/- RIP1Tag2 tumors. Actin was used as a loading control.

Supplemental Figure S6



Supplemental Figure S6. Processing of ALCAM, neuropilin 1 and CD44 in soluble RIP1-Tag2 tumor extracts.

Immunological detection of processed ALCAM, neuropilin 1 and CD44 in soluble extracts from six additional tumors is shown, in addition to the experiment shown in Figure 4d. In the last replicate (lower panels), tumor samples were prepared in the presence of 20 μM E-64 in the homogenization buffer. Actin was used as a loading control. In both replicates, substrate processing was almost completely abolished in the absence of cathepsin S, while the absence of cathepsin B showed much lesser effect.

Supplemental Table S1

protein name	**protein ID**	**gene name**	**number of identified peptides**	**SCR (cathepsin L)**			**SCR (cathepsin S)**			**SCR (cathepsin B)**	
activated leukocyte cell adhesion molecule	IPI00015102	ALCAM	16	18	4.4	10	3.3	4.2	6.6	-	-
alkaline phosphodiesterase 1	IPI00184311	ENPP1	14	11.5	15	39	4.6	4.5	24	-	-
CD44 antigen	IPI00305064	CD44	3	15	4.4	4.2	5.25	20	26	-	-
cell surface glycoprotein MUC18	IPI00016334	MCAM	5	7	-	10	12	8	6	-	-
decay-accelerating factor CD55	IPI00382926	DAF	12	20	15	59	12	11	14	-	-
epidermal growth factor receptor	IPI00018274	EGFR	8	14	10	26	16	5	-	-	-
ephrin type A receptor 2	IPI00021267	EPHA2	6	25	41	53	5	35	15	-	-
L1 cell adhesion molecule	IPI01013306	CAML1	20	14	2	28.3	14	1	10.5	-	-
nectin-like protein 5	IPI00299158	PVR	2	-	7	11	3.5	13	6	-	-
neuropilin 1	IPI00299594	NRP1	16	30	12.75	15.8	23	6	6.8	4.6	7.5
plexin A1	IPI00552671	PLXNA1	9	14	12	23	8	6	8	-	-
plexin B2	IPI00853369	PLXNB1	11	15	23	22	12	27	6	-	-
transferrin receptor protein 1	IPI00022462	TFRC	25	8	2	8.5	9	5.6	12.3	-	-

Supplemental Table S1. Spectral count ratio (SCR) values of identified substrate candidates in cathepsin treated MDA-MB-231 cells.

A group of 13 identified substrate candidates is listed with their peptide ID, gene name and the total number of peptides identified. For all identified substrate candidates an SCR ratio of all biological replicates is shown. Experiments with cathepsins L and S were done in three biological replicates, while the experiment with cathepsin B was performed in two biological replicates, with only one substrate candidate (neuropilin 1) identified after cathepsin B treatment. The SCR ratios were reproducibly >3.0 for the majority of identified substrates, showing that they were released from the cell surface with high reproducibility. Epidermal growth factor receptor, MUC18 and nectin-like protein 5 were not identified in one biological replicate, while L1CAM and transferrin receptor protein 1 had SCR values <3.0 in one (transferrin receptor protein 1) or two (L1CAM) out of six biological replicates.

Supplemental Table S2

PANC1

protein name	protein IDs	gene name	peptides (catL)	peptides (catS)	SCR (catL)	SCR (catS)
alkaline phosphatase	IPI00007289	ALPP	2	2	4	4
intercellular adhesion molecule 1	IPI00008494	ICAM1	5	2	5	4
ephrin type A receptor 2	IPI00021267	EPHA2	6	2	4	13
perlecan	IPI00024284	HSPG2	4	19	6	21.5
nidogen 1	IPI00026944	NID1	3	15	6	18.5
CD109 antigen	IPI00152540	CD109	31	8	6.5	5
ephrin type B receptor 4	IPI00289342	EPHB4	3	2	5	6
leucyl-cystinyl aminopeptidase	IPI00307017	LNPEP	6	3	5	7
agrin	IPI00374563	AGRN	2	4	4	7
laminin subunit alpha 5	IPI00783665	LAMA5	13	56	10	85
plexin B2	IPI00853369	PLXNB2	4	3	5	8

HT144

protein name	protein IDs	gene name	peptides (catL)	peptides (catS)	SCR (catL)	SCR (catS)
4F2 cell-surface antigen heavy chain	IPI00554481	SLC3A2	5	3	8	5.7
activated leukocyte cell adhesion molecule	IPI00015102	ALCAM	4	2	10	4
CD44 antigen	IPI00305064	CD44	1	2	127	191
cell surface glycoprotein MUC18	IPI00016334	MCAM	6	6	5.25	28
discoidin, CUB and LCCL domain-containing protein 2	IPI00433138	DCBLD2	3	3	6	8
EMILIN1	IPI00013079	EMILIN1	8	7	7	7
endoglin	IPI00017567	ENG	3	4	11	14
ephrin type B receptor 4	IPI00289342	EPHB4	3	5	7	6
melanotransferrin	IPI00029275	MFI2	14	10	23.7	41
neural cell adhesion molecule L1-like protein	IPI00299059	CHL1	2	4	3.3	19
neuronal cell adhesion molecule	IPI00873446	NRCAM	8	9	5.5	27
neuropilin 2	IPI00029693	NRP2	7	7	18	14
perlecan	IPI00024284	HSPG2	27	37	8.25	55.5
plexin B2	IPI00853369	PLXNB2	10	9	28	23
receptor-type tyrosine-protein phosphatase F	IPI00107831	PTPRF	13	17	23	67
roundabout homolog 1	IPI00740934	ROBO1	4	7	5	7.5
tenascin	IPI00031008	TNC	13	12	23	45
teneurin 3	IPI00398020	TNM3	10	6	12	12
transferrin receptor protein 1	IPI00022462	TFRC	3	2	12	7
transforming growth factor-beta-induced protein ig-h3	IPI00018219	TGFBI	3	3	11	5

T98-G

protein name	protein ID	gene name	peptides (catL)	peptides (catS)	SCR (catL)	SCR (catS)
4F2 cell-surface antigen heavy chain	IPI00554481	SLC3A2	10	8	6.1	4.6
activated leukocyte cell adhesion molecule	IPI00015102	ALCAM	2	1	6	-
collagen alpha-1(XII) chain	IPI00329573	COL12A1	8	11	12	7
C-type mannose receptor 2	IPI00005707	CLEC13E	10	12	4.2	6.75
ephrin type A receptor 2	IPI00021267	EPHA2	2	2	5	5
fibronectin	IPI00855785	FN1	32	45	7.2	3.6
galectin-3-binding protein	IPI00023673	LGALS3BP	11	8	4.2	14
laminin subunit alpha 4	IPI00329482	LAMA4	10	7	5	15
neuropilin 1	IPI00299594	NRP1	10	6	27	12
perlecan	IPI00024284	HSPG2	30	31	69	68
plexin B2	IPI00853369	PLXNB2	12	7	11	8.5
receptor-type tyrosine-protein phosphatase F	IPI00107831	PTPRF	7	6	7.5	13
receptor-type tyrosine-protein phosphatase gamma	IPI00011651	PTPRG	3	3	9	14
sodium bicarbonate cotransporter 3	IPI00926820	SLC4A7	3	3	11	7
transferrin receptor protein 1	IPI00022462	TFRC	7	6	4.75	4.75
vasorin	IPI00395488	VASN	10	9	27	40

MCF-7

protein name	protein ID	gene name	peptides (catL)	peptides (catS)	SCR (catL)	SCR (cat S)
activated leukocyte cell adhesion molecule	IPI00015102	ALCAM	4	2	14	8
cadherin EGF LAG seven-pass G-type receptor 2	IPI00015346	CELSR2	11	12	23	27
ephrin type-B receptor 4	IPI00289342	EPHB4	4	3	10	7
FRAS1-related extracellular matrix protein 2	IPI00180707	FREM2	2	4	5	6
galectin-3-binding protein	IPI00023673	LGALS3BP	3	2	10	6
L1 cell adhesion molecule	IPI01013306	CAML1	7	6	13	14
neuronal cell adhesion molecule	IPI00873446	NRCAM	2	6	6	6.5
plexin B2	IPI00853369	PLXNB2	2	0	4	-
receptor-type tyrosine-protein phosphatase F	IPI00107831	PTPRF	13	12	27	23

Supplemental Table S2. Lists of cell surface proteins identified in the supernatant after cathepsin treatment of cell lines PANC1, HT144, T98-G and MCF-7.

Proteins released from the cell surface and identified in the supernatants of four tested cell lines treated with cathepsins L and S are listed. For each cathepsin treatment, the number of identified peptides and the corresponding spectral count ratio between treated sample and negative control are provided.