ABRF PRG2008 Method Summary

1D PAGE

The samples were dissolved and reduced in 1X Laemli buffer, vortexed, heated up to 100C for 5 minutes. 85% of sample A and 70% of sample B was loaded onto two separate lanes of a pre-cast Novex Tris-Glycine 1D midi-gel (Invitrogen). The gel was run according to manufacturer's instructions, fixed and stained with Colloidal Coumassie Blue according to a standard protocol. Both lanes of the gel were identically cut into 17 slices corresponding to major protein bands and portions of the gel between the bands. The slices were washed, destained, alkylated with iodoacetoamide and digested overnight with trypsin (Promega) at 37C according to standard protocols.

Protein Identification by Nano- LC ESI-MS/MS

Upon digestion, the samples were concentrated to a volume of 10 µL on a SpeedVac and diluted to 33 µL of aqueous solvent containing 2% formic acid 2% acetonitrile. A 10 µL aliquot of each digest was subjected to three replicate analyses by nano-LC MS/MS The digests were injected onto a C18 solid phase extraction (SPE) trapping column (100 µm i.d. x 25 mm) in house packed in an IntegraFrit fused silica capillary (New Objective, MA) and connected to a transfer capillary (75 µm i.d. x 12 cm) using a PicoClear union (New Objective). The trapping column was connected online to a self-packed 100 µm i.d. x 13 cm nano-LC reversed-phase (Magic C18, 3 µm) PicoTip column (NewObjective). The eluent was introduced into a LTQ XT Orbitrap mass spectrometer (ThermoElectron) by nanoelectrospray from a 10 µm i.d. pulled fused silica tip of the nano-LC column. A 30-minute long gradient was applied on the column from a 2-D LC (Eksigent) HPLC system to separate the digests. 0. 1% formic acid 2% acetonitrile in deionized water was used as an HPLC solvent "A" and 0. 1% formic acid 5% isopropanol 80% acetonitrile was used as an HPLC solvent "B".

In data-dependent MS/MS scanning mode, a full MS scan between 350 and 1700 m/z was followed by seven full MS/MS scans for the five most intense ions from the MS scan. The target resolution was set to 60,000. The normalized collision energy was set to 35 %. Parent ions were isolated using isolation width of 2.0 m/z units and fragmented at default charge state set to +3, activation energy to 0.250 and activation time to 30 ms.

Database searches.

MS/MS data-dependent acquisition, followed by database searching using SEQUEST and X!Tandem algorithms on a Sorcerer IDA II searching engine (SageN Research, CA) allowed protein identification. At least three rounds of searches were performed for all LC MS runs using either a fully tryptic peptide database and limited number of potential differential modifications (Cys alkylation; Met, His and Trp oxidation) or fully tryptic peptide database and extended list of potential differential modifications (Cys alkylation; Met, His and Trp oxidation; Lys acetylation; Ser, Tre and Tyr phosphorylation; Lys ubiquitylation; N-terminal thrombin cleavage site modification, Asn N-GlcNAc glycosylation), or a semitryptic database with limited number of potential differential modifications (Cys alkylation; Met, His and Trp oxidation). The *fasta* database included concatenated forward and reverse protein sequences of the latest (November 2007) Uniprot human, E. coli databases and an in-house compiled database of common contaminants. The validation of database searching results was done using ProteinProphet, PeptideProphet, Scaffold software platforms and by tuning the filtering parameters to keep the estimated false positive rate bellow 0.5%. Identification matches corresponding to common contaminants (keratins, BSA, caseins, etc.) and common ubiquitous proteins (glyceraldehydes-phosphate dehydrogenase, etc.) were manually removed from the list of filtered results. Identification matches corresponding to peptides containing post-translational modifications

were manually validated. Such differential modifications as HMW oxidation and C alkylation were not reported while they could be introduced *in vitro* during the sample preparation. Identifications of RAGE isoforms was validated using sequence coverage, peptide identification and 1D gel data.

Comments: Alternative proteolytic enzymes in combination or separately, as well as any *de novo* sequence identification software tools were not used because the experiment was mistakenly planned as a quantitative study.

Detected differential modifications are shown in red. "Phos "stays for phosphorylation; "Ac" stays for acetylation; "Ub" stays for ubiquitylation; oxidation and alkykation events are not shown.

Protein Identification Results:

Sequence	**Found in sample A or B?**	**Entry name**	**Protein name**	**Accession #**	**MW (Da)**	**# of unique peptides**	**% sequence coverage**	**Gel slice #**
maagtavgaw vlvlslwgav vgaqnitarI GEPLVLK(Ac)CKG apkkppqrLE WK(Ac) LNTGRTEA WK(Ac)VLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRcq amnrngketk snyrvrVYQI PGKPEIVDSA SELTAGVPNK vveesrrsrk rpceqeggdm cvrgklpcrd s	B	A6NKF0_HUMAN	Uncharacterized protein AGER - Homo sapiens	A6NKF0	**18549**	22	42.7	16
maagtavgaw vlvlslwgav vgaqnitarI GEPLVLK(Ub)CK(Ub)G apkKPPQRLE WKLNTGRTEA WKVLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRcq amnrngketk snyrslrtft itasdwifpp seipgkPEIV DSASELTAGV PNKVGTCVSE GSYPAGTLSW HLDGKPLVPN EKGVSVKEQT RRHPETGLFT LQSELMVTPA RGGDPRPTFS CSFSPGLPRh ralr	B	Q5SSZ3_HUMAN	Advanced glycosylation end product-specific receptor - Homo sapiens	Q5SSZ3	**25177**	48	67.9	13
maagtavgaw vlvlslwgav vGAQNITARI GEPLVLKckg apkKPPQRLE WK(Ac)LNTGRTEA WKVLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRcq amnrngketk snyrvrVYQI PGKPEIVDSA SELTAGVPNK vgtc VSEGSY PAGTLSWHLD GKPLVPNEKG VSVKEQTRRH PETGLFTLQS	A	Q86SN1_HUMAN	Soluble form of receptor for advanced glycation endproducts precursor - Homo sapiens	Q86SN1	**37050**	56	66.9	11

ELMVTPARGG DPRPTFScsF SPGLPRhral rTAPIQPRVW EPVPLEEVQL VVEPEGGAVA PGGTVTLTce vpaqpspqih wmkDGVPLPL PPSPVLILPE IGPQDQGTYs cvathsshgp qesrAVSISI IEPGEEGpta gegfdkvrea edspqhm								
maagtavgaw vlvlslwgav vGAQNITARI GEPLVLKckg apkKPPQRLE WKLNTGRTEA WKVLSPQGGG PWDSVARVLPNGSLFLPAVG IQDEGIFRcq amnrngketk snyrvrVYQI PGKPEIVDSA SELTAGVPNK vgtc VSEGSY PAGTLSWHLD GKPLVPNEKG VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFScsF SPGLPRhral rTAPIQPRVW EPVPLEEVQL VVEPEGGAVA PGGTVTLTce vpaqpspqih wmkDGVPLPL PPSPVLILPE IGPQDQGTYs cvathsshgp qesrAVSISI IEPGEEGpta gegfdkvrea edspqhm	B	Q86SN1_HUMAN	Soluble form of receptor for advanced glycation endproducts precursor - Homo sapiens	Q86SN1	**37050**	47	**69.5**	11
maagtavgaw vlvlslwgav v GAQNITARI GEPLVLKCK(Ac)G apkkppqr LE WK(Ac)LNTGRTEA WKVLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRcq amnrngketk snyrvrVYQI PGK(Ac)PEIVDSA SELTAGVPNK vveesrrsrk rpceqeVGTC VSEGSYPAGT LSWHLDGKPL VPNEKGVSVK EQTRRHPETG LFTLQSELMV TPARGGDPRP TFSCSFSPGL PRhralrTAP IQPRVWEPVP LEEVQLVVEP EGGavapggt vtltcevpaq pspqihwmk D GVPLPLPPSP VLILPEIGPQ DQGTYSCVAT HSSHGPQES(phos)R AVSISIIEPG EEGPTAGsvg gsglgtlala lgilgglgta alligvilwq rrqrrgeerk apenqeeeee raelnqseep eagesstggp	A	Q3L1R8_HUMAN (indistinguishable from Q15109 using our experimental data)	Receptor for advanced glycosylation end-products intron 4 variant - Homo sapiens	Q3L1R8	**44773**	60	58.1	10
maagtavgaw vlvlslwgav v GAQNITARI GEPLVLKckg apkkppqrle wklntgrTEA WKVLSPQGGG	B	Q3L1R8_HUMAN (indistinguishable	Receptor for advanced glycosylat	Q3L1R8	**44773**	50	57.4	10

PWDSVARVLP NGSLFLPAVG IQDEGIFRcq amnrngketk snyrvrVYQI PGKPEIVDSA SELTAGVPNK vveesrrsrk rpceqeVGTC VSEGSYPAGT LSWHLDGKPL VPNEKGVSVK EQTRRHPETG LFTLQSELMV TPARGGDPRP TFSCSFSPGL PRhralrTAP IQPRVWEPVP LEEVQLVVEP EGGavapggt vtltcevpaq pspqihwmk D GVPLPLPPSP VLILPEIGPQ DQGTYSCVAT HSSHGPQESR AVSISIIEPG EEGPTAGsvg gsglgtlala lgilgglgta alligvilwq rrqrrgeerk apenqeeeee raelnqseep eagesstggp		from Q15109 using our experimental data)	ion end-products intron 4 variant - Homo sapiens					
maagtavgaw vlvlslwgav vGAQNITARI GEPLVLKCK(Ac)G apkkppqrLE WK(Ac)LNTGRTEA WK(Ac)VLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRcq amnrngketk snyrvrVYQI PGK(Ac)PEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLD GKPLVPNEKG VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRhral RTAPIQPRVW EPVPLEEVQL VVEPEGGava pggtvtltce vpaqpspqih wmkDGVPLPL PPSPVLILPE IGPQDQGTYS CVATHSSHGP QES(phos)RAVSISI IEPGEEGPTA Gsvggsglgt lalalgilgg lgtaalligv ilwqrrqrrg eerkapenqe eeeeraelnq seepeagess tggp	A	RAGE_HUMAN (indistinguishable from Q86SN1 (and possibly Q3L1R8) using our experimental data)	Advanced glycosylation end product-specific receptor precursor - Homo sapiens	Q15109	**42803**	61	62.6	10
maagtavgaw vlvlslwgav vGAQNITARI GEPLVLKckg apkkppqrle wklntgrTEA WKVLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRcq amnrngketk snyrvrVYQI PGKPEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLD GKPLVPNEKG VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRhral RTAPIQPRVW EPVPLEEVQL VVEPEGGava pggtvtltce vpaqpspqih	B	RAGE_HUMAN (indistinguishable from Q86SN1 (and possibly Q3L1R8) using our experimental data)	Advanced glycosylation end product-specific receptor precursor - Homo sapiens	Q15109	**42803**	50	56.9	10

wmkDGVPLPL PPSPVLILPE IGPQDQGTYS CVATHSSHGP QESRAVSISI IEPGEEGPTA Gsvggsglgt lalalgilgg lgtaalligv ilwqrrqrrg eerkapenqe eeeeraelnq seepeagess tggp								
mpepaksapa pkkgskkavt kaqkkdgkkr krsrkesysv yvykvlkqvh pdtgisskAM GIMNSFVNDI FERiageasr lahynkrsti tsreiqtavr LLLPGELAKh avsggtkavt kytssk	A	Q0D2M2 _HUMAN	Histone H2B - Homo sapiens	Q0D2M2	**13834**	2	19	17
msgrgkggkg lgkggakrhr kvlrdniqgi tkpairrlar rggvkrISGL IYEETRgvlk vflenvirda vtytehakrk tvtamdvvya lkrqgrtlyg fgg	A	H4_HUM AN	Histone H4 – Homo sapiens	P62805	**11367**	1	9.7	17
mtggkagkds gkaktkavsr cqrAGLQFPV GRihlhlksr ttnhgrvgat aavysaailk yltaevlela gnaskdlkvk rsttghlqla irgdeeldsl ikatiagggv iphihksltg kkgqqktv	A	A6NKS5_ HUMAN	Histone H2A - Homo sapiens	A6NKS5	**13449**	1	7	17
marplctlll lmatlagala ssskeenrII PGGIYDADLN DEWVQRALHF AISEYNKate deyyrRPLQV LRareqtfgg vnyffdvevg rtictkSQPN LDTCAFHEQP ELQKkQLCSF EIYEVPWEDR mslvnsrcqe a	B	CYTS_H UMAN	Cystatin-S precursor - Homo sapiens	P01036	**16214**	5	48.9	10
maqhlstlll llatlavala wspkeedrII PGGIYNADLN DEWVQRALHF AISEYNKatk ddyyrrplrv lrarqqtvgg vnyffdvevg rtictkSQPN LDTCAFHEQP ELQKkqlcsf eiyevpwenr rslvksrcqe s	B	CYTN_H UMAN	Cystatin-SN precursor - Homo sapiens	P01037	**16362**	3	33.3	10
mklfwllfti gfcwaqyssn tqqgrTSIVH LFEWRwvdia lecerylapk gfggvqvspp nenvaihnpf rpwweryqpv syklctrSGN EDEFRnmvtr cnnvgvriyv davinhmcgn avsagtsstc gsyfnpgsrD FPAVPYSGWD FNDGKckTGS GDIENYNDAT QVRdcr LSGL LDLALGKdyv rskiaeymnh lidigvagfr idaskhmwpg dikaildklh nlnsnwfpeg skpfiyqevi dlggepikSS DYFGNGRvte	B	AMY1_H UMAN	Alpha-amylase 1 precursor - Homo sapiens	P04745	**57768**	9	25.0	10

fkygaklgtv irkwngekms ylknwgegwg fmpsdralvf vdnhdnqrGH GAGGASILTF WDARlykmav gfmlahpygf trvmssyrwp ryfengkdvn dwvgppndng vtkEVTINPD TTCGNDWVCE HRwrqirnmv nfrNVVDGQP FTNWYDNGSN QVAFGRgnrg fivfnnddwt fsltlqtglp agtycdvisg dkingnctgi kiyvsddgka hfsisnsaed pfiaihaesk l								
mdtlcstlll ltipswvlsq itlkesgpal vkptqtltlt ctfsgfslss sglsvgwirq ppgkalewla liywnddkrh rpslksrlti tkdtsknqvv ltmtnmdpvd tatyycahky sgswnafdiw gqgtmvtvss asptspkvfp lslcstqpdg nvviaclvqg ffpqeplsvt wsesgqgvta rnfppsqdas gdlyttssql tlpatqclag ksvtchvkhy tnpsqdvtvp cpvpstpptp spstpptpsp scchprlslh rpaledlllg seanltctlt glrdasgvtf twtpssgkSA VQGPPERdlc gcysvssvlp gcaepwnhgk TFTCTAAYPE SKTPLTATLS Ksgntfrpev hllpppseel alnelvtltc largfspkdv lvrWLQGSQE LPRekyltwa srQEPSQGTT TFAVTSILRv aaedwkkgdt fscmvgheal plaftqktid rlagkpthvn vsvvmaevdg tcy	B	Q569J1_HUMAN	IgG protein group: IGHA1 protein - Homo sapiens	Q569J1 (indistinguishable from Q96KX8, Q6GMX2 and some other IgGs)	**53158**	5	11.6	10
mqaprelavg idlgttyscv gvfqqgrvei landqgnrTT PSYVAFTDTE Rlvgdaaksq aalnphntvf dakrligrkf adttvqsdmk hwpfrvvseg gkpkvrvcyr gedktfypee issmvlskmk etaeaylgqp vkhavitvpa yfndsqrqat kDAGAIAGLN VLRIINEPTA AAIAYGLDRr gagernvlif dlgggtfdvs vlsidagvfe vkatagdthl ggedfdnrlv nhfmeefrrk hgkdlsgnkr alrrlrtace rakrtlssst qatleidslf egvdfytsit rarfeelcsd lfrstlepve kalrdakldk aqihdvvlvg gstripkvqk llqdffngke lnksinpdea vaygaavqaa vlmgdkcekv qdllllldvap lslgletagg vmttliqrna tiptkqtqtf ttysdnqpgv fiqvyegera mtkdnnllgr FELSGIPPAP Rgvpqievtf didangilsv tatdrstgka nkititndkg rlskeeverm vheaeqykae deaqrdrvaa knsleahvfh vkgslqeesl	B	HSP76_HUMAN	Heat shock 70 kDa protein 6 - Homo sapiens	P17066	**71028**	4	8.1	5

rdkipeedrr kmqdkcrevl awlehnqlae keeyehqkre leqicrpifs rlyggpgvpg gsscgtqarq gdpstgpiie evd								
makaaaigid lgttyscvgv fqhgkVEIIA NDQGNRTTPS YVAFTDTERl igdaaknqva lnpqntvfda krligrkfgd pvvqsdmkhw pfqvindgdk pkvqvsykge tkafypeeis smvltkmkei aeaylgypvt navitvpayf ndsqrqatkd agviaglnvl rIINEPTAAA IAYGLDRtgk gernvlifdl gggtfdvsil tiddgifevk atagdthlgg edfdnrlvnh fveefkrkhk kdisqnkrav rrlrtacera krtlssstqa sleidslfeg idfytsitra rfeelcsdlf rstlepveka lrdakldkaq ihdlvlvggs tripkvqkll qdffngrdln ksinpdeava ygaavqaail mgdksenvqd llldvapls lgletaggvm talikrnsti ptkqtqiftt ysdnqpgvli qvyegeramt kdnnllgrFE LSGIPPAPRg vpqievtfdi dangilnvta tdkstgkank ititndkgrl skeeiermvq eaekykaede vqrervsakn alesyafnmk savedeglkg kiseadkkkv ldkcqevisw ldantlaekd efehkrkele qvcnpiisgl yqgaggpgpg gfgaqgpkgg sgsgptieev d	B	HSP71_HUMAN	Heat shock 70 kDa protein 1 - Homo sapiens	P08107	**70052**	4	8.0	5

Detected PTMs for RAGE_HUMAN
(Q15109 and Q86SN (and possibly Q3L1R8) were indistinguishable from our experimental sequence coverage data)

maagtavgaw vlvlslwgav vGAQNITARI GEPLVLK(Ub, Ac)CK(Ub, Ac)G apkkppqrLE WK(Ac)LNTGRTEA WK(Ac)VLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRcq amnrngketk snyrvrVYQI PGK(Ac)PEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLD GKPLVPNEKG VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRhral RTAPIQPRVW EPVPLEEVQL VVEPEGGava pggtvtltce vpaqpspqih wmkDGVPLPL PPSPVLILPE IGPQDQGTYS CVATHSSHGP QES(phosph)RAVSISI IEPGEEGPTA Gsvggsglgt lalalgilgg lgtaalligv ilwqrrqrrg eerkapenqe eeeeraelnq seepeagess tggp

unique peptides: 61
% sequence coverage: 62.6%

Detected PTMs for Q86SN1_HUMAN:

maagtavgaw vlvlslwgav vGAQNITARI GEPLVLK(Ub, Ac)CK(Ub, Ac)G apkKPPQRLE WK(Ac)LNTGRTEA WK(Ac)VLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRcq amnrngketk snyrvrVYQI PGK(Ac)PEIVDSA SELTAGVPNK vgtc VSEGSY PAGTLSWHLD GKPLVPNEKG VSVKEQTRRH PETGLFTLQS ELMVTPARGG DPRPTFScsF SPGLPRhral rTAPIQPRVW EPVPLEEVQL VVEPEGGAVA PGGTVTLTce vpaqpspqih wmkDGVPLPL PPSPVLILPE IGPQDQGTYs cvathsshgp QES(phos)RAVSISI IEPGEEGpta gegfdkvrea edspqhm

unique peptides: 57
sequence coverage: 73.8%

All combined isoforms of Q15109 were detected with sequence coverage 55.7% and 61%, number of unique peptides 62 and 61, total number of peptides 1319 and 1391 for samples A and B accordingly.