

Identification of the GATA factor TRPS1 as a repressor of the osteocalcin promoter

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Online Supplemental Data for Piscopo, et al.

Supplemental Figures

Supplemental Figure S1. MASCOT settings for database searching of peptide ion masses detected by mass spectrometry.

Supplemental Figure S2. Peptide coverage maps for high scoring protein matches.

Supplemental Figure S3. Product ion scan of the triply charged molecular ion at m/z 570.6 detected in sample Ros 5-6 that was authenticated as 187TMVEIADHPAELVR201 from Runt-related transcription factor 2[RUNX2_MOUSE(Q08775)].

Supplemental Tables

Supplemental Table 1. Primers used to make *Trps1*-shRNA lentivirus

Supplemental Table 2. Primers used for Sybr Green qRT-PCR

Supplemental Table 3. Primers and probes used for Taqman qRT-PCR

Supplemental Table 4. Primers used for Chromatin Immunoprecipitation analysis

Piscopo et al., Figure S1

Supplemental Figure S1. MASCOT settings for database searching of peptide ion masses detected by mass spectrometry.

MS/MS Data Analysis

Mascot (Matrix Science)

SETTINGS

Database: Sprout 50.0 (222289 sequences; 81585146 residues)
Taxonomy: Homo sapiens (human) (14099 sequences) **and** Rodentia (Rodents) (17299 sequences)
Type of search: MS/MS Ion Search
Enzyme: Trypsin
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Deamidation (NQ), Oxidation (M), Pyro-glu (N-term Q)
Mass values: Monoisotopic
Protein Mass: Unrestricted
Peptide Mass Tolerance: ± 150 ppm
Fragment Mass Tolerance: ± 0.15 Da
Max Missed Cleavages: 2
Instrument type: ESI-QUAD-TOF

Probability Based Mowse Score

Individual peptides represented by their MS/MS data are assigned ion scores. Ion score is $-10 \cdot \log(P)$, where P is the probability that the observed match is a random event. **Individual ion scores > N indicate identity or extensive homology ($p < 0.05$).** Protein scores are derived from ion scores as a non-probabilistic basis for ranking protein hits. Reported protein scores and protein sequence coverage include peptides identified with scores below identity/extensive homology. Matched peptides with highly significant scores are shown in **Bold Red**, those with scores **below N** are shown in **Bold Black**.

Supplemental Figure S2. Peptide coverage maps for high scoring protein matches.Match to: **TRPS1_HUMAN, Zinc finger transcription factor Trps1 (Zinc finger protein GC79)**Primary accession number: **Q9UHF7**Score: **249**Nominal mass (M_r): **143656**; Calculated pI value: **7.53**Sequence Coverage: **6%**Found in sample: 1-C_1-6 **Significant Score N=31**

```

1 MVRKKNPPLR NVASEGEGQI LEPIGTESKV SGKNKEFSAD QMSENTDQSD
51 AAELNHKEEH SLHVQDPSSS SKKDLKSAVL SEKAGFNYES PSKGGNFPSF
101 PHDEVTDNRN LAFSSPAAGG VCEPLKSPQR AEADDPQDMA CTPSGDSLET
151 KEDQKMSPKA TEETGQAQSG QANCQGLSPV SVASKNPQVP SDGGVRLNKS
201 KTDLLVNDNP DPAPLSPELQ DFKCNICGYG YGNDPTDLI KHFRKYHLGL
251 HNRTRQDAEL DSKILLALHNM VQFHSKDFQ KVNRSVFSGV LQDINSSRPV
301 LLNGTYDVQV TSGGTFIGIG RKTPTDCQNT KYFRCKFCNF TYMGNSSTEL
351 EQHFLQTHPN KIKASLPSSS VAKPSEKNSN KSIPALQSSD SGDLGKWQDK
401 ITVKAGDDTP VGYSVPIKPL DSSRQNGTEA TSYWCKFCS FSCSSSSSLK
451 LLEHYGKQHG AVQSGGLNPE LNDKLSRGSV INQNDLAKSS EGETMTKTDK
501 SSSGAKKKDF SSKGAEDNMV TSYNCQFCDF RYSKSHGPDV IVVGPLLRHY
551 QQLHNIHKCT IKHCPFCPRG LCSPEKHLGE ITYPFACRKS NCSHCALLLL
601 HLSPGAAGSS RVKHQCHQCS FTTPDQDVLV FHYESVHESQ ASDVKQEANH
651 LQGSQDQSVV KESKEHSCTK CDFITQVEEE ISRHYRRAHS CYKCRQCSFT
701 AADTQSLLEH FNTVHCQEQD ITTANGCEDG HAISTIKEEP KIDFRVYNLL
751 TPDCKMGEV SVSEVVKREKL EEKDGKLEKV WTESSSDDL NVTWRGADIL
801 RGSPTSQTAS LGLLTPVSGT QEQTKTLRDS PNVEAAHLAR PIYGLAVETK
851 GFLQGAPAGG EKSGALPQQY PASGENKSKD ESQSLRRRRR GSGVFCANCL
901 TTKTSLWRKN ANGGYVCNAC GLYQKLHSTP RPLNIIKQNN GEQIIRRRTR
951 KRLNPEALQA EQLNKQQRGS NEEQVNGSPL ERRSEDHLTE SHQEIPLPS
1001 LSKYEAQGSL TKSHSAQQPV LVSQTLDIHK RMQPLHIQIK SPQESTGDPG
1051 NSSSVSEGKG SSERGSPIEK YMRPAKHPNY SPPGSPIEKY QYPLFGLPFV
1101 HNDFQSEADW LRFWSKYKLS VPGNPHYLSH VPGLPNPCQN YVPYPTFNLP
1151 PHFSAVGSND DPLDLAIKH SRPGPTANGA SKEKTKAPPN VKNEGPLNVV
1201 KTEKVDRSTQ DELSTKCVHC GIVFLDEVMY ALHMSCHGDS GPFQCSICQH
1251 LCTDKYDFTT HIQRGLHRNN AQVEKNGKPK E

```

Start - End	Observed	Mr (expt)	Mr (calc)	ppm	Miss	Sequence
405 - 424	691.99	2072.96	2073.04	-37	0	K.AGDDTPVGYSVPIKPLDSSR.Q (Ions score 54)
478 - 488	579.78	1157.56	1157.60	-42	0	R.GSVINQNDLAK.S (Ions score 10)
535 - 548	486.93	1457.78	1457.84	-38	0	K.SHGPDVIVVGPLLR.H (Ions score 58)
851 - 862	566.28	1130.54	1130.57	-29	0	K.GFLQGAPAGGK.S (Ions score 34)
953 - 965	734.37	1466.72	1466.77	-35	0	R.LNPEALQAEQLNK.Q (Ions score 42)
995 - 1003	492.27	982.53	982.57	-36	0	R.EIPLPSLSK.Y (Ions score 31)
1193 - 1201	485.25	968.50	968.53	-35	0	K.NEGPLNVVK.T (Ions score 23)

Match to: **SIPI1_HUMAN, Zinc finger homeobox protein 1b (Smad-interacting protein 1) (SMADI1)**

Primary accession number: **O60315**

Score: **124**

Nominal mass (M_r): **137844**; Calculated pI value: **5.90**

Sequence Coverage: **3%**

Found in sample: 1-C_1-6 **Significant Score N=31**

```
1 MKQPIMADGP RCKRRKQANP RRKNVVNYDN VVDTGSETDE EDKLVHIAEDD
51 GIANPLDQET SPASVPNHES SPHVSQALLP REEEEDEIRE GGVEHPWHNN
101 EILQASVDGP EEMKEDYDTM GPEATIQTAI NNGTVKNANC TSDFEYFAK
151 RKLLEERDGHV VSIEEYLQRS DTAIYPEAP EELSRLGTPE ANGQEENDLP
201 PGTPDAFAQL LTCPYCDRGY KRLTSLKEHI KYRHEKNEEN FSCPLCSYTF
251 AYRTQLERHM VTHKPGTDQH QMLTQGAGNR KFKCTECGKA FKYKHHLLKEH
301 LRIHSGEKPY ECPNCKKRFV HSGSYSSHIS SKKCIGLISV NGRMRNNIKT
351 GSSPNSVSSS PTNSAITQLR NKLENGKPLS MSEQTGLLKI KTEPLDFNDY
401 KVLMAHGFV GTSPFMNGGL GATSPLGVHP SAQSPMQLHG VGMEAPLLGF
451 PTMNSNLSEV QKVLQIVDNT VSRQKMDCKA EEISKLKGYH MKDPCSQPEE
501 QGVTPSNIPP VGLPVVSHNG ATKSIIDYTL EKVNEAKACL QSLTTDSRRQ
551 ISNIKKEKLR TLIDLVTDDK MIENHNISTP FSCQFCKESF PGPIPLHQHE
601 RYLCKMNEEI KAVLQPHENI VPNKAGVFVD NKALLLSSVL SEKGMTSPIN
651 PYKDHMSVLK AYYAMNMEPN SDELLKISIA VGLPQEFVKE WFEQRKVYQY
701 SNSRSPSLER SSKPLAPNSN PPTKDSLPR SPVKPMDISIT SPSIAELHNS
751 VTNCDPPLRL TKPSHFTNIK PVEKLDHSRS NTPSPNLSS TSSKNSHSSS
801 YTPNSFSSEE LQAEPLDLSL PKQMKEPKSI IATKNKTKAS SISLDHNSVS
851 SSENSDEPL NLTFIKKEFS NSNNLDNKST NPVFSMNPFS AKPLYTALPP
901 QSAFPPATFM PPVQTSIPGL RPYPLDQMS FLPHMAYTYP TGAATFADMQ
951 QRRKYQRKQG FQGELLDGAQ DYMSGLDDMT DSDSCLSRKK IKKTESGMYA
1001 CDLCDKTFQK SSSLRHKYE HTGKRPHQCQ ICKKAFKHKH HLIEHSRLHS
1051 GEKPYQCDK GKRFSHSGSY SQHMNHRYSY CKREAEEREA AEREAREKGH
1101 LEPTLLMNR AYLQSIPTQG YSDSEERESM PRDGESEKEH EKEGEDGYGK
1151 LGRQDGDEEF EEEEESEENK SMDTDPETIR DEEETGDHSM DDSSEDGKME
1201 TKSDHEEDNM EDGM
```

Start - End	Observed	Mr (expt)	Mr (calc)	ppm	Miss	Sequence
463 - 473	622.33	1242.64	1242.69	-43	0	K.VLQIVDNTVSR.Q (Ions score 52)
561 - 570	566.79	1131.56	1131.60	-37	0	R.TLIDLVTDDK.M (Ions score 16)
612 - 624	486.92	1457.75	1457.80	-37	0	K.AVLQPHENIVPNK.A (Ions score 4)
633 - 643	580.33	1158.65	1158.69	-31	0	K.ALLLSSVLSEK.G (Ions score 53)

Match to: **TOP2A_RAT**, DNA topoisomerase 2-alpha (EC 5.99.1.3) (DNA topoisomerase II, alpha isozyme)

Primary accession number: **P41516**

Score: **305**

Nominal mass (M_r): **173853**; Calculated pI value: **8.83**

Sequence Coverage: **6%**

Found in sample: Ros_1-6 **Significant Score N=32**

```
1  MELSP LQPVN  ENMLLNKKKN  EDGKKRLSVE  RIYQKKTQLE  HILLRPDTYI
51  GSVELVTQQM  WYDEDEVGIN  YREVTFVPGGL  YKIFDEILVN  AADNKQRDPK
101 MSCIRVTMMR  NNLISIWNNG  KGIPVVEHKV  EKMYVPALIF  GQLLTSSNYD
151 DDEKKVTGGR  NGYGAKLCNI  FSTKFTVETA  SREYKKMFQK  TWMDNMGRAG
201 DMELKPFSGE  DYTCITFQPD  LSKFKMQSLD  KDIVALMVRR  AYDIAGSTKD
251 VKVFLNGNRL  PVKGFERSYVD  MYLKDKVDET  GNALKVVHEQ  VNPRWEVCLT
301 MSEKGFQQIS  FVNSIATSKG  GRHVDYVADQ  IVSKLVDVVK  KKNKGGVAVK
351 ADQVKNHMI  FGNAVIENPT  FDSQTKENMT  LQAKSFGSTC  QLSEKFIKAA
401 ICGGIVESIL  NWWKFKAQIQ  LNKKCSAVKH  NRIKGIPKLD  DANDAGSRNS
451 AECTLILTEG  DSAKTLAVSG  LGVVGRDKYG  VFPLRGKILN  VREASHKQIM
501 ENAEINNIK  IVGLQYKKNY  EDEDSLKTLR  YGKIMIMTDQ  DQDGSHIKGL
551 LINFIIHNWP  SLLRHRFLEE  FITPIVKVSK  NKQEI AFYSL  PEFEEWKSTN
601 PNHKKWKVKY  YKGLGTSTSK  EAKEYFANMK  RHRIQFKYSG  PEDDAAISLA
651 FSKKQVDDRK  EWLTFNFMEDR  RQRKLLGLPE  DYLYGQTTMY  LTYNDFINKE
701 LILFNSDNE  RSIPSMVDGL  KPGQRKVLFT  CFKRNDKREV  KVAQLAGSVA
751 EMSSYHHGEM  SIMMTIINLA  QNFVGSNNLN  LLQPIGQFGT  RLHGGKDSAS
801 PRYIFTMLSP  LARLLFP SKD  DHTLRFLYDD  NQRVEPEWYI  PIIPMVLING
851 AEGIGTGWSC  KIPNFDVREV  VNNIRRLLDG  EEPLPMLPSY  KNYKGTIEEL
901 ASNQVINGE  VAILNSTTIE  ITELPIRTWT  QTYKEQVLEP  MLNGTEKTPP
951 LITDYREYHT  DTTVKFVIKM  TEEKLAEAEER  VGLHKVFKLQ  TSLTCNSMVL
1001 FDHVGCLKKY  DTVLDILRDF  FELRLKYYGL  RKEWLLGMLG  AESSKLNQQA
1051 RFILEKIDGK  IVIENKPKKE  LIKVLIQRGY  DSDPVKAWKE  AQQKVPEEEEE
1101 NEENESESE  STSPAAESGP  TFNYLLDMPL  WYLTKEKKDE  LCKQRDEKEQ
1151 ELNTLKKKTP  SDLWKEDLAA  FVEELEVVEA  KEKQDEQVGL  PGKGVKAKGK
1201 KAQISEVLPS  PVGKRVIPQV  TMEMRAEAEK  KIRRKIKSEN  VEGTPAEDGA
1251 EPGLRQRLEK  RQKREPQTRA  KKQTTLPFKP  IKKAQKQNPW  SDESSEDMSSN
1301 ESNFDVPPRE  KEPRIAATKA  KFTADLSDSD  DFSGLDEKDE  DEDFFPLDDT
1351 PPKTKMPPKN  TKKALKPQKS  STSVDLESDG  KDSVPASPGA  SAADVPAETE
1401 PSKPSSKQTV  GVKRTITKGQ  SLTSTAGTKK  RAVPKETKSD  SALNAHVSKK
1451 PAPAKAKNSR  KRMPSSSDSS  DSEFEKAISK  GATSKKLGKE  ERDFHVDLDD
1501 TVAPRAKSGR  ARKPIKYLEE  SDDDLF
```

Start - End	Observed	Mr (expt)	Mr (calc)	ppm	Miss	Sequence
73 - 82	576.80	1151.59	1151.62	-32	0	R.EVTFVPGLYK.I (Ions score 4)
83 - 95	731.36	1460.70	1460.75	-36	0	K.IFDEILVNAADNK.Q (Ions score 28)
465 - 476	564.82	1127.63	1127.67	-31	0	K.TLAVSGLGVVGR.D (Ions score 47)
479 - 485	426.23	850.45	850.47	-26	0	K.YGVFPLR.G (Ions score 39)
567 - 577	668.36	1334.70	1334.75	-38	0	R.FLEEFITPIVK.V (Ions score 36)
712 - 725	500.92	1499.73	1499.78	-32	0	R.SIPSMVDGLKPGQR.K Oxidation (M) (Ions score 32)
862 - 868	430.72	859.43	859.46	-33	0	K.IPNFDVR.E (Ions score 24)
948 - 956	538.28	1074.54	1074.57	-32	0	K.TPPLITDYR.E (Ions score 28)
1010 - 1018	554.29	1106.56	1106.60	-37	0	K.YDTVLDILR.D (Ions score 71)

Match to: **TRPS1_MOUSE, Zinc finger transcription factor Trps1**

Primary accession number: **Q925H1**

Score: **177**

Nominal mass (M_r): **143170**; Calculated pI value: **7.53**

Sequence Coverage: **6%**

Found in sample: Ros_1-6 **Significant Score N=32**

1 MVRKKHPPPLR NVASEGEGQT LEPTATESKV SGKNKELSAD QMSENTDQSD
51 VAELNSKEEH STHGQEPSSS GKKDLQISGL SEKAGFNYES PSKGGSLVSF
101 PHDEVTDNRN LAFSSPAAGG VCEPLKSPQR AEADDPQDMA CTPSGDSLET
151 KEEHKMSPKA TEETGPVQSG QANCQGLSPV SVASK**NPQVP SDGGVRLSKP**
201 KGDLLVNDNP DPAPLSPELQ DFKCNICGYG YYGNDPTDLI KHFRKYHLGL
251 HNRTRQDAEL DSKILALHNM VQFSHSKDFQ KVNRSVLSGV LQDISSSRPA
301 LLNGTYDVQV TSGGTFIGIG RKTTPDCQNT KYFRCKFCNF TYMGNSSTEL
351 EQHFLQTHPN KIKVSLPSSE GVKPSEKNSN KSIPALRASD SGDVGKWQDK
401 M**TVKAGDDTP VGYSVPIKPL DSSR**QNGTEA TSYYWCKFCS F**SC**ESSSSLK
451 LLEHYGKQHG AVQSGGLNPE LNDKLPRGSV **INQNDLAKSV** EGEPLTKPEK
501 GLSGAKKKDF PSKGAEDNMV TSYNCQFCDF RYSK**SHGPDV IVVGP**LLRHY
551 QQLHNIHKCT IKHCPFCPRG LCSPEKHLGE ITYPFACRKS NCSHCALLLL
601 H**L**SPGVAGSS RVKHQCHQCS FSTPDVDVLL FHYETVHESQ ASDVKQEANH
651 LLGSDGQQAV RDSKEHSCTK CDFITQVEEE ISRHYRRAHS CYKCRQCSFT
701 AADTQSLLEH FNTVHCQEQE ITTANGEEGG HAIPTIKEEP KIDLKVSLL
751 NPDSKMGETV PESIVKREKL DDKEGLKDKI WTESSTDDL R GVAWRGADIL
801 R**G**SPSYTQAS LGLLTPVSSS QEQT~~KT~~LRDS PNVEAAHLAR PMYGLAVDTK
851 GFLQGAPAGS EKSASLTQQY PASGESKTKD ESQSLRRRRR GSGVFCANCL
901 TTKTSLWRKN ANGGYVCNAC GLYQKLHSTP RPLNIIKQNN GEQIIRRRTR
951 **KRLNPEALQA EQLNK**QQRGS GEEQVNGSPL ERRSEDHLSE SHPR**EIPLPS**
1001 **LSK**YEAQGS L TKSHSAQQPV LVSQALDIHK RMQPLHIQIK SPQESTGDPG
1051 NSSSVSDGKG SSERGSPIEK YMRPAKHPNY SPPGSPIEKY QYPLFGVVPFV
1101 HND**FQ**SEADW LRFWSKYKLS VPGNPHYLSH VPGLPNPCQN YVPYPTFNL
1151 PHFS**AVG**SDN DIPLDLAIKH SRPGPTANGA SKEKTKAPPT VKNEDPLNVV
1201 KTEK**VDR**STQ DELSTKCVHC GIVFLDEVMY ALHMSCHGDS GPFQCSICQH
1251 LCTDKYDFTT HIQRGLHRNN AQA**E**KNGKPK E

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
186 - 196	563.77	1125.52	1125.54	-19	0	K.NPQVP SDGGVRL .L Deamidated (NQ) (<u>Ions score 2</u>)
405 - 424	691.99	2072.96	2073.04	-37	0	K. AGDDTPVGYSVPIKPLDSSR .Q (<u>Ions score 58</u>)
478 - 488	579.79	1157.57	1157.60	-31	0	R. G SVIN QNDLAK .S (<u>Ions score 1</u>)
535 - 548	486.94	1457.79	1457.84	-33	0	K. SHGPDVIVVGP LLR.H (<u>Ions score 58</u>)
953 - 965	734.37	1466.73	1466.77	-32	0	R. LNPEALQA EQLNK.Q (<u>Ions score 27</u>)
995 - 1003	492.27	982.54	982.57	-35	0	R. EIPLPSLSK .Y (<u>Ions score 32</u>)

Match to: **TOP2B_MOUSE, DNA topoisomerase 2-beta (EC 5.99.1.3) (DNA topoisomerase II, beta isozyme)**

Primary accession number: **Q64511**

Score: **145**

Nominal mass (M_r): **182707**; Calculated pI value: **8.46**

Sequence Coverage: **3%**

Found in sample: Ros_1-6 **Significant Score N=32**

```
1 MAKSSLAGSD GALTWVNNAT KKEELETANK NDSTKKLSVE RVYQKKTQLE
51 HILLRPTDYI GSVEPLTQLM WVYDEDEVGMN CREVTFVPGGL YKIFDEILVN
101 AADNKQRDKN MTCIKVSI DP ESNIISIWNN GKGIPVVEHK VEKVYVPALI
151 FGQLLTSSNY DDDEKKVTGG RNYGAKLCN IFSTKFTVET ACKEYKHSEFK
201 QTWMNMMKT SEAIKIKHFDG EDYTCITFQP DLSKFKMEKL DKDIVALMTR
251 RAYDLAGSCK GVKVMFNGKK LPVNGFRSYV DLYVKDKLDE TGVALKVIHE
301 LANERWDVCL TLSEKGFQQI SFVNSIATTK GGRHVDYVVD QVVS KLIEVV
351 KKKNKAGVSV KPFQVKNHIW VFINCLIENP TFDSQTKENM TLQPKSFGSK
401 CQLSEKFFKA ASNCGIVESI LNWWKFKAQ TQLNKKCSSVK YSKIKGIPKL
451 DDANDLAGGKH SLECTLILTE GDSAKSLAVS GLGVIGRDRY GVFPLRGKIL
501 NVREASHKQI MENAEINNII KIVGLQYKKS YDDAESLKT L RY GKIMIMTD
551 QDQDGSHIKG LLINFIIHNN PSL LKHGFLE EFITPIVKAS KNKQELSFYS
601 IPEFDEWKKH IENQKAWKIK YYKGLGTSTA KEAKEYFADM ERHRILFRYA
651 GPEDDAAITL AFSK KIDDR KEWLTNFMED RRQRRLHGLP EQFLYGTATK
701 HLTYNDFINK ELILFSNSDN ERSIPSLVDG FKPGQRKVL F TCFKRNDKRE
751 VKVAQLAGSV AEMSAYHHGE QALMMTIVNL AQNFVGSNNI NLLQPIGQFG
801 TR LHGGK DAA SPRYIF TMLS SLARLLFP AV DDNLLKFLYD DNQRVEPEWY
851 IPIIPMVLIN GAEGIGT GWA CKLPNYDARE IVNNVRRMLE GLDPHPMLPN
901 YKNFKGTIQE LGQNQYAVSG EIFVVD RNTV EITELPVRTW TQVYKEQVLE
951 PMLNGTDKTP ALISDYKEYH TDTTVKFVVK MTEEKLAQAE AAGLHKVFKL
1001 QTTLTCNSMV LFDHMGCLKK YETVQDILKE FFDLRLSYYG LRKEWLVGML
1051 GAESTKLNNQ ARFILEKIQQ KITIENRSKK DLIQMLVQRG YESDPVKAWK
1101 EAQEKA AEEEE DSQNQHDDSS SDSGTPSGPD FNYILNMSLW SLTKEKVEEL
1151 IKQRDTKGRE VNDLKRKSPS DLWKEDLAAF VEELDKVEAQ EREDILAGMS
1201 GKAIKGVGK PKVKKLQLEE TMPSPYGRRI VPEITAMKAD ASRKL LKKKK
1251 GDPDTTVVKV EFDEEFSGTP AEGTGEETLT PSAPVNKGPK PKREKKEPGT
1301 RVRKTPTSTG KTNAKKVKKR NPWSDDESKS ESDLEEAEPV VIPRDSL LRR
1351 AAAERP KYTF DFSEEE DDDA AAADDSNDLE ELKVKASPI T NDGEDEFVPS
1401 DGLDKDEYAF SSGKSKATPE KSSNDKKSQD FGNLFSFPSY SQKSEDDSAK
1451 FDSNEEDTAS VFAPSFGLKQ TDKLPSKTVA AKKGKPPSDT APKAKRAPKQ
1501 KKIVETINS DSDSEFGIPKK TTPKGKGRG AKKRKASGSE NEG DYNPGRK
1551 PSKTASKKPK KTSFDQSDV DIFPSDFTSE PPALPRTGRA RKEVKYFAES
1601 DEEEDVDFAM FN
```

Start - End	Observed	Mr (expt)	Mr (calc)	ppm	Miss	Sequence
83 - 92	576.80	1151.59	1151.62	-32	0	R.EVTFVPGLYK.I (<u>Ions score 4</u>)
93 - 105	731.36	1460.70	1460.75	-36	0	K.IFDEILVNAADNK.Q (<u>Ions score 28</u>)
490 - 496	426.23	850.45	850.47	-26	0	R.YGVFPLR.G (<u>Ions score 39</u>)
814 - 824	659.32	1316.63	1316.68	-37	0	R.YIF TMLSSLAR.L Oxidation (M) (<u>Ions score 39</u>)
825 - 836	679.37	1356.73	1356.77	-27	0	R.LLFPVDDNLLK.F (<u>Ions score 26</u>)
1081 - 1089	566.26	1130.50	1130.61	-98	0	K.DLIQMLVQR.G Oxidation (M) (<u>Ions score 10</u>)

Match to: **NPM_HUMAN, Nucleophosmin, Nucleolar phosphoprotein B23, Numatrin, Nucleolar protein NO38.**

Primary accession number: **P06748**

Score: **109**

Nominal mass (M_r): **32726**; Calculated pI value: **4.64**

Sequence Coverage: **18%**

Found in sample U-4-6 **Significant Score N=44**

1 MEDSMDMDMS PLRPQNYLFG CELKADKDYH FKVDNDENEH QLRLRTVSLG
51 AGAK**DELHIV EAEAMNYEGS PIK**VTLATLK **MSVQPTVSLG GFEITPPVVL**
101 **RLKCGSGPVH** ISGQHLVAVE EDAESEDEEE EDVKLLSISG KRSAPGGGSK
151 VPQKKVKLAA DEDDDDDDEE DDEDDDDDD FDDEEAEEKA PVKKSIRDTP
201 AKNAQKSNQN GKDSKPSSTP RSKGQESFKK QEKTPKTPKG PSSVEDIKAK
251 MQASIEKGGG LPKVEAKFIN YVKNCFR**MTD QEAIQDLWQW** RKSL

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
55 - 73	720.97	2159.89	2160.00	-53	0	K.DELHIVEAEAMNYEGS PIK.V Oxidation (M) (Ions score 50)
81 - 101	743.06	2226.16	2226.21	-23	0	K. MSVQPTVSLGGFEITPPVVL.R.L (Ions score 59)
81 - 101	748.36	2242.06	2242.20	-62	0	K. MSVQPTVSLGGFEITPPVVL.R.L Oxidation (M) (Ions score 54)
278 - 291	612.59	1834.75	1834.83	-41	0	R. MTDQEAIQDLWQWR.K Oxidation (M) (Ions score 21)

Match to: **ACTB_RAT, Actin, cytoplasmic 1 (Beta-actin)**

Primary accession number: **P60711**

Score: **305**

Nominal mass (M_r): **42052**; Calculated pI value: **5.29**

Sequence Coverage: **42%**

Found in sample Ros Mix-02 **Significant Score N=43**

1 MDDDIAALVV DNGSGMCKAG **FAGDDAPRAV** FPSIVGRPRH QGVMVGMGQK
51 DSYVGDEAQS KRGILTLKYP IEHGIVTNWD DMEKIWHHTF YNELRVAPEE
101 **HPVLLTEAPL** **NPKANREKMT** **QIMFETFNT** **AMYVAIQAVL** **SLYASGR**TTG
151 IVMDSGDGVV HTVPIYEGYA LPHAILRLDL AGRDLTDYLM **KILTERGYSE**
201 **TTTAEREIVR** DIKEKLCYVA **LDFEQEMATA** **ASSSSLEKSY** **ELPDGQVITI**
251 **GNERFRCPEA** LFQPSFLGME SCGIHETTFN SIMKCDVDIR **KDLYANTVLS**
301 **GGTMYPGIA** **DRMQKEITAL** **APSTMKIKII** APPERKYSVW IGGSILASLS
351 TFQQMWISK**Q** **EYDESGPSIV** **HRKCF**

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
19 - 28	488.68	975.35	975.44	-96	0	K.AGFAGDDAPR.A (<u>Ions score 39</u>)
19 - 28	488.68	975.35	975.44	-91	0	K.AGFAGDDAPR.A (<u>Ions score 18</u>)
19 - 28	488.69	975.37	975.44	-70	0	K.AGFAGDDAPR.A (<u>Ions score 38</u>)
96 - 113	651.96	1952.87	1953.06	-95	0	R.VAPEEHPVLLTEAPLNPK.A (<u>Ions score 24</u>)
96 - 113	651.97	1952.89	1953.06	-84	0	R.VAPEEHPVLLTEAPLNPK.A (<u>Ions score 11</u>)
119 - 147	1101.10	3300.29	3300.59	-89	0	K.MTQIMFETFNTPAMYVAIQAVLSLYASGR.T 3 Oxidation (M) (<u>Ions score 43</u>)
119 - 147	1101.77	3302.29	3302.55	-80	0	K.MTQIMFETFNTPAMYVAIQAVLSLYASGR.T 2 Deamidation (NQ); 3 Oxidation (M) (<u>Ions score 31</u>)
184 - 191	507.70	1013.39	1013.47	-86	0	R.DLTDYLMK.I Oxidation (M) (<u>Ions score 9</u>)
197 - 206	566.72	1131.42	1131.52	-85	0	R.GYSFTTTAER.E (<u>Ions score 42</u>)
216 - 238	855.98	2564.92	2565.16	-94	0	K.LCYVALDFEQEMATAASSSSLEK.S Oxidation (M) (<u>Ions score 26</u>)
239 - 254	895.88	1789.74	1789.88	-80	0	K.SYELPDGQVITIGNER.F (<u>Ions score 17</u>)
292 - 312	744.29	2229.86	2230.06	-90	0	K.DLYANTVLSGGTMYPGIADR.M Oxidation (M) (<u>Ions score 28</u>)
316 - 326	590.26	1178.51	1178.61	-84	0	K.EITALAPSTMK.I O18 (C-term); Oxidation (M) (<u>Ions score 19</u>)
360 - 372	750.28	1498.54	1498.67	-88	0	K.QEYDESGPSIVHR.K Pyro-glu (N-term Q) (<u>Ions score 59</u>)
360 - 372	751.28	1500.55	1500.67	-83	0	K.QEYDESGPSIVHR.K O18 (C-term); Pyro-glu (N-term Q) (<u>Ions score 56</u>)
360 - 372	506.20	1515.57	1515.70	-85	0	K.QEYDESGPSIVHR.K (<u>Ions score 25</u>)

Match to: **TPM1_RAT, Tropomyosin 1 alpha chain (Alpha-tropomyosin)**

Primary accession number: **P04692**

Score: **216**

Nominal mass (M_r): **32718**; Calculated pI value: **4.69**

Sequence Coverage: **15%**

Found in sample Ros 5-6 **Significant Score N=32**

1 MDAIKKKMQM LKLDKENALD RAEQAEADKK AAEDRSK**QLE** **DELVSLQK**KL
51 KGTEDELDKY SEALKDAQEK LELAEKKATD AEADVASLNR **RIQLVEEELD**
101 **RAQERL**TAL QKLEEAEKAA DESERGMKVI ESRAQKDEEK **MEIQEIQLKE**
151 **AKHIAE**DADR KYEEVAR**KL**V **IESDLER**AE ERAELSEGKC AELEEEELKTV
201 TNNLKSLEAQ AEKYSQKEDK YEEEIKVLSD KLKEAETRAE FAERSVTKLE
251 KSIDDLEDEL YAQKLKYKAI SEELDHALND MTSI

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
38 - 48	642.81	1283.61	1283.66	-43	0	K.QLEDELVSLQK.K Gln->pyro-Glu (N-term Q) (<u>Ions score 27</u>)
38 - 48	651.32	1300.63	1300.69	-40	0	K.QLEDELVSLQK.K (<u>Ions score 15</u>)
91 - 101	467.24	1398.69	1398.75	-40	1	R.RIQLVEEELDR.A (<u>Ions score 51</u>)
92 - 101	622.31	1242.60	1242.65	-37	0	R.IQLVEEELDR.A (<u>Ions score 69</u>)
141 - 149	574.28	1146.55	1146.60	-43	0	K.MEIQEIQLK.E Oxidation (M) (<u>Ions score 22</u>)
141 - 152	492.58	1474.72	1474.77	-35	1	K.MEIQEIQLKEAK.H Oxidation (M) (<u>Ions score 8</u>)
168 - 178	438.91	1313.70	1313.76	-42	1	R.KLVIIESDLER.A (<u>Ions score 41</u>)

Match to: **H12_RAT, Histone H1.2 (H1d)**

Primary accession number: **P15865**

Score: **156**

Nominal mass (M_r): **21843**; Calculated pI value: **11.10**

Sequence Coverage: **15%**

Found in sample Ros 5-6 **Significant Score N=32**

1 SEAAPAAPAA APPAEKAPAK KKAACKPAGV RR**KASGPPVS ELITK**AVAAS
51 KER**SGVSLAA LKALAAAGY DVEK**NNSRIK LGLKSLVSKG ILVQTKGTGA
101 SGSEFKLNKKA ASGEAKPQAK KAGAAKAKKP AGAAKKPKKA TGAATPKKAA
151 KKTPKKAKKP AAAAVTKKVA KSPKKAKVTK PKKVKASASKA VKPKAAKPKV
201 AKAKKVAACK K

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
33 - 45	442.90	1325.68	1325.76	-55	1	R.KASGPPVSELITK.A (Ions score 44)
34 - 45	599.81	1197.62	1197.66	-38	0	K.ASGPPVSELITK.A (Ions score 35)
54 - 62	423.24	844.47	844.50	-41	0	R.SGVSLAALK.K (Ions score 44)
64 - 74	554.26	1106.50	1106.56	-51	0	K.ALAAAGYDVEK.N (Ions score 32)

Match to: **RUNX2_MOUSE, Runt-related transcription factor 2 (Core-binding factor, alpha 1 subunit)**

Primary accession number: **Q08775**

Score: **47**

Nominal mass (M_r): **66563**; Calculated pI value: **9.37**

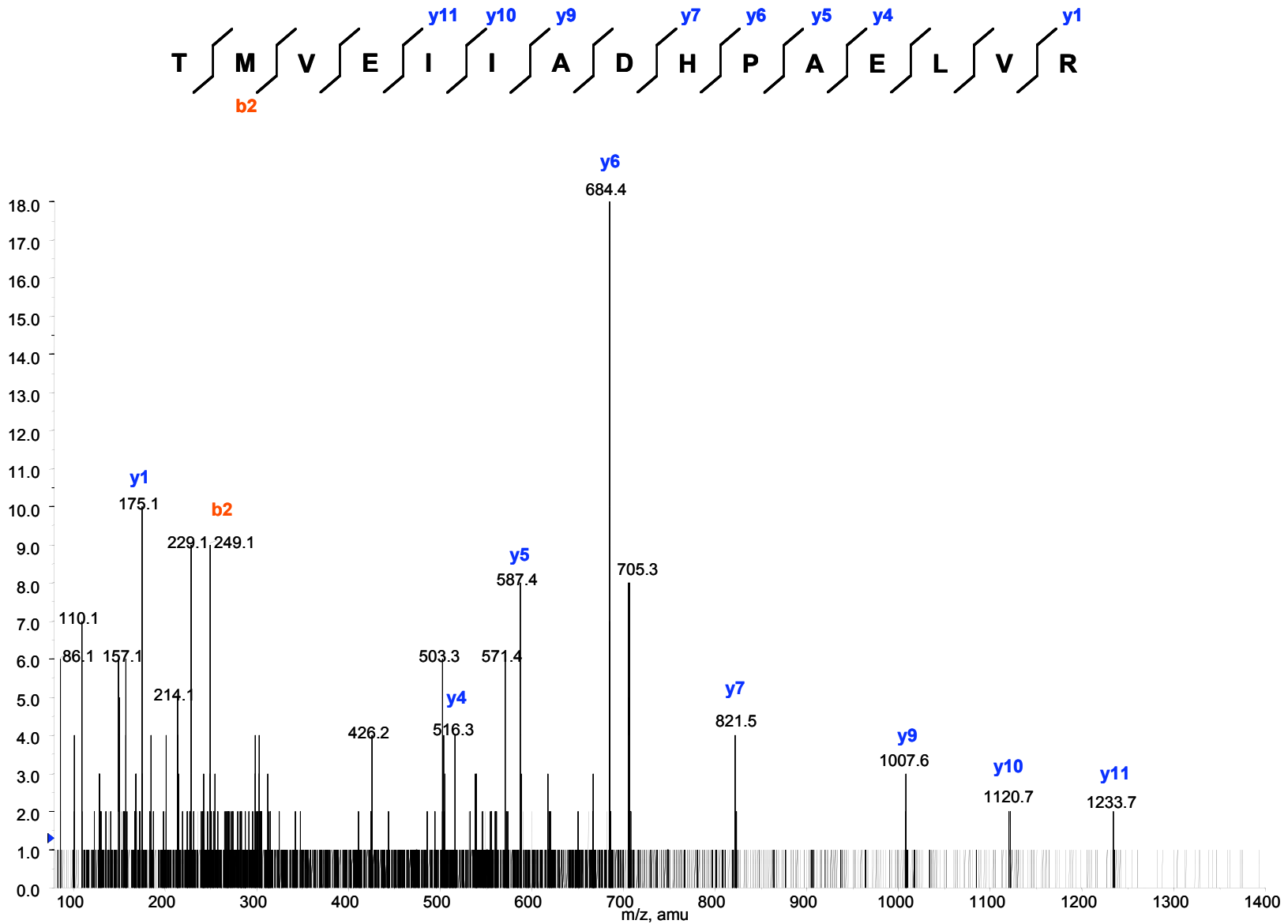
Sequence Coverage: **4%**

Found in sample Ros 5-6 **Significant Score N=32**

```
1 MLHSPHKQPQ NHKCGANFLQ EDCKKALAFK WLISAGHYQP PRPTESVSAL
51 TTVHAGIFKA ASSIYNRGHK FYLEKKGGTM ASNSLFSAVT PCQQSFFWDP
101 STSRRFSPPS SSLQPGKMSD VSPVVAQQQ QQQQQQQQQQ QQQQQQQQQQ
151 QQQQQQEAAA AAAAAAAAAA AAAAAVPLR PPHDNRTMVE IIADHPAELV
201 RTDSPNFLCS VLPSHWRCNK TLPVAFKVVA LGEVPDGTVV TVMAGNDENY
251 SAELRNASAV MKNQVAREFND LRFVGRSGRG KSFTLTITVF TNPPQVATYH
301 RAIKVTVDGP REPRRHRQKL DDSKPSLFSR RLSDLGRIPH PSMRVGVPPQ
351 NPRPSLNSAP SPFNPQQSQS ITDPRQAQSS PPWSYDQSY S YLSQMTSPS
401 IHSTPLSST RGTGLPAITD VPRRISDDDT ATSDFCLWPS SLSKKSQAGA
451 SELGPFSDPR QFPSISLSTE SRFSNPRMHY PATFTYTPPV TSGMSLGMSA
501 TTHYHTYLPP PYPGSSQSQS GPFQTSSTPY LYYGTSSASY QFPMVPGGDR
551 SPSRMVPPCT TTSNGSTLLN PNLPNQNDGV DADGSHSSSP TVLNSSGRMD
601 ESVWRPY
```

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence	
187 - 201	570.61	1708.82	1708.88	-38	0	R.TMVEIIADHPAELVR.T	Oxidation (M) (Ions score 37)
263 - 272	412.21	1233.61	1233.61	0	1	K.NQVAREFNDLR.F	2 Deamidated (NQ) (Ions score 10)

Product Ion Scan of ion of m/z 570.6@ 26.55 min of the Ros 5-6 Sample Gradient



Supplemental Figure S3. Product ion scan of the triply charged molecular ion at m/z 570.6 detected in sample Ros 5-6 that was authenticated as 187TMVEIADHPAELVR201 from Runt-related transcription factor 2[RUNX2_MOUSE(Q08775)]. Data were acquired during LC MS/MS analysis at elution time 26.55 min. Fragment ions of y - and b -series are annotated in blue and red, respectively. As illustrated by the peptide sequence drawing on the top, the majority of peptide bonds generated discernable fragmentation ions providing solid evidence as to the peptide identity. No differentiation between leucine and isoleucine amino acid residues was possible under conditions of the low energy CID (collision induced dissociation) experiment; their annotation as I and L in the peptide sequence is based on protein database information and was not established in the course of this analysis.

Supplemental Table 1. Primers used to make *Trps1*-shRNA lentivirus

shRNA1

5' -TGGAATTCAATAGCTTGTAATTCAAGAGATTACAAGCTATTGAATTCCTTTTTTC-3'

5' -TCGAGAAAAAAGGAATTCAATAGCTTGTAATCTCTTGAATTACAAGCTATTGAATTCCA-3'

shRNA2

5' -TGGATGCACTGTCAGATTAATTCAAGAGATTAATCTGACAGTGCATCCTTTTTTC-3'

5' -TCGAGAAAAAAGGATGCACTGTCAGATTAATCTCTTGAATTAATCTGACAGTGCATCCA-3'

shRNA3

5' -TGGGTGTTCCCTGACGATTAATTCAAGAGATTAATCGTCAGGAACACCCTTTTTTC-3'

5' -TCGAGAAAAAAGGGTGTTCCTGACGATTAATCTCTTGAATTAATCGTCAGGAACACCCA-3'

Supplemental Table 2. Primers used for Sybr Green qRT-PCR

rat *Trps1*

5' -GTGCCTTATCCCACCTTCAA-3'
5' -ATGCCACAGTGCACACATTT-3'

rat *GAPDH*

5' -ATGTGTCCGTCGTGGATCTGA -3'
5' -TTGAAGTCGCAGGAGACAACCT-3'

rat Osteocalcin

5' -GGTGCAAAGCCCAGCGACTCT-3'
5' -GGAAGCCAATGTGGTCCGCTA-3'

rat *Runx2*

5' -CACCGAGACCAACCGAGTCA-3'
5' -TGCTCGGATCCCAAAGAAG-3'

mouse *Trps1*

5' -AGCCCAGGGTTCATTGACTAAAAG-3'
5' -AAGCCAGGCACATGACTCAAGTAG-3'

mouse *RPL19*

5' -ATGTATCACAGCCTGTACCTG-3'
5' -TTCTTGGTCTCTTCCTCCTTG-3'

mouse Collagen 1A1

5' -CAACCTGGACGCCATCAAG-3'
5' -ATCGGTCATGCTCTCTCCAAA-3'

mouse Osteocalcin

5' -AGCTTAACCCTGCTTGTGACG-3'
5' -GGAGGATCAAGTCCCGGAGA-3'

mouse *Runx2*

5' -CACCGAGACCAACCGAGTCA-3'
5' -TGCTCGGATCCCAAAGAAG-3'

mouse *OPG*

5' -AGAGCAAACCTTCCAGCTGC-3'
5' -CTGCTCTGTGGTGAAGTTTCG-3'

mouse *RankL*

5' -CACCATCAGCTGAAGATAGT-3'
5' -CCAAGATCTCTAACATGACG-3'

mouse Osteopontin

5' -GTATTGCTTTTGCCTGTTTGG-3'
5' -TGAGCTGCCAGAATCAGTCACT-3'

mouse Alkaline Phosphatase

5' -GTGCAGTCTGTGTCTTGCCTG-3'
5' -CCTTGCCTGTATCTGGAATCCT-3'

mouse *MMP13*

5' -CGGGAATCCTGAAGAAGTCTACA-3'
5' -CTAAGCCAAAGAAAGATTGCATTTTC-3'

Supplemental Table 3. Primers and probes used for Taqman qRT-PCR

RPL19

5'-GCGGATTCTCATGGAACACATCCACA-3'

5'-CTGGTCAGCCAGGAGCTT-3'

TaqMan probe: 5'-AGCTGAAGGCAGACAAGGCCCGCAA-3'

TRPS1

5'-TCTGAGTGAGAAGGCTGGCTTCAA-3'

5'-TGTCTGTCACCTCATCATGCGGAA-3'

TaqMan probe: 5'-CCAGTAAGGGAGGAAACTTTCCCTCCT-3'

Osteocalcin

5'-ACCCAGGCGCTACCTGTATCAAT-3'

5'-ATAGGCCTCCTGAAAGCCGATGT-3'

TaqMan probe: 5'-TGTGTGAGCTCAATCCGGACTGTGA-3'

RUNX2

5'-CCCGTGGCCTTCAAGGT-3';

5'-TGACAGTAACCACAGTCCCATCTG-3';

TaqMan probe: 5'-ACCTCTCCGAGGGCTA-3'

Supplemental Table 4. Primers used for Chromatin Immunoprecipitation analysis

mouse IgC- α

5' -GGAATTCGCTGGAGTCCTGGCCCCATCA-3'

5' -CTCCACGTCATGTCTGGCC-3'

mouse Osteocalcin

5' -CCGCTCTCAGGGGCAGAC-3'

5' -AGGGGATGCTGCCAGGACTAAT-3'