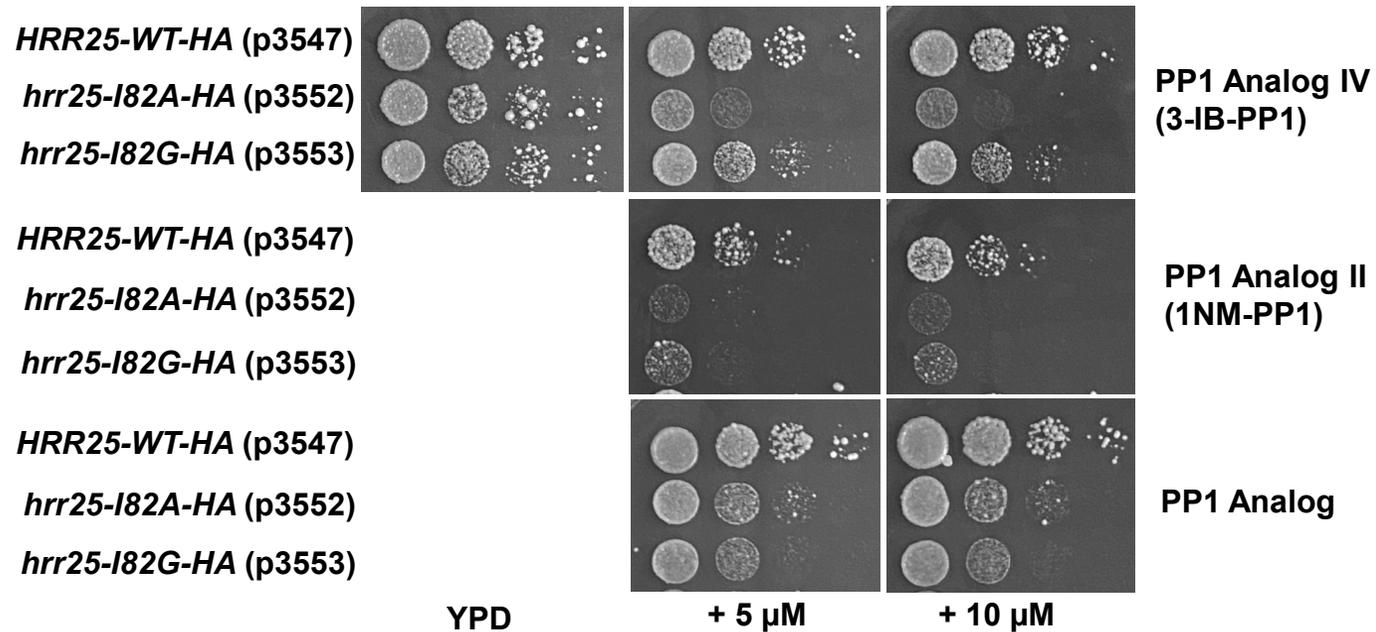


**Supplemental Figure S1.** HU treatment induces association of Pkc1 with Hrr25. Wild-type cells (DL100) co-expressing Pkc1-HA (from p813) and Hrr25-GFP (from p3357), or Pkc1-HA alone, were treated with 250 mM HU for 4 h. Hrr25-GFP was immunoprecipitated (IP) from extracts with GFP-trap beads and samples were tested by immunoblot analysis for co-IP of Pkc1-HA. Input Pkc1-HA from extracts is shown at bottom. Non-specific binding of Pkc1-HA to beads was not detected.



**Supplemental Figure S2. Sensitivity of *hrr25* “gatekeeper” mutants to growth inhibition by inhibitory ATP analogs.** Plasmids with the indicated alleles of *HRR25* were introduced into strain DL4290, which bears a chromosomal *hrr25*Δ mutation maintained by a plasmid-borne copy of *HRR25*<sup>degron</sup> under the inducible control of the *GAL1* promoter. This strain is only viable on galactose-containing medium. Transformants were plated at 10-fold dilutions (from left to right) on YPD, or YPD plus the indicated concentration of one of three protein kinase inhibitors and incubated for 3 days at 30 °C.



**Supplemental Figure S3. Pkc1 phosphorylation sites identified in this study.** Ser and Thr residues that are shown in bold indicate identified phosphorylation sites according to the following confidence levels: black: >95% site confidence; red: >85% site confidence; yellow: >75% site confidence. Red asterisks indicate sites that have only been found in experiments in which cells were grown in YPD (rather than in SILAC experiments). Black lines indicate sequence coverage.



Supplemental Table S2. Pkc1-associated proteins without HU only

Description

RP59A SGDDID:5000006002, Chr XVI from 404950-404956,405458-406044, Genome Release 64-1-1, Verified ORF, ""Protein component of the small (40S) ribosomal subunit; nearly identical to Rps9Bp and has similarity to E. coli S4 and rat S9 ribosomal proteins

RPL2B SGDDID:5000001280, Chr IX from 316768-316771,317172-317932, Genome Release 64-1-1, Verified ORF, ""Protein component of the large (60S) ribosomal subunit, identical to Rpl2Ap and has similarity to E. coli L2 and rat L8 ribosomal proteins; expres

PET10 SGDDID:5000001754, Chr XI from 525074-524223, Genome Release 64-1-1, reverse complement, Verified ORF, ""Protein of unknown function that co-purifies with lipid particles; expression pattern suggests a role in respiratory growth; computational a

RPL27B SGDDID:5000002879, Chr IV from 1401770-1401800,1402185-1402564, Genome Release 64-1-1, Verified ORF, ""Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl27Ap and has similarity to rat L27 ribosomal protein""

RPL13A SGDDID:5000002240, Chr IV from 308424-308427,308793-309388, Genome Release 64-1-1, Verified ORF, ""Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl13Bp; not essential for viability; has similarity to rat L13 ribos

YRA1 SGDDID:5000002789, Chr IV from 1236558-1236842,1237609-1238004, Genome Release 64-1-1, Verified ORF, ""RNA binding protein required for export of poly(A)+ mRNA from the nucleus; proposed to couple mRNA export with 3' end processing via its interna

RPL8B SGDDID:5000003968, Chr XII from 48629-47859, Genome Release 64-1-1, reverse complement, Verified ORF, ""Ribosomal protein L4 of the large (60S) ribosomal subunit, nearly identical to Rpl8Ap and has similarity to rat L7a ribosomal protein; mutati

RP52 SGDDID:5000003091, Chr VII from 277617-278381, Genome Release 64-1-1, Verified ORF, ""Protein component of the small (40S) subunit, essential for control of translational accuracy; phosphorylation by C-terminal domain kinase I (CTDK-I) enhances t

ENO1 SGDDID:5000003486, Chr VII from 1000927-1002240, Genome Release 64-1-1, Verified ORF, ""Enolase I, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction d

RPS1B SGDDID:5000004528, Chr XIII from 146482-147249, Genome Release 64-1-1, Verified ORF, ""Ribosomal protein 10 (rp10) of the small (40S) subunit; nearly identical to Rps1Ap and has similarity to rat S3a ribosomal protein""

HTA2 SGDDID:5000000099, Chr II from 235792-235394, Genome Release 64-1-1, reverse complement, Verified ORF, ""Histone H2A, core histone protein required for chromatin assembly and chromosome function; one of two nearly identical (see also HTA1) subtyp

YGR161C-D SGDDID:5000007368, Chr VII from 821709-817747,823015-821711, Genome Release 64-1-1, reverse complement, transposable element, gene, ""Retrotransposon TYA Gag and TYB Pol genes; transcribed/translated as one unit; polyprotein is processed to m

RP531 SGDDID:5000004157, Chr XII from 498947-499405, Genome Release 64-1-1, Verified ORF, ""Fusion protein that is cleaved to yield a ribosomal protein of the small (40S) subunit and ubiquitin; ubiquitin may facilitate assembly of the ribosomal prot

ERG27 SGDDID:5000004090, Chr XII from 341810-342853, Genome Release 64-1-1, Verified ORF, ""3-keto sterol reductase, catalyzes the last of three steps required to remove two C-4 methyl groups from an intermediate in ergosterol biosynthesis; mutants ar

ZRT1 SGDDID:5000003224, Chr VII from 20978-22108, Genome Release 64-1-1, Verified ORF, ""High-affinity zinc transporter of the plasma membrane, responsible for the majority of zinc uptake; transcription is induced under low-zinc conditions by the Zap1

DPM1 SGDDID:5000006387, Chr XVI from 900755-901558, Genome Release 64-1-1, Verified ORF, ""Dolichol phosphate mannosyl synthase of the ER membrane, catalyzes the formation of Dol-P-Man from Dol-P and GDP-Man; required for glycosyl phosphati

EPL1 SGDDID:5000001870, Chr VI from 90345-87847, Genome Release 64-1-1, reverse complement, Verified ORF, ""Component of NuA4, which is an essential histone H4/H2A acetyltransferase complex; homologous to Drosophila Enhancer of Polycomb""

RP58A SGDDID:5000001068, Chr II from 89125-88523, Genome Release 64-1-1, reverse complement, Verified ORF, ""Protein component of the small (40S) ribosomal subunit; identical to Rps8Bp and has similarity to rat S8 ribosomal protein""

RPL21B SGDDID:5000006000, Chr XVI from 406636-406646,407068-407539, Genome Release 64-1-1, Verified ORF, ""Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl21Ap and has similarity to rat L21 ribosomal protein""

ERG1 SGDDID:5000003407, Chr VII from 848423-846933, Genome Release 64-1-1, reverse complement, Verified ORF, ""Squalene epoxidase, catalyzes the epoxidation of squalene to 2,3-oxidosqualene; plays an essential role in the ergosterol-biosynthesis pathway

KGD1 SGDDID:5000001387, Chr IX from 122689-125733, Genome Release 64-1-1, Verified ORF, ""Component of the mitochondrial alpha-ketoglutarate dehydrogenase complex, which catalyzes a key step in the tricarboxylic acid (TCA) cycle, the oxidative decarbo

RP527B SGDDID:5000010663, Chr VIII from 148116-147871,148669-148667, Genome Release 64-1-1, reverse complement, Verified ORF, ""Protein component of the small (40S) ribosomal subunit; nearly identical to Rps27Ap and has similarity to rat S27 ribosomal

SEF1 SGDDID:500000162, Chr II from 100115-96669, Genome Release 64-1-1, reverse complement, Verified ORF, ""Putative transcription factor, has homolog in Kluyveromyces lactis""

RPL43A SGDDID:5000006247, Chr XVI from 654166-654167,654571-654847, Genome Release 64-1-1, Verified ORF, ""Protein component of the large (60S) ribosomal subunit, identical to Rpl43Bp and has similarity to rat L37a ribosomal protein; null mutation con

SACT SGDDID:5000002797, Chr III from 1252537-1254501, Genome Release 64-1-1, Verified ORF, ""GTPase activating protein (GAP) for Rho1p, involved in signaling to the actin cytoskeleton, null mutations suppress tor2 mutations and temperature sensitive mu

PAT1 SGDDID:5000000673, Chr III from 252628-250238, Genome Release 64-1-1, reverse complement, Verified ORF, ""Topoisomerase II-associated deadenylation-dependent mRNA-decapping factor; also required for faithful chromosome transmission, maintenance o

MSS116 SGDDID:5000002602, Chr IV from 847946-845952, Genome Release 64-1-1, reverse complement, Verified ORF, ""DEAD-box protein required for efficient splicing of mitochondrial Group I and II introns; non-polar RNA helicase that also facilitates stran

SGF73 SGDDID:5000003034, Chr VII from 377609-379582, Genome Release 64-1-1, Verified ORF, ""SAGA complex subunit; has a role in anchoring the deubiquitination module into SAGA and SLIK complexes; involved in preinitiation complex assembly at promoters

RP517A SGDDID:5000004486, Chr XIII from 225889-225891,226290-226697, Genome Release 64-1-1, Verified ORF, ""Ribosomal protein 51 (rp51) of the small (40s) subunit; nearly identical to Rps17Bp and has similarity to rat S17 ribosomal protein""

HSC82 SGDDID:5000004798, Chr XIII from 632355-634472, Genome Release 64-1-1, Verified ORF, ""Cytoplasmic chaperone of the Hsp90 family, redundant in function and nearly identical with Hsp82p, and together they are essential; expressed constitutively a

CDC48 SGDDID:5000002284, Chr IV from 238664-236157, Genome Release 64-1-1, reverse complement, Verified ORF, ""ATPase involved in ubiquitin-mediated protein degradation; Cdc48p-Npl4p-Ufd1p complex participates in ER-associated degradation (ERAD) while

SL51 SGDDID:5000004129, Chr XII from 423473-421542, Genome Release 64-1-1, reverse complement, Verified ORF, ""Mitochondrial membrane protein that coordinates expression of mitochondrially-encoded genes; may facilitate delivery of mRNA to membrane-bou

OSH6 SGDDID:5000001711, Chr XI from 445381-446727, Genome Release 64-1-1, Verified ORF, ""Member of an oxysterol-binding protein family with overlapping, redundant functions in sterol metabolism and which collectively perform a function essential for

SSO1 SGDDID:5000006153, Chr XVI from 107275-108147, Genome Release 64-1-1, Verified ORF, ""Plasma membrane t-SNARE involved in fusion of secretory vesicles at the plasma membrane and in vesicle fusion during sporulation; forms a complex with Sec9p tha

ROG1 SGDDID:5000003112, Chr VII from 234507-232450, Genome Release 64-1-1, reverse complement, Verified ORF, ""Protein with putative serine active lipase domain""

MSH6 SGDDID:5000002504, Chr IV from 643837-640109, Genome Release 64-1-1, reverse complement, Verified ORF, ""Protein required for mismatch repair in mitosis and meiosis, forms a complex with Msh2p to repair both single-base & insertion-deletion mismatch

THO2 SGDDID:5000005083, Chr XIV from 365717-360924, Genome Release 64-1-1, reverse complement, Verified ORF, ""Subunit of the THO complex, which is required for efficient transcription elongation and involved in transcriptional elongation-associated r

YMR185W SGDDID:5000004797, Chr XIII from 629025-631970, Genome Release 64-1-1, Uncharacterized ORF, ""Putative protein of unknown function; conflicting evidence on whether null mutant is viable with elongated buds, or inviable""

Supplemental Table S3. SILAC mass spectrometry of Pkc1 phosphorylation sites, HU treated versus untreated.

peptide	start	phospho number	phospho sites	protein	silac01 - Ratio H/L (untreated vs. HU)	silac02 - Ratio H/L (untreated vs. HU)	silac03 - Ratio H/L (untreated vs. HU)
_(ac)S(ph)FSQLEQNIK_	2	1	2S	PKC1	0.40	0.43	0.30
_(ac)SFSQLEQNIKK_	2	0		PKC1	0.79		1.27
_(ac)SFSQLEQNIK_	2	0		PKC1	0.83	0.82	
_KIAVEENIIR_	13	0		PKC1	0.91		
_IAVEENIIR_	14	0		PKC1			0.99
_KKTSNVM(ox)VIQK_	29	0		PKC1		0.82	
_KKTSNVMVIQK_	29	0		PKC1		0.85	1.17
_KTSNVM(ox)VIQK_	30	0		PKC1	0.83	0.76	
_KTSNVMVIQK_	30	0		PKC1	0.81	0.83	
_TSNVM(ox)VIQK_	31	0		PKC1	0.85	0.86	
_TSNVMVIQK_	31	0		PKC1	0.82	0.87	
_QNLEYEDSLK_	49	0		PKC1	0.84	0.84	1.17
_(g)QNLEYEDSLKK_	49	0		PKC1	0.86		1.08
_(g)QNLEYEDSLK_	49	0		PKC1	0.88	0.92	
_QNLEYEDSLK_	49	0		PKC1	0.90	0.86	
_EYGFSTK_	85	0		PKC1	0.82	0.87	1.27
_IQYM(ox)LQLEFK_	116	0		PKC1	0.82	1.08	1.71
_IQYMLQLEFK_	116	0		PKC1	0.83	0.88	1.15
_(g)IQYQEAANTK_	132	0		PKC1	0.85		
_SSAAEGGAMESK_	152	0		PKC1	0.86	0.80	1.13
_SSAAEGGAM(ox)ESK_	152	0		PKC1	0.85		
_KYQAINVDFDQFK_	176	0		PKC1	0.80	0.86	0.54
_YQAINVDFDQFK_	177	0		PKC1	0.82		1.27
_HQPND(de)DIM(ox)DNQQPK_	189	0		PKC1	0.81	0.86	
_HQPNDIM(ox)DNQQPK_	189	0		PKC1	0.93	0.87	
_HQPNDIMDNQQPK_	189	0		PKC1	0.88	0.89	1.76
_RKQLTGVLTIGITAAR_	204	0		PKC1			1.15
_DVDHIQSPM(ox)FAR_	220	0		PKC1	0.89	0.98	1.48
_KPESYVTIK_	232	0		PKC1	0.82	0.82	1.20
_WSEDFQIPEVK_	257	0		PKC1	2.62	0.91	1.29
_GNEIETVYDK_	268	0		PKC1	0.96	1.50	
_QLMGGLHR_	391	0		PKC1			1.25
_(g)QLM(ox)GGLHR_	391	0		PKC1	0.76	0.92	
_KEEIEFHGHFVQK_	406	0		PKC1	0.81	0.88	1.23
_EEIEFHGHFVQK_	407	0		PKC1	0.89	0.83	1.21
_YTFGQCQDCK_	437	0		PKC1	0.91		
_KCYTNVVTK_	452	0		PKC1	1.14		
_CYTNVVTK_	453	0		PKC1	0.78		
_TSTDTPDEAK_	465	0		PKC1	0.89		1.40
_TVPSAQLGSSIGTANGSDLS(ph)PSKLAER_	558	1	577S	PKC1	0.85		0.75
_TVPSAQLGSSIGTAN(de)GSDLS(ph)PSK_	558	1	577S	PKC1	0.80	0.63	
_TVPSAQLGSSIGTANGSDLS(ph)PSK_	558	1	577S	PKC1	0.77	0.61	
_TVPSAQLGSSIGTAN(de)GSDLS(ph)PSKLAER_	558	1	577S	PKC1	0.78	0.44	
_TVPSAQLGSSIGTAN(de)GSDLSPSK_	558	0		PKC1	1.00	3.54	4.46
_TVPSAQLGSSIGTANGSDLSPSK_	558	0		PKC1	1.04		
_ANAPLPQPR_	585	0		PKC1	0.98		1.57
_KHKDKT(ph)PS(ph)PQK_	595	2	599T / 601S	PKC1			0.62
_KHKDTPS(ph)PQK_	595	1	601S	PKC1	0.89	0.83	1.12
_KHKDTPSPQK_	595	0		PKC1	1.04	3.09	4.30
_VGRDPS(ph)PTKQHDPIIDK_	605	1	609S	PKC1	1.00		
_DSPTKQHDPIIDKR_	608	0		PKC1		3.75	3.21
_QHDPIDK_	613	0		PKC1	1.17		
_(g)QHDPIDK_	613	0		PKC1	1.07		
_FIDENEAYLN(de)FTEGAQQTAEFSS(ph)PEK_	635	1	657S	PKC1	0.55		
_FIDENEAYLNFTEGAQQTAEFSS(ph)PEK_	635	1	657S	PKC1	0.76	0.52	0.69
_FIDENEAYLNFTEGAQQTAEFSSPEK_	635	0		PKC1	1.08		
_TLDPTSNR_	661	0		PKC1	0.96		
_TLDPTSND(de)RR_	661	0		PKC1	0.87		
_S(ph)LGLTDLSEHSQTWESK_	670	1	670S	PKC1	0.83		1.62
_SLGLTDLSEHSQTWESK_	670	0		PKC1	1.21		
_AQREEM(ox)EIEIKQDSGEIQEDLEVHDIDLETK_	700	0		PKC1		0.90	
_EEMELEIK_	703	0		PKC1	0.90		
_QDSGEIQEDLEVHDIDLETK_	711	0		PKC1	0.89		
_(g)QDSGEIQEDLEVHDIDLETK_	711	0		PKC1	0.87		
_(g)QKLDWENK_	731	0		PKC1	0.97	1.02	
_QKLDWENK(de)DFR_	731	0		PKC1	0.89		
_QKLDWENK_	731	0		PKC1	0.87	0.98	
_(g)EADLTIDSTHTNPFR_	743	0		PKC1	7.48		
_DM(ox)NS(ph)ETFQIEQDHASK_	758	1	761S	PKC1	1.03		
_DMNS(ph)ETFQIEQDHASK_	758	1	761S	PKC1	0.88		
_DMN(de)SETFQIEQDHASK_	758	0		PKC1	0.80		
_DM(ox)N(de)SETFQIEQDHASK_	758	0		PKC1	0.87		
_DMNSETFQIEQDHASK_	758	0		PKC1	0.89	0.97	
_DM(ox)NSETFQIEQDHASK_	758	0		PKC1	0.95	0.99	
_DMNSETFQIEQDHASKEVLQETVSLAPTSTHASR_	758	0		PKC1			1.81
_EVLQET(ph)VSLAPTSTHASR_	774	1	779T	PKC1	1.07		1.18
_EVLQETV(ph)LAPTSTHASR_	774	1	781S	PKC1	0.98	1.18	
_TTDQQS(ph)PQKSQSTSAK_	792	1	797S	PKC1	0.81	0.52	0.79
_TTDQQS(ph)PQKS(ph)QTSTSAK_	792	2	797S / 801S	PKC1	0.85		
_KVS(ph)LDNFVLLK_	818	1	820S	PKC1	0.94		1.73
_KVSLDNFVLLK_	818	0		PKC1	0.92	1.06	0.94
_VSLDN(de)FVLLK_	819	0		PKC1	0.97	1.06	
_VSLDNFVLLK_	819	0		PKC1	0.89	1.03	1.12
_KDNIIQNHDIESARAEEK_	857	0		PKC1		0.60	
_KDNIIQNHDIESAR_	857	0		PKC1	0.98		26.96
_DNIIQNHDIESAR_	858	0		PKC1	0.84	1.11	
_DNIIQNHDIESAR_	858	0		PKC1	0.91		
_DNIIQ(de)NHDIESAR_	858	0		PKC1	1.01		1.23
_DNIIQNHDIESARAEEK_	858	0		PKC1		0.83	3.59
_KVFLLATK_	874	0		PKC1	0.92	1.00	0.66
_VFLLATK_	875	0		PKC1	0.91		1.15
_AKFYAAEVLALK_	926	0		PKC1	1.11	1.11	1.22

(g) Pyro-glu  
(ac) acetylation  
(ox) oxidation  
(de) deamidation  
(ph) phosphorylation

_FYAAEVLLALK_	928	0		PKC1	0.93	1.06	1.12
_YFHDNGVYR_	939	0		PKC1	1.03		0.93
_DLKLENIITPEGHIK_	949	0		PKC1	0.98	1.00	1.05
_LENIITPEGHIK_	952	0		PKC1	0.94	0.97	1.16
_T(ph)STFCGTPEFM(ox)APEILK_	981	1	982S	PKC1	1.20		
_T(ph)STFCGTPEFMAPEILKEQYTK_	981	1	983T	PKC1	0.94		
_T(ph)STFCGTPEFM(ox)APEILKEQYTK_	981	1	983T	PKC1		0.92	
_TST(ph)FCGTPEFMAPEILK_	981	1	983T	PKC1	1.00		
_TST(ph)FCGTPEFM(ox)APEILKEQYTK_	981	1	983T	PKC1	0.93		
_TST(ph)FCGTPEFM(ox)APEILK_	981	1	983T	PKC1	0.98	1.05	
_DADEVN(ox)EEPFRR_	1073	0		PKC1		1.19	
_NIN(de)FDDILNLR_	1085	0		PKC1	0.98		
_VKPPYIPEIK_	1096	0		PKC1	1.02	1.03	1.25
_PPYIPEIK_	1098	0		PKC1	0.74	0.89	
_SPEDTSYFEQFTSAPPT(ph)LTPLPSVLTTSQEFFF	1106	1	1125T	PKC1		1.21	
_GFSFM(ox)PDDLDSGSAAR_	1141	0		PKC1		0.92	
_AADITS(ph)LYK_	1161	1	1166S	PKC1	1.43	1.03	0.78
_AADITSLYKIK_	1161	0		PKC1		1.11	
_AADITSLYK_	1161	0		PKC1	1.00	1.18	1.16
_TAALAQHDEAAGK_	1224	0		PKC1	1.04	1.06	1.04
_TAALAQHDEAAGKAGEGEIPAPLAGTVSK_	1224	0		PKC1	1.18	1.17	1.17
_AGEGEIPAPLAGTVSK_	1237	0		PKC1	0.99	1.08	1.18
_ILVKEGDTVK_	1253	0		PKC1	1.11	1.14	1.07
_DAVQGGGLIK_	1296	0		PKC1	1.10	1.11	1.12