Phosphorylation by Akt within the ST loop of AMPK-α1
down-regulates its activation in tumor cells

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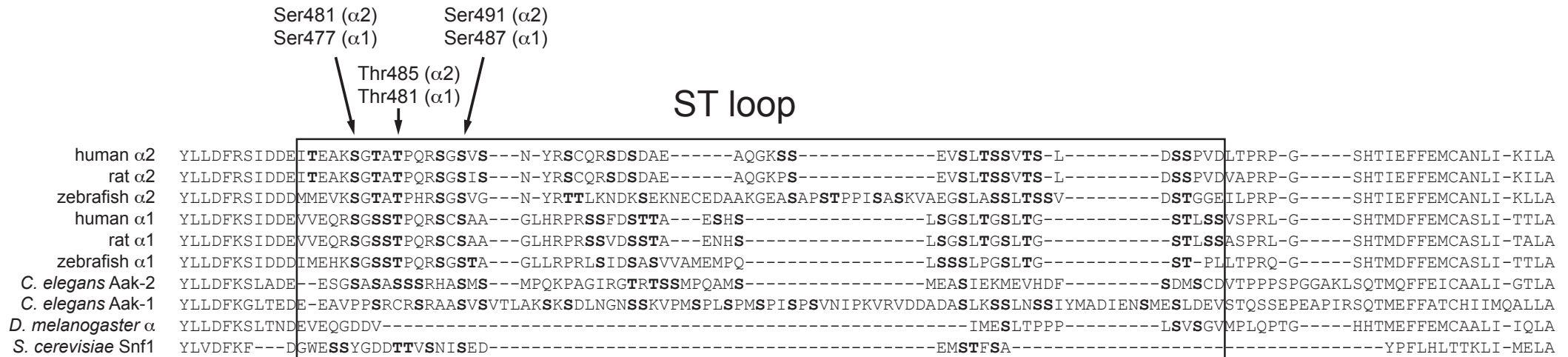SUPPLEMENTARY FIGURE LEGENDS

Legend to Supplementary Figures

Figure S1: C-terminal sequences of selected vertebrate and non-vertebrate AMPK-α subunits, showing the location of the ST loop (boxed). Sequences were aligned using CLC Main Workbench 6 using a "gap open cost" of 10 and a "gap extension cost" of 1. Note that the ST loop is well conserved between vertabrates and *C. elegans*, but poorly conserved and truncated in the sequences from insects *(D. melanogaster)* and fungi *(S. cerevisiae)*. Serine and threonine residues within the ST loop are highlighted in bold font. The positions of Ser487/491, Thr481/485 and Ser477/481 (α1/α2 respectively, human numbering; see main text for details) are indicated.

Figure S2: Some AMPK activators inhibit Akt in an AMPK-independent manner. (A) DBTRG-05MG cells were incubated for 60 min with vehicle (DMSO), berberine (300 µM), A769662 (300 µM), phenformin (10 mM) quercetin (300 µM) or A23187 (10 µM) and lysates analyzed by Western blotting using anti-pS473 and anti-Akt antibodies. (B) WT MEFs were incubated in duplicate dishes with DMSO, berberine, A769662, phenformin, quercetin or A23187 as in (A), and lysates analyzed by Western blotting using the indicated antibodies. (C) As (B), but using AMPK KO MEFs.

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Ser481 (α2)   Ser491 (α2)
Ser477 (α1)   Ser487 (α1)

Thr485 (α2)
Thr481 (α1)

ST loop

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human α2           YLLDFRSIDDEITEAKSGTATPQRSGSVS---N-YRSCQRSDSDAE------AQGKSS------------EVSLTSSVTS-L---------DSSPVDLTPRP-G-----SHTIEFFEMCANLI-KILA
rat α2             YLLDFRSIDDEITEAKSGTATPQRSGSIS---N-YRSCQRSDSDAE------AQGKPS------------EVSLTSSVTS-L---------DSSPVDVAPRP-G-----SHTIEFFEMCANLI-KILA
zebrafish α2       YLLDFRSIDDDMMEVKSGTATPHRSGSVG---N-YRTTLKNDKSEKNECEDAAKGEASAPSTPPISASKVAEGSLASSLTSSV---------DSTGGEILPRP-G-----SHTIEFFEMCANLI-KLLA
human α1           YLLDFKSIDDEVVEQRSGSSTPQRSCSAA---GLHRPRSSFDSTTA---ESHS---------------LSGSLTGSLTG-----------STLSSVSPRL-G-----SHTMDFFEMCASLI-TTLA
rat α1             YLLDFKSIDDEVVEQRSGSSTPQRSCSAA---GLHRPRSSVDSSTA---ENHS---------------LSGSLTGSLTG-----------STLSSASPRL-G-----SHTMDFFEMCASLI-TALA
zebrafish α1       YLLDFKSIDDDIMEHKSGSSTPQRSGSTA---GLLRPRLSIDSASVVAMEMPQ---------------LSSSLPGSLTG-----------ST-PLLTPRQ-G-----SHTMDFFEMCASLI-TTLA
C. elegans Aak-2   YLLDFKSLADE--ESGSASASSSRHASMS---MPQKPAGIRGTRTSSMPQAMS--------------MEASIEKMEVHDF--------SDMSCDVTPPPSPGGAKLSQTMQFFEICAALI-GTLA
C. elegans Aak-1   YLLDFKGLTEDE-EAVPPSRCRSRAASVSVTLAKSKSDLNGNSSKVPMSPLSPMSPISPSVNIPKVRVDDADASLKSSLNSSIYMADIENSMESLDEVSTQSSEPEAPIRSQTMEFFATCHIIMQALLA
D. melanogaster α  YLLDFKSLTNDEVEQGDDV-----------------------------------------------------IMESLTPPP---------LSVSGVMPLQPTG-----HHTMEFFEMCAALI-IQLA
S. cerevisiae Snf1 YLVDFKF---DGWESSYGDDTTVSNISED----------------------------------------------EMSTFSA--------------------------------YPFLHLTTKLI-MELA
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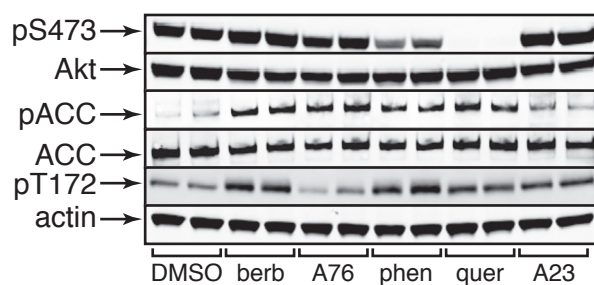
A) Phosphorylation of Akt (DBTRG-05MG cells)

pS473 →
Akt →

DMSO:	+					
berberine:		+				
A769662:			+			
phenformin:				+		
quercetin:					+	
A23187:						+
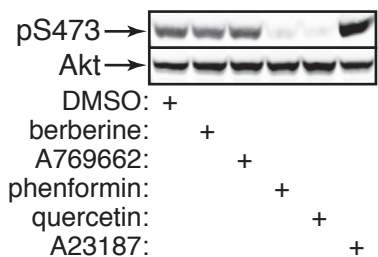
B) Phosphorylation of Akt, ACC, AMPK (WT MEFs)

pS473 →
Akt →
pACC →
ACC →
pT172 →
actin →

DMSO   berb   A76   phen   quer   A23

C) Phosphorylation of Akt, ACC (AMPK KO MEFs)

pS473 →
Akt →
pACC →
ACC →
pT172 →
actin →

DMSO   berb   A76   phen   quer   A23