

## Evidence for conserved phospho-sites

The following figures show supporting evidence for (A) MAP2K phosphorylation by MAP3K and GSK3. We also show conserved S6K phosphorylation sites for (B) GSK3, (C) TOR and (D) PDK1. (E) The conserved CK2 phosphorylation motif for CCA1 is also shown.

A) Conservation of the *O. tauri* MAP2K (Ot04g04050) residues for phosphorylation by MAP3K at S178 (orange) and GSK3 phosphorylation at S182 and T186 (black).

<i>O.tauri</i>  Ot04g04050+A4RWEB	G	H	L	T	D	A	S	-	-	K	C	H	S	W	V	G	T	V	T	Y	M	S	P	E	R	I
<i>O.lucimarinus</i>  OSTLU_4731 A4RWEB	G	H	L	V	D	A	S	-	-	K	C	H	S	W	V	G	T	V	T	Y	M	S	P	E	R	I
<i>A.thaliana</i>  MKK1 At4g26070.2	K	I	L	T	S	-	T	S	S	L	A	N	S	F	V	G	T	Y	P	Y	M	S	P	E	R	I
<i>A.thaliana</i>  MKK2 At4g29810.2	T	V	M	T	N	-	T	A	G	L	A	N	T	F	V	G	T	Y	N	Y	M	S	P	E	R	I
<i>A.thaliana</i>  MKK7 At1g18350.1	K	I	I	T	R	-	S	L	D	Y	C	N	S	Y	V	G	T	C	A	Y	M	S	P	E	R	F
<i>A.thaliana</i>  MKK9 At1g73500.1	K	I	L	V	R	-	S	L	D	S	C	N	S	Y	V	G	T	C	A	Y	M	S	P	E	R	F
<i>A.thaliana</i>  MKK8 At3g06230.1	K	I	V	V	R	-	S	L	N	K	C	N	S	F	V	G	T	F	A	Y	M	S	P	E	R	L
<i>A.thaliana</i>  MKK4 At1g51660.1	R	I	L	A	Q	-	T	M	D	P	C	N	S	S	V	G	T	I	A	Y	M	S	P	E	R	I
<i>A.thaliana</i>  MKK5 At3g21220.1	R	I	L	A	Q	-	T	M	D	P	C	N	S	S	V	G	T	I	A	Y	M	S	P	E	R	I
<i>A.thaliana</i>  MKK3 At5g40440.1	A	G	L	E	N	-	S	M	A	M	C	A	T	F	V	G	T	V	T	Y	M	S	P	E	R	I
<i>A.thaliana</i>  MKK6 At5g56580.1	A	S	L	A	S	-	S	M	G	Q	R	D	T	F	V	G	T	Y	N	Y	M	S	P	E	R	I
Human MP2K1 Q02750	G	Q	L	I	D	-	S	-	-	M	A	N	S	F	V	G	T	R	S	Y	M	S	P	E	R	L
Human MP2K2 P36507	G	Q	L	I	D	-	S	-	-	M	A	N	S	F	V	G	T	R	S	Y	M	A	P	E	R	L
Human MP2K5 Q13163	T	Q	L	V	N	-	S	-	-	I	A	K	T	Y	V	G	T	N	A	Y	M	A	P	E	R	I
<i>S.cerevisiae</i>  STE7 P06784	K	K	L	I	N	-	S	-	-	I	A	D	T	F	V	G	T	S	T	Y	M	S	P	E	R	I
<i>S.cerevisiae</i>  MKK1 P32490	G	E	A	V	N	-	S	-	-	L	A	T	T	F	T	G	T	S	F	Y	M	A	P	E	R	I
<i>S.cerevisiae</i>  MKK2 P32491	G	E	A	V	N	-	S	-	-	L	A	M	T	F	T	G	T	S	F	Y	M	A	P	E	R	I

B) Conservation of residues for GSK3 mediated (S371) phosphorylation of S6K (Ot07g02590), which is part of the TORC1 pathway, in *A. thaliana* and *O. tauri*. The proposed GSK3 phosphorylation site is highlighted in black. The upstream conserved S/T residue which could act as the GSK3 primer site is highlighted in red. The GSK3 target site is not conserved in yeast.

<i>Ostreococcus_lucimarinus</i>  XP_001418716.1/1-320	T	D	V	D	P	V	-	-	-	-	-	-	-	-	-	-	D	S	V	A	A	T	P	V	S
<i>Ostreococcus_tauri</i>  Ot07g02590/1-441	T	D	V	D	P	V	-	-	-	-	-	-	-	-	-	-	D	S	V	A	A	T	P	V	S
<i>Arabidopsis_thaliana</i>  S6K2 AT3G08720.1/1-471	T	D	M	S	V	L	-	-	-	-	-	-	-	-	-	-	D	S	P	A	S	S	P	N	S
<i>Arabidopsis_thaliana</i>  S6K1 AT3G08730.1/1-465	T	D	M	S	V	L	-	-	-	-	-	-	-	-	-	-	D	S	P	A	S	S	P	S	S
Human p70S6K/1-525	T	R	Q	T	P	V	-	-	-	-	-	-	-	-	-	-	D	S	P	D	D	S	T	L	S
Human p70S6Kb/1-482	T	R	Q	T	P	V	-	-	-	-	-	-	-	-	-	-	D	S	P	D	D	T	A	L	S
Yeast SCH9 P11792/1-824	T	T	A	S	T	S	-	-	-	-	-	-	-	-	-	-	F	M	M	T	A	T	P	L	S

C) Conservation of the residues for TORC1 mediated (S389) phosphorylation of S6K (Ot07g02590), and subsequent PDK1 binding, in *A. thaliana* and *O. tauri*.

