PI3 Kinase/Akt Signaling

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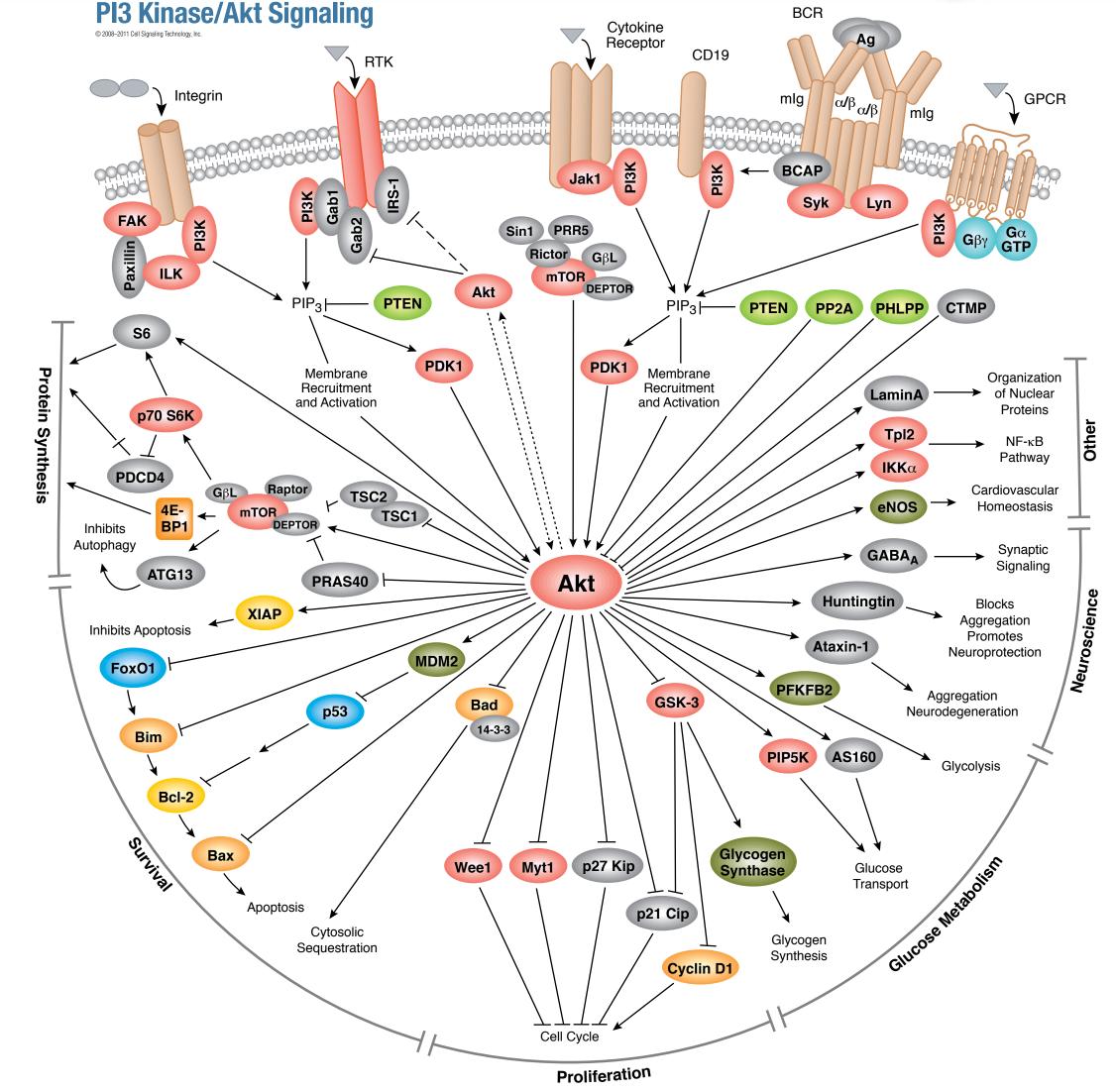
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These Akt signaling reference materials were created by research scientists at Cell Signaling Technology and reviewed by the foremost scientists in the field. Visit www.cellsignal.com for additional reference materials and comprehensive validation data for over 4,000 antibodies and related reagents.

Akt Substrates

This Substrate Table provides a comprehensive list of demonstrated downstream targets of Akt phosphorylation, along with the relevant phosphorylation site, publication reference, substrate function, and effect of phosphorylation. This information was generated using PhosphoSitePlus®, our online systems biology resource providing comprehensive information and tools for the study of protein post-translational modifications, available on the web at www.phosphosite.org.

Akt Cubetrato	Akt	Phosphorylation	Dubling of ID #	Outstand Francisco and Effect of Physical and Alice
Akt Substrate Acinus	Isoform 1	Site (Human) S1180	PubMed ID # 16177823	Substrate Function and Effect of Phosphorylation induces chromatin condensation during apoptosis; phosphorylation inhibits this process
APS	1	S598	16141217	adaptor protein; recruited to the insulin receptor to signal insulin-stimulated glucose transport; phosphorylation promotes membrane localization
AR (Androgen Receptor)	1	S213, S791	11404460, 11156376	nuclear receptor; phosphorylation suppresses AR activation, expression of AR target genes, and AR-mediated apoptosis
Arfaptin 2	1	S260	15809304	protein levels are elevated in Huntington's Disease; phosphorylation inhibits polyQ- Huntingtin induced neurotoxicity; promotes cell survival and neuroprotection
AS160	1	S588, T642	11994271	insulin stimulated Rab GTPase-activating protein, structurally and functionally similar to TBC1D1; phosphorylation results in increased Glut4 translocation
ASK1	1, 2	S83	11154276, 14500571, 15911620	MAPKKK; induces apoptosis via JNK pathway; phosphorylation inhibits activity and promotes survival
Ataxin-1	1	S776	12757707, 17540008	14-3-3 binds to and stabilizes ataxin-1, which forms polyglutamine aggregates and
Bad	1	S75, S99, S118	9381178, 11723239,	neurodegeneration; phosphorylation promotes 14-3-3 binding pro-apoptotic protein; phosphorylation inhibits function and promotes survival
BcI-xL	1	S106	9346240, 10949026 18951975	prevent apoptosis through binding to apoptotic protein; phosphorylation promotes VDAC
Bim	1	S87	16282323	binding pro-apoptotic protein; phosphorylation promotes 14-3-3 binding/inactivation and cell
B-Raf	1, 3	S365, S429	10869359	survival signaling intermediate in Erk1/2 pathway; phosphorylation causes inhibition
BRCA1	1	T509	10542266	breast cancer susceptibility gene product, tumor suppressor; phosphorylation alters function, perhaps by preventing nuclear localization
CACNB2	1	S630	15311280	voltage-dependent calcium channel; phosphorylation regulates channel trafficking to plasma membrane
CaRHSP1 Caspase-9	1	S52 S196	15910284 9812896	RNA binding protein; phosphorylation effect currently unknown protease, initiates apoptosis; phosphorylation inhibits protease activity
CBP	1	T1871	17166829	acetylates histone and non-histone proteins; phosphorylation increases activity
CCT2 Cdc25B	1	S260 S353	19332537 17554083	member of protein chaperone complex; effect of phosphorylation currently unknown protein phosphatase responsible for cdc2 activation; phosphorylation promotes
CDK2	1	T39	18354084	activation of M-phase promoting factor cyclin-dependent kinase functioning in S-phase; phosphorylation increases cyclin A
CENTB1 (ACAP1)	1	S554	16256741	binding GTPase-activating protein (GAP) for ARF proteins; phosphorylation prevents recycling of
Chk1	1	S280	15107605, 12062056	β1-integrin containing endosomes and cell migration DNA damage effector; regulates G2/M transition during DNA damage; phosphorylation
CK1-D	1	S370	17594292	inhibits function by preventing phosphorylation by ATM/ATR kinase and core component of circadian clock; phosphorylation inhibits kinase activity
Connexin 43	1, 2	\$372 \$400	17008717 12138205	gap junction protein; phosphorylation allows 14-3-3 binding oncogene; phosphorylation induces NF-kB-dependent transcription
Cot (Tpl2) CSP	1	S400 S10	12138205 16243840	oncogene; phosphorylation induces NikB-dependent transcription exocytosis; phosphorylation regulates the kinetics of late stage exocytosis
CTNNB1 (β-Catenin) CTNND2 (Catenin	1, 2	S552 T457	17237784 17993462	Wnt signaling pathway protein; phosphorylation causes nuclear localization transcriptional activator, plays a role in adhesion molecule regulation; phosphorylation
δ-2)				promotes binding to p190RhoGEF, dendritic morphogenesis
CUGBP1 DLC1 (p122RhoGAP)	1	S28 S329	18570922 16338927	RNA-binding protein; phosphorylation enhances interaction with cyclin D1 mRNA tumor suppressor and insulin stimulated phosphoprotein; may play role in Glut4
EDC3	2	S161	20051463	translocation; phosphorylation may inhibit its GAP activity involved in removal of the mRNA 5' cap structure; phosphorylation induces 14-3-3
				protein interaction and promotes ED3 mediated post-transcriptional regulation through \ensuremath{mRNA}
EDG-1	1	T236	11583630	G protein-coupled receptor; phosphorylation activates signaling to promote cell migration
elF4B	1	S422	18836482	necessary for binding of mRNA to ribosomes; phosphorylation increases transcriptional activity
eNOS	1	S1176	10376602, 10376603, 10514497	enzyme that catalyzes the production of nitric oxide (NO); phosphorylation results in enzyme activation, NO production, and cardiovascular homeostasis (vasodilation,
ER-α	1, 2	S167	11139588, 16113102	vascular remodeling, angiogenesis) nuclear receptor and transcription factor; phosphorylation activates the receptor and
(Estrogen Receptor-α) Ezh2	1	S21	16224021	increases gene expression, causing mammary and uterine cell proliferation methyltransferase; phosphorylation decreases histone H3 methylation of Lys27 and
Ezrin	2	T567	15531580	increases gene expression plasma membrane/cytoskeletal linker protein; phosphorylation promotes actin binding
FANCA	1	S1149	11855836	and cytoskeletal organization ATPase involved in DNA repair; phosphorylation is negatively regulated by Akt
FLNC	1, 2	S2233	15461588	muscle-specific filamin functioning in muscle cells; phosphorylation effect currently
F0XA2	1	T156	14500912	unknown transcription factor involved in embryonic development and differentiation;
	1	T279	17435750	phosphorylation results in nuclear exclusion and inhibition of FoxA2-dependent transcriptional activity
FOXG1				transcriptional repression factor involved in brain development; phosphorylation promotes nuclear export
Fox01a	1	T24, S256, S319	10358075, 10377430, 11237865	transcription factor involved in cell cycle arrest, apoptosis, and glucose metabolism; phosphorylation causes export from the nucleus and inhibits activity
Fox03a	1	T32, S253	11154281, 10102273	transcription factor involved in cell cycle arrest and apoptosis; phosphorylation causes export from the nucleus and inhibits activity
Fox04	1	T32, S197, S262	11313479, 10217147, 14690436	phosphorylation causes export from the nucleus and inhibits activity
Gab2	1	S159	11782427	docking/scaffolding protein; proto-oncogene; RTK signaling intermediate; phosphorylation inhibits activity
GATA-1	1	\$310	16107690	transcription factor; phosphorylation increases activity and promotes blood cell differentiation
GATA-2	1	S401	15837948	transcription factor; phosphorylation inhibits activity to promote adipogenesis and reduce inflammation
Girdin GOLGA3	1	S1417 S174, S389	16139227 17888676	actin binding protein; phosphorylation promotes cell migration golgi auto-antigen; phosphorylation results in reduced apoptosis
GSK-3β	1 1	S21 S9	11520785, 8524413 12095987, 12900420,	cell cycle, glycogen synthesis, and apoptosis; phosphorylation inhibits activity cell cycle, glycogen synthesis, and apoptosis; phosphorylation inhibits activity
H2B	1	S37	15073173 8985174	core component of the nucleosome; phosphorylation effect currently unknown
HMOX1 hnRNP A1	1	S188 S199	15581622 18562319	heme oxigenase involved in stress response; phosphorylation regulates binding affinity involved in pre-mRNA packaging into hnRNP particles and transport of poly(A) mRNA
IIIINP AT	,	3199	10302319	from cytoplasm to nucleus; phosphorylation regulates role in cyclin D1 and c-Myc IRES activity
hnRNP E1	1, 2	S43	20154680	binds to single-stranded nucleic acid; phosphorylation results in disruption of BAT
HtrA2	1, 2	S212	17311912	element binding and translational activation of Dab2 and ILEI mRNA protease released during apoptosis; phosphorylation inhibits activity and attenuates its
Huntingtin	1	S421	12062094, 15843398	pro-apoptotic function Huntington's Disease; Akt phosphorylation blocks nuclear aggregation and provides peurografication
IKK-a	1, 2	T23	10485710	neuroprotection NF-кВ signaling intermediate; phosphorylation activates NF-кВ and immune/stress
IP3R1	1	S2690	16332683	response Ca ²⁺ release and signaling; phosphorylation induces resistance to apoptosis, possibly through caspase. 3 inactivation
IRS-1	1	\$527	17579213	through caspase-3 inactivation insulin receptor signaling intermediate; phosphorylation inhibits function
Kv11.1 iso5	1	T897	18791070	pore-forming subunit of voltage-gated potassium channels, essential for rhythmic excitability of cardiac muscle and endocrine cells; phosphorylation inhibits channels
Lamin A/C Mad1	1	S301, S404 S145	18808171 18451027, 19526459	component of nuclear lamina; phosphorylation regulates function of nuclear lamina component of spindle-assembly checkpoint; phosphorylation results in ubiquitination
MDM2	1	S166, S186, S188	11504915, 15169778,	and degradation through 26S proteasome pathway ubiquitin ligase involved in p53 degradation; phosphorylation results in translocation to
MLK3	1	S674	11850850 12458207	the nucleus and inhibition of p53 JNK-mediated neuronal cell death; phosphorylation inhibits activity
METTL1 MST1	1	S27 T120	15861136 19940129	catalyzes the formation of m7G46 in tRNA; phosphorylation results in inactivation pro-apoptotic kinase; phosphorylation inhibits kinase activity and nuclear translocation
	1	S2448		resulting in inhibition of pro-apoptotic signaling protein synthesis and cell growth; phosphorylation increases activity
mTOR MY05A	2	S1652	10567225, 15208671 17515613	actin-based motor protein with a role in cytoplasmic vesicle transport and anchorage;
Myt1	1	S83	11802161	phosphorylation promotes insulin-mediated Glut4 vesicle translocation Weet family member and cell cycle regulator; phosphorylation downregulates Myt1 and
Ndrg2	1	T348	14985363	initiates M-phase insulin-stimulated phosphoprotein; phosphorylation promotes insulin signaling
NFAT90 NMDAR2C	1	S647 S1081	18097023 19477150	translation inhibitory protein; phosphorylation required for nuclear export Glutamate receptor channel subunit; phosphorylation promotes binding to 14-3-3ε and
NuaK1	1	S600	12409306	leads to increased surface expression of cerebellar MMDA receptors AMPK family member activated under nutrient starvation; mediates cell survival during
Nur77	1	S351	11274386, 11438550	glucose starvation; phosphorylation increases kinase activity nuclear receptor, transcription factor; T cell apoptosis; phosphorylation inhibits
p21 Waf1/Cip1	1	T144, S145	11756412, 11231573,	transcriptional activity
p300	1	S1834	15173090 11116148, 16024795	transcriptional co-activator; phosphorylation can either activate or suppress
Palladin	1	S507	20471940	transcriptional activity depending on cell type and physiological stimuli actin-bundling protein; phosphorylation promotes F-actin bundling and inhibits cell
PDCD4	1	S67, S457	16357133	migration tumor suppressor protein that is strongly induced during apoptosis; phosphorylation
PDCD4 PDE3A	1	\$292, \$293, \$294	17124499	inhibits tumor suppressor function
				regulates levels of cAMP and cGMP; insulin-dependent oocyte maturation; phosphorylation increases activity regulates levels of cAMP and cGMP; activated by insulin to regulate linelysis;
PDE3B	1	S295	17560660	regulates levels of cAMP and cGMP; activated by insulin to regulate lipolysis; phosphorylation increases activity
Peripherin	1	\$59	17569669	neuronal intermediate filament protein; phosphorylation promotes motor nerve regeneration
PFKFB2	1	S466, S483	9211863, 10521487, 16829026	glycolytic enzyme; insulin-mediated glucose metabolism; phosphorylation increases activity
PGC-1	1, 2	S571	17554339	regulates gluconeogenesis and fatty acid oxidation; phosphorylation inhibits function



Pathway Description: Since its initial discovery as a protooncogene, the serine/threonine kinase Akt (also known as protein kinase B or PKB) has become a major focus of attention because of its critical regulatory role in diverse cellular processes, including cancer progression and insulin metabolism. The Akt cascade is activated by receptor tyrosine kinases, integrins, B and T cell receptors, cytokine receptors, G protein-coupled receptors and other stimuli that induce the production of phosphatidylinositol 3,4,5

triphosphates (PtdIns(3,4,5)P3) by phosphoinositide 3-kinase (PI3K). These lipids serve as plasma membrane docking sites for proteins that harbor pleckstrin-homology (PH) domains, including Akt and its upstream activator PDK1. There are three highly related isoforms of Akt (Akt1, Akt2, and Akt3) and these represent the major signaling arm of PI3K. For example, Akt is important for insulin signaling and glucose metabolism, with genetic studies in mice revealing a central role for Akt2 in these processes. Akt regulates cell growth through its effects on the mTOR and p70 S6 kinase pathways, as well as cell cycle and cell proliferation through its direct action on the CDK inhibitors p21 and p27, and its indirect effect on the levels of cyclin D1 and p53. Akt is a major mediator of cell survival through direct inhibition of pro-apoptotic signals such as Bad and the Forkhead family of transcription factors. T lymphocyte trafficking to lymphoid tissues is controlled by the expression of adhesion factors downstream of Akt. In addition, Akt

has been shown to regulate proteins involved in neuronal function including GABA receptor, ataxin-1, and huntingtin proteins. Akt has been demonstrated to interact with Smad molecules to regulate TGF- β signaling. Finally, lamin A phosphorylation by Akt could play a role in the structural organization of nuclear proteins. These findings make Akt/PKB an important therapeutic target for the treatment of cancer, diabetes, laminopathies, stroke and neurodegenerative disease.

Akt Substrate	Akt Isoform	Substrate Phosphorylation Site (Human)	PubMed ID #	Substrate Function and Effect of Phosphorylation
PRPK	1	S250	17712528	p53 binding protein and kinase; phosphorylation causes activation and results in p53 phosphorylation
PTP1B	1	S50	11579209	protein tyrosine phosphatase that dephosphorylates the insulin receptor; phosphorylation inhibits activity
QIK	2	S358	17805301	AMPK related protein; phosphorylation leads to kinase activation and promotes ubiquitination/degradation of TORC2
Rac1	1	S71	10617634	Rho-GTPase; actin cytoskeletal organization; phosphorylation inhibits GTP-binding activity
Raf1 (c-Raf)	1	S259	11443134, 12087097	signaling intermediate in Erk1/2 pathway; phosphorylation inhibits activity
RANBP3	1	S126	18280241	RAN binding protein 3 functions in nuclear transport; phosphorylation mediates Ran binding and regulates nuclear transport
Ron	1	S1394	12919677, 14505491	receptor tyrosine kinase for macrophage stimulating protein (MSP); cell adhesion, proliferation and migration; phosphorylation causes 14-3-3 binding
S6	1, 2	S235, S236	12151408, 15358595	S6 ribosomal protein; phosphorylation activates the protein and promotes protein synthesis
SEK1	1	S80	11707464, 15911620	signaling intermediate of the JNK/SAPK pathway; stress/inflammation; phosphorylation inhibits activity
SH3BP4	1	S246	19122209	controls selective internalization of the transferrin receptor through endocytosis; phosphorylation promotes 14-3-3 binding at the plasma membrane
SH3RF1	1, 2	S304	17535800	scaffolding protein that binds to activated Rac and promotes apoptosis via JNK activation; phosphorylation reduces ability to bind Rac, promoting apoptosis
Skp2	1	S72	19270695	component of SCF-Skp2 E3 ubiquitin ligase complex; phosphorylaion regulates Skp2 stability and cytoplasmic localization
SKI	1	T458	19875456	negative regulator of TGF-β signaling by binding to Smads; phosphorylation causes its destabilization and reduces Ski-mediated inhibition of expression of Smad7
SSB	1	T302	18836485	RNA binding protein, plays a role in processing of RNA polymerase III transcripts; phosphorylation promotes export to cytoplasm where it binds polysomes and regulates expression of a specific set of mRNAs
TAL-1	1	T90	15930267	transcription factor; phosphorylation inhibits transcriptional repressor activity and regulates intracellular localization
TBC1D4 (AS160)	1	T642, T649	11994271, 16880201 16935857	Rab GTPase-activating protein involved in insulin-stimulated Glut4 trafficking; phosphorylation promotes glucose transport
TERT	1	S227, S824	10224060	telomerase reverse transcriptase, chomosome length maintenance; phosphorylation enhances telomerase activity
TOPBP1	1	S1159	19477925	induces a large increase in the kinase activity of ATR; phosphorylation prevents the enhanced association of ATR with TopBP1 after DNA damage
TRF1	1	T273	19160102	controls telomere structure; phosphorylation decreases telomere length
TTC3	1	\$378	20059950	E3 ligase to Akt; phosphorylation promotes TTC3 function, such as ability to ubiquitinylate and destabilize Akt
Tuberin (TSC2)	1	S939, S981, T1462	12150915, 12172553 15342917, 16636147	tumor suppressor that inhibits mTOR; phosphorylation inhibits function and allows protein synthesis to occur
USP8	1	T945	17210635	deubiquitinating enzyme that plays a role in growth factor receptor trafficking and degradation; phosphorylation increases protein stability
VCP	1	S351	16551632	ATPase and molecular chaperone; phosphorylation may impair its pro-apoptotic effect and promote cell survival
WNK1	1	T60	14611643, 16081417	regulates ion channels; phosphorylation of WNK1 causes SGK1 activation and regulation of sodium ion transport
XIAP	1, 2	\$87	14645242	inhibitor of apoptosis; phosphorylation prevents ubiquitination/degradation and causes increased cell survival
YAP1	1	S127	12535517	transcriptional co-activator; phosphorylation causes 14-3-3 binding and inhibits pro-apoptotic gene expression
YB1	1	S102	15806160	regulates transcription by binding to Y-box promoter sequences; phosphorylation causes nuclear translocation and activation
Zyxin	1	S142	17572661	pro-survival focal adhesion molecule; phosphorylation causes nuclear localization and

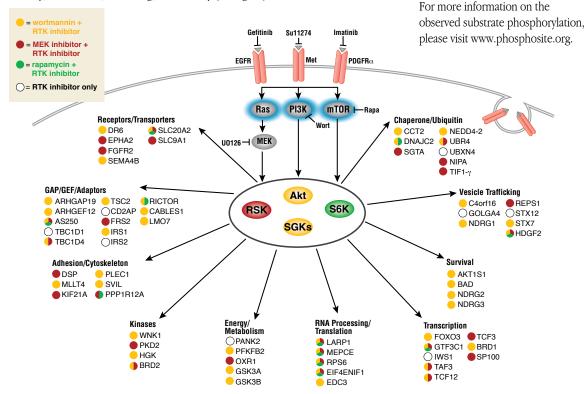
suppression of apoptosis

Putative Akt Substrates

Identified by Scientists at Cell Signaling Technology

MAPK, mTOR, and the PI3K/Akt pathways are key signaling pathways activated downstream of oncogenic receptor tyrosine kinases (RTKs). All of these pathways activate AGC kinase family members, including Akt, RSK, and p70 S6 kinases, whose protein substrates are phosphorylated at the RxRxxS/T motif.

In a recent phosphoproteomic study authored by scientists in the Cell Signaling Technology (CST) Site Discovery Group [Sci. Signal. (2010) 24;3(136):ra64], over 300 putative substrates for these AGC family kinases were identified in three cell lines, each driven by either EGFR, c-Met, or PDGFR. The experimental approach involved the use of PTMScan® technology, a proprietary methodology for antibody-based peptide enrichment combined with tandem mass spectrometry for quantitative profiling of post-translational modifications. A key step was the development of an RxRxxS/T Motif Antibody, which was then used as an affinity reagent to selectively immunoprecipitate phosphorylated substrates of Akt, RSK, and p70 S6 kinases. Use of targeted cancer drugs with inhibitors specific for PI3K, mTOR, and MEK allowed for mapping of the signaling network downstream of these RTKs. Substrates included proteins involved in many cellular functions, including scaffolding, protein stability, metabolism, trafficking, and motility (see figure).



Pathway Description: Connections identified using RTK inhibitors and pathway specific inhibitors. Proteins inhibited by two or more RTK inhibitors and the indicated pathway inhibitor(s) are grouped by protein function. Color coding indicates sensitivity to pathway inhibitor.

Reference Moritz A., Li Y., Guo A., Villén J., Wang Y., MacNeill J. Kornhauser J., Sprott K., Zhou J., Possemato A., Ren J.M., Hornbeck P., Cantley L.C., Gygi S.P., Rush J., Comb M.J. (2010) Akt-RSK-S6 kinase signaling networks activated by oncogenic receptor tyrosine kinases. Sci Signal. 3(136):ra64.

Direct Stimulatory Modification Direct Inhibitory Modification

16525023

12524439

1 T246

PLCy1

PRAS40 (Akt1S1)

1 S1248

→ Multistep Stimulatory Modification

results in increased protein synthesis

catalyzes PI 4.5 bisphosphate to IP3 and DAG; increases intracellular Ca²⁺ levels:

binds to and inhibits mTOR; phosphorylation causes 14-3-3 binding/inhibition and

phosphorylation increases activity and enhances EGF-stimulated cell motility

── Tentative Stimulatory Modification — — Tentative Inhibitory Modification

Transcriptional Stimulation Transcriptional Inhibition

Separation of Subunits or Cleavage Products Joining of Subunits ----→ Translocation





