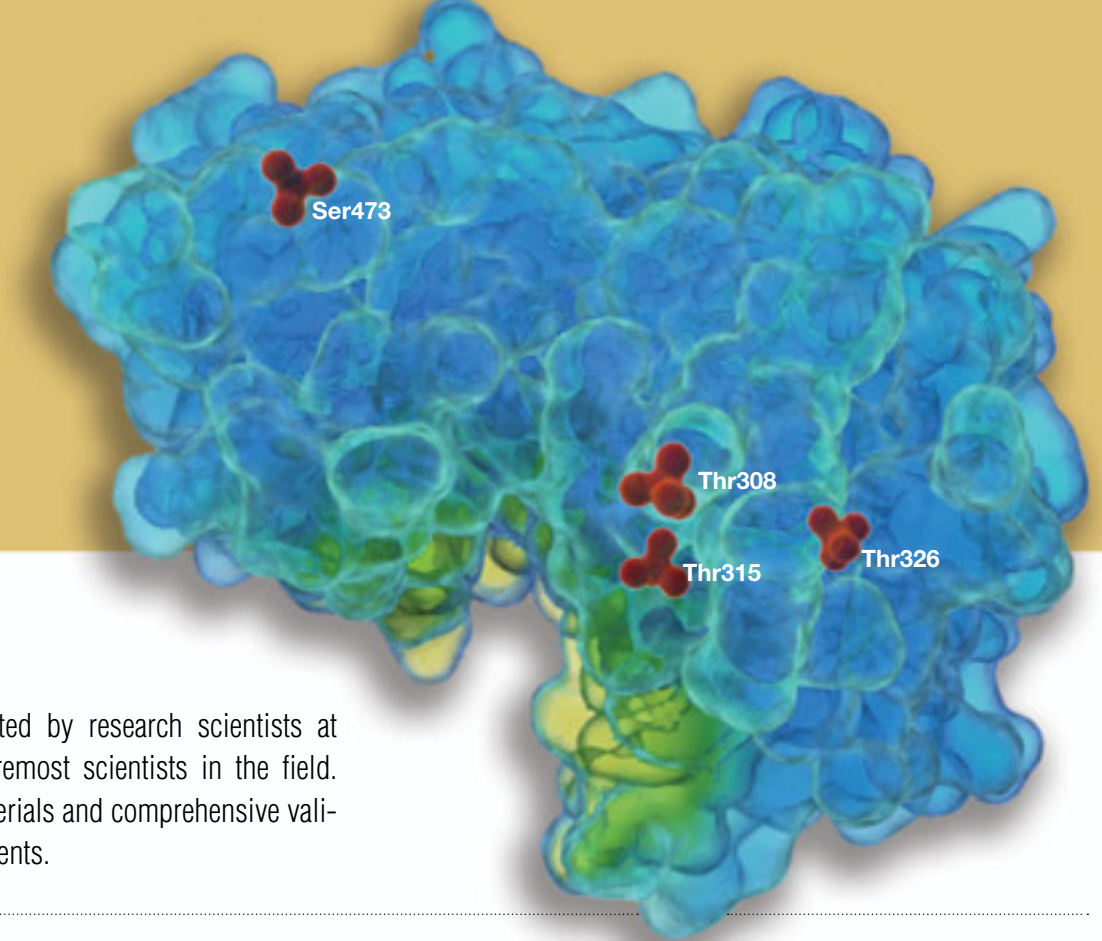


# PI3 Kinase/Akt Signaling

Reference Materials from Cell Signaling Technology



## Our Commitment to You

As a company driven by science, our goal is to accelerate biomedical research by developing a "research tool box" that enables researchers to monitor and measure protein activity. We strive to meet contemporary and future research challenges by creating the highest quality, most specific and thoroughly validated antibodies and related reagents.

As a committed member of the research community, we practice responsible and sustainable business methods and invest heavily in research and development. We also encourage thoughtful use of our limited natural resources by highlighting environmental issues in our catalog and by promoting conservation and recycling.

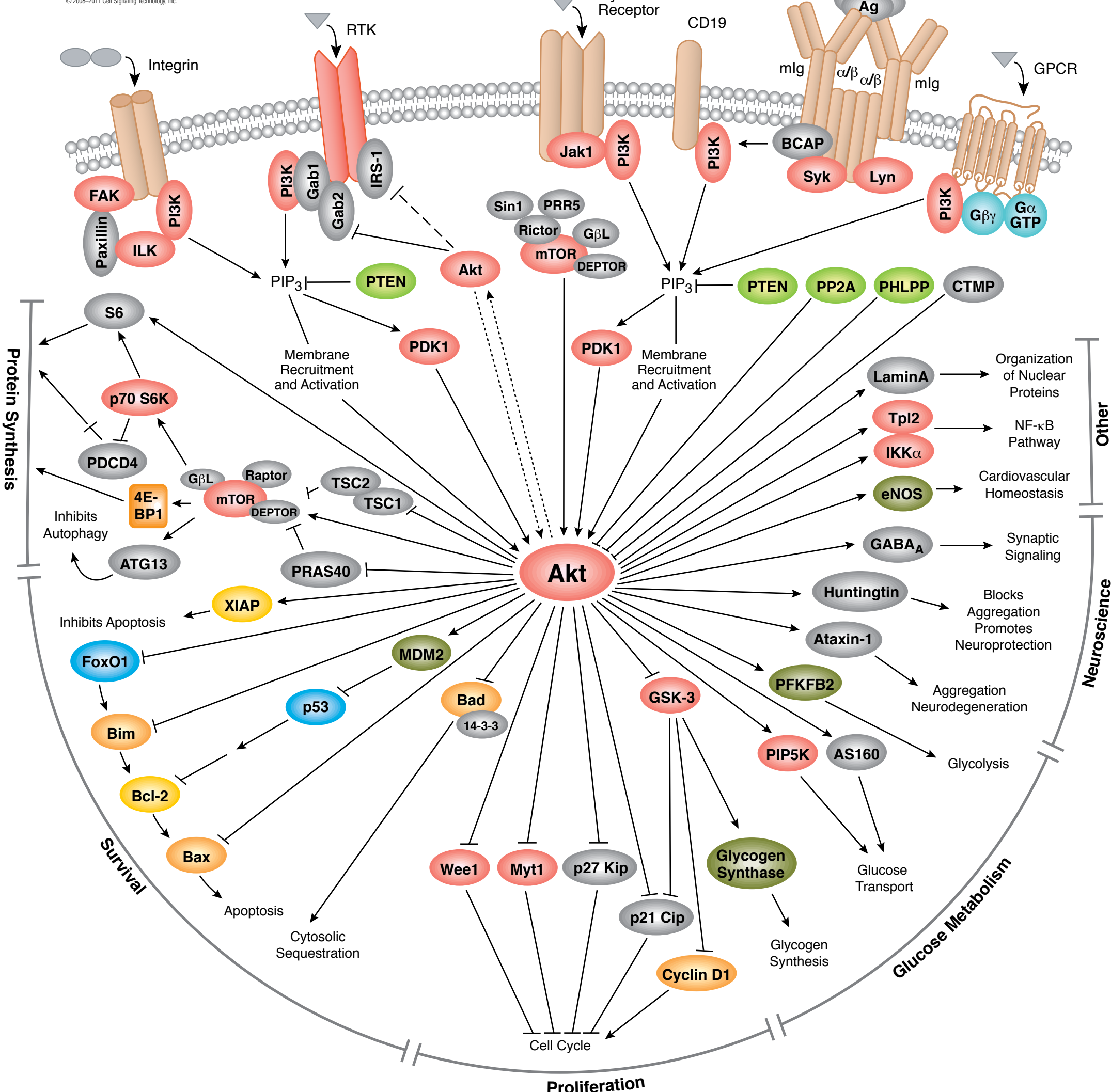
These Akt signaling reference materials were created by research scientists at Cell Signaling Technology and reviewed by the foremost scientists in the field. Visit [www.cellsignal.com](http://www.cellsignal.com) for additional reference materials and comprehensive validation data for over 4,000 antibodies 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](http://www.phosphosite.org).

Akt Substrate	Akt Isoform	Substrate Phosphorylation Site (Human)	PubMed ID #	Substrate Function and Effect of Phosphorylation
<b>Acinus</b>	1	S1180	1617823	induces chromatin condensation during apoptosis; phosphorylation inhibits this process
<b>APC</b>	1	S598	1641217	adaptor protein; recruited to the insulin receptor to signal insulin-stimulated glucose transport; phosphorylation promotes membrane localization
<b>AR (Androgen Receptor)</b>	1	S213, S791	11404460, 11156376	nuclear receptor; phosphorylation suppresses AR activation, expression of AR target genes, and AR-mediated apoptosis
<b>Arp1ap2</b>	1	S260	15809304	protein levels are elevated in Huntington's Disease; phosphorylation inhibits polyQ-Huntingtin induced neurotoxicity; promotes cell survival and neuroprotection
<b>AS160</b>	1	S588, T642	11994271	insulin-stimulated Rab GTPase-activating protein; structurally and functionally similar to TBC1D1; phosphorylation results in increased Glu4 translocation
<b>ASK1</b>	1, 2	S83	11154276, 14500571, 15911620	MAPK3; induces apoptosis via JNK pathway; phosphorylation inhibits activity and promotes survival
<b>Ataxin-1</b>	1	S776	12757707, 17540008	14-3-3 binds to and stabilizes ataxin-1, which forms polyglutamine aggregates and neurodegeneration; phosphorylation promotes 14-3-3 binding
<b>Bad</b>	1	S75, S99, S118	9281178, 11722339, 9046240, 10949026	pro-apoptotic protein; phosphorylation inhibits function and promotes survival
<b>Bcl-xL</b>	1	S106	18951975	prevents apoptosis through binding to apoptotic protein; phosphorylation promotes VDAC binding
<b>Bim</b>	1	S87	16282323	pro-apoptotic protein; phosphorylation promotes 14-3-3 binding/inactivation and cell survival
<b>B-Raf</b>	1, 3	S265, S429	10869259	signaling intermediate in Erk1/2 pathway; phosphorylation causes inhibition
<b>BRCA1</b>	1	S1509	10542266	breast cancer susceptibility gene product; tumor suppressor; phosphorylation alters function, perhaps by preventing nuclear localization
<b>CACNB2</b>	1	S630	15311280	voltage-dependent calcium channel; phosphorylation regulates channel trafficking to plasma membrane
<b>CaRHP1</b>	1	S52	15910284	RNA binding protein; phosphorylation effect currently unknown
<b>Caspase-9</b>	1	S196	9812896	protease; initiates apoptosis; phosphorylation inhibits protease activity
<b>CBP</b>	1	T1871	17166829	acetylates histone and non-histone proteins; phosphorylation increases activity
<b>CCT2</b>	1	S260	19325237	member of protein chaperone complex; effect of phosphorylation currently unknown
<b>Cdc25B</b>	1	S353	17554083	protein phosphatase responsible for cdc2 activation; phosphorylation promotes activation of M-phase promoting factor
<b>CDK2</b>	1	T39	18354084	cyclin-dependent kinase functioning in S-phase; phosphorylation increases cyclin A binding
<b>CENT1 (ACAP1)</b>	1	S554	16256741	GTPase-activating protein (GAP) for ARF proteins; phosphorylation prevents recycling of β1-integrin containing endosomes and cell migration
<b>Chk1</b>	1	S280	11507605, 12062056	DNA damage effector; regulates G2M transition during DNA damage; phosphorylation inhibits function by preventing phosphorylation by ATM/ATR kinase and core component of cdc2/cyclin B complex; phosphorylation inhibits kinase activity
<b>CK1-D</b>	1	S370	17594292	gap junction protein; phosphorylation allows 14-3-3 binding
<b>Connexin 43</b>	1, 2	S372	17008717	gap junction protein; phosphorylation allows 14-3-3 binding
<b>Cot (Tlp2)</b>	1	S400	12138205	oncogene; phosphorylation induces NF-κB-dependent transcription
<b>CSP</b>	1	S10	16243840	ecocyst; phosphorylation regulates the kinetics of late stage ecocystosis
<b>CTNNB1 (β-Catenin)</b>	1, 2	S552	17237784	Wnt signaling pathway protein; phosphorylation causes nuclear localization
<b>CTNND2 (Catenin δ-2)</b>	1	S457	17933462	transcriptional activator; plays a role in adhesion molecule regulation; phosphorylation promotes binding to p190RhoGEF; dendritic morphogenesis
<b>CIGBP1</b>	1	S28	18570922	RNA-binding protein; phosphorylation enhances interaction with cyclin D1 mRNA
<b>DLC1 (p12RhoGAP)</b>	1	S329	16338927	tumor suppressor and insulin stimulated phosphoprotein; may play role in Glu4 translocation; phosphorylation may inhibit its GAP activity
<b>EDC3</b>	2	S161	20051463	involved in removal of the mRNA 5' cap structure; phosphorylation induces 14-3-3 protein interaction and promotes ED3 mediated post-transcriptional regulation through mRNA
<b>EDG-1</b>	1	T236	11583630	G protein-coupled receptor; phosphorylation activates signaling to promote cell migration
<b>eIF4B</b>	1	S422	18836482	necessary for binding of mRNA to ribosomes; phosphorylation increases transcriptional activity
<b>eNOS</b>	1	S1176	10376932, 10376933, 10514497	enzyme that catalyzes the production of nitric oxide (NO); phosphorylation results in enzyme activation, NO production, and cardiovascular homeostasis (vasodilation, vascular remodeling, angiogenesis)
<b>ER-α (Estrogen Receptor-α)</b>	1, 2	S167	11139588, 16113102	nuclear receptor and transcription factor; phosphorylation activates the receptor and increases gene expression, causing mammary and uterine cell proliferation
<b>Ezh2</b>	1	S21	16224021	methyltransferase; phosphorylation decreases histone H3 methylation of Lys27 and increases gene expression
<b>Ezrin</b>	2	T567	15531580	plasma membrane/cytoskeletal linker protein; phosphorylation promotes actin binding and cytoskeletal organization
<b>FANCA</b>	1	S1149	11855836	ATPase involved in DNA repair; phosphorylation is negatively regulated by Akt
<b>FLNC</b>	1, 2	S2233	15461588	muscle-specific filamin functioning in muscle cells; phosphorylation effect currently unknown
<b>FOXA2</b>	1	T156	14500912	transcription factor involved in embryonic development and differentiation; phosphorylation results in nuclear exclusion and inhibition of Foxo2-dependent transcriptional activity
<b>FOXG1</b>	1	T270	17435750	transcriptional repression factor involved in brain development; phosphorylation promotes nuclear export
<b>FoxO1a</b>	1	T24, S256, S319	10388075, 10377430, 11237865	transcription factor involved in cell cycle arrest, apoptosis, and glucose metabolism; phosphorylation causes export from the nucleus and inhibits activity
<b>FoxO3a</b>	1	T32, S253	11154281, 10102273	transcription factor involved in cell cycle arrest and apoptosis; phosphorylation causes export from the nucleus and inhibits activity
<b>FoxO4</b>	1	T32, S197, S262	11313479, 10217147, 14690436	transcription factor involved in cell cycle arrest, apoptosis, and insulin signaling; phosphorylation causes export from the nucleus and inhibits activity
<b>Gab2</b>	1	S159	11782427	docking/scaffolding protein; proto-oncogene; RTK signaling intermediate; phosphorylation inhibits activity
<b>GATA-1</b>	1	S310	16107690	transcription factor; phosphorylation increases activity and promotes blood cell differentiation
<b>GATA-2</b>	1	S401	15837948	transcription factor; phosphorylation inhibits activity to promote osteogenesis and reduce inflammation
<b>Girdin</b>	1	S1417	16139227	actin binding protein; phosphorylation promotes cell migration
<b>GOLGA3</b>	1	S174, S389	17888676	golgi auto-antigen; phosphorylation results in reduced apoptosis
<b>GSK-3α</b>	1	S21	11520785, 8524413	cell cycle, glycogen synthesis, and apoptosis; phosphorylation inhibits activity
<b>GSK-3β</b>	1	S9	12065987, 12900420, 15037173	cell cycle, glycogen synthesis, and apoptosis; phosphorylation inhibits activity
<b>H2B</b>	1	S37	8985174	core component of the nucleosome; phosphorylation effect currently unknown
<b>HMOX1</b>	1	S188	15581622	heme oxygenase involved in stress response; phosphorylation regulates binding affinity involved in pre-mRNA packaging into hnRNP particles and transport of poly(A) mRNA from cytoplasm to nucleus; phosphorylation regulates role in cyclin D1 and c-Myc IRES activity
<b>hnRNP A1</b>	1	S199	18652319	RNA binding protein; phosphorylation regulates role in cyclin D1 and c-Myc IRES activity
<b>hnRNP E1</b>	1, 2	S43	20154680	binds to single-stranded nucleic acid; phosphorylation results in disruption of BAT element binding and translational activation of Da2c2 and ILE1 mRNA
<b>Hra2</b>	1, 2	S212	17139112	protease released during apoptosis; phosphorylation inhibits activity and attenuates its pro-apoptotic activity
<b>Huntingtin</b>	1	S421	12062094, 15843398	Huntingtin's Disease; Akt phosphorylation blocks nuclear aggregation and promotes neuroprotection
<b>IKK-α</b>	1, 2	T23	10485710	NF-κB signaling intermediate; phosphorylation activates NF-κB and immune/stress response
<b>IP3R1</b>	1	S2690	16332683	Ca <sup>2+</sup> release and signaling; phosphorylation induces resistance to apoptosis, possibly through caspase-3 inactivation
<b>IRS-1</b>	1	S527	17579213	insulin receptor signaling intermediate; phosphorylation inhibits function
<b>Kv11.1 iso5</b>	1	T897	18791070	pot-forming subunit of voltage-gated potassium channels; essential for rhythmic excitability of cardiac muscle and endocrine cells; phosphorylation inhibits channels
<b>Lamin A/C</b>	1	S301, S404	18808171	component of nuclear lamina; phosphorylation regulates function of nuclear lamina
<b>Mad1</b>	1	S145	18451027, 19526459	component of spindle-assembly checkpoint; phosphorylation results in ubiquitination and degradation through 26S proteasome pathway
<b>MDM2</b>	1	S166, S186, S188	11504915, 15169778, 11895050	ubiquitin ligase involved in p53 degradation; phosphorylation results in translocation to the nucleus and inhibition of p53
<b>MLK3</b>	1	S674	12458207	JNK-mediated neuronal cell death; phosphorylation inhibits activity
<b>METTL1</b>	1	S27	15861136	catalyzes the formation of m7G46 in tRNA; phosphorylation results in inactivation
<b>MST1</b>	1	T120	19940129	pro-apoptotic kinase; phosphorylation inhibits kinase activity and nuclear translocation resulting in inhibition of pro-apoptotic signaling
<b>mTOR</b>	1	S248	10567225, 15208071	protein synthesis and cell growth; phosphorylation increases activity
<b>MYO5A</b>	2	S1662	17515613	actin-based motor protein with a role in cytoplasmic vesicle transport and anchorage; phosphorylation promotes insulin-mediated Glu4 vesicle translocation
<b>Myt1</b>	1	S83	111802161	Wee1 family member and cell cycle regulator; phosphorylation downregulates Myt1 and inhibits M-phase
<b>Ndr2</b>	1	T348	14985363	insulin-stimulated phosphoprotein; phosphorylation promotes insulin signaling
<b>NFAT9</b>	1	S647	18097023	transcription inhibitor protein; phosphorylation required for nuclear export
<b>NMDAR2C</b>	1	S1081	19477150	Glutamate receptor channel subunit; phosphorylation promotes binding to 14-3-3c and leads to increased surface expression of cerebellar NMDA receptors
<b>NuaK1</b>	1	S600	12403036	AMPK family member activated under nutrient starvation; mediates cell survival during glucose starvation; phosphorylation increases kinase activity
<b>Nur77</b>	1	S351	11274386, 11438350	nuclear receptor; transcription factor; T cell apoptosis; phosphorylation inhibits transcriptional activity
<b>p21 Waf1/Cip1</b>	1	T144, S145	11756412, 11231573, 15173900	regulates cell cycle and cell survival; phosphorylation increases protein stability
<b>p300</b>	1	S1834	11116148, 16024795	transcription co-activator; phosphorylation can either activate or suppress transcriptional activity depending on cell type and physiological stimuli
<b>Palladin</b>	1	S607	20471940	actin-binding protein; phosphorylation promotes F-actin bundling and inhibits cell migration
<b>PDCD4</b>	1	S67, S457	16357133	tumor suppressor protein that is strongly induced during apoptosis; phosphorylation inhibits tumor suppressor function
<b>PDE3A</b>	1	S292, S293, S294	17124499	regulates levels of cAMP and cGMP; insulin-dependent oocyte maturation; phosphorylation increases activity
<b>PDE3B</b>	1	S295	10454575	regulates levels of cAMP and cGMP; activated by insulin to regulate lipolysis; phosphorylation increases activity
<b>Peripherin</b>	1	S59	17569669	neuronal intermediate filament protein; phosphorylation promotes motor nerve regeneration
<b>PFKFB2</b>	1	S466, S483	9211863, 10521487, 18689056	glycolytic enzyme; insulin-mediated glucose metabolism; phosphorylation increases activity
<b>PGC-1</b>	1, 2	S571	17554339	regulates gluconeogenesis and fatty acid oxidation; phosphorylation inhibits function
<b>PLCγ1</b>	1	S1248	16525023	catalyzes PI 4,5 bisphosphate to P3 and DAG; increases intracellular Ca <sup>2+</sup> levels; phosphorylation increases activity and enhances EGF-stimulated cell motility
<b>PRAS40 (Akt1S1)</b>	1	T246	12524439	binds to and inhibits mTOR; phosphorylation causes 14-3-3 binding/inhibition and results in increased protein synthesis

## PI3 Kinase/Akt Signaling



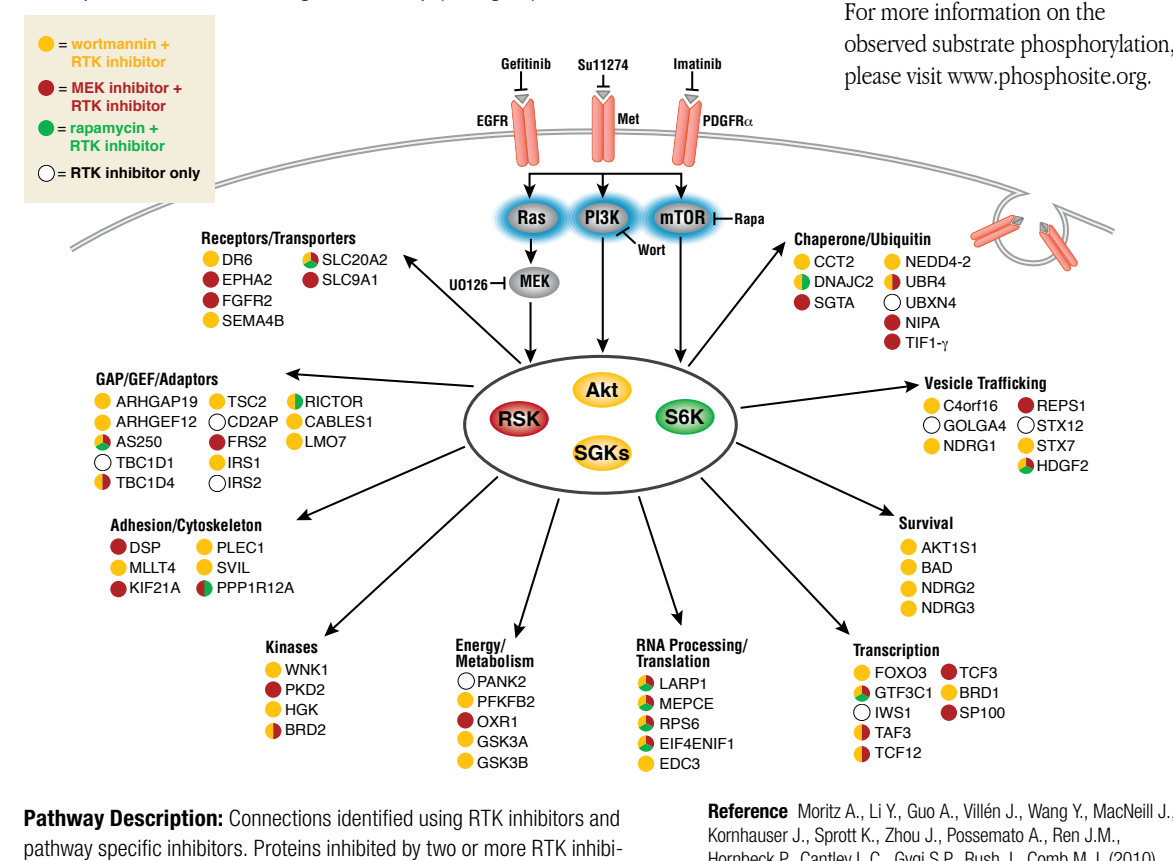
**Pathway Description:** Since its initial discovery as a proto-oncogene, the serine/threonine kinase Akt (also known as protein kinase B or PKB) has become a major focus of attention because of its critical regulatory role in diverse cellular processes, including cancer progression and insulin metabolism. 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-triphosphate (PIP3) 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.

## 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 RxxSx/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 RxxSx/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 inhibitor(s) are grouped by protein function. Color coding indicates sensitivity to pathway inhibitor.