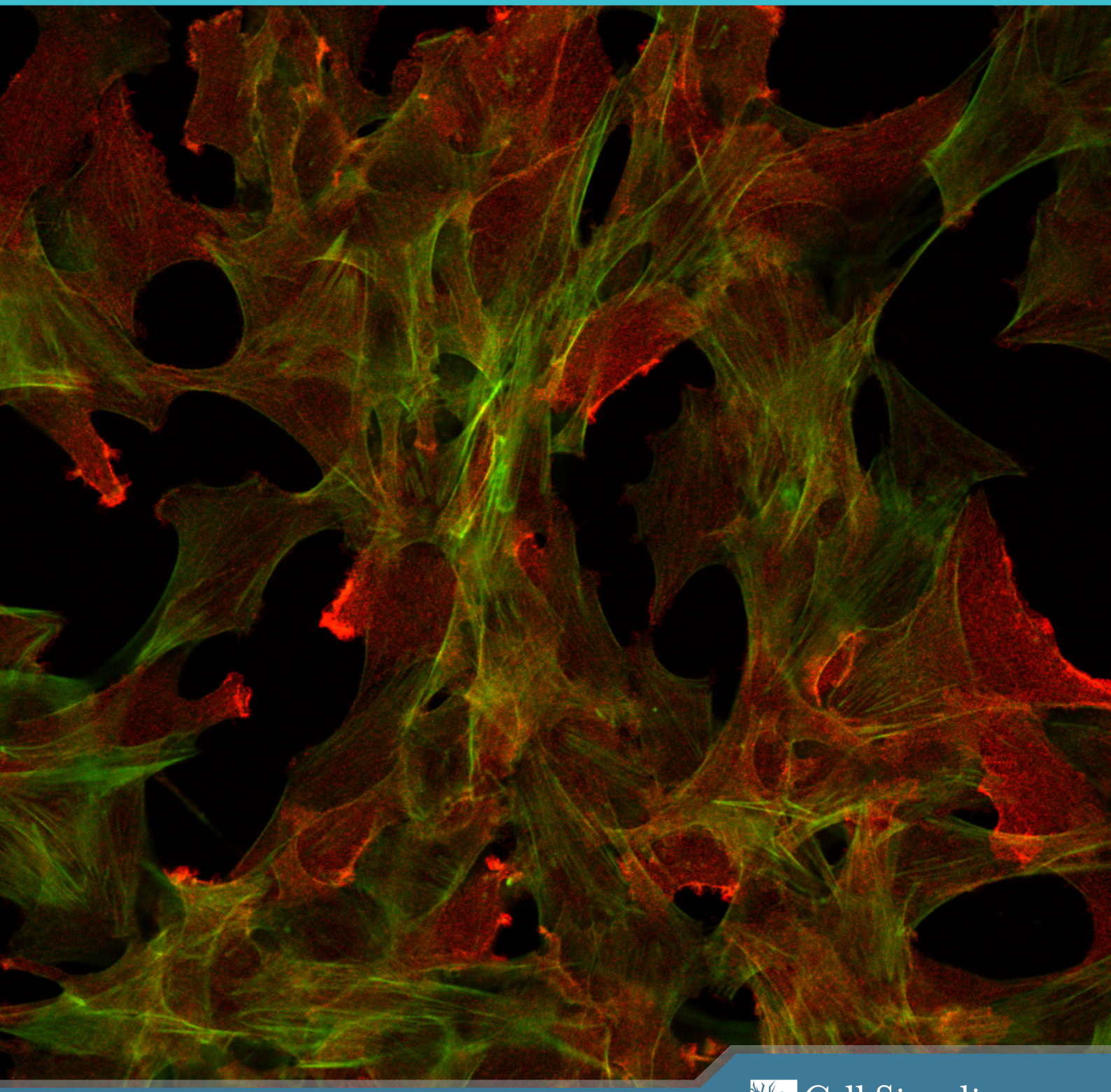
RESEARCH FOCUS

Akt Substrates Guide

A comprehensive table of Akt Substrates identified using PTMScan® Technology.



Cell Signaling TECHNOLOGY®

Akt Substrates Guide

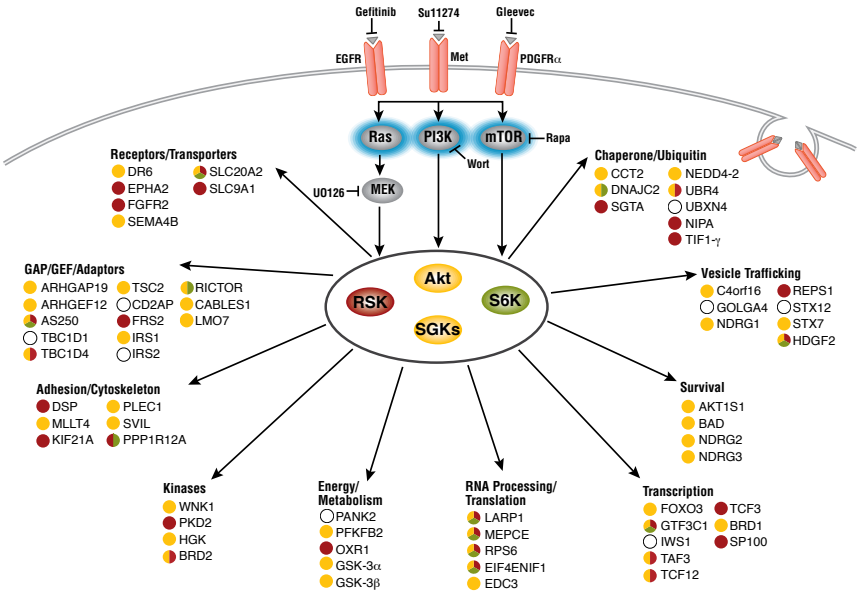
Since its initial discovery as a proto-oncogene, the serine/threonine kinase Akt (also known as protein kinase B or PKB) has become a major focus of attention because of its critical regulatory role in diverse cellular processes, including cancer progression and insulin metabolism. The Akt cascade is 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 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. The tumor suppressor PTEN is recognized as a major inhibitor of Akt and is frequently lost in human tumors. Recently, there has been increased focus on phosphatases that can inactivate Akt, including PHLLP.

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. In addition,

A phosphoproteomic study co-authored by Cell Signaling Technology (CST) scientists (Moritz et al. (2010) *Sci. Signal.* 136, ra64) identified 300 novel downstream substrates for the PKA, PKG, PKC (AGC) family of kinases. The scientists used PTMScan® Technology, CST's 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 a phospho-Akt substrate (RxRxxS/T) antibody, which was used to selectively immunoprecipitate phosphorylated substrates of Akt, p70 S6, and RSK AGC kinase family members. PTMScan® allowed for mapping of the signaling network targets downstream of oncogenic RTKs. Novel identified substrates included proteins involved in many cellular functions, including cellular scaffolding, protein stability, metabolism, trafficking, and motility.

Moritz, A., Li, Y., Guo, A., Villen, 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., and Comb, M.J. (2010) *Sci. Signal.* 136, ra64



Pathway Key

- wortmannin + RTK inhibitor
- rapamycin + RTK inhibitor
- MEK inhibitor + RTK inhibitor
- RTK inhibitor only

Substrate	Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
14-3-3 ζ	Akt1	human	S58	S58	VVGARRSsWRVVssI	11956222	a key regulatory protein in signal transduction, checkpoint control, apoptotic, and nutrient-sensing pathways; effect of phosphorylation is unknown
acinus	Akt1	human	S1180	S1180	GPRsRsRsRDRRRKE	18559500, 16177823	induces chromatin condensation during apoptosis; phosphorylation inhibits this process
	Akt1	rat	S1329	S1331	HSRSRSRsTPVRDRG	16177823	induces chromatin condensation during apoptosis; phosphorylation inhibits this process
ACLY	Akt1	mouse	S455	S455	PAPSRtAsFsESRAD	16007201	catalyzes the formation of acetyl-CoA and oxaloacetate (OAA) in the cytosol; phosphorylation enhances the catalytic activity of the enzyme
ADRB2	Akt1	human	S346	S346	LLCLRRssLKAyGNG	11809767	a receptor that binds epinephrine and norepinephrine, acting as a neuromodulator in the central nervous system and as a hormone in the vascular system; phosphorylation in response to insulin stimulation leads to sequestration of ADBR2
Akt1	Akt1	human	S246, T72	S246, T72	LSRERVFsEDRARFY, TERPRPNtFIIRCLQ	16549426	activated by insulin and various growth and survival factors to function in a wortmannin-sensitive PI3 kinase-involved pathway controlling survival and apoptosis; autophosphorylation activates the kinase
	Akt1	mouse	S473	S473	RPHFPQFsYsAsGtA	11570877, 10722653	activated by insulin and various growth and survival factors to function in a wortmannin-sensitive PI3 kinase-involved pathway controlling survival and apoptosis; autophosphorylation activates the kinase
AMPKA1	Akt1	rat	S485	S485	ATPQRSGsISNYRSC	16340011	heterotrimeric complex that plays a key role in the regulation of energy homeostasis; phosphorylation regulates AMPK activity
AMPKA2	Akt1	rat	S491	S491	STPQRSCsAAGLHRP	16340011	heterotrimeric complex that plays a key role in the regulation of energy homeostasis; phosphorylation regulates AMPK activity
APS	Akt1	rat	S588	S598	SARSRSNsTEHLLEA	16141217	an adaptor protein recruited to the insulin receptor to signal insulin-stimulated glucose transport; phosphorylation promotes membrane localization
AR	Akt1	human	S213, S791	S213, S791	SGRAREAsGAPTsSK, CVRMRHLsQEFGWLQ	11404460, 14555644, 17470458, 11156376	nuclear receptor; phosphorylation suppresses AR activation, expression of AR target genes, and AR-mediated apoptosis
arfaptin 2	Akt1	human	S260	S260	GTRGRLEsAQATFQA	15809304	ADP ribosylation factor-interacting protein, implicated as a factor in Huntington's disease; phosphorylation promotes neuronal cell survival and neuroprotection
ARHGAP22	Akt1	human	S16	S16	ARRARSKsLVMGEQS	21969604	a Rho GTPase activator that inhibits Rac1; phosphorylation allows 14-3-3 binding and regulation of cell motility
AS160	Akt1	human	T642	T642	QFRRRAHtFsHPPss	16880201, 11994271, 16935857	insulin stimulated Rab GTPase-activating protein, structurally and functionally similar to TBC1D1; phosphorylation results in increased Glut4 translocation
ASK1	Akt1, Akt2	human	S83	S83	ATRGRGssVGGGSRR	11154276, 15782121, 15911620, 14500571, 12697749	MAPKKK, induces apoptosis via JNK pathway; phosphorylation inhibits activity and promotes survival
ataxin-1	Akt1	human	S775	S775	ATRKRRWsAPESRKL	17540008, 12757707	14-3-3 binds to and stabilizes ataxin-1, which forms polyglutamine aggregates and neurodegeneration; phosphorylation promotes 14-3-3 binding

germline mutations of Akt have been identified in pathological conditions of cancer and insulin metabolism.

Akt regulates cell growth through its effects on the TSC1/TSC2 complex and mTOR 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 the pro-apoptotic regulator Bad and the FoxO and Myc 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-□ 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.

Substrate	Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
B-Raf	Akt1, Akt3	human	S365, S429	S365, S429	GQRDRsssAPNVHIN, PQRERKsssSsEDRN	10869359, 18451171	signaling intermediate in Erk1/2 pathway; phosphorylation causes inhibition
BAD	Akt1	human	S99	S99	PFRGRsRsAPPNLWA	11020382, 10558990, 19667065	pro-apoptotic protein; phosphorylation inhibits function and promotes survival
	Akt1	mouse	S112, S155	S75, S118	ETRsRHssyPAGtEE, GRELRRMsDEFEGSF	9381178, 11723239, 10983986, 15123689, 10949026	pro-apoptotic protein; phosphorylation inhibits function and promotes survival
Bcl-10	Akt1	human	S218, S231	S218, S231	EEGTCANsSEMFLPL, PLRSRtVsRQ_____	16280327	a CARD (caspase recruitment domain) containing protein shown to induce apoptosis and activate NF-κB; phosphorylation induces nuclear translocation
Bcl-xL	Akt1	rat	S106	S106	LRYRRAFsDLTSQLH	18951975	prevents apoptosis through binding to apoptotic proteins; phosphorylation promotes VDAC binding
Bex1	Akt1	rat	S105	S102	KLRERQLsHSLRAVS	16498402	a neurotrophin and nerve growth factor signaling adaptor molecule involved in promoting cell cycle progression; phosphorylation prevents degradation by the proteasome
Bim	Akt1	human	S87	S87	FIFMRRssLLSRSss	16282323	pro-apoptotic protein; phosphorylation promotes 14-3-3 binding/inactivation and cell survival
BRCA1	Akt1	human	S694, T509	S694, T509	QTSKRHDsDTFPELK, LKRKRRPtsGLHPED	20085797, 10542266	breast cancer susceptibility gene product, tumor suppressor; phosphorylation alters function, perhaps by preventing nuclear localization
BRF1	Akt1	human	S92, S203	S92, S203	RFRDRsFsEGGERLL, PRLQHsFsFAGFPSA	17030608, 15538381	a CCCH zinc-finger protein that binds to AU-rich elements (ARE) found in the 3'-untranslated regions of mRNAs and promotes de-adenylation and rapid degradation by the exosome; phosphorylation results in binding by 14-3-3 protein and inactivation of BRF1
CACNB2	Akt1	rat	S625	S630	KQRSRHKsKDRYCDK	15311280	voltage-dependent calcium channel; phosphorylation regulates channel trafficking to plasma membrane
CaRHSP1	Akt1	human	S52	S52	tRRtRtFsAtVRASQ	15910284	RNA binding protein; phosphorylation effect currently unknown
Casp9	Akt1	human	S196	S196	KLRRRFssLHFMVEV	9812896	protease, initiates apoptosis; phosphorylation inhibits protease activity
CBP	Akt1	mouse	T1872	T1871	LMRRRMAtMNTRNVP	17166829	acetylates histone and non-histone proteins; phosphorylation increases activity
CBY1	Akt1, Akt2	human	S20	S20	TPPRKSAsLSNLHsL	18573912	an inhibitor of the Wnt signaling pathway; phosphorylation allows 14-3-3 binding and β-catenin sequestration in the cytoplasm
CCT2	Akt1	human	S260	S260	GsRVRVDstAkVAEI	19332537	member of the protein chaperone complex; effect of phosphorylation currently unknown
CD34	Akt2	mouse	S343	S346	yssGPGAsPETQGKA	21499536	a type I transmembrane glycophosphoprotein expressed by hematopoietic stem/progenitor cells, vascular endothelium and some fibroblasts as a negative regulator of cell adhesion; effect of phosphorylation currently unknown
Cdc25B	Akt1	mouse	S351	S353	VQSKRRKsVtPLEEQ	17554083	protein phosphatase responsible for cdc2 activation; phosphorylation promotes activation of M-phase promoting factor
CDK2	Akt1	human	T39	T39	LkKIRLDtETEGVPs	18354084	cyclin-dependent kinase functioning in S-phase; phosphorylation increases cyclin A binding
CELF1	Akt1	human	S28	S28	GQVPRTWsEKDLREL	18570922	RNA-binding protein; phosphorylation enhances interaction with cyclin D1 mRNA
CENTB1	Akt1	human	S554	S554	SIRPRPGsLRSKPEP	16256741	GTPase-activating protein (GAP) for ARF proteins; phosphorylation prevents recycling of b1-integrin containing endosomes and cell migration
CENTG1	Akt1	human	S985	S985	THLSRVRsLDLDDWP	19176382	a GTPase activating protein for ARF1 and ARF5; phosphorylation enhances CENTG1 GTP binding and NF-κB activity
CFLAR	Akt1	human	S273	S273	LLRDTFTsLGYEVQK	19339247	a regulator of apoptosis; phosphorylation targets CFLAR for degradation
Chk1	Akt1	human	S280	S280	AKRPRVtsGGVsEsP	15107605, 12062056	DNA damage effector that regulates G2/M transition during DNA damage; phosphorylation inhibits function by preventing phosphorylation by ATM/ATR
CK1-D	Akt1	rat	S370	S370	MERERKVsMRLHRGA	17594292	kinase and core component of circadian clock; phosphorylation inhibits kinase activity
CLK2	Akt1	human	S34, T127	S34, T127	HKRRRSRsWSSSSDR, RRRRRSRtFSRSSSQ	20682768	a dual specificity serine/threonine and tyrosine kinase; phosphorylation increases cell survival after ionizing radiation
Cot	Akt1	human	S400	S400	EDQPRCQsLDSALLE	12138205	oncogene; phosphorylation induces NF-κB-dependent transcription
CREB	Akt1	rat	S133	S133	EILsRRPsYRkILND	9829964	bZIP transcription factor that activates target genes through cAMP response elements; activated by phosphorylation
CTNNB1	Akt1, Akt2	human	S552	S552	QDtQRRtsMGGtQQQ	17287208	Wnt signaling pathway protein; phosphorylation causes nuclear localization
CTNND2	Akt1	mouse	T454	T457	tGTFRtstAPssPGV	17993462	transcriptional activator, plays a role in adhesion molecule regulation; phosphorylation promotes binding to p190RhoGEF, dendritic morphogenesis
Cx43	Akt1	rat	S369	S369	RPssRAssRAssRPR	18163231	gap junction protein; phosphorylation allows 14-3-3 binding
	Akt1, Akt3	rat	S373	S373	RAssRAssRPRPDDL	17008717, 18163231	gap junction protein; phosphorylation allows 14-3-3 binding

AKT SUBSTRATES

Substrate	Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
DLC1	Akt1	rat	S330	S766	VTRTRSLsTCNKRVG	16338927	tumor suppressor and insulin stimulated phosphoprotein, may play role in Glut4 translocation; phosphorylation may inhibit its GAP activity
DNAJC5	Akt1	rat	S10	S10	DQRQRsLsTSGESLY	16243840	exocytosis; phosphorylation regulates the kinetics of late stage exocytosis
DNMT1	Akt1	human	S143	S143	RtPRRsksDGEAkPE	21151116	a maintenance methyltransferase, transferring proper methylation patterns to newly synthesized DNA during replication; phosphorylation increases DNMT1 stability and prevents methylation
EDC3	Akt1, Akt2	human	S161	S161	sFRRRHNsWssSsRH	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
EDG-1	Akt1	human	T236	T236	RTRSRRLtFRKNISK	11583630	G protein-coupled receptor; phosphorylation activates signaling to promote cell migration
eIF4B	Akt1	mouse	S422	S422	RERsRtGsEssQtGA	18836482	necessary for binding of mRNA to ribosomes; phosphorylation increases transcriptional activity
ENaC-α	Akt1	rat	S621	S594	RFRSRYWsPGRGARG	21220922	an amiloride sensitive epithelial sodium channel that mediates sodium reabsorption; phosphorylation increases ENaC specific activity
eNOS	Akt1	human	S615, S1177	S615, S1177	SYKIRFNsISCSDPL, TsRIRtQsFsLQERQ	12511559, 12446767, 10376603, 18622039, 12171920	enzyme that catalyzes the production of nitric oxide (NO); phosphorylation results in enzyme activation, NO production, and cardiovascular homeostasis (vasodilation, vascular remodeling, angiogenesis)
EphA2	Akt1	human	S897	S897	RVsIRLPstsGsEGV	19573808	receptor tyrosine kinase that binds to a GPI-anchored ephrin A ligand for regulation of cell adhesion, cell migration, axon guidance, and homeostasis; phosphorylation regulates EphA2 induced cell migration and invasion
ER-α	Akt1, Akt2	human	S167	S167	GGRERLAsTNDKGSM	11139588, 16113102, 11507039	nuclear receptor and transcription factor; phosphorylation activates the receptor and increases gene expression, causing mammary and uterine cell proliferation
	Akt1	human	S305	S305	IkRSkkNsLALSLtA	20101208	nuclear receptor and transcription factor; phosphorylation activates the receptor and increases gene expression, causing mammary and uterine cell proliferation
ER-β	Akt1	mouse	S236	D236	VRRQRSAsEQVHCLN	17166829	nuclear receptor and transcription factor; phosphorylation prevents cofactor binding and decreases activity
EZH2	Akt1	human	S21	S21	CWRKRVKsEYMRLRQ	16224021	methyltransferase; phosphorylation decreases histone H3 methylation of Lys27 and increases gene expression
ezrin	Akt2	human	T567	T567	QGRDKYKtLRQIRQG	15531580	plasma membrane/cytoskeletal linker protein; phosphorylation promotes actin binding and cytoskeletal organization
FANCA	Akt1	human	S1149	S1149	CLRSRDPsLMVDFIL	11855836	ATPase involved in DNA repair; phosphorylation is negatively regulated by Akt
FLEG1	Akt1	human	S486	S486	GLEtRRLsLPSsKAK	17256767	a chaperone protein involved in directing specific histones to the centromere; phosphorylation allows binding to 14-3-3
FLNC	Akt1, Akt2	human	S2233	S2233	LGRERLGsFGsItRQ	15461588	muscle-specific filamin functioning in muscle cells; phosphorylation effect currently unknown
FOXA2	Akt1	human	T156	T156	KTYRRSYtHAKPPYS	14500912	transcription factor involved in embryonic development and differentiation; phosphorylation results in nuclear exclusion and inhibition of FoxA2-dependent transcriptional activity
FOXG1	Akt1	human	T279	T279	KLRRRSttsRAKLAF	17435750	transcriptional repression factor involved in brain development; phosphorylation promotes nuclear export
FOXO1A	Akt1	human	S256, S319, T24	S256, S319, T24	sPrRrAAsMDNNSkF, TFRPRtssNAsTlsG, LPRPRSCtWPLPRPE	15668399, 10358075, 11237865, 16076959, 11311120	transcription factor involved in cell cycle arrest, apoptosis, and glucose metabolism; phosphorylation causes export from the nucleus and inhibits activity
FOXO3A	Akt1	human	S253, T32	S253, T32	APRRRAVsMDNSNKY, QSRPRsCtWPLQRPE	10910908, 10995739, 10102273, 11154281	transcription factor involved in cell cycle arrest and apoptosis; phosphorylation causes export from the nucleus and inhibits activity
FOXO4	Akt1	human	S197, S262, T32	S197, S262, T32	APRRRAAsMDSSSKL, TFRPRSssNASSVST, QSRPRsCtWPLPRPE	11313479, 11313479, 10217147, 16272144	transcription factor involved in cell cycle arrest, apoptosis, and insulin signaling; phosphorylation causes export from the nucleus and inhibits activity
Gab2	Akt1	human	S159	S159	LLRERKSsAPSHsSQ	11782427	docking/scaffolding protein, proto-oncogene, RTK signaling intermediate; phosphorylation inhibits activity
GABRB2	Akt1	rat	S434	S434	SRLRRRAsQLKITIP	12818177	receptor that mediates fast inhibitory synaptic transmission in the brain; phosphorylation increases the number of receptors on the cell surface
GAPDH	Akt2	human	T237	T237	GMAFRVPtANVSVVD	21979951	catalyzes the phosphorylation of glyceraldehyde-3-phosphate during glycolysis; phosphorylation decreases nuclear translocation and GAPDH induced apoptosis
GATA1	Akt1	human	S310	S310	QTRNRKAsGkGkkkR	16107690	transcription factor; phosphorylation increases activity and promotes blood cell differentiation
GATA2	Akt1	human	S401	S401	QTRNRKMsNKSKKSK	15837948	transcription factor; phosphorylation inhibits activity to promote adipogenesis and reduce inflammation
girdin	Akt1	human	S1417	S1417	INRERQKsLtLTPTR	16139227	actin binding protein; phosphorylation promotes cell migration
GOLGA3	Akt1	mouse	S174, S385	S174, S389	VKRHRERsSQPAtKM, EVRsRRDsICsSVSM	17888676	golgi auto-antigen; phosphorylation results in reduced apoptosis
Grb10	Akt1	mouse	S455	S428	NAPMRsVsENsLVAM	15722337	an adaptor protein that interacts with many receptor tyrosine kinases as well as downstream signal molecules; phosphorylation allows binding to 14-3-3
GSK-3α	Akt1	human	S21	S21	SGRARtssFAEPGGG	11340086, 11563975, 11577096	serine/threonine protein kinase that phosphorylates and inactivates glycogen synthase; phosphorylation inhibits activity
GSK-3β	Akt1	human	S9	S9	SGRPRttsFAESCKP	12900420, 15457186, 11563975, 11340086, 11577096, 8985174	serine/threonine protein kinase that phosphorylates and inactivates glycogen synthase; phosphorylation inhibits activity
H2B	Akt1	human	S37	S37	RKRsRkEsyslyVyk	8985174	core component of the nucleosome; phosphorylation effect currently unknown
H3	Akt1	mouse	S10	S10	tKQTARksTGGkAPR	12529330	core component of the nucleosome; phosphorylation is correlated with chromosome condensation during mitosis and meiosis

Substrate	Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
HMOX1	Akt1	human	S188	S188	LYRSRMNsLEMtPAV	15581622	heme oxygenase involved in stress response; phosphorylation regulates binding affinity
hnRNP A1	Akt1	human	S199	S199	sQrGrsGsGNFGGGr	18562319	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
hnRNP E1	Akt1, Akt2	mouse	S43	S43	VKRIREEsGARINIS	20154680	binds to single-stranded nucleic acid; phosphorylation results in disruption of BAT element binding and translational activation of Dab2 and ILEI mRNA
HSP27	Akt1	human	S82	S82	RALsRQLssGVSEIR	12740362	heat shock protein that confers cellular resistance to stress and adverse environmental change; phosphorylation alters tertiary structure, modulates actin polymerization, and reorganization
HTRA2	Akt1, Akt2	human	S212	S212	RVRVRLLsGDTYEAV	17311912	protease released during apoptosis; phosphorylation inhibits activity and attenuates its pro-apoptotic function
Huntingtin	Akt1	human	S421	S421	GGRsRsGsIVELIAG	12062094, 14725621, 15843398, 16452687	Huntington's disease; Akt phosphorylation blocks nuclear aggregation and provides neuroprotection
IKK-α	Akt1, Akt2	human	T23	T23	EMRERLGtGGFGNVC	18515365, 12048203, 10485710, 19609947	NF-κB signaling intermediate; phosphorylation activates NF-κB and immune/stress response
IP3R1	Akt1	rat	S2682	S2690	FPRMRAMsLVSSDSE	16332683	Ca^{2+} release and signaling; phosphorylation induces resistance to apoptosis, possibly through caspase-3 inactivation
IRAK1	Akt1	human	T100	T100	LRARDIItAWHPPAP	11976320	a serine/threonine-specific IL-1 receptor-associated kinase involved in Toll signaling; phosphorylation inhibits IRAK mediated NF-κB activation
IRS1	Akt1	human	S629	S629	VPSGRKGsGDyMPMs	17640984	insulin receptor signaling intermediate; phosphorylation inhibits function
	Akt1	rat	S522	S527	RFRKRTHsAGTSPTI	17579213	insulin receptor signaling intermediate; phosphorylation inhibits function
KHSRP	Akt1, Akt2	human	S193	S193	GLPERSVsLTGAPES	17177604	recruits degradation machinery, activates mRNA turnover, regulates splicing; phosphorylation inhibits RNA turnover by degradation
Kv11.1 iso5	Akt1	human	T897	T897	SFRRRtDtDtEQPGE	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	Akt1	rat	S301, S404	S301, S404	RSRGRASsHSSQSQG	18808171	component of nuclear lamina; phosphorylation regulates function of nuclear lamina
LTB4R2	Akt1	human	T355	T355	GGRsREGtMELRTTP	22044535	a low-affinity leukotriene receptor involved in chemotaxis; phosphorylation regulates activation of chemotactic responses
Mad1	Akt1	human	S145	S145	IERIRMDsIGSTVSS	18451027, 19526459	component of spindle-assembly checkpoint; phosphorylation results in ubiquitination and degradation through 26S proteasome pathway
MDM2	Akt1	human	S166, S186, S188	S166, S186, S188	SsRRRAIsEtEENsD, RQRKRHKsDsIsLsF, RKRHKsDsIsLsFDE	11715018, 15169778, 11504915, 11850850, 11923280, 15527798, 11960368	ubiquitin ligase involved in p53 degradation; phosphorylation results in translocation to the nucleus and inhibition of p53
MDM4	Akt1	human	S367	S367	PDCRRtIsAPVVRPK	18356162	RING-finger domain protein involved in p53 degradation and apoptosis; phosphorylation stabilizes MDM4 and MDM2
METTL1	Akt1	human	S27	S27	yYRQrAHsNPMADHT	15861136	catalyzes the formation of m7G46 in tRNA; phosphorylation results in inactivation
MKK4	Akt1	human	S80	S80	IERLRtHsIEsSGKL	15911620, 11707464	signaling intermediate of the JNK/SAPK pathway involved in stress/inflammation; phosphorylation inhibits activity
MLK3	Akt1	human	S674	S674	PGRERGEsPTtPPTP	12458207	JNK-mediated neuronal cell death; phosphorylation inhibits activity
MST1	Akt1	human	T120	T120	IIRLRNktLTEDEIA	19940129	pro-apoptotic kinase; phosphorylation inhibits kinase activity and nuclear translocation resulting in inhibition of pro-apoptotic signaling
MST2	Akt1	human	T117, T384	T117, T384	IIRLRNktLIEDEIA, GTMKRNAtsPQVQRP	20231902, 20086174	upstream activator of the MAPK pathway that regulates apoptosis, morphogenesis, and cytoskeletal rearrangements; phosphorylation inhibits pro-apoptotic activity
mTOR	Akt1	human	T2446, S2448	T2446, S2448	RsRtRtDsysAGQsV	15208671, 10910062, 10567225	protein synthesis and cell growth; phosphorylation increases activity
MYO5A	Akt2	mouse	S1650	S1652	GLRKRtssIADEGty	17515613	actin-based motor protein with a role in cytoplasmic vesicle transport and anchorage; phosphorylation promotes insulin-mediated Glut4 vesicle translocation
Myt1	Akt1	starfish	S75	S83	ESRPRAVsFRQSEPS	11802161	Wee1 family member and cell cycle regulator; phosphorylation downregulates Myt1 and initiates M-phase
NDRG2	Akt1	human	S332, T348	S332, T348	LsRsRtAsLtsAAsV, GNRsRsRtLsQssEs	15461589	insulin-stimulated phosphoprotein; phosphorylation promotes insulin signaling
NFAT90	Akt1	human	S647	S647	rGrGRGGsIRGRGRG	18097023, 20870937	translation inhibitory protein; phosphorylation required for nuclear export
NHE1	Akt1	human	S648, S703, S796	S648, S703, S796	KTRQRLRsyNRHTLV, MsRARIGsDPLAyEP, QRIQRCLsDPGPHPE	18757828, 20026127	sodium/hydrogen exchanger involved in pH regulation and signal transduction; phosphorylation inhibits activity
NMDAR2C	Akt1	mouse	S1084	S1081	GPRPRHAsLPSSVAE	19477150	Glutamate receptor channel subunit; phosphorylation promotes binding to 14-3-3ε and leads to increased surface expression of cerebellar NMDA receptors
	Akt1	rat	S1083	S1081	GPRPRHAsLPSSVAE	19477150	Glutamate receptor channel subunit; phosphorylation promotes binding to 14-3-3ε and leads to increased surface expression of cerebellar NMDA receptors
NuaK1	Akt1	human	S600	S600	PARQRIRsCVSAENF	15060171, 12409306	AMPK family member activated under glucose starvation that mediates cell survival; phosphorylation increases kinase activity
Nur77	Akt1	human	S351	S351	GRRGRLPsKPKQPPD	16434970, 11274386	a nuclear receptor and transcription factor regulating T cell apoptosis; phosphorylation inhibits transcriptional activity

AKT SUBSTRATES

Substrate	Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
p21 Cip1	Akt1	human	S146, T145	S146, T145	GRkRRQtsMTDFYHs, QGRkRRQtsMTDFYH	17855660, 11231573, 11756412, 15173090, 11463845, 116982699	regulates cell cycle and cell survival; phosphorylation increases protein stability
p27Kip1	Akt1	human	S10, T157, T198	S10, T157, T198	NVRVsNGsPsLErMD, GIRkrPAtDDSSTQN, PGLRRRQt_____	18710949, 12042314, 12244302	a cyclin-dependent kinase inhibitor that enforces the G1 cell cycle restriction point; phosphorylation promotes 14-3-3 binding and cytoplasmic localization
p300	Akt1	human	S1834	S1834	MLRRRMAsMQRTGVV	16024795, 11116148	transcriptional co-activator; phosphorylation can either activate or suppress transcriptional activity depending on cell type and physiological stimuli
p47phox	Akt1	human	S304, S328	S304, S328	GAPPRRssIRNAHSI, QDAYRRNsVRFLQQR	12734380	a component of the phagocytic NADPH oxidase multiprotein enzyme that catalyzes the reduction of oxygen to superoxide in response to pathogenic invasion; phosphorylation regulates p47hox respiratory burst activity
PAK1	Akt1	mouse	S21	S21	APPMRNTsTMIGAGS	14585966	a p21-activated kinase engaged in cytoskeletal reorganization, MAPK signaling, apoptotic signaling, control of phagocyte NADPH oxidase, and growth factor-induced neurite outgrowth; phosphorylation at Ser21 regulates binding with the adaptor protein Nck
palladin	Akt1	human	S1118	S1118	VRRPRsRsRDsGDEN	20471940	actin-bundling protein; phosphorylation promotes F-actin bundling and inhibits cell migration
PAR-4	Akt1	rat	S249	N257	SRHNRDTsAPANFAS	16209943	a pro-apoptotic factor that activates the Fas-FADD-caspase-8 pathway as well as inhibits the NF-κB pro-survival pathway; phosphorylation prevents nuclear translocation, promoting cell survival
PDCD4	Akt1	human	S67, S457	S67, S457	kRRLRKNssRDsGRG, RGRKRFVsEGDGGRL	16357133	tumor suppressor protein that is strongly induced during apoptosis; phosphorylation inhibits tumor suppressor function
PDE3A	Akt1	mouse	S290, S291, S292	S290, S291, S292	GWKRRRRsssVVAGE, WKRRRRsssVVAGEM, KRRRRsssVVAGEMS	17124499	regulates levels of cAMP and cGMP, insulin-dependent oocyte maturation; phosphorylation increases activity
PDE3B	Akt1	mouse	S273	S295	VIRPRRRssCVsLGE	10454575	regulates levels of cAMP and cGMP, activated by insulin to regulate lipolysis; phosphorylation increases activity
PEA-15	Akt1	human	S116	S116	KDIIRQPsEEEIIKL	12808093	a phosphoprotein shown to coordinate cell growth, death, and glucose utilization; phosphorylation mediates binding to FADD or Erk and further regulates the Erk and apoptosis signaling pathways
peripherin	Akt1	mouse	S66	S59	SSSARLGsFRAPRAG	17569669	neuronal intermediate filament protein; phosphorylation promotes motor nerve regeneration
PFKFB2	Akt1	human	S466, S483	S466, S483	PVRMRRNsFtPLSSS, IRRPRNysVGSRPLK	12853467	glycolytic enzyme, insulin-mediated glucose metabolism; phosphorylation increases activity
PFKFB3	Akt1	human	S461	S461	NPLMRRNsVtPLAsP	15896703	synthesis and degradation of fructose 2,6-bisphosphate; phosphorylation decreases sensitivity to inhibition
PGC-1 α	Akt1, Akt2	mouse	S570	S571	RMRSRsRsFsRHRSC	17554339	regulates gluconeogenesis and fatty acid oxidation; phosphorylation inhibits function
PIP5K	Akt1	human	S307	S307	PARNRsAsItNLsLD	15546921	a protein/ lipid kinase involved in membrane trafficking; phosphorylated in response to insulin
	Akt1	mouse	S105	S105	EELHRRSsVLENTLP	20513353	a protein/ lipid kinase involved in membrane trafficking; phosphorylated in response to insulin
PLB	Akt1	rat	S16	S16	RSAIRRAstIEMPQQ	18838385	a major phosphoprotein calcium regulation component of the sarcoplasmic reticulum; phosphorylation causes release of inhibition and increases calcium uptake by the sarcoplasmic reticulum
PLCG1	Akt1	human	S1248	S1248	HGRAREGsFEsRyQQ	16525023	catalyzes PI 4,5 bisphosphate to IP_3 and DAG, increases intracellular Ca^{2+} levels; phosphorylation increases activity and enhances EGF-stimulated cell motility
PPP1CA	Akt1	human	T320	T320	NPGGRPItPPRNSAK	14633703	a serine/threonine phosphatase involved in cell cycle regulation; phosphorylation inhibits activity
PRAS40	Akt1	human	T246	T246	LPRPRLNtsDFQKLK	12524439, 17277771, 18372248	binds to and inhibits mTOR; phosphorylation causes 14-3-3 binding/inhibition and results in increased protein synthesis
PRPF19	Akt1	human	T193	T193	ERKKRGKtVPEELVK	20629186	a member of the splicesome that also functions in DNA double strand break repair; phosphorylation allows 14-3-3 binding
PRPK	Akt1	human	S250	S250	RLRGRKRsMVG____	17712528	p53 binding protein and kinase; phosphorylation causes activation and results in p53 phosphorylation
PTP1B	Akt1	human	S50	S50	RNRyRDVsPFDHsRI	11579209	protein tyrosine phosphatase that dephosphorylates the insulin receptor; phosphorylation inhibits activity
QIK	Akt2	mouse	S358	S358	DGRQRRPstIAEQTV	17805301	AMPK related protein; phosphorylation leads to kinase activation and promotes ubiquitination/ degradation of TORC2
Rac1	Akt1	human	S71	S71	yDRLRPLsYPQTDVF	10617634	Rho-GTPase, actin cytoskeletal organization; phosphorylation inhibits GTP-binding activity
Raf1	Akt1	mouse	S259	S259	SQRQRStsTPNVHMV	12087097, 12087097	signaling intermediate in Erk1/2 pathway; phosphorylation inhibits activity
	Akt1	rat	S259	S259	SQRQRSTsTPNVHMV	11443134	signaling intermediate in Erk1/2 pathway; phosphorylation inhibits activity
RANBP3	Akt1	human	S126	S126	VKRERtssLtQFPPs	18280241	RAN binding protein 3 functions in nuclear transport; phosphorylation mediates Ran binding and regulates nuclear transport
RARA	Akt1	human	S96	S96	FVCQDKSsGYHYGVS	16417524	nuclear receptor for retinoic acid that acts as a direct regulator of gene expression, phosphorylation of the DNA binding domain inhibits RARA activity
RGC32	Akt1	human	S65	S65	ERMKRRSsAsVSDSS	19162005	a regulator of cell cycle-specific kinases in response to DNA damage; phosphorylation leads to activation and regulation of growth factors
RNF11	Akt1	human	T135	T135	DWLMRSFtCPSCMEP	16123141	a member of a ubiquitin editing complex that modulates transient inflammatory signaling; phosphorylation allows 14-3-3 binding
Ron	Akt1	human	S1394	S1394	VRRPRPLsEPPRPT_	12919677, 14505491	receptor tyrosine kinase for macrophage stimulating protein (MSP), cell adhesion, proliferation and migration; phosphorylation causes 14-3-3 binding
RPS3	Akt1	human	T70	T70	GrrIrELtAVVQkRF	20605787	a member of the 40S ribosomal subunit that also induces neuronal apoptosis and acts as an endonuclease; phosphorylation inhibits proapoptotic function, increases nuclear import/accumulation, and increases DNA repair

Substrate	Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
S6	Akt1	mouse	S236	S236	AKRRRLssLRAstsK	12151408	S6 ribosomal protein; phosphorylation activates the protein and promotes protein synthesis
	Akt1, Akt2	rat	S235, S236	S235, S236	IAKRRRLssLRAsts, AKRRRLssLRAstsK	15358595	S6 ribosomal protein; phosphorylation activates the protein and promotes protein synthesis
SFRS5	Akt2	rat	S86	S86	GRGRGRYsDRFSSRR	15684423	a member of the splicesome involved in constitutive and alternative splicing; phosphorylation activates alternative splicing exon inclusion
SH3BP4	Akt1	mouse	S245	S246	FRSKRSysLsELsVL	19122209	controls selective internalization of the transferrin receptor through endocytosis; phosphorylation promotes 14-3-3 binding at the plasma membrane
SH3RF1	Akt1, Akt2	human	S304	S304	KNTKKRHsFtsLTMA	17535800	scaffolding protein that binds to activated Rac and promotes apoptosis via JNK activation; phosphorylation reduces ability to bind Rac, promoting apoptosis
SKI	Akt1	human	T458	T458	QPRKRKLtVDTPGAP	19875456	negative regulator of TGF-β signaling by binding to Smads; phosphorylation causes its destabilization and reduces SKI-mediated inhibition of expression of Smad7
SOX2	Akt1	mouse	T118	T116	kYRPRRktkTLMkKD	20945330	a transcription factor required for early embryogenesis and embryonic stem cell pluripotency; phosphorylation stabilizes SOX2, increasing transcriptional activity
SRPK2	Akt1	human	T492	T492	PSHDRSRtVsAsstG	19592491	a protein kinase targeting the serine/arginine family of splicing factors; phosphorylation causes nuclear translocation and upregulation of targets regulating cell cycle progression and apoptosis
SSB	Akt1	mouse	T301	T302	LLRNKKVtWKVLEGH	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
STXBP4	Akt2	mouse	S99	S99	RAKLRsEsPWEIAFI	15753124	inhibits formation and translocation of intracellular vesicles; insulin-stimulated phosphorylation of STXBP4 releases inhibition
SYTL1	Akt1	human	S241	S241	RMLSSSSsVSSLNSS	15998322	a secretory factor family member that is involved in granule exocytosis; phosphorylation regulates SYTL1 subnuclear localization
TAL1	Akt1	human	T90	T90	EARHRVPttELCRPP	15930267, 19406989	transcription factor; phosphorylation inhibits transcriptional repressor activity and regulates intracellular localization
TBC1D1	Akt1	human	T596	T596	AFRRRANtLsHFPIE	17995453	Rab GTPase-activating protein involved in insulin-stimulated Glut4 trafficking; phosphorylation promotes glucose transport
TERT	Akt1	human	S227, S824	S227, S824	GARRRGGsASRSLPL, AVRIRGKsYVQCQGI	10224060	telomerase reverse transcriptase, chromosome length maintenance; phosphorylation enhances telomerase activity
THOC4	Akt1	human	S34, T219	S34, T219	RGRGRAGsQGGrGGG, GGGtrRGtRGGARGR	18562279	an RNA binding and export protein that also acts as a chaperone for dimerization of transcription factors; phosphorylation regulates THOC4 subnuclear localization and activates mRNA export and cell proliferation
TOPBP1	Akt1	human	S1159	S1159	EERARLAsNLQWPSC	19477925	induces a large increase in the kinase activity of ATR; phosphorylation prevents the enhanced association of ATR with TopBP1 after DNA damage
TRF1	Akt1	human	T273	T273	SKRTRTItSQDKPSG	19160102	controls telomere structure; phosphorylation decreases telomere length
TSC2	Akt1	human	S939, S981, T1462	S939, S981, T1462	sFRARstsLNERPKs, AFRCRSIsVSEHVVR, GLRPRGytIsDSAPs	15342917, 12150915, 16636147	tumor suppressor that inhibits mTOR; phosphorylation inhibits function and allows protein synthesis to occur
	Akt1	rat	S1130, S1132	S1130, S1132	GARDRVRsMsGGHGL, RDRVRsMsGGHGLRV	12172553	tumor suppressor that inhibits mTOR; phosphorylation inhibits function and allows protein synthesis to occur
TTC3	Akt1	human	S378	S378	AYTPRsLsAPIFTTS	20059950	E3 ligase to Akt; phosphorylation promotes TTC3 function, such as ability to ubiquitinylate and destabilize Akt
TWIST1	Akt1	human	S42, S123	S42, S123	GGRKRRSsRRSAGGG, RERQRTQsLNEAFAA	20400976	a regulatory basic helix-loop-helix anti-apoptotic transcription factor; phosphorylation activates TWIST1, causing inhibition of p53 and promotion of cell survival
USP8	Akt1	mouse	T907	T945	TCRRRSRtFEAFMYL	17210635	deubiquitinating enzyme that plays a role in growth factor receptor trafficking and degradation; phosphorylation increases protein stability
VCP	Akt1	human	S352, S746, S748	S352, S746, S748	AAtNRPNsIDPALRR, AMRFARRsVsDNDIR, RFARRsVsDNDIRky	16551632, 16027165	ATPase and molecular chaperone; phosphorylation may impair its pro-apoptotic effects and promote cell survival
Vimentin	Akt1	human	S39	S39	ttsTrtysLGsALRP	20856200	a cytoskeletal intermediate filament protein; phosphorylation induces cellular motility and invasion by protection from proteolysis
Wee1	Akt1	human	S642	S642	KKMNRsVsLTIy___	15964826	a protein kinase that inhibits cell cycle progression by phosphorylation inhibition of cdc2 kinase; phosphorylation promotes a change in Wee1 localization from nuclear to cytoplasmic and is associated with G2/M arrest
WNK1	Akt1	human	T60	T60	EYRRRRHtMDKDSRG	14611643, 16081417	regulates ion channels; phosphorylation of WNK1 causes SGK1 activation and regulation of sodium ion transport
XIAP	Akt1, Akt2	human	S87	S87	VGRHRKVsPNCRFIN	14645242, 17537996	inhibitor of apoptosis; phosphorylation prevents ubiquitination/degradation and causes increased cell survival
YAP1	Akt1	human	S127	S127	PQHVRAHssPAsLQL	12535517	a transcriptional co-activator of PEBP2 and other transcription factors; phosphorylation suppresses p73-mediated apoptosis
YB-1	Akt1	human	S102	S102	NPRKyLRsVGDGEtV	22417301	a transcription/translation factor involved in mRNA stability and expression; phosphorylation induces activation and translocation to the nucleus
zyxin	Akt1	human	S142	S142	PQPREKVssIDLEId	17572661	a focal adhesion molecule that moves between the cytoplasm and nucleus; phosphorylation promotes an association with acinus and anti-apoptotic activity

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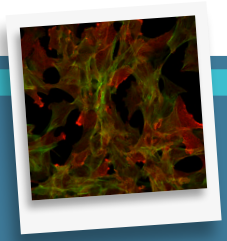

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Phospho-Akt (Ser473) Antibody #4058: Confocal IF analysis of C2C12 cells.

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