

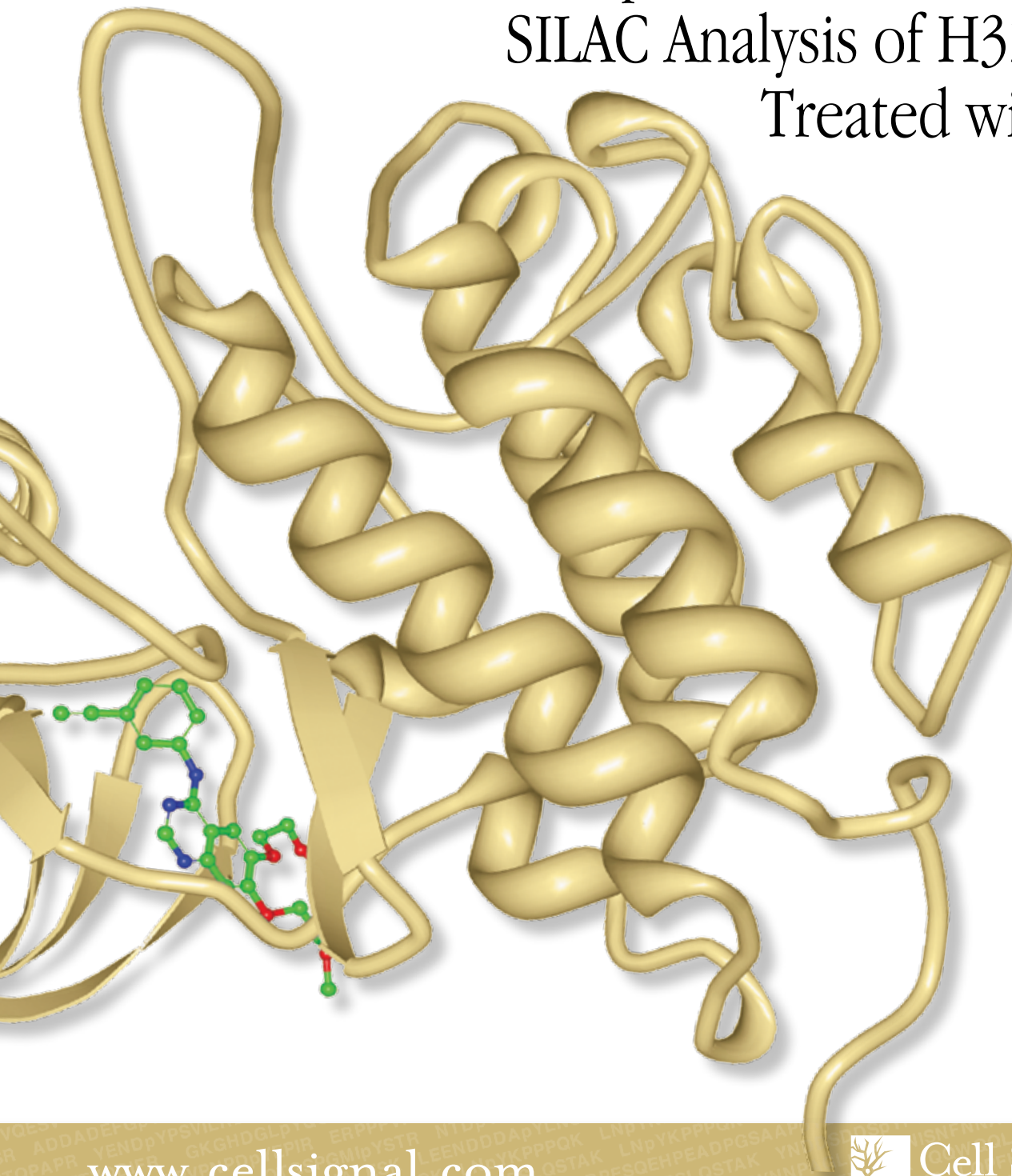
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PhosphoSignature™

...from Cell Signaling Technology

PhosphoScan® Profiling Table: SILAC Analysis of H3255 Cells Treated with Iressa



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Study Background

In many forms of non-small-cell lung cancer, the receptor tyrosine kinase EGFR (epidermal growth factor receptor) is overexpressed. Iressa (Gefitinib) is an EGFR inhibitor approved by the FDA for treatment of patients with advanced disease, and the majority of patients who respond to Iressa treatment have tumors with mutant EGFR genes (Lynch et al., 2004). The molecular mechanisms underlying Iressa-responsiveness are still largely unknown.

Study Description

For PhosphoScan® analysis (Guo et al., 2007), quantification of Iressa-regulated phosphorylation was performed with SILAC, (Ong and Mann, 2006). Briefly the non-small-cell lung cancer cell line H3255 was grown in normal culture media, serum starved overnight, and treated with 1 µM Iressa for 3 hours. To produce an isotope-coded untreated control sample, H3255 was grown in media formulated to contain heavy isotope-labeled forms of arginine and lysine. The cells were harvested and lysed (2×10^8 cells, corresponding to 10 mg cellular protein), and equal amounts of Iressa-treated (normal isotope coded) and untreated (heavy isotope coded) lysate were combined. The proteins were reduced and alkylated to stabilize cysteine-containing peptides, and the extract was digested with trypsin. The digested cellular protein extract was taken through the PhosphoScan® procedure using the phospho-tyrosine antibody P-Tyr-100 to isolate phospho-tyrosine-containing peptides. The isolated peptides were analyzed by liquid chromatography-mass spectrometry using

a high-resolution, high mass accuracy mass spectrometer. From this one sample, one LC-MS analysis gave 930 phospho-tyrosine-peptide assignments, corresponding to 234 phospho-tyrosine sites. This included 63 phospho-tyrosine-peptide assignments to EGFR and 20 to the receptor tyrosine kinase Met. The effect of Iressa on the level of each phospho-tyrosine site was reflected in the ratio of normal- and heavy-isotope labeled phosphopeptide ions. Iressa treatment lowered the levels of 110 phospho-tyrosine sites at least 2.5-fold, and several phospho-tyrosine sites in EGFR were at least 30-fold less abundant after Iressa treatment. Other phospho-tyrosine sites responding strongly to Iressa were found in the adaptor proteins DLG3 and Gab1 and the phospholipase PLCG1.

References

- Guo A, Villén J, Kornhauser J, Lee KA, Stokes M, Rikova K, Possemato A, Nardone J, Innocenti G, Wetzel R, Wang Y, MacNeill J, Mitchell J, Gygi SP, Rush J, Polakiewicz RD, Comb MJ. Signaling Networks Assembled by Oncogenic EGFR and c-Met. *Proc Natl Acad Sci U S A*. 2007, in press.
- Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004 May 20;350(21):2129-39.
- Ong SE, Mann M. A practical recipe for stable isotope labeling by amino acids in cell culture (SILAC). *Nat. Protoc*. 2006;1(6):2650-60.

Select Column Descriptions:

Protein Accession Number: Unique protein identifier for parent protein from which the corresponding phospho-peptide is derived. Detailed protein information for each identifier can be found in PhosphoSitePlus® (www.phosphosite.org).

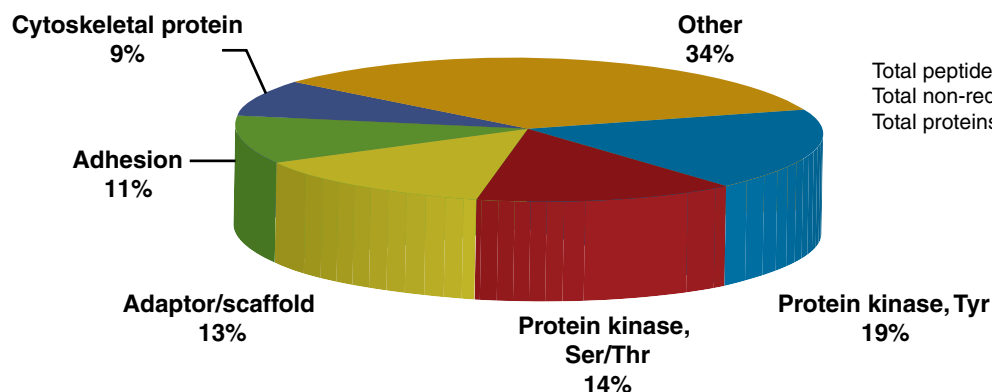
Phospho-Site (Tyr): The phosphorylated (tyrosine) residue number in the protein sequence; the symbol § denotes a published site; all other sites are novel and were discovered in PhosphoScan® studies performed by scientists at Cell Signaling Technology.

Phospho-peptide: Sequence assigned based on mass spectrometry analysis; y indicates the site(s) of tyrosine phosphorylation.

Count in Study: The number of times the phosphopeptide was identified in this PhosphoScan® study.

Count at CST: The number of times the phosphopeptide has been identified in all PhosphoScan® studies conducted at Cell Signaling Technology.

Heavy-to-Light Ratio: The ratio of heavy- and normal-isotope labeled phosphopeptide ions, resulting from SILAC labeling of Iressa-treated cells with light-isotope-labeled forms of arginine and lysine. The ratios correspond to the fold-change in phosphopeptide levels due to Iressa treatment. Positive numbers indicate phosphopeptides that are more abundant in untreated cells than in Iressa-treated cells.



Total peptides = 930 (see pie chart)
Total non-redundant sites = 251
Total proteins = 183

PHOSPHOSCAN-SILAC ANALYSIS OF H3255 CELLS TREATED WITH IRESSA

Gene Name	Description	Accession	kD	Site	Peptide	Count in Study	Count at CST	Heavy-to-Light Ratio Mean	Heavy-to-Light Ratio Range
Actin binding proteins									
CTNNA1	Catenin alpha-1 (Cadherin-associated protein)	P35221	100	177	NAGNEQDLGIQyK	4	282	1.6	1.5-1.8
CTNND1	Catenin delta-1 (Cadherin-associated Src substrate) (p120(cas))	O60716	108	248	YRPSMEGyR	2	44	14.5	7.2-21.9
CTNND1	Catenin delta-1 (Cadherin-associated Src substrate) (p120(cas))	O60716	108	865	SQSSHSyDDSTLPLIDR	1	21	3.9	
CTNND1	Catenin delta-1 (Cadherin-associated Src substrate) (p120(cas))	O60716	108	904	SLDNNySTPNERGDHNR	1	224	5.5	
CTNND1	Catenin delta-1 (Cadherin-associated Src substrate) (p120(cas))	O60716	108	904	SLDNNySTPNER	4	1198	3.0	2.8-3.1
CTNND1	Catenin delta-1 (Cadherin-associated Src substrate) (p120(cas))	O60716	108	§228	HYEDGYPGGSDNyGsLSR	4	4	1.8	1.8-1.9
CTNND1	Catenin delta-1 (Cadherin-associated Src substrate) (p120(cas))	O60716	108	§228	HYEDGYPGGSDNyGSLSR	6	754	1.8	1.7-1.9
CTNND1	Catenin delta-1 (Cadherin-associated Src substrate) (p120(cas))	O60716	108	§257	QDVyGPQPQVR	1	482	1.1	
CTNND1	Catenin delta-1 (Cadherin-associated Src substrate) (p120(cas))	O60716	108	§280	FHPEPyGLEDDQR	3	109	6.1	3.8-10.5
CTNND1	Catenin delta-1 (Cadherin-associated Src substrate) (p120(cas))	O60716	108	§96	LNGPQDHSLLySTIPR	1	400	3.0	
DBNL	Drebrin-like protein (SH3 domain-containing protein 7) (HIP-55)	Q9UJU6	48	§162	FQDVGPQAPVGSVyQK	2	511	11.1	7.3-14.9
Adaptor/scaffold proteins									
ANK3	Ankyrin-3 (ANK-3) (Ankyrin-G)	Q12955	480	533	ADIVQQLLQQGASPNAATTSGyTPLHLSAR	3	163	0.7	0.6-0.8
NEDD9	Enhancer of filamentation 1 (CRK-associated substrate-related protein)(CasL)	Q14511	93	§166	TGHGYyEYPSR	4	325	1.2	1.1-1.3
CRK	Proto-oncogene C-crk (p38) (Adapter molecule crk)	P46108	34	136	QGSyVILRQEEAEyVR	1	2	9.9	
CRK	Proto-oncogene C-crk (p38) (Adapter molecule crk)	P46108	34	136	QEEAEyVR	1	6	>8.4	
DLG3	Disks large homolog 3 (Synapse-associated protein 102) (SAP102)	Q92796	90	673	RDNEVDGQDyHFVVSr	7	597	>58.4	>27.9-65.2
DLG3	Disks large homolog 3 (Synapse-associated protein 102) (SAP102)	Q92796	90	673	DNEVDGQDyHFVVSr	3	67	25.5	18.1-33.5
EPS8	Epidermal growth factor receptor kinase substrate 8	Q12929	92	§485	LSTEHSVSEyHPADGyAFSSNIYTR	2	43	19.9	17.0-22.9
FLOT1	Flotillin-1	O75955	47	203	VSAQyLSEIEMAK	3	116	1.3	1.2-1.3
GAB1	GRB2-associated-binding protein 1 (GRB2-associated binder 1)	Q13480	77	§406	DASSQDCyDIPR	2	937	13.9	9.2-18.5
GAB1	GRB2-associated-binding protein 1 (GRB2-associated binder 1)	Q13480	77	§627	GDKQVEyLDLDDSGK	4	272	>11.0	>5.9->16.7
GAB1	GRB2-associated-binding protein 1 (GRB2-associated binder 1)	Q13480	77	§659	SSGSGSSVADERVDyVVVDQK	3	807	>22.0	>6.6->51.9
ITSN2	Intersectin-2 (SH3 domain-containing protein 1B) (SH3P18)	Q9NZM3	193	§552	LlyLVPEK	2	335	8.7	7.8-9.5
WASL	Neural Wiskott-Aldrich syndrome protein (N-WASP)	O00401	55	§256	VlyDFIEK	2	593	1.0	1.0-1.1
PAG1	Phosphoprotein associated with glycosphingolipid-enriched microdomains 1	Q9NWQ8	47	§227	AEFAEyASVDR	4	340	1.6	1.5-1.7
PAG1	Phosphoprotein associated with glycosphingolipid-enriched microdomains 2	Q9NWQ8	47	§341	SGQSLTVPESTyTSIQGDpQR	1	413	3.2	
PAG1	Phosphoprotein associated with glycosphingolipid-enriched microdomains 3	Q9NWQ8	47	§359	SPSSCNLDyATVK	4	432	2.6	2.0-3.2
PAG1	Phosphoprotein associated with glycosphingolipid-enriched microdomains 4	Q9NWQ8	47	§417	ATLGTNGHHGLVPKENDyESISDLQQGR	4	184	>10.4	>6.0-13.9
PAG1	Phosphoprotein associated with glycosphingolipid-enriched microdomains 5	Q9NWQ8	47	§417	ENDyESISDLQQGR	4	578	3.2	3.0-3.4
PARD3	Partitioning-defective 3 homolog (Atypical PKC isotype-specific-interacting prot)	Q8TEW0	151	388	FSPDSQyIDNR	2	228	1.7	1.5-1.8

PHOSPHOSCAN-SILAC ANALYSIS OF H3255 CELLS TREATED WITH IRESSA

Gene Name	Description	Accession	kD	Site	Peptide	Count in Study	Count at CST	Heavy-to-Light Ratio Mean	Heavy-to-Light Ratio Range
PARD3	Partitioning-defective 3 homolog (Atypical PKC isotype-specific-interacting prot)	Q8TEW0	151	489	DVTIGGSAPlyVK	4	146	1.3	1.2-1.4
PARD3	Partitioning-defective 3 homolog (Atypical PKC isotype-specific-interacting prot)	Q8TEW0	151	719	RISHSLySGIEGLDESPSR	1	67	1.4	
PARD3	Partitioning-defective 3 homolog (Atypical PKC isotype-specific-interacting prot)	Q8TEW0	151	719	ISHSLySGIEGLDESPSR	5	150	0.9	0.7-1.1
PARD3	Partitioning-defective 3 homolog (Atypical PKC isotype-specific-interacting prot)	Q8TEW0	151	719	RIsHSLySGIEGLDESPSR	2	2	0.3	0.3-0.4
PARD3	Partitioning-defective 3 homolog (Atypical PKC isotype-specific-interacting prot)	Q8TEW0	151	§1127	EGHMMDALyAQVK	1	63	0.8	
MPZL1	Myelin protein zero-like protein 1	O95297	29	§263	SESVVlyADIR	3	449	2.1	2.1-2.2
DLG1	Disks large homolog 1 (Synapse-associated protein 97) (SAP-97) (hDlg)	Q12959	100	760	DyHFVTSR	1	15	1.5	
DLG1	Disks large homolog 1 (Synapse-associated protein 97) (SAP-97) (hDlg)	Q12959	100	760	DYEVDGRDyHFVTSR	3	159	1.2	1.2-1.4
DLG1	Disks large homolog 1 (Synapse-associated protein 97) (SAP-97) (hDlg)	Q12959	100	760	RDYEVDRDyHFVTSR	1	111	1.1	
SHB	SH2 domain-containing adapter protein B	Q15464	55	§201	LDyCGSGEGPGVQR	2	511	1.0	0.8-1.1
SHB	SH2 domain-containing adapter protein B	Q15464	55	§333	DKVTIADDySDPFDKAK	4	480	1.3	1.2-1.3
SHB	SH2 domain-containing adapter protein B	Q15464	55	§333	DKVTIADDySDPFDKAKNDLK	5	337	1.1	1.1-1.2
SHB	SH2 domain-containing adapter protein B	Q15464	55	355	AGKGESAGyMEPYEAQR	6	911	1.1	1.1-1.1
SHB	SH2 domain-containing adapter protein B	Q15464	55	355	GESAGyMEPYEAQR	3	542	1.1	1.0-1.3
SHC1	SHC-transforming protein 1 (SH2 domain protein C1)	P29353	63	§427	ELFDPPSyVNVQNLDK	7	1830	>18.1	>10.8-20.7
SPRY1	Sprouty homolog 1	O43609	35	53	GSNEyTEGSPVVK	1	113	>13.9	
SPRY1	Sprouty homolog 1	O43609	35	89	THEIIPINVNNNyEHR	2	2	>11.1	>10.7->11.4
SPRY4	Sprouty homolog 4	Q6QIX2	35	§75	TSHVENDyIDNPSLALTTGPKR	1	6	>15.8	
TNS3	Tumor endothelial marker 6	Q8IZW7	155	§780	KLsLGQyDNDAGGQLPFSK	2	2	>11.3	>9.8->12.8
TNS3	Tumor endothelial marker 6	Q8IZW7	155	§780	LSLGGQyDNDAGGQLPFSK	1	258	8.7	
TNS3	Tumor endothelial marker 6	Q8IZW7	155	§780	KLsLGQyDNDAGGQLPFSK	5	428	7.4	5.5-9.9
TNS1	Tensin-1	Q9HBL0	186	1404	AGSLPNyATINGK	2	698	1.1	1.0-1.2
TJP2	Tight junction protein ZO-2 (Zonula occludens protein 2)	Q9UDY2	134	§1118	IEIAQKHPDlyAVPIK	3	838	1.9	1.8-2.0
Adhesion proteins									
CTNNB1	Catenin beta-1	P35222	86	30	AAVSHWQQQSyLDSGIHSGATTAPSLSGK	2	12	>11.8	>11.8
CDH1	Epithelial cadherin	P12830	98	§753	DNVlyYYDEEGGEEQDFDLSQLHR	2	9	2.6	2.5-2.8
ERBB2IP	Protein LAP2 (ErbB2-interacting protein) (Erbin) (Densin-180-like protein)	Q96RT1	158	§1104	AQIPEGDyLSYR	6	407	2.1	2.0-2.1
ERBB2IP	Protein LAP2 (ErbB2-interacting protein) (Erbin) (Densin-180-like protein)	Q96RT1	158	§1104	RAQIPEGDyLSYR	8	270	3.1	2.2-4.1
ERBB2IP	Protein LAP2 (ErbB2-interacting protein) (Erbin) (Densin-180-like protein)	Q96RT1	158	1164	TMSVDFNySR	2	189	17.8	11.8-23.7
F11R	Junctional adhesion molecule A	Q9Y624	33	280	KVlySQPSAR	4	407	3.0	2.9-3.0
F11R	Junctional adhesion molecule A	Q9Y624	33	280	VlySQPSAR	2	101	2.1	1.9-2.3
ITGB1	Integrin beta-1	P05556	89	§783	WDTGENPlyKSAVTTVVNPK	1	189	33.7	
ITGB1	Integrin beta-1	P05556	89	§783	WDTGENPlyK	4	616	2.4	2.2-2.5
ITGB1	Integrin beta-1	P05556	89	§795	SAVTTVVNPKyEGK	1	41	103.9	
MUC1	Mucin-1	P15941	122	§1203	DTyHPMSEyPYHTHGR	2	220	1.7	1.5-1.9
MUC1	Mucin-1	P15941	122	1209	DTYHPMSEyPYHTHGR	1	1	0.8	
MUC1	Mucin-1	P15941	122	1209, §1212	DTYHPMSEyPYHTHGR	1	300	0.9	
MUC1	Mucin-1	P15941	122	§1212	DTYHPMSEyPYHTHGR	2	395	1.6	1.5-1.8
MUC1	Mucin-1	P15941	122	§1229	YVPPSSDRSPyEK	3	88	1.9	1.8-2.0

Gene Name	Description	Accession	kD	Site	Peptide	Count in Study	Count at CST	Heavy-to-Light Ratio Mean	Heavy-to-Light Ratio Range
PVRL1	Poliovirus receptor-related protein 1	Q15223	57	468	YDEDAKRPyFTVDEAEAR	4	117	1.8	1.7-2.0
PVRL4	Poliovirus receptor-related protein 4	Q96K15	55	445	SySTLITTVR	1	11	0.7	
PVRL4	Poliovirus receptor-related protein 4	Q96K15	55	502	AKPTGNGIyINGR	4	20	0.9	0.9-1.0
OCLN	Occludin	Q16625	59	287	SNILWDKEHlyDEQPPNVEEWVK	1	55	>7.9	
PKP2	Plakophilin-2	Q99959	97	166	AHYTHSDyQYSQR	4	245	6.3	4.4-7.3
PKP2	Plakophilin-2	Q99959	97	631	YSQNlyIQNR	1	22	5.1	
PKP3	Plakophilin-3	Q9Y446	87	§84	GQyHTLQAGFSSR	9	846	6.8	6.1-7.4
PKP3	Plakophilin-3	Q9Y446	87	176	ADyDTLSLR	7	372	2.7	2.6-2.7
PKP3	Plakophilin-3	Q9Y446	87	176	GGVGSRADyDTLSLR	2	91	1.0	0.9-1.1
PKP3	Plakophilin-3	Q9Y446	87	390	NLyDNADNK	1	122	1.1	
PKP4	Plakophilin-4 (p0071)	Q99569	134	157	SSTQMNSYSDSGyQEAGSFHNSQNVSK	1	87	4.5	
PKP4	Plakophilin-4 (p0071)	Q99569	134	372	TVHDMEQFGQQYDlyER	4	293	0.8	0.7-0.8
PKP4	Plakophilin-4 (p0071)	Q99569	134	§415	SAVSPDLHITPIyEGR	3	59	0.7	0.7-0.7
PKP4	Plakophilin-4 (p0071)	Q99569	134	470	NNyALNTTAtyAEPYRPIQYR	1	1	1.6	
PKP4	Plakophilin-4 (p0071)	Q99569	134	470, §478	NNyALNTTAtyAEPYRPIQYR	2	116	1.5	1.3-1.6
PKP4	Plakophilin-4 (p0071)	Q99569	134	§478	NNyALNTTAtyAEPYRPIQYR	2	418	1.1	1.1-1.1
PKP4	Plakophilin-4 (p0071)	Q99569	134	1168	STTNyVDFYSTK	7	374	1.3	1.3-1.3
SDC4	Syndecan-4	P31431	22	197	KAPTNEFyA	2	40	1.7	1.6-1.8
VCL	Vinculin (Metavinculin)	P18206	124	§821	SFLDSGyR	4	1058	0.7	0.7-0.7
Cytoskeletal proteins									
KRT7	Keratin, type II cytoskeletal 7 (Cytokeratin-7) (Sarcolectin)	P08729	51	39	LSSARPGGLGSSsLyGLGASRPR	2	2	1.0	1.0-1.0
KRT7	Keratin, type II cytoskeletal 7 (Cytokeratin-7) (Sarcolectin)	P08729	51	39	LSSARPGGLGSSsLyGLGASRPR	4	344	0.7	0.7-0.7
CLDN3	Claudin-3 (CPE-receptor 2) (HRVP1)	O15551	23	214	STGPGASLGTGyDR	1	158	8.4	
CLDN3	Claudin-3 (CPE-receptor 2) (HRVP1)	O15551	23	219	STGPGASLGTGYDRKdyV	3	66	9.3	9.0-9.5
CFL1	Cofilin-1 (Cofilin, non-muscle)	P23528	19	§139	LTGIKHELQANCyEEVKDR	2	446	5.8	4.9-6.7
CFL1	Cofilin-1 (Cofilin, non-muscle)	P23528	19	§139	HELQANCyEEVKDR	3	906	4.3	4.0-5.1
CTTN	Src substrate cortactin (Amplaxin) (Oncogene EMS1)	Q14247	62	§421	LPSSPVyEDAASFk	2	293	1.2	1.1-1.3
CTTN	Src substrate cortactin (Amplaxin) (Oncogene EMS1)	Q14247	62	§446	GPVSGTEPEPVySMEAADYR	6	1246	1.8	1.8-1.9
JUP	Junction plakoglobin (Desmoplakin-3) (Desmoplakin III) (Catenin gamma)	P14923	82	§19	VTEWQQTyTDSGIHSGANTCVPSVSSK	2	71	159.2	151.0-167.5
EPB41L4B	Band 4.1-like protein 4B (Protein EHM2) (FERM-containing protein CG1)	Q9H329-2	57	479	ASASGDDSHFDyVHDQNQK	2	52	34.3	34.3
VIL2	Ezrin (p81) (Cytovillin) (Villin-2)	P15311	69	423	SQEQLAAELAEyTAK	2	33	>6.3	>6.3
PXN	Paxillin	P49023	65	§88	FIHQQPQSSsPVyGSSAK	3	3	1.6	1.4-1.7
PXN	Paxillin	P49023	65	§88	FIHQQPQSSsSPVyGSSAK	2	2	1.5	1.4-1.6
PXN	Paxillin	P49023	65	§88	FIHQQPQSSSPVyGSSAK	5	1415	0.7	0.7-0.8
PXN	Paxillin	P49023	65	§118	VGEEEHVysFPNK	7	1490	1.0	0.9-1.0
PXN	Paxillin	P49023	65	§118	VGEEEHVysFPNKQK	9	1564	0.9	0.7-1.0
SNIP	p130Cas-associated protein (p140Cap) (SNAP-25-interacting protein) (SNIP)	Q9C0H9	113	136	KEPLyAAFPGSHLTNGDLR	3	78	0.5	0.5-0.5
SNIP	p130Cas-associated protein (p140Cap) (SNAP-25-interacting protein) (SNIP)	Q9C0H9	113	268	GEGLyADPYGLLHEGR	3	41	0.8	0.7-0.8
TAGLN2; TAGLN3	Transgelin-2 (SM22-alpha homolog)	P37802	22	191; 192	GASQAGMTGyGMPR	2	1588	3.9	3.8-3.9
TLN1	Talin-1	Q9Y490	270	70	ALDyYMLR	11	1025	0.9	0.9-1.0
TLN1	Talin-1	Q9Y490	270	127	IGITNHDEySLVR	2	60	9.6	7.0-12.3

PHOSPHOSCAN-SILAC ANALYSIS OF H3255 CELLS TREATED WITH IRESSA

Gene Name	Description	Accession	kD	Site	Peptide	Count in Study	Count at CST	Heavy-to-Light Ratio Mean	Heavy-to-Light Ratio Range
TUBB4; TUBB; TUBB2C; TUBB3	Tubulin beta-4 chain (Tubulin 5 beta)	P04350; P07437; P68371; Q13509	50	340; 340; 340; 340	NSSyFVEWIPNNVK	1	147	1.1	
Enzyme, misc.									
ALDH9A1	4-trimethylaminobutyraldehyde dehydrogenase	P49189	54	476	VTIEyYSQLK	2	35	1.0	1.0-1.0
ALDOA	Fructose-bisphosphate aldolase A (Lung cancer antigen NY-LU-1)	P04075	39	2	PyQYPALTPEQK	3	370	1.3	1.3-1.4
CALM1; CALM2; CALM3	Calmodulin (CaM)	P62158	17	§99	VFDKDGNGyISAAELR	4	1102	2.0	1.7-2.3
CTPS	CTP synthase 1 (UTP--ammonia ligase 1) (CTP synthetase 1)	P17812	67	473	KLYGDADyLEER	2	34	0.3	0.3-0.3
DDX3X	ATP-dependent RNA helicase DDX3X (DEAD box protein 3, X-chromosomal)	O00571	73	§103	GRSDyDGIGSR	1	808	5.4	
ENO1; ENO2; ENO3	Alpha-enolase (2-phospho-D-glycerate hydro-lyase) (Non-neural enolase)	P06733; P09104; P13929	47	§43; 43; §43	AAVPSGASTGlyEALELR	2	1686	1.0	0.9-1.0
G6PD	Glucose-6-phosphate 1-dehydrogenase (G6PD)	P11413	59	§400	VQPNEAVyTK	1	299	0.7	
G6PD	Glucose-6-phosphate 1-dehydrogenase (G6PD)	P11413	59	502	VGFQyEGTyK	2	316	1.5	1.1-1.9
G6PD	Glucose-6-phosphate 1-dehydrogenase (G6PD)	P11413	59	502	RVGFQyEGTyK	3	243	1.5	1.4-1.5
G6PD	Glucose-6-phosphate 1-dehydrogenase (G6PD)	P11413	59	§506	VGFQYEGTyK	5	386	5.0	4.7-5.4
G6PD	Glucose-6-phosphate 1-dehydrogenase (G6PD)	P11413	59	§506	RVGFQYEGTyK	2	78	1.5	1.4-1.5
LDHA	L-lactate dehydrogenase A chain (LDH-A) (LDH muscle subunit) (LDH-M)	P00338	37	§238	QVVESAyEVIK	1	761	1.0	
LDHB	L-lactate dehydrogenase B chain (LDH-B) (LDH heart subunit) (LDH-H)	P07195	37	§239	MVVESAyEVIK	4	856	1.0	1.0-1.0
ATP1A1	Sodium/potassium-transporting ATPase subunit alpha-1	P05023	113	260	GIVVyTGDR	4	1620	2.0	2.0-2.1
PLCG1	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-1	P19174	149	§771	IGTAEPDyGALYEGR	1	181	30.9	
PLCG1	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-2	P19174	149	§783	NPGFyVEANPMPTFK	2	176	44.5	21.0-68.1
GTP binding protein or regulator									
ARHGEF5	FLJ00261 protein	Q6ZML7	182	710	SGRDySTVSASPTALSTLK	2	55	>8.5	>7.3->9.8
ARHGEF5	FLJ00261 protein	Q6ZML7	182	1424	RTEELyLSQK	1	10	0.4	
GNAI3	Guanine nucleotide-binding protein G(k) subunit alpha (G(i) alpha-3)	P08754	41	60	IIHEDGySEDECKQYK	1	3	13.4	
GNAI3	Guanine nucleotide-binding protein G(k) subunit alpha (G(i) alpha-3)	P08754	41	60	IIHEDGySEDECK	2	17	11.1	8.8-13.4
IQGAP1	Ras GTPase-activating-like protein IQGAP1 (p195)	P46940	189	§1510	LQQTyAALNSK	2	595	1.0	1.0-1.1
RAB34	Ras-related protein Rab-34 (Rab-39) (Ras-related protein Rah)	Q9BZG1	29	247	INSDDSNlyLTASK	2	50	21.4	14.4-28.4
Kinase (non-protein)									
GUK1	Guanylate kinase (GMP kinase)	Q16774	22	53	NPRPGEENGKDYyFVTR	1	2	>9.6	
PIK3R1	Phosphatidylinositol 3-kinase regulatory subunit alpha	P27986	84	467	SREYDRlyEEYTR	2	1190	1.1	0.8-1.3
PIK3R2	Phosphatidylinositol 3-kinase regulatory subunit beta	O00459	82	§464	SREYDQlyEEYTR	3	408	1.0	1.0-1.1
PIK3R2	Phosphatidylinositol 3-kinase regulatory subunit beta	O00459	82	§464	EYDQlyEEYTR	2	838	1.0	1.0-1.0
PIK3R2	Phosphatidylinositol 3-kinase regulatory subunit beta	O00459	82	§467	EYDQlyEEyTR	3	119	1.2	1.2-1.2
PIK3R2	Phosphatidylinositol 3-kinase regulatory subunit beta	O00459	82	605	NETEDQyALMEDEDLPHHEER	1	218	17.6	
PI4KA	Phosphatidylinositol 4-kinase alpha (PI4-kinase alpha)	P42356	231	973	DQPyYDIPDAPYR	3	10	0.9	0.8-0.9

Gene Name	Description	Accession	kD	Site	Peptide	Count in Study	Count at CST	Heavy-to-Light Ratio Mean	Heavy-to-Light Ratio Range
Lipid binding proteins									
ANXA1	Annexin A1 (Lipocortin I) (Phospholipase A2 inhibitory protein)	P04083	39	§20	QAWFIENEQEYVQTVK	2	49	>4.9	>4.9
ANXA1	Annexin A1 (Lipocortin I) (Phospholipase A2 inhibitory protein)	P04083	39	38	GGPGSAVSPYPTFNPSDVAALHK	2	167	4.3	3.8-4.9
ANXA2	Annexin A2 (Lipocortin II) (Calpactin I heavy chain)	P07355	39	§23	LSLEGDHSTPPSAyGSVK	4	2210	1.6	0.4-2.1
ANXA2	Annexin A2 (Lipocortin II) (Calpactin I heavy chain)	P07355	39	§29	AyTNFDAERDALNIETAIK	3	234	38.1	15.1-57.9
ANXA2	Annexin A2 (Lipocortin II) (Calpactin I heavy chain)	P07355	39	§29	AyTNFDAER	3	636	6.7	6.1-7.9
ANXA2	Annexin A2 (Lipocortin II) (Calpactin I heavy chain)	P07355	39	§237	SYSPyDMLESIRK	4	246	3.4	3.4-3.5
ANXA2	Annexin A2 (Lipocortin II) (Calpactin I heavy chain)	P07355	39	§237	SYSPyDMLESIR	1	393	2.2	
PLEKHA6	Pleckstrin homology domain-containing family A member 6 (PEPP-3)	Q9Y2H5	117	§492	SEDIyADPAAYVMR	3	273	0.8	0.8-0.9
Other									
AP2B1	AP-2 complex subunit beta-1 (Beta-adaptin)	P63010	105	276	DSDyYNMLLK	2	19	2.0	1.8-2.2
CSTB	Cystatin-B (Stefin-B) (Liver thiol proteinase inhibitor) (CPI-B)	P04080	11	97	AKHDELTYF	2	69	1.9	1.9-1.9
EFNB2	Ephrin-B2	P52799	37	§304	TADSVFCPhyEK	3	100	1.2	1.2-1.2
DNAJA1	DnaJ homolog subfamily A member 1 (Heat shock 40 kDa protein 4)	P31689	45	§381	HYNGEAYEDDEHHPR	2	437	10.7	6.9-14.4
ERRF1	ERBB receptor feedback inhibitor 1 (Mitogen-inducible gene 6 protein) (Mig-6)	Q9UJM3	51	394	VSSTHyYLLPERPPYLDKYEK	2	278	27.3	27.2-27.4
ERRF1	ERBB receptor feedback inhibitor 1 (Mitogen-inducible gene 6 protein) (Mig-6)	Q9UJM3	51	395	VSSTHyYLLPERPPYLDKYEK	1	149	>57.0	
MYO6	myosin VI	CAI19522	145	1114	SVTDyAQQNPAQIPAR	2	2	13.7	11.1-16.2
STX4	Syntaxin-4 (Renal carcinoma antigen NY-REN-31)	Q12846	34	251	NILSSADyVER	2	66	6.9	6.6-7.1
HDLBP	Vigilin (High density lipoprotein-binding protein) (HDL-binding protein)	Q00341	141	§437	MDyVEINIDHK	7	159	2.6	2.4-2.7
VTI1B	Vesicle transport through interaction with t-SNAREs homolog 1B	Q9UEU0	27	115	YGlyAVENEHMNR	1	47	5.0	
Phosphatase									
PTPRA	Receptor-type tyrosine-protein phosphatase alpha precursor (R-PTP-alpha)	P18433	91	§798	VVQEYIDAFSDyANFK	5	1904	1.4	1.2-1.6
INPPL1	Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 2 (SHIP-2)	O15357	139	§886	ERLyEWISIDKDEAGAK	1	239	0.7	
INPPL1	Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 2 (SHIP-2)	O15357	139	§1135	TLSEVDyAPAGPAR	2	756	4.2	3.6-4.8
PTPN11	Tyrosine-protein phosphatase non-receptor type 11 (PTP-2C)(SHP-2)	Q06124	68	§62	IQNTGDyYDLYGGEK	4	1318	0.9	0.9-0.9
PTPN11	Tyrosine-protein phosphatase non-receptor type 11 (PTP-2C)(SHP-2)	Q06124	68	§580	VyENVGLMQQK	2	1279	7.3	5.4-9.2
Protease									
CPD	Carboxypeptidase D	O75976	153	1376	LLSHEFQDETDEETLySSKH	4	10	2.9	2.3-3.6
CPD	Carboxypeptidase D	O75976	153	1344	LRQHHDyEDEIR	2	19	4.1	3.6-4.6
PSMA2	Proteasome subunit alpha type-2 (Proteasome component C3)	P25787	26	56	SIyDER	1	37	0.7	
PSMA2	Proteasome subunit alpha type-2 (Proteasome component C3)	P25787	26	75	HIGLVySGMGPDYR	2	448	0.6	0.6-0.6
Protein kinase, dual-specificity									
DYRK1A; DYRK1B	Dual specificity tyrosine-phosphorylation-regulated kinase 1A	Q13627; Q9Y463	86	§321; §273	IYQyIQSR	8	6195	0.9	0.9-0.9
DYRK2; DYRK4	Dual specificity tyrosine-phosphorylation-regulated kinase 2	Q92630; Q9NR20	60	§309; 286	VYTyIQSR	2	533	1.3	1.3-1.4

PHOSPHOSCAN-SILAC ANALYSIS OF H3255 CELLS TREATED WITH IRESSA

Gene Name	Description	Accession	kD	Site	Peptide	Count in Study	Count at CST	Heavy-to-Light Ratio Mean	Heavy-to-Light Ratio Range
Protein kinase, Ser/Thr (non-receptor)									
CDC2	Cell division control protein 2 homolog (CDK1)	P06493	34	§15	IEKIGEGTyGVVYK	5	1966	1.5	1.3-1.7
CDC2	Cell division control protein 2 homolog (CDK1)	P06493	34	§15	IGEGTyGVVYKGR	3	3	0.8	0.8-0.9
CDC2	Cell division control protein 2 homolog (CDK1)	P06493	34	§15	IEKIGEGTyGVVYK	6	6	0.7	0.7-0.8
CDC2	Cell division control protein 2 homolog (CDK1)	P06493	34	§19	IGEGTyGVVYKGR	1	341	0.8	
CDC2; CDK2; CDK3	Cell division control protein 2 homolog (CDK1)	P06493; P24941; Q00526	34	§15; §15; 15	IGEGTyGVVYK	6	8531	1.2	1.2-1.2
CDC2; CDK2; CDK3	Cell division control protein 2 homolog (CDK1)	P06493; P24941; Q00526	34	§15; §15; 15	IGEGTyGVVYK	8	8	0.8	0.8-0.8
CDC2; CDK2; CDK3	Cell division control protein 2 homolog (CDK1)	P06493; P24941; Q00526	34	§19; 19; 19	IGEGTyGVVYK	2	872	0.8	0.7-0.8
CDK2; CDK3	Cell division protein kinase 2 (p33 protein kinase)	P24941; Q00526	34	§15; 15	VEKIGEGTyGVVYK	1	1	0.6	
CDK2; CDK3	Cell division protein kinase 2 (p33 protein kinase)	P24941; Q00526	34	§15; 15	VEKIGEGTyGVVYK	4	1817	1.0	1.0-1.1
CDK5	Cell division protein kinase 5	Q00535	33	§15	IGEGTyGTVFK	3	594	1.0	1.0-1.1
CDKL5	Cyclin-dependent kinase-like 5 (Serine/threonine-protein kinase 9)	O76039	116	§171	NLSEGNANANYTEyVATR	3	411	1.2	1.2-1.3
MAPK3	Mitogen-activated protein kinase 3 (ERK-1) (MAPK 1)	P27361	43	§204	IADPEHDHTGFLIEyVATR	1	1	8.7	
MAPK3	Mitogen-activated protein kinase 3 (ERK-1) (MAPK 1)	P27361	43	§204	IADPEHDHTGFLIEyVATR	5	2886	6.9	6.7-7.2
MAPK1	Mitogen-activated protein kinase 1 (ERK-2) (MAPK 2)	P28482	41	§186	VADPDHDHTGFLIEyVATR	6	6	10.7	8.0-23.4
MAPK1	Mitogen-activated protein kinase 1 (ERK-2) (MAPK 2)	P28482	41	§186	VADPDHDHTGFLIEyVATR	9	3918	8.2	7.6-11.1
MAPK7	Mitogen-activated protein kinase 7 (ERK-5)	Q13164	89	§220	GLCTSPAEHQYFMTEyVATR	2	801	6.8	6.5-7.1
GSK3A; GSK3B	Glycogen synthase kinase-3 alpha (GSK-3 alpha)	P49840; P49841	51	§279; §216	GEPNVSYICSR	19	12701	0.9	0.9-0.9
GSK3A; GSK3B	Glycogen synthase kinase-3 alpha (GSK-3 alpha)	P49840; P49841	51	§279; §216	GEPNVSYICsR	8		0.9	0.8-0.9
HIPK1; HIPK2	Homeodomain-interacting protein kinase 1	Q86202; Q9H2X6	131	§352; §361	AVCSTYLQSR	3	3767	1.2	1.1-1.2
HIPK3	Homeodomain-interacting protein kinase 3 (FIST) (ANPK)	Q9H422	134	§359	TVCSTYLQSR	1	2296	4.8	
ICK	Serine/threonine-protein kinase ICK (MRK) (LCK2)	Q9UPZ9	71	§159	SKPPYtDyVSTR	2	2	0.9	0.9-1.0
MINK1	Misshapen-like kinase 1 (MAPK/ERK kinase kinase 6) (MEKKK 6)	Q8N4C8	150	906	LLHADSNGyTnLPDyVQPSHSPTENSK	2	72	>8.4	>7.9->9.0
MAPK14	Mitogen-activated protein kinase 14 (MAP kinase p38 alpha)	Q16539	41	§181	HTDDEMTGyVATR	9	13834	0.8	0.7-0.9
MAPK14	Mitogen-activated protein kinase 14 (MAP kinase p38 alpha)	Q16539	41	§181	HTDDEMTGyVATR	3	3	0.8	0.7-0.8
MAPK13	Mitogen-activated protein kinase 13 (MAP kinase p38 delta)	O15264	42	182	HADAEMTgyVVTR	5	1265	0.5	0.4-0.6
PRKCD	Protein kinase C delta type (nPKC-delta)	Q05655	78	§313	RSDSASSEPVGIyQGFEEK	1	569	1.1	
PRPF4B	Serine/threonine-protein kinase PRP4 homolog (PRP4 kinase)	Q13523	117	§849	LCDFGSASHVADNDITPyLVSR	6	6709	1.4	1.0-1.8
Protein kinase, tyrosine (non-receptor)									
PTK2	Focal adhesion kinase 1 (FADK 1) (pp125FAK) (Protein-tyrosine kinase 2)	Q05397	119	§397	THAVSVSETDDyAEIIDEEDTYTMPSTR	1	974	1.2	
PTK2	Focal adhesion kinase 1 (FADK 1) (pp125FAK) (Protein-tyrosine kinase 2)	Q05397	119	§397, §407	THAVSVSETDDyAEIIDEEDTYTMPSTR	1	509	1.3	
PTK2	Focal adhesion kinase 1 (FADK 1) (pp125FAK) (Protein-tyrosine kinase 2)	Q05397	119	§576	YMEDSTyYK	7	3860	0.9	0.8-1.0
PTK2	Focal adhesion kinase 1 (FADK 1) (pp125FAK) (Protein-tyrosine kinase 2)	Q05397	119	§576, §577	YMEDSTyYKASK	2	885	0.5	0.5-0.5
PTK2	Focal adhesion kinase 1 (FADK 1) (pp125FAK) (Protein-tyrosine kinase 2)	Q05397	119	§577	YMEDSTyYK	1	1337	0.9	
FRK	Tyrosine-protein kinase FRK (FYN-related kinase)	P42685	58	46	HGHYFVALFDYQAR	5	444	0.9	0.8-0.9
LCK; FYN; YES1; SRC	Proto-oncogene tyrosine-protein kinase LCK (LSK)	P06239; P06241; P07947; P12931	61	§393; §419; §425; §418	LIEDNEYTAR	2	3823	0.6	0.6-0.6

Gene Name	Description	Accession	kD	Site	Peptide	Count in Study	Count at CST	Heavy-to-Light Ratio Mean	Heavy-to-Light Ratio Range
FYN; YES1	Proto-oncogene tyrosine-protein kinase Fyn (SLK)	P06241; P07947	61	212; 221	KLDNGGyYITTR	2	868	0.7	0.7-0.7
LYN; HCK	Tyrosine-protein kinase Lyn	P07948; P08631	59	§396; §410	VIEDNEyTAR	2	1902	0.9	0.8-0.9
LYN	Tyrosine-protein kinase Lyn	P07948	59	§192	SLDNGGyYISPR	2	689	0.6	0.5-0.7
PTK2B	Protein tyrosine kinase 2 beta (Focal adhesion kinase 2) (FADK 2)	Q14289	116	§579, §580	YIEDEDyyKASVTRLPIK	2	370	1.1	1.1-1.1
TYK2	Non-receptor tyrosine-protein kinase TYK2	P29597	134	§292	LLAQAEGEPCyIR	3	734	1.3	1.2-1.4
Protein kinase, tyrosine (receptor)									
EGFR	Epidermal growth factor receptor	P00533	134	§869	LLGAEKEyHAEGGKVPIK	3	56	11.2	9.4-12.1
EGFR	Epidermal growth factor receptor	P00533	134	§998	MHLPSPTDSNFyR	1	1	52.1	
EGFR	Epidermal growth factor receptor	P00533	134	§998	MHLPSPTDSNFyR	10	99	4.7	3.6-10.7
EGFR	Epidermal growth factor receptor	P00533	134	§1110	RPAGSVQNPVyhNQPLNPAPSR	7	324	42.0	41.3-42.8
EGFR	Epidermal growth factor receptor	P00533	134	§1172	GSHQISLDNPDyQQDFFPK	14		39.0	33.1-48.5
EGFR	Epidermal growth factor receptor	P00533	134	§1172	GSHQISLDNPDyQQDFFPK	17	850	38.2	13.9-76.9
EGFR	Epidermal growth factor receptor	P00533	134	§1172	GsHQISLDNPDyQQDFFPK	1	1	36.4	
EGFR	Epidermal growth factor receptor	P00533	134	§1197	EAKPNGIFKSTAEAEyLR	2	119	>25.3	>20.8->29.8
EGFR	Epidermal growth factor receptor	P00533	134	§1197	GSAENAEyLR	1	1	>10.4	
EGFR	Epidermal growth factor receptor	P00533	134	§1197	GSTAENAEyLR	4	1322	11.5	11.4-11.6
EGFR; ERBB2; ERBB4	Epidermal growth factor receptor	P00533; P04626; Q15303	134	§727; 735; 733	VLGSGAFGTyK	3	65	0.9	0.8-0.9
EPHA1	Ephrin type-A receptor 1	P21709	108	§781	LLDDFDGyTETQGGKIPIR	3	132	1.3	1.2-1.6
EPHA2	Ephrin type-A receptor 2	P29317	108	575	QSPEDVyFSKSEQLKPLK	2	31	1.0	0.9-1.0
EPHA2	Ephrin type-A receptor 2	P29317	108	575	QSPEDVyFSK	2	283	0.8	0.7-0.8
EPHA2	Ephrin type-A receptor 2	P29317	108	§588	SEQLKPLKTyVDPHTYEDPNQAVLK	2	271	1.4	1.3-1.5
EPHA2	Ephrin type-A receptor 2	P29317	108	§588, §594	SEQLKPLKTyVDPHTYEDPNQAVLK	3	502	1.5	1.4-1.5
EPHA2	Ephrin type-A receptor 2	P29317	108	§588, §594	TyVDPHTyEDPNQAVLK	3	83	0.8	0.6-1.0
EPHA2	Ephrin type-A receptor 2	P29317	108	§594	TYVDPHTyEDPNQAVLK	5	682	1.0	0.9-1.0
EPHA2	Ephrin type-A receptor 2	P29317	108	§772	VLEDDPEATyTSSGGKIPIR	6	360	1.0	1.0-1.0
EPHA2	Ephrin type-A receptor 2	P29317	108	§772	VLEDDPEATyTSSGGK	5	713	1.0	0.9-1.0
EPHA2	Ephrin type-A receptor 2	P29317	108	§921	MQQyTEHFMAAGyTAIEK	2	12	1.2	1.2-1.3
EPHA2	Ephrin type-A receptor 2	P29317	108	§921, §930	MQQyTEHFMAAGyTAIEK	1	2	0.9	
EPHA2	Ephrin type-A receptor 2	P29317	108	§930	MQQyTEHFMAAGyTAIEK	2	1	3.2	1.6-4.8
EPHA3; EPHA5; EPHA4	Ephrin type-A receptor 3	P29320; P54756; P54764	110	§779; 833; 779	VLEDDPEAAyTTR	2	445	1.0	1.0-1.0
EPHA4	Ephrin type-A receptor 4	P54764	110	§596, §602	TyVDPFTyEDPNQAVR	2	82	0.9	0.9-0.9
EPHB3; EPHB4	Ephrin type-B receptor 3	P54753; P54760	110	§614; 596	VYIDPFYEDPNEAVR	1	171	1.4	
EPHB4	Ephrin type-B receptor 4	P54760	108	574	EAEySDKHGQYLIGHGTK	2	41	2.2	2.2-2.2
EPHB4	Ephrin type-B receptor 4	P54760	108	774	FLEENSSDPTyTSSLGGKIPIR	3	130	2.4	1.9-2.7
EPHB4	Ephrin type-B receptor 4	P54760	108	774	FLEENSSDPTyTSSLGGK	4	146	1.7	1.6-1.8
EPHB4	Ephrin type-B receptor 4	P54760	108	987	SQAKPGTGGTGGPAPQy	3	219	1.0	0.8-1.1
ERBB2	Receptor tyrosine-protein kinase erbB-2	P04626	138	§877	LLDIDETyHADGGKVPIK	3	98	1.1	1.1-1.1
ERBB3	Receptor tyrosine-protein kinase erbB-3	P21860	148	1328	SLEATSAFDNPDyWHSR	1	201	>17.7	
TYRO3; MERTK	Tyrosine-protein kinase receptor TYRO3	Q06418; Q12866	97	686; §754	IYSGDyYR	1	1	1.3	
TYRO3; MERTK	Tyrosine-protein kinase receptor TYRO3	Q06418; Q12866	97	685; §753	IYSGDyYR	1	12	1.3	
TYRO3; MERTK	Tyrosine-protein kinase receptor TYRO3	Q06418; Q12866	97	685; §753	KIYSGDyYR	1	86	1.5	
MET	Hepatocyte growth factor receptor	P08581	156	§1003	SVSPTTEMVSNESVDyR	4	552	1.4	1.4-1.5
MET	Hepatocyte growth factor receptor	P08581	156	§1234	DMYDKEySVHNK	11	1195	2.7	2.3-3.9
MET	Hepatocyte growth factor receptor	P08581	156	§1234, §1235	DMYDKEySVHNK	4	628	8.9	7.9-9.7
MET	Hepatocyte growth factor receptor	P08581	156	§1235	DMYDKEySVHNK	1	178	2.8	

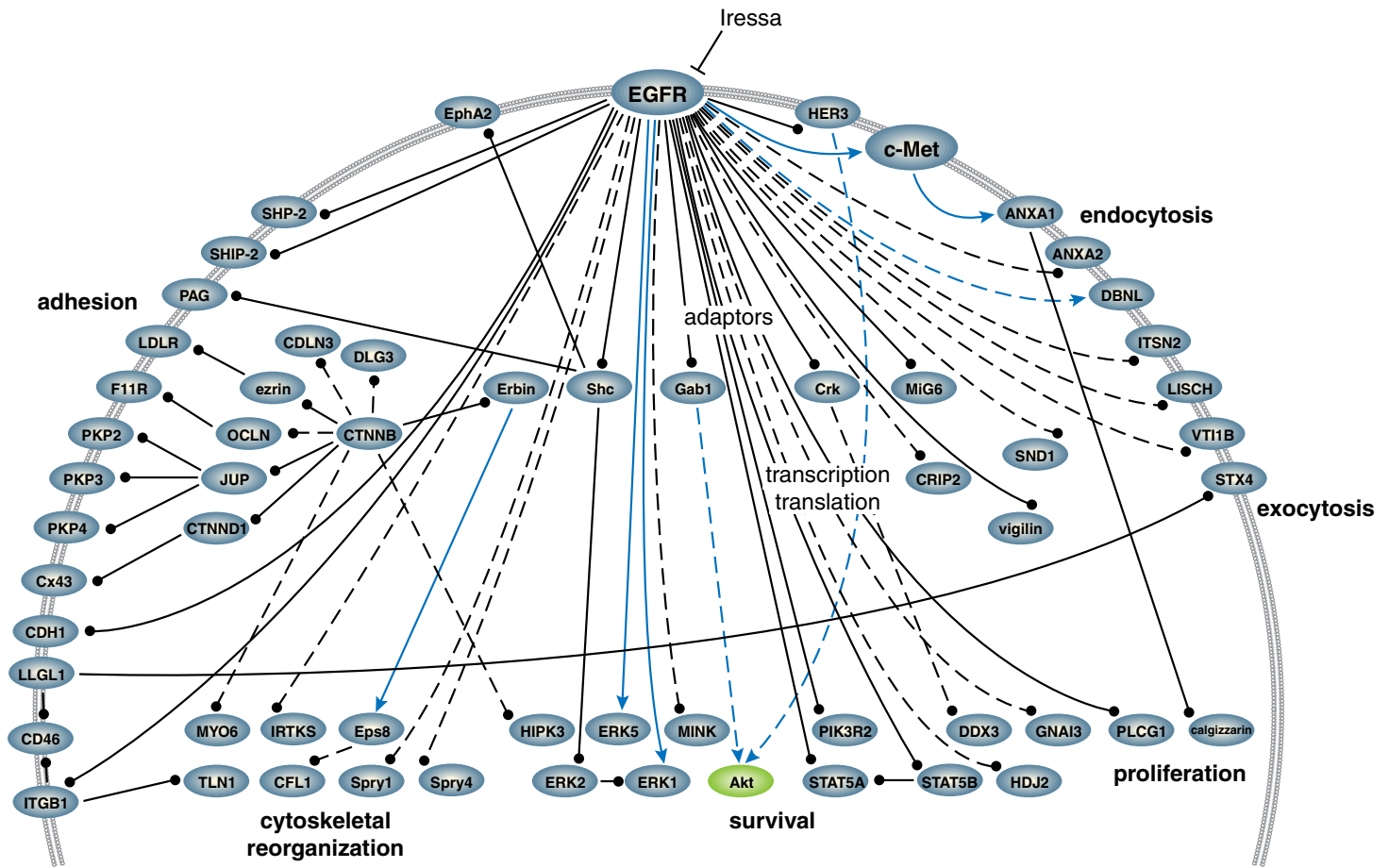
PHOSPHOSCAN-SILAC ANALYSIS OF H3255 CELLS TREATED WITH IRESSA

Gene Name	Description	Accession	kD	Site	Peptide	Count in Study	Count at CST	Heavy-to-Light Ratio Mean	Heavy-to-Light Ratio Range
Receptor, channel, or other cell surface protein									
APP	Amyloid beta A4 protein precursor (APP) (Alzheimer disease amyloid protein)	P05067	87	§757	MQQNGyENPTYK	1	682	1.6	
APLP2	Amyloid-like protein 2	Q06481	87	750	MQNHGyENPTYK	2	604	1.1	0.9-1.3
APLP2	Amyloid-like protein 2	Q06481	87	755	MQNHGyENPTyK	1	532	0.6	
CD46	Membrane cofactor protein	P15529-4	41	§354	GKADGGAEyATyQTK	2	223	3.9	3.7-4.1
CD46	Membrane cofactor protein	P15529-4	41	§354	ADGGAEyATyQTK	3	669	3.8	3.6-3.8
CD46	Membrane cofactor protein	P15529-4	41	§357	GKADGGAEyATyQTK	2	94	3.9	3.7-4.1
CD46	Membrane cofactor protein	P15529-4	41	§357	ADGGAEyATyQTK	4	240	3.7	3.6-3.8
CD46	Membrane cofactor protein	P15529	44	378	GyLTDETHR	2	2	>9.1	>8.5->9.6
GJA1	Gap junction alpha-1 protein (Connexin-43)	P17302	43	§246	SDPyHATSGALSPAK	2	40	>6.8	>6.8->6.9
GPRC5C	G-protein coupled receptor family C group 5 member C	Q9NQ84	48	387	VPSEGAyDIILPR	2	119	1.4	1.3-1.5
GPRC5C	G-protein coupled receptor family C group 5 member C	Q9NQ84	48	414	AEDMySAQSQHAATPPKDGK	2	104	1.0	0.9-1.0
LDLR	Low-density lipoprotein receptor	P01130	95	§845	TTEDEVHICHNQDGySYPSR	2	314	3.8	3.1-4.5
GPRC5A	Retinoic acid-induced protein 3 (Orphan G-protein-coupling receptor PEIG-1)	Q8NFJ5	40	§347	AHAWPSPyKDYEVK	2	353	0.7	0.6-0.7
GPRC5A	Retinoic acid-induced protein 3 (Orphan G-protein-coupling receptor PEIG-1)	Q8NFJ5	40	§347, §350	AHAWPSPyKDYEVKK	2	570	0.6	0.6-0.6
GPRC5A	Retinoic acid-induced protein 3 (Orphan G-protein-coupling receptor PEIG-1)	Q8NFJ5	40	§347, §350	AHAWPSPyKDYEVKKEGS	1	647	0.5	
GPRC5A	Retinoic acid-induced protein 3 (Orphan G-protein-coupling receptor PEIG-1)	Q8NFJ5	40	§350	AHAWPsPYKDYEVKKEGS	1	1	0.6	
SLITRK6	SLIT and NTRK-like protein 6	Q9H5Y7	95	801	KVLVEQTKNEyFELK	2	11	29.7	26.9-32.6
TFRC	Transferrin receptor protein 1 (CD71 antigen) (T9) (p90)	P02786	85	§20	SAFSNLFGEPLSyTR	4	733	2.0	1.9-2.1
Transcriptional regulation									
S100A11	Protein S100-A11 (S100 calcium-binding protein A11) (Calgizzarin) (MLN 70)	P31949	12	30	YAGKDGyNYTLsk	1	77	28.8	
SND1	Staphylococcal nuclease domain-containing protein 1	Q13122	102	304	IWRDyVAPTANLDQKDK	1	3	>8.6	
SND1	Staphylococcal nuclease domain-containing protein 1	Q13122	102	304	IWRDyVAPTANLDQK	2	2	>10.4	>9.6->11.2
SND1	Staphylococcal nuclease domain-containing protein 1	Q13122	102	§883	ADDADEFGySR	1	376	37.6	
GRLF1	Glucocorticoid receptor DNA-binding factor 1 (GRF-1) (Rho GAP p190A)	Q9NRY4	172	§1087	SVSSSPWLPQDGFDPsDyAEPMDAVVKPR	1	765	1.6	
GRLF1	Glucocorticoid receptor DNA-binding factor 1 (GRF-1) (Rho GAP p190A)	Q9NRY4	172	§1105	NEEENlySVPHDSTQgK	11	2944	1.9	1.8-1.9
STAT3	Signal transducer and activator of transcription 3	P40763	88	§705	YCRPESQEHPEADPGSAAPyLK	4	2422	0.7	0.7-0.8
STAT3	Signal transducer and activator of transcription 4	P40763-2	88	§704	YCRPESQEHPEADPGAAPyLK	3	1416	0.7	0.7-0.7
STAT5A; STAT5B	Signal transducer and activator of transcription 5A	P42229; P51692	91	§694; §699	AVDGYVKPQIK	2	1363	>44.7	>32.2->57.2
Translation initiation complex									
EEF1A1; EEF1A2	Elongation factor 1-alpha 1 (Elongation factor Tu)	P68104; Q05639	50	§29; §29	STTTGHLLyK	2	1167	0.9	0.8-0.9
EEF1A1; EEF1A2	Elongation factor 1-alpha 1 (Elongation factor Tu)	P68104; Q05639	50	§141; §141	EHALLAyTLGvK	2	917	0.8	0.7-0.8
RPS27	40S ribosomal protein S27 (Metallopan-stimulin 1) (MPS-1)	P42677	10	30	LVQSPNSyFMDvK	10	955	0.9	0.8-0.9
Transporter									
PITPNA	Phosphatidylinositol transfer protein alpha isoform	Q00169	32	139	HVEAVyIDIADR	3	2	0.7	0.7-0.8
SLC38A2	Amino acid transporter system A	Q9HAV3	56	41	SHyADVDPENQNFLLESNLGK	1	454	7.3	
SLC38A2	Amino acid transporter system A	Q9HAV3	56	41	SHyADVDPENQNFLLESNLGKK	3	273	4.0	3.9-4.3

Gene Name	Description	Accession	kD	Site	Peptide	Count in Study	Count at CST	Heavy-to-Light Ratio Mean	Heavy-to-Light Ratio Range
Unknown function									
TANC2	Amino acid transporter system A	Q9HCD6	206	1793	TNNAQNGHLEDDYSPHGMLANGSR	1	26	>5.8	
TANC2	Amino acid transporter system A	Q9HCD6	206	1794	TNNAQNGHLEDDYSPHGMLANGSR	1	2	18.6	
C21orf13	Uncharacterized protein C21orf13	O95447	77	98	yNVSKIIsQSK	1	1	>9.0	
CRIP2	Cysteine-rich protein 2 (CRP2) (Protein ESP1)	P52943	23	196	GVNTGAVGSyLYDRDPEGK	5	17	>29.5	>10.5->54.4
CRIP2	Cysteine-rich protein 2 (CRP2) (Protein ESP1)	P52943	23	198	GVNTGAVGSyLYDRDPEGK	3	16	5.6	4.0-8.2
CRIP2	Cysteine-rich protein 2 (CRP2) (Protein ESP1)	P52943	23	196, 198	GVNTGAVGSyLYDRDPEGK	1	1	>6.6	
TMEM106B	TMEM106B	Q8N353	35	90	NGDVSQFPyVEFTGR	2	178	2.9	2.8-3.0
FAM62A	Protein FAM62A (Membrane-bound C2 domain-containing protein)	Q9BSJ8	123	§822	HLSPyATLTVGDSSHK	6	1178	0.8	0.7-1.0
BAIAP2L1	Brain-specific angiogenesis inhibitor 1-associated protein 2-like protein 1	Q9UHR4	57	163	EIEyVETVTSR	2	18	10.2	4.5-15.9
KIAA1217	Sickle tail protein	Q9ULK3	146	239	NVYyELNDVR	6	359	1.2	1.1-1.2
KIAA1217	Sickle tail protein	Q9ULK3	146	§388	NEGFyADPYLYHEGR	6	283	0.9	0.9-1.1
LSR	Lipolysis-stimulated lipoprotein receptor	Q86X29	71	324	SSSAGGQGSyVPLLR	2	304	7.1	5.8-8.5
LSR	Lipolysis-stimulated lipoprotein receptor	Q86X29	71	487	SRDDLyDQDDSRDFPR	1	210	>7.6	
LSR	Lipolysis-stimulated lipoprotein receptor	Q86X29	71	503	SRDPHYDDFR	2	382	5.4	5.2-5.5
LSR	LSR protein	Q9BWS2	64	304	NSSAGGQGSyVPLLR	2	2	6.3	4.2-8.5
LLGL1	Lethal(2) giant larvae protein homolog 1	O00188	112	509	KVGC FDPySDDPR	2	52	4.1	4.1-4.1
LLGL1	Lethal(2) giant larvae protein homolog 1	O00188	112	509	VGCFDPySDDPR	4	85	2.5	2.5-2.6
LMBRD2	LMBR1 domain-containing protein 2	Q68DH5	81	290	NMDDyEDFDEK	1	14	>5.2	
PLEKHA7	Pleckstrin homology domain-containing family A member 7	Q86VZ7	85	282	SADDTyLQLKK	2	3	0.9	0.8-1.0
LRBA	Lipopolysaccharide-responsive and beige-like anchor protein	P50851	319	1110	SIVEEEEDDyVELK	2	19	>6.0	>6.0
MPP7	Membrane protein, palmitoylated 7 (MAGUK p55 subfamily member 7)	Q81Y28	66	417	SQESDGEyEYIFISK	2	17	7.5	7.3-7.7
MPP7	Membrane protein, palmitoylated 7 (MAGUK p55 subfamily member 7)	Q81Y28	66	417	RSQESDGEyEYIFISK	1	7	23.2	
NAALADL2	N-acetylated alpha-linked acidic dipeptidase-like protein 2	Q58DX5	89	106	LQEESDyITHYTR	4	20	4.8	4.5-5.0
NAALADL2	N-acetylated alpha-linked acidic dipeptidase-like protein 2	Q58DX5	89	106, 110	LQEESDyITHYTR	1	5	34.5	
PROSC	Proline synthetase co-transcribed bacterial homolog protein	O94903	30	69	TFGENyVQELLEK	4	37	0.8	0.7-0.9
PTTG1IP	Pituitary tumor-transforming gene 1 protein-interacting protein	P53801	20	174	KYGLFKEENPyAR	1	216	1.3	
PTTG1IP	Pituitary tumor-transforming gene 1 protein-interacting protein	P53801	20	174	YGLFKEENPyAR	7	920	1.3	1.3-1.4
SLITRK5	SLIT and NTRK-like protein 5	O94991	108	945	LNVEPDyLEVLEK	2	1	>12.6	>10.0->15.2
STEAP1	Metalloreductase STEAP1 (Six-transmembrane epithelial antigen of prostate 1)	Q9UHE8	40	27	NLEEDDyLHKDTGETSMLK	1	6	368.9	
STEAP1	Metalloreductase STEAP1 (Six-transmembrane epithelial antigen of prostate 1)	Q9UHE8	40	27	RNLEEDDyLHKDTGETSMLK	3	16	>13.8	>10.6->17.0



Regulatory Networks Sensitive to Iressa in H3255 Cells Revealed by PhosphoScan®



KEY	
	phosphorylation
	binding
	direct interaction
	indirect interaction
	protein not in dataset

Pathway diagram of proteins shown by PhosphoScan to have Iressa-responsive phospho-tyrosine sites. Each protein has one or more phospho-tyrosine sites that was made at least 2.5-fold less abundant by treatment with 1 μ M Iressa for 3 hours. Guo et al., Figure S5. Original pathway diagram generated using Pathway Studio® (Ariadne Genomics).

