Supplemental Text File.

Accompanies manuscript "Global impact of oncogenic Src on a phosphotyrosine proteome" by Luo et al.

Predicted Src sites frequently identified in both nontransformed and Src-transformed cells. An anticipated characteristic of major and biologically-relevant sites of tyrosine phosphorylation by Src is their ready detection using the shotgun pTyr proteomics approach in both the nontransformed and Src-transformed cell populations. Our study identified 47 distinct pTyr sites representing 36 different proteins that met the criteria of having 4 or more independent identifications (as Sequest top-scoring peptides) from both populations. Brief overviews are presented below for 32 of these proteins (sites on CMGC kinases being excluded), with emphasis on protein function, evidence for phosphorylation by Src, and functional significance of tyrosine phosphorylation. Many of the overviews make reference to the PhosphoSite Database of *in vivo* phosphorylation sites (www.phosphosite.org), a useful resource provided by Cell Signaling Technology, Inc. The identification frequency profiles of the sites are shown in Table 4 of the main manuscript. Known Src substrates and phosphorylation sites are highlighted in red.

1. Src (UniProt ID: Q80XU2)

pTyr-418 (representative peptide: R.LIEDNEY@TAR.Q)

Tyr-418 (numbered according to mouse nonneuronal form) is an autophosphorylation site in the kinase domain activation loop and the main site of *in vivo* tyrosine phosphorylation of v-Src (Smart et al., 1981). Phosphorylation of this site is critical for transforming ability (Kmiecik and Shalloway, 1987) and can be achieved through both inter- and intramolecular mechanisms (Cooper and MacAuley, 1987). In the inactive conformation, the activation loop forms a helix that helps stabilize the closed conformation, blocks the peptide substrate-binding site, and prevents Tyr-418 phosphorylation (Xu et al., 1999). Disruption of the intramolecular interactions by SH2 or SH3 ligands, or by dephosphorylation of pTyr-529, could lead to exposure and phosphorylation of Tyr-418. Conversely, Tyr-418 phosphorylation promotes reorientation of kinase domain helix C into the active conformation and increases the accessibility of the SH3 domain for ligands (Xu et al., 1999; Gonfloni et al., 2000).

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Xu W, Doshi A, Lei M, Eck MJ, Harrison SC (1999) *Mol Cell* 3:629-638.

2. FAK (P34152-3)

pTyr-576 (R.YM#EDSTY@YK.A)

pTyr-577 (R.YM#EDSTY@Y@K.A)

FAK (focal adhesion kinase) is a 120 kDa nonreceptor tyrosine kinase that localizes prominently to focal adhesions, undergoes tyrosine phosphorylation in response to integrin-mediated adhesion, and exhibits elevated phosphotyrosine in Src-transformed cells (Hanks et al., 1992; Schaller et al., 1992). FAK autophosphorylates on Tyr-397 to create a high affinity binding site for the Src SH2 domain (Schaller et al., 1994), and this interaction is implicated in Src-mediated phosphorylation of the FAK Tyr-576/577 sites that lie in the kinase domain activation loop (Calalb et al., 1995; Ruest et al., 2000). Maximal FAK autophosphorylation activity and the

ability of FAK to efficiently promote cell migration requires the Tyr-576/577 sites (Owen et al., 1999; Sieg et al., 1999). Thus Src-mediated phosphorylation of the FAK activation loop tyrosines may contribute to a positive feedback loop important for efficient assembly of a FAK/Src complex within cell/ECM adhesions. Signaling through the FAK/Src complex promotes cell motility and invasion, with paxillin and p130Cas being major substrates of the complex (reviewed in Schlaepfer et al., 2004).

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3. SgK269 (Q8BX56)

pTyr-632 (K.NAIKVPIVINPNAY@DNLAIYK.S)

pTyr-638 (K.NAIKVPIVINPNAY@DNLAIY@K.S)

SgK269 ("Sugen Kinase 269") is an uncharacterized putative protein kinase of the NKF3 family that was first described in the analysis of the human "kinome" (Manning et al., 2002). The UnitProt entry for SgK269 (Q8BX56) indicates it is a tyrosine kinase, but there is no apparent supportive evidence for this claim other than the fact that it can be phosphorylated on tyrosine residues. The PhosphoSite database lists many sites of SgK269 tyrosine phosphorylation, including the Tyr-632 and Tyr-638 sites, which were first reported in Rush et al. (2005).

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4. SHP2 (P35235)

pTyr-62 (K.IQNTGDY@YDLYGGEK.F)

SHP2 (reviewed in Neel et al., 2003) is a 68 kDa non-transmembrane protein-tyrosine phosphatase containing tandem SH2 domains that mediate interactions with receptor tyrosine kinases, adapter/docking proteins, and cell adhesion components. SHP2, like its Drosophila ortholog Corkscrew, has been characterized as an enhancer of receptor tyrosine kinase signaling leading to activation of the Ras > ERK pathway (Neel et al., 2003). Multiple mechanisms have been implicated in this paradoxical role, including stimulation of Src activity through dephosphorylation of proteins that bind to the Src negative regulator Csk (Ren et al., 2004; Zhang et al., 2004). Supporting a role for SHP2 as a positive regulator of Src activity in integrin signaling, SHP2-deficient fibroblasts are defective in adhesion-stimulated FAK and ERK activation as well as cell spreading and migration (Yu et al., 1998; Oh et al., 1999) and exhibit increased stress fibers and focal adhesions linked to increased RhoA activity (Schoenwaelder et al., 2000). In Src-transformed cells, SHP2 exhibits constitutive tyrosine-phosphorylation (Feng et al, 1993) and is required for full morphological transformation and podosome formation (Hakak et al., 2000). Tyrosine phosphorylation of SHP2 Tyr-62 could be a novel mechanism for promoting SHP2 signaling. Notably, the D′E turn of the N-terminal SH2 domain (Asn58-Tyr62) inserts into the catalytic cleft to autoinhibit phosphatase activity (Hof et al., 1998). While binding of the N-terminal SH2 domain to a phosphopeptide ligand can dissociate the

autoinhibitory interaction to promote SHP2 signaling, Tyr-62 phosphorylation could also act to stabilize the active conformation. SHP2 Tyr-62 phosphorylation has been documented in other proteomics studies (PhosphoSite), and implicated in cell migration in response to HER2 (Kumar et al., 2006; Wolf-Yadlin et al. 2006).

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5. PI3K p85α (P26450)

pTyr-467 (K.SREYDRLY@EEYTR.T)

Phosphatidylinositol 3' kinase (PI3K) type 1A is a heterodimer of regulatory (p85) and catalytic (p110) subunits. PI3K phosphorylates the inositol ring D3 position to produce phosphatidylinositol-3,4,5-trisphosphate (PI-3,4,5-P3) that acts as a second messenger to affect cell growth, survival, and movement (reviewed by Cantley, 2002). PI3K activity is stimulated by mitogens (Auger et al., 1989) and is elevated in Src–transformed cells (Whitman et al., 1988). PI-3,4,5-P3 functions in the plasma membrane to recruit proteins containing pleckstrin homology domains including the serine-threonine kinase Akt. Signals downstream of PI3K > Akt have been implicated in transformation by oncogenic Src (Penuel and Martin, 1999; Johnson et al., 2000). The two p85 isoforms (alpha and beta) share a domain structure of N-terminal SH3 domain, RhoGAP-related domain, and two SH2 domains. Under quiescent conditions the p85 inter-SH2 region interacts with the p110 subunit to inhibit catalytic activity, and the inhibition is relieved when p85 SH2 domains interact with phosphotyrosine residues on activated receptor tyrosine kinases or other docking proteins (Yu et al., 1998; Jimenez et al., 2002). The Src SH3 domain interacts with p85 (Liu et al., 1993; Pleiman et al., 1994), correlating with PI3K activation and transformation (Wages et al., 1992). Phosphorylation of Tyr-688 (numbering for p85alpha) in the C-terminal SH2 domain, a known target of Src- and Abl-family kinases (von Willebrand et al., 1998), neutralizes the inhibitory function of p85 (Cuevas et al., 2001, Chan et al., 2001). Oncogenic Src can also promote PI3K activation by indirect mechanisms such as promoting Ras interaction with p110 (Rodriguez-Viciana et al., 1994). Tyr-688 phosphorylation has yet to be detected in phosphotyrosine proteomics studies, while many other sites in the interSH2 region and C-terminal SH2 domain have been detected (PhosphoSite), including pTyr-467 in the inter-SH2 region. Since Tyr-467 is part of a coiled-coiled domain important for the inhibitory interaction with p110 (Dhand et al., 1994), phosphorylation of this residue could influence enzyme activity.

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6. SHIP2 (Q9JLL7)

pTyr-987 (K.NSFNNPAY@YVLEGVPHQLLPLEPPSLAR.A)

SHIP2 is an SH2-containing inositol 5'-phosphatase. In addition to an N-terminal SH2 domain, SHIP2 has a proline-rich region, a single NPXY motif, and a C-terminal SAM domain. SHIP2 localizes to focal contacts during attachment and lamellipodia in spreading cells, and the SH2 domain of SHIP2 interacts with p130Cas (Prasad et al., 2001). Tyr-987 is the tyrosine of the NPXY motif. During cell attachment and spreading on collagen I (but not fibronectin, collagen IV, or laminin) there is good evidence that Src phosphorylates this residue to promote an interaction with Shc, via the Shc PTB domain (Prasad et al., 2002). Expression of a SHIP2 Y987F/Y988F mutant results in irregular membrane protrusions and actin spikes (Prasad et al., 2002), suggesting that the SHIP2-Shc interaction has a significant impact on the organization of the actin cytoskeleton. Suppression of SHIP2 levels by RNAi causes F-actin deformities associated with delayed cell spreading, absence of cell/ECM adhesions, and defects in endocytosis (Prasad and Decker, 2005).

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7. GIT1 (Q68FF6)

pTyr-554 (R.LQPFHSTELEDDAIY@SVHVPAGLYR.I)

GIT1 was first characterized as an ARF-GAP that binds G-protein-coupled receptor kinases and inhibits their internalization (Premont et al., 1998; Claing et al., 2000). GIT1, and the related protein GIT2, have since been shown to also have major functions as regulators of adhesion and cytoskeletal organization (reviewed in Hoefen and Berk, 2006). GIT proteins interact strongly with the PIX family of GEFs for Rac1/Cdc42 (Premont et al., 2004) and have also been detected in complex with other signaling proteins, including paxillin, FAK, phospholipase C, MEK1, and ERK1/2 (Hoefen and Berk, 2006). GIT1 promotes adhesion disassembly through a PIX-dependent mechanism (Zhao et al., 2000) and there is evidence that GIT1, PIX, paxillin, and the Rac/Cdc42 effector kinase p21PAK exist as a motile signaling complex that targets to adhesions and lamellipodia leading edges to regulate cell migration (Manabe et al., 2002). GIT1 is a recognized Src substrate (Bagrodia et al., 1999; Haendeler et al., 2003; Yin et al., 2005), and the scaffolding activity of FAK may play a role in Src-mediated GIT1 phosphorylation in cell/ECM adhesions (Bagrodia et al., 1999). From proteomics approaches several sites of GIT1 tyrosine

phosphorylation have been identified (Webb et al., 2006; PhosphoSite Database), including Tyr-554. Tyr-554 is not conserved in GIT2, nor is a nearby GIT1 phosphorylation site (Tyr-563) that was detected only in Src-transformed cells in our study. There are no published studies on the significance of GIT1 tyrosine phosphorylation. However, tyrosine phosphorylation of GIT2 by Src and/or FAK (on sites equivalent to mouse GIT2 Tyr-285, -391, and -541) was implicated in recruitment to focal complexes (Brown et al., 2005). Although GIT1 tyrosine phosphorylation was not similarly implicated in adhesion targeting (Brown et al., 2005), all three of these sites are conserved in mouse GIT1 (as Tyr-293, -392, and -607). Of these sites, only GIT2 Tyr-541 and GIT1 Tyr-392 phosphorylation were detected in our analysis, and only in nontransformed cells.

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8. p190RhoGAP (Q91YM2)

pTyr-943 (R.NEEENIY@SVPHDSTQGK.I)

p190RhoGAP was first recognized in Src transformed cells as a highly tyrosine phosphorylated protein that also binds p120RasGAP (Ellis et al., 1990; Bouton et al., 1991; Settleman et al., 1992a,b). p190RhoGAP accounts for ~60% of cellular GAP activity towards Rho (Vincent and Settleman, 1999). Tyr-943 (equivalent to Tyr-1105 in the human protein), is the major site of p190RhoGAP phosphorylation by Src, and this site mediates the interaction with p120RasGAP (Roof et al., 1998). The interaction with p120RasGAP may stimulate the GAP activity of p190RhoGAP by promoting its recruitment to the plasma membrane (Bradley et al., 2006). Tyrosine phosphorylation of p190RhoGAP, via a Src-dependent mechanism, is an early response to integrin-mediated cell/ECM adhesion and is a mechanism for transient suppression of RhoA activity necessary for lamellipodia extension during cell motility (Arthur et al., 2000). RhoA inhibition via p190RhoGAP phosphorylation is also a likely mechanism for v-Src-induced cytoskeletal disruption (Fincham et al., 1999). The pTyr-943 site is also phosphorylated by the Abl-related kinase Arg, and the adhesion-dependent stimulation of p190RhoGAP phosphorylation is not observed in arg(-/-) cells (Hernandez et al., 2004). Since Src can also activate Arg by phosphorylating the Arg activation loop (Plattner et al., 1999; Tanis et al., 2003), it is possible that Tyr-943 phosphorylation occurs through a Src >Arg/Abl > p190RhoGAP pathway.

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9. RIN1 (Q921Q7)

pTyr-35 (K.EKPSTDPLY@DTPDTR.G)

RIN1 (Ras and Rab interactor 1) was originally identified by its ability to physically bind to and interfere with activated Ras (Han and Colicelli, 1995). RIN1 competes with Raf1 for Ras-binding, and can inhibit cellular transformation by activated mutant Ras (Han et al., 1997; Wang et al., 2002). Other studies have demonstrated functions for RIN1 as a GEF for a G-protein (Rab5) involved in facilitating Ras-activated receptor endocytosis (Tall et al., 2001) and in activating Abl-family tyrosine kinases including Bcr-Abl (Afar et al., 1997; Han et al., 1997; Hu et al., 2005). RIN1 potentiates the oncogenic activity of BCR-Abl and accelerates BCR-Abl-induced leukemias in mice (Afar et al., 1997). Abl activation by RIN1 involves a stable association of the proteins resulting from an initial interaction of the Abl SH3 domain with a RIN1 proline-rich motif, Abl-mediated phosphorylation of RIN1 Tyr-36 (equivalent to mouse RIN1 Tyr-35), and finally binding of the Abl SH2 domain to this site (Afar et al., 1997; Hu et al., 2005). Moreover Abl activation by RIN1 is enhanced when RIN1 interacts with activated Ras, supporting a Ras > RIN1 > Abl > Crk signaling pathway involved in cytoskeletal remodeling (Hu et al., 2005).

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10. Annexin A2 (P07356)

pTyr-23 (K.LSLEGDHSTPPSAY@GSVKPYTNFDAER.D)

The annexins are a large family of calcium-dependent phospholipid-binding proteins (reviewed in Moss and Morgan, 2004). Annexin A2, among other functions, has been implicated in membrane transport events including both endocytic and secretory pathways (Kwon et al., 2005). Annexin A2 was first recognized as a major (36 kDa) tyrosine-phosphorylated protein in v-Src-transformed cells (Radke and Martin, 1979; Erikson and Erikson, 1980) and initially named "calpactin I" (Saris et al., 1986) or "lipocortin II" (Huang et al., 1986) when first described at the sequence level. Tyr-23 is the major site of annexin A2 phosphorylation by Src (Isacke et al., 1986). In addition to being phosphorylated, the N-terminal tail of annexin A2 interacts with a member of the S100 family of calcium binding proteins, p11, that enhances membrane phospholipid binding affinity (Thiel et al., 1992). A heterotetramer of two annexin A2 heavy chains and two p11 light chains is the predominant form in many cell types. By bringing p11 to the cell surface, the heterotetramer is implicated in plasminogen regulation (reviewed in Kwon et al., 2005). Heat stress induced translocation of annexin A2 to the cell surface was shown to require both p11 and Tyr-23 phosphorylation (Deora et al., 2004). On the other hand, tyrosine

phosphorylation of annexin A2 was implicated as a negative regulatory event for heterotetramer function by inhibiting F-actin binding and complex formation with the plasma membrane and chromaffin granules (Hubaishy et al., 1995). In addition to Tyr-23, our study also detected annexin A2 phosphorylation on tyrosines 237, 274, 310, 315, and 316. The 237, 274, 315, and 316 sites were all detected frequently but only in the Src-transformed cells. Tyr-310 was detected infrequently and only in the nontransformed cells.

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11. ANKS1/Odin (P59672)

pTyr-471 (L.AVRPRIQSSAPQEEEEHPY@EL.L)

ANKS1 (also known as Odin) was first characterized as a cytoplasmic protein that undergoes tyrosine phosphorylation in response to growth factor (EGF or PDGF) stimulation (Pandey et al., 2002). Odin contains six tandem ankyrin repeats near the N-terminus, two sterile alpha motifs and a C-terminal PTB domain. Overexpression of Odin had a negative effect on the mitogenic response to growth factors (Pandey et al., 2002). Consistent with its role as a negative regulator of growth factor receptor signaling, MEFs generated from Odin-deficient mice exhibit a hyperproliferative phenotype (Kristiansen et al., 2004). In the original report of Odin, tyrosine phosphorylation sites were not identified, and the functional significance of Odin tyrosine phosphorylation sites are unknown. Tyr-471 lies in the central region of the protein, between the ankyrin repeats and sterile alpha motifs. The same site has been detected on the human protein (as pTyr-472) from a quantitative phosphotyrosine proteomics study as undergoing increased phosphorylation in response to insulin (Schmelzle et al., 2006).

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Schmelzle K, Kane S, Gridley S, Lienhard GE, White FM (2006) *Diabetes* 55:2171-2179.

12. Caveolin-1 (P49817)

pTyr-14 (K.YVDSEGHLY@TVPIR.E)

Caveolin-1 is an essential coat protein of caveolae, but it also localizes to other cellular compartments and appears to have multiple functions in lipid transport, membrane trafficking, and as a negative regulator of proliferative cell signaling (reviewed in Liu et al., 2002; Williams and Lisanti, 2005; Schwencke et al., 2006). Caveolin-1 was first identified as a 21-22 kDa protein exhibiting elevated tyrosine-phosphorylation in Src-transformed cells (Glenney, 1989, Rothberg, 1992). Tyr-14 was later shown to be the principal site of caveolin-1 phosphorylation by Src (Li et al., 1996). The pTyr-14 site is reported to mediate caveolin-1 interactions with Grb7 (Lee et al., 2000), Csk (Cao et al., 2002), and MT1-MMP (Labrecque et al., 2004). Tyr-14-phosphorylated caveolin-1 appears to localize primarily to cell/ECM adhesions of adherent

fibroblasts but, upon cell detachment, translocates to caveolae where the phosphorylation site appears to be important for the internalization of the cholesterol-enriched caveolae (del Pozo et al., 2005). This mechanism could account for the inhibition of ERK, PI3K, and Rac signaling pathways observed upon cell detachment (Echarri and del Pozo, 2006). It is not known if Tyr-14-phosphorylated caveolin-1 has specific functions in focal adhesions. Indeed, there are specificity concerns regarding the observed focal adhesion staining by a phosphospecific antibody against the pTyr-14 site (Hill et al., 2007).

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13. Dok1 (P97465)

pY314 (R.IPPGPSQDSVY@SDPLGSTPAGAGEGVHSK.K)

pY361 (K.TKLTDSKEDPIY@DEPEGLAPAPPR.G)

pY408 (R.LKEEGYELPYNPATDDY@AVPPPR.S)

Dok1 was first described as a major tyrosine-phosphorylated protein in Bcr-Abl-transformed cells (Carpino et al., 1997; Yamanashi and Baltimore, 1997) and subsequent studies revealed this docking protein (characterized by N-terminal PH and PTB domains and a C-terminal phosphorylation domain) to be a target of various other nonreceptor and receptor tyrosine kinases. Both Abl (Woodring et al., 2004) and Src (Noguchi et al., 1999) activities have been linked to increased Dok1 tyrosine phosphorylation in response to integrin-mediated cell-ECM adhesion. Tyr-361 is a major site of Dok1 tyrosine phosphorylation, leading to recruitment of the adaptor Nck to stimulate F-actin reorganization and cell motility (Noguchi et al., 1999; Woodring et al., 2004). A chemical genetic screen identified Dok1 tyrosines 361, 295 and 450, as direct phosphorylation targets of v-Src (Shah and Shokat, 2002). Including these three sites, our study detected 10 different sites of Dok1 tyrosine phosphorylation including pTyr-314 and pTyr-408 that were frequently detected in both populations. Tyrosine-phosphorylated Dok1 is also known to interact with RasGAP (Carpino et al., 1997; Yamanashi and Baltimore, 1997) and Csk (Neet and Hunter, 1995). Mutation of Dok1 Tyr-450 abolishes the interaction with Csk (Shah and Shokat, 2002). The RasGAP and Csk interactions act to inhibit Ras/MAPK and Src/c-Myc pathways, respectively, contributing to the negative effect of Dok1 on PDGF-induced mitogensis (Zhao et al., 2006). Src-mediated tyrosine phosphorylation also prevents entry of Dok1 into the nucleus (Niu et al., 2006).

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Zhao M, Janas JA, Niki M, Pandolfi PP, Van Aelst L (2006) *Mol Cell Biol* 26:2479–2489.

14. Nck1 (Q99M51)

pTyr-105 (K.PSVPDTASPADDSFVDPGERLY@DLNMPAFVK.F)

Nck1 is a 47 kDa SH2/SH3 domain-containing adapter protein implicated in actin cytoskeleton organization, cell adhesion and migration (reviewed in Li et al., 2001; Buday et al., 2002). EGFR and PDGFR activation, as well as v-Src cellular transformation are associated with phosphorylation of Nck1 at a single tyrosine, and Src was shown capable of directly phosphorylating Nck1 at the same site (Meisenhelder and Hunter, 1992). The site has been identified as Tyr-105 (T. Hunter, personal communication). The same site was reported from other proteomics studies (PhosphoSite), including two that linked the Nck1 Tyr-105 phosphorylation to Bcr-Abl signaling (Griswold et al., 2006; Goss et al., 2006). The functional significance of Nck1 Tyr-105 phosphorylation is unknown.

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Meisenhelder J, Hunter T (1992) *Mol Cell Biol* 12:5843-5856.

15. Shc1 (P98083-1, p66 isoform)

pTyr-423 (R.ELFDDPSY@VNIQNLDK.A)

Shc1 is an adaptor protein first identified as a protooncogene involved in growth factor signaling (Pelicci et al., 1992). Three isoforms of Shc1 (p46, p52 and p66 kDa) are generated through alternative splicing, and all contain PTB and SH2 domains and a central region containing tyrosine phosphorylation sites (Pelicci et al., 1996). Shc1 tyrosine phosphorylation has been observed in response to many extracellular stimuli including growth factors, cytokines, hormones, and cell/ECM adhesion (reviewed in Ravichandran, 2001) and in human tumors (Pelicci et al., 1995). Shc proteins have elevated tyrosine-phosphorylated in v-Src transformed cells (McGlade et al., 1992). Tyr-317 (numbered for the p52 isoform (eq. to Tyr-423 in p66) is phosphorylated by Src and other tyrosine kinases to create a high affinity binding site for Grb2 (Salcini et al., 1994), establishing a link to mitogenic signaling via the Ras/MAPK pathway. Phosphorylated tyrosines 239 and 240 have also been identified and implicated in Grb2 binding (van der Geer et al., 1996), and in our study these sites were also frequently identified in the Src-transformed cells, but detected infrequently from the nontransformed cells. Elevated Shc1 phosphorylation is a likely cause of Ras activation required for transformation by oncogenic Src (Smith et al., 1986, Penuel and Martin, 1999). Targetted knockout of Shc1 in mice supports a role for this signaling adaptor in growth factor-induced MAP kinase activation, as well as in proper organization of focal adhesions and the actin cytoskeletal network (Lai and Pawson, 2000).

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16. p130Cas (Q61140)
pTyr-253 (R.HLLAPGPQDIY@DVPPVR.G)
pTyr-271 (R.GLLPNQYGQEVY@DTPPMAVK.G)
pTyr-414 (R.VLPPEVADGSVVDDGVY@AVPPPAER.E)

p130Cas (Crk-associated substrate) was first recognized as a major tyrosine-phosphorylated protein in cells transformed by v-Crk (Mayer et al., 1988) and v-Src (Kanner et al., 1990). p130Cas is a nonenzymatic docking protein with an N-terminal SH3 domain that interacts with FAK, Src-binding domain (SBD) near the C-terminus, and interior substrate domain (SD) defined by 15 YxxP motifs. p130Cas tyrosine phosphorylation is achieved primarily by Src-family kinases with the SD YxxP tyrosines representing major sites (Sakai et al., 1997; Ruest et al., 2001; Fonseca et al., 2004). Mapping studies identified 14 YxxP tyrosines (all but the most N-terminal) as subject to direct phosphorylation by Src in vitro (Goldberg et al., 2003; Shin et al., 2004). p130Cas SD phosphotyrosine is detected at sites of integrin-mediated adhesion (Fonseca et al., 2004) and has been linked to integrin signaling pathways promoting both cell motility and survival (Klemke et al., 1998; Honda et al., 1999; Cho and Klemke, 2000; Goldberg et al, 2003; Shin et al., 2004). Recruitment of Crk adaptor proteins to phosphorylated YxxP sites is implicated as a relevant downstream signaling event (Klemke et al., 1998; Cho and Klemke, 2000). In PDGF-treated cells, p130Cas SD tyrosine phosphorylation leading to recruitment of Nck adaptors is implicated in membrane ruffling (Rivera et al., 2006). Elevated p130Cas YxxP site phosphorylation has been linked to invasive/metastatic behavior of Src-transformed fibroblasts (Brabek et al., 2004, 2005). Tyrosines 253, 271, and 414 (representing the 7th, 8th, and 15th SD YxxP sites) were frequently identified in our study in both nontransformed and Src-transformed cells, while YxxP sites 2, 6, 9, 10, 11, and 14 were also detected.

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Ruest PJ, Shin NY, Polte TR, Zhang X, Hanks SK (2001) *Mol Cell Biol* 21:7641-7652.
Sakai R, Nakamoto T, Ozawa K, Aizawa S, Hirai H (1997) *Oncogene* 14:1419-1426.

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17. LPP (Q8BFW7)

pY245 (H.YMAGPSSGQIY@GPGPRGY.N)
pY301 (R.YYEPY@YAAGPSYGGR.S)
pY302 (R.YYEPYY@AAGPSYGGR.S)

LPP, discovered as the product of a preferred translocation partner in a subset of lipomas (Petit et al., 1996), is a member of the zyxin family characterized by the presence of three C-terminal LIM domains. The LPP LIM domains target the protein to focal adhesions (Petit et al., 2000, 2003). LPP is also present in the cytoplasm, cell-cell contacts, and shuttles to the nucleus. LPP expression can be regulated by FAK and contribute to efficient cell spreading (Gorenne et al., 2006). LPP tyrosines 245, 301, and 302 are among a dozen tyrosines in the central region of the protein (N-terminal to the LIM domains) that have been detected by phosphotyrosine proteomics studies (PhosphoSite Database). However there are no published reports on the functional significance of any of these sites or the responsible tyrosine kinase(s). Src is reported to phosphorylate the zyxin family member TRIP6 as part of the signaling response to LPA (Lai et al., 2005), but the major site of TRIP6 phosphorylation by Src is not conserved in LPP -- nor are the LPP phosphotyrosine sites conserved in TRIP6.

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Petit M, Mols R, Schoenmakers E, Mandahl N, van de Ven W (1996) *Genomics* 36:118-129.
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18. Paxillin (Q8VI36)

pTyr-118 (R.AGEEEHVY@SFPNKQK.S)

Paxillin is a docking protein first characterized as being tyrosine-phosphorylated protein in Src-transformed cells (Glenney and Zokas, 1989). Paxillin prominently localizes to focal adhesions (Turner et al., 1990) and undergoes tyrosine phosphorylation in response to integrin-mediated adhesion (Burridge et al., 1992). Within the N-terminal two-thirds of the protein are five LD motifs, while four tandem LIM domains are found in the C-terminal region. LIM domains 2 and 3 are crucial for recruitment of paxillin to focal adhesions, while FAK interacts with paxillin *via* LD motifs 2 and 4 (Brown et al., 1996). Studies on paxillin-null fibroblasts demonstrated critical roles for paxillin in focal adhesion and cortical cytoskeleton organization, integrin-mediated tyrosine kinase signaling, and cell spreading and motiltiy (Hagel et al., 2002). Paxillin tyrosine phosphorylation occurs on multiple residues in the N-terminal region, and appears to be mediated by FAK- and/or Src-family kinases (reviewed in Schaller and Schaefer, 2001). Tyrosines 31 and 118 were first described as the major sites of phosphorylation with a function in recruitment of Crk-family adaptors (Schaller and Parsons, 1995; Bellis et al., 1995) important for cell migration (Petit et al., 2000; Vindis et al., 2004). These two sites have also been implicated in binding of the tandem SH2 domains of p120RasGAP, providing a mechanism for localized suppression of RhoA (Tsubouchi et al., 2002). A recent study indicates that Tyr-31/Tyr-118 phosphorylation stimulates assembly of cell/ECM adhesions while also inducing the turnover of adhesions through recruitment of FAK (Zaidel-Bar et al., 2007). Tyrosines 40, 88, and 181 have also been reported as minor sites of phosphorylation (Schaller and Parsons, 1995;

Bellis et al., 1995, Nakamura et al., 2000; Schaller and Schaefer, 2001). In addition to pTyr-118, our study also identified the Tyr-31 and Tyr-88 sites.

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Vindis C, Teli T, Cerretti DP, Turner CE, Huynh-Do U (2004) J Biol Chem 279:27965-27970.
Zaidel-Bar R, Milo R, Kam Z, Geiger B (2007) J Cell Sci 120:137-148.

19. Talin1 (P26039)

pTyr-26 (K.TMQFEPSTMVY@DAC*R.M)
pTyr-70 (K.ALDY@YMLR.N)

Talin1 is a 235 kDa focal adhesion protein that functions in establishing the physical linkage between integrins and F-actin (reviewed in Critchley, 2000; Nayal et al., 2004). Studies with talin1-null cells indicate a role for talin1 in the force-dependent formation of early cell-ECM adhesion sites (reinforcement of initial integrin-cytoskeleton bonds) (Giannone et al., 2003). Talin undergoes enhanced tyrosine phosphorylation in Src-transformed fibroblasts (Pasquale et al., 1986) irrespective of the morphology induced (DeClue and Martin, 1987). In nontransformed cells talin is also phosphorylated at multiple tyrosine residues, some of which appear to be distinct from the sites elevated in response to oncogenic Src (DeClue and Martin, 1987). Our study identified six sites of talin1 tyrosine phosphorylation, including Tyr-26 and Tyr-70 found frequently in both nontransformed and Src-transformed cells. All of these sites have been identified in other proteomics studies (PhosphoSite), and the functional significance of any of the sites is unknown.

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Pasquale EB, Maher PA, Singer SJ (1986) *Proc Natl Acad Sci USA* 83:5507-5511.

20. p120 catenin (P30999)

pTyr-96 (K.LNGPQDHNHLLY@STIPR.M)
pTyr-228 (R.HYEDGYPGGSDNY@GSLSR.V)

p120 catenin is a multiple armadillo domain-containing protein first described as a major tyrosine-phosphorylated protein in Src-transformed cells and subsequently characterized as a cadherin-interacting protein required for E-cadherin stability at the cell surface (reviewed by Reynolds, 2007). p120 is further implicated as a modulator of Rho-family GTPases, through multiple mechanisms. p120 overexpression causes a dendritic cell phenotype linked to the ability of p120 to bind to either RhoA and act as a Rho-GDI (Anastasiadis et al., 2000) and/or to a Rho-family GEF (Vav2) to increase the activity of Cdc42 and Rac1 (Noren et al., 2000). More recently, p120 was implicated in coordinating antagonism between Rac and Rho by interacting with p190RhoGAP at cadherin complexes (Wildenberg et al., 2006). Localized RhoA inhibition

at cadherin complexes via p190RhoGAP (Noren et al., 2003) could contribute to cadherin stability at the cell surface. While a large number of p120 tyrosine phosphorylation sites have been reported in the "regulatory domain" N-terminal to the armadillo repeats, the functional significance of these modifications is uncertain (Alemà and Salvatore, 2007). Tyr-96 and -228 are among 8 residues identified as *in vitro* Src sites (Mariner et al., 2001). Tyr-96 is found only in splice isoform 1, the predominant form in mesenchymal cells, and is not present in isoform 3 that is the major form expressed in epithelial cells. Using a phosphospecific antibody, Tyr-228 phosphorylation was observed in response to EGF stimulation in a Src-independent manner (Mariner et al., 2004). p120 tyrosine phosphorylation by Src-family kinases was reported to regulate the interaction with RhoA and regulation of RhoA activity by p120, with surprising differential effects of phosphorylation mediated by Fyn versus Src (Castano et al., 2007).

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Wildenberg GA, Dohn MR, Carnahan RH, Davis MA, Lobdell NA, Settleman J, Reynolds AB (2006) *Cell* 127:1027-1039.

21. ZO-1 (P39447)

pTyr-1164 (R.HEEQPAPAY@EVHNR.Y)

ZO-1 (zonula occludens-1) is a 220 kDa tight junction protein and member of the MAGUK family (Stevenson et al., 1986; Itoh et al., 1993; Willott et al., 1993). MAGUKs are molecular scaffolds containing PDZ, SH3, and guanylate kinase homology domains and act to recruit signaling proteins to plasma membrane structural specializations, including epithelial cell tight junctions, synaptic junctions, and neuromuscular junctions (Kim, 1995; Dimitratos et al., 1999). In fibroblasts, ZO-1 localizes to cell-cell contacts (Itoh et al., 1993). In epithelial cells ZO-1 has a role in efficient tight junction assembly (Umeda et al., 2004, 2006; McNeil et al., 2006). In endothelial cells ZO-1/alpha-catenin complexes regulate barrier integrity while ZO-1/cortactin complexes regulate chemotaxis (Lee et al., 2006). ZO-1 undergoes tyrosine phophorylation in MDCK cells following v-Src activation, correlating with weakened cell-cell adhesion (Takeda et al., 1995). ZO-1 also becomes tyrosine phosphorylated in EGF stimulated A431 cells correlating with junctional localization (Van Itallie et al., 1995) and in stressed Caco-2 cells correlating with decreased transepithelial resistance (Rao et al., 2002). Our study identified 5 sites of ZO-1 tyrosine phosphorylation (and 6 sites on the related protein ZO-2), most of which were found only in the Src-transformed cells. PhosphoSite lists 12 pTyr sites for ZO-1 (including Tyr-1164) and 12 sites for ZO-2. The functional significance of any of these phosphorylation events is unknown.

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Willott E, Balda MS, Fanning AS, Jameson B, Van Itallie C, Anderson JM (1993) *Proc Natl Acad Sci USA* 90:7834-7838.

22. Cofilin-1 (P18760)

pTyr-139 (K.HELQANC*Y@EEVKDR.C)

Cofilin-1 is a member of the ADF/cofilin family of small (15-18 kDa) actin-binding proteins that act to increase the rate of actin filament turnover (by both severing and depolymerizing F-actin) to help drive cell motility (Bamburg, 1999; Carlier et al., 1999; Ghosh et al. 2004; Hotulainen et al., 2005). In mammals there are three family members: ADF expressed mainly in epithelial and neuronal cells, cofilin-1 expressed in most cell types, and cofilin-2 expressed specifically in muscle. ADF/cofilin function is known to be regulated by phosphorylation (reviewed in Huang et al., 2006). LIM-kinase, a downstream effector of Rho, phosphorylates cofilin at a conserved residue (Ser-3), which inhibits binding to F-actin leading to F-actin stabilization. The Rho > ROCK > LIMK > cofilin pathway thus contributes to Rho-mediated stress-fiber stabilization (Yang et al., 1998; Maekawa et al., 1999). In Src-transformed cells, cofilin serine phosphorylation is reduced (cofilin activated) (Pawlak and Helfman, 2002), consistent with the ability of oncogenic Src to disrupt the Rho > ROCK > LIMK pathway. Cofilin has also been recognized as a direct v-Src substrate in a chemical genetic screen (Shah and Shokat; 2002), suggesting another possible mechanism by which Src could regulate coflin. Tyr-139 lies near the "ADF homology" domain, and the significance its phosphorylation is unknown. The site has been found in other phosphoproteomics studies (Phosphosite).

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23. Cortactin (Q60598)

pTyr-466 (Y.TSEPVY@ETTEAPGHY.Q)

Cortactin was first characterized as a Src substrate associated with the cortical actin cytoskeleton (Wu et al., 1991, Wu and Parsons, 1993). Cortactin interacts directly with F-actin and the Arp2/3 complex (Weed et al., 2000) and acts to stabilize Arp2/3-mediated branched actin filament formation (Weaver et al., 2001; Weed and Parsons, 2001). Tyr-466 lies in the central proline-rich region and is one of three major sites of phosphorylation reported for Src, along with Tyr-421 and Tyr-482 (Huang et al., 1998). Studies with phosphospecific antibodies indicate that cortactin tyrosine phosphorylation requires Rac1 activity and association with the cortical actin cytoskeleton (Head et al., 2003). Src-mediated cortactin phosphorylation appears to negatively

affect its ability to enhance actin polymerization (Huang et al., 1997; Martinez-Quiles et al., 2004). Consistent with this notion, tyrosine phosphorylation of cortactin has been implicated in regulating turnover of podosomes in osteoclasts (Luxenburg et al., 2006) and smooth muscle cells (Zhou et al., 2006). Cortactin tyrosine phosphorylation appears to occur in a progressive manner, with Tyr-421 phosphorylation required for phosphorylation of Tyr-466 (Head et al., 2003). In our study peptides containing Tyr-421 were frequently detected (in Src-transformed cells only), but Tyr-421 was not the site of phosphorylation assigned by Sequest. Our study also identified cortactin pTyr-442 in the Src-transformed cells, while pTyr-482 was not detected.

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24. Septin-2 (P42208)

pTyr-17 (K.QQPTQFINPETPGY@VGFANLPNQVHR.K)

Septins are a conserved family of GTPases implicated in diverse cellular processes including cytokinesis, vesicle trafficking and exocytosis (Kinoshita, 2003; Spiliotis and Nelson; 2006). Septin-2 (originally called Nedd5) is a 45 kDa ubiquitously expressed protein belonging to the acidic septin subgroup consisting of a short N-terminus, a conserved GTPase domain and a C-terminal coiled-coil structure (Kinoshita et al., 1997; Kinoshita, 2003). Septin-2 is involved in cytokinesis, and in interphase cells septin-2 fibers are seen in association with actin bundles and focal adhesions (Kinoshita et al., 1997). During cytokinesis, septin-2 localizes to the contractile ring and midbody and may have a role in formation of the midbody diffusion barrier (Schmidt and Nichols, 2004). In nonmotile interphase cells, septin-2 plays a role in stabilizing actin bundles (Kinoshita et al., 2002; Schmidt and Nichols, 2004), while in migrating and ruffling cells septin-2 assembles into dynamic actin-independent ring structures (Schmidt and Nichols, 2004). Septin-2 Tyr-17 phosphorylation was found in a previous phosphotyrosine proteomics study (Rush et al., 2005), and the functional significance of this modification is unknown.

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Spiliotis A, Nelson WJ (2006) *J Cell Sci* 119:4-10.

25. VASP (P70460)

pTyr-39 (R.VQIY@HNPTANSFR.V)

VASP ("vasodilator-stimulated phosphoprotein"), first characterized as a substrate of cGMP- and cAMP-dependent kinases in platelets (Waldmann et al., 1987), is a founding member of the Ena/VASP family of proteins, along with *Drosophila* Enabled (Ena) discovered in a genetic

screen for dominant suppressors of Abl (Gertler et al., 1990). Mena and EVL are other vertebrate family members. Ena/VASP proteins share an N-terminal EVH1 domain, a C-terminal EVH2 domain, and central polyproline-rich core. VASP localizes prominently to lamellipodial leading edges and filopodial tips where it functions in actin-based motility (Krause et al., 2003). The VASP EVH2 domain binds to F-actin free barbed ends, antagonizing the capping proteins that inhibit filament elongation, and thereby promoting lamellipodial extension (Bear et al., 2002). Other studies indicate VASP aids in the supply of actin monomers for filament nucleation by recruiting profilin (Krause et al., 2003). VASP Tyr-39 is a conserved residue in the EVH1 domain (Prehoda et al., 1999). EVH1 functions (similarly to WW and SH3 domains) in binding type II poly-proline helical sites (Prehoda et al., 1999). The EVH1 domain is involved in targeting Ena/VASP proteins to focal adhesions (Gertler et al., 1996) and filopodia/lammelipodia (Krause et al., 2004), and interacts with vinculin (Brindel et al., 1996), zyxin (Drees et al., 2000), the zyxin-related LPP (Petit et al., 2000), and lammellipodin (Krause et al., 2004). Tyr-39 phosphorylation may regulate targeting involving these interactions. Abl and Src can directly interact with the VASP proline-rich region (Cormer et al., 1998), and interaction with Abl was shown to occur in a manner dependent on cell-ECM adhesion (Howe et al., 2002). While there is no published evidence that either Abl or Src phosphorylates VASP, Drosophila Abl was shown to phosphorylate Ena on six sites clustered in the proline-rich region (Cormer et al., 1998) none of which are conserved in vertebrate Mena or VASP.

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26. Enolase (P17182, alpha form)

pTyr-43 (R.AAVPSGASTGIY@EALELR.D)

Enolase catalyzes the first common step of glycolysis and gluconeogenesis, the interconversion of 2-phospho-D-glycerate and phosphoenolpyruvate. Mammalian enolase is composed of 3 isozyme subunits, alpha, beta and gamma, which can form homodimers or heterodimers. Enolase was one of the first identified v-Src substrates (Cooper et al., 1983) and Tyr-43 was identified as the principle site of phosphorylation by Src (Cooper et al., 1984). Tyr-43 lies within a loop that undergoes movement after 2-phosphoglycerate binding to help create the active site (Pancholi, 2001). Thus Tyr-43 phosphorylation could influence the catalytic activity of the enzyme. However due to low phosphorylation stoichiometry and the fact that enolase is not a rate-limiting step in glycolysis, it is unlikely that enolase phosphorylation by Src contributes to the increased glycolytic flux observed in transformed cells (Hunter and Cooper, 1986). Enolase has roles in addition to metabolism, including hypoxia tolerance (Graaven et al.,

1993) and autoimmunity and microbial diseases (Pancholi, 2001). Enolase overexpression is a diagnostic marker for many tumors (Eriksson et al., 2000), and could contribute to increased invasiveness through its ability to serve as a receptor and activator of plasminogen (Miles at al., 1991). Recently, enolase was reported to be phosphorylated by Src during capacitation of spermatozoa (Baker et al., 2006).

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Pancholi V (2001) *Cell Mol Life Sci* 58:902-920.

27. GAPDH (P16858)

pTyr-315 (K.LISWYDNEY@GYSNR.V)

GAPDH (glyceraldehyde-3-phosphate dehydrogenase) catalyses the first step of the second part of glycolysis: NAD+ dependent reversible oxidative phosphorylation of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate. In addition to its established role in metabolism, GAPDH has been implicated in non-glycolytic activities, including transcriptional regulation (Zheng et al., 2003), DNA repair (Meyer-Siegler et al., 1991), and telomeric DNA binding (Sundararaj et al., 2004). Specific association of GAPDH with viral RNA may be involved in the regulation of viral gene expression and/or translation (De et al., 1996). GAPDH has also been implicated in membrane fusion, tubulin bundling, nuclear RNA export and apoptosis (reviewed in Kim and Dang, 2005). GAPDH was identified as a major component of actin-rich tumor cell pseudopodia (Nguyen et al., 2000), raising the possibility that localized glycolysis regulates plasma membrane protrusion. GAPDH has been previously described as a substrate for receptor tyrosine kinases EGFR (Reiss et al., 1986) and Tkl (Gartner et al., 1992), and the pTyr-315 site has been identified in several other phosphotyrosine proteomics studies (PhosphoSite). Tyr-315 is located in the alpha 4 helix close to the C-terminus in the NAD+ binding region (Eyschen et al., 1999). It is likely the helix must unfold to allow Tyr-315 phosphorylation. In human GAPDH, Tyr-315 is substituted by phenylalanine. However two other conserved tyrosine residues in this region (corresponding to mouse Tyr-311 and Tyr-317), are reported as unpublished sites of human GAPDH phosphorylation in PhosphoSite. The significance of any of these phosphorylation events is unknown.

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Zheng L, Roeder RG, Luo Y (2003) *Cell* 114:255-266.

28. eEF1A (P10126, isoform 1)

pTyr-29 (K.STTTGHLIY@K.C)

pTyr-141 (R.EHALLAY@TLGVK.Q)

Eukaryotic elongation factor 1 alpha (eEF1A) catalyzes the GTP-dependent binding of aminoacyl-tRNAs to their acceptor sites on the ribosome and accounts for 1-2% of total protein in normal growing cells (second in abundance only to actin). eEF1A has functions in addition to its core role in translation (Ejiri, 2002; Lamberti et al., 2004). Notably, eEF1A associates with cytoskeletal components including microfilaments and microtubules (Condeelis, 1995). By binding simultaneously to F-actin and beta-actin mRNA, EF1A acts as a scaffold to direct beta-actin to the protrusive leading edges of cells and thereby contribute to polarity and directional movement (Liu et al., 2002; Condeelis and Singer, 2005). eEF1A2 is frequently overexpressed in some cancers and has the capacity to promote anchorage-independent growth and tumorigenesis (Anand et al., 2002; Tomlinson et al., 2005; Kulkarni et al., 2006). The oncogenic properties of eEF1A2 may relate to its ability to promote the PI3K > Akt pathway resulting in filopodia formation, cell migration, and invasion (Amiri et al., 2006). eEF1A2 was recognized as a Src substrate through an approach involving chemical rescue of a Src mutational variant (Qiao et al., 2006). In our study, eEF1A tyrosines 29 and 141 (peptides identical for both isoforms) were among the sites frequently identified in both nontransformed and Src-transformed cells. No published studies have addressed the functional significance of eEF1A tyrosine phosphorylation.

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29. Cbl (P22682)

pTyr-672 (K.PSSSANAIY@SLAARPLPMPK.L)

First characterized as an oncogene product (Langdon et al., 1989), Cbl is a 120 kDa E3 ubiquitin ligase that acts as a negative regulator of tyrosine kinases (Thien and Langdon, 2001; Peschard and Park, 2003). Through its N-terminal PTB domain that binds phosphotyrosine residues and central RING domain that binds E2 ubiquitin-conjugating enzymes, Cbl acts as an adaptor to promote receptor tyrosine kinase ubiquitination and degradation. Oncogenic forms of Cbl behave as dominant-negative mutants to impair this activity. The Cbl PTB domain interacts with Src pTyr-418 to inhibit kinase activity (Sanjay et al., 2001), while in another model Src-mediated Cbl tyrosine phosphorylation leads to the displacement of E2 and ubiquitination of both Src and Cbl to promote destriction of both proteins (Yokouchi et al., 2001). By promoting Cbl destruction, Src may enable EGFR to evade downregulation (Bao et al., 2003). Cbl is tyrosine phosphorylated in Src-transformed cells (Tanaka et al., 1995; Odai et al., 1995) and in response to integrin-mediated adhesion (Ojaniemi et al., 1997). In T cells, the major sites of human Cbl

tyrosine phosphorylation were determined to be Tyr-700, -731, and -774, and Src-family kinases are capable of phosphorylating these residues (Feshchenko et al., 1998). The ability of Cbl to stably interact with Src is reduced in a 3YF mutant with phenylalanine substitutions for the Tyr-700, -731, and -774 sites (Bao et al., 2003). These three sites have been further characterized as binding sites for SH2-containing signaling proteins including Crk-family adaptors (Andoniou et al., 1996), PI3K-p85 (Ueno et al., 1998), and Vav (Marengere et al., 1997), which could contribute to a role for Cbl in cell migration (Meng and Lowell, 1998; Uemura and Griffin, 1999). In our study mouse equivalents of these three sites were not detected, while pTyr-672 (equivalent to human Cbl Tyr-674) was frequently detected in both nontransformed and Src-transformed cells. The Tyr-672 site has been detected in other proteomics studies (PhosphoSite), but no published studies have addressed the functional significance of this site.

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30. Intersectin-2 (Q9Z0R6)

pTyr-921 (R.GEPEALY@AAVTK.K)

Intersectin-2 and the related protein intersectin-1 are Dbl-family GEFs for Rho GTPases that function in clathrin-mediated endocytosis (Pucharcos et al., 2000). These proteins are characterized by two N-terminal Eps15 homology (EH) domains, a coiled-coil domain, five SH3 domains, a Dbl-homology (DH) domain, a pleckstrin homology (PH) domain, and a C2 domain. The tandem DH-PH cassette is the hallmark of the Dbl family. The DH domain stimulates GDP/GTP exchange while the PH domain promotes interaction with the plasma membrane. Alternative splicing generates short forms of both intersectins that lack the DH, PH and C2 domains. The long form of intersectin-1 was shown to be a Cdc42-specific GEF that regulates actin assembly via N-WASP (Hussain et al., 2001). Similary, there is evidence that intersection-2 functions cooperatively with N-WASP and Cdc42 to promote clathrin-mediated endocytosis of the T-cell receptor complex (McGavin et al., 2001). A recent study demonstrated a role for intersectin-1 in EGFR internalization, but also in the ubiquitination and degradation of the receptor that was linked to the interaction of intersectin-1 SH3 domains with the E3 ubiquitin ligase Cbl (Martin et al., 2006). Intersectin-2 Tyr-921 lies between the 2nd and 3rd SH3 domains. The site has been reported in several other proteomics studies (PhosphoSite). Five other pTyr sites on intersectin-2 are also reported in PhosphoSite, while there are no reported

sites of intersectin-1 tyrosine phosphorylation. There are no published studies on the significance of tyrosine phosphorylation of intersectin-2.

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Pucharcos C, Estivill X, de la Luna S (2000) *FEBS Lett* 478:43-51.

31. CDV3 (Q920I4, isoform A)

pTyr-213 (R.KTPQGPPEIY@SDTQFPSLQSTAK.H)

CDV3 ("*c*arnitine *d*eficiency-associated gene expressed in *v*entricle") was first described as the product of a gene upregulated in hypertrophied ventricles of "juvenile visceral steatosis" (JVS) mice (Fukumaru et al., 1999). JVS mice are deficient in carnitine, a key molecule for β-oxidation of long chain fatty acids in mitochondria. Two splice isoforms encode proteins (CDV3A and CDV3B) with predicted masses of 24.4 and 29.7 kDa, respectively. CDV3B has a C-terminal extension, relative to CDV3A. The CDV3 proteins were independently identified as novel Abl tyrosine kinase substrates in a B cell progenitor line, and given the alternative names TPP36 ("tyrosine-phosphorylated protein of apparent molecular weight 36 kDa") and TPP32 (Tsuchiya et al., 2003). In a coexpression assay, several nonreceptor tyrosine kinases were shown to promote tyrosine phosphorylation of CDV3/TPP proteins, including Src and Bcr-Abl (Tsuchiya et al., 2003). Tyr-120 was described as a major site of TPP36 phosphorylation by Bcr-Abl (Tsuchiya et al., 2003). The CDV3/TPP proteins are predominantly cytoplasmic (Tsuchiya et al., 2003) although they contain predicted nuclear translocation signals. Otherwise, the biological functions of CDV3 proteins, or their tyrosine phosphorylation, are unknown. CDV3A/B Tyr-213 was also reported in the phosphotyrosine proteomics study of Rush et al. (2005).

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32. PREDICTED: Similar to Oligophrenin 1 (UPI00005125B7)

pTyr-371 (K.LWLEAMDGKEPIY@TLPAIISK.K)

This uncharacterized protein, predicted from genome sequence data, is related to oligophrenin-1 (OPHN1, mouse entry UniRef100_Q99J31) but is clearly a distinct protein. OPHN1 is a Rho-family GAP encoded by a gene mutated in a form of nonspecific X-linked mental retardation associated with dendritic abnormalities and impaired synaptic transmission and information processing (Billuart et al., 1998). In glial and neuronal cells, OPHN1 colocalizes with actin, notably at the tip of growing neurites (Fauchereau et al., 2003). Overexpression studies suggest that the OPHN1 RhoGAP domain regulates actin cytoskeletal organization by inactivating Rho and Cdc42 (Fauchereau et al., 2003). Knocking down OPHN1 by siRNA significantly decreased dendritic spine length, related to its ability to repress RhoA and Rho-kinase activities (Govek et al., 2004). "Similar to Oligophrenin 1" pTyr-371 site lies in a small linker between PH and RhoGAP domains. The equivalent residue of human OPHN1 is reported in PhosphoSite as an unpublished site of tyrosine phosphorylation. The FAK-interacting protein "Graf " shares the

central PH-RhoGAP organization while also posessing a C-terminal SH3 domain. A C-terminal SH3 domain is also present in "Similar to Oligophrenin 1" but not in OPHN1. "PSGAP" (Ren et al., 2001) is fourth mammalian member of this RhoGAP family.

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