

Supplemental Figure 1. Amino acid sequences of constructs.

Supplemental Figure 2. Activation loop phosphorylation of c-Src and mutants.

Experiments were carried out as described in the legend to Fig. 2A. Results shown in Fig. 2A are at the top, and results from two additional independent experiments are shown.

Supplemental Figure 3. Cas phosphorylation by c-Src and mutants. Experiments were carried out as described in the legend to Fig. 5A. Results shown in Fig. 5A are at the top, and results from two additional independent experiments are shown.

Supplemental Figure 4. Sam68 phosphorylation by c-Src and mutants. Experiments were carried out as described in the legend to Fig. 7. Results shown in Fig. 7 are at the top, and results from two additional independent experiments are shown.

Supplemental Figure 1
Amino acid sequences of wild-type and mutant forms of Src

WT

MDYKDHDGDYKDHDIDYKDDDDDKLAAAMGSNKS KPKDASQRRRSLEPSE
NVHGAGGAFPASQTPSKPASADGHRGPSAAFVPPAAEPKLFGGFNSSDTV
TSPQRAGALAGNSGGVTTFFVALYDYESRTETDLSFKKGERLQIVNNT
EGDWLAHSLSTGQTGYIPSNYVAPSDSGQDLWYFGKITRRESERLLLNAEN
PRGTFLVRESETTKGAYCLSVSDFDNAKGLNVKHYKIRKLDSGGFYITSRT
QFNSLQQLVAYYSKHADGLCHRLTTVCGISPTSKPQTQGLAKDAWEIPRE
SLRLEVKLGQGC FGEVWMGTWNGTTRVAIKTLKPGTMSPEAFLQEAQVMK
KLRHEKLVQLYAVVSEEPYIVTEYMNKGSLLDFLKGETGKYLRLPQLVD
MSAQIASGMAYVERMNYVHRDLRAANILVGENLVCKVADFGLARLIEDNE
YTARQGAKFPIKWTAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYPG
MVNREVLDQVERGYRMP CPPECPESLHDLMCQCWRKEPEERPTFEYLQAF
LEDYFTSTEPQYQPGENL

U23C

MDYKDHDGDYKDHDIDYKDDDDDKLAAAMGSNKS KPKDASQRRRSLEPSE
NVHGAGGAFPASQTPSKPASADGHRGPSAAFVPPAAEPKLFGGFNSSDTV
TSPQRAGALAGNSWYFGKITRRESERLLLNAENPRGTFLVRESETTKGAY
CLSVSDFDNAKGLNVKHYKIRKLDSGGFYITSRTQFNSLQQLVAYYSKHA
DGLCHRLTTVCGQDLGGVTTFFVALYDYESRTETDLSFKKGERLQIVNTE
GDWWLAHSLSTGQTGYIPSNYVAPSDSGISPTSKPQTQGLAKDAWEIPRE
SLRLEVKLGQGC FGEVWMGTWNGTTRVAIKTLKPGTMSPEAFLQEAQVMK
KLRHEKLVQLYAVVSEEPYIVTEYMNKGSLLDFLKGETGKYLRLPQLVD
MSAQIASGMAYVERMNYVHRDLRAANILVGENLVCKVADFGLARLIEDNE
YTARQGAKFPIKWTAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYPG
MVNREVLDQVERGYRMP CPPECPESLHDLMCQCWRKEPEERPTFEYLQAF
LEDYFTSTEPQYQPGENL

UC32

MDYKDHDGDYKDHDIDYKDDDDDKLAAAMGSNKS KPKDASQRRRSLEPSE
NVHGAGGAFPASQTPSKPASADGHRGPSAAFVPPAAEPKLFGGFNSSDTV
TSPQRAGALAGNSPTSKPQTQGLAKDAWEIPRESLRLEVKLGQGC FGEVW
MGTWNGTTRVAIKTLKPGTMSPEAFLQEAQVMK KLRHEKLVQLYAVVSEE
PIYIVTEYMNKGSLLDFLKGETGKYLRLPQLV DMSAQIASGMAYVERMNY
VHRDLRAANILVGENLVCKVADFGLARLIEDNEY TARQGAKFPIKWTAPE
AALYGRFTIKSDVWSFGILLTELTTKGRVPYPGMVNREVLDQVERGYRMP
CPPECPESLHDLMCQCWRKEPEERPTFEYLQAF LEDYFRSGGVTTFFVALY
DYESRTETDLSFKKGERLQIVNNT
EGDWLAHSLSTGQTGYIPSNYVAPSDSIQAEWYFGKITRRESERLLLNAENPRGTFLVRESETTKGAYCLSVSDFDNAKGLNVKHYKIRKLDSGGFYITSRTQFNSLQQLVAYYSKHADGLCHRLTTVCSR PQYQPGENL

3U2C

MDYKDHDGDYKDHDIDYKDDDDDKLAAAGGVTTFFVALYDYESRTETDLSF
KKGERLQIVNNTEGDWWLAHSLSTGQTGYIPSNYVAPSDSGGNSMGSNKS
KPKDASQRRRSLEPSENVHGAGGAFPASQTPSKPASADGHRGPSAAFVPP
AAEPKLFGGFNSSDVTSPQRAGALAQDLWYFGKITRRESERLLLNAENP
RGTFLVRESETTKGAYCLSVSDFDNAKGLNVKHYKIRKLDSGGFYITSRT
QFNSLQQLVAYYSKHADGLCHRLTTVCGISPTSKPQTQGLAKDAWEIPRE
SLRLEVKLGQGCFCGEVWMGTWNGTTRVAIKTLKPGTMSPEAFLQEAQVMK
KLRHEKLVQLYAVVSEPIYIVTEYMNKGSLLDFLKGETGKYLRPLQLVD
MSAQIASGMAYVERMNYVHRDLRAANILVGENLVCKVADFGLARLIEDNE
YTARQGAKFPIKWTAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYPG
MVNREVLQVERGYRMPCPPECPELHDLMCQCWRKEPEERPTFEYLQAF
LEDYFTSTEPQYQGENL

32UC

MDYKDHDGDYKDHDIDYKDDDDDKLAAAGGVTTFFVALYDYESRTETDLSF
KKGERLQIVNNTEGDWWLAHSLSTGQTGYIPSNYVAPSDSGGNSWYFGKI
TRRESERLLLNAENPRGTFLVRESETTKGAYCLSVSDFDNAKGLNVKHYK
IRKLDSGGFYITSRTQFNSLQQLVAYYSKHADGLCHRLTTVCGQDLMGSN
KSKPKDASQRRRSLEPSENVHGAGGAFPASQTPSKPASADGHRGPSAAFV
PPAAEPKLFGGFNSSDVTSPQRAGALAGISPTSKPQTQGLAKDAWEIPR
ESLRLEVKLGQGCFCGEVWMGTWNGTTRVAIKTLKPGTMSPEAFLQEAQVM
KKLRHEKLVQLYAVVSEPIYIVTEYMNKGSLLDFLKGETGKYLRPLQLV
DMSAQIASGMAYVERMNYVHRDLRAANILVGENLVCKVADFGLARLIEDN
EYTARQGAKFPIKWTAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYP
GMVNREVLQVERGYRMPCPPECPELHDLMCQCWRKEPEERPTFEYLQA
FLEDYFTSTEPQYQGENL*GS

32CU

MDYKDHDGDYKDHDIDYKDDDDDKLAAAGGVTTFFVALYDYESRTETDLSF
KKGERLQIVNNTEGDWWLAHSLSTGQTGYIPSNYVAPSDSGGNSWYFGKI
TRRESERLLLNAENPRGTFLVRESETTKGAYCLSVSDFDNAKGLNVKHYK
IRKLDSGGFYITSRTQFNSLQQLVAYYSKHADGLCHRLTTVCGQDLPTSK
PQTQGLAKDAWEIPRESLRLEVKLGQGCFCGEVWMGTWNGTTRVAIKTLK
GTMSPEAFLQEAQVMKLRHEKLVQLYAVVSEPIYIVTEYMNKGSLLDF
LKGETGKYLRPLQLVDMSAQIASGMAYVERMNYVHRDLRAANILVGENLV
CKVADFGLARLIEDNEYTARQGAKFPIKWTAPEAALYGRFTIKSDVWSFG
ILLTELTTKGRVPYPMVNREVLQVERGYRMPCPPECPELHDLMCQCW
RKEPEERPTFEYLQAFLEDYFTSTEPQYQGENLEGISMGSNKS
KPKDASQRRRSLEPSENVHGAGGAFPASQTPSKPASADGHRGPSAAFVPPAAEPK
LFGFNSSDVTSPQRAGALAGS

2UC3

MDYKDHDGDYKDHDIDYKDDDDDKLAAA WYFGKITRRESERLLLNAENPR
GTFLVRESETTKGAYCLSVSDFDNAKGLNVKHYKIRKLDSGGFYITSRTQ
FNSLQQLVAYYSKHADGLCHRLTTVCGNSMGSNKS KPKDASQRRRSLEPS
ENVHGAGGAFPASQTPSKPASADGHRGPSAAFVPPAAEPKLFGGFNSSDT
VTSPQRAGALAQDLPTSKPQTQGLAKDAWEIPRESLRLEVKLGQGCFGEV
WMGTWNGTTRVAIKTLKPGTMSPEAFLQEAQVMKKLRHEKLVQLYAVVSE
EPIYIVTEYMNKGSLLDFLKGETGKYLRPLQVDMSAQIASGMAYVERMN
YVHRDLRAANILVGENLVCKVADFGLARLIEDNEY TARQGAKFPIKWTAP
EAALYGRFTIKSDVWSFGILLTELTTKGRVPYPGMVNREVL DQVERGYRM
PCPECPESLHDLMCQCWRKEPEERPTFEYLQAFLEDYFTSTEPQYQPGE
NLEGISGGVTTFFVALYDYESRTETDLSFKKGERLQIVNNT EGDWWLAHSL
STGQTGYIPSNYVAPSDSGS

3UC2

MDYKDHDGDYKDHDIDYKDDDDDKLAAAGGVTTFFVALYDYESRTETDLSF
KKGERLQIVNNT EGDWWLAHSLSTGQTGYIPSNYVAPSDSGGNSMGSNKS
KPKDASQRRRSLEPSENVHGAGGAFPASQTPSKPASADGHRGPSAAFVPP
AAEPKLFGGFNSSDTVTSPQRAGALAQDLPTSKPQTQGLAKDAWEIPRES
LRLEVKLGQGCFGEVWMGTWNGTTRVAIKTLKPGTMSPEAFLQEAQVMKK
LRHEKLVQLYAVVSE EPIYIVTEYMNKGSLLDFLKGETGKYLRPLQVDM
SAQIASGMAYVERMNYVHRDLRAANILVGENLVCKVADFGLARLIEDNEY
TARQGAKFPIKWTAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYPGM
VNREVL DQVERGYRMPCPECPESLHDLMCQCWRKEPEERPTFEYLQAF
EDYFTSTEPQYQPGENLEGISWYFGKITRRESERLLLNAENPRGTFLVRE
SETTKGAYCLSVSDFDNAKGLNVKHYKIRKLDSGGFYITSRTQFNSLQQL
VAYYSKHADGLCHRLTTVCGS

RL/WA

MDYKDHDGDYKDHDIDYKDDDDDKLAAAMGSNKS KPKDASQRRRSLEPSE
NVHGAGGAFPASQTPSKPASADGHRGPSAAFVPPAAEPKLFGGFNSSDTV
TSPQRAGALAGNSGGVTTFFVALYDYESRTETDLSFKKGERLQIVNNT EGD
AWLAHSLSTGQTGYIPSNYVAPSDSGQDLWYFGKITRRESERLLLNAENP
RGTFLVLESETTKGAYCLSVSDFDNAKGLNVKHYKIRKLDSGGFYITSRT
QFNSLQQLVAYYSKHADGLCHRLTTVCGISPTSKPQTQGLAKDAWEIPRE
SLRLEVKLGQGCFGEVWMGTWNGTTRVAIKTLKPGTMSPEAFLQEAQVMK
KLRHEKLVQLYAVVSE EPIYIVTEYMNKGSLLDFLKGETGKYLRPLQVD
MSAQIASGMAYVERMNYVHRDLRAANILVGENLVCKVADFGLARLIEDNEY
YTARQGAKFPIKWTAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYPG
MVNREVL DQVERGYRMPCPECPESLHDLMCQCWRKEPEERPTFEYLQAF
LEDYFTSTEPQYQPGENL

Catalytic domain

MDYKDHDGDYKDHDIDYKDDDDKLAANSSIDLISPTSKPQTQGLAKDAW
EIPRESLRLEVKLGQGCFCGEVWMGTWNGTTRVAIKTLKPGTMSPEAFLQE
AQVMKKLRHEKLVQLYAVVSEEPYIVTEYMNKGSLDFLKGETGKYLRL
PQLVDMSAQIASGMAYVERMNYVHRDLRAANILVGENLVCKVADDFGLARL
IEDNEYTARQGAKFPIKWTAPEAALYGRFTIKSDVWSFGILLTELTTKGR
VPYPGMVNREVLDQVERGYRMPCPPECPESLHDLMCQCWRKEPEERPTFE
YLQAFLEDYFTSTEPQYQGENL





