Supporting Information

Supporting Materials and Methods

Pragmin Purification. *Escherichia coli* Rosetta2 (DE3) was transformed with pGEX6P2-Pragmin-WT-His or pGEX6P2-Pragmin-Y391F-His and was cultured with LB medium. Protein expression was induced by addition of 0.1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) and additional culture at 18°C for 16 h. GST-fused Pragmin-WT-His or GST-fused Pragmin-Y391F-His was purified using Ni Sepharose excel (GE Healthcare). Ni-binding buffer is (8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl, 400 mM NaCl, 0.1% TritonX-100, 0.3 mg/ml Benzamidine). Ni-wash buffer is (8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl, 400 mM NaCl, 1% TritonX-100, 30 mM Imidazole). Ni-elution buffer is (8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl, 400 mM NaCl, 500 mM Imidazole). For tyrosine-phosphorylated Pragmin Purification, *E. coli* BL21 (DE3) was co-transformed with pGEX6P2-Pragmin-WT-His or pGEX6P2-Pragmin-Y391F-His and pACYCDuet1-v-Src.[28] Subsequent procedure was same as non-phosphorylated Pragmin. However, Ni-binding buffer contained 0.2 mM Na_3VO_4.

Csk Purification. *E. coli* BL21 (DE3) was transformed with pGEX6P2-Csk-WT-His and was cultured with LB medium. Protein expression was induced by addition of 0.1 mM IPTG and additional culture at 25°C for 16 h. GST-fused Csk-His was purified using Glutathione Sepharose 4B (GE Healthcare). GST-binding buffer is (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 10 mM β-mercaptoethanol, 0.3 mg/ml Benzamidine). GST-wash buffer-W1 is (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% TrionX-100, 10 mM β-mercaptoethanol). GST-wash buffer-W2 is (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM

β-mercaptoethanol). Then GST tag was excised by treating the GST-Csk-His protein with PreScission Protease (GE Healthcare).

Src-tail Purification. *E. coli* BL21 (DE3) was transformed with pGEX6P2-Src-tail and cultured with LB medium. Protein expression was induced by addition of 0.4 mM IPTG for 7 h at 37°C. GST-fused Src-tail was purified using Glutathione Sepharose 4B (GE Healthcare). GST-binding buffer is (50 mM Tris-HCl, pH 7.3, 150 mM NaCl, 5 mM EDTA, 2 mM DTT). GST-elution buffer is (50 mM Tris-HCl, pH 7.3, 150 mM NaCl, 5 mM EDTA, 2 mM DTT, 20 mM Glutathione).

Table S1. Expression vectors used in this study.

Expression vectors
Mammalian expression vectors
pCMV-Myc-Pragmin-WT
pCMV-Myc-Pragmin-1-829 amino acid residues
pCMV-Myc-Pragmin-830-1368 amino acid residues
pCMV-Myc-Pragmin-Y391F
pCMV-Myc-Pragmin-K997R
pCMV-Myc-Pragmin-Y66/119/146/238/343/368/391/465/ 599F (9F)
pCMV-Myc-Pragmin-Y66/119/146F
pCMV-Myc-Pragmin-Y238/343/368F
pCMV-Myc-Pragmin-Y391/465/599F
pCMV-Myc-Pragmin-Y238F
pCMV-Myc-Pragmin-Y343F
pCMV-Myc-Pragmin-Y368F
pCMV-Myc-Pragmin-Y238/391F
pCMV-Myc-Pragmin-Y343/391F
pCMV-Myc-Pragmin-Y368/391F
pCMV-Myc-Pragmin-Y238/343/391F
pSP65SRα-Csk-WT-Flag
pSP65SRα-Csk-K222R-Flag
Bacterial Expression Vectors
pGEX6P2-Pragmin-WT-His
pGEX6P2-Pragmin-Y391F-His
pGEX6P2-Csk-WT-His
pGEX6P2- c-Src-524-536 amino acid residues
pACYCDuet1-v-Src

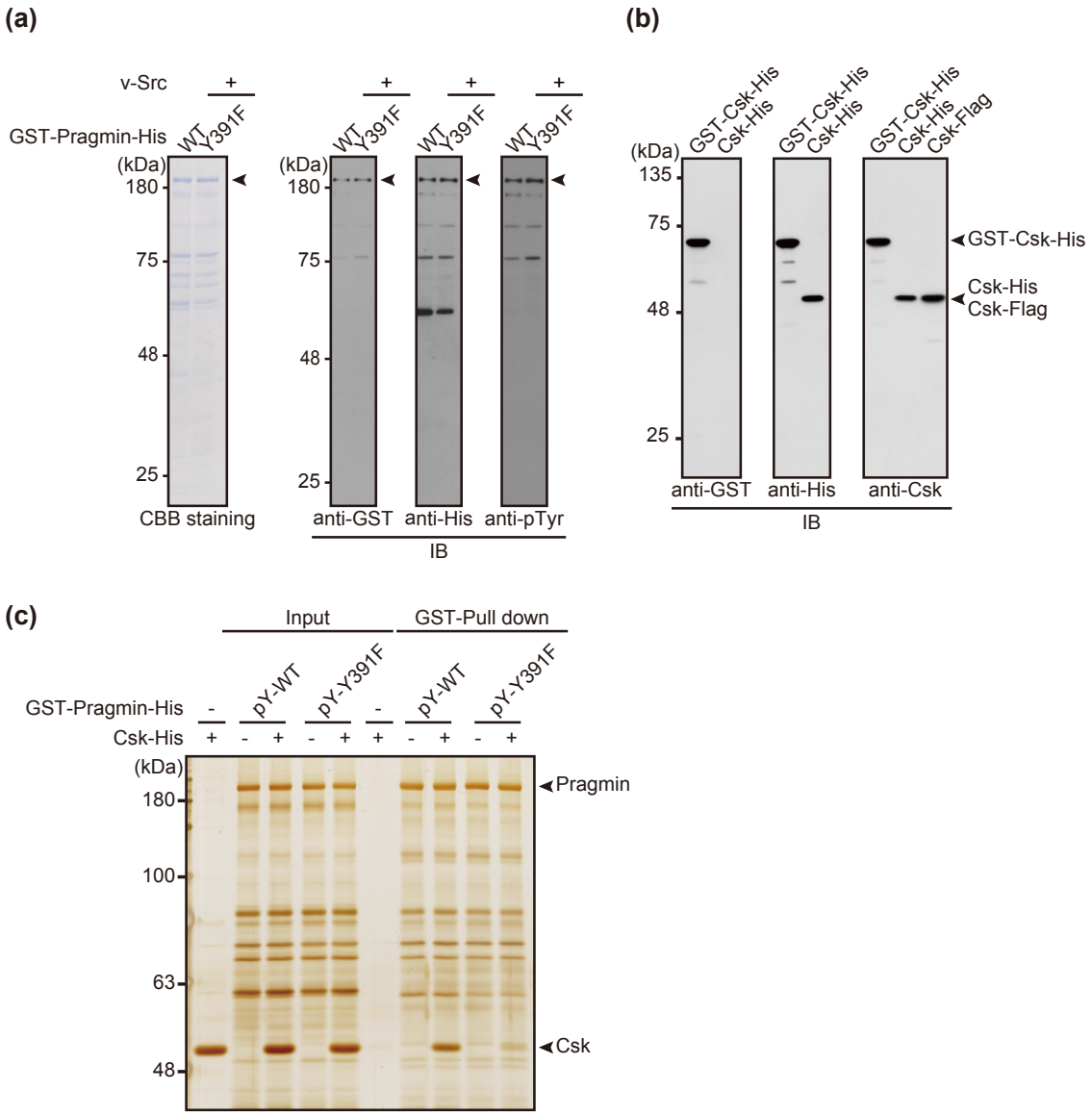

Fig. S1. ***In vitro*** **interaction of Pragmin with Csk.**

(a) CBB staining and IB analysis of recombinant tyrosine-phosphorylated GST-fused Pragmin proteins. Arrowheads indicate the positions of full-length GST-Pragmin-His. (b) IB analysis of recombinant Csk protein. Csk-Flag was expressed in AGS cells. (c) Silver staining analysis of GST pull-down assay using recombinant proteins. pY means tyrosine-phosphorylated.

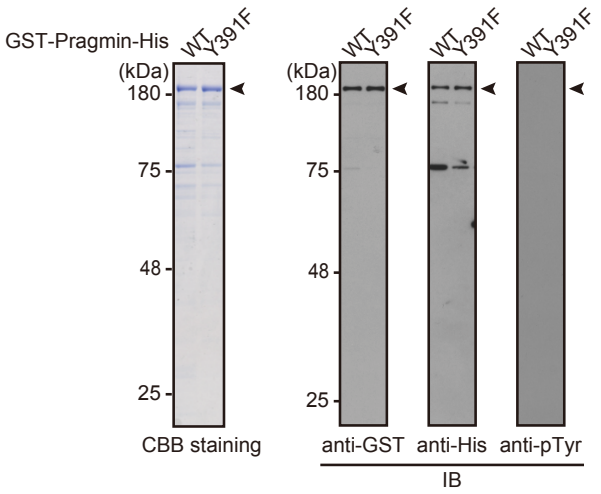

Fig. S2. **Purification of recombinant Pragmin.**

CBB staining and IB analysis of recombinant GST-fused Pragmin proteins. Arrowheads indicate the positions of full-length GST-Pragmin-His.

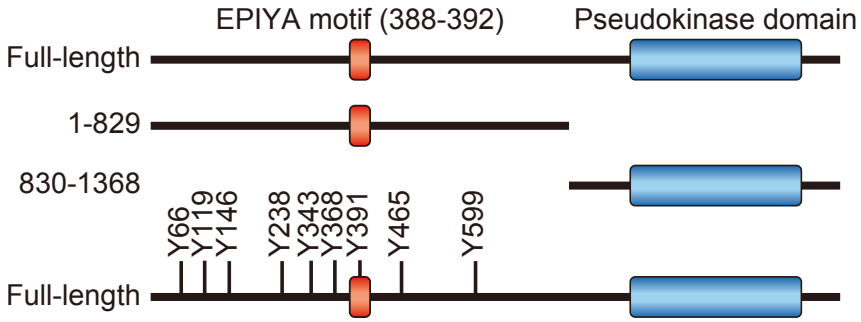

Fig. S3. **Schematic representation of full-length and truncated mutants of Pragmin.**