

SUPPLEMENTARY FIGURE LEGEND

Supplementary Figure S1. The Src family kinase member Yes interacts with cortactin in pancreatic acini. (a) The anti-pan-Src antibody immunoprecipitates Yes from resting pancreatic acini. Whole cell lysates or lysates from pancreatic acini subjected to immunoprecipitation using an anti-pan-Src antibody were analyzed by Western blot for the Src family members Yes, Fyn, c-Src, and Lyn. All four Src family members were detected in whole cell lysates; however, Yes appears to be the only Src family kinase member immunoprecipitated by the anti-pan-Src antibody from pancreatic acini. (b) Yes co-immunoprecipitates with cortactin from acinar cell lysates. Lysates from pancreatic acini were subjected to immunoprecipitation using an anti-cortactin antibody and then analyzed by Western blot for cortactin (top lane) and the indicated Src family members. (c) Yes is activated in a dose-dependent manner in response to CCK stimulation. Whole cell lysates from unstimulated acini (Con) or acini stimulated with the indicated concentrations of CCK (10 pM–10 nM) were analyzed by Western blot using an antibody that recognizes activated forms of multiple Src family kinase members (PY416) and subsequently re-probed using an antibody specifically against Yes. In addition, whole cell lysates from these same samples were subjected to immunoprecipitation using an anti-Yes antibody (bottom panel) and analyzed by Western blot for activated Src family kinases (PY416) and Yes. The ‘activated Src’ (*i.e.* PY416) profile observed in the samples immunoprecipitated for Yes was identical to that obtained upon analysis of whole cell lysates, supporting that Yes is the Src family kinase member activated in response to SS of pancreatic acini with CCK. (d-g’) Fluorescence micrographs of unstimulated control acini or acini stimulated for 30 min with physiologic (0.1 nM) or supra-physiologic (10 nM) concentrations of CCK alone or in the presence of PP2 (20 μ M) and stained for cortactin

and Yes. Cortactin and Yes co-localize at the apical membrane domain near the lumen under resting conditions or following stimulation with physiologic CCK concentrations (d-e', arrows). In contrast, SS disrupts this co-localization and induces a redistribution of both proteins to different cellular domains, with cortactin localizing to the necks of basal membrane blebs (f, arrow) and Yes remaining apical (f', arrow) while also becoming somewhat cytoplasmic. Importantly, pretreatment of acini with the Src family kinase inhibitor PP2 prevents this SS-induced dissociation, and cortactin and Yes remain co-localized at the apical membrane domain (g,g', arrows). These results are consistent with those observed using the anti-pan-Src antibody (Figure 2, main text). Scale bar, 10 μm (d-g').

