Supplementary Figure S2

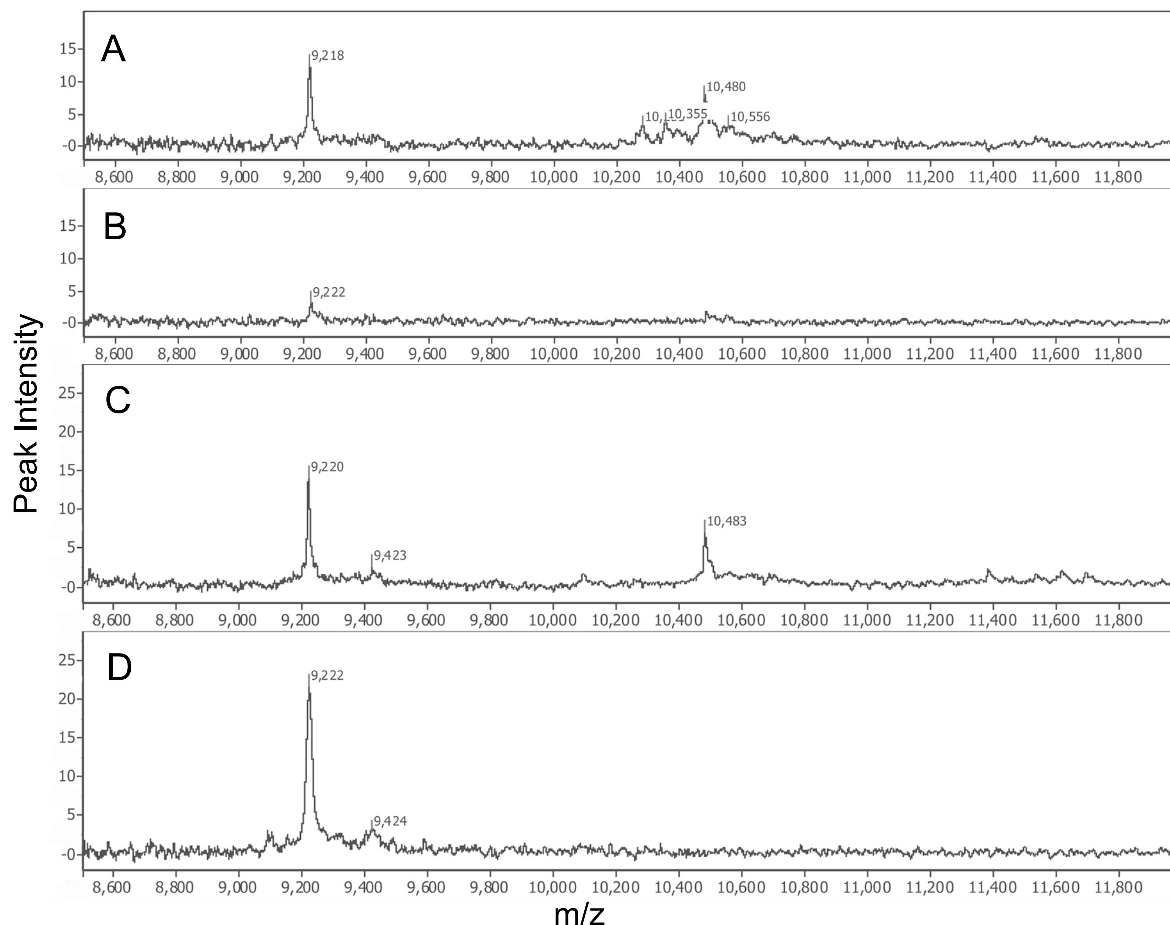

Figure S2. SELDI-TOF MS profiles on normal-phase (NP20) chips, showing protein peaks immunoprecipitated from MCF-7 breast cancer cell lysates using three different S100P antibodies. A: Rabbit monoclonal (Epitomics); B: Mouse polyclonal (Abnova); C: Rabbit polyclonal (Invitrogen). D: Immunoprecipitate from rabbit polyclonal antibody (Invitrogen) after further purification by reverse-phase HPLC.

Methods

Immunoprecipitation. MCF-7 cells were lysed in buffer containing 20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100. To isolate S100P, 25 µl (0.75 mg) of Protein G Dynabeads (Invitrogen) were incubated with S100P antibody (rabbit monoclonal #5263-1, 5.45 µg, Epitomics; MaxPab mouse polyclonal #H0006286-B01P, 5 µg, Abnova; or rabbit polyclonal #18-0046, 4.68 µg, Invitrogen) for 30 min at room temperature with rotation. Following two washes with PBS pH 7.4 containing 0.02% Tween 20, the bound antibody-bead complex was added to 50 µl of cell lysate, mixed by vortexing, and incubated for 2 h at room temperature with rotation. The beads were washed three times with PBS pH 7.4 and bound proteins eluted with 100 mM glycine, pH 2.8. Fractions were monitored by SELDI-TOF MS on normal-phase NP20 protein chips (Bio-Rad).

Reverse-phase HPLC. Immunprecipitated proteins were applied to a 4.6 x 250 mm C18 column (Jupiter, 5 µm, 300 Å) and eluted at 1.5 ml/min with a 30-min gradient from 15% to 60% acetonitrile in 0.1% trifluoroacetic acid.