

Supplementary Figure. PP2 totally blocked chronic morphine-induced Src kinase activation and Tyrphosphorylation. (A) HEKMT cells were treated with 1 μ M morphine for 2 hrs followed by treatment with or without 10 μ M naloxone for 15 min. Src kinase inhibitor PP2 (2 μ M) was added 1 hr prior to the assay. Then lipid raft preparation and immunoprecipitation analyses were performed as described in the Experimental Procedures. The IP antibody was anti-HA (1:200) and the IB antibodies were anti-phospho-Tyr antibody (Upstate, 1:500), anti-pSrc (Tyr-416, Upstate, 1:500), anti-cSrc (Santa Cruz, 1:500) or anti-Gß (Santa Cruz, 1:500). (B) *Quantitative analysis of panels (A)*. The density of pSrc versus the density of cSrc in control cells (without drug treatment) was considered to be 100%. All the experiments were repeated three times. ** denotes P<0.01 and *** denotes P<0.001 when compared with control using unpaired *t*-test.