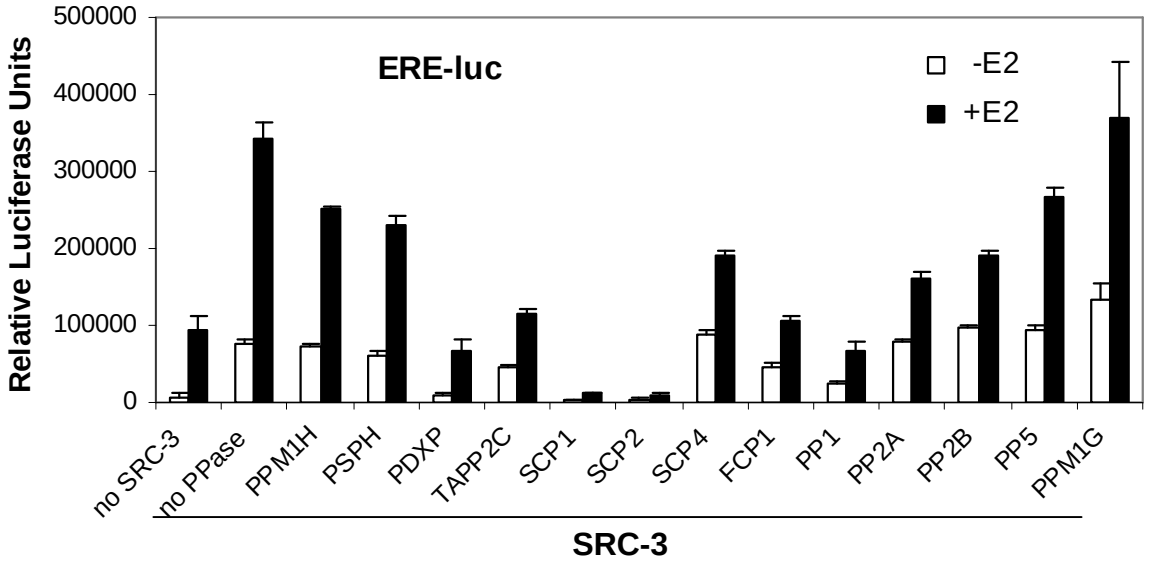
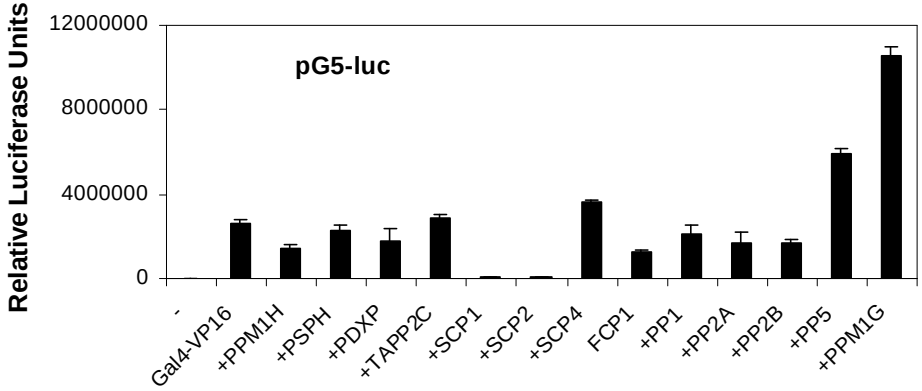
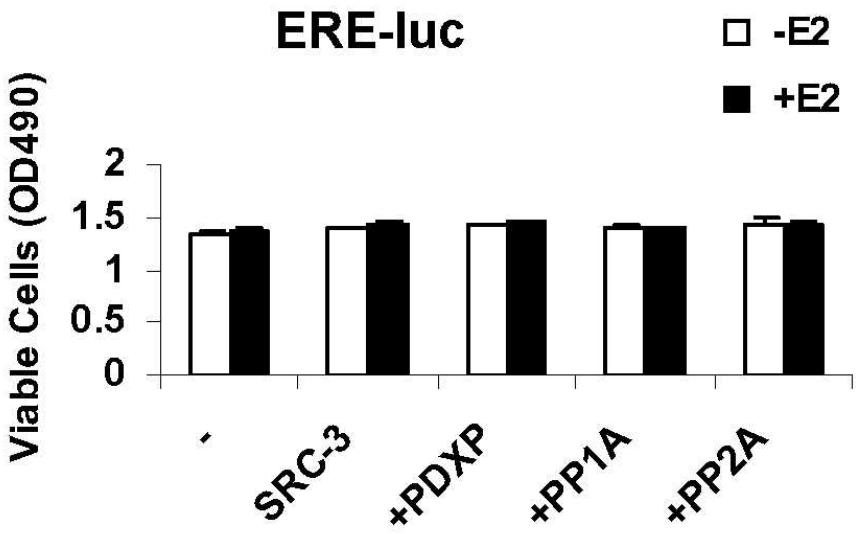


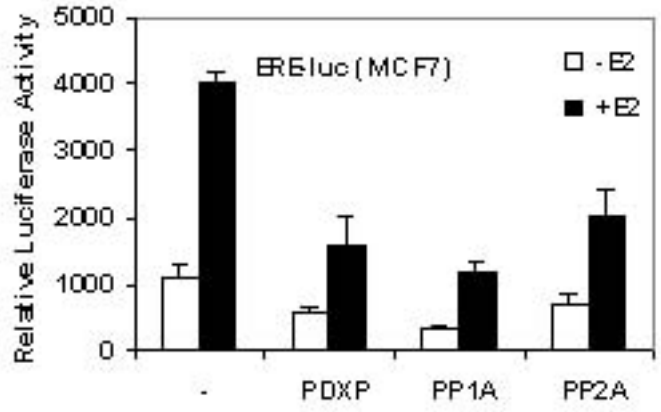
Suppl. Figure S1A. Most Ser/Thr Phosphatases Targeting SRC-3 Inhibit its Transcriptional Activities
Most identified phosphatases dephosphorylating SRC-3 inhibit the Estrogen Responsive Element (ERE) luciferase reporter gene. Transient transfections of HeLa cells were carried out with plasmids for ERE-luc and ER only (no SRC-3), or together with SRC-3 (no PPase), and an additional phosphatase as indicated. After treatment of cells with (+E2) or without estradiol (-E2) overnight, luciferase assays were performed. Error bars indicate S.E.M. with N=3.



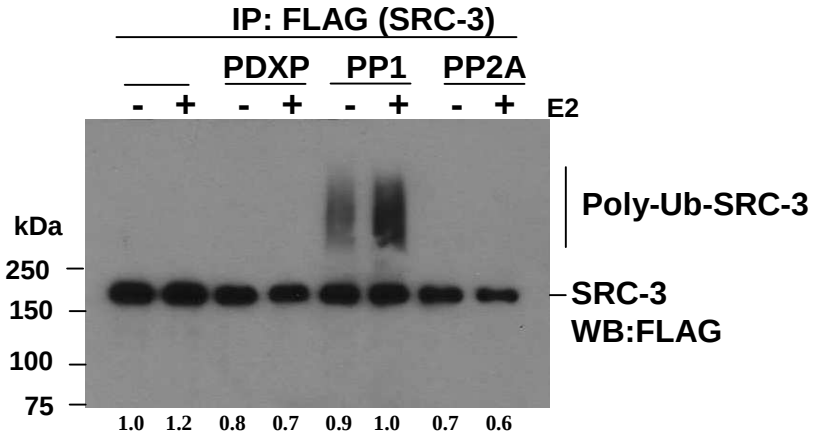
Suppl. Figure S1B. Effects of phosphatases on VP-16 activated transcription.
Assays were performed by transfecting HeLa cells with a luciferase reporter driven by a promoter containing Gal4 binding sites (pG5-luc) in the presence (Gal4-VP16) or absence (-) of Gal4-VP16, or Gal4-VP16 together with each phosphatase as indicated by (+). SCP1 and SCP2 affected transcription in an SRC-3-independent manner. Error bars indicate S.E.M. with N=3.



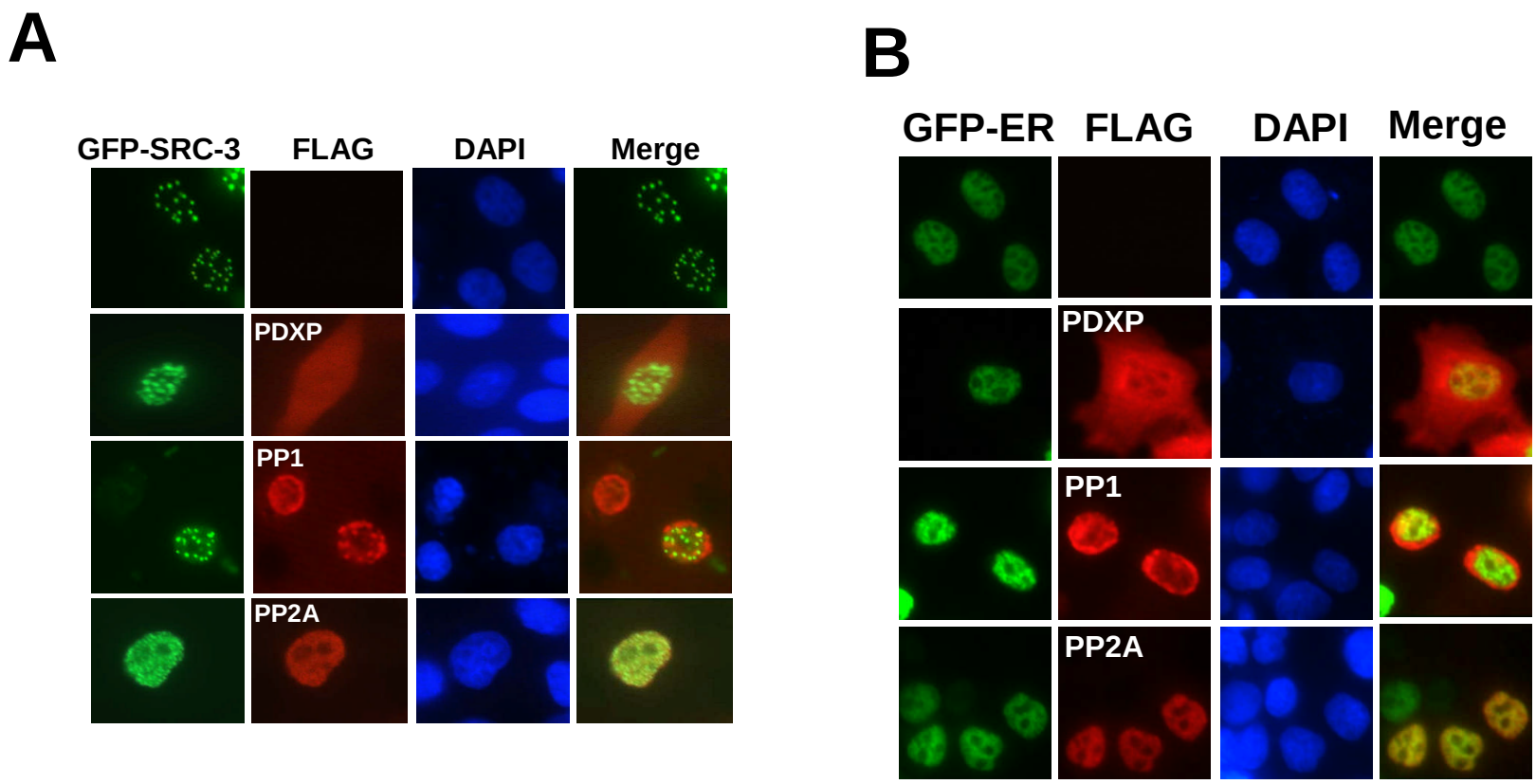
Suppl. Figure S2. Cell viability in ERE-luc assays remains unchanged.
The same experiment was performed as Fig 1A, but in the final assays the HeLa cell viability was determined by the method described in Experimental Procedures. Error bars indicate S.E.M. with N=3.



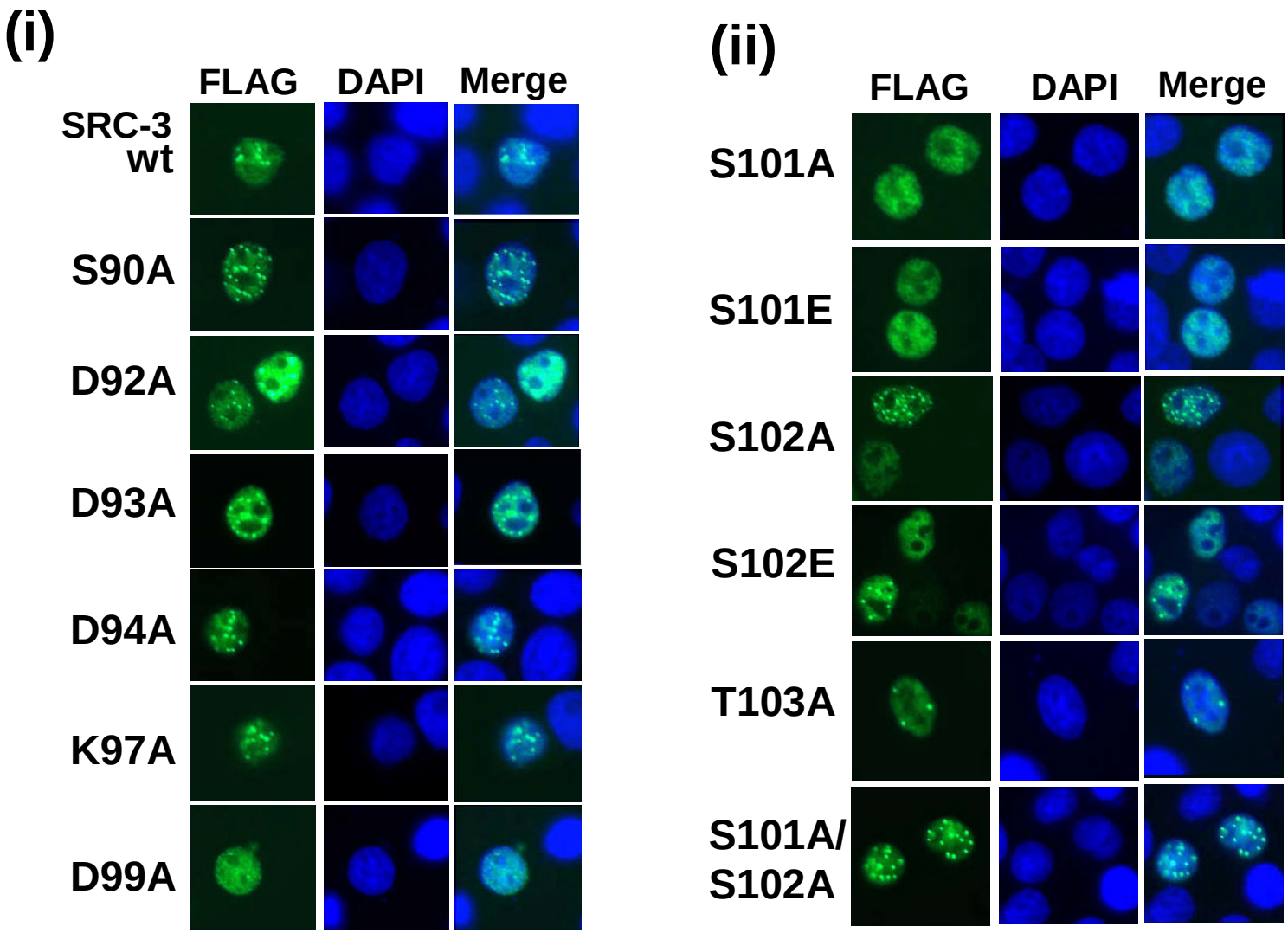
Suppl. Figure S3. Inhibitory function of the PPases in ERE-luc assays using MCF7 cells..
The experiment was performed as basically described in Fig 1A, but in MCF7 cells without transfection ER and SRC-3. Error bars indicate S.E.M. with N=3.



Suppl. Figure S4. PP1 overexpression leads to increased of poly-Ub-SRC-3 levels. The experiment was performed as described in (A). IP products were loaded in the same amounts into a gel before Western blotting using anti-FLAG antibodies to detect SRC-3.



Suppl. Figure S5.
PDXP, PP1, and PP2A do not change SRC-3 and ER subcellular localization in complete medium.
GFP-SRC-3 or GFP-ER along with each FLAG-tagged phosphatase as indicated were expressed into HeLa cells followed by fluorescence microscopy. PDXP, PP1 and P2A were detected by FLAG antibodies and are shown in red. DAPI staining showed the cell nucleus.



Suppl. Figure S6. Single or double point mutants in SRC-3's degron do not affect subcellular compartmentations. FLAG-tagged SRC-3 wt or each mutant was expressed in HeLa cells followed by immunofluorescence staining using anti-FLAG antibodies.