Expanded View Figures

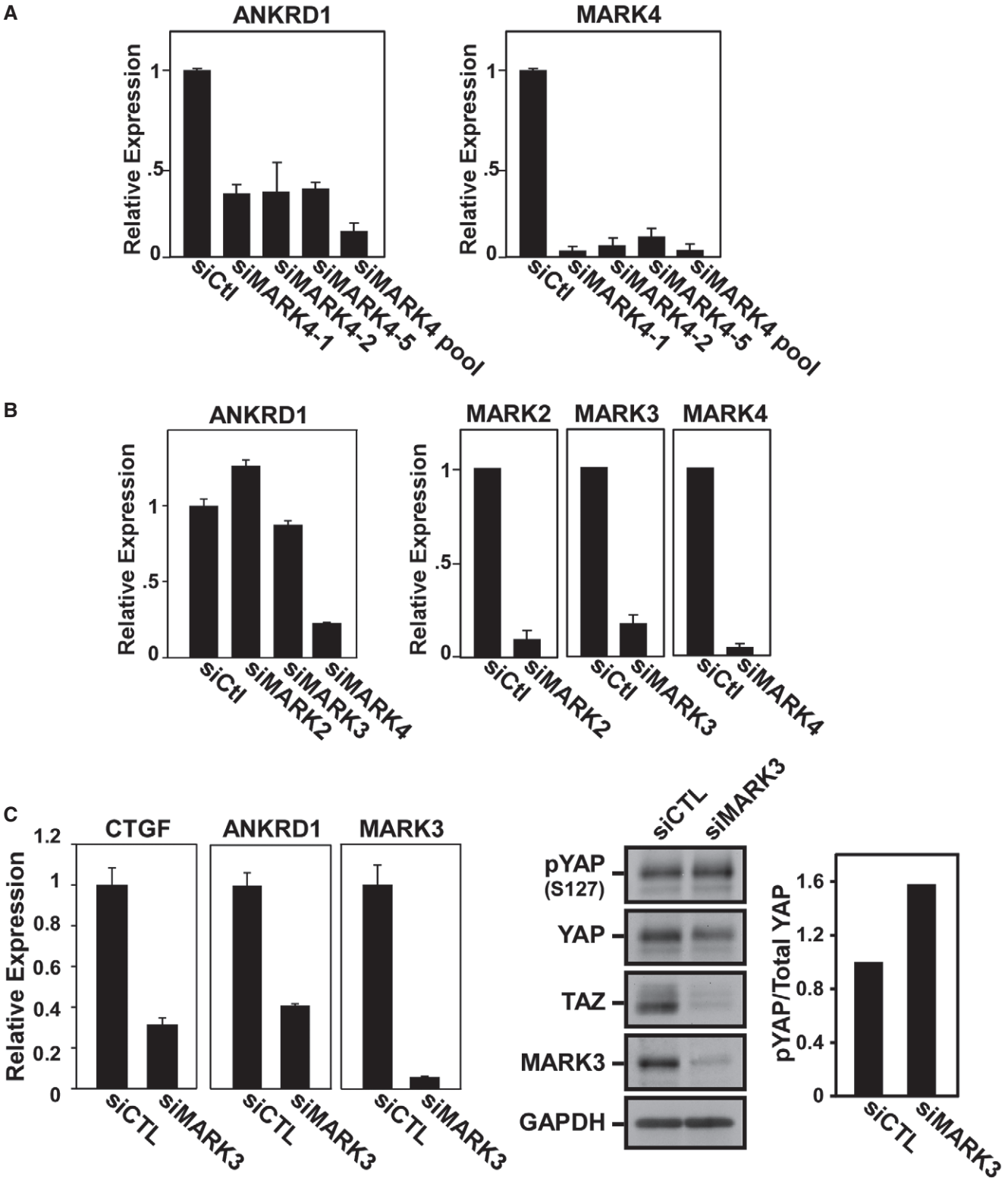

Figure EV1.

Figure EV1. Loss of MARK leads to decreased expression of YAP/TAZ target genes.

A Deconvolution of MARK4 siRNA. MDA-MB-231 cells were transfected with single siMARK4 oligonucleotides that comprise the pool. The expression of YAP/TAZ target gene, ANKRD1, and MARK4 knockdown efficiency was determined by real-time qPCR and is plotted as the mean ± SD of three independent experiments each performed with three technical replicates.

B MDA-MB-231 cells were transfected with siRNAs targeting MARK2, MARK3, and MARK4. The expression of YAP/TAZ target gene, ANKRD1, and MARK4 knockdown efficiency was determined by real-time qPCR and is plotted as the mean ± SD of three independent experiments each performed with three technical replicates.

C MDA-MB-468 cells were transfected with siControl or siMARK3. The expression of YAP/TAZ target genes, ANKRD1, CTGF, and MARK3 knockdown efficiency was determined by real-time qPCR and plotted as the mean ± range (left panels) of a representative of two independent experiments each with three technical replicates. YAP phosphorylation and YAP/TAZ total levels were analyzed by immunoblotting using anti-phospho-YAP (S127), YAP, and TAZ antibodies, respectively. Knockdown efficiency was determined using MARK3 antibody, and GAPDH was used and the loading control. YAP phosphorylation in blots was quantified by measuring the ratio of phospho-YAP (S127) to total YAP (right panels). The data are representative of two independent experiments.

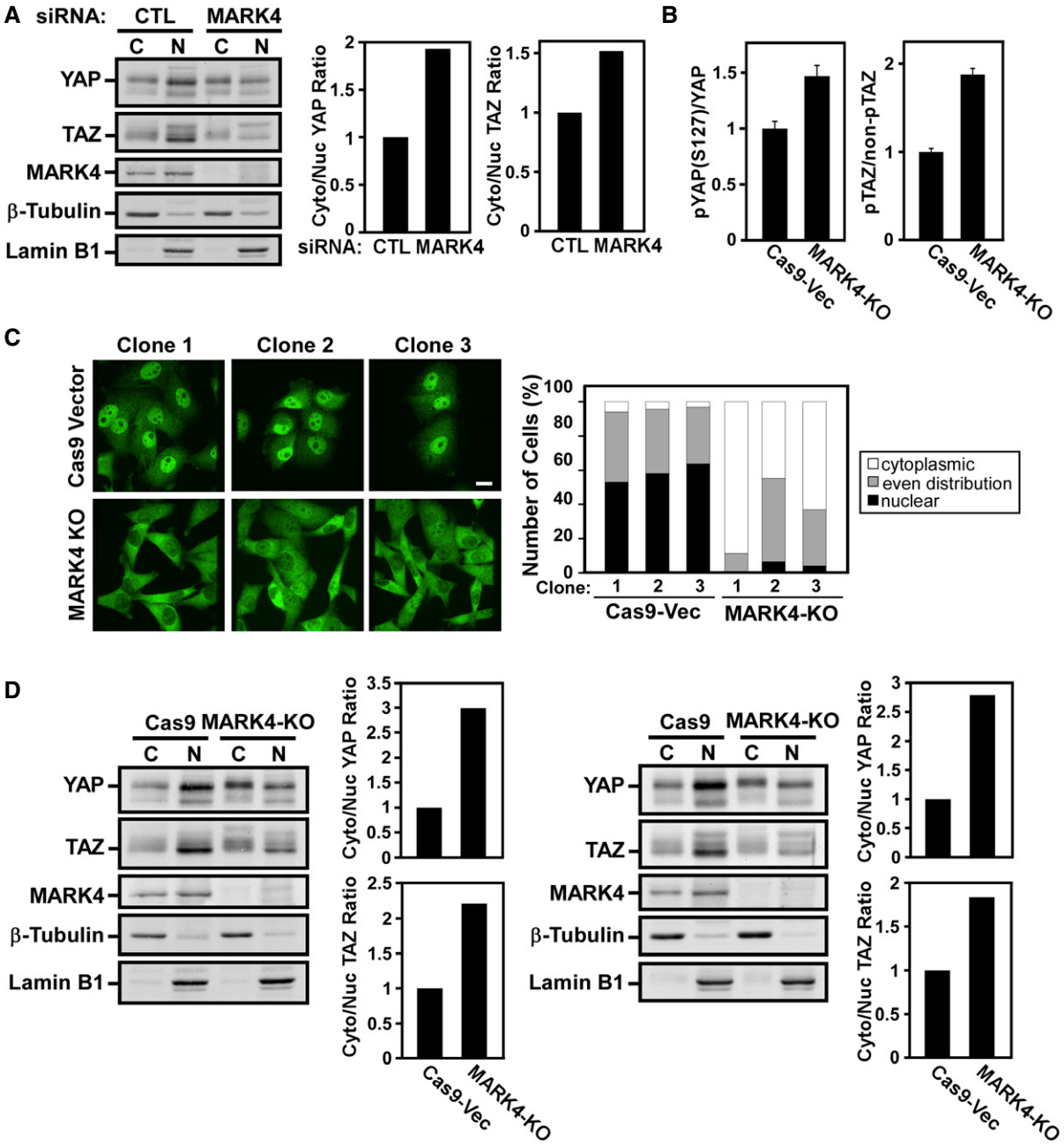

Figure EV2.

Figure EV2. MARK4 abrogation promotes nuclear to cytoplasmic translocation of YAP/TAZ.

A MDA-MB-231 cells were transfected with siControl or siMARK4, and subcellular localization of YAP/TAZ was analyzed by immunoblotting after biochemical fractionation. β-tubulin and lamin B1 were used as cytoplasmic and nuclear markers, respectively. The ratio of cytoplasmic to nuclear YAP and TAZ in blots was quantified. C, cytoplasmic; N, nuclear. The data are representative of two independent experiments.

B Related to Fig 3B. The ratio of phospho-YAP (S127) to total YAP and phosphorylated TAZ (upper band on PhosTag gel) to the un-phosphorylated TAZ (lower band) were quantified from blots and plotted as the mean of 3 control (Cas9-Vector) versus 3 MARK4 knockout (KO) clones ± SD.

C YAP localization (green) was assessed in control and MARK4-KO cells by immunofluorescence confocal microscopy, and subcellular localization of YAP in at least 30 cells per clone was quantified and plotted as percentage of cells displaying the indicated distribution in a representative of two independent experiments. Scale bar 15 μm.

D Subcellular localization of YAP/TAZ was evaluated in two control (Cas9) and two MARK4 knockout (KO) clones by immunoblotting after biochemical fractionation, and the cytoplasmic to nuclear YAP/TAZ ratio in the blots was quantified (right panels) for each independent experiment.

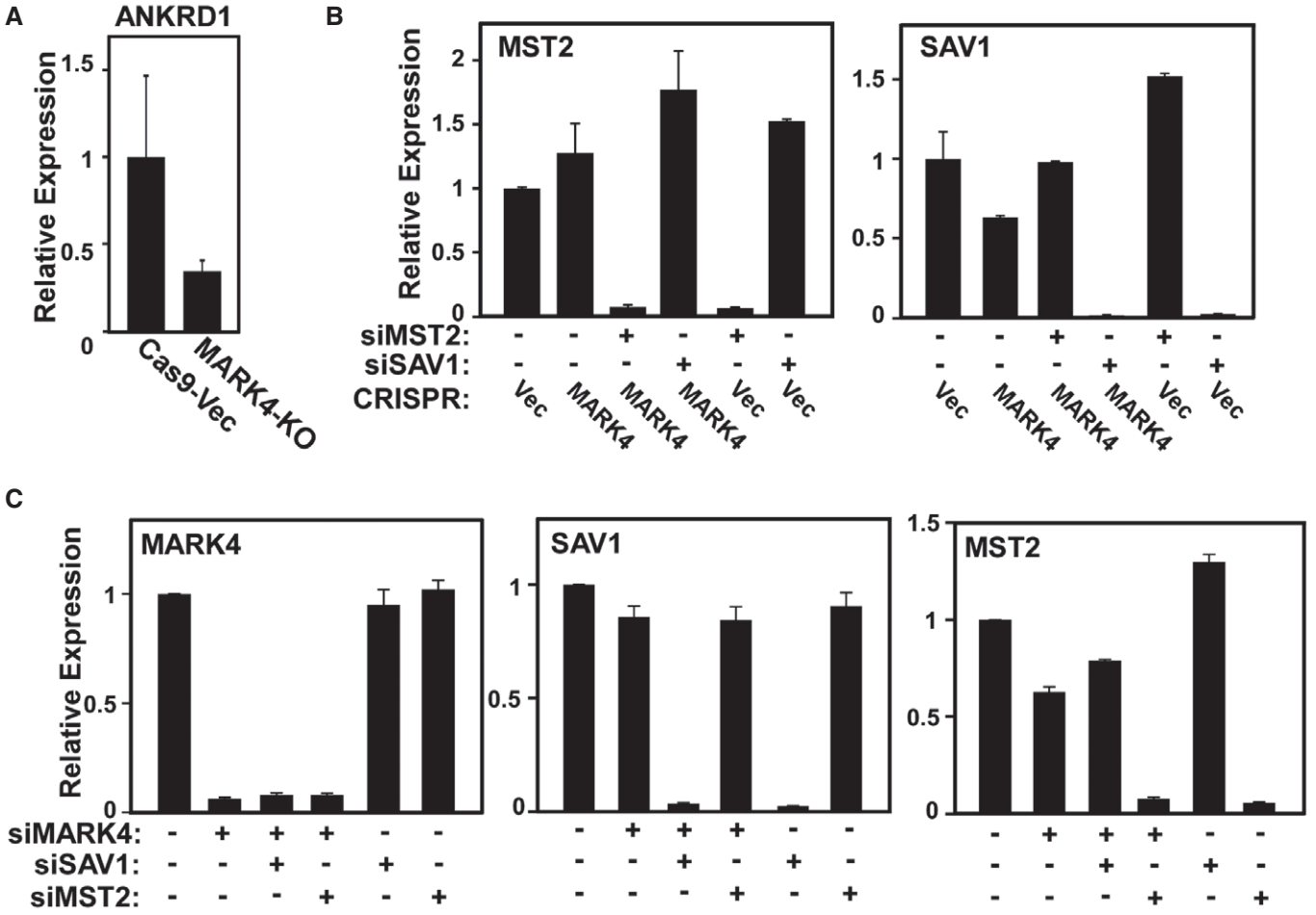

Figure EV3. MARK4 knockout results in a decrease in YAP/TAZ target gene expression.

A The expression of the YAP/TAZ target, ANKRD1, was assessed in two control and two MARK4-KO clones by real-time qPCR and is plotted as mean ± SEM of three independent experiments performed with three technical replicates.

B Related to Fig 3D. MST2 and SAV1 knockdown efficiencies were determined by real-time qPCR and plotted as mean ± SEM from three independent experiments each performed with three technical replicates.

C Related to Fig 3E. MARK4, SAV1, and MST2 knockdown efficiencies were determined by real-time qPCR and plotted as mean ± SEM of six independent experiments each performed with three technical replicates.

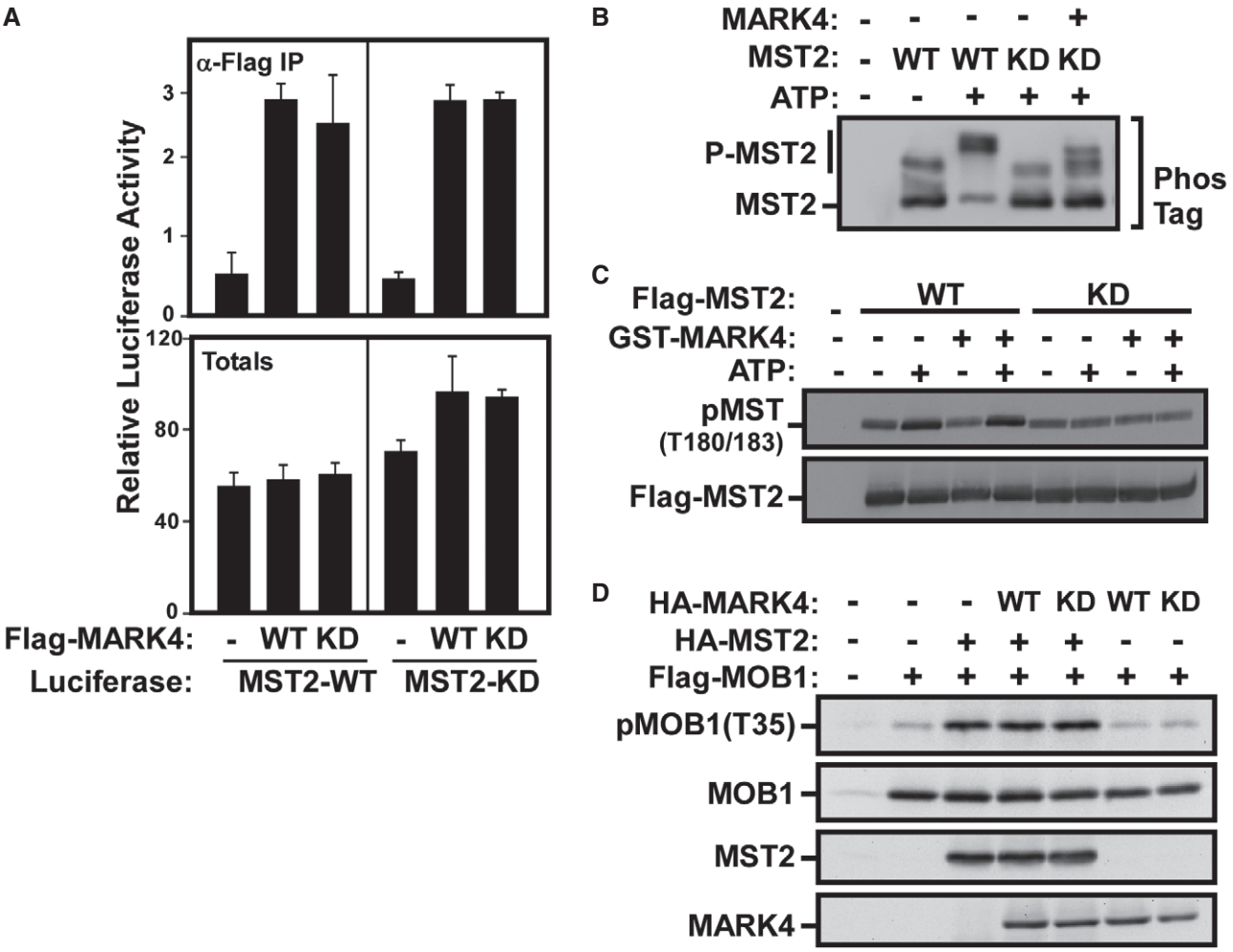

Figure EV4. MARK4 interacts with and phosphorylates MST2 kinase.

A HEK293T cells were transfected with either wild-type (WT) or kinase-dead (KD) Flag-MARK4 and firefly luciferase-tagged wild-type (WT) or kinase-dead (KD) variants of MST2. Cell lysates were subject to anti-Flag immunoprecipitation, and the presence of MST2 was assessed by luciferase assay and plotted as the mean of triplicate samples ± SD of a representative of two independent experiments. Cells transfected with luciferase-tagged MST2 alone were used as negative controls, and total expression was confirmed by luciferase assay.

B–D MST2 phosphorylation and kinase activity in the presence of MARK4 were assessed by an *in vitro* kinase assay using purified GST-MARK4. HEK293T cells were transfected with wild-type (WT) and kinase-dead (KD) Flag-MST2, and cell lysates were subjected to anti-Flag immunoprecipitation. Immunoprecipitated Flag-MST2 was incubated with GST-MARK4 in the presence or absence of ATP, as indicated. MST2 phosphorylation was analyzed by immunoblotting using PhosTag SDS–PAGE gels (B), and MST2 kinase activity was assessed by immunoblotting for autophosphorylation using phospho-MST1/2 (T180/183) antibody (C). The data are representative of three independent experiments. (D) The effect of MARK4 expression on MST2 kinase activity was further analyzed by evaluating MOB1 phosphorylation as the MST1/2 direct substrate. HEK293T cells were transfected with Flag-MOB1 and HA-MST2 along with wild-type (WT) or kinase-dead (KD) versions of HA-MARK4. MOB1 phosphorylation at the MST1/2 phospho-site Thr35 was assessed by immunoblotting using the specific antibody. The data are representative of two independent experiments.

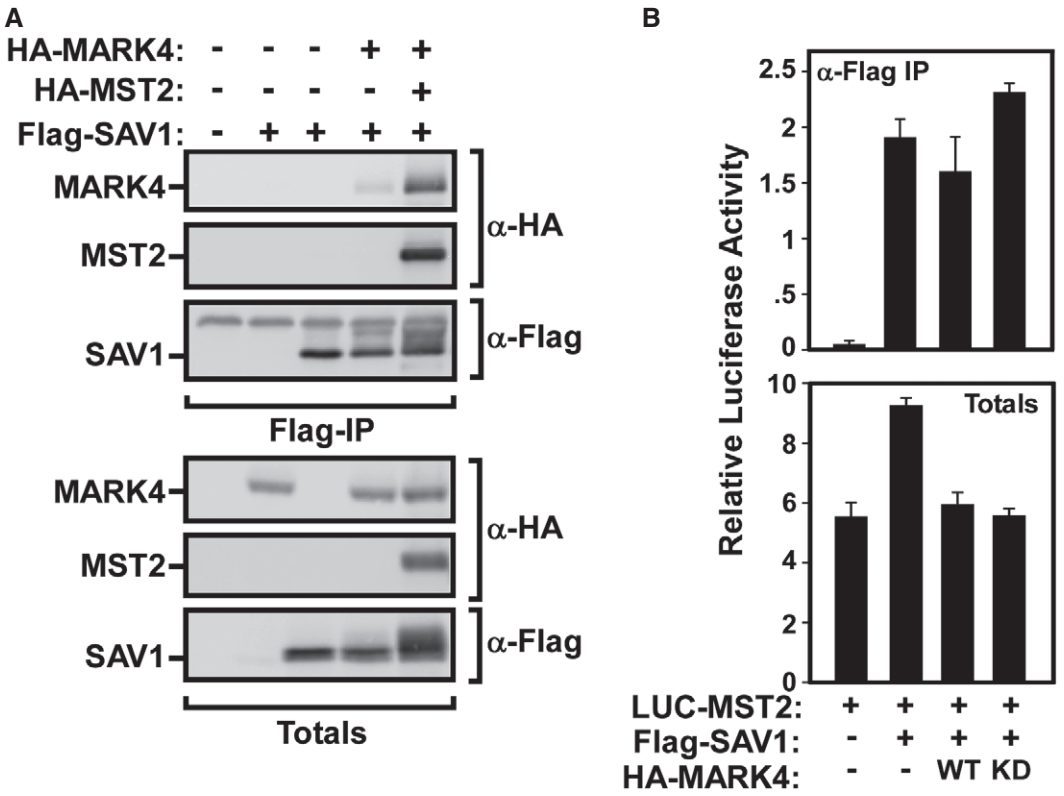

Figure EV5. MARK4 forms a multiprotein complex with MST/SAV.

A SAV1 binds to MARK4, and the presence of MST2 kinase enhances SAV1 interaction with MARK4. HEK293T cell was transfected with Flag-SAV1, HA-MARK4, and HA-MST2. Cell lysates were subject to anti-Flag immunoprecipitation, and the presence of HA-MARK4 and HA-MST1/2 was determined by anti-HA immunoblotting. Equal protein expression levels were confirmed (Totals). The data are representative of three independent experiments.

B MARK4 expression has no effect on MST/SAV binding. HEK293T cells were transfected with Flag-SAV1, firefly luciferase-tagged MST2, and wild-type (WT) or kinase-dead (KD) versions of HA-MARK4. Cell lysates were subjected to anti-Flag immunoprecipitation, and the MST2 binding to SAV was assessed by luciferase assay and plotted as the mean of three biological samples ± SD from one experiment. Cells transfected with luciferase-tagged MST2 alone were used as negative controls, and total expression was confirmed by luciferase assay.