



Suppl. Fig. 4. LIMK1(WT), but not LIMK1(S310A/S3232A), is activated by active MK2 in cell-free assays and by active MKK6 or VEGF in cultured cells. (A) LIMK1(WT), but not LIMK1(S310A/S3232A), is phosphorylated and activated by active MK2 in cell-free assays. Myc-LIMK1(WT) and Myc-LIMK1(S310A/S323A) were expressed in 293T cells, immunoprecipitated, and incubated with [γ -³²P]-ATP and active GST-MK2-Myc, with or without active GST-p38. Both ³²P-incorporation into Myc-LIMK1 and LIMK1 activity were analyzed, as in Fig. 6C. Relative values of ³²P-incorporation into LIMK1 and relative kinase activities are indicated in the bottom panels. (B) LIMK1(WT), but not LIMK1(S310A/S3232A), is activated by active MKK6 in cultured cells. Myc-LIMK1 and its mutant were coexpressed with HA-MKK6(DE) plus Flag p38 in 293T cells. Myc-LIMK1 proteins were immunoprecipitated and subjected to an *in vitro* kinase assay. Relative kinase activities are indicated in the bottom panel, as means \pm SD of triplicate experiments. (C) LIMK1(WT), but not LIMK1(S310A/S3232A), is activated by VEGF in cultured cells. MSS31 cells transfected with Myc-LIMK1(WT) or Myc-LIMK1(S310A/S3232A) were unstimulated or stimulated with VEGF for 15 min. Myc-LIMK1 proteins were precipitated with anti-Myc antibody and subjected to an *in vitro* kinase assay. Relative kinase activities are shown in the bottom panel, as means \pm SD of triplicate experiments.