SUPPLEMENTAL FIGURE LEGENDS

Figure S1 Autoradiography of MST1

A and B) The activity of MST1-FL and MST1-KD towards FoxO1 is shown. The gel shows the incorportation of the γP32 label as a function of concentration. All reactions were carried out for 15 minutes and then stopped with SDS loading buffer.

C and D) Autoradiography gel showing the activity of MST1-KD and MST1-FL towards histone H2B. The signal depicts the incorporation of the γP32 label onto histone H2B. The autophosphorylation signal of MST1 is also visible as indicated.

E) Autoradiograph showing the phosphorylation of the C-terminal regulatory and dimerization domain (residues 338-end) at indicated concentrations using MST-FL as the enzyme.

F) Autoradiograph showing the autophosphorylation of the N-terminal kinase domain at indicated concentrations using MST-FL as the enzyme. A kinase dead form of the pseudosubstrate harboring mutation K59R was used to quantatively measure the phosphorylation signal.

Figure S2 Autoradiography of mutant MST1 constructs

A) Phosphorylation of FoxO1 by the dimerization defective MST1 mutant encompassing residues 1-380 (MST-KD/RR) is shown. The gel shows the incorportation of the γP32 label as a function of FoxO1 concentration.

B) Autoradiograph gel depicting the activity of the MST1 phosphorylation defective triple mutant (MST1-TM) towards FoxO1.

Figure S3 Phosphoactivation of activation loop threonine 183

Western blot depicting the T183 phosphorylation status of the various MST1 constructs. The reaction was performed in duplicate. Equimolar amounts of enzyme was used.for each reaction.

Supplementary Figure 1
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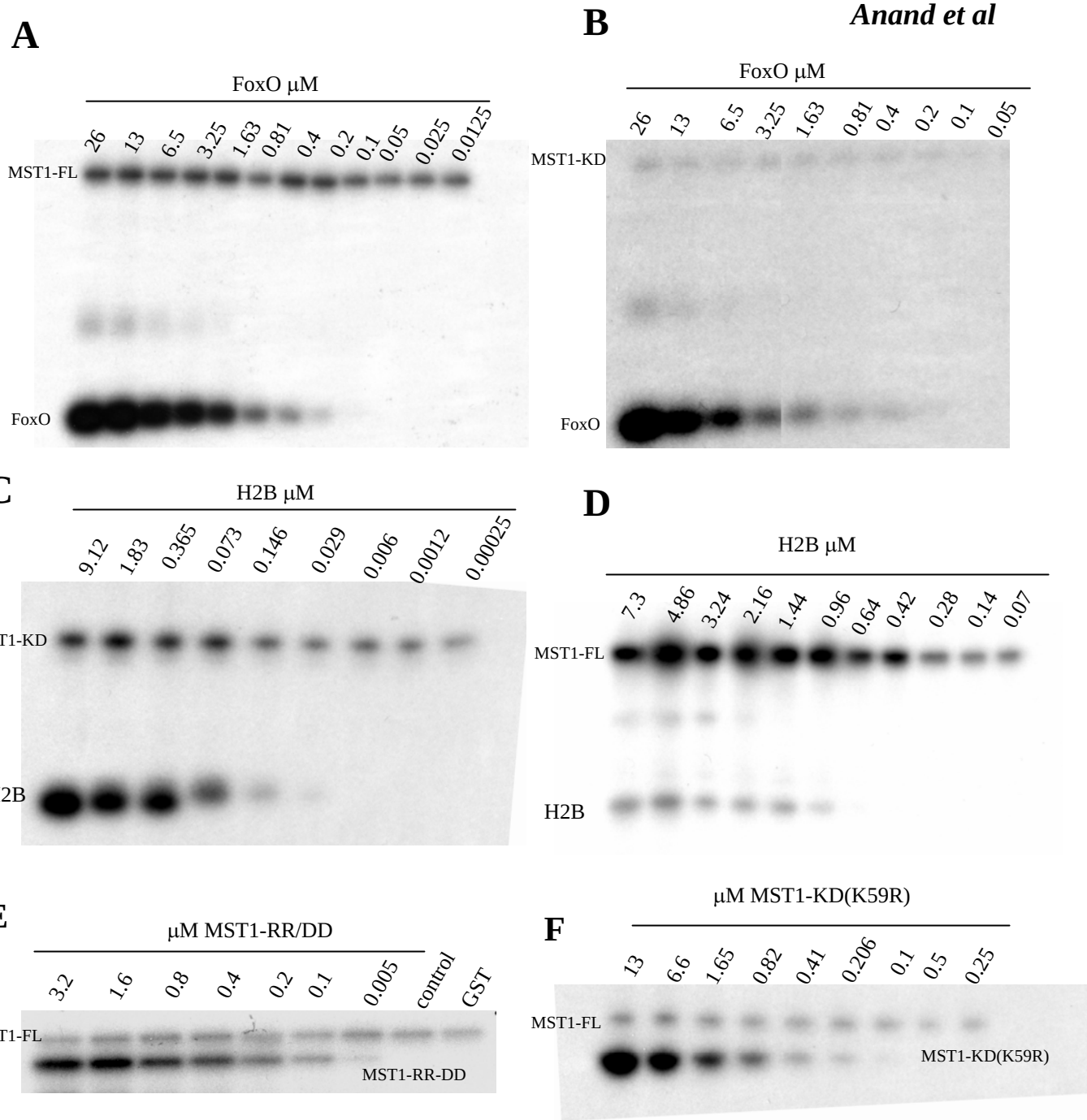

Supplementary Figure 2
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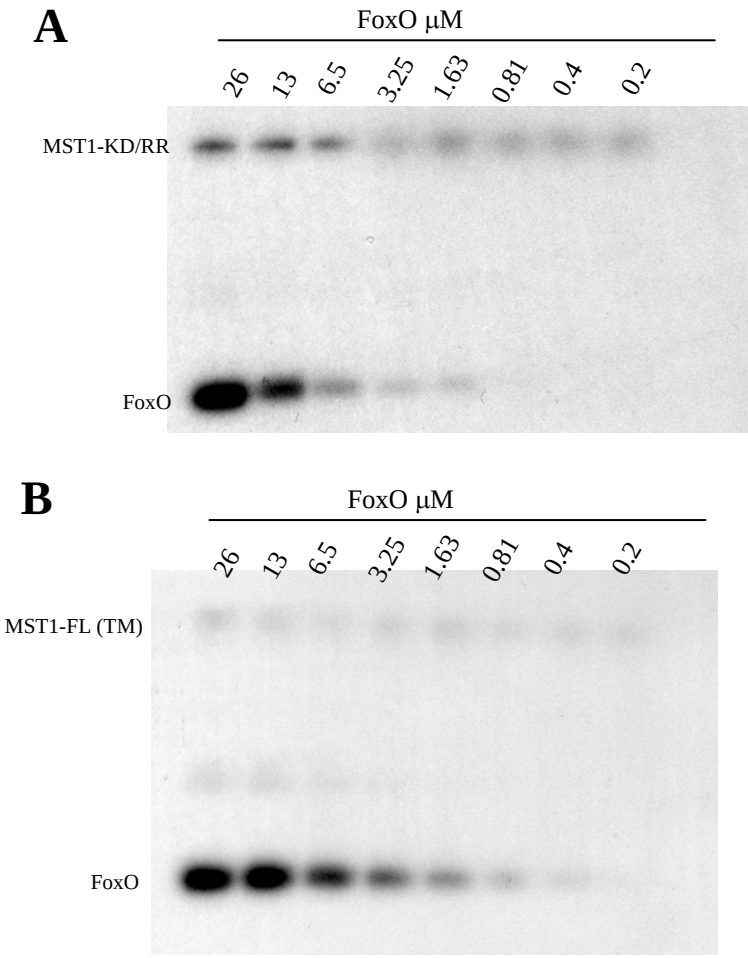

Supplementary Figure 3

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