Osmotic stress induces the phosphorylation of WNK4 Ser575 via the p38MAPK-MK pathway

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Legends for the Supplementary Figures

Supplementary Figure S1. Osmotic stress-dependency of the ASK3-WNK4 interaction

Osmotic stress-dependency of the ASK3-WNK4 interaction. HEK293A cells were transiently transfected with FLAG-hASK3 and hWNK4-HA. Cells were lysed at 48 h after transfection. Before the lysis, cells were stimulated with the osmotic stimulation buffer as indicated for the indicated times. Lysates were immunoprecipitated with anti-FLAG antibody beads and subjected to IB.

Supplementary Figure S2. Profile of the interaction between WNK4 fragments and ASK3

HEK293A cells were transiently transfected with HA-hASK3 and FLAG-hWNK4 fragments. Cells were lysed at 48 h after transfection. Lysates were immunoprecipitated with anti-FLAG antibody beads and subjected to IB.

Supplementary Figure S3. Recombinant MK2 phosphorylates WNK4 Ser575 *in vitro*

0.5 μg recombinant MK2 (R&D systems) were incubated with 0.5 μg GST-hWNK4 (567-586) and Mg-ATP for 15 min at 30ºC. GST was used as a negative control for recombinant MK2 kinase. The reaction was terminated by the addition of SDS sample buffer, and samples were subjected to IB. Phosphorylation of Ser575 residue in GST-hWNK4 (567-586) was analyzed by IB using phospho-Ser575 antibody. Protein amount of recombinant MK2, GST and GST-hWNK4 (567-586) were visualized by Ponceau S staining.

Supplementary Figure S4. Involvement of WNK4 Ser575 phosphorylation in the KLHL3-dependent WNK4 regulation

(a) Interaction between WNK4 mutants and KLHL3. HEK293A cells were transiently transfected with FLAG-hWNK4 mutants and HA-KLHL3. Cells were lysed at 48 h after transfection. Lysates were immunoprecipitated with anti-FLAG antibody beads and subjected to IB.

(b) KLHL3-dependent degradation of WNK4 mutants. HEK293A cells were transiently transfected with FLAG-hWNK4 mutants and HA-KLHL3. Cells were lysed at 48 h after transfection. Before lysis, cells were treated with 5 μg/ml cycloheximide for the indicated times to inhibit the protein synthesis. Cell lysates were subjected to IB.

Supplementary Figure S5. Full-length blots of Figure 1

(a) Full-length blots of Figure 1b.

(b) Full-length blots of Figure 1c.

Supplementary Figure S6. Full-length blots of Figure 2

(a) Full-length blots of Figure 2b.

(b) Full-length blots of Figure 2f.

Supplementary Figure S7. Full-length blots of Figure 3a-e

(a) Full-length blots of Figure 3a.

(b) Full-length blots of Figure 3b-c.

(c) Full-length blots of Figure 3d.

(d) Full-length blots of Figure 3e.

Supplementary Figure S8. Full-length blots of Figure 3f

Full-length blots of Figure 3f.

Supplementary Figure S9. Full-length blots of Figure 4

(a) Full-length blots of Figure 4a.

(b) Full-length blots of Figure 4b.

(c) Full-length blots of Figure 4c.

(d) Full-length blots of Figure 4d.

Supplementary Figure S10. Full-length blots of Figure 5a-c

(a) Full-length blots of Figure 5a.

(b) Full-length blots of Figure 5b.

(c) Full-length blots of Figure 5c.

Supplementary Figure S11. Full-length blots of Figure 5d,f

(a) Full-length blots of Figure 5d.

(b) Full-length blots of Figure 5f.

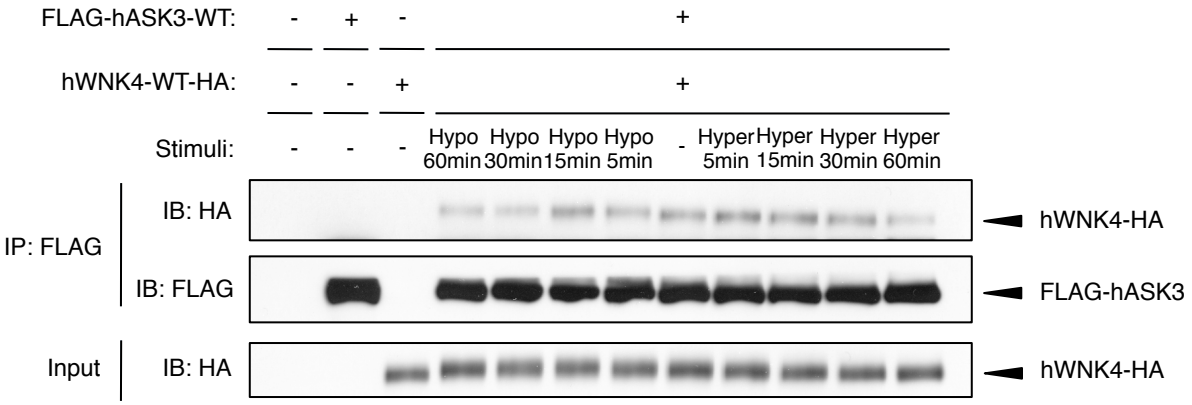
Supplementary Figure S12. Full-length blots of Figure 6

(a) Full-length blots of Figure 6a.

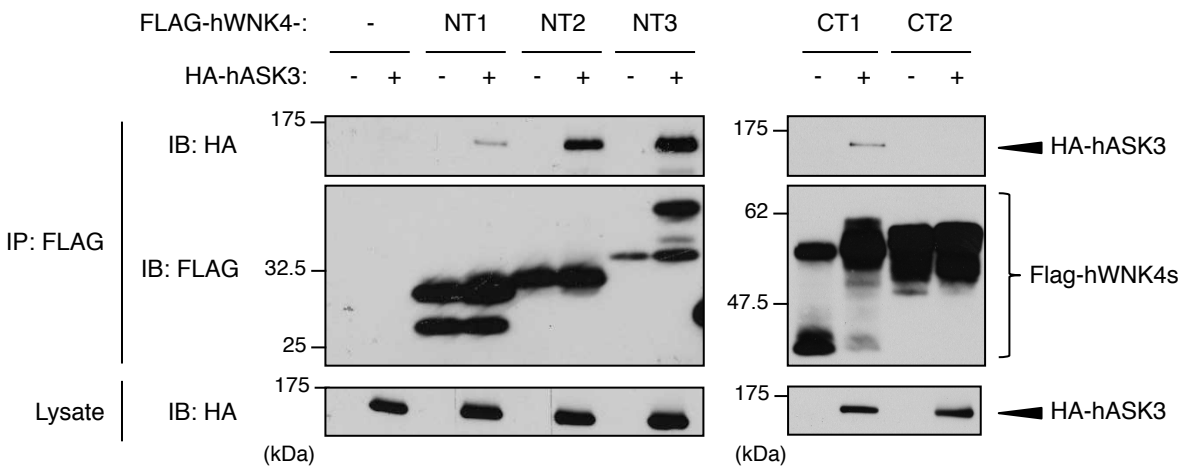
(b) Full-length blots of Figure 6b.

(c) Full-length blots of Figure 6d.

Supplementary Figure S1



Supplementary Figure S2



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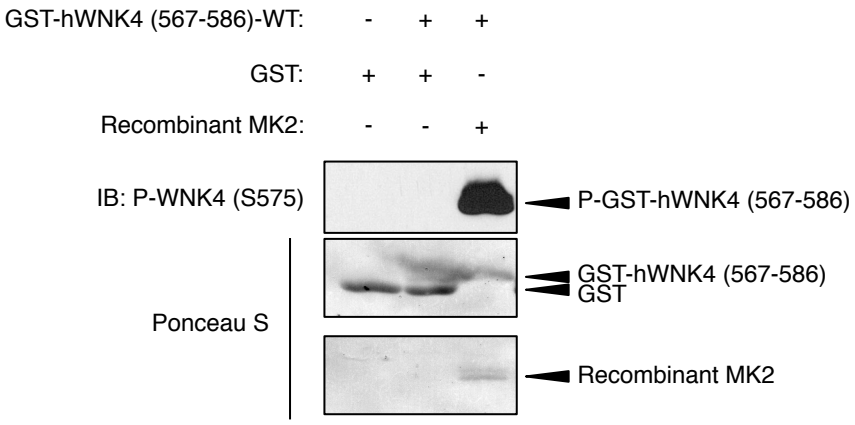

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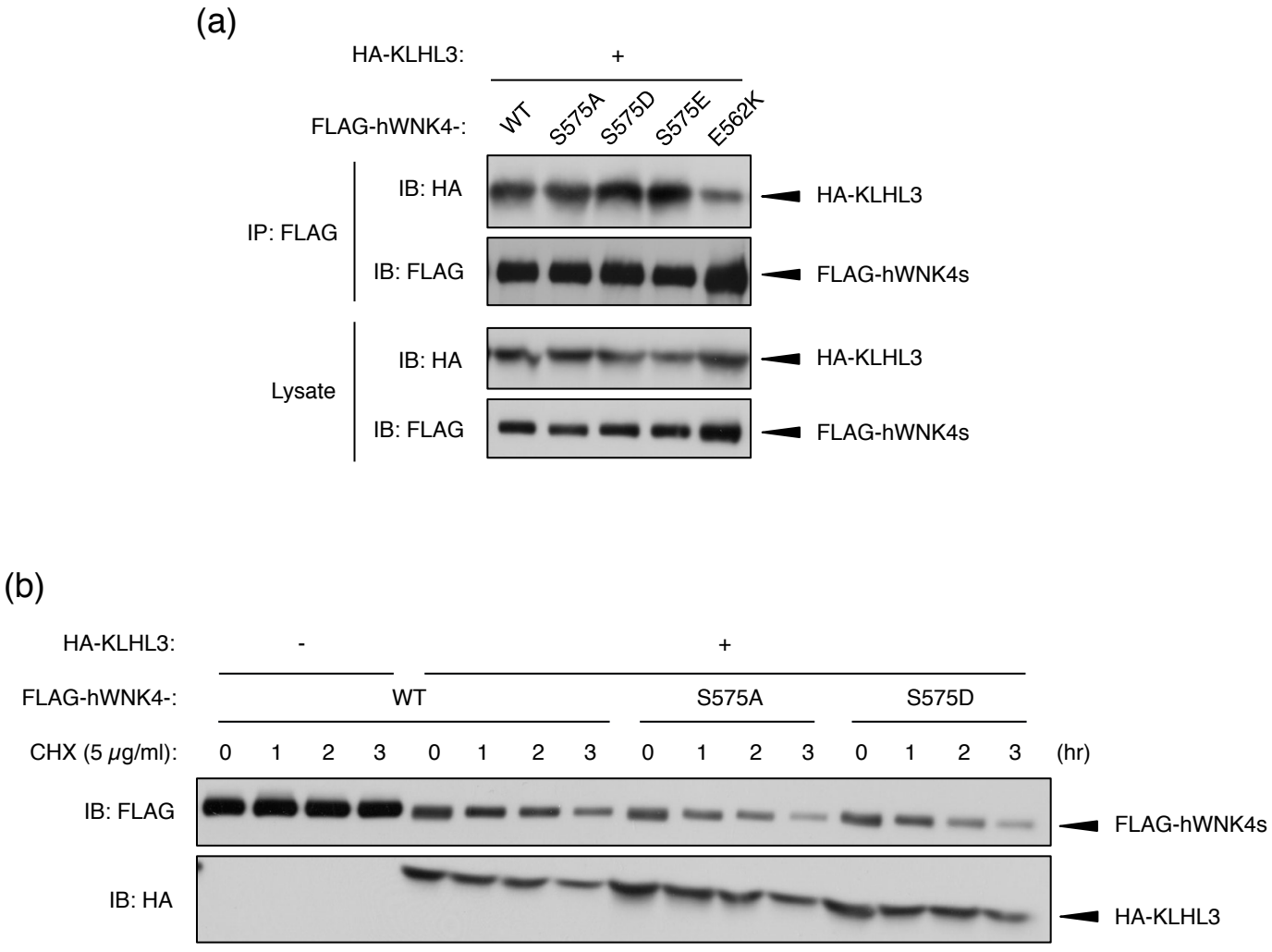
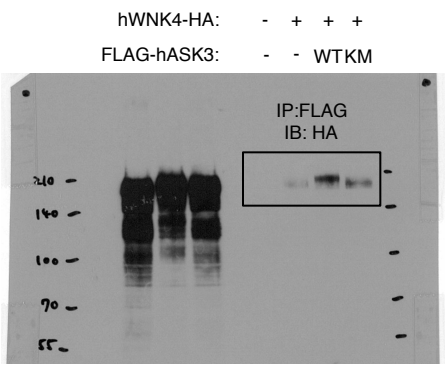
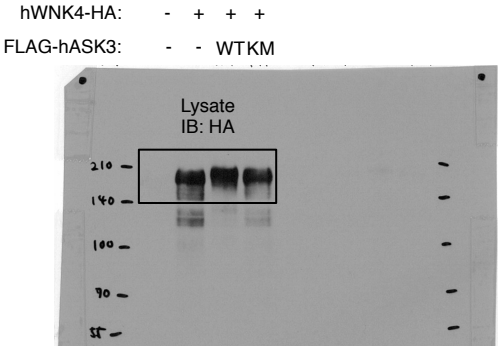
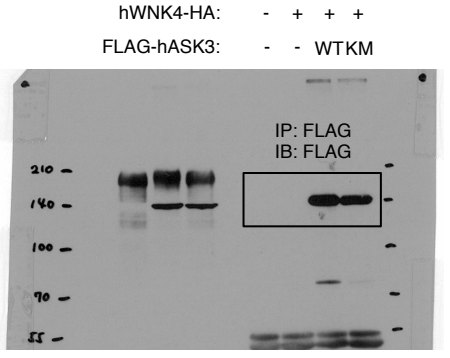


Supplementary Figure S5

(a)







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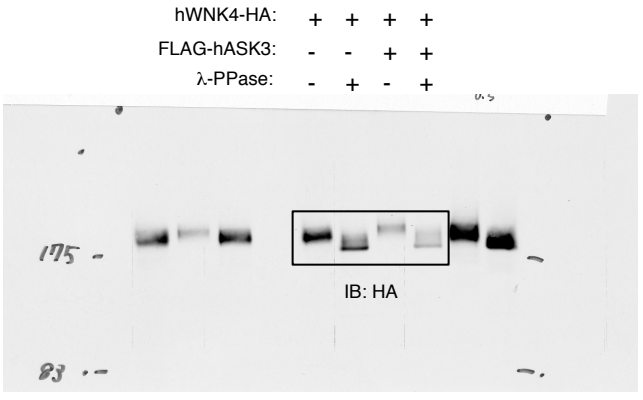
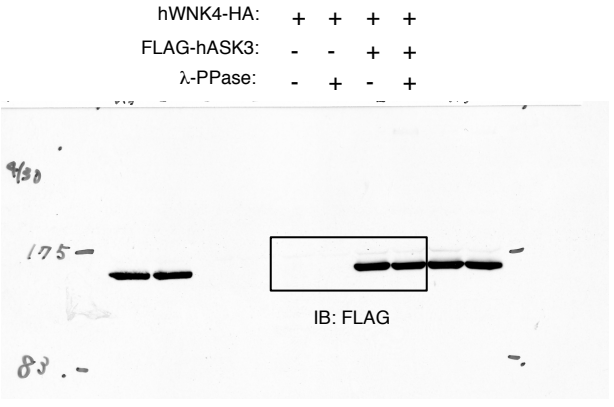
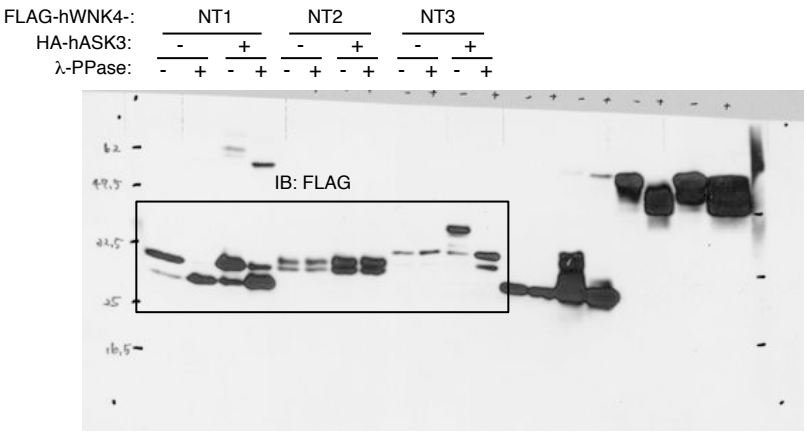
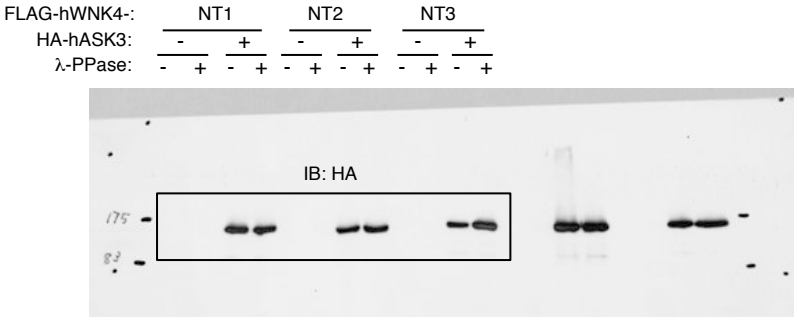


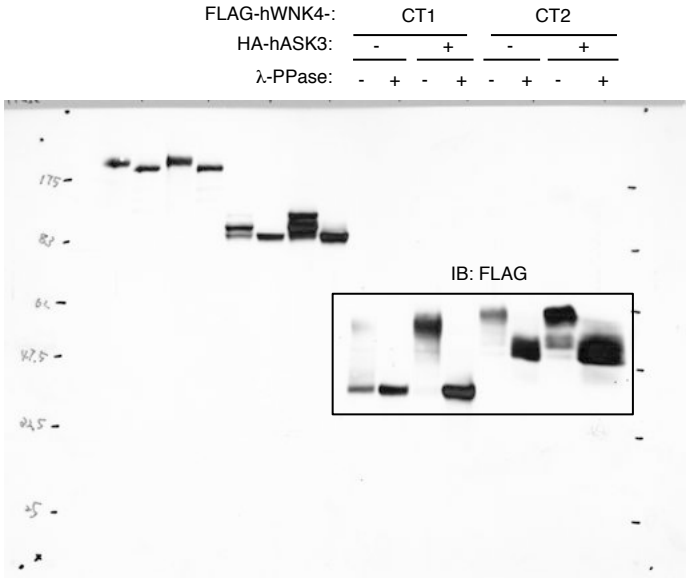


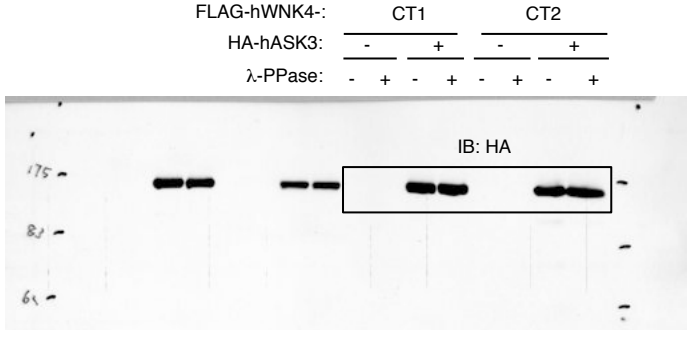
Supplementary Figure S6

(a)









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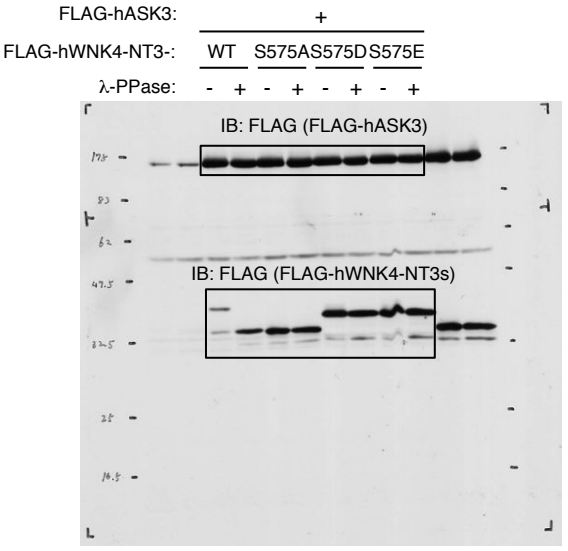

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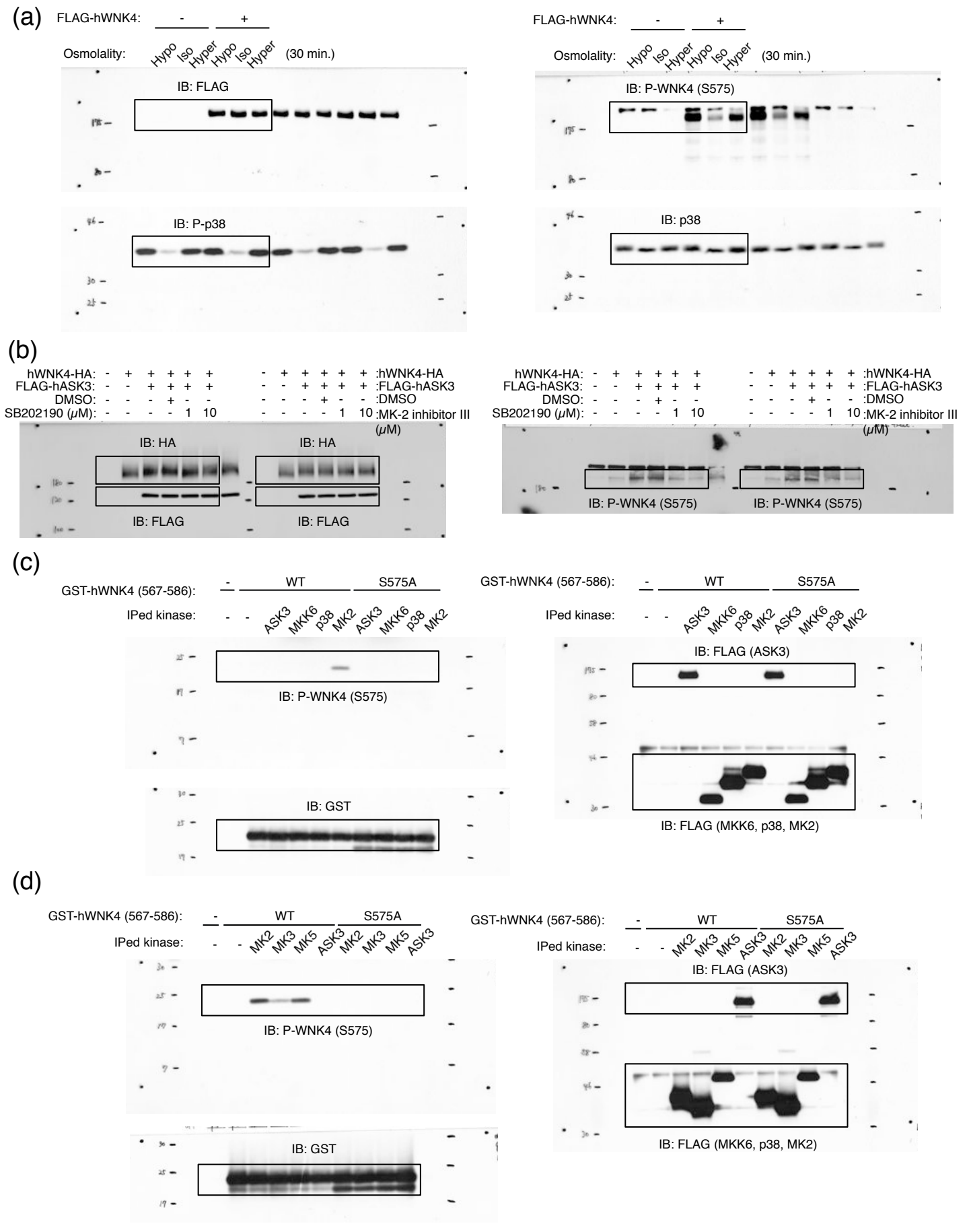

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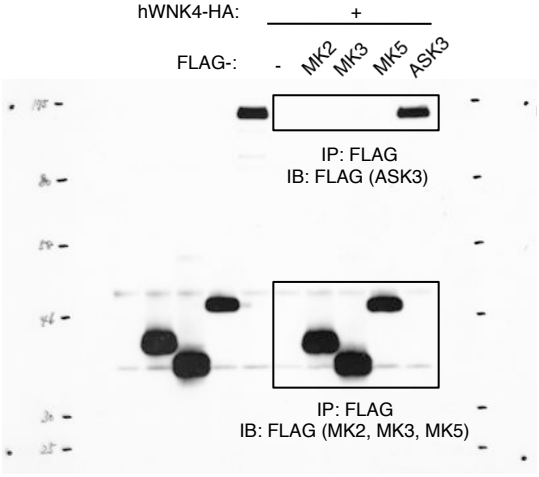

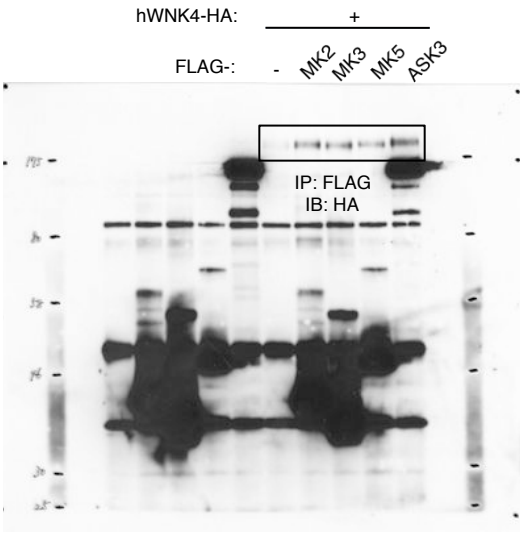

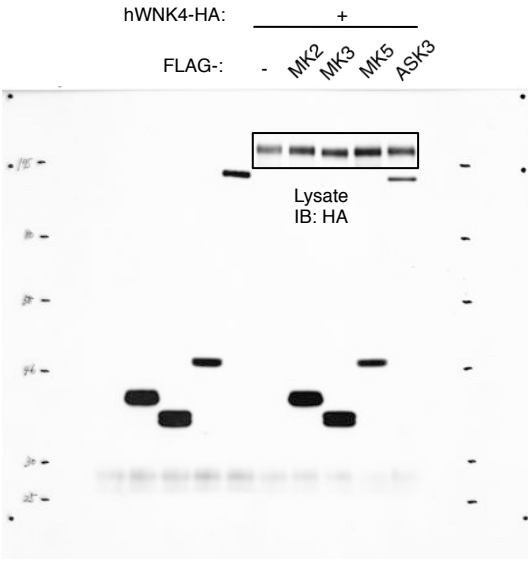

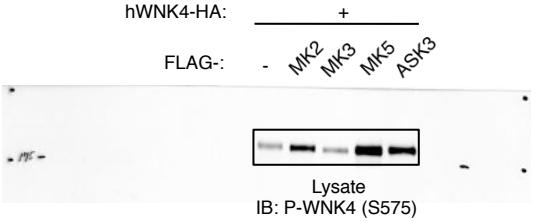

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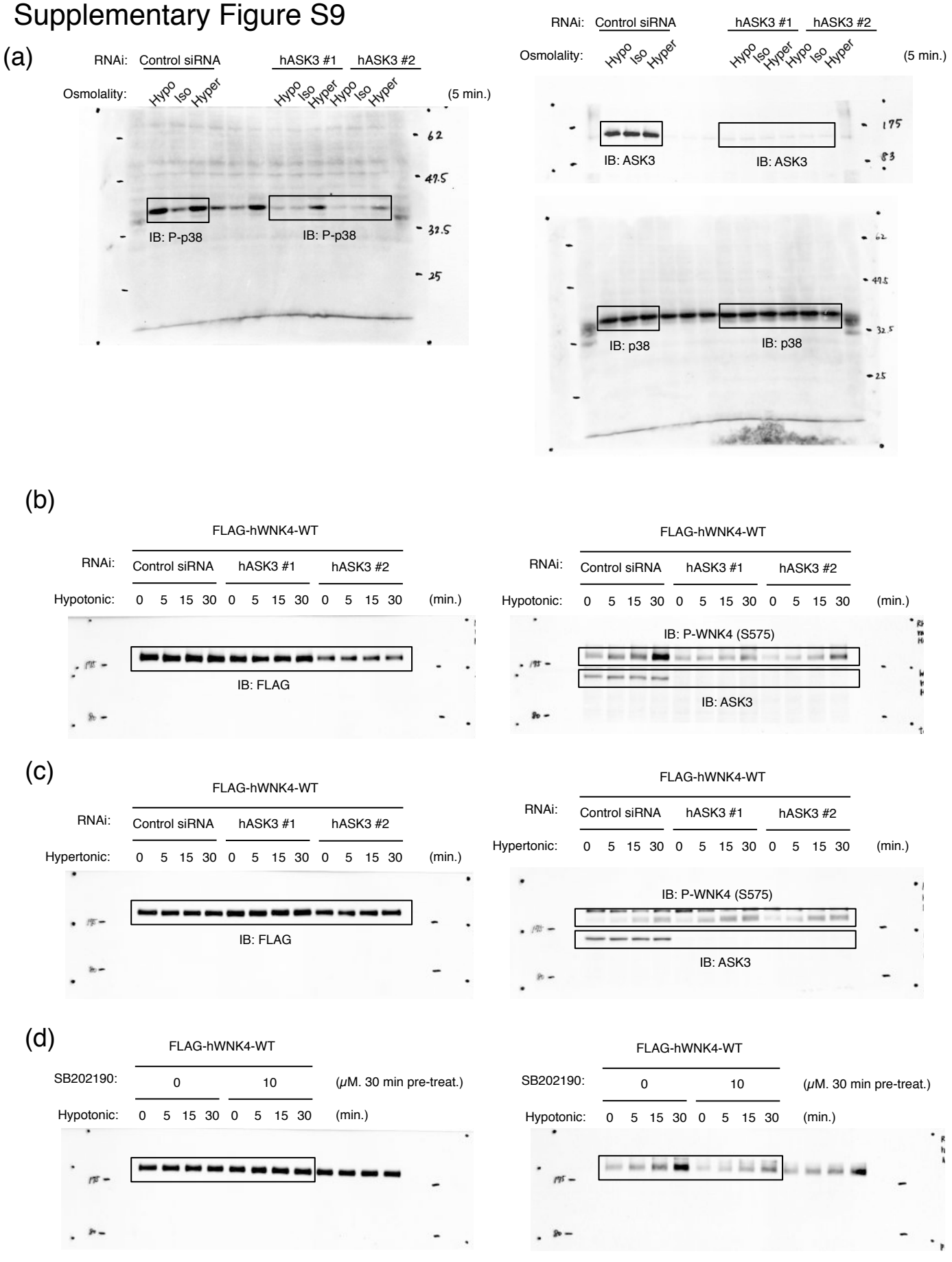
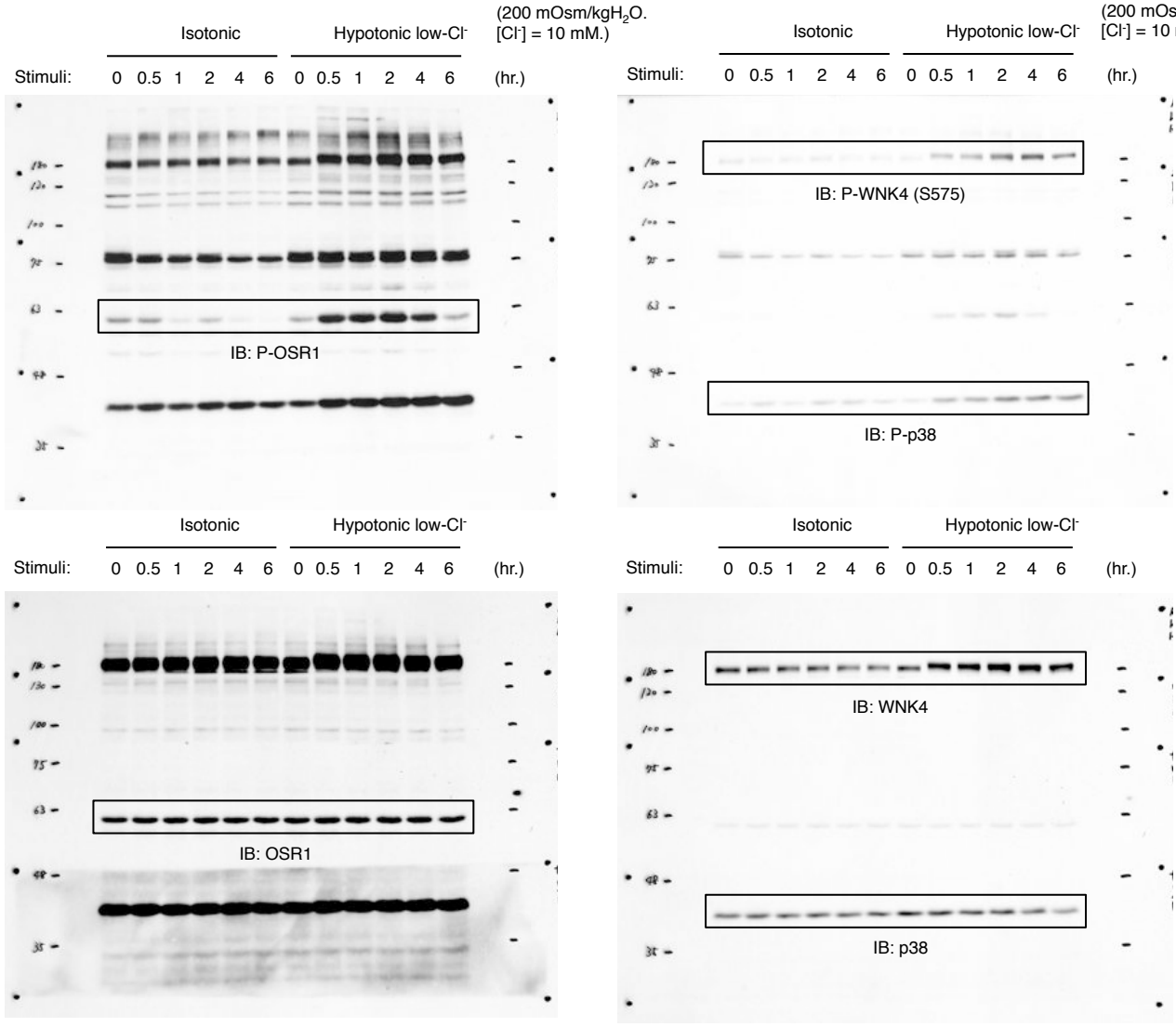


Supplementary Figure S10

(a)



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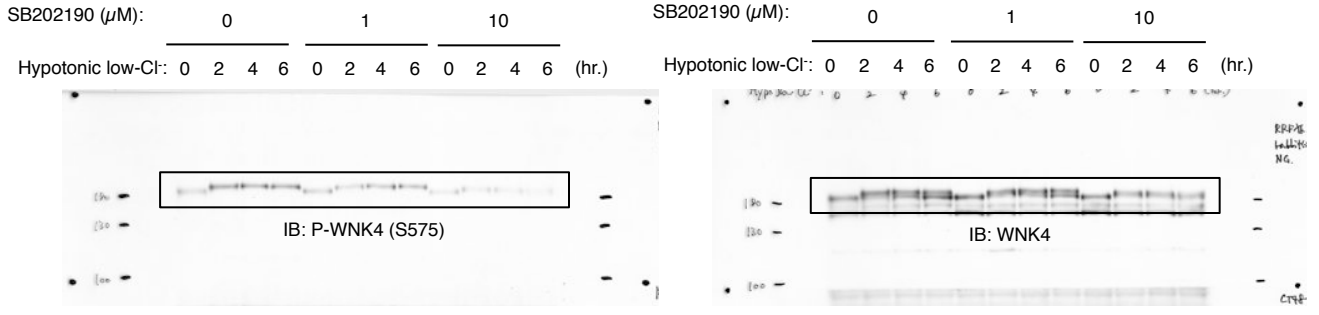

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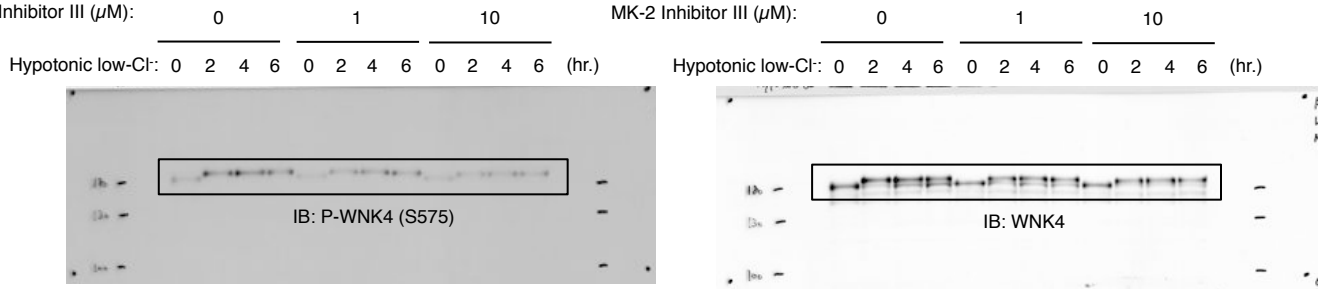
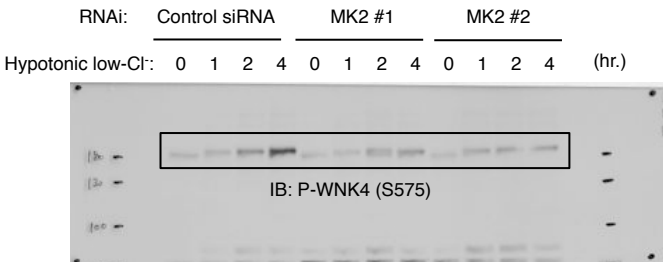
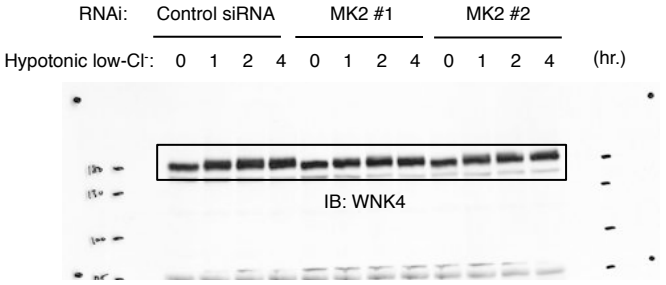


Supplementary Figure S11

(a)





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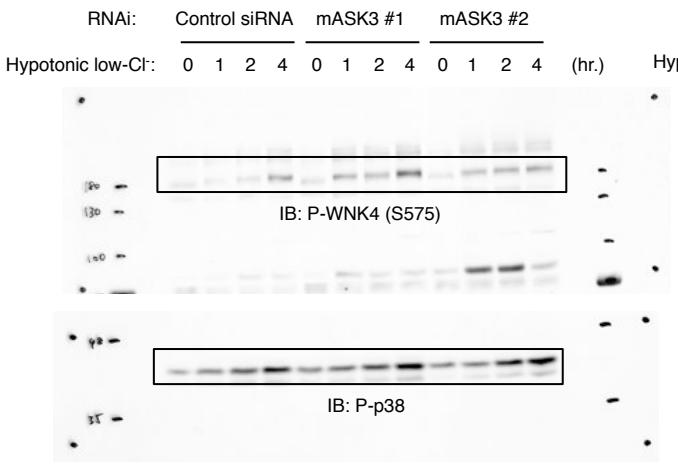
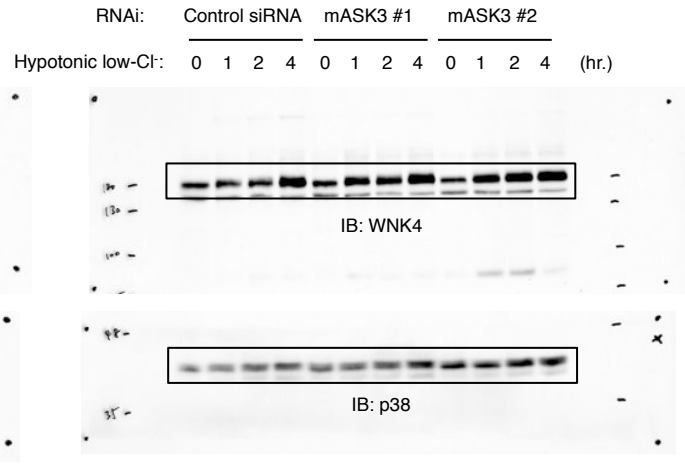
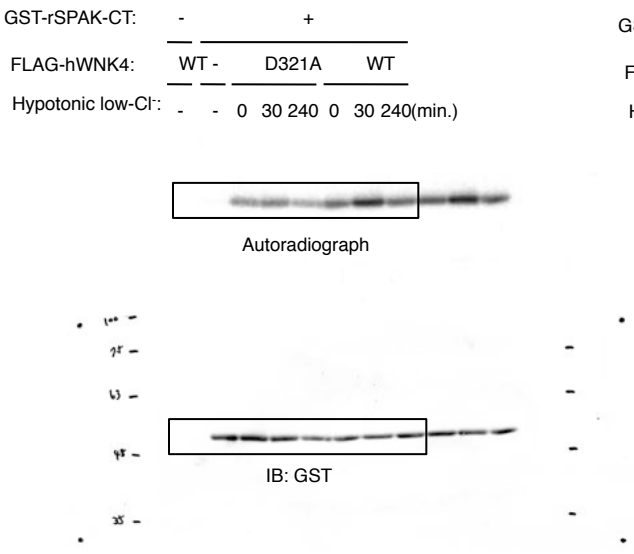
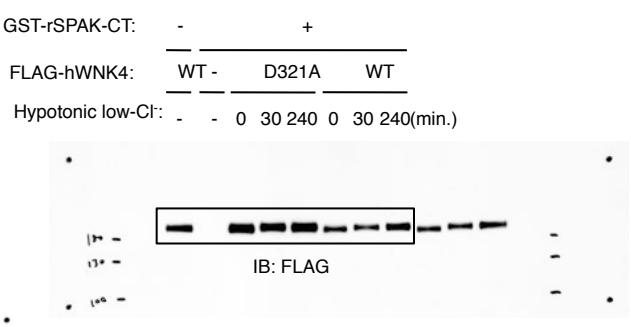




Supplementary Figure S12

(a)





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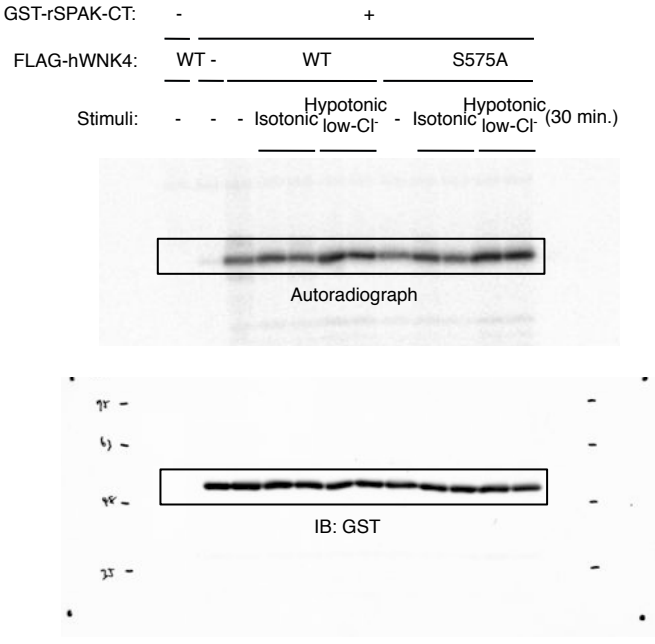

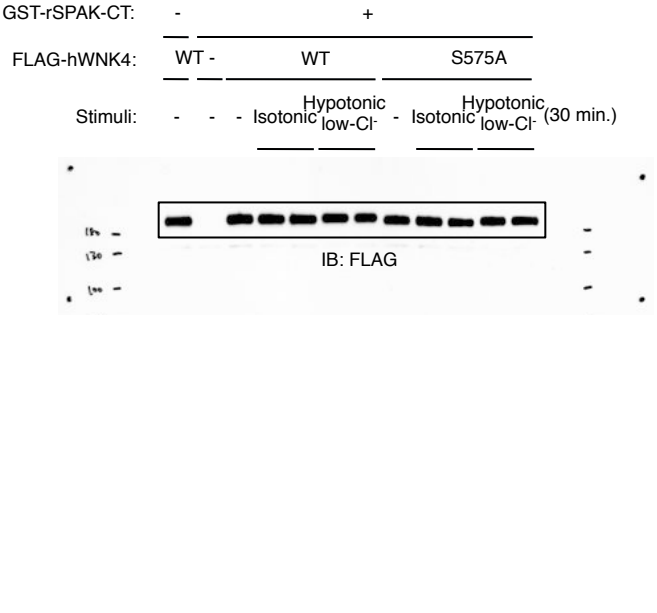

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