## **Supplementary figure legends**

Figure S1. HeLa cells cultured on glass coverslips were fixed and immunostained with anti-MINK1 and anti-alpha-tubulin antibodies. Hoechst was used to visualize the nucleus.

Figure S2. (A) GFP-STRN4 was transiently expressed in 293T cells with HA or HA-MINK1 and immunoprecipitated with anti-HA antibody. The immunoprecipitates were subjected to immunoblot with anti-HA and anti-GFP antibodies. (B) HA-MINK1 was transiently expressed in 293T cells with GFP or GFP-STRN4 and immunoprecipitated with anti-GFP antibody. The immunoprecipitates were subjected to immunoblot with anti-HA and anti-GFP antibodies.

Figure S3. HeLa cells cultured on glass coverslips were fixed and immunostained with anti-STRN4 and anti-alpha-tubulin antibodies. Hoechst was used to visualize the nucleus.

Figure S4. (A) Immunoprecipitated GFP-MINK1 and different amount of recombinant protein of GST-MINK (aa346-431) were immunoblotted with anti-MINK1 antibody. Approximate amount of GFP-MINK1 was measured from the band intensity of recombinant proteins. Note that anti-MINK1 antibody does not react with GST because anti-GST antibody was eliminated by recombinant GST protein. (B) Purified FLAG-STRN4 and different amount of recombinant protein of GST-STRN4 (aa1-147) were immunoblotted with anti-STRN4 antibody. Approximate amount of FLAG-STRN4 was measured from the band intensity of recombinant proteins. Note that anti-STRN4 antibody does not react with GST because anti-GST antibody was eliminated by recombinant of FLAG-STRN4 was measured from the band intensity of recombinant proteins. Note that anti-STRN4 antibody does not react with GST because anti-GST antibody was eliminated by recombinant GST protein. (C) GFP-STRN4 was transiently expressed in 293T cells together with or without FLAG-PPP2R1A. Cells were lysed, immunoprecipitated with anti-FLAG antibody, followed by immunoblot. (D) Immunoprecipitated GFP-MINK1 (1 ng) was subjected to in vitro kinase assay with the different amount of the FLAG-PPP2R1A/GFP-PPP2CA complex in the presence of 10 ng of FLAG-STRN4.



Figure S2

| Α  |   | В   |  |
|--|---|---|--|
| HA<br>HA-MINK1<br>GFP-STRN4<br>100-<br>KDa | IP:HA<br>+ -<br>- +<br>+ +<br>IB:HA<br>IB:GFP | GFP + -<br>GFP-STRN4 - +<br>HA-MINK1 + + +<br>100-<br>220-<br>KDa | IP:GFP<br>+ -<br>- +<br>+ +<br>BB:GFP<br>IB:HA |



