Supporting Information

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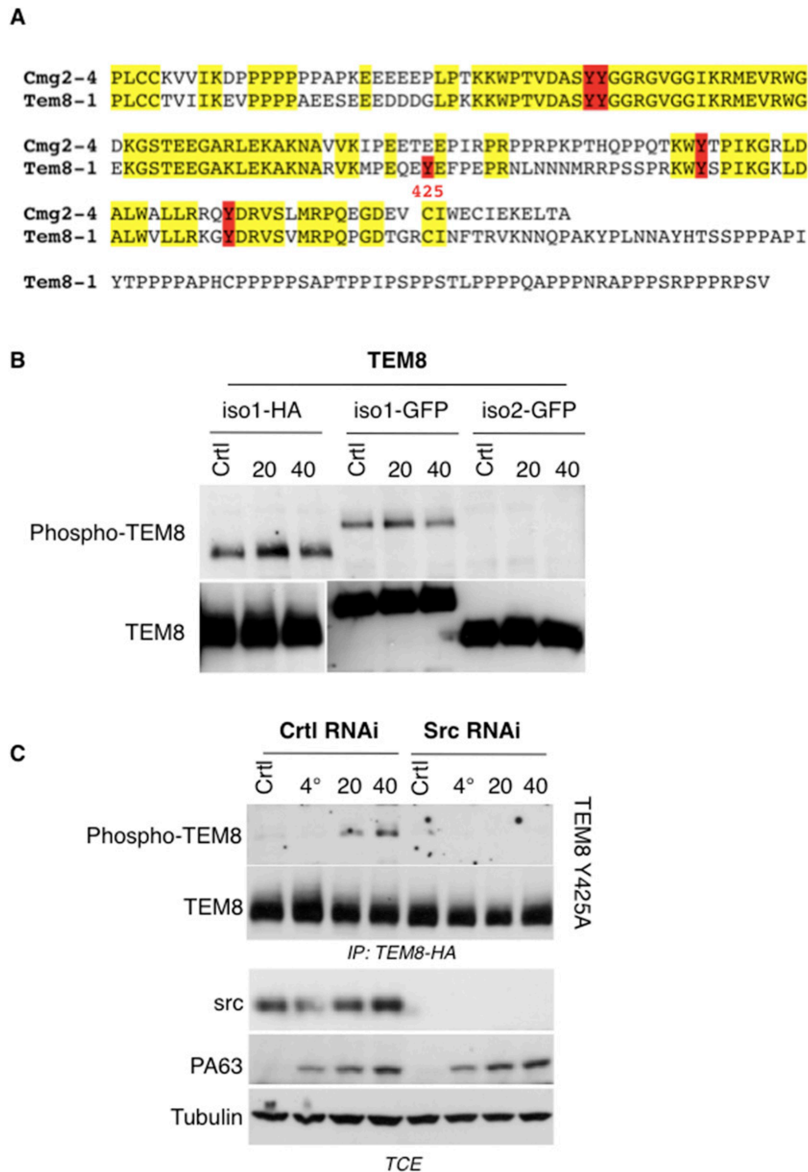

Fig. S1. (*A*) Alignment of CMG2 isoform 4 and TEM8 isoform 1. Red background represents tyrosine residues (Y) and yellow background conserved residues. (*B*) HeLa cells were transfected for 48 h with TEM8-1 tagged with HA or fused to GFP or with TEM8-2 fused to GFP. Cells were treated or not with 1 μg/ml of PA63 for 1 h at 4 °C followed by 20 and 40 min at 37 °C. Immunoprecipitates against HA or GFP were analyzed by SDS/PAGE and Western blotting against phospho-tyrosine proteins. (*C*) HeLa cells were transfected for 48 h with HA-tagged TEM8-1 Y425A, as well as with RNAi duplexes against an irrelevant protein (G protein of VSV) or *src*. Cells were subsequently treated as in *B* to reveal TEM8 phosphorylation. Total cell extracts were analyzed to follow PA and to confirm RNAi efficiency by Western blotting against *src*. Tubulin was used as an equal loading marker.

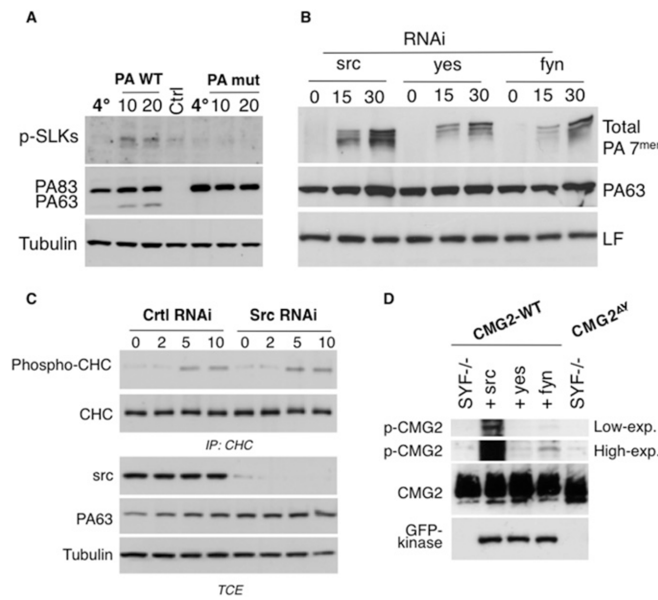

Fig. S2. (*A*) Hela cells were treated with 500 ng/ml of WT PA83 or PA SNKE, deficient in furin cleavage, for 1 h at 4 °C and incubated for the indicated time at 37 °C. Cell extracts (40 μg of proteins) were analyzed by SDS-PAGE and western blotting to reveal phosphotyrosine src-like kinases (Tyr-416), and PA. (*B*) Hela cells were transfected 72 h with siRNAs against src, yes or fyn. Cells were incubated with 500 ng/ml PA63 and 50 ng/ml LF for 1 h at 4 °C and different times at 37 °C and cell extracts were treated 10 min at room temperature with acid buffer and analyzed by SDS-PAGE and western blotting to reveal all PA heptamers (at the surface and intracellular). These samples correspond to the same as those analyzed in Fig. 4B. (*C*) Hela cells were transfected for 72 h with siRNAs against src or an irrelevant RNAi. Cells were incubated with 500 ng/ml PA63 for 1 h at 4 °C and different times at 37 °C. Immunoprecipitation against CHC were performed and samples were analyzed by SDS-PAGE and Western blotting against phospho-CHC, CHC. Total cell extracts were also analyzed and blotted for src, PA and tubulin, as an equal loading marker. (*D*) SYF-MEF cells were transfected or not 48 h with src-GFP, fyn-GFP or yes-GFP, as well as with CMG2-WT-HA or CMG2 Δ Y-HA mutated on 4 tyrosines : Y380A, Y381A, Y445A, Y463A. Cell were then treated with 500 ng/ml of PA63 for 1 h at 4 °C and 20 min at 37 °C. Immunoprecipitates against HA were analyzed by SDS-PAGE and western blotting against phospho-tyrosine proteins, CMG2-HA and GFP.