Supplemental Information

Supplemental Figure Legends

Figure S1. Alignment of protein phosphatase 5 of different species. The protein sequence of PPH-5 of *Caenorhabditis elegans* (CE), PP5 of *Rattus norvegicus* (RN), of *Mus musculus* (MM) and of *Homo sapiens* (HS) and Ppt1 of *Saccharomyces cerevisiae* (SC) were aligned using ClustalW. The alignment was processed by Jalview and Blosum62 for visualization of the sequence homology.

Figure S2. Dose-dependent activation of PPH-5 by the identified compounds. Titration of PPH-5 with P5SA-1 (A), P5SA-2 (B), P5SA-4 (C) and P5SA-5 (D) in a pNPP-based phosphatase assay in a buffer of 40 mM HEPES, pH 7.5, 20 mM KCl, 5 mM $MnCl_2$, 1 mM DTT and 60 mM pNPP. With P5SA-3 no saturation curve could be obtained due to low affinity and stability.

Figure S3. The P5SAs neither influence the ATPase activity of *C. elegans* Hsc70/HSP-1 and *C. elegans* Hsp90/DAF-21 nor interfere with the interaction sites between the chaperones and their cochaperones. Concentrations well above the K_D of P5SA-1 (yellow), P5SA-2 (orange), P5SA-4 (red) and P5SA-5 (blue) were tested for their influence on the ATPase rates of the nematode chaperone systems. DMSO controls are shown in grey. Addition of the nucleotide exchange factor BAG-1 and the J protein DNJ-13 increases the activity of Hsc70/HSP-1, but the P5SAs have no effect on this system. The activator of Hsp90 AHA-1 acts as predicted by increasing the ATPase rate. P5SAs have again no effect on this chaperone system. Results are expressed as mean ± SD ($n \geq 3$).

Figure S4. The P5SAs only weakly influence the apparent K_M of pNPP. Substrate titrations of pNPP were done with 100 nM of PPH-5. The substrate binding curve in absence of activators is depicted in black. P5SAs were applied at their maximal soluble concentrations. Results are expressed as mean ± SD ($n \geq 3$).

Figure S5. The P5SAs are not able to activate in an additive manner. Concentrations of three times the K_D were used for each compound (P5SA-1 was 40μM, P5SA-2 was 23 μM, P5SA-4 was 70 μM and P5SA-5 was 16 μM. P5SA-3 was not used for solubility problems). Compunds were added in different combinations as indicated. Results are expressed as mean ± SD ($n \geq 3$).

Figure S6: Overlay of the human PP5 with the structures of the rat PP5. Stereo view of the backbone superposition of three molecules shown as ribbon plots: human apo PP5 (dark grey) with αJ-helix in cyan, rat apo PP5 (intermediate grey) with αJ-helix in green and PP5:P5SA-2 with αJ-helix in light grey and yellow.

Figure S1.

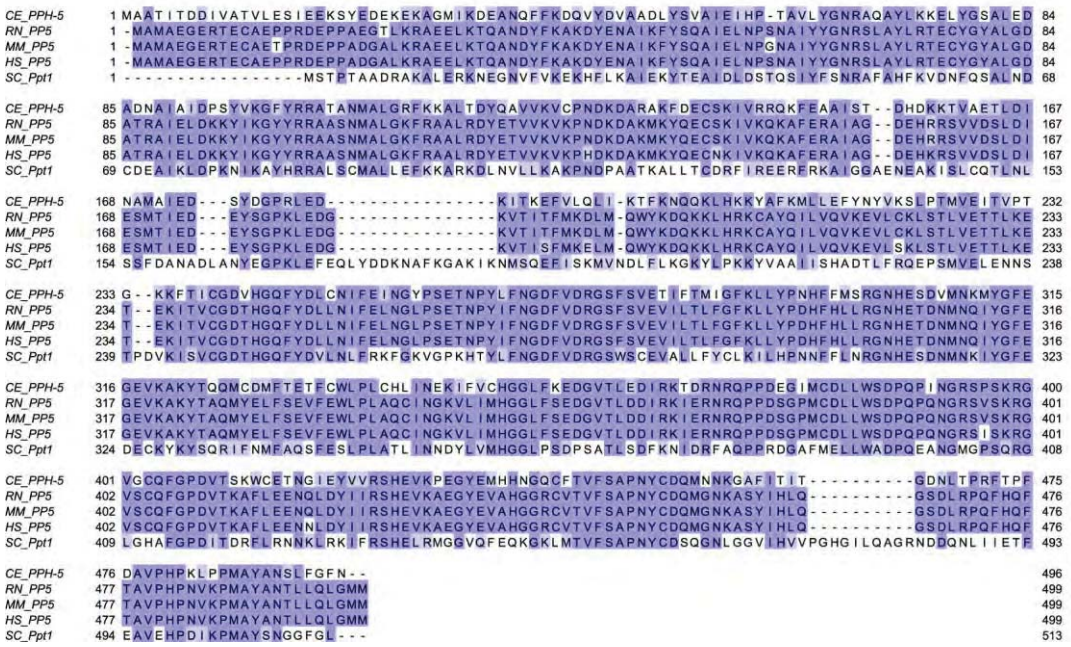
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Figure S2.

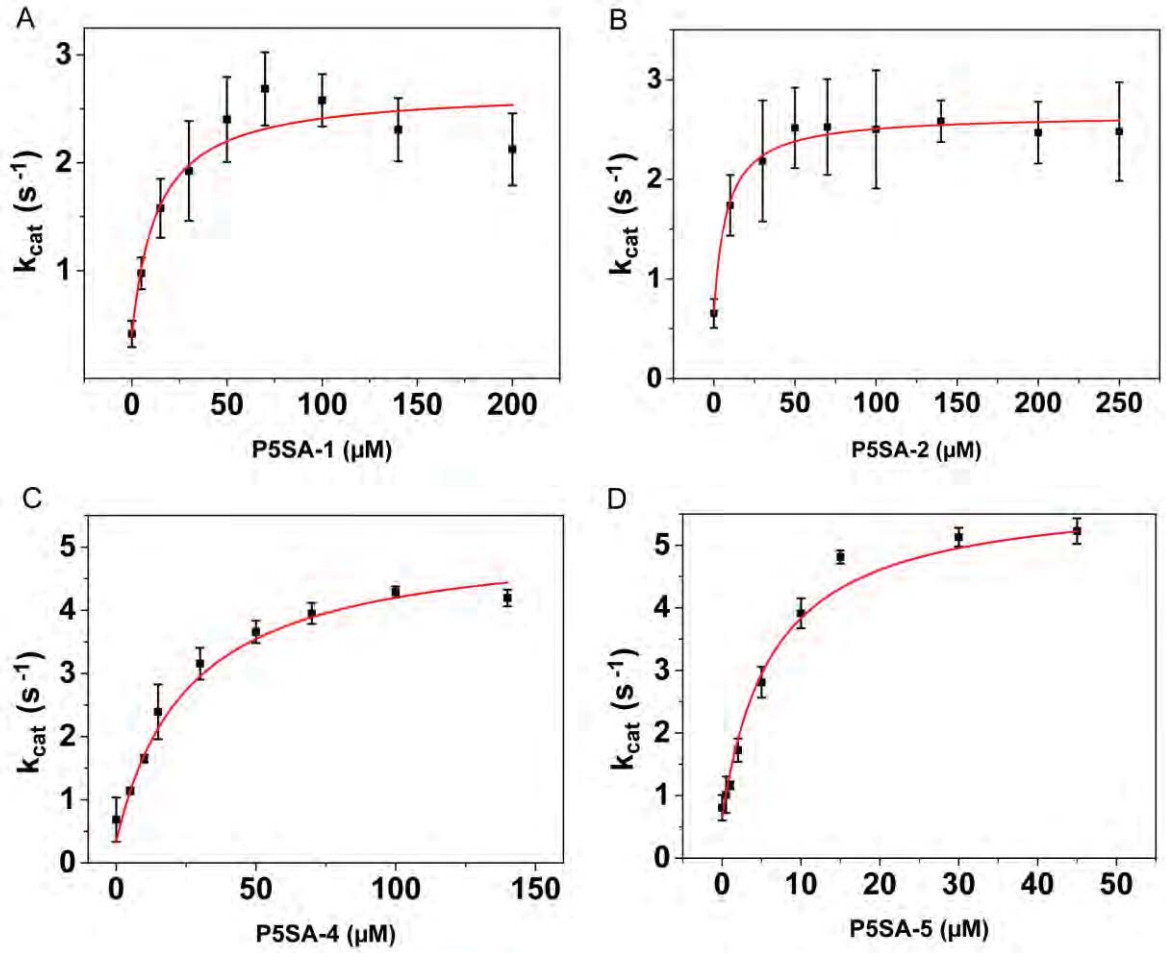

Figure S3.

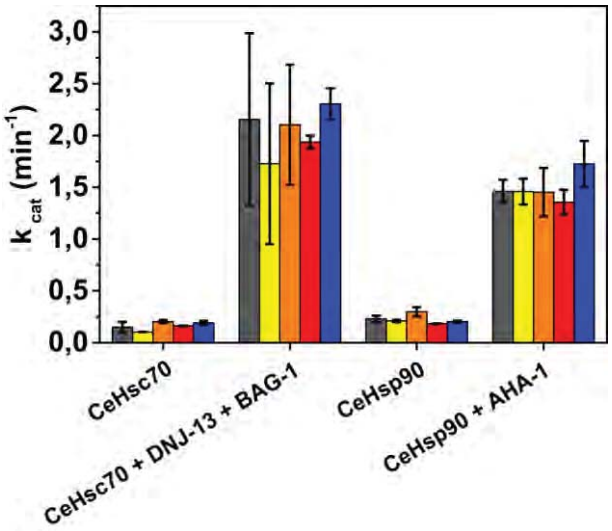

Figure S4.

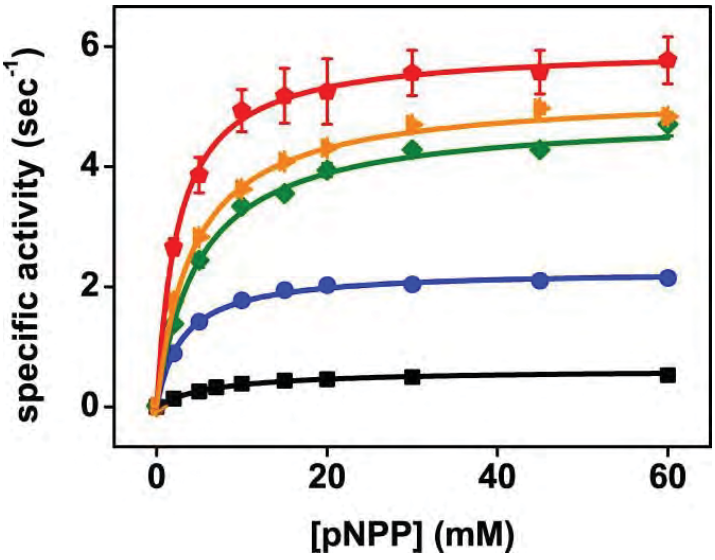

Figure S5.

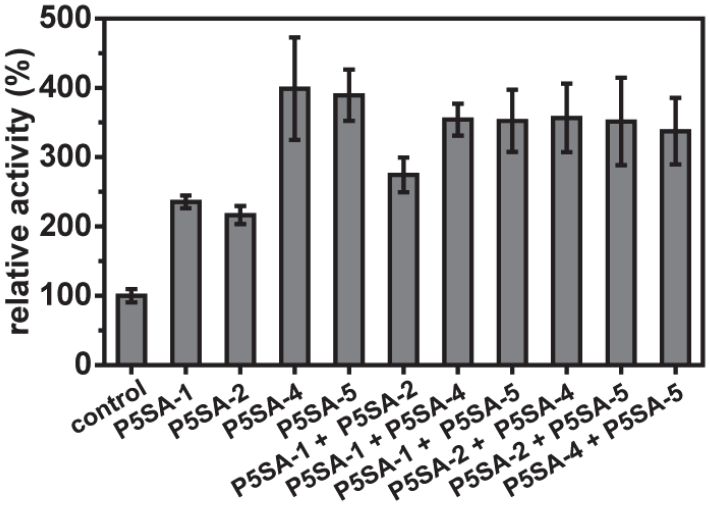

Figure S6.