Supplemental Figure legends (Supplemental data include six figures)

Figure S1A. Modeled structure of Hpo C-terminal coiled coil domain. NMR structure of Mst1 coiled coil domain was used as a template for modeling (25). The C-terminus of Hpo dimerize via an antiparallel coiled coil with kinked N-terminus.

Figure S1B. Quantification of Hpo mutants' wings. Quantification of wing size area in 10^5 pixel for Hpo and the mutants (N=20). N is the number of wings analyzed. Error bars indicate standard error of the mean (SEM).

Figure S2. FRET assay of S2 cells expressing CFP/YFP tagged Hpo constructs. S2 cells were transfected with indicated CFP-tagged or YFP-tagged Hpo constructs in FRET assay. CFP signal was obtained once before and once after photobleaching YFP using the 80% power of 514 nm laser line to bleach 12 times at the selected area, leaving the other part as internal control.

Figure S3A. Hpo with N terminal mutation still interact with each other. Extracts from S2 cells expressing indicated Flag-Hpo-N mutations were incubated with GST or indicated GST fusion proteins. The bound proteins were analyzed by Western blot with Flag antibody.

Figure S3B. Hpo N-terminal truncated mutants interact with the endogenous Hpo, but Hpo-C-I634D barely binds endogenous Hpo. S2 cells were transfected with indicated Hpo N- or C-terminal truncated mutants, and the Flag-Hpo-N or -C was immunoprecipitated with anti-Flag antibody. Western blots were done to detect the endogenous Hpo using the endogenous Hpo antibody. Wild type Hpo-N interact endogenous Hpo weakly because of the lower protein level.

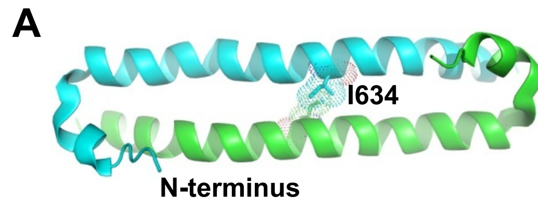
Figure S4. Conformational perturbation of the homo-dimeric Hpo kinase domain affects Hpo kinase activity. S2 cells were transfected with the full length Hpo with various homo-dimeric interface mutations respectively (H240A, M242E, M242P, R243P, R243A, I245P) or transfected with the N-terminal homo-dimeric interface mutations of Hpo-N (H240A-N, M242E-N, M242P-N, R243P-N, R243A-N, I245P-N) together with Sd, Yki plus the *3×Sd2-Luc* reporter genes, and the cell lysates were subjected to dual luciferase assay. H240A is a control. Error bars indicate standard deviation.

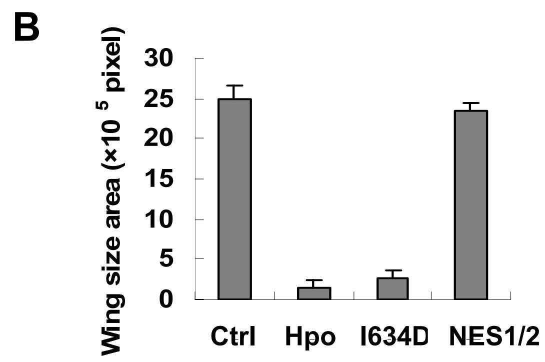
Figure S5. Induced dimer of Hpo kinase domain could promote higher pathway activity. S2 cells were transfected with the indicated kinase domain of Hpo or Hpo hybrid variants together with Sd, Yki plus the *3×Sd2-Luc* reporter gene, and the cell lysates were subjected to dual luciferase assay. Error bars indicate standard deviation (triplicate wells). AP20187 treatment did not influence the activity of Hpo-N. N terminal or C terminal of Hpo kinase domain fused with Fv2 domain (Fv2-HpoN, HpoN-Fv2) exhibit higher activity when treated with AP20187 to induce dimer. Also HpoN-T195E-Fv2 showed inducible activity by AP20187 but not for M242E

or T195A.

Figure S6. Hpo variants genetically interact with Yki. Front views of adult flies from wild type eyes of *GMR* (A), *GMR-Gal4 UAS-Yki* (B), *GMR-Gal4 UAS-Yki* plus Hpo wild type (C), *GMR-Gal4 UAS-Yki* plus Hpo-KD (D), *GMR-Gal4 UAS-Yki* plus Hpo variants (E-I). (J) Quantification of wing size area in 10^5 pixel for Hpo and the mutants (N=20). N is the number of wings analyzed. Error bars indicate standard error of the mean (SEM).

Jin_Figure S1





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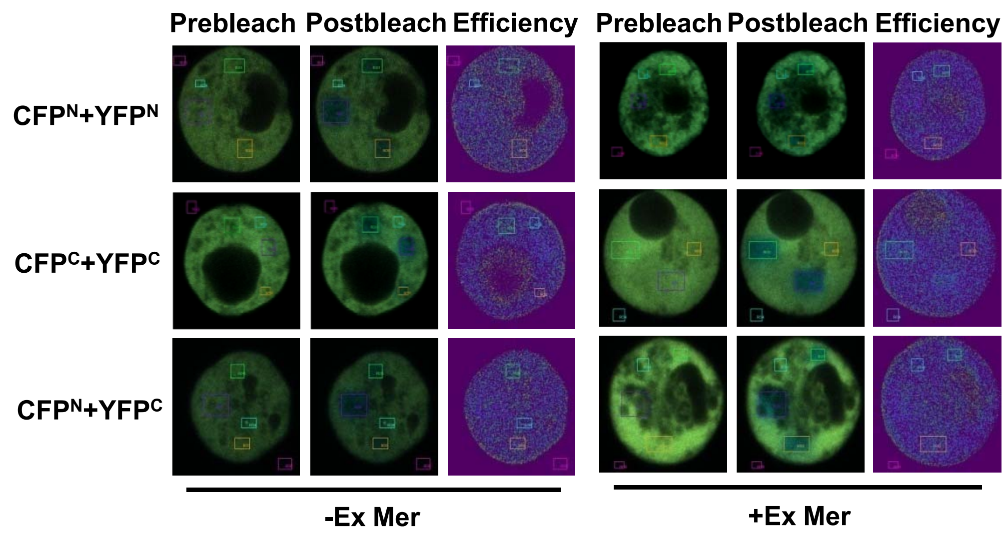

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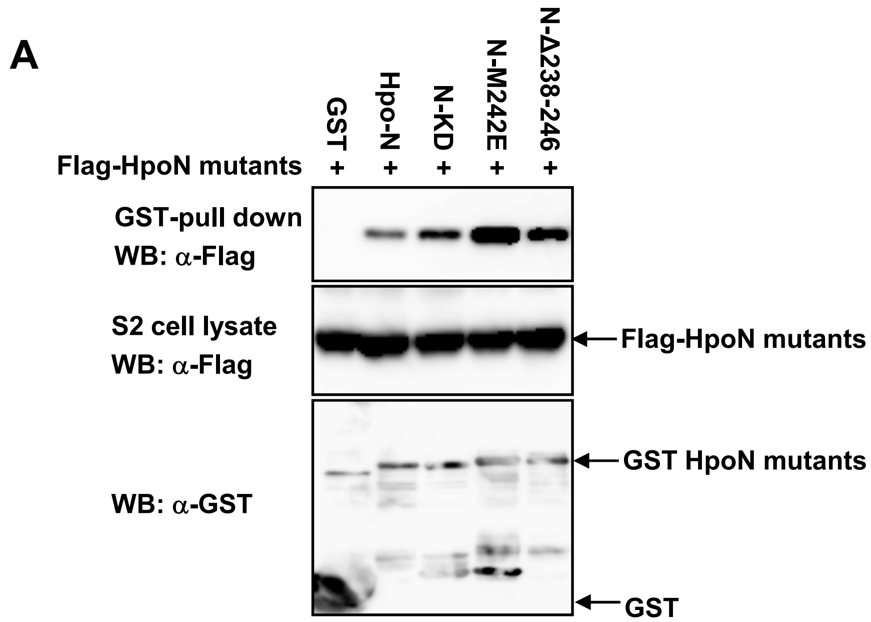

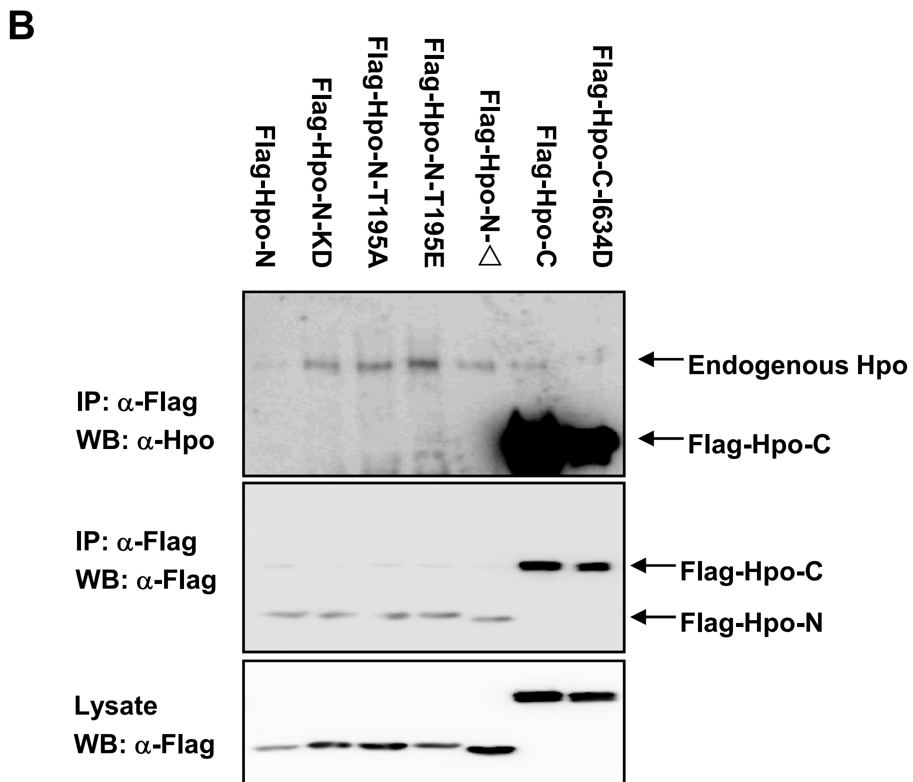

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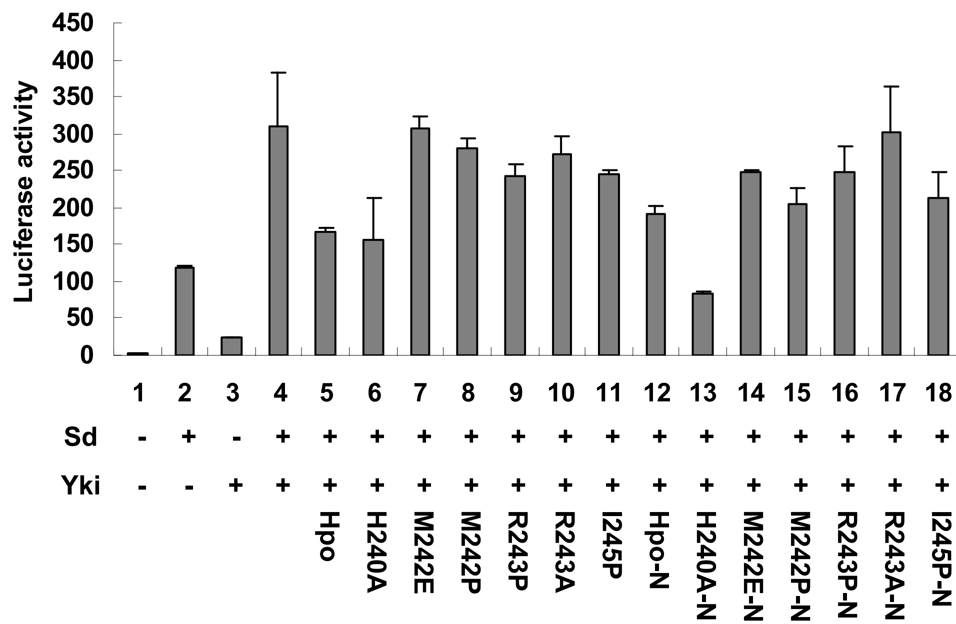

Jin_Figure S5

Luciferase activity

1800
1600
1400
1200
1000
800
600
400
200
0

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
				HpoN		Fv2-HpoN		HpoN-Fv2		N-T195A-Fv2		N-T195E-Fv2		N-M242E-Fv2	
Sd,Yki	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AP20187	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+

Jin_Figure S6