

## Supplementary Figure Legends

Fig. S1. KLHL20 interacts with DAPK. 293T cells were transfected with DAPK and/or KLHL20 expression constructs as indicated. Cells were lysed for immunoprecipitation and/or Western blot analyses with antibodies as indicated.

Fig. S2. Characterization of the anti-KLHL20 antiserum. HeLa cells or HeLa cells transfected with KLHL20-Flag were lysed for Western blot with the KLHL20 anti-serum.

Fig. S3. The catalytic activity of DAPK does not affect its binding to KLHL20. 293T cells were co-transfected with KLHL20-Myc and/or DAPK-Flag or its mutant. Cells were lysed for immunoprecipitation and/or Western blot analyses with antibodies as indicated.

Fig. S4. Generation of a KLHL20 mutant that cannot bind Cul3. (A) Superimposition of the structure of Skp1 (cyan) with that of KLHL20 BTB domain (green). The structure of KLHL20 BTB domain is predicted by SWISS-MODEL. The six residues in Skp1 that contact Cul1 and the corresponding residues in KLHL20 BTB domain are shown in red. These six residues in the predicted structure of KLHL20 are indicated on the right and mutated to generate the KLHL20m6 mutant. (B) KLHL20m6 mutant fails to bind Cul3. HeLa cells transfected with KLHL20 or KLHL20m6 mutant were lysed for immunoprecipitation and/or Western blot analyses with antibodies as indicated.

Fig. S5. Purification of the KLHL20-based Cul3 complex by GST pull down analysis. 293T cells transfected with various constructs were lysed for GST pull down analysis. Bound proteins were resolved on SDS-PAGE and then subjected to Western blot with antibodies as indicated. The expression levels of various proteins were analyzed by Western blot (bottom

panel).

Fig. S6. IFN- $\gamma$  does not affect the formation of ROC1-Cul3-KLHL20 complex. 293T cells transfected with various constructs were treated with or without IFN- $\gamma$  for 18 h. Cells were lysed for GST pull down analysis and/or Western blot analysis with antibodies as indicated.

Fig. S7. IFN- $\alpha$  induces an enrichment of KLHL20 in PML-NBs. HeLa cells were treated with or without IFN- $\alpha$  for 18 h. Cells were fixed and stained as in Fig. 5B. The box area was amplified to show the colocalization of KLHL20 and PML. Bar, 20  $\mu$ m.

Fig. S8. DAPK and PML compete for binding KLHL20. Myc-KLHL20 purified from transfected cells were incubated with a fixed amount of baculovirally purified DAPK and an increasing amount of baculovirally purified PML-I. Proteins coprecipitated with KLHL20 were analyzed by Western blot.

Fig. S9. Depletion of PML abolishes IFN- $\gamma$ -induced relocation of KLHL20 in PML-NBs. HeLa cells stably expressing PML siRNA#2 were treated with or without IFN- $\gamma$  for 18 h. Cells were then fixed and stained with DAPI, anti-KLHL20 antibody and anti-PML antibody. Bar, 20  $\mu$ m.

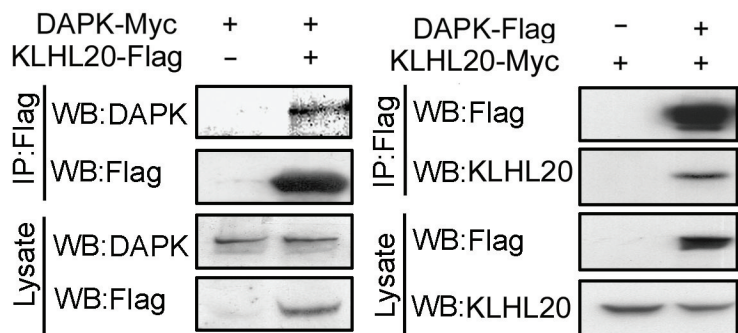
Fig. S10. IFN- $\gamma$  treatment of MCF7 cells induces the concentration of KLHL20 to PML-NBs. MCF7 cells were treated with or without IFN- $\gamma$ , fixed and stained as in Fig. 5B. Bar, 20  $\mu$ m.

Fig. S11. IFN- $\gamma$  does not increase the amount of S308-phosphorylated DAPK. HeLa cells were treated with or without IFN- $\gamma$  for 18 h and then lysed for Western blot analysis with

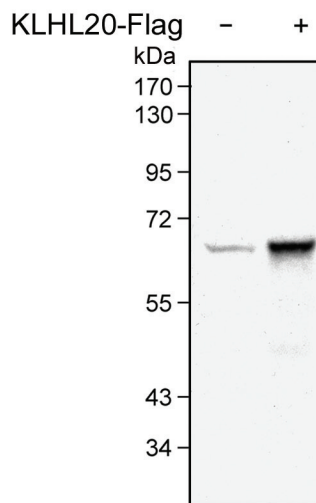
anti-DAPK and anti-pS308-DAPK antibodies.

# Supplementary information

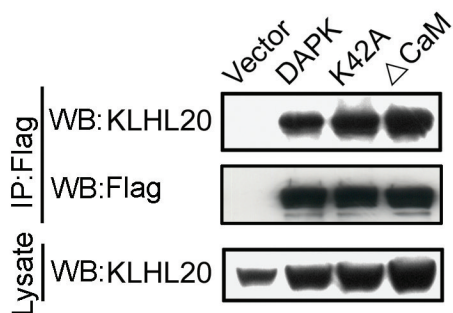
## Fig. S1



## Fig. S2

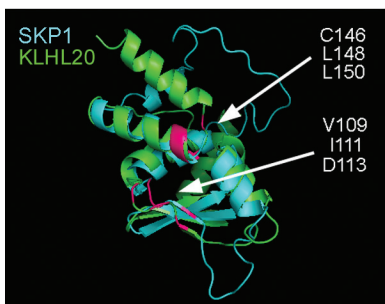


## Fig. S3

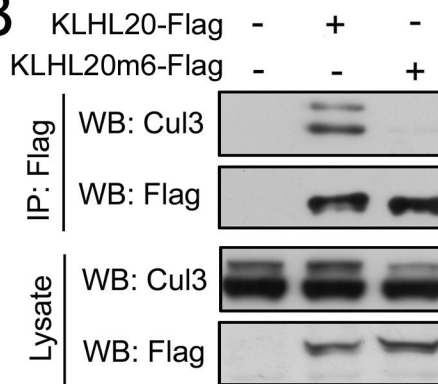


## Fig. S4

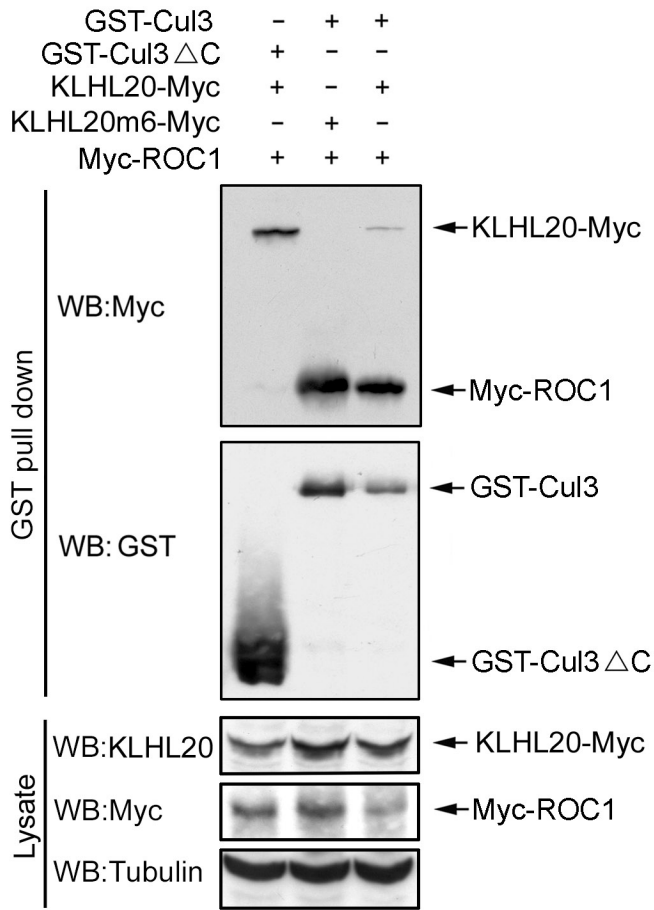
### A



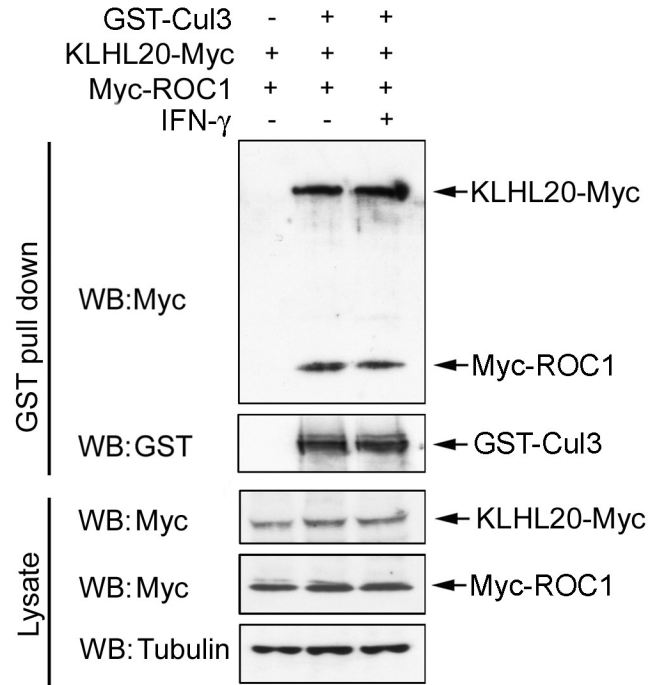
### B



# Fig. S5



# Fig. S6



# Fig. S7

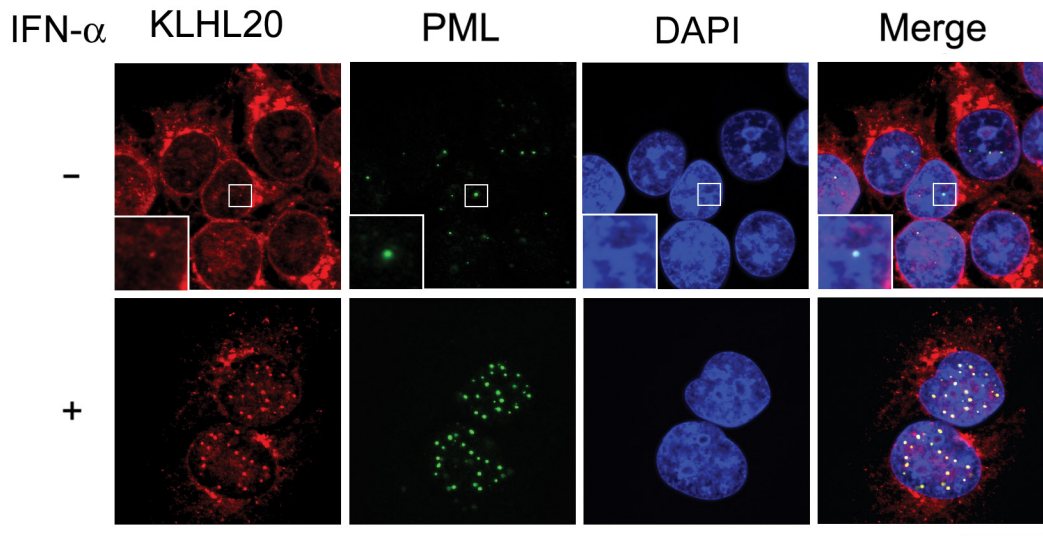


Fig. S8

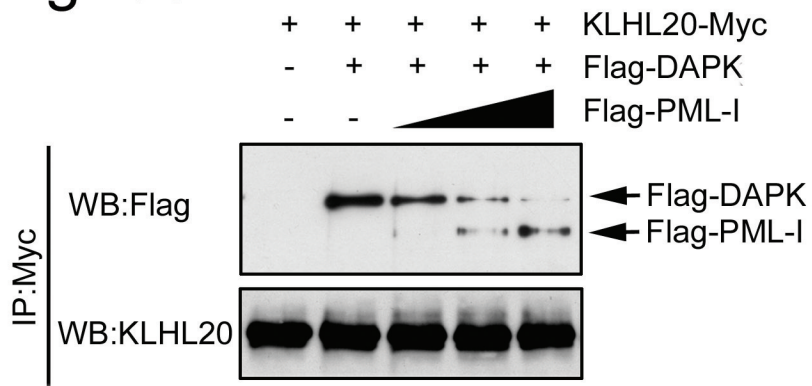


Fig. S11

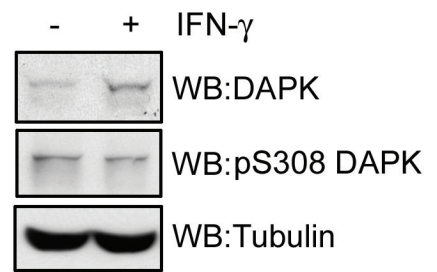


Fig. S9

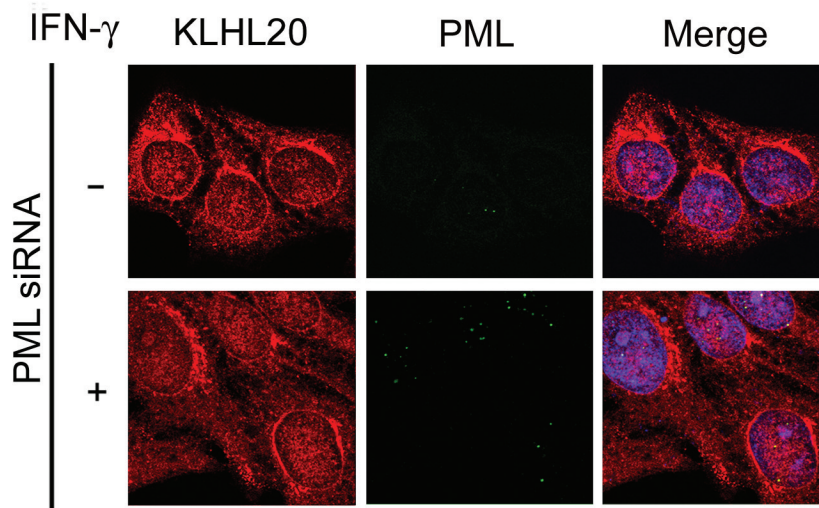


Fig. S10

