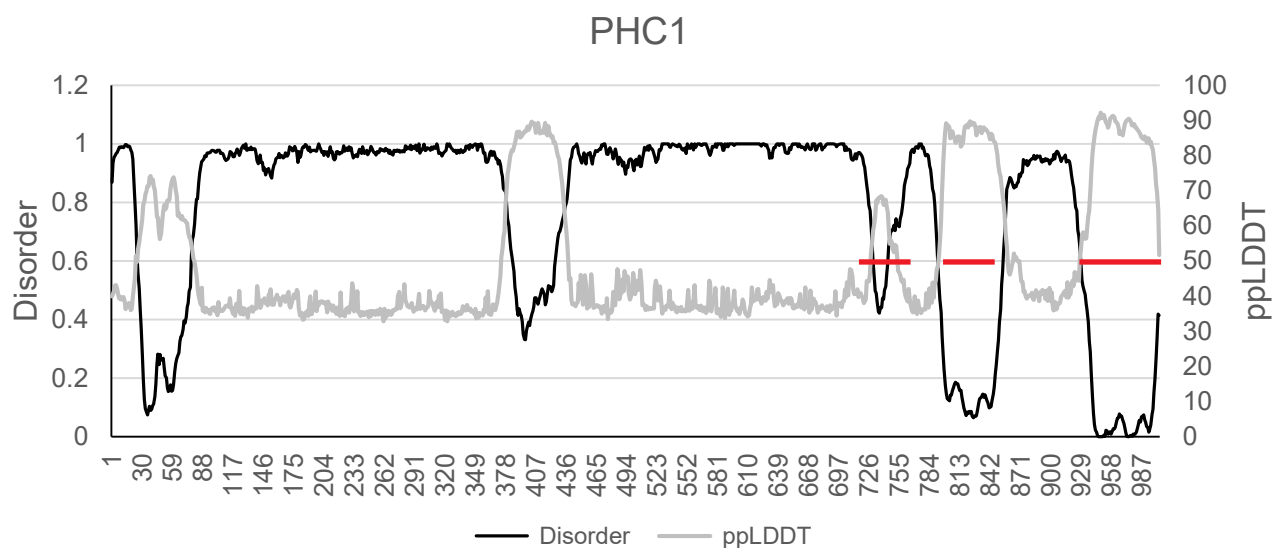
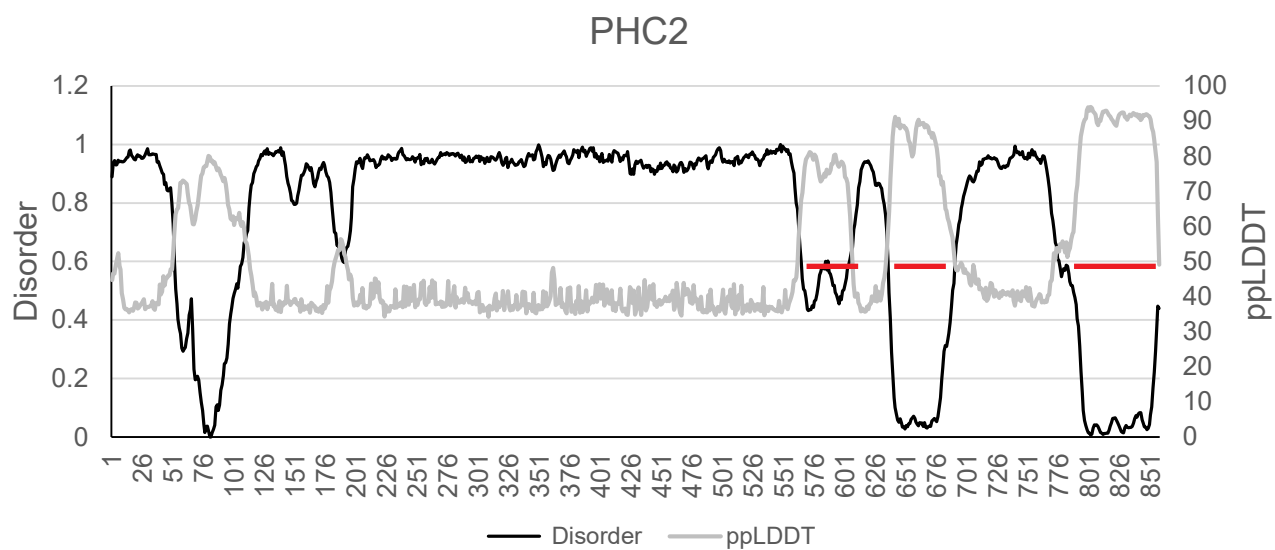


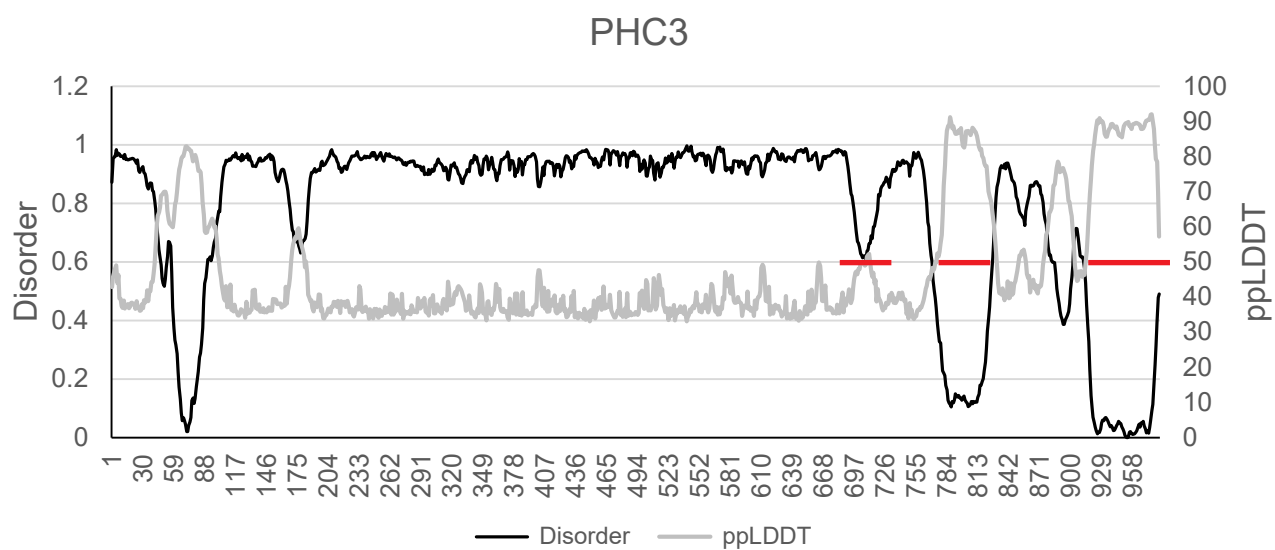
A



B



C



**Figure S1 Predicted disorder in Polyhomeotic-like Ph homologues. (A-C)** Metapredict2 analysis of PHC1-3. Red lines indicate positions of SAM, FCS, and HD1 domains, from C- to N-terminus. X-axis is amino acid position.

positively charged  
negatively charged  
polar  
aliphatic  
aromatic  
proline

### Ph 1

M D R R A L K F M Q K R A D T E S D T T T P V S T T A S Q Q I S A S A I L A G G T L P L K D N S N I  
R E K P L H H N Y N H N N N S S Q H S H S H Q Q Q Q Q Q Q V G G K Q L E R P L K C L E T L A Q K A  
G I T F D E K Y D V A S P P H P G I A Q Q Q A T S G T G P A T G S G S V T P T S H R H G T P P T G R  
R Q T H T P S T P N R P S A P S T P N T N C N S I A R H T S L T L E K A Q N P G Q Q V A A T T T V P  
L Q I S P E Q L Q Q F Y A S N P Y A I Q V K Q E F P T H T T S G S G T E L K H A T N I M E V Q Q Q L  
Q L Q Q Q L S E A N G G G A A S A G A G G A A S P A N S Q Q S Q Q Q Q H S T A I S T M S P M Q L A A  
A T G G V G G D W T Q G R T V Q L M Q P S T S F L Y P Q M I V S G N L L H P G G L G Q Q P I Q V I T  
A G K P F Q G N G P Q M L T T T T Q N A K Q M I G G Q A G F A G G N Y A T C I P T N H N Q S P Q T V

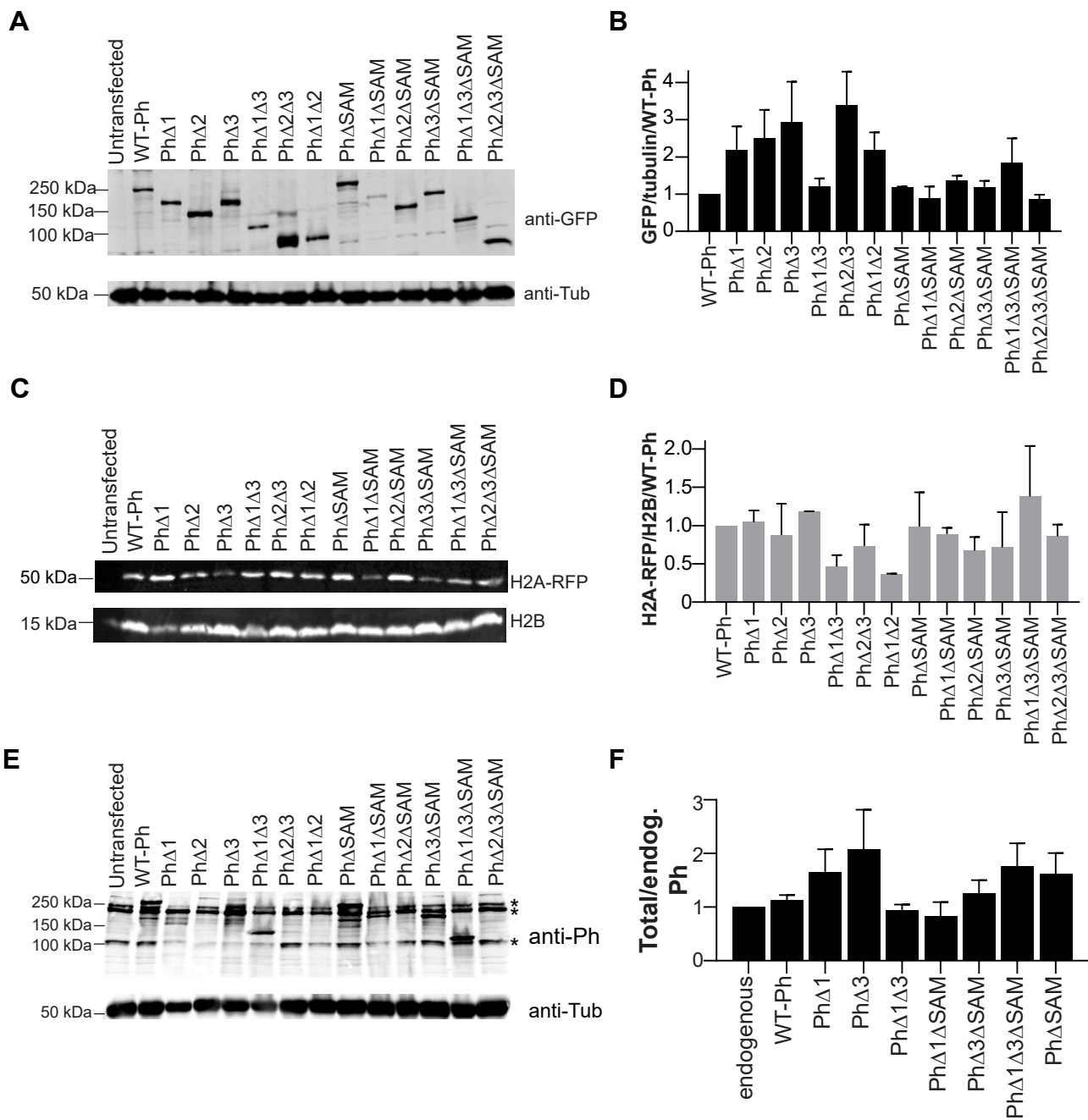
### Ph2

L F S P M N V I S P Q Q Q Q N L L Q S M A A A A Q Q Q Q L T Q Q Q Q Q F N Q Q Q Q Q Q L T Q Q Q Q Q  
L T A A L A K V G V D A Q G K L A Q K V V Q K V T T T S S A V Q A A T G P G S T G S T Q T Q Q V Q Q  
V Q Q Q Q Q Q T T Q T T Q Q C V Q V S Q S T L P V G V G G Q S V Q T A Q L L N A G Q A Q Q M Q I P W  
F L Q N A A G L Q P F G P N Q I L R N Q P D G T Q G M F I Q Q Q P A T Q T L Q T Q Q N Q I I Q C N  
V T Q T P T K A R T Q L D A L A P K Q Q Q Q Q Q V G T T N Q T Q Q Q Q L A V A T A Q L Q Q Q Q Q Q  
L T A A A L Q R P G A P V M P H N G T Q V R P A S S V S T Q T A Q N Q S L L K A K M R N K Q Q P V R  
P A L A T L K T E I G Q V A G Q N K V V G H L T T V Q Q Q Q Q A T N L Q Q V V N A A G N K M V V M S  
T T G T P I T L Q N G Q T L H A A T A A G V D K Q Q Q Q L Q L F Q K Q Q I L Q Q Q Q M L Q Q Q I A A  
I Q M Q Q Q Q A A V Q A Q Q Q Q Q Q V S Q Q Q Q V N A Q Q Q Q A V A Q A Q Q Q Q A V A Q A Q Q Q Q R E  
Q Q Q Q V A Q A Q A Q H Q Q A L A N A T Q Q I L Q V A P N Q F I T S H Q Q Q Q Q Q Q L H N Q L I Q Q  
Q L Q Q Q A Q A Q V Q A Q V Q A Q A Q Q Q Q Q Q R E Q Q Q N I I Q Q I V V Q Q S G A T S Q Q T S Q Q

### Ph3

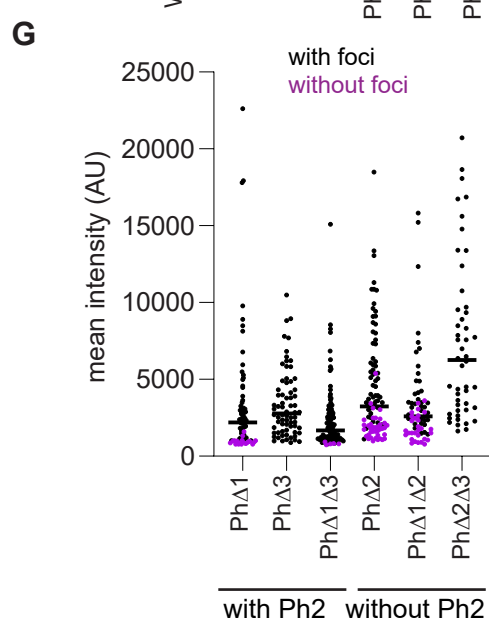
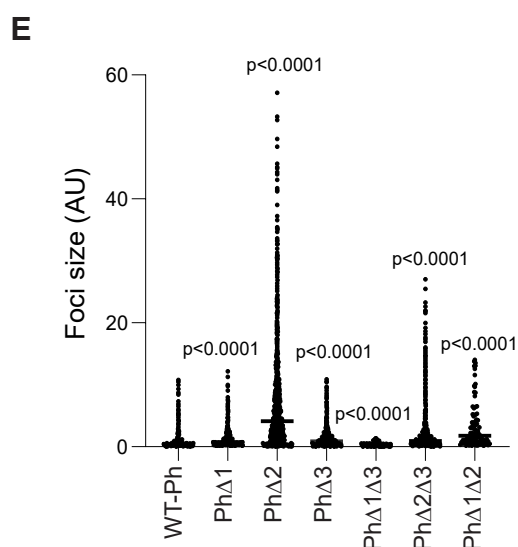
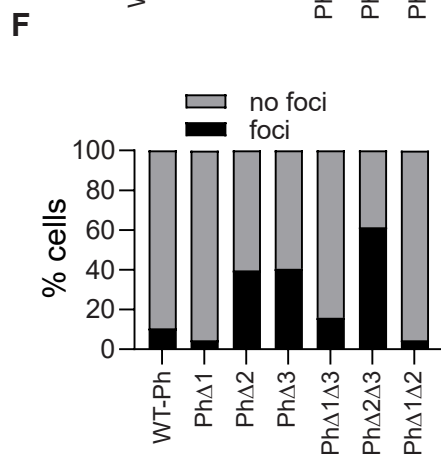
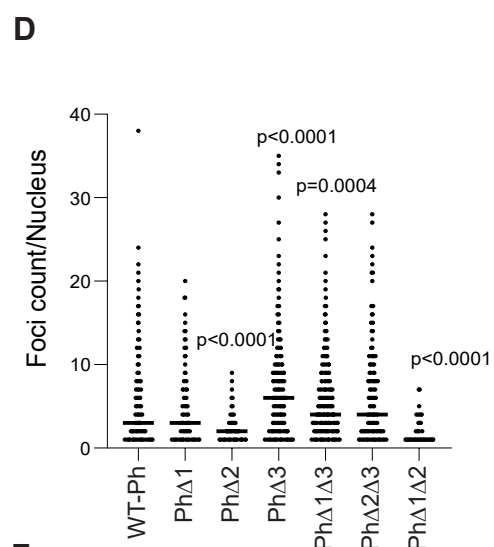
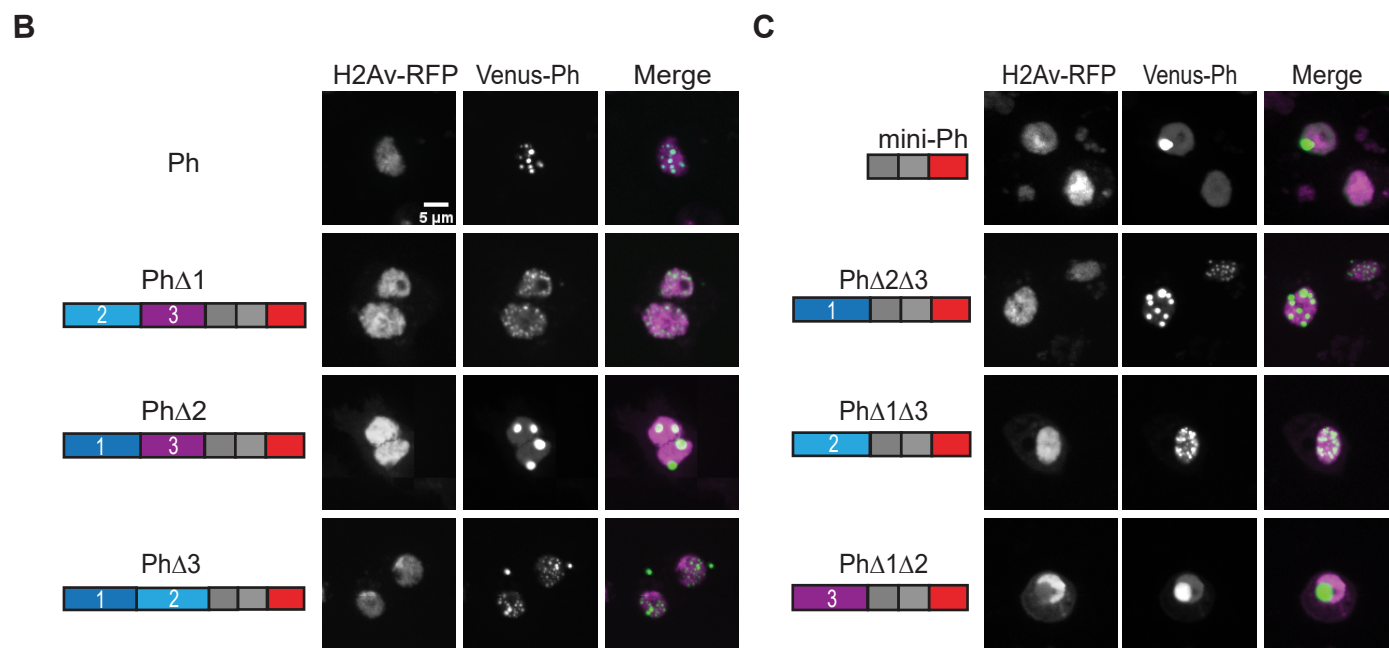
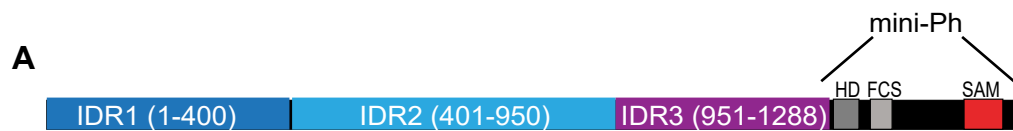
Q Q H H Q S G Q L Q L S S V P F S V S S S T T P A G I A T S S A L Q A A L S A S G A I F Q T A K P G  
T C S S S S P T S S V V T I T N Q S S T P L V T S S T V A S I Q Q A Q T Q S A Q V H Q H Q Q L I S A  
T I A G G T Q Q Q P Q G P P S L T P T T N P I L A M T S M M N A T V G H L S T A P P V T V S V T S T  
A V T S S P G Q L V L L S T A S S G G G G S I P A T P T K E T P S K G P T A T L V P I G S P K T P V  
S G K D T C T T P K S S T P A T V S A S V E A S S S T G E A L S N G D A S D R S S T P S K G A T T P  
T S K Q S N A A V Q P P S S T T P N S V S G K E E P K L A T C G S L T S A T S T S T T T T I T N G I  
G V A R T T A S T A V S T A S T T T T S S G T F I T S C T S T T T T T S S I S N G S K D L P K

**Figure S2 Complete sequences of Ph IDRs.** Sequences are coloured based on amino acid type for Ph1(A), Ph2 (B), and Ph3(C). Figures were generated using Local Cider (<http://pappulab.wustl.edu/CIDER/analysis/>).



**Figure S3 Transfected proteins are (over)expressed as full-length proteins. (A)**

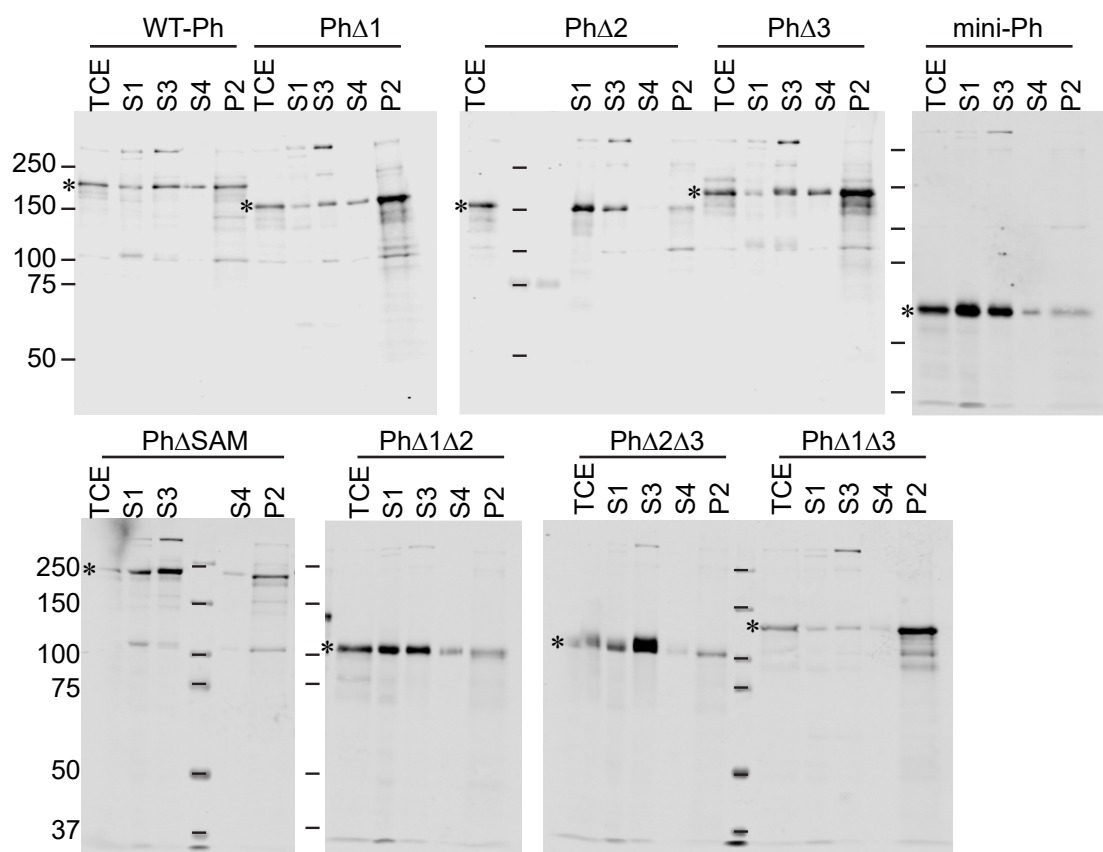
Representative Western blots of cells transfected with Venus-Ph proteins. **(B)** Quantification of Venus-Ph levels. Anti-GFP signal was normalized to Tubulin signal and then to WT-Ph from the same experiment. **(C)** H2Av-RFP, detected with anti-RFP, to measure transfection efficiency. **(D)** Quantification of H2Av-RFP, which was normalized to H2B and then to signal from WT-Ph from the same experiment. **(E)** Representative anti-Ph Western blot to measure total Ph levels (endogenous + transfected). Asterisks next to blots indicate the position of endogenous Ph bands. Note that the antibody epitope is in Ph2 so that proteins lacking this region are not detected. Venus-Ph $\Delta$ 1 and Venus-Ph $\Delta$ 3 migrate similarly to Ph-d. **(F)** Quantification of anti-Ph Western blots. Ph signals were normalized to tubulin, and then normalized to the signal from untransfected cells. Bars show the mean, and error bars the SEM. n=3 (B, D); n=2 (F).



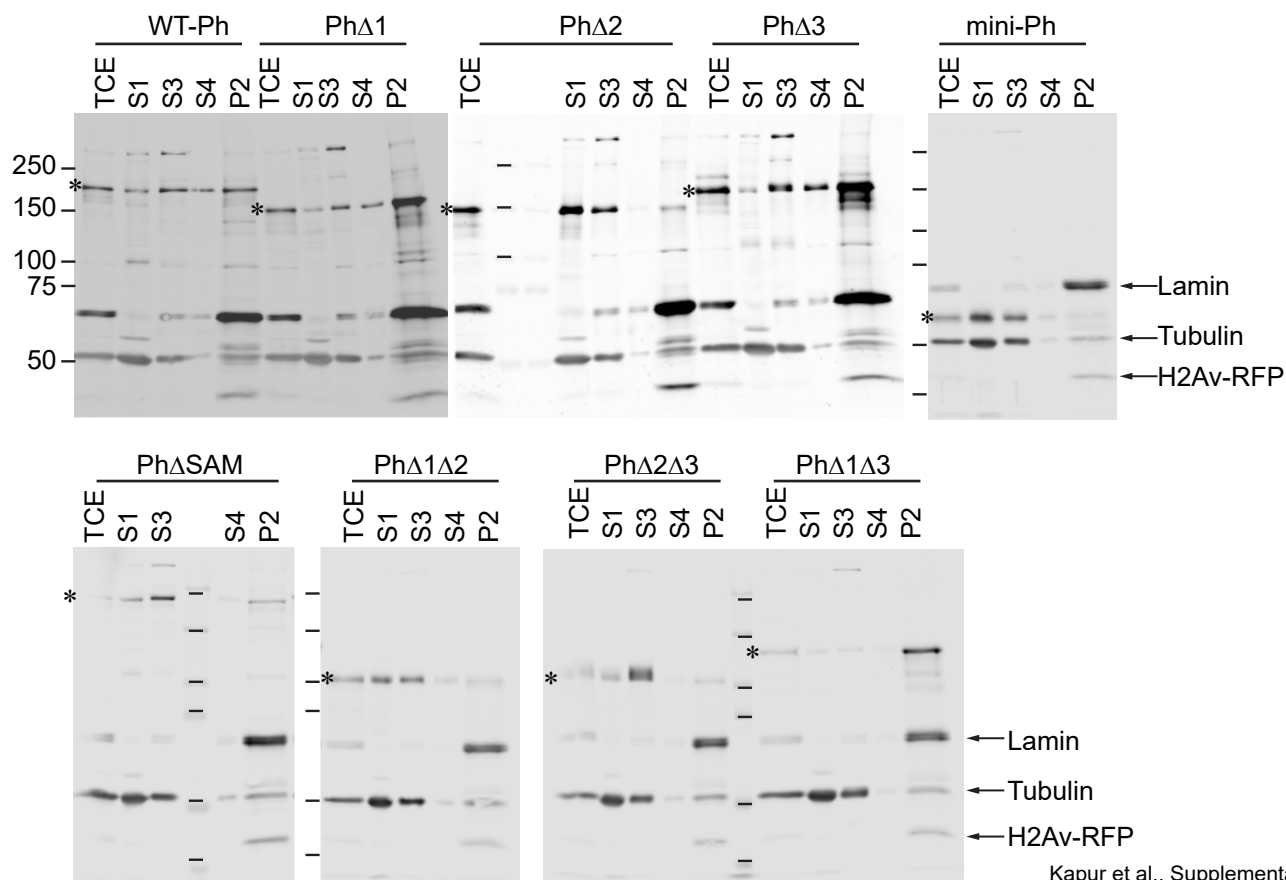
**Figure S4 Summary of effects of Ph IDRs on condensate formation.** (A) Schematic of Ph, indicating IDR boundaries. (B, C) Representative images showing effects of removing one (B), or two (C) IDRs. (D, E) Graphs of number of foci per nucleus (D) and size (E). (F) % of H2Av-RFP positive cells for which foci were identified. (G) Comparison of mean intensities measured for each Ph construct. WT-Ph and mini-Ph are not included in this comparison because the images were collected with different parameters so the intensities are not comparable. Data are taken from Fig. 3-5, but repeated to allow full comparison. See main figure legends for n-values.



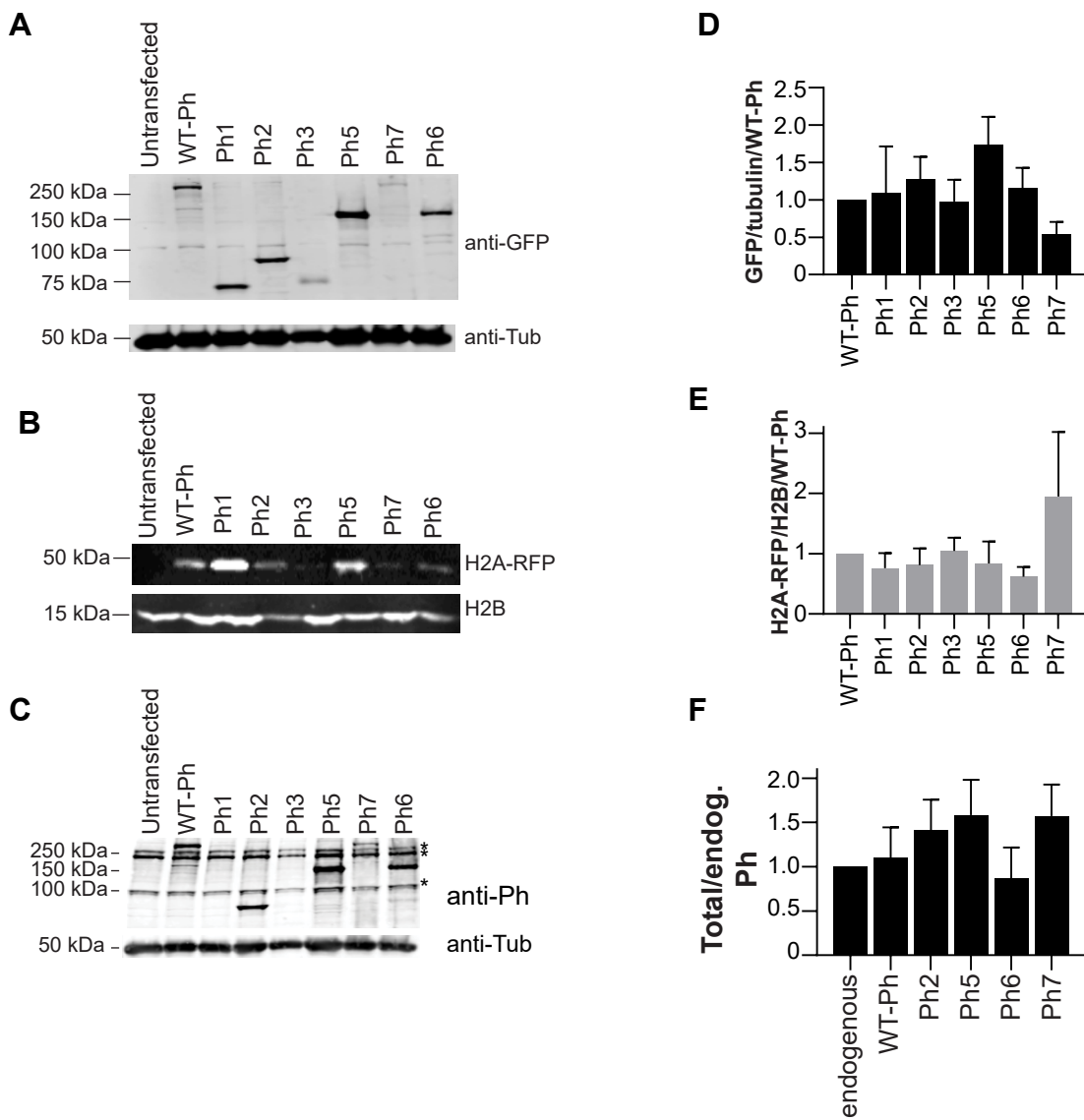
**A** anti-GFP \*Venus-Ph



**B** anti-Lamin, anti-Tubulin, anti-RFP \*Venus-Ph



**Figure S5 Full gels of Western blots of cell fractionation.** (A) Full blots of anti-GFP Western blots used to quantify Venus-Ph proteins in different cellular fractions (Fig. 6). TCE=total cell extract. Half as much TCE and twice as much P2 were loaded to avoid signal saturation. Asterisks indicate position of each Venus-Ph protein. (B). The same blots as in A were reprobed with anti-Lamin, anti-Tubulin, and anti-RFP (to detect H2Av-RFP). The anti-GFP signal is still visible, and indicated with an asterisk. Anti-Lamin and anti-Tubulin were probed with different secondaries, but the images were merged.



**Figure S6 Quantification of Ph IDR expression levels in transfected cells. (A-C)**

Representative Western blots of cells transfected with Venus-Ph IDR constructs. anti-GFP is used to quantify Venus (A) and anti-RFP to quantify H2Av-RFP (B) (to measure transfection efficiency). anti-Ph (C) indicates the amount of IDR+ Ph in cells. Because the antigen for the anti-Ph antibody is in Ph2, only proteins containing this region can be measured (Ph2, Ph5, Ph6, Ph7). **(D-E)** Quantification of Western blot signals. D and F are normalized to tubulin, and E to H2B. Signals are then normalized to WT-Ph from the same experiment (D, E), or untransfected (F) (endogenous Ph levels). Bars are mean and error bars are SEM. n=3 (D); n=2 (E, F).