

1 **Supplementary Figures**

2

3 **Figure S1.** Auto-activation tests of all Giles proteins in pGBKCg (vs. empty pGADT7g). The  
4 baits gp11, gp25 and gp77 were identified as auto activators as they show growth at 50mM 3-  
5 AT. These baits were not screened in that particular vector combination.

6

7 **Figure S2. (A)** A representative Y2H screen showing a specific protein-protein interaction is  
8 shown (red arrow and square). No 3-AT was used here and no background growth is visible,  
9 indicating the specificity of the interacting bait and prey pair (here: gp7 vs. gp77). A negative  
10 control (empty prey vector) is also shown.

11 **(B)** A representative Y2H screen in the presence of 3-AT in selective media. The use of 3-AT in  
12 media (lower plate) can suppress background on -LTH plates (upper plate). Many non-specific  
13 false positives were seen in the absence of 3-AT whereas in the presence of 3-AT, only a specific  
14 interaction (shown by red arrow) was detected (here: gp14 vs. gp61).

15

16 **Figure S3.** A representative Y2H retest screen is shown at different 3-AT concentrations. A  
17 series of 3-AT concentrations (0-50mM) was used to quantify the strength of PPIs. The non-  
18 interacting prey proteins and empty prey vector were used as controls, to confirm the specificity  
19 of interacting pairs. The non-interacting prey proteins for specific baits were identified from the  
20 raw interactions data set.

21

22 **Figure S4.** Flowchart illustrating calculation of the IScore.

23

24 **Figure S5.** Excess retention for essential vs. non-essential proteins, by each K-core within the

25 Giles interactome network.

26

27 **Figure S6.** A diagram of gene/Pham conservation across mycobacteriophages, using Giles as a  
28 guide. Giles genome map after Morris et al. (2008). **(A)** Conservation of phage protein Phams  
29 across other mycobacteriophage clusters. Square nodes indicate mycobacteriophage clusters  
30 containing the specified gene in at least one phage genome. “Single” denotes a singleton (non-  
31 clustered) phage.

32

33 **Figure S7.** Protein interactions from the Giles interactome in the context of conserved Giles  
34 genes. Lines indicate binary protein-protein interactions. This figure contains the subset of high-  
35 confidence interactions with %IScore values above 30.

36

37 **Figure S8.** Raw PPIs were filtered using assignment of a % IScore. Only PPIs with (IScore  $\geq 0$ )  
38 were included in the high confidence PPIs data set. See **Materials and Methods** for details.

39

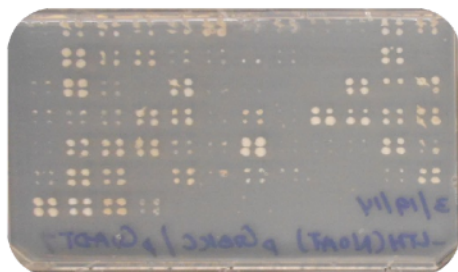
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41

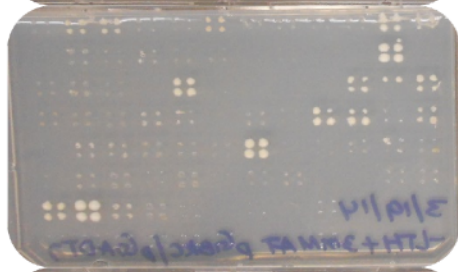
Fig. S1

Bait activation tests

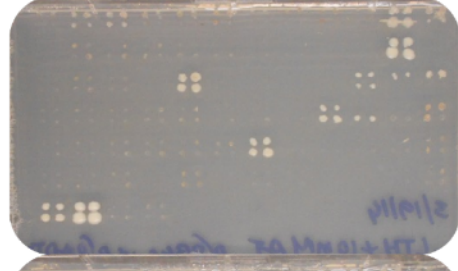
pGBKCg vs pGADT7g



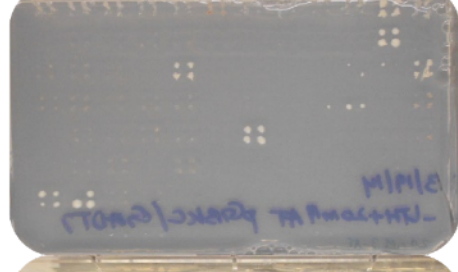
0mM 3AT



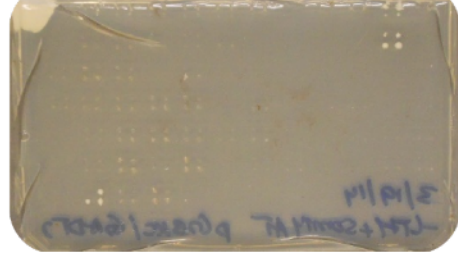
3mM 3AT



10mM 3AT



20mM 3AT



50 mM 3AT

pGBGT7g vs pGADCg

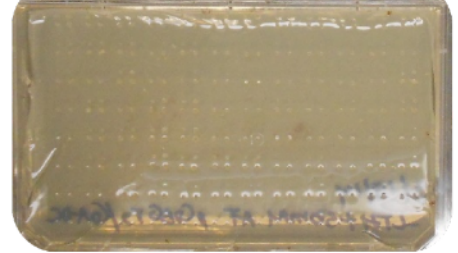
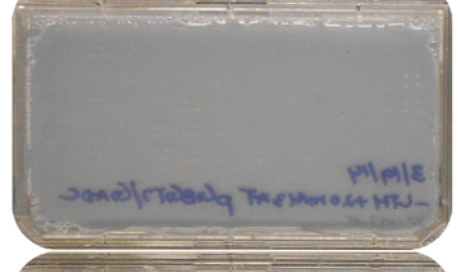
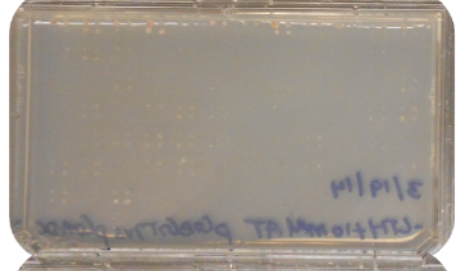
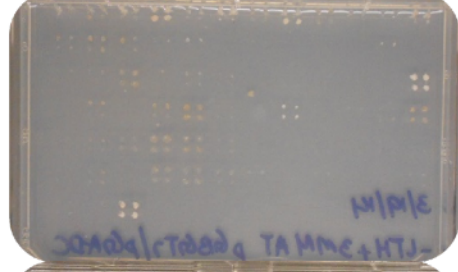
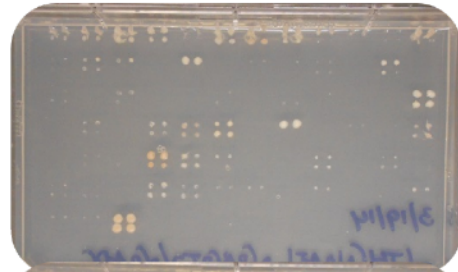
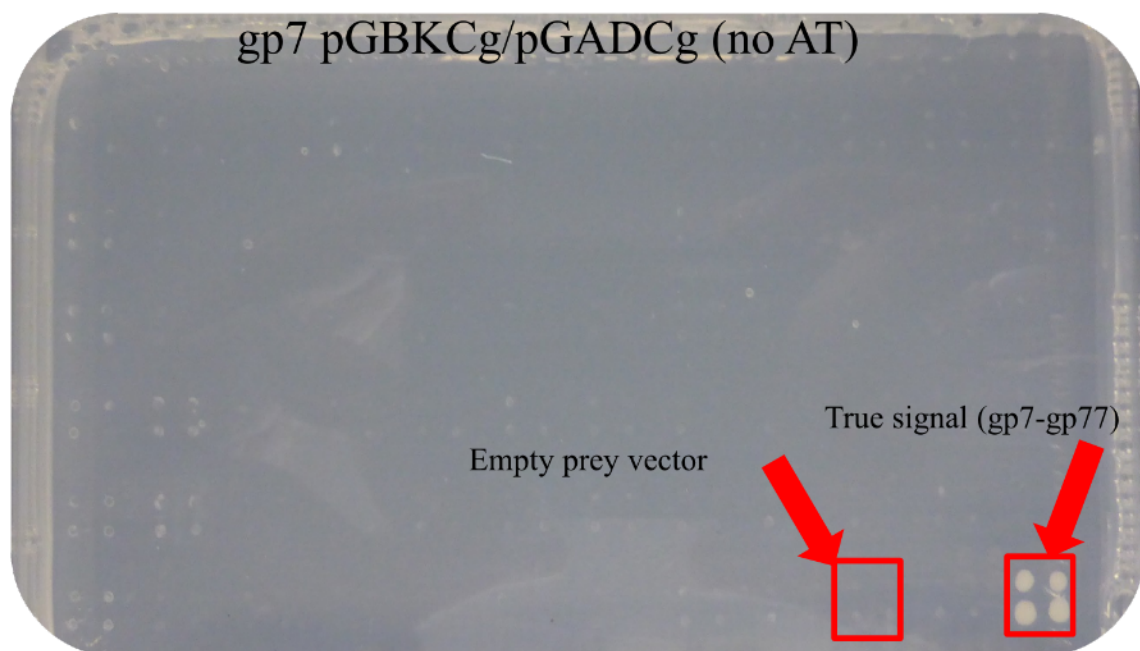


Fig. S2

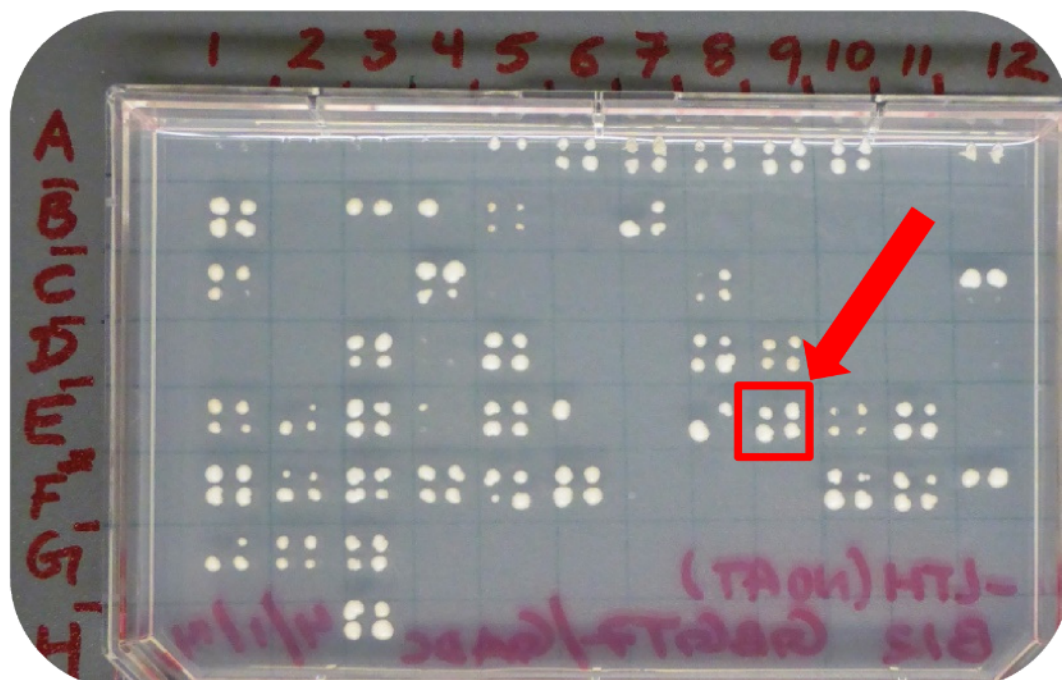
A

gp7 pGBKCg/pGADCg (no AT)

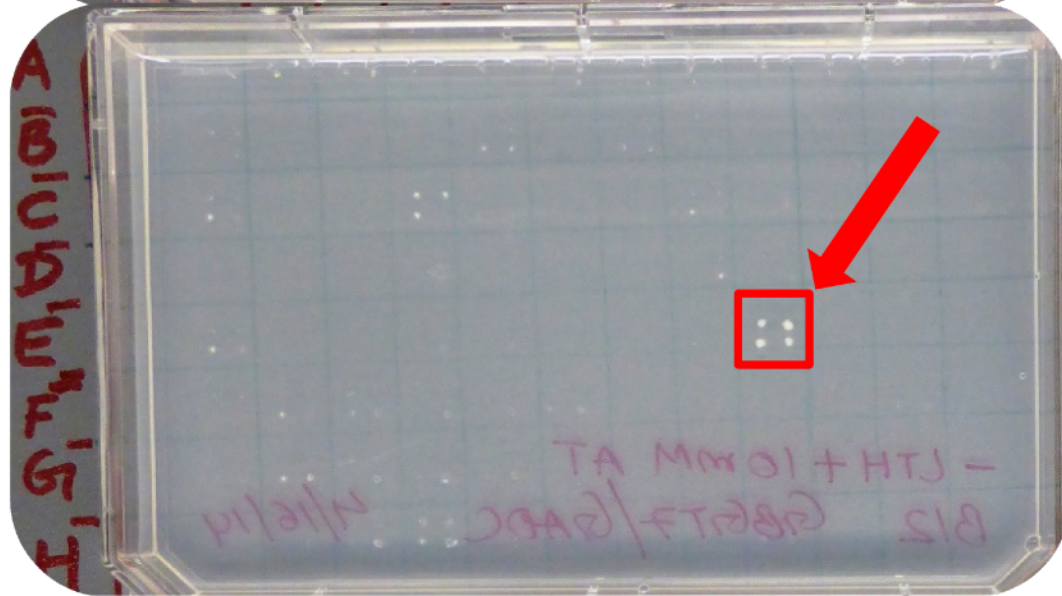


B

gp14, pGBGT7g/pGADCg  
(No 3-AT)

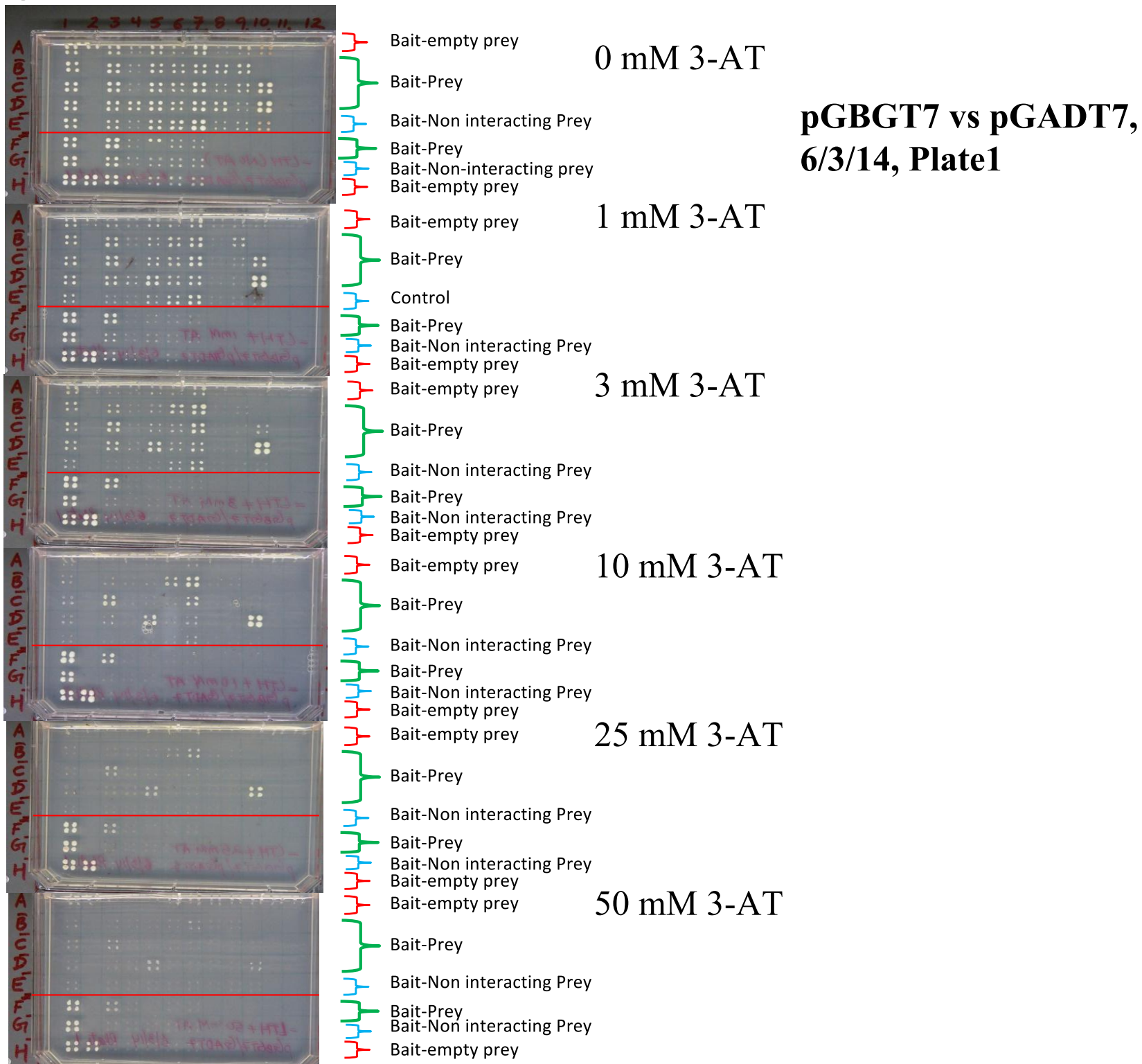


gp14, pGBGT7g/pGADCg  
(10 mM 3-AT)

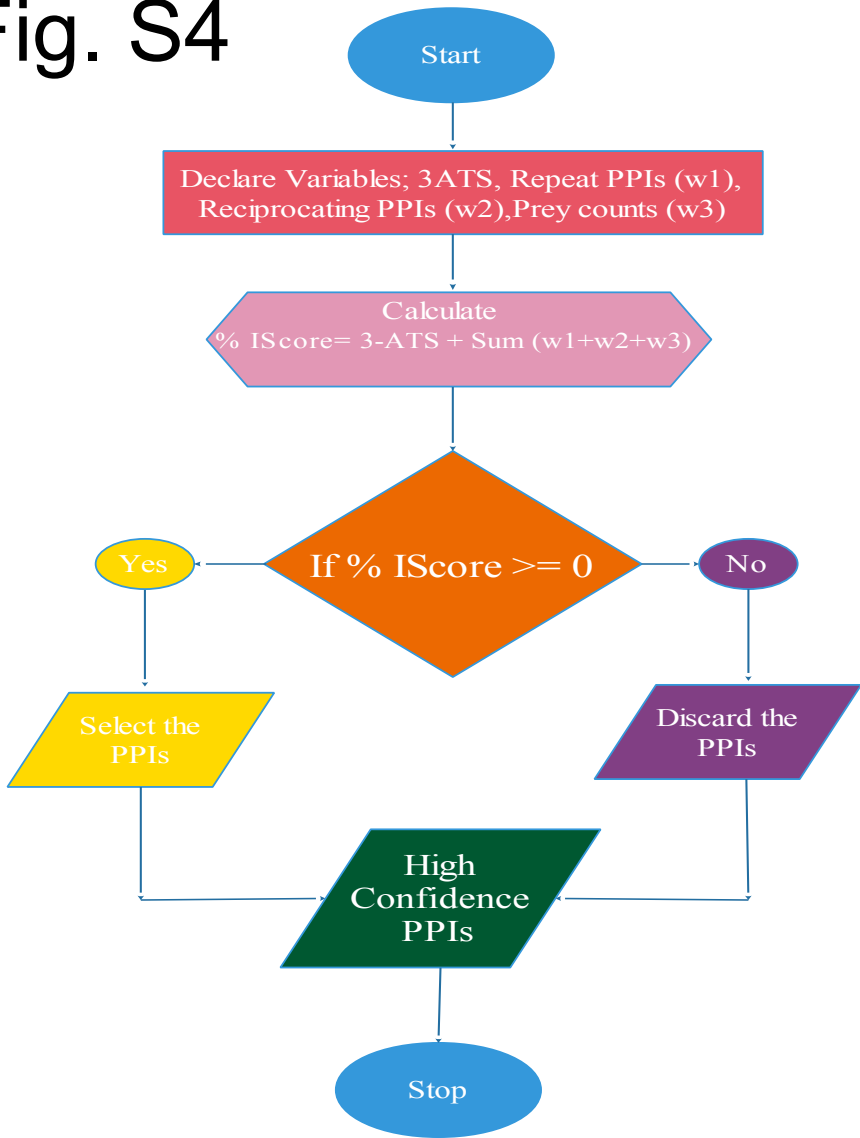




# Fig S3



# Fig. S4



# Fig. S5

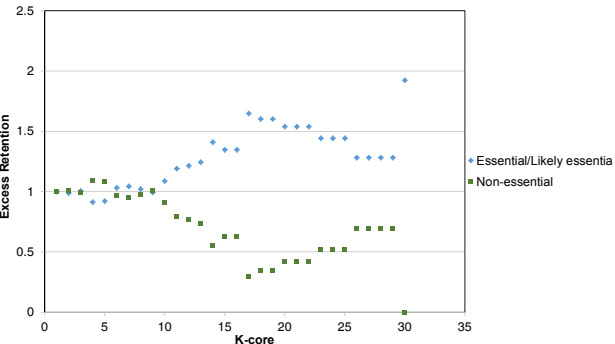


Fig. S6

## Clusters

% identity &gt; 32.5

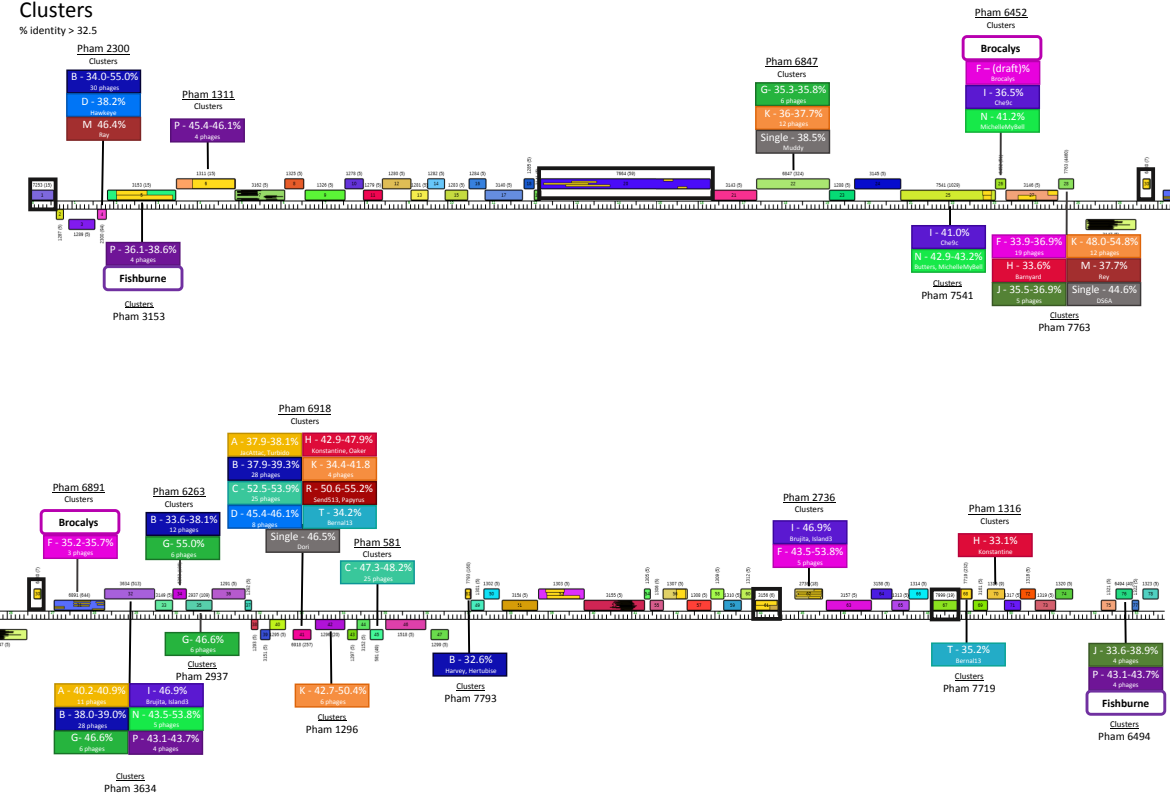




Fig. S7

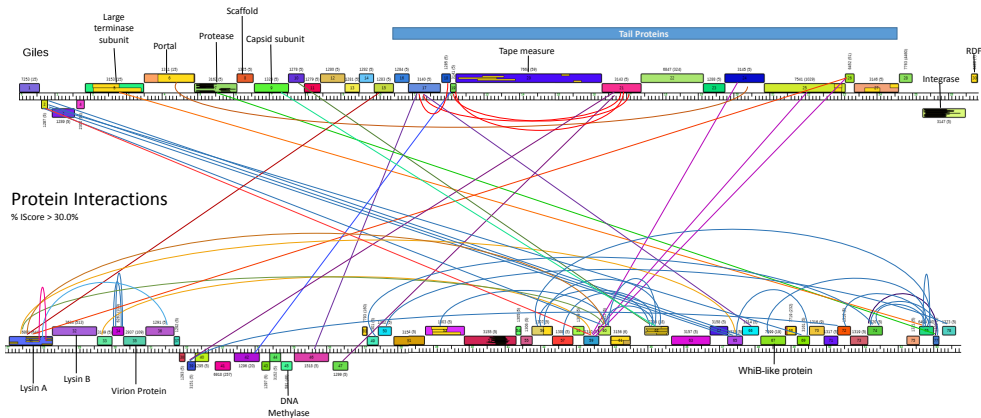


Fig. S8

