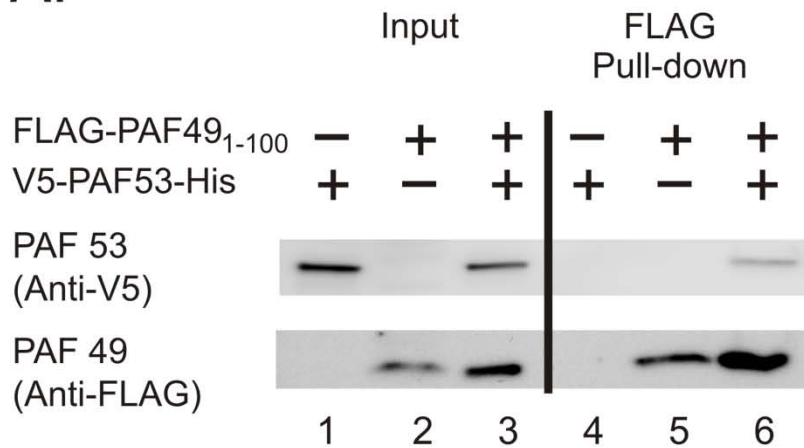


Supplemental Data: Characterization of the Interactions of Mammalian RNA
Polymerase I Associated Proteins PAF53 and PAF49

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A.



B.

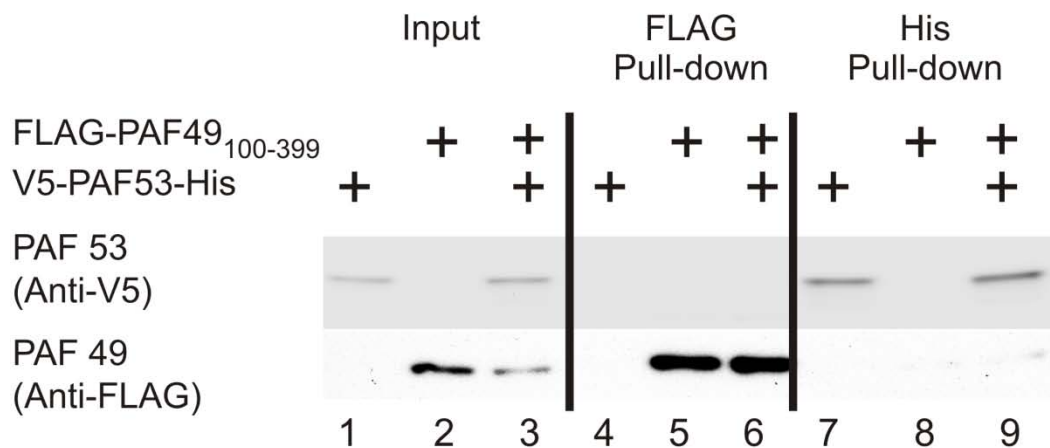


Figure 1. Amino acids 1-100 of PAF49 are sufficient (A) and necessary (B) for the interaction with PAF53. HEK 293T cells were cotransfected with the indicated deletion mutants of PAF49 and V5-tagged PAF53-His. The interacting proteins were pulled down with either immobilized anti-FLAG antibody (FLAG-tagged PAF49 mutants) or by IMAC (V5- and His-tagged PAF53) and were analyzed by SDS-PAGE and western blotting with the indicated antibodies as described in Figure 1.

A.

PFAM finds conserved sequences between *M. musculus* PAF49 and the rpa34.5 family:

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#HMM          lgkkelWlIkaPasvplskLkelpldlsgsqkgeevlkhnngkkYriseeslaqsdeseltlllpskkk
#MATCH        +++++lWlI+aPa++++++L+++ ++lsgs+++++ +l+ +++++Yr+ ++s  +++++etll++s+++
M. musculus  GPDELWLIQAPADFAPQCLNGRRVPLSGSKTVKGKLDGKKHRYRVFTSS---PQAREATLLASSEA
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B.

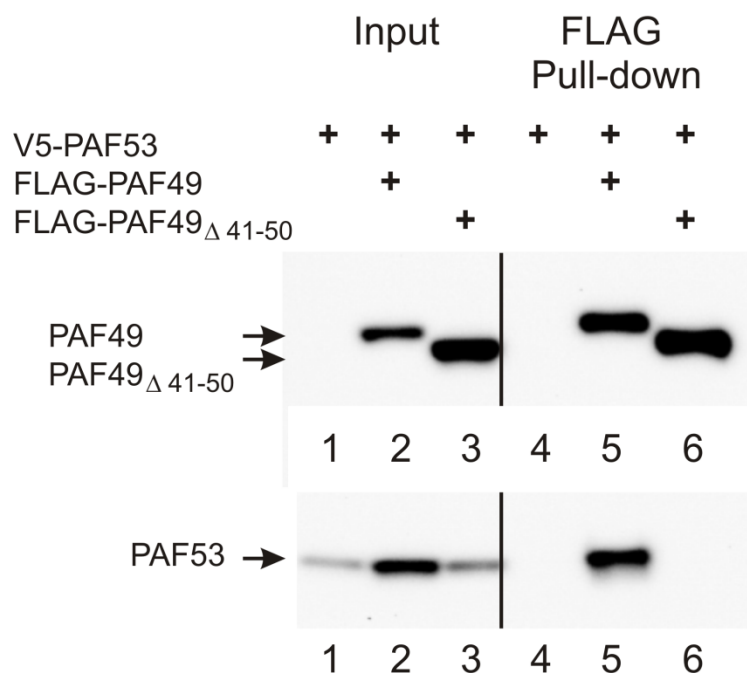


Figure 2. Amino acids 41-50 of PAF49 are essential for the interaction with PAF53. A. Pfam analysis of the A34.5 family and mouse PAF49 identifies an array of nine conserved amino acids in mouse PAF49, amino acids 41-49 (red) between amino acids 41-86 of mouse PAF49. B. Deletion of amino acids 41-50 abrogates the interaction between PAF49 and PAF53. HEK 293 cells were cotransfected with the indicated deletion mutants of PAF49 and V5-tagged PAF53. The interacting proteins were analyzed as described in the legend to Figure 1. Amino acids 41-50 are deleted in FLAG-PAFA49 Δ .

Seq	ELWLIQAPADFAPQCLNGRRVPLSGSKTVKGKLDGKKHRYRVFTS
Sec.Str	CCSSSSCCCCCHHHCCSSCCCCSSSSCCCCSSSSSSCCC

Secondary structure elements are shown as:
H for Alpha helix, S for Beta sheet and C for coil.

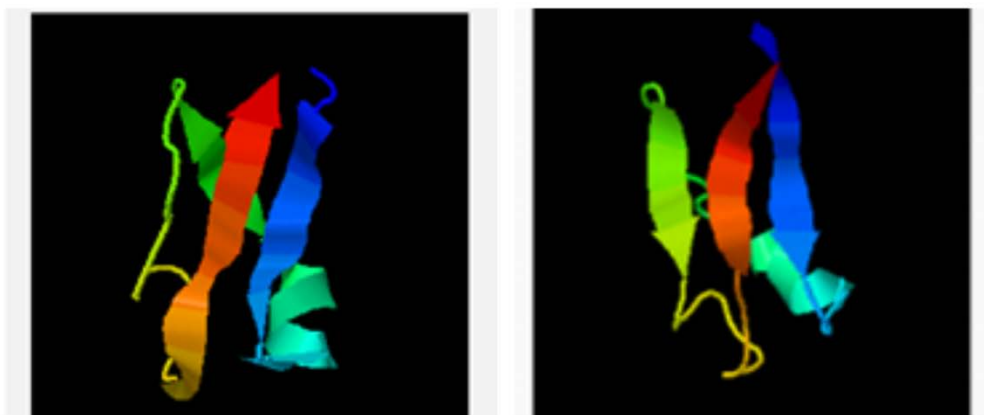


Figure 3. The predicted structure of amino acids 41-86 of mouse PAF49 is very similar to that predicted for that part of the region of *Candida* A34.5 that interacts with *Candida* A49. Amino acids 41-86 were analyzed using I-TASSER. The top panel presents the predicted secondary structure for amino acids 41-86. Secondary structure elements are shown as H for Alpha helix, S for Beta sheet & C for Coil. The lower panel presents two predicted tertiary structures for amino acids 41-86. The two top templates for the structure were found in *Candida glabrata* A34.5^{43, 44}. There were only 17 identical amino acids within this region when the mouse and *Candida* proteins were aligned using the Jotun Hein alignment method.