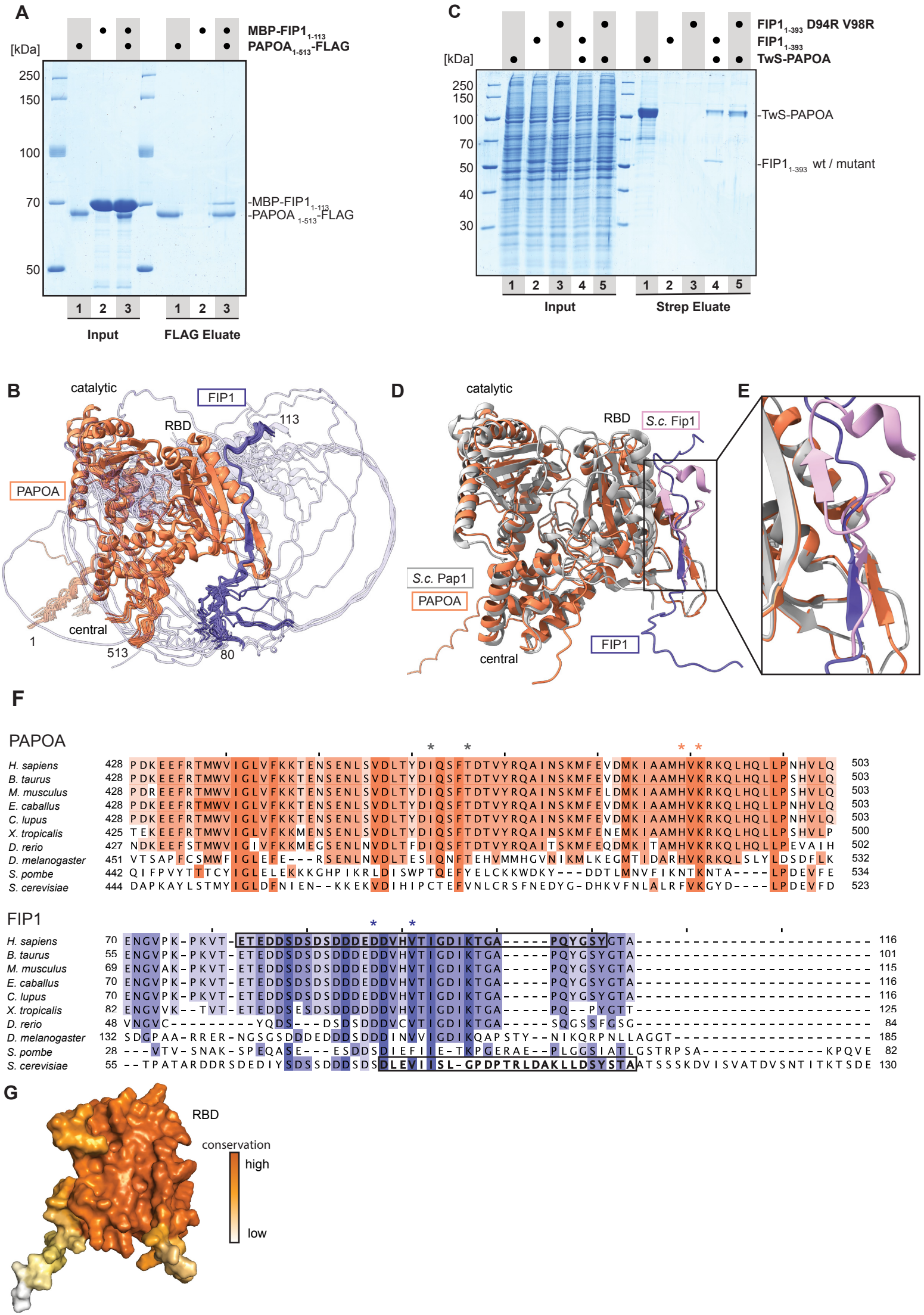
A
[kDa]
MBP-FIP1 1-113
PAPOA 1-513-FLAG
250
150
100
70
50
-MBP-FIP1 1-113
-PAPOA 1-513-FLAG
1 2 3 1 2 3
Input
FLAG Eluate
C
[kDa]
FIP1 1-393 D94R V98R
FIP1 1-393
TwS-PAPOA
250
150
100
70
50
40
30
-TwS-PAPOA
-FIP1 1-393 wt / mutant
1 2 3 4 5 1 2 3 4 5
Input
Strep Eluate
B
catalytic
FIP1
RBD
113
PAPOA
central
1
513
80
D
catalytic
RBD
S.c. Fip1
S.c. Pap1
PAPOA
FIP1
central
E
F
PAPOA
H. sapiens 428 PDKEEFRTMWVIGLVFKKTENSENLSVDLTYDIQSFTDTVYRQAINSKMFEVDMKIAAMHVKRKQLHQLLPNHVLQ 503
B. taurus 428 PDKEEFRTMWVIGLVFKKTENSENLSVDLTYDIQSFTDTVYRQAINSKMFEVDMKIAAMHVKRKQLHQLLPSHVLQ 503
M. musculus 428 PDREEFRTMWVIGLVFKKTENSENLSVDLTYDIQSFTDTVYRQAINSKMFELDMKIAAMHVKRKQLHQLLPSHVLQ 503
E. caballus 428 PDKEEFRTMWVIGLVFKKTENSENLSVDLTYDIQSFTDTVYRQAINSKMFEVDMKIAAMHVKRKQLHQLLPNHVLQ 503
C. lupus 428 PDKEEFRTMWVIGLVFKKTENSENLSVDLTYDIQSFTDTVYRQAINSKMFEVDMKIAAMHVKRKQLHQLLPNHVLQ 503
X. tropicalis 425 TEKEEFRTMWVIGLVFKKMENSENLSVDLTYDIQSFTDTVYRQAINSKMFENEMKIAAMHVKRKQLHQLLPSHVLP 500
D. rerio 427 NDKEEFSTMWVIGIVFKKMEGSENLNVDLTFDIQSFTDTVYRQAITSKMFEQDMKITAMHVKRKQLHQLLPEVAIH 502
D. melanogaster 451 VTSAPFCSMWFIGLEFE---RSENLNVDLTESIQNFTEHVMMHGVNIKMLKEGMTIDARHVKRKQLSLYLDSDFLK 532
S. pombe 442 QIFPVYTTTCYIGLELEKKKGHPIKRLDISWPTQEFYELCKKWDKY---DDTLMNVFIKNTKNTA----LPDEVFE 534
S. cerevisiae 444 DAPKAYLSTMYIGLDFNIEN--KKEKVDIHIPCTEFVNLCRSFNEDYG-DHKVFNLALRFVKGYD----LPDEVFD 523
FIP1
H. sapiens 70 ENGVPK-PKVT-ETEDDSDSDSDDDEDDVHVTIGDIKTGA-----PQYGSYGTA---------- 116
B. taurus 55 ENGVPK-PKVT-ETEDDSDSDSDDDEDDVHVTIGDIKTGA-----PQYGSYGTA---------- 101
M. musculus 69 ENGVAK-PKVT-ETEDDSDSDSDDDEDDVHVTIGDIKTGA-----PQYGSYGTA---------- 115
E. caballus 70 ENGVPK-PKVT-ETEDDSDSDSDDDEDDVHVTIGDIKTGA-----PQYGSYGTA---------- 116
C. lupus 70 ENGVPK-PKVT-ETEDDSDSDSDDDEDDVHVTIGDIKTGA-----PQYGSYGTA---------- 116
X. tropicalis 82 ENGVVK--TVT-ETEDDSESDSDDDDDDVHVTIGDIKTGA-----PQ--PYGTT---------- 125
D. rerio 48 VNGVC---------YQDS---DSDSDDDVCVTIGDIKTGA-----SQGSSFGSG---------- 84
D. melanogaster 132 SDGPAA-RRER-NGSGSDDDEDDDSDDDINVVIGDIKQAPSTY--NIKQRPNLLAGGT---------- 185
S. pombe 28 ---VTV-SNAK-SPEQASE---ESDDSDIEFIIE-TKPGERAE--PLGGSIATLGSTRPSA---------- 82
S. cerevisiae 55 --TPATARDDRSDEDIYSDSSDDDSDSDLEVIISL-GPDPTRLDAKLLDSYSTAATSSSKDVISVATDVSNTITKTSDE 130
G
RBD
conservation
high
low

**Figure S1: Characterization of the PAPOA-FIP1 interaction**

(A) Coomassie-stained SDS-PAGE analysis of pull-down experiment showing that PAPOA core directly interacts with FIP1 N-terminus.

(B) Overlay of 25 computationally predicted models of the $PAPOA_{1-513}$-$FIP1_{1-113}$ complex. The conformational heterogeneous FIP1 N-terminal residues (1-79) are colored in grey. The PAPOA structured domains are indicated.

(C) Coomassie-stained SDS-PAGE analysis of pull-down experiment using HEK cell lysates showing that positively-charged FIP1 mutations in the predicted PAPOA-FIP1 interface disrupt the interaction.

(D) Overlay of a computationally predicted model of $PAPOA_{1-513}$-$FIP1_{80-113}$ (in orange and purple) with experimentally derived structure of yeast $Pap1_{1-537}$-$Fip1_{80-105}$ (in grey and pink) (PDB: 3C66). The PAPOA/Pap1 structured domains are indicated.

(E) Close-up of the binding interfaces of $PAPOA_{1-513}$-$FIP1_{80-113}$ overlayed with yeast $Pap1_{1-537}$-$Fip1_{80-105}$ (PDB: 3C66).

(F) Multiple sequence alignment of PAPOA and FIP1 from different eukaryotic organisms around the PAPOA-FIP1 binding sites, with residues colored by identity. Minimal FIP1 binding peptides from human (this study) or yeast (Meinke et al. 2008) are in bold and boxed, while mutated residues leading to a disruption of the interaction are labeled with colored asterisks (human PAPOA in orange, yeast Pap1 in grey, human FIP1 in purple).

(G) The PAPOA RBD shown in surface representation with residues colored by sequence conservation, from low conservation in white to high conservation in orange.