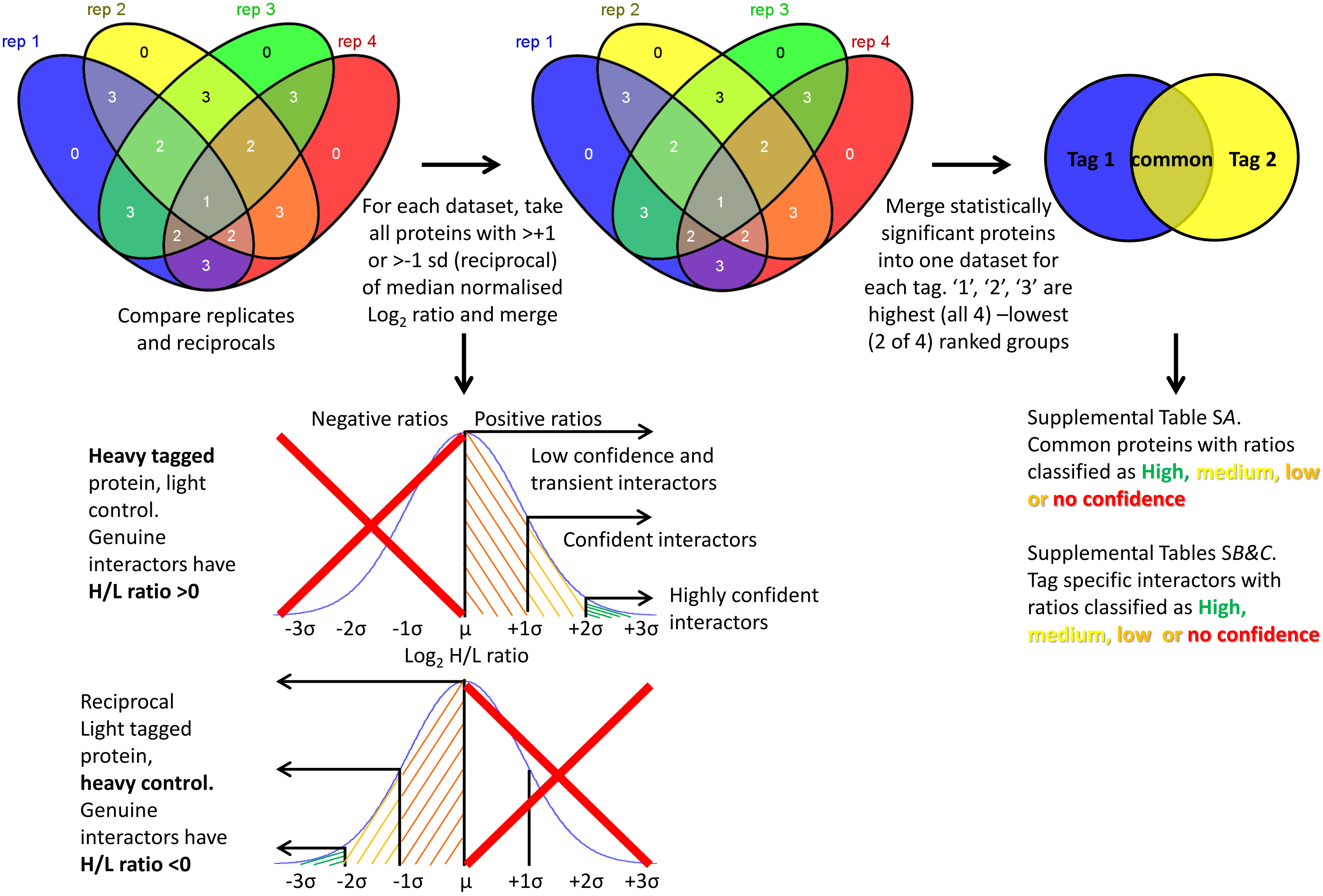
Supplemental Fig. S1. Mass Spectrometry data workflow and protein acceptance criteria.



Compare replicates and reciprocals

For each dataset, take all proteins with >+1 or >-1 sd (reciprocal) of median normalised Log$_2$ ratio and merge

Merge statistically significant proteins into one dataset for each tag. ‘1’, ‘2’, ‘3’ are highest (all 4) –lowest (2 of 4) ranked groups

Supplemental Table S*A*. Common proteins with ratios classified as High, medium, low or no confidence

Supplemental Tables S*B*&*C*. Tag specific interactors with ratios classified as High, medium, low or no confidence

Heavy tagged protein, light control. Genuine interactors have **H/L ratio >0**

Low confidence and transient interactors

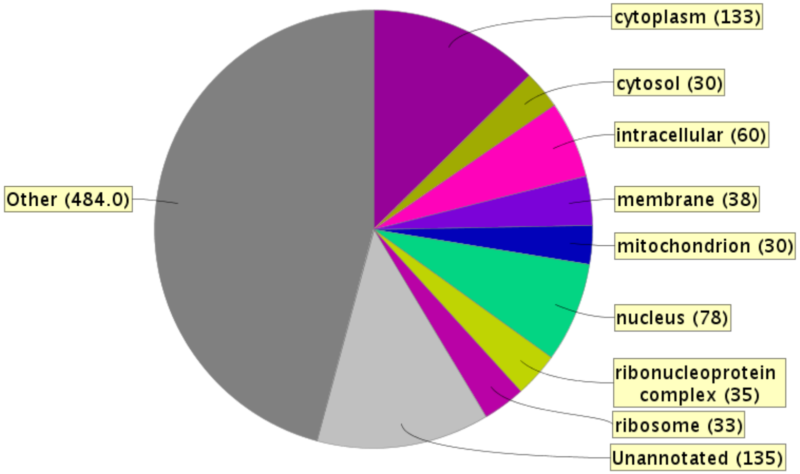
Confident interactors

Highly confident interactors

Reciprocal Light tagged protein, **heavy control.** Genuine interactors have **H/L ratio <0**

Supplemental Fig. S2. GO annotation and proteins over- and under-represented in DT40 lysate.

A) Pie chart showing the GO cellular components classification of DT40



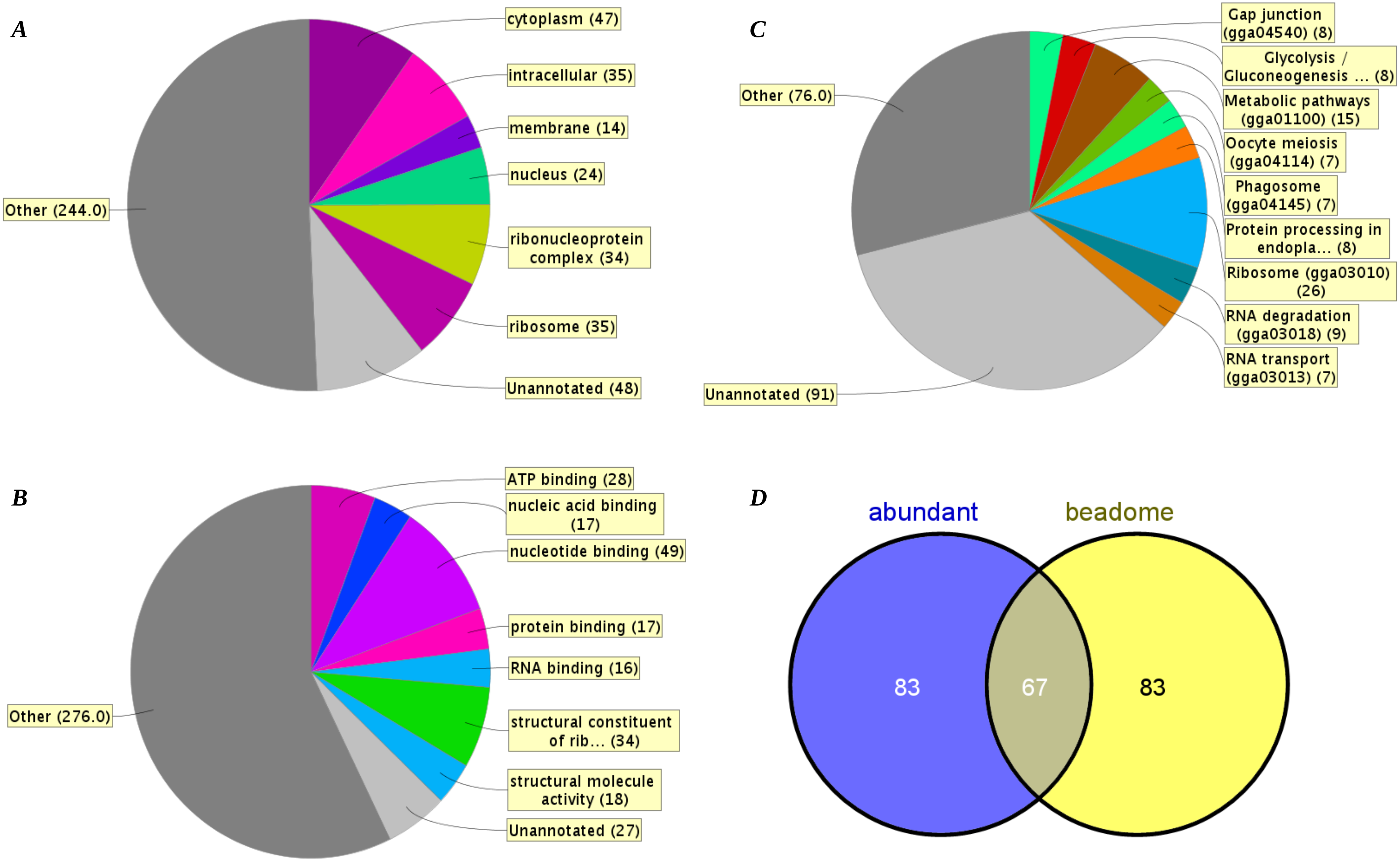
B) Proteins under-represented in DT40 compared to the *Gallus gallus* SwissProt proteome

Description	Count [%]	Ref. Count [%]	Count	Ref. Count	Raw p-value	FDR p-value
cellular_component	73.38	89.85	306	2143	7.29E-28	3.99E-24
intrinsic to membrane	6.95	22.39	29	534	4.20E-20	3.28E-17
integral to membrane	6.95	21.3	29	508	4.17E-18	1.14E-15
extracellular region	2.16	12.87	9	307	5.62E-17	1.28E-14
membrane	17.75	33.88	74	808	9.93E-16	2.05E-13
membrane part	12.47	25.83	52	616	2.70E-13	4.62E-11
plasma membrane	5.04	14.68	21	350	1.18E-11	1.90E-09
cell periphery	6.24	15.51	26	370	2.92E-10	4.31E-08
nucleus	21.1	33	88	787	3.29E-09	4.61E-07
intracellular membrane-bounded organelle	34.53	46.96	144	1120	1.14E-08	1.52E-06
membrane-bounded organelle	34.53	46.96	144	1120	1.14E-08	1.52E-06
extracellular matrix	0	3.35	0	80	1.58E-07	1.96E-05
proteinaceous extracellular matrix	0	3.1	0	74	5.21E-07	5.82E-05
extracellular region part	1.68	6.16	7	147	1.98E-06	2.04E-04
chromosomal part	0.96	4.07	4	97	5.37E-05	3.65E-03
chromosome	1.44	4.86	6	116	5.40E-05	3.65E-03
cell junction	0.72	3.4	3	81	1.41E-04	8.45E-03
cell	66.19	73.5	276	1753	1.60E-04	9.14E-03
cell part	66.19	73.5	276	1753	1.60E-04	9.14E-03
extracellular matrix part	0	1.59	0	38	6.32E-04	2.91E-02
chromatin	0.24	2.1	1	50	7.13E-04	3.20E-02
synapse	0.24	2.1	1	50	7.13E-04	3.20E-02
plasma membrane part	2.16	5.12	9	122	8.39E-04	3.70E-02

C) Proteins over-represented in DT40 compared to the *Gallus gallus* SwissProt proteome

Description	Count [%]	Ref. Coun	Count	Ref. Coun	Raw p-value	FDR p-valu
ribosome	7.91	1.34	33	32	<4.90E-324	<4.90E-324
virion	3.12	0.13	13	3	<4.90E-324	<4.90E-324
virion part	3.12	0.13	13	3	<4.90E-324	<4.90E-324
viral capsid	2.88	0.04	12	1	<4.90E-324	<4.90E-324
cytosolic large ribosomal subunit	1.68	0.25	7	6	<4.90E-324	<4.90E-324
proteasome core complex	1.68	0.13	7	3	<4.90E-324	<4.90E-324
clathrin coat	1.2	0.13	5	3	<4.90E-324	<4.90E-324
proteasome core complex, alpha-subunit complex	0.96	0.08	4	2	<4.90E-324	<4.90E-324
proton-transporting ATP synthase complex, catalytic core F(1)	0.96	0.04	4	1	<4.90E-324	<4.90E-324
chaperonin-containing T-complex	0.72	0	3	0	<4.90E-324	<4.90E-324
clathrin coat of coated pit	0.48	0.04	2	1	<4.90E-324	<4.90E-324
clathrin coat of trans-Golgi network vesicle	0.48	0	2	0	<4.90E-324	<4.90E-324
clathrin vesicle coat	0.48	0	2	0	<4.90E-324	<4.90E-324
septin complex	0.48	0.04	2	1	<4.90E-324	<4.90E-324
trans-Golgi network transport vesicle	0.48	0	2	0	<4.90E-324	<4.90E-324
trans-Golgi network transport vesicle membrane	0.48	0	2	0	<4.90E-324	<4.90E-324
fatty acid beta-oxidation multienzyme complex	0.24	0	1	0	<4.90E-324	<4.90E-324
mitochondrial fatty acid beta-oxidation multienzyme complex	0.24	0	1	0	<4.90E-324	<4.90E-324
mitochondrial proton-transporting ATP synthase complex, catalytic core F(1)	0.24	0	1	0	<4.90E-324	<4.90E-324
perikaryon	0.24	0	1	0	<4.90E-324	<4.90E-324
ribonucleoside-diphosphate reductase complex	0.24	0	1	0	<4.90E-324	<4.90E-324
ribosomal subunit	0.24	0	1	0	<4.90E-324	<4.90E-324
signal recognition particle receptor complex	0.24	0	1	0	<4.90E-324	<4.90E-324
ribonucleoprotein complex	10.07	2.98	42	71	1.27E-15	8.47E-15
ribosomal subunit	3.84	0.67	16	16	5.99E-13	3.87E-12
cytosolic part	4.32	1.05	18	25	2.44E-09	1.52E-08
cytosol	10.55	4.82	44	115	4.54E-08	2.76E-07
large ribosomal subunit	2.16	0.38	9	9	1.42E-07	8.34E-07
proteasome complex	2.16	0.38	9	9	1.42E-07	8.34E-07
cytosolic ribosome	2.64	0.55	11	13	2.32E-07	1.34E-06
macromolecular complex	29.5	20.46	123	488	7.79E-07	4.40E-06
cytoplasmic part	35.01	25.49	146	608	1.14E-06	6.41E-06
cytoplasm	52.28	42.22	218	1007	3.41E-06	1.89E-05
small ribosomal subunit	1.68	0.29	7	7	4.79E-06	2.61E-05
coated membrane	1.68	0.34	7	8	3.25E-05	1.71E-04
membrane coat	1.68	0.34	7	8	3.25E-05	1.71E-04
Golgi-associated vesicle	1.2	0.21	5	5	1.60E-04	8.00E-04
Golgi-associated vesicle membrane	1.2	0.21	5	5	1.60E-04	8.00E-04
proton-transporting ATP synthase complex	0.96	0.17	4	4	9.23E-04	3.99E-03
vesicle coat	0.96	0.17	4	4	9.23E-04	3.99E-03
intracellular non-membrane-bounded organelle	21.58	16.44	90	392	1.47E-03	6.21E-03
non-membrane-bounded organelle	21.58	16.44	90	392	1.47E-03	6.21E-03
proton-transporting two-sector ATPase complex	1.68	0.5	7	12	1.68E-03	7.07E-03
coated vesicle membrane	1.44	0.42	6	10	3.08E-03	1.28E-02
coated vesicle	1.68	0.55	7	13	3.10E-03	1.29E-02
proton-transporting two-sector ATPase complex, catalytic domain	0.96	0.21	4	5	3.98E-03	1.61E-02
AP-type membrane coat adaptor complex	0.72	0.13	3	3	5.31E-03	2.06E-02
clathrin adaptor complex	0.72	0.13	3	3	5.31E-03	2.06E-02
COPI coated vesicle membrane	0.72	0.13	3	3	5.31E-03	2.06E-02
COPI-coated vesicle	0.72	0.13	3	3	5.31E-03	2.06E-02
phosphopyruvate hydratase complex	0.72	0.13	3	3	5.31E-03	2.06E-02
cytoskeletal part	9.35	6.37	39	152	5.70E-03	2.18E-02

Supplemental Fig. S3. Non-specific binding proteins associated with ≥2 of 4 affinity resins: FLAG, Talon, Calmodulin and IgG sepharose. *A*) Pie chart showing GO_Cellular component annotation. *B*) GO_Molecular function annotation and *C*) Kegg pathway analysis. ‘Other’ represents proteins other categories with single protein entries. Data analysed using ProteinCenter. *D*) Overlap of proteins identified as top 150 abundant and the top 150 beadome.



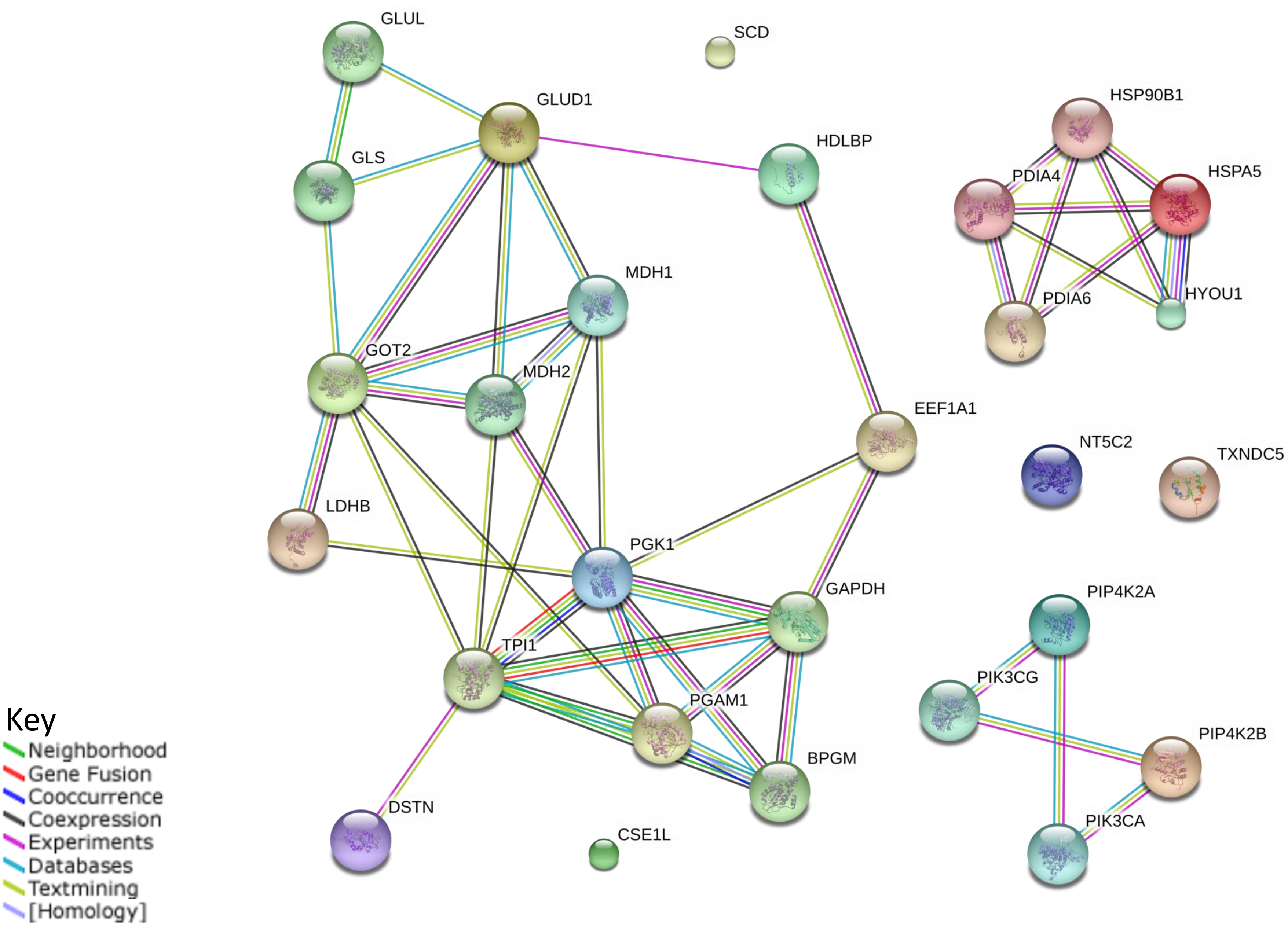
Supplemental Fig. S4. PI5P4K2α and β alignment. PI5P4K2α has unique swissprot (spQ5F356) and trembl (trF1NKL5) accessions, both included in UniprotKB *Gallus gallus* database 2012. Highlighted in purple and blue are unique peptides to PI5P4K2 α and β respectively, and in yellow are conserved peptides identified in replicate SILAC-iPAC screenings. Green are positively charged residues where K and L indicate ends of peptides that can be quantified.

```
1     -------------------------------------AAWVWVLGHNINELSNVPVPVML   23   F1NLK4 β subunit
1     -----------------------------------------------INELSHVQIPVML   13   F1NKL5 α subunit
1     MAAPGTVASVMASKTKTKKKHFVVQKVKLFRASDPLLSVLMWGVNHSINELSHVQIPVML   60   Q5F356 α subunit
                                                     *****.* :****

24    MPDDFKAYSKIKVDNHLFNKENLPSRFKFKEYCPLVFRNLERFGIDDQDYQNSVTRSAP   83   F1NLK4
14    MPDDFKAYSKIKVDNHLFNKENMPSHFKFKEYCPMVFRNLERFGIDDQDFQNSLTRSAP   73   F1NKL5
61    MPDDFKAYSKIKVDNHLFNKENMPSHFKFKEYCPMVFRNLERFGIDDQDFQNSLTRSAP   120  Q5F356
      **********************:**:********:***************:***:*****

84    VNSDSQGRCGARFLTTYDRRFVIKAVSSEDVAEMHNILKKYHQFIVECHGNTLLPQFLGM   143  F1NLK4
74    LANDSQARSGARFHTSYDKRYIIKTITSEDVAEMHNILKKYHQFIVECHGNTLLPQFLGM   133  F1NKL5
121   LANDSQARSGARFHTSYDKRYIIKTITSEDVAEMHNILKKYHQFIVECHGNTLLPQFLGM   180  Q5F356
      : .***.*.**** *:**:*::**:::*********************************

144   YRLTVDGVETYMVVTRNVFSHRLTVHRKYDLKGSTVSREASDKEKAKDLPTFKDNDFLNE   203  F1NLK4
134   YRLTVDGVEIYMIVTRNVFSHRLSVYRKYDLKGSTVAREASDKEKAKELPTFKDNDFIND   193  F1NKL5
181   YRLTVDGVEIYMIVTRNVFSHRLSVYRKYDLKGSTVAREASDKEKAKELPTFKDNDFIND   240  Q5F356
      ********* **:**********:*:**********:**********:*********:*:

204   GQKLHVGEESKKNFLEKLKRDVEFLAQLKIMDYSLLVGIHDVDRAEQEEMEVEDRAEDEE   263  F1NLK4
194   GQKIHIDENNKRMFLEKLKKDVEFLAQLKLMDYSLLVGIHDVERAEQEEVECEENDGEDE   253  F1NKL5
241   GQKIHIDENNKRMFLEKLKKDVEFLAQLKLMDYSLLVGIHDVERAEQEEVECEENDGEDE   300  Q5F356
      ***:*: *:.*: ******:*********:************:******:* *:.  ::*

264   CENDGLGGNPISSYGTPPDSPGNLLNYPRFFGPGEFDPSVDVYAMKSHDSAPKKEVYFMA   323  F1NLK4
254   GESDG-----THPIGTPPDSPGNTLNSSLPLAPGEFDPAIDVYGIKSHESAPRKEVYFMA   308  F1NKL5
301   GESDG-----THPIGTPPDSPGNTLNSSLPLAPGEFDPAIDVYGIKSHESAPRKEVYFMA   355  Q5F356
       *.**         ********* **    :.******::***.:***:***:*******

324   IIDILTPYDAKKKAAHAAKTVKHGAGAEISTVNPEQYSKRFNEFMSNILT   373   F1NLK4    F1NLK4 β subunit
309   IIDILTHYDAKKKAAHAAKTVKHGAGAEISTVNPEQYSKRFLDFIANILT   358   F1NKL5    F1NKL5 α subunit
356   IIDILTHYDAKKKAAHAAKTVKHGAGAEISTVNPEQYSKRFLDFIANILT   405   Q5F356    PI42A_ α subunit
      ****** **********************************  :*::****
```

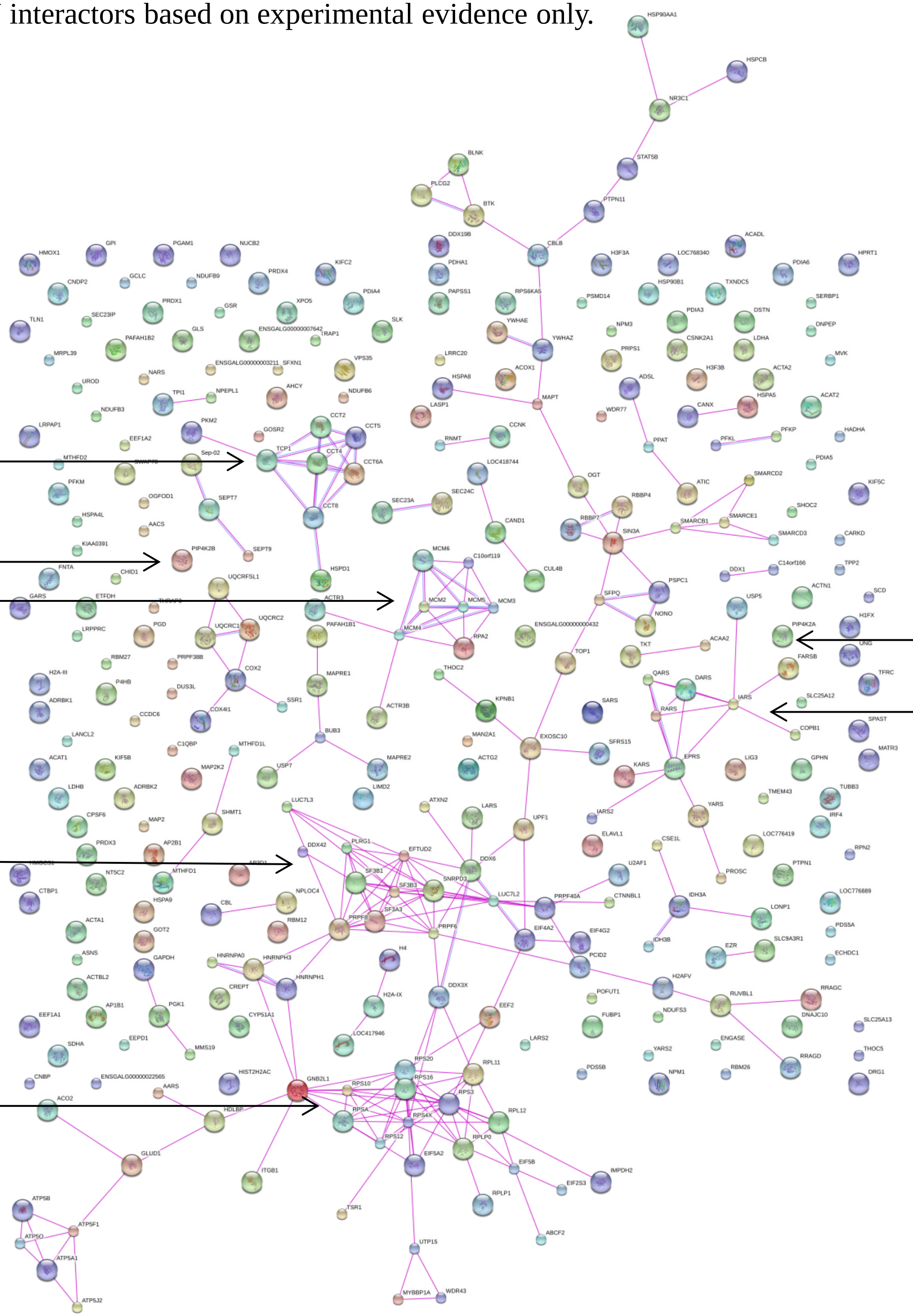
Supplemental Fig. S5. *A.* Fanconi anemia complementation group C *Gallus gallus* UniprotKB accessions alignment with peptides identified in both Calmodulin and IgG pulldowns replicate SILAC-iPAC screenings highlighted in green and IgG only in orange.

```
1      MAQDTTVPKLNFKFWLDKAIEWGQDTTLESQKDVCLHLPQLQEFLHQTYETLKHMNSIAA   60   Q5F3H0
1      MAQDTTVPKLNFKFWLDKAIEWGQATTLESQKDVCLHLPQLQEFLHQTYETLKHMNSIAA   60   Q60GM0
1      MAQDTTVPKLNFKFWLDKAIEWGQATTLESQKDVCLHLPQLQEFLHQTYETLKHMNSIAA   60   F1NYH1
       ************************ ***********************************

61     IQQFPLIGQLLGRLCWNPFVVGYDESQKTLMWCLCCLYSSEPQNPVELKANSWIRSLLCH   120  Q5F3H0
61     IQQFPLIGQLLGRLCWNPFVVGYDESQKTLMWCLCCLYSSEPQNPVELKANSWIRSLLCH   120  Q60GM0
61     IQQFPLIGQLLGRLCWNPFVVGYDESQKTLMWCLCCLYSSEPQNPVELKANSWIRSLLCH   120  F1NYH1
       ************************************************************

121    LLSSSKWERIEAETSTFISALGYTSADYYCHLVENIVSSLVTELRENQFNELNIQERISV   180  Q5F3H0
121    LLSSSKWERIEAETSTFISALGYTSADYYCHLVENIVSSLVTELRENQFNELNIQERISV   180  Q60GM0
121    LLSSSKWERIEAETSTFISALGYTSADYYCHLVENIVSSLVTELRENQFNELNIQERISV   180  F1NYH1
       ************************************************************

181    SRVNAVSLFCLPLITLPDLTPLLETLLLYHGGASKEILSSEFLEAVNDAVLKKKISLSES   240  Q5F3H0
181    SRVNAVSLFCLPLITLPDLTPLLETLLLYHGGASKEILSSEFLEAVNDAVLKKKISLSES   240  Q60GM0
181    SRVNAVSLFCLPLITLPDLTPLLETLLLYHGGASKEILSSEFLEAVNDAVLKKKISLSES   240  F1NYH1
       ************************************************************

241    AIFSLWHRHLPSLESATLSLLDQLTSLQINSLEKVACVIKDSLLPQAASHPAIFRIVNEI   300  Q5F3H0
241    AIFSLWHRHLPSLESATLSLLDQLTSLQINSLEKVACVIKDSLLPQAASHPAIFRIVNEI   300  Q60GM0
241    AIFSLWHRHLPSLESATLSLLDQLTSLQINSLEKVACVIKDSLLPQAASHPAIFRIVNEI   300  F1NYH1
       ************************************************************

301    FKNALLETDGAPEVMTIIQVFIQLFIQVQQNENKQHKFPLKAYFPHHHQPLVIALLRRPF   360  Q5F3H0
301    FKNALLETDGAPEVMTIIQVFIQLFIQVQQNENKQHKFPLKAYFPHHHQPLVIALLRRPF   360  Q60GM0
301    FKNALLETDGAPEVMTIIQVFIQLFIQVQQNENKQHKFPLKAYFPHHHQPLVIALLRRPF   360  F1NYH1
       ************************************************************

361    ELPSTHWLQHLKHISDTLKSIVEDTNVSSFADLYEIWFLVACFGEWLDIAAEQLMKAAVA   420  Q5F3H0
361    ELPSTHWLQHLKHISDTLKSIVEDTNVSSFADLYEIWFLVACFGEWLGYCTKQLMKAAVA   420  Q60GM0
361    ELPSTHWLQHLKHISDTLKSIVEDTNVSSFADLYEIWFLVACFGEWLDIAAEQLMKAAVA   420  F1NYH1
       ***********************************************  .::********

421    PDALLWLLAFYYCPQNENQQRTQTMVESQAVYSRLMMLYNRTVLSFGDLQASVNNAADTE   480  Q5F3H0
421    PDALLWLLAFYYCPQNENQQRTQTMVESQAVYSRLMMLYNRTVLSFGDLQASVNNAADTE   480  Q60GM0
421    PDALLWLLAFYYCPQNENQQRTQTMVESQAVYSRLMMLYNRTVLSFGDLQASVNNAADTE   480  F1NYH1
       ************************************************************

481    QCHTQCLITHLLTNFLLFSSGGHMIAQEFISHITETTDMSKEVCSLLIRTAYRIKHNGGE   540  Q5F3H0
481    QCHTQCLITHLLTNFLLFSSGGHMIAQEFISHITETTDMSKEVCSLLIRTAYRIKHNGGE   540  Q60GM0
481    QCHTQCLITHLLTNFLLFSSGGHMIAQEFISHITETTDMSKEVCSLLIRTAYRIKHNGGE   540  F1NYH1
       ************************************************************

541    NQRTVKLLNELLQKLAVEV   559  Q5F3H0     Q5F3H0_CHICK
541    NQRTVKLLNELLQKLAVEV   559  Q60GM0     Q60GM0_CHICK
541    NQRTVKLLNELLQKLAVEV   559  F1NYH1     F1NYH1_CHICK
       *******************
```

Supplemental Fig. S6. Expanded PIP4Kin2 STRING network (+10) to identify potential missing interactors in our lists



Supplemental Fig. S7. All TALON interactors based on experimental evidence only.



CCT (Chaperone) cluster

PI5P4K2B bait

MCM cluster

PI5P4K2A

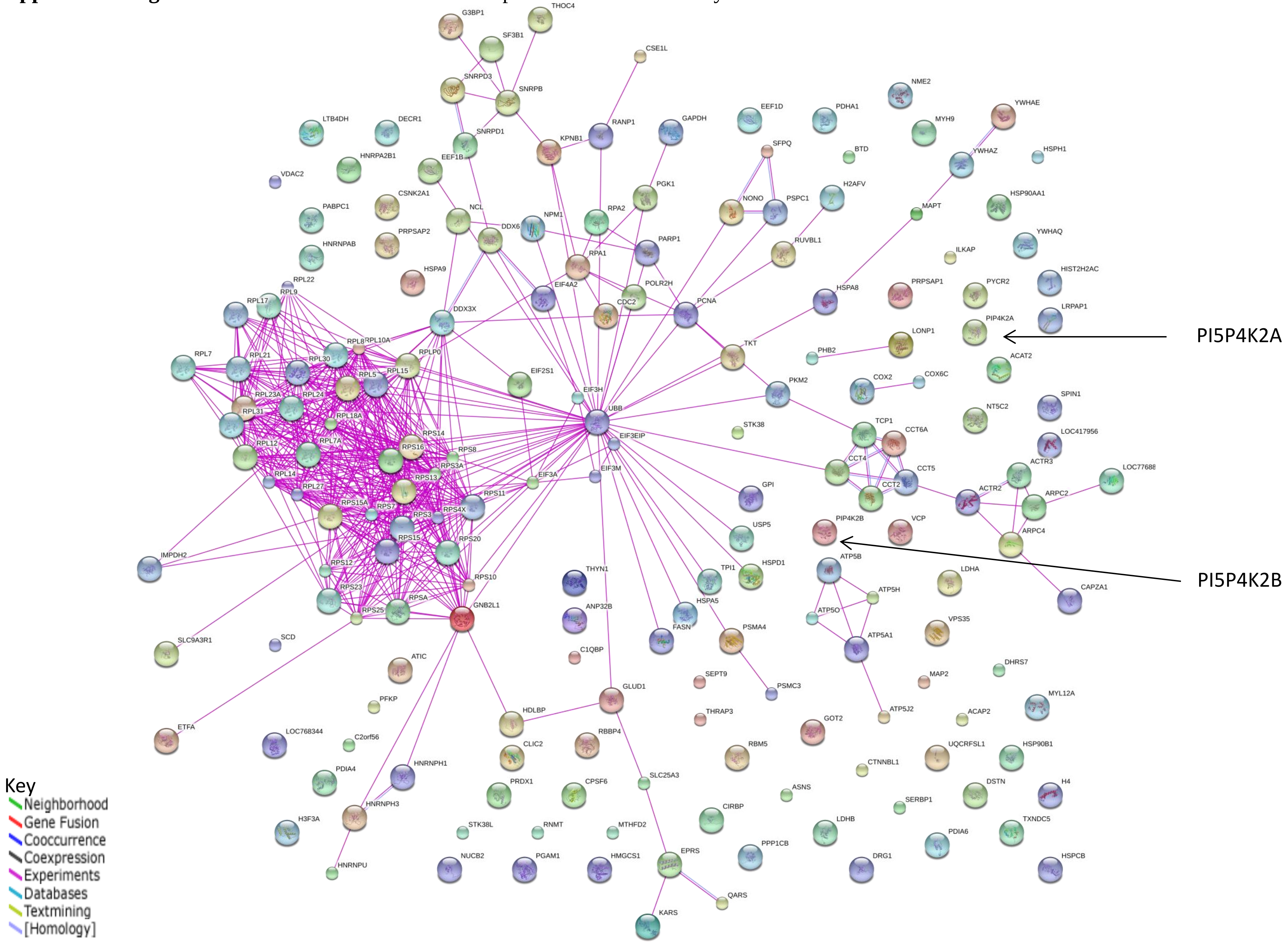
tRNA synthetase cluster

Splicing Factor cluster

Ribosomal and protein synthesis cluster

Key
- Neighborhood
- Gene Fusion
- Cooccurrence
- Coexpression
- Experiments
- Databases
- Textmining
- [Homology]

Supplemental Fig. S8. All FLAG interactors based on experimental evidence only.



Supplemental Fig. S9. Proteins interacting with FA proteins using human accessions *(A)*, *Gallus* accessions specific to only Calmodulin (*B*) or IgG (*C*) resins