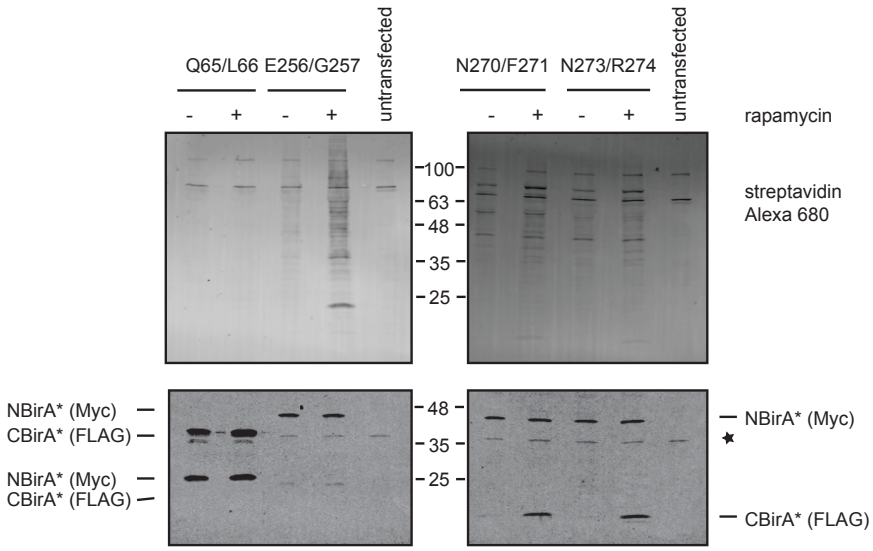
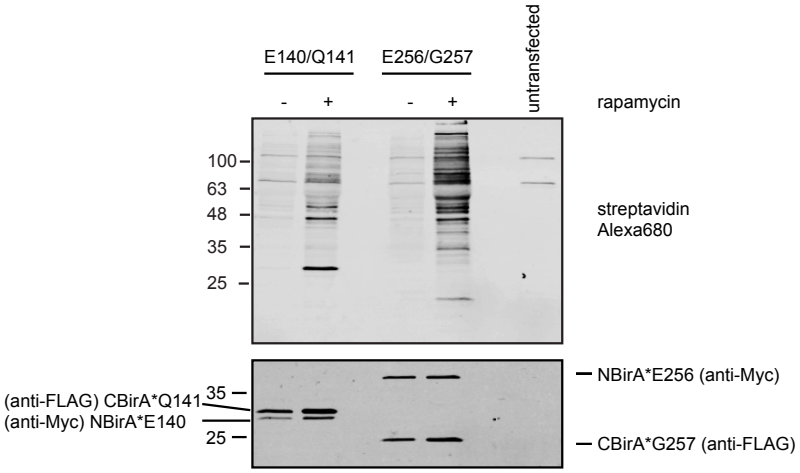
## SUPPLEMENTARY FIGURES

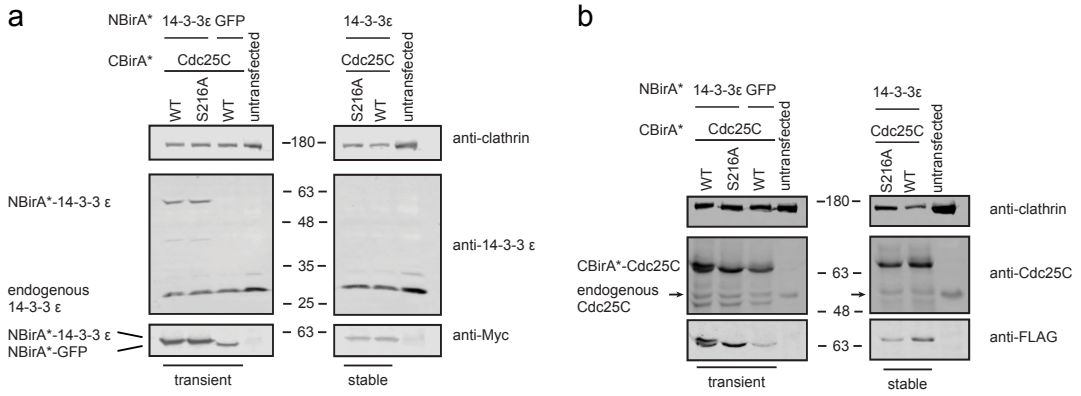



**Supplementary Figure 1: Different split sites tested for suitability for a PCA.**
Lysates of cells transiently transfected with fusions of NBirA* and CBirA* fragments with FKBP and FRB respectively and incubated with or without rapamycin were analyzed by Western blot for interaction-induced biotinylation. Expression levels of the fusion proteins were detected with the anti-Myc and -FLAG antibodies. The band marked with a star is a non-specific signal. Split sites, as depicted in Fig. 1a, are indicated. Splitting BirA* between E256 and G257 yields two inactive fragments that can reassemble an active enzyme upon interaction of the fusion partners.



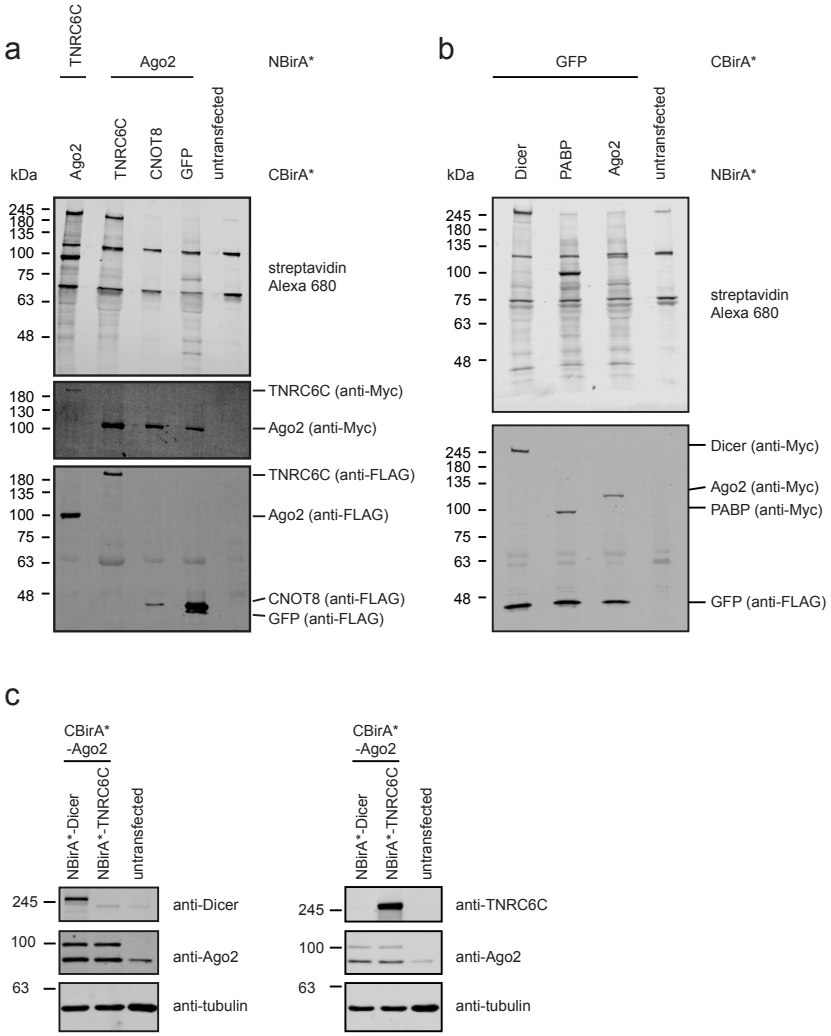

**Supplementary Figure 2: Comparison of E140/Q141 and E256/G257 splitBirA* using FRB-FKBP fusions.**

Lysates of cells transiently transfected with NBirA*-FKBP and CBirA*-FRB fusions corresponding to the splitting site from De Munter et al. (E140/Q141) or ours (E256/G257) were incubated with or without rapamycin and analyzed by Western blot for interaction-induced biotinylation. Expression levels of the fusion proteins were detected with the anti-Myc and -FLAG antibodies. A stronger biotinylation signal at similar expression levels, indicating a stronger regained activity, is observed in rapamycin-treated cells expressing the E256/G257 construct as compared to the E140/Q141 construct.



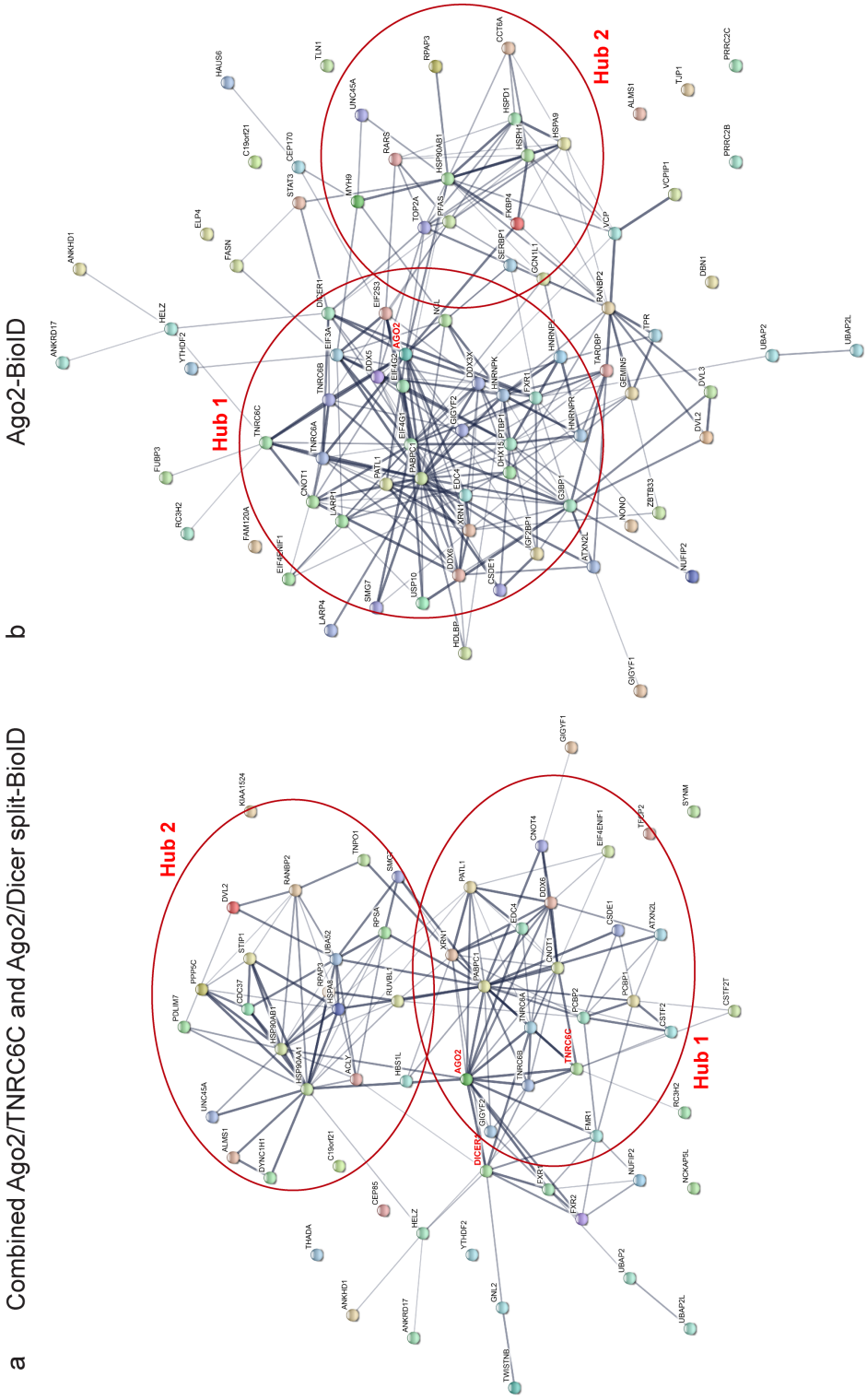

**Supplementary Figure 3: Expression levels of BirA* fusion proteins of Cdc25C and 14-3-3ε**

Comparison of endogenous and fusion protein expression levels in transiently or stably transfected cells expressing the indicated proteins. (a) Endogenous 14-3-3ε shows higher expression than the corresponding BirA* fusion protein. In stable cell lines NBirA*-14-3-3ε is below the detection limit of the anti 14-3-3ε antibody but expression can be confirmed by Myc detection. (b) CBirA*-Cdc25C protein shows higher expression levels than endogenous Cdc25c in both transient and stable cells. Clathrin detection is used as a loading control.

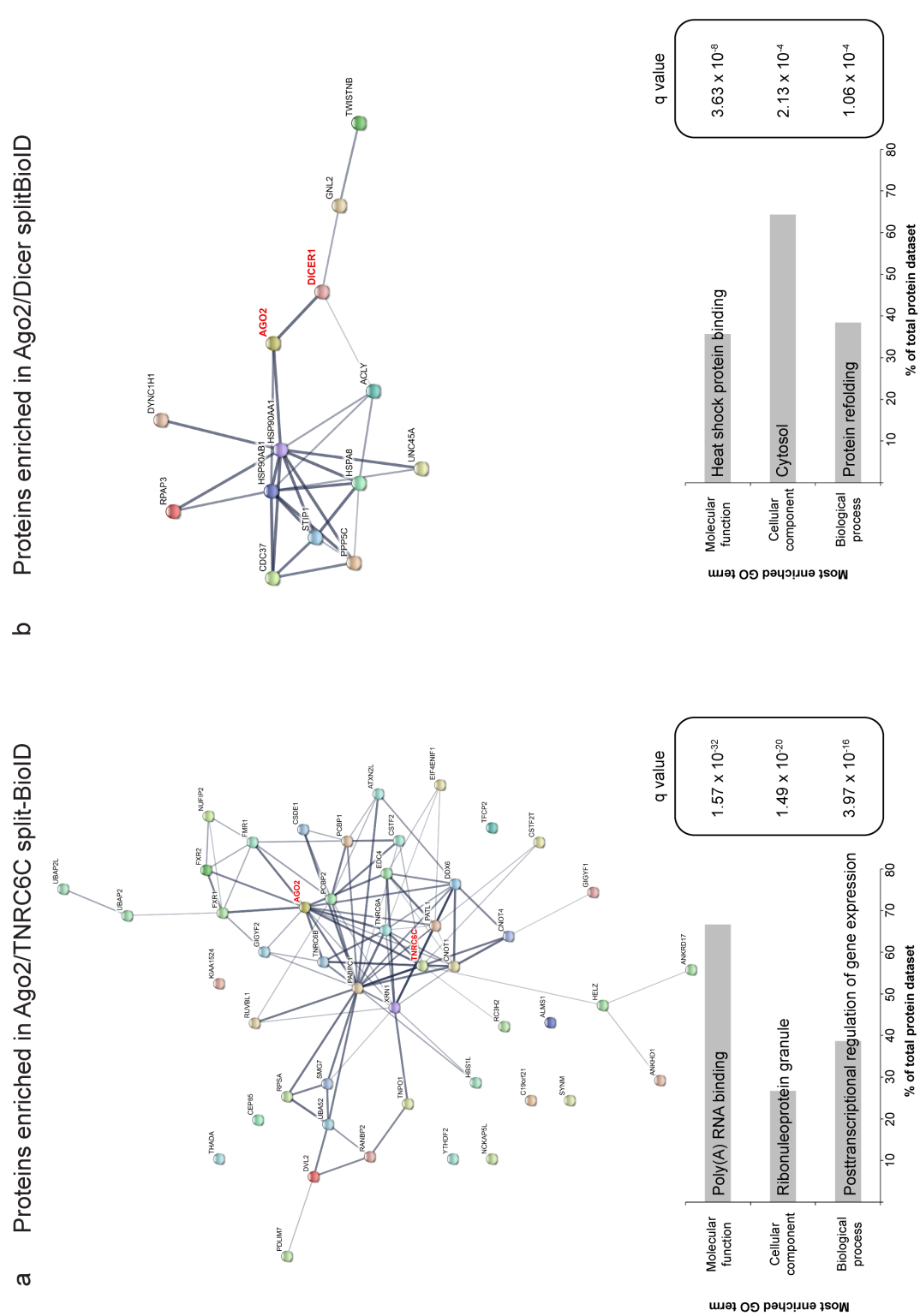



**Supplementary Figure 4: Test of various fusion protein orientations for optimal split-BioID of Ago2, Dicer and TNRC6C and comparison of their expression levels with the endogenous proteins**

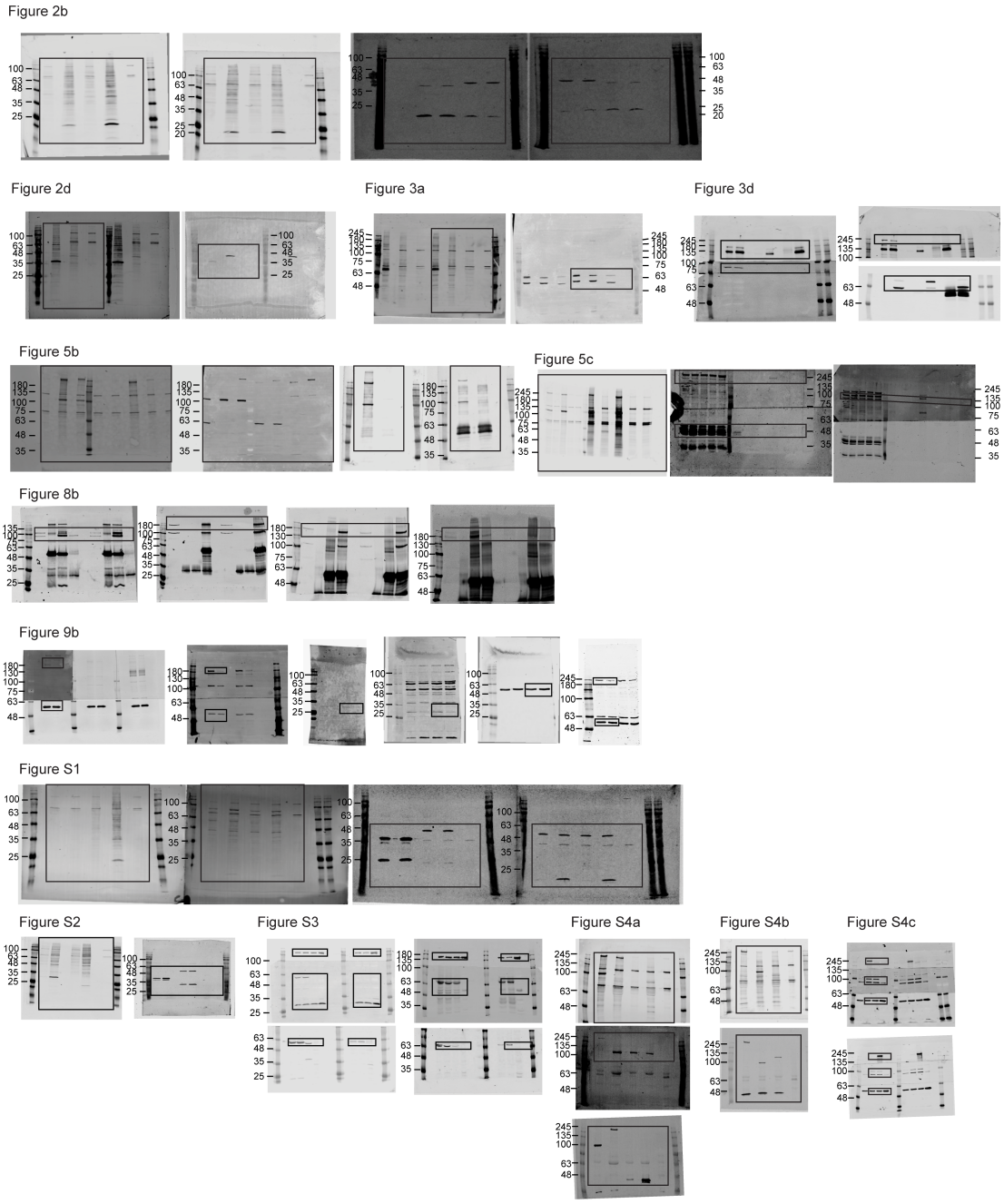
(a) Blot of lysates of cells transiently transfected with the indicated constructs. The NBirA*-TNRC6C/CBirA*-Ago2 combination yielded more biotinylation than the NBirA*-Ago2/CBirA*-TNRC6C counterpart (compare lane 1 and 2) and CBirA*-GFP showed unexpectedly high biotinylation signals (lane 4). (b) Background signals resulting from CBirA*-GFP were further investigated by combining the fusion protein with NBirA* fused to Dicer, Ago2 or PABP. In all cases, strong biotinylation signals could be observed that might be explained by the high expression levels of the CBirA*-GFP fusion. As an NBirA*-GFP fusion yielded background biotinylation levels (Fig. 5b) and the NBirA*-TNRC6C/CBirA*-Ago2 combination resulted in strong biotinylation (a), this orientation was chosen for further experiments. (c) Expression levels of endogenous and fusion proteins in cells transiently transfected with the indicated constructs. Endogenous TNRC6C is not detectable, while endogenous Dicer signals are weaker then the corresponding BirA* fusion protein. Endogenous and CBirA* fusion Ago2 are expressed to the same or lower extent.




**Supplementary Figure 5: String network analysis of the combined Ago2/TNRC6C and Ago2/Dicer split-BioID hits compared to Ago2-BioID.** (a) Predicted PPI for the proteins identified in the Ago2/TNRC6C and Ago2/Dicer split-BioID and significantly enriched over the Ago2/GFP split-BioID. Two main interaction hubs centered on PABPC1 (Hub1) and Hsp90 (Hub2) can be observed. (b) Predicted PPI for the proteins identified using standard Ago2-BioID (See Supplementary Data 3). Proteins cluster around the same two hubs as in (a).




**Supplementary Figure 6: String network analysis on the proteins enriched in Ago2/TNRC6C or Ago2/Dicer split-BioID.**
(a) Up, predicted PPI for the proteins enriched in the Ago2/TNRC6C split-BioID (see Supplementary Data 2). Down, the corresponding most enriched GO terms are indicated. (b) same as (a) for the proteins enriched in the Ago2/Dicer split-BioID.




**Supplementary Figure 7: Full scans of blots used to prepare the figures.** Figures and corresponding cropped areas are indicated.

## SUPPLEMENTARY METHODS

### Supplementary table 1: Primers and oligos used in this study

| Plasmid | Forward primer/oligo | Reverse primer/oligo |
|---|---|---|
| pEYFP- Mitotrap (addgene 46942) | CAGCTTGGCCGGCCATGC GAGTGGCCATCCTCTG | ACGAATGCGGCCGCTTAATTA ACTGCTTTGAGATTCGTC |
| | GTTTAAACATGATCCTCTG GCATGAGAT | ACGAACGATCGCTATTTAATTA ACTGCTTTGAGATTCGTCG |
| pLXIN-Ap1g1-FKBP (addgene 46946) | CAGCTAGTCGACGGCGCG CCTGGAGTGCAGGTGGAA AC | CATCGTCGGATCCCTACAGTTT TAGAAGCTCCA |
| | CGTTATATCGATATGGGAG TGCAGGTGGAAAC | TACTCTACGCGTTTCCAGTTTT AGAAGCTCCAC |
| pSRA-PABP GFP (Stoecklin group) | ATCTTGATCGATATGAACC CCAGTGCCCCC | ATCTTGGGCGCGCCTTAAACA GTTGGAACACCGG |
| pIRESneo –FLAG/ HA Ago2 (Meister group) | CGAATAATCGATATGTACT CGGGAGCCGG | CGTTAGACGCGTTCAAGCAAA GTACATGGTGC |
| pCIneo –NHA-TNRC6C (Chekulaeva group) | CATGGCTACAGGGAGTGC | CTTAATTTAATTAACAGGGACT CCCCGCTGAG |
| | TCCCTAACGCGTCTAATAA TTTACAGGGACTCCCCGCT GAG | TCCTACGCCTCCCGGGGCATT GAGATGGCTACAGGGAGTGCC |
| | ATCGGATCCCGTGCCAAAT CTGACAGTG (CED) | AAAAGCGGCCGCTAGCAGGGA CTCCCCGCTGAG (CED) |
| | GCACCCACGAGGCCAGCT GCAGGGTTAACCAATC (AAGL mutant) | GATTGGTTAACCCTGCAGCTG GCCTCGTGGGTGC (AAGL mutant) |
| pGST- cdc25c (addgene 10969) | CATGTCCTCTACGGAACTC TTC | CGATTGTTAATTAATGGGCTCA TGTCCTTCACC |
| pQTEV-YWHAE (addgene 31562) | TCGCATATCGATATGGATG ATCGAGAGGATCTGG | CTGTAGACGCGTCTGATTTTCG TCTTCCACG |
| pMS2-GFP | ATTCTATCGATATGGTGAG CAAGGGCGAGG | CAGAATTTAATTAACGCGTTGG CTTGTACAGCTCGTC |
| pSF3-BirA* (unpublished data) | CATAGGGTCGACATGGAAC AAAAACTCATC (BirA* fwd) | AACATGGGATCCGGCGCGCCC AGCTGGATAGGCTCAG (N65) |
| | | AACATGGGATCCGGCGCGCCG TTGTCCAGCTTCTCCC (N270) |
| | | AACATGGGATCCGGCGCGCCG TTGATGAAGTTGTCCA (N273) |
| | CATTCGGGCCGGCCTTAAT TAACCTGAACGCCAAGCAG ATC (C66) | CGGTAAGATCTTTACGGCAGG GTCGCGCGTTCC (BirA* rev) |
| | CATTCGGGCCGGCCTTAAT TAACTTCATCAACAGACCT GTG (C271) | |
| | CATTCGGGCCGGCCTTAAT TAACAGACCTGTGAAGCTG AT (C274) | |
| | TGACAAGGCCGGCCTTCA GGGACCTGCTGCTGCC (C141) | CGTAGGAGATCTCCTCACGCT CGAGCTTCTC |
| pSF3-NFKBP-CFRB-2 (created in this study) | CGTTATATCGATATGGGAG TGCAGGTGGAAAC | GATGGCAGCAGCGGATCCCTA CTCCAGTCTCCAG (N140) |

| Plasmid | Forward primer/oligo | Reverse primer/oligo |
|---|---|---|
| pCMV-5-HA-CNOT8 (Winkler group) | TATGCCTGCAGCACTTGTGGAG | GCCTGATTAATTAACTGCTGCATGTTGTTGATAA |
| pCMV- Dicer (Grimm group) | TGAGATGAAAAGCCCTGCTTTG<br>TCCCTAACGCGTCTAATAA<br>TAGCTATTGGGAACCTGAG | CGAATATTAATTAAGCTATTGGGAACCTGAGG<br>TCCTACGCCTCCCGGGGCATT<br>GAGATGAAAAGCCCTGCTTTG |
| pSF3-NBirA*YWHAE-CBirA*cdc25c (created in this study) | CTATATCGCTCCCCGGCGATGCCAGAGAACT | AGTTCTCTGGCATCGCCGGGGAGCGATATAG |
| pEGFP-GIGYF2 (Freund group) | AAACAATTGATGCAGAAGTGGTATTACAAAGATC (532)<br>AAACAATTGATGCAGGAACGACTGACCAGG (607) | GCGGCCGCTCACTCTTGCTCTAGCTTTGCAGCTTTGG (740) |
| Linker 1 | AACCAGATCTCCTACGCCTCCCGGGGCGGCGGCTCCTCCGGCGGCGGCTTAAT | TAAGCCGCCGCCGGAGGAGCCGCCGCCCCGGGAGGCGTAGGAGATCTGGTTAAT |
| Linker 2 | GCGCCTGGCGGCGGCTCCTCCGGCGGCGGCCAGATCTCCTACGCCTCCCGGGGCGG | CGCGCCGCCCCGGGAGGCGTAGGAGATCTGGCCGCCGCCGGAGGAGCCGCCGCCAGG |
| FLAG | ATTCATGGACTACAAGGACGACGATGACAAGGCCGG | CCTTGTCATCGTCGTCCTTGTAGTCCATG |

## Supplementary Table 2: Plasmids generated in this study

| Plasmid | Promotor/Resistance | Description |
|---|---|---|
| pSF3-BirA*-Ago2 | CMV/Amp | BirA*-Ago2 |
| pSF3-BirA*-Rab11a | CMV/Amp | BirA*-Rab11a |
| pSF3-BirA*-Sec61β | CMV/Amp | BirA*- Sec61β |
| pSF3-BirA*-RHD4 | CMV/Amp | BirA*-RHD4 |
| pSF3-BirA*-GRASP65 | CMV/Amp | BirA*-GRASP65 |
| pSF3-BirA*-TGN38 | CMV/Amp | BirA*-TGN38 |
| pSF3-BirA*-Lamp1 | CMV/Amp | BirA*-Lamp1 |
| pSF3-NBirA*65-CBirA*66 | CMV/Amp | NBirA*-FKBP; FRB-CBirA* |
| pSF3-NBirA*270- CBirA*271 | CMV/Amp | NBirA*-FKBP; FRB-CBirA* |
| pSF3-NBirA*273- CBirA*274 | CMV/Amp | NBirA*-FKBP; FRB-CBirA* |
| pSF3-NFKBP-CFRB-1 | CMV/Amp | NBirA*-FKBP; CBirA*-FRB |
| pSF3-NFKBP-CFRB-2 | CMV/Amp | FKBP-NBirA*, CBirA*-FRB |
| pSF3-NFKBP-CFRB-3 | CMV/Amp | NBirA*-FKBP; FRB-CBirA* |
| pSF3-NFKBP-CFRB-4 | CMV/Amp | FKBP- NBirA*; FRB-CBirA* |
| pSF3-NBirA*E140-CBirA*Q141 | CMV/Amp | FKBP-NBirA*, CBirA*-FRB |
| pSF3-NAgo2-CGFP-1 | CMV/Amp | NBirA*-Ago2; CBirA*-GFP |
| pSF3-NAgo2-CCNOT8-1 | CMV/Amp | NBirA*-Ago2; CBirA*-Cnot8 |
| pSF3-NAgo2-CTNRC6C-1 | CMV/Amp | NBirA*-Ago2; CBirA*-TNRC6C |
| pSF3-NAgo2-CDicer-1 | CMV/Amp | NBirA*-Ago2; CBirA*-Dicer |
| pSF3-NGFP-CTNRC6C-1 | CMV/Amp | NBirA*-GFP; CBirA*-TNRC6C |
| pSF3-NGFP-CDicer-1 | CMV/Amp | NBirA*-GFP; CBirA*-Dicer |
| pSF3-NGFP-CAgo2-1 | CMV/Amp | NBirA*-GFP; CBirA*-Ago2 |
| pSF3-NPABP-CGFP-1 | CMV/Amp | NBirA*-PABP; CBirA*-GFP |
| pSF3-NDicer-CGFP-1 | CMV/Amp | NBirA*-Dicer; CBirA*-GFP |
| pSF3-NDicer-CAgo2-1 | CMV/Amp | NBirA*-Dicer; CBirA*-Ago2 |
| pSF3-NTNRC6C-CAgo2-1 | CMV/Amp | NBirA*-TNRC6C; CBirA*-Ago2 |
| pSF3-NYWHAE-Ccdc25C-1 | CMV/Amp | NBirA*-YWHAE; CBirA*-cdc25c |
| pSF3-NYWHAE-Ccdc25C S216A-1 | CMV/Amp | NBirA*-YWHAE; CBirA*-cdc25c S216A |
| pSF3-NGFP-Ccdc25C-1 | CMV/Amp | NBirA*-GFP; CBirA*-cdc25c |
| pMal-c2x-CED $His_6$ | P-lac/Amp | MBP- TNRC6C-CED $His_6$ |
| pMal-c2x-CED-AAGL-$His_6$ | P-lac/Amp | MBP- TNCR6C-CED-AAGL-$His_6$ |
| pGEX-6p-GIGYF2-[532-740] | tac/Amp | GST-GIGYF2 (532-740) |
| pGEX-6p-GIGYF2-[607-740] | tac/Amp | GST_GIGYF2 (607-740) |

## Supplementary table 3: gBlocks used in this study

| gBlock name | Sequence |
|---|---|
| FLAG- C257Bir – linker 1 – X | CGGCCGGCCTCAATTATGGACTACAAGGACGACGATGA<br>CAAGATTAACGGACTGGCTCCTTACCTGAGCAGATGGGA<br>GAAGCTGGACAACTTCATCAACAGACCTGTGAAGCTGAT<br>CATCGGCGACAAGGAAATCTTCGGCATCTCCAGAGGAAT<br>CGACAAGCAGGGAGCTCTGCTGCTGGAGCAGGACGGAA<br>TCATCAAGCCCTGGATGGGCGGAGAAATCTCCCTGAGAA<br>GCGCAGAGAAGCTCGAGGCGAACCAGATCTCCTACGCC<br>TCCCGGGGCGGCGGCTCCTCCGGCGGCGGCGTTTAAAC<br>ACCCTGCCGTATTAATTAATAACGAGGATCC |
| X – FLAG – linker 1 – C257Bir | CGGCCGGCCTCAATTGTTTAAACTATGTTAATTAACGACT<br>ACAAGGACGACGATGACAAGCAGATCTCCTACGCCTCCC<br>GGGGCGGCGGCTCCTCCGGCGGCGGCATTAACGGACT<br>GGCTCCTTACCTGAGCAGATGGGAGAAGCTGGACAACTT<br>CATCAACAGACCTGTGAAGCTGATCATCGGCGACAAGGA<br>AATCTTCGGCATCTCCAGAGGAATCGACAAGCAGGGAGC<br>TCTGCTGCTGGAGCAGGACGGAATCATCAAGCCCTGGAT<br>GGGCGGAGAAATCTCCCTGAGAAGCGCAGAGAAGCTCG<br>AGCGGCCGCCACTGTGCTGTCGCGAACGCCGCGCGATG<br>GAACGCGCGACCCTGCCGTAATAACGAGGATCC |
| X – linker 2 – myc –N256Bir | CTCGAGCTAATCGATTATATGACGCGTGGCGGCGGCTCC<br>TCCGGCGGCGGCCAGATCTCCTACGCCTCCCGGGGCAA<br>ACATGAACAAAAACTCATCTCAGAAGAGGATCTCGACAA<br>GGACAACACCGTGCCCCTGAAGCTGATCGCCCTGCTGG<br>CCAACGGCGAGTTCCACTCTGGCGAGCAGCTGGGAGAG<br>ACCCTGGGAATGAGCAGAGCCGCCATCAACAAGCACAT<br>CCAGACACTGAGAGACTGGGGAGTGGACGTGTTCACCG<br>TGCCTGGCAAGGGCTACAGCCTGCCTGAGCCTATCCAG<br>CTGCTGAACGCCAAGCAGATCCTGGGACAGCTGGATGG<br>CGGAAGCGTGGCCGTGCTGCCTGTGATCGACTCCACCA<br>ATCAGTACCTGCTGGACAGAATCGGAGAGCTGAAGTCCG<br>GCGACGCCTGCATCGCCGAGTACCAGCAGGCTGGCAGA<br>GGAGGCAGAGGACGGAAGTGGTTCAGCCCATTCGGAGC<br>CAACCTGTACCTGTCCATGTTCTGGAGACTGGAGCAGGG<br>ACCTGCTGCTGCCATCGGACTGAGTCTGGTGATCGGAAT<br>CGTGATGGCCGAGGTGCTGAGAAAGCTGGGAGCCGACA<br>AGGTGAGAGTGAAGTGGCCTAATGACCTGTACCTCCAGG<br>ACCGCAAGCTGGCTGGCATCCTGGTGGAGCTGACAGGC<br>AAGACAGGCGATGCCGCTCAGATCGTGATCGGAGCCGG<br>AATCAACATGGCCATGAGAAGAGTGGAGGAGAGCGTGG<br>TGAACCAGGGCTGGATCACCCTGCAGGAGGCTGGCATC<br>AACCTGGACCGGAACACCCTGGCCGCCATGCTGATCAG<br>AGAGCTGAGAGCCGCTCTGGAGCTGTTCGAGCAGGAGA<br>AACATGGATCCTAGGGCGCGCC |

| gBlock name | Sequence |
|---|---|
| Myc- N256Bir – linker 2 – X | CTCGAGATGGAACAAAAACTCATCTCAGAAGAGGATCTC<br>GACAAGGACAACACCGTGCCCCTGAAGCTGATCGCCCT<br>GCTGGCCAACGGCGAGTTCCACTCTGGCGAGCAGCTGG<br>GAGAGACCCTGGGAATGAGCAGAGCCGCCATCAACAAG<br>CACATCCAGACACTGAGAGACTGGGGAGTGGACGTGTT<br>CACCGTGCCTGGCAAGGGCTACAGCCTGCCTGAGCCTA<br>TCCAGCTGCTGAACGCCAAGCAGATCCTGGGACAGCTG<br>GATGGCGGAAGCGTGGCCGTGCTGCCTGTGATCGACTC<br>CACCAATCAGTACCTGCTGGACAGAATCGGAGAGCTGAA<br>GTCCGGCGACGCCTGCATCGCCGAGTACCAGCAGGCTG<br>GCAGAGGAGGCAGAGGACGGAAGTGGTTCAGCCCATTC<br>GGAGCCAACCTGTACCTGTCCATGTTCTGGAGACTGGAG<br>CAGGGACCTGCTGCTGCCATCGGACTGAGTCTGGTGAT<br>CGGAATCGTGATGGCCGAGGTGCTGAGAAAGCTGGGAG<br>CCGACAAGGTGAGAGTGAAGTGGCCTAATGACCTGTACC<br>TCCAGGACCGCAAGCTGGCTGGCATCCTGGTGGAGCTG<br>ACAGGCAAGACAGGCGATGCCGCTCAGATCGTGATCGG<br>AGCCGGAATCAACATGGCCATGAGAAGAGTGGAGGAGA<br>GCGTGGTGAACCAGGGCTGGATCACCCTGCAGGAGGCT<br>GGCATCAACCTGGACCGGAACACCCTGGCCGCCATGCT<br>GATCAGAGAGCTGAGAGCCGCTCTGGAGCTGTTCGAGC<br>AGGAGAAACATGGCGGCGGCTCCTCCGGCGGCGGCCA<br>GATCTCCTACGCCTCCCGGGGCATCGATTATTAGACGCG<br>TTAGGGATCCGGCGCGCC |

## Supplementary table 4: Antibodies used in this study

| Antibody | Host | Dilution | Buffer |
|---|---|---|---|
| anti-Myc (DSHB, 9E10-c) | mouse | WB,1:1000; | 1 % BSA/ 0.1% PBS – Tween 20 |
| | | IF, 1:50 | 5% BSA, 2% donkey serum, 2% goat serum 0.02% PBS-Triton-X 100 |
| anti-Myc (Proteintech, 66004-1-Ig) | mouse | IP, 2.5 µg | IP wash buffer |
| anti-FLAG M2 (Sigma, F1804) | mouse | WB, 1:500<br>IP, 5 µg | 2% BSA / 0.1% PBS – Tween 20 |
| anti-FLAG (Sigma, F7425) | rabbit | IF, 1:50 | 5% BSA, 2% donkey serum, 2% goat serum 0.02% PBS-Triton-X 100 |
| anti-CNOT1 (Proteintech, 14276-1-AP) | rabbit | WB, 1:500 | 5% milk / 0.2% PBS –Tween 20 |
| anti-CNOT7 (Abnova, H00029883-M01) | mouse | WB, 1:500 | 3% BSA / 0.1% TBS –Tween 20 |
| anti-CNOT8 (LSBio, LS-C99242) | rabbit | WB, 1:500 | 2% BSA / 0.1% PBS – Tween 20 |
| anti-TRBP (Proteintech, 15753-1-AP) | rabbit | WB, 1:1000 | 2% BSA / 0.1% PBS – Tween 20 |
| anti-LMO7 (Santa Cruz, sc-376807) | mouse | WB, 1:200 | 2% BSA / 0.1% PBS – Tween 20 |
| anti-CKAP5 (Santa Cruz, sc-374394) | mouse | WB, 1:200 | 2% BSA / 0.1% PBS – Tween 20 |
| anti-rabbit IgG IRDye 800CW (LI-COR, 926-32211) | goat | WB, 1:10000 | 2% BSA / 0.1% PBS – Tween 20 |
| anti-mouse IgG Alexa Fluor 680 (Thermo scientific, A-21057) | goat | WB, 1:10000 | 2% BSA / 0.1% PBS – Tween 20 |
| Streptavidin protein, DyLight 680 conjugate (Thermo scientific, 21848) | - | WB, 1:150000 | 2% BSA / 0.1% PBS – Tween 20 |
| anti-Ago2 (Ascenion, 11A9) | rat | WB, 1:3000;<br>IP, 5 µg;<br>IF, 1:500 | 5% milk / 0.1% PBS –Tween<br>5% BSA, 2% donkey serum, 2% goat serum 0.02% PBS-Triton-X 100 |
| anti-TNRC6A (Bethyl, A302-329A) | rabbit | WB, 1:3000 | 5% milk / 0.1% PBS –Tween |
| anti-TNRC6A (Bethyl, A302-330A) | rabbit | IP, 5 µg | |
| anti-TNRC6C (Bethyl, A303-969A) | rabbit | WB, 1:1000 | 2% BSA / 0.1% PBS – Tween 20 |
| anti- GIGYF2 (Novusbio, NBP2-12812) | rabbit | WB, 1:3000;<br>IP, 5 µg;<br>IF, 1:500 | 5% milk / 0.05% PBS –Tween<br>5% BSA, 2% donkey serum, 2% goat serum 0.02% PBS-Triton-X 100 |
| anti-α-tubulin (Sigma, T5168) | mouse | WB, 1:10000 | 2% BSA / 0.1% PBS – Tween 20 |
| anti-Cdc25c (cell signaling, #4688) | rabbit | WB, 1:1000 | 5% BSA / 0.1% TBS – Tween 20 |
| anti-14-3-3 ε (cell signaling, #9635) | rabbit | WB, 1:1000 | 5% BSA / 0.1% TBS – Tween 20 |

| Antibody | Host | Dilution | Buffer |
|---|---|---|---|
| anti-Clathrin (BD, 610499) | mouse | WB, 1:2000 | 2% BSA / 0.1% PBS – Tween 20 |
| IgG from rabbit serum (Sigma, I5006) | rabbit | IP, 5µg | |
| anti-mouse Alexa 488 (Jackson ImmunoResearch, 715-545-150) | donkey | IF, 1:1000 | 5% BSA, 2% donkey serum, 2% goat serum 0.02% PBS-Triton-X 100 |
| anti-rat Alexa 647 (Invitrogen, A11077) | goat | IF, 1:1000 | 5% BSA, 2% donkey serum, 2% goat serum 0.02% PBS-Triton-X 100 |
| anti-rabbit Alexa 568 (Invitrogen, A10042) | donkey | IF, 1:1000 | 5% BSA, 2% donkey serum, 2% goat serum 0.02% PBS-Triton-X 100 |
| streptavidin Cy5 (Jackson ImmunoResearch, 016-170-084) | - | IF, 1:200 | 5% BSA, 2% donkey serum, 2% goat serum 0.02% PBS-Triton-X 100 |

## Supplementary table 5: IF min/max displayed values

| **Hela 11ht** (Fig 3) | **Cy5** | **Alexa488/GFP** | **Dapi** | **Alexa568** |
|---|---|---|---|---|
| untransfected | 556/2612 | 654/889 | 566/827 | 1063/3396 |
| CBirA*-Ago2<br>NBirA*-TNRC6C | | 654/889 | | |
| CBirA*-Ago2<br>NBirA*-Dicer | | 654/1807 | | |
| CBirA*-Ago2<br>NBirA*-GFP | | 654/889 | | |
| **Hela 11ht** (Fig 2) | **Cy5** | **Alexa488/GFP** | **Dapi** | **Alexa568** |
| untransfected | 493/710 | 426/872 | 494/827 | 477/578 |
| CBirA*-Cdc25C<br>NBirA*-14-3-3 **ε** | 428/982 | 426/872 | | |
| CBirA*-Cdc25C<br>S216A<br>NBirA*-14-3-3 **ε** | | 494/972 | | |
| CBirA*-Cdc25C<br>NBirA*-GFP | | 462/546 | | |
| **Hela 11ht** (Fig 4) | **Alexa647** | **-** | **Dapi** | **Alexa568** |
| untransfected | 479/692 | | 527/1342 | 468/761 |