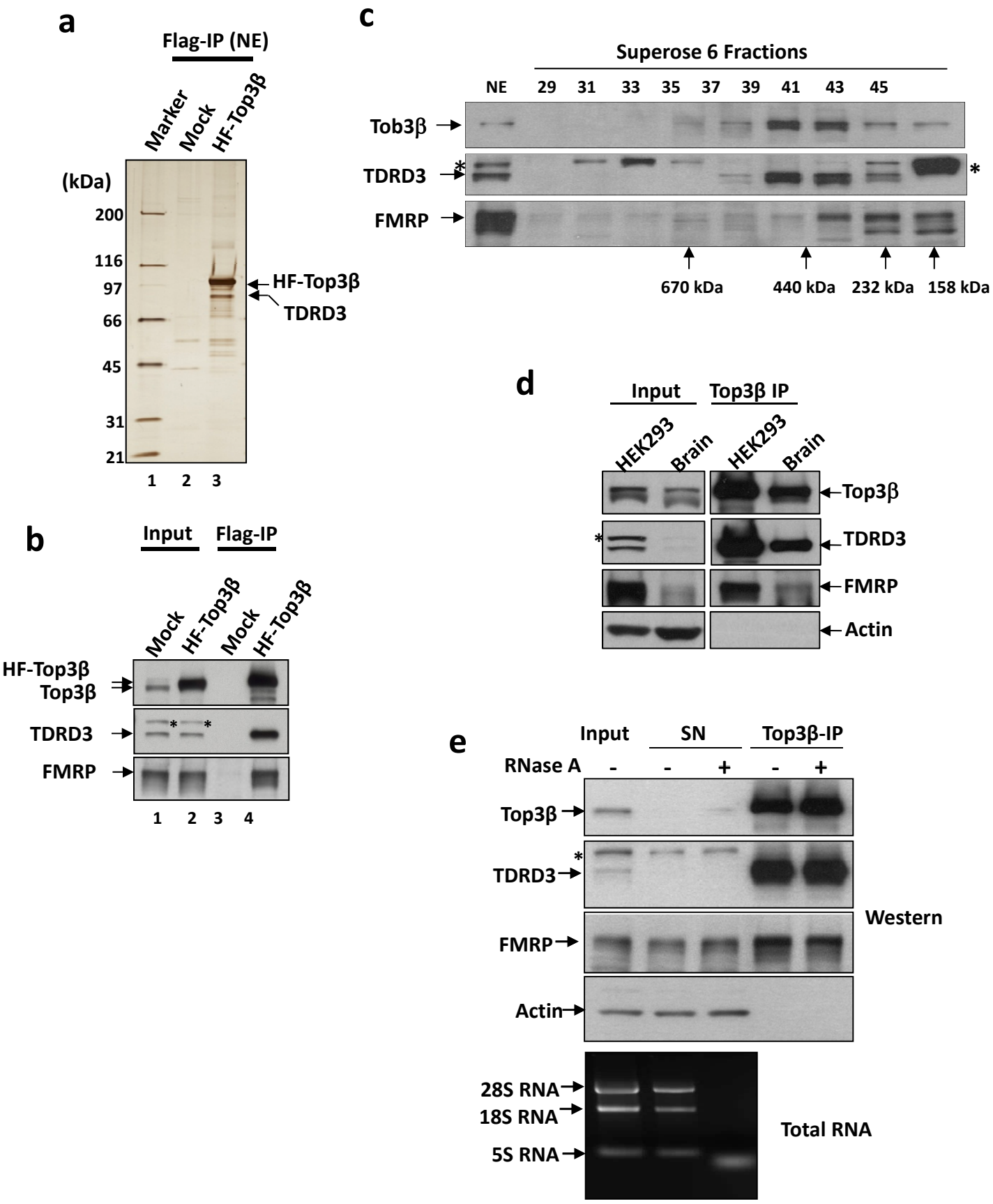
Supplementary Information

Title

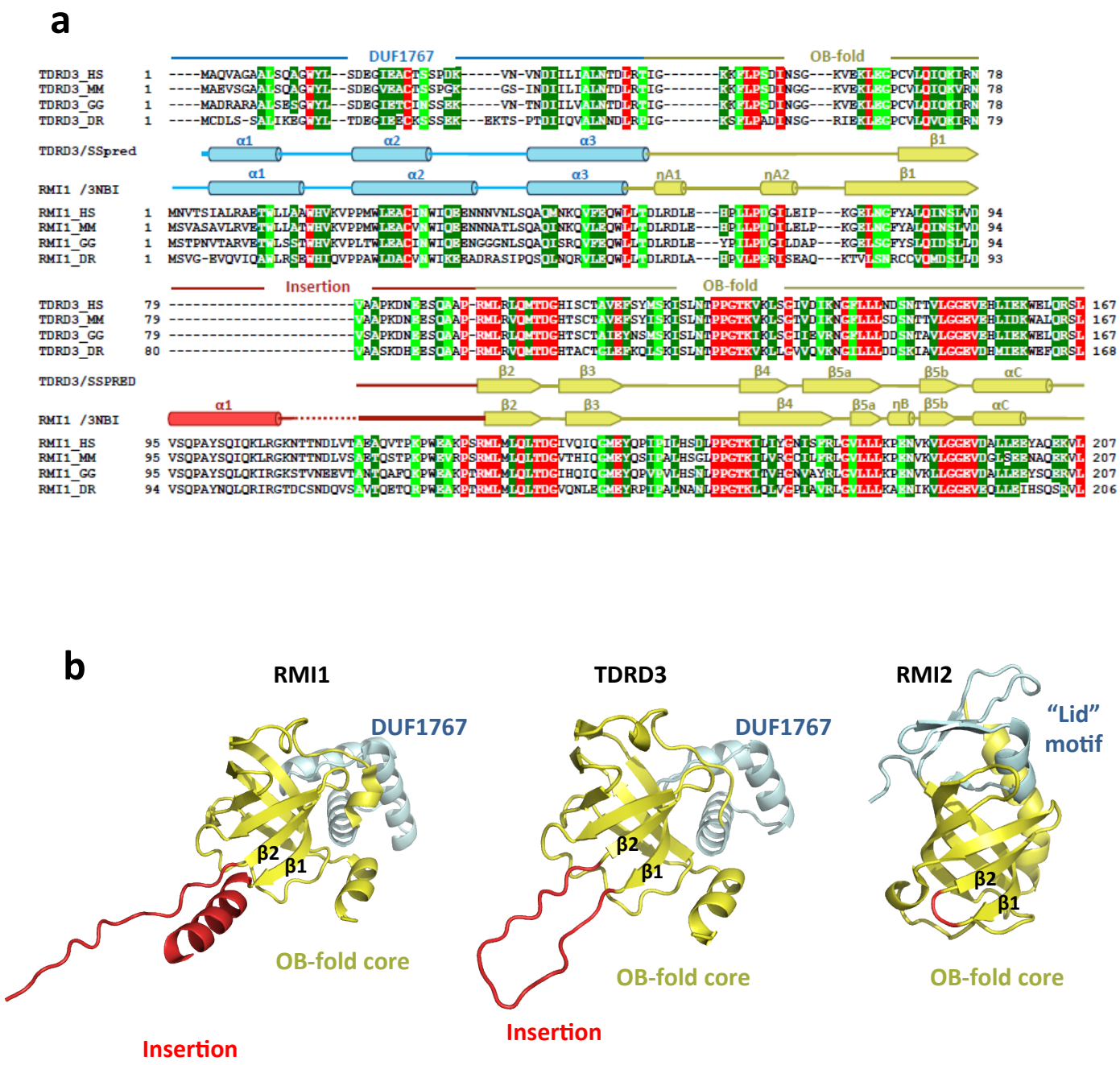
Top3β is an RNA topoisomerase that works with Fragile X syndrome protein to promote synapse formation

Authors

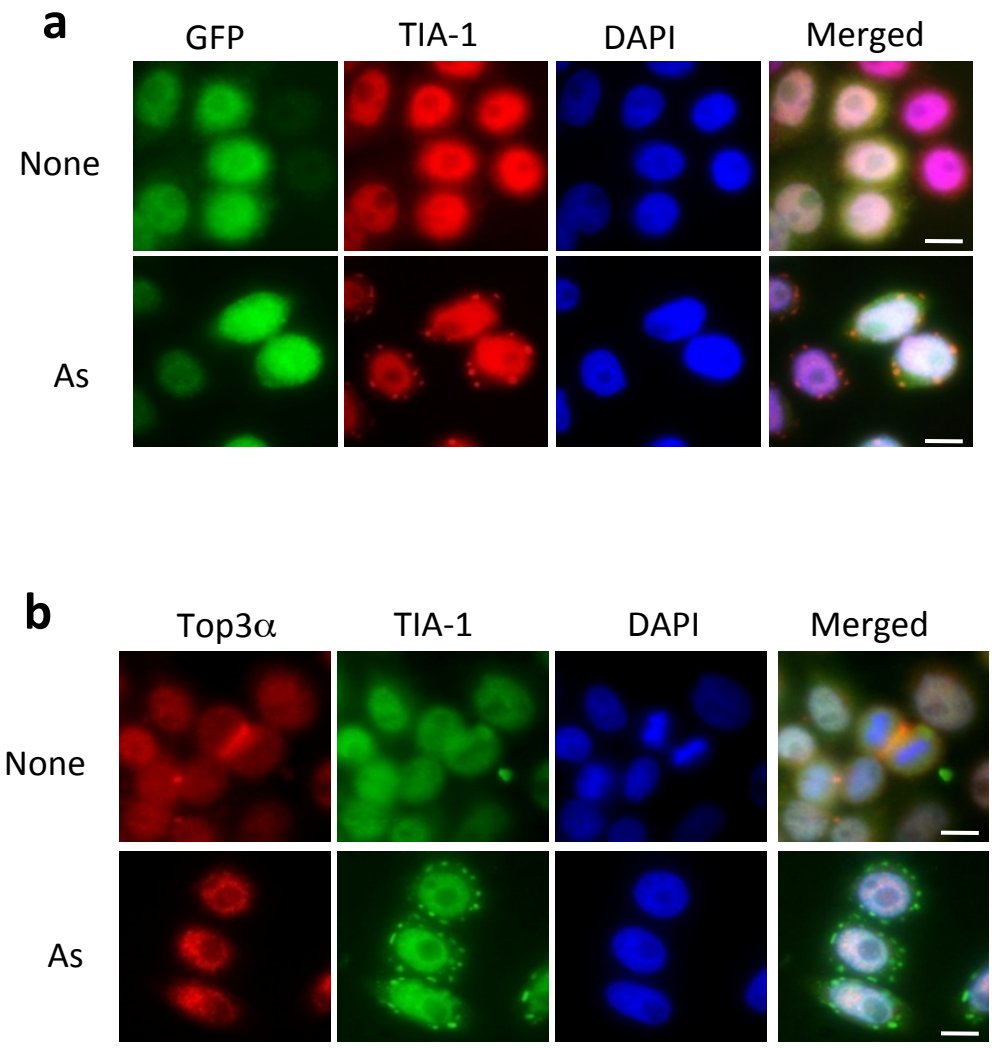
Dongyi Xu, Weiping Shen, Rong Guo, Yutong Xue, Wei Peng, Jian Sima, Jay Yang, Alexei Sharov, Subramanya Srikantan, Jiandong Yang, David Fox, 3rd, Yong Qian, Jennifer L. Martindale, Yulan Piao, James Machamer, Samit R. Joshi, Subhasis Mohanty, Albert C. Shaw, Thomas E. Lloyd, Grant W. Brown, Minoru S.H. Ko, Myriam Gorospe, Sige Zou, Weidong Wang.


a
Flag-IP (NE)
Marker
Mock
HF-Top3β
(kDa)
200
116
97
66
45
31
21
HF-Top3β
TDRD3
1 2 3
b
Input
Flag-IP
Mock
HF-Top3β
Mock
HF-Top3β
HF-Top3β
Top3β
TDRD3
FMRP
1 2 3 4
c
Superose 6 Fractions
NE 29 31 33 35 37 39 41 43 45
Tob3β
TDRD3
FMRP
670 kDa
440 kDa
232 kDa
158 kDa
d
Input
Top3β IP
HEK293
Brain
HEK293
Brain
Top3β
TDRD3
FMRP
Actin
e
Input
SN
Top3β-IP
RNase A - - + - +
Top3β
TDRD3
FMRP
Actin
Western
28S RNA
18S RNA
5S RNA
Total RNA

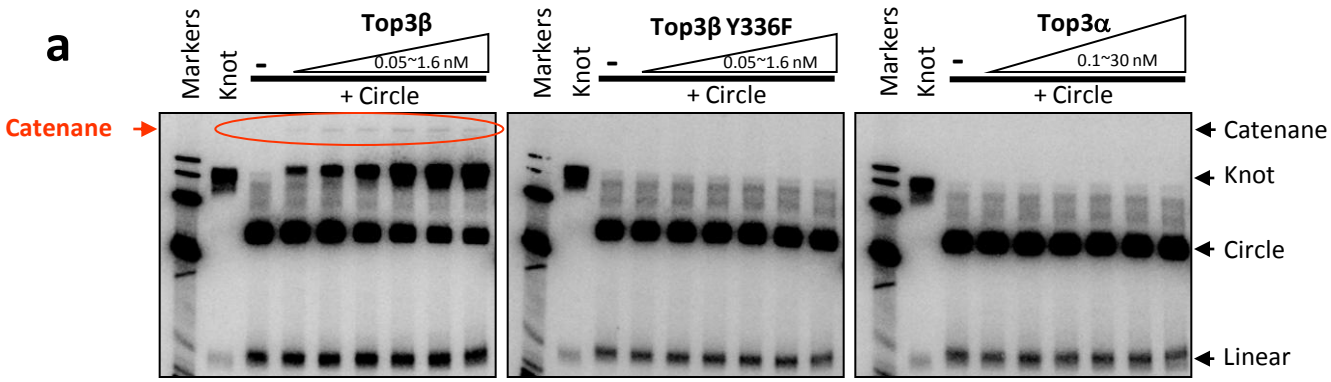
Supplementary Figure 1. Top3β and TDRD3 form a complex that associates with FMRP in the presence or absence of RNA. (a,b) A silver-stained SDS gel (a) and immunoblotting (b) show that 6-histidine and Flag double-tagged Top3β (HF-Top3β) coimmunoprecipitated with TDRD3 and FMRP from HeLa nuclear extract. The major polypeptides were identified by mass spectrometry. As a control, mock IP (Mock) was performed by using regular HeLa cells that do not express HF-Top3β. IP was performed using a Flag antibody. (C) Immunoblotting shows that the Superose 6 fractionation profile of the Top3β-TDRD3 complex overlaps with that of FMRP in HeLa nuclear extract. The fractions corresponding to molecular weight standards are indicated at the bottom. The asterisks mark a crossreactive polypeptide. The peak fraction of Top3β is co-incidental with that of TDRD3 (fractions 39 and 41), consistent with the notion that the two proteins fractionate as a complex. However, this peak only partially overlaps with that of FMRP (fractions 41-43), suggesting that only a fraction of Top3β-TDRD3 complex associates with FMRP and vice versa. (d) IP-Western shows that Top3b co-immunoprecipitated with TDRD3 and FMRP from whole cell extracts of mouse brain. Human HEK293 cell extracts were included as a positive control. An asterisk marks a crossreactive polypeptide. (e) Top: IP-Western shows that TDRD3 and FMRP coimmunoprecipitate with Top3β in whole cell extract untreated or treated with RNase A (0.1 mg/ml). RNase-out (0.4 unit/ml) was added to the untreated extract to inhibit RNA degradation by endogenous RNases. Bottom: an ethidium bromide-stained agarose gel shows that RNase A treatment eliminates 28S, 18S and 5S rRNAs (top) in the supernatant (SN) after immunoprecipitation.

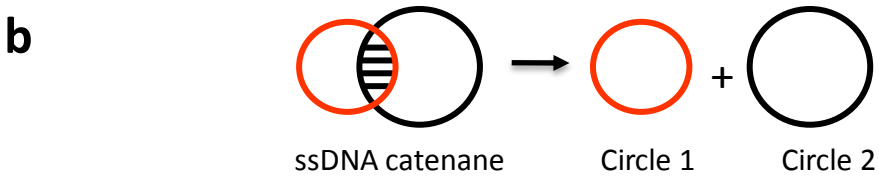

a
DUF1767
OB-fold
TDRD3_HS
TDRD3_MM
TDRD3_GG
TDRD3_DR
TDRD3/SSpred
RMI1 /3NBI
RMI1_HS
RMI1_MM
RMI1_GG
RMI1_DR
Insertion
OB-fold
TDRD3/SSPRED
RMI1 /3NBI
b
RMI1
TDRD3
RMI2
DUF1767
DUF1767
"Lid" motif
β2
β1
β2
β1
β2
β1
OB-fold core
OB-fold core
OB-fold core
Insertion
Insertion

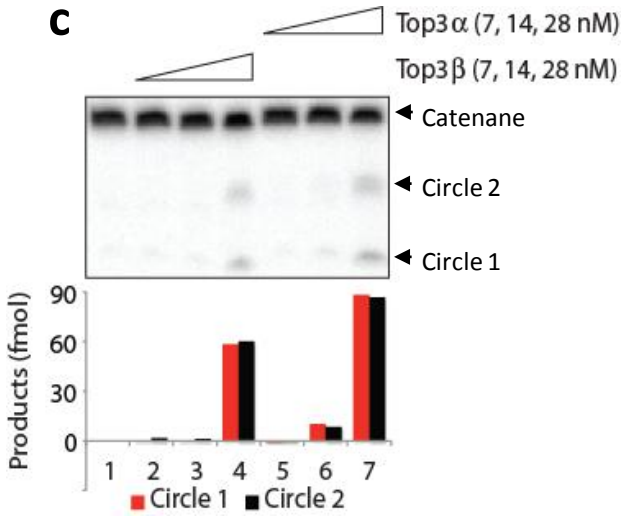
Supplementary Figure 2. The N-terminal regions of TDRD3 and RMI1 have the same domains, including an OB-fold with a unique intervening region. (a) Multiple sequence alignment with secondary and tertiary structure prediction for TDRD3 N-terminal region. The protein and their species are: TDRD3 and RMI1from human (HS; Q9H7E2 [TDRD3], Q9H9A7 [RMI1]), mouse (MM; Q91W18 [TDRD3], Q10160 [RMI1]), chicken (GG; Q5ZMS6 [TDRD3], Q5ZHV8 [RMI1]) and fish (DR; Q6NYG6 [TDRD3], Q7ZVM9 [RMI1]) N-terminal sequences were aligned using Clustal-W with manual adjustments for secondary structure. Conservation between TDRD3 and RMI1 is indicated as follows: Red – identical residues; dark green – strong group semi-conserved; light green – weak group semi-conserved. RMI1 secondary structure was derived from the deposited pdb file (3NBI). TDRD3 secondary structure was predicted with PredictProtein and Phyre servers, and is shown above the RMI1 secondary structure for comparison. DUF1767 (blue) and OB-fold (yellow) domains for RMI1 and TDRD3 are shown above the alignment. The helix/loop insertion between β1-β2 in the RMI1 OB-fold, which is required for interaction with Top3α, is shown in red. TDRD3 is predicted to contain a shorter and unstructured loop, also shown in red, inserted at the same position as in RMI1. Dashes indicate missing residues in the RMI1 crystal structure. (b) Structural comparison of OB-fold loop insertions. Left, the RMI1 crystal structure (3NBI); Center, a TDRD3 homology model based on the RMI1 crystal structure (3BNI) and the structure-based alignment in S2a and generated using the alignment mode in SWISS-MODEL; Right, the RMI2 crystal structure (3NBH). The DUF1767, OB-fold, and loop insertions are colored as in (a). RMI2 is shown as comparison for an OB-fold lacking an insertion between strands β1-β2. Dashes indicated missing residues in the RMI1 crystal structure. Structures were visualized using the PyMol software package v0.99 .

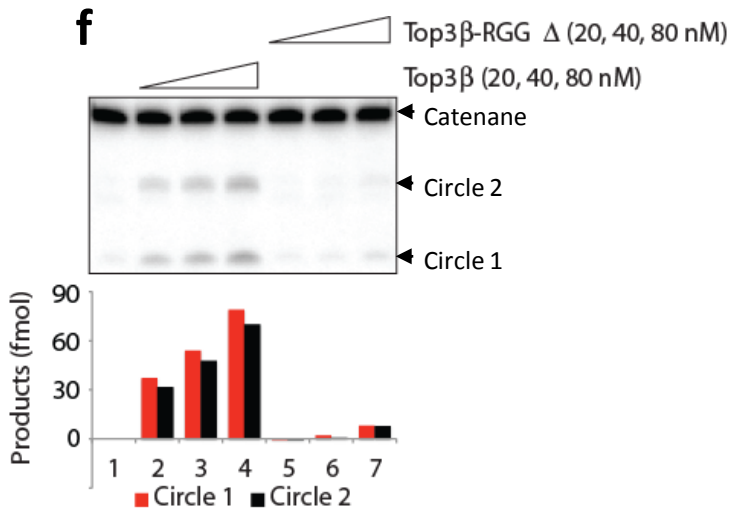


Supplementary Figure 3. GFP and Top3a do not localize in RNA stress granules. (a, b)
Immunofluorescence shows that GFP **(a)** and Top3α **(b)** are not localized in RNA stress granules in HeLa cells induced by arsenite (As). TIA-1 is a marker for stress granules, and the nucleus is stained with DAPI. The scale bars are 100 μm.

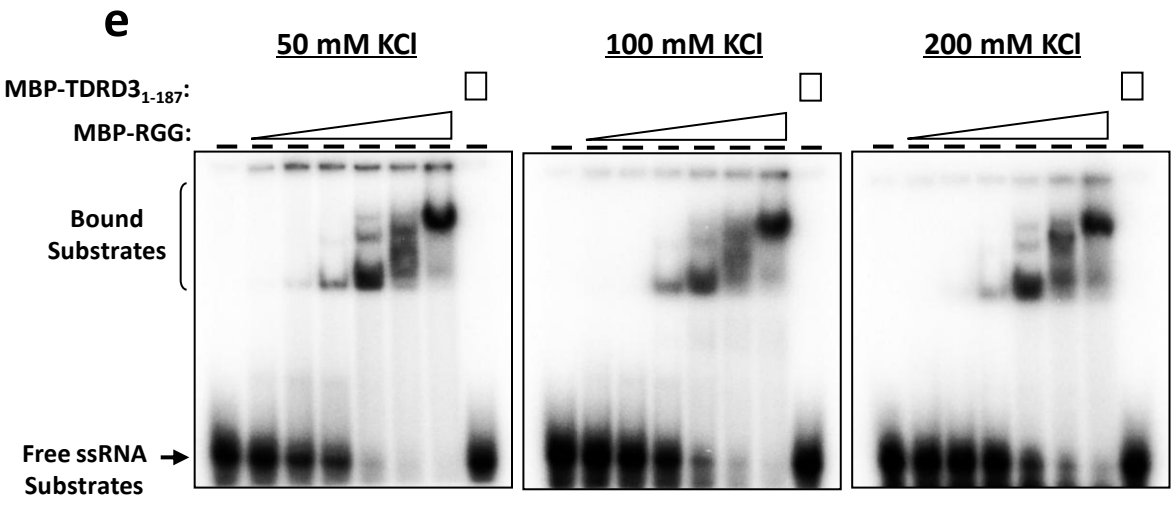

a
Markers
Knot
Top3β
0.05~1.6 nM
+ Circle
Top3β Y336F
Top3α
0.1~30 nM
Catenane
Knot
Circle
Linear


b
ssDNA catenane
Circle 1
Circle 2

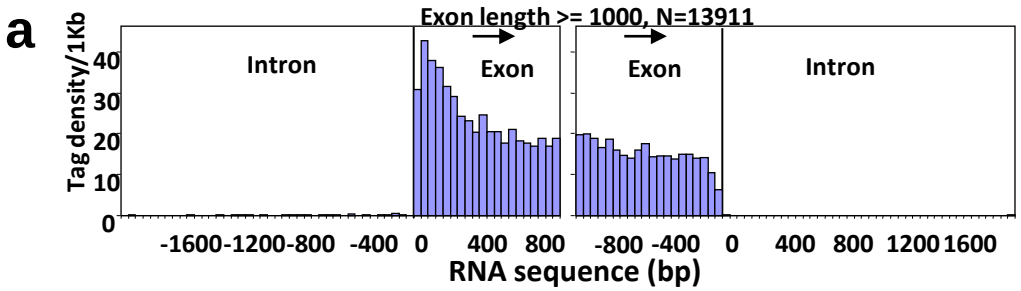

c
Top3α (7, 14, 28 nM)
Top3β (7, 14, 28 nM)
Catenane
Circle 2
Circle 1
Products (fmol)
Circle 1
Circle 2


f
Top3β-RGG Δ (20, 40, 80 nM)
Top3β (20, 40, 80 nM)
Catenane
Circle 2
Circle 1
Products (fmol)
Circle 1
Circle 2

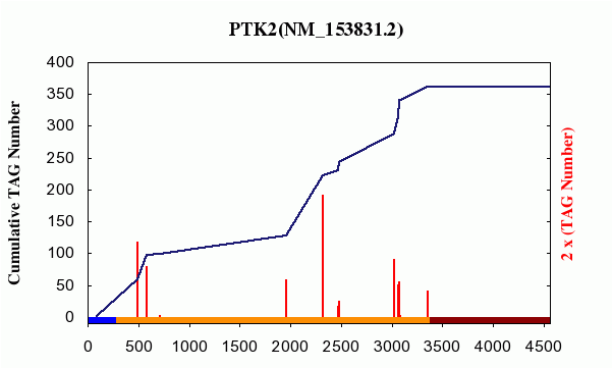

d
Markers
MBP-RGG

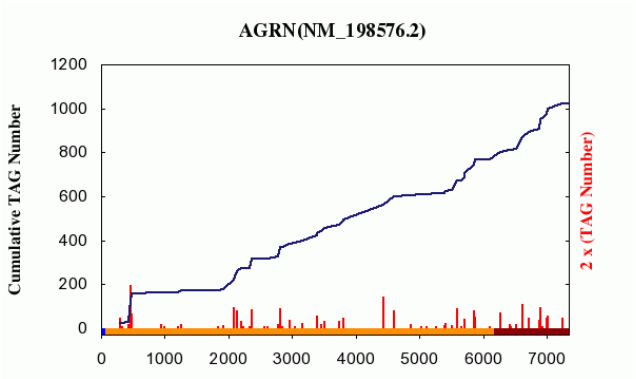

e
50 mM KCl
100 mM KCl
200 mM KCl
MBP-TDRD3 1-187:
MBP-RGG:
Bound Substrates
Free ssRNA Substrates

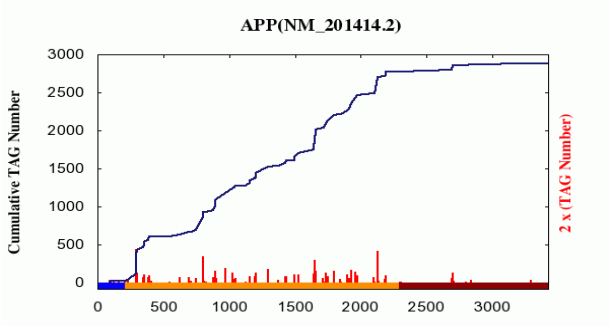
Supplementary Figure 4. The Top3β–catalyzed RNA topoisomerase reaction produces a small amount of catenane; the RGG domain of Top3β binds RNA at different salt concentrations; the RGG domain of Top3β is important for its ssDNA decatenase activity. (a) PhosphoImager analyses at long exposure to show that the RNA catenane products are produced by Top3β wildtype protein, but not by its catalytic inactive mutant Y336F or Top3α. The catenane products are highlighted by a red ellipse. The light exposure version is shown in Figure 4C. (**b**) Schematic representation of the single-stranded DNA (ssDNA) decatenase assay. (**c**) ssDNA decatenase assay (top panel) and its quantification (bottom panel) show that Top3α and Top3β have similar ssDNA decatenase activity. (**d**) A Coomassie blue stained SDS gel shows the purified fusion protein containing maltose-binding protein and the RGG domain of Top3β (MBP-RGG). (**e**) Gel-shift assays to show that MBP-RGG binds a single-stranded RNA substrate at different salt concentrations as indicated above each image. Reaction contains 1 nM single-stranded RNA and 62.5 nM, 125 nM, 250 nM, 500 nM, 1000 nM and 2000 nM MBP-RGG protein under different salt conditions as indicated . A different MBP-fusion protein containing the OB-fold domain of TDRD3 (MBP-$TDRD_{31\text{-}187;}$ 2000 nM) was included as a negative control. (**f**) ssDNA decatenase assay (top panel) and its quantification (bottom panel) show that the RGG motif deletion mutant of Top3β (RGGΔ) has reduced ssDNA decatenase activity compared to the wild type protein.



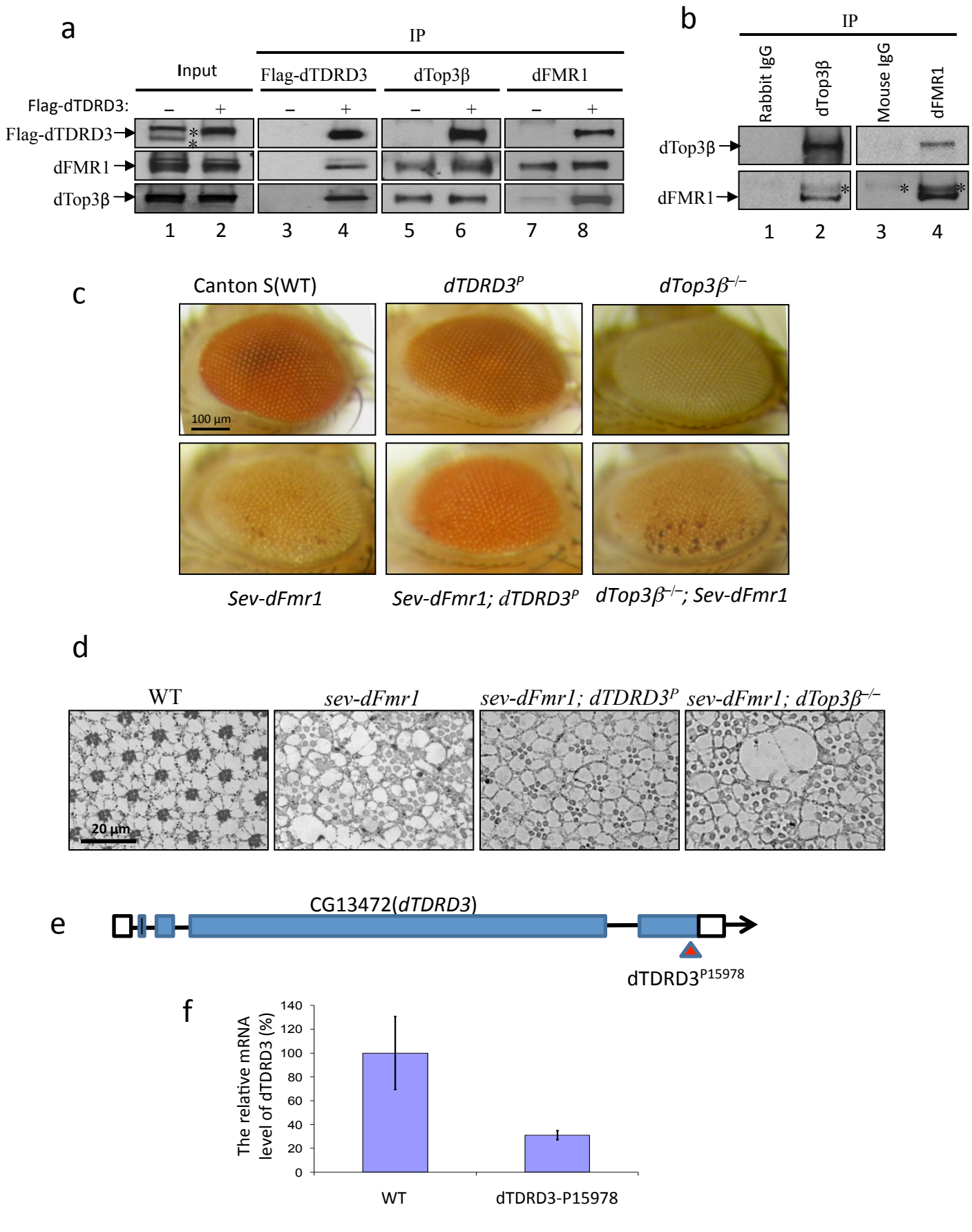
b





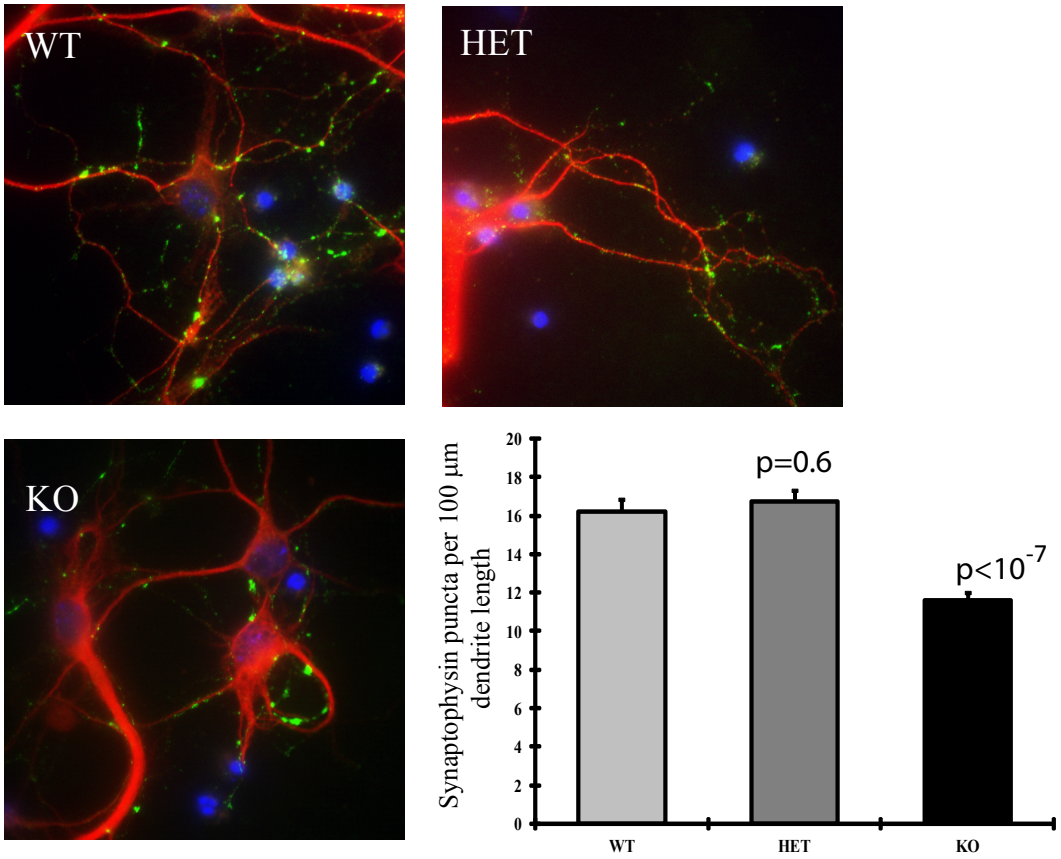


Supplementary Figure 5. Top3β binding sites in RNAs in vivo are enriched in exons; Top3β binding tags are enriched in protein coding regions—a pattern similar to that of FMRP. (**a**) Histograms illustrate that Top3-beta binding sites, represented by sequence tags identified by HITS-CLIP, preferentially map to exons, but not introns, suggesting that Top3β mainly binds to mature mRNAs. The number of sequence tags was counted at specific distances from exon start (left graph) and exon end (right graph) and then converted to tag density per 1 Kb. The lengths of exons and their numbers are indicated on top of each histogram. (**b**) Cumulative graphs show that the Top3β binding tags are enriched in protein coding regions (marked in orange) over 5' or 3' untranslated regions (marked in blue and purple, respectively). This pattern is highly similar to that described for FMRP (Darnell et al. Cell 2011).

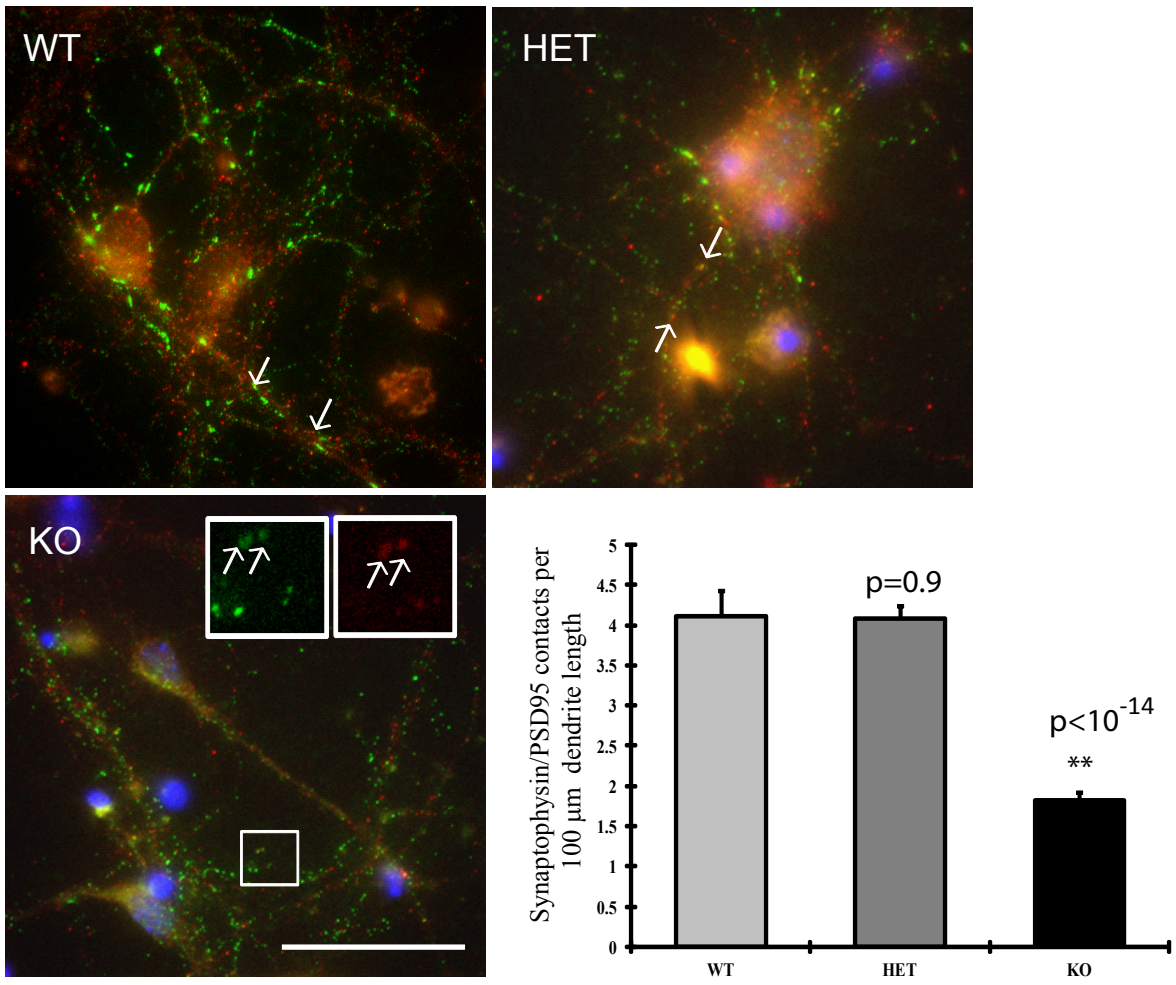

a
IP
Input
Flag-dTDRD3
dTop3β
dFMR1
Flag-dTDRD3:
– + – + – + – +
Flag-dTDRD3
dFMR1
dTop3β
1 2 3 4 5 6 7 8
b
IP
Rabbit IgG
dTop3β
Mouse IgG
dFMR1
dTop3β
dFMR1
1 2 3 4
c
Canton S(WT)
dTDRD3P
dTop3β−/−
100 μm
Sev-dFmr1
Sev-dFmr1; dTDRD3P
dTop3β−/−; Sev-dFmr1
d
WT
sev-dFmr1
sev-dFmr1; dTDRD3P
sev-dFmr1; dTop3β−/−
20 μm
e
CG13472(dTDRD3)
dTDRD3P15978
f
The relative mRNA level of dTDRD3 (%)
140
120
100
80
60
40
20
0
WT
dTDRD3-P15978

Supplementary Figure 6. The association between Top3β-TDRD3 and FMR1 is conserved in *Drosophila;* mutations of *Top3β* and *TDRD3* in Drosophila modify the rough eye phenotype induced by ectopic expression of FMR1. (**a**) IP-Western shows co-immunoprecipitation of dTop3β, dTDRD3 and dFMR1 from extracts of the *Drosophila* S2 cells. The cells without or with ectopically expressed Flag-tagged dTDRD3 are indicated on top. Immunoprecipitations (IP) were performed using anti-Flag, anti-dTop3β and anti-dFMRP1 antibodies, as illustrated. A mock immunoprecipitation was performed using S2 cells that do not express Flag-dTDRD3. The crossreactive polypeptides are indicated with asterisks. Notably, over-expression of dTDRD3 increased the amount of dTop3β that co-immunoprecipitated with dFMR1 (compare lanes 8 and 7), consistent with the notion that TDRD3 serves as a bridge between the other two proteins. (**b**) Control experiments to show that dFMR1 and dTop3β were not immunoprecipitated by rabbit or mouse IgG from extracts of the *Drosophila* S2 cells. (**c**) Images from light microcopy (scale Bar 100 μm), and (**d**) images from tangential sections (scale bar 20 μm), of *Drosophila* compound eyes show genetic interactions of dFMR1 with Top3β and TDRD3. Images of *Drosophila* compound eyes of different genotypes are indicated on top. The rough eye phenotype induced by ectopic expression of *dFMR1* in the eye (*sev-dFmr1*) is suppressed by a reduction of function mutant of *dTDRD3* (*dTDRD3-P*), and is enhanced by *Top3β null* mutant. The scar-like spots in (c) are necrotic ommatidia which are quantified in Figure 6b. TDRD3 and Top3b mutant flies in the wildtype background have normal eye morphology (c). (e) Schematic view of the P-element insertion site at dTDRD3 genomic locus in dTDRD3^{P15978} strain. (f) qRT-PCR chart shows that the dTDRD3 transcript level is reduced by about 70% in dTDRD3^{P15978} line. The relative mRNA level of dTDRD3 is normalized by the dRpl32 mRNA level. Mean and s.d. from three independent experiments are shown.

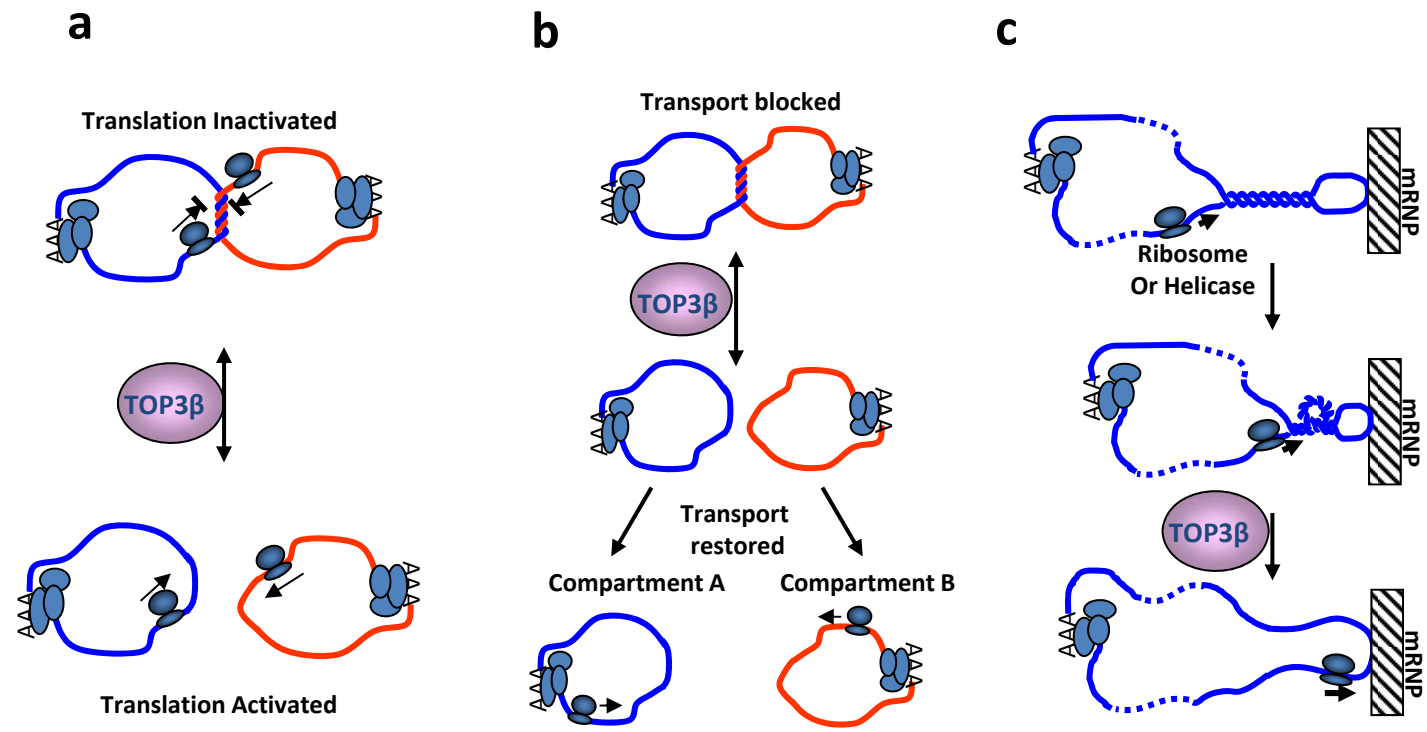
a

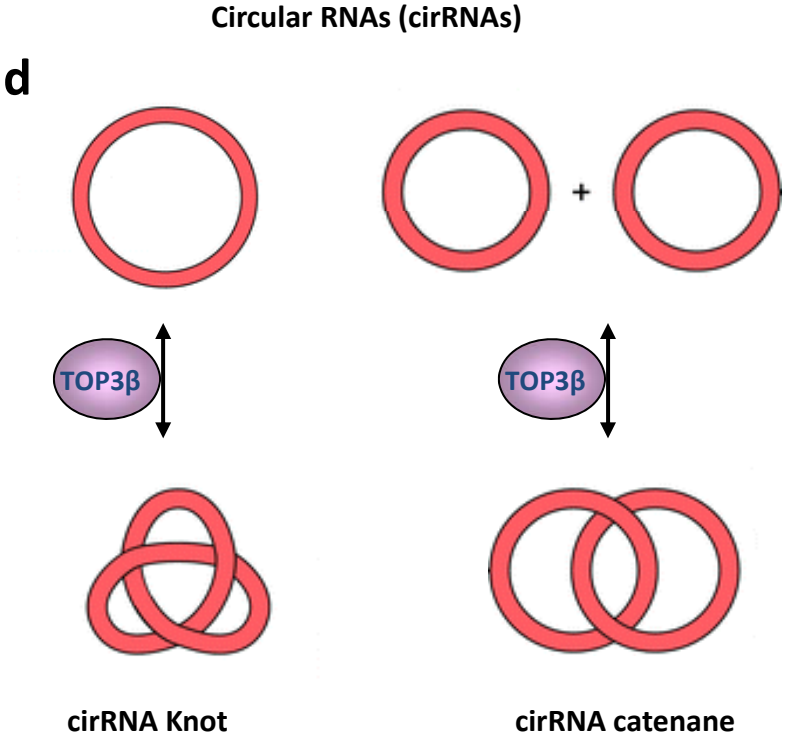

WT
HET
KO
Synaptophysin puncta per 100 µm dendrite length
20
18
16
14
12
10
8
6
4
2
0
p=0.6
p<10-7
WT
HET
KO

b


WT
HET
KO
Synaptophysin/PSD95 contacts per 100 µm dendrite length
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
p=0.9
p<10-14
**
WT
HET
KO

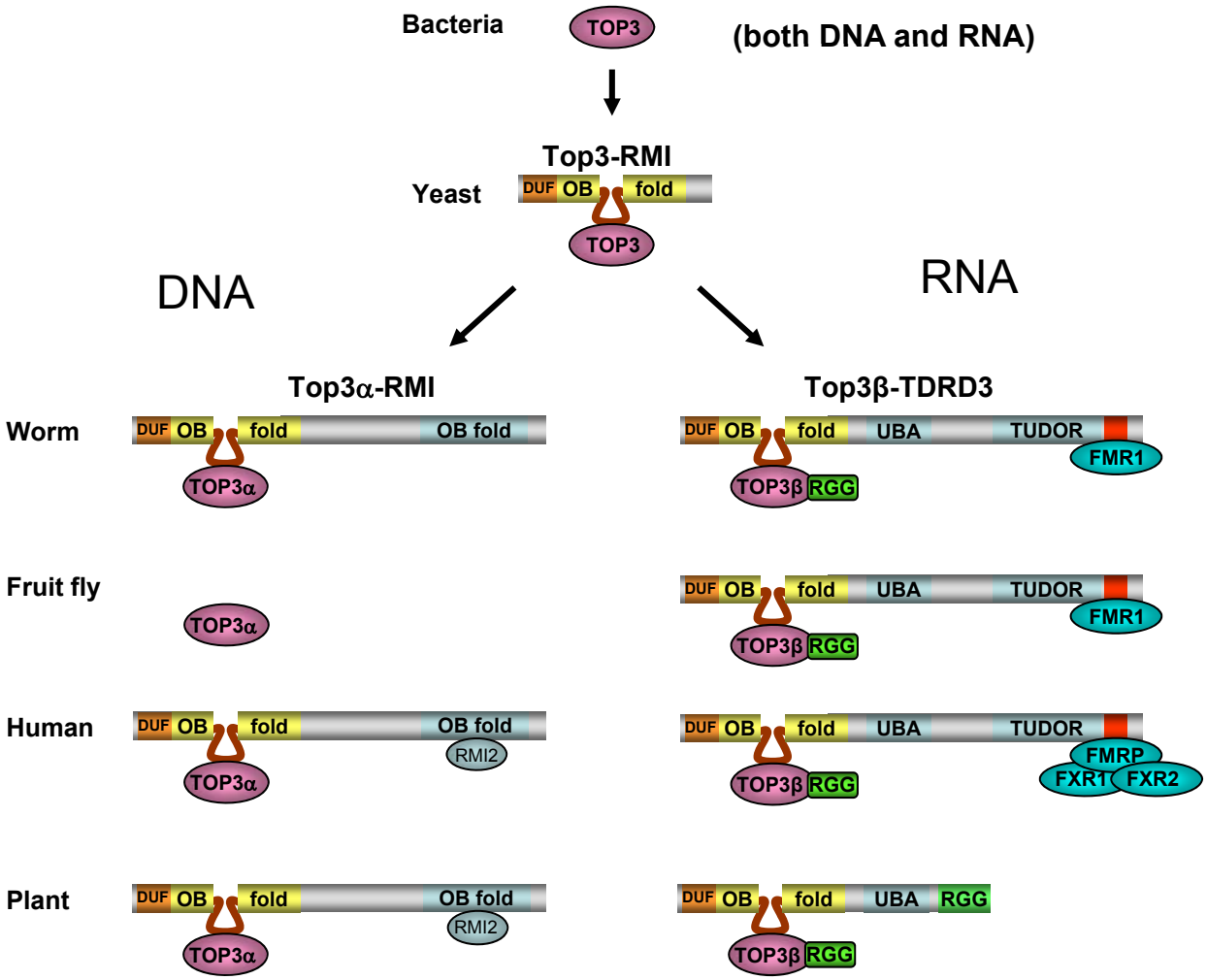
Supplemental Figure 7. Synaptogenesis is abnormal in cultured cortical neurons from Top3β-knockout mice. (a) Immunostaining shows reduced presynaptic vesicles in cultured cortical neurons of Top3β-knockout mice. Synaptophysin (green) and MAP2 (red) antibodies were used to stain presynaptic vesicles and dendrites, respectively, in cultured neurons (10 days) from wide type (WT), heterozygous (HET) and knockout (KO) mice. The number of synaptophysin puncta along a defined length of a dendrite was counted. Quantified data were obtained from one WT, two HET and four KO mice from the same litter. *P* values between WT and HET, and between WT and KO, were calculated using Student T-test; and the results were listed on top of the data bar. Scale bar, 100 μm. **(b)** Immunostaining shows reduced number of synapses in cultured cortical neurons of Top3β-knockout mice. Synaptophysin (green) and PSD95 (red) were co-stained to indicate synapses (merged) in cultured neurons (10 days) from the same mice as in (**a)**. The arrows indicate co-localized puncta of synaptophysin and PSD95, which were counted along neurites as (**a)**. The small islet boxes showed an example of co-localized synaptophysin and PSD95 using two separate light channels. Quantitative data indicate reduced synaptogenesis in Top3β-deficient cortical neurons. *P* values between WT and HET, and between WT and KO, were calculated using Student T-test; and the results were listed on top of the data bar. Scale bar, 100 μm.





Supplementary Figure 8. Models for why RNA metabolism may create topological problems that need an RNA topoisomerase to solve.

(a)(b) Models to illustrate that Top3β may catalyze catenation or decatenation of two mRNA circles, thus enhancing or inhibiting translation (b) or transport of mRNAs (c). It has been shown that mRNA circles can form when two ends of mRNA interacting with a common protein. (c) A model illustrates that helical torsions may arise when a translating ribosome or a helicase unwinds a duplex region in an mRNA hairpin. If the hairpin is bound to an immobile mRNP or cellular matrix, the helical torsion will not be relieved by rotation. Such torsion may impede progression of ribosomes or RNA helicases during translation or transport. Top3β can relax the helical torsion through its strand passage activity. (d) Top3b may interconvert cirRNAs to knot or catenane structures, which may affect the function of these RNAs.


Bacteria
TOP3
(both DNA and RNA)
Top3-RMI
Yeast
DUF
OB
fold
TOP3
DNA
RNA
Top3α-RMI
Top3β-TDRD3
Worm
OB fold
TOP3α
UBA
TUDOR
FMR1
TOP3β
RGG
Fruit fly
Human
RMI2
FMRP
FXR1
FXR2
Plant

Supplementary Figure 9. Top3 has evolved to become two homologous complexes with distinct functions in RNA and DNA metabolism.

Schematic presentations to show how Top3 has evolved to become two homologous complexes, Top3α-RMI1 and Top3β-TDRD3, which have distinct functions in DNA and RNA metabolism, respectively.

Bacteria have a single copy of Top3 that can catalyze both DNA and RNA strand passage reactions, but it has no orthologs of RMI1.

Yeast have a single copy of Top3 and RMI. They form a complex that is required for maintenance of genomic stability. It remains unclear whether it also functions in RNA metabolism.

In higher eukaryotes, Top3 has evolved into two paralogous complexes: the Top3α-RMI1 complex, which retains its function in the maintenance of genomic stability but lost the ability to catalyze RNA strand passage reactions, and the Top3β-TDRD3 complex, which works in RNA metabolism. Top3β may have acquired an RGG-box RNA binding motif through gene fusion, and retained the ability to resolve topological problems for RNA. The two Top3 complexes not only have a homologous catalytic subunit in Top3α and Top3β, but also a homologous partner subunit, RMI1 and TDRD3, respectively. In fact, RMI1 and TDRD3 are the only metazoan proteins containing both a DUF1767 domain and a unique OB-fold with an intervening region, indicating that they are paralogs derived from a common ancestor. This ancestor likely resembles the single RMI1 protein from yeast, which consists of only the DUF1767 and OB-fold domains. The unique C-terminal domains in RMI1 and TDRD3 of higher eukaryotes are likely acquired through gene fusions later in evolution. These domains provide unique protein-binding surfaces to allow their respective topoisomerases to interact with specific regulators, such as FMRP or RMI2.

Interestingly, the Top3β-TDRD3 complex can associate with FMRP through the C-terminal region of TDRD3 and modulate FMRP function in animals. In contrast, plants do not have an ortholog of FMR1 and the C-terminal region of TDRD3 is similar to those found in several protozoans, which encodes a C-terminal RGG-box motif but lacks both the metazoan UBA and TUDOR domains.

Xu et al. Supplementary Figure 10

Full-length pictures of the blots in the main figures

Fig. 1c.

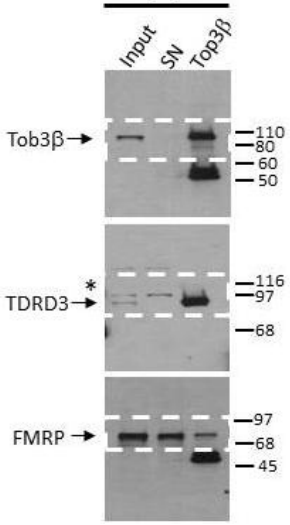


Fig. 1e.

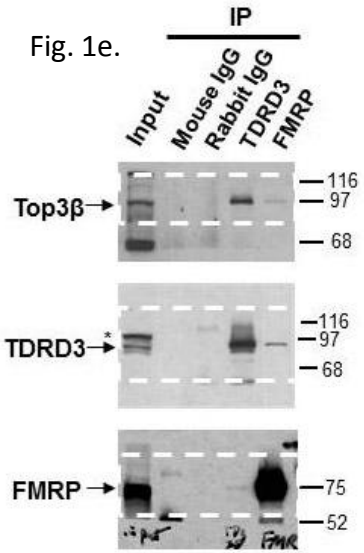


Fig. 1f.

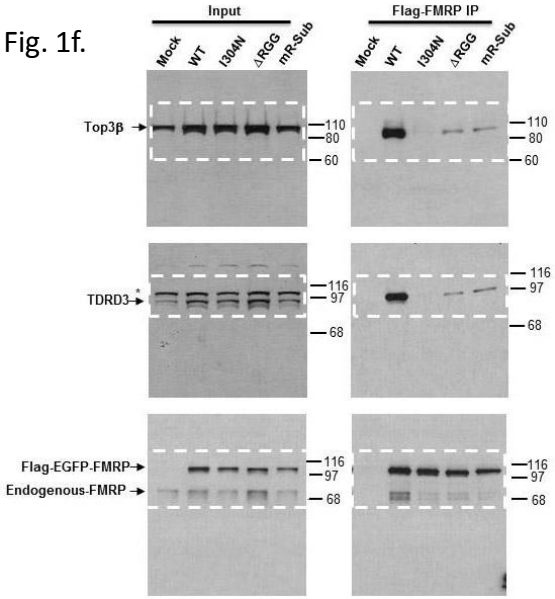


Fig. 2a.

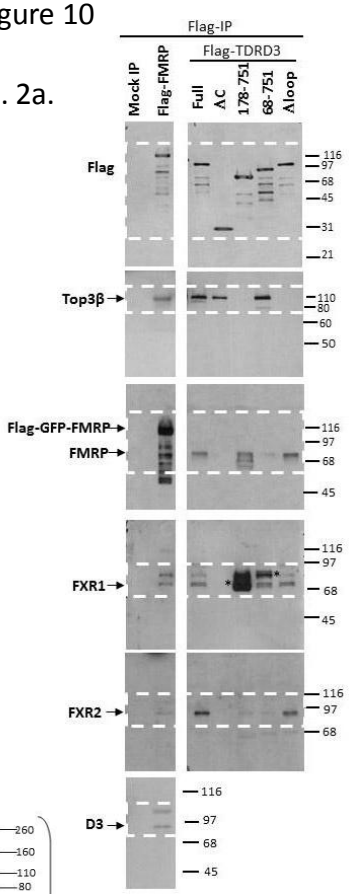


Fig. 2c.

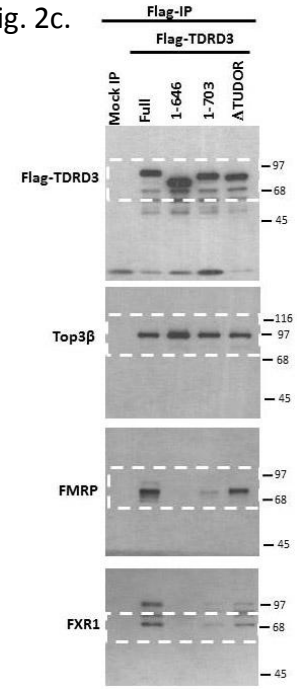


Fig. 2d.

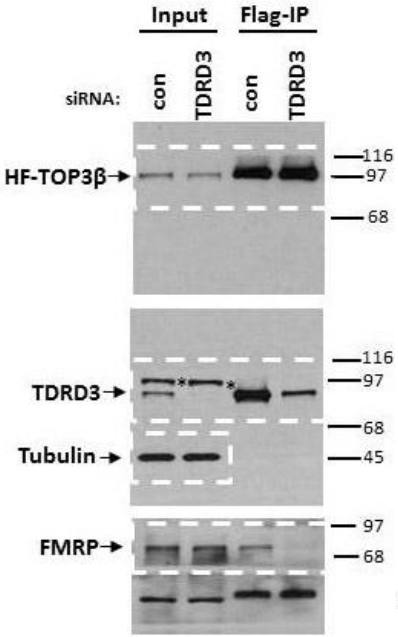
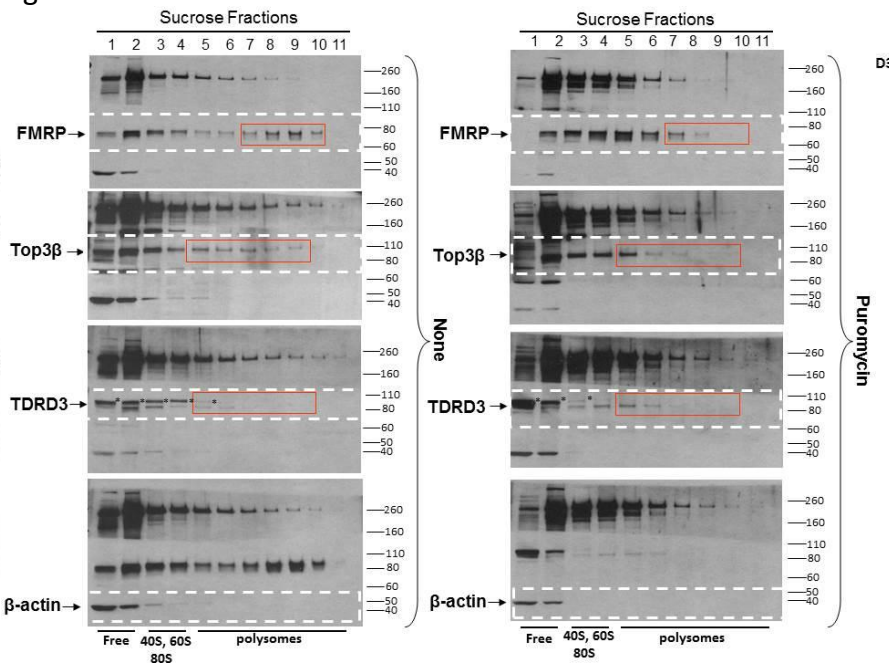


Fig. 3d.



Supplementary Table 1. The number of peptides identified by mass spectrometry analyses of the entire Top3β immunoprecipitates from cells untreated or treated with RNase A

Proteins	Number of peptides (- RNase)	Number of peptides (+RNase)
Top3β	33	42
TDRD3	36	48
FMRP	15	11
FXR2	20	10
FXR1	17	7
IGF2BP1*	17	0
EIF4A3*	21	0
PABPC1*	29	0
TUBB2C**	13	13
HSPA9**	16	17

Legend. A partial list of the peptides identified by mass spectrometry analyses of the entire Top3β immunoprecipitates from whole cell extracts. The silver-stained SDS-gel analyses of the immunoprecipitate was shown in Figure 1b. Cells were either treated or untreated with RNase A as indicated. The single asterisk marks several RNA binding proteins whose presence in Top3β IP depends on RNA. The double asterisks indicate two contaminating polypeptides whose presence does not depend on RNA. Notably, TDRD3, FMRP and its homologs do not depend on RNA for their presence.

Supplementary Table 2. Number of sequenced tags in Top3β HITS-CLIP

Category of Top3β tags	Total	Sense	Antisense	%sense
Total tags	21774326			
Tags not matched to hg18	10761490			
Tags matched to hg18	11012836			
unique	2773915			
2-5 matches	7959287			
>5 matches	279634			
Tags matched to RefSeq (directly)	2285222	2211865	32579	98.55
Tags matched to RefSeq genes	2422267	2321609	100658	95.84
Tags matched to RefSeq exons	1847106	1835428	11678	99.37
Tags matched to RefSeq introns	575161	486181	88980	84.53

Supplementary Table 3. The top Top3β-bound mRNAs that match the FMRP targets

#GID-GSE23316	gene Human	gene Mouse	Ntags (Top3b CLIP)	Npeaks	mRNA_len	RNA-seq rep1 (GSM591682)	RNA-seq rep2 (GSM591659)	Average RNAseq	ORF_len,aa	Ratio1	MatchFMRP(Darnell)
1952	CELSR2	Celsr2	3667	187	10540	8.11	5.74	6.925	2923	11.04874	1
488	TNRC18	Tnrc18	1340	87	10586	0.05	0.04	0.045	2968	8.661025	1
3897	PKD1	Pkd1	2861	152	14138	12.2	9.42	10.81	4303	7.304181	1
5591	PCNXL3	Pcnxl3	2659	130	6584	10.1	13.43	11.74	2034	5.979761	1
871	LPHN1	Lphn1	1392	67	7874	1.69	2.02	1.855	1587	5.651624	1
23250	MLL2	Mll2	2510	113	19432	9.03	11	10.015	5537	5.645766	1
7916	NOMO3	Nomo1	1852	71	4315	2.48	2.93	2.705	1222	5.588351	1
729086	TTYH3	Ttyh3	1330	68	4838	4.05	3.22	3.635	523	4.987165	1
1362	PTPRS	Ptprs	1678	91	7347	11	7.33	9.185	1948	4.743289	1
2799	LMTK2	Lmtk2	982	51	8961	1.8	2.04	1.92	1503	4.278523	1
8940	KIAA0100	2610507B1	4642	155	7456	21.3	34.08	27.675	2235	4.114134	1
7132	PTPRJ	Ptprj	1647	68	7854	5.29	8.4	6.845	1451	4.036806	1
250	ODZ3	Odz3	1013	41	10810	0.56	0.53	0.545	2699	3.888099	1
286077	NDST1	Ndst1	829	50	8030	3.21	4.24	3.725	882	3.642987	1
79158	PCNX	Pcnx	1196	58	12936	6.86	5.82	6.34	2341	3.549572	1
975	ATP2B4	Atp2b4	1482	58	8911	8.21	5.65	6.93	1170	3.425871	1
10908	MACF1	Macf1	1699	56	19271	8.2	7.15	7.675	5938	3.168317	1
24145	BIRC6	Birc6	1361	51	15718	6.01	7.16	6.585	4857	3.075068	1
3340	PLXNA1	Plxna1	1095	61	9071	8.99	11.14	10.065	1896	3.04012	1
1122	PLXND1	Plxnd1	1403	56	6977	11.2	7.52	9.355	2070	2.893309	1
6522	BAT2	Bat2	1365	74	6921	16.2	18.31	17.27	2157	2.713605	1
5420	FASN	Fasn	2519	120	8481	31.6	37.7	34.64	2511	2.688172	1
4035	NOMO2	Nomo1	1842	72	4317	13.8	20.12	16.935	1222	2.673102	1
6489	MLL3	Mll3	655	30	16872	1.22	1.34	1.28	4911	2.659574	1
1889	EXTL3	Extl3	1007	42	6172	4.76	6.84	5.8	919	2.658228	1
84220	DST	Dst	737	33	16652	2.44	2.59	2.515	5171	2.636836	1
10130	SCAP	Scap	1222	68	4255	13.2	18.96	16.075	1279	2.607862	1
7846	PTPRF	Ptprf	4251	170	7733	58.6	55.42	56.985	1907	2.537882	1
92140	WNK1	Wnk1	1986	68	11232	17.7	16.14	16.93	2642	2.525065	1
2802	KIAA0802	1110012J1	570	30	6092	2.76	1.7	2.23	1586	2.452984	1
4105	INTS1	Ints1	890	44	7000	7.2	8.84	8.02	2190	2.441731	1
26100	HERC2	Herc2	1129	44	15334	8.46	8	8.23	4834	2.413604	1
221955	MYH10	Myh10	502	30	7619	2.47	2.66	2.565	1976	2.387585	1
2192	ANKRD11	Ankrd11	1237	61	9326	12.3	20.65	16.465	2663	2.304931	1
57634	MYO10	Myo10	935	39	11436	6.11	8.27	7.19	2058	2.268761	1
55714	GCN1L1	Gcn1l1	2187	83	8699	23.2	30.07	26.645	2671	2.264975	1
22856	LRP1	Lrp1	1593	77	14905	40.1	8.1	24.105	4544	2.257733	1

10079	PCDH7	Pcdh7	503	29	8728	3.02	2.92	2.97	1255	2.235929	1
10613	MLL	Mll1	1004	37	16608	6.53	6.65	6.59	3972	2.230259	1
23224	NSD1	Nsd1	940	37	12998	5.59	8.25	6.92	2696	2.186761	1
66008	LRRN2	Lrrn2	432	21	3227	0	0.01	0.005	713	2.098951	1
6046	ARHGEF17	Arhgef17	552	31	7830	7.51	5.37	6.44	2063	1.885645	1
1282	TRRAP	Trrap	688	28	12596	4.97	4.89	4.93	3830	1.875419	1
3678	NLGN2	Nlgn2	968	42	4642	17	8.35	12.665	995	1.853077	1
2137	SPTBN1	Spnb2	2017	70	10238	28.5	27.44	27.96	2364	1.844046	1
23420	DYNC1H1	Dync1h1	1933	77	14361	29.8	33.72	31.77	4646	1.843428	1
10682	DGCR2	Dgcr2	765	34	4504	7.99	9.17	8.58	550	1.829925	1
140707	NAV1	Nav1	233	20	13029	0.9	1.05	0.975	1877	1.822323	1
9780	NOMO1	Nomo1	1849	69	4261	21.6	34.33	27.975	1222	1.816985	1
80020	TNS3	Tns3	574	22	7697	2.28	2.49	2.385	1540	1.776342	1
10555	SHANK3	Shank3	396	18	7145	0.31	0.33	0.32	1852	1.744186	1
444	EP300	Ep300	552	31	8761	7.12	9.2	8.16	2414	1.707048	1
399687	NCOR1	Ncor1	641	17	9809	0	0	0	2440	1.7	1
9793	MAPK4	Mapk4	307	17	4736	0.39	0.06	0.225	587	1.662592	1
6520	ATP2A2	Atp2a2	3177	119	8329	53.6	73.18	63.395	1157	1.621364	1
1499	ANAPC1	Anapc1	651	31	6329	9.09	9.78	9.435	1944	1.59506	1
6840	AGPAT3	Agpat3	654	27	6555	6.41	7.64	7.025	376	1.585903	1
412	NAT8L	Nat8l	295	19	5894	1.92	2.21	2.065	459	1.574803	1
8712	PDZD8	Pdzd8	623	25	3853	5.73	6.22	5.975	1154	1.564945	1
23326	SPEN	Spen	771	31	12227	9.63	10.5	10.065	3664	1.544979	1
23524	PLXNA2	Plxna2	349	17	11457	1.04	1.22	1.13	1909	1.527403	1
55636	SETD5	Setd5	839	35	6827	14.1	12.36	13.205	1442	1.508296	1
57142	EP400	Ep400	636	28	12265	8.04	9.15	8.595	3122	1.505781	1
1059	TLN2	Tln2	350	18	11650	1.14	2.79	1.965	2542	1.504388	1
135114	HUWE1	Huwe1	2136	93	14734	46.4	57.69	52.06	4374	1.49855	1
27173	TRIO	Trio	933	34	10244	13	12.81	12.91	3097	1.484068	1
5797	C20orf117	9830001H(	483	18	8036	2.43	1.87	2.15	1866	1.481481	1
83639	POLR2A	Polr2a	1189	64	6738	26.6	40.01	33.315	1970	1.477548	1
949	PCDH1	Pcdh1	621	24	4779	5.27	7.66	6.465	1237	1.457637	1
9343	ITPR1	Itpr1	749	22	10197	7.39	3.33	5.36	2743	1.432292	1
57622	ARHGAP21	Arhgap21	631	25	7185	7.41	7.61	7.51	1958	1.427756	1
78999	KIAA0947	BC018507	684	35	7966	13.2	16.17	14.665	2266	1.419015	1
10067	TNRC6B	Tnrc6b	535	16	18297	1.38	1.53	1.455	1833	1.39677	1
8943	ARNT2	Arnt2	518	24	6576	10.4	4.08	7.225	825	1.393324	1
27	FOXK2	Foxk2	787	32	5262	13.1	12.84	12.975	660	1.392818	1
6745	ASH1L	Ash1l	678	27	11784	9.12	9.68	9.4	2964	1.391753	1
6609	TULP4	Tulp4	310	15	11123	0.78	0.91	0.845	1543	1.383126	1
1718	FSCN1	Fscn1	1439	77	2819	43.9	47.91	45.895	493	1.377583	1
9695	OGDH	Ogdh	595	26	4319	6.73	11.45	9.09	1023	1.36197	1
60528	SKI	Ski	374	25	5707	9.82	7.03	8.425	728	1.356852	1
55699	ADNP	Adnp	988	43	4713	22.3	21.54	21.935	1102	1.346485	1
6747	HCFC1	Hcfc1	573	37	8436	15.9	19.4	17.665	2035	1.33743	1
79827	SREBF2	Srebf2	1216	44	4325	27.2	18.68	22.915	1141	1.336777	1
56052	CABIN1	Cabin1	574	23	7222	7.38	7.78	7.58	2220	1.308305	1

9536	CHD4	Chd4	1455	60	6511	34.3	39.15	36.735	1912	1.283834	1
1191	MFHAS1	Mfhas1	435	20	5255	6.24	5.01	5.625	1052	1.28	1
3753	SIPA1L2	Sipa1l2	350	13	6501	0.17	0.17	0.17	1722	1.278269	1
9703	ATP1A1	Atp1a1	4504	157	3778	119	112.3	115.695	1023	1.249055	1
334	PRPF8	Prpf8	2403	87	7311	55.3	66	60.635	2335	1.231684	1
84975	BCL9L	Bcl9l	453	27	7740	12.3	12.55	12.42	1499	1.204282	1
55624	JAK1	Jak1	972	32	5053	21.7	11.88	16.775	1154	1.195145	1
6830	LRP8	Lrp8	545	22	4528	8.13	9.17	8.65	963	1.179625	1
140453	TLE3	Tle3	604	33	5354	17.9	18.34	18.1	772	1.174377	1
23170	TANC2	Tanc2	436	21	11747	7.89	8.28	8.085	2105	1.161183	1
2677	CHD3	Chd3	774	41	7372	27.3	24.29	25.805	2059	1.145091	1
283149	ZCCHC14	Zcchc14	384	25	6932	12	11.75	11.88	949	1.142596	1
55027	SIPA1L3	Sipa1l3	326	14	7984	2.31	2.26	2.285	1781	1.139601	1
56913	USP34	Usp34	838	26	11326	11.8	13.97	12.89	3546	1.135867	1
84268	ZHX3	Zhx3	313	14	9991	2.52	2.25	2.385	956	1.1304	1
3339	EIF4G1	Eif4g1	2588	101	5686	62.7	96.77	79.715	1600	1.125787	1
23139	CELSR3	Celsr3	197	15	11974	3.56	3.27	3.415	3312	1.118151	1
4043	BAZ2A	Baz2a	623	33	8934	19.8	19.26	19.52	1905	1.117886	1
9618	ATP9A	Atp9a	459	19	7580	8.52	5.57	7.045	1047	1.114696	1
5962	HDAC4	Hdac4	175	12	8980	1.3	0.97	1.135	1200	1.077683	1
1654	REV3L	Rev3l	327	14	10719	2.55	3.53	3.04	3130	1.07362	1
23196	USP9X	Usp9x	555	31	12401	17.7	20.05	18.88	2570	1.073407	1
286410	NCOR2	Ncor2	416	31	8901	19.6	18.59	19.085	2524	1.065841	1
4059	CREBBP	Crebbp	471	20	10197	8.23	9.52	8.875	2442	1.059603	1
1495	LARS2	Lars2	456	18	4203	5.18	8.86	7.02	903	1.057579	1
54583	NPTXR	Nptxr	241	12	5814	1.23	1.57	1.4	599	1.052632	1
7175	HERC1	Herc1	493	17	15216	7.08	5.26	6.17	4861	1.05133	1
55654	SPTBN2	Spnb3	700	31	7884	16.1	23.34	19.7	2390	1.043771	1
51421	GRLF1	Grlf1	511	20	8904	9.11	9.41	9.26	1499	1.038422	1
171023	TTC3	Ttc3	754	28	9021	15.1	19.18	17.115	2025	1.032639	1
2081	NF1	Nf1	457	18	12444	6.82	8.68	7.75	2839	1.014085	1
9550	AAK1	Aak1	220	11	21283	0.79	1.34	1.065	961	0.994126	1
4288	FBXO41	Fbxo41	194	17	6939	7.5	7.17	7.335	875	0.980675	1
10128	DGKZ	Dgkz	439	15	4094	4.11	6.56	5.335	1303	0.978155	1
85002	ARID1A	Arid1a	316	16	8585	6.92	5.96	6.44	2285	0.973236	1
6310	BMPR2	Bmpr2	261	11	12086	1.39	1.31	1.35	1038	0.969163	1
375035	PPP1R9B	Ppp1r9b	534	21	4071	13.3	10.81	12.07	817	0.951518	1
3012	KIAA1109	4932438A1	361	12	15592	2.72	2.83	2.775	5005	0.939335	1
1822	CLCN3	Clcn3	359	15	4058	4.26	7.74	6	866	0.9375	1
79573	ATP13A2	Atp13a2	284	15	3996	5.02	7.27	6.145	1180	0.92908	1
1181	ARHGEF11	Arhgef11	308	14	6904	4.9	5.84	5.37	1696	0.910865	1
9400	BAI2	Bai2	178	10	5434	1.18	0.87	1.025	1693	0.907029	1
51142	AGRN	Agrn	1020	61	7319	67.4	47.22	57.31	2045	0.906255	1
55275	DIDO1	Dido1	455	17	8592	9.04	8.5	8.77	2240	0.905701	1
11196	AFF4	Aff4	483	18	9580	9.29	10.54	9.915	1163	0.903841	1
7378	LRRC8B	Lrrc8b	150	10	7823	1.02	1.2	1.11	803	0.90009	1
25836	CLTC	Cltc	1286	53	8575	39	59.51	49.235	1675	0.894741	1

10319	ADARB1	Adarb1	527	23	7044	19.9	11.81	15.855	845	0.889576	1
3837	KIAA0090	C230096C1	481	26	4253	15.2	23.31	19.245	993	0.889041	1
8370	MYO18A	Myo18a	306	18	7591	11.5	9.21	10.35	2054	0.884521	1
9019	SPTAN1	Spna2	1513	51	7907	47.3	48.23	47.785	2477	0.882582	1
9528	BCR	Bcr	333	16	6927	8.1	8.17	8.135	1271	0.882272	1
6744	ARHGEF12	Arhgef12	491	16	9501	8.31	8.15	8.23	1544	0.877674	1
24148	NAV2	Nav2	243	11	11020	2.59	2.54	2.565	2432	0.875448	1
10813	CDC42BPA	Cdc42bpa	222	12	10527	3.61	4.1	3.855	1719	0.866113	1
79813	VPS13D	Vps13d	163	12	16341	4.45	3.26	3.855	4388	0.866113	1
991	MGAT5B	Mgat5b	309	10	4246	1.5	1.63	1.565	918	0.864678	1
60312	ATG9A	Atg9a	775	29	3795	25	22.42	23.695	839	0.860662	1
84864	DICER1	Dicer1	324	13	10323	5.53	5.31	5.42	1922	0.843061	1
54935	MYST3	Myst3	255	14	9285	6.13	7.12	6.625	2004	0.842105	1
3988	SON	Son	825	32	8412	22.4	34	28.18	2426	0.838135	1
23401	HCN2	Hcn2	210	13	3459	6.75	4.53	5.64	1135	0.831202	1
10783	FBXL19	Fbxl19	203	12	3814	4.34	4.56	4.45	694	0.83045	1
3074	TCF20	Tcf20	329	12	7407	4.5	4.54	4.52	1960	0.826446	1
374986	USP22	Usp22	913	39	5220	32.1	42.5	37.285	525	0.824786	1
2022	KIAA0664	1300001I0	763	38	5252	38	34.21	36.08	1309	0.824653	1
3399	GBF1	Gbf1	442	18	6376	10.6	13.42	12	1859	0.818182	1
58517	CHD6	Chd6	244	13	10389	6.34	5.78	6.06	2715	0.809465	1
8879	MYCBP2	Mycbp2	422	12	15025	4.31	5.35	4.83	4678	0.809171	1
10560	ZFP106	Zfp106	351	20	10487	14.8	14.72	14.775	1883	0.807265	1
5033	UBE3C	Ube3c	372	16	5195	9.71	10.43	10.07	1083	0.79721	1
54552	DAB2IP	Dab2ip	187	13	5472	7.14	5.56	6.35	1132	0.795107	1
55849	PTCH1	Ptch1	219	10	8065	2.75	2.48	2.615	1381	0.792707	1
57648	PHF20	Phf20	478	18	5899	13.7	11.82	12.755	1012	0.791035	1
1652	COBL	Cobl	284	11	5326	4.57	3.31	3.94	1261	0.789096	1
51293	RTN4	Rtn4	668	27	4871	21.3	28.44	24.855	1192	0.774638	1
54443	AP3D1	Ap3d1	504	22	4863	18.8	18.47	18.615	1153	0.768828	1
84516	DIP2B	Dip2b	347	13	8715	6.61	7.77	7.19	1576	0.756254	1
5046	TRAK2	Trak2	304	10	6527	2.78	3.86	3.32	914	0.750751	1
254065	NUP98	Nup98	501	23	6980	18.3	23.04	20.67	1800	0.749918	1
23317	WWC1	Wwc1	295	16	6753	12.7	10.46	11.59	1119	0.741084	1
55784	DLG5	Dlg5	502	21	7493	20.7	16.14	18.44	1919	0.738397	1
9639	ARID1B	Arid1b	335	11	9648	5.29	5	5.145	2249	0.726312	1
1432	CDC42BPB	Cdc42bpb	446	25	6701	26.1	22.9	24.52	1711	0.724218	1
8722	UBE2O	Ube2o	243	17	5395	12.8	14.22	13.485	1292	0.723866	1
93183	RERE	Rere	317	12	8207	6.12	7.43	6.775	1566	0.71535	1
27242	AP2A2	Ap2a2	210	13	4616	8.72	7.89	8.305	939	0.710188	1
445815	BAT3	Bat3	397	22	3832	20.7	21.34	21	1234	0.709677	1
84172	MAST2	Mast2	254	14	5756	10.6	8.86	9.75	1798	0.708861	1
80308	CLASP1	Clasp1	176	10	8118	3.75	4.6	4.175	1650	0.705467	1
64924	BRD4	Brd4	468	21	5198	20.1	19.56	19.835	1362	0.703871	1
57505	HIVEP2	Hivep2	259	10	9732	4.96	3.59	4.275	2531	0.700525	1
26577	GTF3C1	Gtf3c1	352	15	7124	9.24	13.62	11.43	2352	0.699953	1
26020	CLSTN1	Clstn1	579	30	5209	32.8	32.98	32.875	1056	0.699708	1

7343	ACO2	Aco2	528	20	2744	15.4	22.11	18.735	780	0.696015	1
253725	CBX6	Cbx6	181	11	3284	5.92	5.76	5.84	412	0.694444	1
4800	AHDC1	Ahdc1	241	10	6512	4.36	4.6	4.48	1703	0.690608	1
10945	BPTF	Bptf	302	13	10914	7.94	9.71	8.825	2920	0.690571	1
3460	TMEM63B	Tmem63b	284	16	3215	12.2	14.34	13.265	832	0.687728	1
6448	DHX30	Dhx30	586	22	4133	19.4	25.18	22.28	1155	0.681537	1
2175	CAMSAP1L	Camsap1l1	252	13	7359	6.87	11.31	9.09	1478	0.680985	1
9507	ANKRD17	Ankrd17	214	14	9390	9.77	11.45	10.61	2603	0.679282	1
51043	ACLY	Acly	453	29	4450	26.7	40.05	33.375	1101	0.668588	1
3146	DIP2A	Dip2a	346	14	7290	13.4	8.57	10.995	1571	0.666825	1
643314	CTNNB1	Ctnnb1	1164	44	3720	50.5	63.66	57.07	781	0.656031	1
23332	KIAA0284	AW555464	203	12	6681	9.01	8.25	8.63	1554	0.644122	1
7707	USP32	Usp32	300	11	7026	6.95	7.21	7.08	1604	0.644028	1
221002	RUSC2	Rusc2	227	10	5636	5.99	5.32	5.655	1516	0.638774	1
85464	SAMD4B	Samd4b	177	18	4543	16.3	20.57	18.425	694	0.633245	1
5445	SBF1	Sbf1	193	14	8033	12.2	12.92	12.555	1893	0.620705	1
6926	CAMTA2	Camta2	256	10	4852	5.95	6.35	6.15	1241	0.619195	1
1104	MAP7D1	Mtap7d1	154	12	3357	7.56	12.1	9.83	841	0.605144	1
649	YWHAG	Ywhag	881	27	3779	27.1	42.31	34.685	247	0.60423	1
5138	MAP4	Mtap4	577	20	6337	23	23.27	23.155	1152	0.603227	1
23221	ARHGAP23	4933428G2	154	10	6276	5.21	8	6.605	1476	0.602228	1
57418	PHF12	Phf12	188	10	4475	7.7	7.68	7.69	1004	0.565291	1
653489	SUPT6H	Supt6h	527	20	5896	23.5	27.35	25.445	1726	0.564254	1
332	NFIC	Nfic	500	15	2401	19.7	13.74	16.72	499	0.561377	1
5358	MAP4K4	Map4k4	267	12	7495	9.88	13.34	11.61	1320	0.555298	1
5902	PIGQ	Pigq	183	12	2921	12	11.27	11.64	780	0.554529	1
8570	CIC	Cic	180	11	5473	10.5	9.25	9.87	1608	0.553598	1
8355	CNP	Cnp	218	12	5222	12.1	12.08	12.075	421	0.543601	1
20	AP1B1	Ap1b1	229	11	4194	7.39	13.43	10.41	949	0.538951	1
51322	PUM1	Pum1	459	16	5416	20.2	19.55	19.895	1188	0.535207	1
1892	ADD1	Add1	170	10	4068	7.65	9.75	8.7	779	0.534759	1
55206	CIT	Cit	266	12	8576	14.7	10.37	12.525	2027	0.532741	1
1675	EIF2C2	Eif2c2	160	13	3520	14.5	14.5	14.5	859	0.530612	1
55568	XPO6	Xpo6	831	23	4422	28.1	38.76	33.42	1296	0.52971	1
85377	DCTN1	Dctn1	549	19	4518	21.9	30.04	25.98	1278	0.528071	1
80012	IRS2	Irs2	198	16	7014	23.6	17.59	20.615	1338	0.52262	1
23536	NCOA6	Ncoa6	194	10	7062	8.4	11.01	9.705	2063	0.507485	1
56910	PDE2A	Pde2a	658	27	4327	31.2	55.41	43.32	941	0.506377	1
79139	PLD3	Pld3	528	27	2424	49.6	40.3	44.925	490	0.491579	1
23052	CAMSAP1	Camsap1	290	12	7650	14.1	14.77	14.435	1602	0.491099	1
55127	RHOB	Rhob	910	47	2384	61.1	116.4	88.755	196	0.475925	1
9922	PTPN11	Ptpn11	526	16	6300	18.5	29.17	23.835	593	0.472883	1
137964	ATN1	Atn1	479	24	4367	44.7	39.68	42.185	1190	0.459902	1
9197	ZFR	Zfr	447	17	4746	24.9	29.11	26.995	1074	0.459522	1
5833	SYMPK	Sympk	174	12	4188	14.3	18.33	16.315	1274	0.456014	1
53838	DOT1L	Dot1l	198	10	7455	13.7	10.36	12.01	1537	0.454339	1
4241	GIT1	Git1	408	16	3778	26.2	26.36	26.27	770	0.441136	1

3688	APP	App	3007	87	3648	194	184.1	188.875	770	0.437461	1
2873	MADD	Madd	204	12	6031	18	17.03	17.49	1647	0.436522	1
8501	CAND1	Cand1	414	14	5956	21.3	24.57	22.955	1230	0.424822	1
3480	PSAP	Psap	7598	179	2848	432	391.5	411.685	527	0.424487	1
25987	CKAP5	Ckap5	496	20	6714	32.4	42.93	37.67	2032	0.419551	1
79850	ZC3H7B	Zc3h7b	137	10	5868	14.5	13.19	13.84	977	0.419463	1
29894	PTK2	Ptk2	361	13	4453	18.8	23.28	21.04	1052	0.418814	1
80856	SLC25A23	Slc25a23	192	10	3475	14.2	13.81	14.02	468	0.41632	1

Legend. The top Top3β-bound mRNAs identified by HITS-CLIP that match the targets of FMRP.
Column abbreviations are:
mRNA_len = Length of mRNA, nucleotides
ORF_len,aa = Length of open reading frame, amino acids
RNA-seq rep1 (GSM591682) = RNA-seq, GEO acc# GSM591682, reads per 1 Kb
RNA-seq rep2 (GSM591659) = RNA-seq, GEO acc# GSM591659, reads per 1 Kb
Average RNA-seq = average of two replications of RNA-seq
Ntags (Top3b CLIP) = Number of Top3b CLIP tags mapped to mRNA
Ratio 1 = ratio of Top3b-Npeaks/(RNAseq+10)
The FMRP HITS-CLIP data are from Darnell et al. Cell. 2011
Binding (1) to FMRP is indicated.
The complete list is available upon request.
The raw Top3β HITS-CLIP data have been deposited at GEO (accession number GSE47502)

Supplementary Table 4. Top 30 gene ontology (GO) terms enriched in common target transcripts of Top3β and FMRP

Molecular function	(-LOG(p-value))	Categories	genes
histone acetyltransferase complex	20	Cell component	Trrap,Ep300,Crebbp
establishment of nucleus localization	20	Biological Process	Myh10,Cdc42bpa,Ptk2
microtubule binding	20	Molecular function	Macf1,Dst,Apc,Nf1,Vps41,Kif1b,Clasp1,Mast2
transcription factor binding	20	Molecular function	Nsd1,Trrap,Spen,Arnt2,Ep300,Chd4,Crebbp,Hcfc1,Atf7ip,Bptf,Dip2a,Hipk1,Ctnnb1,Nrip1,Ncor2,Arid2,Ncoa6
chromatin modification	14.95	Biological Process	Ncor1,Mll3,Mll1,Nsd1,Trrap,Ash1l,Ep400,Chd4,Crebbp,Hdac4,Rere,Myst3,Bptf,Ehmt2,Npcd,Cbx6,Nptxr
tubulin binding	14.61	Molecular function	Macf1,Dst,Apc,Nf1,Vps41,Kif1b,Clasp1,Mast2
embryonic development	13.49	Biological Process	Celsr2,Mll2,Macf1,Plxnd1,Ndst1,Mll1,Nsd1,Shank3,Myh10,Arnt2,Ep300,Bmpr2,Nf1,Grlf1,Cobl,Dicer1,Ptch1,Ski,Mib1,Myst3,Kif1b,Ctnnb1,Ncor2,Chd8,Gnas,Ncoa6
stem cell development	12.84	Biological Process	Apc,Dicer1,Myst3
nucleus localization	12.84	Biological Process	Myh10,Cdc42bpa,Ptk2
axon cargo transport	12.84	Biological Process	Dst,Kif1b,App
stem cell maintenance	12.84	Biological Process	Apc,Dicer1,Myst3
lysine N-methyltransferase activity	12.26	Molecular function	Mll3,Nsd1,Ash1l,Ehmt2
histone-lysine N-methyltransferase activity	12.26	Molecular function	Mll3,Nsd1,Ash1l,Ehmt2
protein-lysine N-methyltransferase activity	12.26	Molecular function	Mll3,Nsd1,Ash1l,Ehmt2
establishment and/or maintenance of chromatin archit	12.24	Biological Process	Ncor1,Mll3,Mll1,Nsd1,Trrap,Ash1l,Ep400,Chd4,Crebbp,Hdac4,Arid1a,Rere,Chd3,Chd6,Myst3,Bptf,Ehmt2,Npcd,Cbx6,Nptxr
Rho guanyl-nucleotide exchange factor activity	12.09	Molecular function	Trio,Arhgef17,Arhgef12,Arhgef11,Bcr,Arhgef7,Arhgef4
regulation of synapse structure and activity	11.65	Biological Process	Agrn,App,Ptk2

transcription cofactor activity	11.62	Molecular function	Nsd1,Trrap,Spen,Ep300,Crebbp,Hcfc1,Atf7ip,Hipk1,Ctnnb1,Nrip1,Ncor2,Ncoa6
post-translational protein modification	11.01	Biological Process	Lmtk2,Ptprj,Ptprs,Birc6,Ndst1,Wnk1,Hsn2,Mll1,Herc2,Nsd1,Ptprf,Spnb2,Mapk6,Mapk4,Tulp4,Sytl3,Trio,Usp34,Anapc1,Jak1,Herc1,Mycbp2,Bmpr2,Huwe1,Aak1,Crebbp,Ppp1r9b,Ptch1,Cdc42bpa,Ube3c,Wnk2,Usp9x,Mib1,Usp22,Fbxl19,Taok1,Hipk1,Brd4,Ehmt2,Ptpn11,Mast2,Map4k4,Fbxo41,App,Cit,Cdc42bpb,Cand1,Ptk2,Nedd4,Ube2o
stem cell differentiation	10.66	Biological Process	Apc,Dicer1,Myst3
coated pit	10.66	Cell component	Lrp1,Cltc,Ap2a2,App,Sh3bp4
hormone receptor binding	10.66	Molecular function	Nsd1,Ptpn11,Nrip1
regulation of cellular Cell component organization and	10.47	Biological Process	Dst,Mll1,Myh10,Spnb2,Grlf1,Eif4g1,Spna2,Mib1,Rtn4,Mtap4,Clasp1,Agrn,Cit,Eif4g3,Ptk2
chromosome organization and biogenesis	10.31	Biological Process	Ncor1,Mll3,Mll1,Nsd1,Trrap,Ash1l,Ep400,Chd4,Crebbp,Hdac4,Arid1a,Rere,Chd3,Chd6,Myst3,Bptf,Ehmt2,Npcd,Cbx6,Nptxr,Cit
Ras guanyl-nucleotide exchange factor activity	10.22	Molecular function	Trio,Arhgef17,Arhgef12,Arhgef11,Bcr,Arhgef7,Arhgef4
small GTPase regulator activity	10.19	Molecular function	Trio,Arhgef17,Arhgef12,Arhgef11,Bcr,Cdc42bpa,Tbc1d9,Map4k4,Arhgef7,Cit,Cdc42bpb,Arhgef4,Git1
GTPase regulator activity	10.18	Molecular function	Sipa1l2,Trio,Arhgap21,Arhgef17,Arhgef12,Nf1,Grlf1,Arhgef11,Bcr,Cdc42bpa,Tbc1d9,Dab2ip,Map4k4,Arhgef7,Cit,Cdc42bpb,Arhgef4,Git1
morphogenesis of embryonic epithelium	10.02	Biological Process	Celsr2,Shank3,Grlf1,Cobl,Ptch1,Mib1,Ctnnb1
negative regulation of cellular Biological Process	9.96	Biological Process	Ncor1,Birc6,Mll1,Nsd1,Spnb2,Lrp1,Spen,Apc,Atp1a1,Dpm1,Adnp,Nf1,Grlf1,Dicer1,Rc3h1,Ptch1,Spna2,Hdac4,Sash1,Atf7ip,Mib1,Rtn4,Myst3,Bptf,Mtap4,Clasp1,Hipk1,Ctnnb1,Ehmt2,Ptpn11,Nrip1,Ncor2,Dab2ip,Cit,Hivep1,Ptk2

Legend. The top 30 gene ontology (GO) terms that are enriched in 233 common targets of Top3b and FMRP.
These 233 transcripts are from the top 1500 Top3b target transcripts which also match FMRP targets (Darnell et al. 2011).
The highlighted categories may be related to FMRP function in neurons.

Supplementary Table 5. List of Top3b-bound mRNAs that match either schizophrenia or autism related genes

Gene Symbol	Schizophrenia(ALL COMBINED)	Autism	FMRP binding (Darnell)	FMRPbinding (Ascano 2012, score>=500)	Gene Ontology (GO) Terms
EP300	1	1	1	1	axon extension
C4B	1	1	0	0	
JARID2	1	1	0	0	
CTNNB1	1	0	1	1	neuron migration;neuroblast proliferation
NOTCH2	1	0	0	1	
DGCR2	1	0	1	0	
PLXNA2	1	0	1	0	axon guidance;neural tube development
ARHGEF11	1	0	1	0	axon guidance
RTN4	1	0	1	0	axonal fasciculation;axon extension
TTL	1	0	0	1	regulation of axon extension
RTN4R	1	0	1	0	axonogenesis;regulation of axonogenesis
NR3C1	1	0	0	1	postsynaptic density;neuron apoptosis
ARVCF	1	0	1	0	
UHMK1	1	0	0	1	axon;neuron projection development
PTK2	1	0	1	0	neuron migration;axon guidance,synapse formation
YWHAE	1	0	0	0	neuron migration
TGFB1	1	0	0	0	neuroblast proliferation;axon
DDR1	1	0	0	0	regulation of cell growth
COMT	1	0	0	0	synaptic transmission;neurotransmitter
ZDHHC8	1	0	0	0	
CFB	1	0	0	0	
PAM	1	0	0	0	
SYT11	1	0	0	0	synaptic vesicle membrane
SHC1	1	0	0	0	neurotrophin TRKA receptor binding;neuron projection
NUMBL	1	0	0	0	neuroblast division in subventricular zone
HR	1	0	0	0	
ANKRD11	0	1	1	1	multicellular organism growth
NSD1	0	1	1	1	
EP400	0	1	1	1	
HDAC4	0	1	1	1	neuromuscular junction
CREBBP	0	1	1	1	
NF1	0	1	1	1	neuroblast proliferation;neural tube development
AFF4	0	1	1	1	
ARID1B	0	1	1	1	

APC	0	1	1	1	axonogenesis;neuronal cell body
PTPN11	0	1	1	1	axon guidance
MET	0	1	0	1	neuron migration;axon guidance
SHANK3	0	1	1	0	postsynaptic density;neuron differentiation
ARNT2	0	1	1	0	
CHD7	0	1	0	1	
NIPBL	0	1	0	1	brain development
POGZ	0	1	0	1	
RAI1	0	1	0	1	
ADARB1	0	1	1	0	
JMJD1C	0	1	0	1	
TSN	0	1	0	1	
DYRK1A	0	1	0	1	
RB1CC1	0	1	0	1	
UBE3A	0	1	0	1	brain development
XPO1	0	1	0	1	
ROBO1	0	1	0	0	axon guidance
CACNA1H	0	1	0	0	axon guidance
LAMB1	0	1	0	0	axon guidance;neuron projection development
GTF2I	0	1	0	0	neuronal cell body
LAMC3	0	1	0	0	
CDH10	0	1	0	0	
LAMA1	0	1	0	0	axon guidance
CD99L2	0	1	0	0	
SLC16A3	0	1	0	0	
EXT1	0	1	0	0	axon guidance
SLC30A5	0	1	0	0	
NFIA	0	1	0	0	
SPAST	0	1	0	0	
SLC39A11	0	1	0	0	
C3orf58	0	1	0	0	
MARK1	0	1	0	0	neuron migration
PITX1	0	1	0	0	
CMIP	0	1	0	0	
CASC4	0	1	0	0	
SATB2	0	1	0	0	neuron migration
YEATS2	0	1	0	0	
VPS13B	0	1	0	0	
SND1	0	1	0	0	
REEP3	0	1	0	0	
CLTCL1	0	1	0	0	
DCTN5	0	1	0	0	

Legend. The top Top3b-bound mRNAs identifedy by HITS-CLIP that match either Schizophrenia or autism related genes. The matched genes are marked with "1", whereas those that do not match are marked with "0". The schizophrenia related genes are from a combined lists of genes reported by previous studies: The Schizophrenia Psychiatric Genome-Association Study (GWAS) Consortium, 2011; Sun et al. 2009; and Chandrasekaran and Bonchev; 2012. The autism spectrum disorder related genes are downloaded from website: https://gene.sfari.org/autdb/HG_Home.do.

Supplementary Table 6. Oligos used in binding and topoisomerase assays.

DNA oligos	Sequence
K128f	ACTTCGAAATTAATACGACTCACTATAGGGAGATTTTTTTTTTTTTTTTTTTTGTCAGACGGATC TTTTTTTTTTTTTTTTTTTTTCTCCCGACTGGTTTTTTTTTTTTTTTTTTTGATCCGTCTGACTTTT TTTTTTTTTTTTTTTTCCAGTC
K128r	GACTGGAAAAAAAAAAAAAAAAAAAAAGTCAGACGGATCAAAAAAAAAAAAAAAAAAAAA CCAGTCGGGAGAAAAAAAAAAAAAAAAAAAAAAGATCCGTCTGACAAAAAAAAAAAAAAAA AAAAATCTCCCTATAGTGAGTCGTATTAATTTCGAAGT
K128link	GATCCGTCTGACAAAAAAAAAAAAAAAAAAAAATCTCCCGACTGGAAAAA
H1	GTGACCGTCTCCGGGAGCTGGAAACGCGCGAGACGAAAGG
H5	CCTTTCGTCTCGCGCGTTTCCAGCTCCCGGAGACGGTCAC

RNA oligos	
rH1	GUGACCGUCUCCGGGAGCUGGAAACGCGCGAGACGAAAGG
rH5	CCUUUCGUCUCGCGCGUUUCCAGCUCCCGGAGACGGUCAC
rA40	AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA
rU40	UUUUU UUUUU UUUUU UUUUU UUUUU UUUUU UUUUU UUUUU
rC40	CCCCC CCCCC CCCCC CCCCC CCCCC CCCCC CCCCC CCCCC
rG40	GGGGG GGGGG GGGGG GGGGG GGGGG GGGGG GGGGG GGGGG