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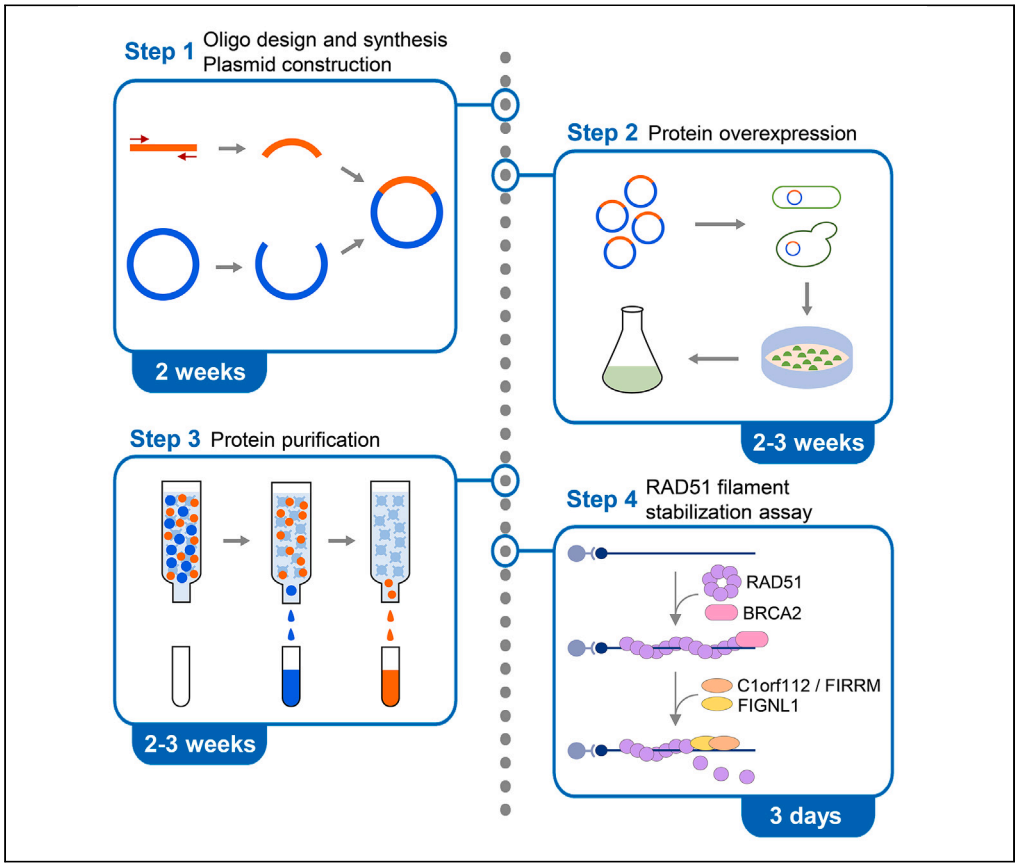
Reconstitution of the antagonistic effect between C1orf112/FIRRM-FIGNL1 and BRCA2 on RAD51 filament stabilization



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Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Expression and purification of C1orf112/FIRRM and RAD51 in bacteria

Expression and purification of FIGNL1 and miBRCA2 in yeast cells

Reconstitution of RAD51 filament disassembly by C1orf112/FIRRM and FIGNL1

Reconstitution of the antagonism between C1orf112/FIRRM-FIGNL1 and miBRCA2

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Reconstitution of the antagonistic effect between C1orf112/FIRRM-FIGNL1 and BRCA2 on RAD51 filament stabilization

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SUMMARY

C1orf112/FIRRM is a recently identified DNA damage repair factor that regulates RAD51 in homologous recombination through interacting with the anti-recombinase FIGNL1. Here, we describe steps for purifying C1orf112/FIRRM, FIGNL1, miBRCA2, and RAD51 proteins from *Escherichia coli* or *Saccharomyces cerevisiae* cells. We then detail procedures for reconstituting the disassembly of RAD51 filament by C1orf112/FIRRM-FIGNL1 *in vitro* and the antagonistic effect between C1orf112/FIRRM-FIGNL1 and miBRCA2 on RAD51 filament stabilization.

For complete details on the use and execution of this protocol, please refer to Zhou et al. (2023).[1]

BEFORE YOU BEGIN

C1orf112 (renamed FIRRM, FIGNL1-interacting regulator of recombination and mitosis) in human functions as a RAD51 regulator mainly in the HR step of Fanconi Anemia (FA) pathway to repair DNA interstrand cross-link (ICL).[1] FIRRM contains 853 amino acid residues with an unknown DUF4487 domain and its biochemical characteristics have not been well studied. In addition, the conserved interactor of FIRRM, FIGNL1, has been identified to have the activity of dissociating RAD51 filament *in vitro*.[2] Another interactor of FIRRM, BRCA2, is an important mediator to help RAD51 assemble onto RPA-coated ssDNA and stabilize RAD51 filament.[3–5] Thus, it will be interesting to investigate whether FIRRM can dissociate RAD51 filament and the relationship among FIRRM, FIGNL1 and BRCA2 in the regulation of RAD51 filament.

We expressed and purified FIRRM and FIGNL1 from *Escherichia coli* and *Saccharomyces cerevisiae* cells, respectively. For BRCA2, we constructed a mini form termed miBRCA2 and purified it from *Saccharomyces cerevisiae* cells. RAD51 without any tag was expressed and purified from *Escherichia coli* cells by multiple steps of ion exchange and gel filtration chromatography. Using these proteins, we reconstituted the assembly and disassembly of RAD51 filaments *in vitro*, and assessed the effects of FIRRM, FIGNL1 and miBRCA2 on RAD51 filament stability. Specifically, we have not detected any anti-recombinase activity in FIRRM but we found that FIRRM can stimulate the anti-recombinase activity of FIGNL1. We also found that miBRCA2 can protect RAD51 filament from FIRRM-FIGNL1-mediated disassembly.

Primer design

⏲ Timing: 2 h

1. Primers to construct FIRRM expressing plasmid (pGEX-MBP-FIRRM-10×His).
   a. FIRRM-Forward: 5′-GGGCCCCTGGGATCCGAATTCATGTTTTTA-3′
      FIRRM-Reverse: 5′-GATGCGGCCGCTCGAAAGCTTTATTAATGG-3′
   b. pGEX-FIRRM-10×His-Forward: 5′-CGCGCAGACTTCGGATCTGGAAGTT-3′
      pGEX-FIRRM-10×His-Reverse: 5′-CGATTTTCATGAATACTGTTTCCTG-3′
   c. MBP-Forward: 5′-TATTCATGAAAATCGAAGAA-3′
      MBP-Reverse: 5′-TCCGAAGTCTGCGCGTCTTT-3′
2. Primers to construct a mini form of BRCA2 (miBRCA2) expressing plasmid (pESC-Flag-miBRCA2-10×His).
   a. pESC-URA-miBRCA2-F: 5′-CTATAGGGCCCGGGCAAAAAAATGTCTGAC-3′
      pESC-URA-miBRCA2-R: 5′-CGGATCTTAGCTAGCTCAGCTTTATTAATG-3′
3. Primers to construct RAD51 expressing plasmid (pET11d-RAD51).
   a. pET11d-RAD51-F: 5′-AGGAGATATACCATGGCAATGCAGATGCAG-3′
   b. pET11d-RAD51-R: 5′-GTTAGCAGCCGGATCCTCAGTCTTTGGCATC-3′
4. Sequencing primers to verify the DNA sequences of FIRRM, FIGNL1, miBRCA2 and RAD51.
   a. MBP-F: 5′-GATGAAGCCCTGAAAGACGCGCAG-3′;
   b. GAL1R: 5′-GTTCTTAATACTAACATAACT-3′;
   c. FIGNL1-1: 5′-CCTACATTCTCAGCACCTGT-3′;
   d. FIRRM-1: 5′-ATTTCATGCCAATACTTGGA-3′
      FIRRM-2: 5′-TCTCTCTACCTGTTCATTTA-3′
   e. pGEX-3: 5′-CCGGGAGCTGCATGTGTCAGAGG-3′;
      pGEX-5: 5′-GGGCTGGCAAGCCACGTTTGGTG-3′;
   f. MBP-1: 5′-TACTTCACCTGGCCGCTGATTGCT-3′;
      MBP-2: 5′-AACCAGGAAGGTCAGACCCGCTTT-3′
   g. miBRCA2-1: 5′-TCACCAGTTTTAGCCATCAATGGG-3′
      miBRCA2-2: 5′-TGTGGATCCAAAGCTTATTTCTAG-3′
      miBRCA2-3: 5′-TTTGAAGAACATGAAGAAAACACA-3′
   h. pYES2-F: 5′-TACCTCTATACTTTAACGTC-3′
   i. RAD51-S1: 5′-ACTGAGGGTACCTTTAGGCCAGAA-3′
      RAD51-S2: 5′-TGATGCTTGATAAAGGAGCTGGGT-3′
5. Oligos for reconstituted reactions.
   a. 5′-biotinylated dT60: biotin-5′-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
      TTTTTTTTTTTTTT-3′
   b. dT60: 5′-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3′

Construction of expression plasmid

⏲ Timing: 2 weeks

6. Construction of pGEX-MBP-FIRRM-10×His. Troubleshooting 1.
   a. Amplify the coding sequence for FIRRM with a C-terminal 10×His by PCR with FIRRM-Forward/Reverse primers. And linearize the Escherichia coli cell expression vector pGEX-6P1 with restriction enzymes (BamHI/XhoI).
   b. Introduce the PCR fragment into pGEX-6P1 to generate pGEX-6P1-FIRRM-10×His using In-fusion cloning kit.
   c. Transform the In-fusion reaction products into Trans1 competent cells and spread the cells on LB medium plate containing ampicillin (0.1 mg/mL). After overnight (approximately 15 h) growth, pick the bacterial clone into LB medium with ampicillin (0.1 mg/mL) to culture.

d. After overnight (approximately 15 h) growth, collect the cells and extract the plasmids from the cells. Then send the plasmids for sequencing using primers pGEX-3/5 and FIRRM-1/2.
e. Amplify MBP tag by PCR with MBP-Forward/Reverse primers. And obtain the vector fragment by PCR with pGEX-6P1-FIRRM-10×His as template and pGEX-FIRRM-10×His-Forward/Reverse as primers.
f. Ligate MBP and vector fragments by In-fusion cloning kit to generate pGEX-MBP-FIRRM-10×His.
g. After transformation, bacterial clone proliferation and plasmid extraction, send the plasmid for sequencing with primers MBP-1/2, MBP-F, FIRRM-1/2 and pGEX-3.

7. Construction of pESC-MBP-FIGNL1-Flag.
   a. Generate the FIGNL1 DNA fragment by digestion with restriction enzymes (BamHI/KpnI). And linearize the *Saccharomyces cerevisiae* cell expression vector pESC-TRP-MBP-Flag with the same restriction enzymes.
   b. Ligate DNA fragments to obtain pESC-MBP-FIGNL1-Flag vector.
   c. Transform the products into Trans1 competent cells and spread the cells on LB medium plate containing ampicillin (0.1 mg/mL).
   d. After bacterial clone proliferation and plasmid extraction, send the plasmid for sequencing by MBP-F, FIGNL1-1, GAL1R primers.
8. Construction of pESC-Flag-miBRCA2-10×His.
   Because of the difficulty to express and purify the full-length BRCA2, we constructed a mini form of BRCA2 (termed miBRCA2,[5] containing the key functional domains of BRC4, HD, OB folds and CTRB) with an N-terminal Flag tag and a C-terminal 10×His tag.
   a. Amplify the miBRCA2 DNA fragment by PCR with pESC-URA-miBRCA2-F/R primers. And linearize the *Saccharomyces cerevisiae* cell expression vector pESC-URA with restriction enzymes (SalI/NheI).
   b. Insert the miBRCA2 DNA fragment into pESC-URA to obtain pESC-Flag-miBRCA2-10×His vector by using In-fusion cloning kit.
   c. After transformation into Trans1 competent cells, bacterial clone proliferation and plasmid extraction, send the plasmid for sequencing by primers pYES2-F, miBRCA2-1/2/3 and GAL1R.
9. Construction of pET11d-RAD51.
   a. Amplify the RAD51 DNA fragment by PCR with primers pET11d-RAD51-F/R. Linearize the vector pET11d with restriction enzymes (NcoI/BamHI).
   b. Insert the PCR products into pET11d vector by using In-fusion cloning kit to obtain pET11d-RAD51 vector.
   c. After plasmid transformation, bacterial culture and plasmid extraction, send the plasmids for sequencing with primers RAD51-S1/2.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-RAD51	Proteintech	Cat#14961-1-AP; RRID: AB_2177083
Bacterial and virus strains		
Trans1-T1 phage-resistant competent cell	TransGen	Cat#CD501
ArcticExpress (DE3) pRARE2 competent cell	Weidibio	Cat#EC2021
BLR (DE3) pLysS competent cell	Novagen	Cat#69956
Chemicals, peptides, and recombinant proteins		
Tryptone	Thermo Fisher Scientific	Cat#LP0042B
Yeast extract	Thermo Fisher Scientific	Cat#LP0021B
Agar powder	Solarbio	Cat#A8190
Peptone	Solarbio	Cat#P8450

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Yeast nitrogen base	BBI Life Sciences	Cat#A600505
Ammonium sulfate	Tongguang Chemicals	Cat#112136
D-glucose	Solarbio	Cat#G8150
Adenine sulfate	BBI Life Sciences	Cat#A600028
L-arginine HCl	Solarbio	Cat#A8220
L-histidine	Solarbio	Cat#H0020
L-isoleucine	Solarbio	Cat#I0010
L-leucine	Solarbio	Cat#L0011
L-lysine HCl	Solarbio	Cat#L0012
L-methionine	Solarbio	Cat#M0010
L-phenylalanine	Ouhe Technology	Cat#1006032
L-tryptophan	Ouhe Technology	Cat#1006033
L-tyrosine	Solarbio	Cat#Y0010
Uracil	Ouhe Technology	Cat#01006149
L-valine	Ouhe Technology	Cat#01008369
D-(+)-galactose	Aladdin	Cat#G100367
Sodium DL-lactate solution	Macklin	Cat#S817880
Glycerol	Tongguang Chemicals	Cat#105132
Ampicillin sodium salt	Solarbio	Cat#A8180
IPTG	Solarbio	Cat#I8070
Lithium acetate dihydrate	Solarbio	Cat#L8710
PMSF	BBI Life Sciences	Cat#A610425
DTT	Solarbio	Cat#D8220
Igepal	Sigma	Cat#I3021
Coomassie brilliant blue	BBI Life Sciences	Cat#A610037
D-(+)-maltose monohydrate	Solarbio	Cat#D8110
Imidazole	Solarbio	Cat#I8090
FLAG peptide	Sigma	Cat#F3290
Bovine serum albumin	Sigma	Cat#A1933
Magnesium chloride hexahydrate	Sigma	Cat#M2670
Calcium chloride dihydrate	Sigma	Cat#21097
ATP	Sigma	Cat#A2383
Tris	LABLEAD	Cat#0497
KCl	Tongguang Chemicals	Cat#112019
Critical commercial assays		
PrimeSTAR Max DNA polymerase	Takara	Cat#R045Q
In-Fusion Cloning Kit	Clontech	Cat#639650
StarPrep DNA Gel Extraction Kit	GenStar	Cat#D205-04
StarPrep Fast Plasmid Mini Kit	GenStar	Cat#D201-04
Coomassie Plus (Bradford) Assay Kit	Thermo Fisher Scientific	Cat#23236
Anti-FLAG M2 affinity beads	Sigma	Cat#A2220
Amylose resin	New England Biolabs	Cat#E8021
Ni-NTA agarose resin	QIAGEN	Cat#30210
Streptavidin Sepharose beads	GE Healthcare	Cat#GE17-5113-01
Other		
Gene amplification instrument	LongGene	Cat#T20
NanoDrop OneC	Thermo Fisher Scientific	Cat#840–317400
Low temperature centrifuge	Eppendorf	Cat#5425R
Horizontal constant temperature shaker	Tianjin Honour Instrument	Cat#HNY-211B
Avanti J-26S XP high performance centrifuge	Beckman	Cat#B22984
High-speed disperser	SCIENTZ	Cat#XHF-DY
Ultrasonic homogenizer	SCIENTZ	Cat#JY92-IIN
Beadbeater	BioSpec	Cat#1107900
Orbit M60 digital shaker	Labnet	Cat#S2020-M60

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Pulsing vortex mixer	OHAUS	Cat#VXMNPS
G:BOX Chemi XRQ	Syngene	N/A
Rotary mixer	MIULAB	Cat#YC-80

MATERIALS AND EQUIPMENT

Materials

LB medium or plate		
Reagent	Final concentration	Amount
Tryptone	1%	10 *g*
Yeast extract	0.5%	5 *g*
NaCl	1%	10 *g*
Agar powder (for plate)	1.5%	15 *g*
ddH_2O	N/A	Add to 1 L

Autoclaved medium can be stored at room temperature (20°C–25°C) for up to 1 month. Plates can be stored at 4°C for up to 2 months.

YPD medium or plate		
Reagent	Final concentration	Amount
Peptone	2%	2 *g*
Yeast extract	1%	1 *g*
D-Glucose	2%	2 *g*
Agar powder (for plate)	1.5%	1.5 *g*
ddH_2O	N/A	Add to 100 mL

Autoclaved medium can be stored at room temperature (20°C–25°C) for up to 1 month. Plates can be stored at 4°C for up to 2 months.

Omission medium mix (-Trp or -Ura)	
Reagent	Amount
Yeast nitrogen base	300 *g*
Ammonium sulfate	900 *g*
Adenine sulfate	5.4 *g*
L-Arginine HCl	3.6 *g*
L-Histidine	3.6 *g*
L-Isoleucine	5.4 *g*
L-Leucine	5.4 *g*
L-Lysine HCl	5.4 *g*
L-Methionine	3.6 *g*
L-Phenylalanine	9.0 *g*
L-Tryptophan	3.6 *g* (0 *g* for -Trp)
L-Tyrosine	5.4 *g*
Uracil	3.6 *g* (0 *g* for -Ura)
L-Valine	27 *g*

Store at room temperature (20°C–25°C) for up to 1 year.

Omission medium or plate		
Reagent	Final concentration	Amount
Omission medium mix	0.72%	4.32 *g*
Dextrose	2%	12 *g*
Agar powder (for plate)	1.5%	9 *g*
ddH_2O	N/A	Add to 600 mL

Autoclaved medium can be stored at room temperature (20°C–25°C) for up to 1 month. Plates can be stored at 4°C for up to 2 months.

2× T buffer		
Reagent	Final concentration	Amount
Tris	50 mM	6.057 *g*
Glycerol	20%	200 mL
EDTA (0.5 M)	1 mM	2 mL
HCl	Adjust to pH 7.5	N/A
ddH_2O	N/A	Add to 1 L
Store at 4°C for up to 6 months.		

T buffer		
Reagent	Final concentration	Amount
2× T buffer	1×	500 mL
DTT (1 M)	1 mM	1 mL
Igepal (10%)	0.01%	1 mL
ddH_2O	N/A	Add to 1 L
Store at 4°C for up to 1 month.		

T1000 buffer		
Reagent	Final concentration	Amount
2× T buffer	1×	500 mL
KCl (3 M)	1 M	333 mL
DTT (1 M)	1 mM	1 mL
Igepal (10%)	0.01%	1 mL
ddH_2O	N/A	Add to 1 L
Prepare buffers with different concentrations of KCl by mixing T and T1000. Store at 4°C for up to 1 month.		

Cell breakage buffer		
Reagent	Final concentration	Amount
Tris-HCl (200 mM, pH 7.5)	50 mM	250 mL
Sucrose	10%	100 *g*
EDTA (0.5 M)	5 mM	10 mL
KCl (3 M)	200 mM	67 mL
DTT (1 M)	2 mM	2 mL
ddH_2O	N/A	Add to 1 L
Store at 4°C for up to 1 month.		

Coomassie blue staining buffer		
Reagent	Final concentration	Amount
Coomassie brilliant blue	0.2%	2 *g*
Methanol	25%	250 mL
Acetic acid	12.5%	125 mL
ddH_2O	N/A	Add to 1 L
Store at room temperature (20°C–25°C) for up to 1 year.		

Destaining buffer		
Reagent	Final concentration	Amount
Methanol	25%	250 mL
Acetic acid	12.5%	125 mL
ddH_2O	N/A	Add to 1 L
Store at room temperature (20°C–25°C) for up to 1 year.		

3× RB-1Mg2Ca buffer		
Reagent	Final concentration	Amount
Tris-HCl (200 mM, pH 7.5)	75 mM	15 mL
$MgCl_2$ (100 mM)	3 mM	1.2 mL
$CaCl_2$ (100 mM)	6 mM	2.4 mL
DTT (1 M)	3 mM	120 μL
Igepal (10%)	0.3%	1.2 mL
ddH_2O	N/A	Add to 40 mL
Store at 4°C for up to 1 month.		

Buffer A with different concentrations of KCl and ATP		
Reagent	Final concentration	Amount
3× RB-1Mg2Ca buffer	1×	8 mL
BSA (10 mg/mL)	100 μg/mL	240 μL
KCl (3 M)	50 or 100 mM	400 or 800 μL
ATP (100 mM, pH7.5)	1 or 2 mM	240 or 480 μL
ddH_2O	N/A	Add to 24 mL
Do not store, use fresh.		

Note: Omission medium mix is a mixture of various powders which need to be thoroughly stirred and mixed well. Medium and plates for cell culture need to be autoclaved before storage. DTT and ATP should be added into buffers just before use.

Equipment

For the shaking culture of bacteria and yeast, we use the horizontal constant temperature shaker (Tianjin Honour Instrument). For the harvest of cell pellet, cell lysis supernatant and the collection of beads from 50 mL tube, we use Avanti J-26S XP high performance centrifuge (Beckman). We use high-speed disperser (SCIENTZ) to disperse the bacteria pellet and use ultrasonic homogenizer (SCIENTZ) to break the bacteria. For the breakage of the yeast, we use the bead-beater (BioSpec) and glass beads with a diameter of 0.5 mm. During the protein purification, we use rotary mixer (MIULAB) and vortex mixer (OHAUS) for affinity beads incubation. During the RAD51 filament stabilization assay, we use Orbit M60 digital shaker (Labnet) to mix beads and substrates.

STEP-BY-STEP METHOD DETAILS

Expression and purification of FIRRM

Timing: 1–2 weeks

This section describes the expression of MBP-FIRRM-10×His in a bacterial system and subsequent purification of MBP-FIRRM-10×His with Amylose beads and Ni-NTA agarose resin.

1. Transform plasmid into competent cells. Troubleshooting 2.
   a. Add 100 ng of pGEX-MBP-FIRRM-10×His plasmid into 20 μL of ArcticExpress (DE3) pRARE2 competent cells and incubate on ice for 30 min.
   b. Heat-shock the competent cells at 42°C for 1 min in a water-bath and then immediately transfer onto ice for 2 min.
   c. Culture the cells in 600 μL of LB medium without antibiotics at 37°C for 1 h with shaking (180 rpm).
   d. Spread 100 μL of the cells on an LB plate containing Ampicillin (100 μg/mL) and incubate overnight (approximately 18 h) at 37°C in a thermostatic incubator.

Note: This step requires evenly spreading the cells on the LB plate.

2. Pick bacterial colonies.
   a. Pick 40–50 colonies from the plate using a 200 μL pipette tip with the front end cut off.
   b. Transfer colonies into 500 mL of LB medium containing Ampicillin (0.1 mg/mL).
   c. Incubate the colonies at 37°C with shaking until the optical density at 600 nm (OD600) reaches 0.9.

   Note: The regular incubation time is around 6 h. However, the incubation time may be longer if the OD value does not reach 0.9, or shorter if the OD is much higher than 0.9.

3. Mix 500 mL of bacterial culture with 700 mL of fresh LB medium containing Ampicillin (0.1 mg/mL), then evenly divide them into three 1 L conical flasks (400 mL per flask) and culture at 37°C with shaking until the OD600 reaches 1.2.

   Note: The regular incubation time is 1.5–2 h.

4. Dilute 1.2 L of bacterial culture with 4.8 L of fresh LB medium containing Ampicillin (0.1 mg/mL), then evenly divide them into twelve 1 L conical flasks (500 mL per flask) and culture at 37°C with shaking. When the OD600 reaches 0.3, adjust the temperature to 16°C, and continue to incubate until the OD600 reaches 0.8.

   Note: The regular incubation time is 2–2.5 h.

   ⚠ CRITICAL: The incubation temperature and time of the bacterial culture are important to the activity of the bacteria. Low or high cell density will result in poor targeting protein expression.

5. Induction of FIRRM expression.
   a. To induce FIRRM expression, add IPTG to a final concentration of 0.1 mM.
   b. Loosen the sealing film of the conical flask, and continue to incubate the bacterial medium at 16°C for 16 h with shaking.
   c. Harvest the bacteria by centrifugation at 5000× *g* for 10 min at 4°C in 500 mL centrifuge bottles. After discarding the supernatant, transfer the cell pellet into a 50 mL centrifuge tube, and store at −80°C. 6 L of bacterial culture usually will yield ~6 *g* of cell pellet.

   Note: To ensure sufficient cell pellet for protein purification, FIRRM expression can be repeated multiple times.

   ⏸ **Pause point:** The cell pellet can be stored at −80°C for up to one month.

6. Purification of FIRRM. Troubleshooting 3.

   Note: All of the purification steps are carried out at 0°C–4°C.

   a. Preparation of cell lysate (taking 6 g cell pellet as an example).
      i. Prepare 50 mL T400 buffer and keep on ice.
      ii. Transfer frozen cell pellet into a 100 mL beaker on ice, add 45 mL T400 to resuspend cells. Then add protease inhibitors (aprotinin, chymostatin, leupeptin and pepstatin A at 5 μg/mL each, 1 mM phenylmethylsulfonyl fluoride) and Igepal (final concentration, 0.05%).
      iii. Homogenize the mixture on ice using high-speed disperser (3000 rpm, 45 s).
      iv. Lyse the bacterial cells by sonication (4 min, 30% power).

Note: After sonication, the lysate should be translucent or clear. If the lysate is turbid and viscous, the sonication time should be increased.

v. Centrifuge the cell lysate at 13000 rpm (17947 × *g*) for 30 min at 4°C and transfer the supernatant into a pre-cooled 100 mL beaker. Then add T buffer to adjust the salt concentration to 300 mM.

b. Affinity purification with Amylose beads.
   i. Pre-wash 400 μL of Amylose beads with 1 mL of T300 and then centrifuge at 10000 × *g* for 1 min at 4°C. Carefully discard the supernatant, ensure the beads pellet is not disturbed. Repeat this step twice.
   ii. Add the washed Amylose beads to the cell lysate and incubate at 4°C for 4 h on a rotary mixer at 10 rpm.
   iii. Centrifuge the cell lysate at 5000 × *g* for 4 min at 4°C to collect the beads. Transfer the beads to a 2 mL centrifuge tube.
   iv. Wash the beads with T300 buffer four times. Centrifuge at 3000 × *g* for 2 min at 4°C and then transfer the beads to a 1.5 mL centrifuge tube.
   v. Elute proteins with 250 μL T300 buffer containing 10 mM maltose at 4°C for 7 min on a rotary mixer at 15 rpm.

Note: Considering the residual buffer in the beads pellet, when performing protein elution for the first time, add 1.5 times of maltose (15 mM).

   vi. Repeat the elution step three additional times. Keep 5 μL of each eluate for SDS-PAGE and Coomassie blue staining.

c. Affinity purification with Ni-NTA agarose resin.
   i. Pre-wash 140 μL of Ni-NTA agarose resin with 1 mL of T300 as described above.
   ii. Mix resin with the eluate (∼3.8 mL) from three times of above affinity purification (∼20 *g* cell pellet in total), and add imidazole to the final concentration of 10 mM. Then incubate at 4°C for 3 h on a rotary mixer at 10 rpm.
   iii. Centrifuge at 3000 × *g* for 2 min at 4°C to collect the resin and transfer the resin to a 1.5 mL centrifuge tube.
   iv. Prepare T300 and T150 buffers containing 10 mM imidazole; wash the resin three times with each buffer. Centrifuge at 3000 × *g* for 2 min at 4°C.
   v. Elute proteins with 120 μL T150 buffer containing 200 mM imidazole at 4°C for 10 min on a vortex mixer at 1100 rpm.

Note: Considering the residual buffer in the resin, when performing protein elution for the first time, add 1.5 times of imidazole (300 mM).

   vi. Repeat the elution step three additional times. Keep 3 μL of each eluate for SDS-PAGE and Coomassie blue staining.

d. Concentrate FIRRM protein with centrifugal filter (30 kDa).
   i. Pre-wash centrifugal filter with T150 buffer twice.
   ii. Pool the eluate (∼480 μL) from the above Ni-NTA affinity purification and add the mixed eluate into centrifugal filter tube.
   iii. Centrifuge at 8000 rpm (6010 × *g*) for 2 min at 4°C. Repeat this step until the eluate is concentrated to approximately 100 μL.
   iv. Transfer the concentrated protein into a 1.5 mL tube. Keep 1.5 μL protein for SDS-PAGE and Coomassie blue staining.
   v. Measure the protein concentration by using Coomassie plus assay reagent.
   vi. Aliquot the protein into small tubes, freeze in liquid nitrogen, and store at −80°C.

Expression and purification of FIGNL1

Timing: 1–2 weeks

The N-terminal truncated form of FIGNL1 (FIGNL1ΔN) has been purified by other researchers and demonstrated to have anti-recombinase activity.[2] In this section, we express and purify the full-length FIGNL1 with N-terminal MBP tag and C-terminal Flag tag in *Saccharomyces cerevisiae* cells.

7. Transformation of pESC-MBP-FIGNL1-Flag plasmid.
   a. Resuscitate the frozen protease-deficient *Saccharomyces cerevisiae* cells and culture them in YPD medium at 30°C with shaking (180 rpm) until the OD660 reaches 1.0–1.2.
   b. Collect the cells from 4 mL culture by centrifugation (1000 × *g*, 3–5 min), and wash the collected cells twice with 1 mL ddH_2O and 1 mL 100 mM LiAc respectively. Then resuspend the cells in 200 μL 100 mM LiAc.
   c. Heat 20 μL herring sperm DNA (10 mg/mL) at 95°C for 5 min and then quickly cool it on ice for 3 min.
   d. Collect the cells from 100 μL cell suspension by centrifugation (10000 × *g*, 15–30 s), and add the reagents in the following table to the cell precipitate in sequence. Then mix the cells and reagents by vortexing.

Reagents for transformation	
50% PEG-3350	240 μL
1 M LiAc	36 μL
Heated herring sperm DNA (10 mg/mL)	10 μL
2 μg plasmid (pESC-MBP-FIGNL1-Flag)	x μL
ddH_2O	Add to 360 μL

Note: To avoid the direct interaction between the cells and high concentration of LiAc, 1 M LiAc should to be carefully added onto 50% PEG-3350.

   e. Heat-shock the cells with transformation reagents in 42°C water bath for 40 min.
   f. After centrifugation (3500 × *g*, 15–30 s), resuspend the cell pellet with 200 μL of ddH_2O and spread on two solid medium plates (-Trp).
   g. Culture the plates at 30°C for 2–4 days, keeping humid.
8. Expression of MBP-FIGNL1-Flag protein.
   a. Transfer the yeast cells into 600 mL omission medium (-Trp) and incubate at 30°C with shaking for 20–24 h, until the OD660 of eight-fold diluted cell culture reaches 0.7–0.8.
   b. Prepare expression medium (-Trp) as described in the following table, then evenly divide them into four 1 L conical flasks (600 mL per flask) and culture at 30°C with shaking for 24 h.

Expression medium (-Trp)	
Omission medium mix (-Trp)	17.3 *g*
Galactose	48 *g*
Glycerol	72 mL
Sodium DL-lactate solution	72 mL
Yeast culture	600 mL
Ampicillin (100 mg/mL)	1.2 mL
ddH_2O (add to 2.4 L)	1656 mL

   c. Harvest the cells by centrifugation (5000× *g*, 10 min, 4°C) and store the cell pellet at −80°C.

Pause point: The cell pellet can be stored at −80°C for up to one month.

9. Purification of MBP-FIGNL1-Flag protein.

a. Add the cell pellet (nearly 9.5 *g*) and 40 mL of T500 buffer containing 0.1% Igepal and protease inhibitors (as described above) into a bead-beater container with approximately 50 mL glass beads (diameter of 0.5 mm).
b. Keep the container in an ice bath and apply it to bead-beater for 3 min.
c. Clarify the cell lysate with ultracentrifugation (53340 × *g*, 30 min, 4°C) to remove the insoluble fractions.
d. Pre-wash 600 μL Amylose beads three times with T300 buffer.
e. Adjust the salt concentration of the supernatant to 300 mM by adding 15 mL T buffer with protease inhibitors (as described above). Then gently mix the adjusted supernatant with pre-washed Amylose beads and incubate on a rotary mixer (10 rpm, 4°C) for 4 h.
f. Collect the beads by centrifugation (4000 × *g*, 3 min, 4°C) and wash four times with T500 buffer and one time with T300 buffer.
g. Elute proteins three times with 600 μL T300 buffer containing 10 mM maltose at 4°C on a rotary mixer at 15 rpm, 7 min for each time.

Pause point: The eluate can be stored at −80°C for up to two weeks.

h. Pre-wash 150 μL anti-Flag affinity beads three times with T300 buffer.
i. Supplement the eluate from Amylose purification step with T300 buffer to 5 mL. Then incubate the mixture with pre-washed anti-Flag affinity beads on a rotary mixer (10 rpm, 4°C) for 4 h.
j. Collect the beads and wash three times with T300 buffer and one time with T150 buffer.
k. Elute proteins three times with 150 μL T150 buffer containing 250 ng/μL Flag peptide.

Note: For each elution, shake the beads on a vortex mixer (1100 rpm, 4°C) for 15 min and collect the eluate by centrifugation (9391 × *g*, 2 min, 4°C).

l. Analyze the eluted protein with SDS-PAGE and Coomassie blue staining. And determine the concentration of FIGNL1 by using Coomassie plus assay reagent.
m. Store the purified protein at −80°C in small aliquots.

⚠ CRITICAL: All of the protein purification steps are carried out at 0°C–4°C. When adjusting the KCl concentration of cell lysate, T buffer should be added slowly and the mixture should be stirred in time to avoid the drastic change of salt concentration. The purified protein should be aliquoted into small tubes to prevent repeated freeze-thaw cycles.

Expression and purification of miBRCA2

Timing: 1–2 weeks

In order to analyze the relationship between BRCA2 and FIRRM-FIGNL1 in the regulation of RAD51, in this section, we express and purify the Flag-miBRCA2-10×His protein in *Saccharomyces cerevisiae* cells.

10. Transformation of pESC-Flag-miBRCA2-10×His plasmid.
    a. The procedures are the same as described in step 7 except that plasmid pESC-Flag-miBRCA2-10×His and -Ura solid medium plates are used.
11. Expression of Flag-miBRCA2-10×His protein.
    a. The procedures to induce the expression of miBRCA2 are the same as described in step 8 except that -Ura medium is used.

Pause point: The cell pellet can be stored at −80°C for up to one month.

12. Purification of Flag-miBRCA2-10×His protein.
    a. Add the cell pellet (nearly 10 *g*) and 40 mL of T500 buffer containing 0.5% Igepal and protease inhibitors (as described above) into a bead-beater container with approximately 50 mL glass beads (diameter of 0.5 mm).
    b. Keep the container in an ice bath and apply it to bead-beater for 3 min.
    c. Clarify the cell lysate with ultracentrifugation (53340 × *g*, 30 min, 4°C) to remove the insoluble fractions.
    d. Pre-wash 500 μL Ni-NTA agarose resin three times with T300 buffer.
    e. Adjust the salt concentration of the supernatant (nearly 35 mL) to 300 mM by adding 15 mL T buffer with protease inhibitors. Then add imidazole to 10 mM and incubate the mixture with Ni-NTA agarose resin on a rotary mixer (10 rpm, 4°C) for 3 h.
    f. Collect the beads by centrifugation (4000 × *g*, 3 min, 4°C) and wash four times with T300 buffer containing 10 mM imidazole.
    g. Elute proteins five times with 500 μL T300 buffer containing 200 mM imidazole at 4°C on a rotary mixer at 15 rpm, 7 min for each time.

    Pause point: The eluate can be stored at −80°C for up to two weeks.

    h. Pre-wash 130 μL anti-Flag affinity beads three times with T300 buffer.
    i. Supplement the eluate from Ni-NTA purification step with T buffer to 4.5 mL. Then incubate the mixture with pre-washed anti-Flag affinity beads on a rotary mixer (10 rpm, 4°C) for 4 h.
    j. Collect the beads and wash three times with T300 buffer and one time with T150 buffer.
    k. Elute proteins three times with 120 μL T150 buffer containing 250 ng/μL Flag peptide.

    Note: For each elution, shake the beads on a vortex mixer (1100 rpm, 4°C) for 15 min and collect the eluate by centrifugation (9391 × *g*, 2 min, 4°C).

    l. Analyze the eluted protein with SDS-PAGE and Coomassie blue staining. And determine the concentration of miBRCA2 by using Coomassie plus assay reagent.
    m. Store the purified protein at −80°C in small aliquots.

Expression and purification of RAD51

Timing: 1–2 weeks

In order to reconstitute the assembly and disassembly of RAD51 filaments *in vitro*, based on the previous study,[6,7] we use a modified procedure to express and purify human RAD51 without any tag in *Escherichia coli* cells.

13. Plasmid transformation, bacterial culture and protein expression.
    a. The procedures are the same as described in steps 1 to 5 except that plasmid pET11d-RAD51 and RecA-deficient *Escherichia coli* strain BLR (DE3) pLysS competent cells are used. 6 L of bacterial culture usually will yield ∼10 *g* of cell pellet.
14. Purification of RAD51 protein.

    Note: All of the purification steps are carried out at 0°C–4°C.

    a. Preparation of cell lysate.
        i. Transfer cell pellet into a beaker on ice, add 80 mL cell breakage buffer with protease inhibitors (as described above) to resuspend cells.
        ii. Homogenize the mixture on ice using high-speed disperser (3000 rpm, 1 min). Then lyse the bacterial cells by sonication (6 min, 30% power).

iii. Centrifuge the cell lysate at 13000 rpm (17947 × *g*) for 30 min at 4°C and transfer the supernatant into a pre-cooled beaker.
iv. Treat the supernatant with ammonium sulfate at 0.23 *g*/mL to precipitate RAD51.
v. Collect the precipitate by centrifugation at 12000 rpm (15292 × *g*) for 20 min at 4°C and store the precipitate at −80°C.

Pause point: The precipitate is stored at −80°C overnight (approximately 15 h).

b. Protein purification with Q Sepharose column.
   i. Dissolve protein precipitate in 60 mL T buffer with protease inhibitors. Then load the solution onto Q Sepharose column (10 mL) equilibrated with T175 buffer.
   ii. Wash the column with 50 mL T175 buffer, and elute proteins with a 100 mL of gradient of 200–600 mM KCl in T buffer. Pool the peak fractions of RAD51 and dialyze against T50 buffer.
c. Protein purification with MHAP column.
   i. Apply the fractions eluted from Q Sepharose column onto MHAP column (2 mL) equilibrated with T100 buffer.
   ii. Wash the column with 10 mL T100 buffer, and elute proteins with a 20 mL of gradient of 60–270 mM KH_2PO_4 in T buffer. Pool the peak fractions of RAD51, freeze in liquid nitrogen and store at −80°C.

Note: If the flowthrough fractions from MHAP column contain a large amount of more pure RAD51 protein, load the flowthrough onto a second MHAP column. This purification step may need to be repeated two or three times.

Pause point: The fractions containing proteins are stored at −80°C overnight (approximately 15 h).

d. Protein purification with Mono Q column and protein concentration.
   i. Thaw the above fractions and load them onto Mono Q column (0.5 mL) equilibrated with T50 buffer.
   ii. Wash the column with 5 mL T50 buffer, and elute proteins with a 10 mL of gradient of 200–500 mM KCl in T buffer. Pool the peak fractions of RAD51 and concentrate protein with centrifugal filter (10 kDa).
e. Protein fractionation with Superdex 200 column and protein concentration.
   i. Apply the concentrated fractions onto Superdex 200 column (24 mL) equilibrated with T300 buffer.
   ii. Elute with 24 mL T300 buffer to separate RAD51 protein. Then pool the peak fractions of RAD51 and concentrate protein with centrifugal filter (10 kDa).
   iii. Check protein with SDS-PAGE and Coomassie blue staining, and measure protein concentration by using Coomassie plus assay reagent.
   iv. Aliquot purified protein into small tubes, freeze in liquid nitrogen, and store at −80°C.

RAD51 filament stabilization assay

Timing: 3 days

In order to more directly assess the effects of various factors on the formation and disruption of RAD51 filament, we modify the protocol to reconstitute RAD51 filament assembly and disassembly according to previous studies.[2,8]

15. Immobilization of ssDNA on streptavidin Sepharose beads.

a. Pre-wash Streptavidin Sepharose beads (15 μL beads per reaction, binding capacity: >300 nM biotin/mL medium or 6 mg biotinylated BSA/mL medium) twice with 800 μL buffer A containing 100 mM KCl.

Note: For each wash, mix the beads and buffer A on a shaker (1200 rpm) at room temperature (20°C–25°C) and collect the beads by centrifugation (3000 × *g*, 2 min, 4°C).

b. Incubate 5′-biotinylated 60 nt ssDNA (oligo dT60, 7.5 nM) with streptavidin Sepharose beads in 30 μL buffer A containing 100 mM KCl for 30 min on a shaker (1200 rpm) at room temperature (20°C–25°C).
c. After incubation, collect the beads with ssDNA by centrifugation (3000 × *g*, 2 min, 4°C) and wash three times with 200 μL buffer A containing 100 mM KCl on a shaker (1200 rpm) for 1 min.

16. Assembly of RAD51 onto ssDNA. Troubleshooting 4.
    a. Add RAD51 (50 nM) or RAD51 (50 nM) and miBRCA2 (50 nM) in 30 μL buffer A containing 100 mM KCl and 1 mM ATP to the beads with ssDNA and incubate on a shaker (1200 rpm) at room temperature (20°C–25°C) for 30 min.
    b. After centrifugation (3000 × *g*, 2 min, 4°C), collect and mix the supernatant with 10 μL 4× SDS loading buffer as unbound protein to analyze the binding efficiency of RAD51 to ssDNA.
    c. Wash the beads with RAD51 filament three times with 200 μL buffer A containing 100 mM KCl and 1 mM ATP.

Note: For each time, mix the beads with buffer A on a shaker (1200 rpm) at room temperature (20°C–25°C) for 1 min and collect the beads by centrifugation (3000 × *g*, 2 min, 4°C).

17. Disassembly of RAD51 filament. Troubleshooting 5.
    a. Mix FIGNL1 (50 nM), FIRRM (50 nM) or FIGNL1 (50 nM) and different concentrations of FIRRM (50 nM or 100 nM) with 100 nM trap ssDNA (oligo dT60) in 30 μL of buffer A containing 50 mM KCl and 2 mM ATP.
    b. Add the mixture to the beads with RAD51 filament and incubate on a shaker (1200 rpm) at 37°C for 15 min to disassemble RAD51 filament.
    c. After centrifugation (3000 × *g*, 2 min, 4°C), collect the supernatant and mix it with 10 μL 4× SDS loading buffer as supernatant fraction to analyze the protein level of dissociated RAD51 from ssDNA.
    d. Wash the beads three times with 200 μL buffer A containing 50 mM KCl and 2 mM ATP.

Note: For each wash, incubate the beads on a shaker (1200 rpm) at room temperature (20°C–25°C) for 1 min and collect the beads by centrifugation (3000 × *g*, 2 min, 4°C).

    e. Elute the residual RAD51 filament on the beads by adding 30 μL buffer A and 10 μL 4× SDS loading buffer and boiling the beads for 5 min as beads fraction.

18. Analysis of RAD51 level in supernatant and beads fractions.
    a. Analyze the samples of supernatant and beads fractions with SDS-PAGE and Western blot.
    b. After the protein is transferred onto NC membrane, incubate the membrane with specific antibodies and followed by incubation with secondary antibodies.

Note: RAD51 antibody is used at 1:3000 dilution.

    c. Wash and expose the membrane.

⚠ CRITICAL: To ensure the activity of RAD51, miBRCA2, FIGNL1 and FIRRM, the proteins should be thawed on ice and prevented from repeated freeze-thaw cycles. And all reactions should be set up on ice.

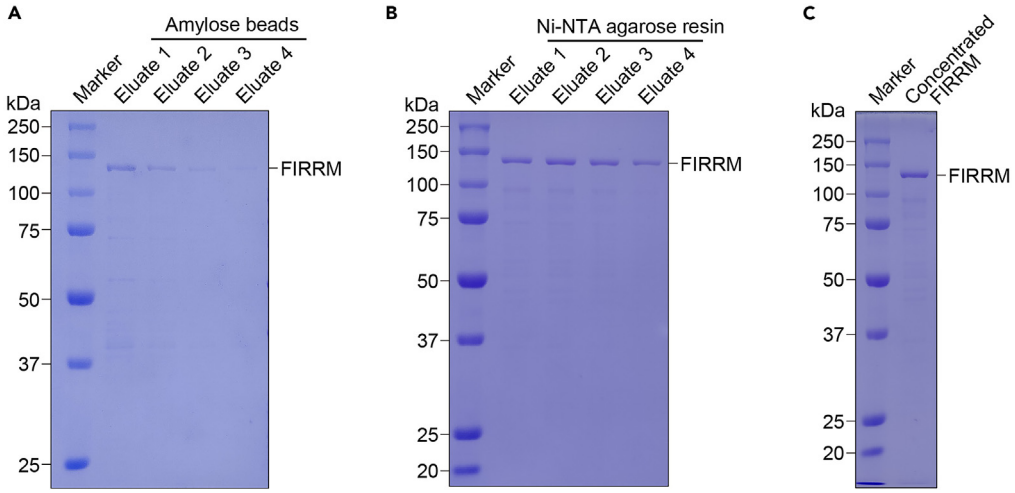

Figure 1. Purification of FIRRM

(A) The elution fractions (5 μL) from Amylose beads were analyzed by SDS-PAGE and Coomassie blue staining.

(B) The elution fractions (3 μL) from Ni-NTA agarose resin were analyzed by SDS-PAGE and Coomassie blue staining.

(C) The concentrated FIRRM protein (1.5 μL) was analyzed by SDS-PAGE and Coomassie blue staining.

Note: In this protocol, the buffer A contains 2 mM Ca^{2+}, because we tried buffer without Ca^{2+} and found that the RAD51 filament is very unstable in the absence of Ca^{2+} during the above experiment.

EXPECTED OUTCOMES

As shown in Figure 1, the MBP-FIRRM-10×His protein expressed in *Escherichia coli* cells is purified to near homogeneity through affinity purification with Amylose beads and Ni-NTA agarose resin. In Figure 2, after affinity purification with Amylose beads and anti-Flag beads, the MBP-FIGNL1-Flag protein expressed in *Saccharomyces cerevisiae* cells is purified to near homogeneity. The expression of Flag-miBRCA2-10×His is induced in *Saccharomyces cerevisiae* cells and the protein is purified to near homogeneity through Ni-NTA agarose resin and anti-Flag beads affinity purification (Figure 3). RAD51 without any tag overexpressed in *Escherichia coli* cells is purified to near homogeneity by multiple steps of ion exchange and gel filtration chromatography (Figure 4).

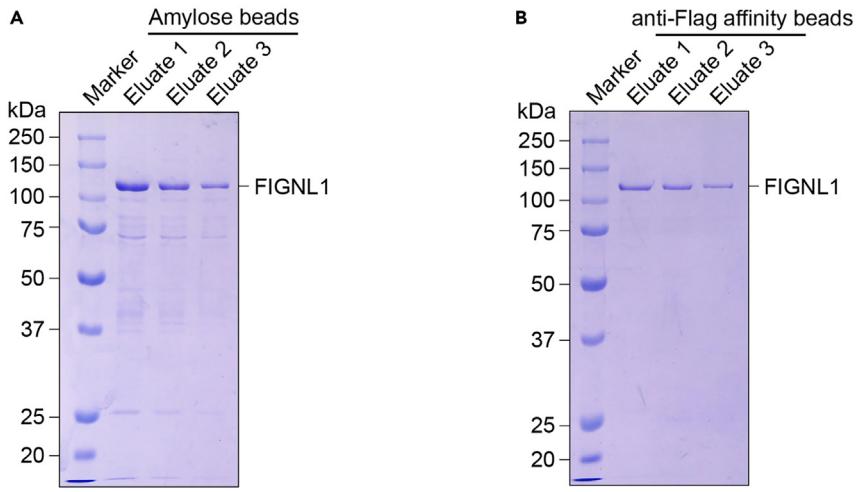

Figure 2. Purification of FIGNL1

(A) The elution fractions (3 μL) from Amylose beads were applied to SDS-PAGE and Coomassie blue staining.

(B) The elution fractions (2 μL) from anti-Flag affinity beads were applied to SDS-PAGE and Coomassie blue staining.

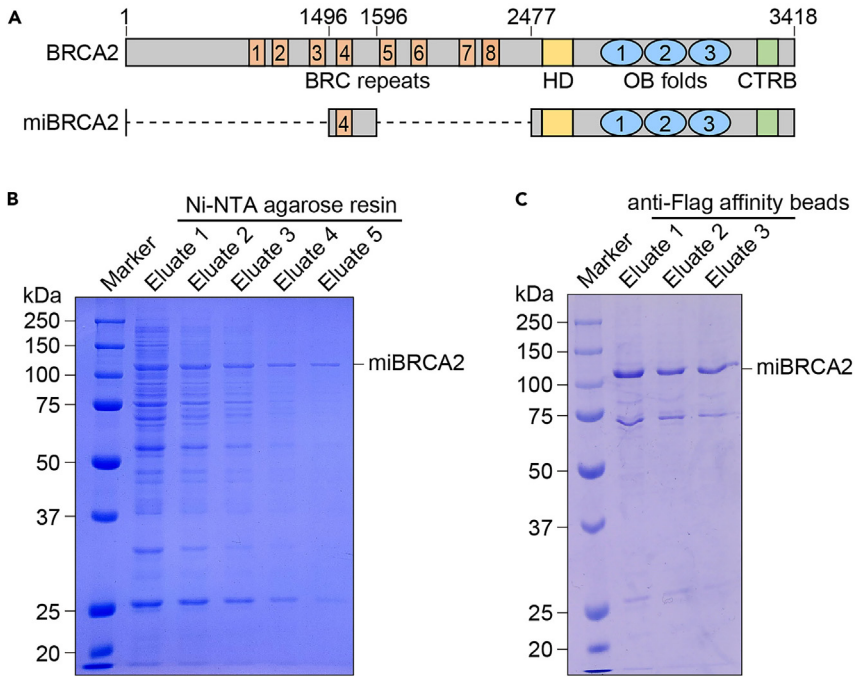

Figure 3. Purification of miBRCA2

(A) Schematic of full-length BRCA2 and its mini form (termed miBRCA2) containing the key functional domains of BRC4, HD (Helical Domain), OB (Oligonucleotide Binding) folds and CTRB (C-terminal Recombinase Binding) as indicated.

(B) The elution fractions (2 μL) from Ni-NTA agarose resin were analyzed by SDS-PAGE and Coomassie blue staining.

(C) The elution fractions (4 μL) from anti-Flag affinity beads were analyzed by SDS-PAGE and Coomassie blue staining.

In the reconstituted reactions of RAD51 filament stabilization assay (Figure 5A), full-length FIGNL1 exhibits obvious anti-recombinase activity to RAD51 (Figure 5B), while FIRRM alone has no such effect (Figure 5C). Further experiments demonstrate that FIRRM can promote the anti-recombinase activity of FIGNL1 (Figure 5D), and that miBRCA2 can protect RAD51 filament from disassembly by FIRRM-FIGNL1 (Figure 5E).

LIMITATIONS

To facilitate the expression and purification of FIRRM and FIGNL1, we introduce an MBP tag at the N-terminal of these proteins. MBP-tagged FIRRM and FIGNL1 function normally in the above assay. However, this still has potential limitations, because the large size of the MBP tag (approximately 42 kDa) may affect FIRRM-FIGNL1 activities. Meanwhile, we use miBRCA2, a truncated form, to study the relationship between BRCA2 and FIRRM-FIGNL1 in the regulation of RAD51 filament. Thus, full-length form of proteins with MBP tag cut off may be needed to confirm the outcomes. In addition, there are still many other assays to assess the anti-recombinase activity to RAD51, such as nuclease protection assay[9] and nucleoprotein filament disruption assay.[10] It would be better to further confirm the above results by using other assays.

TROUBLESHOOTING

Problem 1

Bacterial colonies with pGEX-MBP-FIRRM-10×His plasmid grow very slowly and many colonies carry incorrect plasmids (related to construction of pGEX-MBP-FIRRM-10×His).

Potential solution

Incubate the plate with bacterial colonies at room temperature (20°C–25°C) for ~20 h or a longer time. The small bacterial colonies usually carry the correct plasmid.

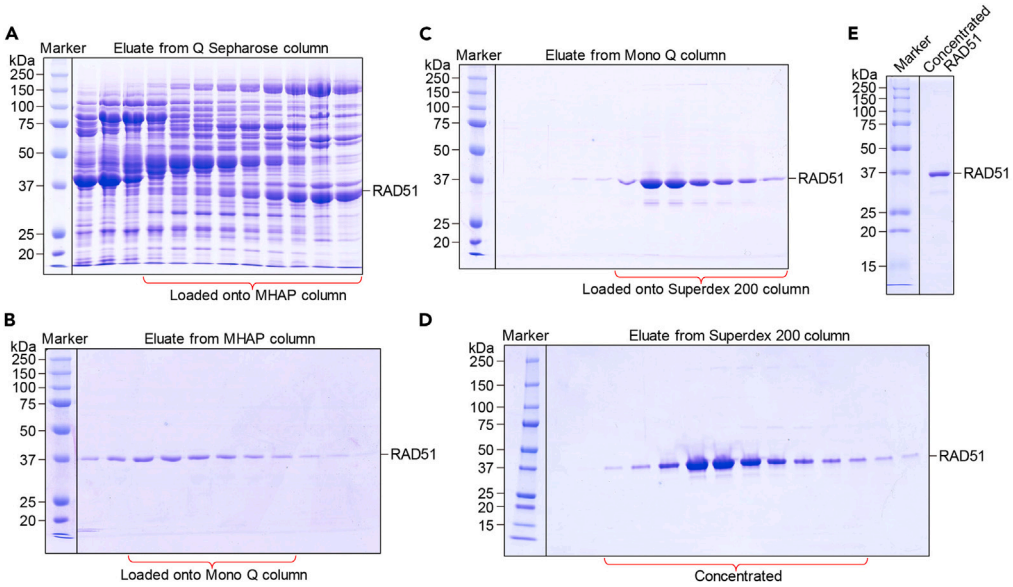

Figure 4. Purification of RAD51

(A) The elution fractions from Q Sepharose column were subjected to SDS-PAGE and Coomassie blue staining. The fractions were pooled as indicated and applied onto MHAP column.

(B) The elution fractions from MHAP column were subjected to SDS-PAGE and Coomassie blue staining. The fractions were pooled as indicated and applied onto Mono Q column.

(C) The elution fractions from Mono Q column were subjected to SDS-PAGE and Coomassie blue staining. The fractions were pooled as indicated and applied onto Superdex 200 column.

(D) The elution fractions from Superdex 200 column were analyzed by SDS-PAGE and Coomassie blue staining. The fractions were pooled as indicated and concentrated.

(E) Final concentrated RAD51 was analyzed by SDS-PAGE and Coomassie blue staining.

Problem 2

FIRRM protein is difficult to overexpress (related to steps 1 and 5 of expression and purification of FIRRM).

Potential solution

FIRRM may tend to aggregate when overexpressed. We tried several bacterial strains and found that the ArcticExpress (DE3) pRARE2 cells show a relatively better result. And the overexpression condition should strictly conform to our protocol.

Problem 3

FIRRM protein is difficult to purify (related to step 6 of expression and purification of FIRRM).

Potential solution

The yield of the first step affinity purification is usually low, maybe because of the low expression and low affinity between protein and Amylose resin. To increase the yield of FIRRM protein, the first step affinity purification with Amylose resin can be repeated multiple times before the second step purification.

Problem 4

RAD51 filament is unstable and RAD51 is easy to dissociate from ssDNA (related to step 16 of RAD51 filament stabilization assay).

Potential solution

In the above assay, appropriate amount of Ca^{2+} is added to facilitate RAD51 filament formation.

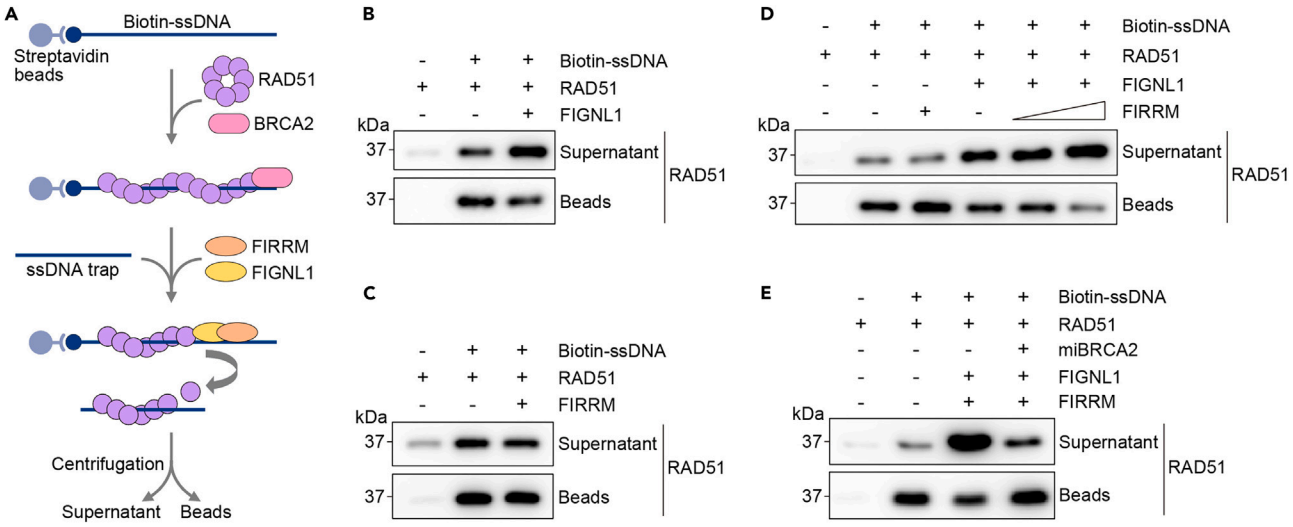

Figure 5. Effects of FIGNL1, FIRRM, and miBRCA2 on RAD51 filament stabilization

(A) Schematic of RAD51 filament stabilization assay used to analyze the effects of FIRRM-FIGNL1 and miBRCA2 proteins *in vitro*.

(B) The effect of FIGNL1 on RAD51 filament disassembly was assessed by biochemical experiment as indicated above. Immunoblotting was used to analyze the protein levels of RAD51 in supernatant and on beads.

(C) The effect of FIRRM on RAD51 filament disassembly was assessed as in (B).

(D) FIGNL1 and increasing concentrations of FIRRM were incubated with RAD51 filament, followed by the analysis as in (B).

(E) RAD51 was pre-incubated with miBRCA2 protein, followed by the addition of FIGNL1 and FIRRM. The analysis was carried out as in (B).

Problem 5

RAD51 filament is difficult to disassemble (related to step 17 of RAD51 filament stabilization assay).

Potential solution

If RAD51 filament is too stable, appropriately reduce the concentration of Ca^{2+} in reaction buffer, increase the amount of anti-recombinase, or adjust the incubation time with anti-recombinase.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Weibin Wang (weibinwang@bjmu.edu.cn).

Technical contact

Technical questions on executing this protocol should be directed to and will be answered by the technical contact, Weibin Wang (weibinwang@bjmu.edu.cn).

Materials availability

The reagents generated in this study are available from the lead contact with a completed materials transfer agreement.

Data and code availability

This study did not generate any datasets or code.

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AUTHOR CONTRIBUTIONS

Z.Z. and H.Y. performed the experiments. X.L., T.Z., and Q.L. provided technical and experimental assistance. Z.Z., H.Y., and W.W. designed the experiments and wrote the manuscript. J.W. and W.W. proofread and revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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