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Supplementary information

iDRiP for the systematic discovery of proteins bound directly to noncoding RNA

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SUPPLEMENTARY METHODS

Immuno-RNA-FISH

Cells were cytospun, CSKT treated (10 mM PIPES, pH 6.8, 100 mM NaCl, 3 mM MgCl2, 0.3 M sucrose, 0.5% Triton X-100, adjust to pH 6.8), and fixed in 4 % paraformaldehyde and stored at 70% EtOH at -20°C. Samples were blocked at 1% BSA/PBS with 1 mM EDTA and 0.8 U/μl of RNase inhibitor at 4°C for 1 hr. Cells were incubated with primary antibodies in blocking solution at 4°C for 2 hr, and washed with 0.2% Tween20/PBS 3 times at RT. Followed by incubation of secondary antibodies (in blocking solution) at 4°C for 30 min, cells were washed with PBS 3 times and fixed in 4 % paraformaldehyde for 10 minutes. RNA FISH was then performed. For TERRA: $(TAACCC)_7$-Alexa488-3'. DNA oligo probes for RNA-FISH were mixed at the final concentration 0.5 pmol/μl in hybridization solution (50% formamide, 2×SSC, 2 mg/ml BSA, 10% Dextran Sulfate-500K). Hybridization was carried out at 42°C overnight for RNA FISH. Slides were washed with 2×SSC/50% formamide for 5 min three times at 44°C, and then wash with 2×SSC for 5 min twice at 44°C. Antibodies were used against ATRX (Cat#: sc-15408, Santa Cruz) and PML (Cat#: 05-718, Millipore).

UV-RIP

ES cells were trypsinized, spun, and resuspended in 1X cold PBS. About $1X10^8$ cells in 5ml of PBS were added to 15 cm^2 plate to form a thin layer, and exposed to 200 mJ/cm^2 UV light (254nm) to crosslink RNA-protein complexes. Cells were collected, spun, and resuspended in 10ml of ice-cold CSKT buffer (10 mM PIPES, pH 6.8, 100 mM NaCl, 3 mM $MgCl_2$, 0.3 M sucrose, 0.5% Triton X-100, 1 mM PMSF) with protease inhibitor for 3 minutes for nuclear extraction. Cells were washed with cold PBS once, and the cell pellets were snap-frozen and stored at -80°C. Cells (1.0×10^7) were resuspended in 226.24 μl DNase I buffer (50 mM Tris pH 7.5, 0.5% Nonidet-P 40, 0.1% sodium lauroyl sarcosine, 1x protease inhibitors, SuperaseIn, 40U DNase I), and incubated at 37°C for 15 min. The proper amount of lysis buffer was added into samples to reach to the final concentration (1XPBS, 1% NP-40, 1% sodium deoxycholate, 2% RNase Inhibitor (superaseIN), and 1X protease inhibitor) in 600 μl. Samples were incubated at 4°C for 25 minutes with rotation. Insoluble materials were then removed by centrifugation at the highest speed for 10 min. Antibodies (5 ug) were incubated with Dynabeads® Protein G (ThermoFishe) at 4°C for 1 hr. After two washes with DNase I buffer, antibody (Ab)-beads were added into lysates, and incubated at 4°C overnight. Beads are washed three times with ice-cold RIPA-1-500 mM (PBS containing 1% NP40, 0.5% sodium deoxycholate and 500 mM NaCl) or RIPA-1-200 mM (PBS containing 0.5% NP40, 0.5% sodium deoxycholate and 200 mM NaCl (only for POT1 IP)). Ab-beads were washed with 1X cold PBS, and then were treats with Turbo DNase (30 U) for

15 min at 37°C. Ab-beads were washed with three more times with RIPA-2 (50 mM HEPES. 10 mM EDTA, 0.5% sodium deoxycholate, 0.5% NP-40, 50 mM NaCl). To elute RNA-protein complex, Ab-beads were incubated in 100 µl proteinase K buffer (100 mM Tris-HCl pH 7.5, 50 mM NaCl, 10 mM EDTA, 20 µg/µl Proteinase K, and 0.5% SDS) for 30 min at 55°C. RNA was extracted using TRizol (ThermoFisher). Antibodies were used against EZH2 (D2C9, #5246,Cell Signaling), POT1 (GTX64574, GeneTex), CHD8 (ab84527, Abcam), EHMT1 (Ab41969, Abcam), CENP-C (ABE1957, Merck) and AuroraB (NB100-294, Novus).

RNA slot blot

After UV-RIP (RNA immunoprecipitation), RNA was extracted using Trizol reagent. RNA samples were denatured at 65°C. After denaturation and cooling on ice, equal amount of 20X SSC was added to RNA samples reach the final concentration to 10X SCC. Hybond-XL membrane and 3M filter papers were immersed in 10X SSC and assembled to BioRad Bio-Dot SF microfiltration apparatus. RNA samples in 10X SSC were loaded into BioRad Bio-Dot SF microfiltration apparatus. RNA was further crosslinked to membrane using ultra-violet light. Northern probe GAPDH: 5′-GTAGACCCACGACATACTCAGCACCGGCCTCACCCCATT-3′. Northern probe TERRA: 5′-$(TAACCC)_5$-3′ All oligo probes were end labeled with ^{32}P using T4 polynucleotide kinase. Hybridization was carried out at 42°C overnight using ULTRAhyb-Oligo hybridization buffer (ThermoFisher).