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**Item title:** The NORAD lncRNA assembles a topoisomerase complex critical for genome stability

**Link back to the item:** <https://hdl.handle.net/1721.1/126584>



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1 **Supplementary Notes**

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3 **Note S1: Proteins interacting with NORAD *in vitro***

4 While this paper was under review, an independent study reported an *in vitro* association  
5 between NORAD and the partially cytoplasmic protein KHDRBS1, inferred from mixing of  
6 exogenous NORAD fragments with cytoplasmic extracts<sup>1</sup>. Our quantitative RAP-MS data  
7 demonstrates that endogenous NORAD interacts strongly with the nuclear protein KHDRBS3 (a  
8 paralog of KHDRBS1) in intact cells.

9  
10 **Note S2: Assessing statistical significance in RAP-MS and co-IP MS data**

11 We have used a moderated *t*-test for determining statistical significance in RAP-MS and co-IP  
12 MS experiments. The limma library we have used for this purpose applies an empirical Bayes  
13 approach to effectively use variance shrinkage to make the test robust even with very few  
14 samples<sup>2,3</sup>. Ritchie *et al.*, 2015<sup>3</sup> demonstrate that this method is applicable to a small number of  
15 samples — in fact the example presented in their study uses two groups with two replicates each.  
16 This approach is widely used for the analysis of quantitative mass spectrometry experiments<sup>4,5</sup>.

17  
18 **Note S3: Promiscuous RNA binders in RAP-MS data**

19 Promiscuous RNA binders were defined by intersecting the results of 9 different lncRNA RAP  
20 experiments, including MALAT1, U1, CRNDE, NORAD, RMRP, PVT1, DANCR, RN7SK,  
21 RPPH1.

22  
23 **Note S4: Detection of NARC1 components by size-exclusion chromatography and western  
24 blot**

25 ALYREF is the only NORAD-dependent RBMX interacting protein that was also identified as a  
26 direct NORAD binding protein in RAP-MS experiments. CLIP further demonstrated that  
27 ALYREF binds to the 5' end of NORAD. Hence, we suspect that the interaction between  
28 ALYREF and RBMX is mediated by the NORAD lncRNA and may not involve direct protein-  
29 protein interactions. Consistent with these findings, we did not observe ALYREF when purifying  
30 the Benzonase digested NARC1 complex by size-exclusion chromatography.

31  
32 While we detected PRPF19 as a component of NARC1 by size-exclusion chromatography and  
33 western blot, we did not manage to probe for the PRPF19/CDC5L complex subunit CDC5L due  
34 to a lack of high quality antibodies. With available antibodies the detection of CDC5L in cellular  
35 extracts or CDC5L enriched immunoprecipitates proved challenging.

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38 **References**

- 39  
40 1. Tichon, A., Perry, R. B.-T., Stojic, L. & Ulitsky, I. SAM68 is required for regulation of  
41 Pumilio by the NORAD long noncoding RNA. *Genes & Development* **32**, 70–78 (2018).  
42 2. Smyth, G. K. Linear models and empirical bayes methods for assessing differential  
43 expression in microarray experiments. *Stat Appl Genet Mol Biol* **3**, Article3–25 (2004).  
44 3. Ritchie, M. E. *et al.* limma powers differential expression analyses for RNA-sequencing and  
45 microarray studies. *Nucleic Acids Research* **43**, e47–e47 (2015).

- 46 4. Huttlin, E. L. *et al.* The BioPlex Network: A Systematic Exploration of the Human  
47 Interactome. *Cell* **162**, 425–440 (2015).
- 48 5. Krönke, J. *et al.* Lenalidomide induces ubiquitination and degradation of CK1 $\alpha$  in del(5q)  
49 MDS. *Nature* **523**, 183–188 (2015).
- 50