SUPPLEMENTARY MATERIAL

Contents:

Supplemental Table 1: Oligonucleotides used in this study.

Primer name	Sequence (5' to 3')
GAPDH_Fw	TGCACCACCAACTGCTTAGC
GAPDH_Rv	GGCATGGACTGTGGTCATGAG
FANCA-qPCR_Fw	TTGCTGCCTTTGGGTCTTCC
FANCA-qPCR_Rv	CAGCGCCTCAGAAGAGAGGACG
FANCC-qPCR_Fw	GCTGGCACTCTTTCAGTTGAC
FANCC-qPCR_Rv	CGTCTTCATGGAAGTAGGAGAGA
miR-20-qPCR	TAAAGTGCTTATAGTGCAGGTAGAAA
Let-7-qPCR	TGAGGTAGTAGGTTGTATAGTTAAA
SNORD44_qPCR	GCAAATGCTGACTGAACATGAA

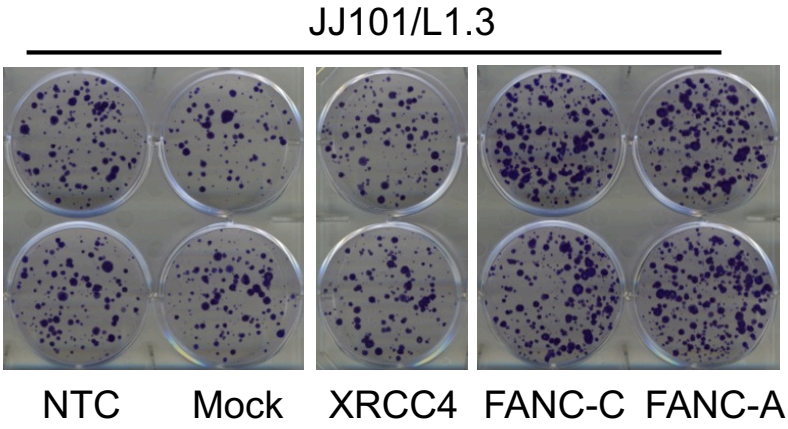
Supplemental Table 2: Human genes regulated by miRNA-20. Column 1 indicates if the gene was experimentally validated (blue, [1]) or identified *in silico* in this study (green). Columns 2 and 3 show the maximum effect detected for each gene in K562 and HeLa cells, using data from [2].

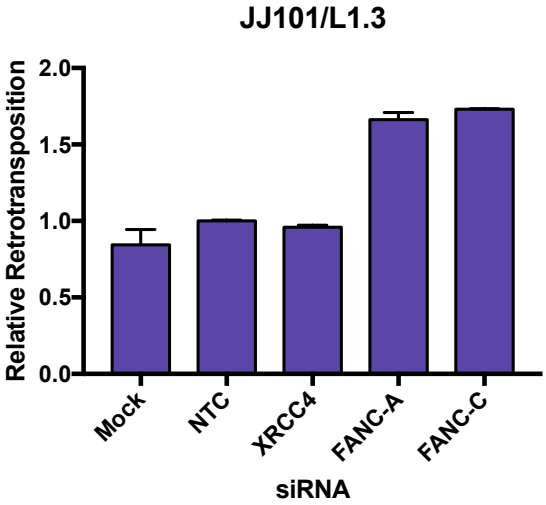
Trompeter et al. PLOS One 2011	**K562**--Maximum Effect (95% Credible Interval)	**HeLa**--Maximum Effect Estimate
CCND1	1,9	1,7
CCND2	2	1,1
CDKN1A	2,3	1,5
E2F1	1	3,8
PTEN	3	4,6
RBL1	1	2
RBL2	4,2	2,2
(experimetally validated!)		
Found by 3 softwares		
CFL2	4,3	1,3
DYNC1LI2	1,7	3,5
ENPP5	0,9	3,1
EPHA4	-0,1	2,8
EZH1	3	2,7
FBXL5	4,3	1,6
GPR6	5,8	3,5
MYT1L	1,1	2,6
PKD2	2	2,6
PLEKHA3	0,5	2,9
RRAGD	4	2,1
RUFY2	1,7	6,2
VLDLR	2,7	1,8
ZNF800	2,1	3,9
ZNFX1	5,6	4

Supplemental Figure 1 (Related to Figure 2): Mir-20a binding sites in human RC-L1s. The table describe the binding sites for miR-20a identified in the consensus sequence of human active L1s, after sequence analyses using the rna22 online tool (https://cm.jefferson.edu/rna22/). From left to right, columns 1 and 2 indicate the name of the miRNA and gene, respectively; column 3, indicates the leftmost position of the predicted binding site, with respect to L1.3 [[3], accession number L19088.1]; column 4, indicates the folding energy (in Kcal/mol); column 5, contains the alignment of the miRNA and gene; and column 6, indicates the p value of the findings.

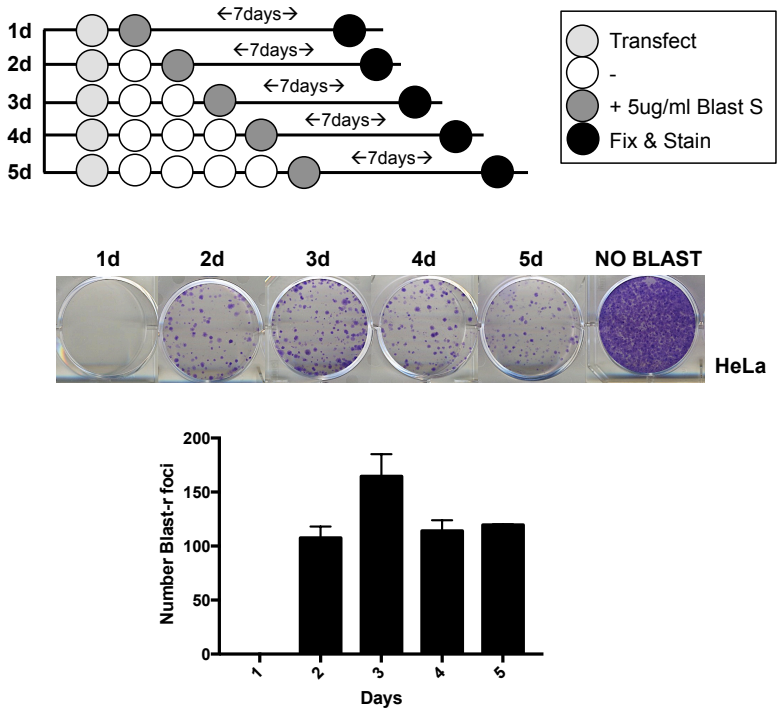
miR name	Transcript	Leftmost position of predicted target site	Folding energy (Kcal/mol)	Heteroduplex alignment	p value
hsa-miR-20a	L1Hs	607	-10.00	GTCCCTGT-CTG--ACAGCTTTG ||||: ||: :||||: GATGGACGTGATATTCGTGAAAT	5.68E-3
hsa-miR-20a	L1Hs	5910	-10.10	ATACCTAATGCTAGATGACACATTA ||||| :||| | ||| ||| GATGGA-CGTGATATTC-GTGAAAT	3.74E-1

Supplemental Figure 2 (Related to Figure 3): siRNA/L1-retrotransposition reporter assay controls. Shown are representative retrotransposition assays conducted in HeLa cells using plasmid JJ101/L1.3 in the presence of the indicated siRNA. The graph at the bottom plots the relative retrotransposition rate of the above assays, where cells co-transfected with a non-targeting control siRNA (NTC) were assigned 1 for comparison. The SD of the assay is indicated (duplicate).

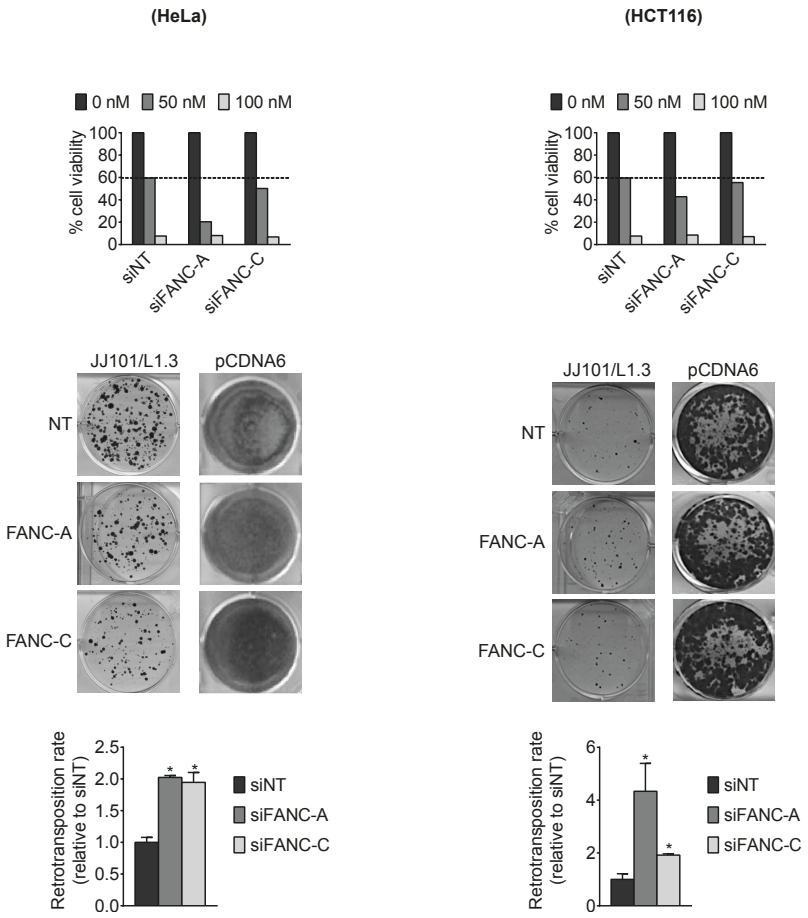




Supplemental Figure 3 (Related to Figure 3): Kinetic of L1 retrotransposition using *mblastI*-tagged RC-L1s. The top panel shows a scheme of the assay, where circles indicate days. Below are shown representative retrotransposition results in HeLa cells using plasmid JJ101/L1.3. Indicated in the top is the day where blasticidin selection was started. NO BLAST, no antibiotic was used, as a control. The graph in the bottom shows the number of blasticidin resistant foci (triplicate) generated upon JJ101/L1.3 transfection in HeLa cells, and starting blasticidin selection in the indicated day.



Supplemental Figure 4 (Related to Figure 3): siRNA/L1-retrotransposition reporter assays in HeLa (left side) and HCT116 (right side) cells. The graphs at the top show results from mitomycin C (MMC) sensitivity assays upon siRNA treatment (for FANC-A and FANC-C). The graph plots the percentage of cell viability detected upon treatment of siRNA transfected cells with 0 (black bars, assigned 100%), 50 (dark grey) and 100 nM MMC (light grey bars). Below are shown representative retrotransposition (JJ101/L1.3) and toxicity/clonability (pCDNA6) results in HeLa (left) and HCT116 (left) cells transfected with the indicated FANC siRNA (A or C) or with a NT control (siNT), used as an internal negative control. The graphs at the bottom plot the retrotransposition rate upon siRNA treatment (FANC-A and FANC-C, dark and light grey bars, respectively) relative to siNT. The SD of the assay is indicated. Unpaired two-sided t-test, *P<0.05.



Supplementary References

1 Trompeter, H. I., Abbad, H., Iwaniuk, K. M., Hafner, M., Renwick, N., Tuschl, T., Schira, J., Muller, H. W., Wernet, P. 2011 MicroRNAs MiR-17, MiR-20a, and MiR-106b act in concert to modulate E2F activity on cell cycle arrest during neuronal lineage differentiation of USSC. *PloS one*. **6**, e16138. (10.1371/journal.pone.0016138)

2 Liu, N., Lee, C. H., Swigut, T., Grow, E., Gu, B., Bassik, M. C., Wysocka, J. 2018 Selective silencing of euchromatic L1s revealed by genome-wide screens for L1 regulators. *Nature*. **553**, 228-232. (10.1038/nature25179)

3 Sassaman, D. M., Dombroski, B. A., Moran, J. V., Kimberland, M. L., Naas, T. P., DeBerardinis, R. J., Gabriel, A., Swergold, G. D., Kazazian, H. H., Jr. 1997 Many human L1 elements are capable of retrotransposition. *Nat Genet*. **16**, 37-43. (10.1038/ng0597-37)