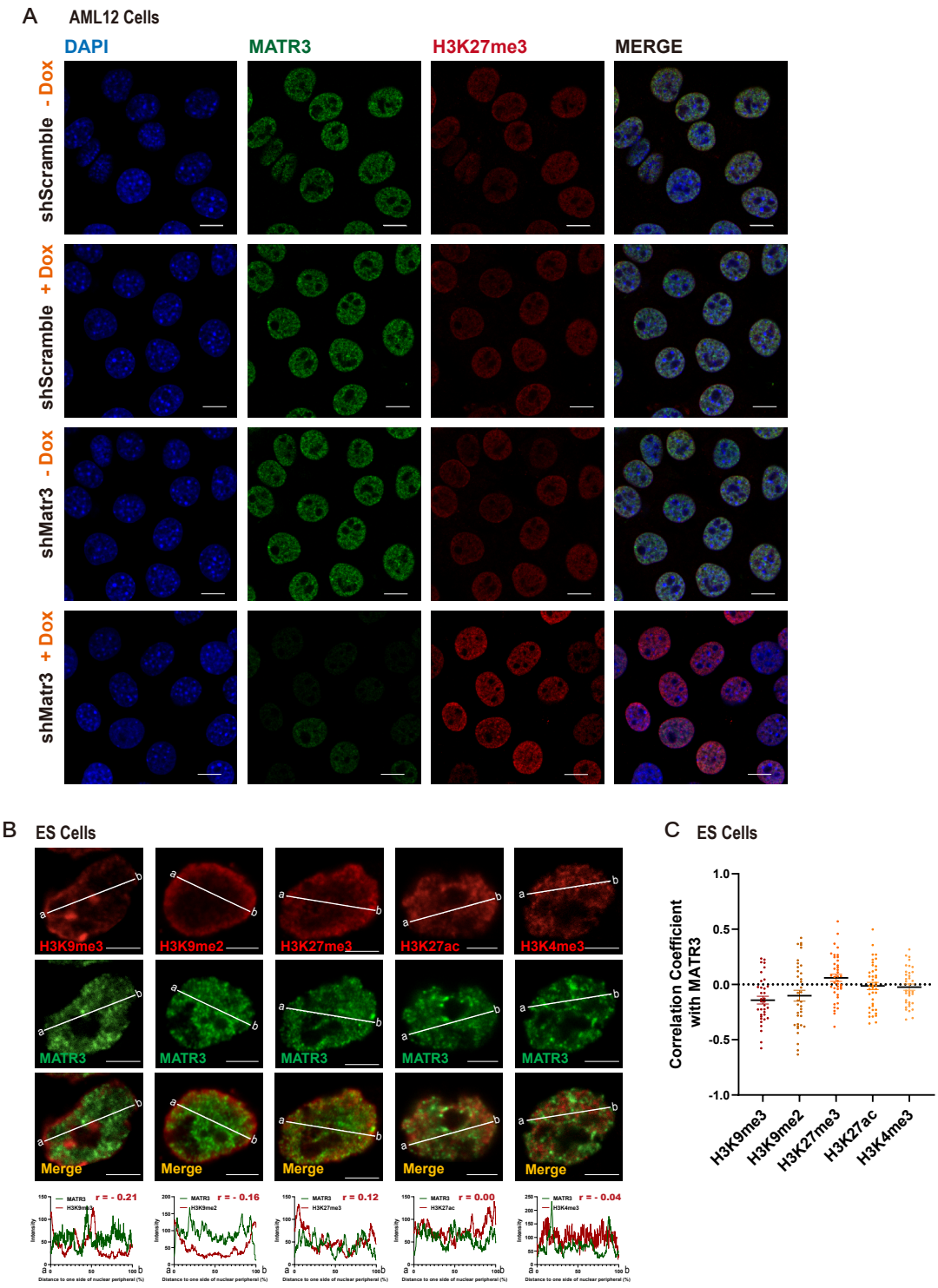
MATR3-antisense LINE1 RNA meshwork scaffolds higher-order chromatin organization

Yuwen Zhang, Xuan Cao, Zehua Gao, Xuying Ma, Qianfeng Wang, Xiaoxuan Xu, Xiumei Cai, Yan Zhang, Zhao Zhang, Gang Wei, Bo Wen

Appendix Contents

Appendix Figure S1



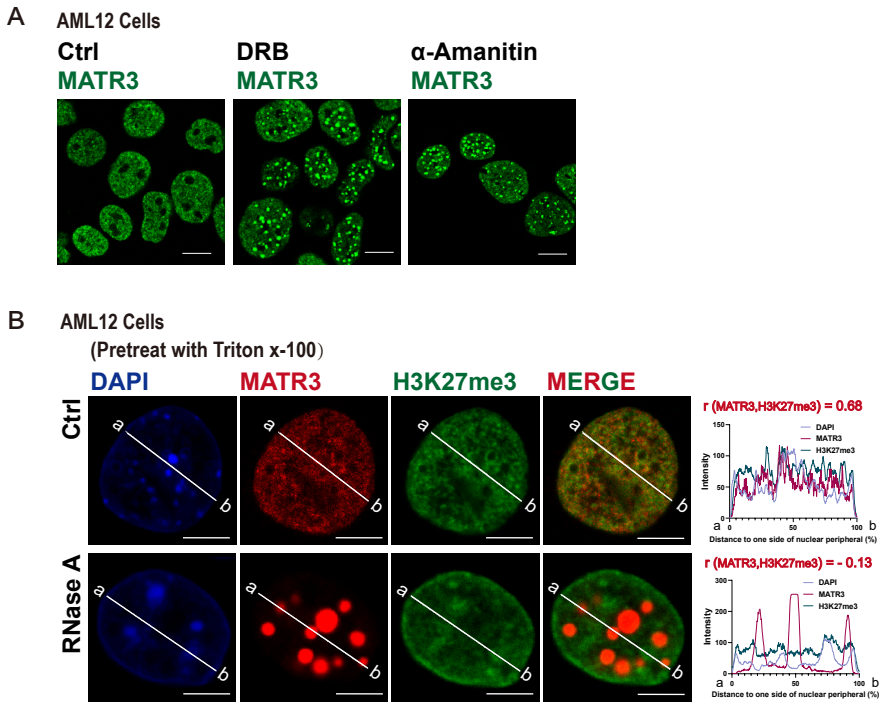
Appendix Figure S1. The correlation between MATR3 and histone modification markers.

A Representative cross-section images showing nuclear distribution of MATR3 and H3K27me3 in cells transfected with inducible-shRNA targeting to scrambled sequence (shScramble) or *Matr3* gene (shMatr3) before (-Dox) and after (＋Dox) 72h treating with 1μg/ml Dox. Scale bars, 10μm.

B (Upper) Representative cross-section images showing relative distribution between MATR3 and histone modifications (H3K9me3, H3K9me2, H3K27me3, H3K27ac and H3K4me3) in ES cells. (Lower) Line charts showing pixel intensity of each channel on the regions of interest (ROI). r, coefficient of correlation. Scale bars, 5μm.

C Coefficient of correlation between MATR3 and histone modification H3K9me3 (n=35), H3K9me2 (n=36), H3K27me3 (n=40), H3K27ac (n=40) and H3K4me3 (n=36) in ES cells. Quantifications were performed on randomly selected ROIs in cell nuclei. Each point represents one cell.

Appendix Figure S2



Appendix Figure S2. RNAs help maintaining the meshwork structure of MATR3 proteins in nuclei.

A The representative cross-section image showing nuclear distribution of MATR3 after 24h treating with 75μM DRB or 24h treating with 50μg/mL α-amanitin in AML12 cells. Scale bars, 10μm.

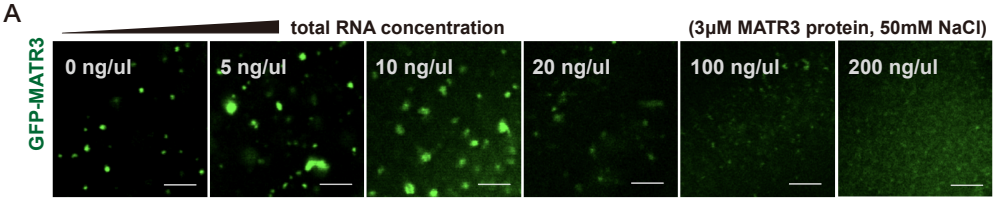
B (Left) The representative cross-section image showing nuclear distribution of DAPI, MATR3 and H3K27me3 before and after RNase A treatment (pretreat with 0.05% Triton x-100 for 30s, followed by 10μg/ml RNase A for 1h) in AML12 cells. Ctrl cells were treated with 0.05% Triton x-100 for 30s. (Right) Line charts showing pixel intensity of each channel on the ROIs. r, coefficient of correlation. Scale bars, 5μm.

C The representative cross-section image showing nuclear distribution of GFP-tagged MATR3-WT, MATR3-△RRM1 and MATR3-△RRM2. Scale bars, 5μm.

D and **E** Heatmap of MATR3 RIP-seq sense and antisense signal in repetitive elements in AML12 cells (**D**) and ES cells (**E**). All RE copies with the RIP (MATR3 -IgG) count number >= 10 are kept. For each RE family, RE copies from antisense and sense of two replicates are merged. Then compute the RPM of RIP (MATR3 -IgG) signal for RE copies and compute the mean RPM of each sample. The color indicates the mean density of RIP (MATR3 -IgG) for each RE family.

Appendix Figure S3

A



B

Sense L1 RNA derived from 3' truncation of L1 Md_F2 ORF2 element (0 MATR3-binding motifs)

AAAAGCACUCUGGAAAUCAGUCUGGCAGUUCCUCAGAAAAUUGGACA
UACUACUACUGGAGGAUCCCGCAAUACCUCUCCUGGGCAUAUCCAGA
AGAUUUCCCAACCGGUAAGAAGGACCCAUGCUCCACUAUGUUCAUAG
UAGCCUUGUUUAUAAUAGCCAGAAGCUGGAAAGAACCCAGAUGCGCC
UCAACAGAGGAAUGGAUACAGAAAAUGUGGUACAUUUACACAAUGGU
AUACUACUCAGCUAUUUUAAAAAAAUGUAUUUAUGAAAUUCCUAGGCA
AAUGGAUGGACCUGGAGGGUAUCAUCCUGAGUGAG

Antisense L1 RNA derived from 3' truncation of L1 Md_F2 ORF2 element (4 MATR3-binding motifs)

CUCACUCAGGAUGAUACCCUCCAGGUCCAUCCAUUUGCCUAGGAAUU
UCAUAAAUACAUUUUUUUAAAAUAGCUGAGUAGUAUACCAUUGUGUAA
AUGUACCACAUUUUCUGUAUCCAUUCCUCUGUUGAGGCGCAUCUGGG
UUCUUUCCAGCUUCUGGCUAUUAUAAACAAGGCUACUAUGAACAUAGU
GGAGCAUGGGUCCUUCUUACCGGUUGGGAAAUCUUCUGGAUAUGCCC
AGGAGAGGUAUUGCGGGAUCCUCCAGUAGUAGUAUGUCCAAUUUUCU
GAGGAACUGCCAGACUGAUUUCCAGAGUGCUUUU

Sense B1 RNA (0 MATR3-binding motifs)

GCCGGGCAUGGGUGGCGCACGCCUUUAAUCCCAGCACUUGGGAGGCA
GAGGCAGGCGGAUUUCUGAGUUCGAGGCCAGCCUGGUCUACAAAGUG
AGUUCCAGGACAGCCAGGGCUACACAGAGAAACCCUGUCUCGA

Antisense B1 RNA (0 MATR3-binding motifs)

UCGAGACAGGGUUUCUCUGUGUAGCCCUGGCUGUCCUGGAACUCACU
UUGUAGACCAGGCUGGCCUCGAACUCAGAAAUCCGCCUGCCUCUGCC
UCCCAAGUGCUGGGAUUAAAGGCGUGCGCCACCCAUGCCCGGC

Sense MajSAT RNA (0 MATR3-binding motifs)

GGACCUGGAAUAUGGCGAGAAAACUGAAAAUCACGGAAAAUGAGAAAU
ACACACUUUAGGACGUGAAAUAUGGCGAGGAAAACUGAAAAAGGUGGA
AAAUUUAGAAAUGUCCACUGUAGGUCGUGGAAUAUGGCAAGAAAACUG
AAAAUCAUGGAAAAUGAGAAACAUCCACUUGACGACUUGAAAAAUGAC
GAAAUCACUAAAAAACGUGAAAAAUGAGAAAUGCACACUGAA

Appendix Figure S3. MATR3 proteins interplay with RNAs in vitro.

A Representative images of droplet formation assays with 3 μM GFP-MATR3 proteins and different concentration of total RNAs. NaCl concentration, 50mM.

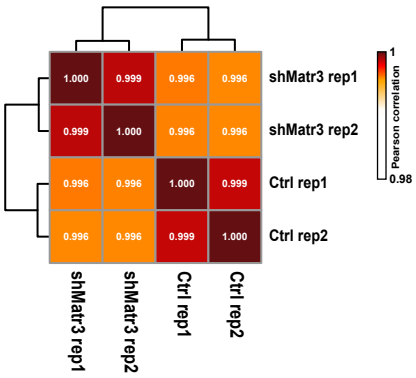
B Sequences of sense L1 RNAs, antisense L1 RNAs, sense B1 RNAs, antisense B1 RNAs and sense MajSAT RNAs used in droplet formation assays. The 7-mer MATR3-binding motifs are highlighted.

Appendix Figure S4

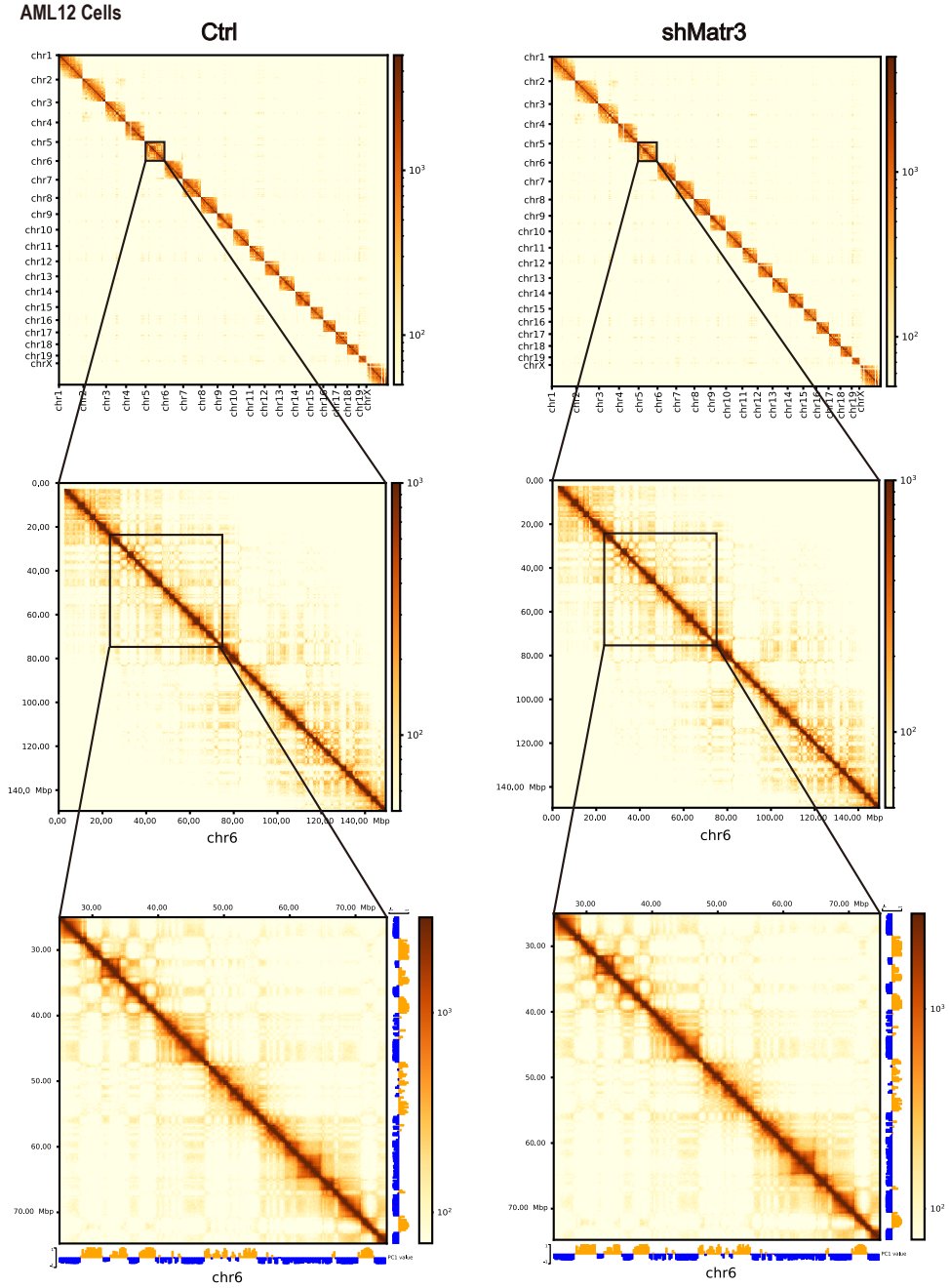
A AML12 Cells

	Ctrl rep1	Ctrl rep2	shMatr3 rep1	shMatr3 rep2
Total reads	595,419,066	611,165,131	601,429,299	617,926,024
Mapped R1	554,454,338	569,274,150	563,446,803	579,826,895
Mapped R2	529,893,326	532,821,932	524,714,852	555,806,207
Unique pairs	323,705,058	323,766,223	323,563,215	340,495,760
Dangling ends pairs	6,426,405	8,405,944	6,603,828	5,462,148
Self circle pairs	141,066	124,164	124,614	146,389
Dumped pairs	5,217	5,780	8,317	5,989
Valid pairs	311,080,734	308,726,195	310,825,335	329,239,157
Unique valid pairs	207,957,571	208,370,315	201,305,924	234,105,937
Cis interaction	171,414,301	172,223,273	164,160,012	190,299,049
Trans interaction	36,543,270	36,147,042	37,145,912	43,806,888
Contacts>20k	148,113,808	148,178,911	141,917,458	164,786,892

B AML12 Cells



C AML12 Cells



Appendix Figure S4. The overall view of Hi-C datasets.

A Mapping statistics of Hi-C sequencing data of two replicates in Ctrl and shMatr3 AML12 cells.

B Pearson correlation coefficients of PC1 values at 250 kb resolution between replicates.

C Hi-C contact maps in Ctrl and shMatr3: whole genome at 1MB resolution (top); Chr6 at 250kb resolution (middle); chr6:27-73 Mb at 250kb resolution (down).

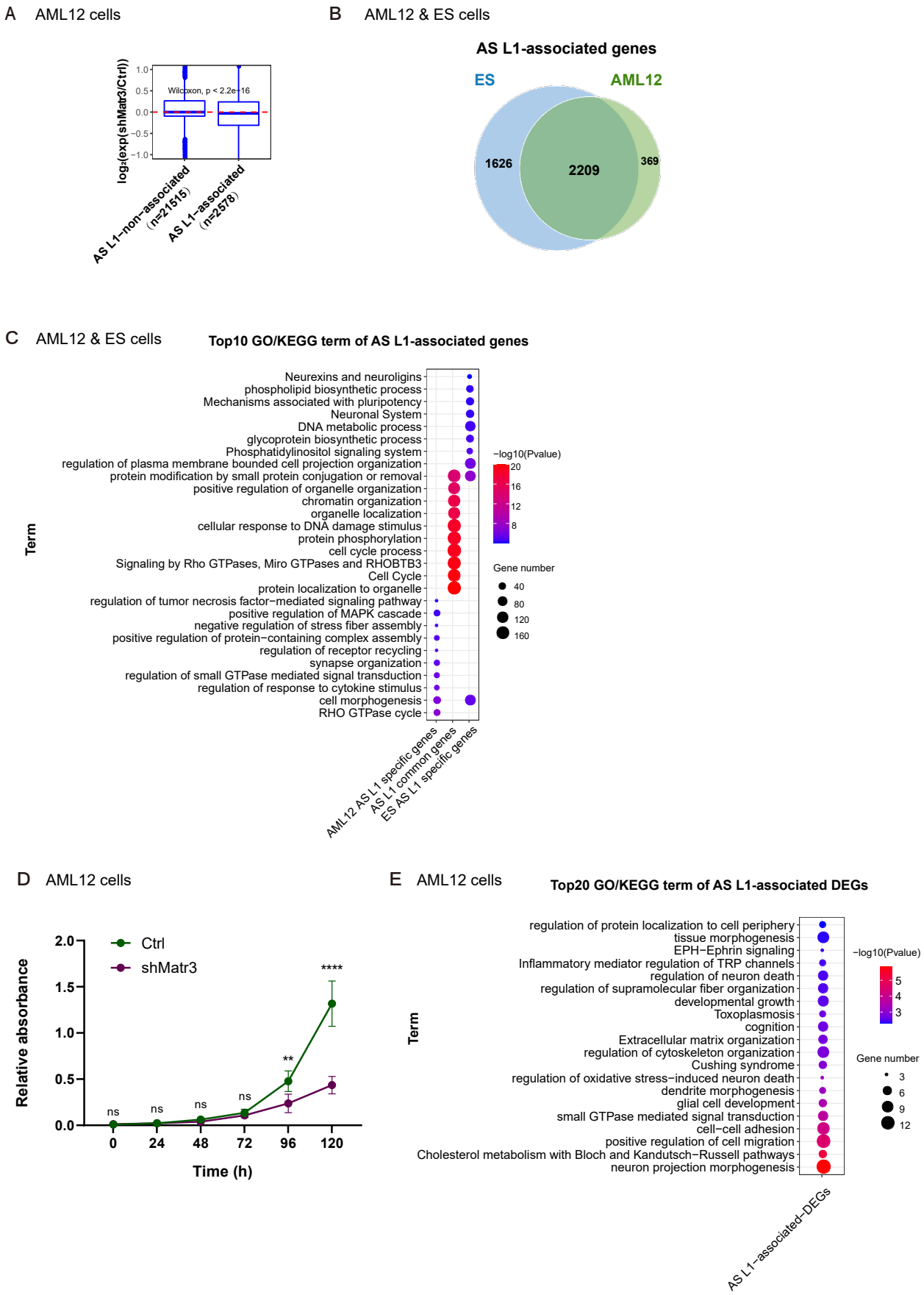
Appendix Figure S5

ES Cells

	Ctrl rep1	Ctrl rep2	MATR3-AID rep1	MATR3-AID rep2
Total reads	428,727,232	459,542,741	436,359,212	461,177,436
Mapped R1	393,627,743	432,545,672	402,625,219	425,730,135
Mapped R2	361,654,965	388,843,588	365,601,945	390,736,864
Unique pairs	216,540,949	235,260,893	224,293,542	238,756,442
Dangling ends pairs	3,160,315	3,909,828	4,964,669	4,360,881
Self circle pairs	100,579	102,052	99,640	117,170
Dumped pairs	4,424	4,003	3,786	4,162
Valid pairs	210,093,154	228,351,131	215,776,060	230,817,355
Unique valid pairs	157,272,810	180,497,484	169,680,300	178,880,239
Cis interaction	127,341,407	141,054,726	136,915,485	144,208,459
Trans interaction	29,931,403	39,442,758	32,764,815	34,671,780
Contacts>20k	112,570,546	126,060,303	121,273,440	127,885,199

Appendix Figure S5. Mapping statistics of Hi-C sequencing data of two replicates in Ctrl and MATR3-AID ES cells.

Appendix Figure S6



Appendix Figure S6. Functional relevance of MATR3-AS L1 RNA associated genes

A Box plot shows gene expression changes in MATR3-AS L1 RNAs non-associated TADs and associated TADs.

B Genes that enriched with MATR3-AS L1 RNAs overlap between ESC and AML12 cells.

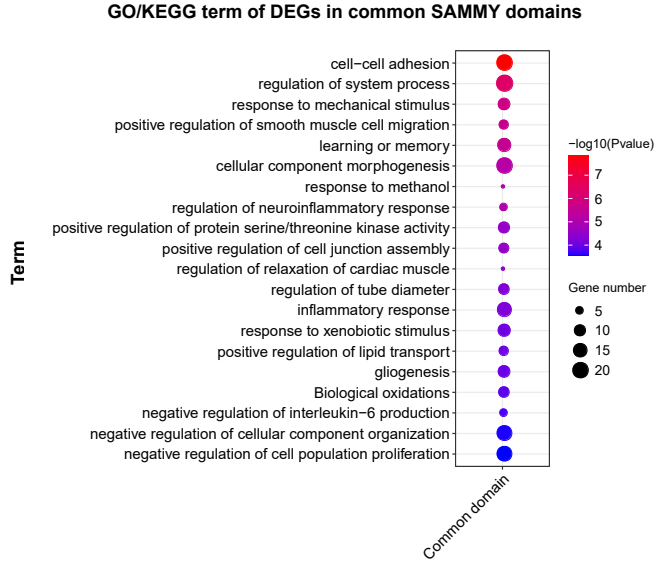
C Top enriched GO/KEGG terms for genes from groups in **B**. Bubble colours represent the corrected P value. Bubble sizes indicate the number of of genes involved in each term.

D Viability of AML12 cells before and after MATR3 knockdown as detected by CCK-8 assay (n = 7). The P values were calculated using unpaired two-tailed Student's t test; *$p<0.05$, ****$p<0.0001$. Error bars indicate mean ± s.e.m.

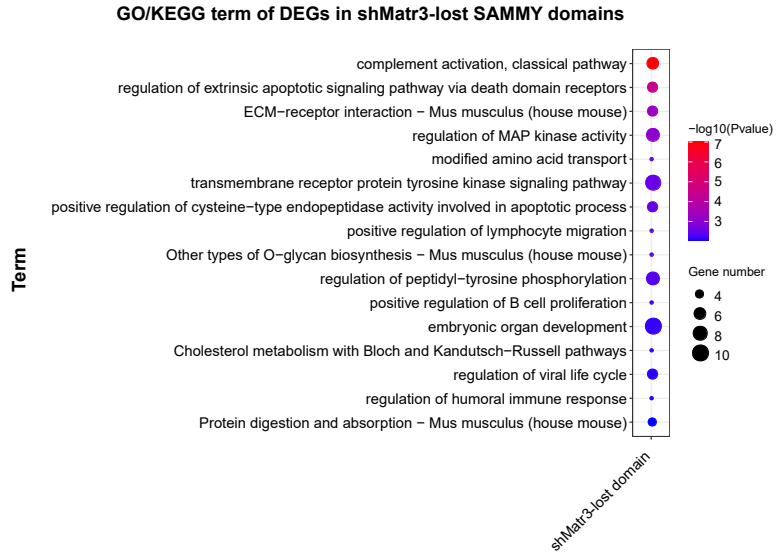
E Top enriched GO/KEGG terms for AS L1- associated DEGs (Ctrl vs shMatr3) from AML12 cells. Bubble colours represent the corrected P value. Bubble sizes indicate the number of of genes involved in each term.

Appendix Figure S7

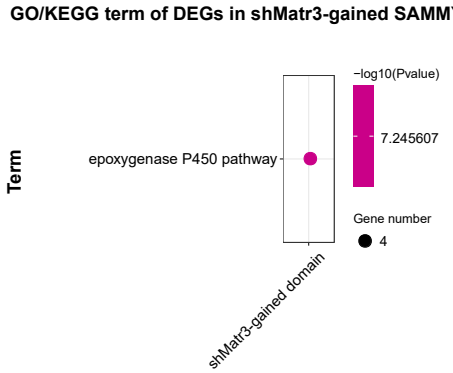
A AML12 cells



B AML12 cells



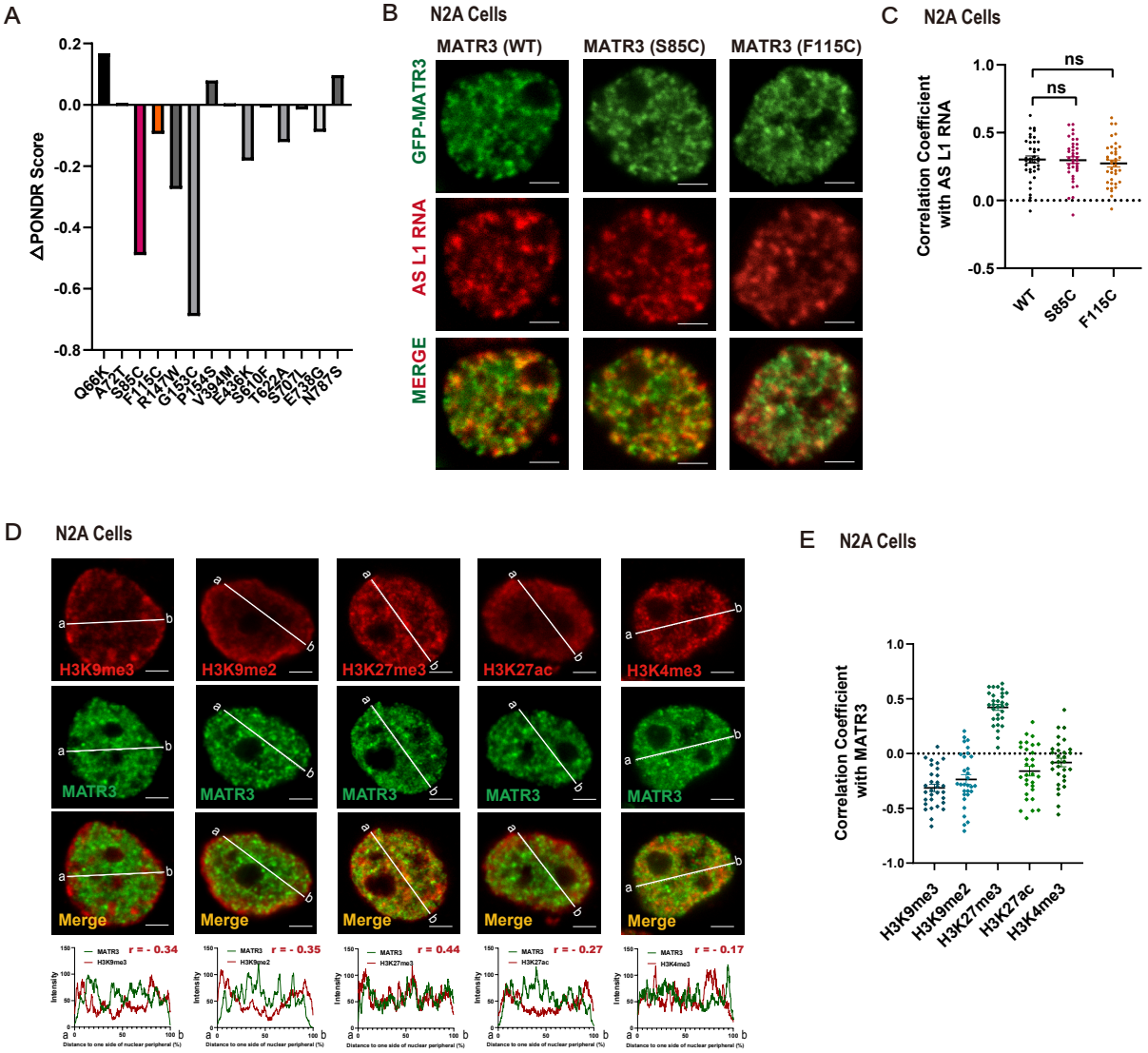
C AML12 cells



Appendix Figure S7. Biological consequences caused by the structure changes of SAMMY domains

Bubble plots showing top enriched GO/KEGG terms for DEGs from common (**A**), shMatr3-lost (**B**) and shMatr3-gained (**C**) SAMMY domains. Bubble colours represent the corrected P value. Bubble sizes indicate the number of of DEGs involved in each term.

Appendix Figure S8



Appendix Figure S8. Nuclear distribution pattern of ALS associated mutants in N2A cells.

A The difference of PONDR score at the mutation point between wild-type MATR3 and degenerative-disease-associated MATR3 mutants.

B The representative images showing nuclear colocalization of AS L1 RNAs with wild-type (WT) and mutant (S85C/F115C) GFP-MATR3 proteins in N2A cells. Scales bar, 5μm.

C Coefficient of correlation between AS L1 RNA with wild-type and mutant GFP-MATR3 proteins. WT (n=38), S85C (n=38), F115C (n=38). The P values were calculated using unpaired two-tailed Student's t test; ns, not significant. Error bars indicate mean ± s.e.m.

D (Upper) Representative cross-section images showing relative distribution between MATR3 and histone modifications (H3K9me3, H3K9me2, H3K27me3, H3K27ac and H3K4me3) in N2A cells. (Lower) Line charts showing pixel intensity of each channel on the regions of interest (ROI). r, coefficient of correlation. Scales bar, 5μm.

E Coefficient of correlation between MATR3 and histone modification H3K9me3 (n=30), H3K9me2 (n=30), H3K27me3 (n=30), H3K27ac (n=30) and H3K4me3 (n=30) in N2A cells. Quantifications were performed on randomly selected ROIs in cell nuclei. Each point represents one cell. Error bars indicate mean ± s.e.m.