Expanded View Figures

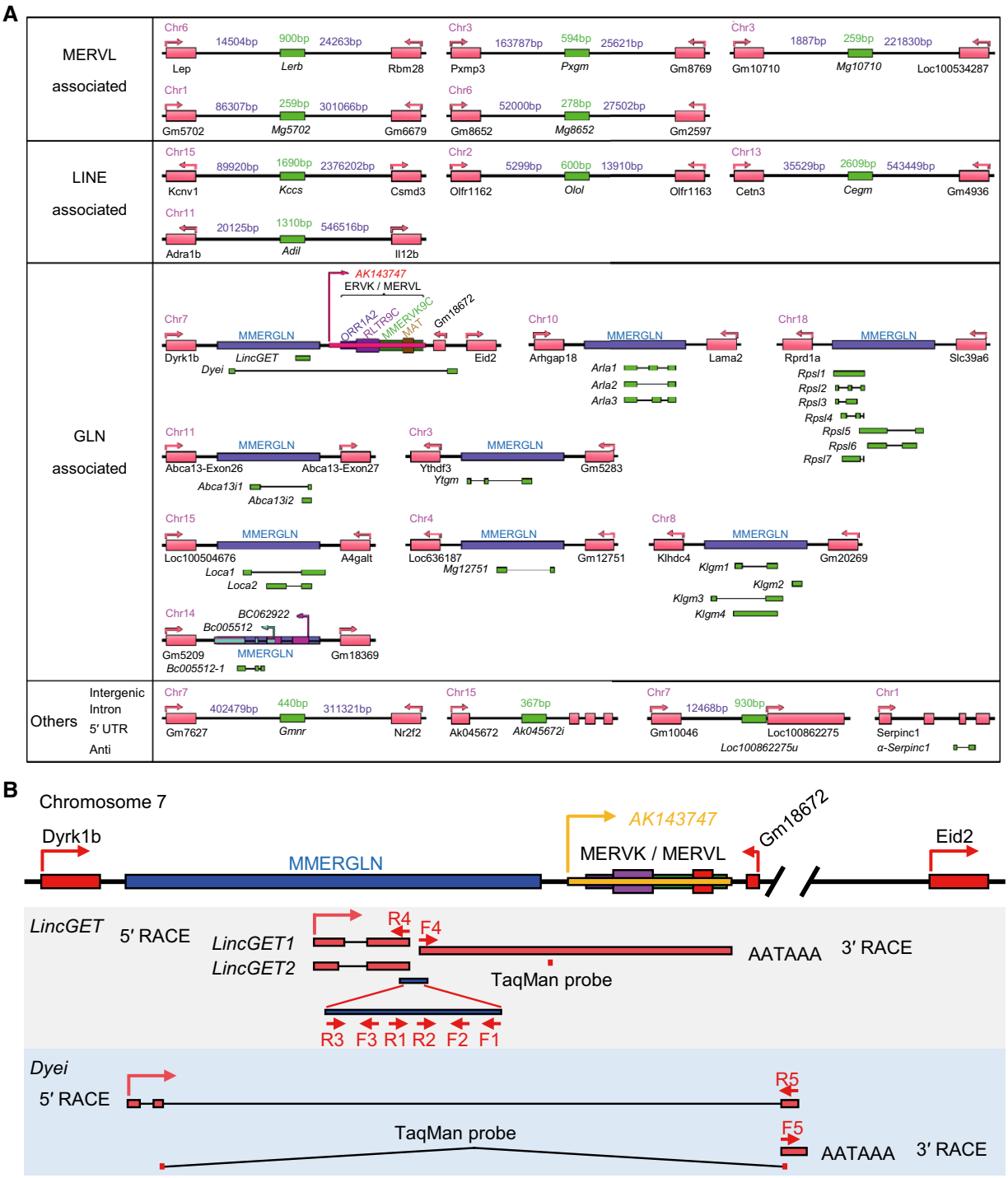

Figure EV1. *LincGET* and *Dyei* are ERV-associated transcripts.

A Gene loci of 36 novel transcripts in mouse preimplantation embryos (GenBank accession numbers are shown in Table EV2). Twenty-three are GLN-associated, 5 are MERVL-associated, 4 are LINE-associated, 1 is in the intergenic region between Gm7627 and NR2F2, 1 is located in the intron of Ak045672, 1 is 5′ UTR-associated, and 1 is an antisense transcript of *Serpinc1*.

B Gene locus of *LincGET* and *Dyei*. Primers and TaqMan probes are shown. RACE, rapid amplification of cDNA end. AATAAA is the polyadenylated signal site.

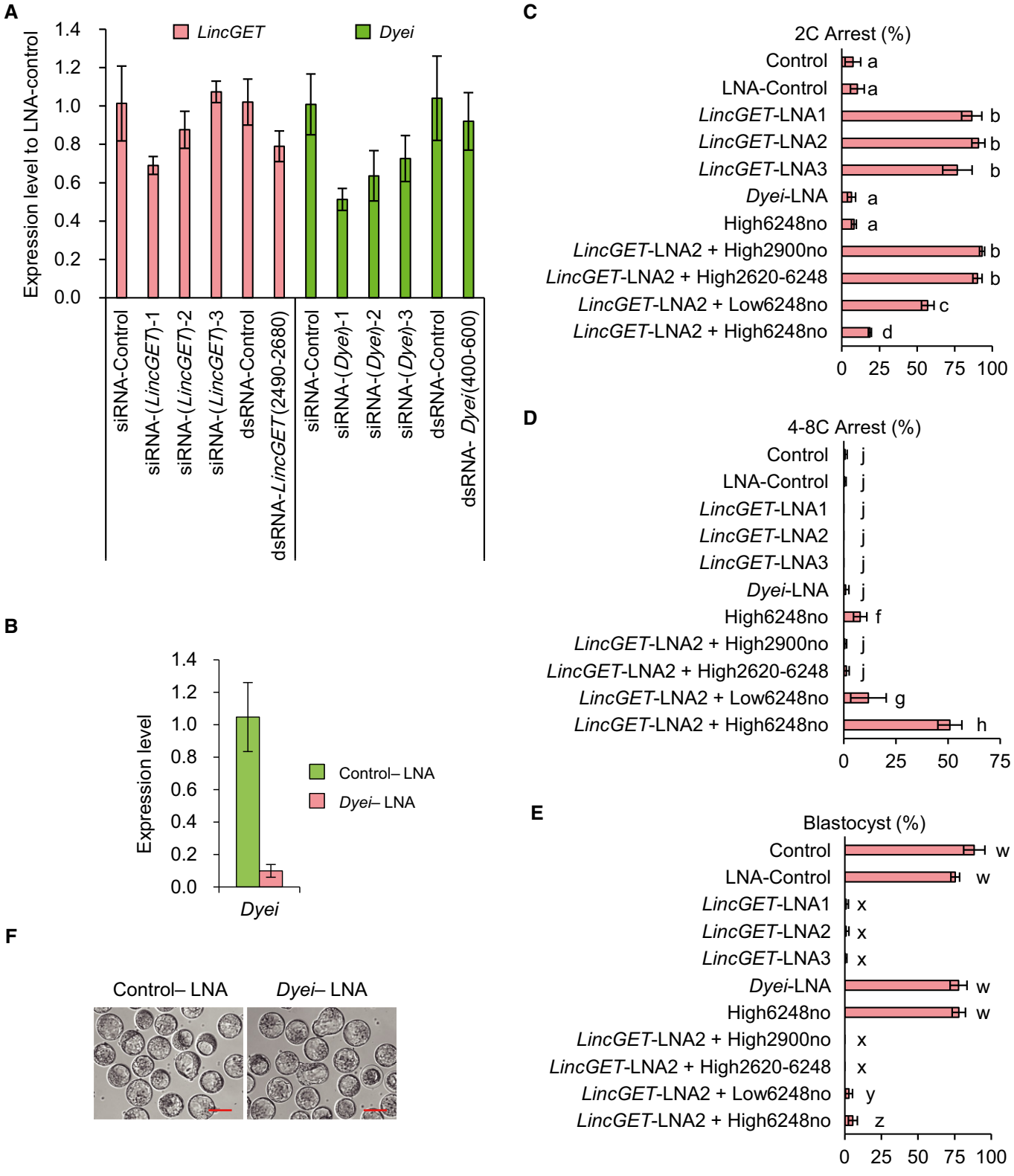

Figure EV2.

Figure EV2. *LincGET* depletion results in developmental arrest at the late G2 phase of two-cell stage, while *Dyei* depletion has no effect on the preimplantation development.

A siRNA or dsRNA do not efficiently interfere with nuclear *LincGET* and *Dyei*. siRNA or dsRNA was injected at phCG 25 h, and embryos were collected at phCG 48 h at the late two-cell stage for TM-qPCR analysis. The error bars represent s.e.m. Three experimental replicates were performed, and about 50 embryos were used for each time.

B *Dyei*-LNA can efficiently deplete nuclear *Dyei*. LNA was injected at phCG 25 h, and embryos were collected at phCG 48 h at the late two-cell stage for TM-qPCR analysis. The error bars represent s.e.m. Three experimental replicates were performed, and about 50 embryos were used for each time.

C–E *LincGET* depletion leads to developmental arrest at the late G2 phase of two-cell stage and the co-injection of full-length of *LincGET1* lacking *LincGET*-LNA2 target site, but not that of partial sequences can partially rescue the embryonic development. *Dyei* depletion has no effect on the preimplantation development. 2C, two-cell stage; 4–8C, four- to eight-cell stage; BL, blastocyst stage; 6,248no, full-length *LincGET*1 with LNA target sites mutation; 2,900no, 1–2,900 nt of *LincGET1* with LNA target sites mutation; 2,620–6,248, 2,620–6,248 nt of *LincGET*1. High, 400 ng/μl; low, 150 ng/μl. Different letters in same panel indicate significant difference ($P < 0.001$).

F Injection of *Dyei*-LNA has no significant impact on preimplantation development. LNA was injected at phCG 25 h, and photographs were taken at phCG 114 h at the late blastocyst stage. Scale bar, 50 μm.

Figure EV3. *LincGET* depletion results in developmental arrest at the late G2 phase of two-cell stage with no effect on ZGA initiation and pericentric rings reorganization.

A *LincGET* depletion results in developmental arrest at the G2 phase of two-cell stage without affecting DNA integrity and replication. We used BrdU to visualize S and G2 phases, CAF-1 to visualize S phase, and PI to visualize the M phase. Cyclin B1 and histone H3 serine 10 phosphorylation (H3S10ph) are markers of the G2 stage, and H2A.X is a marker of DNA damage. Aphidicolin-treated embryos arrested at S phase without DNA replication. LNA was injected at phCG 25 h, and embryos were collected at phCG 48 h at late two-cell stage for IF analysis. Scale bar, 50 μm. Three experimental replicates were performed, and about 15 embryos were used in each group.

B Embryos injected with *LincGET*-LNA were arrested at the G2 phase, and no DNA replication was observed. Results showed that embryos injected with control-LNA were BrdU positive and reached the four-cell stage, while embryos injected with *LincGET*-LNA were BrdU negative and arrested at the two-cell stage. BrdU was added at the late two-cell stage (phCG 48 h) and measured at the late four-cell stage (phCG 62 h). Scale bar, 50 μm. Three experimental replicates were performed, and about 15 embryos were used in each group.

C EU staining indicated the normal major ZGA process after *LincGET* depletion. EU was added to the culture medium at phCG 40 h, and EU signals were detected at phCG 48 h. Scale bar, 50 μm. Three experimental replicates were performed, and about 15 embryos were used in each group.

D DNA-FISH analysis of major transcripts shows that the pericentric domain reorganization toward chromocenters is not affected by *LincGET* depletion. Scale bar, 50 μm. Three experimental replicates were performed, and about 15 embryos were used in each group.

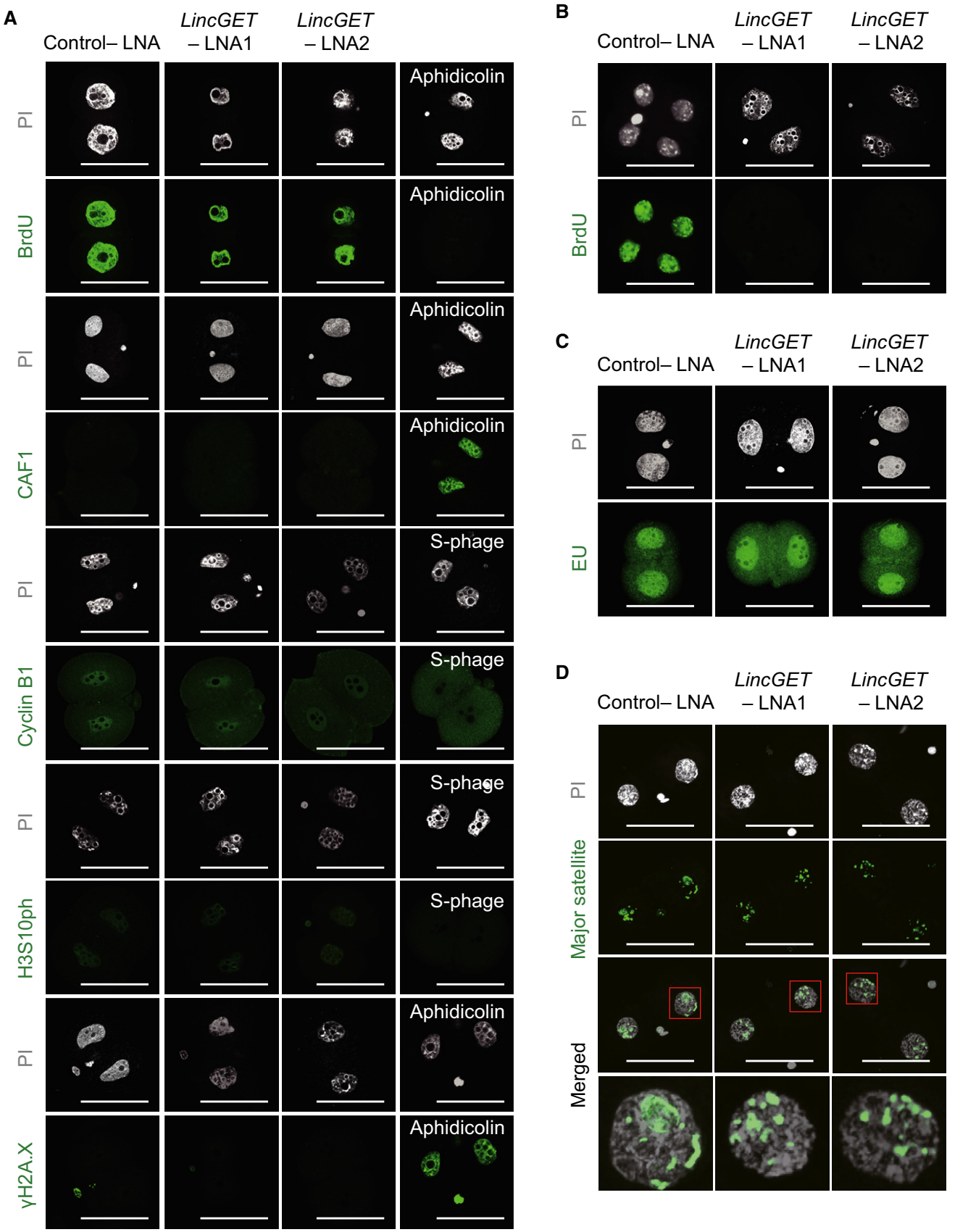
A
Control– LNA
LincGET – LNA1
LincGET – LNA2
PI
Aphidicolin
BrdU
CAF1
S-phage
Cyclin B1
H3S10ph
γH2A.X
B
C
EU
D
Major satellite
Merged

Figure EV3.

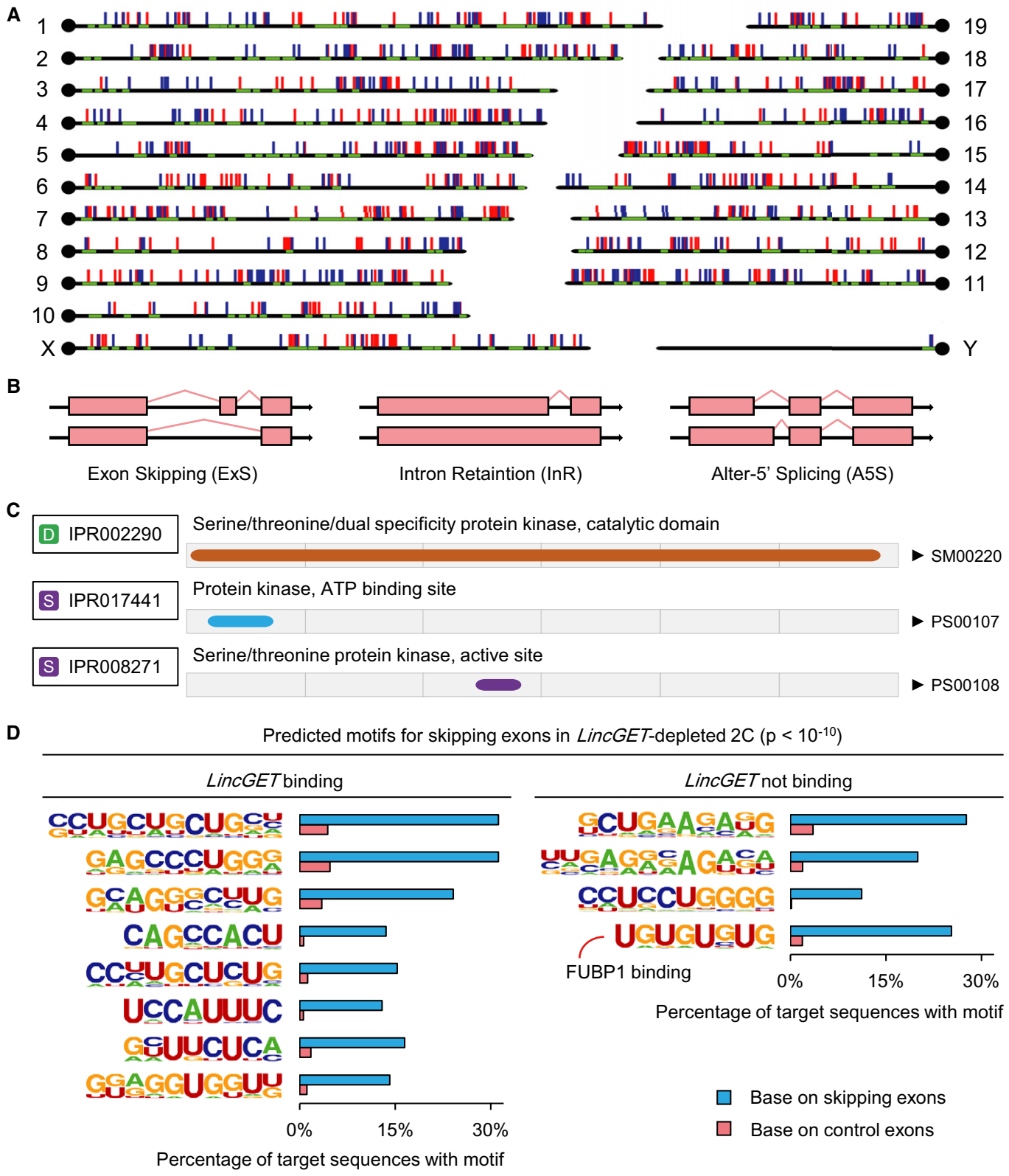

Figure EV4.

Figure EV4. Some features of DEGs and splicing events in *LincGET*-depleted 2C.

A Loci of DEGs and GLKLTRs. Red lines represent upregulated genes; blue lines represent downregulated genes; green blots represent GLKLTRs.
B Three types of alternative splicing events were observed and different between control-LNA L2C and *LincGET*-depleted 2C.
C *CDK1* protein domain prediction by InterPro (http://www.ebi.ac.uk/interpro/). An ATP binding domain is located at the N-terminus.
D Predicted motifs for skipping exons in *LincGET*-depleted 2C. We analyzed the sequences of skipping exons in *LincGET*-depleted 2C with flanked 200-bp intron sequences, and 12 motifs that were enriched in these skipping exons relative to random control exons ($P < 10^{-10}$) were detected. Eight of these 12 motifs (left) are affiliated to *LincGET*. One of the other 4 *LincGET*, which are not binding motifs (right, the UGUGUGUG motif), is the FUBP1 targeting sequence in Dmd intron 38 (UUGUGUGUGU) required for exon 39 skipping splicing.

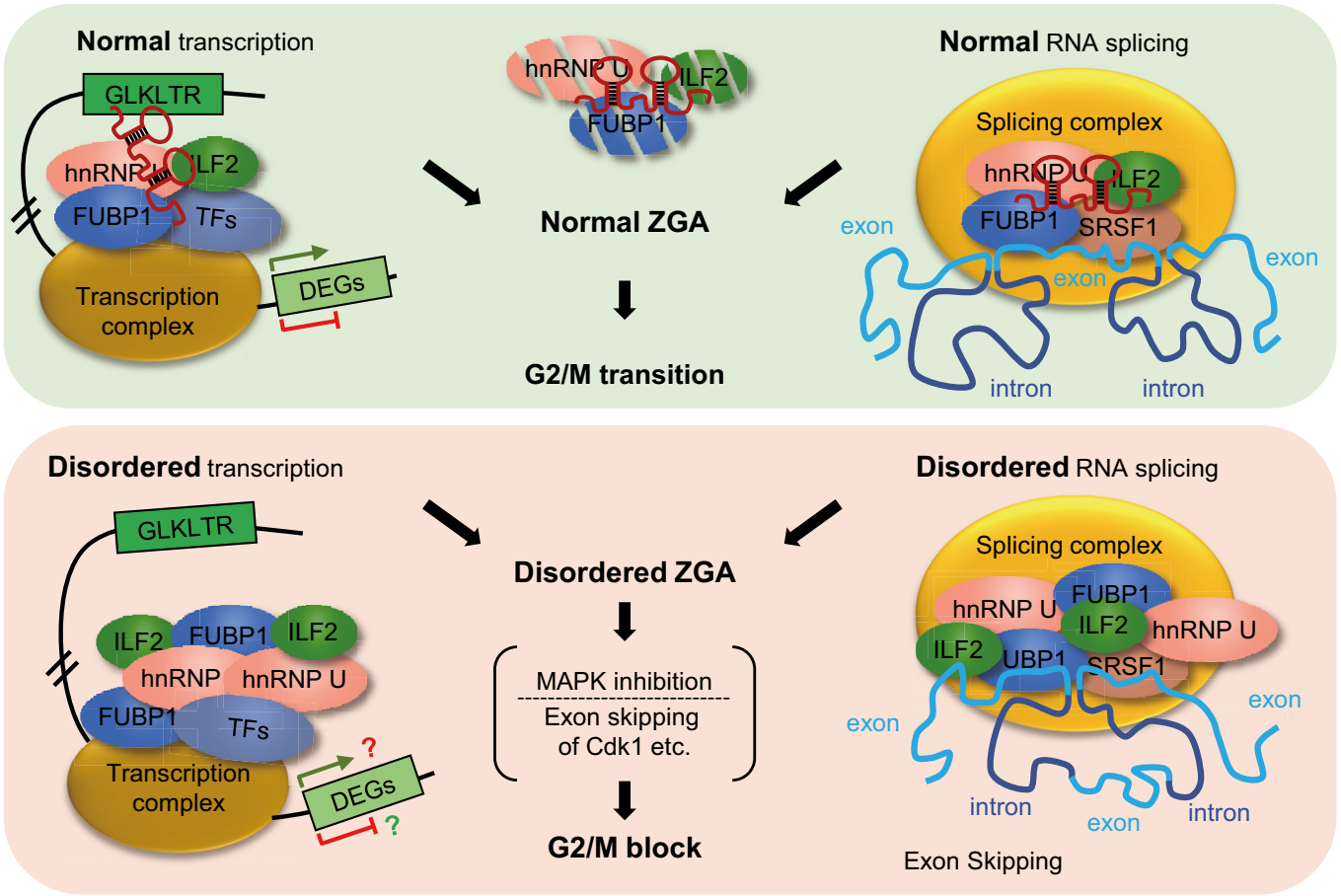

Figure EV5. Model representing *LincGET* function.

In normal two-cell mouse embryos, *LincGET* works as a transcription factor, mediating the *cis*-regulatory activity of GLKLTRs, and an inhibitor of exon skipping, partially through decreasing hnRNP U, FUBP1, and ILF2 protein levels (up). However, when *LincGET* is depleted, disordered transcription and disordered RNA splicing would result in disordered ZGA, MAPK signaling inhibition, and exon skipping of some M-phase-associated genes, including *Cdk1*, leading to G2/M block (down).