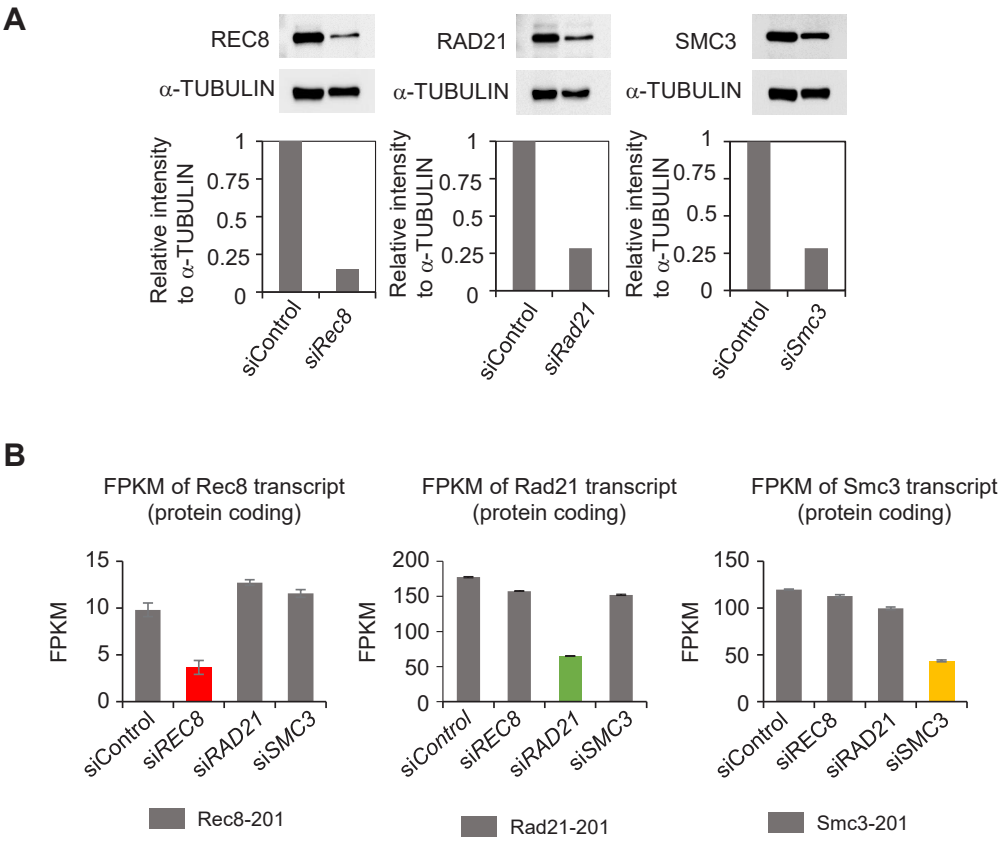
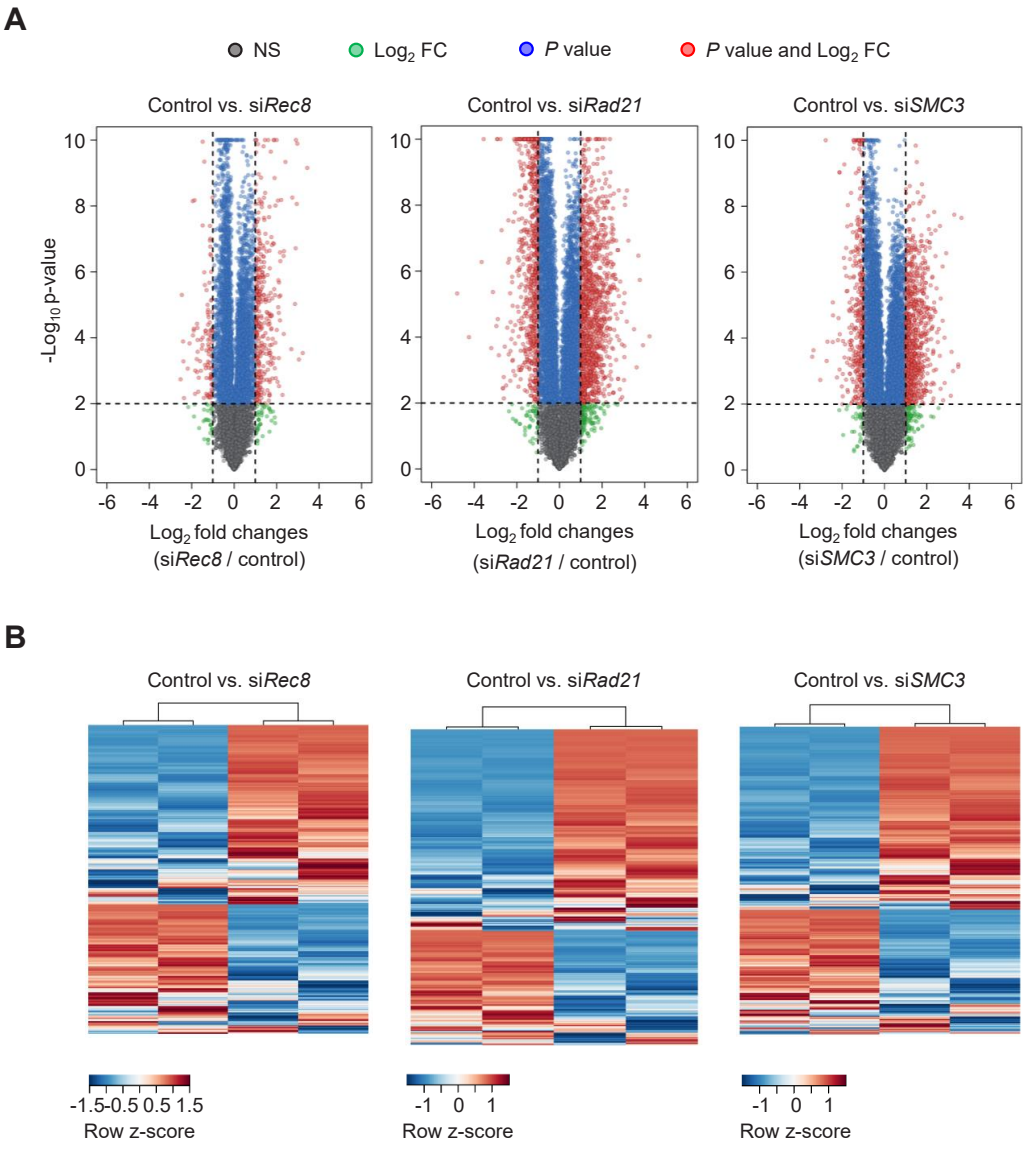
Supplementary Table S1. List of primers used in this study

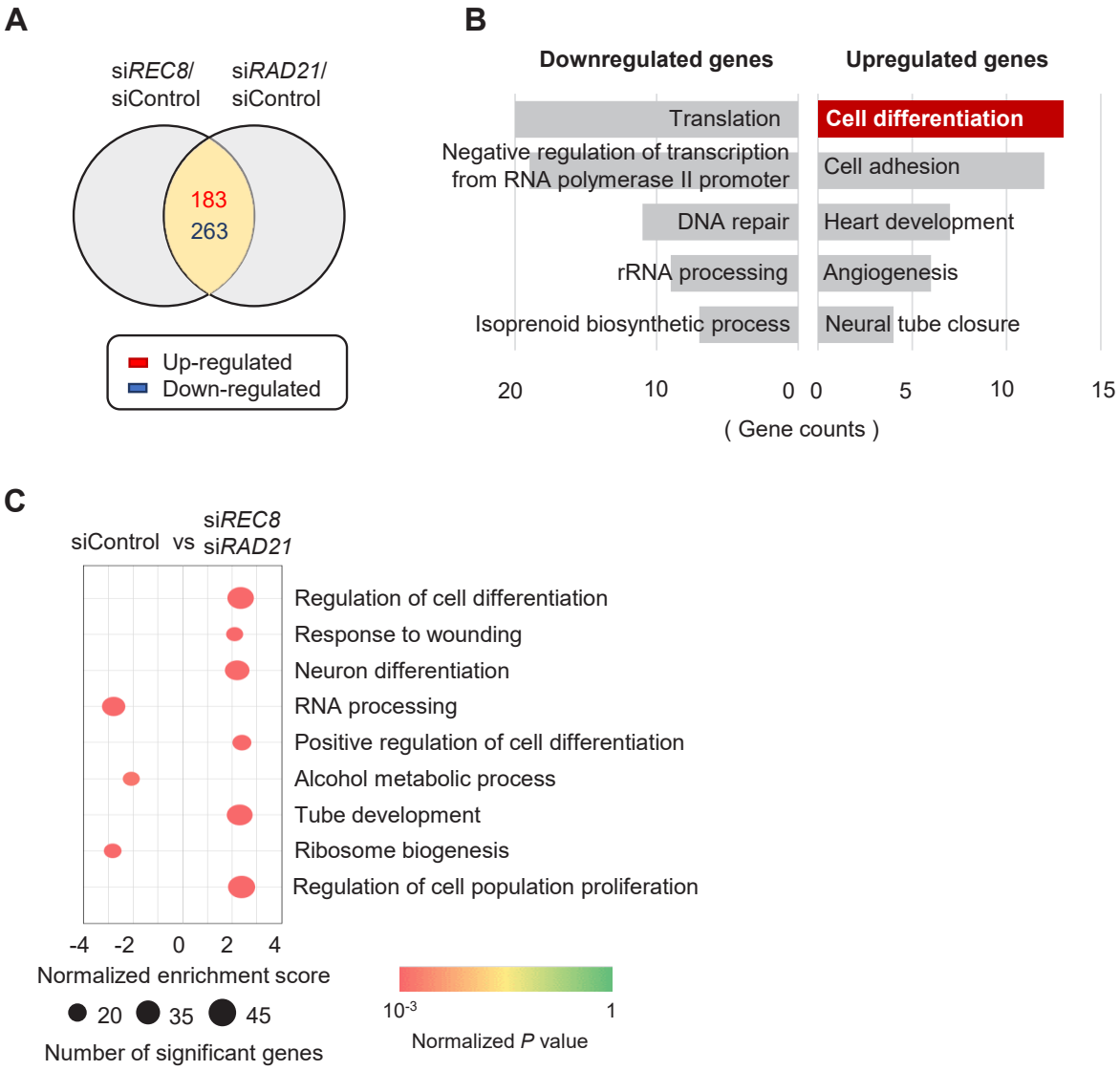
Genes	Primer sequence
OCT3/4	Forward: 5'-TCTTTCCACCAGGCCCCCG-3'
	Reverse: 5'-GGCGGACATGGGGAGATCC-3'
NANOG	Forward: 5'-CAGGAGTTTGAGGGTAGCTC-3'
	Reverse: 5'-CGGTTCATCATGGTACAGTC-3'
KLF4	Forward: 5'-TGGGGGTTTTGGTTTGAGGT-3'
	Reverse: 5'-CCAGGTGGCTGCCTCATTA-3'
SOX2	Forward: 5'-GCGGAGTGGAAACTTTTGTCC-3'
	Reverse: 5'-GGGAAGCGTGTACTTATCCTTCT-3'
ENG	Forward: 5'-GCACCTTGTCCCAGGAAGTC-3'
	Reverse: 5'-GGAGGCTTGGGATACTCACG-3'
GATA4	Forward: 5'-GTAAGTGACCAACTGCTCGTGAAT-3'
	Reverse: 5'-TCCTCTGCATCCTCACTATCACA-3'
NESTIN	Forward: 5'-TCCCGACTTCCCTTCACCATAC-3'
	Reverse: 5'-CCTGCGACAAGGGCTTGTTTAG-3'
NEUROD1	Forward: 5'-AACCTTTTAACAACAGGAAGTGGA-3'
	Reverse: 5'-CTCATCTGTCCAGCTTGGGG-3'
DDX4	Forward: 5'-GGAGCGGAGAGGAACCTGA-3'
	Reverse: 5'-TCCAGAAGGCCCATCTTCCA-3'
STRA8	Forward: 5'-ATATCACAGCCTCAAAGTGGCA-3'
	Reverse: 5'-GACCTCCTCTAAGCTGTTGGG-3'
18s rRNA	Forward: 5'-GTAACCCGTTGAACCCCATT-3'
	Reverse: 5'-CCATCCAATCGGTAGTAGCG-3'



Supplementary Fig. S1. Knockdown efficiency of each cohesin subunit in ESCs. (A) Western blot analysis of cohesin knockdown efficiency in ESC line. Protein levels of each cohesin subunits transfected with pooled siRNAs were tested by western blot. The bands were quantified using Bio-Rad Image Lab 6.0 software. (B) RNA-Seq analysis of cohesin knockdown efficiency in ESCs. From the results of RNA sequencing, FPKM values of protein-coding transcripts were quantified. Knocking down each cohesin factor did not influence the expression levels of the other.



Supplementary Fig. S2. Gene expression profile data in normal and cohesin knockdown conditions. (A) Volcano plot (Log_2 fold change vs $-Log_{10}$ *P* value) of genes that are differentially expressed in cohesin-knockdown conditions compared to siControl condition. Red points indicate genes that are significantly increased or decreased in the cohesin-knockdown condition by having the absolute value of Log_2 fold change > 1 and $-Log_{10}$ (*P* value). (B) A hierarchical clustering heatmap illustrating DEGs in cohesin-knockdown conditions. Mapping grids are colored according to their row *z*-scores.



Supplementary Fig. S3. Distribution of differentially expressed genes (DEGs) in ESC following knockdown of REC8 and RAD21. (A) Venn diagram of all unique and common differentially expressed genes (DEGs) that are co-regulated in si*REC8*/siControl and si*RAD21*/siControl (fold change > 1.5, *P* value < 0.05). Red: number of upregulated genes; Blue: number of downregulated genes. (B) Analyses of the DEGs performed by the DAVID database. Enriched biological themes, especially gene ontology terms, were identified in both upregulated and downregulated genes. The numbers on the x-axis indicate gene counts that are involved in each gene ontology term. (C) GSEA analysis for DEGs in REC8 and RAD21 knockdown condition. GSEA was analyzed with mRNA-seq data from two independent experiments. To adjust the data, we used normalized *P* values. The cut-off value to reject the null hypothesis was set at 0.05, which means the extreme value for the test statistic is expected to be <5% of the time. NOM *P* value, normalized *P* value.