**Supplementary Figure 1. Single nucleotide frequencies for 19-30nt-long reads obtained by XR-seq.** The enrichment of TC, CT and TT for (6-4)PP XR-seq and that of mainly TT for CPD XR-seq are at position 6 nucleotide from the 3' end of the excised oligos starting from the 28nt in length through 19nt in length. For downstream analysis, we selected 19-24nt-long reads.

Supplementary Figure 2. TT and TC dinucleotides in the transcribed and non-transcribed strands. (A) The number of TT dinucleotides in the transcribed strand is highly correlated with that in the non-transcribed strand. **(B)** The number of TC dinucleotides in the transcribed strand is highly correlated with that in the non-transcribed strand. Gene length is a good proxy for the number of TT and TC dinucleotides, and thus we use RPKM for normalization for XR-seq repair read counts.

A

B
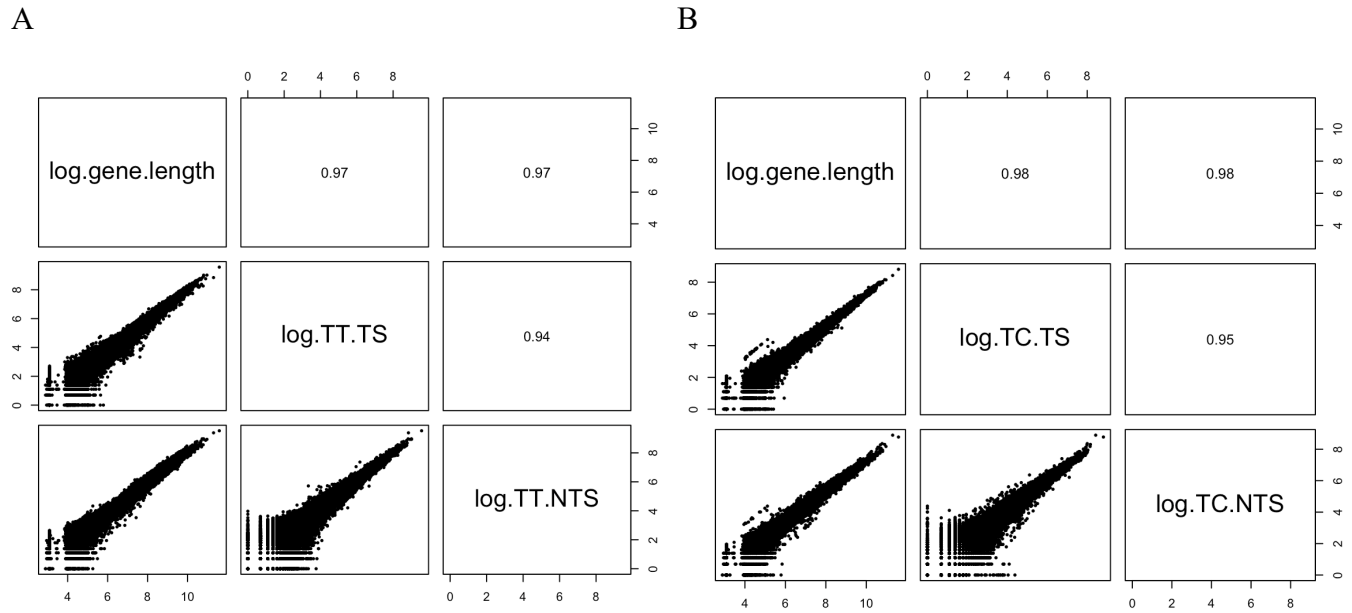
Supplementary Figure 3. High reproducibility between each pair of XR-seq replicates. Normalized gene-specific repair is shown as each dot. Spearman correlation coefficient is shown.
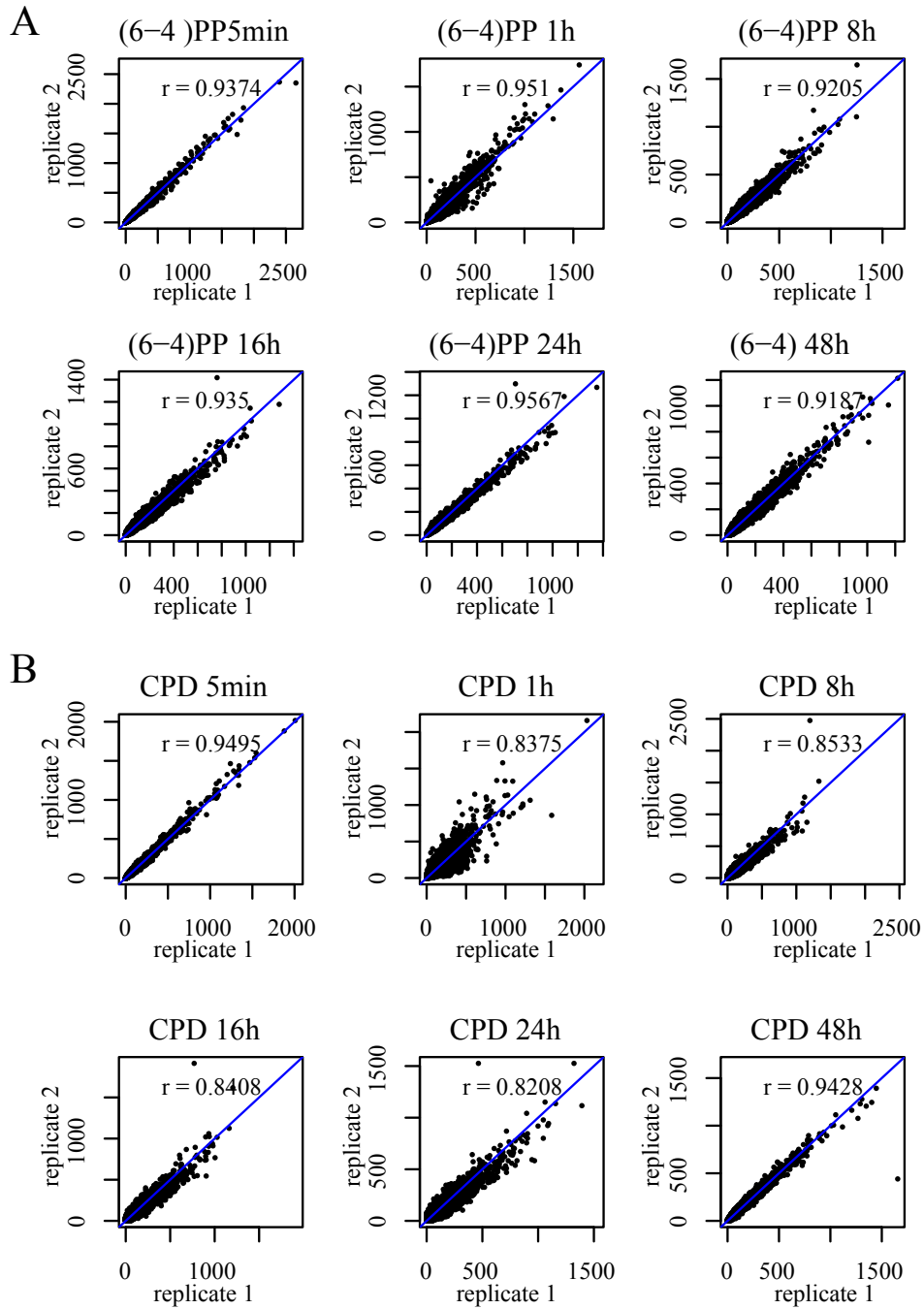
Supplementary Figure 4. Pairwise correlation of transcription-coupled repair from the transcribed strand as measured by XR-seq. Spearman correlation coefficient is calculated between each pair of the XR-seq samples using the normalized read counts. Samples from the (6-4)PP and CPD XR-seq cluster together, with temporal changes observed at different timepoints.
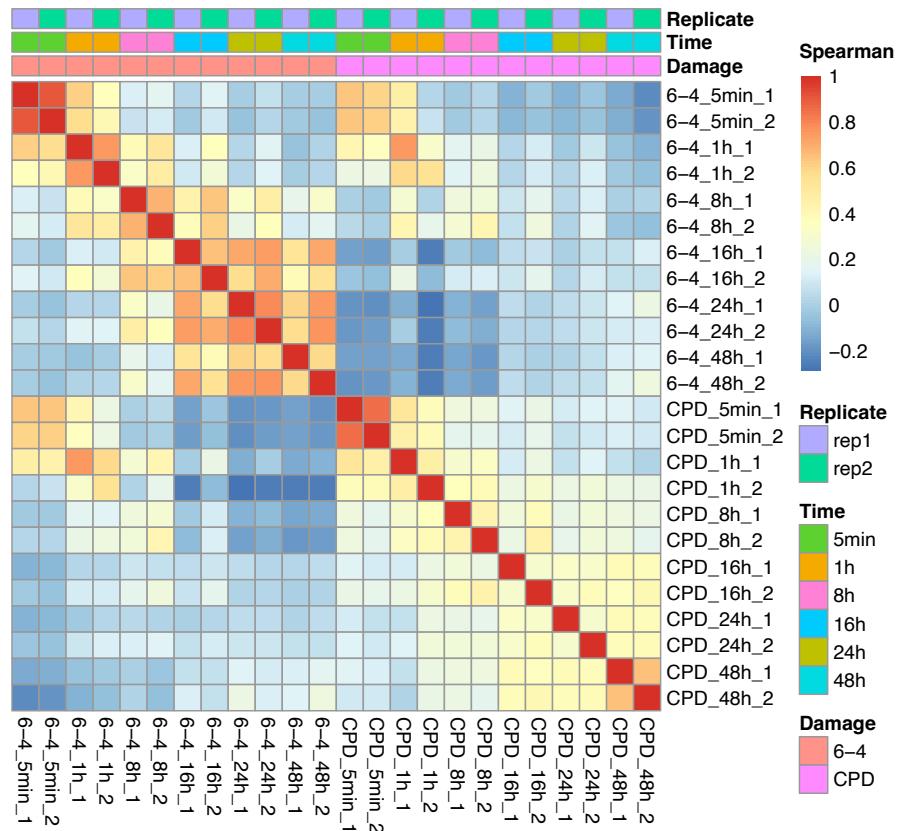
Supplementary Figure 5. Average profile of two repeats (6-4)PP (A) and CPD (B) XR-seq on the transcribed strand (TS) and non-transcribed strand (NTS) over all reference genes at 5 minutes, 1, 8, 16, 24 and 48h after UV treatment. RPKM is reads per kilobase per million total reads. Data for each strand were scaled to a unit gene (as detailed in Materials and Methods) to represent average repair in RNA Polymerase II-transcribed genes and the 2 kb upstream and downstream.
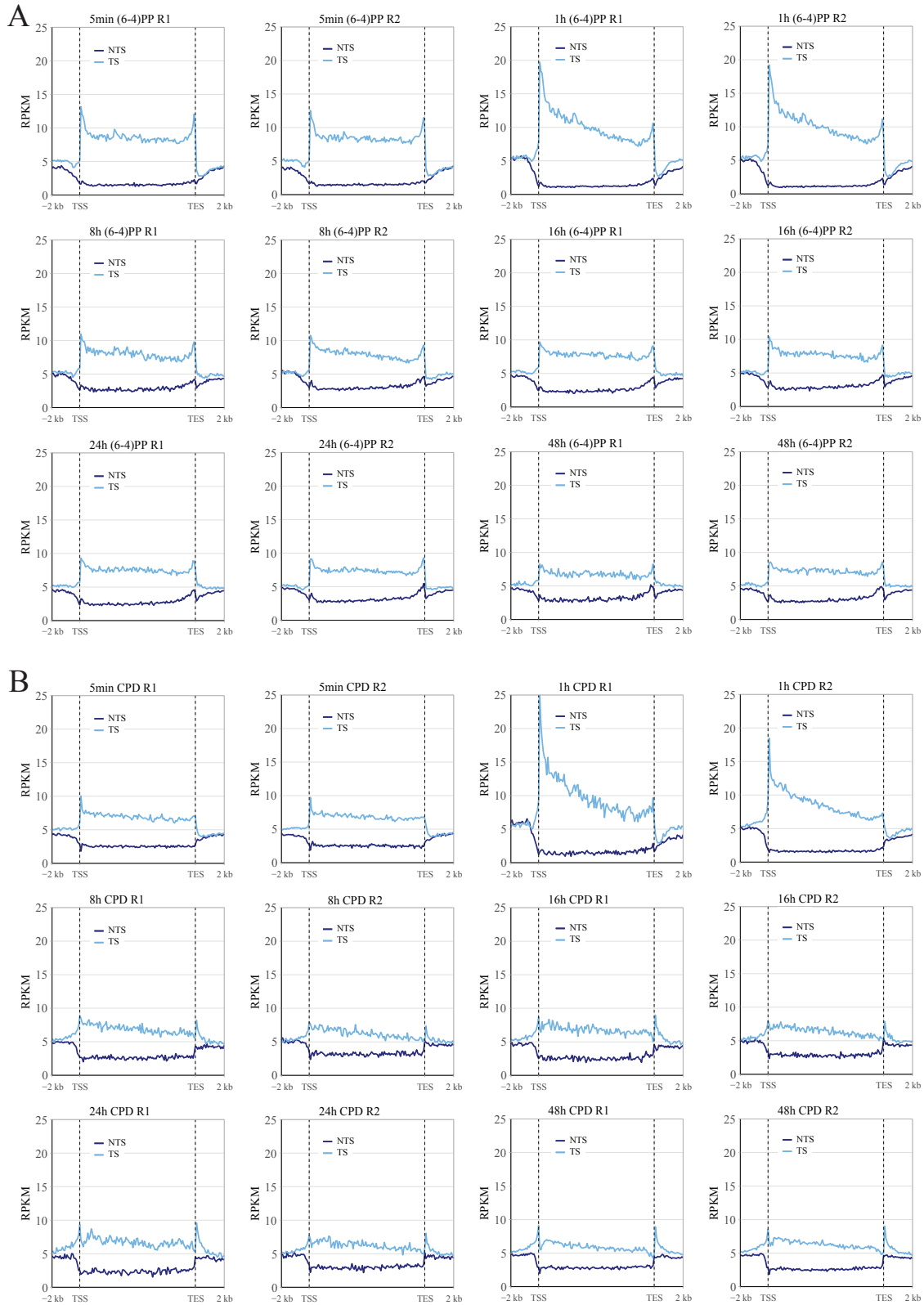
Supplementary Figure 6. Transcription events captured by XR-seq is highly correlated with the transcription captured by capped RNA-seq. XR-seq repair signals correlate with short- and long-capped RNA-seq signals much better than conventional RNA-seq. Pairwise smooth scatterplots are shown on the lower triangle; Spearman correlation coefficients are shown on the upper triangle, with text size proportionate to the absolute value of the coefficient.
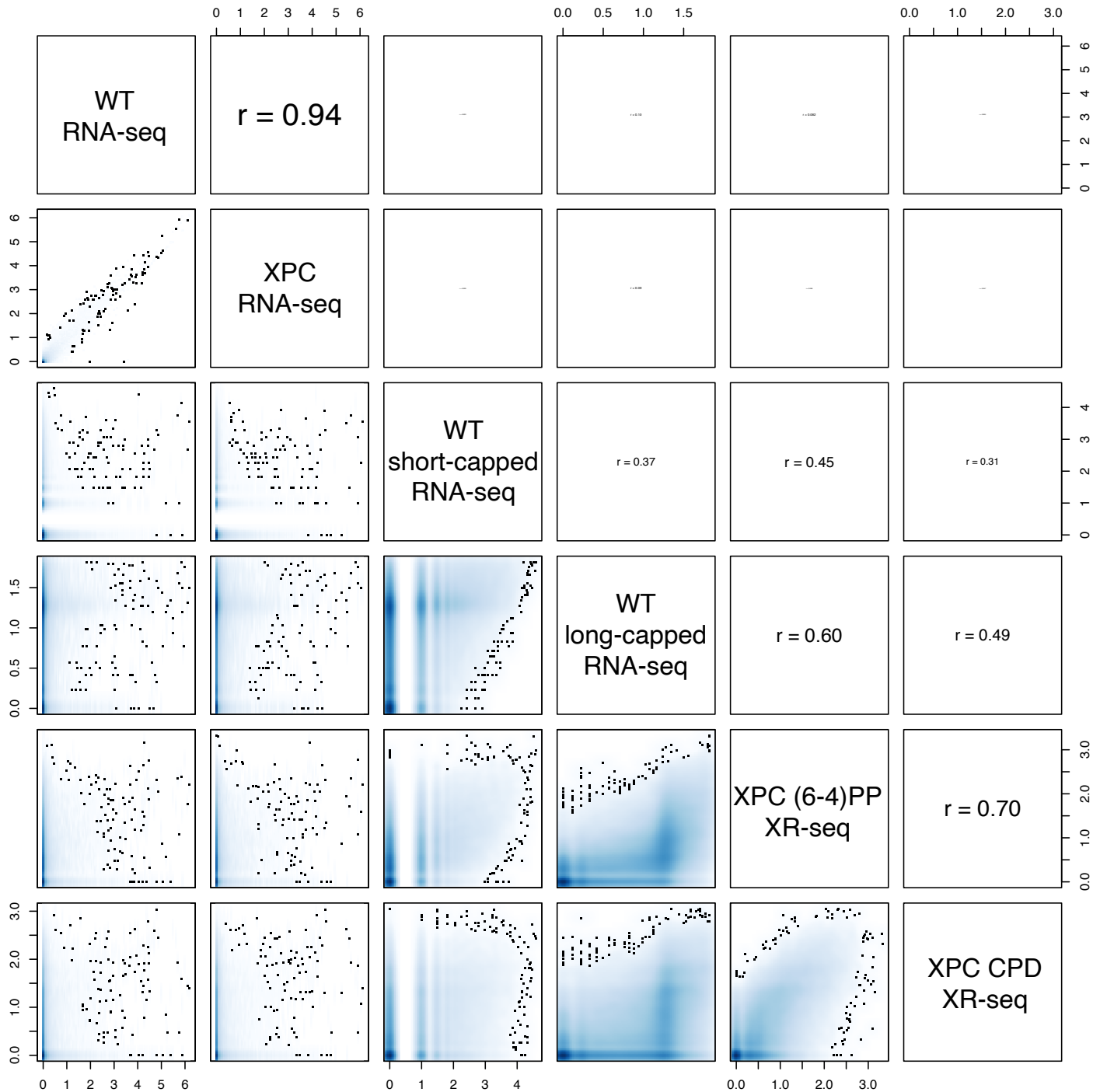
Supplementary Table 1. XR-seq sample information. Summary of C. elegans (6-4)PP and CPD XR-seq samples across different timepoints and replicates. Total_mapped: total mapped reads. Dedup: deduplicated reads. Mapq: reads with mapping quality > 20 (reads that are equally mapped to multiple genomic locations are removed with this QC). Chr: reads mapped to chrI, II, III, IV, V, X. Qwidth: reads with lengths 19-24. GenebodyPromoter: reads mapped to genes and 2 Kb upstream of transcription start sites (i.e., promoters). Genebody: reads mapped to genes.

Sample_name	Damage	Repair_time	Replicate	total_mapped	dedup	mapq	chr	qwidth	genebodypromoter	genebody
CExpcL15m4KR164_TTAGGC_S12_L001_R1_001	6-4	5min	1	3354876	3329717	2751808	2747753	1420219	1316728	1088150
CExpcL15m4KR264_TGACCA_S14_L001_R1_001		5min	2	3434248	3411177	2863138	2857944	1284786	1190326	981346
CEL1xpc64_GATCAG_S1_L008_R1_001		1h	1	9492312	8950194	6729601	6707813	3164856	2933350	2355648
XPCL11h64_CAGGCG_S69_L006_R1_001		1h	2	6559541	6460451	5346772	5339079	2576659	2380790	1907932
xpcL1B4k8hR164_CTCAGA_S28		8h	1	2286434	2252038	1077462	1075440	525603	477677	373260
xpcL1B4k8hR364_GATCAG_S9		8h	2	8370958	8186326	5851038	5824176	1409732	1279984	1000554
xpcL1B4k16hR164_GACGAC_S20		16h	1	2895387	2830981	1852461	1848471	943683	856688	668563
xpcL1B4k16hR364_TAGCTT_S1		16h	2	5684434	5486207	4583648	4561086	1192213	1081847	844950
xpcL1B4k24hR164_TAATCG_S22		24h	1	4946336	4810633	3599295	3588403	1638515	1481981	1151039
xpcL1B4k24hR364_GGCTAC_S3		24h	2	10815325	10583435	8104453	8072503	2203131	1990334	1548442
xpcL1B4k48hR164_TACAGC_S24		48h	1	1825849	1782186	1174881	1168156	474893	425263	321796
xpcL1B4k48hR364_CTTGTA_S5		48h	2	4987318	4804692	3823173	3806272	1502307	1356136	1049418
CExpcL15m4KR1CPD_GCCAAT_S13_L001_R1_001	CPD	5min	1	5837318	5763808	4072843	4053858	1452895	1300422	981492
CExpcL15m4KR2CPD_CAGATC_S15_L001_R1_001		5min	2	4302562	4264267	2958176	2944500	1067358	954165	719738
CEL1xpcCPD_ACTTGA_S2_L008_R1_001		1h	1	1717268	1710614	524924	522437	227281	211003	166995
XPCL11hCPD_CATTTT_S70_L006_R1_001		1h	2	5843021	5804098	4092344	4077700	1921303	1731004	1274284
xpcL1B4k8hR1CPD_CATTTT_S29		8h	1	1340986	1327771	746362	743063	314802	280403	201968
xpcL1B4k8hR3CPD_TGACCA_S10		8h	2	3094513	3042372	1355727	1346939	403994	355247	250147
xpcL1B4k16hR1CPD_CCAACA_S21		16h	1	1082126	1072088	590778	588731	259173	230254	165860
xpcL1B4k16hR3CPD_ACAGTG_S2		16h	2	1704743	1656693	982544	976155	357955	315907	224116
xpcL1B4k24hR1CPD_CGGAAT_S23		24h	1	684978	679498	419344	417964	196339	173972	123874
xpcL1B4k24hR3CPD_GCCAAT_S4		24h	2	1404716	1389973	814922	810876	316865	278957	197723
xpcL1B4k48hR2CPD_CGGAAT_S29		48h	1	2943738	2844139	1969532	1952041	1023631	897604	619118
xpcL1B4k48hR3CPD_CAGATC_S6		48h	2	5145059	5055556	3671400	3654971	1685725	1484541	1041861

Supplementary Table 2. Epigenomic data of L1 C. elegans adopted in this study.

Epigenomics		Measures	C. elegans stage	Accession + bigwig
ATAC-seq		Chromatin accessibility	L1	GSE114439_atac_wt_l1.bw
DNase-seq		DNase I hypersensitivity	L1	GSM3142662_dnase_wt_l1_rep1_100U_ml.bw
ChIP-seq	H3K4me1	Histone modification (activation)	L1	GSE114440_H3K4me1_wt_l1.bw
	H3K4me3	Histone modification (activation)	L1	GSE114440_H3K4me3_wt_l1.bw
	H3K27me3	Histone modification (repression)	L1	GSE114440_H3K27me3_wt_l1.bw