Figure S1

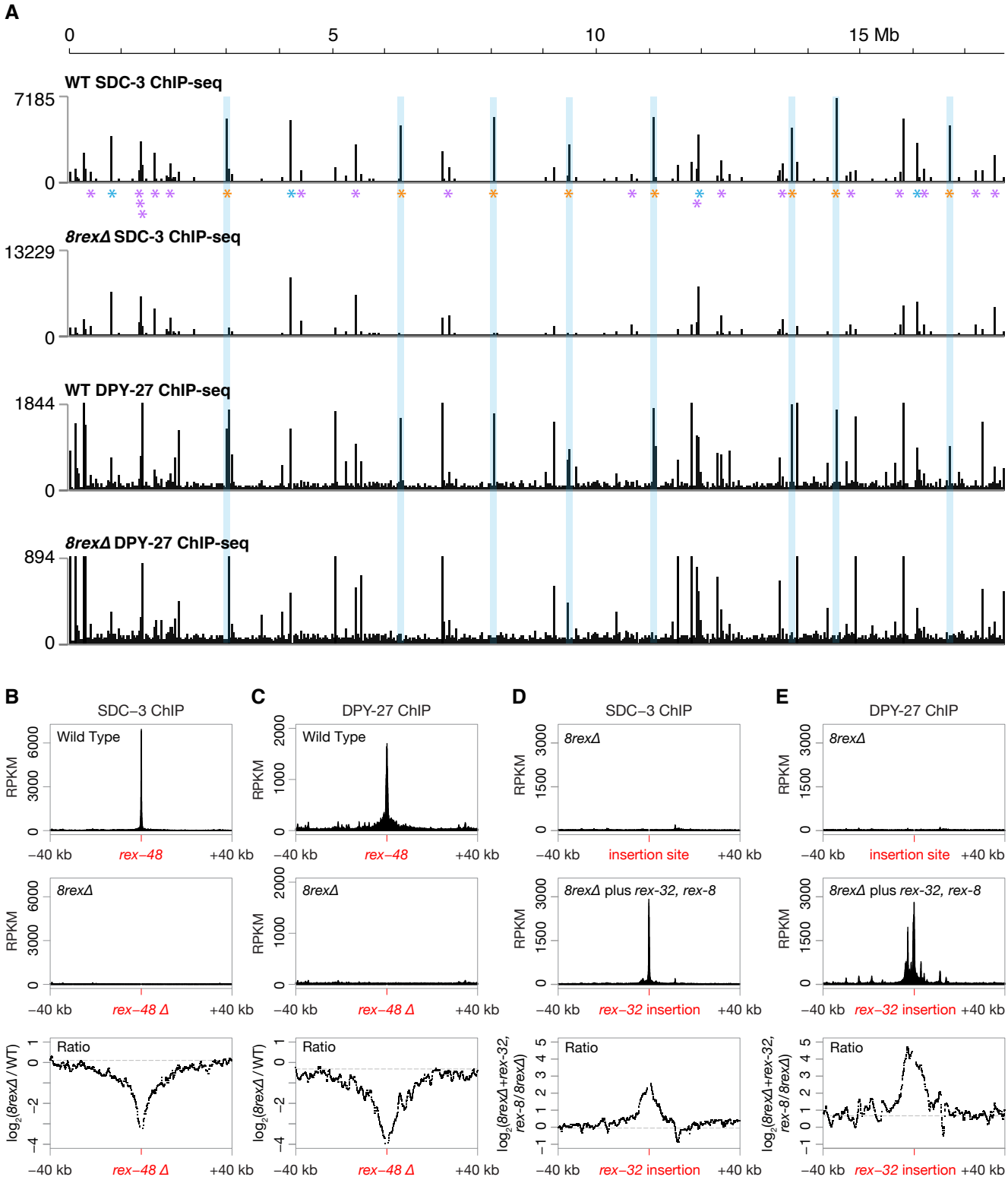
A
0
5
10
15 Mb
WT SDC-3 ChIP-seq
7185
0
8rexΔ SDC-3 ChIP-seq
13229
0
WT DPY-27 ChIP-seq
1844
0
8rexΔ DPY-27 ChIP-seq
894
0
B
SDC–3 ChIP
Wild Type
8rexΔ
RPKM
−40 kb
rex–48
rex–48 Δ
+40 kb
Ratio
log2(8rexΔ / WT)
C
DPY-27 ChIP
D
SDC–3 ChIP
8rexΔ plus rex-32, rex-8
insertion site
rex–32 insertion
log2(8rexΔ+rex-32, rex-8/8rexΔ)
E
DPY-27 ChIP

Figure S1. DCC Binding at Deleted *rex* Sites Is Abolished But Most DCC Binding on X Persists, Related to Figure 1

(A) SDC-3 and DPY-27 ChIP-seq profiles on X chromosomes of wild-type and *8rexΔ* embryos. Plots are scaled based on the binding at four strong *rex* sites that were not deleted (*rex-40*, *rex-23*, *rex-16*, and *Prex-30*, blue asterisks left to right, respectively). Other *rex* sites that were not deleted are marked with purple asterisks. Blue stripes and orange asterisks highlight the locations of the eight deleted *rex* sites.

(B and C) SDC-3 and DPY-27 ChIP-seq RPKM (reads per kilobase of transcript per million mapped reads) in the 80 kb region surrounding *rex-48* in wild-type embryos (top) and surrounding the *rex-48* deletion in *8rexΔ* embryos (middle). Red ticks show the location of the 408 bp *rex-48* deletion. Bottom plots display the $\log_2$ ratio of ChIP-seq reads in *8rexΔ* versus wild-type embryos. Ratios were not calculated for the deleted region. Gray dashed lines show the median ratio of signal between genotypes for the entire X chromosome. Binding of SDC-3 and DPY-27 was reduced immediately adjacent to the deletions but returned to wild-type levels within 20 kb.

(D and E) SDC-3 and DPY-27 ChIP-seq RPKM in the 80 kb region surrounding the location of the inserted *rex-32* in *8rexΔ* and in *8rexΔ* plus *rex-32*, *rex-8* embryos. Red ticks show the location of the 592 bp *rex-32* insertion. As above, bottom plots show the $\log_2$ ratio of DCC binding between genotypes. Binding of SDC-3 and DPY-27 was increased immediately adjacent to the insertions but returned to wild-type levels within 15 kb.

Figure S2

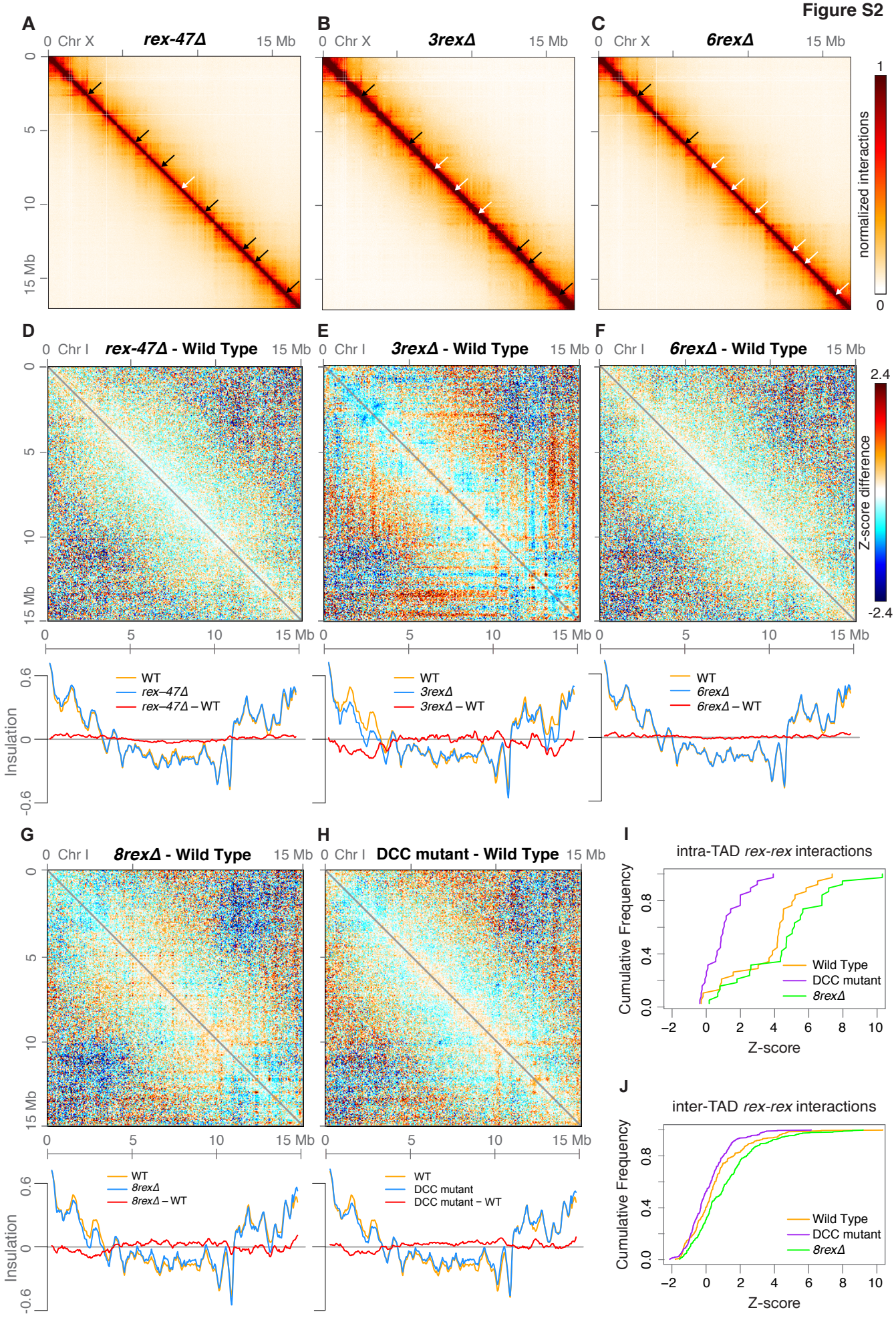
A
0 Chr X
rex-47Δ
15 Mb
B
3rexΔ
C
6rexΔ
normalized interactions
1
0
D
0 Chr I
rex-47Δ - Wild Type
E
3rexΔ - Wild Type
F
6rexΔ - Wild Type
Z-score difference
2.4
-2.4
Insulation
WT
rex–47Δ
rex–47Δ – WT
3rexΔ
3rexΔ – WT
6rexΔ
6rexΔ – WT
G
8rexΔ - Wild Type
H
DCC mutant - Wild Type
8rexΔ
8rexΔ – WT
DCC mutant
DCC mutant – WT
I
intra-TAD rex-rex interactions
Cumulative Frequency
Z-score
Wild Type
DCC mutant
8rexΔ
J
inter-TAD rex-rex interactions

Figure S2. *rex* Deletions Cause X-Specific Changes in Chromosome Structure, Related to Figure 1

(A-C) X chromosome heatmaps binned at 20 kb show Hi-C interactions in *rex-47Δ*, *3rexΔ*, and *6rexΔ* embryos. White arrows mark the positions of deleted *rex* sites, and black arrows mark remaining DCC-dependent boundaries.

(D-H) Z-score subtraction heatmaps of chromosome I binned at 50 kb show increased (red) and decreased (blue) Hi-C interactions in four strains with different numbers of *rex* deletions and in a DCC mutant strain [*sdc-2(y93, RNAi)*] compared to the wild-type strain. Lower plots show insulation scores across chromosome I in *rex* deletion or DCC mutant (blue) and in wild-type (orange) strains and the insulation score difference between genotypes (red).

(I and J) Cumulative frequency of interaction Z-scores for interactions between *rex* sites within the same TAD (I) or between *rex* sites in separate TADs (J). In *8rexΔ* embryos, higher interactions occur between *rex* sites located across deleted boundaries and between *rex* sites within regions defined as TADs on the wild-type X. Only the 22 non-boundary *rex* sites with the highest SDC-3 binding were analyzed, and only interactions between sites within 4 Mb were included.

Figure S3

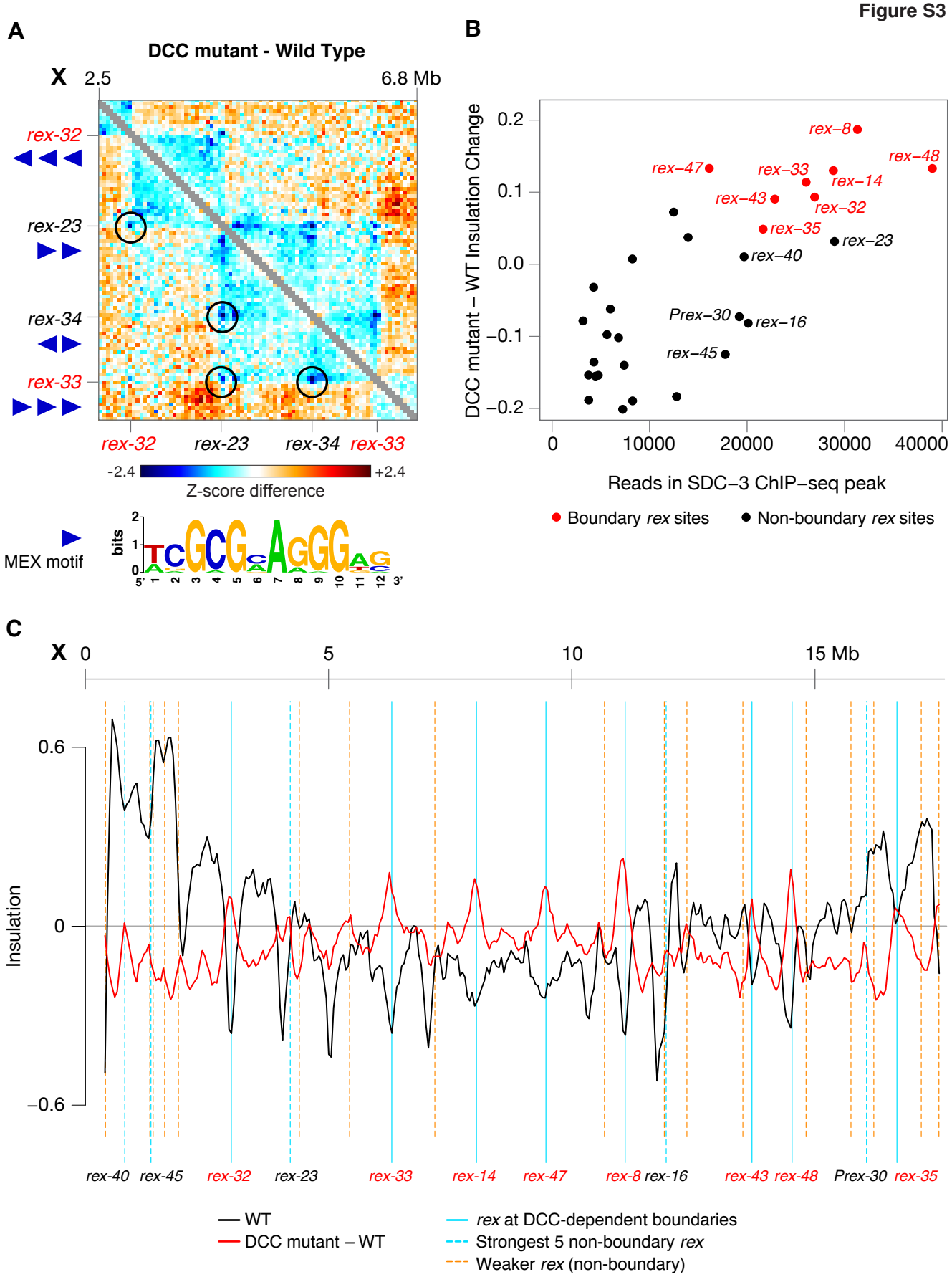
A
DCC mutant - Wild Type
X
2.5
6.8 Mb
rex-32
rex-23
rex-34
rex-33
-2.4
+2.4
Z-score difference
MEX motif
bits
B
DCC mutant – WT Insulation Change
Reads in SDC–3 ChIP–seq peak
Boundary rex sites
Non-boundary rex sites
rex–8
rex–48
rex–47
rex–33
rex–14
rex–43
rex–32
rex–35
rex–23
rex–40
Prex–30
rex–16
rex–45
C
X
0
5
10
15 Mb
Insulation
0.6
0
–0.6
rex-40
rex-45
rex-32
rex-23
rex-33
rex-14
rex-47
rex-8
rex-16
rex-43
rex-48
Prex-30
rex-35
WT
DCC mutant – WT
rex at DCC-dependent boundaries
Strongest 5 non-boundary rex
Weaker rex (non-boundary)

Figure S3. Only High-Occupancy *rex* Sites Establish TAD Boundaries, Related to Figure 1
(A) Examples showing that *rex* sites interact regardless of the orientation in these sites of known X-enriched motifs (MEX) important for DCC binding. Z-score subtraction heatmap of a portion of the X chromosome shows increased (red) and decreased (blue) interactions in DCC mutant versus wild-type embryos. Circles mark DCC-dependent interactions among four *rex* sites. Loss of a specific interaction in DCC mutants is reflected in a blue corner peak. For each *rex* site, blue triangles show the orientations of MEX motifs with a score less than -12 (Jans et al., 2009). *rex-23* (plus strand motifs) interacts with sites that have minus strand motifs (*rex-32*), plus strand motifs (*rex-33*), or both (*rex-34*). *rex-34* (both orientations) also interacts with *rex-23* (plus strand motifs).
(B) Correlation between insulation score change and DCC binding. For each of the 30 highest-occupancy *rex* sites, the insulation score difference in DCC mutant versus wild-type strains is plotted against the level of SDC-3 binding. SDC-3 levels were calculated by summing ChIP-seq reads mapping within 200 bp of the summit. *rex* sites at DCC-dependent boundaries are colored red. Five *rex* sites have more binding than *rex-47* but do not create boundaries (*rex-23*, *rex-16*, *rex-40*, *Prex-30*, and *rex-45*).
(C) Insulation profile of X chromosomes in the wild-type strain and insulation profile difference between wild-type and DCC mutant strains. Insulation scores were calculated by summing interactions in a 250 kb sliding window. (Plots in all other figures used a 500-kb window.) All DCC-dependent boundaries (solid cyan lines) contain one of the 13 highest-occupancy *rex* sites. Five *rex* sites that have the same or more DCC binding as boundary *rex* sites (dotted cyan lines) are not at boundaries (not at local minima in the wild-type insulation profile, black line) but do show insulation changes in the DCC mutant (local maxima in the insulation profile difference, red line). That is, in DCC mutants, interactions between loci flanking these non-boundary *rex* sites increased compared to interactions across neighboring loci. Insulation score changes also occur at some *rex* sites of rank 14-30 (dotted orange lines).

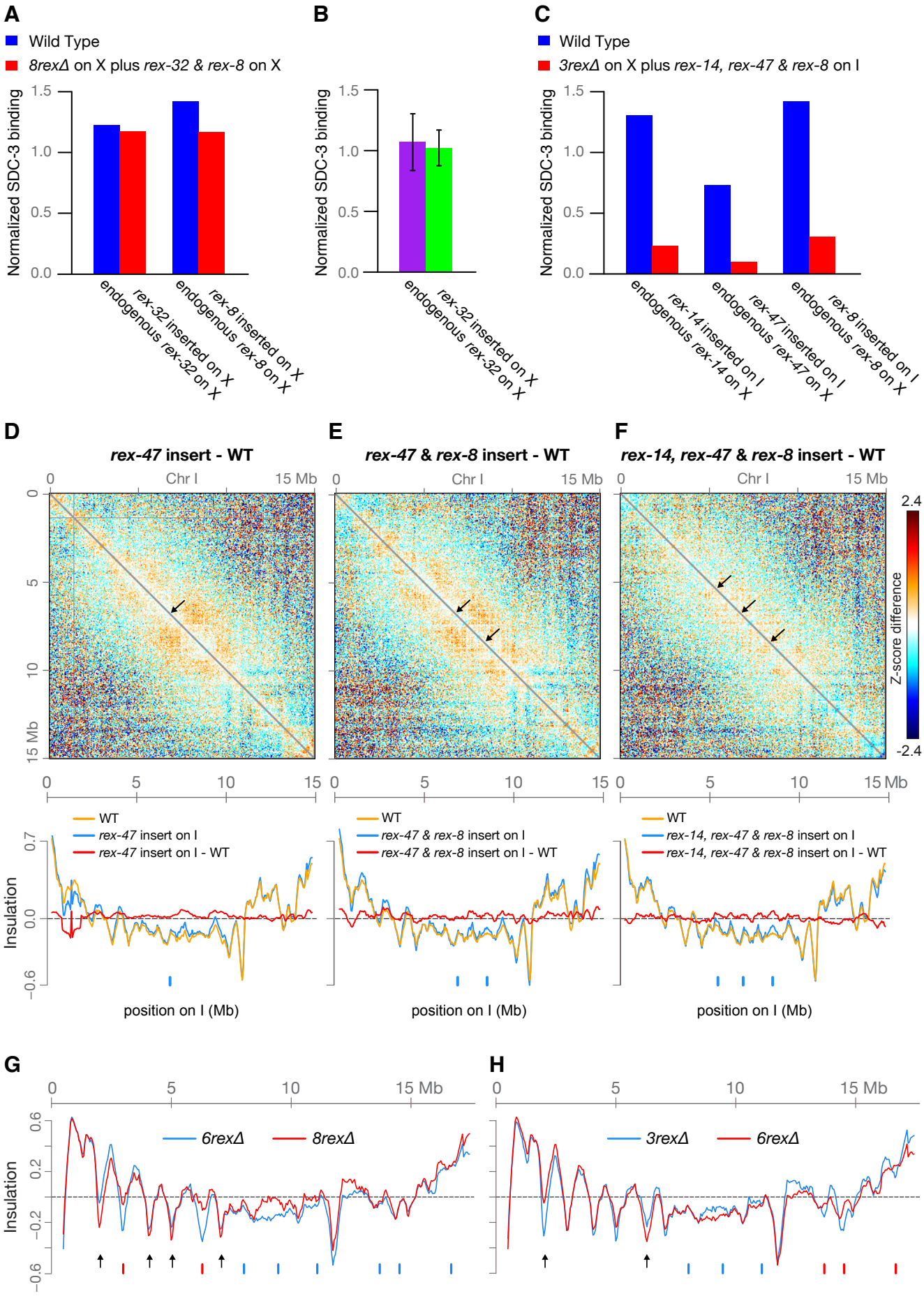
A
Wild Type
8rexΔ on X plus rex-32 & rex-8 on X
Normalized SDC-3 binding
1.5
1.0
0.5
0.0
endogenous rex-32 on X
rex-32 inserted on X
endogenous rex-8 on X
rex-8 inserted on X
B
Normalized SDC-3 binding
endogenous rex-32 on X
rex-32 inserted on X
C
Wild Type
3rexΔ on X plus rex-14, rex-47 & rex-8 on I
Normalized SDC-3 binding
endogenous rex-14 on X
rex-14 inserted on I
endogenous rex-47 on X
rex-47 inserted on I
endogenous rex-8 on X
rex-8 inserted on I
D
rex-47 insert - WT
E
rex-47 & rex-8 insert - WT
F
rex-14, rex-47 & rex-8 insert - WT
0
Chr I
15 Mb
5
10
2.4
Z-score difference
-2.4
WT
rex-47 insert on I
rex-47 insert on I - WT
rex-47 & rex-8 insert on I
rex-47 & rex-8 insert on I - WT
rex-14, rex-47 & rex-8 insert on I
rex-14, rex-47 & rex-8 insert on I - WT
Insulation
0.7
0.0
−0.6
position on I (Mb)
G
H
6rexΔ
8rexΔ
3rexΔ
0.6
0.2
−0.2

Figure S4. *rex* Sites Inserted on Chromosome I Weakly Recruit the DCC But Do Not Create TAD Boundaries, Related to Figure 2

(A) SDC-3 binding at *rex-32* and *rex-8* inserted at new locations on X is similar to binding at the endogenous sites. Binding at *rex-32* and *rex-8* was normalized to the average binding at four strong non-boundary *rex* sites within the same dataset (*rex-40*, *rex-23*, *rex-16*, and *Prex-30*). Binding was calculated by summing ChIP-seq reads mapping within 200 bp of the summit.

(B) SDC-3 binding at *rex-32* inserted at 15.5 Mb on the wild-type X chromosome is equivalent to binding at the endogenous *rex-32* site as measured by ChIP-qPCR. Binding was normalized to the binding at five strong *rex* sites (*rex-32*, *rex-8*, *rex-16*, *rex-48*, and *rex-35*). Error bars show standard deviation of three biological replicates.

(C) ChIP-seq experiments show that SDC-3 binding at three *rex* sites inserted on chromosome I is lower than binding at the endogenous sites on X. Binding was calculated and normalized as in (A).

(D-F) Z-score subtraction heatmaps binned at 50 kb show increased (red) and decreased (blue) Hi-C interactions in strains with one, two, or three *rex* sites inserted on chromosome I of the *3rexΔ* strain. This strain lacked the sites on X to allow unique mapping of reads to the new insertion locations. Arrows mark positions of inserted *rex* sites. *rex* insertions have no effect on chromosome I structure. Plots (below) show insulation scores across chromosome I in *rex* insertion (blue) and wild-type (orange) strains and the insulation score difference between genotypes (red). Chromosome I data used as the wild-type reference were from the strain carrying the *3rexΔ* X chromosome.

(G) Insulation profiles comparing X chromosomes in *6rexΔ* and *8rexΔ* strains. Blue ticks show *rex* sites deleted in both mutants, and red ticks show *rex* sites deleted only in the *8rexΔ* strain. Boundaries adjacent to the *rex* sites deleted only in the *8rexΔ* strain are strengthened (arrows).

(H) Insulation profiles comparing X chromosomes in *3rexΔ* and *6rexΔ* strains. Blue ticks show *rex* sites deleted in both mutants and red ticks show *rex* sites deleted only in *6rexΔ*. Insulation scores change at boundaries far from the deleted boundaries (arrows).

Figure S5

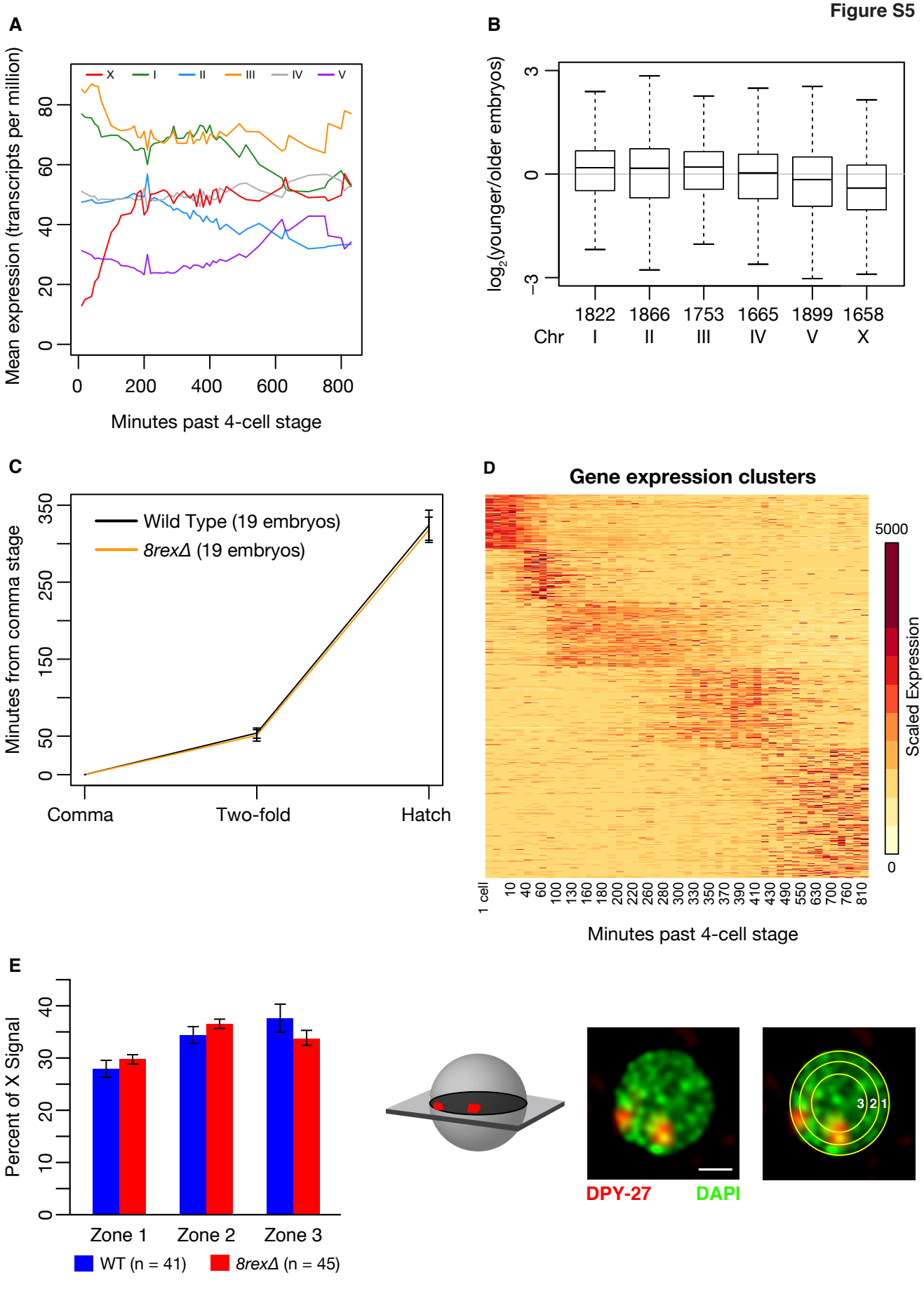
A
Mean expression (transcripts per million)
X
I
II
III
IV
V
0
20
40
60
80
0
200
400
600
800
Minutes past 4-cell stage
B
log2(younger/older embryos)
3
0
−3
1822
1866
1753
1665
1899
1658
Chr
I
II
III
IV
V
X
C
Minutes from comma stage
0
50
150
250
350
Wild Type (19 embryos)
8rexΔ (19 embryos)
Comma
Two-fold
Hatch
D
Gene expression clusters
5000
0
Scaled Expression
1 cell
10
40
60
100
130
160
180
200
220
260
280
300
330
350
370
390
410
430
490
550
630
700
760
810
Minutes past 4-cell stage
E
Percent of X Signal
0
10
20
30
40
Zone 1
Zone 2
Zone 3
WT (n = 41)
8rexΔ (n = 45)
3
2
1
DPY-27
DAPI

Figure S5. Analysis for Matching Age Distribution of Embryo Populations, Related to Figure 3

(A) Average expression from RNA-seq data (Hashimshony et al., 2015) of all the genes on each chromosome plotted at each time point during embryogenesis.

(B) Chromosome-by-chromosome comparison of gene expression in younger versus older wild-type embryos. Two replicates are included for each age. Because X chromosome silencing is gradually lost during early development, genes on X have lower expression in the younger population.

(C) The average time for development from comma stage to two-fold stage and then to hatching is equivalent in wild-type and *8rexΔ* embryos. Error bars show standard deviation.

(D) Expression for genes whose transcript levels change during development. Using data from (Hashimshony et al., 2015), we selected genes that have >1 transcript per million at any measured time point during embryogenesis and also change at least 20-fold in expression. We scaled each gene's expression by subtracting its mean and dividing by its standard deviation. The genes were divided into five groups by k-means clustering.

(E) X chromosome radial positioning is similar in nuclei of wild-type and *8rexΔ* embryos. (right) X chromosomes were stained with DPY-27 antibody (red) and DNA was stained with DAPI (green). For each nucleus, the Z section with the highest DPY-27 signal was selected. The nucleus was divided into three concentric zones of equal area, and the percentage of DPY-27 signal in each zone was calculated. (left) The graph shows the average percentage of X in each zone for wild-type and *8rexΔ* nuclei. Error bars show SEM. Scale bar is 1 μm.

Figure S6

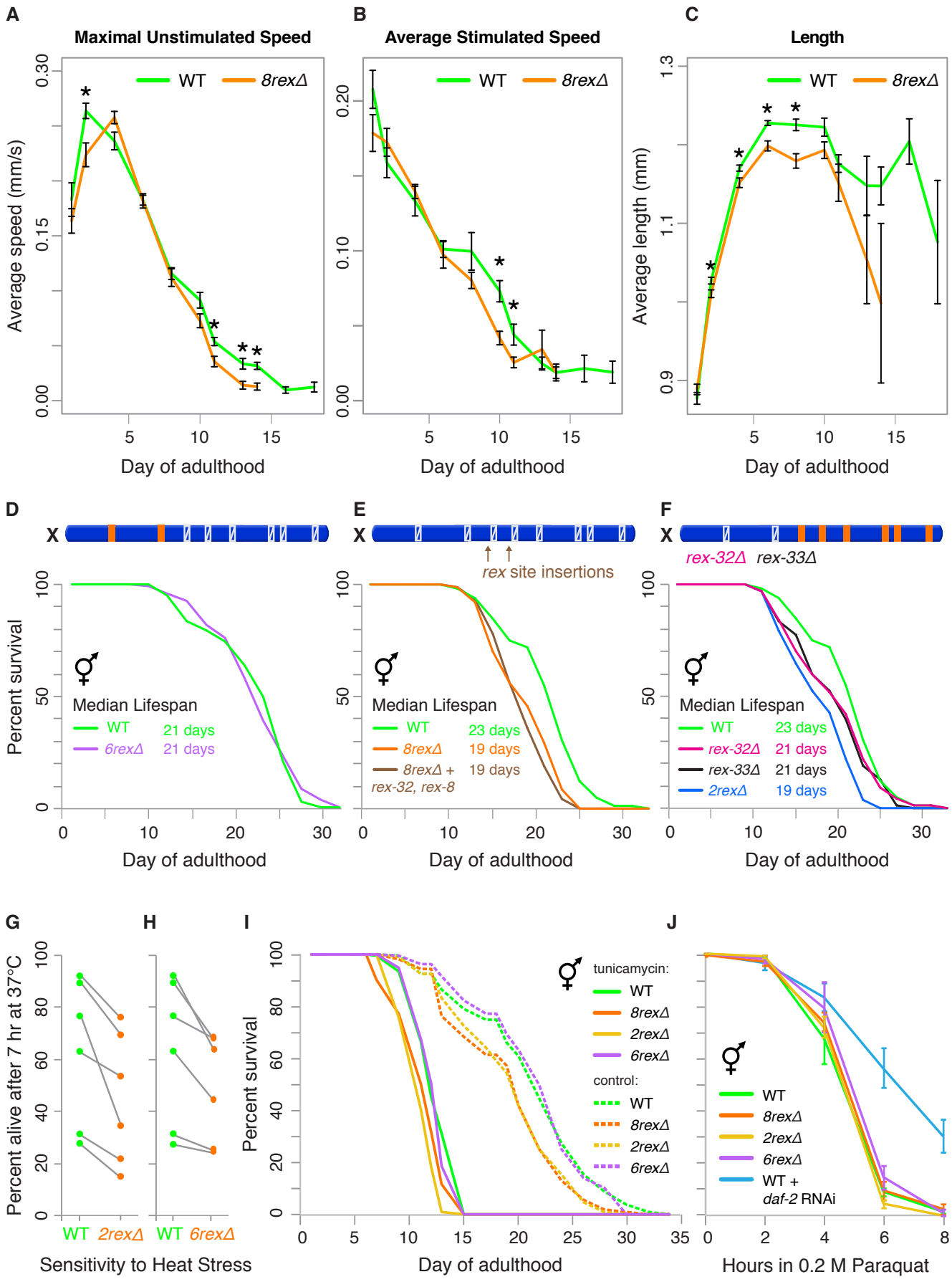
A
Maximal Unstimulated Speed
Average speed (mm/s)
WT
8rexΔ
Day of adulthood
B
Average Stimulated Speed
C
Length
Average length (mm)
D
X
Percent survival
Median Lifespan
WT 21 days
6rexΔ 21 days
E
rex site insertions
Median Lifespan
WT 23 days
8rexΔ 19 days
8rexΔ + rex-32, rex-8 19 days
F
rex-32Δ rex-33Δ
Median Lifespan
WT 23 days
rex-32Δ 21 days
rex-33Δ 21 days
2rexΔ 19 days
G
Percent alive after 7 hr at 37°C
WT 2rexΔ
H
WT 6rexΔ
Sensitivity to Heat Stress
I
Percent survival
tunicamycin:
WT
8rexΔ
2rexΔ
6rexΔ
control:
WT
8rexΔ
2rexΔ
6rexΔ
Day of adulthood
J
WT
8rexΔ
2rexΔ
6rexΔ
WT + daf-2 RNAi
Hours in 0.2 M Paraquat

Figure S6. Shortened Lifespan, Accelerated Aging, and Reduced Thermotolerance of Hermaphrodites with *rex* Deletions, Related to Figure 5

(A) Maximal unstimulated movement of wild-type and *8rexΔ* hermaphrodites during aging. For each genotype, the speed of 50 worms on each of eight plates was measured throughout adulthood. The measurement only included moving worms, not immobile or dead worms. We calculated the maximum speed of worms on each plate and plotted the mean ± SEM of eight plates. Asterisks indicate significant differences ($p < 0.05$, t test). mm/s, millimeters per second

(B) Average movement speed of wild-type and *8rexΔ* hermaphrodites in response to a mechanical stimulus (plate tap) during aging. Mean ± SEM of all eight plates are plotted as in (A).

(C) Body length of wild-type and *8rexΔ* hermaphrodites during aging. Mean ± SEM of all eight plates are plotted as in (A).

(D) Lifespans of wild-type versus *6rexΔ* hermaphrodites ($p = 0.8$, logrank test).

(E) Lifespans of wild-type versus *8rexΔ* hermaphrodites ($p < 0.0001$) or versus *8rexΔ* + *rex-32, rex-8* hermaphrodites ($p < 0.0001$).

(F) Lifespan of wild-type hermaphrodites versus *rex-32Δ* ($p = 0.06$) or versus *rex-33Δ* ($p = 0.03$) or versus *2rexΔ* ($p < 0.0001$). For (D-F), values from replicate experiments are presented in Table S6.

(G and H) Percent survival of wild-type and *2rexΔ* or *6rexΔ* day 1 adult worms after 7 hr at 37°C in each of six trials shows reduced thermotolerance in both *2rexΔ* and *6rexΔ* adults ($p= 0.0135$ and $p = 0.0183$, respectively, paired t test). Fifty worms of each genotype were counted in each trial. A gray line links the measurements of the two genotypes in the same trial. Replicate experiments and statistics are in Table S4.

(I) Lifespans were scored for wild-type, *8rexΔ*, *2rexΔ*, and *6rexΔ* hermaphrodites transferred on day 1 of adulthood to plates containing either 20 ng/μL tunicamycin to induce ER unfolded protein stress (solid lines) or DMSO as a control (dashed lines). Lifespans of all strains grown on tunicamycin were reduced. As in control experiments, lifespans of *8rexΔ* and *2rexΔ* adults grown on tunicamycin were shorter than those of wild-type and *6rexΔ* adults on tunicamcyin. The degree of lifespan reduction was similar across all genotypes, indicating similar sensitivity to ER unfolded protein stress. Replicate experiments and statistics are in Table S5.

(J) Percent survival of day 1 adult wild-type, *8rexΔ*, *2rexΔ*, and *6rexΔ* hermaphrodites in 0.2 M paraquat to induce reactive oxygen species in mitochondria. Wild-type worms subjected to *daf-2* RNAi were used as a control for increased oxidative stress tolerance. For each genotype, the average ± SEM of at least three replicates is plotted.

Table S1. Locations of *rex* site deletions and insertions, Related to Figure 1; Figure 2

***rex* site**	**Deletion location**	**Deletion size**	**Genomic location**	**Distance to nearest protein-coding gene**	**Cas9 guide RNA target sequence (protospacer)**
rex-32	X:2996734 to 2997626	892 bp	intergenic	677 bp *Y41G9A.6*	constructed using TALENs Lo et al., 2013
rex-33	X:6296494 to 6297792	1298 bp	genic	last intron of *mom-1* (Thorpe et al., 1997)	ccatttacatttggcgcagg and taacttattttacagaaaac
rex-14	X:8035969 to 8037090	1121 bp	intergenic	423 bp *xol-1*	atccacattactgtggttgg and cctttcacaacactcttttc
rex-47	X:9465542 to 9465960	418 bp	intergenic	817 bp *ZK899.6*	gtagtcacaccgaattgata Crane et al., 2015
rex-8	X:11093785 to 11094686	901 bp	intergenic	2395 bp *W02H3.3*	agttgaaacaccatggagcgg and gcaacttatcggtgtcgcgg
rex-43	X:13700642 to 13701123	481 bp	intergenic	4842 bp *adm-2*	ttggattgtgttcatcgtgg and aatgtcattaggttaaatg
rex-48	X:14525672 to 14526080	408 bp	intergenic	491 bp *nspc-17*	ccagcatttttgagtgctt
rex-35	X:16681790 to 16682252	462 bp	intergenic	1967 bp *F22H10.2*	ctatatacatgtttgaaac and tgttattctatttctaaag

***rex* site**	**Insertion location**	**Insertion size**	**Inserted sequence**	**Distance to nearest protein-coding gene**	**Cas9 guide RNA target sequence (protospacer)**
rex-32	X:7812122	592 bp	X:2996832 to 2997424	2988 bp *R03G5.6*	gtagaatgctccgtgtatgg
rex-8	X:9198531	754 bp	X:11093928 to 11094681	4149 bp *Y81B9A.1*	agtggactccatcacactgg
rex-14	I:5448650	500 bp	X:8036158 to 8036657	1795 bp *F27C1.13*	atttactgccaaacaggggg
rex-47	I:6858675	419 bp	X:9465601 to 9466019	1146 bp *glh-1*	ttccaaatcaggccgtaggg
rex-8	I:8507023	796 bp	X:11093891 to 11094686	2018 bp *sec-22*	cgtggtagtggtagaagcgg
rex-32	X:15574677	592 bp	X:2996832 to 2997424	984 bp *dyn-1*	ttatgtagtctctttcagtg

The genes nearest each deleted *rex* site do not show significant expression changes in *8rexΔ* compared to wild-type embryos (all q values > 0.99 and fold changes < 2).

Table S2. Statistics for Hi-C libraries, related to Figure 1; Figure 2; Figure S2; Figure S4.

Genotype	sequencing ID	Read pairs sequenced	Mapped paired-end reads	Hi-C contacts	cis contacts	% cis contacts	contacts ≤20kb	contacts >20kb	trans contacts
Wild type, replicate 1	BMEA008	345,019,879	282,509,400	165,106,057	108,180,356	65.5	18,537,295	89,643,061	56,925,701
Wild type, replicate 2	BMQB011	347,282,747	285,963,877	174,488,311	120,023,946	68.8	19,440,906	100,583,040	54,464,365
DCC mutant, replicate 1	BMEA014	343,219,970	282,454,019	212,570,004	162,743,891	76.6	28,524,650	134,219,241	49,826,113
DCC mutant, replicate 2	BMEA015	335,462,364	275,027,515	226,964,909	172,371,804	75.9	29,473,750	142,898,054	54,593,105
rex-47Δ, replicate 1	BMEA007	294,081,104	243,068,670	153,945,373	125,150,766	81.3	28,931,142	96,219,624	28,794,607
rex-47Δ, replicate 2	BMEA009	338,247,895	276,996,740	165,220,501	110,622,381	67.0	17,894,594	92,727,787	54,598,120
3rexΔ	BMEA027	318,171,138	258,390,604	148,851,059	118,655,892	79.7	27,718,268	90,937,624	30,195,167
6rexΔ, replicate 1	BMEA005	357,347,563	295,322,718	104,132,475	71,384,861	68.6	13,393,596	57,991,265	32,747,614
6rexΔ, replicate 2	BMEA010	346,012,880	283,577,018	156,033,638	107,310,063	68.8	19,405,386	87,904,677	48,723,575
8rexΔ, replicate 1	BMEA011	332,906,702	279,292,784	122,811,741	109,070,728	88.8	31,604,873	77,465,855	13,741,013
8rexΔ, replicate 2	BMEA012	321,206,155	263,925,332	174,496,009	138,646,425	79.5	37,428,958	101,217,467	35,849,584
8rexΔ plus *rex-32*	BMEA034	289,222,774	238,607,345	147,738,802	123,184,375	83.4	30,757,996	92,426,379	24,554,427
8rexΔ plus *rex-32* & *rex-8*	BMEA038	312,384,765	254,262,569	179,289,194	122,937,082	68.6	16,183,574	106,753,508	56,352,112
rex-32 insert	BMAS001	340,895,823	273,682,301	204,321,406	158,275,799	77.5	25,154,746	133,121,053	46,045,607
3rexΔ plus *rex-47* on I	BMEA028	291,839,702	236,564,530	132,176,851	107,531,507	81.4	25,669,424	81,862,083	24,645,344
3rexΔ plus *rex-47* & *rex-8* on I	BMEA029	330,676,073	279,160,255	132,500,704	110,416,797	83.3	26,804,337	83,612,460	22,083,907
3rexΔ plus *rex-14, rex-47* & *rex-8* on I	BMEA035	295,667,838	240,271,869	147,744,551	113,255,151	76.7	28,417,402	84,837,749	34,489,400

For each Hi-C experiment, the table gives the total number of paired end reads, the number of pairs for which both ends mapped, the number of valid Hi-C contacts after filtering (as in Brejc et al., 2017, Imakaev et al., 2012), and the number of contacts between pairs of loci on the same chromosome (within 20 kb or greater than 20 kb) and on different chromosomes.

Table S3. Wild-type and *8rexΔ* hermaphrodites have similar brood sizes and viability, Related to Figure 3

Genotype	Total embryos	Total adults	Broods counted	Viability	Average Brood Size	Brood Size Standard Deviation
Trial 1						
WT	2572	2593	10	1.01	257	37
8rexΔ	2256	2310	9	1.02	251	27
Trial 2						
WT		2798	9		311	53
8rexΔ		2685	9		298	36
Trial 3						
WT		2980	9		331	29
8rexΔ		3115	10		312	29

Wild-type and *8rexΔ* brood sizes are not significantly different (Trial 1 p = 0.87, Trial 2 p = 0.56, Trial 3 p = 0.16, two-tailed t test).

Table S4. *8rexΔ, 2rexΔ, and 6rexΔ* have significantly lower thermotolerance than wild-type adults, Related to Figure 5

	Percent alive after 37°C heat stress			
Trial (hours at 37°C)	**WT**	***8rexΔ***	***2rexΔ***	***6rexΔ***
Trial 1 (5 hr)	82%	68%		
Trial 2 (7 hr)	74%	46%		
Trial 3 (7 hr)	49%	30%		
Trial 4 (7 hr)	88%	54%		
Trial 5 (7 hr)	53%	37%		
Trial 6 (7 hr)	43%	34%		
Trial 7 (6 hr)	83%	76%	68%	57%
Trial 8 (6 hr)	38%	28%	36%	31%
Trial 9 (7 hr)	27%	30%	14%	24%
Trial 10 (7 hr)	31%	30%	21%	25%
Trial 11 (7 hr)	89%	80%	69%	68%
Trial 12 (7 hr)	76%	31%	34%	68%
Trial 13 (7 hr)	63%	40%	53%	44%
Trial 14 (7 hr)	92%	62%	76%	63%
Trial 15 (9.5 hr)	70%	34%	63%	33%
p value compared to WT (all Trials)	-	0.0001	0.0043	0.0024
p value compared to *8rexΔ* (all Trials)	0.0001	-	0.6175	0.9671
p value compared to *2rexΔ* (all Trials)	0.0043	0.6175	-	0.7036
p value compared to WT (7 hr Trials)	-	0.0014	0.0135	0.0183
p value compared to *8rexΔ* (7 hr Trials)	0.0014	-	0.8564	0.6761
p value compared to *2rexΔ* (7 hr Trials)	0.0135	0.8564	-	0.571

Sensitivity to heat stress was assessed by counting the percentage of day 1 adults that survive at 37°C for the indicated number of hours. P values were calculated using a two-tailed paired t test. WT, wild type

Table S5. Wild-type, *8rexΔ, 2rexΔ,* and *6rexΔ* hermaphrodites have similar tunicamycin sensitivity, Related to Figure 5

Genotype	Median Lifespan (Days)	Worms counted	Total Worms	P value compared to WT with tunicamycin	P value compared to *8rexΔ* with tunicamycin	P value compared to WT without tunicamycin
Trial 1						
WT	19	96	120	< 0.0001	< 0.0001	
8rexΔ	17	94	120	< 0.0001	< 0.0001	0.0004
WT tunicamycin	13	98	120		0.2939	< 0.0001
8rexΔ tunicamycin	12	102	120	0.2939		< 0.0001
Trial 2						
WT	24	92	120	< 0.0001	0.3192	
8rexΔ	20	84	120	0.3192	< 0.0001	< 0.0001
WT tunicamycin	19	110	120		< 0.0001	< 0.0001
8rexΔ tunicamycin	19	105	120	< 0.0001		< 0.0001
Trial 3						
WT tunicamycin	12	103	120		0.0002	< 0.0001
8rexΔ tunicamycin	12	106	120	0.0002		< 0.0001
2rexΔ tunicamycin	11	109	120	< 0.0001	0.0188	< 0.0001
6rexΔ tunicamycin	12	109	120	0.338	0.002	< 0.0001
WT	22	100	120	< 0.0001	< 0.0001	
8rexΔ	20	97	120	< 0.0001	< 0.0001	0.0018
2rexΔ	20	104	120	< 0.0001	< 0.0001	0.001
6rexΔ	22	99	120	< 0.0001	< 0.0001	0.9858
Trial 4						
WT tunicamycin	12	101	120		0.0084	< 0.0001
8rexΔ tunicamycin	11	94	120	0.0084		< 0.0001
2rexΔ tunicamycin	12	100	120	0.3653	0.0605	< 0.0001
6rexΔ tunicamycin	12	98	120	0.0086	< 0.0001	< 0.0001
WT	18	101	120	< 0.0001	< 0.0001	
8rexΔ	16	94	120	< 0.0001	< 0.0001	< 0.0001
2rexΔ	16	100	120	< 0.0001	< 0.0001	< 0.0001
6rexΔ	18	98	120	< 0.0001	< 0.0001	0.1227

Sensitivity to ER unfolded protein stress was assessed by measuring hermaphrodite lifespan on plates containing 20 ng/µl tunicamycin, which causes accumulation of unfolded glycoproteins in the ER. “Worms counted” is the number of worm deaths tallied. “Total worms” is the initial number of worms, including those that were censored from the experiment because they crawled off the plate. P values were calculated using the logrank test. Tunicamycin exposure reduced the lifespan of all genotypes. As in control experiments, lifespans of *8rexΔ* and *2rexΔ* adults on tunicamycin were shorter than those of wild-type and *6rexΔ* adults on tunicamcyin. The degree of lifespan reduction was similar across all genotypes, indicating similar sensitivity to ER unfolded protein stress. WT, wild type.

Table S6. Statistics for hermaphrodite and male lifespan experiments, Related to Figure 5

Genotype	Median Lifespan (Days)	Worms counted	Total Worms	P value compared to WT	P value compared to *8rexΔ*
Hermaphrodite Trial 1					
WT	21	77	108		
8rexΔ	17	90	120	< 0.0001	
Hermaphrodite Trial 2					
WT	23	89	120		
8rexΔ	19	102	120	< 0.0001	
rex-32Δ rex-33Δ	19	88	120	< 0.0001	
rex-32Δ	21	98	120	0.055	0.0226
rex-33Δ	21	100	120	0.0304	0.0196
8rexΔ plus rex-32 & rex-8	19	103	120	< 0.0001	0.02218
Hermaphrodite Trial 3					
WT	21	78	120		
8rexΔ	17	100	120	< 0.0001	
6rexΔ	21	92	120	0.7959	< 0.0001
rex-32Δ rex-33Δ	17	109	120	< 0.0001	0.0632
Hermaphrodite Trial 4					
WT	20	92	120		
6rexΔ	18	88	120	0.1974	
rex-32Δ	18	78	120	0.189	
rex-33Δ	20	93	120	0.5062	
Male Trial 1					
WT males	14	169	300		
8rexΔ males	14	133	300	0.2485	
Male Trial 2					
WT males	18	146	310		
8rexΔ males	16	89	300	0.0633	

“Worms counted” is the number of worm deaths tallied. “Total worms” is the initial number of worms, including those that were censored from the experiment because they crawled off the plate. As observed previously, the majority of males crawled off the plate before dying. P values were calculated using the logrank test. WT, wild type