Supplementary figure 1

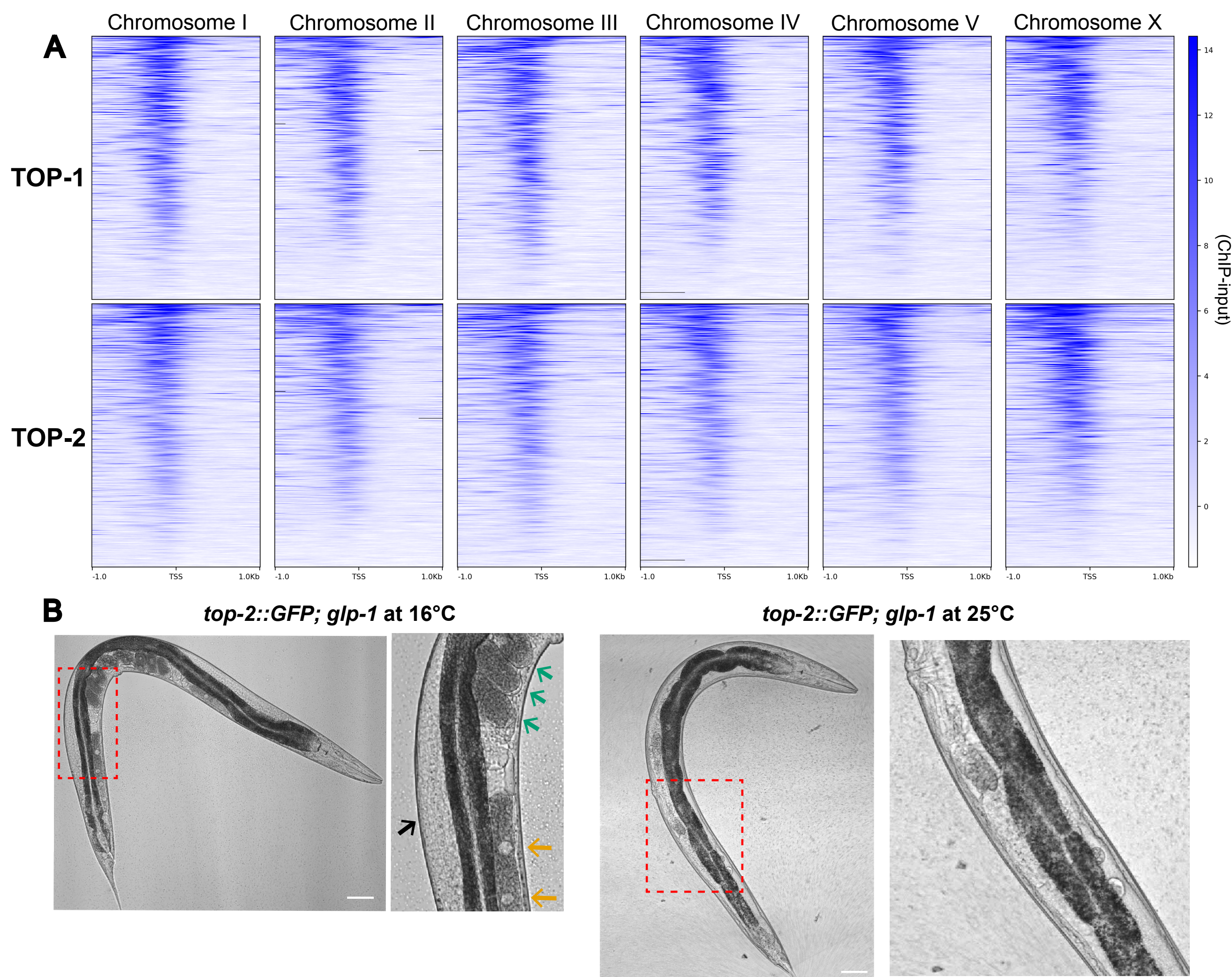


Figure S1. TOP-1 and TOP-2 binding across promoters. Related to Figure 1.

A) Heatmap showing TOP-1 and TOP-2 ChIP-seq signals across TSSs on all chromosomes. The transcription start site annotations are from GRO-seq experiments (Kruesi et al., 2013).

B) Images of *top-2::sfGFP; glp-1(q224)* adults that were grown at the permissive (16°C) and restrictive (25°C) temperatures. *glp-1(q224)* worms lack a germline when grown at the restrictive temperature. Black arrow indicates the germline (surface view). Orange arrows indicate oocytes. Green arrows indicate fertilized embryos. All these structures are missing in worms grown at 25°C. Scale bars: 64.5 μm.

Supplementary Figure 2

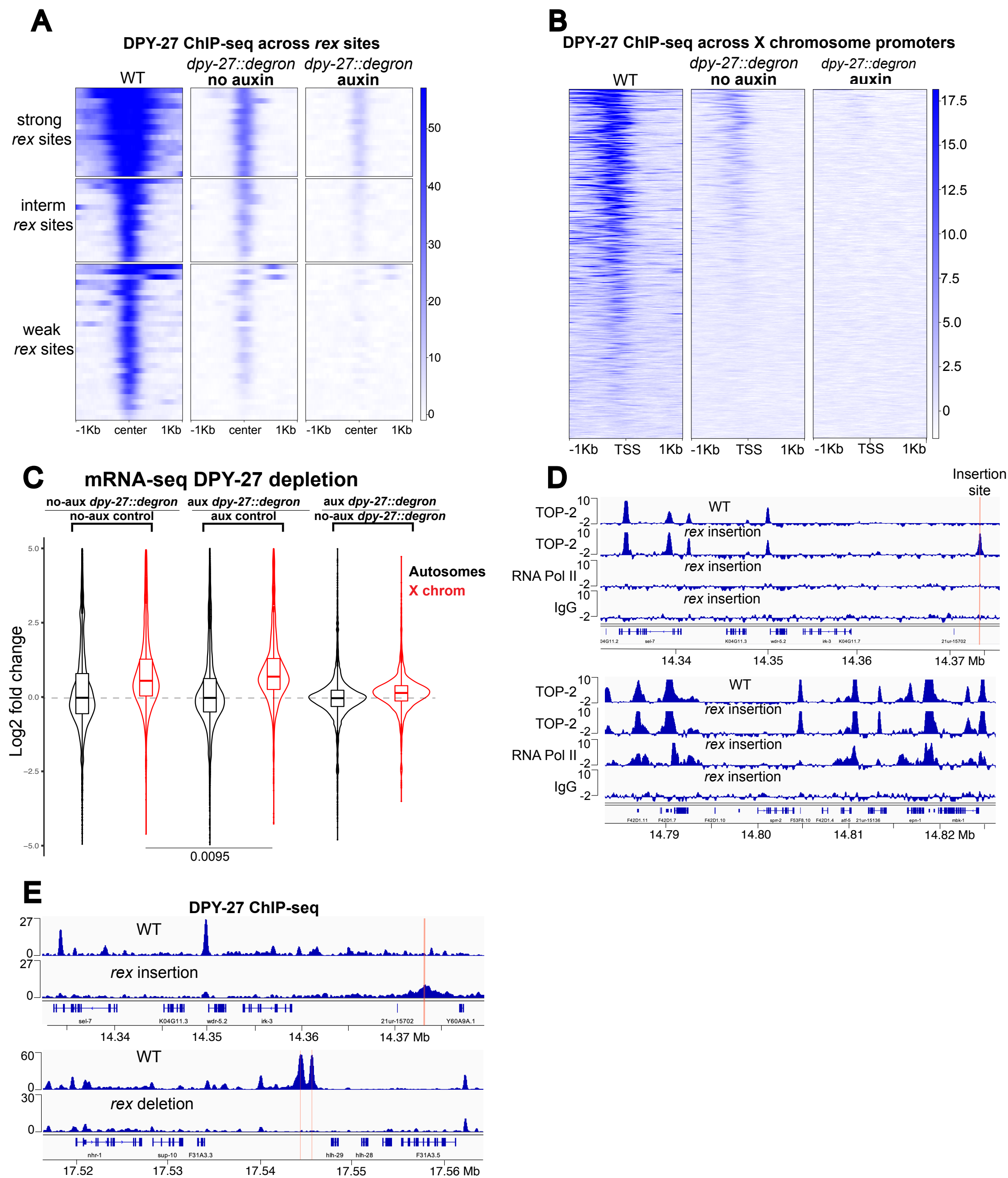


Figure S2. Characterization of *dpy-27::degron* strain. Related to Figure 2.

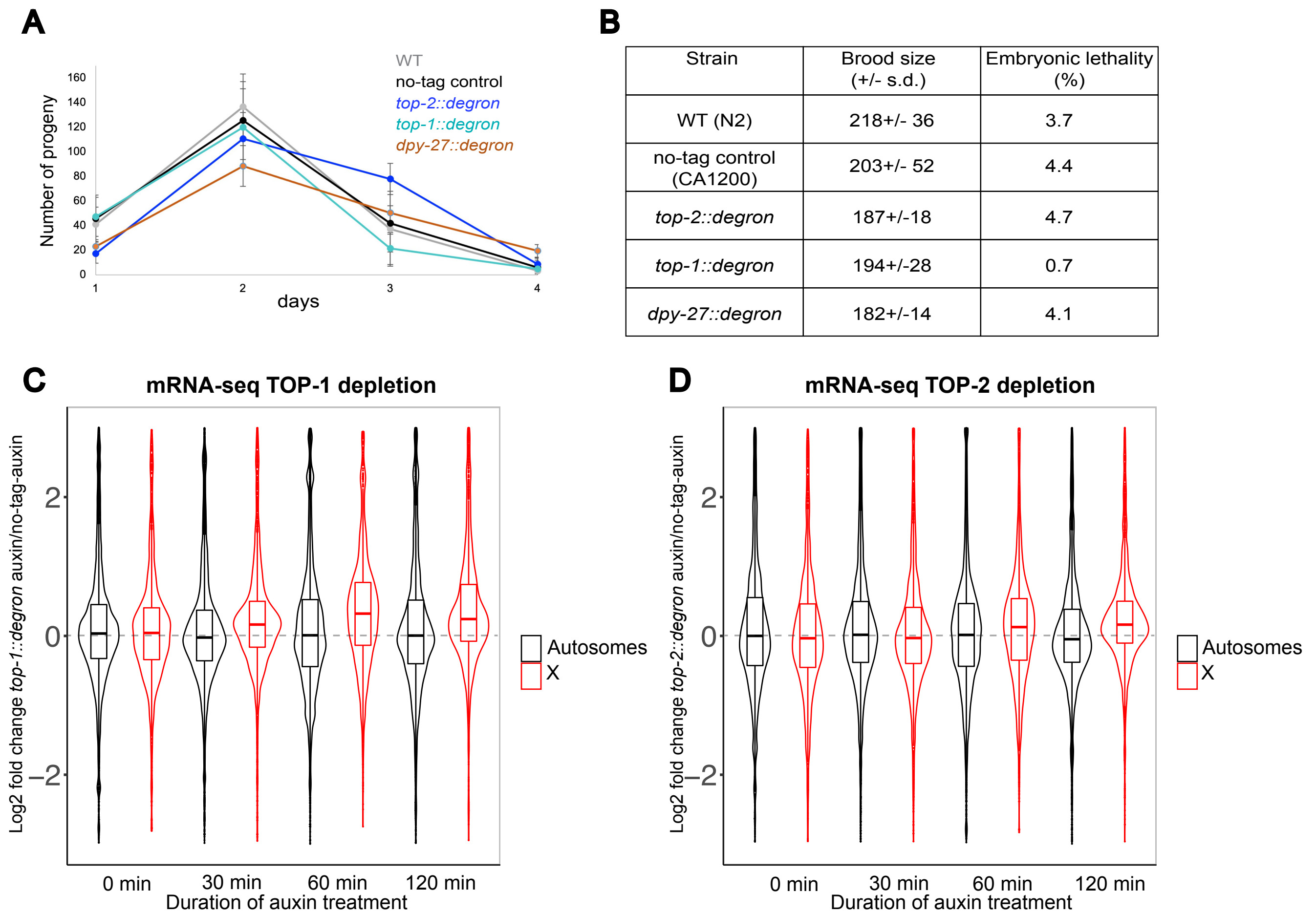
A) Heatmap showing DPY-27 ChIP-seq signal across a 2 Kb window centered around 64 *rex* sites previously described (Albritton et al., 2017). Data are from WT and *dpy-27::degron* L2/L3 worms without auxin and *dpy-27::degron* L2/L3 larvae treated with auxin for 60min. Insertion of the degron tag in the presence of TIR-1 reduced DPY-27 binding to the X chromosome without the addition of auxin. 1 hour of auxin treatment further reduced DPY-27.

B) Heatmap showing DPY-27 ChIP-seq signals across GRO-seq defined TSSs in the WT, *dpy-27::degron* strain without auxin and *dpy-27::degron* worms treated with auxin for 60min.

C) mRNA-seq data of *dpy-27::degron* worms that were treated with or without auxin. Fold changes were calculated between no-auxin *dpy-27::degron* and no-auxin control (left), 120 min auxin *dpy-27::degron* and 120 min auxin control (middle) and 120 min *dpy-27::degron* and no-auxin *dpy-27::degron* (right). The

distributions of log2 fold changes are shown for Autosomes and X chromosome. Two-tailed independent two-sample *t*-test p-values are indicated.
D) ChIP-seq profiles of TOP-2 in WT and TOP-2, RNA Pol-II and IgG in a strain carrying an ectopic *rex-8* insertion on the X chromosome. Profiles around the insertion site (top panel) and an additional representative (bottom panel) region of the X chromosome are shown.
E) DPY-27 ChIP-seq profiles in WT, in a strain carrying an ectopic *rex-8* insertion (top panel) and in a strain in which ~100 bp containing the two recruiting motifs of the endogenous *rex-41* have been deleted (lower panel). Profiles around the insertion and deletion sites are shown. Data in the insertion and deletion strains are from (Jimenez et al., 2021) and (Albritton et al., 2017), respectively.

Supplementary figure 3



Strain	Brood size (+/- s.d.)	Embryonic lethality (%)
WT (N2)	218+/- 36	3.7
no-tag control (CA1200)	203+/- 52	4.4
top-2::degron	187+/-18	4.7
top-1::degron	194+/-28	0.7
dpy-27::degron	182+/-14	4.1

Figure S3. Characterization of *top-1::degron* and *top-2::degron* strains. Related to Figure 3.
A) Number of viable progeny laid by one worm per day. Single L4 worms were placed in individual plates and transferred to a new plate every 24 hours for a total of 4 days. Viable progeny was counted after 48 hours of each transfer. The progeny of 8 worms of each genotype was counted. Mean values and standard deviation are reported.
B) Total brood size and embryonic lethality calculated as the mean percentage of unhatched eggs. Synchronized gravid adults were allowed to laid eggs on a 35mm plate and removed after 2 hours. The eggs laid on each plate were counted immediately after removing the parent and the viable progeny was quantified 48 hours later. The progeny of 8 worms of each genotype was analyzed.
C-D) mRNA-seq was performed after 30 min, 60 min and 120 min auxin-mediated depletion of TOP-1(C) and TOP-2 (D). Distribution of log2 fold changes between the degron-auxin and no-tag-auxin conditions are shown for autosomes and X chromosomes.

Supplementary figure 4

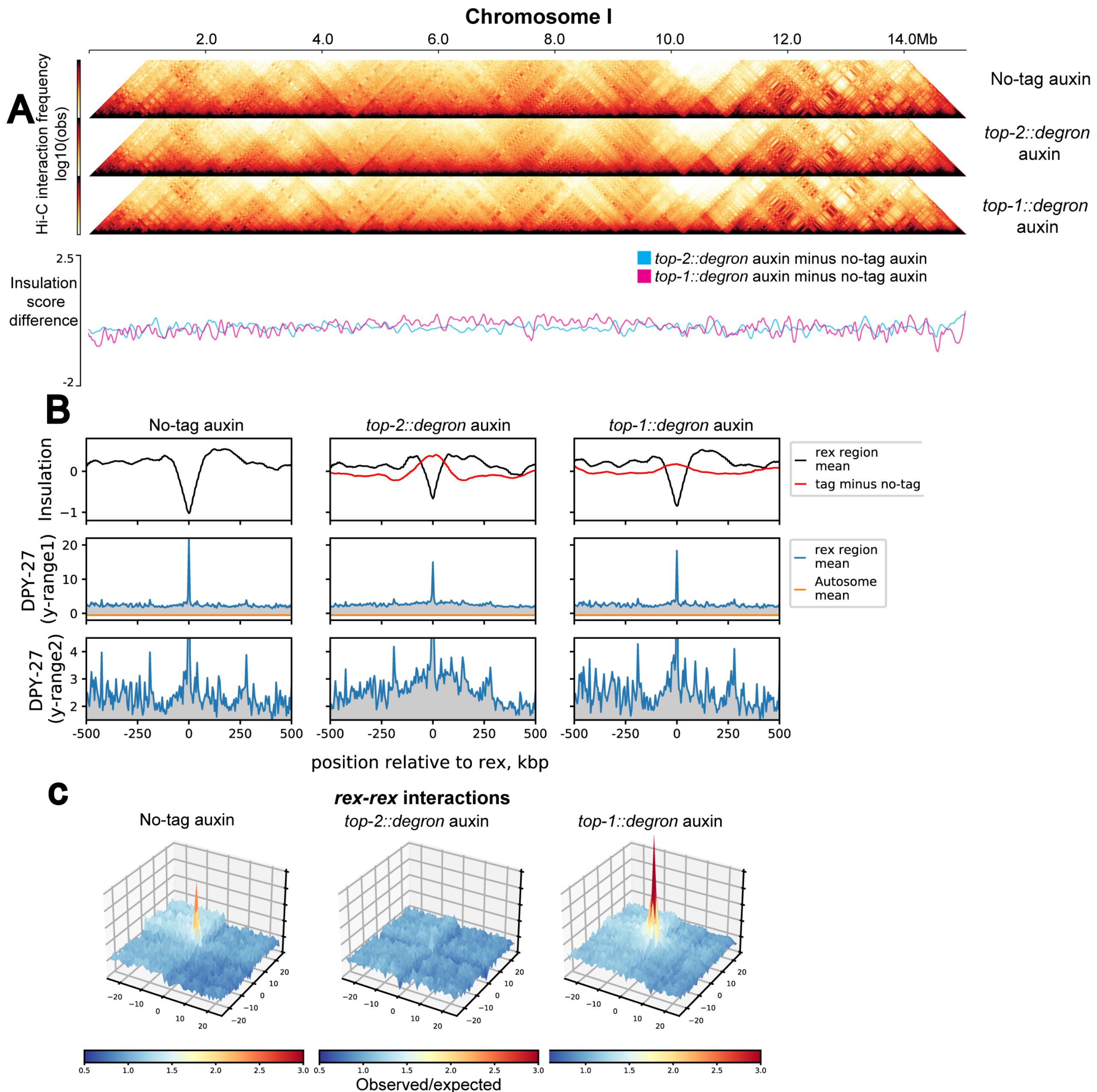


Figure S4. Hi-C analysis upon TOP-1 and TOP-2 depletion. Related to Figure 4.

A) Hi-C heatmap of chromosome I in no-tag auxin, *top-2::degron* auxin, and *top-1*::*degron* L2/L3 worms treated with auxin for 60 min. The difference in the insulation scores (subtraction) between the depletion condition and the control are shown below.

B) Average profile insulation score (black) and its change from control condition (red) across +/- 500 Kb regions surrounding the 17 strong *rex* sites. 500 Kb is chosen based on the mean genomic distance between strong *rex* sites being approximately 1Mb. DPY-27 ChIP-seq is shown below. For DPY-27 ChIP-seq data, normalized ChIP minus input coverage is shown. The blue line indicates its profile surrounding *rex* sites and the orange line indicates the mean value for all autosomes. Two different y-ranges are shown to accentuate changes in DPY-27 distribution pattern around *rex* sites.

C) Meta-dot plot showing the average strength of interactions between 250Kb windows centered at the 17 strong *rex* sites. For 17 strong *rex* sites, a total of 33 *rex-rex* pairs located within 3 Mb of each other were used.

Supplementary figure 5

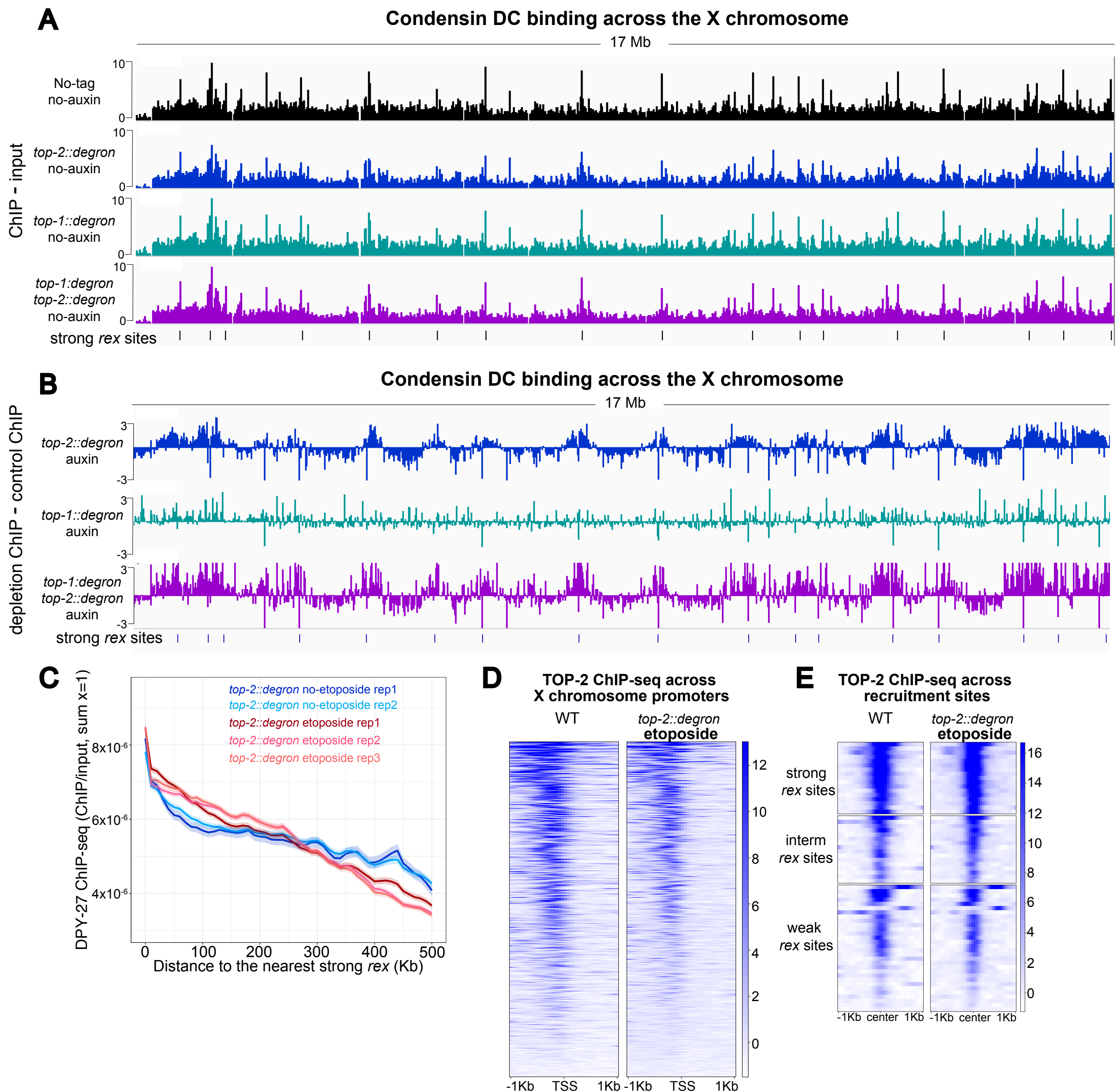


Figure S5. Analysis of DPY-27 binding upon TOP-1 and TOP-2 depletion. Related to Figure 5.

A) X chromosome view of DPY-27 ChIP-seq profile in control (no-tag), *top-2::degron*, *top-1::degron* and double *top-1::degron; top-2::degron* worms that were incubated in no-auxin plates for 60 minutes. Normalized ChIP minus input coverages are shown. Black lines at the bottom indicate the location of strong *rex* sites.

B) X chromosome view of DPY-27 ChIP-seq profile in TOP-2, TOP-1 and TOP-1;TOP-2 depleted worms. Coverages shown were obtained by subtracting the control coverage to the depleted condition coverage. Black lines at the bottom indicate the location of strong *rex* sites.

C) Metaplot of normalized DPY-27 ChIP-seq within a sliding window of 100 Kb and step size of 10 Kb moving away from strong *rex* sites for *top-2::degron* worms that were incubated for 60 min with or without etoposide. The main line indicates the average and the range indicates 95% confidence interval of each window.

D) Heatmap showing TOP-2 ChIP-seq signal across GRO-seq defined TSSs in WT worms and *top-2::degron* worms that were treated with etoposide for 60min. Etoposide treatment did not alter TOP-2 binding at promoters.
E) Heatmap showing TOP-2 ChIP-seq signal across a 2 Kb window centered around 64 *rex* sites previously described (Albritton et al., 2017) in WT worms and *top-2::degron* worms that were treated with etoposide for 60min. Etoposide treatment did not alter TOP-2 binding at *rex* sites.

Methods S1. R code for ChIP-seq sliding window analysis. Related to Figure 5D

```
library(dplyr)
library(ggplot2)
library(slider)
library(reshape2)
library(ggpubr)
library(IRanges)
options(scipen = 999)

## for plotting asthetics
addUnits <- function(n) {
  labels <- ifelse(n < 1000, n,  # less than thousands
                ifelse(n < 1e6, paste0(round(n/1e3), 'kb'),  # in thousands
                      ifelse(n < 1e9, paste0(round(n/1e6), 'Mb'),  # in millions
                            ifelse(n < 1e12, paste0(round(n/1e9), 'B'), # in billions
                                  ifelse(n < 1e15, paste0(round(n/1e12), 'T'), # in trillions
                                        'too big!'
                                  )))))
  return(labels)
}

## 'TSS' does not need to be TSS,
# any bed3-column + n number of value columns additional columns
# second 'rex' is bed3, to which each of 'TSS' rows will be assigned to
# ie. each row of 'TSS' is assigned to single closest row of 'rex'
assignTSStoNearestRex<- function(TSS,rex) {
  TSS_mid <- (TSS[,2]+TSS[,3])/2
  rex_mid <- (rex[,2]+rex[,3])/2
  TSS_rex_pair <- list()
  for (i in 1:nrow(TSS)){
    nearest_rex_i <- rex[abs(TSS_mid[i]-rex_mid)==min(abs(TSS_mid[i]-rex_mid)),]
    TSS_rex_pair[[i]] <- cbind(TSS[i,],nearest_rex_i)
  }
  do.call("rbind",TSS_rex_pair) %>% return
}

## generates submatrix, this is used to compute moving average on assignTSStoNearestRex output
Generate_submatrix <-  function(chrInfo,windowSize,stepSize) {
  output <- list()
  for (i in 1:length(chrInfo)) {
    nSteps <- (floor(as.numeric(chrInfo[[i]][2]))-windowSize)/stepSize
    V2=c(stepSize*(0:nSteps))
    V3=c(stepSize*(0:nSteps))+windowSize
    chr_i <- data.frame(V1=rep(chrInfo[[i]][1],nSteps+1),V2,V3)
    output[[chrInfo[[i]][1]]] <- chr_i
  }
  return(output)
}
#ce10 <- list(c("I",15072423),c("II",15279345),c("III",13783700),
#          c("IV",17493793),c("V",20924149),c("X",17718866))

#two column plus: 1st is position, second plus is value to average
MovingAverage <- function(twocolumn,windowSize,stepSize) {
  chrinfo <- list(c("NA",max(twocolumn[,1])))
  submat <- Generate_submatrix(chrinfo,windowSize,stepSize)$"NA"
  MovingAvg <- list()
  for (i in 1:nrow(submat)) {
```

```
    MovingAvg[[i]]<- data.frame(twocolumn[(twocolumn[,1]>submat[i,2])&(twocolumn[,1]<submat[i,3]),-1])
%>% colMeans
  }
  data.frame(submat[,c(2:3)],do.call("rbind",MovingAvg)) %>% return
}

# same as moving average instead computes confidence interval 95%
MovingCI <- function(twocolumn,windowSize,stepSize,CI_12) {
  chrinfo <- list(c("NA",max(twocolumn[,1])))
  submat <- Generate_submatrix(chrinfo,windowSize,stepSize)$"NA"
  MovingCI <- list()
  for (i in 1:nrow(submat)) {
    submat_i <- data.frame(twocolumn[(twocolumn[,1]>submat[i,2])&(twocolumn[,1]<submat[i,3]),-1])
    MovingCI[[i]]<-sapply(submat_i,function(x) t.test(x)$"conf.int"[CI_12])
  }
  data.frame(submat[,c(2:3)],do.call("rbind",MovingCI)) %>% return
}

# combined above functions into one big one
ChIPdecay_format <- function(rex,bin_bedgraph,windowSize,stepSize) {
  bin_X <- bin_bedgraph[bin_bedgraph[,1]=="chrX",]
  NumOfChIP <- ncol(bin_X)-3
  paired <- assignTSStoNearestRex(bin_X,rex[,c(1:3)])
  bin_mid <- (paired[,2]+paired[,3])/2
  rex_mid <- (paired[,sum(5,NumOfChIP)]+paired[,sum(6,NumOfChIP)])/2
  rexToBin <- abs(bin_mid-rex_mid)
  ChIPdecay <- data.frame(rexToBin,paired[,(4):(3+NumOfChIP)])
  ChIPdecay_cc <- ChIPdecay[complete.cases(ChIPdecay),]
  ChIPdecay_cc_sort <- ChIPdecay_cc[order(ChIPdecay_cc$rexToBin),]
  MovingAverage(ChIPdecay_cc_sort,windowSize,stepSize) %>% return
}


ChIPdecay_format_CI <- function(rex,bin_bedgraph,windowSize,stepSize,CI_12) {
  bin_X <- bin_bedgraph[bin_bedgraph[,1]=="chrX",]
  NumOfChIP <- ncol(bin_X)-3
  paired <- assignTSStoNearestRex(bin_X,rex[,c(1:3)])
  bin_mid <- (paired[,2]+paired[,3])/2
  rex_mid <- (paired[,sum(5,NumOfChIP)]+paired[,sum(6,NumOfChIP)])/2
  rexToBin <- abs(bin_mid-rex_mid)
  ChIPdecay <- data.frame(rexToBin,paired[,(4):(3+NumOfChIP)])
  ChIPdecay_cc <- ChIPdecay[complete.cases(ChIPdecay),]
  ChIPdecay_cc_sort <- ChIPdecay_cc[order(ChIPdecay_cc$rexToBin),]
  MovingCI(ChIPdecay_cc_sort,windowSize,stepSize,CI_12) %>% return
}

# sum chrX = 1
applyUnityX <- function(multi.tab) {
  M <- multi.tab[complete.cases(multi.tab),]
  M.x <- M[M[,1]=="chrX",]
  M.z <- sapply(M.x[,-c(1:3)],function(x) x/sum(x))
  cbind(M.x[,c(1:3)],M.z) %>% return
}
############################################## end of functions
##################################
#rm(list = ls())
```

```
rex <-
read.csv("C:/Users/kimj5/Desktop/annotation/sarah_strong_annot.bed",header=F,sep="\t",quote="",check.
names=F)
# bed file format: three column, chr, start, end

################################# main: strains with auxin or etoposide
######################
sample_orders <-
c("AKM189_AKM211","AKM104_AK148","AKM333_AKM335_AKM337","AKM171_AKM240","AKM219_AK
M221")
sample_labels <- c("No-tag auxin",
                "top-2::degron auxin",
                "top-2::degron etoposide, No-auxin",
                "top-1::degron auxin",
                "top-1::degron; top-2::degron auxin")
auxin_colors <- c("#000000","#0033CC","#AA0000","#009999","#9900CC")

auxin_bins <- read.csv("multibins_auxin_100_inputsubt.tab",header=T,sep="\t",quote="",check.names=T)
# output of deeptools multibigwig summary bins (--outRawCounts, -bs 100)
# applyUnityX divides each data point by the sum of values on X
# ChIPdecay_format params: applyUnityX output, bedfile, windowsize, stepsize
# CI1/2 params: same as ChIPdecay, but additional param 1 or 2 used to compute low or high bounds of
95% confidence interval

auxin_bins_f <- ChIPdecay_format(auxin_bins %>% applyUnityX,rex=rex,windowSize=100000,10000)
auxin_bins_f_CI1 <- ChIPdecay_format_CI(auxin_bins %>%
applyUnityX,rex=rex,windowSize=100000,10000,1)
auxin_bins_f_CI2 <- ChIPdecay_format_CI(auxin_bins %>%
applyUnityX,rex=rex,windowSize=100000,10000,2)

auxin_bins_f.m <- melt(auxin_bins_f[,-2],id.vars="V2")
auxin_bins_f_CI1.m <- melt(auxin_bins_f_CI1[,-2],id.vars="V2")
auxin_bins_f_CI2.m <- melt(auxin_bins_f_CI2[,-2],id.vars="V2")

auxin_combined_f.m <- auxin_bins_f.m %>%
  cbind(auxin_bins_f_CI1.m$value) %>%
  cbind(auxin_bins_f_CI2.m$value)
auxin_combined_f.m %>% head
colnames(auxin_combined_f.m) <- c('V2','variable','value','low','high')
auxin_combined_f.m$variable <- factor(auxin_combined_f.m$variable, levels = sample_orders)

gg_auxin_avg <- ggplot(auxin_combined_f.m %>% subset(V2<=500000),
       aes(V2,value,color=variable))+
  geom_line(aes(color=variable),size=1.2)+
  geom_ribbon(aes(ymin=low,
                ymax=high,fill=factor(variable)),
             linetype=0,alpha=0.2)+
  scale_x_continuous(labels=addUnits)+theme_bw()+
  scale_fill_manual(values=auxin_colors,names("samples"),labels=sample_labels)+
  scale_color_manual(values=auxin_colors,names("samples"),labels=sample_labels)+
  scale_y_continuous(labels = function(x) format(x, scientific = TRUE),
                   limits=c(3*10^-6, 9.5*10^-6))+
  ylab("DPY-27 ChIP-seq (sum X=1)")+
  xlab("Distance to the nearest strong rex")+
  ggtitle("auxin inputsubt: bin=100bp,window=100kb,step=10kb")
ggsave(plot=gg_auxin_avg,"gg_auxin_avg.pdf",device="pdf",dpi=800,width=10,height=6,unit="in")

####### no auxin avg ####
```

```
sample_orders_noAuxin <-
c("AKM188_AKM210","AKM102_AKM147","AKM170_AKM239","AKM218_AKM220")
sample_labels_noAuxin <- c("No-tag No-auxin",
                "top-2::degron No-auxin",
                "top-1::degron No-auxin",
                "top-1::degron; top-2::degron No-auxin")
noAuxin_colors <- c("#000000","#0033CC","#009999","#9900CC")

noAuxin_bins <-
read.csv("multibins_noAuxin_100_inputsubt.tab",header=T,sep="\t",quote="",check.names=T)
noAuxin_bins_f <- ChIPdecay_format(noAuxin_bins %>%
applyUnityX,rex=rex,windowSize=100000,10000)
noAuxin_bins_f_CI1 <- ChIPdecay_format_CI(noAuxin_bins %>%
applyUnityX,rex=rex,windowSize=100000,10000,1)
noAuxin_bins_f_CI2 <- ChIPdecay_format_CI(noAuxin_bins %>%
applyUnityX,rex=rex,windowSize=100000,10000,2)

noAuxin_bins_f.m <- melt(noAuxin_bins_f[,-2],id.vars="V2")
noAuxin_bins_f_CI1.m <- melt(noAuxin_bins_f_CI1[,-2],id.vars="V2")
noAuxin_bins_f_CI2.m <- melt(noAuxin_bins_f_CI2[,-2],id.vars="V2")

noAuxin_combined_f.m <- noAuxin_bins_f.m %>%
  cbind(noAuxin_bins_f_CI1.m$value) %>%
  cbind(noAuxin_bins_f_CI2.m$value)
noAuxin_combined_f.m %>% head
colnames(noAuxin_combined_f.m) <- c('V2','variable','value','low','high')
noAuxin_combined_f.m$variable <- factor(noAuxin_combined_f.m$variable, levels =
sample_orders_noAuxin)

gg_noAuxin_avg <- ggplot(noAuxin_combined_f.m %>% subset(V2<=500000),
      aes(V2,value,color=variable))+
  geom_line(aes(color=variable),size=1.2)+
  geom_ribbon(aes(ymin=low,
               ymax=high,fill=factor(variable)),
            linetype=0,alpha=0.2)+
  scale_x_continuous(labels=addUnits)+theme_bw()+
  scale_fill_manual(values=noAuxin_colors,names("samples"),labels=sample_labels_noAuxin)+
  scale_color_manual(values=noAuxin_colors,names("samples"),labels=sample_labels_noAuxin)+
  scale_y_continuous(labels = function(x) format(x, scientific = TRUE),
                  limits=c(3*10^-6, 9.5*10^-6))+
  ylab("DPY-27 ChIP-seq (sum X=1)")+
  xlab("Distance to the nearest strong rex")+
  ggtitle("No-auxin inputsubt: bin=100bp,window=100kb,step=10kb")
ggsave(plot=gg_noAuxin_avg,"gg_noAuxin_avg.pdf",device="pdf",dpi=800,width=10,height=6,unit="in")


#################### etop effect ################
sample_orders_etop <- c("AKM102", "AKM147", "AKM333", "AKM335", "AKM337")
sample_labels_etop <- c("top-2::degron No-auxin, AKM102",
                   "top-2::degron No-auxin, AKM147",
                   "top-2::degron No-auxin,etoposide AKM333",
                   "top-2::degron No-auxin,etoposide AKM335",
                   "top-2::degron No-auxin,etoposide AKM337")
etop_colors <- c("#0033CC","#00A9FF",
              "#AA0000","#FF68A1","#F8766D")

etop_bins <- read.csv("multibins_etop_100_inputsubt.tab",header=T,sep="\t",quote="",check.names=T)
head(etop_bins)
```

```
etop_bins_f <- ChIPdecay_format(etop_bins %>% applyUnityX,rex=rex,windowSize=100000,10000)
etop_bins_f_CI1 <- ChIPdecay_format_CI(etop_bins %>%
applyUnityX,rex=rex,windowSize=100000,10000,1)
etop_bins_f_CI2 <- ChIPdecay_format_CI(etop_bins %>%
applyUnityX,rex=rex,windowSize=100000,10000,2)

etop_bins_f.m <- melt(etop_bins_f[,-2],id.vars="V2")
etop_bins_f_CI1.m <- melt(etop_bins_f_CI1[,-2],id.vars="V2")
etop_bins_f_CI2.m <- melt(etop_bins_f_CI2[,-2],id.vars="V2")

etop_combined_f.m <- etop_bins_f.m %>%
  cbind(etop_bins_f_CI1.m$value) %>%
  cbind(etop_bins_f_CI2.m$value)

colnames(etop_combined_f.m) <- c('V2','variable','value','low','high')
etop_combined_f.m$variable <- factor(etop_combined_f.m$variable, levels = sample_orders_etop)


gg_etoposide_reps<- ggplot(etop_combined_f.m %>% subset(V2<=500000),
       aes(V2,value,color=variable))+
  geom_line(aes(color=variable),size=1.2)+
  geom_ribbon(aes(ymin=low,
                ymax=high,fill=factor(variable)),
            linetype=0,alpha=0.2)+
  scale_x_continuous(labels=addUnits)+theme_bw()+
  scale_fill_manual(values=etop_colors,names("samples"),labels=sample_labels_etop)+
  scale_color_manual(values=etop_colors,names("samples"),labels=sample_labels_etop)+
  scale_y_continuous(labels = function(x) format(x, scientific = TRUE),
                  limits=c(3*10^-6, 9.5*10^-6))+
  ylab("DPY-27 ChIP-seq (sum X=1)")+
  xlab("Distance to the nearest strong rex")+
  ggtitle("etoposide inputsubt: bin=100bp,window=100kb,step=10kb")

ggsave(plot=gg_etoposide_reps,"gg_etoposide_reps.pdf",device="pdf",dpi=800,width=10,height=6,unit="in
")
```