Note: the bioinformatics scripts conclude shell and R scripts which are given in directories named "shell_scripts"and "R_scripts" respectively. R custom functions used in R scripts are listed in directory named "R_functions". Please source the related funcitons befor perform R script. In this text, shell scripts, R scripts, R custom functions and figure names are marked by green, orange, blue and red color respectively to facilitate understanding. In addition to, the provided scripts here may require additional steps and modifications to perform well on different platforms.

1.analysis of RNAseq data for atino80-5 mutant at white light

1)copy fastq.gz file to the current directory

cp /mnt/USB1/WL_atino80/*.gz ./

2)trim adapters, map reads to genome, sort bam files, get bigwig files and align reads to genes

sh **RNAseq_paired_stranded.sh**

3)get differentially expressed genes comparing mutant with wild-type

Rscript **RNAseq_DEG_atino80_WL.r**

2.overlap between AtINO80-regulated and light-induced genes for Figure 3A-3C

1)use R to get venn diagram and p value for two sets of genes, analyze GO functions for overlap genes

source("**OverlapPlot_function.r**")# import function firstly

Plot("/Volumes/OpheliaData/atino80/atino80_0317/light_regulate_genes/light_INO80_5/","/Volumes/OpheliaData/atino80/atino80_0317/light_regulate_genes/light_regulate/PNAS/up_all.xls","/Volumes/OpheliaData/atino80/atino80_0317/RNAseq/WL_atino80/DEG/15bei_pvalue/WLino80_diff.xls","light_induced","atino80_diff","atino80-diff and light_induced")

2)use R to get bubble figure for GO analysis result

source("**GOBubblePlot_function.r**")# import function firstl

BubblePlot("/Volumes/OpheliaData/atino80/atino80_0317/light_regulate_genes/light_INO80_5/GO/","/Volumes/OpheliaData/atino80/atino80_0317/light_regulate_genes/light_INO80_5/GO/GO_choose_term.txt",c(0,50),c(1,18),c(1,3,6,9))

3.analysis of RNAseq data for atino80-6 mutant at dark

1)copy fastq.gz file to the current directory

cp /mnt/USB1/Dark_atino80/sequence_2/*.gz ./

2)trim adapters, map reads to genome, sort bam files, get bigwig files and align reads to genes

sh **RNAseq_paired_unstranded.sh**

3)get differentially expressed genes comparing mutant with wild-type

Rscript **RNAseq_DEG_atino80_dark.r**

4. overlap between AtINO80-regulated and dark-induced genes for Figure 3D-3F

1)use R to get venn diagram and p value for two sets of genes, analyze GO functions for overlap genes

source("**OverlapPlot_function.r**")# import function firstly

Plot("/Volumes/OpheliaData/atino80/atino80_0317/light_regulate_genes/light_DarkINO80_

5/","/Volumes/OpheliaData/atino80/atino80_0317/light_regulate_genes/light_regulate/PNAS/down_all.xls","/Volumes/OpheliaData/atino80/atino80_0317/RNAseq/Dark_atino80_sequence2/DEG/15bei_pvalue/Darkino80_diff.xls","light_repressed","atino80_diff","atino80-diff and light_repressed")

2)use R to get bubble figure for GO analysis result

source("**GOBubblePlot_function.r**")# import function firstly

BubblePlot("/Volumes/OpheliaData/atino80/atino80_0317/light_regulate_genes/light_DarkINO80_5/GO/","/Volumes/OpheliaData/atino80/atino80_0317/light_regulate_genes/light_DarkINO80_5/GO/GO_choose_term.txt",c(0,20),c(1,14),c(0,3,6))

5.analysis of ChIPseq data for atino80-5 mutant at white light

1)copy fastq.gz file to the current directory

cp /mnt/USB1/ara_ino80_H2AZ_repeat2/*.gz ./

2)map reads to genome, sort bam files, remove duplicates, get bigwig files and bed files

sh **ChIPseq_paired_nocut.sh**

3)get fragment size for Figure S8

sh **FragmentSize.sh**

4)find enriched regions (peaks) of histone variants (H2A.Z and H3)

sh **PeaksSicer.sh**

5)find overlap peaks between two replicates by R

source("**PeakOverlap_function.r**")# import function firstly

setwd("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/overlappeaks/")

Peakover("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/rawpeak/sig_WT1_H2AZ_repeat1.bed","/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/rawpeak/sig_WT1_H2AZ_repeat2.bed","WT1_repeat1","WT1_repeat2","WT1_H2AZ_repeats_overlap_peaks")

Peakover("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/rawpeak/sig_ino80_H2AZ_repeat1.bed","/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/rawpeak/sig_ino80_H2AZ_repeat2.bed","ino80_repeat1","ino80_repeat2","ino80_H2AZ_repeats_overlap_peaks")

Peakover("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/rawpeak/sig_WT1_H3_repeat1.bed","/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/rawpeak/sig_WT1_H3_repeat2.bed","WT1_repeat1","WT1_repeat2","WT1_H3_repeats_overlap_peaks")

Peakover("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/rawpeak/sig_ino80_H3_repeat1.bed","/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/rawpeak/sig_ino80_H3_repeat2.bed","ino80_repeat1","ino80_repeat2","ino80_H3_repeats_overlap_peaks")

6)remove the prefix "chr" of seqnames in overlap enrichment peaks

```
ls -1 *.bed |while read id
do
echo "${id%.bed}"
awk -F "hr" '{print    $2}' ${id} >    ${id%.bed}_nochr.bed
done
```

wc -l *_nochr.bed

7)get merged bam files, bigwig files and bed files

sh **merge.sh**

6.analysis of published ChIPseq data (GSE108450)

1)download data from NCBI, map reads to genome, sort bam files, remove duplicates and get bigwig files and bed files

sh **ChIPseq_single_nocut.sh**

2)find enriched regions (peaks) of H2A.Z

sh **PeakSicer_GSE108450.sh**

3)find overlap peaks between two replicates by R

source("**PeakOverlap_function.r**")# import function firstly

setwd("/Volumes/OpheliaData/atino80/atino80_0317/published_ChIPseq/ara/peaks/overlappeaks/")

Peakover("/Volumes/OpheliaData/atino80/atino80_0317/published_ChIPseq/ara/peaks/repeats/sig_SRR6412321.bed","/Volumes/OpheliaData/atino80/atino80_0317/published_ChIPseq/ara/peaks/repeats/sig_SRR6412322.bed","WT1_repeat1","WT1_repeat2","WT1_H2AZ_repeats_overlap_peaks")

4)remove the prefix "chr" of seqnames in overlap enrichment peaks

```
ls -1 *.bed |while read id
do
echo "${id%.bed}"
awk -F "hr" '{print    $2}' ${id} >    ${id%.bed}_nochr.bed
done
wc -l *_nochr.bed
```

7. compare H2A.Z peaks in our ChIPseq data with published data for Figure S5C

source("**PeakOverlapTest_function.r**")# import function firstly

setwd("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/overlappeaks/compareWithPublised/")

Peakover("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/overlappeaks/WT1_H2AZ_repeats_overlap_peaks_nochr.bed","/Volumes/OpheliaData/atino80/atino80_0317/published_ChIPseq/ara/peaks/overlappeaks/WT1_H2AZ_repeats_overlap_peaks_nochr.bed","our","published","WTH2AZ_enriched_overlap_peaks_our_published")

8.fragment size for Figure S5B

sh **FragmentSize.sh**

9.normolized reads density of histone variants(H2AZ and H3) across genes for Figure 4A-4B and 5A-5C

1)get matrix of normolized reads density on gene body, around TSS and TES

sh **matrix.sh**

2)get matrix for heatmap by R

Rscript **HeatmapMatrix.r**

3)plot heatmap

sh **HeatmapPlot.sh**

4)plot average density profile by R

Rscript **PlotProfile.r**

10.differentially enriched regions of histone modifcations (H2A.Z and H3) for Figure 4C-4E and Figure S6.

1)get fold change of histone variants(H2AZ and H3) on enrichment regions (peaks) in mutant and wild-type

sh **PeaksSicerDiff.sh**

2)find differentially enriched regions by R

source("**DiffPeak_function.r**")# import function firstly

DiffPeak1("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/","H2AZ","/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/FCH2AZ_merge_nochr.summary",1.2,0.05)

DiffPeak1("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/","H3","/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/FCH3_merge_nochr.summary",1.2,0.05)

3)annotate differentially enriched regions by R

source("**PeakAnnotate_function.r**")# import function firstly

setwd("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/annotation/")

peakanno("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff./merge/H2AZ_sigPeakdown.xls","H2AZ_downpeak")

peakanno("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff./merge/H2AZ_sigPeakup.xls","H2AZ_uppeak")

peakanno("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff./merge/H3_sigPeakdown.xls","H3_downpeak")

peakanno("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/H3_sigPeakup.xls","H3_uppeak")

4)plot venn diagram and perform GO analysis for the overlap between H2A.Z, H3 diff-peaks and AtINO80-regulated genes

source("**VennPlot1_function.r**")# import function firstly

VennPlot1("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/venn_histiondiff_with_expressiondiff/atino80_diff/","/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/annotation/H2AZ_diffpeak_annoOvgene_genelist.xls","/Volumes/OpheliaData/atino80/atino80_0317/RNAseq/WL_atino80/DEG/15bei_pvalue/WLino80_diff.xls","H2AZ_diff","atino80-diff","H2AZdiff_and_atino80diff")

VennPlot1("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/venn_histiondiff_with_expressiondiff/atino80_diff/","/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/annotation/H3_diffpeak_annoOvgene_genelist.xls","/Volumes/OpheliaData/atino80/atino80_0317/RNAseq/WL_atino80/DEG/15bei_pvalue/WLino80_diff.xls","H3_diff","atino80-diff","H3diff_and_atino80diff")

5)plot venn diagram for the overlap between H2A.Z diff-peaks and H3 diff-peaks

source("**VennPlot2_function.r**")# import function firstly

VennPlot2("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/venn_histiondiff_with_expressiondiff/H2AZdiff_H3diff/","/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/annotation/H2AZ_diffpeak_annoOvgene_genelist.xls","/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/sicer_diff/merge/annotation/H3_diffpeak_annoOvgene_genelist.xls","H2AZ_diff","H3_diff","H2AZdiff_and_H3diff")

11.differentially enriched regions of H2A.Z/H3 for Figure 4F

1)find differentially enriched regions of H2A.Z considering H3 as input

Rscript **DiffBind_H2AZvsH3.r**

2)plot MAplot by R

Rscript **MAplot.r**

3)annotate differentially enriched regions

source("**PeakAnnotate_function.r**")# import function firstly

setwd("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/diffbind/overlappeak/annotation/")

peakanno("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/diffbind/overlappeak/H2AZ_H3_Down_nochr.xls","H2AZ:H3_downpeak")

peakanno("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/diffbind/overlappeak/H2AZ_H3_Up_nochr.xls","H2AZ:H3_uppeak")

12.enrichment analysis of H2AZ/H3 differentially enriched regions on DEGs in atino80-5 for Figure 4G and 4H

source("**EnrichmentAnalyze_function.r**")# import function firstly

setwd("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/diffbind/overlappeak/relation_with_diffregulatedgenes/")

EnrichAna("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/diffbind/overlappeak/annotation/H2AZ:H3_downpeak_annoOvgene_genelist.xls","/Volumes/OpheliaData/atino80/atino80_0317/RNAseq/WL_atino80/DEG/15bei_pvalue/WLino80_up.xls","downH2AZ:H3","up-regulated genes","H2AZ:H3down_genes_with_upregulated")

EnrichAna("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/diffbind/overlappeak/annotation/H2AZ:H3_downpeak_annoOvgene_genelist.xls","/Volumes/OpheliaData/atino80/atino80_0317/RNAseq/WL_atino80/DEG/15bei_pvalue/WLino80_down.xls","downH2AZ:H3","down-regulated genes","H2AZ:H3down_genes_with_downregulated")

EnrichAna("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/diffbind/overlappeak/annotation/H2AZ:H3_uppeak_annoOvgene_genelist.xls","/Volumes/OpheliaData/atino80/atino80_0317/RNAseq/WL_atino80/DEG/15bei_pvalue/WLino80_up.xls","upH2AZ:H3","up-regulated genes","H2AZ:H3up_genes_with_upregulated")

EnrichAna("/Volumes/OpheliaData/atino80/atino80_0317/ChIPseq2/peaks_sicer2/diffbind/overlappeak/annotation/H2AZ:H3_uppeak_annoOvgene_genelist.xls","/Volumes/OpheliaData/atino80/atino80_0317/RNAseq/WL_atino80/DEG/15bei_pvalue/WLino80_down.xls","upH2AZ:H3","down-regulated genes","H2AZ:H3up_genes_with_downregulated")