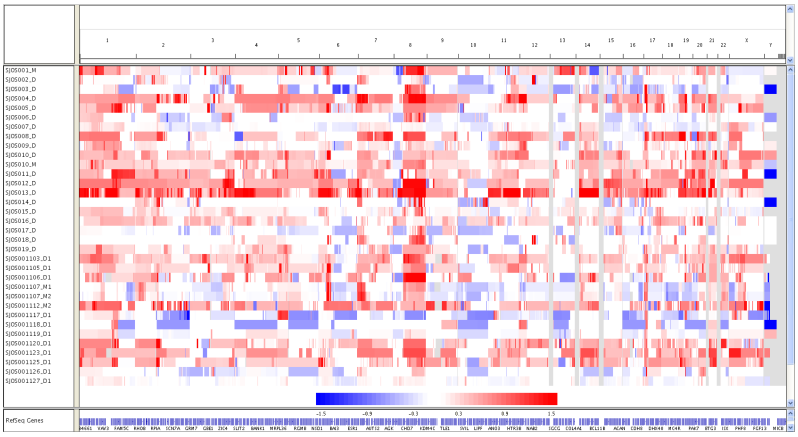
SUPPLEMENTAL DATA

A



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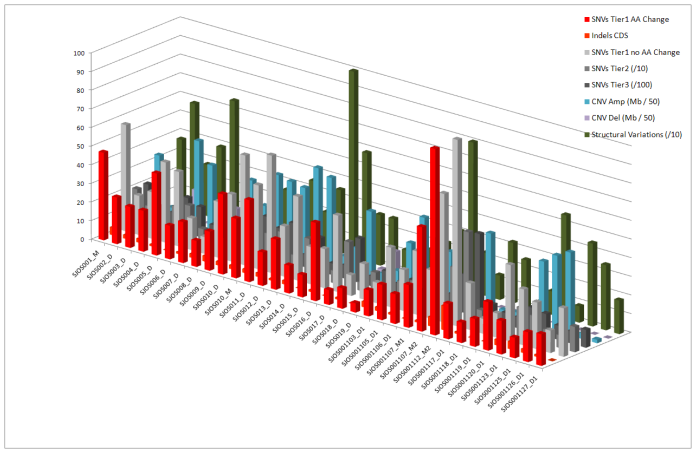

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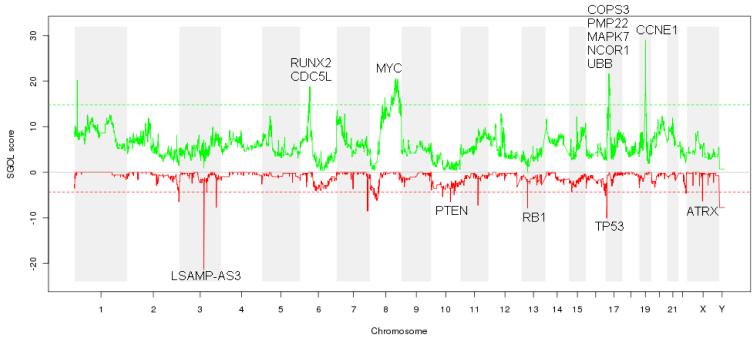

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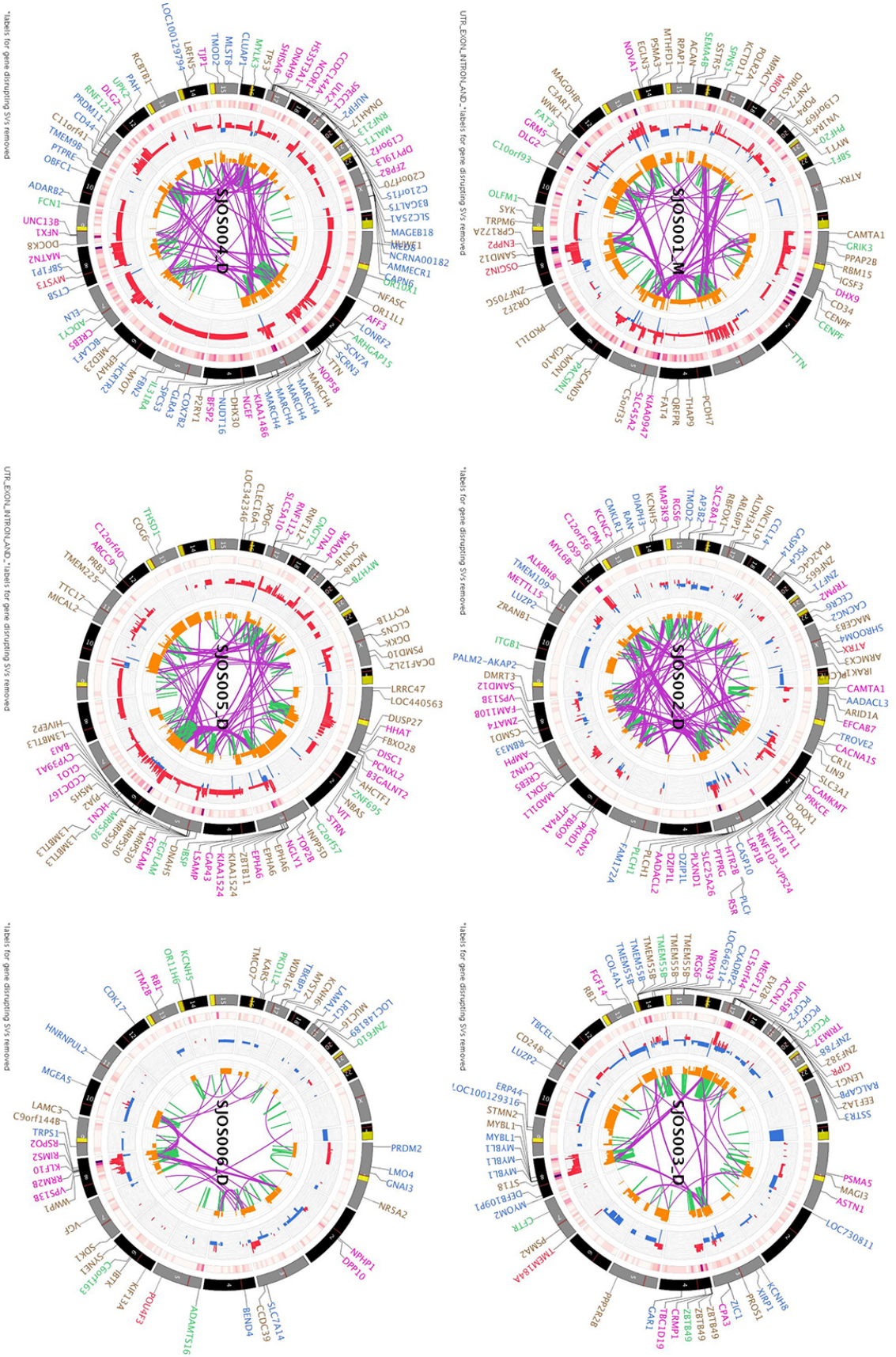

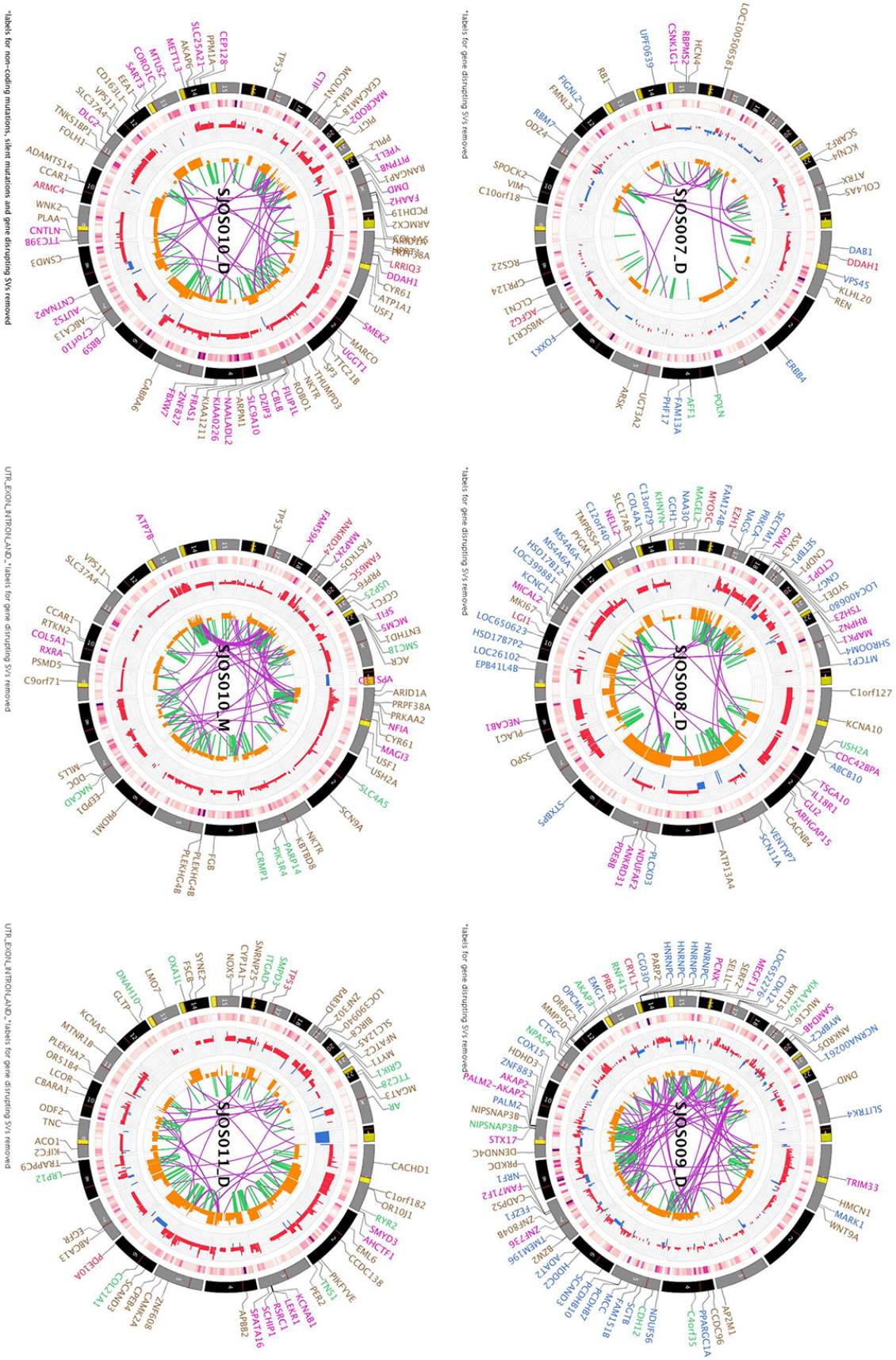
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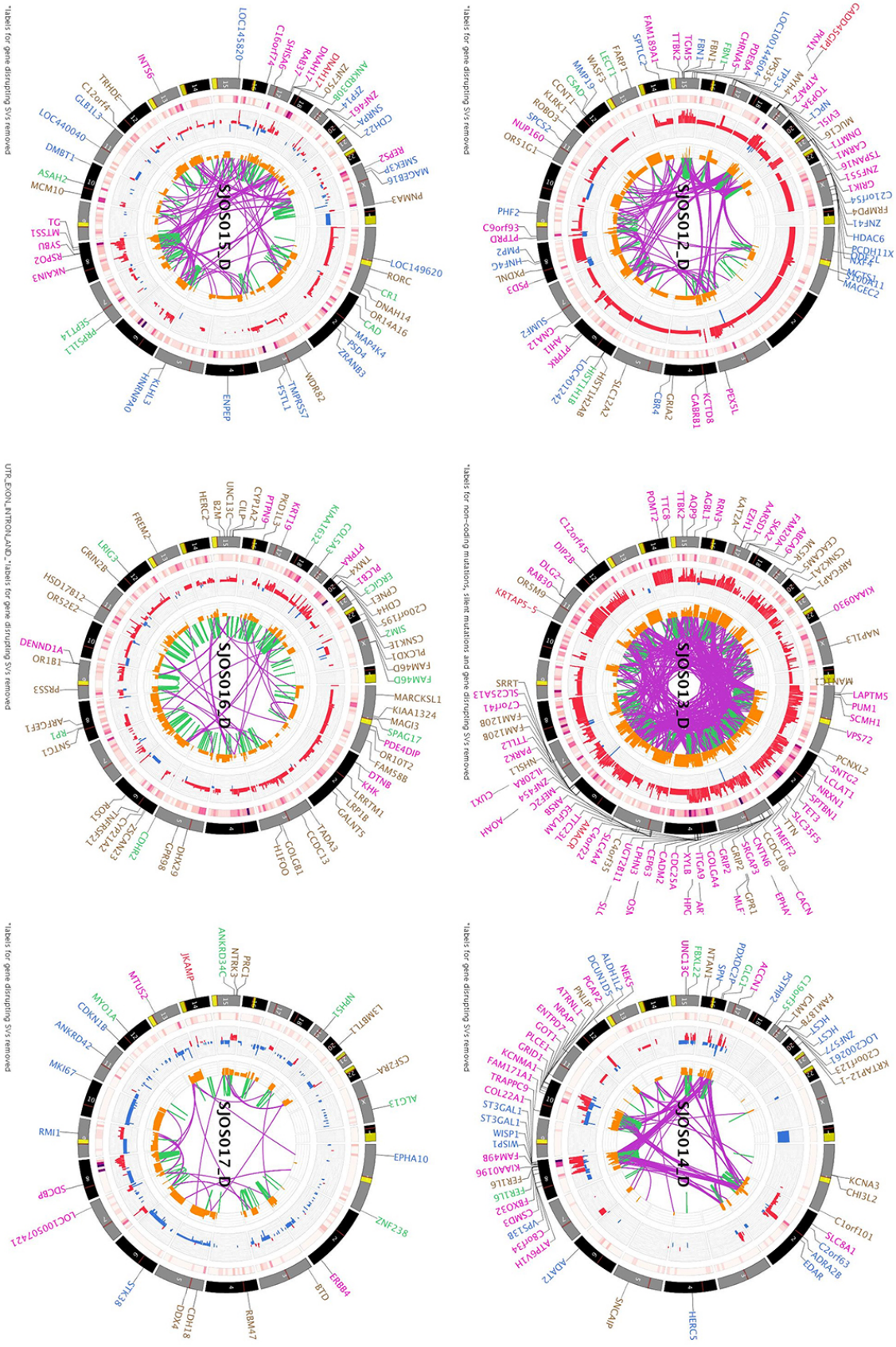
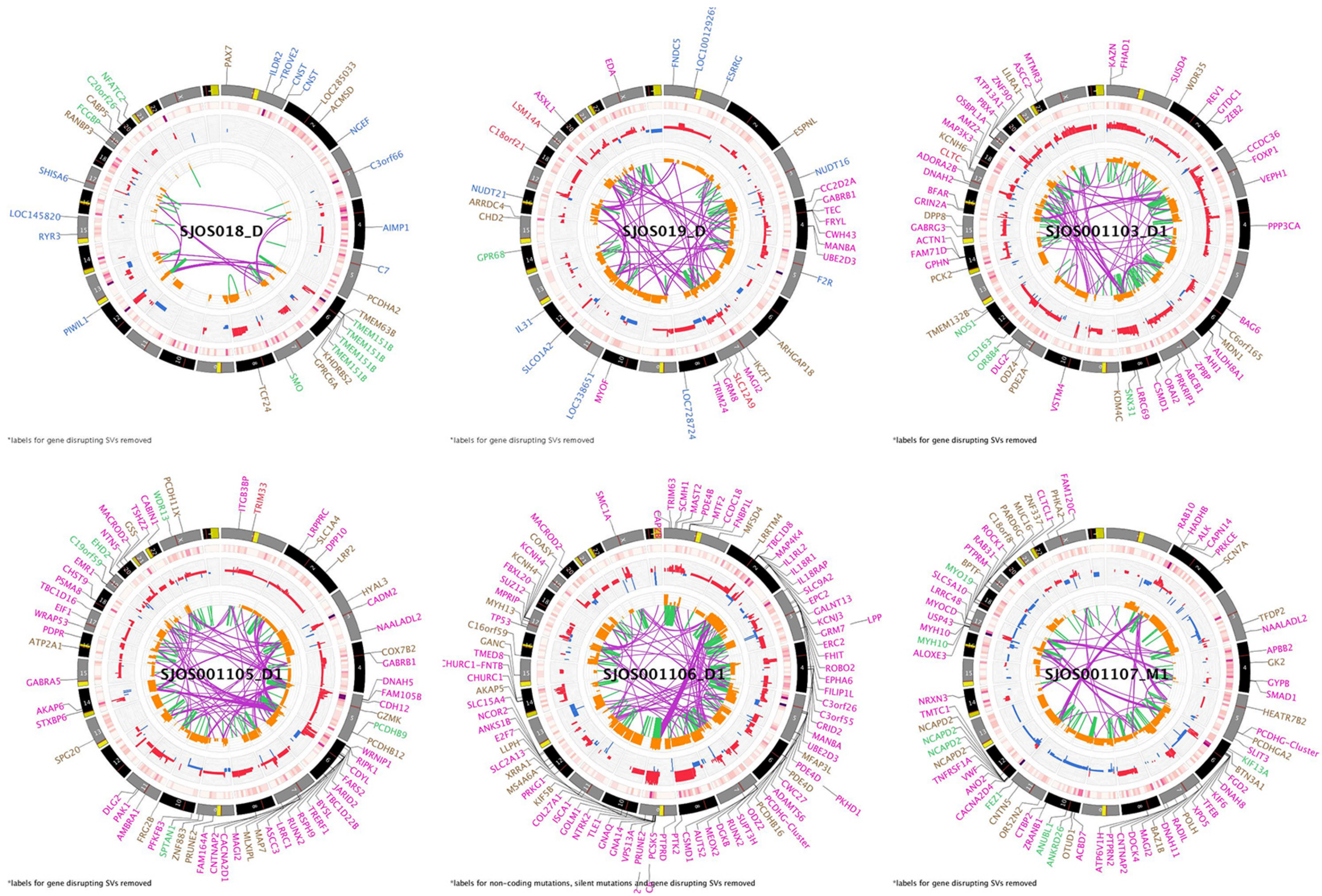
*labels for gene disrupting SVs removed
SJOS007_D
UTR_EXON_INTRON_LAND, *labels for gene disrupting SVs removed
SJOS010_M
*labels for gene disrupting SVs removed
SJOS008_D
UTR_EXON_INTRON_LAND, *labels for gene disrupting SVs removed
SJOS011_D
*labels for gene disrupting SVs removed
SJOS009_D


*labels for gene disrupting SVs removed
SJOS012_D
*labels for gene disrupting SVs removed
SJOS015_D
*labels for non-coding mutations, silent mutations and gene disrupting SVs removed
SJOS013_D
UTR_EXON_INTRON_LAND; *labels for gene disrupting SVs removed
SJOS016_D
*labels for gene disrupting SVs removed
SJOS014_D
*labels for gene disrupting SVs removed
SJOS017_D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*labels for gene disrupting SVs removed
SJOS019_D
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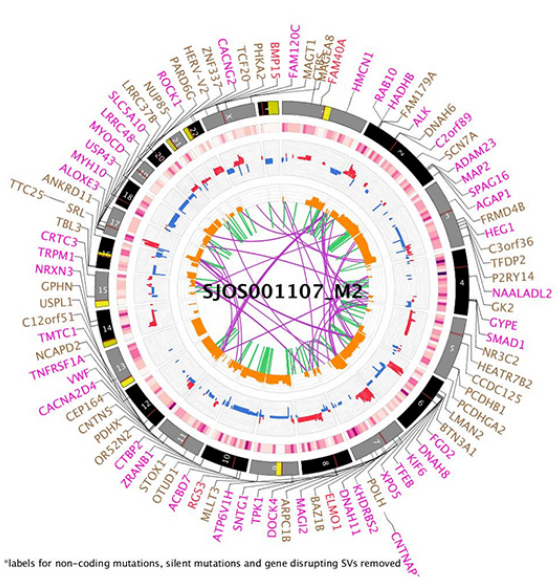
SJOS001103_D1
*labels for gene disrupting SVs removed
SJOS001105_D1
*labels for gene disrupting SVs removed
SJOS001106_D1
*labels for non-coding mutations, silent mutations and gene disrupting SVs removed
SJOS001107_M1
*labels for gene disrupting SVs removed



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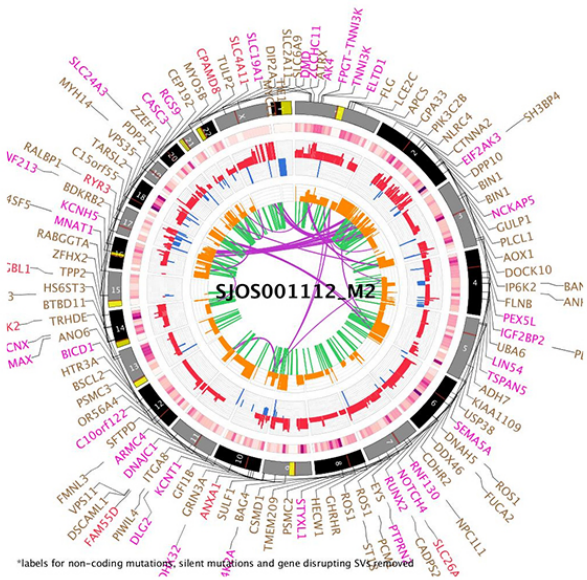
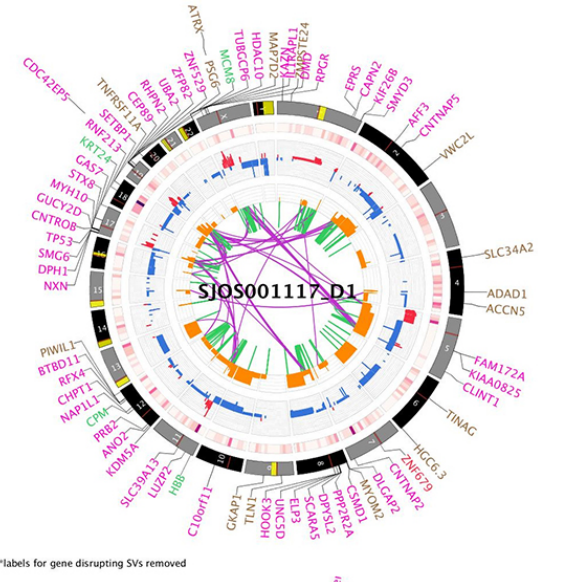
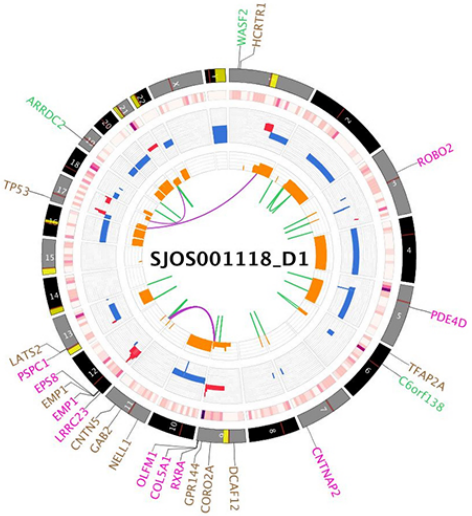


*labels for non-coding mutations, silent mutations and gene disrupting SVs removed



*labels for gene disrupting SVs removed



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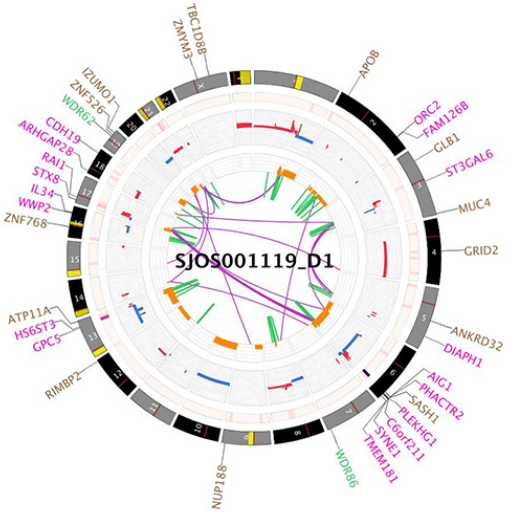
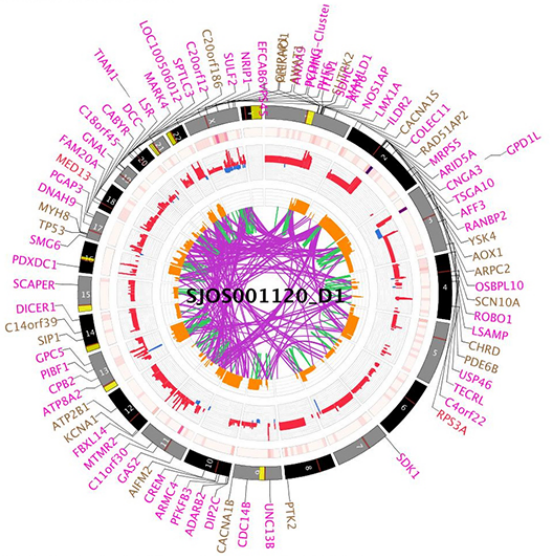


*labels for gene disrupting SVs removed



*labels for non-coding mutations, silent mutations and gene disrupting SVs removed

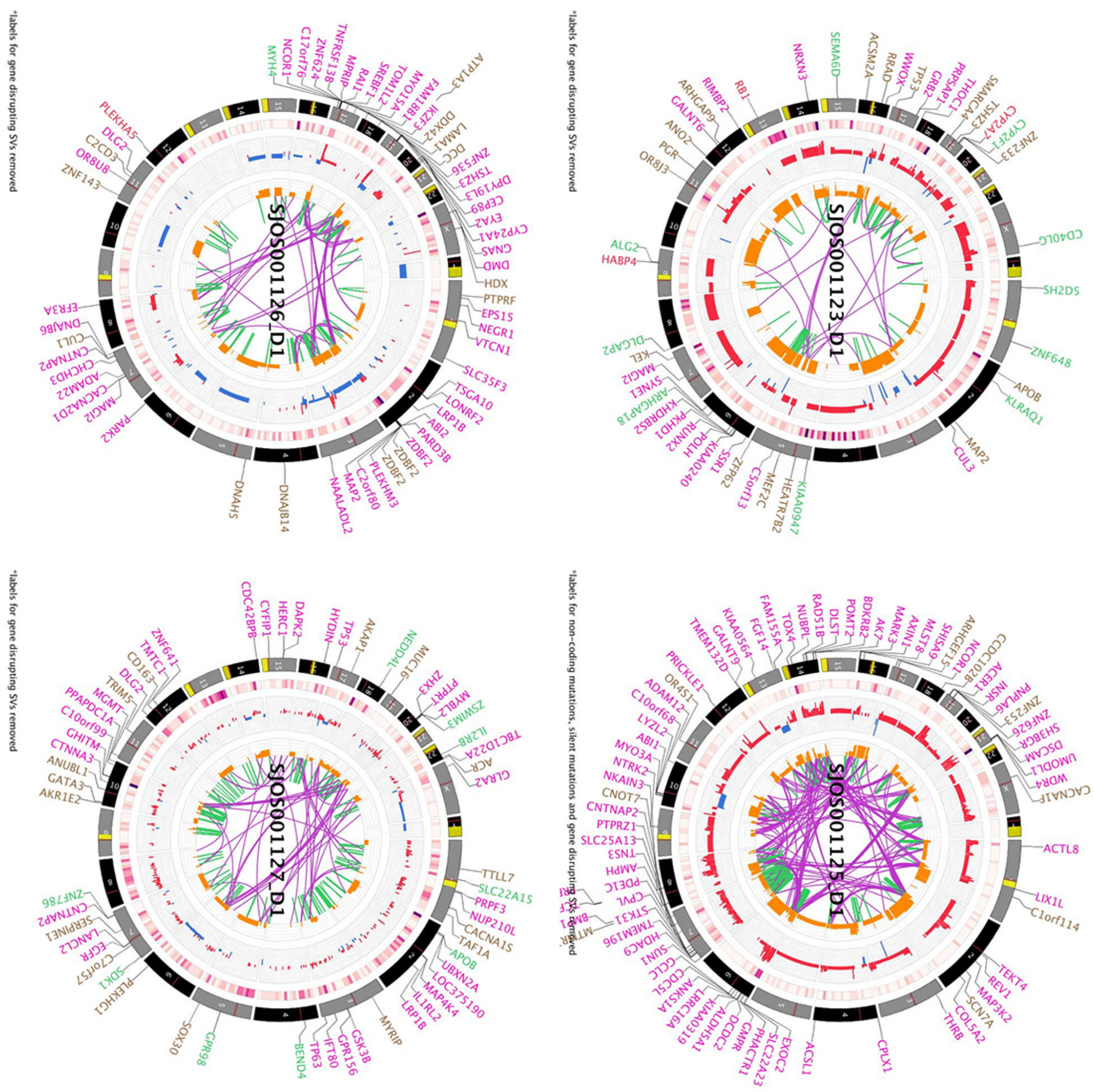

SJOS001126_D1
*labels for gene disrupting SVs removed
SJOS001123_D1
*labels for gene disrupting SVs removed
SJOS001127_D1
*labels for gene disrupting SVs removed
SJOS001125_D1
*labels for non-coding mutations, silent mutations and gene disrupting SVs removed

Figure S1 related to Figure 1. Copy number analysis of osteosarcoma discovery cohort. A) Copy number analysis for all 34 osteosarcomas in the discovery cohort with red indicating gain and blue indicating loss. **B)** Histogram of the type of mutations (SNV, CNV, SV, indel) across the osteosarcoma tumors in the discovery cohort. **C)** GISTIC analysis of the copy number changes in the osteosarcoma discovery cohort with green indicating gains and red indicating loss. The dashed lines represent cutoff for statistical significance and the individual chromosomes are labeled along the bottom of the plot. **D)** CIRCOS plots of all 34 tumors sequenced by WGS in this study.

Table S1 related to Figure 1. Clinical features of discovery and validation cohorts.

Provided as a separate file.

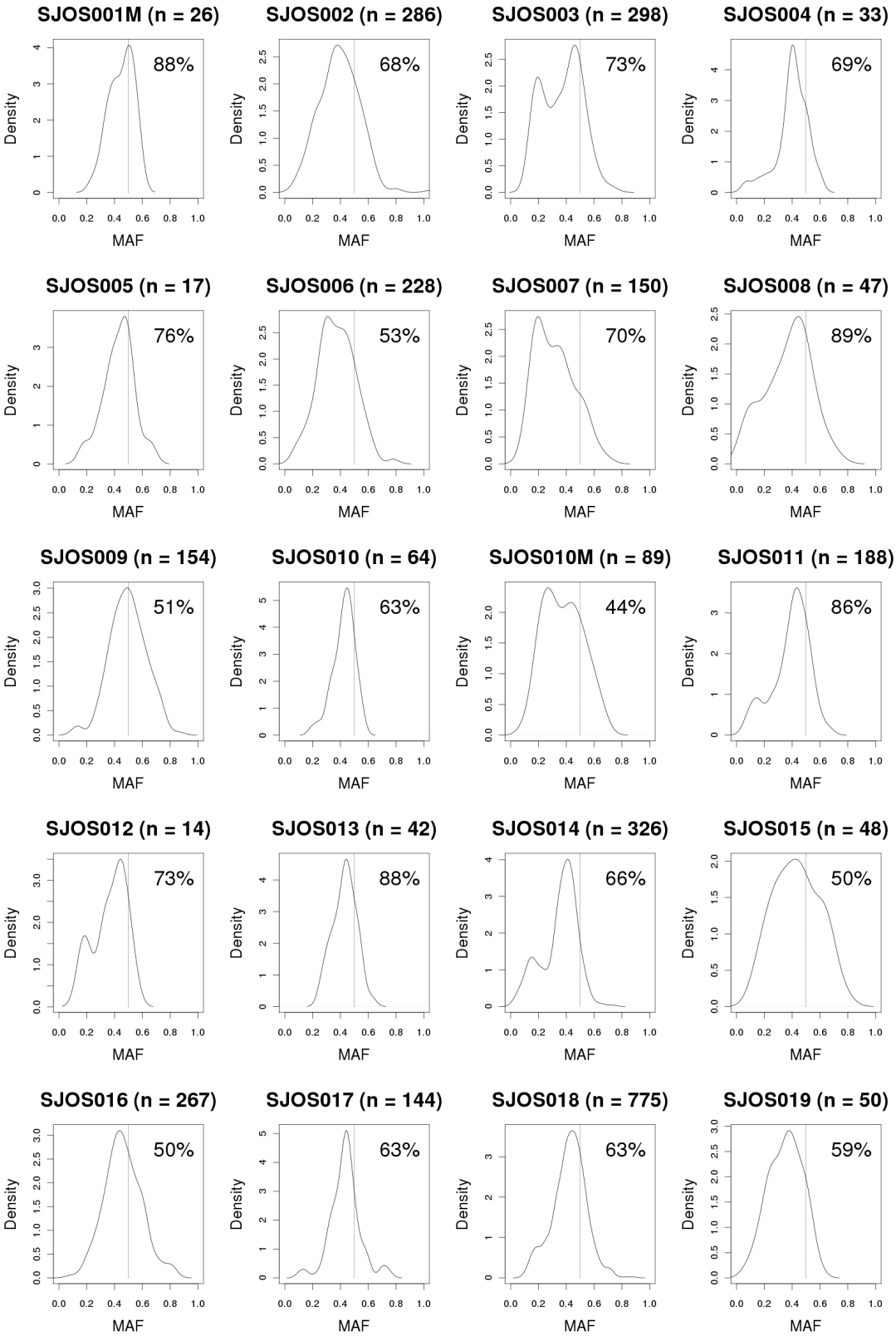
Table S2 related to Figure 1. Sequence coverage data.

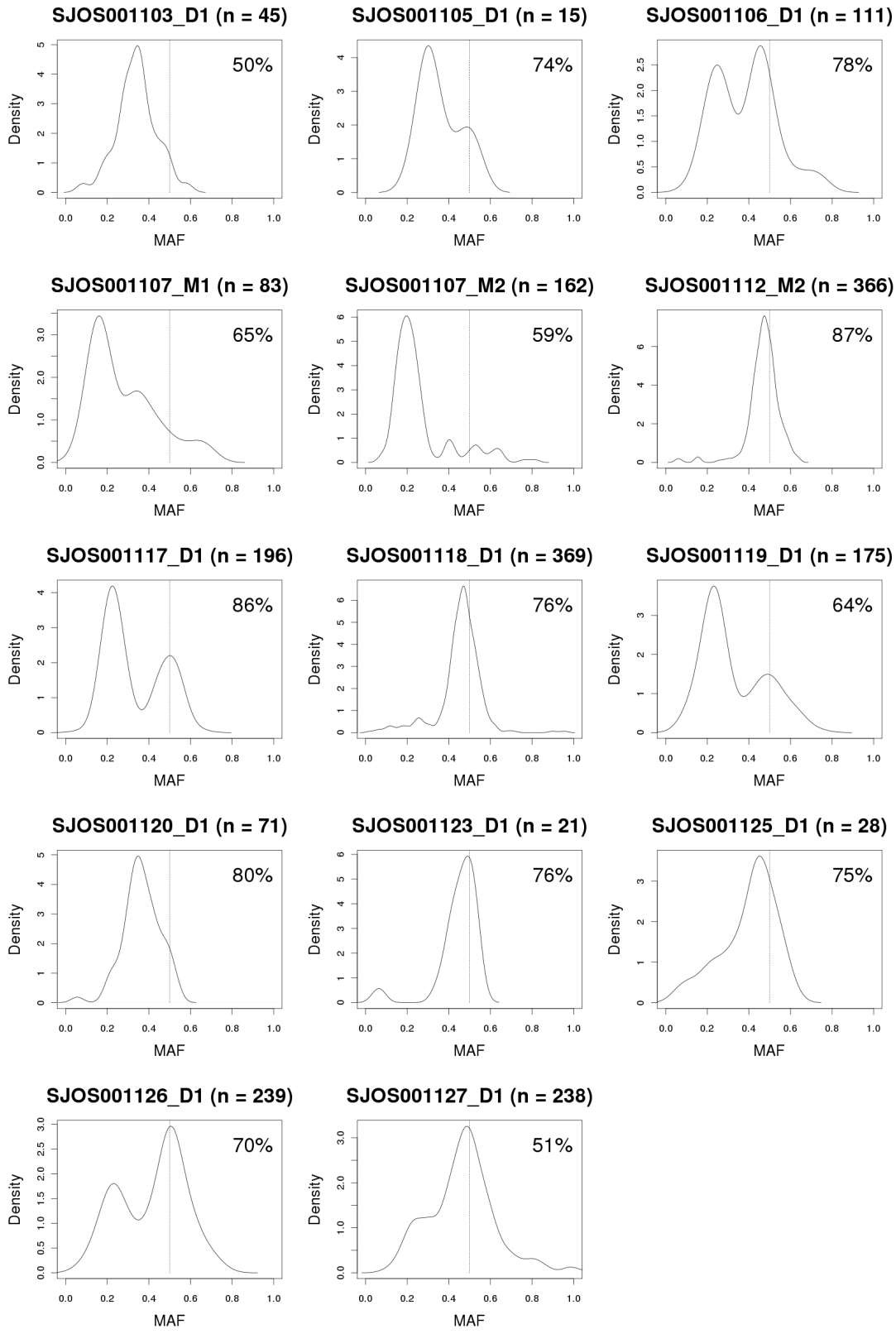
Provided as a separate file.

Table S3 related to Figure 1. Validated mutations.

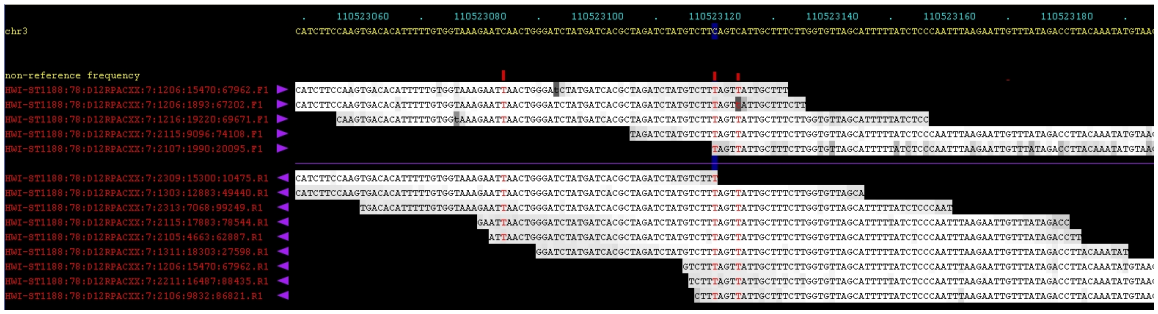
Provided as a separate file.

A

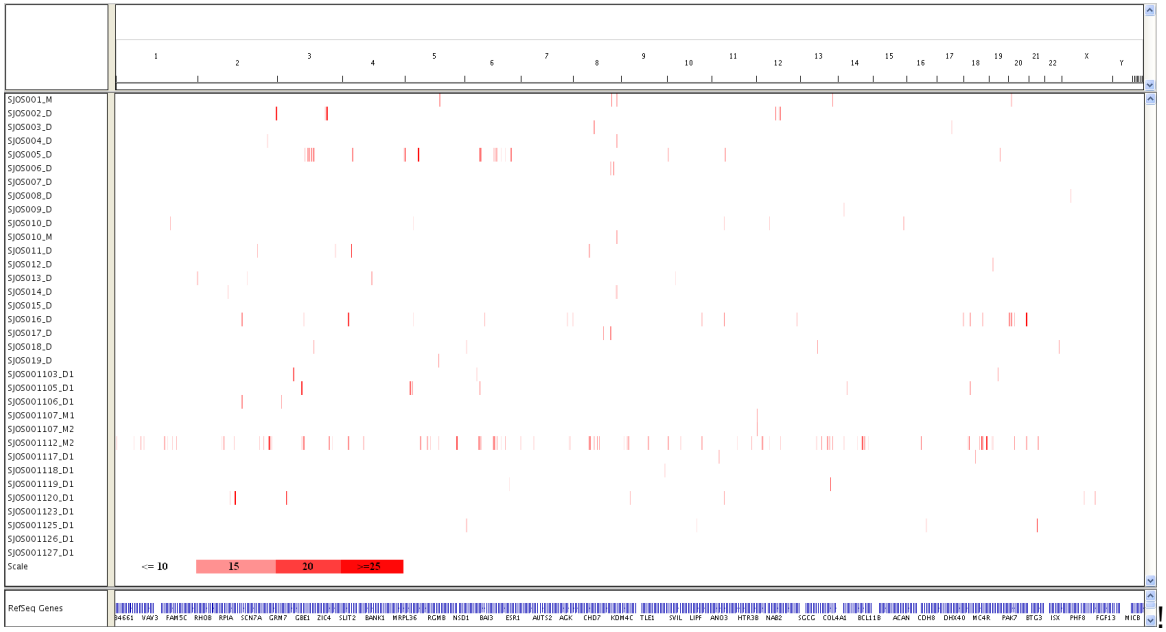

SJOS001M (n = 26)
88%
SJOS002 (n = 286)
68%
SJOS003 (n = 298)
73%
SJOS004 (n = 33)
69%
SJOS005 (n = 17)
76%
SJOS006 (n = 228)
53%
SJOS007 (n = 150)
70%
SJOS008 (n = 47)
89%
SJOS009 (n = 154)
51%
SJOS010 (n = 64)
63%
SJOS010M (n = 89)
44%
SJOS011 (n = 188)
86%
SJOS012 (n = 14)
73%
SJOS013 (n = 42)
88%
SJOS014 (n = 326)
66%
SJOS015 (n = 48)
50%
SJOS016 (n = 267)
50%
SJOS017 (n = 144)
63%
SJOS018 (n = 775)
63%
SJOS019 (n = 50)
59%
Density
MAF


SJOS001103_D1 (n = 45)
50%
SJOS001105_D1 (n = 15)
74%
SJOS001106_D1 (n = 111)
78%
SJOS001107_M1 (n = 83)
65%
SJOS001107_M2 (n = 162)
59%
SJOS001112_M2 (n = 366)
87%
SJOS001117_D1 (n = 196)
86%
SJOS001118_D1 (n = 369)
76%
SJOS001119_D1 (n = 175)
64%
SJOS001120_D1 (n = 71)
80%
SJOS001123_D1 (n = 21)
76%
SJOS001125_D1 (n = 28)
75%
SJOS001126_D1 (n = 239)
70%
SJOS001127_D1 (n = 238)
51%
Density
MAF

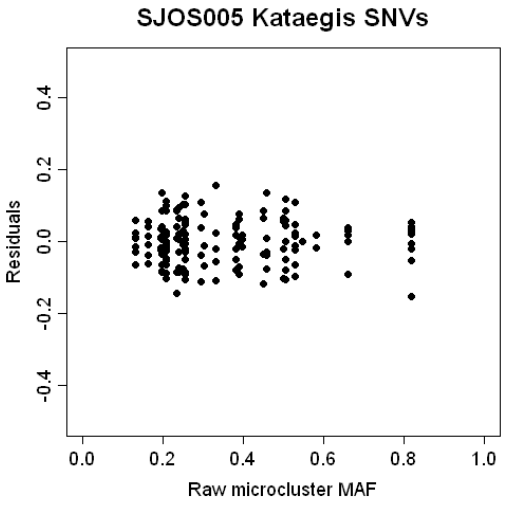
B


chr3
110523060
110523080
110523100
110523120
110523140
110523160
110523180
non-reference frequency

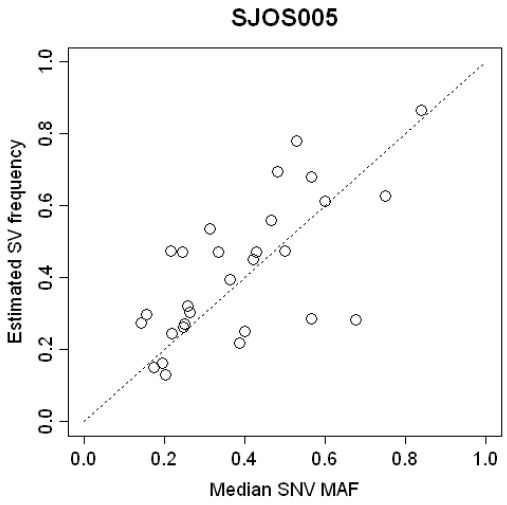
C

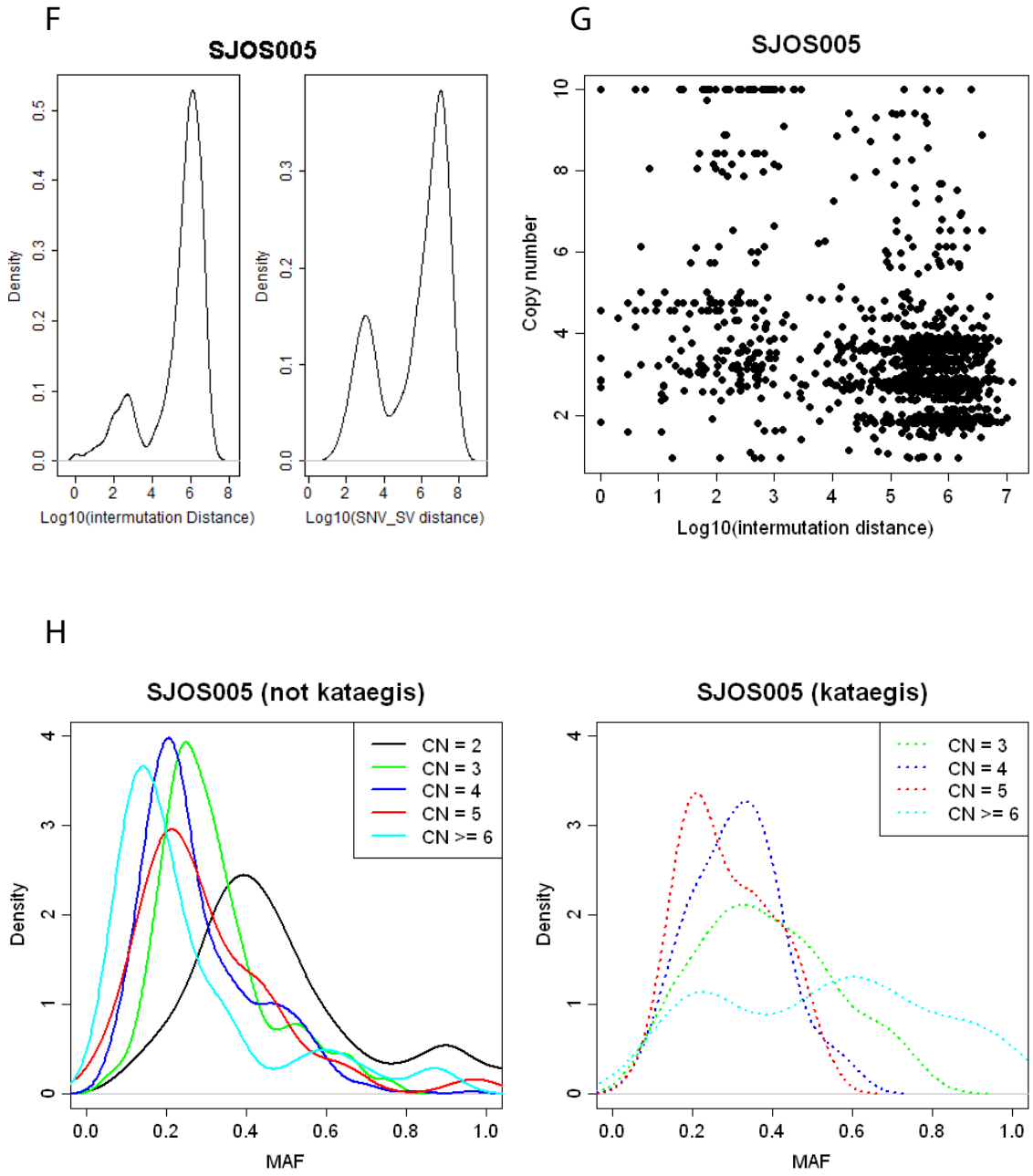

Scale
<= 10
15
20
>=25
RefSeq Genes

D

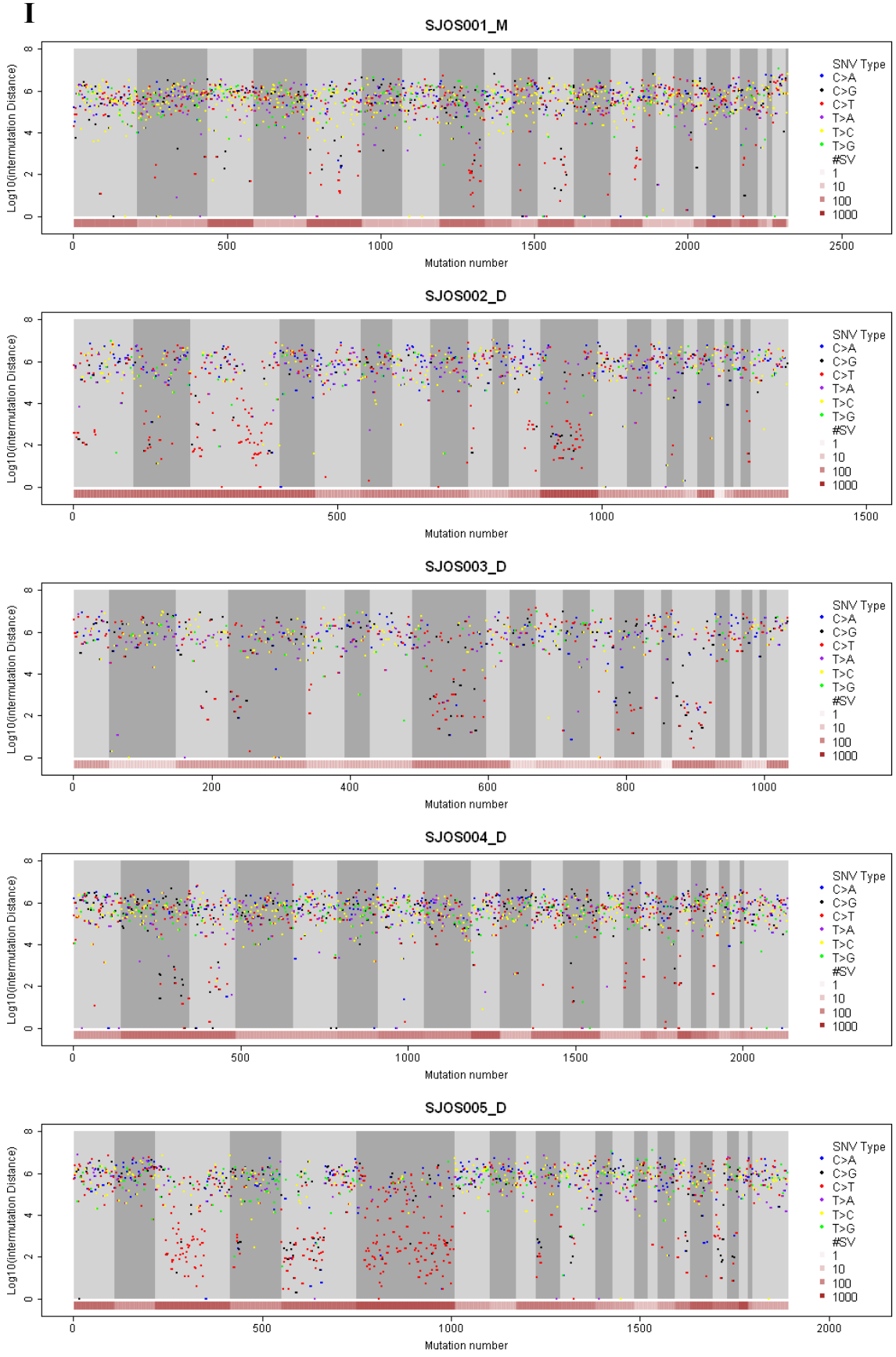

SJOS005 Kataegis SNVs
Residuals
Raw microcluster MAF

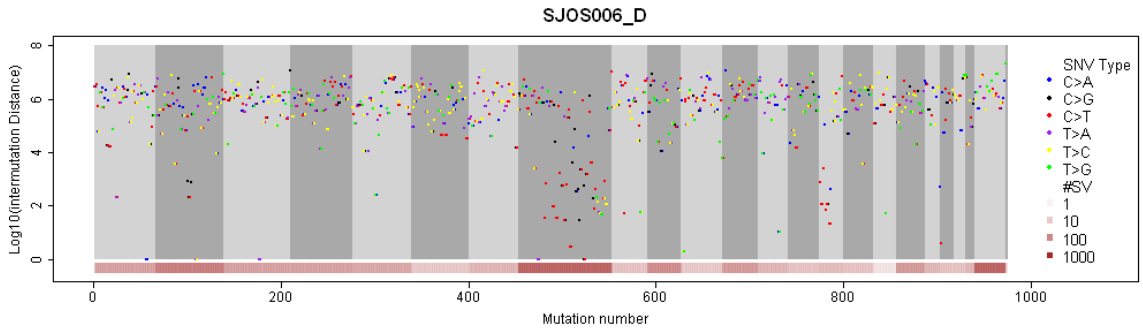
E

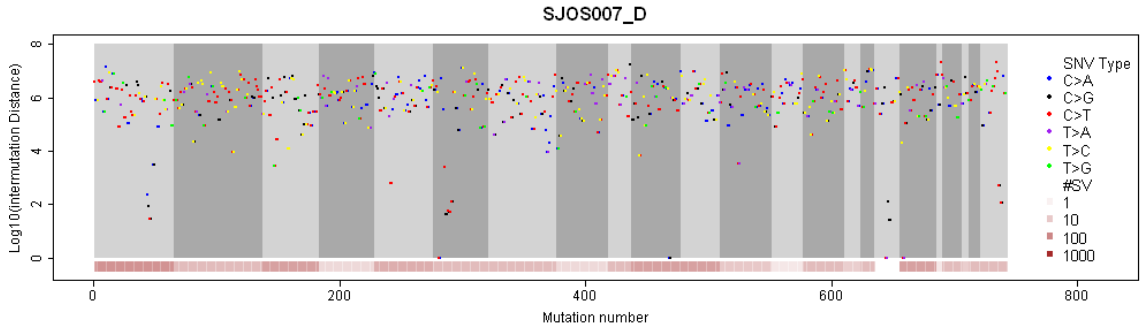

SJOS005
Estimated SV frequency
Median SNV MAF


F
SJOS005
Density
Log10(intermutation Distance)
Log10(SNV_SV distance)
G
SJOS005
Copy number
Log10(intermutation distance)
H
SJOS005 (not kataegis)
CN = 2
CN = 3
CN = 4
CN = 5
CN >= 6
Density
MAF
SJOS005 (kataegis)
CN = 3
CN = 4
CN = 5
CN >= 6
Density
MAF

I


SJOS001_M
SJOS002_D
SJOS003_D
SJOS004_D
SJOS005_D
Log10(intermutation Distance)
Mutation number
SNV Type
C>A
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000


SJOS006_D
Log10(intermutation Distance)
Mutation number
SNV Type
C>A
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000


SJOS007_D
Log10(intermutation Distance)
Mutation number
SNV Type
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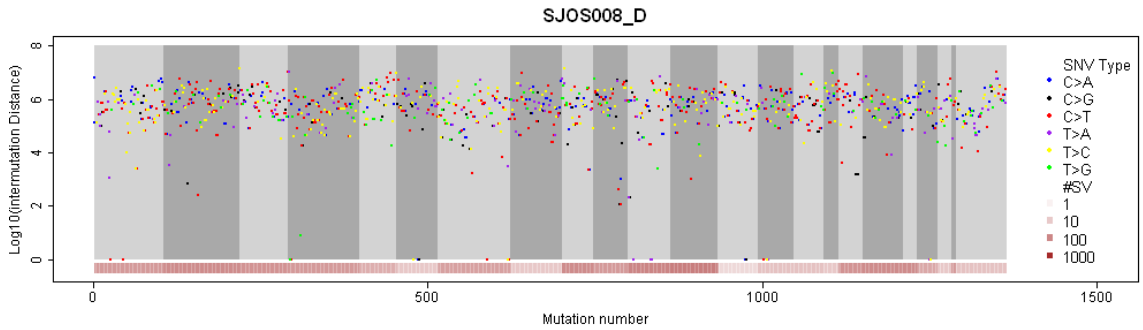
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000


SJOS008_D
Log10(intermutation Distance)
Mutation number
SNV Type
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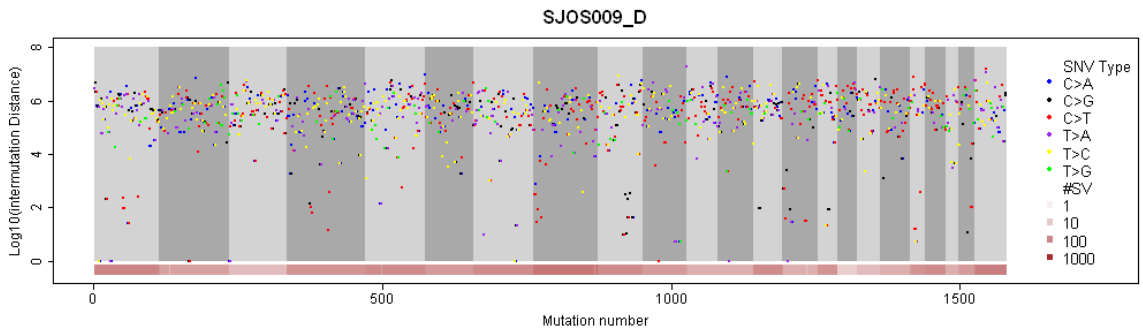
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000


SJOS009_D
Log10(intermutation Distance)
Mutation number
SNV Type
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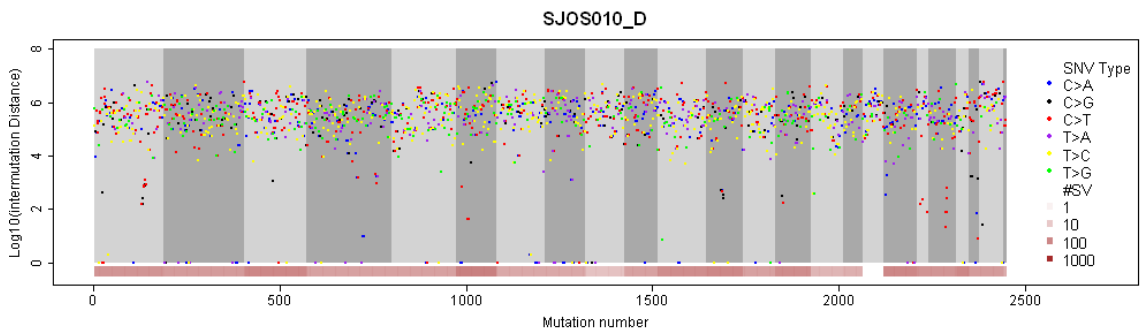
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000


SJOS010_D
Log10(intermutation Distance)
Mutation number
SNV Type
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C>T
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T>C
T>G
#SV
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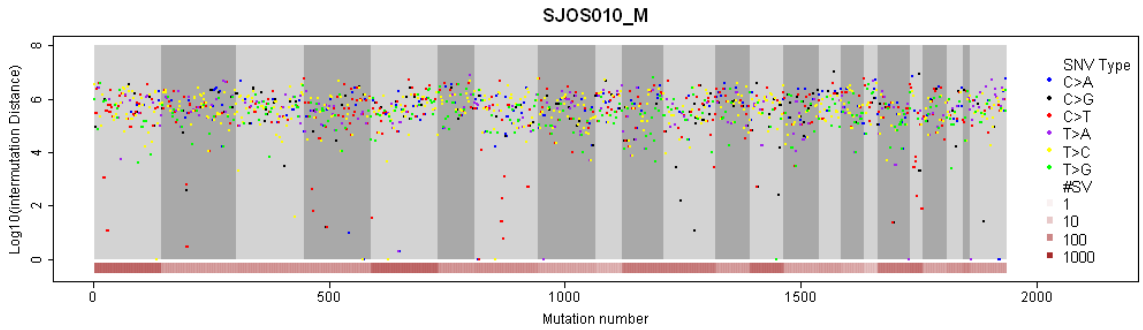
100
1000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Log10(intermutation Distance)
Mutation number
SNV Type
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C>G
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T>C
T>G
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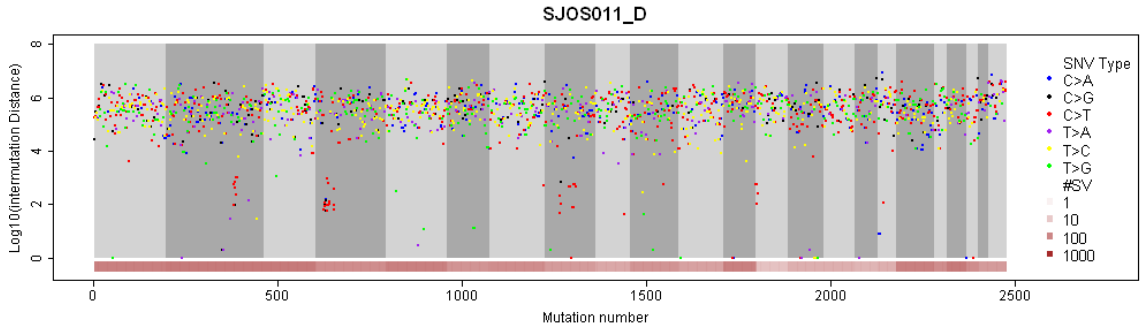
1000


SJOS011_D
Log10(intermutation Distance)
Mutation number
SNV Type
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C>T
T>A
T>C
T>G
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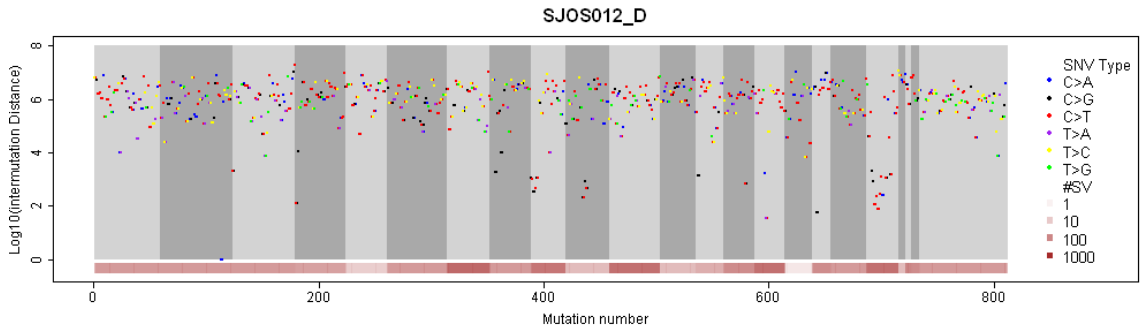
100
1000


SJOS012_D
Log10(intermutation Distance)
Mutation number
SNV Type
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C>G
C>T
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T>C
T>G
#SV
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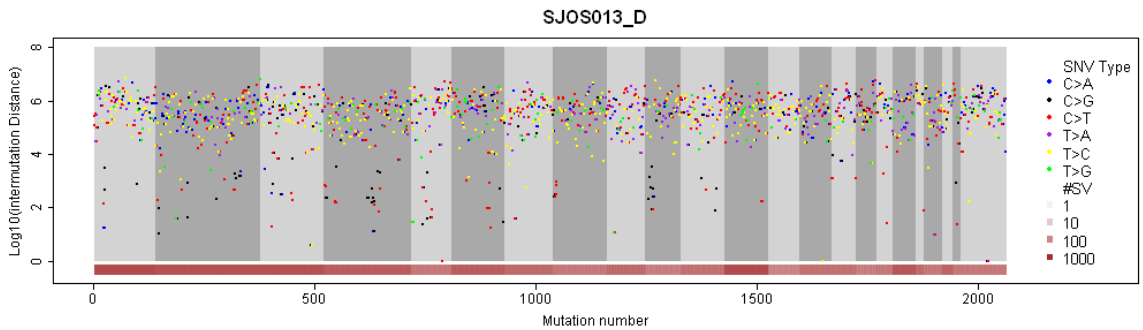
100
1000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Log10(intermutation Distance)
Mutation number
SNV Type
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C>G
C>T
T>A
T>C
T>G
#SV
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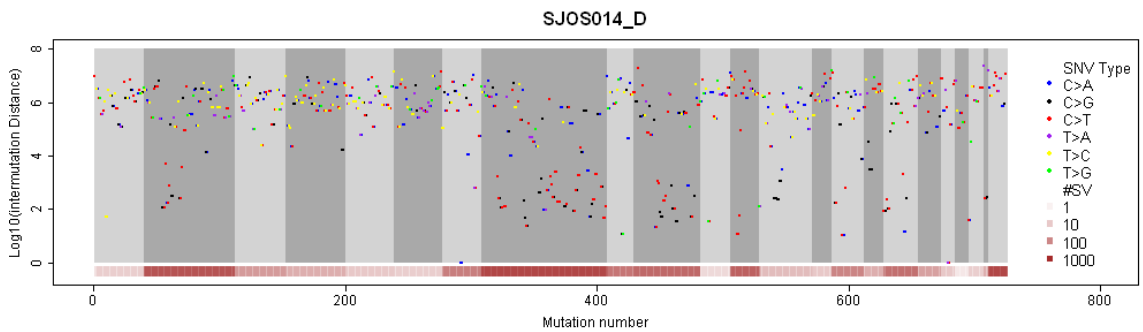
100
1000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Log10(intermutation Distance)
Mutation number
SNV Type
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C>G
C>T
T>A
T>C
T>G
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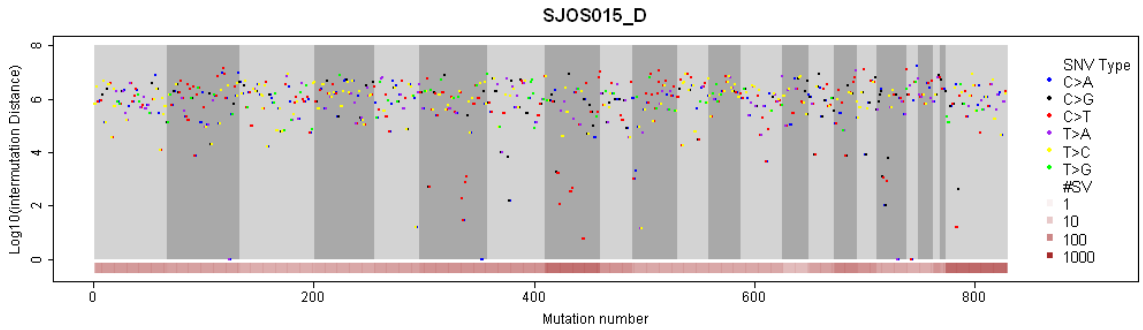
100
1000


SJOS015_D
Log10(intermutation Distance)
Mutation number
SNV Type
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C>G
C>T
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T>C
T>G
#SV
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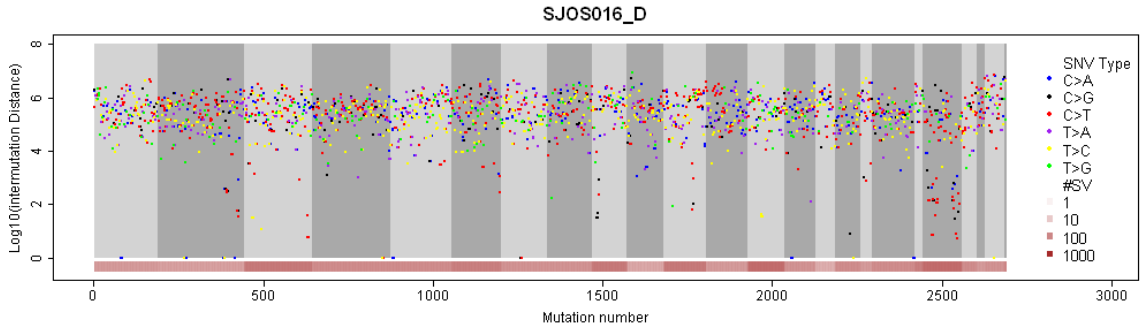
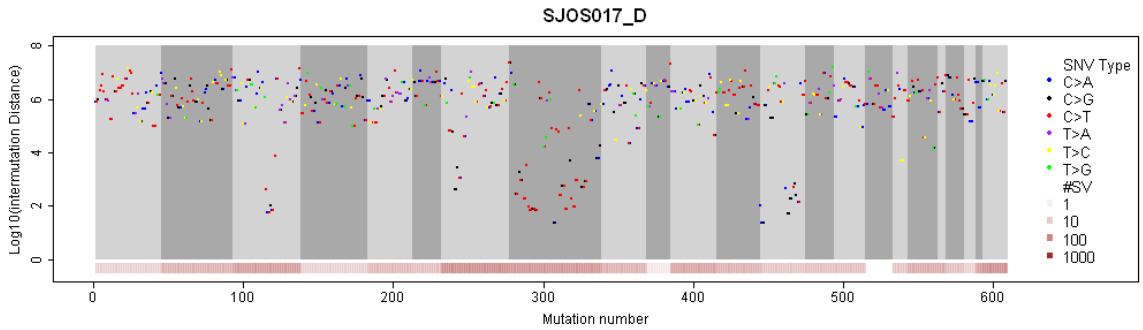
10
100
1000


SJOS016_D
Log10(intermutation Distance)
Mutation number
SNV Type
C>A
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000


SJOS017_D
Log10(intermutation Distance)
Mutation number
SNV Type
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C>G
C>T
T>A
T>C
T>G
#SV
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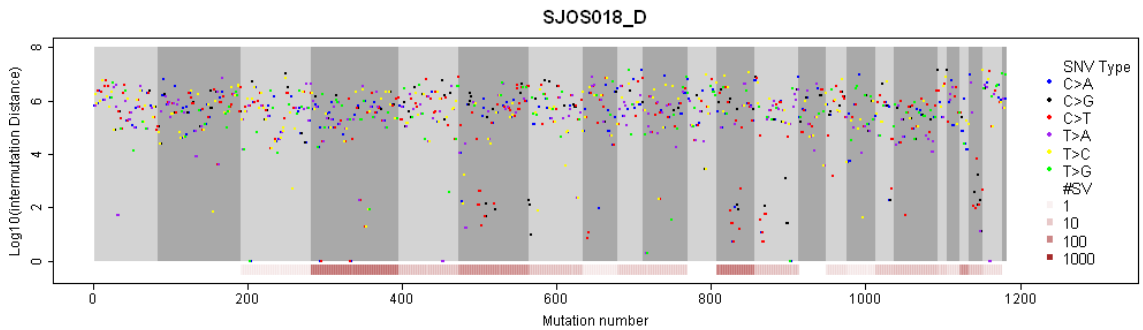
10
100
1000


SJOS018_D
Log10(intermutation Distance)
Mutation number
SNV Type
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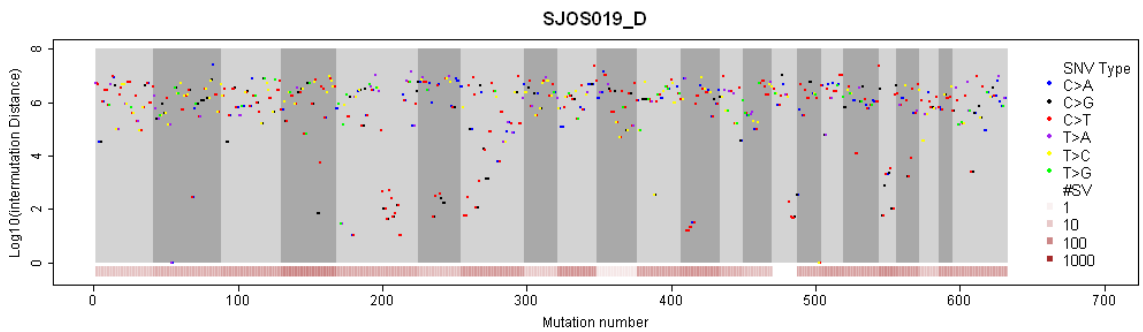
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000


SJOS019_D
Log10(intermutation Distance)
Mutation number
SNV Type
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C>G
C>T
T>A
T>C
T>G
#SV
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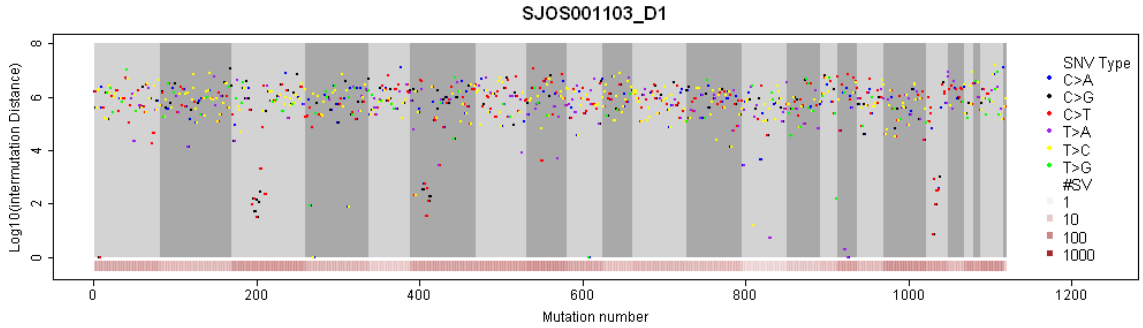
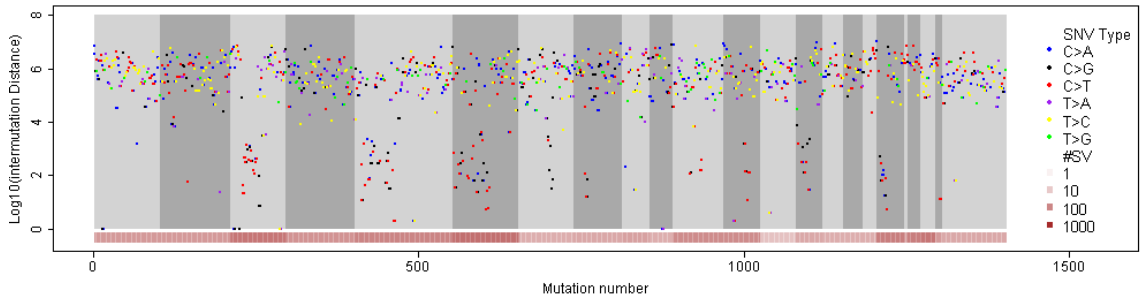
10
100
1000


SJOS001103_D1
Log10(intermutation Distance)
Mutation number
SNV Type
C>A
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000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Log10(intermutation Distance)
Mutation number
SNV Type
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C>G
C>T
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T>C
T>G
#SV
1
10
100
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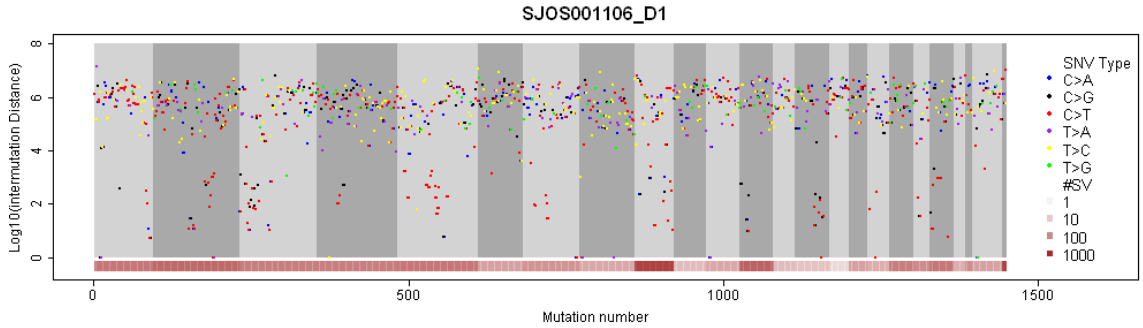
SJOS001106_D1
Log10(intermutation Distance)
Mutation number
SNV Type
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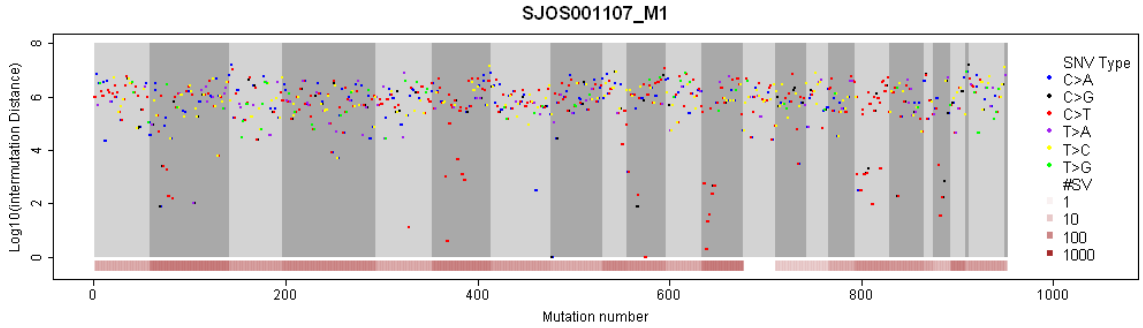
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000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Log10(intermutation Distance)
Mutation number
SNV Type
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T>A
T>C
T>G
#SV
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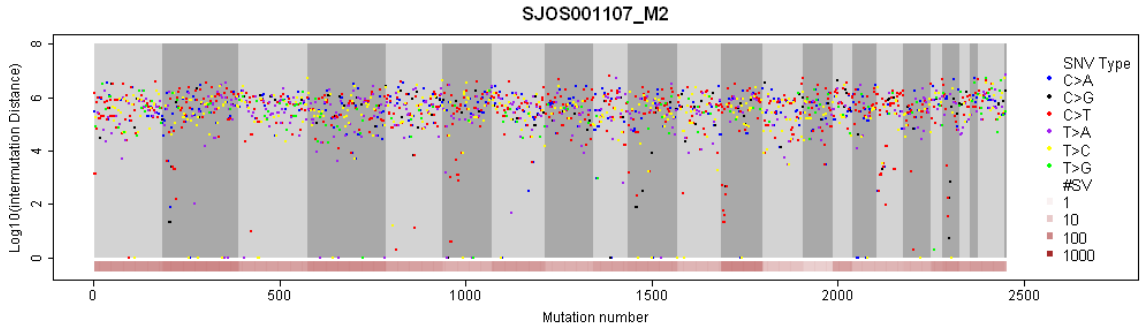
100
1000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Log10(intermutation Distance)
Mutation number
SNV Type
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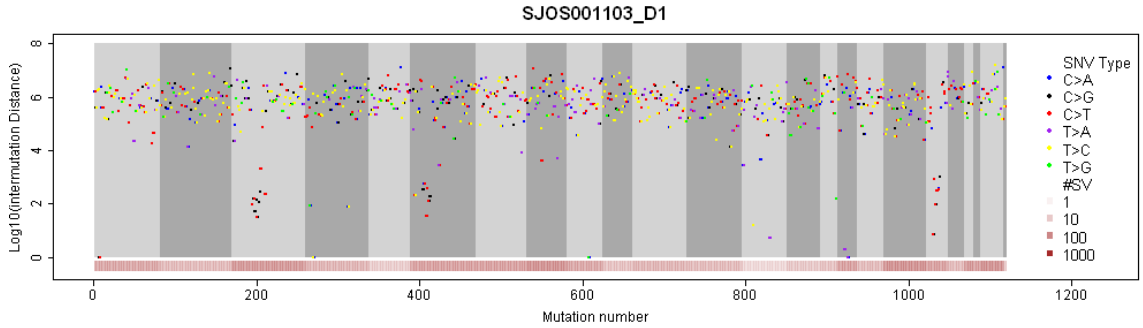
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000


SJOS001103_D1
Log10(intermutation Distance)
Mutation number
SNV Type
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C>G
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T>A
T>C
T>G
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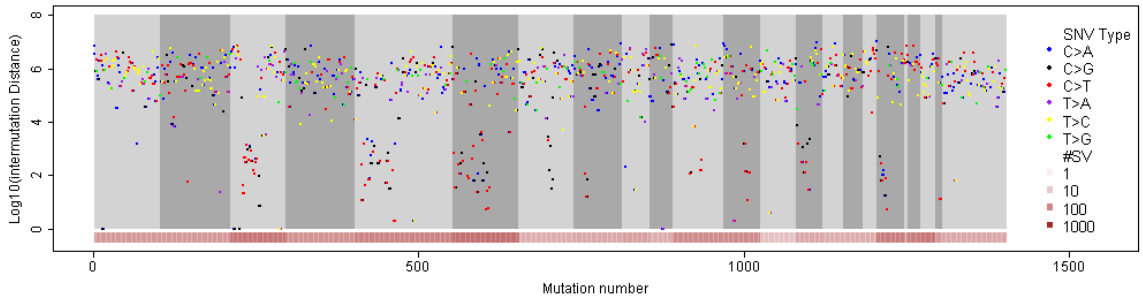
100
1000


SJOS001105_D1
Log10(intermutation Distance)
Mutation number
SNV Type
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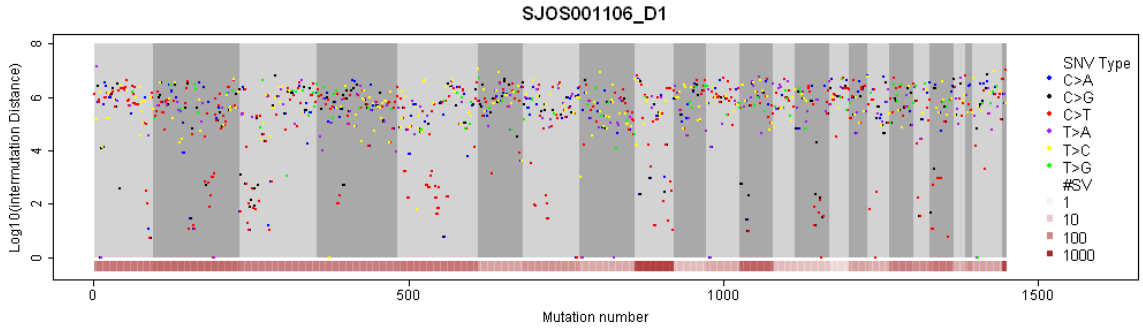
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000


SJOS001106_D1
Log10(intermutation Distance)
Mutation number
SNV Type
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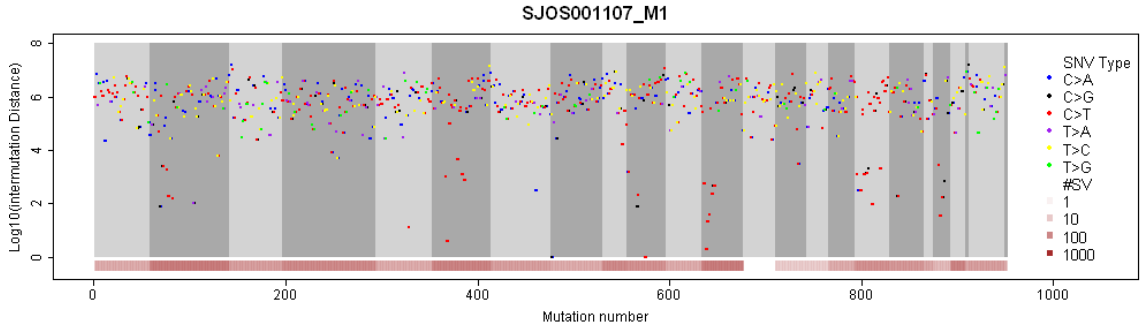
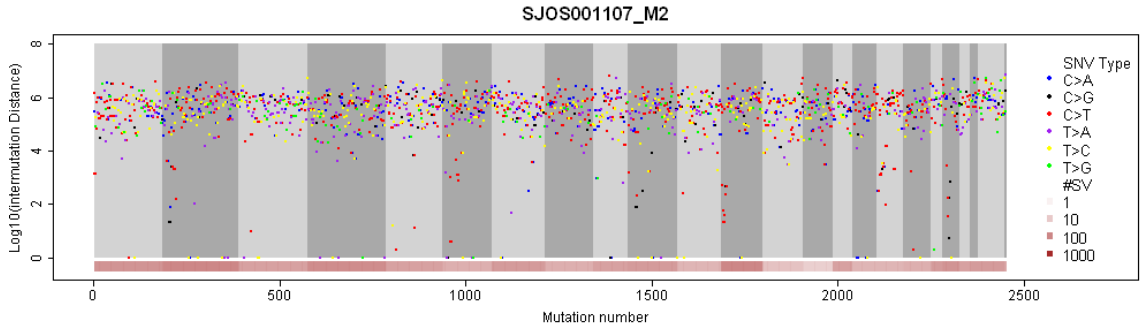
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000


SJOS001107_M1
Log10(intermutation Distance)
Mutation number
SNV Type
C>A
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000


SJOS001107_M2
Log10(intermutation Distance)
Mutation number
SNV Type
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C>G
C>T
T>A
T>C
T>G
#SV
1
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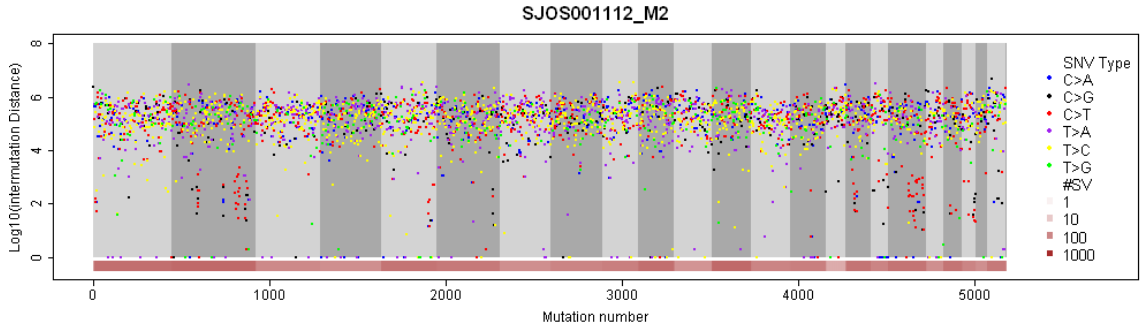
100
1000


SJOS001112_M2
Log10(intermutation Distance)
Mutation number
SNV Type
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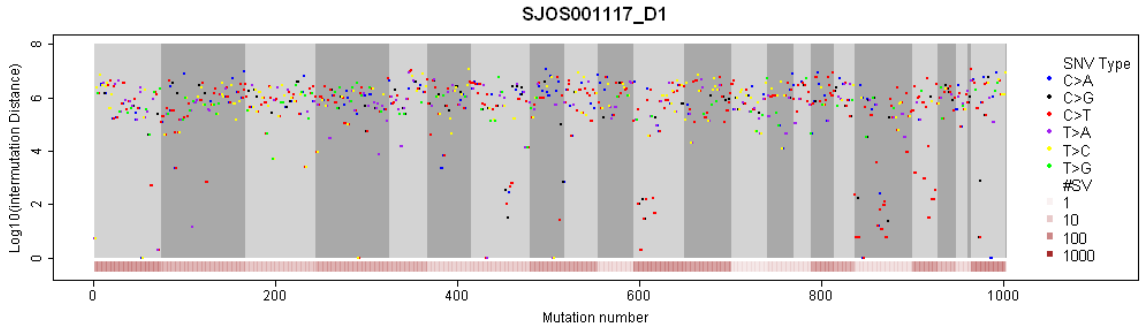
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000


SJOS001117_D1
Log10(intermutation Distance)
Mutation number
SNV Type
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C>G
C>T
T>A
T>C
T>G
#SV
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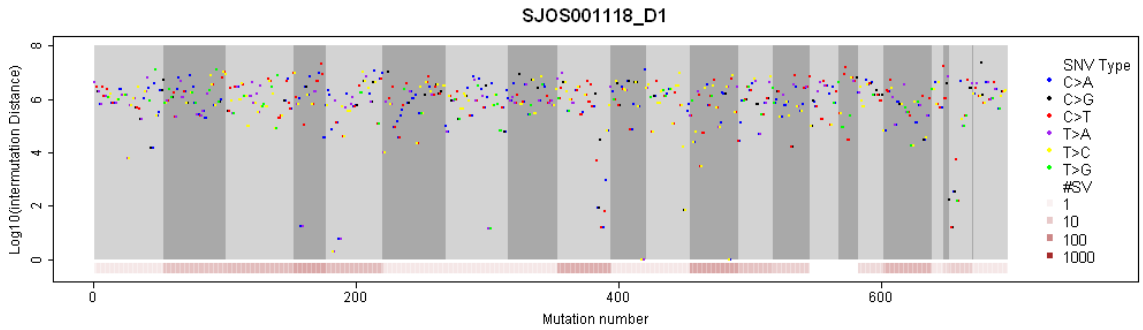
10
100
1000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Log10(intermutation Distance)
Mutation number
SNV Type
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T>A
T>C
T>G
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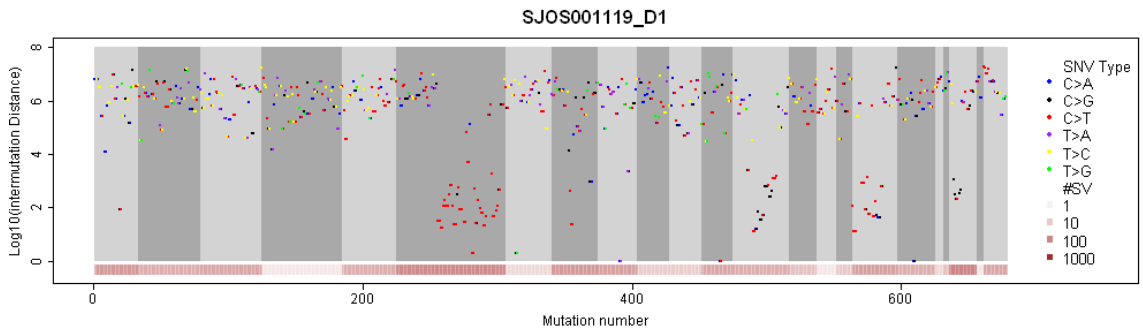
100
1000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Log10(intermutation Distance)
Mutation number
SNV Type
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T>C
T>G
#SV
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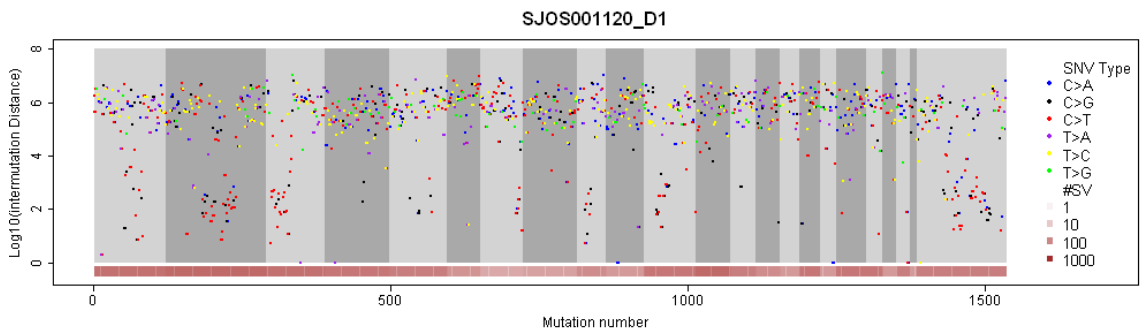
10
100
1000


SJOS001120_D1
Log10(intermutation Distance)
Mutation number
SNV Type
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T>C
T>G
#SV
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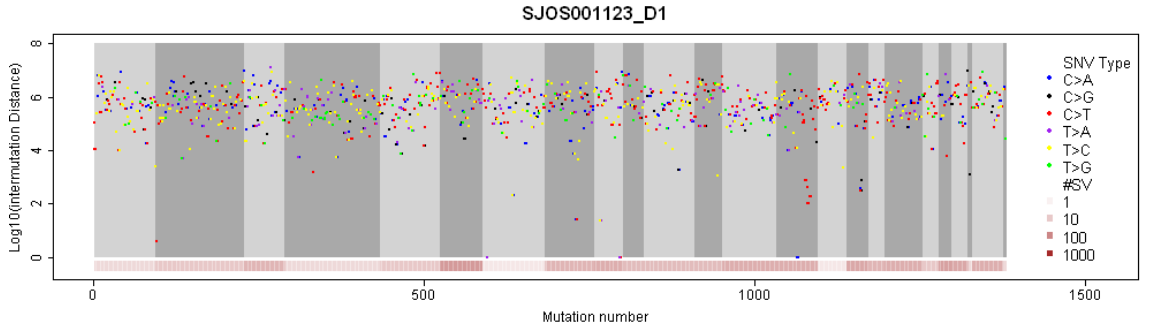
100
1000


SJOS001123_D1
Log10(intermutation Distance)
Mutation number
SNV Type
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T>C
T>G
#SV
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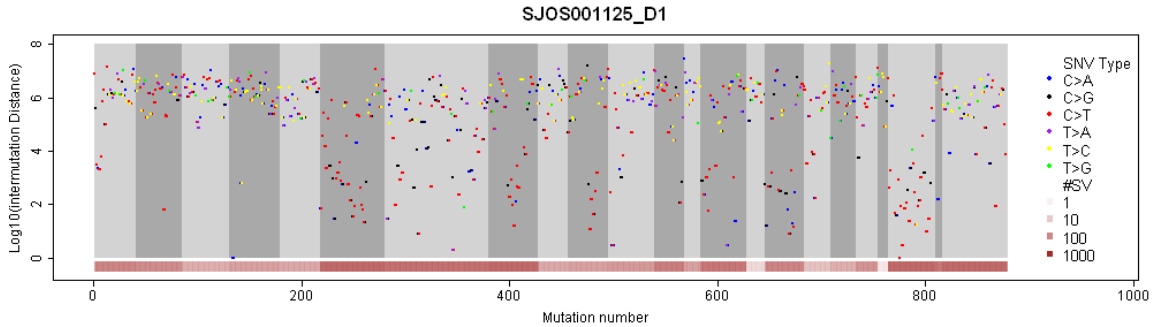
10
100
1000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Log10(intermutation Distance)
Mutation number
SNV Type
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T>C
T>G
#SV
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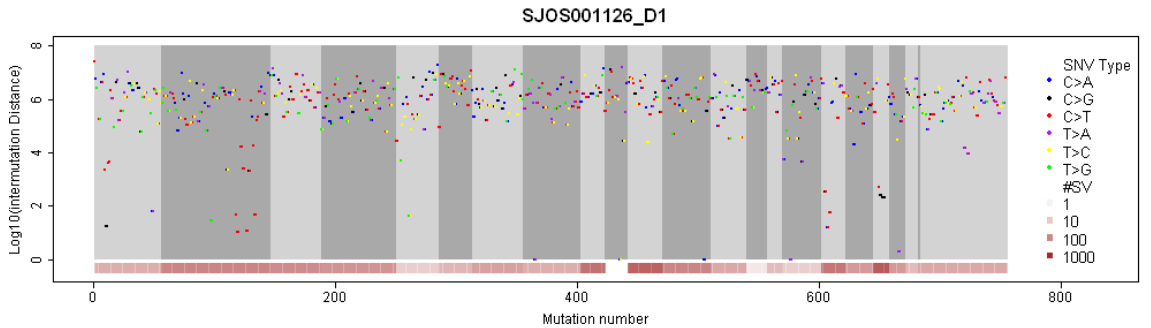
10
100
1000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Log10(intermutation Distance)
Mutation number
SNV Type
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T>C
T>G
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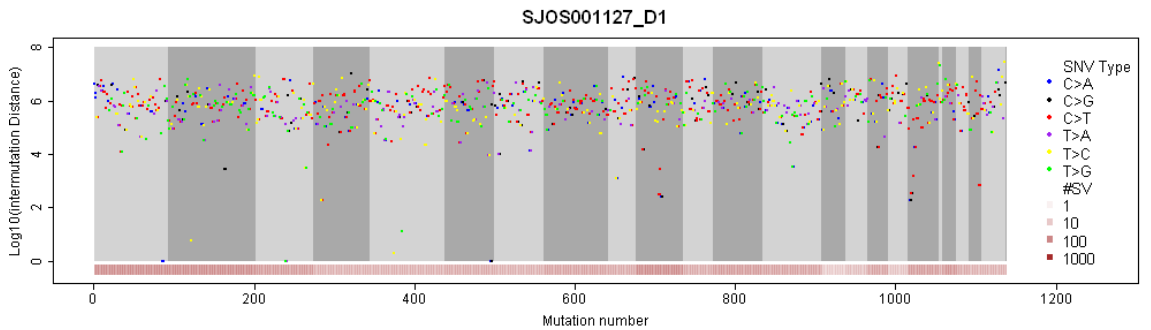
10
100
1000


SJOS001127_D1
Log10(intermutation Distance)
Mutation number
SNV Type
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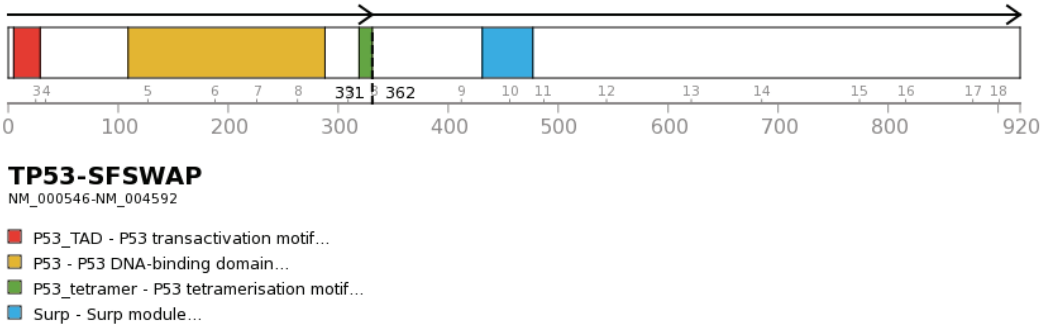
C>G
C>T
T>A
T>C
T>G
#SV
1
10
100
1000

Figure S2 related to Figure 2. Analysis of tumor heterogeneity and kataegis. A) Tumor purity adjusted mutant allele fraction (MAF) for samples analyzed by whole genome sequencing. The actual tumor purity that was used to adjust the MAF is shown as a percentage for each tumor. The number of qualifying SNVs (n) used to plot the density plot is shown for each tumor. **B)** Screenshot of WGS data with individual sequence reads showing kataegis hypermutation on the same DNA strand. **C)** Genomewide distribution of mutation hotspots in osteosarcoma shown as the ratio between observed mutation rate in the window over the genomewide mutation rate within a 3.2 Mb window across the genome for the 34 samples analyzed by WGS. **D)** Kataegis SNVs showed different MAFs among different microclusters although SNVs within a microcluster shared similar MAFs. **E)** Comparison of MAFs of SVs and SNVs in kataegis regions. **F)** The distribution of intermutation distance and distance from a SNV to nearest SV breakpoint in SJOS005, showing majority of mutations in the genome have a intermutation distance around 1 Mb while a small portion of SNVs have an intermutation distance smaller than 10 kb. **G)** Distribution of copy number variation and SNV intermutation distance (log10) which shows higher proportion of closely spaced SNVs (i.e. kategis SNVs) occur in amplified regions. **H**)Distribution of mutant allele fraction of non-kataegis SNVs (left panel) and kataegis SNVs (right panel) in regions of different copy number state in SJOS005. SNVs acquired before amplification show multiple mutant allele fraction (MAF) peaks, depending on whether the mutant allele or the reference allele is amplified while mutations acquired after amplification showed a single MAF corresponding to mutation on a single copy. **I)** Rainfall plot of all 34 genomes in the WGS cohort.

A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6
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8
331
362
9
10
11
12
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14
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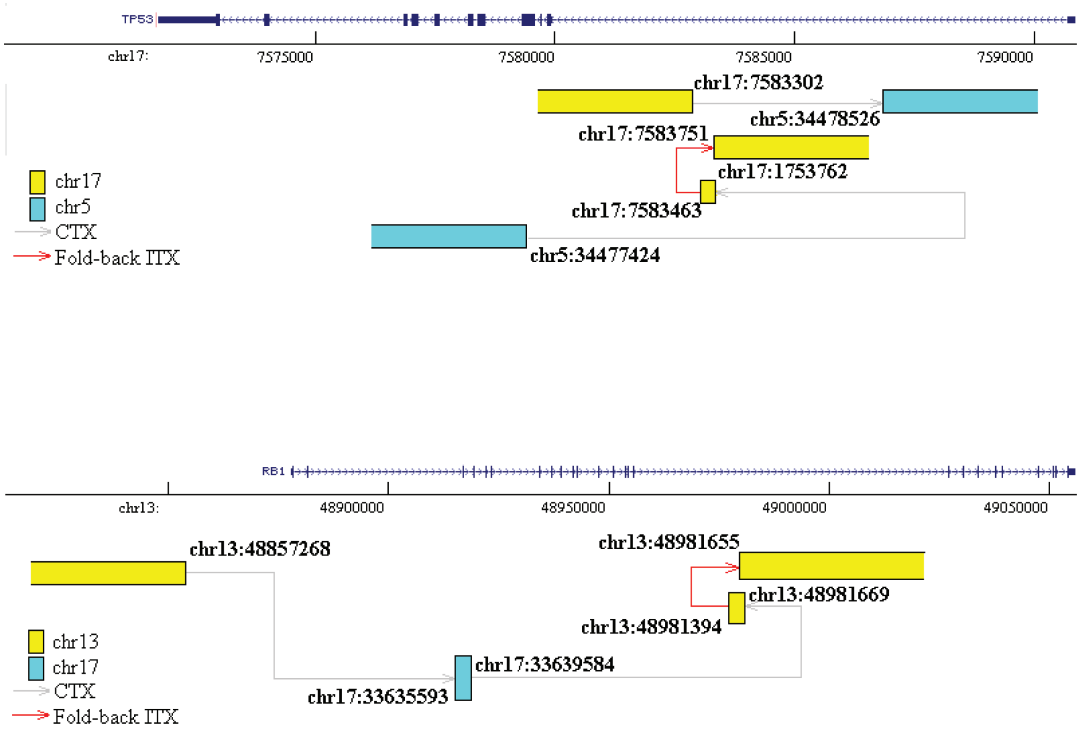
16
17
18
0
100
200
300
400
500
600
700
800
920
TP53-SFSWAP
NM_000546-NM_004592
P53_TAD - P53 transactivation motif...
P53 - P53 DNA-binding domain...
P53_tetramer - P53 tetramerisation motif...
Surp - Surp module...

B


TP53
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7580000
7585000
7590000
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chr5:34478526
chr17:7583751
chr17:1753762
chr17:7583463
chr5:34477424
chr17
chr5
CTX
Fold-back ITX
RB1
chr13:
48900000
48950000
49000000
49050000
chr13:48857268
chr13:48981655
chr13:48981669
chr13:48981394
chr17:33639584
chr17:33635593
chr13
chr17
CTX
Fold-back ITX

Figure S3 related to Figure 3. ***TP53* translocations in osteosarcoma**. **A)**Diagram of the predicted fusion gene generated by the interchromosomal translocation between the *TP53* gene and the *SFSWAP* gene in SJOS007_D. **B)** Diagram of the fold-back translocation in the *TP53* gene in SJOS001_M (upper panel). Diagram of the fold-back translocation in the *RB1* gene in SJOS015_D (lower panel). The translocations are indicated by the red arrows.

Table S4 related to Figure 3. Analysis of p53 mutations in osteosarcoma.

Provided as a separate file.

Table S5 related to Figure 4. Analysis of p53 analysis and clinical features.

Provided as a separate file.

Table S6 related to Figure 5. ATRX analysis in osteosarcoma.

Provided as a separate file.

Table S7 related to Figure 5. Cancer gene mutations in osteosarcoma.

Provided as a separate file.