Patient and Specimen Information

Patient and Specimen Information	Observed Value
Accession Number	TN25-
Primary Tumor Site	Cerebellum, NOS
Histology	Glioma, malignant
Specimen Site	Cerebral hemisphere
Dissection Type	Microdissection
Tissue Area Dissected (mm^2)	120

Next Generation Sequencing Metrics

Metric	Observed Value
Total DNA Reads (Million)	56.4
Mapped Reads (Million)	51.5 (91.34%)
Low Quality Reads (Million)	4.8 (8.50%)
Unmapped Reads (Million)	0.1 (0.16%)
Average Depth (WES, Targeted Panel)	53.0, 303.2
Insert Size, Mode	112
Insert Size, 20th Percentile	86
Insert Size, 50th Percentile	114
RNA Total Reads (Million)	6.7
RNA Mapped Reads (Million)	6.4
RNA Unique Reads (Million)	5.0

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Tumor Mutational Burden (TMB) Variants

Variant	Variant	Variant
chr1: PRAMEF11 (L41P)	chr1: PRAMEF19 (V202I)	chr11: KMT5B (S107L)
chr1: PRAMEF11 (P39_P40insS)	chr1: PRAMEF19 (R176G)	chr11: OR8D4 (I126T)
chr1: PRAMEF2 (S183G)	chr1: PRAMEF17 (P413H)	chr11: ROBO4 (I753L)
chr1: PRAMEF2 (K184R)	chr1: PRAMEF17 (L419I)	chr12: ETNK1 (V110fs)
chr1: PRAMEF2 (K184N)	chr1: PRAMEF20 (E21Q)	chr12: MUC19 (T4669K)
chr1: PRAMEF6 (K440R)	chr1: PRAMEF20 (S328R)	chr12: MUC19 (L5804Q)
chr1: PRAMEF6 (K440E)	chr1: PRAMEF20 (T330I)	chr12: MUC19 (I5822T)
chr1: PRAMEF6 (A436P)	chr1: PRAMEF20 (V366I)	chr12: MUC19 (S5824P)
chr1: PRAMEF6 (I434L)	chr1: PRAMEF20 (S374N)	chr12: KRT1 (P171L)
chr1: PRAMEF6 (Y413H)	chr1: PRAMEF20 (R385C)	chr14: REM2 (A17T)
chr1: PRAMEF6 (L408I)	chr1: PRAMEF20 (T391M)	chr15: APBA2 (Q525*)
chr1: PRAMEF6 (K406N)	chr1: PRAMEF20 (L407I)	chr16: EME2 (Y188H)
chr1: PRAMEF6 (I404R)	chr2: XDH (A1094T)	chr16: ORAI3 (N128I)
chr1: PRAMEF6 (S400C)	chr2: SPHKAP (G450R)	chr16: C16orf58 (P251S)
chr1: PRAMEF27 (L14H)	chr3: FRG2C (S149C)	chr16: SLC7A6OS (E246G)
chr1: PRAMEF9 (R6W)	chr4: GAK (P943A)	chr16: MTHFSD (L367R)
chr1: PRAMEF9 (A15V)	chr4: SPARCL1 (T138I)	chr17: C17orf97 (E270K)
chr1: PRAMEF9 (R17Q)	chr5: ADAMTS12 (W1469*)	chr17: DNAH2 (L1576F)
chr1: PRAMEF9 (M27I)	chr5: PIK3R1 (E443_L449del)	chr17: NDEL1 (V269M)
chr1: PRAMEF8 (V329I)	chr5: MCC (S28_S29insGSSS)	chr17: LRRC75A (R288C)
chr1: PRAMEF8 (C315R)	chr6: HIST1H2AM (A114T)	chr17: GAS2L2 (N611D)
chr1: PRAMEF8 (S307L)	chr6: SLC18B1 (*457*)	chr17: UBTF (G764D)
chr1: PRAMEF8 (S293P)	chr7: THSD7A (R390*)	chr18: DLGAP1 (N164S)
chr1: PRAMEF15 (L14H)	chr7: CALN1 (R84Q)	chr19: SAFB2 (E637K)
chr1: PRAMEF15 (C76W)	chr7: MUC3A (L1220V)	chr19: LRFN1 (P241L)
chr1: PRAMEF15 (M244V)	chr7: BPGM (Y92*)	chr20: PANK2 (E554K)
chr1: PRAMEF15 (E253del)	chr8: PINX1 (S226G)	chr20: SLC24A3 (E406K)
chr1: PRAMEF15 (Q254K)	chr8: CLU (A129fs)	chr20: NCOA3 (I492M)
chr1: PRAMEF15 (K255Q)	chr9: SPATA31A7 (L97V)	chr22: KIAA1671 (P818L)
chr1: PRAMEF15 (I258F)	chr9: EPB41L4B (N234K)	chr22: MORC2 (R807C)

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Variant	Variant	Variant
chr1: PRAMEF14 (M28V)	chr9: TTC16 (E410Q)	chr22: PACSIN2 (E367K)
chr1: PRAMEF14 (S24A)	chr9: ADAMTSL2 (V996I)	chrX: SHROOM2 (R206L)
chr1: PRAMEF14 (G14V)	chr9: CAMSAP1 (G1281S)	chrX: KLHL34 (G489R)
chr1: PRAMEF19 (I277V)	chr9: CCDC187 (S296L)	chrX: ZNF157 (H212D)
chr1: PRAMEF19 (M274I)	chr10: LYZL2 (A29T)	chrX: DGKK (S715R)
chr1: PRAMEF19 (Y268N)	chr10: WASHC2A (S288L)	chrX: ATRX (T480fs)
chr1: PRAMEF19 (A222V)	chr11: SOX6 (E449K)	chrX: CSAG3 (W40*)

Tumor Mutational Burden by Whole Exome Sequencing (WES) Methods:
Samples with a depth of coverage on reportable genes (720 genes) lower than 100x are considered indeterminate. On samples with sufficient coverage, variants with at least 5% allele frequency identified across the whole exome are filtered to remove low quality, low depth, non-coding, synonymous, and other types that have been determined to be unreliable or unassociated with TMB. Any presumed germline variants which belong to gnomAD (AC>0), dbSNP 151 common or were found in at least 10% of training samples are also excluded. From the filtered list, missense, nonsense, in-frame INDEL, and frameshift variants in selected coding regions (25.5 Mb) with sufficient depth in training samples are counted. The final value is the variant count (displayed in the table above) divided by the length of the selected coding regions, presented as mutations/Mb. We consider samples containing 10 per Mb to be TMB high.

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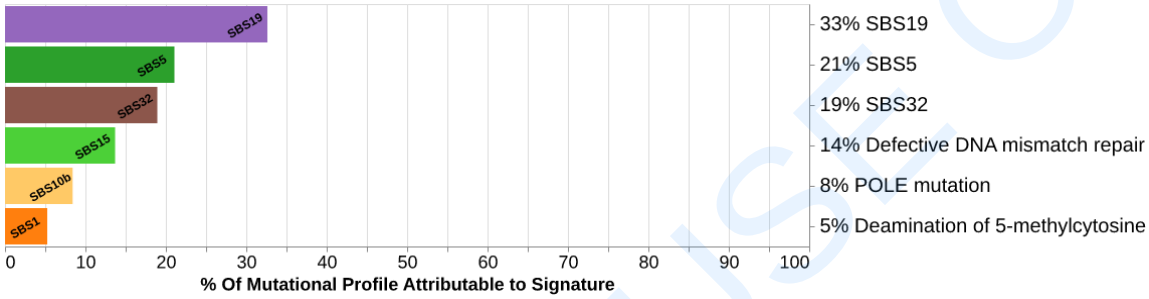
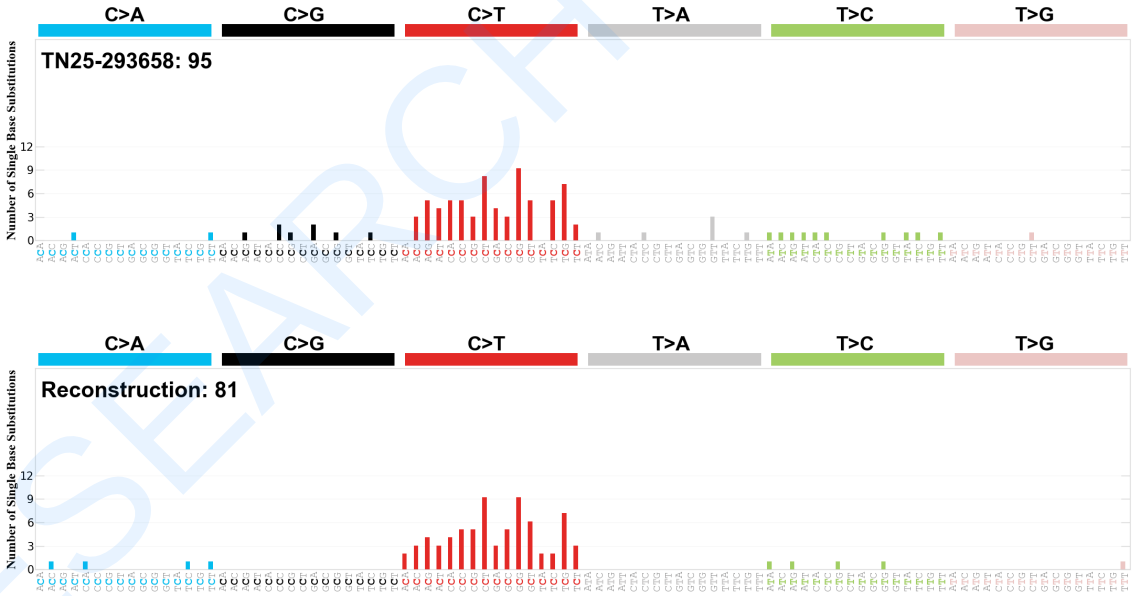
COSMIC Signatures

Metric	Result
TMB	4 per Mb
SBS Count	95
Reconstruction Accuracy	0.92

Signature Decomposition



Signature Reconstruction



COSMIC Methods:
Single base substitutions detected by Whole Exome Sequencing were used to evaluate similarity between the patient's mutation profile and established molecular phenotypes.

Alexandrov LB, Kim J, Haradhvala NJ, et al. The repertoire of mutational signatures in human cancer. Nature 578, 94-101 (2020).

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HRD - Genomic Scar Score

Component	Score
gLOH	5
LST	2
HRD-GSS	7

HRD-Genomic Scar Score (HRD-GSS) methods:
A measure of accumulated genomic instability in cells and is calculated by combining the total levels of genome-wide loss of heterozygosity (LOH) and large-scale chromosomal transitions (LST). To calculate LOH, the 22 autosomal chromosomes are split into 552 segments and the LOH of single nucleotide polymorphisms (SNPs) within each segment is calculated. Caris data consist of approximately 250k SNPs spread across the genome. SNP alleles with frequencies skewed towards 0 or 100% indicate LOH (heterozygous SNP alleles have a frequency of 50%). LST are detected when chromosomal breakages generate chromosomal gains or losses of 10 Mb or greater.

NOTE: The threshold for a high HRD-GSS (gLOH+LST) was determined and validated in a cohort consisting only of ovarian cancer patients. Caris' HRD-GSS cutoff for ovarian cancer is HRD-GSS ≥ 46, with an inconclusive range of 38-45. The clinical significance of this HRD-GSS measurement outside of ovarian cancer has not been established.

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Pathogen Identification

Pathogen	Reads	Threshold	Result
EBV	0	100,000	Negative
HPV16	0	300	Negative
HPV18	0	300	Negative
HPV31	0	300	Negative
HPV33	0	300	Negative
HPV34	0	---	---
HPV39	0	---	---
HPV45	0	300	Negative
HPV51	0	---	---
HPV52	0	---	---
HPV56	0	---	---
HPV58	0	---	---
HPV59	0	---	---
HPV66	0	---	---
HPV70	0	---	---
KSHV (HHV-8)	0	---	---
MCPYV	0	1,000	Negative

--- Denotes that the value is not clinically reportable and does not have an associated qualitative value or threshold.

Epstein-Barr virus (EBV) detection by Whole Exome Sequencing (WES) Methods:
The DNA of EBV must be detected for a tumor to be considered positive for EBV. This EBV-detection assay determines EBV-status using WES to enumerate the number of sequencing reads specific to EBV. EBV is commonly found in gastric/esophageal junction carcinomas (EJC) and nasopharyngeal carcinoma (NPC) of the head and neck. EBV-positive gastric/EJC and NPC tumors exhibit molecular hallmarks of potential sensitivity to cancer immunotherapy: EBV-positivity has been associated with partial responses to immunotherapy in gastric/EJC cancer, and define an undifferentiated subtype with superior prognosis and an immune-dense microenvironment in NPC.

Human papilloma virus (HPV) 16/18/31/33/45-detection by Whole Exome Sequencing (WES) Methods:
The DNA of HPV types 16, 18, 31, 33 or 45 must be detected for a tumor to be considered positive for HPV. This HPV-detection assay determines HPV-status using WES to enumerate the number of sequencing reads specific to aforementioned HPV types. HPV 16, 18, 31, 33 and 45 are among the most prevalent HPV genotypes associated with oral and anogenital cancers (including cervical, vulvar, anal, penile and oropharyngeal). Generally, HPV-associated etiology has been associated with improved prognosis, de-intensified treatment protocols and tumor subclassification. Tumors commonly ascribed to HPV etiology are most common in squamous cell carcinomas including cervical, vulvar, anal, penile and oropharyngeal. If positive, HPV has been associated with improved prognosis, de-intensified treatment protocols and tumor subclassification. Other HPV subtypes may not have sufficient coverage to be reliably detected and therefore should not be considered negative results.

Merkel Cell Polyomavirus (MCPyV) detection by Whole Exome Sequencing (WES) Methods:
This MCPyV detection assay determines MCPyV status using WES to enumerate the number of sequencing reads specific to MCPyV. MCPyV positivity is associated with the pathogenesis of Merkel Cell Carcinoma (MCC). MCC tumors that are MCPyV-positive typically have more stable genomes and lower mutational burden than those that are MCPyV-negative.

Kaposi sarcoma-associated herpesvirus (KSHV, HHV-8) detection by Whole Exome Sequencing (WES) Methods:
Kaposi sarcoma-associated herpesvirus (KSHV), also known as Human Herpesvirus-8 (HHV-8) is commonly found in Kaposi sarcoma tumors.

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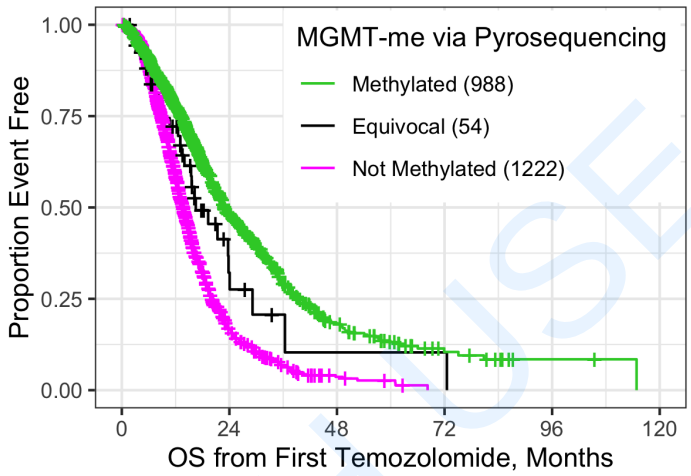
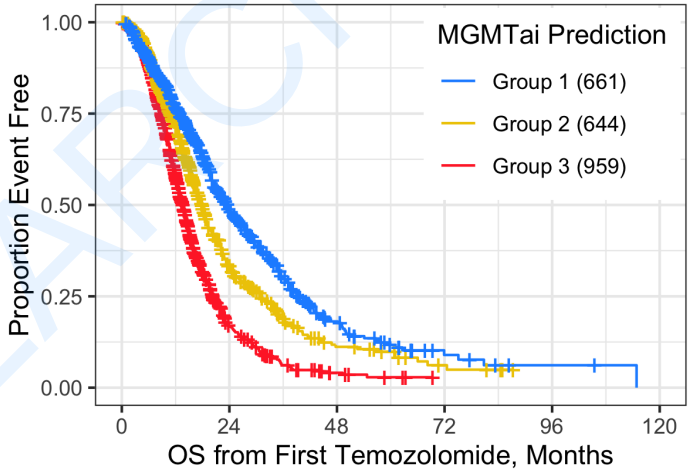
Caris AI Insights™

MGMTai: Prediction of MGMT Promoter Methylation via Whole Exome and Whole Transcriptome

Assay	Result
MGMT Promoter Methylation via Pyrosequencing (%)	Methylated (9)
MGMTai Score via Whole Exome and Whole Transcriptome Sequencing	Group 1 (0.843)





MGMTai Methods:
Caris MGMTai is a Next Generation Profiling (NGP) signature that uses Whole Exome (WES) and Whole Transcriptome Sequencing (WTS) data to predict response to temozolomide. The training cohort is comprised of more than 5,700 IDH1 and IDH2 wild type glioblastoma cases with valid MGMT-methylation status via pyrosequencing which yields a well-established survival profile in advanced glioblastoma patients. The signature uses a gradient boosted framework to predict methylation status identified via pyrosequencing using the WES and WTS data. The model was not informed of any patient survival data. A subset of the training cohort had temozolomide outcomes data available, and the results of the cross-validated training cohort were found to split into three survival profiles with statistically significant hazard ratios via a Cox proportional hazards model: Group 1 (MGMTai score above 0.8), Group 2 (0.2-0.8), and Group 3 (below 0.2). We present the Kaplan-Meier curves using the pyrosequencing results (top) compared to the MGMTai score cohorts (bottom). The efficacy of MGMTai accuracy or its ability to identify successful response to temozolomide outside of IDH1/2 wild type glioblastoma has not been established.

TN25-

Tumor Infiltrating Immune Cells

Cell Type	QuantiSeq Fraction	Epic Fraction	MCP Counter Percentile
B cell	0.111	0.000	33
Cancer associated fibroblast	-	0.014	13
Cytotoxicity score	-	-	72
Endothelial cell	-	0.127	60
Macrophage	-	0.022	-
Macrophage M1	0.052	-	-
Macrophage M2	0.093	-	-
Monocyte	0.051	-	91
Myeloid dendritic cell	0.086	-	7
NK cell	0.051	0.000	17
Neutrophil	0.000	-	31
T cell	-	-	12
T cell CD4+	-	0.074	-
T cell CD4+ (non-regulatory)	0.000	-	-
T cell CD8+	0.000	0.053	15
T cell regulatory (Tregs)	0.000	-	-
Uncharacterized cell	0.557	0.711	-

Tumor Infiltrating Lymphocytes methods:
Tumor Infiltrating Lymphocytes in the immune microenvironment is a gene expression-based calculation for quantitating the immune context in tumor cells. By counting the expression of certain immune cell markers and expression profiles, a fraction of infiltrating immune cells can be estimated. quanTIseq methodology is utilized for the calculation.

Reference: Finotello F, Mayer C, Plattner C, et al. Molecular and pharmacological modulators of the tumor immune contexture revealed by deconvolution of RNA-seq data Genome Med. 2019 Jul 29;11(1):50.

Reference: Racle J, de Jonge K, Baumgaertner P, et al. Simultaneous enumeration of cancer and immune cell types from bulk tumor gene expression data. ELife 2017.

Reference: Becht E, Giraldo N, Lacroix L, et al. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. Genome Bio. 2016 Oct 20;17(1):218.

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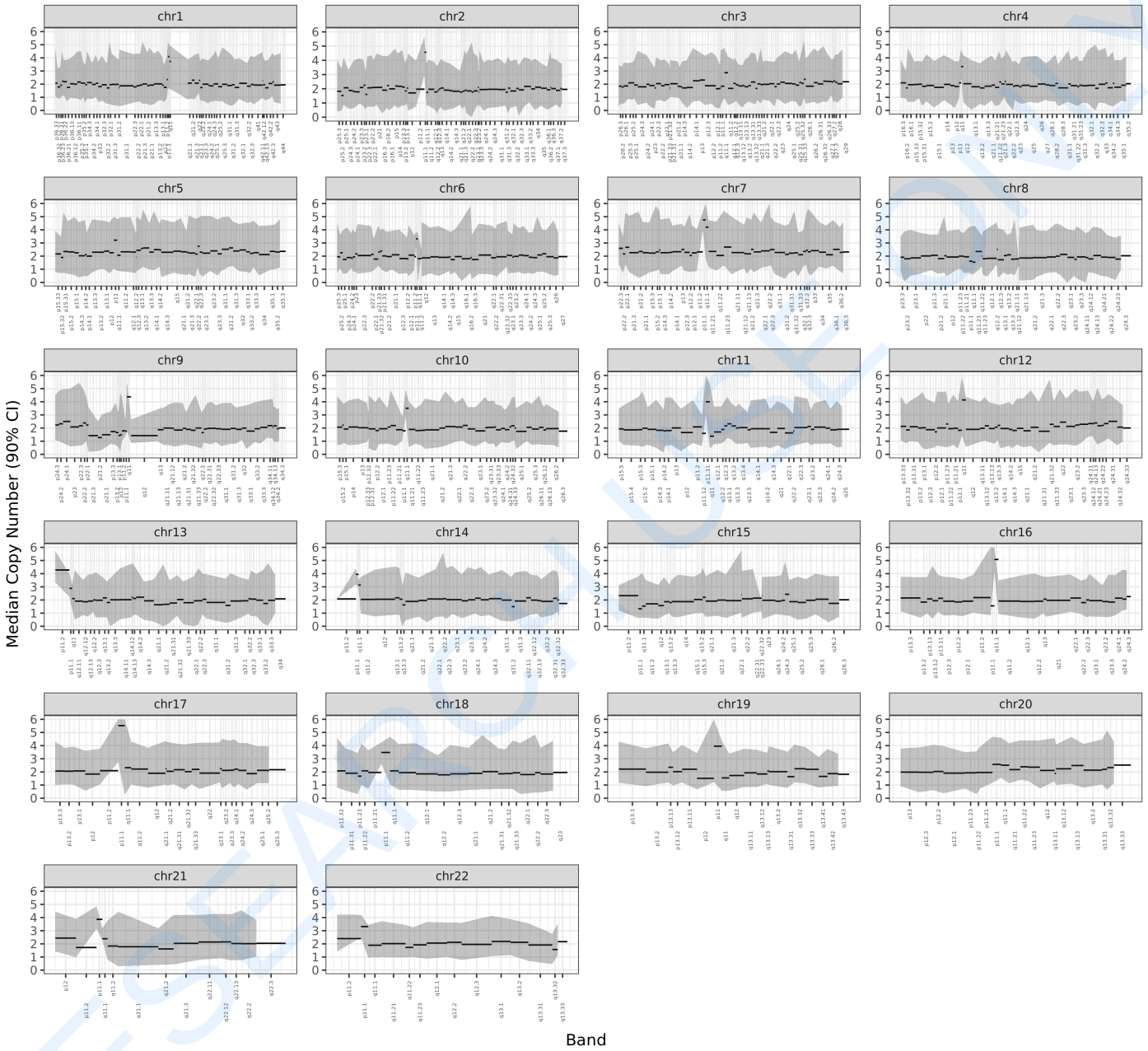
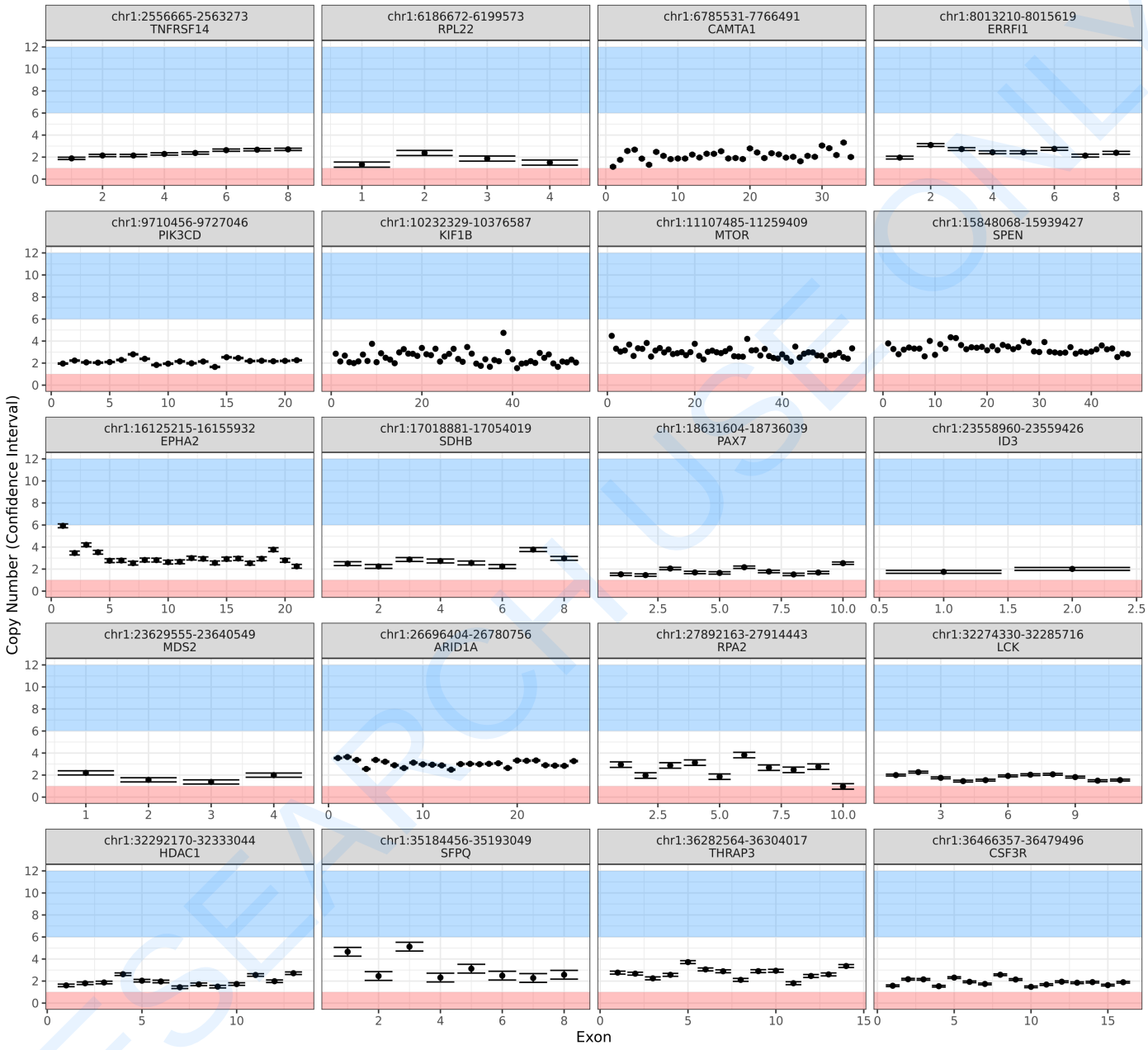
Band-Level Karyotype



Karyotyping using Copy Number Analysis by Whole Exome Sequencing (WES) Methods:
Band-level karyotyping is the process of analyzing chromosomes to identify structure, number and potential abnormalities via cytogenetic staining techniques. Band-level analysis was performed here to estimate copy number across the karyotype: y-axis - median copy number (90% confidence interval range) along the length of each chromosome (x-axis).

Copy Number Alterations



Copy number alterations by whole exome sequencing (WES):
The following plots provide estimated copy number values (black circle, y-axis) of exons per gene (x-axis), organized by chromosome (grey headers). Respective confidence intervals of each copy number call is also plotted for examining statistical error associated with next generation sequencing. Values are currently capped at 12 copies.

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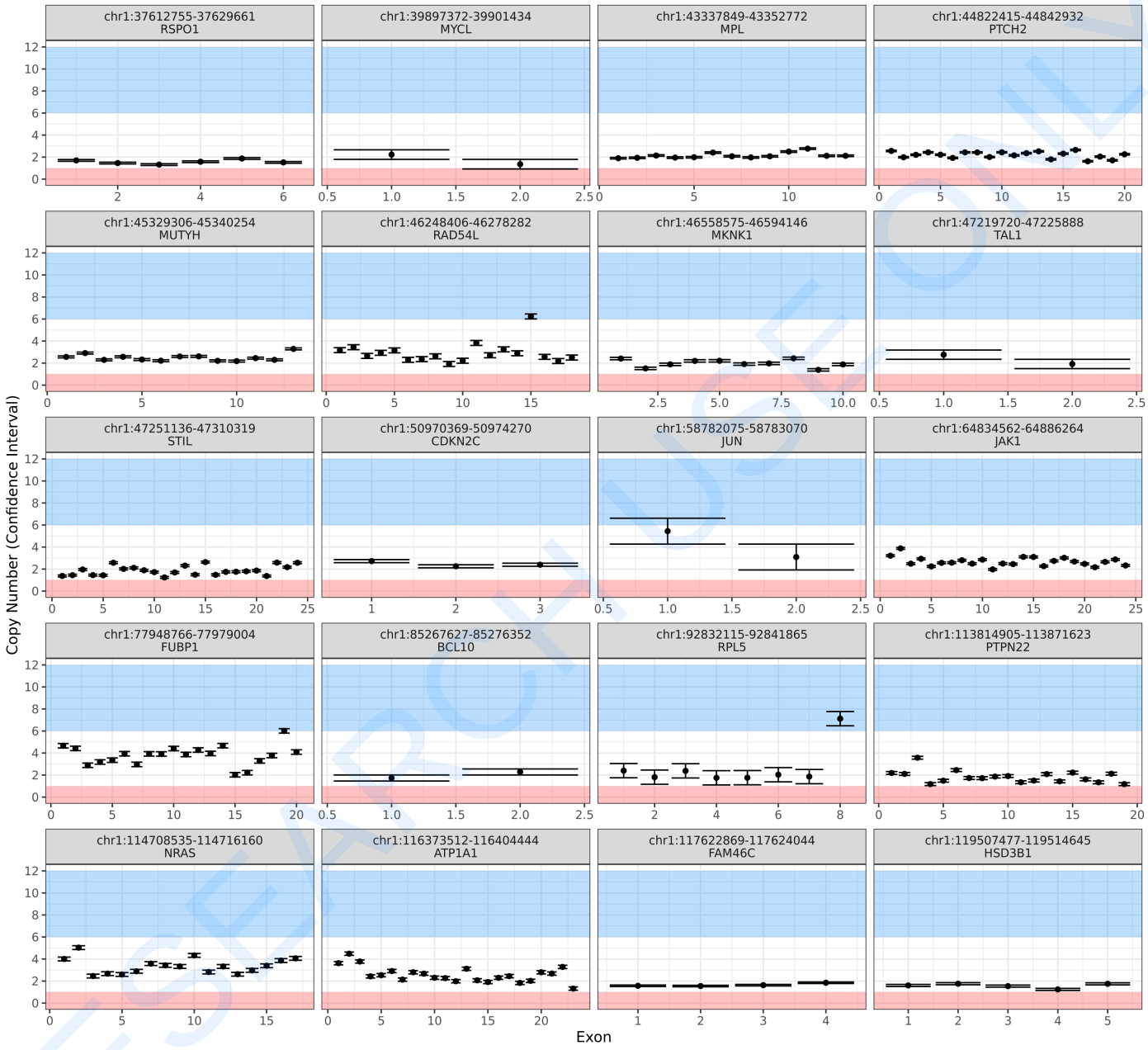

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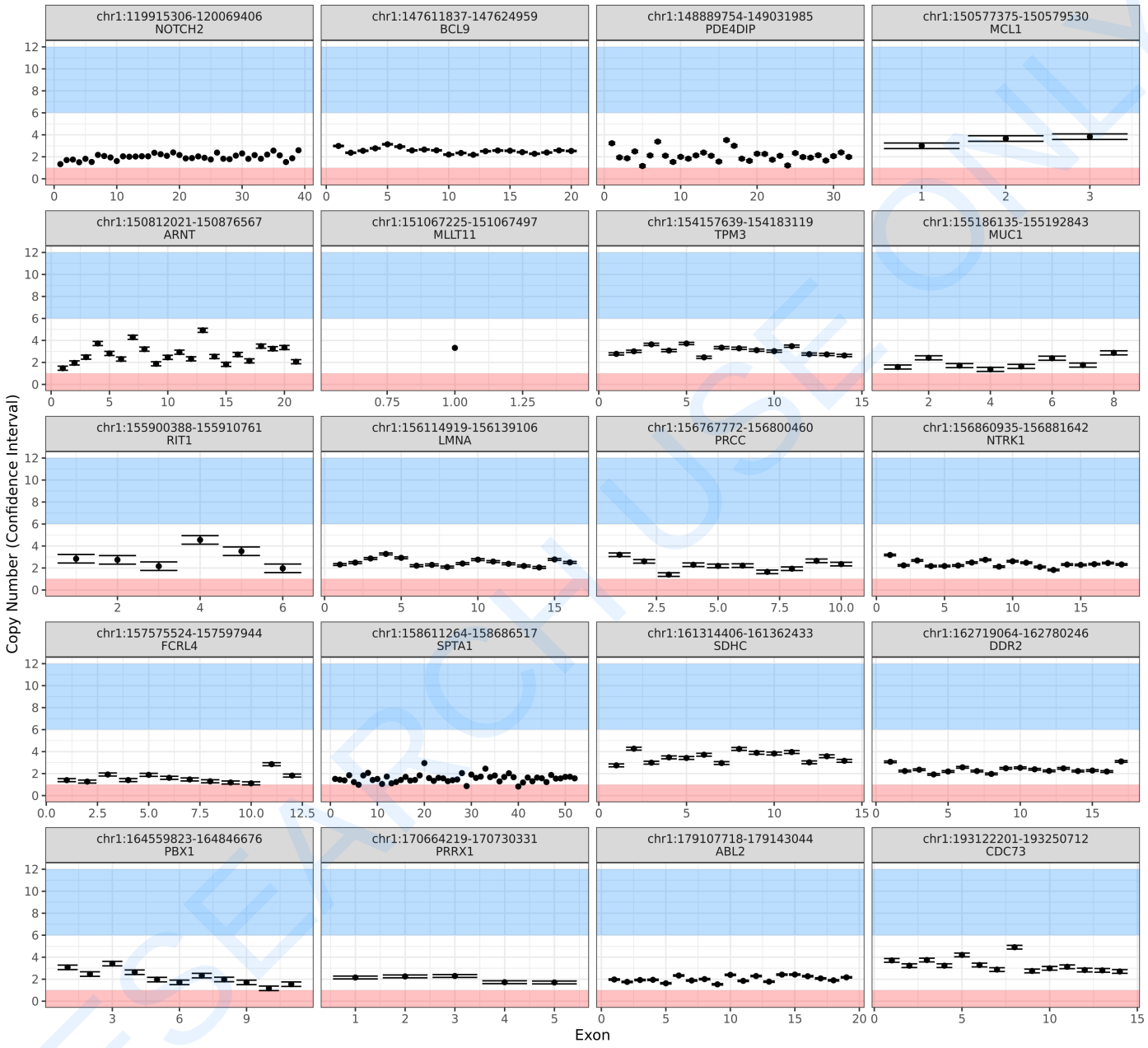

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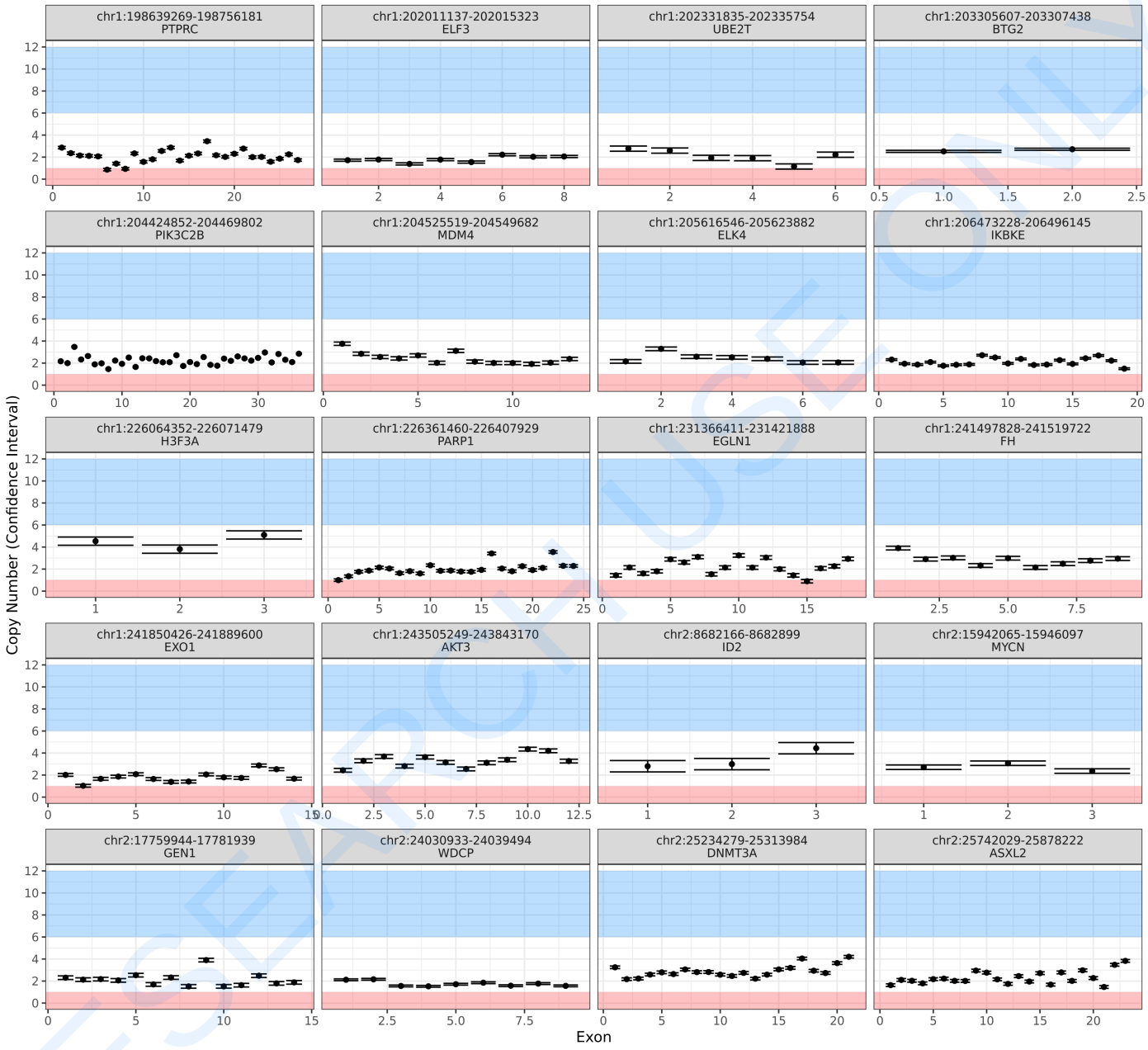

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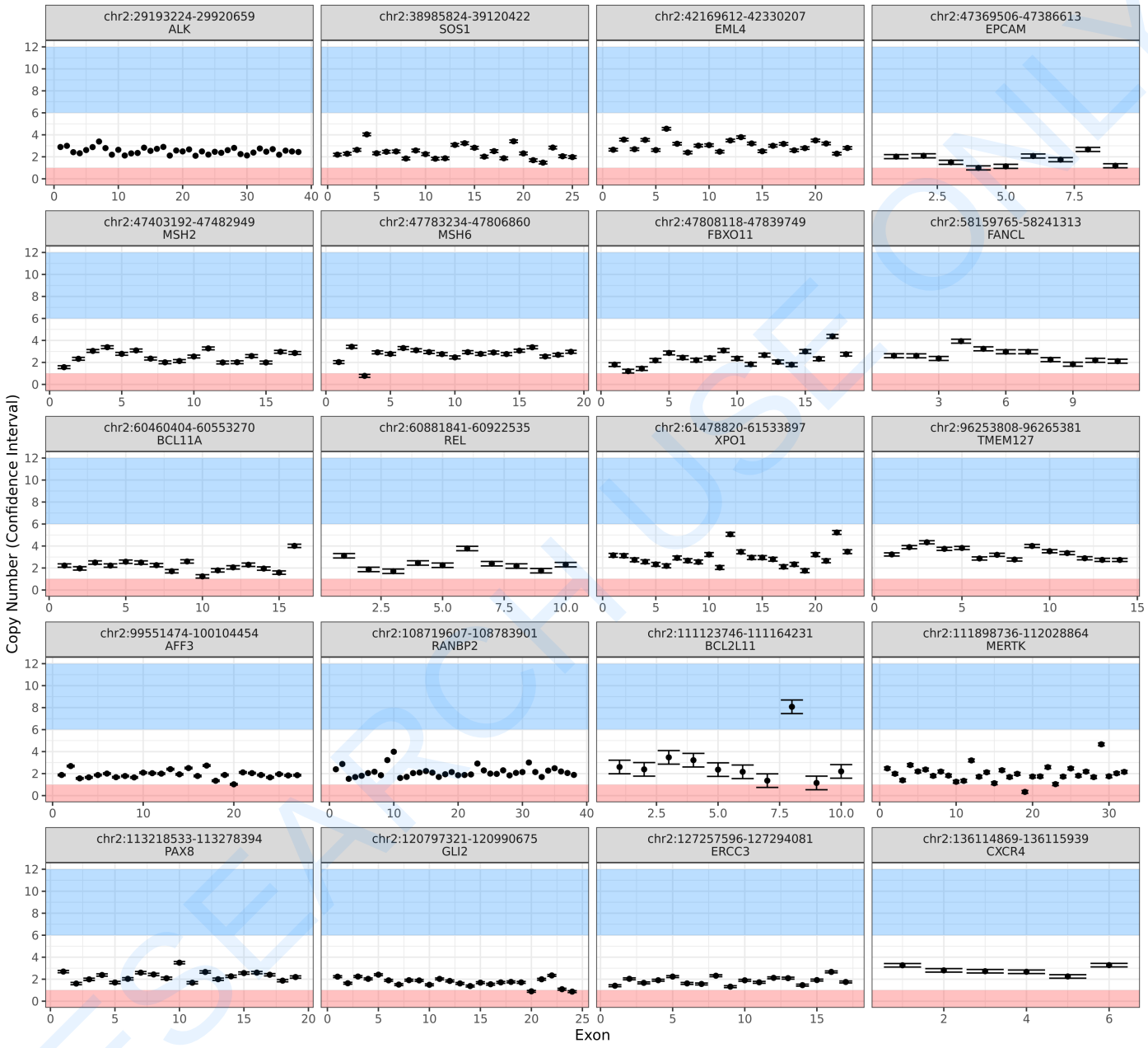

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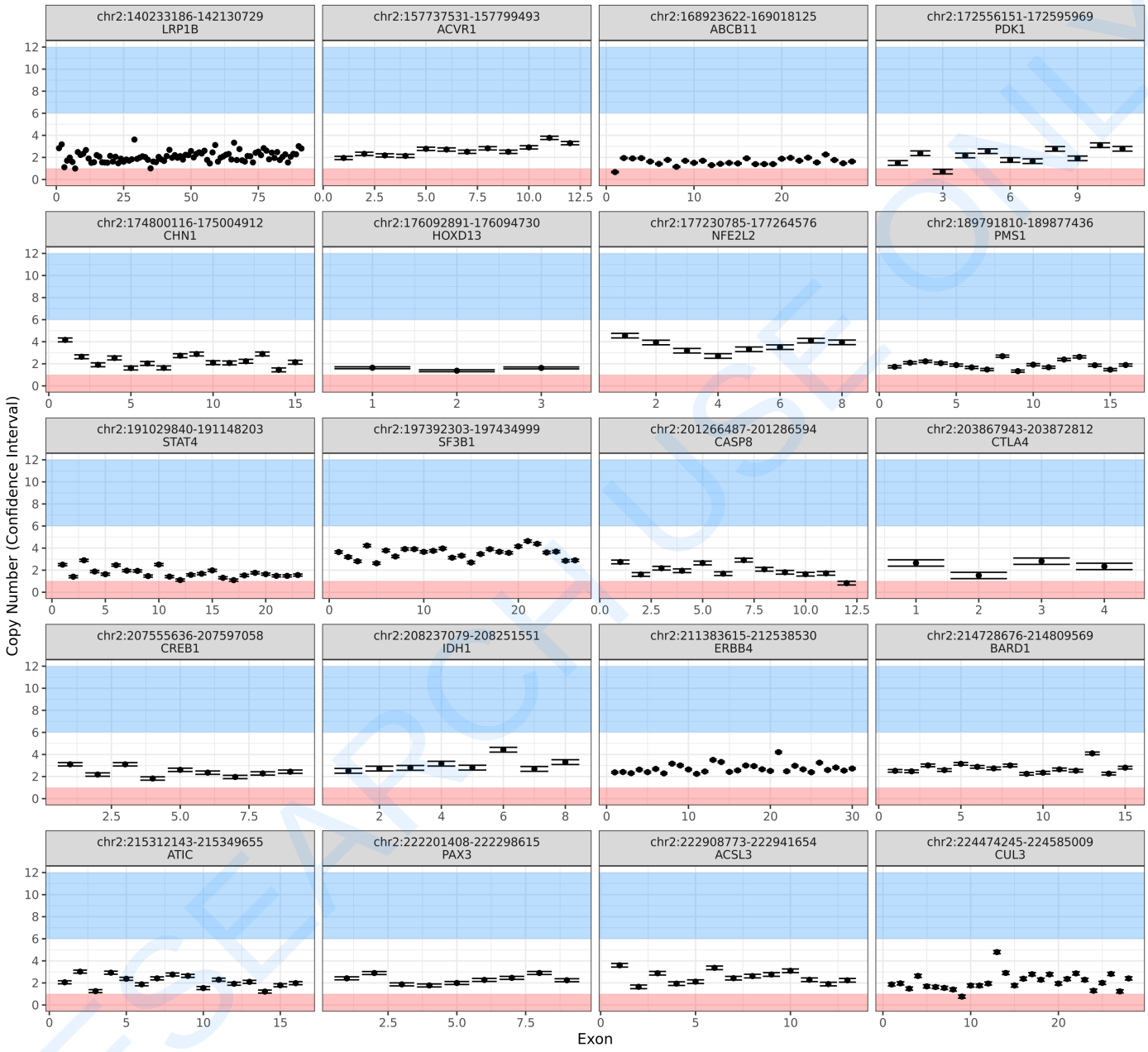

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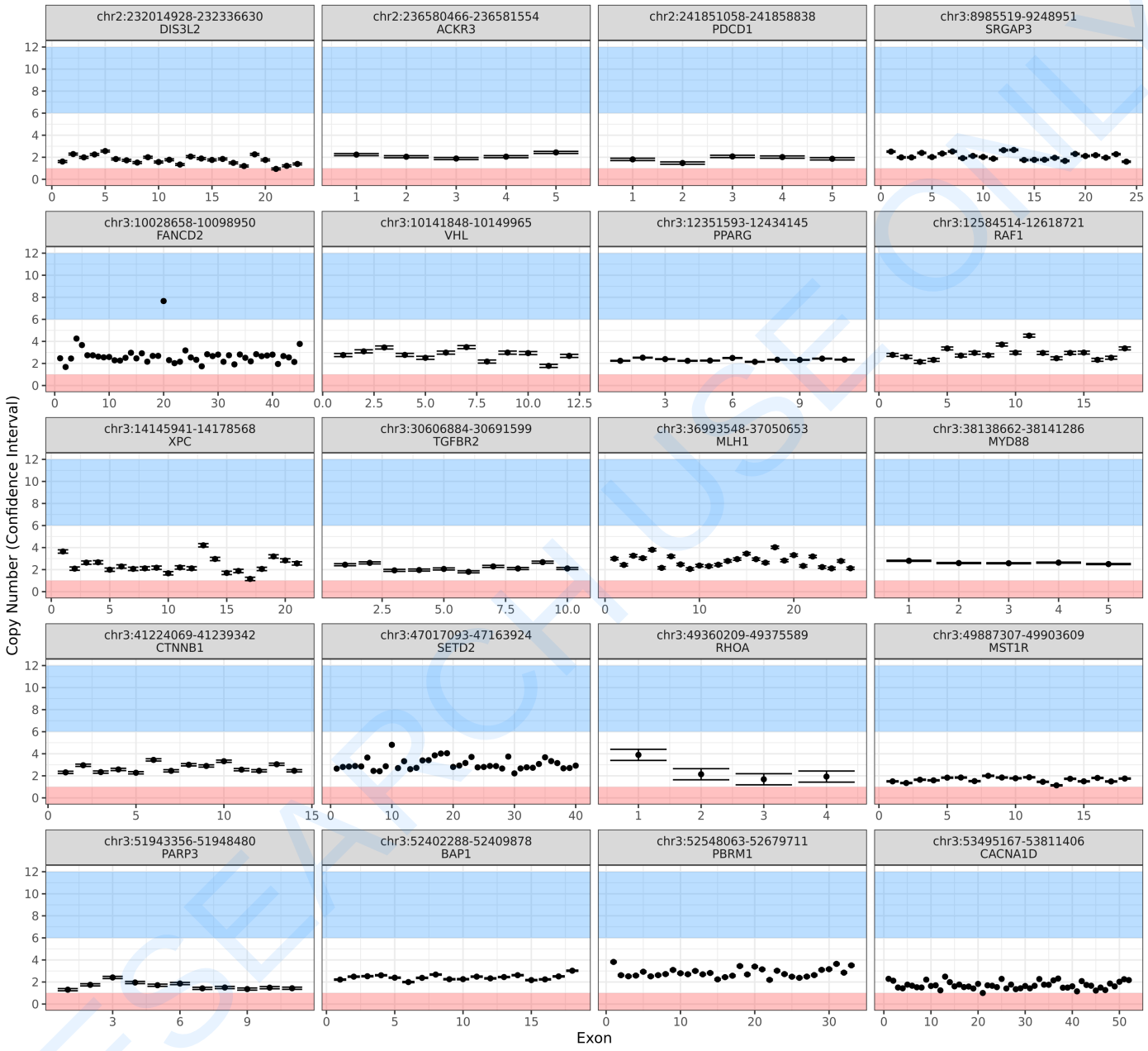

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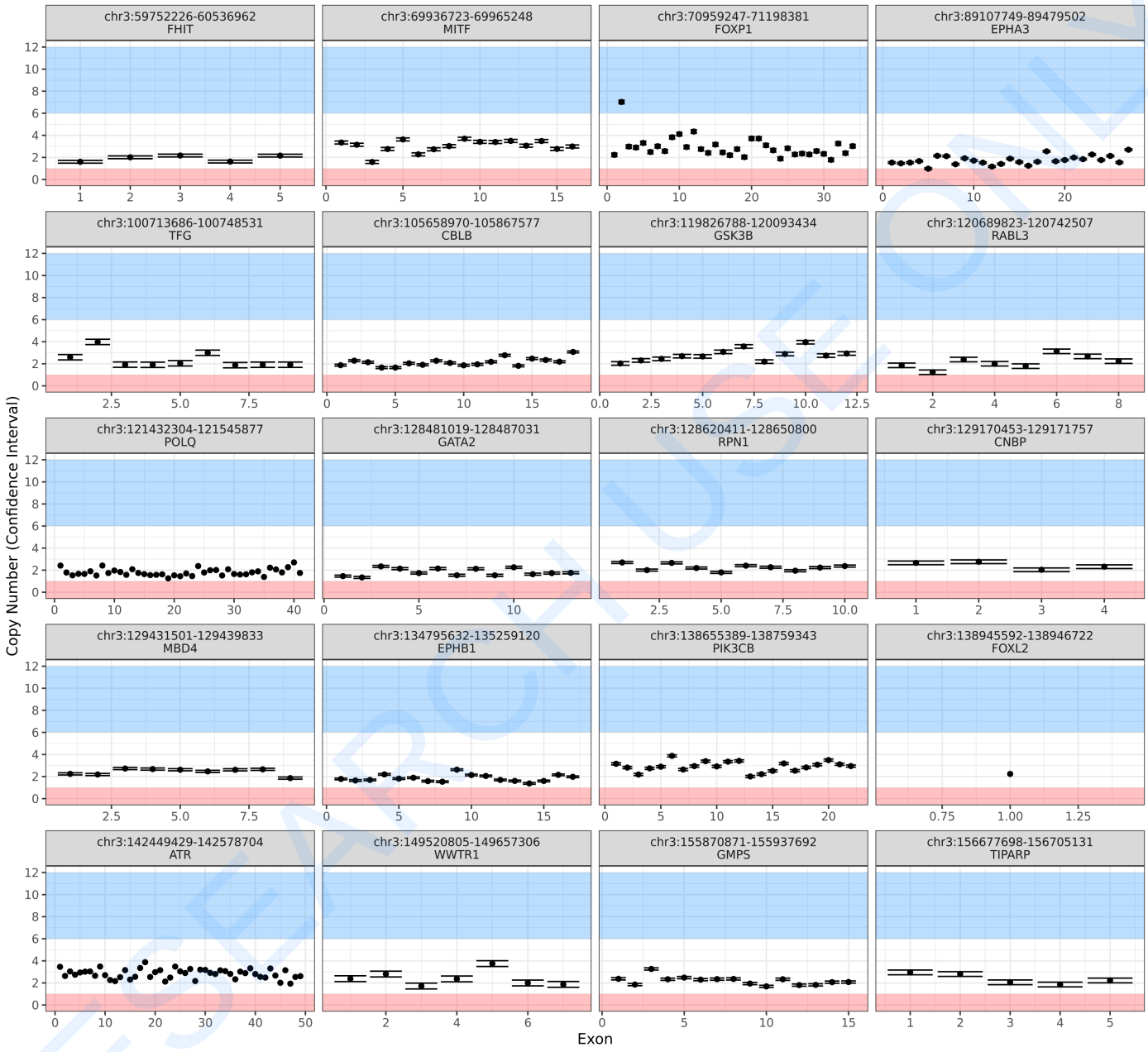

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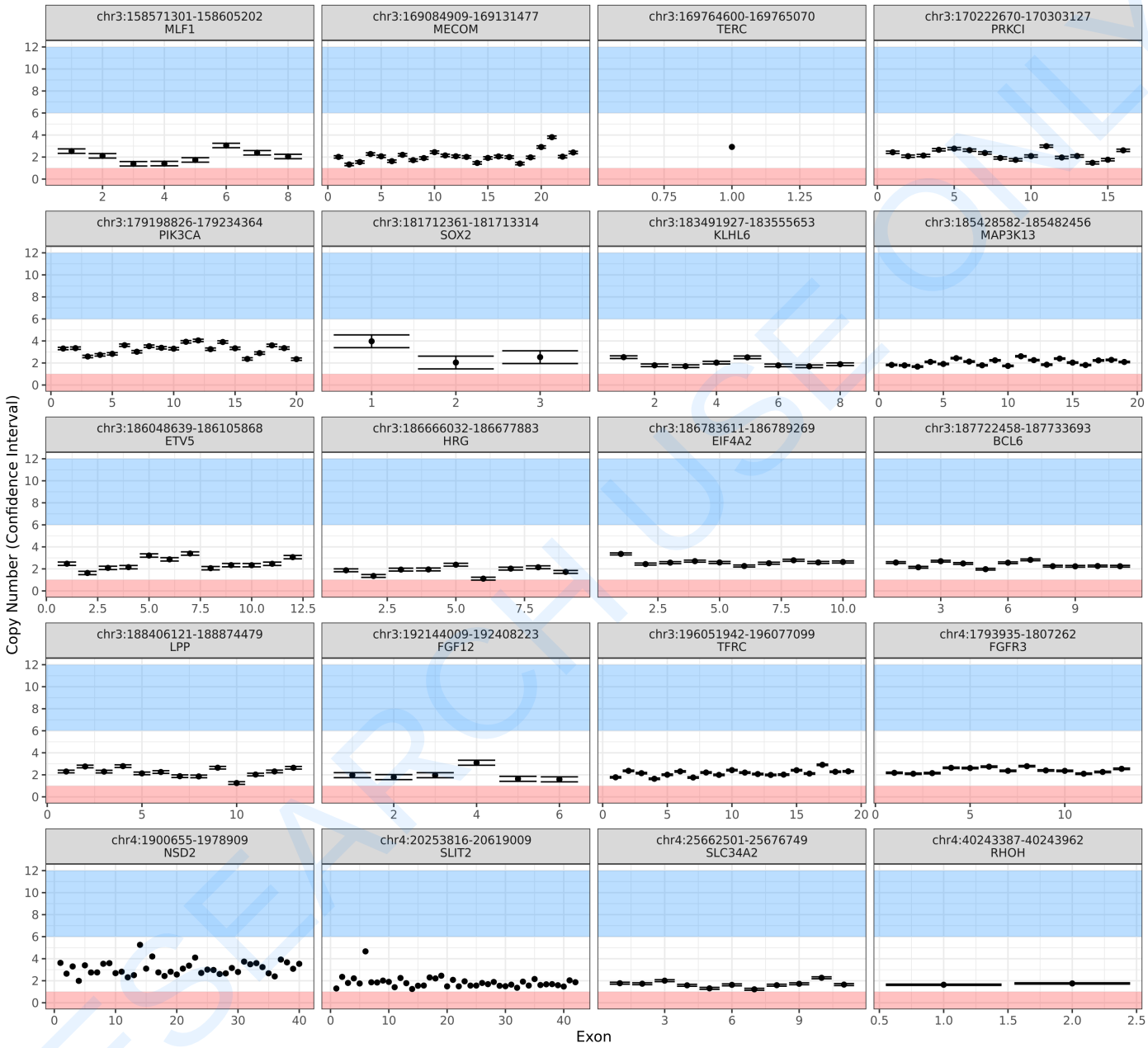

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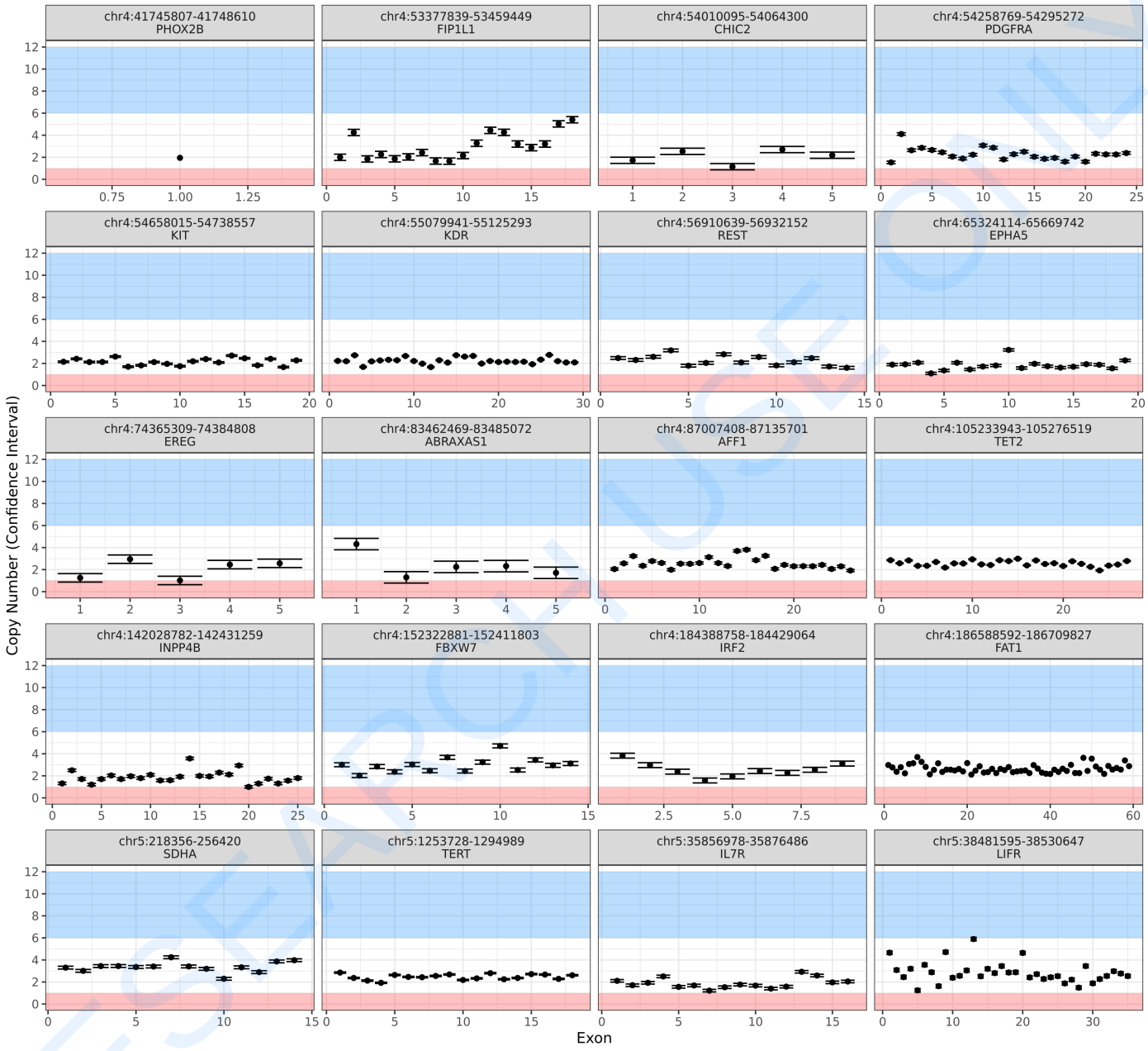

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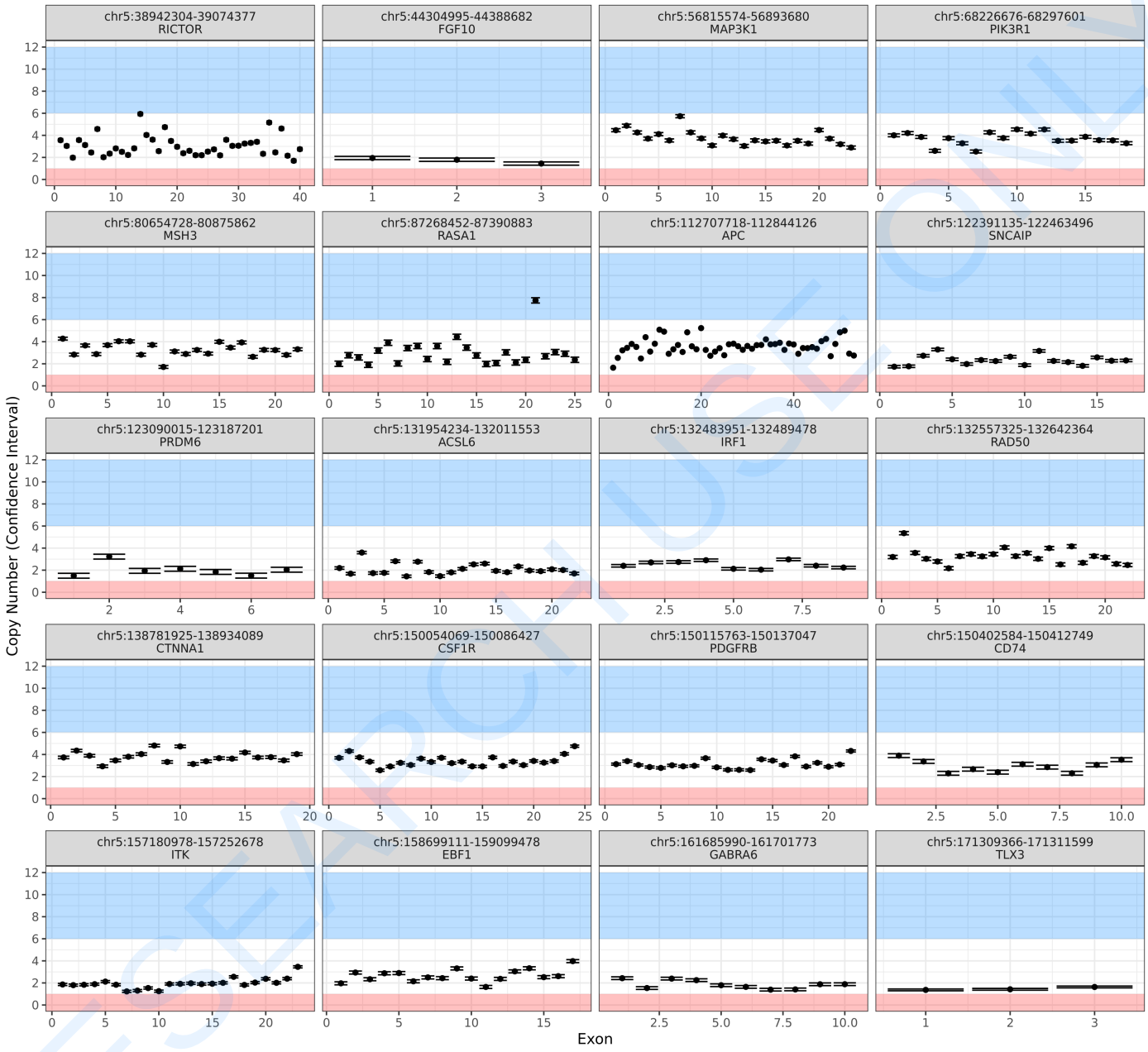

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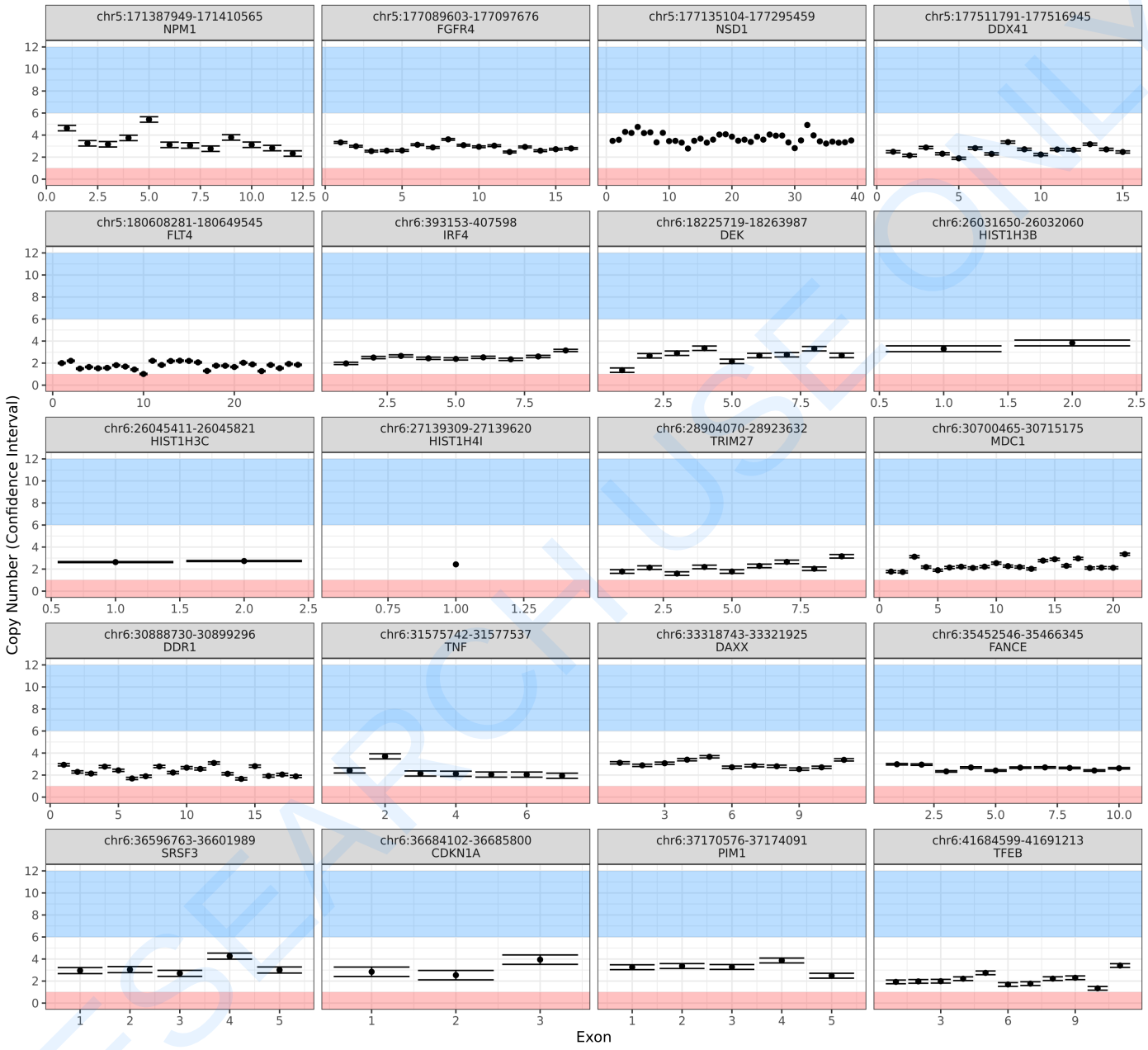

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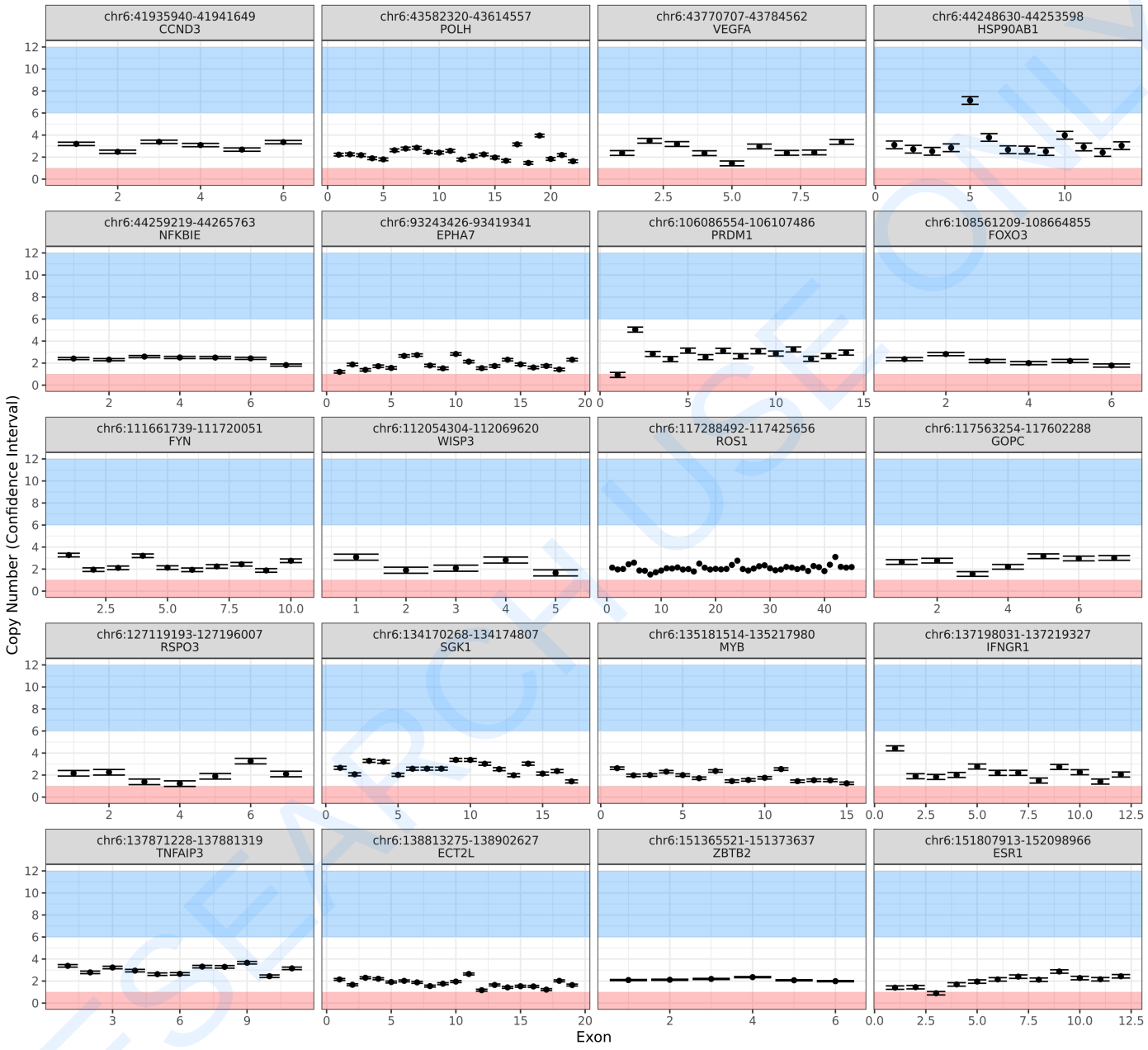

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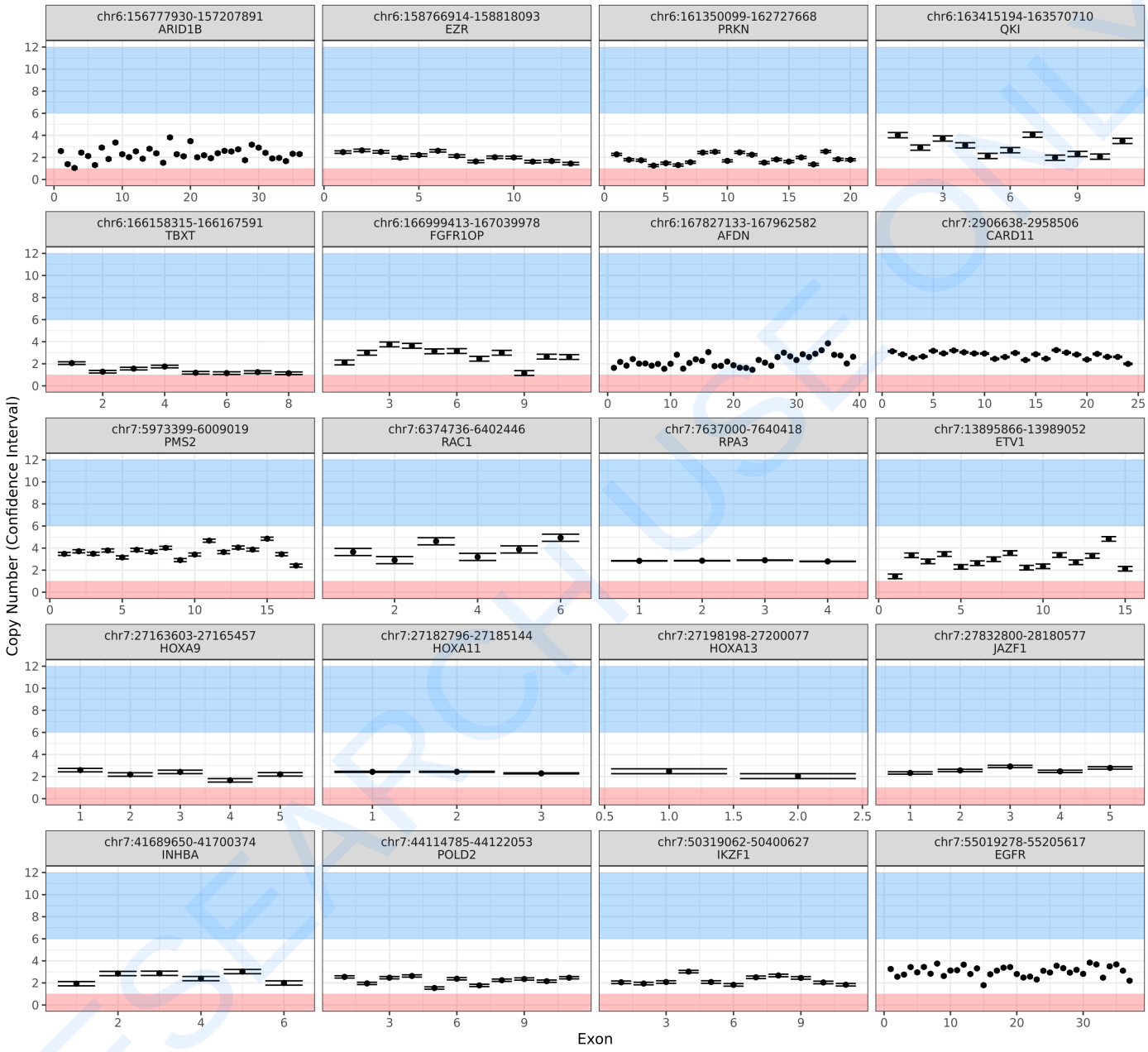

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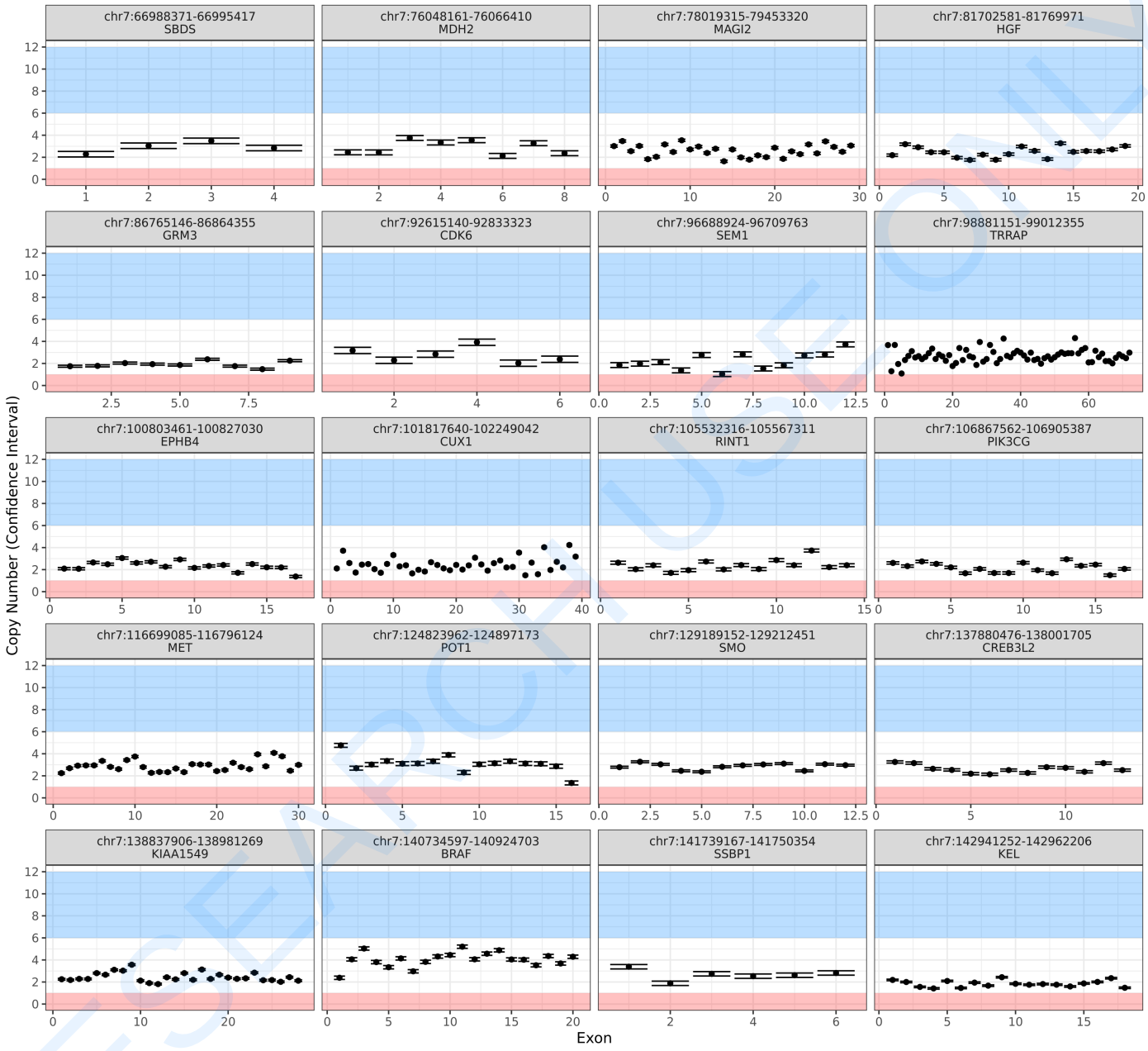

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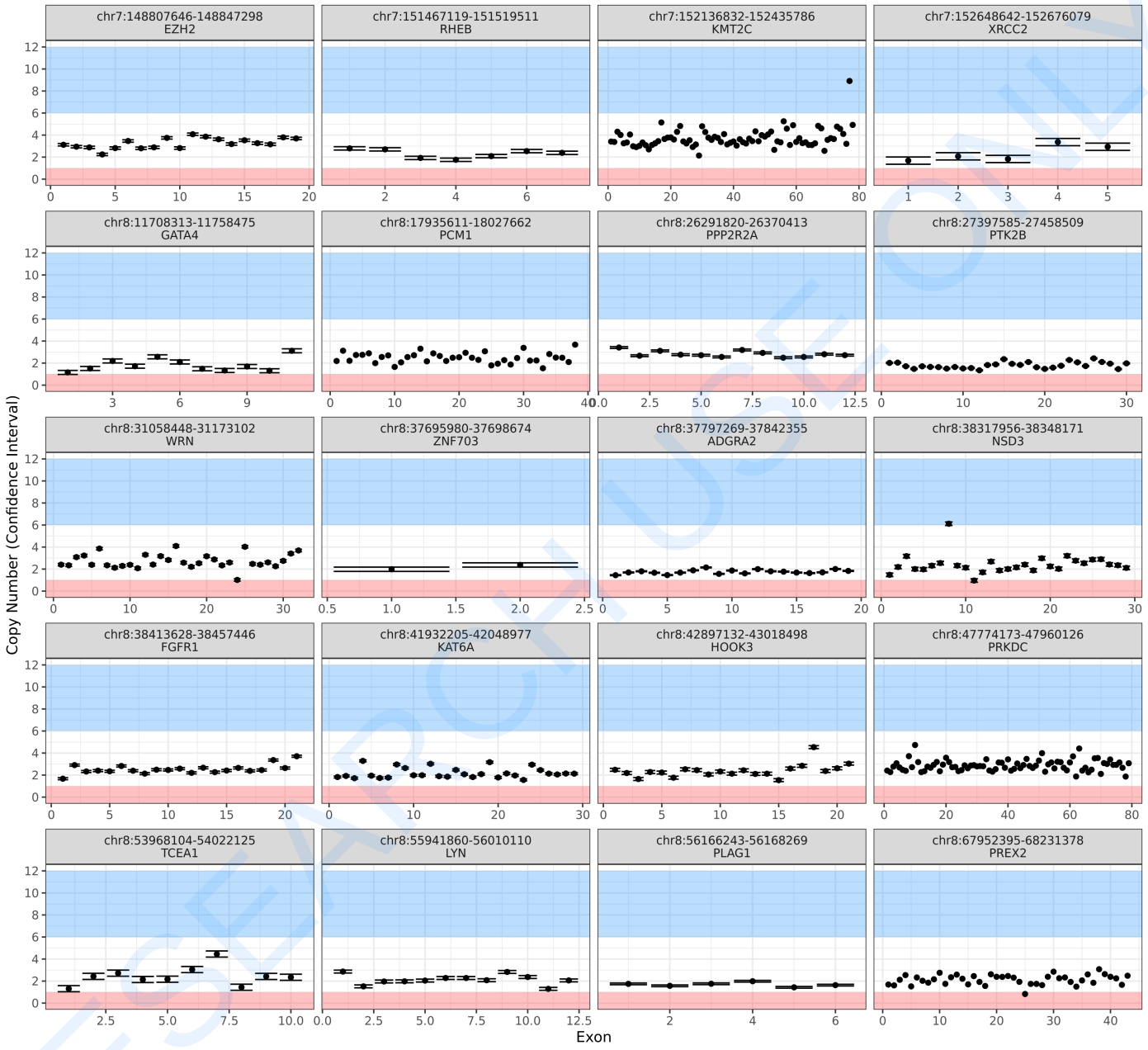

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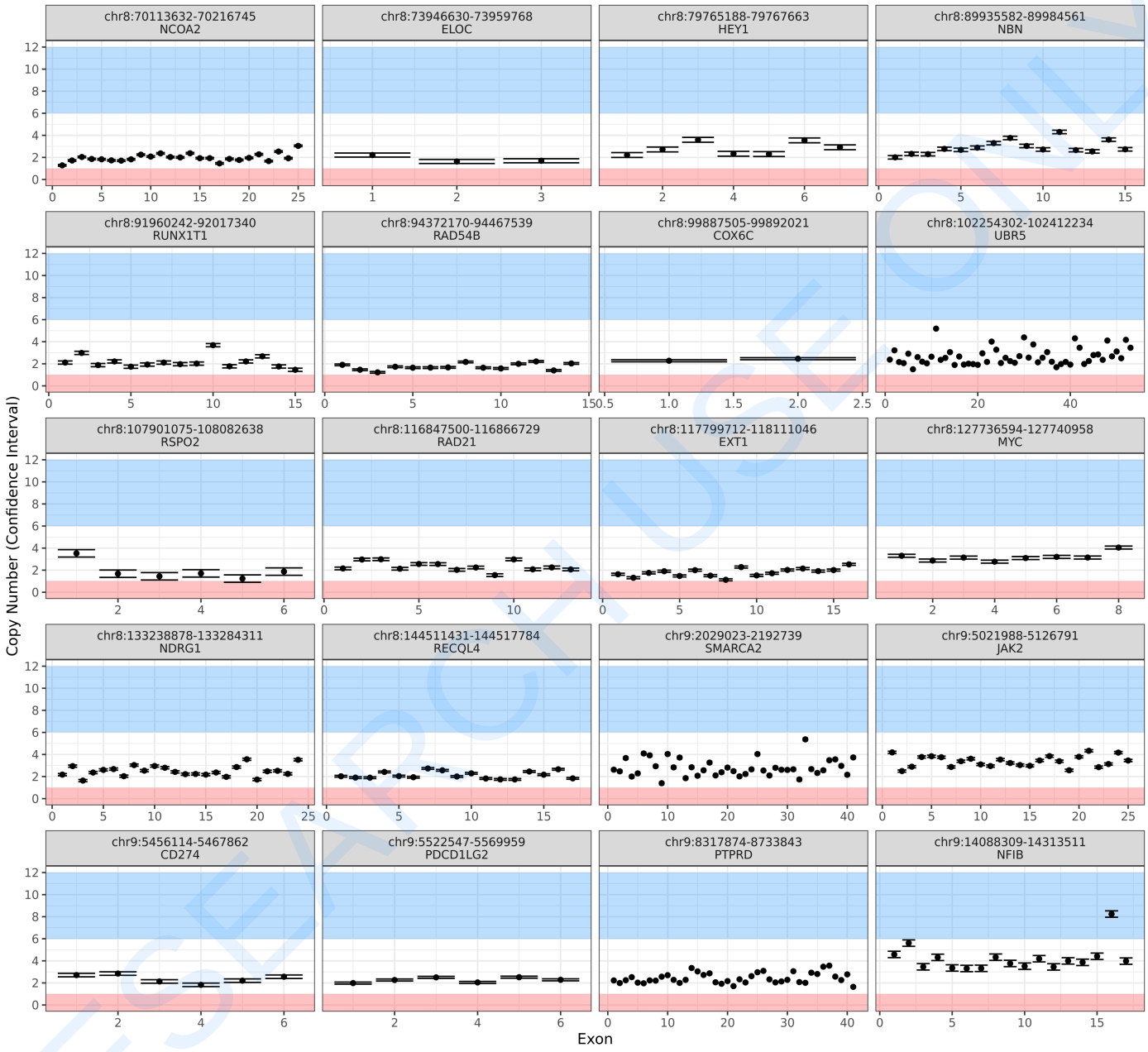

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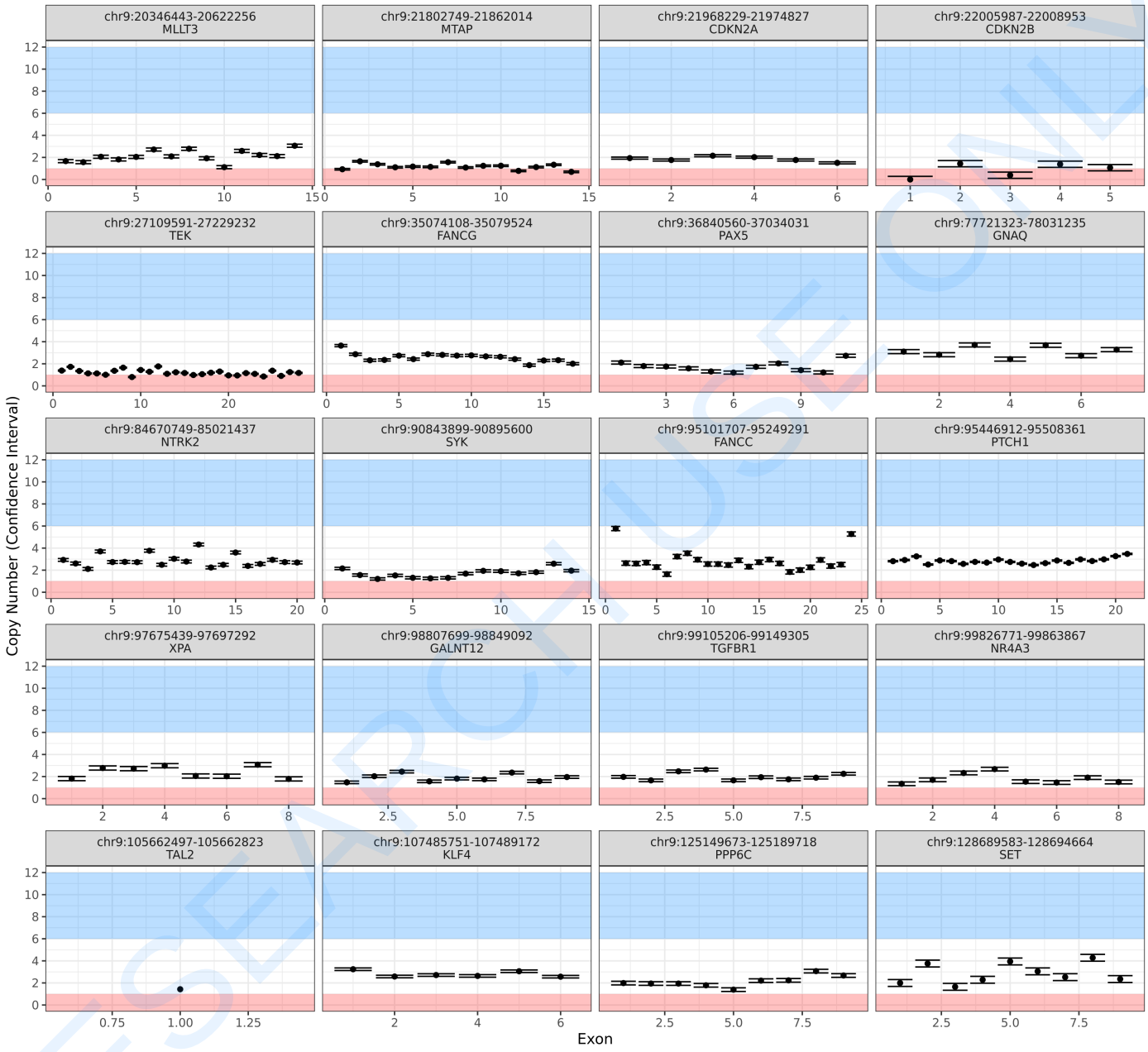

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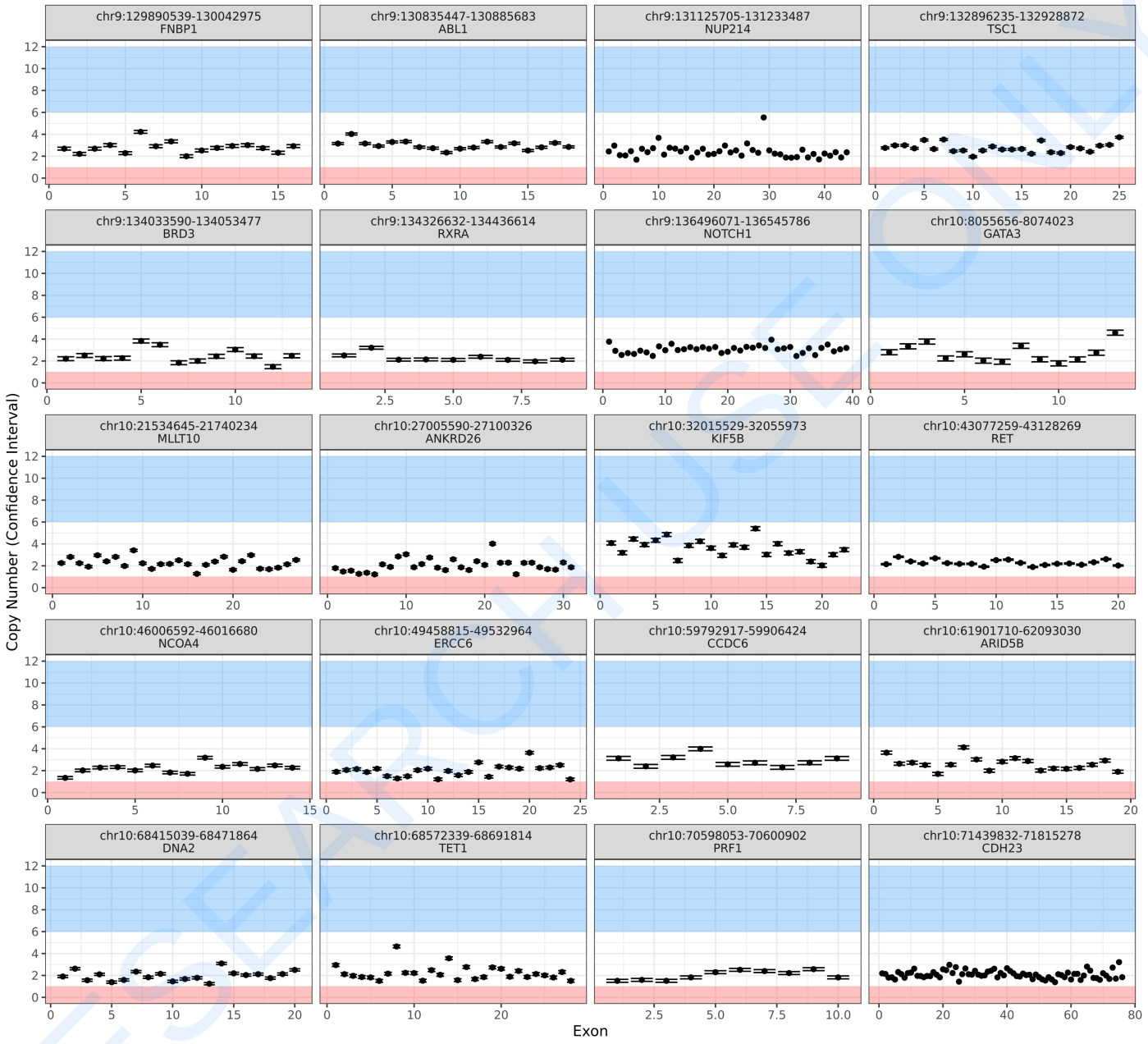

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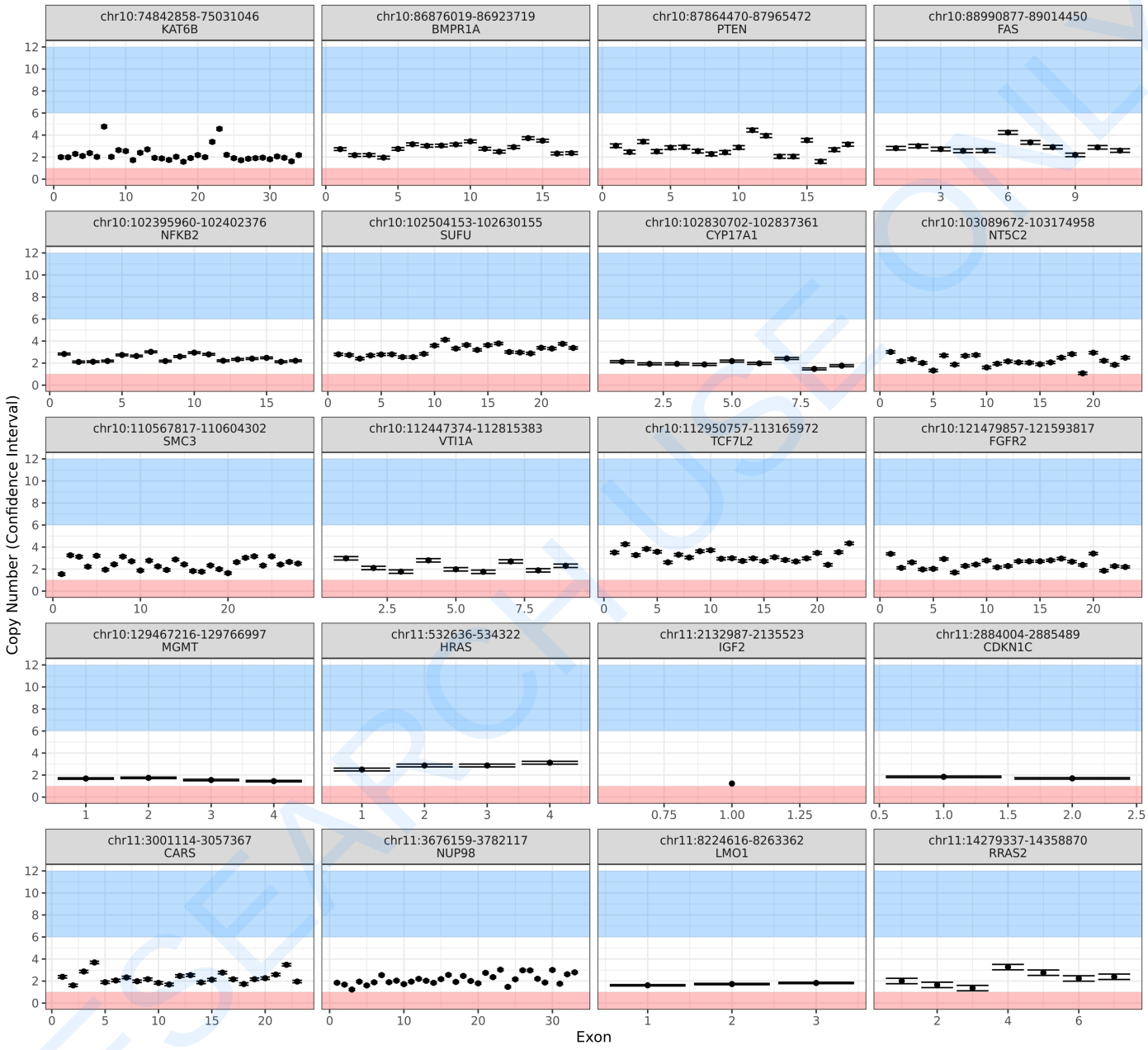

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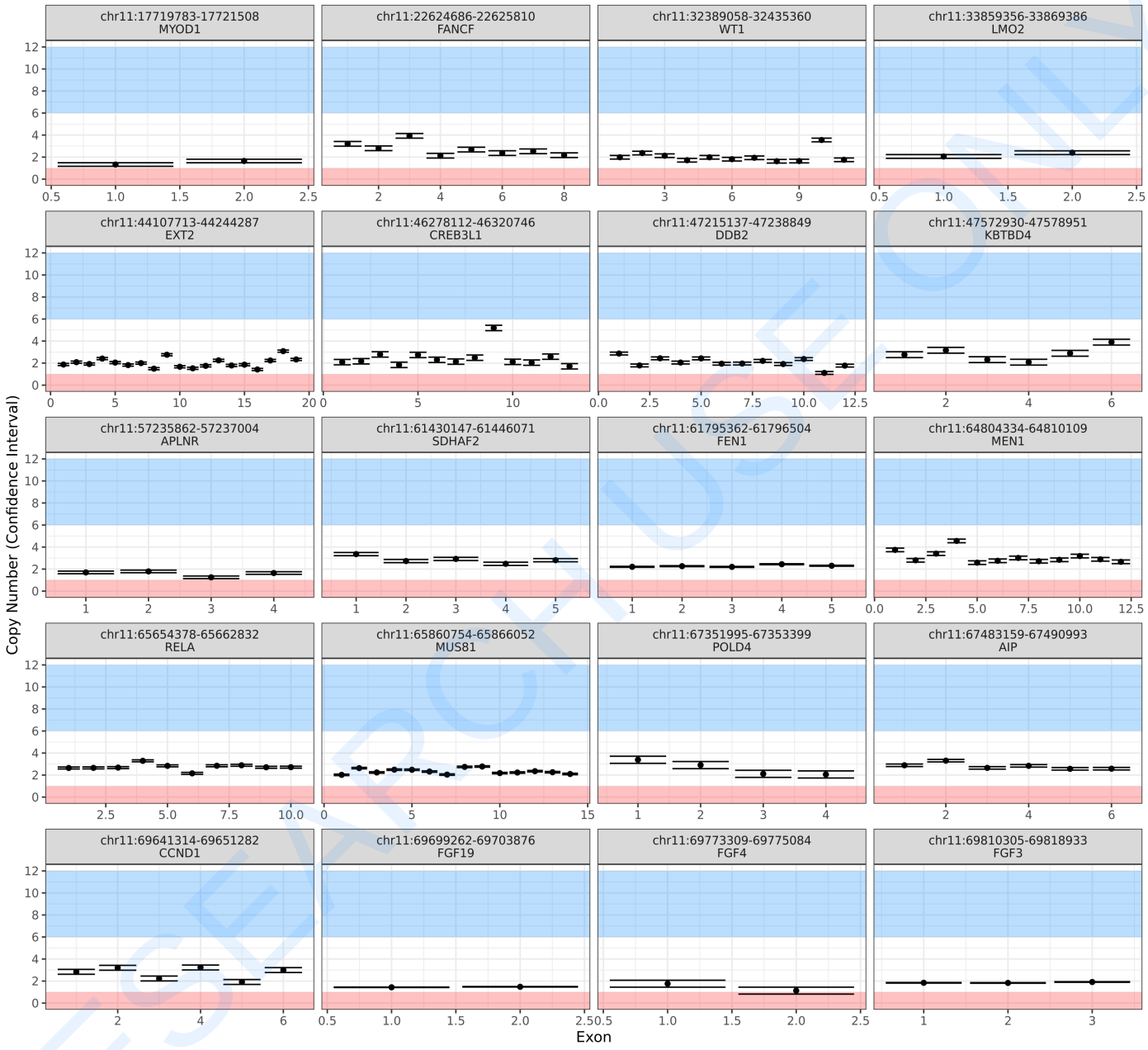

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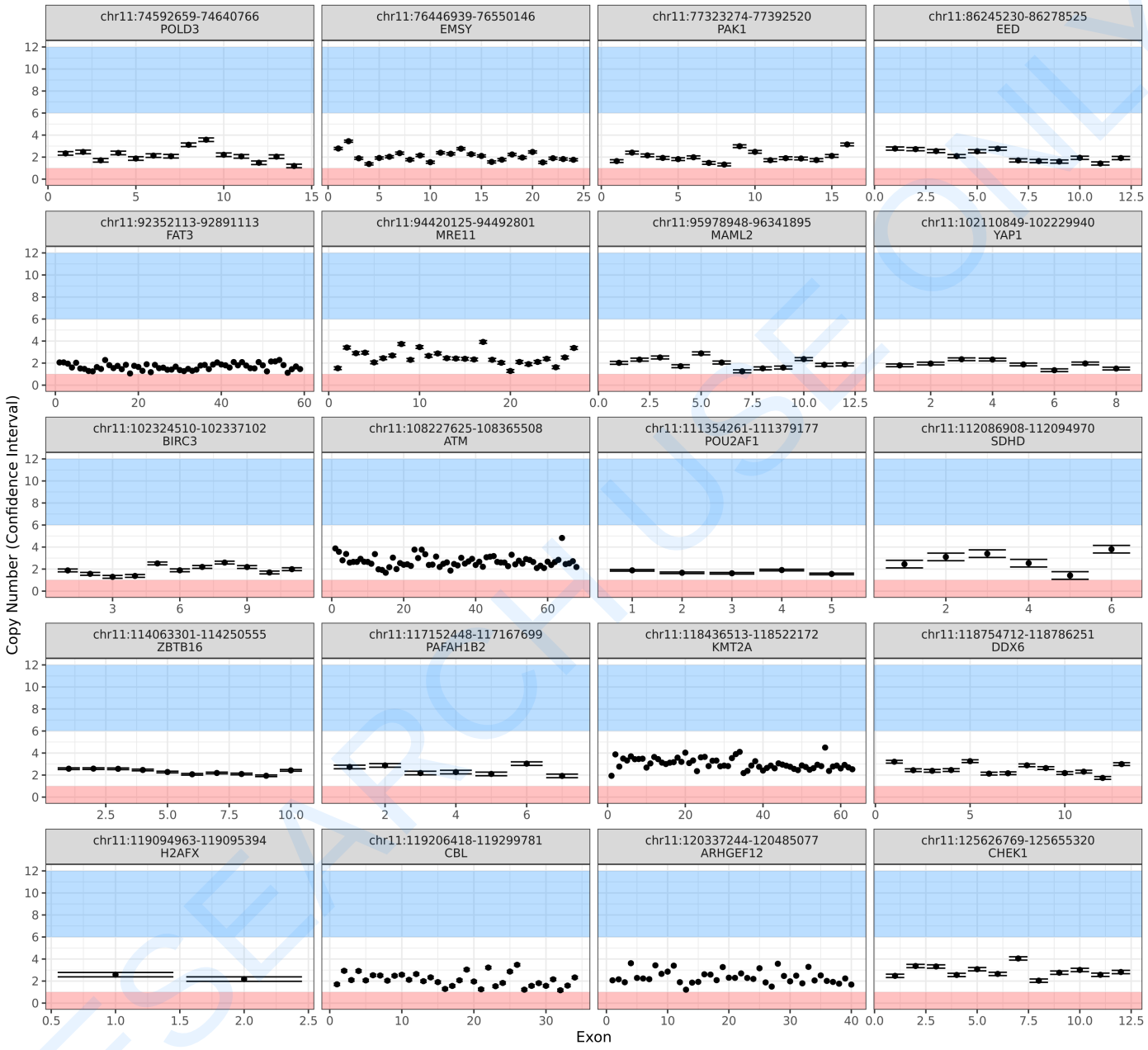

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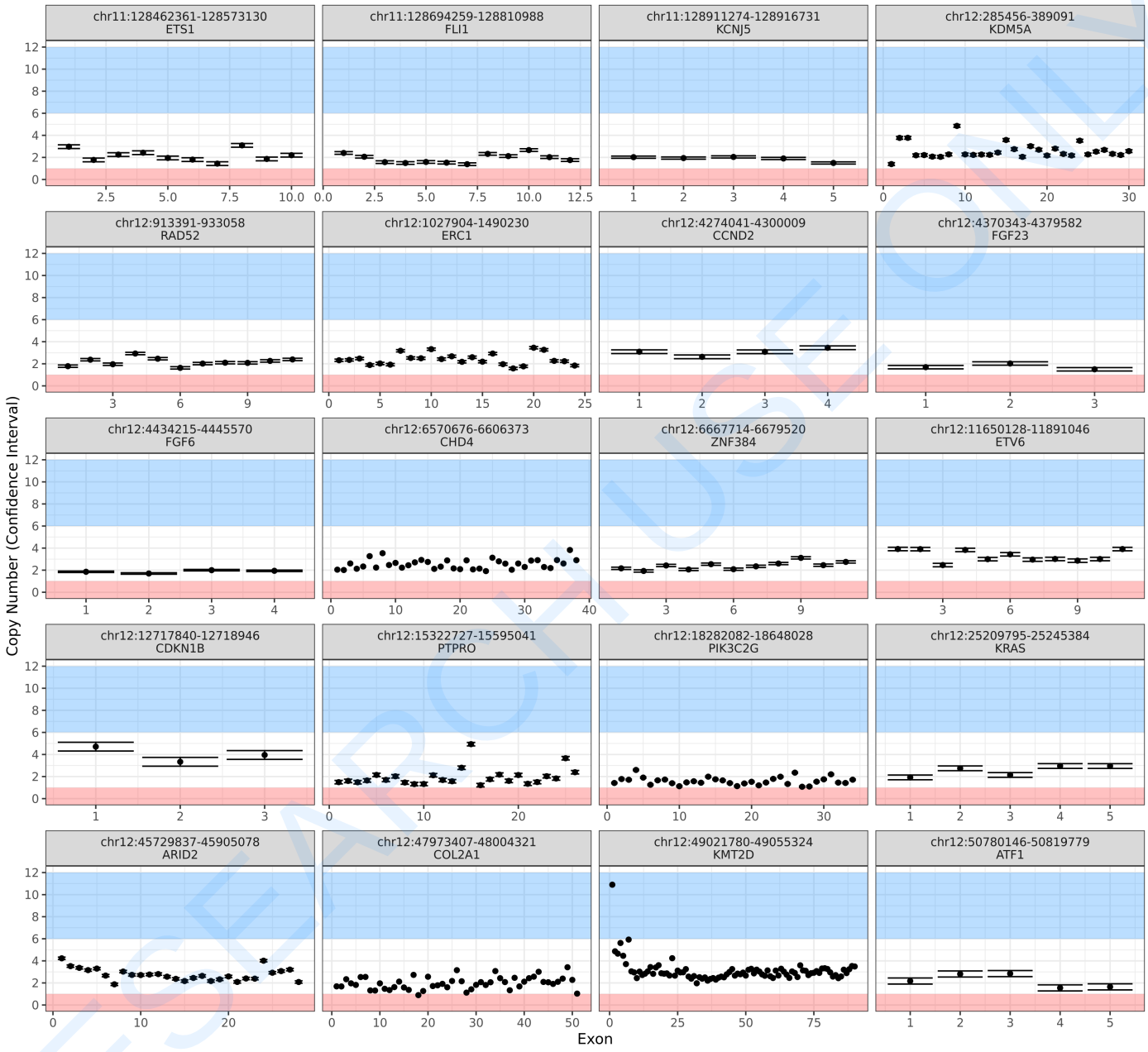

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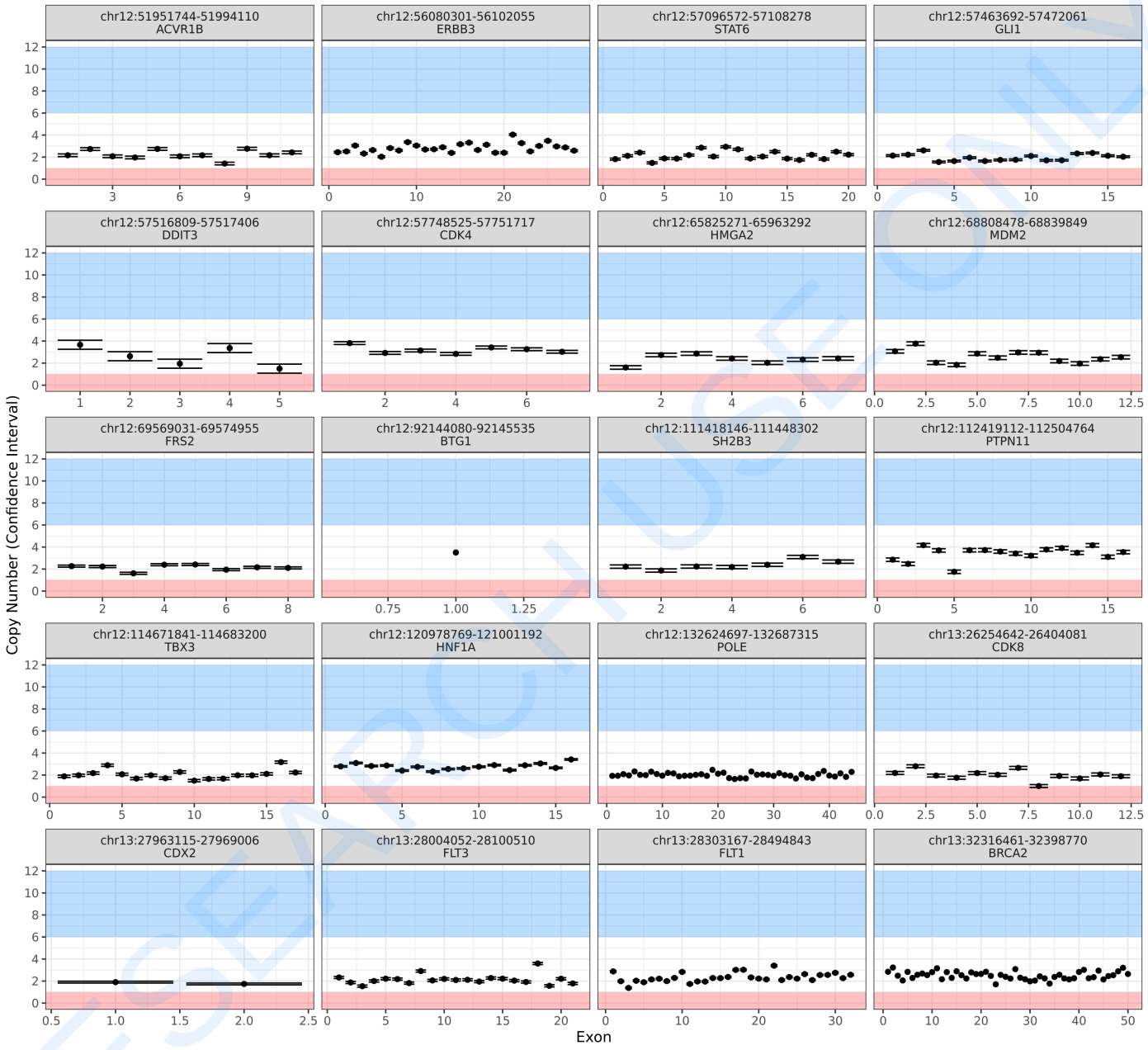

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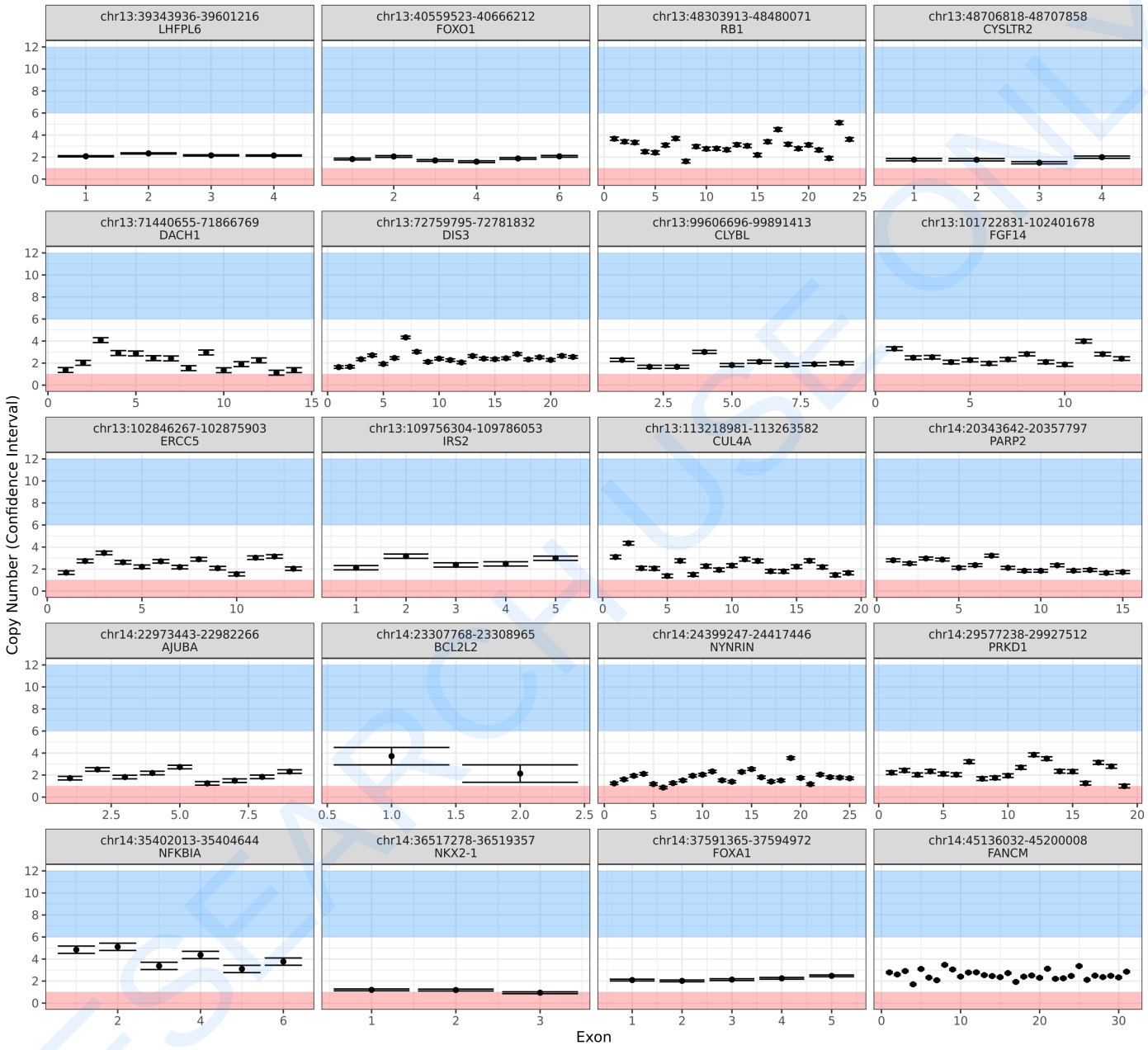

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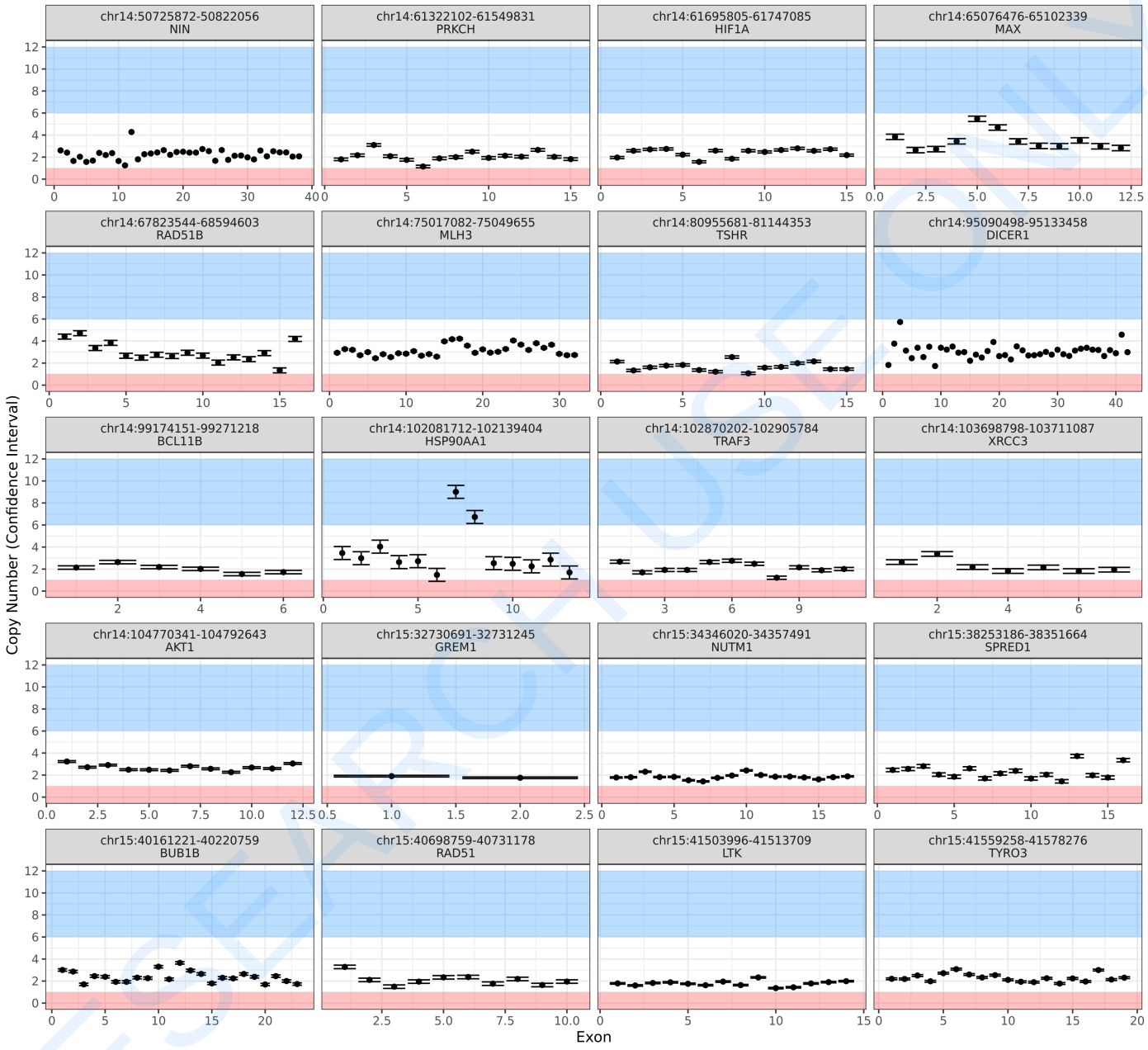

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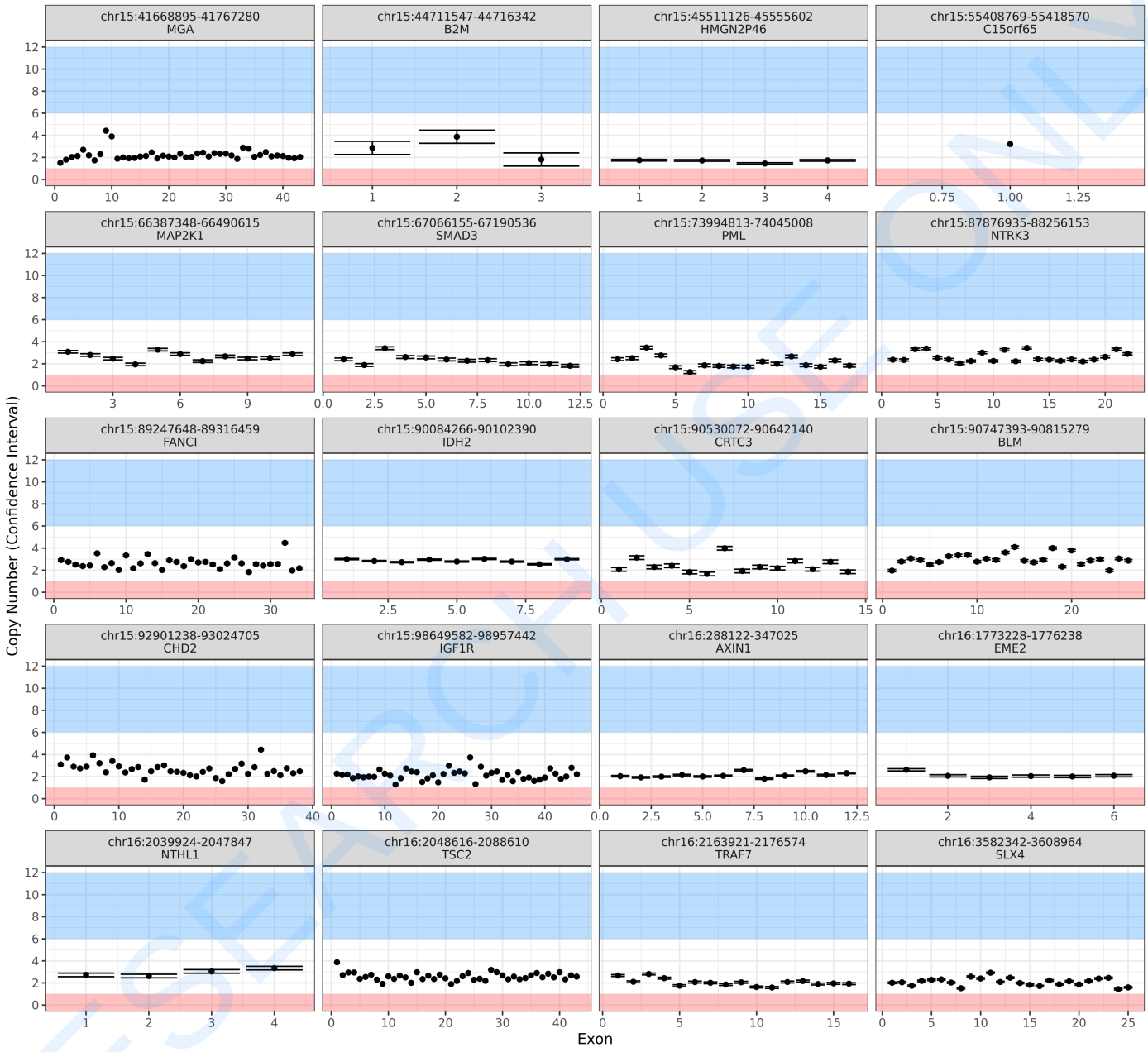

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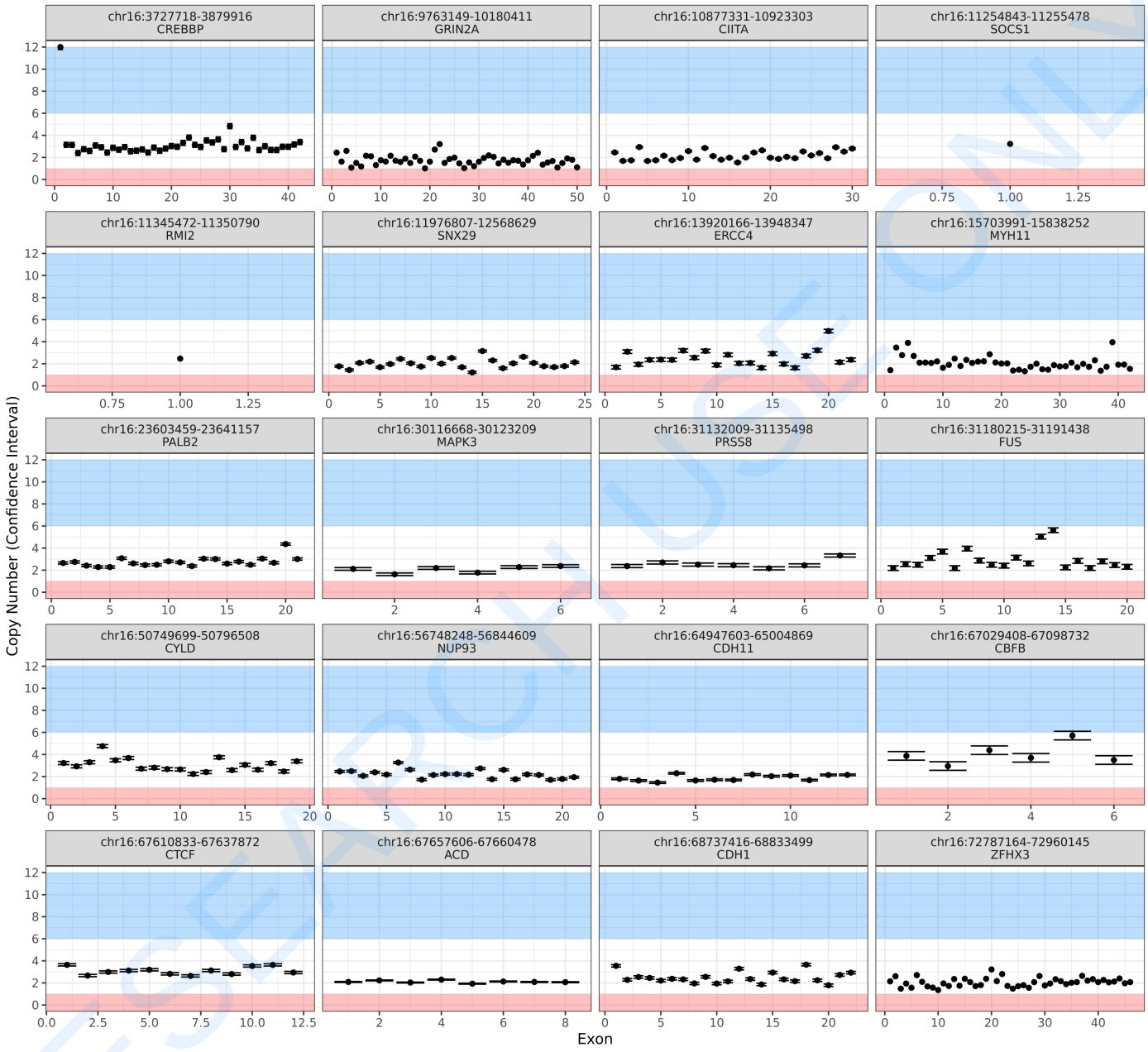

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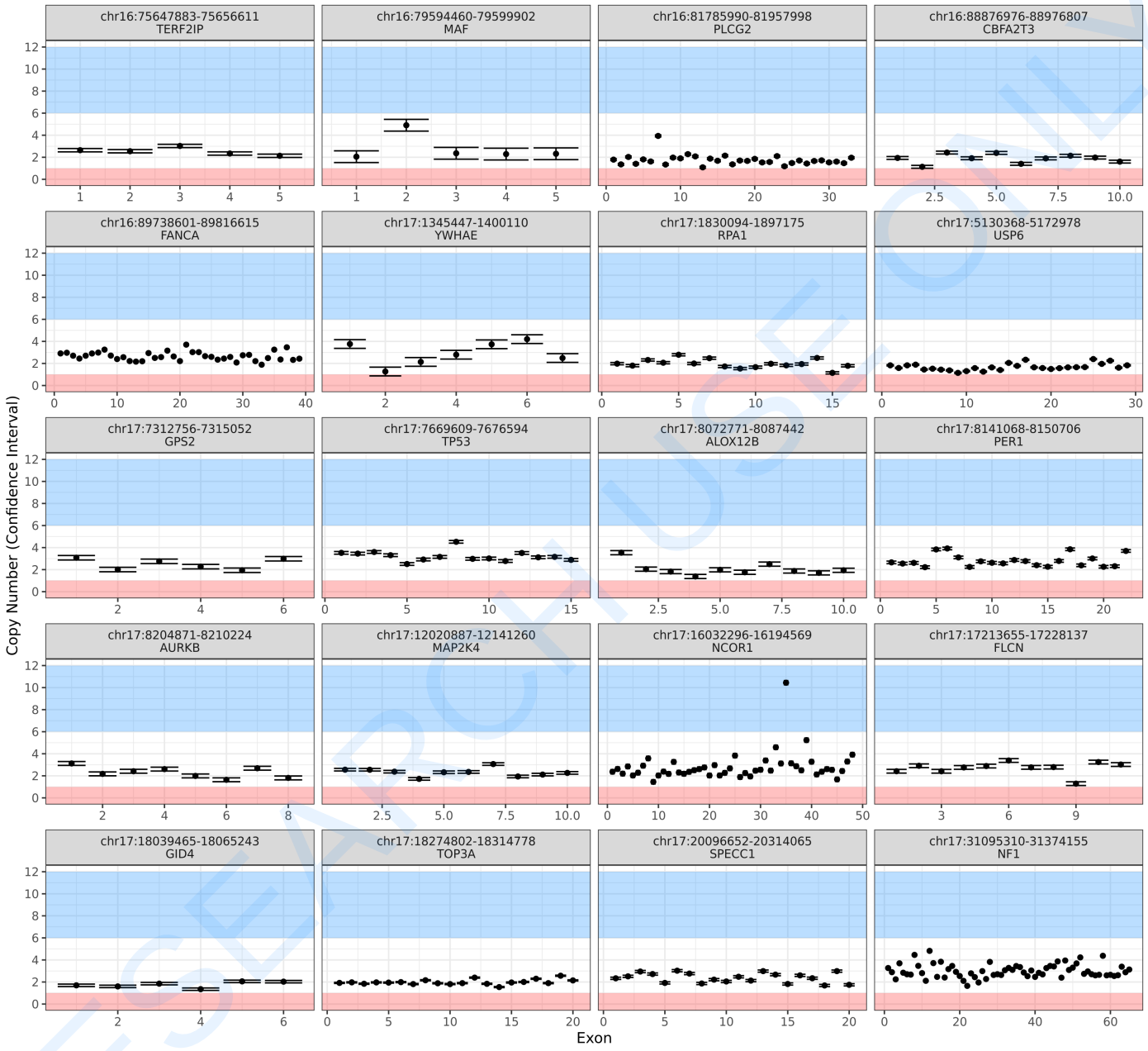

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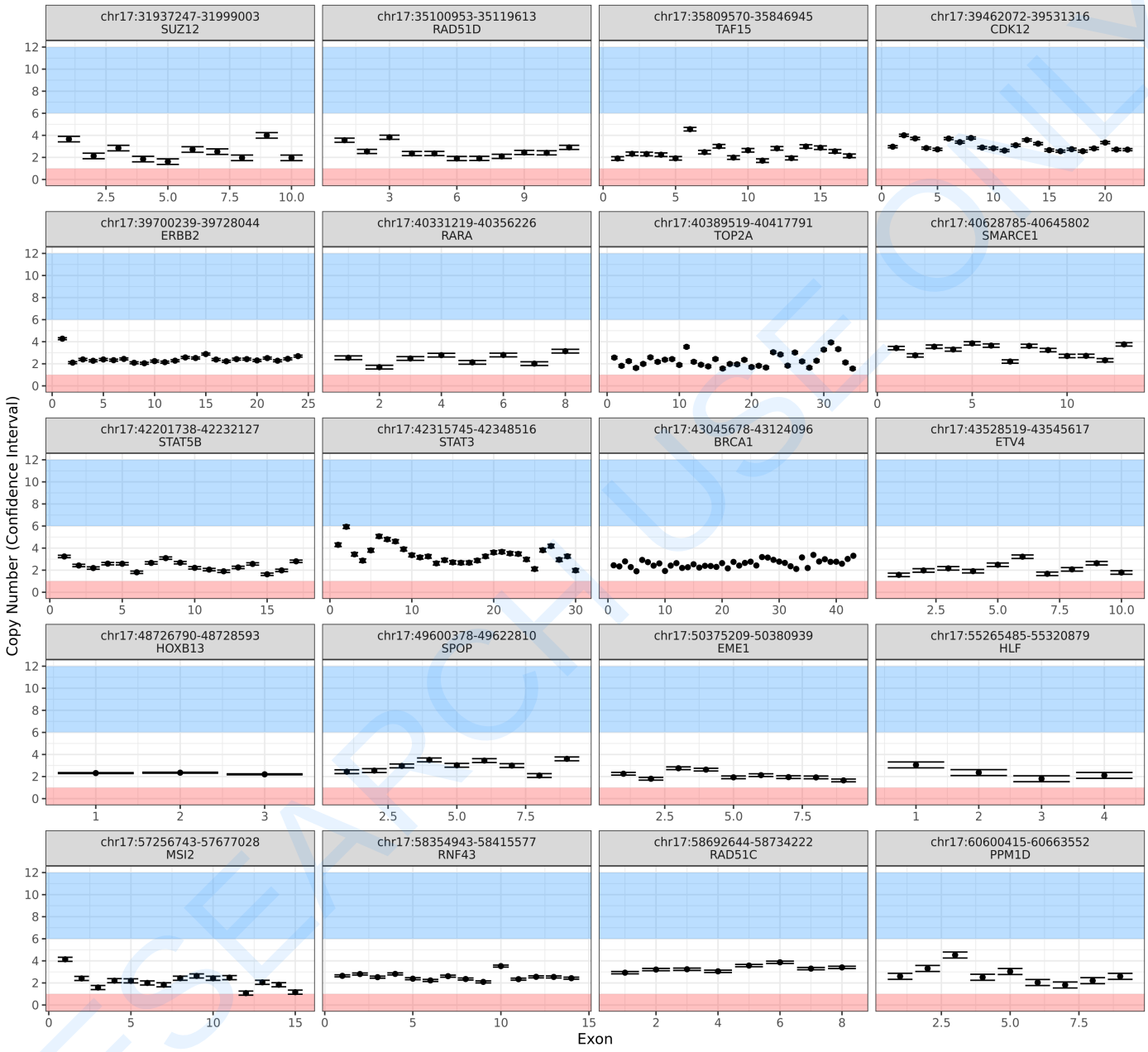

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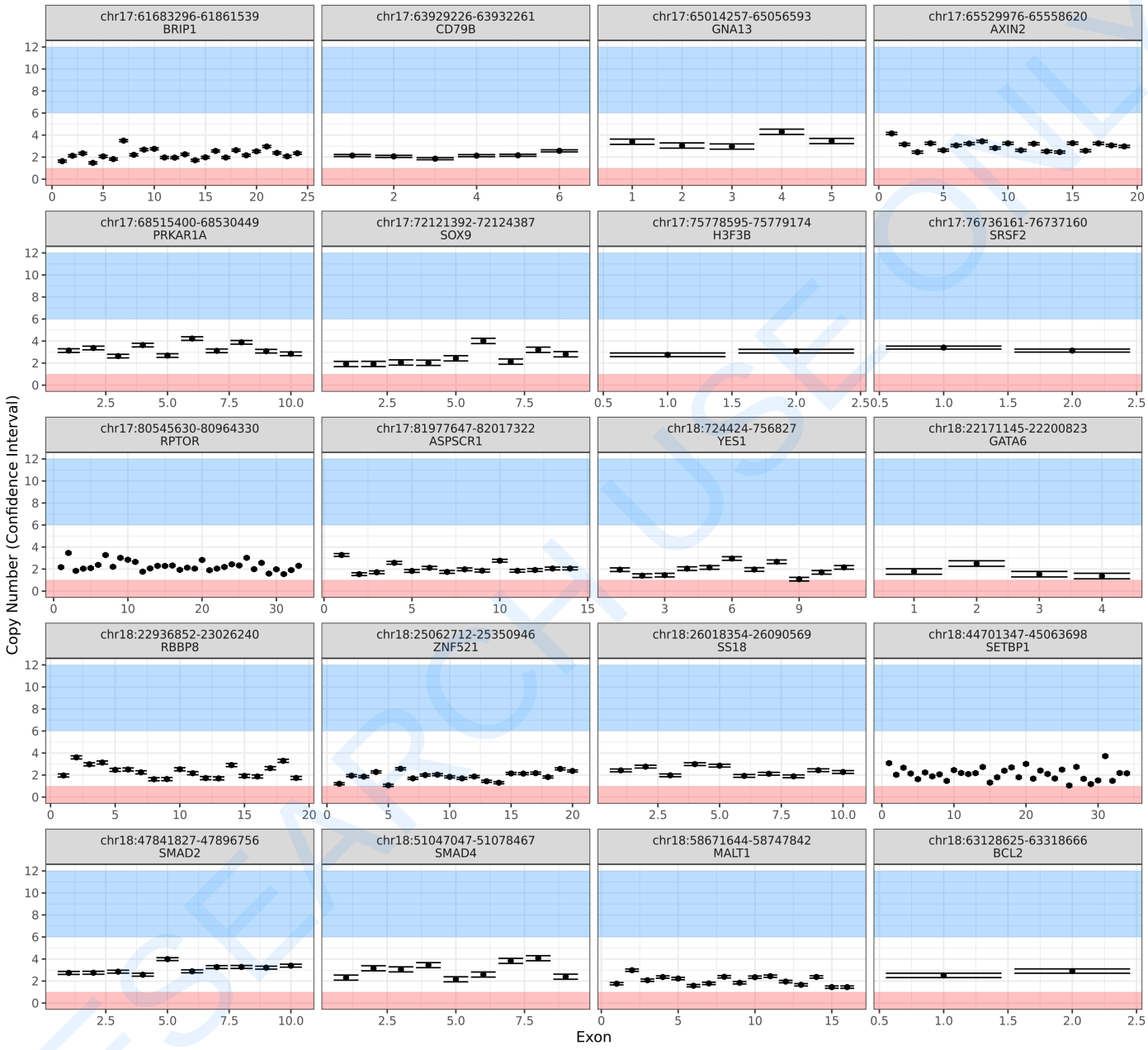

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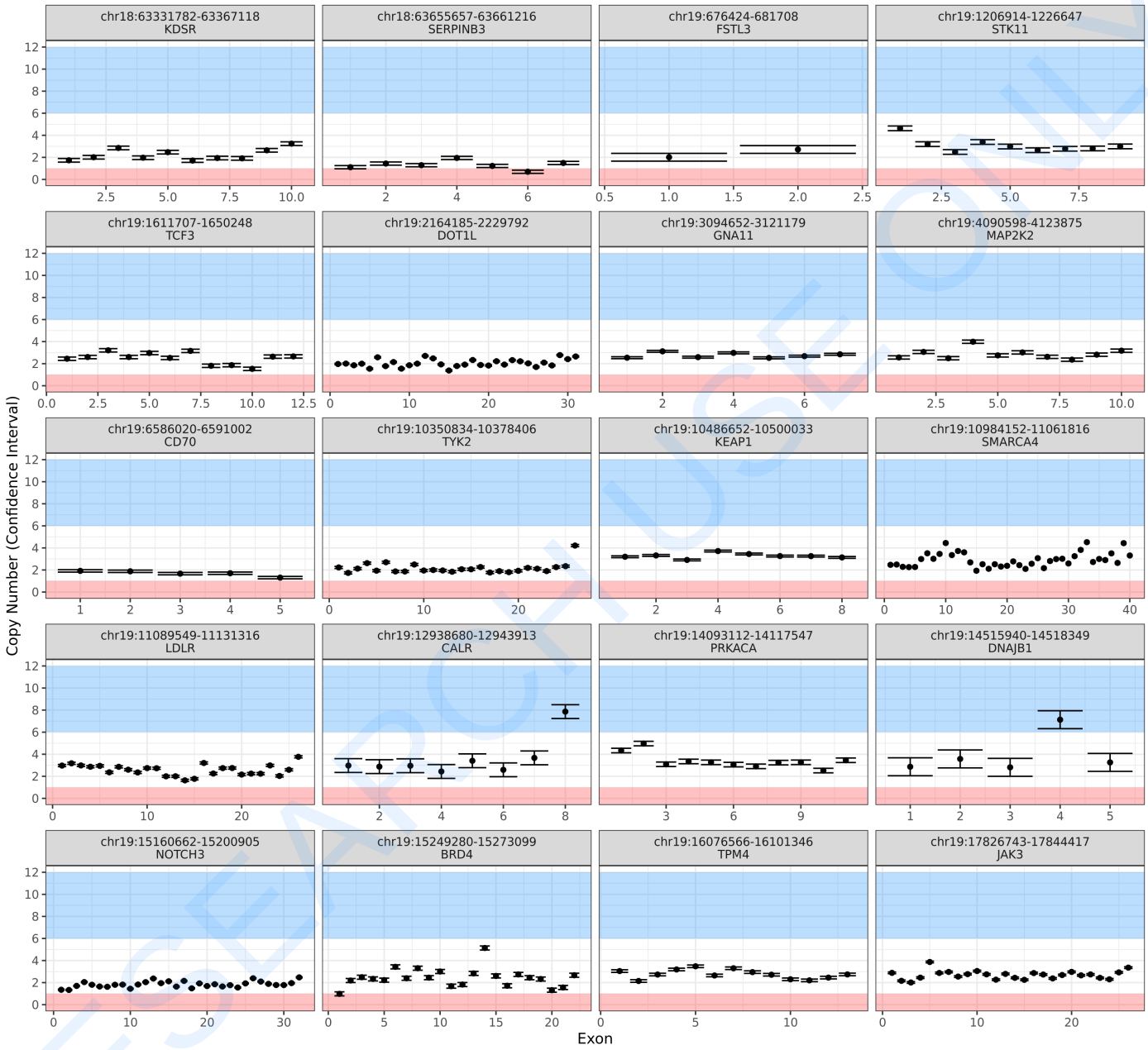

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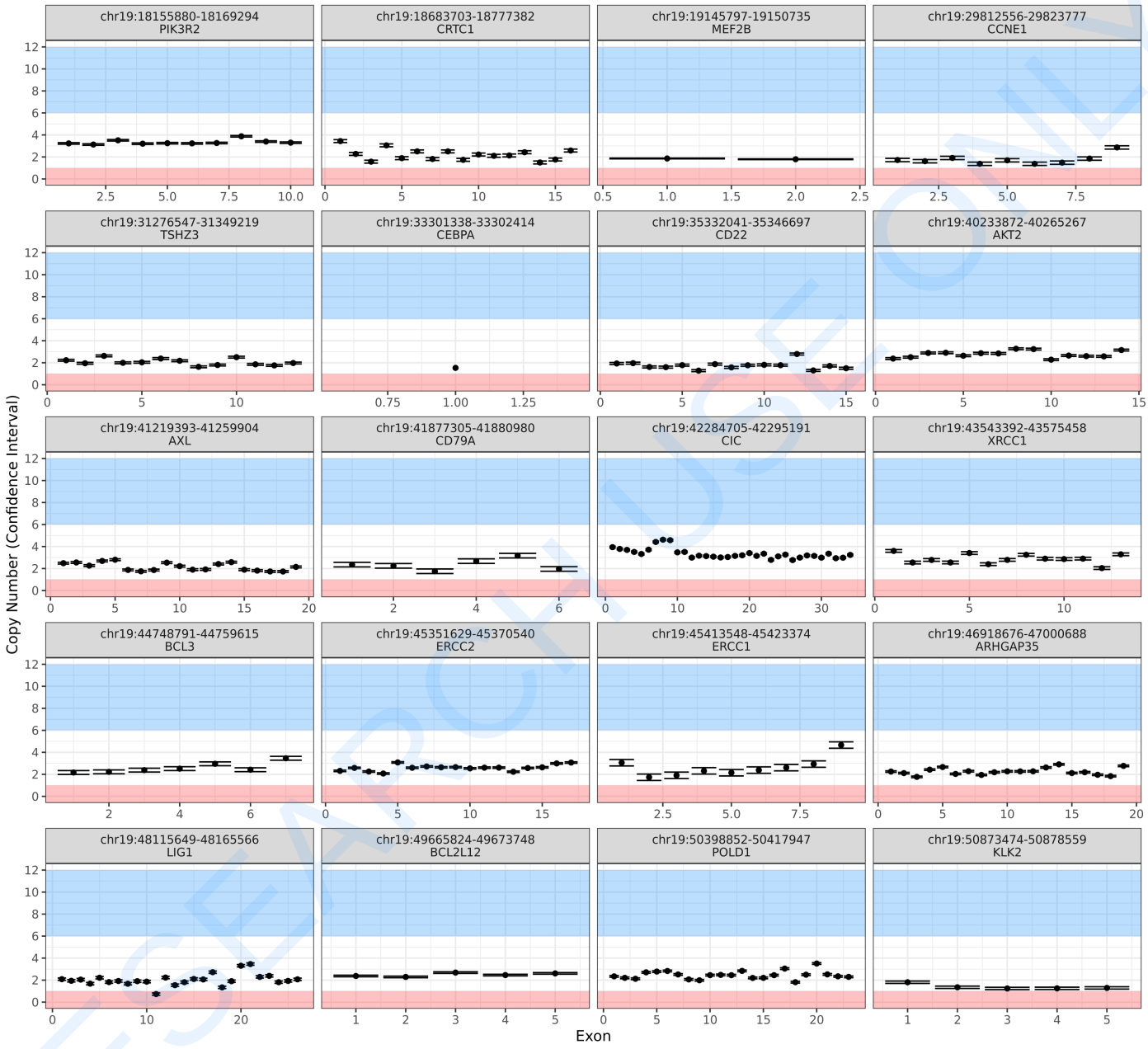

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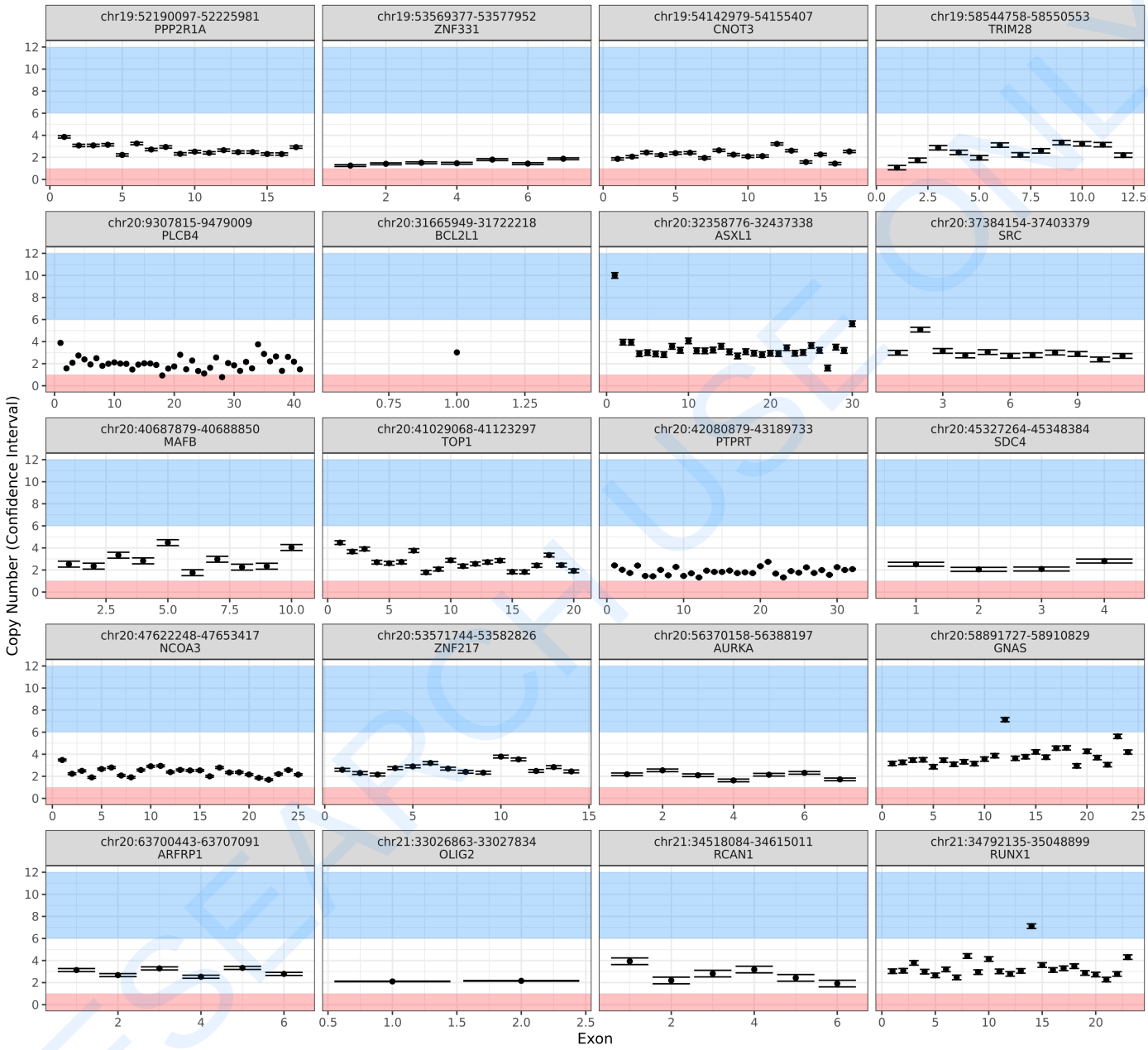

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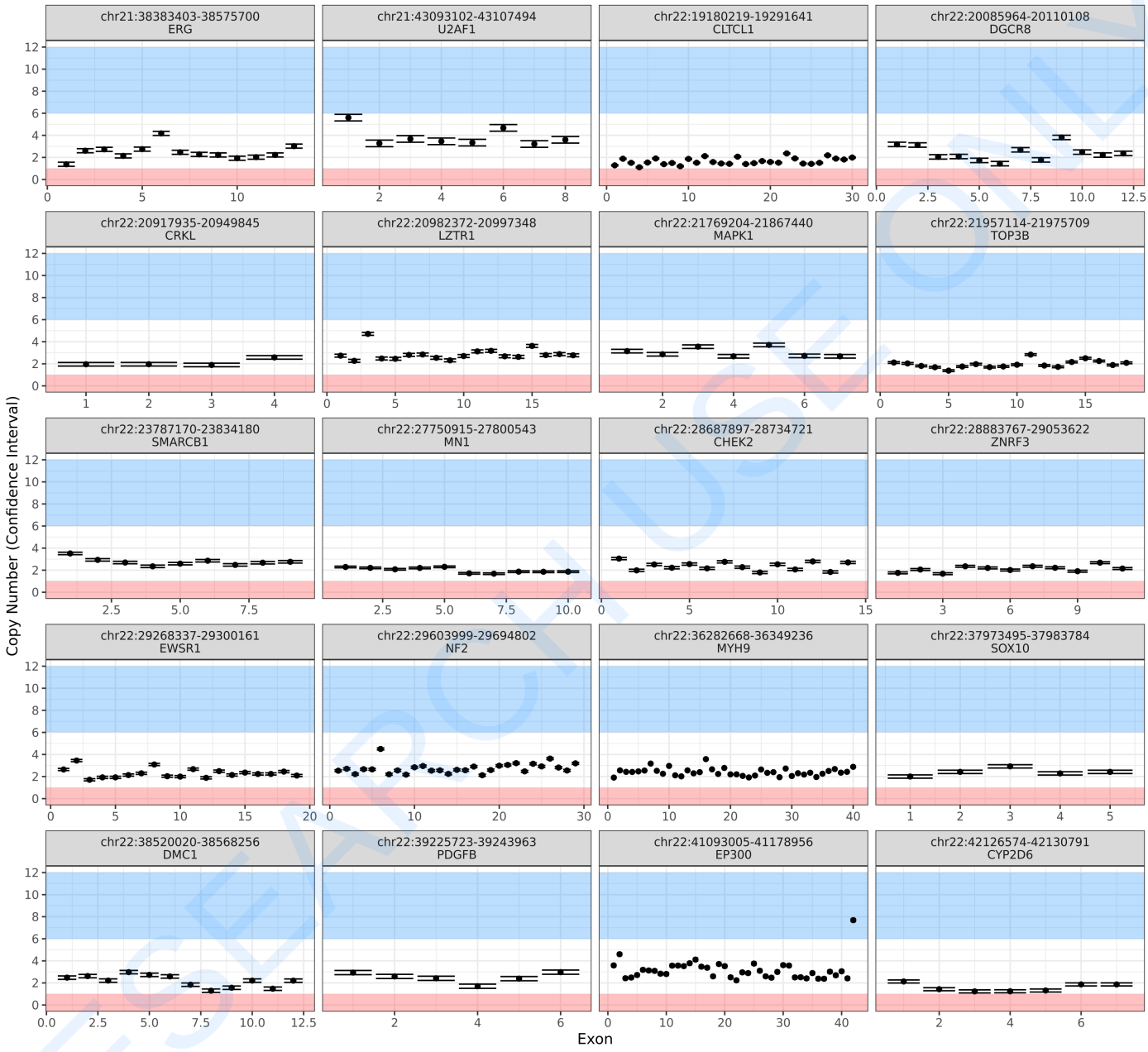

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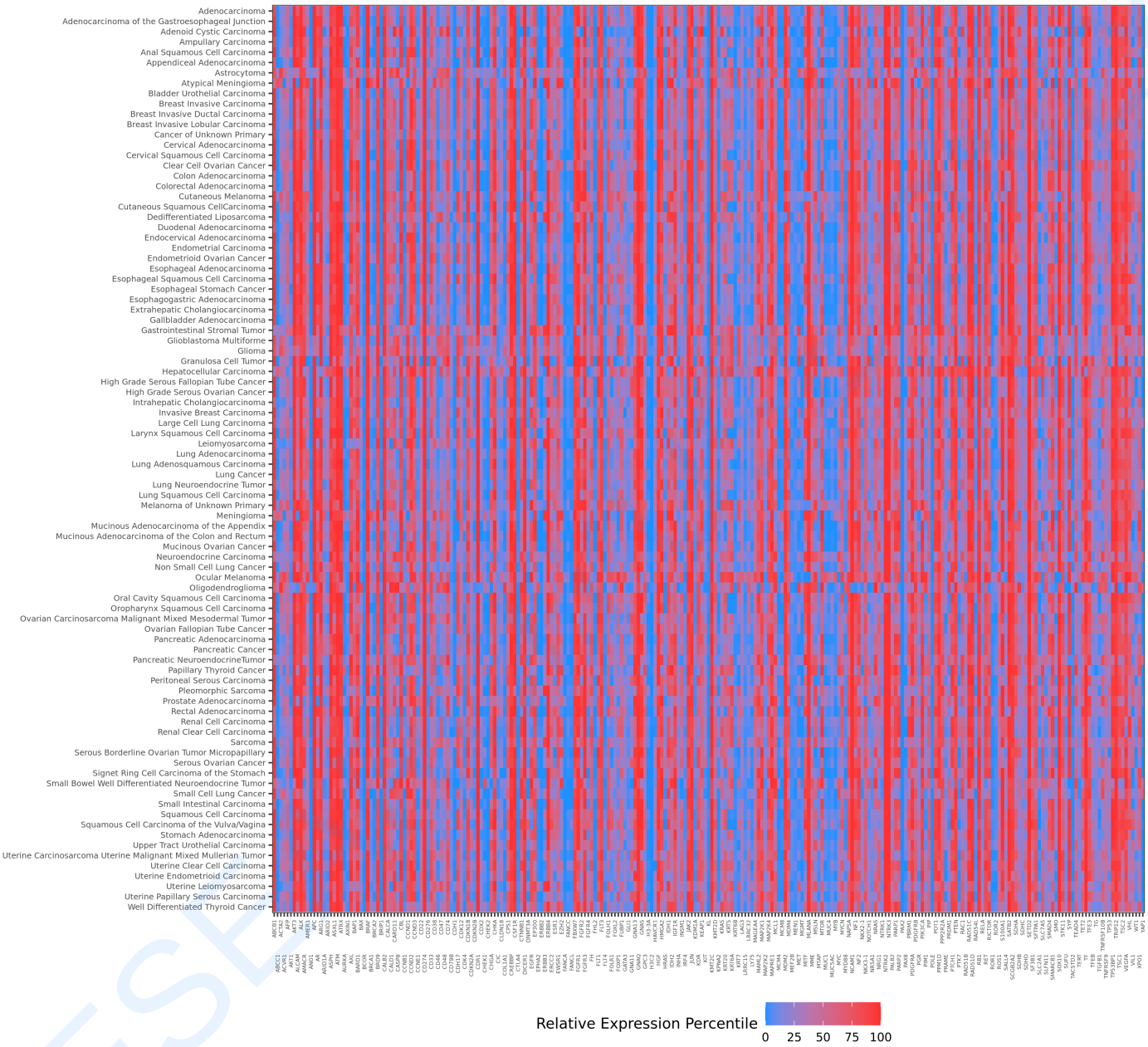
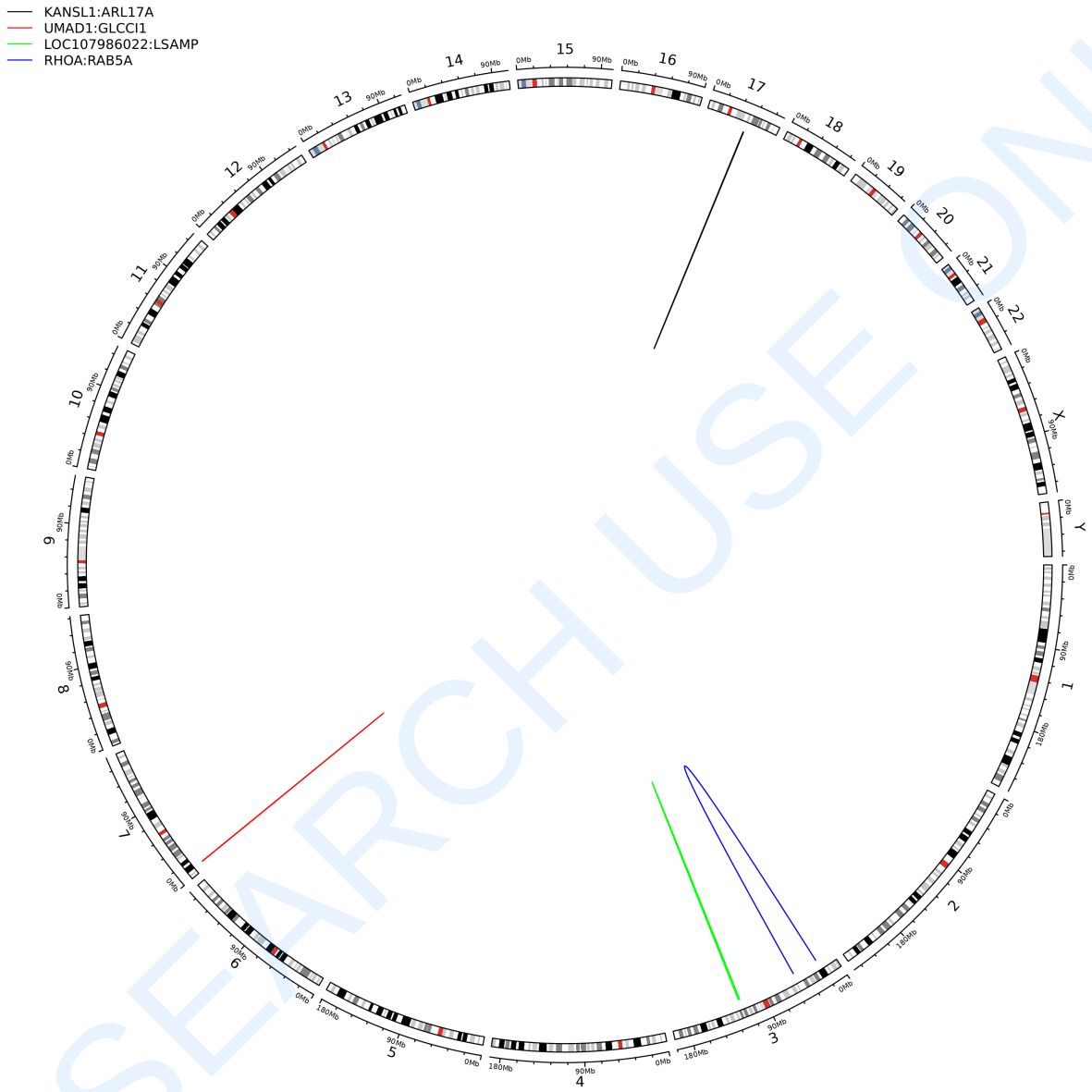
Relative RNA Expression



Gene expression is derived from whole transcriptome sequencing (WTS).
Relative expression of genes is calculated as normalized values using Transcripts per Million Molecules (TPM), for which a percentile is derived by comparison to a distribution of Caris data across multiple tumor types. The blue-red gradient represents the relative expression percentile of a specific gene (x-axis) across these different tumor types (y-axis). Darker red indicates the sample exhibits overexpression of the gene relative to all other samples analyzed within that tumor type.

TN25-

RNA Gene Fusions



RNA Fusions via Whole Transcriptome Sequencing:
RNA read alignments are created using FPGA-adapted STAR Aligner. Fusions are detected by FPGA-adapted STAR-Fusion which is a component of the Trinity Cancer Transcriptome Analysis Toolkit (CTAT). The STAR alignment software maps junction reads and spanning reads to a reference annotation set (hg38). STAR-Fusion uses the aligned output from STAR to produce fusion calls and read statistics. STAR-Fusion performs a fast mapping of fusion evidence to reference transcript structure annotations and filters likely artifacts to report accurate fusion predictions. We have developed and optimized a new normalized reads score called nReads which takes the total fusions reads found and modifies it based on an exponential distribution that matures a classic saturation curve. This score weighs both junction and spanning reads equally and also normalizes for the total RNA mapped reads. A threshold of this score has been optimized to call fusion events with highest sensitivity, PPV, and specificity. The displayed fusions are not necessarily in-frame.

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