

Supplementary information for

Long-read sequencing identifies full-length circular RNAs linked with therapy resistance of pediatric cancers

Chloé Bessière, Loélia Babin, Elissa Andraos, Johannes Markus Riepl, Annabell Szymansky, Marco Lodrini, Hedwig E. Deubzer, Angelika Eggert, Cyril Quivoron, Charlotte Rigaud, Véronique Vêrgé, Stéphane Pyronnet, Laurence Lamant, Fabienne Meggetto, Christine Gaspin, Steffen E. Fuchs

Correspondence: steffen.fuchs@charite.de; fabienne.meggetto@inserm.fr

Figure S1| Sensitive and resistant cell models do not show differences in sequencing quality, while more circRNAs are detected in resistant models

a, b, RNA of cell models from the ALKi sensitive (left panel) and resistant group (right panel) was treated enzymatically to enrich for circRNAs and deplete other RNAs. Expression levels were detected by qRT-PCR and enrichments are shown relative to the control samples (n=4 biologically independent samples each, data are presented as mean \pm SD).

c, Number of reads in the 2 groups of cell models (n=4 biologically independent samples each). Data are represented as boxplot (blue: sensitive group; red: resistant group).

d, Distribution of read length in the different samples (n=8 biologically independent samples). Data are represented as boxplot (blue: sensitive group; red: resistant group).

e,f, Number of circRNAs detected by CIRI-Long and circFL in ALKi resistant cell models (n=4 biologically independent samples) and sensitive models (n=4 biologically independent samples).

In boxplots the center line represents the median, boxes indicate the interquartile range, the whiskers show the 1.5 interquartile range. Source data are provided as a source data file.

Figure S2| Novel exons are more prominent in resistant cell models

a, The upset plot shows the total number of novel exon events identified by Nanopore sequencing per group of cell models (blue, ALKi sensitive and red, ALKi resistant, n=4 biologically independent samples each) and the events specific or common (indicated by a connecting line) to the different cell models.

b, The genome browser view shows circARNTL2(2,3,4,NE,5) with a novel exon (NE) between exon 4 and exon 5. The event was validated by Sanger sequencing of the junction in Karpas-299 cells. Details on the circRNA ID (circATLAS 3.0), length and coordinates are given. Source data are provided as a source data file.

Figure S3| Exon skipping events have a higher frequency in resistant models

a, The upset plot shows the total number of exon skipping events by group of cell models (blue, ALKi sensitive and red, ALKi resistant, n=4 biologically independent samples each) and the events specific or common (indicated by a connecting line) to the different cell models. Events were identified by Nanopore sequencing.

b, The genome browser view shows circZNF207(7,8,10) with skipped exon 9. The event was validated by Sanger sequencing of the junction between exon 10 and 8 in Karpas-299 cells. Details on the circRNA ID (circATLAS 3.0), length and coordinates are given.

c, Validation of the circular character of RNAs where the splicing event was detected, was performed by treatment with RNaseR of Karpas-299 cells and subsequent qRT-PCR in comparison to linear controls (n=3 biologically independent experiments. Data are presented as mean \pm SD, Two-way ANOVA test). Stable events are marked in bold. Source data are provided as a source data file.

Figure S4| Resistant models show a higher expression of circRUNX1 and circRAD23B

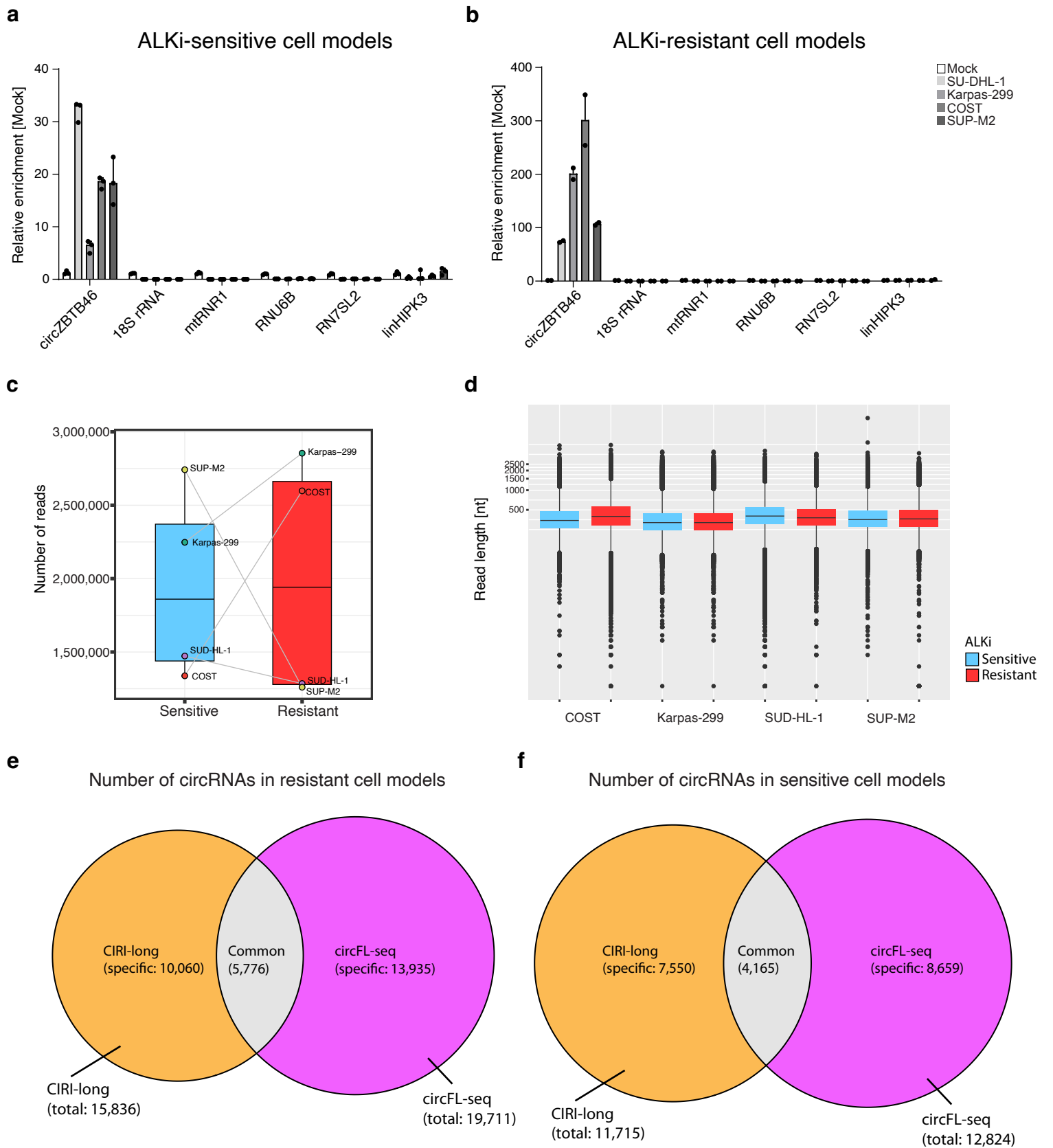
a, The genome browser view shows circRUNX1(6,7) and circRAD23B(3,4,5) with read coverage in ALKi sensitive (blue) and ALKi resistant (red) groups of cell models (n=4 biologically independent samples each).

b, c, Validation of circRUNX1(6,7) and circRAD23B(3,4,5) by Sanger sequencing in Karpas-299 cells. Details on the circRNA ID (circATLAS 3.0), length and coordinates are given. Source data is provided as a source data file.

d, The expression of cognate linear RNAs was quantified in short-read sequencing data of the same sensitive and resistant cell models as in **a**.

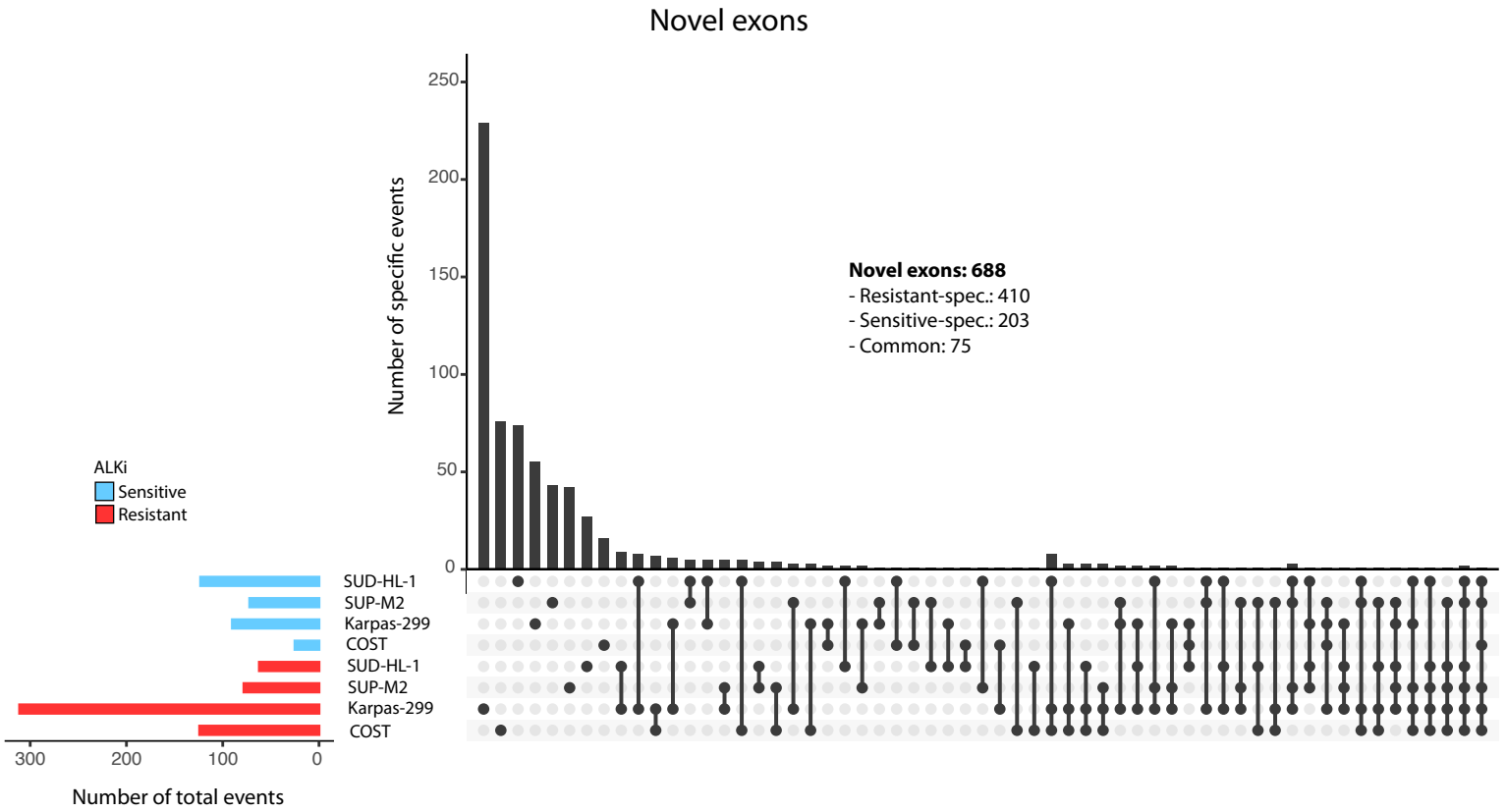
In boxplots the center line represents the median, boxes indicate the interquartile range, the whiskers show the 1.5 interquartile range. Data are represented as a boxplot. In boxplots the center line represents the median, boxes indicate the interquartile range, the whiskers show the 1.5 interquartile range. Source data are provided as a source data file.

Supp Fig. 1



Supp Fig. 2

a



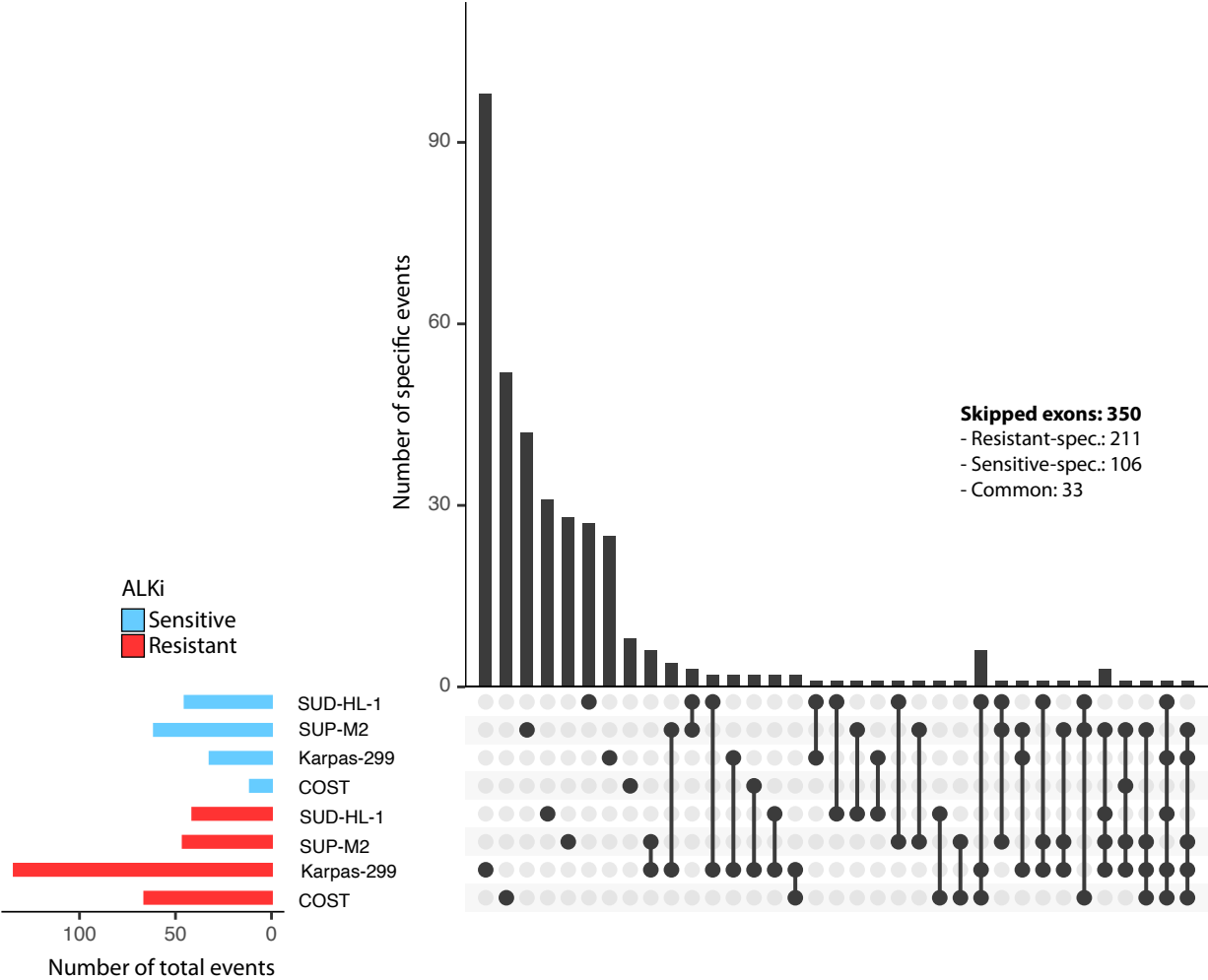
b



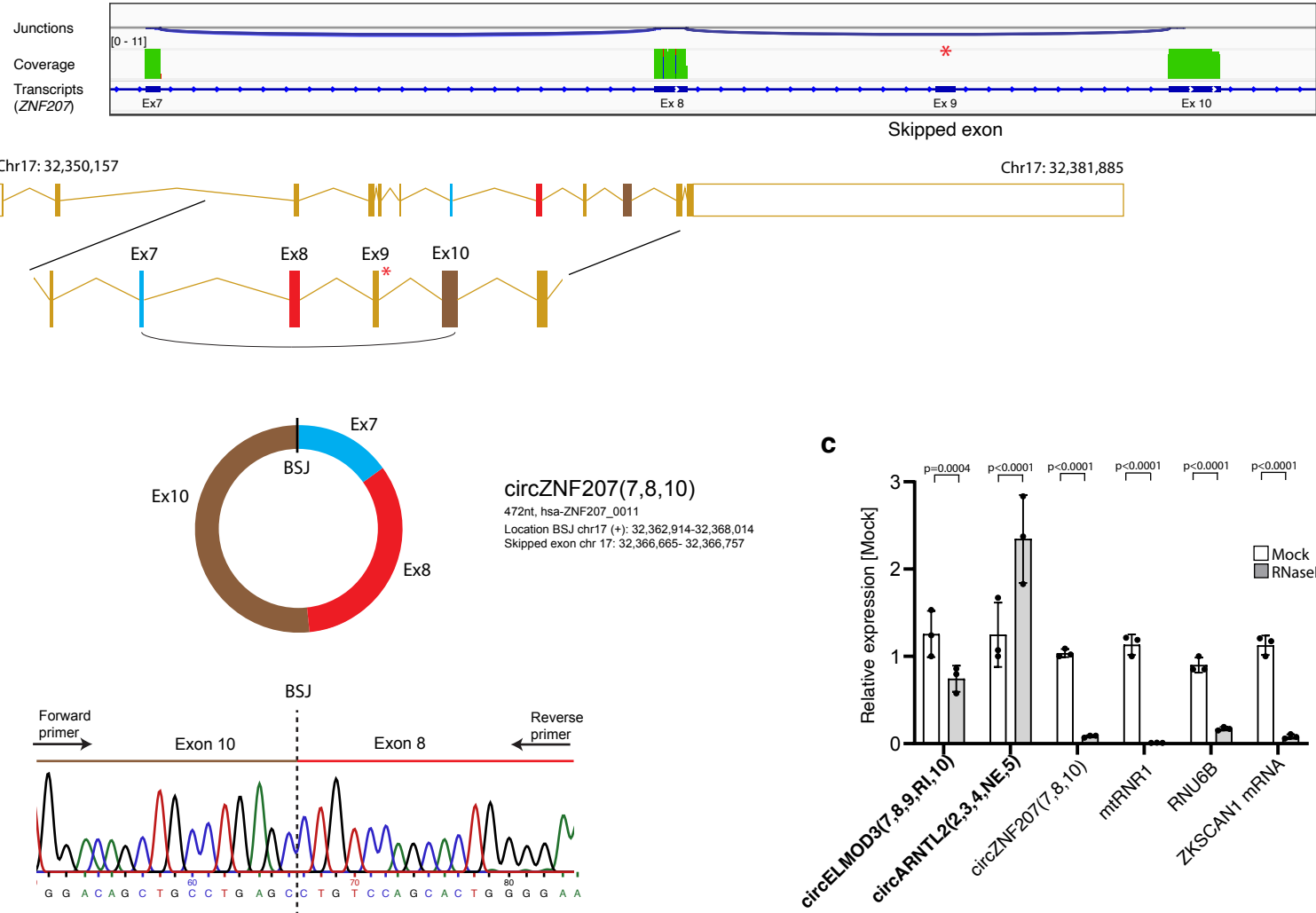
Supp Fig. 3

Exon skipping

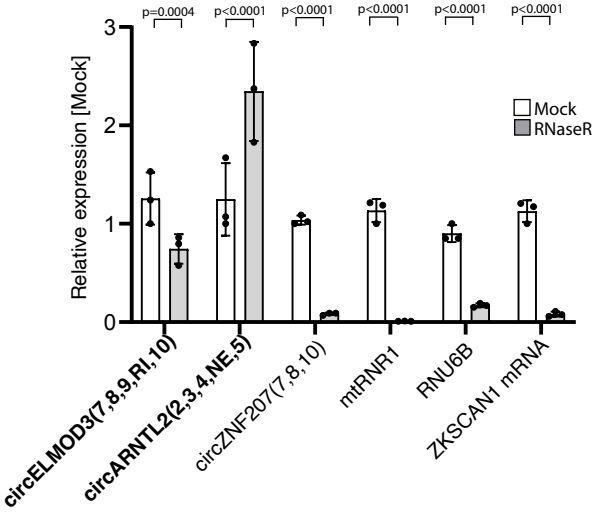
a



b

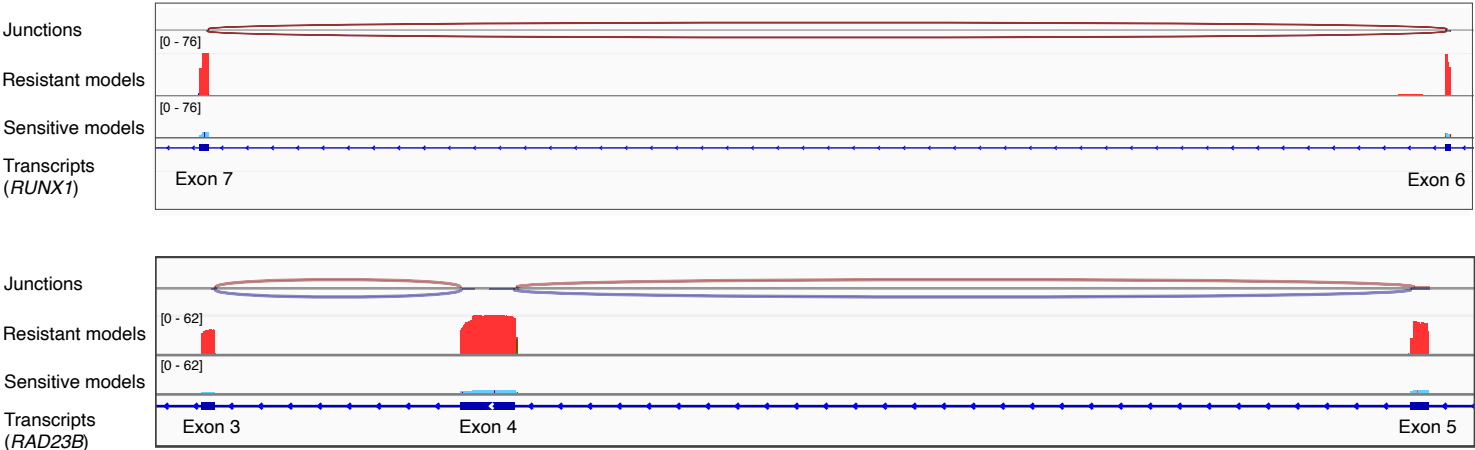


c

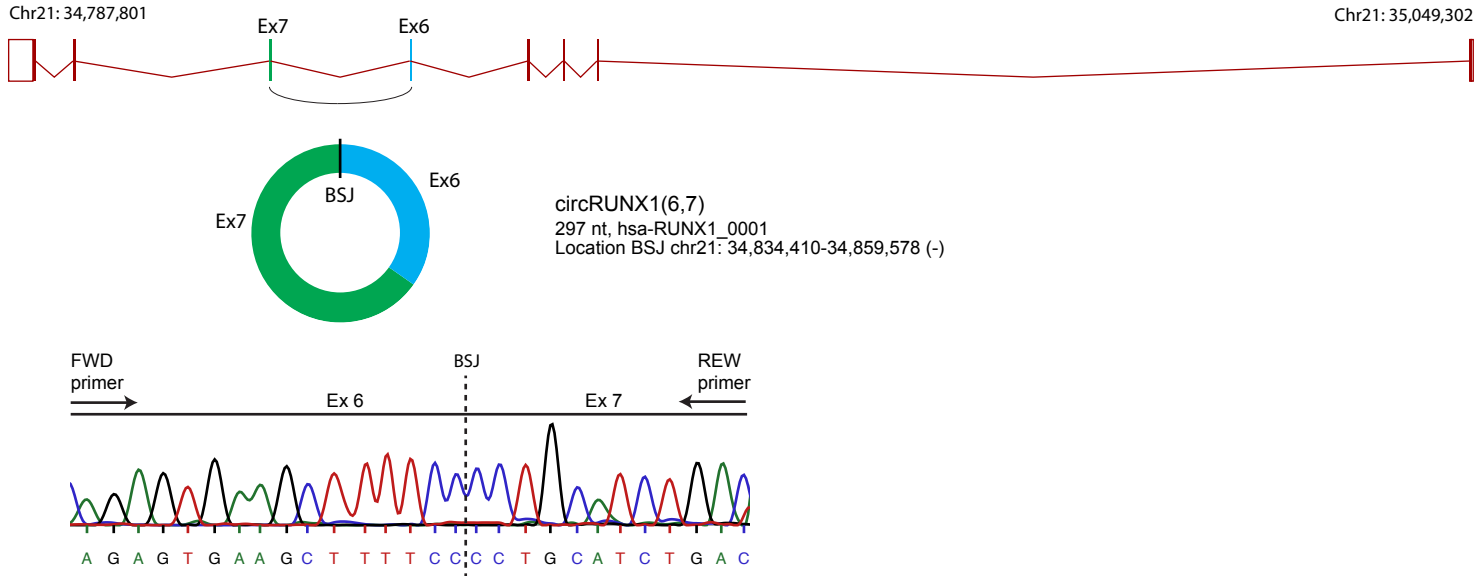


Supp Fig. 4

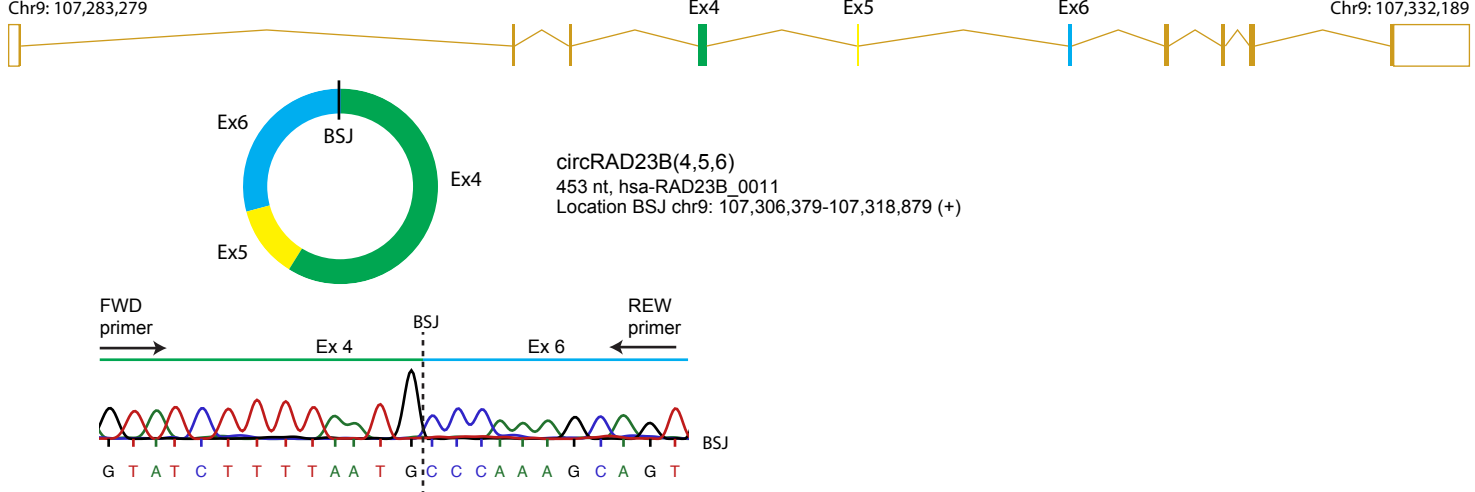
a



b



c



d

