Supplementary Figures

Fig. S1 Sequences at early EPSs corresponding to gRNA ends in GAP1 RNAi contain junctions suggesting failure in gRNA exchange. **A** EPSs at ESS22 and ESS40 in RPS12 correspond to the ends of gRNA-1 and gRNA-2, respectively. Sequences from GAP1 RNAi cells show an increase in junction length zero at these sites, consistent with complete utilization of the 3' gRNA, but no utilization of the 5' gRNA. **B** The two EPSs caused by GAP1 RNAi in ND7-5' occur at the ends of gRNA-1 and gRNA-2, which overlap in the regions they guide. The major junction sequence at ESS33 is not junction length zero, but the two major junction sequences match full utilization of gRNA-1 with only a variation in whether the terminal U's guide editing, as shown in C. **C** The two sequences depicted and aligned with gRNA-2 comprise ~60% of all sequences at ESS33 in both replicates (top: 48% and 42%, bottom: 22% and 26% in replicates 1 and 2, respectively). Red, fully edited sequence; green, junction sequence. **D** The major junction sequence exacerbated at ESS34 is junction length zero, which corresponds to the end of gRNA-2.

Fig. S2 Abundance of sequences at EPSs. The number of sequence at all EPSs shown in Figures 4A and 4B are graphed for each knockdown cell line. The bars are split sections indicated by different colors that indicate the number of sequences in each junction length category (black, no junction; orange, junction length 1-10; yellow, junction length 11-50; green, junction length >50). RPS12, **A**; ND7-5', **B**.

Fig. S3 Mis-editing at deletion sites immediately 5' of EPSs show evidence of deletion RECC recruitment. All sequences from EPSs whose junction start site is a U deletion site in the fully edited sequence were analyzed to determine how often the deletion site was skipped (“no deletion”, yellow), partially deleted (green), or contained inappropriately added Us (orange). These parameters were determined for each RNAi cell line, either uninduced (-TET) or induced for RNAi (+TET), with replicates 1 and 2 combined. Each row shows an EPS (label far right), and each column is a distinct RNAi cell line (label top of first row). To the right of the graphs are shown the deletion actions canonically required at each junction start site.

Fig. S4 Proportion of sequences with each junction length at all ESS in RPS12, A, and ND7-5', B. The sequences at all ESS across each transcript were grouped by junction length as in Fig. S2 and an average of replicates 1 and 2 was taken for each group. The proportion of sequences in each group for each ESS in all samples was graphed (top) and the same proportion overlayed on the total number of sequences with each ESS is also shown (bottom) for comparison of the relative abundance of each ESS. Each rowof graphs shows a different condition or cell line. Data from wild type 29-13 cells from a previous publication (*Simpson et al*, 2016) was included as a control for uninduced samples. White circles above the graphs show the location of EPSs in a given cell line. Black circles

above the graphs show the location of significantly increased junction 0 sequences (increased counts) in each cell line. Blue bars below the graph show the locations of the gRNAs depicted in Figs. 4A and 4B.

Table S1 MIQE Checklist for quantitative RT PCR. The standard MIQE checklist obatained from http://www.rdml.org/miqe.php is shown. In the column titled "checklist" a "Y" indicates the information is given in the text of the materials and methods section, "N" indicates data is not shown and other explanations not provided in the text of the materials and methods section are provided here.

Table S2 Total fragments and unique sequences in the partially edited sequence libraries A shows the a breakdown of the number of fragments in each sample (induced (+) and uninduced (-)) and how many were "standard alignments" (i.e. no non-T insertions, deletions or SNPs) and how many were "non-standard" (i.e. with non-T errors) with a breakdown of how many mismatches are in sequences in this category (1, 2, >3). **B** shows for the same data set the number of unique sequences represented in each category. **C** and **D** are ND7-5' replicate 2 and **E-H** contain the same information for RPS12 replicates 1 and 2.

Table S3 Determination of Exacerbated Pause Sites (EPS) in RPS12. The number of sequences at each Editing Stop Site (ESS) in RPS12, replicates 1 and 2 is shown in this table. The first column shows the ESS numbers. The second two columns show the average number of sequences that share each ESS in the uninduced samples of replicate 1 and 2 (n=4 for each replicate). The number of sequences at each ESS in replicate 1 and 2 for each RNAi induced sample is shown (norm. count) with the associated p and q values in the remaining columns. Sites are considered significantly increased if $p < 0.05$ and $q < 0.05$ and the norm. count is greater than the average of uninduced for the respective replicate. If an ESS is significantly increased in both replicates 1 and 2, this is an EPS and is shown in bold in the table.

Table S4 Determination of Exacerbated Pause Sites (EPS) in ND7-5'. The number of sequences at each Editing Stop Site (ESS) in ND7-5', replicates 1 and 2 is shown in this table as in Table S3.

Table S5 Fisher's Exact Tests of EPSs to editing site characteristics. This table shows all p-values for the Fisher's Exact Tests used to test correlations between EPSs and editing site characteristics.

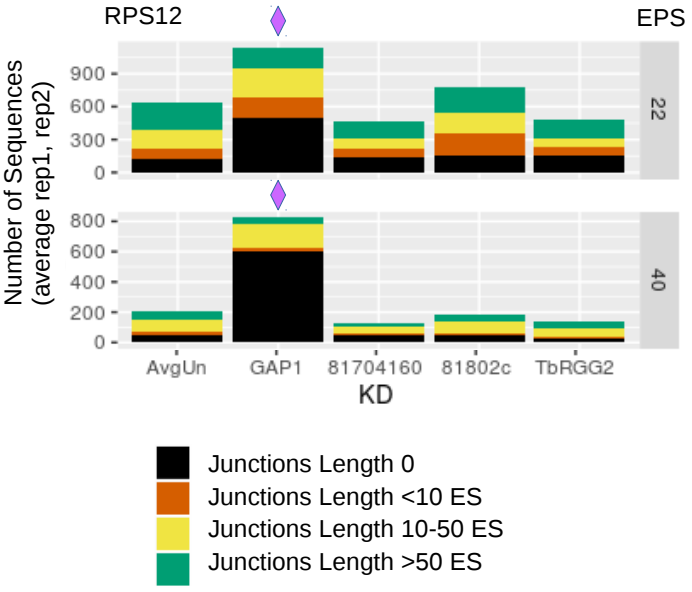
Table S6 Sequences with a single modification beyond ESS15 The column "KD" shows the name of the protein that was knocked down in a given RNAi line (n=2) or AvgUn for data for the average of the uninduced samples (n=8). The column "Axn1" shows a shorthand of the single ES that has been modified beyond ES15 as Axn*xy* where *xy* is the ES number. The column "AvgNm" is the average of the normalized sequence counts taken as a sum for each single action group (n=2 for induced, n=8 for uninduced). The sequences included could have canonical or non-canonical editing modifications at

the site in question and thus can be potentially heterogeneous (*e.g.*, Axn16 could have one or more u's added though canonical calls for only a single u insertion). The number of unique sequences represented in this calculation is shown in column “unq”. The average total number of sequences with a single action beyond ESS15 in each RNAi are shown in the column “tot”, and the percentage of the single action sites occupied by each ES is shown in the column “Perc” (Perc = AvgNm/Tot *100). “NA” in a column means that no sequences with this ES modification were recovered in any replicate of the sample (*e.g.*, AvgUn Axn21 AvgNm = NA means that no sequences in any uninduced samples had canonical editing to ESS15 with a single additional modification at ES21).

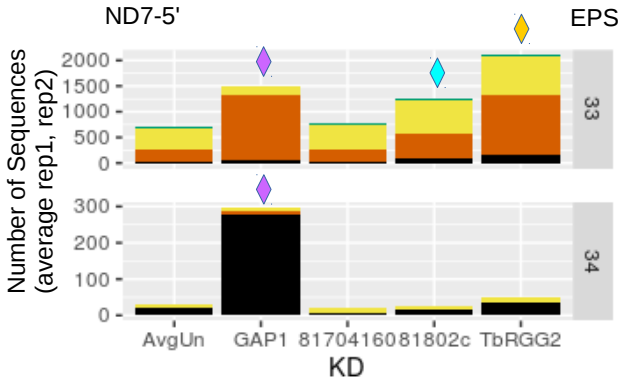
Table S7 Sequences with two modifications beyond ESS15 This table is structured as Table S6; however, Axn1 here is notated to show the only two ES that have been modified beyond canonical editing to ESS15. For example, axn18_20 means that editing in this sequence was canonical up to ES15 and beyond that ES18 and ES20 have been modified in some way.

Table S8 Sequences with three modifications beyond ESS15 This table is structured as Tables S6 and S7. However, Axn1 here is notated to show three ESs that have been modified beyond canonical editing to ESS15. For example, axn18_20_22 means that editing in this sequence was canonical up to ES15 and beyond that ES18, ES20 and ES22 have all been modified in some way but all other ES match pre-edited.

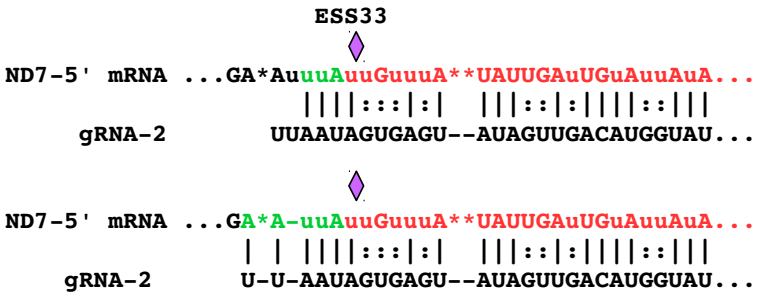
A



B



C



D

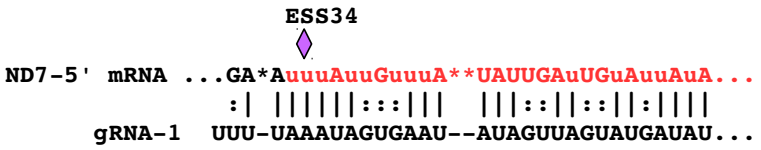
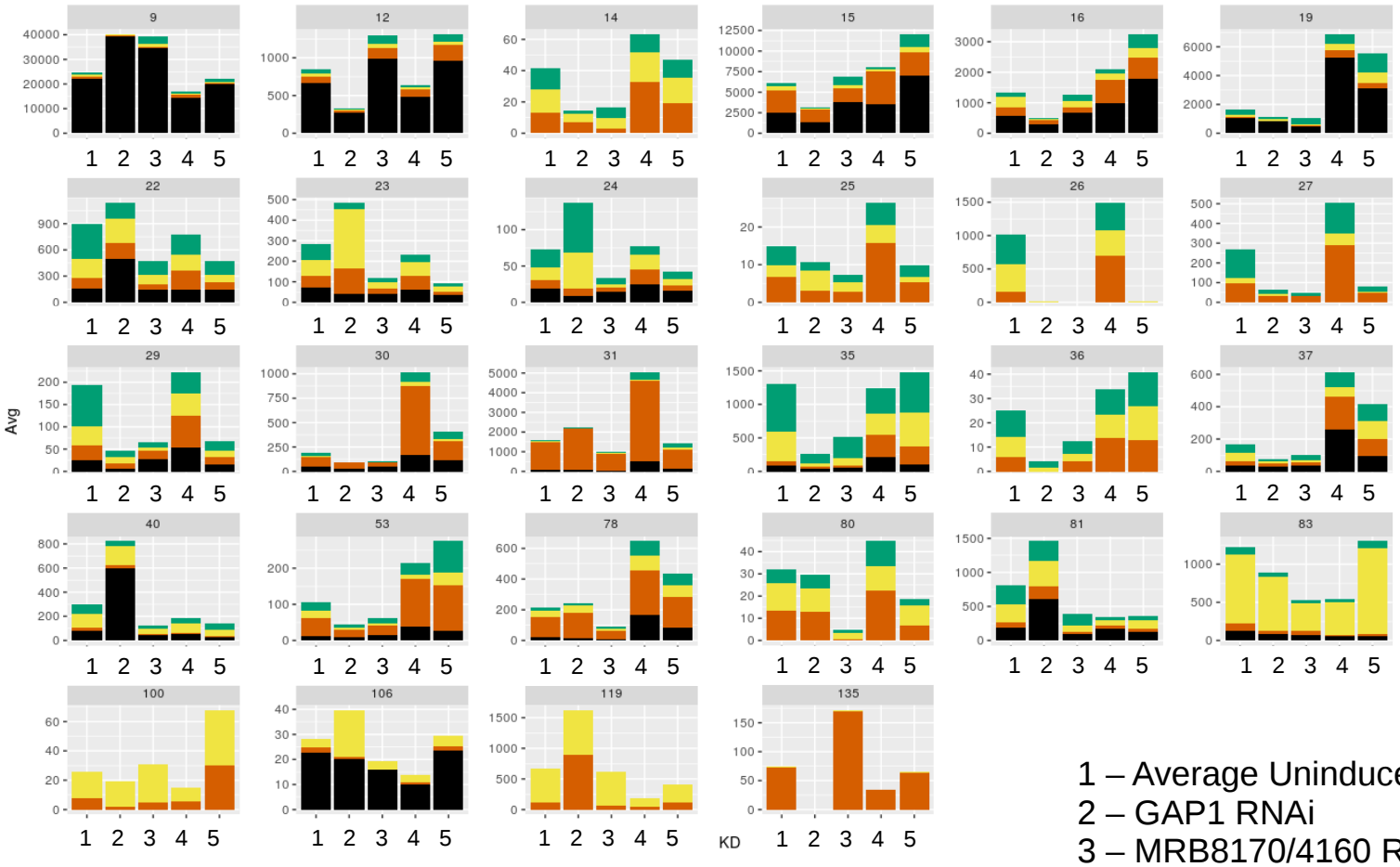


Figure S1



1 – Average Uninduced
2 – GAP1 RNAi
3 – MRB8170/4160 RNAi
4 – MRB8180 RNAi
5 – TbRGG2 RNAi

Figure S2A: RPS12

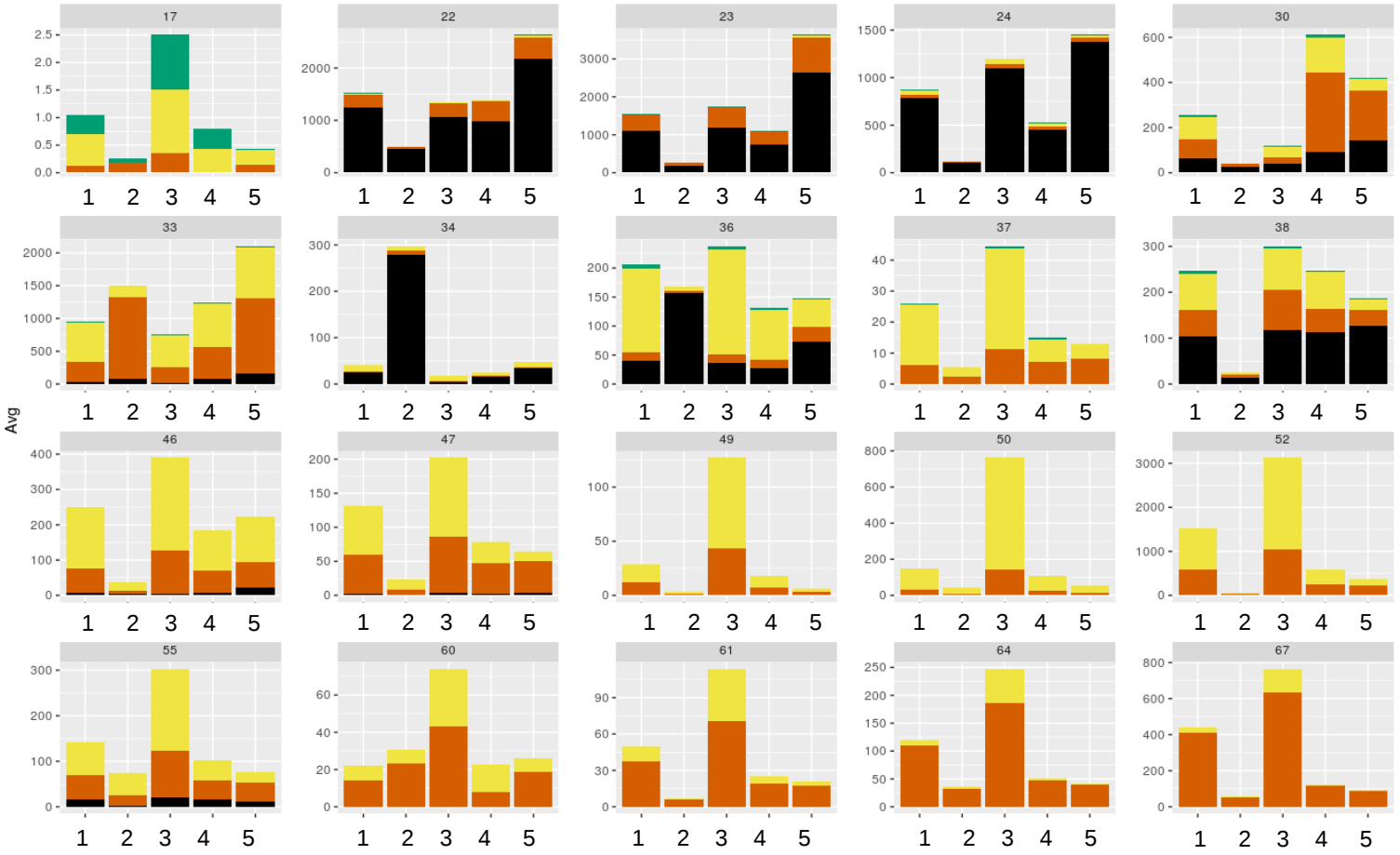

17
22
23
24
30
33
34
36
37
38
46
47
49
50
52
55
60
61
64
67
Avg
JL
0
1-10
11-50
>50

Fig. S2B: ND7-5'

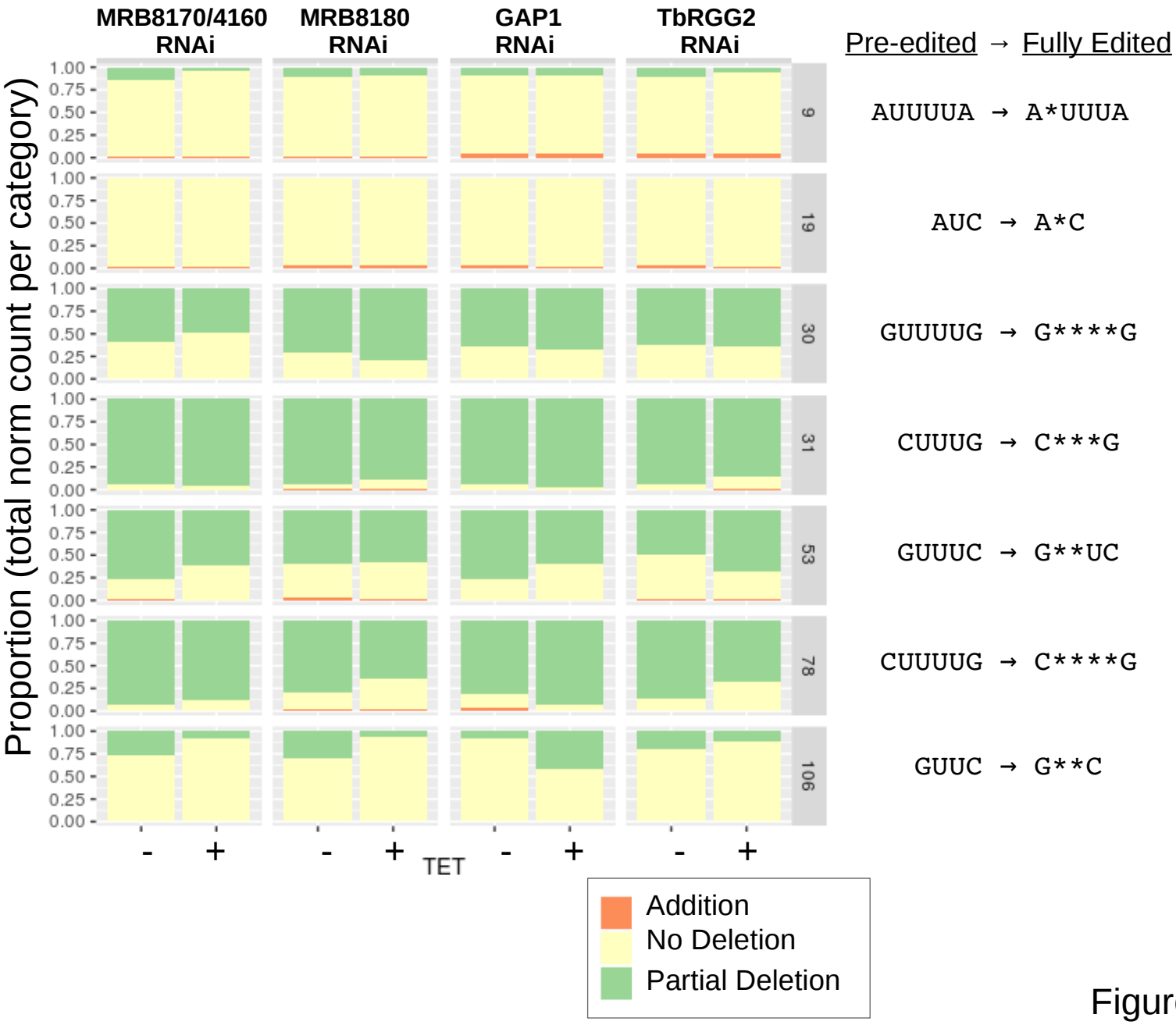

MRB8170/4160 RNAi
MRB8180 RNAi
GAP1 RNAi
TbRGG2 RNAi
Pre-edited → Fully Edited
AUUUUA → A*UUUA
AUC → A*C
GUUUUG → G****G
CUUUG → C***G
GUUUC → G**UC
CUUUUG → C****G
GUUC → G**C
Proportion (total norm count per category)
1.00
0.75
0.50
0.25
0.00
9
19
30
31
53
78
106
-
+
TET
Addition
No Deletion
Partial Deletion

Figure S3

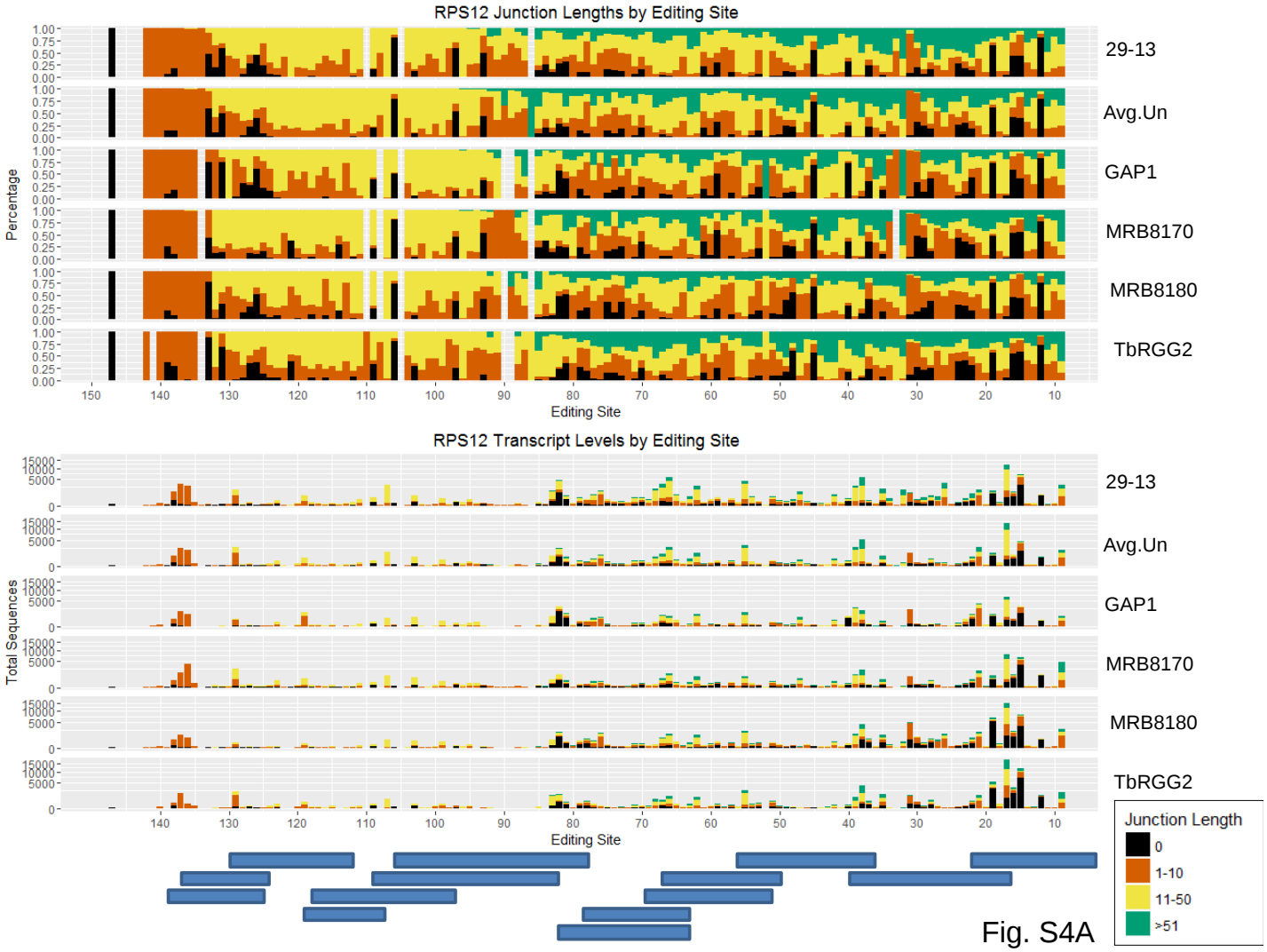

RPS12 Junction Lengths by Editing Site
Percentage
29-13
Avg.Un
GAP1
MRB8170
MRB8180
TbRGG2
Editing Site
RPS12 Transcript Levels by Editing Site
Total Sequences
Editing Site
Junction Length
0
1-10
11-50
>51

Fig. S4A

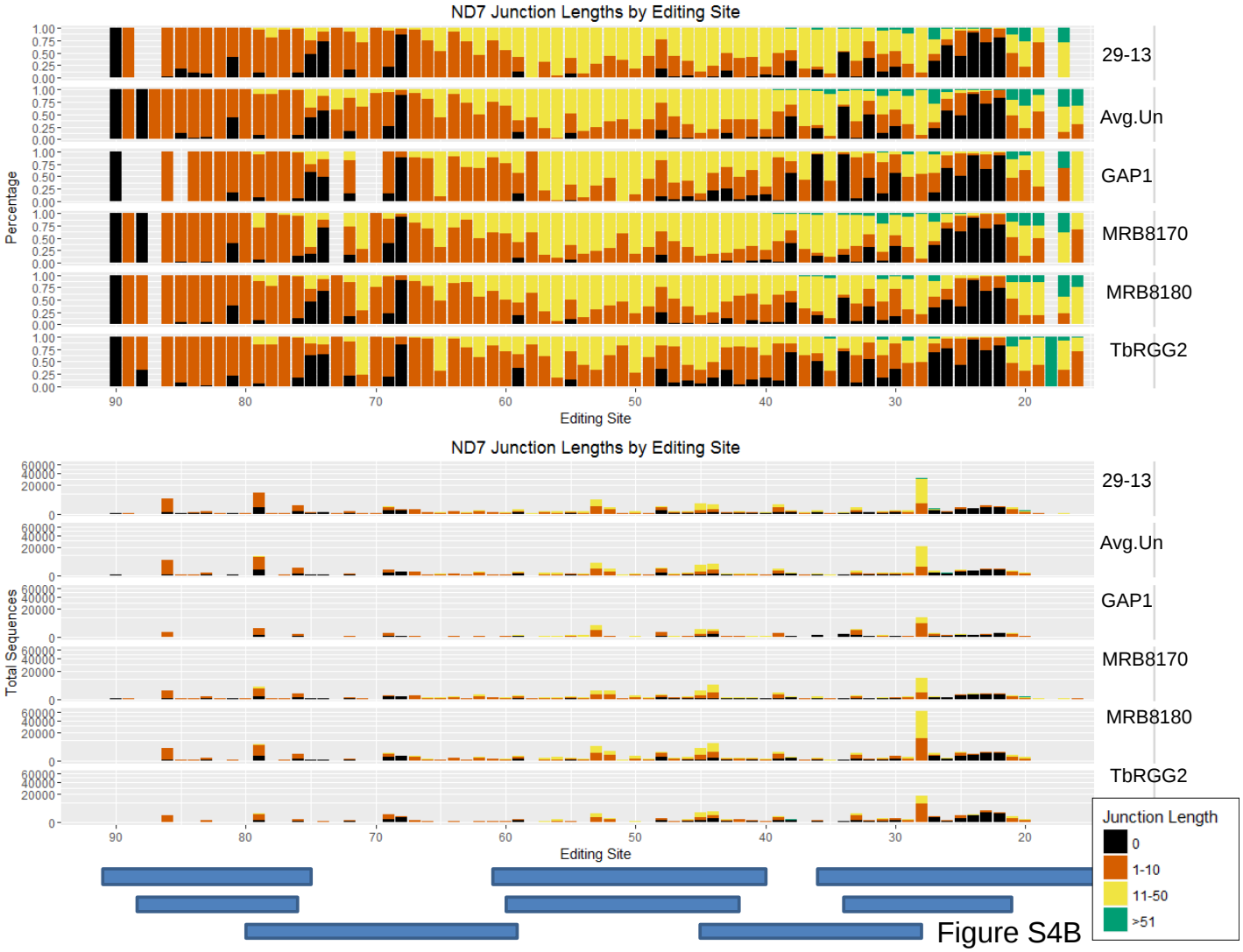

ND7 Junction Lengths by Editing Site
Percentage
29-13
Avg.Un
GAP1
MRB8170
MRB8180
TbRGG2
Editing Site
ND7 Junction Lengths by Editing Site
Total Sequences
Editing Site
Junction Length
0
1-10
11-50
>51

Figure S4B