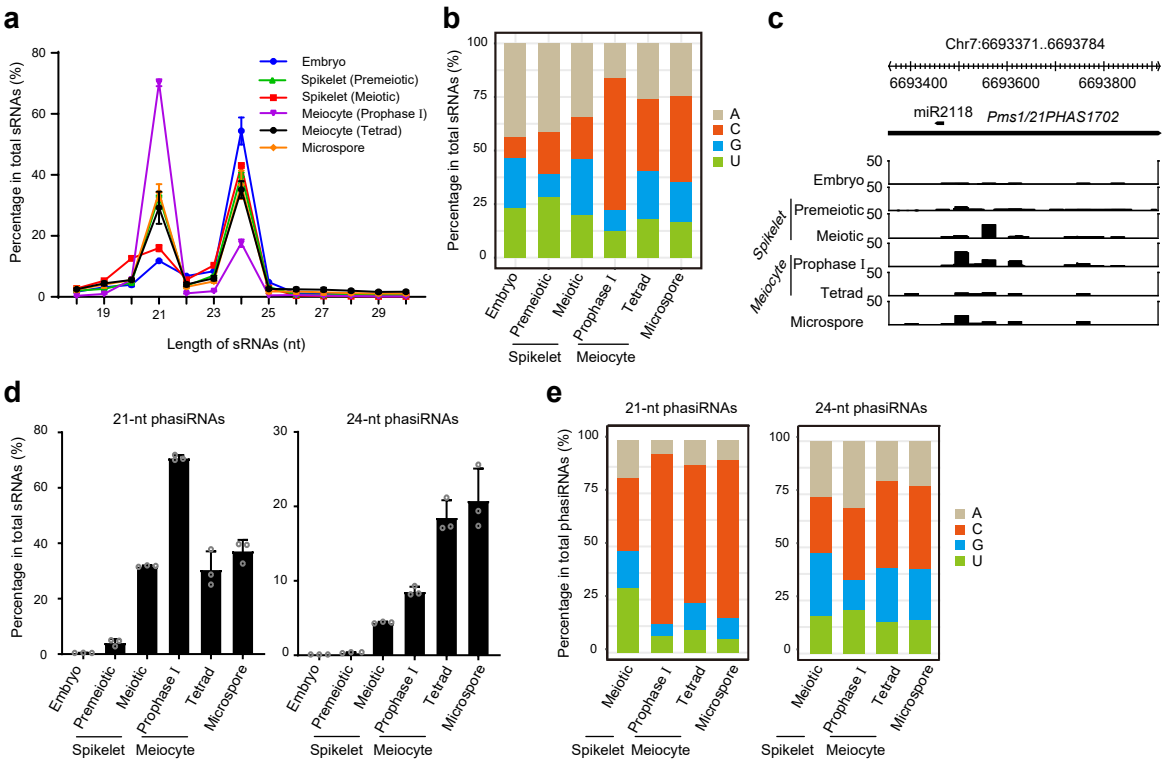
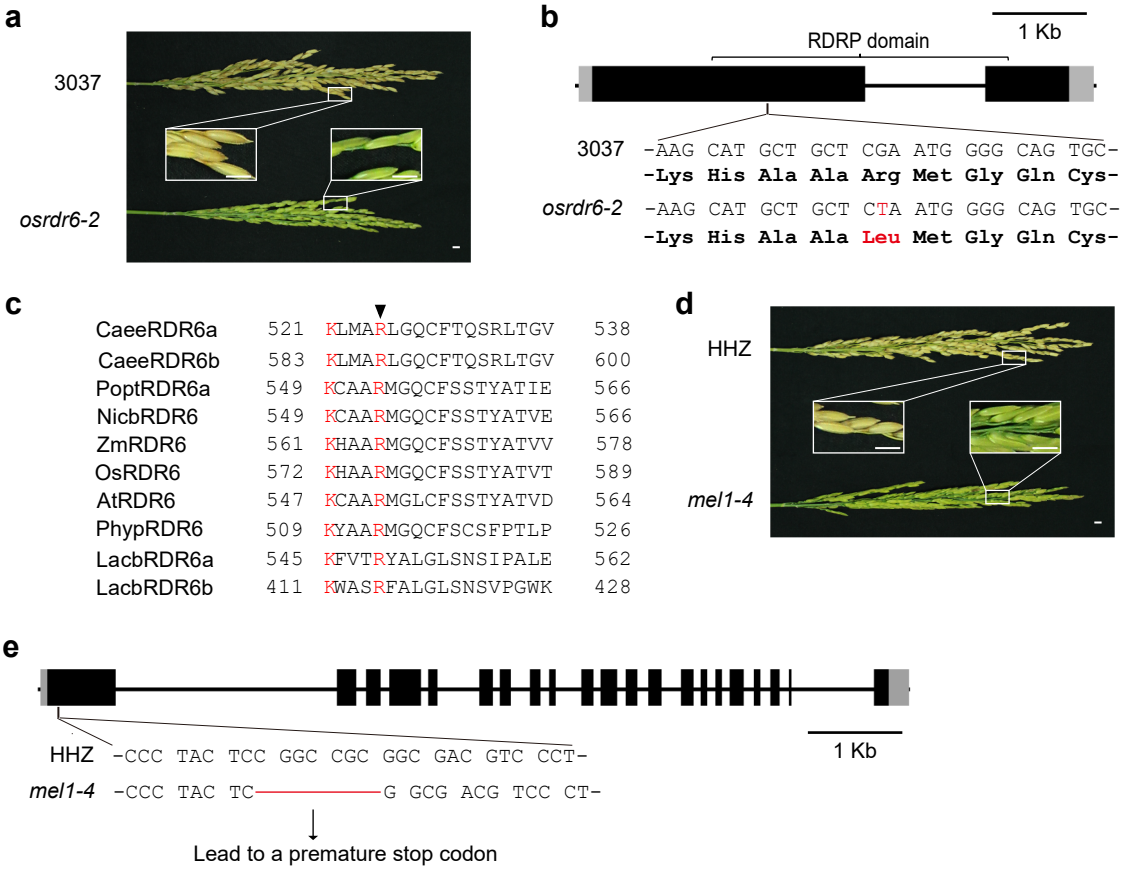
Supplementary Information

Supplementary Figures



Supplementary Fig. 1. Profiling of phasiRNAs in rice spikelets and purified germ cells. a, Length distribution of genome-matched sRNAs in embryos, spikelets, meiocytes and microspores. **b**, 5' terminal nucleotide frequency of genome-matched sRNAs in embryos, spikelets, meiocytes and microspores. **c**, Genome browser views of 21-nt phasiRNA signals at the *Pms1* locus in embryos, spikelets, meiocytes and microspores. The y-axis represents sRNA abundance (RPM). **d**, Percentages of 21- and 24-nt phasiRNAs in total genome-matched sRNAs in embryos, spikelets, meiocytes and microspores. The error bars represent standard deviation of three biological replicates. **e**, 5' terminal nucleotide frequency of 21- and 24-nt phasiRNAs in meiotic spikelets, meiocytes and microspores.

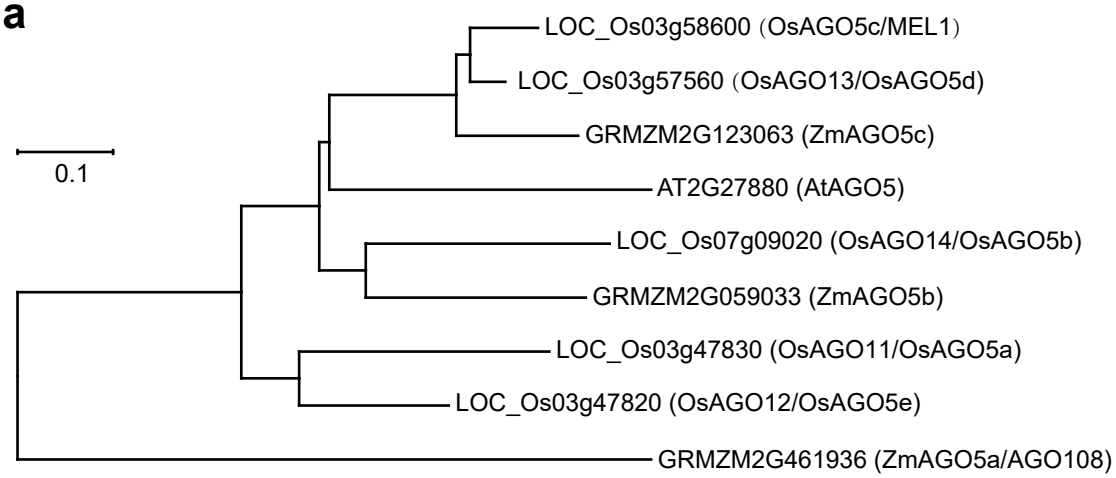


Supplementary Fig. 2. Characterization of the *osrdr6-2* and *mel1-4* mutants.

a, Panicles of *osrdr6-2* and 3037 rice plants at the grain-filling stage. Note that the seeds of *osrdr6-2* are unfilled and green. Scale bars, 0.5 cm. **b**, Gene structures of *OsRDR6*. Exons are indicated by boxes and introns are indicated by lines. *osrdr6-2* carries point mutation that leads to an amino acid change. **c**, Alignment of partial RDR6 protein sequences. The sequences were aligned with ClustalX. Conserved residues are shown in red. **d**, Panicles of *mel1-4* and HHZ rice plants at the grain-filling stage. Note that the seeds of *mel1-4* are unfilled and green. Scale bars, 0.5 cm. **e**, Gene structures of *MEL1*. Exons are

indicated by boxes and introns are indicated by lines. *mel1-4* has a deletion mutation that results in a premature stop codon. **f,** Relative accumulation levels of *TAS3*-derived tasiRNAs in early prophase I meiocytes of 3037 and *osrdr6-2*. The error bars represent standard deviation of three biological replicates. Asterisks indicate significant differences between *osrdr6-2* and the corresponding wild-type 3037 plants. *, Student's t test，*p*-value ≤ 0.05.

a



b

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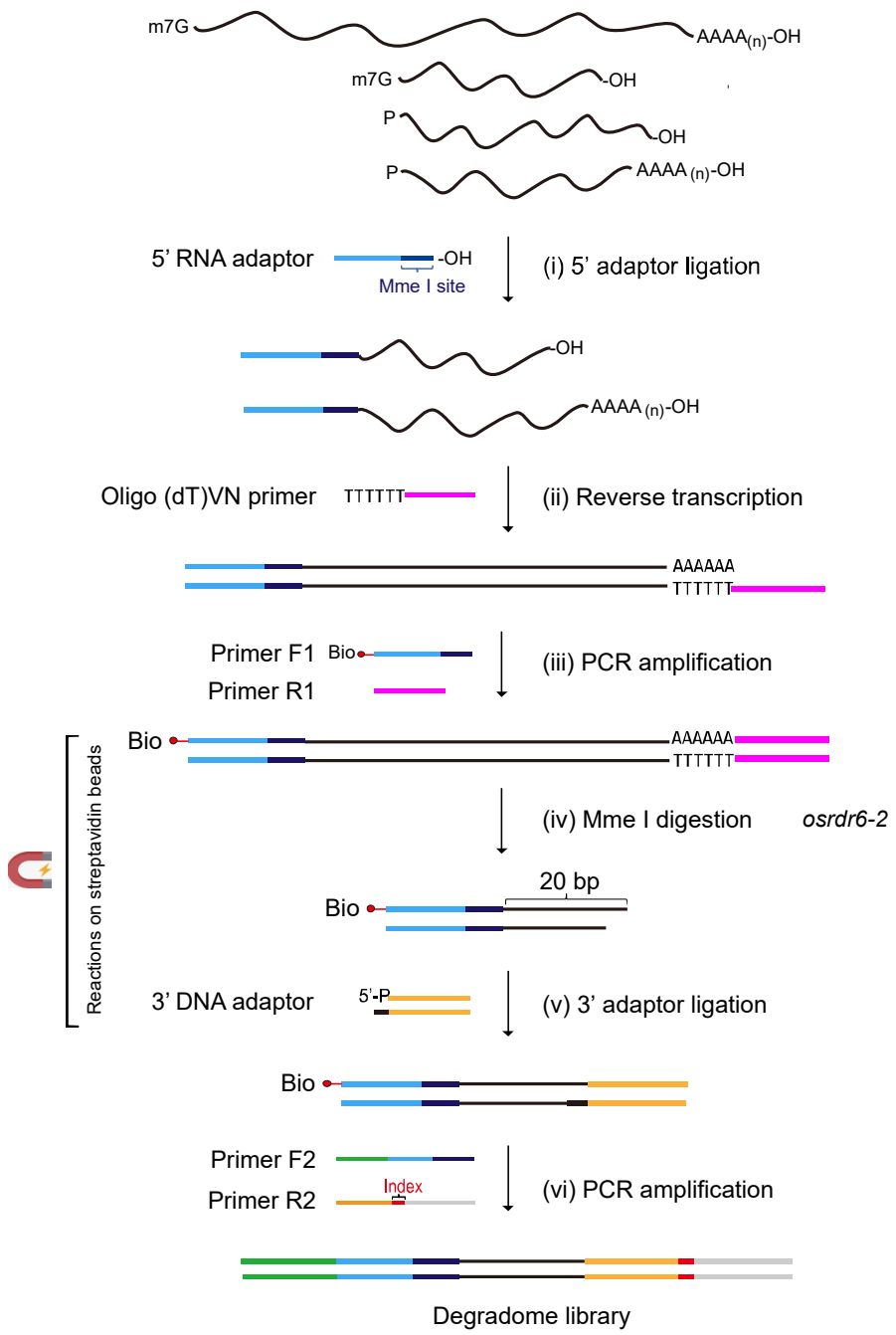
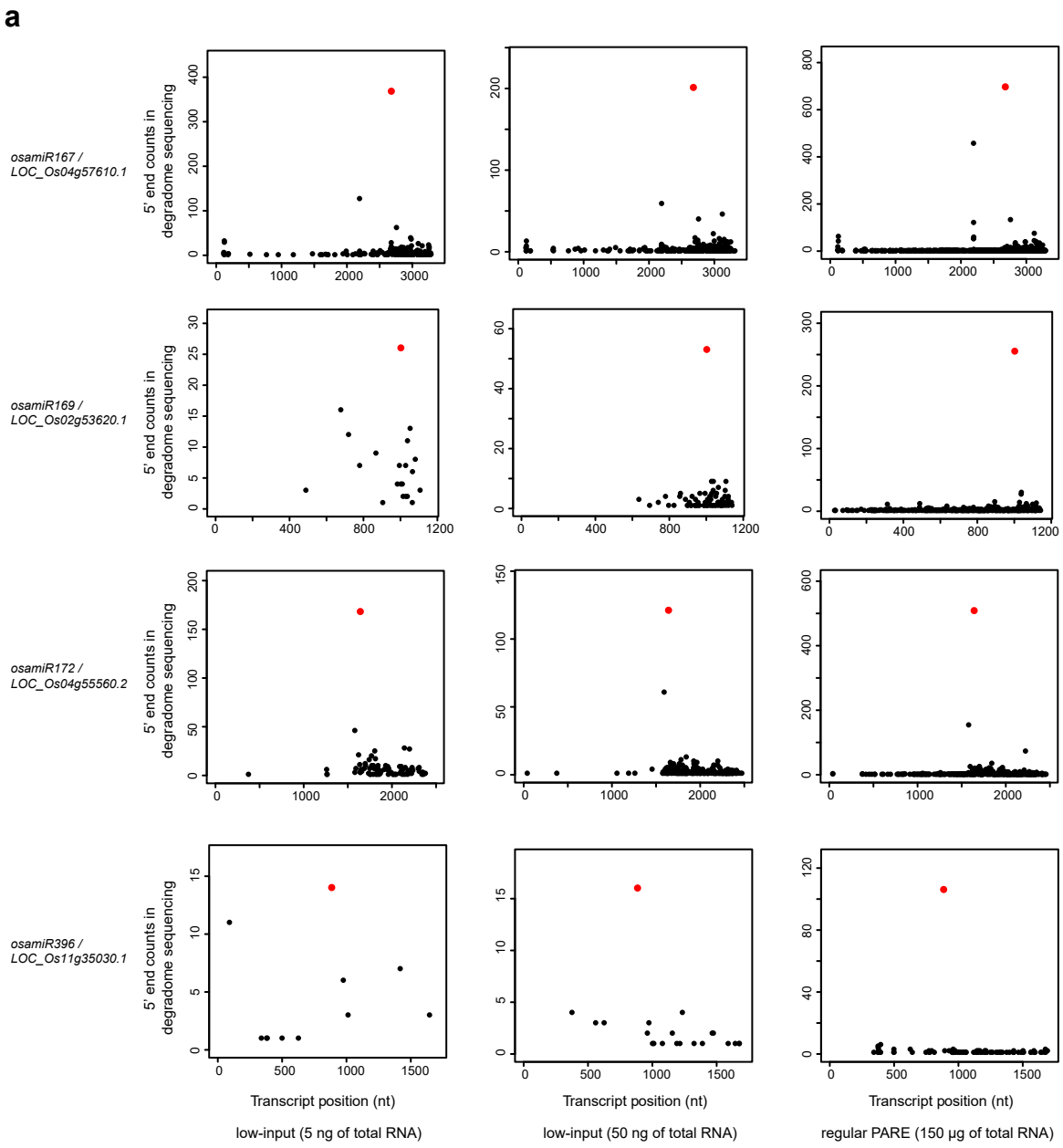
MEL1/OsAGO5c	773	PTIIFGADVTHP.....IIFYRDGVSEG.....PAYYAHLAAFRA	1011
OsAGO5d	402	-------LMSHT.....IIFYRDGVSEG.....PAYYSHLAASHA	633
OsAGO5e	779	PTIVFGADVTHP.....IIFYRDGISEG.....PAYYAHLGAFRA	1016

Supplementary Fig. 3. AGO5 subclade proteins in rice, maize and *Arabidopsis*. **a**, Phylogenetic analysis of AGO5 proteins in rice, maize and *Arabidopsis*. The neighbor joining tree was constructed using the MEGAX software. At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Zm, *Zea mays*. Scale bars, 0.1 substitutions per amino acid position. **b**, Alignment of AGO5 protein sequences. The sequences were aligned with ClustalX. Residues forming the catalytic DDH motif are shown in red.

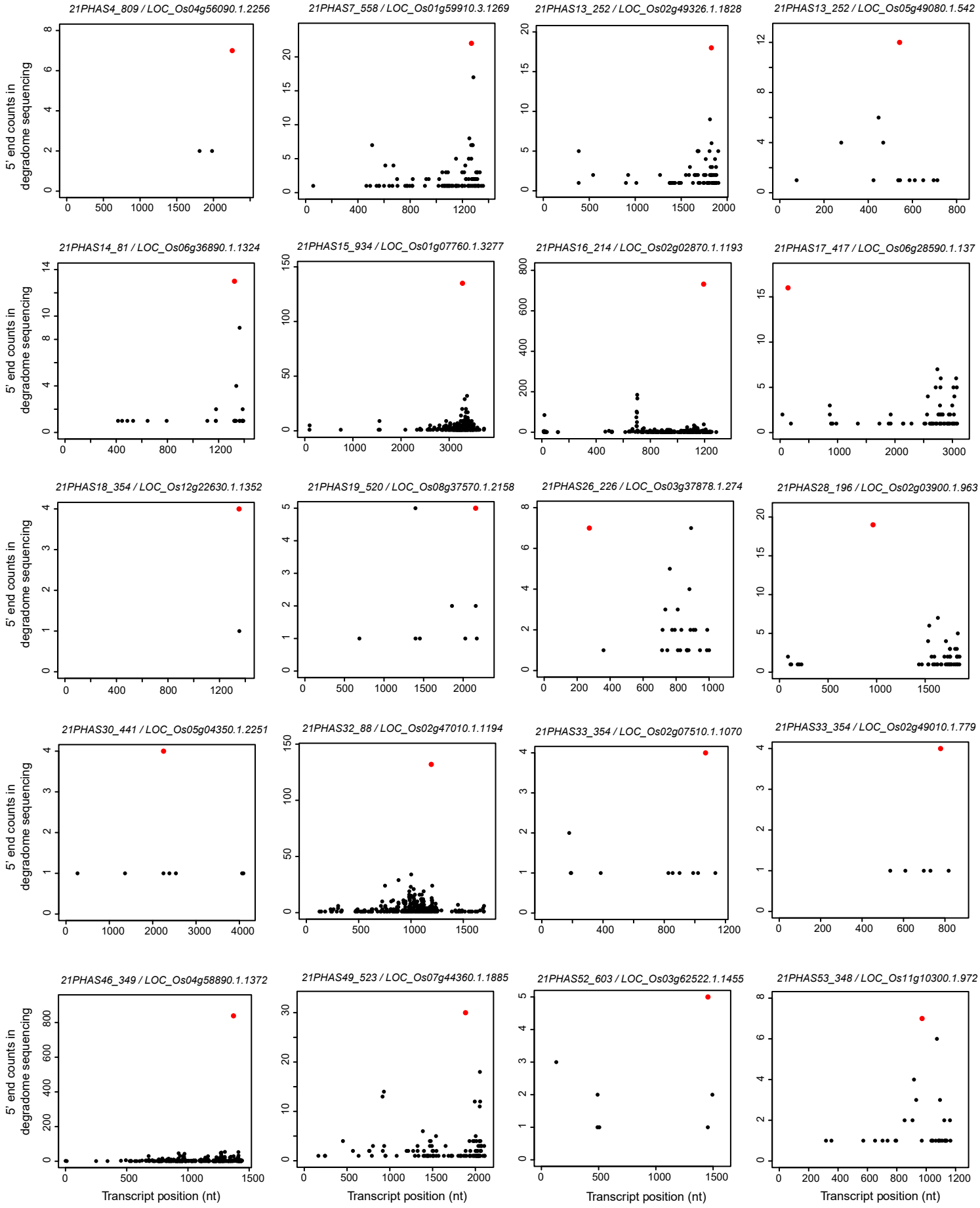


Supplementary Fig. 4. Schematic depiction of low-input degradome library construction. Construction of a degradome library for low-input RNAs includes the following steps: (i) RNA with 5'-monophophates group in total RNA is ligated to the 5' RNA adaptor; (ii) the ligated product with 3' poly-A is reverse-transcribed with Oligo-dT$_{30}$VN primer; (iii) the reverse transcription product is purified and amplified with biotin-labelled primer F1 and primer R1; (iv) the PCR product was purified and digested with MmeI; (v) the digested product is purified and ligated to the 3' DNA adaptor; (vi) the final ligated product is purified and amplified with primer F2 and primer R2.



Supplementary Fig. 5. Comparison of the specificity of the low-input and regular PARE protocols. a, Plots showing the distribution of the *degradome* tags *along miRNA targets*. The x-axis represents the nucleotide position of each target mRNA (5'-3'). The y-axis represents the

relative frequency of degradome tags. The red dot represents degradome tag at +1 position of miRNA-guided cleavage site. **b**, Venn diagram showing the overlap between miRNA targets identified by the low-input protocol (with 5 ng and 50 ng of total RNA) and the regular PARE protocol (with 150 μg of total RNA).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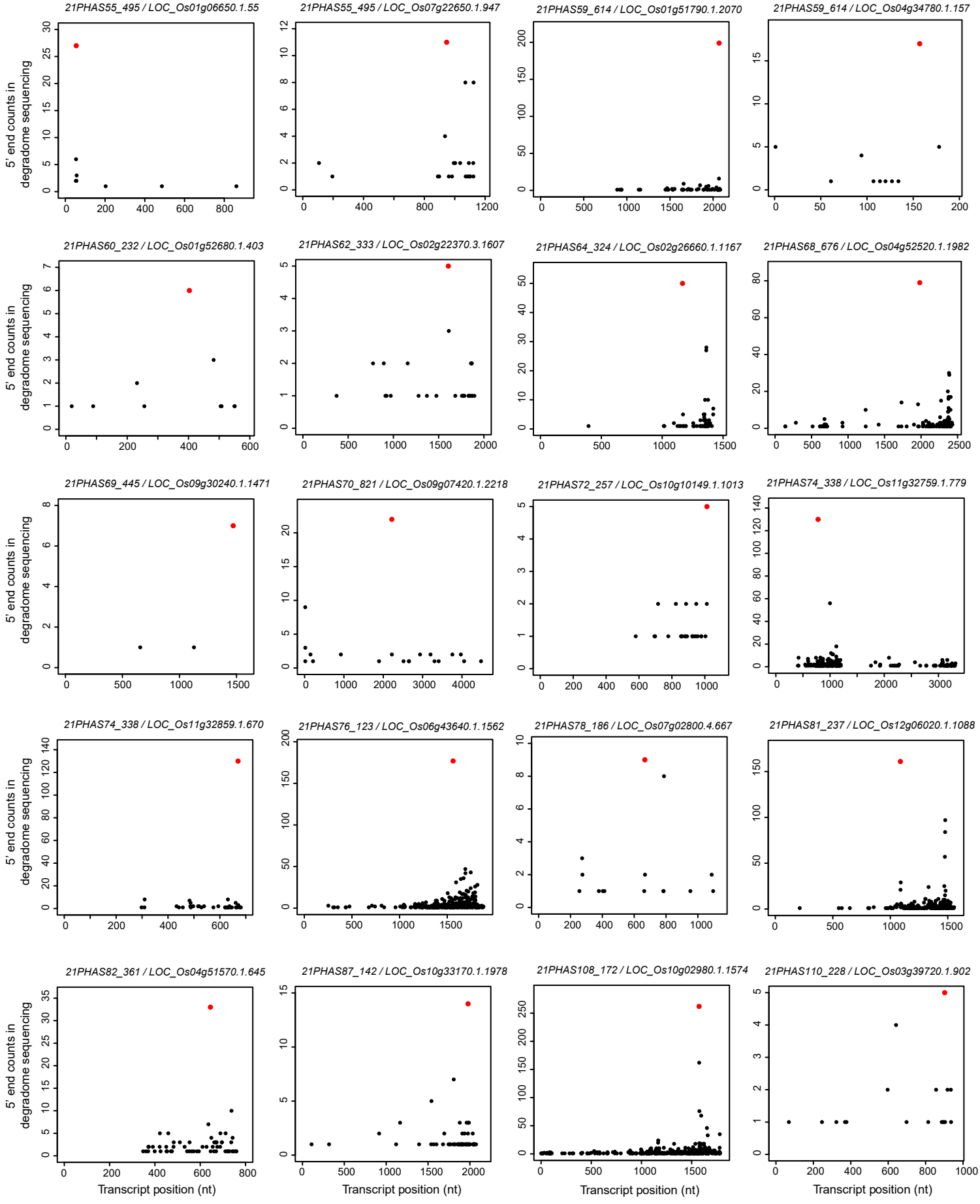
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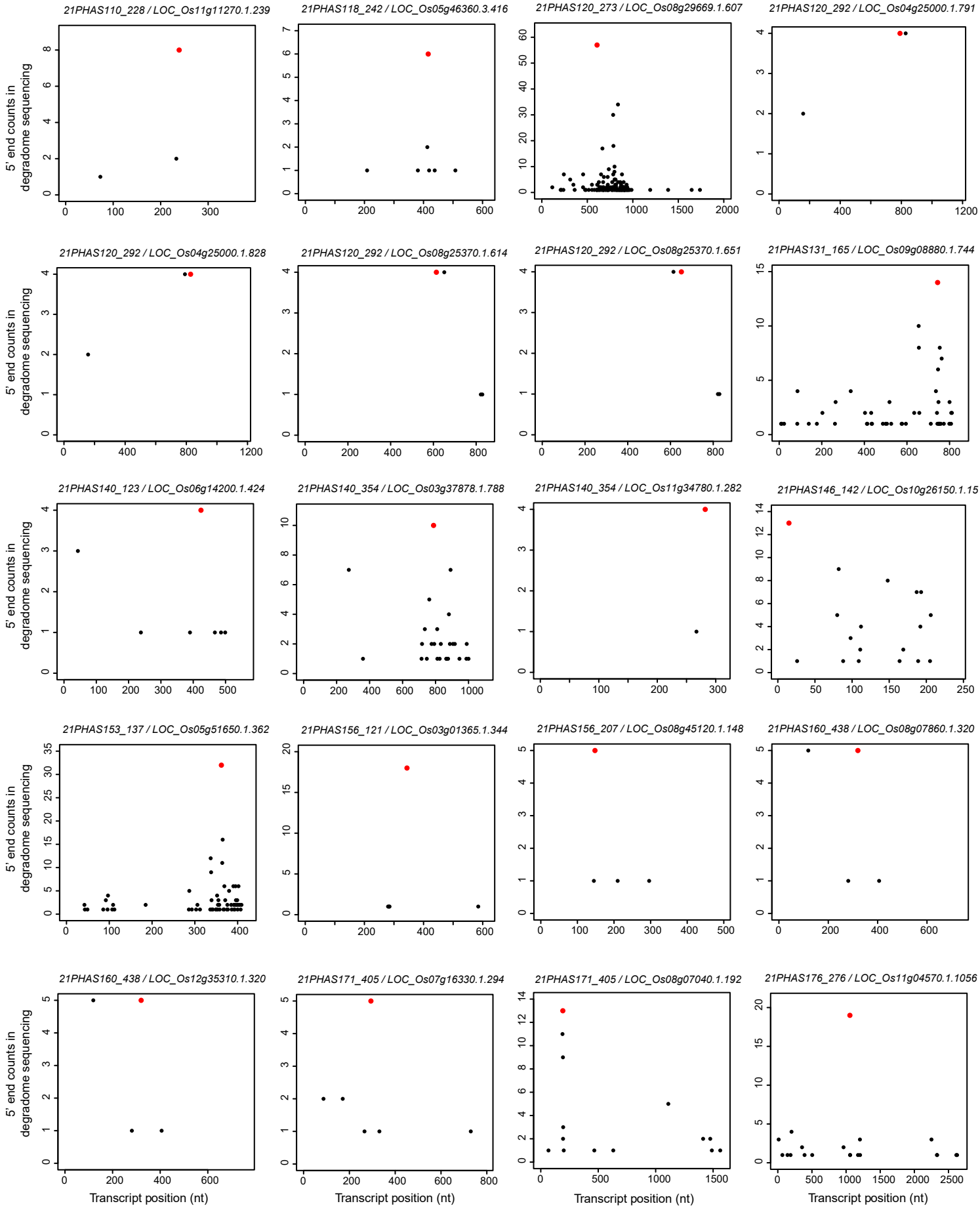
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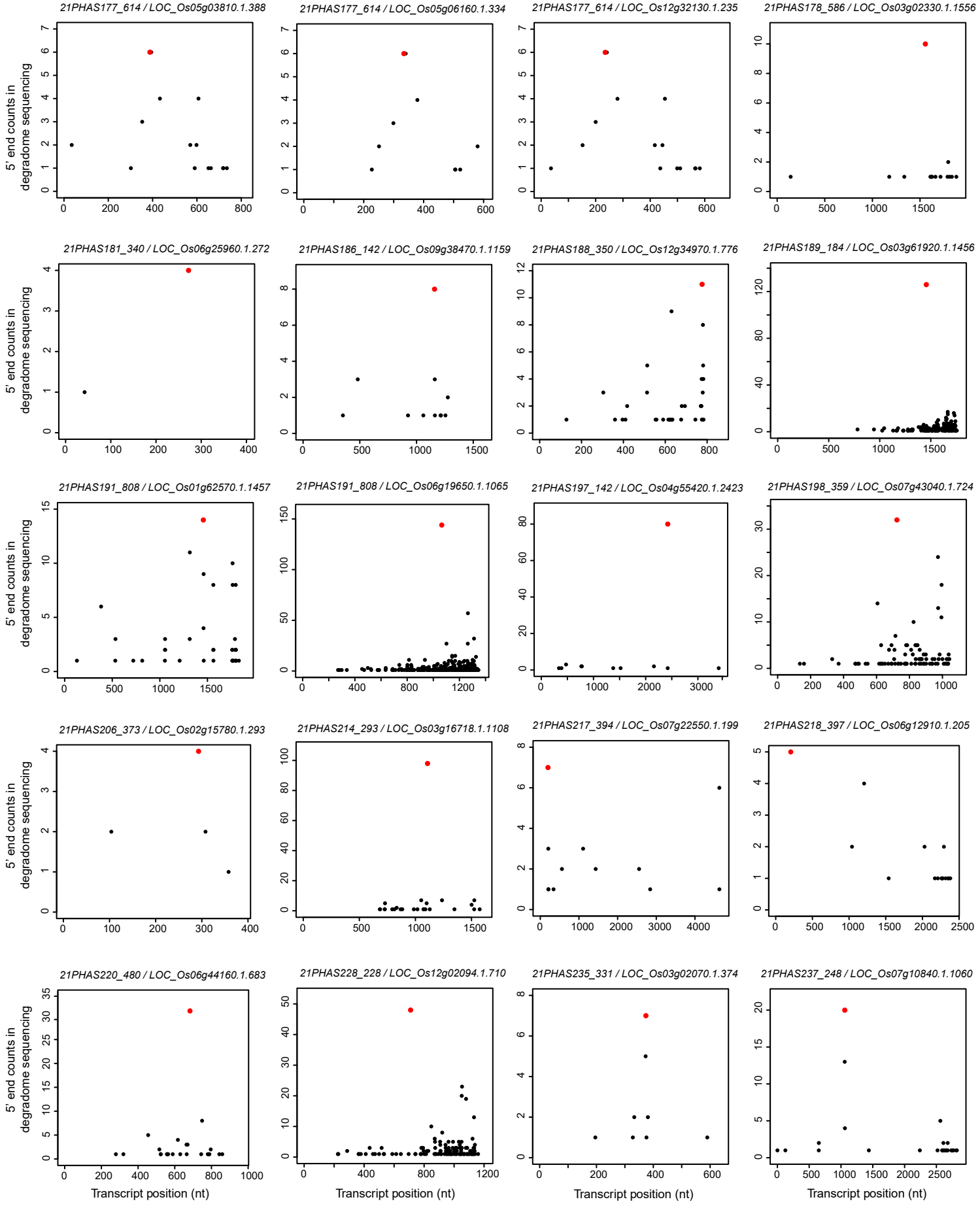
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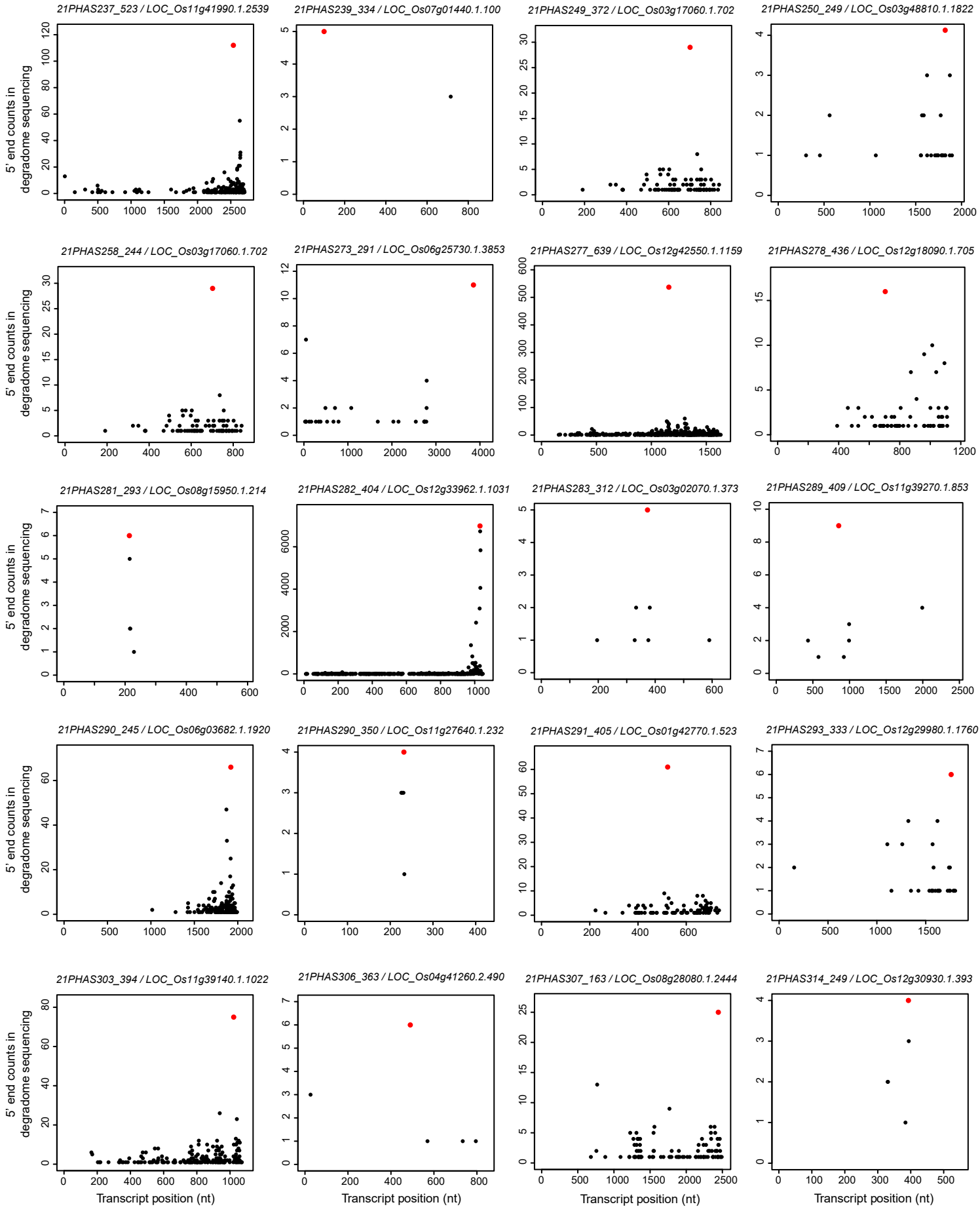
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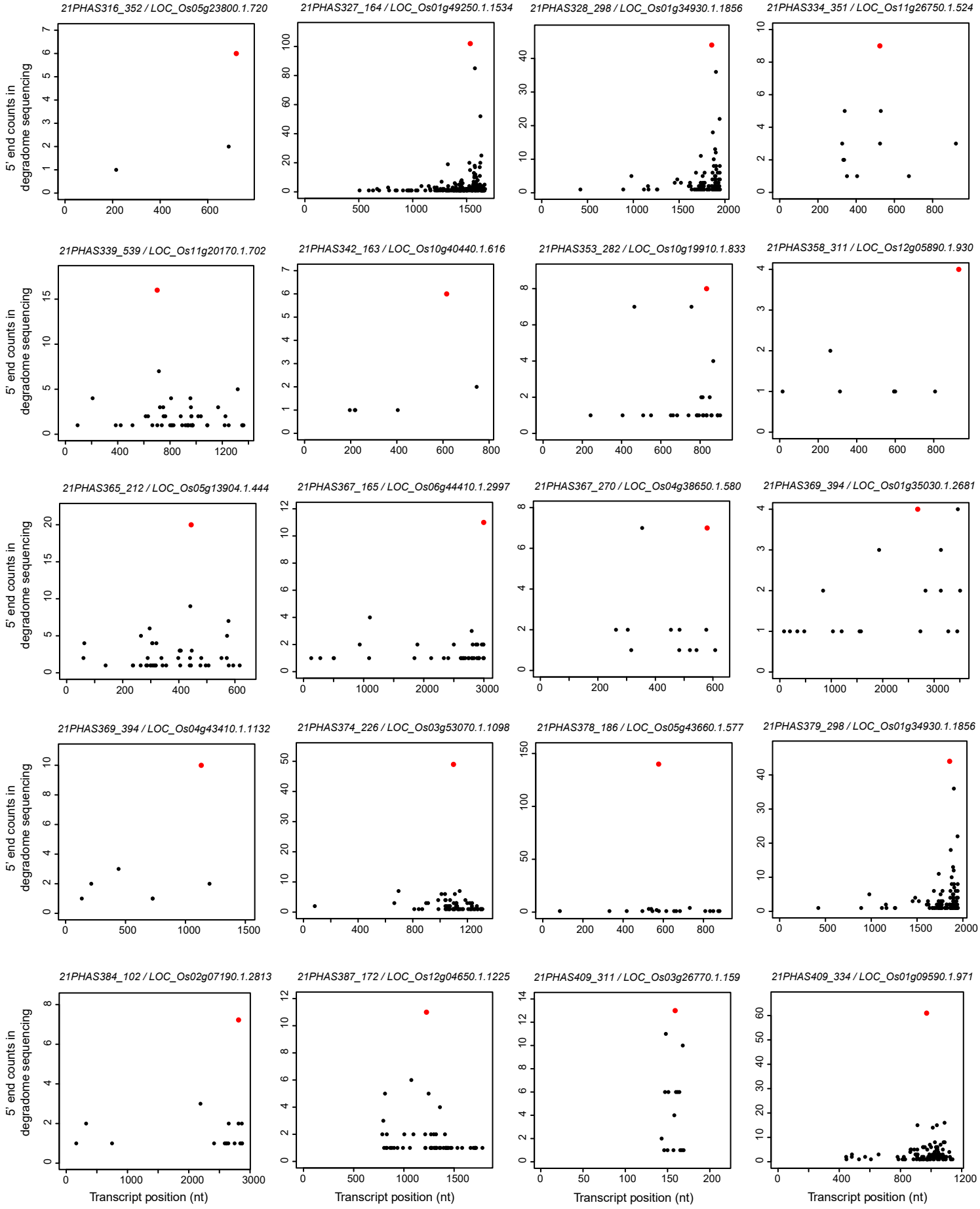
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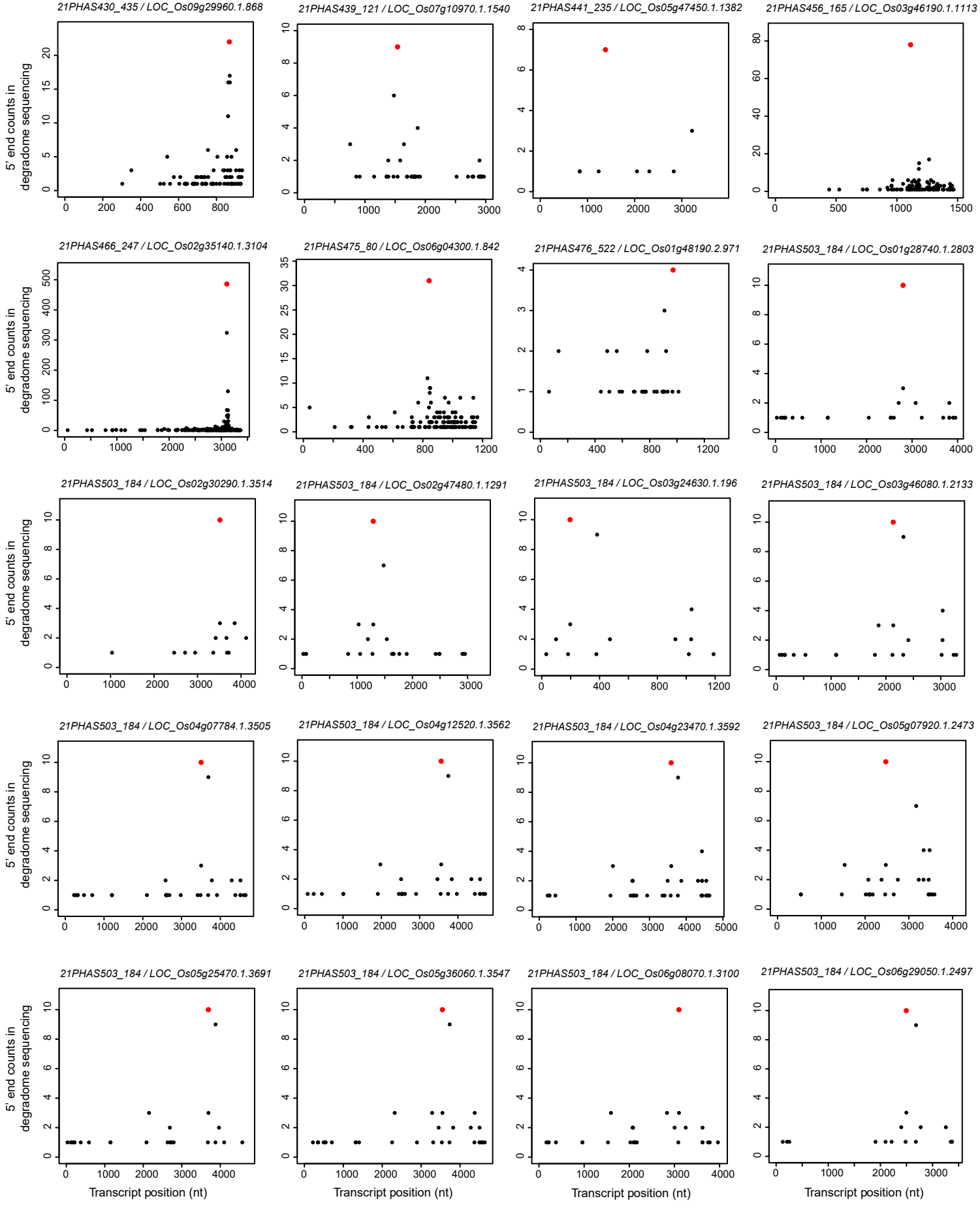
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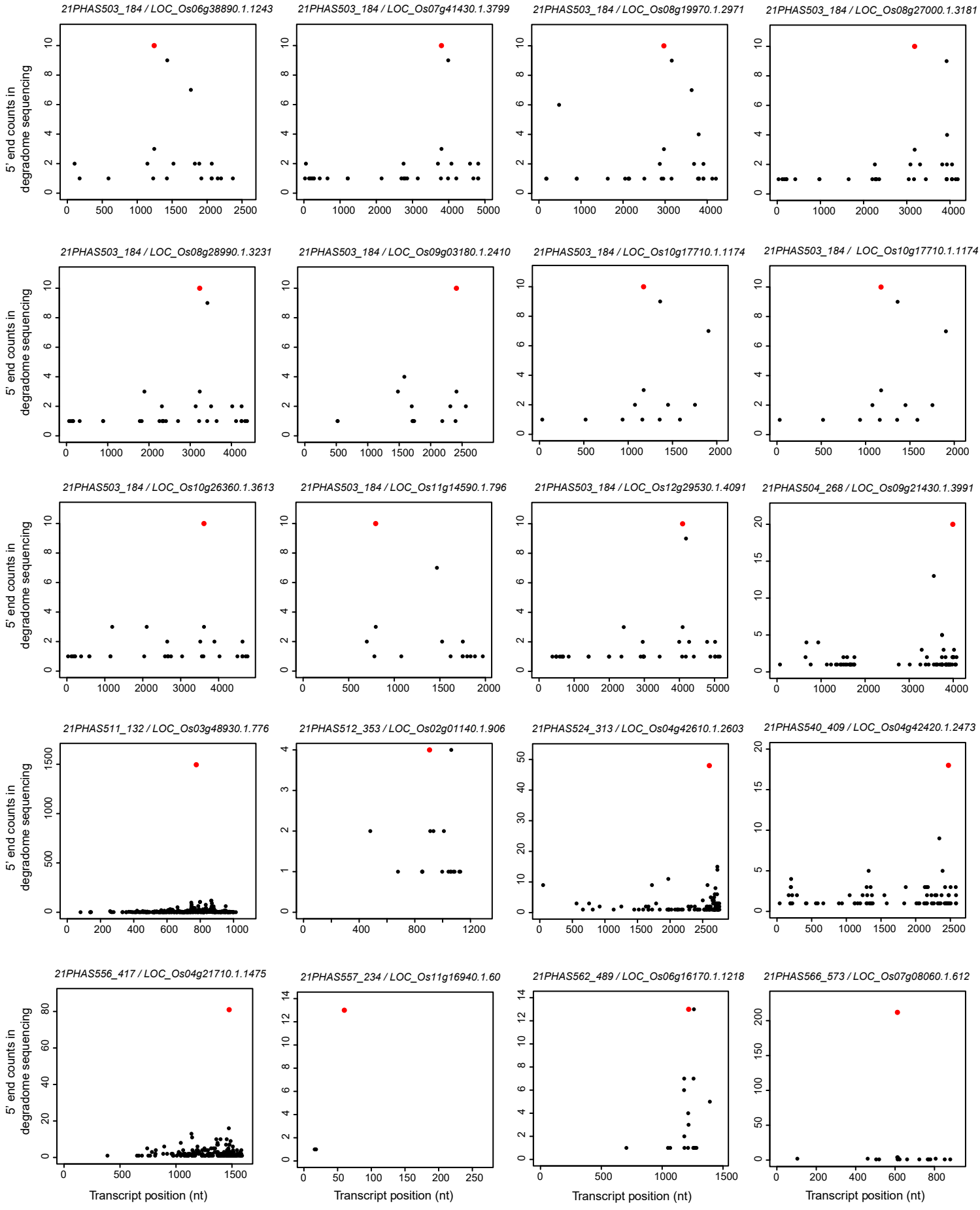
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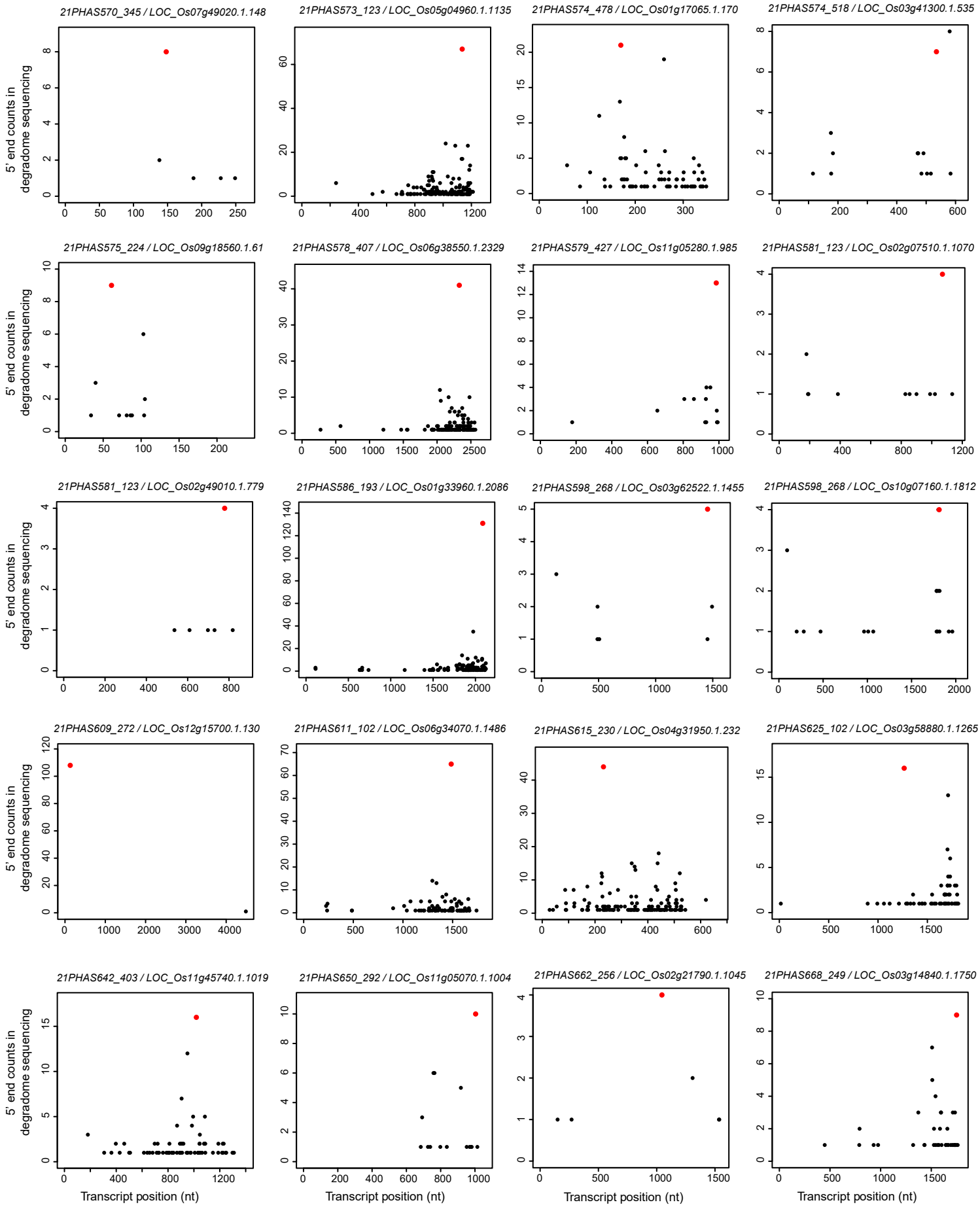
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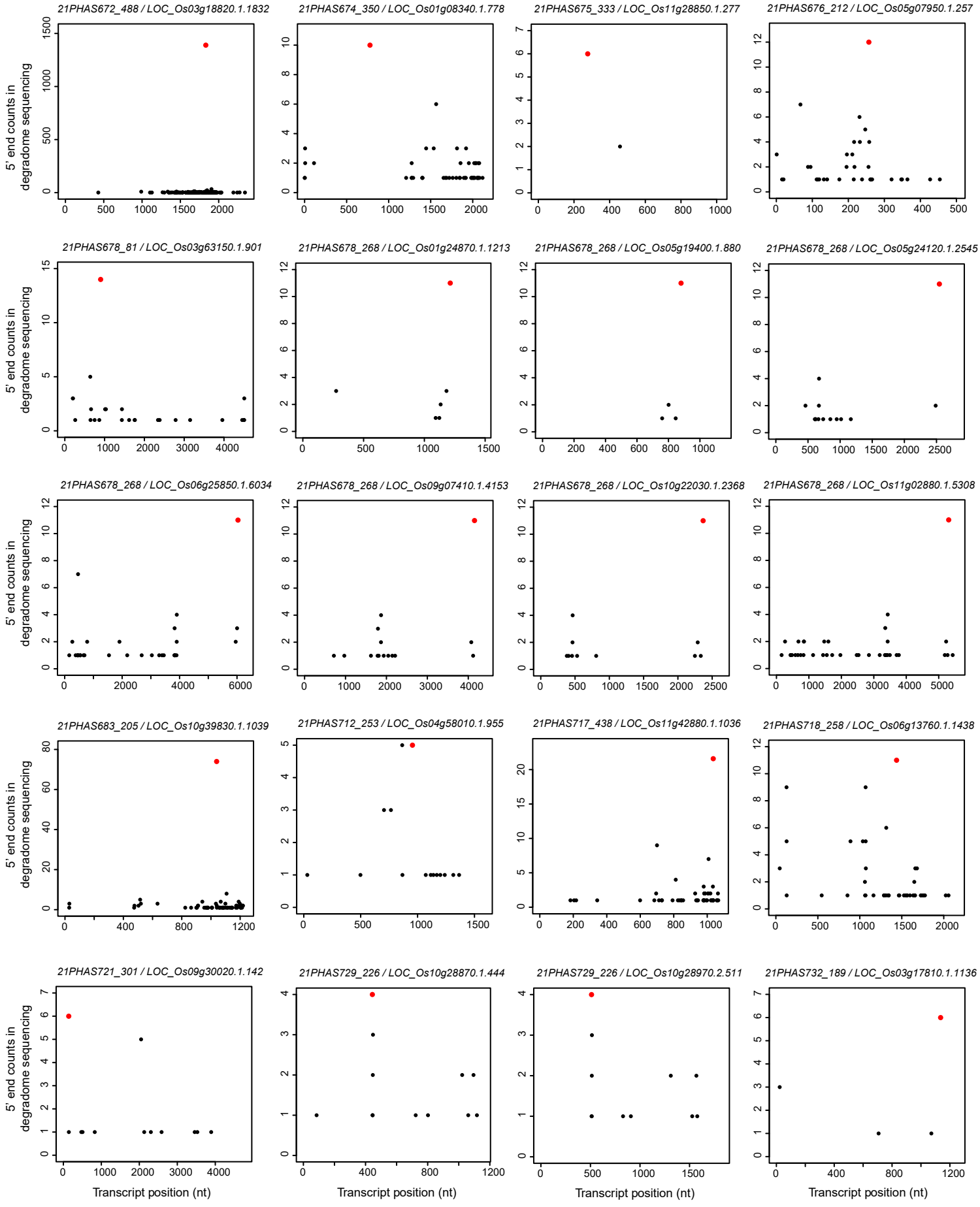
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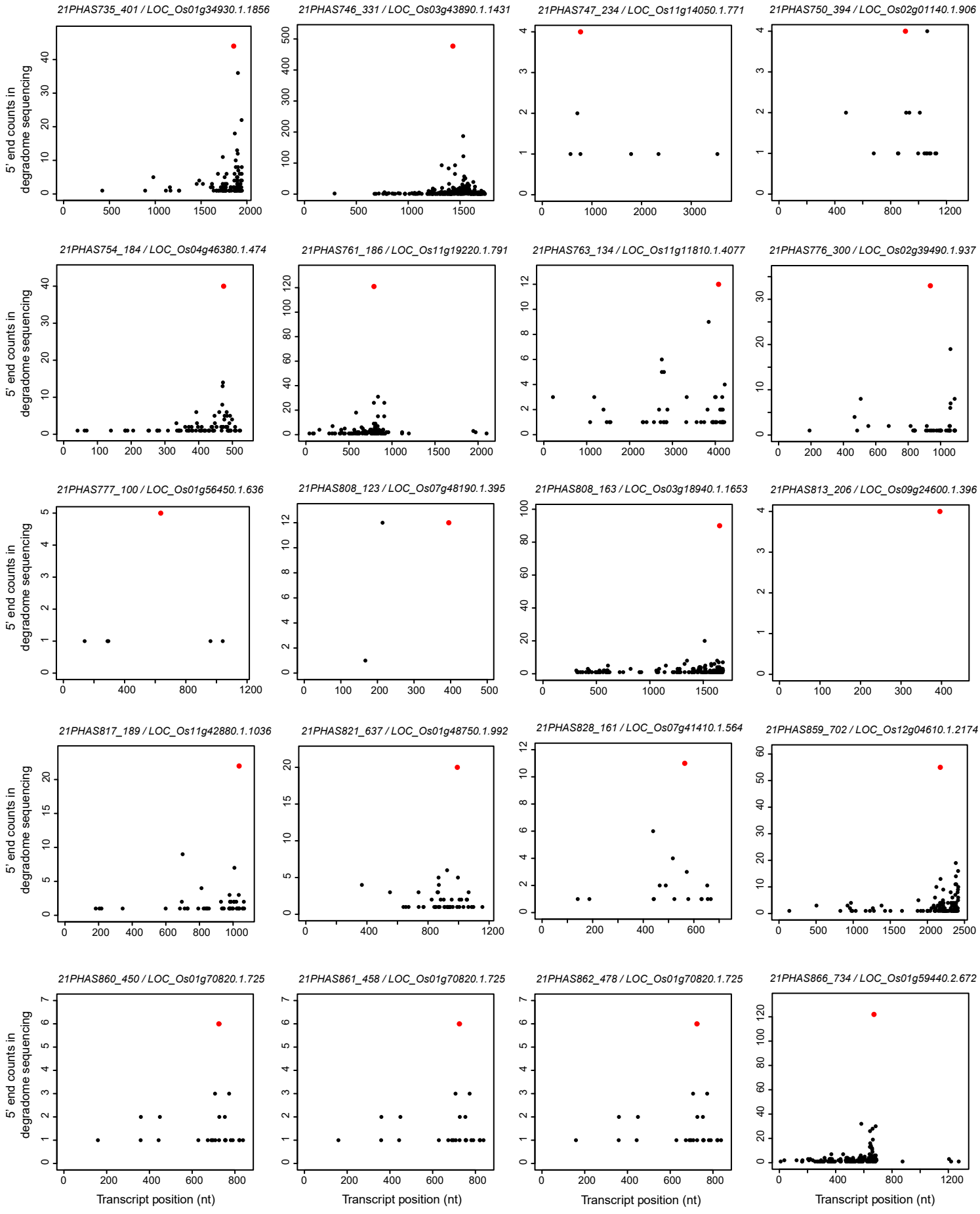
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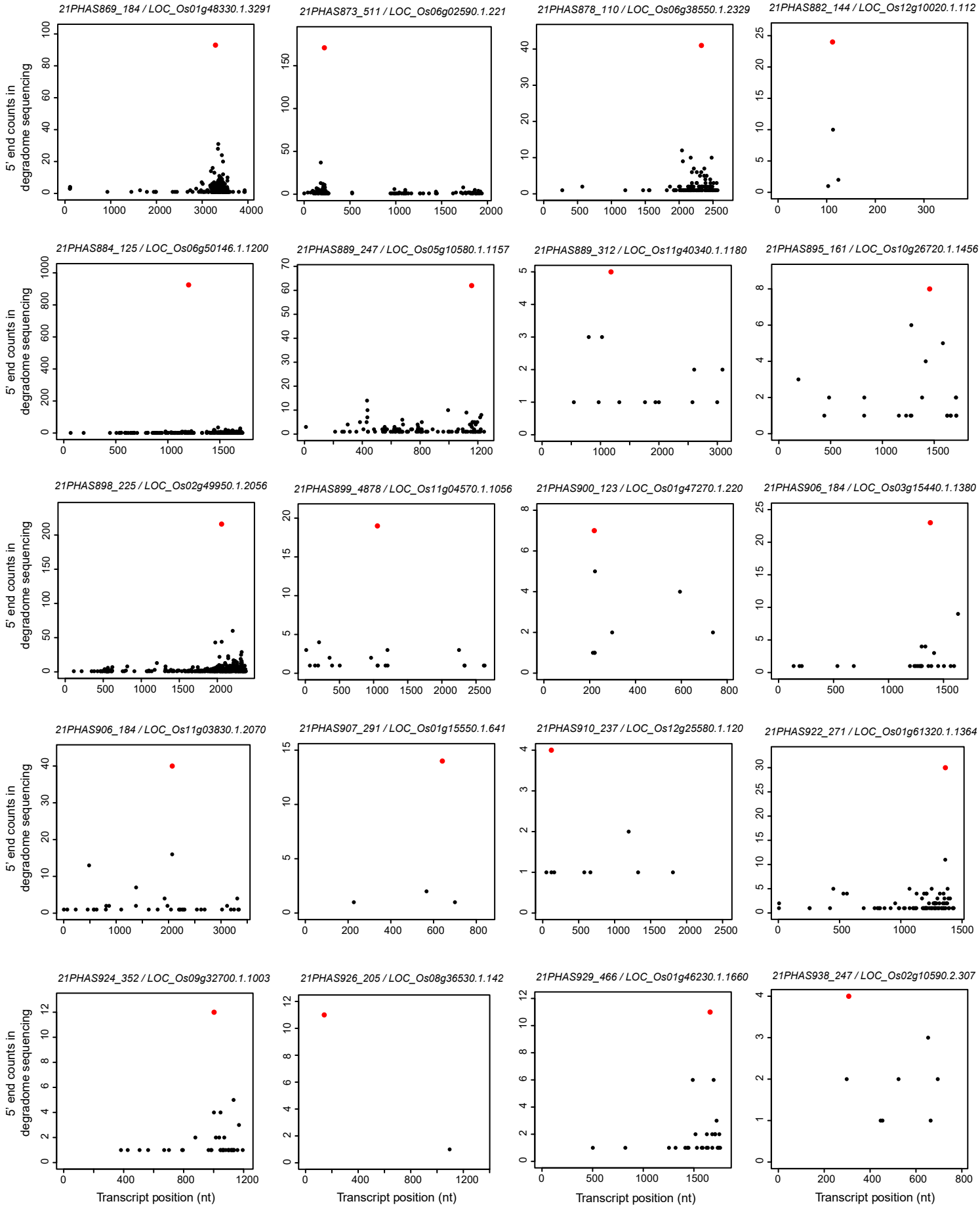
5' end counts in degradome sequencing
Transcript position (nt)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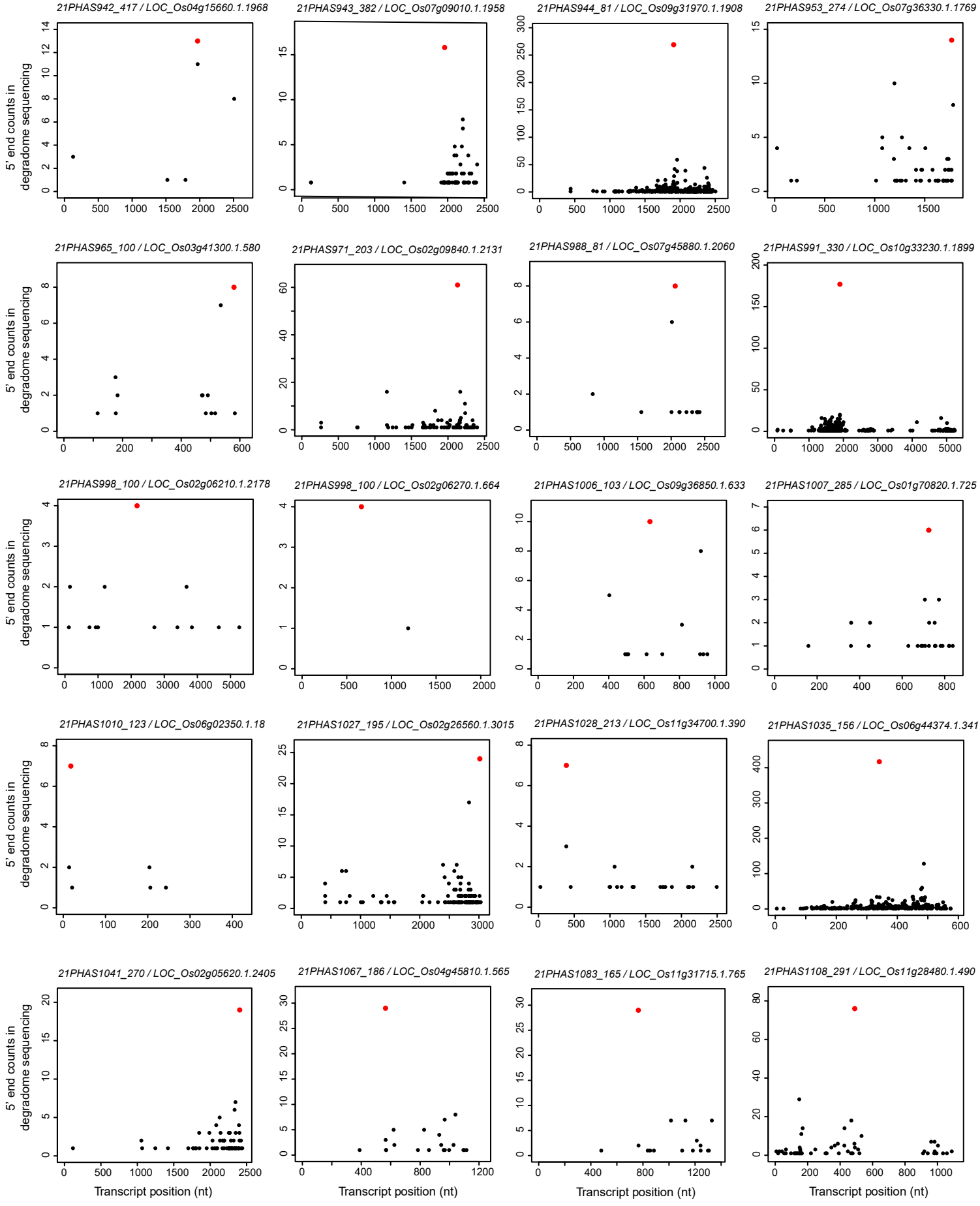
Transcript position (nt)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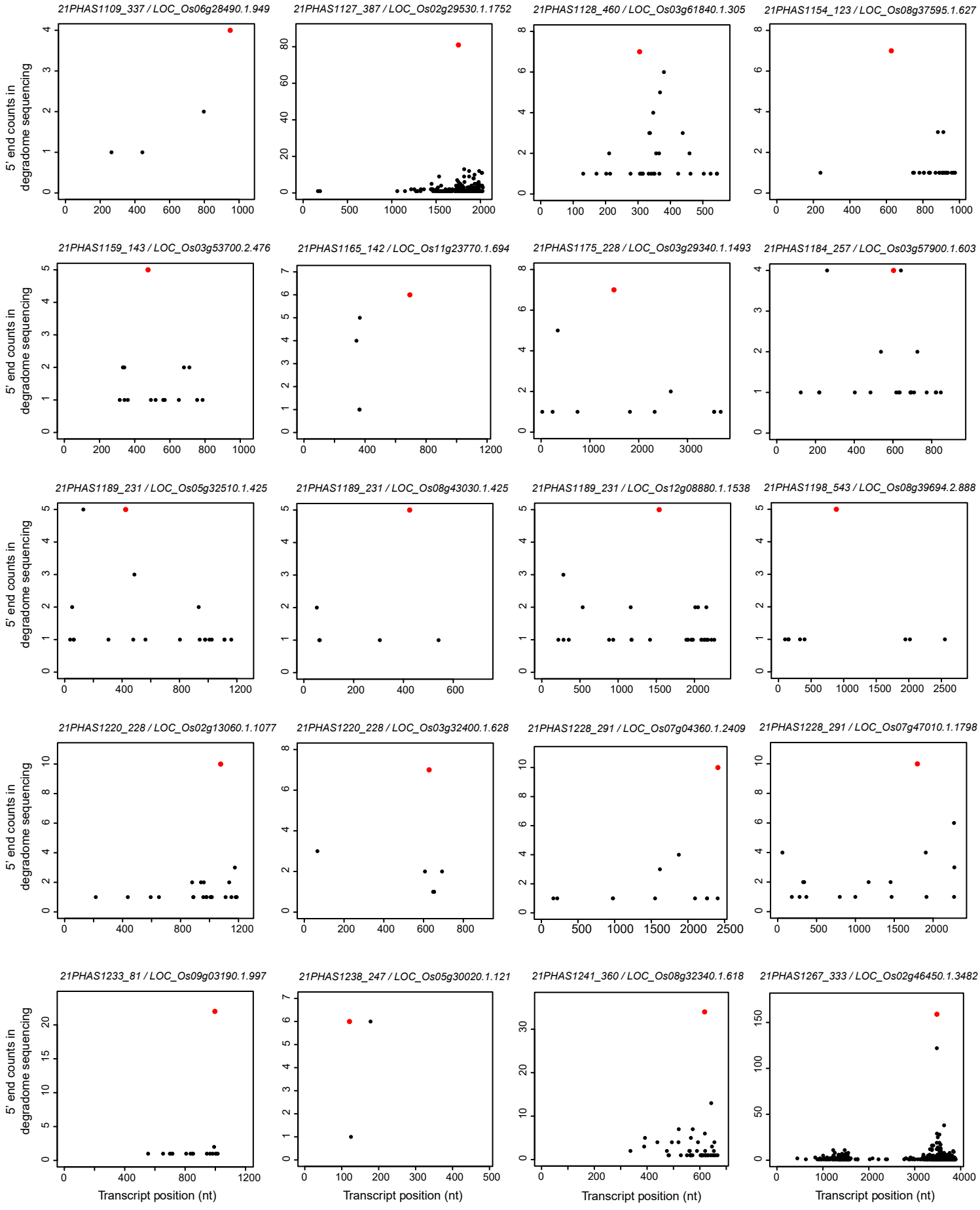
Transcript position (nt)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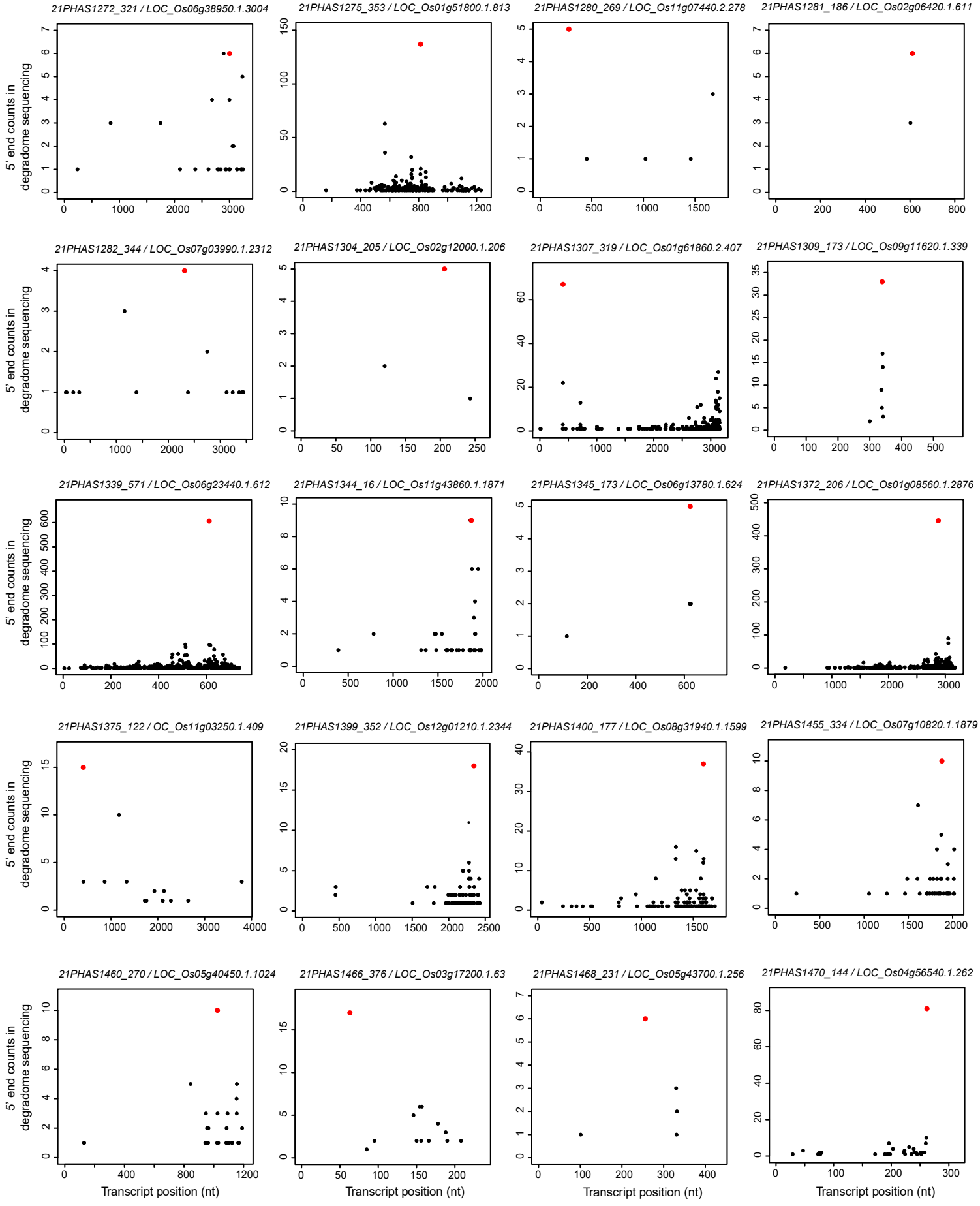
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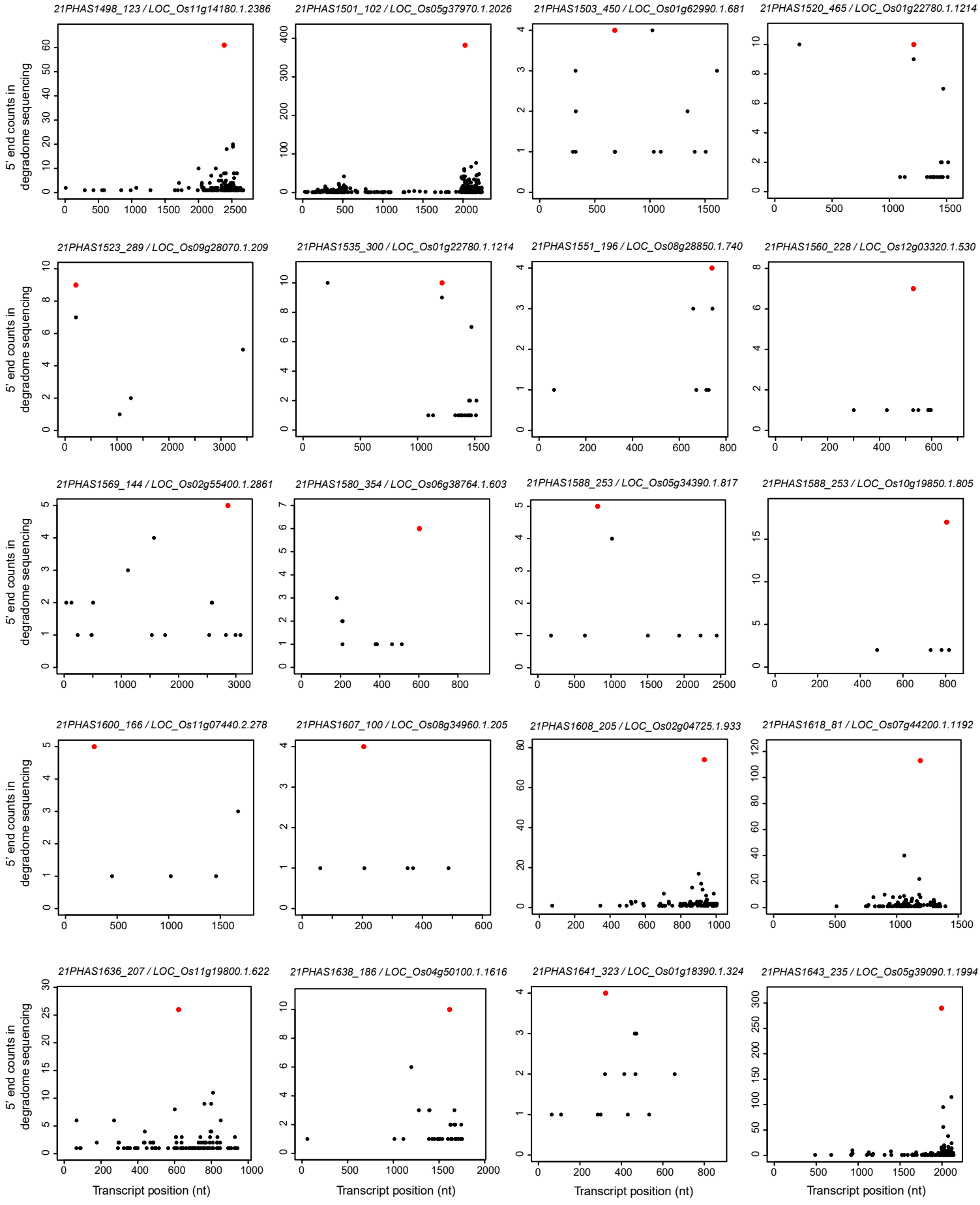
5' end counts in degradome sequencing
Transcript position (nt)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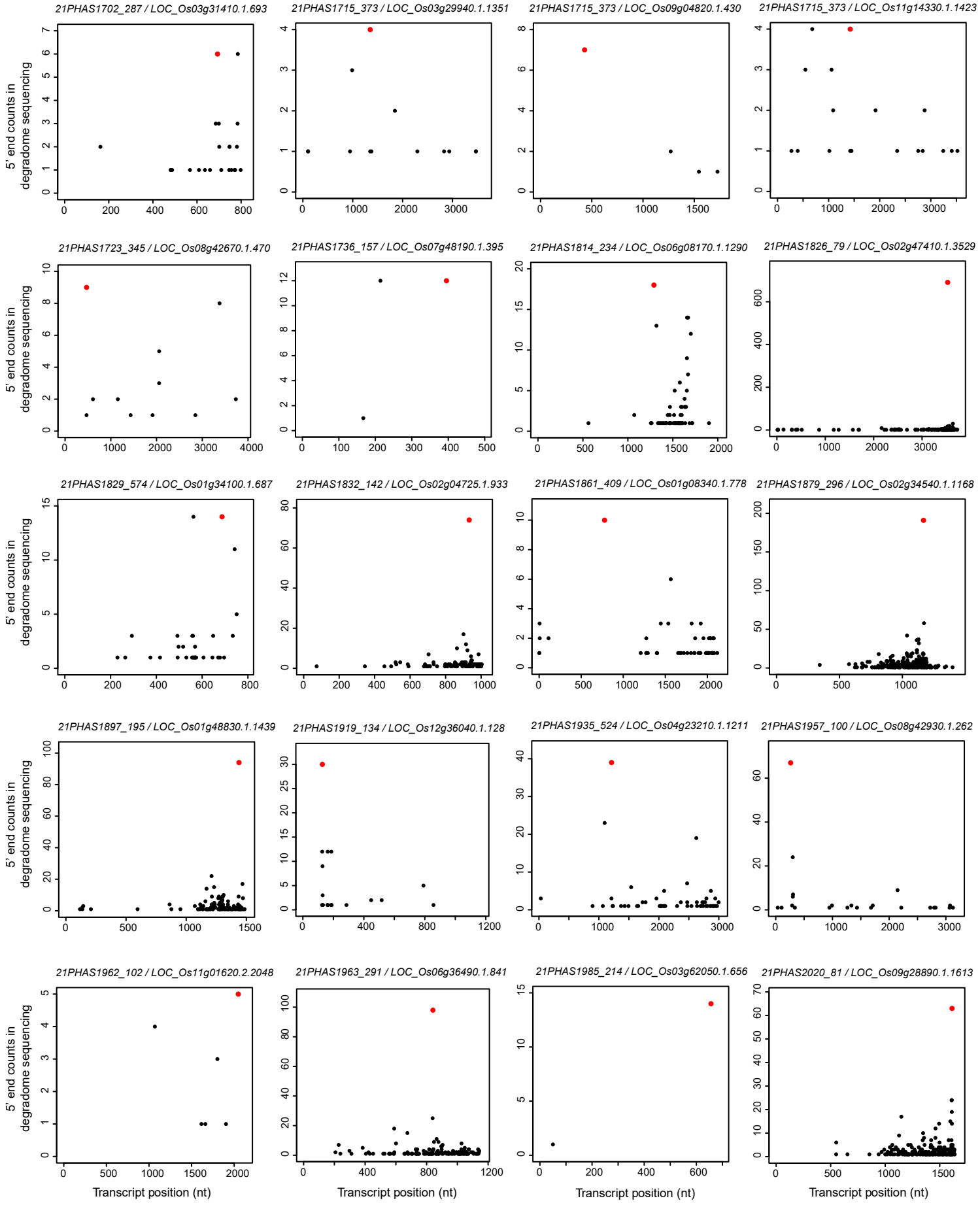
5' end counts in degradome sequencing
Transcript position (nt)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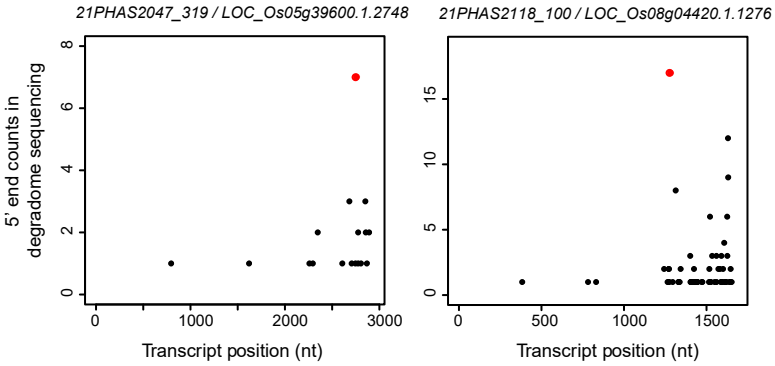
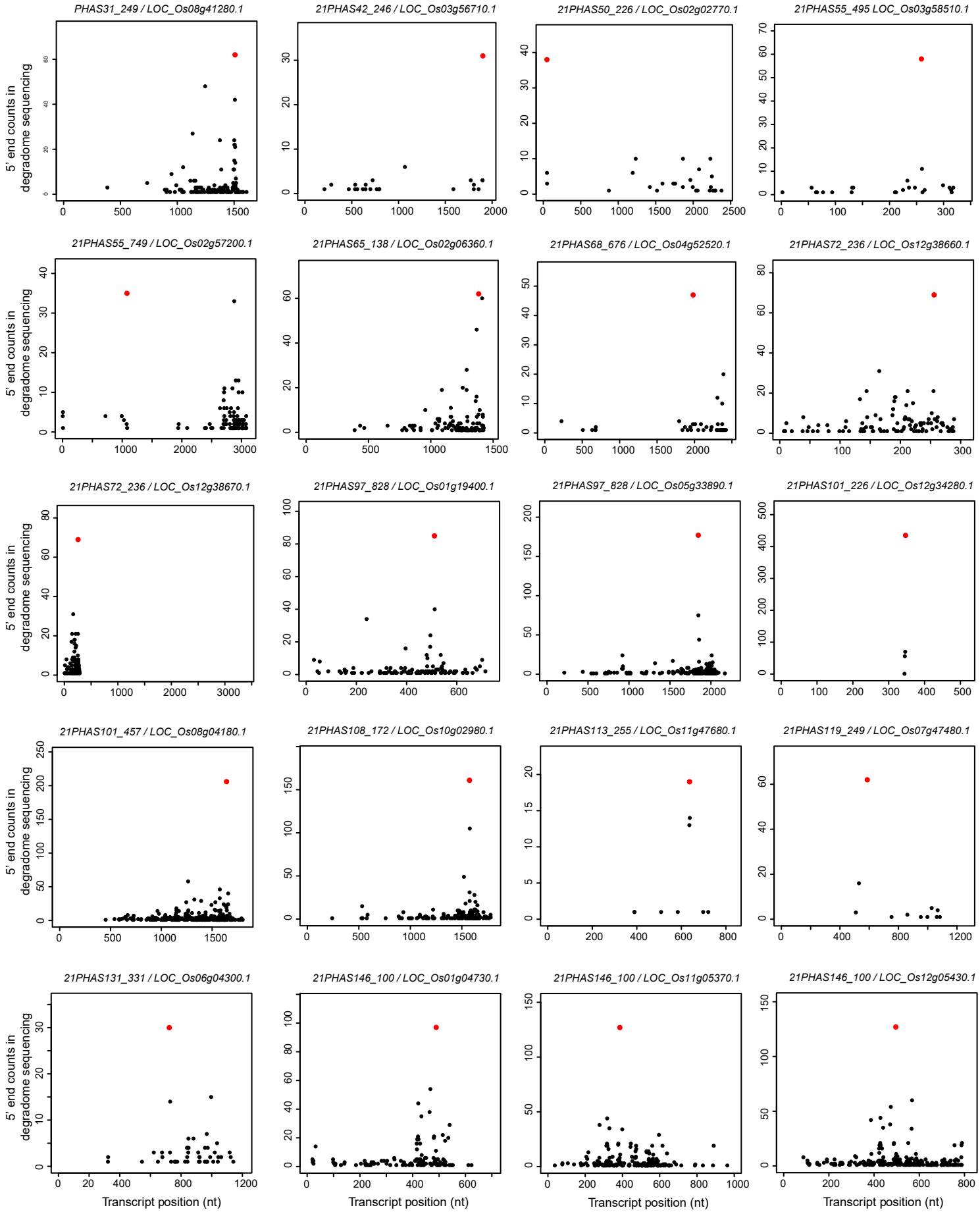
5' end counts in degradome sequencing
Transcript position (nt)



Supplementary Fig. 6 Validation of 21-nt phasiRNA targets in early prohase I meiocytes by degradome sequencing. Plots showing the distribution of the degradome tags along 21-nt phasiRNA targets. The x-axis represents the nucleotide position of each target mRNA (5'-3'). The y-axis represents the relative frequency of degradome tags. The red dot represents degradometag at +1 position of 21-nt phasiRNA-guided cleavage site.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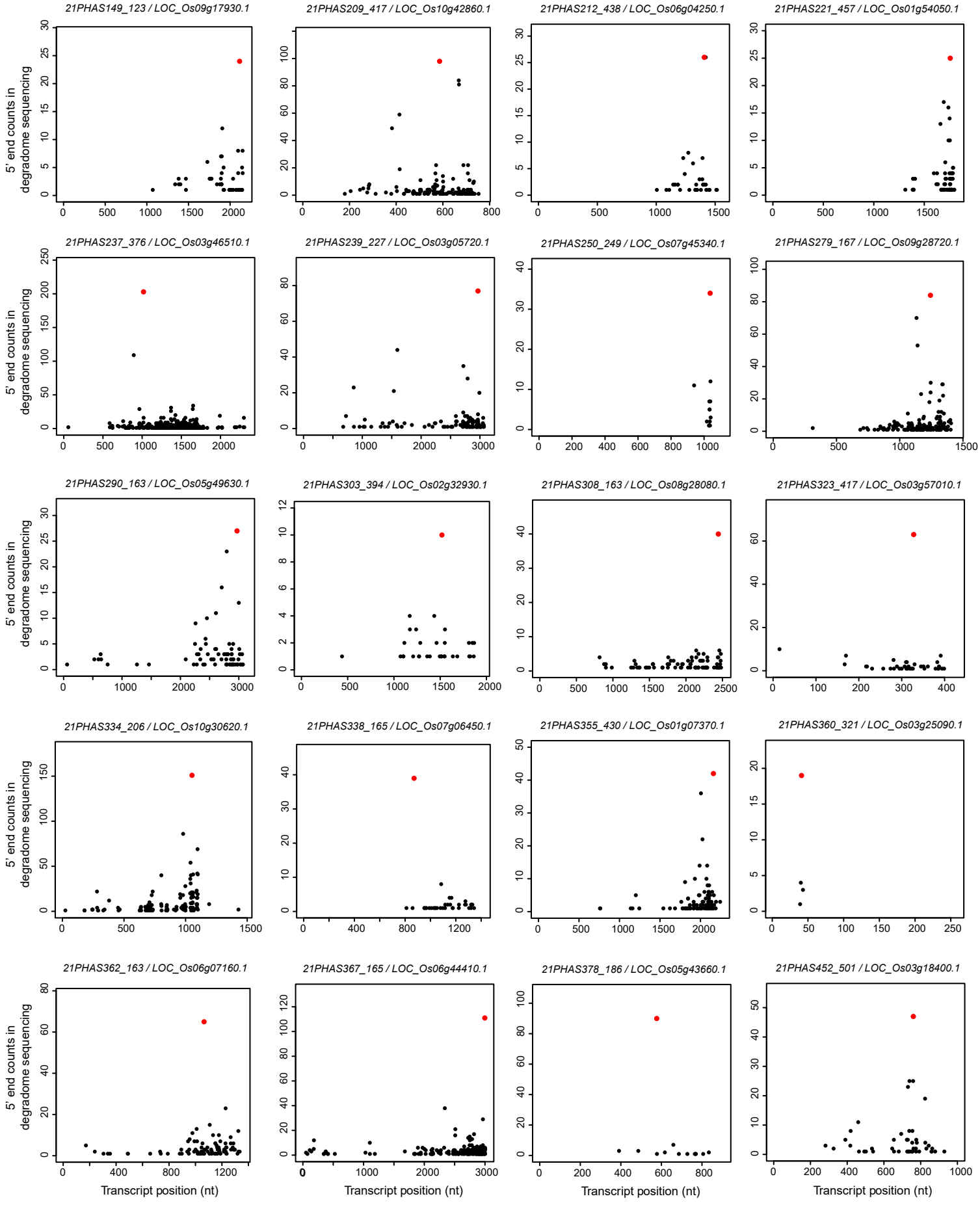
5' end counts in degradome sequencing
Transcript position (nt)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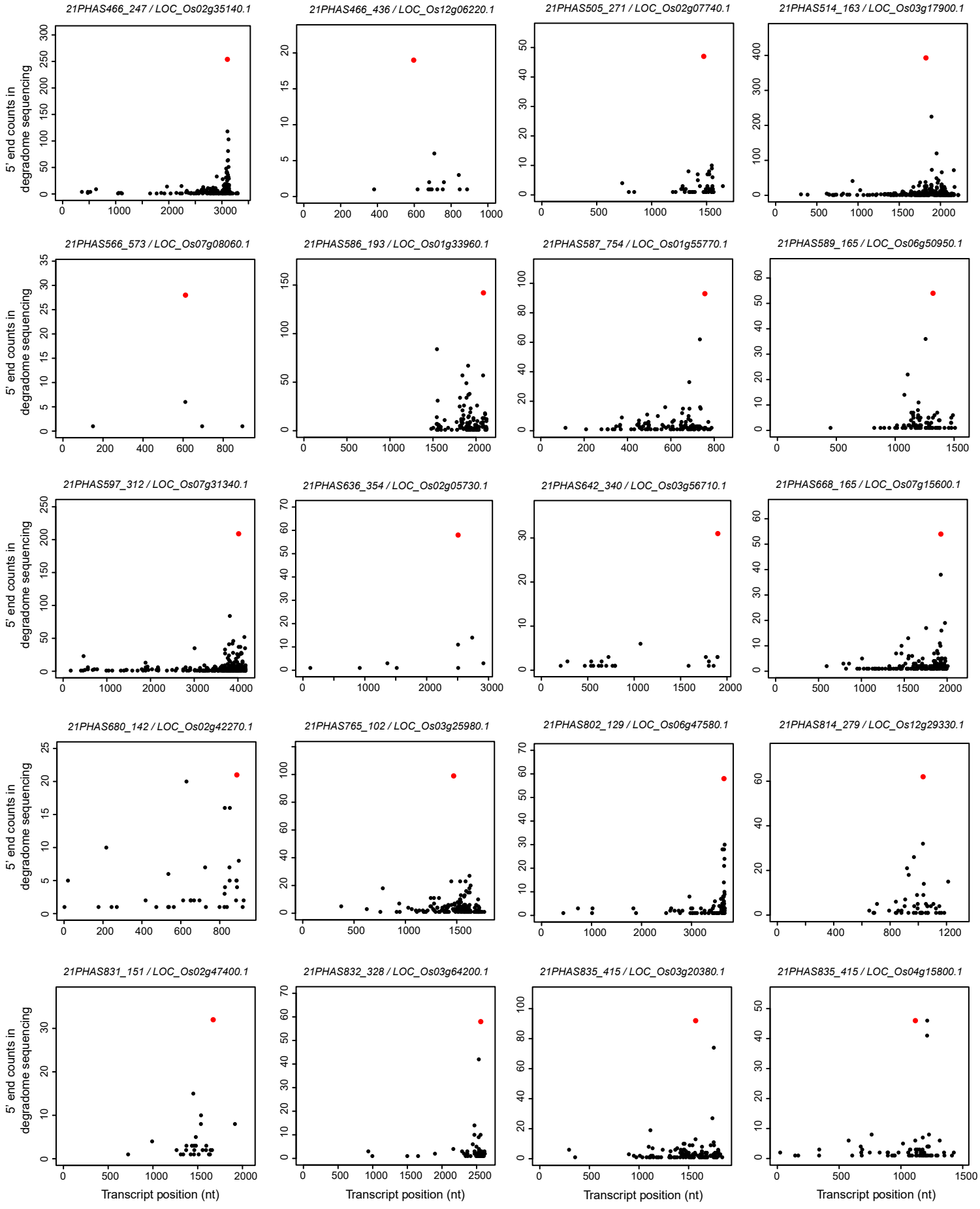
Transcript position (nt)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5' end counts in degradome sequencing
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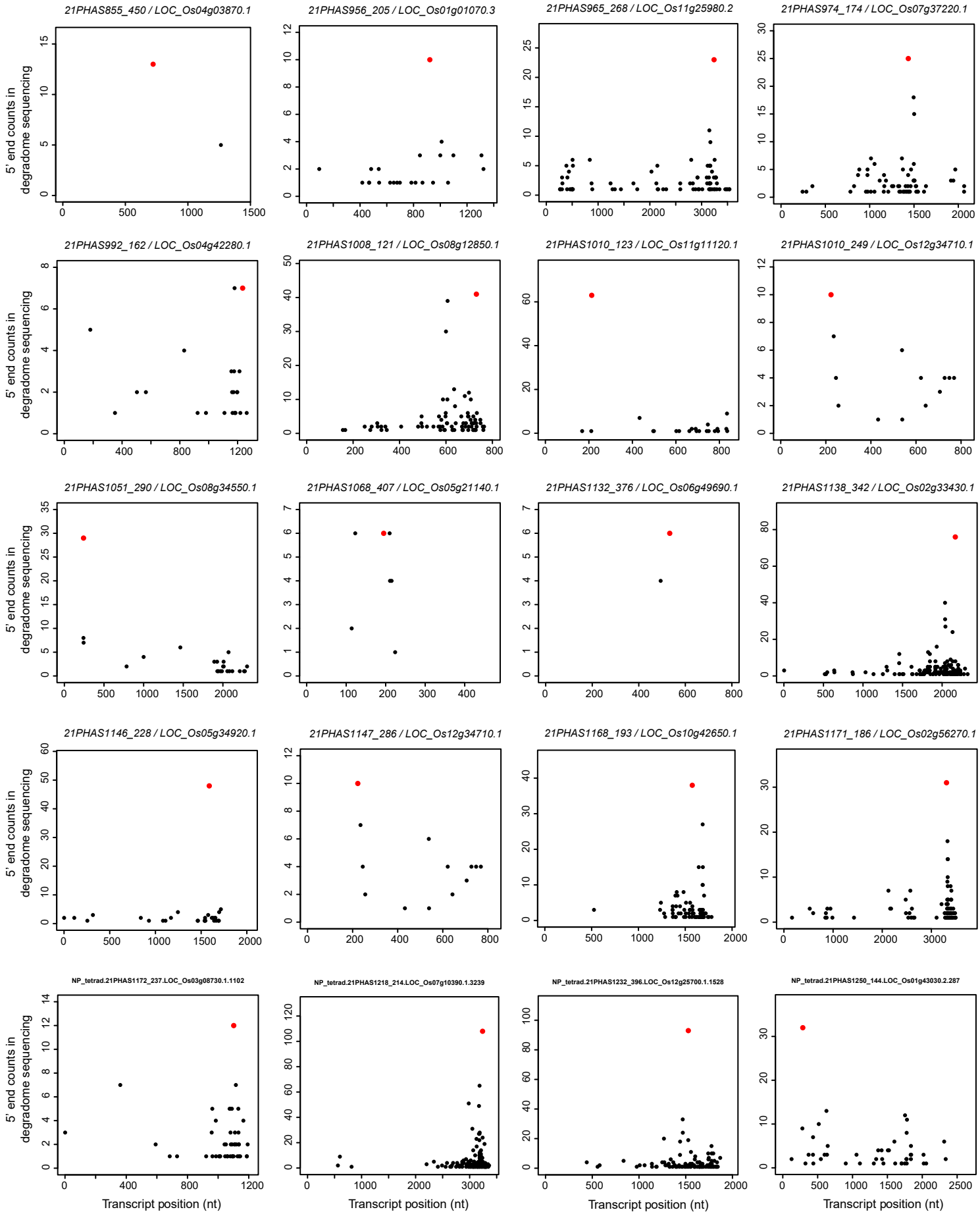
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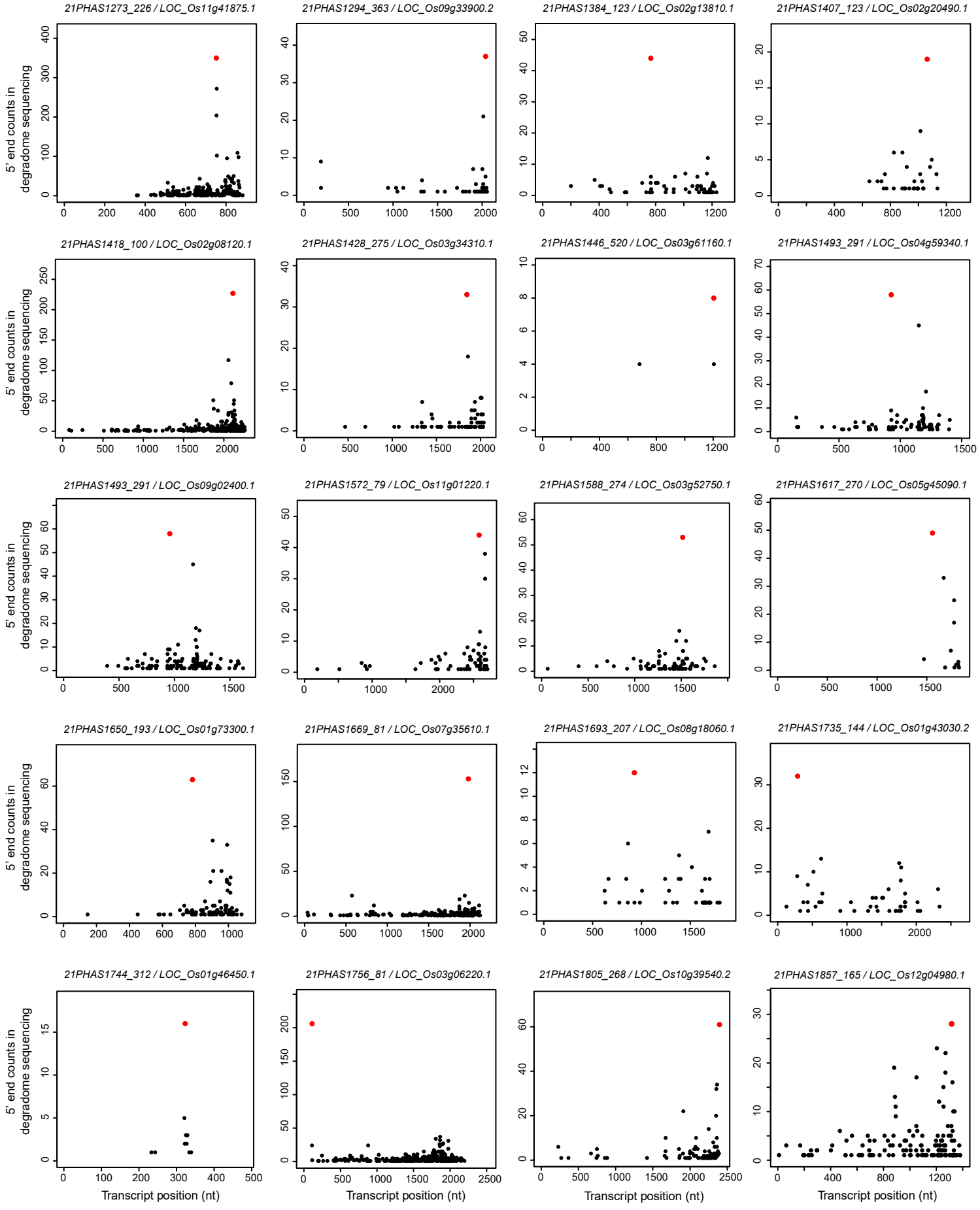
5' end counts in degradome sequencing
Transcript position (nt)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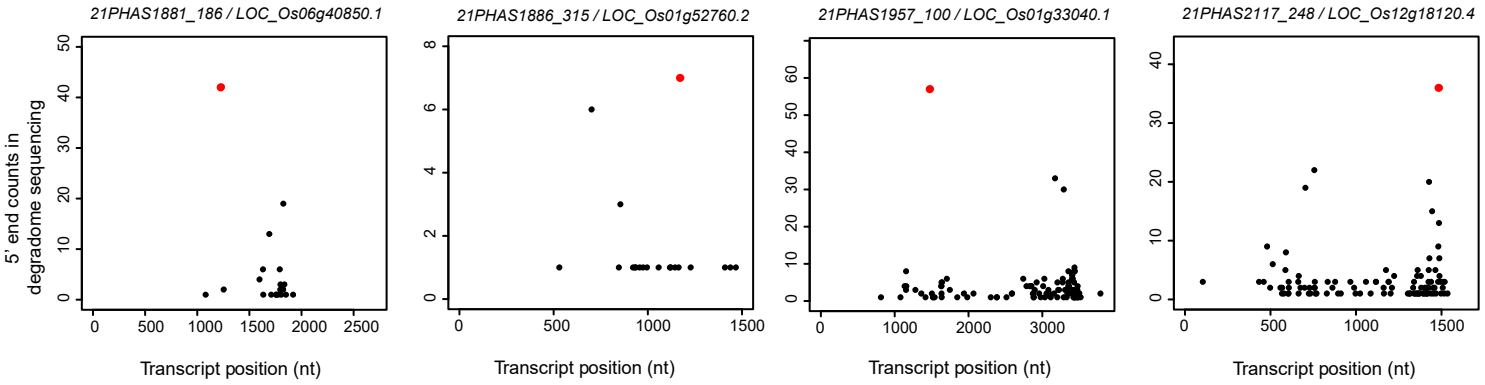
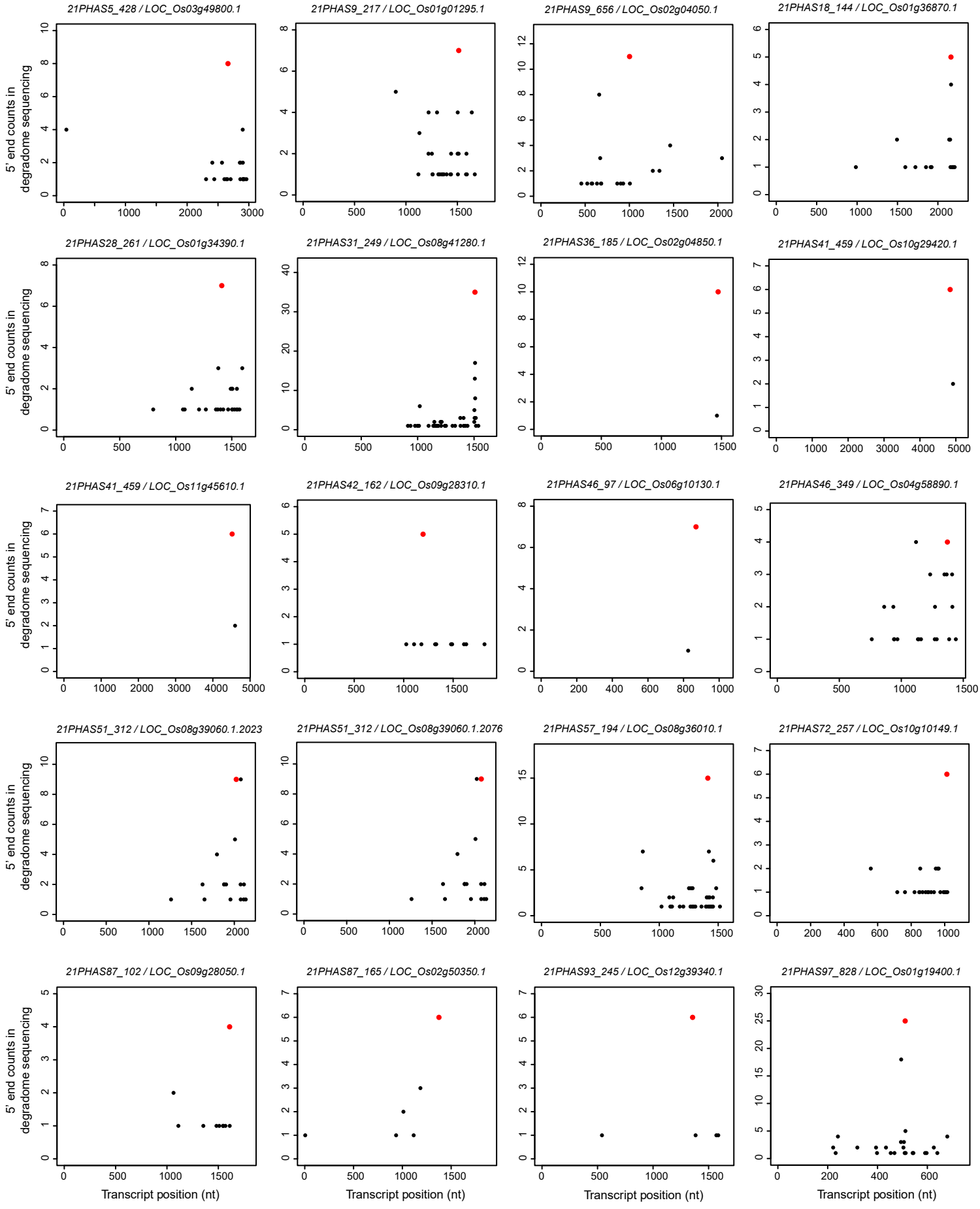
5' end counts in degradome sequencing
Transcript position (nt)



Supplementary Fig. 7 Validation of 21-nt phasiRNA targets in tetrads by degradome sequencing. Plots showing the distribution of the degradome tags along 21-nt phasiRNA targets. The x-axis represents the nucleotide position of each target mRNA (5'-3'). The y-axis represents the relative frequency of degradome tags. The red dot represents degradome tag at +1 position of 21-nt phasiRNA-guided cleavage site.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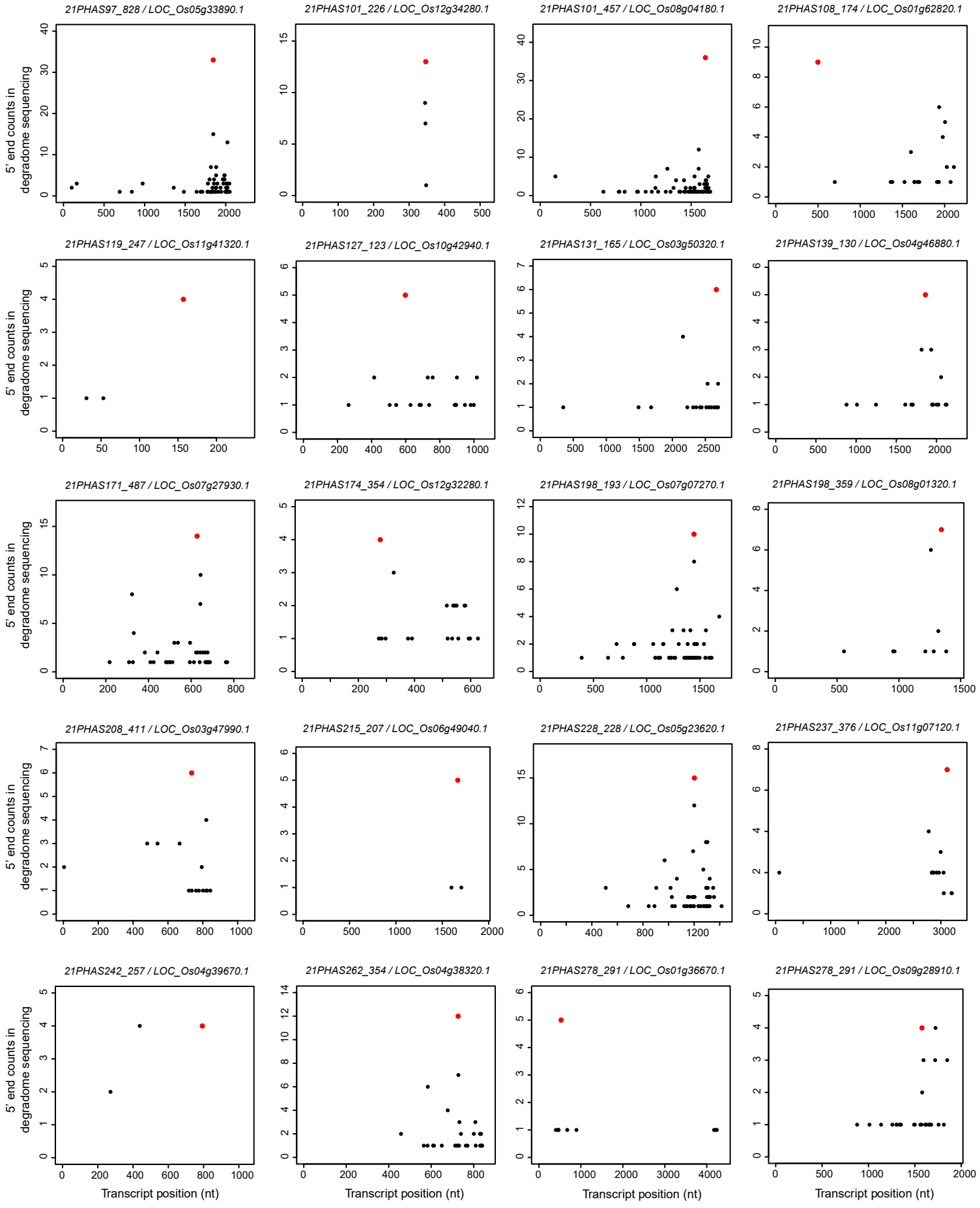
Transcript position (nt)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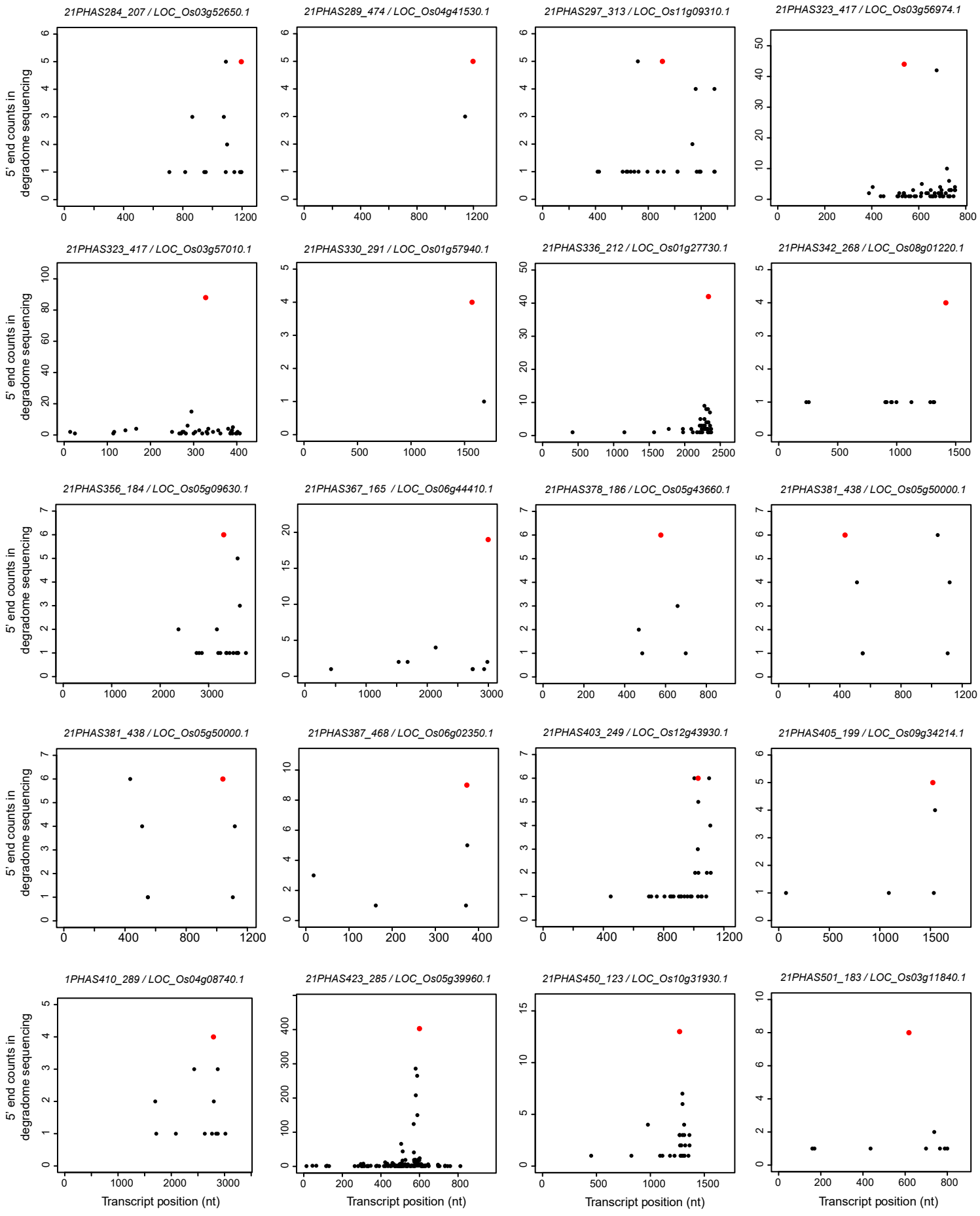
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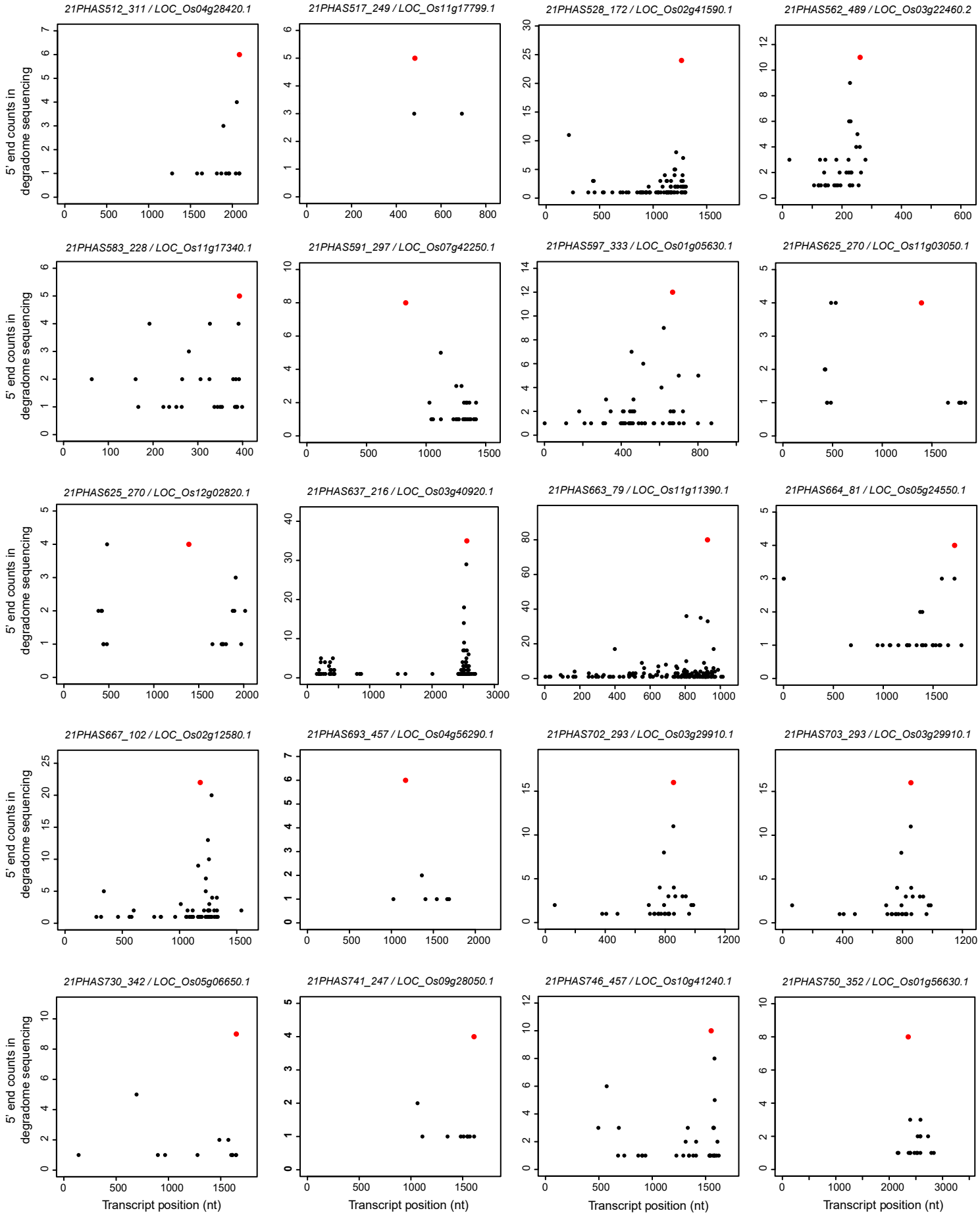
5' end counts in degradome sequencing
Transcript position (nt)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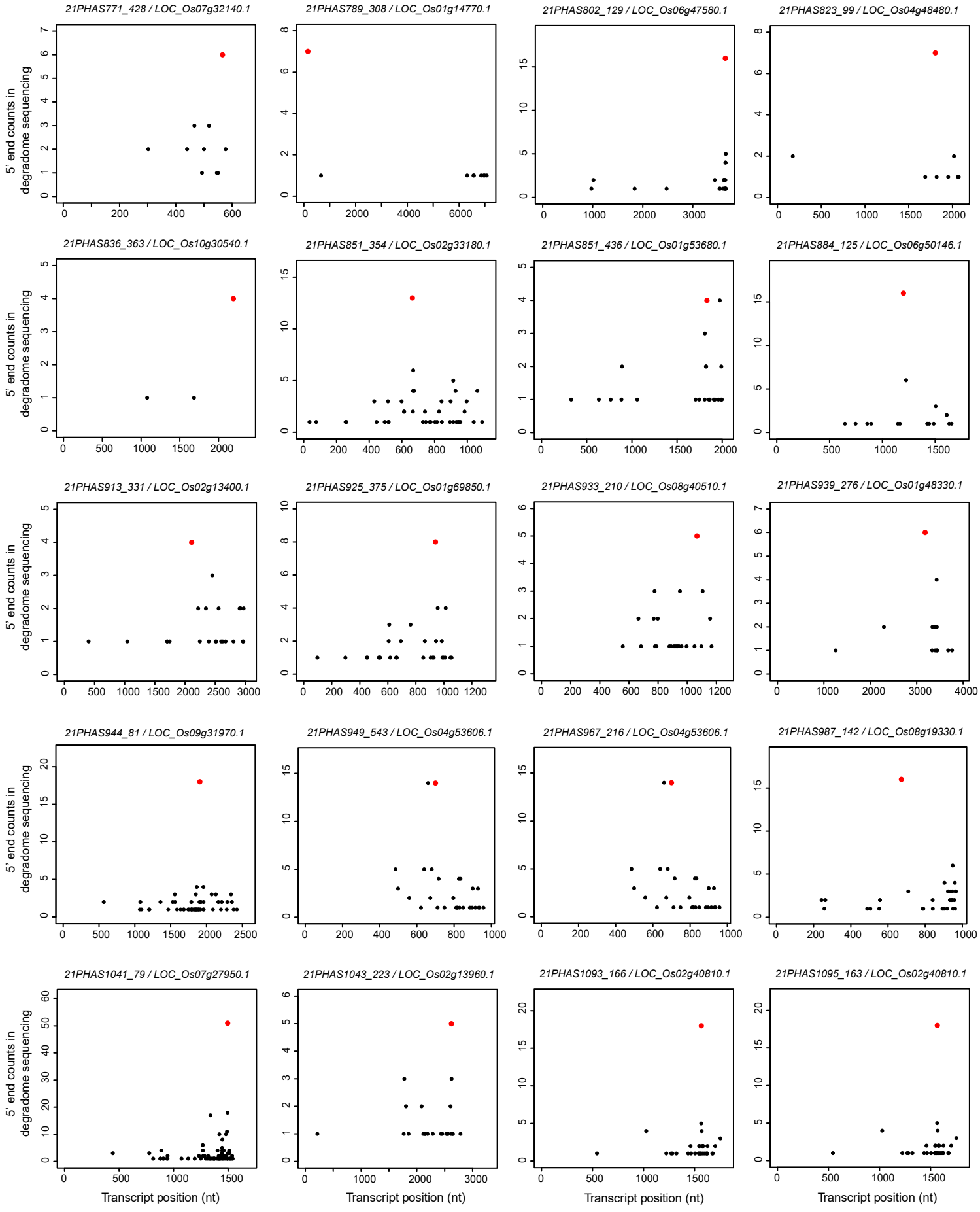
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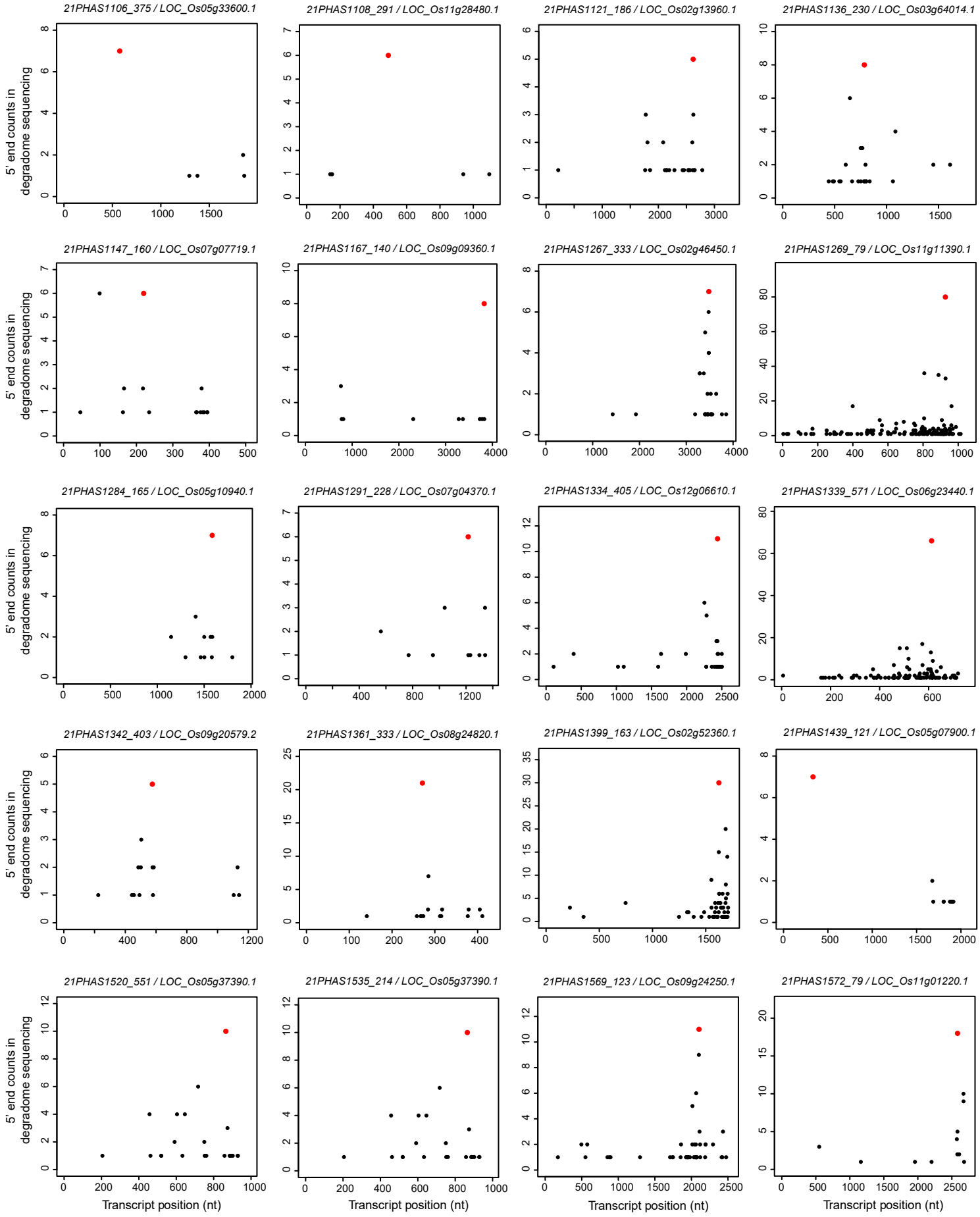
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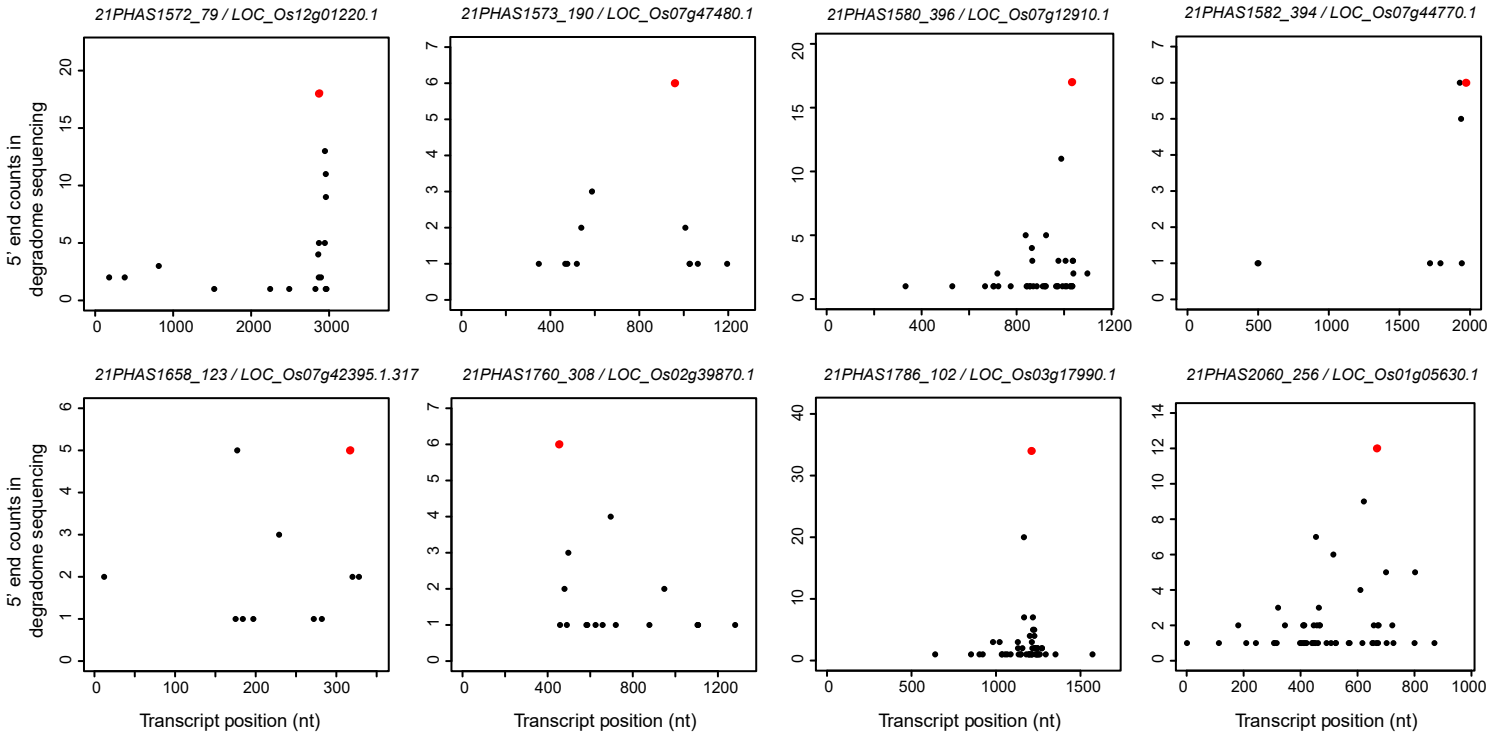
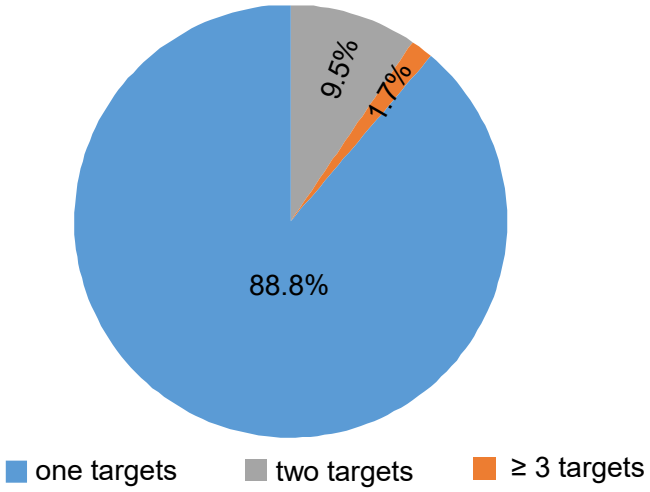
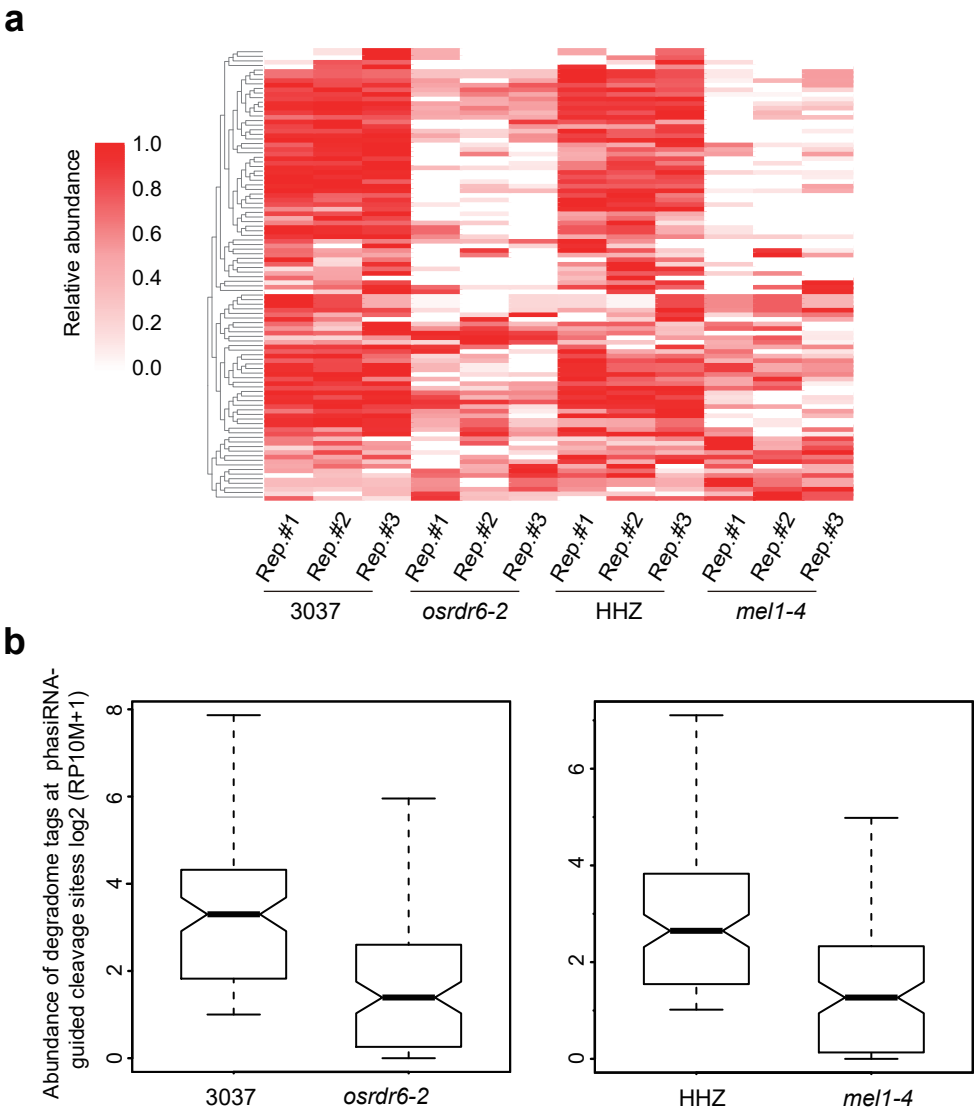
Transcript position (nt)



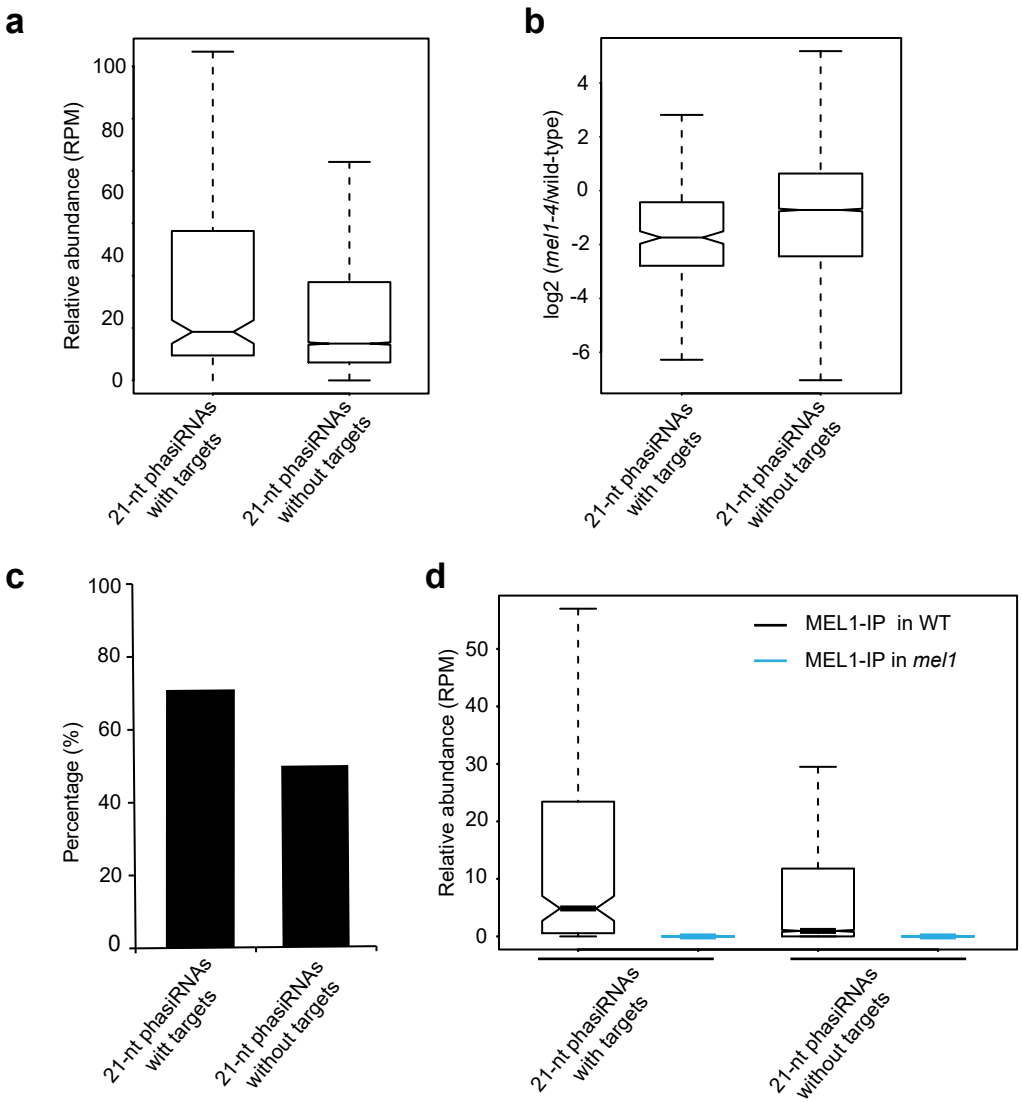
Supplementary Fig. 8 Validation of 21-nt phasiRNA targets in microspores by degradome sequencing. Plots showing the distribution of the degradome tags along 21-nt phasiRNA targets. The x-axis represents the nucleotide position of each target mRNA (5'-3'). The y-axis represents the relative frequency of degradome tags. The red dot represents degradome tag at +1 position of 21-nt phasiRNA-guided cleavage site.



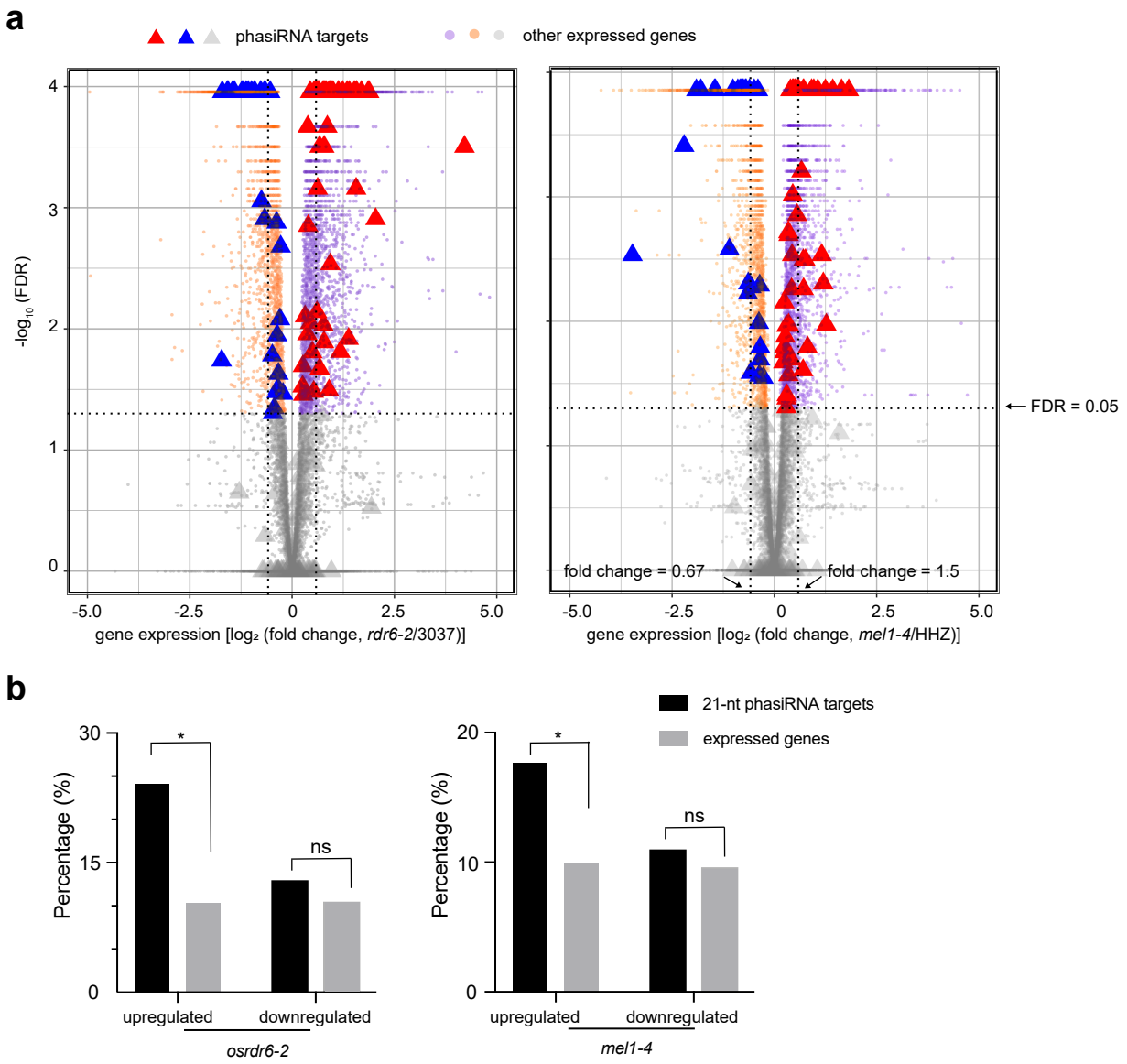
Supplementary Fig. 9. Analysis of *PHAS* loci with targets. Pie chart showing the percentages of *PHAS* loci that produce phasiRNAs targeting one, two and three or more genes, respectively.



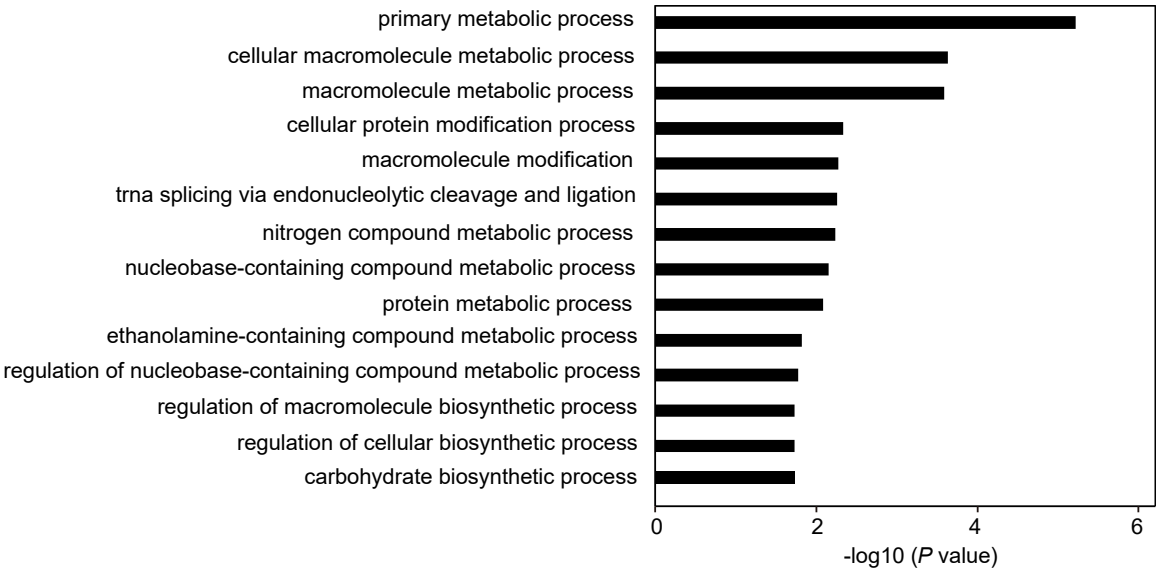
Supplementary Fig. 10. Relative abundances of degradome tags at phasiRNA-guided cleavage sites in 3037, *osrdr6-2*, HHZ and *mel1-4*. a, Heatmap showing the abundances of degradome tags at +1 positions of 21 -nt phasiRNA-guided cleavage sites in early prophase I meiocytes of *osrdr6-2*, *mel1-4* and their respective wild-type plants. Colors intensity represents the fractional density across the row of log2-transformed (RP10M+1) values. **b,** Box plots showing the log2-tranformed (RP10M+1) values in 3037, *osrdr6-2*, HHZ and *mel1-4*. The central line of the box represents the median while two bounds represent 25% quartile and 75% quartile, respectively. The whisker represents 1.5 × interquartile range of the lower or upper quartile.



Supplementary Fig. 11. Comparison of the stability of phasiRNAs with and without targets. a, Relative abundances of phasiRNAs with and without cleavage targets in Nipponbare rice plants as determined by sRNA-seq. **b,** Log2 (fold change) of phasiRNA abundance in *mel1-4* relative to its wild-type HHZ. **c,** Percentages of enriched phasiRNAs with and without cleavage targets in MEL1-IP sRNA-seq library. **d,** Relative abundances of phasiRNAs with and without targets in MEL1-IP sRNA-seq library. The central line of the box represents the median while two bounds represent 25% quartile and 75% quartile, respectively. The whisker represents 1.5 × interquartile range of the lower or upper quartile.



Supplementary Fig. 12. Differential expression of phasiRNA targets in *osrdr6-2* and *mel1-4*. **a**, Volcano plots showing differential expression of phasiRNA targets and other expressed genes in *osrdr6-2* and *mel1-4*. **b**, Comparison of the percentages of significantly upregulated and downregulated targets in total targets and the percentages of significantly upregulated and downregulated genes in total expressed genes. Fisher's exact test, p-value ≤ 0.05



Supplementary Fig. 13. Gene ontology enrichment analysis of the 21-nt phasiRNA targets derepressed in early prophase I meiocytes in *osrdr6-2* or *mel1-4*. GO biological process terms significantly enriched in the targets derepressed in *osrdr6-2* or *mel1-4* are shown. The *P* values were calculated by Fisher's exact test.