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Supplemental information

Transcriptome-wide Cas13 guide RNA design for model organisms and viral RNA pathogens

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Figure S1

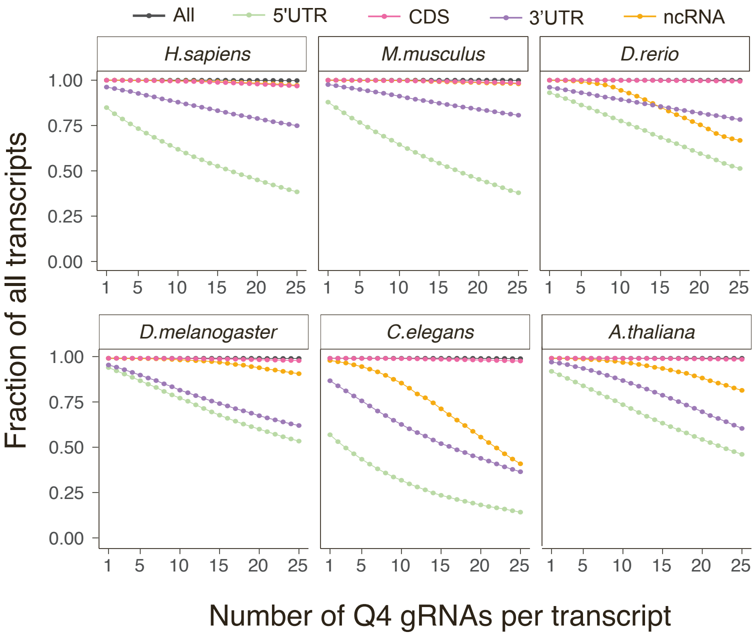

Figure S1. Reference transcripts with Q4 gRNAs. Related to Figure 1. The fraction of processed transcripts that contain at least 1 (up to 25) Q4 gRNAs (predicted high-scoring gRNAs) was calculated for each reference transcriptome.

Figure S2

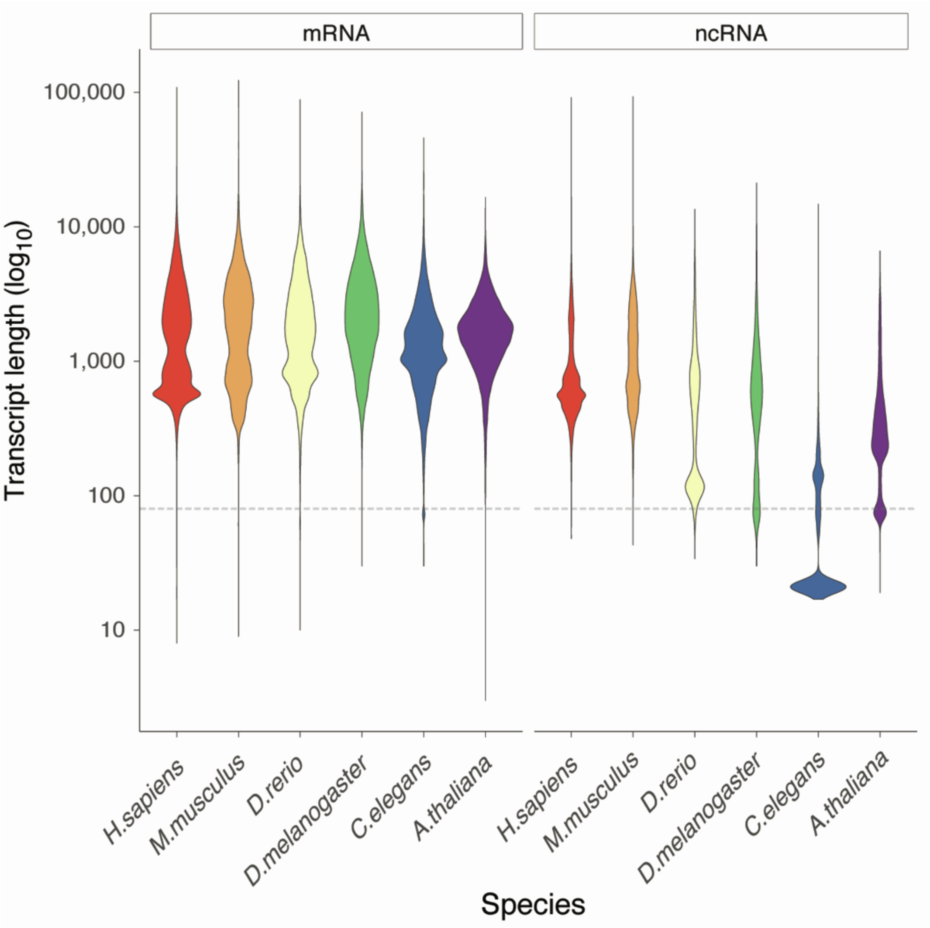

Figure S2. Transcript length for mRNAs and ncRNAs across species. Related to Figure 1.

Dotted line indicates the minimal input length requirements (> 80 bp) for Cas13d design software. Transcript lengths were derived from corresponding gene annotation references.

Figure S3

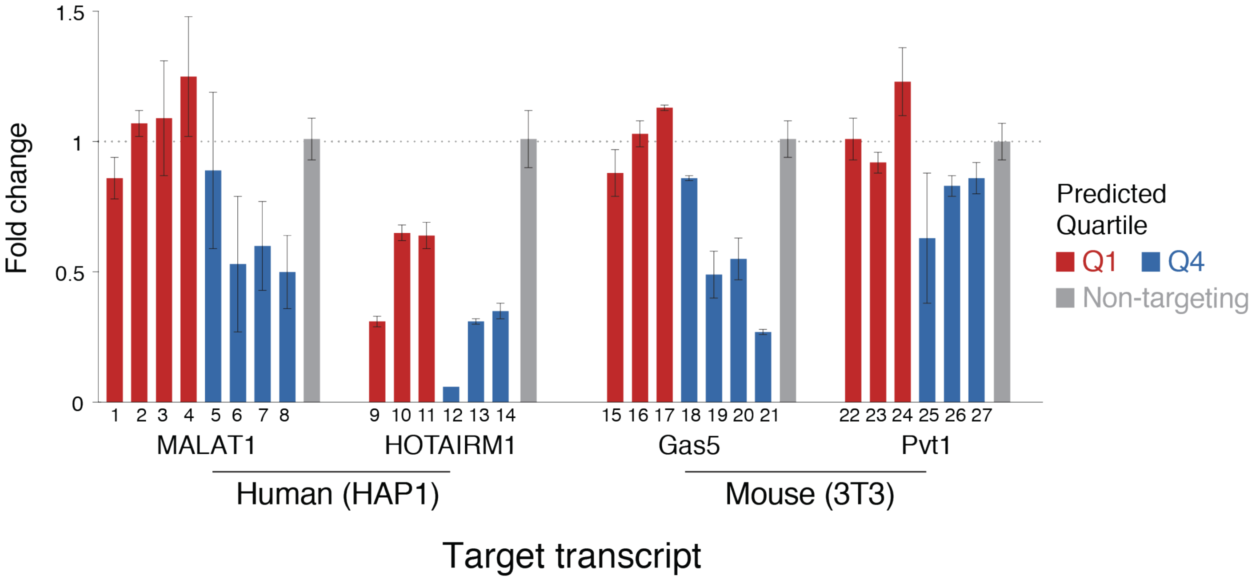

Figure S3. Knockdown by individual Cas13 guide RNA for long non-coding RNAs (lncRNAs). Related to Figure 1. For human and mouse lncRNA targets, we measured knockdown using at least 3 Q4 or Q1 gRNAs via lentiviral transduction and then, after 72 hours, quantitative PCR. Each column shown is an individual Cas13 guide RNA. Mean ± s.e.m, *n* = 3 transduction replicates.

Figure S4

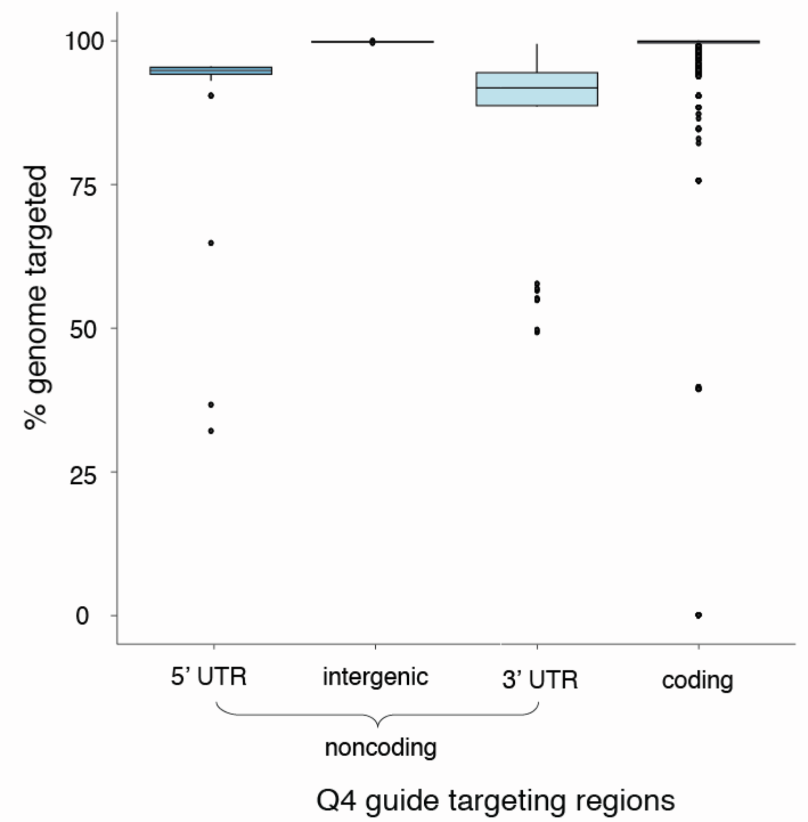

Figure S4. Q4 gRNAs targeting coding SARS-CoV-2 regions verses noncoding SARS-CoV-2 regions. Related to Figure 2. Classification of coding vs. noncoding regions was based on NCBI annotation of SARS-CoV-2 reference strain.

Table S1. Comparison of Cas13 gRNA design webtools. Related to STAR Methods.

	Cas13design	**CHOPCHOP v3**	**CRISPR-RT**
Cas13 variant	*Rfx*Cas13d	C2c2/Cas13a	C2c2/Cas13a
On-target scores	Yes	No	No
Reference target transcript input	Can search with gene symbol, transcript ID, gene ID, or transcript type	Can search with gene symbol and transcript ID	FASTA only
Isoform usage	Per transcript	Transcript or consensus	Per transcript
Output gRNA display	Interactive distribution of all gRNAs across the input transcript	Scrollable gene plot with all isoforms and a few gRNAs, plus link to UCSC genome browser	Graphical display is available per gRNAs
Organisms	6 model organisms and 4 RNA viruses	2 model organisms	26 organisms
Off-target scores	Count per guide for up to 2 mismatches	Count per guide for up to 3 mismatches	Count per guide for perfect match
High-throughput design	Command-line tool available	Command-line tool available	Not available
Website	https://cas13design.nygenome.org/	https://chopchop.cbu.uib.no/	http://bioinfolab.miamioh.edu/CRISPR-RT/interface/C2c2.php

Table S2. lncRNA-targeting gRNA sequences and predicted scores. Related to Figure 1.

Species	Target gene		gRNA sequence	Score	Quartile
Human	*MALAT1*	1	CCGGGCTTCTGCGTTGCTAAAAT	0.15249	1
Human	*MALAT1*	2	GGGCCGTTGGCTGCCAGTGGCCC	0.15034	1
Human	*MALAT1*	3	GTTCAGGGCTTTACTTTCCATTA	0.16100	1
Human	*MALAT1*	4	AGTTCTTCGGTTAAAAATAGGTT	0.16123	1
Human	*MALAT1*	5	GACAGCTAAGATAGCAGCACAAC	0.95725	4
Human	*MALAT1*	6	GAAAACGCCTCAATCCCACACCA	0.82996	4
Human	*MALAT1*	7	TTAGAAACGTGAAAACCCACTCT	0.79093	4
Human	*MALAT1*	8	GATTAAAGTGTGATAGTTCAGGG	0.80126	4
Human	*HOTAIRM1*	9	AGGGCTGCGCAGCTCCTGGATGC	0.16104	1
Human	*HOTAIRM1*	10	ACTAAGTTATCAACAATTCATTT	0.15709	1
Human	*HOTAIRM1*	11	ATGAGTAACACGGAGTTTCTTTA	0.16042	1
Human	*HOTAIRM1*	12	AAAGAGATCAATTGCAGGAACAC	0.93766	4
Human	*HOTAIRM1*	13	AGGGCTGCGCAGCTCCTGGATGC	0.95672	4
Human	*HOTAIRM1*	14	ACAGTCTAAGATTTGGGCCAAGC	0.90240	4
Mouse	*Gas5*	15	CCGGGCCGCGTCTGGGTTGCAGC	0.16049	1
Mouse	*Gas5*	16	AATACACTTTAATGGTAAAATTT	0.15376	1
Mouse	*Gas5*	17	TCATGTTATAATACACTTTAATG	0.15805	1
Mouse	*Gas5*	18	CCCTCCCAGCACTCGGCGACCTT	0.78533	4
Mouse	*Gas5*	19	TTTCAAACTGTCTATCCAGCCTC	0.72260	4
Mouse	*Gas5*	20	TCAGAAACAAAGGTGCAGTCACT	0.90981	4
Mouse	*Gas5*	21	AACACAATATATCTGACACCATC	0.87841	4
Mouse	*Pvt1*	22	TTACTTGGCATCTCTTAAGTCAA	0.15917	1
Mouse	*Pvt1*	23	CATAGGCTCCATCCTTAATGTGC	0.15735	1
Mouse	*Pvt1*	24	CGTAGGGCCCACATTCAATGTAT	0.15424	1
Mouse	*Pvt1*	25	TATCCACCAGAATTACTCCCCAG	0.84689	4
Mouse	*Pvt1*	26	AGCACTTGTAACTTCCAAGCCAT	0.96328	4
Mouse	*Pvt1*	27	TCAGAAAGTCAAAATGTCCAAGG	0.93282	4
-	Non-targeting		TTCTTCGGCTTAGACCAGTGCGG	N/A	N/A

Table S3. qPCR primers for gene expression quantification. Related to Figure 1.

Species	Target gene	Primer type	Primer sequence (5' to 3')
Human	*MALAT1*	Forward	GCTCTGTGGTGTGGGATTGA
Human	*MALAT1*	Reverse	GTGGCAAAATGGCGGACTTT
Human	*HOTAIRM1*	Forward	TTAAATCAACCCGCCCCACA
Human	*HOTAIRM1*	Reverse	TCAACCCCCTCCCCCATAAA
Human	*GAPDH*	Forward	ACAACTTTGGTATCGTGGAAGG
Human	*GAPDH*	Reverse	TATTTGGCAGGTTTTTCTAGACG
Mouse	*Gas5*	Forward	AGCTGGATAACAGAGCGAGC
Mouse	*Gas5*	Reverse	GCAGTGCCTTCACTTGAGGT
Mouse	*Pvt1*	Forward	GCTGGATCCACCCTCTTGTC
Mouse	*Pvt1*	Reverse	CCAGGCAAGCCAGGTGTAAT
Mouse	*Gapdh*	Forward	GGAGAGTGTTTCCTCGTCCC
Mouse	*Gapdh*	Reverse	ATGAAGGGGTCGTTGATGGC