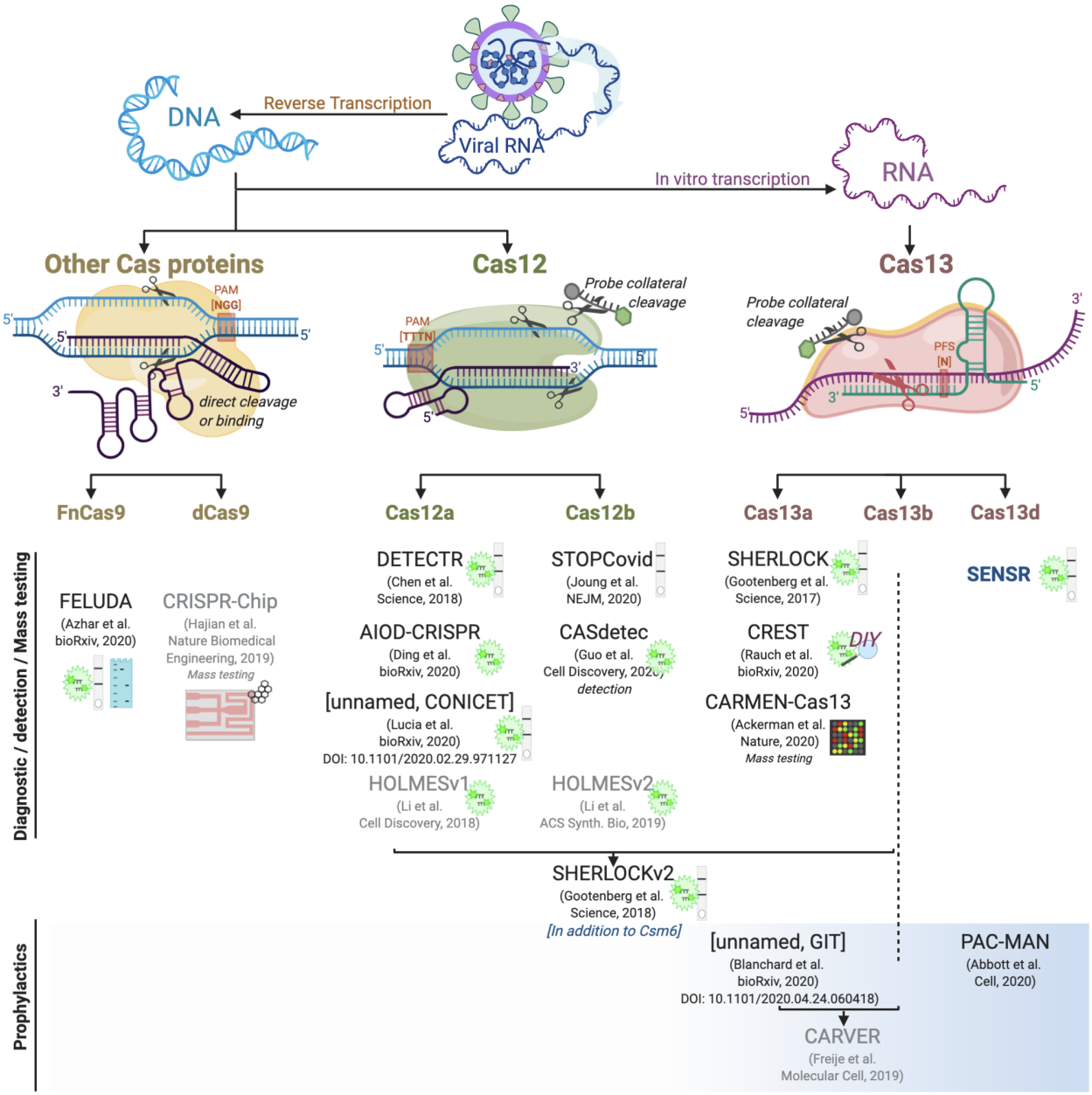
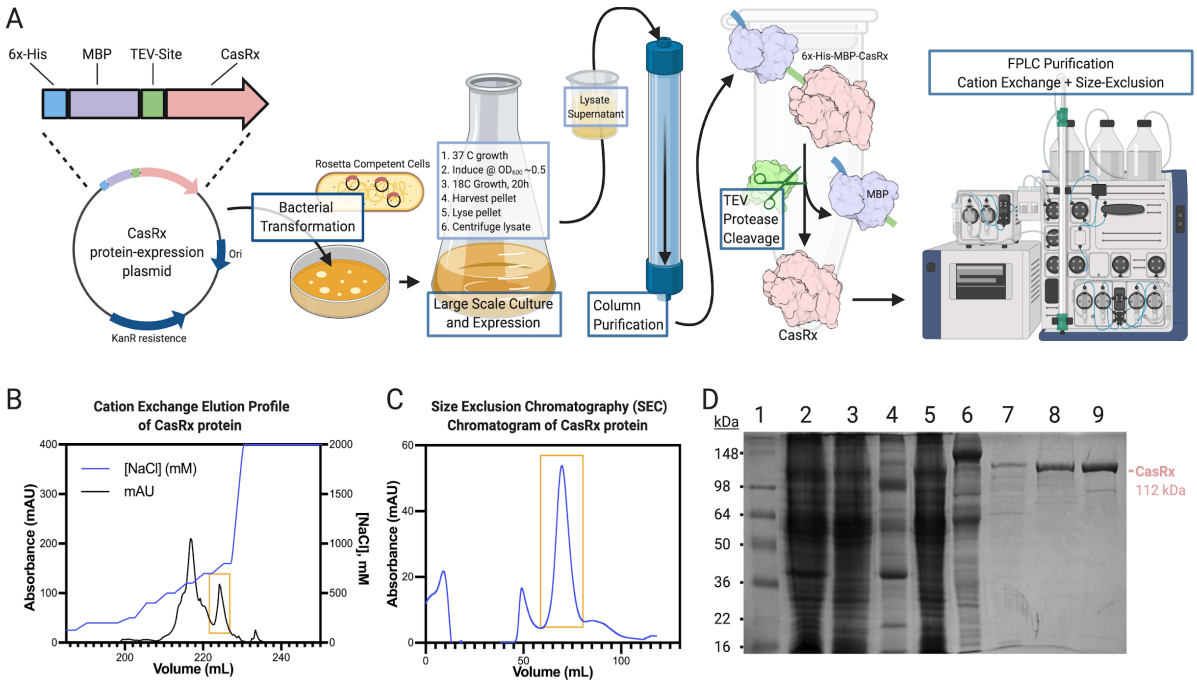
SUPPLEMENTARY FIGURES

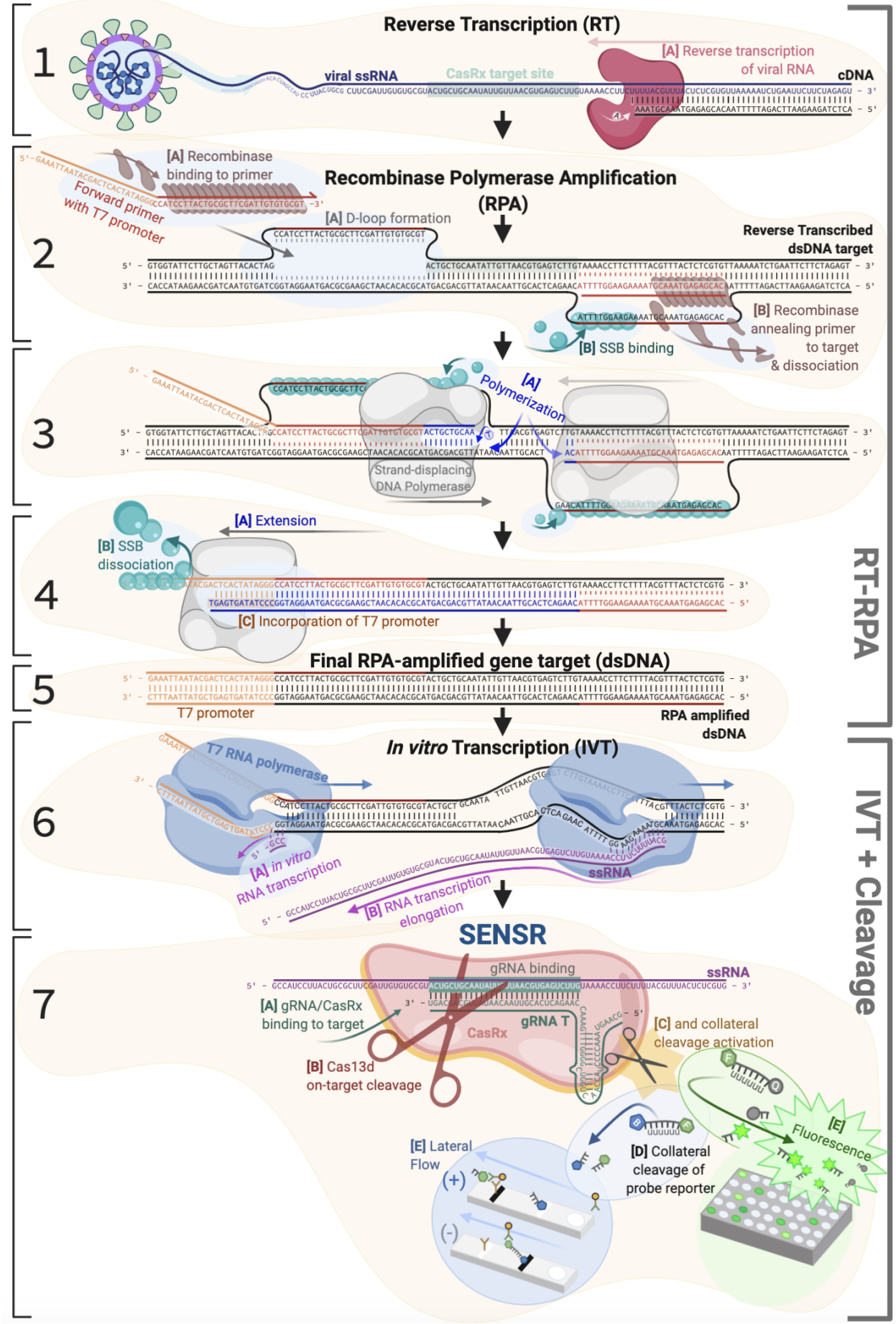


Supplementary Figure 1. Summary of current CRISPR-based anti-Covid technologies organized by Cas enzyme used and by role as diagnostic or detection tool, or as a putative prophylactic. Those technologies shown in black font have been demonstrated to have explicit activity against SARS-CoV-2, while those technologies shown in grey font have been publicly discussed or proposed to be technological candidates for SARS-CoV-2 detection or prophylaxis, however have not yet been fully demonstrated/optimized for said purpose as of the publication of this manuscript. Some technologies have not yet been given a formal name by their authors, and are therefore denoted as 'unnamed' followed by the acronym for the primary institutional affiliation behind the work. To better identify and distinguish these technologies, the DOI has been provided. **[Diagnostics/detection systems/Mass testing]** For all technologies viral RNA is extracted, reverse transcribed into cDNA, followed by template amplification by

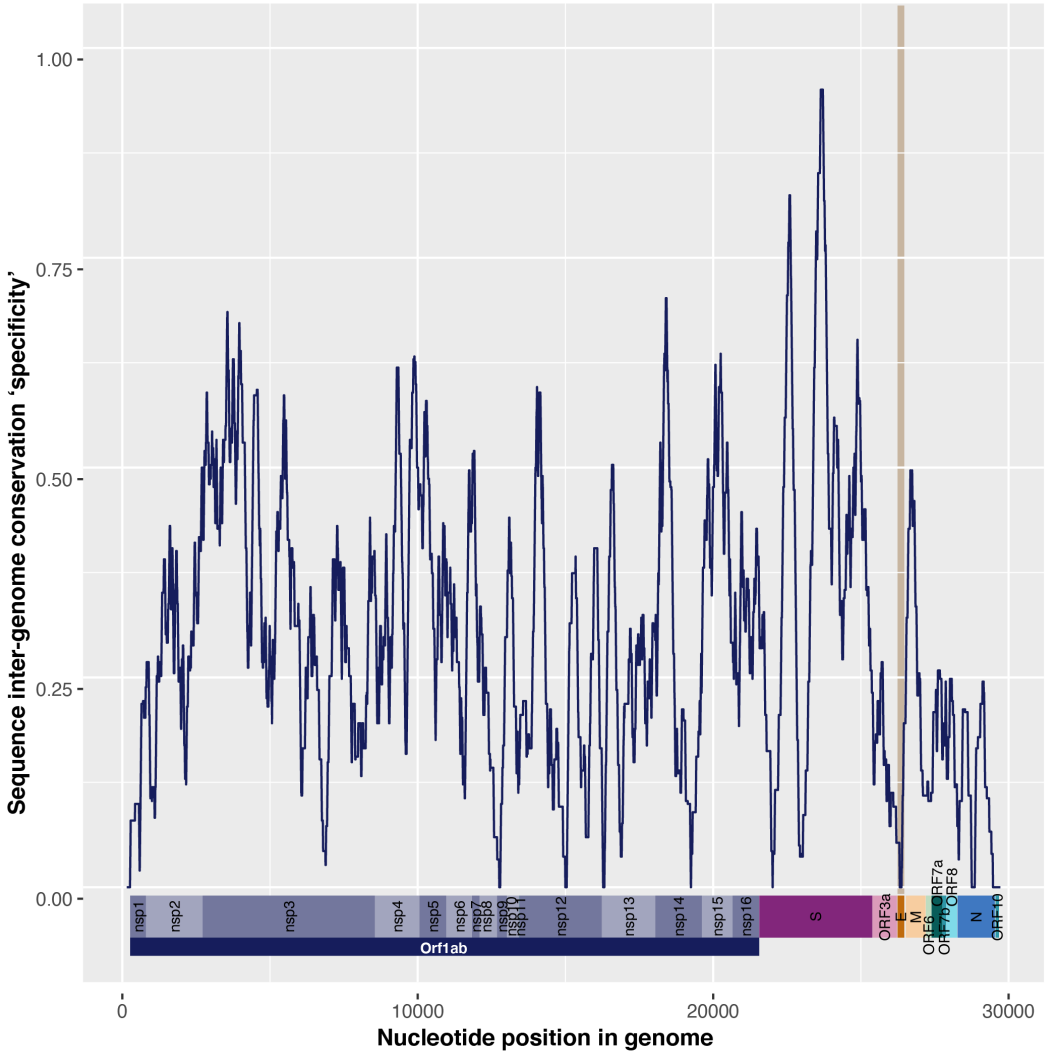
either PCR, RPA or LAMP, then input into subsequent reactions with the exception of CRISPR-Chip which does not require an amplification step. Cas12-based enzymes, as well as many other Cas proteins (including Cas9) recognize DNA species, while Cas13-based enzymes recognize ssRNA, and all can be used to detect evidence of specific sequences by fluorescence or lateral flow. The detection method for each technology is noted with the presence of an icon adjacent. The majority of the technologies summarized here use fluorescence or lateral flow or both. The lime glow icon indicates readout by fluorescence, while the grey bar indicates read-out by lateral flow. Some of the other technologies can be read out by different detection methods. Notably because FELUDA relies on direct sequence cleavage, and not the collateral cleavage property shared amongst the Cas12- and Cas13-based technologies, it can also be analyzed by gel electrophoresis as evidence of distinct band cleavage (aqua gel icon). Also, CRISPR-Chip has been discussed as a SARS-CoV-2 mass detection candidate, though these findings have not yet been made publicly available. Read-out by this technology is achieved via a graphene-based transistor (icon is red, grey with graphene structure adjacent). Other different technologies include CREST, which achieves detection via fluorescence using a distinctly do-it-yourself (DIY) BioHacking approach, by utilizing equipment easily procured and operated by novice scientists, as well as CARMEN-Cas13 which detects evidence of SARS-CoV-2 sequences by microarray. The technology outlined here is an addition to current SHERLOCK detection utilizing Cas13d (CasRx). The technical details of most technologies is summarized in Table S1.



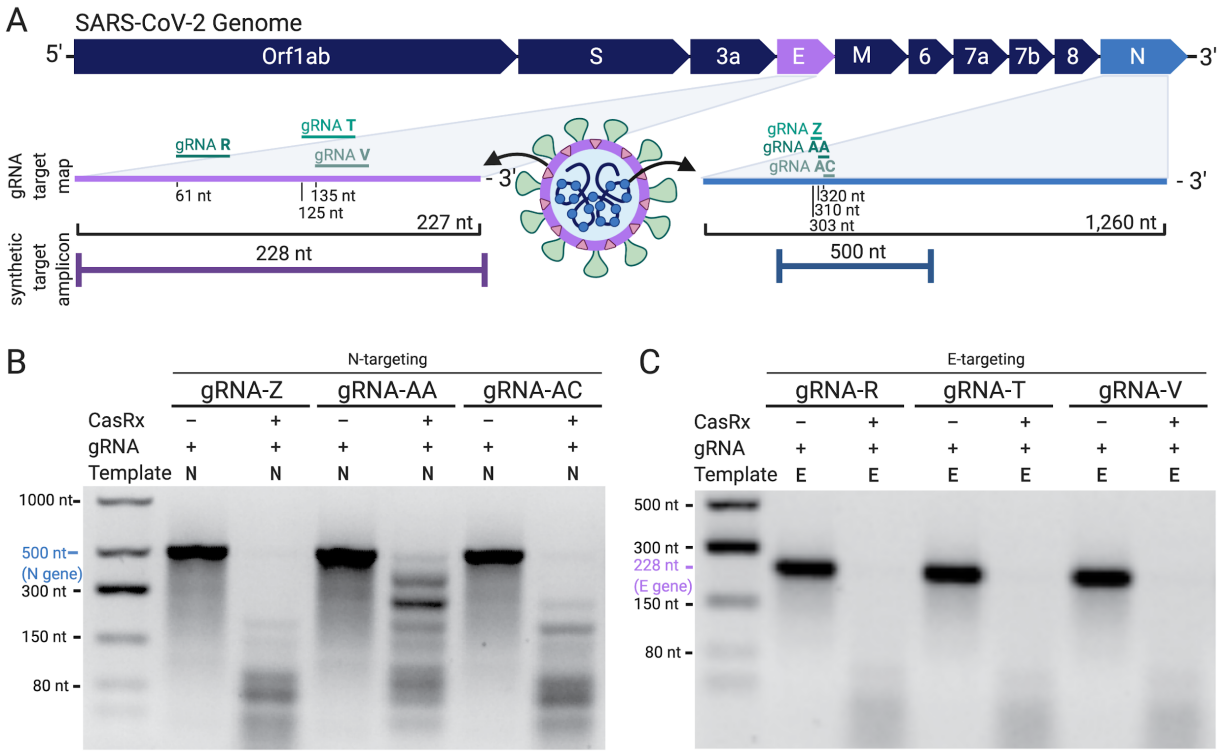
Supplementary Figure 2. CasRx protein purification workflow and quality control. [A] CasRx protein was produced and purified essentially as described in (Konermann et al. 2018). A CasRx protein-expression plasmid was generated with CasRx (pink) downstream of a Maltose-binding protein (MBP, purple) domain with an N-terminal 6xHis tag (blue), connected by a TEV protease cleavage sequence (green). Origin of replication and KanR cassette shown in navy. Transformation of the plasmid into Rosetta2™ (DE3) Competent *E. coli* was followed by large scale culture growth. Cell lysate supernatant containing 6xHis-MBP-CasRx soluble hybrid protein was run on a Ni-NTA column for purification by affinity chromatography. CasRx protein was then released from the 6xHis-MBP moiety by TEV protease cleavage during O/N dialysis, and was further purified by cation exchange and size exclusion using Fast Protein Liquid Chromatography (FPLC). **[B]** The cation exchange elution profile of CasRx with the concentration of NaCl (mM) shown in blue. Peak containing CasRx recombinant protein (boxed in gold) elutes at ~700 mM NaCl. **[C]** The SEC elution profile of CasRx recombinant protein following Size Exclusion Chromatography (SEC) (blue). Peak containing CasRx recombinant protein highlighted boxed in gold. **[D]** A 10% SDS-PAGE gel showing protein species present at different stages of the purification protocol. Lane 1 is SeeBlue™ Plus2 Pre-stained Protein Standard with the predicted final CasRx protein size marked at right in pink at approximately 112 kDa. Lane 2 is a resuspended cell pellet. Lane 3 is cell lysate supernatant. Lane 4 is cell lysate pellet. Lane 5 is Ni-NTA flow through. Lane 6 is Ni-NTA Elution. Lane 7 is the sample post O/N Dialysis. Lane 8 is the sample after IEC, and Lane 9 is the concentrated Final Sample post-SEC.



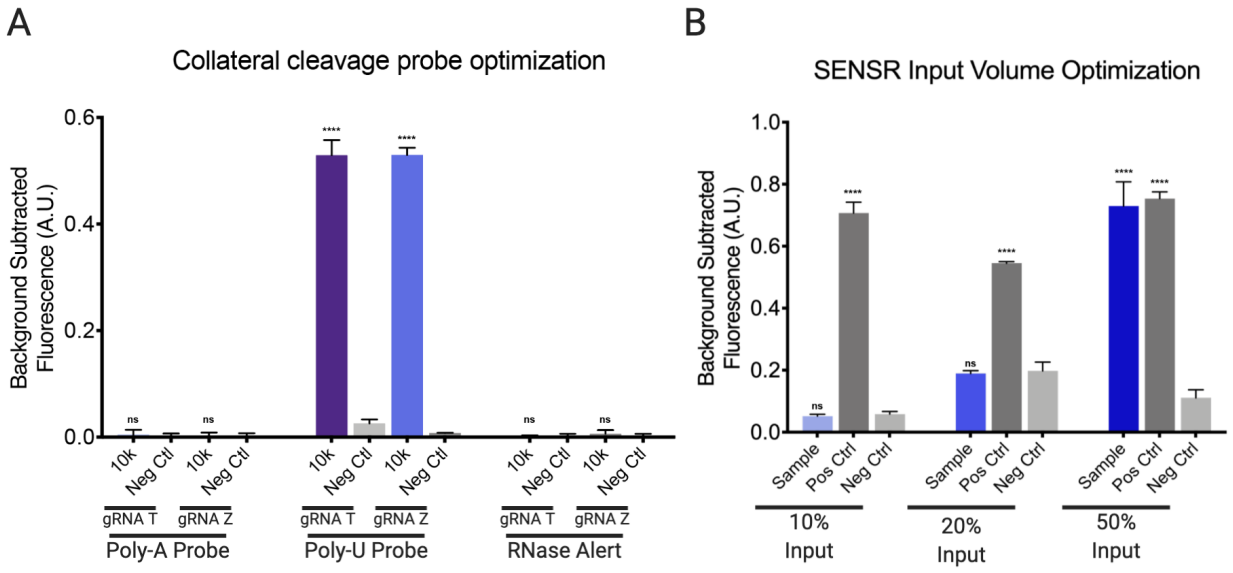
Supplementary Figure 3. Molecular scale overview of CasRx detection protocol workflow. Bracketed into the two base component reactions, RT-RPA or IVT + cleavage. [**Panel 1A**] Viral ssRNA is extracted and reverse transcribed into cDNA. A fragment of the viral E-gene ssRNA (navy), with the final CasRx target site highlighted (mint). Reverse transcriptase (raspberry) actively reverse transcribes the viral template into cDNA. [**Panel 2A**] In the simultaneous RPA reaction, recombinase (brown) binds to the primer (red and orange) with D-loops formed at binding sites. [**2B**] Recombinase helps anneal the primer to the target site, and single strand binding protein (SSB, teal) begins annealing to ssDNA to stabilize the strand and reaction. [**Panel 3A**] Strand-displacing DNA polymerase (grey) amplifies the target DNA, with continued binding of SSB to ssDNA for stabilization. [**Panel 4A**] DNA amplicon extension is completed, [**4B**] while polymerase simultaneously dislodges SSB, and [**4C**] the T7 promoter region (orange) is added to the amplicon via primer extension. [**Panel 5**] The final product of the RT-RPA reaction is a small amplified fragment of target DNA encompassing the CasRx target site, with a T7 promoter added for subsequent IVT. [**Panel 6A**] T7 RNA polymerase-based *in vitro* transcription (blue) occurs, initiated from the T7 promoter (orange) in order to generate ssRNA (magenta) required as the activation substrate of CasRx. [**6B**] Elongation of the ssRNA template takes place. **[Panel 7**] CasRx detection of on-target sequence, activation of collateral cleavage activity, and detection. [**7A**] The CasRx/gRNA complex (pink and green) recognize, [**7B**] bind to, and cleave (red scissors) the ssRNA viral target template (magenta). [**7C**] This action activates the collateral cleavage property of CasRx (gold, black scissors). [**7D**] Following activation, collateral cleavage of an included small ssRNA probe can be analyzed via [**7E**] fluorescence or lateral flow assay.



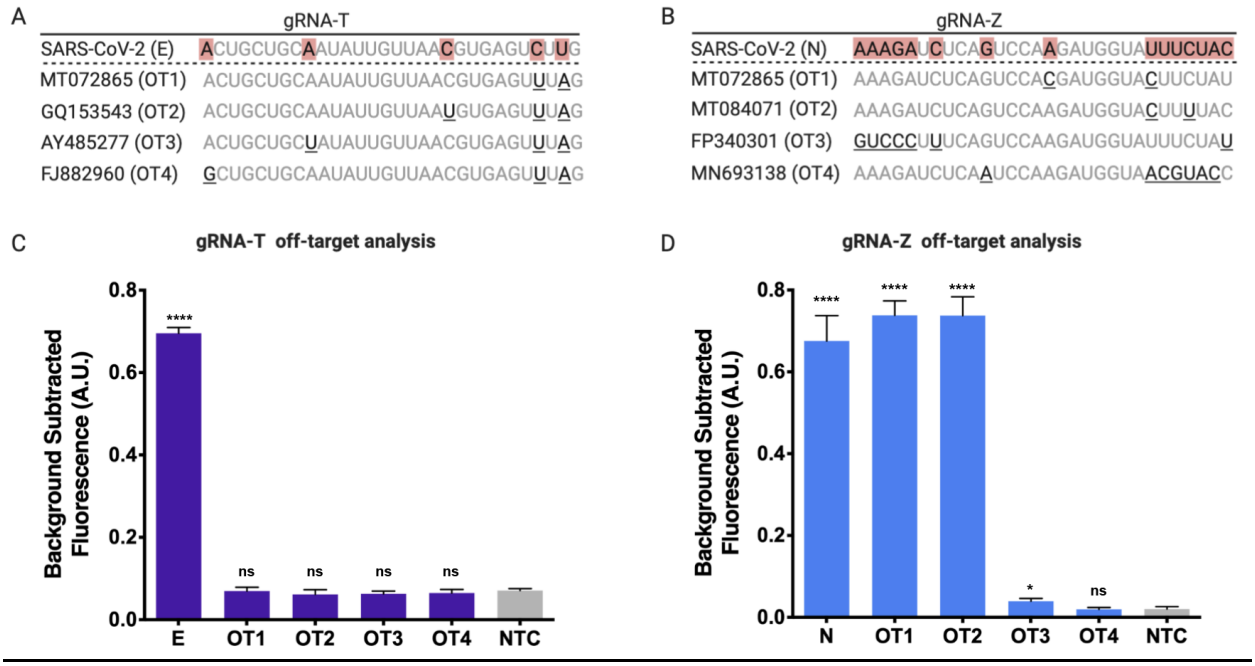
Supplementary Figure 4. Depiction of unique gRNA target sequences across SARS-CoV-2 genome. Spread of unique and specific 30 nt putative gRNA target sequences (Table S3-S5) displayed across the SARS-CoV-2 genome, smoothened over a 301 nt window. Higher specificity score indicates higher density of unique and conserved targets. Representative genome map shown at bottom with ORFs and genes annotated. E-gene ORF highlighted in orange with beige vertical bar highlighting the zero specificity score of the gene.



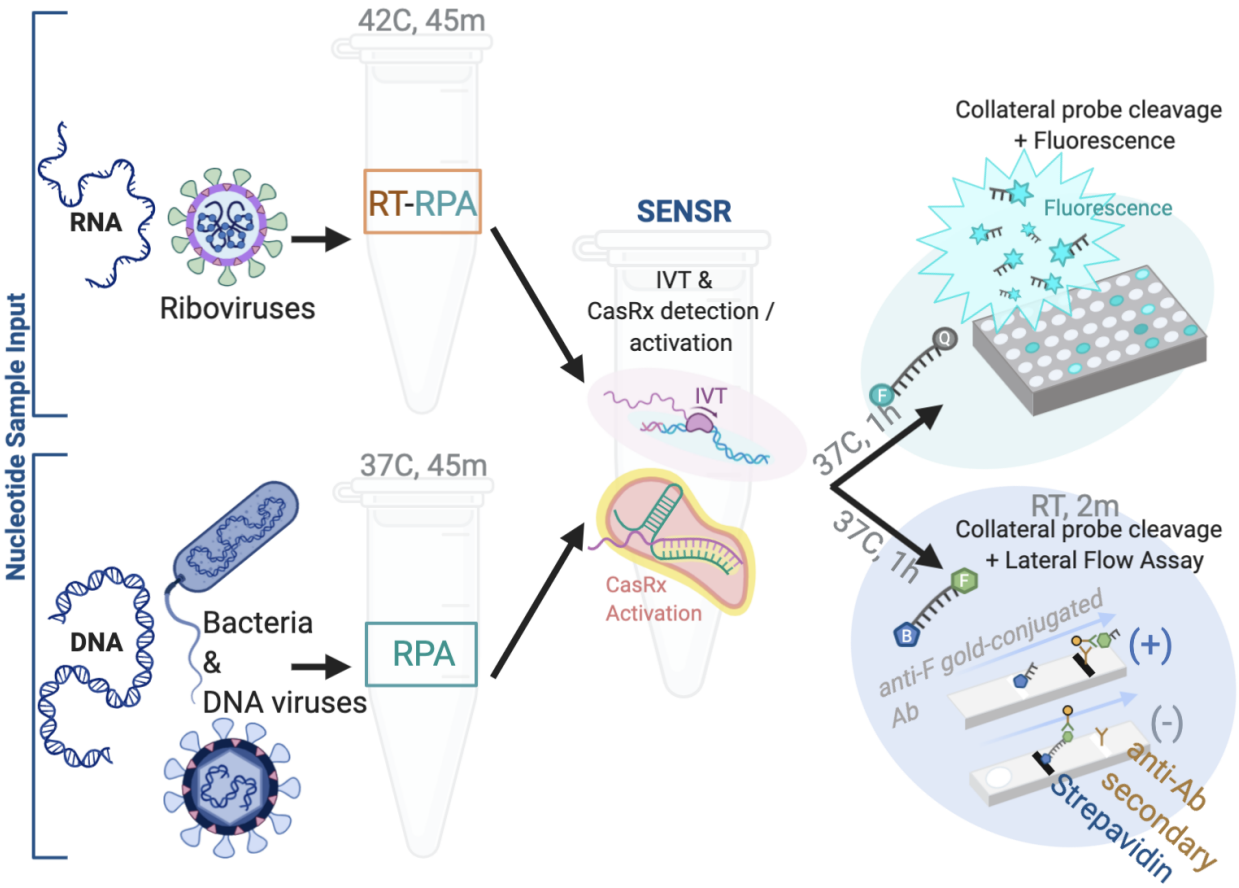
Supplementary Figure 5. Preliminary *in vitro* cleavage assays for guides tested throughout this work. **[A]** Summary map of gRNA target location within the SARS-CoV-2 genome. gRNA-R, -T, -V target the Envelope (E) gene, and gRNA-Z, -AA, -AC target the Nucleocapsid (N) gene. **[B]** *In vitro* on-target cleavage assays for the six guides developed in the first cohort (R, T, V). Equimolar amounts of N and E synthetic RNA fragments (500 nt and 228 nt respectively) were incubated at room temperature (RT) in the presence or absence of their on-target gRNA, named above. The on-target gene is denoted with (E) or (N) for each gRNA, and is shown mapped in **[A].** The presence of the gRNA initiates cleavage of both the on-target template in addition to collateral cleavage of the off-target template. **[C]** On-target *in vitro* cleavage assays for the conserved and specific N-targeting gRNA-Z, -AA, -AC. In each reaction the 500 nt N-gene synthetic RNA fragment is provided, and the presence or absence of CasRx initiates on-target cleavage of the template into smaller fragments.



Supplementary Figure 6. Optimizing SENSR Fluorescence detection assay. [A] Determining optimal sequence for collateral cleavage probe. Collateral cleavage of Poly-A probe is tested in the first group, Poly-U is tested in the second, and RNase Alert in the third group. Within each group, gRNA-T and gRNA-Z are incubated in a reaction saturated with 10,000 (10k) target copies, or 0 (Neg Ctrl) as a negative control. Performing a two-tailed t-test of unequal variance comparing each 10k group to the respective Neg Ctrl groups a significant increase in fluorescence was only found for the Poly-U probe 10k groups (gRNA-T 10k: $p<0.0001$, gRNA-Z 10k: $p<0.0001$). No significant increase in fluorescence was observed for the 10k Poly-A or 10k RNase Alert groups compared to the respective Neg Ctrl groups for gRNA-T (Poly-A: $p=0.5953$, RNase Alert: $p=0.4294$) or gRNA-Z (Poly-A: $p=0.7935$, RNase Alert: $p=0.1510$). Performing a one-way ANOVA followed by a Dunnett's test comparing the 10k groups of the Poly-A and RNase Alert groups to the 10k Poly-U groups a significant difference was found for both gRNA-T (Poly-A: $p<0.0001$, RNase Alert: $p<0.0001$) and gRNA-Z (Poly-A: $p<0.0001$, RNase Alert: $p<0.0001$). Bars indicate mean ± SD of background subtracted fluorescence from three technical replicates. **[B]** Determining the optimal input volume of RT-RPA sample into SENSR reaction. In all cases the trial sample is equivalent to 100 copies/μL (blue), the positive control is established with 10,000/μL (dark grey), and the negative control is no-template control (pale grey). In the left most grouping the RT-RPA template sample input into the SENSR reaction comprises 10% of the preamplification reaction, in the middle grouping it composes 20% of the preamplification reaction, and in the final grouping it comprises 50% of the preamplification reaction. Performing a one-way ANOVA followed by a Dunnett's test within each % input group significance was only found for both the sample ($p<0.0001$) and positive control ($p<0.0001$) in the 50% group (10%: Sample - $p= 0.8925$, Pos Ctrl - $p<0.0001$; 20%: Sample - $p=0.8160$, Pos Ctrl - $p<0.0001$). Performing a second one-way ANOVA followed by a Dunnett's test comparing the 50% sample group to the 10% and 20% sample groups, a significant increase in fluorescence was observed (10%: $p<0.0001$, 20%: $p<0.0001$). Bars indicate mean ± SD of background subtracted fluorescence from three technical replicates.



Supplementary Figure 7. Assessment of gRNA specificity within the SENSR system. Schematic representation of gRNA mismatches for the four most closely related sequences (off-targets, OT1-OT4) identified via BLAST for **[A]** gRNA-T and **[B]** gRNA-Z. **[C]** gRNA-T demonstrated no off-target activity on the 4 closely related sequences. Bars indicate mean ± SD of background subtracted fluorescence from three technical replicates. Performing a one-way ANOVA followed by a Dunnett's test comparing to the NTC fluorescence signal was found to be significant for the E-gene compared ($p<0.0001$), however no significant fluorescence signal increase was found for the four off-targets (OT1: $p=0.9998$, OT2: $p=0.5242$, OT3: $p=0.6633$, OT4: $p=0.8475$). Performing a one-way ANOVA followed by a Dunnett's test comparing to the E-gene positive control significant fluorescence signal increase was found when targeting the E-gene compared to the four off-targets (OT1, OT2, OT3, OT4: $p<0.0001$). **[D]** Collateral cleavage was observed for two of the closely related off-targets for gRNA-Z . Bars indicate mean ± SD of background subtracted fluorescence from three technical replicates. Performing a one-way ANOVA followed by a Dunnett's test comparing to the NTC fluorescence signal increase was found to be significant for the N-gene positive control, OT1, and OT2 (N, OT1, OT2: $p<0.0001$), however no significant fluorescence signal increase was found for OT3 and OT4 (OT3: $p=0.8877$, OT4: $p>0.9999$). Performing a one-way ANOVA followed by a Dunnett's test comparing to the N-gene positive control, significant fluorescence signal increase was found ~~to~~ when targeting the N-gene compared to OT3 and OT4 (OT3, OT4: $p<0.0001$), but no significant difference was found targeting the N-gene compared to OT1 and OT2 (OT1: $p=0.0786$, OT2: $p=0.0833$).



Supplementary Figure 8. In addition to RNA-based templates (top row), SENSR technology could also be used for DNA-based templates, including, but not limited to, bacterial samples (shown) as well as DNA viruses such as herpes (shown, bottom, navy). DNA-based sample templates can be input directly into the RPA amplification reaction, negating the need for a simultaneous reverse transcription (RT) reaction as is required for RNA-based templates. Like SENSR on RNA-based templates, the reaction occurs in two steps. The first amplification step, RT-RPA or RPA, is followed by the SENSR reaction which is identical regardless of initial input molecular species. This reaction is composed of simultaneous *in vitro* transcription (IVT) as well as CasRx cleavage and activation of collateral cleavage activity. The assay can be read out by fluorescence or lateral flow in the same fashion regardless of DNA or RNA sample input.

----------SUPPLEMENTARY TABLES----------

Supplementary Tables

Table S1. Summary Table of current, and possible future, CRISPR-based anti-Covid technologies.

Table S2. Analysis identifying putative 30nt gRNA target sites conserved across, and specific to the SARS-CoV-2 genome.

Table S3. Predicted unique and conserved 30nt CasRx gRNA target sequences to SARS-CoV-2.

Table S4. Analysis of Inter-SARS-CoV-2 Conservation (433 genomes) and Pan-coronavirus Specificity (3164 genomes) on the three E-targeting gRNAs (R,T,V).

Table S5. List and sequences of reagents generated and used throughout this work. Primers for cloning, gRNA prep, and RT-RPA, as well as gRNA sequences, viral gene templates, plasmid sequences and probes.

Table S6. Top four naturally-occurring off-target sequences for gRNA T and gRNA Z.

Table S7. Data from RT-qPCR and SENSR fluorescence analysis of patient samples for detection of SARS-CoV-2.