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## Supplemental information

# Engineering highly thermostable Cas12b via *de novo* structural analyses for one-pot detection of nucleic acids

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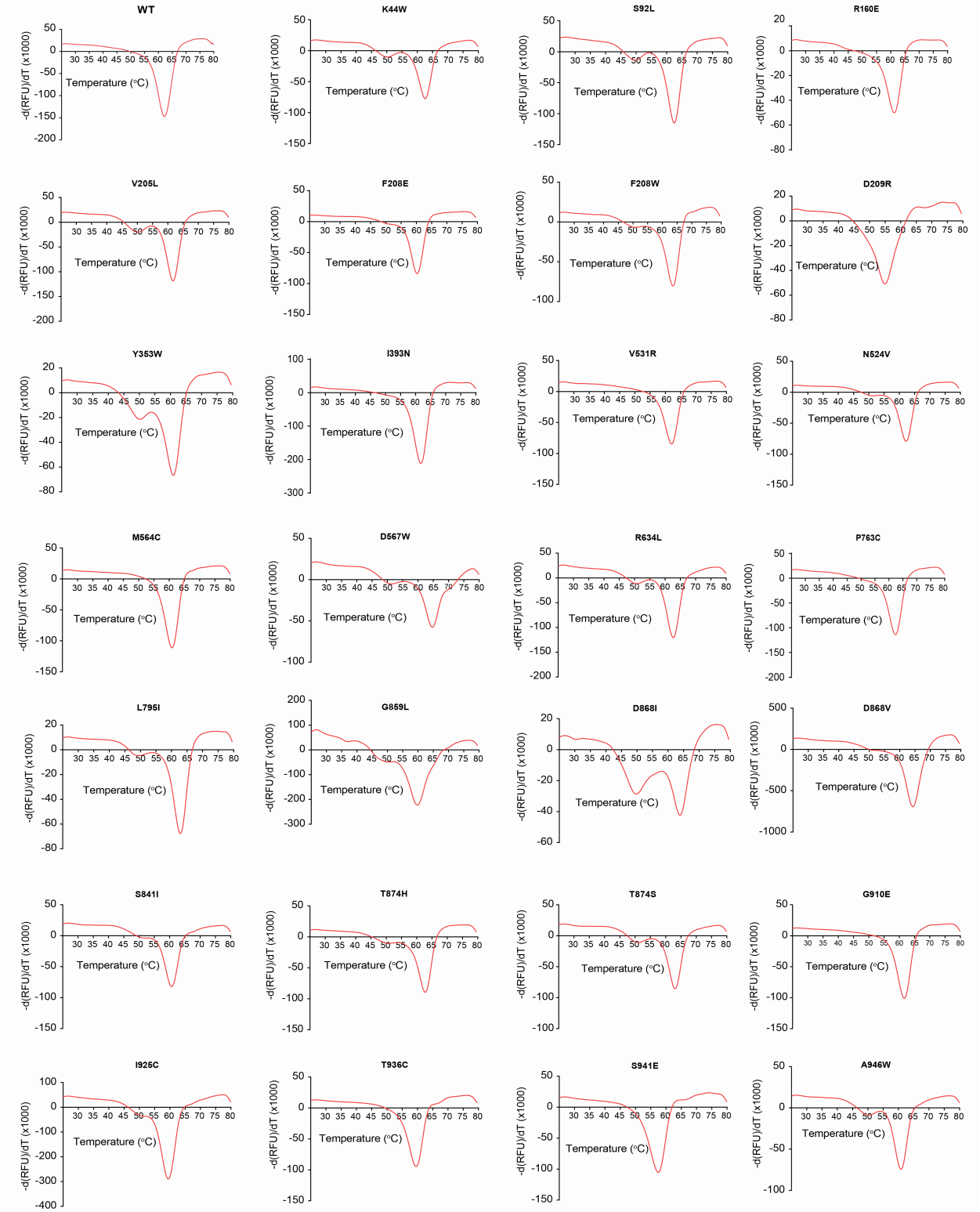

WT
K44W
S92L
R160E
V205L
F208E
F208W
D209R
Y353W
I393N
V531R
N524V
M564C
D567W
R634L
P763C
L795I
G859L
D868I
D868V
S841I
T874H
T874S
G910E
I925C
T936C
S941E
A946W
-d(RFU)/dT (x1000)
Temperature (°C)

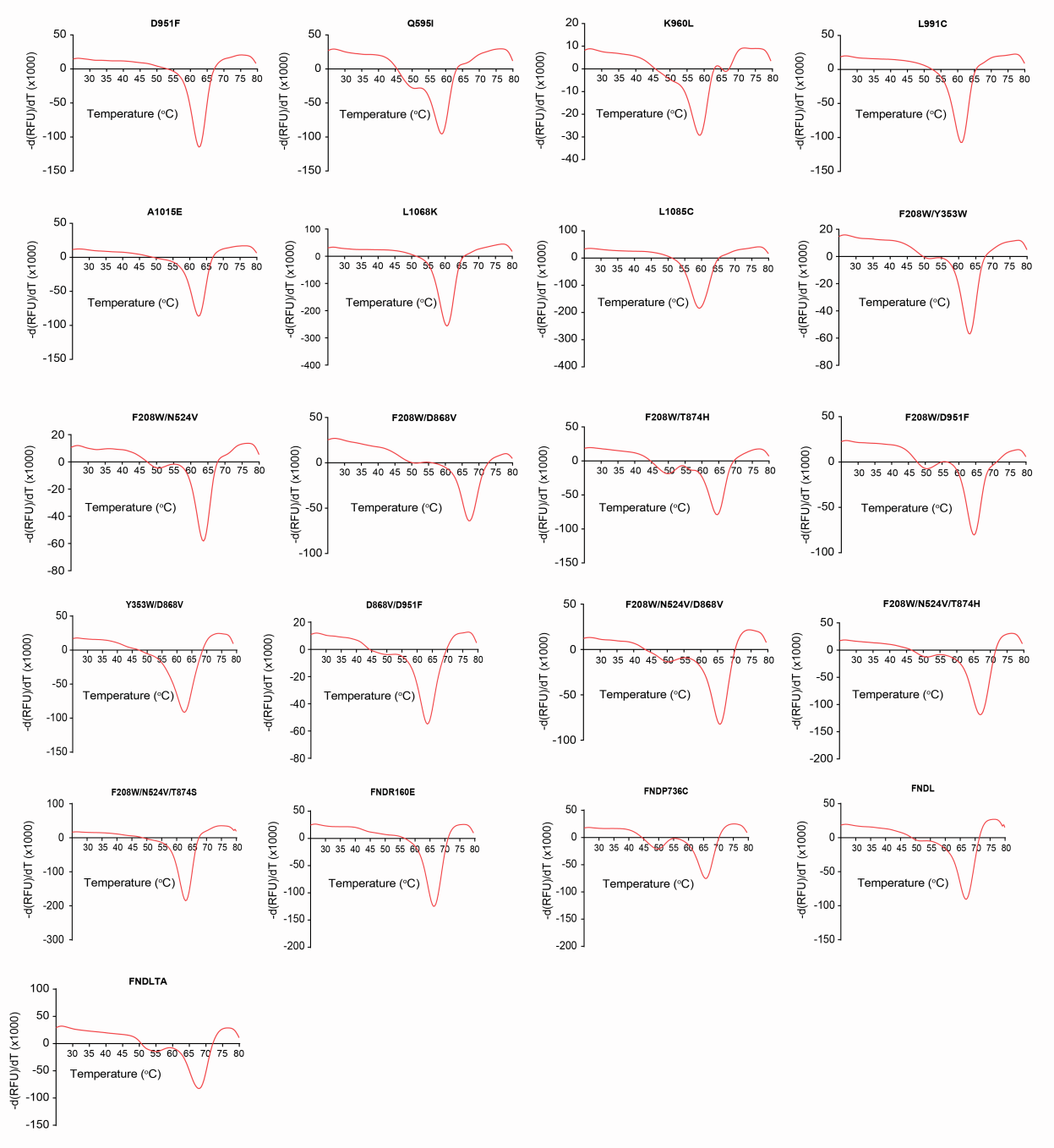
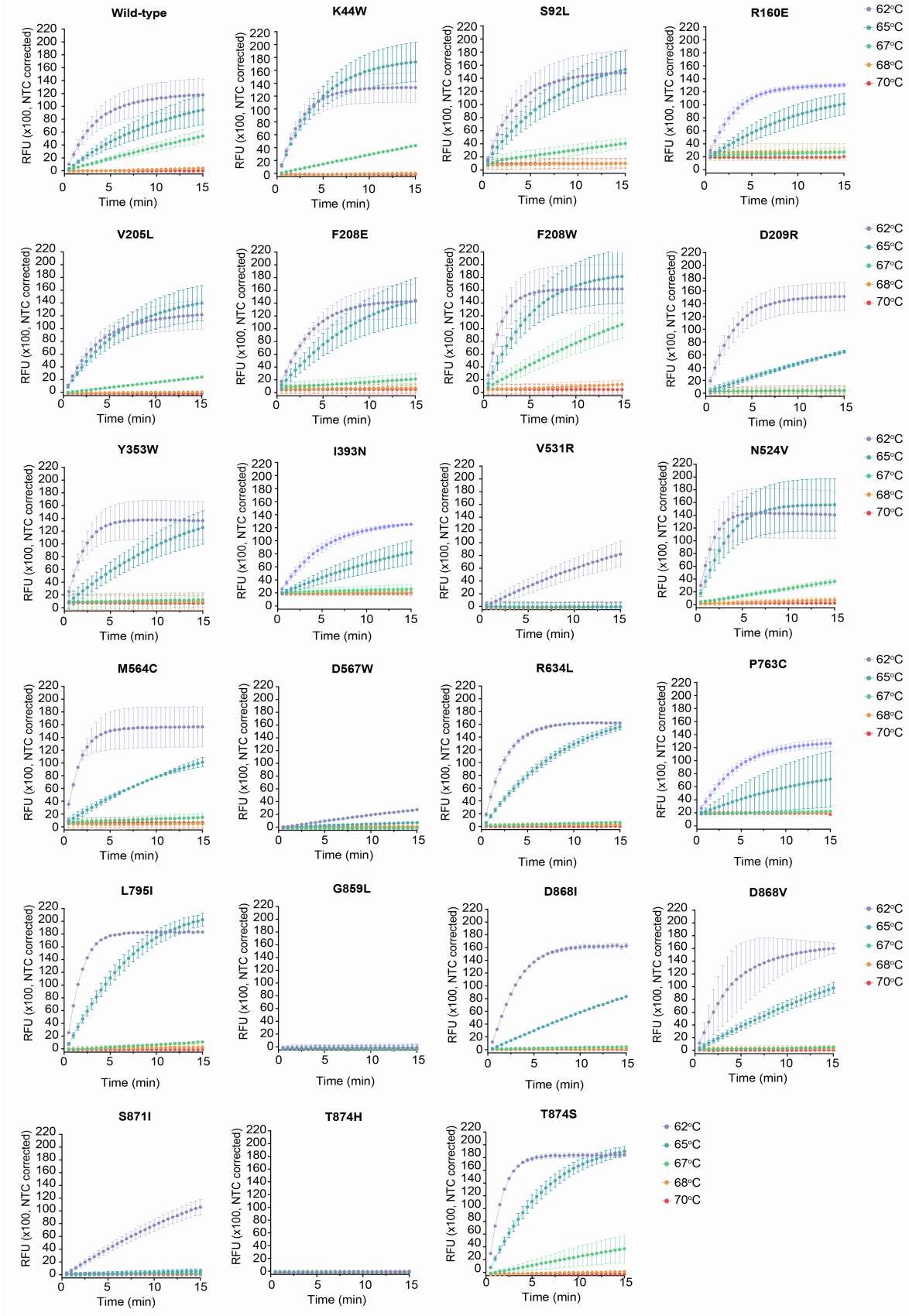



**Figure S1**. **Differential Scanning Fluorometric Measurements of All BrCas12b Variants Used in the Study, Related to Figure 2** . The curve at each temperature point represents the average of fluorescence over 4 replicates (n = 2 technical replicates per experiment over two experiments). The melting point ($T_m$) was determined as a global minimum of the derivative curve.


Wild-type
K44W
S92L
R160E
V205L
F208E
F208W
D209R
Y353W
I393N
V531R
N524V
M564C
D567W
R634L
P763C
L795I
G859L
D868I
D868V
S871I
T874H
T874S
RFU (x100, NTC corrected)
Time (min)
62°C
65°C
67°C
68°C
70°C

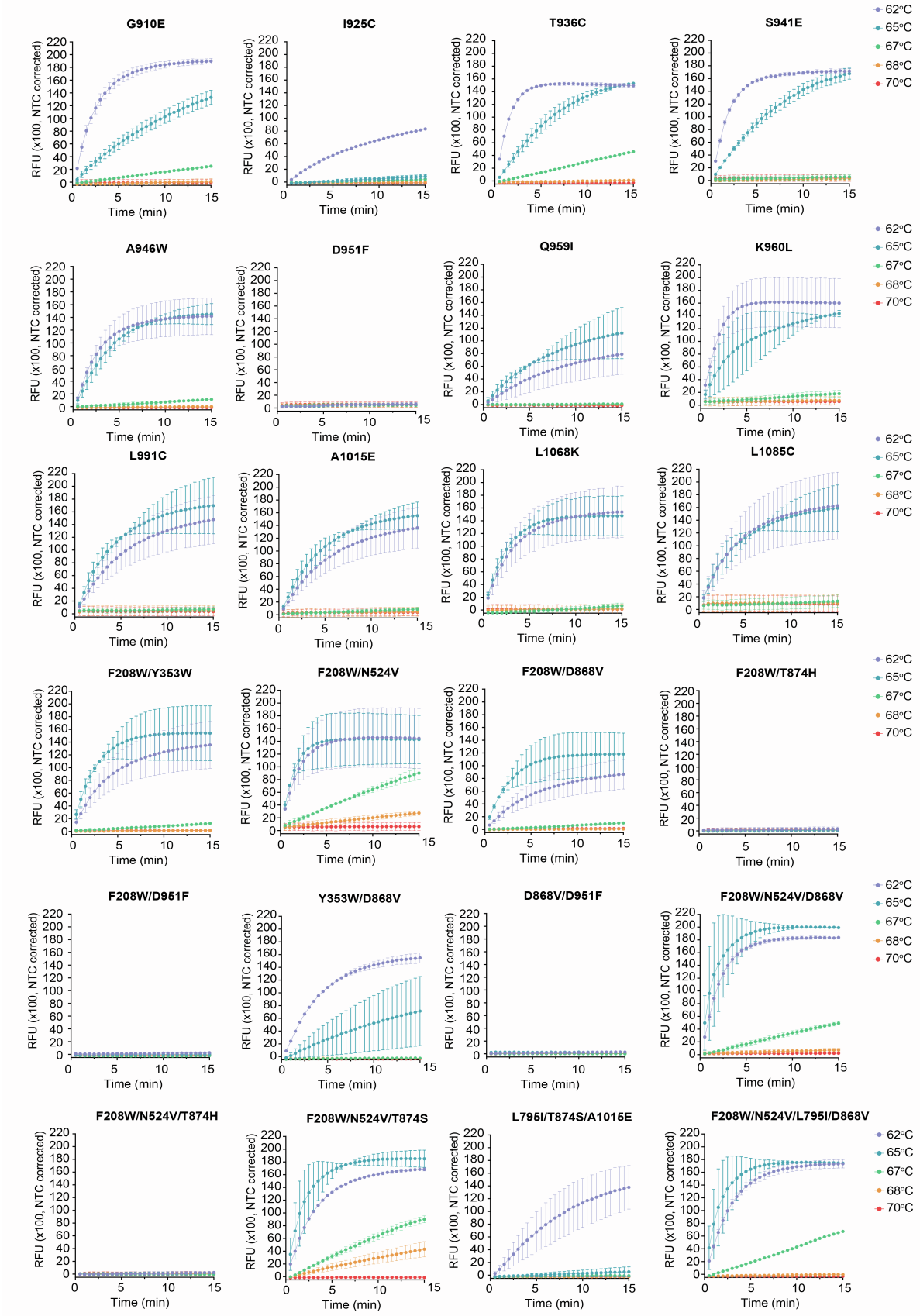

G910E
I925C
T936C
S941E
A946W
D951F
Q959I
K960L
L991C
A1015E
L1068K
L1085C
F208W/Y353W
F208W/N524V
F208W/D868V
F208W/T874H
F208W/D951F
Y353W/D868V
D868V/D951F
F208W/N524V/D868V
F208W/N524V/T874H
F208W/N524V/T874S
L795I/T874S/A1015E
F208W/N524V/L795I/D868V
RFU (x100, NTC corrected)
Time (min)
62°C
65°C
67°C
68°C
70°C

**Figure S2. Time- and Temperature-dependent Trans-cleavage Activity of BrCas12b Variants Used in the Study, Related to Figure 2**. The ribonucleoprotein complex and trans-cleavage reaction were incubated at 62°C, 65°C, 67°C, 68°C, and 70°C, respectively. Error bars represent mean ± s.d., where n = 2 biological replicates.

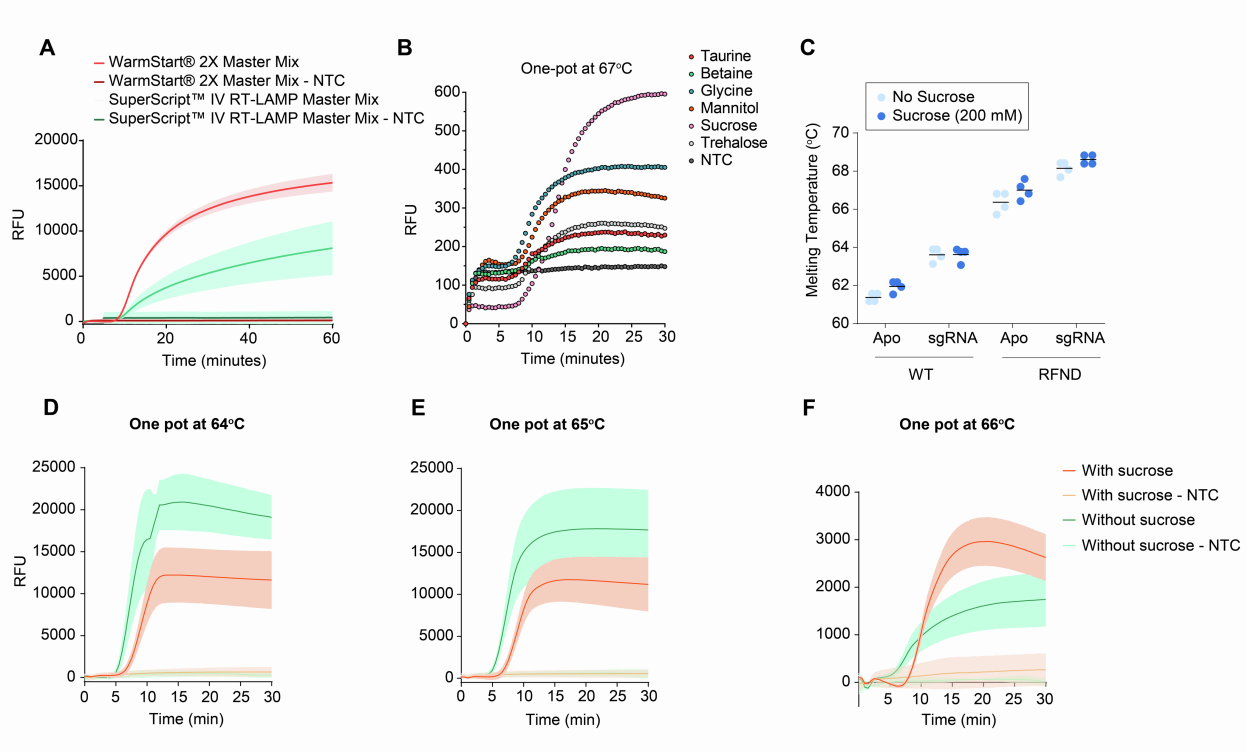



**Figure S3**. **BrCas12b Detection Optimization, Related to Figure 4**. (**A**) Compatibility of BrCas12b-based detection reaction with commercially available RT-LAMP master mixes. (**B**) Additive optimization of the one-pot reaction with additives for the FNDLTA variant. Additives were mixed to a final concentration of 50 mM, and the reaction was incubated isothermally at 67ºC. Each data point represents an average of triplicates (n = 3 biological replicates). (**C**) Effects of sucrose on the thermostability of wild-type BrCas12b and the thermally improved RFND variant. Melting temperatures were determined from differential scanning fluorimetry (n = 2 technical replicates per experiment over two experiments). The melting point ($T_m$) was identified as a global minimum of the derivative RFU curve with respect to temperature. (**D**), (**E**), and (**F**) SPLENDID assay using RFND variant in the presence and absence of sucrose at 64ºC, 65ºC, 66ºC, respectively. The SARS-CoV-2 genomic RNA input was 25,000 copies. The fluorescence measurements were taken at t = 30 minutes. Each curve represents the average of fluorescence signals. The shaded area represents standard deviation, n = 3.

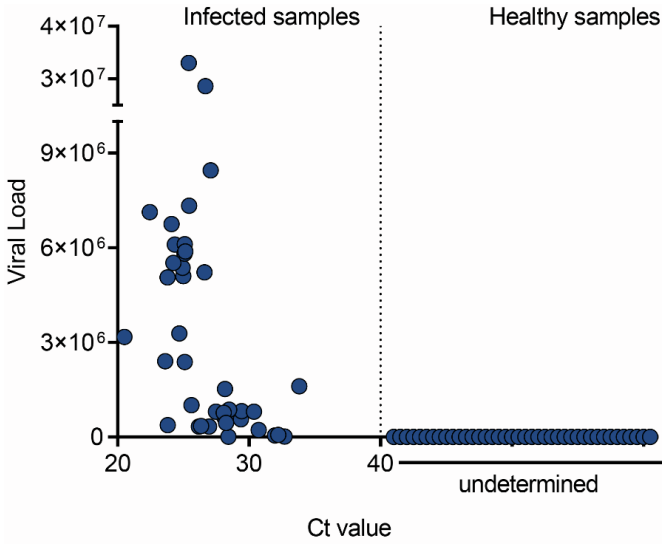



**Figure S4. Correlation between In-house RT-qPCR and Clinically Validated Viral Loads for 80 HCV Patient Samples, Related to Figure 5.**

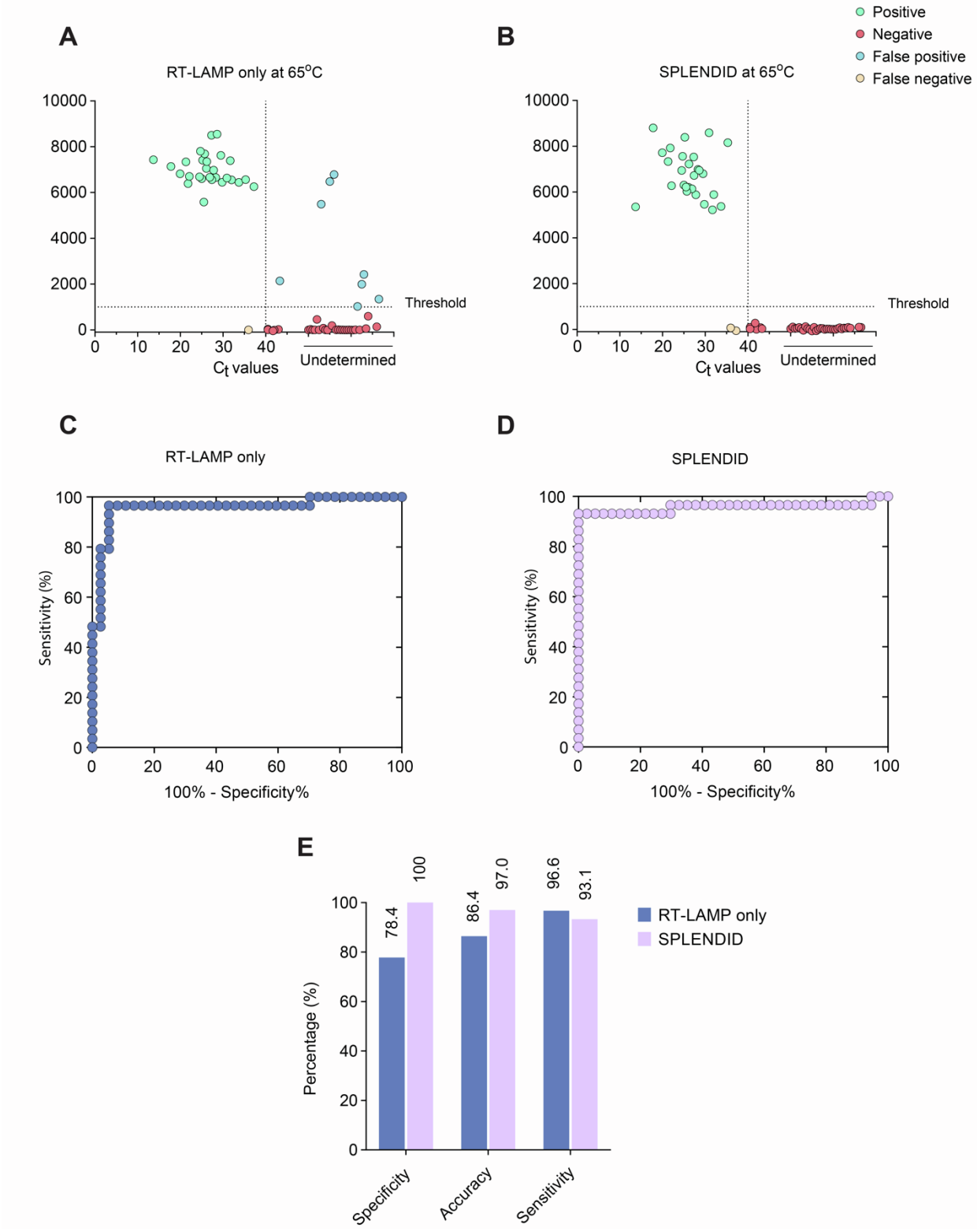

A
RT-LAMP only at 65°C
10000
8000
6000
4000
2000
0
0 10 20 30 40
Ct values
Undetermined
Threshold
B
SPLENDID at 65°C
Positive
Negative
False positive
False negative
Threshold
Undetermined
C
RT-LAMP only
Sensitivity (%)
100% - Specificity%
D
SPLENDID
E
Percentage (%)
78.4
100
86.4
97.0
96.6
93.1
RT-LAMP only
SPLENDID
Specificity
Accuracy
Sensitivity

**Figure S5**. **Clinical Validation of SPLENDID and RT-LAMP Only in SARS-CoV-2 Infected Samples, Related to Figure 5.** (**A**) and (**B**) Fluorescence measurements of 66 SARS-CoV-2 clinical samples (29 negative and 37 positive samples) from the RT-LAMP only or SPLENDID assay, respectively. Signal readouts were taken at t = 30 minutes. (**C**) and (**D**) Receiver operating characteristic curve (ROC) at t = 30 minutes of RT-LAMP only and SPLENDID assays, respectively. (**E**) Summary of clinical validation of SPLENDID and RT-LAMP only in terms of sensitivity, specificity, and accuracy.

**Table S1. Sequences Used in the Study, Related to Figure 1-5.**

**RT-LAMP primers**

| Target | Name | Sequence |
|---|---|---|
| N gene of SARS-CoV-2 | N_gene_F3 | AACACAAGCTTTCGGCAG |
| | N_gene_B3 | GAAATTTGGATCTTTGTCATCC |
| | N_gene_FIP | TGCGGCCAATGTTTGTAATCAGCCAA GGAA ATTTTGGGGAC |
| | N_gene_BIP | CGCATTGGCATGGAAGTCACTTTGAT GGC ACCTGTGTAG |
| | N_gene_LF | TTCCTTGTCTGATTAGTTC |
| | N_gene_LB | ACCTTCGGGAACGTGGTT |
| 5' UTR of Hepatitis C[1] | UTR_F3 | CGGGAGAGCCATAGTGGT |
| | UTR_B3 | WGGAWGTGTGCTCATGATGCACG |
| | UTR_FIP | AAATCTCCAGGCATTGAGCGTTTTTGCGGAACCGGTGAGTAC |
| | UTR_BIP | CCGCRAGACYGCTAGCCGAGTTTTACCCTATCAGGCAGTACCAC |
| | UTR_LF | TCGTCCYGGCRATTCCGG |
| | UTR_LB | TAGTGTTGGGTCGCGAAAG |

**Single-guide RNAs**

| Target | Name | Sequence |
|---|---|---|
| N gene of SARS-CoV-2 | Br_sgN_CoV2 | GAAGGUGGUUAGCUACAGGCUGACCAGUGCAGUUGUGUCAUGUGCUACGG UGACCUAACACGUCACUCAGUCACAACGGCUAUCUAUAUUUCCACUAACCA AAGUUAGUGGAAAUGUAGAUGGUUAGCAC**CGAAGAACGCUGAAGCGCUG** |
| 5' UTR of Hepatitis C | Br_sgUTR_HCV | GAAGGUGGUUAGCUACAGGCUGACCAGUGCAGUUGUGUCAUGUGCUACGG UGACCUAACACGUCACUCAGUCACAACGGCUAUCUAUAUUUCCACUAACCA AAGUUAGUGGAAAUGUAGAUGGUUAGCAC**UCCAAGAAAGGACCCGGUCG** |

**RT-qPCR primers[2]**

| Target | Name | Sequence |
|---|---|---|
| 5' UTR of Hepatitis C | HCV_UTR_FOR | AGCGTCTAGCCATGGCGTT |
| | HCV_UTR_REV | GCAAGCACCCTATCAGGCAGT |
| | HCV_UTR_Probe | /56-FAM/TCTGCGGAA/ZEN/CCGGTGAGT/3IABkFQ/ |

Reporters

| Target | Name | Sequence |
|---|---|---|
| Universal | Reporter 1 | /5HEX/TTTTTTTT/3IABkFQ/ |
| | Reporter 2 | /5FAM/TTTTTTTTTT/3IABkFQ/ |

**Protein sequences**

| Name | Sequence |
|---|---|
| AapCas12b | MAVKSIKVKLRLDDMPEIRAGLWKLHKEVNAGVRYYTEWLSLLRQENLYR RSPNGDGEQECDKTAEECKAELLERLRARQVENGHRGPAGSDDELLQLA RQLYELLVPQAIGAKGDAQQIARKFLSPLADKDAVGGLGIAKAGNKPRWV RMREAGEPGWEEEKEKAETRKSADRTADVLRALADFGLKPLMRVYTDSE MSSVEWKPLRKGQAVRTWDRDMFQQAIERMMSWESWNQRVGQEYAKL VEQKNRFEQKNFVGQEHLVHLVNQLQQDMKEASPGLESKEQTAHYVTGR ALRGSDKVFEKWGKLAPDAPFDLYDAEIKNVQRRNTRRFGSHDLFAKLAE |

| | |
|---|---|
| | PEYQALWREDASFLTRYAVYNSILRKLNHAKMFATFTLPDATAHPIWTRFD<br>KLGGNLHQYTFLFNEFGERRHAIRFHKLLKVENGVAREVDDVTVPISMSE<br>QLDNLLPRDPNEPIALYFRDYGAEQHFTGEFGGAKIQCRRDQLAHMHRRR<br>GARDVYLNVSVRVQSQSEARGERRPPYAAVFRLVGDNHRAFVHFDKLSD<br>YLAEHPDDGKLGSEGLLSGLRVMSVDLGLRTSASISVFRVARKDELKPNS<br>KGRVPFFFPIKGNDNLVAVHERSQLLKLPGETESKDLRAIREERQRTLRQL<br>RTQLAYLRLLVRCGSEDVGRRERSWAKLIEQPVDAANHMTPDWREAFEN<br>ELQKLKSLHGICSDKEWMDAVYESVRRVWRHMGKQVRDWRKDVRSGER<br>PKIRGYAKDVVGGNSIEQIEYLERQYKFLKSWSFFGKVSGQVIRAEKGSRF<br>AITLREHIDHAKEDRLKKLADRIIMEALGYVYALDERGKGKWVAKYPPCQLI<br>LLEELSEYQFNNDRPPSENNQLMQWSHRGVFQELINQAQVHDLLVGTMY<br>AAFSSRFDARTGAPGIRCRRVPARCTQEHNPEPFPWWLNKFVVEHTLDA<br>CPLRADDLIPTGEGEIFVSPFSAEEGDFHQIHADLNAAQNLQQRLWSDFDI<br>SQIRLRCDWGEVDGELVLIPRLTGKRTADSYSNKVFYTNTGVTYYERERG<br>KKRRKVFAQEKLSEEEAELLVEADEAREKSVVLMRDPSGIINRGNWTRQK<br>EFWSMVNQRIEGYLVKQIRSRVPLQDSACENTGDI |
| BrCas12b | MPVRSFKVKLVTRSGDAEHMLQLRRGLWKTHEIVNQGIAYYMNKLALMR<br>QEPYAGKSREVVRLELLHSLRAQQKRNNWTGDAGTDDEILNLSRRLYELL<br>VPSAIGEKGDAQMLSRKFLSPLVDPNSEGGKGTAKSGRKPRWMKMREE<br>GHPDWEAEREKDRAKKAADPTASILNDLEAFGLRPLFPLFTDEQKGIQWL<br>PKQKRQFVRTFDRDMFQQALERMLSWESWNRRVAEEYQKLQAQRDELY<br>AKYLADGGAWLEALQSFEKQREVELAEESFAAKSEYLITRRQIRGWKQVY<br>EKWSQLPEHAAQEQFWQVVADVQTSLPGAFGDPKVYQFLSQPEHHHIW<br>RGYPNRLFHYSDYNGVRKKLQRARHDATFTLPDPVEHPLWIRFDARGGNI<br>HDYEISQNGKQYQVTFSRLLWPENETWVERENVTVAIGASQQLKRQIRLD<br>GYADKKQKVRYRDYSSGIELTGVLGGAKIQFDRRHLRKASNRLADGETGP<br>VYLNVVVDIEPFLAMRNGRLQTPIGQVLQVNTKDWPKVTGYKPAELISWIQ<br>NSPLAVGTGVNTIEAGMRVMSVDLGQRSAAAVSIFEVMRQKPAEQETKLF<br>YPIAVTGLYAVHRRSLLLRLPGEKISDEIEQQRKIRAHARSLVRYQIRLLADV<br>LRLHTRGTAEQRRAKLDELLATLQTKQELDQKLWQTELEKLFDYIHEPAER<br>WQQALVAAHRTLEPVIGQAVRHWRKSLRIDRKGLAGMSMWNIEELEETR<br>KLLIAWSKHSRVPGEPNRLDKEETFAPQQLQHIQNVKDDRLKQMANLLVM<br>TALGYKYDEAEKQWKEAYPACQMILFEDLSRYRFALDRPRRENNRLMKW<br>AHRSIPRLVYLQGELFGIQVGDVYSAYTSRFHAKTGAPGIRCHALKEEDLQ<br>PNSYVVKQLIKDGFIREDQTGSLKPGQIVPWSGGELFVTLADRSGSRLAVI<br>HADINAAQNLQKRFWQQNTEIFRVPCKVTTSGLIPAYDKMKKLFGKGYFA<br>KINQTDTSEVYVWEHSAKMKGKTTPADPAEEGVFDESLTDEMEELEDSQ<br>EGYKTLFRDPSGFFWSSDRWLPQKEFWFWVKRRIEKKLREQLQ |

**Table S2. Genotype Specific Sensitivity for HCV-patient Samples, Related to Figure 5**

| Genotype | #samples | True Positive | False Negative | Sensitivity |
|---|---|---|---|---|
| GT1-(confirmed) | 34 | 31 | 3 | 91% |
| GT2-(confirmed) | 4 | 2 | 2 | 50% |
| GT3-(confirmed) | 2 | 0 | 2 | 0% |

**Table S3. Summary of Detection Platforms, Related to Figure 5.**

| | Target | Accuracy | Specificity | Sensitivity | Source |
|---|---|---|---|---|---|
| RT-qPCR | SARS-CoV-2 | 95.8 | 91.3 | 99.5 | Banko *et al*.[3] |
| | HCV | 97% | 100% | NA | Chen *et al*.[4], Zhang *et al*.[5] |
| STOP-Covid | SARS-CoV-2 | 95.8% | 98.5% | 93.1% | Joung *et al*.[6] |
| SPADE | SARS-CoV-2 | 96.7% | 99.4% | 92.8% | Nguyen *et al*.[7] |
| SHERLOCK | SARS-CoV-2 | NA | 100.0% | 96.0% | Patchsung *et al*.[8] |
| DETECTR | SARS-CoV-2 | 97.6% | 100.0% | 95.0% | Broughton *et al*.[9] |
| ENHANCE | SARS-CoV-2 | 95.0% | 96.8% | 96.8% | Nguyen *et al*.[10] |
| PMID: 35885430 | HCV | 97.0% | 100.0% | 96.0% | Kham-Kjing *et al*.[11] |
| SPLENDID | HCV | 90.0% (overall) | 97.5% (overall) | 82.5% (overall) | This study |
| | | 94.6% (GT1) | 97.5% (overall) | 91.1% (GT1) | |
| | SARS-CoV-2 | 97.0% | 100.0% | 93.1% | |

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