1 **Supplemental tables**
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3 TABLE S1. Cross-resistance profile of GS-9669 and other reference NS5B inhibitors against non-Thumb Site
4 II NS5B mutations

| Compound | $EC_{50}$ (nM)[a,b] | Fold resistance[c] against NS5B mutant: | | | |
|---|---|---|---|---|---|
| | WT (1b) | S282T | M414T | P495L | Y448H/Y452H |
| GS-9669 | 11 ± 1.9 | 0.7 | 2 | 1.3 | 1 |
| Lomibuvir | 11 ± 3.0 | 0.8 | 2.8 | 2 | 0.9 |
| Filibuvir | 80 ± 28 | 0.7 | 0.9 | 3.3 | 0.9 |
| 2'-C-Me-A | 206 ± 86 | 25 | 1.2 | 1.1 | 0.5 |
| Benzothiadiazine Palm Site I inhibitor | 370 ± 50 | NT[d] | >15 | NT | NT |
| Benzimidazole Thumb Site I inhibitor | 344 ± 102 | NT | NT | >14 | NT |
| Tegobuvir | 1.5 ± 0.8 | 0.7 | NT | NT | 231 |

5 [a] 96-well assay manual format.
6 [b] $EC_{50}$s are the arithmetic mean of at least two experiments.
7 [c] Resistance fold is calculated as the ratio of mutant $EC_{50}$ to wild-type $EC_{50.}$
8 [d] NT=Not Tested.
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10 TABLE S2. Cross-resistance profile of GS-9669 and other reference inhibitors against NS3 and NS5A
11 resistance mutations

| Compound | $EC_{50}$ (nM)[a,b] | Fold resistance[c] against mutant: | | |
|---|---|---|---|---|
| | WT (1b) | NS3 R155K | NS3 D168V | NS5A Y93H |
| GS-9669 | 11 ± 1.9 | 0.8 | 1 | 1.3 |
| Lomibuvir | 11 ± 3.0 | 1.1 | 1.4 | 2.0 |
| Filibuvir | 80 ± 28 | 1.1 | 0.9 | 3.3 |
| 2'-C-Me-A | 206 ± 86 | 1.1 | 0.7 | 1.1 |
| BILN-2061 | 1.7 ± 1.0 | 1,981 | 3,137 | NT |
| Daclatasvir | 0.003 ± 0.001 | NT[d] | NT | 32 |

12 [a] 96-well assay manual format.
13 [b] $EC_{50}$s are the arithmetic mean of at least two experiments.
14 [c] Resistance fold is calculated as the ratio of mutant $EC_{50}$ to wild-type $EC_{50.}$
15 [d] NT=Not Tested.
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