**SUPPLEMENTARY DATA**

## Optimized high-throughput screen for Hepatitis C viral translation inhibitors

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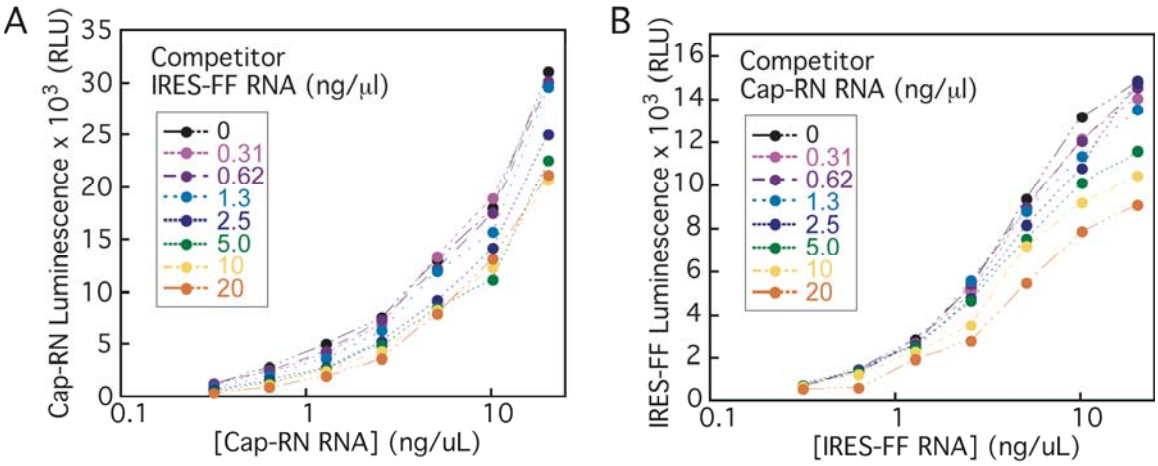
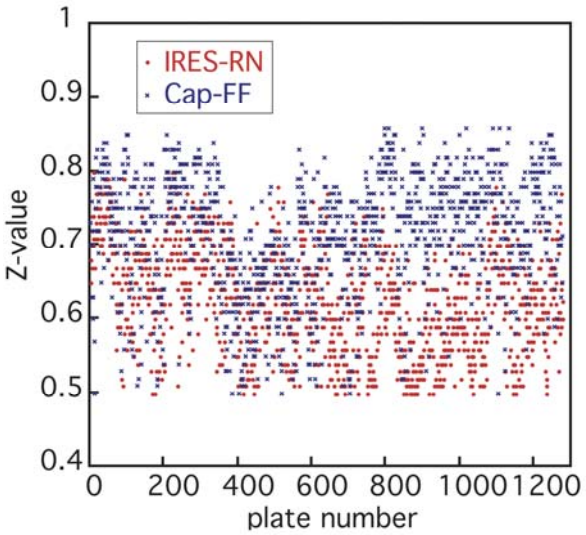



**Supplementary Figure 1**. Two-dimensional titrations of IRES-FF and 5′ cap-RN mRNAs in adjusted RRL. The concentration of each of the two reporter mRNAs was varied between 0 ng/μl and 20 ng/μl and the absolute luminescence activities of (A) 5′ cap-RN and (B) IRES-FF mRNA are plotted. Luciferase activities were measured using the BrightGlo system.



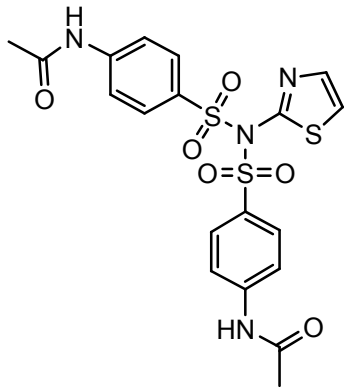
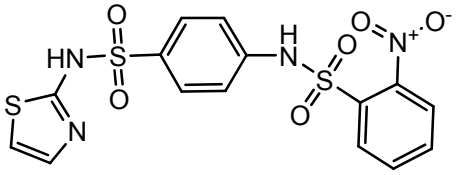
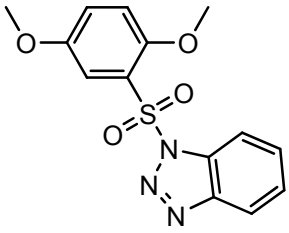
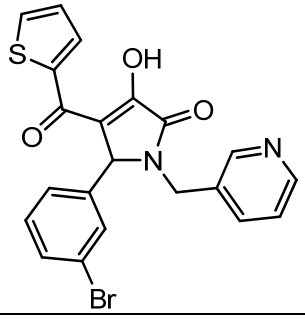

**Supplementary Figure 2**. Z values of all HTS plates. Z-factors were calculated as described in the materials and methods section. Average values for Z-factors were 0.625 and 0.713 for IRES-RN and 5′ cap-FF translation, respectively.

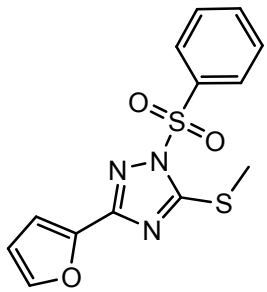
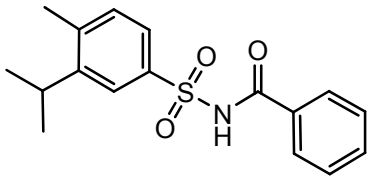
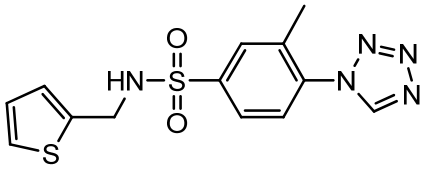
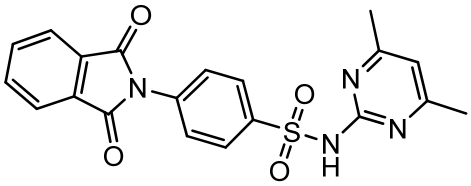
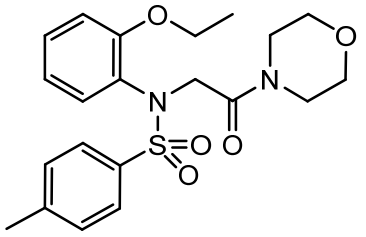
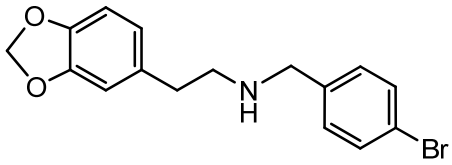
**Supplemental Table 1.** NPI values of known translation inhibitors in the screen. Three known inhibitors of eukaryotic protein synthesis were present in the library. The NPI (%) of these compounds from the primary screen was calculated as described in the Materials and Methods section.

| | NPI (%) | |
|---|---|---|
| **Inhibitor** | **IRES-RN** | **5′ cap-FF** |
| puromycin* (positive control) | 100% | 100% |
| emitine | 92.2% | 95.1% |
| madumycin derivative | 41.7% | 39.3% |

* Puromycin was used as the positive control for the screen. $IC_{50}$ values were determined to be 0.41 μM against IRES-RN and 1.1 μM against 5′ cap-FF.

**Supplemental Table 2.** Examples of potent *Renilla* luciferase-specific inhibitors from the secondary screen. These compounds showed *Renilla* luciferase-specific inhibition to both *in vitro* translated *Renilla* luciferase (regardless of whether translation was driven by the IRES or a 5′ cap) and recombinant *Renilla* luciferase. These compounds showed no inhibition of firefly luciferase at concentrations up to 100 μM under the same conditions.

| | $IC_{50}$ (REN) μM | $IC_{50}$ (FF) μM |
|---|---|---|
|  | 0.02 | > 100 |
|  | 0.10 | > 100 |
|  | 0.73 | > 100 |
|  | 1.44 | > 100 |

| | | |
|---|---|---|
|  | 2.02 | > 100 |
|  | 2.24 | > 100 |
|  | 2.45 | > 100 |
|  | 2.98 | > 100 |
|  | 3.46 | > 100 |
|  | 5.12 | > 100 |