

| Plate_Well | 3A_1 | 3B_1 | 3A_2 | 3B_2 | 3A_3 | 3B_3 | 3A_4 | 3B_4 | 4A_1 | 4B_1 |
|-------------------------|-----------|-------|-------|-------|-------|-------|-------|-------|---------|------|
| Experimental | | | | | | | | | | |
| Library | LINCS2 | | | | | | | | Selleck | |
| Compound | Ibrutinib | | | | | | | | | |
| HTRF Channel 1 (665 nm) | 28311 | 29201 | 31038 | 31679 | 28019 | 29138 | 30006 | 31815 | 7529 | 7468 |
| HTRF Channel 2 (620 nm) | 5736 | 5840 | 5859 | 6111 | 6125 | 5780 | 5791 | 5828 | 8631 | 9073 |
| HTRF Ratio | 49357 | 50002 | 52975 | 51839 | 45745 | 50412 | 51815 | 54590 | 8723 | 8231 |
| Positive Control | | | | | | | | | | |
| HTRF Channel 1 (665 nm) | 7755 | 7906 | 7755 | 7906 | 7755 | 7906 | 7755 | 7906 | 6702 | 6602 |
| HTRF Channel 2 (620 nm) | 9029 | 9081 | 9029 | 9081 | 9029 | 9081 | 9029 | 9081 | 10035 | 9897 |
| HTRF Ratio | 8594 | 8707 | 8594 | 8707 | 8594 | 8707 | 8594 | 8707 | 6787 | 6776 |
| Negative Control | | | | | | | | | | |
| HTRF Channel 1 (665 nm) | 28217 | 28235 | 28217 | 28235 | 28217 | 28235 | 28217 | 28235 | 7418 | 7183 |
| HTRF Channel 2 (620 nm) | 5531 | 5672 | 5531 | 5672 | 5531 | 5672 | 5531 | 5672 | 8219 | 8063 |
| HTRF Ratio | 51077 | 49837 | 51077 | 49837 | 51077 | 49837 | 51077 | 49837 | 9028 | 8912 |

S4 Table. HTRF ratio and FRET emissions for ibrutinib-treated HCMV NEC.

Of the libraries screened, two, LINCS2 and Selleck, contained ibrutinib. Each plate was screened in duplicate (i.e., Plates 3A and 3B, and plates 4A and 4B) and well number (i.e., 1, 2, 3, 4). The concentration of ibrutinib was 10 mM, 2 mM, 400 μ M and 80 μ M in LINCS2 wells 1 to 4 respectively, so the concentrations in the assay were 50, 10, 2, and 0.4 μ M, respectively, and 10 mM in the Selleck's well for an assay concentration of 50 μ M. Positive and negative controls were included on each plate. FRET emissions from the acceptor were measured at 665 nm (Channel 1) and donor emissions at 620 nm (Channel 2). The HTRF ratio was calculated as follows:

$[(\text{Abs}_{665\text{nm}}/\text{Abs}_{620\text{nm}})*10,000]$. The Selleck plate showed a narrower difference between the positive and negative controls than the LINCS plate, but the Z' score was

0.6, with 14% and 32% inhibition observed. Essentially no inhibition was observed from any of the LINCS wells.