Table S1: The presence of NB-DNJ or PEGylated PERLs does not affect DENV titers as determined by plaque assay

<table>
<thead>
<tr>
<th>Virus spiked with:</th>
<th>Viral titre (pfu/ml)(^a)</th>
<th>Fold-difference from titre of virus spiked with media alone(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XVIVO10 media</td>
<td>2.97 x 10^6</td>
<td></td>
</tr>
<tr>
<td>100uM NB-DNJ</td>
<td>1.9 x 10^6</td>
<td>0.6</td>
</tr>
<tr>
<td>200uM PERLs</td>
<td>3.13 x 10^6</td>
<td>1.05</td>
</tr>
<tr>
<td>50uM PERLs encapsulating NB-DNJ</td>
<td>5.4 x 10^6</td>
<td>1.8</td>
</tr>
</tbody>
</table>

\(^a\) DENV2 strain 16681 was diluted to 6.0 x 10^6 pfu/ml and spiked with a 2.5% volume of NB-DNJ, PERLs or media. The titres of infectious virus in these samples were determined (in triplicate) by plaque assay on LLC-MK\(_2\) cells as described in Materials and Methods.

\(^b\) Neither the presence of NB-DNJ nor liposomes in cell supernatants affected DENV titres within 2-fold, a range not considered quantitative for this plaque assay.
<table>
<thead>
<tr>
<th>Cell</th>
<th>CC&lt;sub&gt;50&lt;/sub&gt; (µM drug/lipid)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NB-DNJ</th>
<th>PERLs</th>
<th>PERLs + 5mM NB-DNJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>U937</td>
<td>&gt;5,000</td>
<td>303 +/- 91</td>
<td>275 +/- 22</td>
<td></td>
</tr>
<tr>
<td>HL60</td>
<td>&gt;5,000</td>
<td>290 +/- 71</td>
<td>303 +/- 27</td>
<td></td>
</tr>
<tr>
<td>Primary monocytes</td>
<td>&gt;5,000</td>
<td>424&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Primary macrophages</td>
<td>&gt;31,600</td>
<td>479 +/- 133</td>
<td>520&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Cells (2 x 10<sup>5</sup> cells/well) were incubated for 2 days with serial dilutions of NB-DNJ or PERLs, alongside medium-only controls. Cell viability was measured using CellTitre 96 Aqueous One Solution Cell Proliferation assay according to manufacturer’s instructions and calculated as a percentage of untreated cells. CC<sub>50</sub> values were calculated using the line of best fit on graphs of drug concentration versus percentage cell viability, as a percentage of control (mean +/- SD shown). n=3 for all unless otherwise stated.

<sup>b</sup> n=1

<sup>c</sup> ND = not determined

<sup>d</sup> n=2
Supplemental Experimental Procedures

All data operations, analyses, and curve fits on *in vitro* data were performed in KaleidaGraph 4.0.3 (Synergy Software, Reading, PA, USA).

**Cell Cytotoxicity (MTT/MTS Assays)**

**Data Analysis**

Raw absorbance values at 490 nm (A490) were obtained as per manufacturer's instructions. Where available, "media only" blanks were subtracted to yield adjusted A490. Adjusted A490 values of no drug controls (untreated cells) were averaged and standard deviations determined. The adjusted A490 for each technical replicate of each drug concentration was divided by the untreated cell average to yield a percent viability. Percent viabilities were averaged and standard deviations determined for the set of technical replicates of each drug concentration. Drug dilutions were generally carried out in 0.5 log₁₀ steps (e.g. 31.6 mM, 10 mM, 3.16 mM, 1 mM, etc.) Average percent viability as the dependent variable was plotted along a linear y-axis (from 0 to 100) against a logarithmic x-axis (drug concentration). The x-value of untreated cells was adjusted to 1.5 log₁₀ steps lower than the global minimum drug concentration tested (i.e. lowest concentration tested of 0.0316 μM, therefore untreated concentration set to 0.001 μM) to allow for plotting along a logarithmic axis (log₁₀(0)=undefined).

The coefficient of variation (Cᵥ) for a given data point was calculated to determine precision of the observed value. Briefly, Cᵥ is defined as the standard deviation of an estimator divided by the estimator:

\[ Cᵥ = \frac{s}{\bar{x}} \]

where s is the sample standard deviation and \( \bar{x} \) is the sample mean. Relative Cᵥ was subsequently determined by dividing each Cᵥ by the minimum Cᵥ for a given donor/drug experiment. This operation sets the most precise Cᵥ to 1 with all less precise measures set greater to 1.

**Curve Fitting**

Based on the above plots for each donor/drug combination, several curve types were fit to the data sets. KaleidaGraph General Curve Fits using an iterative method (the Levenberg-Marquardt algorithm) for \( \chi^2 \) minimization were run. Data points were weighted based on the inverse of the square of relative Cᵥ. As such, the most precise measure has a weight equal to 1/1²=1 while less precise measures have lower weight (e.g. for an estimate of relative Cᵥ=2, 1/2²=0.25). The following methods were applied to curves:

**Moddoseresplgst**:

\[ y = A + \frac{B}{1 + \left( \frac{x}{C} \right)^D} \]

Such that initial guesses for: A=-70; B=186; C=5; D=0.2

**Lgstsigmoid**:

\[ y = A + \frac{B - A}{1 + e^{-C(x-D)}} \]

Such that initial guesses for: A=100; B=0.01; C=0.05; D=20
Dosersplgst:

\[ y = A + \frac{B - A}{1 + \left(\frac{X}{C}\right)^D} \]

Such that initial guesses for: \(A=95.9\); \(B=-0.86\); \(C=0.04\); \(D=-0.95\)

In addition, KaleidaGraph’s built-in exponential curve fit was applied to all cytotoxicity curves. If \(R^2\) of this exponential curve indicated better curve fit than error values for the above non-linear curve fits, \(CC_{50}\) was determined by solving this equation setting \(y=50\). Alternatively, the non-linear curve fit that produced the lowest \(\chi^2\) was solved for \(y=50\) to determine the \(CC_{50}\). For curves that did not approach 50 percent reduction in cell viability, the \(CC_{50}\) was defined as greater than the maximum concentration tested. \(CC_{10}\) was similarly assessed with curves solved for \(y=90\) only for curves demonstrating significant cytotoxicity (minimally approaching 50% at the highest concentration tested). Values across donors for each drug were averaged and standard deviations determined.

**Infection of MDMØ (Immunofluorescence Assays)**

**Data Analysis**

Immunofluorescence using anti-DV E antibody (3H5) was performed for each donor/drug combination in technical triplicate as previously described (1). Approximately 800 MDMØ were examined for infection for each sample. For a given donor/drug experiment, the average of percent infection of untreated samples (i.e. no drug) was set to 100 percent infection and used to normalize all other samples. Percent infection of technical replicates of each drug concentration was averaged and standard deviations determined. Drug dilutions were generally carried out in 0.5log₁₀ steps (e.g. 31.6 mM, 10 mM, 3.16 mM, 1 mM, etc.) Average percent infection as the dependent variable was plotted along a linear y-axis (from 0 to 100) against a logarithmic x-axis (drug concentration). The x-value (drug concentration) of untreated cells was adjusted to 1.5log₁₀ steps lower than the global minimum drug concentration tested (i.e. lowest concentration tested of 0.0316μM, therefore untreated concentration set to 0.001μM) to allow for plotting along a logarithmic axis (log₁₀(0)=undefined). Standard deviation error bars were included for each data point. \(C_V\) and relative \(C_V\) were calculated as described above.

In addition to individual drug/donor experiment graphs, the percent infection for each drug concentration for all donors of a given drug treatment (biological summary graph, Figure 1 E). This process was carried out for every concentration tested of all 3 drugs. Standard deviation, \(C_V\), and relative \(C_V\) for each point were determined as measures of biological variation and precision.

**Curve Fitting**

Curve fits as described above were applied to each donor/drug graph as well as to each biological summary graph with the exception of the exponential curve type. The non-linear curve fit that best fit the data (produced the lowest \(\chi^2\)) was solved for \(y=50\) to determine the \(IC_{50}\) and for \(y=10\) to determine the \(IC_{90}\). Values across donors for each drug were averaged and standard deviations determined. These mean \(IC_{50}\) and \(IC_{90}\) value were compared to estimates solved based on the biological summary graphs. All estimates for each drug were comparable, thus only the mean value +/- standard deviation is reported.
**Infectious Virus Release (Plaque Assays)**

Data Analysis
LLC-MK2 plaque assays were performed for each donor/drug combination in technical triplicate as previously described (1). Three log$_{10}$ dilutions of supernatant were plaqued for each donor/drug technical replicate in further triplicate. The dilution of virus yielding an easily countable range of plaques (~5-50) was retained for data analysis providing 9 technical replicate plaque counts for a given donor/drug dilution. For each donor/drug experiment, the average of viral titre of untreated samples (i.e. no drug) was set to 100 percent infectious virus release and used to normalize all other samples. Viral titre of technical replicates of each drug concentration were averaged and standard deviations determined. Drug dilutions were generally carried out in 0.5log$_{10}$ steps (e.g. 31.6 mM, 10 mM, 3.16 mM, 1 mM, etc.) Average percent infection as the dependent variable was plotted along a linear y-axis (from 0 to 100) against a logarithmic x-axis (drug concentration). The x-value (drug concentration) of untreated cells was adjusted to 1.5log$_{10}$ steps lower than the global minimum drug concentration tested (i.e. lowest concentration tested of 0.0316μM, therefore untreated concentration set to 0.001μM) to allow for plotting along a logarithmic axis (log$_{10}$(0)=undefined). Standard deviation error bars were included for each data point. CV and relative CV were calculated as described above.

In addition to individual drug/donor experiment graphs, the percent infection for each drug concentration for all donors of a given drug treatment (biological summary graph, Figure 1 D). This process was carried out for every concentration tested of all 3 drugs. Standard deviation, CV, and relative CV for each point were determined as measures of biological variation and precision.

Curve Fitting
Curve fits as described above were applied to each donor/drug graph as well as to each biological summary graph with the exception of the exponential curve type. The non-linear curve fit that best fit the data (produced the lowest $\chi^2$) was solved for $y=50$ to determine the IC$_{50}$ and for $y=10$ to determine the IC$_{90}$. Values across donors for each drug were averaged and standard deviations determined. These mean IC$_{50}$ and IC$_{90}$ value were compared to estimates solved based on the biological summary graphs. All estimates for each drug were comparable, thus only the mean value +/- standard deviation is reported.