Supplemental Information

Mechanism of Enhanced Cellular Uptake and Cytosolic Retention of MK2 Inhibitory Peptide Nano-polyplexes

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Abbreviated title for running head: Uptake and Trafficking of MK2i Peptide Nano-polyplexes

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**Supplemental figure S1**

**Relative MK2i NP (10 uM) Uptake by flow cytometry**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Inhibitor Dose</th>
<th>Relative Uptake</th>
<th>P=</th>
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</thead>
<tbody>
<tr>
<td><strong>Macropinocytosis</strong></td>
<td>Wortmannin 100 nM</td>
<td>0.694277929</td>
<td>0.023836914</td>
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<tr>
<td></td>
<td>Cytochalasin D 5 uM</td>
<td>0.53133515</td>
<td>0.001915453</td>
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<tr>
<td></td>
<td>EIPA 100 uM</td>
<td>0.531880109</td>
<td>0.003708863</td>
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<tr>
<td><strong>Dynamin</strong></td>
<td>Dynasore 100 uM</td>
<td>0.60027248</td>
<td>0.002711403</td>
</tr>
<tr>
<td><strong>Lipid Raft</strong></td>
<td>Nystatin 50 ug/mL</td>
<td>0.839374317</td>
<td>0.053130527</td>
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<tr>
<td></td>
<td>Methyl Beta Cyclodextrin 5mM</td>
<td>0.977983988</td>
<td>0.634434414</td>
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<tr>
<td><strong>Scavenger Receptors</strong></td>
<td>Polyinosinic Acid 50ug/mL</td>
<td>0.922458629</td>
<td>0.170255514</td>
</tr>
<tr>
<td></td>
<td>Dextran Sulfate 100 ug/mL</td>
<td>1.063211125</td>
<td>0.769427695</td>
</tr>
</tbody>
</table>
**Supplemental figure S2:**
DLS Measurements confirm gold sol loading into MK2i-NP.

**Au loading into Au-MK2i-NP**
*(DLS, hydrodynamic diameter, nm)*

![Graph](image)

*Shows DLS trace for:*

- **Au** - Gold sol only (10 nm nominal)
- **Au-MK2i-NP** - Gold loaded into MK2i-NP (PPAA, Au, then 2x MK2i)

*Peaks labeled with nominal max.*

*Disappearance of gold trace in Au-MK2i-NP indicates Au loading into NP.*
Supplemental figure S3:
MK2i NP concentrates at cell membranes during uptake

Fig S3. Laser scanning confocal microscopy; cell nuclei labeled with N. Composite image, DIC (grey) and MK2i-NP (green). White arrow shows membrane association; red arrow indicates individual endosomes. T=30 min.