Supplemental Information:

**Nanoparticle Encapsulation of Non-genotoxic p53 Activator Inauhzin-C for Improved Therapeutic Efficacy**

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**Figure S1. NMR characterization of n-INZ-C**

A. Structure of INZ-C molecule along with the identification of hydrogens for the NMR peaks presented in Figure 1B

B. NMR spectra of Heptakis(2,6-di-O-methyl)-β-cyclodextrin

C. NMR spectra of Inauhzin-C (INZ-C)

D. NMR spectra of the complex of INZ-C and CD

**Figure S2. DLS and Zeta Potential of n-INZ-C**

A. DLS of nanoparticles immediately following synthesis and after 1 month of storage in -20°C

B. Zeta potential of the nanoparticles immediately following synthesis and after 1 month of storage in -20°C

**Figure S3. TEM histogram and UV-VIS Calibration**
A. Histogram of nanoparticle sizes from TEM
B. UV Vis absorbance of INZ-C standards at different concentrations
C. Calibration curve generated from absorbance measurements in Figure S3B

**Figure S4. Optimization of p53 activation by n-INZ-C**

A. IB of HCT116\(^{p53^+/+}\) cells treated for 18 h. with n-INZ-C containing different ratios of CD/INZ-C ranging from 1:10, 1:15, and 1:20.
B. IB of dose dependent p53 activation of n-INZ-C. Cells were treated with either 1, 0.5 and 0.1 \(\mu\text{M}\) of n-INZ-C or INZ-C and the p53 and p21 activation was compared.
C. IB of cells treated with 0.5 \(\mu\text{M}\) of n-INZ-C initially and after 1 month of storage of n-INZ-C in -20ºC.

**Figure S5. In Vitro activity of n-INZ-C in colon and lung cancer cell lines**

A. IB of HCT116\(^{p53^{-/-}}\) and H1299 cells treated with 0.5 \(\mu\text{M}\) of INZ-C, n-INZ-C, CD, NPC and DMSO control for 18 h. IB was probed for p53, p21 and PUMA.
B. Cell proliferation of HCT116\(^{p53^{-/-}}\) and H1299 cells treated with 0.5 \(\mu\text{M}\) INZ-C, n-INZ-C, DMSO control and NPC for 3 days. Images were taken using incucyte and cell proliferation was calculated using incucyte S3 software. Data represents the average of triplicate measurements of the cell confluence.
C. Images taken using Incucyte of HCT116\(^{p53^+/+}\) and H460 cells after 68 hrs of treatment with 0.5 \(\mu\text{M}\) INZ-C, n-INZ-C, DMSO control and NPC.
Figure S6. *In vitro* activity in melanoma and breast cancer cell lines

A. IB of SKMEL 103, SKMEL 147 and MCF7 cells treated with 0.5 µM of INZ-C, n-INZ-C, CD, NPC and DMSO control. IB was probed with p53, p21 and PUMA.

B. Cell proliferation of SKMEL 103, SKMEL 147 and MCF7 cells treated with INZ-C, n-INZ-C, NPC and DMSO control. All measurements of cell confluence were calculated from incucyte images using the incucyte S3 software. Results represent the average of triplicate measurements of cell confluence.

Figure S7. IC$_{50}$ curves for NPC, INZ-C and n-INZ-C in colorectal and lung cancer cell lines

Dose dependent cell proliferation of NPC, INZ-C and n-INZ-C in (A) HCT116$^{p53+/+}$, (B) H460, (C) HCT116$^{p53/-}$, (D) H1299. Measurements of cell confluence were calculated from incucyte images using the incucyte S3 software. IC$_{50}$ was determined using the equation provided in materials and methods.

Figure S8. Mice weight and organ staining images from *in vivo* toxicity studies

A. H/E staining images of liver, kidney, spleen and lungs of mice treated with high dose (50 mg/kg) and low dose (25 mg/kg) of NPC for 7 days.

B. Weight of mice treated with n-INZ-C, high (50 mg/kg) and low dose NPC (25 mg/kg) and PBS control for 7 days

Figure S9. Calibration data for *in vivo* pharmacokinetic analysis
A. Peak areas of internal standard and n-INZ-C in samples of mice blood determined from MS analysis. Protein in blood samples was precipitated using acetonitrile precipitation as explained in the materials and methods. Samples were then diluted and subjected for MS analysis.

B. Calibration curve for MS detection of INZ-C in plasma data presented in Figure S9A.

Figure S10. Mice weight and tumor imaging from H460 Xenograft model

A. Mice weight during 18-day treatment with 30 mg/kg of n-INZ-C and NPC and 30 mg/kg of INZ-C and PBS + 5% DMSO control.

B. Images of tumors from mice treated with 30 mg/kg NPC and n-INZ-C
Figure S1

A

B

CD

C

INZ-C

D

Complex
**Figure S2**

### A

**NUMERICAL GAUSSIAN DISTRIBUTION**

**Immediate**

**1 month of storage**

### B

|                      | Immediately after Synthesis | After 1 month storage |
|----------------------|-----------------------------|-----------------------|
| Zeta Potential (mV)  | -10 mV                      | -35 mV                |
Figure S3

A. Histogram of Nanoparticle Sizes

B. UV VIS Absorbance of INZ-C Standards

C. Calibration Curve

\[ y = 0.0117x + 0.248 \]

\[ R^2 = 0.9993 \]
Figure S4

A

|        | Control | INZ C | INZ CH CD NP 1:15 | INZ CH CD NP 1:20 | INZ CH CD NP 1:10 |
|--------|---------|-------|-------------------|-------------------|-------------------|
| CONC. µM | 0.5  | 1  | 2 | 0.5 | 1 | 2 | 0.5 | 1 | 2 |
| 55      |      |    |    |    |    |    |    |    |    |
| 34      |      |    |    |    |    |    |    |    |    |

p53

GAPDH

B

|        | Control | Ch NP | CD | INZ C | 1:20 n-INZ-C |
|--------|---------|-------|----|-------|--------------|
| CONC (µM) | 0 | 2 | 40 | 0.1 | 0.5 | 1 |
| 55      |      |    |    |    |    |    |
| 34      |      |    |    |    |    |    |
| 25      |      |    |    |    |    |    |

p53

GAPDH

p21

C

|        | Control | CD | NPC initial | N-INZ_C 1 month |
|--------|---------|----|-------------|-----------------|
| CONC   | 55      |    | 34          |                 |

p53

GAPDH
Figure S5

A

HCT116 -/-

| CONC. µM | 0.5 | 40 | 0.5 | 0.5 |
|----------|-----|----|-----|-----|
| p53      | 55- |    |    |     |
| GAPDH    | 34- |    |    |     |
| PUMA     | 26- |    |    |     |
| p21      | 17- |    |    |     |

H1299

| CONC. µM | 0.5 | 40 | 0.5 | 0.5 |
|----------|-----|----|-----|-----|
| INZ-C    | 55- |    |    |     |
| n-INZ-C  | 34- |    |    |     |
| NPC      | 26- |    |    |     |
| INZ-C    | 17- |    |    |     |

B

HCT116 -/-

% Cell Confluence vs. Time (h)

H1299

% Cell Confluence vs. Time (h)

C

HCT116 +/-

Control NPC 0.5 µM INZ 0.5 µM 1:20 0.5 µM

H460

Control NPC 0.5 µM INZ 0.5 µM 1:20 0.5 µM
Figure S7

A. HCT116 +/+  

B. H460

C. HCT116 -/-  

D. H1299
A

| Weight of Mice (g) | Days with Injection |
|-------------------|---------------------|
| Control (PBS Buffer) | NPC 25 mg/kg |
| NPC 50 mg/kg | NPC 50 mg/kg |
| NPC 50 mg/kg + INZ C 30 mg/kg |

B

Figure S8

- Intestine
- Stomach
- Liver
- Kidney
- Spleen
- Lung

![Histological images of various organs at different NPC dosages](image_url)
A

| nM  | ANA   | INZ-C  | Ratio |
|-----|-------|--------|-------|
| 0   | 29422 | 8617.123 | 0.292 |
| 18.25 | 26418 | 8152.34 | 0.308 |
| 37.5 | 34657 | 14940 | 0.431 |
| 75   | 29808 | 16550 | 0.555 |
| 150  | 32918 | 20659 | 0.627 |
| 300  | 23265 | 22338 | 0.960 |
| 1000 | 30279 | 66637 | 2.200 |

B

\[
y = 0.0019x + 0.3441\]

\[R^2 = 0.9941\]
Figure S10

A

Mice Weight

Days

Weight (g)

Control

INZ-C

B

Mouse 2-1

Mouse 2-2

Mouse 3-1

Mouse 3-2

Control Mice

n-INZ-C