SUPPORTING INFORMATION

Bioorthogonal uncaging of cytotoxic paclitaxel through Pd nanosheet-hydrogel frameworks

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1. General Methods

NMR spectra were recorded at ambient temperature on a 500 MHz Bruker Avance III spectrometer. Chemical shifts are shown in parts per million (ppm) relative to the solvent peak. Compounds purity was >95% for cell studies, as measured by TLC, NMR or HPLC using an Agilent 1200 system. HPLC method: eluent A: water and formic acid (0.1 %); eluent B: methanol and formic acid (0.1 %); A/B = 95:5 to 5:95 in 4min, isocratic 2 min (flow = 1 mL / min). Prodrug-into-drug conversion experiments were conducted in a LCMS/MS (Agilent 1200) using a micrOTOF II detector or an Orbitrap XL mass spectrometer from Thermo Fisher. LCMS method A: eluent A: water and formic acid (0.1 %); eluent B: acetonitrile and formic acid (0.1 %); A/B = 95:5 isocratic 0.5 min, 95:5 to 0:100 in 4.5 min, isocratic 2 min, 0:100 to 95:5 in 0.5 min, and isocratic 2.5 min (flow = 0.2 mL / min). LCMS method B: eluent A: water and formic acid (0.1 %); eluent B: acetonitrile and formic acid (0.1 %); A/B = 80:20 to 0:100 in 10 min, isocratic 3 min, 0:100 to 80:20 in 5 min (flow = 0.3 mL / min). Stock solutions (100 mM) were prepared in DMSO. ICP-OES measurement were carried out in a Varian 715 ICP optical emission spectrometer.

2. Characterization of compounds

2.1. NMR of *N*-(propargyloxycarbonyl)-*N*,*N*'-dimethylethylenediamine (4)

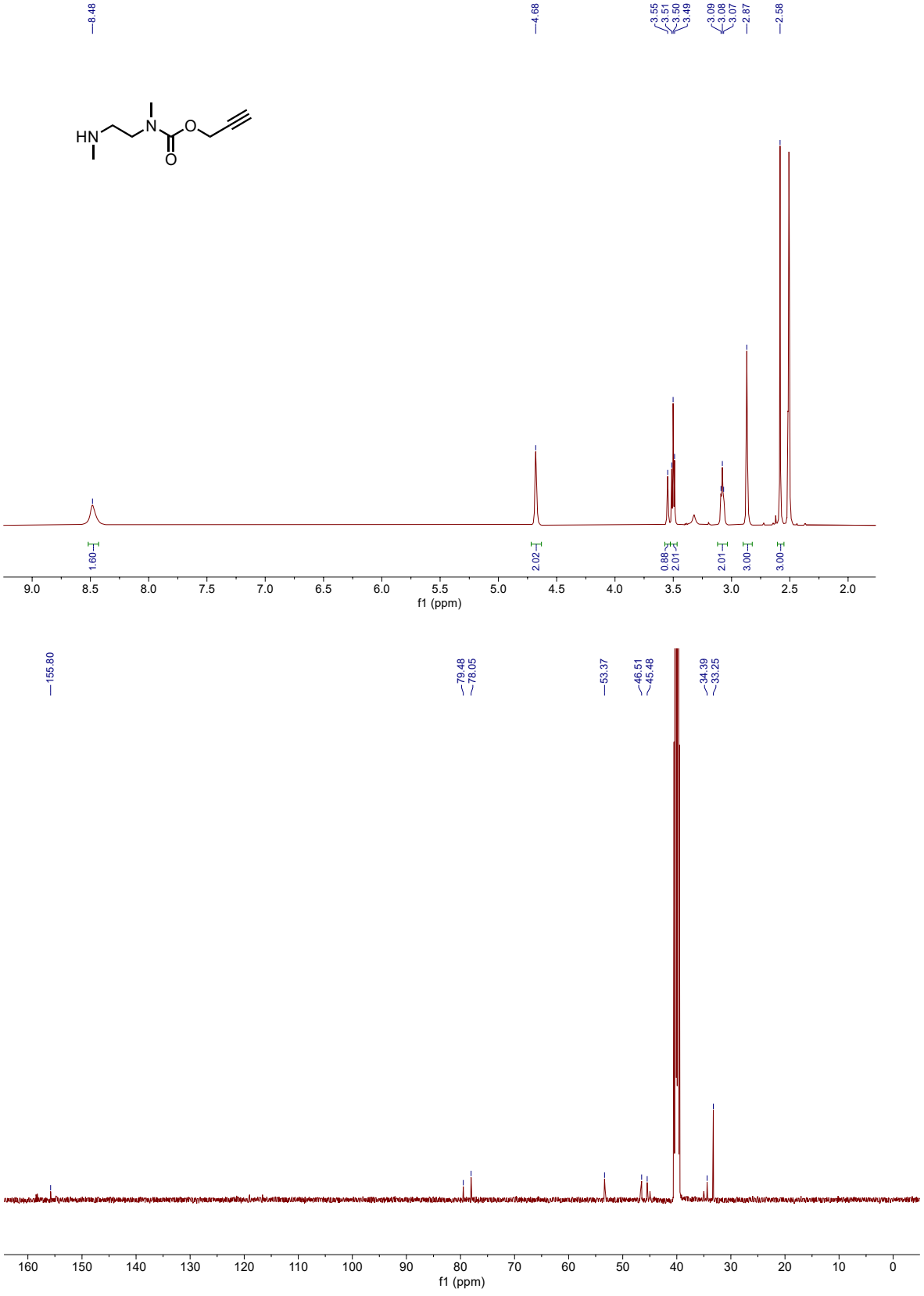

Fig. S1. H and C-NMR of compound **4**.

2.2. NMR of Pro-PTX

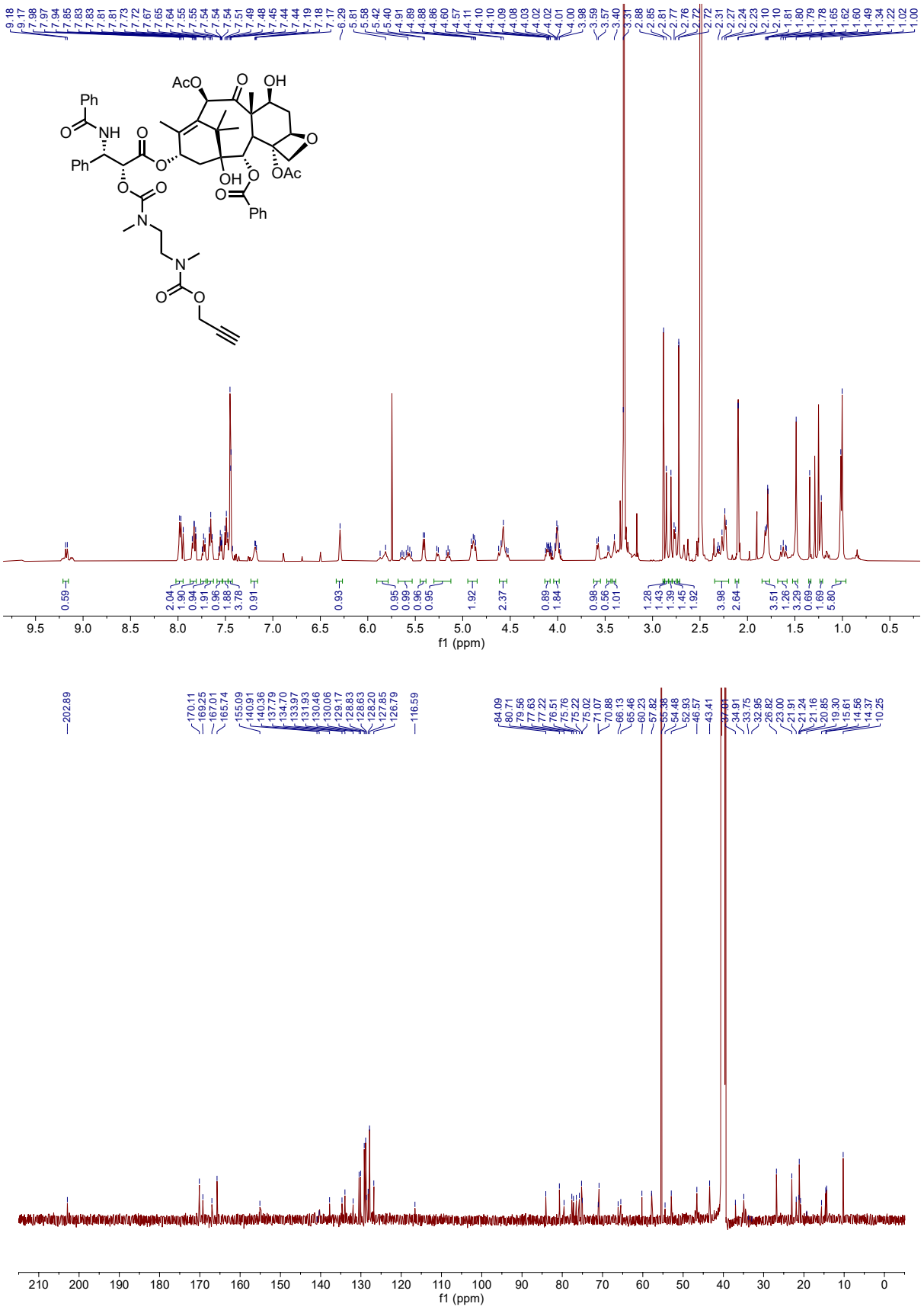

Fig. S2. H and C-NMR of **Pro-PTX**.

3. Fluorogenic assay and cell viability assays with Pd nanosheets

Pro-Res (100 μM) was dissolved in a PBS solution (0.2 mL) with a range of concentrations of **Pd nanosheets** (5-40 μg/mL) in triplicates. The mixtures were shaken at 700 rpm and 37°C in a Thermomixer for 24 h. Reaction crudes were centrifuged (13,000 rpm, 30 min) and fluorescence intensity of 50 μL of supernatants transferred to a 96-well plate format was measured in a PerkinElmer EnVision 2101 multilabel reader (Ex / Em: 540 nm / 590 nm). The percentage of conversion was calculated based on the fluorescence signal of positive control **Resorufin** at 100 μM.

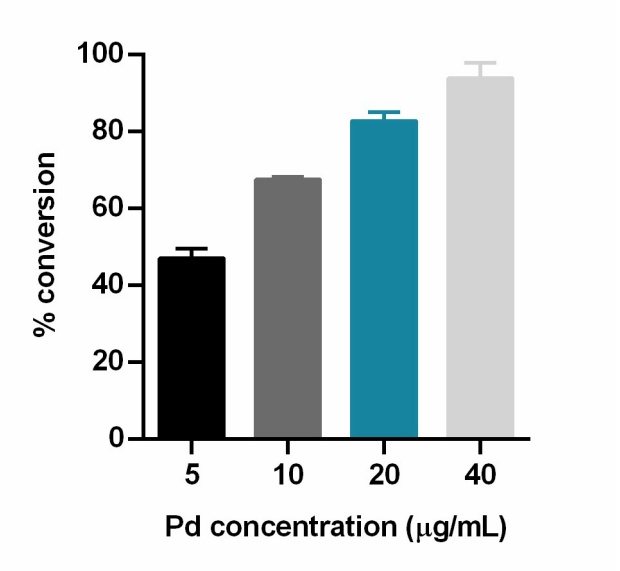

Fig. S3. Conversion of **Pro-Res** (100 μM) into resorufin by **Pd nanosheets** (5-40 μg/mL) after 24 h incubation in PBS at 700 rpm and 37 °C in the Thermomixer. As a negative control, **Pro-Res** was incubated in PBS using same conditions in the Thermomixer. Fluorescence signal was measured at $\lambda_{ex/em}$ 540 / 590 nm. Error bars: ± SD from n = 3.

5 μg/mL of Pd nanosheets= 47 μM in Pd; 10 μg/mL of Pd nanosheets= 94 μM in Pd; 20 μg/mL of Pd nanosheets=188 μM in Pd; 40 μg/mL of Pd nanosheets=376 μM in Pd

This means that, apart from the reaction with 5 μg/mL of **Pd nanosheets**, the rest of them used equal or higher concentration of metallic Pd than that of probe. However, for heterogeneous catalysts, it is important to note that the total amount of metal atoms can be considerably higher than the amount of active metal sites, since usually only

the exposed surface atoms are active. In this case, each nanosheet is formed by ca. 9 layers of atoms, and those in inside layers would not be in contact with the liquid phase.

The full methodology of the cell viability studies is described in the main manuscript.

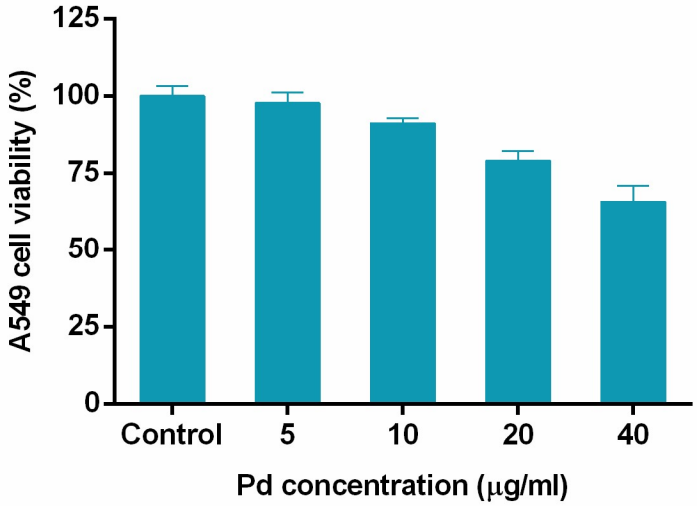

Fig. S4. A549 cell viability study after treatment with **Pd nanosheet** at different concentrations. Cell viability was measured at day 3 using PrestoBlue™ reagent. Error bars: ± SD from n = 3.

4. Prodrug-into-drug conversion studies using Pd nanosheets

Pro-PTX (100 μM) was dissolved in PBS (1 mL) with 20 of **Pd nanosheets** and incubated at 37 °C in a Thermomixer at 1,200 rpm during 6 and 24 h. Reaction crudes were centrifuged (13,000 rpm, 30 min) and filtered through StageTips to remove the **Pd nanosheets** and supernatants were analyzed by LCMS (Agilent 1200) using an Orbitrap XL mass spectrometer (Thermo Fisher, Ion source ESI). **PTX** (100 μM) and **Pro-PTX** (100 μM) in PBS were used as analytical controls. Column: Grom-Sil-120-ODS-4-HE (Grace), length 50mm, ID 2mm, 3μm. Method: **B**.

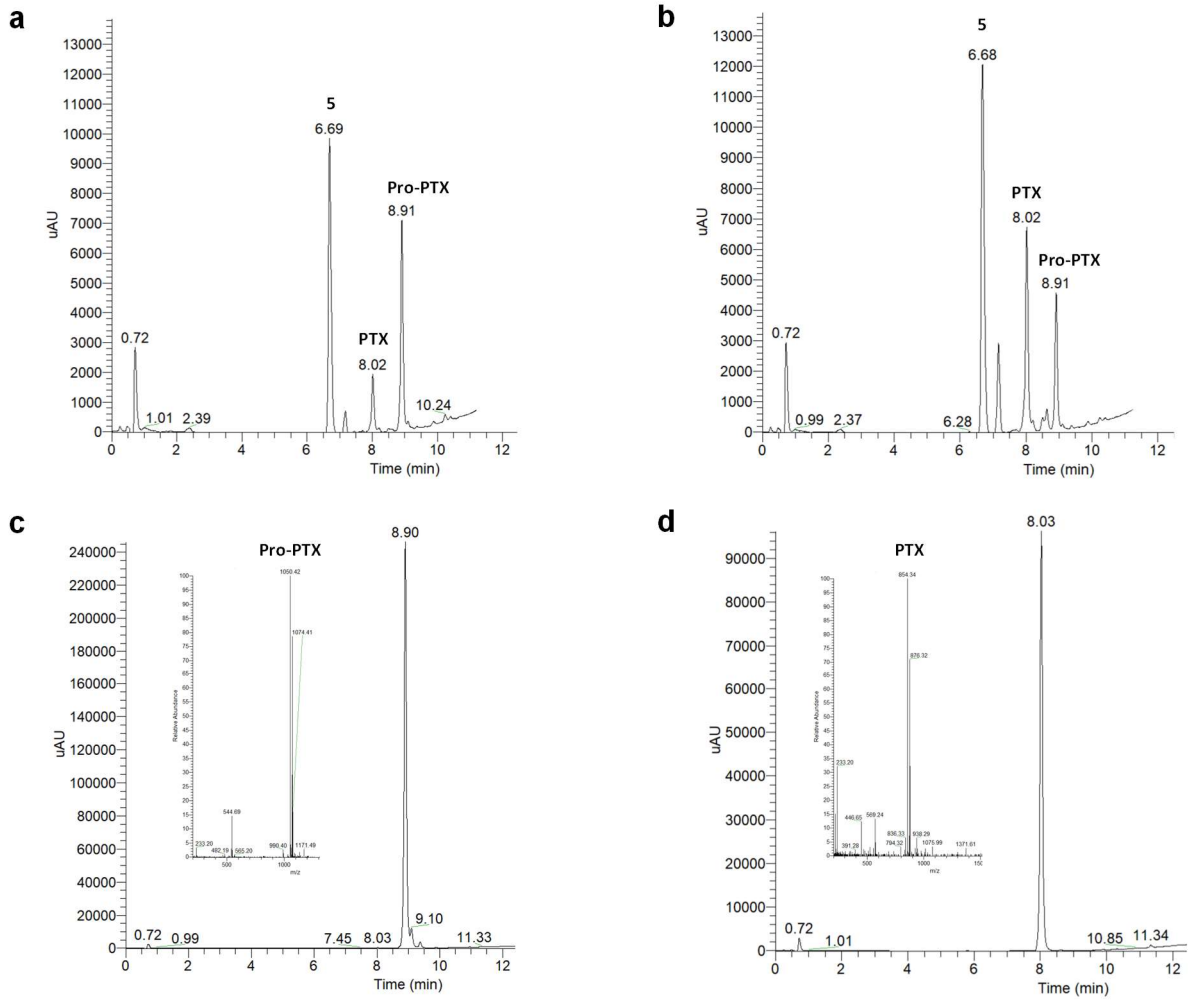

Fig. S5. LCMS analysis of the reaction of **Pro-PTX** with **Pd nanosheets** after (a) 6 h and (b) 24 h. (c) **Pro-PTX**. (d) **PTX**.

5. Characterization of Pd Agarose hydrogels

SEM images were performed using a FEI Quanta 400 ESEM equipped with an EDX analytical system (Figure S6). A portion of hydrogel was supercriticaly dried and coated with a fine carbon layer. Afterwards, the samples were examined by SEM using secondary and backscatter electron detectors.

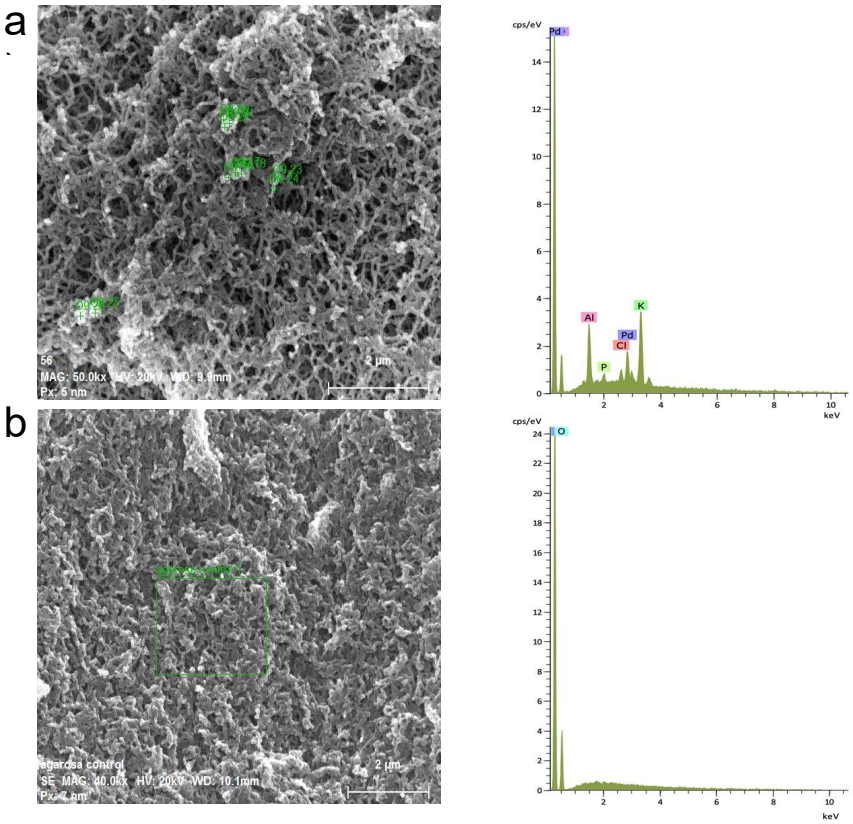

Fig. S6. SEM (left, showing analyzed areas in green) and elemental composition EDX spectrum (right) of representative (a) **Pd-Agarose** hydrogel and (b) Agarose hydrogel.

6. Prodrug activation with Pd Alginate in U87 cell culture

U87 cells were seeded in a 24-well plate format (at 9,000 cells / well) and incubated for 24 h before treatment. Each well was replaced with fresh culture media containing **Pro-PTX** or **PTX** (0.3 μM). Untreated cells were incubated with DMSO (0.1 % v/v). **Pd Alginate** hydrogels were prepared following the procedure previously described. **Pd Alginate** hydrogels were added at 3 beads/well (=20 μg/mL of metal). After 3 d of incubation, cell viability was determined as described above. Experiments were performed in triplicates.

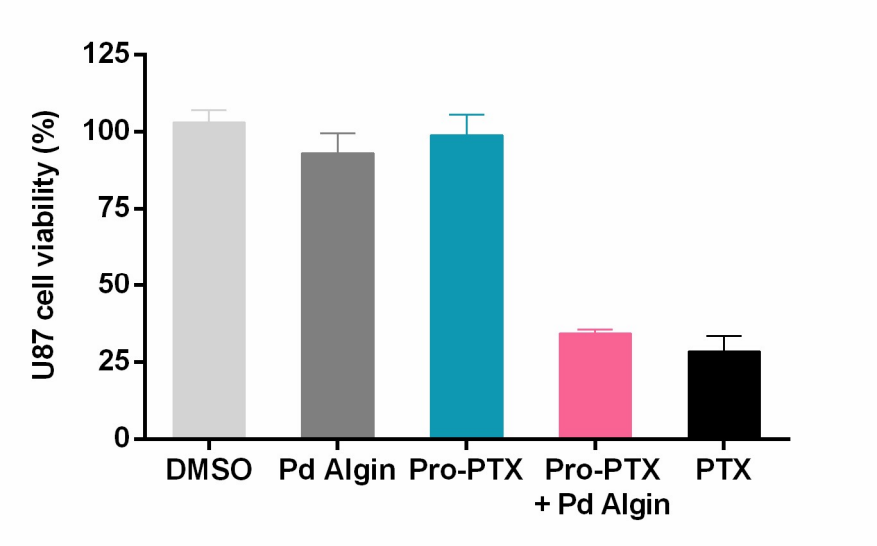

Fig S7. Prodrug activation assays in U87 cells with **Pd Alginate**. Experiments: 0.1% DMSO (untreated control, light grey); **Pd Alginate** (–ve control, dark grey); 0.3 μM **Pro-PTX** (–ve control, blue); **Pd Alginate** + 0.3 μM **Pro-PTX** (activation assay, pink); 0.3 μM **PTX** (+ve control, black). Cell viability was measured at day 3 using PrestoBlue. Error bars: ± SEM, n = 3.

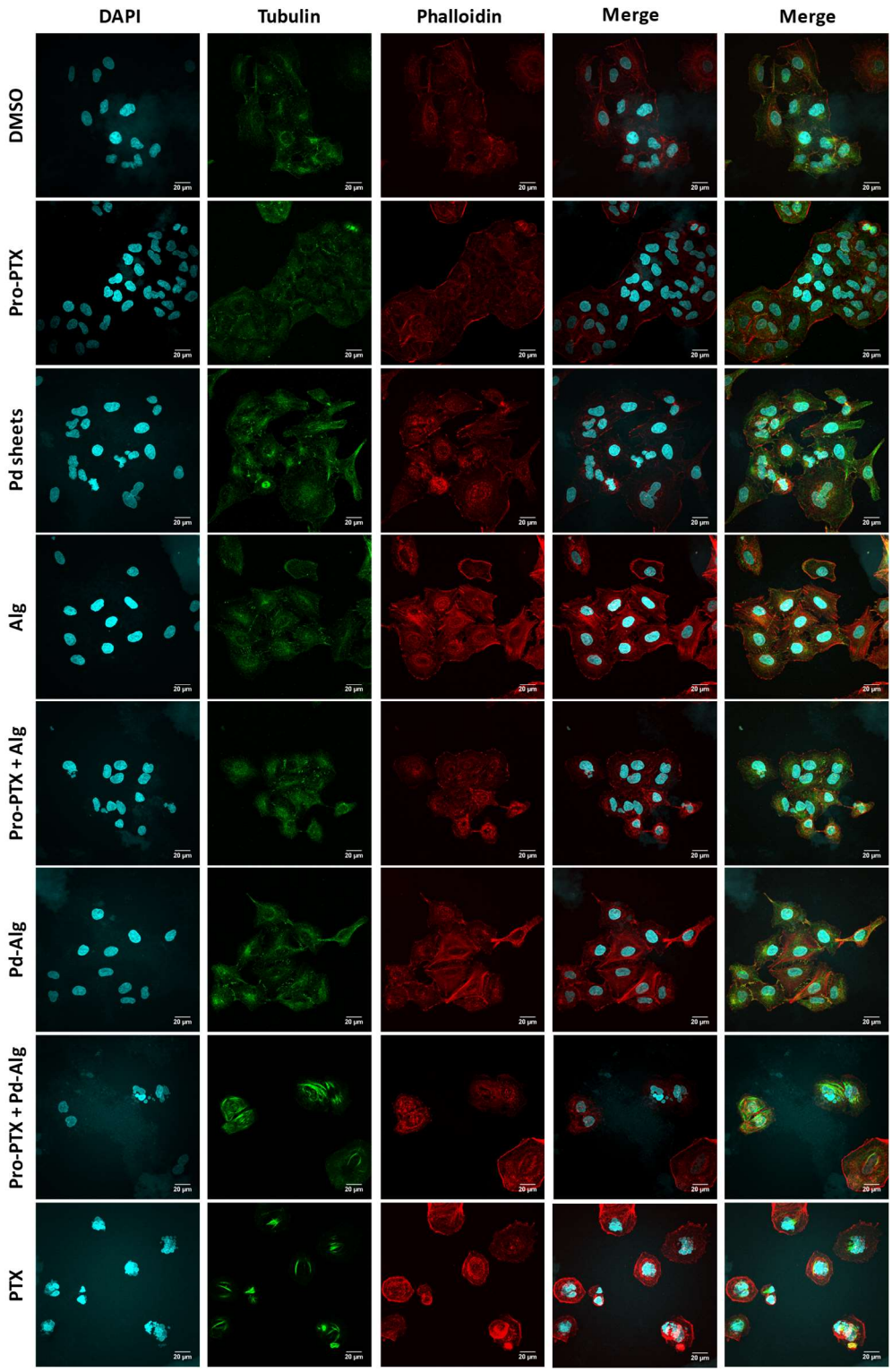

Fig. S8. Full immunofluorescence study in A549 cells. DMSO control (DMSO); 0.75 μM **Pro-PTX**; Pd nanosheets 20 μg/mL (Pd sheets); Alginate (no Pd) (Alg); 0.75 μM **Pro-PTX** + Alginate (no Pd) **(Pro-PTX** + Alg); Pd Alginate 20 μg/mL (Pd Alg); (c) Pd Alginate + 0.75 μM **Pro-PTX** (prodrug activation) (**Pro-PTX** + Pd-Alg); 0.75 μM **PTX**. Staining: anti-α-tubulin, TRITC-phalloidin and DAPI. Scale bar = 20 μm.