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SUPPLEMENTAL DATA

IC_{50}: an unsuitable measure for large-sized prostate cancer spheroids in drug sensitivity evaluation

1. Evaluation of LNCaP cells/spheroids growth in medium with variable FBS concentrations

We firstly evaluated the LNCaP cells/spheroids growth in different FBS concentrations (2%, 5%, 7.5% and 10%) for 18 days. We found that no living cells/spheroids could be observed after the day 10 (Figure S1 A3), when the LNCaP cells/spheroids were cultured in medium with 2% FBS. But for the LNCaP cells/spheroids cultured in the medium with 5% or 7.5% FBS, the timepoint for no living cells/spheroids observed was the day 14 (Figure S1 B4-C4). For the LNCaP cells/spheroids cultured in medium with 10% FBS, living cells/spheroids were observed, and the size of the cells/spheroids were growing over time (Figure S1 D1-D5). These results show that lower FBS concentrations are not suitable for the embedded cultured LNCaP spheroids' survival and growth. Therefore, all the following experiments based on 3D cultured LNCaP cells/spheroids were maintained in RPMI + 10% FBS + 1% PS.

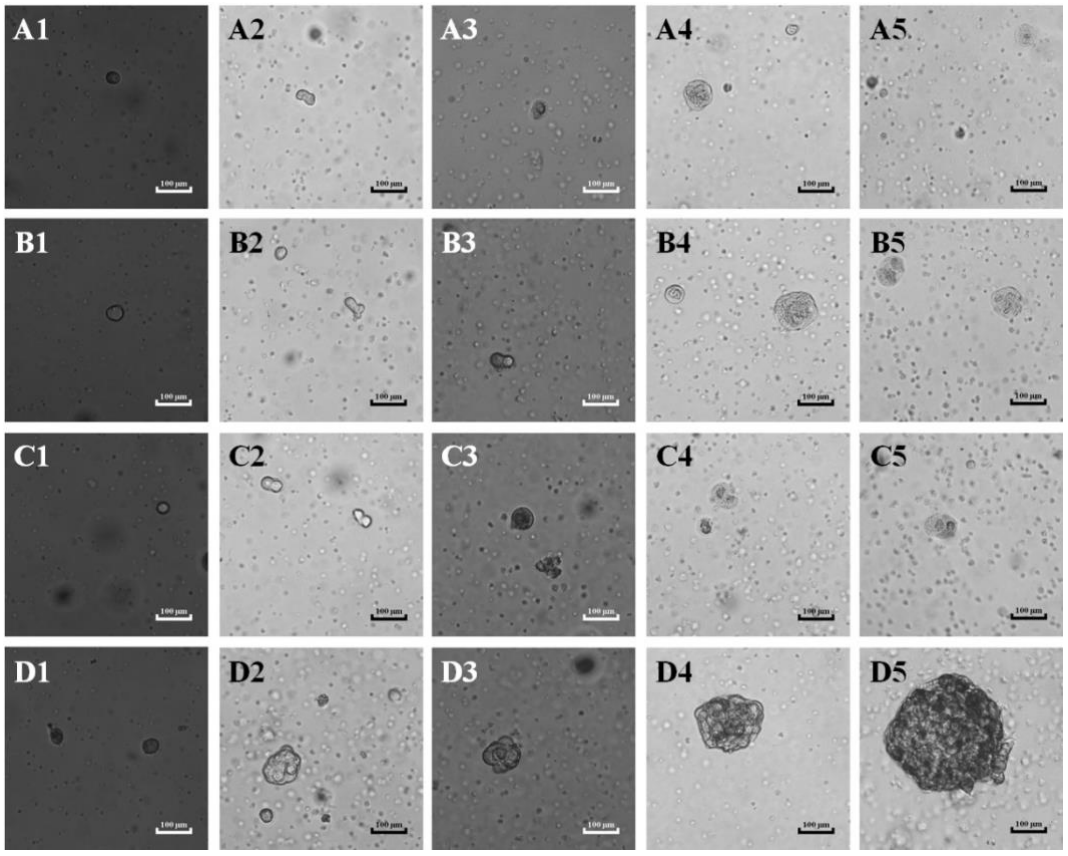

Figure S1. Representative images of LNCaP cells/spheroids cultured in varying FBS concentration. (A1-A5) The images of LNCaP cells/spheroids cultured in 2% FBS at day 3, 7, 10, 14, and 18; (**B1-B5)** The images of LNCaP cells/spheroids cultured in 5% FBS at day 3, 7, 10, 14, and 18; (**C1-C5)** The images of LNCaP cells/spheroids cultured in 7.5% FBS at day 3, 7, 10th, 14, and 18; (**D1-D5)** The images of LNCaP cells/spheroids cultured in 10% FBS at day 3, 7, 10, 14, and 18.

2. Susceptibility of LNCaP cells/spheroids exposed to docetaxel treatment

2.1 Evaluation of the LNCaP cell viability exposed to DMSO: CellTiter Glo assays were performed to analyze the LNCaP cell viability exposed to different DMSO concentrations, which represented the DMSO concentrations in the following drug testing experiments. We found that DMSO did not significantly inhibit the cell viability in concentrations ranging from 5×10^{-7} % to 5×10^{-2} % (Figure S2).

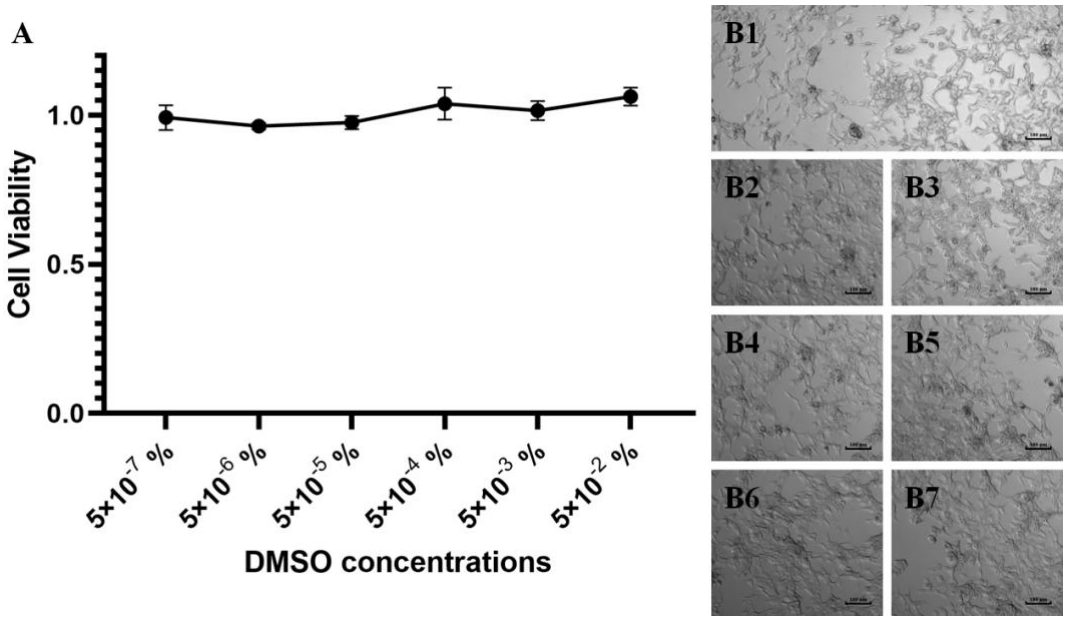

Figure S2. LNCaP cell viability exposed to DMSO. (A) LNCaP cell viability exposed to different DMSO concentrations. (**B1-B7)** The images of the LNCaP cells exposed to different DMSO concentrations for 5 days: B1: 0 (NC), B2: 5×10^{-7} %, B3: 5×10^{-6} %, B4: 5×10^{-5} %, B5: 5×10^{-4} %, B6: 5×10^{-3} %, B7: 5×10^{-2} %.

2.2 Susceptibility of 2D/3D LNCaP cells/spheroids exposed to docetaxel treatment

2.2.1 Susceptibility of LNCaP cells cultured in Matrigel for 4 days: Based on our previous results, LNCaP cells remained single cells in Matrigel for four days, and we compared the susceptibility of the LNCaP cells cultured in Matrigel for two days with 2D-cultured LNCaP cells, which showed similar IC_{50} values but different R^2 /maximum inhibition values (Subheading S3). In this part, we first cultured the LNCaP cells in the Matrigel for 4 days and then exposed the cells to docetaxel. IC_{50} values were 4.140, 5.553, and 3.630 nM (Figure S3 A-C). The images also show that the LNCaP spheroids formation was inhibited by docetaxel (Figure S3 D0-D9).

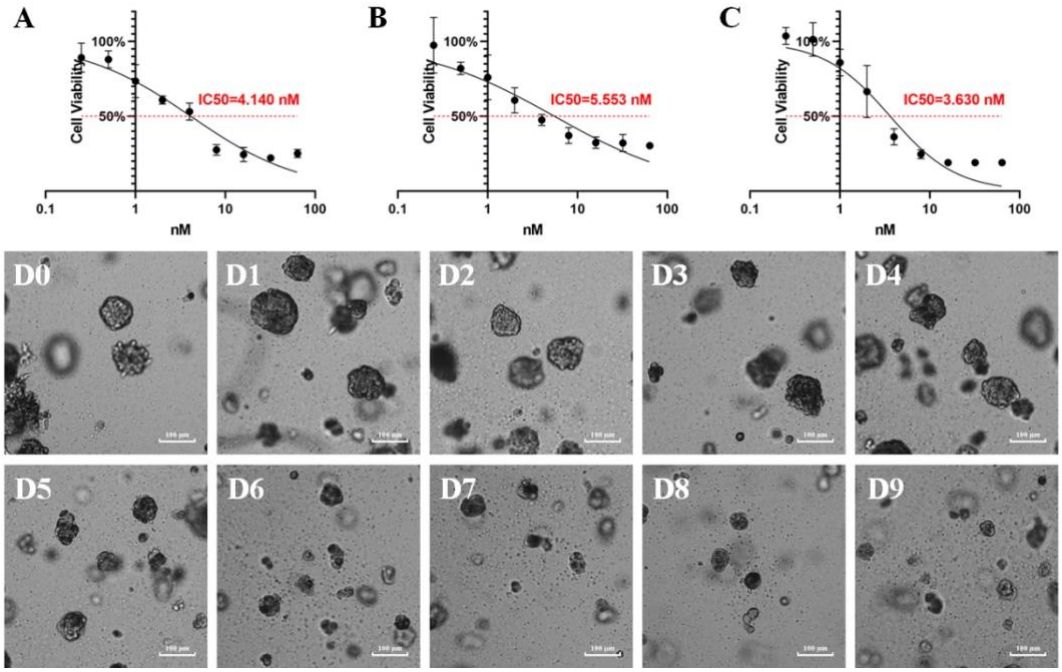

Figure S3. Susceptibility of LNCaP cells cultured for four days in Matrigel to docetaxel treatment. (A-C) The drug testing results. (**D0-D9)** The images of drug testing experiments of LNCaP cells cultured for four days in Matrigel exposed to varying docetaxel concentrations: D0: The image of negative control without docetaxel; D1-D9: The images of the LNCaP cells exposed to varying docetaxel concentrations for 5 days (0.25 nM, 0.5 nM, 1 nM, 2 nM, 4 nM, 8 nM, 16 nM, 32 nM, and 64 nM).

2.2.2 Susceptibility of LNCaP spheroids cultured in Matrigel for seven days: Our previous results indicated that small-sized LNCaP spheroids were formed in Matrigel when LNCaP cells were cultured for seven days. The drug testing experiments in this part were based on these small-sized spheroids instead of single LNCaP cells. The IC_{50} values in this experiment were 9.903, 16.07, and 15.23 nM (Figure S4 A-C). The images show that the growth of LNCaP spheroids was inhibited by docetaxel, and some LNCaP spheroids exposed to higher docetaxel concentrations appeared loose and flat (Figure S4 D0-D9).

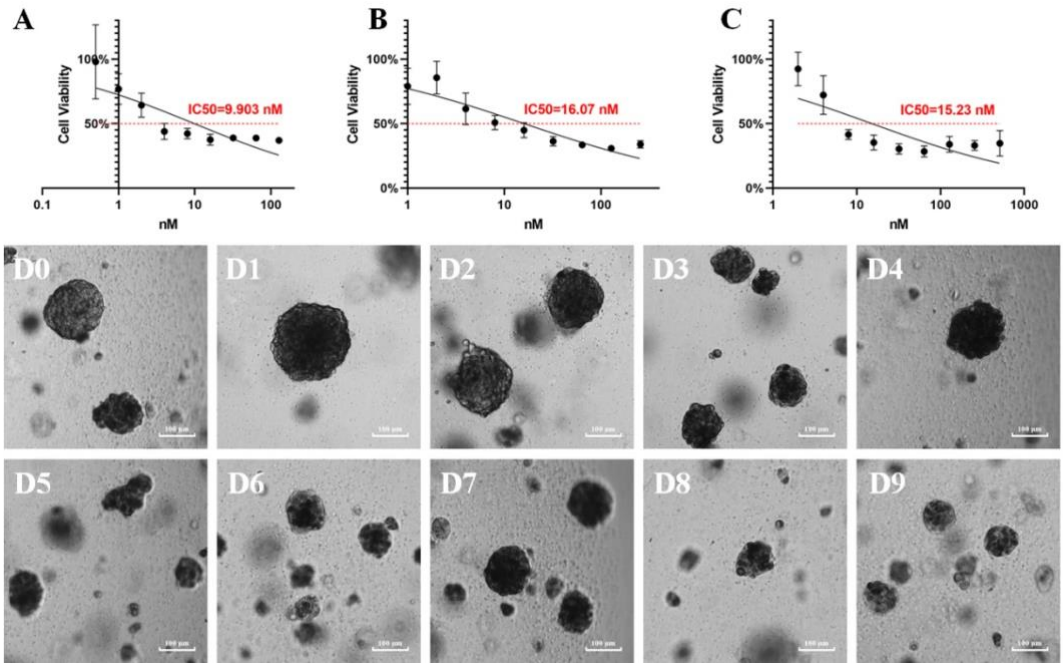

Figure S4. Susceptibility to docetaxel treatment of LNCaP spheroids cultured for seven days in Matrigel. (A-C) The drug testing results. (**D0-D9)** The images of drug testing experiments in LNCaP spheroids cultured for 7 days in Matrigel exposed to varying docetaxel concentrations: D0: The image of negative control without docetaxel; D1-D9: The images of the LNCaP spheroids exposed to varying docetaxel concentrations for 5 days (1 nM, 2 nM, 4 nM, 8 nM, 16 nM, 32 nM, 64 nM, 128 nM, and 256 nM).

2.2.3 Susceptibility of LNCaP spheroids cultured in Matrigel for 14 days: Our previous results indicated that LNCaP spheroids were formed in Matrigel when the LNCaP cells were cultured for 14 days. The drug testing experiments in this part were based on these LNCaP spheroids, and the IC_{50} values in this experiment were 31.38, 38.35, and 28.13 nM (Figure S5 A-C), much higher than those of 2D cells, 3D cells, and smaller spheroids. No significant differences in the spheroids were observed in the images, while the LNCaP spheroids exposed to higher docetaxel concentrations appeared loose and flat (Figure S5 D0-D9).

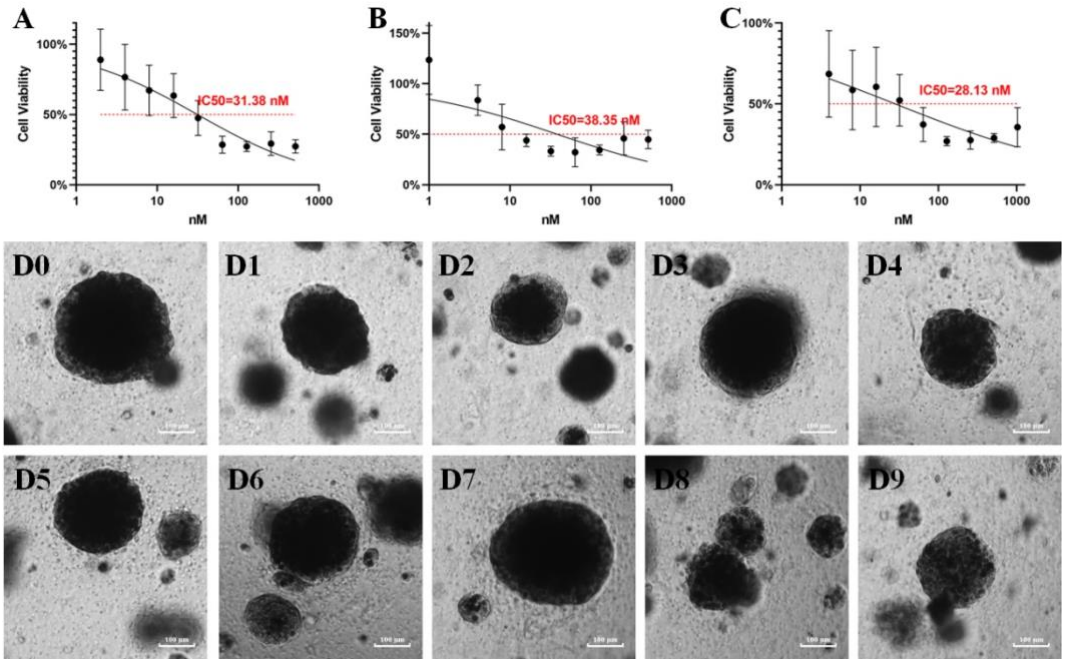

Figure S5. Susceptibility of LNCaP spheroids cultured for 14 days in Matrigel to docetaxel treatment. (A-C) The drug testing results. (**D0-D9)** The images of drug testing experiments in LNCaP spheroids cultured for 14 days in Matrigel exposed to varying docetaxel concentrations: D0: The image of negative control without docetaxel; D1-D9: The images of the LNCaP spheroids exposed to varying docetaxel concentrations for 5 days (2 nM, 4 nM, 8 nM, 16 nM, 32 nM, 64 nM, 128 nM, 256 nM, and 512 nM).

2.2.4 Susceptibility of LNCaP spheroids cultured in Matrigel for 21 days: The LNCaP spheroids cultured in Matrigel for three weeks were also used to evaluate the susceptibility to docetaxel treatment. The mean values of the cell viability were all higher than 50%. Though we elevated the maximum docetaxel concentration in the drug testing experiments from 256 nM to 2048 nM, cell viability curves reached the second plateau phase before 50% (Figure S6 A-C). No significant differences in the spheroids' formation were observed in the images, and the LNCaP spheroids exposed to higher docetaxel concentrations did not appear loose and flat in these experiments (Figure S6 D0-D9).

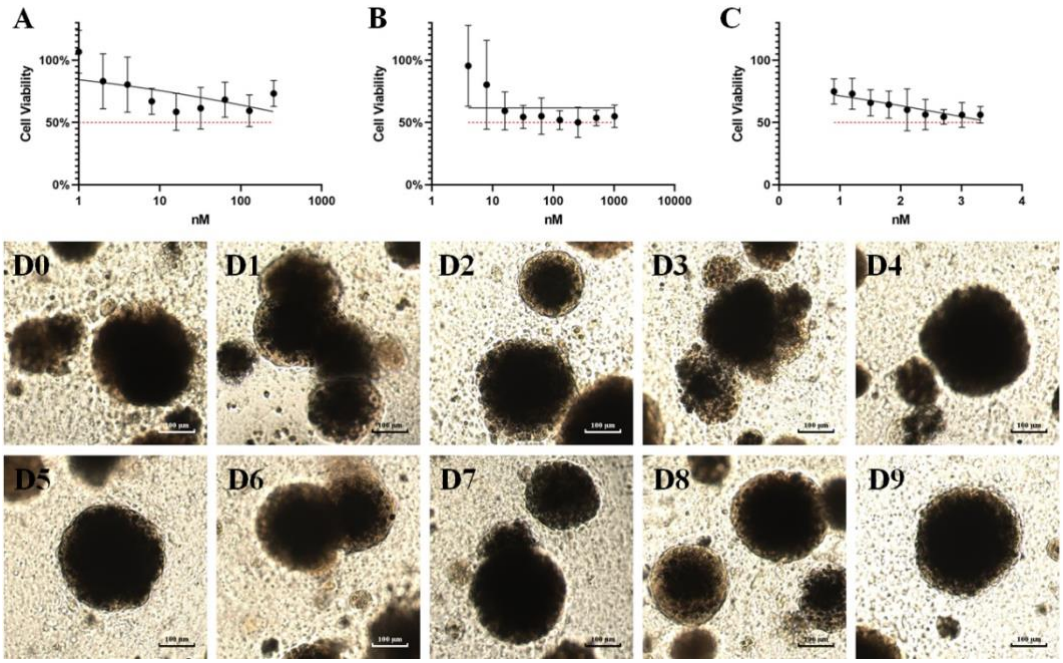

Figure S6. Susceptibility of LNCaP spheroids cultured for 21 days in Matrigel to docetaxel treatment. (A-C) The drug testing results. (**D0-D9)** The images of drug testing experiments in LNCaP spheroids cultured for 21 days in Matrigel exposed to varying docetaxel concentrations: D0: The image of negative control without docetaxel; D1-D9: The images of the LNCaP spheroids exposed to varying docetaxel concentrations for 5 days (4 nM, 8 nM, 16 nM, 32 nM, 64 nM, 128 nM, 256 nM, 512 nM, and 1024 nM).

2.2.5 Susceptibility of floating LNCaP spheroids of variable-sized exposed to docetaxel treatment:

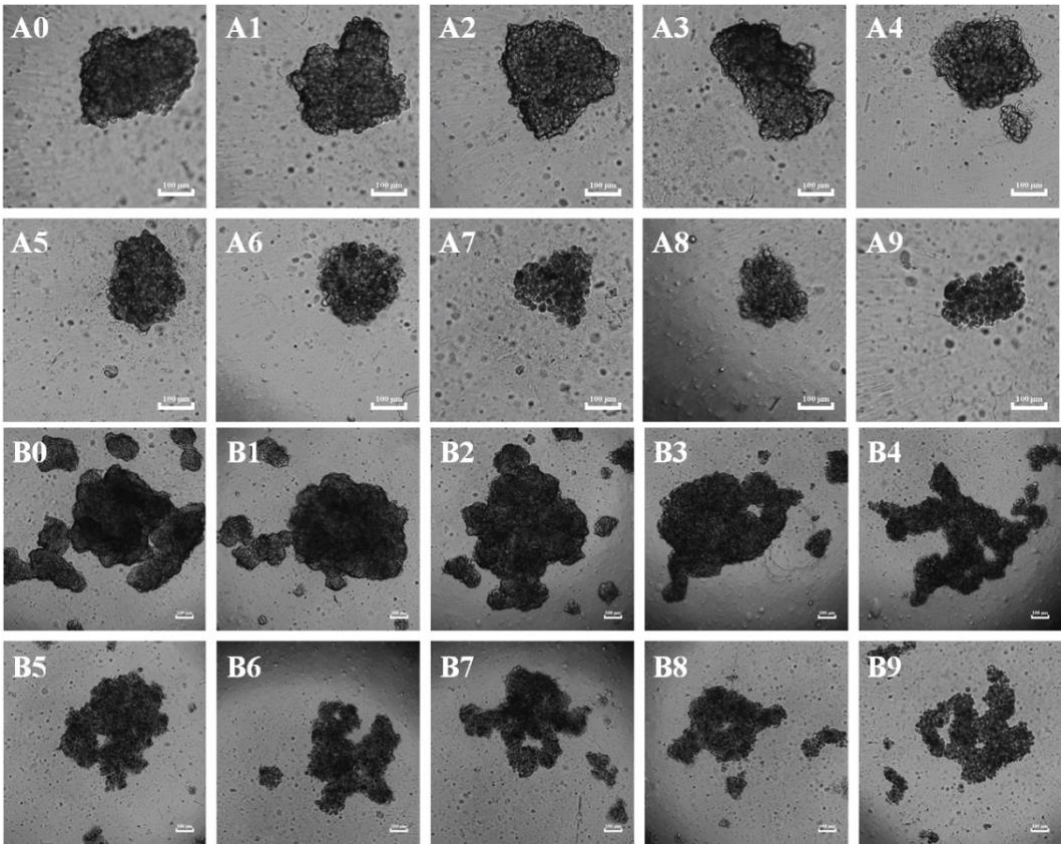

Figure S7. Images of the drug testing experiments based on floating LNCaP spheroids of variable sizes exposed to docetaxel treatment. (A0-A9) The images of the drug testing experiments based on smaller LNCaP spheroids (300 cells/well while plating) exposed to varying docetaxel concentrations: A0: The image of negative control without docetaxel; A1-A9: The images of the LNCaP cells exposed to varying docetaxel concentrations for 5 days (0.25 nM, 0.5 nM, 1 nM, 2 nM, 4 nM, 8 nM, 16 nM, 32 nM, and 64 nM). (**B0-B9)** The images of the drug testing experiments based on bigger LNCaP spheroids (3000 cells/well while plating) exposed to varying docetaxel concentrations: B0: The image of negative control without docetaxel; B1-B9: The images of the LNCaP cells exposed to varying docetaxel concentrations for 5 days (1 nM, 2 nM, 4 nM, 8 nM, 16 nM, 32 nM, 64 nM, 128 nM, and 256 nM).