Supplementary information

The analysis of the PI3K-AKT-mTOR pathway and mitochondria modulation by a 2-aminopyridine compound using the metastatic prostate cancer cell line PC-3

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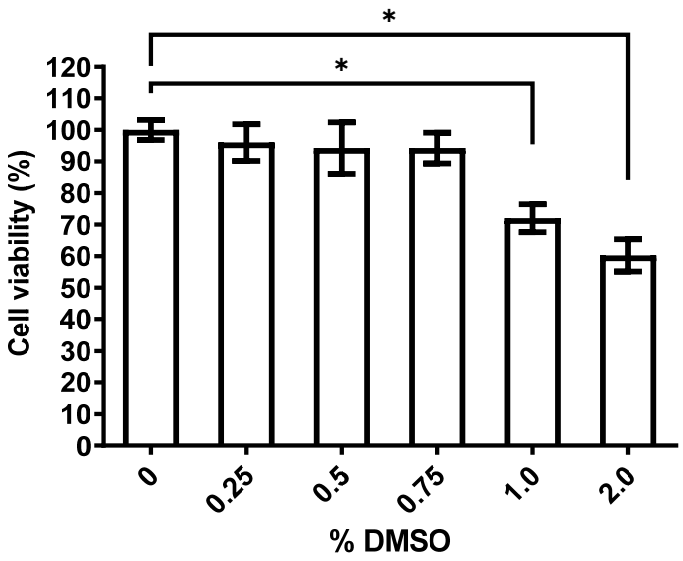


Figure S1. DMSO screening against PC-3 cells. Experiments were performed using MTT as described in the main text. Statistical significance using one-way ANOVA with Dunnett post-hoc study: P value < 0.001.

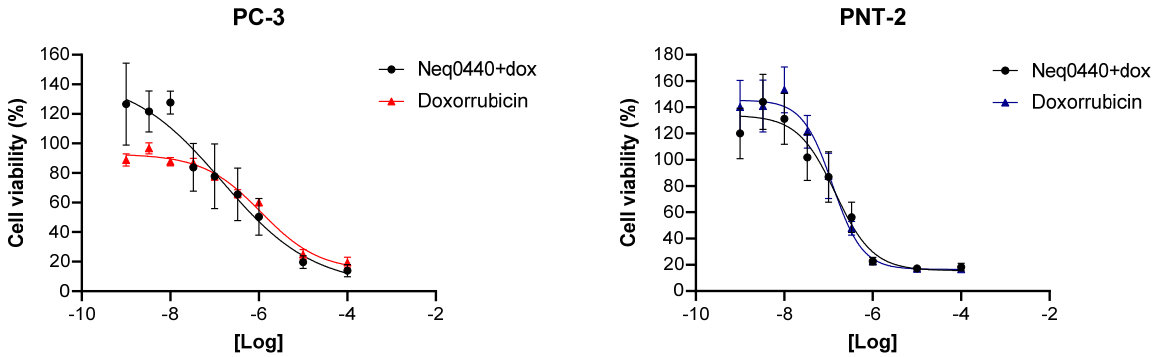
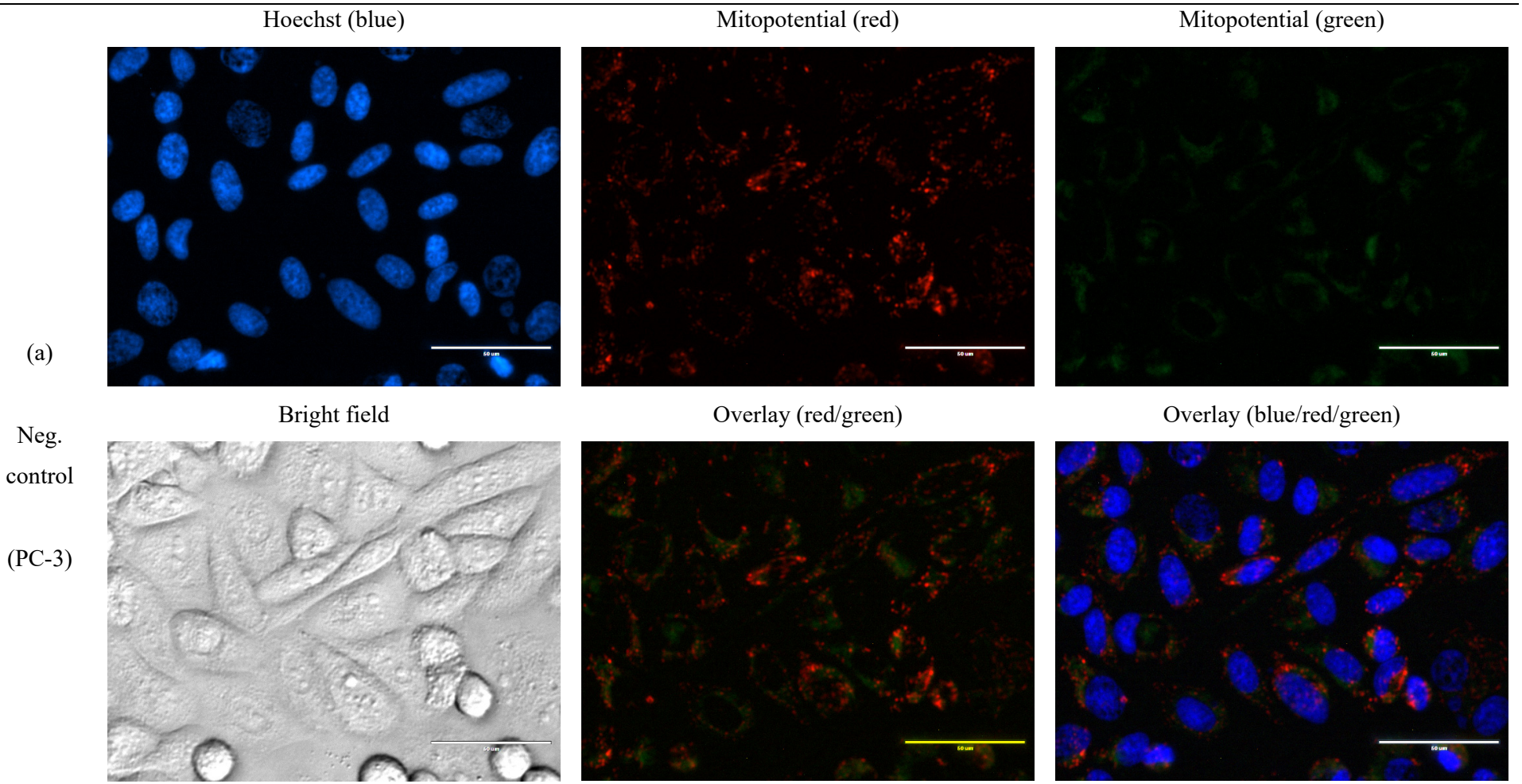
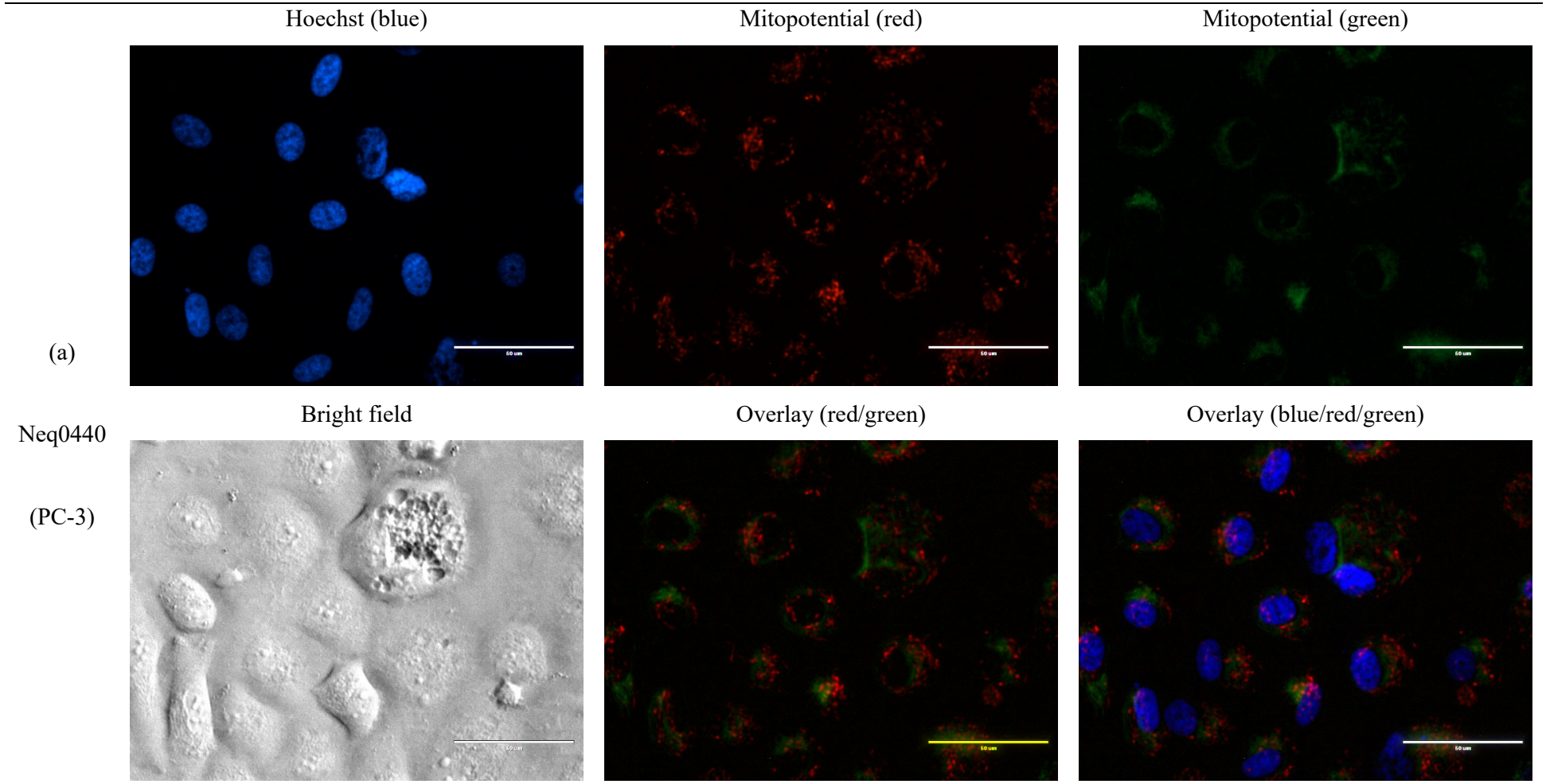
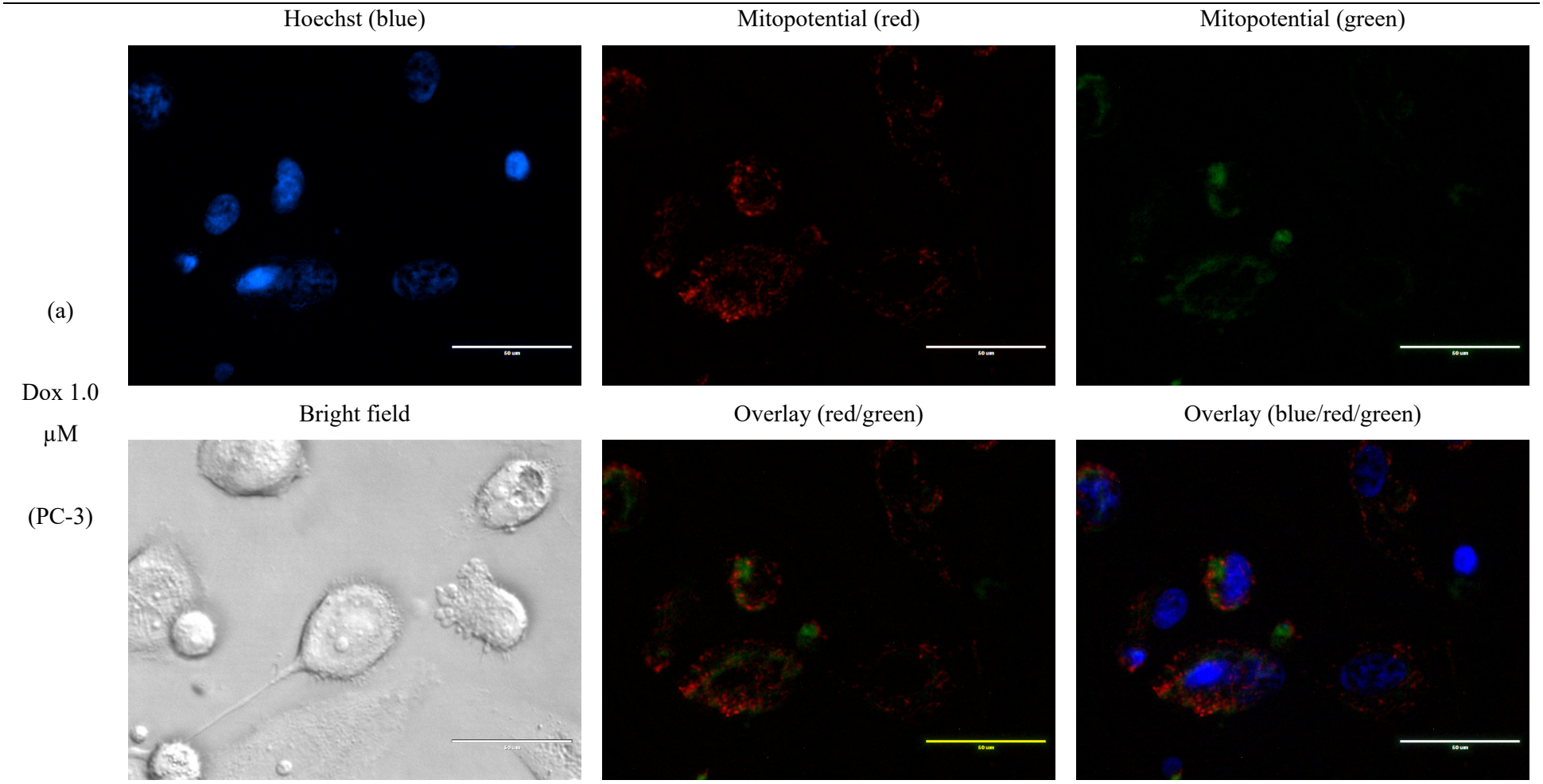
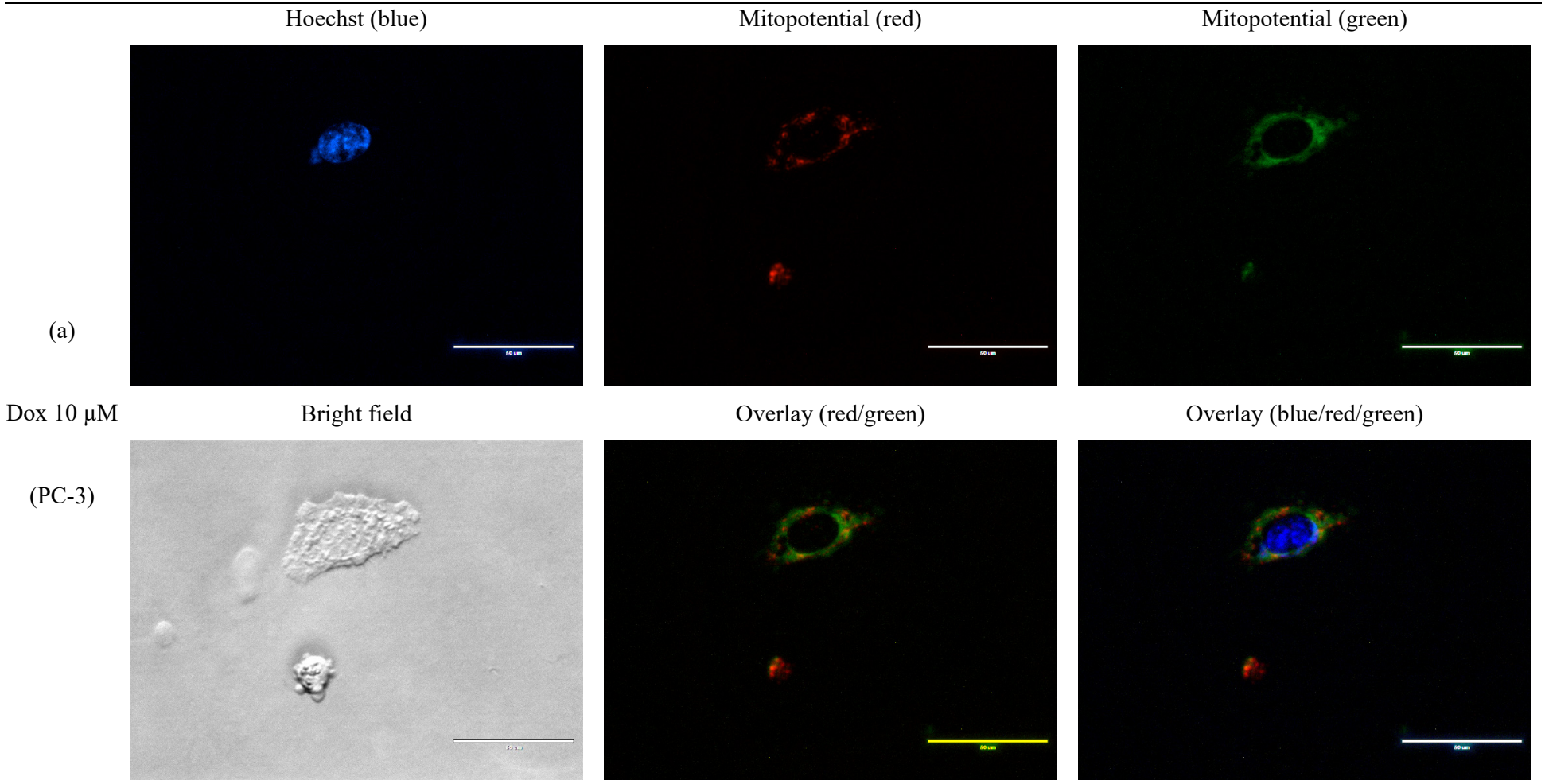


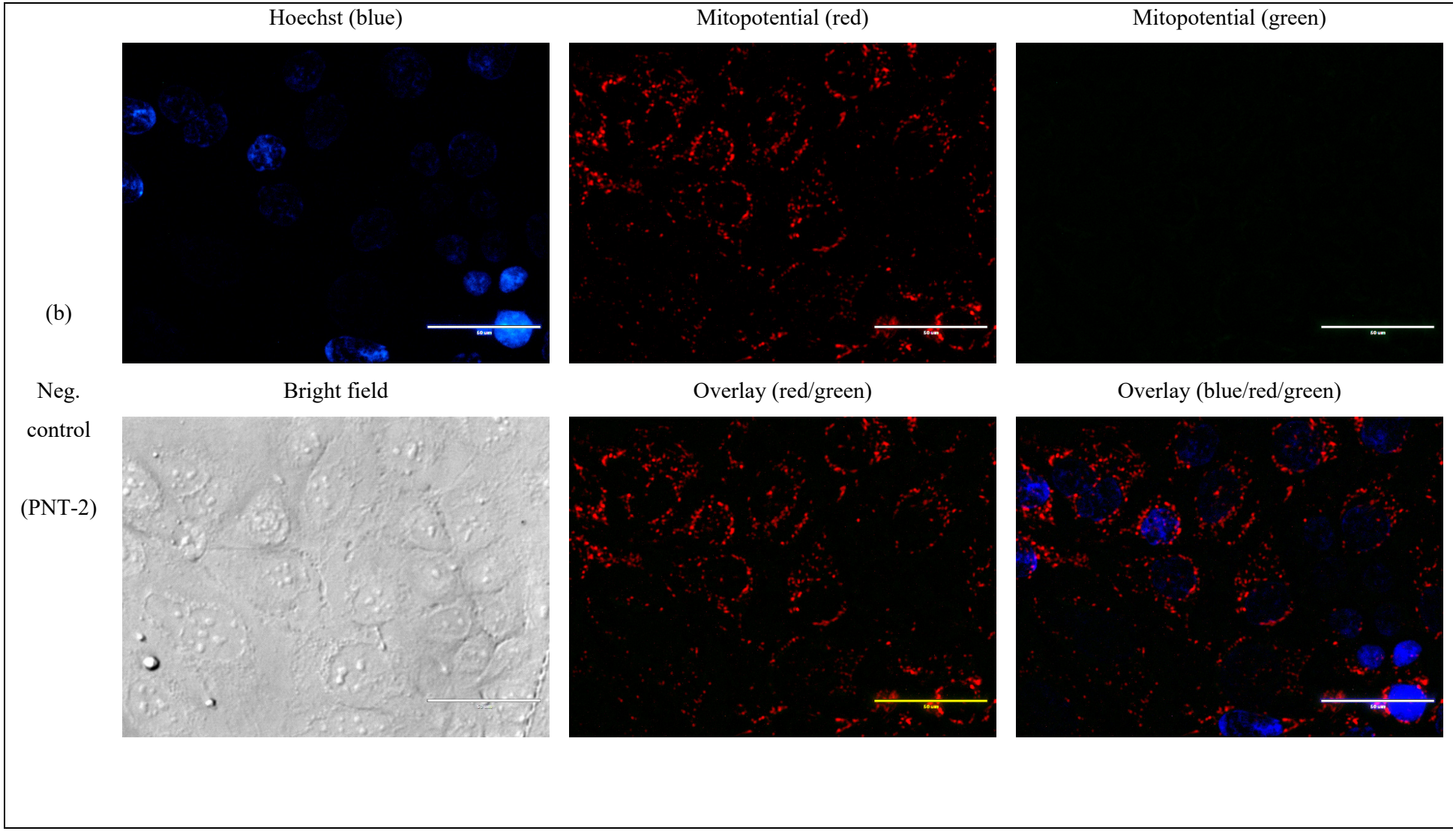
Figure S2. Concentration-response curves for doxorubicin monotherapy and the combination therapy with Neq0440 at 1.0 μM for the PNT-2 (nontumoral) and PC-3 (tumoral) prostate cell lines.

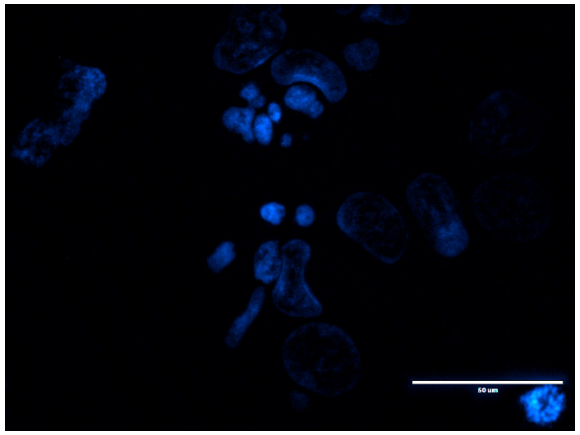
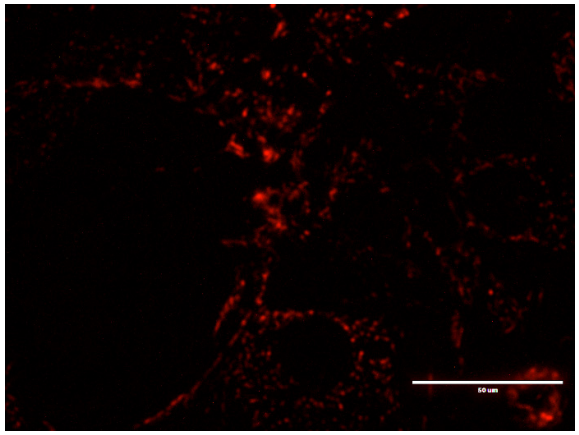
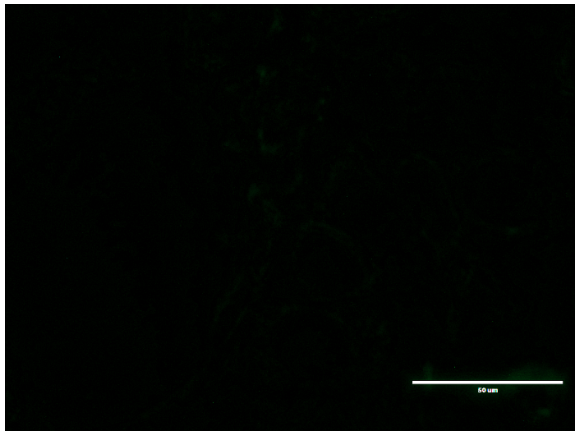
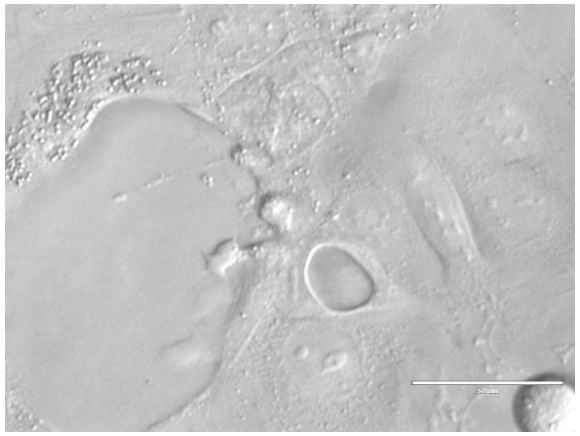
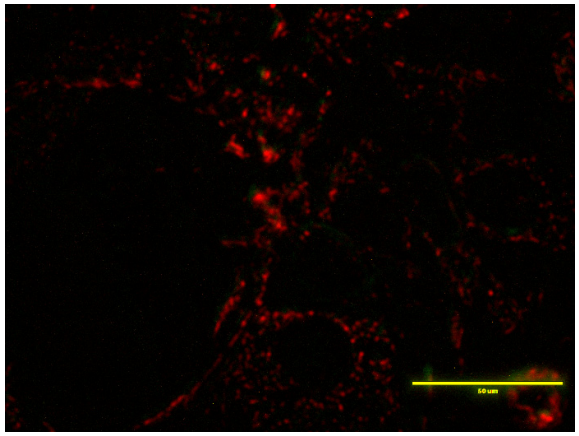
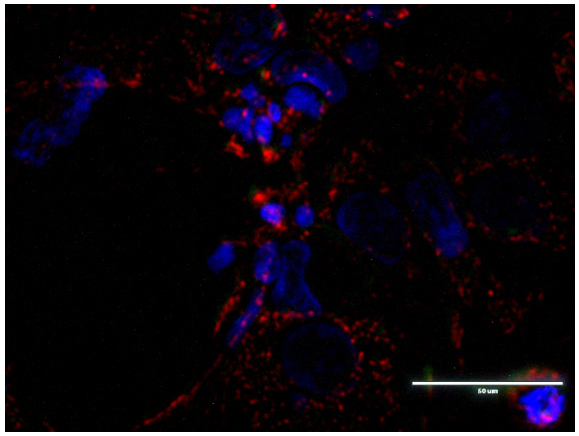

Hoechst (blue)
Mitopotential (red)
Mitopotential (green)
(a)
Neg. control (PC-3)
Bright field
Overlay (red/green)
Overlay (blue/red/green)


Hoechst (blue)
Mitopotential (red)
Mitopotential (green)
(a)
Neq0440
(PC-3)
Bright field
Overlay (red/green)
Overlay (blue/red/green)


Hoechst (blue)
Mitopotential (red)
Mitopotential (green)
(a)
Dox 1.0 μM
(PC-3)
Bright field
Overlay (red/green)
Overlay (blue/red/green)


Hoechst (blue)
Mitopotential (red)
Mitopotential (green)
(a)
Dox 10 µM
Bright field
Overlay (red/green)
Overlay (blue/red/green)
(PC-3)


Hoechst (blue)
Mitopotential (red)
Mitopotential (green)
(b)
Neg. control
(PNT-2)
Bright field
Overlay (red/green)
Overlay (blue/red/green)

	Hoechst (blue)	Mitopotential (red)	Mitopotential (green)
(b)			
Neq0440	Bright field	Overlay (red/green)	Overlay (blue/red/green)
(PNT-2)			

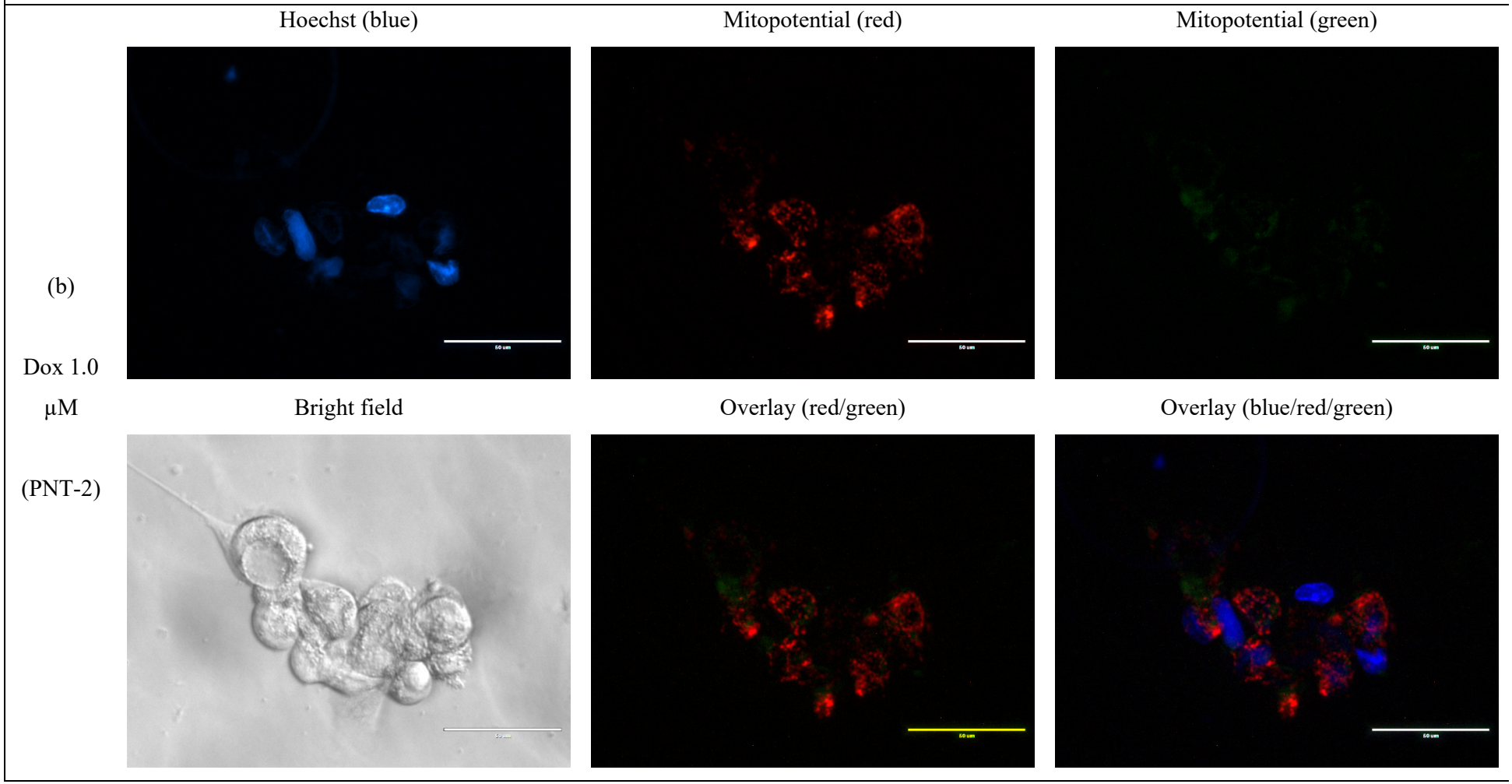

Hoechst (blue)
Mitopotential (red)
Mitopotential (green)
(b)
Dox 1.0
μM
(PNT-2)
Bright field
Overlay (red/green)
Overlay (blue/red/green)

Figure S3. Morphology and the mitochondrial membrane potential of PC-3 (a) and PNT-2 (b) cells (bright-field microscopy) after 72 h incubation of Neq0440 at the IC_{50}, and doxorubicin (Dox) at 1.0 and 10 µM. The cell constituents were labeled in blue by Hoechst 33452 (nuclei), red and green by the Mitopotential (polarized and depolarized mitochondria, respectively). Neg. control: negative control. The white bar represents 50 µm.

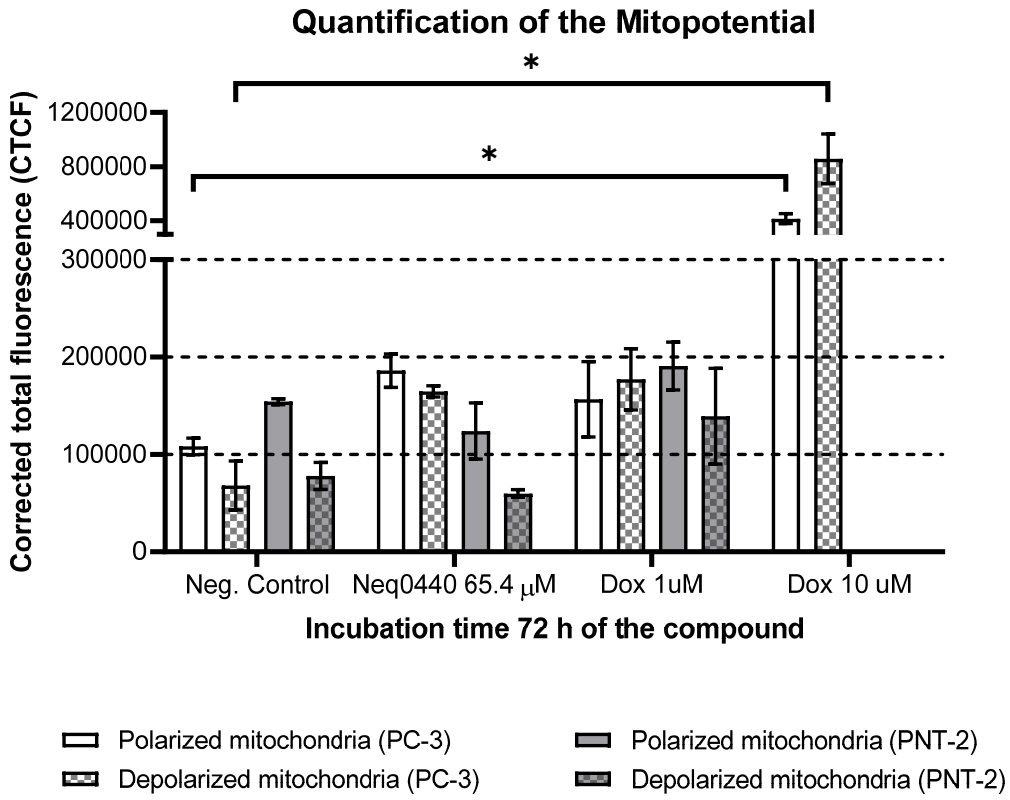
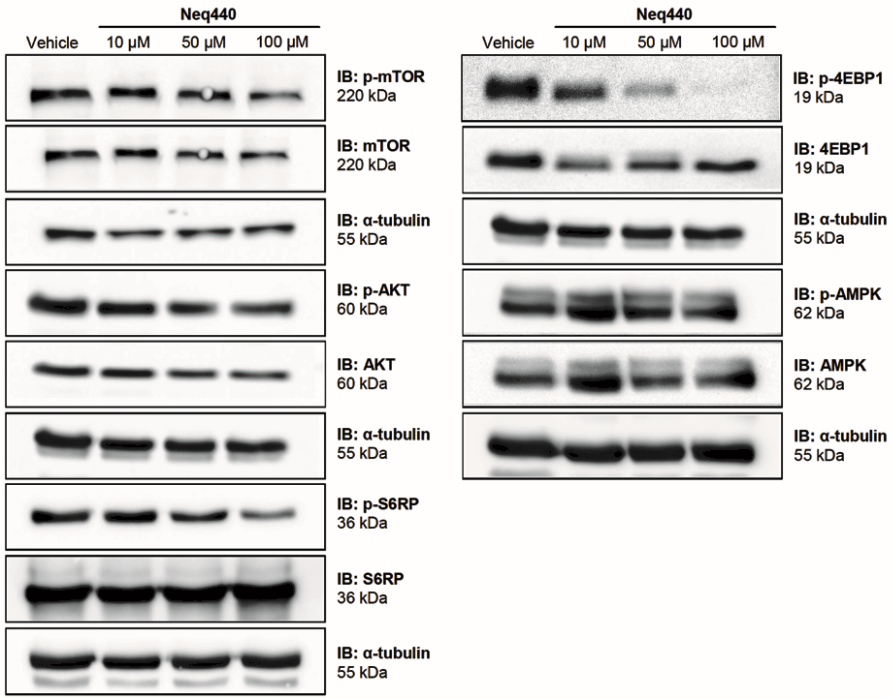


Figure S4. MitoPotential quantification for PC-3 and PNT-2 cells after 3 days of incubation.

(a)


Neq440
Vehicle
10 μM
50 μM
100 μM
IB: p-mTOR
220 kDa
IB: mTOR
220 kDa
IB: α-tubulin
55 kDa
IB: p-AKT
60 kDa
IB: AKT
60 kDa
IB: α-tubulin
55 kDa
IB: p-S6RP
36 kDa
IB: S6RP
36 kDa
IB: α-tubulin
55 kDa
Neq440
Vehicle
10 μM
50 μM
100 μM
IB: p-4EBP1
19 kDa
IB: 4EBP1
19 kDa
IB: α-tubulin
55 kDa
IB: p-AMPK
62 kDa
IB: AMPK
62 kDa
IB: α-tubulin
55 kDa

(b)

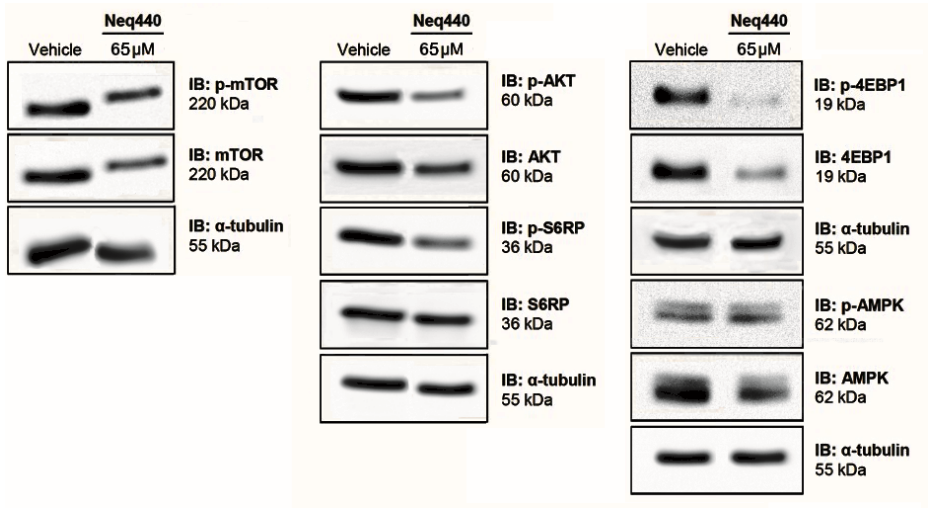
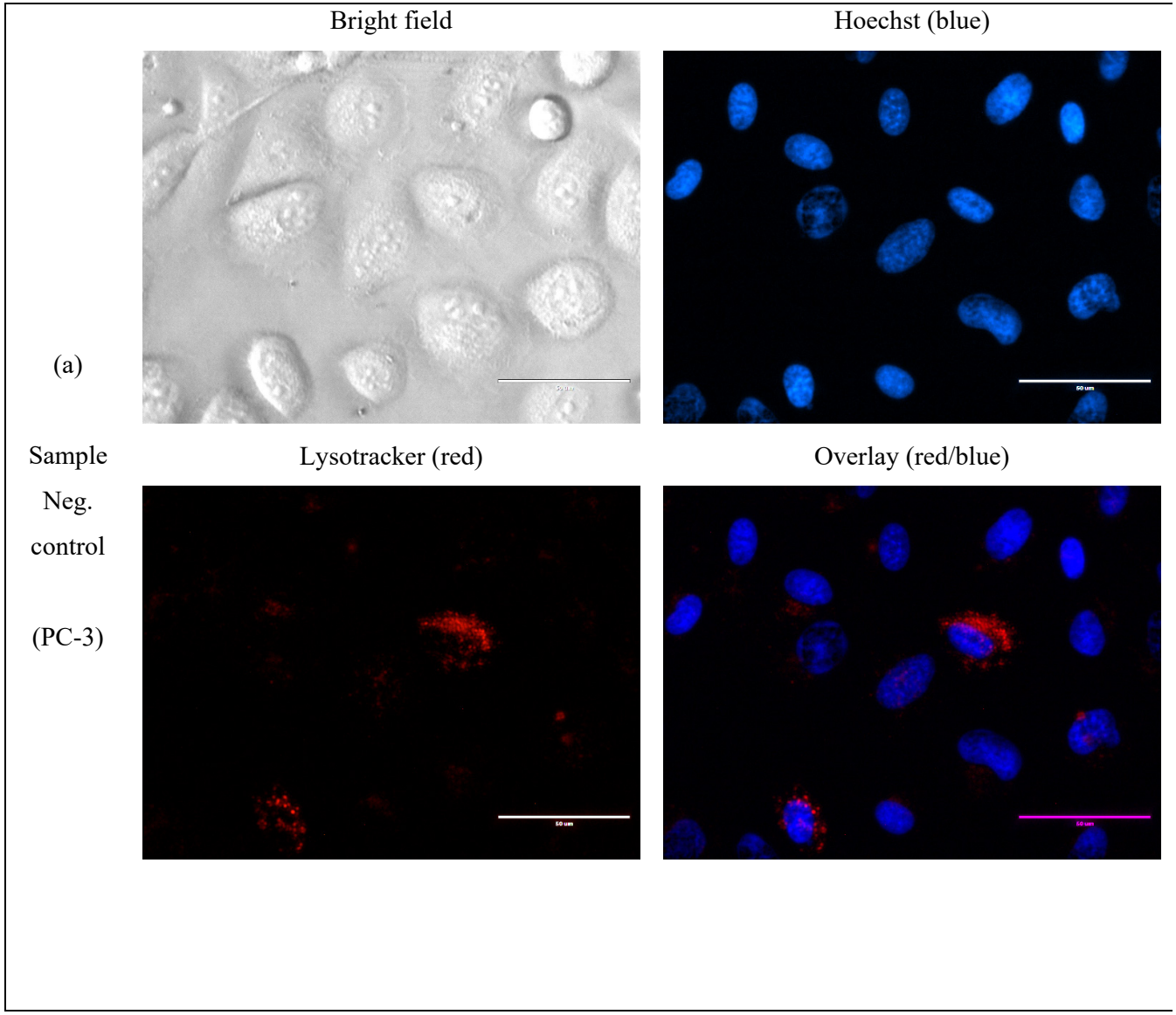
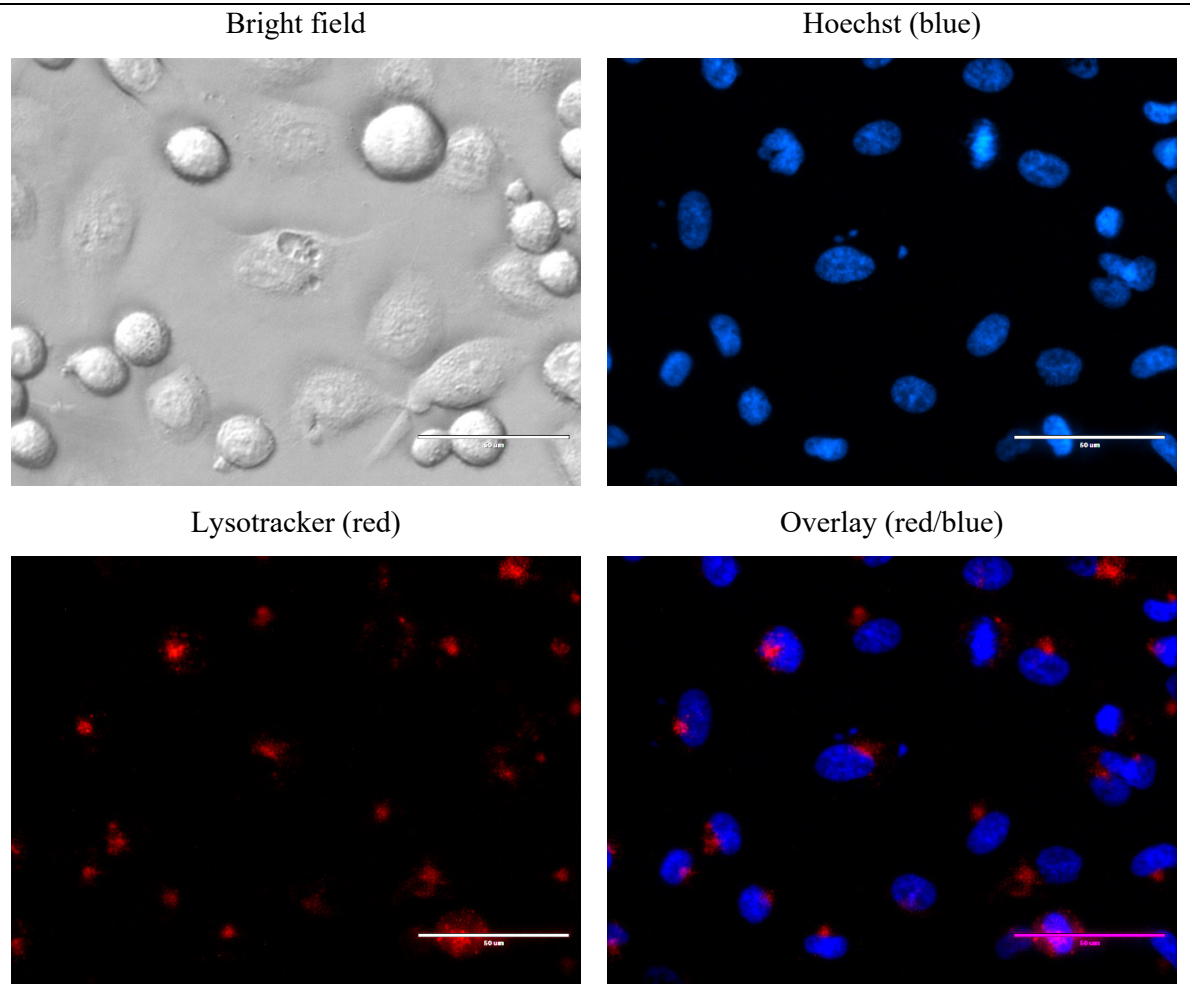
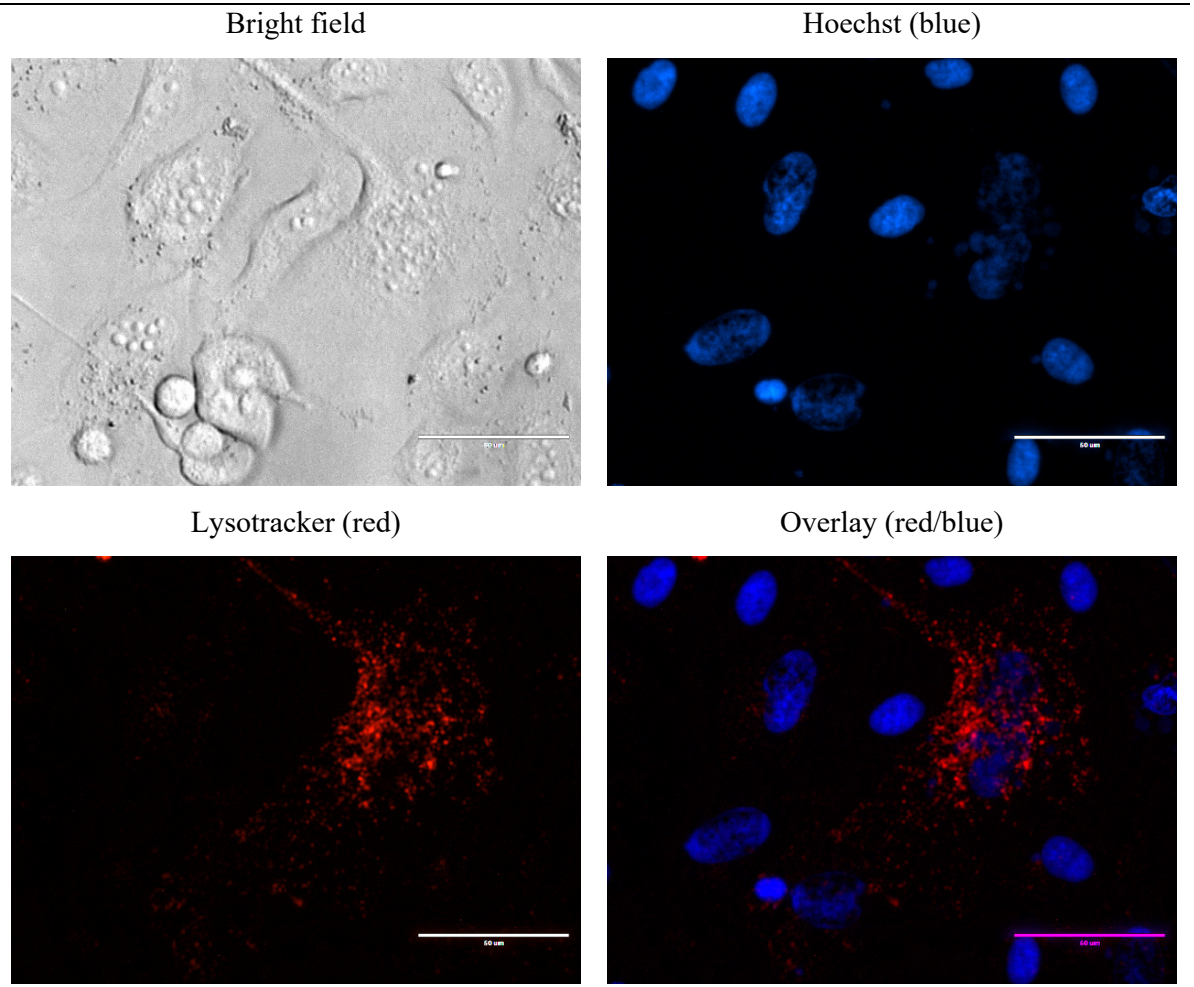
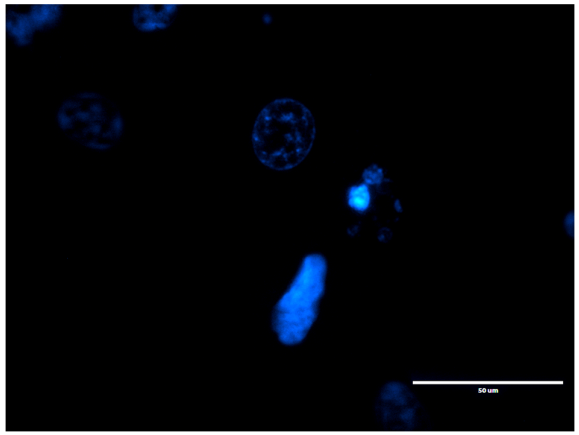
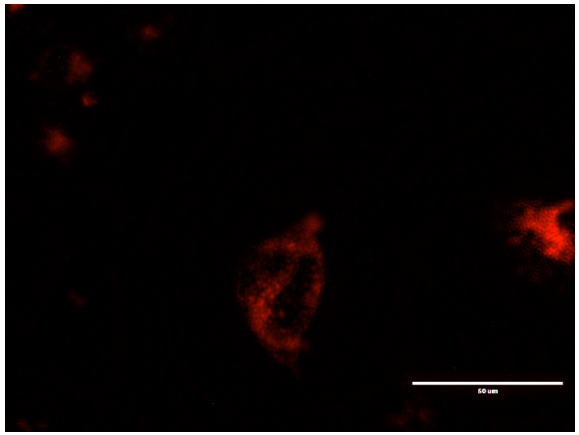
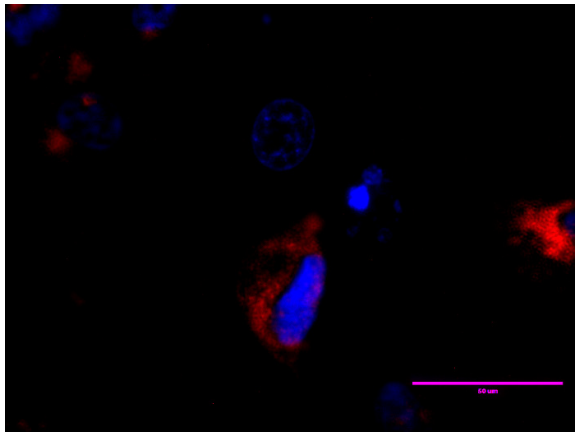


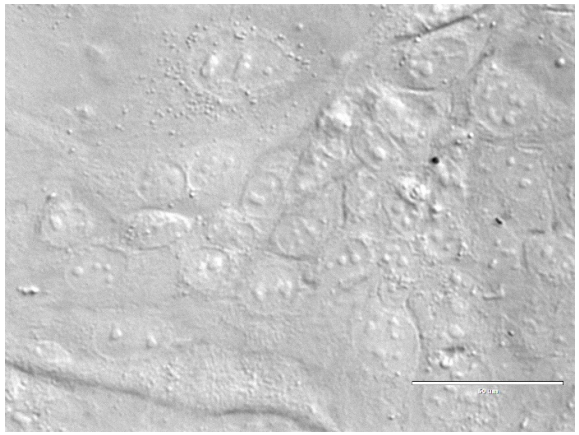
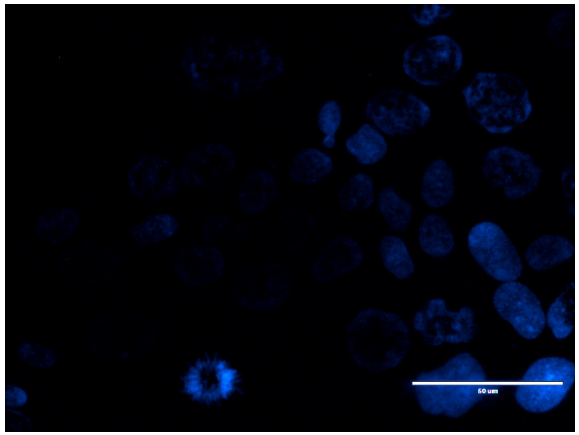
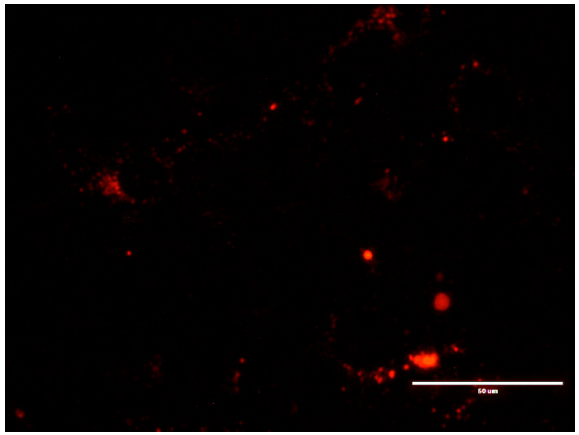
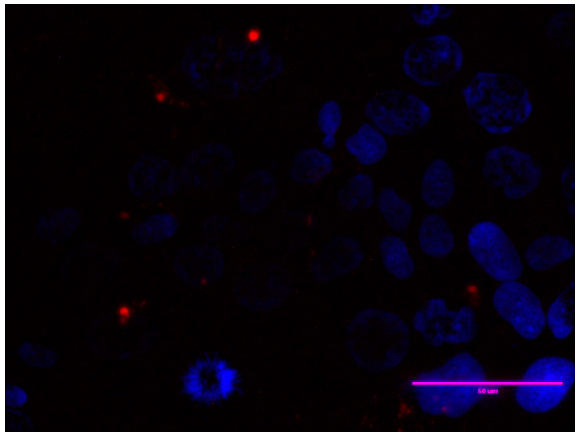
Figure S5. Western blot analyses of the kinase phosphorylation modulation in PC-3 cells after 24 h (a) and 48 h (b) of incubation with compound Neq0440.


Bright field
Hoechst (blue)
(a)
Sample
Lysotracker (red)
Overlay (red/blue)
Neg.
control
(PC-3)


Bright field
Hoechst (blue)
(a)
Sample
Neq0438
(PC-3)
Lysotracker (red)
Overlay (red/blue)


Bright field
Hoechst (blue)
(a)
Lysotracker (red)
Overlay (red/blue)
Sample
Neq0440
(PC-3)

	Bright field	Hoechst (blue)
(a)		
Sample	Lysotracker (red)	Overlay (red/blue)
Dox 1.0 μM (PC-3)		

	Bright field	Hoechst (blue)
(b)		
Sample	Lysotracker (red)	Overlay (red/blue)
Neg. control (PNT-2)		

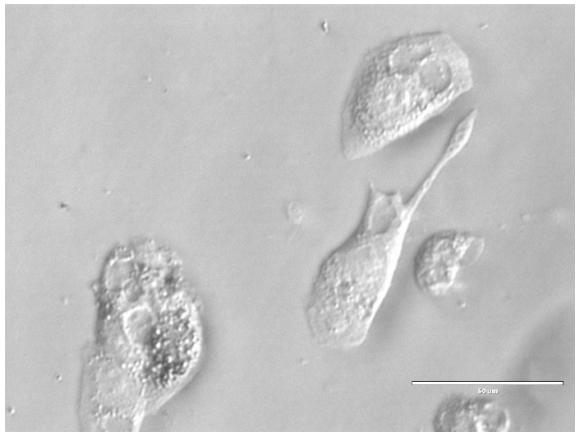
(b)

Sample

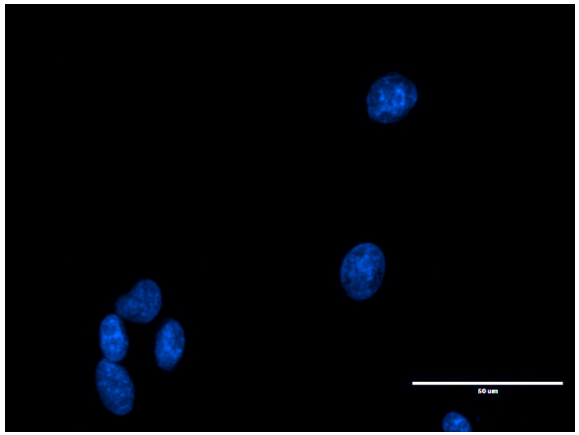
Neq0440

(PNT-2)

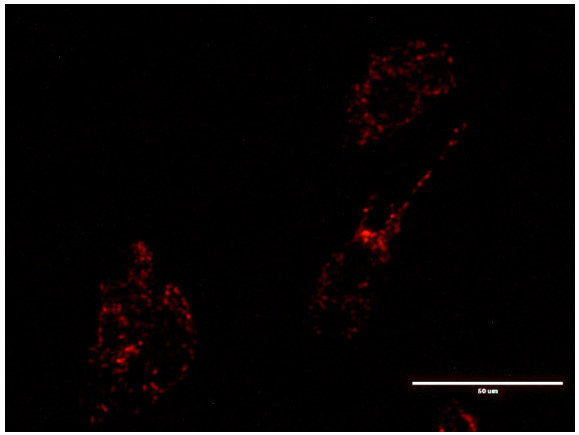
Bright field



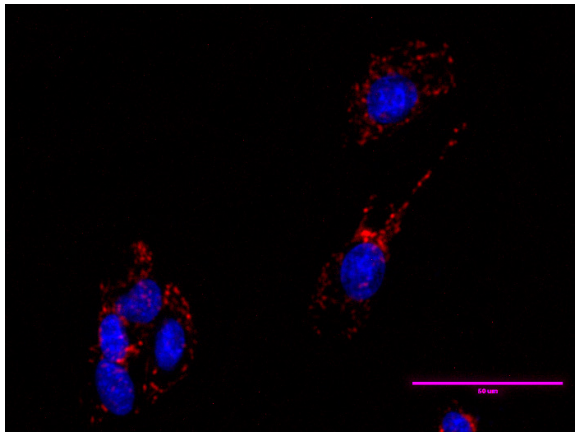
Hoechst (blue)



Lysotracker (red)



Overlay (red/blue)



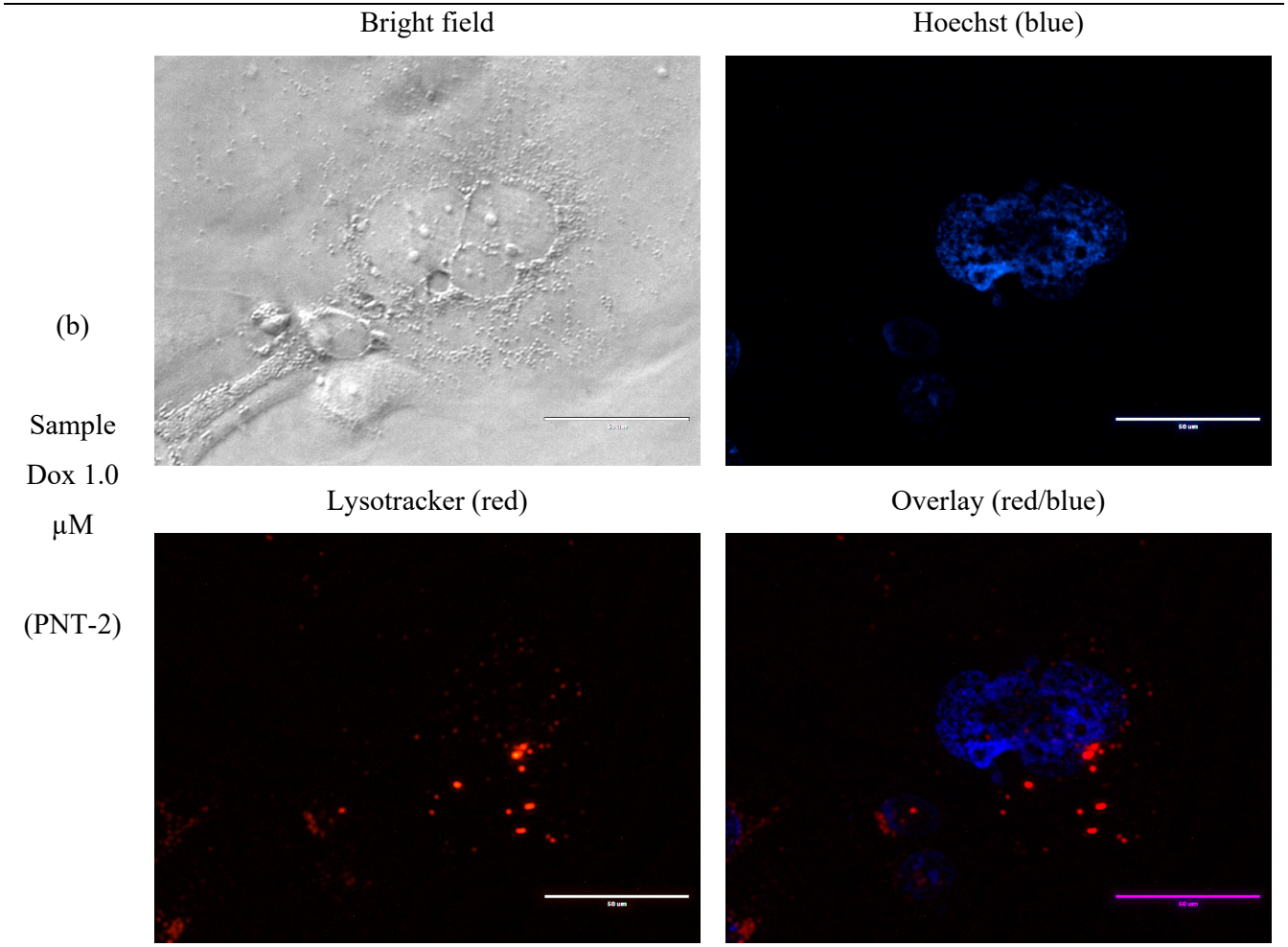


Figure S6. Morphological aspects and the fluorescence markers of nucleus and lysosomes for the PC-3 (a) and PNT-2 (b) cells after 72 h incubation with Neq0438, Neq0440, and doxorubicin. Negative control comprised the culture medium with 0.5% (v/v) DMSO. Hoechst33342 (blue) and Lysotracker (red) labeled nucleus and lysosomes, respectively. Scale bars represent 50 µm.

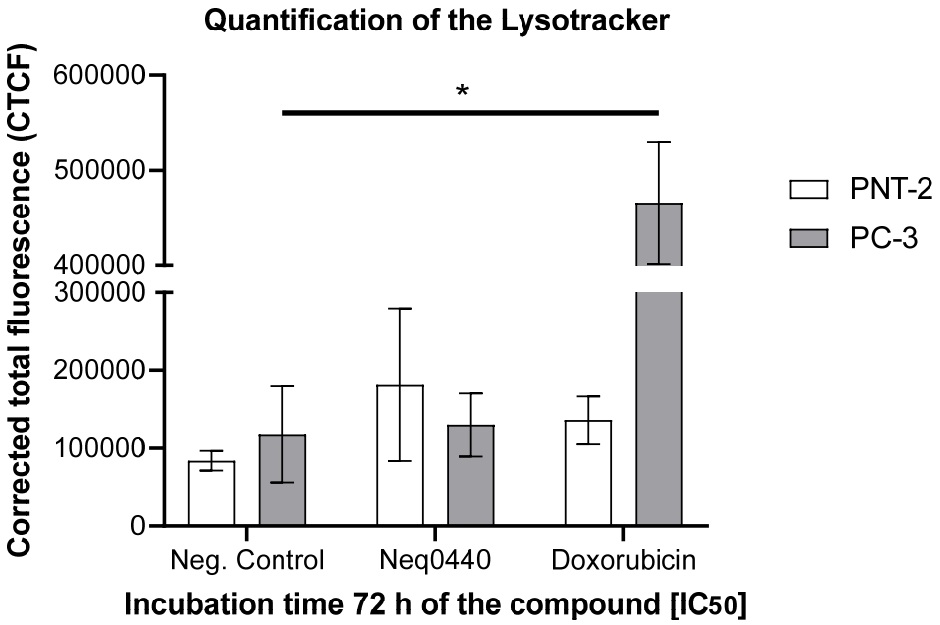


Figure S7. Lysotracker marker quantification after 72 h incubation of Neq0440 with PNT-2 and PC-3 cell lines.

The two-way ANOVA was applied with multiple comparisons using the Dunnett test ($p < 0.05$) concerning the negative control for each cell type.