

# Supplementary Information

## Fucoidan Independently Enhances Activity in Human Immune Cells and Has a Cytostatic Effect on Prostate Cancer Cells in the Presence of Nivolumab

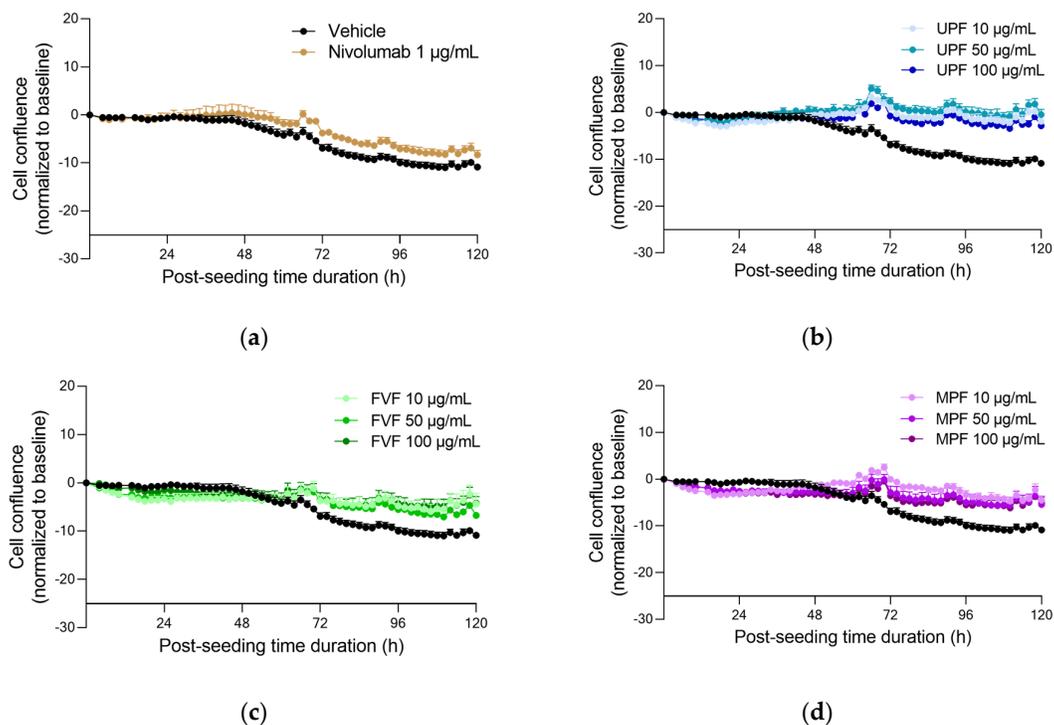
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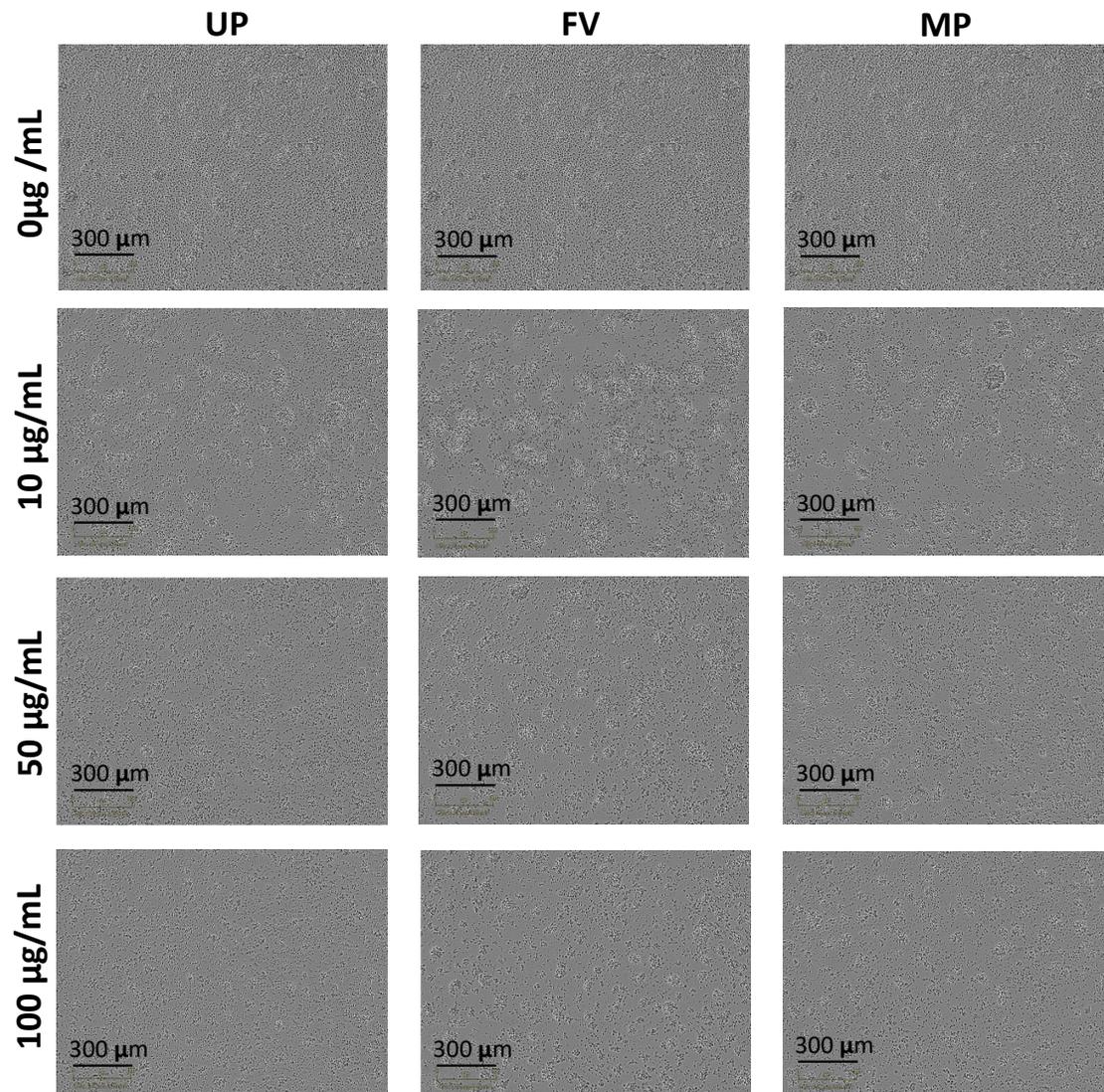
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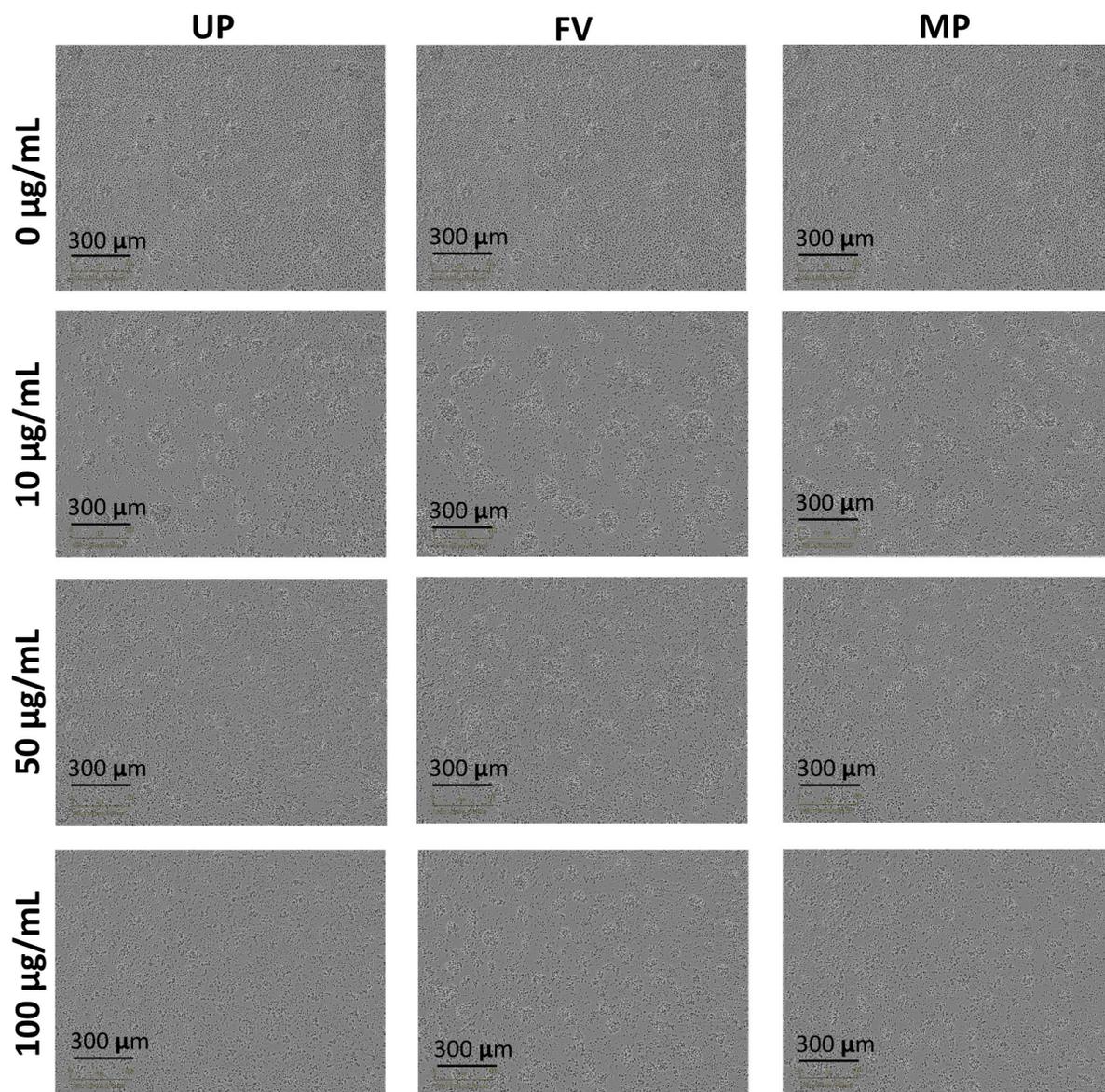
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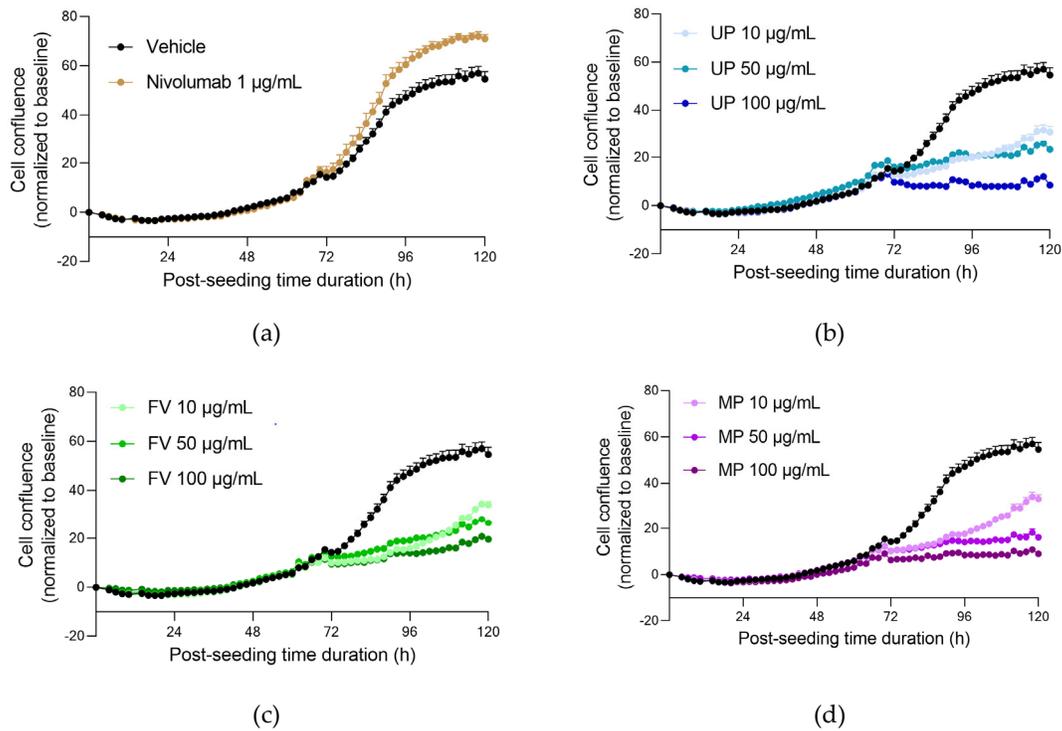
**Figure S1.** Real-time live cell monitoring of inactivated PBMCs, under treatment with fucoidans. PBMCs were seeded in the absence of  $\alpha$ CD3, and treated with increasing doses of fucoidans (a) Nivolumab, as positive control (b) UP (c) FV and (d) MPF. Cell confluence was monitored and quantified – as surrogate of cell proliferation, over a period of ~5 days. Data were normalized and corrected to the baseline and are expressed as means  $\pm$  SEM.



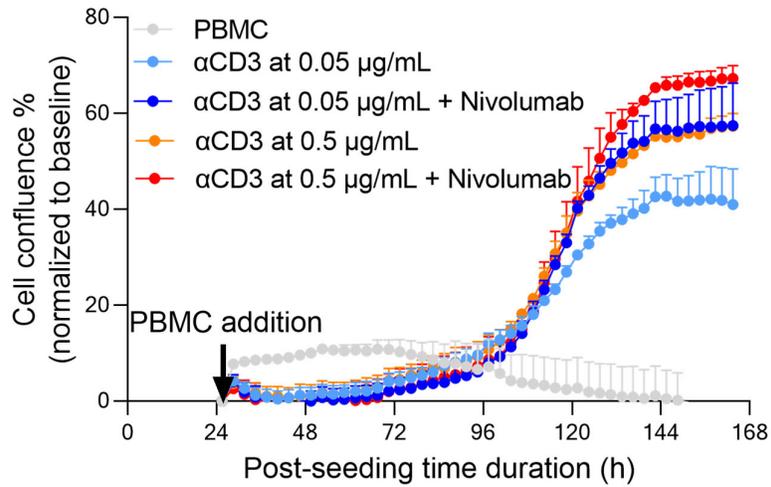
**Figure S2.** Images of cell proliferation and clusterization of activated PBMCs in the presence of  $\alpha\text{CD3}$  ( $0.25 \mu\text{g}/\text{mL}$ ) and fucoidans ( $0\text{--}100 \mu\text{g}/\text{mL}$ ). PBMCs were seeded in the presence of  $\alpha\text{CD3}$  ( $0.25 \mu\text{g}/\text{mL}$ ), and treated with increasing doses of fucoidans. Representative image fields of cell proliferation and clusterization induced upon 96 h treatment with test fucoidan extracts.



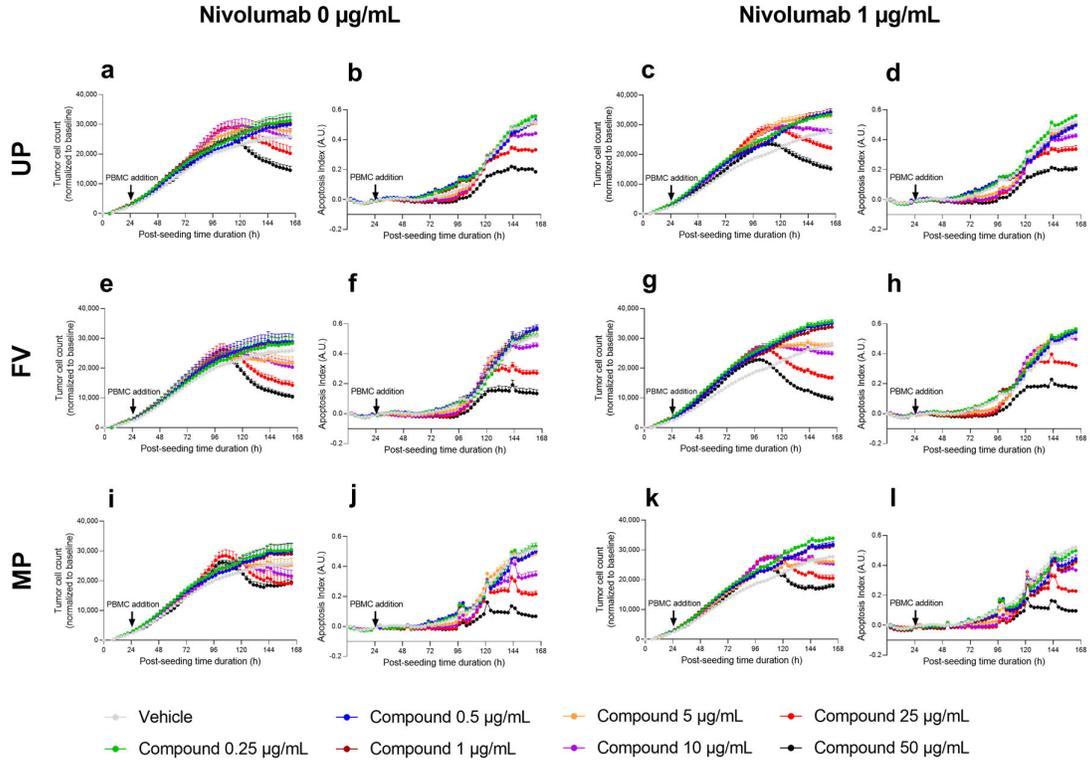
**Figure S3.** Images of cell proliferation and clusterization of activated PBMCs in the presence of  $\alpha$ CD3 (0.5  $\mu$ g/mL) and fucoidans (0–100  $\mu$ g/mL). PBMCs were seeded in the presence of  $\alpha$ CD3 (0.5  $\mu$ g/mL), and treated with increasing doses of test compounds. Representative image fields of cell proliferation and clusterization induced upon 96 h treatment with test fucoidan extracts.



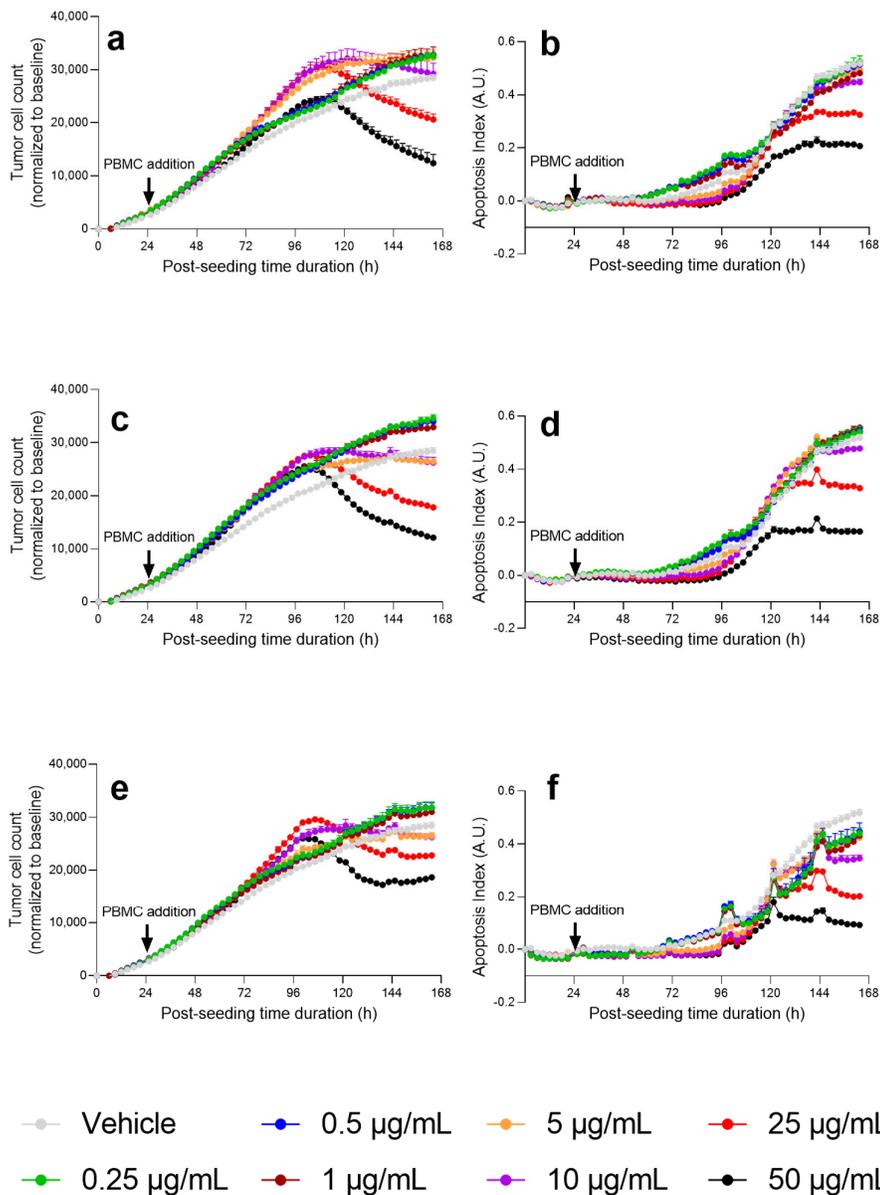
**Figure S4.** Changes in cell confluence of PBMCs in the presence of fucidans. PBMCs were seeded in the presence of  $\alpha$ CD3 at 0.25  $\mu$ g/mL and treated with increasing dose of (a) Nivolumab as positive control, (b) UP, (c) FV and (d) MP. Cell confluence was monitored and quantified – as surrogate of cell proliferation over a period of ~5 days. Data were normalised and corrected to the baseline and are expressed as means  $\pm$  SEM.



**Figure S5.** Real-time live cell monitoring of inactivated and  $\alpha$ CD3-activated PBMCs in the presence and absence of Nivolumab. PBMCs were seeded either in the absence or the presence of  $\alpha$ CD3, with and without Nivolumab at 1  $\mu$ g/mL. Cell confluence was monitored and quantified – as surrogate of cell proliferation, over a period of ~5 days. Data were normalized and corrected to the baseline and are expressed as means  $\pm$  SEM.



**Figure S6.** Real-time live cell monitoring of prostate PC3 tumor cell killing mediated by activated PBMCs, under treatment with fucoidans. Prostate PC3 tumor cells were seeded and 24h later were cocultured with PBMCs, in the presence of  $\alpha$ CD3 (0.05  $\mu$ g/mL), and in the presence and absence of Nivolumab (1  $\mu$ g/mL), and treated with increasing doses of each of test compounds – UP (a–d), FV (e–h), and MP (i–l) under the different conditions of treatments with compounds. Tumor cell count (a, e, i, c, g, k) and apoptosis (b, f, j, d, h, l) were monitored and quantified over a period of ~5 days, by mean of a NucRed probe expression and caspase 3/7 fluorescent probe, respectively, as surrogate measures of immune cell killing activity towards tumor cells. Apoptosis is represented as an index evaluated with respect to the apoptosis events and cell number in each condition. Data were normalized and corrected to the baseline and are expressed as means  $\pm$  SEM.



**Figure S7:** Real-time live cell monitoring of prostate PC3 tumor cell killing mediated by activated PBMCs in the presence of  $\alpha$ CD3 (0.5  $\mu$ g/mL), under treatment with fucoidans. Prostate PC3 tumor cells were seeded and 24h later were cocultured with PBMCs, in the presence of  $\alpha$ CD3 (0.5  $\mu$ g/mL), and in the absence of Nivolumab, and treated with increasing doses of each of test compounds – UP (a–b), FV (c–d), and MP (e–f) under the different conditions of treatments with compounds. Tumor cell count (a, c, e) and apoptosis (b, d, f) were monitored and quantified over a period of ~5 days, by mean of a NucRed probe expression and caspase 3/7 fluorescent probe, respectively, as surrogate measures of immune cell killing activity towards tumor cells. Apoptosis is represented as an index evaluated with respect to the apoptosis events and cell number in each condition. Data were normalized and corrected to the baseline and are expressed as means  $\pm$  SEM.