Different Susceptibilities of Human Melanoma Cell Lines to G2/M Blockage and Cell Death Activation in Response to the Estrogen Receptor β agonist LY500307.

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Supplementary file Legends

Fig. S1 LY500307 effects on tumor-cell proliferation/viability. Melanoma cell lines (Me1402/R, Me665/1 and A375M), and normal human fibroblasts (HFs) were treated with increasing concentrations of LY500307 (2μM, 4μM, 8μM) at different time points and cell viability measured using an XTT assay in comparison with untreated controls (CTR). Data are represented as mean ± SD of at least three independent experiments. Asterisks indicate the level of significance: * p<0.05, ** p<0.01, *** p<0.001. Western blotting insert shows ER expression in HFs, with β–actin as loading control.

Fig. S2 Effect of ERβ selective agonist LY500307 treatment on melanoma cell cycle. (A) Cell cycle analysis of Me665/1 cultured in 2% charcoal and treated or not (CTR) with increased concentrations of LY500307 (2μM, 4μM, 8μM) for 24 hours. (B) Representative Western blot analysis of Cyclin B1 and Wee1 from total proteins extracted from the same experiment. β-Actin was utilized as internal loading control. Asterisks indicate the level of significance: ** p<0.01.

Fig. S3 Effect of ERβ selective agonist LY500307 treatment on NRAS metastatic melanoma SK-MEL-30 cell cycle. (A) Cell cycle analysis of SK-MEL-30 cultured treated or not (CTR) with LY500307 (4μM, 8μM) for 24 hours. (B) Representative Western blot analysis of p21 and Cyclin B1 from total proteins extracted from the same experiment. β-Actin was utilized as internal loading control. Asterisks indicate the level of significance: * p<0.01, ** p<0.001.
Me1402/R

Cell growth (490 nm)

- CTR
- 2μM
- 4μM
- 8μM

Me665/1

Cell growth (490 nm)

- CTR
- 2μM
- 4μM
- 8μM

A375M

Cell growth (490 nm)

- CTR
- 2μM
- 4μM
- 8μM

Human Fibroblast (HF)

Cell growth (490 nm)

- CTR
- 2μM
- 4μM
- 8μM

**Note:** The diagrams show time-course cell growth measurements at different time points (t0, 24h, 48h, 72h) for different concentrations of compounds in each cell line.
**Figure A**

Bar graph showing the cell cycle percentage for Me665/1 treated with different concentrations of LY500307. The x-axis represents the concentration levels (CTR, 2μM, 4μM, 8μM), and the y-axis represents the cell cycle percentage. The bars are color-coded to represent G1 (black), S (white), and G2/M (gray) phases. Significant differences are indicated by "**".

**Figure B**

Western blot analysis for Me665/1 treated with different concentrations of LY500307 and LY500307 alone. The blot shows bands for Cyclin B1, β-Actin, Wee1, and β-Actin. The intensity of the bands suggests different levels of expression or activity under the indicated conditions.
**A**

SK-MEL-30

![Bar chart showing cell cycle distribution at different concentrations of LY500307.](chart)

- **CTR**: 60% G1, 20% S, 20% G2/M
- **4μM**: 60% G1, 20% S, 20% G2/M
- **8μM**: 60% G1, 20% S, 20% G2/M

**B**

SK-MEL-30

![Western blots showing protein levels at different concentrations of LY500307.](blots)

- **CTR**: p21, β-Actin, Cyclin B1, β-Actin
- **4μM**: p21, β-Actin, Cyclin B1, β-Actin
- **8μM**: p21, β-Actin, Cyclin B1, β-Actin

**LY500307**
**Table S1. Melanoma cell lines analyzed in the current study**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Mutation</th>
<th>Histological features</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me1007</td>
<td>WT</td>
<td>Primary tumor VGP*</td>
<td>[1, 2, 3]</td>
</tr>
<tr>
<td>Me501</td>
<td>WT</td>
<td>Primary tumor VGP*</td>
<td>[4]</td>
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<tr>
<td>WM983A</td>
<td>BRAF V600E</td>
<td>Primary tumor VGP*</td>
<td>[4, 5]</td>
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<tr>
<td>Me1402/R</td>
<td>BRAF V600E</td>
<td>Recurrence of primary tumor</td>
<td>[1, 2]</td>
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<tr>
<td>A375M</td>
<td>BRAF V600E</td>
<td>Metastatic melanoma</td>
<td>[6, 7]</td>
</tr>
<tr>
<td>Me665/1</td>
<td>NRAS Q61R</td>
<td>Metastatic melanoma</td>
<td>[2, 8]</td>
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<tr>
<td>SK-MEL-30</td>
<td>NRAS Q61K</td>
<td>Metastatic melanoma</td>
<td>[9]</td>
</tr>
</tbody>
</table>

*VGP: vertical growth phase