

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

Giada Pontecorvi<sup>1</sup>, Maria Bellenghi<sup>1</sup>, Sabrina Tait<sup>1</sup>, Valentina Tirelli<sup>2</sup>, Paola Matarrese<sup>1</sup>, Gianfranco Mattia<sup>1\*</sup>, Alessandra Carè<sup>1\*\*</sup> and Rossella Puglisi<sup>1#</sup>

<sup>1</sup> Center for Gender-Specific Medicine, Istituto Superiore di Sanità, Rome, Italy

<sup>2</sup> Core facilities, Istituto Superiore di Sanità, Rome, Italy

\*Correspondence to: A.Carè and G.Mattia, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: [alessandra.care@iss.it](mailto:alessandra.care@iss.it) and [gianfranco.mattia@iss.it](mailto:gianfranco.mattia@iss.it)

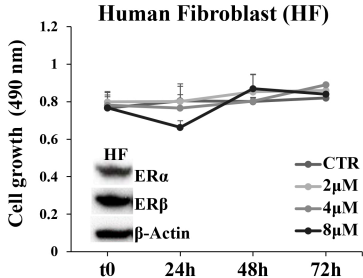
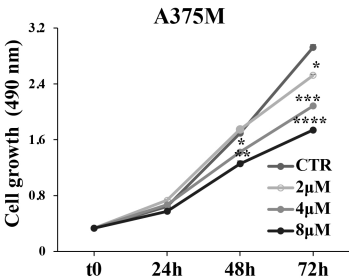
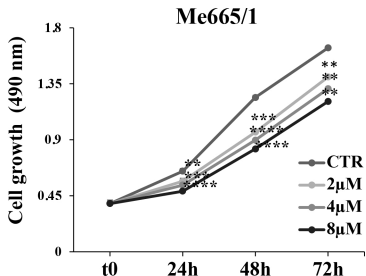
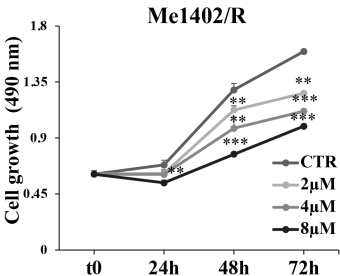
#Equal senior authorship

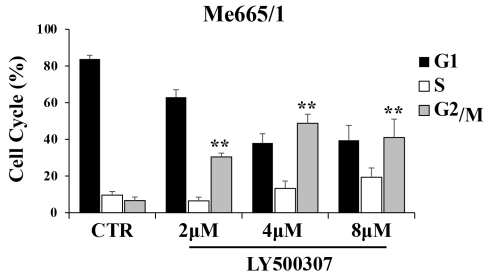
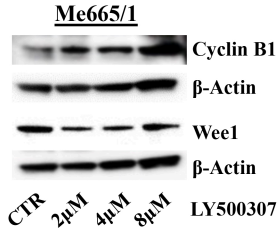
## Supplementary file Legends

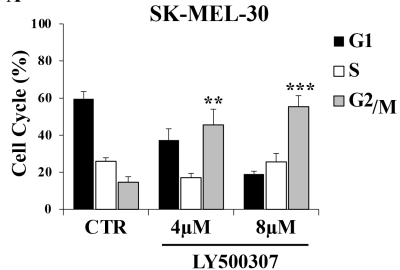
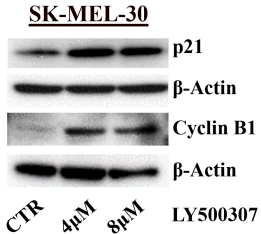
**Fig. S1 LY500307 effects on tumor-cell proliferation/viability.** Melanoma cell lines (Me1402/R, Me665/1 and A375M), and normal human fibroblasts (HFs) were treated with increasing concentrations of LY500307 (2 $\mu$ M, 4 $\mu$ M, 8 $\mu$ M) at different time points and cell viability measured using an XTT assay in comparison with untreated controls (CTR). Data are represented as mean  $\pm$  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  $\beta$ -actin as loading control.

**Fig. S2 Effect of ER $\beta$  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 (2 $\mu$ M, 4 $\mu$ M, 8 $\mu$ M) for 24 hours. **(B)** Representative Western blot analysis of Cyclin B1 and Wee1 from total proteins extracted from the same experiment.  $\beta$ -Actin was utilized as internal loading control. Asterisks indicate the level of significance: \*\* p<0.01.

**Fig. S3 Effect of ER $\beta$  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 (4 $\mu$ M, 8 $\mu$ M) for 24 hours. **(B)** Representative Western blot analysis of p21 and Cyclin B1 from total proteins extracted from the same experiment.  $\beta$ -Actin was utilized as internal loading control. Asterisks indicate the level of significance: \*\* p<0.01, \*\*\* p<0.001.



**A****B**

**A****B**

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

Cell line	Mutation	Histological features	References
<b>Me1007</b>	WT	Primary tumor VGP*	[1, 2, 3]
<b>Mel501</b>	WT	Primary tumor VGP*	[4]
<b>WM983A</b>	BRAF V600E	Primary tumor VGP*	[4, 5]
<b>Me1402/R</b>	BRAF V600E	Recurrence of primary tumor	[1, 2]
<b>A375M</b>	BRAF V600E	Metastatic melanoma	[6, 7]
<b>Me665/1</b>	NRAS Q61R	Metastatic melanoma	[2, 8]
<b>SK-MEL-30</b>	NRAS Q61K	Metastatic melanoma	[9]

\*VGP: vertical growth phase

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