

## Breast Cancer Research and Treatment

### **PI3K/mTOR inhibition can impair tumor invasion and metastasis in vivo despite a lack of antiproliferative action in vitro: implications for targeted therapy**

*Seth A. Wander<sup>1,2</sup>, Dekuang Zhao<sup>1,2</sup>, Alexandra H. Besser<sup>1,2</sup>, Feng Hong<sup>1</sup>,  
Jianqin Wei<sup>5</sup>, Tan A. Ince<sup>1,3</sup>, Clara Milikowski<sup>3</sup>, Nanette H. Bishopric<sup>5</sup>,  
Andy J. Minn<sup>6</sup>, Chad J. Creighton<sup>7</sup>, and Joyce M. Slingerland<sup>1,4,5,\*\*</sup>*

**Authors' Affiliations:** <sup>1</sup>Braman Family Breast Cancer Institute, Sylvester Comprehensive Cancer Center, <sup>2</sup>S. and D. Fuente Graduate Program in Cancer Biology, Depts of <sup>3</sup>Pathology, <sup>4</sup>Biochemistry & Molecular Biology, <sup>5</sup>Medicine, U of Miami Miller School of Medicine, Miami, FL 33136, USA; <sup>6</sup>Dept of Radiation Oncology, Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; <sup>7</sup>Division of Biostatistics, Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX, USA

**\*\*Corresponding Author:** Joyce M. Slingerland; Braman Family Breast Cancer Institute at Sylvester; Miller School of Medicine, University of Miami, Miami, FL 33136, USA. Tel: + 1 305 243 6788; Fax: +1 305 243 6170; E-mail: [jslingerland@med.miami.edu](mailto:jslingerland@med.miami.edu)

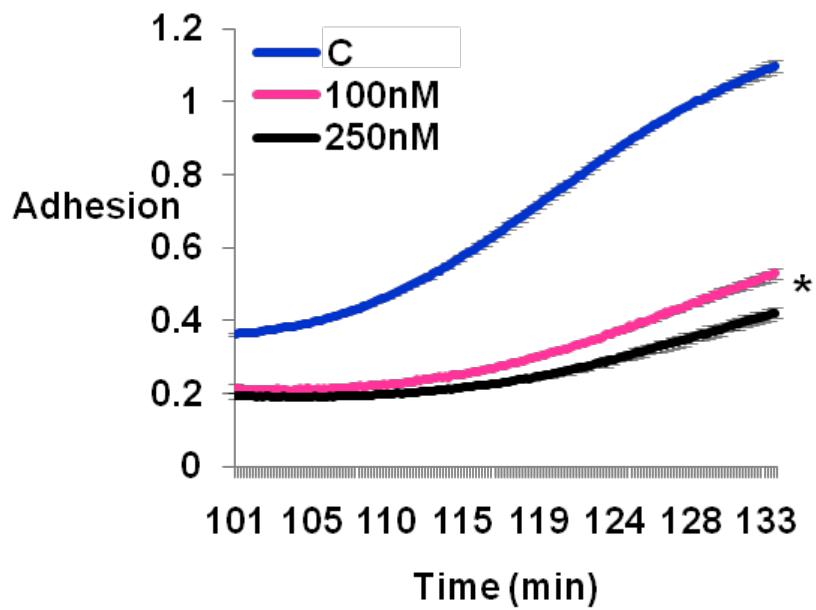


Figure S1. As for MDA-MB-231-1833, PF1502 treatment impairs cell adhesion in parental MDA-MB-231. Cell adhesion assayed +/- drug using real time Xcelligence visualization (values plotted +/-SEM, T-test at final data point \* p=0.0002).

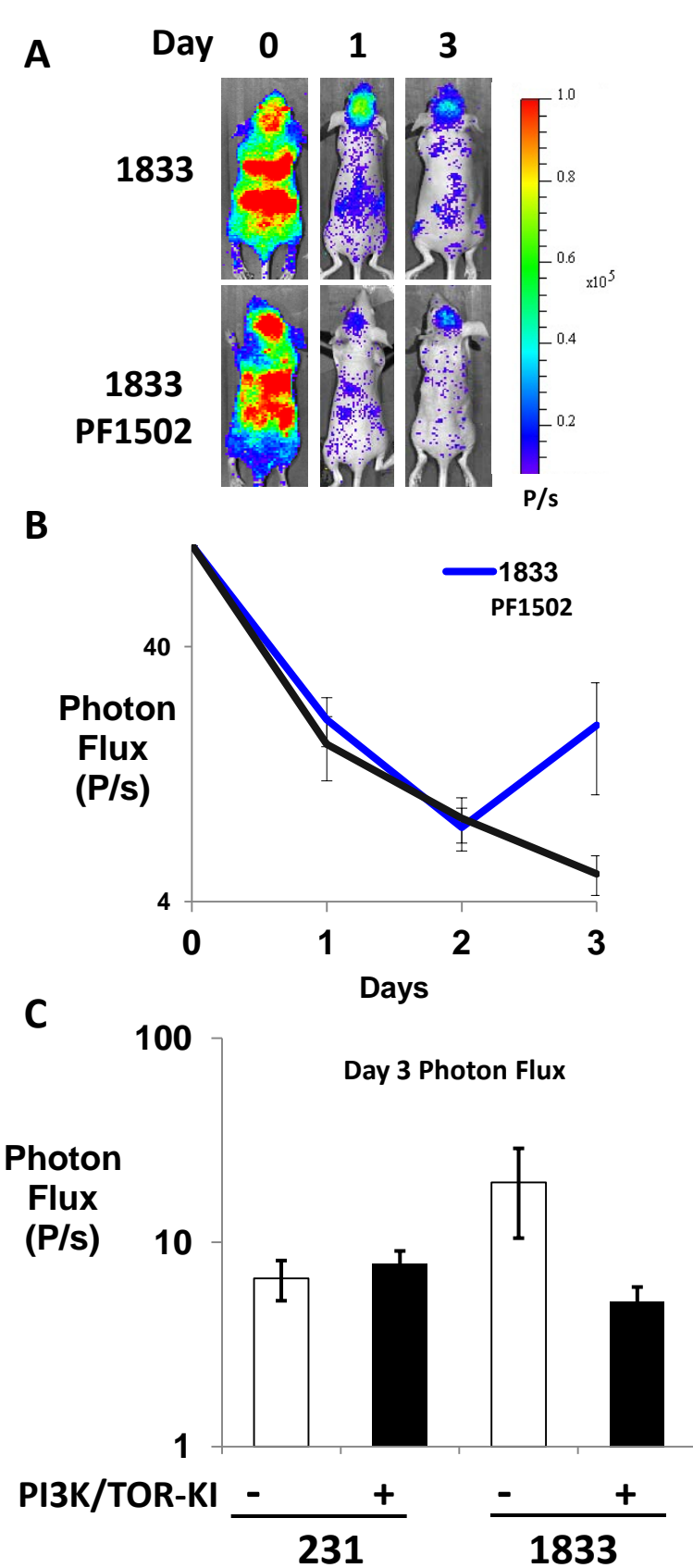


Figure S2. PI3K/mTOR inhibition impairs early establishment of micrometastatic foci. MDA-MB-231 or 1833 cells were pre-treated with vehicle control or 250nM PF1502 for 7 days prior to intracardiac injection. A) Representative IVIS images of systemic tumor burden during the first 72 hours following inoculation. B,C) Quantitative photon flux data for the first 72 hours following intracardiac injection. Average normalized photon flux is presented +/- SEM.

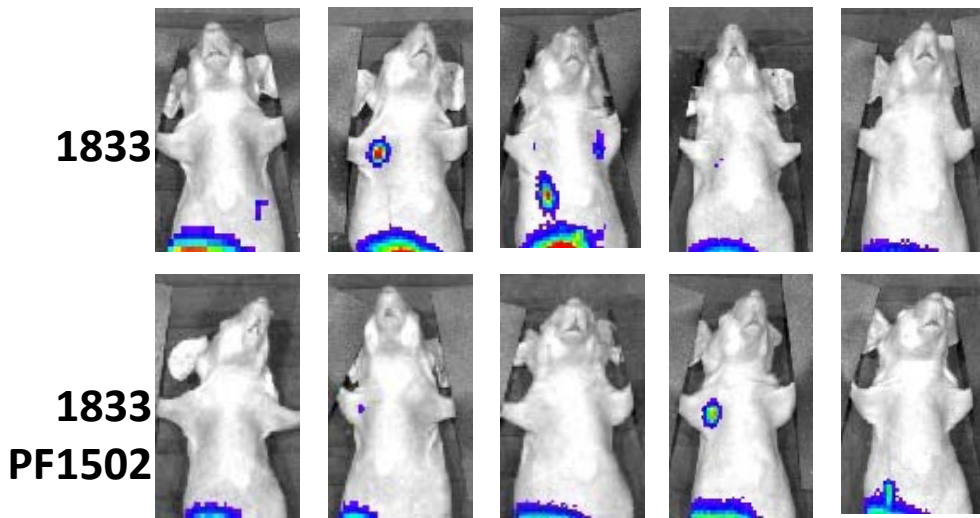


Figure S3. Metastases to regional lymph nodes are demonstrated for mice inoculated in the mammary fat pad with MDA-MB-1833 cells with or without 7-day pre-treatment with PF1502. While PF1502 treatment over one week prior to injection into the mammary fat pad had little effect on primary tumor formation (Fig 5E), lymph nodal metastasis from orthotopic primary mammary fat pad tumors were reduced. Four of five animals receiving untreated cells developed metastasis while only 2/5 drug pre-treated cells yielded tumors that gave rise to nodal metastasis.