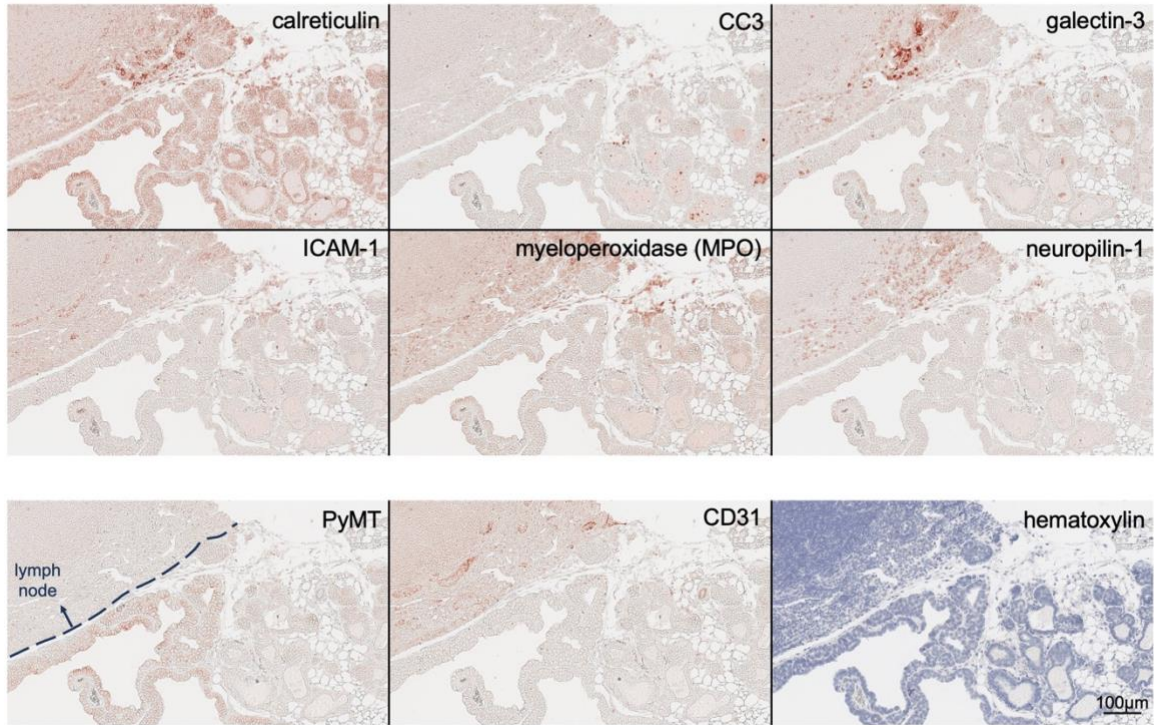


**Panobinostat induced spatial *in situ* biomarkers predictive of anti-PD-1 efficacy in mouse mammary carcinoma**

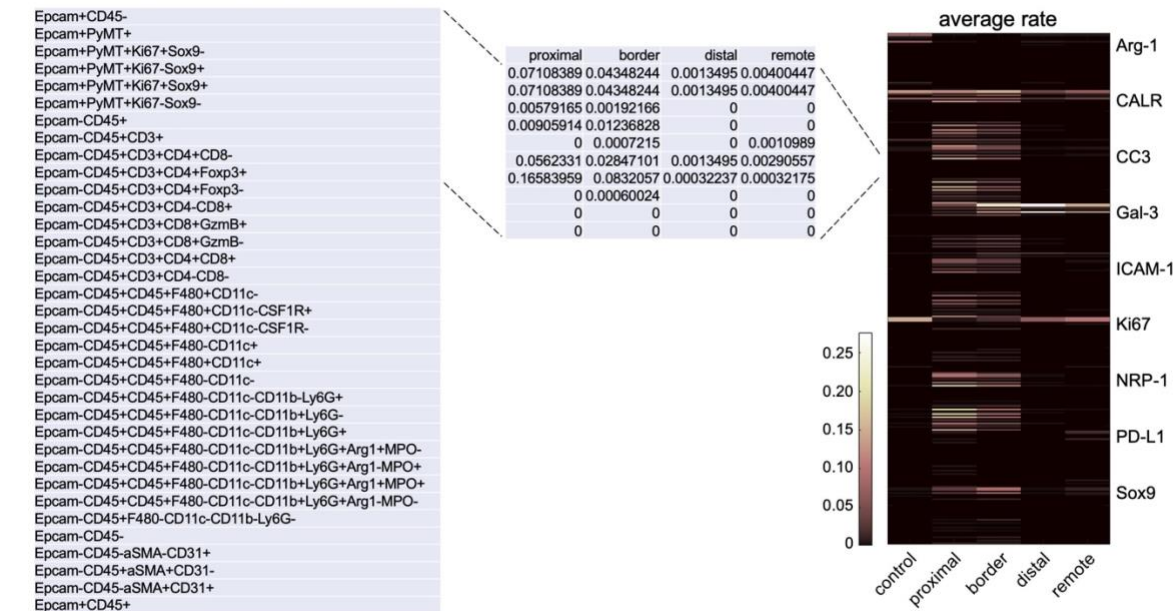
**Zuzana Tatarova <sup>1,2,3,\*</sup>, Dylan C. Blumberg <sup>1,3</sup>, AeSoon Bensen <sup>1,4</sup>, Lisa M. Coussens <sup>2,4</sup>, Gordon B. Mills <sup>5</sup>, Oliver Jonas <sup>3,\*</sup>**

Supporting Fig. 1



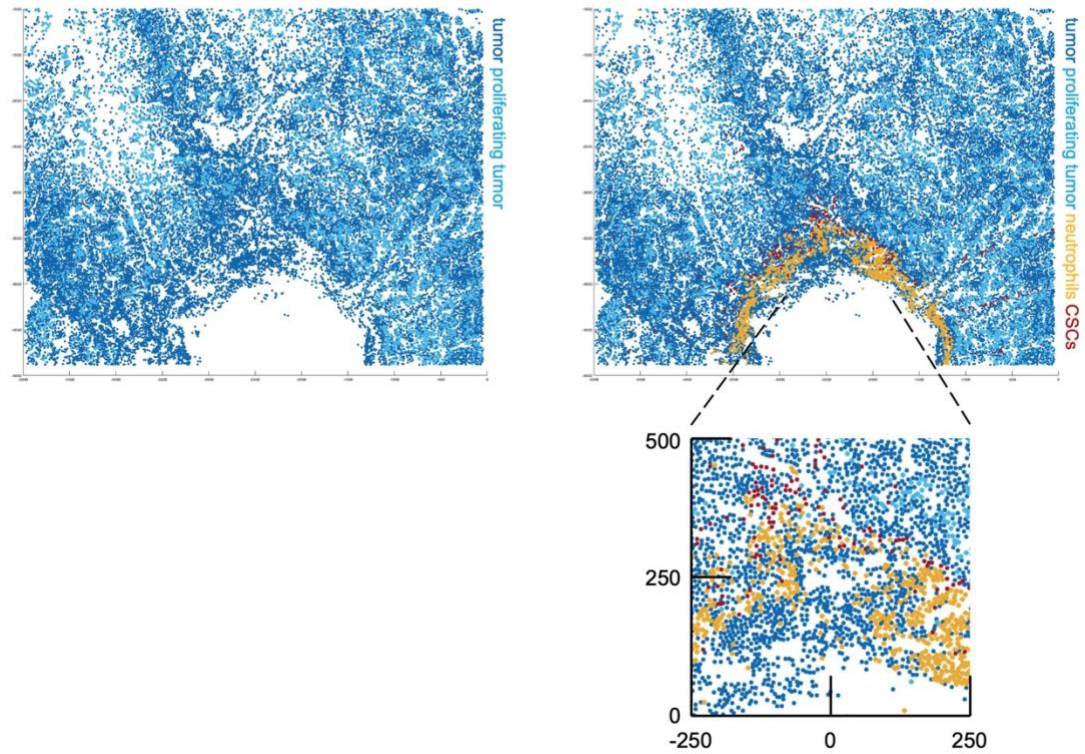
**Supporting Figure S1. Multiplex IHC (mIHC) of candidate markers using positive control FFPE tissue.** Brightfield images of stated markers using mIHC (red signal from 3-amino-9ethylcarbazol, AEC, precipitate). FFPE tissue section containing an early tumor (<0.5cm in the longest dimension) from MMTV-PyMT mouse model of breast cancer and adjacent lymph node (border depicted by dashed line; bottom left). Hematoxylin staining; bottom right image. Scale bar; shown.

Supporting Fig. 2



**Supporting Figure S2. Marker combinations and their spatial quantification at the panobinostat drug-releasing site.** Expression of each candidate ICD marker was measured within “standard cell type” population as defined by marker combination; left list. Zoomed view of quantitative presentation of average cell rates in different panobinostat zones; middle table. Quantification of all 324 marker combinations per panobinostat zone (x-axis) presented in form of a heatmap; right. Average rate and colorbar; presented.

Supporting Fig. 3



**Supporting Figure S3. Zoom view of cell types in XY coordinate space.** Each dot represents a single cell as color-coded on the right. Neutrophil and cancer stem cells (CSCs) are localized into non-proliferating tumor regions; top. Magnified view; bottom right.