Supplementary File 1

3D melanoma spheroid model for the development of positronium biomarkers

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Supplementary Figure 1. Comparison of the mean o-Ps lifetime and intensity for different melanoma cell lines in relation to spheroid metabolism.

The lifetime of o-Ps in the WM266-4 (blue dot) and WM115 (red square) spheroids on two different days (4 and 8) after cell seeding compared with the hypoxia distribution in the spheroids represented as the distance from the spheroid center in WM266-4 (gren line) and WM115 (turqoised line).

Oxygen uptake rate distribution was determined using a hypoxia kit, Image-ITTM Green Hypoxia Reagent (Thermo Fisher Scientific, cat. no I14834). To assess the oxygen uptake rate distribution in the spheroids, WM266-4 and WM115 cells were seeded at densities of 1000 and 2000 cells/drop. On days 4 and 8, the spheroids were transferred to glass-bottom dishes, and 20 μ l of Image-ITTM Hypoxia Reagent (10 μ M) was added to each spheroid. The spheroids were incubated at 37°C for 1 hour. The hypoxia dye was exchanged with fresh growth medium, and the spheroids were placed again in a cell culture incubator for the next 4 hours. Spheroid images were taken with a Nikon Eclipse Ti-E microscope coupled to an A1 scanning confocal system (Nikon, Japan) at a 488 nm/520 nm fluorescent wavelength.