Electronic Supplementary Material (ESI) for Integrative Biology
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Supporting Materials

Zhang, et al "Comparative study of 3D morphology and functions on genetically engineered mouse melanoma cells"

Acquisition of Image Stacks. The emitted fluorescence light from the Syto 61 stain was saved in the red channel while that from the MitoTracker Orange was saved in the green channel of the output image files. In both channels we chose 12-bit digitization for pixel intensity to increase the precision of image processing. A careful examination of these images reveals that in respective channel the average intensity of pixels in the nucleus is higher than that in mitochondria, which further decreases to an even lower value for pixels in cytoplasm but all are of higher values than the average intensity of pixels in the medium. The differences among the average pixel intensities provide the basis to select thresholds for segmentation of different organelles through histogram analysis and other algorithms. A set of Matlab (MathWorks) codes have been developed for 3D reconstruction using the confocal image stack data and subsequent quantitative analysis of cell morphology based on our previous efforts for modeling of light scattering by cells (1-3). The key to the success of reconstruction lies in the accurate segmentation of each image slice into four mutually exclusive regions of nucleus, mitochondria, cytoplasm and background or the area outside of the cell membrane.

Time-Lapse Videos. Attachment of the B16/vector and B16/GPR4 cells was recorded respectively for 1 hr after cell plating with a phase contrast microscope and one image acquired every 30 seconds. Similarly migration of the B16/vector and B16/GPR4 cells was recorded for 16 hr after wounds were generated on the monolayers with one image acquired every 5 minutes.

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One video file was generated from each set of the image files using the ImageJ software to obtain the video files from S1 to S4.

Video Captions

Video S1 The time-lapse video of attachment for the B16/vector cells over an observation period of 1 hour and each frame separated by 30 seconds after the cells were plated.

Video S2 The time-lapse video of attachment for the B16/GPR4 cells over an observation period of 1 hour and each frame separated by 30 seconds after the cells were plated.

Video S3 The time-lapse video of migration for the B16/vector cells over an observation period of 16 hours and each frame separated by 5 minutes after a wound was generated in the monolayer.

Video S4 The time-lapse video of migration for the B16/GPR4 cell monolayer over an observation period of 16 hours and each frame separated by 5 minutes after a wound was generated in the monolayer.