Article

GAP43-dependent mitochondria transfer from astrocytes enhances glioblastoma tumorigenicity

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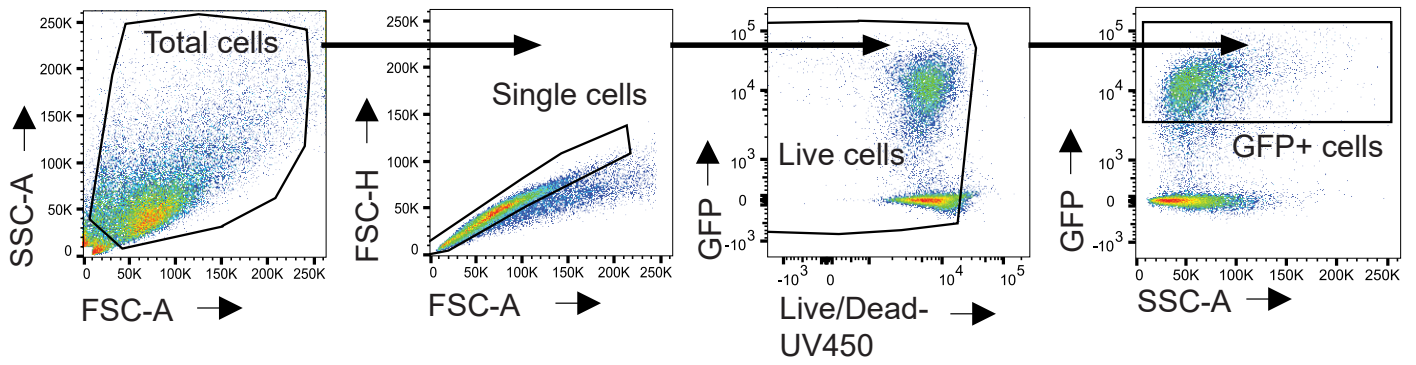
Supplementary Note

<u>GL261 model</u>

(1) Define GL261 objects:
      a. Channel 3 (GFP) threshold
            i. intensities: 11-185
            ii. Minimum object size = 10000 cubic microns
      b. Fill holes in objects
      c. Separate touching objects
            i. Object size guide = 1200 cubic microns
(2) Define mitochondria objects
      a. Channel 1 (mito::mKate2) threshold
            i. Intensities 20-255
            ii. Minimum object size = 1 cubic micron
      b. Separate touching objects
            i. Object size guide = 25 cubic microns
      c. Filter population
            i. Volume >= 0.1 cubic microns
(3) Compartmentalize mitochondria objects inside GL261 objects

<u>SB28 model</u>

(1) Define SB28 objects:
      a. Channel 3 (GFP) threshold
            i. intensities: 20-255
            ii. Minimum object size = 10000 cubic microns
      b. Fill holes in objects
      c. Separate touching objects
            i. Object size guide = 1200 cubic microns
      d. Filter population
            i. Volume > 600 cubic microns
(2) Define mitochondria objects
      a. Channel 1 (mito::mKate2) threshold
            i. Intensities 20-255
            ii. Minimum object size = 1 cubic micron
      b. Separate touching objects
            i. Object size guide = 25 cubic microns
      c. Filter population
            i. Volume >= 0.1 cubic microns
(3) Compartmentalize mitochondria objects inside SB28 objects

In-vitro gating strategy

In-vivo gating strategy
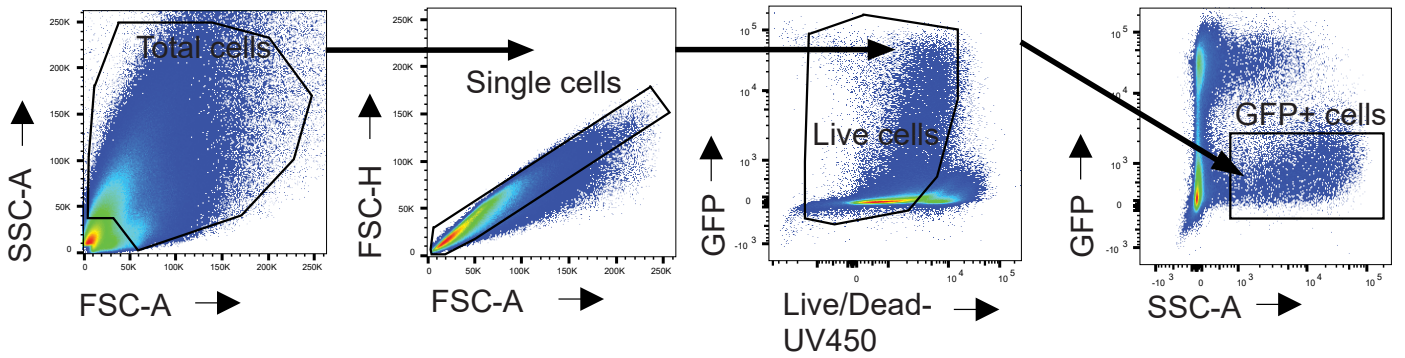
Supplementary Fig. 1. Gating strategy

Flow cytometry gating strategy used for *in vitro* and *in vivo* experiments.