

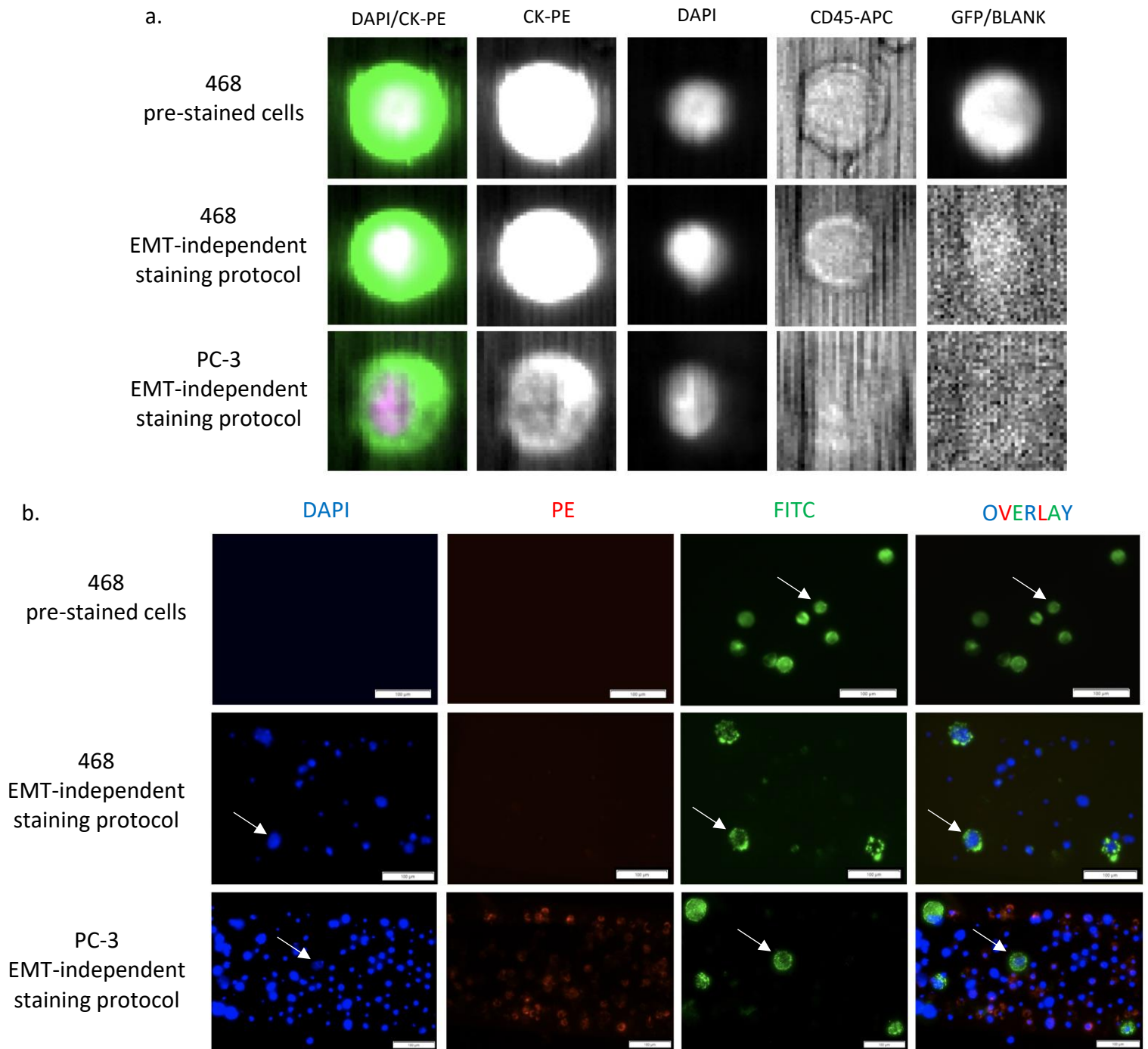
CLINICAL & EXPERIMENTAL METASTASIS

EMT-independent detection of circulating tumor cells in human blood samples and pre-clinical mouse models of metastasis

Jenna Kitz^{1,2}, David Goodale¹, Carl Postenka¹, Lori E. Lowes³, and Alison L. Allan^{1,2,4,5*}

London Regional Cancer Program¹ and Flow Cytometry³, London Health Sciences Centre; Departments of Anatomy & Cell Biology² and Oncology⁴, Western University; and Lawson Health Research Institute⁵, London, Ontario CANADA

***Corresponding author;** alison.allan@lhsc.on.ca or 519-685-8600 x55134



Online Resource 2 Representative images of positive CTCs isolated from mouse blood using CellSearch® and VyCap. For baseline recovery experiments, MDA-MB-468 human breast cancer cells (epithelial) were prestained with the CellTrace™ carboxyfluorescein succinimidyl ester (CFSE) Cell Proliferation Kit. For all other experiments, human MDA-MB-468 cells or human PC-3 mesenchymal cells were stained using the mouse staining protocols outlined in the Materials & Methods. (a) Representative positive CTCs isolated using the mouse CellSearch® protocol. DAPI⁺/CK-PE⁺/CD45⁺/GFP(CFSE)⁺ cells are considered to be positive CTCs in pre-stained samples. DAPI⁺/CK-PE⁺/CD45⁺ cells are considered to be positive CTCs in samples processed with the CellSearch® CTC staining protocol. (b) Representative positive CTCs isolated using the mouse VyCap protocol (white arrows). FITC (CFSE)⁺ are considered to be positive CTCs in pre-stained samples. For samples stained with the EMT-independent staining protocol, DAPI⁺/PE⁺/FITC⁺ cells are considered positive (DAPI (blue) = nuclear stain, PE (red) = CD45, FITC (green) = HLA). Data are presented as the mean ± SEM (n ≥ 3), * = significantly different than CellSearch® (p ≤ 0.05)