## Supplementary Information

## Microfluidic filter device with nylon mesh membranes efficiently dissociates cell aggregates and digested tissue into single cells

Xiaolong Qiu,<sup>#a</sup> Jeremy A. Lombardo,<sup>#a</sup> Trisha M. Westerhof,<sup>d</sup> Marissa Pennell,<sup>a</sup> Anita Ng,<sup>a</sup> Hamad Alshetaiwi,<sup>e</sup> Brian M. Luna,<sup>a</sup> Edward L. Nelson,<sup>cdf</sup> Kai Kessenbrock,<sup>ef</sup> Elliot E. Hui,<sup>ag</sup> and Jered B. Haun<sup>\*abfg</sup>

# These authors contributed equally to this work.



**Figure S1. Single filter device experiments using MCF-7 cells.** (A) Live single cell numbers for experiments performed under direct filtration mode and 12.5 mL/min flow rate. Values were ~40% higher than the control for each of the 5, 10, and 15  $\mu$ m pore sizes. (B) Cell populations obtained for direct filtration experiments at 0.25, 1, and 4 mL/min flow rates. (C-E) Cell populations obtained for tangential filtration experiments using 12.5 mL/min total flow rate and cross-flow ratios of (C) 80%, (D) 60%, and (E) 40%. Error bars represent standard errors from at least three independent experiments. \* indicates p < 0.05 and \*\* indicates p < 0.01 relative to the control.



**Figure S2. Double filter device experiments using MCF-7 cells.** (A) Live single cell number for double filter device experiments performed under direct filtration mode and 12.5 mL/min flow rate. Values were lowest for membrane all combinations that included the 5  $\mu$ m pore size. (B) Live single cell numbers for double filter device experiments performed under tangential filtration mode, 12.5 mL/min total flow rate, and 60% cross-flow ratio. Values were close to 2-fold greater than control in all cases. (C-F) Double filter device experiments performed under tangential filtration mode, 12.5 mL/min total flow rate, and 80% cross-flow ratio. Results for (C) cell populations, (D) single cell recovery, (E) viability, and (F) live single cell recovery were similar to 60% cross-flow ratio experiments. Error bars represent standard errors from at least three independent experiments. \* indicates p < 0.05 and \*\* indicates p < 0.01 relative to the control.



**Figure S3. Filter device optimization using murine kidney tissue.** (A,B) Experiments performed using two single membrane filter devices connected in series. Recoveries are shown for (A) red blood cells and (B) leukocytes, which both increased with both digestion time and device processing in a manner consistent with single tissue cell recovery results in Fig. 4C. (C,D) Experiments performed using the integrated dual membrane filter device with kidney tissue that was digested for 60 min. (C) Single tissue cell number increased by ~60% after device processing relative to the control. (D) Viability remained at >85%, similar to control. Error bars represent standard errors from at least three independent experiments. \* indicates p < 0.05 and \*\* indicates p < 0.01 relative to the control at the same digestion time.



Figure S4. Red blood cell and leukocyte recoveries for murine liver and tumor tissue samples. Results are shown for (A,B) liver and (C,D) mammary tumor cell suspensions. Red blood cell and leukocyte cell counts increased with both digestion time and device processing in all cases. Recoveries increased with digestion time and device processing in a manner consistent with single tissue/epithelial cell results in Fig. 5. Error bars represent standard errors from at least three independent experiments. \* indicates p < 0.05 and \*\* indicates p < 0.01 relative to the control at the same digestion time.