Supporting Information

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SI Text

Phage Display. The random linear phage library (X_7, X = random amino acid; diversity $\sim 5 \times 10^8$) and individual phage (G7 and RPARPAR) were constructed with the T7-Select Phage Display System (EMD Biosciences). Phage were purified by precipitation with PEG-8000/NaCl (Sigma-Aldrich) followed by CsCl gradient ultracentrifugation and dialysis. For library screening and phage binding experiments, cultured cells were grown in cell culture slide chambers to nearly 90% confluence.

Cell Recovery Experiments for Conventional Suspension Biopanning. To investigate cell loss during centrifugation separation in conventional suspension biopanning, we subjected 10^6, 10^5, and 10^4 suspended cells to different numbers of fixed-volume centrifuge washes (1 mL of DMEM + 1% BSA) (350 × *g*, 5 min), with each combination performed in duplicate. The resulting cell pellets were resuspended in 1 mL of DMEM + 1% BSA, and cell number was determined by flow cytometry (Accuri C6 Flow Cytometer from Accuri Cytometers Inc.). Finally, cell recovery was calculated based on the initial sample size.

Dissociation Constant (K_d) Measurements of Biotinylated Peptides. We quantified the K_d of several selected peptides against NRP-1 protein (R and D Systems) using an ELISA-based immunochemical method. Microtiter wells were coated with 50 μL of 5 μg/mL of purified NRP-1 protein overnight at 4 °C. After washing with PBST (PBS + 0.01% Tween 20), the wells were incubated for 1 h at room temperature with 50 μL of various concentrations of biotinylated peptide in PBS. After washing with PBST, we added streptavidin-conjugated horseradish peroxidase (Vector Laboratories) to the wells and incubated for 30 min at room temperature. Horseradish peroxidase binding was quantified using 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) as a substrate. Using *Origin* (OriginLab), we obtained apparent dissociation constants (K_d) for the peptide-NRP-1 interaction by fitting the dependence of the OD_{405} value of specific binding on the concentration of peptides to the one-site binding saturation equation $Y = B_{max}X/(K_d + X)$.

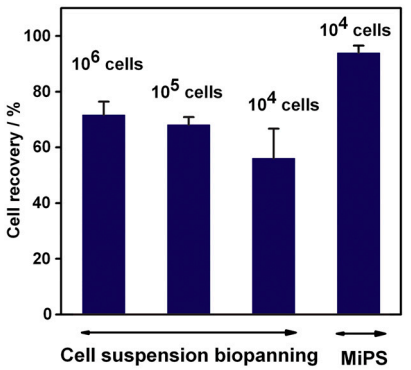

Fig. S1. MiPS chip selection enables high cell recovery even under highly stringent washing conditions. Recovery experiments were performed to count PPC-1 cells before and after the library binding and microfluidic continuous washing steps, and an average of 93.9% recovery was obtained for the MiPS assay over eight experiments. Four trials were performed, each consisting of a pair of slides from before and after library binding and continuous washing steps. For comparison, we performed conventional cell suspension biopanning with starting amounts of 10^6, 10^5, and 10^4 cells in order to investigate cell loss during washing, and obtained average recovery of 71.7%, 68.1%, and 56.1%, respectively, after four fixed-volume washes.

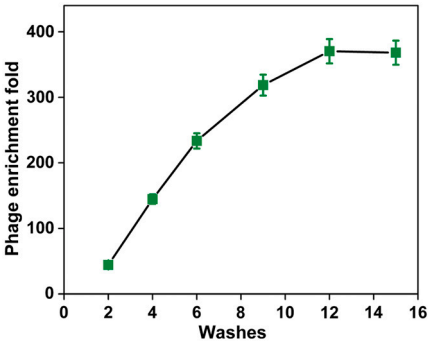

Fig. S2. We performed control biopanning experiments with 2, 4, 6, 9, 12, and 15 fixed-volume washes using Method A (static incubation, fixed-volume washing) and observed that RPARPAR enrichment reaches a plateau after 12 washes (~300-fold enrichment). Further washing did not significantly improve enrichment.

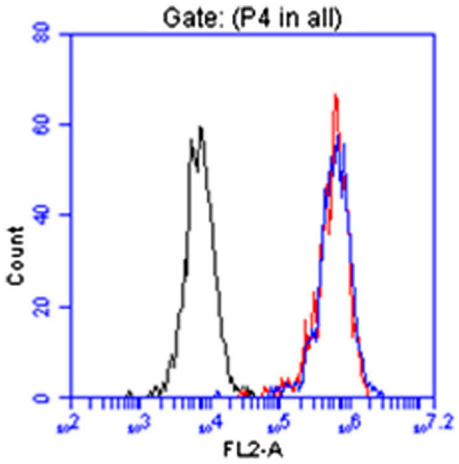

Fig. S3. Flow cytometry analysis of the NRP-1 expression level on PPC-1 cells. Black: cells without antibody labeling. Red: cells labeled with anti-NRP-1-PE, before the incubation and washing. Blue: cells labeled with anti-NRP-1-PE, after incubation and washing in the MiPS device.

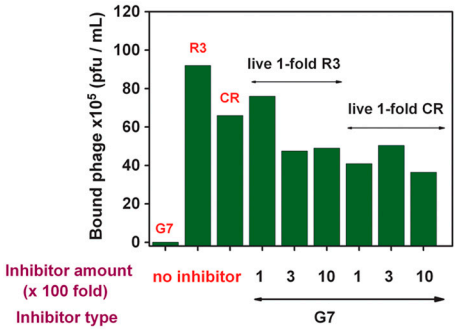

Fig. S4. Inhibitor binding assays screening the affinity of the R3 phage pool and RPARPAR (CR) phage for PPC-1 cell suspensions vs. a 100-, 300-, or 1,000-fold excess of noninfectious G7 phage. Binding of the R3 phage pool to PPC-1 cells was only minimally affected by the presence of a large excess of G7 phage.

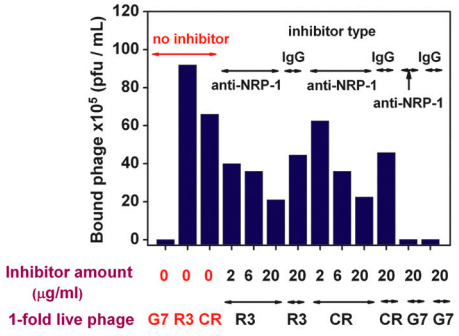

Fig. S5. The R3 pool selected with the MiPS chip binds specifically to PPC-1 cells via recognition of NRP-1. The anti-NRP-1 antibody, but not the control IgG antibody, reduced binding of both R3 pool and RPARPAR phage (CR) to PPC-1 cells.

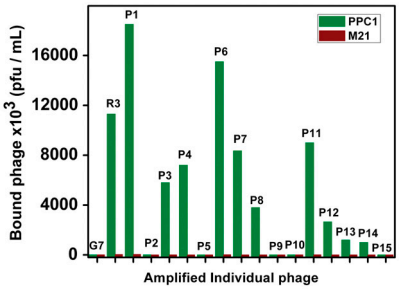

Fig. S6. Individual phage clones within the R3 pool showed strong specificity for PPC-1 cells. The binding data presented here clearly demonstrate that peptides containing R/KXXR motifs (P1, P3, P4, P6, P7, P8, P11, and P12) exhibit significantly stronger binding towards PPC-1 compared to those containing XXXR motifs (P2, P13, and P14); all 15 clones have minimal binding affinity for M21 cells. Sequences of individual clones are presented in Fig. 6.

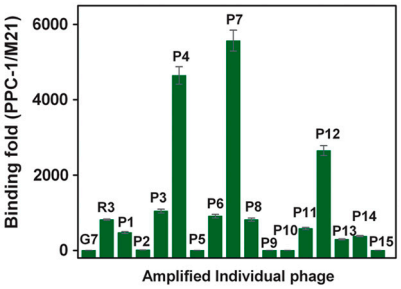

Fig. S7. Relative binding fold of the selected clones against PPC-1 cells relative to M21 cells. These experiments indicate that strongly binding CendR motif sequences (P1, P3, P4, P6, P7, P8, P11, and P12) exhibit high specificity for PPC-1 cells, while phage sequences with C-terminal arginine within XXXR motifs (P2, P13, and P14) achieve only moderate-to-weak binding. Notably, the different CendR motif-containing sequences show different levels of binding and specificity to PPC-1 cells over M21 cells.

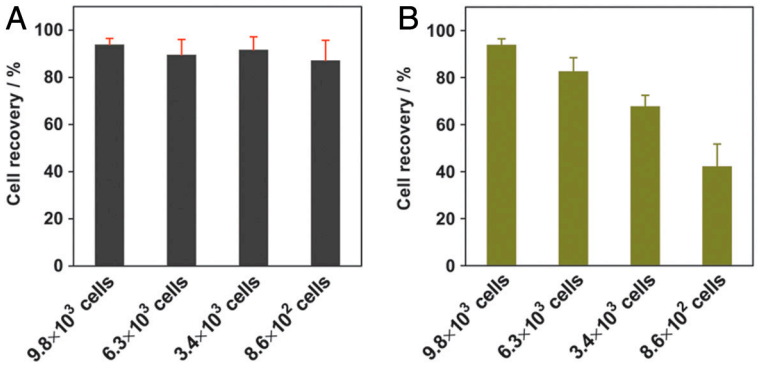

Fig. S8. The cell recovery of MiPS chip is high even when small number of cells are used. Smaller devices with a range of channel widths: 5 mm, 3.5 mm, 2 mm, and 0.5 mm were constructed to accommodate smaller number of cells, 9.8×10^3, 6.3×10^3, 3.42×10^3, and 8.6×10^2 cells respectively. Recovery experiments were performed by counting PPC-1 cells before and after the library binding and microfluidic continuous washing steps. *A*) When the shear stress was kept constant (3 dyn/cm^2) by adjusting the flow rates, an average recovery of 93.9%, 89.5%, 91.7%, 87.2% were obtained. *B*) Cell recovery decreases due to the increased shear stress if the flow rate was kept constant (1 mL/ min), an average recovery of 93.9%, 82.5%, 67.7%, 42.2% were obtained. All experiments were performed in triplicate.

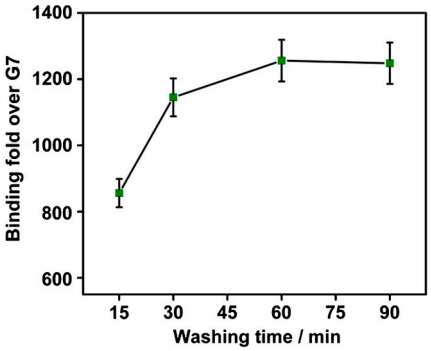

Fig. S9. Binding of RPARPAR phage relative to G7 control phage as a function of washing time in the MiPS chip with smaller channel (1.5 cm × 0.5 mm × 250 μm) which can accommodate ~1,000 cells. The enrichment fold of RPARPAR over G7 increases monotonically with washing time until a plateau (~1,200-fold) is reached after 60 min of continuous washing.