Supporting Information

Aryl-diazonium salts offer a rapid and cost-efficient method to functionalize plastic microfluidic devices for increased immunoaffinity capture

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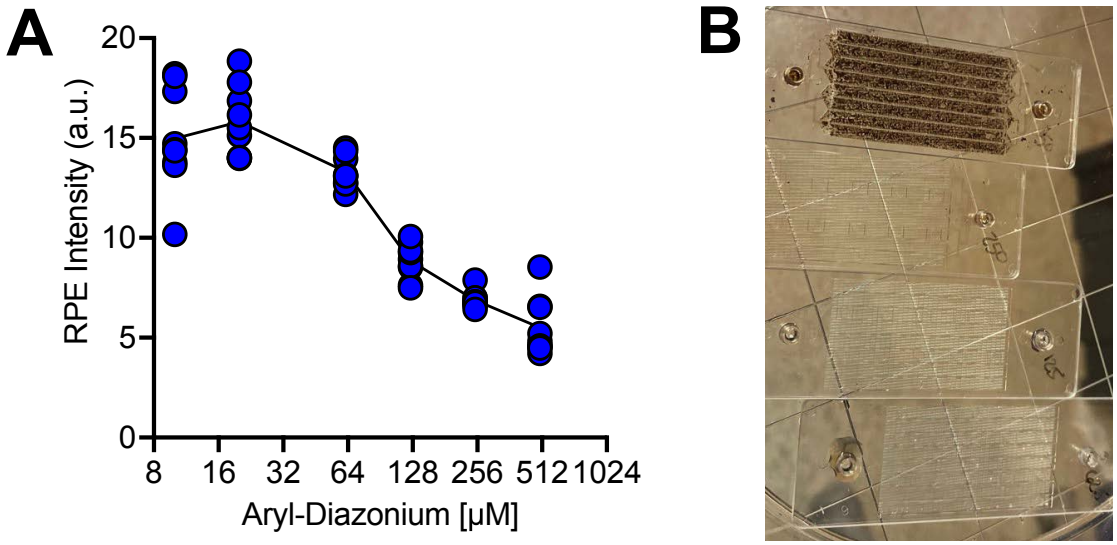

Figure S1: Aryl-diazonium functionalization decreases with concentration. A) Early experiments were conducted by first reacting aryl diazonium with the surface of the chip using various concentrations ranging from 10 to 500 µM. Following deposition of aryl-diazonium to the chip surface, NHS-Ester biotin was reacted within the chip to the surface and the binding capacity measured using our R-PE assay. We found that increasing amounts of aryl-diazonium led to a decrease in binding capacity. N=1 chip per concentration and 9 measurements per chip. B) A visual inspection of the chips following treatment showed a large deposition of aryl-diazonium (brown, 500 µM, top) that clogged the chips and leading to less deposition of biotin on the surface. Images taken with an iPhone.

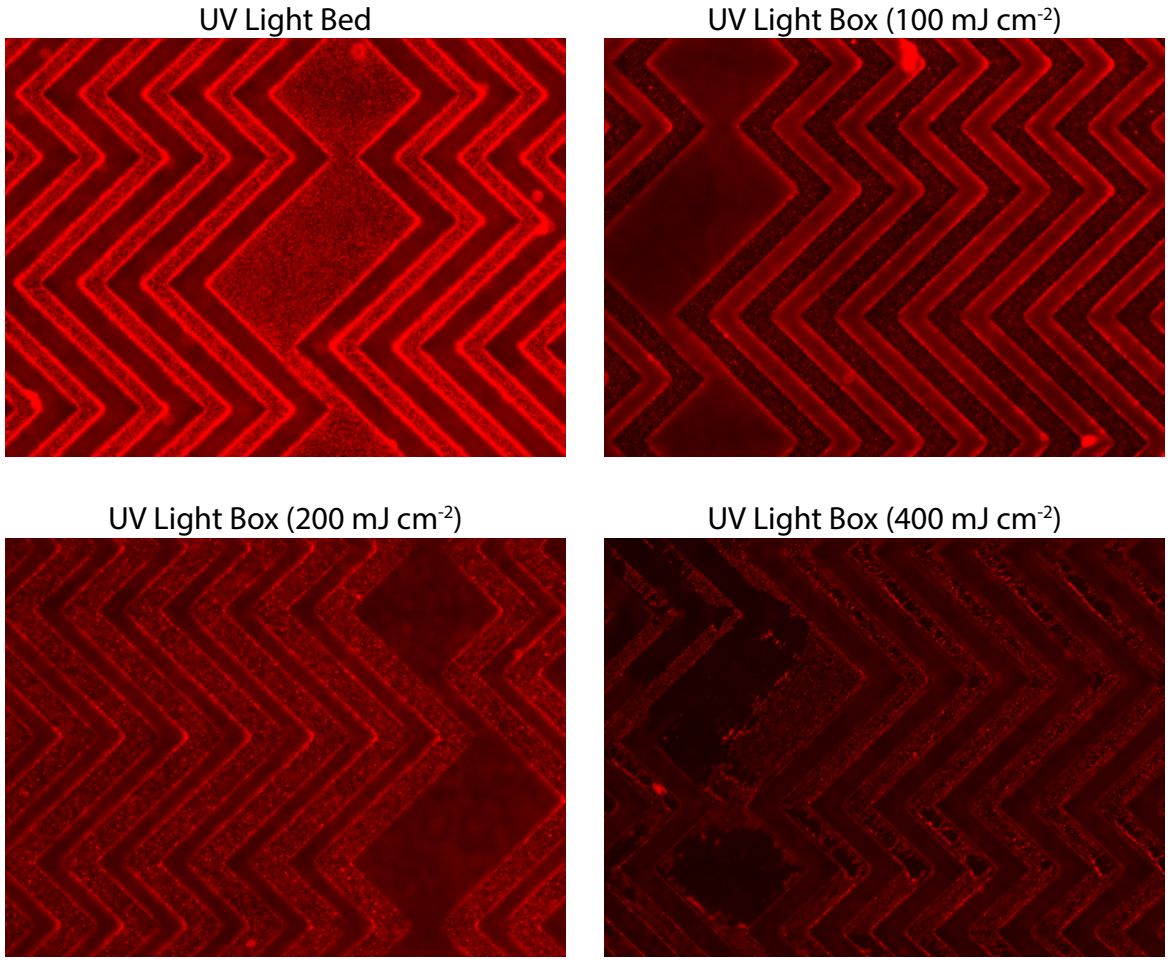

Figure S2: Representative images (main figure 2B) from UV light bed vs UV light box functionalization. Devices were functionalized for 15 minutes using a UV light bed at high **(top left)** or a UV light box with differing energies: 100 mJ cm^{-2} **(top right)**, 200 mJ cm^{-2} **(bottom left)**, or 400 mJ cm^{-2} **(bottom right)**. All images were taken for 100 ms using a 10X lens. UV light box treatment, particularly at higher energies showed many dark patches of unfunctionalized surface. Because the UV light bed showed better more consistent quality, it was utilized moving forward.

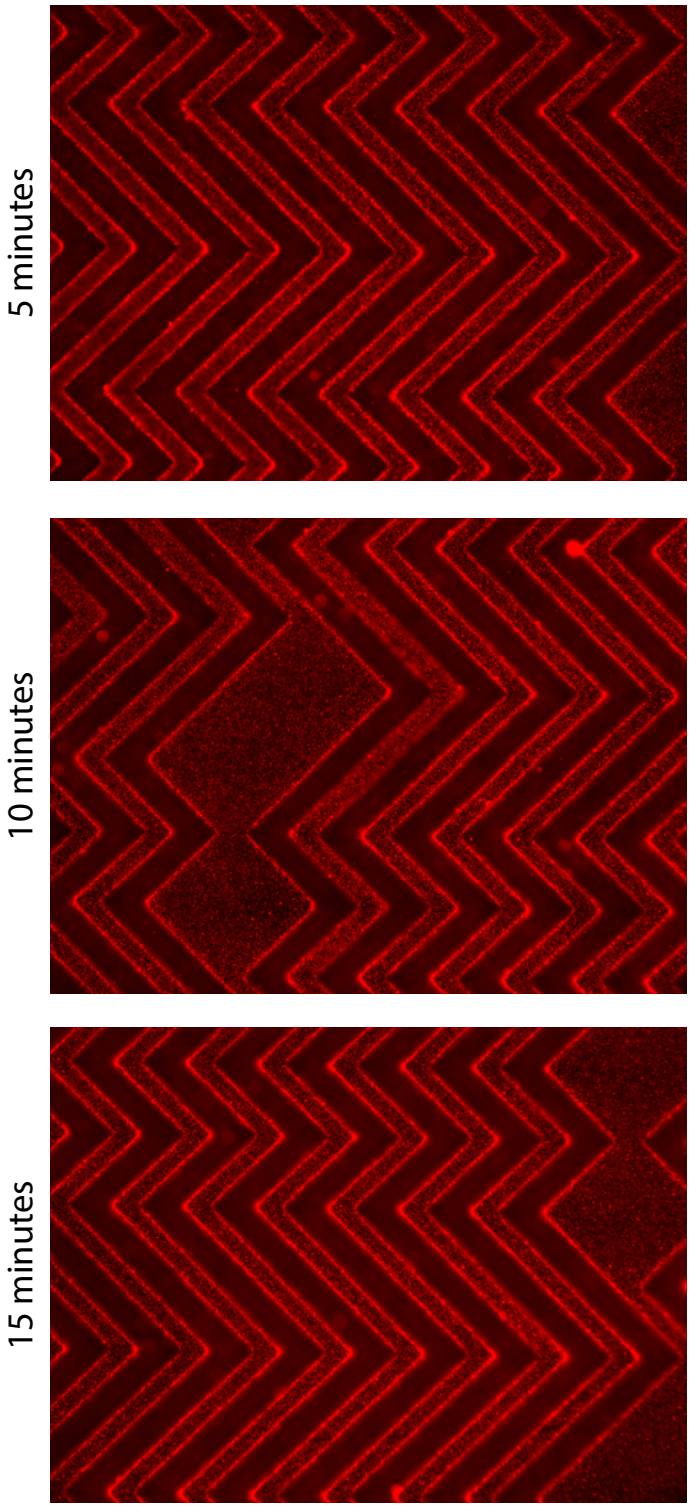

Figure S3: Representative images (main figure 2C) for different times exposed to UV light. Devices were functionalized for 5 minutes **(top)**, 10 minutes **(middle)**, or 15 minutes **(bottom)** using a UV light bed set to high. No difference seen between 10 and 15 minutes, so 10 minutes was used moving forward. All images were taken for 100 ms using a 10X lens.

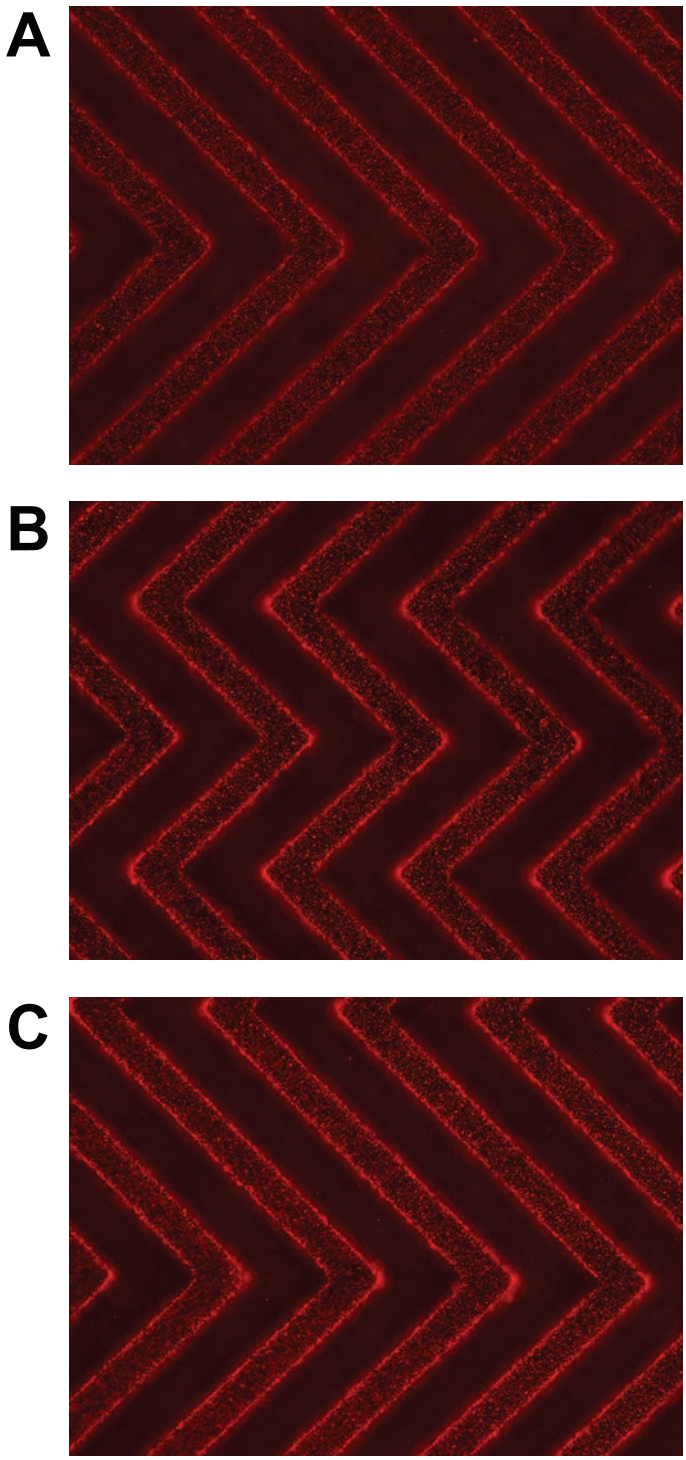

Figure S4: Representative images (main figure 2D) from different NHS-biotin to aryl-diazonium ratios. Devices were functionalized for 15 minutes using a UV light bed using either a reaction ratio of 1:2 **(A)**, 1:1 **(B)**, or 2:1 **(C)** of biotin to aryl-diazonium. All images were taken for 100 ms using a 20X lens. No difference seen between 1:1 and 2:1 ratio, so 1:1 ratio was used moving forward.

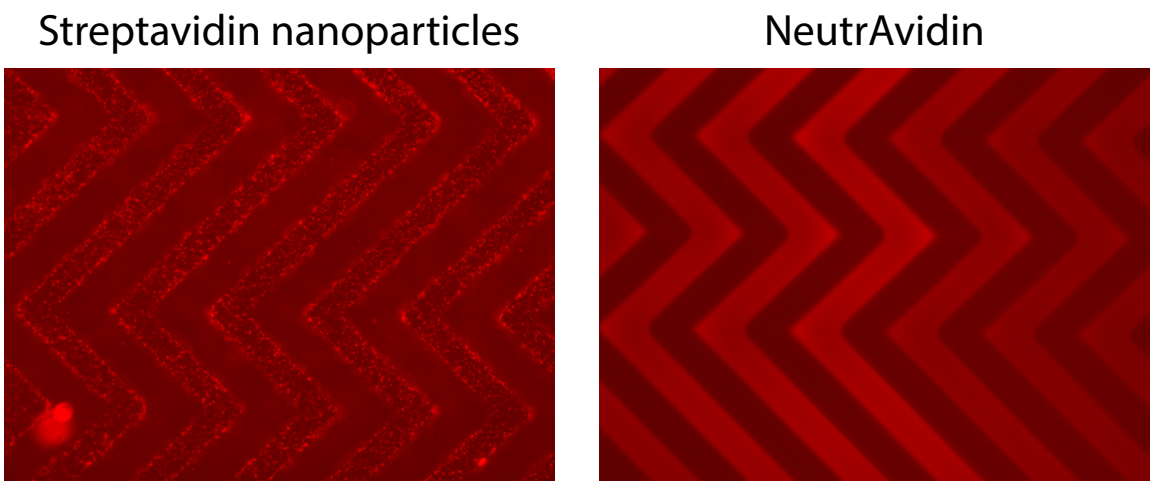

Figure S5: Representative images (main figure 2E) from herringbone chips with either Streptavidin nanoparticles or NeutrAvidin. Devices were functionalized as described, followed by addition of Streptavidin nanoparticles or NeutrAvidin (five device volumes of 20 µg/mL NeutrAvidin through the inlet, then the outlet). All images were taken for 100 ms using a 20X lens. Streptavidin nanoparticles showed an increase fluorescent signal compared to NeutrAvidin and were used for all subsequent experiments.

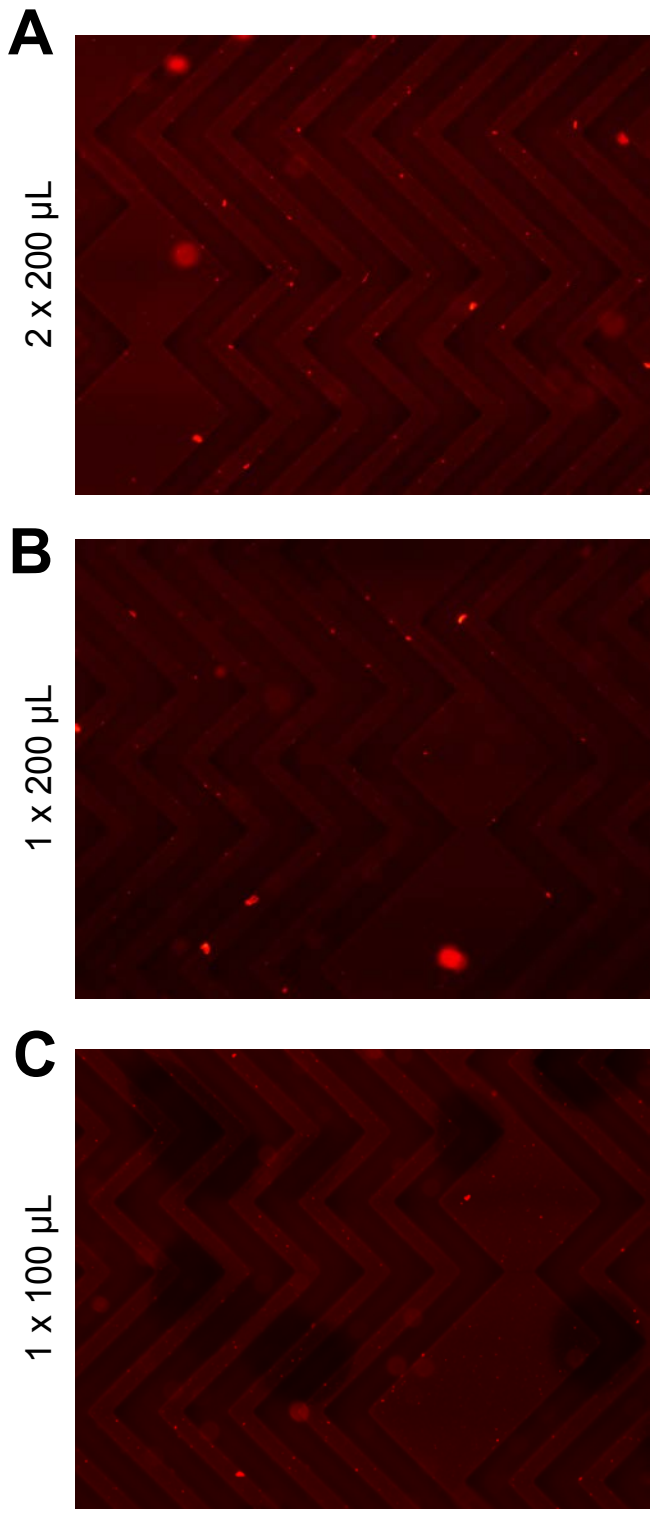

Figure S6: Representative images (main figure 2F) from herringbone chips functionalized with different amounts of biotin aryl-diazonium solution. All images were taken for 100 ms using a 20X lens. One device volume resulted in dark spots with no biotin (likely where NO_2 bubbles formed during initial UV treatment). Two device volumes (200 µL) of solution flown through the chips twice showed the most consistent results and was used moving forward.

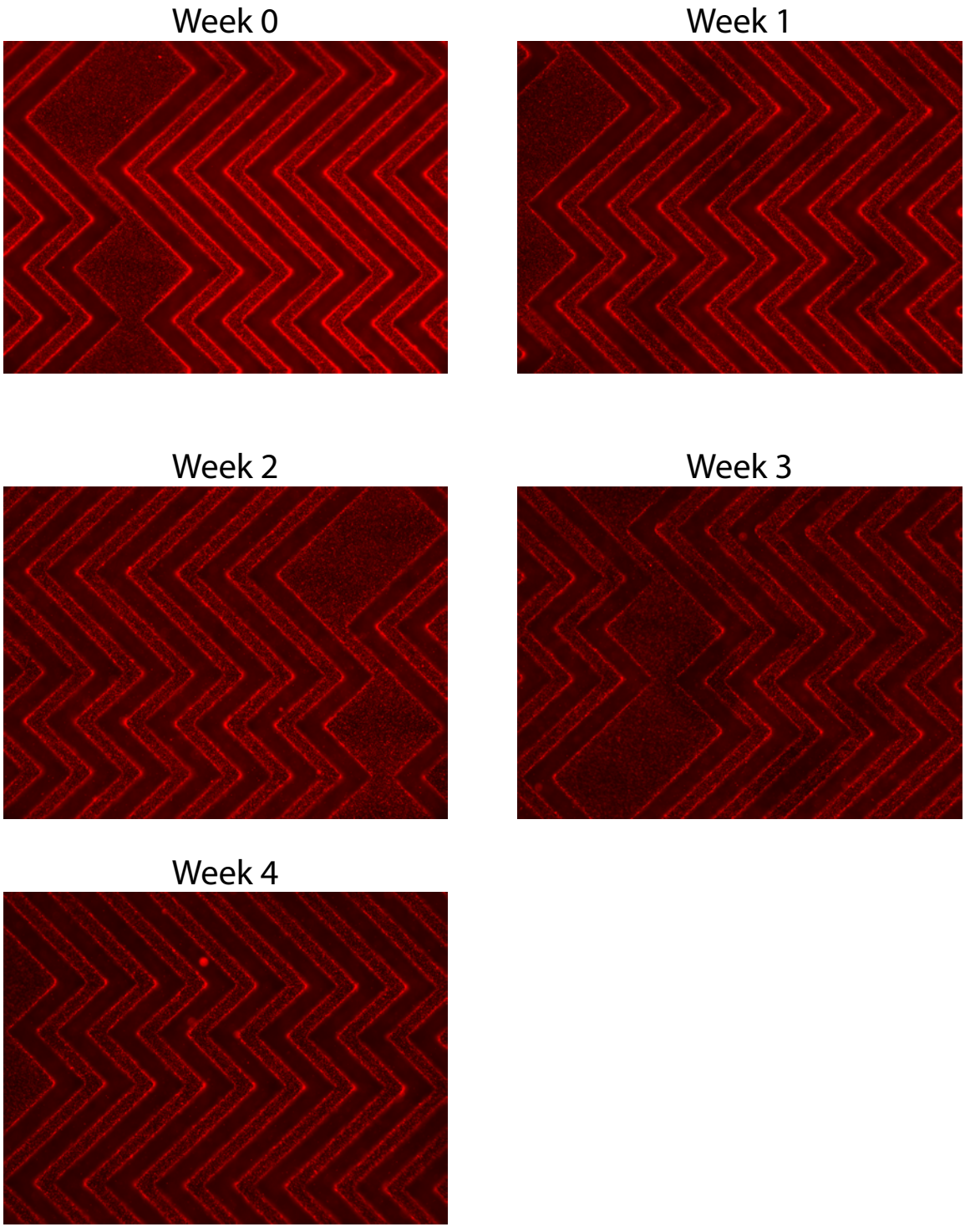

Figure S7: Representative images (main figure 3B) from devices stored in desiccant at 25 °C for up to four weeks. Devices were stored in a desiccator box over 4 weeks. All images were taken for 100 ms using a 10X lens. A 29% drop is seen in chips stored at week one versus week zero. No additional degradation was seen in the following weeks. We moved forward with storing chips in desiccant, but for longer term storage a vacuum desiccator was used.

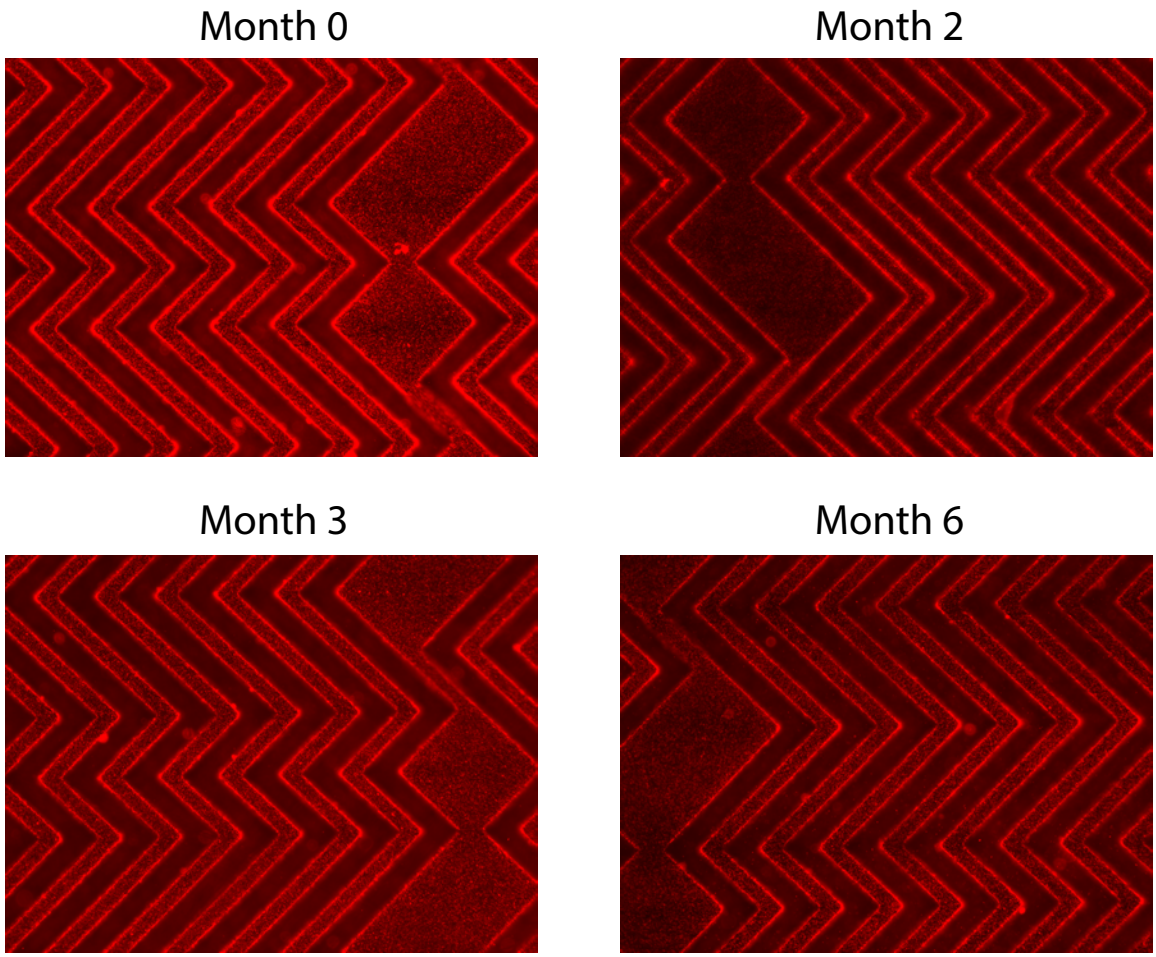

Figure S8: Representative images (main figure 3C) from devices stored in a vacuum desiccator at 25 °C for up to six months. Devices were stored in a vacuum desiccator box until use for up to six months. All images were taken for 100 ms using a 10X lens. No degradation was seen over a six-month period when stored in a vacuum desiccator.

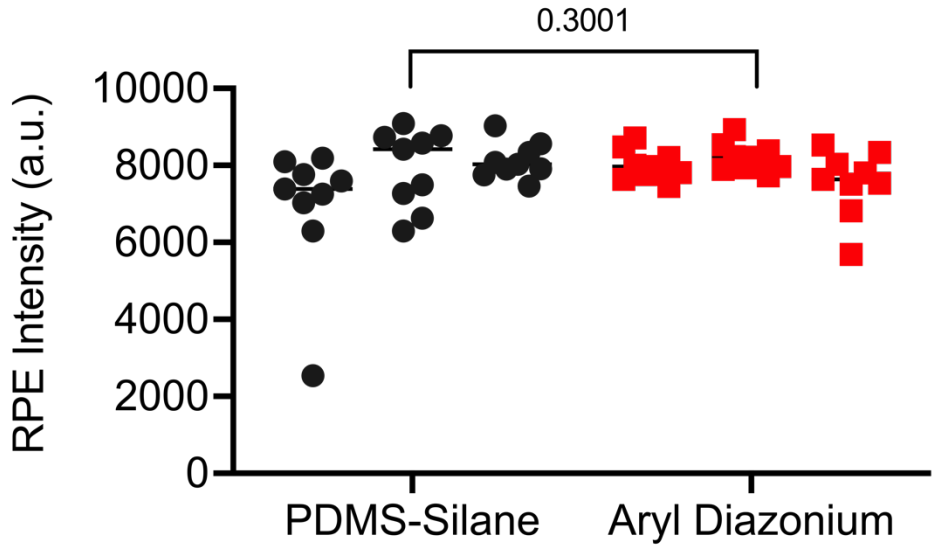

Figure S9: PDMS-Silane devices stored in vacuum bags sealed with desiccant compared to aryl diazonium plastic devices. PDMS-Silane devices (black circles) were stored in a vacuum sealed bag with desiccant for ~10 years and compared to newly made plastic aryl diazonium devices (red boxes) using our R-PE assay. All images were taken for 100 ms using a 10X lens. No difference is seen in chips stored in a dry, vacuum sealed environment, even 10 years later.

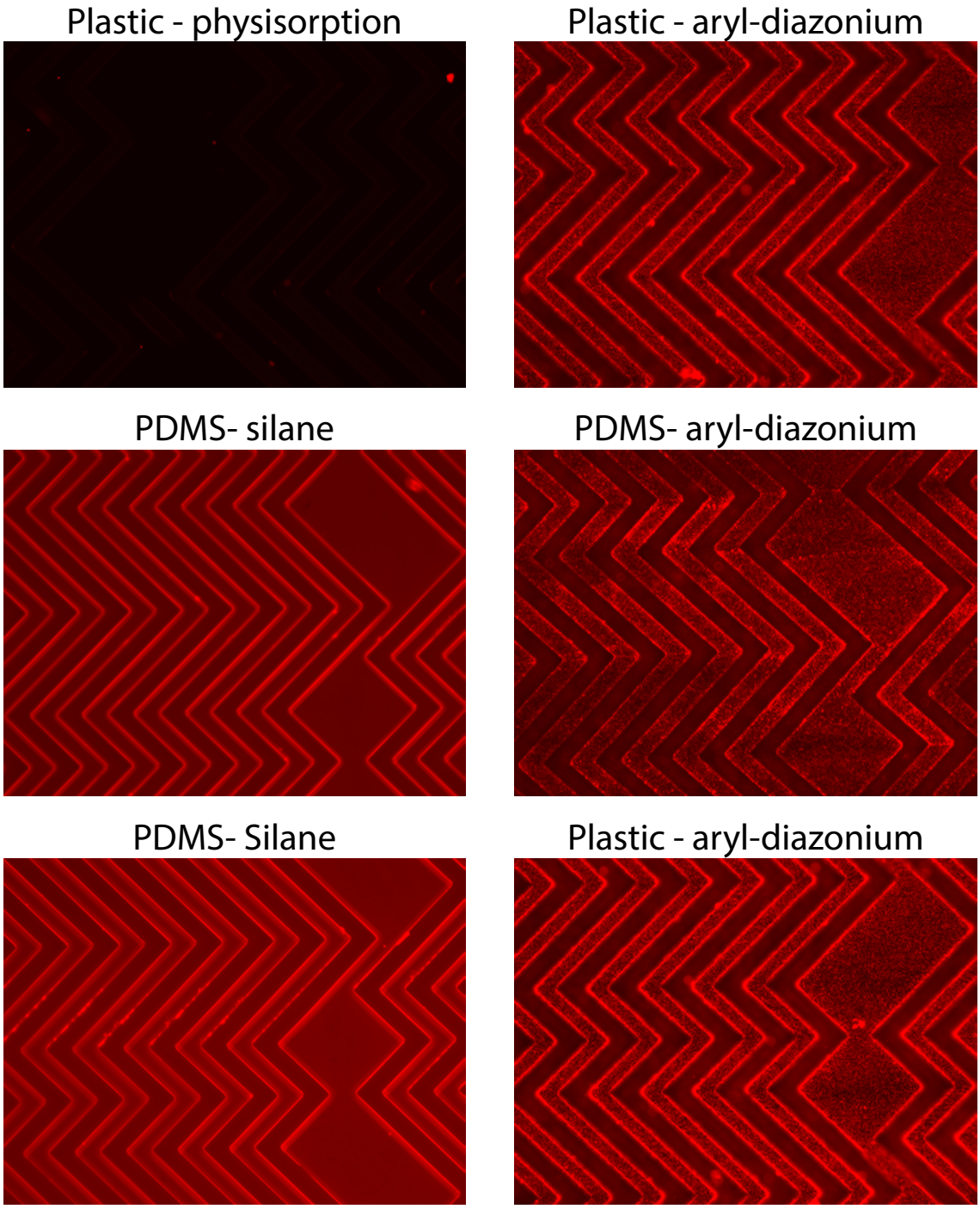

Figure S10: Representative images (main figure 4) from different device types and functionalization strategies. All images were taken for 100 ms using a 10X lens.

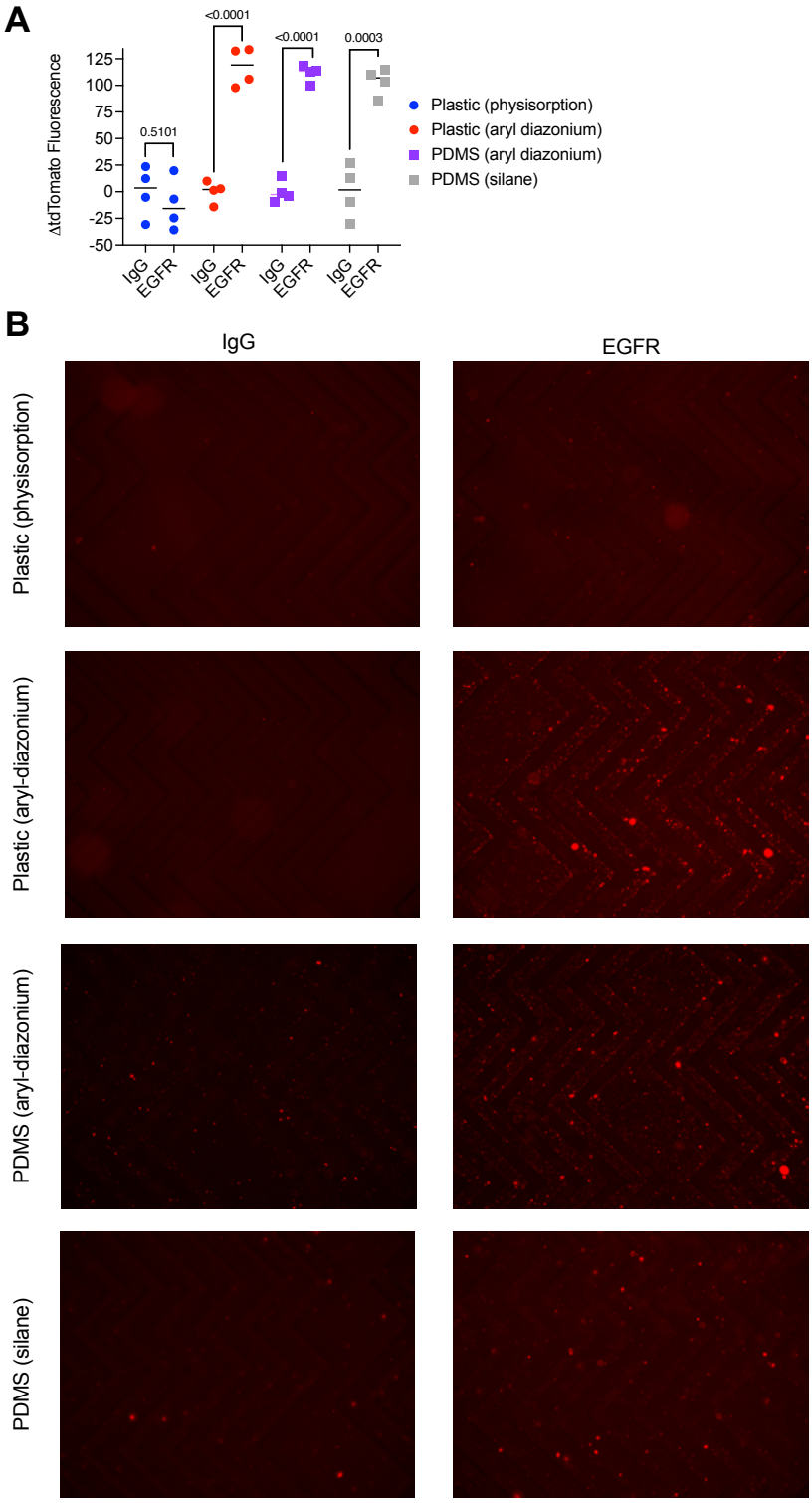

Figure S11: Fluorescent intensity of EVs captured on the ^{EV}HB-Chip. Concentrated serum-free conditioned media containing palm-tdTomato labelled EVs from either MDA-MB-231-BM1 were flown through and captured on devices containing an IgG antibody or an anti-EGFR antibody (Cetuximab). **(A)** Fluorescent intensity of captured palm-tdTomato+ EVs was measured by taking 9 images at 10x zoom for 1 second. P-values were calculated using a two-way ANOVA with correction for multiple comparisons. **(B)** Representative images of each chip type with IgG or EGFR capture

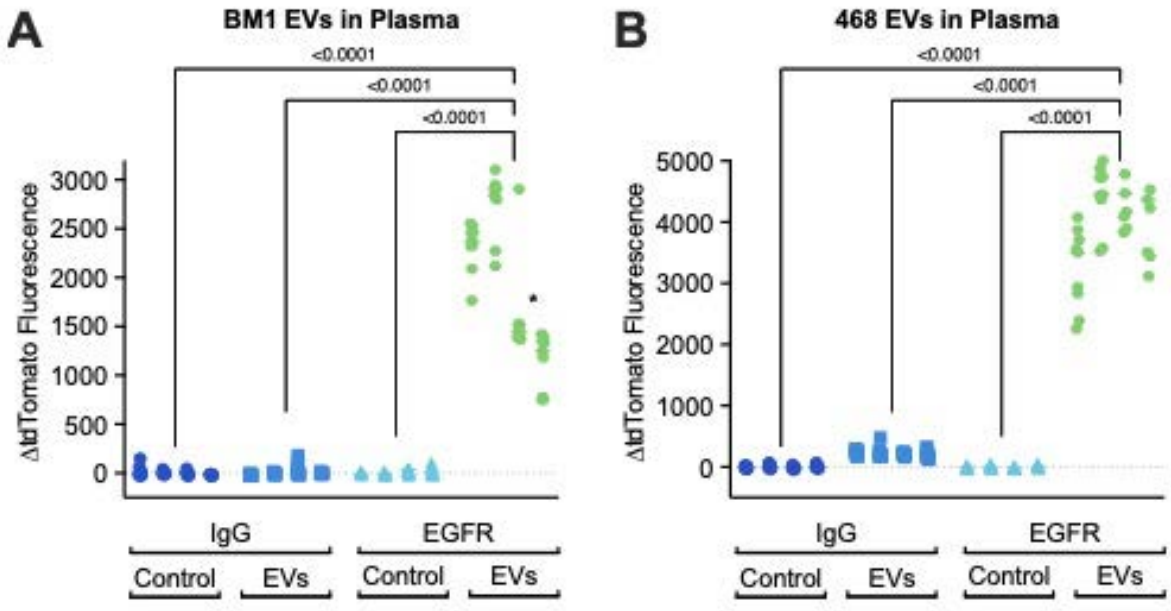

Figure S12: Fluorescent intensity of EVs in plasma captured on the ^{EV}HB-Chip. Concentrated serum-free conditioned media containing palm-tdTomato labelled EVs from either MDA-MB-231-BM1 **(A)** or MDA-MB-468 **(B)** tumor cells or PBS (Control) were spiked into normal plasma, then flown through and captured on devices containing an IgG antibody or an anti-EGFR antibody (Cetuximab). Fluorescent intensity of captured palm-tdTomato+ EVs was measured by taking 9 images at 10x zoom for 1 second. P-values were calculated using a two-way ANOVA with correction for multiple comparisons. *These devices were measured using a 20X lens resulting in lower fluorescent intensity compared to other devices.

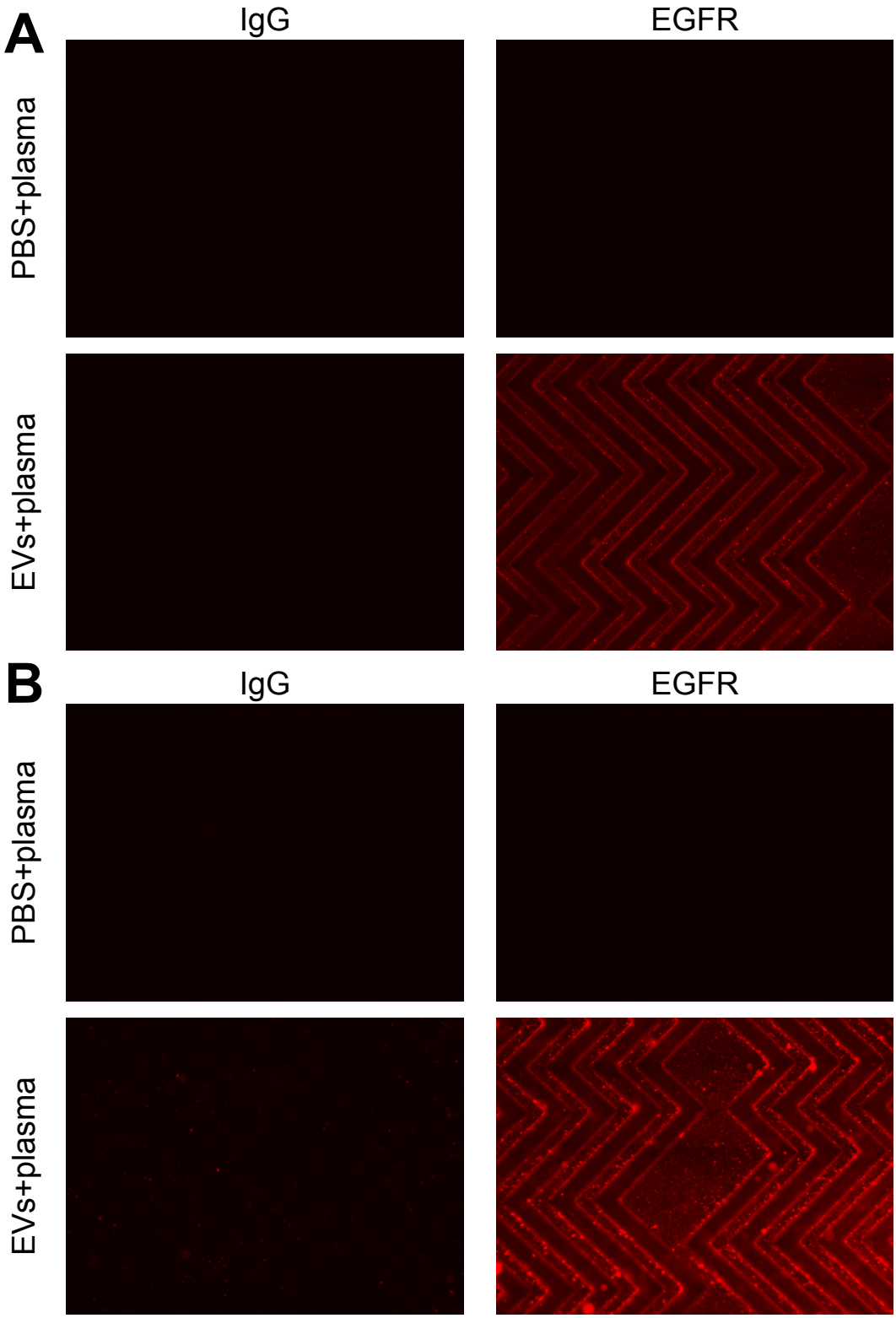

Figure S13: Representative images of fluorescent intensity (supplemental figure S11) of EVs in plasma captured on the ^{EV}HB-Chip. Concentrated serum-free conditioned media containing palm-tdTomato labelled EVs from either MDA-MB-231-BM1 **(A)** or MDA-MB-468 **(B)** tumor cells or PBS (Control) were spiked into normal plasma, then flown through and captured on devices containing an IgG antibody or an anti-EGFR antibody (Cetuximab). Fluorescent intensity of captured palm-tdTomato+ EVs was measured by taking 9 images using a 10X lens with 1 second capture.