# Supplementary Information

# Dramatic enhancement of the detection limits of bioassays via ultrafast deposition of polydopamine

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**ABBREVIATIONS**

EASE: enzyme-accelerated signal enhancement; HRP: horseradish peroxidase; BSA: bovine serum albumin; QD: quantum dot; PBS: phosphate-buffered saline; TBS: tris-buffered saline; IHC: immunohistochemistry; IF: immunofluorescence; ELISA: enzyme-linked immunosorbent assay; Ab: antibody; IgG: immunoglobulin G; HIV p24: HIV capsid antigen p24; KLK3: kallikrein 3, CRP: c-reactive protein; VEGF: vascular endothelial growth factor; EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; sulfo-NHS: N-hydroxysulfosuccinimide.

**CHEMICALS AND MATERISALS**

All chemicals and biochemicals (unless specified) were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. 96-well plastic microplates (each microplate consists of twelve removable strips of wells and a frame) were purchased from R&D Systems (Minneapolis, MN). Nitrocellulose membranes were purchased from EMD Millipore (Billerica, MA). Human cervical cancer (HeLa) cell line was purchased from ATCC (Manassas, VA). Glass-bottom 24-well plates (black wall) were purchased from Greiner Bio-One (Monroe, NC). Fetal bovine serum was purchased from PAA laboratories (Dartmouth, MA). Casein (5% solution) was purchased from Novagen (Billerica, MA). Anti-HSP90 antibody raised in rabbit (LOT: SAB4300541), anti-Lamin A antibody raised in rabbit (LOT: L1293), and anti-GAPDH antibody raised in rabbit (LOT: G9545) were purchased from Sigma-Aldrich (St. Louis, MO). CRHR1/CRF1 antibody was purchased from Novos Biologicals (LOT: NLS1778, Littleton, CO). Monoclonal rabbit antibodies raised against Ki-67 was purchased from Epitomics (LOT: 42031, Burlingame, CA). Monoclonal rabbit antibodies against Cox4 (REF: 4850s), and mouse programmed death ligand-1 expression (PD-L1) (REF: 29122S) were purchased from Cell signaling Technology (Danvers, MA). Goat anti-rabbit IgG (H+L) HRP-2'Ab (LOT: RA230590), goat anti-mouse IgG (H+L) HRP-2'Ab (LOT: 31430), nitrocellulose membranes for dot-blotting (0.45 μm pore size) with high binding affinity, MEM culture medium with L-glutamine, Pierce™ DAB Substrate Kit, QDs (525nm emission) functionalized with secondary Ab fragments (Qdot goat F(ab')2 anti-rabbit IgG conjugates (H+L)) (LOT: 1738599), amine-functionalized QDs (Qdot® 525 ITK™ Amino (PEG) Quantum Dots) (LOT: 1763984), amine functionalized QDs (Qdot® 605 ITK™ Amino (PEG) Quantum Dots) (LOT: 1630058), streptavidin functionalized QDs (Qdot® 605 Streptavidin Conjugate) (LOT: Q10101MP), and HRP-conjugated streptavidin (LOT: 1012719A) were purchased from ThermoFisher. Cy3 labelled donkey anti-mouse IgG (H+L) (LOT: 715165150) and Cy3 labelled donkey anti-rabbit IgG (H+L) (LOT: 711165152) were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Fluorescent beads (carboxylic groups on surface) 5 μm in diameter with three colors (green 480/520nm excitation/emission maxima, yellow 525/565nm, red 660/690 nm) were purchased from Bangs Laboratories (LOT: 11534; 9920; 11376, Fishers, IN). All antibodies were obtained in PBS without carrier proteins or stabilizing reagents. Mouse IgG, HIV p24, KLK3, CRP and VEGF ELISA kits were either purchased from Abcam (REF: ab151276, Cambridge, MA) or R&D Systems (LOT: DHP240; DKK300; DCRP00; DVE00). Seroconversion plasma samples from HIV infected patients were purchased from SeraCare (LOT: 06000237; 06000230; 06000227; 06000262, Milford, MA). Serial bleeds were collected from patients during the development of an HIV infection. All HIV patients' plasma samples were tested and found negative to HBsAg and HCV. Heathy patient plasma samples (age, 25-65) were purchased from Discovery Life Sciences (Los Osos, CA). All plasma samples were tested and found negative to HBV, HCV, HIV and RPR.

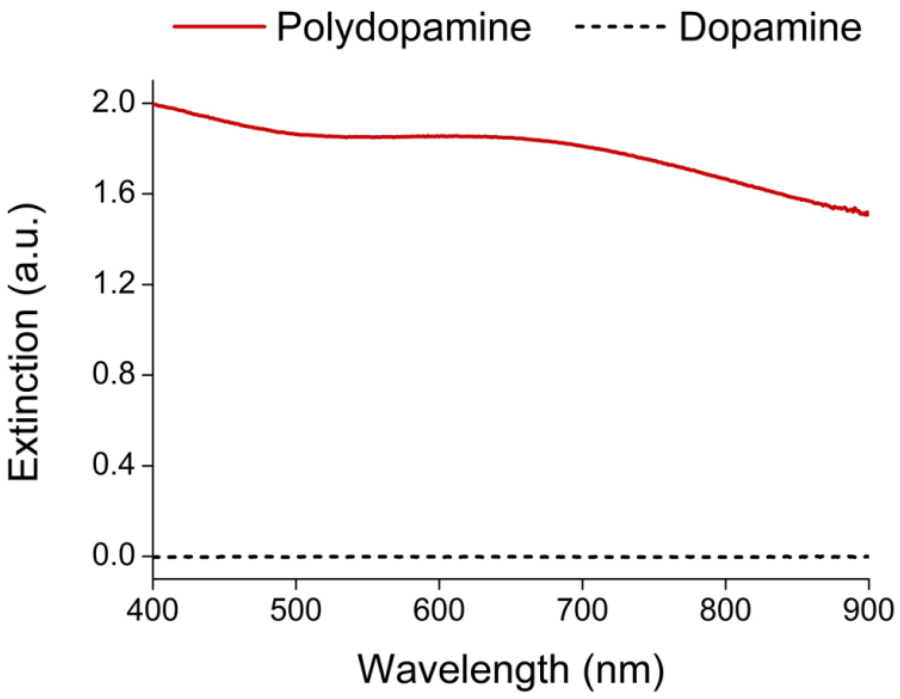
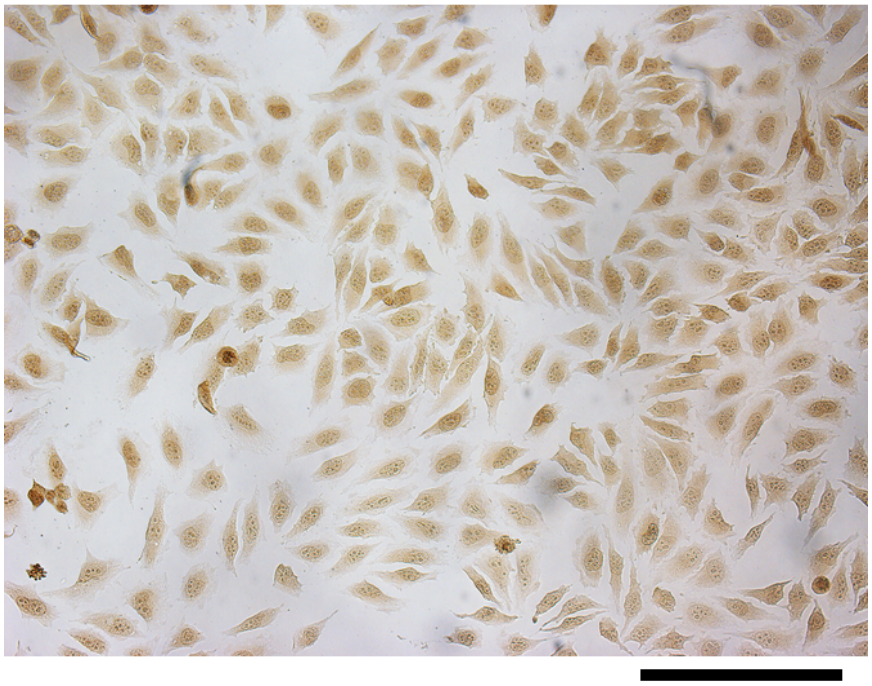



**Figure S1. Normalized extinction spectra of polydopamine and dopamine.**

| Assay | Format | |
|---|---|---|
| IHC / IF | Primary antibody (1'Ab) | Rabbit anti-Lamin A (HSP90, Ki67, Cox-4 or GAPDH) IgG |
| | Secondary antibody (2'Ab) | *EASE*: 2'Ab-HRP (IHC and IF) |
| | | *Conventional*: 2'Ab-HRP (IHC); 2'Ab-QD (IF) |
| | Signal development | *EASE*: EASE substrate (IHC); EASE substrate / QD-$NH_2$ (IF) |
| | | *Conventional*: DAB substrate (IHC); 2'Ab-QD (IF) |
| Suspension microarray | Bead | Coated with IgG (mouse or rabbit) |
| | Target | Biotinylated 2'Ab |
| | Signal development | *EASE*: Streptavidin-HRP / EASE substrate / QD-$NH_2$ |
| | | *Conventional*: Streptavidin-QD |
| ELISA | First layer of sandwich | Capture Ab |
| | Second layer of sandwich | Target (Mouse IgG, HIV p24, KLK3, CRP or VEGF) |
| | Third layer of sandwich | Detection Ab-HRP |
| | Signal development | *EASE*: EASE substrate / HRP/ TMB substrate |
| | | *Conventional*: TMB substrate |
| Lateral flow test | First layer of sandwich | Capture Ab |
| | Second layer of sandwich | Target (HIV p24) |
| | Third layer of sandwich | Detection Ab-HRP |
| | Signal development | *EASE*: EASE substrate / HRP / DAB substrate |
| | | *Conventional*: DAB substrate |

**Figure S2. Assay formats**. Dopamine is used as the EASE substrate.

HSP90



Lamin A

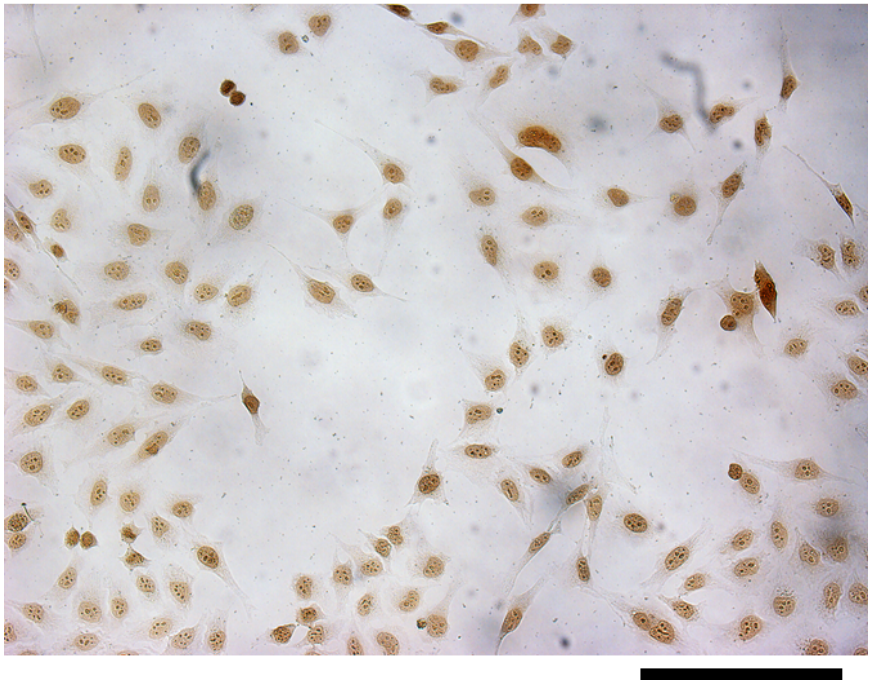


**Figure S3. IHC-EASE single cell staining.** Bright field images of a large population of cells stained with the IHC-EASE reagents showing specific cytoplasmic localization of HSP90 and nuclear localization of Lamin A. Scale bar, 200 µm.

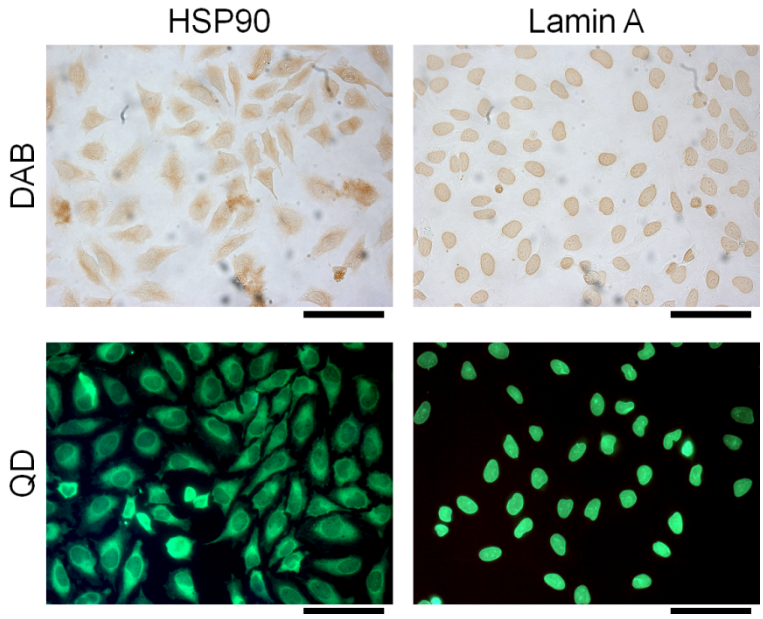



**Figure S4. Comparison of staining patterns before and after QD adsorption.** The top panels show bright field micrographs of conventional IHC cell staining (DAB, 3,3'-diaminobenzidine as the substrate). The bottom two panels show fluorescence micrographs of conventional IF cell staining using QD-labeled 2'Ab (positive control). Scale bar, 100 μm.

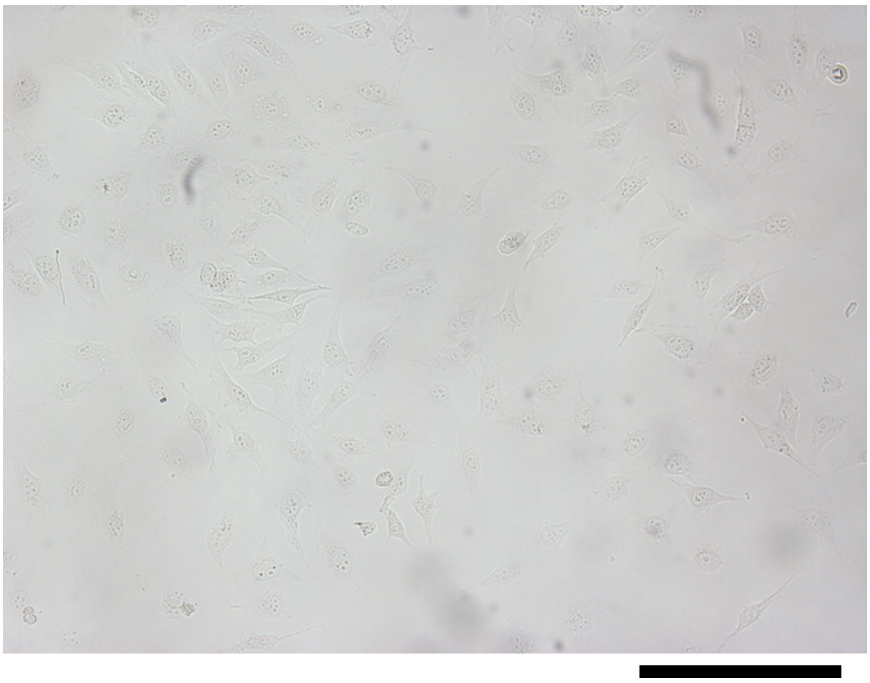


**Figure S5. Control experiment using isotype antibody in IHC-EASE**. Bright field image of a large population of cells stained with IHC-EASE while using an isotype 1'Ab as the control (rabbit IgG). Negligible signals were observed. Scale bar, 200 μm.

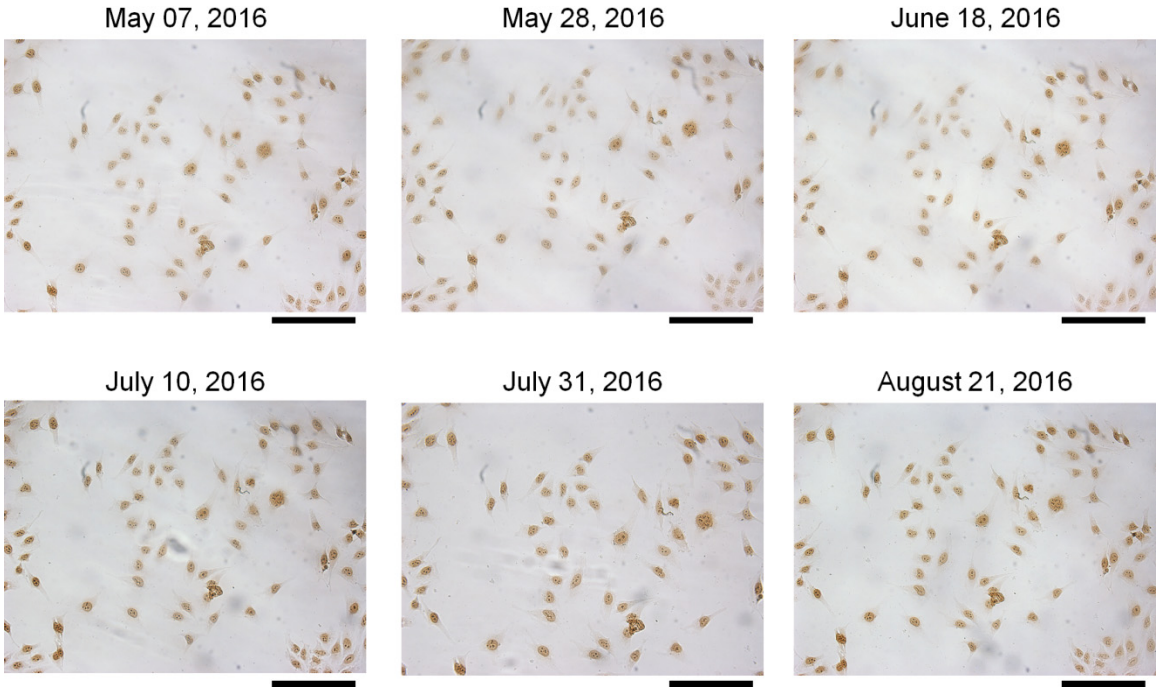



**Figure S6. IHC-EASE staining stability after storage.** Bright field images of the same group of cells were imaged periodically over ~100 days. The stains stored in 1X PBS at 4 °C show no decay over time. Scale bar, 200 µm.

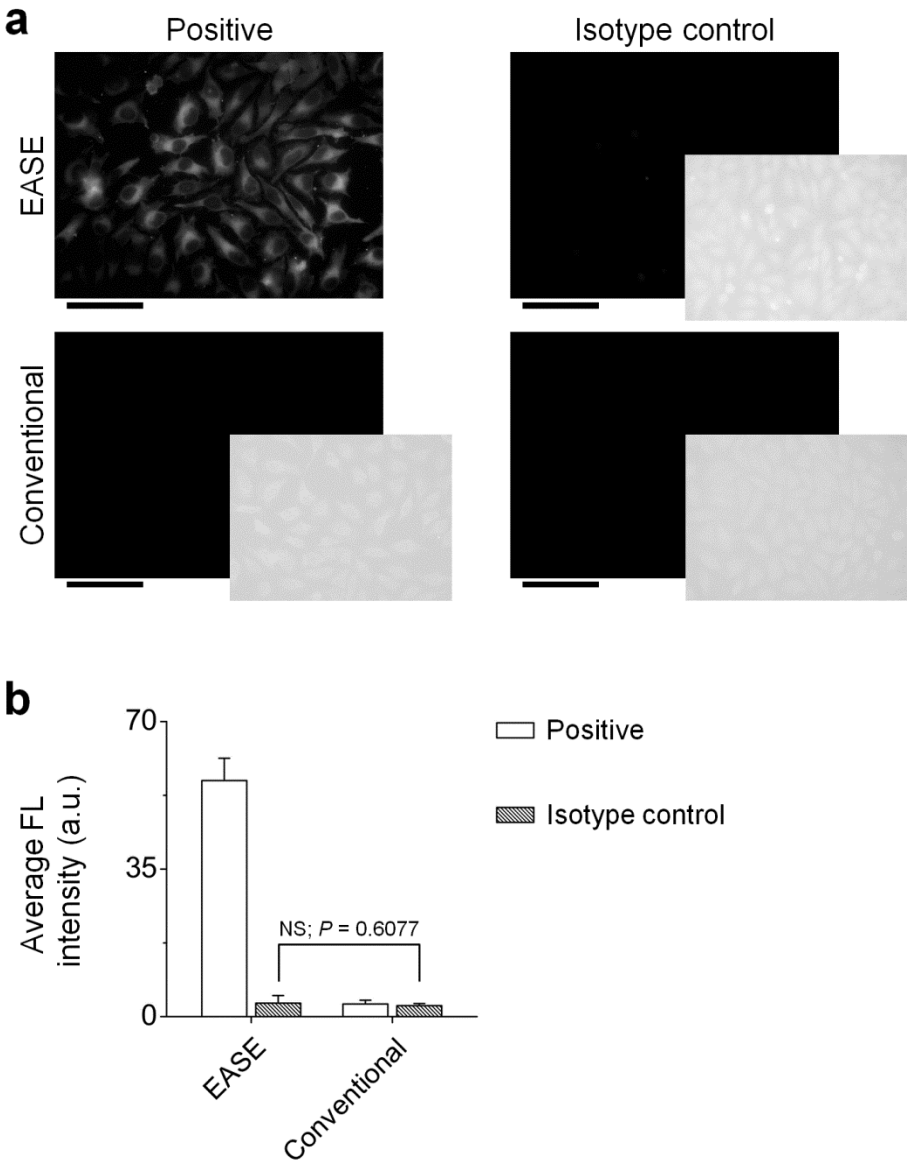



**Figure S7. Verification of IF-EASE staining specificity.** (**a**) Fluorescence micrograph showing HSP90 (88 pM 1'Ab) staining under various conditions: experiment group (left panels) and control group using isotype rabbit IgG as the 1'Ab (right panels), using both IF-EASE (top panels) and conventional IF (bottom panels). Scale bar, 100 μm, exposure time 100 ms. To better illustrate the background levels, long exposure (2 seconds) images were also shown for the control panels. (**b**) By comparing the isotype Ab control for IF and IF-EASE, no significant background increase was observed. $P > 0.1$, not significant by two-tailed *t*-test. Error bars, s.d. over four different images.

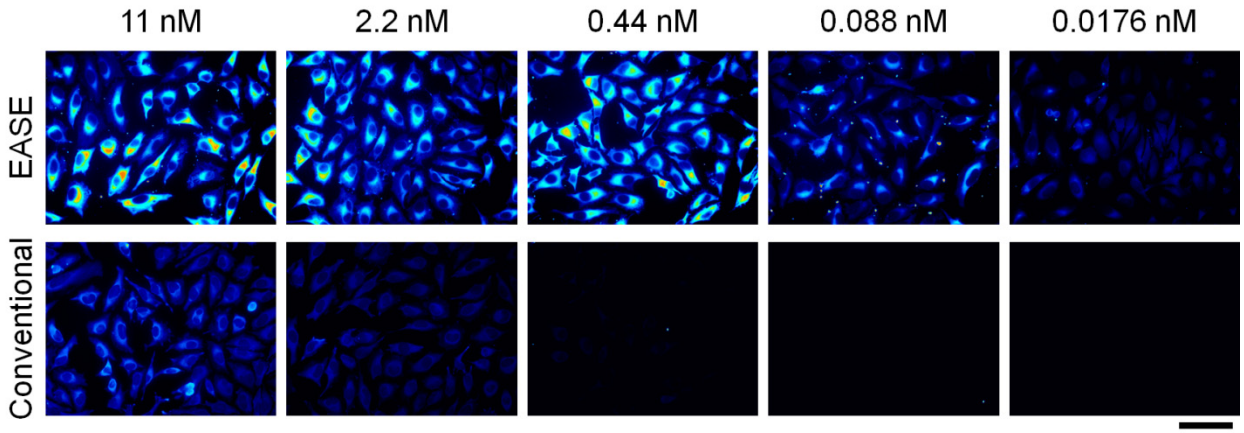



**Figure S8. Signal strength comparison for IF and IF-EASE in single cells.** False-color (heat map) fluorescence imaging of cells stained with various concentrations of primary antibody. Scale bar, 100 µm.

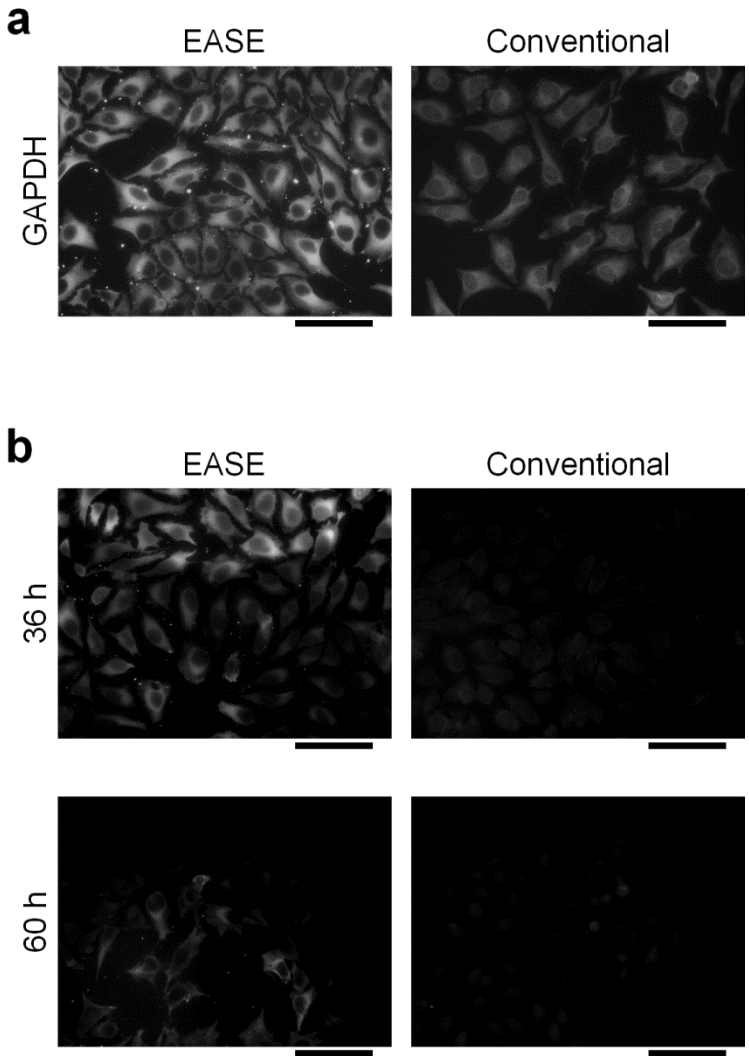



**Figure S9. Imaging low-abundance protein in cells**. GAPDH expression in HeLa cells was suppressed by RNAi. (**a**) Fluorescence images of GAPDH stained by IF-EASE and conventional IF before RNAi. Scale bar, 100 µm. (**b**) Fluorescence images showing GAPDH staining 36 h and 60 h post RNAi. Despite majority of GAPDH protein is degraded, trace amount left is still picked up by IF-EASE but not by conventional IF. Scale bar, 100 µm.

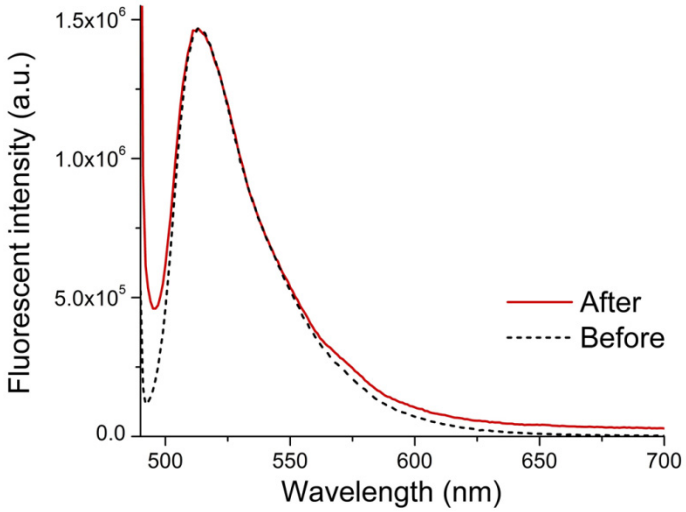



**Figure S10. Effect of PDA on the fluorescence of microspheres.** Fluorescence spectra of green fluorescent beads before (broken line) and after PDA coating (EASE process). The two samples contain the same concentration of beads.

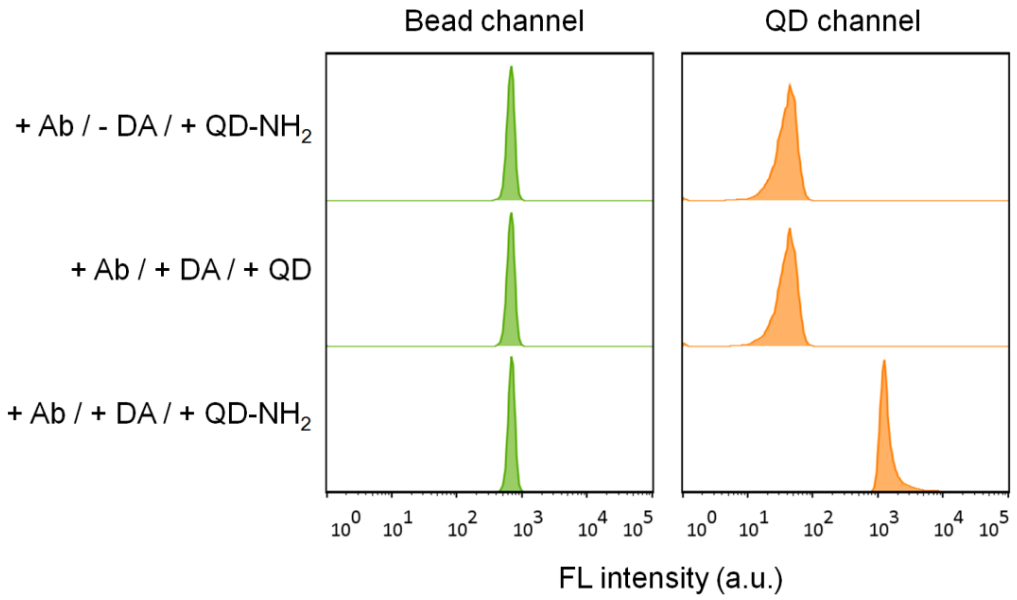



**Figure S11. Specificity of QD-PEG-$NH_2$ deposition on fluorescent microspheres**. Quantitative flow cytometry histograms show that only when dopamine is polymerized on the microsphere surface and aminated QDs are used, QDs bind onto the bead surface. The green channel (left panels) shows the fluorescence from the dye-doped microsphere, and the red channel (right panels) shows QD fluorescence.

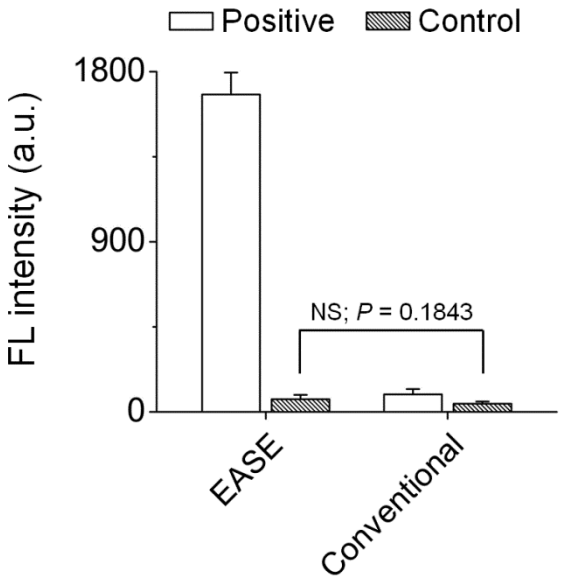



**Figure S12. Verification of suspension microarray specificity**. At a target (biotinylated 2'Ab) concentration of 12 pM, the EASE technology can help conventional suspension microarray to easily detect the target (two blank bars). When the target is missing (control, dashed bars), the background signal intensity suspension microarrays with or without EASE are statistically indistinguishable. $P > 0.1$, NS, not significant by two-tailed *t*-test. Error bars, s.d. over three replicates.

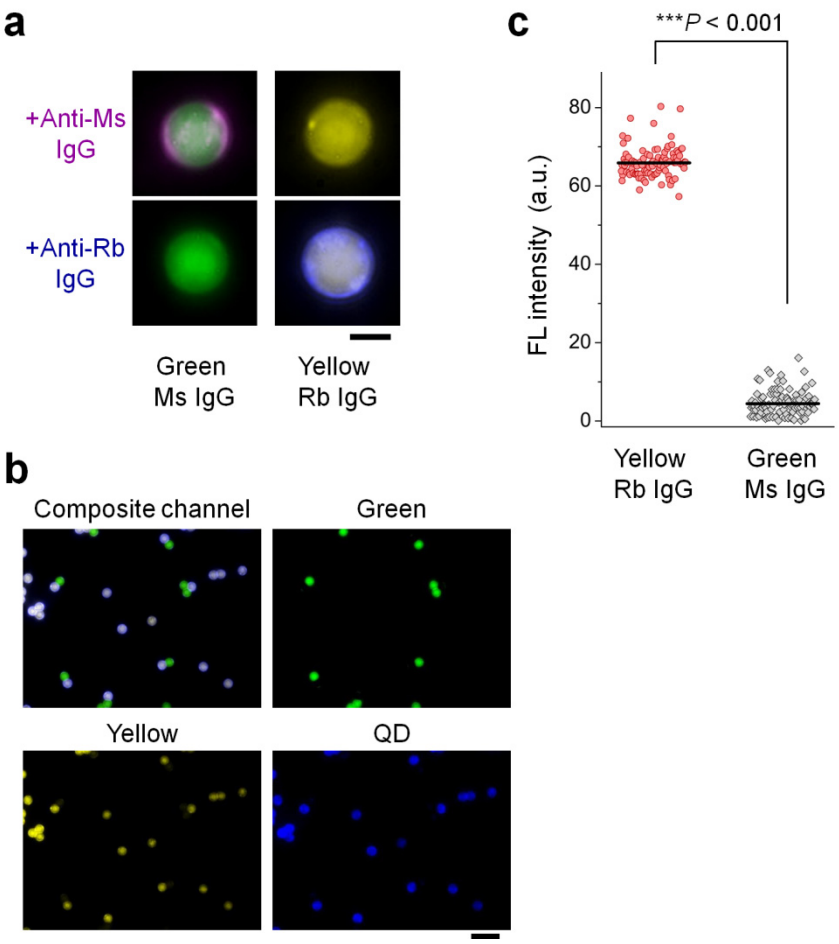



**Figure S13. Assessment of detection crosstalk using multicolor microspheres**. Mouse IgG and Rabbit IgG are immobilized on green and yellow microspheres separately. (**a**) When matching anti-mouse IgG and anti-rabbit IgG were used as the detection probes, QD fluorescence on beads surface (following PDA deposition) is detected by microscopy. Mismatched antibody pairs do not produce QD fluorescence. Scale bar, 3 µm. (**b**) Two-color microspheres mixture incubated with only one target, anti-rabbit IgG. QD deposition only occurs on the yellow microspheres. Scale bar, 15 µm. (**c**) Single bead counting show detection of the anti-rabbit IgG at 100% accuracy (counted 100 beads for each color).

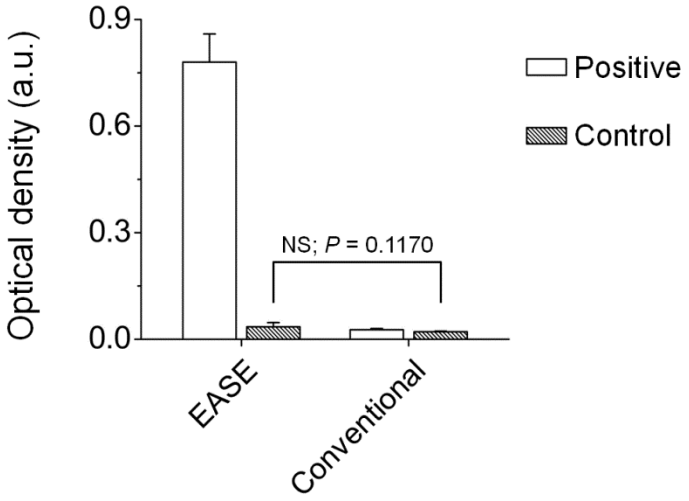



**Figure S14. Verification of ELISA-EASE specificity**. At a target (mouse IgG) concentration of 154 pg $ml^{-1}$, the target presence can be detected by ELISA-EASE easily, but not by ELISA alone. Without the target molecule, the signal intensity difference of ELISA with or without EASE is statistically insignificant. $P > 0.1$, NS, not significant by two-tailed *t*-test. Error bars, s.d. over three replicates.

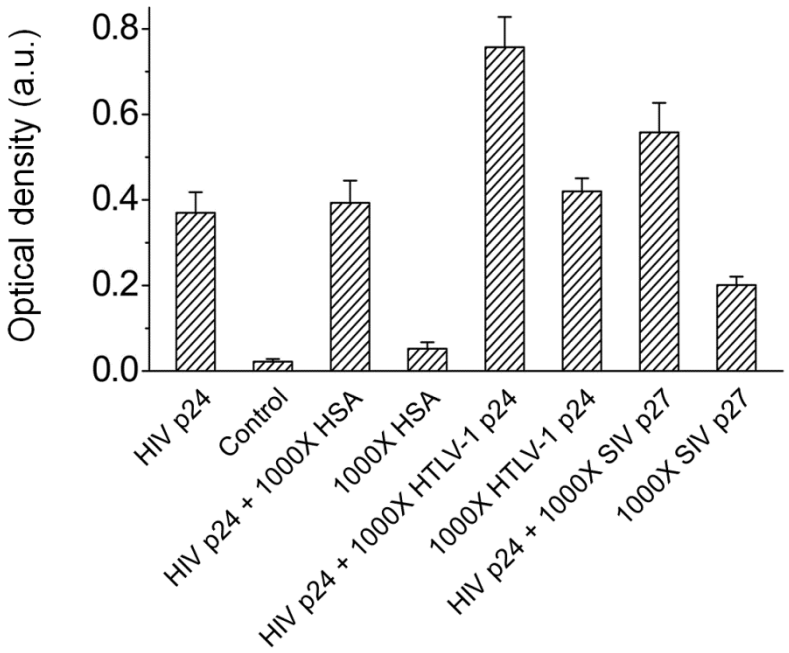



**Figure S15. Confirmation of ELISA-EASE specificity and cross-reactivity**. At the target (HIV p24) concentration of 60 fg $ml^{-1}$, the target presence can be detected by ELISA-EASE easily with very low background from the controls (without target molecule). To further test the selectivity, 1,000 X more concentrated proteins (60 pg $ml^{-1}$) including human serum albumin (HSA), HTLV-1 p24, SIV p27 were spiked into 1X (60 fg $ml^{-1}$) HIV p24 solution, and probed by ELISA-EASE. No significant cross-reactivity was observed for HSA. The non-specific proteins (HTLV-1 p24 and SIV p27) that are more similar to p24 only produce appreciable signals when their concentrations are 1,000 x higher than p24.

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. | Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. |
| 3.8125E-6 | 0.0251 | 0.0042 | 0.0039 | 0.0270 | 0.0030 |
| 7.6250E-6 | 0.0467 | 0.0099 | 0.0078 | 0.0524 | 0.0050 |
| 1.5250E-5 | 0.1052 | 0.0205 | 0.0156 | 0.0995 | 0.0205 |
| 3.0500E-5 | 0.1880 | 0.0237 | 0.0313 | 0.2081 | 0.0196 |
| 6.1000E-5 | 0.3706 | 0.0469 | 0.0625 | 0.3698 | 0.0298 |
| 1.2200E-4 | 0.6985 | 0.1253 | 0.1250 | 0.7083 | 0.0693 |

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration KLK3 (ng ml$^{-1}$) | OD | S.D. | Concentration KLK 3 (ng ml$^{-1}$) | OD | S.D. |
| 4.5875E-4 | 0.0262 | 0.0033 | 0.4688 | 0.0246 | 0.0023 |
| 9.1750E-4 | 0.0531 | 0.0049 | 0.9375 | 0.0509 | 0.0045 |
| 0.0018 | 0.1196 | 0.0291 | 1.8750 | 0.0987 | 0.0123 |
| 0.0037 | 0.2730 | 0.0430 | 3.7500 | 0.1966 | 0.0184 |
| 0.0073 | 0.5882 | 0.0581 | 7.5000 | 0.3790 | 0.0349 |
| 0.0147 | 1.1994 | 0.1967 | 15.000 | 0.7601 | 0.1600 |

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration CRP (ng ml$^{-1}$) | OD | S.D. | Concentration CRP (ng ml$^{-1}$) | OD | S.D. |
| 3.8125E-4 | 0.0312 | 0.0039 | 0.3905 | 0.0323 | 0.0026 |
| 7.6250E-4 | 0.0662 | 0.0105 | 0.7810 | 0.0720 | 0.0098 |
| 1.5250E-3 | 0.1311 | 0.0160 | 1.5620 | 0.1531 | 0.0155 |
| 3.0500E-3 | 0.2998 | 0.0620 | 3.1240 | 0.3697 | 0.0377 |
| 6.1000E-3 | 0.5913 | 0.1238 | 6.2480 | 0.7716 | 0.0992 |
| 1.2200E-2 | 1.2022 | 0.0929 | 12.496 | 1.5002 | 0.3503 |

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration VEFG (ng ml$^{-1}$) | OD | S.D. | Concentration VEGF (ng ml$^{-1}$) | OD | S.D. |
| 1.5283E-5 | 0.0260 | 0.0030 | 0.0157 | 0.0253 | 0.0021 |
| 3.0567E-5 | 0.0504 | 0.0053 | 0.0313 | 0.0460 | 0.0055 |
| 6.1133E-5 | 0.0925 | 0.0190 | 0.0625 | 0.0902 | 0.0067 |
| 1.2227E-4 | 0.1789 | 0.0200 | 0.1250 | 0.1690 | 0.0122 |
| 2.4453E-4 | 0.3297 | 0.0401 | 0.2500 | 0.3266 | 0.0702 |
| 4.8906E-4 | 0.7190 | 0.1208 | 0.5000 | 0.6594 | 0.0528 |

**Figure S16**. Raw data of ELISA working curves for the four targets, HIV p24, KLK3, CRP, and VEGF, with or without EASE.

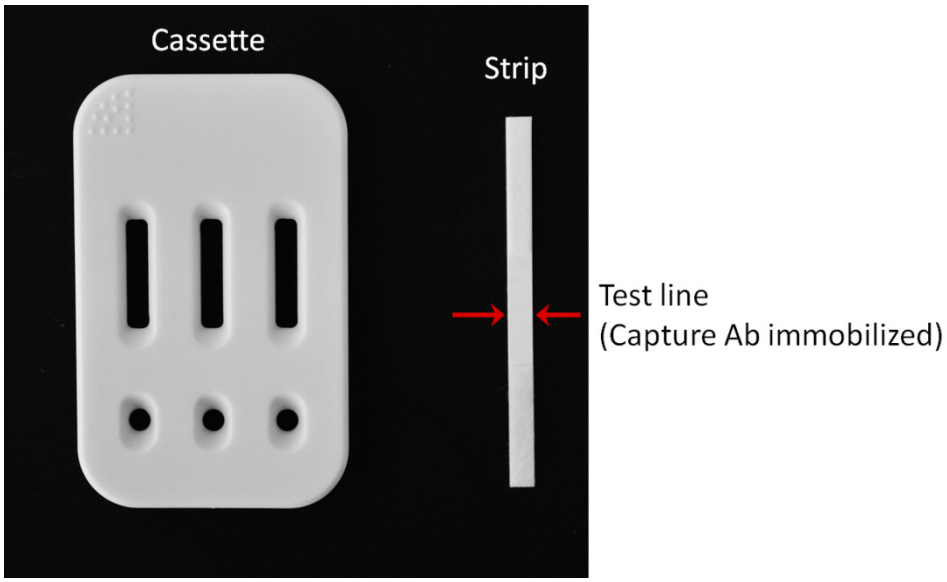



**Figure S17. Construction of the strip.** Each cassette contains three strips. Capture antibody is immobilized along the test line of each strip. Detailed strip fabrication procedure is described in the Methods section.

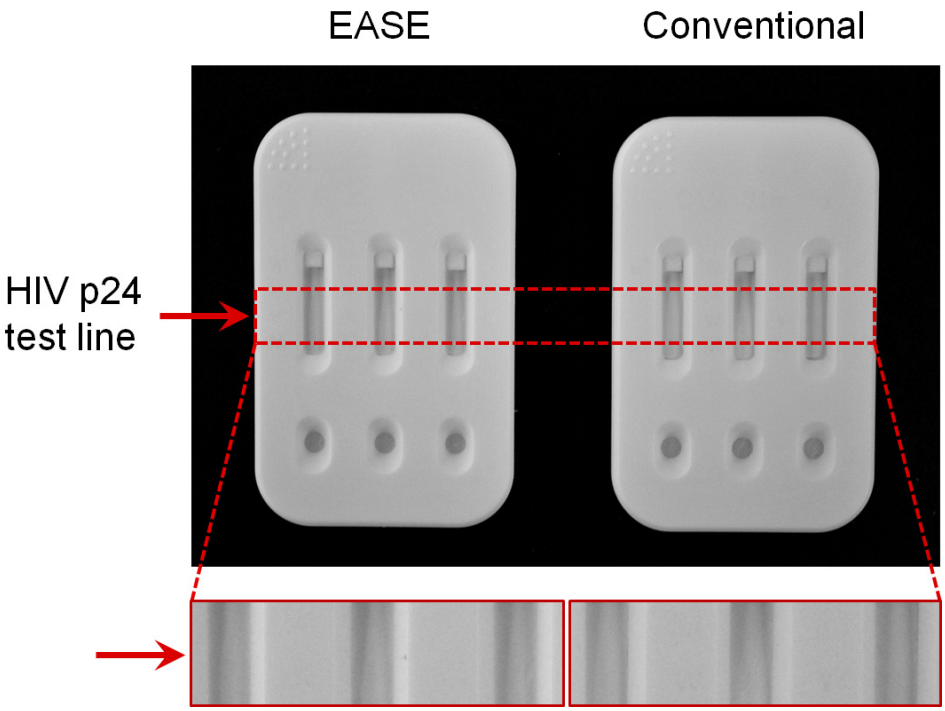



**Figure S18. Verification of lateral flow test specificity.** Control experiments where the target P24 protein is absent show no detectable signals with or without EASE.

| Patient ID | EASE-ELISA | Standard ELISA | PCR |
|---|---|---|---|
| R301520 | ↓ | ↓ | ↓ |
| R301522 | ↓ | ↓ | ↓ |
| R301525 | ↓ | ↓ | ↓ |
| R301527 | ↓ | ↓ | ↓ |
| R301537 | ↓ | ↓ | ↓ |
| R301538 | ↓ | ↓ | ↓ |
| R301548 | ↓ | ↓ | ↓ |
| R301549 | ↓ | ↓ | ↓ |
| R301551 | ↓ | ↓ | ↓ |
| R301555 | ↓ | ↓ | ↓ |
| R301556 | ↓ | ↓ | ↓ |
| R301558 | ↓ | ↓ | ↓ |
| R301560 | ↓ | ↓ | ↓ |
| R301563 | ↓ | ↓ | ↓ |
| R301564 | ↓ | ↓ | ↓ |
| R301566 | ↓ | ↓ | ↓ |
| R301571 | ↓ | ↓ | ↓ |
| R301572 | ↓ | ↓ | ↓ |
| R301573 | ↓ | ↓ | ↓ |
| R301574 | ↓ | ↓ | ↓ |

↓, blow the quantitation range.

**Figure S19. ELISA-EASE diagnosis of HIV infection in plasma from healthy blood donors.** No positive detection is made using ELISA, ELISA-EASE, or PCR, showing detection specificity cross the board.

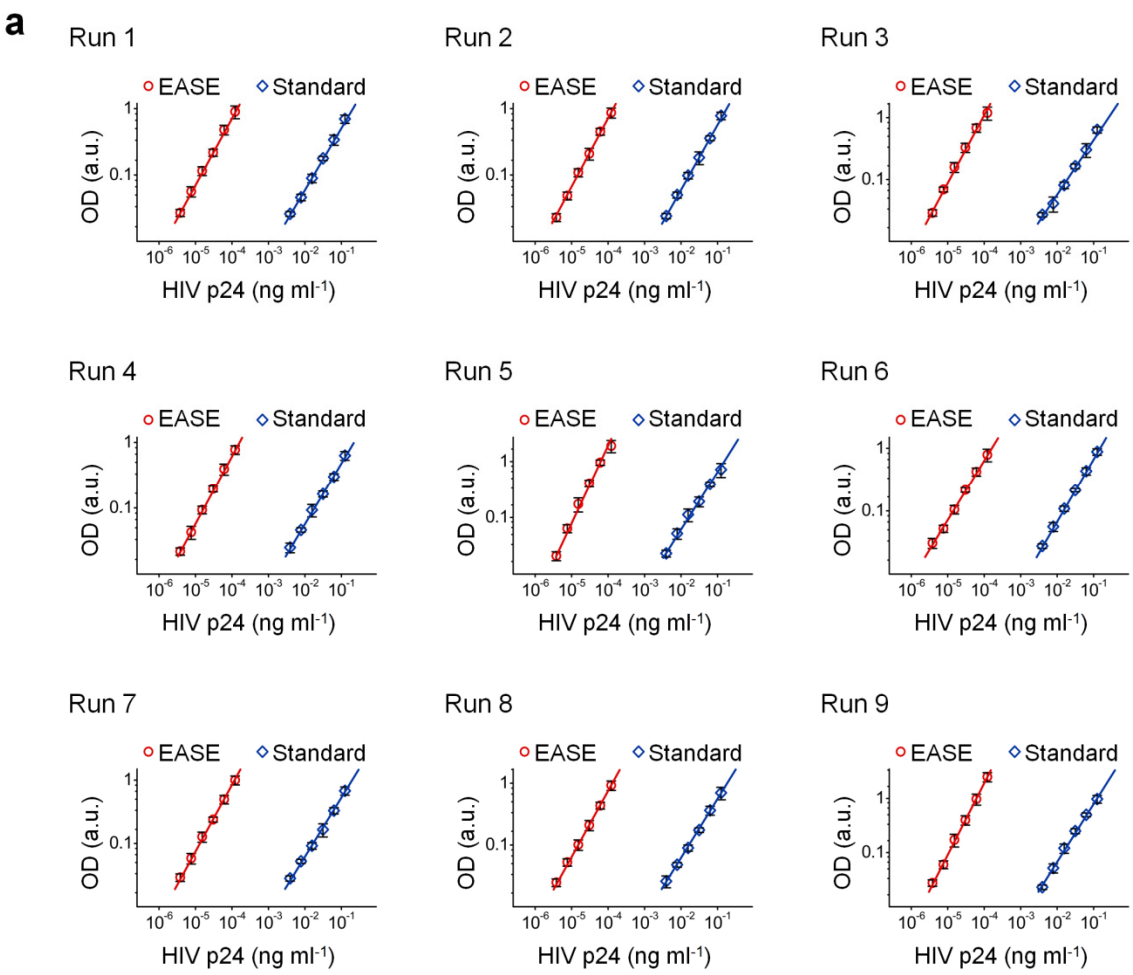



**Figure S20a. HIV p24 detection sensitivity using ELISA and ELISA-EASE.** (**a**) ELISA working curves obtained through 9 calibration runs (performed on different days) with or without the EASE technology. Error bars, s.d. over three replicates.

**b**

Run 1

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. | Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. |
| 3.8125E-6 | 0.0273 | 0.0033 | 0.0039 | 0.0262 | 0.0022 |
| 7.6250E-6 | 0.0568 | 0.1001 | 0.0078 | 0.0461 | 0.0054 |
| 1.5250E-5 | 0.1155 | 0.0175 | 0.0156 | 0.0899 | 0.0130 |
| 3.0500E-5 | 0.2181 | 0.0266 | 0.0313 | 0.1780 | 0.0126 |
| 6.1000E-5 | 0.4825 | 0.0799 | 0.0625 | 0.3395 | 0.0597 |
| 1.2200E-4 | 0.8988 | 0.1953 | 0.1250 | 0.6986 | 0.0993 |

Run 2

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. | Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. |
| 3.8125E-6 | 0.0232 | 0.0029 | 0.0039 | 0.0241 | 0.0020 |
| 7.6250E-6 | 0.0488 | 0.0066 | 0.0078 | 0.0510 | 0.0052 |
| 1.5250E-5 | 0.1089 | 0.0152 | 0.0156 | 0.0982 | 0.0110 |
| 3.0500E-5 | 0.2099 | 0.0402 | 0.0313 | 0.1830 | 0.0399 |
| 6.1000E-5 | 0.4507 | 0.0523 | 0.0625 | 0.3590 | 0.0300 |
| 1.2200E-4 | 0.8685 | 0.1500 | 0.1250 | 0.7814 | 0.1008 |

Run 3

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. | Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. |
| 3.8125E-6 | 0.0290 | 0.0035 | 0.0039 | 0.0272 | 0.0021 |
| 7.6250E-6 | 0.0692 | 0.0062 | 0.0078 | 0.0409 | 0.0112 |
| 1.5250E-5 | 0.1589 | 0.0291 | 0.0156 | 0.0812 | 0.0100 |
| 3.0500E-5 | 0.3293 | 0.0507 | 0.0313 | 0.1639 | 0.0151 |
| 6.1000E-5 | 0.6801 | 0.1028 | 0.0625 | 0.3025 | 0.0758 |
| 1.2200E-4 | 1.1912 | 0.2877 | 0.1250 | 0.6343 | 0.0733 |

Run 4

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. | Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. |
| 3.8125E-6 | 0.0221 | 0.0028 | 0.0039 | 0.0251 | 0.0041 |
| 7.6250E-6 | 0.0430 | 0.0096 | 0.0078 | 0.0466 | 0.0036 |
| 1.5250E-5 | 0.0937 | 0.0126 | 0.0156 | 0.0927 | 0.0200 |
| 3.0500E-5 | 0.1955 | 0.0205 | 0.0313 | 0.1622 | 0.0170 |
| 6.1000E-5 | 0.3956 | 0.0777 | 0.0625 | 0.3023 | 0.0353 |
| 1.2200E-4 | 0.7755 | 0.1159 | 0.1250 | 0.6309 | 0.0955 |

Run 5

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. | Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. |
| 3.8125E-6 | 0.0209 | 0.0038 | 0.0039 | 0.0231 | 0.0033 |
| 7.6250E-6 | 0.0639 | 0.0103 | 0.0078 | 0.0520 | 0.0111 |
| 1.5250E-5 | 0.1745 | 0.0488 | 0.0156 | 0.1123 | 0.0286 |
| 3.0500E-5 | 0.4099 | 0.0590 | 0.0313 | 0.1921 | 0.0380 |
| 6.1000E-5 | 0.9633 | 0.1022 | 0.0625 | 0.3924 | 0.0403 |
| 1.2200E-4 | 1.8988 | 0.4633 | 0.1250 | 0.7233 | 0.1995 |

Run 6

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. | Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. |
| 3.8125E-6 | 0.0311 | 0.0057 | 0.0039 | 0.0278 | 0.0025 |
| 7.6250E-6 | 0.0524 | 0.0068 | 0.0078 | 0.0564 | 0.0091 |
| 1.5250E-5 | 0.1052 | 0.0166 | 0.0156 | 0.1086 | 0.0106 |
| 3.0500E-5 | 0.2154 | 0.0177 | 0.0313 | 0.2130 | 0.0124 |
| 6.1000E-5 | 0.4249 | 0.0649 | 0.0625 | 0.4360 | 0.0576 |
| 1.2200E-4 | 0.7890 | 0.1781 | 0.1250 | 0.8801 | 0.1031 |

Run 7

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. | Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. |
| 3.8125E-6 | 0.0290 | 0.0039 | 0.0039 | 0.0284 | 0.0027 |
| 7.6250E-6 | 0.0584 | 0.0111 | 0.0078 | 0.0522 | 0.0039 |
| 1.5250E-5 | 0.1276 | 0.0233 | 0.0156 | 0.0920 | 0.0110 |
| 3.0500E-5 | 0.2388 | 0.0209 | 0.0313 | 0.1651 | 0.0390 |
| 6.1000E-5 | 0.4967 | 0.0757 | 0.0625 | 0.3299 | 0.0373 |
| 1.2200E-4 | 0.9999 | 0.1604 | 0.1250 | 0.6789 | 0.1002 |

Run 8

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. | Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. |
| 3.8125E-6 | 0.0248 | 0.0035 | 0.0039 | 0.0260 | 0.0056 |
| 7.6250E-6 | 0.0526 | 0.0071 | 0.0078 | 0.0480 | 0.0044 |
| 1.5250E-5 | 0.1012 | 0.0195 | 0.0156 | 0.0897 | 0.0104 |
| 3.0500E-5 | 0.2097 | 0.0396 | 0.0313 | 0.1744 | 0.0151 |
| 6.1000E-5 | 0.4355 | 0.0552 | 0.0625 | 0.3638 | 0.0598 |
| 1.2200E-4 | 0.9240 | 0.1596 | 0.1250 | 0.6987 | 0.1594 |

Run 9

| EASE | | | Conventional | | |
|---|---|---|---|---|---|
| Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. | Concentration HIV p24 (ng ml$^{-1}$) | OD | S.D. |
| 3.8125E-6 | 0.0276 | 0.0038 | 0.0039 | 0.0233 | 0.0023 |
| 7.6250E-6 | 0.0604 | 0.0099 | 0.0078 | 0.0515 | 0.0099 |
| 1.5250E-5 | 0.1724 | 0.0455 | 0.0156 | 0.1206 | 0.0236 |
| 3.0500E-5 | 0.3998 | 0.0784 | 0.0313 | 0.2502 | 0.0269 |
| 6.1000E-5 | 0.9756 | 0.2248 | 0.0625 | 0.4996 | 0.0403 |
| 1.2200E-4 | 2.5063 | 0.4858 | 0.1250 | 0.9753 | 0.1596 |

**Figure S20b. HIV p24 detection sensitivity using ELISA and ELISA-EASE.** (**b**) Raw data of ELISA working curves obtained through 9 calibration runs (performed on different days) with or without the EASE technology.

| Patient ID | Phlebotomy date | EASE (pg ml$^{-1}$) | EASE CV (%) | Standard (pg ml$^{-1}$) | Standard CV (%) |
|---|---|---|---|---|---|
| PRB 946 | 0 | 0.006 | 15.8 | ↓ | N/A |
| | 4 | 0.807 | 9.96 | ↓ | N/A |
| | 7 | 26.86 | 10.2 | 19.22 | 8.48 |
| | 11 | 39.70 | 17.5 | 50.63 | 10.1 |
| PRB 949 | 0 | ↓ | N/A | ↓ | N/A |
| | 6 | 0.029 | 13.6 | ↓ | N/A |
| | 9 | 0.561 | 9.48 | ↓ | N/A |
| | 18 | 22.05 | 18.1 | 17.22 | 10.9 |
| PRB 953 | 0 | 0.043 | 11.2 | ↓ | N/A |
| | 3 | 1.320 | 17.3 | ↓ | N/A |
| | 7 | 23.36 | 8.79 | 16.01 | 7.39 |
| | 10 | 39.99 | 18.8 | 50.97 | 15.7 |
| PRB 977 | 0 | 0.009 | 12.0 | ↓ | N/A |
| | 2 | 0.121 | 7.65 | ↓ | N/A |
| | 13 | >100 | N/A | >100 | N/A |
| | 15 | >100 | N/A | >100 | N/A |

↓ blow the quantitation range.

**Figure S21. Variation of p24 detection in sera using ELISA with or without EASE.** Measurement variabilities were calculated based on coefficient of variation (CV), which was lower than 20% in all measurements.

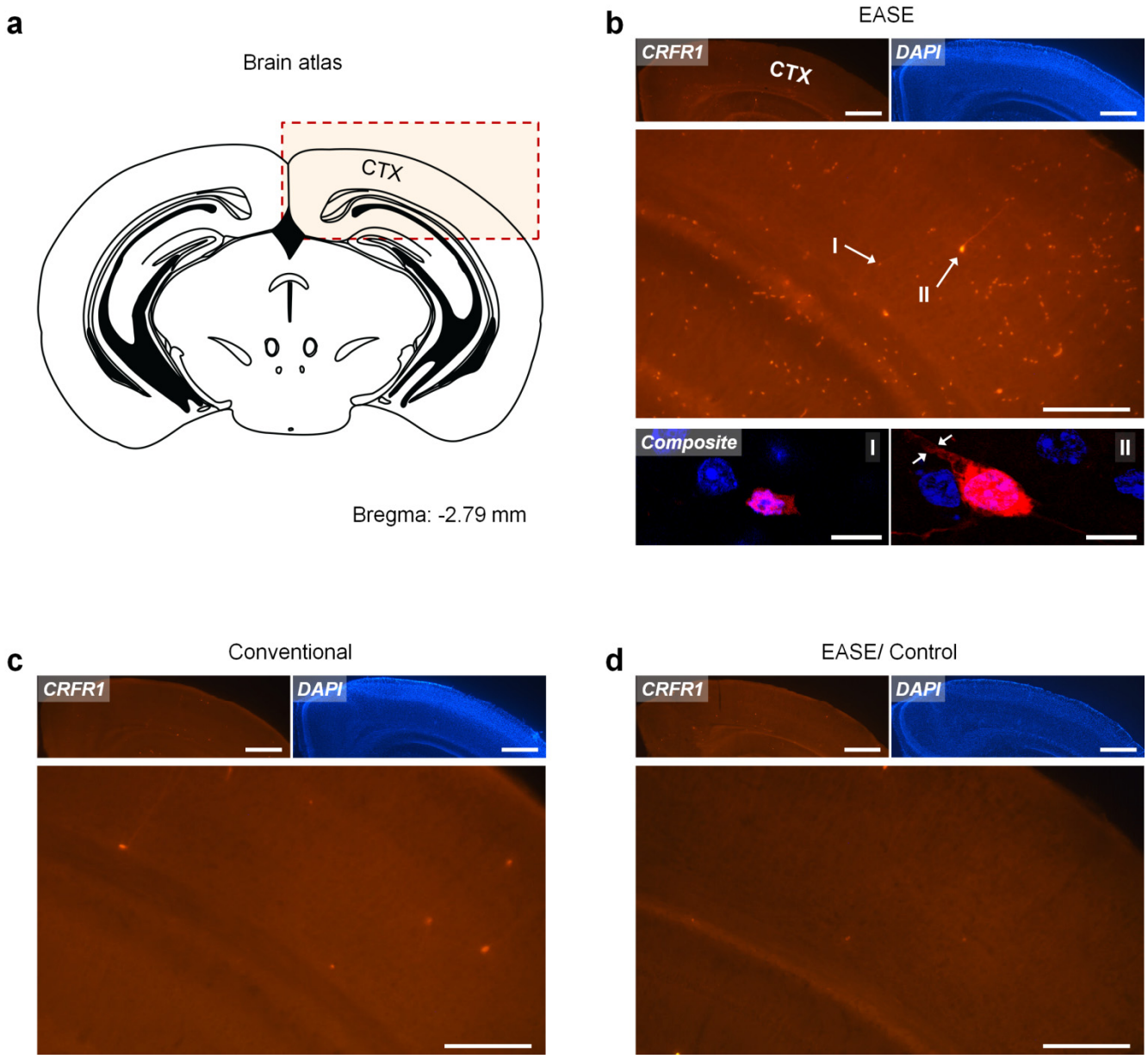



**Figure S22. Resolving the distribution of CRFR1 in the brain.** (**a**) Schematic of cerebral cortex (CTX) in the mouse brain (Bregma: -2.79 mm). (**b-d**) Representative fluorescence images of CRFR1 neurons in CTX, counter stained with DAPI. Scale bar for the CRFR1 and DAPI images (top panels), 200 µm. Scale bar for the zoom-in images (middle panels), 100 µm. Scale bar for the composite confocal images (bottom panels), 5 µm. A large number of CRFR1-positive cells are observed through IF-EASE staining, but not with the conventional IF. Staining specificity is also confirmed by including the control experiment (without primary Ab). Interneurons (I) and pyramidal neurons (II) are indicated by arrows in the CRFR1 image of (**b**). Apical dendrites of pyramidal neurons are shown by the arrows in the composite image (II).

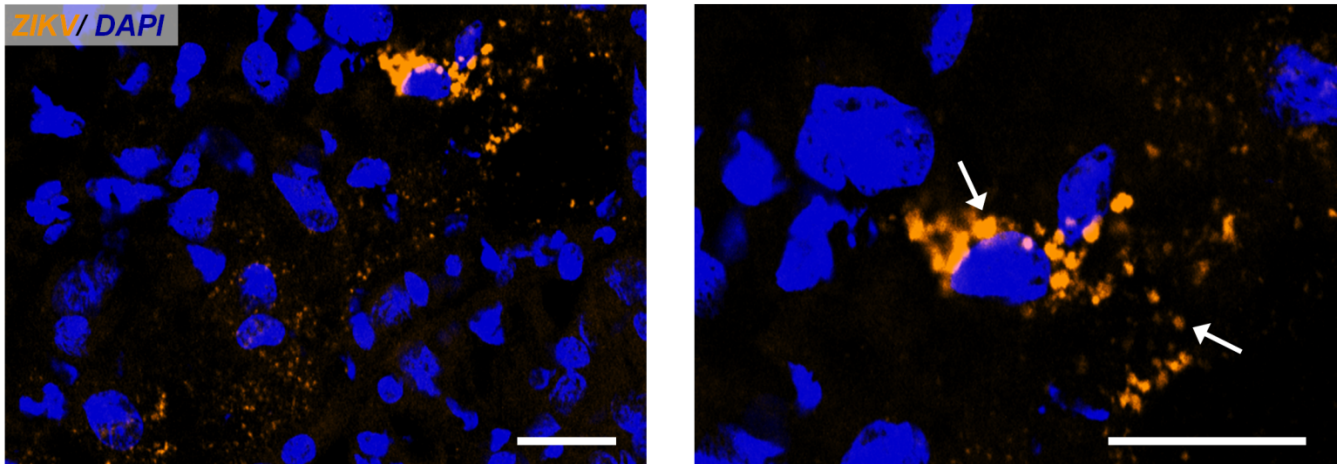



**Figure S23. ZIKV Imaging in placenta.** Representative confocal fluorescence images showing the distribution of ZIKV in the tissue sections (left panel) and single cells (right panel). Dashed lines, cytotrophoblast cell layer (identified by morphology). Infected cells appear within the chorionic villus core and villi beneath in close proximity to the cytotrophoblast cell layer. Scale bar, 50 µm.

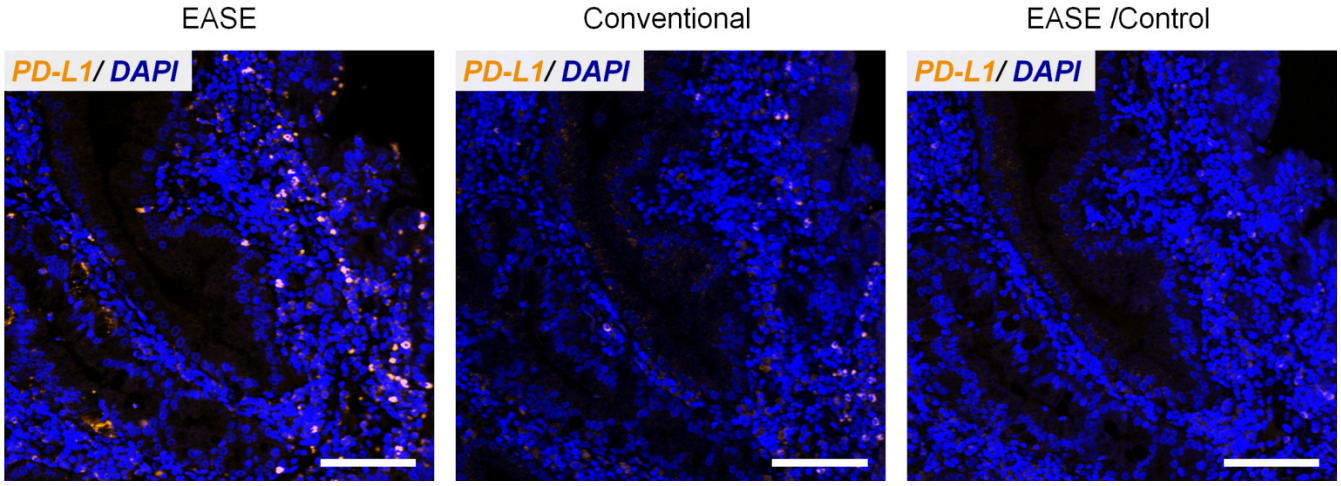



**Figure S24. Sensitive imaging of PD-L1 in FFPE pancreatic tumor specimens.** Representative fluorescence micrographs of PD-L1 expression in pancreatic specimens from the patient (SU-10-26808), samples counter-stained with DAPI. Scale bar, 100 μm. PD-L1 staining can only be observed through the IF-EASE technology, but not the conventional IF. The control experiment (without primary Ab) did not show detectable signals either.