

Jun Hyeok Lim¹, Euna Jeong², Junghyun Choi^{2,4}, Jaehyeon Ok^{2,4}, Donghyung Kim⁴, Taek Lee⁵, JiYoon Bu^{2,3},

¹ Department of Internal Medicine, Inha University Hospital, Inha University College of Medicine, Incheon, Korea

² Department of Biological Sciences and Bioengineering, Inha University, Incheon, Korea

³ Department of Biological Engineering, Inha University, Incheon, Korea

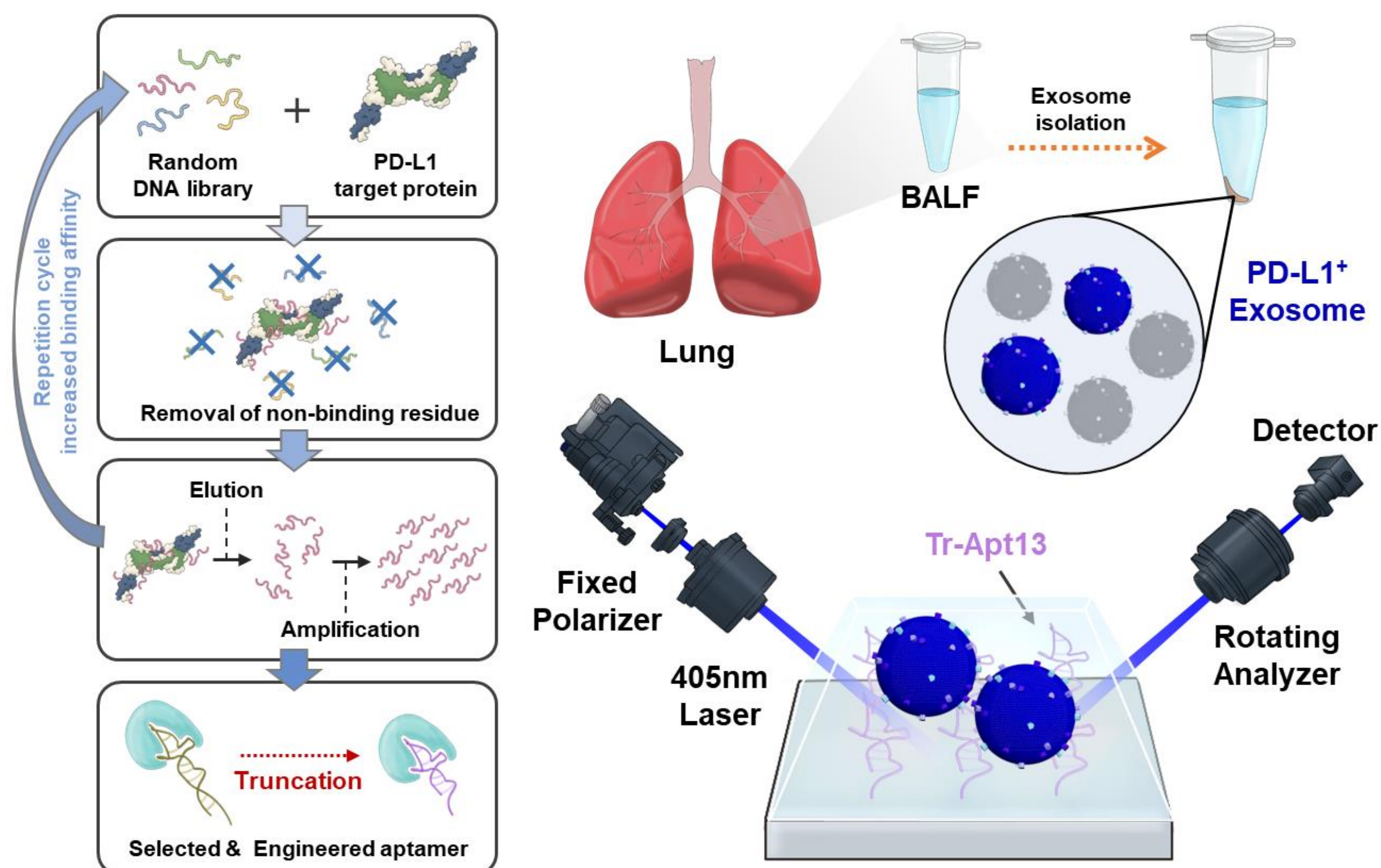
⁴ Division of Biomedical Metrology, Korea Research Institute of Standards and Science, Daejeon, Korea

⁵ Department of Chemical Engineering, Kwangju University, Seoul, Korea

Introduction

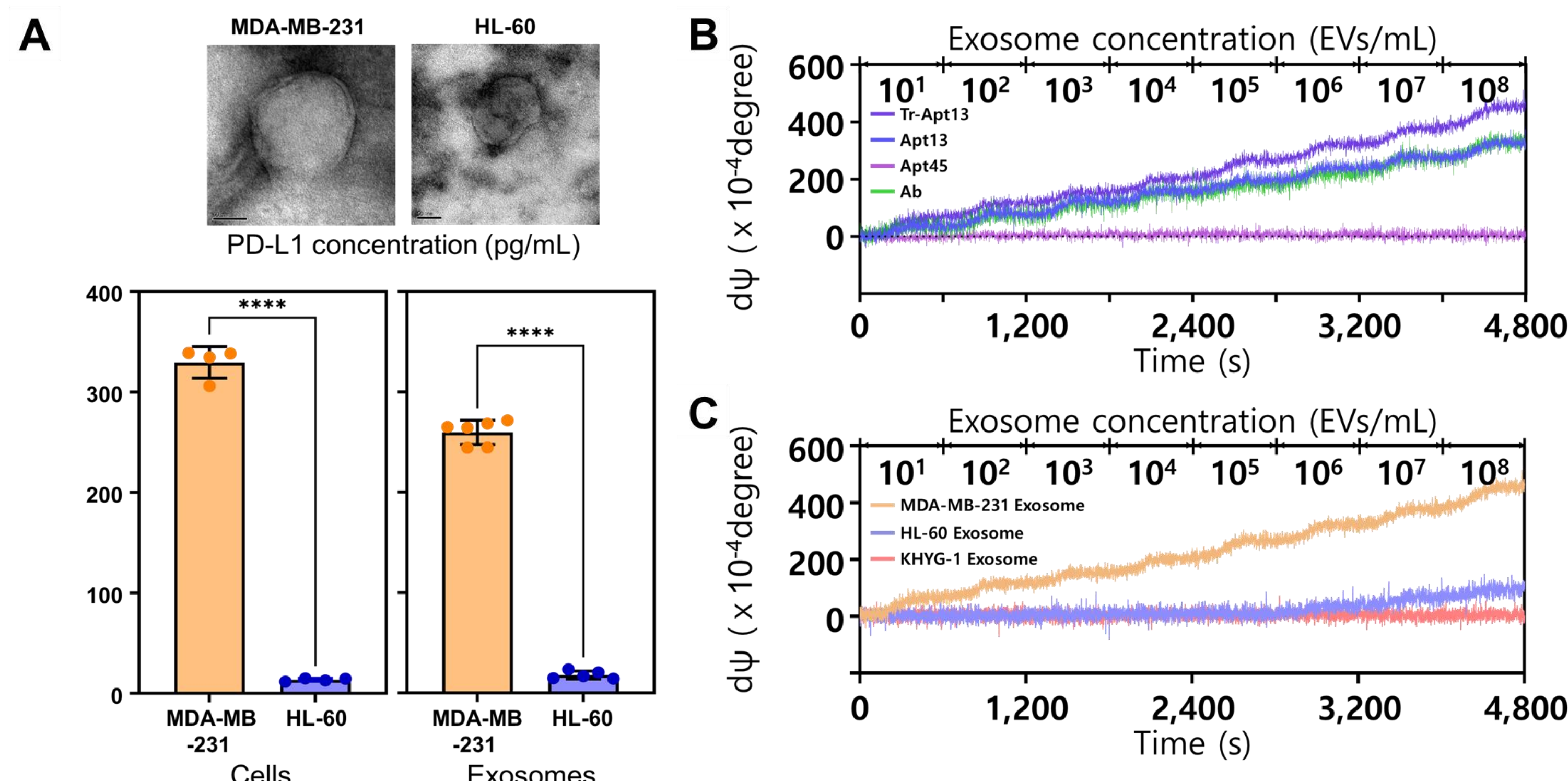
Exosomes have emerged as promising biomarkers of the tumor microenvironment, but their clinical use for predicting immunotherapy responses remains limited by insufficient assay sensitivity, specificity, and sampling relevance. Here, we developed a multi-level engineering strategy integrating PD-L1 aptamer optimization, dual-prism solution-immersed silicon (DP-SIS) ellipsometry, and tumor-proximal bronchoalveolar lavage fluid (BALF) sampling. PD-L1-specific aptamers were identified by systematic evolution of ligands by exponential enrichment (SELEX) and optimized through truncation and computational refinement, yielding Tr-Apt13 with enhanced target recognition compared with conventional antibodies. Integrated into the DP-SIS platform, Tr-Apt13 enabled real-time, label-free detection of PD-L1⁺ exosomes with a detection limit of **9.8 particles mL⁻¹**. In BALF samples from lung cancer patients, Tr-Apt13–DP-SIS distinguished immune checkpoint inhibitor (ICI) responders from non-responders at exosome concentrations as low as **1,000 particles mL⁻¹**, outperforming serum-based analysis, ELISA, and tissue PD-L1 immunohistochemistry. Together, this strategy links aptamer engineering, ultrasensitive optical sensing, and BALF-based sampling to improve exosome-guided immunotherapy response prediction in lung cancer.

Aptamer Engineered DP-SIS Platform Overview



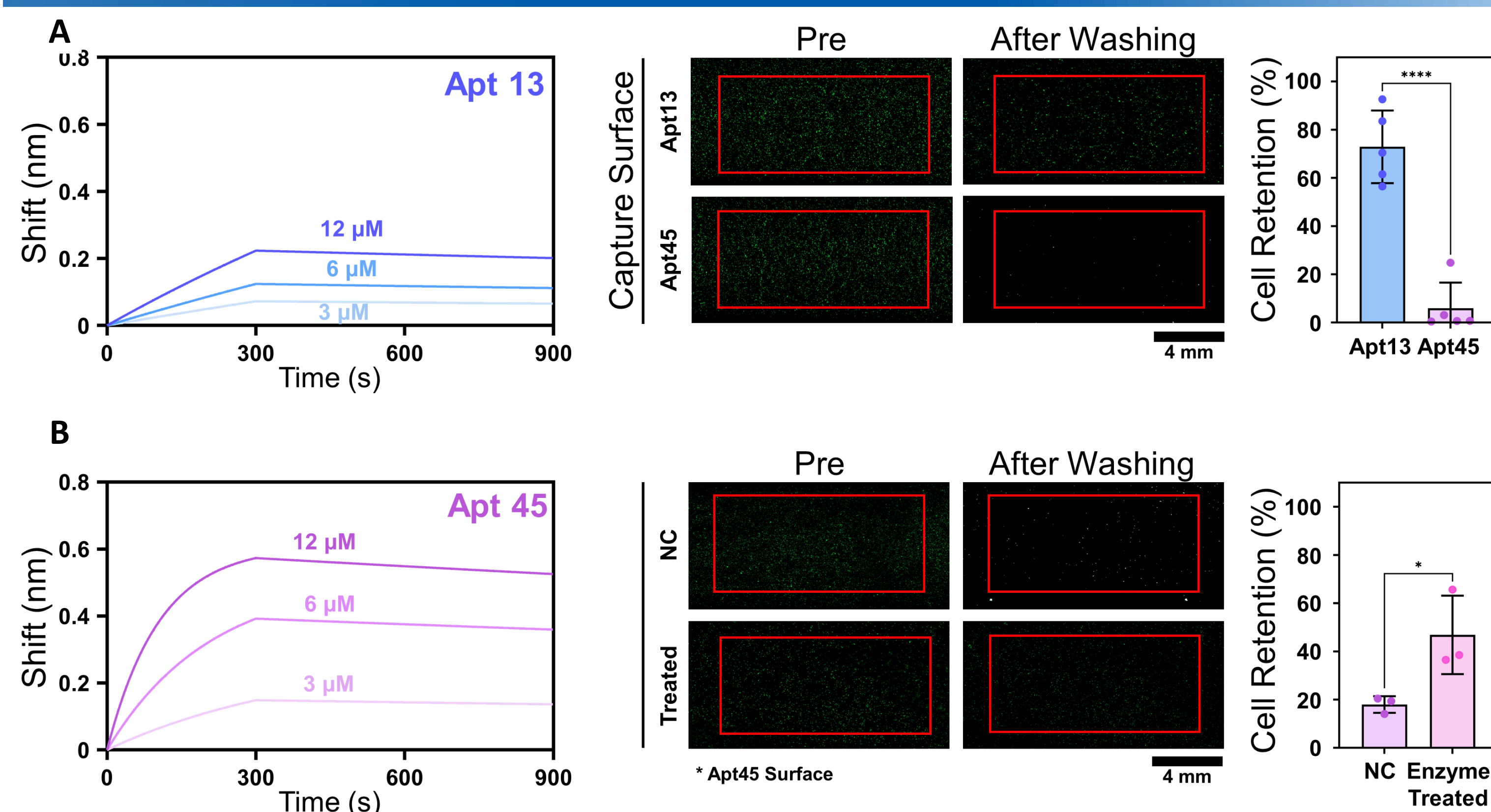
- PD-L1 specific aptamers were isolated by SELEX and optimized by truncation to generate Tr-Apt13.
- Tr-Apt13 was immobilized on the DP-SIS sensor surface for ultrasensitive detection of BALF-derived PD-L1⁺ exosomes.

Specific Detection of PD-L1⁺ Exosomes Using Tr-Apt13–DP-SIS



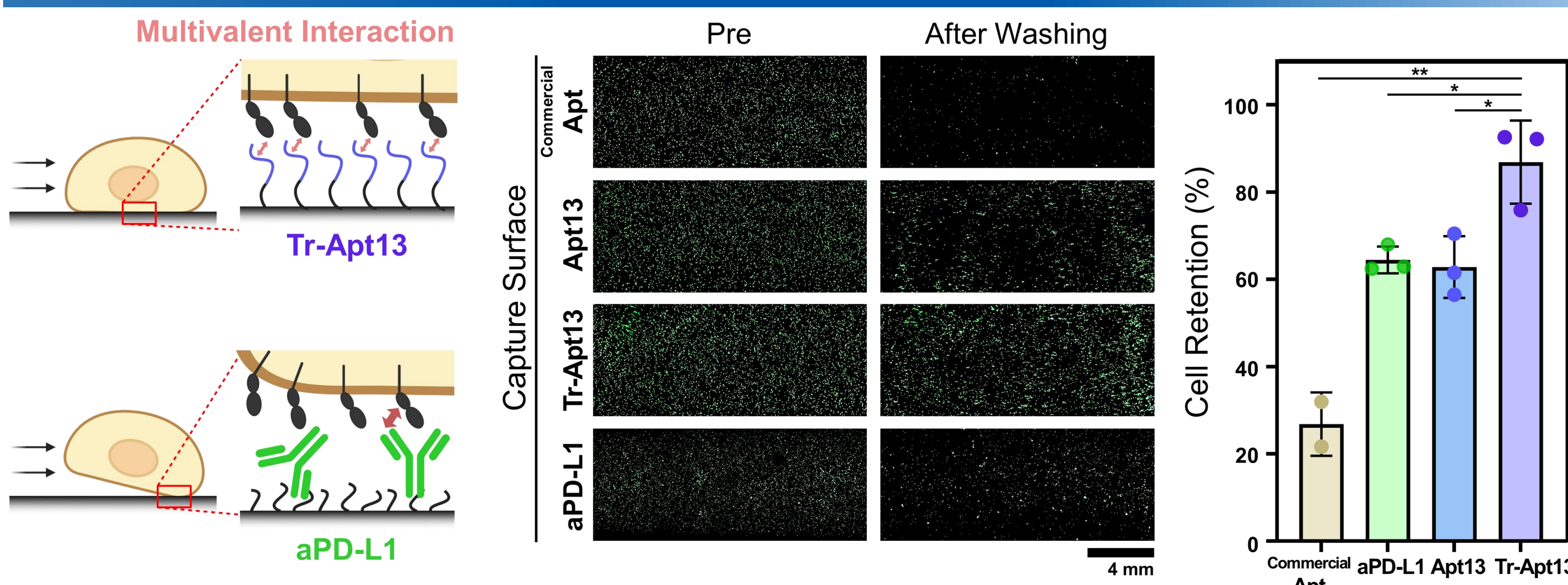
- PD-L1^{negative} HL-60–derived exosomes showed typical exosomal morphology but negligible PD-L1 expression.
- Tr-Apt13 showed the steepest concentration-dependent dΨ response and the lowest limit of detection among the tested aptamer surfaces.
- Tr-Apt13–DP-SIS selectively detected PD-L1⁺ MDA-MB-231 exosomes, with no detectable response from PD-L1^{negative} HL-60–derived exosomes.

Selection and Functional Validation of PD-L1 Aptamers



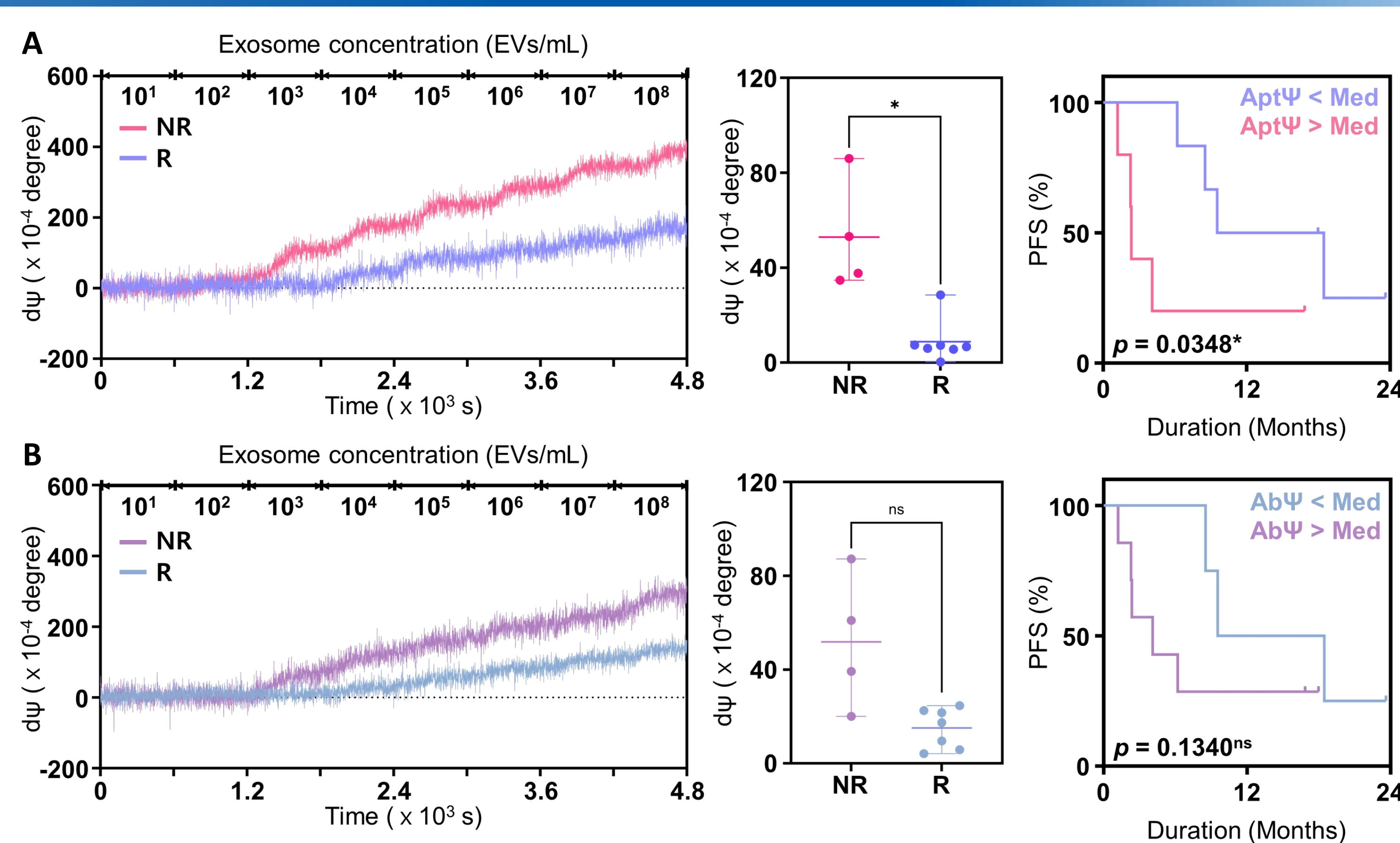
- Apt13 retained 72.9 ± 13.5% of PD-L1⁺ MDA-MB-231 cells, compared with only 5.9 ± 10.0% for Apt45.
- PNGase F deglycosylation restored Apt45 cell binding, indicating that membrane-bound PD-L1 glycosylation limits Apt45 targeting efficiency.

Enhanced Multivalent Capture by Tr-Apt13



- High-density, uniformly oriented Tr-Apt13 enhanced multivalent PD-L1 binding by reducing steric hindrance and improving target accessibility.
- Tr-Apt13 captured **86.8 ± 7.8%** of MDA-MB-231 cells, outperforming antibody, Apt13, and a commercial PD-L1 aptamer.

Clinical Validation Using BALF-Derived Exosomes



- Tr-Apt13–functionalized DP-SIS sensitively detected BALF–derived PD-L1⁺ exosomes, showing stronger sensor responses in non-responders than in responders. Quantitative dΨ analysis further confirmed significantly elevated PD-L1⁺ exosome levels in non-responders, even at **lower total exosome concentrations**.
- aPD-L1–functionalized DP-SIS showed the same overall trend, detecting elevated PD-L1⁺ exosome signals in non-responders. However, its clinical separation was weaker than that of Tr-Apt13–DP-SIS, supporting the superior discrimination achieved by the aptamer-based sensor.

Conclusion

Tr-Apt13–functionalized DP-SIS enabled ultrasensitive, label-free, and specific detection of PD-L1⁺ exosomes by integrating optimized aptamer engineering with a high-performance optical sensing platform. Compared with antibody-based sensing, Tr-Apt13 enhanced PD-L1 recognition through multivalent target binding and showed superior detection performance. In clinical BALF samples, PD-L1⁺ exosome profiling distinguished ICI responders from non-responders and improved prognostic stratification compared with tissue PD-L1 IHC and antibody-based assays. These results support Tr-Apt13–DP-SIS as a tumor-proximal liquid biopsy platform for immunotherapy response prediction in lung cancer.