SUPPORTING INFORMATION

An ultrasensitive colorimetric test for the detection of somatic rare mutations in DNA

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SUPPORTING FIGURE

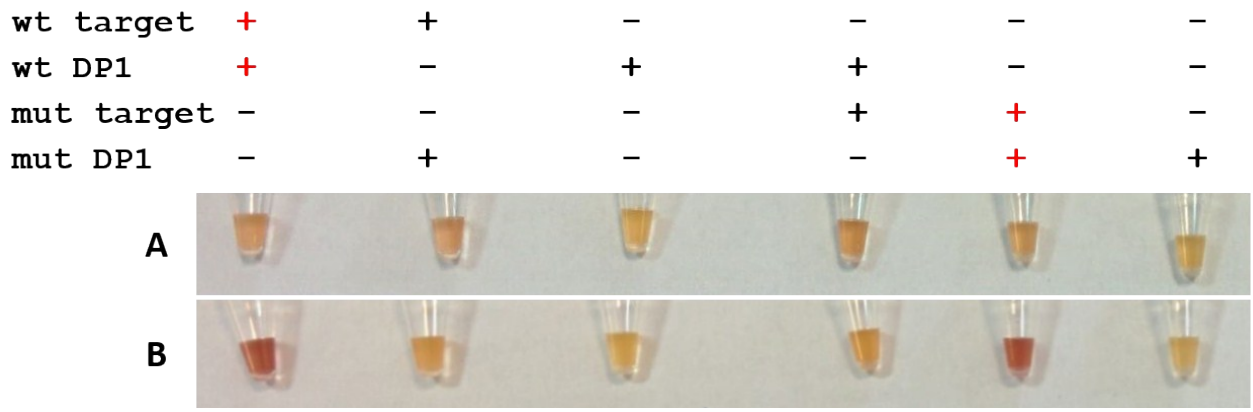

Figure S1. Assay optimization: washing steps. A) In the presence of an excess of DP2, the signal of matched target (positive samples) is undistinguishable from background, due to saturation of the AuNP probes with unbound excess DP2 (starting from left, positive samples n°1 and n°5 are undistinguishable from negative samples n° 2 and n°4). At the same time, when performing washing steps at room temperature, a small background signal was observed, due to a mild unspecific binding (pale pink in negative control samples n°2 and n°4, different from yellow of blank controls (no target) n° 3 and n°6). These limitations resulted in no discrimination of the point mutation. **B)** In the optimized assay, excess DP2 was magnetically separated before the addition of the AuNP probes, markedly increasing the sensitivity (see dark red in positive sample 1 and 5). Moreover, by increasing the temperature of the last washing step at 52°C, any background signal was completely eliminated, markedly increasing the assay specificity ((negative control samples n°2 and n°4 are as yellow as blank controls (no target) n° 3 and n°6).

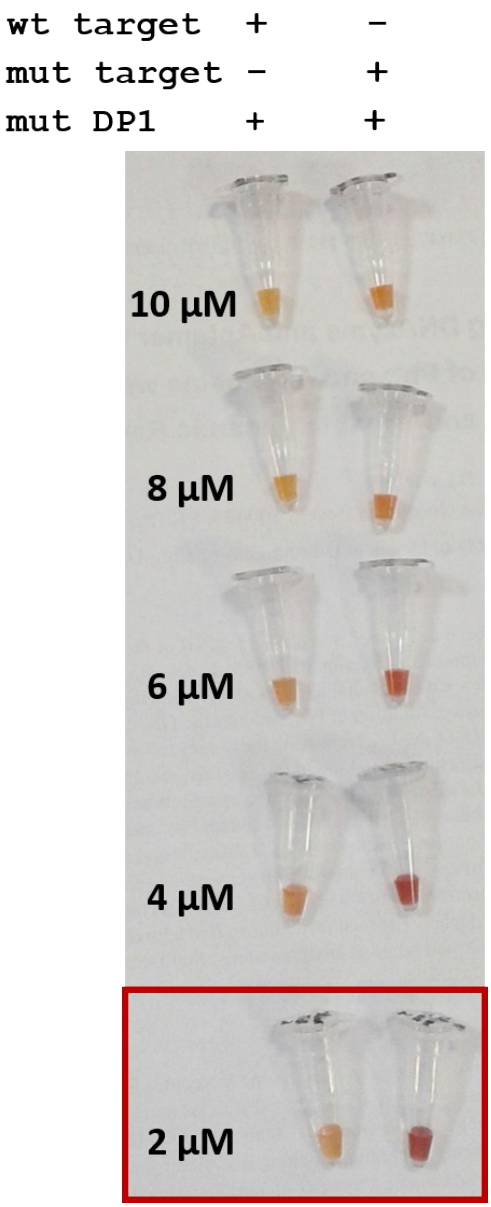

Figure S2. Assay optimization: concentration of DP2. The protocol used in figure S1, including an additional washing step after the incubation with DP2 and a final washing step at 52 °C, was repeated at different concentrations of DP2, in order to further decrease the interference from excess DP2 and increase the sensitivity of the assay. A range of DP2 concentrations from 10 to 2 µM was assayed. The clearest signal discrimination was obtained with the minimum concentration of DP2 tested (2 µM), further proving that excess DP2 (not bound to the target) decrease the sensitivity of the assay by binding the AuNP probes and hindering their binding to target-bound DP2.

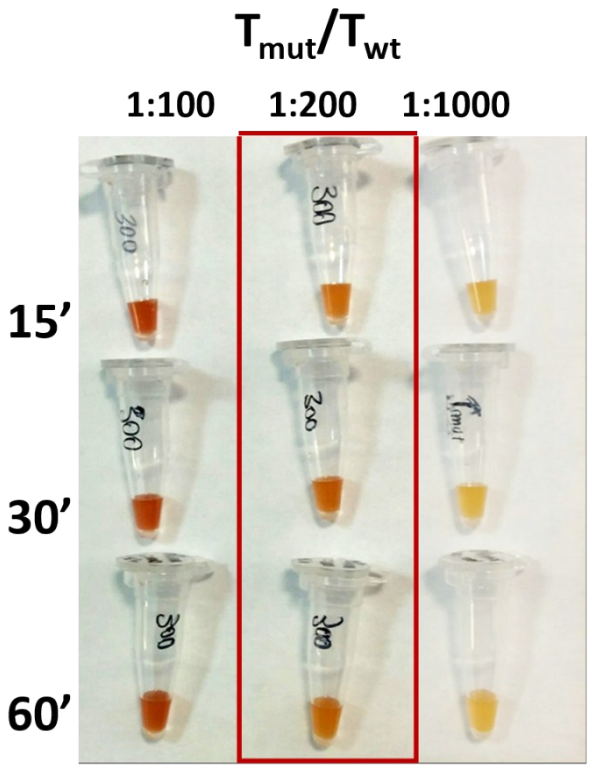

Figure S3. Assay optimization: target hybridization time. The target incubation time was extended up to 1 hour and decreased down to 15 minutes. While a shorter incubation (15') did not significantly affected the discrimination, a prolonged incubation (up to 1 hour) did not improve the limit of discrimination of the assay. Therefore, 30 minutes was chosen as the appropriate time for the target capture step.

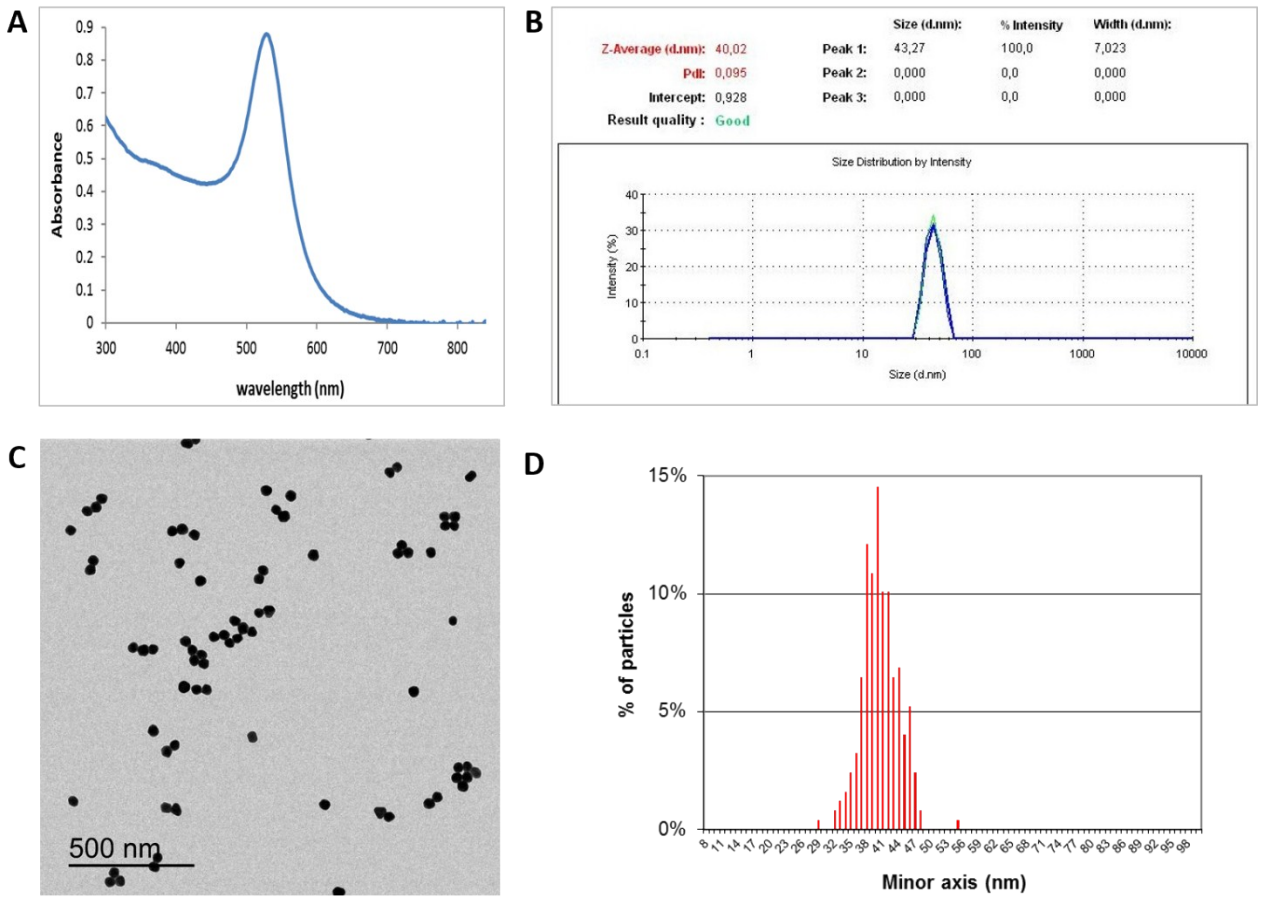

Figure S4. AuNPs characterization. **A)** UV-vis spectra of 40 nm AuNPs. **B)** DLS characterization. **C)** Transmission Electron Microscopy representative image of 40 nm AuNPs. **D)** Statistics of size distribution of AuNPs as measured by TEM.

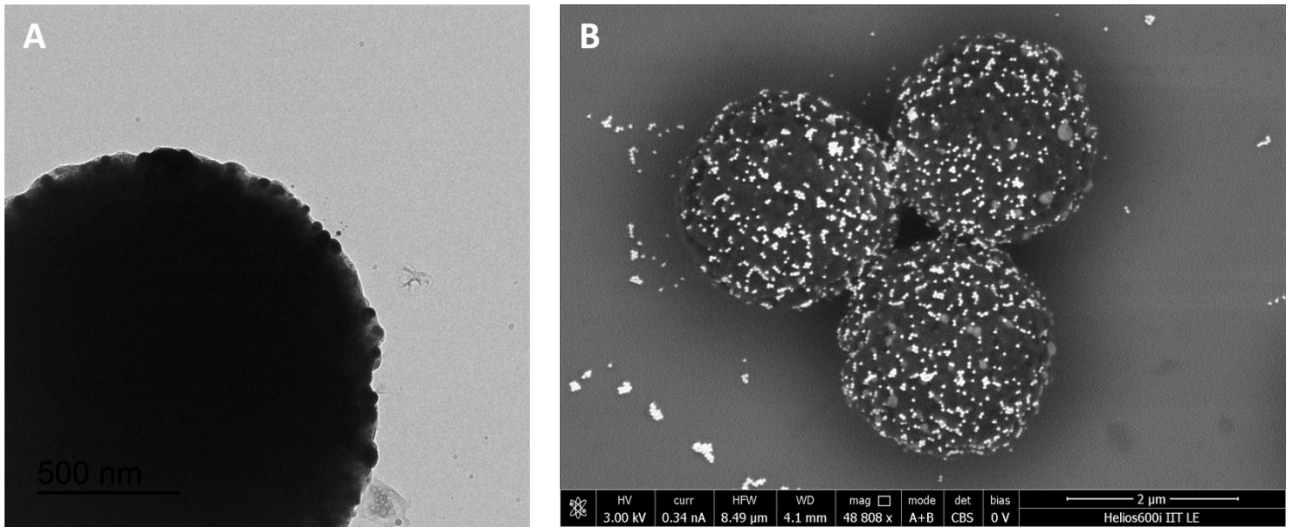

Figure S5. Characterization of paramagnetic microparticles complexed with AuNPs. **A)** Transmission Electron Microscopy (TEM) images of microparticles complexed with AuNPs. The small black dots visible on the periphery of the microparticle are AuNPs. **B)** Scanning Electron Microscopy (SEM) images of microparticles complexed with AuNPs. The brilliant dots on the surface of the microparticles are AuNPs.