



Supplementary Figure 25. Second round of screening of newly developed “3rd generation” assays using dimeric nanobody detectors. Affinity reagent pairs selected from a first round of screening in plasma samples (Supplementary Figure 9) were screened in 25 healthy and 25 GE cancer patient plasma samples. Each assay is denoted by the capture/-detector reagent pair. Second-generation assays are run for comparison (gray boxes). Blue dashed lines indicate assay limits of detection, accounting for four-fold dilution. Blue boxes indicate the final three selected third-generation assays. MT1032 and MT1035 are Nb5-5 homodimers with different linkers. MT1036 is a Nb2-Nb9 heterodimer, MT1037 a Nb5-Nb9 heterodimer, and MT1038 a Nb9-Nb9 homodimer.