**Figure 4. Optimization of coreTIA components.** Example assay runs underlining the relative importance of assay parameters.

**(A)** Heat inactivation: ND50 values for a human serum sample tested against AAV9-NLuc (MOI 100) under untreated vs. heat-inactivated (56°C for 30 minutes) conditions. Heat inactivation significantly reduces the measured ND50 (from ~1/8 to ~1/4), indicating lower detected neutralizing activity.

**(B)** *Transduction Mix* incubation: ND50 values for a different human serum sample estimated after 15, 30, or 60 minutes of incubation at 37°C in a *Transduction Mix* containing the human serum, FBS, and AAV9-NLuc (MOI 100).

**(C)** Post-transduction duration: ND50 values for the same serum sample as in panel B measured against AAV9-NLuc (MOI 100) at 24 and 48 hours post-transduction.

In all panels, bars represent ND50 estimates calculated using the Hill-MCMC method, higher serum dilution values indicate greater neutralizing activity, reflecting higher assay sensitivity. Error bars show 95% credible intervals from Hill-MCMC fits. Statistical significance was determined using Bayesian Practical Equivalence Test with the previously established practical equivalence threshold of θ =0.3 log2 units: "\*" indicates a difference above this threshold, while "ns" indicates no significant difference (i.e., practical equivalence,Methods).