**Figure 5. Hill-MCMC Estimation of ND50 with Uncertainty When Dilution Series Do Not Bracket the 50% Inhibition Point**

**(A)** Precision of ND50 estimation during extrapolation using synthetic data. The dotted gray curve represents the synthetic ground truth (ND50 = 1/32). Grey dots show examples of noisy measurements (CV = 10%) sampled only at dilutions 1/4, 1/8, and 1/16. The solid blue line represents the mean of all 90 noisy samples. Colored vertical dashed arrows show the posterior mean ND50 estimates derived via Hill-MCMC using 1 (teal), 2 (grey), or 3 (pink) randomly chosen technical replicates from the 90 available noisy curves (legend indicates grouping for one example estimate). Horizontal bars at the bottom represent the composite 95% CI-of-CIs intervals (Methods) across 30 independent simulations for each replicate condition, illustrating improved precision (narrower intervals) with more replicates.

**(B)** Hill-MCMC extrapolation applied to real neutralization data. Curves show results from one mouse serum sample tested on different days with different dilution ranges: Day 1 (D1, teal) and Day 2 (D2, orange) used dilutions (1/64–1/4096) that did not bracket the 50% inhibition point, while Day 3 (D3, purple) used an adjusted range (1/1024–1/65536) that did. Points show technical replicate means; solid lines show Hill-MCMC fits. Vertical dashed arrows indicate the posterior mean ND50 estimates derived via Hill-MCMC, with horizontal bars representing the corresponding 95% credible intervals. Note the extrapolation required for D1 and D2.

**(C)** Comparison of posterior ND50 estimates across days. Bars represent the posterior mean ND50 estimates derived via Hill-MCMC for Day 1 (D1), Day 2 (D2), and Day 3 (D3). Error bars represent the 95% credible intervals. Numerical annotations indicate the difference (in log2 units) between the posterior means for the indicated comparisons (e.g., D1 vs D3 ≈ 0.19 log2 units).