Supporting Information

Sensitive detection of glucagon aggregation using amyloid fibril-specific antibodies

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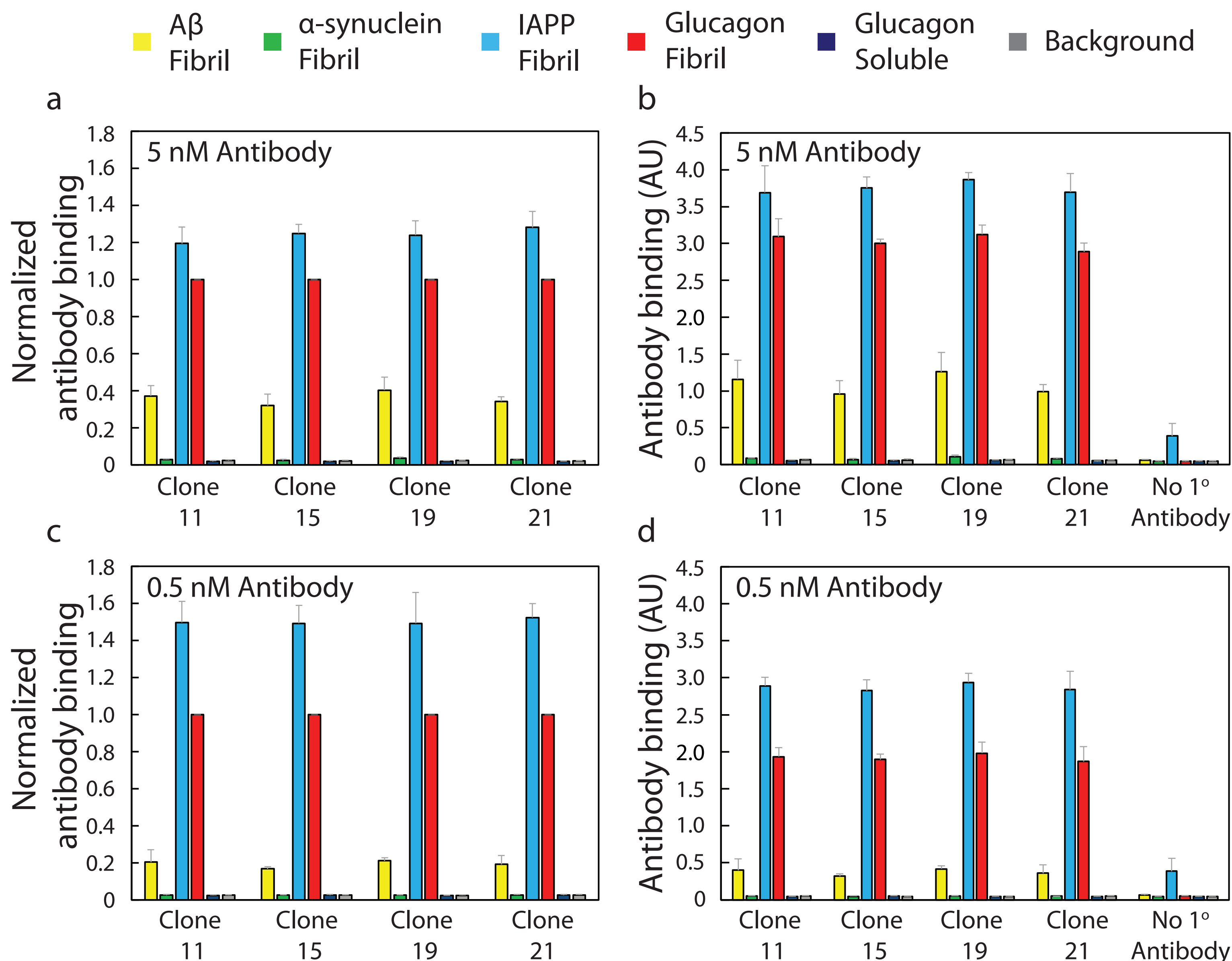

FIGURE S1. Glucagon antibodies cross-react with other amyloid-forming peptides. Sequence specificity of the glucagon antibodies was assessed using Aβ1-42, α-synuclein, and IAPP fibrils. Antigens were immobilized overnight (4 °C) at 2.5 μM using untreated MaxiSorp plates. Two different primary antibody concentrations were evaluated, namely (a,b) 5 nM and (c,d) 0.5 nM. The data reported in (a) and (c) represent the binding of each antibody to the different antigens relative to glucagon fibrils. In (b) and (d), the raw absorbance values are reported. The reported values represent the mean for three independent experiments, and the error bars are standard errors.

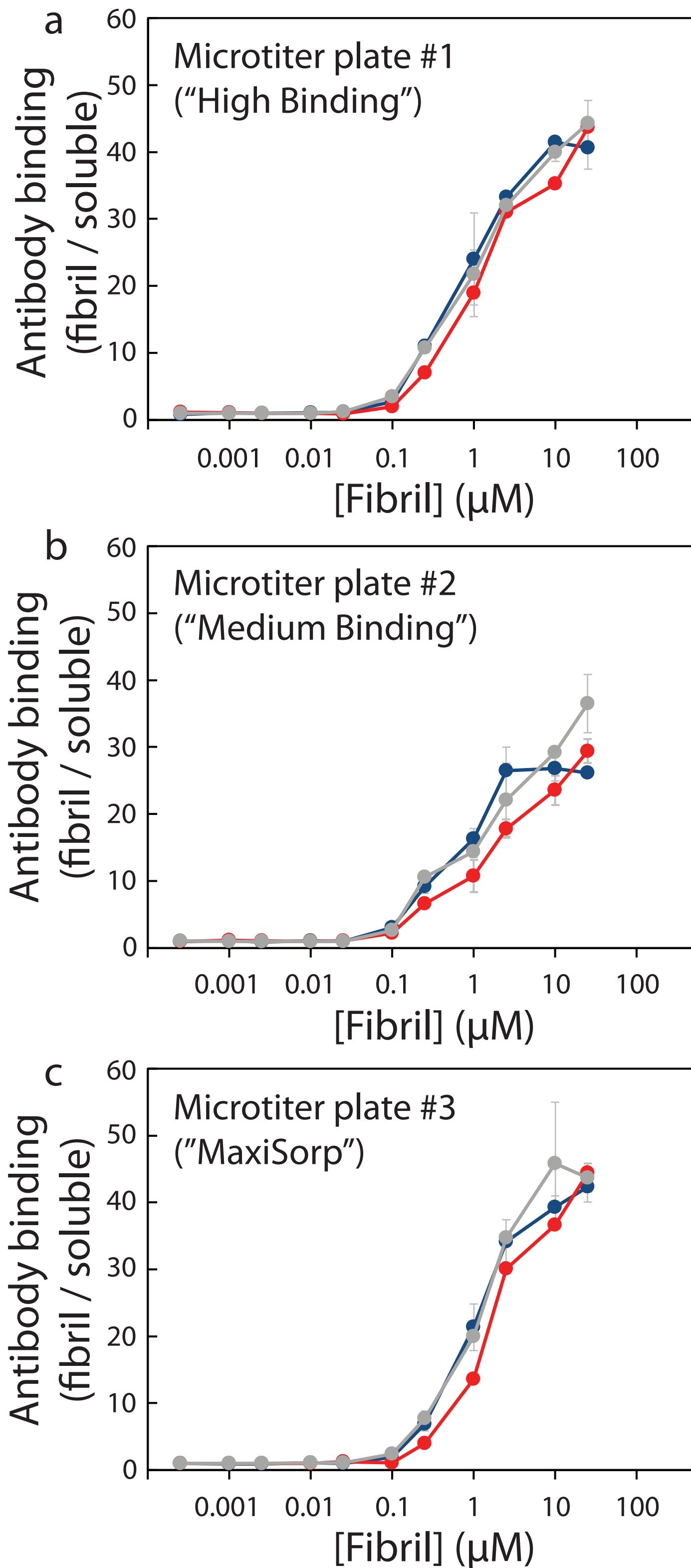

FIGURE S2. Sensitivity of ELISA method is weakly impacted by the type of microtiter plate. Soluble and fibrillar glucagon were separately immobilized in (a) Greiner "High Binding", (b) Greiner "Medium Binding", and (c) Nunc "MaxiSorp" ELISA plates for 2 h at room temperature, and then were detected using glucagon antibodies (0.5 nM). The reported values are normalized signals for antibody binding to glucagon fibrils relative to soluble peptide for two independent experiments. The error bars represent standard errors.

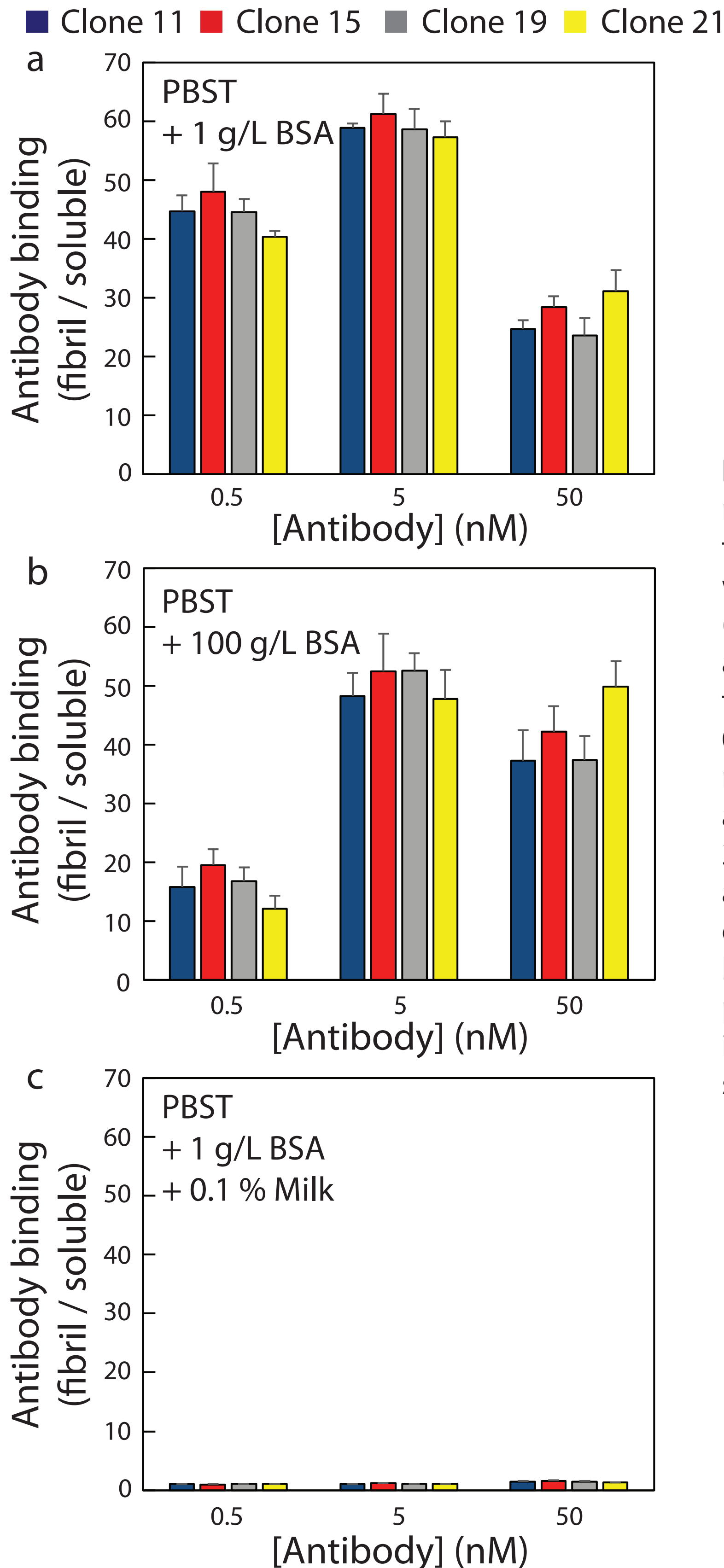

FIGURE S3. Impact of the solution environment on antibody recognition of glucagon fibrils. Soluble and fibrillar glucagon (2.5 μM) were immobilized in MaxiSorp plates (overnight, 4 °C). Glucagon antibodies (0.5, 5, and 50 nM) were allowed to bind to glucagon fibrils for 1 h in (a) PBS supplemented with 0.1% Tween 20 and 1 g/L BSA, (b) PBS supplemented with 0.1% Tween 20 and 100 g/L BSA, and (c) PBS supplemented with 0.1% Tween 20, 1 g/L BSA, and 0.1% milk prior to secondary antibody incubation and substrate development. The mean values are for antibody binding to glucagon fibril relative to soluble peptide, and they were obtained from three independent experiments. The error bars are standard errors.

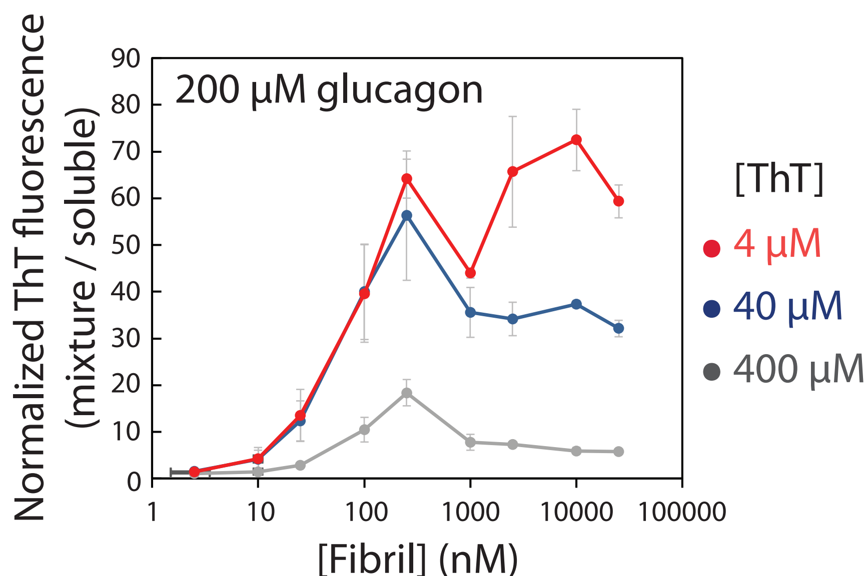

FIGURE S4. Impact of ThT concentration on its sensitivity for detecting glucagon fibrils. Mixtures of soluble and fibrillar glucagon were prepared at a total concentration of 245 μM, and the final peptide concentration was 200 μM after the addition of ThT. The soluble and fibrillar glucagon mixtures were incubated in microcentrifuge tubes for 2 h at room temperature prior to addition of ThT. ThT fluorescence was evaluated (λ_{ex}=444 nm, λ_{em}=482 nm) using black, 384-well plates. The data represent the mean values for three independent experiments, and the error bars are standard errors.