Supporting Information

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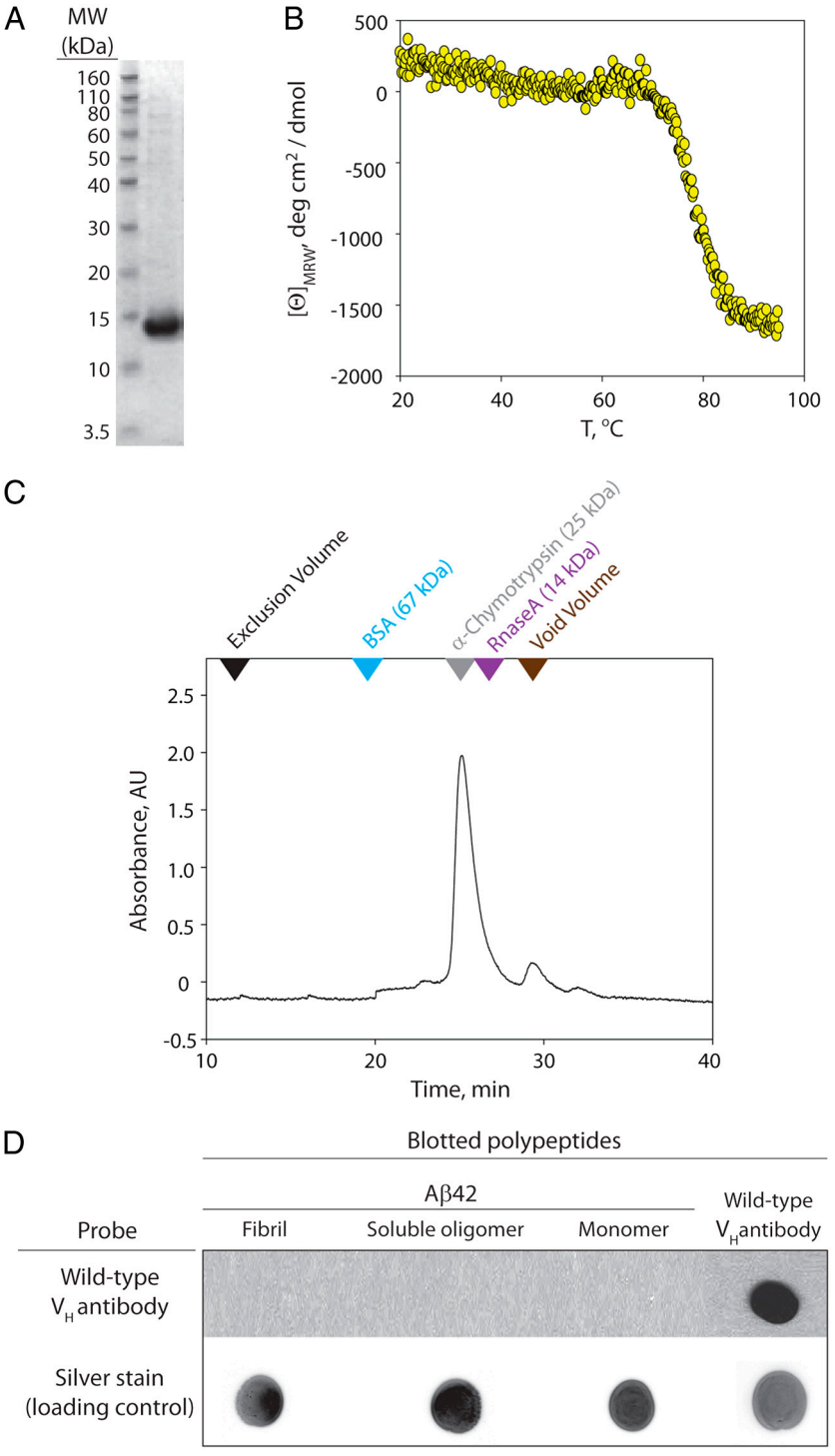

Fig. S1. Biophysical characterization of the wild-type V_H antibody. (*A*) SDS/PAGE analysis (10% 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol gel) of the wild-type antibody (50 μM) using Novex LDS (lithium dodecyl sulfate) sample buffer (50 mM β-mercaptoethanol; Sigma-Aldrich) and MES running buffer (all reagents from Invitrogen unless otherwise specified). (*B*) Circular dichroism thermal unfolding transition of the wild-type antibody (10 μM, PBS, pH 7.4) recorded at 235 nm using a Jasco 815 Spectrometer (0.5 nm bandwidth, 1 s integration time, 1 °C/ min heating rate). The *y* axis reports the mean ellipticity (Θ_{MRW}) where MRW denotes mean residue weight. (*C*) Size-exclusion chromatography analysis of the wild-type antibody (25 μM) using an analytical TSK Gel G3000SW$_{xl}$ column (0.78 × 30 cm, Tosoh Bioscience). (*D*) Wild-type V_H antibody fails to bind to Aβ conformers. Aβ42 peptide was deposited on ni-

trocellulose (220 ng per spot), and probed with the wild-type antibody (biotinylated) and peroxidase-conjugated streptavidin (Pierce), as well as colloidal silver stain (Sigma). The V_H antibody (500 ng) was also deposited as a detection control.

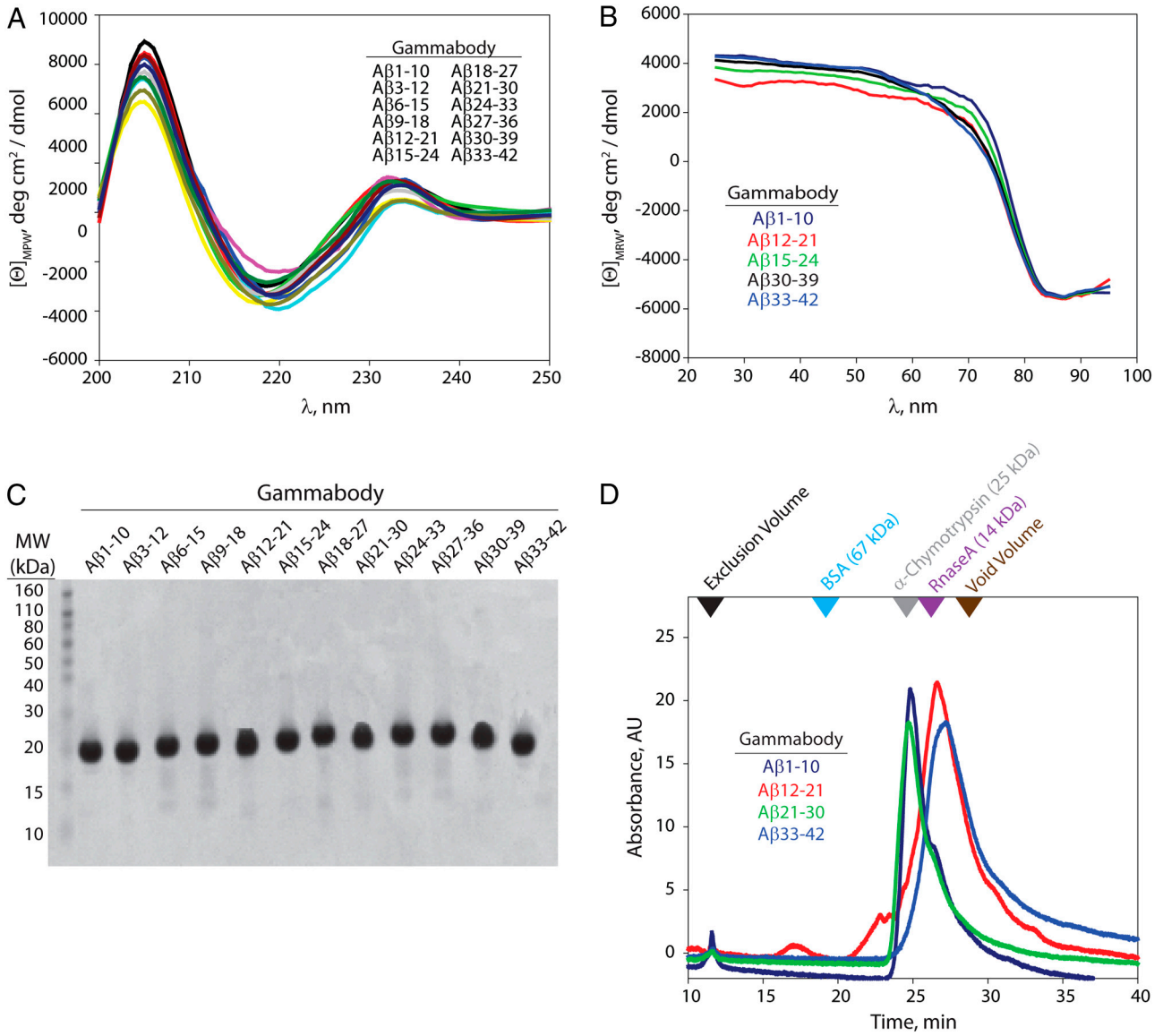

Fig. S2. Aβ gammabodies possess similar biophysical properties. (*A*) Circular dichroism spectra and (*B*) thermal unfolding transitions of Aβ gammabodies (10 μM, PBS, pH 7.4), which were measured as described in Fig. S1. (*C*) SDS/PAGE analysis (10% 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol gel) of the grafted antibodies (50 μM), as described in Fig. S1. (*D*) Size-exclusion chromatography analysis of the Aβ gammabodies (25 μM), as described in Fig. S1. The molecular weight of the grafted antibodies is 17.6–17.9 kDa. The gammabodies displaying hydrophilic Aβ motifs (Aβ1–10 and Aβ 21–30) elute between α-chymotrypsin (25 kDa) and ribonuclease A (14 kDa), as expected. The gammabodies displaying hydrophobic loops (Aβ12–21 and Aβ33–42) elute between ribonuclease A and the void volume, suggesting that these antibodies stick to the chromatography particles. MRW, mean residue weight.

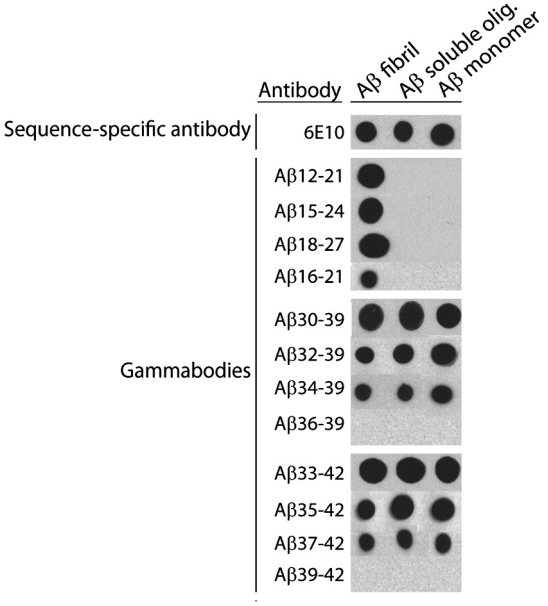

Fig. S3. Identification of minimal Aβ motifs that mediate binding of gammabodies to Aβ conformers. Aβ42 conformers were deposited on nitrocellulose membranes (220 ng) and probed with gammabodies (0.6 μM). As a loading control, the same blots were probed with the sequence-specific monoclonal antibody 6E10 (specific for Aβ residues 1–17).

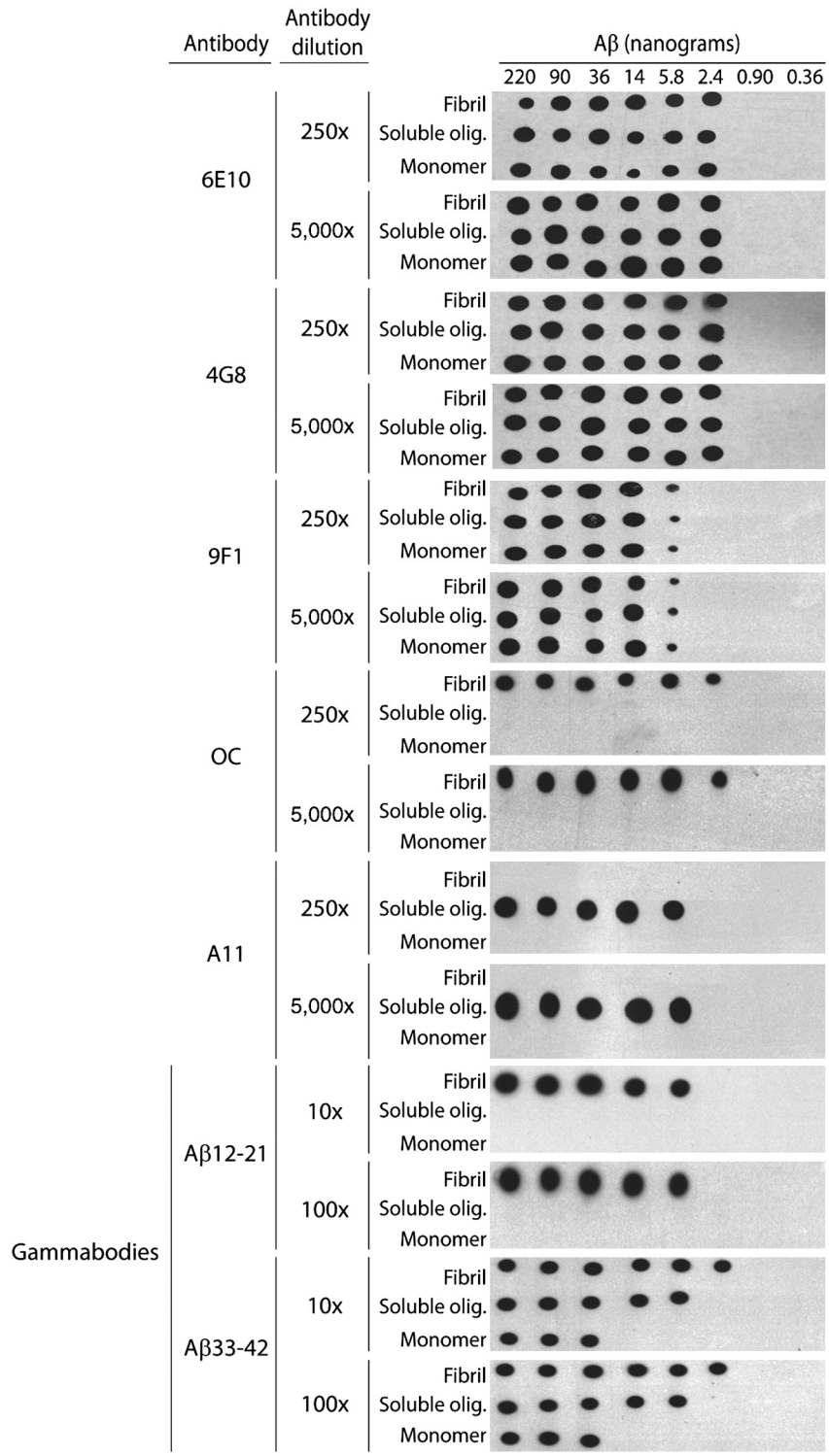

Fig. S4. Antibody binding to Aβ conformers is invariant over a range of antibody concentrations. Aβ42 conformers were deposited on nitrocellulose membranes (0.36–220 ng). The resulting blots were probed with Aβ gammabodies, as well as sequence-specific monoclonal antibodies (6E10-specific for Aβ1–17, 4G8-specific for Aβ residues 18–22, and 9F1-specific for Aβ residues 34–39), fibril-specific antibodies (WO1 and OC), and a prefibrillar oligomer-specific antibody (A11). The initial concentration of each gammabody prior to dilution was 1.0 mg/mL for Aβ12–21 and 1.5 mg/mL for Aβ33–42. The initial concentrations of the reference sequence- and conformation-specific antibodies were 5 mg/mL for 6E10, 2 mg/mL for 4G8, 1 mg/mL for 9F1, 3 mg/mL for OC, 1 mg/mL for WO1, and 1.5 mg/mL for A11.

A

Gammabody	Antigen: Aβ fibril K_D, nM	Aβ fibril IC_{50}, nM	Aβ soluble oligomer K_D, nM	Aβ soluble oligomer IC_{50}, nM
Aβ12-21	370 ± 45	343± 10	-	-
Aβ15-24	370 ± 30	345 ± 15	-	-
Aβ18-27	380 ± 55	344± 15	-	-
Aβ30-39	490 ± 65	520 ± 15	595 ± 30	605 ± 10
Aβ33-42	335 ± 20	350 ± 10	420 ± 60	425 ± 10

B

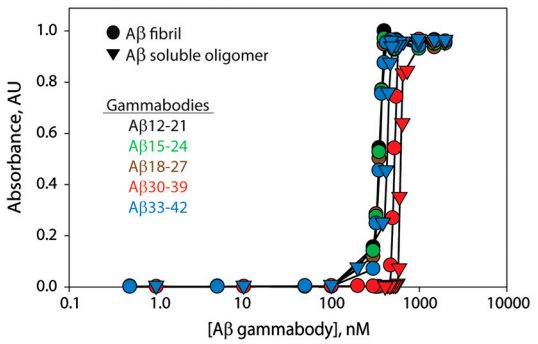

Fig. S5. Measurements of binding affinity of Aβ gammabodies for Aβ soluble oligomers and fibrils. (*A*) Dissociation constants (±standard deviation) of grafted antibodies measured using competitive ELISA binding analysis as well as IC_{50} (±standard deviation) values measured by noncompetitive binding analysis. Importantly, the dissociation constants and IC_{50} values are within error of each other. (*B*) IC_{50} measurements for grafted antibodies against Aβ soluble oligomers and fibrils.

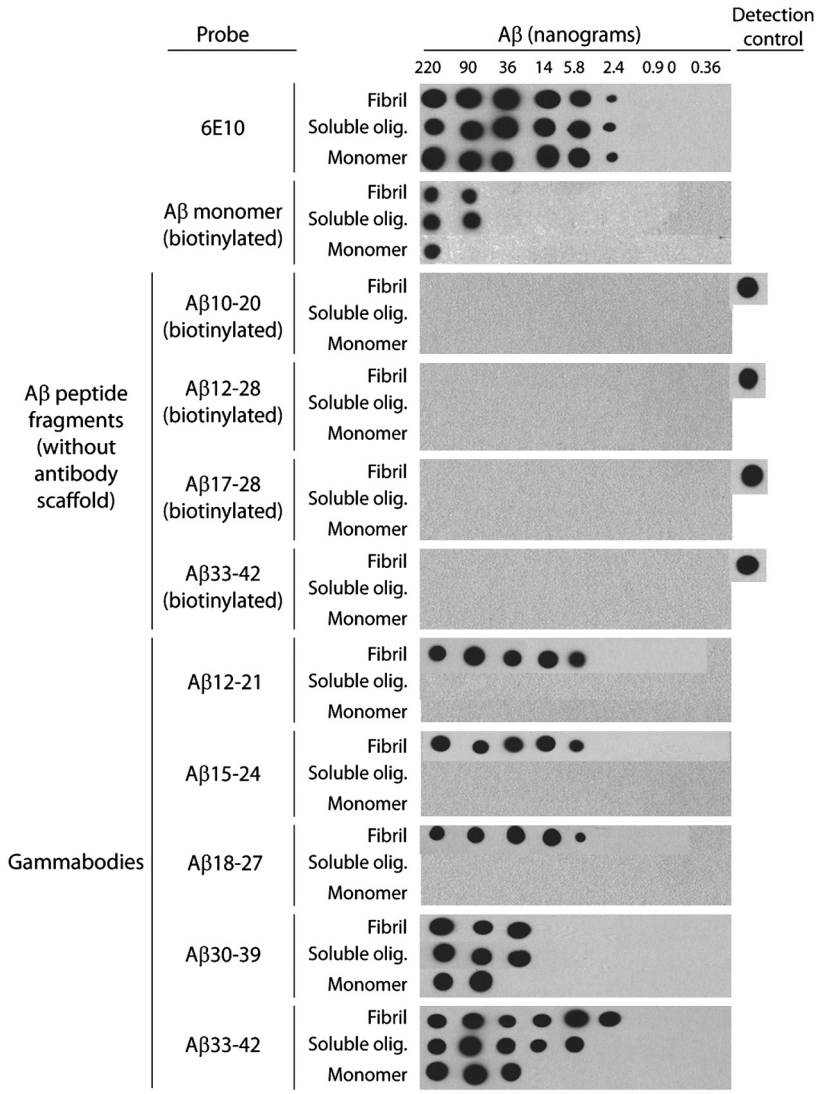

Fig. S6. Aβ42 monomer and Aβ peptide fragments are significantly less sensitive for detecting immobilized Aβ conformers relative to Aβ gammabodies. Aβ42 conformers were deposited on nitrocellulose membranes over a range of loadings (0.36–220 ng). The blots were probed with biotinylated Aβ42 monomer and Aβ peptide fragments (10 μM), and detected using peroxidase-conjugated streptavidin. As a reference, the 6E10 monoclonal antibody (1:1,000 dilution of 5 mg/mL) and grafted Aβ antibodies (1:10 dilution of 1 mg/mL) were incubated with the same blots, and developed using the appropriate peroxidase-conjugated secondary antibodies.

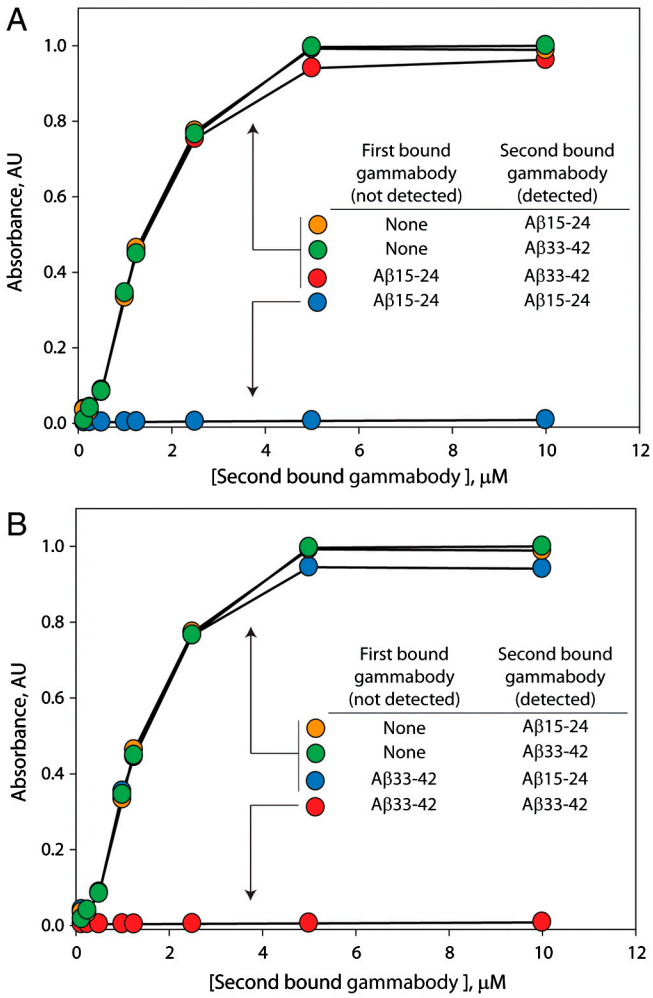

Fig. S7. Aβ gammabodies bind noncompetitively to Aβ fibrils. (*A*) Aβ15–24 and (*B*) Aβ33–42 grafted, nonbiotinylated antibodies (10 μM) were bound to immobilized fibrils (2 μM) in 96-well microtiter plates (Nunc Maxisorb; Thermo Fisher). Next, biotinylated Aβ15–24 and Aβ33–42 gammabodies were bound to the same immobilized fibrils over a range of antibody concentrations (0–10 μM), and detected using peroxidase-conjugated streptavidin. As a control, biotinylated antibodies were bound to Aβ fibrils without binding nonbiotinlyated antibodies.

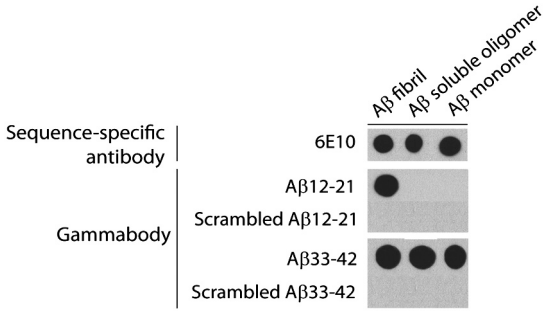

Fig. S8. Scrambling Aβ motifs eliminates binding activity of Aβ gammabodies. Aβ42 conformers (220 ng) were deposited on nitrocellulose membranes and probed with gammabodies (6 μM) displaying scrambled motifs relative to the unscrambled motifs. The scrambled motif for Aβ12–21 was ^{12}FHVQKFAHVL^{21}, and the scrambled motif for Aβ33–42 was ^{33}IVGMGLVGAV^{42}. As a control, loading of the Aβ42 peptide was confirmed using the sequence-specific monoclonal antibody 6E10 (specific for Aβ1–17).

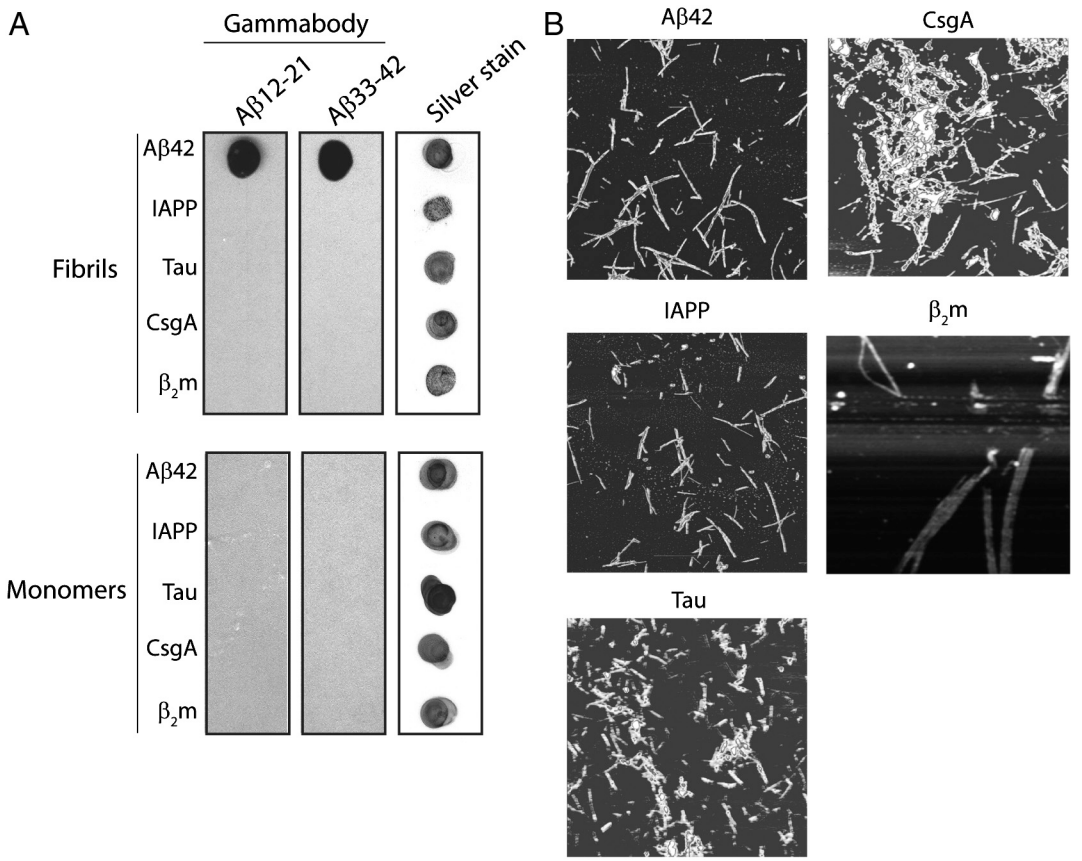

Fig. S9. Aβ gammabodies fail to recognize other misfolded proteins. (*A*) Fibrils and monomers of Aβ42, IAPP (amylin, provided by Daniel Raleigh, Stony Brook University, Stony Brook, NY), Tau (K18 fragment, residues 244–372, provided by Martin Margittai, University of Denver, Denver, CO), CsgA (provided by Matthew Chapman, University of Michigan, Ann Arbor, MI), and β_2-microglobulin (β_2m, provided by Sheena Radford, University of Leeds, Leeds, UK) were deposited on nitrocellulose (5–30 ng), and probed with the Aβ12–21 and Aβ33–42 grafted antibodies (0.5 μM). (*B*) Atomic force microscopy images (5×5 μm except 10×10 μm for Tau and 2×2 μm for β_2m) of fibrils of Aβ42, IAPP, Tau, CsgA, and β_2m.

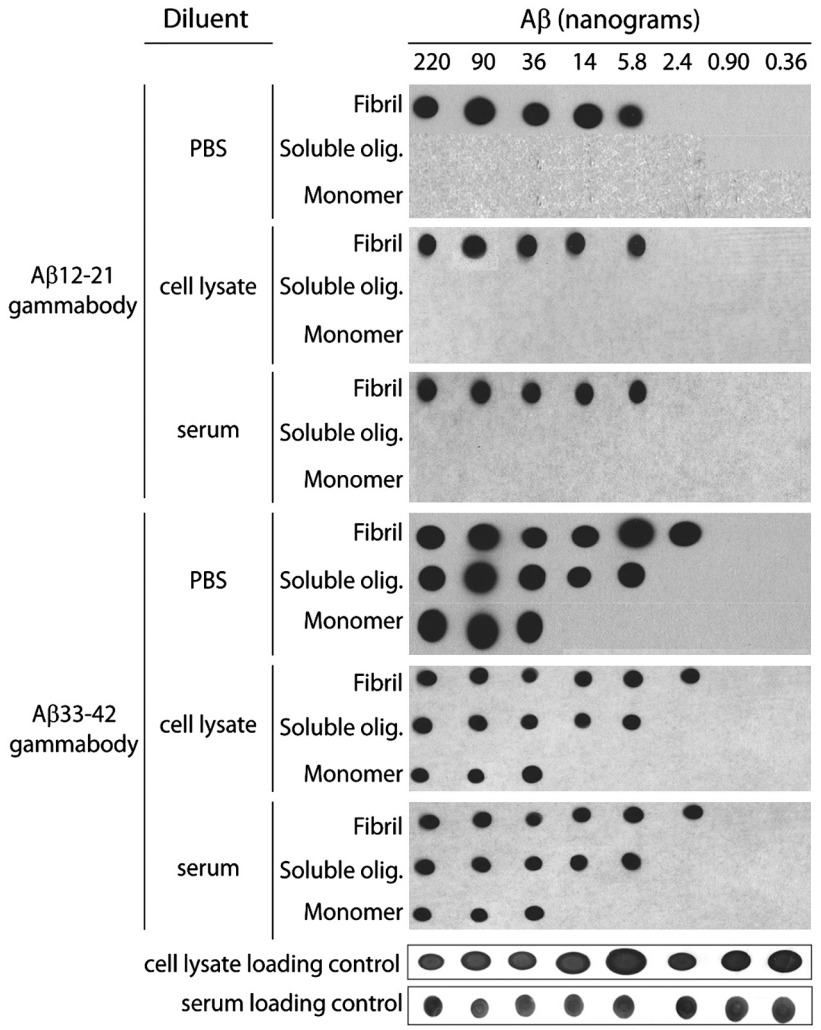

Fig. S10. Detection sensitivity of Aβ gammabodies for Aβ conformers is unchanged in the presence of serum and cell lysate. Aβ42 monomers, soluble oligomers, and fibrils were diluted in a fixed concentration of buffered solution (1:1 PBS), mammalian cell lysate (120,000 PC12 cells/mL) and serum (50% FBS serum; Invitrogen), and deposited on nitrocellulose membranes (0.36–220 ng Aβ42). The blots were probed with Aβ gammabodies (0.5 μM), as well as with Ponceau S (Sigma-Aldrich).