Supporting Information

Ding et al. 10.1073/pnas.1100901108

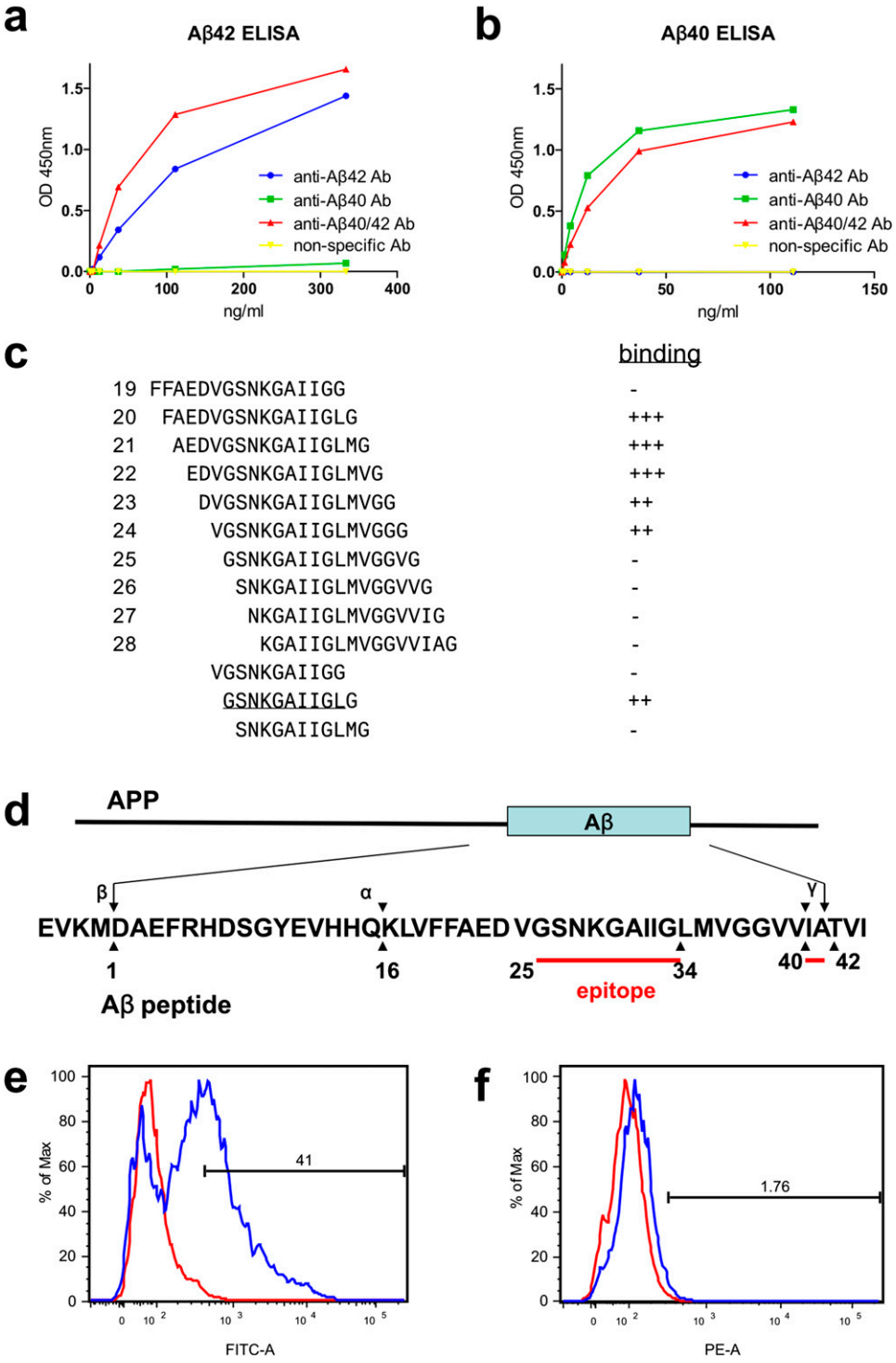

Fig. S1. Characterization of anti-amyloid β (Aβ) 40/42 antibody targeting to the C-terminal region of Aβ peptide. (*A* and *B*) Antibody binding to either Aβ peptide 40 (Aβ40) or 42 (Aβ42) was shown by peptide-specific ELISA with serial dilution of a panel of antibodies: Aβ42-specific antibody in blue, Aβ40-specific antibody in green, Aβ40/42 bispecific antibody in red, and a nonspecific antibody control in yellow. (*C* and *D*) Localization of the minimal epitope for binding of the Aβ40/42 antibody was determined by ELISA using a panel of partially overlapping peptides. +++, strongest signal on ELISA (OD > 2); ++, moderate signal (OD = 1–2); +, modest signal (OD < 1); −, minimal or no signal. The underlined peptide in *C* indicated the minimal required sequence for antibody binding. The relative location of the epitope (underlined in red) within the full-length human amyloid precursor protein (APP) is illustrated in *D*. (*E* and *F*) Cell surface binding of N-terminal antibody, 6E10 (*E*, blue curve), to human APP (hAPP) -transfected 293 cells was significantly above that of a secondary only control (*E*, red curve). There was no appreciable binding of Aβ40/42 antibody (*F*, blue curve) to hAPP-expressing cells compared with a secondary only control (*F*, red curve).

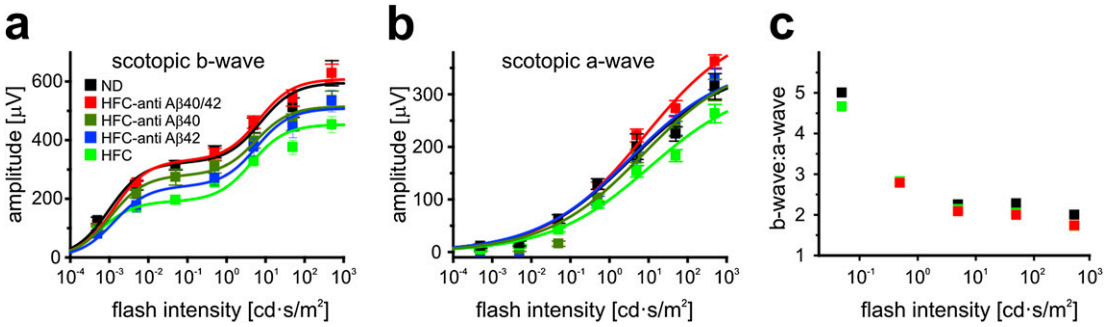

Fig. S2. Visual function is protected in anti-Aβ40/42–treated apolipoprotein E (*APOE4*) high-fat, cholesterol-enriched diet (HFC) mice. (*A* and *B*) Scotopic electroretinogram (ERG) flash responses. (*A*) Stimulus response curves of b-wave amplitudes. Baseline ERGs obtained from normal *APOE4*-normal diet (ND) controls (black, ND) and affected *APOE4*-HFC vehicle-treated controls (green, HFC). b-Wave amplitudes were fully preserved in *APOE4*-HFC mice that received weekly 3 mg/kg i.p. anti-Aβ40/42 antibody injections (red, HFC-anti Aβ40/42), with no significant difference from *APOE4*-ND controls. b-Waves in mice treated with anti-Aβ40 antibody (olive green, HFC-anti Aβ40) during HFC diet exposure are partially protected, which was found previously in a pilot study (1), whereas in the anti-Aβ42–treated mice (blue, HFC-anti Aβ42), there did not seem to be protection from visual function loss compared with normal *APOE4*-ND controls (black) and affected *APOE4*-HFC vehicle-treated controls (green). (*B*) a-Wave amplitudes from control and treated animals as in *A* were determined from the a-wave peaks of ERG responses and plotted as a function of flash intensity. Similar to the trend observed for b-wave amplitudes, mice on HFC diet displayed a reduction in a-wave amplitudes (green) compared with the ND group (black), an effect that was not obvious in the antibody-treated groups. Data are expressed as mean ± SEM. (*C*) The ratios of a- and b-wave amplitudes as a function of flash intensity are plotted for normal control (black), anti-Aβ40/42 antibody-treated (red), and HFC control (green) *APOE4* animals. No significant differences in the ratios were detected between the three groups, indicating that the attenuation in b-wave amplitudes originated in the photoreceptors.

1. Ding JD, et al. (2008) Targeting age-related macular degeneration with Alzheimer's disease based immunotherapies: Anti-amyloid-beta antibody attenuates pathologies in an age-related macular degeneration mouse model. *Vision Res* 48:339–345.

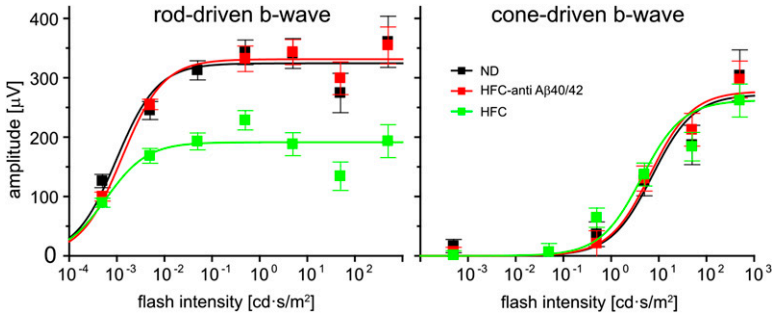

Fig. S3. The rod ERG response is attenuated in the *APOE4*-HFC mouse. The rod- and cone-driven components of the b-wave stimulus-response curves shown in Fig. S2*A* are from *APOE4* mice aged ≥65 wk that were fed a normal diet (black, ND) or a high-fat, cholesterol-enriched diet (green, HFC) and age-matched *APOE4*-HFC mice injected with 3 mg/kg anti-Aβ40/42 (red, HFC–anti-Aβ40/42). Data are expressed as mean ± SEM.

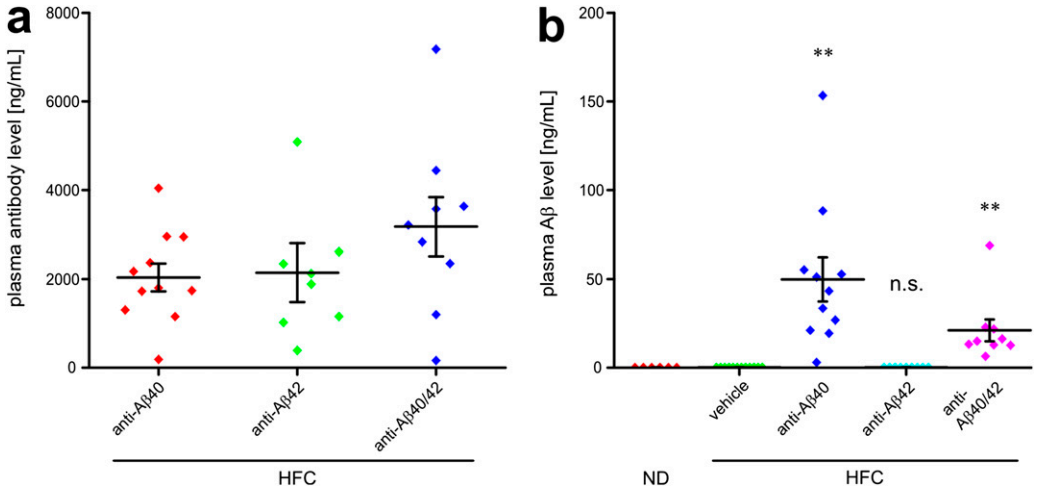

Fig. S4. Plasma levels of Aβ and anti-Aβ antibodies. (*A*) Plasma levels of three anti-Aβ antibodies (nanograms per milliliter) 3 d after the last injection were quite similar to one another (one-way ANOVA; $P = 0.23$). (*B*) Plasma levels of total (bound/unbound) Aβ in all groups were analyzed by unpaired Student *t* test with Welch's correction. Consistent with the peripheral sink hypothesis, plasma from animals treated with anti-Aβ40 (**$P = 0.0026$) or anti-Aβ40/42 (**$P < 0.0096$) antibodies showed increased plasma levels of Aβ40. In contrast, in the anti-Aβ42–treated group, there was no statistically significant elevation in plasma Aβ42 levels (n.s., not significant at $P = 0.32$).

Table S1. Human donor eyes used for retinal pigmented epithelium flat-mount analyses

Donor number	Age (y)	Sex	Death to procurement (h:min)	Cause of death	Ocular diagnosis	Ocular phenotype
03–01	49	M	4:56	Myocardial infarction	Normal	Few small drusen
01–03	77	M	3:30	COPD and respiratory failure	Normal	Few small drusen
03–03	78	F	4:40	Myocardial infarction	AMD	Moderate drusen load
02–05	86	F	8:53	COPD and respiratory failure	AMD	Abundant small drusen
2004–06	77	M	8:20	No data	AMD	Very heavy drusen load
0967–07	75	F	4:39	MI and pneumonia	AMD	Moderate drusen load
2707–09	85	F	3:35	Acute coronary syndrome	Normal	Few small drusen
3024–09	72	F	5:17	Renal failure	Normal	Few small drusen
1276–08	76	M	6:00	CVA	Normal	Few small drusen

M, male; F, female; COPD, chronic obstructive pulmonary disease; AMD, age-related macular degeneration; MI, myocardial infarction; CVA, cerebrovascular accident.

Table S2. Distribution of mice within groups used for testing different anti-Aβ antibodies

Animals (number and genotype)	Diet	Injection*	Group designation
9, *APOE4*	ND	Vehicle injection	*APOE4*-ND
9, *APOE4*	HFC	Vehicle injection	*APOE4*-HFC
9, *APOE4*	HFC	Anti-Aβ42 (7G10-D)	*APOE4*-HFC + anti-Aβ42
12, *APOE4*	HFC	Anti-Aβ40 (2H6-D)	*APOE4*-HFC + anti-Aβ40
12, *APOE4*	HFC	Anti-Aβ40/42 (RN6G)	*APOE4*-HFC + anti-Aβ40/42

*One time per week i.p. injections coinciding with the switch to HFC diet for 8–9 wk.

Table S3. Distribution of mice within groups used for testing anti-Aβ40/42 antibody dose response

Animals (number and genotype)	Diet	Injection*	Dose (mg/kg)	Group designation
11, *APOE4*	ND	Vehicle injection	0	*APOE4*-ND
10, *APOE4*	HFC	Vehicle injection	0	*APOE4*-HFC
11, *APOE4*	HFC	Anti-Aβ40/42 (RN6G)	3	HFC + 3 mg/kg
11, *APOE4*	HFC	Anti-Aβ40/42 (RN6G)	0.3	HFC + 0.3 mg/kg
10, *APOE4*	HFC	Anti-Aβ40/42 (RN6G)	0.03	HFC + 0.03 mg/kg

*One time per week i.p. injections coinciding with the switch to HFC diet for 8–9 wk.