Figure S1

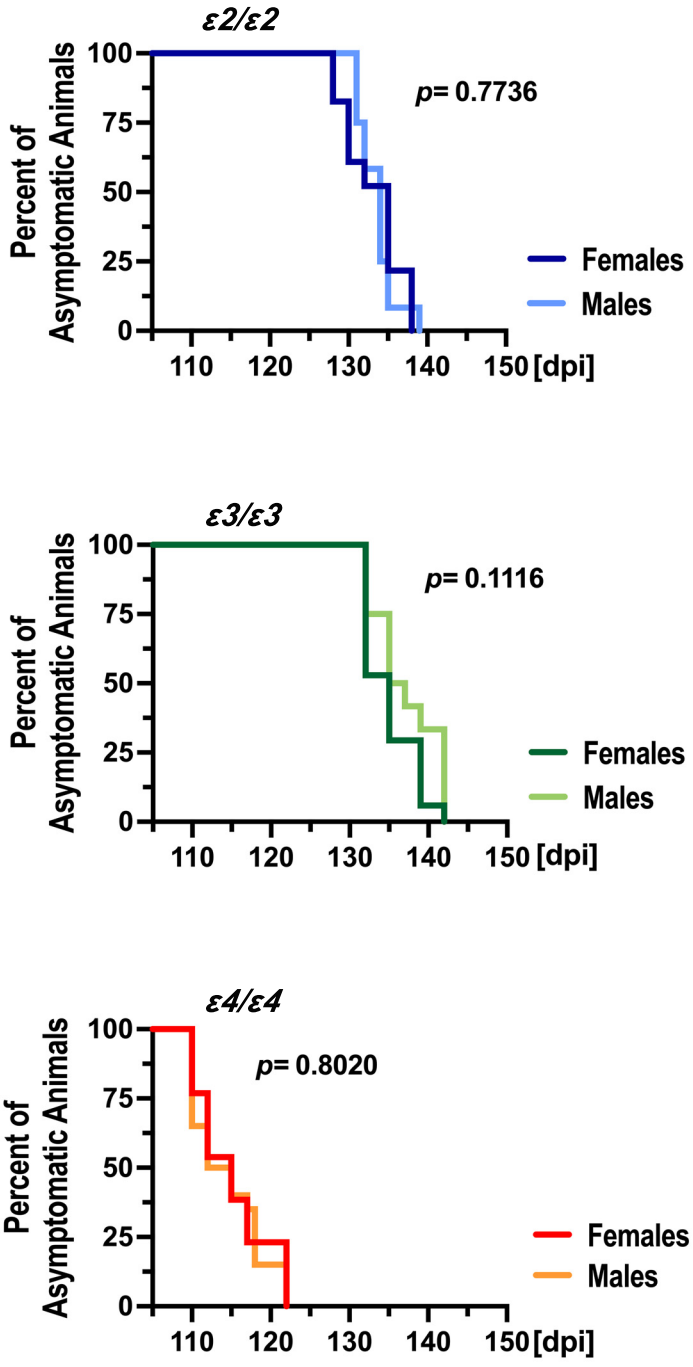

ε2/ε2
Percent of Asymptomatic Animals
100
75
50
25
0
110
120
130
140
150 [dpi]
p= 0.7736
Females
Males
ε3/ε3
Percent of Asymptomatic Animals
100
75
50
25
0
110
120
130
140
150 [dpi]
p= 0.1116
Females
Males
ε4/ε4
Percent of Asymptomatic Animals
100
75
50
25
0
110
120
130
140
150 [dpi]
p= 0.8020
Females
Males

Figure S2

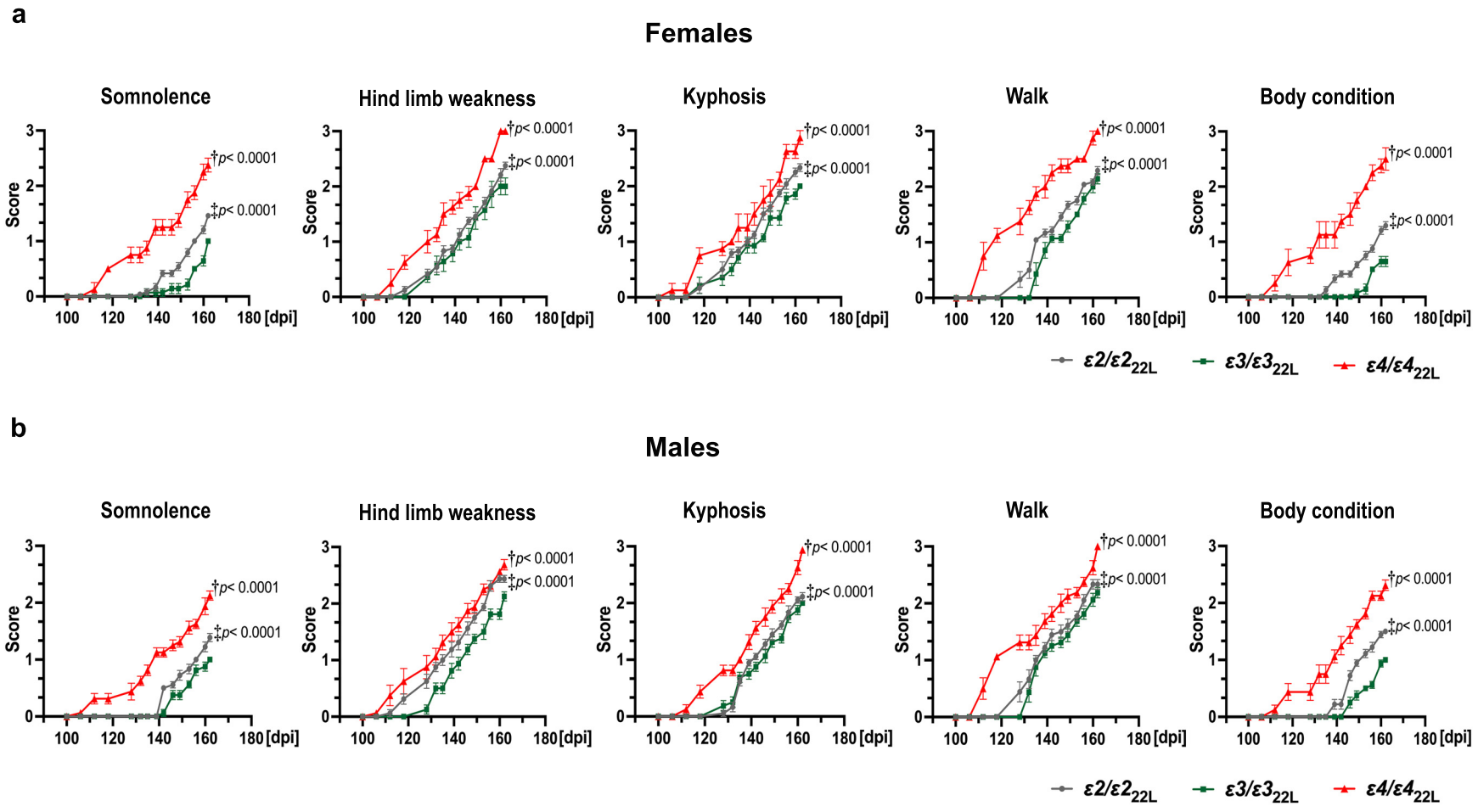

a
Females
Somnolence
Hind limb weakness
Kyphosis
Walk
Body condition
Score
†p< 0.0001
‡p< 0.0001
100 120 140 160 180 [dpi]
ε2/ε2 22L
ε3/ε3 22L
ε4/ε4 22L
b
Males
Somnolence
Hind limb weakness
Kyphosis
Walk
Body condition
Score
†p< 0.0001
‡p< 0.0001
100 120 140 160 180 [dpi]
ε2/ε2 22L
ε3/ε3 22L
ε4/ε4 22L

Figure S3

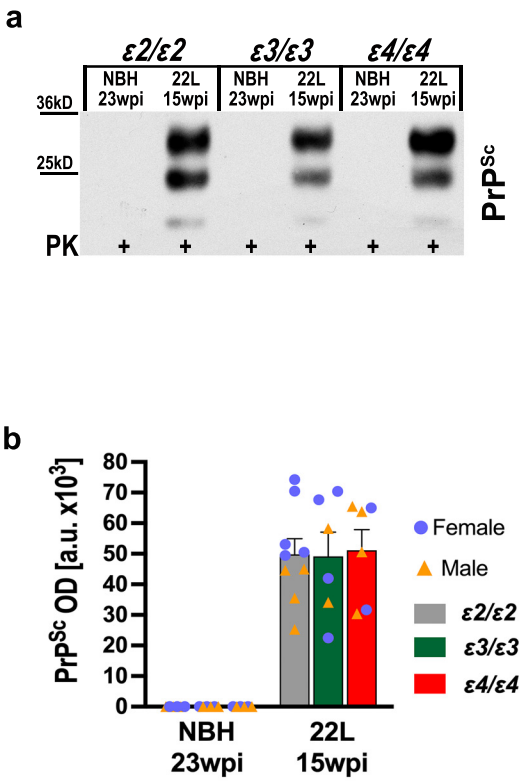
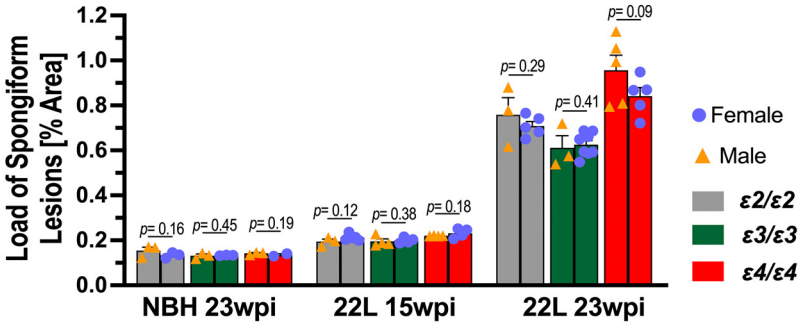

a
ε2/ε2
ε3/ε3
ε4/ε4
NBH 23wpi
22L 15wpi
NBH 23wpi
22L 15wpi
NBH 23wpi
22L 15wpi
36kD
25kD
PK + + + + + +
PrPSc
b
PrPSc OD [a.u. x10³]
80
70
60
50
40
30
20
10
0
NBH 23wpi
22L 15wpi
Female
Male
ε2/ε2
ε3/ε3
ε4/ε4

Figure S4

a


Load of Spongiform Lesions [% Area]
1.2
1.0
0.8
0.6
0.4
0.2
0.0
p= 0.16
p= 0.45
p= 0.19
p= 0.12
p= 0.38
p= 0.18
p= 0.29
p= 0.41
p= 0.09
NBH 23wpi
22L 15wpi
22L 23wpi
Female
Male
ε2/ε2
ε3/ε3
ε4/ε4

b

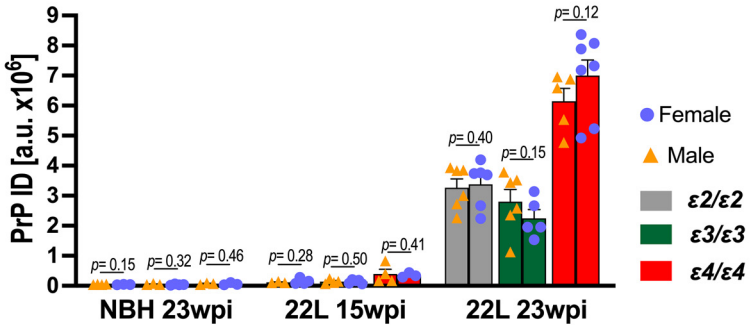
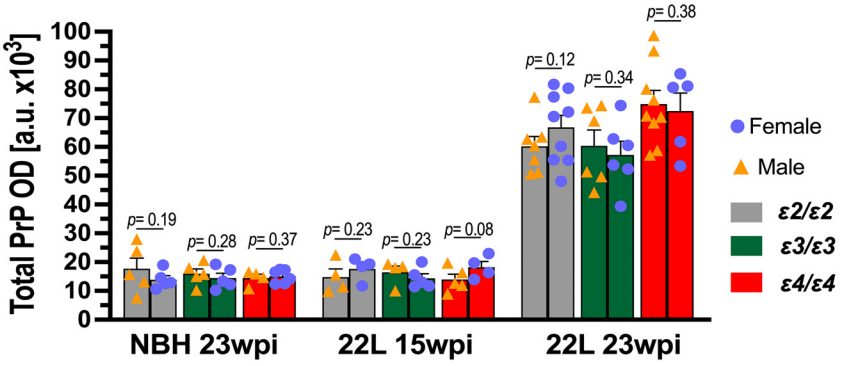
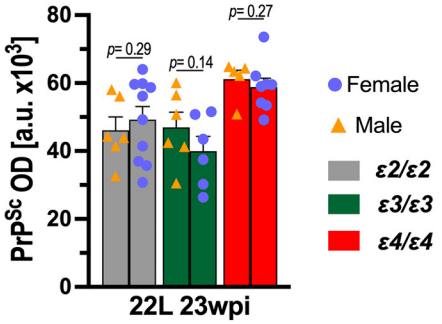

PrP ID [a.u. x10^6]
9
8
7
6
5
4
3
2
1
0
p= 0.15
p= 0.32
p= 0.46
p= 0.28
p= 0.50
p= 0.41
p= 0.40
p= 0.15
p= 0.12
NBH 23wpi
22L 15wpi
22L 23wpi
Female
Male
ε2/ε2
ε3/ε3
ε4/ε4

Figure S5

a



b



c

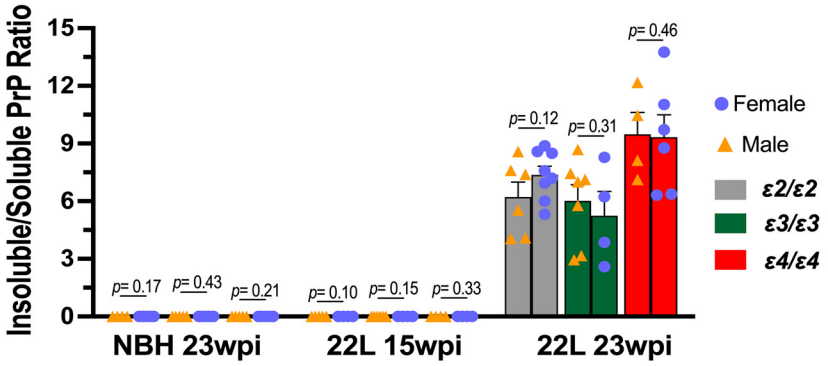


Figure S6

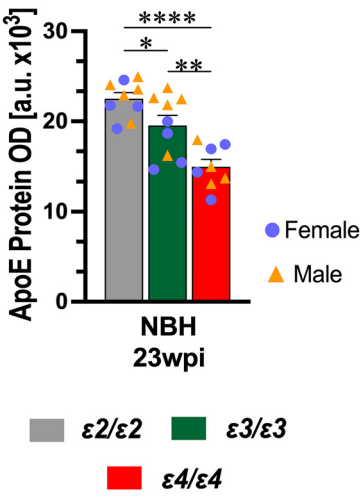

ApoE Protein OD [a.u. x10^3]
30
20
10
0
****
*
**
NBH
23wpi
Female
Male
ε2/ε2
ε3/ε3
ε4/ε4

Figure S7

a

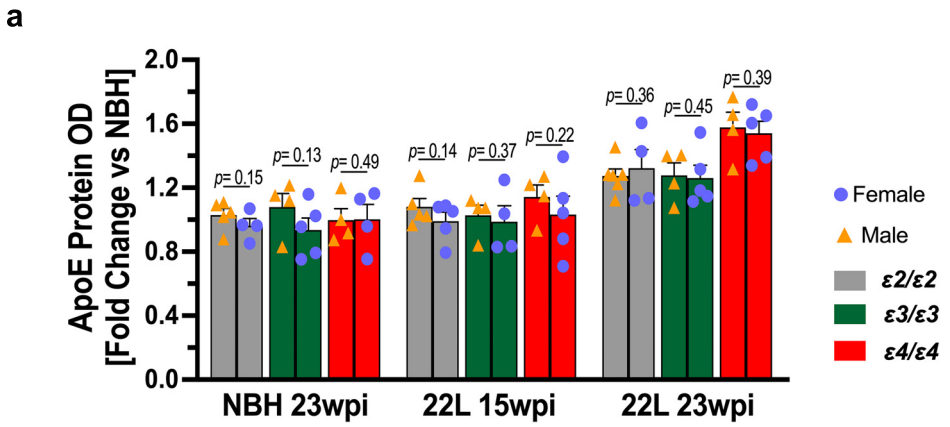
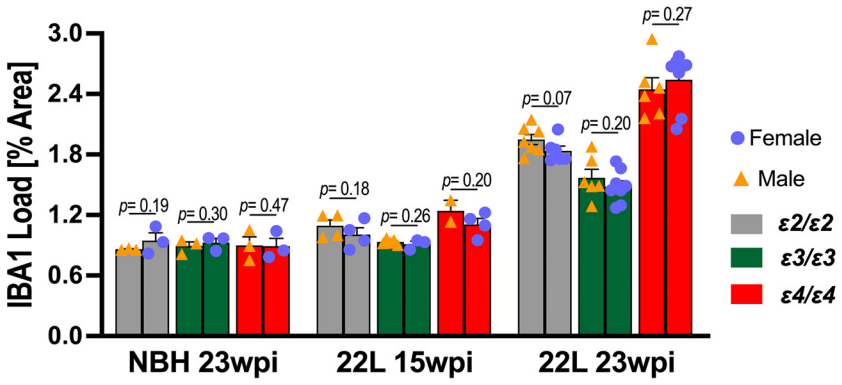

ApoE Protein OD
[Fold Change vs NBH]
2.0
1.6
1.2
0.8
0.4
0.0
p= 0.15
p= 0.13
p= 0.49
p= 0.14
p= 0.37
p= 0.22
p= 0.36
p= 0.45
p= 0.39
NBH 23wpi
22L 15wpi
22L 23wpi
Female
Male
ε2/ε2
ε3/ε3
ε4/ε4

Figure S8

a


IBA1 Load [% Area]
3.0
2.4
1.8
1.2
0.6
0.0
p= 0.19
p= 0.30
p= 0.47
p= 0.18
p= 0.26
p= 0.20
p= 0.07
p= 0.20
p= 0.27
NBH 23wpi
22L 15wpi
22L 23wpi
Female
Male
ε2/ε2
ε3/ε3
ε4/ε4

b

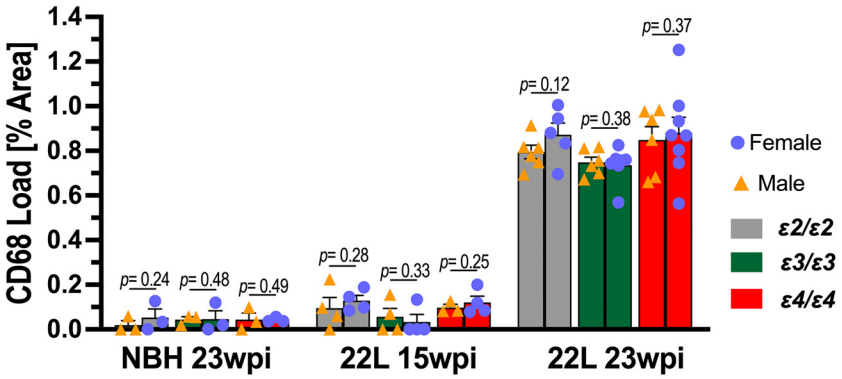
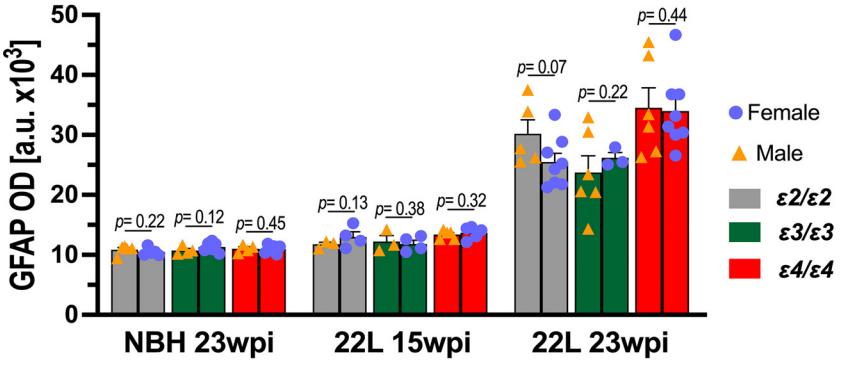
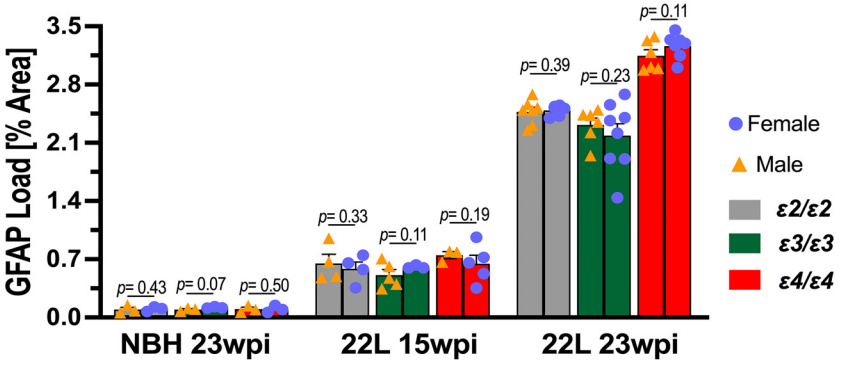

CD68 Load [% Area]
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
p= 0.24
p= 0.48
p= 0.49
p= 0.28
p= 0.33
p= 0.25
p= 0.12
p= 0.38
p= 0.37
NBH 23wpi
22L 15wpi
22L 23wpi
Female
Male
ε2/ε2
ε3/ε3
ε4/ε4

Figure S9

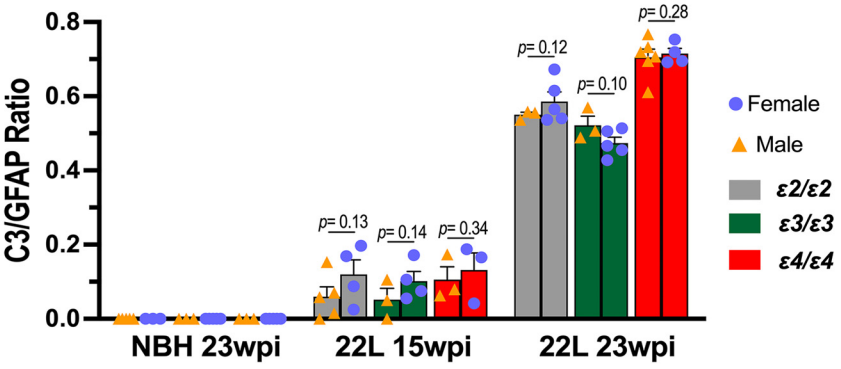
a



b



c



Supplemental Figure Legend

Figure S1. Animal sex does not affect prion disease latency time. Shown are plots of Kaplan-Meier estimates of the prion disease latency time in female and male mice of indicated *APOE* genotypes, which were inoculated with 22L scrapie strain. The x-axis in all graphs displays the number of days post inoculation (dpi). The Kaplan-Meier estimates were derived from 12 to 23 mice per group. *p* values of the Log-Rank test comparing 22L inoculated female and male mice for matching *APOE* genotypes are indicated in the graphs.

Figure S2. *APOE* genotype differentially affects severity and progression of prion disease symptoms. Shown are plots of scorable behavioral characteristics, which contribute to the Total Scrapie Score in 22L infected **(a)** female and **(b)** male mice of indicated *APOE* genotypes. These characteristics include somnolence, hind limb weakness, kyphosis, walk, and body condition and they are scored based on the following criteria: 0 = normal, 1 = subtle, 1.5 = mild, 2 = moderate, 2.5= advanced, and 3 = severe. Their sum makes the Total Scrapie Score shown in Fig. 1a. The x-axis in all graphs displays number of days post inoculation (dpi). Mice were serially assessed starting from the 100th dpi by two independent examiners blinded to the animal genotype. Values represent mean ± SEM from 4 - 12 animals per group. †p< 0.0001 denotes the significance for pairwise comparison between $\varepsilon4/\varepsilon4_{22L}$ and $\varepsilon3/\varepsilon3_{22L}$ or $\varepsilon2/\varepsilon2_{22L}$ animals, while ‡p< 0.0001 for the comparison between $\varepsilon2/\varepsilon2_{22L}$ and $\varepsilon3/\varepsilon3_{22L}$ animals (repeated measures ANOVA).

Figure S3. *APOE* genotype does not affect accumulation of PrP^{Sc} in the lymphoreticular system in prion infected animals. (a) Immunoblot analysis of proteinase K (PK) resistant PrP^{Sc} and **(b)** densitometric quantification of PrP^{Sc} band optical densities (OD) in the spleen homogenate in mice of indicated *APOE* genotypes, which were infected with 22L scrapie strain and killed 15 weeks post inoculation (wpi). Included are PK-treated samples of spleen

homogenate from animals inoculated with normal brain homogenate (NBH), which show no evidence for the PK-resistant PrP^{Sc} conformer. **(b)** Values represent mean + SEM from 6 to 9 mice per group along with data points for single female and male animals. p= 0.9779 (one-way ANOVA).

Figure S4. Animal sex does not significantly affect pathology burden in prion disease Shown is comparison of **(a)** the spongiform lesion load in the M1 cortex and **(b)** values of the integrated density (ID) of anti-PrP immunostaining in the S1 cortex between female and male mice for matching *APOE* genotype, inoculum, and survival time. Unpaired *t*-test with Welch's correction was used to test for inter-sex significance. Values represent mean + SEM from 2 to 8 animals per group along with data points for single female and male animals. *t*-test values are shown directly above the groups compared.

Figure S5. Animal sex does not influence brain levels of the PrP protein and PrP solubility. Shown is comparison of the protein band optical density (OD) values for **(a)** the total PrP protein, **(b)** the PrP^{Sc} conformer, and **(c)** the insoluble / soluble PrP ratio between female and male mice for matching *APOE* genotype, inoculum, and survival time. Unpaired *t*-test with Welch's correction was used to test for inter-sex significance. Values represent mean + SEM from 4 to 10 animals per group along with data points for single female and male animals. *t*-test values are shown directly above the groups compared.

Figure S6. *APOE* genotype differentially affects apoE protein level in the brain in non-infected animals. Shown is densitometric quantification of apoE protein band optical densities (OD) in the brain homogenate, in mice of indicated *APOE* genotypes, which were inoculated with normal brain homogenate (NBH). Corresponding immunoblot analysis of the apoE protein is presented in Figure 4a. Shown are mean values + SEM from 8 to 9 mice per *APOE* genotype

along with data points for single female and male animals. $p < 0.0001$ (one-way ANOVA); *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$ (Holm's-Sidak's post hoc test).

Figure S7. Animal sex does not influence changes in the apoE protein level during prion infection. Shown is comparison of the apoE protein band optical density (OD) values between female and male mice for matching *APOE* genotype, inoculum, and survival time. Unpaired *t*-test with Welch's correction was used to test for inter-sex significance. Values represent mean + SEM from 4 to 6 animals per group along with data points for single female and male animals. *t*-test values are shown directly above the groups compared.

Figure S8. Animal sex does not significantly affect microglia activation during prion infection. Shown is comparison of **(a)** IBA1 and **(b)** CD68 positive microglia load in the S1 somatosensory cortex between female and male mice for matching *APOE* genotype, inoculum, and survival time. Unpaired *t*-test with Welch's correction was used to test for inter-sex significance. Values represent mean + SEM from 3 to 8 animals per group along with data points for single female and male animals. *t*-test values are shown directly above the groups compared.

Figure S9. Animal sex does not significantly affect prion-related astrogliosis. Shown is comparison of **(a)** GFAP band optical densities (OD) values, **(b)** GFAP positive astrocyte load in the S1 somatosensory cortex, and **(c)** the C3/GFAP load ratio between female and male mice for matching *APOE* genotype, inoculum, and survival time. Unpaired *t*-test with Welch's correction was used to test for inter-sex significance. Values represent mean + SEM from 3 to 8 animals per group along with data points for single female and male animals. *t*-test values are shown directly above the groups compared.