A conformational switch controlling the toxicity of the prion protein

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Prion infections cause conformational changes of the cellular prion protein (PrPC) and lead to progressive neurological impairment. Here we show that toxic, prion-mimetic ligands induce an intramolecular R208-H140 hydrogen bond ('H-latch'), altering the flexibility of the α2-α3 and β2-α2 loops of PrPC. Expression of a PrP2Cys mutant mimicking the H-latch was constitutively toxic, whereas a PrP207A mutant unable to form the H-latch conferred resistance to prion infection. High-affinity ligands that prevented H-latch induction repressed prion-related neurodegeneration in organotypic cerebellar cultures. We then selected phage-displayed ligands binding wild-type PrPC, but not PrP2Cys. These binders depopulated H-latched conformers and conferred protection against prion toxicity. Finally, brain-specific expression of an antibody rationally designed to prevent H-latch formation prolonged the life of prion-infected mice despite unhampered prion propagation, confirming that the H-latch is an important reporter of prion neurotoxicity.

The neurotoxicity of prions requires the interaction of the misfolded prion protein PrP<sup>Sc</sup> with its cellular counterpart PrPC (ref. 1), which ultimately leads to depletion of the PIKfyve kinase<sup>2</sup> and to spongiform encephalopathy. Prion toxicity is initiated by unknown mechanisms that require membrane-bound PrPC (refs. 1,2). PrPC is a glycosylphosphatidylinositol (GPI)-anchored protein composed of an amino-terminal, unstructured 'flexible tail' (FT) and a carboxy-terminal, structured 'globular domain' (GD).<sup>3</sup> Mice lacking the protein gene Prnp do not succumb to prion diseases.<sup>4</sup> Antibodies binding the globular domain (GD) of PrP<sup>Sc</sup> can halt this process,<sup>5</sup> but they can also activate toxic intracellular cascades.<sup>6–9</sup> Similar events occur in prion-infected brains, and substances that counteract the damage of infectious prions can also alleviate the toxicity of anti-PrP<sup>C</sup> antibodies, such as POM1 (ref. 4). POM1 exerts its toxicity without inducing the formation of infectious prions,<sup>10</sup> arguing that toxicity is independent of prion replication. Accordingly, toxicity can very effectively be prevented by the therapeutic co-stabilization of FT and GD through bispecific antibodies.<sup>4,11,12</sup> These findings suggest that POM1 and prions exert their toxicity through similar mechanisms.

To explore the causal links between the binding of POM1 to PrP<sup>C</sup> and its neurotoxic consequences, we performed structural and molecular studies in silico, in vitro and in vivo. We found that the induction of an intramolecular hydrogen bond between R208 and H140 of the globular domain of human PrP<sup>C</sup> (hPrP<sup>C</sup>) is an early molecular reporter of prion toxicity.

**Results**

POM1 introduces an intramolecular hydrogen bond in PrP<sup>C</sup>-GD. Structural analysis and molecular dynamics (MD) simulations indicated that POM1 induces an intramolecular hydrogen bond in both human and murine PrP<sup>C</sup> between R208 and H139 in murine PrP<sup>C</sup> (ref. 12). This ‘H-latch’ constrains the POM1 epitope while sterically increasing the flexibility of the β2-α2 and α2–α3 loops (Fig. 1 and Extended Data Fig. 1). To explore its role in prion toxicity, we generated a murine PrP<sub>D207A</sub> mutant that prevents the H-latch without altering the conformation of PrP<sup>C</sup> (Extended Data Fig. 1). We stably expressed murine PrP<sub>D207A</sub> (mPrP<sub>D207A</sub>) in Prnp<sup>−/−</sup> CAD5 cells<sup>11</sup> and Prnp<sub>210/219</sub> cerebellar organotypic cultured slices (COCS; Fig. 2a–c and Extended Data Fig. 2a–c). A panel of conformation-specific anti-PrP antibodies showed similar staining patterns for PrPC and mPrP<sub>D207A</sub>, confirming that both proteins folded properly but had reduced POM1 binding (Extended Data Fig. 2a,c), as expected from the structure of PrP–POM1 co-crystals<sup>12</sup>. Prnp<sup>−/−</sup> CAD5 cells expressing mPrP<sub>D207A</sub> were resistant to POM1 toxicity and, notably, showed impaired prion replication (Fig. 2d–f), pointing to common toxic properties.

Lack of the H-latch confers resistance to prion and POM1 toxicity. To test whether its presence can induce toxicity even in the absence of ligands, we designed an R207C-I138C double-cysteine PrP<sup>C</sup> mutant (PrP<sub>2Cys</sub>)<sup>Fig. 3a,b</sup>, with the goal of replicating the structural effects of the H-latch in the absence of POM1 binding. Nuclear magnetic resonance (NMR) and MD analysis of recombinant mPrP<sub>D207A</sub><sup>Cys</sup> were consistent with a folded protein resembling the
H-latch conformation (Fig. 3a–c). PrP\textsuperscript{2Cys} expressed in a Prnp\textsuperscript{–/–} CAD5 cell line showed correct glycosylation and topology and did not trigger unfolded protein responses (Extended Data Fig. 3b,c). Surface-bound PrP\textsuperscript{2Cys} was detected by POM8 and POM19, which bind to a conformational epitope on the α1–α2 and β1–α3 regions, respectively\textsuperscript{7}, but not by POM1 (Extended Data Fig. 2d,e). The POM1-induced H-latch allosterically altered the β2–α2 loop; similarly, binding of mPrP\textsuperscript{2Cys} to POM5 (recognizing the β2–α2 loop) was impaired (Extended Data Fig. 2a). Taken together, these results suggest that mPrP\textsuperscript{2Cys} adopts a conformation similar to that induced by POM1 (Fig. 3c).

We transduced Prnp\textsuperscript{ZH3/ZH3} COCS with adeno-associated virus-based vectors (AAV) expressing either PrPC or PrP\textsuperscript{2Cys}. Wild-type and mutant proteins showed similarly robust expression levels (Extended Data Fig. 3d). COCS expressing mPrP\textsuperscript{2Cys} developed spontaneous, dose-dependent neurodegeneration 4 weeks after transduction (Fig. 3d–f and Extended Data Fig. 3e,f), suggesting that induction of the H-latch is sufficient to generate toxicity. In agreement with this view, MD simulations showed that human, hereditary PrP mutations responsible for fatal prion diseases favor H-latch formation and altered flexibility in the α2–α3 and β2–α2 loops (Extended Data Fig. 4).

‘Pomologs’ rescue prion-induced neurodegeneration. If POM1 toxicity requires the H-latch, antibody mutants that are unable to induce it should be innocuous. POM1 immobilizes R208 by salt bridges with its heavy-chain (hc) residue hcD52, whereas hcY104 contributes to the positioning of H140 (Fig. 1a). To prevent H-latch formation, we thus replaced eleven of these residues with alanine. For a control, we similarly substituted interface residues that are predicted to have no impact on R208. Resulting ‘pomologs’ were produced as single-chain variable fragments (scFv), three of which retained high affinity, that is a dissociation constant (K\textsubscript{D}) of about 10 nM, for PrP\textsuperscript{C} (Table 1 and Extended Data Fig. 5).
As expected, all pomologs were innocuous to Prnp<sup>ZH1/ZH1</sup> COCS not expressing PrP<sup>C</sup> (ref. 5) (Extended Data Fig. 6a and Supplementary Fig. 1a). hC<sub>Y104A</sub> reduced H-latch formation, according to MD simulations (Fig. 1b and Supplementary Fig. 2) and exerted no neurotoxicity onto COCS from tga20 mice overexpressing PrP<sup>C</sup> (ref. 16), whereas POM1 and all H-latch inducing mutants (hD<sub>52A</sub>, hY<sub>101A</sub> and all light-chain pomologs) were neurotoxic (Fig. 4a and Extended Data Fig. 6b). As with POM1, the toxicity of pomologs required PrPC, featured neuronal loss, astrogliosis and elevated levels of microglia markers (Extended Data Fig. 6c and Supplementary Fig. 1b), and was ablated by co-administration of the antibody POM2, which targets the flexible tail (FT) of PrPC.
Additionally, h\textsuperscript{b}Y104A inhibited POM1 toxicity (Extended Data Fig. 6d). POM1 does not induce de novo prions\textsuperscript{19} but triggers similar neurotoxic cascades\textsuperscript{8}, plausibly by replicating the docking of prions to PrP\textsuperscript{C}. If so, h\textsuperscript{b}Y104A may prevent the neurotoxicity of both POM1 and prions by competing for their interaction with PrP\textsuperscript{C}. Indeed, h\textsuperscript{b}Y104A protected RML6 and 22L prion-inoculated tga20 and C57BL/6 COCS from prion neurodegeneration (Fig. 4b–d and Extended Data Fig. 6g–i), repressed the vacuolation of chronically prion-infected cells (Fig. 4e and ref. 1\textsuperscript{)} and diminished PrP\textsuperscript{Sc} levels ex vivo (Fig. 4f). In contrast to other antiprion antibodies\textsuperscript{17}, h\textsuperscript{b}Y104A did not reduce levels of PrP\textsuperscript{C} (Fig. 4g), corroborating the conjecture that neuroprotection results from interfering with the docking of incoming prions.

The antibody ICSM18 was found to ameliorate prion toxicity in vivo\textsuperscript{18}, although dose-escalation studies have shown conspicuous neuronal loss\textsuperscript{9}. The ICSM18 epitope is close to that of POM1 (ref. 12), and MD simulations indicated that it facilitates the R208-H140 interaction, albeit less so than POM1 does (Fig. 1c).
Table 1 | a, Computational alanine scanning indicates which residues of POM1 and PrP contribute to binding. Positive numbers in the third column suggest loss of binding energy. b, On the basis of these results (Table 1a), we prepared 11 single mutations of POM1 (in each CDR loop) as scFv constructs. Colors (yellow to red) visualize the impact on binding affinity. The mutated residues are shown as sticks on the cartoon POM1 structure in Extended Data Figure 5b.

### Table 1

| Res N° | chain | ΔG(complex) |
|--------|-------|-------------|
| 32     | L     | 0.44        |
| 50     | L     | 1.96        |
| 91     | L     | 2.09        |
| 92     | L     | 0.27        |
| 93     | L     | 0.28        |
| 94     | L     | 1.39        |
| 96     | L     | 0.35        |
| 33     | H     | 2.88        |
| 52     | H     | 2.47        |
| 54     | H     | -0.02       |
| 55     | H     | 1.37        |
| 57     | H     | 1.76        |
| 59     | H     | -0.01       |
| 101    | H     | 0.59        |
| 103    | H     | -0.03       |
| 104    | H     | 4.91        |
| 139    | A     | -0.01       |
| 146    | A     | -0.01       |
| 138    | A     | 0.11        |
| 143    | A     | -0.21       |
| 145    | A     | 0.31        |
| 141    | A     | 0.39        |
| 212    | A     | 0.78        |
| 140    | A     | 0.93        |
| 147    | A     | 1.9         |
| 144    | A     | 3.44        |
| 208    | A     | 3.86        |

### Table 2

| Protein | $k_1$ (1/Ms) | $k_2$ (1/s) | $K_o$ (nM) |
|---------|--------------|-------------|------------|
| POM1    | 6.4 x 10^-4  | 3.1 x 10^-4 | 4.8        |
| hcW33A  | no binding   | no binding  | no binding |
| hcD52A  | 3.3 x 10^-5  | 4.6 x 10^-2 | 103        |
| hcD55A  | 1 x 10^-5    | 3.7 x 10^-2 | 372        |
| hcY57A  | 7.7 x 10^-4  | 3.1 x 10^-2 | 406        |
| hcY101A | 6.3 x 10^-4  | 7.1 x 10^-4 | 11         |
| hcY104A | 3.6 x 10^-4  | 2.1 x 10^-4 | 8.8        |
| lcS32A  | 6.4 x 10^-4  | 5.9 x 10^-4 | 10         |
| lcY50A  | 5.7 x 10^-4  | 8.2 x 10^-3 | 313        |
| lcS91A  | 1.3 x 10^-4  | 1.8 x 10^-2 | 1430       |
| lcW94A  | 3.1 x 10^-5  | 2.7 x 10^-2 | 201        |
| lcY96A  | 6.2 x 10^-4  | 7.4 x 10^-3 | 123        |

**Figure**: Cartoon representations of POM1 and PrP with sticks indicating the mutated residues. The mutated residues are color-coded based on the ΔG values, with yellow indicating a decrease in binding affinity and red indicating an increase. The figures show denaturation and folding processes, illustrating how mutations affect the stability and conformation of the proteins. The diagrams are designed to help visualize the impact of mutations on the structural and functional properties of the proteins, providing insights into their role in binding and other biological processes.
Antibody binding causes conformational changes in GD and FT. Protective pomolog hY104A failed to induce the H-latch, which was induced by toxic mutations (Fig. 1c and Extended Data Fig. 1). MD simulations showed that POM1 rigidified its epitope but increased the flexibility of α2–α3 and β2–α2 loops (Fig. 1c). Conversely, the conformation of PrP attached to the protective hY104A resembled that of free PrP. In accordance with MD simulations, NMR spectra, which are sensitive to local effects and transient populations, of rmPrP90–231 in complex with POM1 revealed long-range alterations in the GD and in the adjacent FT (Fig. 5a). When bound to hY104A instead, rmPrP90–231 elicited spectra similar to those of free PrP. Circular-dichroism (CD) spectroscopy showed that the full rmPrP (rmPrP90–231)–POM1 complex had more irregular structure content than its free components (Fig. 5b), whereas no difference was observed when POM1 was complexed to partially FT-deficient rmPrP90–231. We did not observe any changes in the secondary structure of the hY104A-bound rmPrP23–231 complex. This suggests that POM1 can alter the FT conformation with two possible mechanisms. Either the secondary structure of the FT itself is changed, probably through a shift in the population of conformers (FT-changes), or the...
secondary structure of the GD is altered in a FT-dependent manner, with FT-GD interactions stimulated by POM1 binding. Hence H-latch induction leads to subtle alterations of the structure of both GD and FT, whose presence correlates with toxicity.

We performed animal experiments to confirm that (1) hcY104A by itself is not neurotoxic in vivo, in contrast to POM1, and (2) it protects from prion-dependent neurodegeneration. When produced as IgG holoantibody, hcY104A exhibited subnanomolar affinity to full-length, murine, recombinant PrP (rmPrP23–231, Supplementary Fig. 2). We injected POM1 or holo-hcY104A into the hippocampus of C57BL/6 mice. Histology and volumetric-diffusion-weighted magnetic resonance imaging showed that POM1 (6µg) elicited massive neurodegeneration that was repressed by pre-incubation with recPrP in threefold molar excess, whereas the same amount of holo-hcY104A did not elicit any tissue damage (Fig. 6a–g and Extended Data Figs. 7 and 8). A benchmark dose analysis yielded an upper safe-dose limit of ≥12µg for intracerebrally injected holo-hcY104A (Extended Data Fig. 8a). This indicates that the FT changes conformation upon POM1 binding. Conversely, no differences were detected with the protective pomolog hcY104A.

**Fig. 5** | Antibody binding causes allosteric conformational changes in the GD and FT. 

**a.** Comparison between the [15N,1H]-TROSY spectra of free rmPrP90–231 versus that bound to the hcY104A pomolog. Chemical-shift differences, reflecting subtle alterations of the local chemical structure, were visible not only in the epitope but also at distant sites in the GD and FT. Residues affected by antibody binding are in color on PrPC (GD and part of the FT are shown on a MD model of PrP). Differences between toxic and protective antibodies are evident in the α2–α3 loop (the Y104A complex is identical to free PrPc) and in the FT region closer to the GD. 

**b.** Content of secondary structure estimated from CD spectra of the rmPrP–pomologs complexes. ’Calculated’ indicates the secondary structure content if the rmPrP and pomolog did not change upon binding. POM1 displayed increased content of irregular structure (measured versus calculated) when in complex with full rmPrP23–231, but identical content when in complex with a construct lacking the FT (rmPrP90–231). This indicates that the FT changes conformation upon POM1 binding. Conversely, no differences were detected with the protective pomolog hcY104A.

We then transduced tga20 mice with hcY104A by intravenous injection of a neurotropic AAV-PHLP vector. Two weeks after AAV injection, mice were inoculated intracerebrally with 3 × 10^5 ID_{50} units of RML6 prions. hcY104A expression levels correlated with both survival times and PrP_{Sc} deposition (Fig. 6h–j), suggesting that hcY104A acts downstream of prion replication.

**Phage displayed antibody fragments confer neuroprotection.** If the same toxic PrP conformation is induced by both the H-latch and infectious prions, anti-PrP antibodies unable to bind the H-latch conformers could depopulate them by locking PrP_{C} in its innocuous state, thus preventing prion neurotoxicity. Using phage display (Extended Data Fig. 9a), we generated four antigen-binding fragments (Fabs), three of which bound the globular domain of PrP_{C} preferentially over PrP_{Sc}, with one binding PrP and PrP_{Sc} similarly (Fig. 7a and Extended Data Fig. 9b). When administered to
**Fig. 6 | The holo-IgG antibody **\(^{14}Y104A\) **is innocuous after intracerebral injection.**

(a) Representative magnetic resonance diffusion-weighted images (DWI) 24 hours after stereotactic injection of holo-\(^{14}Y104A\) (left). Contralateral injections of holo-\(^{14}Y104A\) + rmPrP\(_{23-231}\) (right). A small area of hyperintensity was found in one mouse after injection of 12 \(\mu\)g holo-\(^{14}Y104A\) (white arrowhead). White asterisks: needle tract.

(b) Hematoxylin and eosin (HE)-stained sections from mice shown in a. Asterisks: needle tract. Rectangles denote regions magnified in c, HE sections (CA4). Left, holo-\(^{14}Y104A\) injections (6, 9 and 12 \(\mu\)g). Right, holo-\(^{14}Y104A\) + rmPrP\(_{23-231}\). Asterisk (9 \(\mu\)g): neurons with hyper eosinophilic cytoplasm and nuclear condensation in the vicinity of the needle tract. Asterisk (12 \(\mu\)g): These neurons were diffusely distributed among numerous healthy neurons. White arrowhead: vacuoles indicative of edema along the needle tract.

d) DWI images of 6 \(\mu\)g holo-POM1 ± rmPrP\(_{23-231}\), revealing a hyperintense signal at 24 hours. e) HE-stained section from a mouse shown in d. Asterisks: needle tract. Rectangles: areas in f.

(f) HE sections (CA4). Holo-POM1 injections revealed damaged neurons with condensed chromatin and hyper eosinophilic cytoplasm.

g) Volumetric quantification of lesions on DWI imaging 24 hours after injection revealed no significant lesion induction by holo-\(^{14}Y104A\). One datapoint corresponds to an animal. \(P\) values are adjusted for multiple comparisons. n.s.: not significant, \(P > 0.05\), ordinary one-way ANOVA with Šidák's multiple comparisons test.

(h) Antibody expression levels, as determined by Myc-Tag western blot, showed a positive correlation with survival. One datapoint corresponds to one animal. Pearson correlation coefficient \(r = 0.72\), 95% confidence interval 0.099–0.94, \(P = 0.03\). a.u., arbitrary units.

(i) Significant correlation of PrP\(^{Sc}\) and antibody expression levels (representative images depicted in j, aggregated correlation across all brain regions). Different colors represent 3 brain regions from 9 independent animals. Pearson correlation coefficient \(r = 0.53\), 95% confidence interval 0.18–0.76, \(P = 0.0048\). j) Representative images from quantification of i. Sagittal brain sections stained with SAF84, highlighting PrP\(^{Sc}\), and basal ganglia immunofluorescent micrographs marking \(^{14}Y104A\)-Myc-tag. Scale bar SAF84: 1 mm. Scale bar \(^{14}Y104A\)-Myc-tag: 500 \(\mu\)m.
prion-infected tga20 COCS, FabA10 and FabD9 decreased prion neurotoxicity, whereas FabE2, which binds both PrPC and mPrP2Cys, had no beneficial effect (Fig. 7b,c). NMR epitope mapping followed by computational docking and MD showed that FabA10 binds to PrP encompassing the H-latch and partially overlapping with the POM1 epitope (Fig. 7d,e and Extended Data Fig. 10). MD showed that the H-latch is not stable in the presence of FabA10, even if the simulations were started from a POM1-bound PrP conformation with the R208-H140 H-bond present (Extended Data Fig. 10a).

Discussion

In summary, the evidence presented here suggests that H-latch formation is an important feature of prion toxicity. The H-latch was induced by the toxic anti-PrP antibody POM1, PrP mutants unable to form the H-latch conferred resistance to POM1 toxicity, and a PrP mutant mimicking the H-latch was constitutively neurotoxic. Conversely, POM1 mutants retaining their affinity and epitope specificity, but abolishing H-latch formation, proved to be neuroprotective. We observed that formation of the H-latch and its structural effects on PrPC-GD were not only innocuous, but also protective against prion neurotoxicity in vitro and in vivo. The MD predictions were confirmed in vivo using both cerebellar slice cultures and mouse models of prion disease. POM1 mutants or other rationally selected Fabs that were unable to induce the H-latch protected from the deleterious effects of prion infection ex vivo and in vivo. Furthermore, hereditary PrP mutations leading to human prion disease are associated with the absence of the H-latch (12) and this study is consistent with the hypothesis that the H-latch is a common feature of prion neurotoxicity.

Fig. 7 | Phage-displayed antibody fragments differentially binding wild-type PrP, but not PrP2Cys, confer neuroprotection. a, Preferential binding of the selected Fabs to rmPrP23–231 over rmPrP2Cys. With the exception of FabE2, the Fabs show higher apparent affinity for rmPrP23–231 than rmPrP2Cys. One datapoint corresponds to the mean ± s.e.m. of two technical replicates. The experiment was repeated twice. b, FabA10 and FabD9 conferred neuroprotection in prion-infected tga20 COCS. c, Quantification of NeuN fluorescence intensity from b, expressed as percentage of untreated (–) NBH. Scale bar: 500µm. One datapoint corresponds to an independent, organotypic cultured slice. Two-way ANOVA with Dunnett’s multiple comparison test, P values are adjusted for multiple testing: RML untreated (–) versus RML A10: P = 0.006, RML untreated (–) versus RML D9: P = 0.009, **P < 0.01, n.s.: not significant, P > 0.05. d, Structure of PrP (white) in complex with FabA10 (violet) obtained by NMR-validated docking and MD. mPrP90–231 residues whose NMR signal is affected by FabA10 binding are colored blue; residues with no NMR information are gray; residues mutated to Cys are yellow. e, There is partial overlap (green) between the epitopes of POM1 (red) and FabA10 (blue). The 2Cys are in yellow. PrP23–231 is depicted in different orientations in d and e.
diseases favor the H-latch, according to MD simulations. These observations suggest that the H-latch is not only involved in the toxicity of anti-PrP antibodies, but also in the pathogenesis of prion diseases.

Spongiform change, that is endolysosomal hypertrophy through UPR activation and subsequent PIKfyve depletion, is shared in both prion and POM1 toxicity. Multiple toxic cascades are activated in prion infections and in cells treated with POM1 (ref. 18). Cells that stably express PrP\textsuperscript{Cys} are not affected by UPR in the current experimental paradigm, suggesting that either the protein dosage is insufficient to observe UPR or its toxicity is independent of PIKfyve depletion. Besides neuronal loss, which is shared among prion, POM1 and PrP\textsuperscript{Cys} toxicity, it will be interesting to investigate the overlap of toxic cascades between the different prion disease models, which could provide important knowledge of early disease-associated changes.

The above findings hold promise for therapeutic interventions. First, the POM1 binding region includes a well-defined pocket created by the α₁–α₃ helix of PrP\textsuperscript{C}, which may be targeted by therapeutics compounds including antibodies, small molecules, cyclic peptides or aptamers. Second, Y104A halted progression of prion toxicity even when it was already conspicuous, whereas the anti-FT antibody POM2 exerted neuroprotection only when applied directly after prion inoculation. This suggests that Y104A halts prion toxicity upstream of FT engagement. Thirdly, tga20 COCS (which are much more responsive to toxic pomologs than wild-type COCS, and can therefore be regarded as a sensitive sentinel system) tolerated prolonged application of Y104A at concentrations around 150 × K\textsubscript{D}. Finally, intracerebrally injected Y104A was innocuous, and AAV-transduced Y104A extended the lifespan of prion-infected mice, despite elevated PrP\textsuperscript{Cys} levels, suggesting that it acts downstream of PrP\textsuperscript{Cys} replication, possibly by blocking a PrP\textsuperscript{Cys}-PrP\textsuperscript{C} interaction at the POM1 epitope. These findings suggest that blockade of the POM1 epitope by agents that do not induce the H-latch has good in vivo tolerability. In view of the reports that PrP\textsuperscript{C} may mediate the toxicity of disparate amyloids, the relevance of the above findings may extend to proteotoxic diseases beyond spongiform encephalopathies.

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Methods
Adeno-associated virus production and in vivo transduction. Single-stranded adeno-associated virus (ssAAV) vector backbones with AAV2 inverted terminal repeats (ITRs) were kindly provided by B. Schneider (EPFL). Herein, expression of the monomeric NeonGreen fluorophore was driven by the human Synapsin 1 (BSY1) promoter. A P2A sequence (GGCATNNLSFLQKADVENVPGP) was introduced between mNG and PrPfl for bi-cistrionic expression. For mPrPflΔ707 and mPrPflΔ14 expression, a synthetic gene block (gBlock, IDT, full sequence is given in the Supplementary Information) was cloned between the BsrGI and HindIII site of the vector. The resulting linear type PrPfl vector backbone combination of constructs was tested using Smal digestion prior to virus production. The viral vectors and viral vector plasmids were produced as hybrid AAV2/6 (AAV6 capsid with AAV2 ITRs) by the Vector Facility (VVF) of the Neuroscience Center Zurich. The identity of the packaged genomes was confirmed by Sanger DNA-sequencing (identity check). Mutation of mNG-positive cells from confocal images was done using the Spot software (Diagnostic Instruments, USA).

Neurotropic AAV variants for ScFv antibody expression were constructed from a synthetic gene fragment, Nhel-II-L2-scFv-Myc-EcorV (produced by Genscript Biotech), which contained 3′ Y104A sequences preceded by the signal peptide from interleukin-2 (IL-2)5. Nhel and EcoRV restriction-enzyme digestion was performed on Nhel-II-L2-scFv-Myc-EcorV synthetic gene fragments, which were then inserted into the aSAAV vector backbone. ScFv expression was under the control of the strong, ubiquitously active CAG promoter. A WPRE (woodchuck hepatitis virus post-transcriptional regulatory element) sequence was also included, downstream of the transgene, to enhance transgene expression. Production, quality control and determination of vector titer were performed by Viger Biociences. Rep2 and CapPHHPrB plasmids were provided under a Material Transfer Agreement (MTA). Further details about packaging and purification strategies can be found on the company's website (http://www.vigenebio.com).

Allen Mouse Brain Atlas data. Images from in situ hybridization for calbindin and synapsin 1 expression were taken from the Allen Mouse Brain Atlas (www.brain-map.org). The first dataset retrieved by the R package allenbrain (https://github.com/ogarrn/allenBrain) with the closest atlas image to the center of the region (regionID = 512, settings: planeOfSection = coronal, probeOrientation = antisense) was downloaded (dataset ID nos. for calb1 = 71717/640, synl = 227540).

Animals and in vivo experiments. We conducted all animal experiments in strict accordance with the Swiss Animal Protection law and dispositions of the Swiss Federal Office of Food Safety and Animal Welfare (BLV). The Animal Welfare Committee of the Canton of Zurich approved all animal protocols and experiments performed in this study (animal permits 123, ZH190/2013, ZH1210/16, ZH139/16). Genetically modified mice from the following genotypes were used in this study: Zurich I mice homozygous for disrupted Prnp genes (PrnpflΔ707, denoted as PrnpflΔ707), Zurich III PrnpflΔ14 (denoted as PrnpflΔ14), and PrnpflΔ14 (ref. 10). For in vivo transduction with the neurotropic AAV-PHP.B construct, mice received a total volume of 100 µL (1 × 1012 viral vector genomes) by intravenous injection into the tail vein. Fourteen days after AAV transduction, the left hemisphere of the brain was dissected and its ipsilateral cortex (COCS) was infected with 100 µg ml–1 Rocky Mountain Laboratory scrapie prions (RML6) (passage 6; the Rocky Mountain Laboratory strain mouse-adapted scrapie prions) or 22L (mouse-adapted scrapie prions) brain homogenate from terminally sick, prion-infected mice. Brain homogenate from CD1-inoculated mice was used as nonpathogenic brain homogenate (NBH). Sections were incubated with brain homogenates diluted in physiological Gey’s Balanced Salt Solution for 1 hour at 4 °C, then washed as previously described on PTFE membrane inserts. Antibody treatments were given thrice weekly, e.g. with medium change. In naive slices, antibody treatments were initiated after a recovery period of 10–14 days.

For testing of innocuity of pomologs (Fig. 2c, Supplementary Fig. 7c and Supplementary Fig. 10), Pom1 and pomolog antibodies were added at 400 nm for 24 hours. Supplementary Figures 7c and 10 represent aggregated data. In multiple experiments with COCS from mice of identical genotype and age; compounds were administered at identical timepoints and dosage. When added to RML-infected tga20 COCS (Fig. 2d and Supplementary Fig. 7d), 5′-Y57A was added from 20 to 45 dpi, 3′-Y104A was added from 21 to 45 dpi and both antibodies were given at 400 nm. Antibody treatment with 5′-Y57A and 3′-Y104A of RML-infected tga20 COCS used for determination of PrPfl by western blot, see detailed protocol below, was initiated and stopped at 21 and 45 dpi, respectively. dsDNA was added to RML-infected tga20 COCS at either 1 (800 nm, Supplementary Fig. 12d) or 21 (400 nm, Fig. 2d and Supplementary Fig. 7d) dpi. When added to C57BL/6 COCS (Fig. 2e and Supplementary Fig. 7e), 5′-Y57A and 3′-Y104A were added at 21 dpi. In 22L-infected COCS, 3′-Y104A was administered at 21 dpi, and slices were collected at 44 dpi. Phage-derived Fab were added to RML-infected COCS (Fig. 4b–g) from 1 dpi until 45 dpi at 550 nm.

ELISA. PrPfl levels were measured by ELISA using monoclonal anti-PrPfl antibody pairs POM19/POM3 or POM1/POM2 (all as holo–antibodies), as described previously7. First, 384-well SpectraPlates (Perkin Elmer) were coated with 400 ng ml–1 POM19 (POM3) in PBS at 4 °C overnight. Plates were washed 3 times in 0.1% PBS-Tween 20 (PBS-T) and blocked with 80 µL 5% skim milk in 0.1% PBS-T per well for 1 hour at room temperature. Blocking buffer was discarded, and samples and controls were dissolved in 1% skim milk in 0.1% PBS-T for 1 hour at 37 °C. Twofold dilutions of rmPrP23–231, starting at a dilution of 100 ng/mL in 1% skim milk in 0.1% PBS-T, were used as a calibration curve. Biotinylated POM3 (POM2) was used to detect PrPfl (200 ng/ml in 1% skim milk in 0.1% PBS-T), and biotinylated antibodies were detected with streptavidin–HRP (1:1000 in 1% skim milk in 0.1% PBS-T, BD Biosciences). Chromogenic reaction and reading of plates were performed as described in ref. 7. Unknown PrPfl concentrations were interpolated from the linear range of the calibration curve using linear regression (GraphPad Prism, GraphPad Software).

ELISA screening of phage display. Single colonies were picked and cultured in a 384-well plate (Nunc) in 2Y, ampicillin and 1% glucose medium overnight at 37 °C, 150 rpm. The DNA recovered in 2Y was cloned into a phagemid vector. Phages were expressed by co-transfection of cDNA (pmPrP23Δ14) into E.coli DH5α cells, using the calcium chloride method. Phage plaques were picked and propagated in 2Y, and DNA was isolated and sequenced to select those phages expressing the desired PrPfl sequence. In parallel, colonies were grown in 2Y and screened for expression of PrPfl protein using the anti-PrPfl antibody pair POM19/POM3. After overnight incubation, colonies were washed with PBS-T and blocked with 5% milk in 0.1% PBS-T and incubated with the antibody pair at 37 °C. After washing with PBS-T, colonies were developed with 3,3′-diaminobenzidine (DAB).
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K \cdot P_{\text{FRET}} = \left( \frac{[F_{\text{tot}}]}{K_{\text{D}}} \right) \cdot \left( \frac{[\text{Ap}]}{[\text{PrP}]_{\text{tot}}} \right)

This system of equations is solved to give \( F_{\text{t}} \) as a function of \( \text{Ap} \). To relate the concentration of bound holo-PrP in FRET measurements, this equation was rescaled to 100 for the fully bound and 10 for the fully unbound limit. An additional complication in interpreting the experimental data stems from the fact that a FRET signal will appear only if both a POM1-Eu\(^{3+}\) and holo-PrP3-APC are bound to the same PrP. We assume that the binding of POM1 and POM3 is independent, so we can approximate the concentration of PrP\(^{3+}\) bound to a holo-PrP3-APC as the effective PrP concentration, \( P_{\text{PrP}} \), in the above equations. The binding constant of holo-PrP3-APC was determined to be 0.23 M\(^{-1}\), giving an effective concentration of PrP of 1.64 nM (compared with the total PrP concentration of 1.75 nM). To verify the robustness of these results, we fitted the data assuming a much weaker binding of holo-PrP3-APC, with a binding constant of 1 nM. The obtained \( K_{\text{D}} \) values of the single-chain fragments were within the error of the ones determined with a holo-PrP3-APC binding constant of 0.23 M\(^{-1}\).

Immunohistochemical staining and analysis of immunofluorescence. COCs were washed twice in PBS and fixed in 4% paraformaldehyde for at least 2 days at 4°C and were washed again twice in PBS prior to blocking of unspecific binding by incubation in blocking buffer (0.05% Triton X-100 vol/vol, 0.3% goat serum vol/vol in PBS) for 1 hour at room temperature. For visualization of neuronal nuclei, the monoclonal mouse anti-NeuN antibody conjugated to Alexa Fluor 488 (clone A60, Life Technologies) was dissolved at a concentration of 1.6 μg/mL\(^{-1}\) into blocking buffer and incubated for 3 days at 4°C. Further primary antibodies used were recombinant anti-calbindin antibody (1 μg/mL\(^{-1}\), ab108404, Abcam), anti-gial fibrillary acidic protein (1.500, Z0334, DAKO) and anti-F4/80 (1 μg/mL\(^{-1}\), MCA497G, Serotec). Unconjugated antibodies were diluted in blocking buffer and incubated for 3 days at 4°C. After 3 washes with PBS for 30 minutes, COCs were incubated for 3 days at 4°C with secondary antibodies Alexa-Flour-594-conjugated goat anti-rabbit-IgG (Life Technologies) or Alexa-Fluor-647-conjugated goat anti-rat-IgG (Life Technologies) at a dilution of 1:1,000 in blocking buffer. Slices were then washed with PBS for 15 minutes and incubated in 0.05% DAPI (1 μg/mL\(^{-1}\)) in PBS at room temperature for 30 minutes. Two subsequent washes in PBS were performed, and COCs were mounted with fluorescence mounting medium (DAKO) on glass slides. NeuN, GFAP, F4/80 and calbindin morphometry was performed by image acquisition on a fluorescence microscope (BX-61, Olympus), and analysis was performed using gray-level analysis of labeling fraction in ImageJ (www.ImageJ). Cell numbers in Figure 3f were determined using the ‘Spots’ function in Imaris (Oxford Instruments).

Morphometric quantification was done on unprocessed images with identical exposure times and image thresholds between compared groups. Representative fluorescent micrographs in the main and supplementary figures have been processed (linear adjustment of brightness and contrast) for better interpretability. Immunofluorescent images of CAD5 cells, cells were seeded on 18-well µ-slides (ibidi) and fixed with 4% paraformaldehyde for 5 minutes at room temperature. Unspecific reactions were blocked using 3% goat serum in PBS for 1 hour at room temperature. Mouse monoclonal anti-PrP\(^{3+}\) antibodies POM1, POM5, POM8 and POM19 (all holo-antibodies) were used before,\(^2\) POM\(^3\) antibodies were incubated at 4 μg/mL\(^{-1}\) in 3% goat serum in PBS at 4°C, followed by 3 washes in PBS. Antibodies were detected using Alexa-Flour-488-conjugated goat anti-mouse-IgG at 1:250 dilution, followed by nuclear counterstain with DAPI (1 μg/mL\(^{-1}\)) in PBS for 5 minutes at room temperature. Image analysis was performed using SP5 confocal microscope (Leica) with identical exposure times across different experimental groups.

In vitro toxicity assessment. Quantification of POMI toxicity on CAD5 Pnpp\(^{3-}\) cells stably transfected with mPrP, mPrP\(^{3-}\) or empty control vector, as described above, was measured as percentage of PI-positive cells using flow cytometry, as described before.\(^2\)

CAD5 cells were cultured with 20 mL Corning Basal Cell Culture Liquid Media–DMEM and Ham’s F-12, 50/50 Mix, supplemented with 10% FBS, Gibco MEM Non-Essential Amino Acids Solution 1 and MEM Non-Essential Amino Acids Solution 2, 100 μM L-Arginine, 50 μM L-Ornithine, 50 μM L-Citrulline and 100 μM D-Lysine. Media–DMEM and Ham’s F-12, 50/50 Mix, supplemented with 10% FBS, Gibco MEM Non-Essential Amino Acids Solution 1, Gibco GlutaMAX Supplement 1x and 0.5 mg/mL of Geneticin in T75 Flasks (Thermo Fisher) at 37 °C, 5% CO2. Sixteen hours before treatment, cells were split into 96-well plates at 25,000 cells/well in 100 μL. POM1 alone was prepared at 5 μM final concentration in 20 mM HEPES pH 7.2 and 150 mM NaCl, and 100 μL of each sample, including buffer control, was added to CAD5 cells, in duplicates.

After 48 hours, cells were washed 2 times with 100 μL MACS buffer (PBS + 1% FBS + 0.05% EDTA) and resuspended with 100 μL MACS buffer. Three minutes before FACS measurements, PI (1 μg/mL\(^{-1}\)) was added to the cells. Measurements were performed using a BD LSRFortessa. The percentages of PI-positive cells were plotted in columns as mean ± s.d. The gating strategy is depicted in Extended Data Figure 3a.

In vivo toxicity assessment. The in vivo toxicity assessment was performed as described. In brief, mice were intraperitoneally injected using a motorized stereotaxic frame (Neurostar) at the following bregma coordinates (AP –2 mm, ML ±1.7 mm, DV 2.2 mm, angle in ML/DV plane 15°). Antibodies (2 μL) were injected at a flow rate of 0.5 μL/minute. After termination of the injection, the needle was left in place for 3 minutes.

Twelve-four hours after stereotactic injection, mice were placed on a bed equipped with a mouse whole-body radio frequency transmitter coil and a mouse head surface-coil receiver and then transferred into the 4.7 Bruker Pharma scan. For DWI, routine gradient echo sequences with the following parameters were...
Molecular dynamics. Experimental structures were used as a basis for MD simulations when available (scPOM1–mPrP complex, Protein Data Bank (PDB) 4H88; free mPrP, PDB 1XXY). The structures of full-length mPrP, mPrPΔ23–231, and the polymers were predicted by homology modeling on the TASSER webserver, on the basis of the structural envelopes of the extracellular domain (aa 120–231), and were further validated with MD.

In the simulation system, all was set up and equilibrated through standard MD protocols: proteins were centered in a triclinic box, 0.2 nm from the edge, and filled with TIP3 water model and 0.15 M NaCl ions using the AMBER99SB-ILDN protein force field; energy minimization followed. Temperature (298 K) and pressure (1 bar) equilibration steps (100 ps each) were performed. Three independent replicates of 500–ns MD simulations were run with the above-mentioned force field for each protein or complex. MD trajectory files were analyzed after removal of periodic boundary conditions. The overall stability of each simulated complex was verified by root mean square deviation, radius of gyration, and other measures of structural rearrangements. Structural clustering was done using GROMACS28 and standard structural biology tools. RMSF provides a qualitative indication of residue level flexibility, as shown in Figure 1c.

The presence of H-bonds or other interactions between GD residues was adjusted of contrast and brightness) for better visualization.

Production of recombinant proteins and antibodies. Bacterial production of recombinant, full-length mouse PrPΔ23–231, recombinant fragments of human and mouse PrP, and recombinant, biotinylated human PrP-AviTag (not shown) and mouse PrP-AviTag was achieved as previously described30–32. Production of scFv and the IgG POM1 antibodies used in this manuscript was described as before3. Production of holo-αY104A was performed as follows: POM1 IgG, heavy chain containing a Y104A mutation and POMI kappa light chain were ordered as a bicistronic synthetic DNA block (gBlock, IDT) separated by a T7 promoter. The synthetic block was inserted in pGEX-6P-1 (GE Healthcare) in-frame with a 23–230_AviTag (in solution) for positive selection. At the fourth round of selection, DNA minipreps were prepared from the panning output pools by QiAprep Spin Miniprep kit (Qiagen) and the whole anti-PrP Fab enriched library was subcloned in expression vector pP2e2 (kindly provided by Norovits). DNA was then used to transform electrocompetent non-amber suppressor MC10516 bacteria (Lubio Science) to produce soluble Fabs and perform ELISA screening.

Protein analysis. COCs were washed twice in PBS and scrapped off the PTFE membranes with PBS. Homogenization was performed with a Tissuemaxer LT (Qiagen) for 2 minutes at 50 Hz. A biocinachinonic acid assay (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific) was used to determine protein concentrations. PrPΔ23–231 levels were determined through digestion of 20μg of COCs homogenates with 25 μg/mL of protease K (PK, Roche) at a final volume of 20 μL. In PBS for 30 minutes at 37 °C. PK was deactivated by addition of sodium-dodecyl-sulfate-containing NuPAGE LDS sample buffer (Thermo Fisher Scientific) and boiling of samples at 95 °C for 5 minutes. Equal sample volumes were loaded on Nu-PAGE BisTris precast gels (Life Technologies) and PrPΔ23–231 was detected by western blot using the monoclonal anti-PrP antibodies POM1, POM2 or POM1 at 0.4 μg/mL (all holo-antibodies), as established elsewhere3. Further primary antibodies used for western blots in this manuscript are as follows: monoclonal NeuroGent (1:1,000, 32F6, Chromotek), phospho-eIF2α (1:1,000, clone no. D9G8, Cell Signaling Technologies), eIF2α clone no. D9G8, Cell Signaling Technologies), eIF2α (1:1,000, clone no. C4, Millipore), GFAP (1:100, clone no. 2F5, Cell Signaling Technologies), and Myc-tag (1:500, catalog no. ab9106, Abcam). After incubation of primary antibodies at 4 °C overnight, membranes were washed and detected with goat polyclonal anti-mouse (1:1,000; clone no. A15–035–045, Jackson ImmunoResearch antibodies for 1 hour at room temperature. For PNGaseF digestion, 20 μg of samples was processed using a commercially available kit (New England Biolabs), and PrPΔ23–231 detection was performed using the monoclonal anti-PrP antibody POM2, as described above. Western blots were quantified on native photographs (uncropped, naive images are available in the Source Data; representative western blots in the main and supplementary figures have been processed (linear adjustment of contrast and brightness) for better visualization.)
Surface plasmon resonance (SPR). The binding properties of the complexes between rmPrP, POM1 and pomologs were measured at 298 K on a ProteOn XPR-36 instrument (Bio-Rad) using 20 mM HEPES pH 7.2, 150 mM NaCl, 3 mM EDTA and 0.005% Tween-20 as running buffer. mPrP was immobilized on the surface of GLC sensor chips through standard amide coupling. Serial dilution of antibodies (full IgG, Fab or single-chain versions) in the nanomolar range were injected at a flow rate of 100 µL/min (contact time 6 minutes); the dissociation phase was then observed for 5 minutes. Analyte responses were corrected for unspecific binding and buffer responses by subtracting the signal of both a channel where no PrP was immobilized and a channel with no antibody was added. Curve fitting and data analysis were performed with Bio-Rad ProteOn Manager software (version 3.1.0.6).

Statistical analyses. All biological measurements are taken from distinct samples. Unless mentioned otherwise, the following tests were performed for statistical hypothesis testing: unpaired, two-tailed t-test was used for comparison between two groups, one-way ANOVA with Dunnett’s multiple-comparison test was used for comparison of multiple groups with a control group, and ordinary one-way ANOVA with Sidák’s multiple comparisons test was used for comparison of preselected pairs of groups. Statistical analysis and visualization were performed using Prism 8 (GraphPad). No statistical methods were used to pre-determine sample sizes, but our sample sizes are similar to those reported in previous publications.19,20 Except for in vivo prion inoculation experiments and NeuN morphology, data collection and analysis were not performed blind to the conditions of the experiments.

Synchrotron radiation circular dichroism. Secondary structure content of complexes between rmPrP and POM1 and Y57 and Y104A was analyzed with synchrotron radiation circular dichroism (SRCD) spectroscopy. Experiments were performed using a nitrogen-flushed B23 beamline for SRCD at Diamond Light Source or ChiracLasPlus CD spectrophotometer (Applied Photophysics). With both instruments, scans were acquired at 20 °C using an integration time of 1 second and 1 nm bandwidth. Demountable cuvette cells with a pathlength of 0.00335 cm were used in the far-UV region (180–260 nm) to measure the CD of the protein concentration, varying from 10 to 102 µM protein in 10 mM NaP pH 7 and 150 mM NaCl. Mixtures were prepared to a stoichiometric molar ratio of 1:1. SRCD data were processed using CDApps11 and OriginLab. Spectra have been normalized using an average amino acid molecular weight of 113 for secondary structure estimation from SRCD, and CD spectra were created using CDApps with the Continll algorithm11. For comparison of calculated and observed spectra, the full molecular weight of sample and complex were used. Measurements of free mPrP and free antibodies were taken as a reference.

Data availability All source data, for example numeric source data, uncorrected western blot gels including annotations thereof, as well as unique DNA sequences, accompany this manuscript as supplements. The following publicly available data was used: Allen Mouse Brain Atlas, entries 71717640 and 227540 (https://mouse.brain-map.org); Biological Magnetic Resonance Data Bank, entry 16671 (https://bbrmbr.isu.edu/); RCSB Protein Data Bank, entries 1NYX and 4H88 (https://www.rcsb.org). Additionally, all unique biological materials used in the manuscript are readily available from the authors. Source data are provided with this paper.

Code availability New code was generated for analysis of Allen Brain Atlas data and can be found in the Supplementary Software.

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24. Herrmann, U. S. et al. Structure-based drug design identifies polythiophenes as mimickers of prion protein-detergent micelle interactions studied by NMR in solution. J. Biol. Chem. 284, 22713–22721 (2009).
**Extended Data Fig. 1 | Distances between the R207 and H139 residues in MD simulations of PrP-antibody complexes.** The simulation of the PrP-POM1 complex (red) in reproduced in all charts to facilitate comparisons. When PrP is bound to POM1, the H-bond between R207 and H139 (termed H-latch) is always present, with distance between the centroid of their sidechains around 0.3 nm. Greater distances indicate loss of hydrogen interactions and consequently absence of the H-latch. The complex of PrP with the Y104A pomolog never shows formation of the H-latch, whereas FabA10 shows intermediate values. Simulations were run three times, but only representative traces are shown; aggregated analyses are shown in Fig. 1c.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Robust expression and conformation of the PrPR207A point mutant. (a) Representative images of expression levels of Synapsin 1 (Syn1, upper) and Calbindin 1 (Calb1, lower) show predominant (Syn1) or almost exclusive (Calb1) expression in Purkinje cells (pc) in the cerebellar cortex. Image credit: Allen Institute. Scale bar = 100 µm. (b) Fluorescent micrographs of Prnp^{ZH3/ZH3} COCS transduced with the AAV outlined in panel (A) show mNeonGreen expression predominantly in calbindin 1-expressing Purkinje cells. Scale bar = 50 µm. cgI = cerebellar internal granular layer, pc = Purkinje cell layer, ml = molecular layer. These findings were repeated in three independent experiments. (c) Left panel: Stably transfected CAD5-mPrP^{c} and CAD5-mPrP^{R207A} cells show similar PrP^{c} expression levels. Representative PrP^{c} levels of one cell culture passage are shown. Right panel: POM19 immunoreactivity is divided by actin immunoreactivity, values are given as percentages of PrP^{c}. One datapoint corresponds to one passage of CAD5 cells. (d) Surface plasmon resonance (SPR) traces showing binding of POM1 to recombinant mPrP^{R207A} (mPrP^{R207A}, k_{a}=3.8×10^{5} 1/ Ms, k_{d}=1.8×10^{-4} 1/s, K_{D}=4.7×10^{-10} M; for comparison binding to recombinant wild-type murine PrP showed k_{a}=3.6×10^{5} 1/ Ms; k_{d}=9.1×10^{-5} 1/s; K_{D}=2.5×10^{-10} M). (e) Immunohistochemistry of CAD5 Prnp^{-/-} cells stably transfected with pcDNA3.1 vector expressing wild-type murine PrPC (mPrPC), mPrPR207A and mPrP2cys. Monoclonal anti-PrP antibodies targeting distinct conformational epitopes on the globular domain of PrP were incubated to assess conformational changes in mPrP^{R207A} (POM1: α1-α3, POM5: β2-α2, POM8: α1-α2, POM19: β1-α3). Except for diminished staining of POM1 in mPrP^{R207A}, we observed robust detection of mPrP^{R207A} by POM5, POM8 and POM19 and mPrP^{cys} by POM8 and POM19. Parts of this experiment, for example POM1 and POM19, were repeated twice. Scale bar = 20 µm.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Expression of the H-latch mimic R207C-I138C in organotypic cultured slices leads to dose-dependent neurotoxicity. (a) Flow cytometry gating strategy of PI positive CAD5 cells. (b) PNGase-F digestion of cell lysates induced a shift in both murine wild-type PrP$^C$ and mPrP2cys, indicating that both moieties had undergone N-linked glycosylation to a similar extent. Non-adjacent lanes were merged from the same gel. (c) CAD5 Prnp$^{-/-}$ cells expressing mPrP2cys did not show an upregulation of the unfolded protein response, suggesting that mPrP2cys did not undergo pathological degradation. Values are given as percentage of empty control vector (p-eIF2α / eIF2α / actin). One datapoint per group corresponds to a different cell culture passage. Two-sided, unpaired t-test. (d) A POM2/POM3 sandwich ELISA of COCS transduced with empty control, mPrP$^C$, mPrP2cys and buffer control shows robust mPrP2cys expression in transduced COCS, albeit significantly less than wild-type mPrP$^C$. Slices were harvested at 28 days post-transduction. One datapoint corresponds to an independent, biological replicate of 6–9 pooled slices. Ordinary, one-way Anova with Šídák's multiple comparisons test, *: adjusted p-value = 0.039 (e) Reduced levels of mNG in Prnp$^{-/-}$ (ZH3) COCS expressing mPrP2cys. mNG immunoreactivity values are divided by actin immunoreactivity and expressed as percentages of empty control. Slices were harvested at 28 days post-transduction. One datapoint corresponds to an independent, biological replicate of 6–9 pooled slices. Ordinary, one-way Anova with Šídák's multiple comparisons test. Raw, uncropped blots can be found in the Source Data supplement. (f) Quantification of mNG and Calb1 fluorescence intensity from experiments shown in Fig. 3d-f. One datapoint corresponds to a biological replicate, e.g. one organotypic cultured slice. Unpaired, two-tailed t-test without adjustment for multiple testing. P-values are as follows: 31 dpt, 5.2x10$^{10}$ vg·ml$^{-1}$, mNG: 0.001; 31 dpt, 5.2x10$^{10}$ vg·ml$^{-1}$, Calb1: 0.0496; 15 dpt, 1.4x10$^{11}$ vg·ml$^{-1}$; mNG: 0.0065; 15 dpt, 1.4x10$^{11}$ vg·ml$^{-1}$; Calb1: 0.001. ***: p ≤ 0.001, **: p < 0.01, * p < 0.05.
Extended Data Fig. 4 | Molecular dynamics simulations show overlapping structural changes of POM1-PrP° complex and pathogenic PRNP mutations.

Extended Data Figure 4. (a) MD simulations of POM1 binding and pathogenic PRNP mutations causing genetic prion disease show the R156-E196 interaction is abolished and induction of the H140-R208 H-latch is established. Each datapoint represents one independent simulation, values are given as mean ± standard deviation. (b) In agreement with this view, POM1 and human, hereditary PrP mutations responsible for fatal prion diseases favor altered flexibility in the α2-α3 and β2-α2 loop.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Scanning alanine mutagenesis of the POM1 paratope. Extended Data Figure 5. (a) Intermolecular contacts between human PrP<sub>120–230</sub> and POM1 Fab variable heavy chain (magenta, left panel), and POM1 Fab variable light chain (green, right panel) as determined by Baral et al., 2012<sup>8</sup>. Reproduced with permission of the International Union of Crystallography from doi:10.1107/S0907444912037328. (b) Schematic representation of a single-chain fragment of wild-type POM. The mutated residues are indicated as stick on the cartoon structure of POM1, color coded as in Supplementary Table 1b. The CDR loops are shown from the perspective of the antigen. (c) Scheme of competition FRET assay to assess the K<sub>D</sub> of various pomologs. In the absence of competing antibody, FRET occurs due to proximity of allophycocyanin (APC)-labeled holo-POM3 and europium (Eu3<sup>+</sup>)-labeled POM1 (left panel). Because of liquid-phase competition, addition of unlabeled pomologs leads to a decrease in FRET signal (right panel). The calculation of binding constants from FRET is detailed in the methods section. (d) The binding constants measured by SPR and by FRET were in good agreement, Spearman r = 0.77, p=0.0074, 95% CI 0.30–0.94) with the exception of W33A, whose binding on SPR was too weak to be precisely measured.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Pomolog hcY104A acts as dominant negative suppressor of prion toxicity. Extended Data Figure 6. (a) Treatment of PrnpZH1/ZH1 COCS shows toxicity of POM1 and toxic pomologs to be dependent on PrPc, see also Supplementary Fig. 1a. **: p=0.003, ordinary one-way Anova with Dunnett’s multiple comparisons test. Innocuous pomologs are highlighted in green, POM1 and toxic pomologs are highlighted in red. (b) Morphometric quantification of Prnp-overexpressing tga20 COCS treated with pomologs, see also Fig. 4. Color coding according to panel (A). 100%=untreated COCS, comparison of untreated versus treated groups. N.s.: not significant, ***: p<0.0001, ** hcY101A: p=0.0035, ** hcY104A: p=0.0019, ordinary one-way Anova with Dunnett’s multiple comparisons test. (c) Morphometric quantification of fluorescence intensity from images depicted in Supplementary Fig. 1b. §: 1 outlier was excluded (y=2046.3%, p<0.05, extreme studentized deviate method). Values = % of untreated control. Pairwise comparison in the presence or absence of rmPrP23–231. ***: p<0.0001, * GFAP-POM1: p=0.0148, ** GFAP-hcY101A: p=0.0009, *** F4/80-POM1: p=0.0005, * F4/80-hcY101A: p=0.0261, ordinary one-way Anova with Šidák’s multiple comparisons test. (d) Toxicity of high-affinity pomolog hS32A ablated by POM2. 100%=POM1+rmPrP23–231 (bars 1–4) or 100%=hS32+rmPrP23–231 (bars 5–7). ***: p=0.0003, ** p<0.0001, ordinary one-way Anova with Šidák’s multiple comparisons test. (e) Titration of minimal toxic dosage of POM1 in tga20 COCS. 100%=POM1+rmPrP23–231. ***: p<0.0001, ordinary one-way Anova with Dunnett’s multiple comparisons test. (f) **Y104A prevented POM1-induced toxicity. 100%=PrnpZH3/ZH3+22L. ***: p=0.0078, *** (left): p=0.0009, *** (right): p<0.0001, ordinary one-way Anova with Šidák’s multiple comparisons test. (g) Quantification of Fig. 4b. 100%=untreated+NBH. ***: p<0.0001, ordinary one-way Anova with Dunnett’s multiple comparisons test. (h) Quantification of Fig. 4c. 100% = PrnpZH3/ZH3 + 22L. *: p=0.032, ordinary one-way Anova with Šidák’s multiple comparisons test. (i) Quantification of Fig. 4d. 100%=untreated +NBH. *: p=0.0203, ** (left): p=0.0036, ** (right): p=0.005, ordinary one-way Anova with Dunnett’s multiple comparisons test. All graphs: one datapoint corresponds to one biological replicate.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Dose-dependent gliosis of hY104A is also conspicuous around needle tracts. (a) Photomicrographs of glial fibrillary acid protein (GFAP) immunohistochemistry on consecutive sections depicted in Fig. 6c. Left column: holo-hY104A injections (6, 9 and 12 µg). Right column: holo-hY104A + rmPrP23–231. GFAP immunoreaction was increased in areas of neuronal damage (white asterisks) and around needle tracts (white arrowheads). (b) Micrographs demonstrating an intensive GFAP immunoreaction in areas with extensive holo-POM1 (6 µg)-induced neurotoxicity. Left panel: POM1 injection (6 µg). Right panel: holo-hY104A + rmPrP23–231. Sections are consecutive to those shown in Fig. 6f.
Extended Data Fig. 8 | Assessing hcy104A dose-dependent toxicity. Extended Data Figure 8. (a) A hypothetical benchmark dose analysis was performed using log10-transformed lesion volumes corresponding to different amounts of holo-hcy104A (data from Fig. 6g). BMR: Benchmark response (0.15 mm³, dashed red line). The benchmark dose (BMD) is defined as the dose at the BMR. The vertical lines indicate the BMD values corresponding to the different dose response values (blue: 21.5 µg, brown line: 20.5 µg). The upper limit of the safe dose is provided by the lower 95% confidence interval of the BMD (horizontal lines below the graph: blue: 12 µg, brown: 12 µg). One datapoint corresponds to one independent animal. (b) Representative DWI images taken 24 h after stereotactic injection of 6 µg holo-hcy104A into male tga20 mice (left half of the image, injected into CA3). Contralateral side: 6 µg holo-hcy104A pre-incubated with an equimolar amount of rmPrP23–230. White asterisks: needle tract. (c) Photomicrograph of HE-stained sections from mouse brain shown in panel B. Asterisks: needle tract. Rectangles correspond to regions magnified in panel C. (d) Higher magnification of the end-plate of the hippocampus. Left panel: holo-hcy104A. Right panel: holo-hcy104A preincubated with rmPrP23–230. Arrow: needle tract. (e) Quantification of lesion volumes after injection of holo-hcy104A in contrast to control injection into tga20 mice (N = 3). One datapoint corresponds to one independent animal.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Generation and validation of a synthetic human Fab phage library. Extended Data Figure 9. (a) A synthetic human Fab phage library was used for panning. For each panning round, the targeted antigens are reported with the respective concentration. Full-length recombinant murine PrP_{23-231} (rmPrP_{23-231}, light blue boxes) was used as a target for the first and the second round of phage panning. At the third and fourth round, phages were depleted of the binders to rmPrP_{2cys} and selected for binding to either rmPrP_{90-231} or rmPrP_{121-231} (recombinant murine PrP fragments lacking the N-terminal flexible tail; light red boxes) or to recombinant human PrP_{23-230-AviTag} (rhPrP_{23-230-AviTag}, purple boxes). In rmPrP_{90-231} or rmPrP_{121-231} panning, Fab-displayed Fab were depleted of binders to rmPrP_{2cys} coated on plates. In rhPrP_{23-230-AviTag} panning, depletion of binders to rmPrP_{2cys} in solution was achieved by capturing Fabs binding to rhPrP_{23-230-AviTag} on neutravidin coated wells. Polyclonal DNA preparation from the selected phages at the third round (rmPrP_{90-231}) and fourth round (rmPrP_{121-231} and rhPrP_{23-230-AviTag}) was used for transformation in bacteria and the screening of single clones by ELISA. (b) ELISA (OD at 450 nm) comparing the reactivity of phage-derived anti-PrP Fabs to full-length rmPrP_{23-231}, FT fragment rmPrP_{23-110} and GD fragments rmPrP_{90-231} and rmPrP_{121-231}. Anti-PrP Fab100 and Fab53 bind within the FT of PrP - the octapeptide repeat region (OR, amino acid 51–90) and the charged cluster 2 (CC2, amino acid 93–100), respectively. FabA10, FabD9, FabE6 and FabE2 bind within the GD. Error bars = standard error of the mean. One datapoint corresponds to a technical replicate in a multi-well plate.
Extended Data Fig. 10 | FabA10 ameliorates the H-latch but shares its paratope with POM1. (a) The R208-H140 interaction is present in POM1-bound PrP (right, red) but not in free PrP (left, white) or in its complex with FabA10 (left, blue). The final state of MD simulations starting from a POM1-bound conformation, with R208-H140 interaction present, is shown for FabA10. (b) Overlap (green) of FabA10 (blue) and POM1 (red) epitopes on murine PrP<sup>-/-</sup>GD. Coloring according to Fig. 7d, e.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a | Confirmed
---|---
☑️☐ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
☑️☐ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☑️☒ | The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
☑️☐ | A description of all covariates tested
☑️☐ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☑️☐ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☑️☐ | For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
☑️☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☑️☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☑️☑️ | Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection | We used RStudio (1.1.383) with R [3.4.2], CDApps (4.0), FlowJo (10)
Data analysis | Custom code was used to analyze Allen Mouse Brain Atlas data, which can be found in the supplementary data.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All source data, e.g. numeric source data, uncropped western blot gels including annotation thereof as well as unique DNA sequences, is available in the manuscript or the supplementary materials. The following, publicly available data was used: Allen Mouse Brain Atlas, entries 7117640 and 227540 [https://mouse.brain-map.org]; Biological Magnetic Resonance Data Bank, entry 16071 [https://bmbd.io/]; RCSB Protein Data Bank, entries 1XYX and 4H88 [https://www.rcsb.org]. Additionally, all unique biological materials used in the manuscript are readily available from the authors.
Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender
Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data where this information has been collected, and consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Population characteristics
Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment
Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight
Identify the organization(s) that approved the study protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose these points even when the disclosure is negative.

Sample size
No statistical methods were used to predetermine sample size, moreover, sample size was chosen based on previously performed experiments, as published before (PMID 26821311, 25710374, 23903654, 23133383)

Data exclusions
ED Fig 6C: immunohistochemical analysis of slice cultures is known to show large variability (Falsig et al., PLOS Pathogens 2012, PMID 23133383). Here, one extreme outlier was excluded from the analysis (y=2046.3%, p<0.05, extreme studentized deviate method).

Replication
Biological replicates from in vitro experiments correspond to different culture passages. Biological replicates from organotypic slice culture correspond to slices from different animals. Experiments depicted in Figures 2D, 3D-F, 4A, 4B, 4D+F, 7A, ED 3E, ED 6A,B,G, I were successfully replicated in two independent runs. Experiments depicted in Figures 1C,D,F, 2B+C, 3B+C, 6D+E, ED 1, ED 4, ED 5D were successfully replicated in three or more independent runs. Experiments depicted in the following images contain multiple, independent, biological replicates, but were only performed once: 2E, 2F, 3A, 3C, 4E, 6G, 5A, ED 3B, ED 6B-F, ED 6H, ED 7B-C. Experiments depicted in ED 3C-D were performed in two independent, biological replicates. POM1 and POM19 of experiment from fig ED 2D were replicated successfully, we did not attempt replication of POM5 and POM8, because it was later orthogonal verified in MDS and NMR. Due to disproportionate effort and in agreement with standard practice, intracerebral/venous injections and in vivo imaging of animals (depicted in Fig. 6, ED 7A+B, although they were performed with several independent, biological replicates), NMR and SFC analyses (Fig. 3, 5, 7D+D) as well as antibody phage display (Fig. ED 9) were only performed once.

Randomization
Animals were randomly assigned to treatment groups. Brain slices were pooled from different animals and randomly selected for treatment. Similarly, allocation of cells in in vitro experiments was random. Sections used for imaging were selected randomly and were analyzed equally with no sub-sampling thus omitting a need for randomization.

Blinding
NeuN morphometry was performed blindly. Prior disease symptoms of AAV-treated mice were assessed by an animal caretaker blinded to the treatment (P.S.). In the case of other experiments, blinding was not attempted, in parts because testing conditions were evident from the experimental data. However, quantifications were performed using computational pipelines applied equally to all conditions and replicates for a given experiment.

Reporting for specific materials, systems and methods
We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | Antibodies            |
| ☒   | Eukaryotic cell lines |
| ☒   | Palaeontology and archaeology |
| ☒   | Animals and other organisms |
| ☒   | Clinical data         |
| ☒   | Dual use research of concern |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | ChiP-seq              |
| ☒   | Flow cytometry        |
| ☒   | MRI-based neuroimaging |

### Antibodies

**Antibodies used**

Please specify the original source (commercial or other). If they are commercially available, please provide information on their supplier name, catalog number, clone name and lot number, as applicable. For all the other POM whole IgG antibodies, here in the reporting summary.

Please ensure all the antibodies referenced in the manuscript are listed here in the reporting summary (with supplier name, catalog number, clone name and lot number, as applicable). For example: Alexa Fluor 594 Rabbit Anti-Goat IgG secondary antibody

Please provide the catalog number, and lot number, as applicable, for all the following antibodies listed, here in the reporting summary. For example: phospho-elf2α (Cell Signaling Technologies), etc.

POM1 and POM2 whole IgG antibodies are available via Merck (POM1 MABN2285, POM2 MABN2298). Other POM whole IgG antibodies are originally derived by immunization of POMP knock-out mice and subsequent monoclonal expansion of hybridomas as laid out in Polymenioudou et al., PLOS ONE 2008, PMID 19060956. Generation of Fab and scFv fragments was performed using papain digestion of holigo-Gs and periplasmic expression, respectively, as reported extensively in Sonati et al., Nature 2013, PMID 23903654. Generation procedures of POM1 holo-IgG and scFv mutants by eukaryotic and periplasmic expression, respectively, is listed in the materials and methods section of the present manuscript, the antibodies can be obtained through the authors.

Antibody sequences are deposited on NCBI (POM1 heavy chain 4DGK_H, POM1 light chain 4DGK_L, POM2 heavy chain 4J8R_D, POM2 light chain 4J8R_C) and in Polymenioudou et al., PLOS One 2008, or are described in Materials and Methods (POM1 mutants) or can be obtained from the authors (Fab fragments displayed in Fig 7A).

Other antibodies used are as follows, given as Name, Catalog #, Company anti-Fd, PC075, Binding Site anti-Myc-tag, A89106, Abcam anti-monomeric NeonGreen, 32F6-100, Chromotek anti-phospho-elf2α, 3398, Cell Signaling Technologies anti-elf2α, 5324, Cell Signaling Technologies anti-Actin Antibody, A89105, Millipore anti-GFAP, 20334, DAKO/Agilent anti-Fb/Fc, MA9497G, Serotec/BioRad anti-human F(ab)’2 alkaline phosphatase conjugated antibody, SAB3701239, Sigma Streptavidin/HRP, 554066, BD Biosciences anti-mouse IgG, 115-035-062, Jackson ImmunoResearch anti-rabbit IgG, 111-035-045, Jackson ImmunoResearch Alexa594-conjugated goat anti-rabbit IgG, A-11012, Thermo Fisher Alexa594-conjugated rabbit anti-goat IgG, A-11037, Thermo Fisher Alexa647-conjugated goat anti-rat IgG, A-21247, Thermo Fisher Alexa488-conjugated goat anti-mouse IgG, A-28175, Thermo Fisher anti-NeuN Antibody, Alexa Fluor®488 conjugated, MAB377X, Merck

### Validation

Validation information of commercial antibodies is extracted from manufacturer’s websites and listed in the format (abbreviations are explained at the bottom of this field): Target/Name, 1*2/1*2 conjugated 1*4/1*4 AB, Host, Reactivity, Applications-Manufacturer, Application-Manufacturer, Catalog #, Clone (if applicable). Dilution used in the manuscript, Company, Citation PMID, Application in paper different from manufacturer’s suggestion (Yes/No). Citation contains validation of application from paper (Yes/No)

anti-Fd, 1, S, H, Immunoelectrophoresis [IEP], Radial Immunodiffusion [RID], ELISA, PC075, n/a, 1:1000, Binding Site, 32776637, Y, Y anti-Myc-tag, 1, Rb, MycTag, IHC-Fr, IP, WB, IHC-P, ICC, Electron Microscopy, WB, A89106, n/a, 1:500, Abcam, 33534797, N, Y anti-monomeric NeonGreen, 1, M, mNeonGreen fluorescent protein derived from Branchiostoma lanceolatum, IF, ELISA, WB, 32F6-100, 32F6-100, Chromotek, 32014414, Y, Y anti-phospho-elf2α, 1, Rb, H M R Mk Dm, WB, IP, IHC, WB, 3398, D96G, 1:1000, Cell Signaling Technologies, 34953853, N, Y anti-elf2α, 1, Rb, H M R Mk, WB, IP, IHC, WB, 5324, D703, 1:1000, Cell Signaling Technologies, 34953853, N, Y
Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)
CAD5 cells were derived from Cath-a-differentiated (CAD) cells (Qi Y et al., J Neurosci 98), were established by Mahal et al., PNAS 2007 and were a kind gift from Charles Weissmann. Generation of CAD5 Prrp knock-out cells was described in Bardelli et al., PLOS Pathogens 2018.

Authentication
CAD5 cells were not authenticated after receipt from Charles Weissmann.

Mycoplasma contamination
Cell lines were tested negative for mycoplasma contamination.

Commonly misidentified lines
No commonly misidentified cell lines were used in the study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals
Please specify the age for all strains of mice used, here in the reporting summary.

Please provide information on housing conditions for the mice, describing dark/light cycle, ambient temperature and humidity in the manuscript.

We used the following animals [both male and female] for slice culture and in vivo toxicity assessment: C57BL/6J, Tgax20 (described in Fischer et al., EMBO J 1996). Additionally, Prnp0/0 (ZH1, described in Büeler et al., Cell 1993) and Prnp0/0 mice (ZH3, described in Nuvolone et al., J Exp Med 2016) were used for slice culture experiments. Mice were bred in high hygienic grade facilities and housed in groups of 3–5, under a 12 h light/12 h dark cycle (from 7 am to 7 pm) at 21±1°C, with sterilized food (Kliba No. 3431, Provinzi Kliba, Kaiseraugst, Switzerland) and water ad libitum.

Animals were not selected for gender. Animals ages were as follows:
Slice culture: 9-12 days
MEMRI: 4 months
AAV and prion injection: 3 months

Wild animals
This study did not involve wild animals

Reporting on sex
We did not adjust for mouse gender because of lack of group-wise comparisons. Specifically, for Memri experiments, treatment and
Flow Cytometry

Plots

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g., CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

CAD5 cells were cultured with 20ml Corning Basal Cell Culture Liquid Media-DMEM and Ham’s F-12, 50/50 Mix supplemented with 1% FBS, Gibco MEM Non-Essential Amino Acids Solution 1X, Gibco GlutaMAX Supplement 1X and 0.5mg/mL of Geneticin in T75 Flasks ThermoFisher at 37°C 5% CO2. 16 hours before treatment, cells were split into 96wells plates at 25000 cells/well in 100uL.
POM1 alone was prepared at 5 μM final concentration, in 20 mM HEPES pH 7.2 and 150 mM NaCl. 100 μL of each sample, including buffer control, were added to CAD5 cells, in duplicates. After 48 hours, cells were washed two times with 100μL MACS buffer (PBS + 1% FBS + 2 mM EDTA) and resuspended in 100 μL MACS buffer. 30” before FACS measurements PI (1 μg/mL) was added to cells.

Instrument

- BD LSRFortessa

Software

- Flowjo (10)

Cell population abundance

- Only CAD5 cells are present in the sample

Gating strategy

- Based on FSC and SSC the CAD5 cells were selected and separated from debris. After the first gating the cells were analyzed for PI presence. The gate to discriminate PI positive and negative cells was selected on control samples [not treated] and applied to all the other samples. A figure exemplifying the gating strategy is provided in ED Figure 3A.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

- No BOLD imaging / fMRI

Acquisition

Imaging type(s)

- Diffusion weighted imaging

Field strength

- 4.7

Sequence & imaging parameters

- TR: 300 ms TE: 28 ms, flip angle: 90 deg, average: 1, Matrix: 350 x 350, Field of View: 3 x 3 cm, acquisition time: 17 min, voxel size: 87x87 μm3, slice thickness: 700 μm3, Isodistance: 1400 μm3 and b values: 13, 816 s/mm2

Area of acquisition

- Converging on the whole hippocampus

Diffusion MRI

- Used

Parameters

- Single shell, b values: 13, 816 s/mm2
### Preprocessing

| Preprocessing software | Biomap software |
|------------------------|-----------------|

#### Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

| Normalization template | n/a |
|------------------------|-----|
| Noise and artifact removal | n/a |
| Volume censoring | n/a |

### Statistical modeling & inference

| Model type and settings | no fMRI |
|-------------------------|--------|
| Effect(s) tested | no fMRI |

Specify type of analysis:

- [ ] Whole brain
- [ ] ROI-based
- [ ] Both

Statistic type for inference

(See [Iklund et al., 2016](#))

| Correction | no fMRI |
|------------|--------|

### Models & analysis

| n/a | Involved in the study |
|-----|-----------------------|
| x   | Functional and/or effective connectivity |
| x   | Graph analysis |
| x   | Multivariate modeling or predictive analysis |