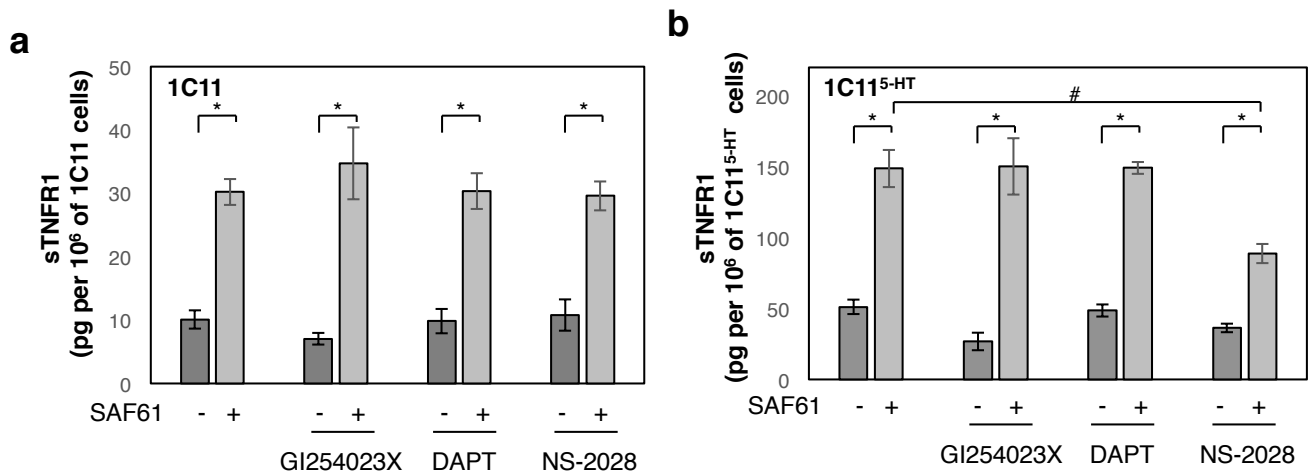


SUPPLEMENTARY INFORMATIONS

Protective role of cellular prion protein against TNF α -mediated inflammation through TACE α -secretase

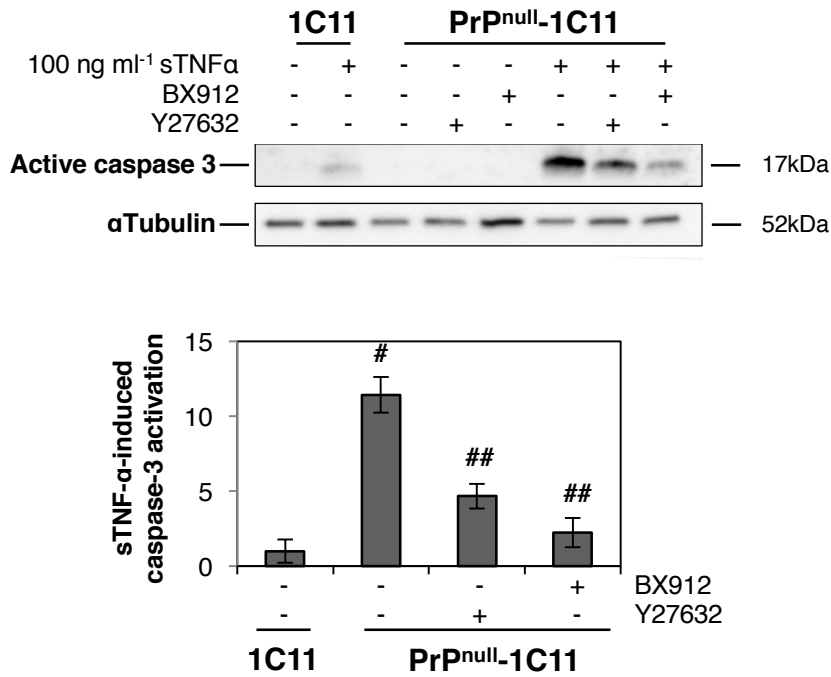
Juliette Ezpeleta, François Boudet-Devaud, Mathéa Pietri, Anne Baudry, Vincent Baudouin, Aurélie Alleaume-Butaux, Nathalie Dagoneau, Odile Kellermann, Jean-Marie Launay and Benoit Schneider

Supplementary Figure 1



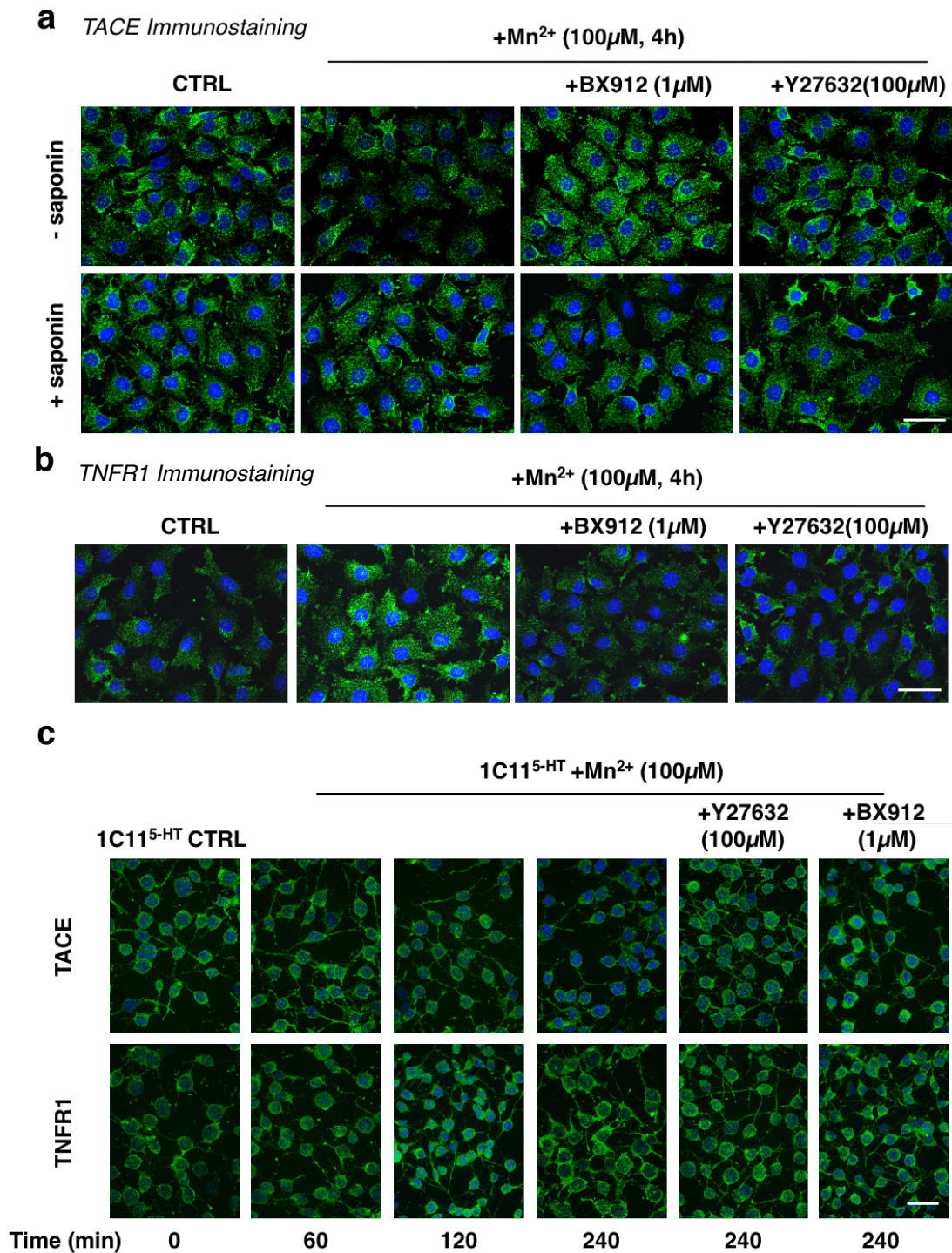
Supplementary Figure 1: Impact of the inhibition of guanylate cyclase, ADAM10 or γ -secretase on PrP^C-stimulated TNFR1 ectodomain shedding. ELISA-based measure of sTNFR1 concentration in the cell culture medium of 1C11 (a) and 1C11^{5-HT} (b) cells treated with SAF61 PrP antibody (10 $\mu\text{g ml}^{-1}$) for 120 min in the presence or not of the guanylate cyclase inhibitor NS-2028 (1 μM), the ADAM10 inhibitor GI254023X (50 nM), or the γ -secretase inhibitor DAPT (10 μM). Data shown are the mean \pm SEM from three experiments performed in triplicate. * p <0.01. # p <0.01.

Supplementary Figure 2



Supplementary Figure 2: Inhibition of ROCK-I or PDK1 protects PrP^{null}-1C11 cells from sTNF α toxicity. Western blots showing a stronger activation of caspase-3 in PrP^{null}-1C11 cells exposed to sTNF α (100 ng ml⁻¹) for 120 min than in 1C11 cells. Antagonizing either ROCK-I activity with Y-27632 (100 μ M for 1h) or PDK1 activity with BX912 (1 μ M for 1h) in PrP^{null}-1C11 cells reduces sTNF α toxic effect. Quantitative data are shown as the mean \pm SEM from three experiments performed in triplicate. # p <0.05 vs. 1C11 cells exposed to sTNF α ; ## p <0.05 vs. PrP^{null}-1C11 cells treated with sTNF α .

Supplementary Figure 3



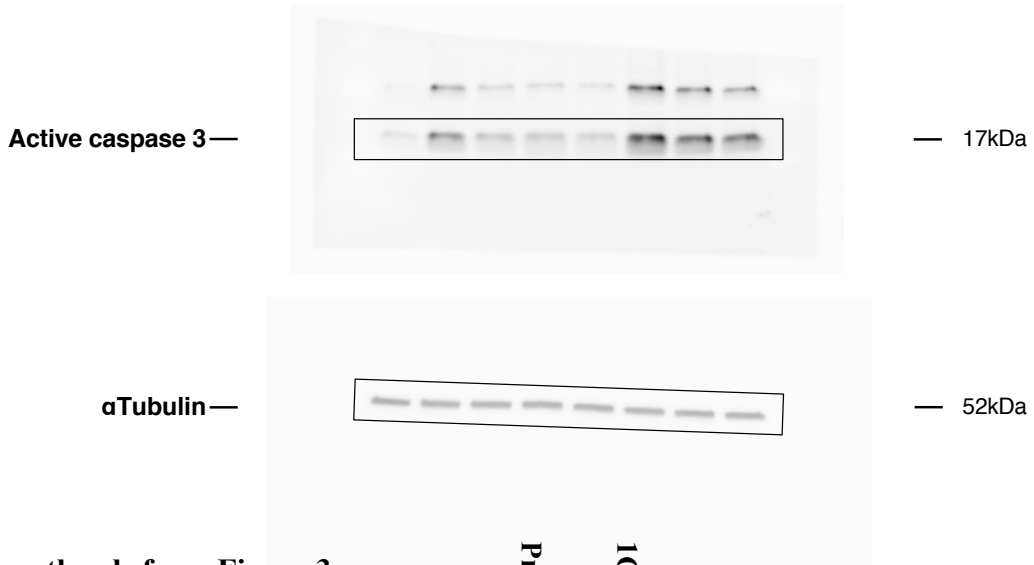
Supplementary Figure 3: Manganese-induced overstimulation of $\beta 1$ integrin signaling triggers TACE internalization and TNFR1 shedding deficit in PrP^C expressing 1C11 and 1C11^{5-HT} cells. Immunolabeling experiments showing internalization of TACE (a) and

increase in plasma membrane TNFR1 staining **(b)** in 1C11 cells exposed to Mn^{2+} (100 μM) for 4h that are counteracted upon inhibition of ROCK-I with Y-27632 (100 μM) or PDK1 with BX912 (1 μM). **(c)** Time-dependent reduction of TACE level and increase of TNFR1 amount at the surface of serotonergic 1C11^{5-HT} neuronal cells exposed to 100 μM Mn^{2+} . The inhibition of ROCK-I with Y-27632 (100 μM) or PDK1 with BX912 (1 μM) blocks Mn^{2+} effects.

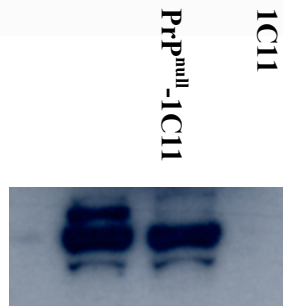
Supplementary Figure 4

Full-length gels from Figure 2e:

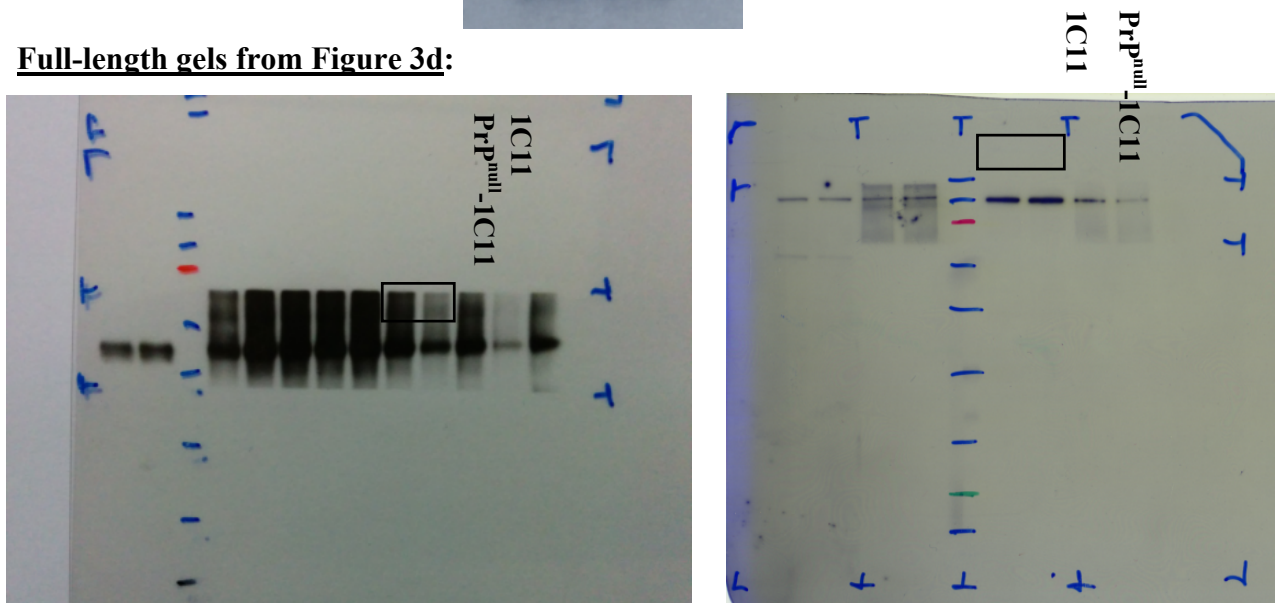
	1C11 ^{5HT}		PrP ^{null} -1C11 ^{5HT}					
10ng ml ⁻¹ sTNF α	-	+	-	-	-	+	+	+
BX912	-	-	-	-	+	-	-	+
Y27632	-	-	-	+	-	-	+	-



Full-length gels from Figure 3a:



Full-length gels from Figure 3d:



Full-length gels from Figure 4e:

