# Online Resource 1

**Article title:** Perineuronal nets enwrap dysmorphic neurons and parvalbumin interneurons at the seizure onset zone of focal cortical dysplasia type IIb

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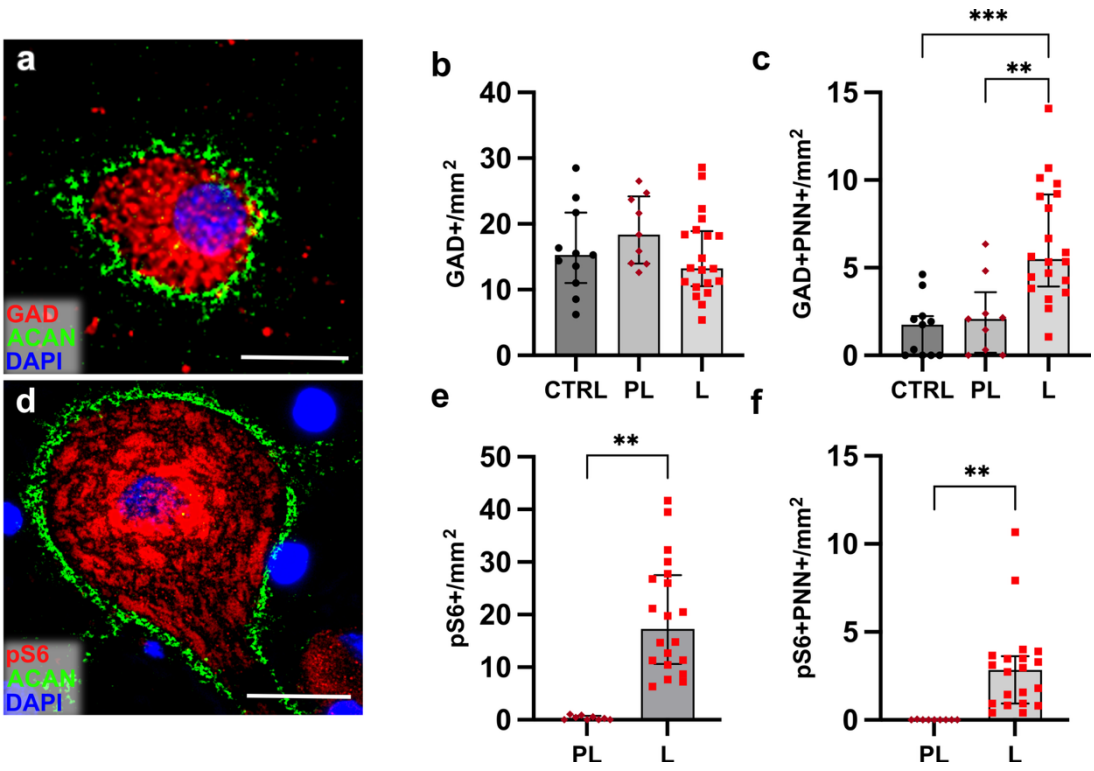
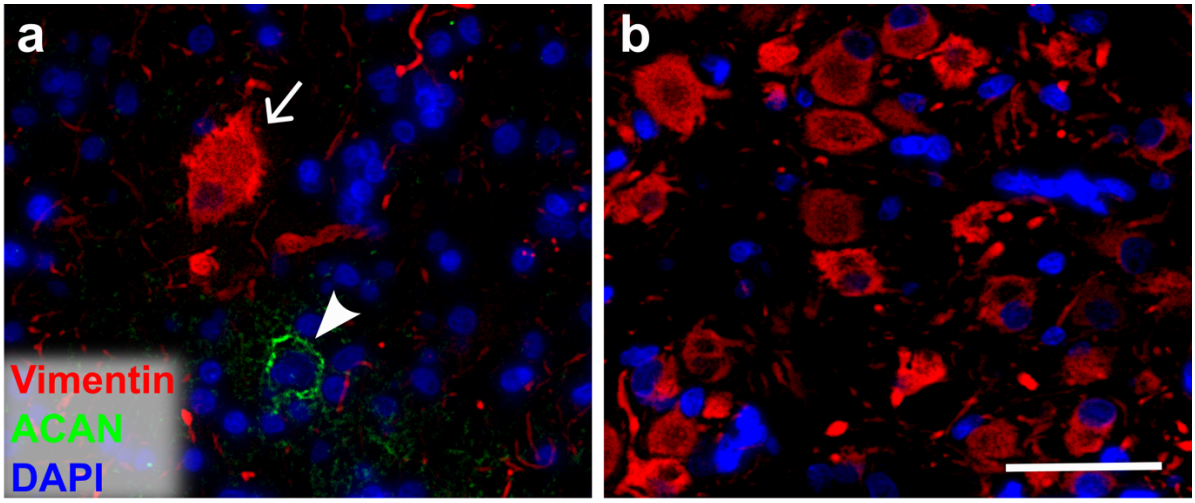
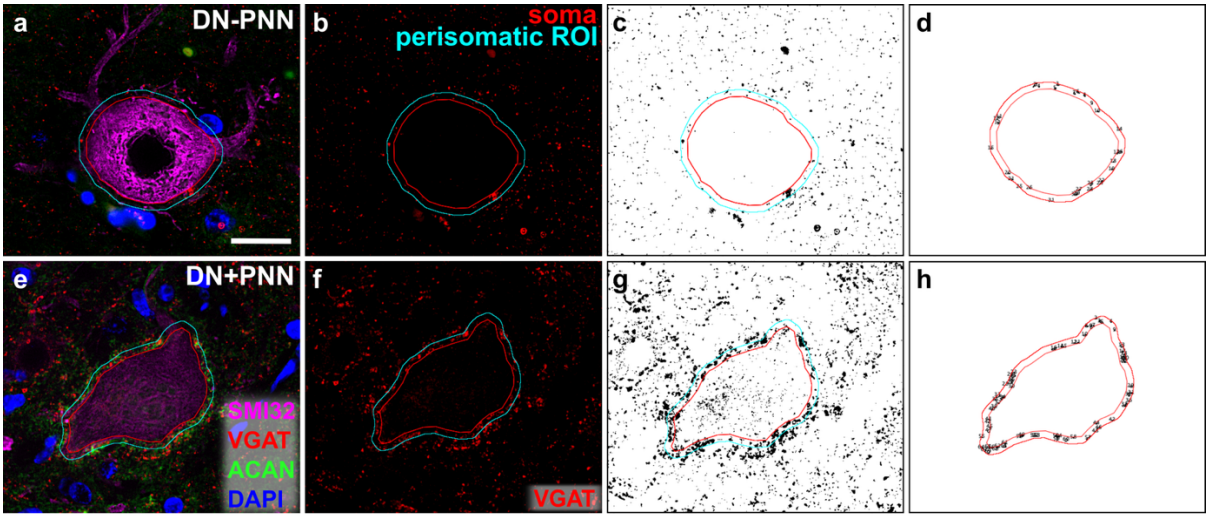
This file contains Supplementary Figures 1–4. The Fiji macro used for the quantification of perisomatic VGAT-positive puncta is provided separately as Online Resource 2.



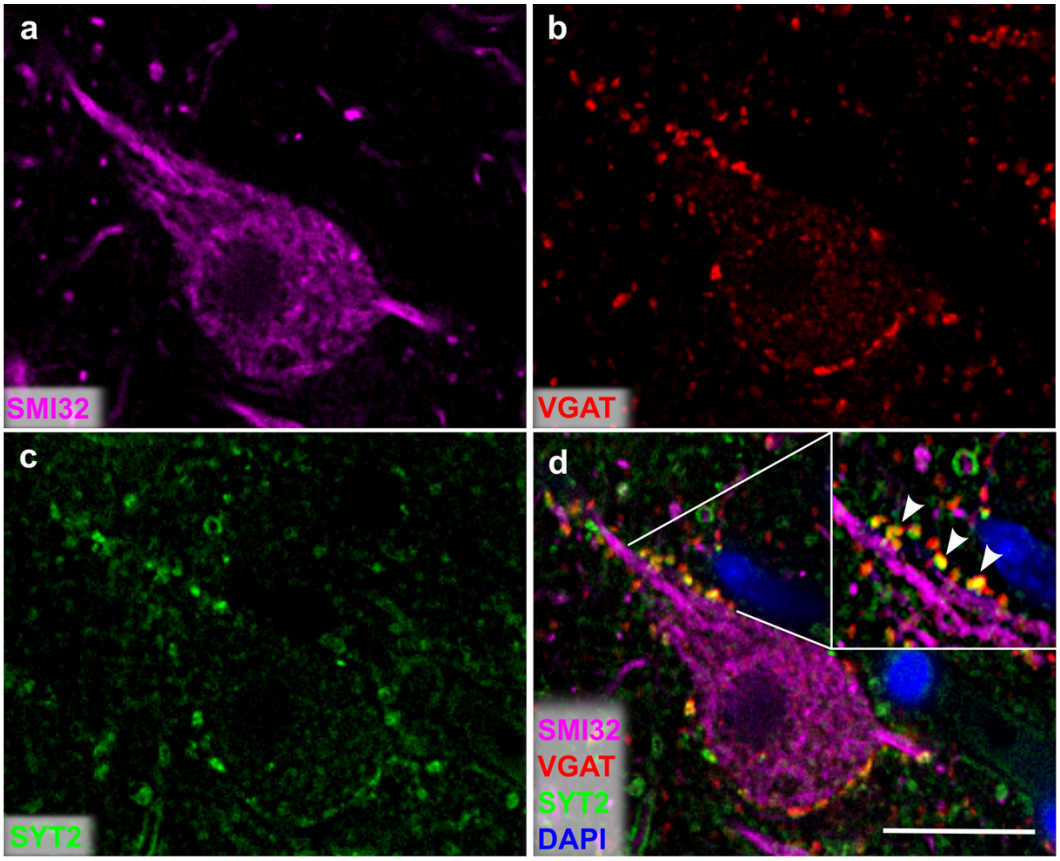

**Suppl. Fig. 1** Perineuronal nets are increased around GABAergic interneurons and phospho-S6-positive dysmorphic neurons in lesional FCDIIb cortex. **a** Aggrecan-positive PNN (ACAN, green) enwrapping a GAD+ interneuron (red); DAPI (blue). Scale bar 10 µm. **b** GAD+ cell density does not differ between control (CTRL, $n = 11$), perilesional (PL, $n = 9$) and lesional cortex (L, $n = 20$). **c** The density of GAD+ cells with PNN is increased in lesional cortex. **d** PNN (green) enwrapping a pS6-positive dysmorphic neuron (red); DAPI (blue). Scale bar 20 µm. **e** Density of pS6-positive DN and **f** of pS6-positive DN with PNN in perilesional and lesional cortex. Data are median with IQR. Statistics: Kruskal–Wallis test with Dunn's correction (**b**, **c**); Wilcoxon matched-pairs signed-rank test (**e**, **f**). $^{**}P < 0.01$; $^{***}P < 0.001$.




**Suppl. Fig. 2** Vimentin-positive balloon cells are not surrounded by aggrecan-positive perineuronal nets in lesional FCDIIb cortex. **a** Vimentin-positive balloon cell (red, arrow) without association with an aggrecan-positive PNN (ACAN, green, arrowhead); DAPI (blue). **b** Accumulation of vimentin-positive balloon cells (red) in the white matter without association with aggrecan-positive PNNs. Scale bar 50 µm (applies to **a** and **b**).




**Suppl. Fig. 3** Workflow for the quantification of perisomatic VGAT-positive puncta on dysmorphic neurons without (a–**d**; DN−PNN) and with PNN (**e**–**h**; DN+PNN). **a**, **e** Neuronal somata were annotated manually on the SMI32 channel (magenta), and each DN was classified as DN−PNN or DN+PNN by the absence or presence of an aggrecan-positive PNN (ACAN, green). **b**, **f** A 3-µm perisomatic region of interest (ROI) was generated automatically around the soma and transferred to the VGAT channel (red). **c**, **g** The VGAT channel was converted to 8-bit and binarized with the Fiji "Default" threshold algorithm. **d**, **h** VGAT-positive puncta within the perisomatic ROI were detected with "Analyze Particles". All steps after manual soma annotation were performed by a custom Fiji macro (Online Resource 2).




**Suppl. Fig. 4** Synaptotagmin-2-positive terminals contribute to the perisomatic inhibitory innervation of dysmorphic neurons in FCDIIb. **a** SMI32-positive DN (magenta). **b** VGAT-positive presynaptic terminals (red) and **c** synaptotagmin-2 (SYT2)-positive puncta (green) surrounding the soma of the DN. d The merged image shows partial overlap of SYT2- and VGAT-positive puncta (inset, arrowheads); DAPI (blue). Scale bar 20 µm (applies to all panels).