SEMA4D upregulation signals neuronal stress and triggers reactive astrogliosis

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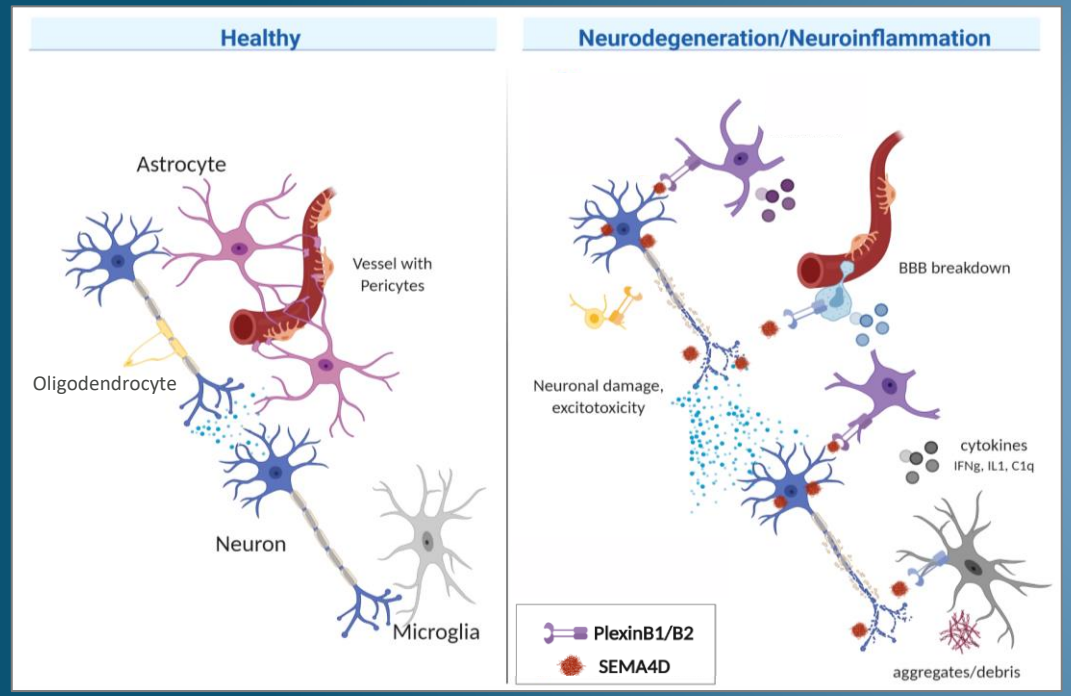
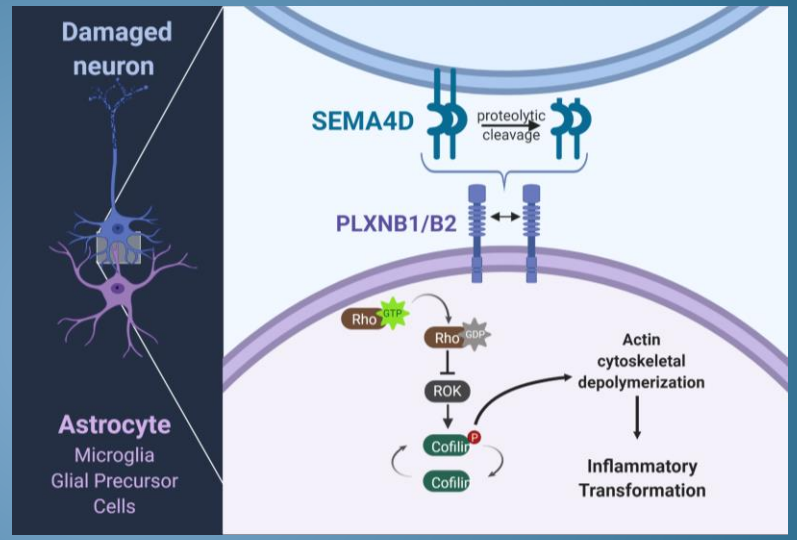


- SEMA4D is upregulated on damaged neurons in HD and AD brains
- Glial cells express receptors for SEMA4D and respond to damage induced by mutant Huntingtin and other neurotoxins
- Reported effects of SEMA4D include microglial activation, survival and differentiation of glial precursor cells, integrity of BBB.
- Chronic activation contributes to and exacerbates neurodegeneration



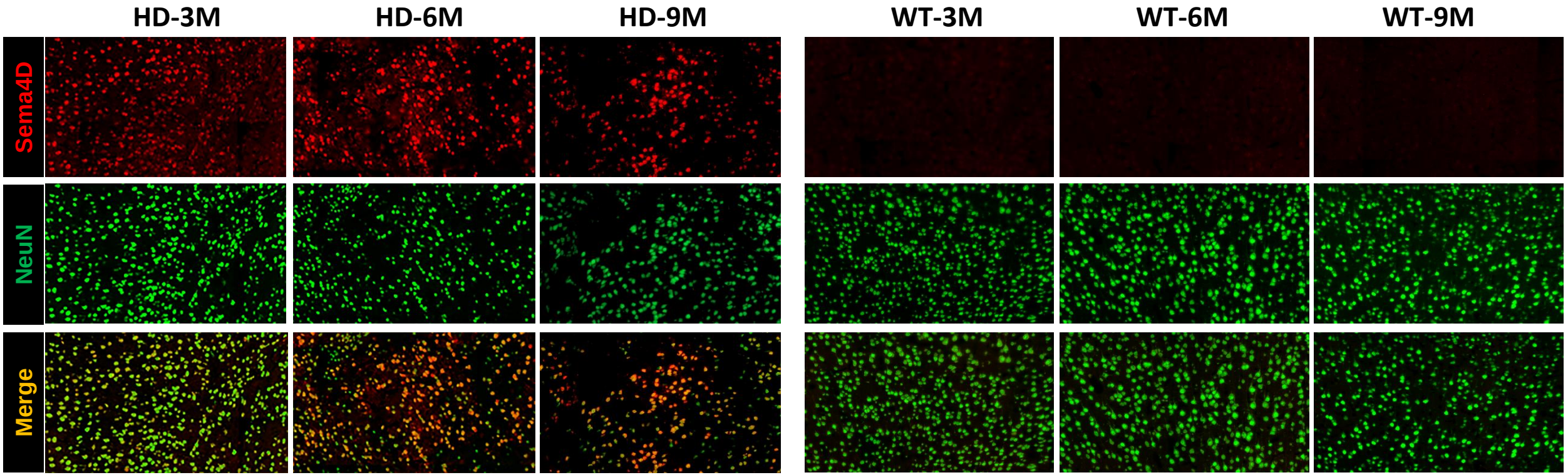
Pepinemab (VX15 antibody) binds to SEMA4D and blocks it's signaling activity.

This preserves and restores normal astrocyte morphology and function

- SEMA4D signals through PLXNB1 and PLXNB2 receptors on astrocytes to trigger dissociation of polymerized F-actin and collapse of cell's cytoskeleton
- The cell cytoskeleton regulates process extensions which enable direct cell to cell interactions.

- **SEMA4D induces changes associated with reactive astrogliosis**
  - **Morphologic rearrangements characterized by hypertrophic cell bodies with retracted short processes and loss of fine processes**
  - **Loss of normal functions, including glucose transport and glutamate recycling**

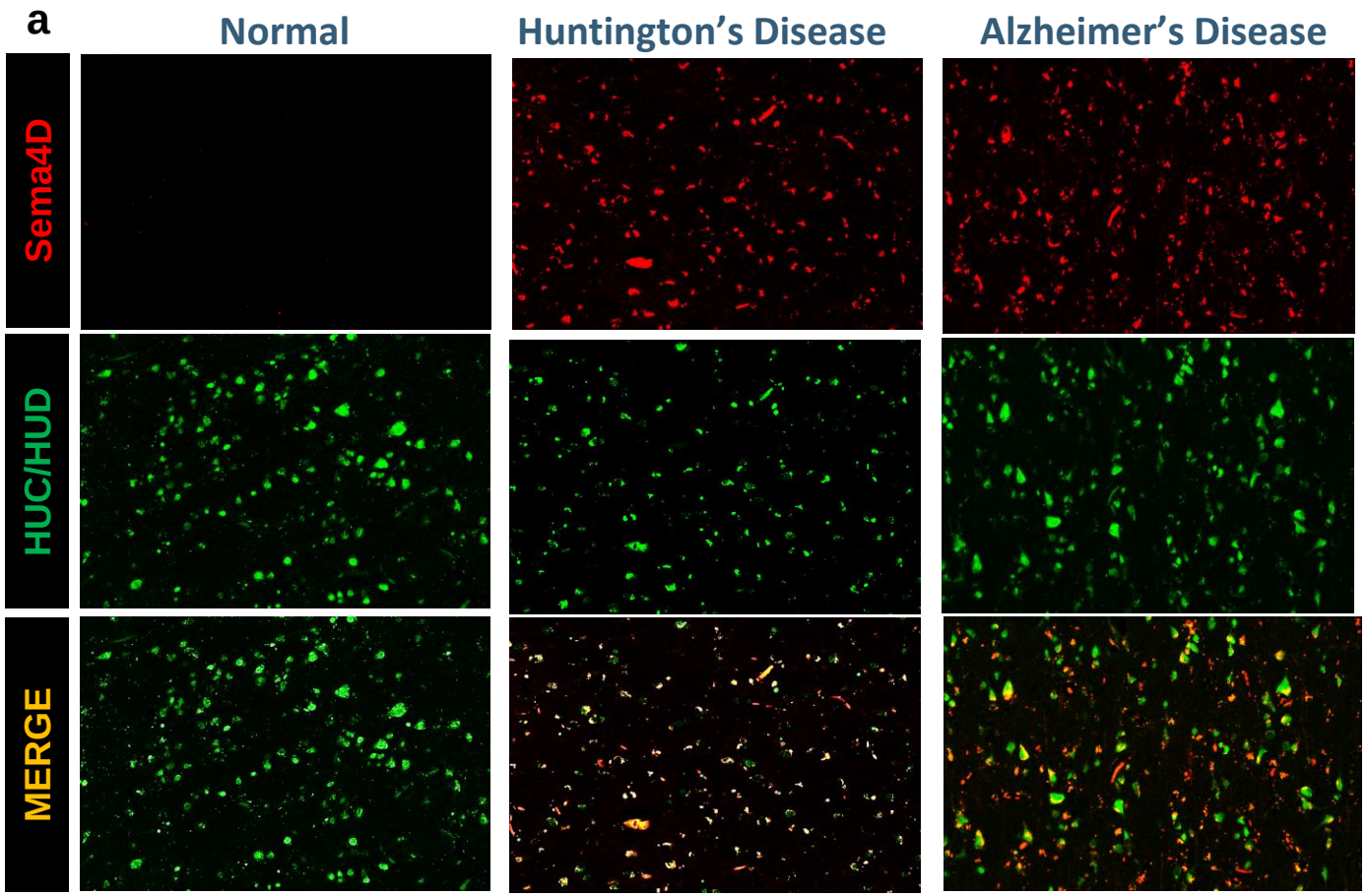
SEMA4D is progressively upregulated in neurons of HD transgenic mice



- **SEMA4D expression is upregulated in HD mice as disease progresses, compared to low expression in WT control.**
  - SEMA4D is upregulated early in disease, prior to onset of symptoms, which occurs ~ 5 months of age in Q175 HD transgenic mice.
- **SEMA4D co-localizes with NeuN+ neurons.**

Figure 1: NeuN/Sema staining of retrosplenial cortex region of Q175 knock-in mouse model of HD and age-matched wild type (WT) littermate controls. Representative images are shown from analysis of 3 mice/time-point. M = months of age.

SEMA4D is upregulated in neurons during Human HD and AD brains



SEMA4D is progressively upregulated with increasing pathologic stages of HD

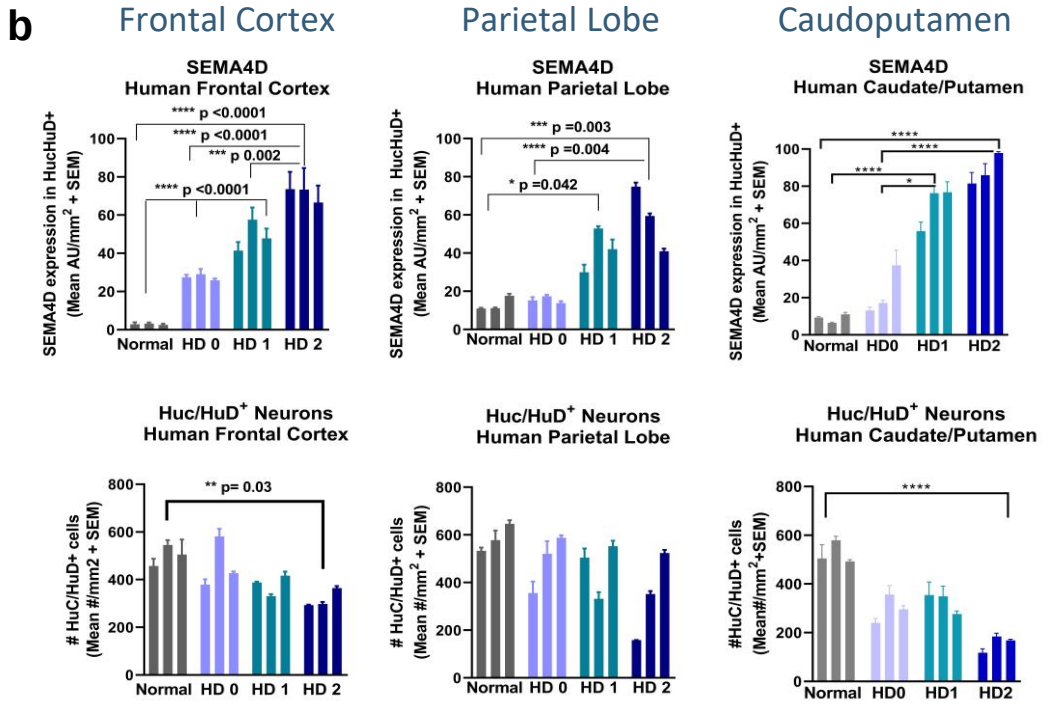
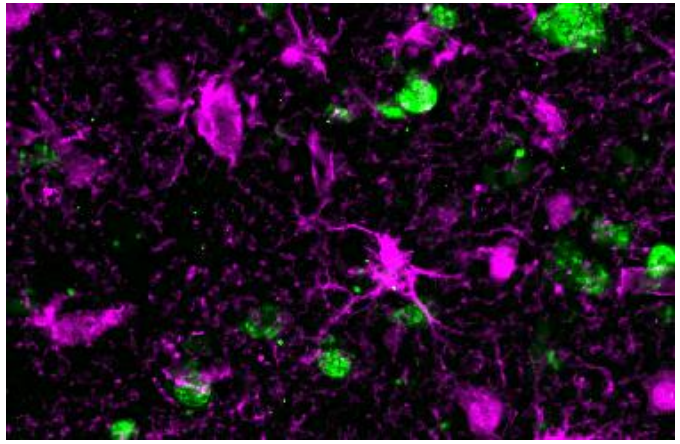


Figure 2: SEMA4D is increasingly upregulated in human HD and AD neurons in parallel with loss of HuC/HuD+ neurons. a. Representative images of frontal lobe are shown. **b.** SEMA4D expression and number of neurons were quantified across entire human autopsy sections of frontal cortex (Inferior frontal gyrus, BA 44-45), parietal lobe (Somatosensory cortex- BA 1,2,3 and part of frontal cortex BA4) and striatum (caudate/putamen) regions. Each bar represents autopsy tissue from one individual, Mean of 3-4 consecutive sections/individual +SEM is shown for each subject/condition. Group differences and statistical significance were determined using one-way ANOVA with Tukey post hoc analysis and is indicated by * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

SEMA4D upregulation is concurrent with reactive astrogliosis in disease

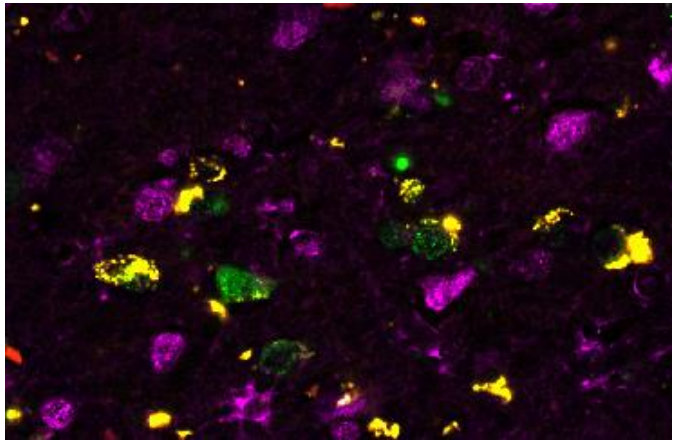
a

Control Human



Glutamine Synthetase expressed in astrocyte cell body and dendritic processes
HuC/HuD expressed in neuronal body
SEMA4D

Huntington's Disease



Glutamine Synthetase expressed in astrocyte cell body

SEMA4D HuC/HuD MERGE

Reactive astrocytes are characterized by

- Collapse of actin cytoskeleton
- Downregulation of glutamine synthetase (GS)
  - GS is required for astrocytic recycling of glutamate and GABA transmitters
- Loss or gain of normal functions

b

Glutamine Synthetase (Astrocytes)

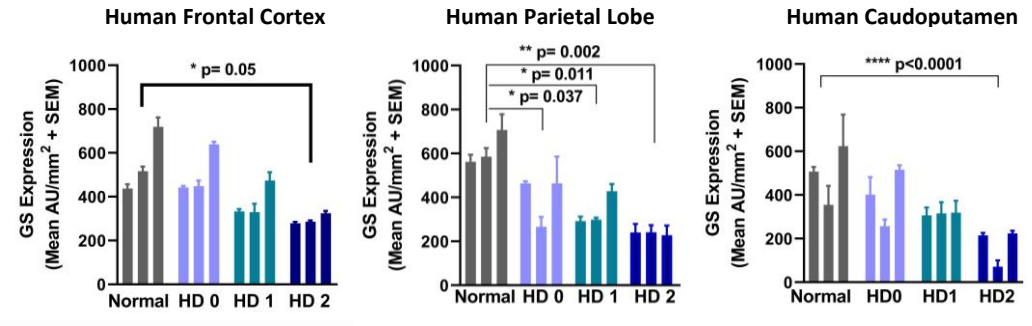
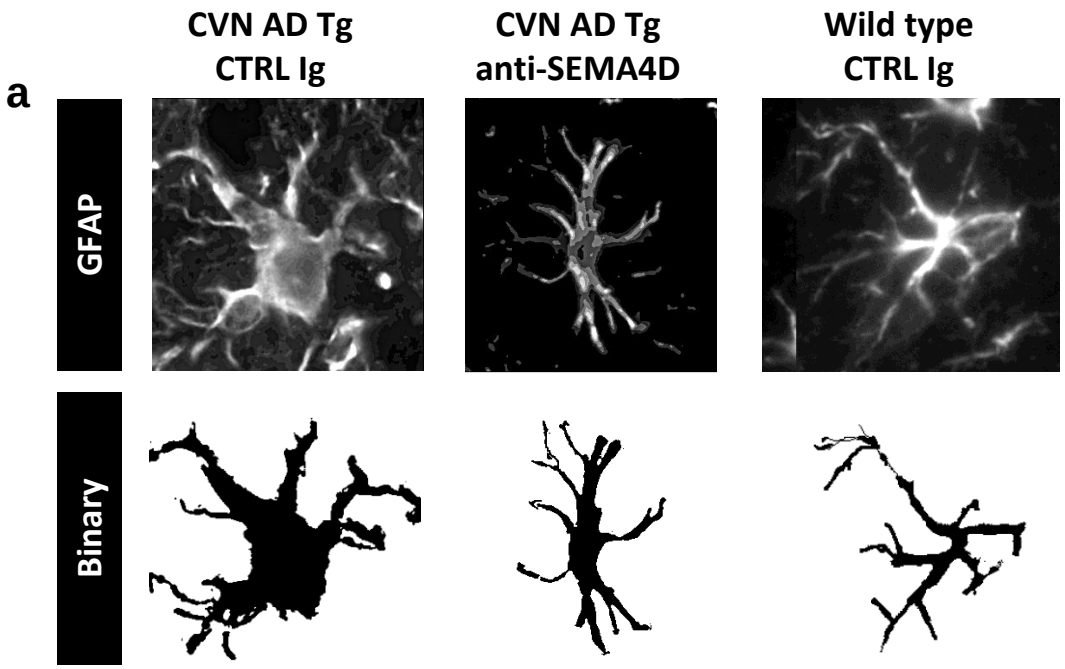
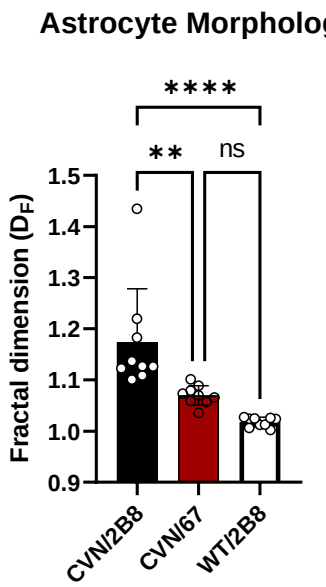


Figure 3. Downregulation of GS in reactive astrocytes is coincident with SEMA4D upregulation in neurons of HD brains. a. Reactive astrocyte morphology, with reduced GS expression and retracted astrocytic endfeet, along with upregulation of neuronally expressed SEMA4D in human HD frontal cortex sections were stained for glutamine synthetase (GS, astrocyte cell body and processes), SEMA4D, and Huc/HuD (neuronal cell body). **b**. Number of GS+ cells were quantified as described in Fig. 2b.

SEMA4D blockade restores loss of GS expression and inhibitory synapses in mouse model of AD

SEMA4D antibody restores normal astrocyte morphology in mouse model of AD





SEMA4D antibody restores loss of astrocytic GS expression

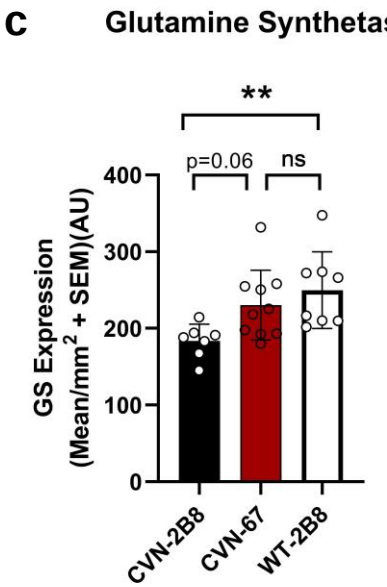


Figure 4. Reactive astrocyte morphologic changes are mediated by SEMA4D and are inhibited with antibody therapy in vivo. CVN (APPSwDI/NOS2-/-) transgenic AD and wild type (WT) mice treated weekly with anti-SEMA4D/67 or isotype control/2B8 antibody from week 26 to 38. Brains were collected at 41 weeks of age, when neurodegeneration and inflammatory responses are evident. CA1 hippocampal region of CVN and wild type mice were stained for GFAP and GS. **a**. Representative images of GFAP stain and conversion to binary images for fractal dimension analysis. Enlarged soma and shorter thicker processes are observed in astrocytes of CVN mice. **b**. Quantification of fractal dimension analysis demonstrates significant morphologic changes in brains of AD mice compared to wild type, which is restored following treatment with anti-SEMA4D antibody. More than 100 GFAP+ cells per section per mouse (3-4 section from 3 mice/treatment group) were analyzed in several optical planes with ApoTome from 10 µm sections; statistics determined from fractal dimension analysis of hippocampal region. **c**. GS expression/nucleated cells was quantified, as described in 5b.

SEMA4D induces morphologic changes in actin cytoskeleton of astrocytes

SEMA4D regulates cytoskeletal changes in human primary astrocyte cultures

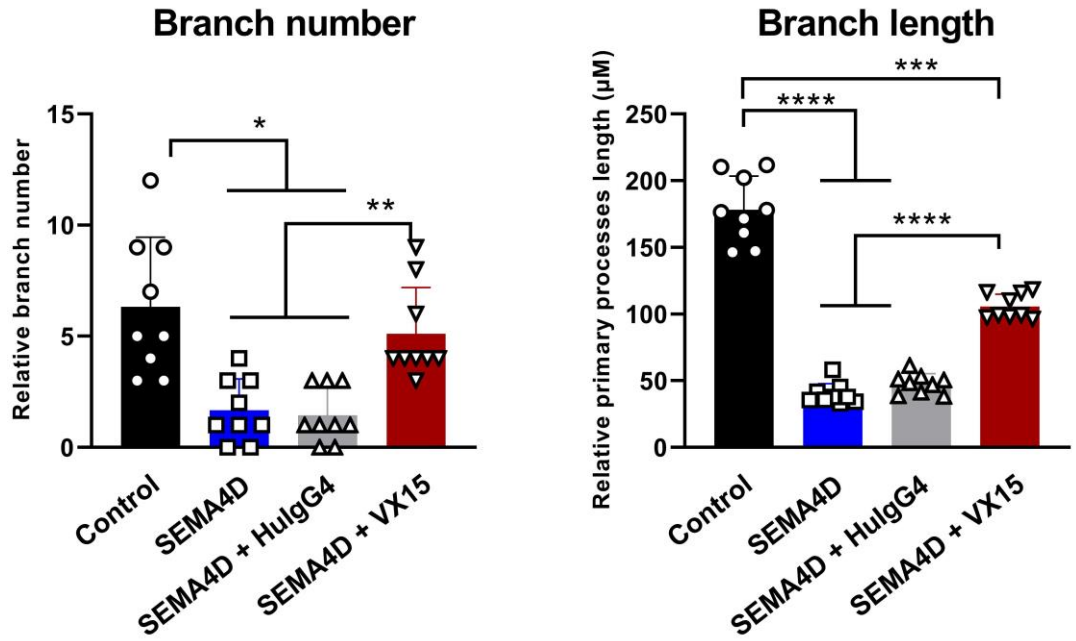


Figure 5. Reactive astrocyte morphologic changes are mediated by SEMA4D and are reversible with antibody blockade of SEMA4D. a. iPSC-derived human astrocytes were cultured in presence of rSEMA4D or control protein. Antibody blocking effect was determined by incubation with rSEMA4D or control protein (5 ug/ml), in presence/absence of anti-SEMA4D antibody/VX15 or isotype control human IgG4 antibody (25 ug/ml) for 48 hours. Morphologic changes in primary branch length and number of branches were quantified in replicate wells.

Astrocytes couple synaptic function and energy metabolism

SEMA4D down regulates glucose and glutamate transporters

Neurotransmitter recycling

Energy metabolism

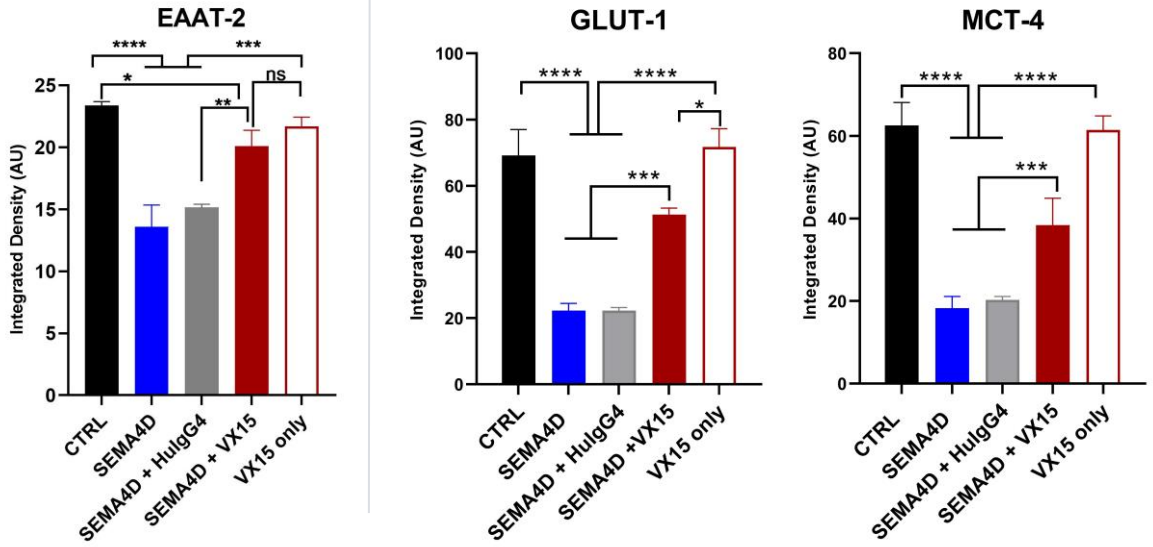
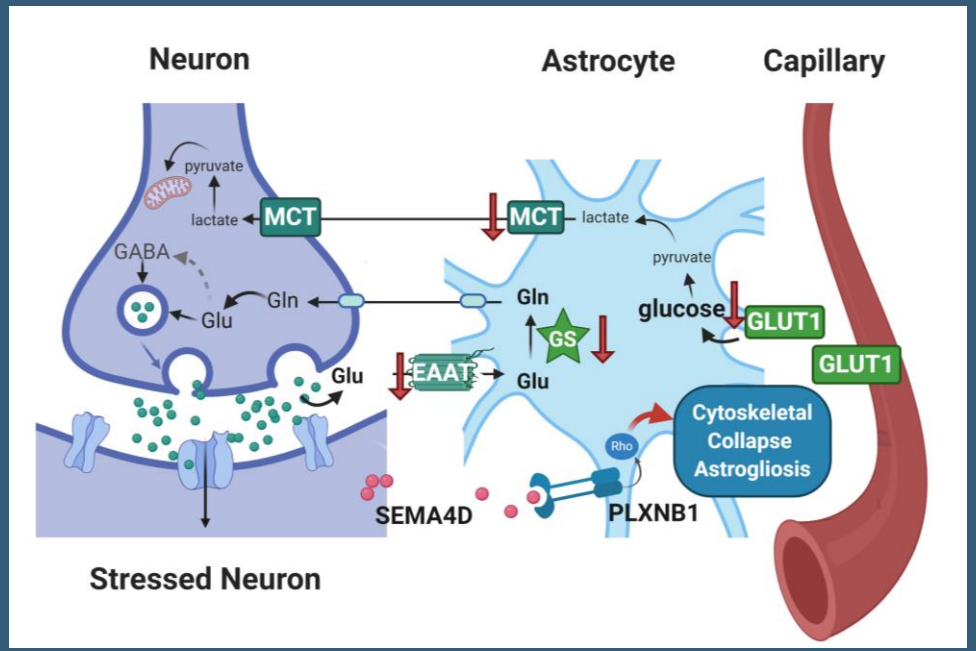


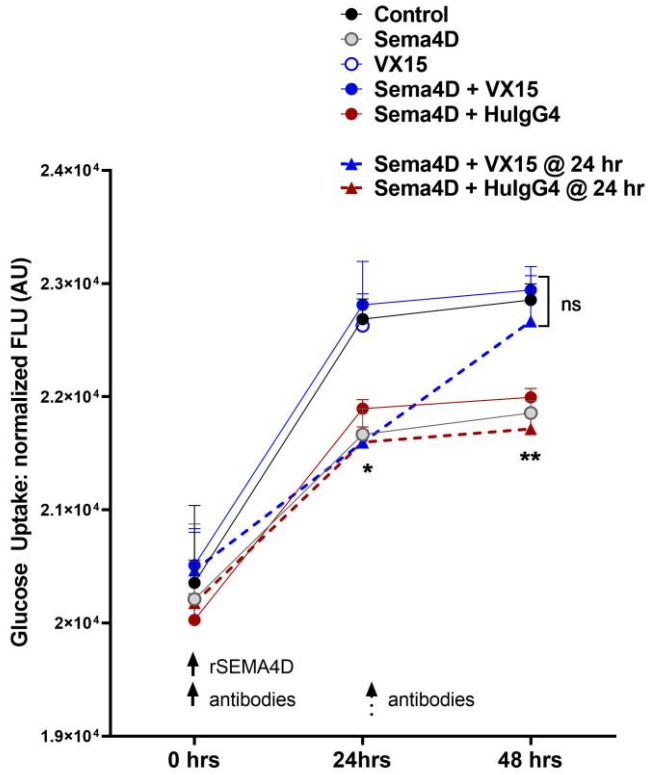
Figure 6. Astrocytic functions of glutamate and glucose transport are mediated by SEMA4D signaling and are reversible with antibody blockade of SEMA4D. iPSC-derived human astrocytes cultures were treated as in Figure 4 and stained for **a**. glutamate transporter (EAAT-2), and glucose (GLUT-1) and lactate (MCT-4) transporters. Antibody blockade of SEMA4D restored transporter expression. Quantification for each condition is shown as average+SEM (bar) from 3 wells (symbol)/condition/timepoint.

Molecular changes and interplay between neuronal damage and astrocyte reactivity: SEMA4D is upregulated on damaged neurons. SEMA4D signals to PLXN receptors on astrocytes and triggers astrocyte activation and associated changes in glutamate recycling and energy transport.



SEMA4D antibody blockade restores loss of glucose uptake

SEMA4D induces loss of glucose uptake *in vitro*
Antibody blockade reverses loss of astrocytic metabolic function



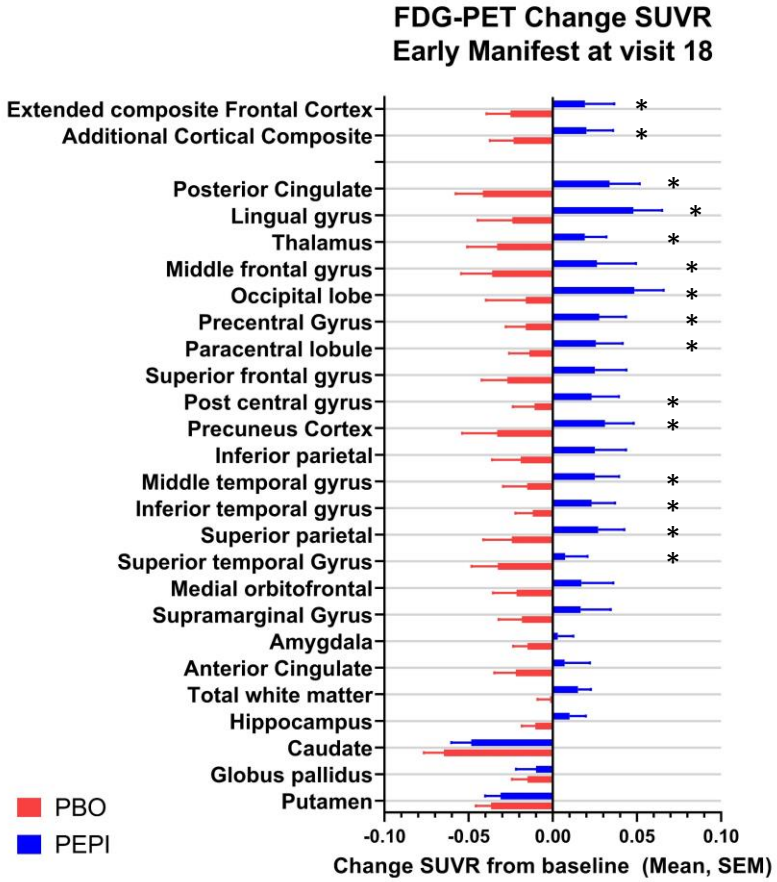
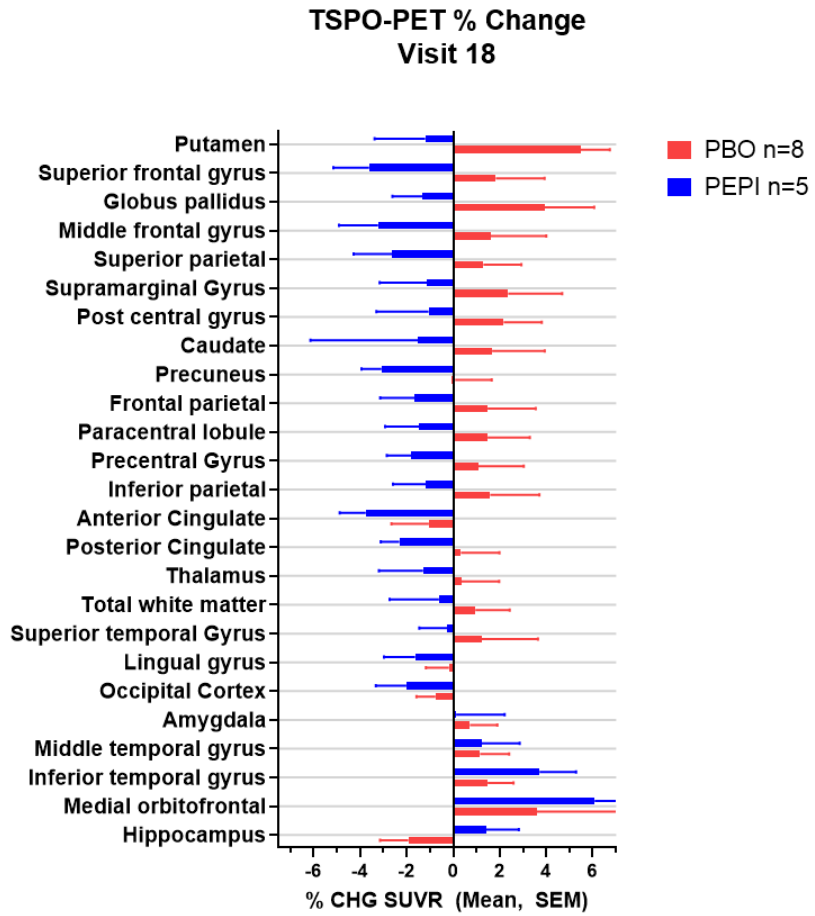


Figure 7. a. Uptake of fluorescent non-metabolizable glucose analog was measured in human primary astrocyte cultures as in 6a.To evaluate reversal of activity, rSEMA4D was added at time 0 and antibodies were added at t=24 hr (dotted lines), anti-SEMA4D/VX15 or isotype control HuIgG4. b. Uptake of radioactive non-metabolizable glucose analog was measured by FDG-PET in pre-manifest HD patients in SIGNAL trial. Mean change in SUVR+SEM from baseline to month 18 is shown for each brain region of interest. PBO (Placebo) n=31, PEPI (pepimenab/VX15) n=28.

SEMA4D antibody blockade appears to reverse the increase in TSPO-PET signal, a biomarker of neuroinflammation



TSPO is expressed by glial cells (microglia and astrocytes) and is upregulated under neuroinflammatory conditions, serving as a biomarker using TSPO radioligands.

Both microglia and astrocytes express receptors for and are activated in presence of SEMA4D.

Pepinemab (PEPI) treatment appears to reverse the increase in TSPO-PET observed in the placebo group (PBO) among early manifest subjects in SIGNAL.

Figure 8. Uptake of radioactive translocator protein 18 kDa (TSPO) was measured by TSPO-PET in HD patients in SIGNAL trial. Mean percent change in SUVR+SEM from baseline to month 18 is shown for each brain region of interest. Group sizes are very small, however, these data suggest a positive trend toward reduced glial activation in the study, which is consistent with mechanism of action of reducing neuroinflammation.

Conclusions and future directions

- We previously reported that an anti-SEMA4D reduced anxiety-like behavior and cognitive deficits in YAC128 HD transgenic mice, as evidenced by rescue of object recognition and location spatial learning deficits in YAC128 mice. Significant preservation of neuropathological brain atrophy and healthy medium spiny neurons was also reported, however, treatment effects with respect to astrocyte phenotype and neuronal function were not characterized in this model.
- SEMA4D is progressively upregulated on damaged neurons in HD. Astrocytes express Plexin receptors and display characteristic properties associated with reactive astrogliosis in response to SEMA4D
    - Morphologic rearrangements characterized by hypertrophic cell bodies with retracted short processes and loss of fine processes
    - Molecular changes, including downregulation of glucose and lactate transporters, GLUT-1 and MCT4, and molecules associated with neurotransmitter recycling, including glutamine synthetase and glutamate transporter EAAT-2
    - Loss of normal functions such as glucose uptake
- Antibody blockade can inhibit and reverse these effects in preclinical *in vitro* and *in vivo* models

- Evidence from SIGNAL clinical trial supports mechanism of action whereby pepinemab appears to reverse neuroinflammation and restores loss of normal functions associated with reactive gliosis
    - Additional clinical data demonstrate treatment benefits in cognition assessments and brain atrophy (vMRI), as reported by Zauderer et al
- The mechanism of action is believed to be applicable to diseases exacerbated by inflammatory glial activation.
    - A Phase 1 study in AD is planned.
    - The mechanism may complement gene therapy or other independent approaches

Smith et al. 2014 Neurobiology of Disease
Southwell et al. 2015. Neurobiology of Disease
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