



TREAT-AD

TaRget Enablement to Accelerate
Therapy Development for AD

SLIT1

(slit guidance ligand 1)

A Target Enabling Package (TEP)

Gene ID / UniProt ID / EC	6585/075093
Target Nominator	TREAT-AD, AMP-AD
TREAT-AD Authors	Katerina Gospodinova ¹ , Ishita Ajith ¹ , Jesse Wiley ² , Gregory Cary ³ , and the Emory-Sage-SGC TREAT-AD Center
Therapeutic Area(s)	Alzheimer's disease
Document version	1.0
Document version date	February 2024
Citation	https://zenodo.org/doi/10.5281/zenodo.10694504
Affiliations	¹ Centre for Medicines Discovery, University of Oxford, Oxford, United Kingdom ² Sage Bionetworks, Seattle, WA, USA ³ The Jackson Laboratory, Bar Harbor, ME, USA

USEFUL LINKS



TREAT-AD

[Visit TREAT-AD](#)

Learn more about the TREAT-AD centers and mission



Agora

[Visit Agora](#)

View Alzheimer's disease target results explorer



AD Knowledge Portal

[Visit AD Knowledge Portal](#)

View available target enabling resources

CONTENTS OF TEP

Bioinformatic analysis: Target source & hypothesis

Protein constructs & expression methods: EGFLike domain of Human SLIT2 (E916-E1534).

TARGET SOURCE & HYPOTHESIS

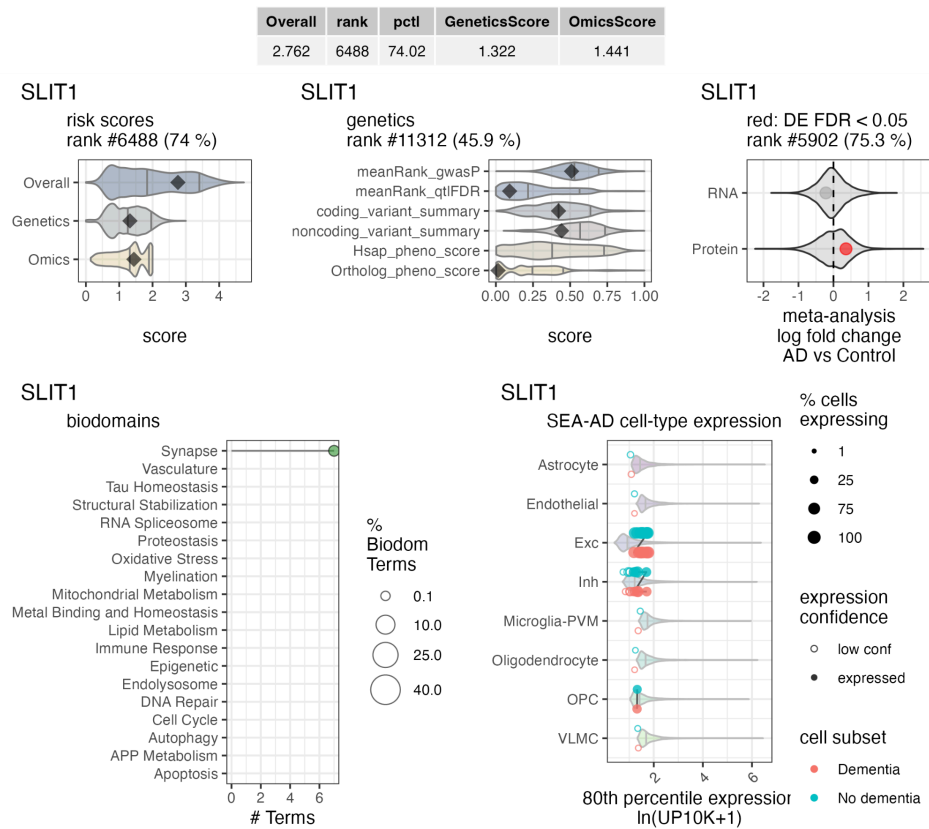
Why was the target selected? This target is found within a TMT proteomics network module that was highly correlated with cognition. This module (Module 42) contains several novel AD targets, including SMOC1 and SFRP1(1,2).

TREAT-AD Target Risk Score: 2.76 (rank #6488, 74th percentile)

The TREAT-AD Center has developed a target ranking score encompassing genomics and genetics evidence. The Target Risk Score represents the gene target’s general relevance to Alzheimer's Disease, and is the sum of the target's Genetics Score and Genomics Score. Target Risk Score values range from 0 to 5, with 5 being evidence of the strongest association with AD. A complete description of the methodology used to calculate these scores is available [here](#). This score was taken from Agora, Data Version syn13363290-v62.

The TREAT-AD Target Risk Score for this target is 2.76 out of 5 (rank #6488, 74th percentile). The individual score components include 1.32 of 3 for genetics, and 1.44 out of 2 for genomics. The meta-analysis of proteomic and transcriptomic data sources used in the genomics score indicates that the expression of SLIT1 protein is significantly increased in brains from patients with AD.

The TREAT-AD Center has also developed a target categorization system specific to AD relevant processes, termed biological domains (biodomains). These biological domains are defined by constituent Gene Ontology (GO) terms and genes are then annotated to specific biological domains via GO term annotations. SLIT1 is annotated to GO terms from the Synapse domain.



Cell-type specific expression is assessed using single-cell expression data from the Seattle Alzheimer’s Disease Brain Cell Atlas (SEA-AD). The distribution of expression values for all genes found in each broad cell type are displayed as violins. The expression of the target in subtypes within each broad class is shown as a point. Blue points show the summary expression of cells from individuals with no dementia, while red points show summary metrics of cells from dementia patients. Open circles reflect low confidence measures of

expression. The expression of SLIT1 is confidently detected in oligodendrocyte precursor cells (OPC) and both excitatory and inhibitory neuron sub-types within this dataset.

SUMMARY OF PROJECT

SLIT1 is a secreted axon guidance factor that through association with the Robo receptor family can direct the axonal repulsion and appropriate developmental synapse formation (3–5). The identification of SLIT1 in a core module identified in proteomic studies of AD brain(4) suggests an involvement in AD, and further investigation, as little is currently known about SLIT1 involvement in AD. The goal of this project is to

develop validated antibodies, purified protein, and a bioinformatics workup as part of the SLIT1 target enablement package (TEP). We hope these resources will be useful to the scientific community in advancing the understanding of its role in AD pathogenesis.

SCIENTIFIC BACKGROUND

Slit guidance ligand 1 (SLIT1) is a large axon guidance and repulsion protein of 1531 amino acids that is secreted into the extracellular space (6,7). The protein is composed of 20 leucine rich repeat regions (LRR) and 9 EGF-like domains (Uniprot: O75093) that enable its signalling properties through its association with the Robo family of single pass transmembrane receptors (6,7). SLIT1 mediated axonal guidance is critical in development to ensure midline partitioning of retinal axons (8–10), the guidance cues from midbrain to cortical connections (3–5), and may facilitate peripheral regeneration of dorsal root ganglion neuronal axons after injury (5,11,12). The combination of SLIT family members and Robo receptors may drive different effects within different biological systems, as there is evidence that both SLIT1 and SLIT2 may regulate angiogenesis and tumor growth (13–15). The inactivation of SLIT1 transcription via promoter hypermethylation is common in tumor cells (15,16), likely to prevent its tumor suppressor during metastasis.

The relationship between SLIT1 and AD is unknown, yet as an axon guidance factor SLIT1 is known to influence neuronal cytoskeletal structure (3), which is disrupted in AD. Further, SLIT1 was identified in a deep proteomic investigation of AD brains versus healthy controls, appearing in a small co-expression module associated with cognitive decline and pathological progression in AD (2). The role of the SLIT family in synapse formation in development may continue in adulthood as synaptic maintenance and plasticity, facilitating new or strengthened neuronal connections (17,18), which could impact synaptic durability and resilience during encroaching pathogenesis in AD. While the relationship between SLIT1 and AD is unknown and purely speculative at the present time, the resources developed here will hopefully help the scientific community explore and elucidate the gene-disease relationship.

RESULTS – THE TEP

Proteins Purified

1. SLIT1A-c003: EGFLike domain of Human SLIT2 (E916-E1534). Contains a C-terminal 6His and AviTag (allowing in vitro biotinylation, if needed). Protein produced in Expi293F cells.

See **Additional information (section 1)** with details of plasmid expression constructs and purification procedures.

CONCLUSION

The tools presented here provide a foundation for further investigation of the role of SLIT1 in AD.

FUNDING INFORMATION

For more information or questions regarding the TEP, please contact treatad.info@sagebionetworks.org

The work performed by the Emory-Sage-SGC TREAT-AD Center has been funded by the National Institute on Aging through grant U54 AG065187.

Additional Information

Materials and Methods

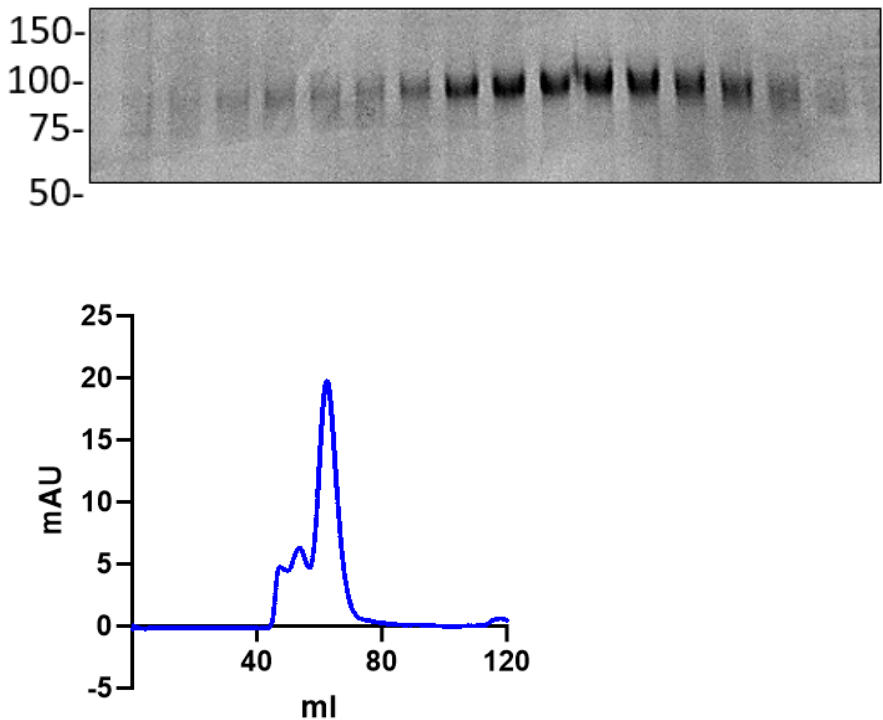
Protein expression constructs and protein purification

All relevant plasmids are available on addgene: [TREAT-AD plasmid collection](#)

1. SLIT1A-c003: EGFlke domain of Human SLIT2 (E921-E1529). Contains a C-terminal 6His and AviTag (allowing in vitro biotinylation, if needed). Protein produced in Expi293F cells.

Construct ID	SLIT1A-c003
Parental vector	pHL-avitag3
Tag	C-terminal His6, C-terminal Avitag
Protein mass (with tag and secretion signal)	73138.8 Da
Extinction Coefficient (M⁻¹ cm⁻¹)	57300
Protein sequence (with tag)	MGILPSPGMPALLSLVSLLSVLLMGCVAETGGPPTLAVQAKCDLCLSSPCQNQGTCHNDPL EVYRCACPSGYKGRDCEVSLDSCSSGPCENGTTCHAQEGEDAPFTCSCTGFEPTCGVNT DDCVDHACANGGVCDGVGNITCQCPLQYEGKACEQLVDLCSPDLNPCQHEAQCVGTP DGPRCECMPGYAGDNCSNQDDCRDHRCQNGAQCMEVNSYSCLCAEGYSGQLCEIPP HLPAPKSPCEGTECQNGANCVDQGNRPVCQCLPGFGGPECEKLLSVNFVDRDTYLQFTDL QNWPRANITLQVSTAEDNGILLYNGDNDHIAVELYQGHVRSYDPGSYPSSAIYSAETINDG QFHTVELVAFDQMVNLSIDGGSPMTMDNFGKHITLNSEAPLYVGGMPVDVNSAAFRWL QILNGTGFHGCIRNLYINNELQDFTKTQMKPGVVPGEPCRKLYCLHGICQPNATPGPMCH CEAGWVGLHCDQPADGPCHGHKCVHGQCVPLDALSYSCQCQDGYSGALCNQAGALAEP CRGLQCLHGHCAQSGTKGAHCVCDPGFSGELCEQESECRGDPVRDFHQVQRGYAICQTTR PLSWVECRGSCPGQGCCQGLRLKRRKFTFECSDGTSFAEEVEKPTKCGCALCAGTGGSGGS GLNDIFEAQKIEWHEGRTKHHHHHH

Purified Protein

Size Exclusion Chromatography: SLIT1A-c003 (with tag)	 <p>The figure displays size exclusion chromatography results for SLIT1A-c003 (with tag). The top panel is a gel image with molecular weight markers at 150, 100, 75, and 50 kDa. A single band is visible at approximately 100 kDa. The bottom panel is a chromatogram showing mAU (mAbsorbance Units) on the y-axis (ranging from -5 to 25) versus ml on the x-axis (ranging from 40 to 120). A single peak is observed at approximately 60 ml, reaching a maximum mAU of about 20.</p>
Intact Mass Deconvolution: GPNMB-c200 (with tag)	N/A
Observed Mass:	N/A
Protein yield:	0.9 mg/L of culture

Expression and Purification Protocol	
Expression host	<i>Mammalian</i> : Expi293F cells (Thermo Fisher Scientific)
Expression medium	FreeStyle 293 Expression Medium (Thermo Fisher Scientific)
Plasmid purification	Transform the construct into the <i>E. coli</i> strain MACH1. Plate on LB-agar plates containing ampicillin (100 µg/ml). Inoculate 5 ml LB broth containing ampicillin (100 µg/ml) with a single colony and grow 6h at 37°C with shaking. Inoculate 800 ml LB broth containing the same antibiotic with 2 ml of starter culture and grow overnight at 37°C with shaking. Split culture into 4 and perform MaxiPrep (Qiagen) with 4 columns according to manufacturer's instructions. Add 0.7 volumes of isopropanol to precipitate the plasmid. Spin (17000g, 30 min, 4°C) and wash pellet with 70% ethanol. Resuspend plasmid with sterile TE buffer under sterile conditions. Yield is typically 2 mg of plasmid.

Transfection	<ol style="list-style-type: none"> 1. Split Expi293F cells to 1×10^6 cells/ml in 1L FreeStyle 293 Expression Medium. Incubate for 24 h until cells are approximately 2×10^6 cells/ml (37°C, 150 rpm, 8% CO₂, 75% rh). 2. Add plasmid to 20mL of pre-warmed Opti-MEM at 0.5 µg/ml final concentration. 3. Add linear PEI (1 mg/ml sterile stock) to another 20mL of pre-warmed Opti-MEM at 3 µg/ml final concentration. 4. Add the mixture from Step 3 to the one in Step 2 and incubate at room temperature for 30 min. 5. Add the plasmid/PEI mixture slowly to the cells. 6. Add sodium butyrate (1 M sterile stock) to 12 mM final concentration. 7. Incubate cells at 30°C (8%CO₂/75% rh) with shaking at 150 rpm. 8. Harvest cell culture supernatant at 5 days post transfection (900g, 20 min, 4°C). 9. Filter supernatant through 0.2 µm filter.
Purification buffers	<ol style="list-style-type: none"> 1. Ni IMAC W10 buffer: 50 mM HEPES (pH 7.5), 200 mM NaCl, 10 mM imidazole, 5% glycerol 2. Ni IMAC W25 buffer: 50 mM HEPES (pH 7.5), 200 mM NaCl, 25 mM imidazole, 5% glycerol 3. Ni IMAC elution buffer: 50 mM HEPES (pH 7.5), 200 mM NaCl, 250 mM imidazole, 2% glycerol 4. Size exclusion chromatography elution buffer : dPBS (from ThermoFisher) 5. Ni-sepharose beads, equilibrated in Ni IMAC W10 buffer.
Purification step 1: IMAC	<ol style="list-style-type: none"> 1. Add imidazole to filtered supernatant to 10 mM final concentration. 2. Add a total of 2.5 ml equilibrated Ni-sepharose beads to the cell supernatant, split into 50-ml falcon tubes. Mix by rotation for 1 hr in a cold room. 3. Spin beads/supernatant slurry (700g, 5 min, 4°C). Decant supernatant and wash beads with 100 ml W10 buffer. Repeat wash with 50 ml W10. Spin again and transfer beads to gravity column in a cold room. 4. Wash column with 25 ml W25. 5. Elute protein with 1x 10 ml elution buffer, followed by 2x5 ml elution buffer. Analyse the EB fractions by SDS-PAGE and determine the protein yield using the Bradford assay. Pool elution fractions containing protein.
Purification step 2: Size Exclusion Chromatography	<ol style="list-style-type: none"> 1. Concentrate pooled IMAC elution fractions to 1 ml using a centrifugal concentrator with the appropriate MWCO. 2. Perform SEC on a HiLoad Superdex S200 HR 16/60 column, equilibrated in DPBS, at 1 ml/min. 3. Analyse fractions by SDS-PAGE. Pool fractions containing protein of desired purity and concentrate to 1-5 mg/ml, as measured by UV spectroscopy. 4. Snap-freeze aliquots in thin-walled PCR tubes in liquid N₂, and store at -80°C.

References

1. Johnson ECB, Dammer EB, Duong DM, Ping L, Zhou M, Yin L, et al. Large-scale proteomic analysis of Alzheimer's disease brain and cerebrospinal fluid reveals early changes in energy metabolism associated with microglia and astrocyte activation. Nat Med. 2020 May;26(5):769–80.
2. Johnson ECB, Carter EK, Dammer EB, Duong DM, Gerasimov ES, Liu Y, et al. Large-scale deep multi-layer

analysis of Alzheimer's disease brain reveals strong proteomic disease-related changes not observed at the RNA level. *Nat Neurosci.* 2022 Feb;25(2):213–25.

3. Round JE, Sun H. The adaptor protein Nck2 mediates Slit1-induced changes in cortical neuron morphology. *Mol Cell Neurosci.* 2011 Aug;47(4):265–73.
4. Bielle F, Marcos-Mondéjar P, Leyva-Díaz E, Lokmane L, Mire E, Mailhes C, et al. Emergent growth cone responses to combinations of Slit1 and Netrin 1 in thalamocortical axon topography. *Curr Biol.* 2011 Oct;21(20):1748–55.
5. Zhang HY, Zheng LF, Yi XN, Chen ZB, He ZP, Zhao D, et al. Slit1 promotes regenerative neurite outgrowth of adult dorsal root ganglion neurons in vitro via binding to the Robo receptor. *J Chem Neuroanat.* 2010 Jul;39(4):256–61.
6. Nakayama M, Nakajima D, Nagase T, Nomura N, Seki N, Ohara O. Identification of high-molecular-weight proteins with multiple EGF-like motifs by motif-trap screening. *Genomics.* 1998 Jul;51(1):27–34.
7. Itoh A, Miyabayashi T, Ohno M, Sakano S. Cloning and expressions of three mammalian homologues of *Drosophila* slit suggest possible roles for Slit in the formation and maintenance of the nervous system. *Brain Res Mol Brain Res.* 1998 Nov;62(2):175–86.
8. Yang JJJ, Bertolesi GE, Hehr CL, McFarlane S. Lhx2/9 and Etv1 Transcription Factors have Complementary roles in Regulating the Expression of Guidance Genes slit1 and sema3a. *Neuroscience.* 2020 May;434:66–82.
9. Jin Z, Zhang J, Klar A, Chédotal A, Rao Y, Cepko CL, et al. Irx4-mediated regulation of Slit1 expression contributes to the definition of early axonal paths inside the retina. *Development.* 2003 Mar;130(6):1037–48.
10. Plump AS, Erskine L, Sabatier C, Brose K, Epstein CJ, Goodman CS, et al. Slit1 and Slit2 cooperate to prevent premature midline crossing of retinal axons in the mouse visual system. *Neuron.* 2002 Jan;33(2):219–32.
11. Chen B, Carr L, Dun XP. Dynamic expression of Slit1-3 and Robo1-2 in the mouse peripheral nervous system after injury. *Neural Regeneration Res.* 2020 May;15(5):948–58.
12. Zhang Q, Zhao J, Shen J, Zhang X, Ren R, Ma Z, et al. The ATP-P2X7 Signaling Pathway Participates in the Regulation of Slit1 Expression in Satellite Glial Cells. *Front Cell Neurosci.* 2019 Sep;13:420.
13. Katoh Y, Katoh M. Comparative genomics on SLIT1, SLIT2, and SLIT3 orthologs. *Oncol Rep.* 2005 Nov;14(5):1351–5.
14. Zheng Y, Xiao M, Zhang J, Chang F. Micro RNA-640 Targeting SLIT1 Enhances Glioma Radiosensitivity by Restraining the Activation of Wnt/ β -Catenin Signaling Pathway. *Br J Biomed Sci.* 2022 Apr;79:10067.
15. Shuai W, Wu J, Chen S, Liu R, Ye Z, Kuang C, et al. SUV39H2 promotes colorectal cancer proliferation and metastasis via tri-methylation of the SLIT1 promoter. *Cancer Lett.* 2018 May;422:56–69.
16. Dickinson RE, Dallol A, Bieche I, Krex D, Morton D, Maher ER, et al. Epigenetic inactivation of SLIT3 and SLIT1 genes in human cancers. *Br J Cancer.* 2004 Dec;91(12):2071–8.
17. Ying Z, Wu J, Jiang W, Zhang G, Zhu W, Li X, et al. Expression of Slit and Robo during remodeling of corticospinal tract in cervical spinal cord in middle cerebral artery occlusion rats. *Mol Biol Rep.* 2021 Dec;48(12):7831–9.

18. Beker M, Caglayan AB, Beker MC, Altunay S, Karacay R, Dalay A, et al. Lentivirally administered glial cell line-derived neurotrophic factor promotes post-ischemic neurological recovery, brain remodeling and contralesional pyramidal tract plasticity by regulating axonal growth inhibitors and guidance proteins. *Exp Neurol*. 2020 Sep;331:113364.

We respectfully request that this document is cited using the DOI value as given above if the content is used in your work.