

Choroid plexus apocrine secretion shapes CSF proteome during mouse brain development

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Protein	Fold Change	Protein	Fold Change	Protein	Fold Change
CFL2	-2.677702396	ANGPT1	1.107722	CAPN2	1.253244
SLMAP	-1.958047138	APBB1	1.122756	SHH	1.254541
MGMT	-1.489150925	EIF3B	1.135808	CHMP3	1.256712
NPM1	-1.465645541	EDAR	1.140927	SULT1B1	1.259806
ACBD4	-1.3126736	TYRP1	1.144411	HSD17B10	1.263076
FAM171A1	-1.306032518	YWHAH.1	1.14687	GFRA1	1.263377
HSPA5	-1.289718269	NTN1	1.149025	COL2A1.1	1.264269
FIBP	-1.239313573	NTN1.1	1.149307	TLL1	1.27252
CPTP.1	-1.216096043	MIF	1.15044	PPP2R1A	1.273816
RBM39	-1.199984307	CADM1	1.156866	EFNA2	1.2745
CYTL1	-1.196602888	MAP2K3	1.16222	DMKN	1.287389
LILRB5	-1.187896776	SYAP1	1.162807	CHST1	1.287997
MYBPC2	-1.187287086	ENTPD6	1.163364	EIF4B	1.30313
TNFRSF25	-1.152109912	DNAJC18	1.164101	NEGR1	1.315286
YTHDC1	-1.13757003	TPST2	1.165798	FGFRL1.1	1.329058
NOSIP	-1.134353999	FAM171B.1	1.166307	IGF2	1.340103
ACVR1	-1.13010118	STK3	1.17156	GLT8D1	1.340113
MET.1	-1.111471542	POMC.2	1.173689	EFNA5	1.348281
TMEM9	-1.094764252	NFASC	1.175271	NLGN2.1	1.351096
IGHG2	-1.093790708	UBXN4.1	1.184758	THSD7A	1.352122
CLUH	-1.085820113	ACLY	1.189366	NEGR1.1	1.354892
LRP11.1	-1.079009834	ANAPC10	1.189717	NUCB1	1.363485
THAP2	1.073949919	CMPK1	1.193424	C11orf68	1.368757
WBP2	1.074126956	PTPRU	1.196621	OTUD7B	1.372287
SHBG.1	1.07450517	EPN1	1.200221	VAPA	1.390194
TMEM52	1.076830491	SLAMF1	1.208397	SBDS	1.395874
DGKB	1.077596117	HAUS1	1.211578	NUCB2	1.400142
MAGEA3	1.081612965	SIGLEC11.1	1.212659	NLGN3.1	1.439247
CGA LHB	1.082951889	ING1	1.216631	ACAT2	1.463587
EPHB3	1.083444976	SERPINE2	1.224738	LDHA.1	1.467336
ULBP3	1.08347554	MCTS1	1.225412	KREMEN1	1.468277
SKP1	1.085563306	TPST1	1.22785	IVD	1.497916
DFFA	1.089705016	HOMER2	1.229924	PTK7	1.617312
FLRT1.1	1.089984573	PARK7.2	1.23303	DSTN	1.751027
DDI1	1.092664998	PCDH9	1.238185	SLITRK6	1.975016
UBE2D3.1	1.094902212	NDRG3	1.24016	TYMS	1.998942
NRG1.1	1.095929315	ANXA6	1.242997	AMIGO2	2.336661
NTN4	1.100043446	GALNT14	1.246098	AMIGO2.1	2.453852
CHST15	1.102332036	HSPH1	1.246523	NLGN4Y	2.932438
ATP23	1.10241375	FTCD	1.253056	NLGN3	3.033497
PDGFA	1.104117096				
CD2AP	1.106712066				
GRIA4	1.10749003				

Supplementary Table 1. Protein list showing differential abundance by SomaLogic analysis. All differentially abundant proteins between control CSF and apocrine CSF at E16.5.

Symbol	Protein	Documented effects on neural progenitor or radial glia cell fate
ANGPT1	Angiopoietin-1	- Promotes survival and differentiation of neural progenitor cells via the PI3K/Akt pathway⁴. - Promotes proliferation and differentiation of neural progenitor cells through the Notch and Wnt signaling pathways⁵.
EFNA2	Ephrin-A2	- Influences proliferation and differentiation, guiding neural progenitor behavior⁶. - Inhibits apical progenitor proliferation in the ventricular zone⁷. - Negatively regulates neural progenitor cell growth in adult mouse SGZ and SVZ⁸.
EFNA5	Ephrin-A5	- Ectopic expression of ephrin-A5 in cortical progenitors caused transient wave of apoptosis, resulting in premature depletion and decreased cortical size⁹. - Overexpression of EFNA5 induced apoptosis in neural epithelial cells and was accompanied by severe brain malformation during embryonic development¹⁰. - Ephrin-A5-deficient mice have more deep-layer and fewer upper-layer cortical neurons¹¹. Also, EphA4 receptors are expressed by cortical progenitors and can be activated by ephrin A5-Fc. - Impaired hippocampal neurogenesis in adult ephrin-A5-deficient mice¹²
FLRT1	Fibronectin Leucine Rich Transmembrane Protein 1	Affects cerebral cortex neuron migration¹³.
IGF2	Insulin-like Growth Factor 2	- IGF2 promotes neural progenitor proliferation and survival¹⁴. - Promotes stemness and self-renewal of neural-restricted precursors¹⁵.
KREMEN1	KREMEN1	- Typically membrane-bound receptor, not well-established that secreted KREMEN1 has effects - Wnt antagonist, affects specification of ectoderm into neural ectoderm cell fate¹⁶.
MIF	Macrophage Migration Inhibitory Factor	- Promotes proliferation and neuronal differentiation of neural stem cells through Wnt/B-catenin signal pathway¹⁷. - Upregulates Sox6 in neural progenitor cells, promoting survival and maintenance and inhibiting differentiation¹⁸. - Increased MIF promotes microglial proliferation¹⁹.
NRG1	Neuregulin 1	- Induces proliferation of neural progenitors via PLC/PKC pathway²⁰. - Activates migration of neuronal precursors expressing ErbB4²¹. - As a cell-extrinsic signal, promotes cell divisions generating neurons from progenitors in zebrafish²². - NRG1 signaling is required for the establishment of radial glia and their transformation into astrocytes in cerebral cortex²³.

		Regulates neural precursor growth and the generation of oligodendrocytes <i>in vitro</i>²⁴.
NTN1	Netrin-1	- Contributes to vascular remodeling in the SVZ and promotes progenitor migration²⁵. - Regulates astroglial proliferation and migration, which is essential for proper telencephalic morphogenesis and corpus callosum formation²⁶.
PDGFA	Platelet-Derived Growth Factor A	- Induces oligodendrocyte precursor cell migration²⁷. - Important for astroglial differentiation from neural progenitors²⁸. - Overexpressing PDGF-A in retinal ganglion cells caused hyperproliferation of astrocytes²⁹.
PTK7	Protein Tyrosine Kinase 7	- Typically a membrane receptor tyrosine kinase, but can undergo proteolytic cleavage and its secreted form can modulate cellular processes by interacting with Wnt pathway components³⁰. - Inhibits canonical Wnt/β-catenin signaling by diverting Wnt ligands away from Frizzled receptors that are necessary for canonical signaling³¹. - Can activate non-canonical Wnt pathways³².
SHH	Sonic Hedgehog	- Maintains progenitor pool, acts as a mitogen for neural progenitors, elevated SHH signaling induces ventral progenitor fate in dorsal cortex (reviewed in ³³) - Required in dorsal progenitors for late embryonic transition to oligodendrogenesis³⁴.

Supplementary Table 2. Proteins secreted by ChP 5HT2C activation with documented effects on the cortical neuroepithelium or WNT, BMP, or SHH signaling.

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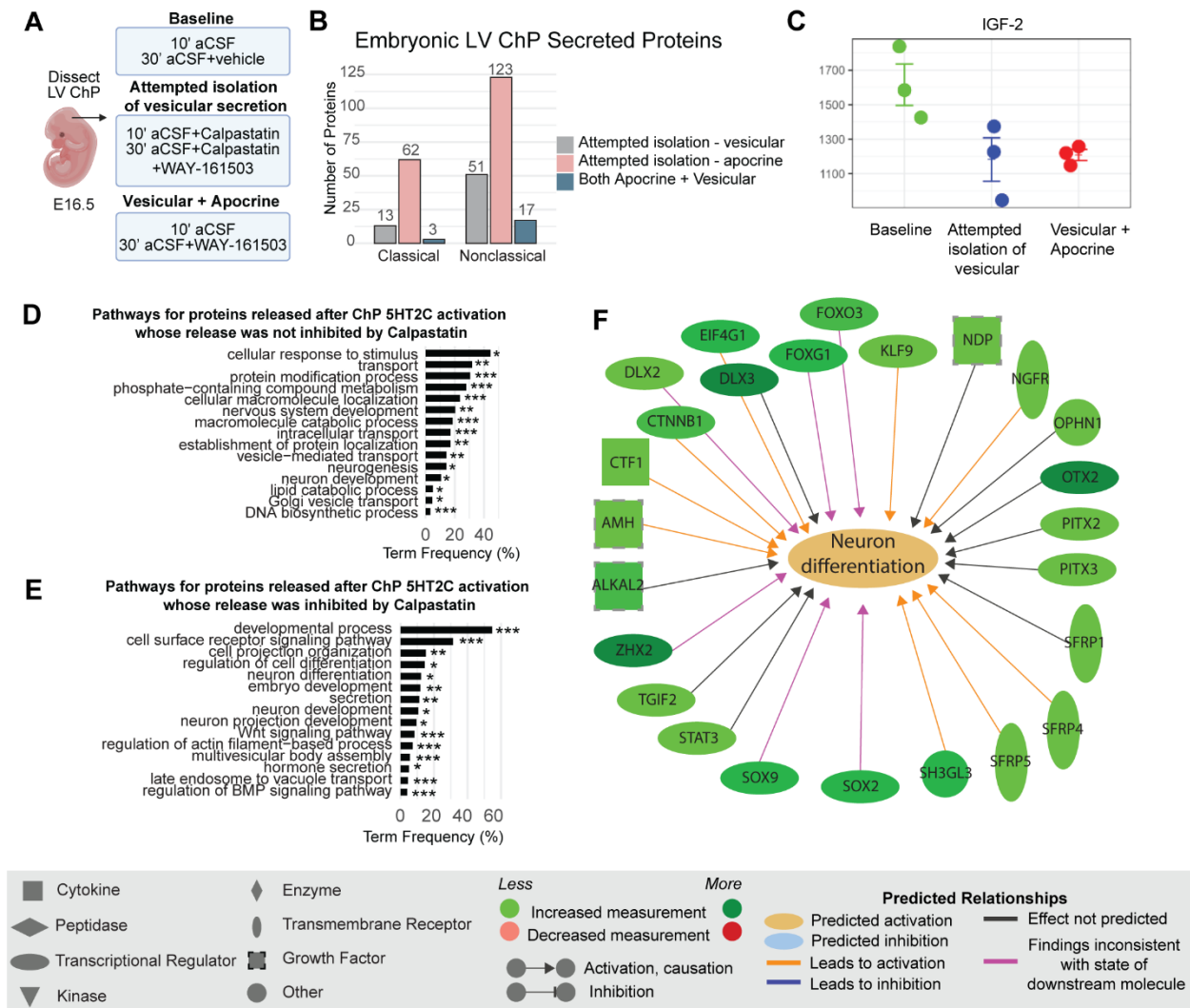
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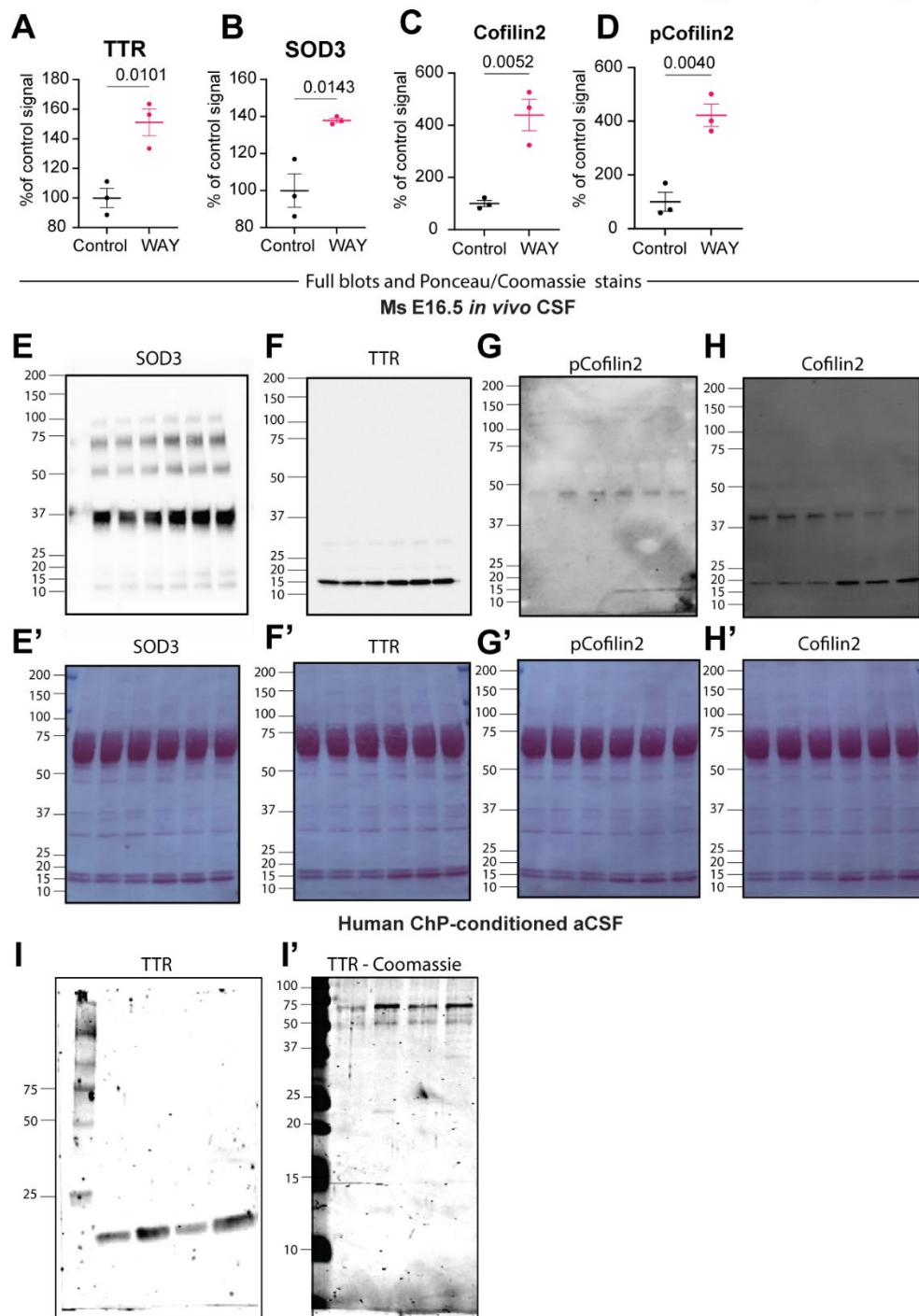
Supplementary Data Fig. 1



Supplementary Figure 1. Probing the contributions of apocrine vs. vesicular secretion to the embryonic choroid plexus secretome. (A) Experimental schematic (B) Bar graph showing number of classically and non-classically secreted proteins preferentially released by our attempt to disambiguate LV ChP vesicular secretion, apocrine secretion, or both. (C) In contrast to increases in IGF-2 shown repeatedly by ELISA after the same experiment, SomaLogic SomaScan intensity levels for IGF-2 suggest a decrease in abundance after WAY-161503.

N = 3 biological replicates per condition. (D) Significant biological pathways identified by GSEA for proteins released by our attempt to isolate ChP apocrine or (E) vesicular secretion. (F) IPA of ChP apocrine secreted proteins demonstrated release of many proteins affecting neuron differentiation. All data presented as mean $\pm$ SEM.

Supplementary Data Fig. 2



Supplementary Figure 2. Immunoblot quantifications, full blots, and full Ponceau stains. (A-D) Quantification of relative protein abundance in CSF from WAY-161503-treated (WAY) and control embryos at E16.5. **(A)** Transthyretin (TTR) levels show significant increases in the WAY group compared to controls ($P = 0.0101$, *two-tailed unpaired t-test*). **(B)** Superoxide dismutase 3 (SOD3) is also elevated in WAY-treated embryos ($P = 0.0143$, *two-tailed unpaired t-test*). **(C)** Cofilin-2 levels and **(D)** phosphorylated Cofilin-2 (pCofilin-2) are markedly increased in the WAY group compared to controls ($P = 0.0052$ and $P = 0.0040$, *two-tailed unpaired t-tests*). **(E-H)** Full immunoblots for SOD3 **(E)**, TTR **(F)**, pCofilin-2 **(G)**, and Cofilin-2 **(H)** in CSF samples collected from control and WAY-treated embryos. **(E'-H')** Corresponding Ponceau stains of total protein loading to confirm equal sample loading and transfer efficiency for each blot. Molecular weight markers (kDa) are indicated on the left. Data are represented as mean $\pm$ SEM. Statistical analysis was performed using two-tailed unpaired t-tests. **(I)** Full immunoblot and **(I')** Coomassie blue stain for TTR in conditioned aCSF samples collected from human ChP tissue with or without exposure to WAY-161503. Data Presentation: $n = 3$ pooled CSF samples for (A)-(D).