

Supplemental Material

The choroid plexus is a source of active renin in the central nervous system

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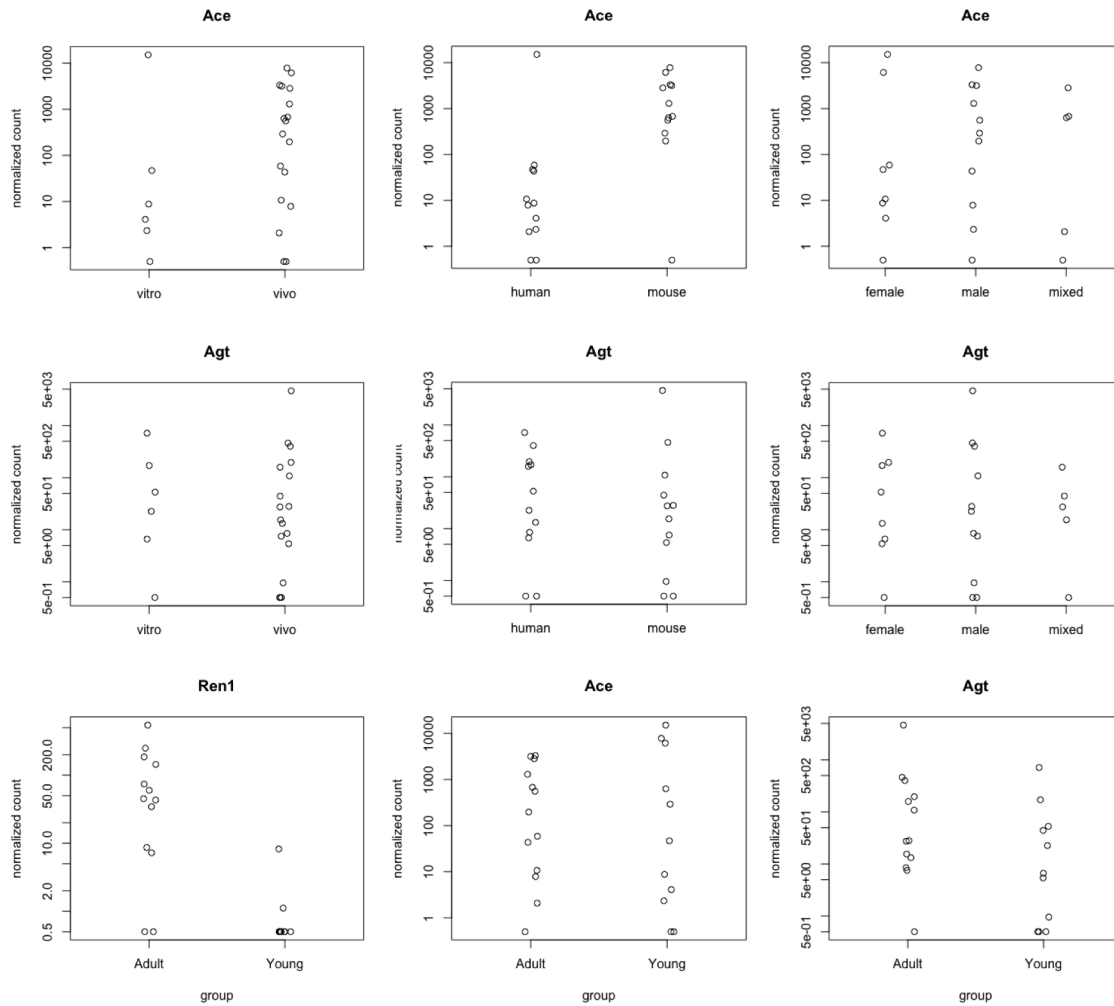
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Study	Species	Sex	Age	Method	System	Choroid Plexus Epithelial Cell (n)	Ren1/REN
Yang 2021 ²⁸	Human	M/F	Adult	Single nuclei	in vivo	21198	Yes
Dani 2021 ²⁴	Mouse	M/F	Adult Embryo	Single nuclei Single cell	in vivo	13356 10759	Yes
Xu 2024 ¹⁷	Mouse	M/F	Adult	Single cell	in vivo	9456	Yes
Verhaege, 2026 ³⁶	Mouse	M/F	Adult	Single Cell	In vivo	5800	Yes
Mathys 2024 ²³	Human	M/F	Adult	Single nuclei	in vivo	4513	Yes
Zhao 2020 ²⁰	Mouse	M	Embryo Adult	Single cell	in vivo	3311	Yes
Tang 2024 ²¹	Mouse	M	Adult	Spatial	in vivo	410	Yes
GSE205511 ²²	Mouse	M	Adult	Single cell	in vivo	430	Yes
Ximerakis 2023 ¹⁹	Mouse	M/F	Adult	Single nuclei	in vivo	180	Yes
Russell 2023 ¹⁸	Mouse	M	Embryo	Single nuclei	in vivo	81	No
Nowakowski 2017 ³⁰	Human	M/F	14 PCW	Single cell	in vivo	14	No
Huang 2022 ²⁶	Human	F	30 div	Single cell	in vitro	11085	No
Pellegrini 2020 ²⁹	Human	F	55 div	Single cell	in vitro	7894	No
Master 2025 ³¹	Human	M	75 div	Single cell	in vitro	3632	Yes
Kanton 2019 ²⁵	Human	M/F	120 div	Single nuclei	in vitro	183	No
Paulsen 2022 ²⁷	Human	F	35 div	Single cell	in vitro	86	No
Marques 2010 ³⁴	Mouse	M	Adult	Bulk	in vivo	n/a	Yes
Lun 2015 ³⁵	Mouse	M/F	Adult	Bulk	in vivo	n/a	No
Rodriguez-Lorenzo 2020 ³²	Human	M/F	Adult	Bulk	in vivo	n/a	No
Parichha 2021 ³³	Human	F	30 div	Bulk	in vitro	n/a	No

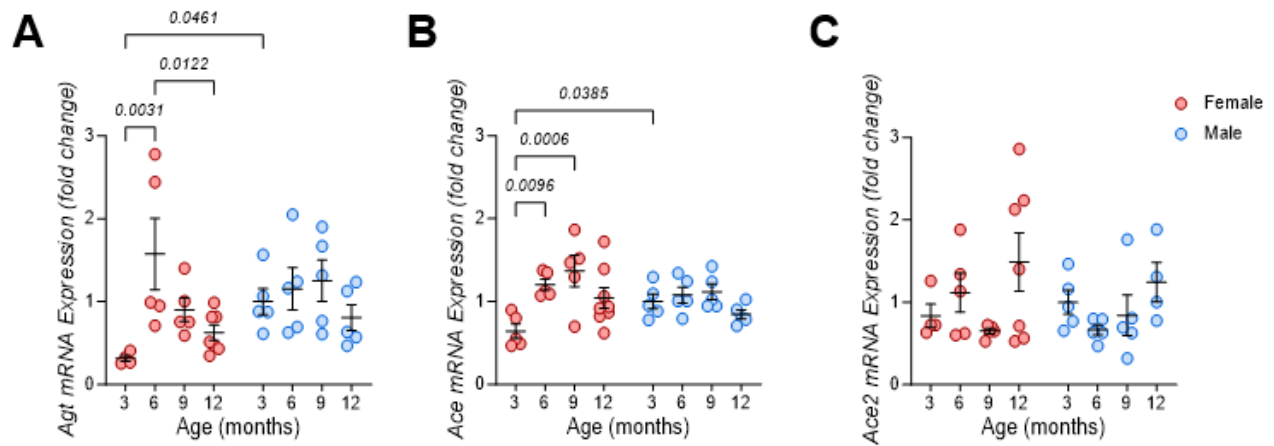
Supplementary Table 1. RNA sequencing dataset characteristics. In vivo single cell/nuclei datasets containing at least 180 choroid plexus epithelial cells contained renin mRNA in their expression matrix. Only one in vitro study contained renin mRNA in the expression matrix.

Outcome	β	CI	p value
CSF renin	1.46	0.17, 2.76	0.03
CSF aldosterone	0.01	-0.05, 0.08	0.68
CSF angiotensinogen	0.45	-0.69, 1.58	0.42
PRA-S	6.51	-8.37, 21.4	0.37
Serum Aldosterone	-0.06	-0.38, 0.26	0.70
Serum Ang II	0.91	-2.39, 4.22	0.57
ACE-S	-0.11	-0.18, -0.04	0.004
CSF/serum renin	-0.002	-0.01, 0.009	0.76

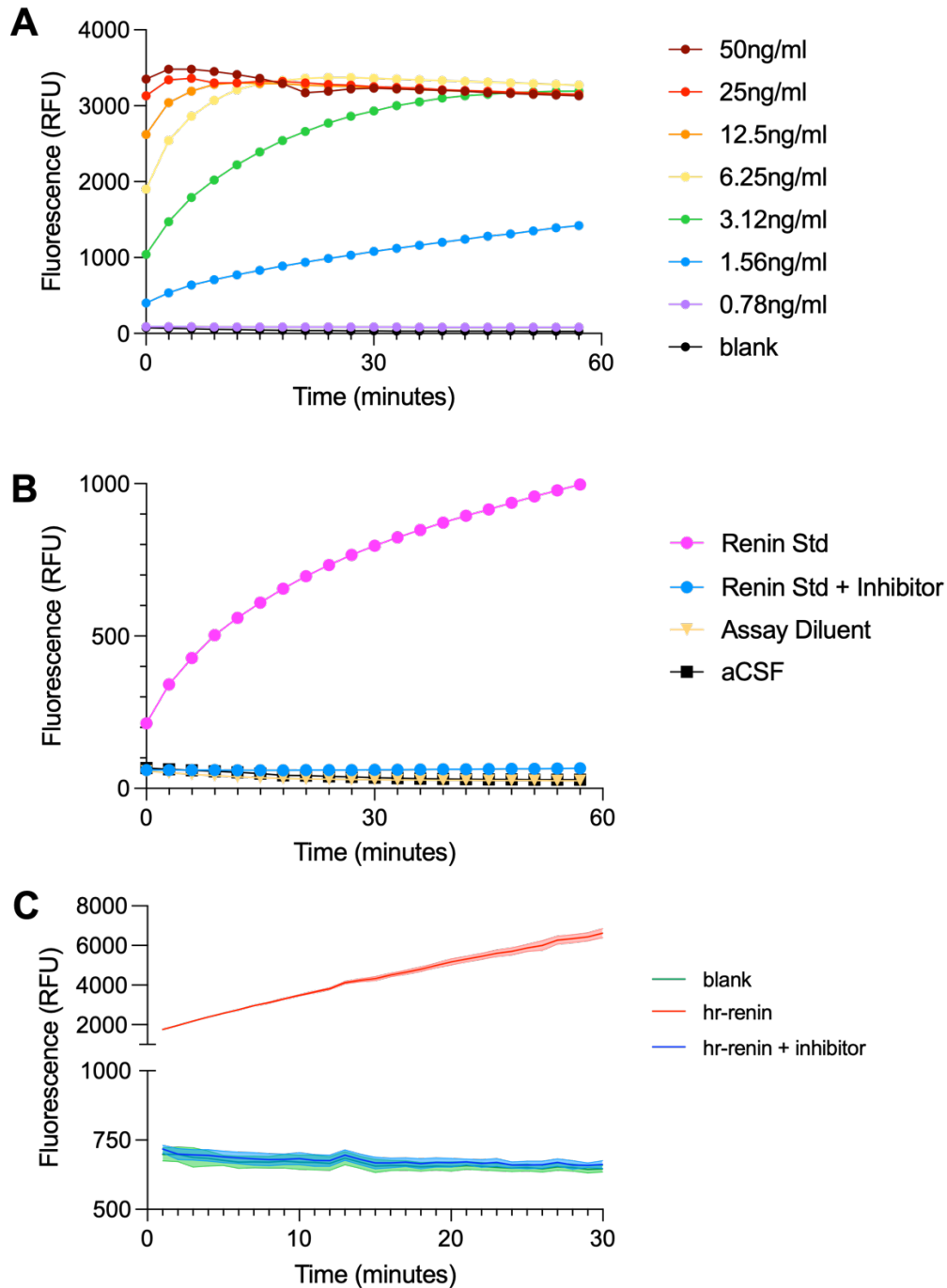
Supplementary Table 2. Association of other RAS measures with aging. Linear regression models adjusting for sex. Predictor: age; Outcomes as listed.



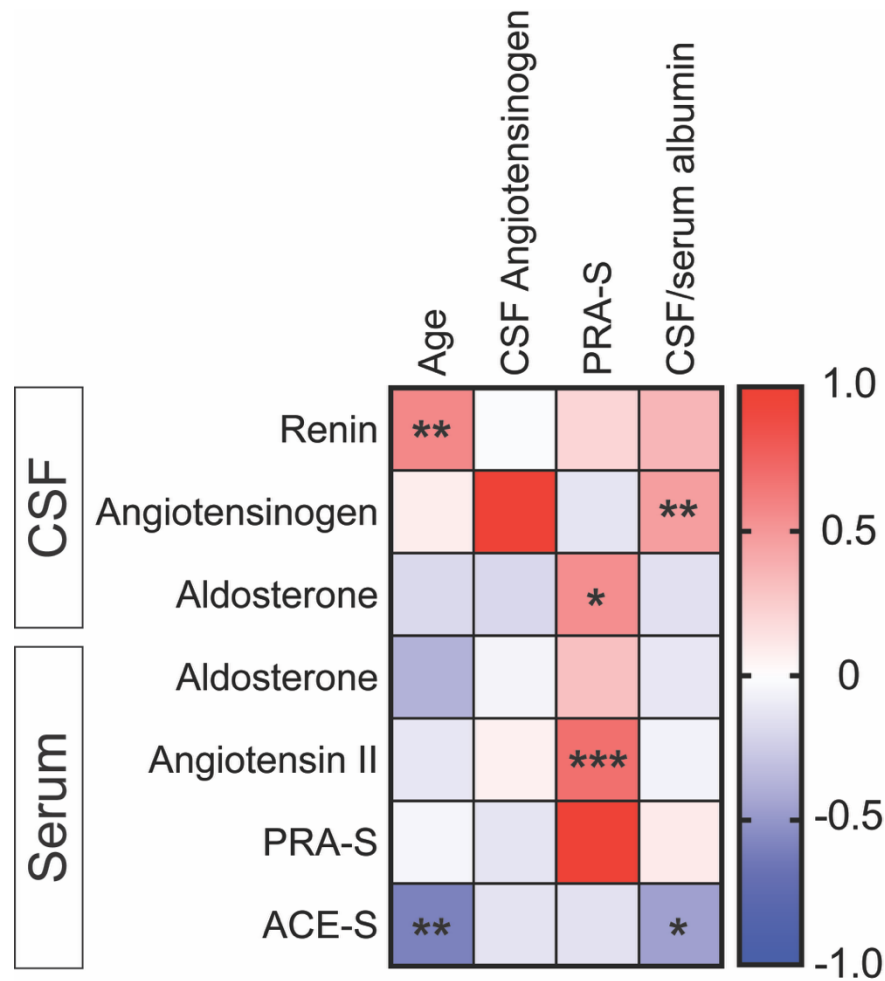
Supp Figure 1. Choroid plexus epithelial cells express Ace and Agt. Pseudobulk analysis of angiotensin converting enzyme (Ace) and angiotensinogen (Agt) expression between species (mice and humans) and sex (female and male), but higher expression *in vivo* than *in vitro*. Renin, Ace, and Agt expression between datasets from adult vs young.



Supp Figure 2. Other RAS members mRNA in the choroid plexus across age. a, Agt, b, Ace, and c, Ace2 mRNA expression across age (3, 6, 9, and 12 months) and sex. Data were analyzed by two-way ANOVA followed by Tukey's multiple-comparison test (n=4-8 animals per group as indicated)



Supp Figure 3. Validation of fluorogenic peptide substrate renin activity assay. **a**, Mouse activated renin was loaded at various concentrations, as indicated, and incubated with FRET-peptide. Fluorescence is shown over 60 minutes of recording. **b**, Mouse recombinant renin standard was loaded with and without renin inhibitor, compared to assay diluent and artificial cerebrospinal fluid (aCSF). **c**, Human recombinant renin standard was loaded with and without renin inhibitor compared to assay diluent (blank). Fluorescence is shown over 30 minutes of recording.



Supp Figure 4. Correlation matrix of baseline predictors and all outcome variables.

Baseline predictors: age, CSF angiotensinogen, plasma renin activity surrogate (PRA-S), and cerebrospinal fluid (CSF)/serum albumin ratio are shown as columns. Outcome variables: CSF renin, CSF angiotensinogen, CSF aldosterone, serum aldosterone, serum angiotensin II, PRA-S, angiotensin converting enzyme activity in the serum (ACE-S) are shown as rows. Red indicates positive correlation; blue indicates a negative correlation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.