SUPPLEMENTAL MATERIALS

Unique expression of the atypical mitochondrial subunit NDUFA4L2 in cerebral pericytes fine tunes HIF activity in response to hypoxia.

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Supplemental table 1: List of primers

Human gene	Forward primers 5'>3'	Reverse primers 5'>3'
28S	GGT AGC CAA ATG CCT CGT CAT	GGA TAG TAG GTA GGG ACA GTG GGA AT
NDUFA4L2	TTC TAC CGG CAG ATC AAA AGA CA	GGG CGA GTC GCA GCA A
NDUFA4	TGC GTC TGG CAT TGT TCA A	TCC AGG GCT CTG GGT TAT TTC
PDGFRB	AGA CAC GGG AGA ATA CTT TTG C	AGT TCC TCG GCA TCA TTA GGG
ACTA2	GTG TTG CCC CTG AAG AGC AT	GCT GGG ACA TTG AAA GTC TCA
MYH11	CAT CTA CTC GGA GAA GAT CGT CG	CGC CTG TGC ATA GAA TGG ACT
GFAP	GCA CGC AGT ATG AGG CAA TG	TAG TCG TTG GCT TCG TGC TT
PECAM1	CCA CTG CAG AGT ACC AGG TG	CCA CCT TGG ATG GCC TCT TT
MAP2	CTC AGC ACC GCT AAC AGA GG	CAT TGG CGC TTC GGA CAA G
PHD2	CCC TCA TGA AGT ACA ACC AGC AT	CAT CTG CAT CAA AAT ACC AAA CAG T
PHD3	GCC GGC TGG GCA AAT ACT A	CCG GAT AGC AAG CCA CCA T

Mouse gene	Forward primers 5'>3'	Reverse primers 5'>3'
28S	GGT AGC CAA ATG CCT CGT CAT	GGA TAG TAG GTA GGG ACA GTG GGA AT
Ndufa4l2	CCT GCG CAG TCC TGA TGT CT	GGT TGA AAC GGC AAG GAA CTT
Ndufa4	AGC AGC ACT GTA TGT GAT GCG C	TGT AGT CCA CAT TCA CAG AGT AGA
Cox4i2	ACA GTG ATG GGC TGC GTC TTC T	CTG TTG GGC TTT CCG TTC TTC C

Supplemental table 2: List of antibodies

Antibody	Host	Company	Reference
α-SMA	Mouse	Dako	M0851
β-ACTIN	Mouse	Sigma	A3854
βIII TUBULIN	Mouse	Santa Cruz	sc-51670
GFAP	Mouse	Chemicon®	Mab3402
HIF1α	Mouse	BD Biosciences	610959
NDUFA4L2	Rabbit	Proteintech	16480
PDGFRβ (28E1)	Rabbit	Cell Signaling	3169
PORIN	Rabbit	Abcam	AB15895
VE-Cadherin	Rabbit	Cell Signaling	D87F2
Cleaved Caspase-3 (Asp175)	Rabbit	Cell Signaling	9661

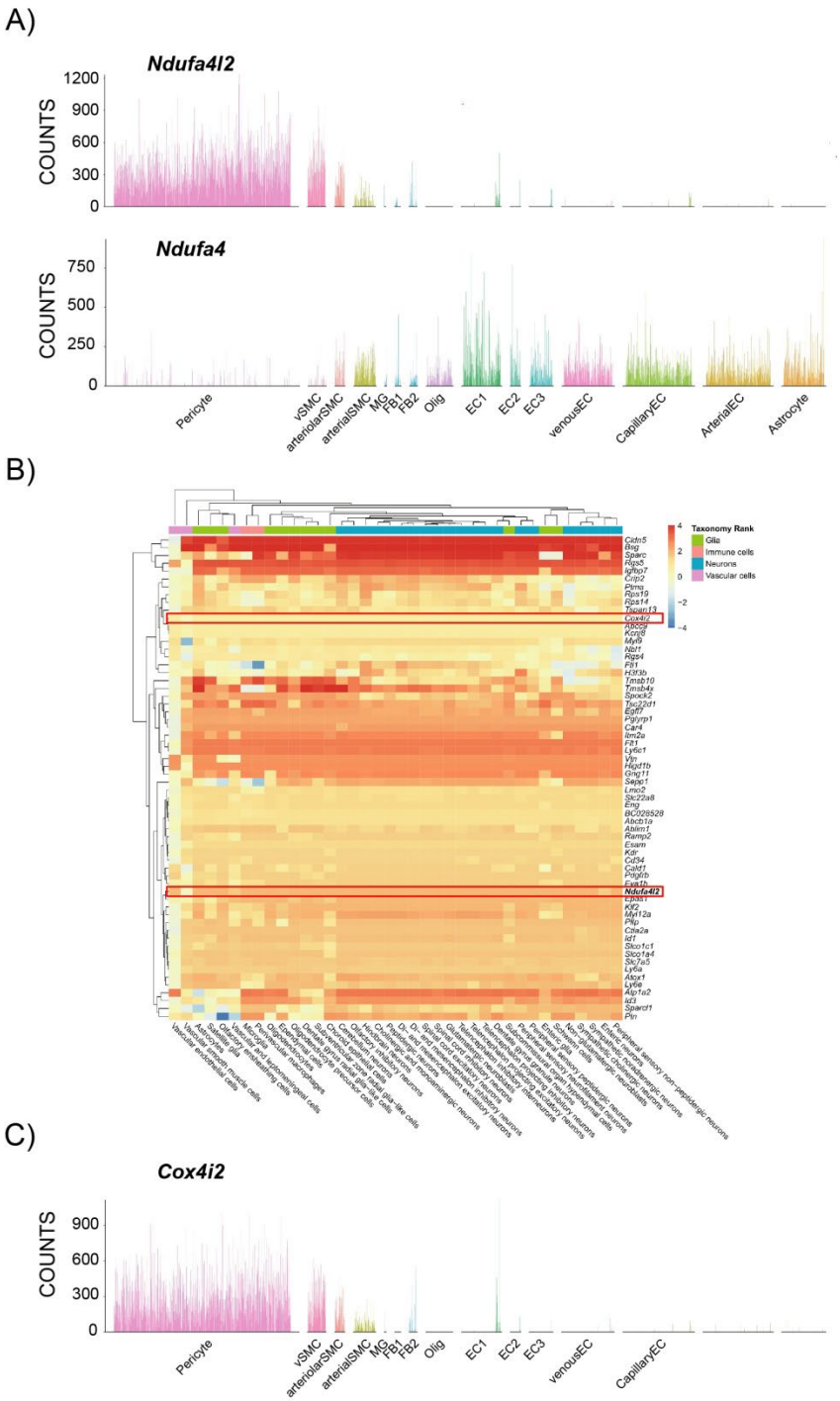

Figure S1: *Ndufa4l2* and *Cox4i2* expression is confined to pericytes and vascular smooth muscle cells in mouse brain. (A) Normalized *Ndufa4l2* and *Ndufa4* expression within the clusters identified in the scRNA-seq data set by Vanlandewijck et al.[1] (B) Heatmap showing top enriched genes expressed in brain pericyte cluster relative to other clusters. Data extracted from scRNA-seq data set (Zeisel et al.[2]) (C) Normalized *Cox4i2* expression within the clusters identified in the scRNA-seq data set by Vanlandewijck et al.[1]

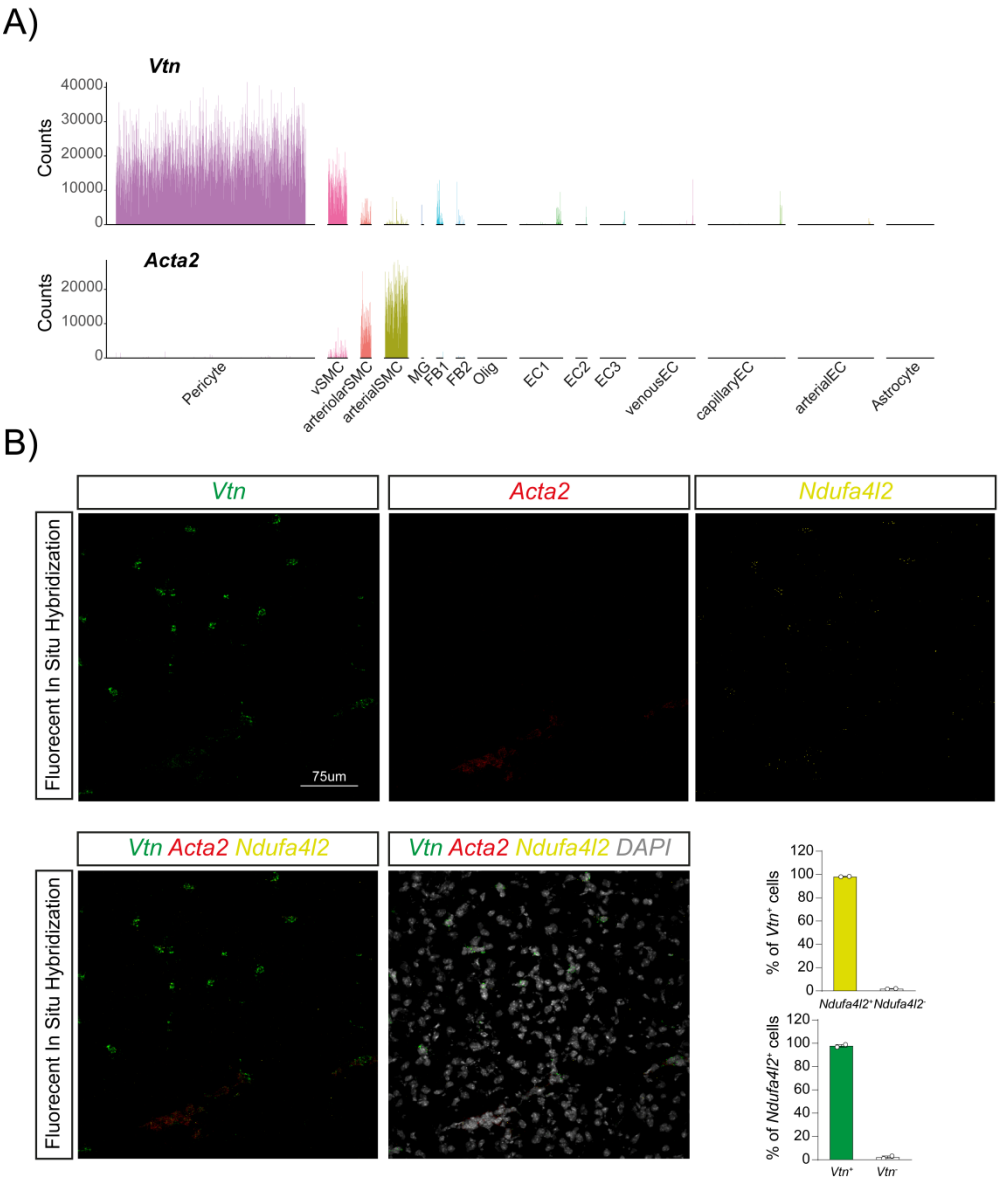

Figure S2: *Ndufa4l2* is mainly expressed by pericytes in mouse brain. (A) Normalized *Vitronectin* (*Vtn*) and *alpha smooth muscle actin* (*Acta2*) expression within the clusters identified in the scRNA-seq data set by Vanlandewijck et al.[1] (B) Representative images of multiplex RNA F.I.S.H. of mouse cerebral cortex detecting the pericyte marker *Vtn* (green fluorescent signal), the smooth muscle cell marker *Acta2* (red fluorescent signal) and *Ndufa4l2*-expressing cells (yellow fluorescent signal) and DAPI nuclear staining (grey signal) (n=2). The graph bars represent the percentage of *Ndufa4l2*$^{+}$ and *Ndufa4l2*$^{-}$ expressing *Vtn* mRNA, and the percentage of *Vtn*$^{+}$ and *Vtn*$^{-}$ expressing *Ndufa4l2* mRNA. Data are represented as mean ± SD.

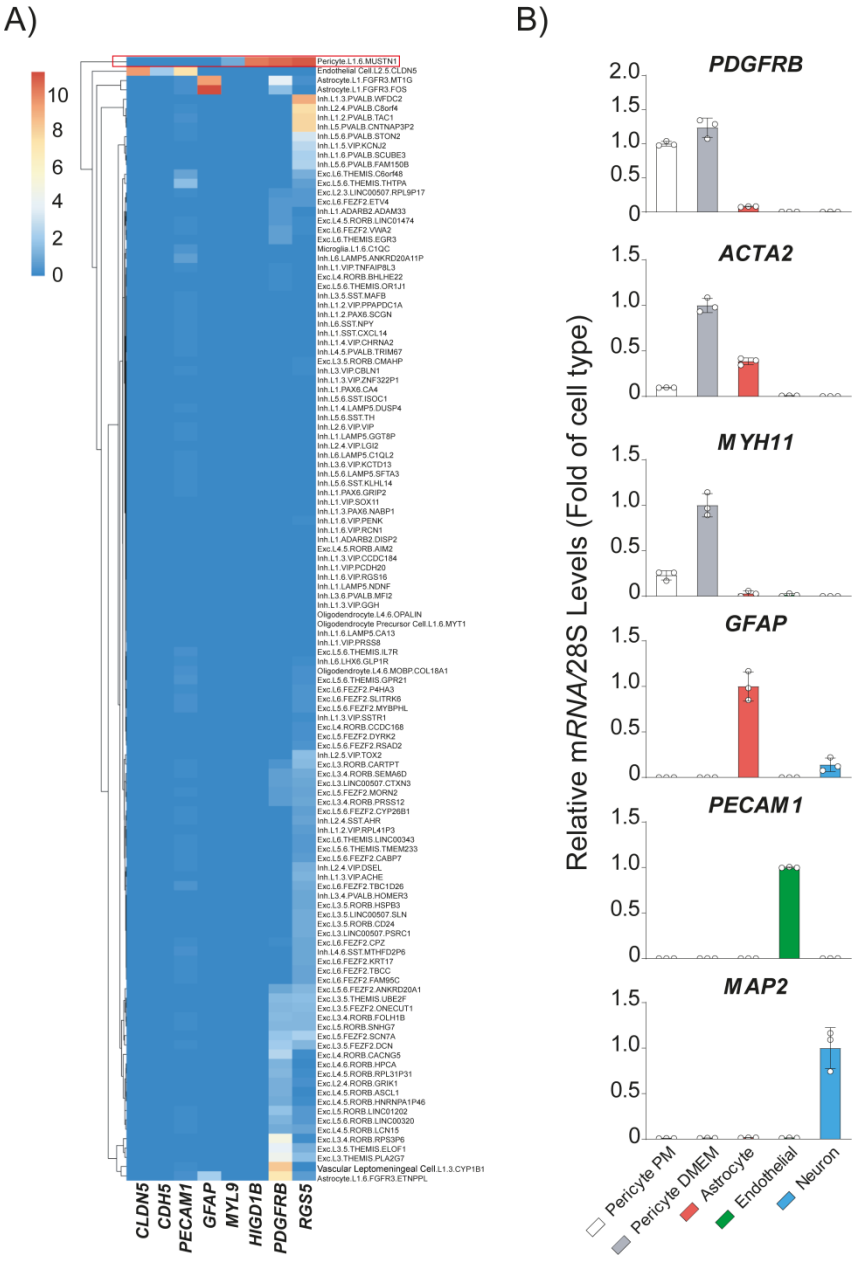

Figure S3: Molecular characterization of human brain pericytes in vivo and in vitro. (A) Heatmap showing that the cluster defined as pericytes (first row from the top) expresses positive well-defined molecular markers of human brain pericytes (*RGS5*, *PDGFRB*, *HIGF1B*, *MYL9*) and absence of markers of astrocytes (*GFAP*) or endothelial cells (*PECAM1*, *CDH5*, *CLDN5*). Data extracted from Allen Multiple Cortical Areas - SMART-seq data set. (B) Relative mRNA levels of marker genes of human brain pericytes cultured in pericytes medium (PM) or complete DMEM (*PDGFRB*, *ACTA2*, *MYH11*), astrocytes (*GFAP*), microvascular endothelial cell (*PECAM1*) and neurons (*MAP2*) (n=3). Note the dramatic increase of markers related to muscularization (*ACTA2*, *MYH11*) of pericytes when cultured in complete DMEM.

Mesa-Ciller et al., Figure S4

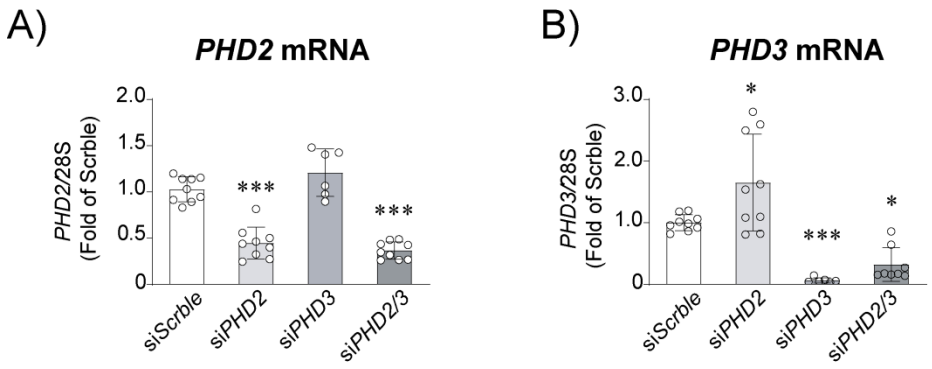

Figure S4: Effect of transient inactivation of *PHD2, PHD3* and double *PHD2/3* in human brain pericytes in vitro. (A) Relative levels of *PHD2* and (B)*PHD3* transcripts in transient *PHD2*-, *PHD3*- and *PHD2/3*-silenced human brain pericytes and their corresponding si*Scrble* cells (n=7-9). Data are represented as mean ± SD; Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc analysis; *P < 0.05, ***P < 0.001, compared with si*Scrble* cells.

Mesa-Ciller et al., Figure S5

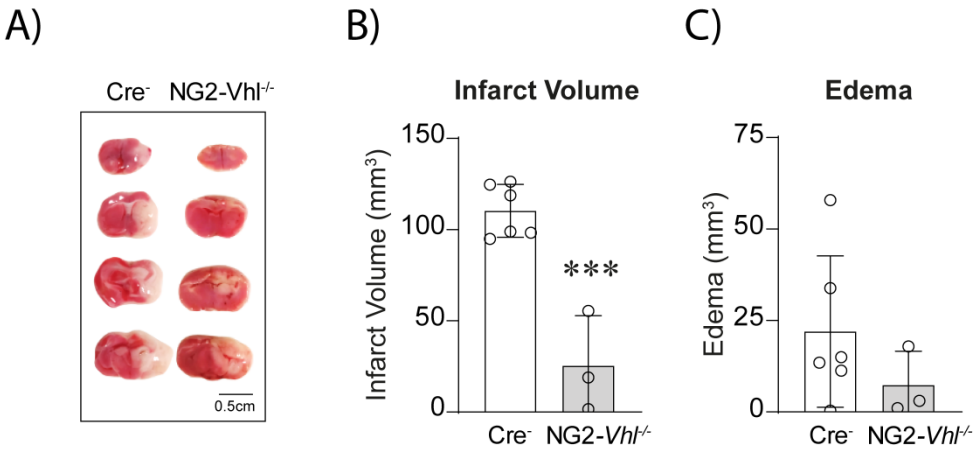

Figure S5: NG2-*Vhl*$^{-/-}$ mice are protected against cerebral ischemic stroke. (A) Representative images of infarct volumes obtained by TTC staining and (B and C) quantification of infarct volume by and edema at 24 h after 1 h of ischemia in Cre^{-} and *NG2-Vhl*$^{-/-}$ mice (n= 6 and 3 respectively). Data are represented as mean ± SD; Statistical analysis was performed using two-tailed Student's t test. ***P < 0.001, compared with Cre^{-} control mice.

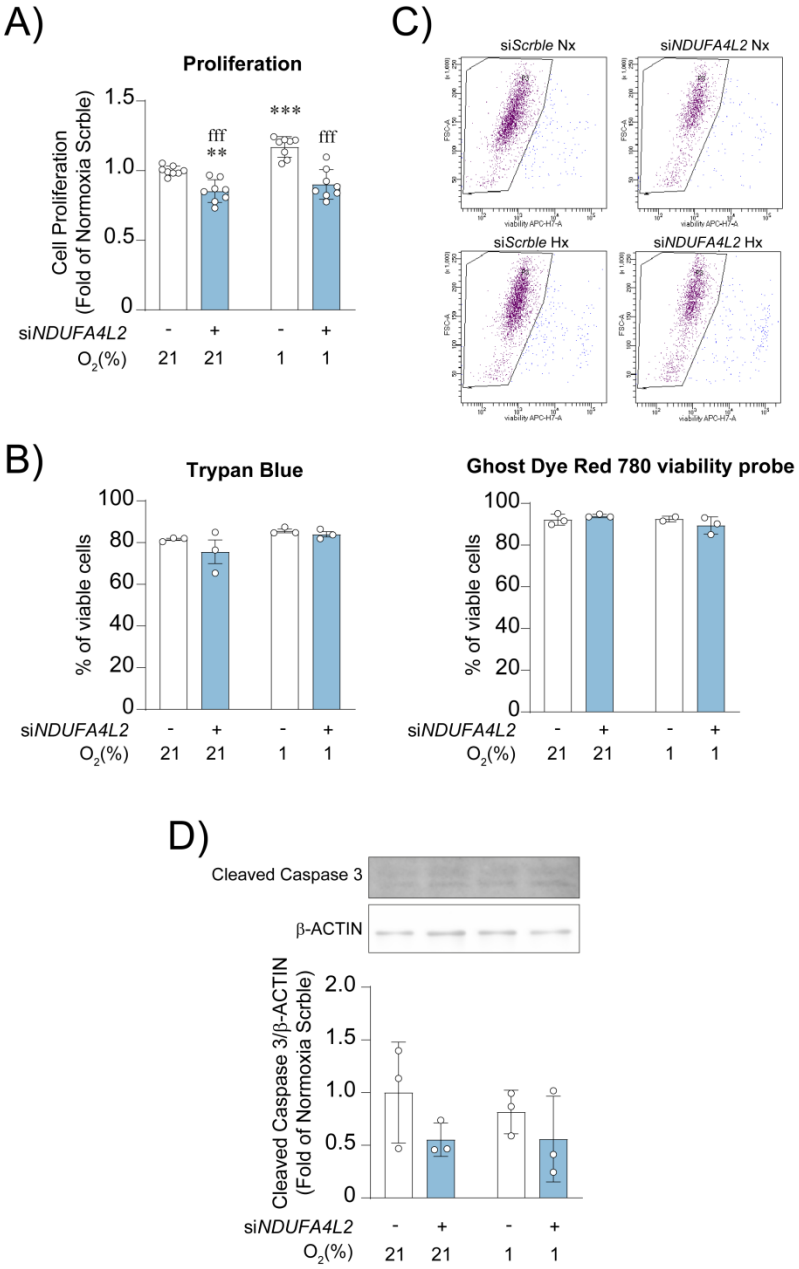

Figure S6: Effect of transient inactivation of *NDUFA4L2* and hypoxia in human brain pericyte proliferation and survival in vitro. (A) Human brain pericyte proliferation after transient inactivation of *NDUFA4L2* and/or exposure to hypoxia (1% O_2) (n=8). (B) Cell viability of human brain pericytes after transient inactivation of *NDUFA4L2* and/or exposure to hypoxia (1% O_2) assed by Trypan Blue staining (n=3) or (C) Ghost Dye Red 780 staining (n=3). (D) Representative western blot and quantification of cleaved caspase-3 after transient inactivation of *NDUFA4L2* and/or exposure to hypoxia (1% O_2) (n=3). Data are represented as mean ± SD; Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc analysis; **P < 0.01, ***P < 0.001, compared with normoxic si*Scrble* cells; ^{fff}P < 0.001 compared with hypoxic si*Scrble*.

URLs to access scRNA seq data:

1. http://mousebrain.org/genesearch.html
2. http://betsholtzlab.org/VascularSingleCells/database
3. https://portal.brain-map.org/atlases-and-data/rnaseq/human-multiple-cortical-areas-smart-seq

References:

1. Vanlandewijck M, He L, Mäe MA, Andrae J, Ando K, Del Gaudio F *et al.* A molecular atlas of cell types and zonation in the brain vasculature. *Nature* 2018; 554(7693)**:** 475-480.

2. Zeisel A, Hochgerner H, Lönnerberg P, Johnsson A, Memic F, van der Zwan J *et al.* Molecular Architecture of the Mouse Nervous System. *Cell* 2018; 174(4)**:** 999-1014.e22.