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Supplemental Information

The Median Eminence, A New Oligodendrogenic Niche in the Adult Mouse Brain

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Supplemental information

Supplementary figures and legends

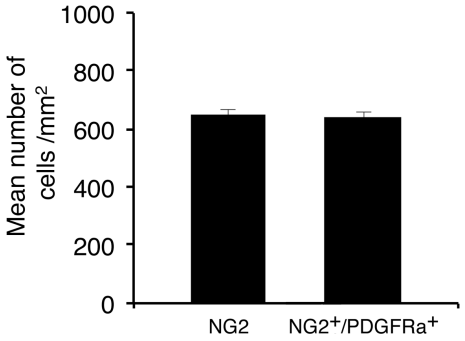

Figure S1 Quantitative analysis of $NG2^+$ and $NG2^+/PDGFRa^+$ in the ME of naïve mice calculated as mean number of marker/s positive cells per mm^2, (n=4 for each group).

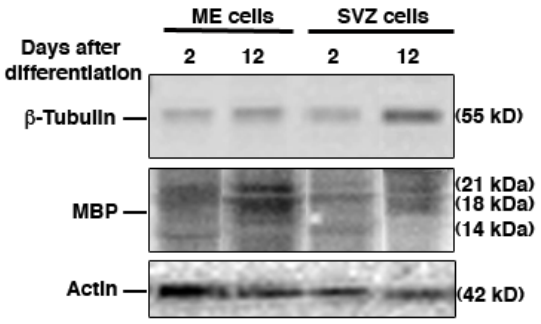

Figure S2 Western blot analysis of neuronal (b-Tubulin) and oligodendrocyte (MBP) markers following differentiation of ME- or SVZ-NSCs.

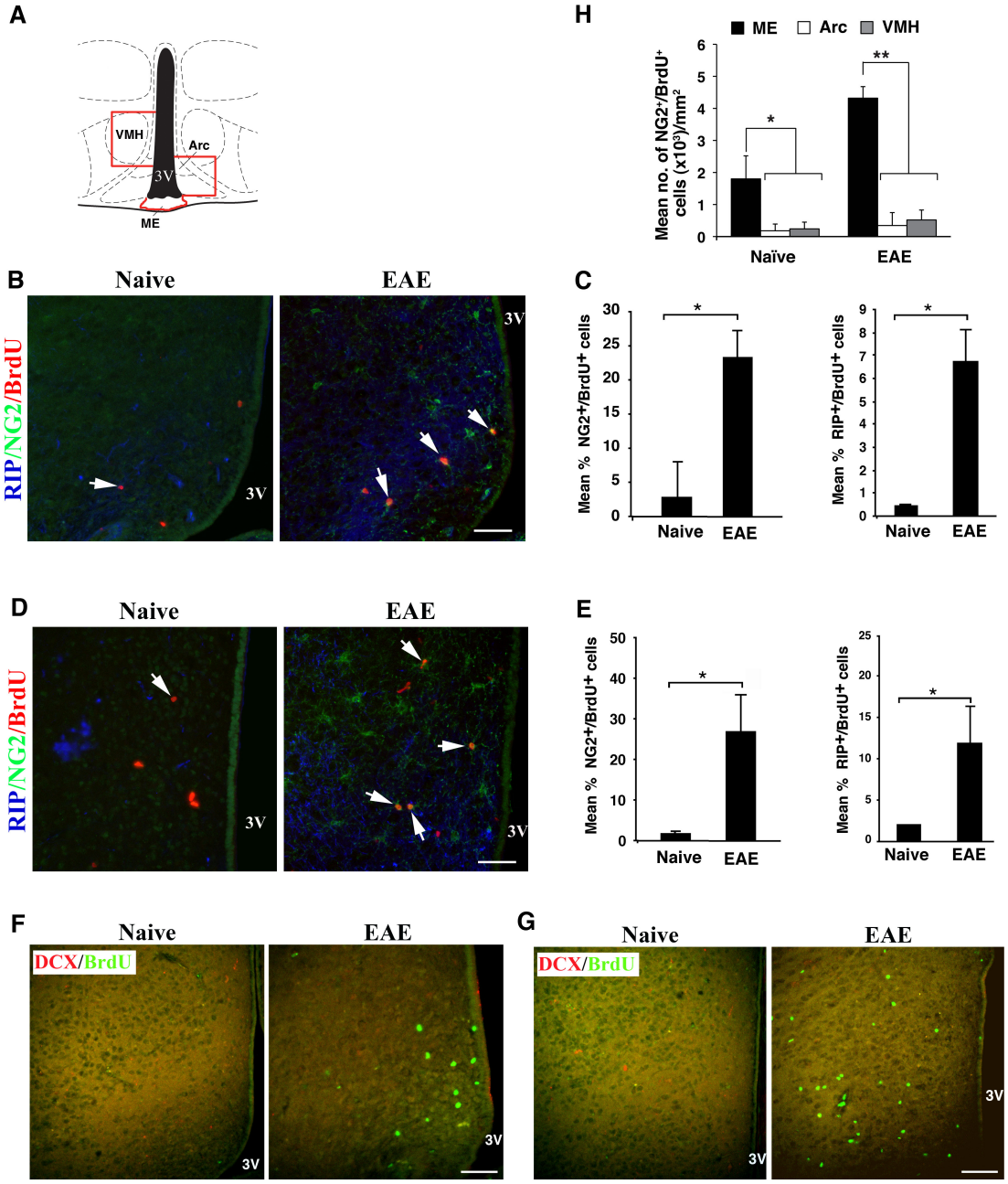

Figure S3 EAE induces oligodendrogenesis in the Arc and VMH of the hypothalamus (**A**) Schematic draw of the hypothalamus regions that were analyzed (**B**) Confocal images of double staining $NG2^+/BrdU^+$ and $RIP^+/BrdU^+$ in the Arc. bar=50μm. (**C**) Quantitative analysis of $NG2^+/BrdU^+$ and $RIP^+/BrdU^+$ in the Arc**.** Left: *p≤0.006, right: *p=0.03 **(D)** Confocal images of double staining $NG2^+/BrdU^+$ or $RIP^+/BrdU^+$ in the VMH. bar=50μm. (**E**) Quantitative analysis of $NG2^+/BrdU^+$ in the VMH *p=0.03, Two-tails student T test. **(F)** Images show no DCX staining in Arc in naïve and EAE

mice. bar=50μm. **(G)** Images show no DCX staining in VMH in naïve and EAE mice. bar=50μm. (H) Quantitative analysis of percentage of $NG2^{+}/BrdU^{+}$ cells in ME compare Arc and VMH in Naïve and EAE mice (n=4) ; * p=0.04 ; ** p=0.01.

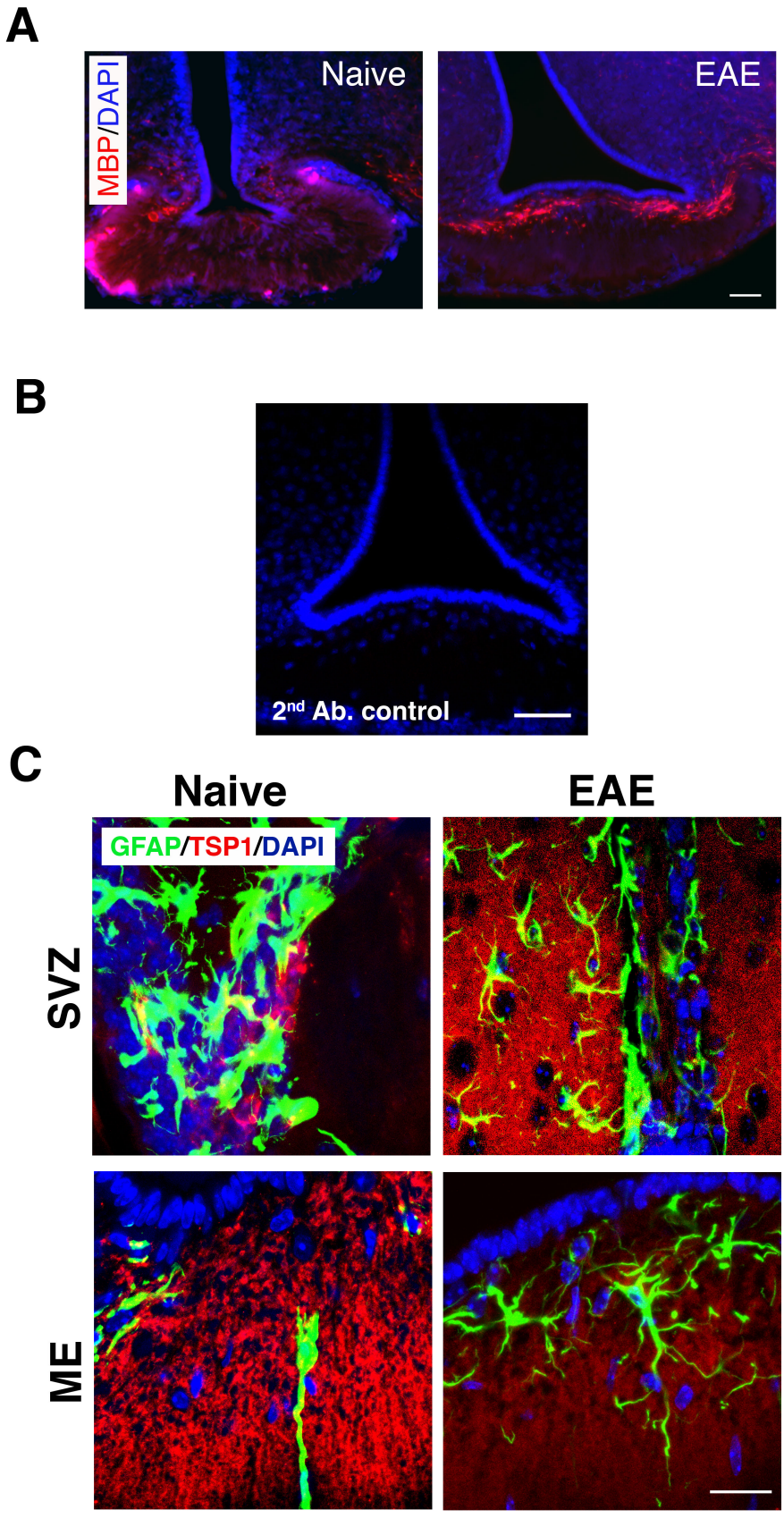

Figure S4 (A) MBP immunostaining in ME of naïve and EAE mice. bar=50μm.

(B) Negative control staining with only secondary antibody (**C**) Low power images of TSP1 from sections shown in Fig. 4F. bar=50μm.

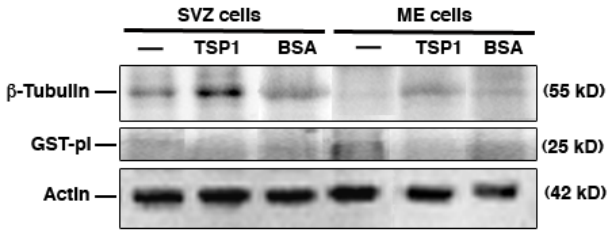

Figure S5 Western blot analysis of neuronal (b-Tubulin) and oligodendrocyte (GST-pI) markers following differentiation of ME- or SVZ-NSCs in presence of TSP1.

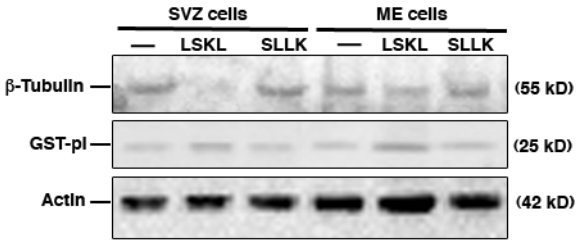

Figure S6 Western blot analysis of neuronal (b-Tubulin) and oligodendrocyte (GST-pI) markers following differentiation of ME- or SVZ-NSCs in presence of TSP1 antagonist (LSKL) or agonist (SLLK).

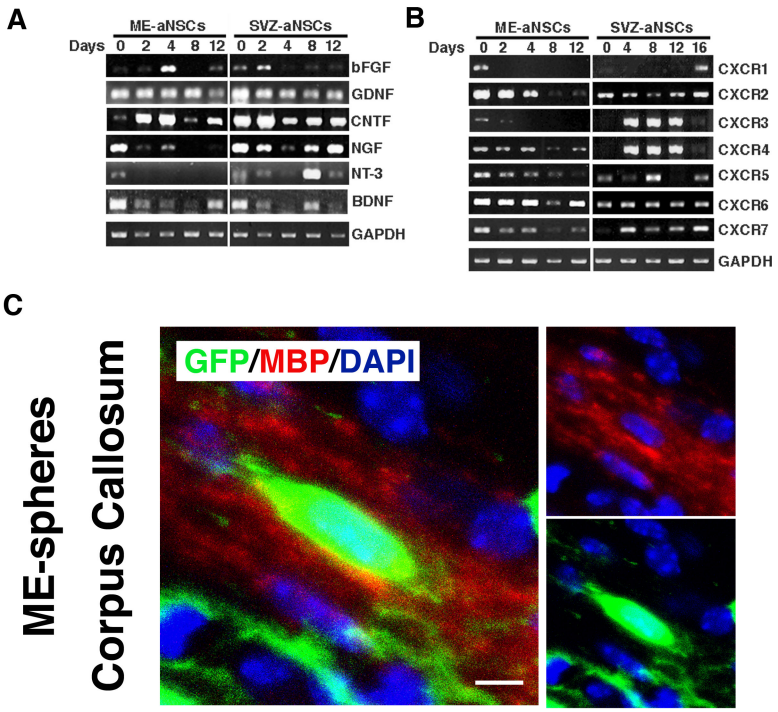

Figure S7 temporal expression profile of chemokine receptors **(A)** and neurotrophic factors **(B)** by ME-originated cells are distinct from these expressed by SVZ-originated cells. Semiquantitative RT–PCR of cDNA reverse transcribed from total RNA extracted from ME-NSCs compare SVZ-NSCs at elevated time points following differentiation. Undifferentiated NSCs (day 0) served as control. GAPDH expression was assessed as an internal standard. Results are representative of three repeats. **(C)** Single GFP^+/MBP^+ cell derived from ME-NS in the CC demonstrating short MBP-positive profiles at the periphery of the GFP-positive soma. bar=20μm.

Supplemental table 1

List of used antibodies

Antibody	Manufacture	Cat. No.
mouse anti-glial fibrillary acidic protein (GFAP)	Sigma	G-3893
monoclonal mouse anti-NeuN	Chemicon	MAB377
rat anti-MBP	Chemicon	MAB386
rabbit anti-NF200	Sigma	N4142
goat anti-DCX	Chemicon	Sc-8066
monoclonal mouse anti β-Tubulin III	Chemicon	MAB1637
rabbit anti NG2	Chemicon	AB5320
rat anti BrdU	Serotec	MCA2060
rat anti-platelet derived growth factor alpha (PDGFRa)	eBioscience	14-1401
rat anti MAC2	Biolegend	125401
rat anti CD45	Biolegend	103101

Supplementary methods

Semiquantitative RT-PCR analysis. Total RNA was purified either from NSCs at time point 0 (undifferentiated NSCs) or from NSCs which were cultured in differentiation medium on PDL for elevated time points, using TRIzol Reagent (Invitrogen Corp.). cDNA was prepared from 1 μg of total RNA using MMLV-

reverse transcriptase (Promega, Madison, WI) and olido-dT (Promega, Madison, WI) according to manufacturer's instructions. The cDNA mixure was diluted 1:5 with PCR-grade water. We examined the expression of specific mRNAs using semi-quantitative reverse transcription PCR (RT-PCR) with selected gene-specific primer pairs. The primers used were: TSP1, sense 5'- CAA GGG CTCAGGGATACTCAGG-3', antisense 5'- AGG TTT TGT CAT AGA TGG GTC C-3' (product size, 270 bp); CXCR1, sense 5'- GGC CGA GGC TGA ATA TTT CAT TCT-3', antisense 5'- GGT GGC ATG GAC GAT GGC CAG TAT-3' (product size, 450 bp); CXCR2, sense 5'- GGA GAA TTC AAG GTG GAT AAG-3', antisense 5'- AGT GTC TCT TCT GGA TCA GTG-3' (product size, 486 bp); CXCR3, sense 5'- GAG GTT AGT GAA CGT CAA GTG-3', antisense 5'- GGG GTC CCT GCG GTA GAT CTG-3' (product size, 482 bp); CXCR4, sense 5'- GGT CTG GAG ACT ATG ACT CC-3', antisense 5'- CAC AGA TGT ACC TGT CAT CC-3' (product size, 525 bp); CXCR6, sense 5'- ACA AAG ATG TTG CTG GCA GA-3', antisense 5'-GGC CTG TTT TCA GTC CCA TA-3' (product size, 219 bp); CXCR7, sense 5'- TTT GAG TTC AGG GGA GGA T-3', antisense 5'-GCT CGC TGA CAC CTA ACC TC-3' (product size, 202 bp); CCR1, sense 5'- ATG GAG ATT TCA GAT TTC ACA GAA-3', antisense 5'- GGC TAC AGG TAC GGT GAG TGA ACT-3' (product size, 510 bp); CCR2, sense 5'- ATG TTA CCT CAG TTC ATC CAC GGC-3', antisense 5'- GTA ATG GTG ATC ATC TTG TTT GGA-3' (product size, 506 bp); CCR3, sense 5'- ATG GCA TTC AAC ACA GAT GAA-3', antisense 5'- GTC ACA GTT CGG GCT CGA AGG-3' (product size, 512 bp); and GAPDH, sense 5'- CCA TCA ACG ACC CCT TCA TTG AC-3', antisense 5'- GGA TGA CCT TGC CCA CAG CCT TG-3' (product size, 580 bp).

The RT-PCR reactions were carried out using 100 ng of cDNA, 20 pmol of each primer, and Taq polymerase (Sigma-Aldrich, Israel) in a total volume of 20 ml. PCR reactions were carried out in a PTC-100 programmable Thermal controller (MJ Research, Inc) PCR system. Amplification included one stage of 5 min at 95ºC followed by 35 cycles of 95ºC for 30 s, 56ºC for 1 min, 72ºC for 1 min and 72ºC for 10 min then reactions were kept at 4ºC. As an internal standard for the amount of cDNA synthesized, we used GAPDH mRNA. PCR products were subjected to agarose gel analysis and visualized by ethidium bromide staining. In all cases one product was observed with each primer set, and the observed product had an amplicon size predicted from published cDNA sequences.

Western blot

Cells were lysed (25 mM Tris–HCl pH 7.6, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM Na3VO4, 100 nM, PMSF and protease inhibitor cocktail (1:50; Sigma--Aldrich). Lysis was done 1h/4°C then lysate was centrifuged 13,000rpm/30min/4°C and supernatant was collected. The protein content of cell lysates was determined using the BCA protein estimation kit (Pierce; Rockford, IL) with BSA as a standard. Proteins were denatured at 95°C for 5 minutes and further diluted in sample buffer (250 mM Tris-HCl pH 6.8 containing 4% SDS, 10% glycerol and 2% β-mercaptoethanol). Equal amounts of proteins were resolved on 10% SDS-PAGE and transferred onto nitrocellulose membrane (Invitrogen kit) for subsequent immuno-blotting with antibodies specific for Tubulin beta (1:1000; Chemicon), MBP (1:2000; Chemicon), GST-pI (1:3000; Abcam). To control protein loading, blots were additionally stained with either anti-β-actin (1:5000; Abcam). Blots were analyzed by standard chemi-luminescence (Supersignal Kit, Pierce, Rockford, IL, USA) and visualization was done by a ChemiDoc™ XRS System (Bio-Rad).