

**Supp. Fig. 1: Cluster annotation. A.** UMAP plots with originally identified (unannotated) cell clusters colored by average module score (expression) of marker genes specific to the different cell types/hippocampal regions. It is important to note that 2-5 marker genes are used to classify different cell types, while the classification of the CA and DG regions is based on 27 and 38 marker genes, respectively (see also Supp. Table 2). Especially the InN2 cluster,

which will be discussed later in our manuscript as a cluster that may reflect a specific state of adult neurogenesis, appears to show some expression of CA-specific genes. However, the mean expression value of CA genes for the InN2 cluster is 0.14 when compared to clusters ExN2, 3, 4, 6, and 10, 12 (ranging from 0.33 to 0.46), which more clearly classify as CA. Thus, the fact that the InN2 cluster expresses some CA genes would not suggest that adult neurogenesis occurs in the CA region but rather indicate that the CA and DG marker gene lists are not suitable to classify the InN2 cluster. Please also see the violin plots in panel C. **B**. UMAP plots with cell clusters colored by annotated cell-type (*top*), sample (mouse) of origin (*middle*) and sequencing batch (*bottom*). **C**. Violin plots showing normalized expression of individual marker genes specific to the different cell types, after cell-type specific annotation of clusters (*ExN1-13: excitatory neurons; InN1-4: inhibitory neurons; ODC: oligodendrocytes; OPC: oligodendrocyte precursor cells; AST: astrocytes; MGL: microglia*).



**Supp. Fig. 2: A.** Violin plots showing average module score (expression) of the top 10 computationally detected marker genes specific to each of the 13 different excitatory neuron clusters (ExN1-13). **B.** Dotplot showing the top 5 significant GO biological process terms enriched among the top 50 marker genes of the excitatory and inhibitory neuron clusters.



**Supp. Fig. 3: A.** Violin plots showing normalized expression of the top marker gene specific to each inhibitory neuron cluster in all cell-types (*ExN1-13: excitatory neurons; InN1-4: inhibitory neurons; ODC: oligodendrocytes; OPC: oligodendrocyte precursor cells; AST:* 

*astrocytes; MGL: microglia*). **B.** Violin plots showing normalized expression of the top marker gene specific to each inhibitory neuron cluster (as above), in only the inhibitory clusters, further split by experimental condition. **C.** Violin plots showing normalized expression of gene markers used for canonical classification of inhibitory neuron subtypes.



**Supp. Fig. 4: A.** Violin plots showing normalized expression of the top 20 differentially upregulated genes in the InN2 cluster, as compared to the other inhibitory neuron clusters. **B.** Violin plots showing normalized expression of the 17 genes comprising the Prdm16 regulon.



**Supp. Fig. 5: A.** UMAP plot depicting the average module score (expression) of the top 50 genes differentially expressed between the ExN11 cluster and the mature DG excitatory neuron cluster (ExN1). **B.** UMAP plot depicting the average module score (expression) of the top 50 genes differentially expressed between the ExN11 cluster and the oligodendrocyte and OPC clusters (ODC and OPC). **C.** UMAP plots depicting normalized expression of selected marker genes for radial glia/immature neurons/exercise-mediated neurogenesis.



Supp. Fig. 6: Network plot with TFs and their respective regulon genes, specific to the InN2 and/or ExN11 cluster, indicating gene regulatory interactions between these two clusters (Larger nodes represent genes/TFs that connect two or more regulon networks).



**Supp. Fig.** <u>7</u>: **A.** Dotplot showing the top (up to 5) significant GO biological process terms enriched among genes making up the regulons with significant differential activity between cells from exercise and control conditions, in specific clusters as indicated in Fig. 4(C). **B.** Violin plots showing average module score (expression) of regulons with significant differential activity upon exercise, in the ExN6 cluster.

#### Genes downregulated upon exercise: ExN6 significant de-ulation: ExN6 Genes upregulat exercise: ExN6 Regulon TFs with upregulated activity upon exercise: ExN6 Regulon TFs with downregulat activity upon exercise: ExN6 Fill colour Npr3 Gap43 Nov Infaip813 Eda Contract of the top 50 Cluster markers: ExN6 TFs of the top 5 cluster Fact of the top 5 cluster Factors: ExN6 Remaining genes in the markers: remaining TFs in the top 50 markers: remaining TFs in the network not regulating the top 5 specific regulating Border colour Pcsk1 Bhlhe40 Smarca1 6430573F11Rik Nr4a2 Shape Fosl2 Rnf207 Zfp423 Stk32a Myo16 Rgs1 Rpas1 Efnb2 FbIn2 3cl6 Ptprf Trhr end4 Ppp2r2b Zfp385b Ldb2 Cpne2 Nr2f1 Rspo3 Camk2a Mmp17 Vwa3a Sidt1 Pkp2 Cwh43 Plcxd3 Syt9 Tctn3 Fxyd6 Cnr1 Golga7b Snrpn Megf11 Skil Rein Chrm1 Hectd2 Rspo2 Cbfa2t3 Inhba Mmd2 Ak4 Dict Gabras Rapgef11 Cnksr2 Sv2b Spata6 Tox ¥. Cntn6 Trpc7 Thsd4 Hes1 Crtac1 Htr2c A Gpc3 Slit2 Tacr3 Slc9a2 Abat Celc42ep3 Vat11 Fam184b Adamtsthem132chfpl3 Kenmb2 Cux2 Nmnat3 Dach1 Sesn3 Hs3st5 Add3 Adarb2Runx3 Esrrg Kcnip1 Mt1 Atp11b Lypd6 Kctd1 Ptpn13 Gabrg3 Slco5a1 Nxph1 Btbd11 Myo1b Gnb4 Elavi2 Dix60s1 Mettil8 Dix1as Syt15 Slc1a3 Kcnq5 Nfia Cbfb Scan2 Kene1 Trps1 Sncaip Foxn3 Alcam KIf9 Col19a1 Vstm2a Rap1gap Arhgap10Rbm20 Dgkb 02b Lhx6 Scn9a Osbp18 Tanc1 Dtna Rbms3 Neto1 Ngf Gpr176 Rbms1 Rnf144b Tox2 Oprd tmem44 Bcan TIII Cacna2d3 Zbtb7c Sema6c Coro6 Fras1 Tbc1d8Kirre Gpring Sv2c Syt2 Arhgap18 Kctd8 Chst8 Wfs1 Cdc14a Adcy5 Slc1a2 Hivep1 Elmo1 Meis2 Deptor Osbpl10 Rorb Pbx3 Eti4 Gulp1 Cdh22 Foxp2 Nrg1 Tle3 Ngef Etv5 Xkr6 Grm8 Rmst Etvi Ndst4 Rait4 Oprm1 Ipcef1 Pbx1 Ankfn1 Celf4 Glra2 Atp2b4 Arhgap25 Sulft

**Supp. Fig. 8**: Network plot with TFs and their respective regulon genes, specific to or showing differential activity in ExN6 upon exercise, indicating gene regulatory interactions in ExN6 cluster (Genes/TFs that are significantly deregulated upon exercise or are specifically overexpressed in ExN6 are represented bigger in size, while selected genes belonging to these regulons which are deregulated in the same direction as the regulon TF are colored in red or blue).