



Fig. 5. Slc22a3 regulates histone serotonylation in OB astrocytes. (A) Immunostaining of H3-serotonin in OBs of Aldh111-GFP mice and quantification of GFP+H3-5HT+ colabeling ($n = 3$, 25 to 45 cells). White dashed lines in the zoom-in views represent the boundary of the astrocyte nucleus. Error bars represent mean \pm SEM. Scale bar, 50 μ m; zoom-in scale bar, 20 μ m. (B) Schematic illustrating viral vectors and timelines for H3-5HT quantification and ChIP-seq. IF, immunofluorescence. (C and D) H3-5HT immunostaining (C) and quantification (D) in control and Slc22a3-cKO OB astrocytes (74 to 79 cells per cohort; * $p = 0.0377$, unpaired Student's two-tailed t test on $n = 4$ mice per cohort). Yellow dashed lines represent the boundary of the astrocyte nucleus. Scale bar, 5 μ m. In the box plot, the center line

represents the median, box limits are upper and lower quartiles, and whiskers are minimum and maximum values. DAPI, 4',6-diamidino-2-phenylindole. (E) Venn diagram depicting the number of H3-5HT ChIP-seq peaks that are specific to or shared between control and Slc22a3-cKO OBs ($n = 4$ OBs per cohort). (F) Venn diagram depicting number of genes that both lose H3-5HT peaks and are down-regulated in Slc22a3-cKO astrocytes. (G) Heatmaps comparing ChIP H3-5HT at 4 kb from the peak center in control versus Slc22a3-cKO OBs. TSS, transcription start site. (H) GO analysis of genes at H3-5HT peaks revealing a loss of H3-5HT regulation at GABA-associated pathways in Slc22a3-cKO OBs. (I) GO analysis of the 538 overlapping genes shown in (F). See table S3 for data summary. Illustrations were created with Biorender.com.

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