

## Supplementary Legends

Extended Data Figure 1. Breathing abnormalities in P0 *Auts2* cKO pups. (a-f) *Nes11-Cre* (pan-CNS) *Auts2* cKO mice. Representative plethysmography traces from control (a; n=7), heterozygous (b; n=7), and homozygous cKO (c; n=3) animals on P0. Abnormal breathing events (gasps, sighs, apneas) are indicated. Breathing frequency (d) was reduced ( $p=0.005$ ); tidal volume (e) was increased ( $p=0.041$ ); and breathing rhythms (f) were more irregular ( $p=0.002$ ) in *Nes11-Cre* *Auts2* cKO pups. (one-way ANOVA,  $P=0.0406$ ). (g-i) *Emx1-Cre* *Auts2* cKOOctx mice. Breathing frequency was reduced ( $p=0.041$ ), but other parameters were not significantly different in heterozygous (n=3) or homozygous (n=3) cKO mice from controls (n=3).

Respiratory metrics represented as mean + S.E.M. \*,  $p<0.05$ ; \*\*,  $p<0.005$ .

Extended Data Figure 2. Histology of P5 control and *Auts2* cKOOctx forebrain. (a-d) DAPI-stained sections (fluorescence inverted) showed no abnormalities of neocortex (arrows) in homozygous *Auts2* cKOOctx brains (b, d) compared to controls (a, c). However, the DG (arrowheads) appeared slightly smaller in *Auts2* cKOOctx brains, as confirmed at higher magnification (c, d). Scale bar: B, 0.5 mm for a, b; 100  $\mu\text{m}$  for c, d.

Extended Data Figure 3. *Tbr2*<sup>+</sup> INPs are decreased and *Penk* mRNA is upregulated in *Auts2* cKOOctx DG (a, b) P0.5 dentate gyrus stained for TBR2 in control (a) and *Auts2* cKOOctx (b) mice, white outlined region indicates region of cell quantification. (c) Boxplot of cell counts from matched sections of three cKO and three control animals (one-tailed wilcox  $p$ -value = 0.05). (d, e) P0.5. Cells in the molecular layer (marginal zone) of the DG (red outlines) expressed *Penk* at higher levels in *Auts2* cKOOctx (e) than in control (d) mice. *Penk* was also increased in marginal zone cells of the adjacent subiculum (asterisks, e). (f, g) P7.5. *Penk* expression remained elevated in *Auts2* cKOOctx (g) as compared to control (f) DG. Scale bars as indicated.

Extended Data Figure 4. Examination of unaffected marker genes at P0 in cortex and dentate gyrus (a, b) P0.5 cerebral cortex stained for layer VI marker TBR1 in control (a) and *Auts2* cKOOctx (b) mice, images are oriented rostral cortex to the right (c, d) P0.5 cerebral cortex stained for layer II-III marker CUX1 in control (c) and *Auts2* cKOOctx (d) mice, images are oriented rostral cortex to the right (e, f) P0.5 dentate gyrus stained for cajal-retzius cell marker Reelin (*Reln*) in control (e) and *Auts2* cKOOctx (f) mice (g, h) P0.5 dentate gyrus stained for glial scaffolding

marker glial fibrillary acidic protein (Gfap) in control (g) and *Auts2* cKOctx (h) mice

Extended Data Figure 5. Interaction network of proteins encoded by DETs bound by AUTS2 or expressed by genes with AUTS2 ChIP-seq peaks. (a) Transcripts that were bound and regulated by AUTS2 in vivo (Table 1) encoded proteins that formed a significant interaction network ( $p=0.01$ ) as determined by STRING-DB. (b) Transcripts that were regulated by AUTS2, and whose cognate genes were bound by AUTS2, encoded proteins that formed no significant interaction network.