



4 Supplementary Fig. 1. RT-PCR validation of RNAseq and small RNAseq data. mRNAs and miRNAs shown to be age regulated by RNA and small RNA sequencing 5 showed the same regulation when assessed by RT-PCR. The data from P10 and 6 P28 visual cortex samples were analyzed using the ddCT methods. (A) Fold change 7 in P28 vs. P10 mice; Tet1, Cxxc4, Dnmt3a, Grin2b, Calb2, Mecp2 (t test, p < 0.0001; 8 9 n =4 for each group and target). (B) Fold change in P28 vs. P10 mice for miR-29a, 10 miR-338, miR-219, miR-132 (t test, p < 0.02; n = 4 for each group and target). Error 11 bars represent SEM. Asterisks indicate statistical significance (p<0.05). 12

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15 **Supplementary Figure 2.** 16 17





Supplementary Fig 2. (A) OSI in wt and mutant mice (ko) at P30 and P60 (wt-P60, 19 20 ko-P60). Error bars represent SEM. (B) ΔO values for wt, ko, wt-P60 and ko-P60 21 animals. Results are analyzed by case. Whiskers represent the average $\Delta O \pm SEM$ 22 for each experimental group. Circles represent individual ΔOs for each animal. 23 Binocular matching is strongly impaired in miR-132/212 null mice both at P27-28 and 24 P60 (Two-way ANOVA, effect of genotype p < 0.001; post hoc Holm-Sidak test, wt ko: P27-28 p < 0.01, P60 p < 0.001). ** p<0.01, *** p<0.001. 25 VS. 26

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33 Supplementary Fig 3. (A) Contralateral bias index (CBI) values for wt and ko mice 34 monocularly deprived (md) or nondeprived (bin). Whiskers represent the average 35 CBI ± SEM for each experimental group; circles represent individual CBIs for each 36 animal. CBI of null mice was not significantly different from that of wt animals (Two-37 way ANOVA, genotype x condition interaction p < 0.05; post-hoc Holm-Sidak test, p 38 = 0.96) and from that of md null mice (post-hoc Holm-Sidak test p = 0.97), whereas 39 md-wt mice displayed the typical ocular dominance (OD) shift towards the open eye 40 (post-hoc Holm-Sidak test, p < 0.05). (B) ΔO values for wt, ko, wt-md and ko-md 41 animals. Whiskers represent the average $\Delta O \pm SEM$ for each experimental group; 42 circles represent individual ΔO for each animal. The effect of MD on binocular 43 matching is occluded by the deletion of miR-132/212 (Two-way ANOVA, genotype x 44 condition interaction p < 0.01; post-hoc Holm-Sidak test: wt vs wt-md p < 0.01, ko vs. 45 ko-md p = 0.795). * p < 0.05.

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49 Supplementary Figure 4.



Supplementary Fig. 4 miR132/212 deletion does not affect visual acuity. 54 Histograms represent the average VA ± SEM for wild-type (n = 3) and miR132/21255 null mice (n = 3). Error bars represent SEM. Examples of VEP curves for single mice 56 are reported on the right.

62 **Supplementary Figure 5**



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Supplementary Fig. 5. (A) Total distance moved (TDM) and (B) velocity in bin and 66 67 mon mice. (C) Average time spent in the shallow side for wt, ko and wt-md animals. Whiskers represent the average % in shallow side ± SEM for each experimental 68 69 group; circles represent individual percentages for each animal. Dotted line 70 represents chance level. (D) No difference was detected between wt and ko animals 71 in the time spent in the center of the visual cliff arena at P30 (t-test, p = 0.126). (E) 72 Average time spent in the shallow side for wt-P60 and ko P60 mice. Whiskers 73 represent the average % in shallow side ± SEM for each experimental group; circles 74 report individual percentages for each animal. (F) Exploration time of the center of

visual cliff arena is totally comparable in wt-P60 and ko-P60 mice (t-test, p = 0.767). * p < 0.05.

Supplementary Figure 6.



Supplementary Fig. 6 Low magnification image of the visual cortex of a miR132/212 mice crossed with GFP expressing line M mice. Please note GFP positive layer V pyramidal cell bodies and dendrites in the visual cortex. Left hand side: scheme with anatomical location of V1 (from Paxinos G and Franklin KBJ The mouse brain in stereotaxic coordinates 2nd edition 2001, Academic Press) Scale bar 500 microns

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101 **Supplementary Fig. 7** Δ O values for emx-wt, flox, and emx-ko animals. Whiskers 102 represent the average Δ O ± SEM for each experimental group; circles represent 103 individual Δ Os for each animal. Binocular matching is significantly impaired in emx-104 ko mice (One-way ANOVA p < 0.05, post hoc Holm-Sidak test, miR-132/212^{fl/fl} vs 105 Emx1:Cre-miR-132/212^{-/-} p < 0.05; Emx1:Cre-wt vs Emx1:Cre-miR-132/212^{-/-} p < 106 0.05; Emx1:Cre-wt vs miR-132/212^{fl/fl} p = 0.808) * p<0.05.

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