

Activation of the medial preoptic area (MPOA) ameliorates loss of maternal behavior in a *Shank2* mouse model for autism

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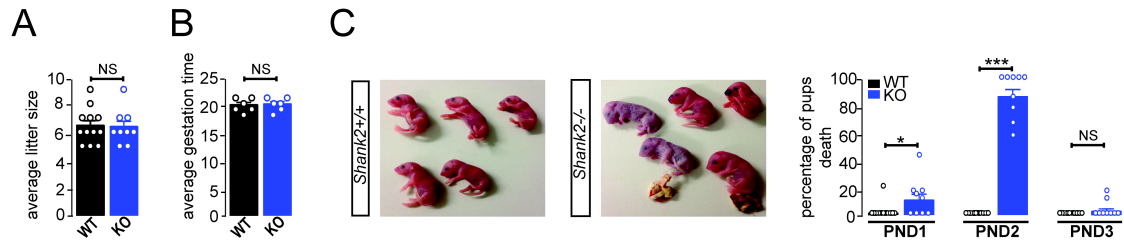
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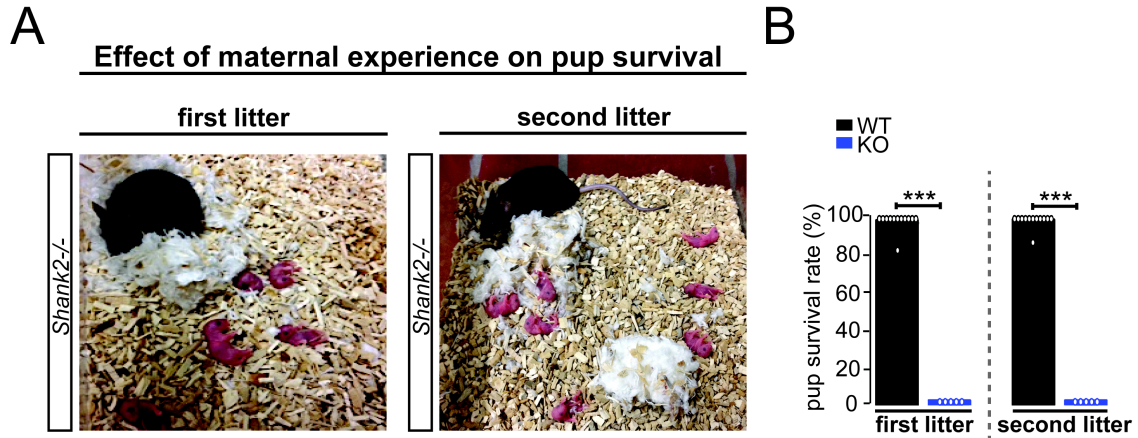
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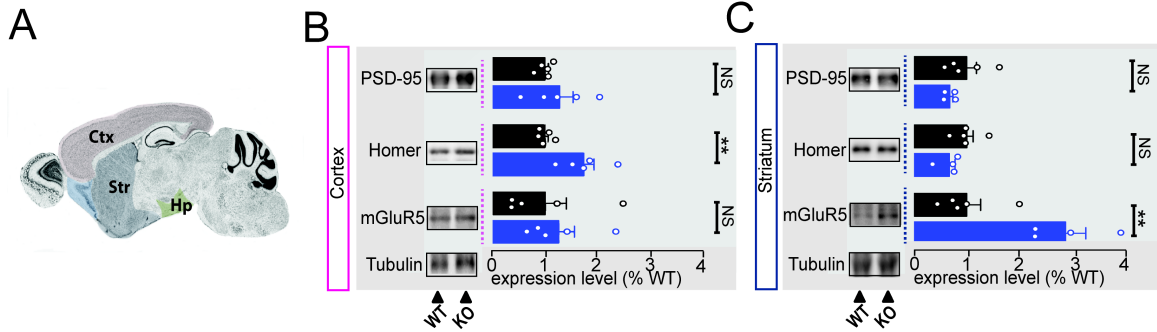


Appendix Figure S1: *Shank2*^{-/-} mice display normal litter size and gestation time

(A) *Shank2*^{-/-} delivered the same number of pups in comparison to *Shank2*^{+/+} mice, unpaired, two-tailed Student's t-test, $P=0.880$, *Shank2*^{+/+} $n = 12$, *Shank2*^{-/-} $n = 9$. **(B)** No significant difference was detected comparing the average gestation time, unpaired, two-tailed Student's t-test, $P=0.813$, *Shank2*^{+/+} $n = 6$, *Shank2*^{-/-} $n = 6$. **(C)** Left panel: Typical litter of pups born to *Shank2*^{+/+} dams. Pup mortality was nearly absent in *Shank2*^{+/+} mice. Right panel: Typical litter of pups born to *Shank2*^{-/-} dams the morning after delivery; note the neglected appearance of the pups. Diagram: Percentage of pup lethality between postnatal day (PND) 1 and 3. The majority of pups born to *Shank2*^{-/-} mice died within 48h after delivery, two-way ANOVA, effect of genotype: $***P<0.001$, effect of day: $***P<0.001$, genotype x day interaction: $***P<0.001$, *Shank2*^{+/+} $n = 12$, *Shank2*^{-/-} $n = 9$. All graphs are presented as mean $\pm$ SEM; NS, not significant.



Appendix Figure S2: Neonatal survival was not enhanced by prior birth experience in *Shank2^{-/-}* dams. (A) *Shank2^{-/-}* mice displayed a similar phenotype independent from the number of pregnancies. The majority of pups remained untouched and appeared neglected at the first (left panel) as well as after the second delivery (right panel) by *Shank2^{-/-}* dams. **(B)** No significant difference was detected comparing the first with the subsequent pup survival rate in *Shank2^{-/-}* dams, two-way ANOVA, effect of genotype: *** $P < 0.001$, effect of trial: $P = 0.834$, genotype x trial interaction: $P = 0.834$. All graphs are presented as mean $\pm$ SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS, not significant, *Shank2^{+/+}* $n = 12$, *Shank2^{-/-}* $n = 5$.



Appendix Figure S3: Synaptic protein levels in crude membrane fractions (P2) of distinct brain areas in *Shank2*^{-/-} mice. Levels of synaptic proteins in the crude synaptosomes fraction (P2) from the **(B)** neocortex and **(C)** Striatum of *Shank2*^{+/+} and *Shank2*^{-/-} mice. Protein levels were normalized to β 3-Tubulin. Representative bands for indicated proteins are shown. *Shank2*^{-/-} mice displayed significant alterations in Homer 1b/c protein levels in the neocortex, unpaired, two-tailed Student's t-test, ***P*=0.007. Additionally, protein levels of mGluR5 were significantly altered in the striatum of *Shank2*^{-/-} mice, unpaired, two-tailed Student's t-test, ***P*=0.004. All graphs are presented as mean $\pm$ SEM, **P*<0.05, ***P*<0.01, ****P*<0.001. *Shank2*^{+/+} *n* = 5, *Shank2*^{-/-} *n* = 5, age 8-12 weeks.