Supplementary Methods to:

Title: Experience-dependent expression of miR132 regulates ocular dominance plasticity.
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Chromatin immunoprecipitation (ChIP)

For chromatin immunoprecipitation experiment, P27 wild type C57BL/6J mice were sacrificed in darkness or at 20 minutes upon light exposure after 3 days of DR. Visual cortices were removed and the fresh tissue was processed immediately. Monocular deprivation was performed in critical period mice (P25) anesthetized with avertin (1ml/50mg) by suturing the eyelids of the right eye. Mice were sacrificed and the binocular part of the visual cortex was removed. The binocular right and left cortices of three different animals were pooled together and processed.

Fresh tissue was chopped and cross-linked using 1% formaldehyde in PBS, shaking it at room temperature for 15 minutes. The cross linking reaction was stopped by adding 0,125 M glycine. Samples were centrifuged (1000 x g; 5 minutes; 4°C). Supernatant was discarded and the pellet was washed once with ice- cold PBS plus protease inhibitors cocktail 10 μl/ml (Cat. n. P8340 SIGMA-Aldrich) and NaBu 20 mM by centrifugation (1000 x g; 5 minutes; 4°C). Pellets were re- suspended in new PBS plus protease inhibitors and NaBu and homogenized to obtain a single cells suspension. The suspension was centrifuged (1000 x g; 5 minutes; 4°C) and the volume of the pellet was estimated. The pellet was re-suspended in 6 X volumes of cell lysis buffer (5 mM PIPES pH 8.0, 85 mM KCl, 0,5% Igepal, plus protease inhibitors and NaBu). The samples were incubated on ice for 10 minutes and centrifuged (1000 x g; 5 minutes; 4°C). Supernatant was discarded and the pellet was re-suspended by vortexing in Nuclear lysis buffer (50 mM TrisHCl, 10 mM EDTA, 1% SDS plus protease inhibitors and NaBu). Samples were incubated on ice for 5 minutes and sonicated to shear the chromatin using the BioruptorTM (Diagenode) for 30 minutes (30 seconds “ON”, 30 seconds “OFF”). The sheared chromatin was diluted with the dilution buffer (0,01% SDS, 1,1% Triton X100, 1.2 mM EDTA, 16,7 mM Tris-Cl pH 8.1, 167 mM NaCl). 100 μl of sheared chromatin was incubated with protein A coated magnetic beads (Dynabeads protein A Cat. n. 100.02D, Invitrogen) previously incubated with 1μg of specific antibody: anti-AcH3 (lys 9-14) (Cat. n. 06-599, Upstate),
anti-phospho(Ser10)Acetyl(Lys14)H3 (Cat. n. 07-081, Upstate), anti- Me2K4H3 (Cat. n. 17-677, Upstate); or 1 μg of control antibodies: normal rabbit IgG (Cat. n. 17-614, Upstate) and normal mouse IgG (Cat. n. 17-677, Upstate). Samples were incubated overnight at 4°C in constant rotation (40 rpm).

The magnetic beads were washed for two times with the dilution buffer and 6 times with the washing buffer (100 mM Tris-Cl, 500 mM LiCl, 1% NP40, 1% Deoxycholic Acid). At the end of the washing beads were incubated with 10% chelex (Chelex 100 Resin, Cat. n. 143-2832, Biorad) in boiled water for 10 minutes. Samples were treated with Proteinase K (Cat. n. P4850, SIGMA-Aldrich) for 30 minutes at 55°C with shacking (1000 rpm). The samples were incubated in boiled water for 10 minutes and after that centrifuged (14000 x g; 1 minutes; 4°C). Part of the supernatant (DNA) was recovered. 100 μl of ChIP grade water was added and the samples were centrifuged again (14000 x g; 1 minutes; 4°C). The supernatant, that contains DNA, was recovered and DNA was ready for PCR reaction.

Quantitation of Chromatin immunoprecipitation by Real time PCR

Levels of specific histone modifications at gene promoters of interest: CREmiR132, CREmiR212 and CREc-fos, were determined by measuring the amount of that promoter in chromatin immunoprecipitates by use of Real time PCR (Step one machine Applied Biosystems, Foster City, CA). Input DNA (non-immunoprecipitated DNA) and immunoprecipitated DNA were PCR amplified in duplicate in the presence of Taqman probes. The relative quantities of immunoprecipitated DNA fragments were calculated by using the threshold cycle number (Ct) obtained during the exponential growth of the PCR products and normalized to the Input Ct. Primer and probe sequences used for Real time PCR were as follows: CREc-fos forward GAGACCCCCTAAGATCCCAA, reverse CCCCCGTCTTTGGCATACA, MGB probe TGAACACTCATAGGTGAAAG; CREmiR132 forward GGGGCCGCGACACT, reverse CGGTTGCCCTGGAGACG, MGB probe CAGGCTGACGTCAGCAC; CREmiR212 forward CCACCGCCGGAAATGC, reverse CGACCGTGACGTCAGAC, MGB probe CTGTGACGTCAAAGATG.

Western blotting

P27 mice were sacrificed in darkness or at 20 minutes upon light exposure after 3 days of DR. Visual cortices were removed and frozen on dry ice. The tissue samples were homogenized in cell disruption buffer (Ambion, Italy). Histones were extracted as previously described in Putignano et al. 2007. Briefly, histones proteins were extracted from the nuclear fraction by
the addition of five volumes of 0.2 M HCl and 10% glycerol and pelleted by centrifugation (18,000 x g; 30 min; 4°C). Histones were precipitated from the acid supernatant by the addition of ten volume of ice-cold acetone followed by centrifugation (18,000 x g; 30 min; 4°C). The obtained pellet was re-suspended in Urea 9 M. Proteins concentration was determined by Bradford assay (Biorad, Italy). For the running, each sample was boiled and 15μg of histones were loaded in each lane of a 12% acrylamide gels, using the Precast gel System (Biorad, Italy). The samples were blotted onto nitrocellulose membrane (Biorad, Italy) and blocked in 4% nonfat dry milk or 4% BSA in Tris-buffered saline (TBS) for 2 hours. The nitrocellulose membrane was incubated with the following antibodies: H3 (Cat. N. 05-499 Upstate, NY) and Me2K4H3 (Cat. N. 17-677 Upstate, NY). Anti-H3 was diluted (1:300) in TTBS and 2% BSA and anti-Me2K4H3 was diluted (1:1000) in TTBS 2% milk, and both incubated overnight at 4°C. Blots were than washed for 3 times in TTBS for twenty minutes, incubated in HRP- conjugated anti-mouse (Biorad, Italy) diluted (1:3000) in 2% BSA or 2% milk in TTBS for two hours at RT. The membranes were than rinsed three times in TTBS and incubated in enhanced chemiluminescent substrate (Biorad, Italy) and exposed to film (hyperfilm, Amersham Biosciences, Europe). Films were scanned and densitometry was analyzed through Imag software. To minimize variability, each sample was loaded in parallel in two lanes and two gels were run simultaneously on the same apparatus. For each gel, the corresponding blotted filters were cut in two in order to obtain in each filter a complete series of samples. One of the filters was reacted with the antibody against the modified histone (Me2K4H3) and the other with the antibody insensitive to the target protein modification (H3). The densitometric quantification of the band corresponding to the methylated histone was normalized to the value obtained for the total amount of histone (H3) from the same gel. For MeCP2 Western blots, Right and left cortex were homogenized in cell disruption buffer (Ambion, Italy) and samples were centrifuged (14, 000 x g; 2 minutes; 4°C). The supernatant was used for the experiment. 25μg of proteins were loaded in each lane of a 10% acrylamide gels, using the Precast gel System (Biorad, Italy). The samples were blotted onto nitrocellulose membrane (Biorad, Italy) and blocked in 4% nonfat dry milk or 4% BSA in Tris-buffered saline (TBS) for 2 hours. The nitrocellulose membrane was incubated with the following antibodies: β-tubulin 1:5000 (Sigma Aldrich) and MeCP2 1:1000 (cat. n. M 9317 Sigma Aldrich). Other steps as in the histone western blot protocol.

**TSA treatment**
TSA (5 mg/ml in DMSO) was administered by means of daily i.p. injection at a dose of 2.5 mg/kg. Adult wild type C57BL/6J mice (P100-120) received four injection of either TSA or the same volume of vehicle beginning from the first day of dark rearing (DR) until the day of light re-exposure (4th day). The last day all the mice received the i.p. injection and after one hour a group of mice were re-exposed to light. A group of mice was sacrificed in darkness and a second group after 105 minutes of visual stimulations. Visual cortices were removed and frozen on dry ice.

**RNA extraction, reverse transcription and Real time PCR**

For RNA extraction and western blotting, P27 mice were killed by decapitation in darkness or at different times after visual stimulation (20 minutes, 105 minutes, 7 hours). P7-P15-P20-P25-P30-P35 standard (STR) mice were sacrificed by decapitation. A group of mice were reared in completely darkness (DRB) from birth and at P7-P15-P20-P25-P30-P35 were decapitated in darkness. The effects of monocular deprivation were assessed after three days of deprivation (MD3d mice). Mice were sacrificed and the binocular visual cortices were removed and frozen on dry ice. The contralateral and the ipsilateral visual cortices were processed separately. Visual cortices were removed and frozen on dry ice. Tissue samples were homogenized in cell disruption buffer (Ambion, Italy). RNA was extracted by the addiction of Phenol/guanidine-based QIAzol Lysis Reagent (Qiagen, Italy). Chloroform was added and the samples were shaken for 15 seconds. The samples were left at RT for 3 minutes and then centrifuged (12000 x g, 15 min, 4°C). The upper phase aqueous solution, containing RNA, was collected in a fresh tube and the RNA was precipitated by the addiction of isopropanol. Samples were mixed by vortexing, left at RT for 10 minutes and then centrifuged (12000 x g, 10 min, 4°C). Supernatant was discarded and the RNA pellet was washed in 75% Ethanol by centrifugation (7500 x g, 5 min, 4°C). Supernatant was discarded and the pellet was re-suspended in Depc (Diethyl pyrocarbonate Cat. N. D5758, Sigma-Aldrich, Germany) water. Total RNA concentrations were determined by measuring absorbance at 260 nm with a SmartSpec Plus Spectrophotometer (Bio-Rad). RNA quality was analyzed through a gel running (1% agarose). Total RNA was reverse transcribed using QuantiTech Reverse Transcription Kit (Cat. n. 205311, Quiagen). MicroRNAs were reverse transcribed with the TaqMan microRNA reverse transcription Kit (Part n. 4366596 Applied Biosystems, CA) using miRNA specific primers for mature miR132 (Cat. n. 457 Applied Biosystems, CA) and SNO234
small nucleolar 234 RNA cat. n. 1234 Applied Biosystems, CA). Pri-microRNA and microRNA expression was analyzed by Real time PCR (Step one, Applied Biosystems, Foster City, CA). Taqman inventoried assays were used for pri-miR132 (Cat. n. Mm 03306275), miR132 (Cat. n. 457 Applied Biosytems) and SNO234 (Cat. n. 1234 Applied Biosytems). Taqman assay was used for Glyceraldehyde phosphate dehydrogenase (GAPDH), GADPHprobe ATCCCATGAAGCTGAACGG, GADPHforward CAAGGCTGTGGGCAAGGT, GADPHreverse GGCCATGAGCTGAGCTT. Quantitative values for cDNA amplification were obtained from the threshold cycle number (Ct) obtained during the exponential growth of the PCR products. Threshold was set automatically by the Stepone software. Data were analyzed using the ΔΔCt methods using GAPDH and SNO234 to normalize primiRNA and mature miRNA cDNA levels, respectively.

In situ hybridization

MD3d critical period mice and age matched nondeprived control mice were transcardially perfused with ice-cold 4% paraformaldehyde in phosphate buffer and brains were post-fixed for two hours before being placed in 30% sucrose in PBS overnight. 40 µm coronal sections of the brains were cut on microtome. In situ hybridization was executed as described in Decembrini et al. 2008, briefly:

1st day: free-floating sections were treated with 4% paraformaldehyde at RT for 10 minutes. Sections were washed with PBS and treated with 1: 40000 proteinase K (20 mg/ml) in PBS 0,1% tween-20 (PBT) for 10 minutes at RT. The reaction was blocked by washing two times with glycine 2mg/ml in PBT at RT. Slices were re-fixed with 4% paraformaldehyde for 15 minutes at RT and washed three times in PBT. Slices were prehybridised 30 minutes at 48°C in hybridization buffer [HB, 50% formammide (Sigma Cat. N. F9037), 5X SSC (20X SSC: 3 M NaCl, 0,3 M trisodium citrate in H2O), 0,1% tween-20, 50 µg/ml Heparin (Sigma Cat. N. H3393), 500 microg/ml torula yeast RNA]. Sections were hybridised at 48°C overnight (0/N) whit 0,3ul/ 150ul of mercury LNA 3’DIG labelled mmu-miR132 probe (Exiqon, Cat. N. 39033-05).

2nd day: Slices were washed twice with 2X SSC and once with 0,2X SSC at RT for 10 minutes and blocked 30 minutes at RT in blocking solution [1% blocking reagent (Roche Cat. N. 11096176001), 1% sheep serum in MABT (100 mM maleic acid, 150 mM NaCl at pH 7,5, 0,1% tween-20]. Sections were incubated with Anti-Dig-AP Fab fragments Ab 1:2000 (Roche Cat. N. 11093274910) in blocking solution O/N at 4°C.
3rd day: Slices were washed five times with PBT and three times with NMNT (100 mM NaCl, 100 mM Tris- HCl ph 9.5, 50 mM MgCl2, 0.1% tween-20, 2 mM tetrakisole. Sections were incubated in darkness with Fast Red tablets (1 tablet in 2 ml 0.1 M Tris- HCl pH 8.2, Roche Cat. N. 11496549001) and the staining reaction was controlled every 10-15 minutes. Slices were washed three times in PBS and mounted on glass slides. Images acquisition was performed by using a Confocal microscope (LEICA DM6000).

MiRNAs mimic treatment
Critical period mice (P25) were anesthetized with avertin (1ml/50mg) and monocularly deprived. Monocular deprivation was achieved by suturing the eyelids of the right eye. The mice were implanted in the cortex contralateral to the deprived eye with a cannula (gauge 30) connected via a PVC tubing to an osmotic minipump (Model 1007D; Alzet, Palo Alto, CA, reservoir volume 100 μl, flux 0.5 μl/hr). The cannula was inserted at a location 4 mm anterior and 3 mm lateral to lambda. Minipumps were filled with 25 μM double strands miR132 mimic (Thermo Scientific Dharmacon) or 25 μM double strands control miRNA mimic (Thermo Scientific Dharmacon) in Depc saline. Double strands miRNAs mimic had the following modification on the sense strand: all ribonucleotides were 2’-O – methyl modified, six phosphorothioate backbone modifications were also included with two phosphorothioates located at the 5’–end and four at the 3’-end and a cholesterol moiety at the 3’-end (Kuhn et al., 2010). The oligonucleotides had been converted 2’-hydroxyl, purified, annealed and desalted duplex. The miRNAs mimic used had the following sequences: miR132 mimic sense UAACAGUCUACAGCCAUGGUCG, antisense CGACCAUGGCUGUAGACUGUUA; control miRNA mimic sense UUGUACUACACAAAAGUACUG, antisense CAGUACUUUUGUGUAGUACAA.

To visualize miR132 mimic infusion in the visual cortex a group of MD3d animals was treated with 5’- biotinylated miR132 mimic. Transcardial perfusion was executed with ice-cold 4% paraformaldehyde in phosphate buffer and brains were post-fixed for two hours before being placed in 30% sucrose in Depc-PBS overnight. Brains were frozen in isobutene and 40 μm coronal sections were cut on cryostat. Free-floating sections were subjected to a 2 hr block (Depc-PBS containing 10% BSA and 0.3% Triton X-100) followed by incubation with ABC kit (Vector laboratories, Burlingame, CA) for 1 hour. The reaction was completed using DAB (Vector Laboratories, Burlingame, CA) as a chromogen.

Spine morphology assessment
To study spine morphology, the visual cortex of critical period Thy-1 GFP transgenic mice (line M, Feng et al., 2000) were infused with miR132 mimic or the control miRNA for three days. At the end of the treatment mice were perfused with 4% paraformaldehyde and brains were post-fixed for two hours and incubated in 30% sucrose in PBS O/N. 200 micron coronal sections were cut on vibratome. Slices were mounted on glass slides and stacks of images (1/0.2 micron) were acquired by using a confocal microscope (LEICA DM6000) using a 100x (NA 1.4) objective (0.09 micron/pixel). Spines were manually counted using ImageJ.

**In vitro miRNAs mimic treatment**

The NIH-3T3 cell line was cultured in modified Dulbecco’s medium supplemented with 10% new born calf serum, 1% L-glutamine and antibiotics (100 units/ml penicillin-streptomycin). The cells were plated on glass disks that were 6 cm in diameter at 60-70% confluence, and transfected using Lipofectamine 2000 (Invitrogen) with 100 nM miR132 mimic or control miRNA. 48 hours after transfections, cells were treated with RIPA buffer (50 mM Tris-Cl, 150 mM NaCl, 1% NP40, 0.5% Sodium Deoxycholate, 0.1% SDS plus protease inhibitors cocktail 10 μl/ml (Cat. n. P8340 SIGMA-Aldrich)) and scraped from the dish to obtain a whole cells protein extract. Proteins concentration was determined by Bradford assay (Biorad, Italy). For the running, each sample was boiled and 35 μg of proteins were loaded in each lane of a 10% acrylamide gels, using the Precast gel System (Biorad, Italy). The samples were blotted onto nitrocellulose membrane (Biorad, Italy) and blocked in 4% BSA in TBS for 2 hours. The nitrocellulose membrane was incubated with the following antibodies: β-tubulin (Sigma Aldrich) and p120RasGAP (Santa Cruz biotechnology). Anti-β-tubulin was diluted 1:3000 and anti-p120RasGAP was diluted 1:500 in TTBS and 2% BSA, and both incubated overnight at 4°C. Blots were than washed for 3 times in TTBS for twenty minutes, incubated in HRP-conjugated anti- mouse (Biorad, Italy) diluted (1:3000) in 2% BSA in TTBS for two hours at RT. The membranes were than rinsed three times in TTBS and incubated in enhanced chemiluminescent substrate (Biorad, Italy) and exposed to film (hyperfilm, Amersham Biosciences, Europe). Films were scanned and densitometry was analyzed through ImageJ software. The densitometric quantification of the band corresponding to p120RasGAP was normalized to the value obtained for β-tubulin from the same gel.

**Single unit recordings**
We analyzed 6 mice untreated mice (nondeprived), 5 MD3d mice, 5 MD3d mice infused with miR132 mimic (MD3d miR132 mimic), 5 MD3d mice infused with control miRNA mimic (MD3d control miR). At the end of the treatments, spiking activity of cortical neurons was recorded under urethane anesthesia (0.6ml/100g i.p., 20% solution in saline) at P28. For each animal, eight-ten cells were recorded in each of at least three tracks spaced evenly (>100 um) across the binocular primary visual cortex, to avoid sampling bias. None of the treatments apparently altered the number of visually responsive cells in that visually driven cells were recorded at an interdistance of 60 microns from each other in control animals as well as in all treatment groups. The position of receptive fields of single units were mapped and only cells with receptive fields within 20° of the vertical meridian were included in our sample. Spontaneous activity, peak response and receptive field (RF) size were determined from peristimulus time histograms (PSTHs) recorded in response to computer-generated bars, averaged over at least 15 stimulus presentations as described in Lodovichi et al. 2000. Binocularity was expressed as the normalized OD score of single neurons (Rittenhouse et al., 1999) and plotted the cumulative distribution for each experimental group. OD score was computed on cells with complete PSTH analysis of peak and baseline spiking activity after closure of either eye. OD score was defined as \[
\frac{\{\text{Peak(ipsi)} - \text{baseline(ipsi)}\} - \{\text{Peak(contra)} - \text{baseline(contra)}\}}{\{\text{Peak(ipsi)} - \text{baseline(ipsi)}\} + \{\text{Peak(contra)} - \text{baseline(contra)}\}}.\]
To express the contralateral bias of the response of each animal we used the contralateral bias index (CBI). This index is defined as \[
\frac{\{N(\text{cells 1}) - N(\text{cells 7})\} + 1/2\{N(\text{cells 2-3}) - N(\text{cells 5-6})\} + N_{\text{tot}}}{2N_{\text{tot}}}.\]

**VEP recordings**

Visual Evoked Potentials (VEPs) recordings were performed using a micropipette inserted into the binocular part of the primary visual cortex (depth 400 μm) in urethane-anesthetized mice as described (Rossi et al., 2001). Eyes were not restrained in a fixed position, but their position remained stable throughout the experiment and was similar between different mice (Schuett et al., 2002). Stimuli consisted of full-field computer-generated horizontal gratings (0.08 c/deg, mean luminance 15 cd/m², 100% contrast, 90 x 72 deg) alternating in time (1 Hz) presented at 20 cm from the animal. To measure VEP receptive field, grating width was 20°, its position was changed by 20° steps along the horizontal meridian, and VEP amplitude was recorded. Contra/Ipsi ratio was assessed by acquiring VEP responses to interleaved
stimulation of each eye in electrode tracks with receptive field centered on the vertical meridian.

**Statistical analysis**

Parametric tests such as t-test, paired t-test, and ANOVA were used as first choice. However, if the assumptions of normality of data distribution and omoschedasticity required by these tests were not satisfied, we adopted non parametric tests such as Mann-Whitney (to compare two groups), signed rank test (to compare two repeated measures on the same subjects), or Kruskall-Wallis ANOVA. Cumulative distributions were compared using the nonparametric Kolmogorov-Smirnov test. P=0.05 was assumed as significance level. Statistical analysis was performed using the Sigma Stat (Systat, USA) software. Data are expressed as mean ± standard error of mean (SEM).

**References**


Suppl. Fig. 1. Visual stimulation increases Lys4 dimethylation of Histone H3 in critical period mice.
Western blot of visual cortex samples of dark reared and 20 minutes visually stimulated (LR20m) juvenile mice. The ratio between the intensity of the band of Me2K4H3 and total H3 was taken as an index of the amount of dimethylated H3. Visual stimulation strongly increased H3 Lys4 dimethylation. (DR3d n=4 vs LR20m n=4, t test p=0.007). Error bars represent SEM.
Suppl. Fig. 2. Fos staining is increased by visual stimulation in the visual cortex of P27 mice. Fos immunohistochemistry was performed as described in Mainardi et al., 2009.

**Suppl. Fig. 3. Specificity of ChIP experiments.** Normal rabbit IgG or normal mouse IgG caused a statistically significant less recovery of chromatin respect to specific posttranslationally modified histone antibodies. CREmiR132 sequence abundance was measured using RT-PCR in cortical samples immunoprecipitated with antibodies specific for AcH3 or Normal rabbit IgG (a) or Me2K4H3 antibodies and Normal mouse IgG (b). Data were normalized to the average recovery obtained with the antibody for the posttranslationally modified histone. a) AcH3 n=7 vs Normal rabbit IgG n=7, Mann-Whitney rank sum test p<0.001. b) Me2KH3 n=4 vs Normal mouse IgG n=4, t test p=0.047. Error bars represent SEM.

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Suppl. Fig. 4. Enhancement of pri-miR132 light induced expression by TSA treatment in adult mice. 105 minutes of light exposure (LR105m) after 3 days of dark rearing in adult control mice (ADCON, P100-120) resulted in a significant induction of pri-miR132 expression (ADDR3d n=5 vs ADLR105m n=5 Mann-Whitney rank sum test p<0.05). However, the induction level present in ADCON mice were significant reduced with respect to juvenile critical period mice (CP, P27, data reproduced from Fig. 1f). Treatment of adult mice with trichostatin A (ADTSA) significantly enhanced light induced pri-miR132 expression (One way ANOVA p=0.026, post hoc Bonferroni t test: ADCON n=5 vs ADTSA n=6 p<0.05; ADCON vs CP P<0.05; ADTSA vs CP p=0.078). Dashed line represents pri-miR132 DR level of each group. Error bars represent SEM.
Suppl. Fig. 5. Monocular deprivation causes decrease in p(Ser10) Ac(Lys14) H3 on specific CRE loci in the visual cortex of juvenile mice. MD3d significantly reduced H3 phosphoacetylation, but not Lys9-14 acetylation and Lys4 dimethylation of H3 on CREmiR212 (a) and CREc-fos (b) sequences in the binocular visual cortex contralateral to the deprived eye (left ctx contra) with respect to the ipsilateral cortex (right ctx ipsi). (a) CREmiR212: p(Ser10) Ac(Lys14) H3 n=13, right ctx ipsi vs left ctx contra paired t test p=0.036; Ac(Lys9-14) H3 n=13, right ctx ipsi vs left ctx contra paired t test p=0.28; Me2(Lys4) H3 n=13, right ctx ipsi vs left ctx contra paired t test p=0.28. b) CREc-fos: p(Ser10) Ac(Lys14) H3 n=13, right ctx ipsi vs left ctx contra paired t test p=0.048; Ac(Lys9-14) H3 n=13, right ctx ipsi vs left ctx contra paired t test p=0.42; Me2(Lys4) H3 n=13, right ctx ipsi vs left ctx contra paired t test p=0.41). Error bars represent SEM. Thus, prolonged deprivation does not merely reverse the histone marks pattern observed with acute visual stimulation (see fig. 1a-c in the main text).
Suppl. Fig. 6. MiR132 mimic treatment in vitro. Western blot for p120RasGAP show a downregulation in NIH-3T3 cultures transfected with 100 nM miR132 mimic with respect to control miRNA (control miR n=3 vs miR132 mimic n=3, t test p<0.05). Error bars represent SEM.
Suppl. Fig. 7. *In vivo* effect of miR132 mimic infusion in the visual cortex of MD3d critical period mice. a) DAB staining of biotinylated miR132 mimic infused for 3 days in the visual cortex contralateral to the deprived eye of MD3d mice. Biotinylated miR132 mimic reached the binocular and monocular visual cortex but did not diffuse into the contralateral hemisphere. b) Western blot of cortical extracts of MeCP2 KO and wild type mice showed the specificity of the antibody used in the experiment. c) MiR132 mimic caused a significant decrease of MeCP2 protein expression in the infused cortex (ctx) with respect to the non infused ctx (upper graph, miR132 mimic n=6, untreated ctx vs treated ctx, Signed rank test p=0.031). The infusion of a control miRNA mimic (control miR) did not significantly change MeCP2 protein levels (lower graph, control miR n=6, untreated ctx vs treated ctx, Signed rank test p=0.31). Error bars represent SEM.
Suppl. Fig. 8. Three days miR132 mimic treatment increases the fraction of mushroom/stubby spines. To assess the effect of miR132 mimic on spine morphology, we infused miR132 mimic or control miRNA in the cortex of line M GFP expressing non deprived juvenile mice, using the same protocol adopted for electrophysiological assessment of ODP. a) No difference in spine density was present between treated and untreated cortex both in miR132 mimic and control miRNA (control miR n=3, paired t test p=0.22; miR132 mimic n=5, paired t test p=0.41). No difference was also present between miR132 mimic and control miRNA treated cortex (t test p=0.07). b) No difference in spine length was present between treated and untreated cortex both in miR132 mimic and control miRNA infused mice (control miR n=3, paired t test p=0.65; miR132 mimic n=5, paired t test p=0.99). No difference was also present between miR132 mimic and control miRNA treated cortex (t test p=0.17). c) Spines were classified as filopodia (protrusions without an enlargement of the tip), thin (protrusions with a head smaller than spine length), mushroom/stubby (large head spines). We found a significant increase (chi-square test with Bonferroni correction for multiple comparisons) in the fraction of mushroom/stubby spines in the cortex treated with miR132 mimic (miR132 treated ctx n=704 spines vs. miR132 untreated ctx n=650 spines, p=0.006; miR132 treated ctx vs. control miR treated ctx n=466 spines, p<0.001; miR132 treated ctx vs. control miR untreated ctx n=598 , p<0.001. Other tests not significantly different, p>0.05).
Suppl. Fig. 9. Mature miR132 levels after miR132 mimic or control miR infusion in the visual cortex of MD3d critical period mice. MiR132 downregulation in the visual cortex contralateral to the deprived eye in MD3d juvenile mice was not present after miR132 mimic treatment (a; MD3d miR132 mimic n=4, right cortex ipsi vs left cortex contra, paired t test p=0.7). Infusion of the visual cortex contralateral to the deprived eye with a control miRNA mimic (control miR) showed the expected decrease of miR132 expression after MD3d (b; MD3d control miR n=4, right ctx ipsi vs left ctx contra, paired t test p=0.041). Error bars represent SEM.
Suppl. Fig. 10. MiRNA mimic treatment does not affect functional properties of visual cortical neurons. a) Receptive field size (RFS) was not altered by miR132 or control miR mimic treatments in MD3d critical periods mice (nondeprived 103 cells; MD3d miR132 mimic 79 cells; MD3d 83 cells; MD3d control miR 95 cells). None of the treatments significantly altered RFS (one way ANOVA p=0.733). Receptive fields were measured on peri-stimulus time histograms elicited by light bars of optimal orientation drifting in the visual field. The width of the part of visual field eliciting cell discharge greater than spontaneous discharge plus two SDs was taken as RFS. b) Peak was measured on peri-stimulus time histograms as the maximum response of each cell. (nondeprived 103 cells; MD3d miR132 mimic 79 cells; MD3d 83 cells; MD3d control miR 95 cells. One way ANOVA p=0.16). c) Cell responsiveness for each unit of the different groups (nondeprived 103 cells; MD3d miR132 mimic 79 cells; MD3d 83 cells; MD3d control miR 95 cells) was expressed as ratio between peak response frequency and baseline discharge frequency. No statistical difference is present between all groups (one way ANOVA on ranks p=0.1). d) Baseline activity of each cell of the different groups was not affected by the miRNA mimic treatments (nondeprived 103 cells; MD3d miR132 mimic 79 cells; MD3d 83 cells; MD3d control miR 95 cells. One way ANOVA on ranks p=0.071). Error bars represent SEM.