Supporting Material for

Dnmt3a1 regulates hippocampus-dependent memory via the downstream target Nrp1

Janina Kupke[1,2], Julien Klimmt[1,3,#], Franziska Mudlaff[1,4,#], Maximilian Schwab[1,5], Pavlo Lutsik[6,7], Christoph Plass[6], Carsten Sticht[8] and Ana M.M. Oliveira[1,9,*]

[1]Department of Neurobiology, Interdisciplinary Center for Neurosciences (IZN), Heidelberg University, 69120 Heidelberg, Germany

[2]Present address: Department of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, 1081 HV, Netherlands

[3]Present address: Institute for Stroke and Dementia Research, University Hospital, Ludwig-Maximilians-University Munich, 81377 Munich, Germany

[4]Present address: Integrated Program in Neuroscience, McGill University, Montreal, QC H3A 2B4, Canada; Centre for Research in Neuroscience, Research Institute of the McGill University Health Centre, Montreal QC, H3G 1A4, Canada

[5]Present address: Department of Medical Oncology, National Center for Tumor Diseases (NCT) Heidelberg, University Hospital Heidelberg, 69120 Heidelberg, Germany

[6]Division of Cancer Epigenomics, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany

[7]Department of Oncology, KU Leuven, 3000 Leuven, Belgium

[8]Next Generation Sequencing Core Facility, Medical Faculty Mannheim, Heidelberg University, 68167, Mannheim, Germany

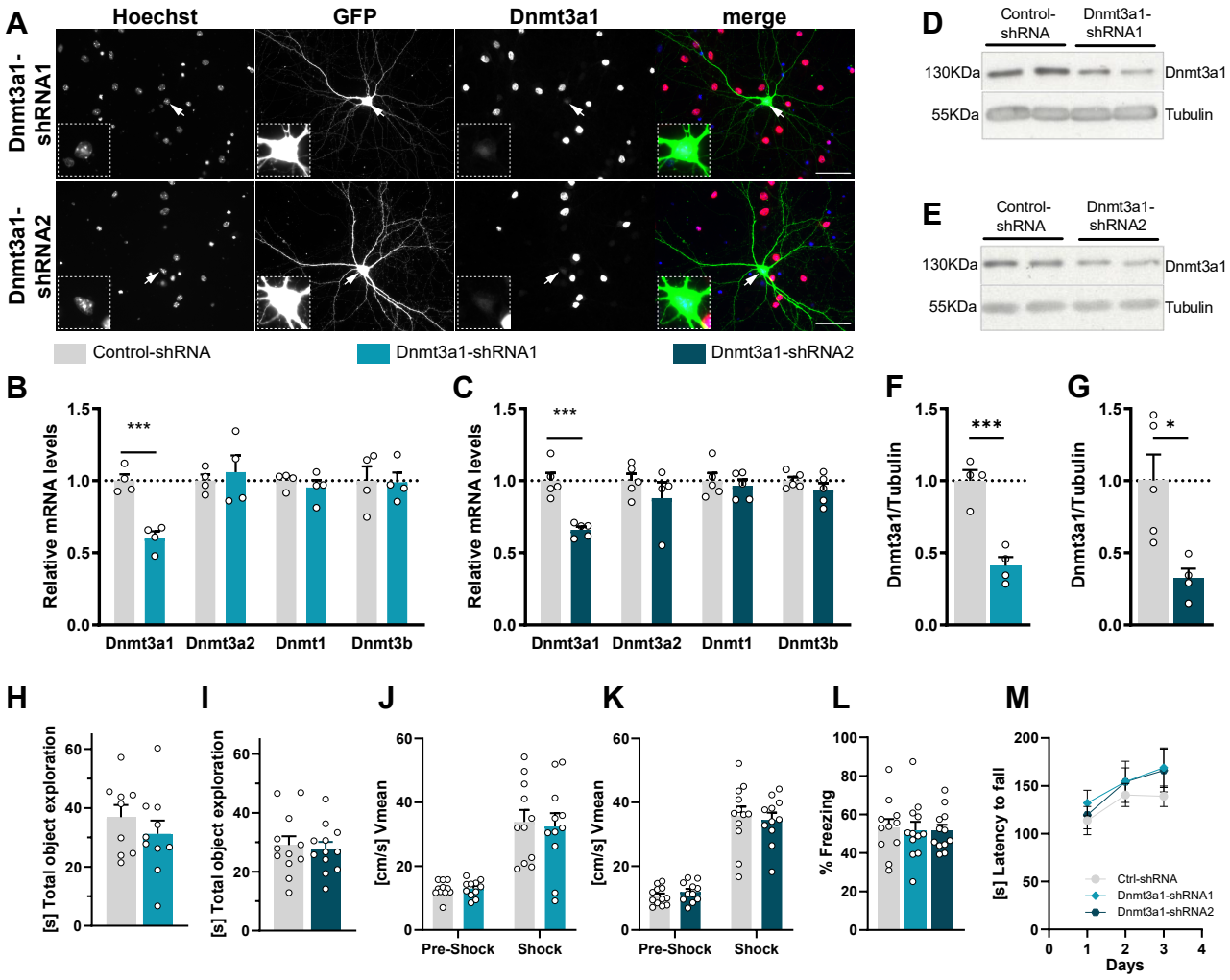
[9]Department of Molecular and Cellular Cognition Research, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, 68159 Mannheim, Germany

[#]equal contribution

*Ana M.M. Oliveira. Department of Molecular and Cellular Cognition Research, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, 68159 Mannheim, Germany. Phone: +49(0)62215416510. Email: oliveira@nbio.uni-heidelberg.de, ana.oliveira@zi-manheim.de

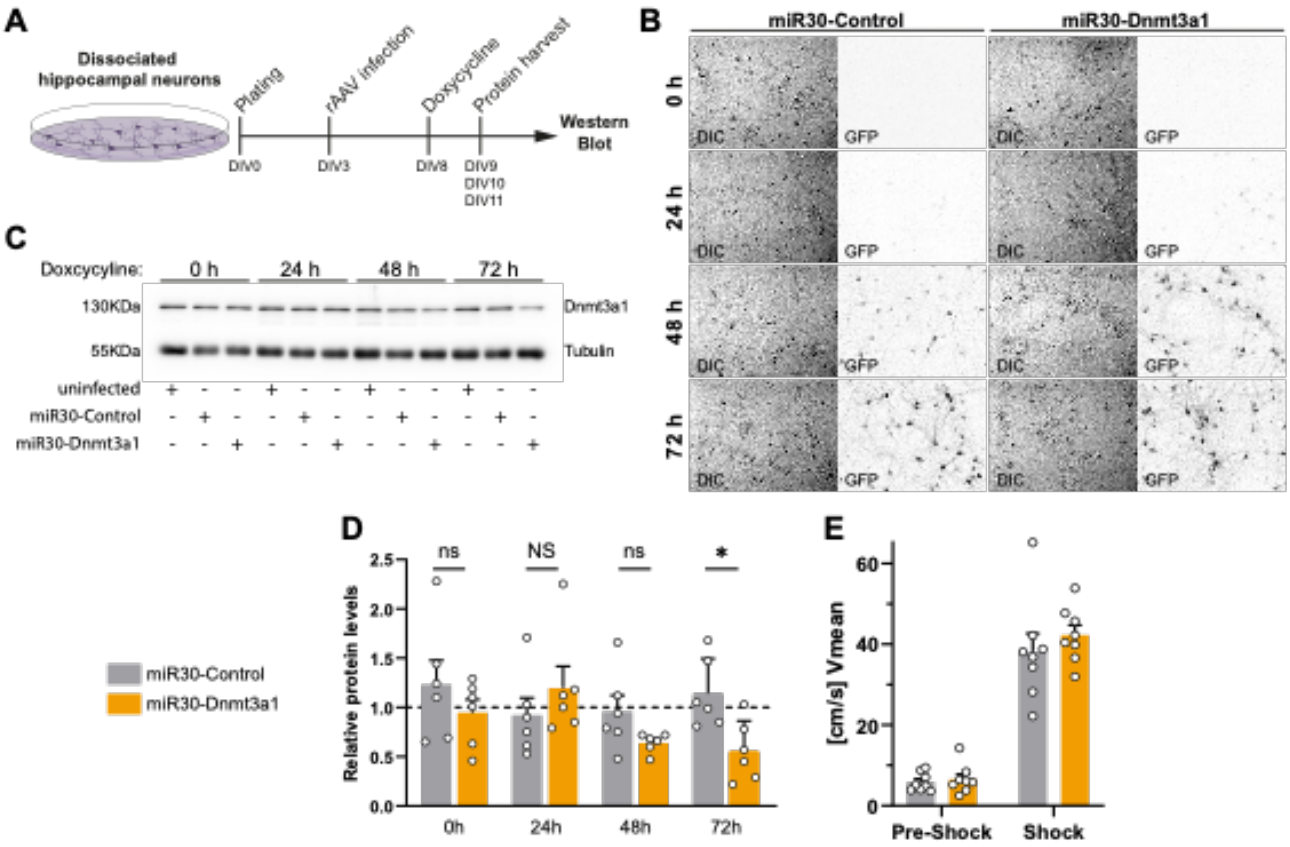
Sup. Table 1. List of primers used for qRT-PCR.

Name		Sequence
Actin	5' Primer	TATCCTGACCCTGAAGTACC
	3' Primer	CTCGGTGAGCAGCACAGGG
Alkal2	5' Primer	TGAAACATCTCACAGGTCCTCT
	3' Primer	GTGCAGTCTCTCGTGTTGTG
Cacng5	5' Primer	CCCTCGTCAGCCTCTTCTTC
	3' Primer	ACCACAAGAGAGAGGCCAGA
Crhbp	5' Primer	CTGAAGGTATTTGATGGTTGGA
	3' Primer	TCTTCATAGTGGGCAGAGGG
Nrp1	5' Primer	GCAAGACTCGAATCCTCCC
	3' Primer	CCAATGTGAGGGCCAACTTC
Npy	5' Primer	CGTGTGTTTGGGCATTCTGG
	3' Primer	TGCCATATCTCTGTCTGGTGA
Trpc6	5' Primer	CCAGCTTCCGGGGTAATGAA
	3' Primer	ACATGTATGCTGGTCCTCGA

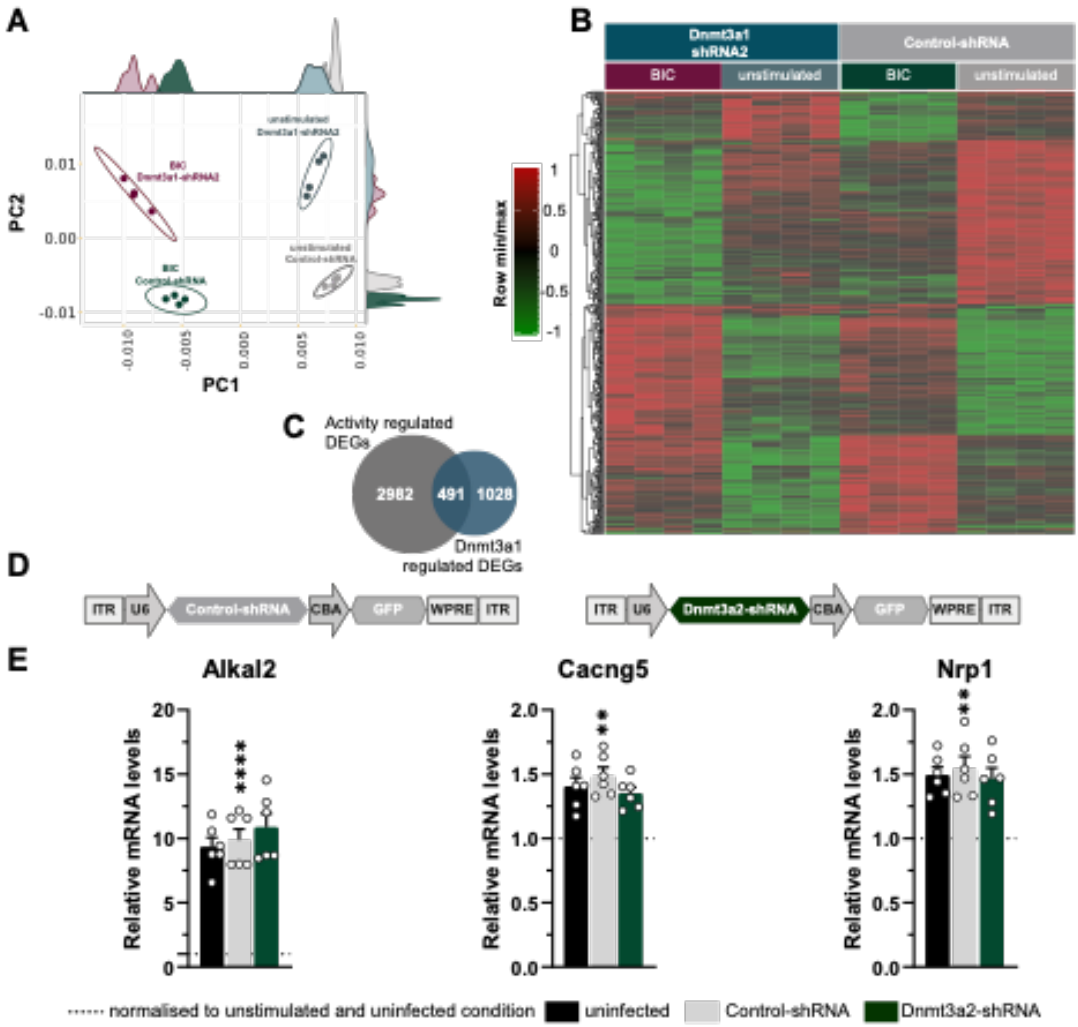


Sup. Figure 1: Validation of Dnmt3a1 shRNA-dependent knock down and behavioural control parameters. (**A**) Representative image of primary hippocampal cultures transfected with Dnmt3a1-shRNA1 or Dnmt3a1-shRNA2 (green) and immunostained against endogenous Dnmt3a levels (red). Scale bar: 50 µm. (**B, C**) Quantitative reverse-transcription PCR (qRT-PCR) analysis of the expression of DNA methyltransferases (Dnmt) in the dHPC of mice infected with rAAVs against Control-shRNA (n=4-5) or either (B) Dnmt3a1-shRNA1 (n=4) or (C) Dnmt3a1-shRNA2 (n=4). Dashed lines represent normalised expression levels to Control-shRNA. ***p≤0.001 by two-tailed, unpaired t-test. (**D, E**) Representative immunoblot scans of the expression of Dnmt3a1 or Tubulin in the dHPC of mice infected with rAAVs against Control-shRNA or either (D) Dnmt3a1-shRNA1 or (E) Dnmt3a1-shRNA2. (**F, G**) Immunoblot quantification of Dnmt3a1 protein levels in the dHPC of mice infected with rAAVs against Control-shRNA (n= 4-5) or either (F) Dnmt3a1-shRNA1 (n=4) or (G) Dnmt3a1-shRNA2 (n=4). Dashed lines represent normalised Dnmt3a1 to Tubulin expression of control group. *p<0.05, ***p≤0.001 by two-tailed, unpaired t-test. (**H, I**) Total object exploration time of mice infected with rAAVs against Control-shRNA (n=9-12) or either (H) Dnmt3a1-shRNA1 (n=10) or (I) Dnmt3a1-shRNA2 (n=12) during the three sessions of spatial object recognition training. No significant changes by two-tailed, unpaired t-test. (**J, K**) Mean velocities during CFC training pre-shock (Pre-Shock) or during shock (Shock) administration of the mice expressing

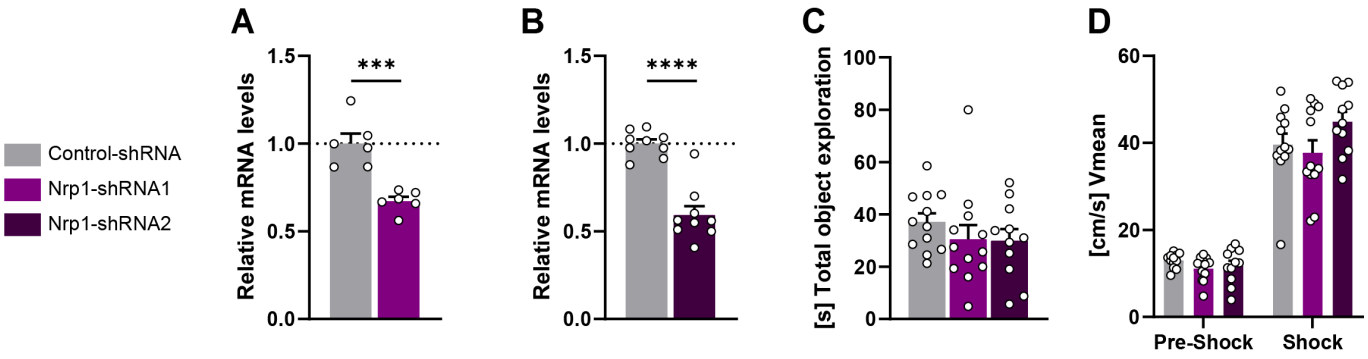
rAAVs against Control-shRNA (n=11-13) or either (J) Dnmt3a1-shRNA1 (n=11) or (K) Dnmt3a1-shRNA2 (n=11). No significant changes by two-tailed, unpaired t-test. (**L**) Long-term cued fear memory of mice expressing Control-shRNA (n=11), Dnmt3a1-shRNA1 (n=12) or Dnmt3a1-shRNA2 (n=12). No significant changes by one-way ANOVA test followed by Dunnett's multiple comparisons test. (**M**) Motor skill learning of mice expressing Control-shRNA (n=11), Dnmt3a1-shRNA1 (n=12) or Dnmt3a1-shRNA2 (n=12). Effect of training day: $p<0.001$; effect of virus: p=ns by two-way repeated measures ANOVA. Ns= no significant.



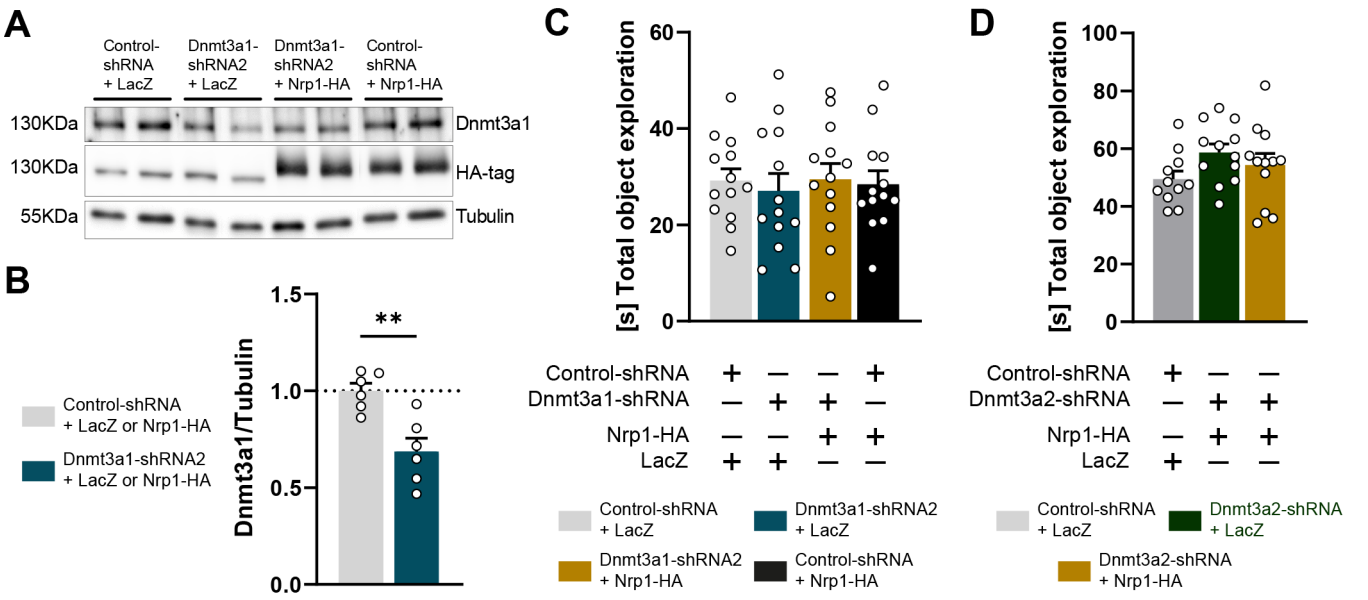
Sup. Figure 2: Validation of mir30-based Dnmt3a1 shRNA-dependent knock down and behavioural control parameters. (**A**) Experimental design used to identify the time-point of knock-down using the TetON-based miR30 system. DIV: Day *in-vitro*. (**B**) Representative images of primary hippocampal cells infected with miR30-Control or miR30-Dnmt3a1-shRNA2 sequence. Cultures were treated with doxycycline for 0h, 24h, 48, or 72h. (**C**) Representative immunoblot scans of the expression of Dnmt3a1 and Tubulin of primary hippocampal cultures infected with miR30-Control or miR30- Dnmt3a1-shRNA2 sequence and treated with doxycycline for either 0h, 24h, 48h or 72h. (**D**) Immunoblot quantification of Dnmt3a1 protein levels of primary hippocampal neurons infected with miR30-Control or miR30- Dnmt3a1-shRNA2 sequence and treated with doxycycline for either 0h, 24h, 48h or 72h. Expression levels were normalised to the uninfected controls (dashed line); (n=6 independent neuronal cultures). *p≤0.05, ns: not significant by two-tailed, unpaired t-test. NS: not significant by Mann-Whitney test. (**E**) Mean velocities during CFC training pre-shock (Pre-Shock) or during shock (Shock) administration of the mice expressing miR30-Control (n=8) or miR30-Dnmt3a1 (n=8) shRNAs. No significant changes by two-tailed, unpaired t-test.



Sup. Figure 3: PCA analysis and heat map of RNA sequencing data and effect of Dnm3a2 levels on the expression of selected genes. (**A**) Principle-component analysis (PCA) of DEGs in primary hippocampal cultures infected with Control-shRNA or Dnmt3a1-shRNA2, that were either stimulated with Bicuculline for 4h or unstimulated; (n=4 independent neuronal culture preparations). (**B**) Heatmap of 491 activity-regulated Dnmt3a1-dependent genes (overlap between Figure 3C with Figure 3D) (n=4 independent neuronal cell preparations). Hippocampal cultures infected with rAAVs expressing Control-shRNA or Dnmt3a1-shRNA2, that were either stimulated with Bicuculline for 4h or unstimulated. (**C**) Venn Diagramm of activity regulated DEGs (Figure 3C) and Dnmt3a1 regulated DEGs (Figure 3D). (**D**) Schematic representation of viral constructs. (**E**) qRT-PCR analysis of Alkal2, Cacng5 and Nrp1 expression levels in hippocampal-cultured cells infected with Control–shRNA or Dnmt3a2-shRNA and stimulated 4h with Bicuculline. Expression levels were normalised to the uninfected controls in baseline conditions (dashed line); (n=6 independent neuronal cultures). Control-shRNA 4h bicuculline versus unstimulated: **p<0.01, ****p≤0.0001 by one-way ANOVA test followed by Sidak's multiple comparisons test. No significant differences between Control-shRNA and Dnmt3a2-shRNA by two-tailed, unpaired t-test.



Sup. Figure 4: Validation of Nrp1 shRNA-dependent knock down and behavioural control parameters. (**A, B**) qRT-PCR analysis of Nrp1 expression in dHPC of mice infected with rAAVs against Control-shRNA (n=6-9), (A) Nrp1-shRNA1 (n=6) or (B) Nrp11-shRNA2 (n=9). Dashed lines represent normalised expression levels to Control-shRNA. ***p≤0.001, ***p≤0.0001, by two-tailed, unpaired t-test. (**C**) Total object exploration time of mice infected with rAAVs against either Control-shRNA (n=12) or Nrp1-shRNA1 (n=12) or Nrp1-shRNA2 (n=11) during the three sessions of spatial object recognition training. No significant changes between Control shRNA and either Nrp1-shRNA1 or Nrp1-shRNA2 by one-way ANOVA test followed by Dunnett's multiple comparisons test. (**D**) Mean velocities during CFC training pre-shock (Pre-Shock) or during shock (Shock) administration of the mice infected with either Control-shRNA (n=12) or Nrp1-shRNA1 (n=12) or Nrp1-shRNA2 (n=11). No significant changes between Control shRNA and either Nrp1-shRNA1 or Nrp1-shRNA2 by one-way ANOVA test followed by Sidaks multiple comparisons test.



Sup. Figure 5: Validation of Nrp1 overexpression and behavioural control parameters. (**A**) Representative immunoblot scans of the expression of Dnmt3a1, HA-tagged Nrp1 or HA-tagged LacZ and Tubulin in the dHPC of mice infected with rAAVs as indicated. (**B**) Immunoblot quantification of Dnmt3a1 protein levels in the dHPC of mice infected with rAAVs against Control-shRNA (n= 6) or Dnmt3a1-shRNA2 (n=6). Dashed lines represent normalised Dnmt3a1 to Tubulin expression of control group. **p≤0.01 by two-tailed, unpaired t-test. (**C**) Total object exploration time of mice infected with Control-shRNA + LacZ (n=13), Dnmt3a1-shRNA2 + LacZ (n=13), Dnmt3a1-shRNA2 + Nrp1-HA (n=13) or Control-shRNA + Nrp1-HA (n=13) during the three sessions of spatial object recognition training. No significant changes by one-way ANOVA test followed by Sidak's multiple comparisons test. (**D**) Total object exploration time of mice infected with Control-shRNA + LacZ (n=11), Dnmt3a2-shRNA + LacZ (n=12), Dnmt3a2-shRNA + Nrp1-HA (n=12) during three sessions of spatial object recognition training. No significant changes by one-way ANOVA test followed by Dunnett's multiple comparisons test.