

SUPPLEMENTARY INFORMATION

[Upregulating endogenous genes by an RNA-programmable artificial transactivator, by Fimiani et al.]

Contents:

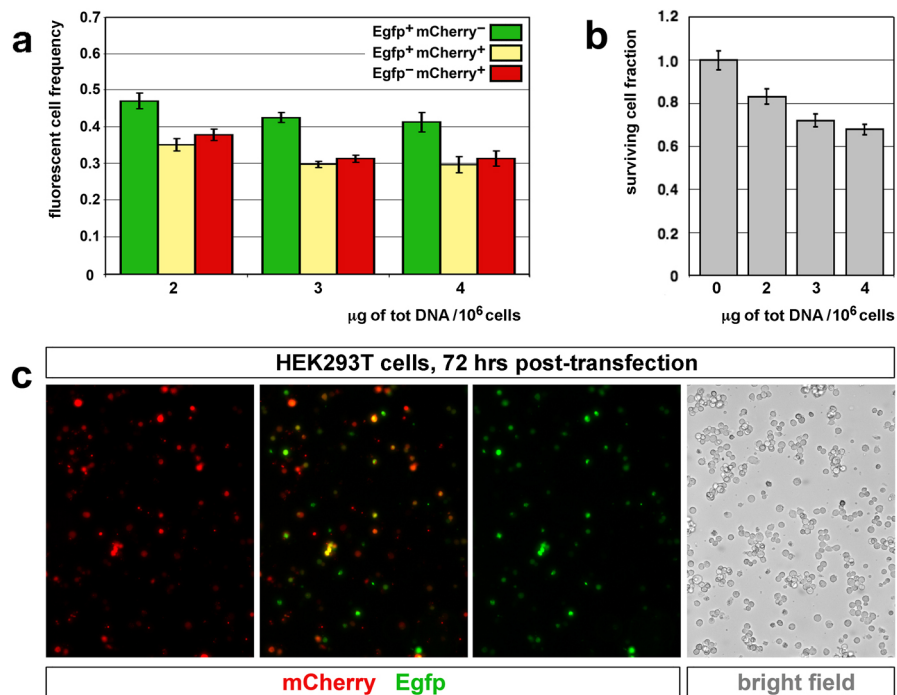
- SUPPLEMENTARY FIGURES S1-S11

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SUPPLEMENTARY FIGURES

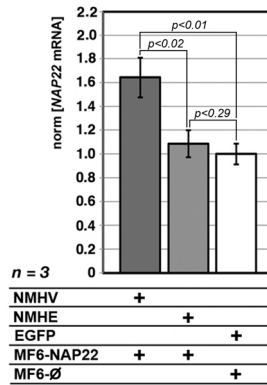
Supplementary Figure S1. Evaluation of HEK293T cell cotransfection efficiency. (a) Absolute frequencies of fluorescent elements, evaluated 72 hours after LipoD co-transfection of HEK293T cells with equimolar amounts of P_{gkp1}-mCherry (8.0kb) and CMVp-EGFP (4.7kb) plasmids. In x, total amounts of DNA delivered to 10⁶ cells are indicated. (b) Surviving fractions of cells referred to in (a). Data normalized against untransfected cells. (c) Dark and bright field pictures of cells referred to in (a), transfected with 2 μg of total DNA /10⁶ cells.

Figure S1



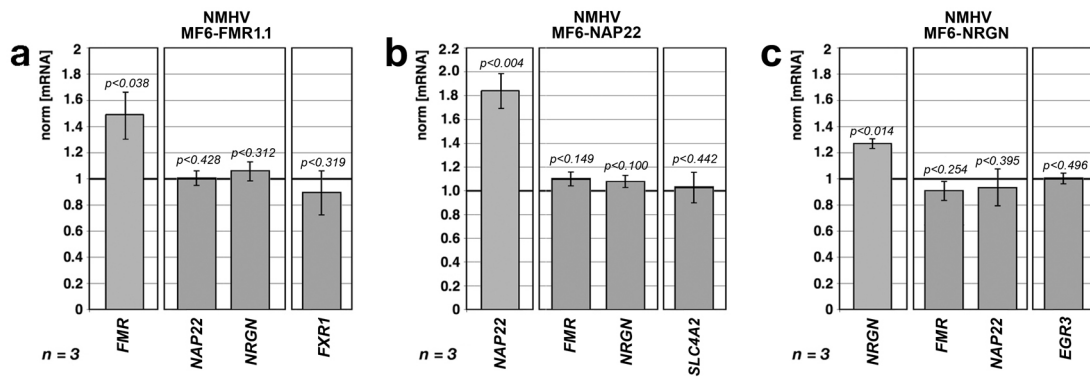
Supplementary Figure S2. Requirement of the VP16 domain for the NMHV function. *NAP22*-mRNA levels in HEK293T cells cotransfected by NMHV, NMHE, EGFP, MF6-NAP22 and MF6-Ø, in different combinations. Results were normalized against *GAPDH* and further normalized against EGFP/MF6-Ø controls. Time-frame of the experiments and statistical parameters were as in Figure 1.

Figure S2



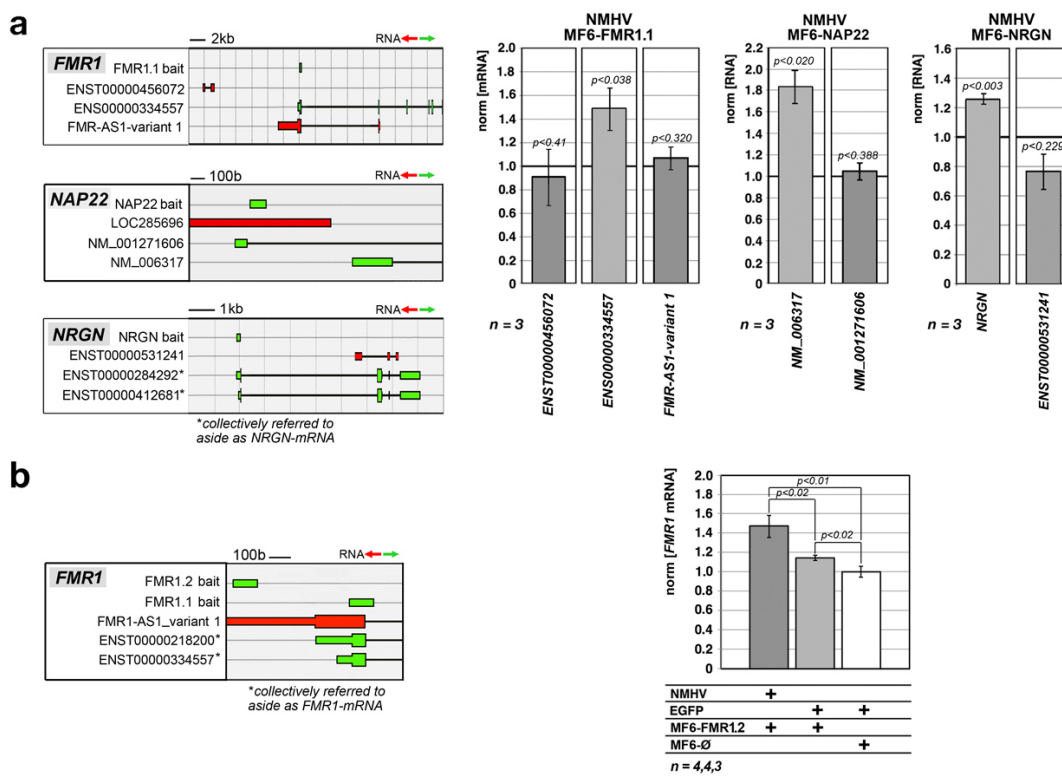
Supplementary Figure S3. Specificity of NMHV-mediated *FMR1*, *NAP22* and *NRGN* transactivation: no perturbation of other active genes and structurally similar, potential offtargets. No aspecific gene upregulation in HEK293T cells expressing NMHV and the MF6-type, *FMR1.1* (a), *NAP22* (b) or *NRGN* (c) baits. *FXR1*, *SLC4A2* and *EGR3* are potential offtargets, with 5' ends displaying remarkable homology to *FMR1.1*, *NAP22* and *NRGN* baits, respectively (see Supplementary Table S5). Results were normalized against *GAPDH* and further normalized against EGFP/MF6- \emptyset controls. Time-frame of the experiments and statistical parameters were as in Figure 1.

Figure S3



Supplementary Figure S4. Specificity of NMHV-mediated *FMR1*, *NAP22* and *NRGN* transactivation: limited cis-propagation and polarity effects. (a) Absent upregulation of transcripts stemming from TSSs >0.5kb far from bait-targets, in HEK293T cells co-expressing NMHV and the MF6-type, *FMR1.1*, *NAP22* or *NRGN* baits. (b) Upregulation of transcripts originating from *FMR1*-TSSs, being <0.5kb far from bait-target, in HEK293T cells co-expressing NMHV and the sense-oriented, MF6-type, *FMR1.2* bait. (c) Variable modulation of transcripts stemming from TSSs <0.5kb apart from bait-targets, in HEK293T cells co-expressing NMHV and the antisense-oriented, MF6-type, *FMR1.1-AS*, *FMR1.2-AS*, *NRGN-AS* or *NAP22-AS* baits. In each case, transcript upregulation by NMHV and the corresponding sense-oriented bait is shown as a reference. Time-frame of the experiments, data normalization and statistical parameters were as in Figure 1.

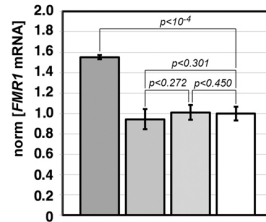
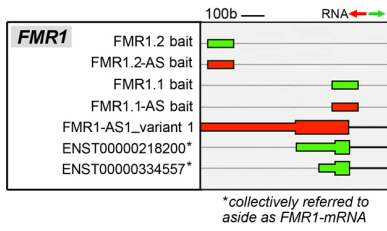
Figure S4



(split picture, to be continued)

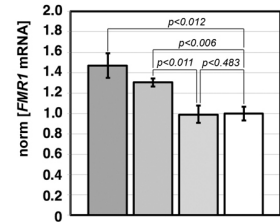
Figure S4

C



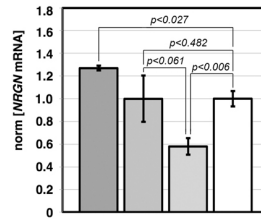
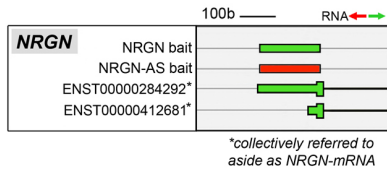
NMHV	+	+		
EGFP			+	+
MF6-FMR11	+			
MF6-FMR11-AS		+	+	
MF6-∅				+

$n = 3,3,3,3$



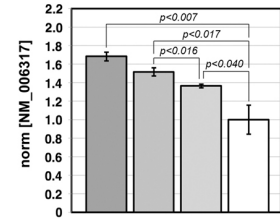
NMHV	+	+		
EGFP			+	+
MF6-FMR12	+			
MF6-FMR12-AS		+	+	
MF6-∅				+

$n = 4,3,3,3$



NMHV	+	+		
EGFP			+	+
MF6-NRGN	+			
MF6-NRGN-AS		+	+	
MF6-∅				+

$n = 3,3,3,3$

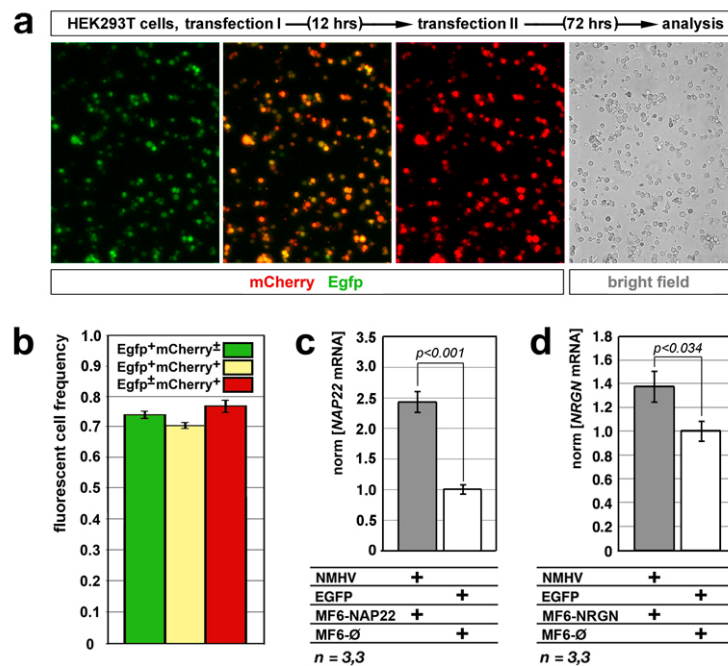


NMHV	+	+		
EGFP			+	+
MF6-NAP22	+			
MF6-NAP22-AS		+	+	
MF6-∅				+

$n = 3,3,3,3$

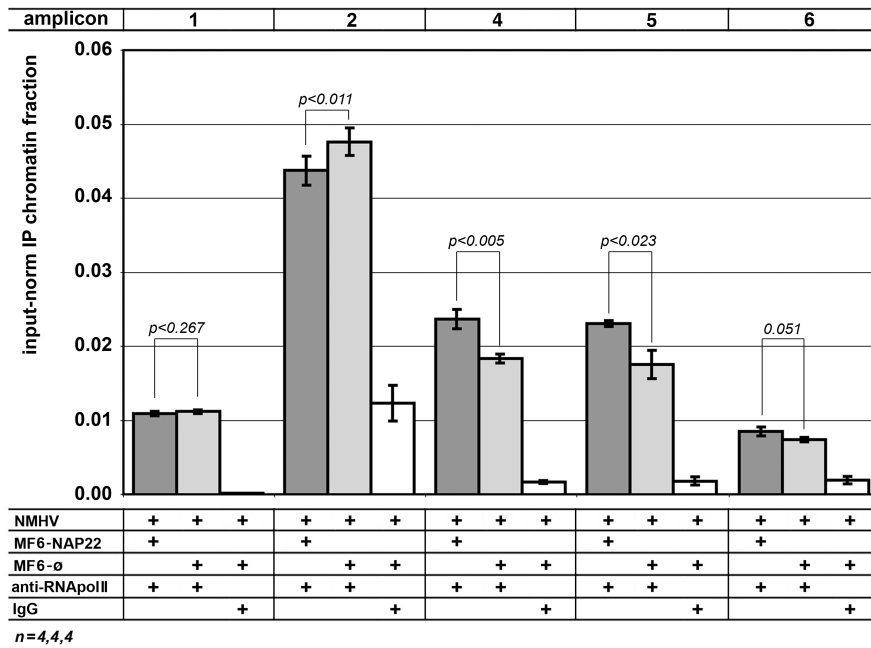
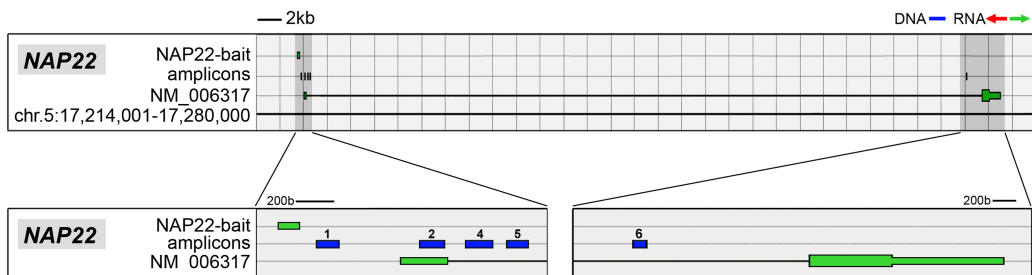
Supplementary Figure S5 Improving the transfection efficiency of HEK293T cells results in a consistent increase of NMHV/MF6-bait-dependent gene transactivation. (a) Dark and bright field pictures of HEK293T cells co-transfected with equimolar amounts of Pgkp1-mCherry (8.0kb) and CMVp-EGFP (4.7kb) plasmids (2.5 μ g of total DNA/ 10^6 cells). Analysis performed 72 hours after completion of the two-steps, Lipofectamine 3000 transfection procedure. **(b)** Absolute frequencies of fluorescent elements, referred to in **(a)**. **(c,d)** Upregulation of *NAP22* and *NRGN* mRNAs in HEK293T cells cotransfected with NMHV- and MF6-bait-encoding plasmids, according to the procedure shown in **(a)**. Pgkp1-EGFP ("EGFP") and MF6- \emptyset plasmids were used as controls. Results were normalized against *GAPDH* and further normalized against the EGFP/MF6- \emptyset combination. Numbers of biological replicates, *n*, are displayed under the graphs. Bars represent s.e.m.'s.

Figure S5



Supplementary Figure S6. Fraction of *NAP22* chromatin immunoprecipitated by anti-RNAPoIII, in HEK293T cells expressing NMHV/MF6-NAP22 or NMHV/MF6- \emptyset . Graphs refer to results of the experiments described in Figure 2d, prior to normalization against NMHV/MF6- \emptyset controls. They include results of additional control tests, run challenging NMHV/MF6- \emptyset cells by IgGs, in place of anti-RNAPoIII. *n* are numbers of biological replicates. Bars represent s.e.m.'s.

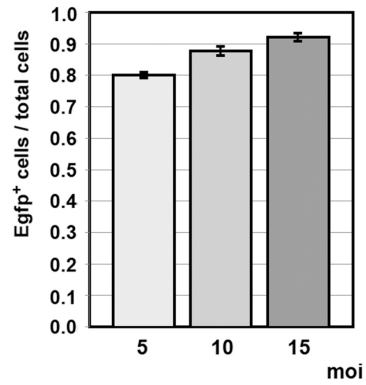
Figure S6



Supplementary Figure S7. Lentivirus dose-dependent infection of rhombo-spinal precursors.

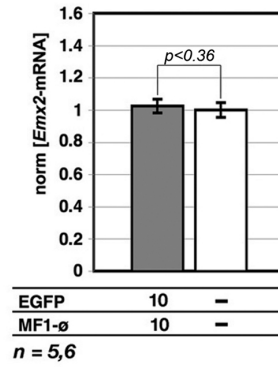
Correlation between multiplicity of infection (moi) and effective transduction rate, upon treatment of dissociated E10.5 rhombo-spinal precursors by the constitutive EGFP expressor pCCLsin.PPT.hPGK.EGFP.Wpre (37), as assessed 4 days upon infection. Tests performed in sextuplicate. Bars represent s.e.m.'s.

Figure S7



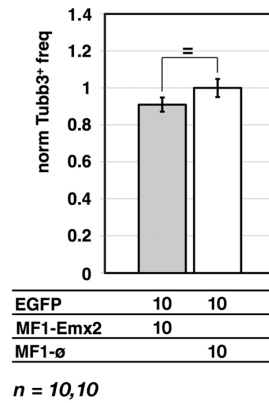
Supplementary Figure S8. Unaltered *Emx2* expression upon infection of cortico-cerebral precursors by control lentiviruses. *Emx2*-mRNA levels in uninfected cells and precursors co-infected with the control EGFP- and MF1-Ø-encoding lentivectors. Results were normalized against *Gapdh* and further normalized against uninfected cells. Time-frame of the experiments as well as representation of moi's and statistical parameters were as in Figure 3

Figure S8



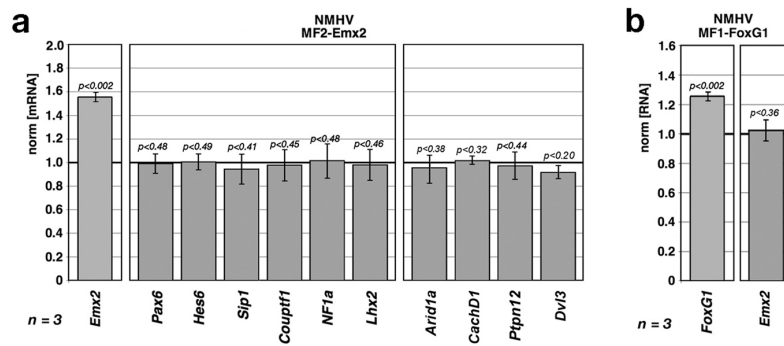
Supplementary Figure S9. Impact of *Emx2*-bait overexpression on neuronogenesis. No modulation of frequency of *Tubb3*⁺ neurons in cultures of EGFP/MF1-*Emx2*-overexpressing precursors, as assayed by immunofluorescence. Results were normalized against EGFP/MF1-∅ controls. Time-frame of the experiments as well as representation of moi's and statistical parameters were as in Figure 3.

Figure S9



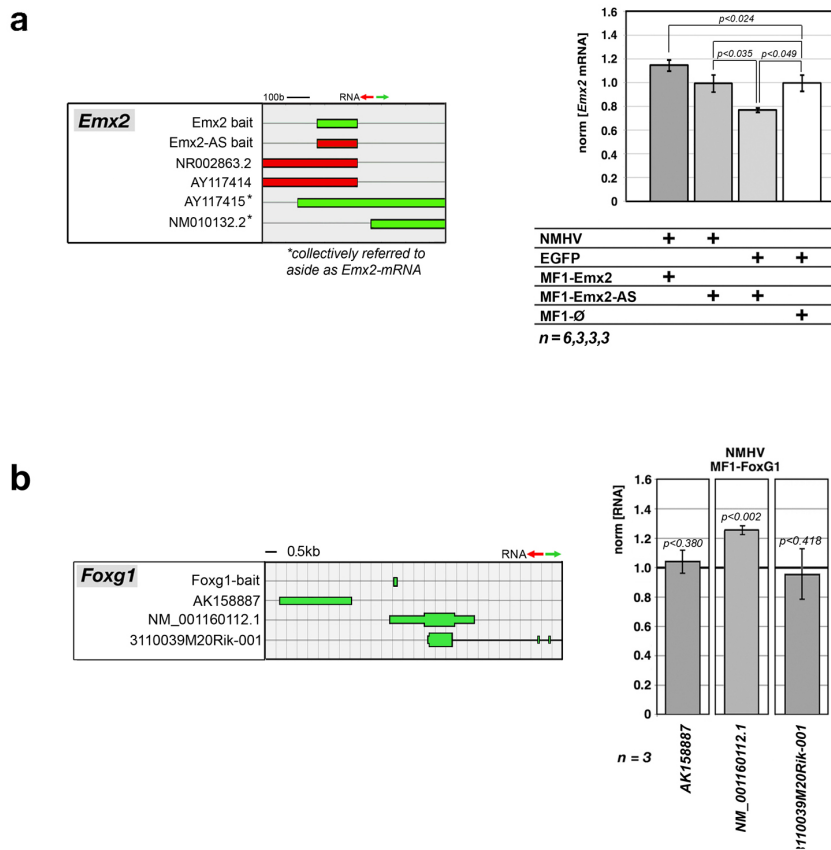
Supplementary Figure S10. Specificity of NMHV-mediated *Emx2* and *FoxG1* transactivation: no perturbation of other active genes and structurally similar, potential offtargets. Absence of aspecific gene transactivation in neural precursors overexpressing *Emx2* (a), upon LV_NMHV / LV_MF2-*Emx2* infection, or *FoxG1* (b), upon LV_NMHV / LV_MF1-*FoxG1* infection. *Pax6*, *Hes6*, *Sip1*, *Couptf1*, *Nf1a* and *Lhx2* are randomly chosen genes active in cortico-cerebral precursors. *Arid1a*, *CachD1*, *Ptpn12* and *Dvl3* are potential offtargets, with 5' ends displaying remarkable homology to the *Emx2* bait (see Supplementary Table S6). Results were normalized against *Gapdh* and further normalized against EGFP/MF2- \emptyset and EGFP/MF1- \emptyset controls, respectively. Time-frame of the experiments as well as representation of moi's and statistical parameters were as in Figure 3.

Figure S10



Supplementary Figure S11. Specificity of NMHV-mediated *Emx2* and *FoxG1* transactivation: limited cis-propagation and polarity effects. (a) No upregulation of *Emx2* mRNA, in cortical precursors co-expressing NMHV and the antisense-oriented, MF1-type, *Emx2*-AS bait. *Emx2* mRNA upregulation by NMHV and the corresponding sense-oriented bait is shown as a reference. **(b)** No upregulation of transcripts stemming from TSSs >1.5kb far from the bait-target, in neural precursors co-expressing NMHV and the MF1-type, *FoxG1* bait. Time-frame of the experiments, representation of moi's, data normalization and statistical parameters were as in Figure 3.

Figure S11



Supplementary Table S1. Wild type baits I

bait name	bait genomic coordinates	bait length	bait orientation vs TSS	bait midpoint distance from TSS	bait CG%	bait cloning oligos - sequence (5'-3')
FMR1.1	hg19_chrX(+)_nt 146,993,634-146,993,748	115b	S	+222b	68%	FMR1b/F - CCCGGATCCCCTCGAGCGCCCGCAGCCCA FMR1b /R - CCCGTCGACCTTGTAGAAAGCGCCATTGGAGCCC
FMR1.2	hg19_chrX(+)_nt 146,993,101-146,993,220	120b	S	-308b	61%	FMR1-UPTSb.S/F - CCCGGATCCCCAGAGAGGCCGAACTGGGATAACC FMR1- UPTSb.S/R - CCCGTCGACCGTGAAGGGCGGTGACAGGTGCGCA
NAP22	hg19_chr5(+)_nt 17,217,026-17,217,142	117b	S	+153b	62%	NAP22b/F - CCCGGATCCCGTCGGGAGCGGGCTGGACTG NAP22b/R - CCCGTCGACGTTGGATAAGAAGTGCTCGGGCTCTC
NRGN	hg19_chr11(+)_nt 124,609,848+124,609,995	148b	S	+92b	74%	NRGRb/F - CCCGGATCCCAGAGCAGAGCTGCTGTTTCGGC NRGRb/R - CCCCTCGAGGGTGCAGCAGTCCATGCTGGTGTC
FMR1.1-AS	hg19_chrX(-)_nt 146,993,634-146,993,748	115b	AS	+222b	68%	FMR1b-AS/F - CCCGGATCCCCTTGTAGAAAGCGCCATTGGAGCCC FMR1b-AS/R - CCCGTCGACCCCTCGAGCGCCCGCAGCCCA
FMR1.2-AS	hg19_chrX(-)_nt 146,993,101-146,993,220	120b	AS	-308b	61%	FMR1-UPTSb.AS/F - CCCGGATCCCGTGAAGGGCGGTGACAGGTGCGCA FMR1- UPTSb.AS/R - CCCGTCGACCCAGAGAGGCCGAACTGGGATAACC
NAP22-AS	hg19_chr5(-)_nt 17,217,026-17,217,142	117b	AS	+153b	62%	NAP22b-AS/F - CCCGGATCCCGTGGATAAGAAGTGCTCGGGCTCTC NAP22b-AS/R - CCCGTCGACCGTCCGGGAGCGGGCTGGACTG
NRGN-AS	hg19_chr11(-)_nt 124,609,848+124,609,995	148b	AS	+92b	74%	NRGRb-AS/F - CCCGGATCCCGTGCAGCAGTCCATGCTGGTGTC NRGRb-AS/R - CCCCTCGAGCGAGAGCAGAGTCTGTTTCGGC
FMR1.1.60L	hg19_chrX(+)_nt 146,993,634-146,993,698	65b	S	+197b	74%	FMR1b/F - CCCGGATCCCCTCGAGCGCCCGCAGCCCA FMR65/R - CCCGTCGACTTCTCTTTCAGCCCTGCTAGCGCC
FMR1.1.60R	hg19_chrX(+)_nt 146,993,688-146,993,748	61b	S	+249b	56%	FMR61/F - CCCGGATCCTGAAGAGAAGATGGAGGAGCTGGTGG FMR1b /R - CCCGTCGACCTTGTAGAAAGCGCCATTGGAGCCC
NAP22.60L	hg19_chr5(+)_nt 17,217,026-17,217,090	65b	S	+126b	65%	NAP22b/F - CCCGGATCCCGTCGGGAGCGGGCTGGACTG NAP65/R - CCCGTCGACGTGATGAATGACACCCCTGGCACC
NAP22.60R	hg19_chr5(+)_nt 17,217,080-17,217,142	63b	S	+181b	54%	NAP63/F - CCCGGATCCTCATTCATCACCTCGAGGCAGTCCCTA NAP22b/R - CCCGTCGACGTTGGATAAGAAGTGCTCGGGCTCTC

Supplementary Table S2. Wild type baits II

bait name	bait genomic coordinates	bait length	bait orientation vs TSS	bait midpoint distance from TSS	bait CG%	bait cloning oligos - sequence (5'-3')
Emx2 (Emx2.179)	mm10_chr19(+)_nt 59,458,457-59,458,635	179b	S	+174b	70%	Emx2.179-S/F - CCCAGATCTGCCGCAGGAGCGAGGGAGCTAGC Emx2.179-S/R - CCCCTCGAGGACTTTTAAAGTGCCTCAGAGGGAGG
Emx2.120	mm10_chr19(+)_nt 59,458,457-59,458,576	120b	S	+144b	74%	Emx2.179-S/F - CCCAGATCTGCCGCAGGAGCGAGGGAGCTAGC E2b.120/R - GGGCTCGAGGGCCAGAATGGAACGGAGTCGG
Emx2.60	mm10_chr19(+)_nt 59,458,457-59,458,516	60b	S	+114b	73%	Emx2.179-S/F - CCCAGATCTGCCGCAGGAGCGAGGGAGCTAGC E2b.60/R - GGGCTCGAGCCGCCGACCTTTGGTGTCTTCGCC
Emx2.30	mm10_chr19(+)_nt 59,458,457-59,458,486	30b	S	+99b	77%	E2b.30/F - GATCTGCCGCAGGAGCGAGGGAGCTAGCCCGGAGCC E2b.30/R - TCGAGGCTCCGGCTAGCTCCCTCGCTCCTGCGGCA
Emx2-AS	mm10_chr19(-)_nt 59,458,457-59,458,635	179b	AS	+174b	70%	Emx2.179-AS/F - CCCAGATCTGACTCTTTAAAGTGCCTCAGAGGGAGG Emx2.179-AS/R - CCCCTCGAGGCCGCAGGAGCGAGGGAGCTAGC
FoxG1	mm10_chr12_nt 49,383,099-49,383,244	146b	S	+165b	59%	FoxG1.146-S/F - CCCAGATCTAGTACTGCCTAGAAAGCTGAAGAGG FoxG1.146-S/R - CCCCTCGAGACCCAACAGTCCCGAAATAAAGC

Supplementary Table S3. qRTPCR oligos

gene	oligos - sequence (5'-3')
<i>3110039M20Rik-001</i>	RykM20.1/F - CCTGCCTCCATACTCCAGCTTCTCC RykM20.1/R - CCCGCACGGGTGAGGCGATCTC
<i>AK158887</i>	AK158887.1108/F - TGGACTGCATTGATTCCCTGGGCAACTAGG AK158887.1250/R - TTGAGCCCCCTGGTAAGTAAGACTCCAG
<i>Arid1a</i>	Arid1a.201/F - GCAGATGGAACACCCAAGACAGAATCCAAATC Arid1a.201/R - TCTCGGGCTCACCACCCAACCTCATACA
<i>CachD1</i>	Cachd1-001/F - GTGAACAGCCGAGCCTTCAACCCAGGA Cachd1-001/R - CCCTTCTTCTGAGCTGAAATATTGCCACTTGAT
<i>Couptf1</i>	Couptf1/F - CTTTCATGGACCACATCCGCATCTTTCAGGAACAG Couptf1/R - TCACATACTCCTCCAGGGCACACTGTGATTTCTC
<i>Dvl3</i>	Dvl3.ex13/F - CTACATCTTTGGCGACCTCTGTGGTAACAT Dvl3.ex14/R - GGAGCCAGTGTGTCTGGTCAGAG
<i>EGR3</i>	EGR3.001/F - CGGCAGCAGCGACTCGGTAGTCCATT EGR3.001/R - GCTGGAAGGAGCCGGAGTAAGAGAGTT
<i>Emx2</i>	Emx2.E2S/N2F - GGAAGAAGCAGCTGGCTCACAGTCTCAGTCTTAC Emx2.E2S/N2R - GTGGTGTGTCCCTTTTCTTCTGTTGAGAATCTGAGCCTTC
<i>ENST00000531241</i>	ENST531241/F - ATTACCTTTGAAAATGCTCAGTTCTTCTTCGGTC ENST531241/R - TCCTATCTCCTGTGTTTGGAGTTCATCCTG
<i>ENST00000456072</i>	ENST456072/F - ATGCCTGCCTTGTTATGGATGTGACAC ENST56072/R - CTGATCGTAGCTCACTGTAGCCTCAAACCTC
<i>FMR1</i>	hFMR1/F - GAAGATTCAATAACAGTTGCATTTGAAAACAACCTGG hFMR1/R - GCTCTGGAATACACCTCAACTTCATCAC
<i>FMR-AS1-v1</i>	NR024503/F - CCATTCCTGAAAAGCACTCAAACCTGGACTTGAA NR024503/R - GGGCTCCAATGGCGCTTTCTACAAGG
<i>FoxG1</i>	FoxG1.2193/F - TGGAGTGTGAGCGAGGTGCAATGTFGG FoxG1.2303/R - TACTGCACACATGGAATCTGGCAGCC
<i>FXR1</i>	FXR1.001/F - GCGGCTCTAACGGGGCTTTCTACAAGG FXR1.001/R - CTTTCATTAATGGAACCTGGCGTTCTGGTTG
<i>Gapdh</i>	Gapdh.5/F - ATCTTCTTGTGTCAGTGCAGCCTCGTC Gapdh.5/R - GAACATGTAGACCATGTAGTTGAGGTCAATGAAGG
<i>Hes6</i>	Hes6/FI - GGAAGCACGGGGGACCGCAAG Hes6/RE - TGCACTGGATGTAGCCAGCAGCGAAG
<i>Lhx2</i>	Lhx2/ForM - GGCAAGCCCTTCTCGGGGACGCGCATC Lhx2/RevM - GCACACAACGCTGCCGGTCACTGCTGATGGACGG
<i>NM_006317 (NAP22-var1)</i>	hNAP22/F - GGGGCTGCATAGGCACCCAGAG hNAP22/R - CCTCGGCCTTCTGTCTTCTCTCTTG
<i>Nf1a</i>	Nf1a/F - GGCCTACTTCTCACACCCAGCCATCCGTTAC Nf1a/R - GTGCACCTTGCCCTTGACTGCTTCCATTGG
<i>NM_001271606 (NAP22-var2)</i>	Nap22/F2 - CTCACCCCAACCTCGCTGCCAG Nap22/R2 - GGCCTTCTGTCTTCTCTCTTGGCTTTC
<i>NRGR</i>	hNRGR/F2 - GGAGACTAGGCCAGAATGAGCATTTTCAA hNRGR/R2 - CAGGGAAGTCTCGTCCGTCGGAAG
<i>Pax6</i>	Pax6/ForM - CCAAGGGCGGTGAGCAGATGTGTGAGATCTTCTATTCTAG Pax6/RevM - CCCGTTGACAAAGACACCACCAAGCTGATTCACCTC
<i>Ptpn12</i>	Ptpn12.001/FN - GCCTTTGGCGAATACAGTCATAGACTTCTGGGA Ptpn12.001/R - CTTTTCTCTCCATCTCAAATCTCGACAGGC
<i>SCL4A2</i>	SCL4A2/F - CTCCTCTCCTTCCGAGTCTCCT SCL4A2/R - CAGAGATGACCATCTGTCCACCAC
<i>Sip1</i>	Sip1/F2 - CGAGAGGCATATGGTGACGCACAAG Sip1/R1 - CACTGTGAATTCAGGTGTTCTTTCAGGT

Supplementary Table S4. ChIP oligos

ChIP amplicon	oligos - sequences (5'-3')
<i>NAP22_amplicon 1</i>	NapIP/F - GAGAAGTCCACGGACAGCCCAGG NapIP/R - GTGCCCCGCCAACGTTTCCTAAATG
<i>NAP22_amplicon 2</i>	Nap225UTR/F - GCTCCAACCTGGCTCCTCGCTCC Nap225UTR/R - GTGCCTATGCAGCCCCTGAGTTAG
<i>NAP22_amplicon 3</i>	Nap22jun/F - GGGGCTGCATAGGCACCCAGAG Nap22jun/R - CTGCTGCCCGCTGCATGAAGGGA
<i>NAP22_amplicon 4</i>	Nap22i1/F - TCCCTTCATGCAGCGGGCAGCAG Nap22i1/R - CCCTCCACCATGCGAGCGGC
<i>NAP22_amplicon 5</i>	Nap22i3/F - GGTGACGGGAGGAATTGCCGAATG Nap22i3/R - CTTTCTCCCAGGCTGGTAAGGGCT
<i>NAP22_amplicon 6</i>	Nap22i2/F - CAGTTCTGCCAGTGGACCTGACCTG Nap22i2/R - GTCCCATAAATGAGAAAAGCCAGGAG
<i>SLC4A2</i>	SLC4IP/F2 - GCTATTGGTCACTGGCCCAGAGG SLC4IP/R2 - CTGAGGCCGCAGGAGGAAGTCCAT
<i>Emx2</i>	Emx2.α/Fint - ATTTACCAGGCTTCGGAGGAAGCTG Emx2.α/Rint - CATGGAAGTGTTCGCTTCTTCACTAGTA
<i>CachD1</i>	CachdIP/F - AAAGTTTGGCGGGCTGCGCCTCC CachdIP/R - CCGGCCTTACGGTCTCCTCTTCCT
<i>Dvl3</i>	Dvl3IP/F - CCGTACCTGGTGAAGCTGCCCT Dvl3IP/R - CCAAAATCGTCCATAGACTGAAGAAG
<i>Ptpn12</i>	PtpnIP/F - GGGACTTCATGGTGAGTGTCCGTTCT PtpnIP/R - CCTGTCCTCGCACCCACACAAA

Supplementary Table S5. Off-target/on-target comparisons

off-target gene (on-target gene)	off-target sequence location: - hg19 coordinates - distance from off-target gene TSS, relative orientation	off-target vs on-target comparison	gap density	homology
<i>SLC4A2</i> (<i>NAP22</i>)	- chr.7(-):150759353-150759431 - circa -0.3kb, antisense	<pre> off 1 GGGTCTGAGACGGGTTGCTGGGTGAGAGACCCA-GGGTTCTCAAAGTTCCTCAACCGCTG 59 on 22 GGGTCTGAGAAGGGTAGCT-GGTG-----CCAGGGGGTGTCA---TTCATC-ACCTC-G 69 off 60 AGGCTAAGCTACCTAGTCT 79 on 70 AGGC--AGCTCCCTAAGTCT 87 </pre>	15/80 (19%)	57/80 (71%)
<i>FXR1</i> (<i>FMR1</i>)	- chr.3(+):180,630,471-180,630,524 - circa +0.3kb, sense	<pre> off 1 AACATGGCGGAGCTGACGGTGGAGGTTTCGCGGCTCTAACGGGGCTTTCTACAAG 54 on 62 AAGATGGAGGAGCTGGTGGTGAAGTGCAGGGGCTCCAATGGCGCTTTCTACAAG 115 </pre>	0/54 (0%)	44/54 (81%)
<i>EGR3</i> (<i>NRGN</i>)	- chr.8(+):22,550,683-22,550,780 - circa +0.05kb, sense	<pre> off 5 GGGTGCCTCAGCTGGTGCGGTATGAGGCTGGGTCGT-GGG-GGGGGTGGGGCGTGTGCGG 62 on 121 GGGT-CCTCTGCGGGGACG--A--AGGCTGG--CGCAGGGCGGGGGTGGGGTCTGT---- 73 off 63 GGGGTG--GGGTGGGGCTGGGCTGGGG 88 on 72 GTGGTGCCGGGTGGGGGCT---CTGGGG 48 off 59 GCGGGGGTGGGGTGGGGCTGG-GCTGGG-GGGGATCT 96 on 89 GC-GGGGGTGGGGTCTGTG-TGGTGCCGGGTGGGGGCTCT 52 off 31 GCTGG-GTCGTGGGGGGGTGGGGCGTGTGCGGGG-GGTGGGGTGGGGGCTGGGCTGGGG 88 on 133 GCTGGTGTGCG---GGGGT----CCTCTGCGGGGACGAAGGCT-GGCGCAGGGCGGGGG 83 off 89 GGGGATCT 96 on 82 TGGGGTCT 75 </pre>	18/88 (20%)	62/88 (70%)
			4/40 (10%)	31/40 (78%)
			11/68 (16%)	46/68 (68%)

Supplementary Table S7. Mutant, NAP22.60L-derivative baits.

<i>bait</i>	<i>sequence (5' → 3')</i>
NAP22.60L	CGTCGGGAGCGGGCTGGACTGGGGTCTGAGAAGGGTAGCTGGTGCCAGGGGGTGTTCATTCATCAC
NAP22.60L.mut30.1	CGTCGGGAGCGGGCTGGACTGGGACTCAGAGGAAACGATCAACGCCAGGGGGTGTTCATTCATCAC
NAP22.60L.mut30.2	CGTCGGGAGCGGGCTAAGTCAAAACCTGAGAAGGGTAGCTAACATTGAAAGGTGTTCATTCATCAC
NAP22.60L.mut30.3	CGTCGGGAGCGAATCAAGCTGGGGTCTGAGAGGAAACAGCTGGTGCCAATAACAATTCATTCATCAC
NAP22.60L.mut30.4	CGTCGGGAGTAAATTTGGACTGGGACTCAAGAAGGGTAATCAATGCCAGGGGACACTATTCATCAC
NAP22.60L.mut15.1	CGTCGGGAGCGGGCTGGACTGGGGTCTCTCTCCCATGCTGGTGCCAGGGGGTGTTCATTCATCAC
NAP22.60L.mut15.2	CGTCGGGAGCGGGCTGGAGACCCGTCTGAGAAGGGTAGCTGGACGGTGGGGGTGTTCATTCATCAC
NAP22.60L.mut15.3	CGTCGGGAGCGGGGACGACTGGGGTCTGAGTTCCGTAGCTGGTGCCAGCCGTGTTCATTCATCAC
NAP22.60L.mut15.4	CGTCGGGAGCGCCGTGGACTGGGGTGAAGAAGGGTAGGAGGTGCCAGGGGCACATTCATTCATCAC
NAP22.60L.mut15.5	CGTCGGGAGCCGCTGGACTGCCGTCTGAGAACCGTAGCTGGTCCGAGGGGGTGTAGATTCATTCATCAC
NAP22.60L.mut15.6	CGTCGCGAGCGCGCTGGTCTGGGCTCTGACAAGGGAAGCTGCTGCCACGGGGTCTCATTGATCAC