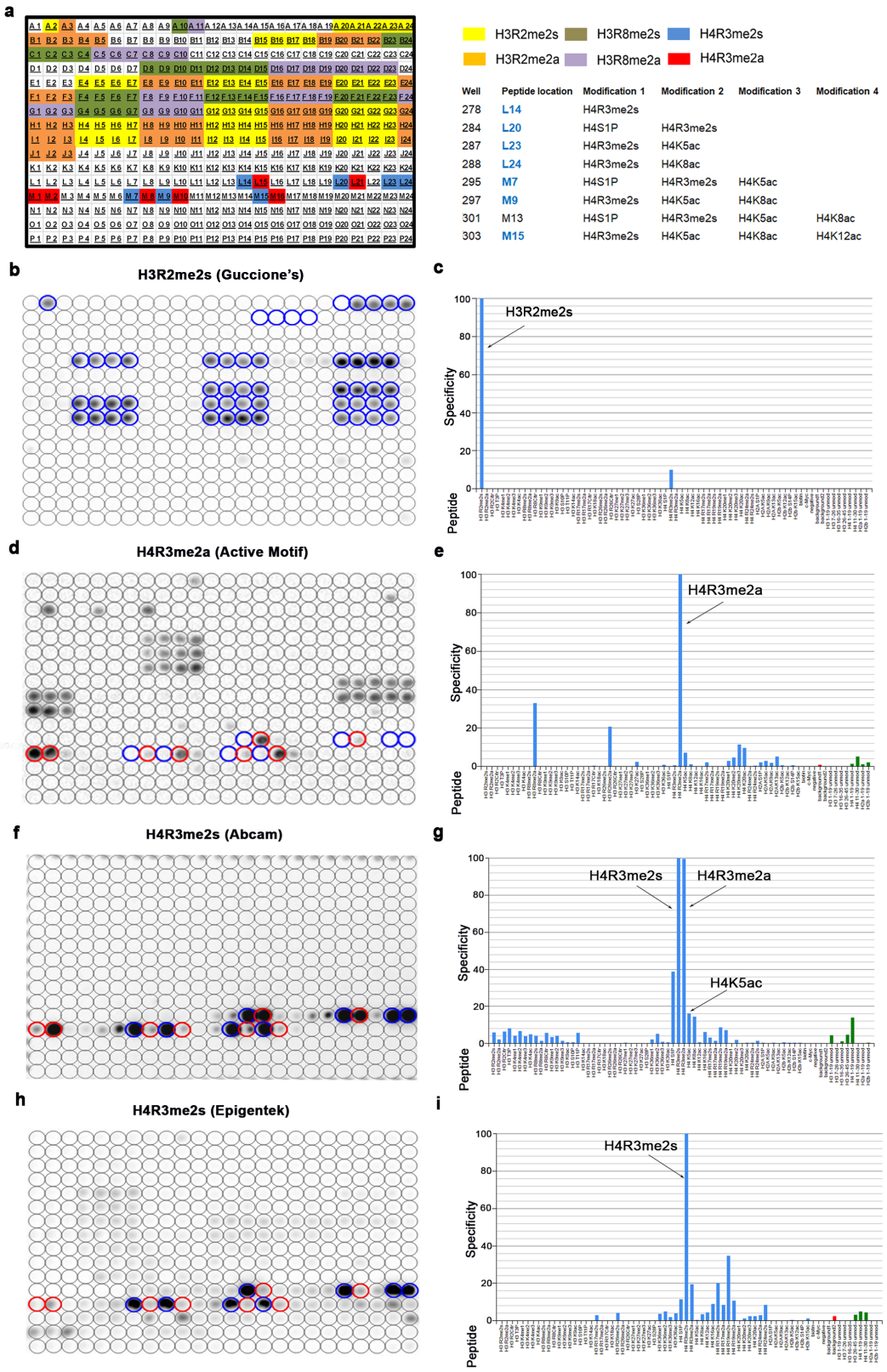
PRMT5-mediated regulation of developmental myelination

Scaglione *et al.*

Supplementary Figures 1-9

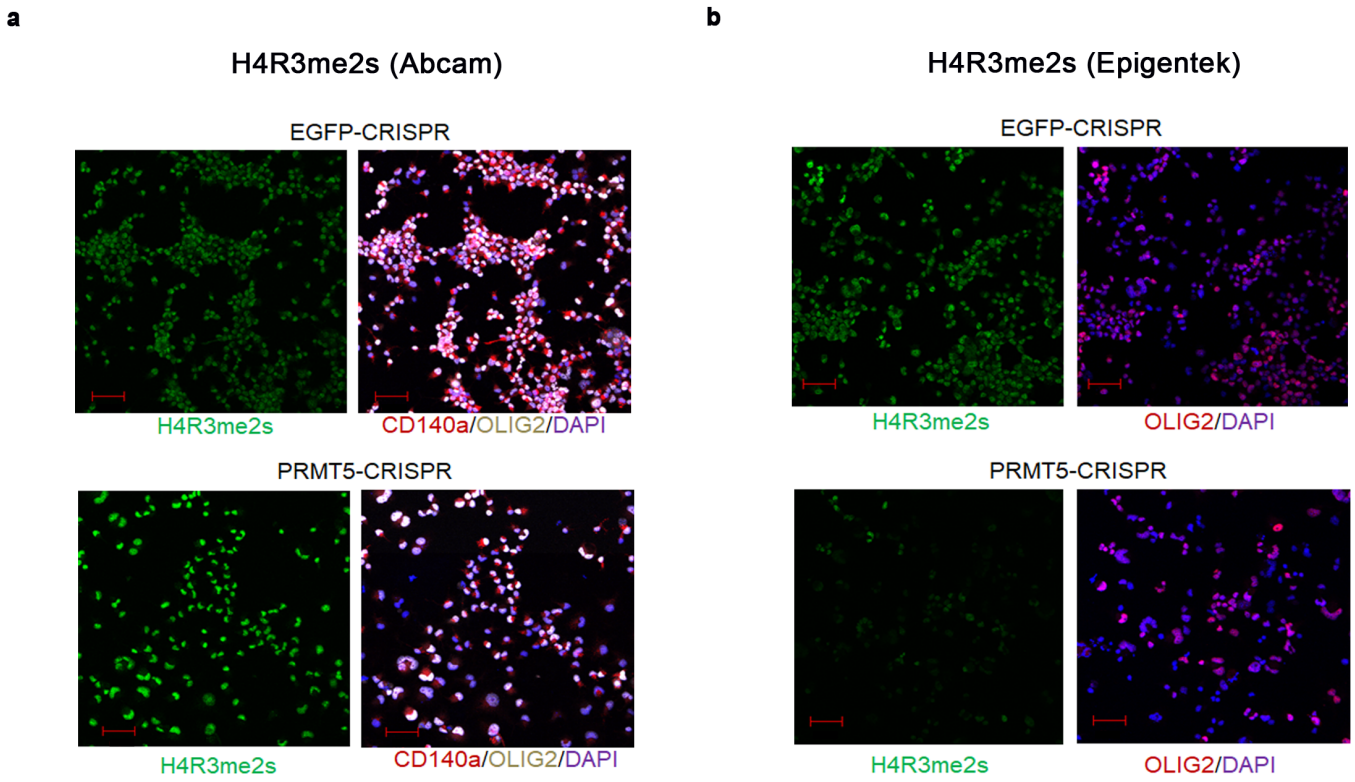
Supplementary Tables 1-4

Supplementary References

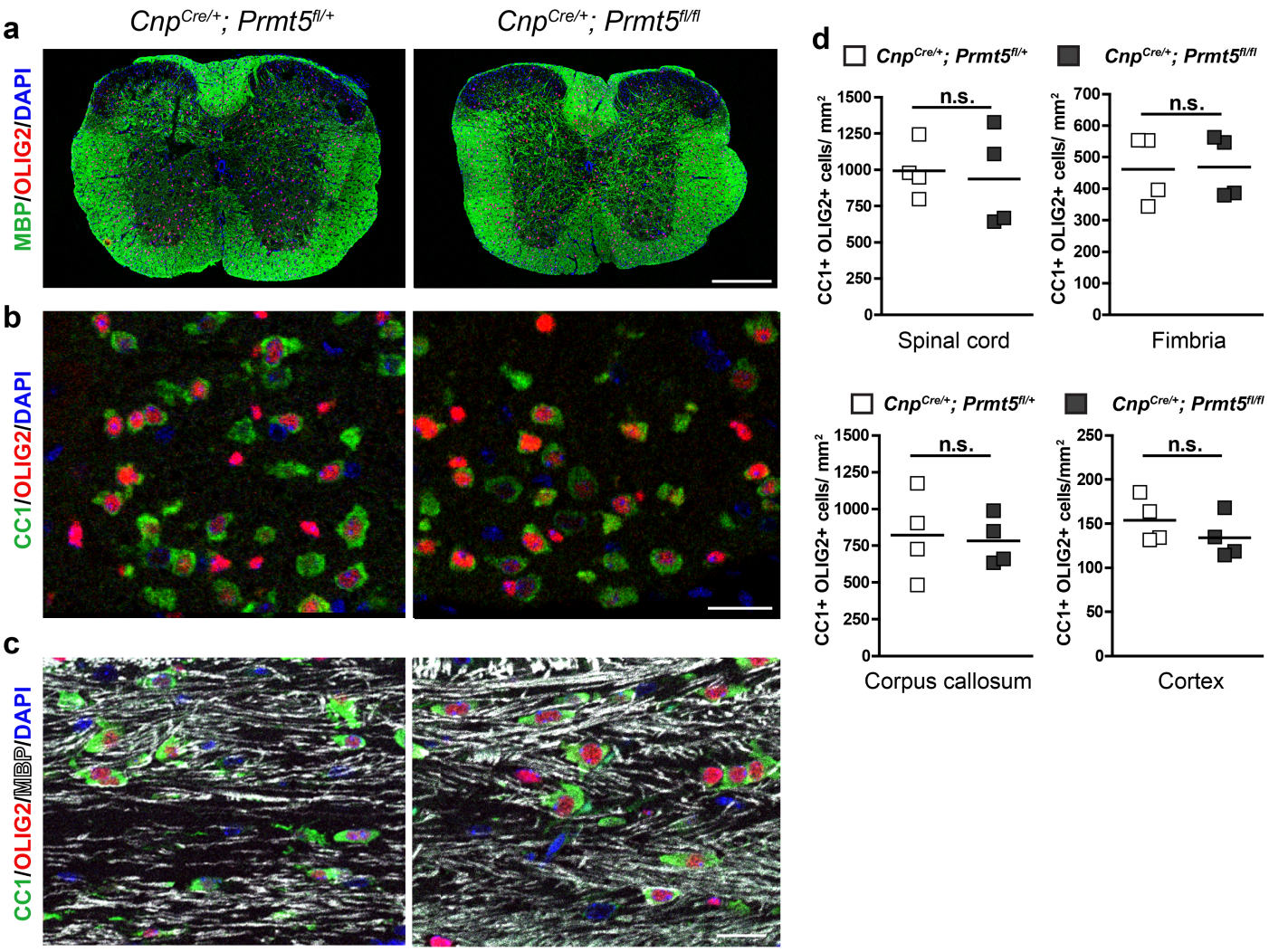


Supplementary Figure 1. Validation of antibody specificity using a modified histone peptide array. (a) Outline of the antibody array from Active Motif. Each well contains a unique combination of histone H3 or H4 peptides with distinctive post translational modifications, as detailed in the Methods section. Histone peptides containing H4 methylated arginine residues or a combination of different post-translational modifications, are highlighted in the table with different colors (yellow for symmetric methylation of R2 in histone H3, red for asymmetric methylation of R3 in histone H4, blue for symmetric methylation of the same residue). The

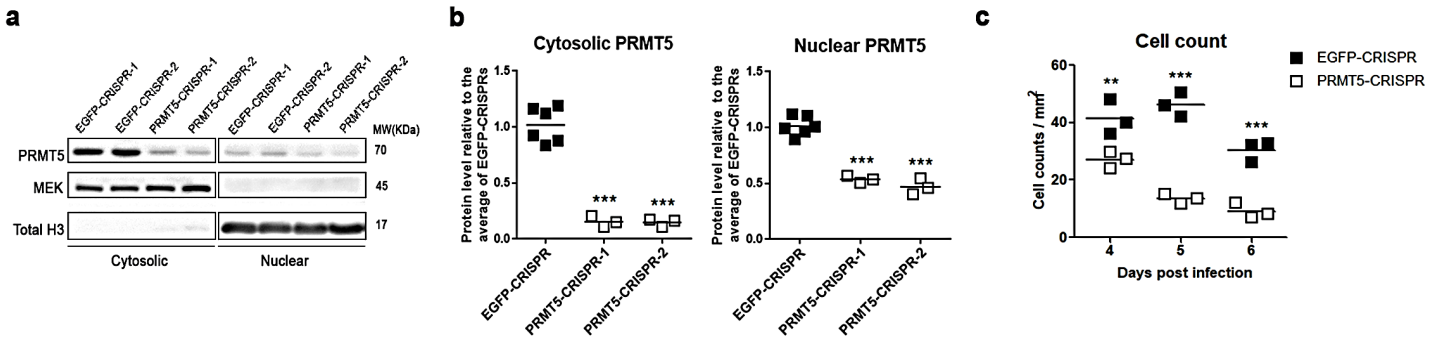
corresponding position in the array with the relative coordinates and specific examples are indicated. For instance H4R3me2s modification is recognized by the corresponding antibody as the only modification in the histone peptide in the well identified by the coordinates L14. The same antibody, can identify the same post-translational modification also if additional modifications are present, such as acetylation of residue H4K5 in well L23 or acetylation of H4K8, as shown in well L24, or in the presence of multiple acetylated lysine residues, as shown for wells M7, M9, M13 and M15. Multiple antibodies specific for symmetrically or asymmetrically methylated arginine residues in histone H3 or histone H4 were tested using the modified histone peptide array. The results for the antibody specific for H3R2me2s provided by Ernesto Guccione are shown in (**b**). The light purple circles, identify positions in the array containing peptides harboring H3R2me2s.The results for the antibodies specific for H4R3me2a, purchased from Active Motif are shown in (**d**). Those for the antibodies specific for H4R3me2s purchased from Abcam are shown in (**f**) and those for the antibodies purchased from Epigentek are shown in (**h**). In (**d**, **f**, **h**) the red circles identify H4R3me2a and the blue circles identify the H4R3me2s immunoreactive dots. In (**c,e,g,i**) the bar graphs represent the output of the “Multiple Peptide Average” option, calculated using the “Array Analyze Software” provided by Active Motif. The specificity of each antibody is calculated by computing the average intensity of all the spots of the dot blot in positions containing a given mark, divided by the average intensity of all the spots of the array not containing the mark.



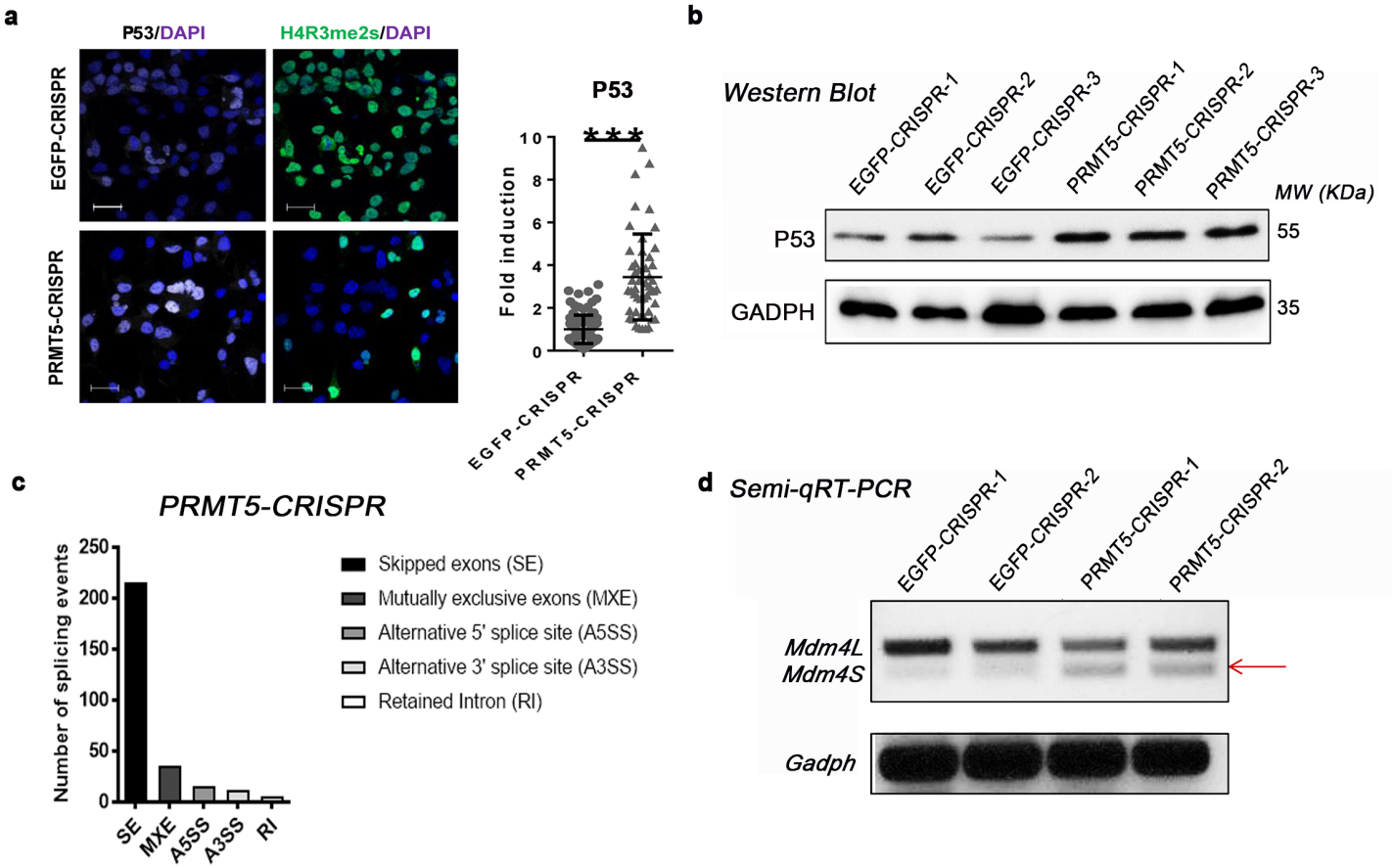
Supplementary Figure 2. Validation of H4R3me2s antibody specificity for immunocytochemistry using cells with CRISPR/Cas9 targeting of PRMT5. Representative confocal images of oligodendrocyte progenitor cells with (PRMT5-CRISPR) or without (EGFP-CRISPR) targeting of PRMT5 using lentiviral vectors. Effective knockdown of PRMT5 in OPC was evaluated by immunocytochemistry using antibodies specific for OLIG2 (gray in **a** and red in **b**), for PDGFRα (CD140a antibody in red in **a**) and for H4R3me2s (green) from Abcam (**a**) or Epigentek (**b**). DAPI (blue) as nuclear counterstain. Note that the Abcam antibody identifies immunoreactive cells even after knockdown of PRMT5, which is enzy placing the H4R3me2s mark, while the Epigentek antibody shows greater selectivity. Scale, 50μm.



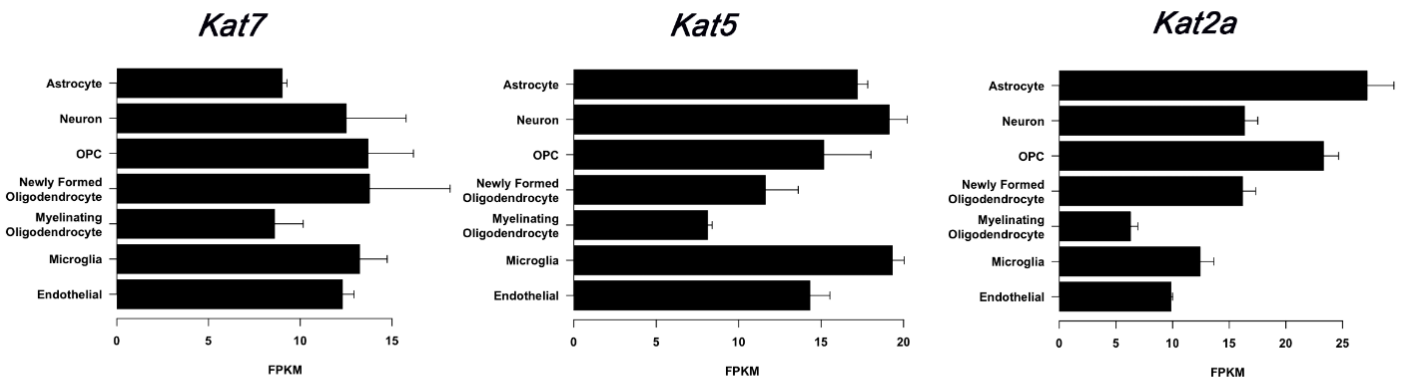
Supplementary Figure 3. Genetic ablation of *Prmt5* in oligodendrocytes using *Cnp-Cre*. Confocal images of P14 spinal cord (**a**,**b**) sections from controls *(Olig1$^{Cnp/+}$;Prmt5$^{fl/+}$)* and *Prmt5* mutants (*Olig1$^{Cnp/+}$;Prmt5$^{fl/fl}$)* stained for (**a**) MBP (green), (**b**) CC1 (green), (**a**, **b**) OLIG2 (red) and DAPI (blue). Scale bar in **a**: 200µm , in **b**: 20µm. (**c**) Confocal images of P14 corpus callosum sections from controls *(Olig1$^{Cnp/+}$;Prmt5$^{fl/+}$)* and *Prmt5* mutants (*Olig1$^{Cnp/+}$;Prmt5$^{fl/fl}$)* stained for MBP (white), CC1 (green), OLIG2 (red) and DAPI as nuclear counterstain (blue). Scale bar: 20µm. (**d**) Scatter plot represents the average number of CC1$^+$ /OLIG2$^+$ cells quantified in in spinal cord, fimbria, corpus callosum, and cortex from four controls *(Olig1$^{Cnp/+}$;Prmt5$^{fl/+}$)* and four *Prmt5* mutants (*Olig1$^{Cnp/+}$;Prmt5$^{fl/fl}$).* Student's *t*-test, n.s.= not significant.



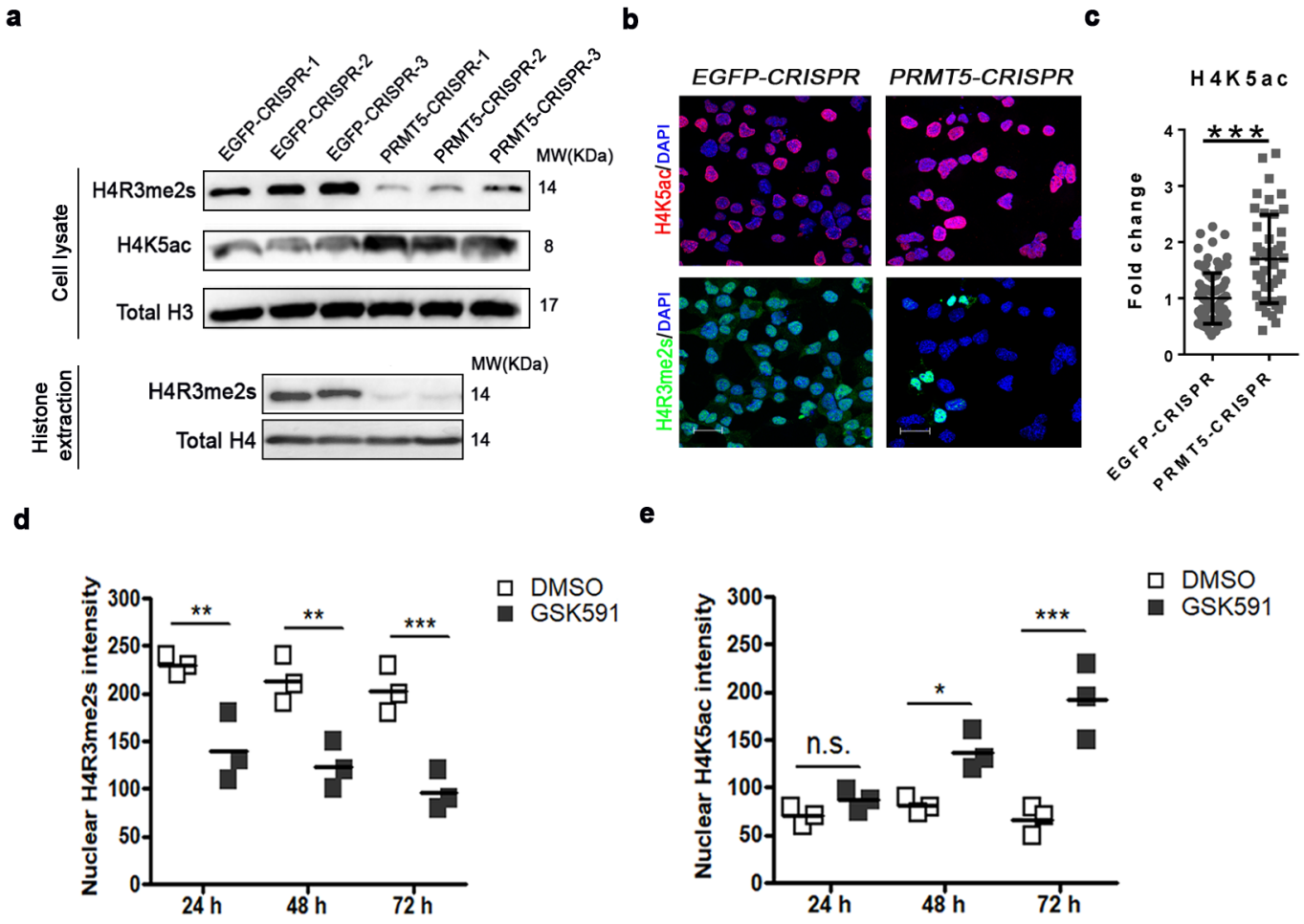
Supplementary Figure 4. Validation of PRMT5 knockdown in oligodendrocytes lineage cells, using CRISPR/Cas9. (**a**) Western blot analysis of cytosolic or nuclear protein extracts from OPC either infected with the control (EGFP-CRISPR) or knockdown (PRMT5-CRISPR) lentiviruses and probed with antibodies for PRMT5, MEK was used as control for cytoplasmic proteins and total H3 as control for nuclear proteins. (**b**) Relative quantification of the PRMT5 protein level in control (EGFP-CRISPR) or knockdown (PRMT5-CRISPR) cytosolic and nuclear extracts is shown. Data were normalized to the appropriate loading controls and are presented as means from three different preparations (one-way ANOVA with Bonferroni's Multiple Comparison Test, *** $p<0.001$). (**c**) Absolute numbers of OPC cells infected with either control (EGFP-CRISPR) or knockdown (PRMT5-CRISPR) lentiviruses, at day 4, 5 and 6 after infection. Scatter plots indicate relative values normalized to the average of the control group (DMSO), (one-way ANOVA, ** $p<0.01$, *** $p<0.001$), n=3 independent preparations.



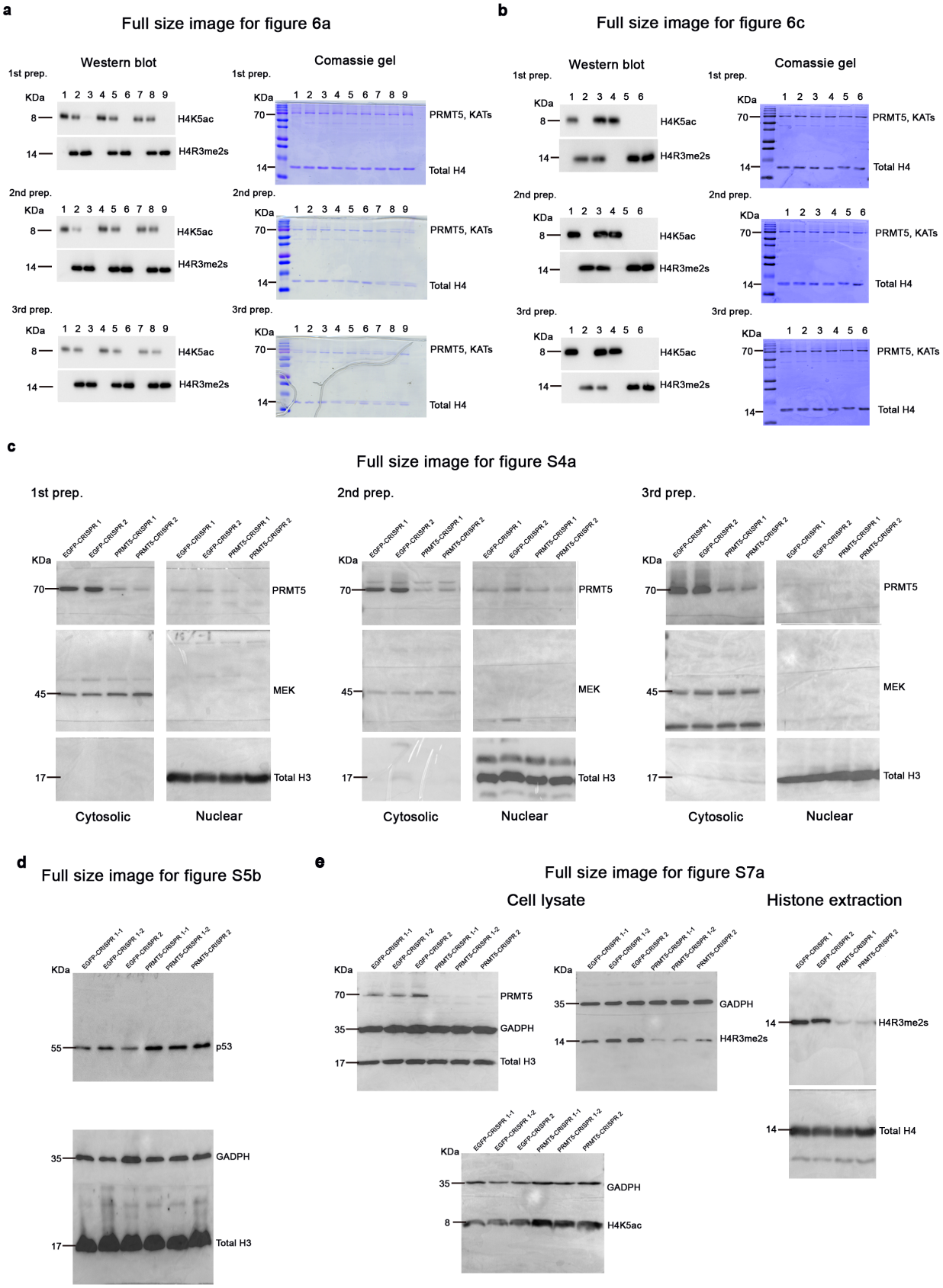
Supplementary Figure 5. CRISPR/Cas9 targeting of PRMT5 in oligodendrocyte progenitors leads to alteration of *Mdm4* splicing and up-regulation of P53 protein levels. (**a**) Representative confocal images of oligodendrocyte progenitor cells with (PRMT5-CRISPR) or without (EGFP-CRISPR) targeting of PRMT5 using lentiviral vectors, processed for immunocytochemistry using antibodies specific for P53 (gray) and for H4R3me2s (green). DAPI (blue) was used as nuclei counter-stain. Scale: 20 μm. Scatter plot identify the intensity of p53 immunoreactivity in H4R3me2s^{+} (EGF-CRISPR) and in H4R3me2s^{-} (PRMT5-CRISPR) cells. Data are shown as normalized values, relative to the average of the EGFP group ± s.e.m. from three independent preparations. *** =p<0.001 Student's *t*-test. (**b**) Whole cell protein lysates extracted from progenitors transduced with control (EGFP-CRISPR) or targeted (PRMT5-CRISPR) lentiviral vectors were processed for Western blot analysis and probed with antibodies specific for P53 and for GAPDH, as loading control. (**c**) Relative distribution of RNA splicing events detected in Olineu cells after PRMT5 knockdown (PRMT5-CRISPR). (**d**) Semi-qRT-PCR detecting the transcripts of the *Mdm4* long and short isoforms in EGFP-CRISPR or PRMT5-CRISPR infected oligodendrocyte progenitors.



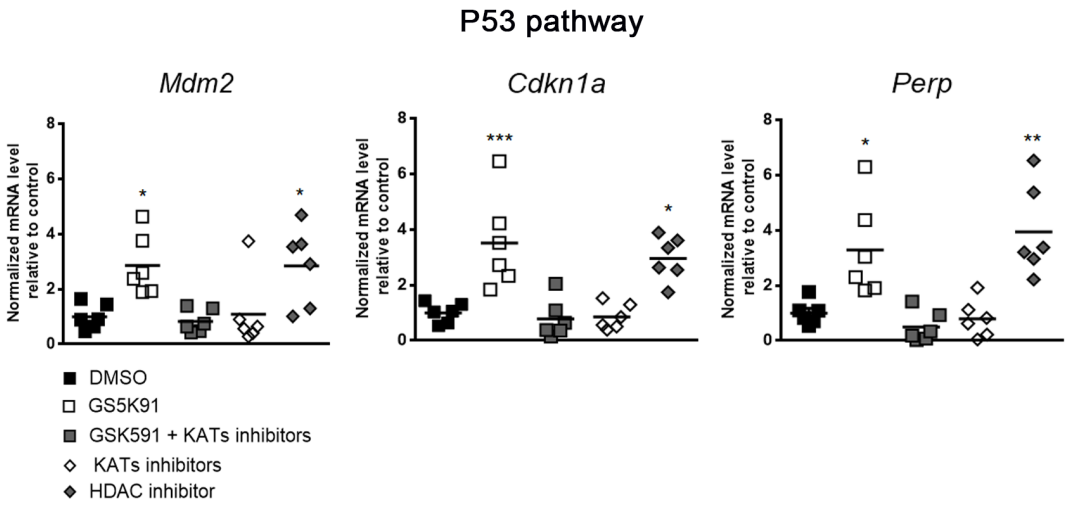
Supplementary Figure 6. Transcript levels of acetyltransferase from available databases. The transcript values of *Kat7*, *Kat5* and *Kat2a* in different cell classes of the brain as reported in RNA-seq transcriptome database[1,2]. Note the enrichment of the three transcripts in the OPC.



Supplementary Figure 7. Increased acetylation of lysine residue K5 in histone H4 after PRMT5 knockdown. (**a**) Western blot analysis of cell lysates and histone extraction from control (EGFP-CRISPR) and knockdown (PRMT5-CRISPR) cultures probed with antibodies specific for H4R3me2s and for H4K5ac. Antibdes for total H3 or total H4 were used as protein loading control for histones. (**b**) Representative confocal images of control (EGFP-CRISPR) and knockdown (PRMT5-CRISPR) OliNeu cells stained with antibodies specific for H4K5ac (red), H4R3me2s (green) and DAPI (blue). (**c**) Scatter plot of the normalized immunoreactivity intensity for H4K5ac staining counted in at least 50 cells per condition and from three independent biological replicates. Scatter plots indicate relative values normalized to the average of the EGFP group ± s.e.m. Student's t-test, *** $p<0.001$, scale 20 µm. (**d-e**) Time course of the effect of the pharmacological inhibitor of PRMT5 (GSK591, 10 nM) treatment on methyl-arginine (H4R3me2s) and acetyl-lysine (H4K5ac) deposition in primary oligodendrocyte progenitors, cultured in presence of growth factors (+GF), for 24 and 72 hours. Scatter plot of the average immunoreactivity intensity of nuclear H4R3me2s and H4K5ac staining. Each dot represents the average of 50 PDGFRα$^+$ cells from three independent preparations. The values werenormalized to the average of the control group (DMSO). One-way ANOVA n.s. $p>0.05$ * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



Supplementary Figure 8. Original western blots of data presented in this manuscript. Uncropped images of immunoblots and coomassie gels displayed in the main and supplementary figures. Antibodies indicated on the right side and size markers are indicated on the left side of each blot.



Supplementary Figure 9. Upregulation of P53-dependent genes induced by PRMT5 inhibition is rescued by co-treatment with KAT inhibitors. qRT-PCR of selected P53-target genes (*Mdm2, Cdkn1a, Perp*) normalized to the geo-mean of three housekeeping genes (*18s*, *Wdr33* and *Pja2*). Differentiating oligodendrocyte progenitors were treated for 48 hours with DMSO, KAT inhibitors (Butyrolactone-3, 100 μM and Nu-9056, 0.2 μM) alone or in combination with the PRMT5 inhibitor GSK591 (10nM) or with the HDAC inhibitor Trichostatin A (TSA, 20 nM). Note that TSA is toxic and induces upregulation while the KAT inhibitors rescue the upregulation induced by GSK591. Scatter plots indicate transcript values for the indicated genes from six independent preparations. One-way ANOVA with Bonferroni Multiple Comparison Test was performed for each gene compared to the value of the control (DMSO) (* $p<0.05$** $p<0.01$ *** $p<0.001$).

Supplementary Tables

Supplementary Table 1. List of primary antibodies used in the study.

Technique	Antibody name	Antibody source	Commercial house and reference	Dilution
Immunopanning	CD140a	Rat	BD Bioscience 558774	1:1000
Immunoprecipitation	PRMT5	Rb	Abcam, ab31751	
Histone peptide array (Active Motif, 13005)	H3R2me2s	Rb	Ernesto Guccione's	
	H4R3me2a	Rb	Active Motif, 39705	
	H4R3me2s	Rb	Abcam, ab5823	
	H4R3me2s	Rb	Epigentek, A-3718	
Western Blot	GADPH	Ms	Abcam, ab8245	1:5000
	H3	Rb	Abcam ab1791	1:20000
	H3K9ac	Ms	Active Motif, 61251	1:1000
	H4	Rb	Active motif, 39269	1:1000
	H4R3me2s	Rb	Epigentek A-3718	1:1000
	H4K5ac	Ms	Active Motif, 61523	1:1000
	H4K5ac	Rb	Abcam, ab51997	1:1000
	H4K8ac	Rb	Millipore, 07-328	1:1000
	H4K12ac	Rb	Millipore, 07-595	1:1000
	MEK	Rb	Sigma, M 5795	1:5000
	PRMT5	Rb	Abcam, ab109451	1:1000
Immunohistochemistry	CC1	Ms	Calbiochem, OP80	1:100
	H4R3me2s	Rb	Diagenode, C15410308	1:200
	H4K5Ac	Ms	Active Motif, 61523	1:200
	Ki67	Rb	Abcam, ab15580	1:500
	MBP	Rat	Millipore MAB386	1:500
	Olig2	Rb	Chemicon, AB9610	1:200
	Olig2	Ms	Millipore, MABN50	1:200
	PDGFRα	Rb	Cell Signalling, 3164S	1:100
	PRMT5	Rb	Abcam, AB109451	1:100

Immunocytochemistry	Cleaved CASPASE-3	Rb	Cell Signalling, 9661	1:200
	CNP	Ms	Covance, E11BF00277	1:200
	H4R3me2s	Rb	Diagenode, C15410308	1:500
	H4K5Ac	Ms	Active Motif, 61523	1:500
	Ki67	Ms	Abcam, ab15580	1:500
	MBP	Rat	Millipore MAB386	1:1000
	Olig2	Rb	Chemicon, AB9610	1:500
	Olig2	Ms	Millipore, MABN50	1:500
	P53	Rb	Cell Signalling, 2524S	1:500
	PRMT5	Rb	Abcam, AB109451	1:200
	PDGFRα	Rat	Millipore, CBL1366	1:1000

Supplementary Table 2. Number of total reads and aligned read pairs for the RNA-seq analysis performed for CRISPR-PRMT5 and CRISPR-EGFP control cells. The table shows the values obtained from four different analysis performed for each condition (CRISPR-PRMT5 and CRISPR-EGFP control)

Sample	Total read pairs	Aligned read pairs	Average of total read pairs	Range of total read pairs	Read type
EGFP-CRISPR-1 (exp.1)	64722813	52660045	61602313.25	56743181 - 66929862	Paired end
EGFP-CRISPR-2 (exp. 1)	66929862	54600276			Paired end
EGFP-CRISPR-1 (exp.3)	56743181	45668535			Paired end
EGFP-CRISPR-2 (exp.4)	58013397	47106242			Paired end
PRMT5-CRISPR-3 (exp. 1)	68576976	55956459	63379315.75	59009841 - 68576976	Paired end
PRMT5-CRISPR-4 (exp. 2)	64201644	52395588			Paired end
PRMT5-CRISPR-3 (exp. 3)	59009841	47970026			Paired end
PRMT5-CRISPR-4 (exp. 4)	61728802	49934101			Paired end

Supplementary Table 3. List of qRT-PCR primers used in the study

Gene	Forward primer	Reverse primer
Prmt5	CTGAGTGTCTGGATGGAGCA	GCATCTCAAACTGTGCCTCA
Cdkn1a	CAGGGCAGAGGAAGTACTGG	CGGTGGAACTTTGACTTCGT
Mdm2	TAAAGTCCGTTGGAGCGCAAA	CTGCTGCTTCTCGTCATATAACC
Perp	ACCCCAGATGCTTGTTTTCCT	CTGCTGCTTCTCGTCATATAACC
Gpr17	ACACATTGTCTGCCTGCAA	TGACCGTGGTGATGAATGGG
Myt1	TGCAGACCTCAGTTGTCCTAC	TCCTCTTGGATACCAGGTGCT
Sox10	GGAGATCAGCCACGAGGTAATG	GTTGGGTGGCAGGTATTGGT
Cnp	ACCCGCAAAAGCCACACA	CACCGTGTCCTCATCTTGAAG
Mbp	ACACACGAGAACTACCCATTATGG	AGAAATGGACTACTGGGTTTTCATCT
m18s	AGTCCCTGCCCTTTGTACACA	GATCCGAGGGCCTCACTAAAC
Pja2	GCC TTG CCA TCA CTT CTT TC	GCA GAT GCG TCA ATA ACT GC
Wdr33	TGA TCT GGT CCC ACC AAT AG	GCA GAT GCG TCA ATA ACT GC

Supplementary Table 4. List of the genes identified in PRMT5 knockdown cells compared to controls for the following alternative splicing event: Skipped Exon (SE), Mutually Exclusive Exon (MXE), Alternative 5' splice event (A5SS), Alternative 3' splice event (A3SS), Retained Intron (RI)

SE	MXE	A5SS	A3SS	RI
Myo5a	*Rsbn1l*	*Zfp691*	*Zfp2*	*Hsf1*
Supl15h	*Sobp*	*Sh3bp5l*	*Epn1*	*Adamts10*
Prpf39	*Vit*	*Adamts10*	*Map4k4*	*Pla2g4b*
Crem	*Zfp346*	*Steap2*	*Tbc1d24*	*Amigo2*
Uspl1	*Ints10*	*Taf1c*	*Fkbp1a*	*Keap1*
Aste1	*Fuk*	*Lzts3*	*Zfp788*	*Tert*
Csnk1g3	*Tmbim6*	*Ciz1*	*Zbtb37*	
Sbno2	*Dap3*	*Ccser1*	*Zfp946*	
Pdp1	*Klhl18*	*Rfx5*	*Baz2b*	
Sobp	*Ptk2*	*Il15ra*	*Tank*	
Map3k4	*Zfp788*	*Adamts10*	*Pms2*	
Atp5s	*Enox2*	*Sema6c*	*Zfp82*	
Pkd1	*Zfp788*	*Apoo*		
Atr	*A730011L01Rik*	*Rsad1*		
edpk	*9530077C05Rik*	*Cant1*		
AK016788	*Tcf7l2*			
B3gnt5	*Pdgfc*			
Eps15l1	*Epb41l4a*			
Enox2	*Smarce1*			
Armcx1	*Sorbs1*			
Trim68	*Ap4e1*			
Nsun3	*Prss36*			
Zfp788	*Senp8*			
Ankrd29	*Lrrc7*			
Fbrsl1	*Kif21a*			

mKIAA1584	*Cyp2r1*			
Kirrel3	*Scyl3*			
1110054O05Rik	*Lrrc7*			
BC006965	*Dnahc9*			
2310005N01Rik	*COX4AL*			
Tmbim6	*Senp8*			
Mllt4	*Dnahc9*			
Sdccag3	*Cnot2*			
Krba1	*Enox2*			
mKIAA1335	*Kif21a*			
Phf16				
Svil				
Fam151b				
Enox2				
Zfp202				
Dos				
Zfp821				
Cep152				
Krba1				
Ocel1				
Acvr1				
Tmtc1				
Zfyve21				
Senp8				
Gal3st4				
Mylk				
Senp8				
Vegfa				

Wdr52				
Acap2				
Trerf1				
Zfp788				
Ccdc77				
Abca8b				
Pla2g4b				
Zfp324				
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St3gal3				
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Slc7a3				
Pbx4				
Rgs19				
4933411K20Rik				
Tmem194b				
Cyp2r1				
Osgepl1				
Tns1				
Elmod3				
Itgav				
4932438A13Rik				
Ppp4r1l-ps				
Nfatc2ip				
Zfp324				
Alkbh1				

Baz2b				
Slmap				
Wdr62				
6720401G13Rik				
Atg16l1				
Atp4a				
Lrrc45				
Brd4				
1810013D10Rik				
Clta				
Sgip1				
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Wnk1				
Accs				
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Polm				
BC046404				
Agbl2				
Zfp655				
2010015L04Rik				
Tcf7l2				
6230409E13Rik				
Anubl1				
Cyp2r1				
Ddb2				
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Fggy				
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Csmd1				
Ttc13				
Dnajc17				
Zfp952				
Wipf3				
Tcf20				
Cyp2r1				
Gprasp2				
2210009G21Rik				
Gbp3				
Ccdc57				

Ptk2				
Cep63				
Prss36				
Gprasp2				
Sh3glb2				
BC004004				
Rgs7				
Rcbtb1				
Ranbp17				
Fbxo25				
Grhl1				
Tec				
Mbd5				
Tmem194b				
Gpr137				
Poli				
Pion				
Mark3				
Scn2a1				
Cyp4f17				
Wdr78				
AI118078				
Abcb4				
Slc39a14				
Lrp8				
Zbtb43				
Slc17a8				
Slc29a3				

Tgfbr2				
Cd99l2				
Ppm1b				
Raph1				
Prpf40b				
2210404J11Rik				
Zfp551, Znf551				
Fggy				
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Gfra1				
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Shc1				
Crem				
Madd				
Lipo1				
Rapgef6				
Rims1				
Dsc2				
Enox2				
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Lrp1b				
Thap6				
Wdfy2				
4930432K21Rik				
Thsd1				

E130303B06Rik				
Fam35a				
Kif24				
Blzf1				
Zfp317				
Rapgefl1				
Tpd52l1				
D2hgdh				
Nr3c2				
Sytl4				
Scly				
Trpm2				
Pgbd1				
Dlg3				
1700012B15Rik				
Zfp420				
Xrcc3				
Tpcn2				
2210009G21Rik				
Thap6				
Slc35a1				
Mfsd9				

Supplementary References

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