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***PM20D1* is a quantitative trait locus associated with Alzheimer's disease**

Jose V. Sanchez-Mut¹, Holger Heyn^{2,3}, Bianca A. Silva¹, Lucie Dixsaut¹, Paula Garcia-Esparcia⁴, Enrique Vidal⁵, Sergi Sayols⁶, Liliane Glauser¹, Ana Monteagudo-Sánchez⁷, Jordi Perez-Tur⁸, Isidre Ferrer⁴, David Monk⁷, Bernard Schneider⁹, Manel Esteller¹⁰ and Johannes Gräff^{1*}

¹Laboratory of Neuroepigenetics, Brain Mind Institute, Faculty of Life Sciences, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.

²Centro Nacional de Análisis Genómico (CNAG), Center for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain. ³Universitat Pompeu Fabra (UPF), Barcelona, Spain. ⁴Department of Pathology and Experimental Therapeutics, University of Barcelona, Institut d'Investigació Biomedica de Bellvitge (IDIBELL), Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Barcelona, Spain. ⁵Center for Genomic Regulation, Barcelona Institute of Science and Technology, Barcelona, Spain. ⁶Bioinformatics Core Facility, Institute of Molecular Biology, Mainz, Germany. ⁷Genomic Imprinting and Cancer Group, Cancer Epigenetics and Biology Program, Institut d'Investigació Biomedica de Bellvitge, Barcelona, Spain. ⁸Institut de Biomedicina de València-CSIC, Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas, Instituto de Investigación Sanitaria 'La Fe', València, Spain. ⁹Neurodegenerative Studies Laboratory, Brain Mind Institute, Faculty of Life Sciences, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland. ¹⁰Cancer Epigenetics Group, Cancer Epigenetics and Biology Program, Institut d'Investigació Biomedica de Bellvitge, Barcelona, Spain. *e-mail: johannes.graef@epfl.ch

Supplementary Table 1. Analysis of whole genome bisulfite sequencing (WGBS) and Illumina 450K methylation arrays (statistical description): GSE57361 WGBS data (GSE47966 GSM1173772 – Control female 64 years old frontal cortex grey matter sample – and GSE57361 GSM1380998 – AD female 81 years old frontal cortex grey matter sample) was analyzed using Bismark software and processed with SAMtools, bedtools, Tabix, and ggplot2 packages as described in material and methods. AVG.Beta values from five additional grey matter control and AD biologically independent samples (GSE57361 – three females and two males aged 68.0±11.9 and three females and two males aged 83.2±3.2 respectively) using Illumina 450K methylation arrays were used for WGBS DMR validation. WGBS promoter DMR showing similar DNA methylation tendencies in Illumina 450K methylation arrays (FDR<0.05) were selected.

TARGETID	CHR	MAP INFO	SYMBOL	FC_C	FC_AD	Delta	FDR p-val
cg06778853	1	6672620	PHF13	0.29949512	0.373405956	0.073910836	0.014140061
cg02900253	1	6673709	PHF13	0.326912196	0.281490387	-0.045421808	0.014140061
cg21291672	1	29451713	TMEM200B	0.549864508	0.611546702	0.061682193	0.004511719
cg09251959	1	32170507	COL16A1	0.382070045	0.477568242	0.095498196	0.008146802
cg02297616	1	40255140	BMP8B	0.340205825	0.27590237	-0.064303456	0.004511719
cg03401114	1	40255440	BMP8B	0.609966722	0.707605992	0.09763927	0.004511719
cg26051619	1	40255555	BMP8B	0.681128228	0.768230296	0.087102068	0.037900087
cg26354017	1	205819088	PM20D1	0.337495448	0.463252047	0.125756599	0.037900087
cg11965913	1	205819406	PM20D1	0.176355415	0.405934702	0.229579288	0.037900087
cg24503407	1	205819492	PM20D1	0.529907126	0.668318423	0.138411297	0.023600884
cg07157834	1	205819609	PM20D1	0.662356628	0.77118093	0.108824303	0.037900087
cg03593823	1	240690404	GREM2	0.724920395	0.8251648	0.100244406	0.008146802
cg16595607	2	63279495	OTX1	0.519666792	0.573252636	0.053585844	0.004511719
cg10271192	2	63279645	OTX1	0.587463345	0.662696704	0.075233359	0.023600884
cg15026634	2	63280097	OTX1	0.540957292	0.569135868	0.028178575	0.037900087
cg00821731	2	219744626	WNT10A	0.48544129	0.574319922	0.088878631	0.004511719
cg12014818	2	219744634	WNT10A	0.473632805	0.545202692	0.071569887	0.023600884
cg25607121	2	219745748	WNT10A	0.293554804	0.223486752	-0.070068052	0.023600884
cg19047707	2	233351525	ECEL1	0.192859694	0.135623028	-0.057236667	0.008146802
cg02932167	2	233352951	ECEL1	0.622872156	0.700960243	0.078088087	0.004511719
cg04083553	3	128778575	GP9	0.812358534	0.854896703	0.042538169	0.037900087
cg06804344	3	128778699	GP9	0.80730098	0.852279469	0.044978489	0.037900087
cg16098726	3	128778738	GP9	0.783917215	0.853837848	0.069920633	0.037900087
cg03389538	3	128779498	GP9	0.662878861	0.786869211	0.12399035	0.008146802
cg19977179	3	128779500	GP9	0.589888308	0.689089367	0.099201059	0.008146802
cg10882599	3	128779586	GP9	0.630504962	0.758703153	0.128198191	0.004511719
cg16275903	3	133524006	SRPRB	0.759051194	0.669245982	-0.089805212	0.004511719
cg01409985	3	133524160	SRPRB	0.548314905	0.498201208	-0.050113697	0.014140061
cg27328673	5	140625750	PCDHB15	0.580923947	0.648220734	0.067296787	0.023600884
cg23974473	5	140625794	PCDHB15	0.629337112	0.682170786	0.052833674	0.023600884
cg07015465	5	167912659	RARS	0.652053286	0.764673761	0.112620475	0.004511719
cg00226923	6	36972027	FGD2	0.676129161	0.724148911	0.04801975	0.037900087
cg08683249	6	43612627	RSPH9	0.736379045	0.777451498	0.041072452	0.037900087
cg01344171	6	43612647	RSPH9	0.554813266	0.632398518	0.077585251	0.037900087
cg16044810	7	151137746	CRYGN	0.526503796	0.580604952	0.054101156	0.037900087
cg23666844	7	151137882	CRYGN	0.539132374	0.602685901	0.063553527	0.014140061

cg08072539	8	496618	C8orf42	0.593552467	0.66899484	0.075442374	0.004511719
cg26280778	8	496739	C8orf42	0.692447232	0.774414713	0.081967481	0.004511719
cg09041527	8	11659497	FDFT1	0.258261859	0.195622367	-0.062639492	0.004511719
cg24123057	8	11660089	FDFT1	0.165658833	0.091811698	-0.073847135	0.037900087
cg23053961	9	4300432	GLIS3	0.489458501	0.586071441	0.09661294	0.008146802
cg13397898	9	133768931	QRFP	0.703100298	0.818101659	0.115001362	0.004511719
cg03924115	9	133768966	QRFP	0.698409708	0.802079425	0.103669717	0.004511719
cg01000188	9	133769184	QRFP	0.574967676	0.667563746	0.09259607	0.004511719
cg03900284	9	133769695	QRFP	0.725576414	0.800767494	0.07519108	0.014140061
cg00404025	10	49892741	WDFY4	0.651707089	0.775994626	0.124287537	0.004511719
cg18768238	10	49892829	WDFY4	0.598515291	0.68058794	0.082072649	0.004511719
cg10887366	10	49892861	WDFY4	0.664192069	0.813478627	0.149286558	0.004511719
cg17526462	10	49892927	WDFY4	0.641517931	0.76915043	0.1276325	0.004511719
cg15164194	10	49892930	WDFY4	0.640044961	0.773400471	0.133355511	0.004511719
cg27459529	10	49892943	WDFY4	0.683280121	0.820613845	0.137333724	0.004511719
cg20504007	10	49892954	WDFY4	0.724877473	0.833355365	0.108477893	0.008146802
cg26246740	10	49893026	WDFY4	0.632100855	0.72046596	0.088365104	0.004511719
cg20434666	10	49893406	WDFY4	0.740515794	0.814548392	0.074032597	0.023600884
cg17967780	10	49893445	WDFY4	0.640182985	0.76976861	0.129585625	0.004511719
cg07345108	10	49893463	WDFY4	0.660939018	0.814146318	0.1532073	0.004511719
cg13447813	10	88756580	AGAP11	0.740614099	0.674369427	-0.066244672	0.014140061
cg07954108	10	127464368	MMP21	0.804670314	0.865945904	0.061275589	0.004511719
cg25319933	10	127464557	MMP21	0.699509499	0.776517867	0.077008367	0.014140061
cg04358037	10	127465013	MMP21	0.778492599	0.68160877	-0.09688383	0.037900087
cg08843492	11	308231	IFITM2	0.465239375	0.539158683	0.073919307	0.008146802
cg12876900	11	320732	IFITM3	0.510480358	0.544181197	0.033700839	0.037900087
cg09554856	11	33914646	LMO2	0.648597251	0.721914611	0.07331736	0.008146802
cg25101936	11	113929164	ZBTB16	0.682092575	0.801323209	0.119230634	0.004511719
cg12526471	11	113929188	ZBTB16	0.613865738	0.686325879	0.072460141	0.004511719
cg09593860	11	113929287	ZBTB16	0.640327462	0.745809653	0.105482191	0.023600884
cg25719851	12	7055452	PTPN6	0.655389416	0.733723832	0.078334415	0.037900087
cg23193870	12	7055657	PTPN6	0.498306022	0.56036803	0.062062008	0.014140061
cg12690127	12	7055683	PTPN6	0.553665174	0.632953909	0.079288736	0.023600884
cg15006627	12	13045938	GPRC5A	0.582255053	0.644463356	0.062208304	0.023600884
cg27409154	12	53490412	IGFBP6	0.615302049	0.712511812	0.097209763	0.008146802
cg04791129	12	53490632	IGFBP6	0.499966189	0.570802466	0.070836277	0.008146802
cg26124597	12	58012960	SLC26A10	0.686032313	0.800158113	0.114125799	0.004511719
cg12883767	12	58013109	SLC26A10	0.692328633	0.805056733	0.1127281	0.004511719
cg03308494	12	58013267	SLC26A10	0.65649414	0.744320244	0.087826103	0.004511719
cg03728296	12	58013271	SLC26A10	0.736397478	0.830381066	0.093983587	0.004511719
cg06631310	12	58013324	SLC26A10	0.612408104	0.718345584	0.105937479	0.004511719
cg26785896	12	58013354	SLC26A10	0.633154063	0.747017969	0.113863907	0.004511719
cg27043188	12	58013414	SLC26A10	0.571537586	0.609082186	0.0375446	0.037900087
cg22844229	12	58013475	SLC26A10	0.574005433	0.615091611	0.041086178	0.014140061
cg20677436	12	58013487	SLC26A10	0.628358808	0.661913808	0.033555	0.008146802
cg09696411	12	58013517	SLC26A10	0.580743487	0.605148712	0.024405226	0.023600884
cg18069290	12	58013539	SLC26A10	0.64104381	0.724157917	0.083114107	0.004511719

cg16567290	12	58013569	SLC26A10	0.590974335	0.636420756	0.045446421	0.014140061
cg14494721	12	58013636	SLC26A10	0.60169478	0.654390033	0.052695253	0.004511719
cg04963424	12	58013645	SLC26A10	0.623979963	0.662278339	0.038298376	0.037900087
cg16577509	12	58013651	SLC26A10	0.509683363	0.567364447	0.057681085	0.008146802
cg08820231	12	58013687	SLC26A10	0.635689848	0.70389314	0.068203291	0.004511719
cg00159243	12	109023799	SELPLG	0.651103051	0.781095287	0.129992236	0.004511719
cg25165932	12	109025846	SELPLG	0.571313334	0.683669249	0.112355915	0.014140061
cg24497819	12	109027683	SELPLG	0.619939993	0.694674094	0.0747341	0.004511719
cg17291826	12	109028356	SELPLG	0.655097524	0.699452266	0.044354742	0.037900087
cg22328644	12	109028385	SELPLG	0.677505609	0.754560122	0.077054513	0.023600884
cg12488585	12	132434050	EP400	0.592100104	0.660900541	0.068800437	0.014140061
cg14912575	14	70038236	C14orf162	0.60418216	0.702680927	0.098498767	0.014140061
cg23920953	14	70038505	C14orf162	0.626676367	0.738630163	0.111953795	0.004511719
cg07139509	14	70038717	C14orf162	0.632265119	0.71804565	0.085780531	0.008146802
cg18477204	14	70038884	C14orf162	0.528138731	0.624775149	0.096636418	0.023600884
cg02380334	14	70038925	C14orf162	0.583184453	0.717583663	0.13439921	0.023600884
cg14539393	14	70038949	C14orf162	0.574127214	0.672479251	0.098352036	0.014140061
cg04571965	14	70039262	C14orf162	0.441893842	0.516797806	0.074903964	0.037900087
cg23515460	14	73361747	DPF3	0.197425206	0.276105977	0.078680771	0.037900087
cg26057784	14	73362214	DPF3	0.703981818	0.750474797	0.046492979	0.023600884
cg10814005	14	91711041	GPR68	0.313927411	0.241754084	-0.072173327	0.023600884
cg15390894	14	91719356	GPR68	0.650032022	0.791495168	0.141463146	0.004511719
cg25054390	14	91720372	GPR68	0.537478134	0.615884407	0.078406273	0.023600884
cg13012868	14	91720578	GPR68	0.804856134	0.862349392	0.057493258	0.014140061
cg02868468	14	105045347	C14orf180	0.675947912	0.768525841	0.092577928	0.014140061
cg25210134	15	40600265	PLCB2	0.54082789	0.597973981	0.057146091	0.004511719
cg05547778	15	40600279	PLCB2	0.633547634	0.752680344	0.11913271	0.014140061
cg14076977	15	40600284	PLCB2	0.655390047	0.753506478	0.098116431	0.014140061
cg21931938	15	40600493	PLCB2	0.624701183	0.742240084	0.117538901	0.004511719
cg02240622	15	40601467	PLCB2	0.678032935	0.794470033	0.116437099	0.008146802
cg06086177	16	73090646	ZFHX3	0.636823457	0.762011475	0.125188018	0.008146802
cg04700292	16	73090723	ZFHX3	0.727543159	0.802120197	0.074577038	0.004511719
cg00614832	16	73092394	ZFHX3	0.274538517	0.195529249	-0.079009268	0.004511719
cg04699663	16	89043171	CBFA2T3	0.559133851	0.635695533	0.076561683	0.004511719
cg06436854	16	89043527	CBFA2T3	0.538936753	0.559130169	0.020193416	0.037900087
cg01404893	16	89043547	CBFA2T3	0.583610306	0.665979855	0.082369548	0.004511719
cg13745346	16	89044523	CBFA2T3	0.673695095	0.762905776	0.089210681	0.004511719
cg08047086	16	89044646	CBFA2T3	0.699452684	0.798223672	0.098770988	0.004511719
cg27292079	16	89044681	CBFA2T3	0.579292498	0.648988595	0.069696097	0.008146802
cg05099708	16	89044855	CBFA2T3	0.599873628	0.66601773	0.066144101	0.008146802
cg00443981	17	58499679	C17orf64	0.72108363	0.809376911	0.088293281	0.004511719
cg12131208	17	58499700	C17orf64	0.723095918	0.809481338	0.086385421	0.004511719
cg04413853	17	58499706	C17orf64	0.724281002	0.834331239	0.110050238	0.004511719
cg06752482	17	58499816	C17orf64	0.603212957	0.674200723	0.070987766	0.008146802
cg17628249	17	58499854	C17orf64	0.748073289	0.840225785	0.092152496	0.014140061
cg19463256	19	46915351	CCDC8	0.576373099	0.678779425	0.102406326	0.037900087
cg19747604	19	46915570	CCDC8	0.580272063	0.649886783	0.06961472	0.014140061

cg17375267	19	46915776	CCDC8	0.516494085	0.579456318	0.062962233	0.014140061
cg18653451	19	46917018	CCDC8	0.337928027	0.417898751	0.079970724	0.037900087
cg03576469	19	46917061	CCDC8	0.360055017	0.463310946	0.103255929	0.037900087
cg11715966	19	46917255	CCDC8	0.825628244	0.900924065	0.075295821	0.004511719
cg22786631	22	47022596	GRAMD4	0.773262725	0.810161818	0.036899093	0.014140061
cg25390635	22	47022618	GRAMD4	0.785744127	0.80752245	0.021778323	0.037900087
cg16617543	22	47022654	GRAMD4	0.65886633	0.714324706	0.055458376	0.008146802

TARGETID: Illumina 450K methylation array code probe, CHR: chromosome, MAPINFO: genomic location, SYMBOL: gene symbol, FC_C: control frontal cortex samples average Beta signal = (Methylated allele intensity (M) / (Unmethylated allele intensity (U) + Methylated allele intensity (M))), FC_AD: AD braak stage V-VI frontal cortex samples average beta signal, Delta: difference of DNA methylation between FC_AD and FC_C, FDR p-val: false discovery rate p-value multiple comparison correction.

Supplementary Table 2. Analysis of Illumina 27K methylation arrays (statistical description): AVG.Beta values from five control (2 females and 3 control males aged 78±2.9 years) and five AD Braak V-VI (1 female and 4 males aged 77.2±0.7 years) hippocampal samples were compared using the Illumina's custom error model with FDR correction for multiple testing. Probes showing Diff.Score above 20 or below -20, corresponding to a p-value of less than 0.01 after FDR-based correction for multiple testing, were selected.

TARGETID	CHR	MAPINFO	SYMBOL	HP_C	HP_AD	Delta	FDR p-val
cg15383120	chr6	236909	DUSP22	0.18264	0.43836	0.25572	<0.01
cg17749961	chr2	30523367	LYCAT	0.05873	0.27719	0.21845	<0.01
cg11235426	chr6	237522	DUSP22	0.17985	0.39291	0.21306	<0.01
cg02512860	chr7	100667471	CLDN15	0.41037	0.59609	0.18572	<0.01
cg00430287	chr6	116489569	FRK	0.50009	0.67685	0.17676	<0.01
cg21880903	chr4	39085060	KLB	0.33804	0.51395	0.17591	<0.01
cg00603172	chr2	242146552	BOK	0.47773	0.64691	0.16918	<0.01
cg14159672	chr1	204085802	PM20D1	0.22493	0.38206	0.15713	<0.01
cg11761535	chr3	150533721	TM4SF18	0.55804	0.69822	0.14018	<0.01
cg16899306	chr6	32840563	HLA-DQB2	0.80168	0.89832	0.09664	<0.01
cg16474696	chr19	13736014	MGC3207	0.08521	0.17543	0.09022	<0.01
cg24794433	chr9	123501993	DAB2IP	0.18311	0.08917	-0.09394	<0.01
cg17977362	chr2	240165186	FLJ45964	0.92023	0.82376	-0.09647	<0.01
cg08128768	chr9	123502003	DAB2IP	0.28507	0.17209	-0.11297	<0.01
cg10832945	chr2	240164816	FLJ45964	0.91617	0.78486	-0.13131	<0.01
cg27244482	chr5	149649681	CAMK2A	0.58588	0.4202	-0.16568	<0.01
cg26537639	chr16	87244875	CYBA	0.57243	0.33124	-0.24119	<0.01

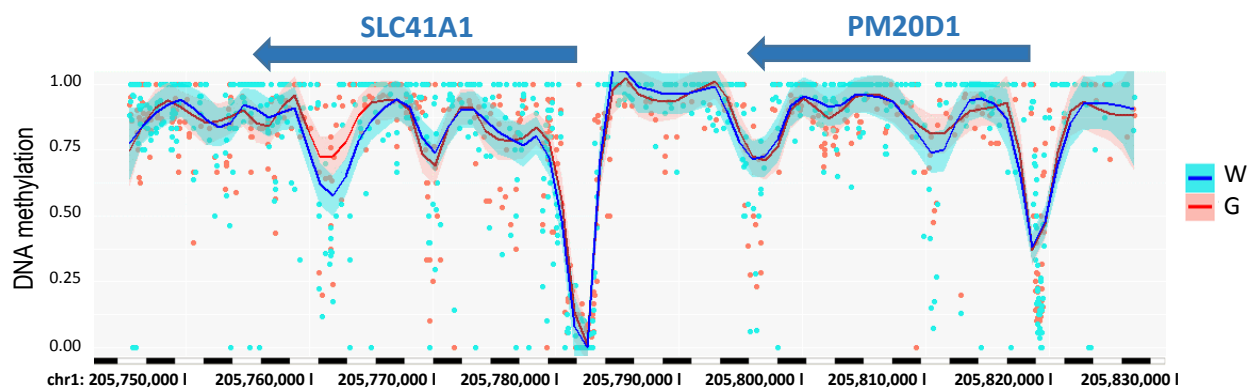
TARGETID: Illumina 27K methylation array code probe, CHR: chromosome, MAPINFO: genomic location, SYMBOL: gene symbol, HP_C: control hippocampus samples average AVG.Beta signal = (Methylated allele intensity (M) / (Unmethylated allele intensity (U) + Methylated allele intensity (M))), HP_AD: AD braak stage V-VI hippocampus samples average, Delta: difference of DNA methylation between HP_AD and HP_C, FDR p-val: false discovery rate p-value multiple comparison correction.

Supplementary Table 3. PM20D1 mQTL/eQTL-associated SNPs and their association with Alzheimer's disease.

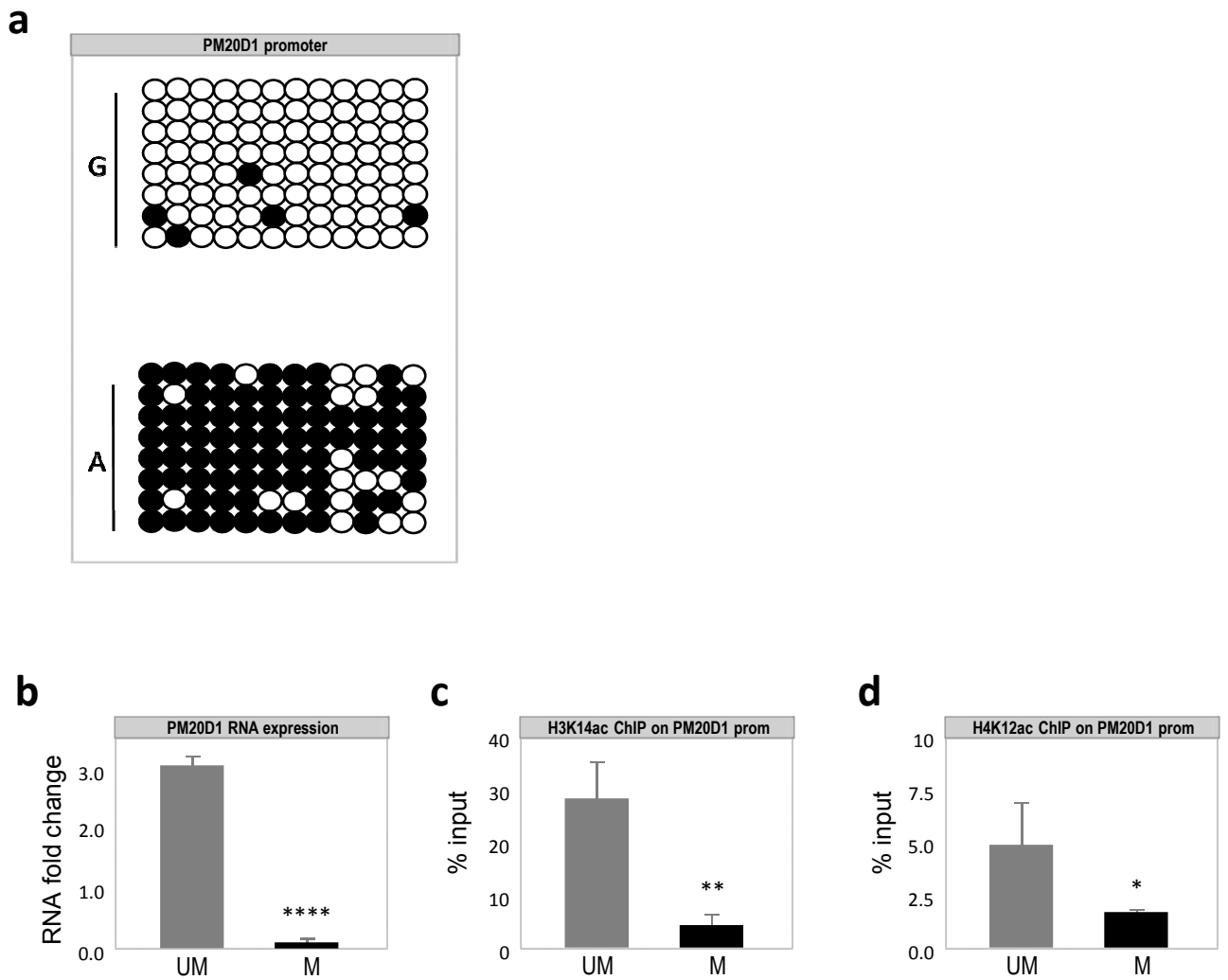
SNP	Position	Risk Hap ¹	Location	Type of mutation	Freq ²	Fetal Pval ³	Adult PFC Pval ³	Adult STR Pval ³	Adult CER Pval ³	GTEx_eQTL Pval ⁴	NIALOAD Pval ⁵
rs1172198	chr1:205662717	A	SLC45A3-NUCKS1	Intergenic	0.43	1.01E-26	1.29E-12	6.49E-13	1.87E-13	3.7E-29	1.72E-01
rs708727	chr1:205767884	A	SLC41A1	CDS synonymous	0.40	1.13E-73	4.27E-34	2.79E-34	2.29E-33	2.3E-49	3.11E-03
rs823082	chr1:205786934	C	SLC41A1-PM20D1	Intergenic	0.52	7.06E-23	7.03E-19	2.9E-18	3.8E-18	7.3E-25	4.54E-02
rs823088	chr1:205794174	T	SLC41A1-PM20D1	Intergenic	0.63	6.11E-14	1.27E-09	2.46E-08	8.77E-09	1.8E-13	4.97E-02
rs1361754	chr1:205801871	G	PM20D1	CDS (I380T)	0.52	7.06E-23	7.03E-19	2.9E-18	3.8E-18	7.3E-25	4.49E-02
rs960603	chr1:205812613	T	PM20D1	Intronic	0.45	4.59E-20	1.36E-13	4.15E-14	1.03E-13	4.8E-21	4.11E-04

1, Haplotype defined according to the HapMap Phase III 1000 Genomes CEU population (<http://www.internationalgenome.org/category/phase-3>) 2, Risk allele frequencies in European population according to HapMap Phase III 1000 Genomes 3, mQTLs from Hannon et al., 2016 Nat Neurosci (<http://epigenetics.essex.ac.uk/mQTL>) 4, eQTLs from GTEx consortium, 2015 Science (<http://www.gtexportal.org/home>) 5, GWAf geepack analysis (Chen and Yang, 2010 Bioinformatics) of phs000168.v1.p1 samples consisting of 607 families - 1,516 affected, 1,306 unaffected. Logistic regression via Generalized Estimation Equation (GEE) -family data - under additive genetic model with phenotype as outcome. Only mQTLs directly interrogated by NIALOAD arrays are included in the table.

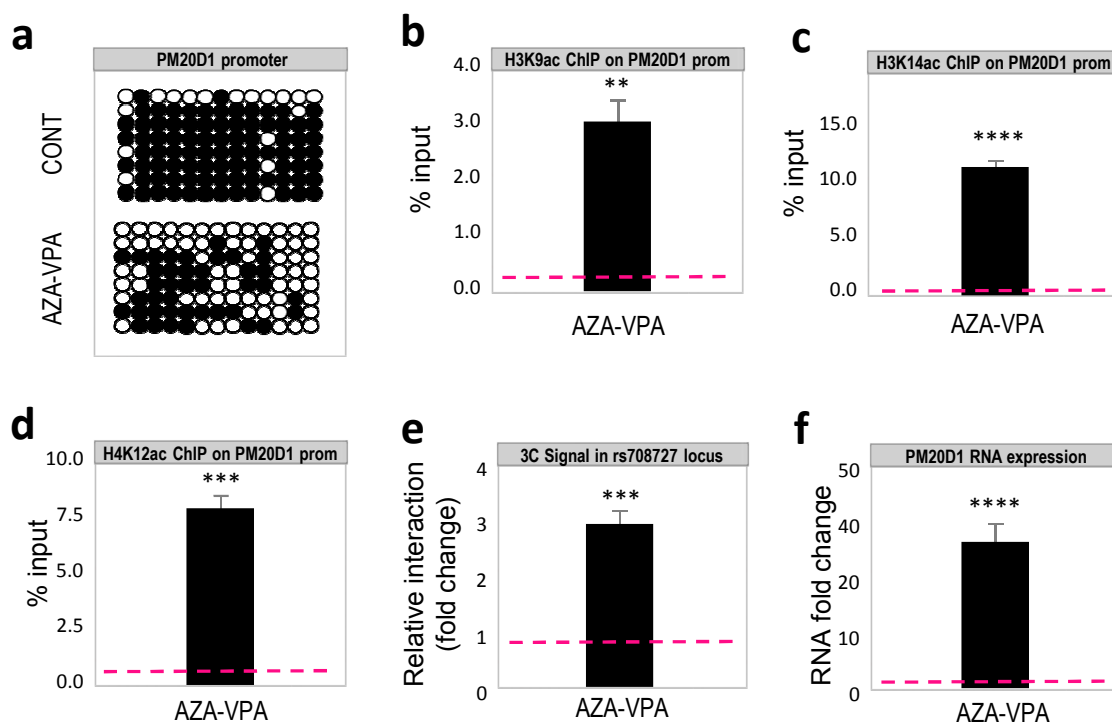
Abbreviations: CDS: coding sequence, CER: cerebellum, Fetal: fetal brain, P: p-value, PFC: prefrontal cortex, SNP: single nucleotide polymorphism, STR: striatum.



Supplementary Figure 1. PM20D1 locus DNA methylation in Grey and White matter. Representative whole genome bisulfite sequencing analysis of DNA methylation in one grey (G) and one white matter (W) human *frontal cortex* obtained from the same donor (GSE47966 GSM1173772—Grey matter—, GSM1279516 GSE52271—white matter). No significant differences in DNA methylation are observed. Dots represents single sequencing reads. Methylation average is represented by smoothed lines, and 95% confidence intervals by shadows.

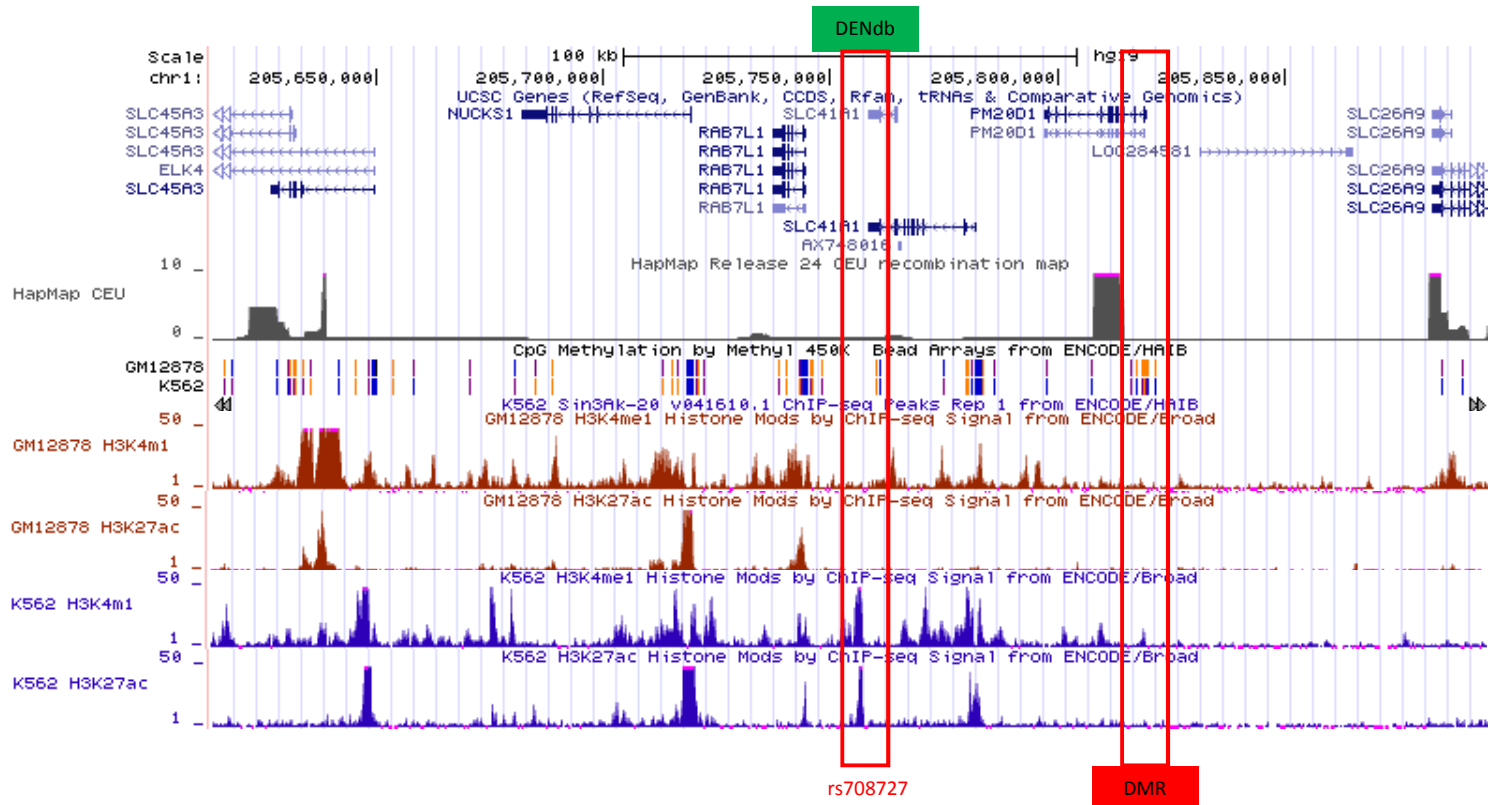


Supplementary Figure 2. Epigenetic characterization of immortalized B cells. **(a)** Bisulfite sequencing/cloning of the *PM20D1* differentially methylated region (DMR) in immortalized B cells from UM and M carriers. **(b)** qRT-PCR experiments showing *PM20D1* RNA levels in immortalized B cells from UM and M samples (n=14 independent experiments; p-val< 0.0001; one-sided Student's t-test). **(c)** Chromatin Immunoprecipitation assay (ChIP) on the *PM20D1* promoter using H3K14ac antibody in UM and M samples (n=3 independent experiments; p-val= 0.0068; one-sided Student's t-test). **(d)** ChIP on *PM20D1* promoter using H4K12ac antibody in UM and M samples (n=3 independent experiments; p-val= 0.0243; one-sided Student's t-test). Data in **b-d** are represented as means plus SEM. *, p<0.05; **,p<0.01; ****,p<0.0001.

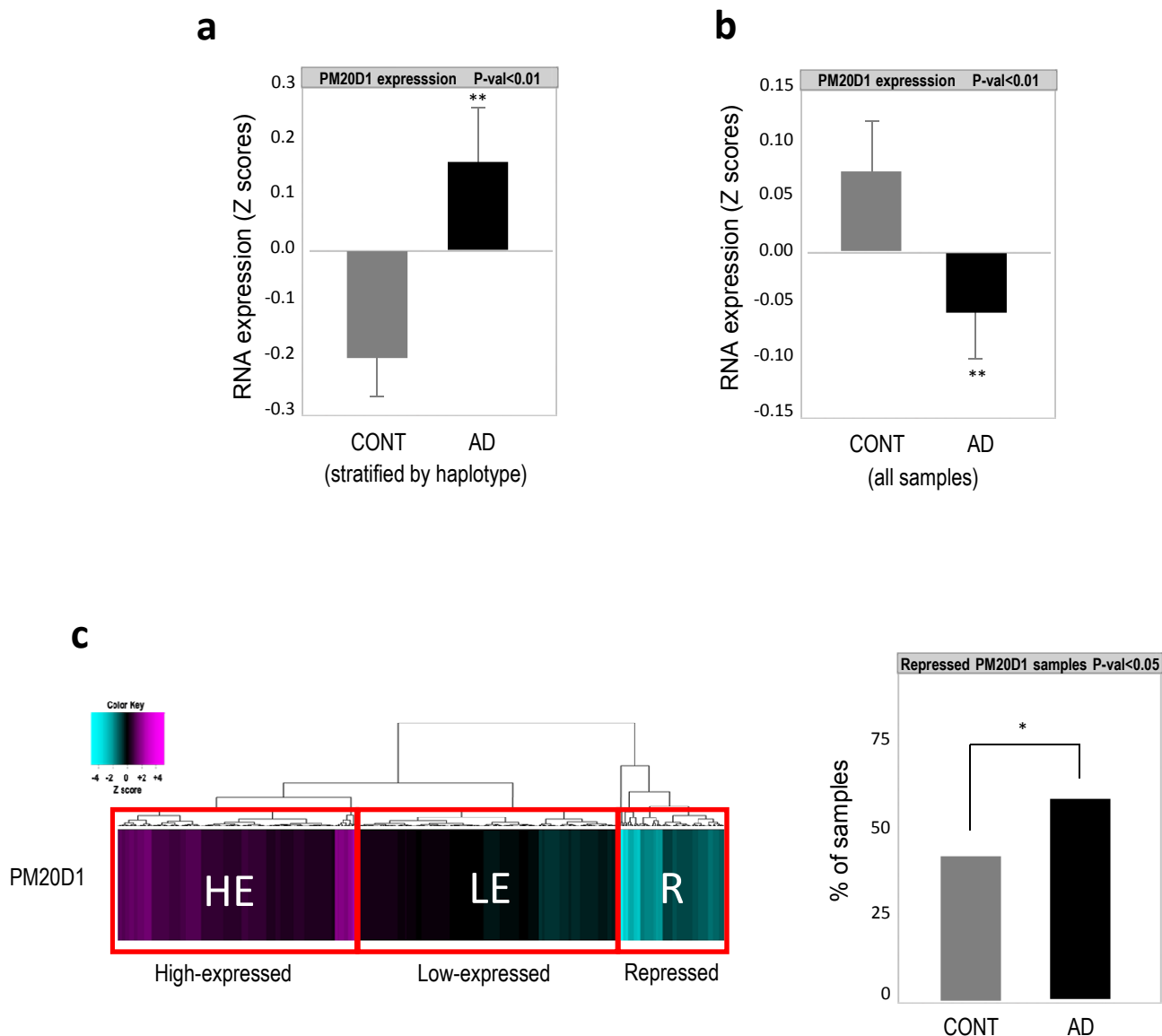


Supplementary Figure 3. Epigenetic regulation of the *PM20D1* 3C DNA loop in B cells. **(a)** Bisulfite cloning/sequencing showing that a combination of the DNA-demethylating agent 5-azacytidine-dC (AZA) and the histone deacetylase (HDAC) inhibitor valproic acid (VPA) in methylated B cells reduces *PM20D1* promoter DNA methylation. **(b)** H3K9ac chromatin Immunoprecipitation assay (ChIP) showing that AZA-VPA treatment increases histone acetylation levels on the *PM20D1* promoter (n=3 independent experiments; p-val= 0.0015; one-sided Student's t-test). **(c)** H3K14ac ChIP showing increase of histone acetylation after AZA-VPA treatment (n=3 independent experiments; p-val< 0.0001; one-sided Student's t-test). **(d)** H4K12ac ChIP showing increase of histone acetylation after AZA-VPA treatments (n=3 independent experiments; p-val= 0.0004; one-sided Student's t-test). **(e)** The 3C DNA loop can be restored by allowing a permissive state of the chromatin with AZA-VPA treatment (n=3 independent experiments; p-val= 0.0003; one-sided Student's t-test). **(f)** *PM20D1* RNA expression can be restored by AZA-VPA treatment (n=3 independent experiments; p-val< 0.0001; one-sided Student's t-test). Dash magenta lines indicate control levels. Data are represented as means plus SEM. *, p<0.05; **, p<0.01; ***, p<0.001; and ****, p<0.0001.

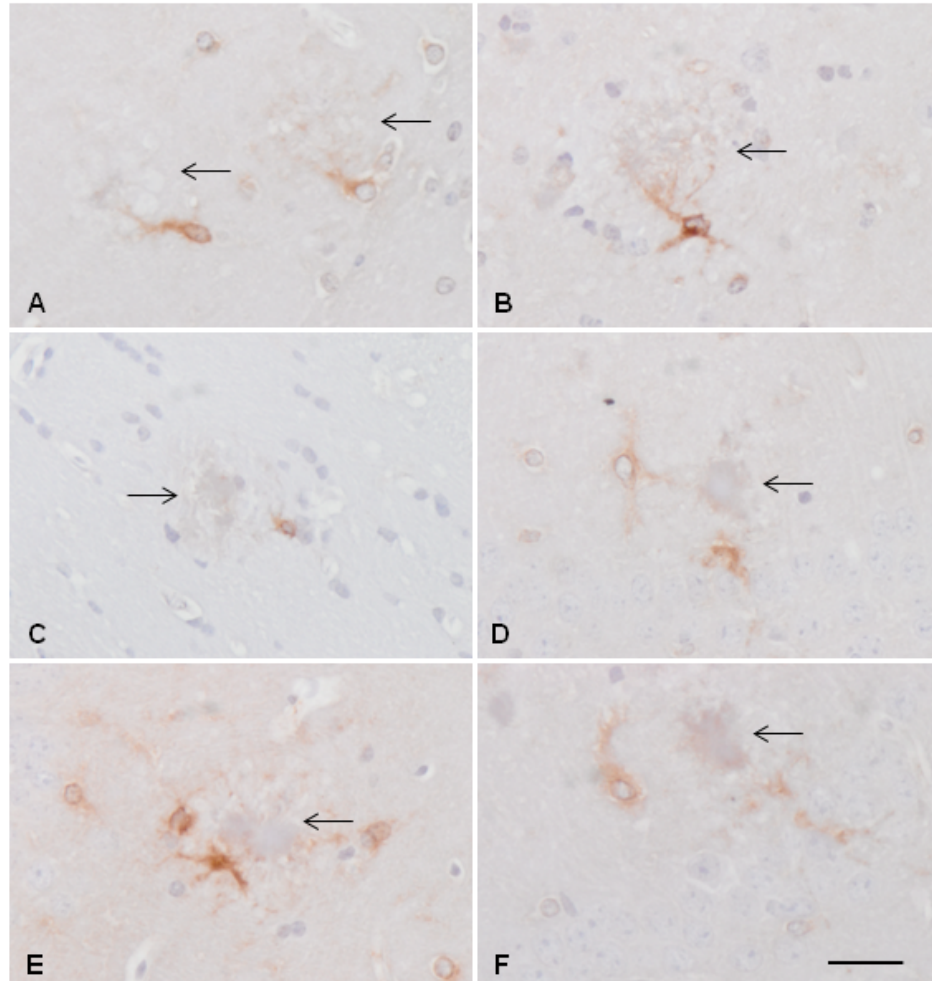
chr1:205,614,110-205,894,696 (hg19)



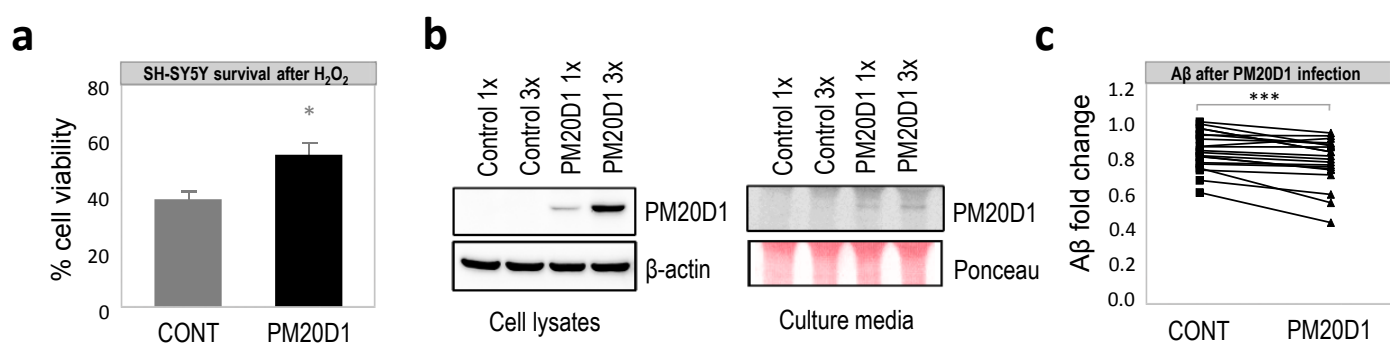
Supplementary Figure 4. UCSC genome browser snapshot showing the rs708727-*PM20D1* locus. Reference genes, HapMap Release 24 CEU recombination map, ENCODE Histone Modifications, Broad ChIP-seq Signal (H3K27ac and H3K4me1) for GM12878 and K562 cells, and GM12878 and K562 Methylation 450K Bead Array from ENCODE/HAIB (orange = methylated; purple = partially methylated, bright blue = unmethylated) tracks are shown. The location of rs708727, *PM20D1* promoter differentially methylated region (DMR; red box) and Dragon enhancer prediction (DENdb; green box) are indicated. Of note, G12878 (methylated) and K562 (partially methylated) cell lines are also differentially methylated in the *PM20D1* promoter and K562 but not G12878 cells show H3K27ac and H3K4me1 peaks in the DENdb region.



Supplementary Figure 5. *PM20D1* RNA expression meta-analysis. **(a)** NCBI GEO RNA expression datasets (GSE33000, GSE15222, and GSE36980) investigating *PM20D1* RNA expression changes between control and AD samples stratified in three segments according to their levels of expression (high-expressed, low-expressed, and repressed samples). High-expressed *PM20D1* samples comparison between controls (n=63) and AD (n=73) (p-val=0.0041 one-sided Student's t-test). **(b)** Overall comparison of *PM20D1* RNA expression levels between control (n=362) and AD (n=501) considering all samples (high-expressed, low-expressed, and repressed samples; p-val=0.0043 two-sided Wilcoxon test). **(c)** Representative heat-map (GSE33000) showing the three clusters of expression levels (left) and cumulative distribution of the *PM20D1*-repressed cluster samples between control and AD subjects (right; n=853 samples; p-val=0.0472 one-sided Fisher's exact test). Data are presented as percentage of methylated (c) and as means $\pm$ SEM (a, b). *, p<0.05; **, p<0.01



Supplementary Figure 6. PM20D1 in APP/PS1 mice. Representative IHC images showing PM20D1 localized in the cytoplasm of neurons and glial cells of APP/PS1 mice, with glial cells in the vicinity of β -amyloid plaque cores (indicated by arrows) showing the strongest immunoreactivity. Hematoxylin staining for PM20D1 in 12 months (A,B,C) and 18 months old mice (D,E,F) (n=8 biologically independent APP/PS1 samples per age; two sections per case). Scale bar, 25 μ m.



Supplementary Figure 7. *PM20D1* overexpression in SH-SY5Y cells and APP/PS1-derived primary hippocampal neuronal cultures. **(a)** Alamarblue cell viability assay showing reduced H_2O_2 -induced cell death in *PM20D1* overexpressing SH-SY5Y cells ($n=4$ independent experiments; p -val= 0.0222; one-sided Student's t-test). Data are presented as means + SEM. **(b)** *PM20D1* overexpression in primary hippocampal cultures. Representative Western Blot images showing that *PM20D1* can be detected in cell lysates (left) as well as in cultured media (right) ($n=3$ independent experiments). **(c)** *PM20D1* transduction decreases A β levels in APP/PS1-derived hippocampal cultures as measured by ELISA after one week of infection. Data represent 15 different paired experiments (mock versus *PM20D1* transduction) performed with three different virus batch productions (five experiments per virus batch) ($n=15$ independent experiments; p -val=0.0009; two-sided Student's t-test). *, $p<0.05$; *** $p<0.001$.