SUPPLEMENTAL FIGURES

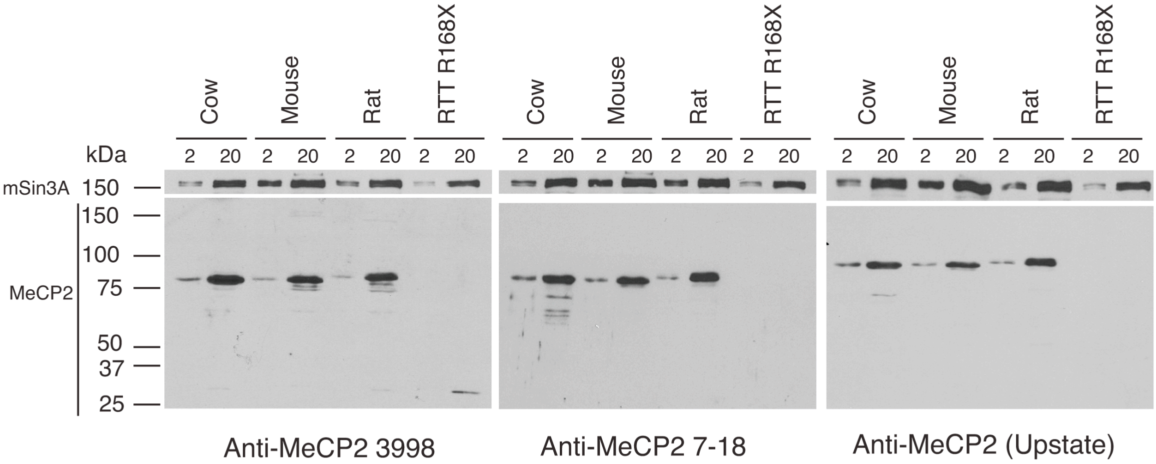

Figure S1. Anti-MeCP2 antibodies react specifically with MeCP2 from multiple protein sources. Antibodies against MeCP2 were used in western blotting of brain nuclear extracts from cow, mouse, and rat, and nuclear extracts from human lymphoblasts expressing the RTT R168X truncation to show their specificities. The Jones lab-generated anti-MeCP2 3998 (left panel) [17], and anti-MeCP2 7-18 (center panel) were compared with an anti- MeCP2 antibody purchased from Upstate Biotech (right panel). All three antibodies react with a single polypeptide migrating at ~80 kDa, consistent with MeCP2's known migration on SDS-PAGE, in brain nuclear extracts (2 or 20 μg/lane) from cow, mouse, and rat. These antibodies show no reactivity to any polypeptides in the human MeCP2 RTT R168X extracts by western blot analysis. These data confirm that the anti-MeCP2 7-17 antibody used in this study is specific for MeCP2. Load controls using an antibody for Sin3A [3] are shown above.

MeCP2 MonoQ flow-thru (QFT): Sequence coverage 41%

MVAGMLGLRKEKSEDQDLQGLKEKPLKFKKVKKDKKEDKEGKHEPLQPSAHH
SAEPAEAGKAETSESSGSAPAVPEASASPKQRRSIIRDRGPMYDDPTLPEGWTRK
LKQRKSGRSAGKYDVYLINPQGKAFRSKVELIAYFEKVGDTSLDPNDFDFTVTG
RGSPSRREQKPPKKPKSPKAPGTGRGRGRPKGSGTGRPKAAASEGVQVKRVLEK
SPGKLLVKMPFQASPGGKGEGGGATTSAQVMVIKRPGRKRKAEADPQAIPKKR
GRKPGSVVAAAAAEAKKKAVKESSIRSVQETVLPIKKRKTRETVSIEVKEVVKPL
LVSTLGEKSGKGLKTCKSPGRKSKESSPKGRSSSASSPPKKEHHHHHHHAESPKA
PMPLLPPPPPPEPQSSEDPISPPEPQDLSSSICKEEKMPRAGSLESDGCPKEPAKTQP
MVAAAATTTTTTTTTVAEKYKHRGEGERKDIVSSSMPRPNREEPVDSRTPVTER
VS

MeCP2 MonoQ bound 2 (QB2): Sequence coverage 23%

MVAGMLGLRKEKSEDQDLQGLKEKPLKFKKVKKDKKEDKEGKHEPLQPSAHH
SAEPAEAGKAETSESSGSAPAVPEASASPKQRRSIIRDRGPMYDDPTLPEGWTRK
LKQRKSGRSAGKYDVYLINPQGKAFRSKVELIAYFEKVGDTSLDPNDFDFTVTG
RGSPSRREQKPPKKPKSPKAPGTGRGRGRPKGSGTGRPKAAASEGVQVKRVLEK
SPGKLLVKMPFQASPGGKGEGGGATTSAQVMVIKRPGRKRKAEADPQAIPKKR
GRKPGSVVAAAAAEAKKKAVKESSIRSVQETVLPIKKRKTRETVSIEVKEVVKPL
LVSTLGEKSGKGLKTCKSPGRKSKESSPKGRSSSASSPPKKEHHHHHHHAESPKA
PMPLLPPPPPPEPQSSEDPISPPEPQDLSSSICKEEKMPRAGSLESDGCPKEPAKTQP
MVAAAATTTTTTTTTVAEKYKHRGEGERKDIVSSSMPRPNREEPVDSRTPVTER
VS

MeCP2 MonoQ bound 3 (QB3): Sequence coverage 33%

MVAGMLGLRKEKSEDQDLQGLKEKPLKFKKVKKDKKEDKEGKHEPLQPSAHH
SAEPAEAGKAETSESSGSAPAVPEASASPKQRRSIIRDRGPMYDDPTLPEGWTRK
LKQRKSGRSAGKYDVYLINPQGKAFRSKVELIAYFEKVGDTSLDPNDFDFTVTG
RGSPSRREQKPPKKPKSPKAPGTGRGRGRPKGSGTGRPKAAASEGVQVKRVLEK
SPGKLLVKMPFQASPGGKGEGGGATTSAQVMVIKRPGRKRKAEADPQAIPKKR
GRKPGSVVAAAAAEAKKKAVKESSIRSVQETVLPIKKRKTRETVSIEVKEVVKPL
LVSTLGEKSGKGLKTCKSPGRKSKESSPKGRSSSASSPPKKEHHHHHHHAESPKA
PMPLLPPPPPPEPQSSEDPISPPEPQDLSSSICKEEKMPRAGSLESDGCPKEPAKTQP
MVAAAATTTTTTTTTVAEKYKHRGEGERKDIVSSSMPRPNREEPVDSRTPVTER
VS

Figure S2. Mass spectrometry MeCP2 peptide identifications. MS identified peptides (in red) are shown in the predicted amino acid sequences for rat MeCP2 from the QF, QB2, and QB3 pools of MeCP2.

EIF2S1 [eukaryotic translation initiation factor 2, subunit 1] Sequence Coverage: 33%

MPGLSCRFYQHKFPEVEDVVMVNVRSIAEMGAYVSLLEYNNIEGMILLSELSRR
RIRSINKLIRIGRNECVVVIRVDKEKGYIDLSKRRVSPEEAIKCEDKFTKSKTVYSI
LRHVAEVLEYTKDEQLESLFQRTAWVFDDKYKRPGYGAYDAFKHAVSDPSILDS
LDLNEDEREVLINNINRRLTPQAVKIRADIEVACYGYEGIDAVKEALRAGLNCST
ETMPIKINLIAPPRYVMTTTTLERTEGLSVLNQAMAVIKEKIEEKRGVFNVQMEP
KVVTDTDETELARQLERLERENAEVDGDDDAEEMEAKAED

EIF2S2 [eukaryotic translation initiation factor 2, subunit 2] Sequence Coverage: 30%

MSGDEMIFDPTMSKKKKKKKKPFMLDEEGDAQTEETQPSETKEVEPEPAEEKDV
EADEEDSRKKDASDDLDDLNFFNQKKKKKKTKKIFDIDEAEEAIKDVKIESDAQE
PAEPEDDLDIMLGNKKKKKKNVKFPDEDEILEKDEALEDEDSKKDDGISFSNQTG
PAWAGSERDYTYEELLNRVFNIMREKNPDMVAGEKRKFVMKPPQVVRVGTKK
TSFVNFTDICKLLHRQPKHLLAFLLAELGTSGSIDGNNQLVIKGRFQQKQIENVLR
RYIKEYVTCHTCRSPDTILQKDTRLYFLQCETCHSRCSVASIKTGFQAVTGKRAQ
LRAKAN

EIF2S3 [eukaryotic translation initiation factor 2, subunit 3] Sequence Coverage: 37%

MAGGEAGVTLGQPHLSRQDLATLDVTKLTPLSHEVISRQATINIGTIGHVAHGKS
TVVKAISGVHTVRFKNELERNITIKLGYANAKIYKLDDPSCPRPECYRSCGSSTPD
EFPTDIPGTKGNFKLVRHVSFVDCPGHDILMATMLNGAAVMDAALLLIAGNESC
PQPQTSEHLAAIEIMKLKHILILQNKIDLVKESQAKEQYEQILAFVQGTVAEGAPII
PISAQLKYNIEVVCEYIVKKIPVPPRDFTSEPRLIVIRSFDVNKPGCEVDDLKGGVA
GGSILKGVLKVGQEIEVRPGIVSKDSEGKLMCKPIFSKIVSLFAEHNDLQYAAPGG
LIGVGTKIDPTLCRADRMVGQVLGAVGALPEIFTELEISYFLLRRLLGVRTEGDKK
AAKVQKLSKNEVLMVNIGSLSTGGRVSAVKADLGKIVLTNPVCTEVGEKIALSR
RVEKHWRLIGWGQIRRGVTIKPTVDDD

Mecp2 [Methyl-CpG-binding protein 2] Sequence Coverage: 23%

MVAGMLGLRKEKSEDQDLQGLKEKPLKFKKVKKDKKEDKEGKHEPLQPSAHH
SAEPAEAGKAETSESSGSAPAVPEASASPKQRRSIIRDRGPMYDDPTLPEGWTRK
LKQRKSGRSAGKYDVYLINPQGKAFRSKVELIAYFEKVGDTSLDPNDFDFTVTG
RGSPSRREQKPPKKPKSPKAPGTGRGRGRPKGSGTGRPKAAASEGVQVKRVLEK
SPGKLLVKMPFQASPGGKGEGGGATTSAQVMVIKRPGRKRKAEADPQAIPKKR
GRKPGSVVAAAAAEAKKKAVKESSIRSVQETVLPIKKRKTRETVSIEVKEVVKPL
LVSTLGEKSGKGLKTCKSPGRKSKESSPKGRSSSASSPPKKEHHHHHHHAESPKA
PMPLLPPPPPPEPQSSEDPISPPEPQDLSSSICKEEKMPRAGSLESDGCPKEPAKTQP
MVAAAATTTTTTTTTVAEKYKHRGEGERKDIVSSSMPRPNREEPVDSRTPVTER
VS

PRPF3 [PRP3 pre-mRNA processing factor 3 homolog] Sequence Coverage: 9%

MALSKRELDELKPWIEKTVKRVLGFSEPTVVTAALNCVGKGMDKKKAADHLKPFLDDSTLRFVDKLFEAVEEGRSSRHSKSSSDRSRKRELKEVFGDDSEISKESSGVKKRRIPRFEEVEEEPEVIPGPPSESPGMLTKLQIKQMMEAATRQIEERKKQLSFISPPAPQPKTPSSSQPERLPIGNTIQPSQAATFMNDAIEKARKAAELQARIQAQLALKPGLIGNANMVGLANLHAMGIAPPKVELKDQTKPTPLILDEQGRTVDATGKEVELTHRMPTLKANIRAVKREQFKQQLKEKPSEDMESNTFFDPRVSIAPSQRQRRTFKFHDKGKFEKIAQRLRTKAQLEKLQAEISQAARKTGIHTSTRLALIAPKKELKEGDIPEIEWWDSYIIPNGFDLTEENPKREDYFGITNLVEHPAQLNPPVDNDTPVTLGVYLTKKEQKKLRRQTRREAQKELQEKVRLGLTPPPEPKVRISNLMRVLGTEAVQDPTKVEAHVRAQMAKRQKAHEEANAARKLTAEQRKVKKVKKLKEDISQGVHISVYRVRNLSNPAKKFKIEANAGQLYLTGVVVLHKDVNVVVVEGGPKAQRKFKRLMLHRIKWDEQTSNTKGDDDEESDEEAVKKTNKCVLVWEGTAKDRSFGEMKFKQCPTENMAREHFKKHGAEHYWDLALSESVLESTD

ABCF1   [PREDICTED: similar to ATP-binding cassette sub-family F member 1 (ATP-binding cassette 50)]
Sequence Coverage: 32%

MPKGPKQQPPEPEWIGDGEGTSPADKVVKKGKKDKKTKKTFFEELAVEDKQAGEEEKLQKEKEQQQQQQQKKKRDTRKGRRKKDVDDDDDGDERVLMERLKQLSVPASDEEDEVPVPVPRGRKKAKGGNVFEALIQDESEEEKEEEEEKPVLKPAKPEKNRINKAVAEEPPGLRNKKGKEEKSKGKAKNKPSATDSEGEDDEDMTKEKEPPRPGKDKDKKGAEQGSEEEKEEKEGEVKANDPYAHLSKKEKKKLKKQMDYERQVESLKAANAAENDFSVSQAEVSSRQAMLENASDIKLEKFSISAHGKELFVNADLYIVAGRRYGLVGPNGKGKTTLLKHIANRALSIPPNIDVLLCEQEVVADETPAVQAVLRADTKRLRLLEEEKRLQGQLEQGDDTAAEKLEKVYEELRATGAAAAEAKARRILAGLGFDPEMQNRPTQKFSGGWRMRVSLARALFMEPTLLMLDEPTNHLDLNAVIWLNNYLQGWRKTLLIVSHDQGFLDDVCTDIIHLDTQRLHYYRGNYMTFKKMYQQKQKELLKQYEKQEKKLKELKAGGKSTKQAEKQTKEVLTRKQQKCRRKNQDEESQDPPELLKRPREYTVRFTFPDPPPLSPPVLGLHGVTFGYEGQKPLFKNLDFGIDMDSRICIVGPNGVGKSTLLLLLTGKLTPTNGEMRKNHRLKIGFFNQQYAEQLHMEETPTEYLQRGFNLPYQDARKCLGRFGLESHAHTIQICKLSGGQKARVVFAELACREPDVLILDEPTNNLDIESIDALGEAINEYKGAVIVVSHDARLITETNCQLWVVEEQSVSQIDGDFDDYKREVLEALGEVMVNRPRD

SDCCA1 [similar to serologically defined colon cancer antigen 1]
Sequence Coverage: 3%

MKTRFSTVDLRAVLAELNANLLGMRVNNVYDVDNKTYLIRLQKPDFKATLLLESGIRIYTTEFEWPKNMMPSSFAMKCRKHLKSRRLVSAKQLGVDRIVDFQFGSDEAAYHLIIELYDRGNIVLTDYEYLILNILRFRTDEADDVKFAVRERYPIDHARAAEPLLTLERLTEVIARAPRGELLKRVLNLLLPYGPALIEHCLIENGFSGNVKVDEKLESKDIEKILVCVQRAEDYLEKTANFNGKGYIIQKREVKPSLDANKPAEDILMYEEFHPFLFSQHLQCPYIEFESFDKEKQALKKLDNVXKDHENRLEALQQAQEIDKLKGEL

IEMNLQIVDRAIQVVRSALANQIDWTEIGVIVKEAQAQGDHVASAIKELKLQTNH
ITMLLRNPYLLSEEEDGDGDGSIENSDAEAPKGKKKAKEQAAAEASEGQAAACR
CGPQPVSLCQCQKVLXSXEVCCXKTTENCRSCXEGIQISREENKANLKRSTNSYF
YPKSKESVLVXEISVVYXFRELSHYRWSRSATEXDYCEKILNTRRHLCACXSSWS
YQLCNXESNRRSHPSSDFDXSRHNGTLLQRGLGCPCYHECLVGAPSSGIXNSTDR
RVLNNWKLHDKRKKEFPSSFIPNDGVXLPFXGRXVLCLETSRXTKRQSAGXRHE
NIDKLHKXTHVRRNGTARRGRQQXRRDRGVMWNHGRSGTQDSGXSRGHCCSQ
WKRGTELXRWRSHQNSHERXRAHWXGEGRGGRISXHHHXLVPSSVPKAPTETD
SKRRIFXFKXQXITEPKTFVSQGEKRNEKEKAPMXLRGFRSDRRKGQRKRKCCA
QXSXPEHKQKCGSWTANEKRPEEXNEKNEGKIQRPGXXRSXTYYEIVGICRFKQ
RRKGEERKERKNKRXTREKKPTETQRWTAGFRCCXRNPVPSGVDSXLTRLCCG
XATXXQGRTXSGSAGKXGKSIXLFDRATTSXRCTNVCYSNMCSLHHHDKLXIQS
ETYSWSSEKGKSCKDSLEQFHALQRSNSKRKRLIPKCEGHRFTKKHSRESESVCP
QSSARKKKI

Figure S3. Mass spectrometry identifications of MeCP2 co-purifying peptides. MS identified peptides (in red) for the MeCP2 co-purifying polypeptides from the QB2 pool of MeCP2.

Table S1: PCR and RT-PCR oligonucleotide primers

Primer	Plasmid Construct	Sequence (5’-3’)
1	pGEM ratAbcf1	ATGCCGAAGGGTCCCAAGCAGC
2	pGEM ratAbcf1	TCAATCCCGAGGTCGGTTGAC
3	pGEM ratEif2s1	ATGCCGGGTCTAAGTTGTAGATTTTATCAACAC
4	pGEM ratEif2s1	TTAATCTTCAGCTTTGGCTTCCATTTCTTCTGC
5	pGEM ratEif2s2	ATGTCCGGGGACGAGATGATTTTGATCCTAC
6	pGEM ratEif2s2	TTAGTTAGCTTTGGCACGGAGCTG
7	pGEM ratEif2s3	ATGGCTGGGGGTGAGGCTG
8	pGEM ratEif2s3	TCAGTCATCATCTACAGTCGGCTTAATG
9	pGEM ratMeCP2	ATGGTAGCTGGGATGTTAGGGC
10	pGEM ratMeCP2	TCAGCTAACTCTCTCGGTCACG
11	pGEM ratPrpf3	ATGGCACTGTCTAAGCGGGAACTGGATG
12	pGEM ratPrpf3	CTACAGAGAACATGGCGCGTGA
13	pGEM ratSdccag1	ATGAAGACCCGCTTCAGCACTGTTGAC
14	pGEM ratSdccag1	CTATTTTCTTTTTACGTGCAGAAGATTGGG
15	pGEM human Prpf3	ATGGCACTGTCAAAGAGGGAGC
16	pGEM human Prpf3	TCAATCAGTGGACTCTAAC
17	pCDNA3.1 ratAbcf1	GCGGCCGCATGCCGAAGGGTCC
18	pCDNA3.1 ratAbcf1	CGCTCGAGTCAATCCCGAGGTCGG
19	pCDNA3.1 ratEif2s1	GCGGCCGCATGCCGGGTCTAAG
20	pCDNA3.1 ratEif2s1	CGCTCGAGTTAATCTTCAGCTTTGGCTTC
21	pCDNA3.1 ratEif2s2	GCGGCCGCATGTCCGGGGAC
22	pCDNA3.1 ratEif2s2	CGCTCGAGTTAGTTAGCTTTGGCACG
23	pCDNA3.1 ratEif2s3	GCGGCCGCATGGCTGGGGGTG
24	pCDNA3.1 ratEif2s3	CGCTCGAGTCAGTCATCATCTACAGTCG
25	pCDNA3.1 ratMeCP2	GCGGCCGCATGGTAGCTGGGATG
26	pCDNA3.1 ratMeCP2	CGCTCGAGTCAGCTAACTCTCTCGGTC
27	pCDNA3.1HA humanMeCP2	GCGGCCGCATGGTAGCTGGGATG
28	pCNA3.1HA humanMeCP2	CGCTCGAGTCAGCTAACTCTCTCGGTC
29	pCDNA3.1 ratPrpf3	GCGGCCGCATGGCACTGTCTAAGC
30	pCDNA3.1 ratPrpf3	CGCTCGAGTCACGCGCCATGTTCTC
31	pCDNA3.1 ratSdccag1	GCGGCCGCATGAAGAGCCGCT
32	pCDNA3.1 ratSdccag1	CGCTCGAGCTATTTCCTTTTTACGTTCAGAAG
33	pCDNA3.1 humanPrpf3	AGAATTCATGGCACTGTCAAAGAG
34	pCDNA3.1 humanPrpf3	CTCGAGTCAATCAGTGGACTCTAAC
35	pGEX5X ratPrpf3	AGAATTCATGGCACTGTCTAAGC
36	pGEX5X ratPrpf3	CTCGAGTCACGCGCCATGTTCTC
37	pGEX5X MeCP2 d1	AGAATTCGCCTCCCCCAAACAG
38	pGEX5X MeCP2 d1	CTCGAGTCAGCTAACTCTCTCGG
39	pGEX5X MeCP2 d2	AGAATTCAAGCAAAGGAAATCTGGC
40	pGEX5X MeCP2 d2	CTCGAGTCAGCTAACTCTCTCGG
41	pGEX5X MeCP2 d3	AGAATTCGGCACCACGAGACC
42	pGEX5X MeCP2 d3	CTCGAGTCAGCTAACTCTCTCGG
43	pGEX5X MeCP2 d4	AGAATTCACGGTCAGCATCGAG
44	pGEX5X MeCP2 d4	CTCGAGTCAGCTAACTCTCTCGG

45	pGEX5X MeCP2 d5	AGAATTCATGGTAGCTGGGATGTTAG
46	pGEX5X MeCP2 d5	CTCGAGGGTCTTGCGCTTCTTG
47	pGEX5X MeCP2 d6	AGAATTCGGCACCACGAGACC
48	pGEX5X MeCP2 d6	CTCGAGACCGAGGGTGGACAC
49	RT-PCR mouseFRG1 5’	AATTGCCCTGAAGTCTGGCTATGG
50	RT-PCR mouseFRG1 3’	CTTTCAATTTGGCTCTCCTGTCCAG
51	RT-PCR mouseCDK10 5’	GGCCAGGGATACCCAGACAG
52	RT-PCR mouseCDK10 3’	CCTCCGAGAAGGGTGTTGGC
53	RT-PCR mouseCasc3 5’	CTACAGTGAAGAGGAGAATTCCAAGGTG
54	RT-PCR mouseCasc3 3’	GATCCTCATCGTCATCCAAGTGC
55	pGEX5X MeCP2 S49X	AGAATTCGCCTCCCCCAAACAG
56	pGEX5X MeCP2 S49X	ACTCGAGTCATGGCTGCACGGGC
57	pGEX5X MeCP2 S68X	AGAATTCGCCTCCCCCAAACAG
58	pGEX5X MeCP2 S68X	ACTCGAGTCACCCTTCTGATGTCTC
59	pGEX5X MeCP2 W104X	AGAATTCGCCTCCCCCAAACAG
60	pGEX5X MeCP2 W104X	ACTCGAGTCAGCCTTCAGGCAGG
61	pGEX5X MeCP2 Y141X	AGAATTCGCCTCCCCCAAACAG
62	pGEX5X MeCP2 Y141X	ACTCGAGTCACGCAATCAACTCC
63	pGEX5X MeCP2 R168X	AGAATTCGCCTCCCCCAAACAG
64	pGEX5X MeCP2 R168X	ACTCGAGTCATCATCTCGCCGGGAGGG
65	pGEX5X MeCP2 S204X	AGAATTCGCCTCCCCCAAACAG
66	pGEX5X MeCP2 S204X	CTCGAGTCACGTGGCCGCCTTG
67	pGEX5X MeCP2 R106W	AGAATTCATGGTAGCTGGGATGTTAGGG
68	pGEX5X MeCP2 R106W	CTCGAGTCAGCTAACTCTCTCGGTC
69	pGEX5X MeCP2 F155S	AGAATTCATGGTAGCTGGGATGTTAGGG
70	pGEX5X MeCP2 F155S	CTCGAGTCAGCTAACTCTCTCGGTC
71	pGEX5X MeCP2 R294X	AGAATTCATGGTAGCTGGGATGTTAGGG
72	pGEX5X MeCP2 R294X	CTCGAGTCATCGGATAGAAGACTCCTTC
73	pGEX5X MeCP2 F155S/R294X	AGAATTCATGGTAGCTGGGATGTTAGGG
74	pGEX5X MeCP2 F155S/R294X	CTCGAGTCATCGGATAGAAGACTCCTTC
75	pGEX5X MeCP2 H370X	AGAATTCATGGTAGCTGGGATGTTAGGG
76	pGEX5X MeCP2 H370X	CTCGAGTCAGTGATGGTGGTGGTGC
77	pGEX5X MeCP2 E397X	AGAATTCATGGTAGCTGGGATGTTAGGG
78	pGEX5X MeCP2 E397X	CTCGAGTCACTCGGAGCTCTCGG
79	pGEX5X MeCP2 R453X	AGAATTCATGGTAGCTGGGATGTTAGGG
80	pGEX5X MeCP2 R453X	CTCGAGTCATCGGTGTTTGTACTTTTCTG

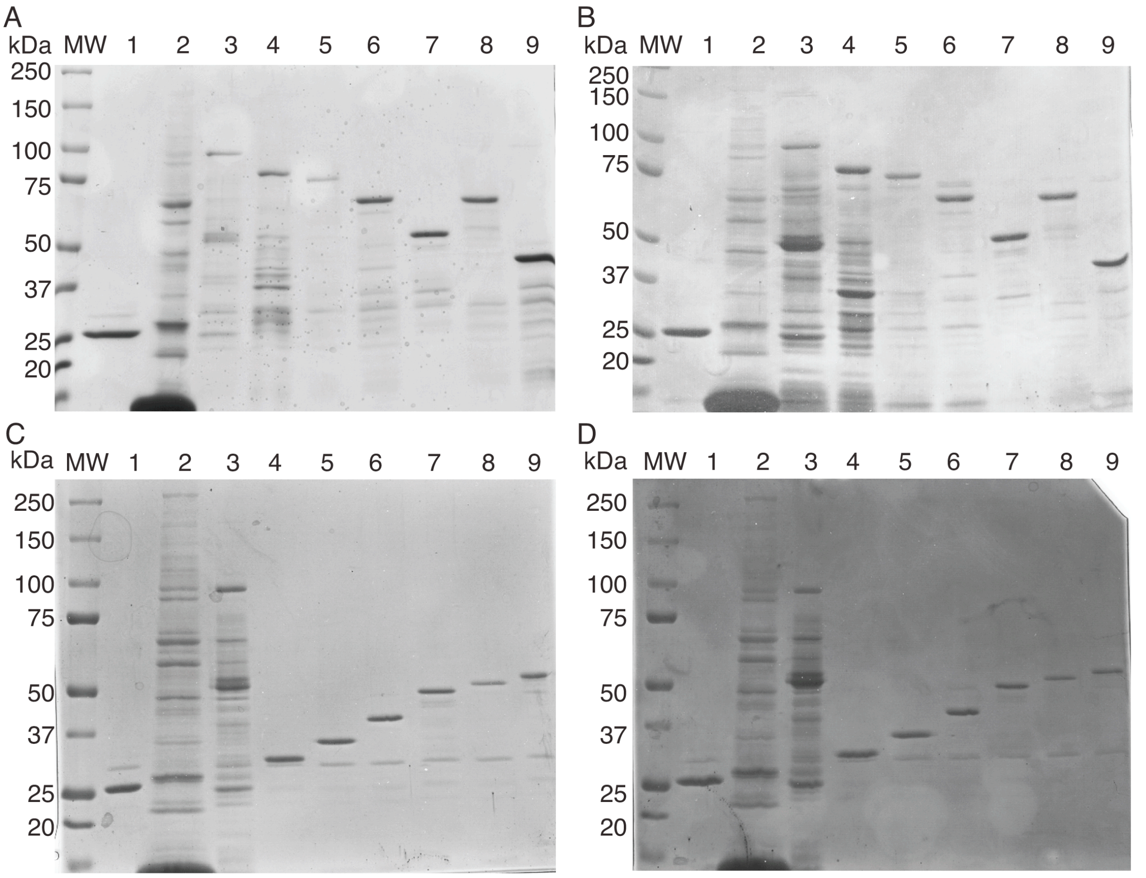

Figure S4. GST input control gels. Coomassie brilliant blue stained SDS-PAGE gels from GST pull-down experiments show equivalent levels of GST-fusion proteins in each pull-down reaction. A) GST-fusion proteins from Figure 4C, Prpf3. B) GST-fusion proteins from Figure 4C, Sdccag1. C) GST-fusion proteins from Figure 6A, Prpf3. D) GST-fusion proteins from Figure 6A, Sdccag1.

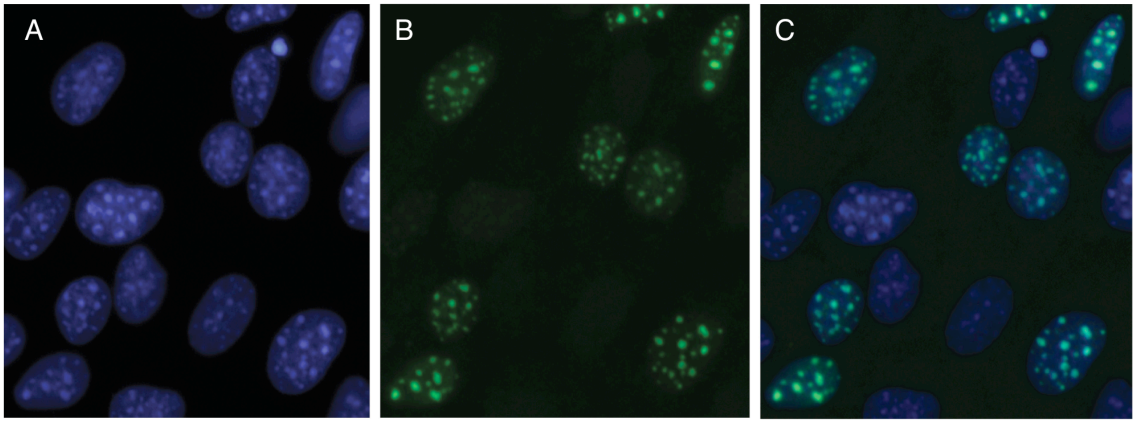

Figure S5. Characterization of HA-MeCP2 expressing HT22 cells. HT22 hippocampal neuronal cells stably transected with HA-MeCP2, visualized by A) DAPI staining and B) indirect immunofluorescence for HA-MeCP2 with C) images merged, show HA-MeCP2 localizes to heterochromatic foci.