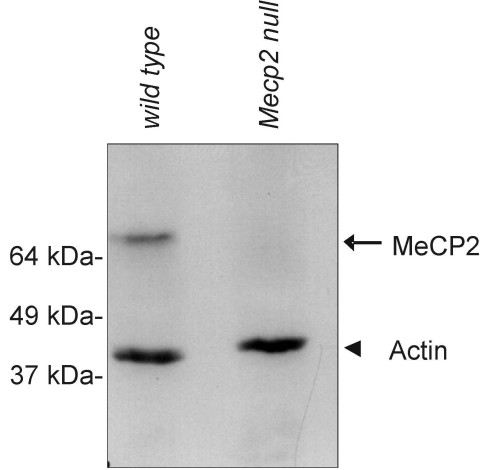
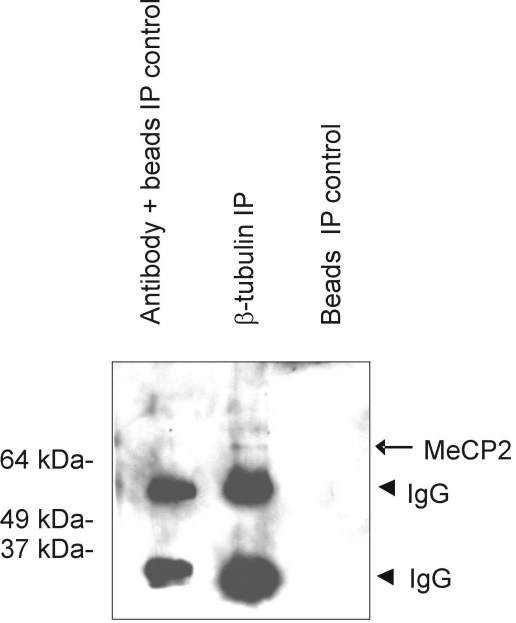
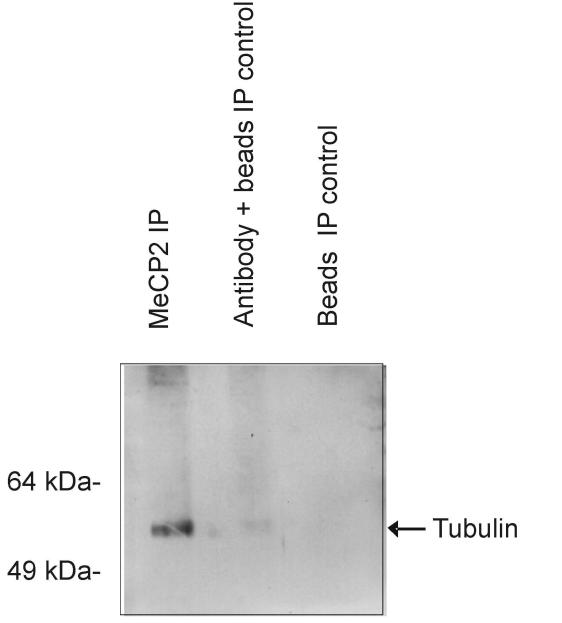


Probed with mouse monoclonal N-terminal antibody against MeCP2 (Sigma) and antibody against actin (Sigma)

Supplemental Fig 1: Specificity of N terminal Sigma antibody against MeCP2
Proteins extracted from wild type and Mecp2 null mouse cerebellum were probed with a mouse monoclonal antibody against MeCP2, as indicated. A single band corresponding to the size of MeCP2 is seen in extract from wild type mouse tissue, not seen in extract from the null mouse.

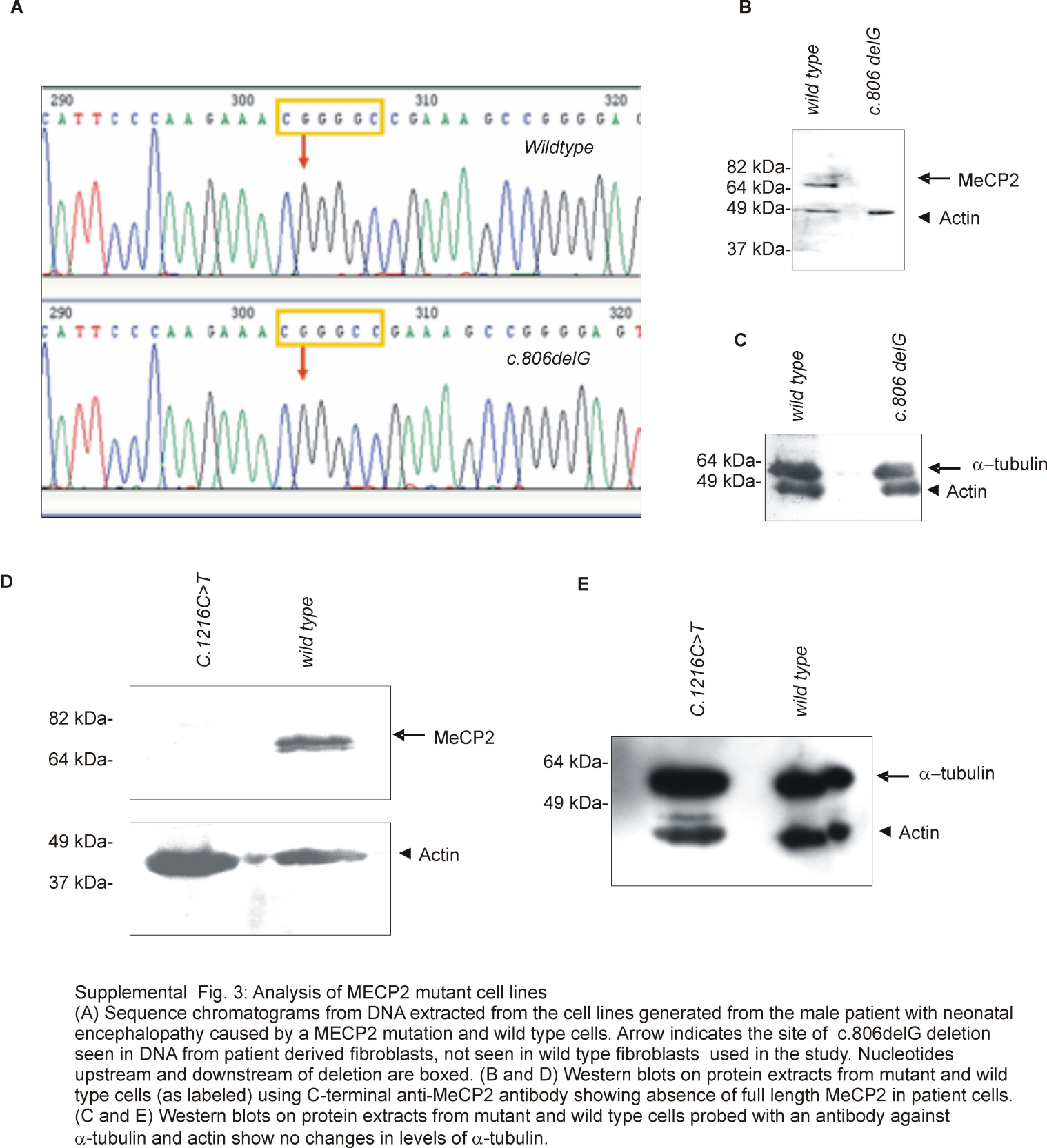


HeLa extract, IP (as indicated),
probed with monoclonal antibody
against MeCP2 (Sigma)



HeLa extract, IP (as indicated),
probed with a sheep polyclonal
antibody against pan tubulin (Cytoskeleton)

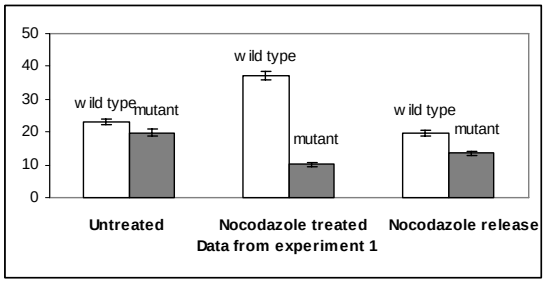
Supplemental Fig 2: Controls for HeLa immunoprecipitation
Total protein extracts from HeLa cells were immunoprecipitated as indicated and probed with antibody against MeCP2 (left panel) or tubulin (right panel). MeCP2 is detected in tubulin immunoprecipitates not seen in controls. Tubulin is seen in MeCP2 immunoprecipitates, not seen in controls.



Supplemental Fig. 3: Analysis of MECP2 mutant cell lines
(A) Sequence chromatograms from DNA extracted from the cell lines generated from the male patient with neonatal encephalopathy caused by a MECP2 mutation and wild type cells. Arrow indicates the site of c.806delG deletion seen in DNA from patient derived fibroblasts, not seen in wild type fibroblasts used in the study. Nucleotides upstream and downstream of deletion are boxed. (B and D) Western blots on protein extracts from mutant and wild type cells (as labeled) using C-terminal anti-MeCP2 antibody showing absence of full length MeCP2 in patient cells. (C and E) Western blots on protein extracts from mutant and wild type cells probed with an antibody against α-tubulin and actin show no changes in levels of α-tubulin.

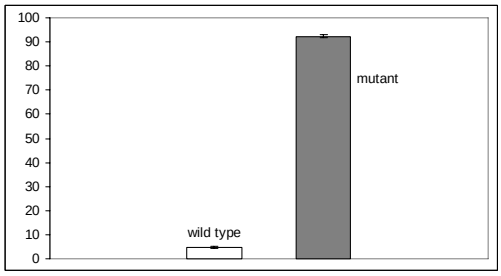
Supplemental table 4*A*: Quantitative analysis of microtubule length showing percentage of pixels located within long microtubules and graphical representation of data from one experiment.
Wild type and mutant fibroblasts were treated in parallel in each experiment. Microtubules were categorized as long if they comprised more than 50 contiguous pixels. The percentage of all pixels located within long microtubules was significantly lower in mutant compared with wild type fibroblasts at each time point, with the difference being accentuated by nocodazole and diminished by release from nocodazole. Graphical representation of data from one experiment is shown on the right.

Experiment	**Percentage of all pixels located within long microtubules**	
	Wild type	**Mutant**
Untreated cells		
Exp1	23.05 (95% CI 22.3 - 23.8)	19.8 (95% CI 18.8 - 20.9)
5 minutes after Nocodazole treatment		
Exp1	37.2 (95% CI 35.9 - 38.4)	10.06 (95% CI 9.4 - 10.8)
Exp2	39.4 (95% CI 38.3 - 40.6)	6.31 (95% CI 6.0 - 6.6)
9 minutes after Nocodazole release		
Exp 1	19.8 (95% CI 18.9 - 20.7)	13.5 (95% CI 12.9 - 14.2)
Exp2	34.7 (95% CI 33.3 - 36.2)	23.7 (95% CI 22.9 - 24.5)



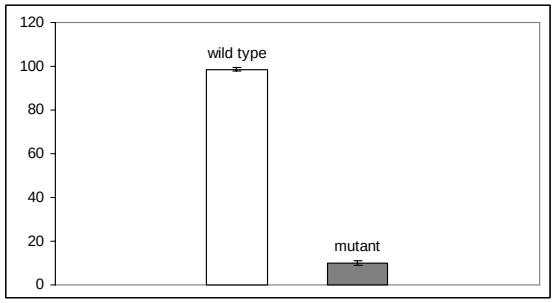
Supplemental table 4*B*: Percentage of cells showing advanced depolymerisation
Cells were visually analyzed after 5 minutes of nocodazole exposure and counted as showing advanced depolymerisation if more than 50% of the tubulin staining was composed of depolymerized tubulin. Graphical representation of the data (n=3, total 300 cells). Error bars show S.E. P value <0.0001

Sample	**Wild type**	**Mutant**
Exp-1	5	*93*
Exp-2	4	*91*
Exp-3	5	*93*
Average	4.67	*92.33*
Std error	*0.33*	*0.67*



Supplemental table 4*C*: Percentage of cells showing complete microtubule reassembly
Cells were treated with nocodazole for 1 hour, stained with β– tubulin 9 minutes after nocodazole release and visually analyzed. Percentage of cells showing complete reassembly and graphical representation of the data (n=3, total 300 cells). Error bars show S.E. P value <0.0001

Sample	**Wild type**	**Mutant**
Exp-1	99	10
Exp-2	100	12
Exp-3	97	8
Average	98.67	10
Std Error	0.88	1.15



Supplemental Table 5*A*: Levels of acetylated tubulin following siRNA transfection

Cells were transfected with siRNA against MeCP2, scrambled siRNA (Neg) or siRNA against GAPDH and extracts were immunoblotted for acetylated tubulin and actin. Data was quantified using Image J software and normalised to actin (shown as raw data). Average level of acetylated tubulin in controls was calculated from datasets of cells transfected with scrambled siRNA (Neg) and siRNA against GAPDH. Decrease in acetylated tubulin levels was expressed as a percentage of the average level in controls. Data from three independent experiments is shown.

	Acetylated tubulin normalized to actin			Percentage of average				
siRNA	**Exp-1**	**Exp-2**	**Exp-3**	**Exp-1**	**Exp-2**	**Exp-3**	**Average**	**Std error**
37	12.24	49.69	43.78	40.91	61.69	69.59	57.39	6.98
36	16.78	28.2	30.55	56.09	35.01	48.56	46.55	5.03
Neg	26.25	86.49	61.86	87.74	107.38	98.33	97.82	4.63
GAPDH	33.58	74.6	63.96	112.25	92.62	101.67	102.18	4.63
Average (Neg + GAPDH)	29.91	80.545	62.91					

Supplemental Table 5*B*: Levels of acetylated tubulin following MeCP2-FLAG transfection

Cells were transfected with MeCP2-FLAG or FLAG-alone or left untreated and extracts were immunoblotted for acetylated tubulin and actin. Data was quantified using Image J software and normalised to actin (shown as raw data). Average level of acetylated tubulin in controls was calculated from datasets of untransfected cells and FLAG-alone transfected cells. Increase in acetylated tubulin levels was expressed as a percentage of the average level in controls. Data from four independent experiments is shown.

	Acetylated tubulin normalized to actin				Percentage of average					
Transfection	**Exp-0**	**Exp-1**	**Exp-4**	**Exp-6**	**Exp-0**	**Exp-1**	**Exp-4**	**Exp-6**	**Average**	**Std error**
MeCP2- FLAG	170.06	573.79	129.34	74.8	118.81	157.98	119.43	189.99	146.55	17.13
FLAG	143.14	363.2	108.3	39.37	100	100	100	100	100	0

Supplemental Fig. 6: Levels of α–tubulin remain unchanged after siRNA knockdown of MeCP2 and over expression of MeCP2 : Protein extracts from siRNA experiments (A) and transfection experiments (B) are shown. Blots were probed with antibodies against α–tubulin and actin.

Supplemental Table 7*:* Percentage of deacetylation in *in vitro experiments*

Percentage of tubulin deacetylation was calculated from the area under the chromatograms in each experiment (n=4). Paired t test P value <0.0225.

Sample	Exp-1	Exp-2	Exp-3	Exp-4	Average	Std error
HDAC6	12	28	14	12	16.5	3.86221
HDAC6+MeCP2	6	7	1.5	0	3.625	1.700184
HDAC6+Control	12	11	8	8	9.75	1.030776