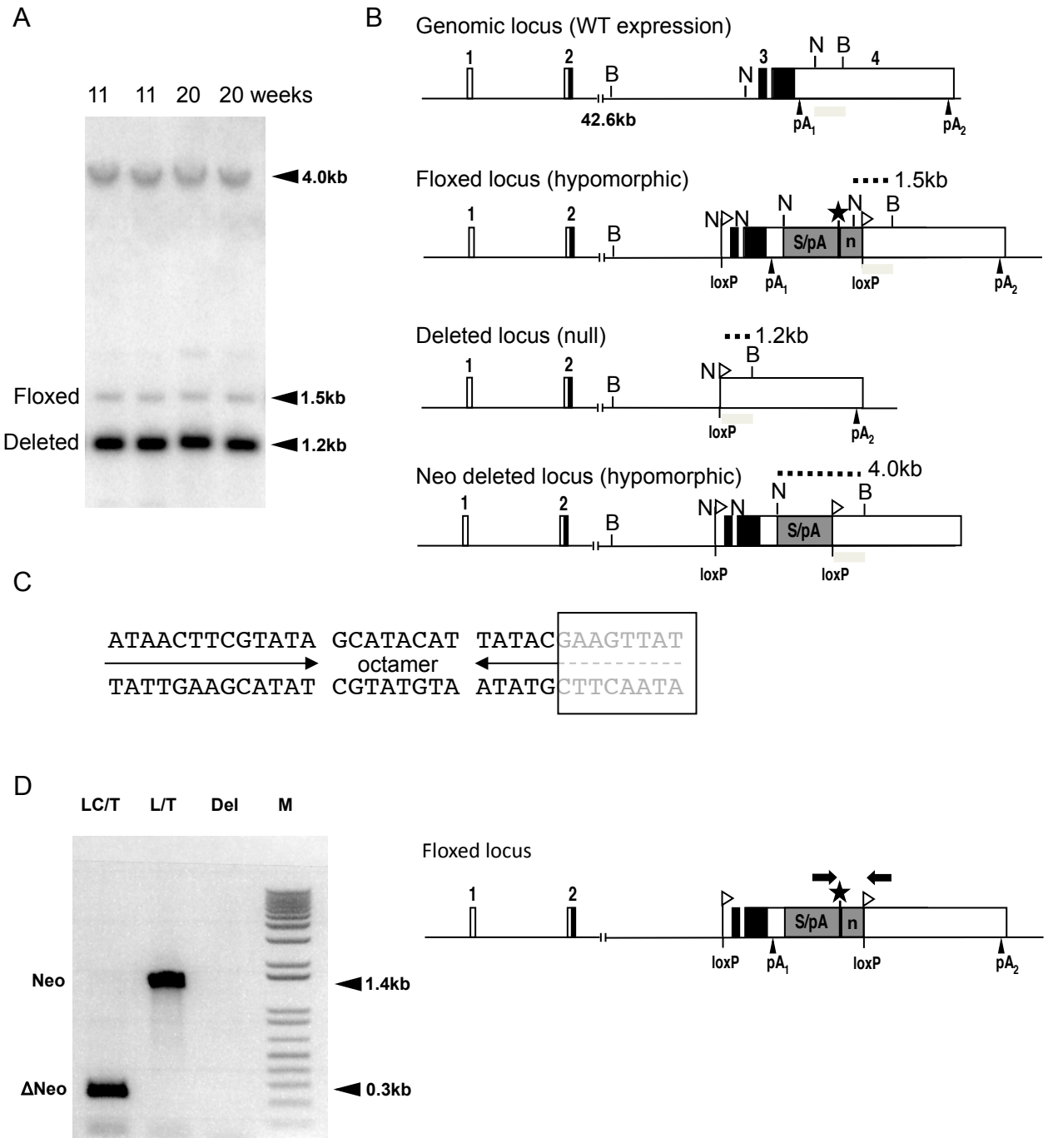
SUPPLEMENTARY MATERIAL



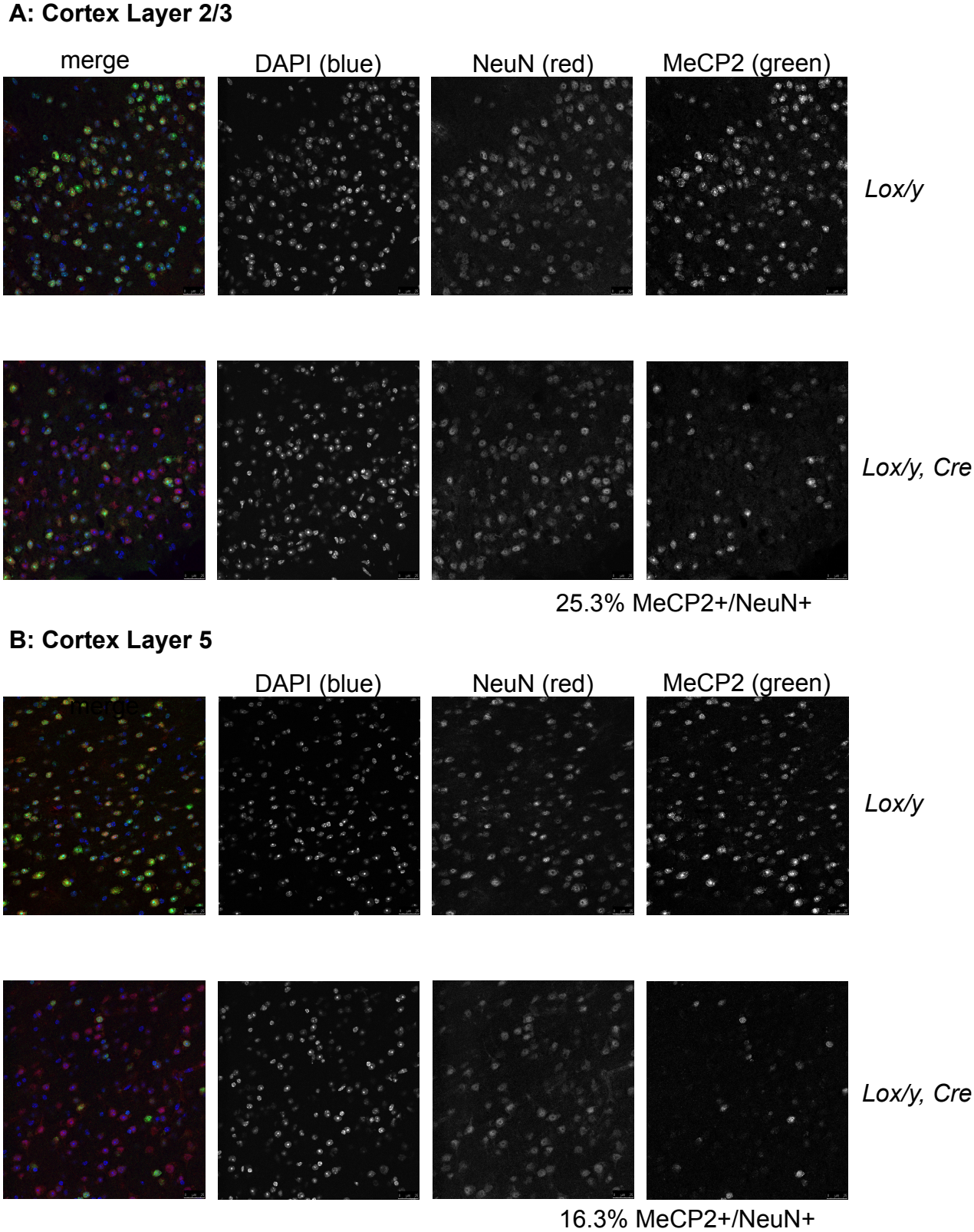
Supplementary material, Fig. S1: Treatment of *lox/y, Cre* animals with tamoxifen causes recombination between both complete and a partial loxP sequence.

A- Southern blot analysis of brain genomic DNA digested with BamHI and NcoI revealed bands of expected sizes for floxed (1.5kb) and deleted alleles (1.2kb) and an additional band at 4.0kb. All samples were taken from *lox/y, Cre* animals treated with tamoxifen at 11 or 20 weeks.

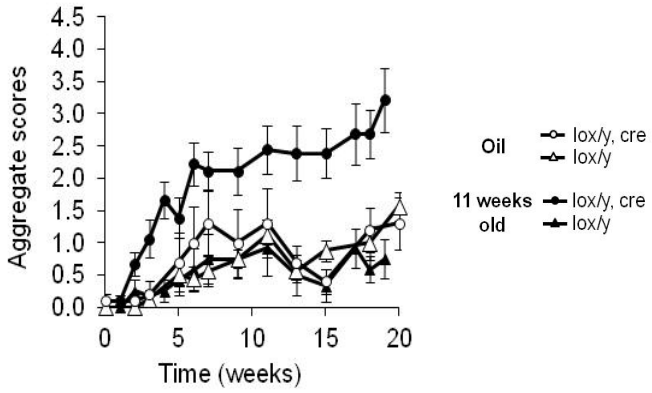
B- Maps of *Mecp2* loci, as in Fig. 1B, with the addition of a 'Neo deleted' allele suspected of causing the 4.0kb Southern blot band. BamHI (B) and NcoI (N) restriction sites are shown. The 1.1kb NcoI-BamHI Southern probe fragment is shown at each locus as a grey bar, and the restriction fragments hybridised by the probe are indicated as dashed lines. The partial loxP site at the 5' end of the Neo resistance gene (n) in the floxed locus is indicated by a star.

C- Sequence of partial loxP site. Nucleotides in black were present, nucleotides shown in grey form part of a complete loxP site, but were absent.

D- PCR analysis of Neo-deleted allele. Forward and reverse primers are shown as black arrows above the floxed locus. The primers amplified a 1.4kb product spanning the *Neo* gene, when the template DNA came from a *lox/y* animal treated with tamoxifen (L/T). A *lox/y, Cre* animal treated with tamoxifen (LC/T) gave a smaller product of approximately 0.3kb. This was found to consist of sequences flanking the *Neo* gene and a complete loxP site, as expected from recombination between a partial and a complete site (data not shown). It is not known why this loxP site fails to recombine with the site upstream of exon 3, although this may be due to the transient presence of CreESR protein in the nucleus. DNA from a *Mecp2*-null brain (Del) gave no product due to the absence of the forward primer sequence.



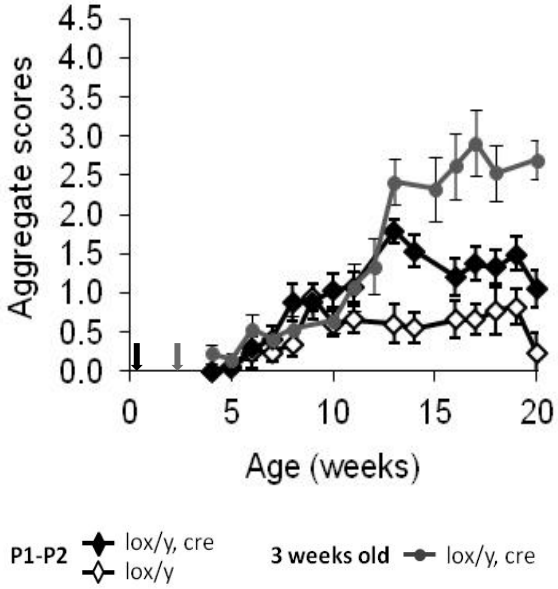
Supplementary material, Fig. 2: Susceptibility of cortical neurons to cre-mediated deletion appears to be typical of the brain as a whole. Sections of layers 2/3 (A) and layer 5 (B) from a single control or tamoxifen-treated mouse (3 week group) were stained with DAPI and antibodies against MeCP2 (Millipore, 07-013) and the neuronal nuclear marker NeuN. Low resolution (2µm) images taken with a Leica SP5 confocal microscope were combined as a z-stack and counts of >400 nuclei were from a maximum projection images of randomly selected fields. Panels show single optical sections. Percent MeCP2- and NeuN-positive neurons remaining is shown below the Cre-positive panels. All NeuN-positive nuclei were also MeCP2-positive in controls.



Supplementary material, Fig. S3: Phenotypic analysis of *lox/y, Cre* and *lox/y* mice injected with tamoxifen or oil.
Lox/y, Cre and *lox/y* mice injected with oil (respectively n=5, open circles and n=8, open triangles) displayed significantly less severe symptoms than *lox/y, Cre* mice injected with tamoxifen (n=9, filled circles) but similar symptoms than *lox/y* mice injected with tamoxifen (n=6, filled circles), as shown by repeated measures ANOVA ($F(3,24)=8.896$, $p=0.0004$, Fisher's PLSD *post hoc* analysis, p values in supplementary table 1).

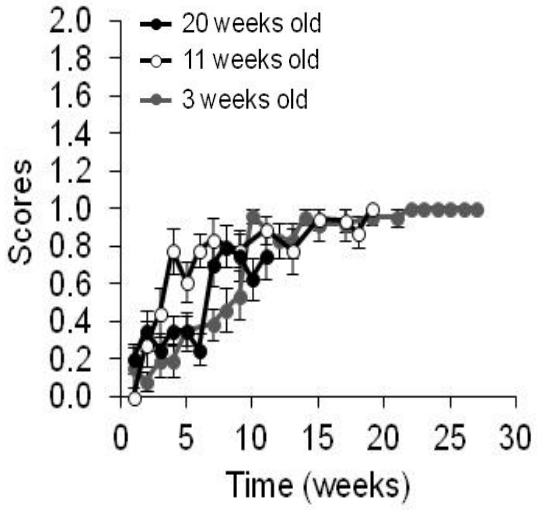
	Fisher's PLSD post hoc analysis, p values for all pairwise combinations					
Time post-treatment (weeks)	***lox/y* oil *vs* *lox/y, Cre* oil**	***lox/y* oil *vs* *lox/y* 11w**	***lox/y, Cre* oil *vs* *lox/y* 11w**	***lox/y, Cre* 11w *vs* *lox/y, Cre* oil**	***lox/y, Cre* 11w *vs* *lox/y* oil**	***lox/y, Cre* 11w *vs* *lox/y* 11w**
2	0.6536	0.2421	0.5271	**0.0146**	**0.0016**	**0.0516**
3	0.8187	0.8930	0.9235	**0.0124**	**0.0025**	**0.0066**
5	0.6546	0.8438	0.5514	0.1239	**0.0266**	**0.0255**
6	0.2903	0.7698	0.4581	**0.0244**	**0.0005**	**0.0023**
7	0.1671	0.7055	0.3271	0.1223	**0.0018**	**0.0090**
9	0.6244	.	0.6448	**0.0337**	**0.0042**	**0.0075**
11	0.7571	0.6977	0.5249	**0.0473**	**0.0107**	**0.0069**
13	0.7881	0.8973	0.7131	**0.0023**	**0.0003**	**0.0005**
15	0.2992	0.2137	0.8897	**0.0001**	**0.0006**	**< 0.0001**

Supplementary material, table 1: Fisher's PLSD *post hoc* analysis related to Fig.S2.
P values < 0.05 are shown in a bold font.

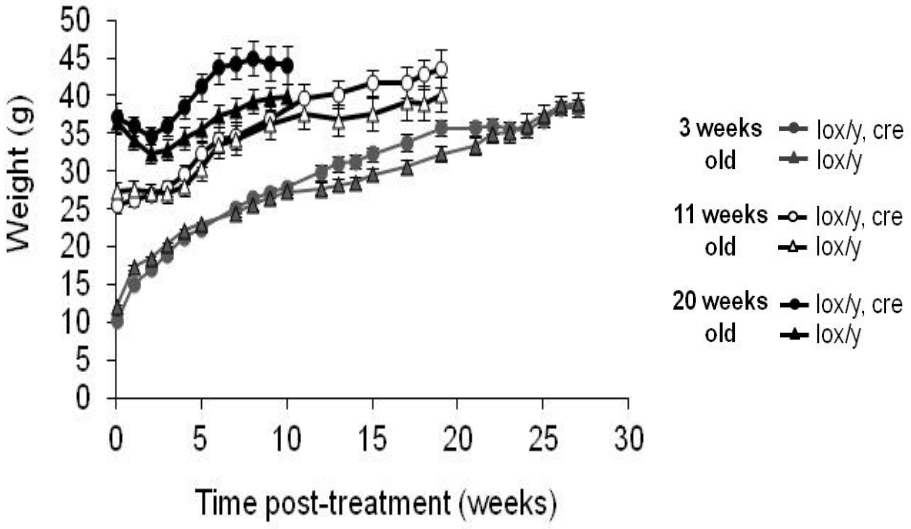


Supplementary material, Fig. S4: Phenotypic analysis of *lox/y, Cre* and *lox/y* mice treated with tamoxifen at P1-P2.

Lox/y, Cre mice treated with tamoxifen at P1-P2 (n=13, black diamonds) developed more severe symptoms compared to their control littermates *lox/y* mice (n=10, open diamonds). Although the symptoms they displayed were milder than those observed in *lox/y, Cre* mice treated with tamoxifen at 3 weeks old (n=12, grey circles), they reached a plateau at the same age (13 weeks old). Arrows indicate the average age of treatment (black, P1-P2; grey, 3 weeks old).



Supplementary material, Fig. S5: Analysis of gait across time after *Mecp2* inactivation.
Lox/y, Cre mice injected with tamoxifen at 3 weeks old (n=11, grey circles), at 11 weeks old (n=9, white circles) and at 20 weeks old (n=8, black circles) exhibited an altered gait, reaching a similar score 9 to 10 weeks after treatment.



Supplementary material, Fig. S6: Weight measures of *lox/y, Cre* and *lox/y* mice after tamoxifen treatment.

In each group, mice from both genotypes gained weight after tamoxifen treatment (repeated measures ANOVA, time effect: 3 weeks old, $F(22,396)=349.457$, $p<0.0001$; 11 weeks old, $F(14,154)=102.833$, $p<0.0001$, 20 weeks old, $F(10,150)=70.097$, $p<0.0001$). *Lox/y, Cre* mice did however show an increased weight gain compared to their control littermates *lox/y* mice. *Lox/y, Cre* mice were indeed slightly underweight than their controls at the beginning of the treatment, but grew faster since their weight was either similar or more important by 30 weeks of age (Interaction time post-treatment–genotype, 3 weeks old, $F(22,396)=3.927$, $p<0.0001$; 11 weeks old, $F(14,154)=2.916$, $p=0.0006$, 20 weeks old, $F(10,150)=5.424$, $p<0.0001$).