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Supplementary Information for

Cas9-expressing chickens and pigs as resources for genome editing in livestock.

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This PDF file includes:

Figures S1 to S9

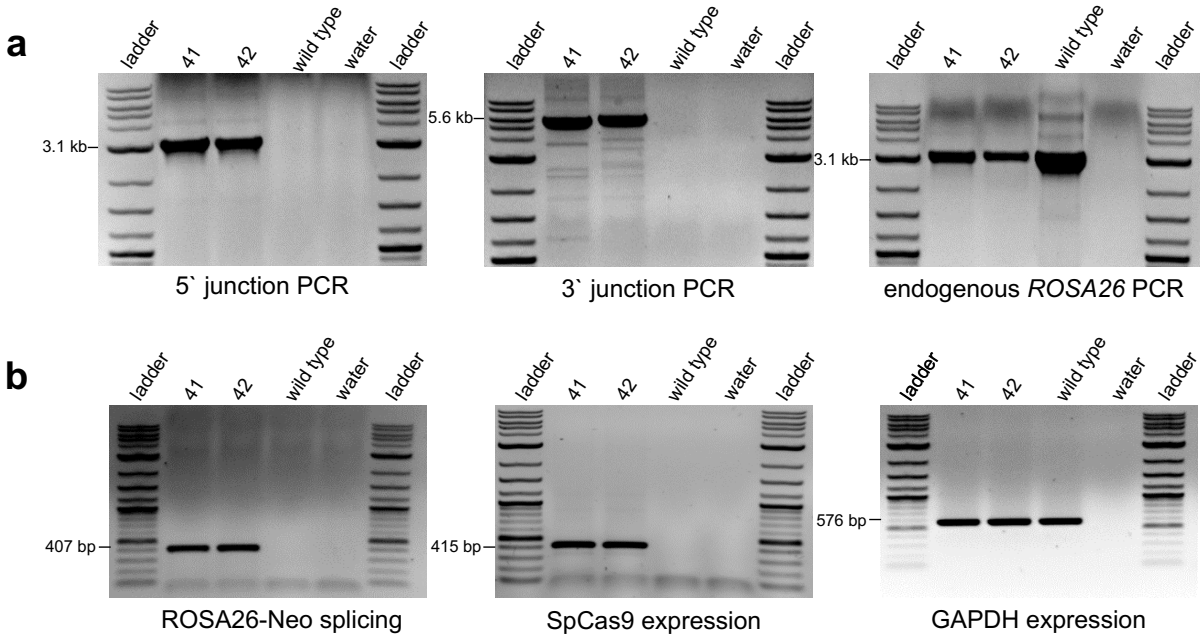


Fig. S1. Targeting PCR and SpCas9 expression analysis of piglets #41 and #42. a) Targeting PCRs performed to reveal correct targeting of 3.1 kb 5´junction, 5.6 kb 3´junction and monoallelic targeting by 3.1 kb endogenous PCR product. Wild type DNA was used as control. b) RT-PCR analysis of PEFs derived from SpCas9-expressing piglets #41 and #42. Correct splicing between *ROSA26* exon one and neomycin, SpCas9 and GAPDH expression reveals a 407 bp, 415 bp and 576 bp amplicon respectively. Wild type DNA was used as control.

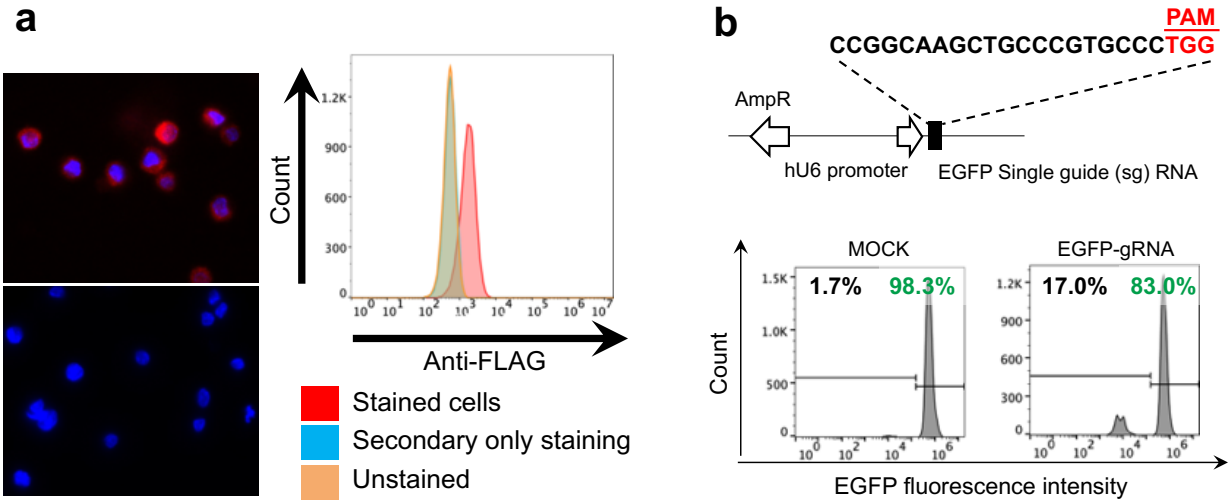


Fig. S2. Stable expression of SpCas9 und functionality in chicken PGCs. a) PGCs stained with anti-FLAG and analyzed by immunofluorescence and flow cytometry. b) Flow cytometry analyses of PGCs electroporated with the pBlueScript II SK (+) vector containing a gRNA against EGFP.

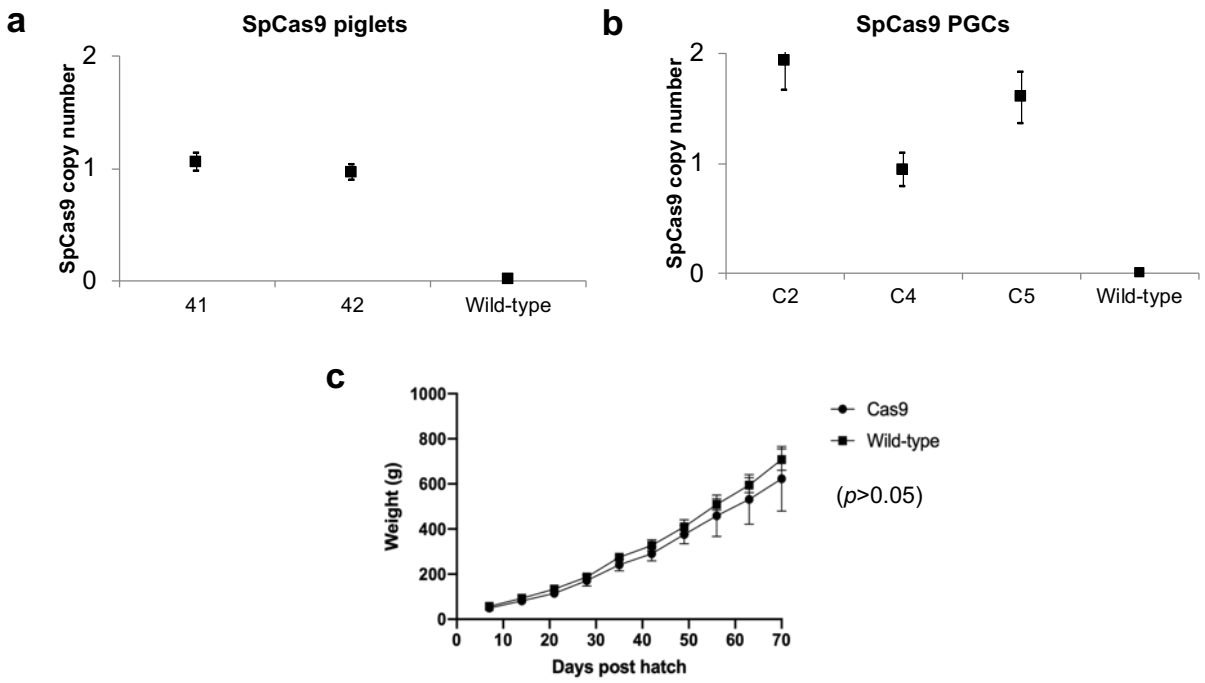


Fig. S3. SpCas9 copy number and development of SpCas9-expressing chickens and pigs a) Determination of SpCas9 copy number by droplet digital PCR in the SpCas9 transgenic founder pigs #41 and #42. Wild-type DNA was used as a negative control. c) Determination of SpCas9 copy numbers by droplet digital PCR in chicken PGC clones C2, C4, and C5. Wild-type DNA was used as a negative control. a) Weight comparison between SpCas9-expressing and wild-type chickens. Body weight was measured weekly for 10 weeks. SpCas9 transgenic chickens were healthy and showed no significant difference in weight gain compared to wild-type birds. Statistics were performed using SPSS24 statistics (version 24.0.0.0) software (IBM, USA).

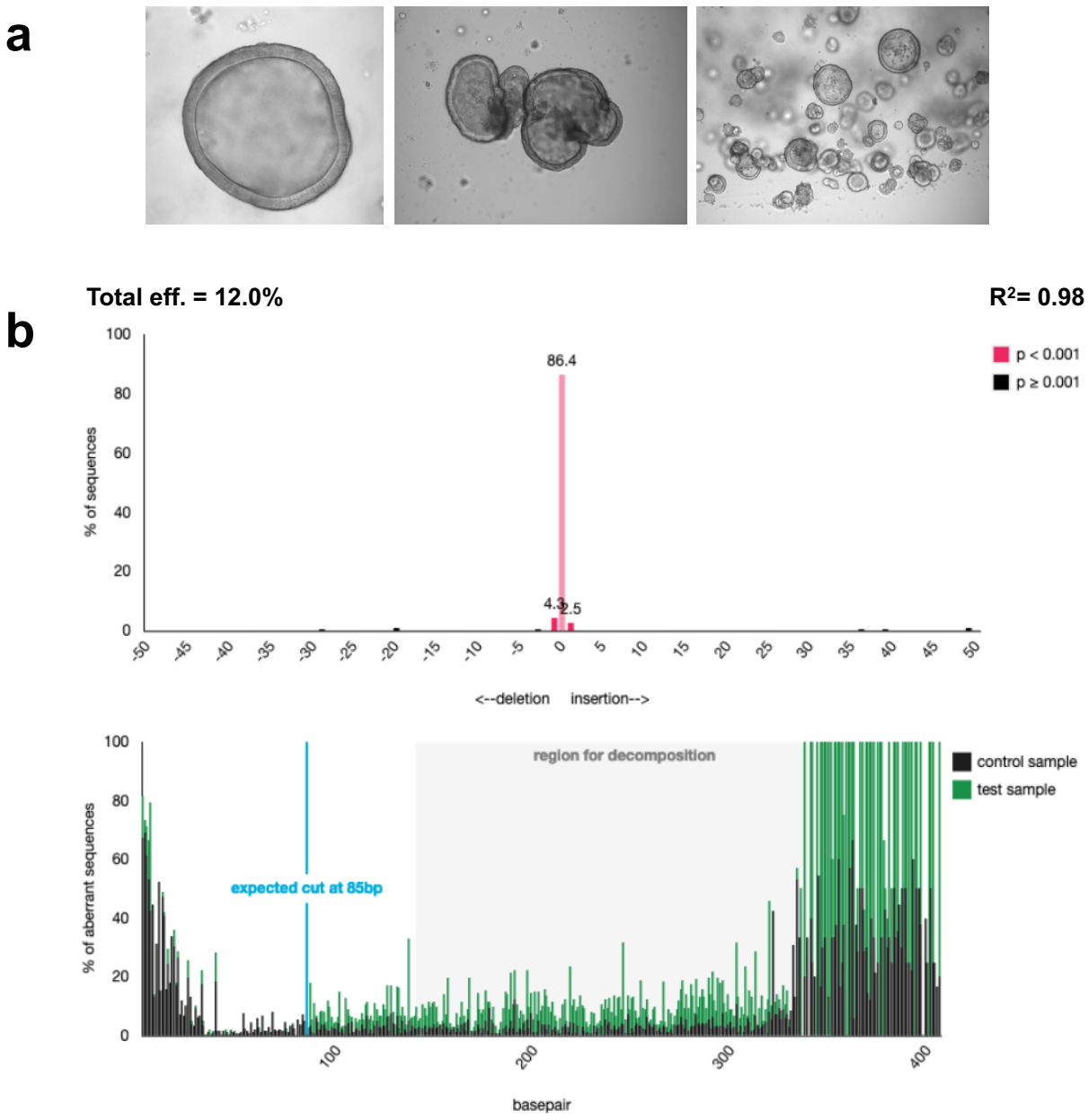
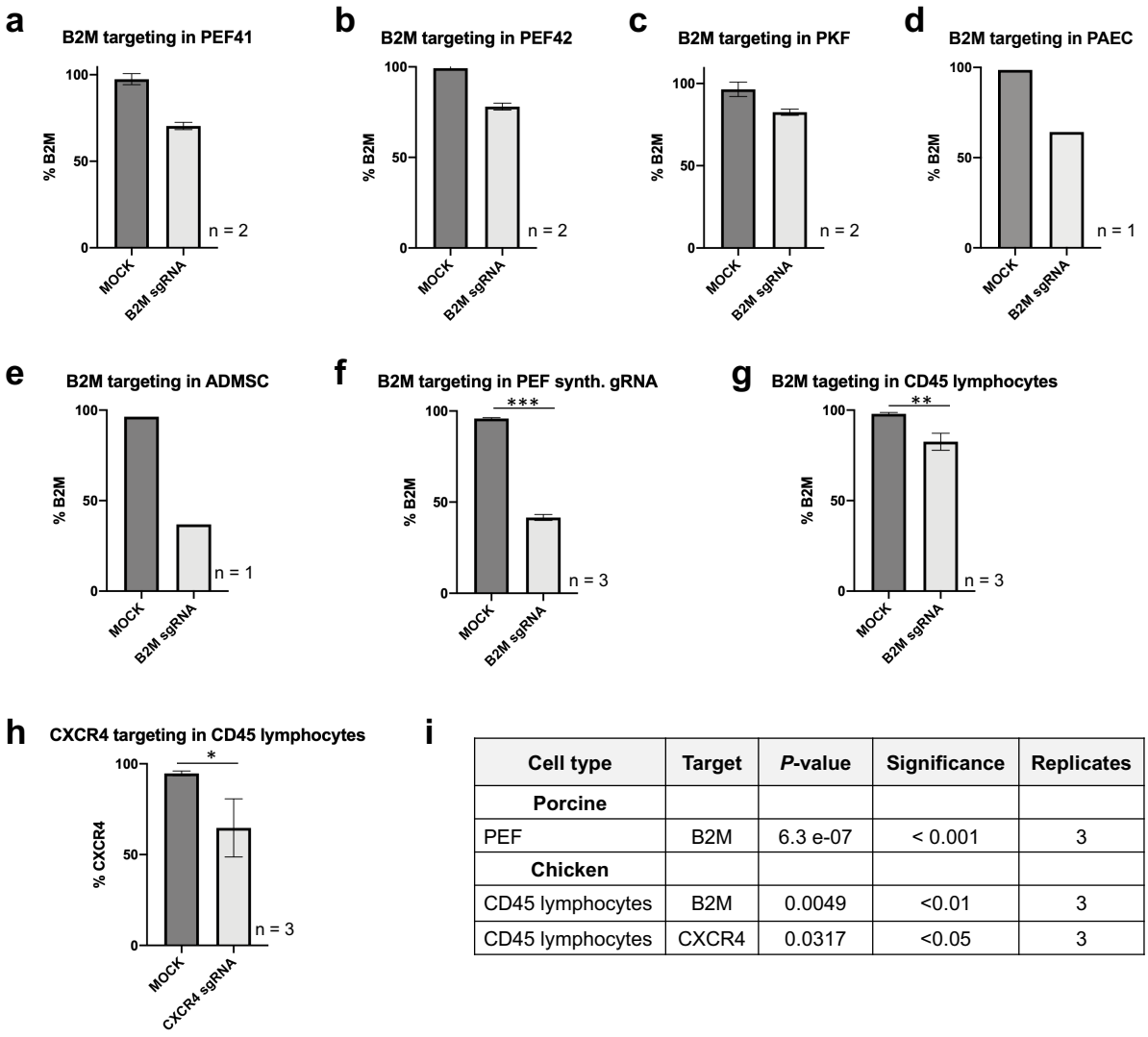


Fig. S4. *In vitro* genome editing in porcine colonic organoids a) Pictures of porcine colonic organoids derived from a SpCas9 transgenic pig and transfected with a construct carrying a gRNA against *GGTA1*. B) Genome editing efficiencies in porcine colonic organoids analyzed by TIDE.



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Cell type	Target	*P*-value	Significance	Replicates
Porcine				
PEF	B2M	6.3 e-07	< 0.001	3
Chicken				
CD45 lymphocytes	B2M	0.0049	<0.01	3
CD45 lymphocytes	CXCR4	0.0317	<0.05	3

Fig. S5. Statistical overview of experiments performed in chicken and porcine cells. a) PEF cells isolated from piglet 41 were transfected with a construct containing a gRNA against B2M resulting in a reduction in B2M expression compared to MOCK control (gRNA against GGTA1) (n = 2). b) PEF cells isolated from piglet 42 were transfected with a construct containing a gRNA against B2M resulting in a reduction in B2M expression compared to MOCK control (gRNA against GGTA1) (n = 2). c) PKF cells were transfected with a construct containing a gRNA against B2M resulting in a reduction in B2M expression compared to MOCK control (gRNA against GGTA1) (n = 2). d) PAEC cells

were transfected with a construct containing a gRNA against B2M resulting in a reduction in B2M expression compared to MOCK control (gRNA against GGTA1) (n = 1). e) ADMSC cells were transfected with a construct containing a gRNA against B2M resulting in a reduction in B2M expression compared to MOCK control (gRNA against GGTA1) (n = 1). f) PEF cells were treated with a synthetic gRNA against B2M. A significant reduction in B2M expression was observed in B2M-gRNA treated but not in MOCK treated control (gRNA against P16) (n = 3). g) CD45+ lymphocytes treated with a chemically modified synthetic gRNA against B2M showed a significant reduction in B2M expression compared to MOCK control (gRNA against CXCR4) (n = 3). h) CD45+ lymphocytes treated with a chemically modified synthetic gRNA against CXCR4 showed a significant reduction in CXCR4 expression compared to MOCK control (gRNA against B2M) (n = 3). i) Table with statistical analysis showing the number of replicates, P-values, and significances. Error bars denote the standard error of mean in all figures. P-values were determined using Student´s t-test. $P<0.05$ was considered as significant.

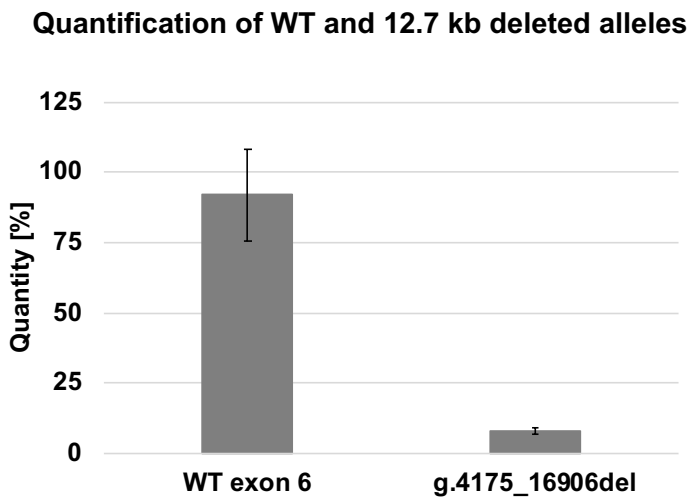

Fig. S6. Quantification of wildtype (WT exon 6) and 12.7 kb deleted (g.4175_16906 del) *MYBPC3* alleles. Samples were measured in triplicates using qPCR.

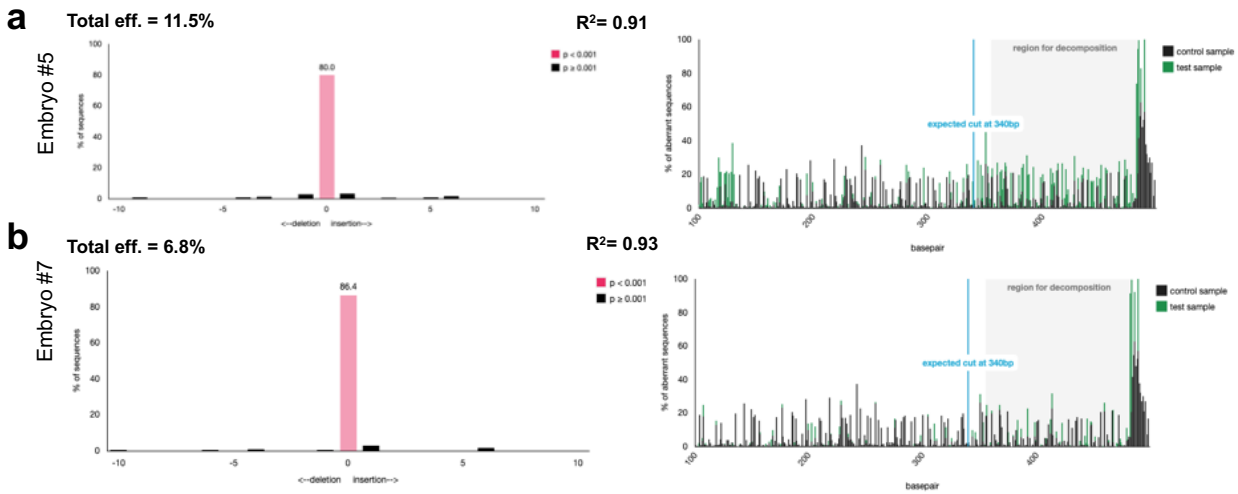

Fig. S7. TIDE analyses of *in ovo*-electroporated embryo midbrains. a, b) Analyses of the midbrain of two Cas9 positive embryos (#5, #7,) electroporated with pBlueScript II SK (+) vector containing a gRNA against EGFP. A fragment across the EGFP target site was amplified by PCR and INDEL analysis was performed.

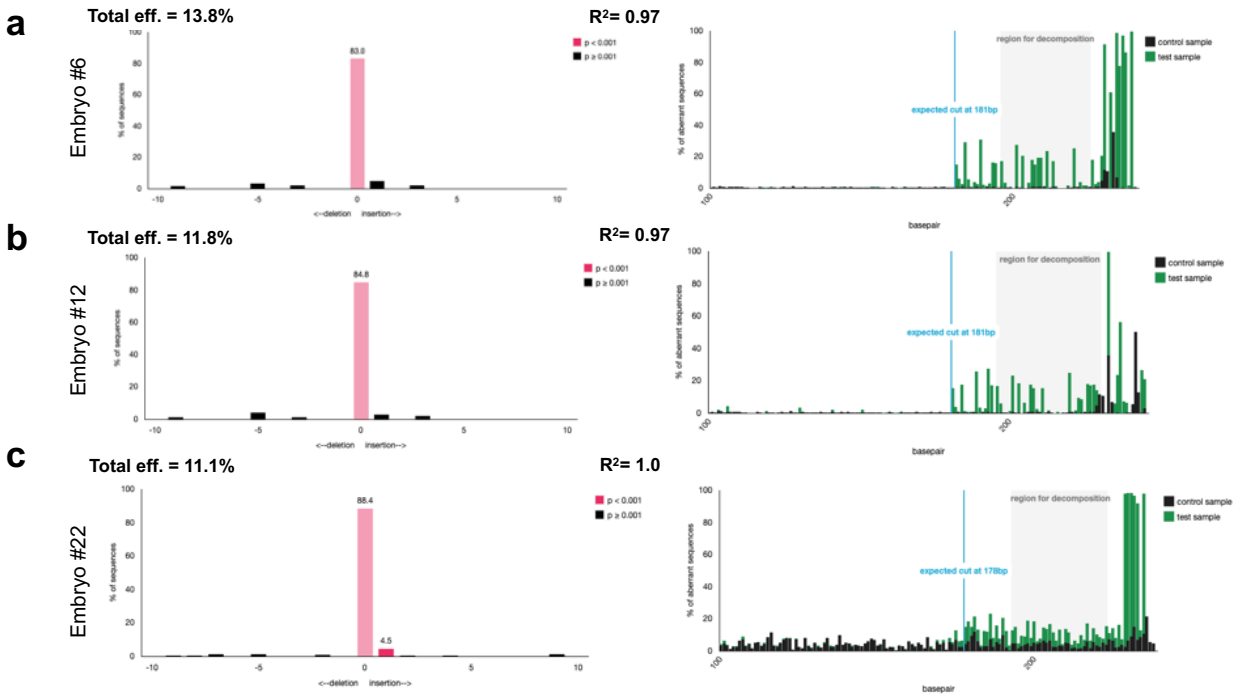

Fig. S8. TIDE analyses of *in ovo*-electroporated embryo midbrains. a, b, c) Analyses the midbrains of three Cas9 positive embryos (#6, #12, #22) electroporated with pBlueScript II SK (+) vector containing a gRNA against *B2M*. A fragment across the B2M target site was amplified by PCR and INDEL analysis was performed.

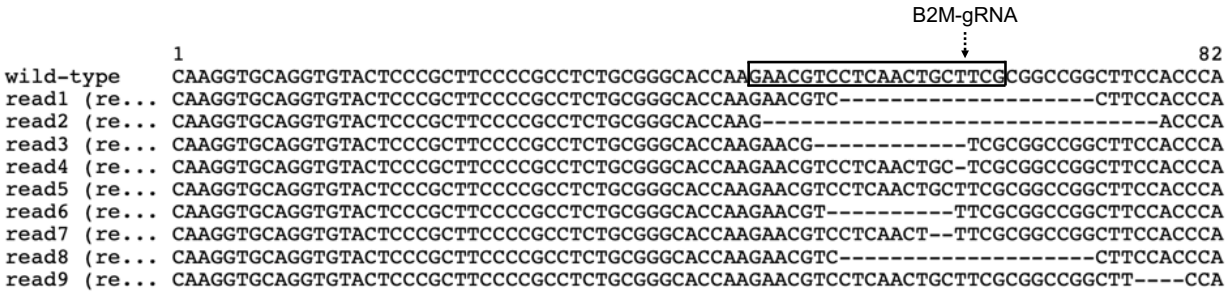

Fig. S9. Analysis of expected cutting sites for *in vivo* B2M targeting in embryonic chicken B cells. Nine example sequences (read1-read9) were aligned to a wild-type sequence (top line) confirming INDEL formation at the expected cutting site of the B2M target region (B2M-gRNA sequence (box); expected cutting site (dashed arrow).