CHEMBIOCHEM

Supporting Information

Sialylation Is Dispensable for Early Murine Embryonic Development in Vitro

Markus Abeln,[a] Kristina M. Borst+,[a] Samanta Cajic+,[b] Hauke Thiesler,[a] Elina Kats,[a] Iris Albers,[a] Maike Kuhn,[c] Volkhard Kaever,[d] Erdmann Rapp,[b, e] Anja Münster-Kühnel,[a] and Birgit Weinhold*[a]

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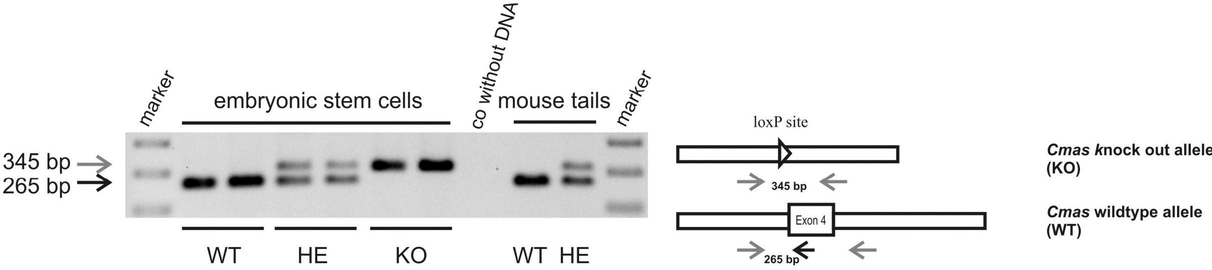
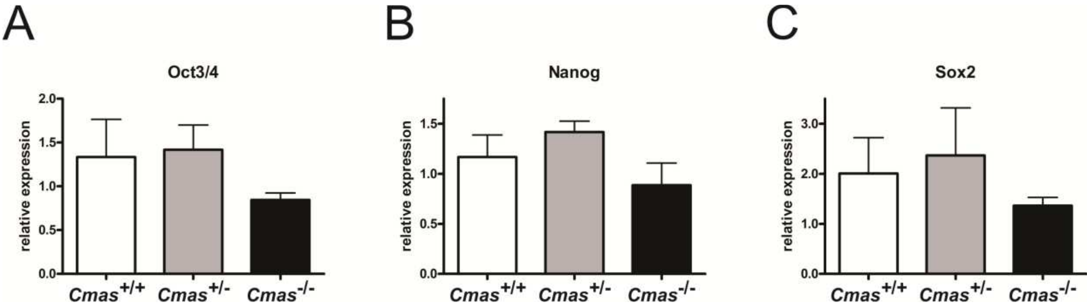
cbic_201700083_sm_M1-wt.mp4
cbic_201700083_sm_M2-ko.mp4

Supplementary Figure S1 Genotyping of embryonic stem cells. Each PCR reaction was performed with three primers: one forward primer located in intron 3 and two backward primers located in exon 4 and intron 4, respectively. A 265 base pair (bp) fragment was generated from wild type (WT) allele, a 345 bp fragment from *Cmas*$^{-/-}$ knock-out (KO) allele and both fragments occurred in heterozygous (HE) mESC. Mouse tail DNA was included as control for the expected fragment length. Water (co without DNA) served as negative control. 100 bp DNA marker at left and right.

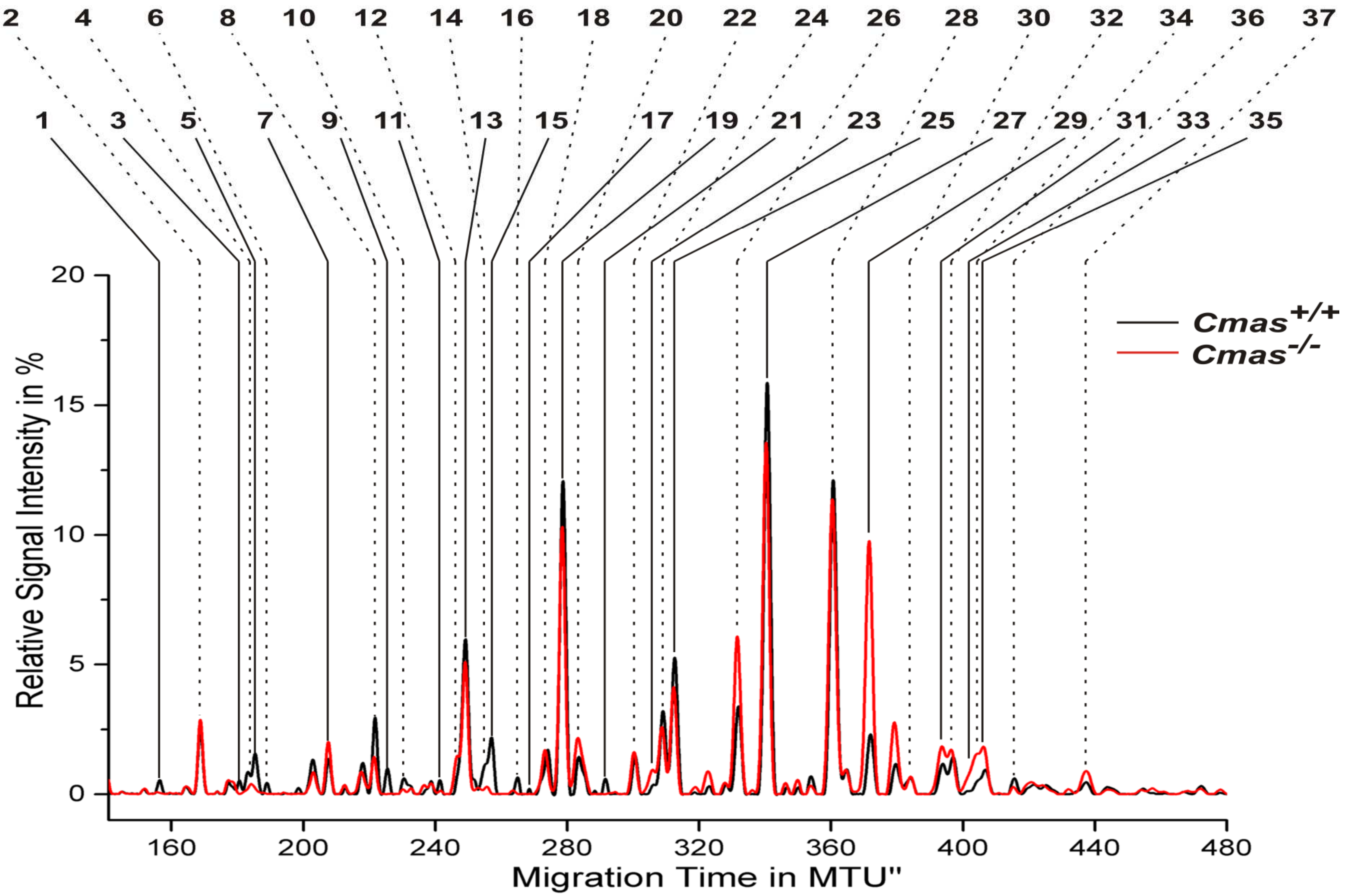


Supplementary Figure S2 Analysis of mRNA expression level of pluripotency transription factors in the isolated mESC lines. Quantitative PCR of *Cmas* mRNA expression from feeder-free cultures of *Cmas*$^{+/+}$, *Cmas*$^{+/-}$ and *Cmas*$^{-/-}$ mESC (n=3 of one representative cell line with the respective genotype, no significant changes, one-way ANOVA with Bonferroni post-test).



Supplementary Figure S3 Differential xCGE-LIF analysis of N-glycans from pluripotent mESC lines. Y-axis of normalized electropherogram is divided by the summed peak height of all quantifiable peaks (signal to noise ratio $S/N \geq 9$) so that the relative signal intensity (in %) of the total peak height is plotted. (A) Electropherogram of N-glycans derived from WT (black) and *Cmas*$^{-/-}$ (red) mESC after labelling with APTS and xCGE-LIF analysis. Glycans are annotated according to comprehensive exoglycosidase digests and migration time matching with our in-house N-glycan database. Glycan structures can be assigned to the corresponding numbers in the electropherogram. Linkage positions of sialic acids are indicated by differing angles. Region from 140 to 480 normalised migration time units (MTU") is shown. High mannose N-glycans represent the predominant species in all analysed mESC lines. (B,C) Electropherograms of APTS-labelled N-glycans derived from B) WT *Cmas*$^{+/+}$ (black) and heterozygous *Cmas*$^{+/-}$ (red) mESC lines and (C) *Cmas*$^{+/+}$ mESC before (black) and after treatment with *A. urefaciens* sialidase (red). Annotated N-Glycan structures with sialylated N-glycans (#1 to #6) are highlighted in purple, galactose capped N-glycans (#7 to #10) are highlighted in yellow and depicted on the right hand side. Region from 140 to 480 normalised migration time units (MTU") is shown. (D) Electropherogram of bovine fetuin before (black) and after treatment with *A. urefaciens* sialidase to confirm enzymatic activity. Region from 110 to 480 normalised migration time units (MTU") is shown.

Supplementary Figure S3 A Electropherogram

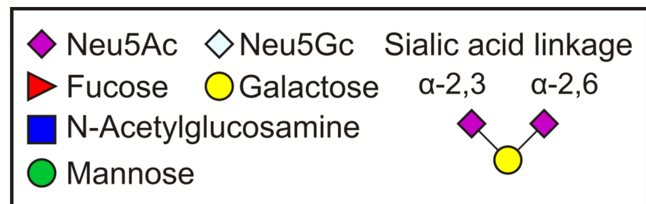


Supplementary Figure S3A Structures

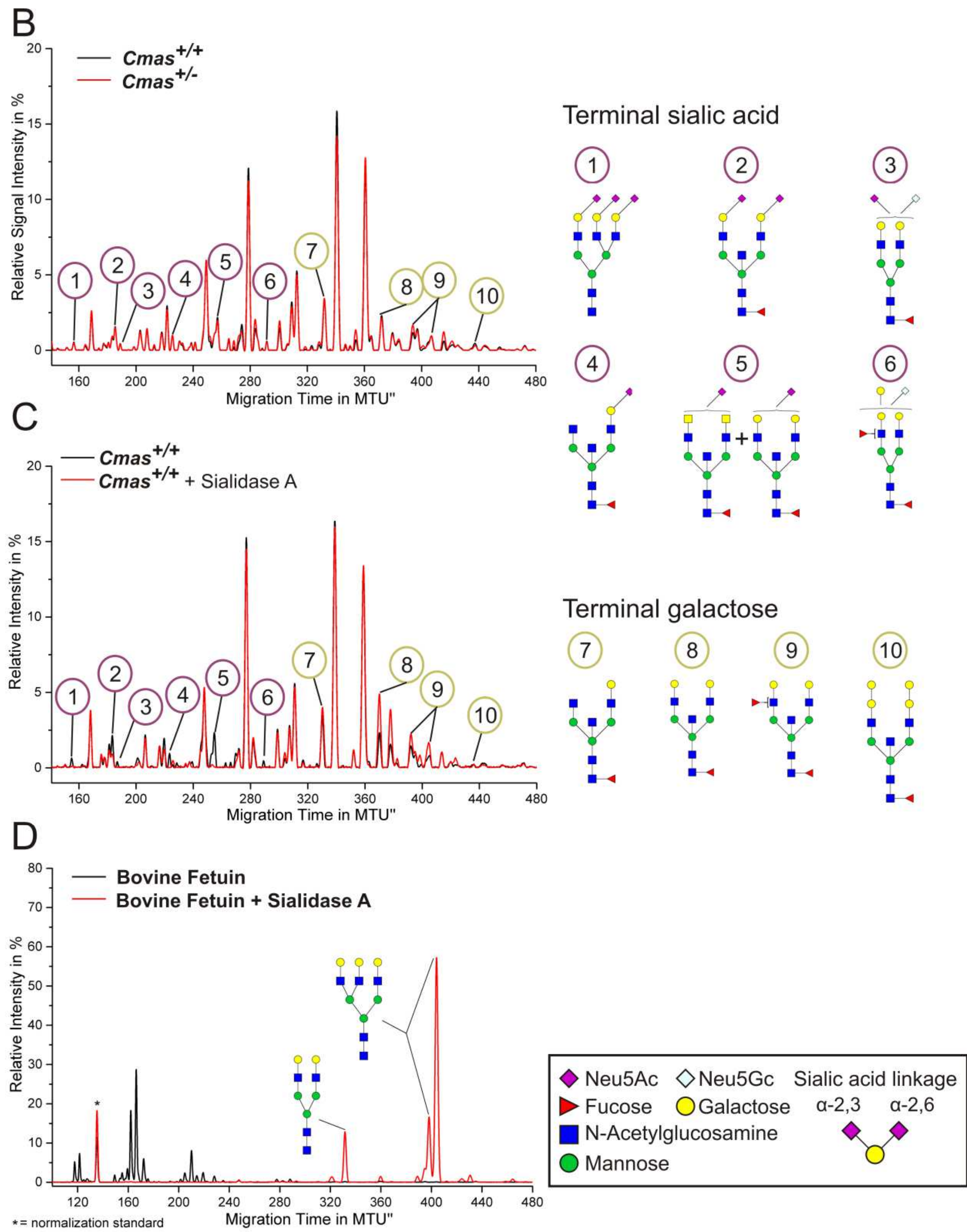
1 2 3 4 5 6 7 8 9 10 11 12

13 14 15 16 17 18 19 20 21 22 23 24

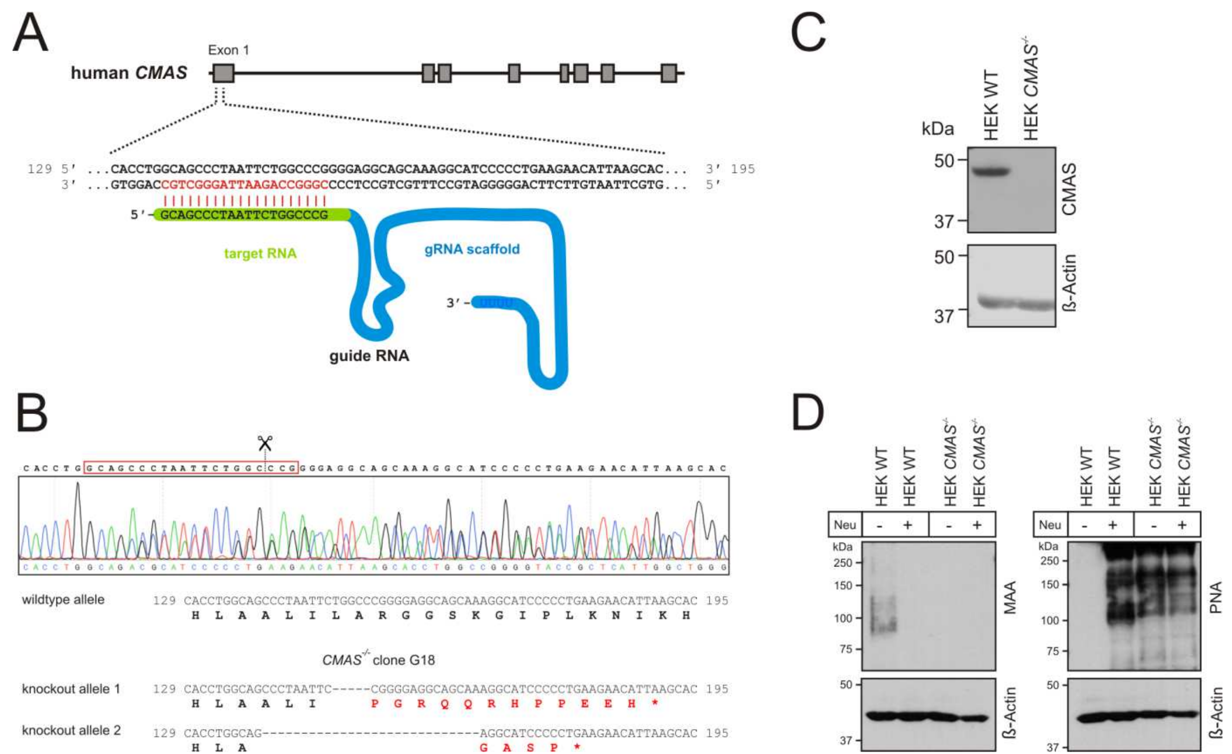
25 26 27 28 29 30 31 32 33 34 35 36 37



Supplementary Figure S3 B-D



Supplementary Figure S4 Generation and biochemical characterization of HEK 293 *CMAS*$^{-/-}$ cells. (A) HEK 293 *CMAS*$^{-/-}$ cells were generated using the CRISPR/Cas9 system. A sequence in exon 1 of the human *CMAS* gene was chosen as target sequence (shown in red) for the Cas enzyme. (B) Sequencing analysis of exon 1 of *CMAS* knockout clone G18 revealed a 5 bp deletion in one allele and a 28 bp in the other allele resulting in frameshifts and a stop codon (*) after a short sequence in both cases. (C) *Cmas*$^{-/-}$ HEK 293 cells lack CMAS protein (~48 kDa) as shown by Western blot analysis using an anti-CMAS antibody. (D) The sialylation status of WT and *Cmas*$^{-/-}$ HEK 293 cell lysates was analyzed by Western Blotting and lectin staining. *Maackia amurensis* agglutinin (MAA) specifically binds to α2,3-linked Sia, while peanut agglutinin (PNA) recognizes the underlying galactose-capped epitope. Lectin specificity was verified by neuraminidase treatment of the cell lysates. Anti-Actin staining was used as loading control.



Supplementary Table 1 Primers for quantitative PCR analysis

Target	Name	Sequence
Klf4	Klf4 forward	GCCACAGACCTGGAGAGT
	Klf4 reverse	GGTTAGCGAGTTGGAAAGGATAA
Nanog	Nanog forward	GCCAGGAAGCAGAAGATG
	Nanog reverse	AGGTTCAGAATGGAGGAGAG
Oct3/4	Oct3/4 forward	CTCTTTGGAAAGGTGTTCAGC
	Oct3/4 reverse	CCTGAAGGTTCTCATTGTTGTCG
Sox2	Sox2 forward	CTCGCAGACCTACATGAAC
	Sox2 reverse	CTCGGACTTGACCACAGA
CMAS	CMAS forward	ACTCTGTCTTCTCCGTTGTGA
	CMAS reverse	AGCCGTTCTCATATAACTCTCCAT