

Supplementary information

Title: Murine transcription factor Math6 is a regulator of placenta development

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Table S1: Breeding statistics of *Math6* WT and KO mice.

<i>Math6</i> genotype		matings	litters	pups	pups / litter
female	male				
+/+	+/+	9	25	168	6,7
+/-	-/-	10	25	140	5,6
-/-	+/+	6	0	0	/
-/-	-/-	6	0	0	/

Table S2: Placenta phenotype and reproductive rate in dependence of the parental *Math6* genotype (E10.5: +/+ x +/+ and -/- x -/- n=7 and -/- x -/- n=5; +/- x -/- n=3).

parental <i>Math6</i> genotype		embryonic <i>Math6</i> genotype	morphology of the decidua at day E10.5	reproductive rate
♀	♂	♀ / ♂		
+/+	+/+	+/+	normal morphology	viable offspring
+/-	-/-	+/-	normal morphology	viable offspring
		-/-		
-/-	+/+	+/-	altered shape, reduced uNK cells, impaired blood vessel remodeling	no offspring, uterine and vaginal bleeding
	-/-	-/-		

Table S3: Uterine implantation statistics in dependence of the maternal *Math6* genotype as evaluated by gross anatomical observation on day E10.5, E13.5 and E18.5.

Maternal <i>Math6</i> genotype	total No. of implantations	total No. of alive implantations	mean No. of implantations	mean No. of alive implantations
+/+	46	42	7,7	7,0
-/-	71	22	6,5	2,0

Table S4: Primer sequences

Gene	Forward primer	Reverse primer	Ref.
Desmin	CGAGGAGAGCAGGATCAACC	TCTCCATCCCAGGTCTCAAT	
Gja1	ATTTTCGTTCCCGTGGAGGTG	TTTTGCCGCCTAGCTATCCC	
Hand2	CAAGATCAAGACTGCGCC	TCTCCGTTCTGGTCGCCTT	
Prl3c1	TGACTCAAGCACGCACCTG	TCCGGTCATATCGTGTGGCA	
Prl8a2	AGCCAGAAATCACTGCCACT	TGATCCATGCACCCATAAAA	57
IGFBP1	CATCCTGTGGAACGCCATCA	AGGGCTCCTTCCATTTCTTGAG	
IGFBP4	CTTCCACCCCAAACAGTGTCA	GTCTTCCGATCCACACACCA	
CD31	GCGGTGGTTGTCATTGGAGTG	GTTGGAGTTCAGAAGTGGAGCAG	57
Mmp9	CTCTGCTGCCCTTACCAG	AGCGGTACAAGTATGCCTCTGC	
Ccnd3	CCTCCTACTTCCAGTGCCTG	GGCAGACGGTACCTAGAAGC	
Klrg1	AGGGAGTGAAGCTGTTTGGAG	CCTCCAGCCATCAATGTTCTT	
Gzma	CCTGAAGGAGGCTGTGAAAGAA	CGCCAGCACAGATGGTATTTG	
Prf1	TCTTGGTGGGACTTCAGCTTTC	TCTGCTTGCAATTCTGACCGA	
Gcm1	TGCTCACCTATGGCTCTCCT	AAAATTCTGCCAAGCCCTTT	46
Hand1	GCAAGCGGAAAAGGGAGTTG	GGTGCGCCCTTTAATCCTCT	
Tpbpa	CCAGCACAGCTTTGGACATCA	AGCATCCAACGCGCTTCA	46
Prl3d	TGGAGCCTACATTGTGGTGGA	TGGCAGTTGGTTTGGAGGA	46
Pcdh12	CCTAAGGGACTCTGCTCACG	CAGTACAGCCAGGCAGATCA	
Gapdh	CAGCCTCGTCCCGTAGACA	CGCTCCTGGAAGATGGTGAT	18
Math6 Primer			
Primer 1-2 (qPCR)	CGTCAATTTACACGTAA	AAGAGACTCACAGTGGTGTCT	18
Primer 3-5	GTGCCGTGCTACTCCTATGG	CTGAGAGATGGTACACGGGC	
Primer 4-5	GAGCCAAGAAGCGGAAGGAG	CTGAGAGATGGTACACGGGC	
Probe	GTTTCTTTTTCTAGCCCCAACCATTC	GGTATAATAAAGCATCCATACTGCCTTGTCC	
Genotyping WT	CATAAGGAGCCAGAGGTGCC	CTTCTACCTCCCACCCGAAC	
Genotyping KO	CATAAGGAGCCAGAGGTGCC	GCATACATTATACGAAGTTATATGCATCAC	

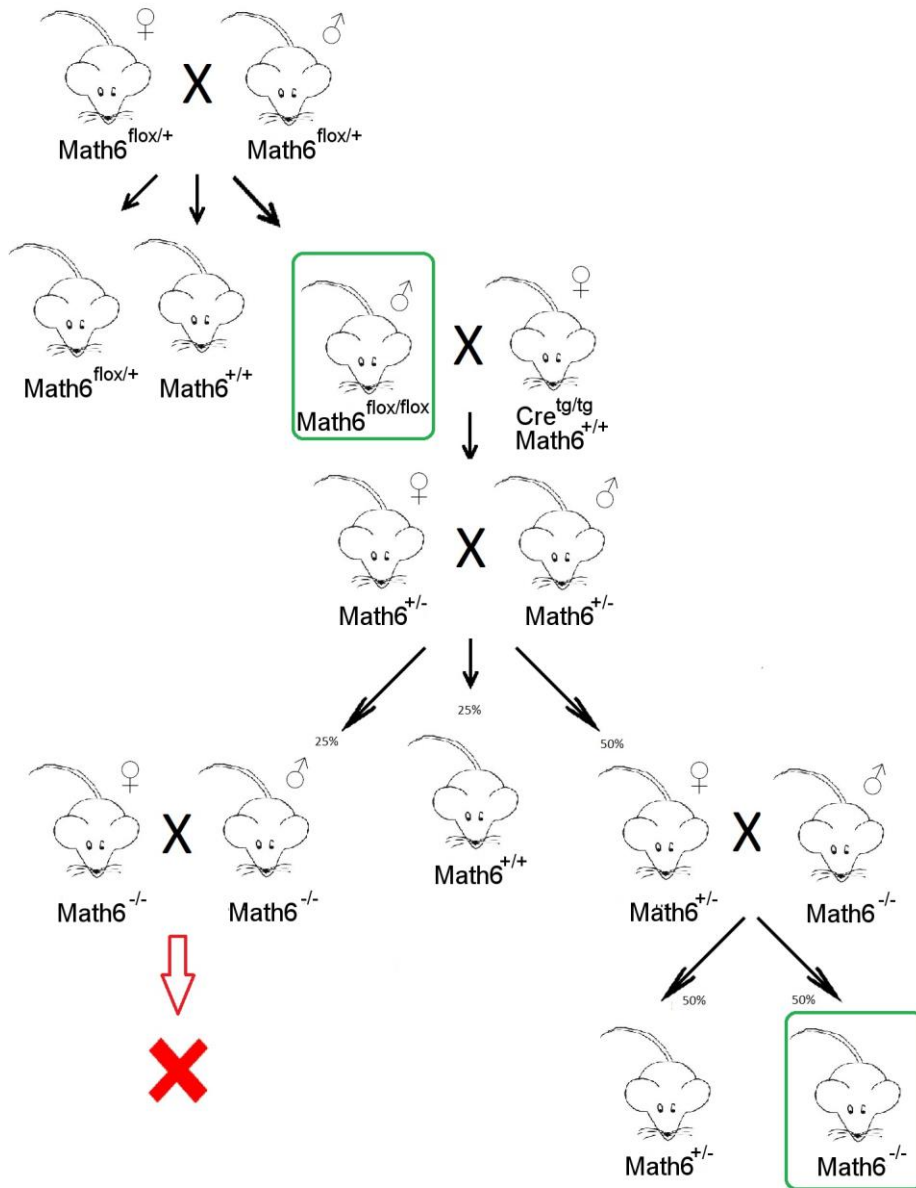


Figure S1: Breeding strategy of *Math6*^{flox/+} mice (*Math6* exon 1 flanked by *loxP*-recombination sites) for receiving and maintaining homozygous *Math6* KO (*Math6*^{-/-}) mice. *Cre*^{tg/tg} = “deleter” mouse homozygous for a ubiquitously expressed *Cre-recombinase* transgene.

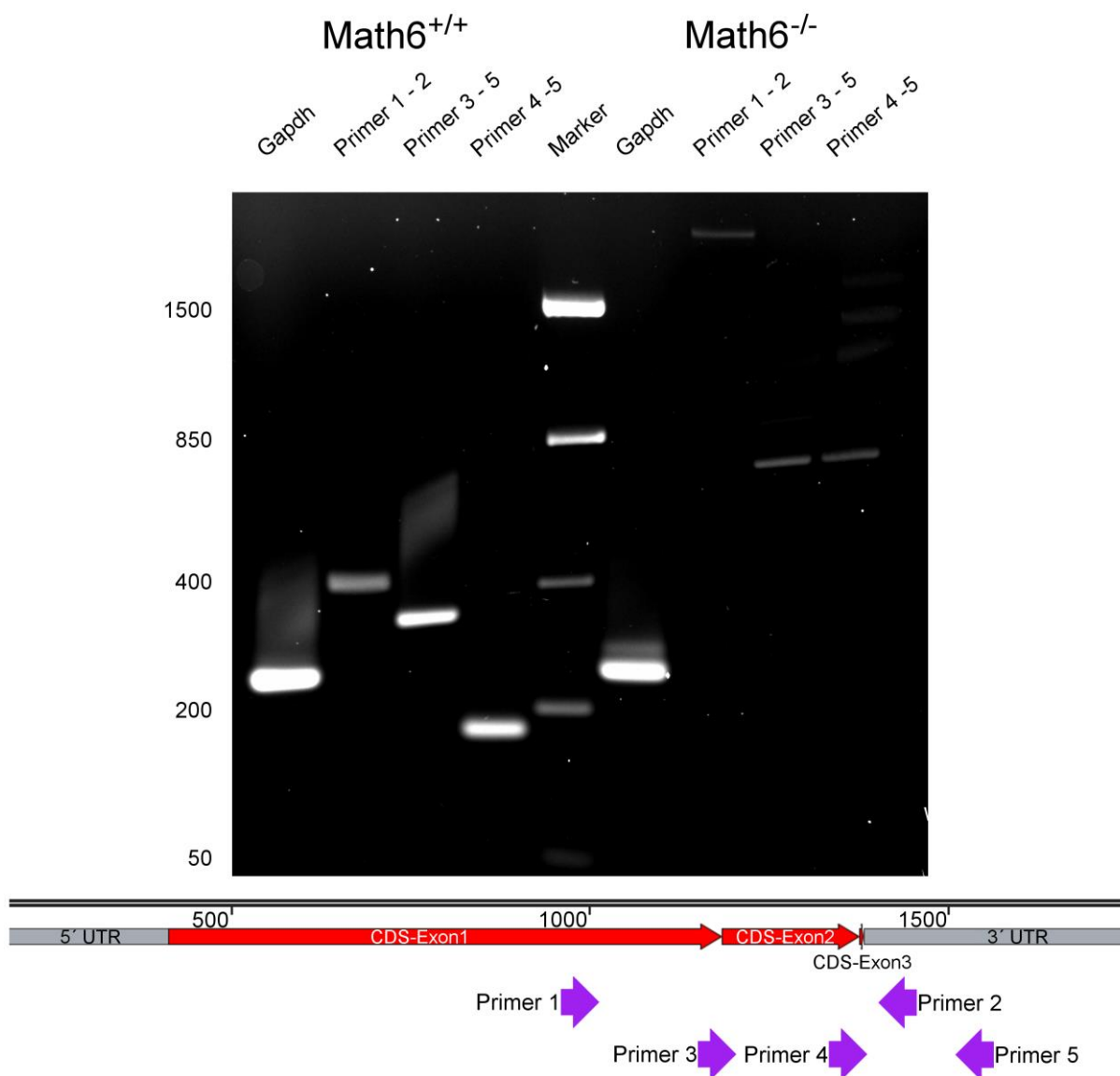


Figure S2: Representative gel-electrophoresis of *Math6* specific RT-PCR samples obtained from WT and KO mice. The localization of the primers used are indicated within the scheme of the *Math6* mRNA below the gel photo (length of the DNA amplificate with primers 1+2: 430 bp; primers 3+5: 346 bp; primers 4+5: 171 bp). In *Math6* KO mice no *Math6* related transcripts could be detected.

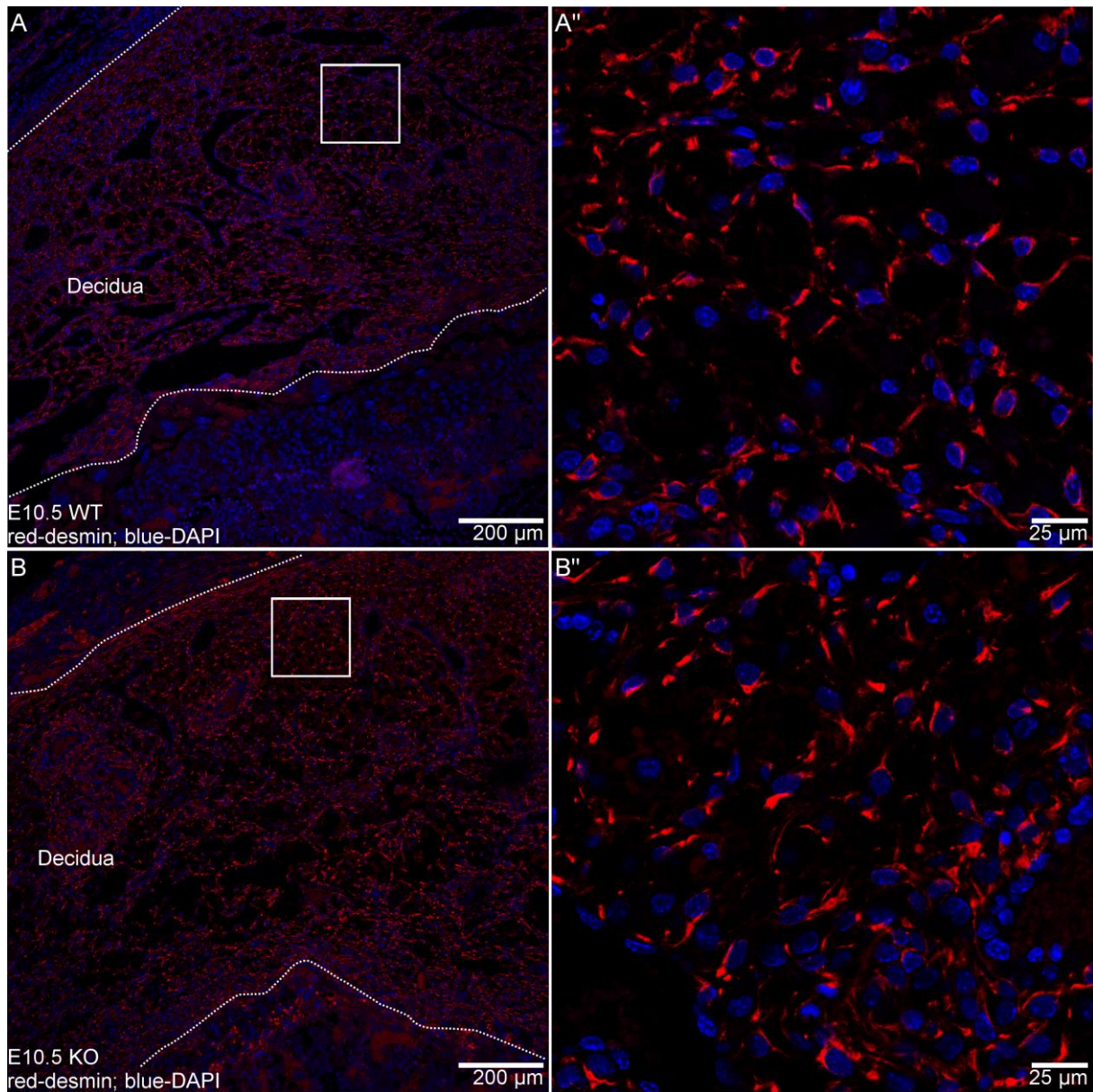


Figure S3: Qualitative analysis of decidual cells as detected by anti-desmin immuno- (red) and nuclear counterstaining by DAPI (blue). The amount of decidual cells is apparently not altered between placentas derived from WT in comparison to those from *Math6*^{-/-} (KO) females.

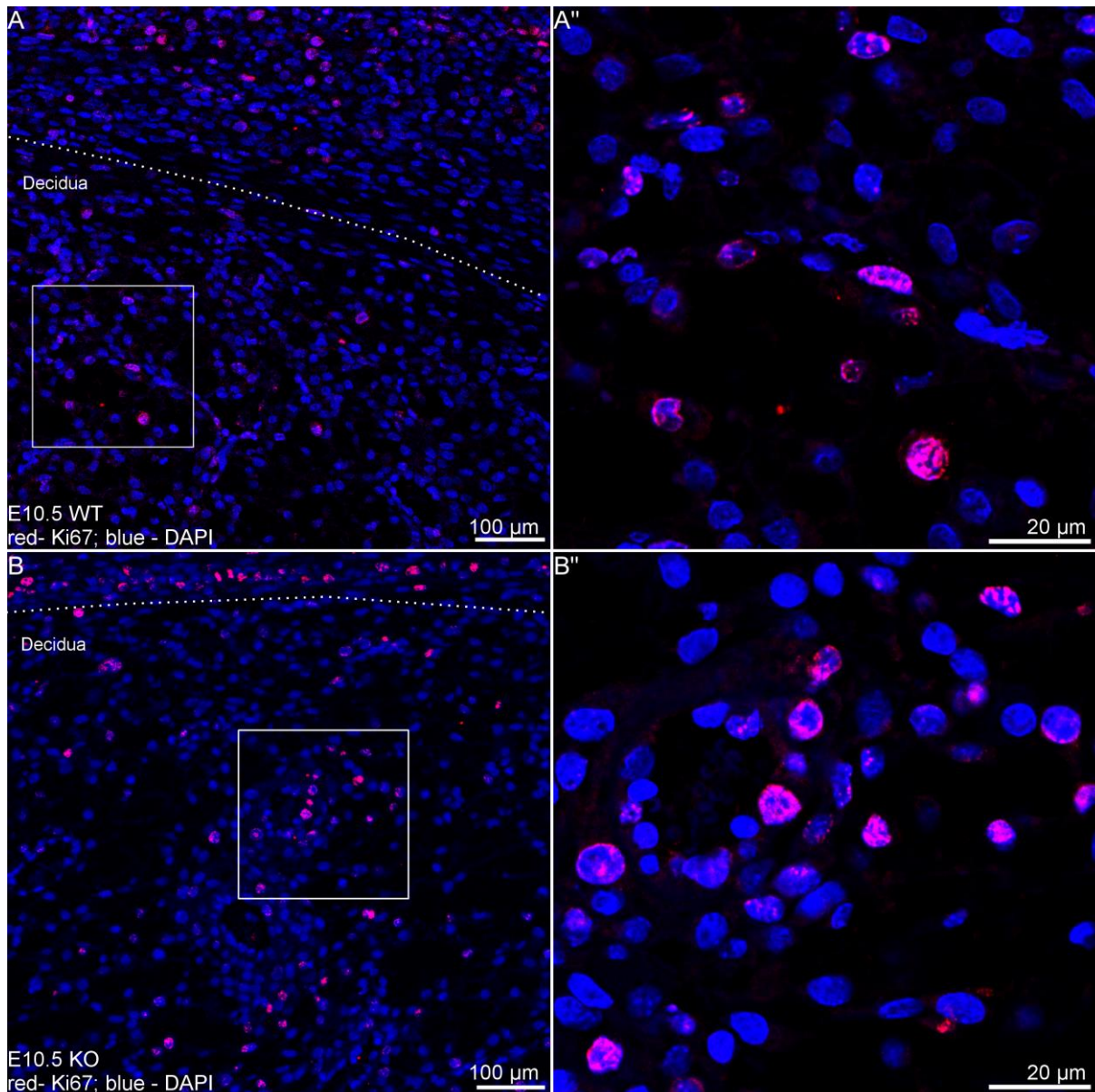


Figure S4: Qualitative analysis of proliferating decidual cells as detected by anti-Ki-67 immuno- (red) and nuclear counterstaining by DAPI (blue). The cell proliferation is apparently not altered between the maternal part of placentas derived from WT in comparison to those from *Math6*^{-/-} (KO) females.

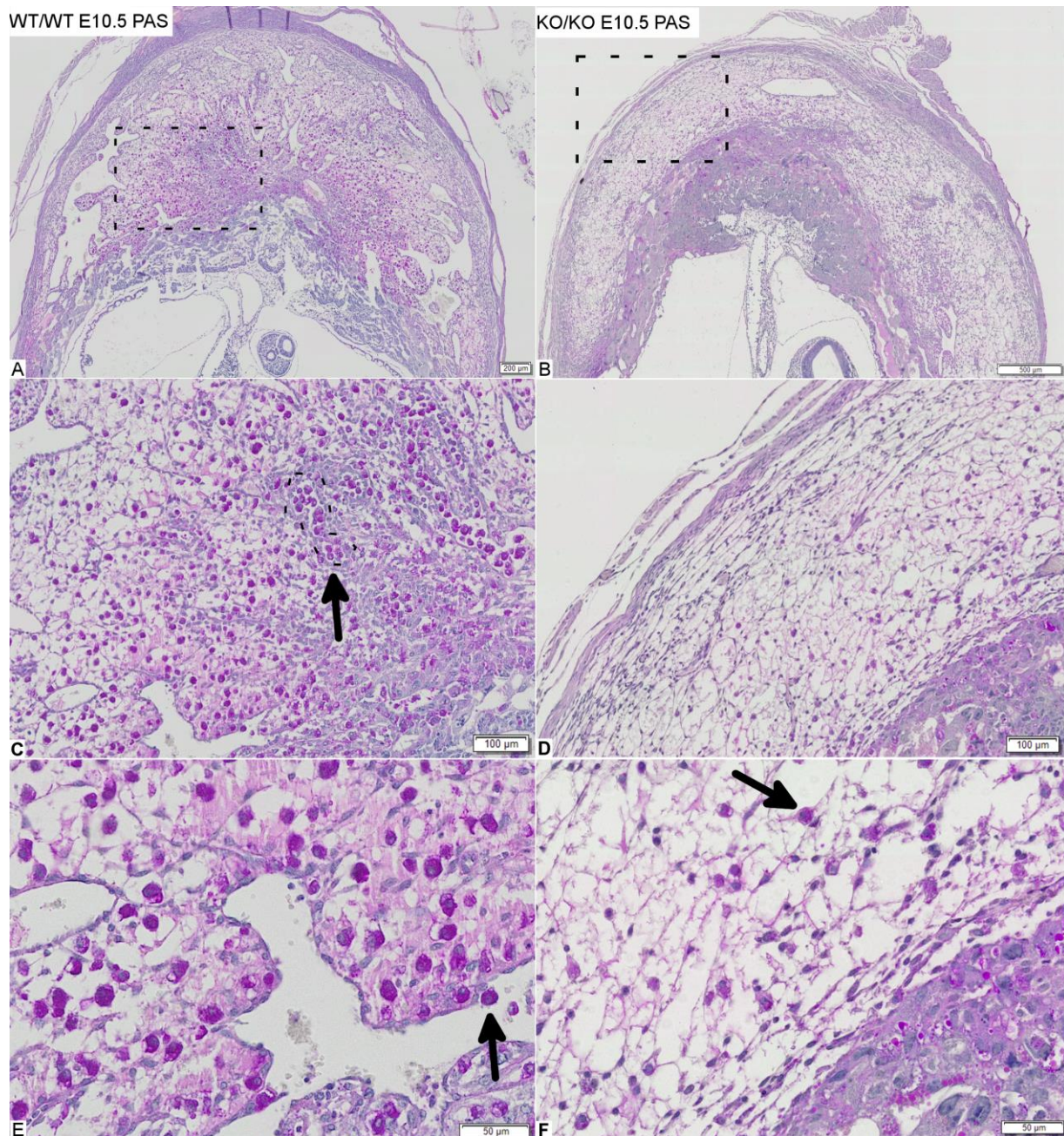


Figure S5: Qualitative analysis of uterine natural killer cells (uNK) in the placenta as detected by PAS-staining. The amount of uNK cells is apparently reduced in the decidua of placentas derived from *Math6*^{-/-} (KO) in comparison to those from WT females.

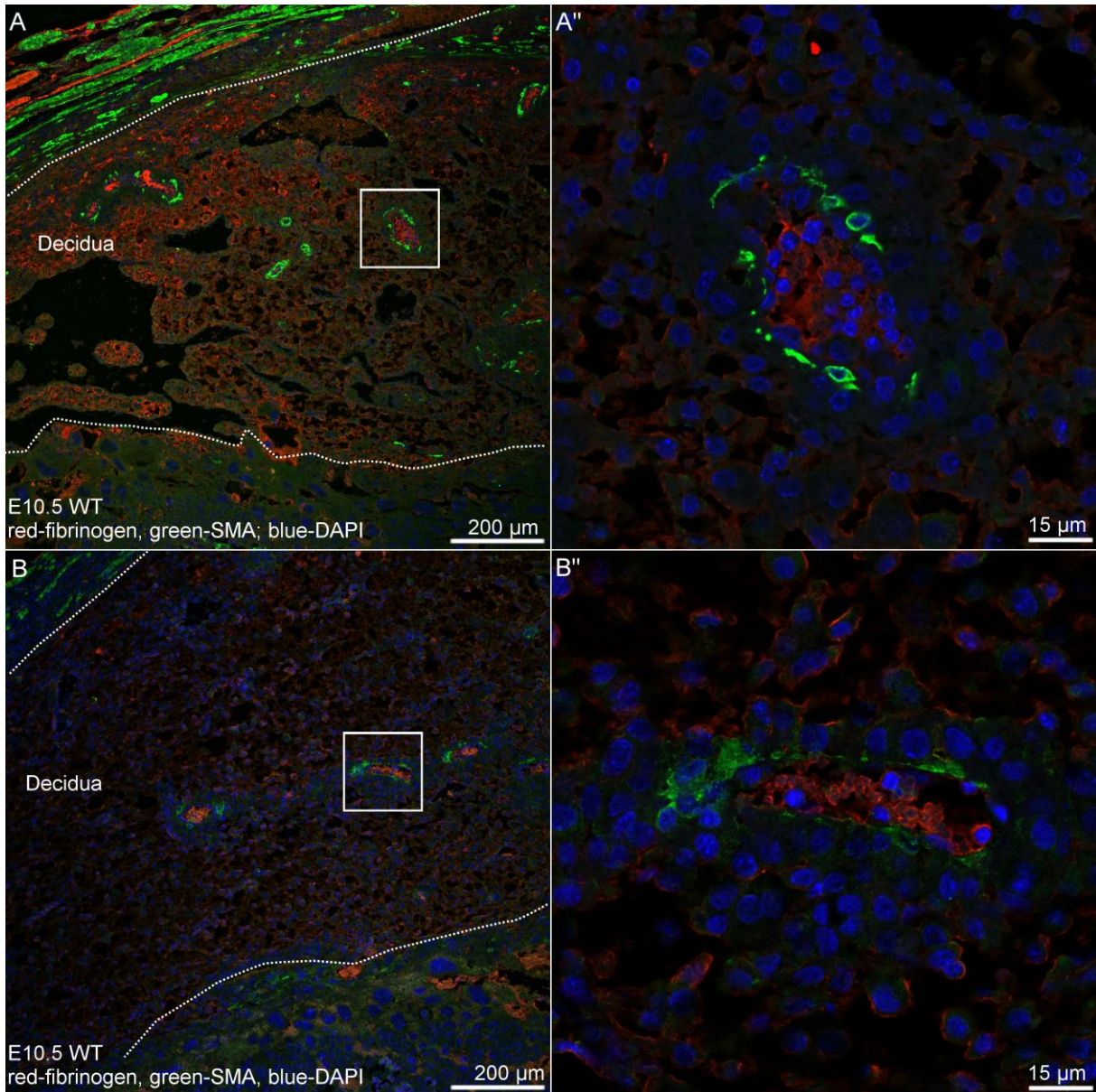


Figure S6: Qualitative analysis of spiral artery morphology detected by anti-alpha smooth-muscle-actin immuno- (green) and nuclear counterstaining by DAPI (blue). The anti-fibrinogen immunostaining (red) serves for better orientation within the sections. The amount of spiral arteries appeared reduced in the decidua of placentas derived from *Math6*^{-/-}(KO) compared to those from WT females. However, the *Tunica media* of these arteries was apparently not altered.

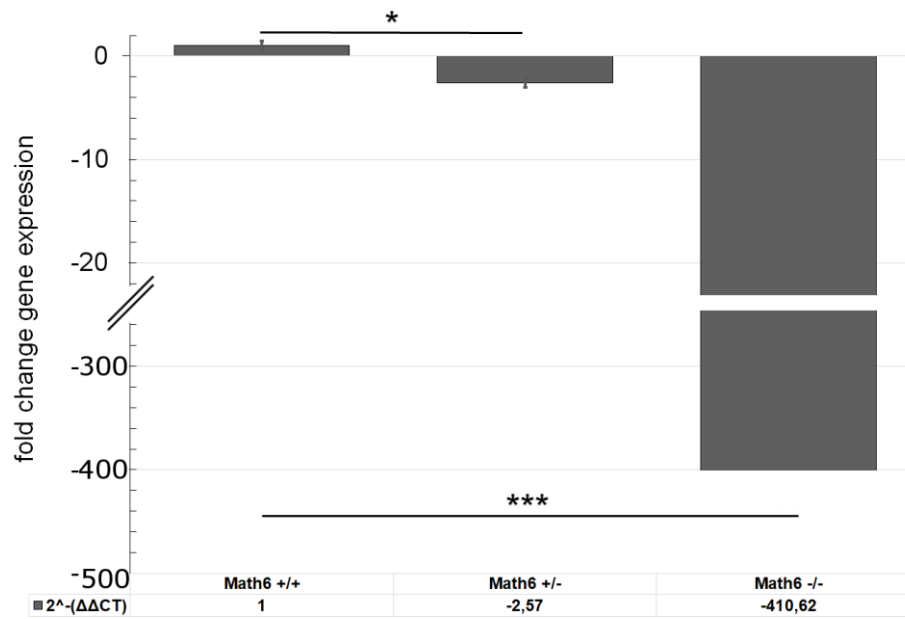


Figure S7: Detection of a *Math6* gene dosage effect with respect to the expression level in the testis of mice of all three genotypes. Heterozygous *Math6* gene expression leads to a 2.57-fold reduction of the mRNA level, whereas in homozygous KO mice the expression level was completely abrogated. Data are presented as mean \pm SEM; n=3; *** \leq 0,001 p; * p \leq 0,05

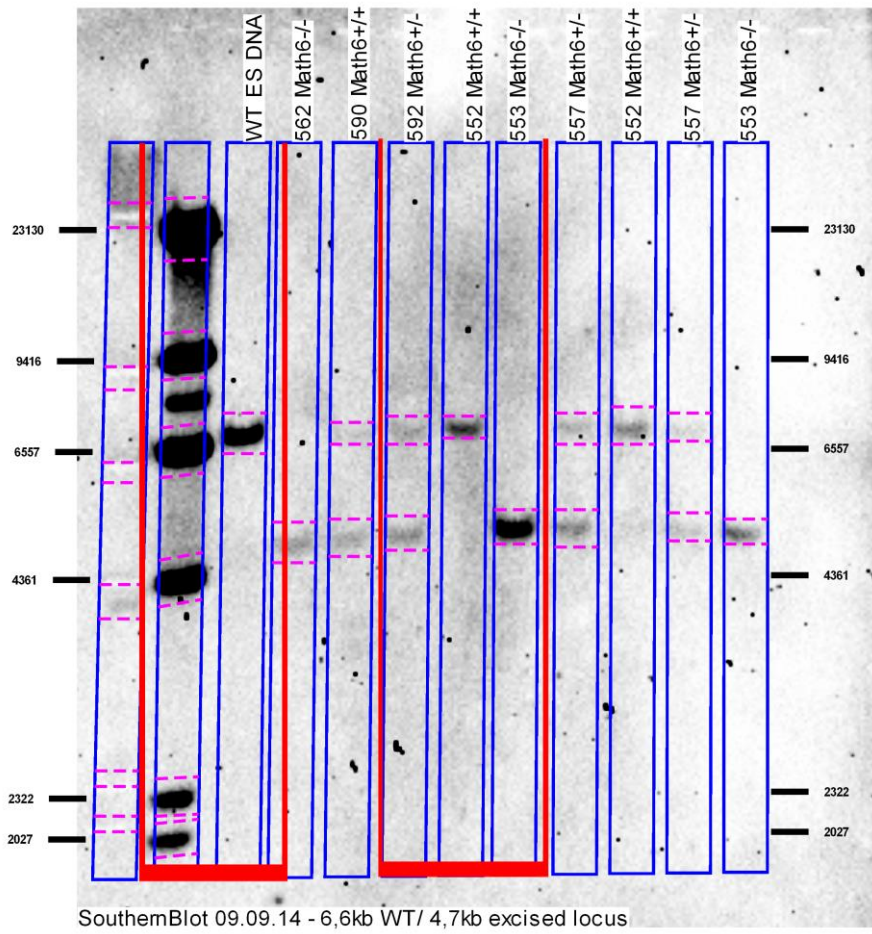


Figure S8: Full picture of the Southern Blot, which is partially presented in Fig.1B.