Supplementary Material for:

Alterations in the balance of tubulin glycylation and glutamylation in photoreceptors leads to retinal degeneration

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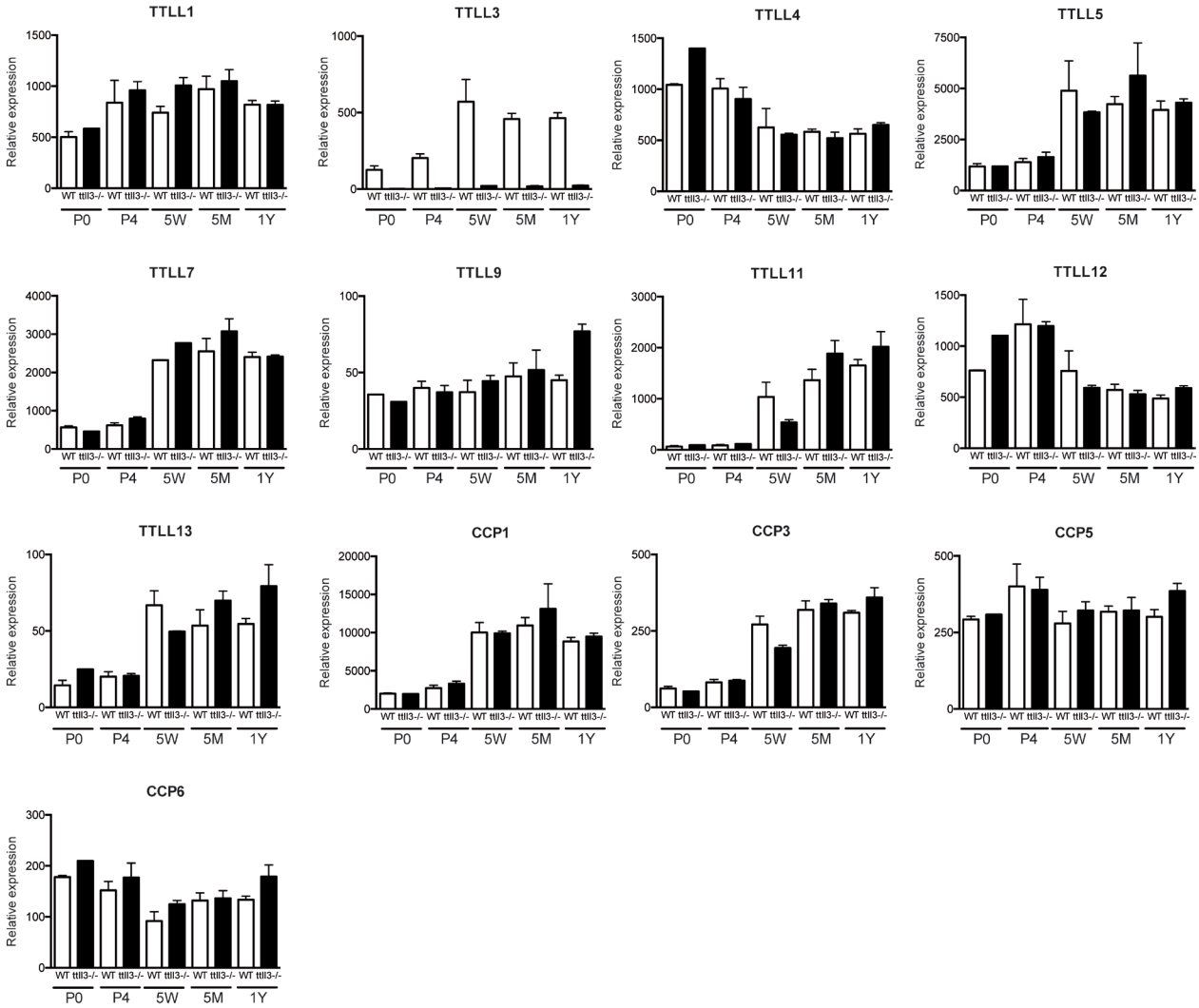
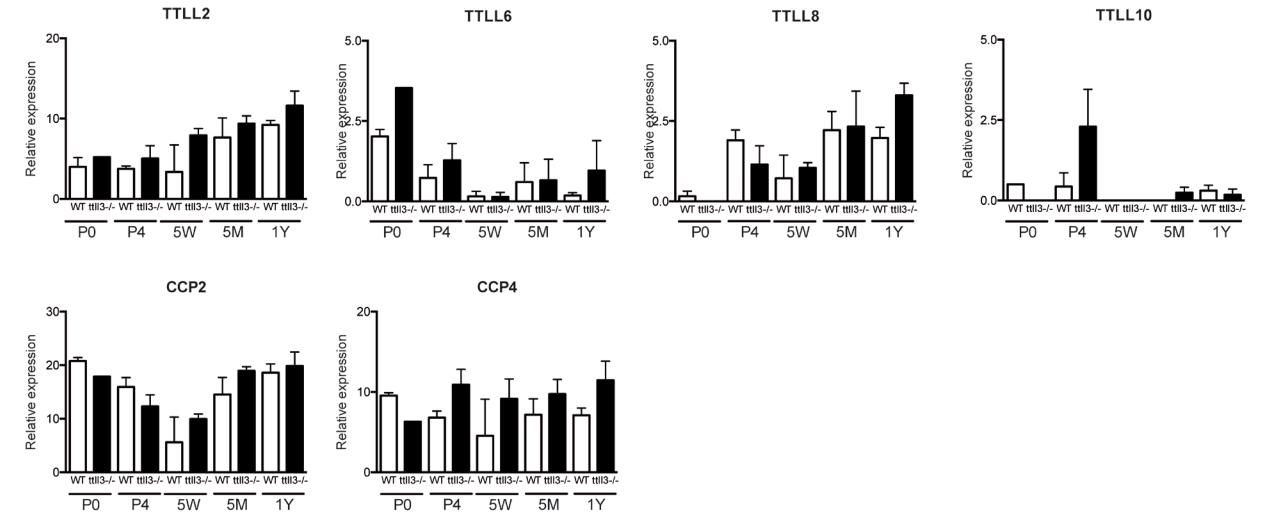
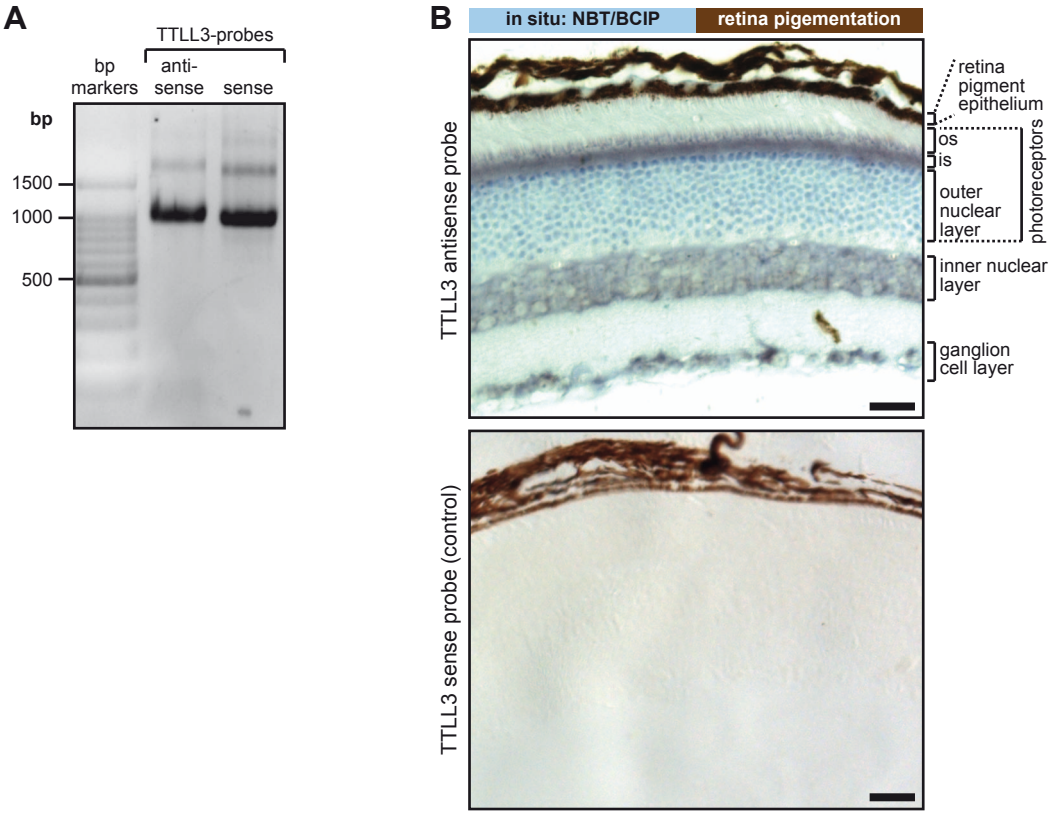
medium and strong expression levels



very low expression levels



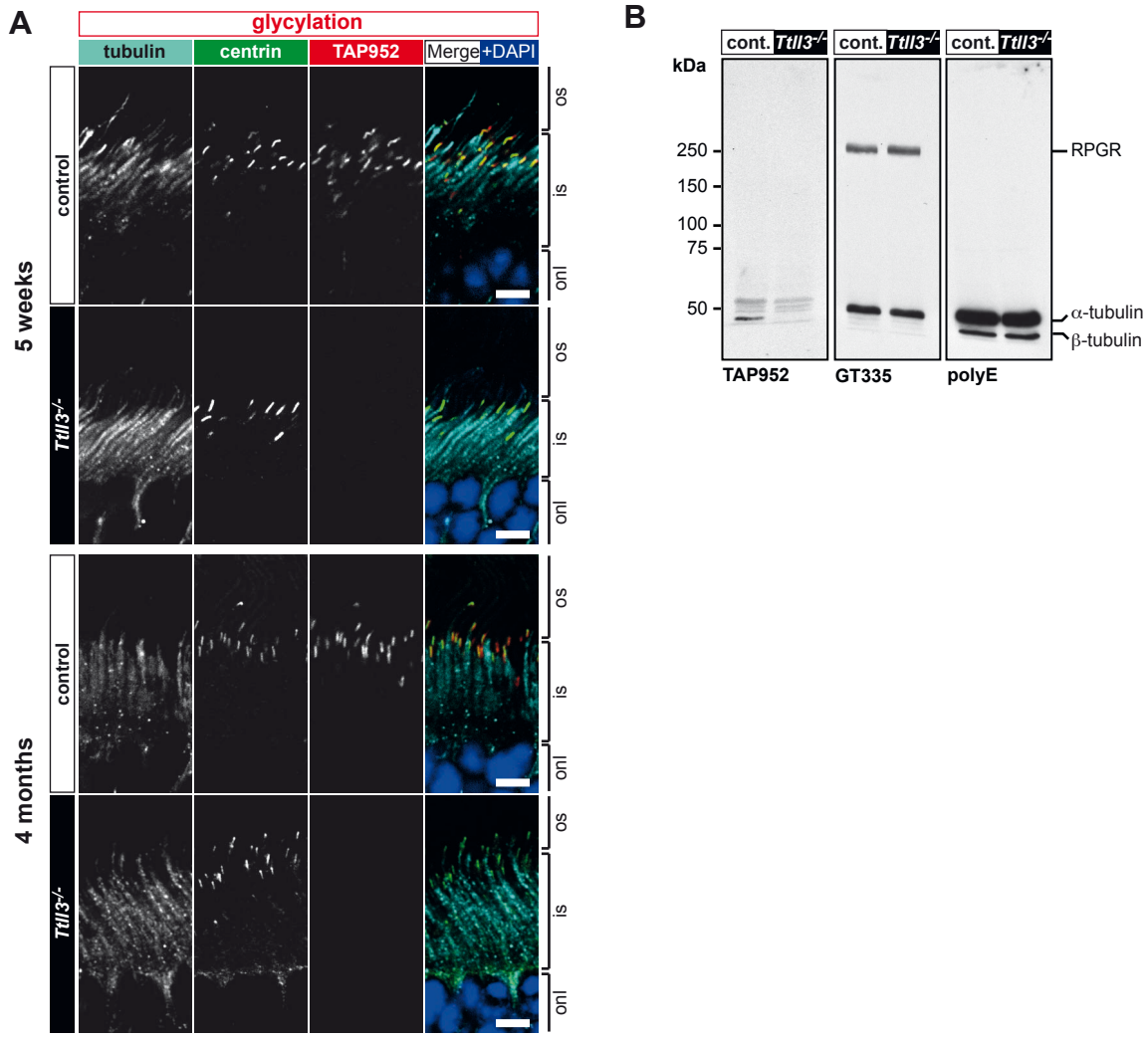
Supplementary Figure S1: TTLL3 is the only initiating glycylase expressed in mouse retina at all developmental stages.
The relative mRNA expression levels of TTLL and CCP genes were analysed by qRT-PCR in retina samples from control and *Ttll3*$^{-/-}$ mice at different postnatal ages. The samples were analysed by qRT-PCR, and values were standardized to expression levels of the TBP gene. Mean values ±SEM of two independent mRNA samples are shown. TTLL and CCP gene expression was classified as “strong/medium” or “very low” expressed. The data of the very-low-expressed genes cannot be considered reliable due to the low values. TTLL3 is not expressed in the *Ttll3*$^{-/-}$ mice retina at any developmental stage. TTLL8 is not reliably quantifiable (only detectable at very low transcript levels). None of the TTLL and CCP genes are significantly up- or down-regulated in the *Ttll3*$^{-/-}$ retina as compared to the control retina.



Supplementary Figure S2: TTLL3 expression in adult mouse retina

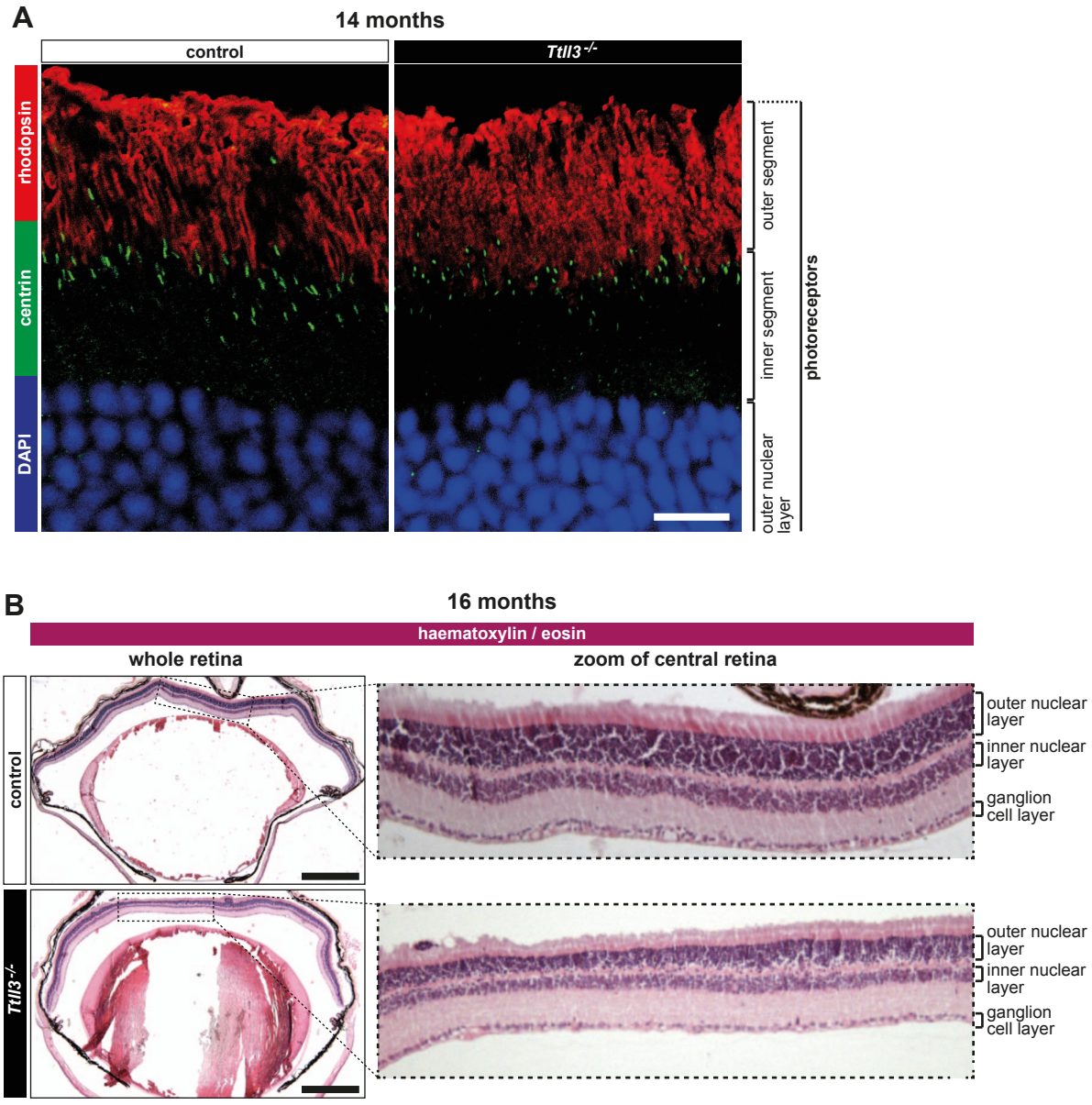
A) RNA probes for in situ hybridization were synthesized, and checked in 0.8% agarose gel electrophoresis stained with ethidium bromide. The size of the molecular weight markers is indicated. **B**) *In situ* hybridization of retinal sections from adult mouse with DIG-labelled antisense and sense (negative control) probes. The probes were detected with anti-DIG antibody. Note the particularly strong labelling of nuclei in the photoreceptors – outer nuclear layer. Scale bar 20 µm. Abbreviations: is: inner segment, os: outer segment.

TTLL3 expression is prominent in the photoreceptor layer, which was consistent with the strong TAP952 immunoreactivity in connecting cilia of the photoreceptors (Fig. 1B). Surprisingly however, TTLL3 transcripts were also found in the cells of the inner nuclear layer, and within ganglion cell layer, both structures in which no obvious glycylation signal was detected with the TAP952 antibody (Fig. 2B). This situation is reminiscent of our previous observations in colon, where TTLL3 is expressed and plays a key role in controlling primary cilia and epithelia proliferation, while no TAP952-positive MT structures were found (Rocha et al., 2014). One possible explanation is that TAP952, which was raised against *Paramecium* tubulin (Callen et al., 1994), does not detect all glycylated epitopes on mammalian tubulin, and might preferentially detect cilia-specific glycylation patterns (Bré et al., 1996).



Supplementary Figure S3: Absence of glycylation in the *Ttll3*$^{-/-}$ mouse retina

A) Co-immunostaining of retina sections from control and *Ttll3*$^{-/-}$ mice at different postnatal ages with pan-centrin antibody (20H5; green), β-tubulin (C105; cyan) and anti-glycylation TAP952 (red). Nuclei were visualized with DAPI (blue). TAP952 labelling is completely absent in the *Ttll3*$^{-/-}$ mouse. Scale bars 5 µm. Abbreviations: is: inner segment, os: outer segment, onl: outer nuclear layer. **B**) Retinas dissected from control and *Ttll3*$^{-/-}$ mice were homogenized and resolved in 7% SDS-PAGE gels, followed by immunoblot. A GT335-reactive protein of 250 kDa, most likely RPGR (Sun et al., 2016) is detected at equal intensity in both, control and *Ttll3*$^{-/-}$ retinas. TAP952 detects a specific protein band (β-tubulin) only in control, but not in *Ttll3*$^{-/-}$ retina. Note that the very strong labelling of α- and β-tubulin with GT335 and polyE most likely originates to a large extent from the neurons in the retina, thus, differences in glutamylation levels that have been detected in the photoreceptors (Fig. 2, 3) are overwritten by the neuronal tubulin in the blot.



Supplementary Figure S4: Verification of connecting cilia length measurements and retina thickness comparison

A) To control for the correct orientation of our retina tissue sections during the length measurements (Fig. 2, 3), control and *Ttll3*$^{-/-}$ retina sections from 12-month-old mice were labelled with pan-centrin (20H5; green) to visualize the connecting cilia and the basal bodies, and with an anti-rhodopsin antibody (red) for the outer segments. Nuclei were labelled with DAPI (blue). Note that the connecting-cilia (20H5) length is reduced in *Ttll3*$^{-/-}$ retina while the outer-segment length is comparable to control. This demonstrates that our measurement procedure for determining the length of connecting cilia was not biased by the orientation of the histological blocks. Scale bar 10 µm.

B) Paraffin-embedded retinal sections of 16-month-old control and *Ttll3*$^{-/-}$ mice were stained with haematoxylin and eosin. In the whole-retina view, outer regions of both retinas appear of similar thickness, while the central part of the retina is thinner. The significant thinning of the outer nuclear layer in the central retina of *Ttll3*$^{-/-}$ mice becomes evident in the zoom images. Scale bar 500 µm.

Supplementary Table S1: Primer pairs used for RT-qPCR

Primer names	Forward primer (5'-3')	Reverse primer (5'-3')
TTLL1-Mm	ATTCCCGACTGCAAGTGGAACA	CTGGGCCAGCTCCTCGTCAT
TTLL2-Mm	GGTCTGCAGAGTTTGGGTGATGT	TCTTCAAAGGCTTGCTTTCCTGTA
TTLL3-Mm	GGAGGGGGATCGGAACATCT	CATCTCATCCAGGCGGTTCAT
TTLL4-Mm	CCATGGAAACAAGCTCAAAGGAGTA	CAGTGGGGCTGAGTCAGAGACAA
TTLL5-Mm	GCGCCTCTATGTGCTCGTGACT	TGCGGATGTTCTTGGAGCCTT
TTLL6-Mm	GGGACGATTCTTGCAGCAGTGT	ATGGCCTGGAAACCCTTGACTT
TTLL7-Mm	GTACATCCCACCAAACGAGTCCA	TGCTGCCTTTGTCTTCAGTTTCAT
TTLL8-Mm	GAATGGAATGACCTGACACAGCAGTA	ACTTTTCGAATCAGTGATGGAAGCA
TTLL9-Mm	CCGCTTCAAGACCACCCTCAT	GCCAGCTGACATCACACCAATAGA
TTLL10-Mm	CCCAGTACCCAAACCAAGGTCCT	AAGCCTGCCTCTCGTCTCTGATGT
TTLL11-Mm	ACCAGCGGGACTCAGGGATGT	CATGGAGAGGTTGCAGCTTGAACT
TTLL12-Mm	CATCCCTCAGTTTGAGAAGCAGTA	GCCTTGAAGATCTCAGCCTGAA
TTLL13-Mm	CCTGGGGTTTGACATCTTACTGGA	AAGCTTGGGGAGTGGTTTACCTCTA
AGBL1-Mm	GCAGCATTGCTGAAATCCAAGTCTA	GCGGCTGTGCCAGTCCTGA
AGBL2-Mm	AATCTGCAGAAAGCCGTCAGAGT	AGTGTGTTTGTCCGTGTAGAGGTCA
AGBL3-Mm	CTGTTTACCCAAACTCCAAGGAAGAT	GGATGTTCGGTTACCCCCAACT
AGBL4-Mm	CCAAGAGTCTTTACCGAGATGGGAT	CTGTGGTCTGGGCAGCGATAGT
AGBL5-Mm	GCACCCAAAAGGTCAGCCAT	GCCGCCTTCTGTCTGAGCA
AGTPBP1-Mm	TTCCACAGAGTCAGATACTGCCAGAT	CAGAACTTCCATGCCTGTAGAACCT
TBP-Mm	CCCTTGTACCCTTCACCAATGAC	TCACGGTAGATACAATATTTTGAAGCTG

References

Callen, A.-M., Adoutte, A., Andrew, J. M., Baroin-Tourancheau, A., Bré, M.-H., Ruiz, P. C., Clérot, J.-C., Delgado, P., Fleury, A., Jeanmaire-Wolf, R. et al. (1994). Isolation and characterization of libraries of monoclonal antibodies directed against various forms of tubulin in Paramecium. *Biol. Cell* **81**, 95-119.

Bré, M. H., Redeker, V., Quibell, M., Darmanaden-Delorme, J., Bressac, C., Cosson, J., Huitorel, P., Schmitter, J. M., Rossier, J., Johnson, T. et al. (1996). Axonemal tubulin polyglycylation probed with two monoclonal antibodies: widespread evolutionary distribution, appearance during spermatozoan maturation and possible function in motility. *J. Cell Sci.* **109**, 727-738.