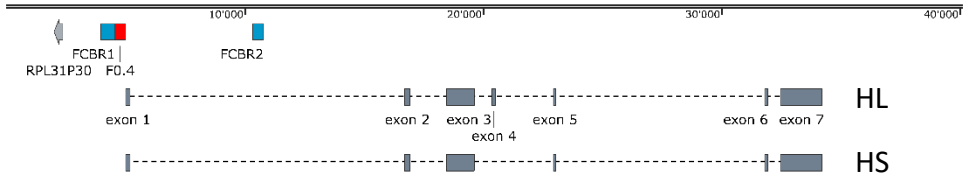
Appendix Table of Content

Appendix Figure S1

A



B

human FAM161A locus
40'070 bp

FCBR1 (enhancer, 607 bp)

GGCTCAGACCTTAGAGACGGGTTCTCGACGATGTGCAGCGCATAACGAGGAAAATACTGACCGGAGGGGGCCGGTCGTAGGGCTGGGG
AGCAGGAGGAGGGCAAAGGATAGAGAGGGGGTAGATAGGGAGTAGATAGGGGGCGGCAGCCTCGGCCTCAGTAAGCAGGGGCTCAGG
GAGCAGATGTCTGAGACTGCACCACGTCCGCTATTTTGAGGTTTGGCTTCTCTTACTGCCACGTGGTGCAGAGGGAAGGCGCCATTTGAT
GCGTCCAAATCCCGAGTGGGAAGAGAAGATACGGATGAGGGAGATGGATGTGTCTGTGGTGACTCTTGGGCACTTGTGCCGTTCAATCCT
GTCACTCTCAAGCTGAAGAACCCAAAGCAAACGCCAGGTTTACAATACCTGCACTATCACTC**ATTA**GTGGGAGGGTCAGGCGCCACCCAA
CTCTGGGCTTTTATTGGTCATGTCAAGAAAAAAGCGAGAGGGACAACTGGGAGCCTCCTTCTGATTGGTGGATTCCTGCTAGCCCTCAAGT
CAATCAGAGTAGAATGTACCGCCCTCCTTCCCACTCTGGCCCCCGCGGGAGACATGTTAGGTAGCGGGACG

FCBR2 (enhancer, 457 bp)

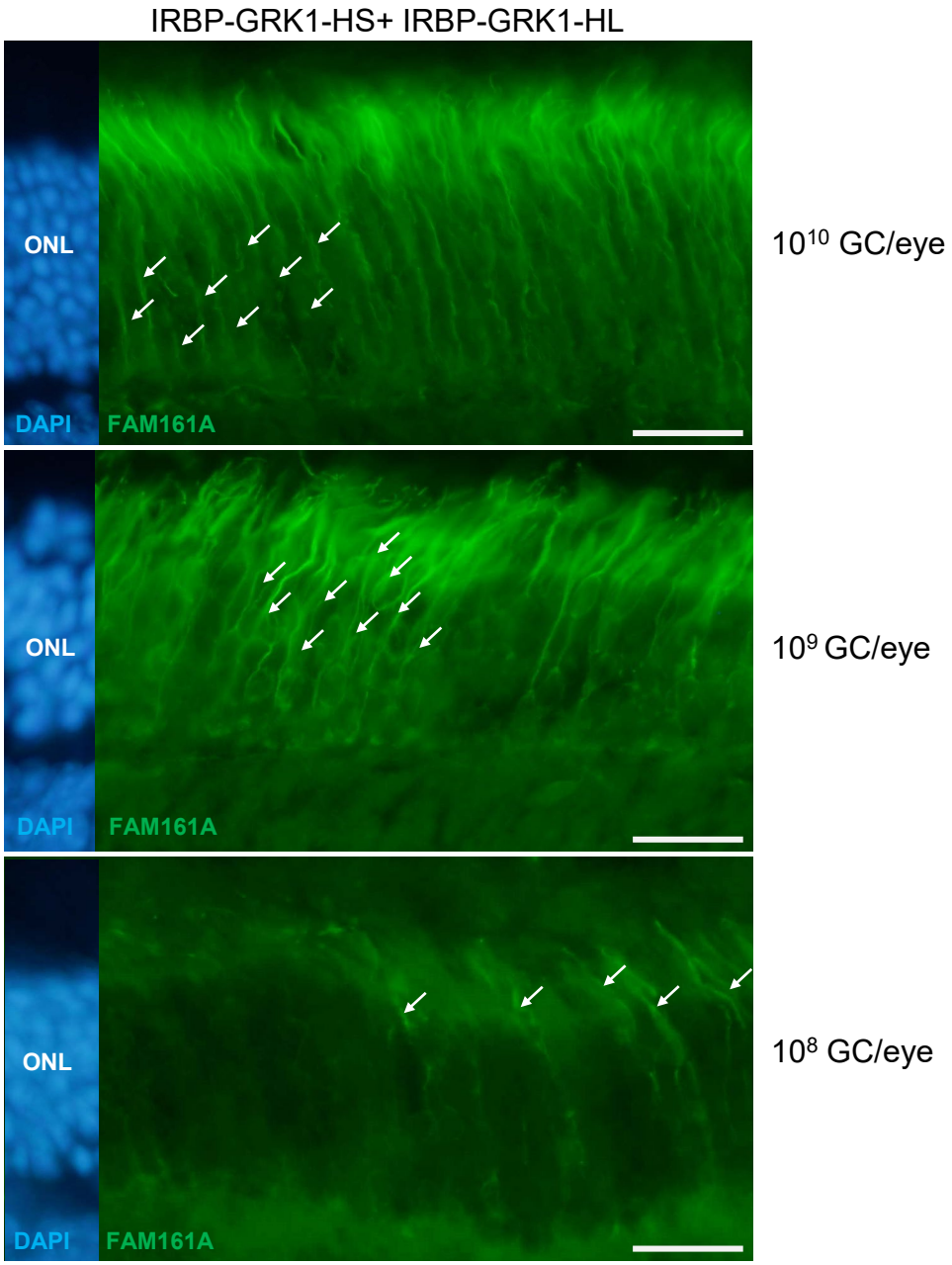
CTCTCTCATTCTTTACAAATTCAGCGAAAAAAATTTTTTCAAAA**ATTA**CAGGTTGCAGATGAAACTATATAGGCTGCAGCACAAA**TAAT**TCTT
CATAGGTGCTG**ATTA**C**TAAT**AAAGCAACAGGTGCTGGAAGGATTC**ATTA**TCTAAATGGGTCAAGCCAATCTAGGGAAG**ATTA**TCCTC**TAAT**
CTGGTGGCTGCATTTACAACATTCCACAAAAAGCCG**ATTA**AAGGGAGAGGATGCTATTTTAGGCTCTTGGAAAAA**TAAT**GTCTATGGCCTA
GGTTATTTTGTATACTAAATATTTTCTCAGTGGAGACA**ATTA**AAG**ATTA**A**ATTA**GAATTGGAGGTCATTTACTAGCCATTCATCTGTTTTAGGT
TAAACACTGTGAGATCAGTTTATCTGAAAAGTTTCGGCTAGCGTTAACAGT**ATTA**AGCTGAAAACAGAATGGATCCAGTGCAAAATCA

F0.4 (promoter, 460 bp)

CCAACCTTGTCACTCTTCTGAGACCGCCACCCAGTAACACGCCCTGCTTTCCTCCGCCGCCGCCGTCTCCTCCATCAACCGTCTCCTTCT
AACATGTTATTGCAGT**TAAT**AAAAAAGCGTTCTTTTCTTCACAAAAGCAGTTGTTTCATTGTG**TAAT**GTGAGAAGTCTGCGTTCAGGCGGTTA
GACGCAAAGGAGTGGAATAAGTGCCCTGTTCAGCCTGCCAACCTTCGGAACCAGCCAACAGACTCGGTACTGACTGCCATGATCCAGTAA
GCACCCGACTCGGGTCTCTGGCAGCCTGAGTGGATTTTGCGTGACTTTTGGTCCCTGGCGGCTCCTGTAGCGTCCCCAGTTACGGCGCC
CATAGCAACCGGCTCCCTAGCTAGGCGCCCCCGGGTTGCCAGGGGCCGCACCAGCTTTCCCGCCCCGGGCCAGCGCAGGCGCTCAGG
CCTCGGAGGCG

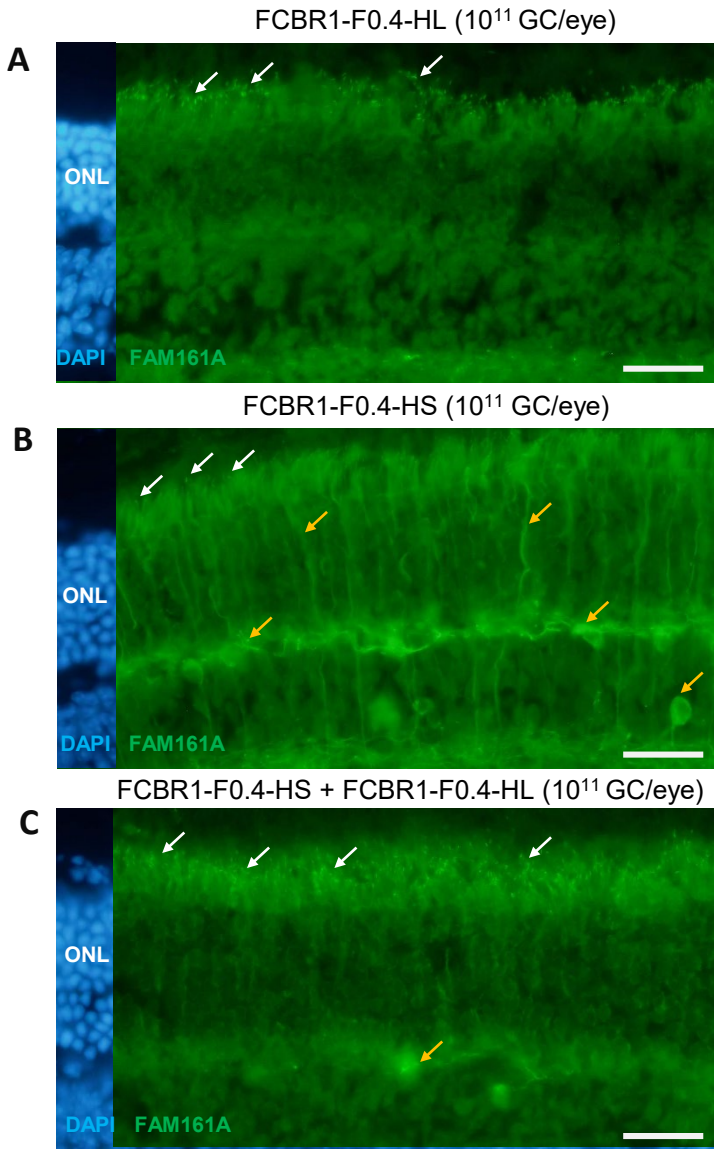
Appendix Figure S1. FAM161A-derived regulatory regions. (**A**) Genomic region of human FAM161A is presented with the localisation of FCBR1, FCBR2 and F0.4 elements used to drive expression of HL or HS isoform via AAV vectors. The map corresponds to 1 to 40000 bp of genbank sequence NG028125.2 comprising the loci RPL31P30 and FAM161A. Gray box correspond to exon sequence, red box to the proximal FAM161A promoter and blue box to FCBR1 and FCBR2 region. (**B**) Sequences of FCBR1, FCBR2 and F0.4 elements with potential crx binding sites in bold and grey boxes.

Appendix Figure S2



Appendix Figure S2. Spread expression in the photoreceptor driven by AAV2/8-IRBP-GRK1-FAM161A vectors is independent of the vector dose. Subretinal injections of 10^8, 10^9, 10^{10} GC/eye of AAV2/8-IRBP-GRK1-FAM161A show the similar pattern of expression in the all photoreceptor body (arrows). The image of 10^{10} GC/eye is a large field of the image presented in Figure 2I. Scale bars are 25 μm.

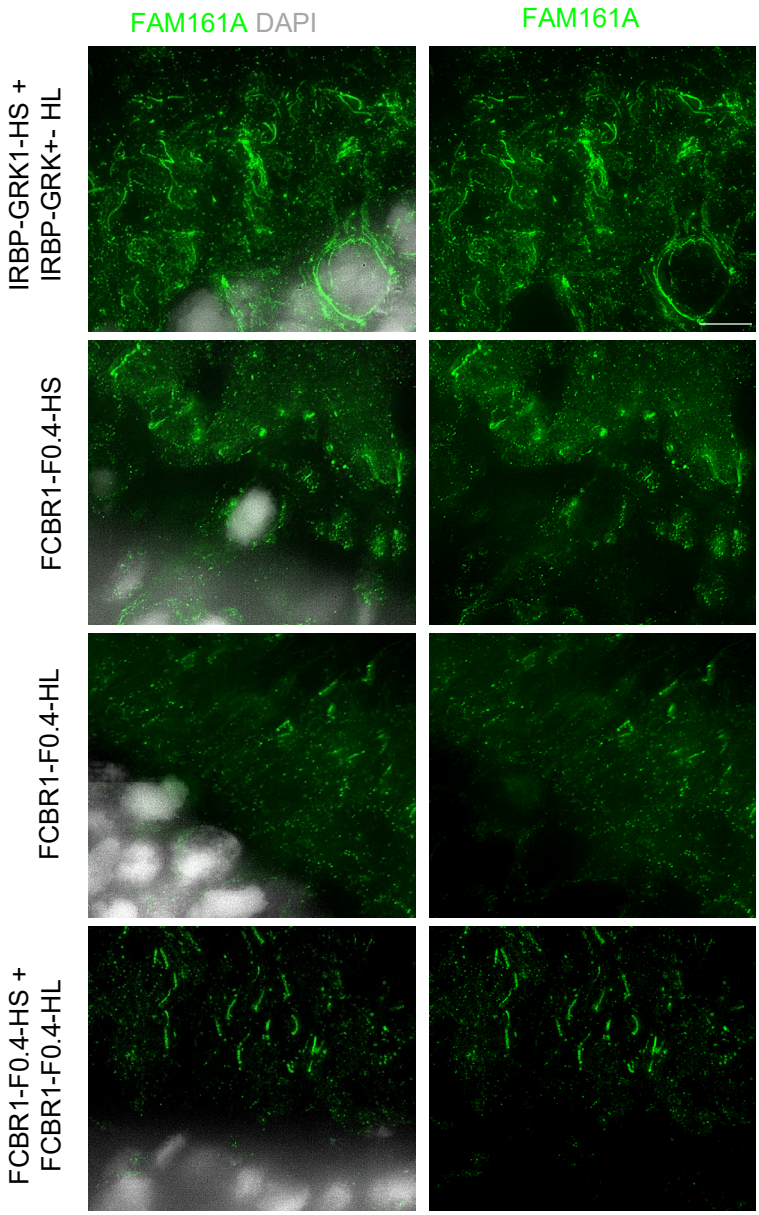
Appendix Figure S3



Appendix Figure S3. Expression pattern of FAM161A using AAV2/8-FCBR1-F0.4 vectors. *Fam161a*$^{tm1b/tm1b}$ mice were subretinally injected with AAV2/8-FCBR1-F0.4-HL (**A**), AAV2/8-FCBR1-F0.4-HS (**B**) or AAV2/8-FCBR1-F0.4-HS + AAV2/8-FCBR1-F0.4-HL (**C**) vectors (10^{11} GC/eye) at PN15 and FAM161A expression was analyzed 3 months later. Note for all conditions, the appearance of FAM161A-positive CC (white arrows), but the expression of the HS isoform induced ectopic expression of the protein (yellow arrows). (**C**) The co-injection of AAV2/8-FCBR1-F0.4-HS and AAV2/8-FCBR1-F0.4-HL produced a targeted and homogenous expression of the FAM161A in CC (white arrows) with also sometimes stronger labeling (orange arrow). ONL: outer nuclear layer; HS: short isoform; HL: human long isoform; white arrows: correct labelling; orange arrows: modified labelling. Scale bars are 25 μm.

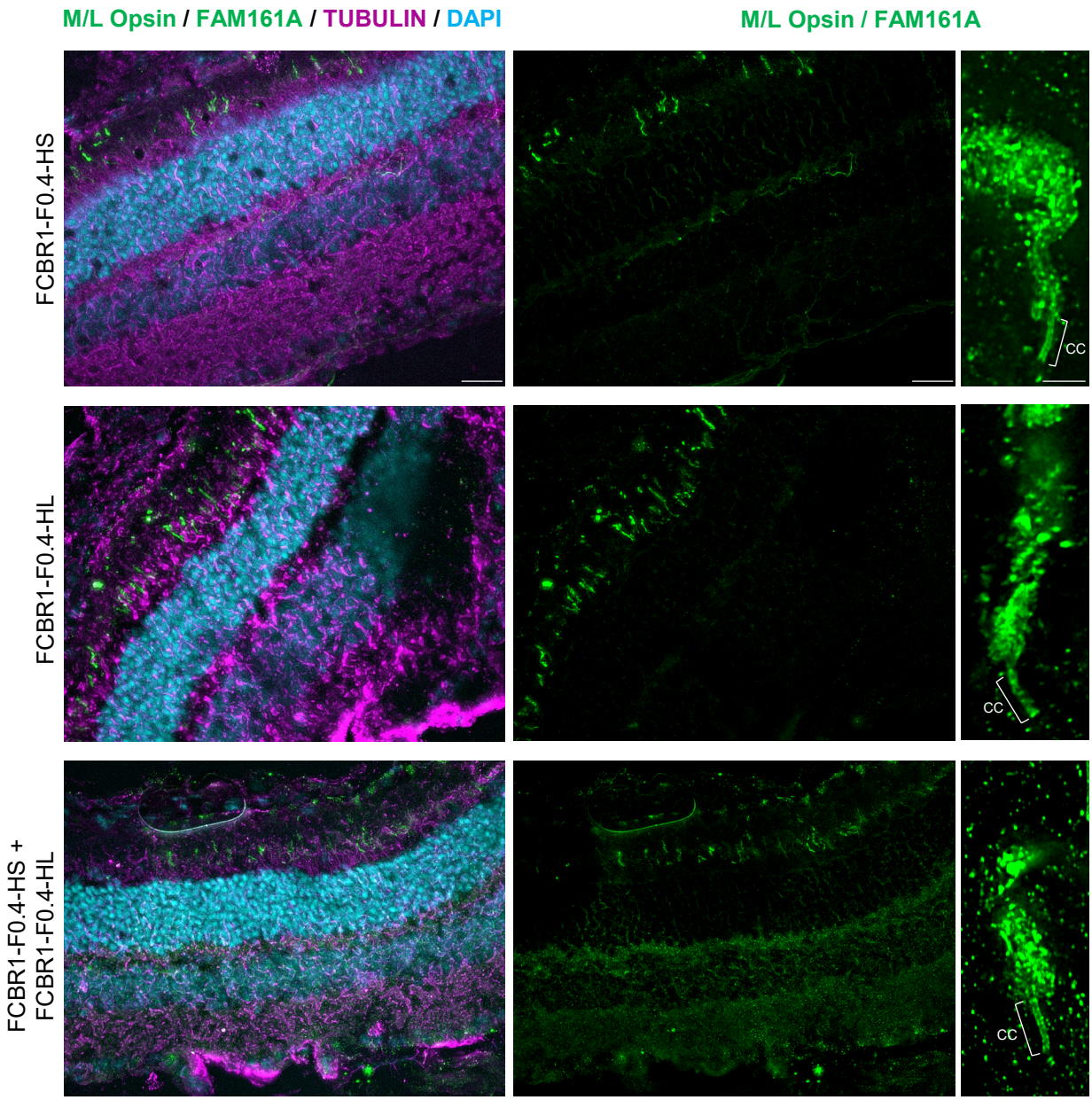
Data information: Results in (A to B) are representative of three (FCBR1-HS 10^{11}), four (FCBR1-HL 10^{11}), and three (FCBR1 HS+HL 10^{11}), independent experiments, n = 5 eyes per group at least.

Appendix Figure S4



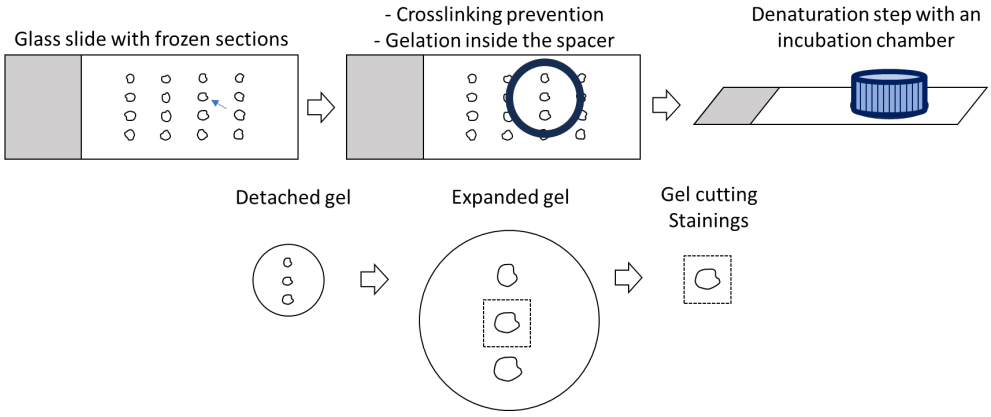
Appendix Figure S4. Abscence of ectopic expression pattern of FAM161A in the ONL after AAV2/8-FCBR1-F0.4-HS + AAV2/8-FCBR1-F0.4-HL treatment. *Fam161a*$^{tm1b/tm1b}$ retina 3 month after treatment with AAV2/8-IRBP-GRK-HS + AAV2/8-IRBP-GRK-HL, AAV2/8-FCBR1-F0.4-HL, AAV2/8-FCBR1-F0.4-HS or AAV2/8-FCBR1-F0.4-HS + AAV2/8-FCBR1-F0.4-HL vectors (10^{11} GC/eye) at PN15 are shown using Uex-M. Note the appearance of FAM161A labeling in the nucleus layer (white), with all treatments except with the combined injection with AAV2/8-FCBR1-F0.4-HS and AAV2/8-FCBR1-F0.4-HL. Scale bar is 20 μm.

Appendix Figure S5



Appendix Figure S5. Restoration of FAM161A expression in the CC of cones. *Fam161a*$^{tm1b/tm1b}$ retina 3 month after treatment with AAV2/8-FCBR1-F0.4-HS, AAV2/8-FCBR1-F0.4-HL or AAV2/8-FCBR1-F0.4-HS + AAV2/8-FCBR1-F0.4-HL vectors (10^{11} GC/eye) at PN15 are shown using Uex-M. Note the appearance of FAM161A labeling in the cc of cones identified by labeling of their outersegment with M/L-opsin antibody (green). Scale bars are 100 μm and 5 μm for low and high magnification respectively.

Appendix Figure S6



Appendix Figure S6. Flow chart of U-Ex-M process of retinal sections.

Appendix Table S1. Primer sequences targeting the regulatory or coding regions included in the different plasmid constructed for the study.

IRBP enhancer	forward	TGGAGGCAGAGGAGAAGG
IRBP enhancer	reverse	GCTTTATGAAGGCCAAAGAGG
hGRK1 promoter	forward	GGGCCCCAGAAGCCTGGTGG
hGRK1 promoter	reverse	GCCCTTGGCCTGTGGCCCG
FCBR1	forward	GGGCTCAGACCTTAGAGACGGG
F0.4	forward	TCCTCCATCAACTGTCTCCTTC
F0.4	reverse	CGCCTCCGAGGCCTGAGC
FCBR2	forward	CTCTCTCATTCTTTACAAATTCAGC
FCBR2	reverse	TGATTTTGCACTGGATCCATTCTG
EFS	forward	GGCTCCGGTGCCCGTCAG
EFS	reverse	TCACGACACCTGTGTTCTGGCG
FAM161A cDNA	forward	ATGGCCACCTCCCACCG
FAM161A cDNA	reverse	TTGAAGAATCACACTGA
WPRE4	forward	GGAATTCGAGCATCTTACCG
WPRE4	reverse	CTTCCCCGACAACACCAC