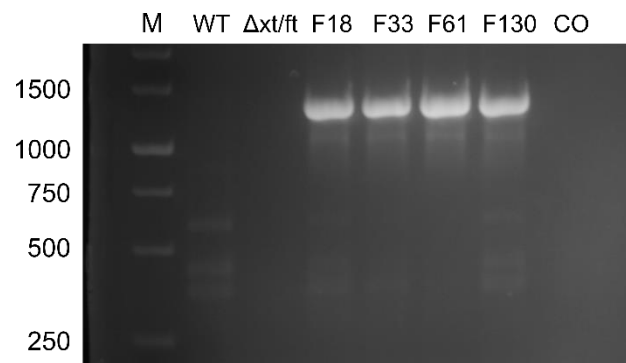


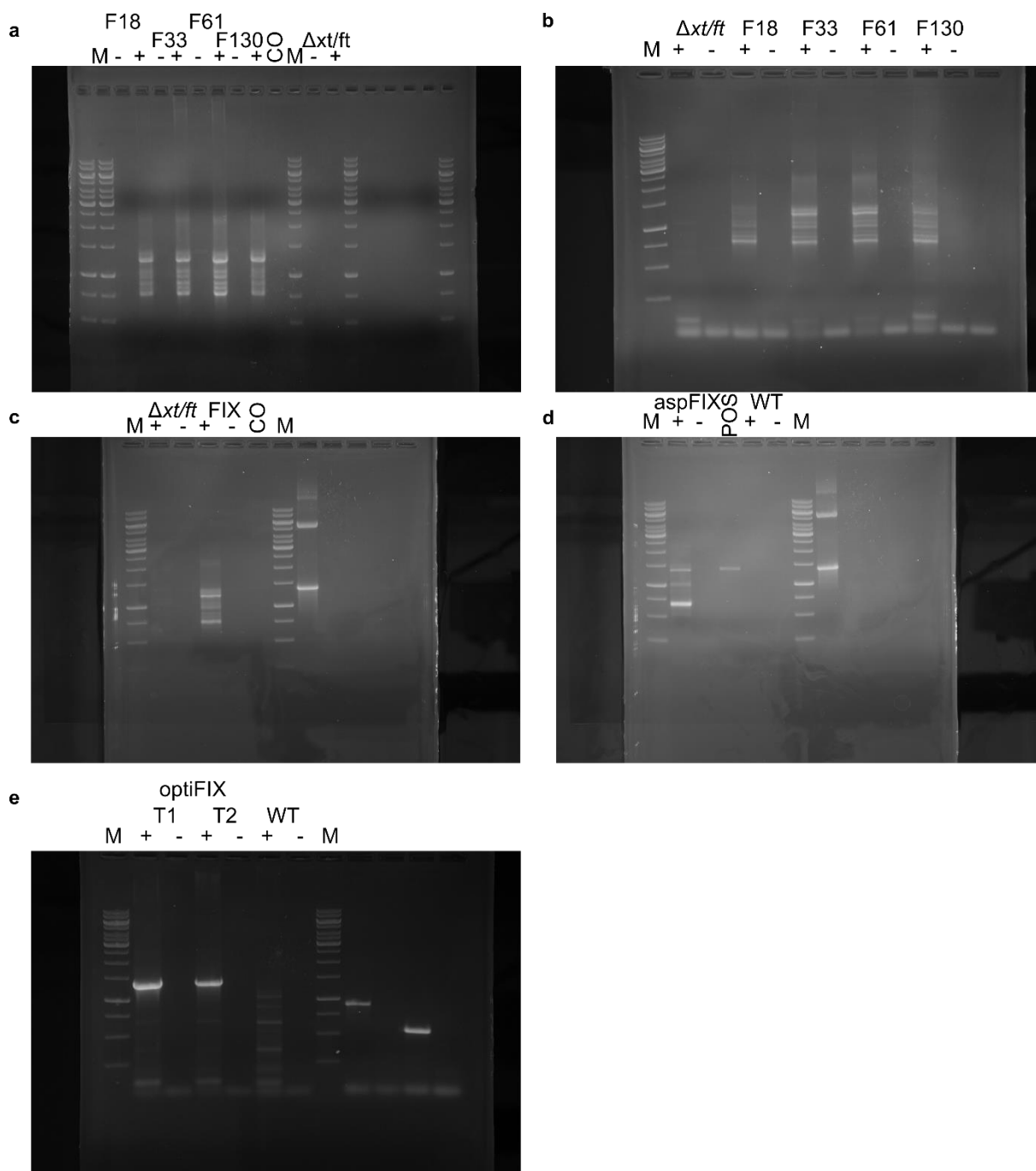
SUPPLEMENTARY INFORMATION

Title: Expression of a human cDNA in moss results in spliced mRNAs and fragmentary protein isoforms

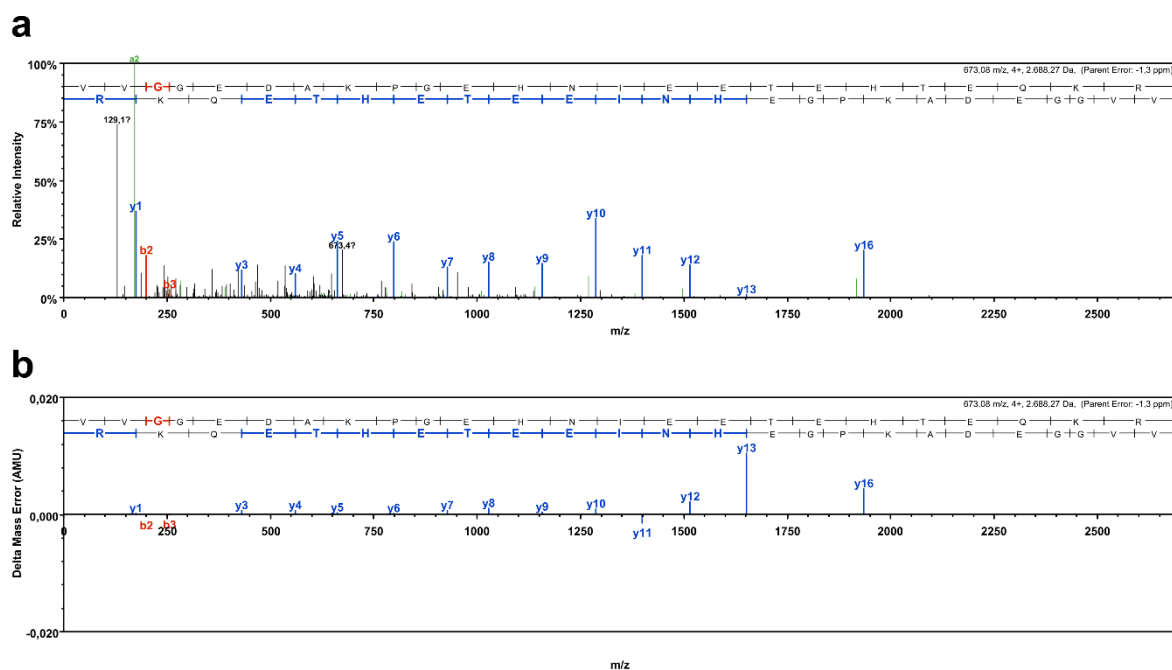
Authors: Oguz Top, Stella W. L. Milferstaedt, Nico van Gessel, Sebastian N. W. Hoernstein, Bugra Özdemir, Eva L. Decker, Ralf Reski



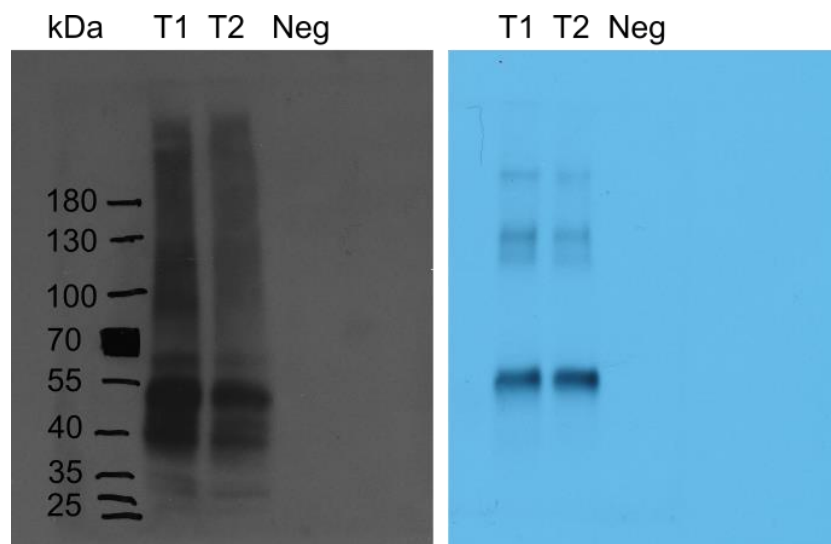
Supplementary Fig. 1: The presence of complete FIX CDS in transgenic lines. Genomic DNA from protonema cultures of 4 FIX-transgenic lines (F), the parental line $\Delta xt/ft$, and WT was prepared and used for PCR using the primers FIXfwdB and FIXrevB. M: 1 kb Marker (Thermo Fisher Scientific), CO: Water control.



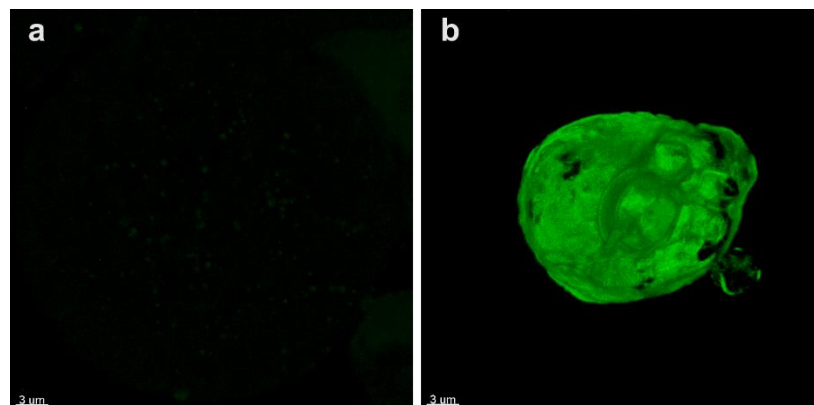
Supplementary Fig. 2: Unedited agarose gel electrophoresis images of Fig. 1a (a), Fig. 1b (b), Fig. 1c (c), Fig. 5a (d) and Fig. 5d (e). M: 1 kb Marker (Thermo Fisher Scientific), -: without reverse transcription, +: with reverse transcription, CO: Water control, Pos: PCR with aspFIX plasmid used as positive control.



Supplementary Fig. 3: Identification of the peptide VVGEDAKPGEHNIEETEHTQKR by MS. a HCD fragment ion spectrum of the identified peptide. **b** Fragment mass error distribution of the b- and y-ion series.



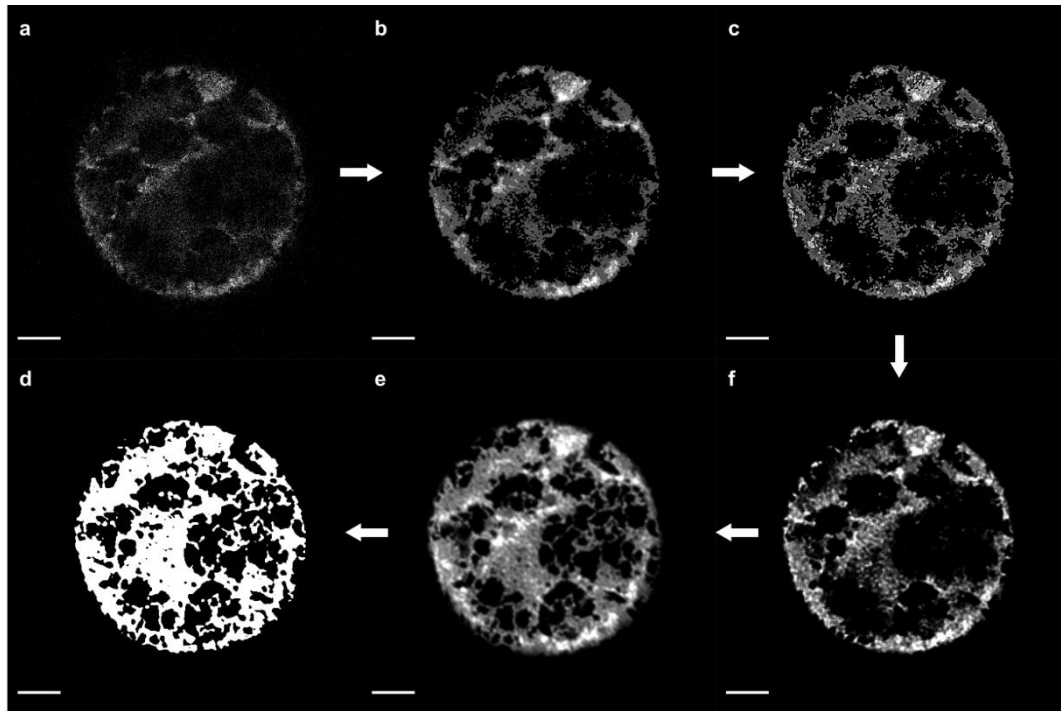
Supplementary Fig. 4: Unedited blots for Fig. 4 (Left) and Fig. 5 (Right). Immunodetection of extracellular FIX produced by two transfections (anti-FIX Ab 1:5,000). Culture supernatant of non-transformed cells was used as negative control (Neg). T1, T2: Respective Two different transient transfections with the FIX cDNA-based expression plasmid (Left) and optiFIX expression plasmid (Right). PageRuler Prestained Protein Ladder (Thermo Fisher Scientific, Catalog Number: 26616) was used and marker bands were marked on the film before scanning.



Supplementary Fig. 5: 3D rendered confocal Z-stacks of Physcomitrella protoplasts transfected with FH-Citrine (a) and optiFH-Citrine (b). Scale bar is 3 μ m.

FIX aa	M G A S R S V R L A F F L V V L V L A A L A E A	25
FIX	ATGGGGGCGATCGAGGAGCTTTCGATTGGCTTTCTTCTGGTTGTTTGGTAGTATTAGCAGCCTTAGCTGAGGCA	75
aspFIX	ATGGGGGCGATCGAGGAGCTTTCGATTGGCTTTCTTCTGGTTGTTTGGTAGTATTAGCAGCCTTAGCTGAGGCA	75
optiFIX	ATGGGGGCGATCGAGGAGCTTTCGATTGGCTTTCTTCTGGTTGTTTGGTAGTATTAGCAGCCTTAGCTGAGGCA	75
consensus	*****	
FIX aa	T V F L D H E N A N K I L N R P K R Y N S G K L E	50
FIX	ACGTTTTTCTTGATCTGAAGAACCCCAACAAATCTGAATCGGCCAAAGAGGTATAATCTCGGAAAATTGGAA	150
aspFIX	ACGTTTTTCTTGATCTGAAGAACCCCAACAAATCTGAATCGGCCAAAGAGGTATAATCTCGGAAAATTGGAA	150
optiFIX	ACGTTTTTCTTGATCTGAAGAACCCCAACAAATCTGAATCGGCCAAAGAGGTATAATCTCGGAAAATTGGAA	150
consensus	*****	
FIX aa	E F V Q G N L E R E C M E E K C S F E E A R E V F	75
FIX	GAGTTTGTCAAGGGAACCTTGAGAGAGAATGTATGGAAGAAAAGTGTAGTTTGAAGAAGCACGAGAAGTTTT	225
aspFIX	GAGTTTGTCAAGGGAACCTTGAGAGAGAATGTATGGAAGAAAAGTGTAGTTTGAAGAAGCACGAGAAGTTTT	225
optiFIX	GAGTTTGTCAAGGGAACCTTGAGAGAGAATGTATGGAAGAAAAGTGTAGTTTGAAGAAGCACGAGAAGTTTT	225
consensus	*****	
FIX aa	E N T E R T T T E F W K Q Y V D G D Q C E S N P C L	100
FIX	GAAACACTGAAGAACAACCTGAATTTGGAAGCAGTATGTTGATGGAGATCAGTGTGATCCATCCATGTTTA	300
aspFIX	GAAACACTGAAGAACAACCTGAATTTGGAAGCAGTATGTTGATGGAGATCAGTGTGATCCATCCATGTTTA	300
optiFIX	GAAACACTGAAGAACAACCTGAATTTGGAAGCAGTATGTTGATGGAGATCAGTGTGATCCATCCATGTTTA	300
consensus	*****	
FIX aa	N G G S C K D D I N S Y E C W C P F C F E G K N C	125
FIX	AATGGCGGCGATTGCAAGGATGACATTAATCTCTATGAATGTGGTGCCCTTTCGATTGAAGGAAAGAACTGT	375
aspFIX	AATGGCGGCGATTGCAAGGATGACATTAATCTCTATGAATGTGGTGCCCTTTCGATTGAAGGAAAGAACTGT	375
optiFIX	AATGGCGGCGATTGCAAGGATGACATTAATCTCTATGAATGTGGTGCCCTTTCGATTGAAGGAAAGAACTGT	375
consensus	*****	
FIX aa	E L D V T C N I K N G R C E O F C K N S A D N K V	150
FIX	GAATTAGATGTAACTGTAACTTAAGAATGCCAGATGCGAGCAGTTTGTAAAAATAGTGTGATAACCAAGCTG	450
aspFIX	GAATTAGATGTAACTGTAACTTAAGAATGCCAGATGCGAGCAGTTTGTAAAAATAGTGTGATAACCAAGCTG	450
optiFIX	GAATTAGATGTAACTGTAACTTAAGAATGCCAGATGCGAGCAGTTTGTAAAAATAGTGTGATAACCAAGCTG	450
consensus	*****	
FIX aa	V C S C T E G Y R L A E N Q K S C E P A V P F P C	175
FIX	GTTTCCGCTGATCGAGGATATCGACTTTCGAGAACCCCTGATGAGCCAGCTGTACCCCTTCCCAAGT	525
aspFIX	GTTTCCGCTGATCGAGGATATCGACTTTCGAGAACCCCTGATGAGCCAGCTGTACCCCTTCCCAAGT	525
optiFIX	GTTTCCGCTGATCGAGGATATCGACTTTCGAGAACCCCTGATGAGCCAGCTGTACCCCTTCCCAAGT	525
consensus	*****	
FIX aa	G R V S V S Q T S K L T R A E T V F P D V D Y V N	200
FIX	GGAAGAGTTTCTGTTTCACAACTTCAAGCTACCCCGTCTGAGACTGTTTTCTGATGTGGACTATGTAAAT	600
aspFIX	GGAAGAGTTTCTGTTTCACAACTTCAAGCTACCCCGTCTGAGACTGTTTTCTGATGTGGACTATGTAAAT	600
optiFIX	GGAAGAGTTTCTGTTTCACAACTTCAAGCTACCCCGTCTGAGACTGTTTTCTGATGTGGACTATGTAAAT	600
consensus	*****	
FIX aa	S T E A E T I L D N I T Q S T Q S F N D F T R V V	225
FIX	TCTAGTGAAGTGAACCAATTTGGATAACATCACTCAAGCACCACCAATCATTAAATGACTTCACTCGGTTGTT	675
aspFIX	TCTAGTGAAGTGAACCAATTTGGATAACATCACTCAAGCACCACCAATCATTAAATGACTTCACTCGGTTGTT	675
optiFIX	TCTAGTGAAGTGAACCAATTTGGATAACATCACTCAAGCACCACCAATCATTAAATGACTTCACTCGGTTGTT	675
consensus	*****	
FIX aa	G G E D A K P C Q F P W Q V V L N G K V D A F C G	250
FIX	GGTGGAGAAGATGCCAAACAGTCAATCCCTTGGCAGCTTGTGTAAGTGAAGTTGATGCATTCTGTGGA	750
aspFIX	GGTGGAGAAGATGCCAAACGGGACATCCCTTGGCAGCTTGTGTAAGTGAAGTTGATGCATTCTGTGGA	750
optiFIX	GGTGGAGAAGATGCCAAACGGGACATCCCTTGGCAGCTTGTGTAAGTGAAGTTGATGCATTCTGTGGA	750
consensus	*****	
FIX aa	G S I V N E K W I V T A A H C V E T G V K I T V V	275
FIX	GGCTCTATCGTTAATGAAAAATGATTGTAAGTCTGCCCACTGTGTGAAGTGGTGTAAAAATACAGTTGTC	825
aspFIX	GGCTCTATCGTTAATGAAAAATGATTGTAAGTCTGCCCACTGTGTGAAGTGGTGTAAAAATACAGTTGTC	825
optiFIX	GGCTCTATCGTTAATGAAAAATGATTGTAAGTCTGCCCACTGTGTGAAGTGGTGTAAAAATACAGTTGTC	825
consensus	*****	
FIX aa	A G E H N I E E T E H T E Q K R N V I R I I P H H	300
FIX	CGAGTGAACATAATTTGAGGAGACAGACATACAGAGCAAAAGCGAAATGATTGCAATTTCTCCACCAC	900
aspFIX	CGAGTGAACATAATTTGAGGAGACAGACATACAGAGCAAAAGCGAAATGATTGCAATTTCTCCACCAC	900
optiFIX	CGAGTGAACATAATTTGAGGAGACAGACATACAGAGCAAAAGCGAAATGATTGCAATTTCTCCACCAC	900
consensus	*****	
FIX aa	N Y N A A I N K Y N H D I A L L E L D E P L V L N	325
FIX	AACACAAATGACGCTATTAAATAGTACAAACATGACATTGCCCTTCTGGAAGTGGACGAACCTTAGTGCTAAAC	975
aspFIX	AACACAAATGACGCTATTAAATAGTACAAACATGACATTGCCCTTCTGGAAGTGGACGAACCTTAGTGCTAAAC	975
optiFIX	AACACAAATGACGCTATTAAATAGTACAAACATGACATTGCCCTTCTGGAAGTGGACGAACCTTAGTGCTAAAC	975
consensus	*****	
FIX aa	S Y V T P I C I A D K E Y T N I F L K F G S G Y V	350
FIX	AGCTACGTTACACCTATTGCTGCTGACAGGAGATACAGAACATCTTCCCTCAATTGGATCTGGCTATGTA	1050
aspFIX	AGCTACGTTACACCTATTGCTGCTGACAGGAGATACAGAACATCTTCCCTCAATTGGATCTGGCTATGTA	1050
optiFIX	AGCTACGTTACACCTATTGCTGCTGACAGGAGATACAGAACATCTTCCCTCAATTGGATCTGGCTATGTA	1050
consensus	*****	
FIX aa	S G W G R V F H K G R S A L V L O Y L R V P L V D	375
FIX	AGTGGCTGGGGAAGAGCTTCCACAAAGGAGATCAGCTTTAGTCTTCACTAGCTTAGAGTCCACTTGTGAC	1125
aspFIX	AGTGGCTGGGGAAGAGCTTCCACAAAGGAGATCAGCTTTAGTCTTCACTAGCTTAGAGTCCACTTGTGAC	1125
optiFIX	AGTGGCTGGGGAAGAGCTTCCACAAAGGAGATCAGCTTTAGTCTTCACTAGCTTAGAGTCCACTTGTGAC	1125
consensus	*****	
FIX aa	R A T C L R S T K F T I Y N N M F C A G F H E G G	400
FIX	CGAGCCACATGCTTTCGATCTACAAAGTTCACCATCTATACAAACATGTTCTGTGCTGCTTCCATGAAGAGGT	1200
aspFIX	CGAGCCACATGCTTTCGATCTACAAAGTTCACCATCTATACAAACATGTTCTGTGCTGCTTCCATGAAGAGGT	1200
optiFIX	CGAGCCACATGCTTTCGATCTACAAAGTTCACCATCTATACAAACATGTTCTGTGCTGCTTCCATGAAGAGGT	1200
consensus	*****	
FIX aa	R D S C Q G D S G C P H V T E V E G T S F L T G I	425
FIX	AGAGATTCAATGTCGAAGGATAGTGGGGACCCCATGTTACTGAAGTGAAGGAGCAGTTTCTTAAGTGAAT	1275
aspFIX	AGAGATTCAATGTCGAAGGATAGTGGGGACCCCATGTTACTGAAGTGAAGGAGCAGTTTCTTAAGTGAAT	1275
optiFIX	AGAGATTCAATGTCGAAGGATAGTGGGGACCCCATGTTACTGAAGTGAAGGAGCAGTTTCTTAAGTGAAT	1275
consensus	*****	
FIX aa	I S W G E E C A M K G K Y G I Y T K V S R Y V N W	450
FIX	ATTAGCTGGGCTGAAGAGTGTGCAATGAAGGTAAGTACGGCATCTATACCAAGGTATCCCGGTATGTCAACTGG	1350
aspFIX	ATTAGCTGGGCTGAAGAGTGTGCAATGAAGGTAAGTACGGCATCTATACCAAGGTATCCCGGTATGTCAACTGG	1350
optiFIX	ATTAGCTGGGCTGAAGAGTGTGCAATGAAGGTAAGTACGGCATCTATACCAAGGTATCCCGGTATGTCAACTGG	1350
consensus	*****	
FIX aa	I K E K T K L T H H H H H H H H -	466
FIX	ATTAGGAAAAAACCAACTAACCCACCACCATCATCATCATCATATAG	1401
aspFIX	ATTAGGAAAAAACCAACTAACCCACCACCATCATCATCATCATATAG	1401
optiFIX	ATTAGGAAAAAACCAACTAACCCACCACCATCATCATCATCATATAG	1401
consensus	*****	

Supplementary Fig. 6: Multiple sequence alignment of FIX, aspFIX and optiFIX CDS. The translated amino acid sequence of FIX (FIX aa) was added for better visualization. Alignment was performed and formatted for publication with MUSCLE via the "MSA" package for R.



Supplementary Fig. 7: The workflow of the image processing steps demonstrated with an exemplary single slice from a FIX-Citrine cell. The original, raw image from the microscope (**a**) was first processed with a median filter (**b**). The resulting image was subjected to an unsharp-mask operation (**c**). Afterwards, a Richardson-Lucy restoration algorithm was implemented (**f**). The pre-processing was completed with a local intensity equalization operation (**e**). The final version of the image was then thresholded using a local adaptive Otsu thresholding method, leading to the binary masks (**d**) that were subsequently used for selection of the voxels for quantification. Scale bar is 5 μm .

Supplementary Table 1: Moss microRNAs targeting human FIX CDS. Analysis was performed using psRNAtarget (http://plantgrn.noble.org/v1_psRNATarget/). The settings of this analysis: Maximum expectation: 5; Length for complementarity scoring (hspsize): 20; # of top target genes for each small RNA: 200; Target accessibility - allowed maximum energy to unpair the target site (UPE): 25; Flanking length around target site for target accessibility analysis: 17 bp in upstream / 13 bp in downstream; Range of central mismatch leading to translational inhibition: 9 – 11 nt. The microRNAs as well as their binding sites, expectation score, maximum energy to unpair the target site (UPE) and mode of action of microRNAs are shown.

MicroRNA	Alignment				Expectation	UPE	Mode of action
miR1044-3P	miRNA	20	UUUUGUUUAUACGUGAUGUU	1	5	13.996	Cleavage
	Target	1304	AAGGCAAAUAUGGAAUAU	1323			
miR1028c-3P	miRNA	21	CGAGA-AUUUGGAUGUUACGGU	1	5	21.847	Translational inhibition
	Target	495	GUCCUGUGAACCAAGCAGUGCCA	516			

Supplementary Table 2: The donor splice sites for all 87,533 annotated transcripts corresponding to the 32,926 protein-encoding genes of the current *P. patens* genome release v3.3.

Motif	Count	Percentage (%)	Motif	Count	Percentage (%)	Motif	Count	Percentage (%)
CAGGT	113,285	22.5460	TAAGT	1,610	0.3204	AGAGC	44	0.0088
AAGGT	84,769	16.8708	AGTGT	1,530	0.3045	GCTGC	44	0.0088
GAGGT	57,630	11.4695	TGAGT	1,492	0.2969	ATCAT	41	0.0082
CTGGT	20,698	4.1193	AAGGC	1,487	0.2959	CGGGC	41	0.0082
ATGGT	18,574	3.6966	CTCGT	1,385	0.2756	GATGC	41	0.0082
TTGGT	15,064	2.9980	GGAGT	1,330	0.2647	TTTGC	40	0.0080
TAGGT	12,835	2.5544	ATCGT	1,273	0.2534	TCGGC	39	0.0078
CAAGT	12,206	2.4292	GACGT	1,273	0.2534	GCGGC	36	0.0072
GTGGT	9,443	1.8793	GTTGT	1,259	0.2506	GGGGC	36	0.0072
ACGGT	9,246	1.8401	TTCGT	985	0.1960	GGAGC	35	0.0070
TGGGT	9,034	1.7980	GAGGC	981	0.1952	CATGC	34	0.0068
AGGGT	8,314	1.6547	ACCGT	914	0.1819	TCAGC	33	0.0066
TCGGT	7,588	1.5102	TACGT	908	0.1807	CCGGC	32	0.0064
CGGGT	6,757	1.3448	CTAGT	879	0.1749	TTCGC	32	0.0064
AAAGT	6,677	1.3289	CGTGT	870	0.1731	ATTGC	31	0.0062
CCGGT	6,223	1.2385	ATAGT	856	0.1704	CTTGC	31	0.0062
GCGGT	6,059	1.2059	CCCGT	804	0.1600	GGTGC	30	0.0060
AATGT	5,632	1.1209	TCCGT	742	0.1477	TCTGC	30	0.0060
CATGT	5,495	1.0936	TGCGT	673	0.1339	AGTGC	28	0.0056
GGGGT	5,371	1.0689	AGCGT	665	0.1323	TGTGC	26	0.0052
GAAGT	4,718	0.9390	GCCGT	516	0.1027	CTCGC	23	0.0046
GATGT	4,086	0.8132	TTAGT	506	0.1007	TGCGC	23	0.0046
CTTGT	3,916	0.7794	GTCGT	406	0.0808	ACTGC	22	0.0044
ATTGT	3,638	0.7240	CGCGT	396	0.0788	GAAAT	22	0.0044
AGAGT	2,987	0.5945	GGTGT	336	0.0669	CCTGC	21	0.0042
CCTGT	2,979	0.5929	GTAGT	300	0.0597	GGCGC	19	0.0038
ACTGT	2,904	0.5780	GGCGT	244	0.0486	TACGC	19	0.0038
CAGGC	2,860	0.5692	CTGGC	221	0.0440	ACAGC	18	0.0036
GCTGT	2,830	0.5632	ATGGC	199	0.0396	GTTGC	18	0.0036
TCTGT	2,772	0.5517	TAGGC	181	0.0360	ATCGC	17	0.0034
TTTGT	2,553	0.5081	TTGGC	89	0.0177	TTAGC	17	0.0034
ACAGT	2,426	0.4828	CAAGC	65	0.0129	TGAGC	16	0.0032
CCAGT	2,286	0.4550	AAAGC	55	0.0109	AACGC	15	0.0030
CACGT	2,273	0.4524	GAAGC	55	0.0109	CCAGC	15	0.0030
AACGT	1,965	0.3911	TGGGC	54	0.0107	GACGC	15	0.0030
GCAGT	1,948	0.3877	AATGC	52	0.0103	GTCGC	15	0.0030
TATGT	1,894	0.3769	GTGGC	52	0.0103	GTCAT	14	0.0028
TCAGT	1,882	0.3746	ACGGC	47	0.0094	TATGC	14	0.0028
CGAGT	1,876	0.3734	AGGGC	46	0.0092	GTAGC	13	0.0026
TGTGT	1,656	0.3296	GCAGC	46	0.0092	TCCGC	13	0.0026

<i>Motif</i>	<i>Count</i>	<i>Percentage (%)</i>	<i>Motif</i>	<i>Count</i>	<i>Percentage (%)</i>
AAGAT	12	0.0024	TGCAT	4	0.0008
AGCGC	12	0.0024	AGAAT	3	0.0006
ATAGC	12	0.0024	ATGAT	3	0.0006
CGTGC	12	0.0024	GCTAT	3	0.0006
CTAGC	12	0.0024	TAATG	3	0.0006
CGAGC	11	0.0022	TTGAT	3	0.0006
AAAAT	10	0.0020	ATAAT	2	0.0004
CAAAT	10	0.0020	CAACT	2	0.0004
CACGC	10	0.0020	CCAAT	2	0.0004
CCCGC	10	0.0020	GACTA	2	0.0004
GCCGC	10	0.0020	GATAT	2	0.0004
ACCAT	9	0.0018	TAATA	2	0.0004
TAAGC	9	0.0018	TGAAA	2	0.0004
TCAAT	9	0.0018	TGGAT	2	0.0004
ATTAT	8	0.0016	CTGAT	1	0.0002
GCAAT	8	0.0016	CTGTG	1	0.0002
TCTAT	8	0.0016	CTTAT	1	0.0002
CATAT	6	0.0012	GGTAA	1	0.0002
TAGAT	6	0.0012	TAAAT	1	0.0002
TATAT	6	0.0012	TAACA	1	0.0002
ACCGC	5	0.0010	TAAGA	1	0.0002
CAGAT	5	0.0010	TAGAG	1	0.0002
CGCGC	5	0.0010	TGATG	1	0.0002
TCGAT	5	0.0010	TGCTT	1	0.0002
TTCAT	5	0.0010	TTGTC	1	0.0002
TTTAT	5	0.0010	TTTCG	1	0.0002
TAAGG	4	0.0008	TTTTA	1	0.0002
TGAGG	4	0.0008	TTTTT	1	0.0002

Supplementary Table 3: The acceptor splice sites for all 87,533 annotated transcripts corresponding to the 32,926 protein-encoding genes of the current *P. patens* genome release v3.3.

<i>Motif</i>	<i>Count</i>	<i>Percentage (%)</i>	<i>Motif</i>	<i>Count</i>	<i>Percentage (%)</i>
<i>CAGGT</i>	90,834	18.0778	<i>AAGTG</i>	669	0.1331
<i>CAGGA</i>	54,132	10.7734	<i>TAGCG</i>	609	0.1212
<i>CAGAT</i>	45,474	9.0503	<i>AAGAG</i>	591	0.1176
<i>CAGGG</i>	36,713	7.3066	<i>AAGAC</i>	547	0.1089
<i>CAGGC</i>	36,350	7.2344	<i>AAGCA</i>	440	0.0876
<i>CAGAA</i>	22,672	4.5122	<i>GAGGT</i>	365	0.0726
<i>CAGAG</i>	21,613	4.3014	<i>AAGCC</i>	315	0.0627
<i>TAGGT</i>	19,555	3.8918	<i>AAGTA</i>	285	0.0567
<i>CAGTT</i>	19,239	3.8290	<i>AAGTC</i>	273	0.0543
<i>CAGCT</i>	18,854	3.7523	<i>AAGCG</i>	238	0.0474
<i>CAGTG</i>	15,897	3.1638	<i>GAGGA</i>	185	0.0368
<i>CAGAC</i>	14,770	2.9395	<i>GAGAT</i>	182	0.0362
<i>CAGCA</i>	11,526	2.2939	<i>GAGGG</i>	168	0.0334
<i>TAGGA</i>	9,029	1.7970	<i>GAGGC</i>	160	0.0318
<i>CAGCC</i>	8,266	1.6451	<i>GAGAA</i>	126	0.0251
<i>CAGTC</i>	7,702	1.5329	<i>GAGTG</i>	108	0.0215
<i>TAGGG</i>	7,378	1.4684	<i>GAGAG</i>	100	0.0199
<i>TAGAT</i>	7,164	1.4258	<i>GAGCT</i>	80	0.0159
<i>CAGTA</i>	6,745	1.3424	<i>GAGTT</i>	77	0.0153
<i>TAGGC</i>	6,040	1.2021	<i>GAGAC</i>	69	0.0137
<i>CAGCG</i>	6,004	1.1949	<i>GAGCA</i>	67	0.0133
<i>AAGGT</i>	4,063	0.8086	<i>CACAT</i>	51	0.0102
<i>TAGAG</i>	2,667	0.5308	<i>GAGTC</i>	48	0.0096
<i>TAGAA</i>	2,624	0.5222	<i>GAGTA</i>	46	0.0092
<i>TAGTT</i>	2,554	0.5083	<i>GAGCG</i>	39	0.0078
<i>TAGTG</i>	2,225	0.4428	<i>GAGCC</i>	37	0.0074
<i>TAGCT</i>	2,183	0.4345	<i>CACGC</i>	26	0.0052
<i>TAGAC</i>	2,119	0.4217	<i>CACAA</i>	16	0.0032
<i>AAGGA</i>	1,792	0.3566	<i>TACCT</i>	14	0.0028
<i>AAGGC</i>	1,483	0.2951	<i>CACAC</i>	11	0.0022
<i>TAGCA</i>	1,480	0.2946	<i>CACGG</i>	10	0.0020
<i>AAGAT</i>	1,342	0.2671	<i>TACAC</i>	10	0.0020
<i>AAGGG</i>	1,107	0.2203	<i>CACGT</i>	8	0.0016
<i>TAGTC</i>	967	0.1925	<i>TACAT</i>	8	0.0016
<i>TAGCC</i>	916	0.1823	<i>CACAG</i>	6	0.0012
<i>AAGAA</i>	784	0.1560	<i>TACAA</i>	6	0.0012
<i>TAGTA</i>	757	0.1507	<i>TACGA</i>	6	0.0012
<i>AAGTT</i>	685	0.1363	<i>TACGC</i>	6	0.0012
<i>AAGCT</i>	671	0.1335	<i>AACGT</i>	5	0.0010

<i>Motif</i>	<i>Count</i>	<i>Percentage (%)</i>	<i>Motif</i>	<i>Count</i>	<i>Percentage (%)</i>
AGAAT	5	0.0010	ACGAT	1	0.0002
CACCT	4	0.0008	AGCAT	1	0.0002
AACAA	3	0.0006	ATAAT	1	0.0002
AGGAT	3	0.0006	CATCT	1	0.0002
CACCA	3	0.0006	CTAAT	1	0.0002
CACTA	3	0.0006	CTGGC	1	0.0002
TACGT	3	0.0006	GATAT	1	0.0002
TACTC	3	0.0006	GCTAT	1	0.0002
AACAG	2	0.0004	GCTTC	1	0.0002
AACTT	2	0.0004	GGAAG	1	0.0002
ACTAA	2	0.0004	GGTAT	1	0.0002
CACTG	2	0.0004	GTGAT	1	0.0002
CACTT	2	0.0004	GTTAT	1	0.0002
GAAAT	2	0.0004	NAGGA	1	0.0002
GAACT	2	0.0004	NAGGG	1	0.0002
GACAA	2	0.0004	NAGTG	1	0.0002
GACAT	2	0.0004	TACCG	1	0.0002
GCAAT	2	0.0004	TACTA	1	0.0002
GCCAT	2	0.0004	TACTG	1	0.0002
GGCAT	2	0.0004	TGCGT	1	0.0002
GGGAT	2	0.0004	TGTGA	1	0.0002
AACAT	1	0.0002	TTATT	1	0.0002
ACCAT	1	0.0002	TTTAC	1	0.0002

Supplementary Table 4: Measured volumes and intensities in images (Supplementary Data 1) that used for Fig. 6c.

Sample	Total volume	Total intensity	Mean intensity
FIX1	533284	2596479	4.868848493
FIX2	882170	2588416	2.93414648
FIX3	1314016	5057256	3.848701994
optiFIX1	994087	27188045	27.34976416
optiFIX2	231369	8712866	37.65787984
optiFIX3	402599	28449343	70.66421675