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Supplemental Information

Engineering of PEDF-Expressing Primary Pigment Epithelial Cells by the *SB* Transposon System Delivered by pFAR4 Plasmids

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Supplemental Figures and Legends

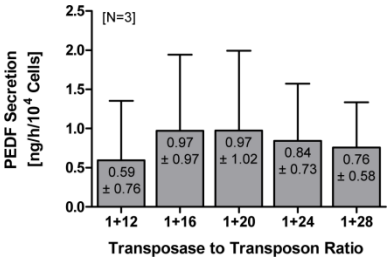

Figure S1. ELISA quantification of total PEDF secreted by primary human RPE cells transfected with varying ratios of SB100X transposase plasmid to PEDF transposon plasmid. For each donor sample, 2 transfections of $1x10^5$ cells with 0.038 µg to 0.017 µg pFAR4-CMV SB100X SV40 transposase and 0.462 µg to 0.483 µg pFAR4-ITRs CMV PEDF BGH plasmid DNA were carried out. PEDF secretion was analyzed in 3 human donor eyes (age: 61.7 ± 13.3 years; gender: 2 males and 1 female; time postmortem: 20.1 ± 1.4 hours; cultivation time before transfection: 45.7 ± 17.0 days). Data are presented as mean ± SD. Statistical analysis by one-way ANOVA with Bonferroni's Multiple Comparison Test showed no significant differences.

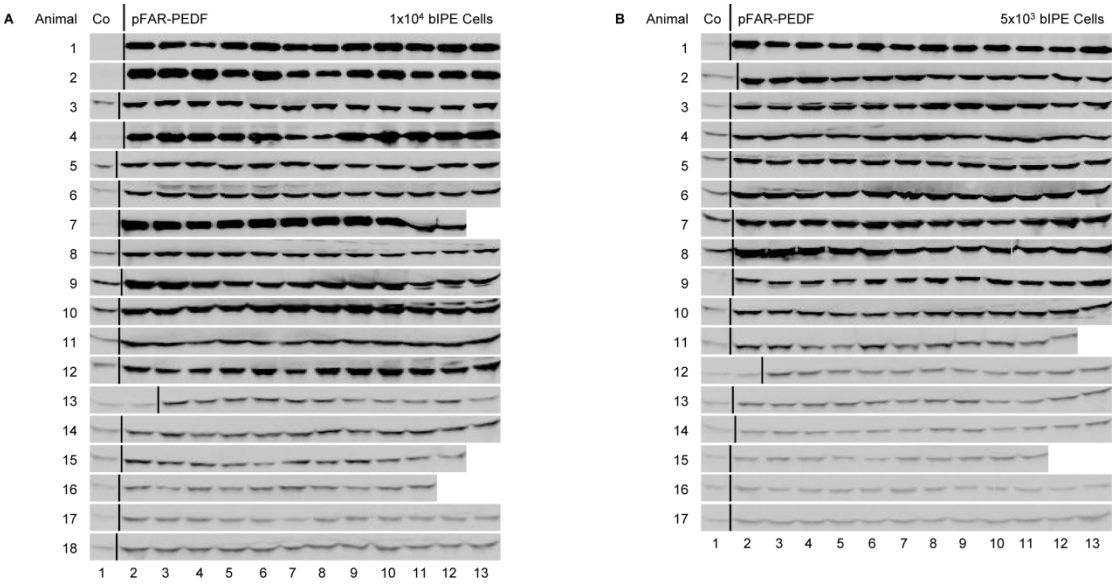

Figure S2. Western blot analysis of PEDF secretion by primary bovine IPE cells transfected with the pFAR4-ITRs CMV PEDF BGH transposon miniplasmid. Each analysis included 1 or 2 control transfections without the addition of miniplasmid DNA (column 1-2) and 10-12 transfections using 0.03 µg pFAR4-CMV SB100X SV40 transposase and 0.47 µg pFAR4-ITRs CMV PEDF BGH transposon miniplasmid DNA (columns 2/(3)-13). Culture supernatants were analyzed for total PEDF secretion using anti-PEDF antibodies 21 days after transfection. (**A**) Western blots of PEDF secreted by $1x10^4$ transfected IPE cells isolated from 18 bovine eyes (cultivation time before transfection: 26.9 ± 9.76 days). (**B**) Western blots of PEDF secreted by $5x10^3$ transfected IPE cells isolated from 17 bovine eyes (cultivation time before transfection: 27.9 ± 13.6 days). Note that the same exposure time was used for all chemiluminescence reactions.

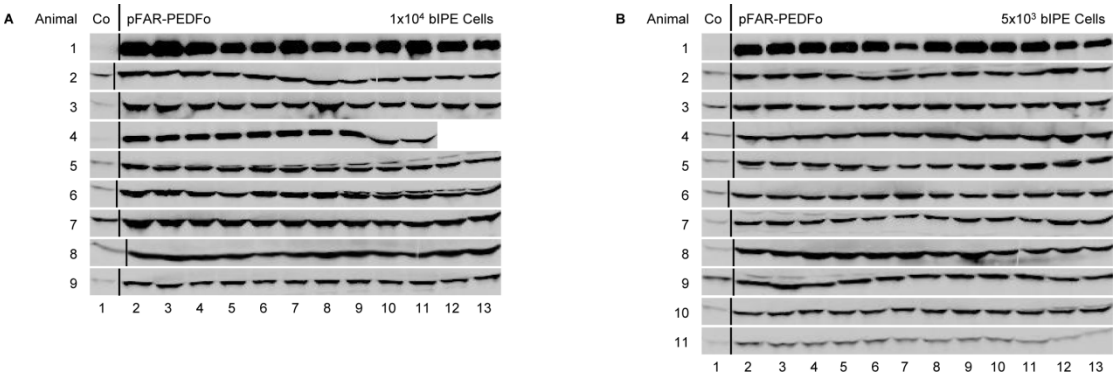

Figure S3. Western blot analysis of PEDF secretion by primary bovine IPE cells transfected with the pFAR4-ITRs CMV PEDFoptimized BGH transposon miniplasmid. Each analysis included 1 control transfection without the addition of miniplasmid DNA (column 1) and 10-12 transfections using 0.03 µg pFAR4-CMV SB100X SV40 transposase and 0.47 µg pFAR4-ITRs CMV PEDFoptimized BGH transposon miniplasmid DNA (columns 2-13). Culture supernatants were analyzed for total PEDF secretion using anti-PEDF antibodies 21 days after transfection. (**A**) Western blots of PEDF secreted by $1x10^4$ transfected IPE cells isolated from 9 bovine eyes (cultivation time before transfection: 22.6 ± 7.15 days). (**B**) Western blots of PEDF secreted by $5x10^3$ transfected IPE cells isolated from 11 bovine eyes (cultivation time before transfection: 29.2 ± 15.2 days). Note that the same exposure time was used for all chemiluminescence reactions.

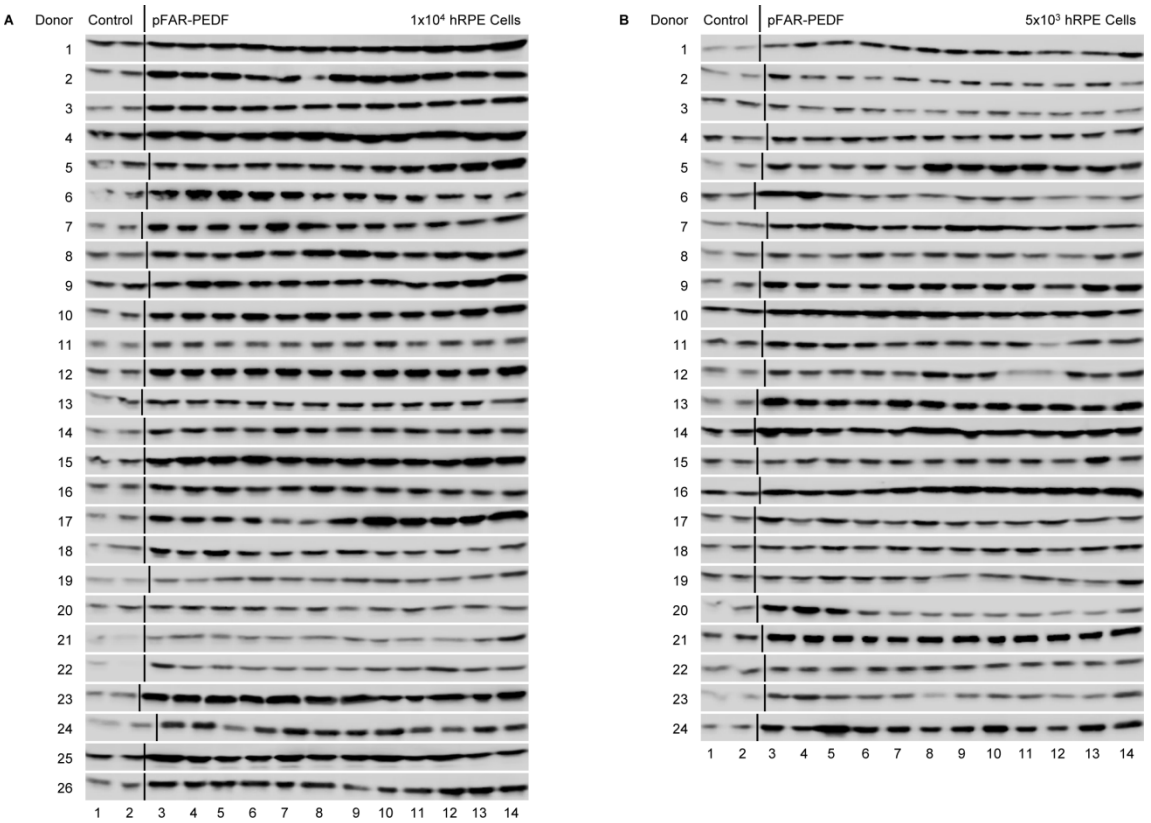

Figure S4. Western blot analysis of PEDF secretion by primary human RPE cells transfected with the pFAR4-ITRs CMV PEDF BGH transposon miniplasmid. Each analysis included 2 control transfections without the addition of miniplasmid DNA (columns 1-2) and 12 transfections using 0.03 µg pFAR4-CMV SB100X SV40 transposase and 0.47 µg pFAR4-ITRs CMV PEDF BGH transposon miniplasmid DNA (columns 3-14). Culture supernatants were analyzed for total PEDF secretion using anti-PEDF antibodies 21 days after transfection. (**A**) Western blots of PEDF secreted by $1x10^4$ transfected RPE cells isolated from 26 human donor eyes (age: 67.5 ± 12.0 years; gender: 13 males and 13 females; time postmortem: 31.7 ± 14.4 hours; cultivation time before transfection: 42.0 ± 14.4 days). (**B**) Western blots of PEDF secreted by $5x10^3$ transfected RPE cells isolated from 24 human donor eyes (age: 66.2 ± 14.6 years; gender: 14 males and 10 females; time postmortem:

32.9 ± 14.5 hours; cultivation time before transfection: 45.2 ± 15.8 days). Note that the same exposure time was used for all chemiluminescence reactions.

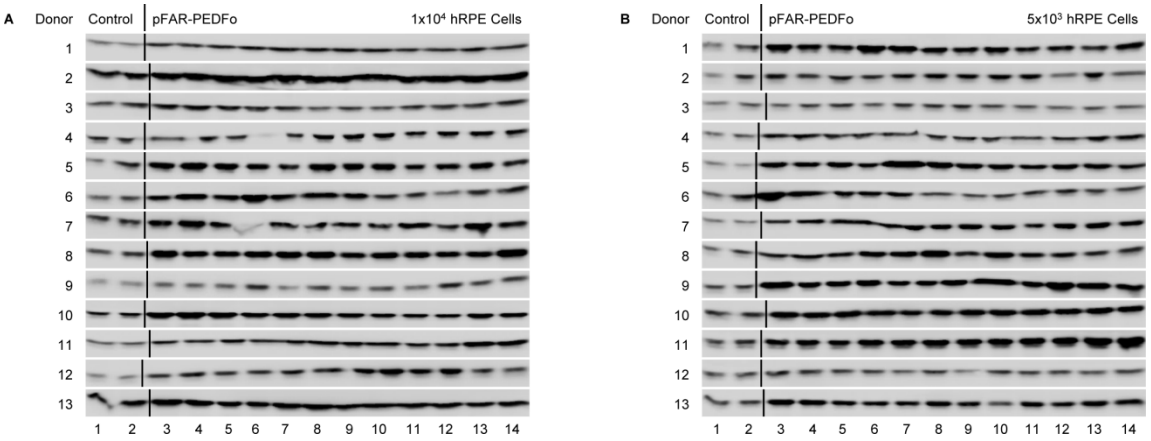

Figure S5. Western blot analysis of PEDF secretion by primary human RPE cells transfected with the pFAR4-ITRs CMV PEDFoptimized BGH transposon miniplasmid. Each analysis included 2 control transfections without the addition of miniplasmid DNA (columns 1-2) and 12 transfections using 0.03 µg pFAR4-CMV SB100X SV40 transposase and 0.47 µg pFAR4-ITRs CMV PEDFoptimized BGH transposon miniplasmid DNA (columns 3-14). Culture supernatants were analyzed for total PEDF secretion using anti-PEDF antibodies 21 days after transfection. (**A**) Western blots of PEDF secreted by $1x10^4$ transfected RPE cells isolated from 13 human donor eyes (age: 61.1 ± 16.3 years; gender: 7 males and 6 females; time postmortem: 33.5 ± 16.2 hours; cultivation time before transfection: 39.8 ± 17.1 days). (**B**) Western blots of PEDF secreted by $5x10^3$ transfected RPE cells isolated from 13 human donor eyes (age: 61.1 ± 16.3 years; gender: 8 males and 5 females; time postmortem: 32.5 ± 16.1 hours; cultivation time before transfection: 42.6 ± 18.6 days). Note that the same exposure time was used for all chemiluminescence reactions.

Supplemental Tables

Table S1. Western blot-based quantification of long-term PEDF secretion by primary bovine IPE cells after SB100X-mediated transfection. Each analysis included 2 control transfections without the addition of miniplasmid DNA and 2 transfections using 0.03 µg pFAR4-CMV SB100X SV40 transposase and 0.47 µg pFAR4-ITRs CMV PEDF BGH or pFAR4-ITRs CMV PEDFoptimized BGH miniplasmid DNA. Culture supernatants were analyzed for total PEDF secretion at times from 5 to 20 weeks. Western blot signal intensities of PEDF-transfected and PEDFo-transfected cells were normalized to the signal intensities of the control cells. Using an unpaired two-tailed t test, a statistical difference was observed between control and transfected cells, but not between cells transfected with pFAR-PEDF and pFAR-PEDFo at any time period.

		Time [Days]	N [Samples]	Signal Intensities			pFAR-PEDF versus pFAR-PEDFo
				Mean ± SD	Range		
$1x10^4$ bIPE Cells	**Control**	49 ± 1	18	1.00 ± 0.25	0.54-1.46		
	pFAR-PEDF			19.9 ± 22.8	1.95-74.9	***P*=0.0013	
	pFAR-PEDFo			17.1 ± 16.1	2.29-58.1	****P*=0.0002	Not significant
	Control	102 ± 9	18	1.00 ± 0.41	0.24-1.76		
	pFAR-PEDF			17.4 ± 13.2	1.34-42.6	*****P*<0.0001	
	pFAR-PEDFo			22.6 ± 18.8	0.89-58.5	*****P*<0.0001	Not significant
	Control	136 ± 10	18	1.00 ± 0.30	0.44-1.56		
	pFAR-PEDF			15.2 ± 14.5	2.93-65.6	****P*=0.0002	
	pFAR-PEDFo			13.5 ± 8.74	2.98-29.7	*****P*<0.0001	Not significant
	Control	221 ± 10	16	1.00 ± 0.36	0.30-1.70		
	pFAR-PEDF			5.97 ± 4.20	1.16-18.4	*****P*<0.0001	
	pFAR-PEDFo			6.00 ± 4.54	1.53-16.9	****P*=0.0001	Not significant
	Control	365 ± 3	16	1.00 ± 0.24	0.49-1.51		
	pFAR-PEDF			13.5 ± 23.7	0.50-73.8	**P*=0.0499	
	pFAR-PEDFo			12.2 ± 22.1	0.19-76.3	Not significant	Not significant
$5x10^3$ bIPE Cells	**Control**	49 ± 1	11	1.00 ± 0.24	0.54-1.46		
	pFAR-PEDF			15.2 ± 14.4	1.75-55.6	*****P*<0.0001	
	pFAR-PEDFo			12.4 ± 12.5	1.28-57.0	*****P*<0.0001	Not significant
	Control	103 ± 9	11	1.00 ± 0.40	0.24-1.76		
	pFAR-PEDF			13.9 ± 12.3	2.56-43.9	*****P*<0.0001	
	pFAR-PEDFo			19.1 ± 17.3	1.00-50.6	*****P*<0.0001	Not significant
	Control	136 ± 9	11	1.00 ± 0.29	0.44-1.56		
	pFAR-PEDF			10.2 ± 6.87	2.08-25.1	*****P*<0.0001	
	pFAR-PEDFo			8.60 ± 7.55	1.84-27.8	*****P*<0.0001	Not significant
	Control	221 ± 9	10	1.00 ± 0.33	0.30-1.70		
	pFAR-PEDF			7.92 ± 7.18	1.09-30.2	****P*=0.0001	
	pFAR-PEDFo			7.88 ± 8.28	0.52-26.1	****P*=0.0007	Not significant
	Control	365 ± 3	10	1.00 ± 0.39	0.00-2.00		
	pFAR-PEDF			8.64 ± 11.1	0.44-37.8	***P*=0.0040	
	pFAR-PEDFo			8.79 ± 19.2	0.20-86.8	Not significant	Not significant

Table S2. Western blot-based quantification of long-term PEDF secretion in primary human RPE cells after SB100X-mediated transfection. Each analysis included 2 control transfections without the addition of miniplasmid DNA and 2 transfections using 0.03 µg pFAR4-CMV SB100X SV40 transposase and 0.47 µg pFAR4-ITRs CMV PEDF BGH or pFAR4-ITRs CMV PEDFoptimized BGH miniplasmid DNA. Culture supernatants were analyzed for total PEDF secretion at the times indicated. Western blot signal intensities of PEDF-transfected and PEDFo-transfected cells were normalized to the signal intensities of the control cells. Statistical analysis by unpaired two-tailed t test showed a significant difference between transfected and non-transfected cells. No statistical significant difference was observed between cells transfected with pFAR-PEDF and pFAR-PEDFo for the first 110 days after transfection. However, at 160 days, a statistical significant decrease in PEDF secretion by pFAR-PEDF transfected cells compared to pFAR-PEDFo transfected cells was observed.

		Time [Days]	N [Samples]	Signal Intensities		pFAR-PEDF versus pFAR-PEDFo	
				Mean ± SD	Range		
$1x10^4$ hRPE Cells	**Control**	31 ± 5	10	1.00 ± 0.15	0.74-1.26		
	pFAR-PEDF			6.36 ± 3.27	2.08-11.3	****P<0.0001	
	Control	27 ± 1	4	1.00 ± 0.22	0.73-1.27		
	pFAR-PEDFo			10.7 ± 4.86	5.07-16.7	**P=0.0071	Not significant
	Control	61 ± 10	30	1.00 ± 0.25	0.56-1.44		
	pFAR-PEDF			6.03 ± 4.57	0.93-19.3	****P<0.0001	
	Control	64 ± 8	14	1.00 ± 0.27	0.61-1.39		
	pFAR-PEDFo			8.07 ± 4.14	1.71-15.5	****P<0.0001	Not significant
	Control	107 ± 8	30	1.00 ± 0.24	0.43-1.57		
	pFAR-PEDF			6.84 ± 7.03	0.41-27.1	****P<0.0001	
	Control	108 ± 7	14	1.00 ± 0.23	0.51-1.49		
	pFAR-PEDFo			8.13 ± 4.98	1.13-15.5	****P<0.0001	Not significant
	Control	162 ± 14	20	1.00 ± 0.15	0.75-1.25		
	pFAR-PEDF			2.51 ± 1.72	0.34-6.03	***P=0.0004	
	Control	154 ± 4	8	1.00 ± 0.17	0.76-1.24		
	pFAR-PEDFo			4.83 ± 2.49	1.66-8.12	***P=0.0007	**P=0.0098
	Control	215 ± 20	10	1.00 ± 0.10	0.85-1.15		
	pFAR-PEDF			2.61 ± 2.12	0.78-7.33	*P=0.0278	
	Control	193 ± 0	2	1.00 ± 0.08	0.94-1.06		
	pFAR-PEDFo			8.10 ± 3.92	5.32-10.9	Not significant	*P=0.0135
$5x10^3$ hRPE Cells	**Control**	33 ± 5	14	1.00 ± 0.24	0.65-1.35		
	pFAR-PEDF			7.51 ± 4.84	2.13-19.4	****P<0.0001	
	Control	31 ± 6	8	1.00 ± 0.28	0.65-1.35		
	pFAR-PEDFo			6.88 ± 4.86	3.30-16.7	**P=0.0042	Not significant
	Control	62 ± 12	30	1.00 ± 0.23	0.56-1.44		
	pFAR-PEDF			7.29 ± 6.92	1.70-33.2	****P<0.0001	
	Control	64 ± 13	20	1.00 ± 0.19	0.76-1.24		
	pFAR-PEDFo			4.79 ± 2.75	0.81-10.7	****P<0.0001	Not significant
	Control	109 ± 10	26	1.00 ± 0.23	0.55-1.45		
	pFAR-PEDF			8.66 ± 11.4	1.32-55.8	**P=0.0012	
	Control	111 ± 11	16	1.00 ± 0.17	0.75-1.25		
	pFAR-PEDFo			8.47 ± 8.03	0.98-26.7	***P=0.0008	Not significant
	Control	162 ± 18	20	1.00 ± 0.25	0.56-1.44		
	pFAR-PEDF			3.20 ± 2.01	0.73-7.79	****P<0.0001	
	Control	153 ± 12	10	1.00 ± 0.29	0.56-1.44		
	pFAR-PEDFo			7.33 ± 7.90	1.35-23.8	*P=0.0208	*P=0.0379
	Control	216 ± 20	12	1.00 ± 0.40	0.43-1.57		
	pFAR-PEDF			2.87 ± 2.02	0.63-6.61	**P=0.0048	
	Control	201 ± 11	4	1.00 ± 0.47	0.43-1.57		
	pFAR-PEDFo			7.53 ± 1.32	6.33-9.40	****P<0.0001	***P=0.0010

Table S3. Primer sequences for integration site profiling of the pFAR4-ITRs CMV PEDF BGH transposon in human RPE cells.

SB_20_hmr	AACTTAAGTGTATGTAAACTTCCGACT
4mer primers	
atcc_asPE	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTHNHNNNatcc
ggat_asPE	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTBBBNNNggat
gaca_asPE	GGACTGGAGTTCAGACGTGTGCTCTTCCGATCTVVVNNNgaca
gcaa_asPE	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTVNVNVNgcaa
tgtc_asPE	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTHHHNNNtgtc
ttgc_asPE	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTHHHNNNttgc
SB-7	cttAAGTGTATGTAAACTTCCGACTTCA
PE_first	Gtgactggagttcagacgtg
SB_PE_noTA_BC_1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATCACGatGTAAACTTCCGACTTCAACTG
SB_PE_noTA_BC_2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATGTatGTAAACTTCCGACTTCAACTG
SB_PE_noTA_BC_3	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTAGGCatGTAAACTTCCGACTTCAACTG
SB_PE_noTA_BC_4	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGACCAatGTAAACTTCCGACTTCAACTG
PE nest	CAAGCAGAAGACGGCATACGAGAT_reverse_complement_of_Illumina_TruSeq_barcode_GTGACTGG AGTTCAGACGTGTGCTCTTCCGATCT
Illumina 1	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT