Murphy et al.:

Comparison of Human Recombinant Protein Coatings and Fibroblast-ECM to Matrigel for Induced Pluripotent Stem Cell Culture and Renal Podocyte Differentiation

Supplementary Data

Adaptation of culture medium for fHDF/TERT166 cells

Initially fHDF/TERT166 were cultured in DMEM (Gibco, cat. 11966-025) and Ham's F12 Nutrient Mix (Gibco, cat. 21765-029, 1:1) enriched with penicillin-streptomycin (100 U/mL and 100 µg/mL, Sigma, cat. P4333), Glutamax (2 mM, Gibco, cat. 35050038) and 10% fetal bovine serum (FBS) (Gibco, cat. 10270-106) at 36.5°C in 5% CO_2. Cells were usually plated at a ratio of 1:3 or 1:4. For this study, cells were switched to 5% FBS (using the same medium as above) in a single step.

Further modifications were made to adapt cells to serum-free media for potential future studies and applications. To change the cells to serum-free conditions, cells cultured in media containing 5% FBS were used as a starting point. Cells were seeded and grown to about 50% confluency, washed with DPBS before adding chemically defined media without FBS. The chemically defined media was prepared as follows, DMEM (Gibco, cat. 11966-025) and Ham's F12 Nutrient Mix (Gibco, cat. 21765-029) (1:1), enriched with penicillin-streptomycin (100 U/mL and 100 µg/mL, Sigma, cat. P4333), Glutamax (2 mM, Gibco, cat. 35050038), 5 µg/mL insulin with 5 µg/mL transferrin and 5 ng/mL sodium selenite (ITS, Sigma, cat. I1884), hydrocortisone (36 ng/mL, Sigma, cat. H0135), TGFβ (30 pg/mL, Peprotech, cat. 100-21) and heat-stable bFGF (10 ng/mL, Gibco, cat. PHG0360). Upon reaching confluency, fibroblasts were subcultured with 0.025% Trypsin-EDTA solution (Sigma, cat. T4049) reducing the incubation time to below 1 min.

Tab. S1:
STR profiles of iPSC lines and TERT-immortalized cell lines

STR	SFC086	SBAD3	SBAD2	fHDF/TERT166	RPTEC/TERT1
D8S1179	14,15	13,13	12,13	13,13	13,15
D21S11	27,31.2	28,31	28,29	28,28	29,30
D7S820	10,13	10,11	10,12	10,11	10,10
CSF1PO	11,13	11,12	10,12	11,11	11,11
D3S1358	14,15	15,17	15,16	15,15	14,16
TH01	6,9	9.3,9.3	6,9	6,9	9,9.3
D13S317	13,14	9,11	11,12	8,11	11,13
D16S539	9,9	13,14	11,14	12,13	11,12
D2S1338	20,2	23,24	17,18	17,17	19,19
D19S433	14,14	13,14	13,14	15,15.2	13,15
vWA	15,17	15,19	16,19	17,17	16,18
TPOX	8,8	8,8	8,12	8,8	8,11
D18S51	13,14	12,18	15,15	14,15	11,20
AMEL	X,X	X,X	X,Y	X,Y	X,Y
D5S818	10,11	13,13	12,13	12,13	9,11
FGA	20,22	23,24	22.2,23	18,24	21,22

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Tab. S2A: Top 50 up-regulated differentially expressed genes comparing iPSC grown on Geltrex with those grown on the four alternative coatings across three cell lines

Vitronectin			Laminin-521			Laminin-511			Fibroblast-ECM		
SBAD2	SBAD3	SFC086	SBAD2	SBAD3	SFC086	SBAD2	SBAD3	SFC086	SBAD2	SBAD3	SFC086
IL24	ADGRE1	FAM71F2	CFC1B	CCL28	FAM71F2	DAZL	DAP3	ETNK1	PAPSS2	CCL28	DIRAS3
ZEB2	MT1H	CCDC62	HES3	CLEC2L	ETNK1	TOX	RPAP2	RPL15	SIAH2	DUXB	CFC1
IL32	ZNF596	RPL15	TOX	GALNT9	CCDC62	ADRA2A	MBTPS1	MTRNR2L9	MT1H	S100A6	SUN3
FOXC2	TRIM43	EDA	HCK	RGS16	RPL15	PTPN2	TSSK4	FAM71F2	MT2A	PTHLH	FAM20A
MFAP5	TRIM43B	ETNK1	SHISA3	FOXN3	MTRNR2L9	CD24	ERBB4	CCDC62	MT1M	SERPINB3	FAM71F2
MT2A	CCL28	TEX54	GRIK3	OPN3	RPL17	CUZD1	RPL15	ST7L	LYPD1	MX2	PITX2
CALHM4	MX2	EDN2	DAZL	CCM2L	TEX54	DLL1	ZNF430	DAP3	DDX3X	SUN3	CALHM4
MFAP5	GALNT9	TMEM161B	PTPN2	TMOD1	ST7L	MAP3K8	OR1P1	USP17L3	MT1E	MT1H	IL13RA2
GBP2	LY6K	GLIPR1	MT1G	HTR1A	EDA	HTR7	ZNF705E	RPL17	HHIP	CIDEB	CER1
GATA6	ZEB2	ZNF430	TXNIP	SOGA1	CYP2C8	SFRP1	NEXMIF	AMY1C	MT1G	SERPINB4	CFC1B
SIAH2	GLIPR1	MTRNR2L9	ID2	ECSCR	ZNF430	BCL6	PRRT3	SMG1	KLF4	PITX2	SLC6A17
EDN2	KRT17	RHOT1	CD24	ZC3H6	C14orf178	PRICKLE3	RDH16	CYP2C8	TCERG1L	DSG3	CYP26A1
MT1H	NG	CFC1B	IFI16	MRAS	COX11	TRPM3	TAMM41	TEX54	VWCE	RGS16	MFAP5
PAPSS2	TMEM255A	C14orf178	MICU3	TCERG1L	TAMM41	YWHAE	EDA	TAMM41	LANCL2	CSHL1	SERPINB4
ACTBP9	CXXC4	CTBP2	MYO18B	LOXL1	RHOT1	LGR4	DLGAP1	ZNF430	PPIH	GLIPR1	KLF4
MYL7	MYL7	CD24	MEDAG	ADAMTS13	RBFOX1	FAM118A	RPL17	CYP2C19	MSANTD2	RASSF9	GLIPR1
DEFB4A	ADAMTS9	UEVLD	GFY	TMEM132B	DLGAP1	COL6A1	SPATA7	EDA	TRPC6	GATA6	LTF
PRSS23	FAM71F2	ZNF208	UEVLD	IL1RAPL2	TMEM161B	LRRC17	FER	TAS2R50	SBDS	NRN1	ANXA1
KCNMB1	GFY	FOXN3	POLM	ZNF790	TAS2R43	HIST1H1A	VEGFD	USP17L2	SPATC1L	MAGEA6	DNMT3L
C11orf88	USP17L19	PABPC3	OXER1	GPC1	USP12	NPIPB4	USP12	COX11	PSMC4	HNMT	ARHGDIB
PSG9	GPR20	TAMM41	SELENOP	ZNF705D	TAS2R50	GDPD2	NPTX1	NPHS1	FOXN3	MFAP5	SPP1
MT1E	FOXN3	ZNF207	NTF3	BACE1	ZNF207	INPPL1	RSL1D1	CFTR	HNRNPH1	TCERG1L	FAM20A
MT1M	FOXB2	FAM20A	PCDH8	MT2A	ANGPT1	.	RRM2	PRLHR	KCNMB1	CSH2	SERPINB3
RGS20	MFAP5	ACTC1	WNT4	POGLUT3	LYRM2	.	MTRNR2L9	ZNF606	WDR24	CSH2	LGALS3
GLIPR1	NPPB	MRGPRF	SIAH2	CELF5	CTBP2	.	TMEM161B	RPAP2	AHNAK	CACNG4	MYL7
FAM167A	MT1G	LYRM2	PLCL2	SPECC1L	CEP135	.	ATP6V1FNB	FBXL13	RALA	MSC	ACTC1
SLC46A1	RAD21L1	SLCO4C1	ADRA2A	MMP16	USP17L2	.	ANKRD20A1	C14orf178	RAB34	CYP26A1	TRPC6
CPLX3	RUFY2	CEP135	HIST1H1A	WDR24	HNRNPK	.	SERPINI2	WDR97	DAZ2	TNIP3	HHIP
LYPD1	RRH	CEP295NL	KIAA0895	ZDHHC14	DERL2	.	GFY	C10orf113	DYNLL2	ACTC1	LYPD1
CSRP1	TAC3	PTPN2	KRCC1	CNTFR	DCAF4L1	.	ISCA1	FKBP14	ARHGAP1	KRT17	RGS5
OR5H1	SLC25A2	BDP1	ZNF76	ZNF322	BDP1	.	CUBN	DLGAP1	MIA3	C19orf33	TRPM2
ARHGAP40	PXDNL	DERL2	YWHAE	TAC3	ZNF273	.	SEMA6D	CTBP2	CYP26A1	CSH1	PTH1R
EBI3	MT2A	PSMG2	CUZD1	ZNF765	ZNF883	.	CEP135	DCAF4L1	YBX1	HHIP	EPHA3
MRGPRF	CD226	DAPK2	ITGAM	COBLL1	ZNF493	.	ETNK1	ADAM23	NPIPB11	FAM20A	MFAP5
ANXA1	ANKRD1	TAS2R43	COPG2	RADX	FKBP14	.	CYP2C8	LRRC66	SERTAD3	TRPM2	KCNMB1
FAM71F1	CYP2U1	MRAS	AHSP	BAG4	MINAR2	.	OR2J3	TMEM161B	TMSB10	CHAD	CR1
KLF5	POGLUT3	ZNF493	DIS3L	TRPV1	FOXN3	.	VHLL	LTB4R2	TTK	MEOX2	KRT17
IGFBP6	PSMG2	ZNF883	DDIT3	DERL2	COX11	.	OR8D4	TSSK4	ZNF296	HSPB2	LIX1
PLAT	HTR1A	HS3ST2	LRRC75B	NEGR1	RWDD4	.	HMCN2	FER	GTF3C5	MFAP5	NPFFR2
KRT80	EDA	HES3	OTX2	MAGOHB	ZNF790	.	TEX54	FAM166A	CD24	HPGD	ANKRD65
LCP1	FAM167A	ZNF784	DRD4	CD24	ZNF107	.	ZNF493	ANKRD30B	SLC25A3	CNR1	EDN1
INSM1	GRPR	MYL7	C12orf57	ZMYND11	FER	.	ZNF273	USP12	DUSP8	FAM20A	SIAH2
ZFPM2	TEDC1	ZNF273	MRAS	CCDC102A	EBLN2	.	EIF3C	CD226	MRAS	GPC3	FEZF1
ANXA8L1	SIRT3	NDUFA9	BOC	SLCO4C1	HLA-DPB1	.	TAS2R31	GABRG1	CGRRF1	MX1	ANGPT1
MT1F	ITGAM	POGLUT3	RIPK2	HIST1H2BB	TREX2	.	TCP11X2	SNX22	SPINT2	TTC29	ZNF784
VGF	MFAP5	HSPD1	ZNF764	NPTX1	SLCO4C1	.	ZNF107	USP17L1	RASSF9	CR1	CFTR
PADI4	ZNF430	FAM189B	DLL1	HBS1L	ANKRD30B	.	ANKRD20A11P	TEX45	POLR2I	GJD2	HPGD
AHNAK	MTMR11	ZNF268	HIST1H3E	KLF15	TAS2R5	.	SYNJ2BP	HAP1	CSRP1	CHODL	FLRT3
TPM1	KIAA0895	HNRNPK	MAP3K8	PCDHGA12	CEP295NL	.	S1PR5	S1PR5	NPIPB4	NCF2	FXYD3
GREM1	CD7	SIAH2	CCM2L	FAM189B	NDUFA9	.	COX11	ZNF705E	AP4B1	MAP2	MAP2

Tab. S2B: Top 50 down-regulated differentially expressed genes comparing iPSC grown on Geltrex with those grown on the four alternative coatings across three cell lines

Vitronectin			Laminin-521			Laminin-511			Fibroblast-ECM		
SBAD2	SBAD3	SFC086	SBAD2	SBAD3	SFC086	SBAD2	SBAD3	SFC086	SBAD2	SBAD3	SFC086
GABRB1	DCAF12L1	SULF1	CYP4F22	DCAF12L1	CYB5A	KLHDC8A	FGFBP1	SERPINB3	SORCS2	DCAF12L1	ONECUT1
POTED	ZNF717	ONECUT1	CER1	ZNF717	JAG1	CER1	HSPA8	LEFTY1	CST1	ADGRL4	INSC
CYP4F22	PDE1B	ONECUT1	SERPINE1	PCDHA6	MARCH11	SERPINE1	LEFTY1	SATB2	OR52A5	PCDHA6	ATP8B3
ZNF248	KCNJ3	RFTN2	MYOCD	NBPF11	SPTB	ANXA1	CER1	BTBD8	LRRC4B	NIPSNAP1	HRC
POTEG	CCDC68	LEFTY2	GRIA1	OC90	LONP2	MFAP5	NODAL	SLC1A4	MYOCD	LRRC4B	FBXL16
GPR139	SGIP1	OC90	OR5P2	NBPF8	SCFD2	AADACL3	ACTG2	CYP2B6	FREM1	LYG1	LRRC4B
NPTX1	DPPA3	LURAP1L	OVCH2	TRPM6	NOVA1	SYTL2	LEFTY2	AADACL3	MROH1	GBX2	PLA2G2A
AMPH	CCDC152	DPPA5	AADACL3	CHCHD2	DYNC1H1	ACTG2	HMGN2	MYH2	NR5A1	TMEM266	RFTN2
PIWIL2	OC90	WNT8B	S100A10	CPNE7	MEIS3	CSH2	LEFTY2	HSPB2	NPTX1	ADGRL4	ONECUT1
DEFB134	NBPF8	DLG2	GALR1	FRMPD4	NBPF8	GALR1	TNFRSF1A	HHLA1	CCDC122	RFTN2	WNT8B
PIWIL3	HHLA1	NEK9	NBPF8	FNDC4	SYN3	CREB5	BHLHE40	SLC7A2	USH1C	HAS3	GABRE
PIWIL2	RGN	TNFSF4	FRMPD4	TRBC1	GNG3	SPP1	S100A10	NODAL	ADGRL4	USP19	SULF1
TSPYL5	DNAJA4	LONP2	SIPA1L2	ZNF16	KLF8	TRPM6	NMNAT3	LEFTY2	OPN3	ARHGAP36	PABPC1L2A
ZNF560	NBPF11	ATP1B2	OR5P3	S100A10	ATPAF2	HAPLN1	SERPINE1	S100A10	HTR1A	LINGO2	PDE8B
VAV1	KDR	AP1G2	UBASH3B	HAPLN1	EFNA3	PDGFB	AADACL3	LEFTY2	PIEZO2	NBPF8	SLC27A1
MEOX2	DSEL	THAP11	ACTA1	GPRC5A	S100A10	UQCRHL	CYP2B6	MYCBP2	LDHAL6B	SCG2	NBPF11
ZNF37A	BCL2L10	LEFTY2	NRK	CHMP4C	RASL12	ADAMTS5	CTHRC1	NIPSNAP1	BGN	HMX2	BTN3A2
CD8B	FRMPD4	MYCT1	ACTG2	CYP51A1	SBF2	PTPN1	CCDC68	ONECUT1	NBPF8	CAPN3	USH1C
ZFP3	AGMO	CYP11A1	CYP51A1	MMP10	PEPD	MYL9	MYL6	GPAT3	WNT8B	TSPAN18	ANKRD34B
TBX6	STC1	ATP8B3	LRRC20	POLD2	LAG3	PITPNM2	MMP10	CBR3	GOLT1A	CCKBR	SNTA1
DAZ2	GPRC5A	GRIN2D	ANXA1	ACO2	NELL2	TNC	PPIAL4H	GLUD1	ADGRL4	TRPC5	VSTM2B
RBM46	LURAP1L	LEFTY1	ADAMTS5	SNTA1	FBXL16	CSH1	DPPA3	COL12A1	NEK9	TNS4	KCNJ10
LMO3	PCDH10	NOVA1	KLHDC8A	HHLA1	PLD5	C1D	CYB5A	ACOT9	ERG	SLC37A2	EFHC2
POTEH	IQSEC2	NIPSNAP1	PXK	ALDH18A1	ACTG2	CLC	OR5H1	ZNF208	MEIOB	TRIM9	SLC29A4
GLB1L2	CAT	CYP1A1	MMP10	ADPRHL2	TFCP2L1	IL6R	PPP1R16B	LONP2	RFX2	NRXN2	DELE1
CCDC144NL	FNDC4	NBPF11	TMPPE	FAH	SNTA1	VIL1	CAV1	FAAH2	CSPG4	NBPF11	AHSP
BTN3A2	CNRIP1	TMEM25	STC1	KDR	AADACL3	LEFTY1	TAGLN2	ACTR8	COL25A1	CST4	KDR
CRYZ	PCDHA6	SETD5	TRIM33	CCDC152	TERF2	NQO1	BNC1	SIPA1L2	SLC25A2	GRAMD1C	GOLGA6D
PCDHB5	SLC7A2	KDR	DNM3	SETD5	ALDH1A3	ETV5	PARP8	CASP10	ARHGAP6	BTN3A2	SEMA5B
CACNA1H	CPNE7	KCNJ3	ZNF37A	HLA-C	CD8B	MYOF	CSH2	FMR1	F7	TLL1	STON1
KCNK2	GAB4	CSNK2B	SYTL2	NACAD	CSNK2A1	CNR1	C7orf57	ZNF438	AKAP14	SPAG4	ATP1B2
FBXO39	CYP2B6	TMEM169	CAV2	SOX15	PDGFB	RBBP8	TMEM102	C19orf33	SLC2A4	FAM24B	NRXN2
SLITRK5	PIK3AP1	SLC26A2	SLC7A10	BUD23	ACTR8	UTF1	TTC39B	KCNH8	LRRC39	STXBP5L	DHRS1
CASR	EPN3	INSC	IL6R	ADCY8	RIPOR1	ANXA3	CLCN3	ZNF200	SLC16A5	APOBEC3F	HMX2
NPAP1	OR52A1	ESRRG	ADAMTS1	UQCRHL	LRRC4B	ADAMTS1	RBM12	MAGEB1	UQCRHL	ACPP	PPP1R13B
DSCAM	SYNPO2	LRRC4B	UQCRHL	MOGS	NIPSNAP1	LAMA3	SOX15	IL1A	MET	BST2	TACR1
ANKRD30B	LAMA3	CST4	TRHDE	NIPSNAP1	HYAL1	GFOD1	SNAP29	RGN	GJA5	BTN3A1	TRAC
RUNDC3B	GLB1L2	AKR1C3	IQSEC2	SERPINE1	CYTL1	NUDT15	RNF13	CSNK2B	STON1	TMPRSS11E	RIPOR1
CNTN1	RGS4	SLC37A2	STARD9	COL7A1	ZNF16	CAV1	SETD4	DTX3L	NBPF11	ANKRD34B	GRIN2D
PIEZO2	NIPSNAP1	RIPOR1	SLC2A13	ANXA3	WBP2	GREM1	CBR3	AJAP1	SLC36A4	KIF4A	PREX1
PCDHB3	LRRC3B	STC1	MOGS	DACT1	FOCAD	LEFTY2	SLC1A1	C7orf57	RWDD4	CCBE1	TSPAN18
TYW3	SEC14L4	UQCRHL	KCNJ3	PPP2R2B	INPP5D	FOCAD	ANXA1	CYTL1	EMILIN3	REXO5	ATP8B3
MAST1	MAP3K5	FOCAD	SPP1	SERTAD4	DDB2	GCNT4	TRAP1	RIPOR1	SLC45A1	CHRD	LZTFL1
PHACTR3	WNT8B	PLA2G2A	ITPRID1	POLA1	ONECUT1	RBMS2	MYOF	KCNJ3	LMO3	DUXAP9	HIST2H3PS2
HS6ST3	MYCT1	CLC	GJC1	ANXA1	SIPA1L2	ARRB1	COCH	ALDH1A3	TMEM232	SSNA1	CLGN
TDRD12	SEPTIN3	NBPF8	TTC29	CER1	EDNRB	SLC25A11	TIMP4	SBF2	CLC	AHSP	GSN
TULP2	CLRN1	MGST2	COL12A1	SORBS2	CSNK2B	HHLA1	F2RL1	KDR	MMRN2	BTBD8	AMPH
FBXL16	FMN1	TMEM106C	FRMD5	CSNK2B	THAP11	AC106795.1	PXN	NQO1	TMEM253	ZNF717	MROH1
COL25A1	TRPC4	PHLPP1	GCNT4	CCNA1	PABPC5	PCDHB5	CCND3	GABRE	LPAR6	PREX1	NFIA
KLK4	TRHDE	CSNK2A1	FAM83G	PRNP	SETD5	MRPS2	LAMA3	LY6H	VPS29	DHRS13	RASGRP2

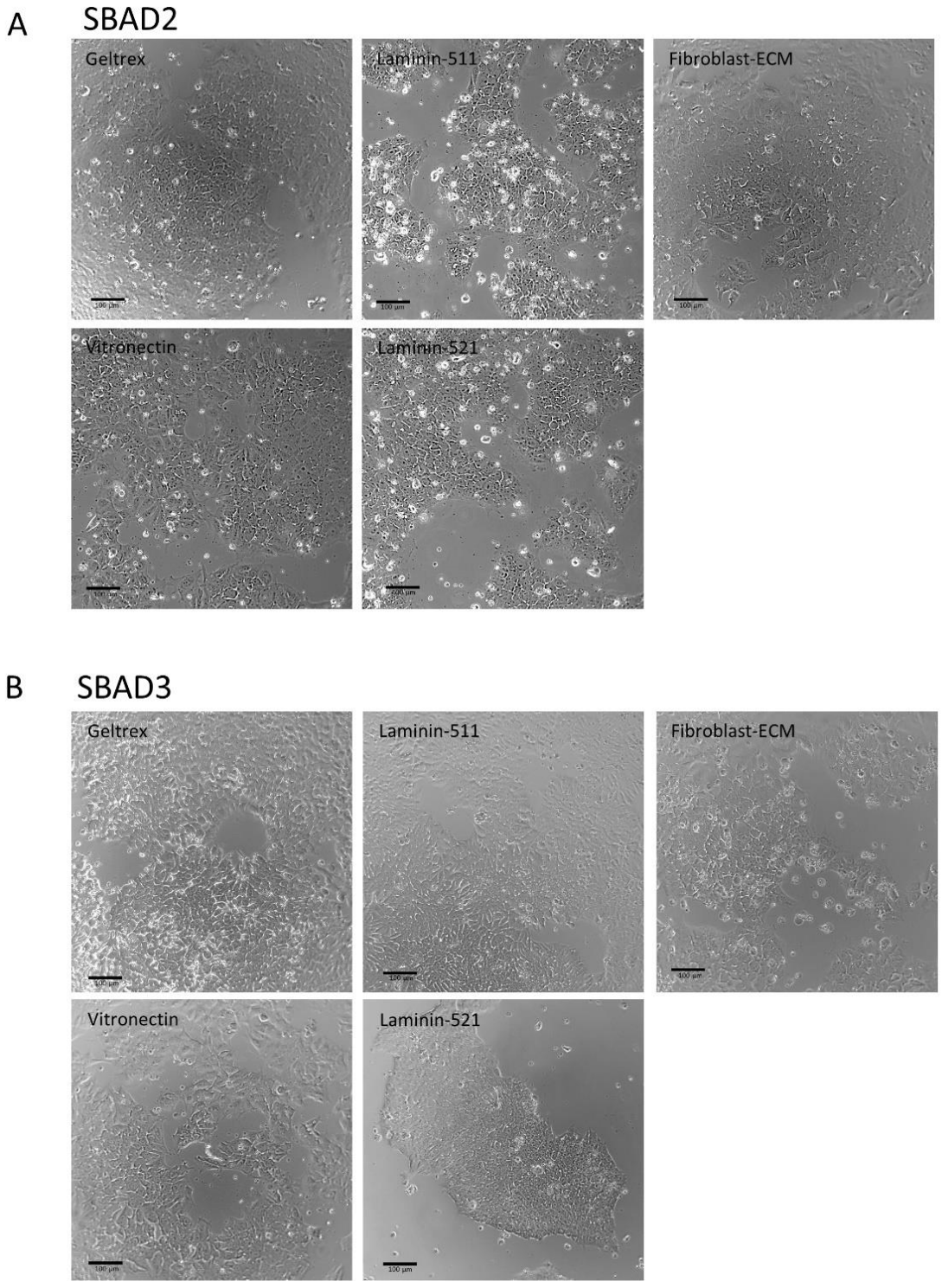
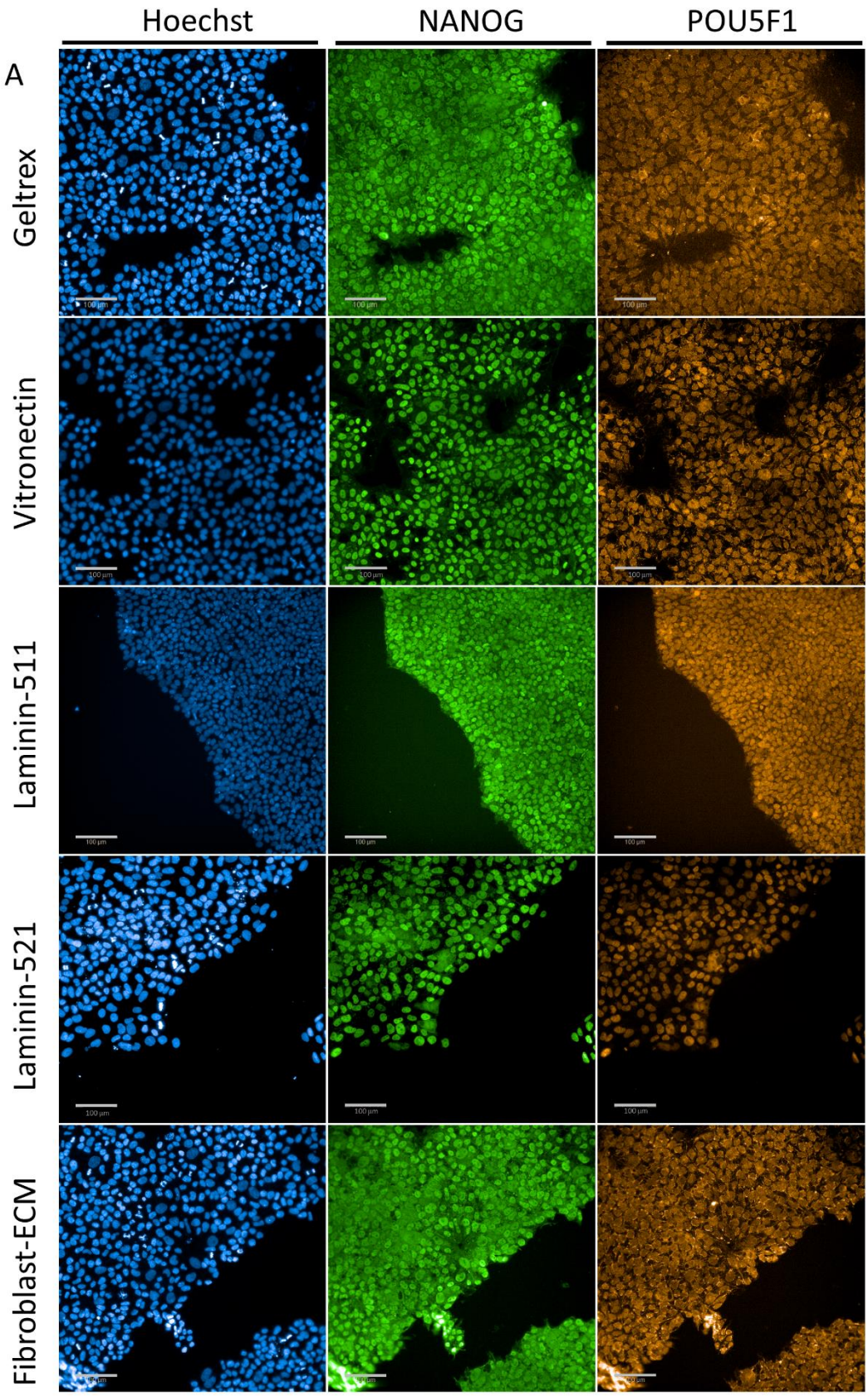


Fig. S1: Morphology of SBAD2 (A) and SBAD3 (B) iPSC on different coatings
Scale bar represents 100 µm.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NANOG
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Geltrex
Vitronectin
Laminin-511
Laminin-521
Fibroblast-ECM

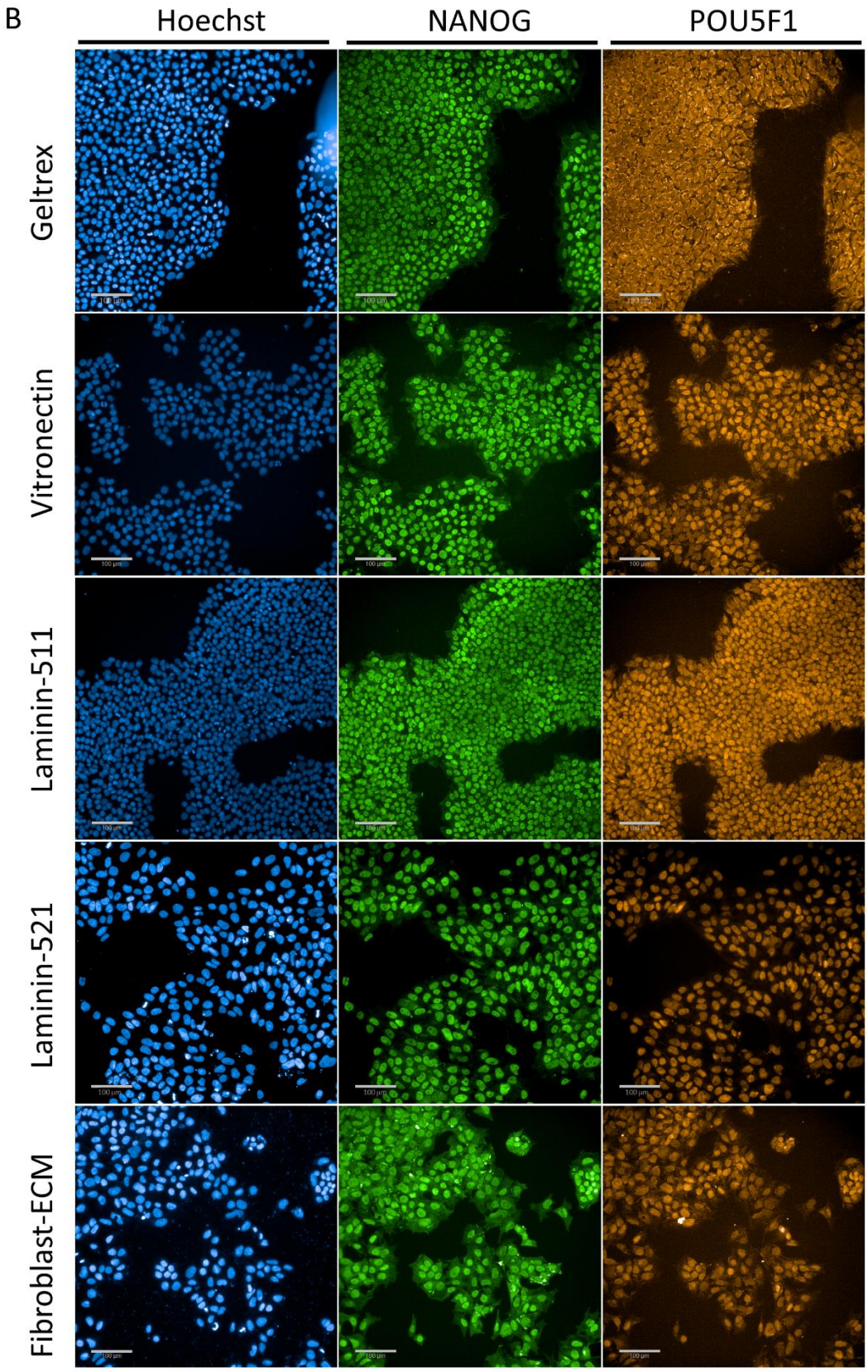
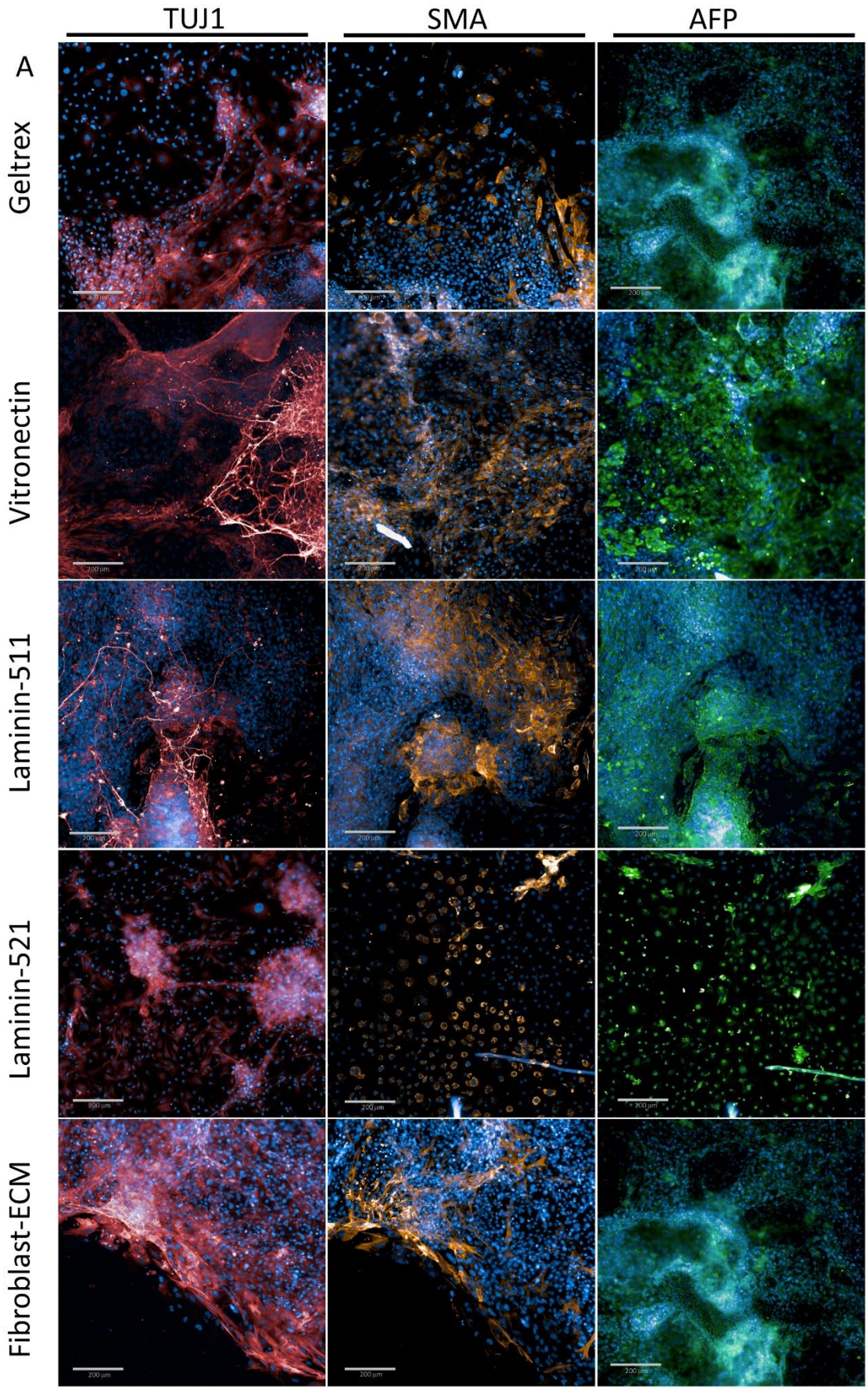


Fig. S2: Pluripotent marker staining of SBAD3 (A) and SFC086 (B) iPSC
Nuclear staining with Hoechst (blue) and antibody staining with POU5F1 (green) and NANOG (orange) are shown. Scale bar represents 100 µm.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AFP
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Geltrex
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Laminin-511
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Fibroblast-ECM

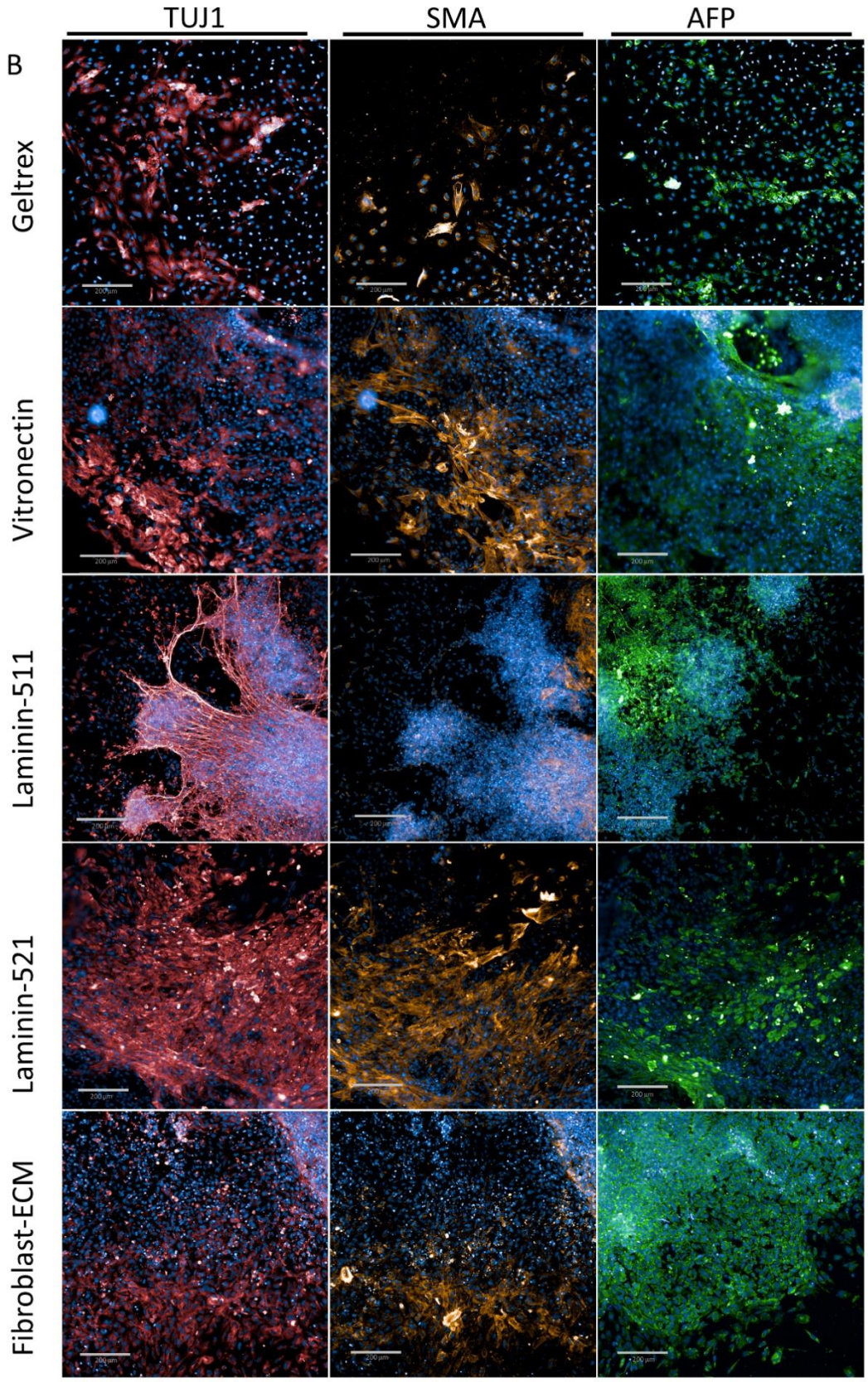
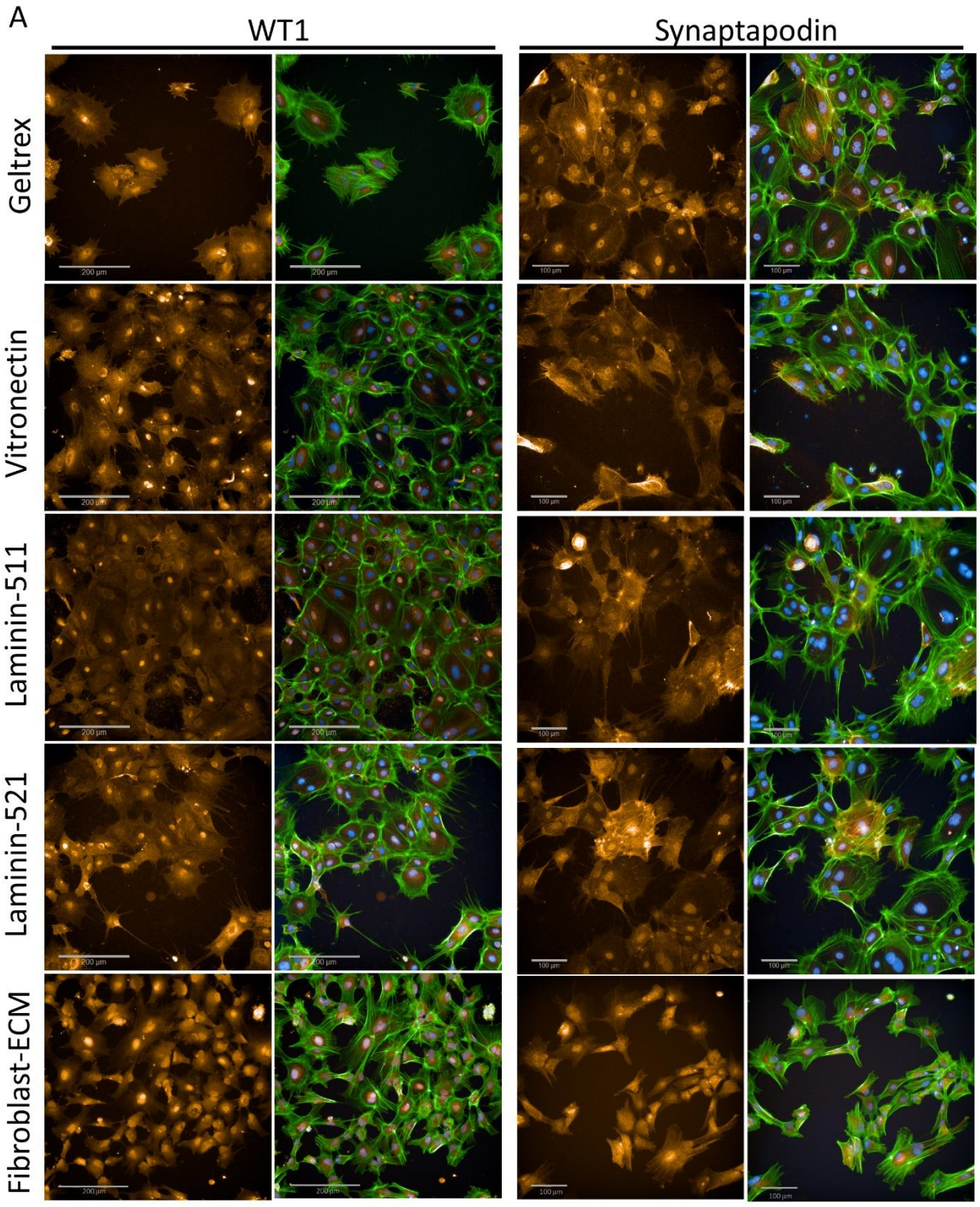


Fig. S3: Embryoid bodies (EBs) derived from SBAD3 (A) and SFC086 (B) iPSC
EBs were fixed and imaged after 3 weeks. Antibody staining for the ectoderm marker TUJ1 (red), the mesoderm marker SMA (orange), and the endoderm marker AFP (green) are shown. Nuclear staining was performed with Hoechst (blue). Scale bar represents 200 µm.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WT1
Synaptapodin
Geltrex
Vitronectin
Laminin-511
Laminin-521
Fibroblast-ECM

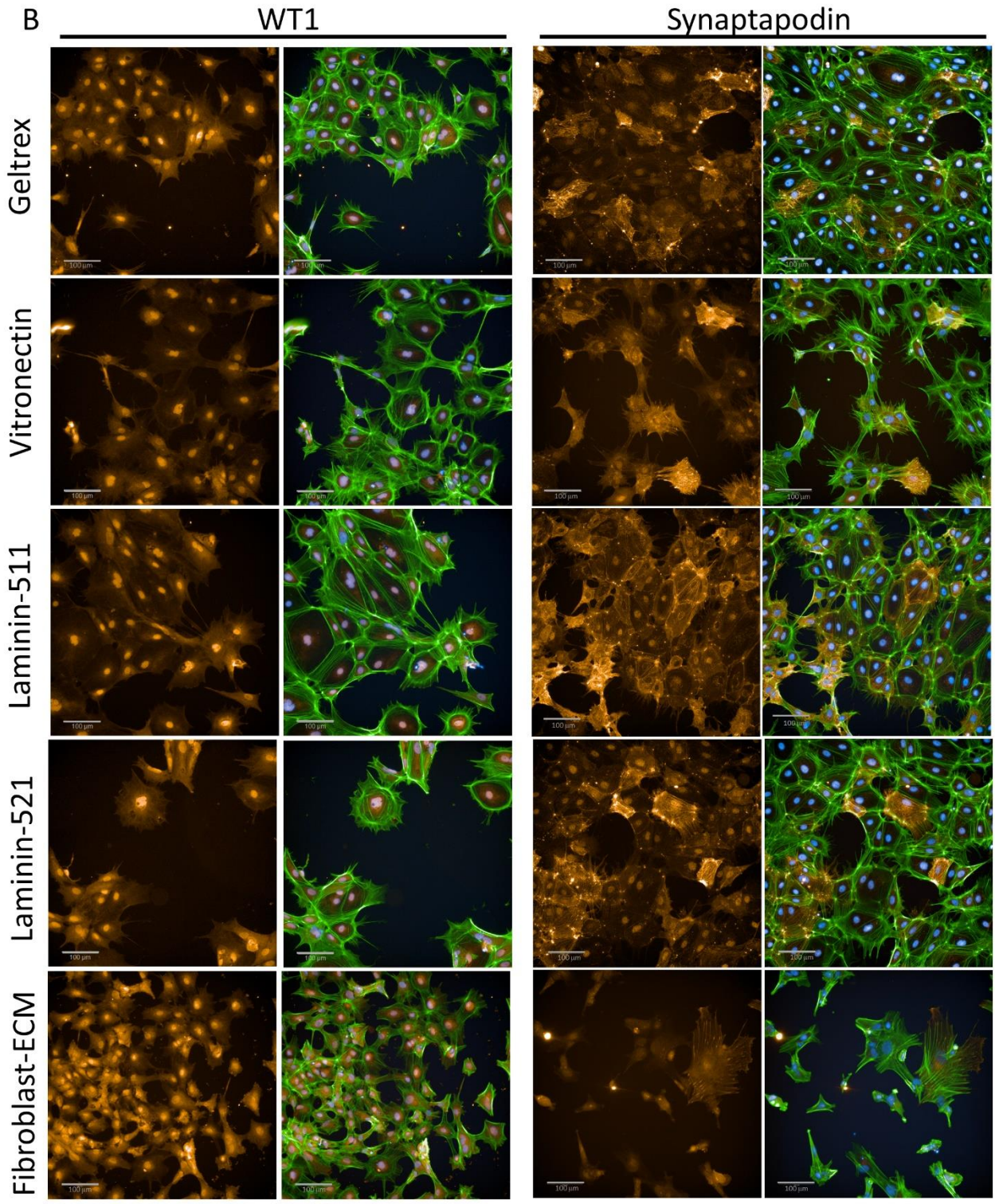


Fig. S4: iPSC differentiation into podocyte-like cells
SBAD3 (A) and SFC086 (B) were differentiated into podocyte-like cells on different coatings and stained on day 14 with the podocyte markers WT1 and synaptopodin (orange). F-actin was visualised with Phalloidin (green). Nuclear staining was performed with Hoechst (blue). Left panel shows antibody staining alone and right panel shows antibody staining overlay with phalloidin and Hoechst. Scale bars represent 100 µm.

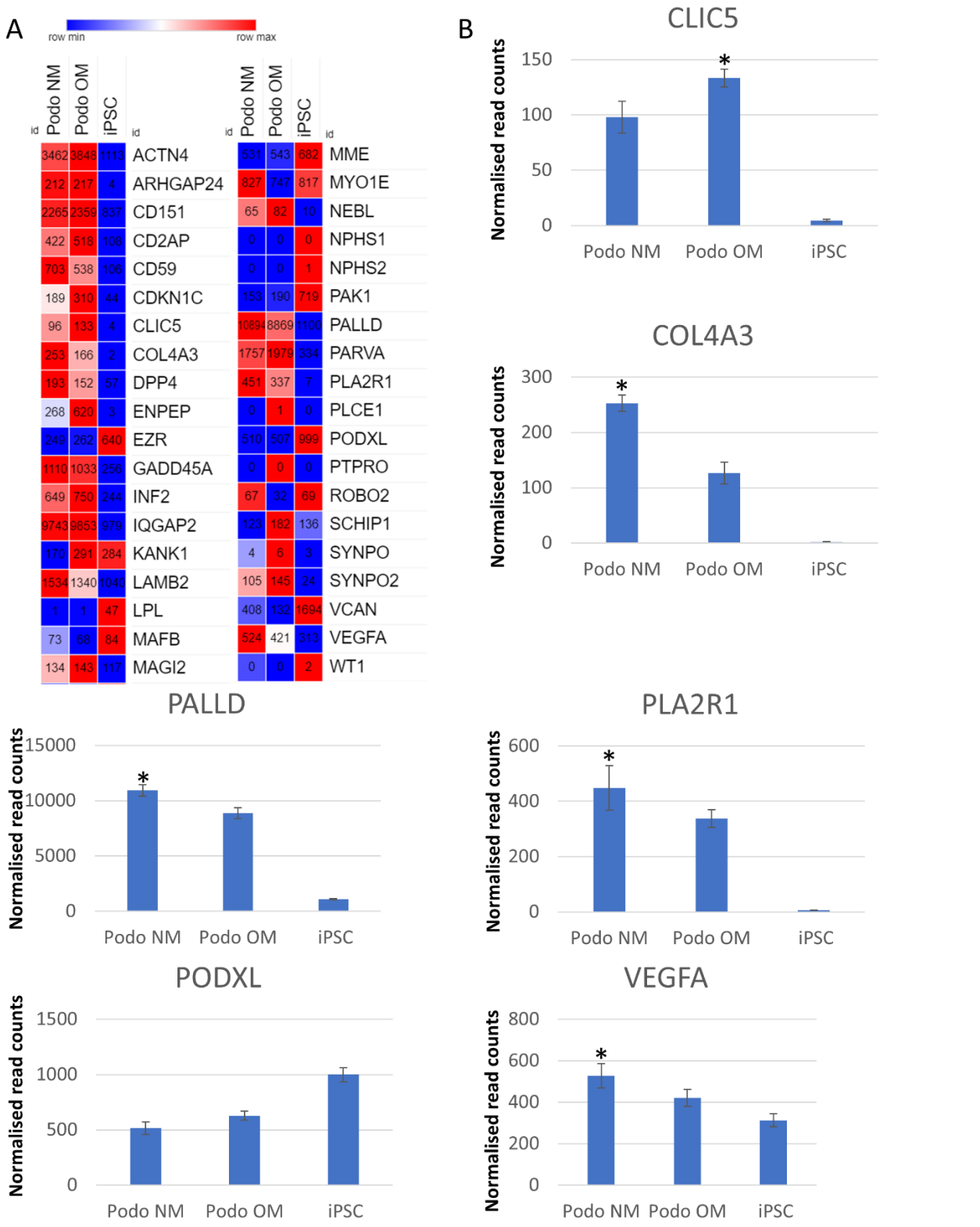
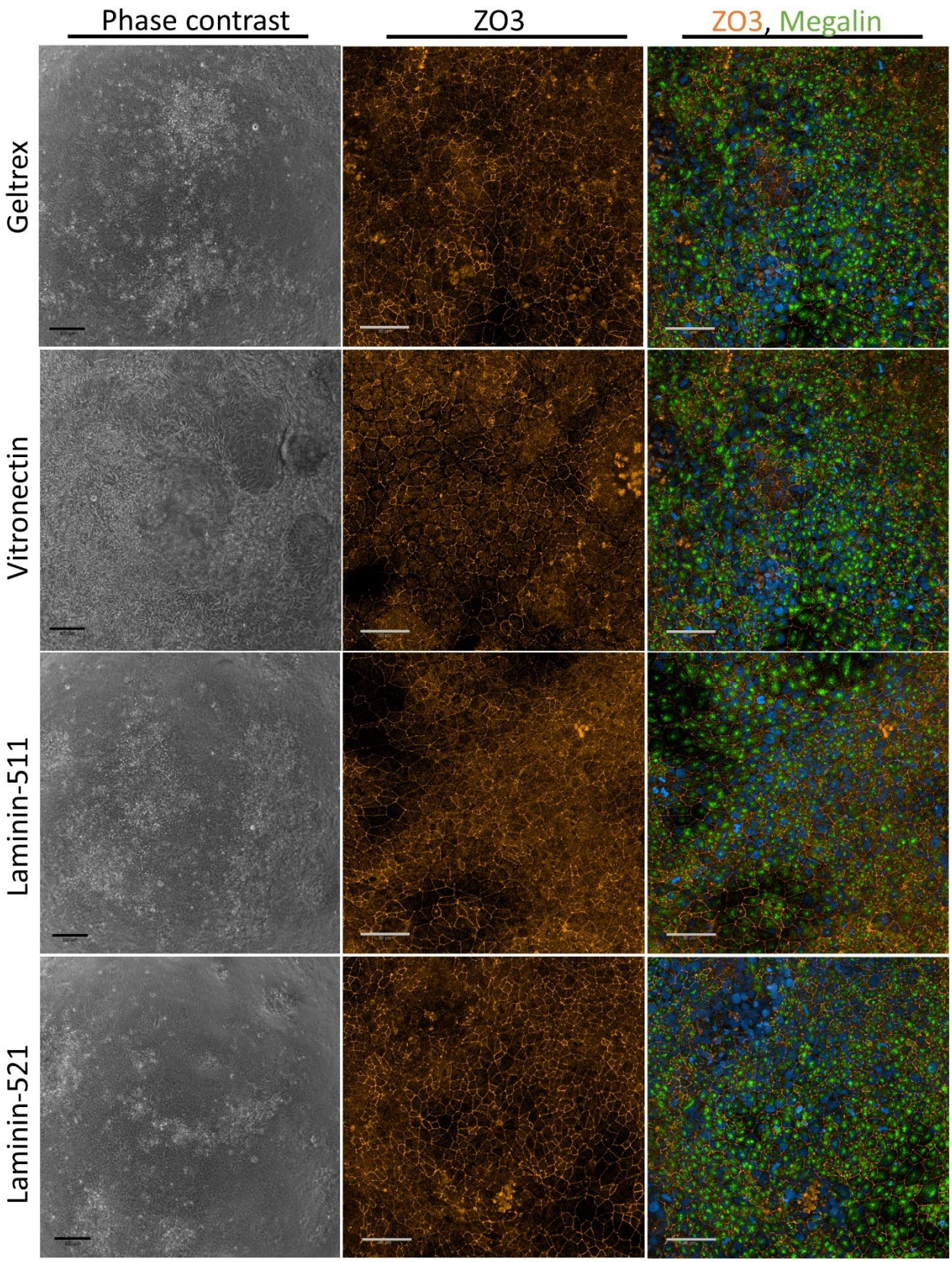


Fig. S5: TempO-Seq analysis of podocyte-related genes for podocyte-like cells grown on Geltrex in original and improved differentiation medium

(A) Heatmap of a range of podocyte-related genes for the average normalized read count for iPSC-derived podocyte like-cells (from SBAD2 line) differentiated and maintained in the original podocyte medium from Rauch et al. (2018) (OM) and in the improved, lower-glucose medium according to the new protocol (NM). Undifferentiated SBAD2 iPSCs grown in mTeSR served as a negative control. Dark red and blue colours represent the highest and lowest gene expression levels per row, respectively. (B) Selected podocyte markers are presented as bar graphs. Statistical analysis was performed with a Student's t-test to compare podocyte-like cells differentiated in the new medium to podocyte-like cells differentiated in the original medium. *, $p < 0.05$, $n = 4$.


Phase contrast
ZO3
ZO3, Megalin
Geltrex
Vitronectin
Laminin-511
Laminin-521

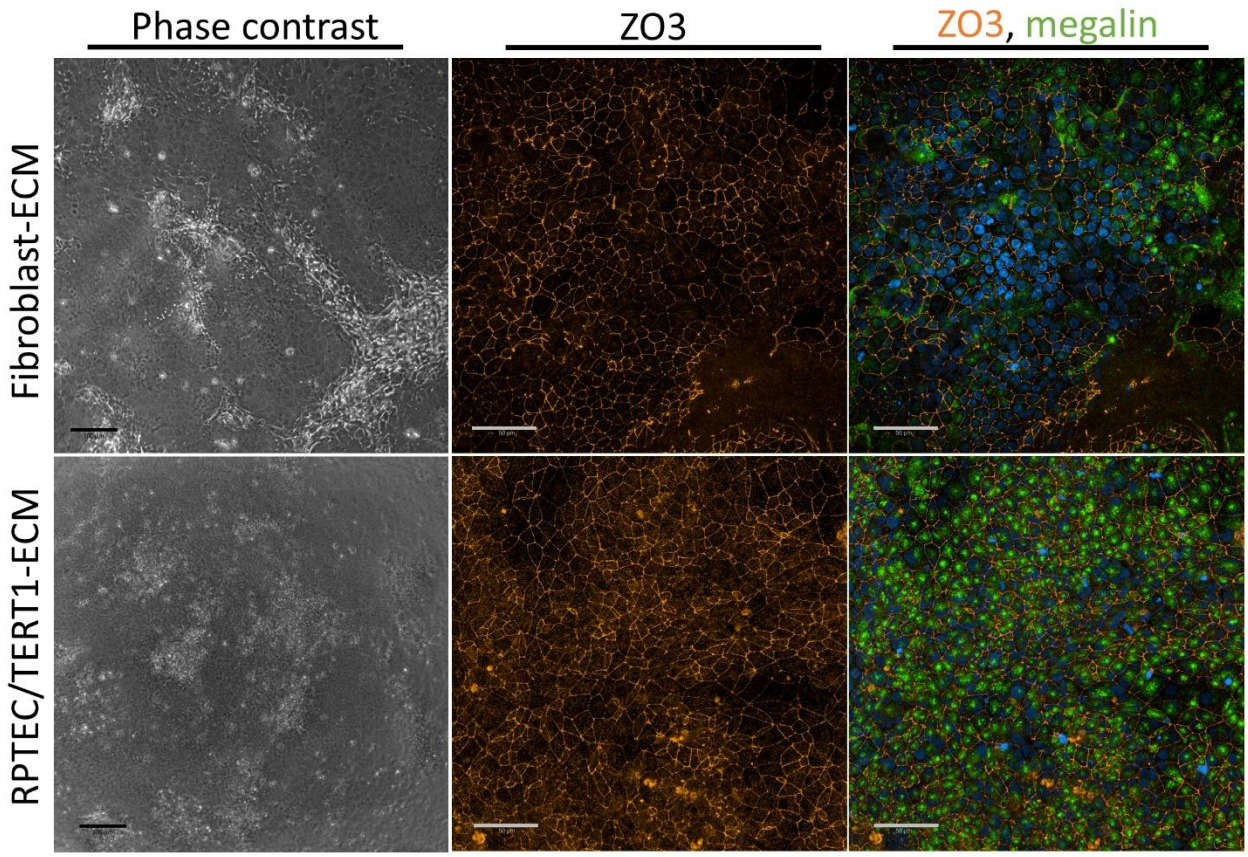


Fig. S6: iPSC differentiation into proximal tubular-like (PTL) cells
SBAD2 were differentiated into PTL cells on (A) different commercial coatings (Geltrex, vitronectin, laminin-511, laminin-521) or (B) on in-house produced RPTEC/TERT1-ECM and Fibroblast-ECM for 14 days. Morphology of the cells is illustrated by phase contrast images (left panel). Cells were fixed and stained with the proximal-tubular marker megalin (green), the epithelial marker zona occludens 3 (ZO3, orange), and the nuclear stain Hoechst 33342 (blue). Single ZO3 staining (middle panel) is displayed to allow better visualization of polarization. Overlay of all markers is shown in the right panel. Scale bar represents 100 and 50 µm for phase contrast and immunofluorescence images, respectively.