

Supplemental Material: Derivation of the Immortalized Cell Line-UM51-PrePodo-hTERT and Its Responsiveness to Angiotensin II and Activation of the RAAS Pathway

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Table S1. antibodies.

Antigen	Company	Dilution	
		ICC	WB
α -ACTININ	Abcam (#108198)	1:200	/
β -ACTIN	Cell Signaling Technology (3700S)	/	1:4000
HA-tag	Proteintech (51064-2-AP)	1:200	1:4000
KI67	Cell Signaling Technology (9449S)	1:200	/
NPHS1	Invitrogen (#PA5-20330)	1:200	1:1000
P53	Merck (OP43-100UG)	1:200	1:1000
pP53	Cell Signaling Technology (9283S)	1:200	/
SYNPO	Thermo Fisher Scientific (PA5-56997)	1:200	1:1000
WT1	Merck (3012219)	1:200	/
Anti-mouse HRP-labeled	Thermo Fisher Scientific #NA931	/	1:4000
Anti-rabbit HRP-labeled	Cell Signaling #7074S	/	1:1000

Table S2. RT-qPCR Primers.

Primer name	Sequence	Annealing temperature (°C)	Product length (bp)
AGTR1 s	5'- tct cag cat tga tcg ata cc -3'	60	80
AGTR1 as	5'- tga ctt tgg cta caa gca tt -3'		
AGTR2 s	5'- tat ggc ctg ttt gtc ctc at -3'	60	114
AGTR2 as	5'- cat tgg gca tat ttc tca gg -3'		
CD106 s	5'- cga acc caa aca aag gca gag ta -3'	60	84
CD106 as	5'- gag gaa ggg ctg acc aag acg -3'		
CD24 s	5'- gcg gac ttt tct ttt ggg ggg -3'	60	174
CD24 as	5'- cca gca gca gcc cca g -3'		
CD2AP s	5'- aac tca tga agc cca gga cga -3'	60	100
CD2AP as	5'- ctg atc cag atg cag ttt cac tca c -3'		
hTERT s	5'- cgg aag agt gtc tgg agc aa -3'	60	145
hTERT as	5'- gga tga agc gga gtc tgg a -3'		
KI67 s	5'- tcg tcc cag tgg aag agt tg -3'	60	144
KI67 as	5'- cag ccc cgc tcc ttt tga t -3'		
NPHS1 s	5'- gcg ggt tct gct acg atg gtg -3'	60	295
NPHS1 as	5'- caa aca cac cag cct cac ccg -3'		
P53 s	5'- cag ggc agc tac ggt ttc c -3'	60	102
P53 as	5'- cag ttg gca aaa cat ctt gtt gag -3'		
RPL s	5'- tcg aca atg gca gca tct ac -3'	60	195
RPL as	5'- atc cgt ctc cac aga caa gg -3'		
SYNPO s	5'- ccc caa cct ctc ctc taa cc -3'	60	116
SYNPO as	5'- atg aca cag gag gca gaa gaa t -3'		
WT1 s	5'- cac agc aca ggg tac gag a -3'	60	133
WT1 as	5'- caa gag tcg ggg cta ctc c -3'		

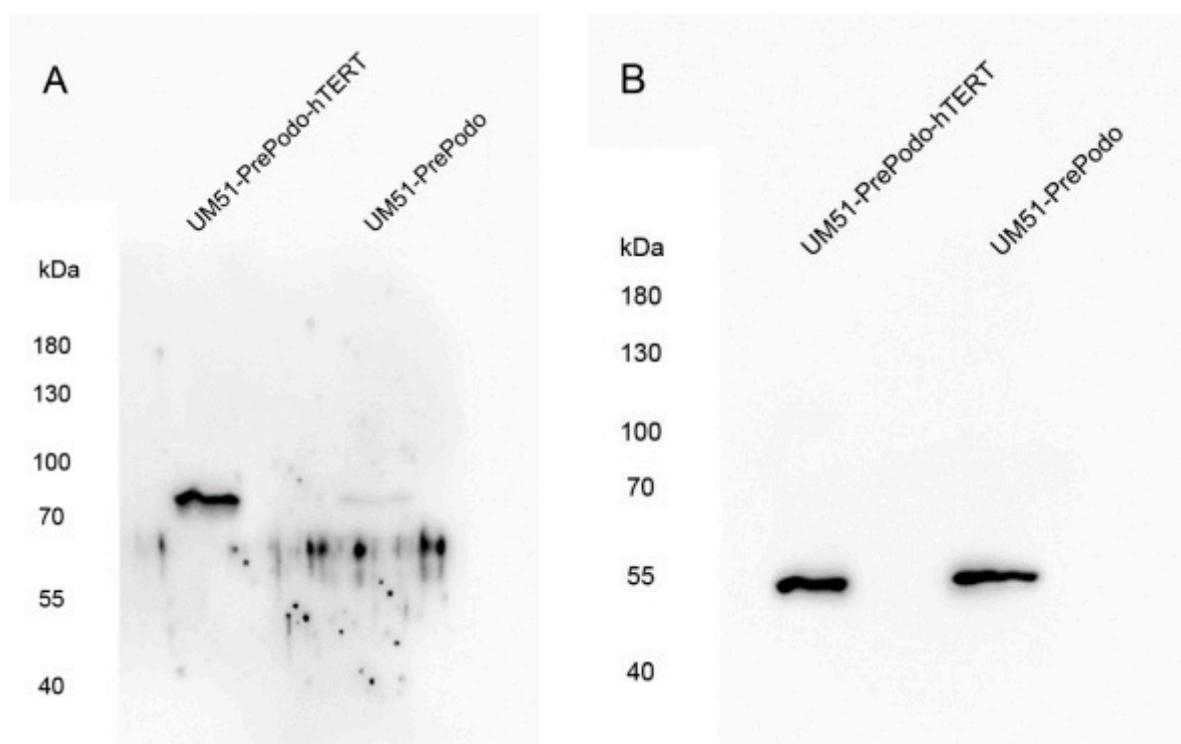


Figure S1. Complete western blot images for the detection of the HA-tag in the cell lines UM51- PrePodo-hTERT and UM51-PrePodo. (A) HA-tag, (B) β -Actin.

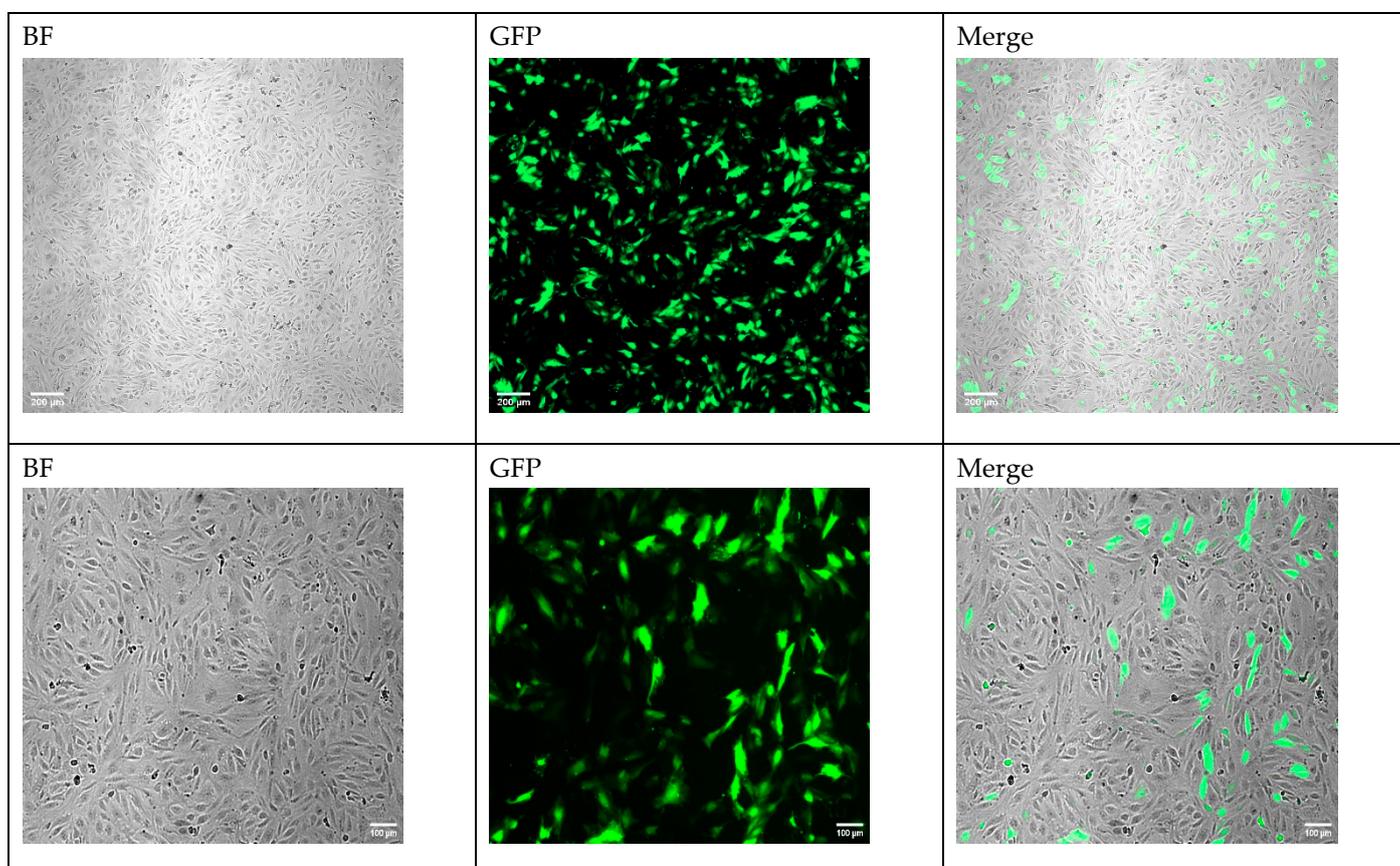


Figure S2. Transfection of UM51-PrePodo-hTERT with the TurboGFP- vector.

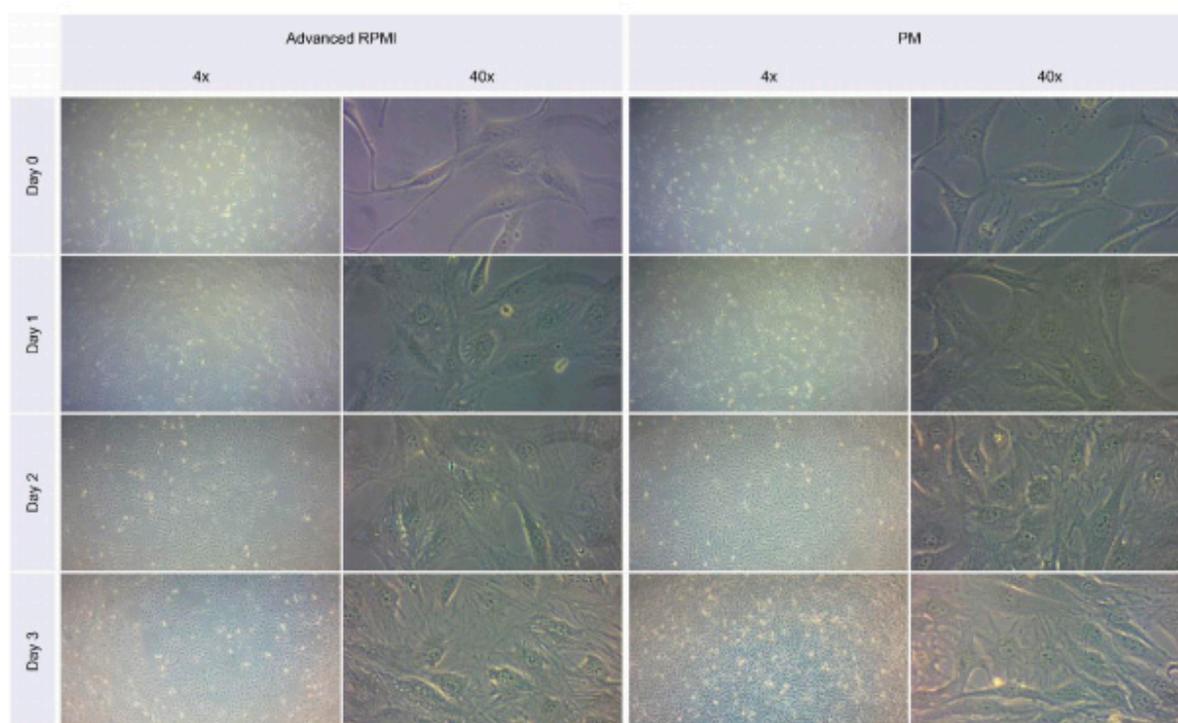
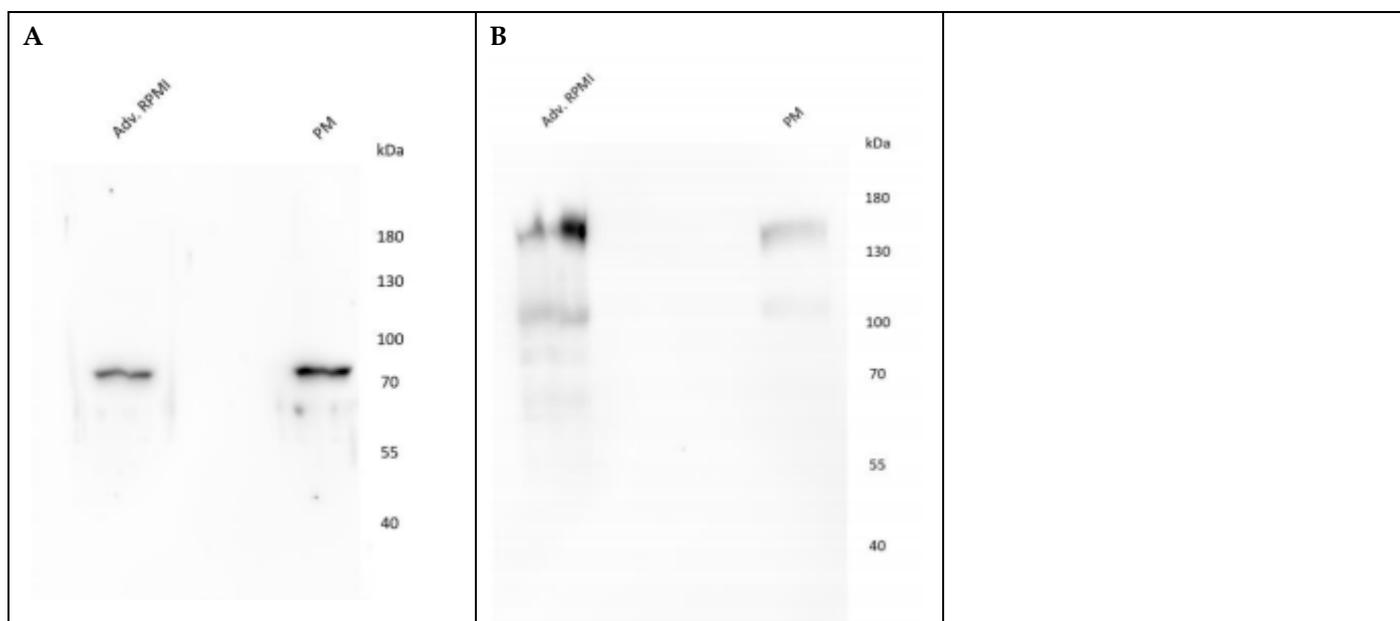


Figure S3. Morphology of UM51-PrePodo-hTERT cells cultured in Proliferation Medium (right) and Advanced RPMI (left) during the resazurin assay.



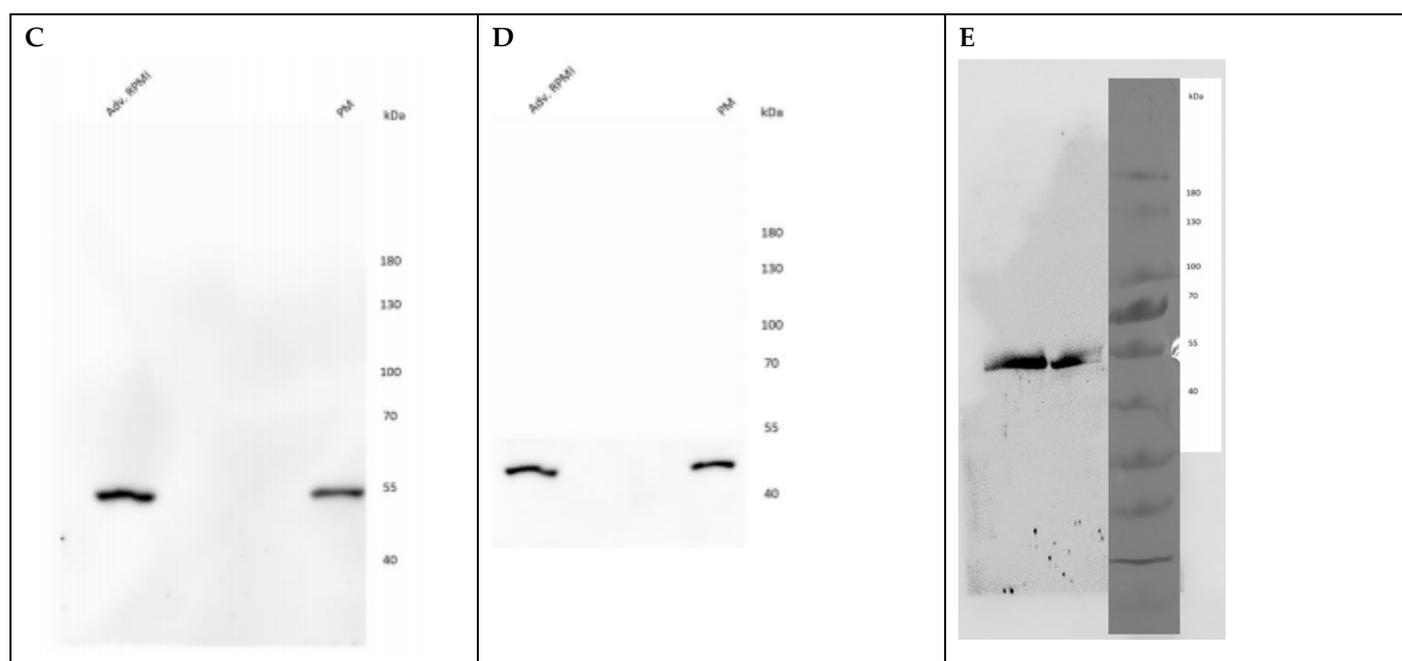
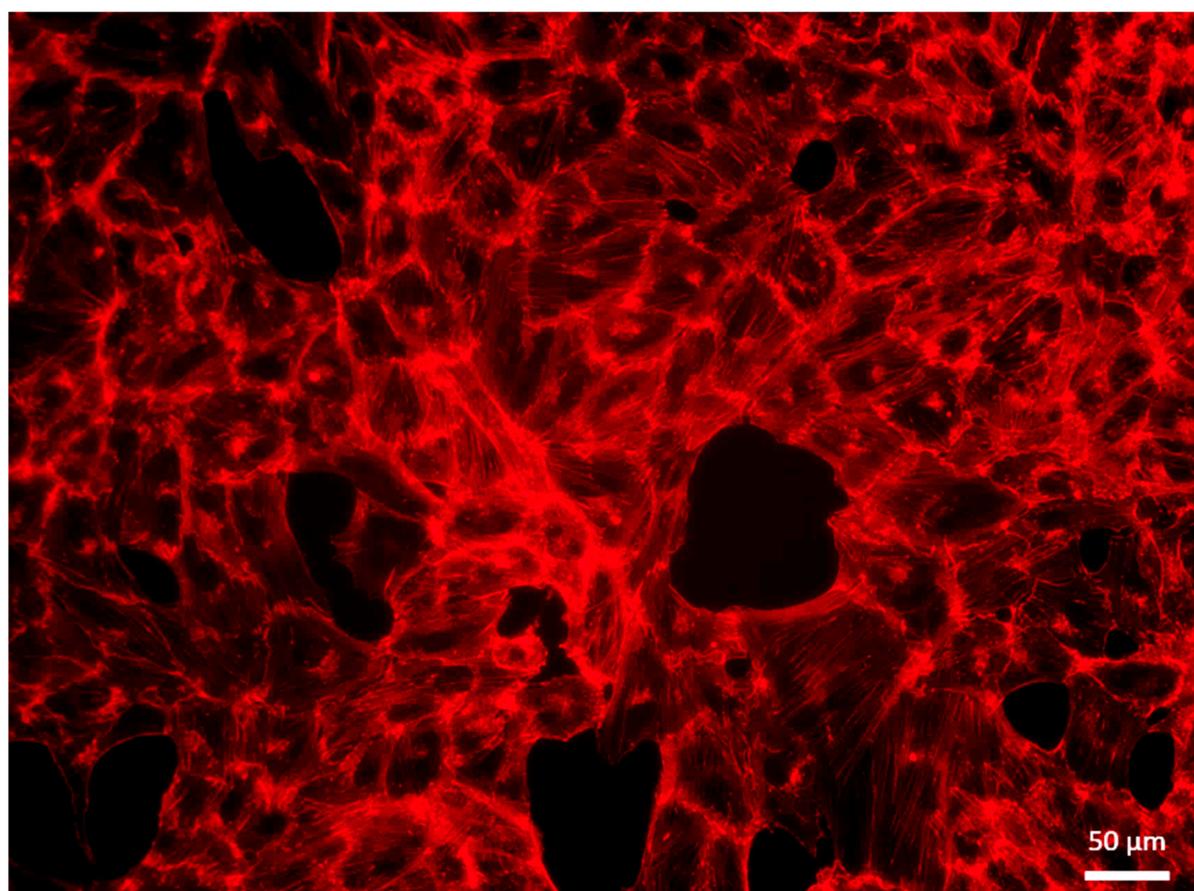


Figure S4. Complete western blot images for the comparison of UM51-PrePodo-hTERT cultured in Proliferation Medium (PM) and Advanced RPMI (Adv. RPMI). (A) HA-tag, (B) SYNPO, (C) P53, (D) β - Actin and (E) LMX1b.

A



B

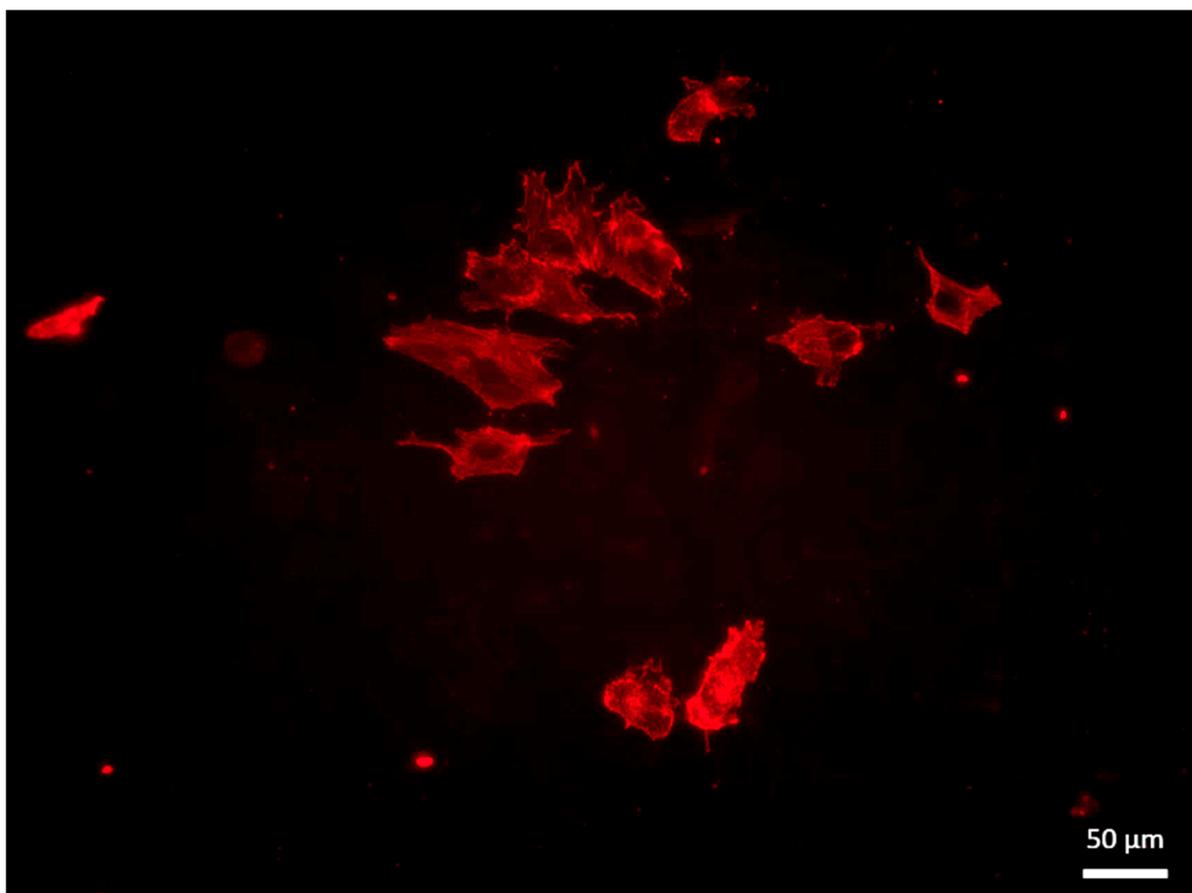
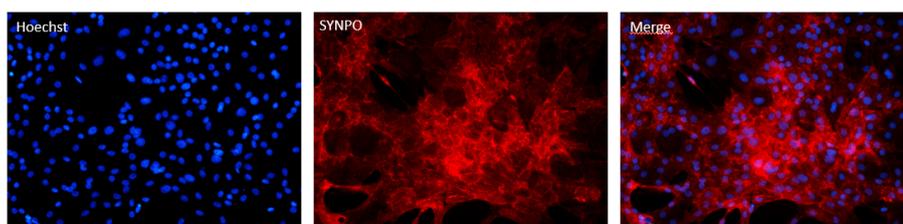


Figure S5. Higher magnification of podocyte cytoskeleton stained with α -Actinin (A). Remodeling of cytoskeleton after treatment with 100 μ M ANGII (B).

A

UM51 hTERT
Podocyte
15.07.2020
P3



B

UM51 hTERT
Podocyte
15.10.2021
P75

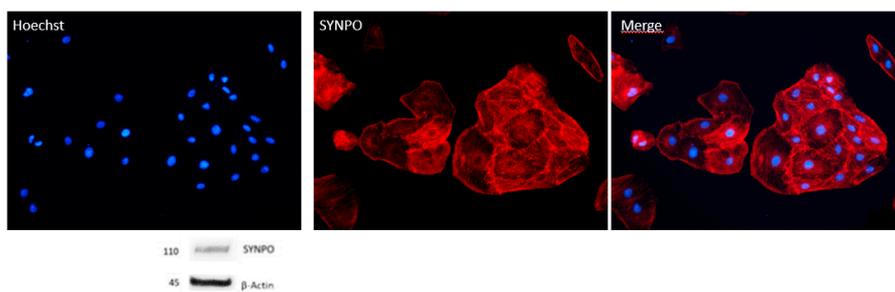


Figure S6. Comparison of UM51 hTERT passage 3 (A) and passage 75 (B). Relative protein expression of Synaptopodin was detected by Immunofluorescent based detection and Western blot analysis.