SUPPLEMENTAL MATERIALS

Everolimus rescues the phenotype of elastin insufficiency in patient iPSC-derived vascular smooth muscle cells

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Running title: Drug screening in elastin deficient patient iPSCs

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Major Resources Table

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration
AFP	R&D Systems	MAB1368	1:200
NANOG	Cell Signaling	4903P	1:400
Nestin	Millipore	MAB5326	1:200
OCT4	Abcam	Ab19857	1:200
SMA	Thermo Fisher Scientific	18-0106 (1A4)	1:200
SM22α	Abcam	Ab14106	1:200
SSEA4	Thermo Fisher Scientific	41-4000	1:100
TRA-1-60	Thermo Fisher Scientific	40-1000	1:100
TUBB3	Chemicon	MAB1637	1:200
Goat anti-mouse	Thermo Fisher Scientific	11001	1:500
Goat anti-rabbit	Thermo Fisher Scientific	11008	1:500

Antibody validation provided in manufacturers data sheets:

AFP: https://resources.rndsystems.com/pdfs/datasheets/mab1368.pdf

NANOG: https://media.cellsignal.com/pdf/4903.pdf

Nestin: https://www.emdmillipore.com/CA/en/product/Anti-Nestin-Antibody-clone-10C2,MM_NF-MAB5326?ReferrerURL=https%3A%2F%2Fwww.google.com%2F#overview

OCT4: https://www.abcam.com/oct4-antibody-chip-grade-ab19857.html#top-100

SMA: https://www.thermofisher.com/content/dam/LifeTech/Documents/PDFs/PG1485-PJ8781-CO36961-Handbook3StemCells-Global-smaller.pdf

SM22α: https://www.abcam.com/tagIntransgelin-antibody-ab14106.html

SSEA4: https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=41-4000&version=98

TRA-1-60: https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=41-1000&version=98

TUBB3: https://www.emdmillipore.com/CA/en/product/Anti-Tubulin-Antibody-beta-III-isoform-CT-clone-TU-20-Similar-to-TUJ1,MM_NF-MAB1637

Goat anti-mouse: https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_secondary&productId=A-11001&version=98

Goat anti-rabbit: https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_secondary&productId=A-11008&version=98

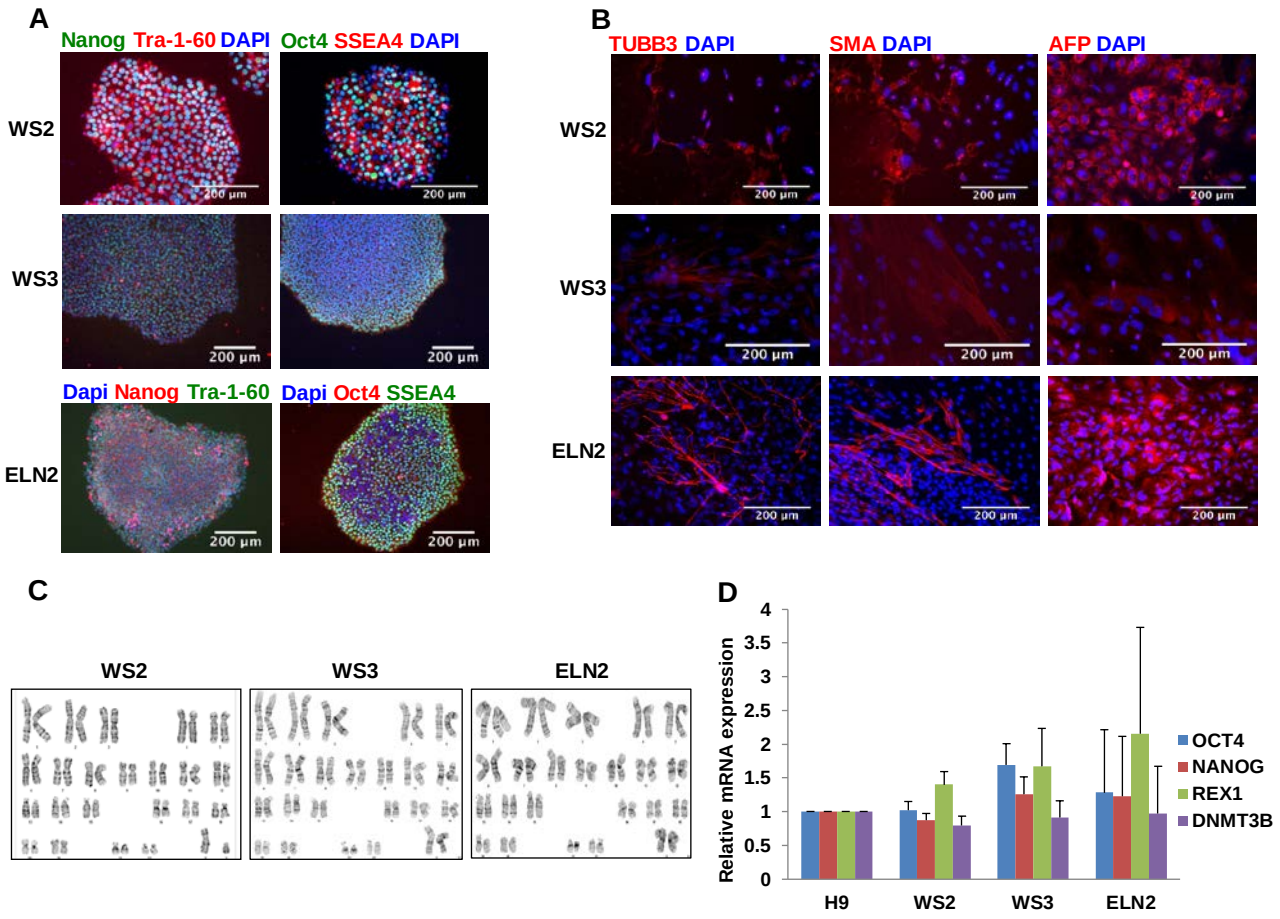

Figure I. Pluripotency characterization of WS2, WS3, and ELN2 iPSCs. (A) iPSCs expressed pluripotency markers NANOG, TRA-1-60, OCT4 and SSEA4. Nuclei were stained with DAPI. (B) iPSCs differentiated in vitro into all three germ layers. Images show TUBB3 staining for neuronal ectoderm, SMA expression as mesoderm, and AFP expression as endoderm. (C) Normal G banding karyotype of the iPSCs. (D) Endogenous pluripotency genes were upregulated following successful reprogramming in H9 and patient iPS cells.
WS, Williams syndrome; ELN, elastin; OCT4, octamer-binding transcription factor 4; DAPI, 4′,6-diamidino-2-phenylindole; TUBB3, tubulin β3; SMA, smooth muscle actin, AFP, α-fetoprotein.

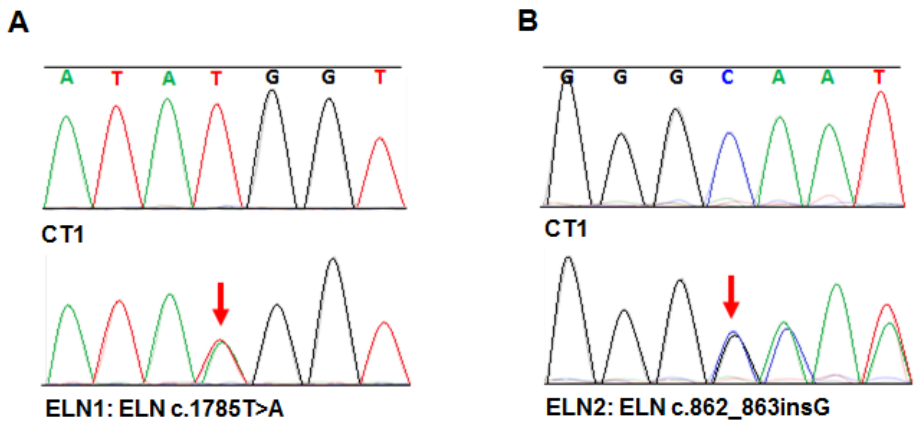

Figure II. Mutation confirmation. (A-B): Chromatographs of control iPSC-SMCs (CT1), heterozygous ELN1 variant (c.1785T>A) and heterozygous ELN2 variant (c.862_863insG). ELN, elastin.

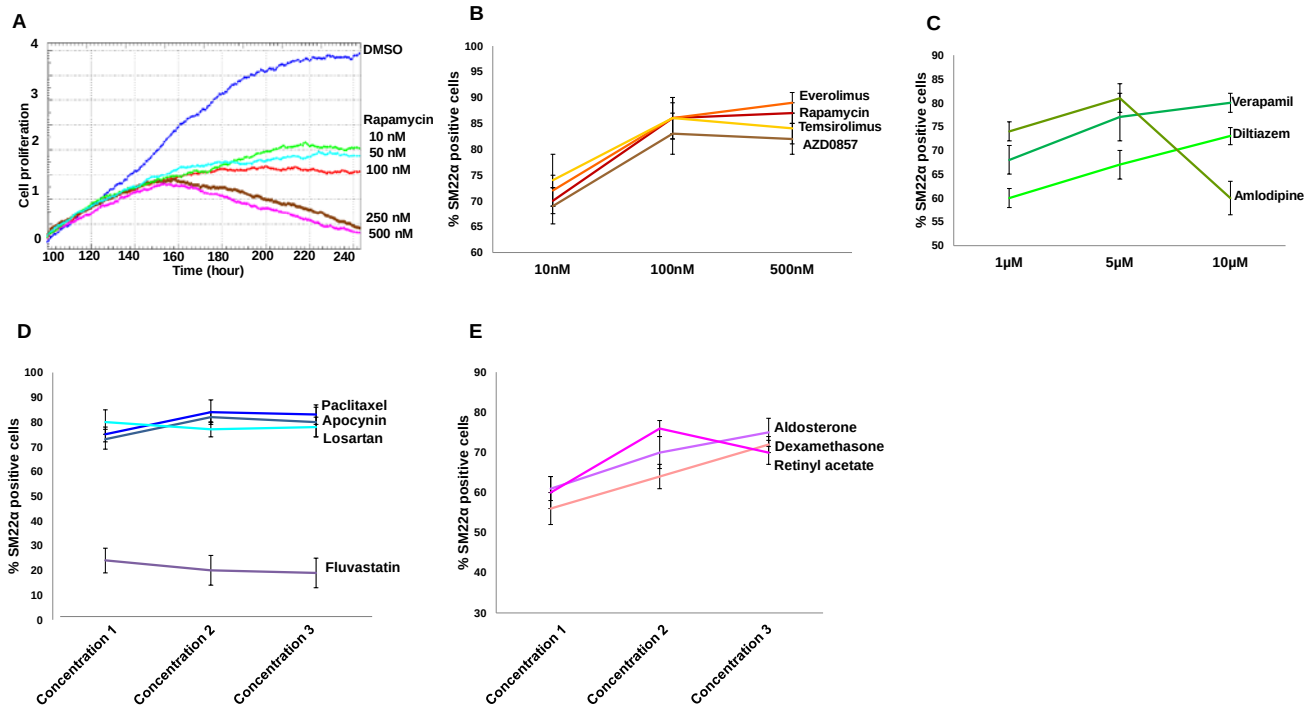

Figure III. Selection of optimal dose for drug assays. (A) xCELLigence graph shows dose-dependent change in cell impedance i.e. SMC proliferation over time in WS patient SMCs with DMSO (blue) and 10-500nM rapamycin. 250nM and 500nM doses induced cytotoxicity. 100nM rapamycin showed greatest effect on proliferation without cytotoxicity. (B) mTOR inhibitors: % of SM22α cells i.e. SMC differentiation showed a dose-dependent increase on treatment with 10-500nM doses of 4 mTOR inhibitors. 100nM dose provided greatest increase after which the effect plateaued. (C) Calcium channel blockers: % of SM22α cells i.e. SMC differentiation showed a dose-dependent increase on treatment with 1-10μM doses of 3 calcium channel blockers. 10μM was chosen as the optimal dose for verapamil and diltiazem and 5 μM for amlodipine which was the maximum tolerated dose. (D) SMC differentiation showed a dose-dependent increase on treatment with paclitaxel (10, 50, 100nM) and apocynin (1, 5, 10μM), but no clear dose response with losartan (1, 5, 10μM). Fluvastatin induced cell toxicity at all doses tested (0.25, 0.5, 1 μM). (E) SMC differentiation showed a dose-dependent increase on treatment with aldosterone (10, 50, 100nM), dexamethasone (10, 50, 100nM), and retinyl acetate (0.1, 1, 10μM). (n=3 technical replicates for each drug).
SMC, smooth muscle cells; WS, Williams syndrome; DMSO, Dimethyl sulfoxide; mTOR, mammalian target of rapamycin; CCB, calcium channel blocker.

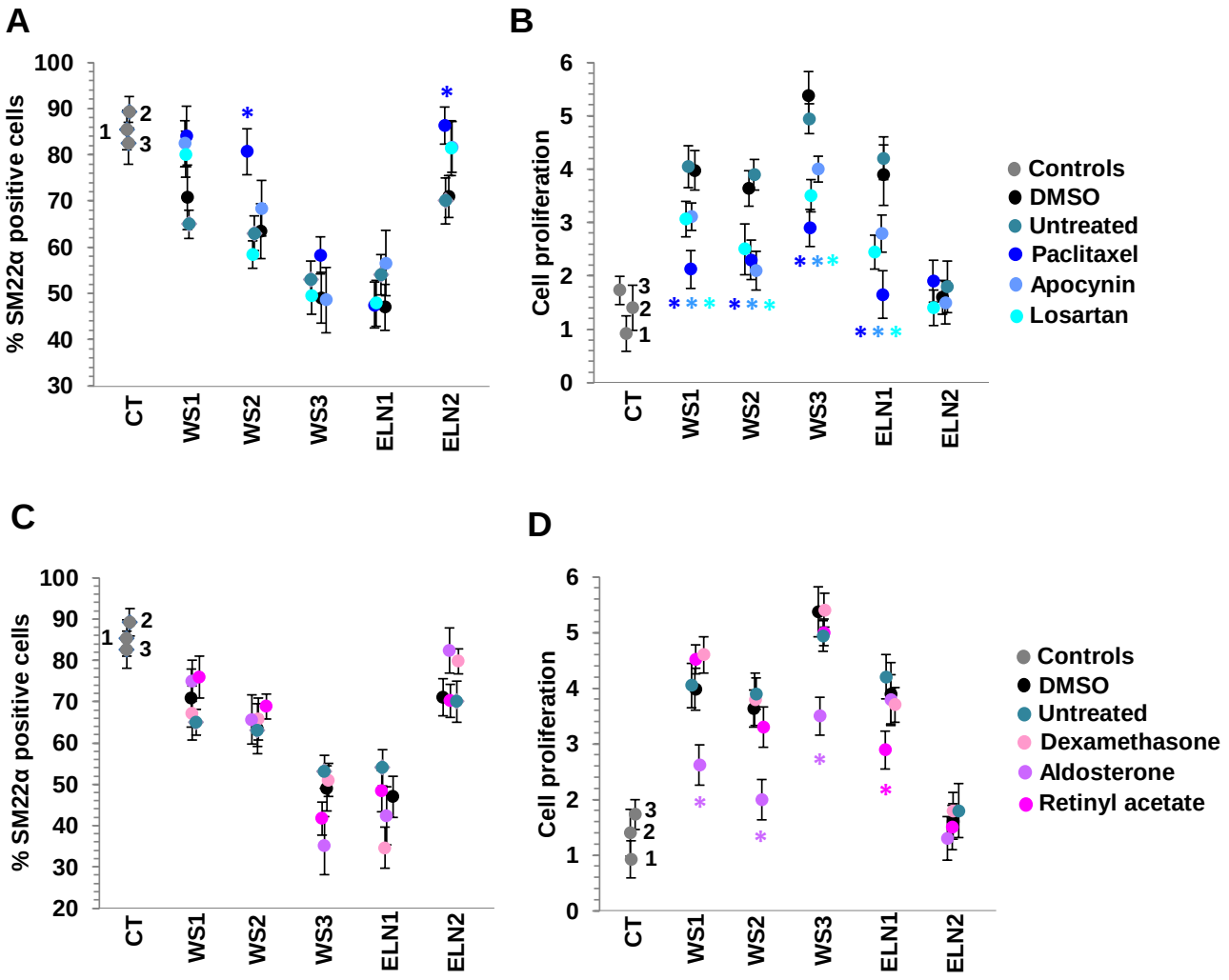

Figure IV. Effect of anti-proliferative and pro-elastogenic drugs on EI iPSC-SMC differentiation and proliferation. (A) The percentage of SM22α positive cells was 50-70% SM22α (black circle) in DMSO treated EI iPSC-SMCs. SMC differentiation was significantly increased with paclitaxel only in WS2 SMCs (81% SM22α, bright blue circle); the other patient SMCs did not respond. None of the patient SMCs responded to apocynin or losartan. Wild-type control (CT1, CT2, and CT3) SMCs are shown in grey. (B): Paclitaxel, apocynin and losartan decreased cell proliferation in all WS and in ELN1 SMCs compared to DMSO treated SMCs although the levels were not consistently restored to control levels in all responders. ELN2 was not hyperproliferative at baseline, therefore no further improvement was seen. (C) SMC differentiation did not improve with dexamethasone, aldosterone and retinyl acetate treatment compared to DMSO treated SMCs. (D) Aldosterone (purple circle) reduced proliferation in all 3 WS patient SMCs compared to DMSO treated SMCs although the levels were not consistently restored to control levels in all responders. Retinyl acetate reduced hyperproliferation only in ELN1 SMCs (pink circle) (n=3 biological replicates, n=3 technical replicates for each experiment) $p<0.05$, drug-treatment vs DMSO.
ELN, elastin; SMC, smooth muscle cells; SM22α, smooth muscle marker 22α; DMSO, Dimethyl sulfoxide; WS, Williams syndrome.

Table I. Short tandem repeat analyses in WS and elastin variant iPSCs in comparison to parental fibroblasts. Results showed the iPSCs have an identical profile to the corresponding parental fibroblasts.

Cell line	Allele	WS2 iPSCs	WS2 Fibroblasts	WS3 iPSCs	WS3 Fibroblasts	ELN1 iPSCs	ELN1 Fibroblasts	ELN2 iPSCs	ELN2 Fibroblasts
Amelogenin	Allele 1	X	X	X	X	X	X	X	X
	Allele 2	Y	Y	X	X	Y	Y	X	X
CSF1PO	Allele 1	12	12	10	10	11	11	10	10
	Allele 2	13	13	12	12	11	11	11	11
D13S317	Allele 1	8	8	11	11	14	14	11	11
	Allele 2	12	12	13	13	14	14	12	12
D16S539	Allele 1	10	10	11	11	11	11	11	11
	Allele 2	12	12	13	13	12	12	13	13
D18S51	Allele 1	12	12	10	10	12	12	12	12
	Allele 2	14	14	14	14	16	16	14	14
D19S433	Allele 1	12	12	13	13	13	13	12	12
	Allele 2	14.2	14.2	15.2	15.2	18	18	14	14
D21S11	Allele 1	29	29	31	31	29	29	29	29
	Allele 2	30	30	31	31	30	30	30.2	30.2
D2S1338	Allele 1	17	17	20	20	20	20	17	17
	Allele 2	18	18	20	20	20	20	17	17
D3S1358	Allele 1	15	15	15	15	14	14	16	16
	Allele 2	16	16	17	17	16	16	18	18
D5S818	Allele 1	9	9	11	11	12	12	11	11
	Allele 2	11	11	13	13	13	13	12	12
D7S820	Allele 1	11	11	10	10	10	10	9	9
	Allele 2	11	11	12	12	10	10	9	9
D8S1179	Allele 1	14	14	10	10	11	11	13	13
	Allele 2	14	14	11	11	14	14	14	14
FGA	Allele 1	22	22	21	21	20	20	22	22
	Allele 2	23	23	23	23	24	24	25	25
THO1	Allele 1	6	6	9	9	8	8	6	6
	Allele 2	9	9	9.3	9.3	9.3	9.3	6	6
TPOX	Allele 1	9	9	8	8	10	10	10	10
	Allele 2	9	9	8	8	11	11	10	10
vWA	Allele 1	16	16	17	17	17	17	18	18
	Allele 2	18	18	19	19	18	18	20	20

WS, Williams syndrome; ELN, elastin

Table II. Drug concentrations used for the candidate drug classes

Drug class	Drug	Concentrations tested	Concentration selected
mTOR inhibitors	Rapamycin	10nM, 100nM, 500nM	100nM
	Everolimus	10nM, 100nM, 500nM	100nM
	Temsirolimus	10nM, 100nM, 500nM	100nM
	AZD8055	10nM, 100nM, 500nM	100nM
CCB inhibitors	Verapamil	1μM, 5μM, 10μM	10μM
	Diltiazem	1μM, 5μM, 10μM	10μM
	Amlodipine	1μM, 5μM, 10μM	5μM
Anti-proliferative drugs	Paclitaxel	10nM, 50nM, 100nM	50nM
	Apocynin	1μM, 5μM, 10μM	5μM
	Losartan	1μM, 5μM, 10μM	1μM
	Fluvastatin	0.25μM, 0.5μM, 1μM	Toxic
Pro-elastogenic drugs	Dexamethasone	10nM, 50nM, 100nM	100nM
	Aldosterone	10nM, 50nM, 100nM	100nM
	Retinyl acetate	100nM, 1μM, 10μM	1μM

CCB, calcium channel blocker

Table III. Phenotyping data of control and patient iPSC-SMCs and response of patient iPSC-SMCs to drugs. This Table is available as a Supplemental Excel file.