ORIGINAL PAPER

Supplementary for "Cytoskeletal and motility changes in human MSCs associated with nuclear-cytoplasmic Rho redistribution during replicative senescence"

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Figure legends

| Figure S1

MSCWJ-1 cells were grown on glasses, fixed and permeabilised at passages: 7, 9, 12, 15, 18, 21, 25, 27, 28, 35, 36. Cells were stained with polyclonal antibodies against synthetic peptide corresponding to amino acids 1949-1960 of human nonmuscle myosin IIA and Alexa 488 secondary anti-rabbit antibodies. Rhodamine phalloidin was used to stain actin cytoskeleton. Colocalization coefficients calculated in Coloc2 ImageJ plugin: Rs, Rval, tM1, tM2, bTau ((Rueden et al. (2017))). Data collected in two channels from manually selected MSCWJ-1 cells as ROIs in confocal images and passed to R for make a matrix of plot with a given data set (Emerson et al. (2013)). PCA factor map (A) and scree plot (B) for myosin-9/F-actin colocolization coefficients. Correlation plots for myosin-9/F-actin (C) and alpha-actinin-4 (D) colocalization coefficients. (E) Pairwise comparison post hoc tests for myosin-9/F-actin bTau colocalization coefficient, In the course of all-pairs comparisons of colocalization data post hoc multiple testing corrections were used to adjust the P-values: Bonferroni method, Scheffe's, and Dunn's tests). The results were visualized using the free Python computing software environment and the scikit-posthocs package (Terpilowski (2019)).

| Figure S2

QQ plots for MSCWJ-1 24 h tracks data. MSCWJ-1 cells were stained with Hoechst33342 and seeded in 6-well plate at passages 9, 15, 36. Then cells were incubated for 4 h for adhesion and and the plate was transferred to CQ1 automated cytometer, where 40 fields of view were recorded within 24 hours from each well. Tracks were manually collected from recorded images in ImageJ and passed to R for analysis with trajr package (McLean and Skowron Volponi (2018)). Observations are summarized for all passages.

| Figure S3

Linear regression for calibration kit FPLC Superose 6 calibration. Elution was performed with elution buffer (150 mM NaCl, 50 mM Tris, pH 7.5, 0.02% NaN_3). The column was calibrated with the set of proteins shown in Table 8.

Tables

Figures

TABLE 1 Cheddock scale for correlation coefficient qualitative estimation as described in Hinkle et al. (2003) and Mukaka (2012)

Value	Colocalization
< 0.1	no link
0.1-0.3	weak
0.3-0.5	moderate
0.5-0.7	noticeable
0.7-0.9	high
0.9-0.99	very high

TABLE 2 Maximum-likelihood factor analysis on the myosin-9/F-actin colocalization data matrix for 2 factors (Lawley and Maxwell (1962)). Cells were grown on glasses, fixed and permeabilised at passages 7, 9, 12, 15, 18, 21, 25, 27, 28, 35, 36. F-actin and myosin-9 were stained with specific antibodies, confocal images were passed to ImageJ. Cells were manually selected on merged images and Coloc2 plugin were used to calculate colocalization coefficients Rval, tM1, tM2, bTau, Rs ((Rueden et al. (2017))). Statistical analysis was performed in R using factanal function (Team et al. (2014)).

	Rval	tM1	tM2	bTau	Rs
Factor 1	0.77	0.91	0.97	0.19	0.23
Factor 2	0.46	0.13	0.17	0.98	0.96
Uniqueness	0.198	0.159	0.039	0.005	0.024

Abbreviations: MSCs, mesenchymal stem cells; τ-Kendall rank correlation coefficient; Rs, Spearman's R correlation coefficient; tM, Manders correlation coefficient; Rval, Pearson correlation coefficient; PCA, principal component analisys

TABLE 3 Kruskal-Wallis rank sum test results for myosin-9/F-actin colocalization coefficients. Cells were grown on glasses, fixed and permeabilised at passages 7, 9, 12, 15, 18, 21, 25, 27, 28, 35, 36. F-actin and myosin-9 were stained with specific antibodies, confocal images were passed to ImageJ. Cells were manually selected on merged images and Coloc2 plugin were used to calculate colocalization coefficients Rval, tM1, tM2, bTau, Rs ((Rueden et al. (2017))). Statistical analysis was performed in R (Team et al. (2014)).

Coefficient	chi-squared	df	p-value
Tau-b	34.669	10	0.0001422
Rs	34.373	10	0.0001596
tM1	16.107	10	0.09661
Pval	15.152	10	0.1266

TABLE 4 Logistic regression with myosin-9/F-actin colocalization coefficients as predictors and WJMSC1 passage number as fitted value. Cells were grown on glasses, fixed and permeabilised at passages 7, 9, 12, 15, 18, 21, 25, 27, 28, 35, 36. F-actin and myosin-9 were stained with specific antibodies, confocal images were passed to ImageJ. Cells were manually selected on merged images and Coloc2 plugin were used to calculate colocalization coefficients Rval, tM1, tM2, bTau, Rs ((Rueden et al. (2017))). Statistical analysis was performed in R (Team et al. (2014)). * $p < 0.05$

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			115	68.13	
Rval	1	0.95	114	67.18	0.3295
tM1	1	0.91	113	66.27	0.3407
tM2	1	1.32	112	64.96	0.2510
bTau	1	4.14	111	60.82	0.0419 *
Rs	1	0.20	110	60.61	0.6509

TABLE 5 Logistic regression with α-actinin-4/Hoechst33342 colocalization coefficients as predictors and passage numbers as fitted values. Cells were grown on glasses, fixed and permeabilised at passages 9, 15, 28, 36. α-actinin-4 was stained with specific antibodies, confocal images were passed to ImageJ. Cells were manually selected on merged images and Coloc2 plugin were used to calculate colocalization coefficients Rval, tM1, tM2, bTau, Rs ((Rueden et al. (2017))). Statistical analysis was performed in R (Team et al. (2014)).

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	3.7063	1.6058	2.31	0.0210
bTau	58.8841	22.4644	2.62	0.0088
tM1	-0.9898	1.5118	-0.65	0.5126
tM2	-1.9521	1.6107	-1.21	0.2256
Rval	-4.3852	1.9107	-2.30	0.0217
Rs	-50.4674	17.4231	-2.90	0.0038

TABLE 6 Logistic regression with RhoA/Hoechst33342 colocalization coefficients as predictors and passage numger as fitted values. Cells were grown on glasses, fixed and permeabilised at passages 9, 15, 28, 36. α-actinin-4 was stained with specific antibodies, confocal images were passed to ImageJ. Cells were manually selected on merged images and Coloc2 plugin were used to calculate colocalization coefficients Rval, tM1, tM2, bTau, Rs ((Rueden et al. (2017))). Statistical analysis was performed in R (Team et al. (2014)). ** p < 0.005, *** p < 0.001

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept) (**)	2.2687	0.7100	3.20	0.0014
bTau (***)	97.1021	27.8353	3.49	0.0005
Rval	0.5242	1.3355	0.39	0.6947
Rs (***)	-78.1599	22.1536	-3.53	0.0004

TABLE 7 Shapiro-Wilk normality test for MSCWJ-1 24 h trajectory analysis data. MSCWJ-1 cells were stained with Hoechst33342 and seeded in 6-well plate at passages 9, 15, 36. Then cells were incubated for 4 h for adhesion and and the plate was transferred to CQ1 automated cytometer, where 40 fields of view were recorded within 24 hours from each well. Tracks were manually collected from recorded images in ImageJ and passed to R for analysis with trajr package (McLean and Skowron Volponi (2018)).

Passage	Parameter	W	P-value
9	mean speed	0.96942	0.0008038
9	max speed	0.97546	0.003996
9	length	0.96942	0.0008038
9	distance	0.94779	6.059e-06
9	sinuosity	0.96901	0.0007248
9	straightness	0.94387	2.806e-06
15	mean speed	0.95258	1.532e-05
15	max speed	0.95519	2.68e-05
15	length	0.95258	1.532e-05
15	distance	0.91826	3.121e-08
15	sinuosity	0.94357	2.491e-06
15	straightness	0.94791	5.855e-06
36	mean speed	0.90753	3.937e-09
36	max speed	0.87963	9.191e-11
36	length	0.93084	1.639e-07
36	distance	0.91003	5.702e-09
36	sinuosity	0.95131	8.383e-06
36	straightness	0.96288	0.0001151

TABLE 8 Molecular weights of Superose 6 gel-chromatography column calibration proteins used in FPLC. Elution buffer: 150 mM NaCl, 50 mM Tris, pH 7.5, 0.02% NaN_3.

Protein	Molecular weight, kDa
Bovine erythrocytes Ovalbumin	43
Horse spleen Thyroglobulin	669
Rabbit muscle Ferritin	440
Chicken egg white Aldolase	158
Bovine lung Ribonuclease A	13.7

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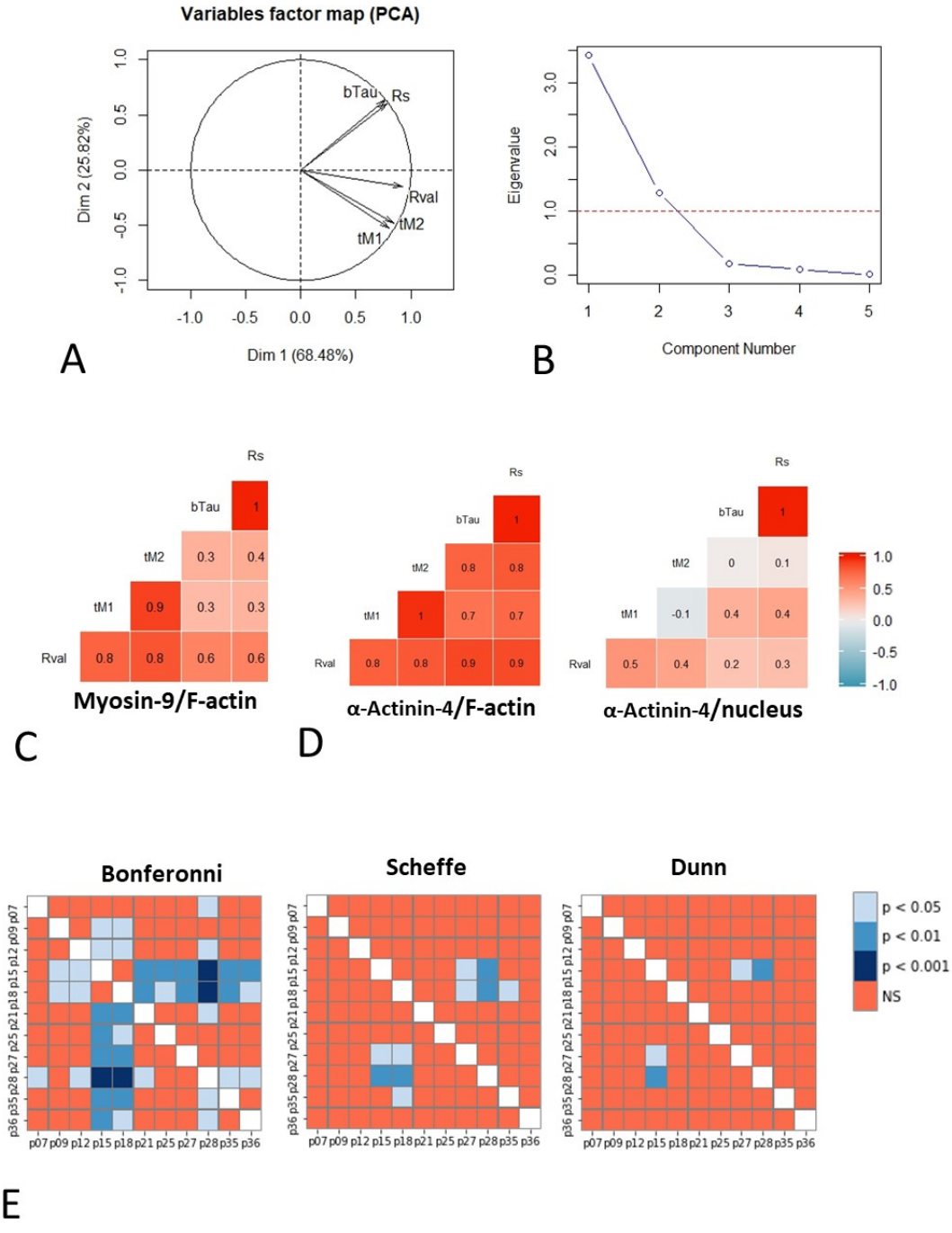
Variables factor map (PCA)
bTau
Rs
Rval
tM2
tM1
Dim 2 (25.82%)
Dim 1 (68.48%)
A
Eigenvalue
Component Number
B
Myosin-9/F-actin
C
α-Actinin-4/F-actin
α-Actinin-4/nucleus
D
Bonferonni
Scheffe
Dunn
p < 0.05
p < 0.01
p < 0.001
NS
E

FIGURE 1

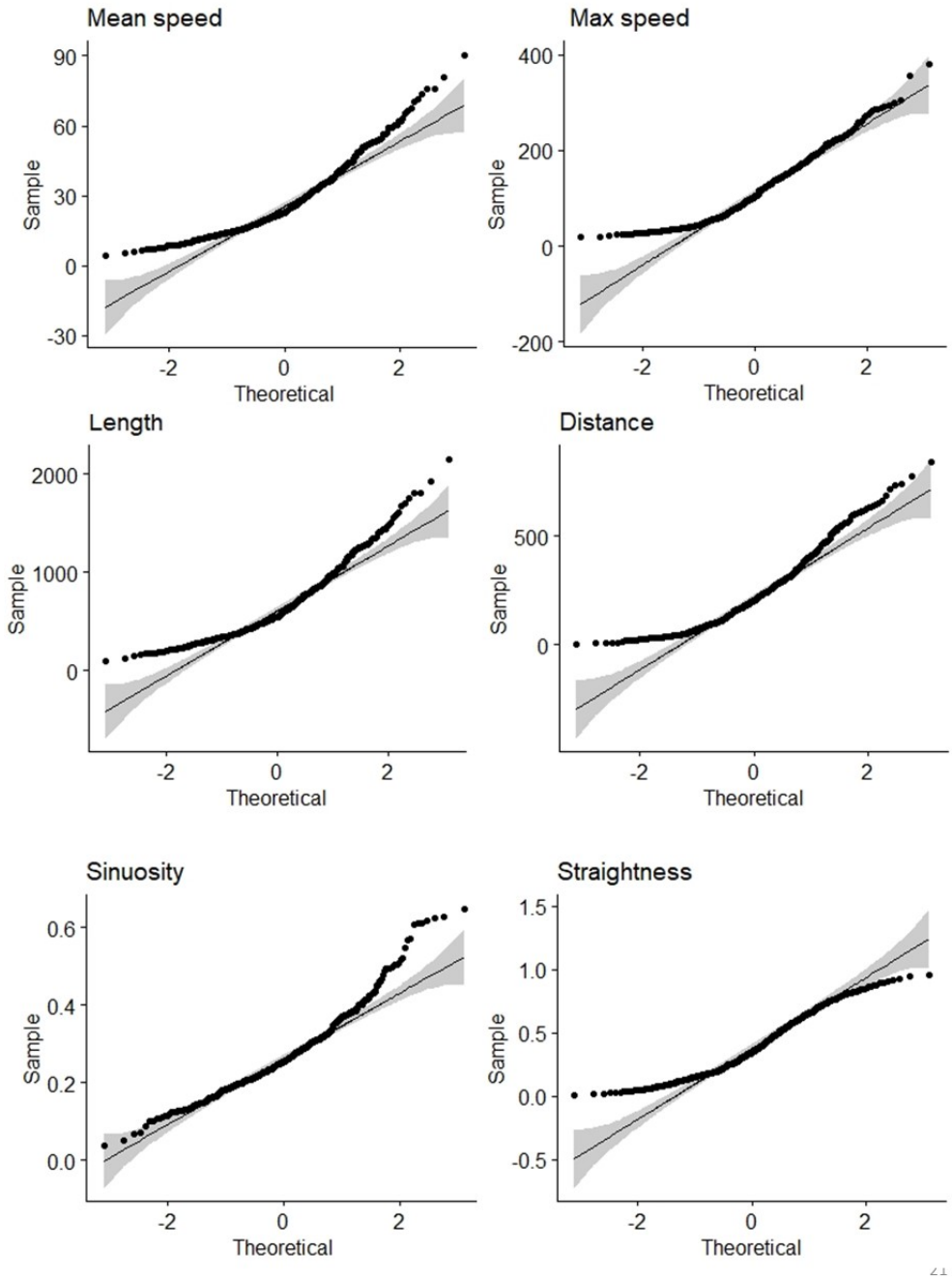
Mean speed
Max speed
Length
Distance
Sinuosity
Straightness
Sample
Theoretical
90
60
30
0
-30
400
200
-200
2000
1000
500
0.6
0.4
0.2
0.0
1.5
1.0
0.5
-0.5
-2
0
2

FIGURE 2

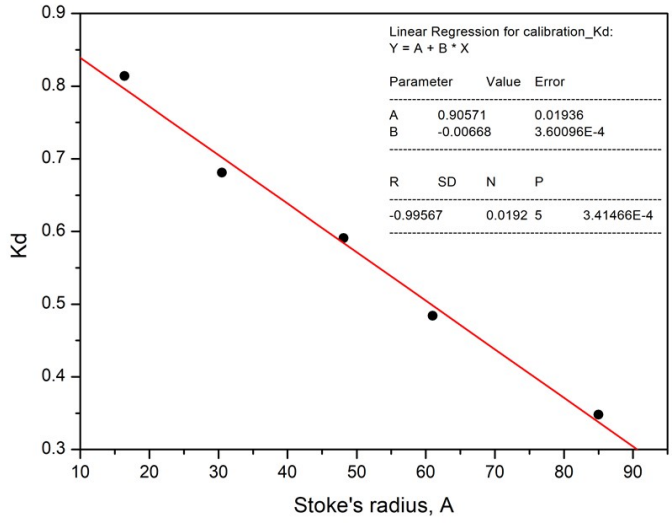
Linear Regression for calibration_Kd:
Y = A + B * X
Parameter Value Error
A 0.90571 0.01936
B -0.00668 3.60096E-4
R SD N P
-0.99567 0.0192 5 3.41466E-4
Kd
Stoke's radius, A
0.9
0.8
0.7
0.6
0.5
0.4
0.3
10
20
30
40
50
60
70
80
90

FIGURE 3