

# 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 Supp.1

Correlation matrix of colocalization coefficients calculated in Coloc2 ImageJ plugin: Rs, Rval, tM1, tM2, bTau ([1]). Cells were grown on glasses, fixed and permeabilised at passages: 7, 9, 12, 15, 18, 21, 25, 27, 28, 35, 36. MSCWJ-1 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. 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 ([2]).

### | Figure Supp.2

Explorative analysis of colocalization data. Cells were grown on glasses, fixed and permeabilised at passages: 7, 9, 12, 15, 18, 21, 25, 27, 28, 35, 36. MSCWJ-1 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. Data collected in two channels from manually selected MSCWJ-1 cells as ROIs in confocal images. PCA factor map (A) and scree plot (B) for myosin-9/F-actin colocalization coefficients. Correlation plots for myosin-9/F-actin (C) and alpha-actinin-4/F-actin 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 ([3]).

Figure Supp.3

QQ plots of MSCWJ-1 24 movement tracks analysis data. Observations are summarized for all passages.

references

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Tables

Figures

TABLE 1 Cheddock scale for Kendall correlation coefficient qualitative estimation as described in [4] and [5]

bTau 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

**Abbreviations:** MSCs, mesenchymal stem cells;  $\tau$ -Kendall rank correlation coefficient; Rs, Spearman's R correlation coefficient; tM, Manders correlation coefficient; Rval, Pearson correlation coefficient; PCA, principal component analysis

**TABLE 2** PCA of myosin-9/F-actin colocalization data loadings for 2 factors

	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

**TABLE 3** Kruskal-Wallis rank sum test results for myosin-9/F-actin colocalization coefficients

Colocalization coefficient	chi-squared	df	p-value
Kendall's Tau-b	34.669	10	0.0001422
Spearman's R	34.373	10	0.0001596
Manders' M	16.107	10	0.09661
Pearson's R	15.152	10	0.1266

**TABLE 4** Logistic regression with myosin-9/F-actin colocalization coefficients as predictors and passage number as fitted values

	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  $\alpha$ -actinin-4/Hoechst33342 colocalization coefficients as predictors and passage numbers as fitted values

	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 number as fitted values

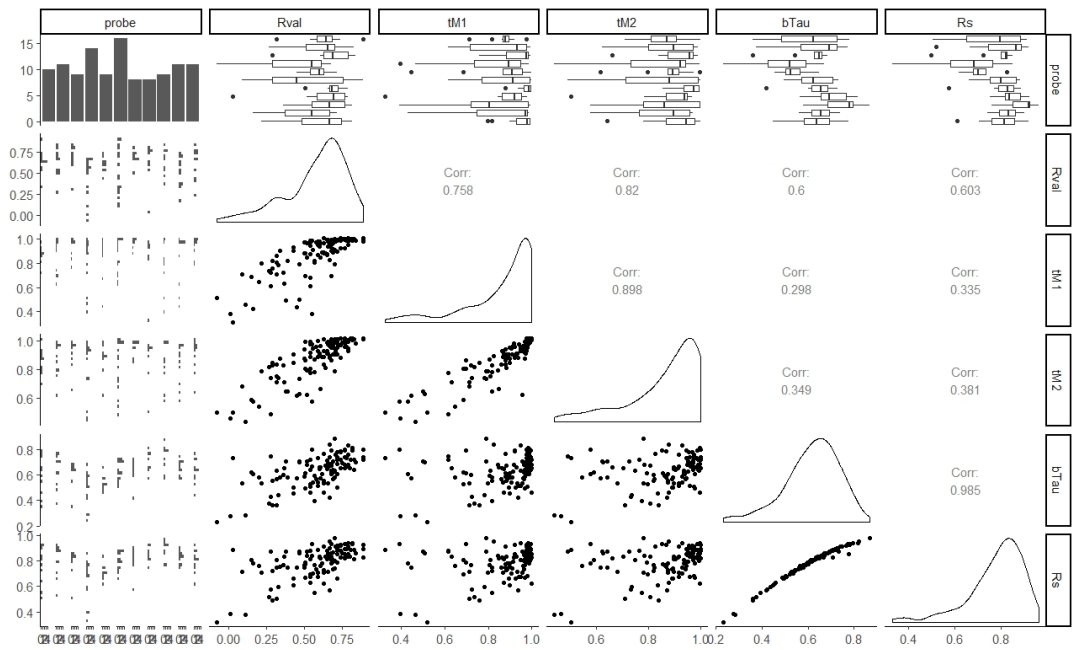
	Estimate	Std. Error	z value	Pr(> z )
(Intercept) (**)	2.2687	0.7100	3.20	0.0014
bTau (***)	97.1021	27.8353	3.49	0.0005
Rval	0.5242	1.3355	0.39	0.6947
Rs (***)	-78.1599	22.1536	-3.53	0.0004

**TABLE 7** Shapiro-Wilk normality test for MSCWJ-1 24 h trajectory analysis data

Passage number	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** Superose 6 column calibration protein set

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



**FIGURE 1**

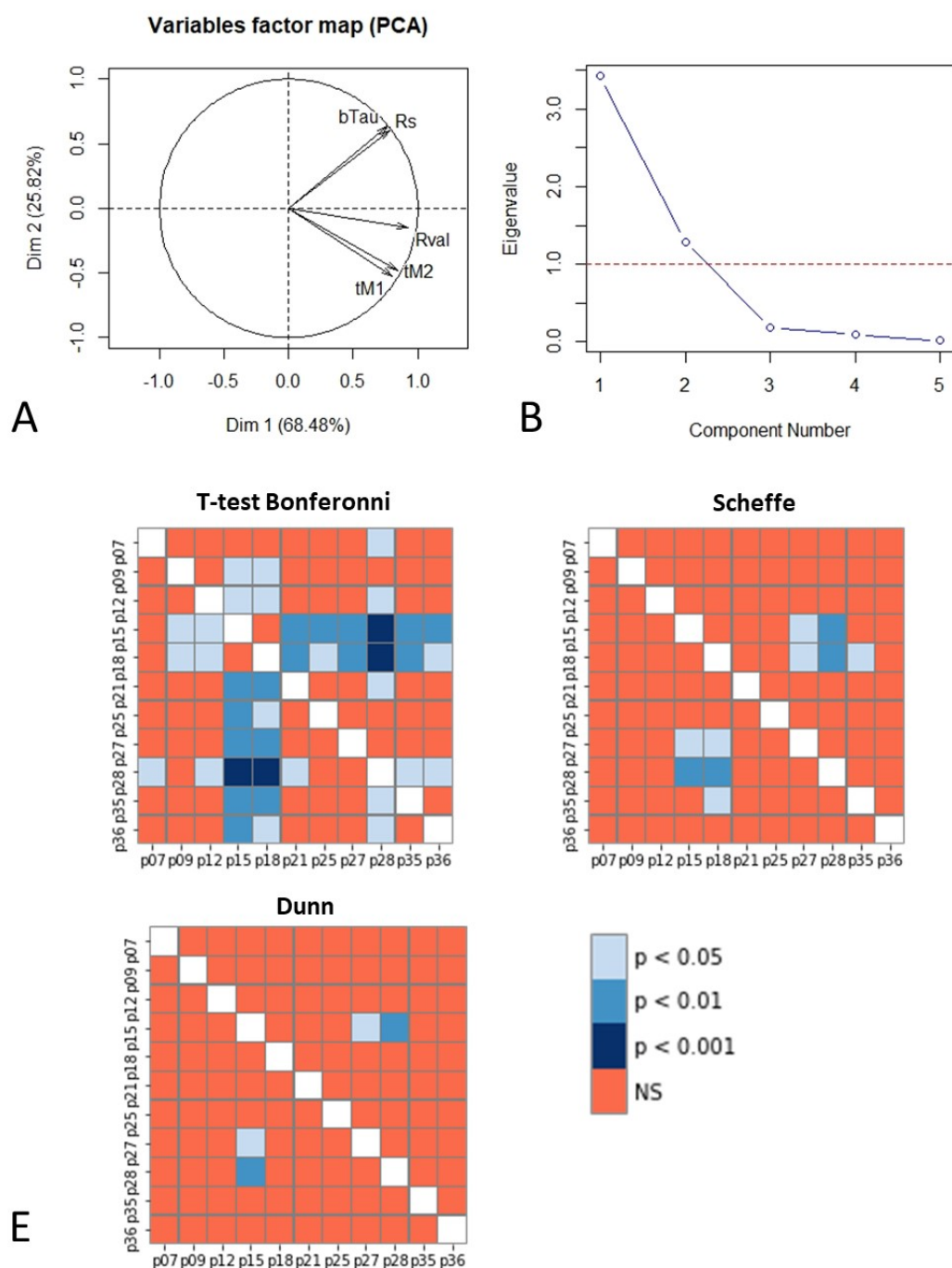


FIGURE 2

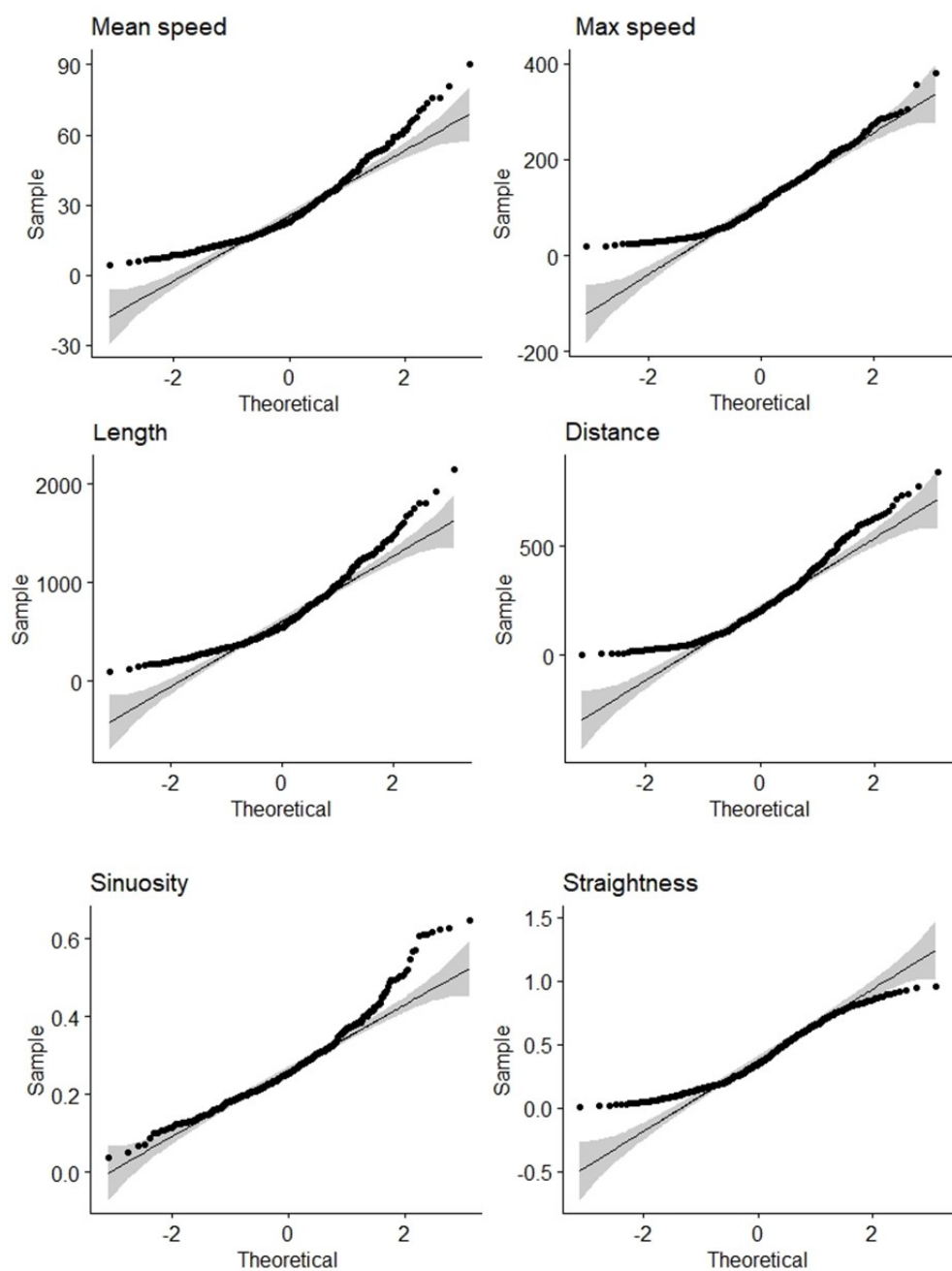


FIGURE 3