



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

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**TABLE 1** Cheddock scale for Kendall correlation coefficient estimation

Tau-Kendall Correlation coeff 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** 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

**Abbreviations:** MSCs, mesenchymal stem cells; PCA, principal component analysis

**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

2 | FIGURES

**TABLE 6** Logistic regression with RhoA/Hoechst33342 colocalization coefficients as predictors and passage number as fitted values

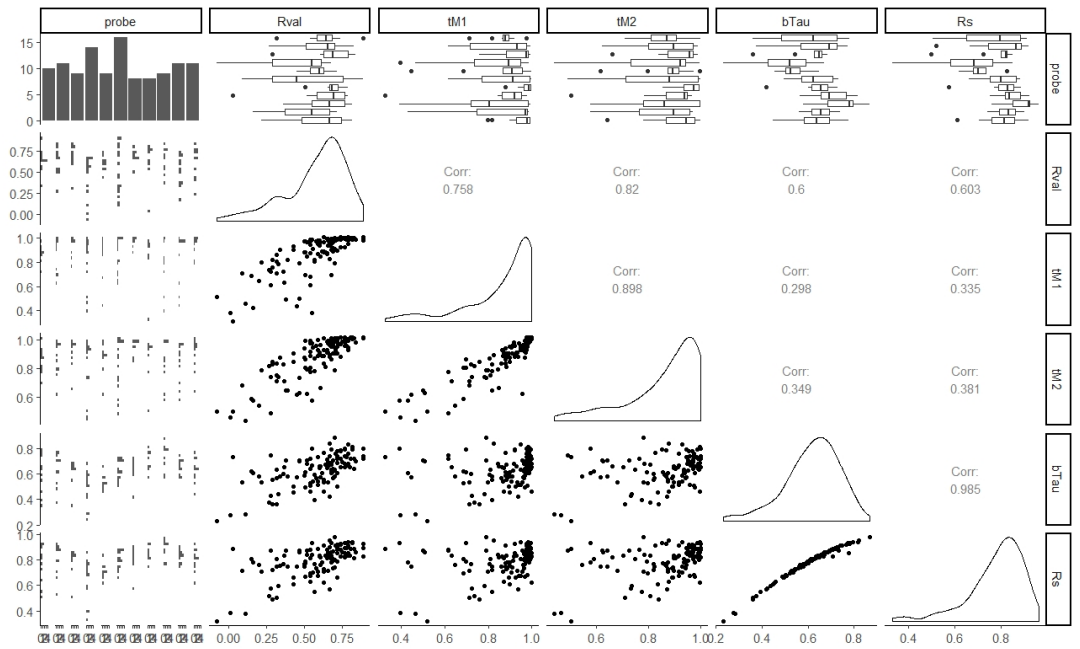
	Estimate	Std. Error	z value	Pr(> z )
(Intercept) (**)	2.2687	0.7100	3.20	0.0014
bTau (***)	97.1021	27.8353	3.49	0.0005
Rval	0.5242	1.3355	0.39	0.6947
Rs (***)	-78.1599	22.1536	-3.53	0.0004

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

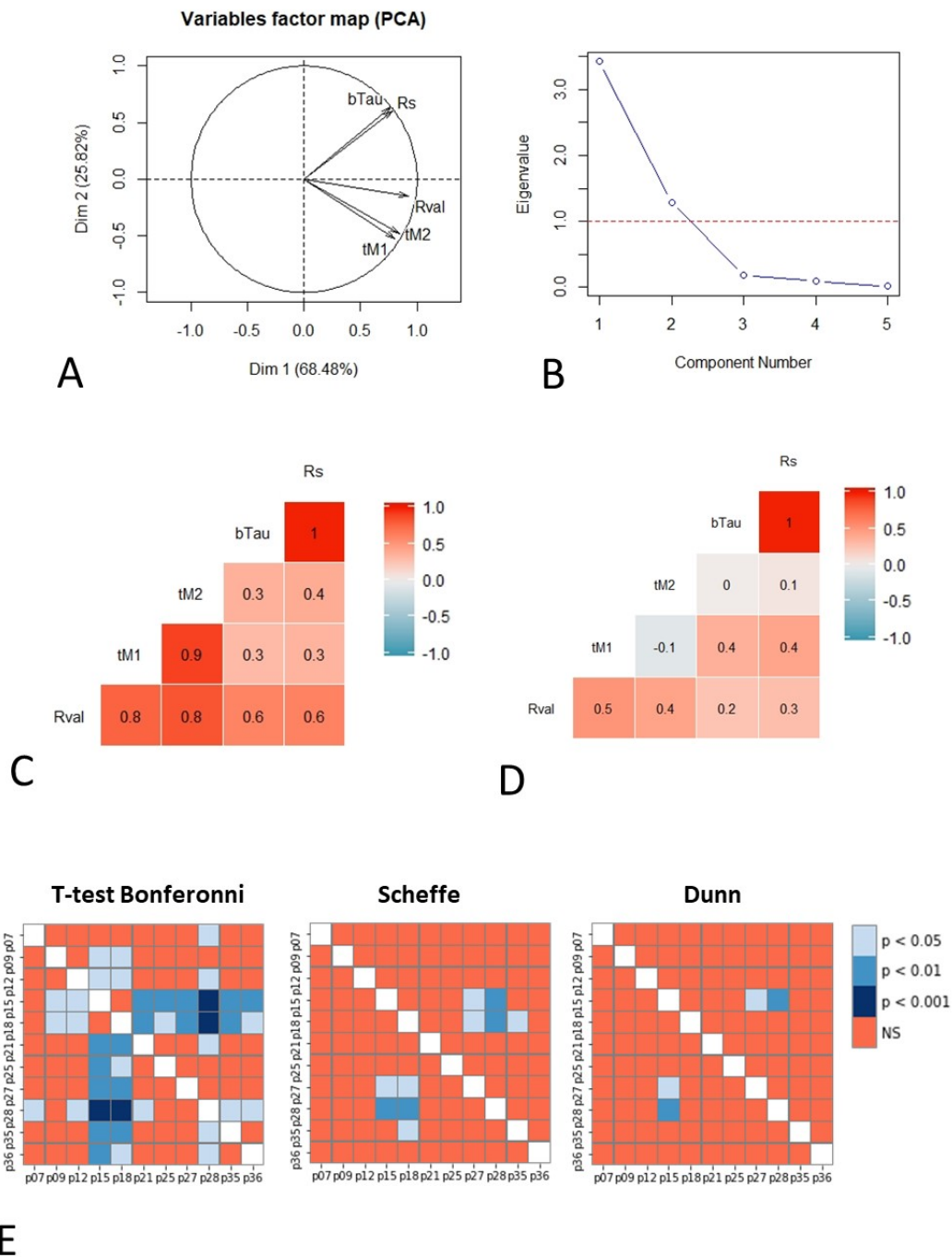
Passage number	Parameter	W	P-value
9	mean speed	0.96942	0.0008038
9	max speed	0.97546	0.003996
9	length	0.96942	0.0008038
9	distance	0.94779	6.059e-06
9	sinuosity	0.96901	0.0007248
9	straightness	0.94387	2.806e-06
15	mean speed	0.95258	1.532e-05
15	max speed	0.95519	2.68e-05
15	length	0.95258	1.532e-05
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 Gel-filtration calibration protein set

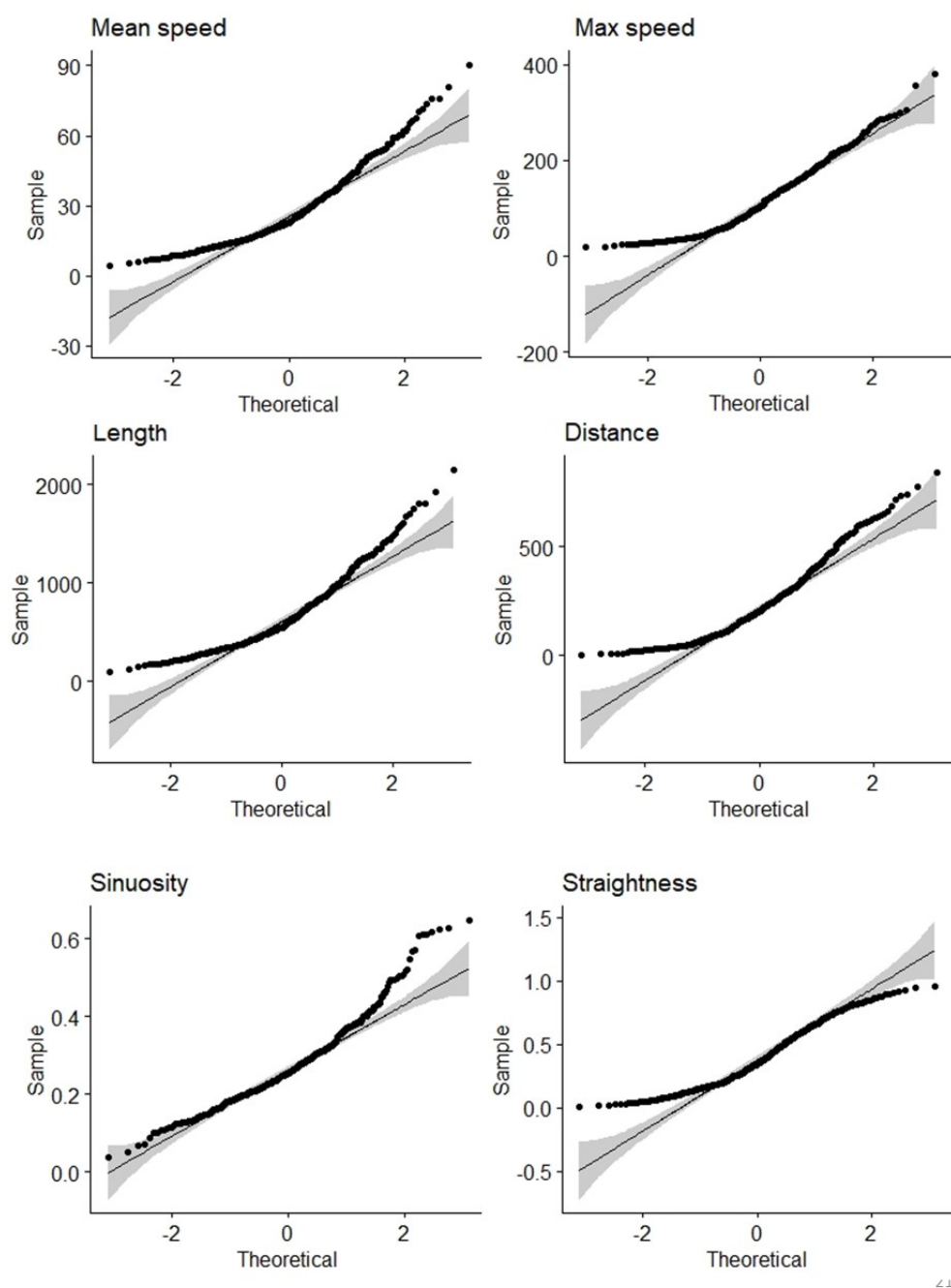
Protein	Molecular weight (Mr), kDa
Ovalbumin	43
Horse spleen Thyroglobulin	669
Rabbit muscle Ferritin	440
Chicken egg white Aldolase	158
Bovine erythrocytes Ovalbumin	43
Bovine lung Ribonuclease A	13.7



**FIGURE 1** Correlation matrix of colocalization coefficients calculated in Coloc2 ImageJ plugin: Rs, Rval, tM1, tM2, bTau. Data collected in two channels from manually selected cells as ROIs in confocal images. MSCWJ-1 cells were stained with polyclonal anti-myosin-9 antibodies and rhodamine phalloidin. Cells were fixed at passages: 7, 9, 12, 15, 18, 21, 25, 27, 28, 35, 36.



**FIGURE 2** Explorative analysis of colocalization data. Data were collected in two channels from manually selected cells as ROIs in confocal images. MSCWJ-1 cells were stained with polyclonal anti-myosin-9 antibodies and rhodamine phalloidin. Cells were fixed at passages: 7, 9, 12, 15, 18, 21, 25, 27, 28, 35, 36. 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.



**FIGURE 3** QQ plots of MSCWJ-1 24 movement tracks analysis data. Observations are summarized for all passages.