ORIGINAL PAPER

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

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1 | TABLES

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 analisysu

 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 α -actinin-4 and nucleus colocalization coefficients as predictors and passage numbers as fitted values

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

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TABLE 6 Logistic regression with RhoA and nucleus 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 |

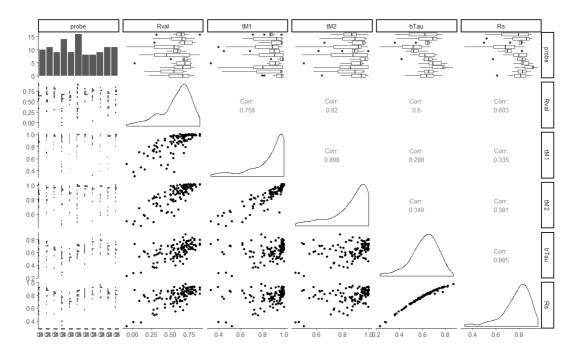
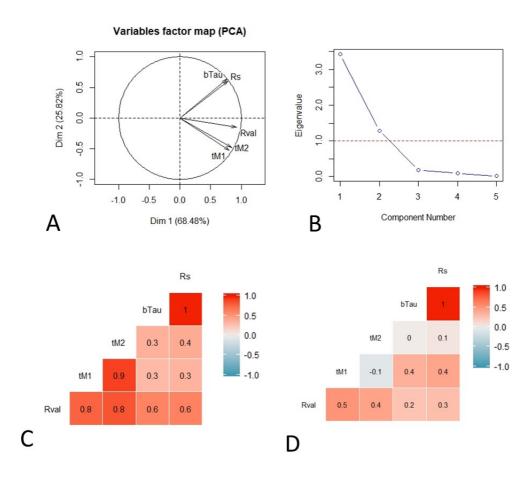
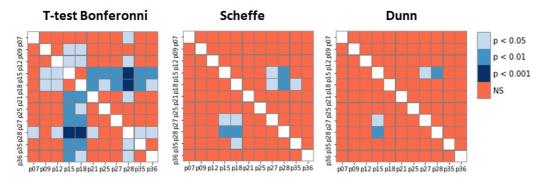


FIGURE 1 Correlation matrix of colocalization coefficients calculated in Coloc2 ImageJ plugin: Rs, Rval, tM1, tM2, bTau. Data collected in two channels from manually selected cells as ROIs in confocal images. MSCWJ-1 cells were stained with polyclonal anti-myosin-9 antibodies and rhodamine phalloidin.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





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FIGURE 2 Explorative analisys 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 colocolization 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.