

# **Cell Shape and Matrix Stiffness Impact Schwann Cell Plasticity via YAP/TAZ and RhoGTPases**

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## **Supplemental Information**

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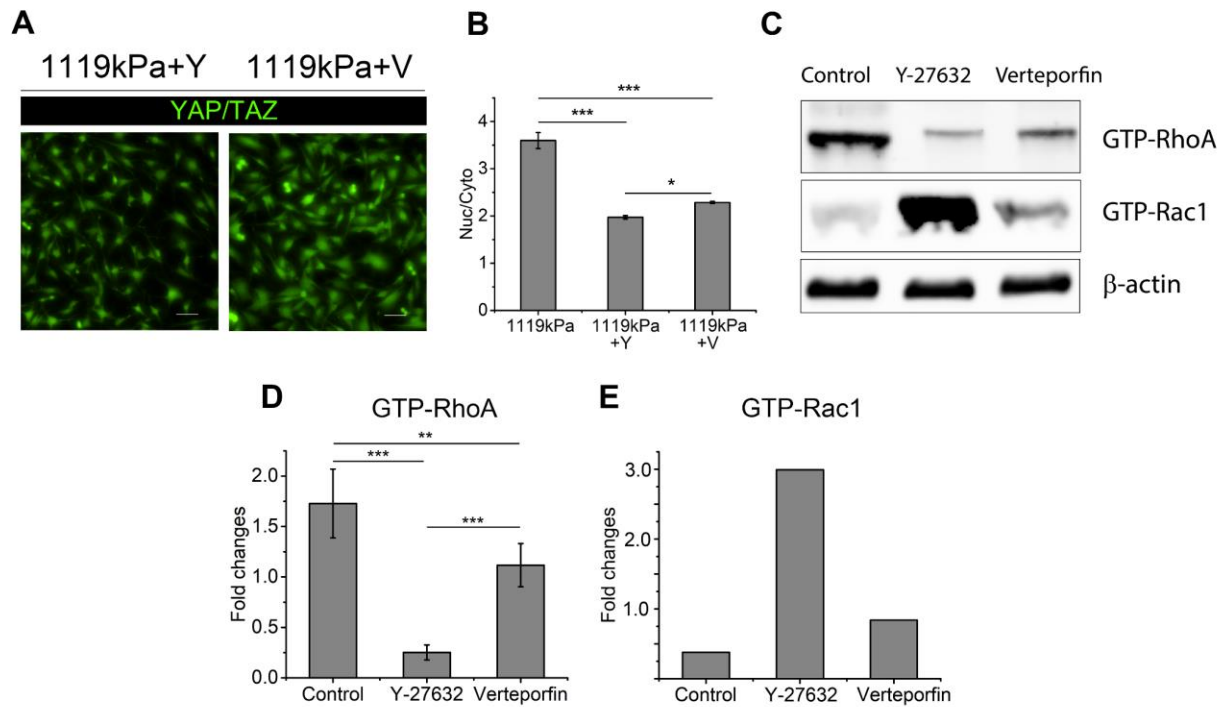
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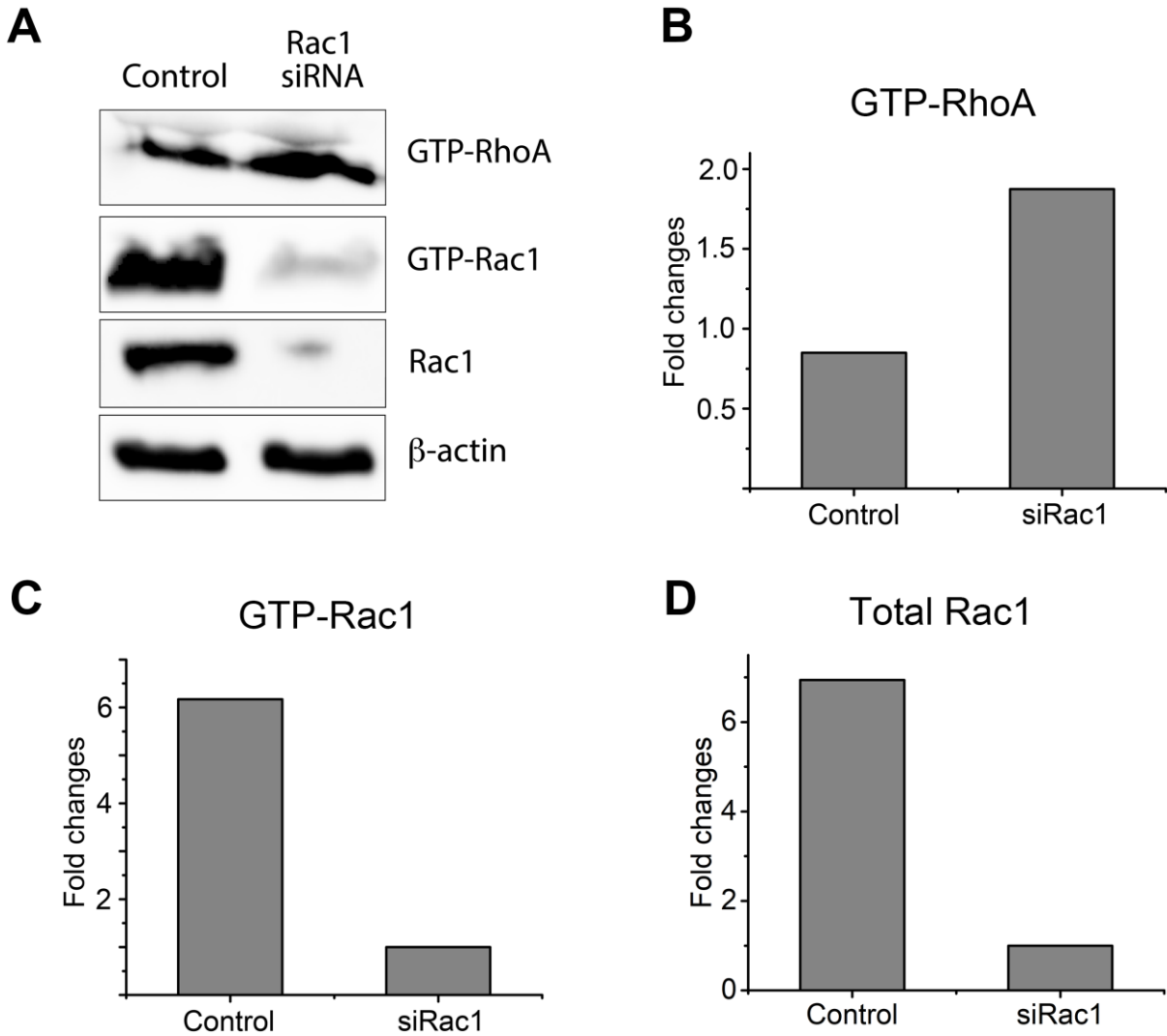
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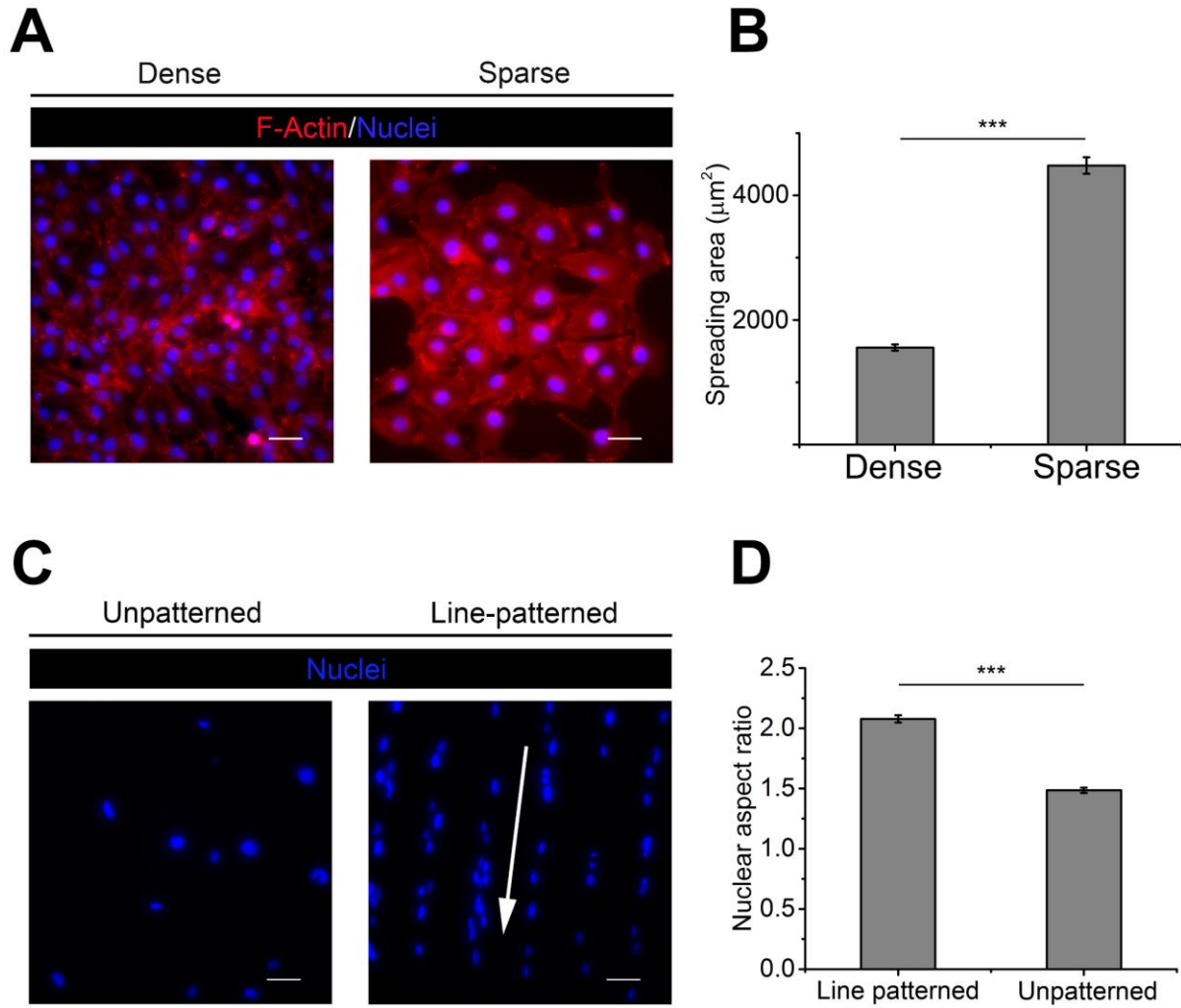
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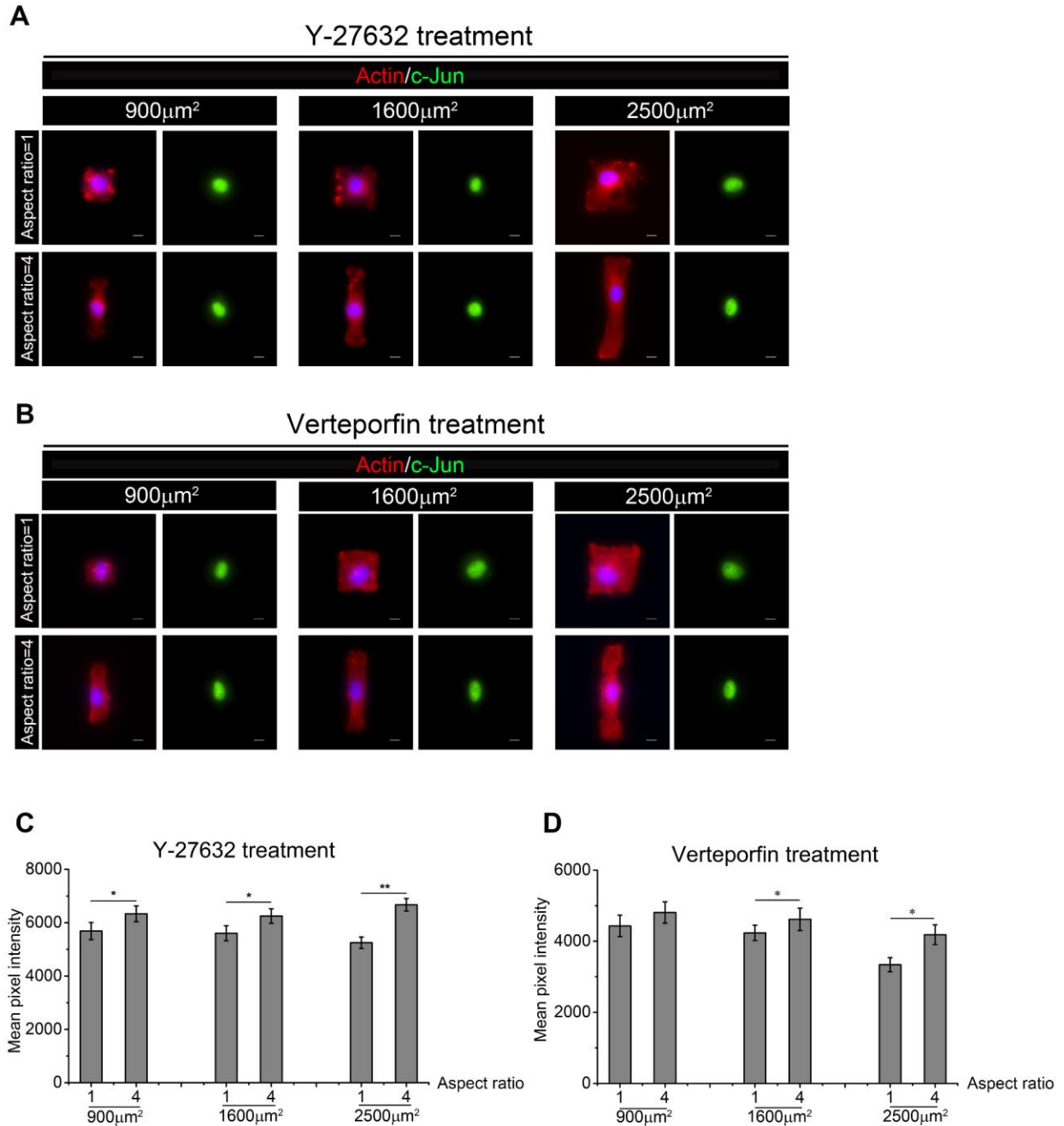
**Figure S1:** (A-B) Nuclear (active) YAP/TAZ is inhibited by Y-27632 (ROCK) and Verteporfin (YAP/TAZ). For YAP/TAZ fluorescent intensity quantification,  $n = 3$  unique trials with a minimum of 35 cells were quantified per trial. Scale bar = 50  $\mu$ m. (C-E) When RhoA and YAP/TAZ are inhibited, active RhoA is downregulated while active Rac1 is upregulated. For Western blot analysis, 3 separate lysates were created to quantify GTP-RhoA and 1 lysate to quantify GTP-Rac1. Data is presented as mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.005$ , \*\*\* $p < 0.0005$ .



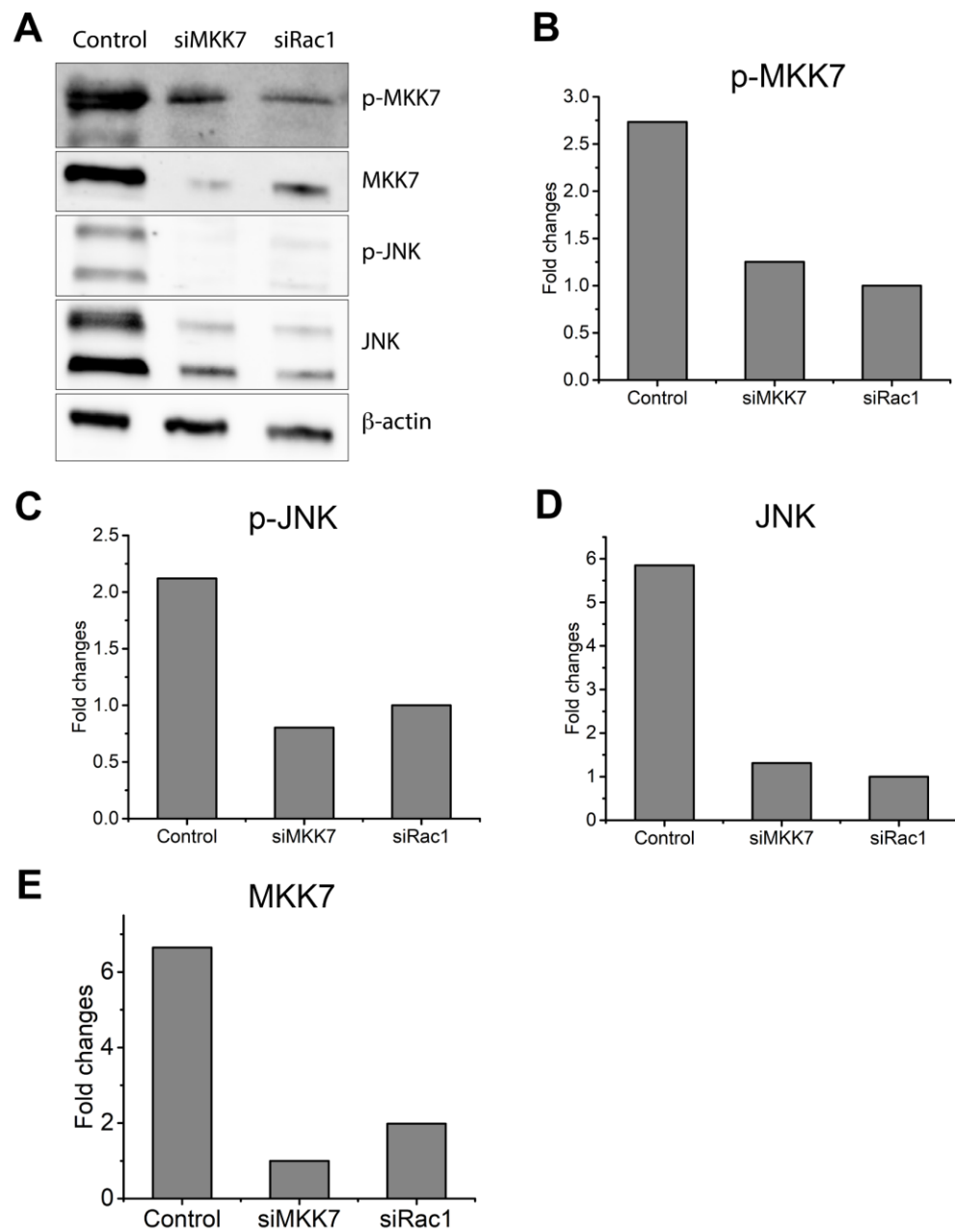
**Figure S2:** (A-D) siRac1 transfection significantly downregulates Rac1 activity while increasing RhoA activity.



**Figure S3:** (A-B) F-actin (red) showing the average cell spreading area was controlled by varying initial cell seeding density. 3 unique trials with a minimum of 74 cells per trial were quantified. Scale bar = 50  $\mu\text{m}$ . (C-D) Nuclear (blue) elongation was promoted using line-patterned substrates when compared to cells on unpatterned surfaces. 6 unique trials with a minimum of 41 cells per trial were quantified. Scale bar = 50  $\mu\text{m}$ . White arrows indicate the direction of the line patterns. Data is presented as mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.005$ , \*\*\* $p < 0.0005$ .



**Figure S4:** SCs on micropatterned, cell adhesive islands with variable spreading area and elongation (A) without treatment and (B) with Y-27632 and verteporfin treatment. SCs are visualized for nucleus (blue), F-actin (red), and c-Jun (green). Scale bar: = 10  $\mu\text{m}$  (C-D) The mean pixel intensity of c-Jun was quantified to show differences in expression across cell area and cell elongation with Y-27632 or verteporfin treatment. 5 unique trials with a minimum of 51 single micropatterned SCs per trial were quantified. Data is presented as mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.005$ , \*\*\* $p < 0.0005$ .



**Figure S5:** (A-D) Western blot analysis of phosphorylated-MKK7, phosphorylated-JNK, and total JNK upon siMKK7 and siRac1 transfection.

**Table S1**

|              |          | Conditions   |              | p value     |
|--------------|----------|--------------|--------------|-------------|
| Western Blot | GTP-RhoA | 1119 kPa     | 8.67 kPa     | 0.001941747 |
|              | GTP Rac1 | 1119 kPa     | 8.67 kPa     | 0.038639998 |
|              | c-Jun    | 1119 kPa     | 8.67 kPa     | 0.024560145 |
|              | p75NTR   | 1119 kPa     | 8.67 kPa     | 0.017519102 |
| Staining     | YAP/TAZ  | 1119 kPa     | 8.67 kPa     | 0           |
| Western Blot | c-Jun    | Control 8.67 | Control 1119 | 0.00333     |
|              |          | Y 1119       | Control 1119 | 0           |
|              |          | Y 1119       | Control 8.67 | 0           |
|              |          | Y 8.67       | Control 1119 | 0           |
|              |          | Y 8.67       | Control 8.67 | 0           |
|              |          | Y 8.67       | Y 1119       | 0.00676614  |
|              |          | V 1119       | Control 1119 | 0.0007288   |
|              |          | V 1119       | Control 8.67 | 0.0008781   |
|              |          | V 1119       | Y 1119       | 1.92738E-07 |
|              |          | V 1119       | Y 8.67       | 0           |
|              |          | V 8.67       | Control 1119 | 3.0885E-08  |
|              |          | V 8.67       | Control 8.67 | 0.000509    |
|              |          | V 8.67       | Y 1119       | 2.97037E-08 |
|              |          | V 8.67       | Y 8.67       | 7.55059E-06 |
|              |          | V 8.67       | V 1119       | 4.70563E-06 |
| Western Blot | p75NTR   | Control 8.67 | Control 1119 | 0.007490434 |
|              |          | Y 1119       | Control 1119 | 0           |
|              |          | Y 1119       | Control 8.67 | 0           |
|              |          | Y 8.67       | Control 1119 | 0           |
|              |          | Y 8.67       | Control 8.67 | 0           |
|              |          | Y 8.67       | Y 1119       | 0.116116524 |
|              |          | V 1119       | Control 1119 | 0.053338261 |
|              |          | V 1119       | Control 8.67 | 0.056898804 |
|              |          | V 1119       | Y 1119       | 0.001990975 |
|              |          | V 1119       | Y 8.67       | 0.001526881 |
|              |          | V 8.67       | Control 1119 | 0.14230723  |
|              |          | V 8.67       | Control 8.67 | 0.19657615  |
|              |          | V 8.67       | Y 1119       | 0.00293879  |
|              |          | V 8.67       | Y 8.67       | 0.002895325 |
|              |          | V 8.67       | V 1119       | 0.001885195 |
|              |          |              |              |             |

**Table S2**

|              |        | Conditions     |                | p value     |
|--------------|--------|----------------|----------------|-------------|
| Western Blot | c-Jun  | Y+Empty        | Y+siRac1       | 0.001110743 |
|              |        | V+Empty        | V+siRac1       | 0.001775254 |
|              |        | Empty          | siRac1         | 0.003378035 |
|              |        | Empty          | V+siRac1       | 0.003868486 |
| Western Blot | p75NTR | Y+Empty        | Y+siRac1       | 0.000139718 |
|              |        | V+Empty        | V+siRac1       | 0.00305854  |
|              |        | Empty          | siRac1         | 0.005522349 |
|              |        | Empty          | V+siRac1       | 0.000255004 |
| Staining     | p75NTR | Y              | Control        | 0           |
|              |        | V              | Control        | 0.001       |
|              |        | V              | Y              | 4.01E-03    |
|              |        | Control+siRac1 | Control        | 0.1005      |
|              |        | Control+siRac1 | Y              | 0           |
|              |        | Control+siRac1 | V              | 5.40E-09    |
|              |        | Y+siRac1       | Control        | 0.15641     |
|              |        | Y+siRac1       | Y              | 0           |
|              |        | Y+siRac1       | V              | 2.00E-08    |
|              |        | Y+siRac1       | Control+siRac1 | 1           |
|              |        | V+siRac1       | Control        | 0.1616      |
|              |        | V+siRac1       | Y              | 0           |
|              |        | V+siRac1       | V              | 2.60E-08    |
|              |        | V+siRac1       | Control+siRac1 | 1           |
|              |        | V+siRac1       | Y+siRac1       | 1           |
| Staining     | Sox-2  | Y              | Control        | 8.03E-09    |
|              |        | V              | Control        | 7.19E-05    |
|              |        | V              | Y              | 8.12E-07    |
|              |        | Control+siRac1 | Control        | 2.55E-08    |
|              |        | Control+siRac1 | Y              | 0           |
|              |        | Control+siRac1 | V              | 4.18E-09    |
|              |        | Y+siRac1       | Control        | 1.77E-08    |
|              |        | Y+siRac1       | Y              | 0           |
|              |        | Y+siRac1       | V              | 2.78E-09    |
|              |        | Y+siRac1       | Control+siRac1 | 0.9999      |
|              |        | V+siRac1       | Control        | 5.41E-08    |
|              |        | V+siRac1       | Y              | 0           |
|              |        | V+siRac1       | V              | 3.93E-09    |
|              |        | V+siRac1       | Control+siRac1 | 0.9925      |
|              |        | V+siRac1       | Y+siRac1       | 0.99909     |



**Table S3**

| 3            |          | Conditions  |                | p value     |
|--------------|----------|-------------|----------------|-------------|
| Western Blot | GTP-RhoA | Sparse      | Dense          | 0.016210457 |
|              | c-Jun    | Sparse      | Dense          | 0.013122015 |
|              | GTP-Rac1 | Sparse      | Dense          | 0.023649123 |
|              | p75NTR   | Sparse      | Dense          | 0.00076223  |
| Western Blot | GTP-RhoA | Unpatterned | Line-patterned | 0.136001196 |
|              | c-Jun    | Unpatterned | Line-patterned | 0.001248187 |
|              | GTP-Rac1 | Unpatterned | Line-patterned | 0.000975385 |
|              | p75NTR   | Unpatterned | Line-patterned | 0.00080756  |
| Staining     | YAP/TAZ  | 900 4:1     | 900 1:1        | 0.99968     |
|              |          | 1600 1:1    | 900 1:1        | 0.40664     |
|              |          | 1600 1:1    | 900 4:1        | 0.71501     |
|              |          | 1600 4:1    | 900 1:1        | 0.422       |
|              |          | 1600 4:1    | 900 4:1        | 0.71563     |
|              |          | 1600 4:1    | 1600 1:1       | 1           |
|              |          | 2500 1:1    | 900 1:1        | 0.000338    |
|              |          | 2500 1:1    | 900 4:1        | 0.02752     |
|              |          | 2500 1:1    | 1600 1:1       | 0.0077539   |
|              |          | 2500 1:1    | 1600 4:1       | 0.83776     |
|              |          | 2500 4:1    | 900 1:1        | 0.00031908  |
|              |          | 2500 4:1    | 900 4:1        | 1.25474E-06 |
|              |          | 2500 4:1    | 1600 1:1       | 0.48494     |
|              |          | 2500 4:1    | 1600 4:1       | 0.000806611 |
|              |          | 2500 4:1    | 2500 1:1       | 0.99943     |

**Table S4**

| 4        |        | Conditions |            | p value     |
|----------|--------|------------|------------|-------------|
| Staining | c-Jun  | 900-1 Y    | 900-1 Con  | 6.29E-05    |
|          |        | 900-1 V    | 900-1 Con  | 0.92292     |
|          |        | 900-1 V    | 900-1 Y    | 0.04283     |
|          |        | 1600-1 Con | 900-1 Con  | 0.8213      |
|          |        | 1600-1 Con | 900-1 Y    | 1.15E-07    |
|          |        | 1600-1 Con | 900-1 V    | 0.16207     |
|          |        | 1600-1 Y   | 900-1 Con  | 2.07E-05    |
|          |        | 1600-1 Y   | 900-1 Y    | 1           |
|          |        | 1600-1 Y   | 900-1 V    | 0.07922     |
|          |        | 1600-1 Y   | 1600-1 Con | 2.60E-08    |
|          |        | 1600-1 V   | 900-1 Con  | 0.9947      |
|          |        | 1600-1 V   | 900-1 Y    | 0.00318     |
|          |        | 1600-1 V   | 900-1 V    | 0.99986     |
|          |        | 1600-1 V   | 1600-1 Con | 0.0042259   |
|          |        | 1600-1 V   | 1600-1 Y   | 0.00196     |
|          |        | 2500-1 Con | 900-1 Con  | 0.003575    |
|          |        | 2500-1 Con | 900-1 Y    | 3.04E-08    |
|          |        | 2500-1 Con | 900-1 V    | 0.00277     |
|          |        | 2500-1 Con | 1600-1 Con | 0.0092224   |
|          |        | 2500-1 Con | 1600-1 Y   | 2.82E-08    |
|          |        | 2500-1 Con | 1600-1 V   | 0.0061      |
|          |        | 2500-1 Y   | 900-1 Con  | 0.00737     |
|          |        | 2500-1 Y   | 900-1 Y    | 0.96333     |
|          |        | 2500-1 Y   | 900-1 V    | 0.4751      |
|          |        | 2500-1 Y   | 1600-1 Con | 2.93E-05    |
|          |        | 2500-1 Y   | 1600-1 Y   | 0.009824    |
|          |        | 2500-1 Y   | 1600-1 V   | 0.11887     |
|          |        | 2500-1 Y   | 2500-1 Con | 2.16E-08    |
|          |        | 2500-1 V   | 900-1 Con  | 0.91699     |
|          |        | 2500-1 V   | 900-1 Y    | 6.41E-05    |
|          |        | 2500-1 V   | 900-1 V    | 0.3792      |
|          |        | 2500-1 V   | 1600-1 Con | 1           |
|          |        | 2500-1 V   | 1600-1 Y   | 4.55E-05    |
|          |        | 2500-1 V   | 1600-1 V   | 0.0059078   |
|          |        | 2500-1 V   | 2500-1 Con | 0.99797     |
|          |        | 2500-1 V   | 2500-1 Y   | 0.0251      |
| Staining | p75NTR | 1600 1 C   | 900 1 C    | 0.96637     |
|          |        | 2500 1 C   | 900 1 C    | 0.000061834 |
|          |        | 2500 1 C   | 1600 1 C   | 0.00732175  |
|          |        | 900 1 Y    | 900 1 C    | 0.027521241 |

|  |  |          |          |             |
|--|--|----------|----------|-------------|
|  |  | 900 1 Y  | 1600 1 C | 0.25442     |
|  |  | 900 1 Y  | 2500 1 C | 0.05647     |
|  |  | 1600 1 Y | 900 1 C  | 0.99608     |
|  |  | 1600 1 Y | 1600 1 C | 0.0034485   |
|  |  | 1600 1 Y | 2500 1 C | 0.2362      |
|  |  | 1600 1 Y | 900 1 Y  | 0.99999     |
|  |  | 2500 1 Y | 900 1 C  | 0.99799     |
|  |  | 2500 1 Y | 1600 1 C | 0.99998     |
|  |  | 2500 1 Y | 2500 1 C | 0.007590934 |
|  |  | 2500 1 Y | 900 1 Y  | 0.0049004   |
|  |  | 2500 1 Y | 1600 1 Y | 0.0085619   |
|  |  | 900 1 V  | 900 1 C  | 0.99995     |
|  |  | 900 1 V  | 1600 1 C | 0.99876     |
|  |  | 900 1 V  | 2500 1 C | 0.8607      |
|  |  | 900 1 V  | 900 1 Y  | 0.0068563   |
|  |  | 900 1 V  | 1600 1 Y | 0.9486      |
|  |  | 900 1 V  | 2500 1 Y | 1           |
|  |  | 1600 1 V | 900 1 C  | 0.19283     |
|  |  | 1600 1 V | 1600 1 C | 0.8849      |
|  |  | 1600 1 V | 2500 1 C | 0.99992     |
|  |  | 1600 1 V | 900 1 Y  | 0.00357     |
|  |  | 1600 1 V | 1600 1 Y | 0.04185     |
|  |  | 1600 1 V | 2500 1 Y | 0.65512     |
|  |  | 1600 1 V | 900 1 V  | 0.44244     |
|  |  | 2500 1 V | 900 1 C  | 0.0397      |
|  |  | 2500 1 V | 1600 1 C | 0.33706     |
|  |  | 2500 1 V | 2500 1 C | 0.83972     |
|  |  | 2500 1 V | 900 1 Y  | 0.00126     |
|  |  | 2500 1 V | 1600 1 Y | 0.00875     |
|  |  | 2500 1 V | 2500 1 Y | 0.017896    |
|  |  | 2500 1 V | 900 1 V  | 0.0010167   |
|  |  | 2500 1 V | 1600 1 V | 0.0395489   |

**Table S5**

| 5            |        | Conditions     |             | p value     |
|--------------|--------|----------------|-------------|-------------|
| Western Blot | p-MKK7 | Line-patterned | Unpatterned | 0.002722371 |
|              | p-JNK  | Line-patterned | Unpatterned | 0.003487516 |
|              | JNK    | Line-patterned | Unpatterned | 0.009162065 |
| siMKK7       | c-Jun  | AR=1           | AR=4        | 0.211790246 |
|              | Sox-2  | AR=1           | AR=4        | 0.264193035 |
|              | p75NTR | AR=1           | AR=4        | 0.598926393 |
| siRac1       | c-Jun  | AR=1           | AR=4        | 0.497195542 |
|              | Sox-2  | AR=1           | AR=4        | 0.409719997 |
|              | p75NTR | AR=1           | AR=4        | 0.509865251 |