Supplemental Materials
Molecular Biology of the Cell
Lövenich et al.
Supplementary Information

**Supplementary Figure S5:** Estimation of confidence intervals. Angles for actin reorientation for all indicated conditions and cell types shown in Figure 3, 4, 5, 6, 7 and Supplementary Figure S1, S2 and S3 were statistically analyzed by using the method for bias-corrected and accelerated bootstrap intervals. Bootstrap sample size was set to 5000 based on the investigated high number of cells which is indicated by n for each sample. Estimated upper and lower 95% confidence intervals and cumulated frequencies are given for classes of 5° each from 5° to 90°.
Table S1. Estimation of the 95% confidence intervals (CI95) for cumulative histograms in Fig. 3

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.027      | 0.051          | 0.079      |
| 10    | 0.062      | 0.092          | 0.13       |
| 15    | 0.100      | 0.13           | 0.17       |
| 20    | 0.150      | 0.20           | 0.24       |
| 25    | 0.200      | 0.25           | 0.30       |
| 30    | 0.240      | 0.29           | 0.35       |
| 35    | 0.340      | 0.40           | 0.46       |
| 40    | 0.380      | 0.44           | 0.49       |
| 45    | 0.420      | 0.49           | 0.54       |
| 50    | 0.490      | 0.55           | 0.60       |
| 55    | 0.540      | 0.60           | 0.65       |
| 60    | 0.590      | 0.64           | 0.70       |
| 65    | 0.650      | 0.70           | 0.75       |
| 70    | 0.730      | 0.78           | 0.82       |
| 75    | 0.780      | 0.83           | 0.87       |
| 80    | 0.880      | 0.92           | 0.95       |
| 85    | 0.910      | 0.94           | 0.97       |
| 90    | 0.990      | 1.00           | 1.00       |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0.0039         | 0.016      |
| 10    | 0          | 0.0039         | 0.016      |
| 15    | 0          | 0.0039         | 0.016      |
| 20    | 0          | 0.0039         | 0.016      |
| 25    | 0          | 0.0039         | 0.024      |
| 30    | 0          | 0.0039         | 0.024      |
| 35    | 0.0039    | 0.016          | 0.035      |
| 40    | 0.0039    | 0.020          | 0.039      |
| 45    | 0.012     | 0.031          | 0.055      |
| 50    | 0.035     | 0.063          | 0.094      |
| 55    | 0.083     | 0.12           | 0.16       |
| 60    | 0.15      | 0.19           | 0.24       |
| 65    | 0.31      | 0.37           | 0.43       |
| 70    | 0.51      | 0.57           | 0.63       |
| 75    | 0.67      | 0.72           | 0.78       |
| 80    | 0.77      | 0.81           | 0.86       |
| 85    | 0.85      | 0.89           | 0.93       |
| 90    | 0.99      | 1.00           | 1.00       |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0.0039         | 0.016      |
| 10    | 0          | 0.0039         | 0.016      |
| 15    | 0          | 0.0039         | 0.016      |
| 20    | 0          | 0.0039         | 0.016      |
| 25    | 0          | 0.0039         | 0.024      |
| 30    | 0          | 0.0039         | 0.024      |
| 35    | 0.0039    | 0.016          | 0.035      |
| 40    | 0.0039    | 0.020          | 0.039      |
| 45    | 0.012     | 0.031          | 0.055      |
| 50    | 0.035     | 0.063          | 0.094      |
| 55    | 0.083     | 0.12           | 0.16       |
| 60    | 0.15      | 0.19           | 0.24       |
| 65    | 0.31      | 0.37           | 0.43       |
| 70    | 0.51      | 0.57           | 0.63       |
| 75    | 0.67      | 0.72           | 0.78       |
| 80    | 0.77      | 0.81           | 0.86       |
| 85    | 0.85      | 0.89           | 0.93       |
| 90    | 0.99      | 1.00           | 1.00       |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0              | 0          |
| 35    | 0          | 0              | 0          |
| 40    | 0          | 0              | 0          |
| 45    | 0          | 0              | 0          |
| 50    | 0.0039    | 0.015          | 0.015      |
| 55    | 0.012     | 0.031          | 0.035      |
| 60    | 0.050     | 0.081          | 0.12       |
| 65    | 0.16      | 0.21           | 0.26       |
| 70    | 0.31      | 0.36           | 0.42       |
| 75    | 0.53      | 0.59           | 0.65       |
| 80    | 0.70      | 0.76           | 0.80       |
| 85    | 0.84      | 0.89           | 0.92       |
| 90    | 0.99      | 1.00           | 1.00       |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0              | 0          |
| 35    | 0          | 0              | 0          |
| 40    | 0          | 0              | 0          |
| 45    | 0          | 0              | 0          |
| 50    | 0          | 0              | 0          |
| 55    | 0          | 0              | 0          |
| 60    | 0          | 0              | 0          |
| 65    | 0.0041    | 0.016          | 0.037      |
| 70    | 0.049     | 0.078          | 0.11       |
| 75    | 0.015     | 0.020          | 0.25       |
| 80    | 0.14      | 0.41           | 0.47       |
| 85    | 0.64      | 0.70           | 0.76       |
| 90    | 0.99      | 1.00           | 1.00       |

Supplementary information Lövenich et al. MBoC
### A7r5 6 h stretched untreated (n=203)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0              | 0          |
| 35    | 0          | 0              | 0          |
| 40    | 0          | 0              | 0          |
| 45    | 0          | 0              | 0          |
| 50    | 0          | 0              | 0          |
| 55    | 0          | 0              | 0          |
| 60    | 0          | 0              | 0          |
| 65    | 0          | 0              | 0          |
| 70    | 0,0049     | 0,025          | 0,049      |
| 75    | 0,11       | 0,15           | 0,21       |
| 80    | 0,29       | 0,35           | 0,42       |
| 85    | 0,56       | 0,62           | 0,88       |
| 90    | 1          | 1              | 1          |

### A7r5 30 min stretched +CQ (n=249)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0,0080         | 0,020      |
| 10    | 0,0040     | 0,016          | 0,036      |
| 15    | 0,0040     | 0,020          | 0,040      |
| 20    | 0,0080     | 0,024          | 0,048      |
| 25    | 0,020      | 0,036          | 0,064      |
| 30    | 0,036      | 0,060          | 0,10       |
| 35    | 0,040      | 0,068          | 0,10       |
| 40    | 0,060      | 0,092          | 0,13       |
| 45    | 0,084      | 0,12           | 0,17       |
| 50    | 0,16       | 0,21           | 0,27       |
| 55    | 0,28       | 0,34           | 0,40       |
| 60    | 0,38       | 0,44           | 0,50       |
| 65    | 0,52       | 0,58           | 0,64       |
| 70    | 0,65       | 0,70           | 0,76       |
| 75    | 0,73       | 0,78           | 0,84       |
| 80    | 0,80       | 0,85           | 0,89       |
| 85    | 0,88       | 0,92           | 0,95       |
| 90    | 1          | 1              | 1          |

### A7r5 1 h stretched +CQ (n=255)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0,012          | 0,027      |
| 35    | 0,0039     | 0,020          | 0,039      |
| 40    | 0,027      | 0,051          | 0,082      |
| 45    | 0,043      | 0,075          | 0,11       |
| 50    | 0,11       | 0,15           | 0,20       |
| 55    | 0,21       | 0,26           | 0,32       |
| 60    | 0,35       | 0,41           | 0,47       |
| 65    | 0,51       | 0,57           | 0,64       |
| 70    | 0,62       | 0,68           | 0,74       |
| 75    | 0,69       | 0,75           | 0,80       |
| 80    | 0,80       | 0,85           | 0,89       |
| 85    | 0,86       | 0,90           | 0,93       |
| 90    | 1          | 1              | 1          |

### A7r5 6 h stretched +CQ (n=199)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0,012          | 0,027      |
| 35    | 0,0039     | 0,020          | 0,039      |
| 40    | 0,027      | 0,051          | 0,082      |
| 45    | 0,043      | 0,075          | 0,11       |
| 50    | 0,11       | 0,15           | 0,20       |
| 55    | 0,21       | 0,26           | 0,32       |
| 60    | 0,35       | 0,41           | 0,47       |
| 65    | 0,51       | 0,57           | 0,64       |
| 70    | 0,62       | 0,68           | 0,74       |
| 75    | 0,69       | 0,75           | 0,80       |
| 80    | 0,80       | 0,85           | 0,89       |
| 85    | 0,86       | 0,90           | 0,93       |
| 90    | 1          | 1              | 1          |
Supplementary information

| A7r5 30 min stretched +MG132 (n=256) | A7r5 1 h stretched +MG132 (n=256) |
|-------------------------------|-------------------------------|
| Class | Lower CI95 | Cum. Frequency | Upper CI95 | Class | Lower CI95 | Cum. Frequency | Upper CI95 |
| 5     | 0           | 0               | 0         | 5     | 0           | 0               | 0         |
| 10    | 0           | 0               | 0         | 10    | 0           | 0               | 0         |
| 15    | 0           | 0,0039          | 0,016     | 15    | 0           | 0               | 0         |
| 20    | 0           | 0,0039          | 0,016     | 20    | 0           | 0               | 0         |
| 25    | 0,0039      | 0,016           | 0,031     | 25    | 0,0039      | 0,020           | 0,039     |
| 30    | 0,012       | 0,027           | 0,051     | 30    | 0,0039      | 0,020           | 0,039     |
| 35    | 0,020       | 0,039           | 0,066     | 35    | 0,020       | 0,039           | 0,066     |
| 40    | 0,039       | 0,066           | 0,10      | 40    | 0,039       | 0,066           | 0,10      |
| 45    | 0,070       | 0,11            | 0,14      | 45    | 0,070       | 0,11            | 0,14      |
| 50    | 0,12        | 0,16            | 0,21      | 50    | 0,12        | 0,16            | 0,21      |
| 55    | 0,23        | 0,28            | 0,34      | 55    | 0,23        | 0,28            | 0,34      |
| 60    | 0,45        | 0,51            | 0,57      | 60    | 0,45        | 0,51            | 0,57      |
| 65    | 0,60        | 0,66            | 0,72      | 65    | 0,60        | 0,66            | 0,72      |
| 70    | 0,70        | 0,75            | 0,80      | 70    | 0,70        | 0,75            | 0,80      |
| 75    | 0,78        | 0,82            | 0,87      | 75    | 0,78        | 0,82            | 0,87      |
| 80    | 0,85        | 0,89            | 0,92      | 80    | 0,85        | 0,89            | 0,92      |
| 85    | 1           | 1               | 1         | 85    | 1           | 1               | 1         |
| 90    | 1           | 1               | 1         | 90    | 1           | 1               | 1         |

| A7r5 6 h stretched +MG132 (n=219) | A7r5 30 min stretched +CQ +MG132 (n=257) |
|-------------------------------|-------------------------------|
| Class | Lower CI95 | Cum. Frequency | Upper CI95 | Class | Lower CI95 | Cum. Frequency | Upper CI95 |
| 5     | 0           | 0               | 0         | 5     | 0           | 0               | 0         |
| 10    | 0           | 0               | 0         | 10    | 0           | 0,0078          | 0,019     |
| 15    | 0           | 0               | 0         | 15    | 0,016      | 0,035           | 0,058     |
| 20    | 0           | 0               | 0         | 20    | 0,023      | 0,047           | 0,074     |
| 25    | 0           | 0               | 0         | 25    | 0,039      | 0,066           | 0,10      |
| 30    | 0           | 0               | 0         | 30    | 0,054      | 0,086           | 0,12      |
| 35    | 0           | 0               | 0         | 35    | 0,078      | 0,12            | 0,16      |
| 40    | 0           | 0               | 0         | 40    | 0,11       | 0,15            | 0,19      |
| 45    | 0           | 0               | 0         | 45    | 0,17       | 0,21            | 0,27      |
| 50    | 0           | 0               | 0         | 50    | 0,25       | 0,30            | 0,37      |
| 55    | 0           | 0               | 0         | 55    | 0,37       | 0,43            | 0,49      |
| 60    | 0           | 0               | 0         | 60    | 0,53       | 0,58            | 0,64      |
| 65    | 0           | 0,0046          | 0,018     | 65    | 0,53       | 0,58            | 0,64      |
| 70    | 0           | 0,014           | 0,032     | 70    | 0,66       | 0,72            | 0,77      |
| 75    | 0,078       | 0,12            | 0,16      | 75    | 0,76       | 0,81            | 0,85      |
| 80    | 0,28        | 0,34            | 0,41      | 80    | 0,83       | 0,87            | 0,91      |
| 85    | 0,53        | 0,60            | 0,66      | 85    | 0,86       | 0,90            | 0,94      |
| 90    | 1           | 1               | 1         | 90    | 0,90       | 0,93            | 0,96      |
### A7r5 1 h stretched +CQ +MG132 (n=252)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0.0079         | 0.020      |
| 35    | 0.012      | 0.012          | 0.028      |
| 40    | 0.028      | 0.052          | 0.083      |
| 45    | 0.052      | 0.083          | 0.12       |
| 50    | 0.11       | 0.15           | 0.19       |
| 55    | 0.19       | 0.25           | 0.30       |
| 60    | 0.35       | 0.41           | 0.47       |
| 65    | 0.52       | 0.58           | 0.64       |
| 70    | 0.64       | 0.70           | 0.75       |
| 75    | 0.73       | 0.78           | 0.83       |
| 80    | 0.82       | 0.87           | 0.91       |
| 85    | 0.87       | 0.91           | 0.94       |
| 90    | 1          | 1              | 1          |

### A7r5 6 h stretched +CQ +MG132 (n=187)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0.0079         | 0.020      |
| 35    | 0.012      | 0.012          | 0.028      |
| 40    | 0.028      | 0.052          | 0.083      |
| 45    | 0.052      | 0.083          | 0.12       |
| 50    | 0.11       | 0.15           | 0.19       |
| 55    | 0.19       | 0.25           | 0.30       |
| 60    | 0.35       | 0.41           | 0.47       |
| 65    | 0.52       | 0.58           | 0.64       |
| 70    | 0.64       | 0.70           | 0.75       |
| 75    | 0.73       | 0.78           | 0.83       |
| 80    | 0.82       | 0.87           | 0.91       |
| 85    | 0.87       | 0.91           | 0.94       |
| 90    | 1          | 1              | 1          |

The lower and upper 95% confidence intervals were analyzed using the method for bias-corrected and accelerated bootstrap intervals. The number of bootstrap intervals was set to 5000 for all histograms based on the indicated high number of cells (n). Cum. Frequency results from mean of upper and lower CI95.
### Table S2. Estimation of the 95% confidence intervals (CI95) for cumulative histograms in Fig. 4

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.036      | 0.060          | 0.086      |
| 10    | 0.065      | 0.10           | 0.13       |
| 15    | 0.10       | 0.14           | 0.17       |
| 20    | 0.13       | 0.17           | 0.21       |
| 25    | 0.17       | 0.21           | 0.26       |
| 30    | 0.21       | 0.26           | 0.30       |
| 35    | 0.25       | 0.30           | 0.35       |
| 40    | 0.29       | 0.34           | 0.39       |
| 45    | 0.34       | 0.39           | 0.44       |
| 50    | 0.39       | 0.44           | 0.49       |
| 55    | 0.42       | 0.47           | 0.52       |
| 60    | 0.48       | 0.54           | 0.59       |
| 65    | 0.53       | 0.59           | 0.64       |
| 70    | 0.60       | 0.65           | 0.70       |
| 75    | 0.68       | 0.72           | 0.77       |
| 80    | 0.74       | 0.78           | 0.82       |
| 85    | 0.84       | 0.87           | 0.91       |
| 90    | 1          | 1              | 1          |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0.011          | 0.025      |
| 10    | 0.014      | 0.032          | 0.054      |
| 15    | 0.021      | 0.046          | 0.071      |
| 20    | 0.032      | 0.061          | 0.089      |
| 25    | 0.043      | 0.071          | 0.10       |
| 30    | 0.057      | 0.089          | 0.12       |
| 35    | 0.075      | 0.11           | 0.15       |
| 40    | 0.10       | 0.14           | 0.18       |
| 45    | 0.13       | 0.18           | 0.22       |
| 50    | 0.18       | 0.23           | 0.28       |
| 55    | 0.22       | 0.27           | 0.32       |
| 60    | 0.28       | 0.33           | 0.39       |
| 65    | 0.44       | 0.50           | 0.55       |
| 70    | 0.57       | 0.63           | 0.68       |
| 75    | 0.66       | 0.71           | 0.77       |
| 80    | 0.75       | 0.80           | 0.85       |
| 85    | 0.85       | 0.89           | 0.92       |
| 90    | 1          | 1              | 1          |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.0051     | 0.015          | 0.028      |
| 10    | 0.0076     | 0.018          | 0.033      |
| 15    | 0.0076     | 0.020          | 0.033      |
| 20    | 0.013      | 0.028          | 0.043      |
| 25    | 0.023      | 0.040          | 0.061      |
| 30    | 0.030      | 0.051          | 0.073      |
| 35    | 0.038      | 0.061          | 0.086      |
| 40    | 0.043      | 0.066          | 0.091      |
| 45    | 0.063      | 0.086          | 0.12       |
| 50    | 0.091      | 0.12           | 0.15       |
| 55    | 0.11       | 0.14           | 0.18       |
| 60    | 0.13       | 0.16           | 0.20       |
| 65    | 0.18       | 0.21           | 0.26       |
| 70    | 0.27       | 0.32           | 0.36       |
| 75    | 0.37       | 0.42           | 0.47       |
| 80    | 0.53       | 0.58           | 0.63       |
| 85    | 0.70       | 0.75           | 0.79       |
| 90    | 1          | 1              | 1          |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0.0069         | 0.016      |
| 15    | 0.009      | 0.021          | 0.037      |
| 20    | 0.011      | 0.023          | 0.039      |
| 25    | 0.014      | 0.027          | 0.043      |
| 30    | 0.027      | 0.046          | 0.066      |
| 35    | 0.032      | 0.053          | 0.076      |
| 40    | 0.046      | 0.069          | 0.094      |
| 45    | 0.057      | 0.082          | 0.11       |
| 50    | 0.076      | 0.10           | 0.13       |
| 55    | 0.092      | 0.12           | 0.15       |
| 60    | 0.14       | 0.18           | 0.21       |
| 65    | 0.20       | 0.24           | 0.28       |
| 70    | 0.31       | 0.35           | 0.40       |
| 75    | 0.47       | 0.52           | 0.57       |
| 80    | 0.61       | 0.66           | 0.70       |
| 85    | 0.75       | 0.79           | 0.83       |
| 90    | 1          | 1              | 1          |
### MEF 10 min stretched +CQ (n=304)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0,0033     | 0,013          | 0,030      |
| 10    | 0,013      | 0,030          | 0,053      |
| 15    | 0,063      | 0,056          | 0,082      |
| 20    | 0,033      | 0,059          | 0,086      |
| 25    | 0,056      | 0,089          | 0,12       |
| 30    | 0,076      | 0,11           | 0,14       |
| 35    | 0,12       | 0,16           | 0,20       |
| 40    | 0,15       | 0,19           | 0,24       |
| 45    | 0,18       | 0,22           | 0,27       |
| 50    | 0,21       | 0,26           | 0,31       |
| 55    | 0,26       | 0,31           | 0,37       |
| 60    | 0,34       | 0,39           | 0,45       |
| 65    | 0,46       | 0,51           | 0,57       |
| 70    | 0,57       | 0,62           | 0,67       |
| 75    | 0,69       | 0,74           | 0,79       |
| 80    | 0,77       | 0,82           | 0,86       |
| 85    | 0,85       | 0,89           | 0,92       |
| 90    | 1          | 1              | 1          |

### MEF 30 min stretched +CQ (n=287)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0,0067         | 0,016      |
| 10    | 0,03       | 0,018          | 0,031      |
| 15    | 0,013      | 0,029          | 0,044      |
| 20    | 0,024      | 0,042          | 0,062      |
| 25    | 0,038      | 0,058          | 0,080      |
| 30    | 0,044      | 0,067          | 0,091      |
| 35    | 0,060      | 0,084          | 0,11       |
| 40    | 0,080      | 0,11           | 0,14       |
| 45    | 0,10       | 0,13           | 0,16       |
| 50    | 0,12       | 0,16           | 0,19       |
| 55    | 0,16       | 0,20           | 0,24       |
| 60    | 0,21       | 0,25           | 0,29       |
| 65    | 0,31       | 0,36           | 0,40       |
| 70    | 0,43       | 0,47           | 0,52       |
| 75    | 0,57       | 0,62           | 0,67       |
| 80    | 0,69       | 0,73           | 0,77       |
| 85    | 0,82       | 0,86           | 0,89       |
| 90    | 1          | 1              | 1          |

### MEF 1 h stretched +CQ (n=270)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0,0044         | 0,011      |
| 10    | 0          | 0,0066         | 0,015      |
| 15    | 0,0022     | 0,011          | 0,022      |
| 20    | 0,013      | 0,026          | 0,042      |
| 25    | 0,026      | 0,042          | 0,064      |
| 30    | 0,037      | 0,057          | 0,081      |
| 35    | 0,048      | 0,070          | 0,095      |
| 40    | 0,059      | 0,081          | 0,11       |
| 45    | 0,075      | 0,10           | 0,13       |
| 50    | 0,090      | 0,12           | 0,15       |
| 55    | 0,13       | 0,16           | 0,19       |
| 60    | 0,16       | 0,19           | 0,23       |
| 65    | 0,22       | 0,26           | 0,30       |
| 70    | 0,29       | 0,34           | 0,38       |
| 75    | 0,42       | 0,46           | 0,51       |
| 80    | 0,56       | 0,60           | 0,65       |
| 85    | 0,73       | 0,77           | 0,80       |
| 90    | 1          | 1              | 1          |

### MEF 10 min stretched +MG132 (n=451)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0,0070     | 0,021          | 0,042      |
| 10    | 0,010      | 0,024          | 0,045      |
| 15    | 0,017      | 0,035          | 0,059      |
| 20    | 0,028      | 0,049          | 0,077      |
| 25    | 0,038      | 0,066          | 0,10       |
| 30    | 0,049      | 0,080          | 0,11       |
| 35    | 0,080      | 0,11           | 0,16       |
| 40    | 0,11       | 0,15           | 0,20       |
| 45    | 0,15       | 0,19           | 0,24       |
| 50    | 0,21       | 0,26           | 0,31       |
| 55    | 0,28       | 0,34           | 0,39       |
| 60    | 0,34       | 0,40           | 0,46       |
| 65    | 0,46       | 0,52           | 0,57       |
| 70    | 0,55       | 0,61           | 0,67       |
| 75    | 0,65       | 0,70           | 0,75       |
| 80    | 0,74       | 0,79           | 0,83       |
| 85    | 0,83       | 0,87           | 0,91       |
| 90    | 1          | 1              | 1          |
### MEF 30 min stretched +MG132 (n=428)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0,0023     | 0,012          | 0,023      |
| 10    | 0,016      | 0,033          | 0,051      |
| 15    | 0,028      | 0,047          | 0,070      |
| 20    | 0,037      | 0,056          | 0,082      |
| 25    | 0,049      | 0,072          | 0,10       |
| 30    | 0,054      | 0,079          | 0,11       |
| 35    | 0,068      | 0,10           | 0,13       |
| 40    | 0,086      | 0,12           | 0,15       |
| 45    | 0,12       | 0,15           | 0,19       |
| 50    | 0,14       | 0,18           | 0,21       |
| 55    | 0,19       | 0,23           | 0,27       |
| 60    | 0,23       | 0,27           | 0,31       |
| 65    | 0,32       | 0,37           | 0,42       |
| 70    | 0,43       | 0,48           | 0,53       |
| 75    | 0,58       | 0,62           | 0,67       |
| 80    | 0,70       | 0,75           | 0,78       |
| 85    | 0,84       | 0,87           | 0,90       |
| 90    | 1          | 1              | 1          |

### MEF 1 h stretched +MG132 (n=438)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0,0047     | 0,014          | 0,028      |
| 10    | 0,0093     | 0,023          | 0,040      |
| 15    | 0,016      | 0,030          | 0,049      |
| 20    | 0,019      | 0,035          | 0,054      |
| 25    | 0,033      | 0,054          | 0,075      |
| 30    | 0,044      | 0,068          | 0,093      |
| 35    | 0,068      | 0,093          | 0,12       |
| 40    | 0,075      | 0,10           | 0,13       |
| 45    | 0,093      | 0,12           | 0,15       |
| 50    | 0,11       | 0,14           | 0,18       |
| 55    | 0,14       | 0,17           | 0,21       |
| 60    | 0,17       | 0,21           | 0,25       |
| 65    | 0,23       | 0,27           | 0,31       |
| 70    | 0,30       | 0,34           | 0,39       |
| 75    | 0,39       | 0,44           | 0,48       |
| 80    | 0,52       | 0,57           | 0,62       |
| 85    | 0,70       | 0,75           | 0,79       |
| 90    | 1          | 1              | 1          |

### MEF 10 min stretched +CQ +MG132 (n=455)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0,0074     | 0,022          | 0,044      |
| 10    | 0,015      | 0,033          | 0,056      |
| 15    | 0,026      | 0,048          | 0,078      |
| 20    | 0,041      | 0,067          | 0,10       |
| 25    | 0,063      | 0,10           | 0,13       |
| 30    | 0,089      | 0,13           | 0,17       |
| 35    | 0,11       | 0,16           | 0,20       |
| 40    | 0,14       | 0,18           | 0,23       |
| 45    | 0,17       | 0,21           | 0,27       |
| 50    | 0,20       | 0,24           | 0,30       |
| 55    | 0,26       | 0,31           | 0,37       |
| 60    | 0,36       | 0,41           | 0,47       |
| 65    | 0,48       | 0,54           | 0,59       |
| 70    | 0,54       | 0,60           | 0,66       |
| 75    | 0,64       | 0,70           | 0,75       |
| 80    | 0,76       | 0,80           | 0,85       |
| 85    | 0,86       | 0,90           | 0,93       |
| 90    | 1          | 1              | 1          |

### MEF 30 min stretched +CQ +MG132 (n=428)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0,0046     | 0,014          | 0,025      |
| 10    | 0,014      | 0,027          | 0,043      |
| 15    | 0,018      | 0,034          | 0,053      |
| 20    | 0,023      | 0,039          | 0,059      |
| 25    | 0,041      | 0,062          | 0,087      |
| 30    | 0,057      | 0,080          | 0,11       |
| 35    | 0,071      | 0,10           | 0,13       |
| 40    | 0,087      | 0,11           | 0,14       |
| 45    | 0,10       | 0,13           | 0,16       |
| 50    | 0,14       | 0,17           | 0,21       |
| 55    | 0,17       | 0,21           | 0,25       |
| 60    | 0,22       | 0,26           | 0,30       |
| 65    | 0,29       | 0,33           | 0,38       |
| 70    | 0,39       | 0,44           | 0,49       |
| 75    | 0,52       | 0,56           | 0,61       |
| 80    | 0,66       | 0,70           | 0,74       |
| 85    | 0,82       | 0,85           | 0,88       |
| 90    | 1          | 1              | 1          |
| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.0023     | 0.0093         | 0.021      |
| 10    | 0.0023     | 0.012          | 0.023      |
| 15    | 0.0093     | 0.021          | 0.037      |
| 20    | 0.016      | 0.030          | 0.049      |
| 25    | 0.023      | 0.040          | 0.061      |
| 30    | 0.028      | 0.047          | 0.070      |
| 35    | 0.044      | 0.065          | 0.091      |
| 40    | 0.063      | 0.086          | 0.12       |
| 45    | 0.084      | 0.11           | 0.14       |
| 50    | 0.093      | 0.12           | 0.15       |
| 55    | 0.12       | 0.15           | 0.19       |
| 60    | 0.15       | 0.19           | 0.22       |
| 65    | 0.21       | 0.24           | 0.29       |
| 70    | 0.31       | 0.36           | 0.40       |
| 75    | 0.44       | 0.48           | 0.53       |
| 80    | 0.58       | 0.63           | 0.67       |
| 85    | 0.76       | 0.80           | 0.84       |
| 90    | 1          | 1              | 1          |

The lower and upper 95% confidence intervals were analyzed using the method for bias-corrected and accelerated bootstrap intervals. The number of bootstrap intervals was set to 5000 for all histograms based on the indicated high number of cells (n). Cum. Frequency results from mean of upper and lower CI95.
Table S3. Estimation of the 95% confidence intervals (CI95) for cumulative histograms in Fig. 5

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.021      | 0.056          | 0.10       |
| 10    | 0.069      | 0.12           | 0.17       |
| 15    | 0.11       | 0.17           | 0.23       |
| 20    | 0.16       | 0.22           | 0.29       |
| 25    | 0.17       | 0.24           | 0.31       |
| 30    | 0.22       | 0.29           | 0.37       |
| 35    | 0.31       | 0.39           | 0.47       |
| 40    | 0.38       | 0.46           | 0.55       |
| 45    | 0.42       | 0.50           | 0.58       |
| 50    | 0.44       | 0.53           | 0.61       |
| 55    | 0.51       | 0.59           | 0.67       |
| 60    | 0.61       | 0.69           | 0.76       |
| 65    | 0.68       | 0.76           | 0.82       |
| 70    | 0.72       | 0.80           | 0.85       |
| 75    | 0.79       | 0.85           | 0.90       |
| 80    | 0.84       | 0.90           | 0.94       |
| 85    | 0.91       | 0.95           | 0.98       |
| 90    | 1          | 1              | 1          |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.005      | 0.025          | 0.050      |
| 10    | 0.040      | 0.070          | 0.11       |
| 15    | 0.070      | 0.11           | 0.16       |
| 20    | 0.10       | 0.15           | 0.20       |
| 25    | 0.14       | 0.20           | 0.25       |
| 30    | 0.16       | 0.22           | 0.28       |
| 35    | 0.18       | 0.25           | 0.31       |
| 40    | 0.21       | 0.28           | 0.33       |
| 45    | 0.26       | 0.33           | 0.39       |
| 50    | 0.32       | 0.39           | 0.45       |
| 55    | 0.35       | 0.43           | 0.49       |
| 60    | 0.40       | 0.48           | 0.54       |
| 65    | 0.47       | 0.55           | 0.61       |
| 70    | 0.59       | 0.66           | 0.72       |
| 75    | 0.72       | 0.78           | 0.83       |
| 80    | 0.82       | 0.87           | 0.91       |
| 85    | 0.88       | 0.93           | 0.96       |
| 90    | 1          | 1              | 1          |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.015      | 0.039          | 0.069      |
| 10    | 0.079      | 0.12           | 0.16       |
| 15    | 0.10       | 0.15           | 0.20       |
| 20    | 0.14       | 0.19           | 0.25       |
| 25    | 0.19       | 0.25           | 0.31       |
| 30    | 0.24       | 0.31           | 0.37       |
| 35    | 0.31       | 0.37           | 0.44       |
| 40    | 0.37       | 0.44           | 0.51       |
| 45    | 0.42       | 0.49           | 0.57       |
| 50    | 0.52       | 0.59           | 0.66       |
| 55    | 0.59       | 0.65           | 0.71       |
| 60    | 0.67       | 0.73           | 0.79       |
| 65    | 0.75       | 0.80           | 0.86       |
| 70    | 0.79       | 0.84           | 0.89       |
| 75    | 0.85       | 0.90           | 0.94       |
| 80    | 0.89       | 0.93           | 0.96       |
| 85    | 0.93       | 0.96           | 0.98       |
| 90    | 1          | 1              | 1          |
## MEF 30 min stretched untreated (n=198)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.005      | 0.020          | 0.045      |
| 10    | 0.020      | 0.045          | 0.076      |
| 15    | 0.020      | 0.045          | 0.076      |
| 20    | 0.045      | 0.081          | 0.12       |
| 25    | 0.051      | 0.091          | 0.13       |
| 30    | 0.076      | 0.12           | 0.17       |
| 35    | 0.10       | 0.15           | 0.20       |
| 40    | 0.11       | 0.15           | 0.20       |
| 45    | 0.13       | 0.18           | 0.23       |
| 50    | 0.15       | 0.20           | 0.26       |
| 55    | 0.21       | 0.26           | 0.32       |
| 60    | 0.31       | 0.37           | 0.44       |
| 65    | 0.39       | 0.46           | 0.53       |
| 70    | 0.52       | 0.59           | 0.66       |
| 75    | 0.65       | 0.72           | 0.77       |
| 80    | 0.73       | 0.79           | 0.85       |
| 85    | 0.82       | 0.87           | 0.91       |
| 90    | 1          | 1              | 1          |

## MEF 30 min stretched +LPA (n=197)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0.010          | 0.025      |
| 25    | 0          | 0.015          | 0.036      |
| 30    | 0.0051     | 0.020          | 0.046      |
| 35    | 0.010      | 0.030          | 0.056      |
| 40    | 0.030      | 0.056          | 0.091      |
| 45    | 0.046      | 0.081          | 0.12       |
| 50    | 0.071      | 0.12           | 0.16       |
| 55    | 0.10       | 0.15           | 0.20       |
| 60    | 0.16       | 0.21           | 0.27       |
| 65    | 0.24       | 0.31           | 0.37       |
| 70    | 0.36       | 0.43           | 0.50       |
| 75    | 0.54       | 0.61           | 0.68       |
| 80    | 0.72       | 0.78           | 0.83       |
| 85    | 0.80       | 0.85           | 0.90       |
| 90    | 1          | 1              | 1          |

## MEF 1 h stretched untreated (n=206)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.0049     | 0.024          | 0.049      |
| 10    | 0.015      | 0.034          | 0.063      |
| 15    | 0.015      | 0.039          | 0.068      |
| 20    | 0.039      | 0.068          | 0.11       |
| 25    | 0.053      | 0.087          | 0.13       |
| 30    | 0.078      | 0.12           | 0.17       |
| 35    | 0.10       | 0.15           | 0.20       |
| 40    | 0.16       | 0.21           | 0.27       |
| 45    | 0.17       | 0.22           | 0.28       |
| 50    | 0.20       | 0.26           | 0.32       |
| 55    | 0.26       | 0.32           | 0.38       |
| 60    | 0.32       | 0.38           | 0.45       |
| 65    | 0.42       | 0.49           | 0.56       |
| 70    | 0.54       | 0.61           | 0.67       |
| 75    | 0.66       | 0.72           | 0.78       |
| 80    | 0.76       | 0.82           | 0.87       |
| 85    | 0.88       | 0.92           | 0.96       |
| 90    | 1          | 1              | 1          |

## MEF 1 h stretched +LPA (n=212)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0.0047         | 0.019      |
| 10    | 0          | 0.0047         | 0.019      |
| 15    | 0          | 0.0094         | 0.024      |
| 20    | 0          | 0.0094         | 0.024      |
| 25    | 0.0047     | 0.019          | 0.042      |
| 30    | 0.0047     | 0.024          | 0.047      |
| 35    | 0.0047     | 0.028          | 0.052      |
| 40    | 0.019      | 0.042          | 0.071      |
| 45    | 0.024      | 0.052          | 0.080      |
| 50    | 0.038      | 0.071          | 0.11       |
| 55    | 0.047      | 0.085          | 0.12       |
| 60    | 0.094      | 0.14           | 0.18       |
| 65    | 0.12       | 0.17           | 0.23       |
| 70    | 0.23       | 0.29           | 0.35       |
| 75    | 0.35       | 0.42           | 0.49       |
| 80    | 0.50       | 0.57           | 0.63       |
| 85    | 0.70       | 0.76           | 0.82       |
| 90    | 1          | 1              | 1          |
The lower and upper 95% confidence intervals were analyzed using the method for bias-corrected and accelerated bootstrap intervals. The number of bootstrap intervals was set to 5000 for all histograms based on the indicated high number of cells (n). Cum. Frequency results from mean of upper and lower CI95.
Table S4. Estimation of the 95% confidence intervals (CI95) for cumulative histograms in Fig. 6

| Class | Lower CI95 | Cum. Frequency | Upper CI95 | Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|-------|------------|----------------|------------|
|       | MEF unstretched +LPA (n=303) |               | MEF unstretched +LPA +CQ (n=292) |
| 5     | 0.056      | 0.086          | 0.12       | 5     | 0.021      | 0.041          | 0.065      |
| 10    | 0.13       | 0.17           | 0.21       | 10    | 0.055      | 0.082          | 0.12       |
| 15    | 0.19       | 0.23           | 0.28       | 15    | 0.10       | 0.14           | 0.18       |
| 20    | 0.24       | 0.28           | 0.34       | 20    | 0.17       | 0.22           | 0.27       |
| 25    | 0.29       | 0.35           | 0.40       | 25    | 0.23       | 0.28           | 0.33       |
| 30    | 0.35       | 0.41           | 0.46       | 30    | 0.28       | 0.34           | 0.39       |
| 35    | 0.41       | 0.47           | 0.52       | 35    | 0.35       | 0.40           | 0.46       |
| 40    | 0.48       | 0.53           | 0.59       | 40    | 0.40       | 0.46           | 0.51       |
| 45    | 0.50       | 0.56           | 0.61       | 45    | 0.47       | 0.53           | 0.59       |
| 50    | 0.55       | 0.61           | 0.66       | 50    | 0.54       | 0.60           | 0.66       |
| 55    | 0.61       | 0.66           | 0.71       | 55    | 0.62       | 0.67           | 0.73       |
| 60    | 0.64       | 0.70           | 0.75       | 60    | 0.66       | 0.72           | 0.77       |
| 65    | 0.74       | 0.78           | 0.83       | 65    | 0.71       | 0.76           | 0.80       |
| 70    | 0.80       | 0.84           | 0.88       | 70    | 0.76       | 0.80           | 0.85       |
| 75    | 0.86       | 0.89           | 0.93       | 75    | 0.79       | 0.84           | 0.88       |
| 80    | 0.90       | 0.93           | 0.96       | 80    | 0.87       | 0.90           | 0.94       |
| 85    | 0.96       | 0.98           | 1          | 85    | 0.91       | 0.94           | 0.97       |
| 90    | 1          | 1              | 1          | 90    | 1          | 1              | 1          |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 | Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|-------|------------|----------------|------------|
|       | MEF 10 min stretched +LPA (n=301) |               | MEF 10 min stretched +LPA +CQ (n=315) |
| 5     | 0.013      | 0.030          | 0.050      | 5     | 0.035      | 0.060          | 0.089      |
| 10    | 0.023      | 0.047          | 0.073      | 10    | 0.054      | 0.083          | 0.11       |
| 15    | 0.030      | 0.056          | 0.083      | 15    | 0.076      | 0.11           | 0.15       |
| 20    | 0.050      | 0.076          | 0.11       | 20    | 0.089      | 0.12           | 0.17       |
| 25    | 0.073      | 0.10           | 0.14       | 25    | 0.12       | 0.16           | 0.20       |
| 30    | 0.10       | 0.13           | 0.17       | 30    | 0.17       | 0.22           | 0.26       |
| 35    | 0.12       | 0.16           | 0.21       | 35    | 0.21       | 0.25           | 0.30       |
| 40    | 0.15       | 0.20           | 0.24       | 40    | 0.24       | 0.29           | 0.34       |
| 45    | 0.21       | 0.26           | 0.31       | 45    | 0.32       | 0.37           | 0.43       |
| 50    | 0.26       | 0.31           | 0.37       | 50    | 0.38       | 0.44           | 0.49       |
| 55    | 0.34       | 0.39           | 0.45       | 55    | 0.43       | 0.49           | 0.54       |
| 60    | 0.41       | 0.47           | 0.52       | 60    | 0.50       | 0.55           | 0.60       |
| 65    | 0.49       | 0.54           | 0.59       | 65    | 0.60       | 0.65           | 0.70       |
| 70    | 0.59       | 0.65           | 0.70       | 70    | 0.70       | 0.75           | 0.79       |
| 75    | 0.72       | 0.77           | 0.82       | 75    | 0.78       | 0.82           | 0.86       |
| 80    | 0.81       | 0.85           | 0.89       | 80    | 0.84       | 0.88           | 0.91       |
| 85    | 0.89       | 0.92           | 0.95       | 85    | 0.92       | 0.95           | 0.97       |
| 90    | 1          | 1              | 1          | 90    | 1          | 1              | 1          |
### MEF 30 min stretched +LPA (n=286)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0.010          | 0.024      |
| 15    | 0.0070     | 0.021          | 0.038      |
| 20    | 0.010      | 0.024          | 0.045      |
| 25    | 0.021      | 0.042          | 0.066      |
| 30    | 0.035      | 0.059          | 0.087      |
| 35    | 0.038      | 0.066          | 0.094      |
| 40    | 0.045      | 0.073          | 0.10       |
| 45    | 0.056      | 0.087          | 0.12       |
| 50    | 0.066      | 0.10           | 0.14       |
| 55    | 0.11       | 0.15           | 0.19       |
| 60    | 0.17       | 0.22           | 0.27       |
| 65    | 0.24       | 0.29           | 0.34       |
| 70    | 0.37       | 0.43           | 0.49       |
| 75    | 0.50       | 0.55           | 0.61       |
| 80    | 0.65       | 0.70           | 0.76       |
| 85    | 0.80       | 0.84           | 0.88       |
| 90    | 1          | 1              | 1          |

### MEF 30 min stretched +LPA +CQ (n=313)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.013      | 0.029          | 0.051      |
| 10    | 0.042      | 0.067          | 0.10       |
| 15    | 0.077      | 0.11           | 0.14       |
| 20    | 0.089      | 0.12           | 0.16       |
| 25    | 0.12       | 0.16           | 0.20       |
| 30    | 0.14       | 0.19           | 0.23       |
| 35    | 0.15       | 0.19           | 0.24       |
| 40    | 0.17       | 0.21           | 0.26       |
| 45    | 0.19       | 0.24           | 0.29       |
| 50    | 0.25       | 0.30           | 0.35       |
| 55    | 0.29       | 0.34           | 0.39       |
| 60    | 0.36       | 0.41           | 0.47       |
| 65    | 0.44       | 0.50           | 0.56       |
| 70    | 0.58       | 0.64           | 0.69       |
| 75    | 0.68       | 0.73           | 0.78       |
| 80    | 0.77       | 0.82           | 0.86       |
| 85    | 0.86       | 0.90           | 0.93       |
| 90    | 1          | 1              | 1          |

### MEF 1 h stretched +LPA (n=290)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.0034     | 0.014          | 0.028      |
| 10    | 0.017      | 0.034          | 0.059      |
| 15    | 0.017      | 0.034          | 0.059      |
| 20    | 0.021      | 0.041          | 0.066      |
| 25    | 0.028      | 0.052          | 0.079      |
| 30    | 0.041      | 0.066          | 0.10       |
| 35    | 0.055      | 0.086          | 0.12       |
| 40    | 0.069      | 0.10           | 0.13       |
| 45    | 0.079      | 0.11           | 0.15       |
| 50    | 0.10       | 0.14           | 0.18       |
| 55    | 0.14       | 0.19           | 0.23       |
| 60    | 0.20       | 0.24           | 0.30       |
| 65    | 0.36       | 0.41           | 0.47       |
| 70    | 0.49       | 0.54           | 0.60       |
| 75    | 0.62       | 0.68           | 0.73       |
| 80    | 0.80       | 0.85           | 0.89       |
| 85    | 1          | 1              | 1          |
| 90    | 1          | 1              | 1          |

### MEF 1 h stretched +LPA +CQ (n=321)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.006      | 0.019          | 0.037      |
| 10    | 0.025      | 0.044          | 0.069      |
| 15    | 0.053      | 0.081          | 0.11       |
| 20    | 0.081      | 0.12           | 0.15       |
| 25    | 0.11       | 0.15           | 0.19       |
| 30    | 0.14       | 0.18           | 0.22       |
| 35    | 0.16       | 0.20           | 0.24       |
| 40    | 0.18       | 0.23           | 0.27       |
| 45    | 0.21       | 0.26           | 0.30       |
| 50    | 0.24       | 0.29           | 0.34       |
| 55    | 0.30       | 0.35           | 0.40       |
| 60    | 0.36       | 0.41           | 0.47       |
| 65    | 0.42       | 0.47           | 0.52       |
| 70    | 0.51       | 0.57           | 0.62       |
| 75    | 0.62       | 0.68           | 0.73       |
| 80    | 0.74       | 0.79           | 0.83       |
| 85    | 0.85       | 0.89           | 0.92       |
| 90    | 1          | 1              | 1          |
The lower and upper 95% confidence intervals were analyzed using the method for bias-corrected and accelerated bootstrap intervals. The number of bootstrap intervals was set to 5000 for all histograms based on the indicated high number of cells (n). Cum. Frequency results from mean of upper and lower CI95.
Table S5. Estimation of the 95% confidence intervals (CI95) for cumulative histograms in Fig. 7

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.037      | 0.064          | 0.091      |
| 10    | 0.074      | 0.10           | 0.14       |
| 15    | 0.11       | 0.15           | 0.19       |
| 20    | 0.15       | 0.19           | 0.24       |
| 25    | 0.17       | 0.21           | 0.27       |
| 30    | 0.21       | 0.26           | 0.32       |
| 35    | 0.26       | 0.30           | 0.36       |
| 40    | 0.30       | 0.35           | 0.41       |
| 45    | 0.36       | 0.41           | 0.47       |
| 50    | 0.42       | 0.47           | 0.53       |
| 55    | 0.48       | 0.53           | 0.59       |
| 60    | 0.56       | 0.61           | 0.67       |
| 65    | 0.62       | 0.67           | 0.73       |
| 70    | 0.68       | 0.73           | 0.78       |
| 75    | 0.77       | 0.81           | 0.86       |
| 80    | 0.86       | 0.89           | 0.93       |
| 85    | 0.93       | 0.95           | 0.98       |
| 90    | 1          | 1              | 1          |

A7r5 BAG3-WT unstretched (n=298)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.045      | 0.079          | 0.12       |
| 10    | 0.09       | 0.13           | 0.17       |
| 15    | 0.14       | 0.18           | 0.23       |
| 20    | 0.18       | 0.23           | 0.29       |
| 25    | 0.23       | 0.28           | 0.34       |
| 30    | 0.26       | 0.31           | 0.38       |
| 35    | 0.33       | 0.39           | 0.45       |
| 40    | 0.34       | 0.40           | 0.47       |
| 45    | 0.42       | 0.48           | 0.55       |
| 50    | 0.49       | 0.55           | 0.61       |
| 55    | 0.56       | 0.62           | 0.68       |
| 60    | 0.63       | 0.69           | 0.74       |
| 65    | 0.67       | 0.73           | 0.79       |
| 70    | 0.74       | 0.79           | 0.84       |
| 75    | 0.79       | 0.83           | 0.88       |
| 80    | 0.86       | 0.90           | 0.93       |
| 85    | 0.93       | 0.95           | 0.98       |
| 90    | 1          | 1              | 1          |

A7r5 BAG3-WA WA unstretched (n=242)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.037      | 0.064          | 0.091      |
| 10    | 0.074      | 0.10           | 0.14       |
| 15    | 0.11       | 0.15           | 0.19       |
| 20    | 0.15       | 0.19           | 0.24       |
| 25    | 0.17       | 0.21           | 0.27       |
| 30    | 0.21       | 0.26           | 0.32       |
| 35    | 0.26       | 0.30           | 0.36       |
| 40    | 0.30       | 0.35           | 0.41       |
| 45    | 0.36       | 0.41           | 0.47       |
| 50    | 0.42       | 0.47           | 0.53       |
| 55    | 0.48       | 0.53           | 0.59       |
| 60    | 0.56       | 0.61           | 0.67       |
| 65    | 0.62       | 0.67           | 0.73       |
| 70    | 0.68       | 0.73           | 0.78       |
| 75    | 0.77       | 0.81           | 0.86       |
| 80    | 0.86       | 0.89           | 0.93       |
| 85    | 0.93       | 0.95           | 0.98       |
| 90    | 1          | 1              | 1          |

A7r5 BAG3-WT 30 min stretched (n=206)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0.010          | 0.029      |
| 10    | 0          | 0.015          | 0.034      |
| 15    | 0.0049     | 0.024          | 0.044      |
| 20    | 0.0049     | 0.024          | 0.044      |
| 25    | 0.015      | 0.034          | 0.063      |
| 30    | 0.034      | 0.058          | 0.10       |
| 35    | 0.034      | 0.063          | 0.10       |
| 40    | 0.073      | 0.11           | 0.16       |
| 45    | 0.11       | 0.16           | 0.21       |
| 50    | 0.18       | 0.24           | 0.30       |
| 55    | 0.30       | 0.36           | 0.43       |
| 60    | 0.46       | 0.53           | 0.60       |
| 65    | 0.54       | 0.61           | 0.67       |
| 70    | 0.63       | 0.69           | 0.75       |
| 75    | 0.75       | 0.80           | 0.85       |
| 80    | 0.82       | 0.87           | 0.92       |
| 85    | 0.91       | 0.95           | 0.98       |
| 90    | 1          | 1              | 1          |

A7r5 BAG3-WA WA 30 min stretched (n=192)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.0052     | 0.026          | 0.052      |
| 10    | 0.021      | 0.047          | 0.078      |
| 15    | 0.042      | 0.078          | 0.12       |
| 20    | 0.078      | 0.11           | 0.17       |
| 25    | 0.13       | 0.17           | 0.23       |
| 30    | 0.19       | 0.24           | 0.31       |
| 35    | 0.24       | 0.30           | 0.38       |
| 40    | 0.28       | 0.34           | 0.41       |
| 45    | 0.33       | 0.40           | 0.47       |
| 50    | 0.42       | 0.49           | 0.56       |
| 55    | 0.54       | 0.61           | 0.68       |
| 60    | 0.65       | 0.71           | 0.77       |
| 65    | 0.71       | 0.78           | 0.83       |
| 70    | 0.79       | 0.84           | 0.89       |
| 75    | 0.82       | 0.87           | 0.91       |
| 80    | 0.86       | 0.91           | 0.94       |
| 85    | 0.91       | 0.94           | 0.97       |
| 90    | 1          | 1              | 1          |
### Supplementary information

 Lövenich et al. MBoC

#### A7r5 BAG3-WT 1 h stretched (n=183)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0,0055         | 0,022      |
| 20    | 0          | 0,0055         | 0,022      |
| 25    | 0          | 0,0055         | 0,022      |
| 30    | 0          | 0,0055         | 0,022      |
| 35    | 0          | 0,0055         | 0,022      |
| 40    | 0          | 0,011          | 0,027      |
| 45    | 0          | 0,016          | 0,038      |
| 50    | 0,033      | 0,066          | 0,10       |
| 55    | 0,11       | 0,16           | 0,22       |
| 60    | 0,19       | 0,25           | 0,31       |
| 65    | 0,38       | 0,46           | 0,53       |
| 70    | 0,54       | 0,61           | 0,68       |
| 75    | 0,66       | 0,73           | 0,79       |
| 80    | 0,75       | 0,81           | 0,87       |
| 85    | 0,84       | 0,89           | 0,93       |
| 90    | 1          | 1              | 1          |

#### A7r5 BAG3-WAWA 1 h stretched (n=195)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0,0051         | 0,021      |
| 10    | 0          | 0,010          | 0,031      |
| 15    | 0          | 0,015          | 0,041      |
| 20    | 0          | 0,026          | 0,051      |
| 25    | 0          | 0,036          | 0,067      |
| 30    | 0          | 0,046          | 0,082      |
| 35    | 0          | 0,10           | 0,14       |
| 40    | 0          | 0,17           | 0,22       |
| 45    | 0          | 0,34           | 0,41       |
| 50    | 0          | 0,52           | 0,66       |
| 55    | 0          | 0,69           | 0,75       |
| 60    | 0          | 0,76           | 0,82       |
| 65    | 0          | 0,84           | 0,93       |
| 70    | 0          | 0,89           | 0,96       |
| 75    | 0          | 0,92           | 0,98       |
| 80    | 0          | 1              | 1          |

#### A7r5 BAG3-WT 4 h stretched (n=275)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0              | 0          |
| 35    | 0          | 0              | 0          |
| 40    | 0          | 0              | 0          |
| 45    | 0          | 0              | 0          |
| 50    | 0          | 0,0036         | 0,015      |
| 55    | 0,0073     | 0,022          | 0,040      |
| 60    | 0,015      | 0,033          | 0,055      |
| 65    | 0,076      | 0,11           | 0,15       |
| 70    | 0,21       | 0,27           | 0,32       |
| 75    | 0,40       | 0,46           | 0,52       |
| 80    | 0,58       | 0,64           | 0,69       |
| 85    | 0,76       | 0,81           | 0,85       |
| 90    | 1          | 1              | 1          |

#### A7r5 BAG3-WAWA 4 h stretched (n=223)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0,013          | 0,031      |
| 20    | 0          | 0,013          | 0,031      |
| 25    | 0          | 0,013          | 0,031      |
| 30    | 0          | 0,013          | 0,031      |
| 35    | 0          | 0,013          | 0,031      |
| 40    | 0          | 0,0090         | 0,027      |
| 45    | 0          | 0,013          | 0,031      |
| 50    | 0          | 0,022          | 0,049      |
| 55    | 0          | 0,040          | 0,072      |
| 60    | 0          | 0,090          | 0,13       |
| 65    | 0          | 0,17           | 0,23       |
| 70    | 0          | 0,29           | 0,41       |
| 75    | 0          | 0,47           | 0,61       |
| 80    | 0          | 0,62           | 0,74       |
| 85    | 0          | 0,78           | 0,87       |
| 90    | 0          | 1              | 1          |
The lower and upper 95% confidence intervals were analyzed using the method for bias-corrected and accelerated bootstrap intervals. The number of bootstrap intervals was set to 5000 for all histograms based on the indicated high number of cells (n). Cum. Frequency results from mean of upper and lower CI95.
Table S6. Estimation of the 95% confidence intervals (CI95) for cumulative histograms in Fig. S1

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.0063     | 0.031          | 0.063      |
| 10    | 0.025      | 0.056          | 0.10       |
| 15    | 0.063      | 0.11           | 0.16       |
| 20    | 0.088      | 0.14           | 0.19       |
| 25    | 0.16       | 0.21           | 0.28       |
| 30    | 0.21       | 0.28           | 0.35       |
| 35    | 0.30       | 0.38           | 0.45       |
| 40    | 0.35       | 0.43           | 0.50       |
| 45    | 0.41       | 0.49           | 0.56       |
| 50    | 0.49       | 0.56           | 0.64       |
| 55    | 0.52       | 0.59           | 0.67       |
| 60    | 0.64       | 0.71           | 0.78       |
| 65    | 0.66       | 0.73           | 0.80       |
| 70    | 0.69       | 0.76           | 0.82       |
| 75    | 0.75       | 0.81           | 0.87       |
| 80    | 0.86       | 0.90           | 0.94       |
| 85    | 0.90       | 0.94           | 0.98       |
| 90    | 1          | 1              | 1          |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.014      | 0.043          | 0.080      |
| 10    | 0.058      | 0.10           | 0.16       |
| 15    | 0.094      | 0.15           | 0.22       |
| 20    | 0.14       | 0.20           | 0.28       |
| 25    | 0.17       | 0.24           | 0.32       |
| 30    | 0.23       | 0.30           | 0.38       |
| 35    | 0.30       | 0.37           | 0.46       |
| 40    | 0.34       | 0.41           | 0.50       |
| 45    | 0.37       | 0.44           | 0.54       |
| 50    | 0.43       | 0.51           | 0.59       |
| 55    | 0.49       | 0.57           | 0.65       |
| 60    | 0.54       | 0.62           | 0.71       |
| 65    | 0.62       | 0.69           | 0.77       |
| 70    | 0.69       | 0.76           | 0.83       |
| 75    | 0.74       | 0.80           | 0.87       |
| 80    | 0.82       | 0.88           | 0.93       |
| 85    | 0.89       | 0.93           | 0.97       |
| 90    | 1          | 1              | 1          |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0.0068     | 0.027          | 0.054      |
| 35    | 0.020      | 0.054          | 0.095      |
| 40    | 0.027      | 0.061          | 0.10       |
| 45    | 0.061      | 0.11           | 0.16       |
| 50    | 0.11       | 0.16           | 0.22       |
| 55    | 0.14       | 0.20           | 0.27       |
| 60    | 0.19       | 0.26           | 0.33       |
| 65    | 0.42       | 0.49           | 0.57       |
| 70    | 0.55       | 0.62           | 0.70       |
| 75    | 0.65       | 0.72           | 0.79       |
| 80    | 0.74       | 0.80           | 0.87       |
| 85    | 0.88       | 0.93           | 0.97       |
| 90    | 1          | 1              | 1          |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0.0068     | 0.027          | 0.054      |
| 35    | 0.020      | 0.054          | 0.095      |
| 40    | 0.027      | 0.061          | 0.10       |
| 45    | 0.061      | 0.11           | 0.16       |
| 50    | 0.11       | 0.16           | 0.22       |
| 55    | 0.14       | 0.20           | 0.27       |
| 60    | 0.19       | 0.26           | 0.33       |
| 65    | 0.42       | 0.49           | 0.57       |
| 70    | 0.55       | 0.62           | 0.70       |
| 75    | 0.65       | 0.72           | 0.79       |
| 80    | 0.74       | 0.80           | 0.87       |
| 85    | 0.88       | 0.93           | 0.97       |
| 90    | 1          | 1              | 1          |
### A7r5 1 h stretched untreated (n=142)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0              | 0          |
| 35    | 0          | 0              | 0          |
| 40    | 0          | 0              | 0          |
| 45    | 0          | 0.021          | 0.049      |
| 50    | 0.0070     | 0.035          | 0.070      |
| 55    | 0.035      | 0.070          | 0.12       |
| 60    | 0.056      | 0.10           | 0.15       |
| 65    | 0.15       | 0.21           | 0.28       |
| 70    | 0.34       | 0.42           | 0.49       |
| 75    | 0.51       | 0.58           | 0.66       |
| 80    | 0.69       | 0.76           | 0.82       |
| 85    | 0.80       | 0.86           | 0.91       |
| 90    | 1          | 1              | 1          |

### A7r5 1 h stretched +LPA (n=158)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0              | 0          |
| 35    | 0          | 0              | 0          |
| 40    | 0          | 0              | 0          |
| 45    | 0          | 0              | 0          |
| 50    | 0.0063     | 0.025          | 0.057      |
| 55    | 0.0063     | 0.025          | 0.057      |
| 60    | 0.013      | 0.038          | 0.070      |
| 65    | 0.089      | 0.14           | 0.20       |
| 70    | 0.25       | 0.32           | 0.40       |
| 75    | 0.42       | 0.50           | 0.58       |
| 80    | 0.59       | 0.66           | 0.73       |
| 85    | 0.73       | 0.79           | 0.85       |
| 90    | 1          | 1              | 1          |

### A7r5 1 h stretched +serum (n=173)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0              | 0          |
| 35    | 0          | 0              | 0          |
| 40    | 0          | 0              | 0          |
| 45    | 0          | 0              | 0          |
| 50    | 0          | 0.0058         | 0.023      |
| 55    | 0          | 0.0058         | 0.023      |
| 60    | 0.0058     | 0.023          | 0.052      |
| 65    | 0.023      | 0.052          | 0.092      |
| 70    | 0.12       | 0.17           | 0.23       |
| 75    | 0.41       | 0.48           | 0.55       |
| 80    | 0.64       | 0.71           | 0.78       |
| 85    | 0.79       | 0.84           | 0.90       |
| 90    | 1          | 1              | 1          |

### A7r5 4 h stretched untreated (n=155)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0              | 0          |
| 35    | 0          | 0              | 0          |
| 40    | 0          | 0              | 0          |
| 45    | 0          | 0              | 0          |
| 50    | 0          | 0.0065         | 0.026      |
| 55    | 0          | 0.0065         | 0.026      |
| 60    | 0          | 0.0065         | 0.026      |
| 65    | 0          | 0.013          | 0.032      |
| 70    | 0.045      | 0.084          | 0.14       |
| 75    | 0.15       | 0.21           | 0.28       |
| 80    | 0.44       | 0.52           | 0.59       |
| 85    | 0.70       | 0.77           | 0.84       |
| 90    | 1          | 1              | 1          |
**A7r5 4 h stretched +LPA (n=160)**

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0              | 0          |
| 10    | 0          | 0              | 0          |
| 15    | 0          | 0              | 0          |
| 20    | 0          | 0              | 0          |
| 25    | 0          | 0              | 0          |
| 30    | 0          | 0              | 0          |
| 35    | 0          | 0              | 0          |
| 40    | 0          | 0              | 0          |
| 45    | 0          | 0              | 0          |
| 50    | 0          | 0              | 0          |
| 55    | 0          | 0              | 0          |
| 60    | 0          | 0              | 0          |
| 65    | 0          | 0              | 0          |
| 70    | 0.031      | 0.069          | 0.11       |
| 75    | 0.21       | 0.28           | 0.35       |
| 80    | 0.46       | 0.54           | 0.62       |
| 85    | 0.72       | 0.78           | 0.85       |
| 90    | 1          | 1              | 1          |

The lower and upper 95% confidence intervals were analyzed using the method for bias-corrected and accelerated bootstrap intervals. The number of bootstrap intervals was set to 5000 for all histograms based on the indicated high number of cells (n). Cum. Frequency results from mean of upper and lower CI95.
Table S7. Estimation of the 95% confidence intervals (CI95) for cumulative histograms in Fig. S2

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0,052      | 0,074          | 0,10       |
| 10    | 0,11       | 0,14           | 0,17       |
| 15    | 0,15       | 0,19           | 0,22       |
| 20    | 0,19       | 0,22           | 0,26       |
| 25    | 0,24       | 0,27           | 0,32       |
| 30    | 0,31       | 0,35           | 0,39       |
| 35    | 0,36       | 0,41           | 0,46       |
| 40    | 0,42       | 0,47           | 0,51       |
| 45    | 0,47       | 0,51           | 0,56       |
| 50    | 0,54       | 0,59           | 0,63       |
| 55    | 0,59       | 0,63           | 0,68       |
| 60    | 0,64       | 0,68           | 0,73       |
| 65    | 0,69       | 0,73           | 0,77       |
| 70    | 0,73       | 0,77           | 0,81       |
| 75    | 0,78       | 0,82           | 0,85       |
| 80    | 0,85       | 0,88           | 0,91       |
| 85    | 0,89       | 0,92           | 0,94       |
| 90    | 1          | 1              | 1          |

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0,0075         | 0,019      |
| 10    | 0,004      | 0,015          | 0,034      |
| 15    | 0,011      | 0,026          | 0,049      |
| 20    | 0,019      | 0,037          | 0,063      |
| 25    | 0,037      | 0,063          | 0,093      |
| 30    | 0,049      | 0,078          | 0,11       |
| 35    | 0,060      | 0,093          | 0,13       |
| 40    | 0,063      | 0,10           | 0,13       |
| 45    | 0,086      | 0,12           | 0,17       |
| 50    | 0,12       | 0,16           | 0,21       |
| 55    | 0,15       | 0,19           | 0,24       |
| 60    | 0,19       | 0,24           | 0,29       |
| 65    | 0,26       | 0,31           | 0,37       |
| 70    | 0,34       | 0,40           | 0,47       |
| 75    | 0,51       | 0,57           | 0,63       |
| 80    | 0,66       | 0,71           | 0,76       |
| 85    | 0,78       | 0,82           | 0,87       |
| 90    | 1          | 1              | 1          |

A7r5 4 h stretched + 10 min stretched (n=180)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0          | 0,0075         | 0,019      |
| 10    | 0,004      | 0,015          | 0,034      |
| 15    | 0,011      | 0,026          | 0,049      |
| 20    | 0,019      | 0,037          | 0,063      |
| 25    | 0,037      | 0,063          | 0,093      |
| 30    | 0,049      | 0,078          | 0,11       |
| 35    | 0,060      | 0,093          | 0,13       |
| 40    | 0,063      | 0,10           | 0,13       |
| 45    | 0,086      | 0,12           | 0,17       |
| 50    | 0,12       | 0,16           | 0,21       |
| 55    | 0,15       | 0,19           | 0,24       |
| 60    | 0,19       | 0,24           | 0,29       |
| 65    | 0,26       | 0,31           | 0,37       |
| 70    | 0,34       | 0,40           | 0,47       |
| 75    | 0,51       | 0,57           | 0,63       |
| 80    | 0,66       | 0,71           | 0,76       |
| 85    | 0,78       | 0,82           | 0,87       |
| 90    | 1          | 1              | 1          |
### A7r5 4 h stretched + 30 min stretched (n=157)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.025      | 0.057          | 0.10       |
| 10    | 0.038      | 0.076          | 0.12       |
| 15    | 0.045      | 0.089          | 0.13       |
| 20    | 0.057      | 0.10           | 0.15       |
| 25    | 0.076      | 0.13           | 0.18       |
| 30    | 0.14       | 0.20           | 0.26       |
| 35    | 0.24       | 0.30           | 0.38       |
| 40    | 0.29       | 0.36           | 0.44       |
| 45    | 0.47       | 0.55           | 0.62       |
| 50    | 0.55       | 0.64           | 0.71       |
| 55    | 0.66       | 0.73           | 0.80       |
| 60    | 0.69       | 0.76           | 0.82       |
| 65    | 0.78       | 0.84           | 0.90       |
| 70    | 0.82       | 0.88           | 0.92       |
| 75    | 0.86       | 0.91           | 0.95       |
| 80    | 0.90       | 0.94           | 0.97       |
| 85    | 0.94       | 0.97           | 0.99       |
| 90    | 1          | 1              | 1          |

### A7r5 4 h stretched + 1 h stretched (n=175)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.040      | 0.080          | 0.13       |
| 10    | 0.086      | 0.13           | 0.18       |
| 15    | 0.20       | 0.26           | 0.33       |
| 20    | 0.31       | 0.38           | 0.46       |
| 25    | 0.55       | 0.62           | 0.69       |
| 30    | 0.74       | 0.81           | 0.86       |
| 35    | 0.90       | 0.95           | 0.97       |
| 40    | 0.94       | 0.98           | 0.99       |
| 45    | 0.96       | 0.99           | 1          |
| 50    | 0.96       | 0.99           | 1          |
| 55    | 0.97       | 0.99           | 1          |
| 60    | 0.97       | 0.99           | 1          |
| 65    | 0.97       | 0.99           | 1          |
| 70    | 1          | 1              | 1          |
| 75    | 1          | 1              | 1          |
| 80    | 1          | 1              | 1          |
| 85    | 1          | 1              | 1          |
| 90    | 1          | 1              | 1          |

### A7r5 4 h stretched + 4 h stretched (n=285)

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0.22       | 0.27           | 0.33       |
| 10    | 0.45       | 0.51           | 0.56       |
| 15    | 0.65       | 0.70           | 0.75       |
| 20    | 0.82       | 0.86           | 0.90       |
| 25    | 0.94       | 0.96           | 0.98       |
| 30    | 0.97       | 0.99           | 1          |
| 35    | 0.98       | 0.99           | 1          |
| 40    | 0.98       | 0.99           | 1          |
| 45    | 1          | 1              | 1          |
| 50    | 1          | 1              | 1          |
| 55    | 1          | 1              | 1          |
| 60    | 1          | 1              | 1          |
| 65    | 1          | 1              | 1          |
| 70    | 1          | 1              | 1          |
| 75    | 1          | 1              | 1          |
| 80    | 1          | 1              | 1          |
| 85    | 1          | 1              | 1          |
| 90    | 1          | 1              | 1          |

The lower and upper 95% confidence intervals were analyzed using the method for bias-corrected and accelerated bootstrap intervals. The number of bootstrap intervals was
set to 5000 for all histograms based on the indicated high number of cells (n). Cum. Frequency results from mean of upper and lower CI95.
Table S8. Estimation of the 95% confidence intervals (CI95) for cumulative histograms in Fig. S3

| Class | Lower CI95 | Cum. Frequency | Upper CI95 |
|-------|------------|----------------|------------|
| 5     | 0,018      | 0,041          | 0,068      |
| 10    | 0,045      | 0,077          | 0,12       |
| 15    | 0,082      | 0,12           | 0,17       |
| 20    | 0,14       | 0,18           | 0,24       |
| 25    | 0,17       | 0,22           | 0,28       |
| 30    | 0,21       | 0,26           | 0,33       |
| 35    | 0,23       | 0,29           | 0,35       |
| 40    | 0,29       | 0,35           | 0,41       |
| 45    | 0,38       | 0,44           | 0,50       |
| 50    | 0,47       | 0,54           | 0,60       |
| 55    | 0,53       | 0,60           | 0,66       |
| 60    | 0,61       | 0,68           | 0,74       |
| 65    | 0,67       | 0,73           | 0,79       |
| 70    | 0,71       | 0,76           | 0,82       |
| 75    | 0,75       | 0,80           | 0,85       |
| 80    | 0,77       | 0,83           | 0,87       |
| 85    | 0,85       | 0,90           | 0,93       |
| 90    | 1          | 1              | 1          |

The lower and upper 95% confidence intervals were analyzed using the method for bias-corrected and accelerated bootstrap intervals. The number of bootstrap intervals was set to 5000 for all histograms based on the indicated high number of cells (n). Cum. Frequency results from mean of upper and lower CI95.
Supplementary Figure S1: RhoA activation in A7r5 cells is neither enhancing actin fibers nor focal adhesions, therefore consequently not strongly amplifying actin reoration. (A) Micrographs of A7r5 cells immune stained for actin (magenta) and paxillin (green) after 30 min treatment with LPA. Scale bar is 50 µm. (B) Actin reorientation of A7r5 cells after 30 min, 1 h and 4 h of cyclic stretch after 30 min pre-treatment with LPA in serum free medium. Control cells were cultivated in serum free medium for 4.5 h. After fixation and staining the angular distributions of actin fibers were evaluated from 0° to 90° to the direction of stretch, cumulative frequency plots are given (n_a = 160, n_b = 138, n_c = 148, n_d = 147, n_e = 142, n_f = 158, n_g = 173, n_h = 155, n_i = 160).

Supplementary Figure S2: Reorientation of actin stress fibers in A7r5 cells is not stable after stretch release and can be inverted after second cyclic stretch in orthogonal direction. (A) Immunofluorescent micrographs of actin cytoskeleton after 4 h cyclic stretch (4 h str.) followed by 4 hours incubation without stretch (4 h str. + 4 h unstr.) or followed by 4 hours additional cyclic stretch in orthogonal direction (4 h str. + 4 h str.). Control cells were cultivated unstretched (unstr.). Arrowheads illustrate stretch directions. Scale bar is 50 µm. (B) After fixation and staining, actin stress fibers were evaluated as angular distribution from 0° to 90° to the direction of the first stretch for all samples and plotted as cumulative frequencies of all analyzed cells (n). (n_a = 299, n_b = 292, n_c = 268, n_d = 285).

Supplementary Figure S3: Actin reorientation and LC3B-spots in A7r5 cells can be likewise induced by uniaxial cyclic stretch on softer 5 kPa elastomer substrates. (A) Immunofluorescent micrographs of actin cytoskeleton after 1 h of cyclic stretch on 5 kPa elastomer substrates. Scale bar is 50 µm. (B) After fixation and staining, actin stress fiber orientations were evaluated as angular distribution from 0° to 90° relative to the direction of stretch and plotted as cumulative frequencies of all analyzed cells (n). (n_a = 220, n_b = 281). (C) LC3B (green) immune stainings of unstretched (unstr.) and 1 h cyclically stretched (1 h str.) A7r5 cells cultivated on 5 kPa elastomeric substrates. Scale bar is 20 µm. (D) Quantification of LC3B-spots per cell shown in (C) in percent of unstretched control. Data represent mean ± S.E.M. of all analyzed cells (n) (n_c = 58, n_d = 52).

Supplementary Figure S4: Workflow of LC3B-spots analysis.

For LC3B-spot analysis cells were immunocytochemically stained against the autophagosomal marker protein LC3B (A). Subsequently, autophagosomes were recorded by of single cells were performed using appropriate settings for excitation and emission. All cells were imaged completely from bottom to top with an optical layer thickness of 159 nm and a layer overlap of 50% based on optimal z-resolution settings in the Zen Black software (Carl Zeiss). Airyscan images were processed with the same software. For image processing, images were smoothed using a Gaussian filter (sigma size 1 pixel) and a cell mask was calculated. Therefore, images were binarized using the mean gray value of the image as a threshold. All values above the threshold were defined as cell mask. This mask
was then post processed by binary closing (disk structuring element with a radius of 10 pixels) and filling of cell mask holes with an area less than 10,000 pixels. To detect functional LC3-spots within the LC3B signal a manually selected threshold was kept constant for each single experiment to separate LC3B-spots from background signal (B). Threshold was typically at least 1.5 fold above average image gray value. All spots with an area of less than 70 pixels for A7r5 and 20 pixels for MEF cells were rejected (C). Signals outside the cell mask (shown by the red line in D) were not counted either. To separate big clusters of spots we used the watershed algorithm. For this purpose a distance transformation of the spot mask was calculated. Local maxima with a minimum distance of at least 5 pixels were detected and used as markers for the watershed algorithm to separate large clusters of spots. The number of remaining spots was then counted as LC3B-spots per cell for further analysis.
