

525 **Supplementary Figure Legends**

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527 **Supplementary Figure 1. Example magnified views of automated and manual**
528 **segmentations.** This magnified view demonstrates that the manual segmentations (performed at
529 lower magnification) oversimplify cell borders, which explains why the automated
530 segmentations consistently have higher perimeters.

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532 **Supplementary Figure 2. Quantitative comparison of automated and manual myocyte**
533 **segmentation for area, perimeter, elongation and circularity.** Automated and manual
534 segmentation for 100 myocytes from a combination control, isoproterenol, and serum-treated
535 conditions were compared. A) Absolute percent error in each phenotypic metric compared with
536 manual segmentation. B) Percent error in in each phenotypic metric compared with manual
537 segmentation. Error bars are +/- SE.

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539 **Supplementary Figure 3. Representative images of myocytes treated with hypertrophic**
540 **agonists.** Below each cell are the values of the phenotypic metrics (area, perimeter, circularity
541 and elongation) for that particular cell, which overall agree well with the median for that
542 condition.

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544 **Supplementary Figure 4. Comparison of manual scoring of sarcomeric organization with**
545 **automated measures of image texture.** Two researchers manually scored for the overall level
546 of sarcomeric organization in each cell (100 myocytes) on a 1-4 scale, and the average result was
547 taken. Manual scores are compared with 3 automated measures of image texture within in each
548 cell: Uniformity, Contrast, and Correlation.

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550 **Supplementary Figure 5. Representative images of sarcomeric organization from myocytes**
551 **treated with hypertrophic agonists.** Above each cell is listed the quantitative value of
552 sarcomeric organization (1-Uniformity) for that cell. Representative cells were chosen by
553 identifying cells close to the median of that population.