Figure S1. Inexpensive fabrication of cell loader with 3D printing.

An inexpensive device for loading cells into the mother machine. The construction involves 3D printing a custom holder/ rotor for a 50mm WillCo dish, on which a mother machine is attached. The holder is printed in three parts (2 blades and a central base) to account for 3D printers with small printing areas. This piece is then assembled and secured to a Honeywell fan from which the original blade has been removed. CAD files and details of the fan centrifuge construction are available at [28].

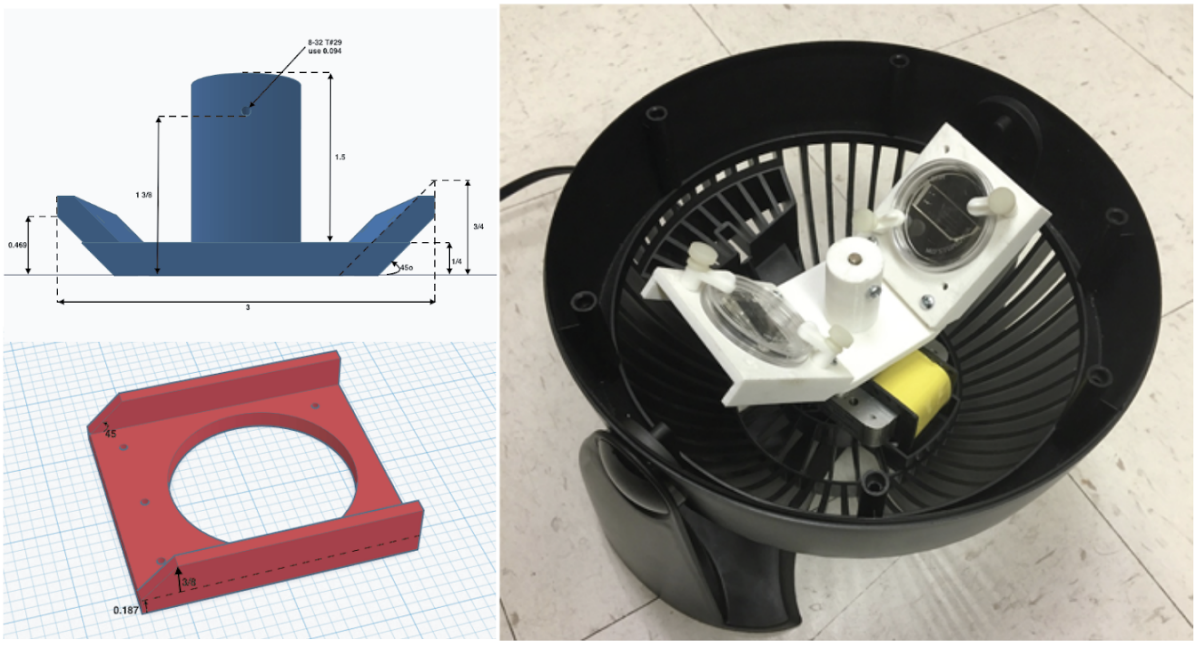

Figure S2: Segmentation accuracy of napari-MM3 Otsu and U-Net methods

To quantify the accuracy of the segmentation masks generated by MM3's Otsu and U-Net segmentation methods, we computed the Jaccard Index [47,50] as a function of the intersection-over-union (IoU) threshold.

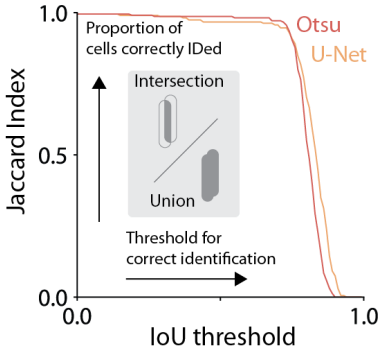

Figure S3: Old-pole aging phenotype is strain specific. Cells imaged with fluorescence often show signs of aging in the old-pole "mother" cell. For instance, in the dataset analyzed in Figure 4 (*E. coli* MG1655 with the fluorescent protein YPet fused to DnaN), we observed systematic differences in cell elongation rate and size between the old-pole cell at the end of the growth channel and its sisters, which inherit the new pole (top center). However, this asymmetry is not universal. Using napari-MM3's Otsu segmentation method, we re-analyzed previously published data obtained without fluorescence illumination [32], and found that the old-pole and new-pole cell elongation rates varied only on the order of 1% (lower center), while in the dataset obtained under fluorescence imaging, the old-pole mother cells grow 7-10% slower than the new pole cells. These results are consistent with a previous survey [62], which found that most evidence for aging in *E. coli* comes from studies utilizing fluorescent proteins for visualization.