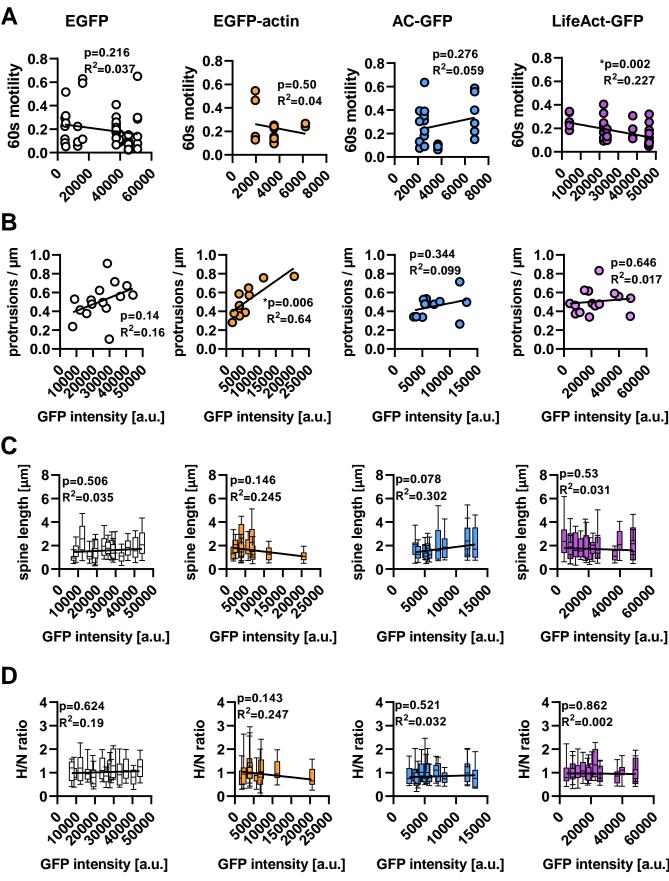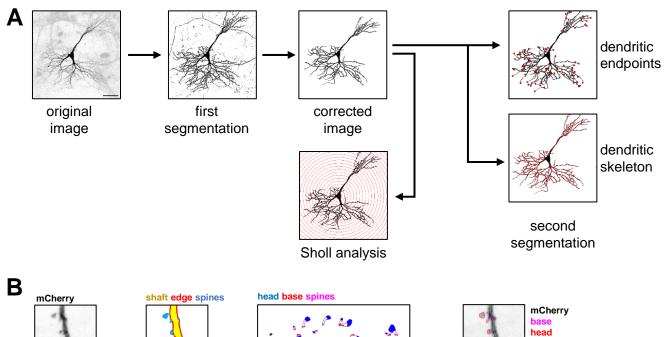Supplementary Materials

Molecular Biology of the Cell

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Supplementary Figure 1. Expression level dependency in the investigated morphometric parameters in dendritic spines. (A) 60sec filopodial motility and (B) total protrusion density plotted against soma GFP intensity. Circles represend individual filopodia. (C) Length and (D) Head/neck diameter ratio of dendritic spines in relation to soma GFP intensity. Box-whisker plots represent data from individual cells. Correlation was calculated from the median values of each cell.

P values show Pearson correlation significance.

**p<0.01 ***p<0.001.

Supplementary Figure 2. Workflow of machine learning based image analysis methods used in the present work. (A) Sholl analysis, dendritic endpoints and skeleton finding. Original and thresholded images are shown in inverted fluorescence, measured properties are shown in red. Scalebar: 50 μm. (B) Dendritic spine morphometric measurements. Original image is shown in inverted fluorescence, the segmentation categories are written in their corresponding colors in the images. Scalebar: 2 μm. (C) Quantification of fluorescence enrichment in dendritic spines. Original image is shown in inverted fluorescence, the segmentation categories are written in their corresponding colors in the images. Scalebar: 2 μm.

A

EGFP — p=0.216, R²=0.037

EGFP-actin — p=0.50, R²=0.04

AC-GFP — p=0.276, R²=0.059

LifeAct-GFP — *p=0.002, R²=0.227

60s motility

B

p=0.14, R²=0.16

*p=0.006, R²=0.64

p=0.344, R²=0.099

p=0.646, R²=0.017

protrusions / µm

GFP intensity [a.u.]

C

p=0.506, R²=0.035

p=0.146, R²=0.245

p=0.078, R²=0.302

p=0.53, R²=0.031

spine length [µm]

GFP intensity [a.u.]

D

p=0.624, R²=0.19

p=0.143, R²=0.247

p=0.521, R²=0.032

p=0.862, R²=0.002

H/N ratio

GFP intensity [a.u.]

A

original image → first segmentation → corrected image

dendritic endpoints

dendritic skeleton

second segmentation

Sholl analysis

B

mCherry — original image

shaft edge spines — first segmentation

head base spines / branch spine length spines — second segmentation

mCherry base head spine length branch length — final reconstruction

C

AC-GFP — original image

branch spines — first segmentation

AC-GFP spine shaft background — spine processing