Fig 1

a powerpoint assets

b input:

t=153, s=1 of IMG32\_S16 (nice, long movie)

left:

imageJ manual render

right, calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Daan code\DAAMparticle\_Shape\_Analysis\ImageAnalysis\_SingleParticleMovie\_BatchStellarisMDJ.m

code blocks:

"Read the data files"

... all up to ...

"Convert secondary signal to mask and align cups"

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\Daanalysis\PlotsBatch\_Miguel\_v3.m

code blocks:

"Initialization"

"Plot triangulated surface of particles"

c input:

same as b

calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Daan code\DAAMparticle\_Shape\_Analysis\ImageAnalysis\_SingleParticleMovie\_BatchStellarisMDJ.m

code blocks:

"Read the data files"

... up to

"Convert secondary signal to mask and align cups"

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\Daanalysis\Old\_Update\_Mask\_GUI

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\Daanalysis\PlotsBatch\_Miguel\_v3.m

code blocks:

"Initialization"

"Plot triangulated 2D surface of particles"

ROI calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\selectROI\_v5.m

code blocks: all

hard-coded demarcation lines based on roi\_position.position

d input:

MPStats file from IMG130\_sgNT, k=70

calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\LineProfileAnalysis\_Batch\_v5.m

code blocks:

"initialization..."

"calculate volume profiles..."

"visualize indentation traces one by one"

e input for graph:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Indentation strength by volumetric profiling\Fig1\_Indents\_CellvsNocell\_20230201.pzfx

with data files in:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Indentation strength by volumetric profiling\Figure 1 - IMG141\_D8\_3 - Cell vs NoCell

calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\LineProfileAnalysis\_Batch\_v5.m

code blocks:

"initialization..."

"calculate volume profiles..."

"plot and save all traces"

f input for graph:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Indentation strength by volumetric profiling\Figure 1 - Kinetics\Frombeginning\Fig1\_IndentsFromBeginning\_IMG147-Day1-7-1-s9\_20230131.pzfx

with data files in:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Indentation strength by volumetric profiling\Figure 1 - Kinetics\Frombeginning

calculated similarly as in d

g input for graph:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Kinetics (movies and time-autocorrelation)

calculated with script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\topography\_timeAutocorr.m

blocks:

"initialize..."

"auto-correlations of absolute topogrpahies..."

using input:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Kinetics (movies and time-autocorrelation)\Autocorrelation grids

gridded synapse input calculated using scripts:

X:\Miguel DJ (Labshare)\Lab life\Daan code\DAAMparticle\_Shape\_Analysis\ImageAnalysis\_SingleParticleMovie\_BatchStellarisMDJ.m

code blocks:

"Read the data files"

... up to

"Convert secondary signal to mask and align cups"

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\Daanalysis\Old\_Update\_Mask\_GUI

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\selectROI\_v5.m

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\save\_GriddedSynapses\_v5.m

code blocks:

"initialize..."

... up to

"interpolate and store gridded data"

h-i input for graph:

D:\Working folder for super-res files\IMG151\_B16vsOTIPrfKO\Annotated\_fromtheStart\Timestamped

h: imageJ (Morgan manually selected and rendered)

i: Imaris (Morgan manually selected and rendered)

j input for graph:

D:\Working folder for super-res files\IMG151\_B16vsOTIPrfKO\Annotated\_fromtheStart\Segment\_masks\done\_contourcurvature

calculated with script:

segment-mask-cellchannel\_20231101.ijm

using input:

D:\Working folder for super-res files\IMG151\_B16vsOTIPrfKO\20240110\_Reanalyze\tifstacks-from-the-start

followed by script:

D:\Working folder for super-res files\IMG151\_B16vsOTIPrfKO\20240110\_Reanalyze

using output from previous step

plotted selected frames

Fig S1

a-d powerpoint, imageJ, and Matlab assets

grids in d calculated as in Fig 1G

e-g input for graph:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Feature analysis (Supervised)

calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\featurePicker\_withPerCell\_v3.m

blocks:

all

using input:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Gridded synapses\IMG141 - Non-synapse reference\MC

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Gridded synapses\IMG141 - P14 D0\_8\_40\_Exh\Grids\_MC\rep3, just the P14D8 data

Fig S2

a powerpoint assets

b input for graph:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Indentation strength by volumetric profiling\IMG155\_Actin-poison\IMG155\_actin-poison-dVROI\_20240311-removeCK666forpaper.pzfx

calculated as in Fig 1E

c-e input for graph:

same as b

calculated as in Fig S1E-G

Fig S3

a powerpoint and imageJ assets

b calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\contour\_curvature\_v2.m

code blocks for contour graph:

"initialization"

"load matrix..."

"parse the matrices..."

code blocks for histograms:

"initialization"

"load matrix..."

"parse the matrices..."

"graph: histogram..."

"graph: manual norm2maxhistogram"

using input data:

D:\Working folder for super-res files\IMG151\_B16vsOTIPrfKO\Annotated\_fromtheStart\Segment\_masks\done\_contourcurvature

D:\Working folder for super-res files\IMG151\_B16vsOTIPrfKO\Annotated\_inSynapseRes\Segment\_masks\done

c similar to b, except code blocks exclude "graph: manual norm2maxhistogram"

Fig 2

a-c imageJ and Matlab assets, Matlab panels calculated as in Fig 1C

d input for graph:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Degranulation topography\20230314\_Degranulation\_Redo\_Debug\IMG147\_Degranulation-ReAligned\_20230316.mat

calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\degranulationROI\_RealSpaceUpdate\_Debug20230314.m

code blocks:

"initialization"

... up to

"Plot: Collect MC & GC and overlay degranulations"

Fig 3 and S4: various manually graphed assets

simulation .csvs calculated using script:

X:\Alex\Code Drop\Abel Simulations Working Folder\SimulationAnalysisBatch.m

code blocks: all

input data for Pareto plots:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Synapse modeling (Abel-Gaetjens)

hard-coded graphing recorded in:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Synapse modeling (Abel-Gaetjens)\20240312\_Record-of-Paretoplots-Matlabcode.txt

Fig 4

a powerpoint assets

b input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Gridded synapses\IMG130 - sgNT Talin WASp WAVE\Mean C

grids calculated as in Fig 1C

c input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Indentation strength by volumetric profiling\IMG130\_sgCSK\IMG130\_sgCSK\_Deform-vs-Complexity\_20230207.pzfx

calculated as in Fig 1E

d forgot which exact grid was used for this

Zernike chart plotted using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\ZernikeChart\_Plotter.m

e hard-coded

f input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Zernike spectra and complexity\IMG130 - sgCSKgenes ZPCA\IMG130\_sgCSKgenes\_coeffstruct.mat

calculated from script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\Zernike\_decomposition\_MultiGridsInput\_v4.m

code blocks:

"initialization"

... up to

"calculate & plot Z-PCA"

"automatically split Z-PCA plot into quantiles" (n=3)

using grids input from:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Gridded synapses\IMG130 - sgNT Talin WASp WAVE\Mean C\withNonSynapse\_forFigureDemo

g hard-coded

h input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Zernike spectra and complexity\IMG130 - sgCSKgenes ZPCA\IMG130\_sgCSKgenes\_ZPCA\_ClusterComp\_20230207Quartiled.pzfx

calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\Zernike\_decomposition\_MultiGridsInput\_v4.m

code blocks:

"initialization"

... up to

"calculate & plot Z-PCA"

"automatically split Z-PCA plot into quantiles" (n=3)

"pattern category disproportionation"

i top panels hard-coded

bottom panel hard-coded

calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\Zernike\_complexity\_v5.m

code blocks:

"import grids..."

"set parameters"

"calculate pattern complexity numbers"

j input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Zernike spectra and complexity\IMG130 - sgCSKgenes ZPCA\IMG130\_sgCSKgenes\_Zernikecomplexity-recalc20240107.pzfx

calculated from script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\Zernike\_complexity\_v5.m

code blocks: same as i, import into Prism

Fig S5

a-c input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Feature analysis (Supervised)\IMG130\_sgCSK\_20230227\_featuresPerCell.pzfx

calculated using script: as in Fig S1E-G

Fig S6

a-b possibly hard coded, Alex Settle may have a script to re-generate these

c input data: same as Fig 4F

calculated using script: same as Fig 4F

code blocks:

"initialization"

... up to

"calculate & plot Z-PCA"

"Highlight cell types separately: scatter"

d import into Prism: score(:,1) data from coeff\_struct data structure

e input data: same as Fig 4F

calculated using script: same as Fig 4F

code blocks:

"initialization"

... up to

"calculate & plot Z-PCA"

"Zernike mode breakdown"

f input data: same as Fig 4F

calculated using script: same as Fig 4I

code blocks:

"import..."

... up to

"assign labels"

"correlation plot of PC1 vs. Settle Complexity"

Fig S7

a hard-coded, as in Fig 4I

b-g input data: same as Fig 4F

calculated using script: same as Fig 4I

code blocks:

"import..."

... up to

"assign labels"

"correlate complexity vs dV", import into Prism

Fig 5

a powerpoint assets

b-c input data:

X:\Miguel DJ (Labshare)\Lab life\Experiments\Flow cytometry\FLEX37\_20240124\_Stiffness-sweep-sgNT-WASp-PTEN\FLEX37\_analysis.pzfx

d left: powerpoint assets

right, input data:

X:\Miguel DJ (Labshare)\Lab life\IMG157\_Analysis-summary.pzfx

(sorry, forgot to organize these guys into their own folders)

calculated using script: same as Fig 1E

e hard-coded

f input data:

X:\Miguel DJ (Labshare)\Lab life\IMG157\_coeff-struct-mc.mat

calculated using script: same as Fig 4F

g input data:

X:\Miguel DJ (Labshare)\Lab life\IMG157\_complex-struct-TopographyMC.mat

calculated using script: same as Fig 4I

Fig S8

a-d input data:

X:\Miguel DJ (Labshare)\Lab life\IMG157\_Analysis-summary.pzfx

calculated using script: same as Fig S1E-G

Fig 6

a SAD powerpoint assets

b input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Indentation strength by volumetric profiling\IMG141\_Endi-experiment\Indent\_IMG141\_LPA\_Stats3D\_20230208.pzfx

calculated using script: same as Fig 1E

c input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Zernike spectra and complexity\IMG141 - Endi expt\ZernikeCoefficients\_IMG141\_20230208.mat

calculated using script: same as Fig 4I

d hard-coded

e input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Zernike spectra and complexity\IMG141 - Endi expt\ZPCA\_IMG141\_20231109-CorrectColor.pzfx

calculated using script: same as Fig 4H

f input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Zernike spectra and complexity\IMG141 - Endi expt\Complexity\_IMG141\_20240107.pzfx

Fig S9

a-b Endi provided from his FlowJo analysis files

input data in:

X:\Miguel DJ (Labshare)\Lab life\Experiments\Flow cytometry\ENDIFLOW ES\_MDJ\_174

Fig S10

a-c input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Feature analysis (Supervised)\FeaturesPerCell\_IMG141\_20230227.pzfx

calculated using script: same as Fig S1E-G

d input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Zernike spectra and complexity\IMG141 - Endi expt\ZernikeCoefficients\_IMG141\_20230208.mat

calculated using script: same as Fig S6E

e-i input data: same as Fig 6F

calculated using script: same as Fig S7B-G

Fig S11

a powerpoint assets

b-e input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Feature analysis (Supervised)\IMG146\_Features\_noTRP1\_20230227Recalc.pzfx

calculated using script: same as Fig S1E-G

f calculated using script: same as Fig 4F

g calculated using script: same as Fig S6E

h hard-coded

i calculated using script: same as Fig 4H

j calculated using script: same as Fig 4J

Fig 7

a powerpoint assets

b hard-coded

c input data in:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Indentation strength by volumetric profiling\IMG144-145\_OTIvsBMDM

(picked files from BlobTrace\_IMG144-OT1.tif and BlobTrace\_IMG145-BMDM.tif)

d X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Indentation strength by volumetric profiling\IMG144-145\_OTIvsBMDM\Indent\_IMG144-145\_OT1vsBMDM\_20230208.pzfx

calculated using script: same as Fig 1E

e input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Radial profiling\IMG145 - MP OTI redo\IMG144-145\_PopRadProf\_recalcwithNonsynapse\_20221215.mat

calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\analyze\_curvaturedistancedata\_v4.m

code blocks:

"initialization..."

... up to

"population-average the radial profiles"

"pairwise difference heatmap plotting"

using distance\_data files calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\save\_GriddedSynapses\_v5.m

code blocks:

"initialize..."

... up to

"create or extend..."

"calculate curvature/distance data"

f-h input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Feature analysis (Supervised)\FeaturesperCell\_IMG144-145\_OT1vsBMDM\_20230227.pzfx

calculated using script: same as Fig S1E-G

i input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Zernike spectra and complexity\IMG144-145 - BMDM vs OTI\ZernikeCoeff\_IMG144-145\_OT1vsBMDM\_20230208.mat

calculated using script: same as Fig 4F

code blocks:

"initialization"

...up to

"replot manual Z-PCA gates..."

Fig S12

a powerpoint assets

b-c calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\analyze\_curvaturedistancedata\_v4.m

code blocks: all

d input data: same as Fig 7I

calculated using script: same as Fig S6E

Fig 8

a-b powerpoint assets

c input data:

X:\Miguel DJ (Labshare)\Lab life\IMG157\_coeff-struct-int.mat

calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Plotting\_Fig7\_update\_20240220.m

code blocks: all

d calculated using script: same as Fig S6E

e hard-coded

f input data:

X:\Miguel DJ (Labshare)\Lab life\IMG157\_Analysis-summary.pzfx

calculated using script:

X:\Miguel DJ (Labshare)\Lab life\Custom scripts\MATLAB\ringratio\_synapse.m

using data from:

X:\Miguel DJ (Labshare)\Lab life\IMG157\_Vertex-data.mat

g powerpoint assets

h input data:

IMG154\_Stiffness-sweep-Ringratio-vs-Stiffness\_20240103.pzfx

calculated using script: same as f

using data from:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Gridded synapses\IMG154\_Grids\Actin

Fig S13

a input data:

X:\Miguel DJ (Labshare)\Lab life\Biomechanotyping\Indentation strength by volumetric profiling\IMG156\_sgNT-WASp-PTEN\_500Pa\IMG156\_sgNT-WASp-PTEN-dVROI\_20240122.pzfx

calculated using script: same as Fig 1E

b input data:

X:\Miguel DJ (Labshare)\Lab life\IMG157\_correlation-Deformation-Zcomplexity.pzfx

calculated using script: same as Fig S7B-G

c input data:

X:\Miguel DJ (Labshare)\Lab life\IMG157\_ZPCA-actin-Disproportionation.pzfx

calculated using script: same as Fig 4H

Fig S14-15 were hard-coded