Expanded View Figures

Figure EV1. MAP3Ks activated by osmotic shock.

A Schematic of the haploid genetic screen workflow to map genetic regulators of p38 activation in response to osmotic shock.
B HAP1 cells and cells deleted for ZAK (ΔZAK) were treated with anisomycin (1 h) or 500 mM sorbitol (1 h). Lysates were analyzed by immunoblotting with the indicated antibodies.
C U2OS cells stably expressing S-HA-ZAKβ were treated with increasing concentrations of sorbitol (1 h). Lysates were analyzed as in (B).
D U2OS cells were pretreated with ZAK inhibitor (ZAKi, 0.5 h) and treated with sorbitol (500 mM, 1 h). Lysates were treated with lambda phosphatase (λ phos.) and phosphatase inhibitor (PPi) as indicated and analyzed as in (B).
E U2OS ΔZAK cells stably expressing wildtype (WT) or kinase-dead (KD) ZAKβ-GFP were treated with sorbitol (500 mM, 1 h) as indicated. Lysates were analyzed as in (B).
F U2OS and ΔZAK cells were treated with sorbitol (500 mM) for the indicated time points and analyzed as in (B).
G U2OS and ΔZAK cells were treated with sorbitol (500 mM), washed out for sorbitol, and allowed to recover for the indicated time points. Lysates were analyzed as in (B).
H U2OS and ΔZAK cells were transfected with siRNAs and treated with sorbitol (500 mM, 1 h) as indicated. Lysates were analyzed as in (B).
I U2OS, ΔZAK, and ΔZAK cells rescued with wildtype (WT) or ribosome-binding deficient (ΔSΔCTD) S-HA-ZAKα constructs were treated with sorbitol (500 mM, 1 h) as indicated. Lysates were analyzed as in (B).

Source data are available online for this figure.

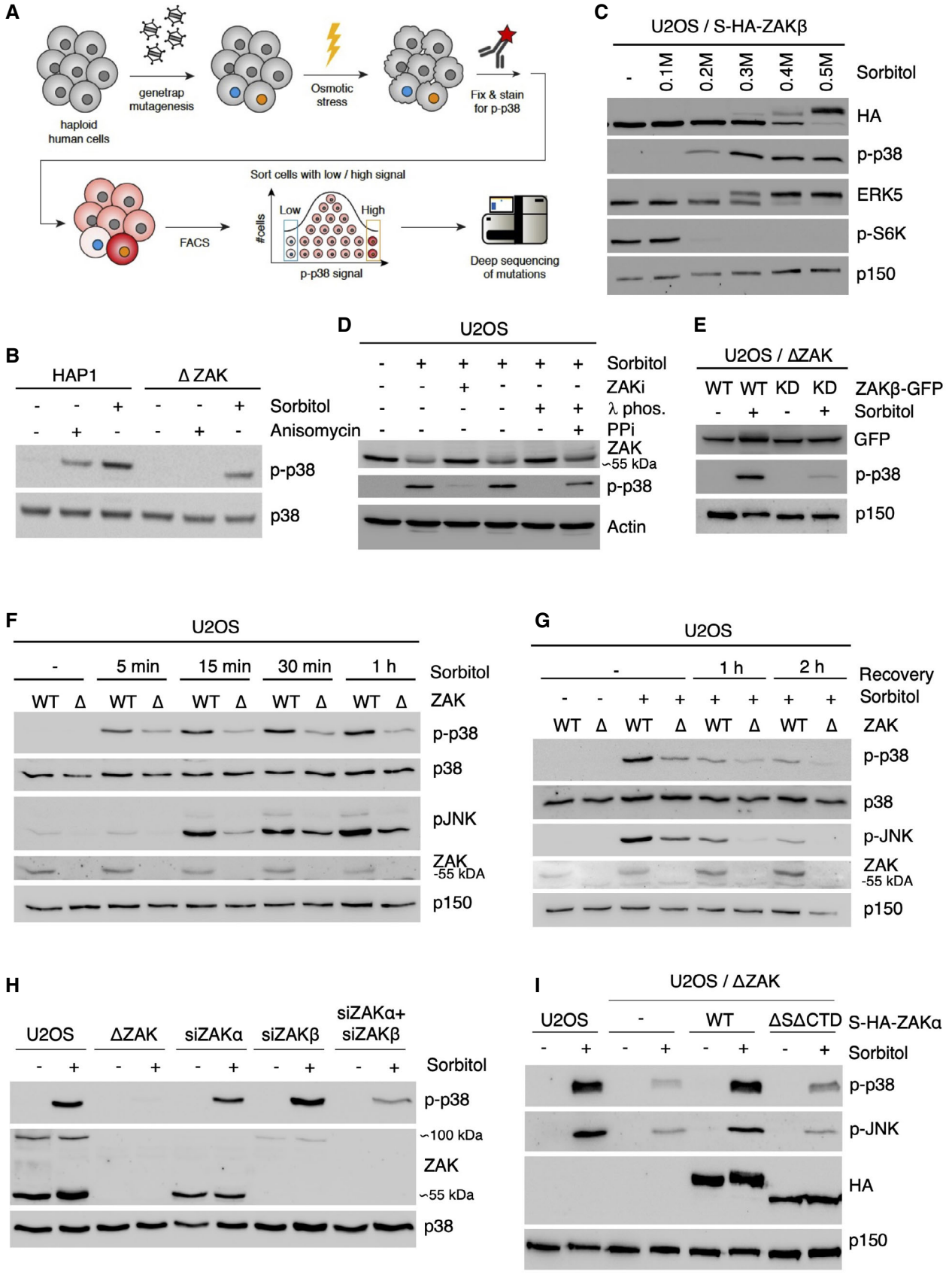
A
haploid human cells
genetrap mutagenesis
Osmotic stress
Fix & stain for p-p38
Sort cells with low / high signal
FACS
#cells
Low
High
p-p38 signal
Deep sequencing of mutations
B
HAP1
Δ ZAK
Sorbitol
Anisomycin
p-p38
p38
C
U2OS / S-HA-ZAKβ
0.1M
0.2M
0.3M
0.4M
0.5M
Sorbitol
HA
p-p38
ERK5
p-S6K
p150
D
U2OS
Sorbitol
ZAKi
λ phos.
PPi
ZAK
~55 kDa
p-p38
Actin
E
U2OS / ΔZAK
WT WT KD KD
ZAKβ-GFP
Sorbitol
GFP
p-p38
p150
F
U2OS
5 min
15 min
30 min
1 h
Sorbitol
WT Δ WT Δ WT Δ WT Δ WT Δ
ZAK
p-p38
p38
pJNK
ZAK
-55 kDA
p150
G
U2OS
1 h
2 h
Recovery
Sorbitol
WT Δ WT Δ WT Δ WT Δ
ZAK
p-p38
p38
p-JNK
ZAK
-55 kDA
p150
H
U2OS
ΔZAK
siZAKα
siZAKβ
siZAKα+ siZAKβ
Sorbitol
p-p38
~100 kDa
ZAK
~55 kDa
p38
I
U2OS / ΔZAK
U2OS
WT
ΔSΔCTD
S-HA-ZAKα
Sorbitol
p-p38
p-JNK
HA
p150

Figure EV1.

Figure EV2. Sorbitol-induced recruitment of ZAKβ to the nuclear lamina and nuclear domains.

A U2OS and ΔZAK cells were transfected with MEKK2 siRNA and treated with sorbitol (500 mM, 1 h) as indicated. Lysates were analyzed by immunoblotting with the indicated antibodies.

B Left: U2OS cells stably expressing ZAKβ-GFP were treated with sorbitol (500 mM, 1 h). Cells were fixed and immunostained with Lamin A/C antibody. Right: Same as left except that Hela cells transiently expressing ZAKβ-GFP were immunostained with 53BP1 antibody. Lower panel: Intensity distribution graphs showing fluorescence intensities along the magenta lines.

C Hela cells transfected with ZAKβ-GFP were treated with sorbitol (500 mM, 1 h) or exposed to ionizing radiation (IR, 4 Gy – 1 h). Cells were fixed and immunostained with γH2AX antibody and counterstained with DAPI.

D As in (C) but immunostained with 53BP1 antibody.

E Quantification of cells from (Fig 2D) with nuclear structure- and/or stress fiber-localized ZAKβ. ****$P < 0.0001$ in the Fisher's exact test.

F U2OS cells stably expressing ZAKβ-GFP were imaged by live-cell fluorescence microscopy. Cells were treated with sorbitol (500 mM, 15 min) before washout. Shown is the last frame before washout, the first frame after washout, and 5 min after washout.

G U2OS cells stably expressing GFP-ZAKα were imaged by live-cell fluorescence microscopy. Sorbitol (final concentration 500 mM) was added after the acquisition of the first frame.

H U2OS cells from (F) were treated with SiR-actin for 15 min prior to sorbitol and imaged as in (G).

I Cells from (F) were co-transfected with mCherry-α-actinin1 and imaged as in (G). Inserts show higher magnification of the yellow regions.

Data information: All scale bars, 10 μm.
Source data are available online for this figure.

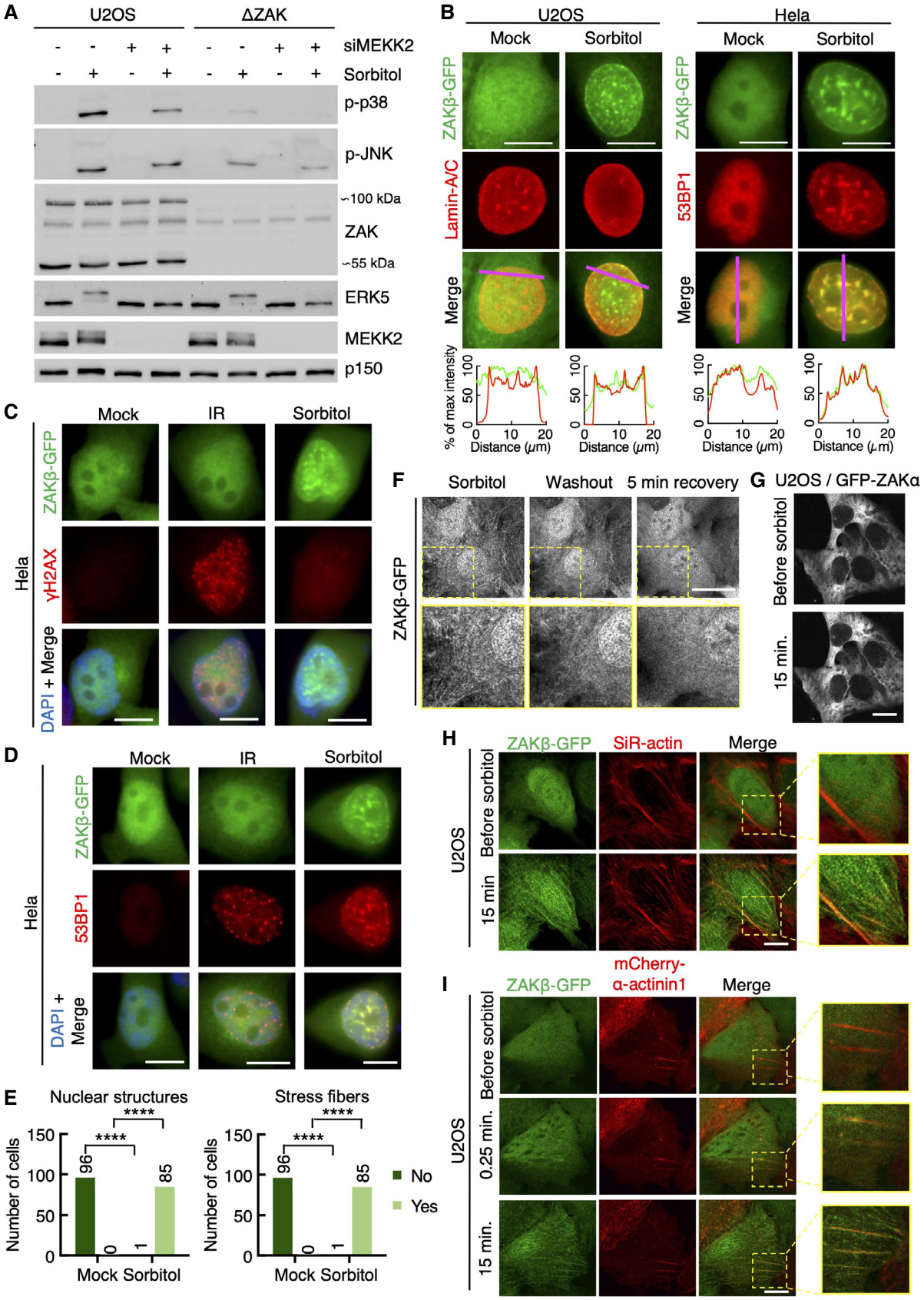
A
U2OS
ΔZAK
siMEKK2
Sorbitol
p-p38
p-JNK
~100 kDa
ZAK
~55 kDa
ERK5
MEKK2
p150
B
U2OS
Hela
Mock
Sorbitol
ZAKβ-GFP
Lamin-A/C
53BP1
Merge
% of max intensity
Distance (μm)
C
Mock
IR
Sorbitol
Hela
ZAKβ-GFP
γH2AX
DAPI + Merge
D
Mock
IR
Sorbitol
Hela
ZAKβ-GFP
53BP1
DAPI + Merge
E
Nuclear structures
Stress fibers
Number of cells
Mock Sorbitol
No
Yes
F
Sorbitol
Washout
5 min recovery
ZAKβ-GFP
G
U2OS / GFP-ZAKα
Before sorbitol
15 min.
H
ZAKβ-GFP
SiR-actin
Merge
U2OS
Before sorbitol
15 min
I
ZAKβ-GFP
mCherry-α-actinin1
Merge
U2OS
Before sorbitol
0.25 min.
15 min.

Figure EV2.

Figure EV3. Cell stretch does not activate ZAKβ.

A Murine C2C12 myoblasts were incubated in myotube differentiation medium with the indicated kinase inhibitors and harvested after 3 and 6 days. Lysates were analyzed by immunoblotting with the indicated antibodies.
B Images of cells from (A) before harvest. Scale bars, 100 μm.
C C2C12 myotubes (6 d differentiation) pretreated with ZAK inhibitor (ZAKi, 0.5 h) were subjected to equibiaxial cyclic stretch (5 min) as indicated. Lysates were analyzed in (A).
D Genotype frequencies for pups born from $ZAK^{+/-}$ intrabreedings.
E Litter sizes from WT and $ZAK^{-/-}$intrabreedings. Error bars represent the standard deviations ($n > 5$ biological replicates). ns—not significant in unpaired *t*-test.
F Weight of 16-18-week-old WT and $ZAK^{-/-}$ male and female mice. Error bars represent the standard deviations ($n > 6$ biological replicates). ns, not significant in *t*-test corrected for multiple comparison using the Bonferroni–Dunn method.
G Mouse embryonic fibroblasts (MEF) isolated from WT and $ZAK^{-/-}$ mice were treated with anisomycin (1 h) or cycloheximide (1 h) as indicated. Lysates were analyzed as in (A).
H Male mice from (F) were subjected to a retroorbital injection of [3H]-2-deoxyglucose followed by 10 min *in situ* contraction of the lower hindlimb. Upon euthanization, TA muscles were isolated, lysed, and analyzed for glucose uptake/clearance and glycogen content. ns, not significant in 2-way ANOVA ($n = 3$ biological replicates).
I Anesthetized 16- to 18-week-old WT and $ZAK^{-/-}$ male mice were subjected to 10 min *in situ* contraction of the lower hindlimb. Extensor digitorum longus (EDL) muscles were isolated, lysed, and analyzed by immunoblotting with the indicated antibodies ($n = 3$ mice).
J Quantification of western blot results in Fig 4E. Error bars represent the standard deviations. ns—not significant, and ***$P < 0.001$ in 2-way ANOVA with multiple comparison using the Tukey's method. FC, fold change ($n = 3$ biological replicates).
K Quantification of (I). Error bars represent the standard deviations. ns—not significant, and ****$P < 0.0001$ in 2-way ANOVA with multiple comparison using the Tukey's method. FC—fold change ($n = 3$ biological replicates).

Source data are available online for this figure.

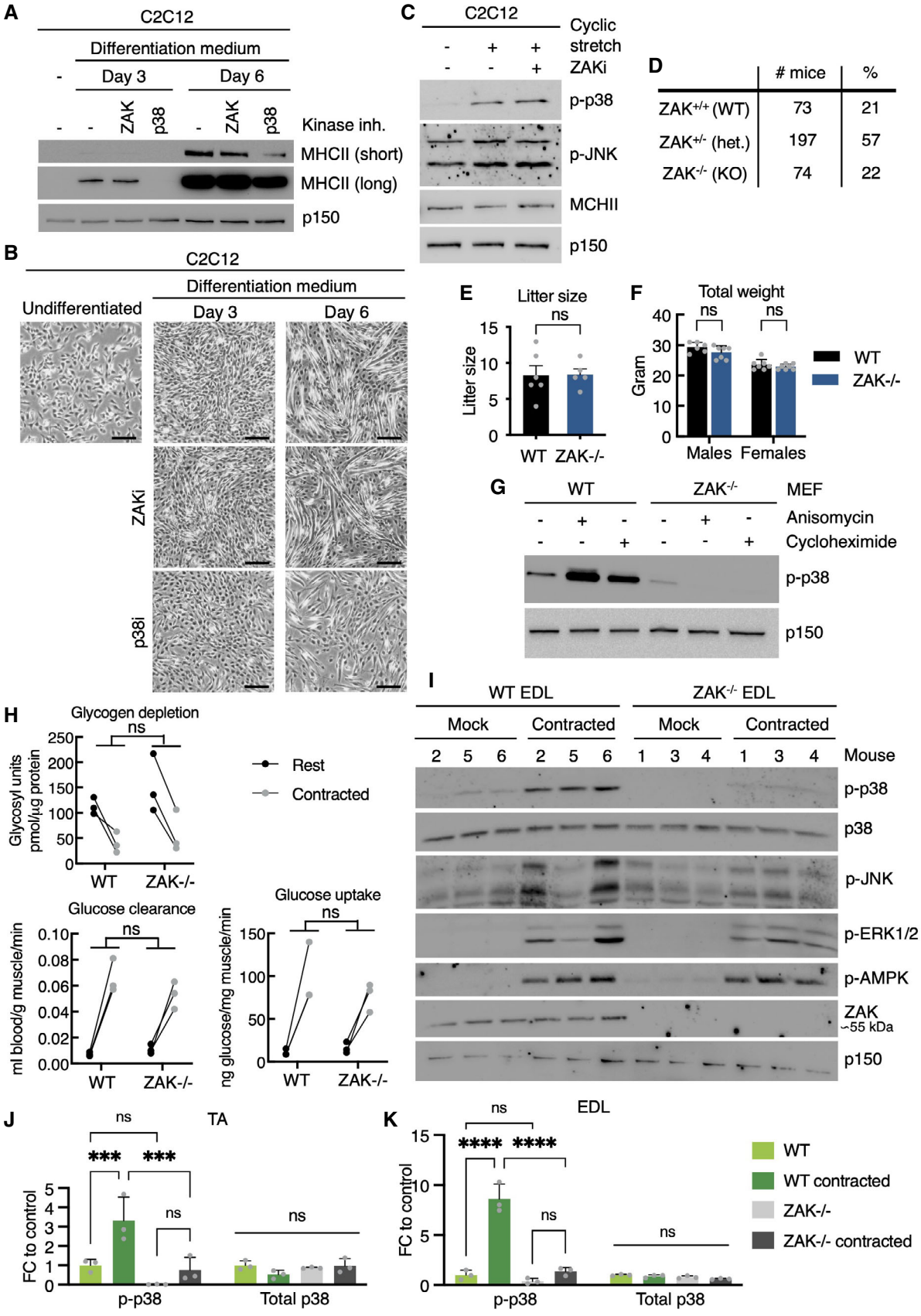
A
C2C12
Differentiation medium
Day 3
Day 6
ZAK
p38
Kinase inh.
MHCII (short)
MHCII (long)
p150
B
C2C12
Differentiation medium
Undifferentiated
Day 3
Day 6
ZAKi
p38i
C
C2C12
Cyclic stretch
ZAKi
p-p38
p-JNK
MCHII
p150
D
mice
%
ZAK+/+ (WT) 73 21
ZAK+/- (het.) 197 57
ZAK-/- (KO) 74 22
E
Litter size
ns
Litter size
WT ZAK-/-
F
Total weight
ns
Gram
Males Females
WT
ZAK-/-
G
WT
ZAK-/-
MEF
Anisomycin
Cycloheximide
p-p38
p150
H
Glycogen depletion
ns
Glycosyl units pmol/µg protein
Rest
Contracted
WT ZAK-/-
Glucose clearance
ml blood/g muscle/min
Glucose uptake
ng glucose/mg muscle/min
I
WT EDL
ZAK-/- EDL
Mock
Contracted
Mouse
p-p38
p38
p-JNK
p-ERK1/2
p-AMPK
ZAK ~55 kDa
p150
J
TA
FC to control
p-p38
Total p38
K
EDL
WT
WT contracted
ZAK-/-
ZAK-/- contracted

Figure EV3.

Figure EV4. ZAKβ mediates muscle contraction-induced MAP kinase activation.

A 16-18-week-old WT and ZAK$^{-/-}$ female mice (n = 3) were subjected to 10 min *in situ* contractions of the lower hindlimb. Tibialis anterior muscles were isolated and phospho- and total proteomes were recorded by mass spectrometry. Phospho-proteomes were subjected to principal component analysis.

B As in (A), except that total proteomes were analyzed.

C Heatmap and clustering of significant phosphorylation changes from data in (A) analyzed by ANOVA. Numbers refer to the mouse-ID. L—left; R—right. Buttom: GO-term enrichment analysis for the cluster with phosphorylation sites showing ZAK-dependent upregulation before and after contraction.

D TA muscles of 12-week-old WT male mice were electroporated with the indicated GFP constructs. After 7 days the muscle was harvested, sectioned longitudinally, and immunostained with an antibody against the Z-disc marker α-actinin1. Lower panel: Intensity distribution graphs showing fluorescence intensities along the magenta lines (10 μm).

E Tibialis anterior (TA), gastrocnemius (GAS), and soleus (SOL) muscles were isolated from 16-18-week-old WT and ZAK$^{-/-}$ male and female mice. Values represent the weight of the muscle from one of the legs and error bars represent the standard deviations (n > 6 biological replicates). ns—not significant in t-test corrected for multiple comparison using the Bonferroni–Dunn method.

F H&E staining of soleus muscle cross-sections from 8- and 22-week-old WT and ZAK$^{-/-}$ female mice. Arrows indicate the presence of centralized nuclei.

G As in (F) except that TA muscles from 8-week-old female mice were analyzed.

H Quantification of (G). Values indicate the percentage of fibers displaying centralized nuclei and error bars represent the standard deviation (n = 6 biological replicates). ns, not significant in unpaired t-test.

Data information: All black scale bars, 50 μm.

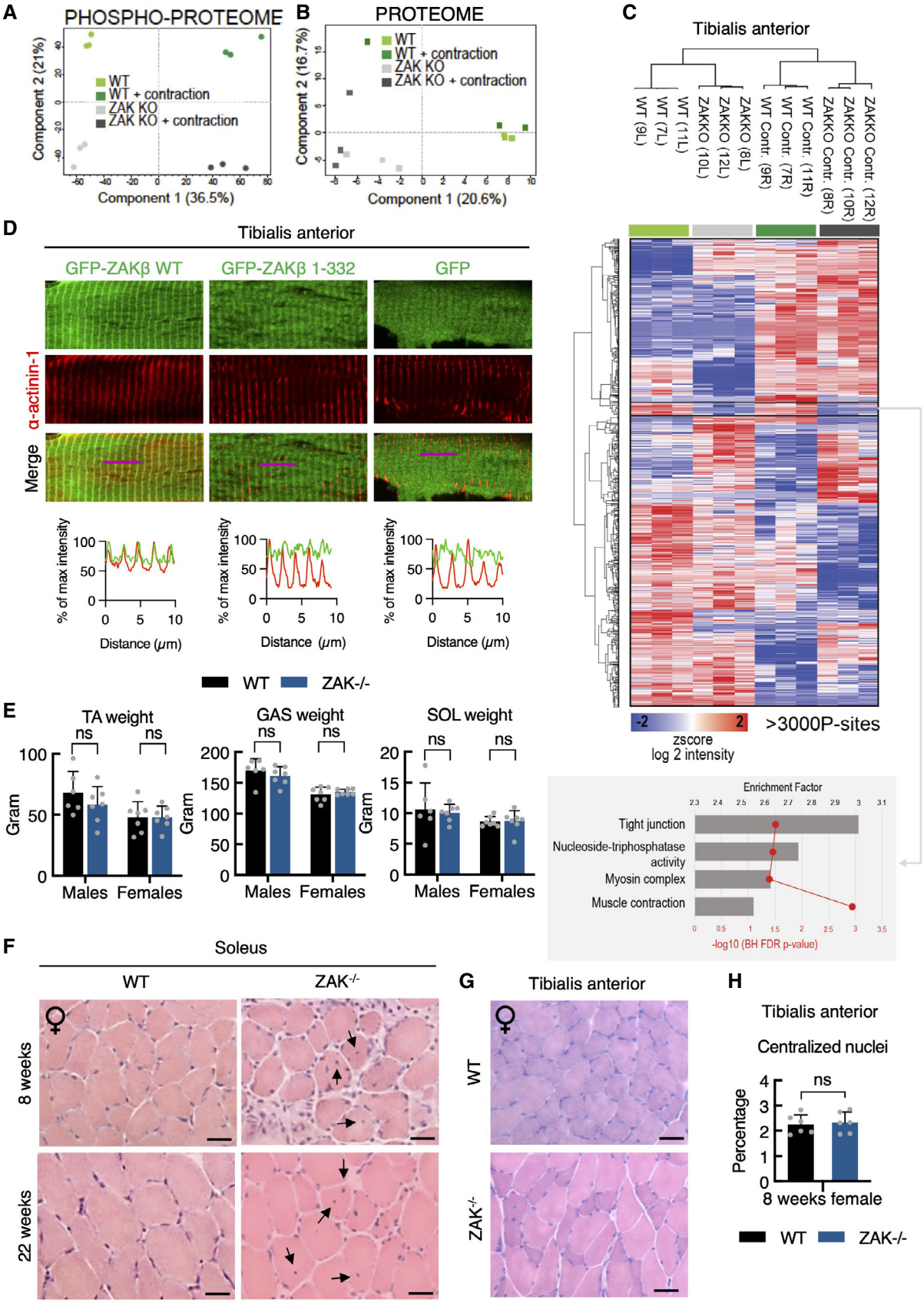
A
PHOSPHO-PROTEOME
Component 2 (21%)
Component 1 (36.5%)
WT
WT + contraction
ZAK KO
ZAK KO + contraction
B
PROTEOME
Component 2 (16.7%)
Component 1 (20.6%)
WT
WT + contraction
ZAK KO
ZAK KO + contraction
C
Tibialis anterior
WT (9L)
WT (7L)
WT (11L)
ZAKKO (10L)
ZAKKO (12L)
ZAKKO (8L)
WT Contr. (9R)
WT Contr. (7R)
WT Contr. (11R)
ZAKKO Contr. (8R)
ZAKKO Contr. (10R)
ZAKKO Contr. (12R)
-2
2
zscore
log 2 intensity
>3000P-sites
Enrichment Factor
Tight junction
Nucleoside-triphosphatase activity
Myosin complex
Muscle contraction
-log10 (BH FDR p-value)
D
Tibialis anterior
GFP-ZAKβ WT
GFP-ZAKβ 1-332
GFP
α-actinin-1
Merge
% of max intensity
Distance (μm)
WT
ZAK-/-
E
TA weight
GAS weight
SOL weight
ns
Gram
Males
Females
F
Soleus
WT
ZAK-/-
8 weeks
22 weeks
G
Tibialis anterior
WT
ZAK-/-
H
Tibialis anterior
Centralized nuclei
ns
Percentage
8 weeks female
WT
ZAK-/-

Figure EV4.

Figure EV5. ZAK$^{-/-}$ mice present with muscle pathology.

A Soleus muscle cross-sections from 8- and 22-week-old WT and ZAK$^{-/-}$ female mice were immunostained for type I (red) and type IIa (green) fibers using myosin isoform-specific antibodies. Scale bars, 500 μm.

B Correlation map of transcriptomes from all individual samples color-coded according to the correlation coefficient.

C Number of differentially expressed genes (DEG) for the indicated group comparisons.

D Volcano plot of up- and downregulated DEGs in soleus muscle dependent on genotype.

E Volcano plot of up- and downregulated DEGs in TA.

F General locomotor activity of 16-18-week-old WT and ZAK$^{-/-}$ male mice was evaluated using a standard open field test. No difference in distance traveled, velocity, time spent moving, or number of entries to the center zone was observed between the genotypes. Error bars represent the standard deviations (n = 5 biological replicates). ns—not significant in unpaired t-test.

G Heat maps illustrating representative moving patterns. TA—tibialis anterior; Sol—soleus.

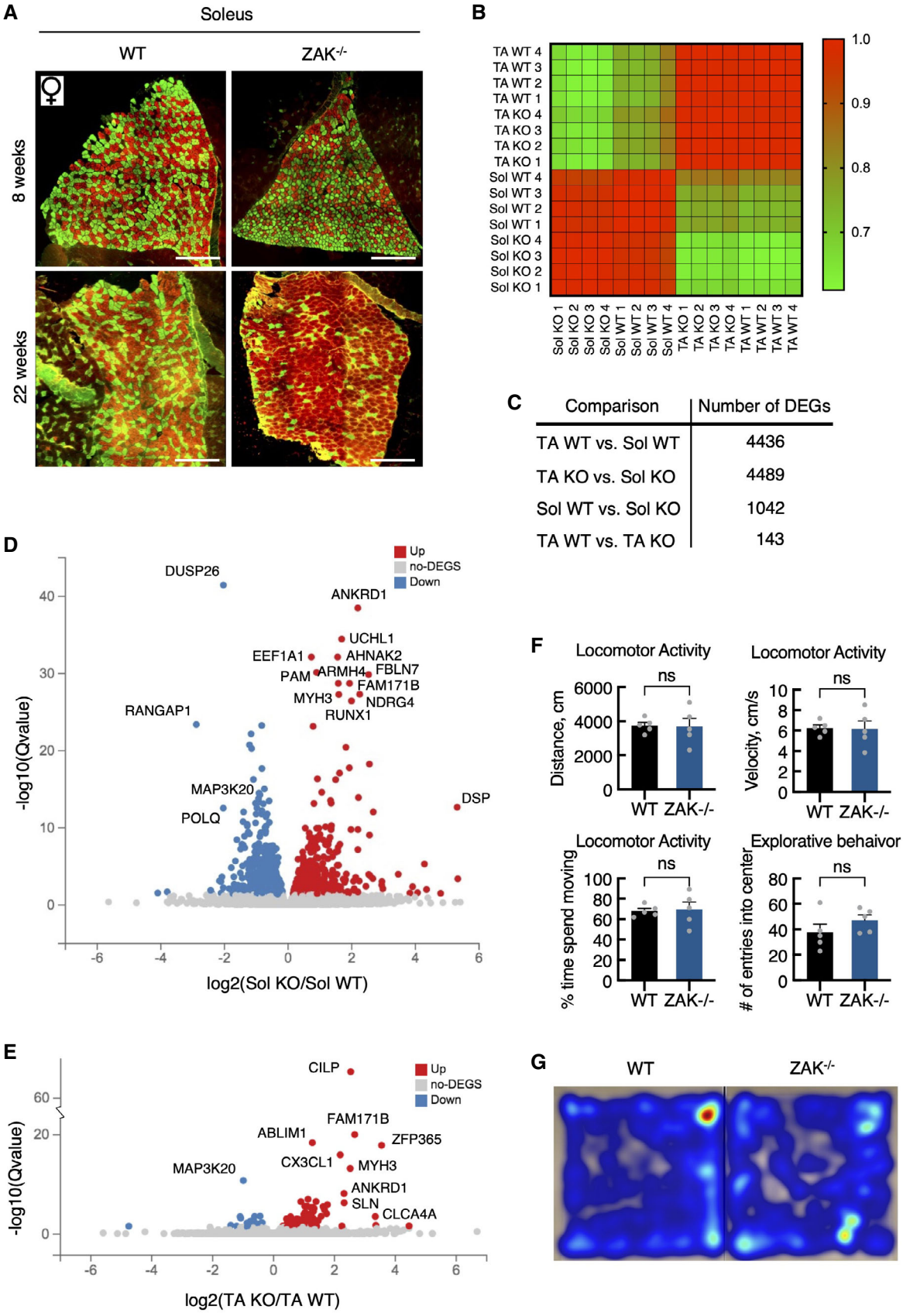
A
Soleus
WT
ZAK-/-
8 weeks
22 weeks
B
TA WT 4
TA WT 3
TA WT 2
TA WT 1
TA KO 4
TA KO 3
TA KO 2
TA KO 1
Sol WT 4
Sol WT 3
Sol WT 2
Sol WT 1
Sol KO 4
Sol KO 3
Sol KO 2
Sol KO 1
1.0
0.9
0.8
0.7
C
Comparison | Number of DEGs
TA WT vs. Sol WT | 4436
TA KO vs. Sol KO | 4489
Sol WT vs. Sol KO | 1042
TA WT vs. TA KO | 143
D
Up
no-DEGS
Down
DUSP26
ANKRD1
UCHL1
EEF1A1
AHNAK2
PAM
ARMH4
FBLN7
FAM171B
MYH3
NDRG4
RUNX1
RANGAP1
MAP3K20
POLQ
DSP
-log10(Qvalue)
log2(Sol KO/Sol WT)
E
CILP
FAM171B
ABLIM1
ZFP365
CX3CL1
MYH3
MAP3K20
ANKRD1
SLN
CLCA4A
-log10(Qvalue)
log2(TA KO/TA WT)
F
Locomotor Activity
Distance, cm
Velocity, cm/s
% time spend moving
Explorative behaivor
# of entries into center
ns
WT
ZAK-/-
G
WT
ZAK-/-

Figure EV5.