

Appendix

Rescue of impaired liver regeneration in aged mice by silencing the Hippo pathway

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Table of Contents

Figure S1 MST1, LATS and MST2 protein expression during liver regeneration

Figure S2 Validation of LATS1 and p-LATS1 antibody specificity by Western blot.

Figure S3 RT-qPCR of regenerating mouse liver tissue sham, 6, 24 and 48 following PH

Figure S4 Western blot detection of p-LATS in young and aged liver post PH

Figure S5 Screening for siRNA sequences targeting the Hippo core kinases MST1 and MST2 in BNL mouse cell line.

Figure S6 Pathway analysis of siMST versus siScr
Genomic microarray was performed on liver samples on day 3 following injection of either siMST or siScr. Pathway analysis was performed using the SetRank method.

Figure S7 Heat maps of genes regulated by siMST compared to siScr

- A Interferon gamma signalling
- B extracellular matrix remodelling
- C mitotic prometaphase

Figure S8 Assessment of liver injury and off target effects from siRNA:liposomes

Figure S9 Measurement of hepatocyte dimensions

Figure S10 Serum transaminases (ALT and AST) and alkaline phosphatase (ALP) in regenerating aged livers \pm MST KD (aged MST)

Appendix Figure S1

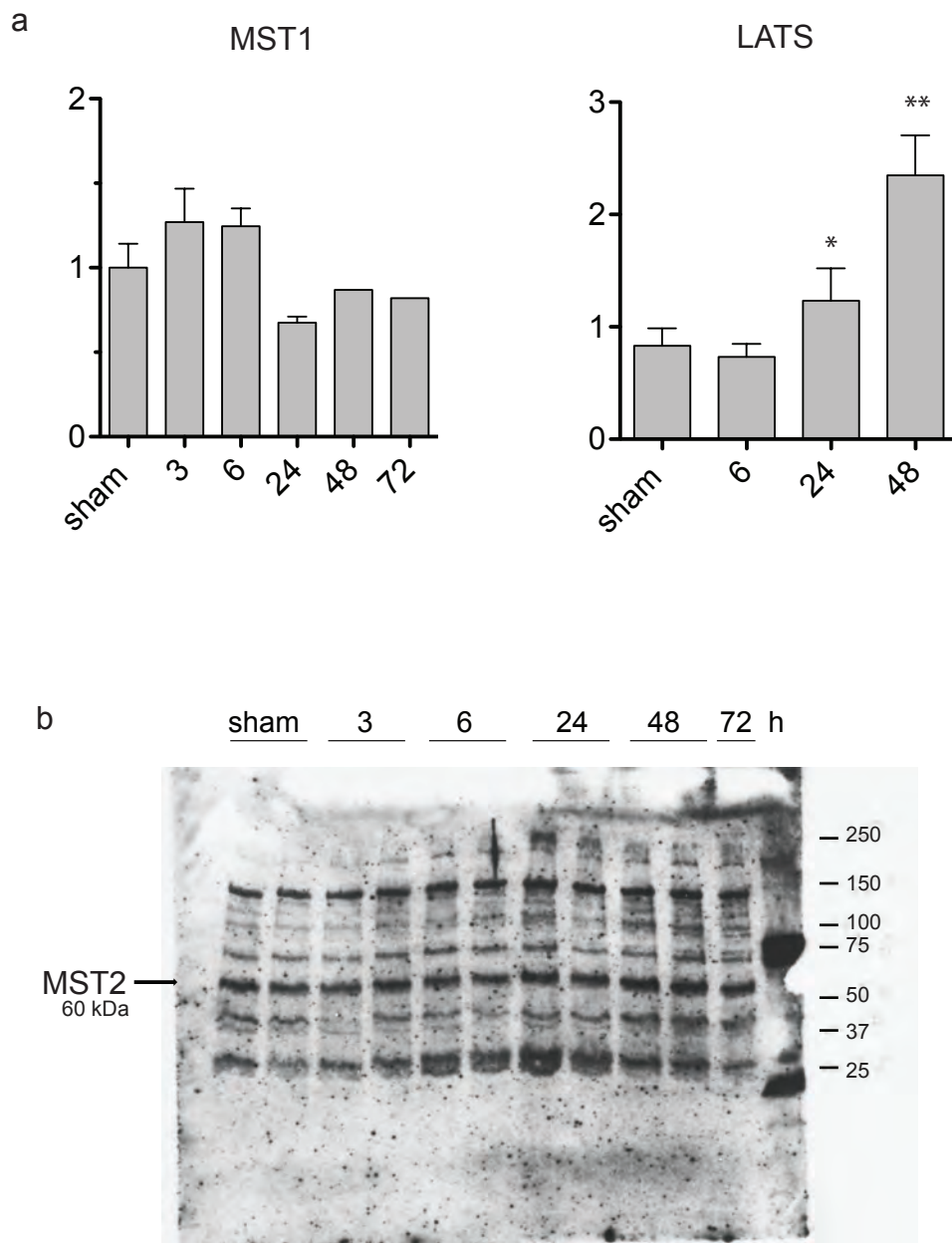


Figure S1 MST1, LATS and MST2 protein expression during liver regeneration

A MST and LATS total protein quantification from Figure 1B.

Data are presented as mean \pm SD. p values were calculated with Student's t-test comparing values at each time point to sham operated control tissue compared to sham controls.

B MST2 Western blot membrane. MST2 migrates at approximately 60kDa.

Appendix Figure S2

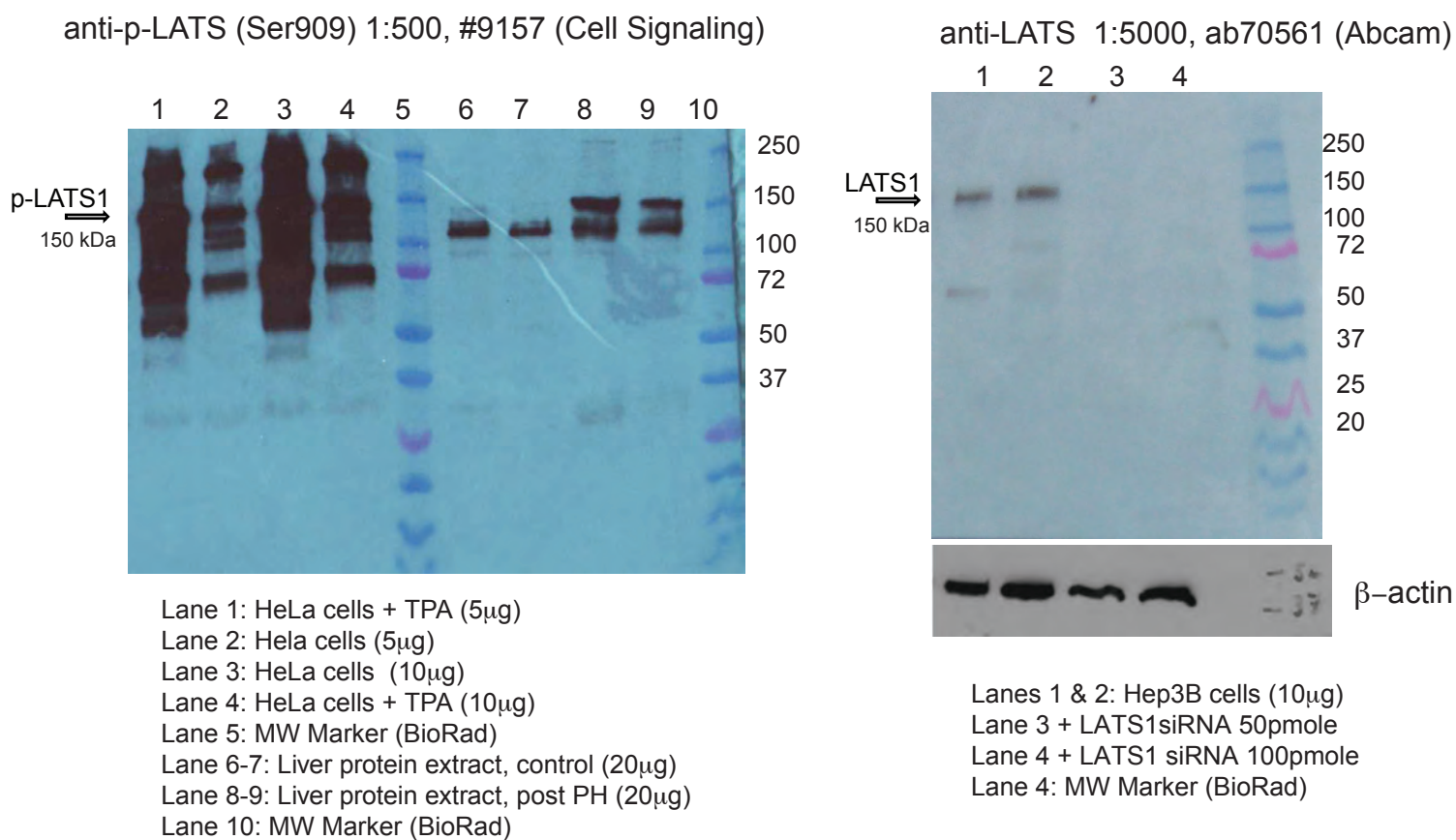


Figure S2 Validation of LATS1 and p-LATS1 antibody specificity by Western blot.

Appendix Figure S3

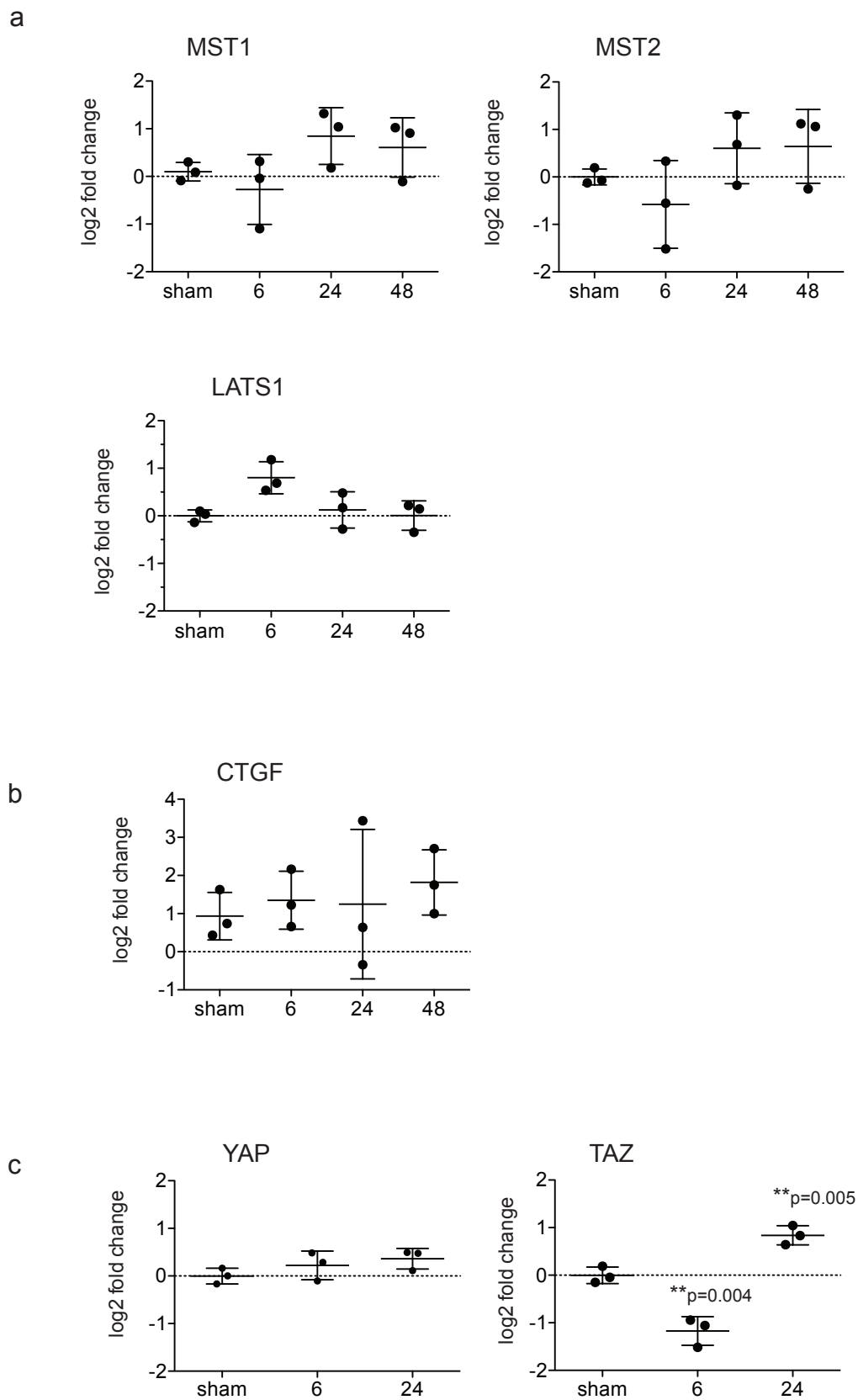


Figure S3 RT-qPCR of regenerating mouse liver tissue sham, 6, 24 and 48 following PH

Data are presented as mean \pm SD. p values were calculated with a student's t-test and time points were compared to sham controls.

Appendix Figure S4

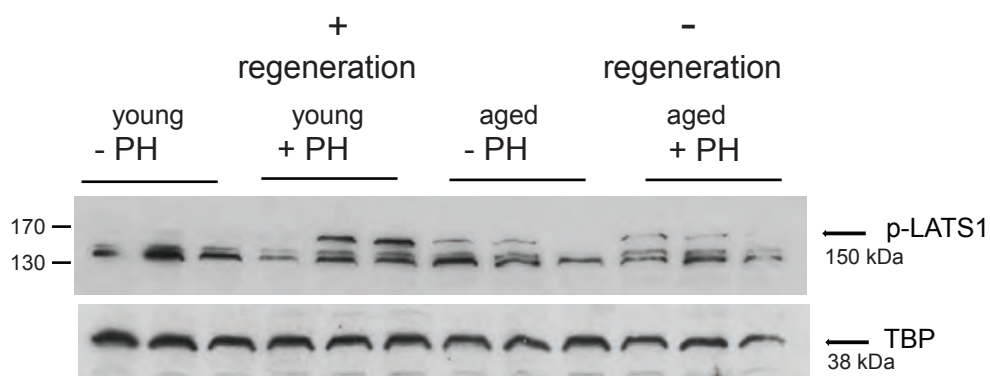


Figure S4 Western blot detection of p-LATS in young and aged liver post PH

Liver protein extracts were isolated from young and aged mice without (-) and with (+) a partial hepatectomy. At 40 h post PH, young mice were determined to be regenerating (+) and aged mice were non-regenerating (-). TBP was used as a loading control. Each band is from an independent animal.

Appendix Figure S5

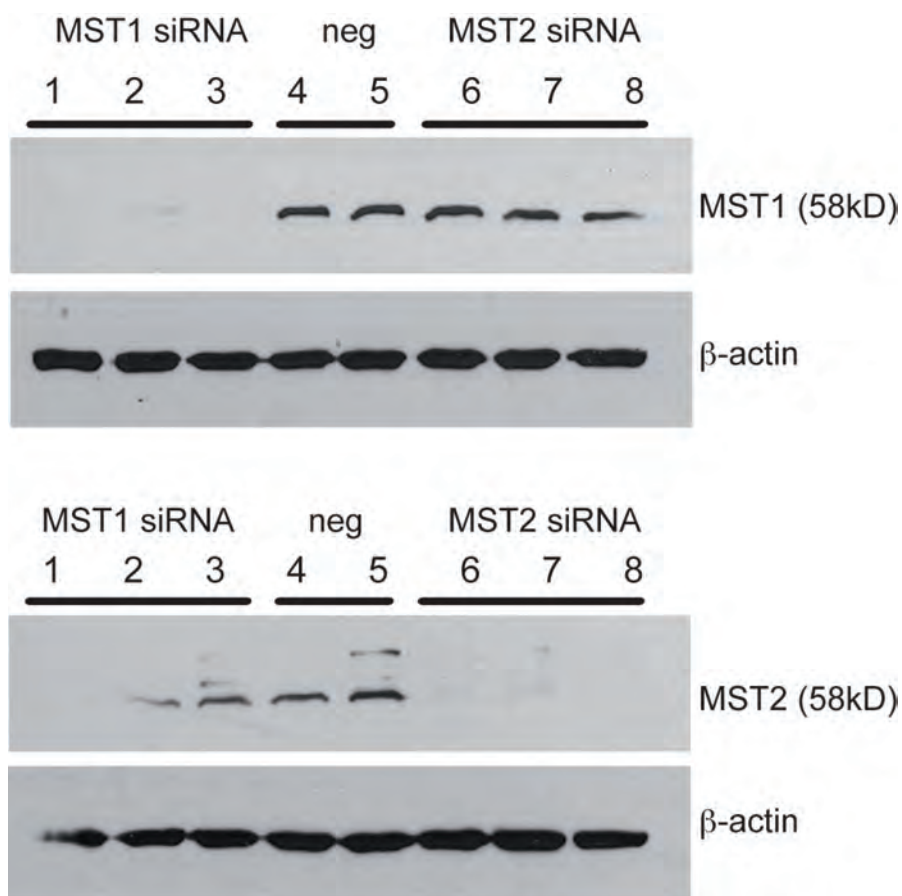


Figure S5 Screening for siRNA sequences targeting the Hippo core kinases MST1 and MST2 in BNL mouse cell line.

(A) MST1 and (B) MST2 protein levels analysed by western blot 24 h after siRNA transfection. β -actin was used as protein loading control.

Lane 1) MST1/STK4 sequence ID: s81598.

Lane 2) MST1/STK4 sequence ID: s81597

Lane 3) MST1/STK4 sequence ID: s81599

Lane 4) negative control sequence

Lane 5) negative control sequence

Lane 6) MST2/STK3 sequence ID:S211749

Lane 7) MST2/STK3 sequence ID:S211750

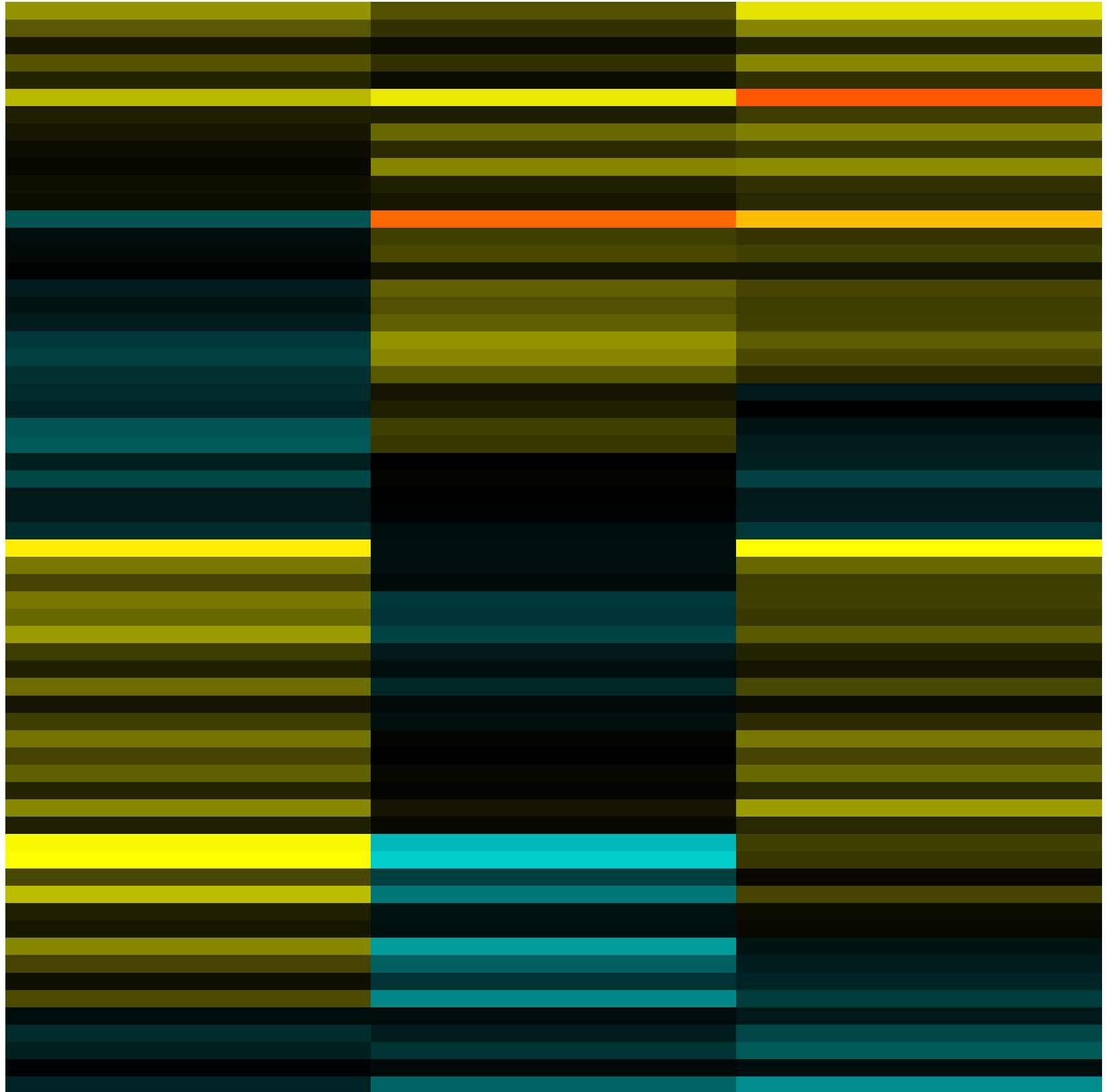
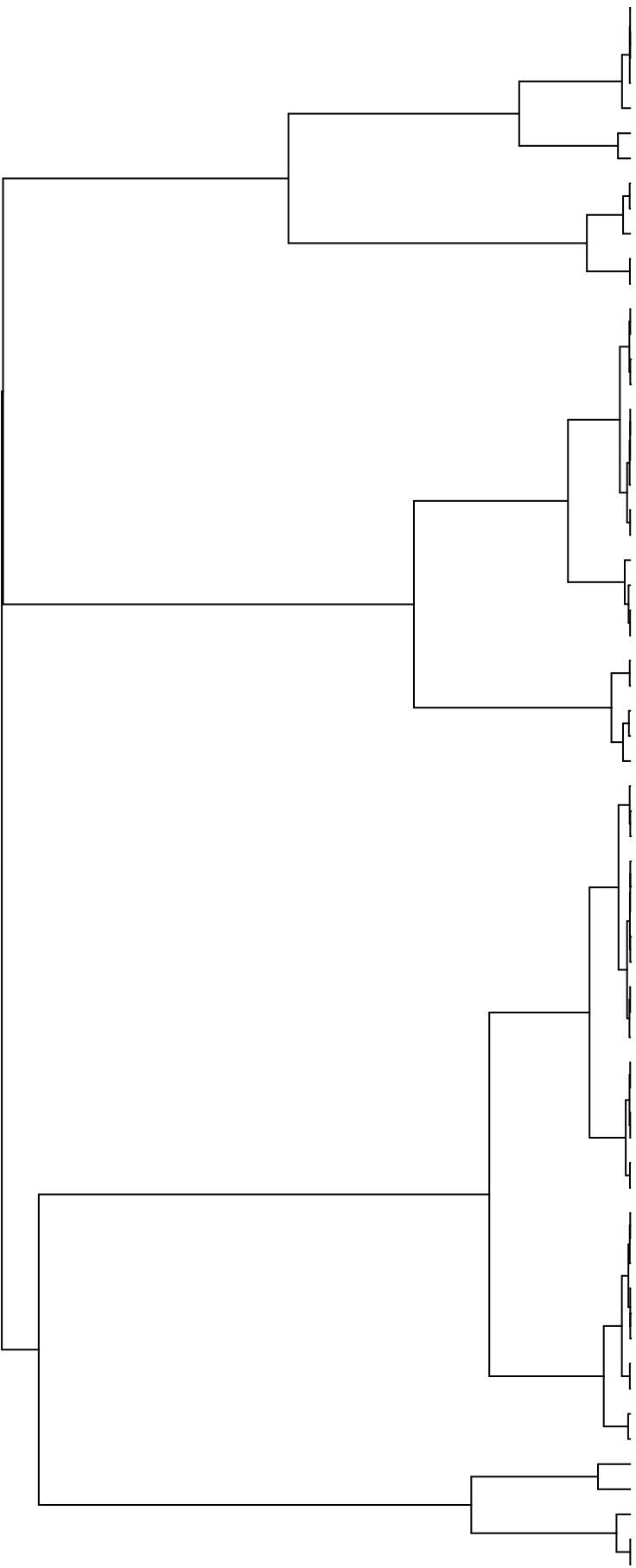
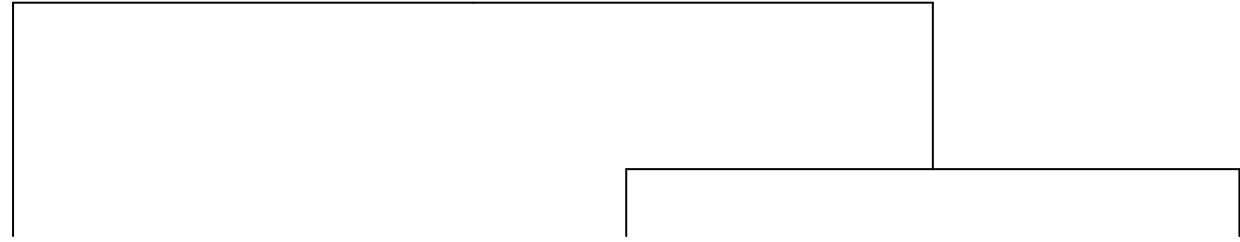
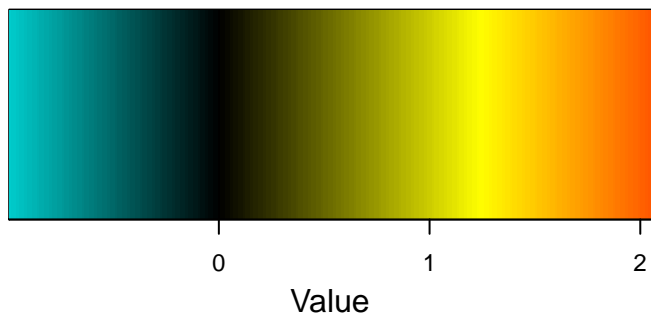
Lane 8) MST2/STK3 sequence ID:S211751

Figure S6 – Pathway analysis of siMST versus siScr

Genomic microarray was performed on liver samples on day 3 following injection of either siMST or siScr. Pathway analysis was performed using the SetRank method.

A Interferon gamma signaling

Appendix Figure S7



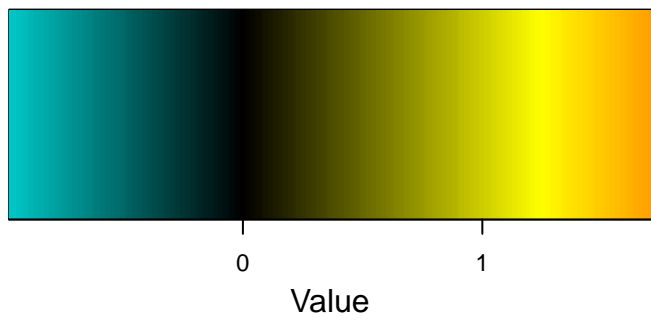
- Gbp2
- Ifi30
- 4933411G06Rik
- Cd44
- Camk2b
- Vcam1
- Prkcd
- Irf8
- Ifngr2
- Ptpn2
- Jak1
- Gm8909
- Socs3
- Irf2
- Socs1
- H2-Eb2
- Ptpn1
- Ncam1
- H2-Q2
- Icam1
- Irf1
- Gm11127
- H2-B1
- Ptpn11
- Ciita
- Oas1d
- Irf3
- Oas1h
- Oas1e
- Camk2g
- H2-Eb1
- Irf7
- H2-Q7
- H2-D1
- Oas2
- Sumo1
- Fcgr1
- H2-Q1
- Pias1
- Irf9
- B2m
- H2-T23
- H2-Q4
- H2-K1
- Oas3
- Ptafr
- Oas1a
- Ifngr1
- Oas1
- Oas1g
- Stat1
- Sp100
- Ptpn6
- Ifng
- Gbp2b
- Oas1b
- Oas1c
- Pml
- H2-Q10
- Camk2a
- H2-Ab1
- Camk2d
- H2-Aa

siMST vs siScr

siScr vs control

siMST vs control

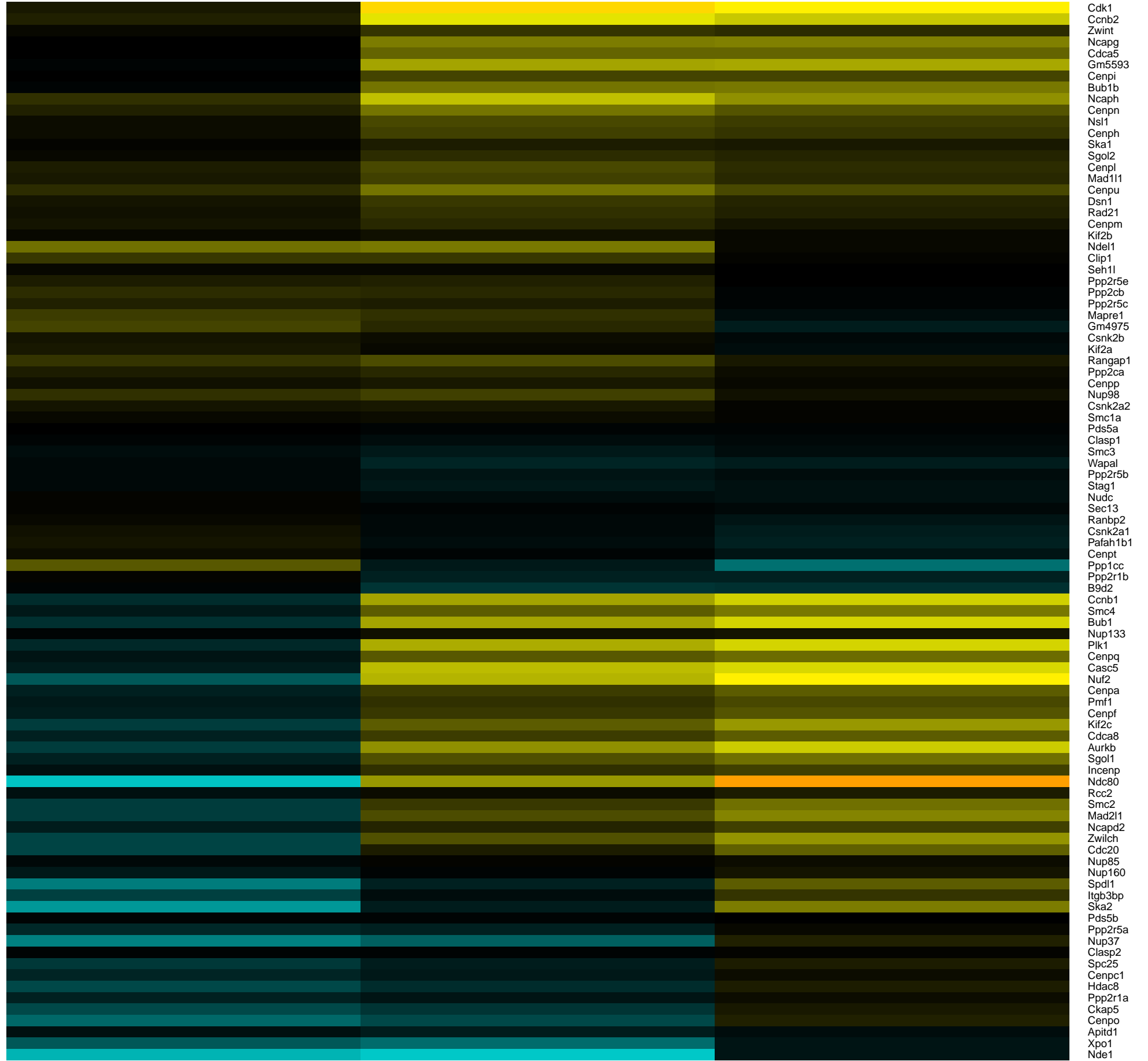
B Mitotic Prometaphase



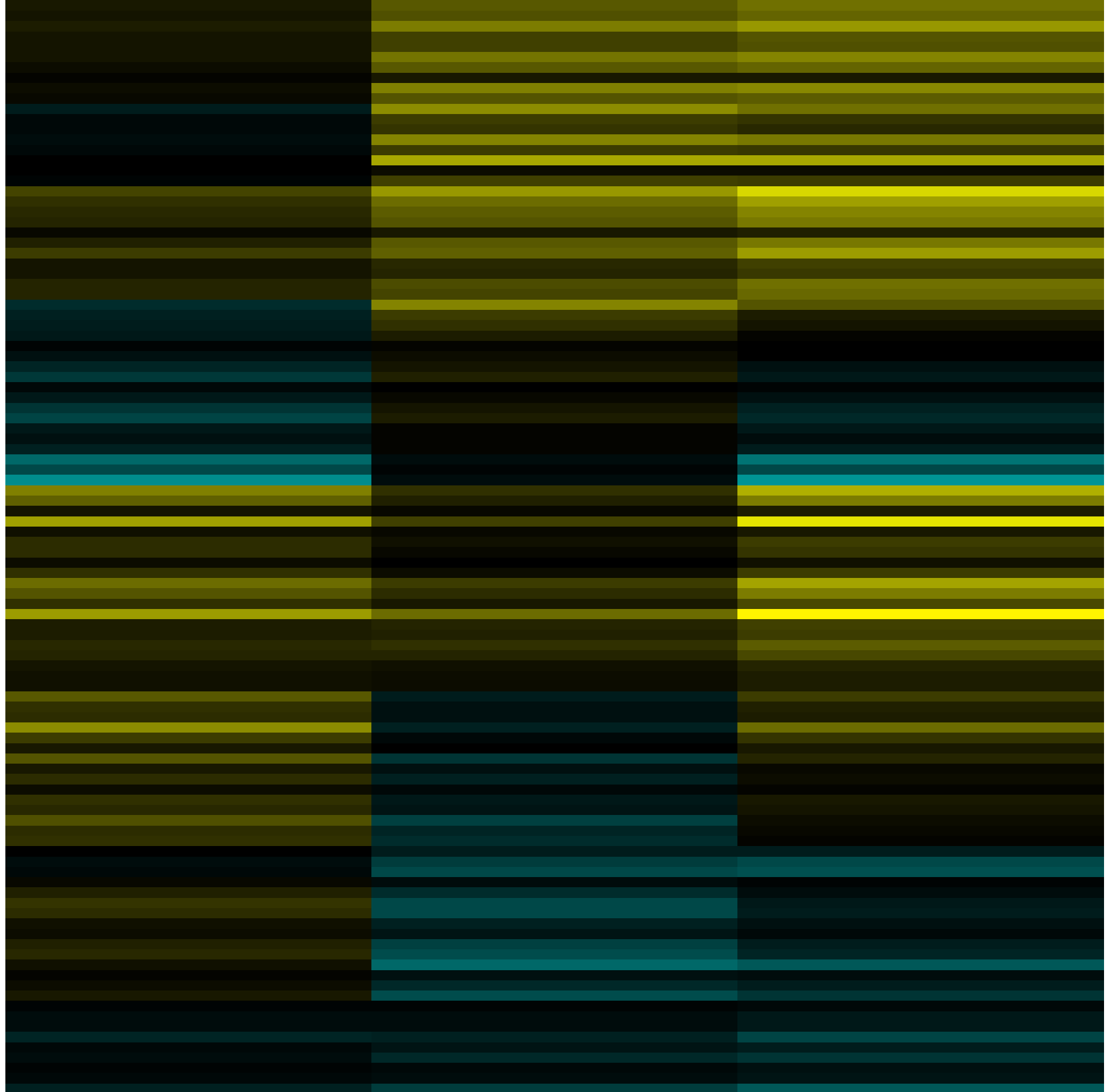
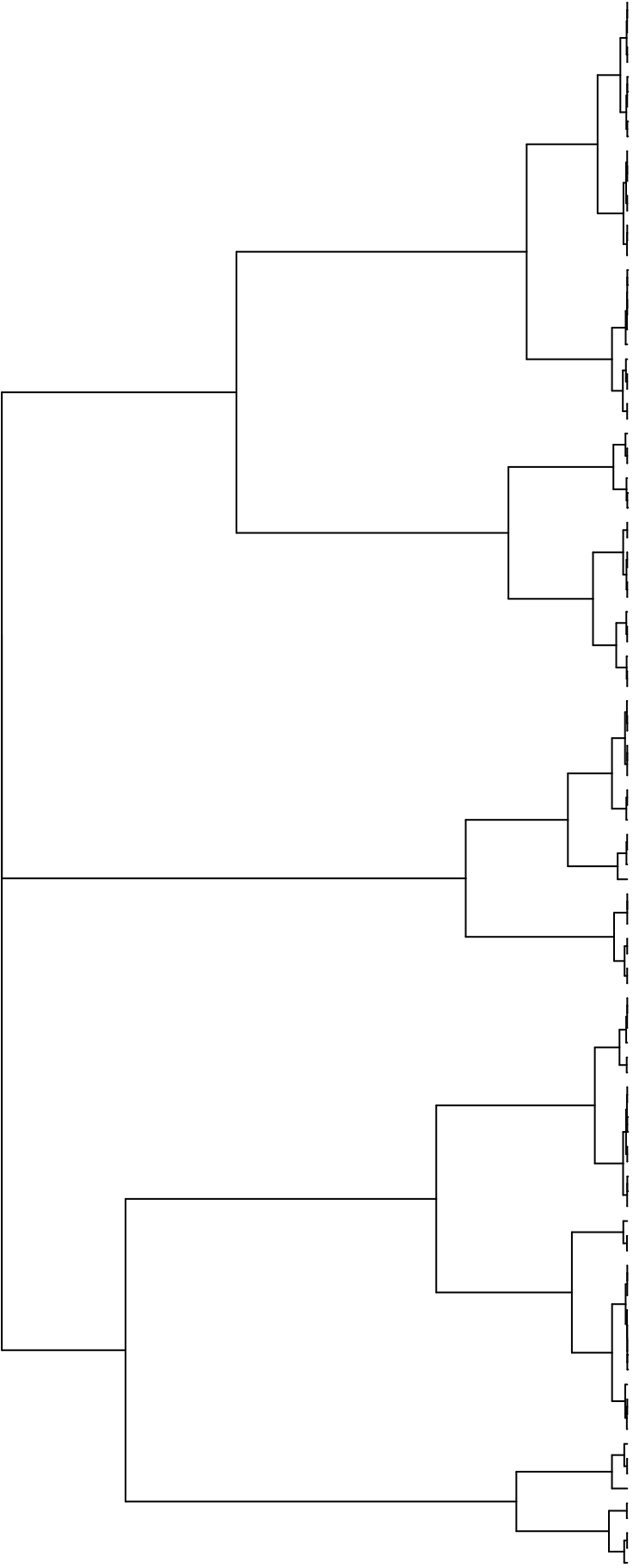
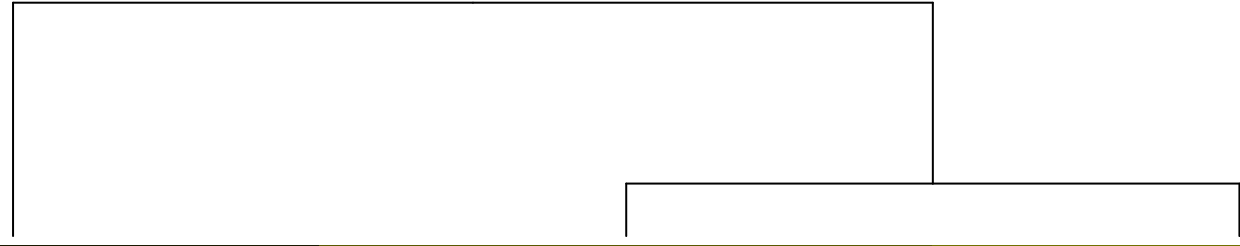
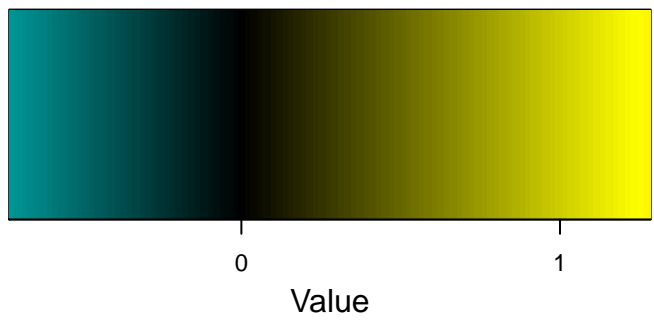
siScr vs control

siMST vs control

siMST vs siScr



C extracellular matrix part



- Mmrn2
- Lamb1
- Loxl2
- Loxl1
- Col1a2
- Thbs2
- Fbln5
- Leprel1
- Sparc
- Ccdc80
- Fbn1
- Lama1
- Ntn1
- Col3a1
- Smoc2
- Col1a1
- Egflam
- Hspg2
- Timp3
- Tnc
- Tgfbj
- Timp2
- Entpd2
- Matn2
- Anxa2
- Lamc1
- Lamb2
- Col12a1
- Nid1
- Lum
- Tinag
- Ntn4
- Amelx
- Hmcn2
- Eln
- Agrn
- Nid2
- Col7a1
- Col18a1
- Col5a3
- Gria3
- Ctdp1
- Frem2
- Frem1
- Lepre1
- Slc1a3
- Lad1
- Timp1
- Col4a2
- Spn
- Mfap4
- Cst3
- Tgfb2
- Ang2
- Rel2
- Smoc1
- Mmp13
- Col4a1
- Runx1
- Entpd1
- Lama4
- Fbln1
- Lama2
- Col5a2
- Col8a2
- Dlg1
- Egfl6
- Col4a5
- Col11a2
- Vwa1
- Adamts1
- Frem3
- Lama5
- Col8a1
- Mfap2
- Col4a4
- Col4a6
- Prss12
- Thbs4
- Lamb3
- Grin1
- Itga6
- Lamc3
- Mfap1a
- Col27a1
- Gsto1
- Papln
- Vwc2
- Rpsa
- Vwa2
- Cask
- Ache
- Mfap5
- Ang
- Fbn2
- Col2a1
- Acan
- Alb
- Smc3
- Amtn
- Col28a1
- Col4a3
- Dag1
- Trf
- Adamts15
- Col15a1

siScr vs control

siMST vs siScr

siMST vs control

Figure S7– Heat maps of genes regulated by siMST compared to siScr

- A Interferon gamma signalling
- B extracellular matrix remodelling
- C mitotic prometaphase

Appendix Figure S8

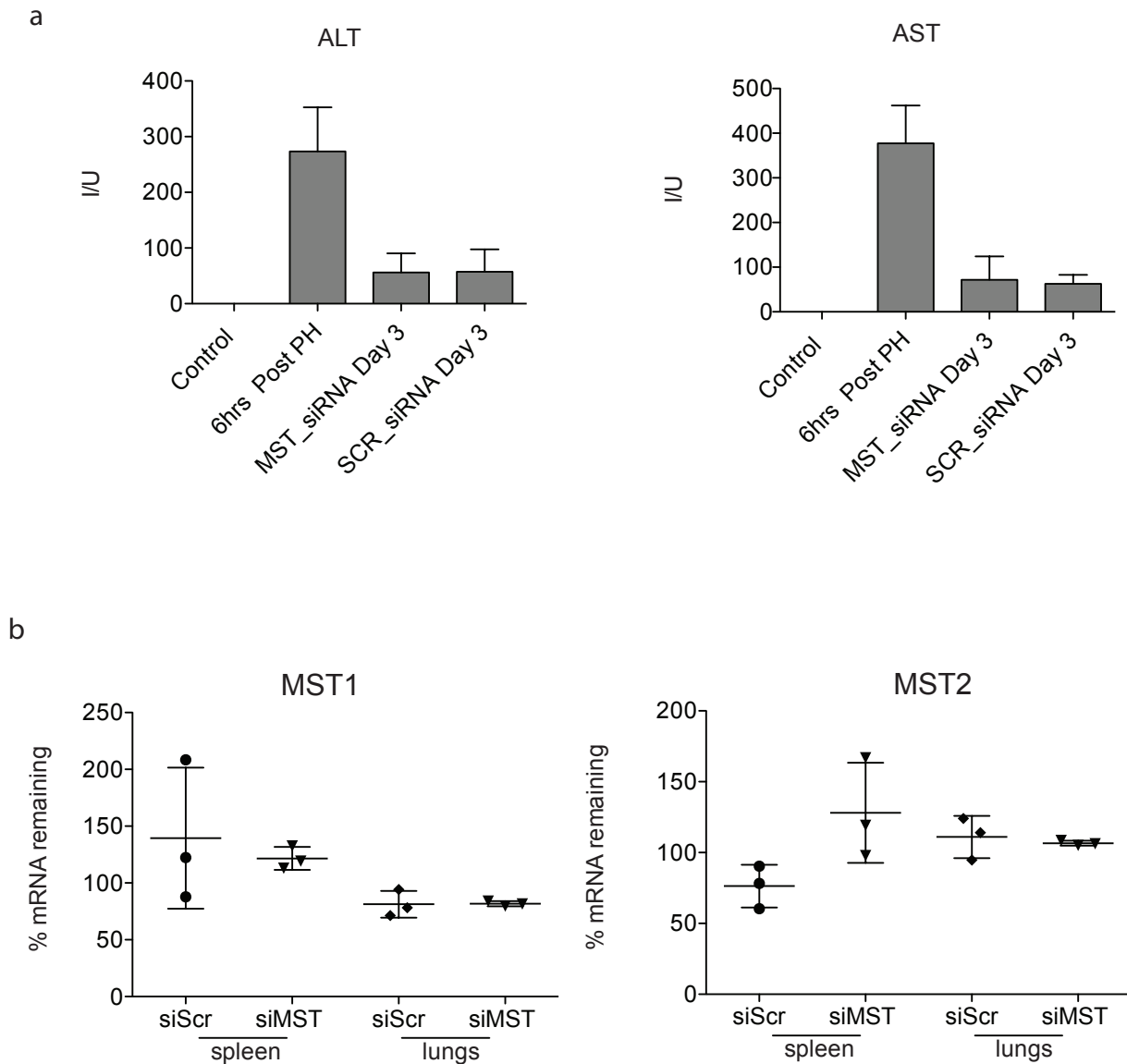


Figure S8 Assessment of liver injury and off target effects from siRNA:liposomes

A Assessment of liver injury by serum levels of AST and ALT 6 h post PH (positive control) and day 3 after injection of liposome coupled siMST or siScr siRNA.

B RT-qPCR of mouse spleen and lung tissue after i.v injection of siScr or siMST liposome complex measured by RT-qPCR. Data are presented as mean \pm SD. Student's t-test was used to calculate p values of siScr to siMST treated animals, with no significant alterations observed.

Appendix Figure S9

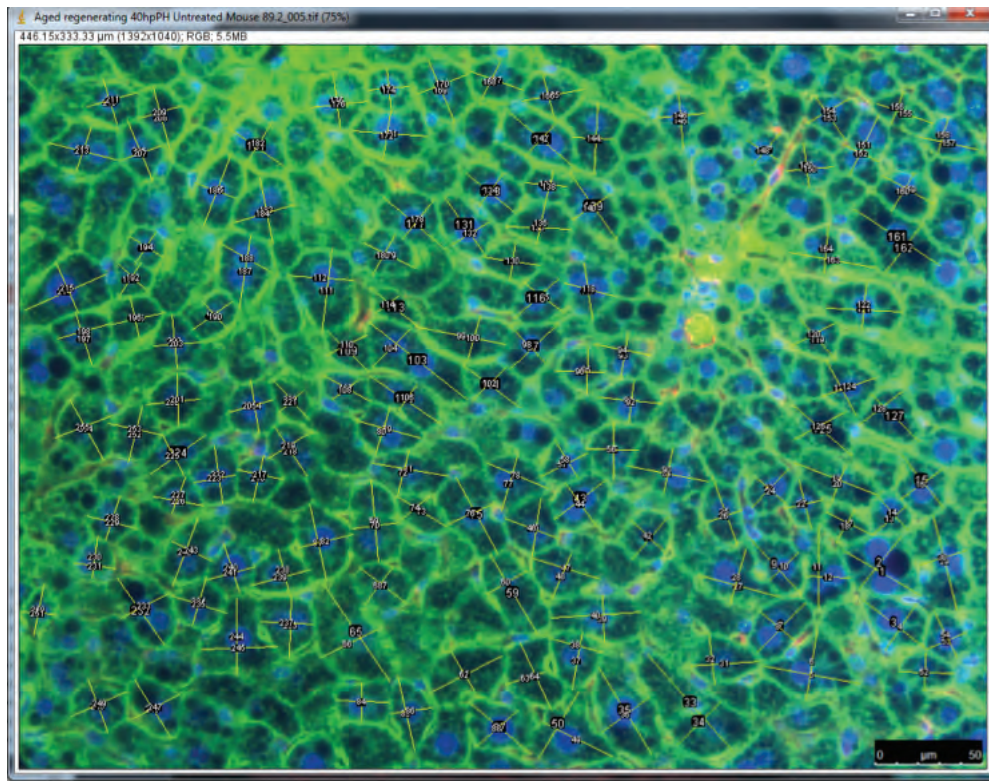


Figure S9 Measurement of hepatocyte dimensions

Representative photomicrograph of hepatocyte size calculation

Appendix Figure S10

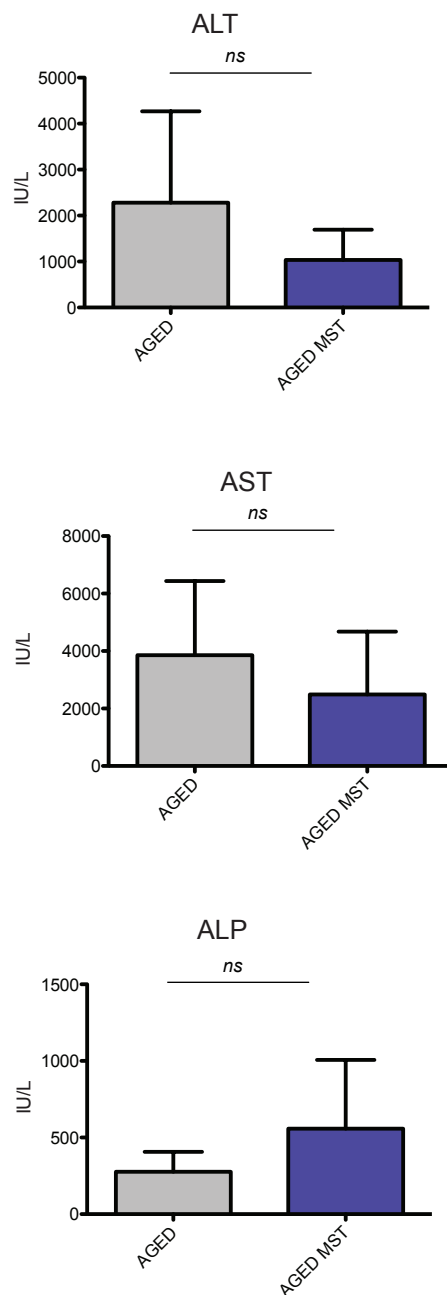


Figure S10 Serum transaminases (ALT and AST) and alkaline phosphatase (ALP) in regenerating aged livers ± MST KD (aged MST)

Data are represented as international units (IU) as measured in mice 40 h post PH in aged and aged + siMST KD mice. Bars are the mean ±SD, n=6 animals per group.