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SUPPLEMENTARY INFORMATION

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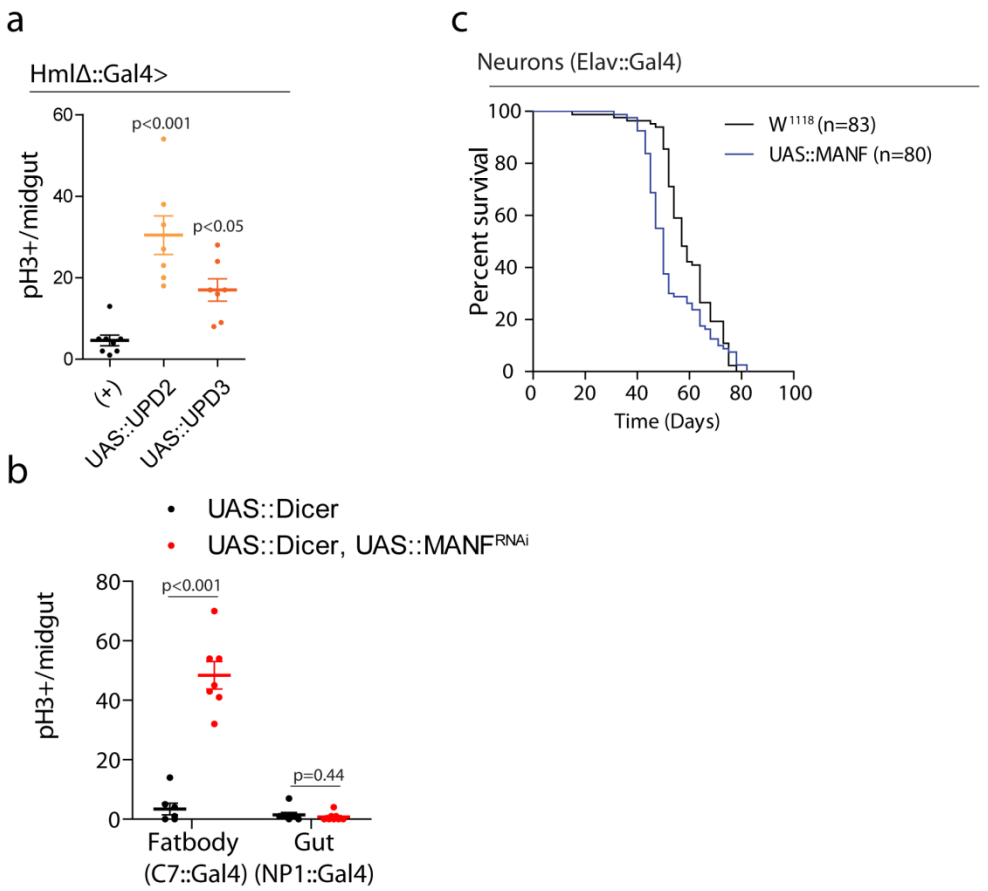
MANF regulates metabolic and immune homeostasis in ageing and protects against liver damage

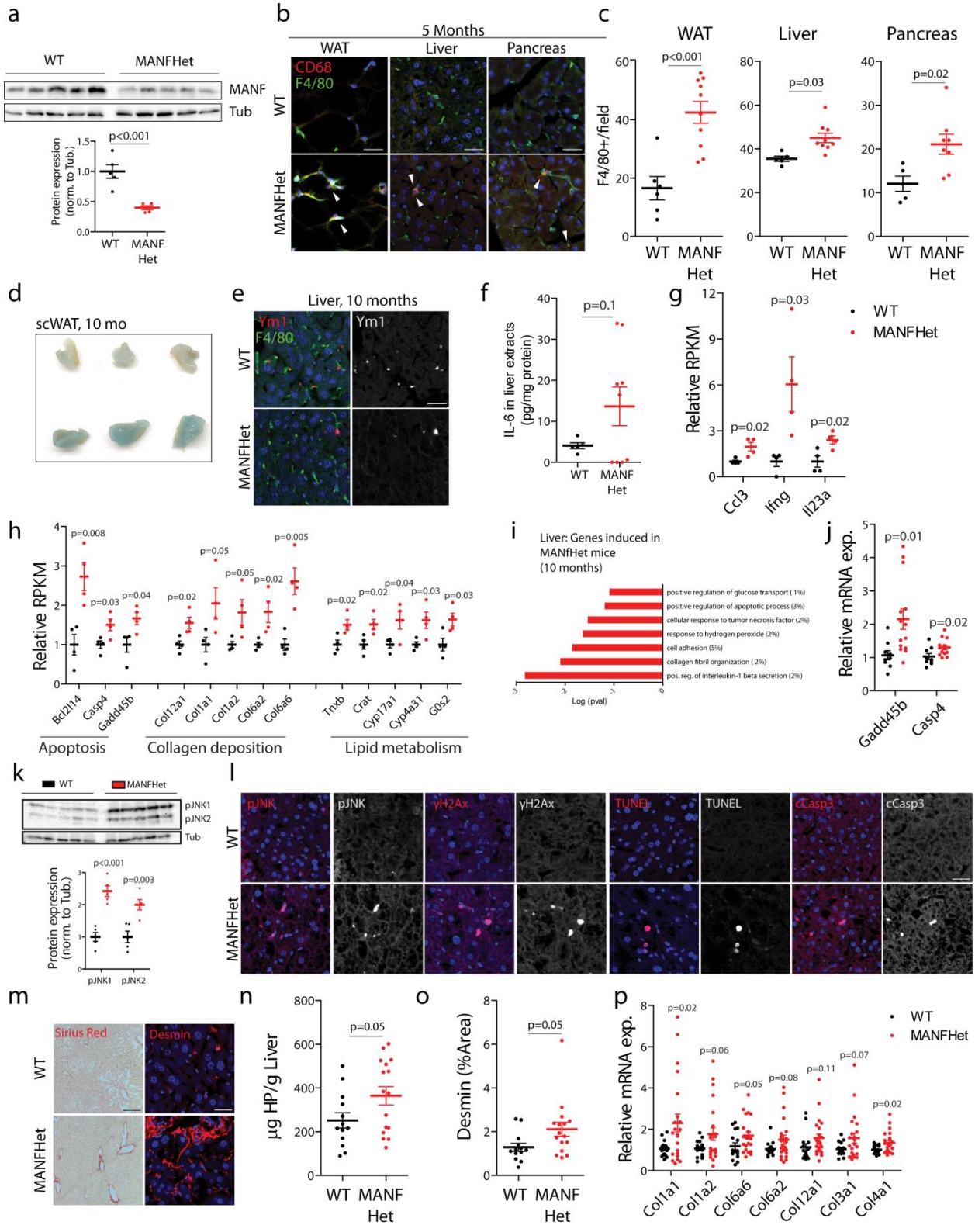
Pedro Sousa-Victor[1,7], Joana Neves[1,7], Wendy Cedron-Craft[1], P. Britten Ventura[2,3], Chen-Yu Liao[1], Rebeccah R. Riley[1], Ilya Soifer[4], Nicholas van Bruggen[4], Ganesh A. Kolumam[4], Saul A. Villeda[2,3], Deepak A. Lamba[1,3,5] and Heinrich Jasper[1,6]*

[1]Paul F. Glenn Center for Biology of Aging Research, Buck Institute for Research on Aging, Novato, CA, USA. [2]Department of Anatomy, University of California, San Francisco, San Francisco, CA, USA. [3]The Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, San Francisco, CA, USA. [4]Calico Life Sciences LLC, South San Francisco, CA, USA. [5]Department of Ophthalmology, University of California, San Francisco, San Francisco, CA, USA. [6]Immunology Discovery, Genentech, South San Francisco, CA, USA. [7]These authors contributed equally: Pedro Sousa-Victor, Joana Neves *e-mail: jasperh@gene.com



Supplementary Figure 1. MANF regulates age-related inflammation and tissue homeostasis in *Drosophila*.

a, Quantification of mitotic figures (pH3+ cells) in midguts of flies overexpressing Upd2 or Upd3 under the control of a hemocyte specific driver (HmlΔ::Gal4, n=7 guts, UAS::UPD2 and UAS::UPD3; n=8 guts, (+)) for 7 days. **b,** Quantification of mitotic figures (pH3+ cells) in midguts of flies expressing MANF-RNAi under the control of fatbody (C7::Gal4, n=7 guts/condition) or gut enterocyte (NP1::Gal4, n=8 guts/condition) specific drivers for 14 days and 7 days, respectively. **c,** Demographics of flies overexpressing MANF in neurons (Elav::Gal4). All flies are female and fly numbers are indicated for each genotype. For a and b data are represented as average ± s.e.m. In a, p values are from one-way ANOVA with Dunnett's multiple comparison post-test. In b, p values are from two-tailed Student’s t-test.

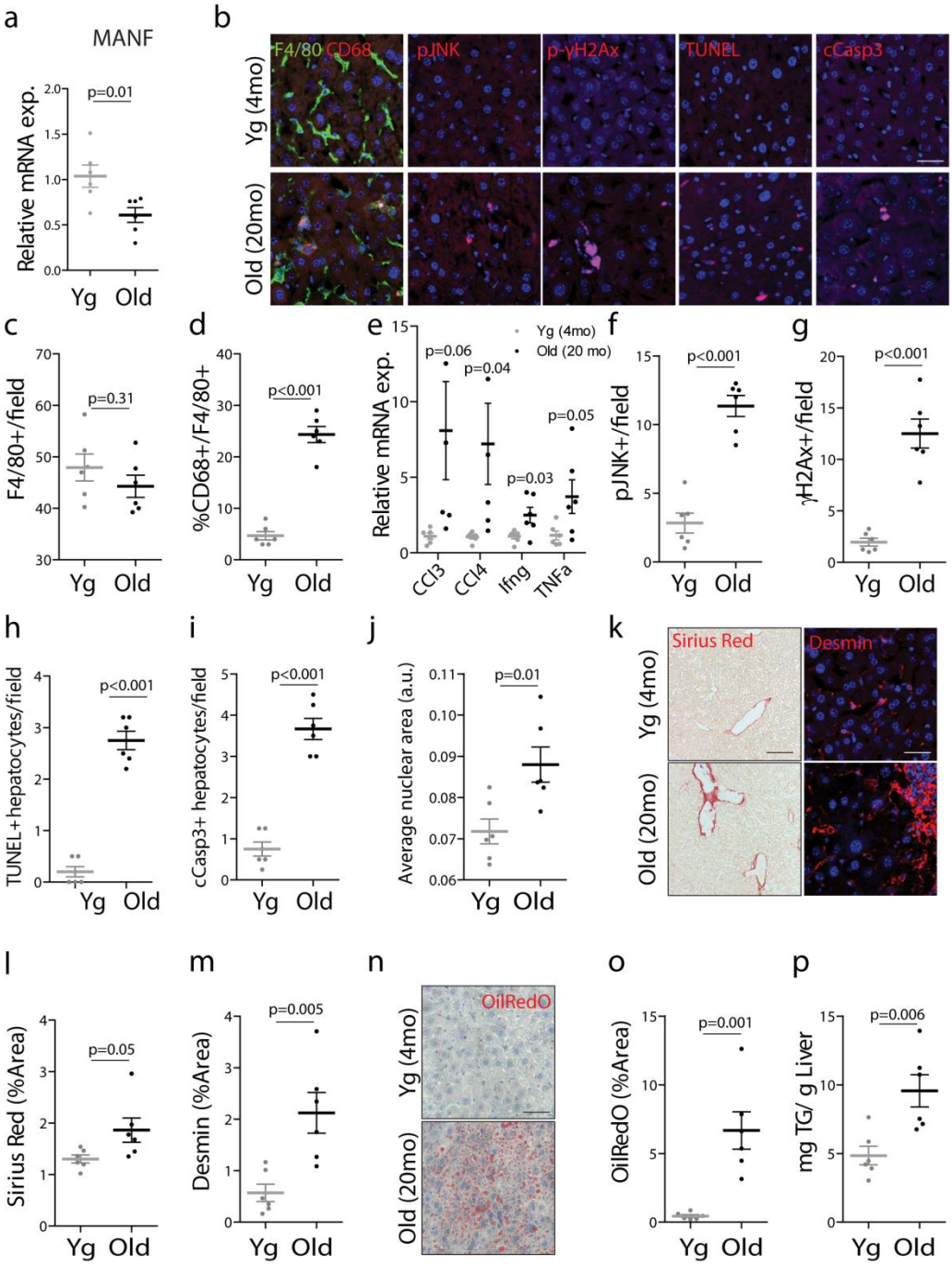

a
WT
MANFHet
MANF
Tub
p<0.001
Protein expression (norm. to Tub.)
WT
MANF Het
b
5 Months
WAT
Liver
Pancreas
CD68
F4/80
WT
MANFHet
c
WAT
Liver
Pancreas
p<0.001
p=0.03
p=0.02
F4/80+/field
WT
MANF Het
d
scWAT, 10 mo
e
Liver, 10 months
Ym1
F4/80
WT
MANFHet
f
p=0.1
IL-6 in liver extracts (pg/mg protein)
WT
MANF Het
g
p=0.03
p=0.02
p=0.02
Relative RPKM
WT
MANFHet
Ccl3
Ifng
Il23a
h
Relative RPKM
p=0.008
p=0.03
p=0.04
p=0.02
p=0.05
p=0.05
p=0.02
p=0.005
p=0.02
p=0.02
p=0.04
p=0.03
p=0.03
Bcl2l14
Casp4
Gadd45b
Col12a1
Col1a1
Col1a2
Col6a2
Col6a6
Tnxb
Crat
Cyp17a1
Cyp4a31
G0s2
Apoptosis
Collagen deposition
Lipid metabolism
i
Liver: Genes induced in MANfHet mice (10 months)
positive regulation of glucose transport (1%)
positive regulation of apoptotic process (3%)
cellular response to tumor necrosis factor (2%)
response to hydrogen peroxide (2%)
cell adhesion (5%)
collagen fibril organization (2%)
pos. reg. of interleukin-1 beta secretion (2%)
Log (pval)
j
Relative mRNA exp.
p=0.01
p=0.02
Gadd45b
Casp4
k
WT
MANFHet
pJNK1
pJNK2
Tub
p<0.001
p=0.003
Protein expression (norm. to Tub.)
pJNK1
pJNK2
l
pJNK
pJNK
γH2Ax
γH2Ax
TUNEL
TUNEL
cCasp3
cCasp3
WT
MANFHet
m
Sirius Red
Desmin
WT
MANFHet
n
p=0.05
µg HP/g Liver
WT
MANF Het
o
p=0.05
Desmin (%Area)
WT
MANF Het
p
Relative mRNA exp.
p=0.02
p=0.06
p=0.05
p=0.08
p=0.11
p=0.07
p=0.02
WT
MANFHet
Col1a1
Col1a2
Col6a6
Col6a2
Col12a1
Col3a1
Col4a1

Supplementary Figure 2. Inflammation and liver damage in MANFHet mice.

a, Western blot analysis of MANF levels in protein extracts from livers of 10 month old MANFHet mice, or WT littermates. Quantification of relative average levels of MANF, normalized to tubulin levels, are represented (n=5/condition). **b,** Representative images of cryosections from white adipose tissue (WAT), liver and pancreas of 5 month old MANFHet mice, or WT littermates, immunostained against F4/80 (green) and CD68 (red). DAPI is used to identify nuclei. Arrowheads indicate double stained cells. Scale bars: WAT, 100µm; liver and pancreas, 50µm. These observations were reproduced in n=4 animals of each genotype. **c,** Quantification of the average number of F4/80+ macrophages in WAT, liver and pancreas of 10 month old MANFHet mice, or WT littermates (vWAT: n=6, WT, n=10, MANFHet; Liver: n=6, WT, n=9, MANFHet; Pancreas: n=5, WT, n=8, MANFHet). **d,** SA-βGal staining in sub-cutaneous WAT of 10 month old MANFHet mice, or WT littermates. These observations were reproduced in two sets of animals, analyzed independently, and containing a total of n=9 WT animals and n=10 MANFHet animals. **e,** Representative images of cryosections from livers of 10 month old MANFHet mice, or WT littermates, immunostained against F4/80 (green) and Ym1 (red). DAPI is used to identify nuclei. Scale bar: 50µm. Quantifications of these stainings, for independent animals, are shown in Fig. 3h. **f,** IL-6 levels in protein samples from livers of 10 month old MANFHet mice, or WT littermates, quantified by ELISA (n=5, WT; n=9, MANFHet). **g-h,** Expression levels of several genes, quantified by RNAseq, in livers of 10 month old MANFHet mice, relative to same age WT littermates (n=4/condition). **i,** GO categories of biological processes showing significant enrichment in the dataset of genes significantly induced in the liver of 10 month old MANFHet mice compared to same age WT littermates (fold induction ≥1.5 and p≤0.05, p values from two-tailed Student's t-test), n=4/condition. Percentage of genes in the whole dataset belonging to each GO category is indicated. **j,** Relative levels of transcripts of the designated genes, quantified by RT-qPCR, in livers of 10 month old MANFHet mice, or WT littermates (n=9, WT; n=14, MANFHet). **k,** Western blot analysis of p-JNK levels in protein extracts from livers of 10 month old MANFHet mice, or WT littermates. Quantification of relative average levels of p-JNK1 and p-JNK2, normalized to tubulin levels, are represented (n=5/condition). **l-m,** Representative images of

cryosections from livers of 10 month old MANFHet mice, or WT littermates, showing the indicated stains. DAPI is used to identify nuclei. Quantifications of these stainings, for independent animals, are shown in Fig. 3i-n and Supplementary Fig. 2o. Scale bars: l and m, Desmin, 50µm; m, Sirius Red, 200 µm. **n,o** Quantification of hydroxyproline content in protein extracts (n) or stellate cell activation, as the percentage of area occupied Desmin stain (o), in livers of 10 month old MANFHet mice, or WT littermates (n=13, WT; n=16, MANFHet). **p,** Relative levels of transcripts of the designated genes, quantified by RT-qPCR, in livers of 10 month old MANFHet mice, or WT littermates (n=16, WT; n=22, MANFHet).

Data are represented as average ± s.e.m. and each n represents one animal. In i, p values are from Fisher Exact test. In all other panels, p values are from two-tailed Student's t-test. Full blots for a and k can be found in Supplementary Figure 12.

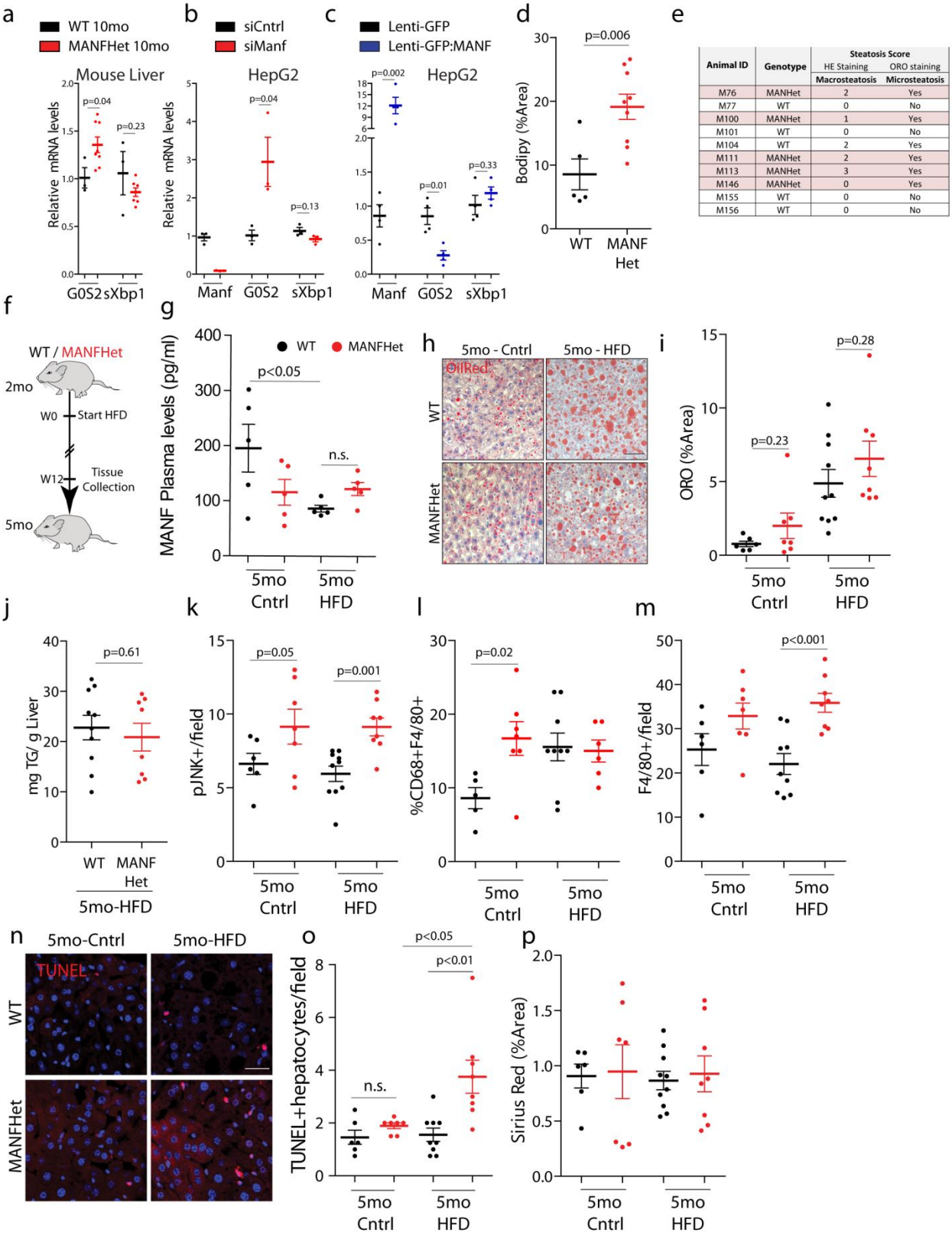


Supplementary Figure 3. Liver aging.

a,e, Relative levels of transcripts of the designated genes, quantified by RT-qPCR, in livers of 4 month old and 20 month old WT mice (n=6/age). **b,k,n,** Representative images of cryosections from livers of 4 month old and 20 month old WT mice, showing the designated stains. DAPI is used to identify nuclei. Scale bars: b, k, Desmin, n,

50µm; k, Sirius Red, 200 µm. Quantifications of these stainings, for independent animals, are shown in Supplementary Fig. 2c,d,f-j,l,m,o. **c,d,f-j,l,m,o,p,** Quantification of the average number of F4/80+ macrophages (c), activated macrophages as the %CD68+ cells within the F4/80+ population (d), p-JNK+ cells (f), γH2Ax+ cells (g), TUNEL+ hepatocytes (h), cleaved caspase3+ hepatocytes (i), average nuclear area (j), collagen deposition, as the percentage of area occupied by Sirius Red stain (l), stellate cell activation, as the percentage of area occupied Desmin stain (m), fat content, as the percentage of area occupied by OilRedO stain (o) and triglyceride content (p), in livers of 4 month old and 20 month old WT mice (n=6/age).

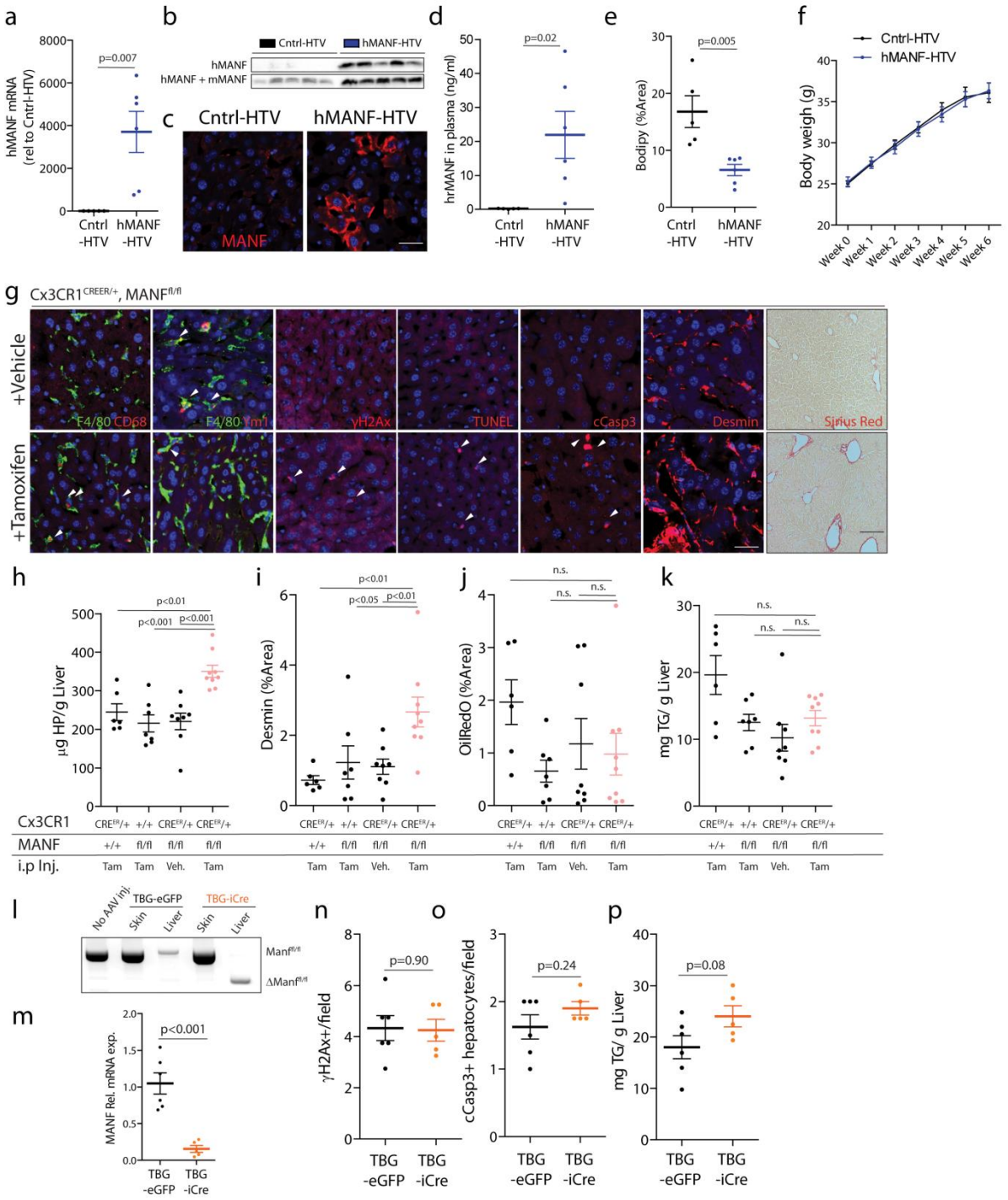
Data are represented as average ± s.e.m. and each n represents one animal. p values are from two-tailed Student's t-test.


a
WT 10mo
MANFHet 10mo
Mouse Liver
Relative mRNA levels
p=0.04
p=0.23
G0S2
sXbp1
b
siCntrl
siManf
HepG2
p=0.04
p=0.13
Manf
G0S2
sXbp1
c
Lenti-GFP
Lenti-GFP:MANF
HepG2
p=0.002
p=0.01
p=0.33
Manf
G0S2
sXbp1
d
p=0.006
Bodipy (%Area)
WT
MANF Het
e
Animal ID
Genotype
Steatosis Score
HE Staining
ORO staining
Macrosteatosis
Microsteatosis
M76 MANHet 2 Yes
M77 WT 0 No
M100 MANHet 1 Yes
M101 WT 0 No
M104 WT 2 Yes
M111 MANHet 2 Yes
M113 MANHet 3 Yes
M146 MANHet 0 Yes
M155 WT 0 No
M156 WT 0 No
f
WT / MANFHet
2mo
W0
Start HFD
W12
Tissue Collection
5mo
g
WT
MANFHet
MANF Plasma levels (pg/ml)
p<0.05
n.s.
5mo Cntrl
5mo HFD
h
5mo - Cntrl
5mo - HFD
OilRed
WT
MANFHet
i
ORO (%Area)
p=0.23
p=0.28
5mo Cntrl
5mo HFD
j
mg TG/ g Liver
p=0.61
WT
MANF Het
5mo-HFD
k
pJNK+/field
p=0.05
p=0.001
5mo Cntrl
5mo HFD
l
%CD68+F4/80+
p=0.02
5mo Cntrl
5mo HFD
m
F4/80+/field
p<0.001
5mo Cntrl
5mo HFD
n
5mo-Cntrl
5mo-HFD
TUNEL
WT
MANFHet
o
TUNEL+hepatocytes/field
p<0.05
p<0.01
n.s.
5mo Cntrl
5mo HFD
p
Sirius Red (%Area)
5mo Cntrl
5mo HFD

Supplementary Figure 4. Hepatosteatosis in MANFHet mice.

a-c, Relative levels of transcripts of the designated genes, quantified by RT-qPCR, in livers of 10 month old MANFHet mice, or WT littermates (a, n=3, WT; n=8, MANFHet); in HepG2 cells 48h after treatment with a siRNA targeting MANF (b, n=3/condition); and in HepG2 cells 48h after infection with a lentivirus expressing hMANF (c, n=4/condition). **d,** Fat content in livers of 10 month old MANFHet mice, or WT littermates, quantified as the percentage of area occupied by Bodipy stain (n=5, WT; n=9, MANFHet). **e,** Summary of hepatosteatosis score of 10 month old MANFHet mice and WT littermates evaluated by a liver pathologist. **f,** Experimental timeline for analysis of animals fed HFD for 12 weeks. **g,** MANF protein levels in plasma of 5 month old MANFHet mice, or WT littermates, fed HFD for 12 weeks (n=5/condition), quantified by ELISA. **h,n** Representative images of cryosections from livers of 5 month old MANFHet mice, or WT littermates, fed HFD for 12 weeks, stained with OilRedO (h) or TUNEL (n). In n, DAPI is used to identify nuclei. Scale bars: 50µm. Quantifications of these stainings, for independent animals, are shown in Supplementary Fig.3i,o. **i,k-m,o,p,** Quantification of fat content, as the percentage of area occupied by OilRedO stain (i), the average number of p-JNK+ cells (k), activated macrophages as the %CD68+ cells within the F4/80+ population (l), F4/80+ macrophages (m), TUNEL+ hepatocytes (o) and collagen deposition, as the percentage of area occupied by Sirius Red stain (p), in livers of 5 month old MANFHet mice, or WT littermates, fed HFD for 12 weeks (CD: n=6, WT, n=7, MANFHet; HFD: n=10, WT; n=8, MANFHet). **j,** Quantification of triglyceride content in livers of 5 month old MANFHet mice, or WT littermates, fed HFD for 12 weeks (CD: n=6, WT, n=7, MANFHet; HFD: n=10, WT, n=8, MANFHet).

Data are represented as average ± s.e.m. and each n represents one animal. In a-d, i-m, p values are from two-tailed Student’s t-test. In g and o, p values are from one-way ANOVA with Bonferroni's multiple comparison post-test.

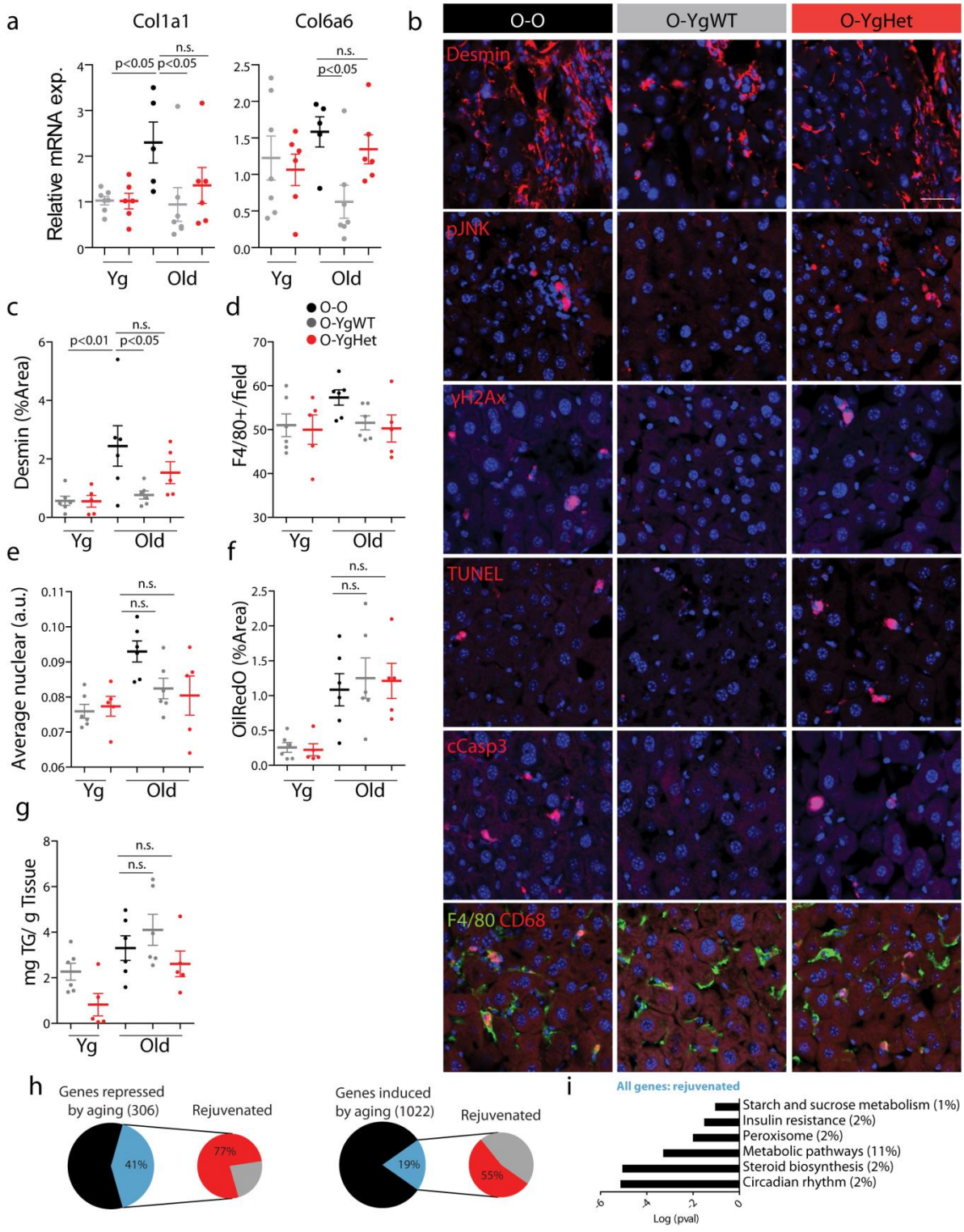


Supplementary Figure 5. Manipulation of MANF in immune cells and hepatocytes

a-c, hMANF mRNA detected by RT-qPCR (a, n=5, Cntrl-HTV; n=6, hMANF-HTV) or protein, detected by Western blot in protein extracts (b, n=5, Cntrl-HTV; n=5, hMANF-HTV) or by immunohistochemistry in cryosections (c, representative images of observations repeated in n=5, Cntrl-HTV and n=6, hMANF-HTV, Scale bar: 50µm) from

livers of 4 month old WT mice fed a HFD for 6 weeks after receiving hMANF injections. Top Western blot in b uses an antibody recognizing only MANF of human origin. Bottom Western blot in b uses an antibody recognizing MANF of human and mouse origin. **d,** hMANF protein, detected by ELISA, in plasma samples of 3 month old WT mice, 2 weeks after receiving hMANF HTV injections (n=5, Cntrl-HTV; n=6, hMANF-HTV). **e,** Fat content in livers of 4 month old WT mice fed a HFD for 6 weeks after receiving hMANF HTV injections, quantified as the percentage of area occupied by Bodipy stain (n=5, Cntrl-HTV; n=6, hMANF-HTV). **f,** Body weight of mice recorded every week after receiving hMANF HTV injections and starting feeding HFD (n=5, Cntrl-HTV; n=6, hMANF-HTV). **g,** Representative images of cryosections from livers of $Cx3cr1^{CRE-ER/+}$,$MANF^{fl/fl}$ mice, 12 weeks after tamoxifen treatment, showing the designated stains. DAPI is used to identify nuclei. Scale bars: Sirius Red, 200 µm; all others, 50µm. Quantifications of these stainings, for independent animals, are shown in Fig. 4g,h,j-m and Supplementary Fig. 5i. **h-k,** Quantification of hydroxyproline content (h), stellate cell activation, as the percentage of area occupied Desmin stain (i), fat content, as the percentage of area occupied by OilRedO stain (j) and triglyceride content (k) in livers of $Cx3cr1^{CRE-ER/+}$,$MANF^{fl/fl}$ mice, 12 weeks after tamoxifen treatment (n=6, Cre^{ER} +Tam; n=7 $Manf^{fl/fl}$ +Tam; n=8, Cre^{ER}/$Manf^{fl/fl}$ +Veh.; n=9, Cre^{ER}/$Manf^{fl/fl}$ +Tam). **l,** PCR gel of genomic DNA extracted from the indicated tissues of $MANF^{fl/fl}$ mice, 6 weeks after injection with AAV-TBGi-Cre, showing amplification of the MANF locus. Bottom band corresponds to the locus after excision of the genomic fragment flanked by LoxP sites. These are representative images of observations repeated in n=6, TBG-eGFP and n=5, TBG-iCre mice. **m,** Relative levels of Manf transcripts, quantified by RT-qPCR, in livers of $MANF^{fl/fl}$ mice, 6 weeks after injection with AAV-TBGi-Cre (n=6, TBG-eGFP; n=5, TBG-iCre). **n-p,** Quantification of the average number of γH2Ax+ cells (n), cleaved caspase3+ hepatocytes (o) and triglyceride content (p), in livers of $MANF^{fl/fl}$ mice, 6 weeks after injection with AAV-TBGi-Cre (n=6, TBG-eGFP; n=5, TBG-iCre).

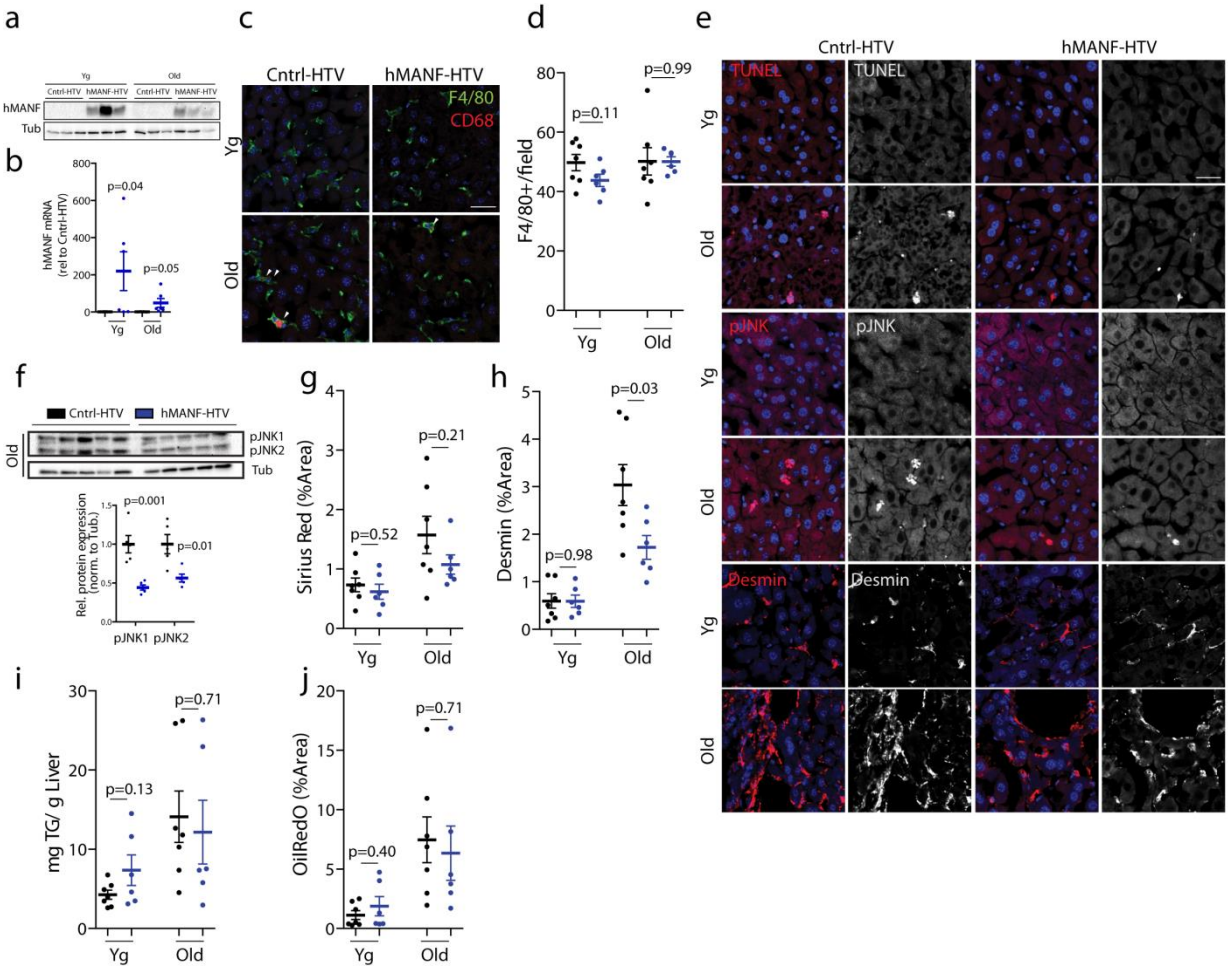
Data are represented as average ± s.e.m. and each n represents one animal. In h-k, p values are from one-way ANOVA with Dunnett's multiple comparison post-test. In a,d,e,m-p, p values are from two-tailed Student's t-test. Full blots for b can be found in Supplementary Figure 13.


a
Col1a1
Col6a6
Relative mRNA exp.
p<0.05
p<0.05
n.s.
p<0.05
n.s.
Yg
Old
b
O-O
O-YgWT
O-YgHet
Desmin
pJNK
γH2Ax
TUNEL
cCasp3
F4/80 CD68
c
Desmin (%Area)
p<0.01
p<0.05
n.s.
d
F4/80+/field
O-O
O-YgWT
O-YgHet
e
Average nuclear (a.u.)
f
OilRedO (%Area)
g
mg TG/ g Tissue
h
Genes repressed by aging (306)
Rejuvenated
41%
77%
Genes induced by aging (1022)
19%
55%
i
All genes: rejuvenated
Starch and sucrose metabolism (1%)
Insulin resistance (2%)
Peroxisome (2%)
Metabolic pathways (11%)
Steroid biosynthesis (2%)
Circadian rhythm (2%)
Log (pval)

Supplementary Figure 6. MANF is required for full liver rejuvenation by heterochronic parabiosis.

a, Relative levels of Col1a1 and Col6a6 transcripts, quantified by RT-qPCR, in livers of young and old animals from each parabiosis pair type (n=5, O-O; n=6, O-Yg Het; n=7, O-Yg WT). **b,** Representative images of cryosections from livers of old animals from each parabiosis pair type, showing the designated stains. DAPI is used to identify nuclei. Scale bar: 50µm. Quantifications of these stainings, for independent animals, are shown in Fig. 5d-h and Supplementary Fig. 6c,d. **c-g,** Quantification of stellate cell activation, as the percentage of area occupied Desmin stain (c) the average number of F4/80+ macrophages (d), average nuclear area (e), fat content, as the percentage of area occupied by OilRedO stain (f) and triglyceride content (g) in livers of young and old animals from each parabiosis pair type (n=5, O-Yg Het; n=6, O-O and O-Yg WT). **h,** Pie chart representing the percentage of genes repressed (left, fold induction O-O/YgWT ≤0.75 and p≤0.05, p values from two-tailed Student's t-test) or induced (right, fold induction O-O/ygWT≥1.5 and p≤0.05, p values from two-tailed Student's t-test) by aging in the liver (left pie charts) rejuvenated by heterochronic parabiosis (blue on left pie charts and whole pie charts on right, p≤0.05 for O-O vs. O-YgWT, p values from two-tailed Student's t-test) in a MANF-dependent manner (red on right pie charts, p>0.05 for O-O vs. O-YgHet, p values from two-tailed Student's t-test), n=5/condition. **i,** Graph showing significantly enriched KEGG pathways in the dataset of liver genes affected by aging and rejuvenated by heterochronic parabiosis, n=5/condition. Percentage of genes in the dataset belonging to each KEGG pathway is indicated.

Data are represented as average ± s.e.m. and each n represents one animal. In a,c-g, p values are from one-way ANOVA with Dunnett's multiple comparison post-test. In i, p values are from Fisher Exact test.

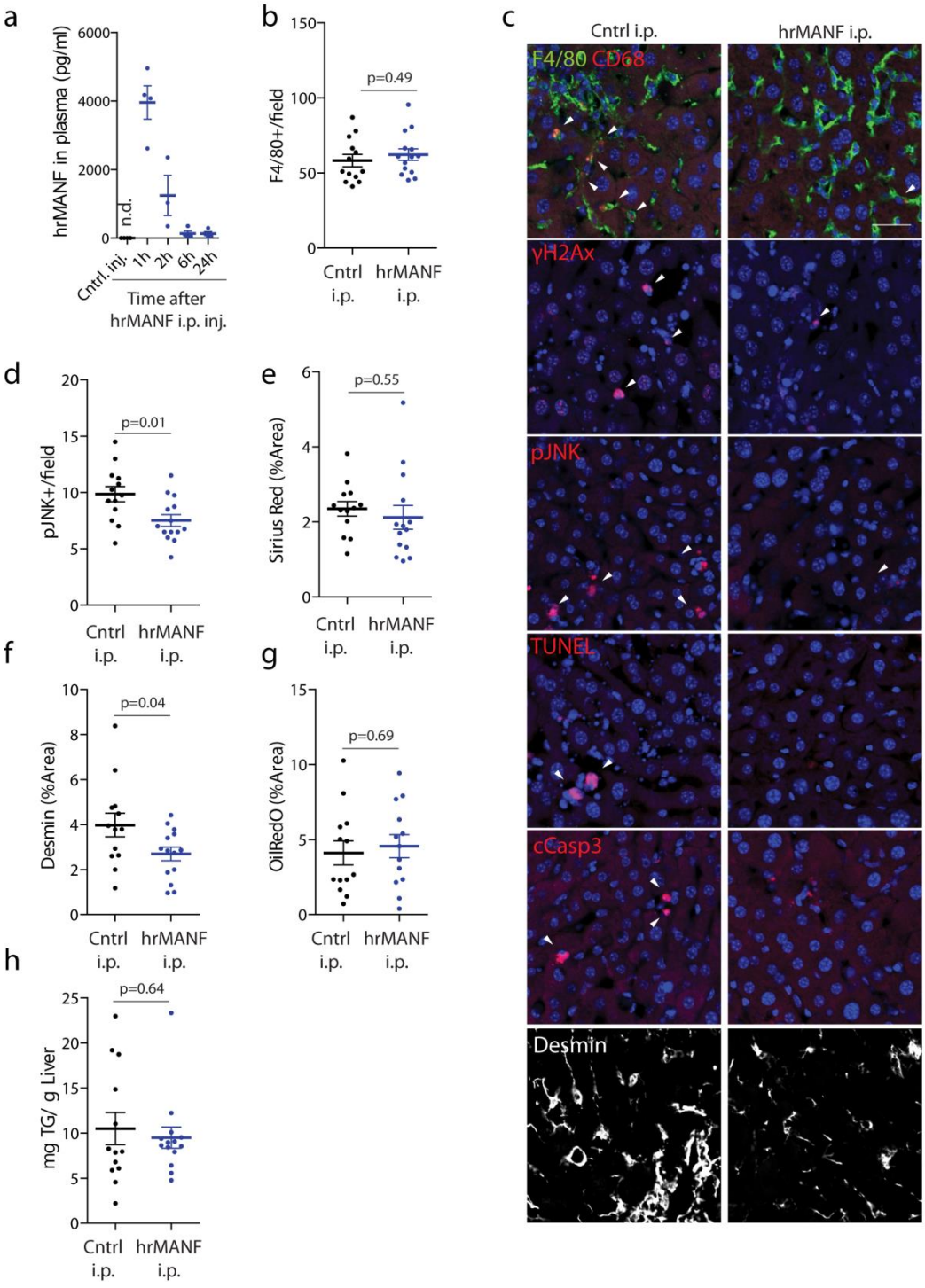


Supplementary Figure 7. MANF HTV injection in old mice.

a-b, hMANF protein detected by Western blot (a, n=3/condition), and mRNA detected by RT-qPCR (b, n=7, Cntrl-HTV, n=6, hMANF-HTV) in livers of young and old WT mice, 5 weeks after receiving hMANF HTV injections. **c,e,** Representative images of cryosections from livers of young and old WT mice, 5 weeks after receiving hMANF HTV injections, showing the designated stains. DAPI is used to identify nuclei. Scale bars: 50µm. Quantifications of these stainings, for independent animals, are shown in Fig. 6b,e,f and Supplementary Fig. 7d,h. **d, g-j,** Quantification of the average number of F4/80+ macrophages (d), collagen deposition, as the percentage of area occupied by Sirius Red stain (g), stellate cell activation, as the percentage of area occupied Desmin stain (h), triglyceride content (i) and fat content, as the percentage of area occupied by OilRedO stain (j) in livers of young and old WT mice, 5 weeks after receiving hMANF

HTV injections (n=7, Cntrl-HTV, n=6, hMANF-HTV). **f,** Western blot analysis of p-JNK levels in protein extracts from livers of old WT mice, 5 weeks after receiving hMANF HTV injections. Quantification of relative average levels of p-JNK1 and p-JNK2, normalized to tubulin levels, are represented (n=5/condition).

Data are represented as average ± s.e.m. and each n represents one animal. p values are from two-tailed Student's t-test. Full blots for a and f can be found in Supplementary Figure 13.

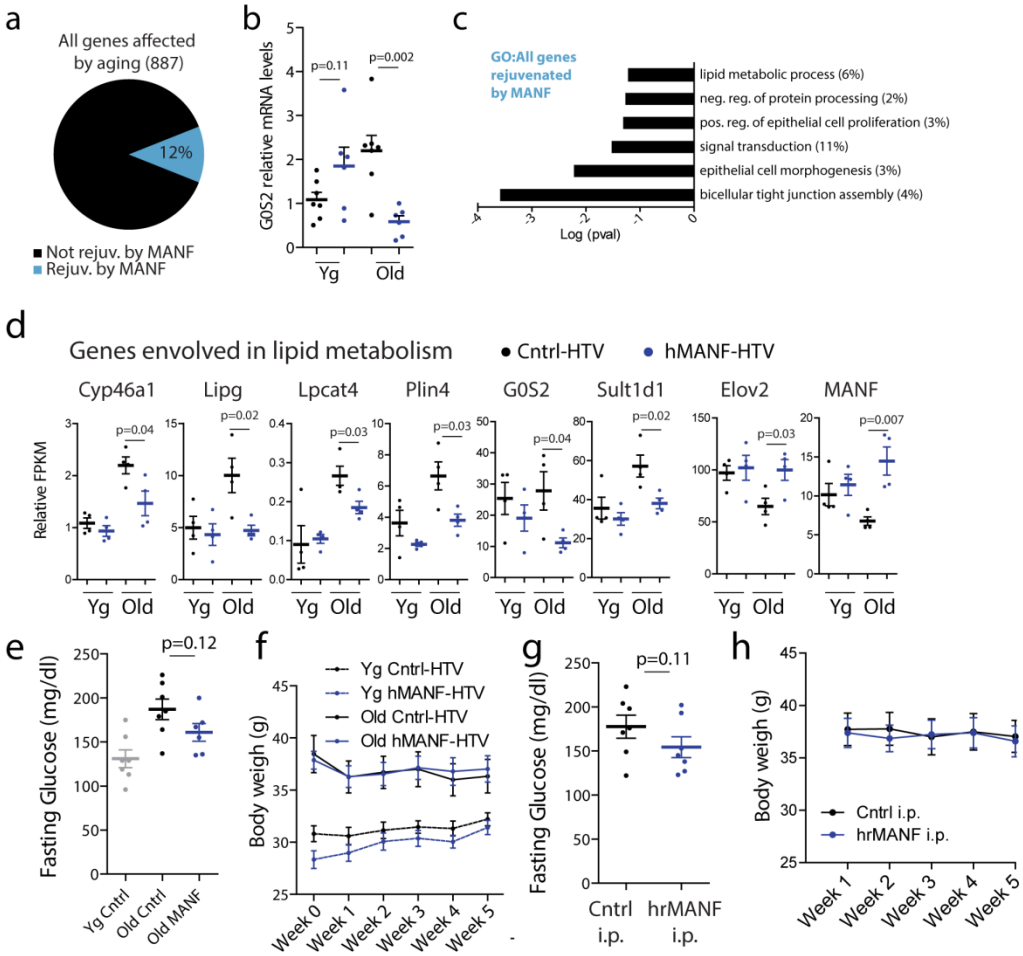


Supplementary Figure 8. MANF protein injection in old mice.

a hMANF protein, quantified by ELISA, in plasma samples of old WT mice at the designated time points after i.p. injection of hrMANF protein (n=3, 2h; n=4, all other time

time points). **b, d-h,** Quantification of the average number of F4/80+ macrophages (b), p-JNK+ cells (d), collagen deposition, as the percentage of area occupied by Sirius Red stain (e), stellate cell activation, as the percentage of area occupied Desmin stain (f), fat content, as the percentage of area occupied by OilRedO stain (g) and triglyceride content (h) in livers of old WT mice, 5 weeks after receiving hrMANF i.p. injections (n=13, Cntrl. i.p.; n=14, hrMANF i.p.). **c,** Representative images of cryosections from livers of old WT mice, 5 weeks after receiving hrMANF i.p. injections, showing the designated stains. DAPI is used to identify nuclei. Scale bar: 50µm. Quantifications of these stainings, for independent animals, are shown in Fig. 6h,j-l and Supplementary Fig. 8b,d,f.

Data are represented as average ± s.e.m. and each n represents one animal. p values are from two-tailed Student's t-test.

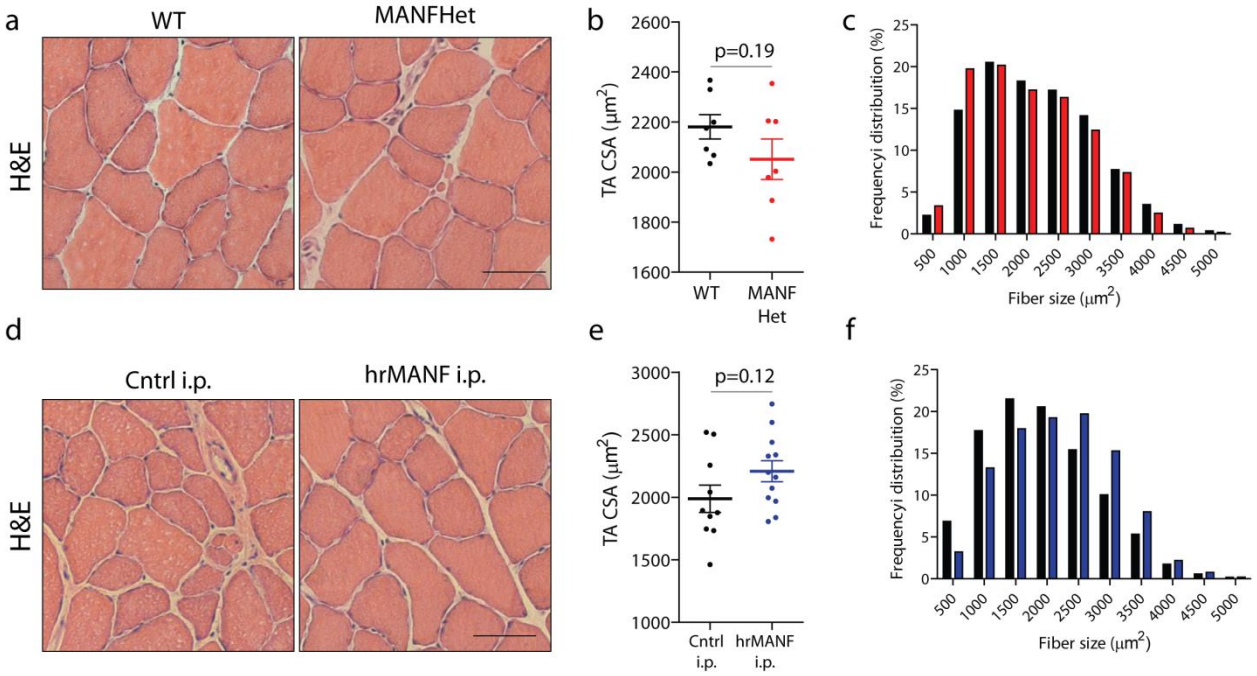


Supplementary Figure 9. Metabolic effects of MANF overexpression in old mice.

a, Pie chart representing the percentage of genes affected by aging (fold induction OldHTVCntrl/YgHTVCntrl ≥1.5 or ≤0.75 and p≤0.05, p values from two-tailed Student's t-test), which were rejuvenated by MANF overexpression (OldHTVMANF vs OldHTVCntrl reversed to young levels with p≤0.05, p values from two-tailed Student's t-test), n=4/condition. **b,** Relative levels of G0S2 transcripts, quantified by RT-qPCR, in livers of young and old WT mice, 5 weeks after receiving hMANF HTV injections (n=7, Cntrl-HTV, n=6, hMANF-HTV). **c,** GO categories of biological processes showing significant enrichment in the dataset of liver genes rejuvenated by MANF overexpression, n=4/condition. Percentage of genes in the dataset belonging to each GO category is indicated. **d,** RPKM of different genes associated with lipid metabolism, in livers of young and old WT mice, 5 weeks after receiving hMANF HTV injections

(n=4/condition). **e,g** Glucose levels in the blood of mice fasted for 4-6h, 4 weeks after receiving hMANF HTV injections (e, n=7, Yg Cntrl and Old Cntrl; n=6 Old hMANF), or 5 weeks after receiving hrMANF i.p. injections (g, n=7/condition). **f,h** Body weight of young and old mice recorded every week after receiving hMANF HTV injections (f, n=7, Cntrl-HTV, n=6, hMANF-HTV), or after receiving hrMANF i.p. injections (h, n=13, Cntrl. i.p.; n=14, hrMANF i.p).

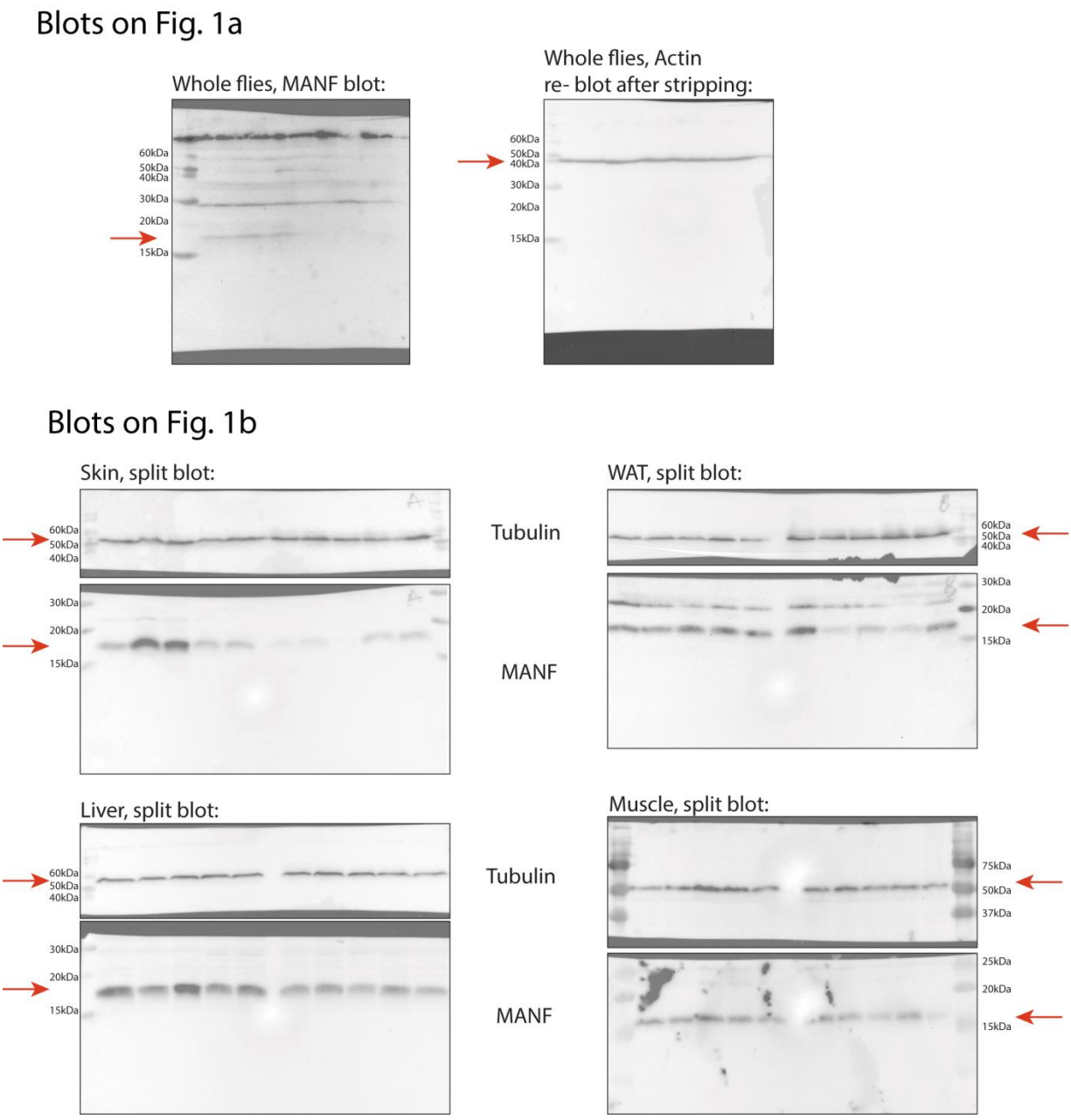
Data are represented as average ± s.e.m. and each n represents one animal. In b,d,e,g, p values are from two-tailed Student's t-test. In c, p values are from Fisher Exact test.



Supplementary Figure 10. The effects of MANF on skeletal muscle.

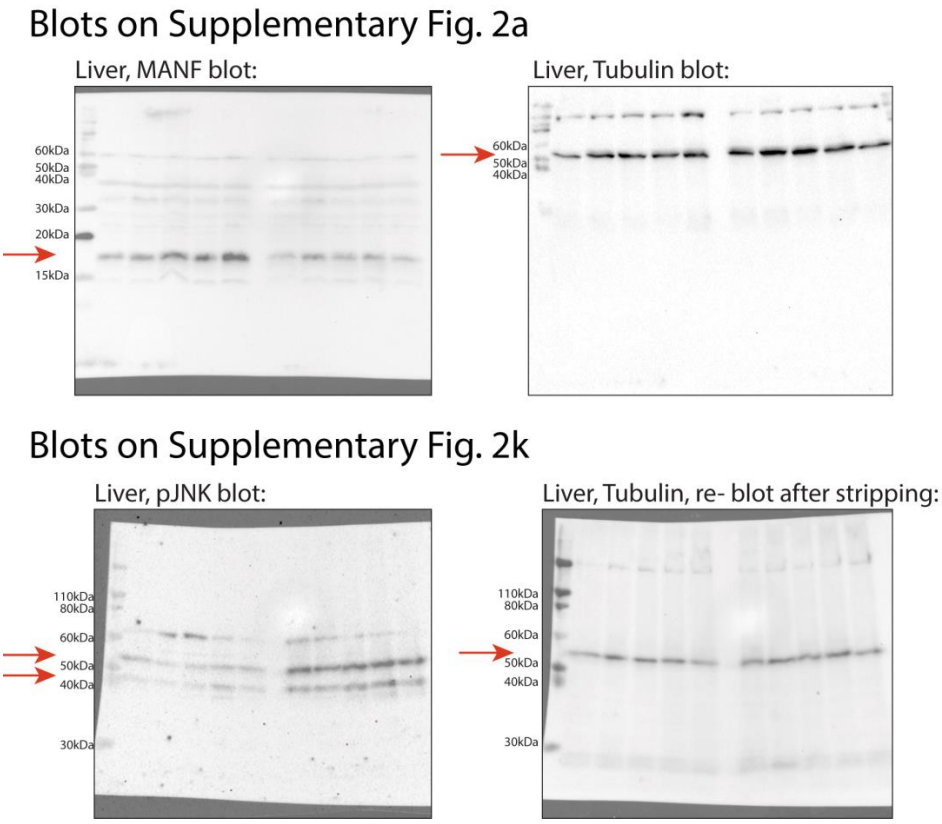
a,d, Representative images of cryosections from tibialis anterior (TA) of 10 month old MANFHet mice and WT littermates (a) or old WT mice, 5 weeks after receiving hrMANF i.p. injections (d), stained with H&E. Scale bars: 50µm. Quantifications of these stainings, for independent animals, are shown in Supplementary Fig. 10b,e. **b,c,e,f** Quantification of the average cross-sectional area of myofibers (b,e) and frequency distribution of myofibers by size (c,f) from TA of 10 month old MANFHet mice and WT littermates (b,c, n=7/condition) or old WT mice, 5 weeks after receiving hrMANF i.p. injections (e,f, n=10, Cntrl i.p.; n=12, hrMANF i.p.).

Data are represented as average ± s.e.m. and each n represents one animal. p values are from two-tailed Student's t-test.



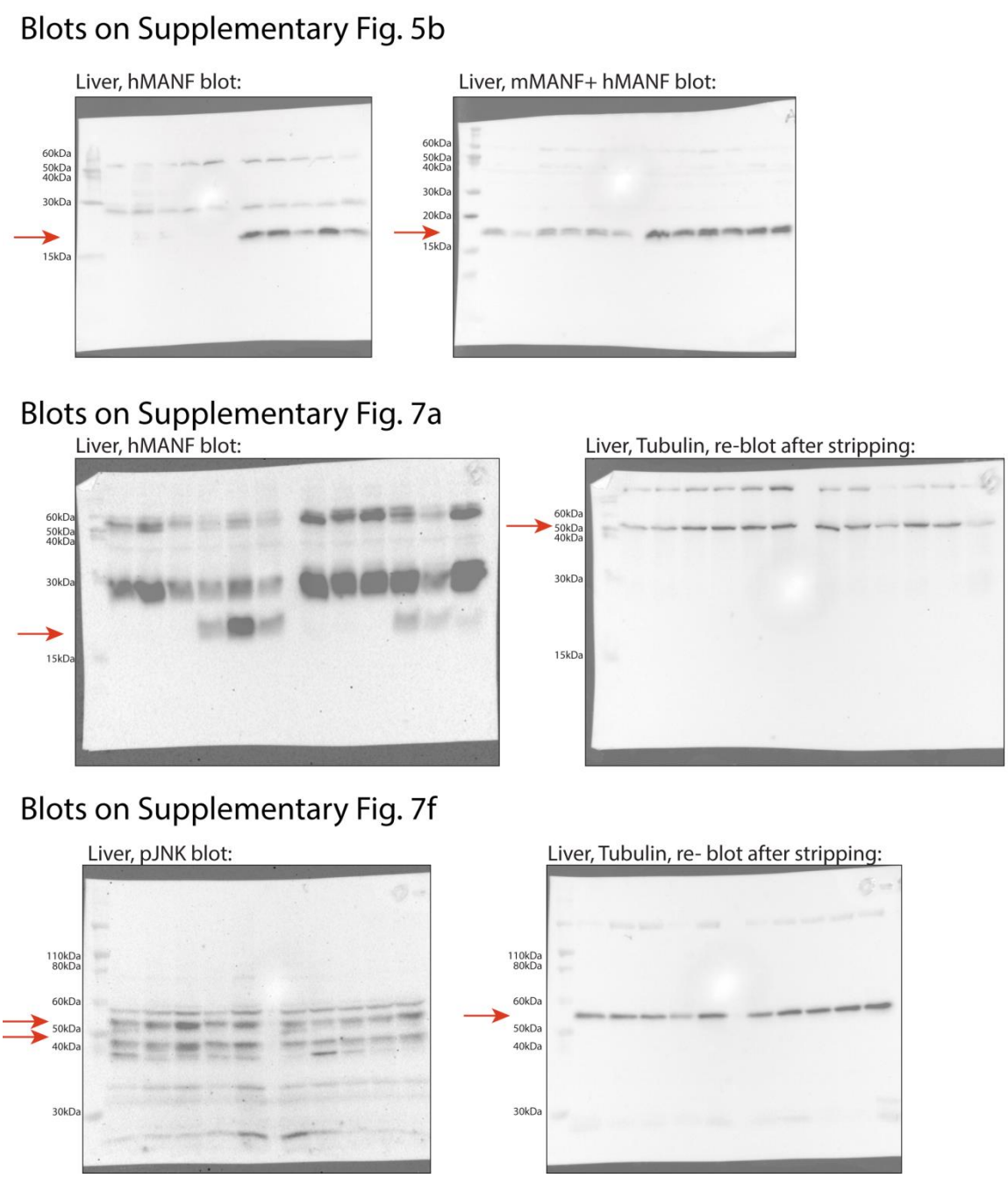
Supplementary Figure 11. Full blots on Figure 1.

Images represent the overlay of the chemiluminescence signal and the bright field image of the membrane where the protein ladder is visible. Red arrows indicate the band of interest.



Supplementary Figure 12. Full blots on Supplementary Figure 2.

Images represent the overlay of the chemiluminescence signal and the bright field image of the membrane where the protein ladder is visible. Red arrows indicate the band of interest. In Fig. 2a, MANF and Tubulin blots are of the same samples run independently in 2 different gels.



Supplementary Figure 13. Full blots on Supplementary Figures 5 and 7.

Images represent the overlay of the chemiluminescence signal and the bright field image of the membrane where the protein ladder is visible. Red arrows indicate the band of interest.

Supplementary Table 2

Primer list for genotyping.

Allele	Size (bp)	Forward Primer	Reverse Primer
MANF Wt	512	TGAAGCAAGAGGCAAAGAGAATCGG	TGCTCAGCTGCAGAGTTAGAGTTCC
MANF null	511	GGGATCTCATGCTGGAGTTCTTCG	TGCTCAGCTGCAGAGTTAGAGTTCC
MANF fl	718	TGAAGCAAGAGGCAAAGAGAATCGG	TGCTCAGCTGCAGAGTTAGAGTTCC
Cx3Cr1 wt	695	AAGACTCACGTGGACCTGCT	CGGTTATTCAACTTGCACCA
Cx3Cr1 Cre-ER	300	AGGATGTTGACTTCCGAGTTG	CGGTTATTCAACTTGCACCA
MANF fl before Cre	>1000	TGAAGCAAGAGGCAAAGAGAATCGG	CCATGGTGATGCTGTAACTGTCACG
MANF fl after Cre	**786**		

Supplementary Table 3

Primary Antibodies for IHC.

Target Gene	Species	Source	Dilution in IHC
MANF	rabbit	Sigma (SAB3500384)	1:300
p-JNK (Thr183/Tyr185)	rabbit	Cell Signaling (9251)	1:400
F4/80	rat	AbD Serotec (ClA3-1)	1:100
CD68	rabbit	Santa Cruz (H-255, sc-9139)	1:100
Ym1	rabbit	Stem Cell Technologies (01404)	1:50
p-Histone H2A.X (Ser139)	rabbit	Cell Signaling (9718)	1:200
Cleaved Caspase 3 (Asp175)	rabbit	Cell Signaling (9664)	1:300
Desmin	rabbit	Abcam (ab8592)	1:100
p-Histone H3 (Ser10)	rabbit	Millipore (06-570)	1:1000

Supplementary Table 4

Primer list for RT-qPCR

Gene	Species	Forward Primer	Reverse Primer
MANF	Drosophila	AGAAGGACGCACAAATCTGC	CGCGTACCTTCAGCTTCTTC
Upd3	Drosophila	GAGCACCAAGACTCTGGACA	CCAGTGCAACTTGATGTTGC
Actin5C	Drosophila	AAGTTGCTGCTCTGGTTGTCG	GCCACACGCAGCTCATTGTAG
MANF	mouse	CACCAGCCACTATTGAAGAAGA	AGCATCATCTGTGGCTCCAA
p16	mouse	CATCTGGAGCAGCATGGAGTC	GGGTACGACCGAAAGAGTTCG
IL-6	mouse	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA
CCl3	mouse	TTCTCAGCGCCATATGGAG	TCAACGATGAATTGGCGTG
CCl4	mouse	CAGCTGTGGTATTCCTGAC	CACTCATGTACTCAGTGACC
Ifng	mouse	CACCTGATTACTACCTTCTTCAG	GTTGTTGACCTCAAACTTGG
TNFα	mouse	TTCTCATTCCTGCTTGTGG	TTGGGAACTTCTCATCCCT
G0S2	mouse	TGACCTTCTCCAGCAAGTG	ATCATCTCCTTGGCCAGAG
sXbp1	mouse	CTGAGTCCGCAGCAGGTG	ACAGTGTCAGAGTCCATGGG
Col1a1	mouse	TGAAGAACTGGACTGTCCC	TTTGGTGATACGTATTCTTCCG
Co1a2	mouse	TTACTGGGAACTTTGCTGC	CTCTGGGTCCCATTAAACC
Col6a6	mouse	TTACTACGTCCATGAGTTCGA	TCTCTGCAGGCTTCTTCAG
Col6a2	mouse	TCTACCGAGATTGACCAGGA	CACCTTGTAGCACTCTCCA
Col12a1	mouse	AGAATGAGATAATCTCGCAGG	GCTCGATGACTTCTTCTCC
Col3a1	mouse	GTGGAACCTGGTTTCTTCTC	TGCATCCCAATTCATCTACG
Col4a1	mouse	CTTCATTAGCAGGTGTGCG	CACTGCGGAATCTGAATGG
Gadd45b	mouse	AAACTGATGAATGTGGACCC	CAGAGCGATATCATCCTCCT
Casp4	mouse	CAACAATTGCCACTGTCCA	TTCTCCAGAGTTCCCACCT
Beta-actin	mouse	GCTCTGGCTCCTAGCACCAT	GCCACCGATCCACACAGAGT
MANF	human	TAAGTATGACAAGCAGATCGAC	TCATCCAGAATCTTCTTCAGC
G0S2	human	TGCCACTAAGGTCATTCCC	ACAGCTTCACCATCTTCCC
sXbp1	human	TGCTGAGTCCGCAGCAGGTG	GCTGGCAGGCTCTGGGGAAG
GAPDH	human	ATCATCAGCAATGCCTCCT	TTCCACGATACCAAAGTTGTC

Supplementary Table 5

Primary antibodies for WB.

Target Gene	Species	Source	Dilution in WB
MANF	rabbit	Sigma (SAB3500384)	1:1000
hMANF	mouse	Origene (AM33442PU-N)	1:1000
p-JNK (thr183/Tyr185)	rabbit	Cell Signaling (9251)	1:1000
Tubulin	mouse	Sigma (T5168)	1:2500
Actin	rabbit	Cell Signaling (4967S)	1:5000