# GARP on hepatic stellate cells is essential for the development of liver fibrosis

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## Supplementary materials and methods

### Mice

C57BL/6 mice were purchased from Jackson Laboratory (Bar Harbor, Maine, USA). Traj18-knockout mice were obtained from La Jolla Institute for Immunology. Lrat-Cre and GARP-floxed mice were generated as previously reported.[1,2] Bmp10-CreER$^{T2}$ mice were developed by our lab as described herein and have been deposited to The Jackson Laboratory under stock number 038492. All animal experiments were approved by the Institutional Animal Care and Use Committee of Harvard Medical School under the protocol IS00001619. All animals were housed in a specific pathogen free (SPF) facility at Harvard Medical School.

### Cell culture

Transfected mink lung epithelial cells (TMLC) were used as active TGF-β reporter cell line. Mouse primary HSCs were cultured in complete DMEM: DMEM supplemented with 10% fetal bovine serum (FBS), 1% Penicillin-Streptomycin-Glutamine, 1% HEPES, and 1% Sodium Pyruvate. TMLC were cultured in complete DMEM supplied with 250 ug/ml Geneticin. Human primary HSCs were cultured in stellate cell basal medium supplemented with 10% FBS. Mouse primary NKT and NK cells were cultured in complete RPMI: RPMI 1640 supplemented with 10% FBS, 1% Penicillin-Streptomycin-Glutamine, and 1% HEPES. All cells were cultured in incubators at 37°C with 5% $CO_2$.

### Fibrosis model

Seven to 10 week-old sex-matched littermates were used for experiments. For the Thioacetamide (TAA) model, mice were fed with TAA in the drinking water (300 mg/L) for 4 months to induce liver fibrosis. TAA water was changed every 4 days. For the non-alcoholic steatohepatitis (NASH) diet model, mice were fed with a 60 kcal% fat, 0.1% methionine and choline deficient (HFD-MCD) diet (Research diets, NJ, USA) for 8 to 10 weeks to induce liver fibrosis without causing body weight loss in animals. HFD-MCD diet was changed every 4 days. In the high-fat diet with high fructose and cholesterol (FPC) model,[3] mice were fed with a diet rich in palmitate and cholesterol (Teklad, TD160785) and drinking water containing 23 g/L fructose and 18.86 g/L glucose for 16 weeks.

**Generation of Bmp10-CreER$^{T2}$ mice**

The development of Bmp10-CreER$^{T2}$ mice was outsourced to Biocytogen (Wakefield, MA, USA). In brief, targeting sequences for the CreER$^{T2}$ construct were generated by PCR and cloned into bacterial artificial chromosomes (BACs). The CreER$^{T2}$-BAC was electroporated into murine embryonic stem (ES) cells, and the Bmp10-CreER$^{T2}$ construct was inserted between the coding region of exon 2 and the 3´ untranslated region (UTR) to avoid disruption of endogenous Bmp10 expression. Unspecific integration into the ES cell genome was prevented by a diphtheria toxin A (DTA) cassette in the targeting vector downstream of the 3´ homologous arm. Mutated ES cells were screened for G418 resistance. Correct integration of the targeting sequence was confirmed by southern blots using probe 1 after KpnI digestion of ES cell DNA (WT: 18.6 kb, mutant: 13.2 kb), or by using probe 2 after digestion with EcoRI (WT: 4.6 kb,

mutant: 4.1 kb). Subsequently, targeted ES cells were injected into blastocysts for chimera breeding and subsequent genotyping of the F1 generation by PCR. Chimera of the desired genotype were bred further with C57BL/6 mice.

**Microarray analysis**

The processing and statistical analyses of the microarrays were performed using the ImmGen platform at Harvard Medical School. Protocols for sample processing, data generation, data pre-processing, and quality control of ImmGen microarray data can be found at http://www.immgen.org/. In brief, the RNA of cells was purified by phenol chloroform extraction and isopropanol precipitation in the presence of glycoblue carrier. RNA pellets were washed with ethanol, air-dried, and dissolved in water. The RNA was converted and amplified into antisense cRNA, prior to conversion to ssDNA and oligonucleotide labeling. Oligonucleotides were prepared for hybridization using the "Hybridization, Wash and Stain kit" from Affymetrix. Subsequently, oligonucleotides were applied to Mouse Gene ST 1.0 arrays, and incubated for 16 hours at 45°C. Finally, microarrays were scanned on an Affymetrix GeneChip scanner, and data were extracted by the Affymetrix Expression Console. Microarray data were pre-processed using the Microarray Suite Software 5.0. The heatmap of gene expression was prepared using Prism GraphPad Version 9.0.2.

Microarray data from human patients were retrieved from Gene Expression Omnibus (GEO) and analyzed using GEO2R provided by GEO with default settings. Gene expression values were prepared using Prism GraphPad and displayed in Robust Multiple-array Average (RMA) form.

**qRT-PCR analysis**

Total RNA was extracted from tissues or cultured cells using TRIzol reagent. 2 ug RNA was reversely transcribed into cDNA using high-capacity RNA-to-cDNA kit. Real-time qPCR was performed using Power SYBR green PCR master mix and corresponding primers. Ct values were detected using a ViiA 7 real-time PCR system (Applied Biosystems). Relative mRNA expression levels were normalized to the housekeeping gene Actb. Primers used for qRT-PCR are listed in Table S1.

**Cell preparation from mouse liver**

HSCs were isolated as previously reported.[4] Briefly, mice were anesthetized with ketamine and xylazine. Subsequently, a catheter was placed into the inferior vena cava, through which the liver was sequentially perfused with EGTA, Protease and Collagenase D solutions in a retrograde manner. Perfused liver was excised and minced in a dish. The liver was further digested with Protease and Collagenase D solution in vitro. Afterwards, cells were pelleted and washed with GBSS/B solution. Then, the single cell suspension was subjected to gradient centrifugation using Nycodenz and GBSS/B. Purified HSCs were collected, washed with GBSS/B, and cultured in complete DMEM.

LSECs were isolated as previously described[5] with minor modifications. Briefly, mice were first anesthetized with ketamine and xylazine. Then, a catheter was placed into the inferior vena cava, through which the liver was sequentially perfused with EGTA and Collagenase type 4 solutions in a retrograde manner. The perfused liver was excised

and minced in a dish. Subsequently, hepatocytes were pelleted and collected by 70 *g* centrifugation for 5 mins, and the remaining non-parenchymal cells were subjected to gradient centrifugation using Optiprep. Resulting cells were used for subsequent LSEC isolation using CD146 MACS beads (Miltenyi). From $CD146^-$ cells, Kupffer cells were isolated using F4/80 MACS beads (Miltenyi).

To isolate NK and NKT cells, mice were sacrificed with $CO_2$, prior to excising the liver and mincing in a dish. Cells were suspended in 20 ml RPMI 1640 supplemented with 2% FBS and mixed with 10 ml Percoll working solution. Cells were then centrifuged at 2,000 rpm for 15 mins at room temperature (RT) without brake. Supernatant was discarded and pellets were treated with 5 ml red blood cell lysis buffer for 5 min at RT and washed once with 15 ml RPMI 1640. For NKT cell isolation, cells were incubated with 13 ug/ml mCD1d-α-GalCer-tetramer coupled to PE (from the NIH tetramer core facility) at 4°C for 30 mins and washed twice with PBS. Subsequently, NKT cells were enriched using anti-PE MACS beads (Miltenyi). Alternatively, for NK cell isolation, cells were first enriched using an NK isolation kit (Miltenyi), and to increase purity, enriched cells were further labeled with PE anti-mouse NK1.1 and positively selected using anti-PE MACS beads.

**Conditioned medium culture**

NKT cells, Kupffer cells, or MDMs were isolated from mouse liver. 3 x $10^4$ isolated cells were cultured in 200 μl cRPMI in 96-well plate at 37°C for 2 days. Conditioned medium was collected afterwards. HSCs were isolated from naïve mice and cultured in cDMEM for 2 days. 1.5 x $10^4$ HSCs were then cultured with 200 μl conditioned medium or cRPMI for 3 days at 37°C. Gene expression in cells was then analyzed by qRT-PCR.

**Immunohistochemistry (IHC) staining**

Mice were sacrificed with $CO_2$, and the liver was excised and fixed with 4% paraformaldehyde (PFA) at 4°C overnight. Subsequently, the liver was dehydrated and embedded with paraffin and cut into 5 μm sections. Sections were deparaffinized and rehydrated through xylene and serial gradient ethanol. Antigens were retrieved with pH 6.0 sodium citrate solution at 95°C for 20 mins. Sections were then blocked with 10% goat serum at RT for 30 mins. Endogenous peroxidase was blocked with 3% $H_2O_2$ for 20 mins at RT. Endogenous biotin was blocked using Avidin/Biotin blocking kit (Vector laboratories). Sections were then incubated with rabbit anti-α-SMA, or rabbit anti-p-Smad2 monoclonal antibodies at 4°C overnight. After washing, sections were incubated with goat anti-rabbit IgG biotin antibodies for 30 mins at RT, followed by Avidin-Biotin-HRP reagent (Vector Laboratories) incubation for 30 mins at RT. Colors were then developed with DAB solution. The cell nucleus was counter-stained with hematoxylin solution. Slides were scanned using an Olympus VS120 slide scanner. Images were processed and analyzed using ImageJ (National Institutes of Health).

**Immunofluorescence (IF) staining**

Liver tissues were dehydrated in 30% sucrose solution at 4°C overnight and embedded in optimal cutting temperature compound (OCT) and cut into 5 um cryosections. Sections were fixed with 4% PFA for 15 mins at RT, then permeabilized with 0.1% Triton X-100 in PBS for 15 mins at RT. Sections were then blocked with 10% donkey serum for 1 hour at RT, followed by incubation with goat anti-mouse Desmin at 4°C

overnight. After washing, sections were incubated with AF647 donkey anti-Goat IgG for 1 hour at RT. Cell nuclei were then stained with Hoechst 33258. Sections were visualized using an Olympus FV1000 confocal microscope, and images were analyzed by ImageJ.

**Histology staining**

Mice were sacrificed with $CO_2$, and livers were excised and fixed with 4% PFA at 4°C overnight. Tissues were embedded with paraffin and cut into 5 μm sections and stained with hematoxylin and eosin (H&E) or Sirius red at the rodent histopathology core at Harvard Medical School. Inflammation was scored by a pathologist at the HMS core based on H&E staining, with 0 = no inflammation, 1 = mild inflammation, 2 = moderate inflammation, 3 = severe inflammation, 4 = very severe inflammation. The Sirius red-positive area was quantified using ImageJ.

**Hydroxyproline assay**

Mice were sacrificed with $CO_2$, livers were excised, and hydroxyproline content in liver tissues was determined using a hydroxyproline assay kit (Sigma) following the manufacturer's instructions.

**Flow cytometry**

Flow cytometry was performed using a BD FACSCanto II analyzer. Raw data were analyzed using Flowjo software version 10.8.1. To assess GARP, LAP, and IL-4Ra expression on the cell surface, 1-day cultured (quiescent) HSCs or 7-day cultured

(activated) cells were disassociated using Accutase solution. Cells were then incubated with APC anti-mouse GARP, PE anti-mouse LAP, PE anti-mouse IL4Ra antibodies as well as viability dye at 4°C for 30 mins. Corresponding isotype antibodies were used as negative control. After washing, cells were analyzed immediately. To assess the impact of IL-4 on GARP expression, 5 x $10^4$ 2-day cultured HSCs were seeded into 12-well plates in complete DMEM and stimulated with 20 ng/ml recombinant murine IL-4 with or without 100 nM STAT6 inhibitor AS1517499 for 3 days. Surface expression of GARP on cells was then determined as described above. To assess GARP expression on human cells, primary human HSCs were cultured for 4 days and then dissociated by Accutase solution and incubated with APC anti-human GARP antibodies and viability dye at 4°C for 30 mins. Cells were then analyzed immediately.

To assess granulocyte frequency in the liver, tissue cells prepared by Percoll centrifugation were incubated with BD Fc Block at 4°C for 5 mins, then incubated with PerCP-Cy5.5 anti-mouse CD45, PE-Cy7 anti-mouse CD11b, Pacific Blue anti-mouse Gr-1 antibodies and viability dye at 4°C for 30 mins. After washing, cells were analyzed immediately. Granulocytes were defined as live $CD45^+CD11b^+Gr\text{-}1^+$ cells.

To assess NK cell frequency and phenotype in the liver, tissue cells prepared by Percoll centrifugation were incubated with BD Fc Block, then incubated with PerCP-Cy5.5 anti-mouse CD45, PE-Cy7 anti-mouse NK1.1, Pacific Blue anti-mouse CD3, FITC anti-mouse NKG2D and viability dye at 4°C for 30 mins. Following wash steps, cells were analyzed without delay. NK cells were defined as live $CD45^+NK1.1^+CD3^-$ cells. To assess cytokine production by NK cells, tissue cells prepared by Percoll centrifugation were stimulated with 10 ng/ml recombinant murine IL-12 in the presence of BD

GolgiPlug and BD GolgiStop in complete RPMI 1640 for 4 hours at 37°C. Cells were then stained for surface markers as described above and fixed using fixation/permeabilization solution (BD). Afterwards, cells were incubated with FITC anti-mouse IFN-γ antibodies at 4°C for 30 mins. Cells were then analyzed after washing. To assess the cytotoxicity of NK cells against HSCs, 1 x $10^4$ 7-day culture-activated HSCs per well were seeded into 96-well U-bottom plates as target cells. 1 x $10^5$ purified NK cells per well were added as effector cells. Cells were cocultured for 20 hours at 37°C. After incubation, supernatant was aspirated, and cells were washed once with PBS. Live target cells were dissociated with trypsin and stained with viability dye and FITC Annexin V. The numbers of live Annexin $V^-$ target cells were compared to the well without NK cells, and cytotoxicity percentages were calculated accordingly.

To assess frequency of Kupffer cells and MDMs, liver homogenate was digested with 0.237 mg/ml collagenase D solution at 40°C for 20 mins with stirring. Then cells were passed through a 70 μm filter and centrifuged at 70 g for 5 mins. The supernatant was collected and centrifuged at 450 g for 10 mins. Resulting cells were washed once with PBS and blocked with BD Fc Block. Kupffer cells ($CD45^+CD11b^{int}F4/80^{high}Siglec\text{-}F^-$) and MDMs ($CD45^+CD11b^{high}F4/80^{int}Siglec\text{-}F^-$) were analyzed or sorted accordingly.

**Tamoxifen administration**

$Bmp10CreER^{T2+/-}$Ai6 reporter mice were given 5 mg tamoxifen dissolved in 250 μl corn oil by oral gavage for 5 consecutive days. Twelve days later, mice were sacrificed for evaluating Zsgreen expression. $Bmp10CreER^{T2+/-}GARP^{fl/fl}$ mice or $GARP^{fl/fl}$ littermates were fed with NASH diet for 4 weeks, then mice were given 5 mg tamoxifen dissolved in

170 µl corn oil by oral gavage on day 1, 2, 3, 5, and 6. Mice were fed with NASH diet for another 4 weeks before experimental read-out.

**OCH administration**

$Lrat^{Cre+/-}$-$GARP^{fl/fl}$ mice or $GARP^{fl/fl}$ littermates were fed with NASH diet for 53 days, mice were then i.p. injected with 2 ug OCH (from the NIH tetramer core facility) dissolved in 200 µl PBS containing 1% DMSO. The same volume of 1% DMSO was i.p. injected into control mice. Mice were fed with NASH diet for another 3 days before analysis.

**TGF-β reporter assay**

Mouse or human HSCs were cultured for 4 to 6 days to induce activation. Activated cells were dissociated with Accutase solution and 1 x $10^4$ HSCs per well were seeded into 96-well flat bottom plates with 1.6 x $10^4$ TMLC in 200 µl complete DMEM. Wells seeded with the same amount of TMLC were used as control. 10 ug/ml purified anti-mouse TGF-β1 antibody, or 100 nM integrin $\alpha_V$ inhibitor CWHM-12, or 20 nM ET-1, or 20 uM Bosentan hydrate, or 50 uM Blebbistatin, or the same volume of PBS were added into the wells. Cells were cocultured for 16 hours at 37°C in a cell culture incubator, then culture medium was aspirated, and luciferase activity in the cells was determined using a luciferase assay system (Promega). Luminescence signal defined as relative light unit (RLU) was detected using a Synergy2 reader (BioTek). Luciferase activity = $RLU_{experiment\ well}$ - $RLU_{blank\ well}$.

**Immunoblot**

Cellular protein was extracted from liver using RIPA buffer supplemented with 1% protease and phosphatase inhibitor. Proteins were quantified using a BCA kit (Thermo Scientific). Protein solutions were diluted with Laemmli loading buffer supplemented with 55 uM 2-Mercaptoethanol, or 100 nM DTT, and boiled at 95°C for 5-10 mins. 25 ug of protein was then loaded onto a 12% SDS gel, prior to performing electrophoresis. Proteins were then transferred onto nitrocellulose membrane. The membrane was blocked with 5% BSA in PBST buffer for 40 mins at RT, then incubated with mouse anti-GARP, rabbit anti-β-actin, rabbit anti-p-Smad2, or rabbit anti-Smad2 antibodies at 4°C overnight. Following wash steps, the membrane was further incubated with HRP-conjugated goat anti-mouse IgG or goat anti-rabbit IgG antibody at RT for 30 mins, and subsequently washed and incubated with chemiluminescent substrate for 1 min. X-ray film was exposed and developed in the dark room.

**Gene knockdown**

HSCs were cultured for 3 days, prior to transfection with mouse GARP siRNA, or human GARP siRNA, or scramble siRNA (Thermo Fisher), using Lipofectamine RNAiMAX Reagent (Invitrogen). After transfection, cells were further cultured for 3 days, prior to analysis by flow cytometry or TGF-β reporter assay. Sequences of siRNA used to knockdown GARP are listed in Table S1.

**Collagen gel contraction assay**

The collagen gel assay was conducted using a cell contraction assay kit (Cell Biolabs) following the manufacturer's instructions. Briefly, HSCs were cultured for 7 days, then

dissociated with Accutase solution. 2 x $10^5$ cells in 100 µl complete DMEM were mixed with 400 µl collagen gel working solution and seeded into a 24-well plate, prior to incubation at 37°C for 1 hour. After collagen polymerization, 1 ml of complete DMEM was added to the top of the gel. Following 2 days incubation, 20 nM ET-1, or 20 uM bosentan hydrate, or 50 uM blebbistatin, or 200 nM integrin $\alpha_V$ inhibitor CWHM-12, or the same volume of PBS were added per well, and gels were gently released from the side of the culture wells using a sterile spatula. The image of the gel was taken at indicated time points, and gel size was quantified using ImageJ software.

**Enzyme-linked immunosorbent assay (ELISA)**

HSCs were cultured for 6 days in complete DMEM and dissociated with Accutase solution. 2 x $10^4$ cells were seeded into 96-well plates in 200 µl complete DMEM and further cultured for 2 days. Then cells were rinsed twice with PBS and cultured with 200 µl serum-free DMEM with or without 10 pM recombinant mouse TGF-β1, or 10 ug/ml purified anti-mouse TGF-β1 for 1 day. Afterwards, supernatant was collected, and ET-1 concentration in supernatant was determined using an endothelin-1 ELISA kit (Enzo) following the manufacturer's instructions.

**Liver enzyme activity assay**

Mice were sacrificed with $CO_2$, and whole blood was collected from the vena cava, prior to serum separation. Alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activity in serum were quantified using an ALT and AST activity assay kit (Sigma), following the manufacturer's instructions.

**Statistical analysis**

Data are presented as mean ± standard error of mean (SEM). Data that passed the normality test were analyzed using two-tailed unpaired t-test or one-way ANOVA. Data that were not normally distributed were analyzed using nonparametric Mann-Whitney U test. $p<0.05$ was considered significant. All statistical analyses were calculated using GraphPad Prism version 9.0.2.

## Supplemental figures and legends

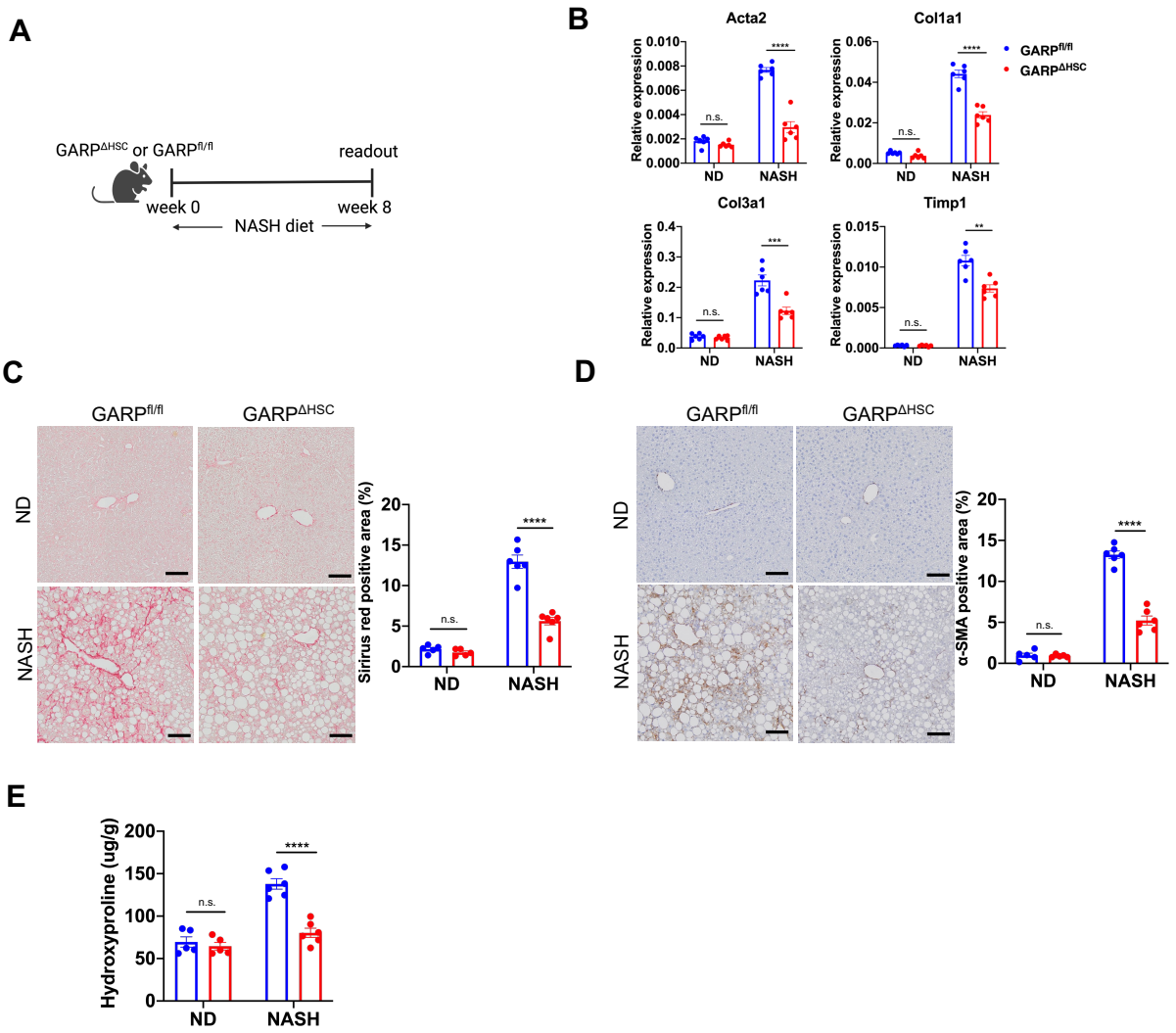


**Fig. S1. GARP on HSCs promotes NASH diet-induced liver fibrosis.** (A) Experimental set-up of NASH diet-induced liver fibrosis. (B) Fibrotic gene expression in the liver of mice determined by qRT-PCR. (C) Representative images of Sirius red staining of mouse liver (left) and quantification of stained area (right). Scale bar 100 μm. (D) Representative images of IHC staining of α-SMA in the liver of mice (left) and quantification of stained area (right). Scale bar 100 μm. (E) Concentration of hydroxyproline in the liver of mice. Each data point represents one mouse. Data are presented as mean ± SEM and are from one experiment representative of three independent experiments with similar results. Unpaired t-test was used to compare two groups. **p<0.01; ***p<0.001; ****p<0.0001.

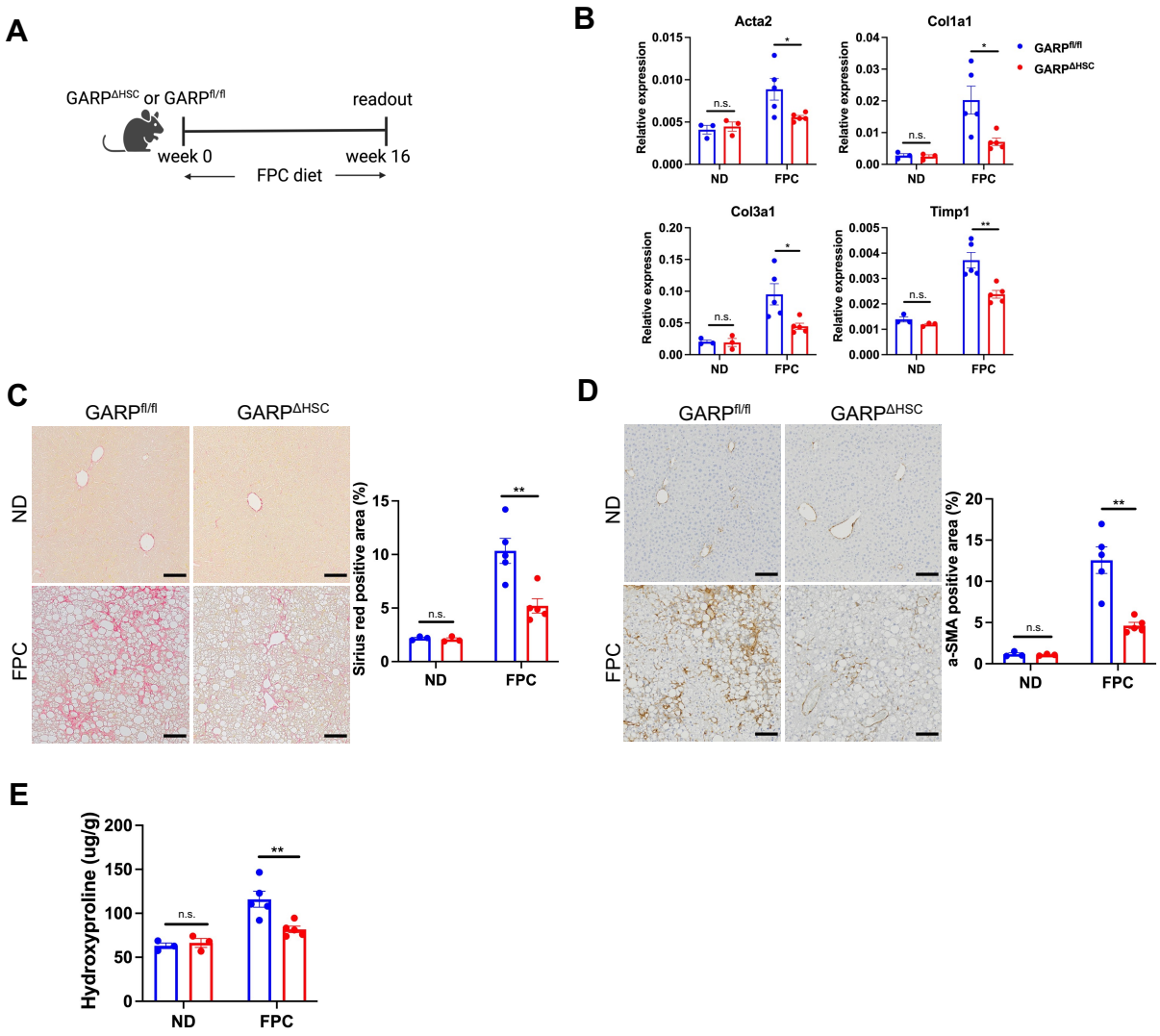


**Fig. S2. FPC diet-induced liver fibrosis is augmented by GARP on HSCs.** (A) Experimental set-up of FPC diet-induced liver fibrosis. (B) Fibrotic gene expression in the liver of mice determined by qRT-PCR. (C) Representative images of Sirius red staining of mouse liver (left) and quantification of stained area (right). Scale bar 100 μm. (D) Representative images of IHC staining of α-SMA in the liver of mice (left) and quantification of stained area (right). Scale bar 100 μm. (E) Concentration of hydroxyproline in the liver of mice. Each data point represents one mouse. Data are presented as mean ± SEM. Unpaired t-test was used to compare two groups. *p<0.05; **p<0.01.

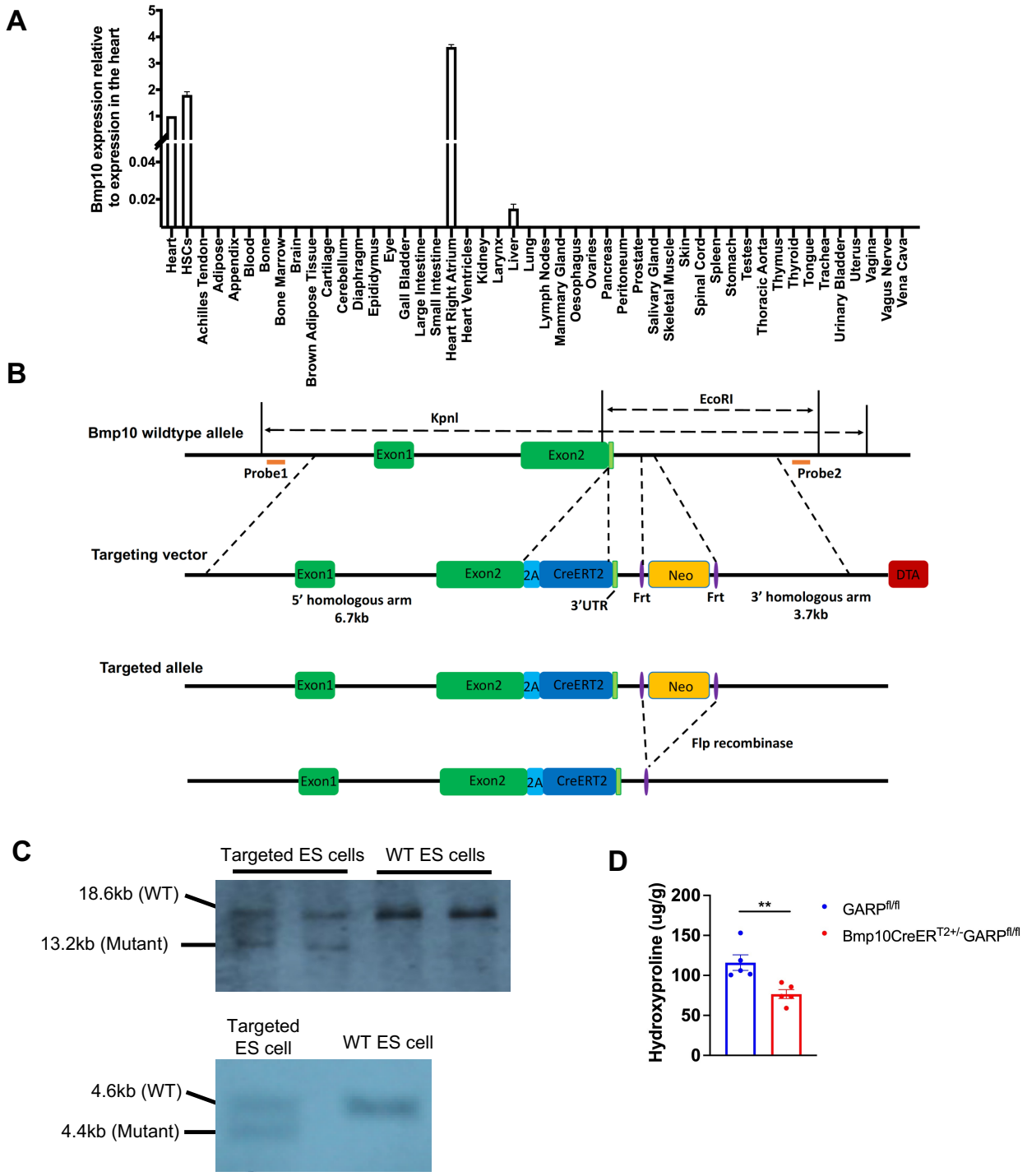


**Fig. S3. Generation of Bmp10-CreER$^{T2}$ mice.** (A) Screening of Bmp10 expression in different tissues of naïve C57BL/6 mice by qRT-PCR. (B) Targeting vectors for generating Bmp10-CreER$^{T2}$ mice. A bacterial artificial chromosome (BAC) targeting vector was electroporated into C57BL/6 embryonic stem (ES) cells in order to target the Bmp10 locus on chromosome 6 with the CreER$^{T2}$ construct. Successful integration of

the vector leads to G418 resistance of ES cells. The neomycin (Neo) cassette is flanked by flippase recognition sequences (Frt) that can be removed by flippase (Flp)-mediated recombination. The targeting vector also carries a diphtheria toxin A (DTA) negative selection cassette to avoid random integration of the vector into the genome. Probe 1 and probe 2 were designed for controlling the integration of the construct by southern blot after KpnI or EcoRI digestion, respectively. 2A, 2A peptide; UTR, untranslated region. (C) Southern blot analysis of targeted ES or WT ES cells using probe 1 after KpnI digestion (upper) or probe 2 after EcoRI digestion (lower). (D) Concentration of hydroxyproline in the liver of mice 8 weeks after NASH diet. Each data point represents one mouse. Data are presented as mean ± SEM. Unpaired t-test was used to compare two groups. **$p<0.01$.

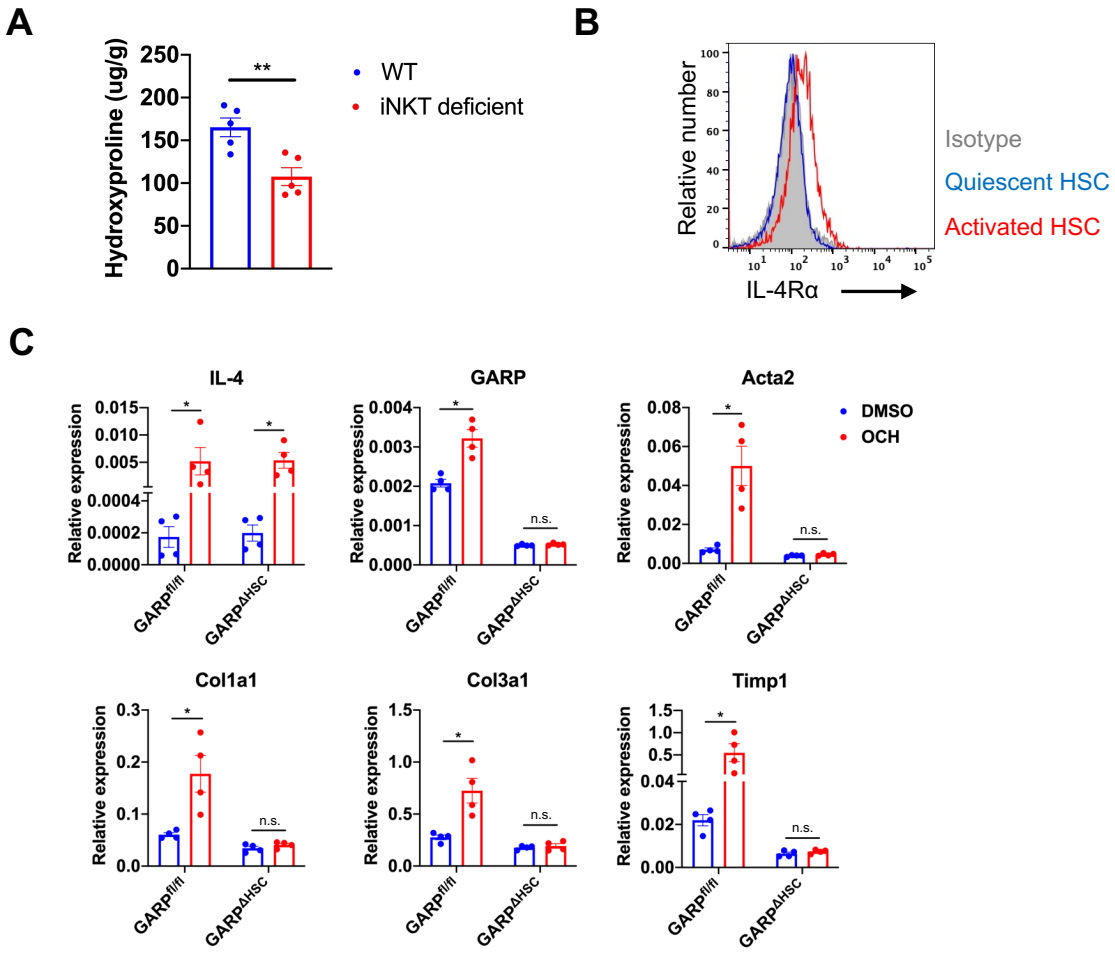


**Fig. S4. iNKT cells promote liver fibrosis by IL-4-mediated GARP induction.** (A) Concentration of hydroxyproline in the liver of iNKT-deficient or WT mice after 8 weeks of NASH diet. (B) FACS analysis of IL-4Rα expression on the surface of quiescent (1-day cultured) or activated (7-day cultured) HSCs. (C) IL-4, GARP, and fibrotic gene expression in the liver of OCH- or DMSO-treated mice determined by qRT-PCR. Each data point represents a single mouse. Data are presented as mean ± SEM. Unpaired t-test in A or Mann-Whitney test in C were used to compare two groups. *p<0.05; **p<0.01.

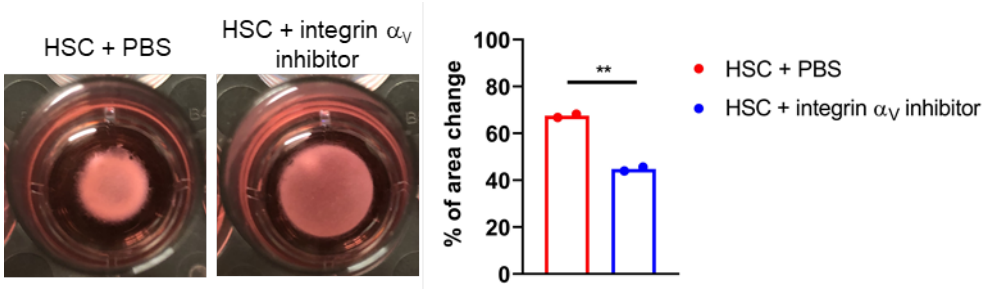


**Fig. S5. Integrin $\alpha_V$ contributes to HSC contraction.** Representative images of collagen gel mixed with WT HSCs in the presence of PBS, or integrin $\alpha_V$ inhibitor CWHM-12, 48 hours after gel release from the well side (left), and statistical analysis of change in gel area (right). Data are presented as mean ± standard error of mean (SEM). Unpaired t-test was used to compare two groups. **p<0.01.

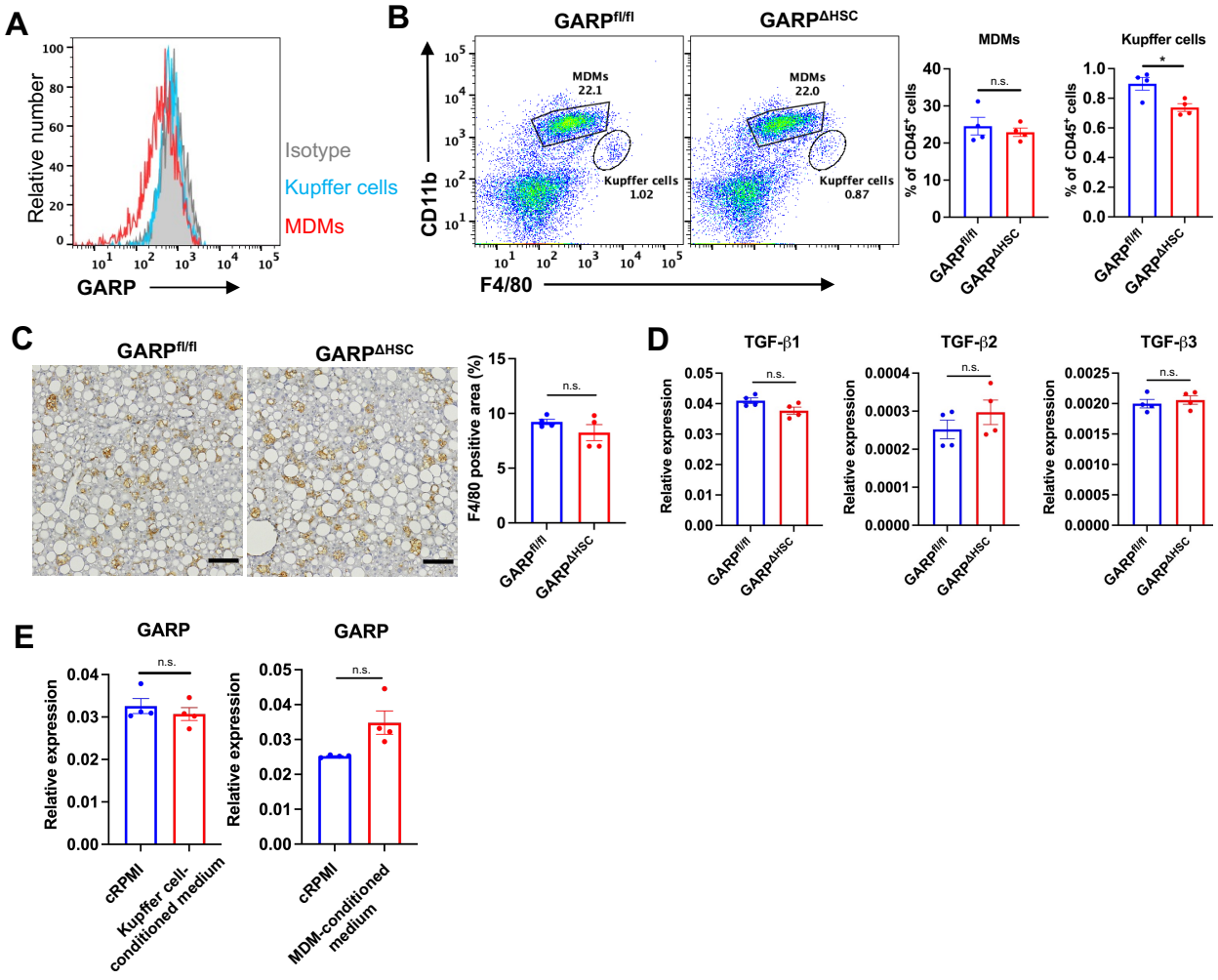


**Fig. S6. Targeting GARP on HSCs does not affect frequency of macrophages in liver.** (A) GARP expression on the surface of Kupffer cells or liver monocyte-derived macrophages (MDMs) detected by flow cytometry. (B) FACS analysis of frequency of Kupffer cells ($CD11b^{Int}F4/80^{high}$) and MDMs ($CD11b^{high}F4/80^{Int}$) in the liver of mice fed with NASH diet for 8 weeks. (C) Representative images of IHC staining of F4/80 in the liver of mice fed with NASH diet for 8 weeks (left) and quantification of stained area (right). Scale bar 100 μm. (D) qRT-PCR analysis of gene expression of liver total macrophages ($CD45^+CD11b^+F4/80^+Siglec\text{-}F^-$) 8 weeks after NASH diet. (E) GARP mRNA expression in HSCs treated with cRPMI, Kupffer cell-conditioned medium, or MDM-conditioned medium. Data are presented as mean ± standard error of mean (SEM). Unpaired t-test was used to compare two groups. *p<0.05.

**Table S1. Sequences of primers and siRNA used for qRT-PCR and gene knockdown.**

| Gene name | Sequence (5' to 3') |
|---|---|
| mActb-F | CAG CCT TCC TTC TTG GGT ATG |
| mActb-R | GGC ATA GAG GTC TTT ACG GAT G |
| mLrrc32-F | CTGTAGGACGGTGAACAAGG |
| mLrrc32-R | GGACAAGTAGAGAGCTTGGATG |
| mBmp10-F | ACCGGACCTCCATGCCGT |
| mBmp10-R | TCCGGAGCCCATTAAAAGTGACTGG |
| mActa2-F | GTTCAGTGGTGCCTCTGTCA |
| mActa2-R | ACTGGGACGACATGGAAAAG |
| mCol1a1-F | TAGGCCATTGTGTATGCAGC |
| mCol1a1-R | ACATGTTCAGCTTTGTGGACC |
| mCol3a1-F | CTGTAACATGGAAACTGGGGAAA |
| mCol3a1-R | CCATAGCTGAACTGAAAACCACC |
| mTimp1-F | GCAACTCGGACCTGGTCATAA |
| mTimp1-R | CGGCCCGTGATGAGAAACT |
| mIl4-F | GGTCTCAACCCCCAGCTAGT |
| mIl4-R | GCCGATGATCTCTCTCAAGTGAT |
| mEdn1-F | GCACCGGAGCTGAGAATGG |
| mEdn1-R | GTGGCAGAAGTAGACACACTC |
| mIl1b-F | CAGGCAGGCAGTATCACTCA |
| mIl1b-R | AGGTGCTCATGTCCTCATCC |
| mIl6-F | GTCTGTAGCTCATTCTGCTCTG |
| mIl6-R | GAAGGCAACTGGATGGAAGT |
| mTnf-F | CCGATGGGTTGTACCTTGTC |
| mTnf-R | AGATAGCAAATCGGCTGACG |
| mTgfb1-F | CTGAACCAAGGAGACGGAATAC |
| mTgfb1-R | GGGCTGATCCCGTTGATTT |

| | |
|---|---|
| mTgfb2-F | CTTCGACGTGACAGACGCT |
| mTgfb2-R | GCAGGGGCAGTGTAAACTTATT |
| mTgfb3-F | CCTGGCCCTGCTGAACTTG |
| mTgfb3-R | TTGATGTGGCCGAAGTCCAAC |
| mLrrc32 siRNA sense | GUACUAGACUUGAGCUGCATT |
| mLrrc32 siRNA anti-sense | UGCAGCUCAAGUCUAGUACCT |
| hLrrc32 siRNA sense | CGAGACUCAUCUACCUGAATT |
| hLrrc32 siRNA anti-sense | UUCAGGUAGAUGAGUCUCGGG |

**Supplementary references**

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