

1 **Supplementary Information for:**

2

3 **LGR5 marks targetable tumor-initiating**
4 **cells in mouse liver cancer**

5 **Cao et al**

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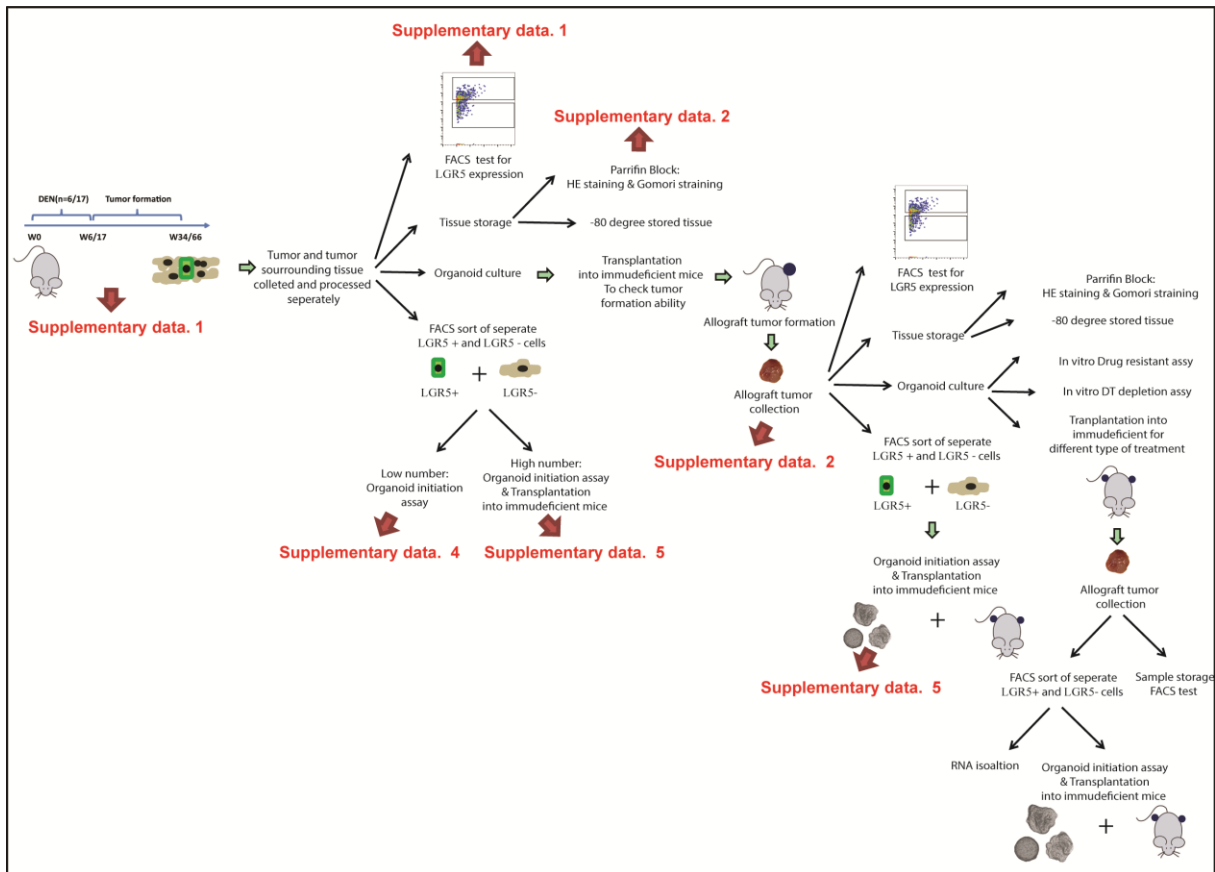
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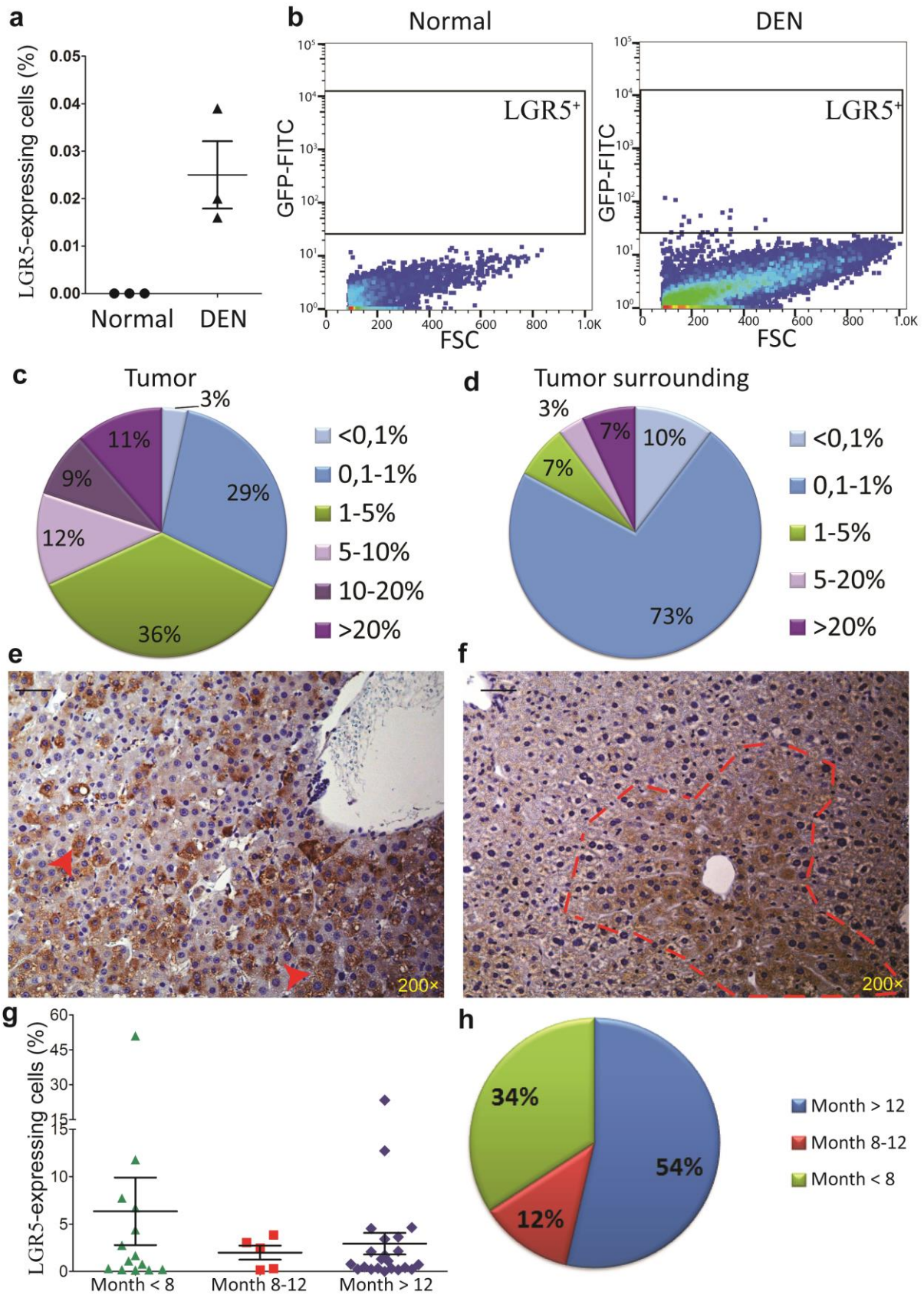
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27

28 **Supplementary Figure 1 | General flowchart of the experimental design.**



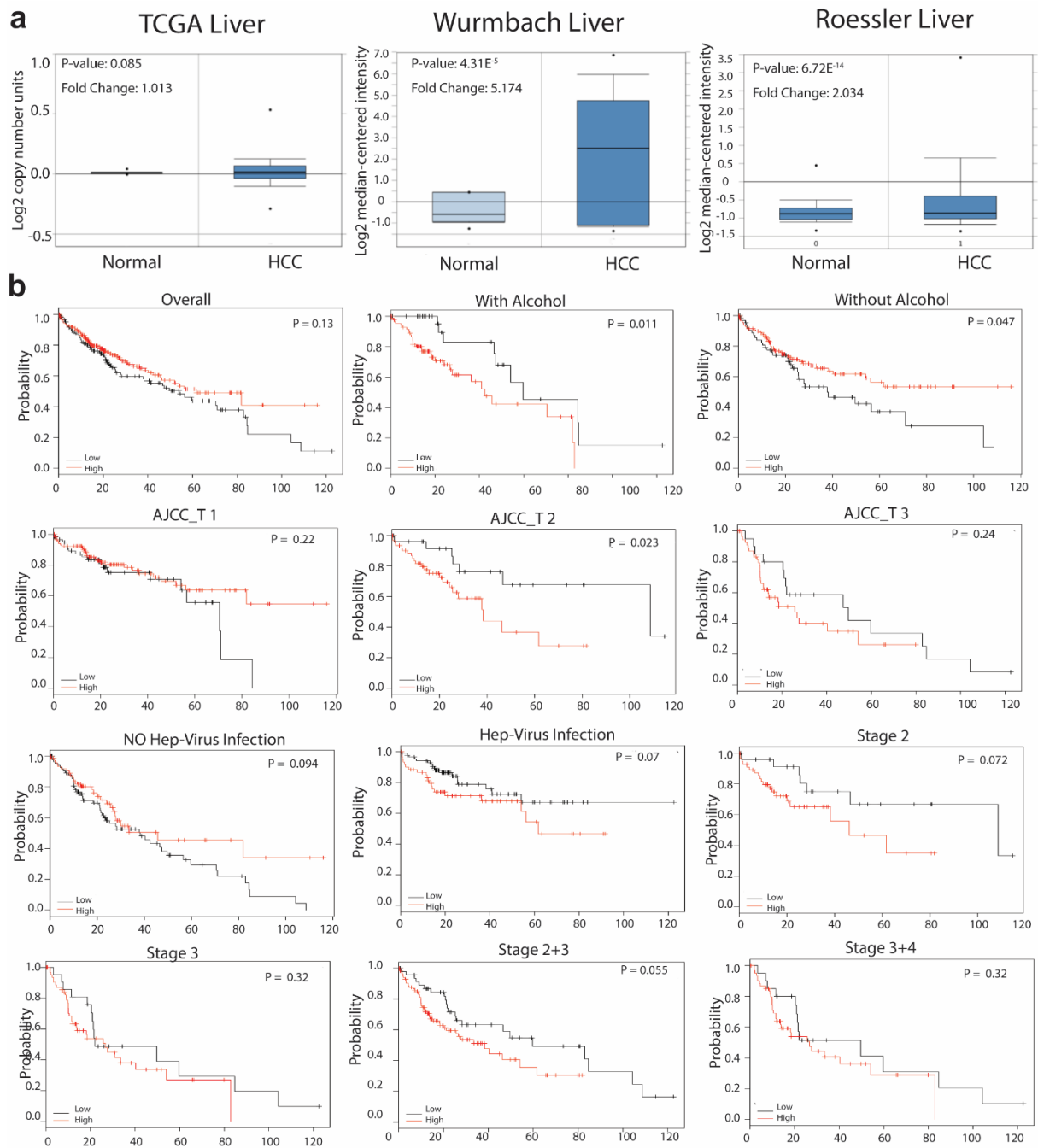
29

30 **Supplementary Figure 2 | LGR5 expressing cells are present in the DEN-induced primary liver tumor.**

31 **a, LGR5⁺ cells are present in the liver upon DEN administration (Post DEN induction day 7). b,**

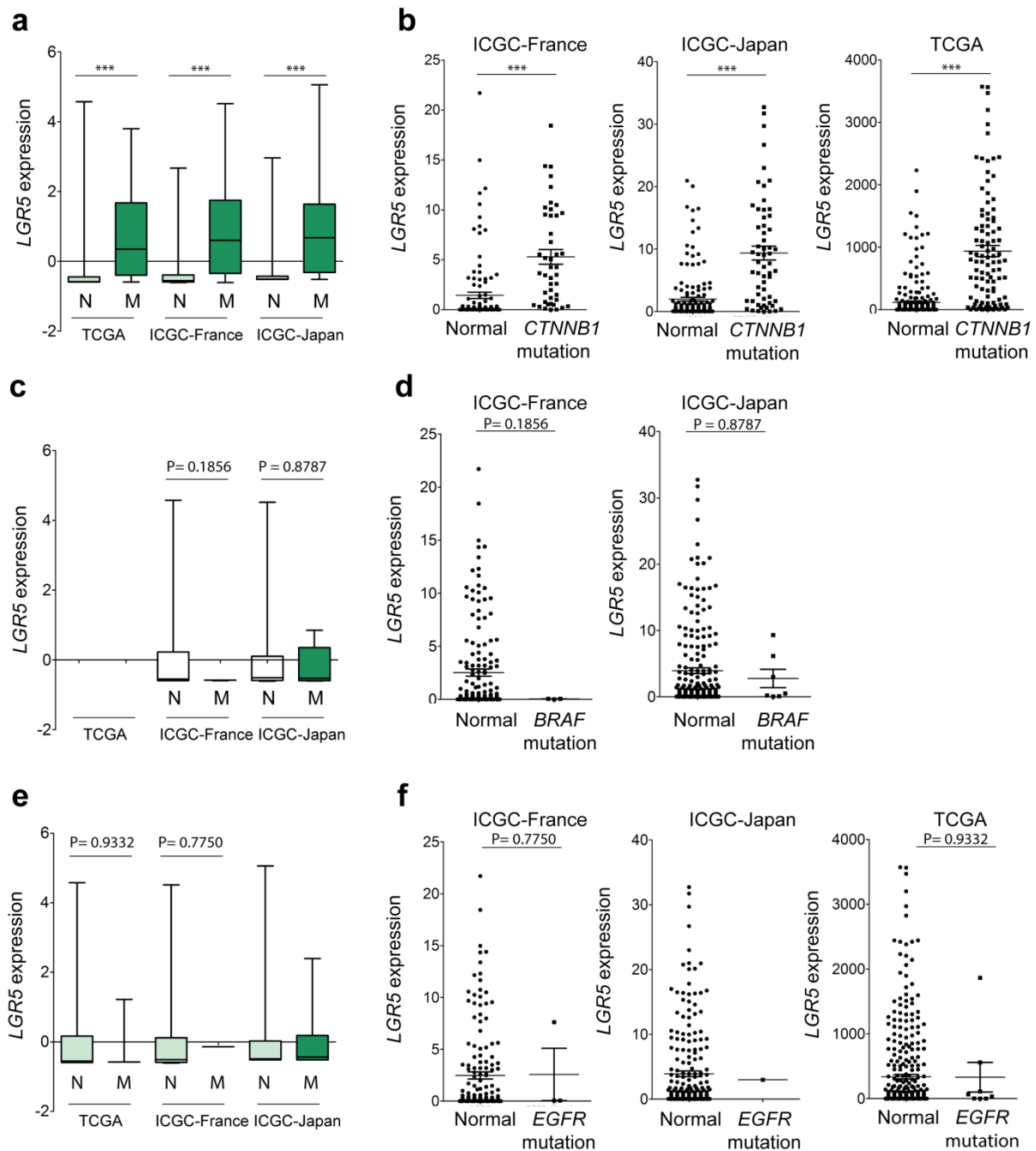
32 Representative FACS plots showing that LGR5⁺ cells are present in the liver following DEN
33 administration (Post DEN induction day 7). **c**, Relative LGR5⁺ fraction in DEN-induced liver tumors. **d**,
34 Relative LGR5⁺ fraction in tissue bordering the DEN-induced liver tumors (denominated as
35 surrounding). **e-f**, Representative immunohistochemistry pictures showing the microscopic aspect of
36 the LGR5⁺ cells in liver cancer. Two types of LGR5⁺ cell distribution patterns are apparent, scattered
37 LGR5⁺ cell distribution (**e**) and LGR5⁺ island-like clusters (**f**). The pictures show an anti-GFP
38 immunohistochemistry staining in which LGR5⁺ cells are brown and nuclei are blue. Scale bar = 50µm.
39 **g**, The percentage of LGR5-expressing cells within each tissue, grouped by tumor collection month
40 (the month is counted since the first administration of DEN). Mean ± SEM. **(h)** Relative mice number
41 fraction in all DEN-induced mice, grouped by tumor collection month. Source data are provided as a
42 Source Data file.

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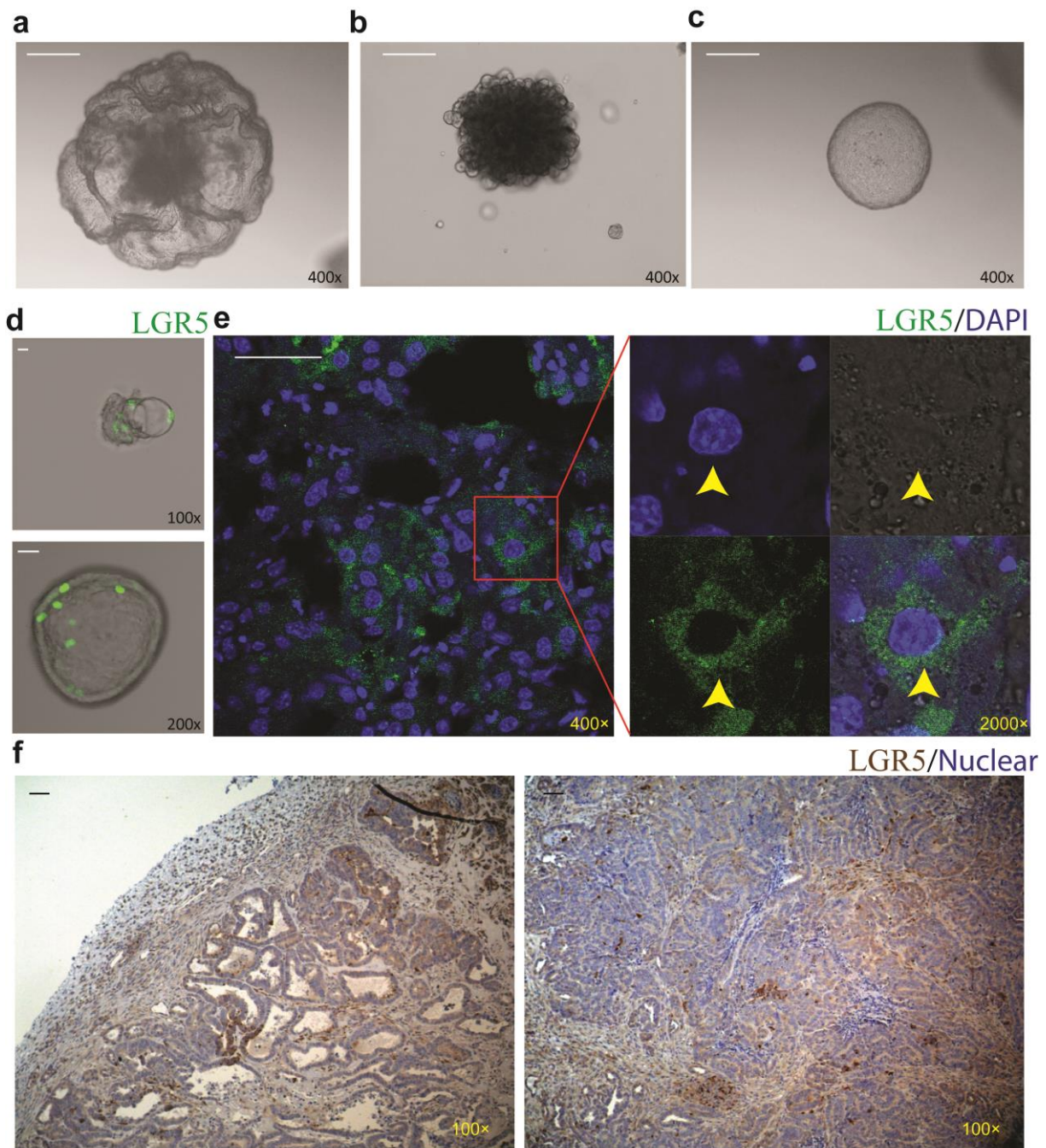
45 **Supplementary Figure 3 | *LGR5* expression in HCC tissues and relation to patient outcome.** **a**, The
 46 upregulated expression of *LGR5* in HCC tissues compared with normal tissues from TCGA Liver cohort
 47 (Normal vs. HCC: $n = 115$ vs. $n = 97$, $P = 0.085$, Fold change: 1.013), Wurmbach liver cohort (Normal vs.
 48 HCC: $n = 10$ vs. $n = 35$, $P = 4.31E^{-5}$, Fold change: 5.174) and Roessler liver cohort (Normal vs. HCC: $n =$
 49 200 vs. $n = 225$, $P = 6.72E^{-14}$, Fold change: 2.034). **b**, Kaplan-Meier curve for HCC of patients based on
 50 different etiology/tumor stage of HCC from TCGA Liver cohort. AJCC_T: American Joint Committee on
 51 Cancer (AJCC) tumor staging system; Stage: Barcelona Clinic Liver Cancer (BCLC) tumor staging system.
 52 Source data are provided as a Source Data file.



53

54 **Supplementary Figure 4 | The expression level of *LGR5* is upregulated in *Cttnb1* mutated HCC tumors.**

55 **a-b**, Upregulation of *LGR5* expression in *Cttnb1* mutated compared to non-*Cttnb1* mutated patient
 56 HCC tumors, in ICGC-France, ICGC-Japan and TCGA cohort. **c-d**, No significant difference of *LGR5*
 57 expression in *Braf* mutated compared to non-*Braf* mutated HCC tumors, in ICGC-France and ICGC-
 58 Japan cohort. **e-f**, No significant difference of *LGR5* expression in *Egfr* mutated compared to non-*Egfr*
 59 mutated HCC tumors, in ICGC-France, ICGC-Japan and TCGA cohort. Mean \pm SEM. Source data are
 60 provided as a Source Data file.

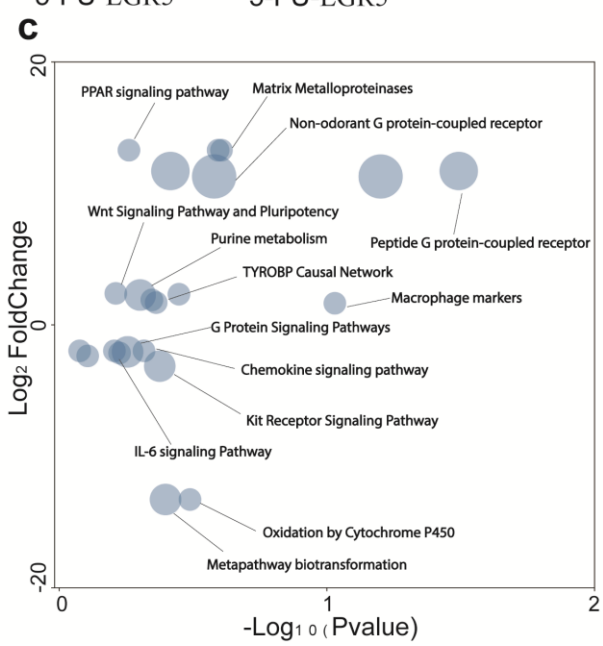
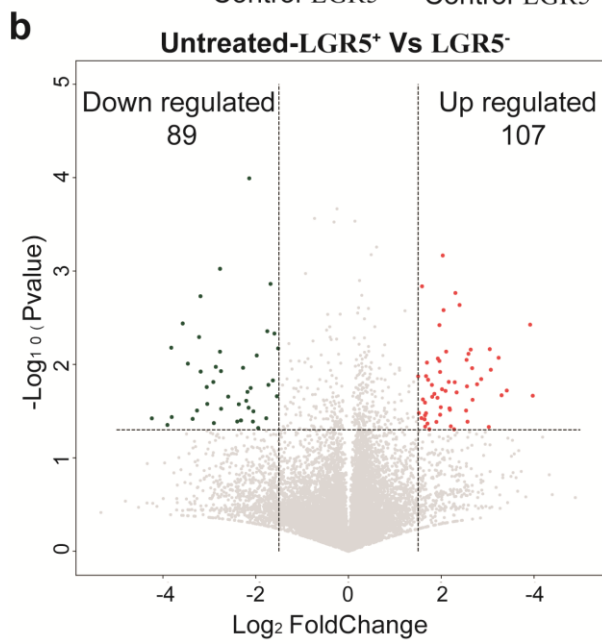
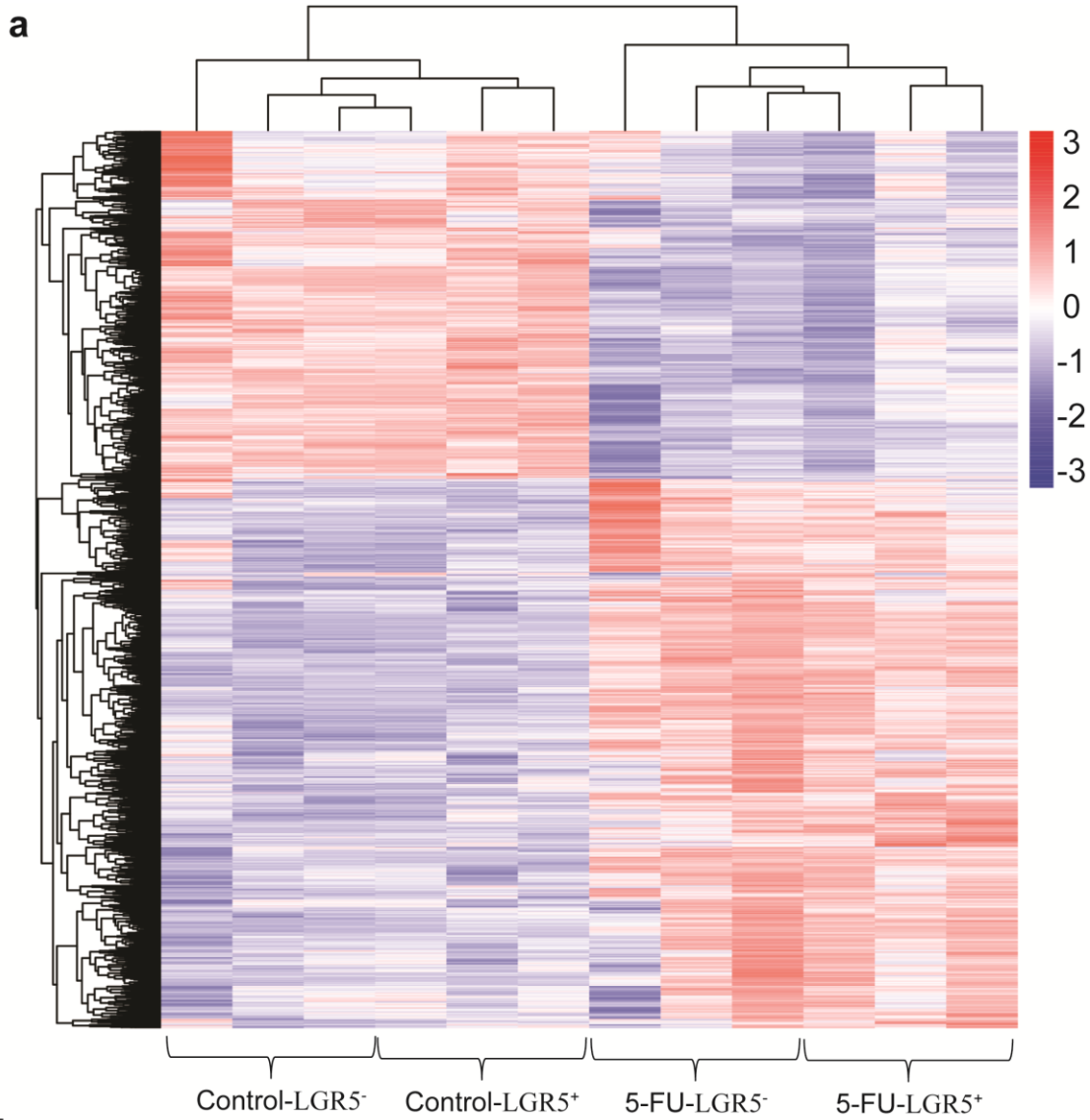


61

62 **Supplementary Figure 5 | Allograft liver tumors maintain an LGR5⁺ compartment.** a-c, 3-7 days after
 63 initiation of allograft tumor-derived cultures, small organoids were observed and subsequently
 64 passage was performed every 4-7 days, employing splitting ratios ranging from 1:4 to 1:10 as
 65 appropriate. Representative pictures show the different morphologies of the tumor organoids
 66 obtained, which include but also flower-like, irregular sheet-like structures (a), and grape-like,
 67 condensed phenotypes (b), and relative normal hollow sphere-like aspects (c). d, Representative
 68 confocal pictures showing the maintenance of an LGR5⁺ compartment in tumor organoids (LGR5-
 69 driven GFP: green). e, Representative immunofluorescent pictures showing an LGR5⁺ compartment

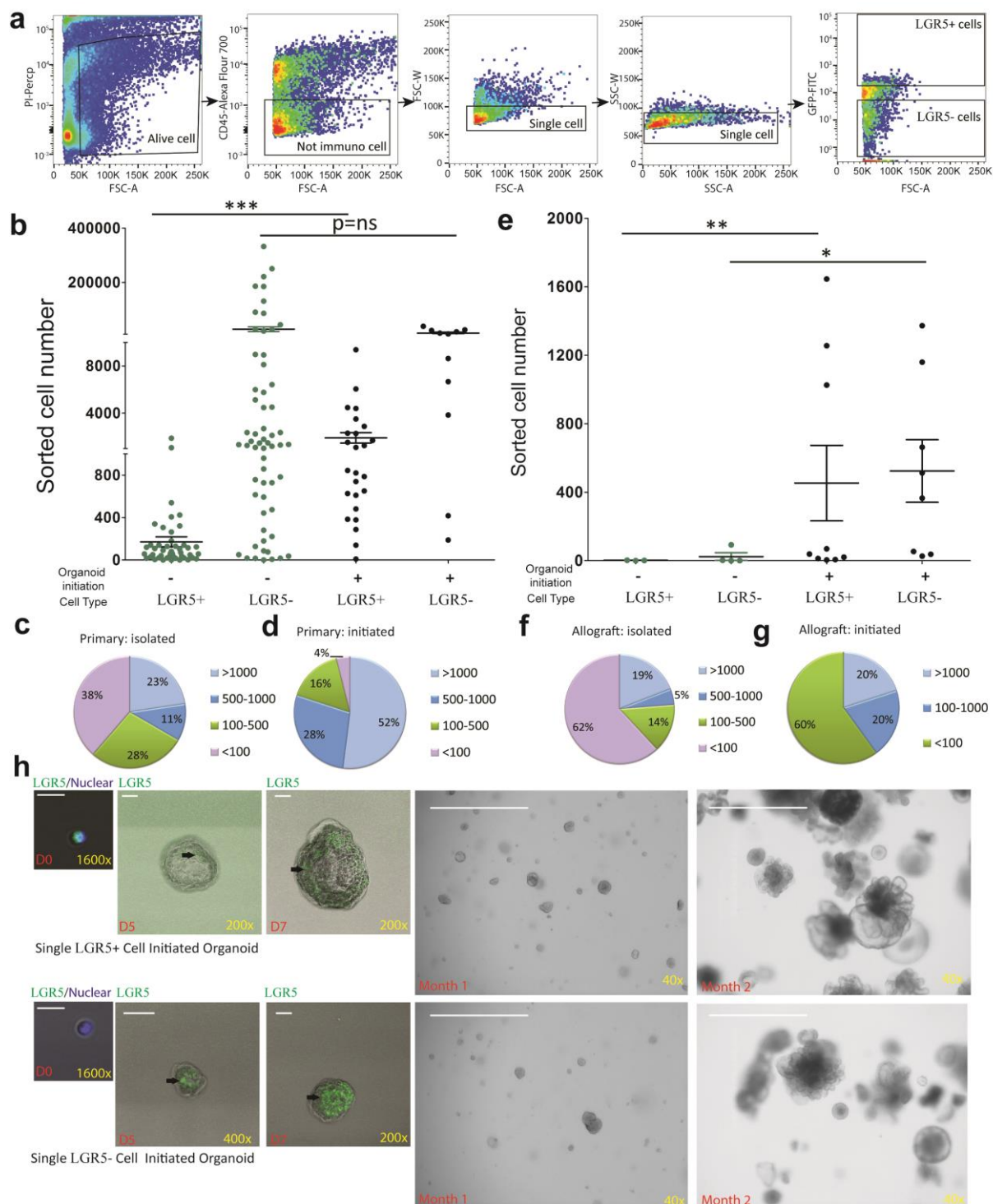
70 in allograft tumors (*LGR5*-driven GFP: green; DAPI: blue). **f**, Representative immunohistochemistry
71 pictures showing expression of *LGR5* promotor-driven GFP in allograft tumors (anti-GFP
72 immunohistochemistry: brown; nuclei: blue). Scale bar = 50 μ m.

73



75 **Supplementary Figure 6 | Genome-wide transcriptomic analysis of LGR5⁺ and LGR5⁻ cells by RNA-**
76 **Seq. a,** Hierarchical clustering showed a separation of all four different groups (Untreated LGR5⁺ cells,
77 Untreated LGR5⁻ cells, 5-FU-treated LGR5⁺ cells, 5-FU-treated LGR5⁻ cells). **b,** A Volcano plot showing
78 the most significantly differentially expressed genes between untreated LGR5⁺ and LGR5⁻ cells. **c,**
79 GSEA with the library of Wiki2019 was performed to reveal the alteration of signaling pathways
80 between untreated LGR5⁺ and LGR5⁻ cells. Source data are provided as a Source Data file.

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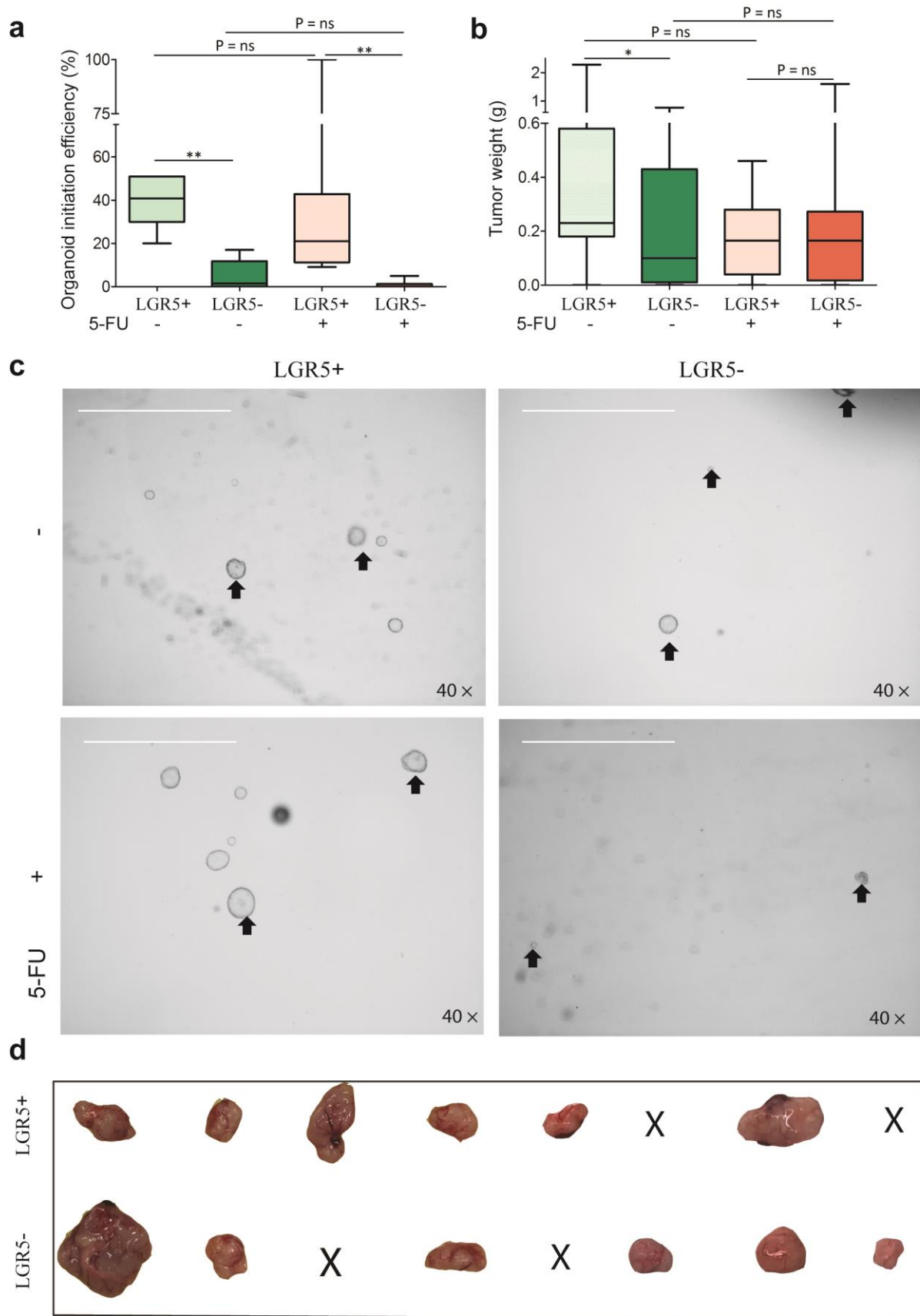


82

83 **Supplementary Figure 7 | LGR5⁺ cells have stronger organoid initiation ability.** **a**, The FACS test/sort
 84 strategy for isolating LGR5-GFP⁺ and LGR5-GFP⁻ cells obtained from liver/allograft tumors/organoids.
 85 **b**, The numbers of sorted LGR5-GFP⁺ and LGR5-GFP⁻ cells yielding successful (black dots) or failure
 86 of organoid initiation (green dots), employing material obtained from DEN-induced murine livers
 87 (LGR5⁺-non-initiation vs. LGR5⁻-non-initiation vs. LGR5⁺-initiation vs. LGR5⁻-initiation: 171.4 ± 47.1,
 88 n = 46 vs. 28350 ± 8914, n = 60 vs. 1906 ± 441.6, n = 25 vs. 13860 ± 3654, n = 11, Mean ± SEM). **c**, The

89 distribution showing the percentage of the number of LGR5⁺ cells which were isolated from primary
90 liver tumors. d, The cell number distribution for the sorted LGR5⁺ cells which can initiate organoid,
91 from primary tumors. e, The exact sorted numbers for LGR5⁺ cells and LGR5⁻ cells , for organoid
92 initiated (black dots) and non-organoid initiated (green dots), from allograft tumors (LGR5⁺-non-
93 initiated vs. LGR5⁻-non-initiated vs. LGR5⁺-initiated vs. LGR5⁻-initiated: 2.0 ± 0.6 , n = 3 vs. 24.3 ± 22.9 ,
94 n = 4 vs. 453.7 ± 220.3 , n = 9 vs. 524.3 ± 182.9 , n = 8). f, Frequency distribution of the relative number
95 of LGR5⁺ cells obtained from allograft liver tumors. g, Frequency distribution of the number of LGR5⁺
96 cells that display successful organoid initiation from material obtained from allograft tumors. h,
97 Representative pictures tracing organoids initiation and growth from LGR5⁺ or LGR5⁻ cells. Black
98 arrow: LGR5 expressing cells. Day0/5/7: Scale bar = 50 μ m; Month1/2: Scale bar = 1000 μ m. Source
99 data are provided as a Source Data file.

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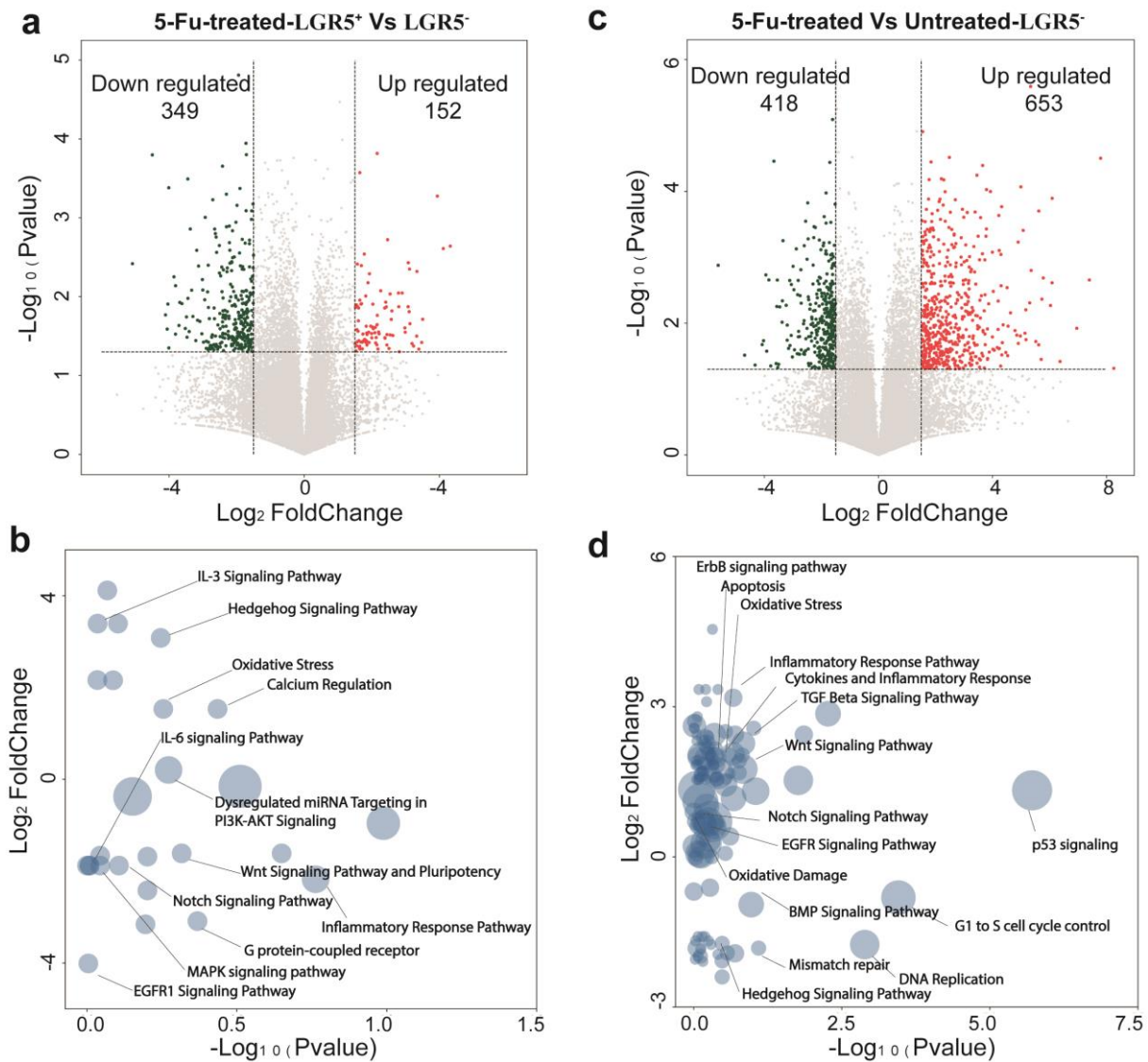


101

102

103 **Supplementary Figure 8 | LGR5⁺ cells from 5-FU treated tumors can initiate organoid and tumor. a,**
104 The organoid initiation ability of untreated LGR5⁺ cells ($39.5 \pm 4.8 \%$, $n = 6$, Mean \pm SEM), untreated
105 LGR5⁻ cells ($5.0 \pm 2.9 \%$, $n = 6$), 5-FU-treated LGR5⁺ cells ($31.2 \pm 14.0 \%$, $n = 6$) and 5-FU-treated LGR5⁻
106 cells ($0.8 \pm 0.8 \%$, $n = 6$). **b,** The tumor initiation ability of untreated LGR5⁺ cells (0.50 ± 0.15 g, $n = 15$),
107 untreated LGR5⁻ cells (0.21 ± 0.06 g, $n = 15$), 5-FU-treated LGR5⁺ cells (0.18 ± 0.05 g, $n = 8$) and 5-FU-
108 treated LGR5⁻ cells (0.31 ± 0.19 g, $n = 8$). **c,** Representative picture showing the organoid initiation
109 ability of above four groups. Black arrow: initiated organoids. Scale bar = 1000 μ m. **d,** Pictures showing
110 5-FU-treated LGR5⁺ cells and 5-FU-treated LGR5⁻ cells initiated tumors. Source data are provided as
111 a Source Data file.

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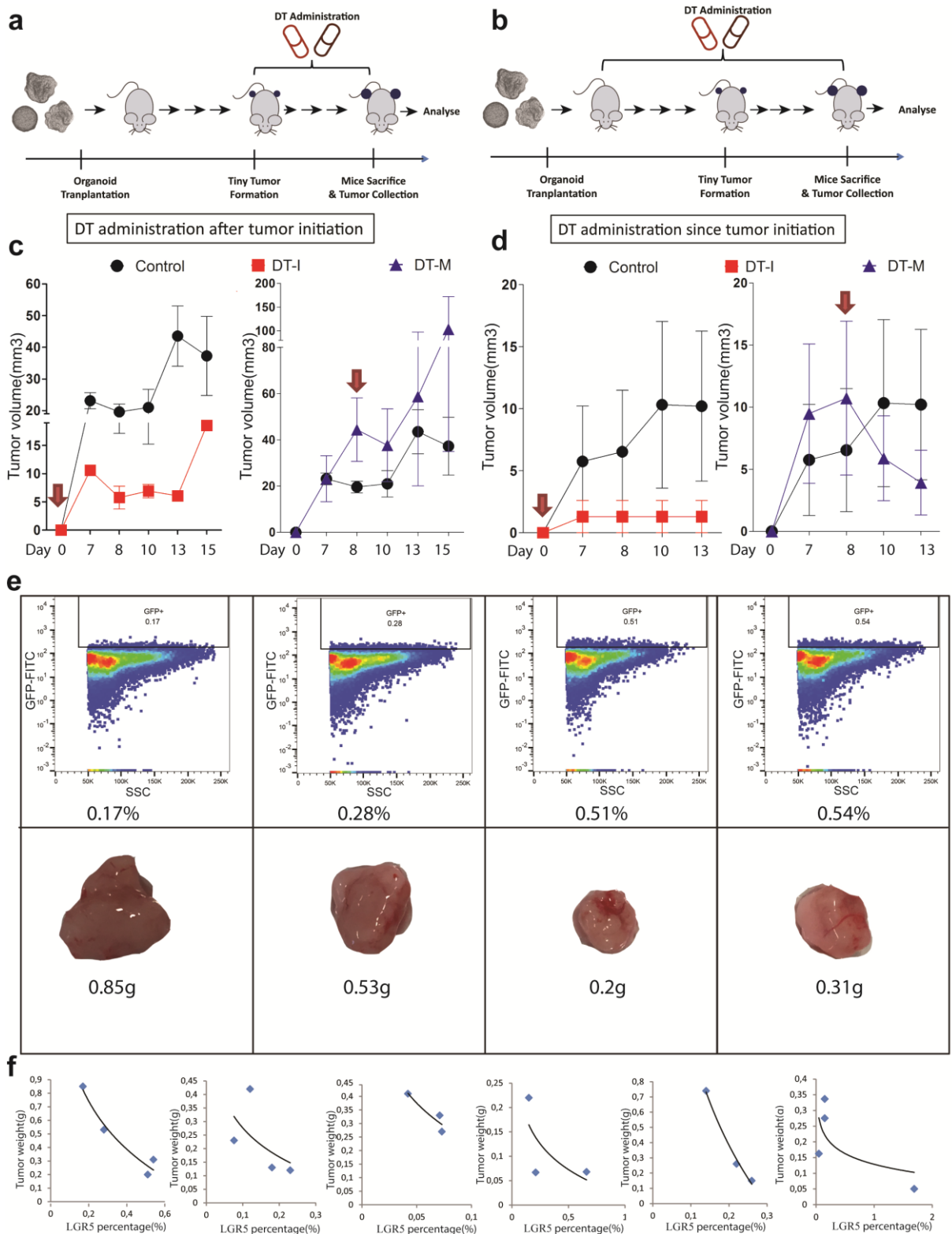


113

114 **Supplementary Figure 9 | Genome-wide transcriptomic analysis of 5-FU treatment on LGR5^{+/-} cells.**

115 **a**, A Volcano plot showing the most significantly differentially expressed genes between 5-FU treated
 116 LGR5⁺ cell/LGR5⁻ cells. **b**, Gene enrichment analysis (Wiki2019) of the differentially expressed genes
 117 between 5-FU treated LGR5⁺ cell/LGR5⁻ cells. **c**, A Volcano plot showing the most significantly
 118 differentially expressed genes between 5-FU treated/untreated LGR5⁻ cells. **d**, Gene enrichment
 119 analysis (Wiki2019) of the differentially expressed genes between 5-FU treated/untreated LGR5⁻ cells.
 120 Source data are provided as a Source Data file.

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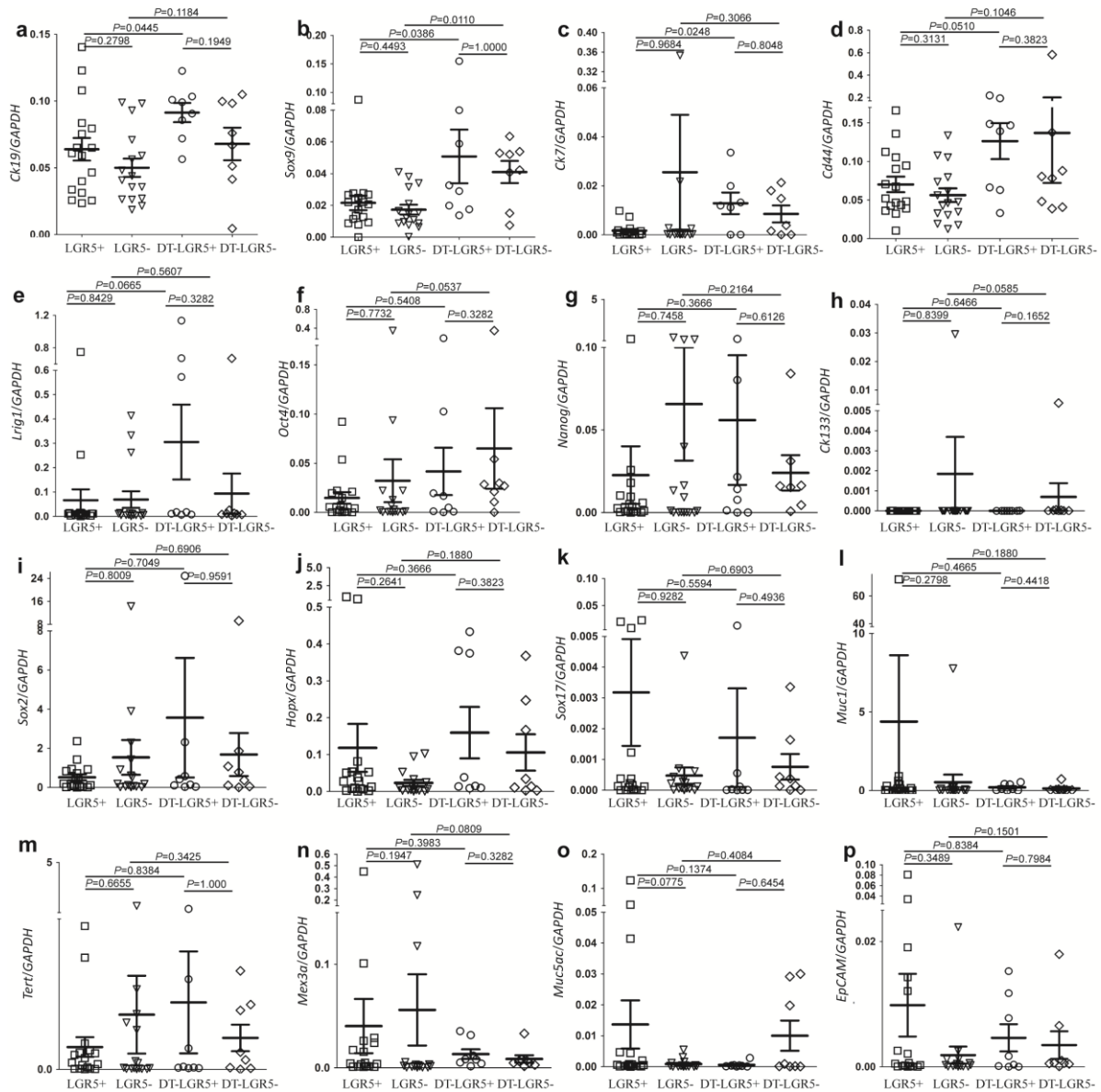


122

123 **Supplementary Figure 10 | Targeting LGR5 impedes the tumor growth.** a-b, Outline of the
 124 experimental strategies used to test the effect of DT administration during tumor growth (a) and at
 125 tumor initiation (b). c-d, Growth curves showing the effects of DT administration during the entire
 126 experimental period (Left, DT-I) and following DT intervention during tumor growth (Right, DT-M) for
 127 organoid strain 1 (c) and strain 2 (d). Mean ± SEM. Red arrow: onset of DT administration (n = 4 for

128 each time point). **e**, Representative FACS pictures (upper channel, with LGR5-GFP⁺ expression) and
129 tumor pictures (lower channel, with tumor weights) showing that the same tumor strains, collected
130 from a single mouse, shows variable LGR5 expression. **f**, tumors collected from six individual mice
131 (transplanted with same strain and same amount of organoid; collected on the same day, non-treated
132 tumors) showing that smaller tumors have relatively higher LGR5 expression. Source data are
133 provided as a Source Data file.

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Supplementary Figure 11 | The expression profile of untreated/DT-treated LGR5⁺ and LGR5⁻ cells.

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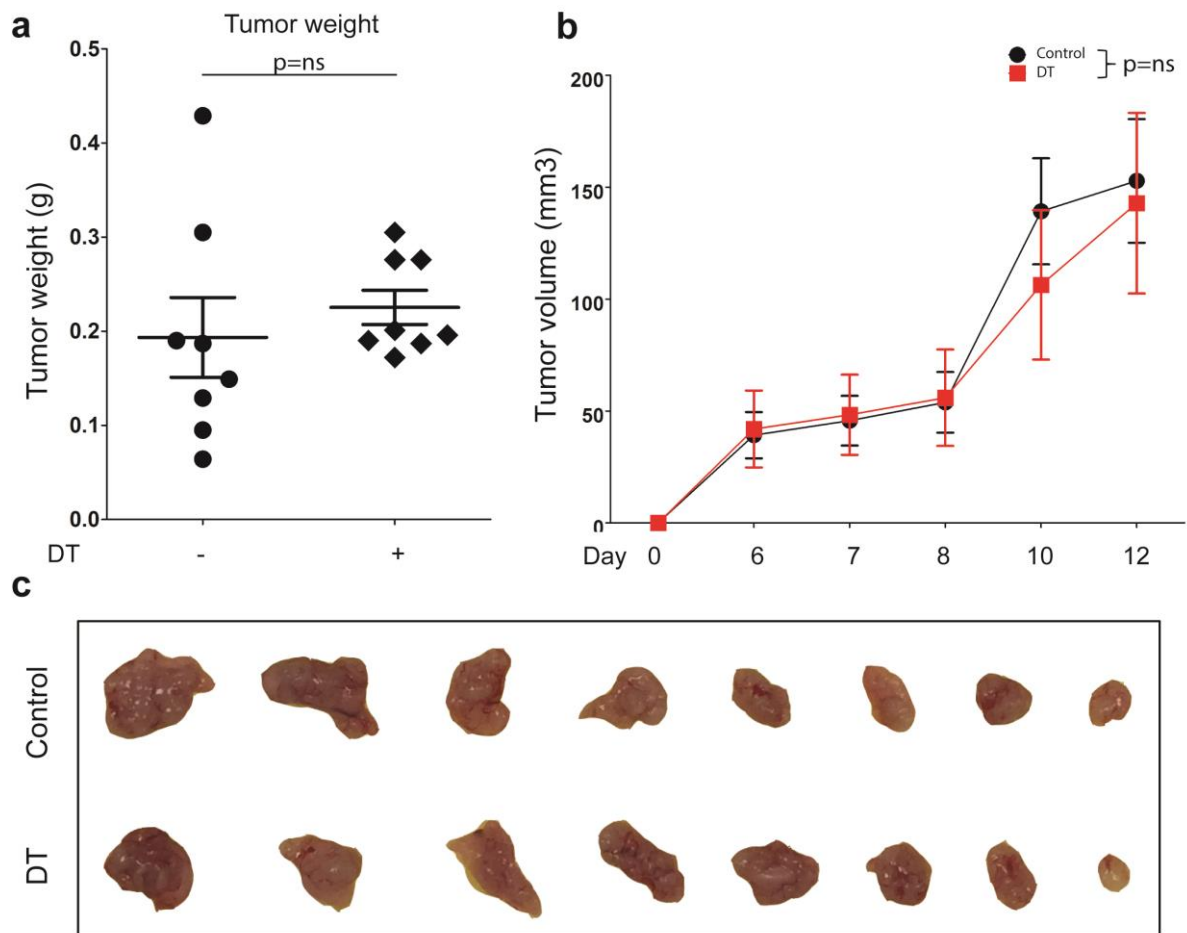
a-p, The expression of stem cell/tumor stem cell markers in LGR5⁺/LGR5⁻ and DT treated tumor isolated LGR5⁺/LGR5⁻ cells was analyzed using qRT-PCR and related to a reference gene. Mean ± SEM.

138

Source data are provided as a Source Data file.

139

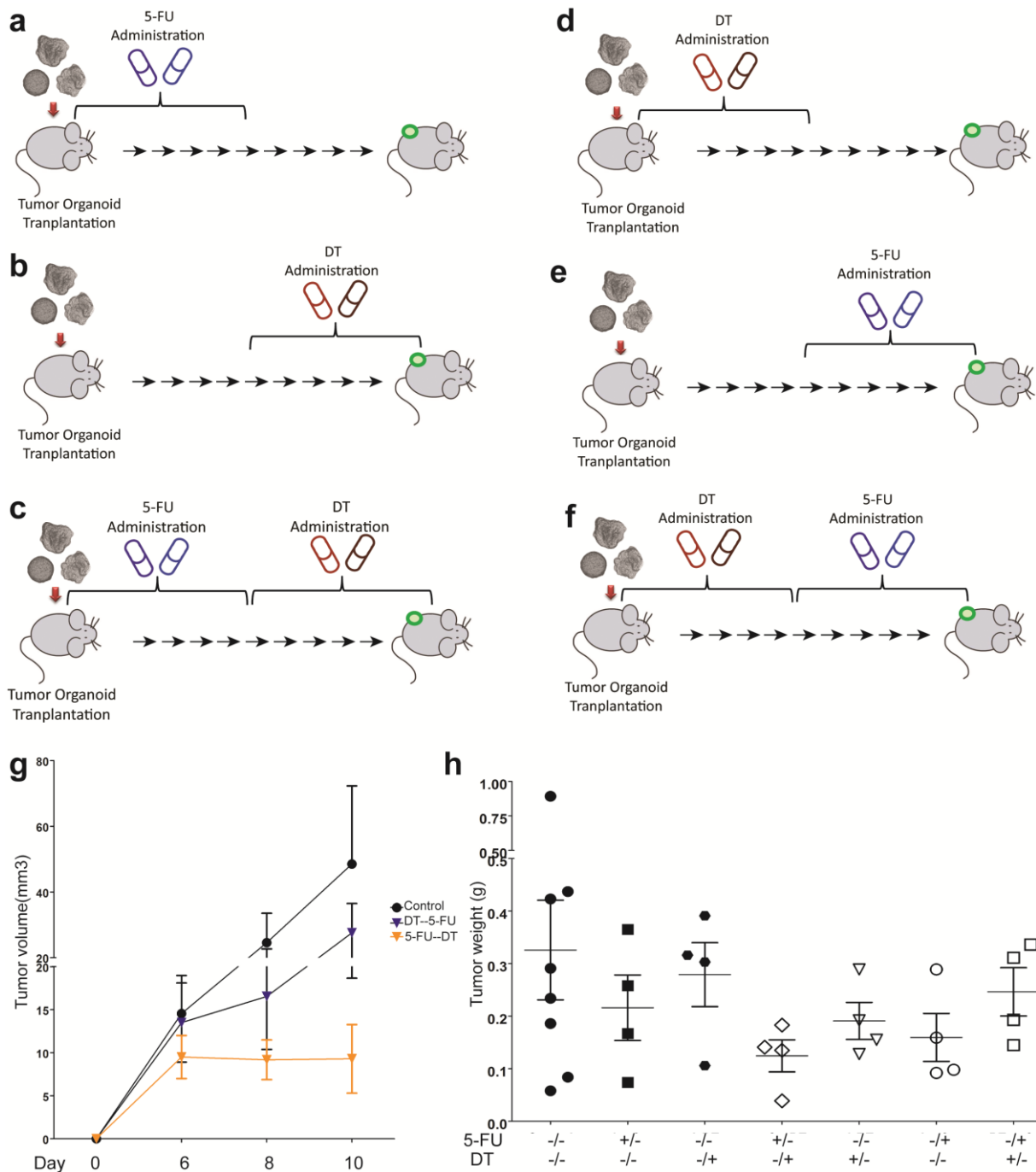
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141

142 **Supplementary Figure 12 | Wide type tumor organoids do not respond to DT administration.** **a**, The
 143 weight of tumors initiated by wild type organoids from control, the DT-treated group following sacrifice
 144 of the animals involved (Control vs. DT: 0.19 ± 0.042 g vs. 0.23 ± 0.018 g, $n = 8$, $P = ns$, Mean \pm SEM). **b**,
 145 Representative growth curve showing tumor volume in the control group and the DT-treated group (n
 146 = 4). **c**, Representative pictures showing the tumors from the control group and DT administrated
 147 group. Source data are provided as a Source Data file.

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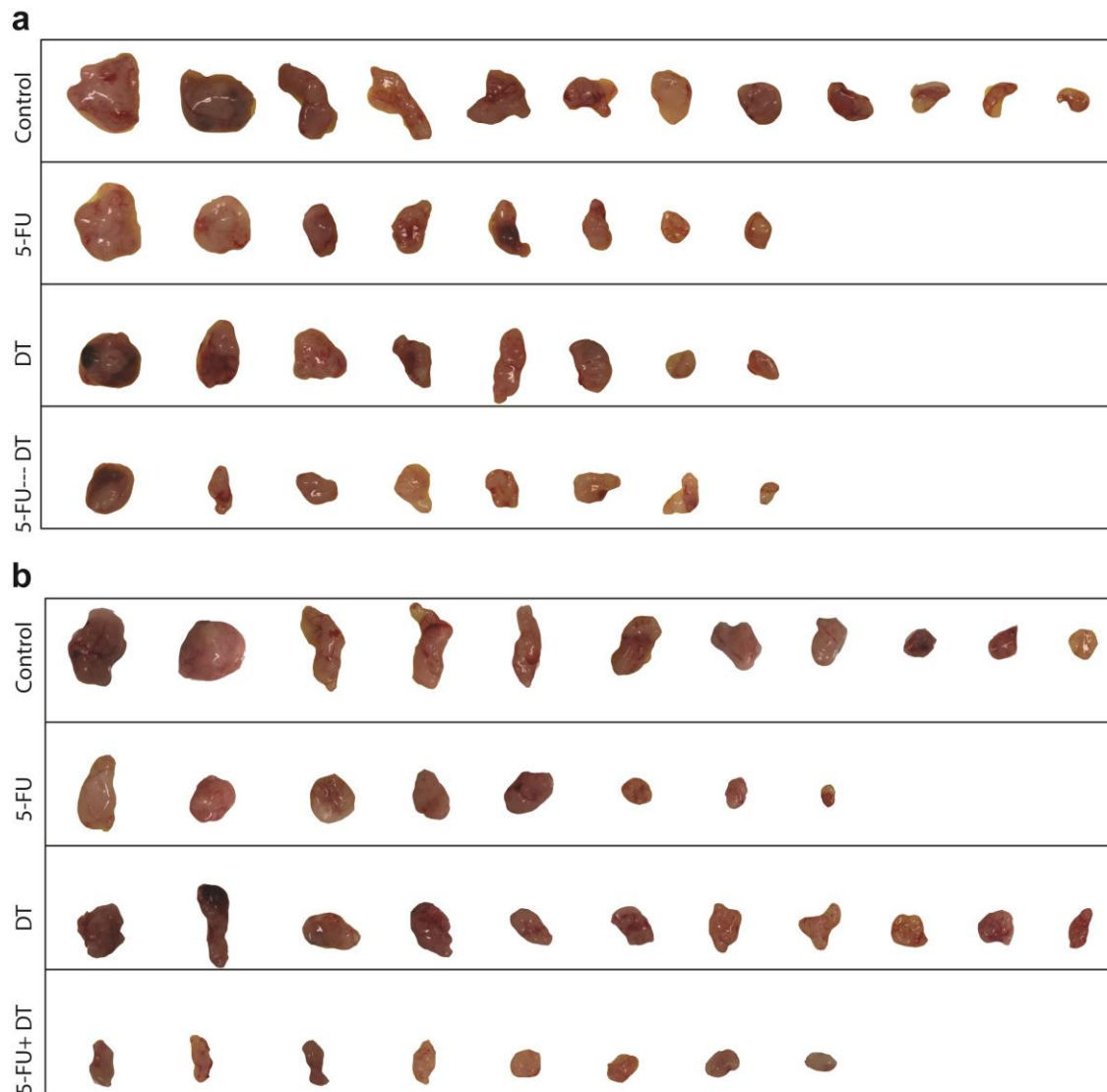
149

150 **Supplementary Figure 13 | Effects of combining DT treatment with conventional anti-cancer therapy.**

151 **a**, 5-FU control: 5-FU was administrated during the first half of the experiment period and tumor
 152 volume was assessed continuously. **b**, DT control: animals were treated with DT during the second half
 153 of the experiment period and tumor volume was assessed continuously. **c**, Combination strategy 1: 5-
 154 FU was administrated during the first half and DT was treated during the second half of the experiment
 155 period. Tumor volume was assessed continuously. (**a** and **b** are the relevant control groups for **c**). **d**,
 156 DT control: DT was administrated during the first half of the experiment period and tumor volume was
 157 assessed continuously. **e**, 5-FU control: 5-FU was administrated during the second half of the

158 experiment period the and tumor volume was assessed continuously. **f**, Combination strategy 2: First
159 DT was applied (first half of the experiment) followed by 5-FU treatment (second half of the
160 experiment) (**d** and **e** are the relevant control groups for **f**). **g**, Representative growth curve showing
161 tumor volumes of the control, Combination strategy 1 (5-FU--DT) and Combination strategy 2 (DT--5-
162 FU) group ($n = 4$). **h**, Weight of tumors from the different groups described above at the end of the
163 experiment. +/-: Treatment was administrated for the first half of the experiment; -/+: Treatment was
164 administrated for the second half of the experiment; -/-: No treatment. Mean \pm SEM. Source data are
165 provided as a Source Data file.

166



167

168 **Supplementary Figure 14 | Combination of LGR5 lineage ablation with conventional therapy. a-b,**
 169 Pictures showing the tumors from the different groups.

170

Allograft Tumor						
Initiated Tumor Code	Isolated Cell Population	Original Strain	Injected Cell Number	Initiated Tumor Weight(g)	Tumor Formation Time	Immunodeficient mice type
1	LGR5+	SAL1	72	0.58	23	Nude mice
	LGR5-		72	0.27	23	Nude mice
2	LGR5+	SAL1	57	0.44	23	Nude mice
	LGR5-		57	0.01	23	Nude mice
3	LGR5+	SAL1	90	0.14	23	Nude mice
	LGR5-		90	0.11	23	Nude mice
4	LGR5+	SAL1	167	0.22	23	Nude mice
	LGR5-		167	0.08	23	Nude mice
5	LGR5+	AL43	66	2.28	38	Nude mice
	LGR5-		66	0.45	38	Nude mice
6	LGR5+	AL43	53	1.11	38	NSG
	LGR5-		53	0.43	38	NSG
7	LGR5+	AL43	57	0.51	38	NSG
	LGR5-		57	0.77	38	NSG
8	LGR5+	SAL2	237	0.8	26	Nude mice
	LGR5-		237	0.05	26	Nude mice
9	LGR5+	SAL2	699	0.23	26	Nude mice
	LGR5-		699	0.1	26	Nude mice
10	LGR5+	SAL1	182	0.53	26	Nude mice
	LGR5-		182	0.09	26	Nude mice
11	LGR5+	SAL1	127	0.18	26	Nude mice
	LGR5-		127	0.61	26	Nude mice

172

173

Table. Patient characteristics	
Characteristic	HCC patients (n=74)
Age at surgery (years)	
Mean \pm SD	60 \pm 15,9
Median (range)	63 (11-82)
Sex – no. (%)	
Male	45 (60,8)
Female	29 (39,2)
Race – no. (%)	
White	61 (82,4)
African	6 (8,1)
Asian	6 (8,1)
Not reported	1 (1,4)
Etiology – no. (%)	
No known liver disease	21 (28,4)
Alcohol	16 (21,6)
Hepatitis B	9 (12,2)
NASH	8 (10,8)
Hepatitis C + Alcohol	6 (8,1)
Hepatitis B + Alc/HepC/NASH	5 (6,8)
Hepatitis C	5 (6,8)
Fibrolamellar HCC	3 (4,1)
Hemochromatosis + NASH	1 (1,4)
Hepatitis status – no. (%)	
Hepatitis B or C positive	25 (33,8)
Chronic Hepatitis B	14 (18,9)
Chronic Hepatitis C	12 (16,2)
Cirrhosis – no. (%)	
Yes	21 (28,4)
No	53 (71,6)
Tumor differentiation – no. (%)	
Good	8 (10,8)
Moderate	40 (54,1)
Poor	14 (18,9)
Unknown	12 (16,2)
Vascular invasion – no. (%)	
Yes	29 (39,2)
No	38 (51,4)
Unknown	7 (9,5)
Number of lesions – no. (%)	
1	40 (54,1)
>1	34 (45,9)
Size of largest lesion (cm)	
Mean \pm SD	7,7 \pm 5,6
Median (range)	6,1 (1-24)
AFP level before resection (ug/l)	
Mean \pm SD	6661,1 \pm 407289,4
Median (range)	8 (1-3118700)

176 **Supplementary Table 3 | Antibody**

Antibody	Antibody clone/ reference	Raised	Origin
CD45	56-0451-82	Mouse	eBioscience
AFP	SAB3500533- 100UG	Goat	Sigma
HNF4a	ab41898	Mouse	Abcam
EpCAM	ab71916	Rabbit	Abcam
Cytokeratin 19	ab52625	Rabbit	Abcam
GFP	A-11122	Rabbit	Invitrogen/Life Technologies
Alexa Fluor® 488 AffiniPure Donkey Anti- Goat IgG (H+L)	705-545-147	Donkey	Bio-Connect
Donkey anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor® 594 conjugate	R37119	Donkey	Thermo fisher
Donkey anti-mouse IgG (H+L) Secondary Antibody, Alexa Fluor® 594 conjugate	R37115	Donkey	Thermo fisher

177

178

Gene name	Gene Symbo	Sequence	
Leucine-rich repeat-containing G-protein coupled receptor 5	Mouse- <i>Lgr5</i>	Fw	CTG ACT TTG AAT GGT GCC TCG
		Re	ATG TCC ACT ACC GCG ATT AC
Cytokeratin-19	Mouse- <i>Krt19</i>	Fw	GTG AAG ATC CGC GAC TGG T
		Re	AGG CGA GCA TTG TC AAT CTG
Transcription factor SOX-9	Mouse- <i>Sox9</i>	Fw	CGA CTA CGC TGA CCA TCA GA
		Re	GAC TGG TTG TTC CCA GTG CT
CD133	Mouse- <i>Prom1</i>	Fw	TCT GTT CAG CAT TTC CTC AC
		Re	TCA GTA TCG AGA CGG GTC
CD44	Mouse- <i>CD44</i>	Fw	CGT CCA ACA CCT CCC ACT AT
		Re	AGC CGC TGC TGA CAT CGT
Keratin, type II cytoskeletal 7	Mouse- <i>Ck7</i>	Fw	ATC CGC GAG ATC ACC ATC
		Re	ATG TGT CTG AGA TCT GCG ACT
Leucine-rich repeats and immunoglobulin-like domains protein 1	Mouse- <i>Lrig1</i>	Fw	AAGGGAACCTCAACTTGGCGAG
		Re	ACGTGAGGCCTTCAATCAGC
Octamer-binding transcription factor 4	Mouse- <i>Oct4</i>	Fw	CTGTAGGGAGGGCTTCGGGCACTT
		Re	CTGAGGGCCAGGCAGGAGCACGAG
Homeobox protein NANOG	Mouse- <i>Nanog</i>	Fw	AGGGTCTGCTACTGAGATGCTCTG
		Re	CAACCACTGGTTTTTCTGCCACCG
SRY (sex determining region Y)-box 2	Mouse- <i>Sox2</i>	Fw	GGCAGCTACAGCATGATGCAGGAGC
		Re	CTGGTCATGGAGTTGTACTGCAGG
Homeodomain-only protein	Mouse- <i>Hopx</i>	Fw	CATCCTTAGTCAGACGCGCA
		Re	AGGCAAGCCTTCTGACCGC
Telomerase reverse transcriptase	Mouse- <i>Tert</i>	Fw	GCAGGTGAACAGCCTCCAGACAG
		Re	TCCTAACACGCTGGTCAAAGGGAAGC
RNA-binding protein MEX3A	Mouse- <i>Mex3a</i>	Fw	ACACCACGGAGTGCGTTC
		Re	GTTGGTTTTGGCCCTCAGA
Mucin 5AC	Mouse- <i>Muc5ac</i>	Fw	GGACCAAGTGGTTTGACACTGAC
		Re	CCTCATAGTTGAGGCACATCCCAG
Epithelial cell adhesion molecule	Mouse- <i>Epcam</i>	Fw	CGCAGCTCAGGAAGAATGTG
		Re	TGAAGTACACTGGCATTGACG
SRY-box 17	Mouse- <i>Sox17</i>	Fw	GGCGCAGCAGAATCCAGA
		Re	CCACGACTTGCCACGAT
Cell surface associated or polymorphic epithelial mucin	Mouse- <i>Muc1</i>	Fw	CCCCAGTTGTCTGTTGGGGTC
		Re	GGATTCTACCACCACGGAGCC
Glyceraldehyde 3-phosphate dehydrogenase	Mouse- <i>Gapdh</i>	Fw	TCACCACCATGGAGAAGGC
		Re	GCTAAGCAGTTGGTGGTGCA
Beta-glucuronidases	Human- <i>GUSB</i>	Fw	CAGGTGATGGAAGAAGTGG
		Re	GTTGCTCACAAGGTCACAG
Hypoxanthine phosphoribosyltransferase 1	Human- <i>HPRT1</i>	Fw	GCTATAAATTCTTTGCTGACCTGCTG
		Re	AATTACTTTTATGTCCCCTGTTGACTGG
Phosphomannomutase 1	Human- <i>PMM1</i>	Fw	CGAGTTCTCCGAAGTGGAC
		Re	CTGTTTTTCAGGGCTTCCAC
Leucine-rich repeat-containing G-protein coupled receptor 5	Human- <i>LGR5</i>	Fw	TCAGTCAGCTGCTCCCGAAT
		Re	CGTTTCCCGCAAGACGTAAC

181 **Supplementary Note:**

182 **Description for Supplementary Data**

183 **[1] Supplementary Data 1**

184 **Content:**

185 The detailed information for *Lgr5-DTRGFP* mice which used to induce liver cancer,
186 including:

187 1)The mice code

188 2)The mice background

189 3)Primary Code: The corresponding code of initiated primary organoid strain.

190 Black mark: the tissue did not initiate an organoid strain or the strain was already
191 lost due to infection in the following culture.

192 4)Post Den Time (Month): The sacrifice time after the induction of DEN.

193 5)DEN Administration (Week): Time passed between administration of DEN and
194 sacrifice.

195 6)Tissue Type: S: tumor surrounding tissue; T: tumor tissue. S—T: initially marked
196 with tumor surrounding tissue and then characterized as tumor.

197 7)Percentage of LGR5-expressing cells (%): The percentage of LGR5-expressing
198 cells within each tissue.

199 8)Allograft Strains: The strain code which initiated allograft tumor in the
200 immunodeficient mice.

201 Remarks: In total, 41 mice were monitored. 10 (mice code:
202 8,12,17,18,21,22,29,31,35,41) out of 41 mice liver did not show obvious tumor
203 formation. After the following characterization, 2 (mice code: 8, 22) out of 10
204 were proven to be liver tumor.

205

206 **[2] Supplementary Data 2**

207 **Content:**

208 The H&E/Gomori/EpCAM/AFP/CK19/GFP staining of primary/allograft tissues
209 for all the allograft strains.

210

211 **[3] Supplementary Data 3**

212 **Content:**

213 Gene enrichment analysis of the differentially expressed genes between
214 Untreated LGR5+ Vs. LGR5-, 5-FU-treated LGR5+ Vs. LGR5-, 5-FU-treated Vs.
215 Untreated LGR5+ and 5-FU-treated Vs. Untreated LGR5- cells.

216

217 **[4] Supplementary Data 4**

218 **Content:**

219 Gene: The differentially expressed genes between untreated LGR5+ cells and
220 LGR5- cells.

221 Gene description: The gene description.

222 Survival Analyses: Online database (The Human Protein Atlas,
223 <http://www.proteinatlas.org/>).

224 P value for Survival Analyses: Online database (The Human Protein Atlas,
225 <http://www.proteinatlas.org/>).

226

227 **[5] Supplementary Data 5**

228 **Content:**

229 The detailed information for single cells isolated from DEN induced murine livers
230 and allograft tumors, then used for organoid initiation, including:

231 1)Code: The corresponding tissues of DEN induced murine livers or allograft
232 tumors. Green mark: the groups did initiate organoids after sorting.

233 2) Initiated organoid number for each group.

234 3) Organoid initiated efficiency (%) for each group.

235 Remarks: In total 89 tissues were collected. Among them, 71 tissues were sorted for
236 following single cell initiation; 18 tissues were failed for the sort due to FACS machine
237 issue or other technical reasons.

238

239 **[6] Supplementary Data 6**

240 **Content:**

241 The detailed information for single cells isolated from DEN induced murine livers,
242 then injected directly into immunodeficient mice for tumor formation, including:

243 1)Code: The corresponding tissues of DEN induced murine livers and initiated
244 allograft tumors. Green mark: the groups did initiate tumors after sorting.

245 2) Injected cell number for each group.

246 3) Pictures of primary tumors and corresponding allograft tumors.

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