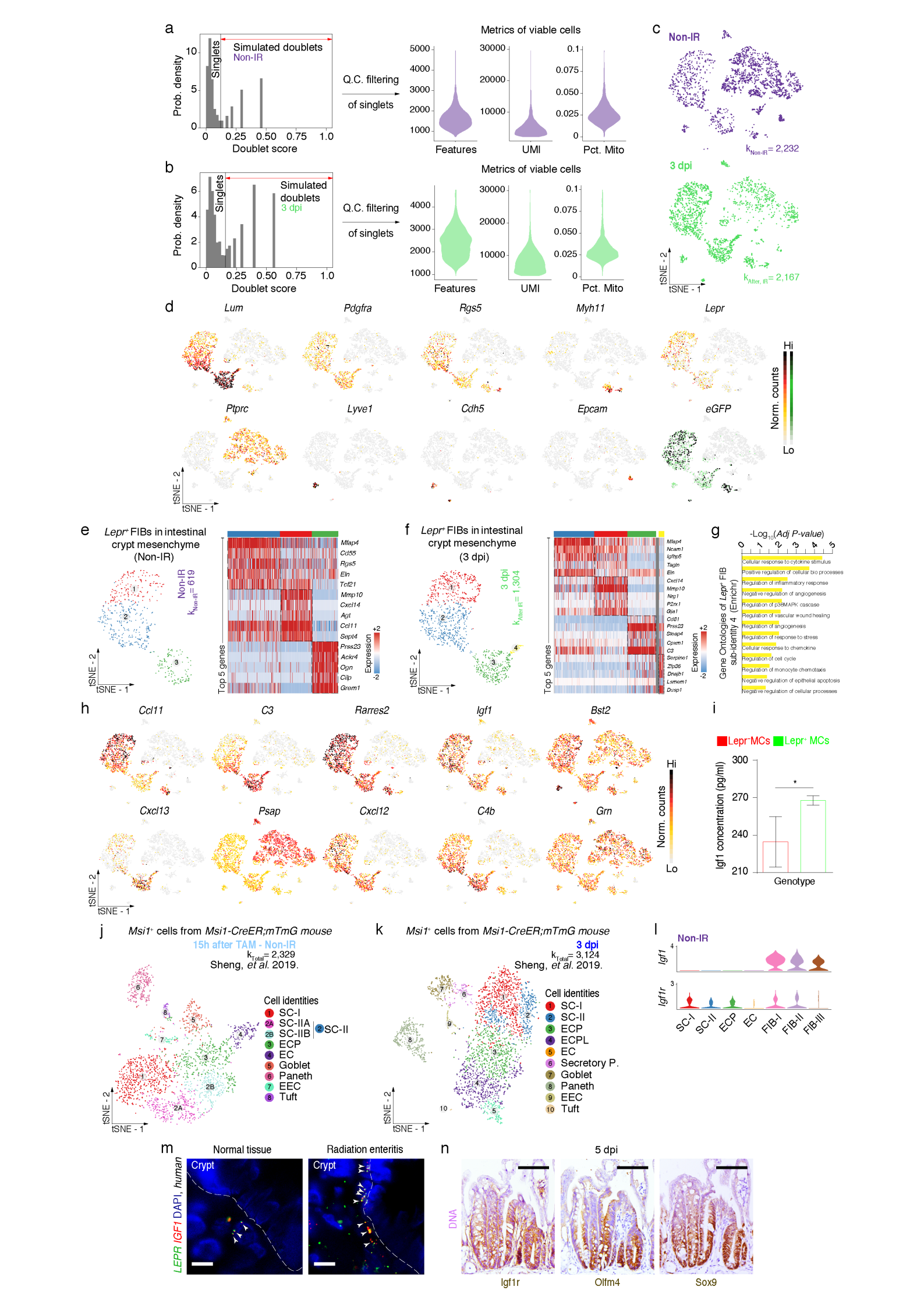
a
Prob. density
Singlets
Simulated doublets
Non-IR
Doublet score
Q.C. filtering
of singlets
Metrics of viable cells
Features
UMI
Pct. Mito
b
3 dpi
c
Non-IR
k_{Non-IR}= 2,232
3 dpi
$k_{After, IR}$= 2,167
tSNE - 1
tSNE - 2
d
Lum
Pdgfra
Rgs5
Myh11
Lepr
Ptprc
Lyve1
Cdh5
Epcam
eGFP
Hi
Lo
Norm. counts
e
Lepr+ FIBs in intestinal crypt mesenchyme (Non-IR)
k_{Non-IR}= 619
Top 5 genes
Expression
f
Lepr+ FIBs in intestinal crypt mesenchyme (3 dpi)
$k_{After IR}$= 1,304
g
-Log10(Adj P-value)
Gene Ontologies of Lepr+ FIB sub-identity 4 (Enrichr)
Cellular response to cytokine stimulus
Positive regulation of cellular bio processes
Regulation of inflammatory response
Negative regulation of angiogenesis
Regulation of p38MAPK cascase
Regulation of vascular wound healing
Regulation of angiogenesis
Regulation of response to stress
Cellular response to chemokine
Regulation of cell cycle
Regulation of monocyte chemotaxis
Negative regulation of epithelial apoptosis
Negative regulation of cellular processes
h
Ccl11
C3
Rarres2
Igf1
Bst2
Cxcl13
Psap
Cxcl12
C4b
Grn
i
Lepr-MCs
Lepr+ MCs
Igf1 concentration (pg/ml)
Genotype
*
j
Msi1+ cells from Msi1-CreER;tmTG mouse
15h after TAM - Non-IR
k_{Total}= 2,329
Sheng, et al. 2019.
Cell identities
SC-I
SC-IIA
SC-IIB
SC-II
ECP
EC
Goblet
Paneth
EEC
Tuft
k
Msi1+ cells from Msi1-CreER;tmTG mouse
3 dpi
k_{Total}= 3,124
Sheng, et al. 2019.
Cell identities
SC-I
SC-II
ECP
ECPL
EC
Secretory P.
Goblet
Paneth
EEC
Tuft
l
Non-IR
Igf1
Igf1r
SC-I
SC-II
ECP
EC
FIB-I
FIB-II
FIB-III
m
Normal tissue
Radiation enteritis
Crypt
LEPR IGF1 DAPI, human
n
5 dpi
DNA
Igf1r
Olfm4
Sox9

Fig. S4 scRNA-seq analysis identified Igf1 as an important niche signal for ISCs. a, b Bimodal distribution of predicted singlet and doublet intestinal crypt mesenchymal cells before and 3 days postirradiation. Straight solid line indicates user-defined doublet score threshold. Singlets were further filtered to remove low-quality cells. Distribution of features/cell, UMI counts/cell, and percentage of mitochondrial genes/cell in valid cells post-filtering. Valid cells were used in downstream query and comparative analysis. **c** Anchored and integrated Lepr$^+$ cells before and 3 days postirradiation (k=1,923 cells) visualized in two-dimensional tSNE embedding. Cells from each condition are color-coded; before irradiation – purple; 3 days postirradiation – green. Circle denotes cluster differences across conditions. **d** Feature plots of biomarker genes in each cell type cluster based on normalized counts. Expression levels for each cell are color-coded. Gray represents low expression and black represents high expression. **e, f** Heatmap of top five genes in Lepr$^+$ fibroblasts before (**e**) and 3 days postirradiation (**f**). Expression levels for each Lepr$^+$ fibroblast population are color-coded. Blue represents downregulated and red represents upregulated gene expression. **g** The gene ontologies of the top 60 specific genes in *Lepr*$^+$ FIB-IV. **h** Feature plots of the identified 10 ligands in each Lepr$^+$ fibroblast cluster based on normalized counts. Expression levels for each cell are color-coded. Gray represents low expression and black represents high expression. **i** Detecting supernatant levels of Igf1 from the culture medium of primary Lepr$^+$ MCs and Lepr$^-$ MCs after 24 hours of culture using ELISA (n=3). **j, k** t-SNE plots revealed nine distinct clusters of *Msi1*$^+$ cells sorted from *Msi1-CreERT2*;*mTmG* mice 15 hours after tamoxifen induction (**j**) and ten distinct clusters of *Msi1*$^+$ cells and their progeny 3 days after γ-IR (**k**). The general identity of each cell cluster is defined on the right. Data sets were adapted from our previous study.[29] **l** Violin plot showing the distributions of *Igf1r* in Msi1$^+$ epithelial cells and Lepr$^+$ cells at homeostasis. **m** *In situ* hybridization for *LEPR* and *IGF1* in intestinal crypts from normal and enteritis human tissues. White arrows represent double-positive cells (n=2). Scale bar: 10 μm. **n** Immunohistochemistry for Igf1r, Olfm4 and Sox9 in intestine from wildtype mice five days postirradiation (n=3). Scale bar: 100 μm. Value in the graph represent means ± SD. Unpaired student's t-test was used for calculating P values in **i**, **P* < 0.005.