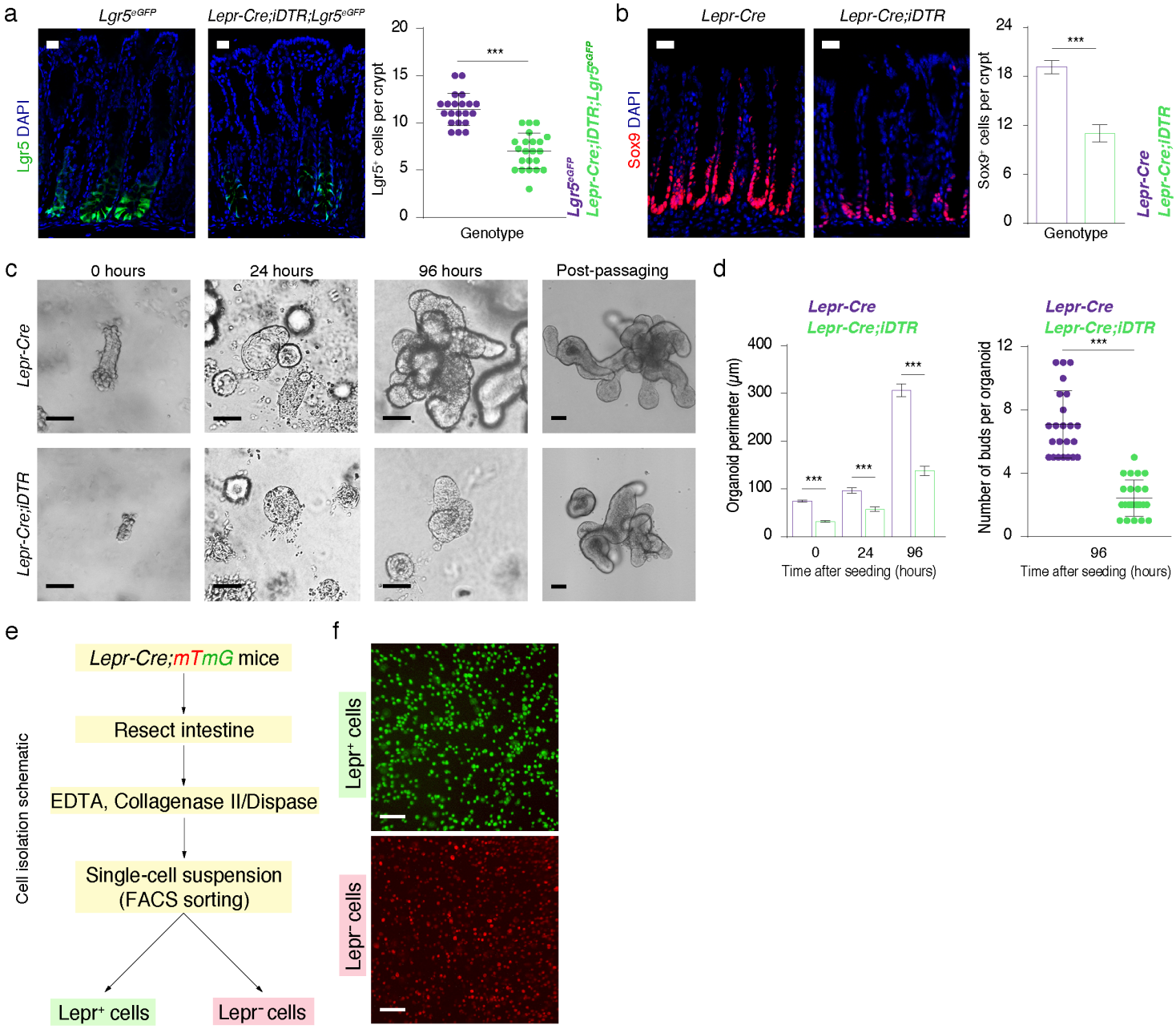
a
Lgr5eGFP
Lepr-Cre;iDTR;Lgr5eGFP
Lgr5 DAPI
Lgr5+ cells per crypt
***
Genotype
Lgr5eGFP
Lepr-Cre;iDTR;Lgr5eGFP
b
Lepr-Cre
Lepr-Cre;iDTR
Sox9 DAPI
Sox9+ cells per crypt
***
Genotype
Lepr-Cre
Lepr-Cre;iDTR
c
0 hours
24 hours
96 hours
Post-passaging
Lepr-Cre
Lepr-Cre;iDTR
d
Lepr-Cre
Lepr-Cre;iDTR
Organoid perimeter (μm)
***
Time after seeding (hours)
Lepr-Cre
Lepr-Cre;iDTR
Number of buds per organoid
***
Time after seeding (hours)
e
Cell isolation schematic
Lepr-Cre;mTmG mice
Resect intestine
EDTA, Collagenase II/Dispase
Single-cell suspension (FACS sorting)
Lepr+ cells
Lepr- cells
f
Lepr+ cells
Lepr- cells

Fig. S3 Ablation of Lepr$^+$ cells led to fewer ISCs in colon. a Immunofluorescence for GFP (Lgr5) in the colons from *Lgr5^{eGFP}* and *Lepr-Cre*;*iDTR*;*Lgr5^{eGFP}* mice treated with DT for 10 consecutive days. Number of Lgr5$^+$ cells per crypt was quantified (n=3). Scale bar: 25 μm. **b** Immunofluorescence for Sox9 in the colon from *Lepr-Cre* and *Lepr-Cre*;*iDTR* mice treated with DT for 10 consecutive days. Number of Sox9$^+$ cells per crypt was quantified (n=3). Scale bar: 25 μm. **c** Intestinal organoid images from *Lepr-Cre* and *Lepr-Cre*;*iDTR* mice at 0, 24 and 96 hours after seeding. Intestinal organoid images from *Lepr-Cre* and *Lepr-Cre*;*iDTR* mice 5 days post-passaging. Mice were treated with DT for 10 consecutive days. Crypt cells were seeded at the same initial density. n=2 biologically independent experiments, 3 replicates each. Scale bar: 50 μm. **d** Quantification of the organoid perimeter at indicated timepoints and the number of buds per organoid at 96 hours. Related to **c**. **e** Schematic depicting physical isolation of Lepr$^+$ (GFP$^+$) and Lepr$^-$ (tdTomato$^+$) cells. **f** Representative images of sorted Lepr$^+$ and Lepr$^-$ cells. Scale: 200 μm. Values in the graphs represent means ± SD. Unpaired student's t-test was used for calculating P values in **a**, **b** and **d**, ***P < 0.001.