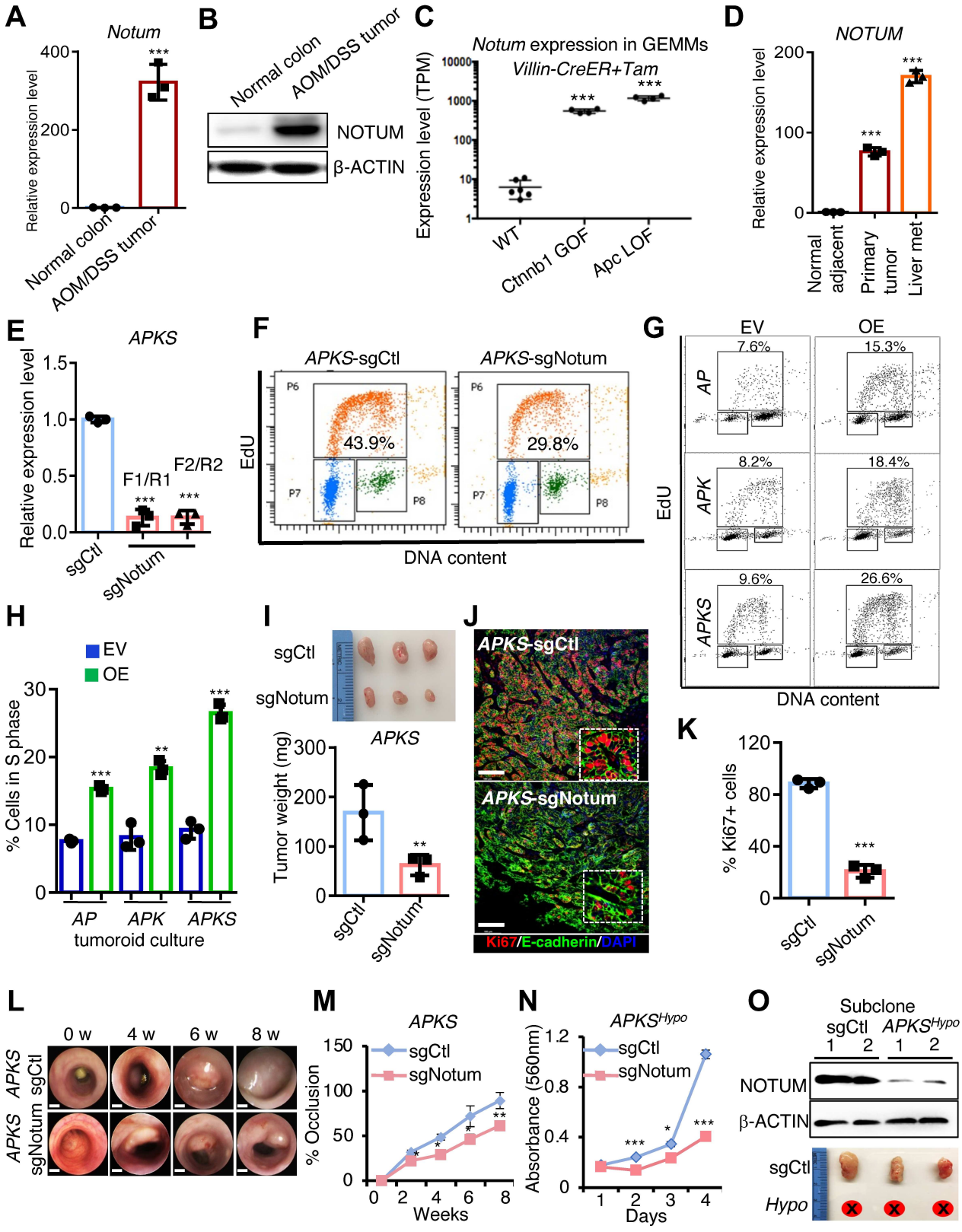

A
Notum
Relative expression level
400
200
0
***
Normal colon
AOM/DSS tumor
B
Normal colon
AOM/DSS tumor
NOTUM
β-ACTIN
C
Notum expression in GEMMs
Villin-CreER+Tam
Expression level (TPM)
10000
1000
100
10
1
***
***
WT
Ctnnb1 GOF
Apc LOF
D
NOTUM
Relative expression level
200
100
0
***
***
Normal adjacent
Primary tumor
Liver met
E
APKS
Relative expression level
1.5
1.0
0.5
0.0
F1/R1
F2/R2
***
***
sgCtl
sgNotum
F
APKS-sgCtl
APKS-sgNotum
P6
P7
P8
43.9%
29.8%
EdU
DNA content
G
EV
OE
7.6%
15.3%
AP
8.2%
18.4%
APK
9.6%
26.6%
APKS
EdU
DNA content
H
EV
OE
% Cells in S phase
30
20
10
0
***
**
***
AP
APK
APKS
tumoroid culture
I
sgCtl
sgNotum
APKS
Tumor weight (mg)
300
200
100
0
**
sgCtl
sgNotum
J
APKS-sgCtl
APKS-sgNotum
Ki67/E-cadherin/DAPI
K
% Ki67+ cells
120
80
40
0
***
sgCtl
sgNotum
L
0 w
4 w
6 w
8 w
APKS sgCtl
APKS sgNotum
M
APKS
sgCtl
sgNotum
% Occlusion
150
100
50
0
Weeks
N
APKHypo
sgCtl
sgNotum
Absorbance (560nm)
1.2
0.8
0.4
0
Days
O
Subclone
sgCtl
APKSHypo
1
2
1
2
NOTUM
β-ACTIN
sgCtl
Hypo

Supplementary Figure 1. Notum is an obligate oncogene in advanced adenocarcinoma models.

(*A*) Quantitative RT-PCR analysis of *Notum* expression in normal mouse colon epithelial cells and AOM/DSS carcinoma cells (n=3 biological replicates).

(*B*) NOTUM protein in normal colon mouse epithelial cells and AOM/DSS carcinoma cells. β-ACTIN was used as a loading control.

(*C*) Expression of *Notum* in colonic epithelium of *Ctnnb1*$^{exon3flx/flx}$*::Villin-CreER* and *Apc*$^{flx/flx}$*::VillinCreER* mice 5 days after tamoxifen-induced gene ablation, relative to controls (n=6 biological replicates).

(*D*) QRT-PCR for *NOTUM* in human primary colorectal adenocarcinoma, cognate liver metastasis, and normal adjacent tissue colon (n=6).

(*E*) Expression of *Notum* in mouse *APKS* tumoroids infected with control sgRNA (sgCtl) or *Notum* sgRNA (sgNotum) (n=3 technical replicates, with one representative of three independent experiments shown).

(*F*) Flow cytometric analysis of EdU incorporation in *APKS* tumoroids infected with control sgRNA (sgCtl) or *Notum* sgRNA (sgNotum) (n=3 technical replicates, with one representative of three independent experiments shown).

(*G*) Flow cytometric analysis of EdU incorporation in mouse *AP*, *APK* and *APKS* after infection with empty lentiviral vector (pUltra-EV) or vector overexpressing Notum (Notum OE) (n=3 technical replicates).

(*H*) Percentage of cells in S phase, from (G).

(*I*) Top: Subcutaneous tumors formed 4 weeks after implantation of mouse *APKS* tumoroids infected with control sgRNA (sgCtl) or *Notum* sgRNA (sgNotum) into syngeneic recipients (n=3 biological replicates). Bottom: Weight of tumors above.

(*J*) Immunofluorescence staining for KI67 and E-CADHERIN on tumors formed from subcutaneous *APKS* injection. Nuclei are visualized by DAPI staining (n=3 biological replicates). Scale bar: 100 μm.

(*K*) Quantification of KI67+ cells in subcutaneous *APKS*-sgCtl and *APKS*-sgNotum mouse tumors.

(*L*) Representative endoscopic pictures showing the development of orthotopically implanted *APKS* tumors with control sgRNA (sgCtl) or *Notum* sgRNA (sgNotum) over 8 weeks of tumor growth (n=5 in sgCtl group, n=4 in sgNotum group). Scale bar: 2 mm.

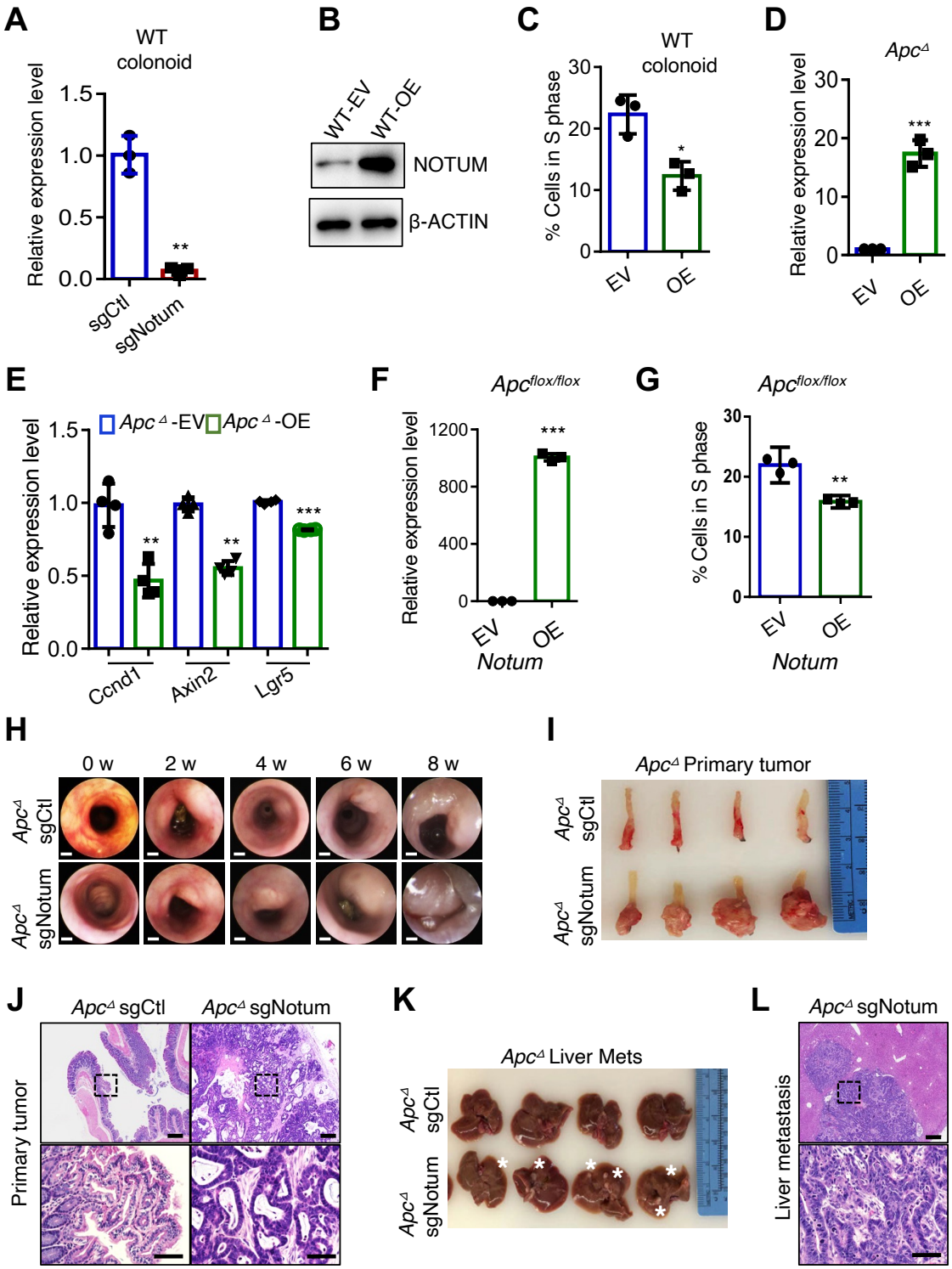
(*M*) The percent of lumen occlusion was measured for each *APKS* tumor from (L) during the course of the experiment.

(*N*) MTT assays in a subclone of mouse *APKS* tumoroids infected with *Notum* sgRNA (sgNotum) and exhibiting hypomorphic NOTUM loss of function (*APKS*^{Hypo}) (n=3 technical replicates).

(*O*) Top: Western blot analysis of mouse NOTUM protein in *APKS*^{Hypo} subclone from (N). β-ACTIN was used as a loading control. Bottom: Subcutaneous tumor formation using the mouse Notum *APKS*^{Hypo} line or control APKS mouse tumoroids.

For all panels: **P*<0.05, ***P*<0.01, ****P*<0.001, Student's *t*-test.

See supplemental figures 8 and 9 for validation of driver mutations, sgRNA knockdown, and overexpression.


A
WT colonoid
Relative expression level
1.5
1.0
0.5
0.0
**
sgCtl
sgNotum
B
WT-EV
WT-OE
NOTUM
β-ACTIN
C
WT colonoid
% Cells in S phase
30
20
10
0
*
EV
OE
D
Apc⊿
Relative expression level
30
20
10
0
***
EV
OE
E
Apc⊿-EV Apc⊿-OE
Relative expression level
1.5
1.0
0.5
0.0
**
**
***
Ccnd1
Axin2
Lgr5
F
Apcflox/flox
Relative expression level
1200
800
400
0
***
EV
OE
Notum
G
Apcflox/flox
% Cells in S phase
30
20
10
0
**
EV
OE
Notum
H
0 w
2 w
4 w
6 w
8 w
Apc⊿ sgCtl
Apc⊿ sgNotum
I
Apc⊿ Primary tumor
Apc⊿ sgCtl
Apc⊿ sgNotum
J
Apc⊿ sgCtl
Apc⊿ sgNotum
Primary tumor
K
Apc⊿ Liver Mets
Apc⊿ sgCtl
Apc⊿ sgNotum
L
Apc⊿ sgNotum
Liver metastasis

Supplementary Figure 2. *Notum* maintains tumor suppressive activity upon APC inactivation.

(*A*) Expression level of *Notum* in wildtype mouse organoids infected with control sgRNA (sgCtl) or *Notum* sgRNA (sgNotum) (n=3 technical replicates).

(*B*) Western blot analysis for NOTUM in wildtype mouse organoids infected with empty vector (EV) or *Notum* overexpression plasmid (OE). β-ACTIN was used as a loading control.

(*C*) The percentage of cells in S-phase (EdU+) in \wildtype mouse organoids infected with empty vector (EV) or *Notum* overexpression plasmid (OE) (n=3 technical replicates).

(*D*) Expression level of *Notum* in *Apc* mutant mouse tumoroids infected with empty vector (EV) or vector overexpressing *Notum* (OE) (n=3 technical replicates).

(*E*) QRT-PCR for canonical Wnt/B-CATENIN pathway target genes in *Apc* mutant tumoroids from (*D*).

(F) Expression level of *Notum* in $Apc^{flox/flox}$*::Villin-CreER* mouse colon tumoroids derived after tamoxifen treatment, infected with empty vector (EV) or vector overexpressing *Notum* (OE) (n=3 technical replicates with one representative of three independent experiments shown).

(*G*) The percentage of cells in S-phase (EdU+) in control or $Apc^{flox/flox}$*::Villin-CreER* organoids (after Apc inactivation with 4OHT *in vitro*) infected with empty vector (EV) or *Notum* overexpressing vector from(*F*).

(*H*) Representative endoscopic pictures showing the development of orthotopic tumors formed in the distal colon by *Apc*-mutant tumoroids infected with control sgRNA (sgCtl) or *Notum* sgRNA (sgNotum) (n=4 biological replicates). Scale bar: 2 mm.

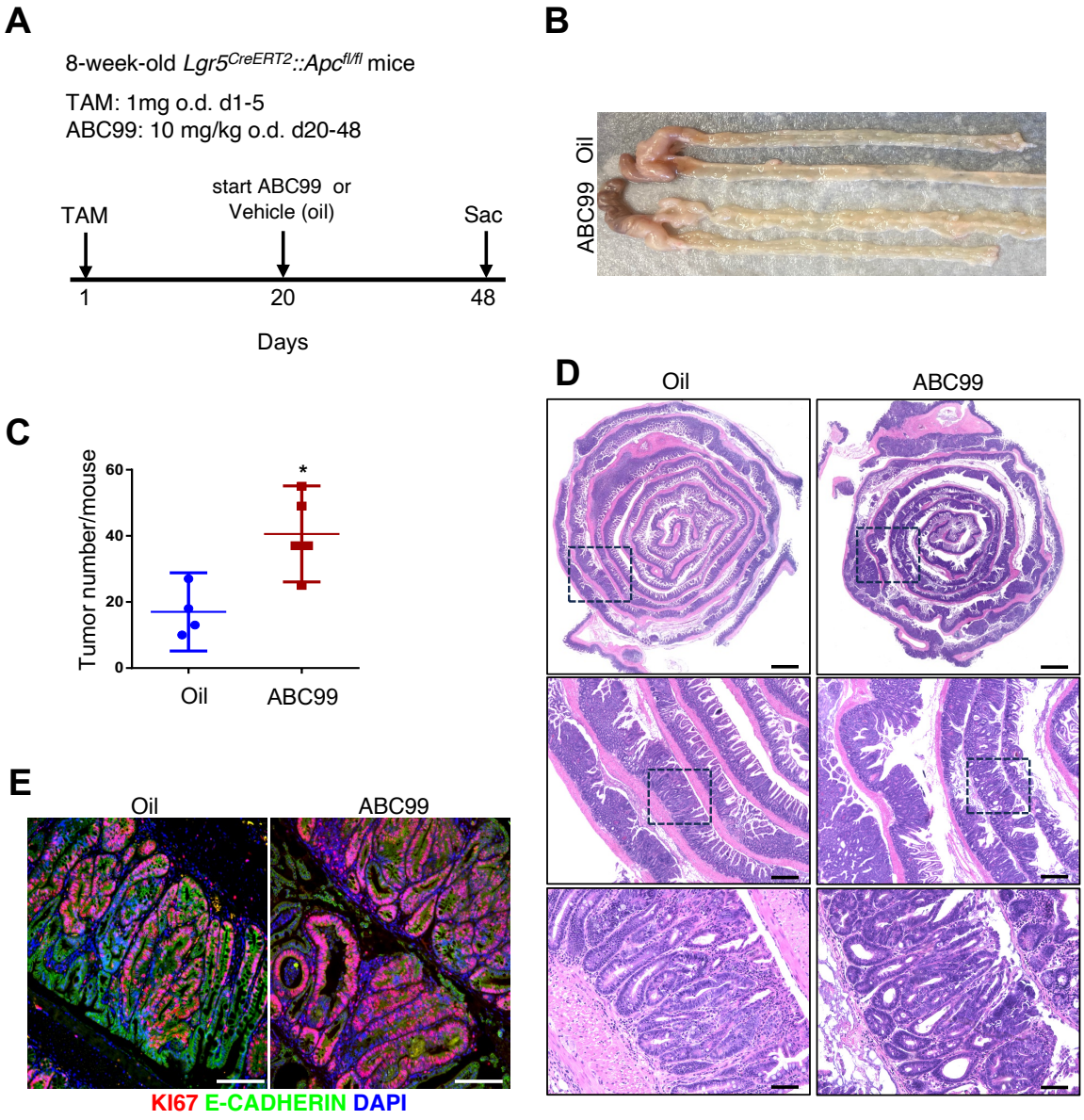
(*I*) Orthotopic primary tumors from (*H*).

(*J*) Hematoxylin and eosin micrographs from tumors in (*I*). Scale bar of top panels: 200 μm. lower panels: 50 μm.

(*K*) Livers of mice from (*H*), With white asterisks indicating macroscopically visible metastatic lesions.

(*L*) Hematoxylin and eosin micrographs from liver metastases in (*K*). Scale bar of top panel: 200 μm. Lower panel: 50 μm.

For all panels: **P*<0.05, ***P*<0.01, ****P*<0.001, Student's *t*-test. See supplemental figures 8 and 9 for validation of driver mutations, sgRNA knockdown, and overexpression.


A
8-week-old Lgr5CreERT2::Apcfl/fl mice
TAM: 1mg o.d. d1-5
ABC99: 10 mg/kg o.d. d20-48
start ABC99 or
Vehicle (oil)
TAM
Sac
1
20
48
Days
B
Oil
ABC99
C
Tumor number/mouse
60
40
20
0
*
Oil
ABC99
D
Oil
ABC99
E
Oil
ABC99
KI67 E-CADHERIN DAPI

Supplementary Figure 3. Pharmacological NOTUM inhibition in a mouse genetic model of adenoma driven by APC loss of function increases tumor burden.

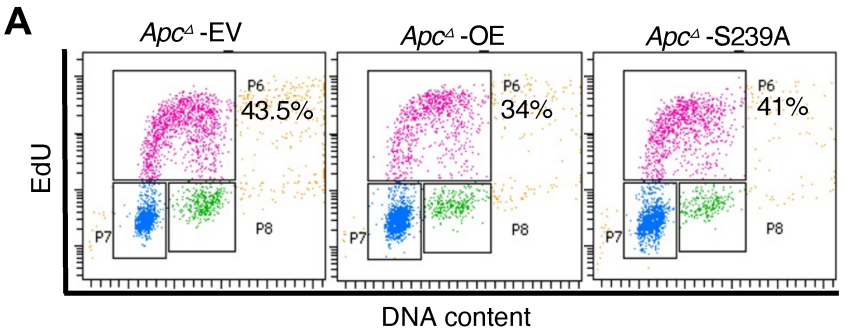
(*A*) Illustration depicting experimental approach for adenoma initiation by tamoxifen (TAM) administration to *Lgr5*^{CreERT2}::*Apc*$^{flx/flx}$ mice followed by treatment with small molecule NOTUM inhibitor ABC99 (n=5) or vehicle control (n=4) twenty days later. ABC99 was administered daily for four weeks until the experimental endpoint.

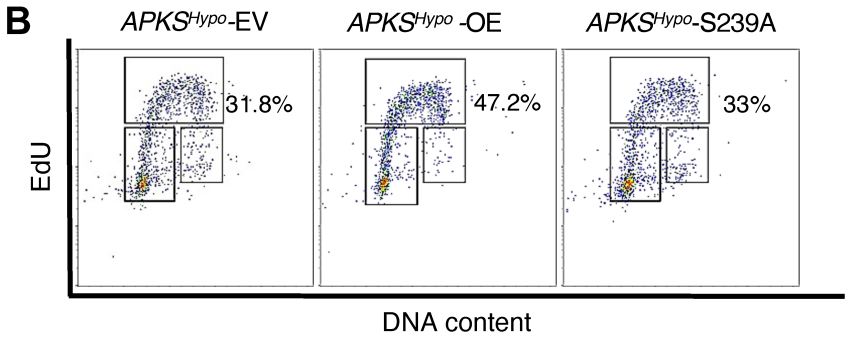
(*B*) Intestine and colon tumors observed in *Lgr5*^{CreERT2}::*Apc*$^{fl/fl}$ mice at the experimental endpoint, comparing ABC99-treated mice with oil-treated mice.

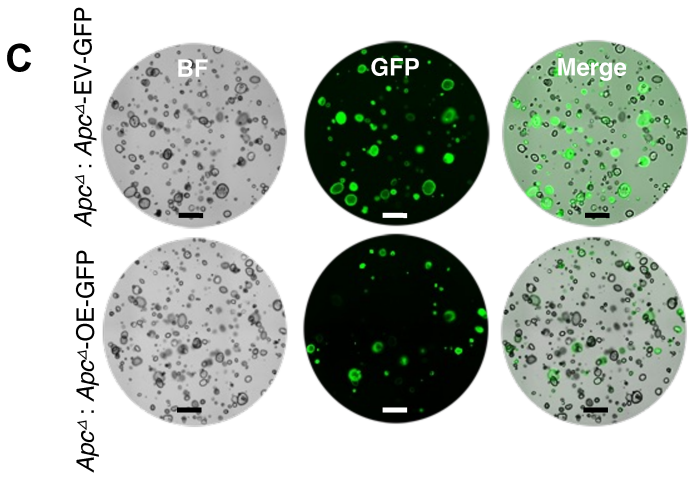
(*C*) Quantification of tumor numbers in the mice described in (B).

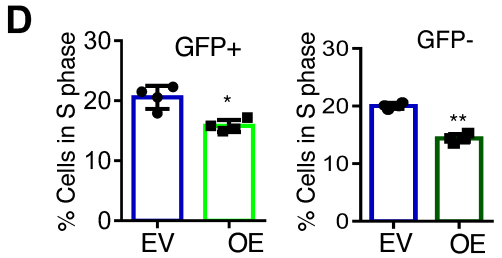
(*D*) Hematoxylin and eosin (H&E) staining of the tumors shown in (B). Scale bar: Top panel - 1000 μm, middle panel - 200 μm, bottom panel - 50 μm.

(*E*) KI67 staining of the tumors shown in (B). Scale bar: 100 μm.


A
Apc⁴ -EV
Apc⁴ -OE
Apc⁴ -S239A
EdU
P6
43.5%
34%
41%
P7
P8
DNA content


B
APKSHypo-EV
APKSHypo -OE
APKSHypo-S239A
EdU
31.8%
47.2%
33%
DNA content


C
Apc⁴ : Apc⁴-EV-GFP
Apc⁴ : Apc⁴-OE-GFP
BF
GFP
Merge


D
GFP+
GFP-
% Cells in S phase
30
20
10
0
*
**
EV
OE

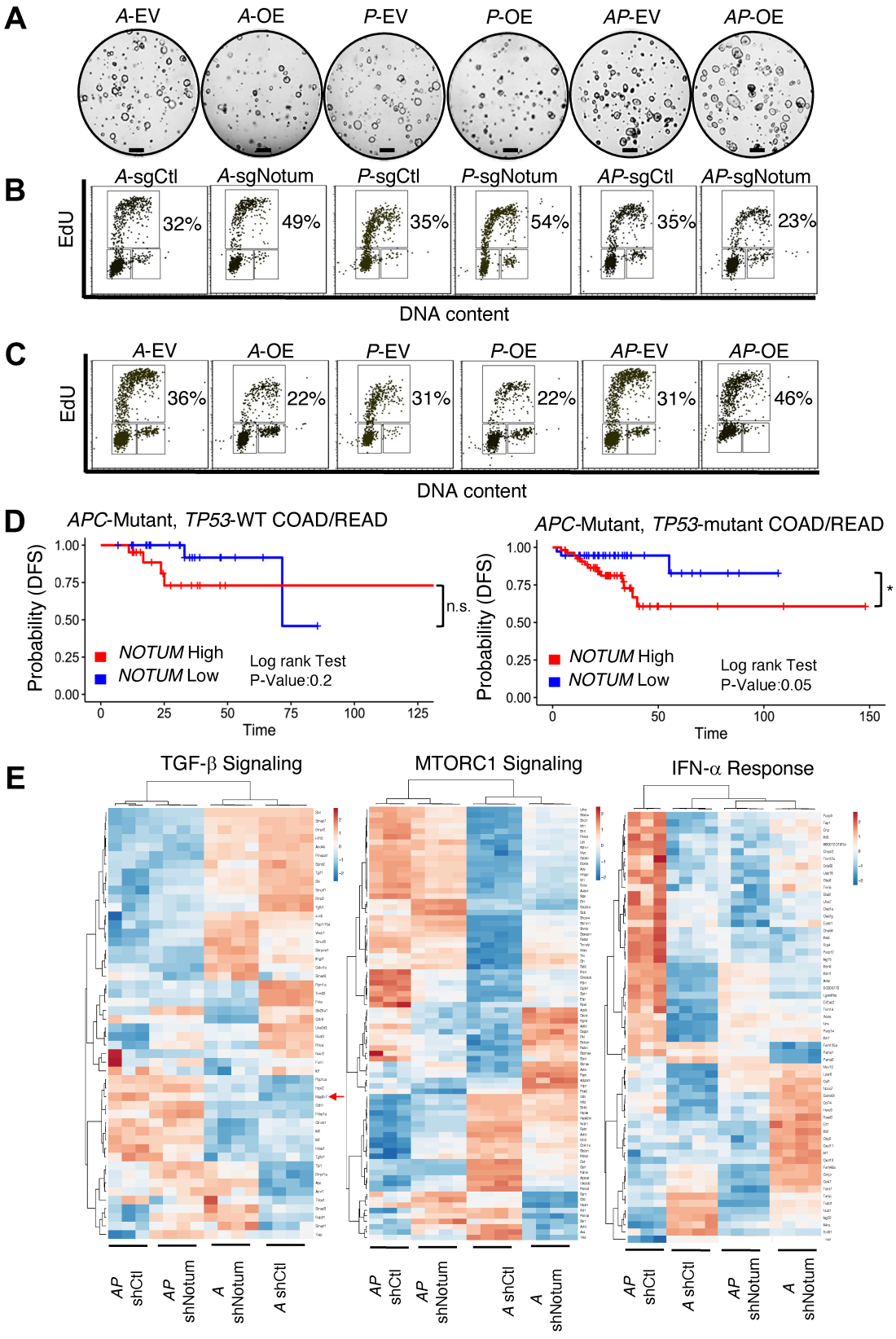
Supplementary Figure 4. Novel tumor suppressive and oncogenic functions of NOTUM require extracellular enzymatic activity.

(*A*) Representative flow cytometric plots of EdU incorporation after a 2-hour pulse, from Figure. 3*A*, *C*: *Apc* mutant mouse tumoroids infected with empty vector (EV), vector expressing wildtype NOTUM (OE), or catalytically dead NOTUM with Ser239Ala mutation (S239A) (n=3 technical replicates).

(*B*) Representative flow cytometric plots of EdU incorporation after a 2-hour pulse, from Figure. 3*D*, *F*: *APKS*^{Hypo} mouse tumoroids infected with empty vector (EV), vector expressing wildtype NOTUM (OE), or catalytically dead NOTUM with Ser239Ala mutation (S239A) (n=3 technical replicates).

(*C*) Images of *Apc*-mutant mouse tumoroid co-cultures, with half of the tumoroids infected with either a vector expressing GFP only (EV-GFP) or expressing GFP and NOTUM (OE-GFP), and the other half uninfected (n=3 technical replicates). Scale bar: 100 μm.

(*D*) EdU assays in the cultures in (*C*), quantifying the percentage of cells in S phase in the GFP+ and GFP-populations. $^{*}P < 0.05$, $^{**}P < 0.01$, Student's *t*-test. See supplemental figures 8 and 9 for validation of driver mutations, sgRNA knockdown, and overexpression.


A
A-EV
A-OE
P-EV
P-OE
AP-EV
AP-OE
B
A-sgCtl
A-sgNotum
P-sgCtl
P-sgNotum
AP-sgCtl
AP-sgNotum
32%
49%
35%
54%
35%
23%
EdU
DNA content
C
A-EV
A-OE
P-EV
P-OE
AP-EV
AP-OE
36%
22%
31%
22%
31%
46%
EdU
DNA content
D
APC-Mutant, TP53-WT COAD/READ
Probability (DFS)
1.00
0.75
0.50
0.25
0.00
n.s.
NOTUM High
NOTUM Low
Log rank Test
P-Value:0.2
0
25
50
75
100
125
Time
APC-Mutant, TP53-mutant COAD/READ
Probability (DFS)
1.00
0.75
0.50
0.25
0.00
*
NOTUM High
NOTUM Low
Log rank Test
P-Value:0.05
0
50
100
150
Time
E
TGF-β Signaling
MTORC1 Signaling
IFN-α Response
AP shCtl
AP shNotum
A shNotum
A shCtl
AP shCtl
AP shNotum
A shCtl
A shNotum
AP shCtl
A shCtl
AP shNotum
A shNotum

Supplementary Figure 5. *Apc* and *Trp53* inactivation synergize to confer oncogenic activity upon NOTUM

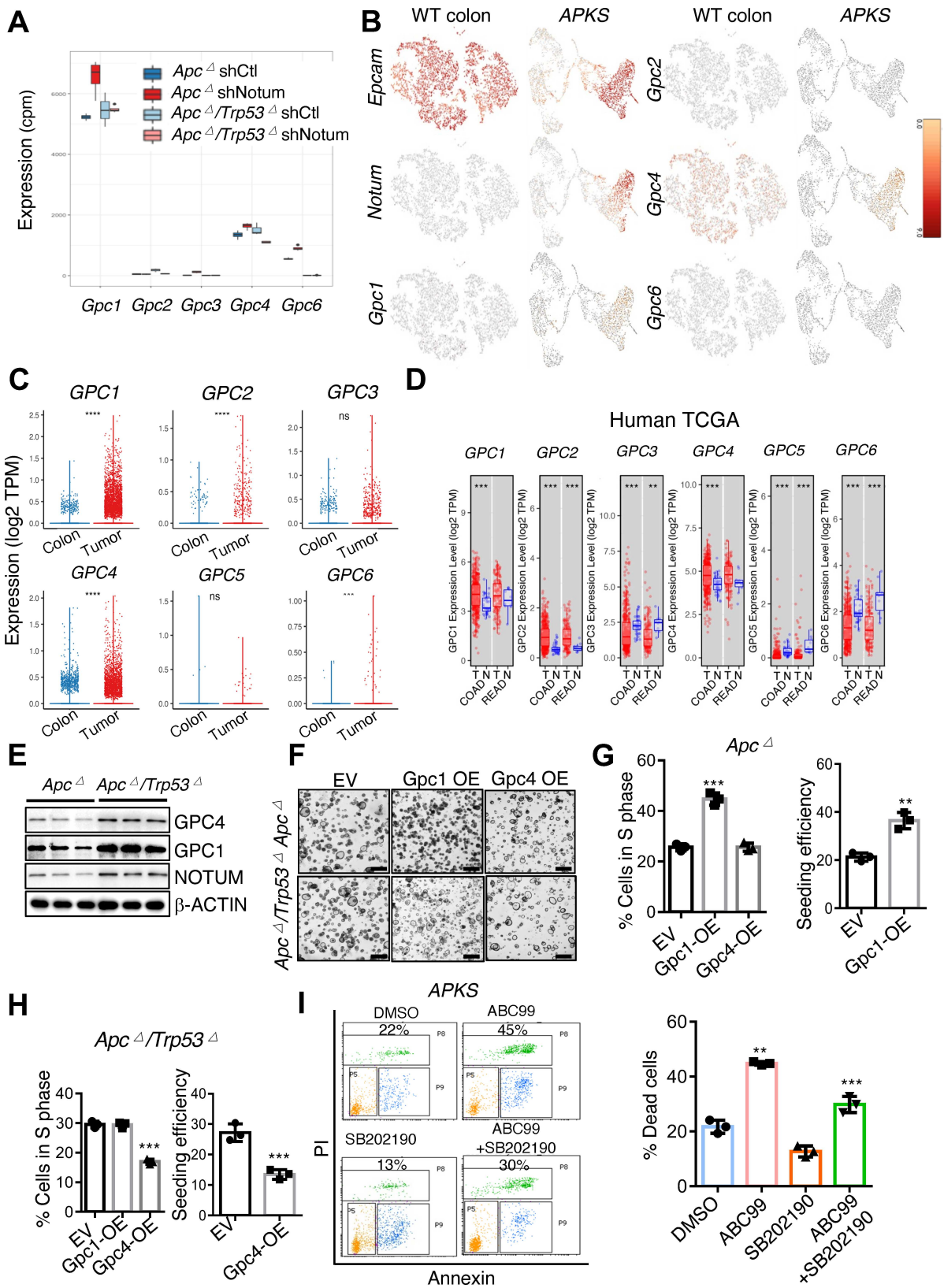
(*A*) Brightfield image of *A*, *P* and *AP* mouse tumoroids infected with vector overexpressing NOTUM (OE) or empty vector control (EV) (n=3 technical replicates with one representative of three independent experiments shown). Scale bar: 100 μm.

(*B*) Representative flow cytometric plots of EdU incorporation (measuring progression though S-phase) after a 2-hour pulse, from Fig. 4b, d: *Apc* (*A*), *Trp53* (*P*), or double mutant (*AP*) tumoroids infected with control sgRNA (sgCtl) or *Notum* sgRNA (sgNotum) (n=3 technical replicates with one representative of three independent experiments shown).

(*C*) Representative flow cytometric plots of EdU incorporation after a 2-hour pulse, from Fig. 4f and Extended Data Fig 5a: *A*, *P* and *AP* tumoroids infected with vector overexpressing NOTUM (OE) or empty vector control (EV) (n=3 technical replicates with one representative of three independent experiments shown).

(*D*) Analysis of disease-free survival (DFS) in human colon and rectal adenocarcinoma patients (COAD, READ) for which gene expression data is available in the Cancer Genome Atlas (TCGA). Left panel shows DFS for *APC* mutant, *TP53* wildtype patients (n=46), binned on *NOTUM* expression above (high) or below (low) the median. Right panel shows DFS for *APC/TP53* double mutant COAD/READ patients (n=93), binned on *NOTUM* expression above (high) or below (low) the median). **P*<0.05, Student's t-test.

(*E*) Heatmaps showing gene expression in gene sets identified by gene set enrichment analysis for pathways affected by NOTUM loss of function in *A* or *AP* mouse tumoroids (related to main figure 4H, I). See supplemental figures 8 and 9 for validation of driver mutations, shRNA/sgRNA knockdown, and overexpression.


A
Expression (cpm)
Apc△ shCtl
Apc△ shNotum
Apc△/Trp53△ shCtl
Apc△/Trp53△ shNotum
Gpc1 Gpc2 Gpc3 Gpc4 Gpc6
B
WT colon
APKS
WT colon
APKS
Epcam
Notum
Gpc1
Gpc2
Gpc4
Gpc6
C
GPC1
GPC2
GPC3
GPC4
GPC5
GPC6
Expression (log2 TPM)
Colon
Tumor
D
Human TCGA
GPC1 GPC2 GPC3 GPC4 GPC5 GPC6
GPC1 Expression Level (log2 TPM)
GPC2 Expression Level (log2 TPM)
GPC3 Expression Level (log2 TPM)
GPC4 Expression Level (log2 TPM)
GPC5 Expression Level (log2 TPM)
GPC6 Expression Level (log2 TPM)
T N T N
COAD READ
E
Apc△ Apc△/Trp53△
GPC4
GPC1
NOTUM
β-ACTIN
F
EV
Gpc1 OE
Gpc4 OE
Apc△
Apc△/Trp53△
G
Apc△
% Cells in S phase
Seeding efficiency
EV
Gpc1-OE
Gpc4-OE
H
Apc△/Trp53△
% Cells in S phase
Seeding efficiency
EV
Gpc1-OE
Gpc4-OE
Gpc4-OE
I
APKS
DMSO
22%
ABC99
45%
SB202190
13%
ABC99
+SB202190
30%
PI
Annexin
% Dead cells
DMSO
ABC99
SB202190
ABC99
+SB202190

Supplementary Figure 6. Glypicans as mediators of NOTUM function in adenoma and adenocarcinoma.

(*A*) Expression level of Glypicans *Gpc1, Gpc2, Gpc3, Gpc4* and *Gpc6* (*Gpc5* was not detected) in RNA sequencing data from *Apc* mutant (*A*) and *Apc/Trp53* double mutant (*AP*) mouse tumoroids infected with control shRNA (shCtl) or *Notum* shRNA (shNotum) (n=4 biological replicates).

(*B*) UMAP plots showing expression of *Epcam, Notum, Gpc1, Gpc2, Gpc4* and *Gpc6* in single cell RNA sequencing data from wildtype mouse colon epithelium and adenocarcinoma derived from orthotopic implantation of *APKS* mouse tumoroids into syngeneic mouse colonic mucosa.

(*C*) Expression level of Glypicans *GPC1, GPC2, GPC3, GPC4, GPC5*, and *GPC6* in single cell RNAseq data from primary human colorectal cancers and normal adjacent colon (n=17).

(*D*) Expression level of *GPC1, GPC2, GPC3, GPC4, GPC5* and *GPC6* in human TCGA colon (COAD) and rectum (READ) adenocarcinoma datasets.

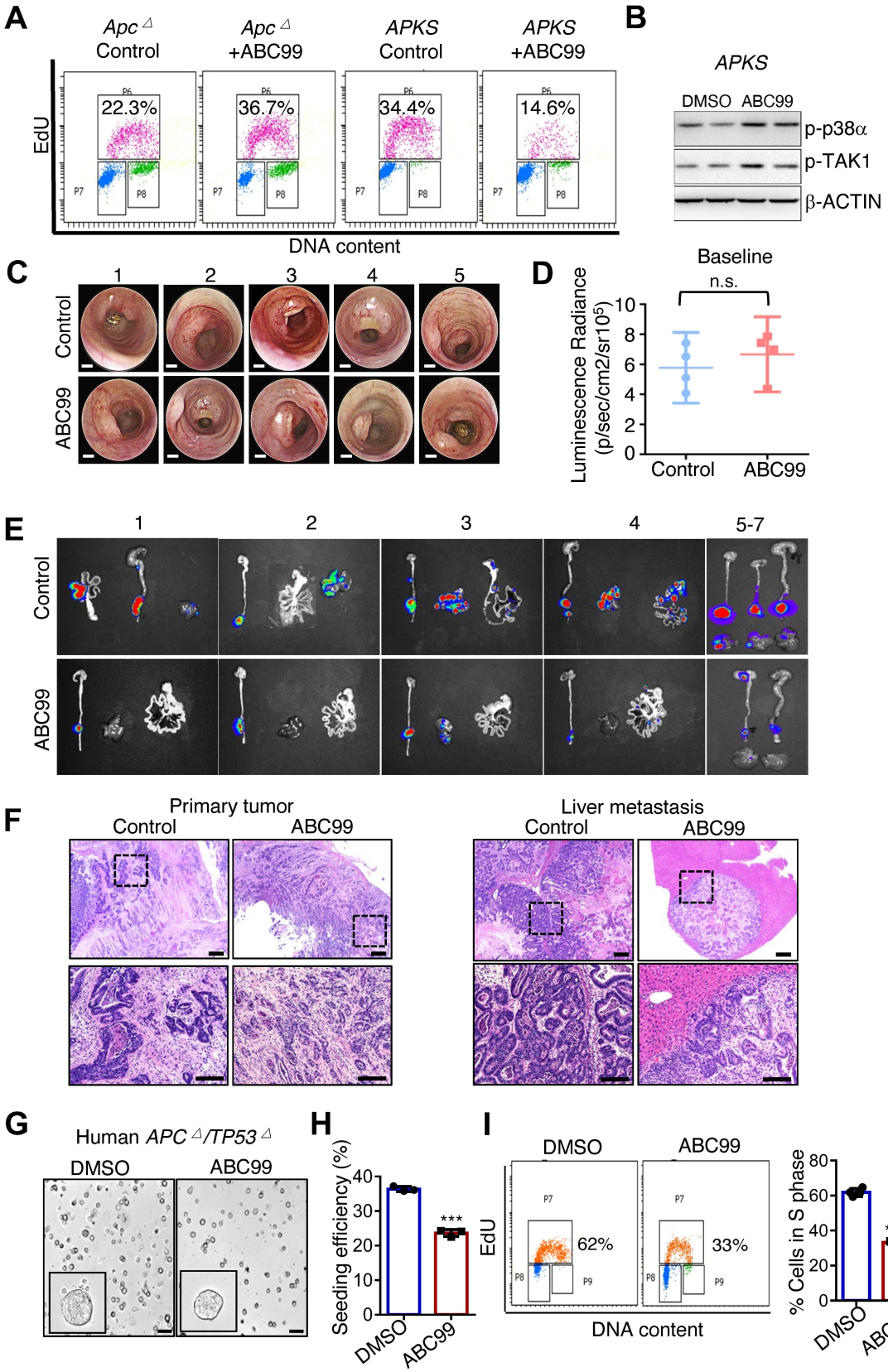
(*E*) Western blotting for GPC1 and 4 in whole cell lysates from *A* and *AP* mouse tumoroids. β-ACTIN was used as a loading control.

(*F*) Brightfield image of *A* and *AP* mouse tumoroids infected with empty vector (EV) or vector expressing GPC1 (Gpc1 OE) or GPC4 (Gpc4 OE) (n=3 technical replicates). Scale bar: 100 μm.

(*G,H*) EdU assays (left) and clonal seeding efficiencies (right) from *Apc* mutant (G) and *Apc/Trp53* double mutant (H) cultures shown in (f) (n=3 technical replicates).

(*I*) Quantification of apoptosis in *APKS* mouse tumoroid cultures after inhibition of NOTUM with the small molecule ABC99, the p38 inhibitor SB202190, or both (n=3 technical replicates).

For all panels: ****P<0.0001, ***P<0.001, **P<0.01, Student's t-test. See supplemental figures 8 and 9 for validation of driver mutations, shRNA/sgRNA knockdown, and overexpression.


A
Apc△ Control
Apc△ +ABC99
APKS Control
APKS +ABC99
EdU
22.3%
36.7%
34.4%
14.6%
P6
P7
P8
DNA content
B
APKS
DMSO ABC99
p-p38α
p-TAK1
β-ACTIN
C
1 2 3 4 5
Control
ABC99
D
Baseline
n.s.
Luminescence Radiance (p/sec/cm2/sr10^5)
Control ABC99
E
1 2 3 4 5-7
Control
ABC99
F
Primary tumor
Liver metastasis
Control ABC99
G
Human APC△/TP53△
DMSO ABC99
H
Seeding efficiency (%)
***
DMSO ABC99
I
DMSO ABC99
EdU
62%
33%
P7 P8 P9
DNA content
% Cells in S phase
***
DMSO ABC99

Supplementary Figure 7. Pharmacological NOTUM inhibition is efficacious in a pre-clinical animal model of metastatic colon cancer.

(*A*) Representative flow cytometric analysis of EdU incorporation in *Apc* mutant and APKS mouse tumoroids treated with the small molecule inhibitor of NOTUM, ABC99, or vehicle control (DMSO), quantified in Figure. 6*A* (n=3 technical replicates).

(*B*) Western blotting for phosphorylation of TAK1 and p38a in APKS tumoroids treated with vehicle or ABC99. β-ACTIN was used as a loading control.

(*C*) Representative endoscopic images of the orthotopic APKS tumors from Figure. 6*B* prior to initiation of treatment, 4 weeks post-implantation. Scale bar: 2 mm.

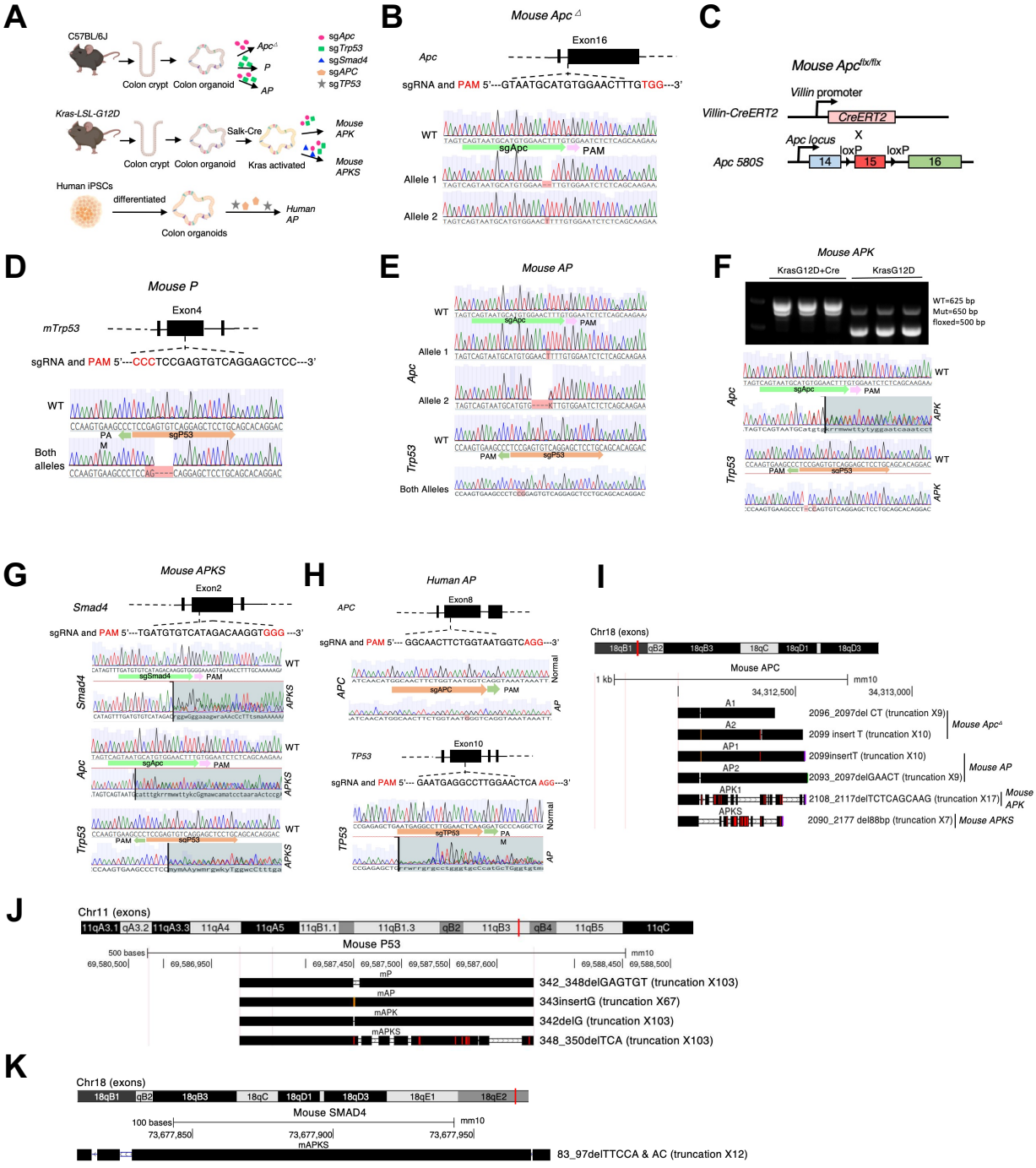
(*D*) Quantification of luminescence radiance from tumors in (*C*) after random assignment to groups and prior to initiation of treatment.

(*E*) Bioluminescence images of dissected intestine, colon, and livers of representative mice from Figure. 6*C* at experimental endpoint.

(*F*) Hematoxylin and Eosin-stained histological sections of representative primary tumors and liver metastases mice from Figure. 6*C* at the experimental endpoint. Scale bar of top panels: 200 μm. Lower panels: 100 μm.

(*G*) Brightfield micrographs of human colon cultures harboring loss-of-function mutations in *APC* and *TP53*, treated with ABC99 or vehicle control (n=3 technical replicates). Scale bar: 100 μm.

(*H*) Clonal seeding efficiency from single cells from human cultures shown in (*G*). I. EdU incorporation assays in cultures shown in (*G*), quantified at right (n=3 technical replicates). For all panels: ***P<0.001, Student's t-test. See supplemental figure 8 for validation of driver mutations.


A
C57BL/6J
Colon crypt
Colon organoid
Apc△
P
AP
sgApc
sgTrp53
sgSmad4
sgAPC
sgTP53
Kras-LSL-G12D
Salk-Cre
Kras activated
Mouse APK
Mouse APKS
Human iPSCs
differentiated
Human AP
Colon organoids
B
Mouse Apc△
Exon16
Apc
sgRNA and PAM 5'---GTAATGCATGTGGAACTTTGTGG---3'
WT
sgApc
PAM
Allele 1
Allele 2
C
Mouse Apcflx/flx
Villin promoter
Villin-CreERT2
CreERT2
Apc locus
Apc 580S
loxP
14
15
16
D
Mouse P
Exon4
mTrp53
sgRNA and PAM 5'---CCCTCCGAGTGTCAGGAGCTCC---3'
WT
PA
M
sgP53
Both alleles
E
Mouse AP
WT
Allele 1
Allele 2
Apc
Trp53
Both Alleles
F
Mouse APK
KrasG12D+Cre
KrasG12D
WT=625 bp
Mut=650 bp
floxed=500 bp
APK
G
Mouse APKS
Smad4
Exon2
sgRNA and PAM 5'---TGATGTGTCATAGACAAGGTGGG ---3'
APKS
H
Human AP
APC
Exon8
sgRNA and PAM 5'--- GGCAACTTCTGGTAATGGTCAGG---3'
Normal
AP
TP53
Exon10
sgRNA and PAM 5'--- GAATGAGGCCTTGGAACTCA AGG---3'
I
Chr18 (exons)
Mouse APC
1 kb
mm10
34,312,500
34,313,000
A1
A2
AP1
AP2
APK1
APKS
2096_2097del CT (truncation X9)
2099 insert T (truncation X10)
2099insertT (truncation X10)
2093_2097delGAACT (truncation X9)
2108_2117delTCTCAGCAAG (truncation X17)
2090_2177 del88bp (truncation X7)
Mouse Apc△
Mouse AP
Mouse APK
Mouse APKS
J
Chr11 (exons)
Mouse P53
500 bases
69,580,500
69,586,950
69,587,450
69,587,500
69,587,550
69,587,600
69,588,450
69,588,500
mP
mAP
mAPK
mAPKS
342_348delGAGTGT (truncation X103)
343insertG (truncation X67)
342delG (truncation X103)
348_350delTCA (truncation X103)
K
Chr18 (exons)
Mouse SMAD4
100 bases
73,677,850
73,677,900
73,677,950
mAPKS
83_97delTTCCA & AC (truncation X12)

Supplementary Figure 8. Genotypes of mouse and human models

(*A*) Schematic representation of mouse and human organoid generation with corresponding mutations.

(*B*) Nucleotide sequences of targeted loci in mouse *Apc* observed in *A* organoids.

(*C*) Schematic representation of $Apc^{flx/flx}$ mouse model utilized in organoid experiments. The same $Apc^{flx/flx}$ allele is used for in vivo experiments, however *in vivo* $Lgr5^{CreER}$ is employed rather than *Villin-CreER*

(*D*) Nucleotide sequences of targeted loci in mouse *Trp53* observed in *P* organoids.

(*E*) Nucleotide sequences of targeted loci in mouse *Apc* and *Trp53* observed in *AP* organoids.

(*F*) PCR and nucleotide sequences of targeted loci in mouse *KrasG12D*, *Apc*, and *Trp53* detected in *APK* organoids.

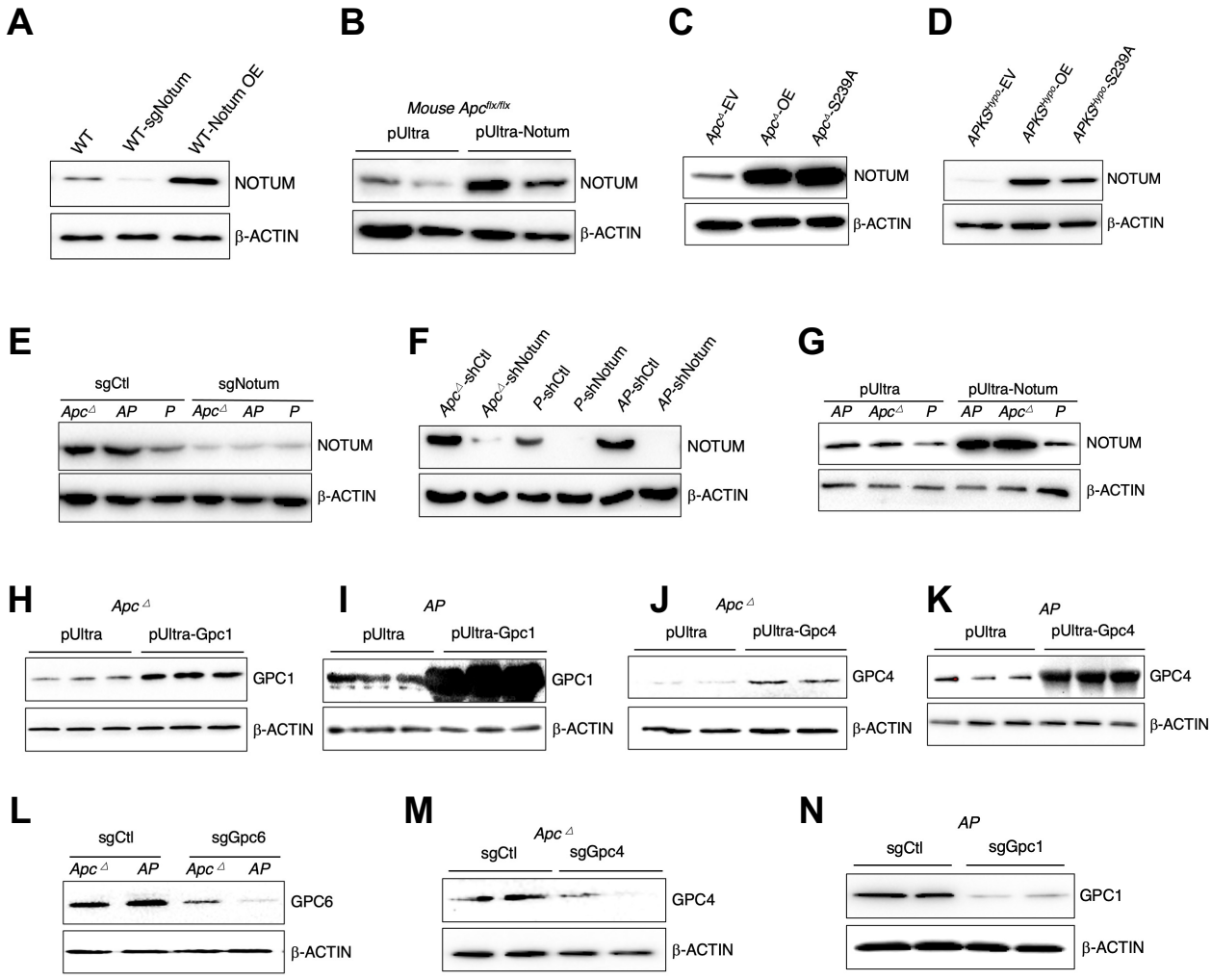
(*G*) Nucleotide sequences of targeted loci in mouse *Apc*, *Trp53* and *Smad4* observed in *APKS* organoids.

(*H*) Nucleotide sequences of targeted loci in human *APC* and *TP53* identified in *AP* organoids.

(*I*) Truncation map of APC in *mouse* Apc^{Δ}, *AP, APK* and *APKS* organoid.

(*J*) Truncation map of P53 in mouse *P*, *AP, APK* and *APKS* organoids.

(*K*) Truncation map of SMAD4 in mouse *APKS* organoids.


A
WT
WT-sgNotum
WT-Notum OE
NOTUM
β-ACTIN
B
Mouse Apc^fix/fix
pUltra
pUltra-Notum
NOTUM
β-ACTIN
C
Apc^Δ-EV
Apc^Δ-OE
Apc^Δ-S239A
NOTUM
β-ACTIN
D
APKS^hypo-EV
APKS^hypo-OE
APKS^hypo-S239A
NOTUM
β-ACTIN
E
sgCtl
sgNotum
Apc^Δ
AP
P
NOTUM
β-ACTIN
F
Apc^Δ-shCtl
Apc^Δ-shNotum
P-shCtl
P-shNotum
AP-shCtl
AP-shNotum
NOTUM
β-ACTIN
G
pUltra
pUltra-Notum
AP
Apc^Δ
P
NOTUM
β-ACTIN
H
Apc^Δ
pUltra
pUltra-Gpc1
GPC1
β-ACTIN
I
AP
pUltra
pUltra-Gpc1
GPC1
β-ACTIN
J
Apc^Δ
pUltra
pUltra-Gpc4
GPC4
β-ACTIN
K
AP
pUltra
pUltra-Gpc4
GPC4
β-ACTIN
L
sgCtl
sgGpc6
Apc^Δ
AP
GPC6
β-ACTIN
M
Apc^Δ
sgCtl
sgGpc4
GPC4
β-ACTIN
N
AP
sgCtl
sgGpc1
GPC1
β-ACTIN

Supplementary Figure 9. Validation of knockout/knockdown and overexpression in organoids.

(*A*) NOTUM protein in WT mouse organoids infected with sgNotum and pUltra-Notum. β-ACTIN served as a loading control.

(*B*) NOTUM protein in *Apc*$^{flx/flx}$ mouse organoids infected with pUltra or pUltra-Notum, after *Apc* inactivation using *Villin-CreER* induction *in vitro*. β-ACTIN was used as a loading control.

(*C*) NOTUM protein in mouse *Apc*$^{\Delta}$ organoids infected with pUltra(EV), pUltra-Notum (OE), or catalytically inactive Notum^{Ser239Ala} (S239A). β-ACTIN was used as a loading control.

(*D*) NOTUM protein in mouse *APKS*^{Hypo} organoids infected with pUltra, pUltra-Notum, or catalytically inactive Notum^{Ser239Ala} (S239A). β-ACTIN served as a loading control.

(*E*) NOTUM protein in mouse *Apc*$^{\Delta}$, *P*, and *AP* organoids infected with sgCtl or sgNotum. β-ACTIN was used as a loading control.

(*F*) NOTUM protein in mouse *Apc*$^{\Delta}$, *P*, and *AP* organoids infected with shCtl or shNotum. β-ACTIN served as a loading control.

(*G*) NOTUM protein in mouse *Apc*$^{\Delta}$, P, and AP organoids infected with pUltra or pUltra-Notum. β-ACTIN was used as a loading control.

(*H*) GPC1 protein in mouse *Apc*$^{\Delta}$ organoids infected with pUltra or pUltra-Gpc1. β-ACTIN served as a loading control.

(*I*) GPC1 protein in mouse *AP* organoids infected with pUltra or pUltra-Gpc1. β-ACTIN was used as a loading control.

(*J*) GPC4 protein in mouse *Apc*$^{\Delta}$ organoids infected with pUltra or pUltra-Gpc4f. β-ACTIN served as a loading control.

(*K*) GPC4 protein in mouse *AP* organoids infected with pUltra or pUltra-Gpc4. β-ACTIN was used as a loading control.

(L) GPC6 protein in mouse *Apc*$^{\Delta}$ and *AP* organoids infected with sgCtl or sgGpc6. β-ACTIN served as a loading control.

(*M*) GPC4 protein in mouse *Apc*$^{\Delta}$ organoids infected with sgCtl or sgGpc4. β-ACTIN was used as a loading control.

(*N*) GPC1 protein in mouse *AP* organoids infected with sgCtl or sgGpc1. β-ACTIN served as a loading control.