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Supplementary Information for

Regnase-1 controls colon epithelial regeneration via regulation of mTOR and purine metabolism

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SI Materials and Methods

In Situ hybridization

Sectioning and ISH were entrusted to GenoStaff (Tokyo, Japan). Briefly, paraffin-embedded Swiss rolled blocks of intestines were sectioned at 6 μm. ISH was performed with the ISH Reagent Kit (Genostaff) according to manufacturer's instructions. The probe for a 422 bp fragment for the ISH analysis was designed from position 2,473 to 2,895 of the mouse Clca1 (Gob5) mRNA. The probes were labeled with a DIG RNA Labeling Kit (Roche Diagnosis). Coloring reactions were performed with NBT/BCIP solution (Sigma) overnight and then washed in PBS. The sections were counterstained with Kernechtrot stain solution (Mutoh) and mounted with G-Mount (Genostaff).

Flow cytometry analysis

Antibodies used in this study were listed in Table S1. Isolated from spleen and perphrial blood and cells were maintained in the dark at 4°C throughout. Cells were washed in ice-cold FACS buffer (2% BSA in PBS), then incubated with each antibody for 15 min and washed twice with FACS buffer. Data were acquired on a BD FACSCanto II, and analyzed using FlowJo software.

Determination of microbiota by deep sequencing

The feces from mice were got from the anus, which were immediately put into dry ice. Amplification of 16S rRNA gene targeting the *V1-V2* region was performed using a primer set (27Fmod: AGRGTTTGATCMTGGCTCAG, 338R: TGCTGCCTCCCGTAGGAGT). DNA libraries were prepared using an Ion Fragment Library Kit (Life Technologies) according to the manufacturer's instructions. Sequencing was performed using a 318 chip and Ion PGM Sequencing 400 Kit (Life Technologies) on the Ion PGM sequencer (Life Technologies). The resulting sequences were analyzed with the QIIME pipeline.

DSS-induced colitis with inosine

Eight- to twelve-week-old (Regnase-$1^{fl/fl}$ vs. Regnase-$1^{\Delta IEC}$) mice were used for DSS-induced colitis experiment. Acute colitis was induced by administration of 2% DSS (36–50 kDa; MP Biomedicals) in the drinking water for 7 days and changed to water for additional 2 to 3 days. Mice were analyzed in body weight. Inosine (Sigma, 10 mg/kg of body weight) was intraperitoneally injected to mice everyday during DSS-administration.

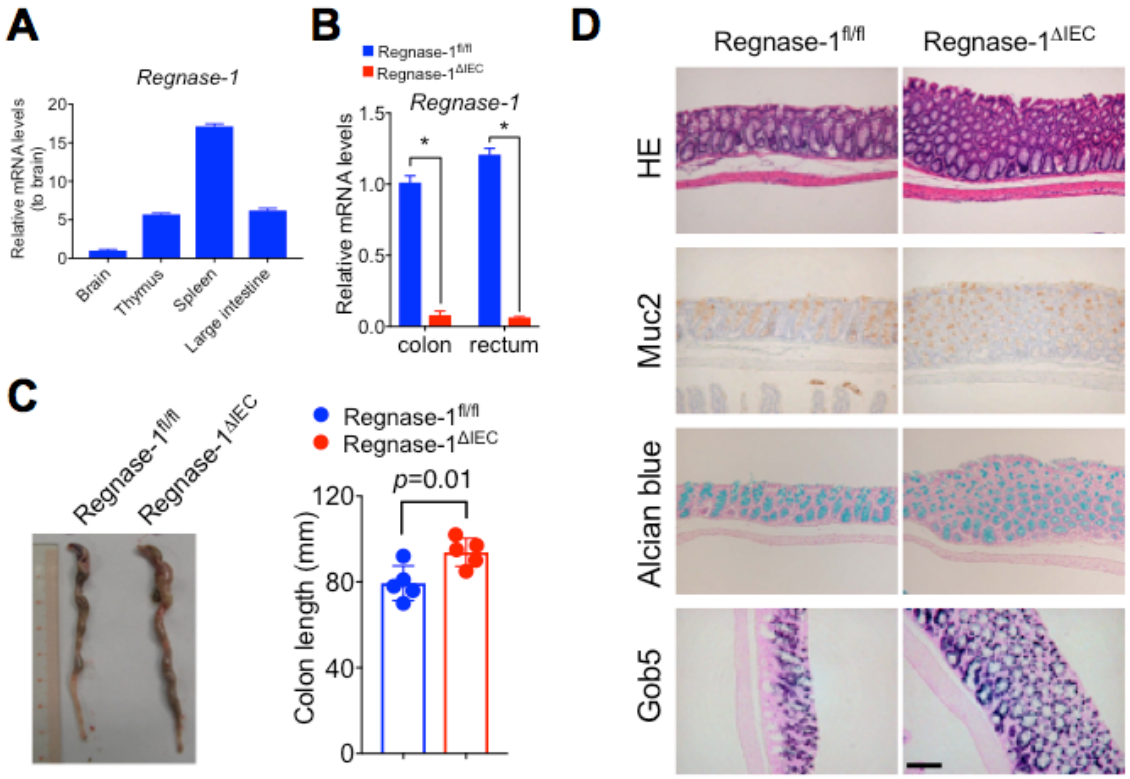

Fig. S1: IEC-specific deletion of Regnase-1 in mice.
(*A*) Regnase-1 expression in tissues. (*B*) Deficiency of murine *Regnase-1* transcripts in both colon and rectum. **P* < 0.05. (*C*) Representative pictures of colons and colon length for Regnase-$1^{fl/fl}$ (*n* = 5) and Regnase-$1^{\Delta IEC}$ mice (*n* = 5). (*D*) Representative colon sections from Regnase-$1^{fl/fl}$ and Regnase-$1^{\Delta IEC}$ mice stained with HE, Muc2 and Alcian blue. *Gob5* transcripts were detected by *in situ* hybridization. Scale bars; 50 μm.

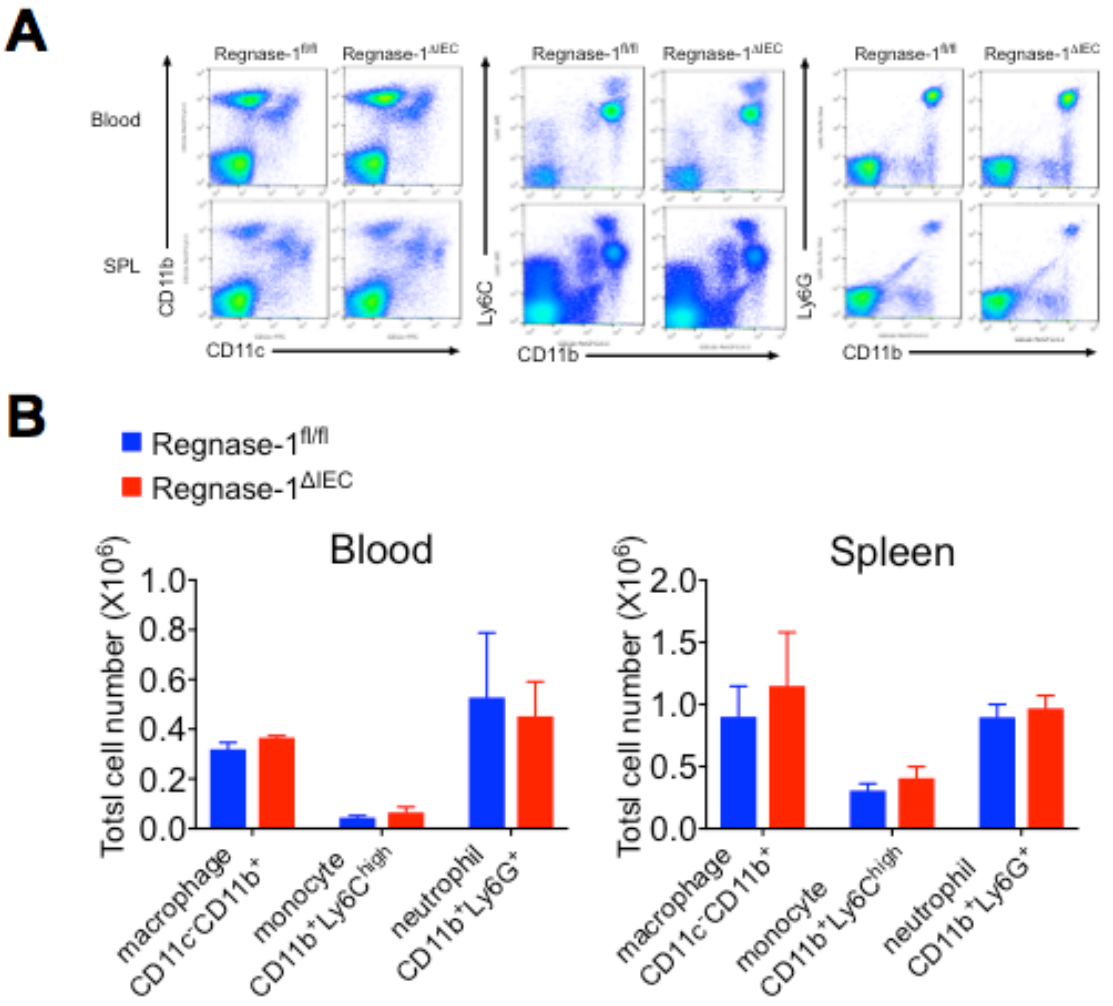

Fig. S2: FACS analysis of spleen and peripheral blood from Regnase-1$^{fl/fl}$ and Regnase-1$^{\Delta IEC}$ mice.

Cells were stained with antibodies to CD11b, CD11c, Ly6C and Ly6G. (*A*) FACS plots. (*B*) The absolute numbers of the different populations are shown. Data are representative of three separate experiments

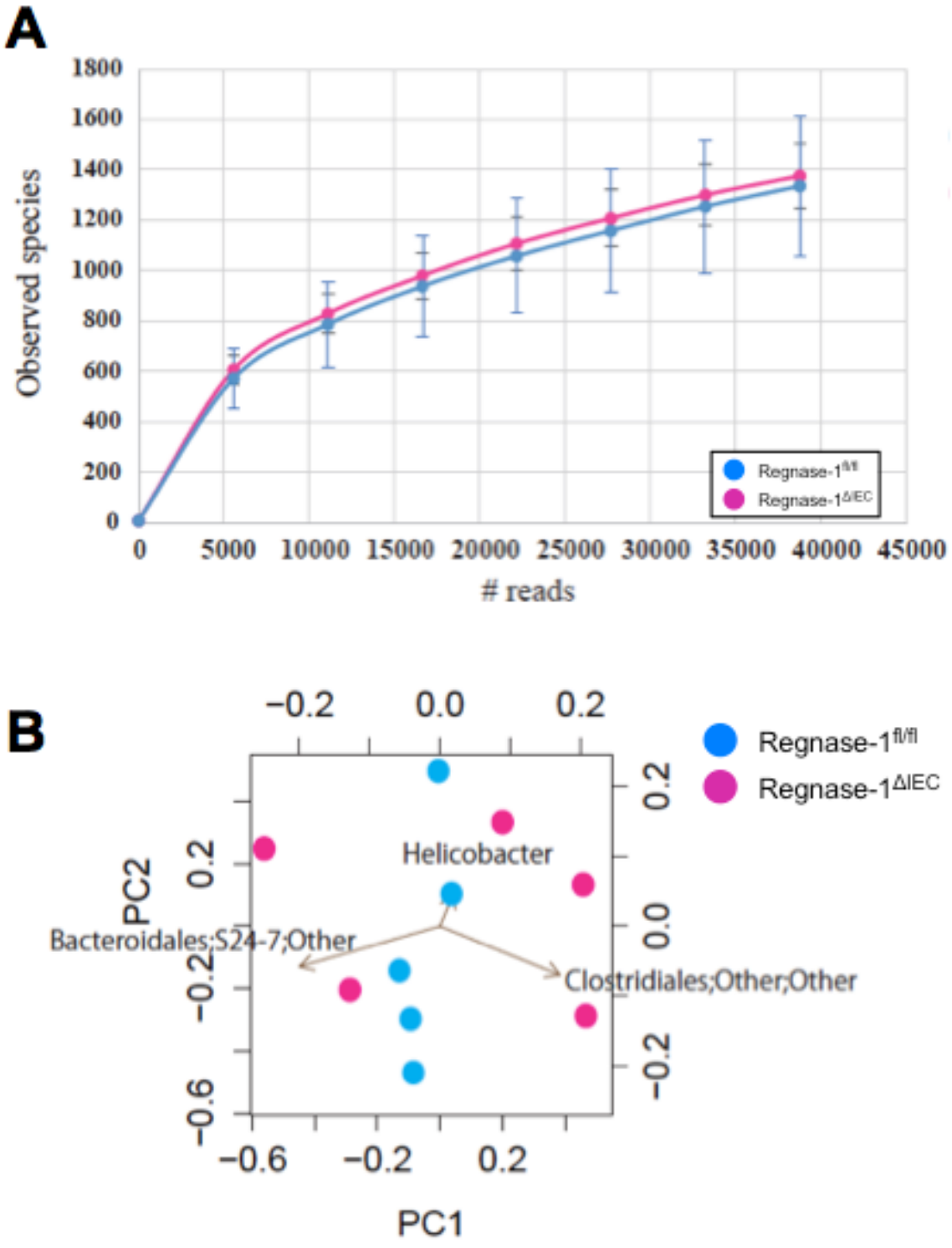

Fig. S3: Bacterial community.
(*A*) The *y*-axis shows the number of observed species that would be expected to be found after sampling the number of sequences shown on the *x*-axis. (*B*) Principal component analysis (PCA) of faecal microbiota in Regnase-1$^{fl/fl}$ and Regnase-1$^{\Delta IEC}$ mice at genus level (each *n*=5 mice).

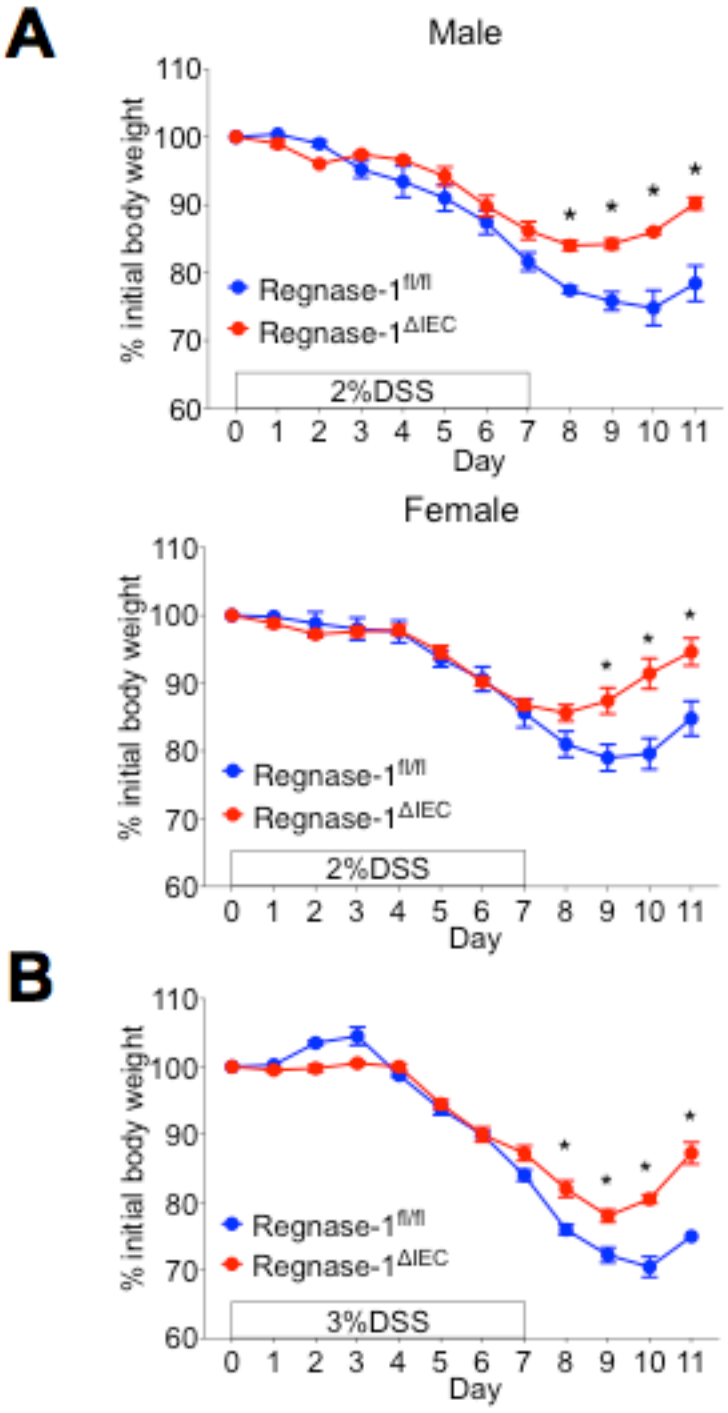

Fig. S4: Percentage change in DSS-treated body weight between Regnase-1$^{fl/fl}$ and Regnase-1$^{\Delta IEC}$ mice.
(*A*) Comparison each male and female group (each 5 mice) in 2% DSS-administration experiment. (*B*) 3% DSS-treated experiment. *$P < 0.05$.

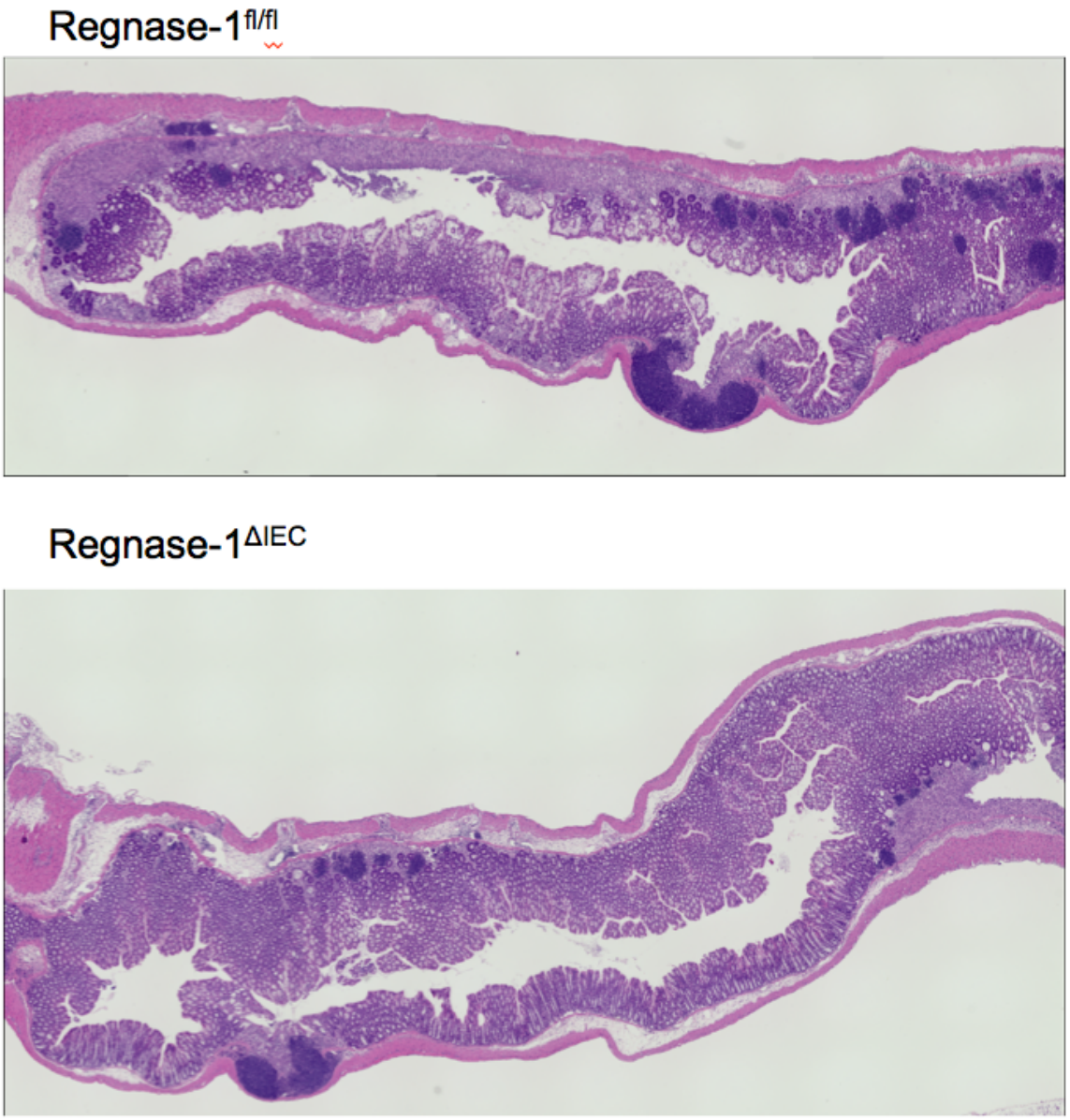

Fig. S5: Colon colitis sections from Regnase-1$^{fl/fl}$ and Regnase-1$^{\Delta IEC}$ mice with HE staining.

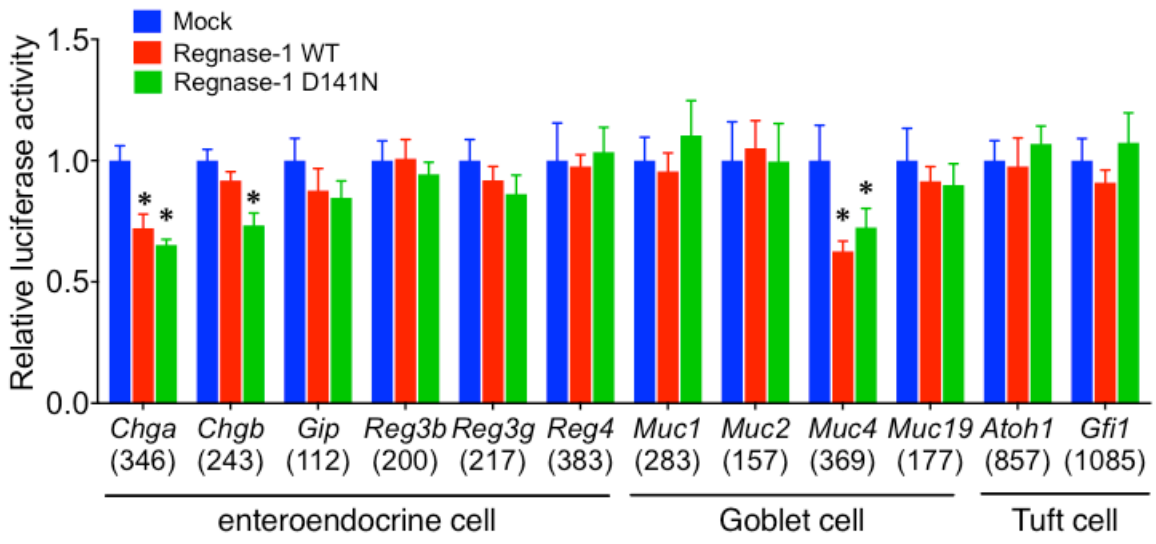

Fig. S6: Luciferase assay for 3'UTR of genes, which are associated with enteroendocrine cell, Goblet cell and Tuft cell.
The length of each 3'UTR is indicated in brackets. *$P < 0.05$.

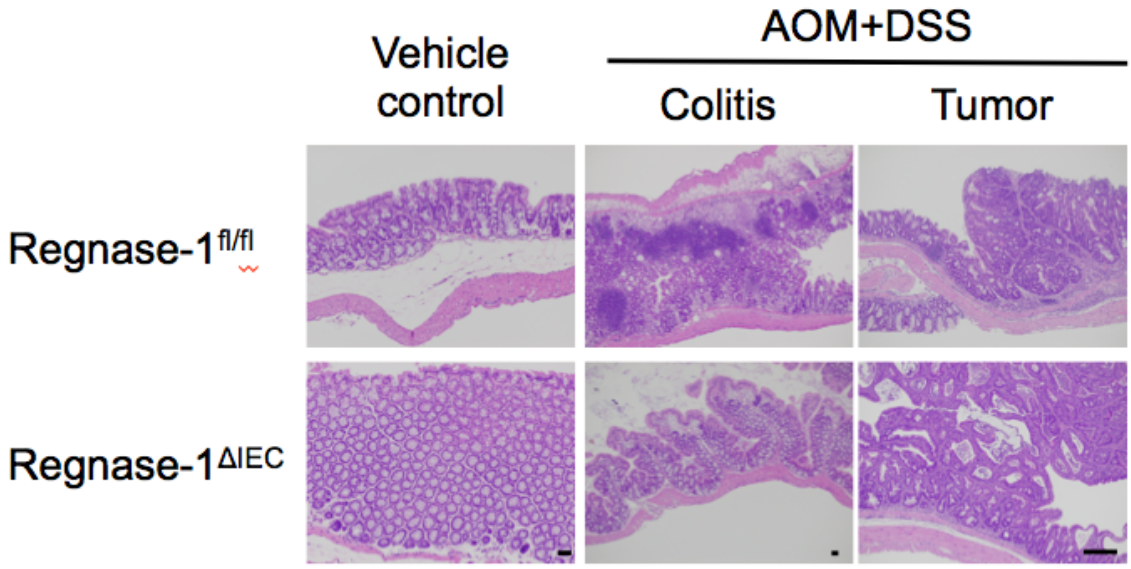

Fig. S7: Colon colitis and tumor sections from Regnase-1$^{fl/fl}$ and Regnase-1$^{\Delta IEC}$ mice with HE staining.
Scale bars, 50 μm.

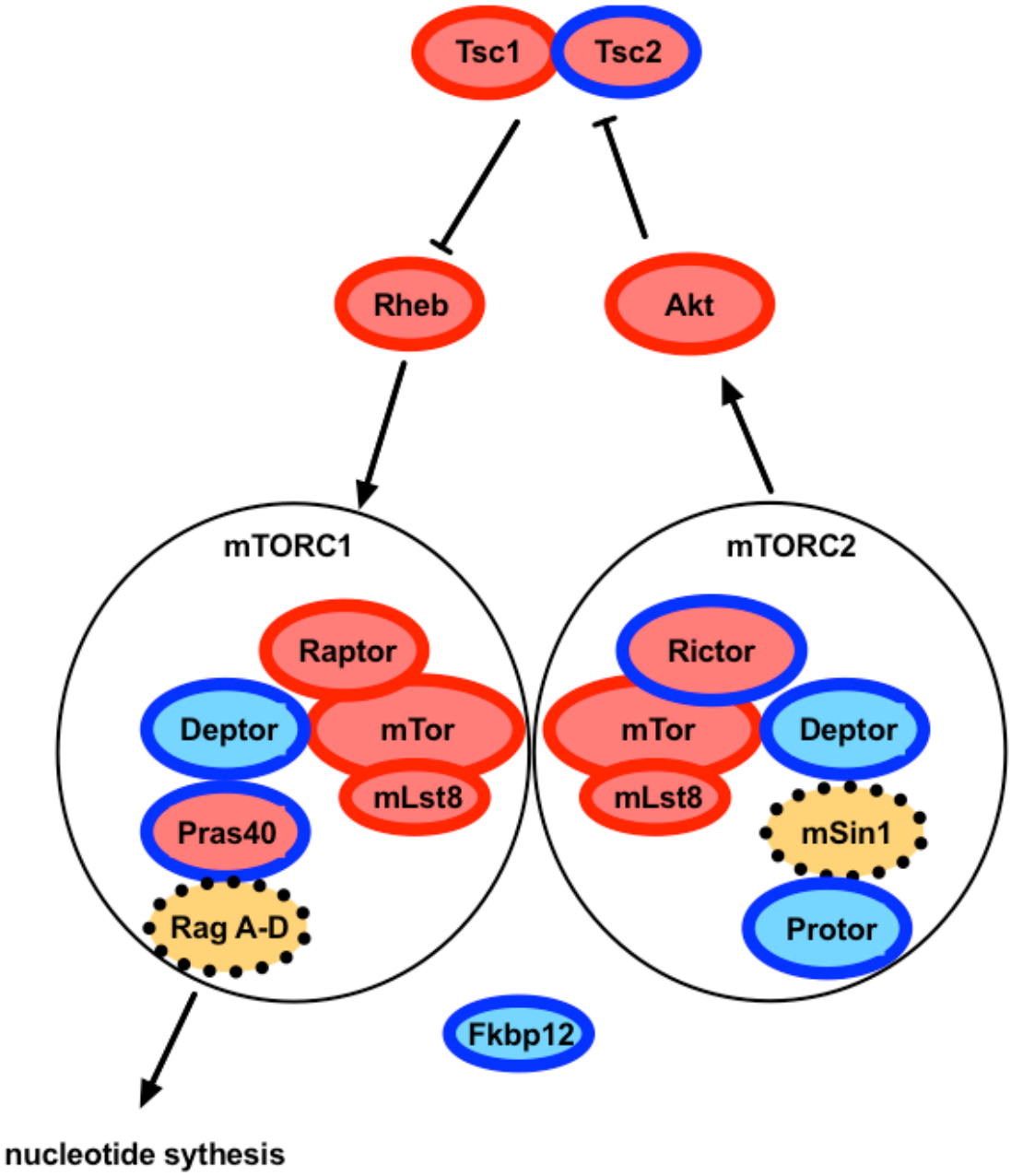

Fig. S8: Target genes of Regnase-1 in mTOR signaling pathway.
Red and blue circles indicate the target and the non-target by 3'UTR luciferase assay, respectively. Dot circles are not tested.

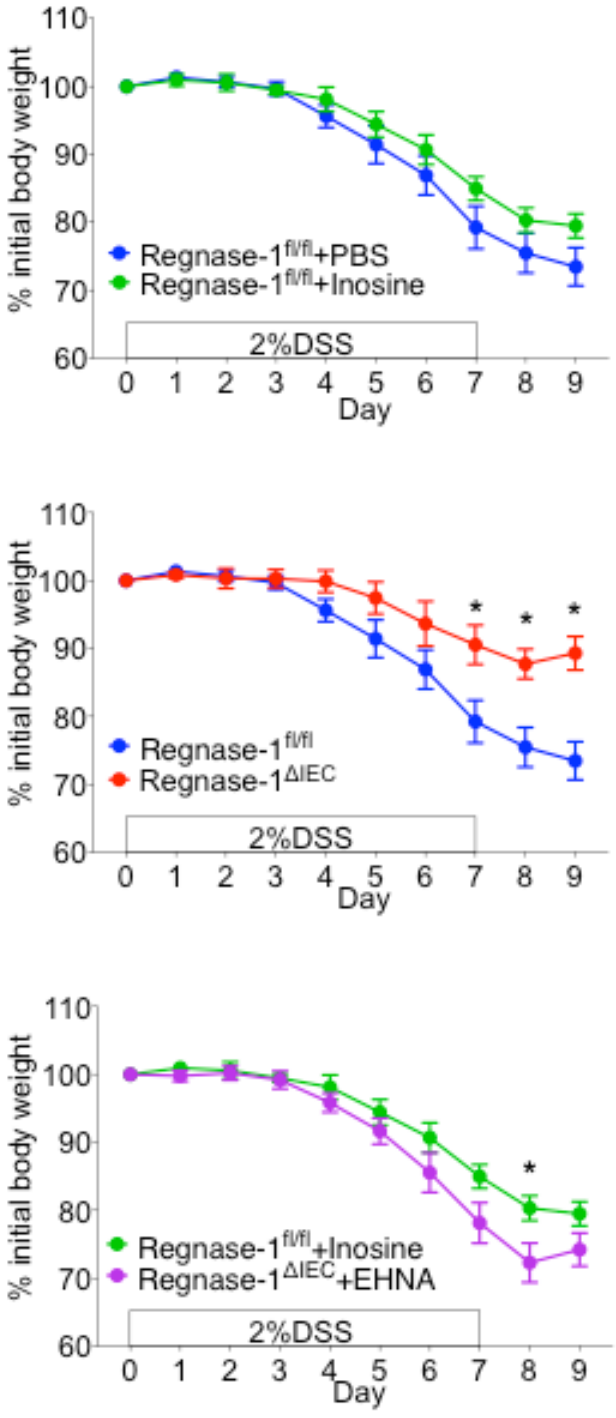

Fig. S9: Percentage change in 2% DSS-induced body weight compared with starting weight of mice with PBS or EHNA (0.3 mg/kg, i.p.) and with inosine (10 mg/kg, i.p.) for 7 days. At least five mice were used for each group. *$P < 0.05$.

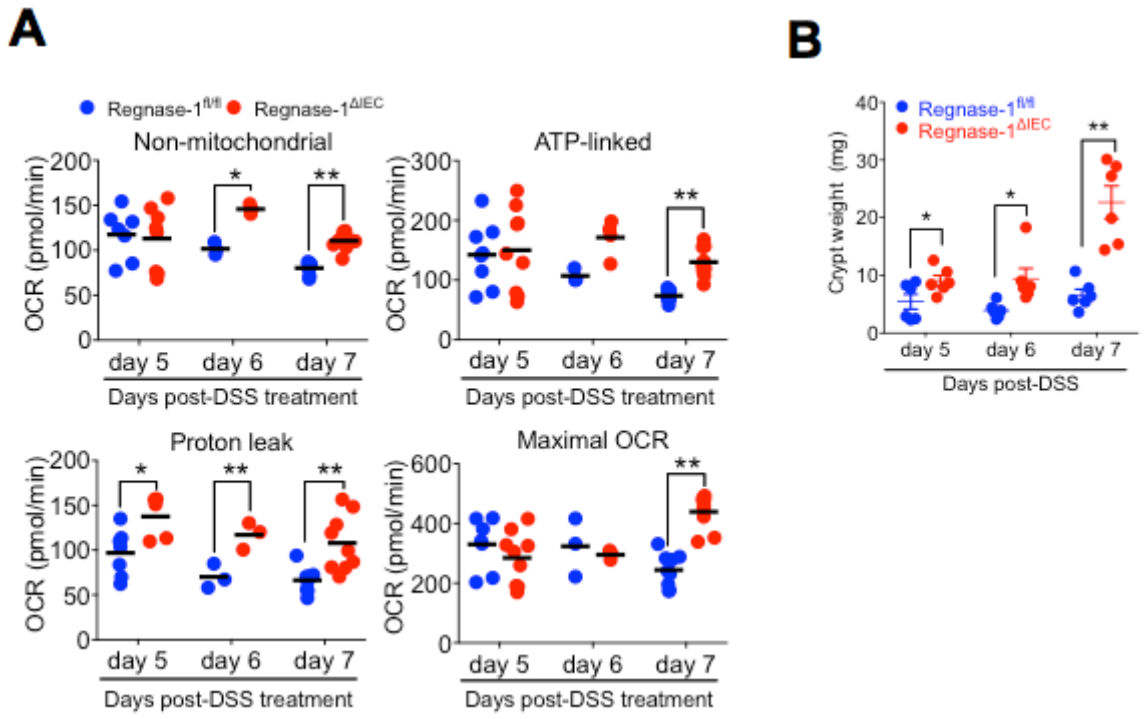

Fig. S10: Seahorse analysis.
(*A*) Basal OCR, ECAR and dependence of each energy formula during DSS-treated time points from Regnase-$1^{fl/fl}$ and Regnase-$1^{\Delta IEC}$ mice. (*B*) The weight of isolated crypts from Regnase-$1^{fl/fl}$ and Regnase-$1^{\Delta IEC}$ mice in each indicated DSS-treated time points. *$P <$ 0.05 and **$P < 0.01$.

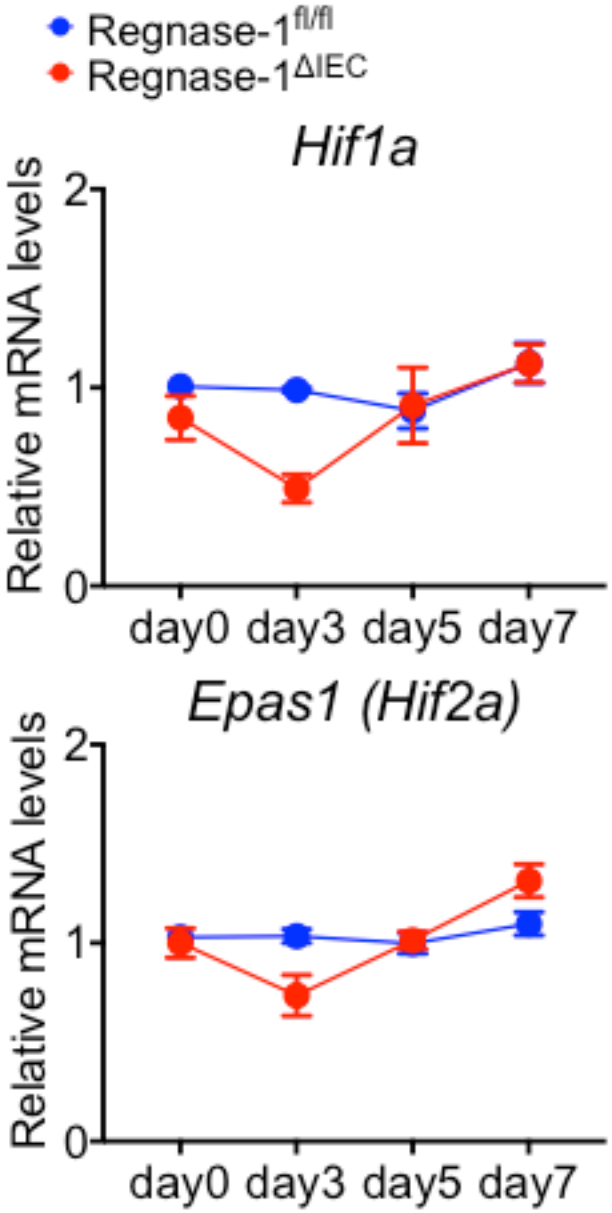

Fig. S11: qPCR analysis of *Hif1a* and *Epas1* (*Hif2a*) in IECs during indicated day points.

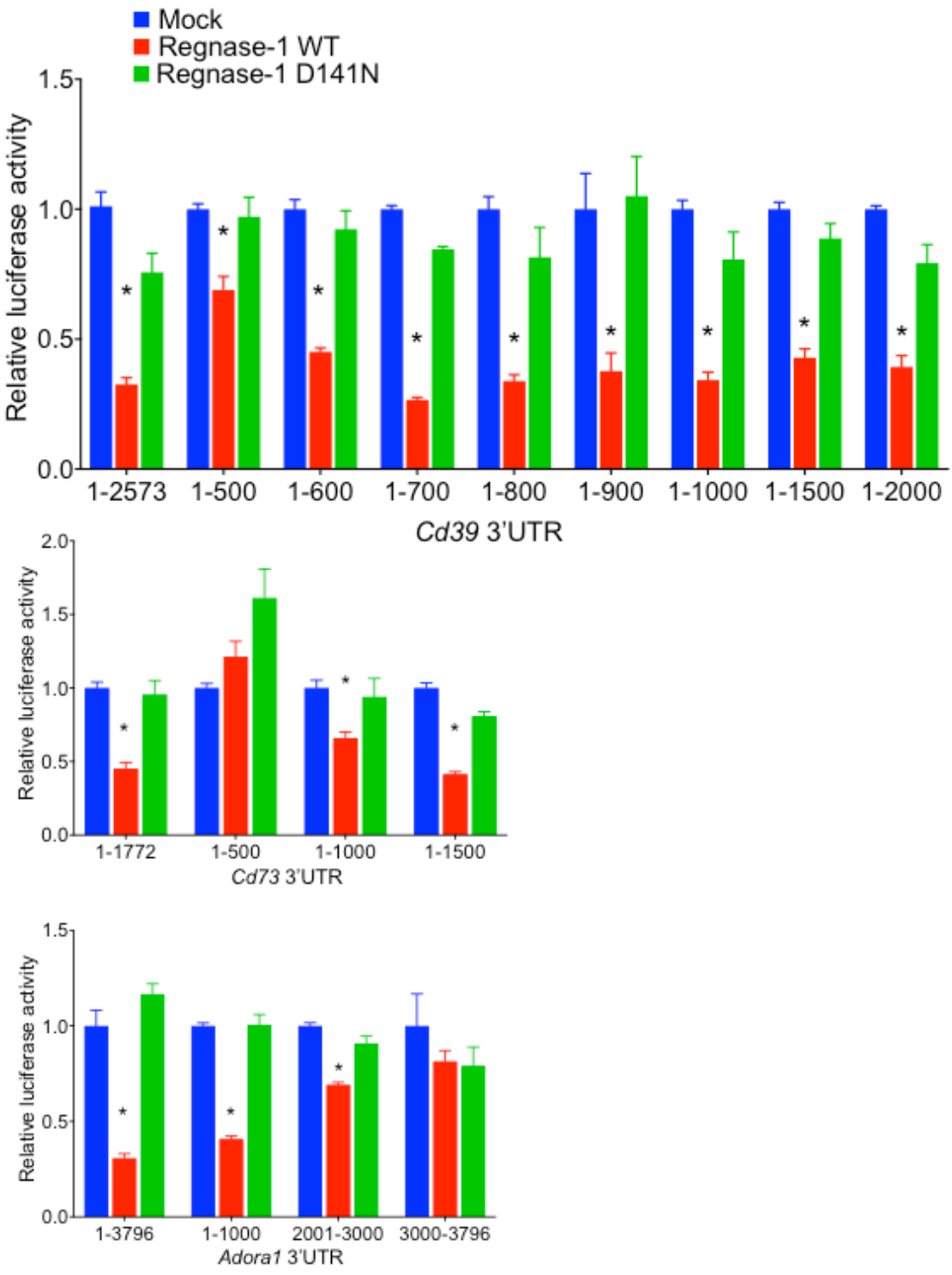

Fig. S12: Luciferase assay for the deletion 3'UTR of *Cd39*, *Cd73* and *Adora1*.
The length of each 3'UTR is indicated in the low axis. *$P < 0.05$.

Table S1: Primers, probes, and antibodies used in this study.

Gentyping primer	Primer sequence (5' to 3')
Vil_Cre_Fw	GTGTGGGACAGAGAACAAACC
Vil_Cre_Rv	ACATCTTCAGGTTCTGCGGG
LAF	GGTTCCTTGGACACACAGCAGATCTGG
MAR	CTCAGAAAGGAGGGTTCCCAGAAGCAG

Gene name	3'UTR primer	Primer sequence (5' to 3')
Tsc1	mTsc1 3UTR-NheI-S	GGGGCTAGCAGAGGGTCTGTCAGTGTTCATTTGCGTCTT
	mTsc1 3UTR-NheI-AS	GGGGCTAGCGGAGTGCAAAACAATAAATCTTTATTTGTT
Tsc2	mTsc2 3UTR-XbaI-S	GGGTCTAGAGGCCAGCAGATCCATGACTAGAGTCCTGTG
	mTsc2 3UTR-XbaI-AS	GGGTCTAGACCCAGCCTCCAAGATGCTAATTTATTCGCC
Rheb	mRheb 3UTR-S	GGGTCTAGACAATTCTGCTGCAGAGCCTGCGGACACTGG
	mRheb 3UTR-AS	GGGTCTAGATGCAGTAAAATACTGTTTACTAGGCATGAC
Akt1	mAkt1 3UTR-S	GGGTCTAGAACCTGATGTTTTGTTTCTCGGATGCGCTGG
	mAkt1 3UTR-AS	GGGTCTAGACAAGCTTGAAAAGCAATTTTTATTGAATTT
mTor	mTor 3UTR-S	GGGGCTAGCCTGAGGCCTGGAAAACCACGTCGTCTCCTC
	mTor 3UTR-AS	GGGGCTAGCTATGCTTTTAAAATTCTGATGTCATTTATT
mLst8	mLst8 3UTR-S	GGGGCTAGCACTTGGCCCTTGAGGCTGCATTGGGCAATC
	mLst8-3UTR-AS	GGGGCTAGCCTGATGGTTCATACACATTTATTGATAGTT
Dptor	mDptor 3UTR-S	GGGTCTAGAGCACAGAGACATCCCTGCTTGTCCCCTATC
	mDptor 3UTR-AS	GGGTCTAGAGGTAATTTTTAAAAATGTAACATTTATTTC
Rptor	mRptor 3UTR-S	GGGTCTAGACGGCACCTAGGACCACTGCCAGGCCGCAGC
	mRptor 3UTR-AS	GGGTCTAGATCAAGATCTCCATGGCAACCAGCATGTCTT
Pras40	mPras40 3UTR-S	GGGTCTAGAGCGAACAGAAAGCCTTGGCCTGCGAGAGGC
	mPras40 3UTR-AS	GGGTCTAGAAACTCACTAGTCAGAGGTAAAACTTTAATC
Rictor	mRictor 3UTR-S	GGGTCTAGACCTCATGCTTATGACGTTTATAGCTGGATA
	mRictor 3UTR-AS	GGGTCTAGATTAAGAATTTTAAGTACATTTTATTAACAA
Protor	mProtor 3UTR-S	GGGTCTAGATGGCCTGAGACTAGTTCATGTTTTTACTGA
	mProtor 3UTR-AS	GGGTCTAGATCTGGGGCTGTAAATTTATTTCATGCCTTC
Cd39	mCD39 3'UTR-NheI-S	GGGGCTAGCCAGGAGCGGCTGAAATGTGCTGGCTGGAGA
	mCD39 3'UTR-NheI-AS	GGGGCTAGCATCACGTTACATTTCTTACTCGTTTATTTA
Cd39 1-500	mCD39 3'UTR-NheI-500AS	GGGGCTAGCAGCACATATTTATCTACTTTAGAAAACTCT
Cd39 1-600	mCD39 3'UTR-NheI-600AS	GGGGCTAGCGAACATACAATGTGTTTGTCTCCACTCTTA
Cd39 1-700	mCD39 3'UTR-NheI-700AS	GGGGCTAGCCTACTGTCACATGCTATGCCCTGAATGCCT

Cd39 1-800	mCD39 3'UTR-NheI-800AS	GGGGCTAGCTTAACATATAAAACCACACAAAACGCTAAA
Cd39 1-900	mCD39 3'UTR-NheI-900AS	GGGGCTAGCCAAATCGTCAATATAGTGATTGTTGTATAA
Cd39 1-1000	mCD39 3'UTR-NheI-1000AS	GGGGCTAGCCCACACATACACACATAACTGAAAGCCAAA
Cd39 1-1500	mCD39 3'UTR-NheI-1500AS	GGGGCTAGCTTTCCATAATAAAAGTCCATAGCCACTTAT
Cd39 1-2000	mCD39 3'UTR-NheI-2000AS	GGGGCTAGCATAATCCTAGCACAGGTGGATCTCTGAGTT
Cd73 full length	mCD73 3'UTR-XbaI-S	GGGTCTAGACAGGGAGTCTCCTTGTCCTTGATGTCAAAC
	mCD73 3'UTR-XbaI-AS	GGGTCTAGAACTATATCTTCAGCTTTATTTTTGGAATTT
Cd73 1-500	mCD73 3'UTR-XbaI-500AS	GGGTCTAGAAAATCTACTTGTAAATTATAATGTTTGTTT
Cd73 1-1000	mCD73 3'UTR-XbaI-1000AS	GGGTCTAGAGAAGGAGAGAGGCAGGGCTTTTGGAGTCGC
Cd73 1-1500	mCD73 3'UTR-XbaI-1500AS	GGGTCTAGACTGCAACAAACTTCATAAAAGGATTGGAAT
Ak1	Ak1 3UTR-XbaI-S	GGGTCTAGACTGGATCCCTTGGCCAGCTCCCAGCCCGCC
	Ak1 3UTR-XbaI-AS	GGGTCTAGATTATAACTGAAATACATTTATTTATTTTAA
Xod	Xdh 3UTR-NheI-S	GGGGCTAGCAAAGGAGGCTCCCCAGTATGGTTTTATACT
	Xdh 3UTR-NheI-AS	GGGGCTAGCTTAACCCCACATTCATTATCATGTTTTATT
Ada	Ada 3UTR-S	GGGTCTAGACCACCACAGACTGACGCAGGGCGGGTCCCC
	Ada 3UTR-AS	GGGTCTAGAACATAAACATAATTAAATATTTAAAAATAA
Adora1 1-1000	Adora1 3UTR-XbaI-S	GGGTCTAGAGCTCTGCCTTGCTCCATCTAGCCCACACCC
	Adora1 3UTR-XbaI-1000AS	GGGTCTAGAGTCTGGTCTGGTCTGGTCTTTCACACTTCT
Adora1 2001-3000	Adora1 3UTR-XbaI-2001S	GGGTCTAGAAAGCTGCTAGAAGAGATAGTGGGGAGTCGG
	Adora1 3UTR-XbaI-3000AS	GGGTCTAGACCAAGATCATCTGGATGAAAAACAAGATGG
Adora1 3001-3736	Adora1 3UTR-XbaI-3001S	GGGTCTAGAGCTGGAAGCAAAGTCAGAGGAGGGTAATGA
	Adora1 3UTR-XbaI-AS	GGGTCTAGATTACTACTCATTTTAATTACATTTATTGGT
Adora1 full length (GeneArt seamless kit)	Adora1_FW2	GATCGCCGTGTAATTCTAGAGCTCTGCCTTGCTCC
	Adora1_RV2	AAGCGGCCGGCCGCCCCGACTTACTACTCATTTTAATTACATTTATTG
Chgb	Chgb UTR-S	GGGTCTAGACAGTTGGAGAGACGAGCCTTTCACTGAAGG
	Chgb UTR-AS	GGGTCTAGACAGGTAAATCTTTTTATTGTTTGTGTTTCA
Gip	Gip 3UTR-XbaI-S	GGGTCTAGACTGACCTAGCCCAGAGCAGGACTGGACTCT
	Gip 3UTR-XbaI-AS	GGGTCTAGAACTTCAGCTTAAGGCTTTATTGGTTTGGTT
Reg3b	mReg3b 3UTR-XbaI-S	GGGTCTAGAACTTATCAGACAGCAAACATCCCGAATTTG
	mReg3b 3UTR-XbaI-AS	GGGTCTAGATGTGACAGAAAGCTTATTTTTATTTTAGAA
Reg3g	mReg3g 3UTR-XbaI-S	GGGTCTAGAAGTACAGAATTGACATCATGGGTTGATATA
	mReg3g 3UTR-XbaI-AS	GGGTCTAGAATGAGCATGAGTTATATACTTTAATATTAT
Reg4	Reg4 3UTR-XbaI-S	GGGTCTAGAAGCAAAAATCAAGCGTCTACCAGCCTTGCA
	Reg4 3UTR-XbaI-AS	GGGTCTAGAGCTGTTTCTTGGCTCCAAAACTTTACTGGT
Muc1	Muc1 3UTR-XbaI-S	GGGTCTAGAGAGCAAGTCACCCCACCCACTTGGGGCAGC

	Muc1 3UTR-XbaI-AS	GGGTCTAGATAACTAACAGGAGGCCATCTTTTATTCAGC
Muc2	Muc2 3UTR-XbaI-S	GGGTCTAGAATGGGTTGTACCGTGCACATTCCTTCGCAT
	Muc2 3UTR-XbaI-AS	GGGTCTAGAGAGTGGCTATGCCTGAGTTTATTATCGGAA
Muc4	Muc4 3UTR-XbaI-S	GGGTCTAGAGGCCCTGTCCCAGATGGGCAGCTGCACCTA
	Muc4 3UTR-XbaI-AS	GGGTCTAGATTATTTAAATAGATTTTAATGAGAAGTAAG
Muc19	Muc19 3UTR-NheI-S	GGGGCTAGCTGCCCGCGTTGCCTTTCCGGTTGCTCTTAT
	Muc19 3UTR-NheI-AS	GGGGCTAGCTCCTTGCACCATGGCGTTTATTTTCCAGCC
Atoh1	Atoh1 3UTR-S	GGGTCTAGAGAAGGCAACAGCTCCCTGAAAACTGAGACA
	Atoh1 3UTR-AS	GGGTCTAGATTCCGAGTTTATTTTATCATTTGTAAAAGA
Gfi1	Gfi1 3UTR-XbaI-S	GGGTCTAGAGTACCCTGGCAGCCCGCAACACCAGCTGTG
	Gfi1 3UTR-XbaI-AS	GGGTCTAGAGTAATAATCTTAATACTTTATTAAGTTAAA
Fkbp2	artificial gene synthesis	
Chga	artificial gene synthesis	

Gene name	Probe ID in Applied Biosystems
Entpd1 (mCd39)	Mm00515447_m1
Nt5e (mCd73)	Mm00501910_m1
A1R (Adora1)	Mm01308023_m1
A2AR (Adora2a)	Mm00802075_m1
A2BR (Adora2b)	Mm00839292_m1
A3R (Adora3)	Mm00802076_m1
Ent1 (Slc29a1)	Mm01270577_m1
Ent2 (Slc29a2)	Mm00432817_m1
Zc3h12a (Regnase-1)	Mm00462535_g1
Hif1a	Mm00468869_m1
Epas1 (Hif2a)	Mm01236112_m1

Antibodies	Company	Identifier
Mucin2	Santa Cruz Biotechnology	sc-15334
Ki67	abcam	ab16667
ZO-1	eBioscience	14-9776-80
pS2448 mTOR	Cell Signaling Technology	5536S
mTOR	Cell Signaling Technology	2972S

T421/S424 S6K	Cell Signaling Technology	9204S
S6K	Cell Signaling Technology	9964S
pT37/46 4E-BP1	Cell Signaling Technology	2855S
4E-BP1	Cell Signaling Technology	9664S
β-actin	Sigma	A5441

Flow Cytometry Antibodies	Company	Identifiers
CD11b-PerCP cy5.5	Biolegend	101230
CD11c-FITC	Biolegend	117306
Ly6C-APC	Biolegend	128016
Ly6G-Pacific Blue	Biolegend	127612
EpCAM (CD326)-FITC	Biolegend	118208
EpCAM (CD326)-PE	Biolegend	118206
CD45-APC	Biolegend	103112