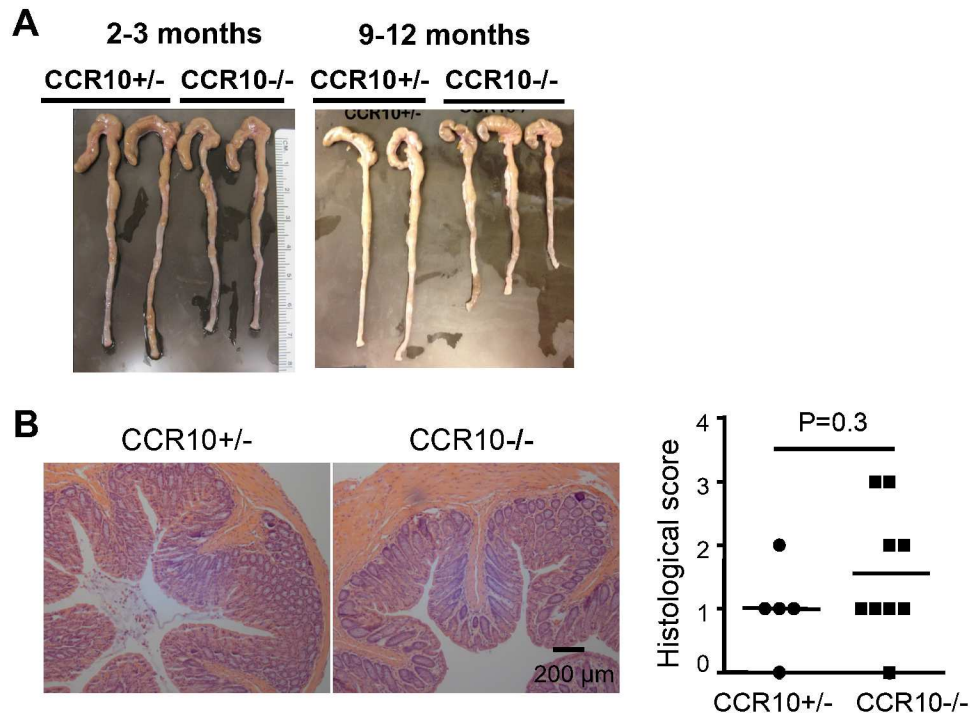
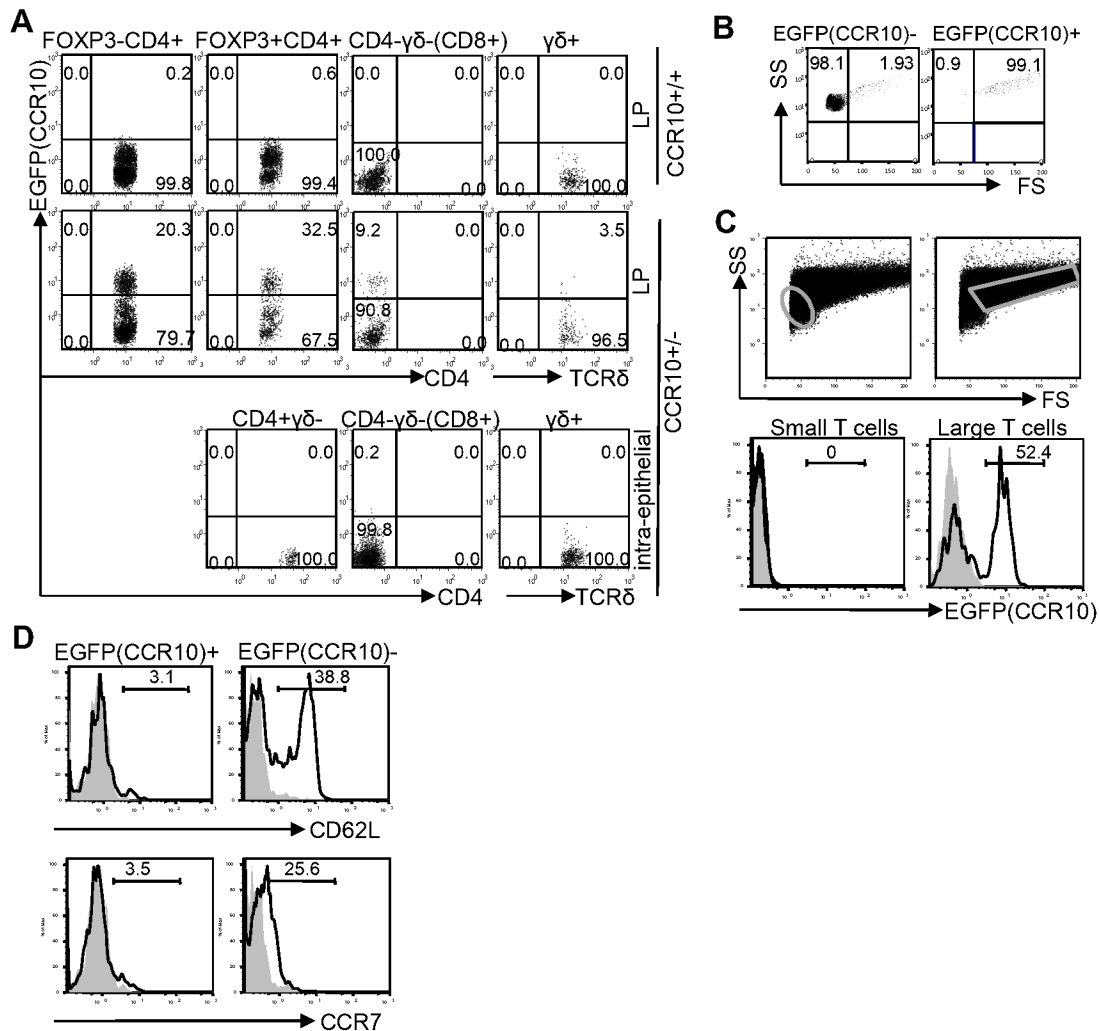


## Supplementary Material

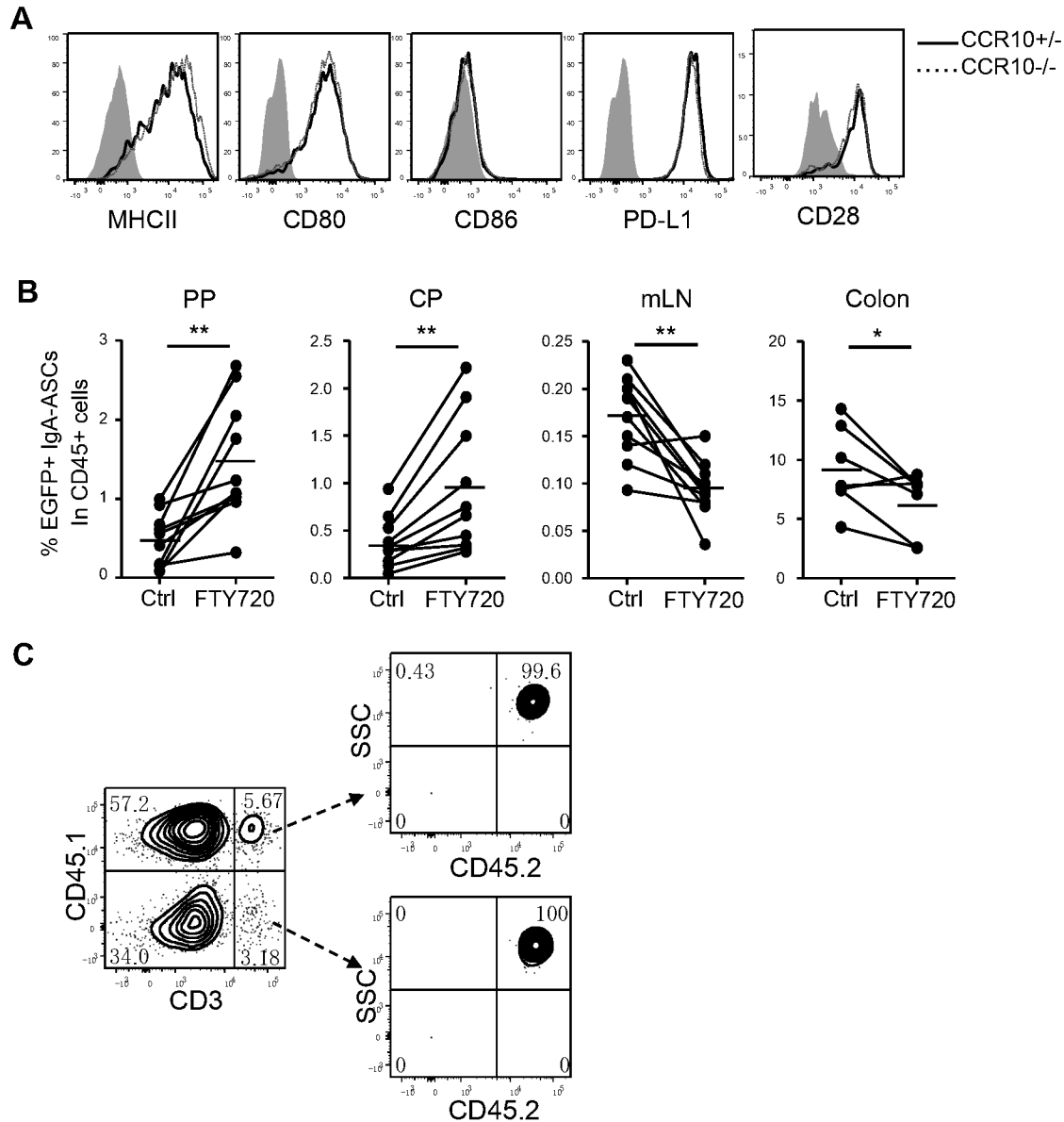
(Supplementary Figures and Tables)



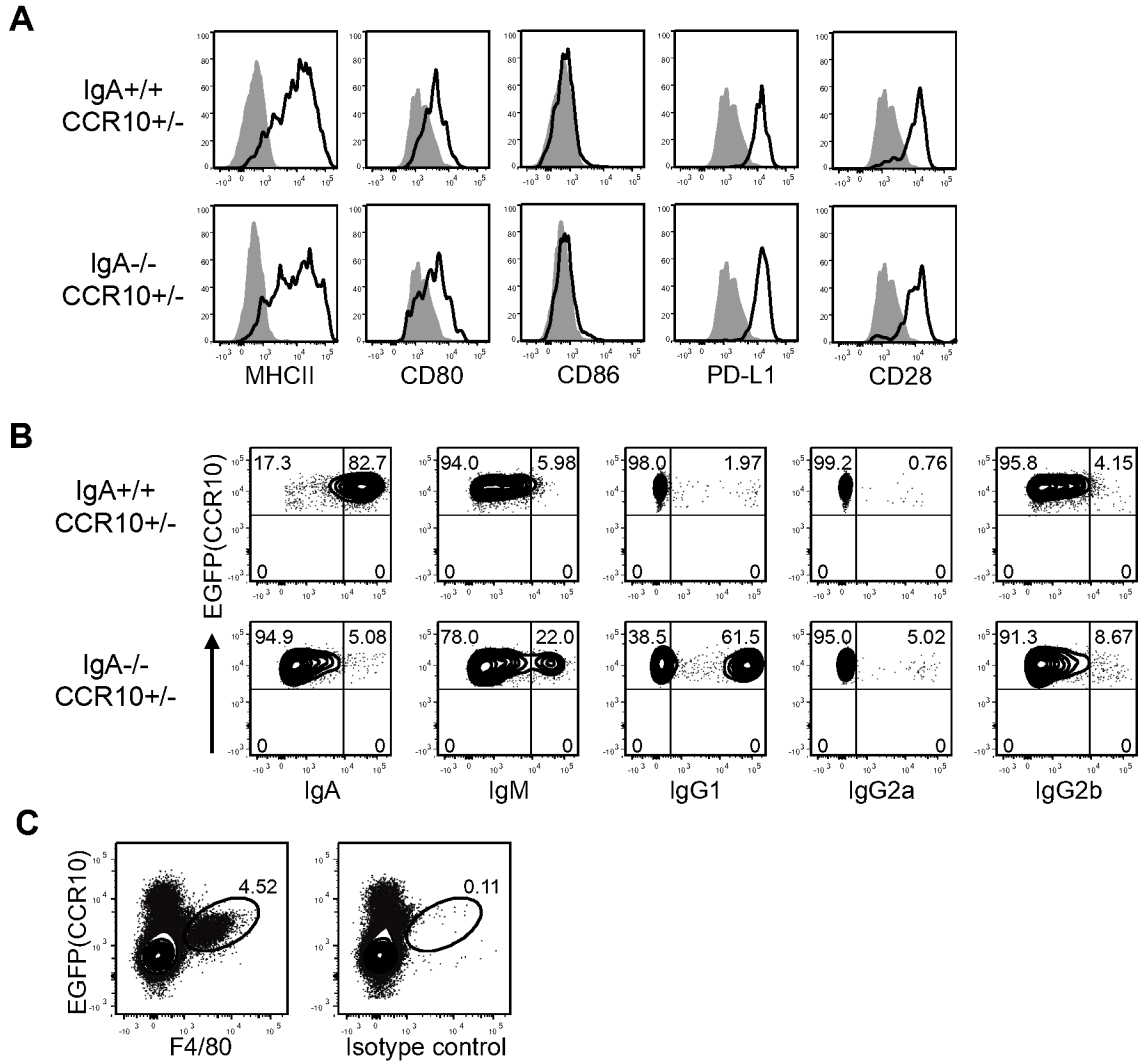
**Supplementary Figure 1.** CCR10<sup>-/-</sup> mice developed colonic inflammation associated with dysregulated T cells. **(A)** Images of colons of CCR10<sup>+/-</sup> and CCR10<sup>-/-</sup> mice at 2-3 and 9-12 months of age. **(B)** Images of H&E-stained colonic sections of CCR10<sup>+/-</sup> and CCR10<sup>-/-</sup> mice at 9-12 months of age and pathological scores of the sections. One dot represents one mouse sample.



**Supplementary Figure 2.** CCR10<sup>+</sup>IgA<sup>+</sup> cells preferentially conjugate with Treg cells in the colon. **(A)** Flow cytometric analysis of EGFP(CCR10) on gated CD45<sup>+</sup>FOXP3<sup>-</sup>CD4<sup>+</sup>, FOXP3<sup>+</sup>CD4<sup>+</sup>, γδ<sup>-</sup>CD4<sup>-</sup> (mostly CD8<sup>+</sup> αβT) and γδ<sup>+</sup> T cells of the colonic LP, and CD4<sup>+</sup>γδ<sup>-</sup>, CD4<sup>-</sup>γδ<sup>-</sup> and γδ<sup>+</sup> T cells of the colonic epithelium of CCR10<sup>+/-</sup> mice. Representative of at least 5 experiments. Staining results of corresponding wild-type CCR10<sup>+/+</sup> cells are included as negative controls for EGFP. **(B)** Flow cytometric analysis of gated EGFP<sup>-</sup> vs. EGFP<sup>+</sup> colonic LP CD45<sup>+</sup>CD3<sup>+</sup>CD4<sup>+</sup> T cells of CCR10<sup>+/-</sup> mice for their forward-scatter (FS) and side-scatter (SS) profiles. Representative of more than 30 experiments. **(C)** Flow cytometric detection of EGFP(CCR10) on gated small and large colonic CD45<sup>+</sup>CD3<sup>+</sup>CD4<sup>+</sup> T cells of CCR10<sup>+/-</sup> mice. Histograms in the top row show gates of small and large cells based on the FS and SS profiles, from which gated CD3<sup>+</sup>CD4<sup>+</sup> T cells are analyzed for CCR10(EGFP) in the bottom row. Gray areas are staining results of corresponding wild-type T cells as negative controls for EGFP. Representative of more than 30 experiments. **(D)** Flow cytometric detection of CD62L and CCR7 on gated EGFP(CCR10)<sup>+</sup> and EGFP(CCR10)<sup>-</sup> colonic CD45<sup>+</sup>CD3<sup>+</sup> T cells. Gray areas are the staining of isotype control antibodies. Representative of at least 3 experiments.



**Supplementary Figure 3.** CCR10 mediates co-migration of IgA-ASCs and conjugating T cells into the colon. **(A)** Representative flow cytometric analysis of gated colonic CD45<sup>+</sup>EGFP<sup>+</sup>intracellular IgA<sup>+</sup> cells of CCR10<sup>+/-</sup> and CCR10<sup>-/-</sup> mice for expression of indicated molecules. The gray graphs are of the staining with isotype control antibodies. **(B)** Comparison of percentages of EGFP(CCR10)<sup>+</sup> IgA-ASCs in the PP, CP, mLN and colons of FTY720-treated (FTY720) and untreated (Ctrl) CCR10<sup>+/-</sup> mice. One dot represents one mouse. Samples analyzed in parallel in the same experiment are linked by a line. **(C)** Representative flow cytometric analysis of EGFP(CCR10)<sup>+</sup> IgA-ASC/T cell conjugates of the PP of  $\mu$ MT<sup>-/-</sup> recipient mouse 2 weeks after receiving co-transfer of CCR10<sup>+/-</sup> (CD45.1/2) and CCR10<sup>-/-</sup> (CD45.2/2) splenic B cells. Gated EGFP(CCR10)<sup>+</sup> cells were first analyzed for CD45.1 and CD3 (Left graph), from which the gated EGFP(CCR10)<sup>+</sup>CD45.1<sup>+</sup>CD3<sup>+</sup> and EGFP(CCR10)<sup>+</sup>CD45.1<sup>-</sup>CD3<sup>+</sup> populations were analyzed for the CD45.2 expression. Representative of 8 mice analyzed.



**Supplementary Figure 4.** CCR10<sup>+</sup> IgG1-ASCs are the major isotype of ASCs that substitute for CCR10<sup>+</sup> IgA-ASCs in the colons of IgA-knockout mice. **(A)** Flow cytometric analysis of gated CD45<sup>+</sup>EGFP(CCR10)<sup>+</sup> colonic plasma cells of IgA<sup>+/+</sup> CCR10<sup>+/-</sup> and IgA<sup>-/-</sup> CCR10<sup>+/-</sup> mice for expression of indicated molecular markers. Gray areas are of the staining of isotype control antibodies. **(B)** Representative flow cytometric analysis of gated CD45<sup>+</sup>EGFP(CCR10)<sup>+</sup> colonic plasma cells of IgA<sup>+/+</sup> CCR10<sup>+/-</sup> and IgA<sup>-/-</sup> CCR10<sup>+/-</sup> mice for intracellular expression of different antibody isotypes. **(C)** Gating strategy to identify colonic CD45<sup>+</sup>F4/80<sup>+</sup> macrophages of IgA<sup>-/-</sup> CCR10<sup>+/-</sup> mice.

**Supplementary Table 1. Clinical Information of Patients with Selective IgA Deficiency\***

Patient	Gender	Age	Ethnicity	Indication for Biopsy	Pathology Diagnosis
1	F	60	Hispanic	Colon cancer screening	Tubular adenoma
2	F	75	African American	Colon cancer screening	Tubular adenoma
3	M	51	Hispanic	Colon cancer screening	Tubular adenoma
4 <sup>#</sup>	F	65	Hispanic	Colon cancer screening	Tubular adenoma
5	F	81	African American	Colon cancer screening	Normal
6	F	43	Hispanic	Colon cancer screening	Tubular adenoma
7	F	49	African American	Colon cancer screening	Juvenile polyp
8	M	36	White	Heme positive stool	Lymphoid aggregates
9	F	57	White	Anemia, diarrhea	Tubular adenoma
10	F	60	African American	Anemia	Lymphoid aggregates
11	F	70	African American	Colon cancer screening	Lymphoid aggregates

Note: \*The composition of plasma cells was evaluated on portions of specimen with normal colonic mucosa other than tubular adenoma, juvenile polyp or lymphoid aggregates. <sup>#</sup>This patient has 4 specimens from different colonic segments.

**Supplementary Table 2. A list of antibodies used**

<b>Name of Antibody (species of origin)</b>	<b>Clone #</b>	<b>Company</b>
anti-mouse CD62L (Rat / IgG2a, kappa)	MEL-14	eBioscience (San Diego, CA)
anti-mouse CD4 (Rat / IgG2b, kappa)	GK1.5	
anti-mouse CD8 $\alpha$ (Rat / IgG2a, kappa)	53-6.7	
anti-mouse TCR $\gamma\delta$ (Armenian hamster / IgG)	GL3	
anti-mouse/rat Foxp3 (Rat / IgG2a, kappa)	FJK-16s	
anti-mouse IgA (Rat / IgG1, kappa)	11-44-2	
anti-mouse TCR $\beta$ (Armenian hamster / IgG)	H57-597	
anti-mouse MHCII (Rat / IgG2b, kappa)	M5/114.15.2	
anti-mouse CD28 (Syrian hamster / IgG)	37.51	
anti-mouse PD-L1 (Rat / IgG2a, lambda)	MIH5	
anti-mouse IL-17A (Rat / IgG1, kappa)	TC11-18H10.1	Biolegend (San Diego, CA)
anti-mouse CD45 (Rat / IgG2b, kappa)	30-F11	
anti-mouse CD45.1 (Mouse / IgG2a, kappa)	A20	
anti-mouse CD45.2 (Mouse / IgG2a, kappa)	104	
anti-mouse CD3 (Rat / IgG2b, kappa)	17A2	
anti-mouse CCR7 (Rat / IgG2a, kappa)	4B12	
anti-mouse IgG1 (Rat / IgG)	RMG1-1	
anti-mouse IgG2a (Rat)	RMG2a-62	
anti-mouse F4/80 (Rat / IgG2a, kappa)	BM8	
anti-mouse CD80 (Armenian hamster / IgG)	16-10A1	

anti-mouse CD86 (Rat / IgG2a, kappa)	GL-1	
anti-mouse IL-10 (Rat / IgG2b, kappa)	JES5-16E3	BD Biosciences (San Jose, CA)
anti-mouse IFN $\gamma$ (Rat / IgG1, kappa)	XMG1.2	
anti-mouse CD3 $\epsilon$ (Armenian hamster / IgG)	145-2C11	
anti-mouse IgA (Rat)	C10-1	
anti-mouse $\alpha 4\beta 7$ (Rat)	DATK32	
anti-mouse IgG2b (Rat)	R12-3	
anti-mouse IgM (Rat / IgG1, kappa)	1B4B1	Southern Biotech
anti-human CCR10 (polyclonal) (Goat)	SKU: CI0126	Capralogics
anti-human IgA (polyclonal) (Rabbit)	A0262	Dako
anti-human IgG (polyclonal) (Rabbit)	A0424	
anti-human IgM (polyclonal) (Rabbit)	A0426	
EnVision+ System-HRP labeled polymer anti-rabbit antibodies (polyclonal)	N/A	
peroxidase affiniPure rabbit anti-goat IgG (H+L) (polyclonal)	N/A	Jackson ImmunoResearch
anti-human IgG2 (Rabbit)	EPR4418	Abcam
anti-human IgG1 (Rabbit)	RM117	RevMAb Biosciences
anti-human IgG3 (Rabbit)	RM119	
anti-human IgG4 (Rabbit)	RM120	

Note: all antibodies are monoclonal unless indicated otherwise.

**Supplementary Table 3. A list of chemicals used**

Name of Chemical	Company
FOXP3/transcription factor staining buffer set	eBioscience (San Diego, CA)
collagenase type I	Worthington Biochemical (Lakewood, NJ)
collagenase type IV	
hyaluronidase	Sigma (St. Louis, MO)
dextran sulfate sodium salt (DSS, M.W. 36,000-50,000)	MP Biomedicals (Solon, OH)
FTY720	Cayman Chemical



**Supplementary Table 4. Sequences of primers used in real-time PCR**

Name of Gene	Sequence of Primer
IL-1 $\alpha$	Forward: GAAGAAGAGACGGCTGAG
	Reverse: GTAGGTGTAAGGTGCTGAT
IL-17A	Forward: CAGCAGCGATCATCCCTCAAAG
	Reverse: CAGGACCAGGATCTCTTGCTG
TNF- $\alpha$	Forward: TTCTATGGCCCAGACCC
	Reverse: GGCACCACTAGTTGGTTGTC
IL-22	Forward: CGTCAACCGCACCTTTAT
	Reverse: AGGGCTGGAACCTGTCTG
IFN $\gamma$	Forward: AGGAACTGGCAAAGGATGGTG
	Reverse: GTGCTGGCAGAATTATTCTTATTG
$\beta$ -actin	Forward: CCCATCTACGAGGGCTAT
	Reverse: TGTCA-CGCACGATTTC