Supplementary Materials and Methods

Southern Blot Analysis

Southern blot analysis was performed as described previously.[1] Briefly, genomic DNA prepared from embryonic stem (ES) cells and tail tips was digested with restriction enzyme *Nco*I, fractionated by agarose gel electrophoresis, transferred to a Hybond-N membrane (GE Healthcare, Piscataway, NJ). The 5′ and 3′ external probes were amplified by polymerase chain reaction (PCR) from genomic DNA using primers Kit-5p-1 and Kit-5p-1r and Kit-3p-1 and Kit-3p-1r, respectively (Supplementary Table 1). These 2 probes were labeled with [α-P^{32}]-dCTP using the Amersham Rediprime II Random Prime Labeling System (GE Healthcare). The membrane was hybridized with 5′ or 3′ DNA probe. Hybridization was performed overnight at 58°C in Perfect Hyb Plus Hybridization Buffer (Sigma-Aldrich). Filters were washed at 58°C in 2X standard saline citrate (SSC) (0.15 mol/L sodium chloride/0.015 mol/L sodium citrate, pH 7.0) with 0.1% sodium dodecyl sulfate (SDS) for 30 minutes, followed by 2 washes in 0.2× SSC containing 0.1% SDS for 30 minutes each. Expected band sizes for the wild-type (WT) and mutant alleles of *Kit*$^{+/copGFP}$ are 18.4 kilobase (kb) and 11.6 kb by the 5′ probe and 18.4 kb and 8.4 kb by the 3′ probe, respectively (Figure 1).

Animals and Tissue Preparation

Kit$^{+/copGFP}$ transgenic mice and WT mice (siblings of transgenic mice) were maintained, and the experiments were performed according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and the American Physiological Society's Guiding Principles in the Care and Use of Animals. All protocols were approved by the Institutional Animal Care and Use Committee at the University of Nevada, Reno, NV. *Kit*$^{+/copGFP}$ transgenic mice and WT mice were anesthetized by isoflurane (AErrane; Baxter Healthcare, Deerfield, IL) inhalation and killed by decapitation. Mutiple tissues, brain, heart, liver, lung, kidney, stomach, small intestine, and colon were diseccted from 4-week-old *Kit*$^{+/copGFP}$ and WT mice. The gastrointestinal (GI) tissues (stomach, small intestine, and colon) were stripped free of mucosa and submucous plexus. The mutiple tissues were used for isolation of total RNAs. Some of small intestine and colon muscularis tissues were used for purification of copGFP^{+} interstitial cells of Cajal (ICC) using fluorescence-activated cell sorter.

Immunohistochemical Identification of copGFP^{+} Cells as ICC

GI tracts were removed and placed in Kreb's Ringer buffer (KRB). Stomachs were opened along the lesser curvature and the small intestine and colon along the mesenteric border. Luminal contents were washed with KRB. Tissues were pinned to Sylgard elastomer (Dow Corning, Midland, MI) in a dissecting dish and stretched to 110% of resting dimension. Mucosa was removed by sharp dissection, and tissues were fixed in paraformaledehyde (4% wt/vol for 30 minutes), as described.[2] After fixation, tissues were washed with phosphate-buffered saline (PBS; 0.01 mol/L, pH 7.4) for several hours and then incubated with bovine serum albumin (BSA; 1% for 1 hour) to reduce nonspecific binding. Tissues were incubated with anti-KIT antibody (goat anti-SCFR; 1:500; R&D Systems, Minneapolis, MN) at 4°C overnight, washed in PBS, and incubated in Alexa fluor 594-coupled donkey anti-goat secondary antibody (Invitrogen; 1:1000 in PBS; 1 hour at room temperature). Control tissues were prepared by omitting either primary or secondary antibodies from the incubation solution. For immunohistochemical studies on cryostat sections, fixed tissues were dehydrated in graded sucrose solutions (5%–20% wt/vol in PBS at 4°C, 1 hour at each concentration). Tissues were incubated overnight in 20% sucrose in PBS at 4°C before being embedded in a 1:1 solution of Tissue-Tek (Miles, IL) and 20% sucrose in PBS and frozen in liquid nitrogen. Cryostat sections were cut at a 7-μm thickness using a Leica CM3050 (Wetzlar, Germany), air-dried for 30 minutes, washed in PBS for 30 minutes, and preincubated in BSA (1% wt/vol in PBS) before being incubated with primary and secondary antibodies as described for whole mounts. Control tissues and sections were prepared by either omitting primary or secondary antibodies from the incubation solutions.

DNA Sequencing and Analysis

DNA sequencing was performed at Nevada Genomics Center (University of Nevada, Reno, NV). The final targeting construct Kit-copGFP/pHW (15.5 kb) was sequenced with each of 30 primers for confirmation. DNA sequences were analyzed and mapped by Vector NTI Suite version 6.0 (Invitrogen). The reverse-transcription PCR products of cell marker genes amplified and purified from the sorted ICC were sequenced and analyzed using the Blast search program in the Vector NTI Suite. All PCR products matched complementary DNA sequence for each gene designated and amplified. Genotyping PCR products (155 bp) of the *Lep* gene were also sequenced for confirmation of a nonsense point mutaion (C to T) in codon 105 in hetrozygote (C/T) and homozygote (T/T).

References

1. Yan W, Ma L, Burns KH, et al. Haploinsufficiency of kelch-like protein homolog 10 causes infertility in male mice. Proc Natl Acad Sci U S A 2004;101:7793–7798.
2. Ward SM, Bayguinov J, Won KJ, et al. Distribution of the vanilloid receptor (VR1) in the gastrointestinal tract. J Comp Neurol 2003; 465:121–135.

Supplementary Table 1. Oligonucleotides Used in This Study

Name	Sequence (5′ to 3′)	Tm (°C)	Gene	Size (bp) of cDNA/genomic	Usage
copGFP-1	CTTCCTGCACGCCATCAACAACG	59	*copGFP*	181/181	Genotyping/qPCR
copGFP-1r	GATGATCTTGTCGGTGAAGATCACG	58	*copGFP*		Genotyping/qPCR
Kit-g1	ACTTGGGCGAGAGCTGTAGCAGA	59	*Kit*	0/159	Genotyping
Kit-g1r	AGAGGGTGCAGTCCTCTTGTCTG	59	*Kit*		Genotyping
Kit-5p-1	CTTCACATTGGGCTGGGATGTTACAAGGG	63	*Kit*	0/166	5’probe
Kit-5p-1r	GTTAACAACTTCTGAGTCTCTTGCCTCAAC	60	*Kit*		5’probe
Lep-1	TGTCCAAGATGGACCAGACTC	54	*Lep*	155/155	Genotyping
Lep-1r	ACTGGTCTGAGGCAGGGAGCA	58	*Lep*		Genotyping
Kit-3p-1	GAGATGTCACGAAAGGAACTGTCCAGTGGAG	64	*Kit*	0/184	3’probe
Kit-3p-1r	GACTAACGGCCACTAAACCAACCTTCCTC	63	*Kit*		3’probe
Kit-2	GGATCAGCAAATGTCACAACAACCTTG	58	*Kit*	158/5512	qPCR
Kit-2r	CAAACCCGAGCACCAGCAGTGGATA	61	*Kit*		qPCR
GAP-2	TCCTGCACCACCAACTGCTTAGC	59	*Gapdh*	110/244	qPCR/RT-PCR
GAP-2r	GTCTTCTGGGTGGCAGTGATGG	59	*Gapdh*		qPCR/RT-PCR
mSMH-2	CTTTTCCGATGGATTCTCAGCCGTG	59	*Myh11*	137/979	RT-PCR
SMH-2r	GTTGATGCACAGCTGCTCGAAGG	59	*Myh11*		RT-PCR
mPGP-2	GTACCATCGGGTTGATCCACGC	59	*Uchl1*	130/2758	RT-PCR
mPGP-2r	TCGAAACACTTGGCTCTATCTTCGG	58	*Uchl1*		RT-PCR
CD34-1	CAGGGTATCTGCCTGGAACTAAGTG	59	*Cd34*	157/7555	RT-PCR
CD34-1r	CTAACCTCAGACTGGGCTAGAAGC	59	*Cd34*		RT-PCR
CD45-2	GATGTCAGTTGGACAACCTTCGTGC	59	*Ptprc*	141/3384	RT-PCR
CD45-2r	CCACAACTAGGCTTAGGCGTTTCTG	59	*Ptprc*		RT-PCR
CD68-1	CAAGGTCCAGGGAGGTTGTGAC	59	*Cd68*	154/290	RT-PCR
CD68-1r	CTGTGGGAAGGACACATTGTATTCC	58	*Cd68*		RT-PCR
Cma1-1	CTTTGTGCTGACTGCAGCTCACTG	59	*Cma1*	159/1160	RT-PCR
Cma1-1r	CATGATGTCGTGGACAACCAAATTCTC	59	*Cma1*		RT-PCR

Supplementary Table 2. Antibodies Used in This Study

Name	Amount added	Company
Anti-mouse CD16/32 (rat IgG2b K, clone 93)	250 ng	eBioscience (San Diego, CA)
Rat IgG	2300 ng	Jackson ImmunoResearch (West Grove, PA)
Bio-anti-CD45 (rat IgG2b K, clone 30-F11)	250 ng	eBioscience
Bio-anti-CD11b (rat IgG2b K, clone M1/70)	125 ng	eBioscience
Bio-anti-CD11c (hamster IgG, clone N418)	63 ng	eBioscience
Bio-anti-F4/80 (rat IgG2a K, clone BM8)	250 ng	eBioscience
PE-TxR-SA	50 ng	BD Biosciences (San Jose, CA)
PE-anti-CD117 (rat IgG2a K, clone ACK2)	125 ng	eBioscience
PC7-anti-Gr-1 (anti-Ly-6C/G) (rat IgG2b K, clone RB6-8C5)	63 ng	eBioscience
7-amino-actinomycin D (7-AAD)	250 ng	eBioscience

PE, R-phycoerythrin; TxR, Texas Red; SA, streptavidin; Bio, biotin; PC7, PE-cyanine 7 tandem.