Supplementary Materials and Methods

Intestinal-specific KLF5 knockdown mice generation

C57BL/6 mice carrying *Klf5* alleles flanked by loxP sites (*Klf5*$^{fl/fl}$ mice) were described previously (1). C57BL/6 mice carrying the Cre recombinase gene under regulation of the *villin* promoter (*Vil-Cre* mice) were purchased from Model Animal Research Center of Nanjing University (Nanjing, China). *Klf5*$^{fl/fl}$ mice were crossed with *Vil-Cre* mice to generate *Vil-Cre; Klf5*$^{fl/+}$ progeny. Littermates or age-matched *Vil-Cre; Klf5*$^{fl/-}$ mice served as controls. Genomic DNA isolation and PCR were used to confirm the genotypes of the mice used.

Antibodies

Antibodies used for Western blotting were KLF5 (1:1000, 07-1580, Millipore, Billerica, MA, USA) and GAPDH (1:1,000, 2251-1, Epitomics, Burlingame, CA, USA). Antibodies used for immunohistochemistry were KLF5 (1:500, 07-1580, Millipore, Billerica, MA, USA); Musashi-1 (1:400, ab21628) and Ki67 (1:100, ab16667) from Abcam (Cambridge, MA, USA). Antibodies used for immunofluorescence were guinea pig monoclonal KLF5 antibody (2) and rabbit monoclonal Ki67 (1:100, ab16667, Abcam, Cambridge, MA, USA).

Horseradish peroxidase-conjugated and FITC-labeled goat-anti rabbit IgG were purchased from Beyotime Institute of Biotechnology (Haimen,

China). Ready-to-use secondary antibody goat-anti rabbit HRP-IgG was purchased from Zhongshan Golden Bridge Biotechnology (Beijing, China). Biotinylated goat-anti guinea pig secondary antibody was purchased from Vector Lab (Burlingame, CA, USA). Cy3-labeled StreptAvidin-Biotin-enzyme Complex was purchased from Wuhan Boster Bio-engineering Co. Ltd. (Wuhan, China).

Morphological analysis

Villus height and crypt depth were determined by taking pictures of H&E-stained sections. The images were analyzed using Image J 1.43 software. At least 30 well-oriented, full-length crypt-villus units per mouse were measured. The measurement in pixels was converted to length in μm with the following conversion factor: 1.46 pixels per μm. Crypts per circumference were counted from 3 separate tubular intestinal H&E-stained slices for each mouse.

Microarray analysis of gene expression

Total RNA was extracted from small intestine tissues with Trizol reagent (Invitrogen, Carlsbad, CA, USA) and quantified using a NanoDrop ND-1000 (Thermo Scientific Inc., Waltham, MA, USA). RNA integrity was assessed by standard denaturing agarose gel electrophoresis. Equal mass amounts of total RNA were pooled from each small intestine to yield a sample representing RNA from 3 separate mice in each group. Microarray-based mRNA expression profiling was performed using Roche-NimbleGen (12x135K) microarrays (Roche NimbleGen, Madison,

WI, USA). The microarrays contained approximately 44,170 assay probes corresponding to all of the annotated mouse mRNA sequences (NCBI MM9, Build 37). Total RNA labeling and array hybridization were performed using standard protocols according to the manufacturers' instructions. An Axon GenePix 4000B scanner was used to scan the probe arrays. Data were extracted and normalized using NimbleScan v2.5 software. mRNA with an absolute fold change of 2 or greater was judged to be differentially expressed. Subsequently, hierarchical clustering was performed using Agilent GeneSpring GX software (version 11.5.1). Gene Ontology (GO) analysis and pathway analysis were performed using the standard enrichment computation method.

Supplemental Results, Figures and Tables

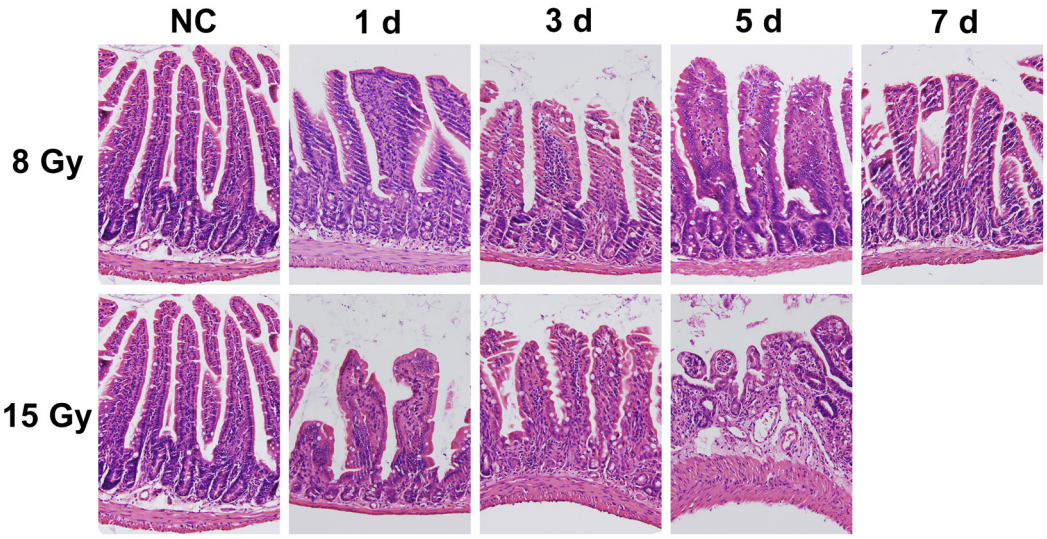

Figure S1. Representative H&E staining of paraffin intestinal sections from WT mice non-irradiated or X-irradiated by 8 or 15 Gy at the indicated times (magnification 200×). NC, non-irradiated control.

Table S1. Primer sequences for real-time PCR analysis.

Gene	Forward primer	Reverse primer
Rev1l	5’- GTTCTCTTCCGCCATCCAC -3’	5’- GGGGAGGACACTGAACGAC -3’
Usp1	5’- TTGCAAGGAGTTGACGAGAAT -3’	5’- CACCAGCGATCCCTCTCC -3’
Lig1	5’- TGTCCTCATTCTGCTCCTCA -3’	5’- ACATCTCCCCATCAGGATTC -3’
Xpc	5’- TTTCCTTAGAGAGCTTTCGGG -3’	5’- CGAGGACAACAAAGTAGCCC -3’
Cul4b	5’- TTGCAGAAATCTTATGAGAACAAA -3’	5’- AAAAGAAGCAGTGGAAGCCA -3’
Ercc5	5’- GCTTGGTTTAGCCAAATGCT -3’	5’- CTGTGGAAGCTGCTGGAGTG -3’
Dclre1c	5’- CTCTTCCTTCTCACCCGAAG -3’	5’- CCCAAAGTACAGATTCTGGGA -3’
Nhej1	5’- CTTGTTCAGCTCCTTGGCTC -3’	5’- ACGGTTATGCCTTGCTGATT -3’
Gapdh	5’- CGTCCCGTAGACAAAATGGT -3’	5’- TTGATGGCAACAATCTCCAC -3’

Table S2. Numbers of up- and down-regulated genes in small intestines of non-irradiated and irradiated mice at 6 h post 15 Gy TBI (*Vil-Cre; Klf5*$^{fl/+}$ mice *vs.* control mice).

Absolute fold change	NC		6h post 15Gy TBI	
	Up-regulated	Down-regulated	Up-regulated	Down-regulated
≥2 and <5	2511	4839	1046	1275
≥5 and <10	583	1404	47	84
≥10 and <50	303	1110	1	13
≥50	24	230	0	0
Total	3421	7583	1094	1372

Note: NC, non-irradiated control.

Table S3. Genes involved in DNA damage repair pathways that are down-regulated by intestinal-specific knockdown of KLF5 (*Vil-Cre; Klf5$^{fl/+}$* mice *vs.* control mice).

Gene name	Fold change	Chromosome	Description
Nucleotide excision repair			
Rbx1	-27.52	chr15	Ring-box 1
Ercc5	-12.61	chr1	Excision repair cross-complementing rodent repair deficiency, complementation group 5
Cul4b	-11.75	chrX	Cullin 4B
Lig1	-7.08	chr7	Ligase I, DNA, ATP-dependent
Rfc3	-6.25	chr5	Replication factor C (activator 1) 3
Xpc	-6.06	chr6	Xeroderma pigmentosum, complementation group C
Cdk7	-5.69	chr13	Cyclin-dependent kinase 7
Rfc1	-5.24	chr5	Replication factor C (activator 1) 1
Pold2	-4.72	chr11	Polymerase (DNA directed), delta 2, regulatory subunit
Rpa3	-4.13	chr6	Replication protein A3
Gtf2h1	-3.82	chr7	General transcription factor II H, polypeptide 1
Gtf2h2	-3.09	chr13	General transcription factor II H, polypeptide 2

Rpa2	-2.98	chr4	Replication protein A2
Ddb2	-2.94	chr2	Damage specific DNA binding protein 2
Gtf2h5	-2.80	chr17	General transcription factor II H, polypeptide 5
Pold1	-2.59	chr7	Polymerase (DNA directed), delta 1, catalytic subunit
Gtf2h4	-2.57	chr17	General transcription factor II H, polypeptide 4
Cetn2	-2.49	chrX	Centrin 2
Cul4a	-2.42	chr8	Cullin 4A
Ercc4	-2.29	chr16	Excision repair cross-complementing rodent repair deficiency, complementation group 4
Rfc5	-2.04	chr5	Replication factor C (activator 1) 5
Fanconi anemia pathway			
Eme2	-12.28	chr17	Essential meiotic structure-specific endonuclease subunit 2
Rev1	-9.41	chr1	REV1 homolog
Poli	-5.35	chr18	Polymerase (DNA directed), iota
Usp1	-5.10	chr4	Ubiquitin specific peptdiase 1
Hes1	-4.66	chr16	Hairy and enhancer of split 1
Telo2	-4.14	chr17	Telomere maintenance 2, homolog

Rpa3	-4.13	chr6	Replication protein A3
Fancc	-3.89	chr13	Fanconi anemia, complementation group C
Top3b	-3.25	chr16	Topoisomerase (DNA) III beta
Polk	-3.17	chr13	Polymerase (DNA directed), kappa
Palb2	-3.16	chr7	Partner and localizer of BRCA2
Fancf	-3.14	chr7	Similar to Fanconi anemia, complementation group F
Blm	-3.14	chr7	Bloom syndrome homolog
Rpa2	-2.98	chr4	Replication protein A2
Fancg	-2.95	chr4	Fanconi anemia complementation group G
Rmi1	-2.80	chr13	RecQ mediated genome instability 1, homolog
Fancl	-2.80	chr11	Fanconi anemia, complementation group L
Rad51	-2.77	chr2	RAD51 homolog
Atrip	-2.43	chr9	ATR interacting protein
C230052I12Rik	-2.36	chr7	RIKEN cDNA C230052I12 gene
Ercc4	-2.29	chr16	Excision repair cross-complementing rodent repair deficiency, complementation group 4
Brca1	-2.01	chr11	Breast cancer 1

Mismatch repair			
Lig1	-7.08	chr7	Ligase I, DNA, ATP-dependent
Rfc3	-6.25	chr5	Replication factor C (activator 1) 3
Rfc1	-5.24	chr5	Replication factor C (activator 1) 1
Mlh3	-5.02	chr12	MutL homolog 3
Pold2	-4.72	chr11	Polymerase (DNA directed), delta 2, regulatory subunit
Rpa3	-4.13	chr6	Replication protein A3
Msh2	-3.95	chr17	MutS homolog 2
Rpa2	-2.98	chr4	Replication protein A2
Pold1	-2.59	chr7	Polymerase (DNA directed), delta 1, catalytic subunit
Msh3	-2.08	chr13	MutS homolog 3
Rfc5	-2.04	chr5	Replication factor C (activator 1) 5

Table S4. Genes involved in the non-homologous end-joining (NHEJ) pathway that are down-regulated by intestinal-specific knockdown of KLF5 at 6 h after 15 Gy TBI (*Vil-Cre; Klf5*$^{fl/+}$ mice *vs.* control mice).

Gene name	Fold change	Chromosome	Description
Nhej1	-4.51	chr1	Nonhomologous end-joining factor 1
Dclre1c	-3.13	chr2	DNA cross-link repair 1C, PSO2 homolog
Rad50	-2.14	chr11	RAD50 homolog

Supplemental references

1. Takeda N, Manabe I, Uchino Y, et al. Cardiac fibroblasts are essential for the adaptive response of the murine heart to pressure overload. J Clin Invest 2010; 120: 254-65.

2. Wan H, Luo F, Wert SE, et al. Kruppel-like factor 5 is required for perinatal lung morphogenesis and function. Development 2008; 135: 2563-72.