Supplemental Information
Supplemental information includes 5 figures and 2 tables.

Supplementary Figure Legends

Figure S1. Distinct temporal regulation of neutrophil recruitment increases tissue I/R injury in males. Male and female rats were subjected to 30min mesenteric ischemia followed by up to 2h reperfusion. (A) Representative images of portions of male and female small intestine at the end of reperfusion, demonstrating redness and edema in males following I/R but no observable change in females. (B-C) Leukocyte/vessel wall interactions throughout reperfusion in mesenteric venules, measured by intravital microscopy: (B) Leukocyte adhesion and (C) number of tissue leukocytes at different distances away from venule (sham: n=5 rats/group, I/R: n=8 rats/group). (D) Circulating CD68+ monocytes in males and females during ischemia and reperfusion. Data are presented as mean ± sem. §P<0.001 by two-way ANOVA, and ‡P<0.05 or #P<0.001 by Bonferroni's post-test. ns denotes P>0.05 by one-way ANOVA.

Figure S2. No effect of sex or ischemia on hemodynamics during reperfusion. Male and female rats were subjected to 30min mesenteric ischemia followed by up to 2h reperfusion. (A) Mean arterial blood pressure (MABP) measured by cannulation of carotid artery. (B) Red blood cell (RBC) velocity and (C) wall shear rate, calculated from RBC velocity and venule diameter, as described in Supplementary Methods. Sham: n=4 rats/group, I/R: n=4 rats/group. Data are presented as mean ± sem. All comparisons P>0.05 by two-way ANOVA.

Figure S3. Increased induction of neutrophil integrins in males. Male and female rats were subjected to 30min mesenteric ischemia followed by 2h reperfusion. Surface expression of integrins β1, α4, αL and αM on RP1+ neutrophils in BM and circulation of male and female rats, measured at 2h reperfusion. Sham: n=3 rats/group, I/R: n=5 rats/group. Data are presented as mean ± sem. *P<0.05, **P<0.01 by one-way ANOVA followed by Bonferroni's post-test.

Figure S4. Sex-specific regulation of Cxcl5 determines magnitude of leukocyte dynamics in I/R. (A-D) Male and female rats were subjected to 30min mesenteric ischemia followed by up to 2h reperfusion. (A) Mesenteric tissue Ccl2 mRNA and (B) plasma protein Ccl2, at 30min and 2h reperfusion. (C-D) Mesenteric tissue Ccl3 and Ccl5 mRNA levels, at 30min and 2h reperfusion. Levels of mRNA are normalized to 18S and calculated as fold expression relative to mean value in sham-operated males. Sham: n=5 rats/group, I/R: n=8 rats/group. (E-F) Male rats were treated with either anti-Cxcl5 (20µg/kg, iv) or control IgG (20µg/kg, iv) 1h prior to mesenteric ischemia (n=5 rats/group). (E) Number of adherent leukocytes and (F) number of emigrated leukocytes within 50µm of blood vessel wall, measured by intravital microscopy during reperfusion. Data are presented as mean ± sem. *P<0.05, by one-way ANOVA followed by Bonferroni's post-test. § P<0.001 by two-way ANOVA and ‡P<0.05, **P<0.01 or #P<0.001 by Bonferroni's post-test.

Figure S5. Cxcl5-induced leukocyte/vessel wall interactions. Male rats were treated with 3µg/kg Cxcl5 (ip, n=5 rats) or PBS (n=3 rats) for 2h. (A) Leukocyte flux, (B) adherent leukocytes, (C) emigrated leukocytes in mesenteric tissues at 2h. Data are presented as mean ± sem. *P<0.05,**P<0.01, ***P<0.001 compared to PBS by Student's t-test.

Table S1. Details of antibodies used for flow cytometry.

Antigen	Clone	Supplier
Rat		
Granulocytes	RP1	BDPharmingen
CD68	ED1	AbD serotec
Integrin β1/CD29	HMβ1-1	Biolegend
Integrin β2/CD18	WT.3	AbD serotec
Integrin αL/CD11a	WT.1	AbD serotec
Integrin αM/CD11b	OX-42	AbD serotec
Integrin α4/CD49d	MRα4-1	Biolegend
L-selectin/CD62L	OX-85	Biolegend
Mouse		
GR1	RB6-8C5	BDPharmingen
Integrin β2/CD18	C71/16	BDPharmingen
Human		
CD14	M5E2	BDPharmingen
CD16	3G8	BDPharmingen
CD3	UCHT1	BDPharmingen
HLA-DR	G46-6	BD Horizon

Table S2. Sequence of primers used for real-time quantitative PCR with SYBR green.

	Forward	Reverse
RAT		
CXCL1/KC/GROα	GGCAGGGATTCACTTCAAGA	GCCATCGGTGCAATCTATCT
CXCL5/LIX	CTCAAGCTGCTCCTTTCTCG	GCGATCATTTTGGGGTTAAT
CCL2/MCP1	ATGCAGTTAATGCCCCACTC	TTCCTTATTGGGGTCAGCAC
CCL3/MIP1α	TTTTGAGACCAGCAGCCTTT	CTCAAGCCCCTGCTCTACAC
CCL5/RANTES	GTGCCCACGTGAAGGAGTAT	ATCCCCAGCTGGTTAGGACT
GCSF	CCTAGCAGGCATTTCCTCTG	GCTGGAAGGCAGAAGTGAAG
CXCR2	CCAAGCTGATCAAGGAGACC	GGGGTTAAGACAGCTGTGGA
18S	AGCCTGCGGCTTAATTTGAC	CAACCTAAGACGGCCATGCA
MOUSE		
CXCL5/LIX	GCATTTCTGTTGCTGTTCACGCT	CCTCCTTCTGGTTTTTCAGTTTAGC