

Supplemental information

**Glucocorticoid receptor-mediated *Nr1d1*
chromatin circadian misalignment
in stress-induced irritable bowel syndrome**

Gen Zheng, Suya Pang, Junbao Wang, Fangyu Wang, Qi Wang, Lili Yang, Mengdie Ji, Dejian Xie, Shengtao Zhu, Yang Chen, Yan Zhou, Gerald A. Higgins, John W. Wiley, Xiaohua Hou, and Rong Lin

Supplementary Information

Supplementary Figures

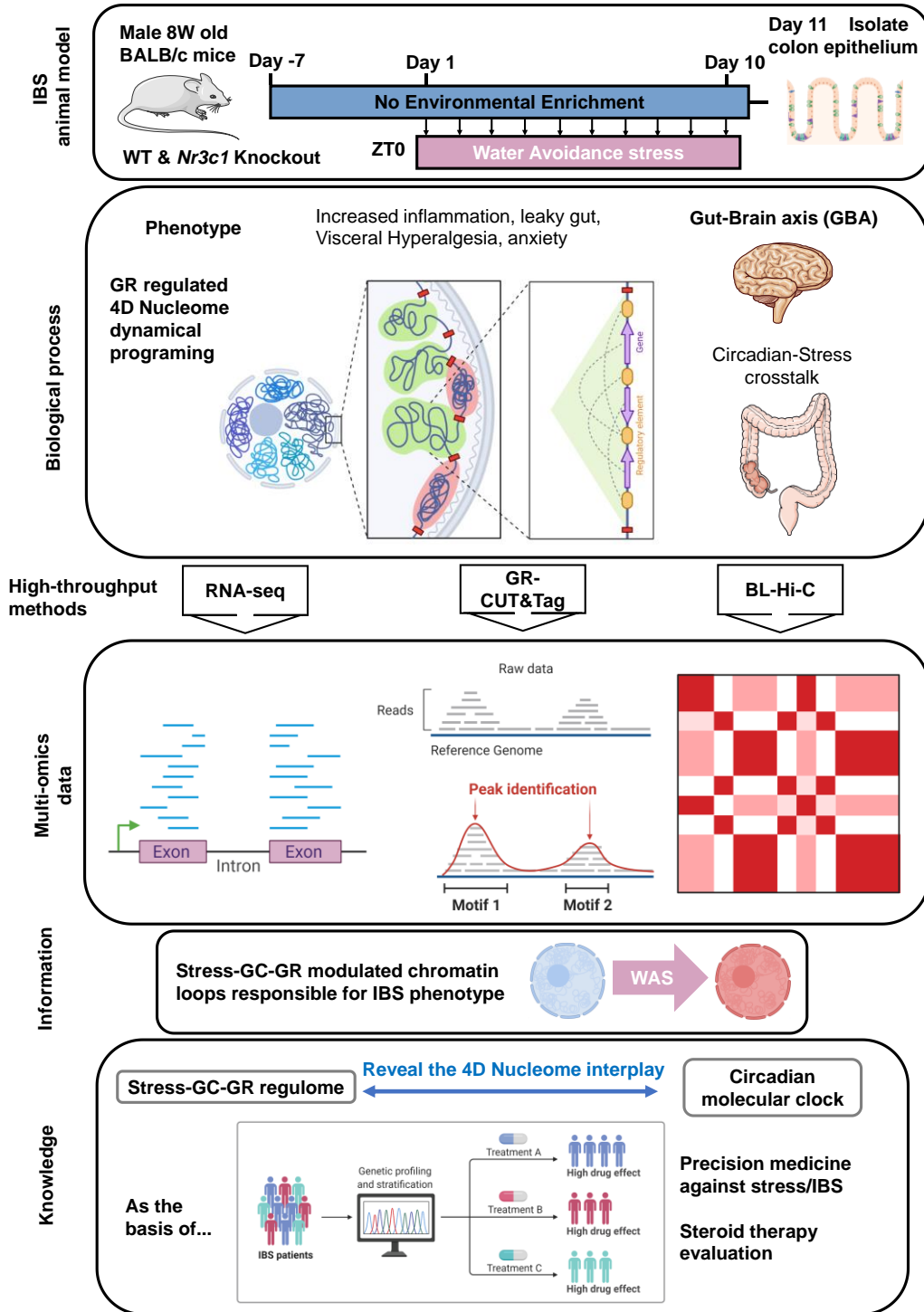


Figure S1. Study design

We used WAS animal model to test our hypothesized IBS 4D nucleome pathogenesis pathway and generated RNA-Seq, GR ChIP-seq, and Hi-C datasets to explore the transcriptional regulation by GR. Conserved circadian-stress crosstalk has translational potential in precision medicine.

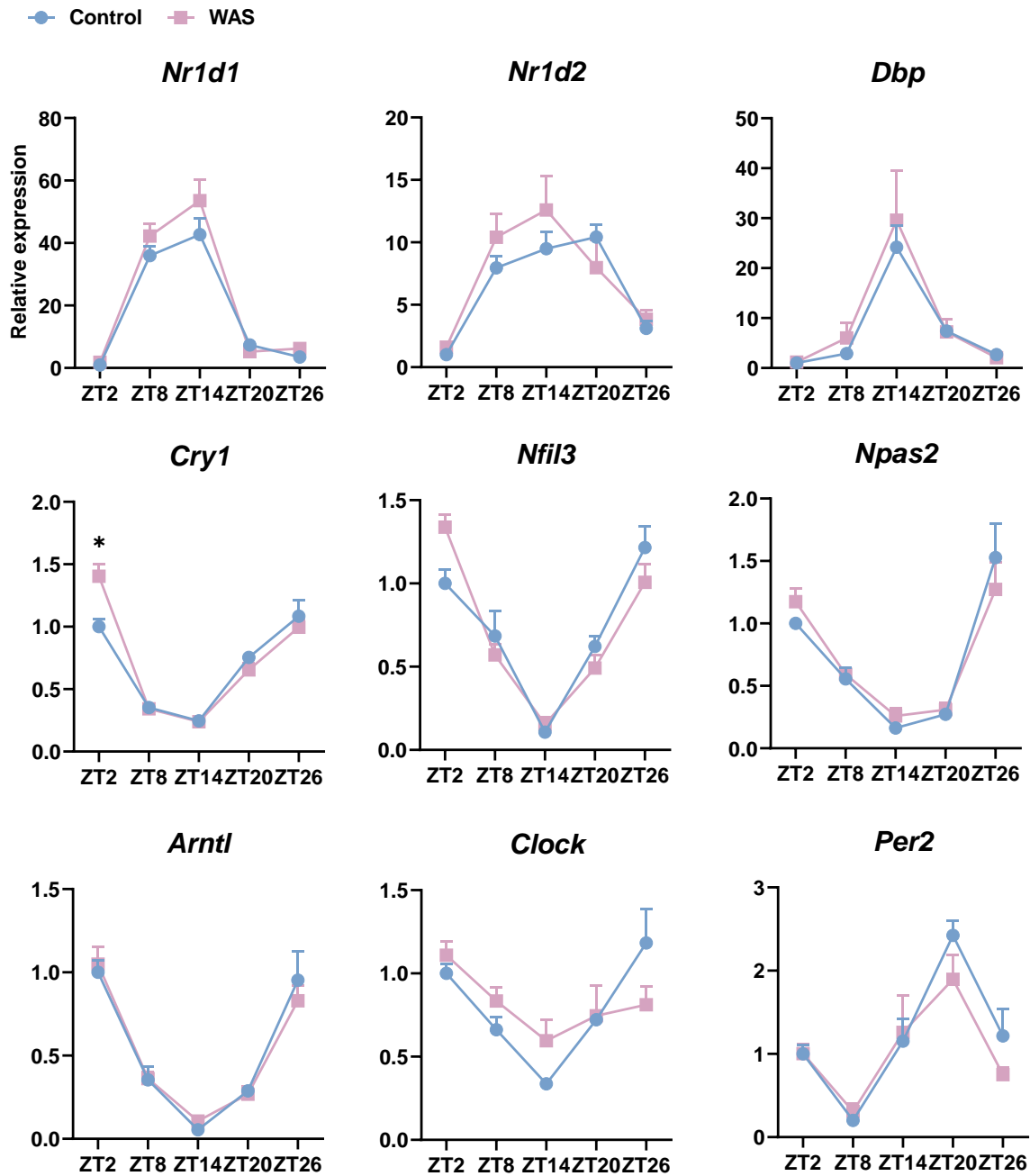


Figure S2. Stress-induced circadian disturbances in colon epithelial cells, Related to Figure

1

Colon epithelial cells of control (CT) and stressed (WAS) mice were isolated for qPCR analysis.

Stress significantly increased *Cry1* at ZT2. Data are expressed as means \pm standard error. Data

from multiple time points were analyzed with the Mann-Whitney test (*, $P < 0.05$; $N = 5$).

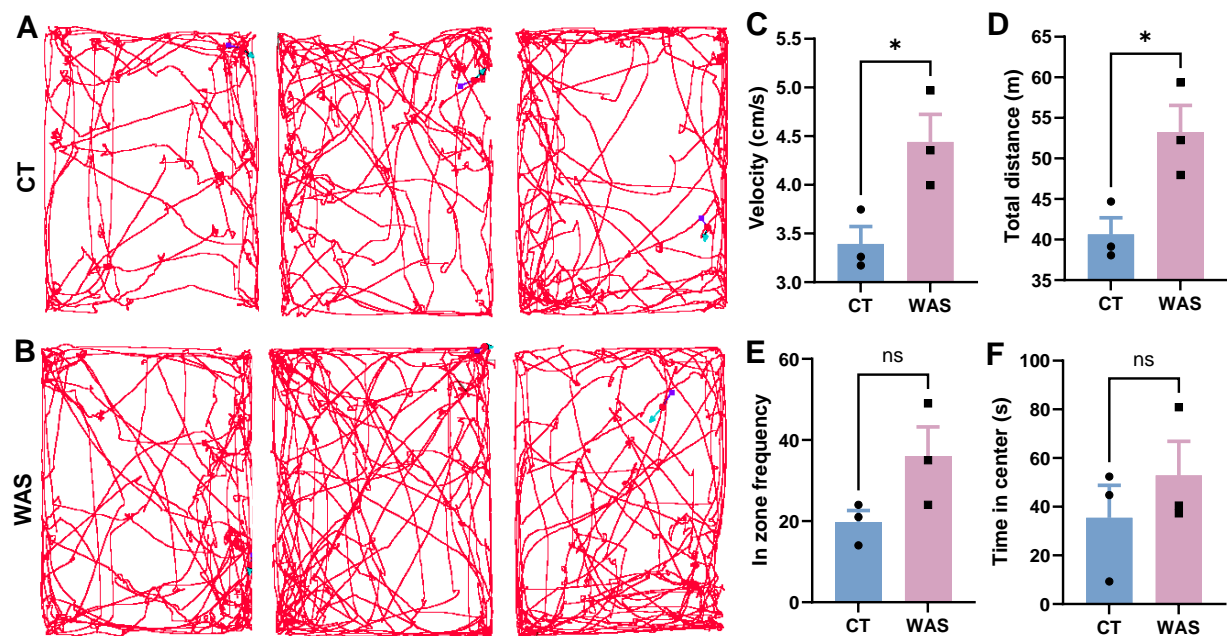


Figure S3. Water avoidance stress (WAS) induced anxiety behavior in the open-field test, Related to Figure 2

(A, B) Representative traces of CT (control) and WAS mice in the open-field arena.

(C-F) Quantification of velocity (C), total distance (D), number of entries into the center (E), and time in the center (F) in the open-field test. Data are expressed as means \pm standard error. Statistical significance was determined using an unpaired *t*-test with Welch's correction (*, $P < 0.05$; $N = 3$).

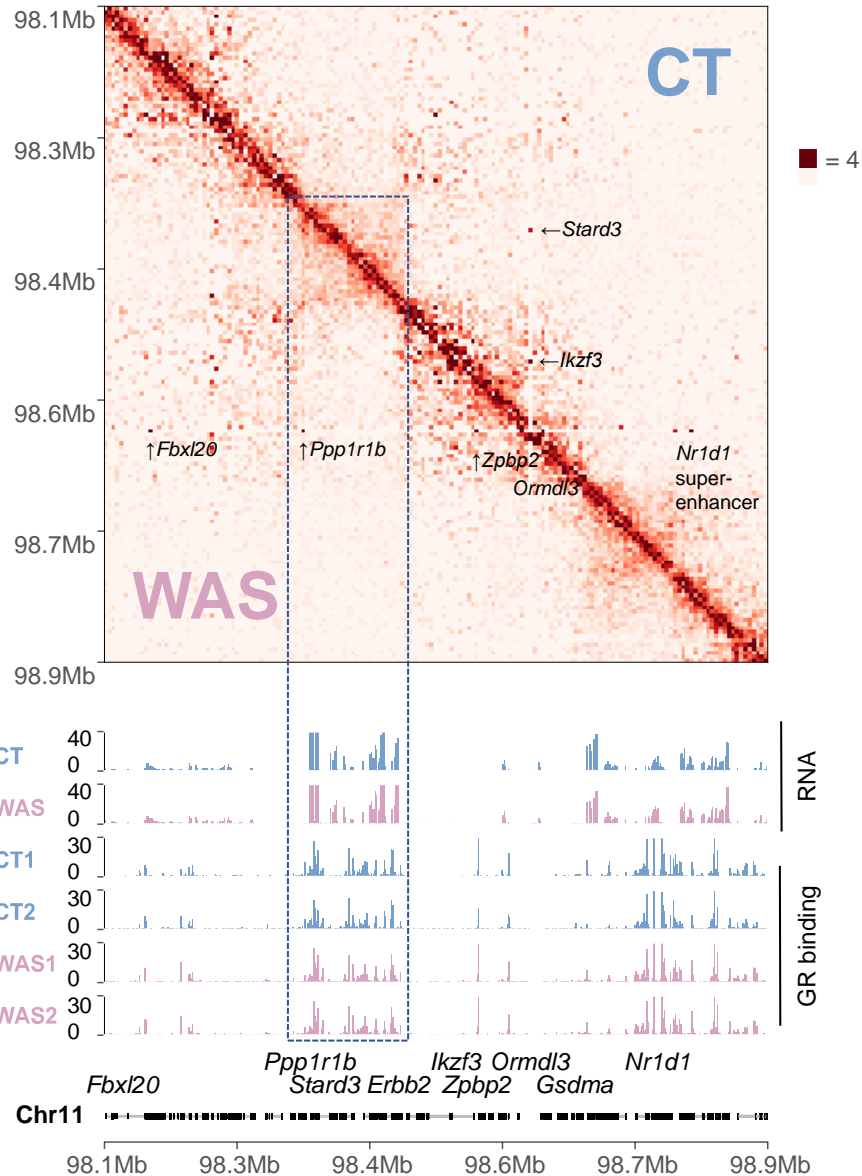


Figure S4. WAS induced *Fbxl20-Ormdl3* and *Ppp1r1b-Ormdl3* chromatin loops replaced the *Stard3-Ormdl3* loop in CT, Related to Figure 4

BL-Hi-C was performed with colon IECs isolated from two control (CT) & two water avoidance stressed (WAS) BALB/c mice; data from each group were combined for visualization; each RNA track represents the combined RNA-Seq data from three mice; GR-CUT&Tag tracks are also shown. Expanded view of Figure 4.

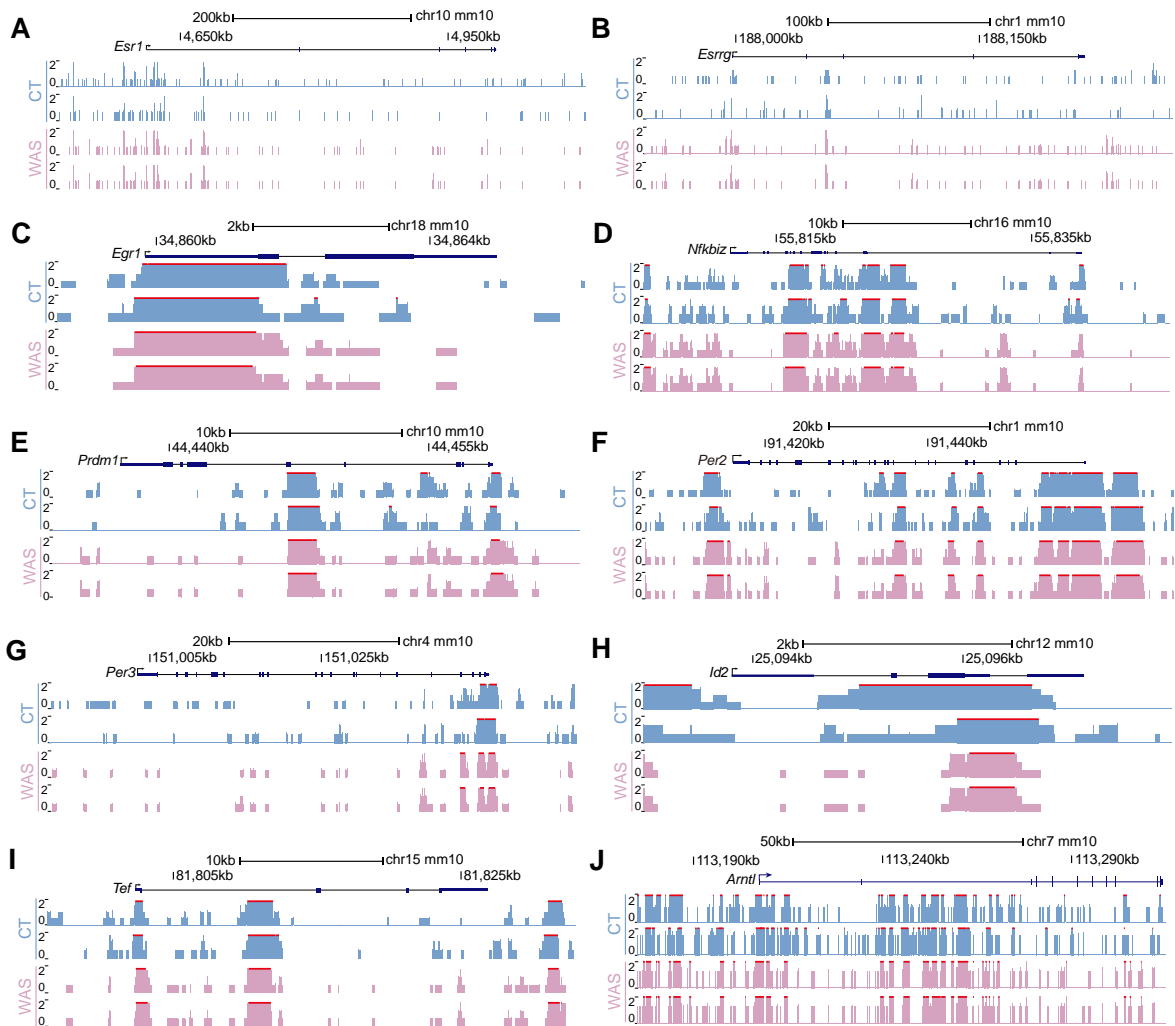


Figure S5. GR binding along the gene bodies of TF DEGs identified in *Nr3c1*^{ΔIEC} mice,

Related to Figure 6

(A-J) GR binding along the *Esr1*, *Esrrg*, *Egr1*, *Nfkbiz*, *Prdm1*, *Per2*, *Per3*, *Id2*, *Tef*, and *Arntl/Bmal1* highlighted GR downstream transcriptional network involved in circadian-stress crosstalk. CT (control) and WAS (water avoidance stress) mice GR cistrome data are visualized.

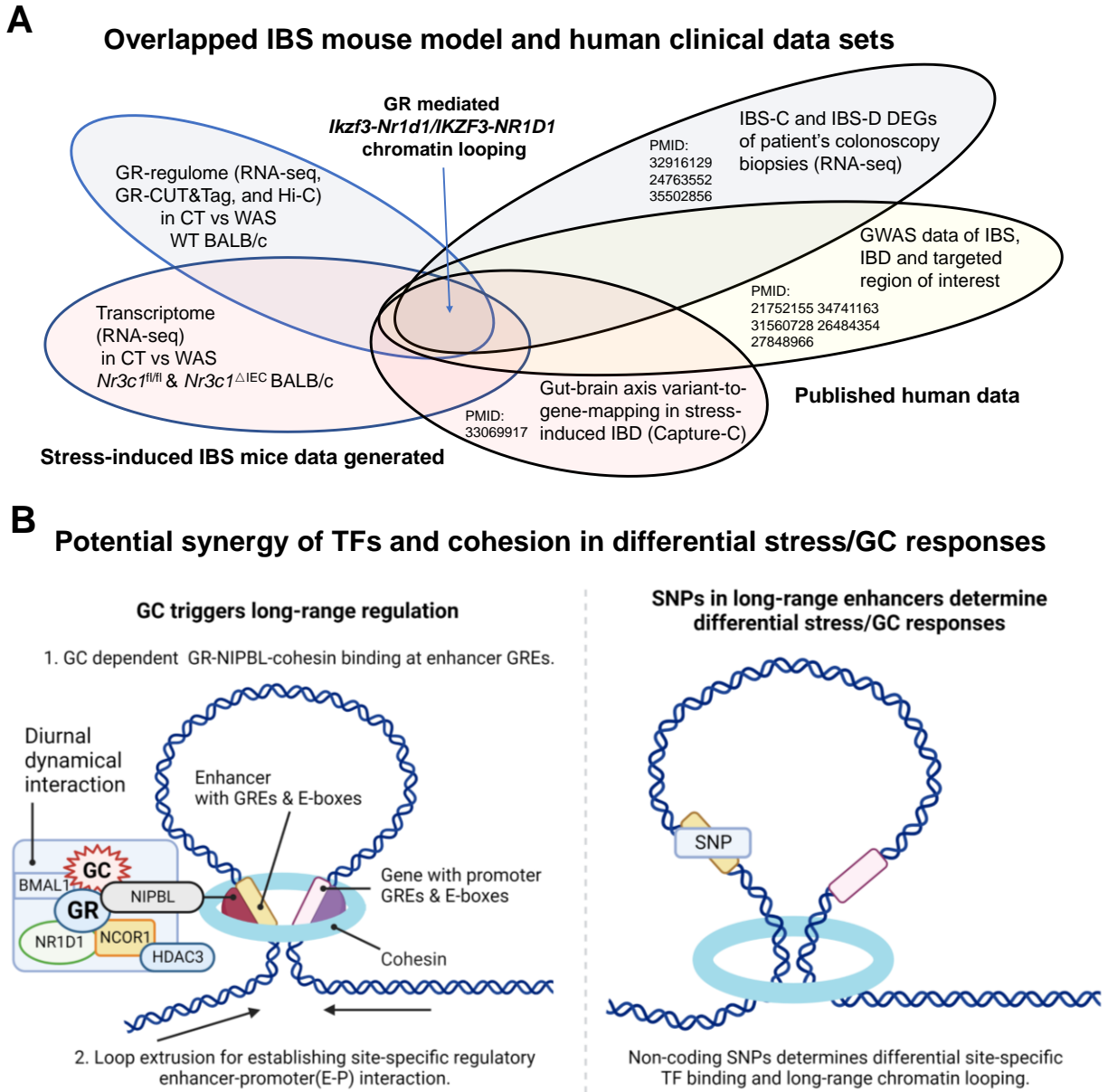


Figure S6. Translation potential of this stress-induced IBS mouse model dataset

(A) Overlapped stress-induced IBS mouse data with human data sets highlighted conserved *Ikzf3-Nr1d1/IKZF3-NR1D1* chromatin circadian misalignment.

(B) Potential mechanism by which long-range enhancer SNPs determine differential stress/glucocorticoid responses. Transcription factors (BMAL1/NR1D1/GR), cohesin loader NIPBL, cofactor (NCOR1), and chromatin remodeler (HDAC3) form protein complex load

cohesin to establish site-specific enhancer-promoter loops for long-range regulation. SNPs in long-range enhancers of GR target genes cause differential transcription factor binding and chromatin looping.

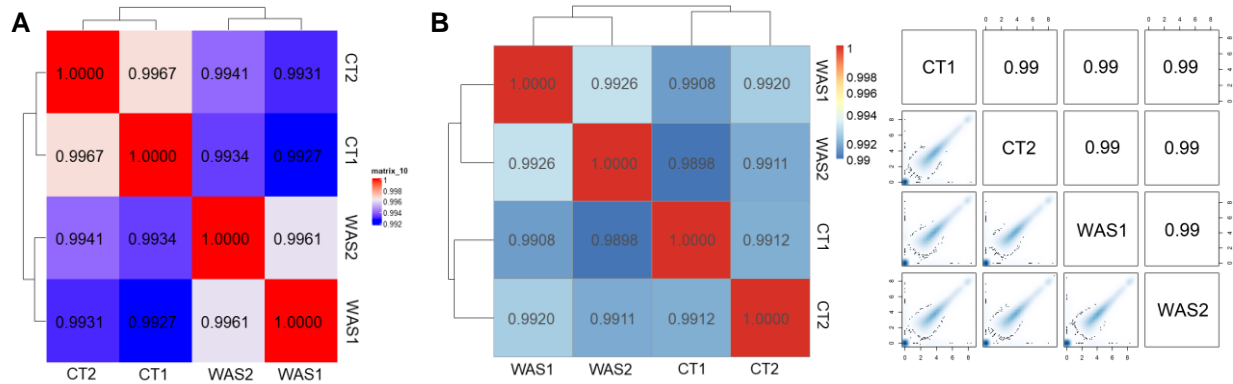


Figure S7. 1 M resolution matrix correlation analysis of BL-Hi-C data generated from two CT (control) and two WAS (water avoidance stress) BALB/c mice, Related to STAR Methods

1 M resolution matrix correlation was performed with two methods (A: hicap; B: hicLibRepeatCor). Differences between each mouse were <1%; however, data within each group showed closer correlations.

Supplementary Tables

Table S1. The histologic scoring system, Related to STAR Methods

Inflammation	Crypt Injury	Ulceration	Score
No significant inflammation	No injury	No ulceration	0
Neutrophilic inflammation in epithelium or lamina propria	Loss of basal one third of crypts	Two or fewer foci of ulceration	1
Inflammatory cells extending into submucosa	Loss of basal two thirds of crypts	Three or four foci of ulceration	2
Transmural inflammation	Loss of full thickness crypts	Diffuse/confluent ulceration	3

Table S2. BL-Hi-C trimming step, Related to STAR Methods

	CT1	CT2	WAS1	WAS2
Total_PETs	1100152888	1104533758	931979552	1133499189
Expect_PETs	982095283	1001781230	863015819	1019164130
Expect_both_PETs	258630025	241737666	252839184	259293712
Chim_PETs	39734122	41877180	27010195	51223027
1Empty_PETs	633625069	662525658	511640710	655985754
2Empty_PETs	89840189	97517906	98535925	103884664
Valid_PETs	547085785	549986750	509131735	615445192

Table S3. BL-Hi-C mapping results, Related to STAR Methods

	CT1	CT1 rate	CT2	CT2 rate	WAS1	WAS1 rate	WAS2	WAS2 rate
Total_pairs_processed	547085785	100	549986750	100	509131735	100	615445192	100
Unmapped_pairs	17142671	3.133	16784184	3.052	15791545	3.102	19071567	3.099
Low_qual_pairs	174552395	31.906	173364376	31.522	162230523	31.864	195446491	31.757
Unique_paired_alignments	219574167	40.135	221269574	40.232	208748132	41.001	243609388	39.583
Multiple_pairs_alignments	0	0	0	0	0	0	0	0
Pairs_with_singleton	135816552	24.825	138568616	25.195	122361535	24.033	157317746	25.562
Low_qual_singleton	0	0	0	0	0	0	0	0
Unique_singleton_alignments	0	0	0	0	0	0	0	0
Multiple_singleton_alignments	0	0	0	0	0	0	0	0
Reported_pairs	219574167	40.135	221269574	40.232	208748132	41.001	243609388	39.583

Table S4. List of valid BL-Hi-C interaction products, Related to STAR Methods

	CT1	CT2	WAS1	WAS2
Valid_interaction_pairs	197904079	198544667	179529211	221337806
Valid_interaction_pairs_FF	49480981	49627423	44865807	55422565
Valid_interaction_pairs_RR	49356184	49529530	44787979	55209141
Valid_interaction_pairs_RF	49275321	49414216	44722878	55083669
Valid_interaction_pairs_FR	49791593	49973498	45152547	55622431
Dangling_end_pairs	19452188	20275202	26889807	19866293
Religation_pairs	2142441	2356423	2263951	2326167
Self_Cycle_pairs	46843	54695	41064	56560
Single-end_pairs	0	0	0	0
Filtered_pairs	0	0	0	0
Dumped_pairs	28616	38587	24099	22562

Table S5. Intra and inter-chromosomal contact maps, Related to STAR Methods

	CT1	CT2	WAS1	WAS2
valid_interaction	197904079	198544667	179529211	221337806
valid_interaction_rmdup	44817997	45210449	66433088	47594763
trans_interaction	28464631	27596849	42461050	31031663
cis_interaction	16353366	17613600	23972038	16563100
cis_shortRange	3124209	3496740	4376450	3021581
cis_longRange	13229157	14116860	19595588	13541519