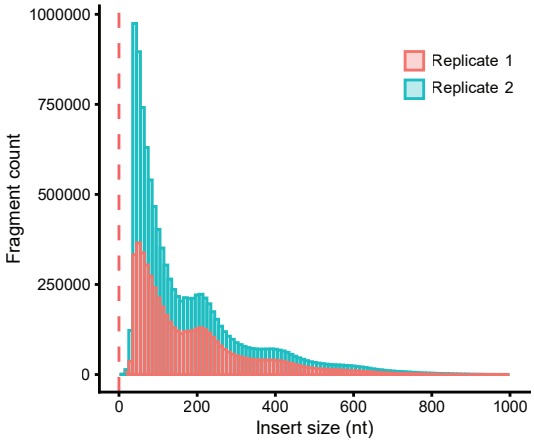

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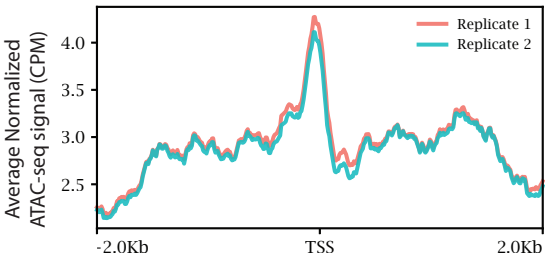

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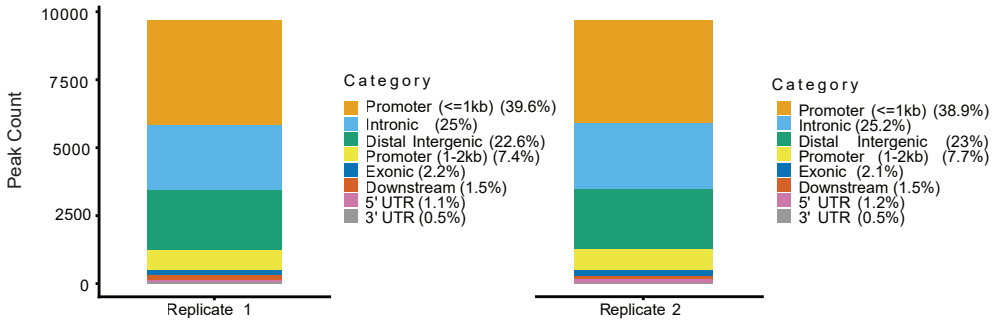

C

Figure S1

Table S2 - Primer sequences

Primer name	Use	Primer sequence 5'-3'
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T7_ds1_R	dsRNA generation	TAATACGACTCACTATAGGCCCAAACCAGCGGGTGACGAA
T7_ds2_F	dsRNA generation	TAATACGACTCACTATAGGAACTTCCCCAACATCTGGCGG
T7_ds2_R	dsRNA generation	TAATACGACTCACTATAGGGGCAGAACTTGTACGACCAA
T7_ds3_F	dsRNA generation	TAATACGACTCACTATAGGGGTTCTTGATATCTCGGCCTA
T7_ds3_R	dsRNA generation	TAATACGACTCACTATAGGGTAGACGTGAAGAATTTATCGCC
T7_7051_F	dsRNA generation	TAATACGACTCACTATAGGGCGCTACCACGTACGCTGCCGG
T7_7051_R	dsRNA generation	TAATACGACTCACTATAGGGCCGTGTACGGGAACTTGTGCG
AGAP007051-qF	measure expression of AGAP007051	CGATCGAAGTCTTCTCCGGG
AGAP007051-qR	measure expression of AGAP007051	AACTACGCTGGGACACACAG
AGAP007051-exon-F	measure expression of AGAP007051 after specific RT	GCTTCGTAGCGGTACAGAGTG
AGAP007051-exon-R	measure expression of AGAP007051 after specific RT	GCTTATTCCACTGCACCAACC
ds1_F_RT	Specific RT forENH_2L_03 candidate eRNA	CCCTTTACCACTGGCCAGCA
AGAP007051-exon-RT	Specific RT for AGAP007051	CACCACCCCTTCGGCTCTTGTC
EN03_Int_F	Detect ENH_2L_03 candidate eRNA after specific RT	CAATTTCGTCACCCGCTGG
ds3_R	Detect ENH_2L_03 candidate eRNA after specific RT	GTAGACGTGAAGAATTTATCGCC
ds1_Conv_F	measure expression of ENH_2L_03 candidate eRNA	CCCTTTACCACTGGCCAGCA
ds2_Conv_R	measure expression of ENH_2L_03 candidate eRNA	GGCAGAACTTGTACGACCAA
EN1_FWD	cloning of enhancer fragments for luciferase activity	GCAGCGACGACTTGTTCAG
EN1_REV	cloning of enhancer fragments for luciferase activity	CATCTCGAACACGCCACG
EN2_FWD	cloning of enhancer fragments for luciferase activity	AATTGGGCACTACACGGGTA
EN2_REV	cloning of enhancer fragments for luciferase activity	AGTTTAGCCTGCCTGCCTAA
EN2_REV2	cloning of enhancer fragments for luciferase activity	AGGATGTAAGTTTAGCCTGCCT
EN3_FWD	cloning of enhancer fragments for luciferase activity	GCCACCCTGAAAATAACGTAGA
EN3_REV	cloning of enhancer fragments for luciferase activity	TTTGCACCGAAGATTTTGAGG
EN4_FWD	cloning of enhancer fragments for luciferase activity	TGGCAAACGAATCATTCCACA
EN4_REV	cloning of enhancer fragments for luciferase activity	ACGAGAAAAGGGCCTGATGA
EN5_FWD	cloning of enhancer fragments for luciferase activity	ATTTTACAACTATCATCGTGTCG
EN5_REV	cloning of enhancer fragments for luciferase activity	CTCACAGCTCCCTCCATTGA
EN6_FWD	cloning of enhancer fragments for luciferase activity	ATAACGGTGCTTATCGG
EN6_REV	cloning of enhancer fragments for luciferase activity	CGTTCGGAAGCACAAAG
EN7_FWD	cloning of enhancer fragments for luciferase activity	ATCGTCATCATCAGCATCGC
EN7_REV	cloning of enhancer fragments for luciferase activity	ACTGCTCATCATGTTGACGG
EN8_FWD	cloning of enhancer fragments for luciferase activity	AACAGTCGTCACACCAGCA
EN8_REV	cloning of enhancer fragments for luciferase activity	CGGCAGGAGACAGATGAATG
EN9_FWD	cloning of enhancer fragments for luciferase activity	AAGTAACGAGGACGAAGCCA
EN9_REV	cloning of enhancer fragments for luciferase activity	AGATGCTGACCAATTCACCAG
LucNrev	seqeunce verification of cloned fragments and SDM	CCTTATGCAGTTGCTCTCC
RVprimer3	seqeunce verification of cloned fragments and SDM	CTAGCAAAATAGGCTGTCCC

Note: underlined sequence correspond to the T7 sequence for the in vitro transcription

fasta sequence of cloned enhancer fragments

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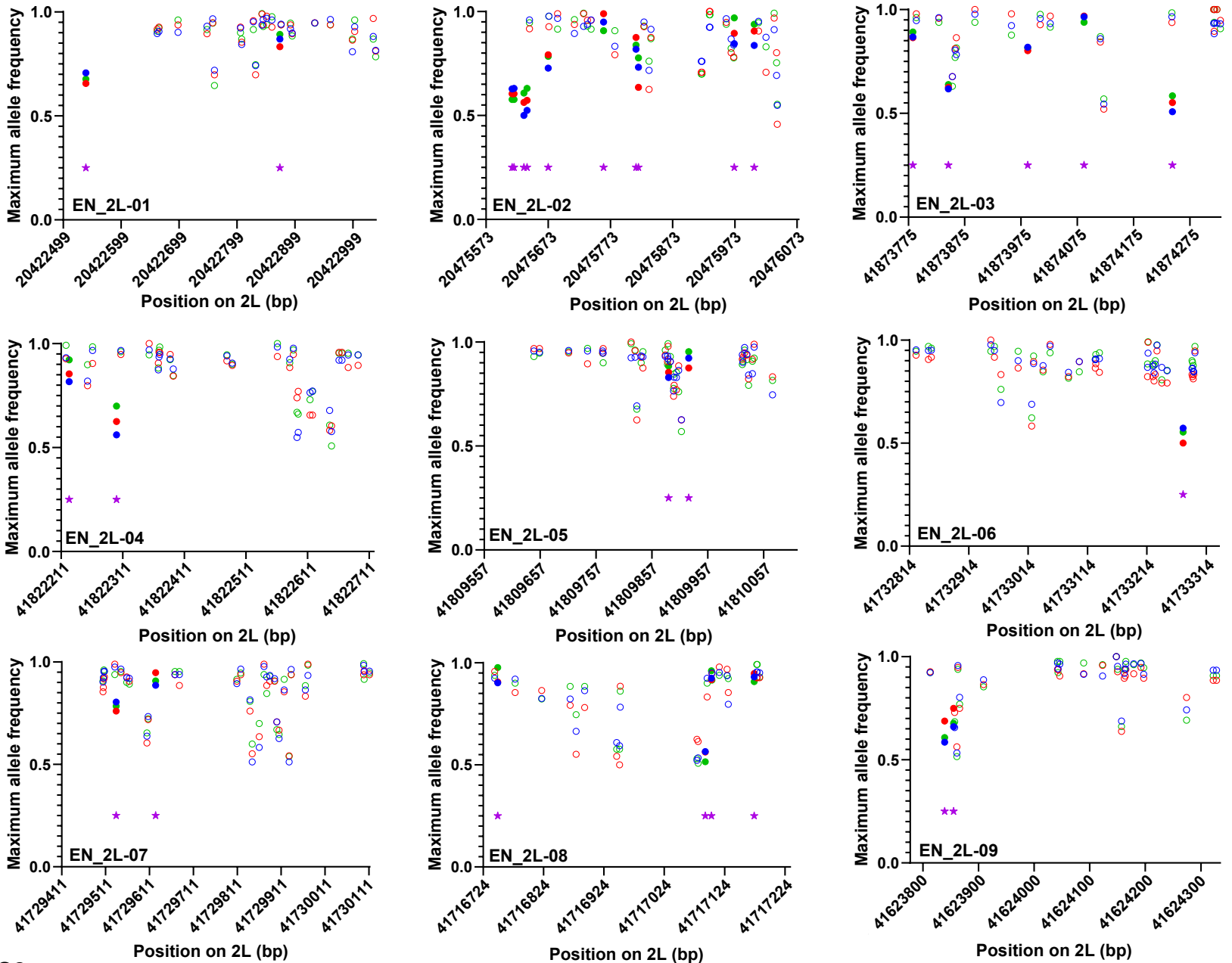
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41624300

Figure S2

Table S3 Overlap of the STARR-seq peaks with other available chromatin accessibility or epigenetic signature data. Overlap was defined as peaks occurring within +/- 1500bp of the published STARR-seq peak. All positions are from the PEST reference genome. Peaks are noted as Chr.PEST position. Note, the 2La inversion brings PEST annotated positions from 42.0MB to 20.5MB, as depicted in Figure 1. For the Ruiz et al. 2021 paper the tissue is given (Midguts or Salivary glands (S. glands) and for Ruiz et al. 2019 the epigenetic marker and the sample are given (control or infected).

Enhancer ID	STARR-seq peak	ATAC-seq	FAIRE-seq peaks from Pérez-Zamorano 2017 [41]	ATAC-seq peaks from Ruiz et al 2021 [42]	Epigenetic Marks/Peaks from Ruiz et al 2019 [27]
ENH_2L-01	2L.20422499-20423038			2L.20422944-20423081- Midguts	
ENH_2L-02	2L.20475573-20476075	2L.20475443-20475824	2L.20476662-20476882	2L.20476654-20476738 - S. glands 2L.20476829-20476885- S. glands	
ENH_2L-03	2L.41873775-41874329	2L.41875558-41875985	2L.41873427-41873627	2L.41873209-41873300- S. glands 2L.41873478-41873644- Midguts 2L.41873502-41873635- S. glands 2L.41873546-41873622- S. glands 2L.41873841-41874087- S. glands 2L.41873883-41873996- S. glands 2L.41874467-41874691- S. glands 2L.41874477-41874700- S. glands 2L.41875537-41875982- S. glands 2L.41875575-41875964- S. glands 2L.41875719-41875803- Midguts 2L.41875749-41875873- Midguts	2L.41873883-41874081 (H3K4me3 Control) 2L.41874349-41874637 (H3K4me3 Infected)
ENH_2L-04	2L.41822211-41822718		2L.41821067-41821267		
ENH_2L-05	2L.41809557-41810116			2L.41810524-41810623- Midguts	
ENH_2L-06	2L.41732814-41733335				
ENH_2L-07	2L.41729411-41730122	2L.41729448-41730046		2L.41728424-41729022- S. glands 2L.41728433-41728640- S. glands 2L.41728706-41728782- Midguts 2L.41728724-41729000- S. glands 2L.41728850-41728925- Midguts 2L.41729179-41729862- S. glands 2L.41729242-41729530- S. glands 2L.41729631-41729803- S. glands	
ENH_2L-08	2L.41716724-41717232		2L.41716827-41717027	2L.41717194-41717246 – S. glands	
ENH_2L-09	2L.41623777-41624334				

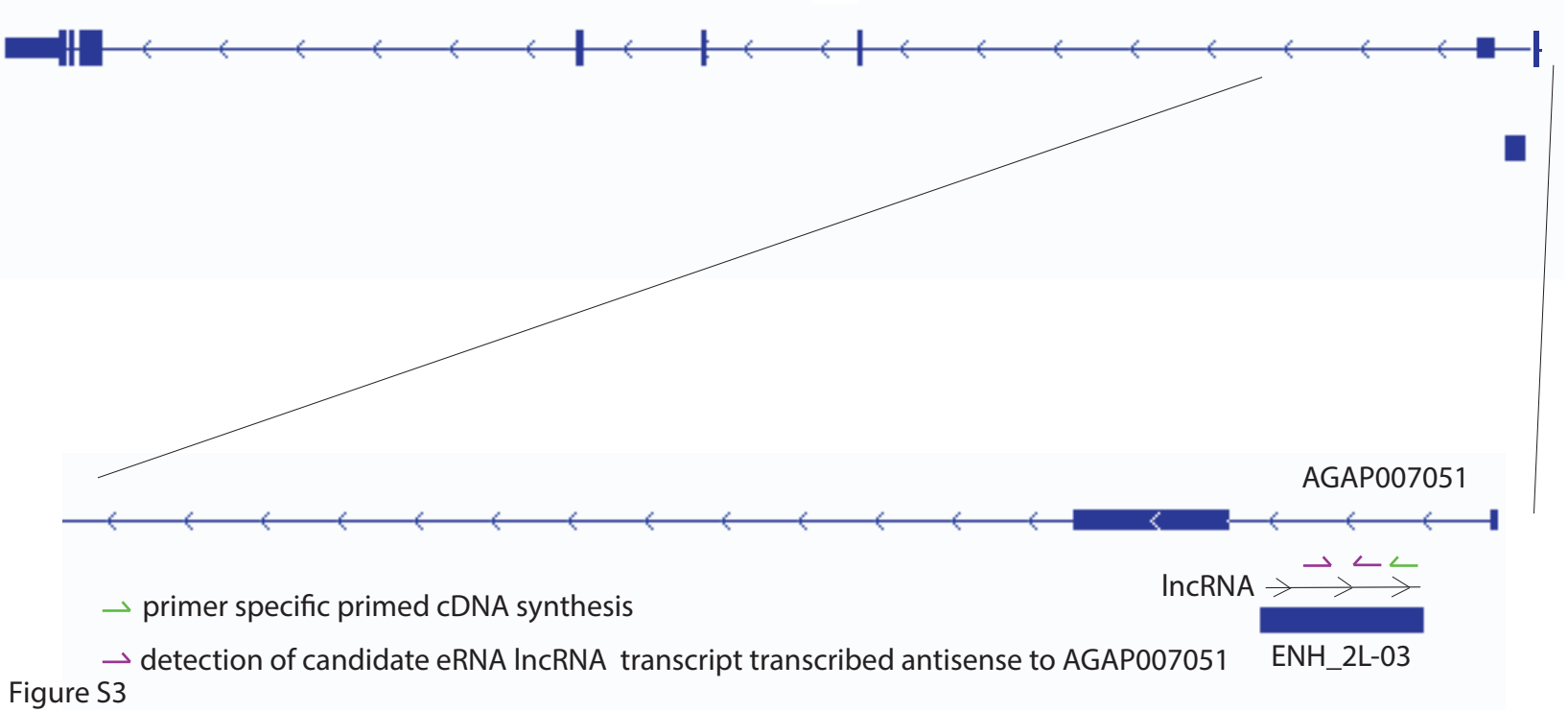

AGAP007051
lncRNA
ENH_2L-03
primer specific primed cDNA synthesis
detection of candidate eRNA lncRNA transcript transcribed antisense to AGAP007051

Figure S3

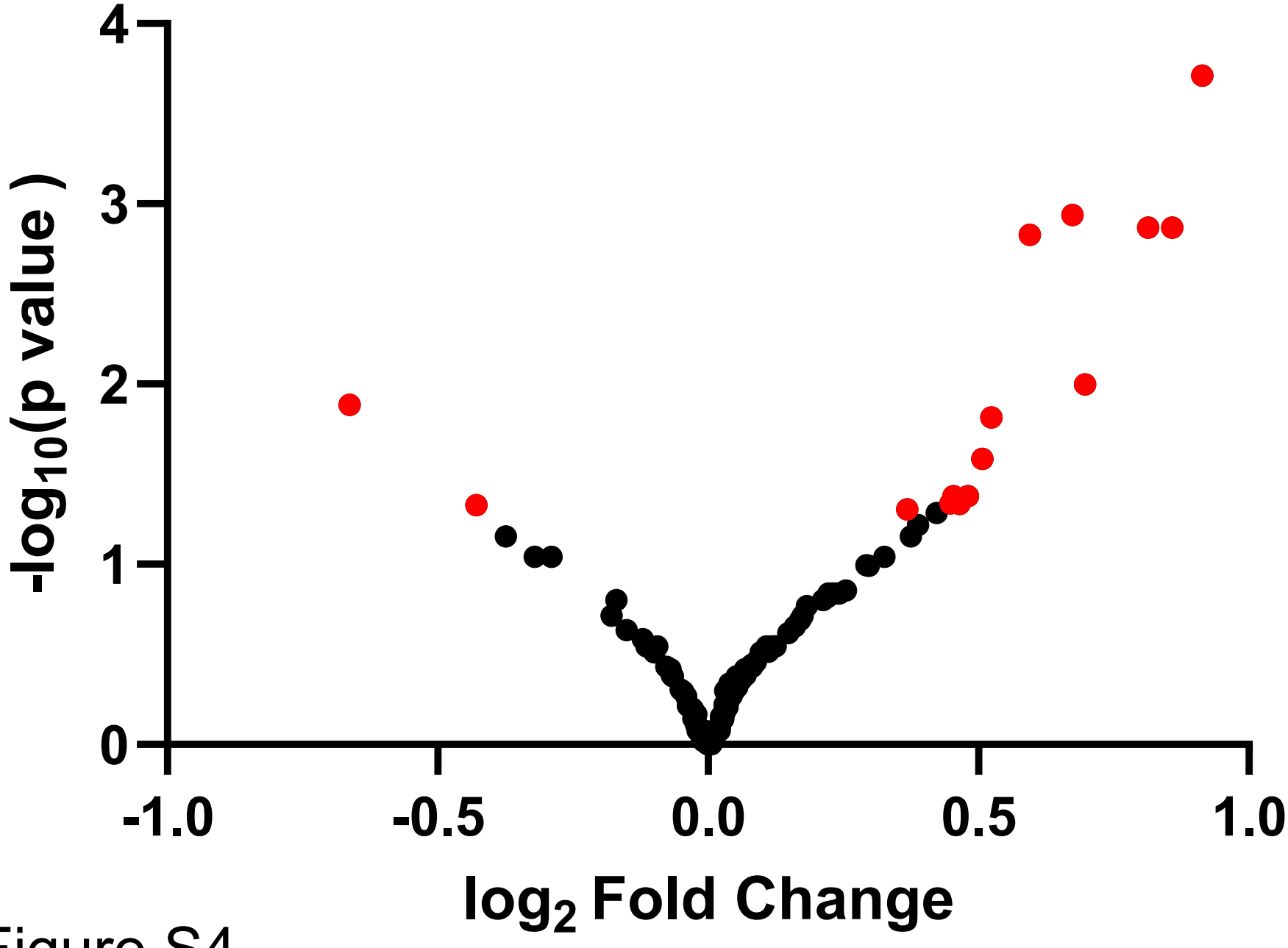


Figure S4

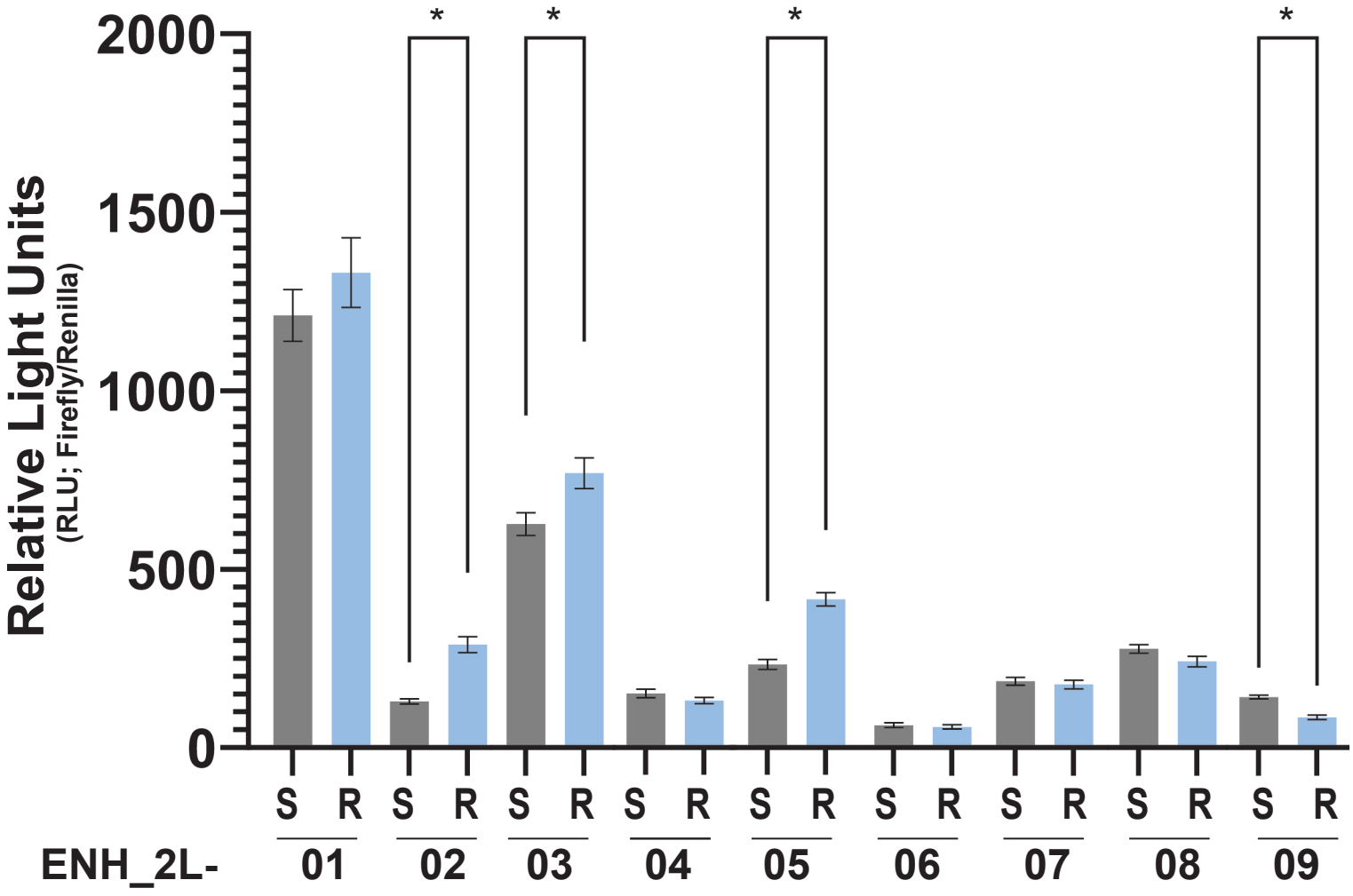
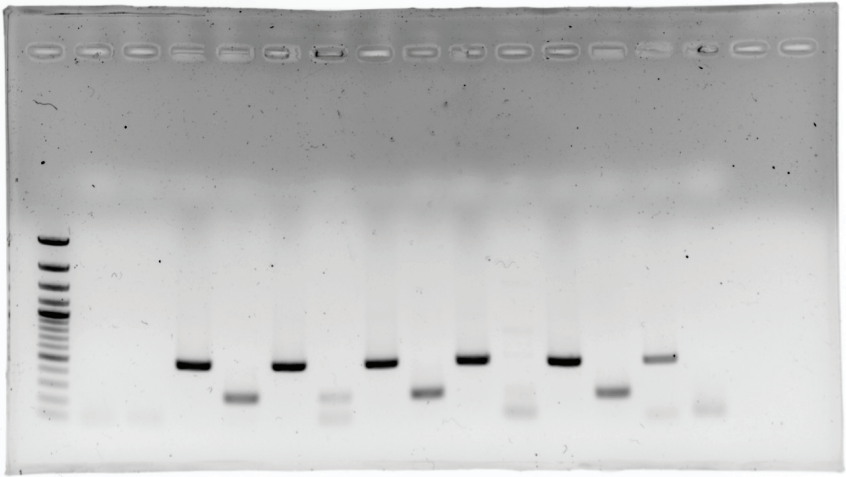
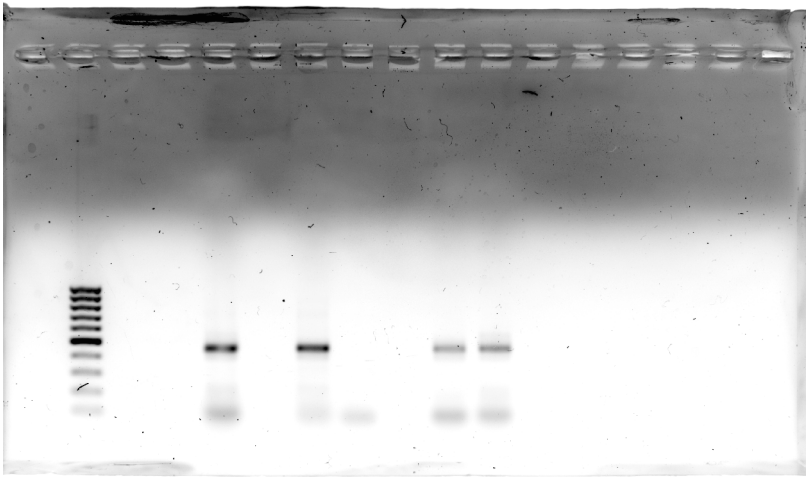

Relative Light Units
(RLU; Firefly/Renilla)
2000
1500
1000
500
0
*
*
*
*
S R S R S R S R S R S R S R S R S R
ENH_2L-
01
02
03
04
05
06
07
08
09

Figure S5



Gel Image from Figure 3C



Gel Image from Figure 3E

Figure S6