

Supplemental Items:

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Figure S1

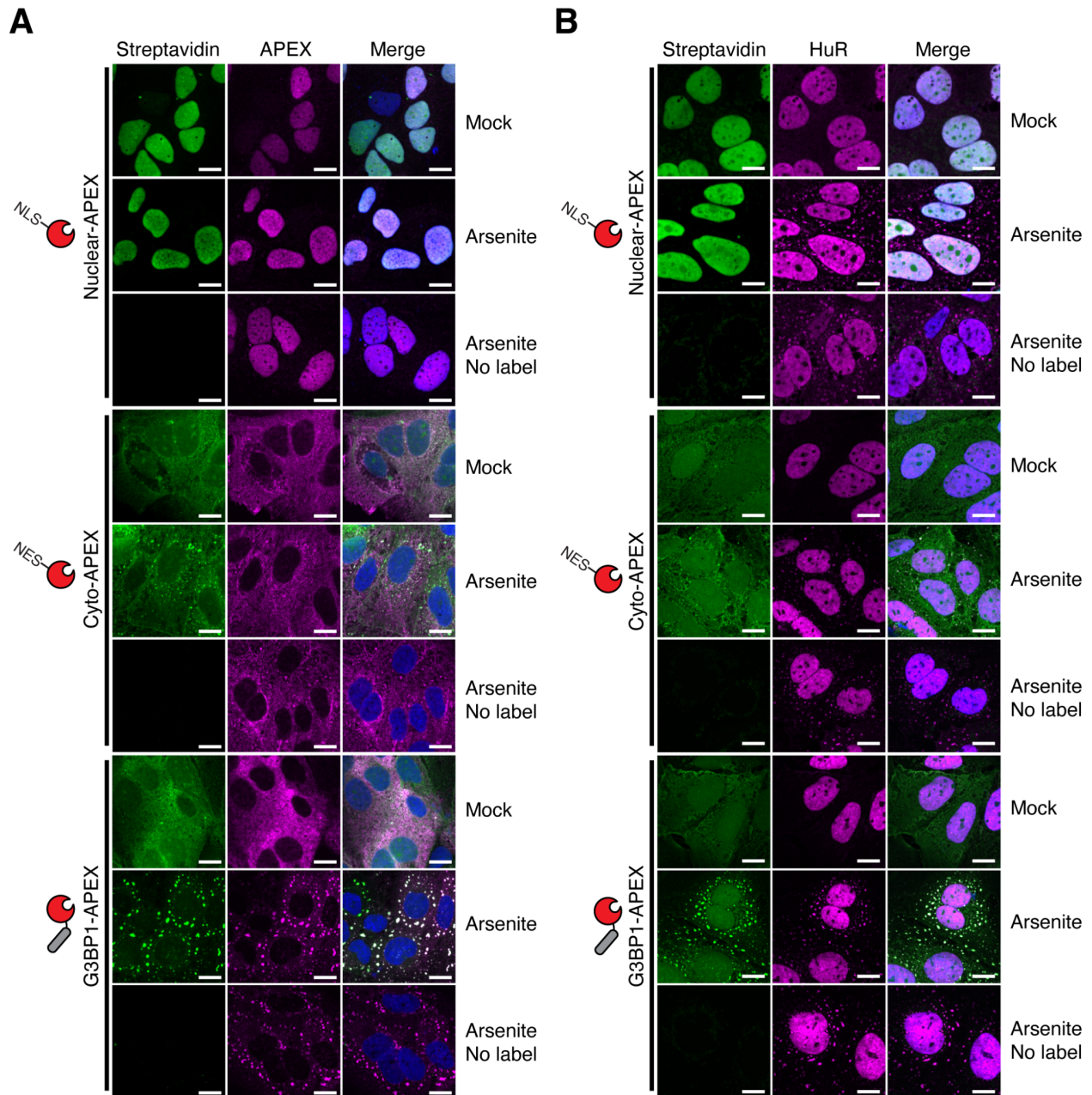


Figure S1: Proximity labeling for CoCLIP in three subcellular regions. (A)

Immunofluorescence staining of Huh7.5 cells stably expressing APEX2 localized to the nucleus, cytoplasm or fused to the stress granule marker G3BP1, in the presence or absence of arsenite stress. Fluorescent streptavidin was used to confirm site-specific labeling, DAPI used as DNA counterstain. Images were captured at 20x magnification. Scale bars = 10 μ m. **(B)**

Immunofluorescence staining as in **(A)** showing HuR relocalization to stress granules upon arsenite stress for all conditions. Scale bars = 10 μ m.

Figure S2

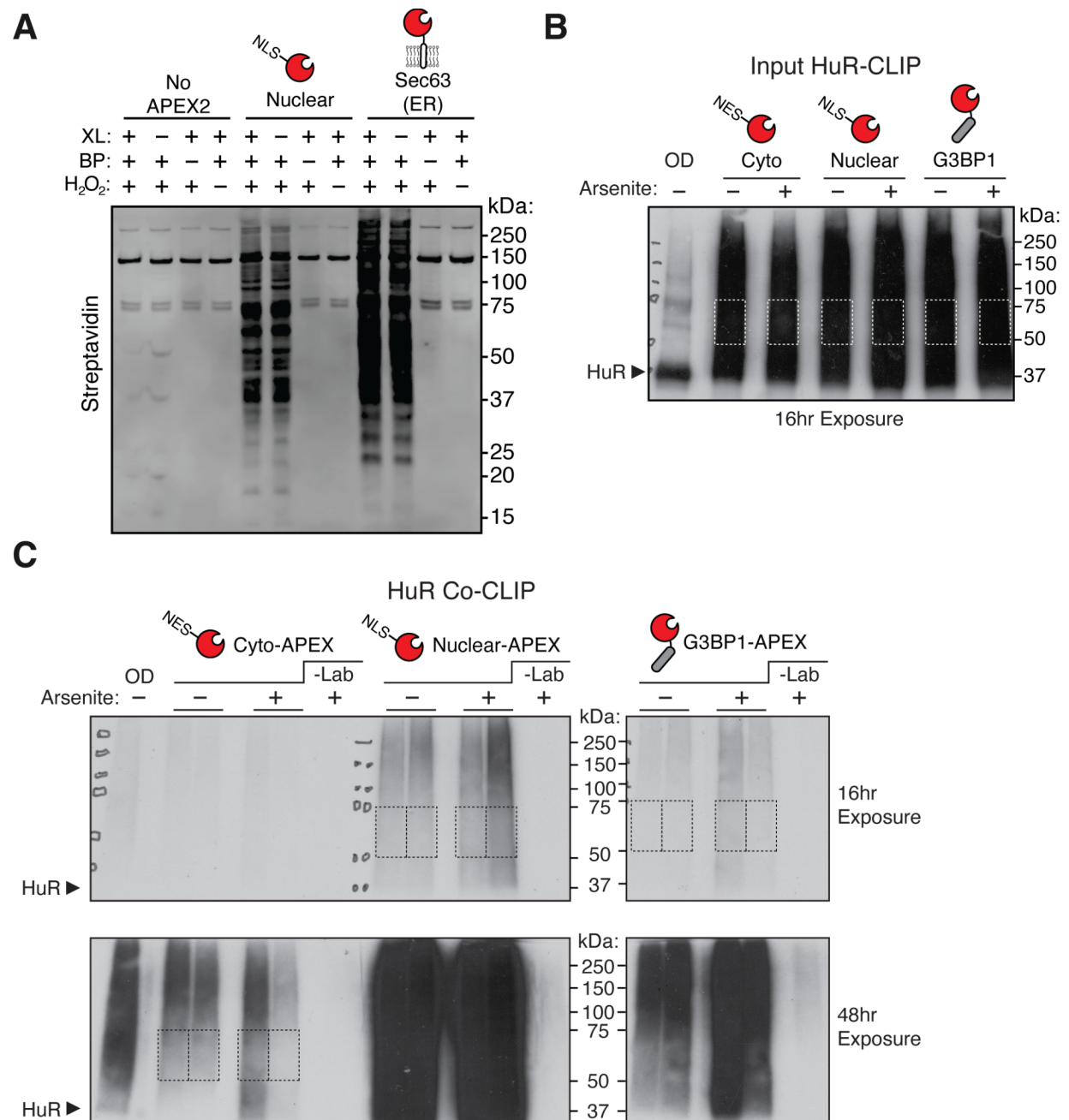


Figure S2: CoCLIP optimization and autoradiograms. (A) UV crosslinking does not induce spurious APEX2 activity. Immunoblot with fluorescent streptavidin in Huh7.5 cells with indicated APEX2 fusions, in the presence or absence of labeling reagents or UV crosslinking (XL) as indicated. BP: Biotin-Phenol. **(B)** Autoradiogram of total HuR CLIP from the conditions in **Figure S1**. OD: RNase overdigest. **(C)** Autoradiogram of HuR coCLIP from the duplicate conditions in **(B)**, presented at two different exposures. Minus label control was performed by omitting hydrogen peroxide pulse.

Figure S3

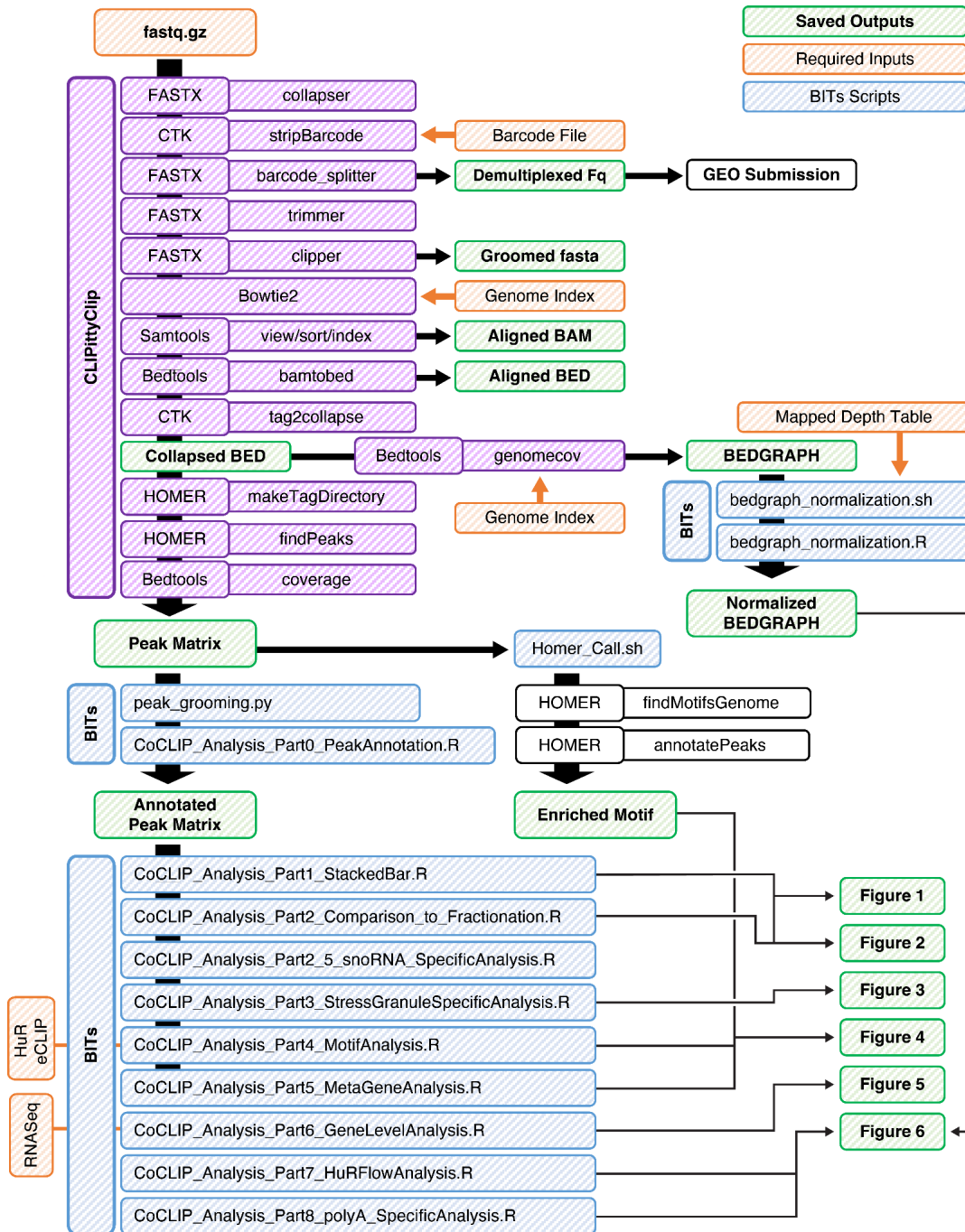


Figure S3: Summary of a coCLIP data analysis pipeline with CLIPittyClip and BITS.

Flowchart diagram of data inputs, outputs, and ordered usage of tools used to process, analyze and visualize the data in this manuscript. CLIPittyClip provides a framework to process raw FASTQ files into a finished peak matrix in a single command line. BITS, for Bioinformatics Tools and Scripts, enables downstream processing of CLIPittyClip outputs for visualization.

Figure S4

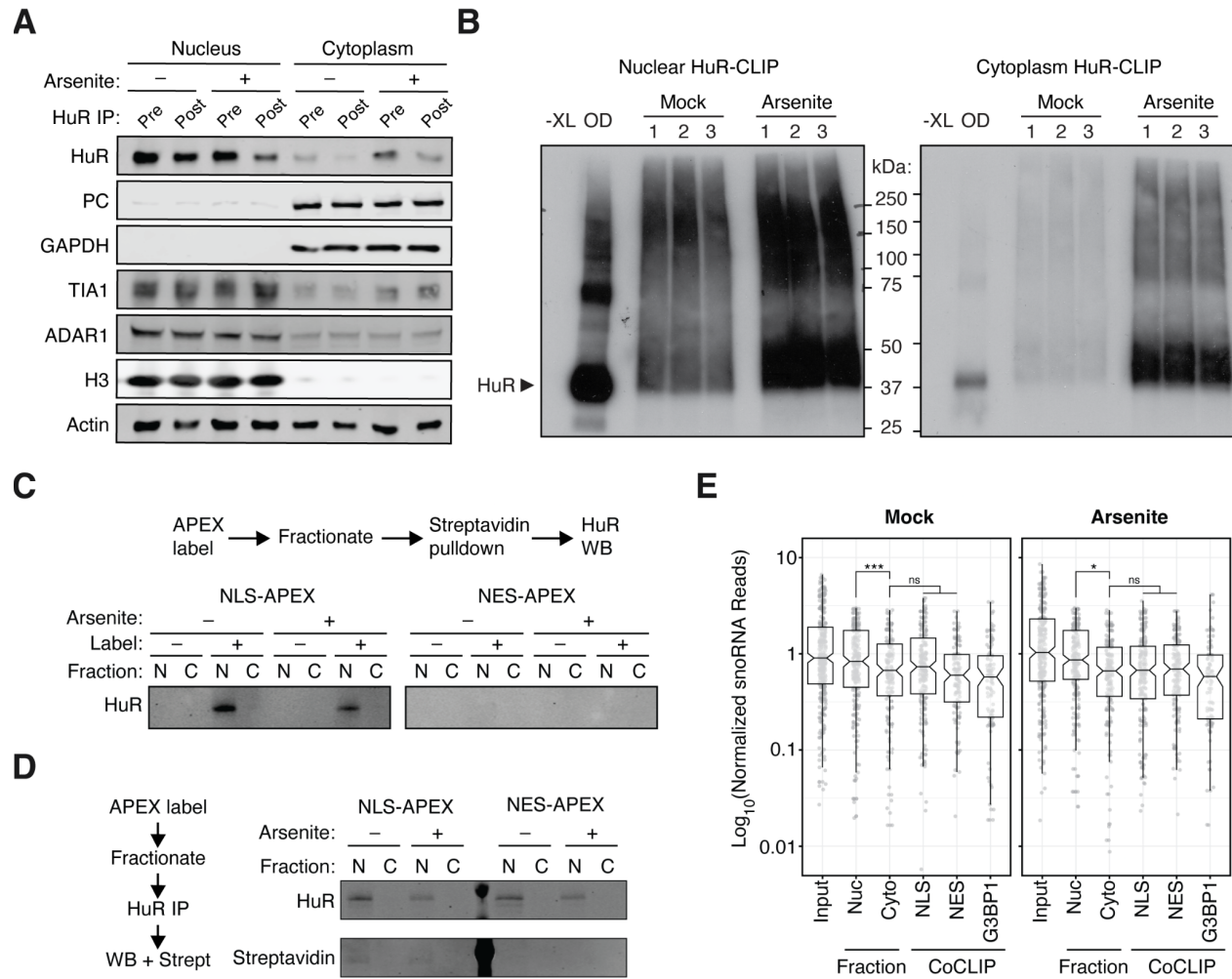


Figure S4: Nuclear and cytoplasmic fractionation for HuR-CLIP. (A) Western blot of nuclear or cytoplasmic fractions before and after HuR immunoprecipitation from Huh7.5 cells. Arsenite treatments as indicated. Pyruvate carboxylase (PC) and GAPDH were used as cytoplasmic markers; TIA1 and ADAR1 were used to mark the nucleoplasm; histone H3 was used to mark chromatin; Actin served as a general loading control. (B) Autoradiograms of HuR-CLIP from nuclear (left) or cytoplasmic (right) fractions as in (A). Exposure time was 30 minutes. -XL: no UV crosslinking control; OD: RNase overdigest. (C) HuR western blot following streptavidin pulldown from NLS or NES-APEX labeled Huh7.5 cells after nuclear or cytosolic fractionation as in (A). (D) The reciprocal experiment from (C) where HuR was immunoprecipitated from NLS or NES-APEX labeled Huh7.5 cells after nuclear and cytosolic fractionation and probed for HuR or biotinylated HuR with streptavidin. (E) Read frequency counts from all detected snoRNAs from each sample. *** $P < 10e-5$, ** $P < 10e-4$, * $P < 10e-3$, ns $P > 10e-2$, two-sided Mann-Whitney U-test.

Figure S5

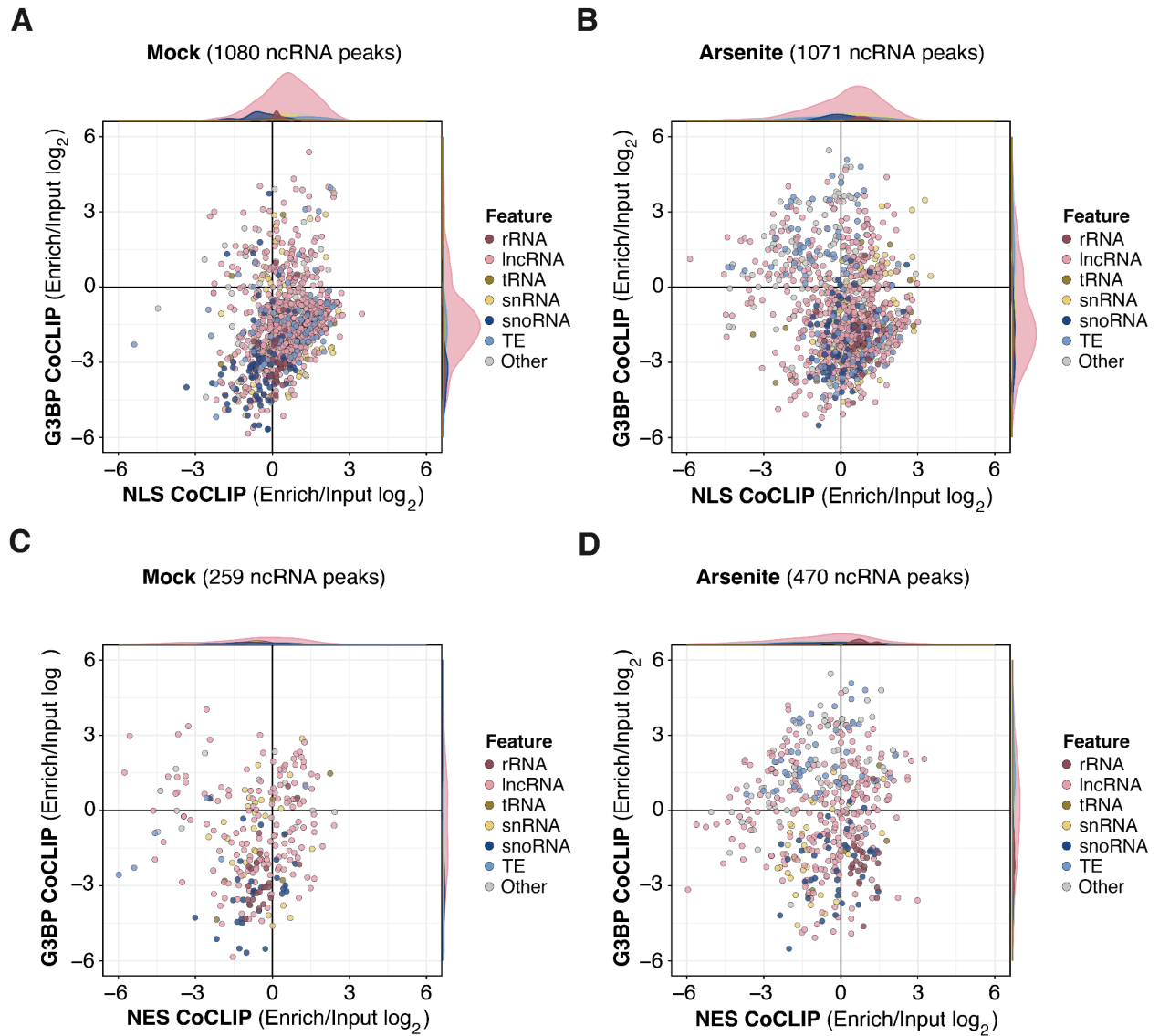


Figure S5: HuR exhibits unique ncRNA binding preferences within stress granules.

(A-B) Scatterplots contrasting enriched coCLIP to input CLIP for ncRNA binding events as log₂ fold changes, comparing G3BP1 coCLIP vs nuclear NLS coCLIP under mock (A) and arsenite (B) stress conditions. Side histograms depict the relative density of each feature and are scaled for comparison within each feature and across mock and arsenite stress. (C-D) Scatterplots as in (C-D) comparing G3BP1 coCLIP vs cytoplasmic NES coCLIP under mock (C) and arsenite (D) stress conditions.

Figure S6

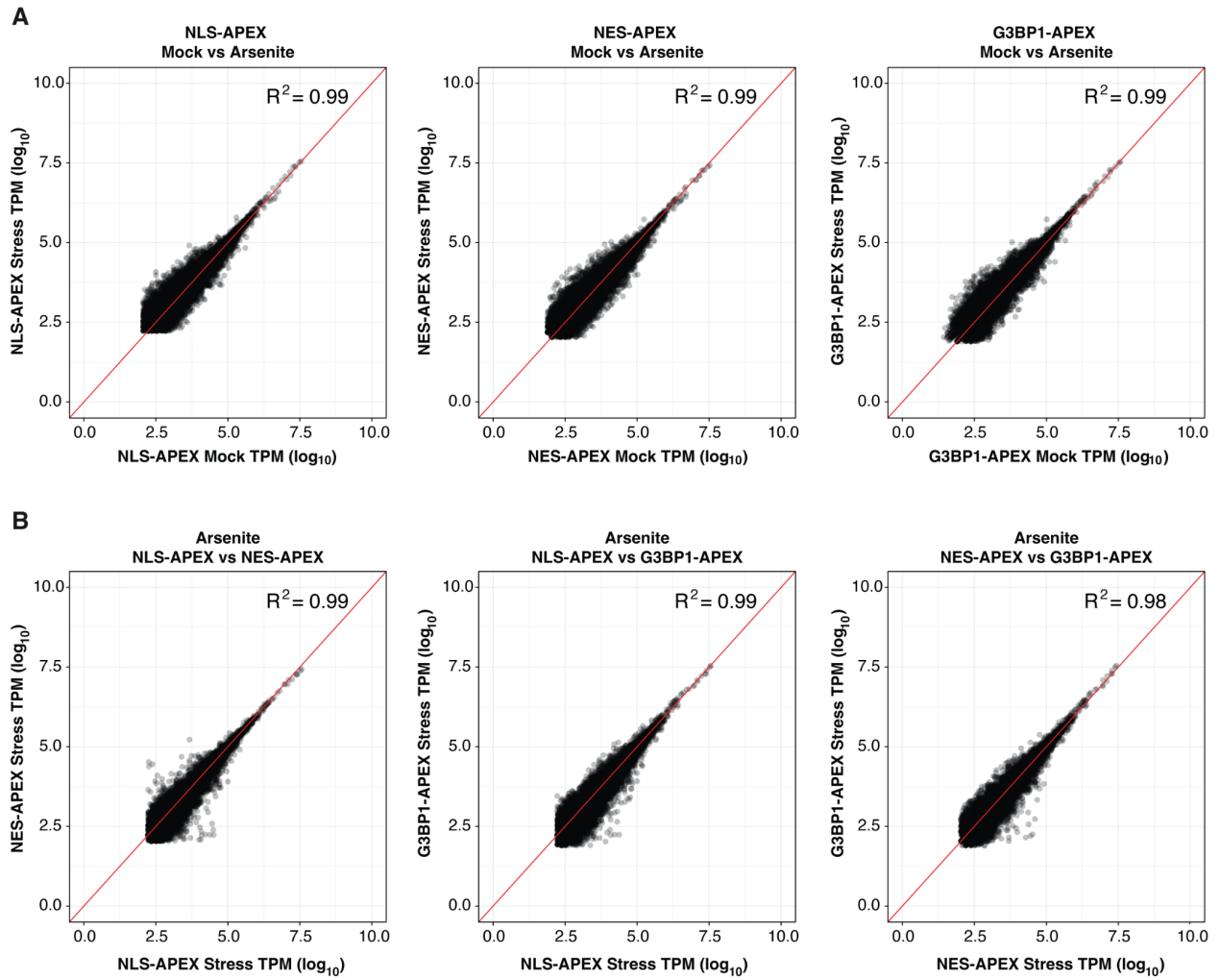


Figure S6: RNA-Seq correlation between APEX expressing cells and conditions. Pair-wise scatterplots of RNAseq transcript per million (TPM) measurements from APEX expressing cells used for coCLIP **(A)** between mock and arsenite conditions for each cell line and **(B)** between cell lines under stress. Pearson correlation coefficients are indicated.

Table S1: Mapping Statistics

Data Source	Sample ID	Mapped Reads	Total Reads	% Mapped
JL0380_Nuc_Fraction_Mock_1	Nuc_F_M_1	686263	703192	97.6
JL0380_Nuc_Fraction_Mock_2	Nuc_F_M_2	1099896	1124929	97.8
JL0380_Nuc_Fraction_Mock_3	Nuc_F_M_3	1035633	1059931	97.7
JL0380_Nuc_Fraction_Ars_1	Nuc_F_S_1	1248888	1277538	97.8
JL0380_Nuc_Fraction_Ars_2	Nuc_F_S_2	1250151	1280660	97.6
JL0380_Nuc_Fraction_Ars_3	Nuc_F_S_3	1154335	1180724	97.8
JL0380_Cyto_Fraction_Mock_1	Cyto_F_M_1	940147	963881	97.5
JL0380_Cyto_Fraction_Mock_2	Cyto_F_M_2	981792	1008995	97.3
JL0380_Cyto_Fraction_Mock_3	Cyto_F_M_3	948354	975909	97.2
JL0380_Cyto_Fraction_Ars_1	Cyto_F_S_1	1385165	1419657	97.6
JL0380_Cyto_Fraction_Ars_2	Cyto_F_S_2	1759090	1797001	97.9
JL0380_Cyto_Fraction_Ars_3	Cyto_F_S_3	1437680	1473264	97.6
JL0388_NLS_Input_Mock_1	NLS_I_M_1	1619414	1642052	98.6
JL1024_NLS_Input_Mock_All	NLS_I_M_2	2622677	3061127	85.7
JL0388_NLS_Input_Ars_1	NLS_I_S_1	2295408	2317050	99.1
JL1024_NLS_Input_Ars_All	NLS_I_S_2	1784078	2092738	85.3
JL0388_NES_Input_Mock_1	NES_I_M_1	834226	844647	98.8
JL1024_NES_Input_Mock_All	NES_I_M_2	120962	163387	74
JL0388_NES_Input_Ars_1	NES_I_S_1	404260	413705	97.7
JL1024_NES_Input_Ars_All	NES_I_S_2	122616	167305	73.3
JL0361_G3BP_Input_Mock_1	G3BP_I_M_1	19748	22417	88.1
JL0361_G3BP_Input_Mock_2	G3BP_I_M_2	210794	218943	96.3
JL0388_G3BP_Input_Mock_1	G3BP_I_M_3	765265	774742	98.8
JL1024_G3BP_Input_Mock_All	G3BP_I_M_4	22476	89907	25
JL0361_G3BP_Input_Ars_1	G3BP_I_S_1	17051	19530	87.3
JL0361_G3BP_Input_Ars_2	G3BP_I_S_2	1109018	1146723	96.7
JL0361_G3BP_Input_Ars_3	G3BP_I_S_3	1206979	1239967	97.3
JL0388_G3BP_Input_Ars_1	G3BP_I_S_4	829192	838876	98.8
JL1024_G3BP_Input_Ars_All	G3BP_I_S_5	180888	270815	66.8
JL0388_NLS_Enrich_Mock_1	NLS_E_M_1	202951	211314	96
JL0388_NLS_Enrich_Mock_2	NLS_E_M_2	146321	151918	96.3
JL1024_NLS_Enrich_Mock_R1	NLS_E_M_3	528719	661200	80
JL1024_NLS_Enrich_Mock_R2	NLS_E_M_4	477565	554969	86.1
JL0388_NLS_Enrich_Ars_1	NLS_E_S_1	72101	75122	96
JL0388_NLS_Enrich_Ars_2	NLS_E_S_2	224335	229473	97.8

JL1024_NLS_Enrich_Ars_R1	NLS_E_S_3	523287	651909	80.3
JL1024_NLS_Enrich_Ars_R2	NLS_E_S_4	344414	426354	80.8
JL0388_NES_Enrich_Mock_1	NES_E_M_1	37831	44483	85
JL0388_NES_Enrich_Mock_2	NES_E_M_2	50343	54651	92.1
JL1024_NES_Enrich_Mock_R1	NES_E_M_3	114360	196682	58.1
JL1024_NES_Enrich_Mock_R2	NES_E_M_4	105485	198586	53.1
JL0388_NES_Enrich_Ars_1	NES_E_S_1	38255	44650	85.7
JL0388_NES_Enrich_Ars_2	NES_E_S_2	76213	80852	94.3
JL1024_NES_Enrich_Ars_R1	NES_E_S_3	482900	656758	73.5
JL1024_NES_Enrich_Ars_R2	NES_E_S_4	564779	777109	72.7
JL0361_G3BP_Enrich_Mock_1	G3BP_E_M_1	6330	11268	56.2
JL0361_G3BP_Enrich_Mock_2	G3BP_E_M_2	151280	175806	86
JL0388_G3BP_Enrich_Mock_1	G3BP_E_M_3	14760	19822	74.5
JL0388_G3BP_Enrich_Mock_2	G3BP_E_M_4	4662	9028	51.6
JL1024_G3BP_Enrich_Mock_R1	G3BP_E_M_5	334236	564930	59.2
JL1024_G3BP_Enrich_Mock_R2	G3BP_E_M_6	944225	1200994	78.6
JL0361_G3BP_Enrich_Ars_1	G3BP_E_S_1	13149	18848	69.8
JL0361_G3BP_Enrich_Ars_2	G3BP_E_S_2	604651	741496	81.5
JL0361_G3BP_Enrich_Ars_3	G3BP_E_S_3	524332	642651	81.6
JL0388_G3BP_Enrich_Ars_1	G3BP_E_S_4	88707	91738	96.7
JL0388_G3BP_Enrich_Ars_2	G3BP_E_S_5	28062	31480	89.1
JL1024_G3BP_Enrich_Ars_R1	G3BP_E_S_6	117098	148123	79.1
JL1024_G3BP_Enrich_Ars_R2	G3BP_E_S_7	66951	106854	62.7

Table S2: HOMER Output Summary

All Libraries:

Motif	p-value
	1e-11810
	1e-1651
	1e-275
	1e-254
	1e-171

* possible false-positive reported by HOMER

CoCLIP Input Mock:

Motif	p-value
	1e-1568
	1e-145
	1e-107
	1e-42
	1e-37

CoCLIP Input Arsenite:

Motif	p-value
	1e-2427
	1e-274
	1e-241
	1e-121
	1e-55

CoCLIP NLS Mock:

Motif	p-value
	1e-95
	1e-10*
	1e-10*
	1e-10*
	1e-9*

CoCLIP NLS Arsenite:

Motif	p-value
	1e-132
	1e-13
	1e-11*
	1e-10*
	1e-9*

CoCLIP NES Mock:

Motif	p-value
	1e-17
	1e-17
	1e-11*
	1e-9*
	1e-8*

CoCLIP NES Arsenite:

Motif	p-value
	1e-64
	1e-13
	1e-10*
	1e-10*
	1e-8*

CoCLIP G3BP Mock:

Motif	p-value
	1e-573
	1e-10*
	1e-9*
	1e-8*
	1e-8*

CoCLIP G3BP Arsenite:

Motif	p-value
	1e-274
	1e-14
	1e-13
	1e-13
	1e-13

Nuclear Fraction CLIP Mock:

Motif	p-value
	1e-3553
	1e-362
	1e-89
	1e-86
	1e-54

Nuclear Fraction CLIP Arsenite:

Motif	p-value
	1e-3964
	1e-262
	1e-121
	1e-56
	1e-48

Cytoplasm Fraction CLIP Mock:

Motif	p-value
	1e-2838
	1e-348
	1e-56
	1e-44
	1e-43

Cytoplasm Fraction CLIP Arsenite:

Motif	p-value
	1e-5355
	1e-515
	1e-362
	1e-62
	1e-36

Table S3: Oligonucleotides

PCR Primers			
Name	Sequence	Content	Primer partner
oJL529	GCTCGGTACCCGGGGATCATCGATGCCACCATGGAC TACAAGGATGACG	ePB_ClaI_APEX2_F	530
oJL578	GTACCCGGGGATCATCGATGCCACCATGGGCCCTAA AAAGAAGCGTAAAGTCGACTACAAGGATGACGACGA TAAGG	ePB_ClaI_nls-APEX2_F	530
oJL579	GTACCCGGGGATCATCGATGCCACCATGTTAGCTTT GAAATTAGCCGGACTAGACATCGACTACAAGGATGA CGACGATAAGG	ePB_ClaI_nes-APEX2_F	530
oJL530	CTTAATCAGCTCGCTCACCATTCCGGATCCGGCATCA GCAAACCCAAGCT	mKate2_BamHI_APEX2_R	529, 578, 579
oJL531	AGCTTGGGTTTGCTGATGCCGGATCCGGAATGGTGA GCGAGCTGATTAAG	APEX2_BamHI_mKate2_F	532
oJL532	GGCTGATTATGATCTAGAGTCGCGGCCGCTCTGTGC CCCAGTTTGCTAG	ePB_NotI_mKate2_R	531
oJL618	GCAAACCTGGGGCACAGAGCTAGCGGAGTGATGGAG AAGCCTAGTCCC	mKate2_NheI_G3BP1_F	619
oJL619	CAGGTTCTCTACTTCTTCTTCTTCTCTCTCTGAGG CTCAGTGACAA	G3BP1_S149E_R	618
oJL620	TTGTCACTGAGCCTCAGGAGGAGGAAGAAGAAGAAG TAGAGGAACCTG	G3BP1_S149E_F	516
oJL516	GGCTGATTATGATCTAGAGTCGCGGCCGCTTACTGC CGTGGCGCAAG	ePB_NotI_G3BP1_R	620
oJL573	GTACCCGGGGATCATCGATGCCACCATGGCCGGGC AGCAGTTCC	ePB-Sec63_F	577
oJL577	TCCTTAATCAGCTCGCTCACCATCGGCCGACGATAC CACATACACCTTCCATATA	mKate2_EagI_Sec63_R	573
oJL578	TATATGGAAGGTGTATGTGGTATCGTTCGGCCGATGG TGAGCGAGCTGATTAAGGA	Sec63_EagI_mKate2_F	574

oJL574	CGTCGTCATCCTTG TAGTCCATTCCGGATCCTCTGTG CCCCAGTTTGCTAGGG	APEX2_BamHI_mKate2_R	578
oJL575	CTAGCAAAC TGGGGCACAGAGGATCCGGAATGGACT ACAAGGATGACGACGATAA	mKate2_BamHI_APEX2_F	576
oJL576	GGCTGATTATGATCTAGAGTCGCGGCCGCTTAGGCA TCAGCAAACCCAAGCT	ePB_NotI_APEX2_R	575

CLIP Oligos

Name	Sequence	Type	Index
L32	/5Phos/GUGUCAGUCACU UCCAGCGG/3ddC/	CLIP RNA linker	N/A
RT-1T	/5Phos/DDDCGTGATNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC	CLIP RT Oligos	ATCAC G
RT-2T	/5Phos/DDDACATCGNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		CGATG T
RT-3T	/5Phos/DDDGCCTAANNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		TTAGG C
RT-4T	/5Phos/DDDTGGTCANNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		TGACC A
RT-5T	/5Phos/DDDCACTGTNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		ACAGT G
RT-6T	/5Phos/DDDATTTGGCNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		GCCAA T
RT-7T	/5Phos/DDDGATCTGNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		CAGAT C
RT-8T	/5Phos/DDDTCAAGTNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		ACTTG A
RT-9T	/5Phos/DDDCTGATCNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		GATCA G
RT-10T	/5Phos/DDDAAGCTANNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		TAGCT T

RT-11T	/5Phos/DDDGTAGCCNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		GGCTA C
RT-12T	/5Phos/DDDTACAAGNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		CTTGT A
RT-13T	/5Phos/DDDCGTGATNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		ATCAC G
RT-14T	/5Phos/DDDGGAACNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		AGTTC C
RT-15T	/5Phos/DDDTGACATNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		ATGTC A
RT-16T	/5Phos/DDDGGACGGNNNNNNNAGATCGGAAGAGCG TCGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGA C		CCGTC C
RT-18T	/5Phos/DDDGCGGACNNNNNNNAGATCGGAAGAGCG TCGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGA C		GTCCG C
RT-19T	/5Phos/DDDTTTCACNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		GTGAA A
RT-20T	/5Phos/DDDGGCCACNNNNNNNAGATCGGAAGAGCG TCGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGA C		GTGGC C
RT-21T	/5Phos/DDDCGAAACNNNNNNNAGATCGGAAGAGCGT CGT/iSp18/CACTCA/iSp18/CCGCTGGAAGTGACTGAC		GTTTC G