Supplementary Figure 1: **(A)** Western blot showing the whole cell protein levels of SUMO-2/3 and Vinculin in heat-shocked (1 h) HeLa cells in presence of DMSO/SUMOi (200nM; added 1 h before HS). **(B)** Z-score normalized cluster heatmap of the differentially regulated proteins obtained from the experiment described in 1A (comparison groups: NHS+SUMOi/NHS, HS/NHS, and HS+SUMOi/NHS; Log2 fold change >0.585; -Log10 *p*-value>1.34). **(C)** Volcano plot showing the relative fold change (Log2 scale) of proteins after HS compared to the non-HS control (data is obtained from the proteomics experiment described in 1A). *P*-values for the fold change (represented in the -Log10 scale in the y-axis) were calculated by Paired Student's t test (n=3). Significantly enriched (Log2 fold change>0.585; -Log10 *p*-value>1.34) and depleted proteins (Log2 fold change<0.585; -Log10 *p*-value>1.34) were coloured in red and blue, respectively. Relevant proteins are highlighted and labelled. **(D)** Z-score normalized cluster heatmap of the differentially regulated proteins obtained from the experiment described in 1C whole cell proteome (comparison groups: HS/NHS, HS Rec./NHS, and HS Rec./HS; Log2 fold change >0.585; -Log10 *p*-value>1.34). Functional clusters are designated. **(E)** Volcano plot showing the relative fold change (Log2 scale) of proteins in the HS Recovery (HS Rec.) group compared to the non-HS control (data is obtained from the proteomics experiment described in 1C). *P*-values for the fold change (represented in the -Log10 scale in the y-axis) were calculated by Paired Student's t test (n=3). Significantly enriched (Log2 fold change>0.585; -Log10 *p*-value>1.34) and depleted proteins (Log2 fold change<0.585; -Log10 *p* value>1.34) were coloured in red and blue, respectively. Relevant proteins are highlighted and labelled. **(F)** Proteins which are significantly increased (Log2 fold change>0.585; -Log10 *p*-value>1.34) in the HS Rec. group over non-HS control were subjected to GO term (Biological Process) enrichment analysis. **(G)** Western blot showing the whole cell protein levels of HSPA6, SUMO-2/3, and Vinculin in heat shock-recovered (2 h) HeLa cells in presence of DMSO/SUMOi (1 hr. pre-priming before 1 h HS) treated at indicated concentrations. **(H)** Western blot showing the whole cell protein levels of HSPA6, SUMO-2/3, and Vinculin in heat shock-recovered (2 h) HeLa cells in presence of DMSO/SUMOi (200nM) treated as indicated. **(I)** Western blot showing the whole cell protein levels of HSPA6, SUMO-2/3, and Vinculin in heat shock-recovered (2, 4, and 8 h) HeLa cells in presence of DMSO/SUMOi (TAK-

981; 200nM) treated as indicated. **(J)** Venn diagram showing the overlap between two sets; Set1: significantly decreased (Log2 fold change<0.585; -Log10 *p*-value>1.34) proteins in the NP-40 soluble fractions of heat–shocked cells over non-HS control, Set2: significantly increased (Log2 fold change>0.585; -Log10 *p*-value>1.34) proteins in the NP-40 insoluble fractions of heat-shocked cells over non-HS control (data obtained from experiment described in 1C NP-40 soluble/insoluble proteome). **(K)** Z-score normalized cluster heatmap of selected proteins obtained from the experiment described in 1C NP-40 insoluble proteome (Log2 fold change >0.263; -Log10 *p*-value>1.34). **(L)** HeLa cells were co-treated with DMSO/MG-132 (5 μM and 10 μM) and DMSO/SUMOi (200 nM) for 6 hours before whole cell lysates were extracted and subjected to SDS-PAGE/immunoblotting to assess protein levels of HSPA6, SUMO-2/3, and Vinculin. **(M)** HeLa cells were pre-primed with DMSO/SUMOi (200 nM) for 1 hour followed by exposure to indicated doses of SA (1 h) and recovery (2 h). Subsequently, whole cell extracts were run on SDS-PAGE and immunoblotting was done to assess protein levels of HSPA6, SUMO-2/3, and Vinculin. **(N)** HeLa cells were co-treated with water/CdCl2 (50 μM) and DMSO/SUMOi (200 nM) for 4 hours before whole cell lysates were extracted and subjected to SDS-PAGE/immunoblotting to assess protein levels of HSPA6, SUMO-2/3, and Vinculin. **(O)** HeLa cells were co-treated with DMSO/GTPP (7.5 μM) and DMSO/SUMOi (200 nM) for 6 hours before whole cell lysates were extracted and subjected to SDS-PAGE/immunoblotting to assess protein levels of HSPA6, SUMO-2/3, and Vinculin. **(P)** Z-score normalized cluster heatmap of selected proteins obtained from the NP-40 insoluble proteome of GTPP (7.5 μM, 6 h)-treated HeLa cells (Log2 fold change >0.263; -Log10 *p*-value>1.34).

Supplementary Figure 2: (A, B) All the proteins which are significantly increased (Log2 fold change>0.263; -Log10 *p* value>1.34) in the HS Rec.+SUMOi group compared to the only HS Rec. (HS rec.+DMSO) group in the NP-insoluble fractions are subjected to Curated Reactome **(A)** and GO term (GO_Biological Process) **(B)** enrichment analyses. **(C)** HSF1 protein levels in HSF1 proficient and KO HeLa cells were shown by immunoblot. **(D)** Gene set enrichment analysis based on curated Reactome and GO term (GO_Biological Process) annotations showing genes associated with protein folding and HSF1-dependent activation being enriched in SUMOi-primed cells during HS (upper

panel) and HS recovery (lower panel) in HSF1 proficient HeLa cells. Data were obtained from the RNAseq experiment described in Figure 2A. (**E**) Heatmap showing unbiased clustering using the normalized transcripts per million (TPM) values of the differentially expressed genes sorted by SUMOi-dependent regulation. (**F**) GO-term (GOBP) analysis of the 8th cluster as shown in Figure S2E. **(G)** HeLa cells were treated with KRIBB11 (5 and 10μM for 6 hrs.)/DMSO and were further co-treated with DMSO/SUMOi (TAK-981; 200nM) for an additional hour before given HS (1 hr.) followed by recovery (2 hrs.). Whole cell lysates were finally run on SDS-PAGE and immunoblotted to assess the protein levels HSPA6, SUMO-2/3, Vinculin and HSF1. **(H)** OPM2 and JJN3 multiple myeloma cell lines were subjected to single or combinatorial treatment of Carfilzomib and Subasumstat (SUMOi) (data obtained from PMID: 35917568). Gene set enrichment analysis based on curated Reactome annotations showing that genes belonging to the “HSF1-dependent transactivation” pathways are significantly enriched in SUMOi+Carfilzomib-primed cells compared to only Carfilzomib treatment. Adjusted *p* value is denoted by q.

Supplementary Figure 3: **(A)** HeLa cells were reverse transfected with either siGL2, siSUMO-1, or siSUMO-2/3 for 72 hours and were further primed with DMSO/SUMOi (TAK-981, 200nM) and/or MG-132 (5 and 10 μM) for another 6 hours. Subsequently, whole cell lysates were prepared and subjected to SDS-PAGE/immunoblotting for checking protein levels of HSPA6, SUMO-1, SUMO-2/3, and Vinculin. **(B, C)** HeLa cells were transfected with HA-SUMO-2 or HIS-SUMO-2 for 36 hours as indicated and further treated with DMSO/SUMOi (200nM; 1 hr.). One set of HIS-SUMO-2 transfected cells was further heat-shocked (1hr.). Subsequently, SUMO-2 conjugated fractions were enriched from the denatured cellular lysates on Ni-NTA magnetic beads and checked for the relative abundance of HSF1 and HIS (SUMO-2) by SDS-PAGE/immunoblotting. 10% cellular lysates were subjected to TCA precipitation and used as “Input” to assess protein levels of **(B)** HSF1, HIS (SUMO-2), and Vinculin or **(c)** FLAG (HSF1), HIS (SUMO-2), and Vinculin. **(D)** HeLa cells were transfected with HA-SUMO-2 or HIS-SUMO-2 and FLAG-HSF1 WT/K298R/5K-R_1/5K-R_2 for 36 hours as indicated. One set of transfected cells was further heat-shocked (1hr.). Subsequently, SUMO-2 conjugated fractions were enriched from the denatured cellular lysates on Ni-NTA magnetic beads and checked for the relative abundance of FLAG (for HSF1) and HIS (for SUMO-2) by SDS-

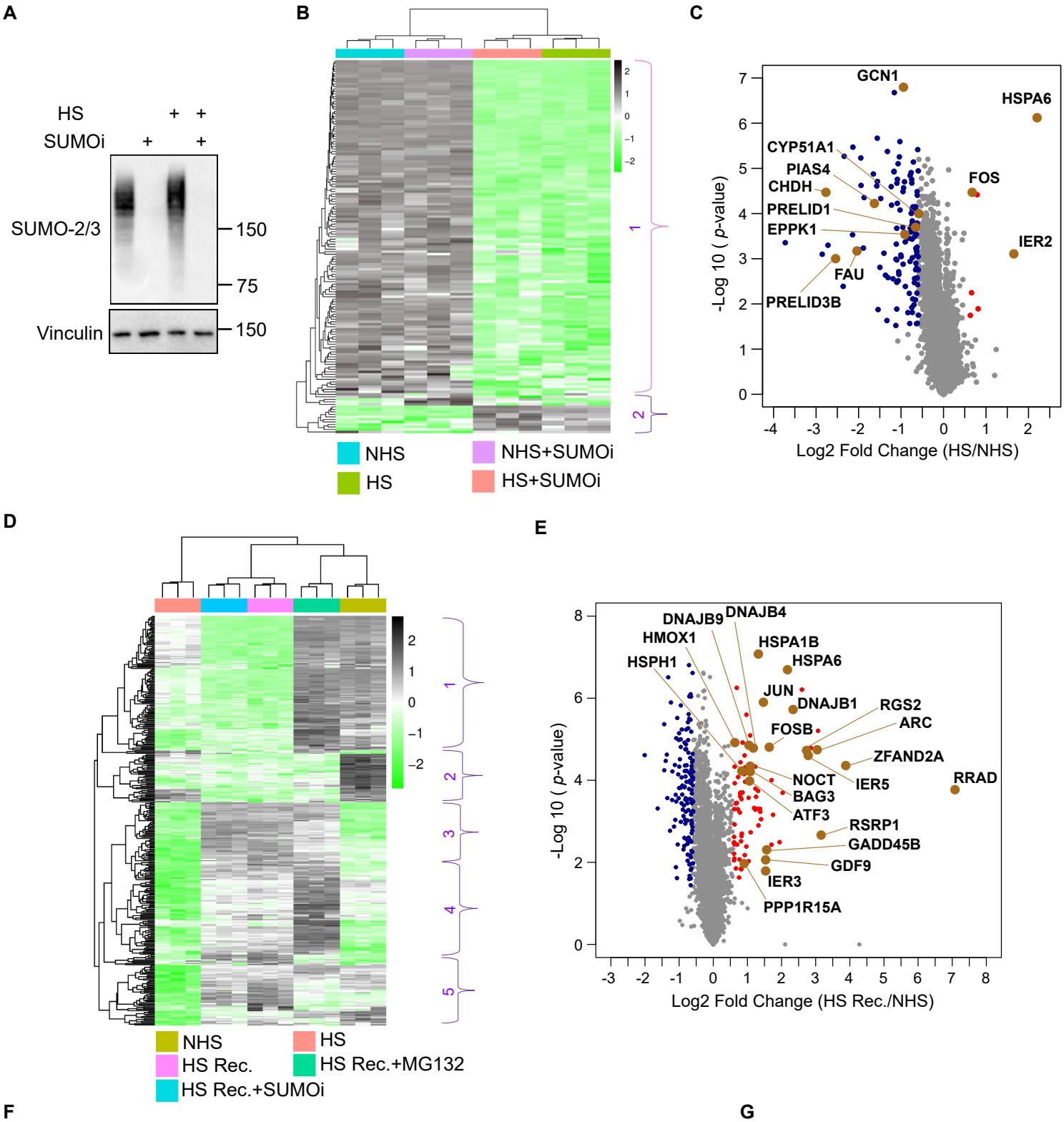
PAGE/immunoblotting. 10% cellular lysates were subjected to TCA precipitation and used as “Input” to assess protein levels of FLAG (HSF1), HIS (SUMO-2), and Vinculin. **(E)** siHSF1 targeting sequence in the coding region of HSF1 is denoted. Site directed mutations (marked with asterisks) were introduced in the siHSF1 targeting sequence of the FLAG-HSF1 plasmid (in the pcDNA5/FRT/TO vector background) to render the plasmid-based HSF1 expression resistant to siHSF1. **(F)** HeLa Flp-In FLAG-HSF1 WT (siHSF1-sensitive and resistant) cells were reverse transfected with siGL2 or siHSF1 and kept for 72 hours. Vehicle control or Doxycycline (1µg/ml) was added for the last 16 hours of transfection. Cells were further heat-shocked (1 hr.) and recovered (2 hrs.) before the whole cell lysates were subjected to SDS-PAGE/immunoblotting for checking protein levels of HSPA6, HSF1, and Vinculin. **(G)** Hela Flp-In FLAG-HSF1 WT (siHSF1-resistant) and FLAG-HSF1^{K298R} (siHSF1-resistant) cells were reverse transfected with siHSF1 and kept for 72 hours. Vehicle control or Doxycycline (1µg/ml) was added for the last 16 hours of transfection. Cells were further treated with DMSO/SUMOi (TAK-981; 200nM) and MG-132 (10µM) for 6 hours. Whole cell lysates were finally prepared and subjected to SDS-PAGE/immunoblotting for checking protein levels of HSPA6, HSF1, SUMO-2/3, and Vinculin.

Supplementary Figure 4: (A) HSF1 proximity proteome was filtered first for the candidates which are significantly enriched (Log2 fold change>0.585; -Log10 *p* value>1.34) in any of the Biotin-primed and 3XHA-ultraID-HSF1 transfected conditions over the both no Biotin control (3XHA-ultraID-HSF1 transfected without Biotin priming) and empty vector control (3XHA-ultraIID transfected with Biotin priming). Subsequently, all these protein candidates were subjected to pathway enrichment analysis based on Curated Reactome annotations, and a cytoscape network of the Curated Reactome pathways are shown. **(B, C)** HeLa cells were treated with A-485 (2.5µM for 12 hrs.)/DMSO **(B)** or AU-15330 (1µM for 1 hr. or 2 hrs.)/DMSO **(C)** and were further co-treated with DMSO/SUMOi (TAK-981; 200nM) for an additional hour before given HS (1 hr.) followed by recovery (2 hrs.). Whole cell lysates were finally run on SDS-PAGE and immunoblotted to assess the protein levels HSPA6, SUMO-2/3, Vinculin **(B, C)**, and SMARCA4 **(C)**. **(D, E)** MG-132-treated (10µM, 6 hours) HeLa cells were primed with DMSO/SUMOi (200nM, 6 hours) and were further co-treated with AU-15330 (1µM for the last 2 hrs. of MG-132

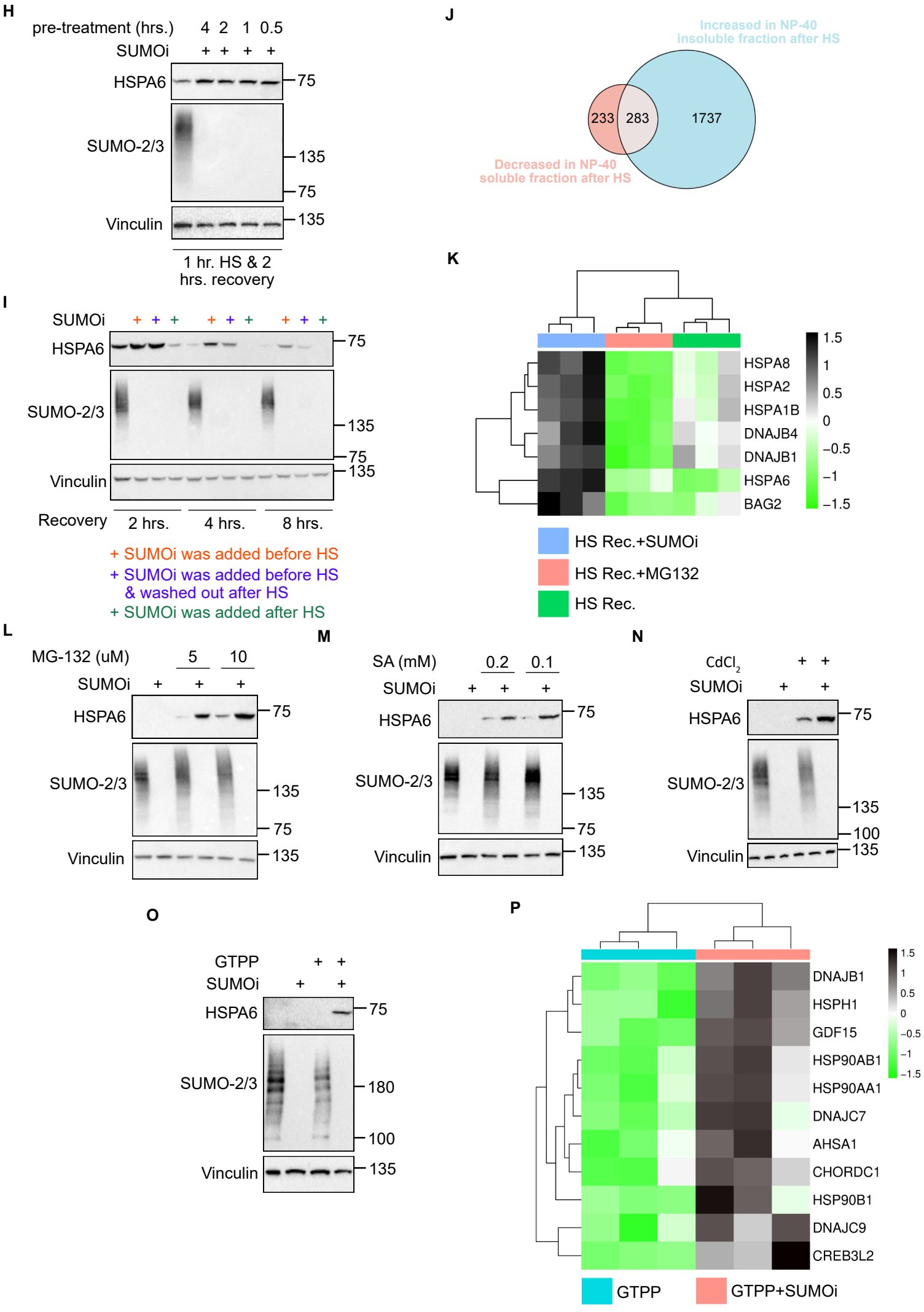
treatment)/DMSO or **(D)** Artepillin C analog A57 (7.5μM for 12 hrs.)/DMSO) **(E).** Whole cell lysates were finally run on SDS-PAGE and immunoblotted to assess the protein levels HSPA6, SUMO-2/3, Vinculin **(D, E)**, and SMARCA4 **(D)**. **(F)** HeLa Flp-In FLAG-HSF1^{K298R} (siHSF1-resistant) cells were reverse transfected with siHSF1 and kept for 72 hours. Vehicle control or Doxycycline (1μg/ml; last 16 hrs. of transfection) as well as A-485 (2.5μM; last 12 hours of transfection)/DMSO were added. As indicated, A-485 treated cells were further co-primed with AU-15330 (1μM for the last 2 hrs. of transfection)/DMSO. Cells were further heat-shocked (1 hr.) and recovered (2 hrs.) before the whole cell lysates were subjected to SDS-PAGE/immunoblotting for checking protein levels of HSPA6, HSF1, SMARCA4, SUMO-2/3, and Vinculin. **(G)** CUT&RUN was performed for HSF1 under four conditions (NHS, HS, NHS+SUMOi, and HS+SUMOi). Reads were aligned to the human reference genome (GRCh38/hg38). Enrichment of HSF1 binding was assessed at transcription start sites (TSS) within a window of ±2 kb. Signal was calculated as log2(IP-HSF1/Input) from normalized coverage tracks. For visualization, genome-wide signal matrices were generated using a reference-point approach centered on TSS coordinates. The resulting matrix spans 4 kb (−2 kb to +2 kb relative to TSS) and includes signal values across all annotated genes. A profile plot displays the average enrichment signal across all regions for each condition, while a heatmap represents the distribution of HSF1 binding across individual loci. Signal intensity corresponds to normalized log2(IP/Input) values across the defined window. **(H)** CUT&RUN was performed for HSF1 under four conditions (NHS, HS, NHS+SUMOi, and HS+SUMOi) and average HSF1 CUT&RUN signal (normalized occupancy, arbitrary units) is shown for *HSPA8*, *DNAJB5*, and *BAG2*. Signal is plotted across gene bodies scaled from 5 kb upstream of the transcription start site (TSS) to 5 kb downstream of the transcription end site (TES). Profiles represent average signal across biological replicates.

Supplementary Figure 5: (A) Representative confocal images (without zooming) of the HSF1 positive nSBs in NHS, heat-shocked and HS-recovered HeLa cells. Scale bar 10μm. **(B)** Quantification for Figure 5A; area for HSF1 positive puncta were calculated by ImageJ and percentage (mean±standard deviation) of nSBs with >1μm^2 area were represented. *P* value was calculated based on two-sided Student's t-tests. At least 250 cells were counted for each condition for the analysis. **(C, D, E)** Representative confocal

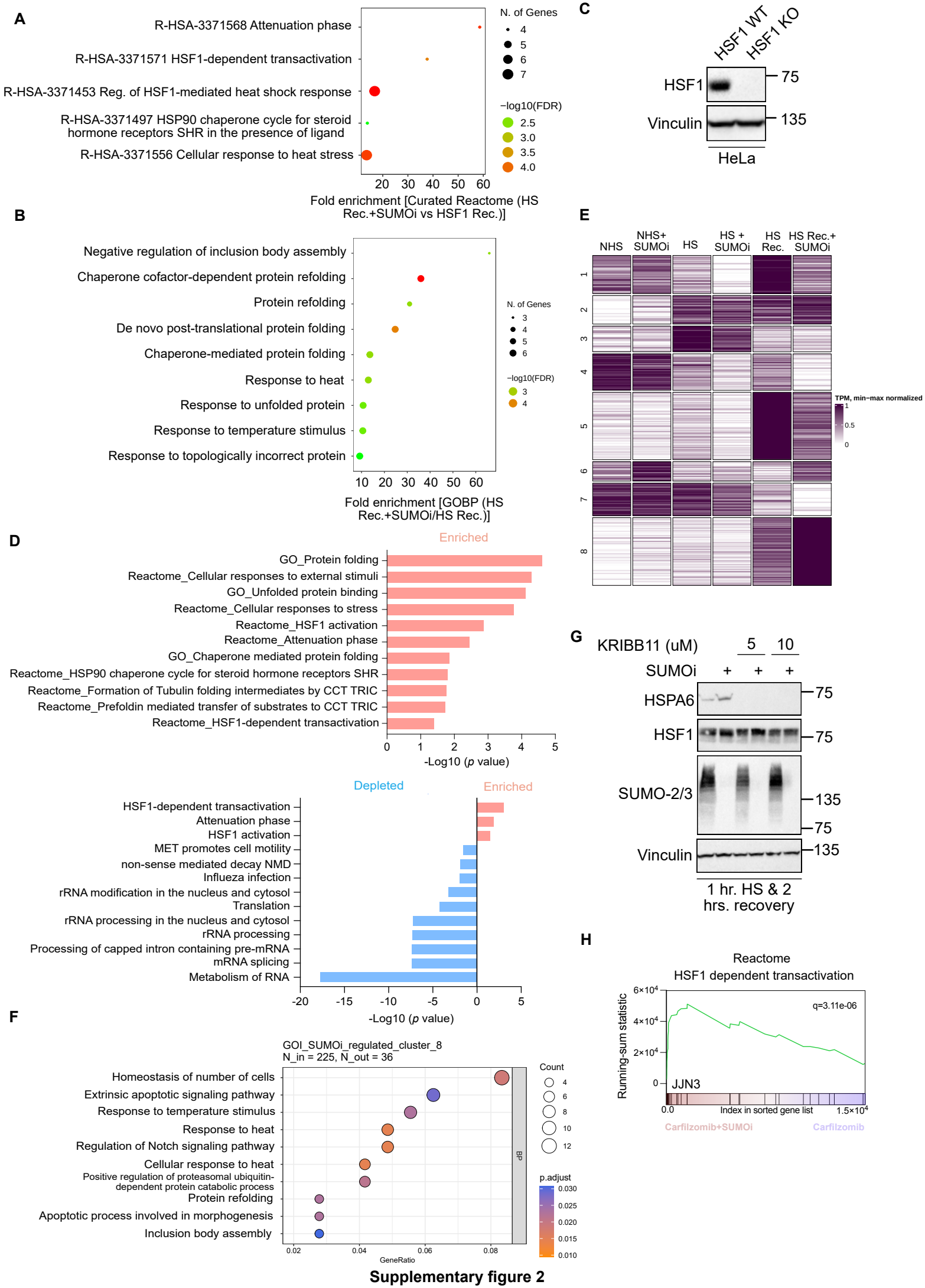
images [zoomed-in **(C, D)**; without zoom **(E)**] of the HSF1 positive nSBs in heat-shocked HeLa cells treated as indicated. Scale bar 10μm. **(F)** Quantification for Figure 5D; area for HSF1 positive puncta were calculated by ImageJ and percentage (mean±standard deviation) of nSBs with >1μm^2 area were represented. *P* value was calculated based on two-sided Student's t-tests. At least 500 cells were counted for each condition for the analysis. (**G**) All the proteins which are significantly increased (Log2 fold change>0.585; -Log10 *p* value>1.34) in the HSF1^{K298R}_HS group compared to the HSF1^{WT}_HS group are subjected to GO term (GO_Cellular Component) enrichment analyses. **(H)** Heatmap of intron-wise PSI (percent spliced-in) values, k-means clustering; n = 15541. **(I)** GO-term analysis of transcripts containing introns that are more retained after SUMO-inhibition. **(J, K)** Comparison of the strength of 5'splice sites (**J**) and GC content (**K**) in introns that were up-regulated (401), down-regulated (165) or not regulated (17259) during heat stress recovery and SUMO inhibition.



Supplementary figure 1



Supplementary figure 1



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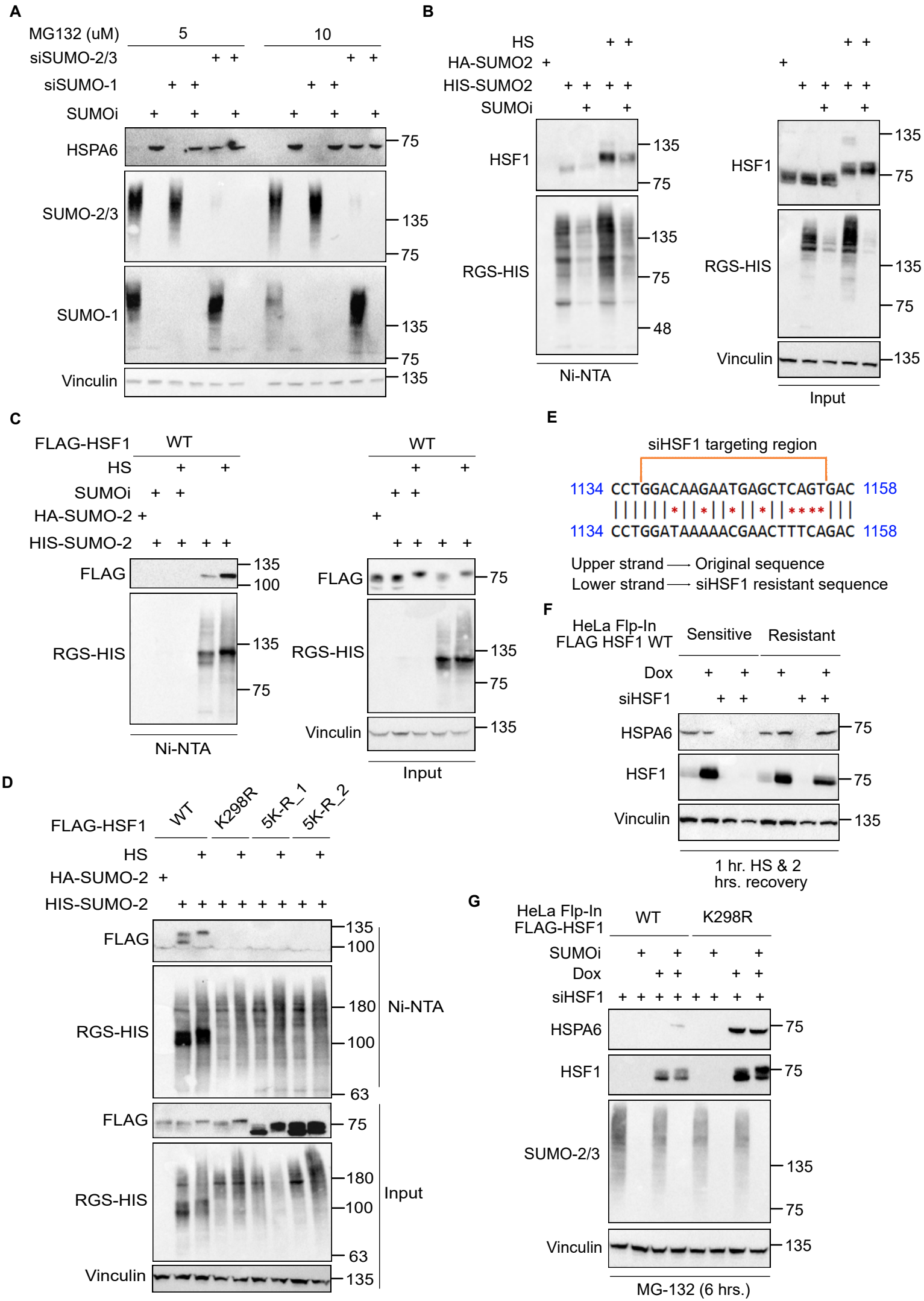

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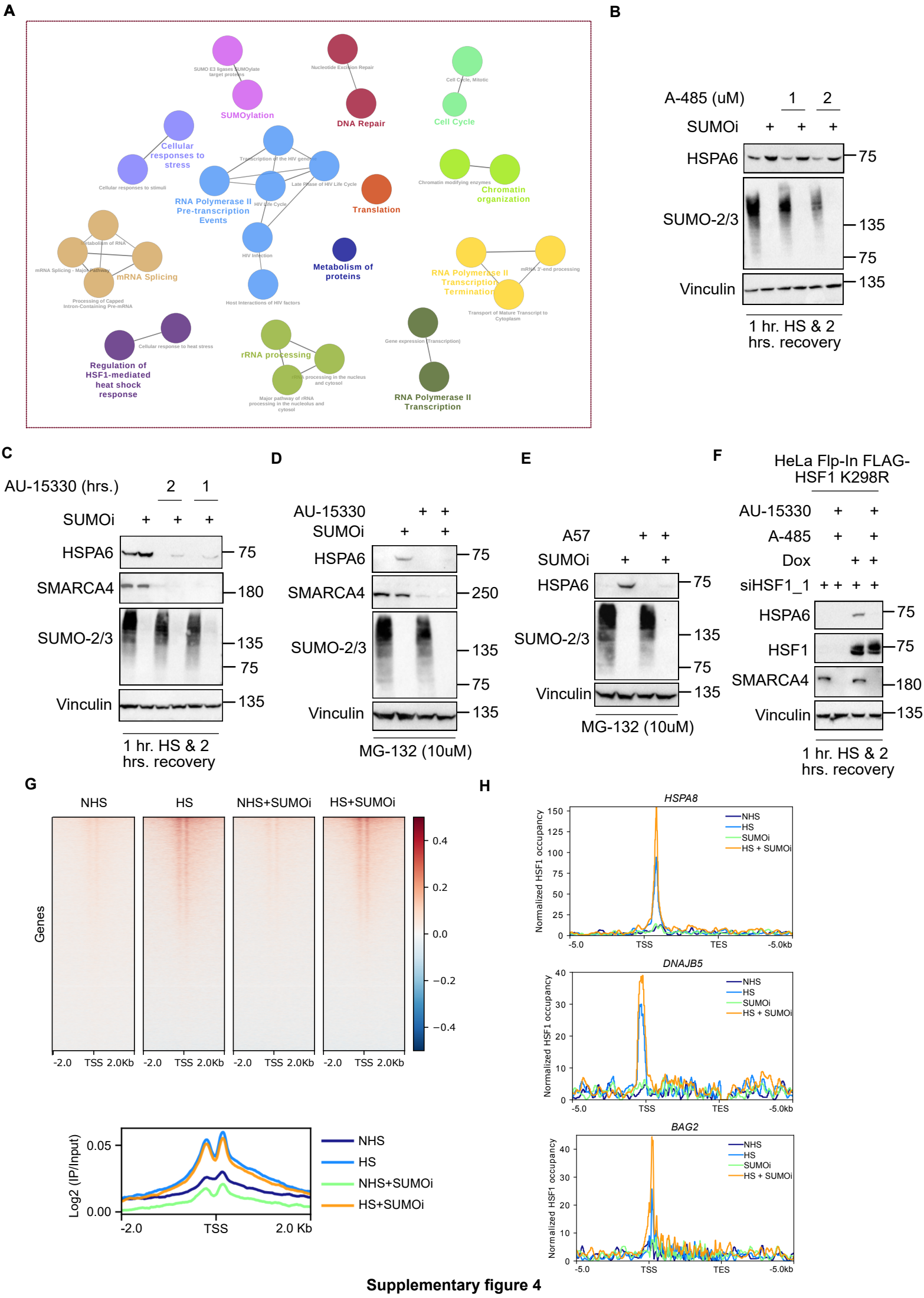
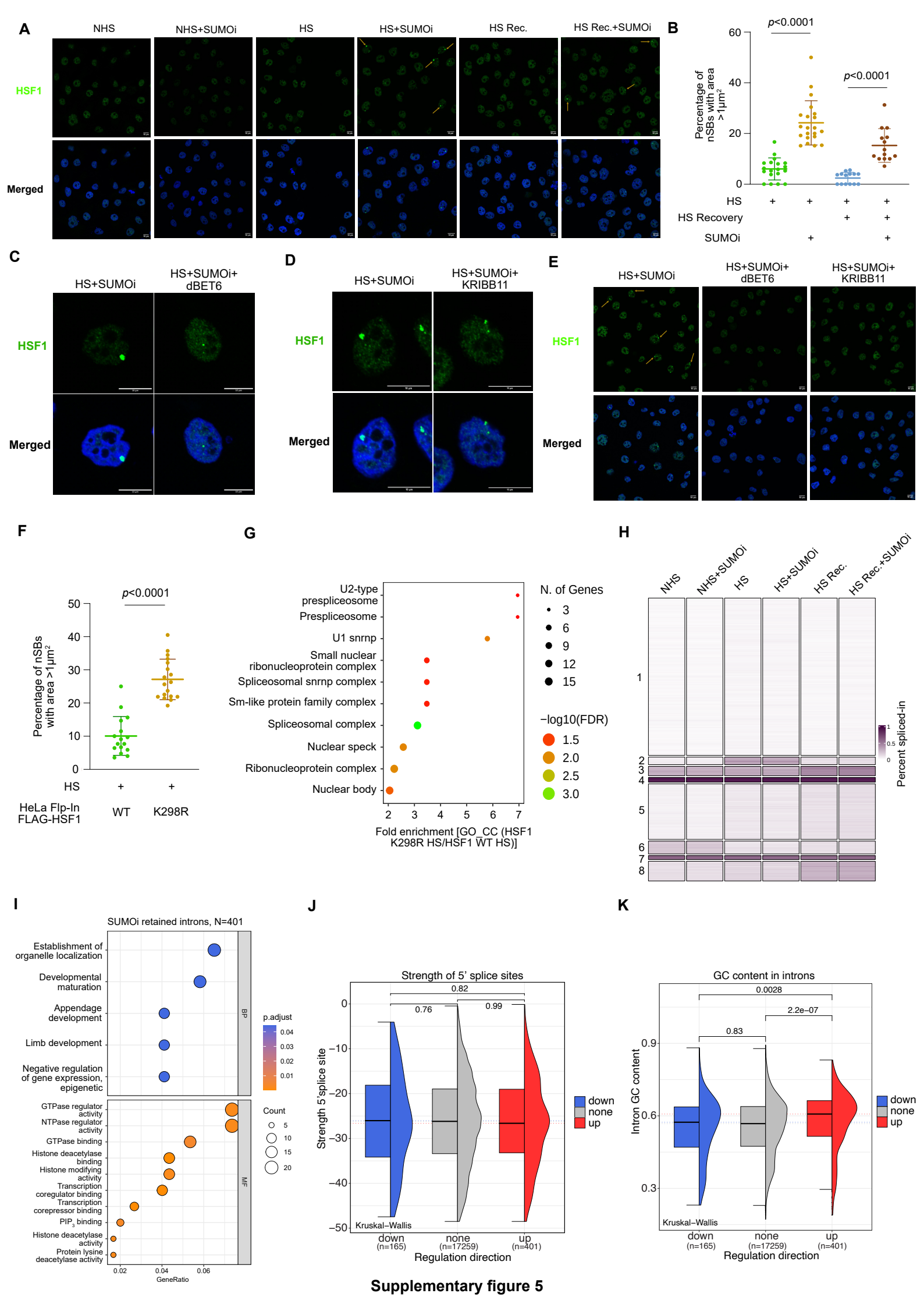


Supplementary figure 4



Supplementary figure 5