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Supplemental figures

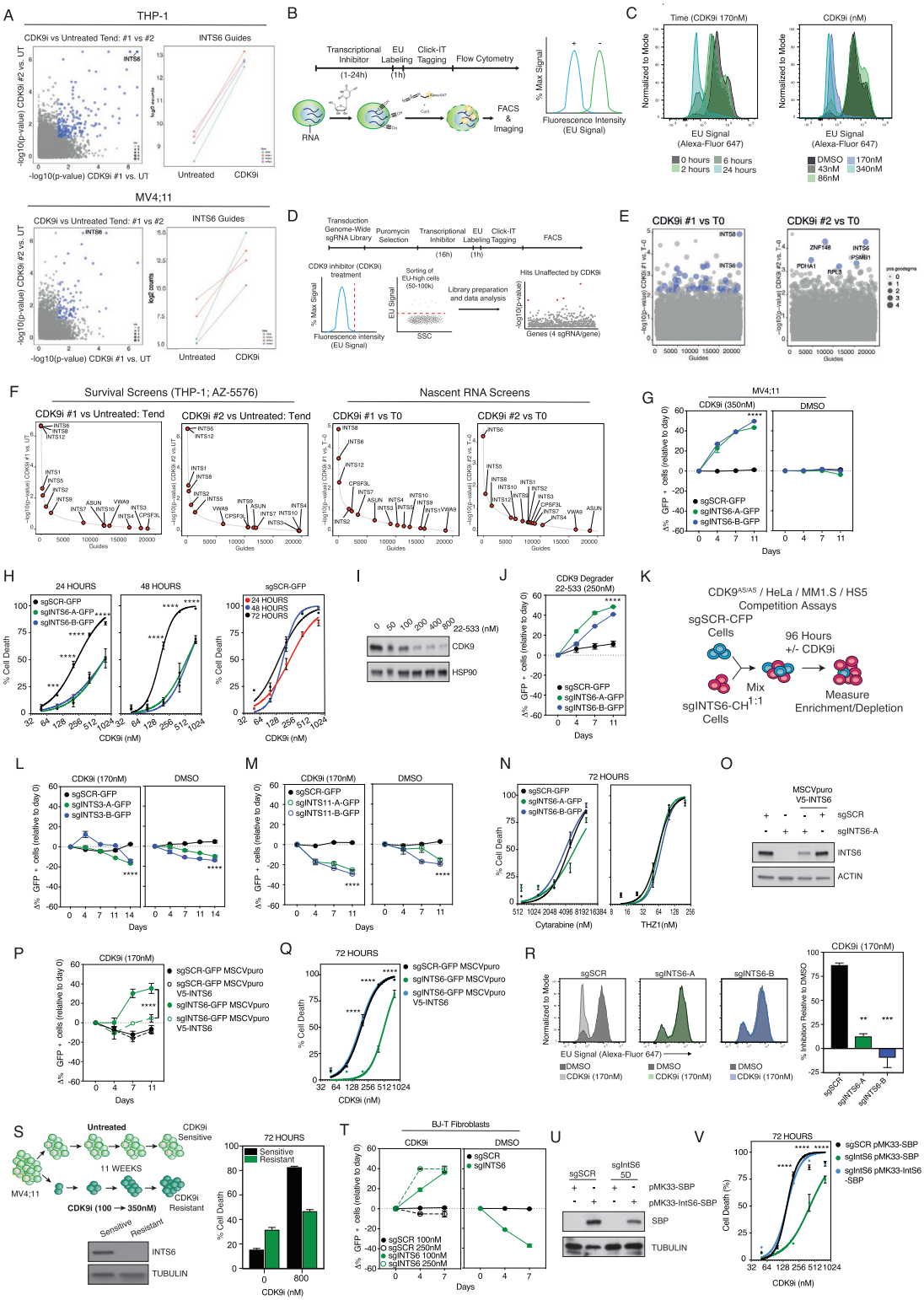

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Figure S1. Loss of INTS6 confers resistance to CDK9 inhibition, related to Figure 1

A. Comparison of enriched sgRNAs for replicate survival screens in THP-1-Cas9 and MV4;11-Cas9 cells (CDK9i versus untreated at Tend) plus enrichment of INTS6 sgRNAs in CDK9i-treated versus untreated cells at Tend. B. Overview of nascent RNA analysis using ClickIT-EU assay and flow cytometry. C. Representative flow cytometry profiles for EU incorporation/Alexa-Fluor647 staining as a measure of nascent RNA production in THP-1 cells treated with CDK9i (170nM) for the indicated time-points (left) or for 24 hours with the indicated dose of CDK9i (right). D. Overview of nascent RNA CRISPR-Cas9 genome-wide screen in THP-1-Cas9 cells; resistant cells were selected by FACS for the maintenance of high-EU signal in the presence of CDK9i. E. Identification of significantly enriched guides in EU-high cells (CDK9i treated relative to T0). F. Enrichment of sgRNAs for Integrator complex subunits in THP-1-Cas9 cells for individual replicate survival screens (significance relative to DMSO-treated cells at Tend) and nascent RNA screens (significance relative to T0). G. Competitive proliferation assay for MV4;11-Cas9 cells expressing indicated *INTS6* targeting sgRNAs in the presence of CDK9i and DMSO. H. Annexin-V analysis of THP-1-Cas9 cells expressing indicated SCR and *INTS6* targeting sgRNAs treated as indicated for 24 and 48 hours, and comparison of THP-1-Cas9 cells expressing SCR sgRNA treated with indicated doses of CDK9i at 24, 48 and 72 hours. (I). Western blot of THP-1 cells treated with 22-533 for 6 hours. J. Competitive proliferation assay for THP-1-Cas9 cells expressing indicated SCR or *INTS6* targeting sgRNAs in the presence of 22-533. K. Overview of THP1 CDK9$^{AS/AS}$, HeLa, MM1.S and HS5 competitive proliferation assays for Figures 1K, 1M, 1O, and 1P: cells expressing sgSCR (CFP-Cas9) and sg*INTS6* (CH-Cas9) were mixed 1:1 and incubated for 96 hours with CDK9i or 1-NA-PP1. Competitive proliferation assay for THP-1-Cas9 cells expressing indicated SCR, *INTS3* (L) or *INTS11* (M) targeting sgRNAs in the presence of CDK9i or DMSO. N. Annexin-V analysis of THP-1-Cas9 cells expressing indicated *INTS6* targeting sgRNAs treated as indicated for 72 hours. O. western blot of THP-1-Cas9 cells expressing SCR or *INTS6*-A targeting sgRNAs plus/minus V5-INTS6 overexpression. P. Competitive proliferation assay and Q. Annexin-V cell death analysis of THP-1-Cas9 cells expressing indicated SCR or *INTS6*-A targeting sgRNAs plus/minus V5-INTS6 overexpression in the presence of A5576 (CDK9i). R. Representative flow-cytometry profiles and quantitation of the relative inhibition of nascent RNA production (as measured by EU incorporation/Alexa-Fluor647 staining) following CDK9i treatment for 24 hours in THP-1-Cas9 cells expressing indicated SCR or *INTS6* targeting sgRNAs. S. Overview of CDK9i acquired resistance; MV4;11 cells were cultured for 11 weeks in the presence or absence of CDK9i as indicated. At endpoint cells were assessed by western blot and cell death was measured by Annexin-V staining following treatment with CDK9i as indicated for 72 hours. T. Competitive proliferation assay for BJ-T-Cas9 cells expressing indicated *INTS6* targeting sgRNAs in the presence of CDK9i and DMSO. U. western blot of *D. melanogaster* S2-Cas9 sgSCR and sg*IntS6*-5D cells plus/minus SBP-IntS6 overexpression. V. Annexin-V analysis of *D. melanogaster* S2-Cas9 cells expressing indicated SCR or *IntS6* targeting sgRNAs plus/minus SBP-IntS6 overexpression treated as indicated for 72 hours. Blue dots (Figures A and E) represent nominal p value < 0.05 or p value < 0.01 respectively. Figures G, H-J, L-R and T-V are representative of 3 independent experiments. Figures G, H, J, L, M, P, Q, R and V were analyzed by 2-way ANOVA, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

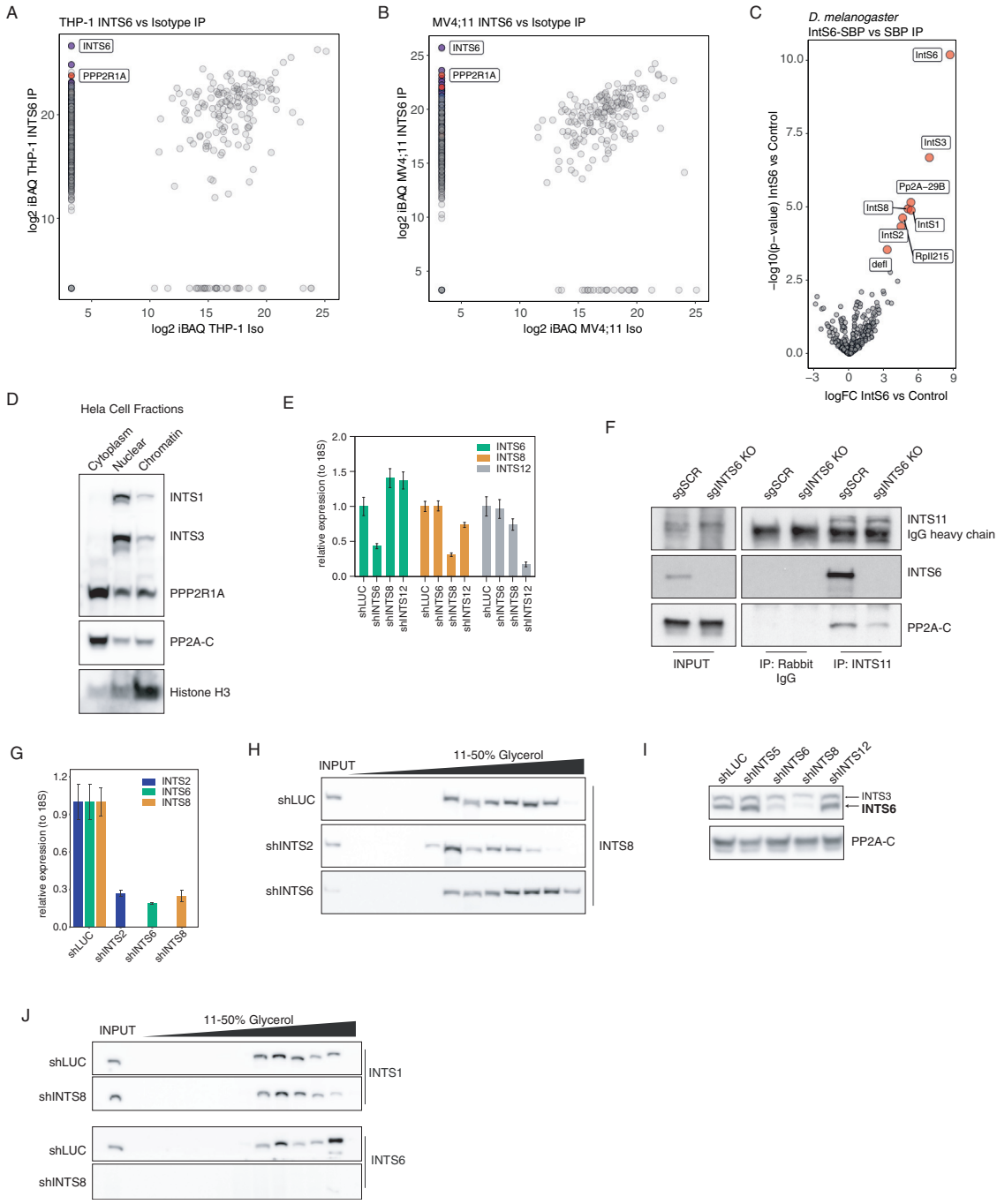

Figure S2. INTS6 bridges the interaction between Integrator and PP2A, related to Figure 2

Log2 iBAQ scores of proteins identified in INTS6 IP versus mouse IgG isotype control IP mass spectrometry experiments for A. THP-1 and B. MV4;11 cells. C. Volcano plot of differentially enriched proteins in SBP-IntS6 versus SBP (isotype control) streptavidin-IP mass spectrometry experiments in *D. melanogaster* S2 cells. D. western blot of HeLa cell cytoplasmic, nuclear, and chromatin fractions. E. Relative expression levels of *INTS6*, *INTS8*, and *INTS12* from shLUC, shINTS6, shINTS8, or shINTS12 infected HeLa cells. Values normalized by expression of ribosomal RNA 18S. RT-PCR results are representative of knockdown validation for all shRNA experiments. F. Co-IP western blot for INTS11 in THP-1-Cas9 sgSCR and sgINTS6-KO cells. G. Relative expression levels of *INTS2*, *INTS6*, and *INTS8* from shLUC, shINTS6, shINTS8, or shINTS2 infected HeLa cells, as presented in Figures 2I and 2J. H. western blot of glycerol gradient fractions of nuclear extracts from shLUC, shINTS2, or shINTS6-infected HeLa cells. I. western blot of nuclear extracts from HeLa cells infected with shLUC, shINTS5, shINTS6, shINTS8, or shINTS12. The same panel of PP2A-C INPUT presented in Figure 2G is re-presented here as a loading control. J. western blot of glycerol gradient fractions of nuclear extracts from shLUC or shINTS8 infected HeLa cells.

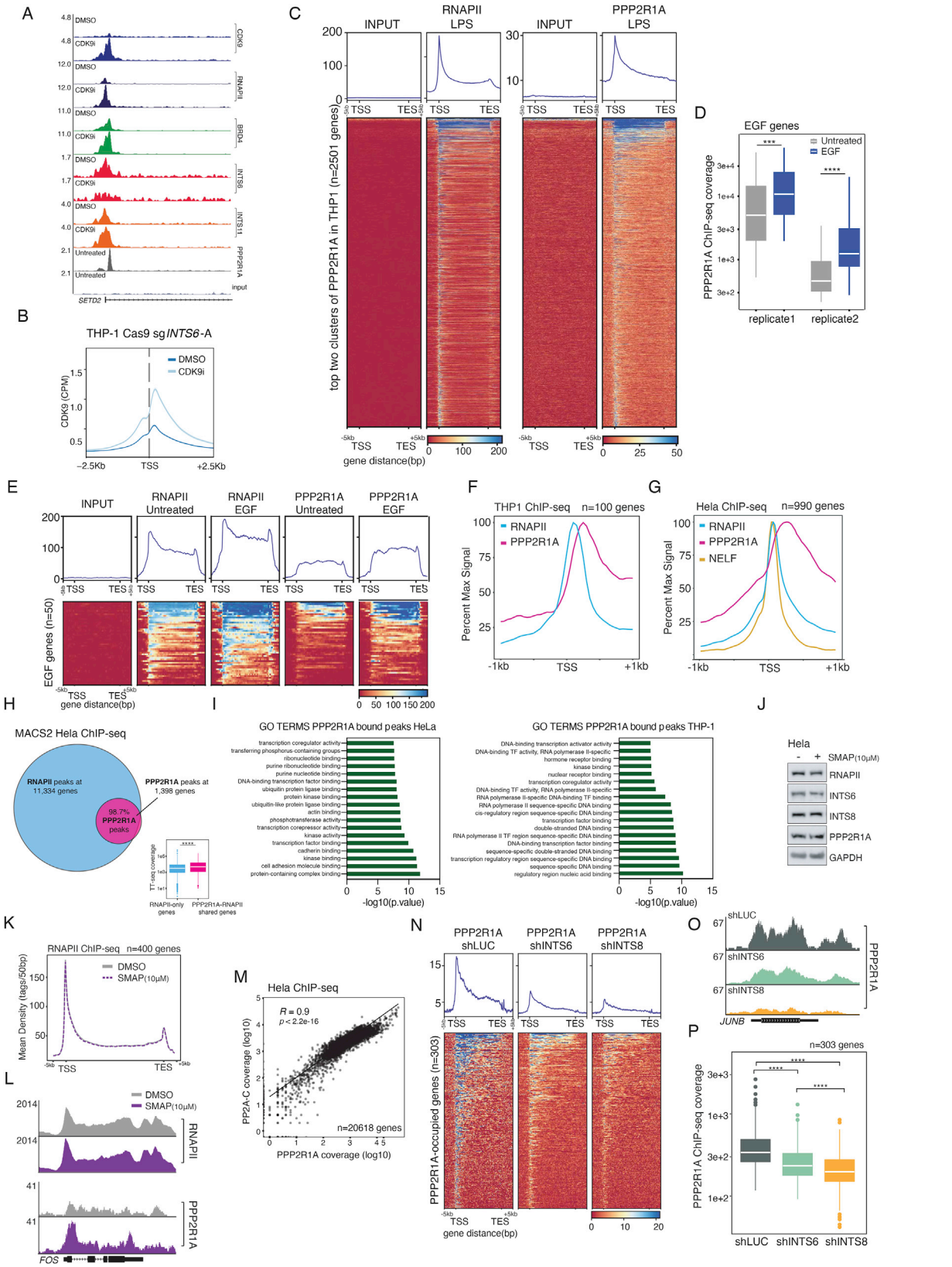

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Figure S3. INTS6-dependent dynamic recruitment of PP2A at actively transcribed genes, related to Figure 3

A. ChIP-seq signal at the *SETD2* locus for indicated proteins in THP-1 cells treated as indicated for 2 hours. B. Average profiles of ChIP-seq signal for CDK9 around the TSS, THP-1-Cas9 cells expressing *INTS6* targeting sgRNA and treated as indicated for 2 hours. C. Heatmap with average profiles of RNAPII and PPP2R1A ChIP-seq, including inputs, from THP1 cells treated with acutely with LPS. The geneset is from the top two (out of five) clusters from k-means clustering of all four replicates of PPP2R1A ChIP-seq in THP1, n = 2501. (D). Quantification of PPP2R1A ChIP-seq coverage in HeLa cells untreated or acutely treated with EGF, at EGF genes (n = 50) in two replicates. E. Heatmap with average profiles of RNAPII and PPP2R1A ChIP-seq at EGF genes (n = 50) in HeLa cells treated as indicated. F. Average profiles of ChIP-seq signal for RNAPII and PPP2R1A around TSS profiles at highest-expressed genes (n = 100) in untreated THP1, presented as percent of maximum read density to visualize the location of the peaks. G. Average profiles of ChIP-seq signal for RNAPII, PPP2R1A, and NELF around TSS profiles at highest-expressed genes (n = 990) in HeLa cells treated acutely with EGF (0.1μg/mL; 15 minutes). NELF ChIP-seq tracks are from a previously published dataset (Lai et al., 2015). H. Visualization of MACS2 broad peak calling results for HeLa RNAPII and PPP2R1A ChIP-seq. After selecting for genes that had peaks called in four replicates for each antibody, there were 1398 robust PPP2R1A-bound genes, of which 98.7% overlapped with RNAPII-occupied genes. Inset boxplot represents quantification of gene expression by TT-seq coverage for each gene set. I. GO term analysis was performed with ToppGene Suite for PPP2R1A-bound genes in HeLa (n = 1398 genes) and in THP1 (n = 564 genes) – defined as above. J. western blot of HeLa cell lysates after treatment with DMSO or SMAP (10μM, 2 hours). K. Average profile of RNAPII ChIP-seq in HeLa after treatment with DMSO or SMAP (10μM, 2 hours) at PPP2R1A-occupied genes (n = 1398). L. RNAPII and PPP2R1A ChIP-seq signal at the *FOS* locus after treatment with DMSO or SMAP (10μM, 2 hours). M. Coverage of PP2A-C and PPP2R1A ChIP-seq plotted against each other at all RNAPII-transcribed genes (n = 20618), with Pearson correlation. N. Heatmap with average profiles of PPP2R1A ChIP-seq in HeLa infected with shLUC, shINTS6, or shINTS8 at 303 PPP2R1A-occupied genes. O. PPP2R1A ChIP-seq signal at the *JUNB* locus in HeLa infected with shLUC, shINTS6, or shINTS8. P. Quantification of PPP2R1A ChIP-seq coverage shown in N. Scale bar for A represents 10kb. Figures D, H, M, and P analyzed by unpaired, two-sided Student's t test, ***$p < 0.001$, ****$p < 0.0001$.

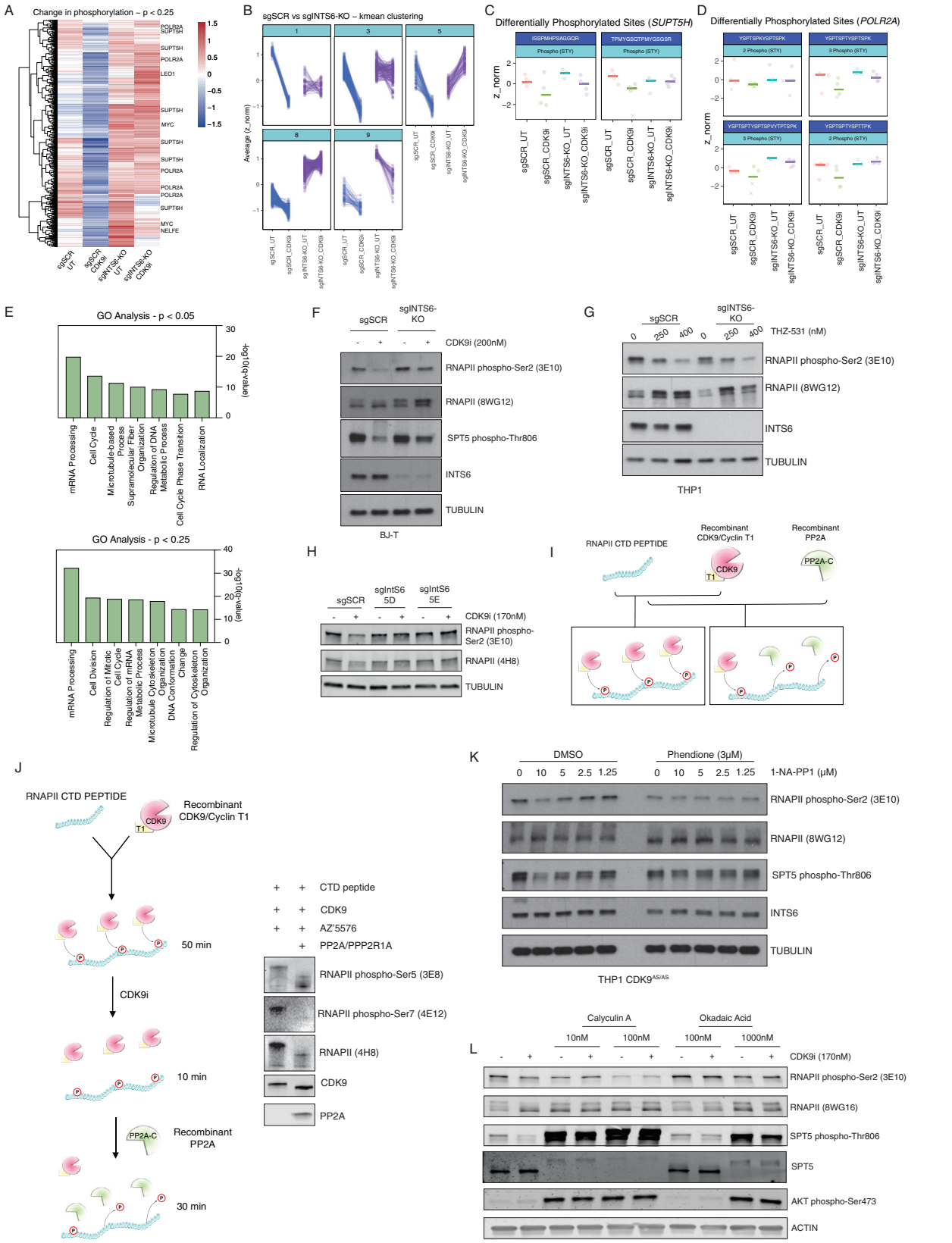

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Figure S4. Loss of INTS6/PP2A results in decreased turnover of CDK9 substrates, related to Figure 4

A. Heatmap of Z-scores of phospho-peptides in THP-1-Cas9 sgSCR and sgINTS6-KO cells treated as indicated for 2 hours, p value < 0.25. B. K-means clustering of differentially phosphorylated peptides identified by phospho-peptide mass spectrometry in THP-1-Cas9 sgSCR and sgINTS6-KO cells treated as indicated for 2 hours. Additional differentially phosphorylated peptides C. *SUPT5H* and D. *POL2RA* peptides in THP-1-Cas9 sgSCR and sgINTS6-KO cells treated as indicated for 2 hours. E. GO-analysis of proteins with differentially phosphorylated peptides in CDK9i-treated THP-1-Cas9 sgSCR (compared to DMSO-treated THP-1-Cas9 sgSCR and CDK9i-treated THP-1-Cas9 sgINTS6-KO cells; $p < 0.05$ and $p < 0.25$). F. western blot of BJ-T cells electroporated with recombinant Cas9 and SCR or *INTS6* targeting sgRNAs and treated with CDK9i as indicated for 2 hours. G. western blot of THP-1-Cas9 sgSCR and sg*INTS6*-KO cells treated with THZ531 as indicated for 6 hours. H. western blot of *D. melanogaster* S2-Cas9 cells expressing indicated sgRNAs and treated as indicated for 2 hours. I. Overview of *in vitro* recombinant CDK9/Cyclin-T1 and PP2A kinase / phosphatase assay related to Figure 4H. J. Overview of *in vitro* recombinant CDK9/Cyclin-T1 and PP2A kinase / phosphatase assay. RNAPII CTD peptide was incubated with CDK9/CyclinT1 for 50 minutes prior to the addition of CDK9i for 10 minutes. PP2A was added to the reaction for 30 minutes and RNAPII CTD peptide phosphorylation was assessed by western blot. K. western blot of THP-1 $CDK9^{AS/AS}$ cells treated as indicated (15 minutes pre-treatment with the PP2A inhibitor Phendione and 2 hours with 1-NA-PP1). L. western blot of THP-1 cells treated as indicated (15 minutes pre-treatment with Calyculin A or Okadaic acid and 2 hours with CDK9i). Ten biological replicates were analyzed for phospho-peptide mass spectrometry. Western blots are representative of 3 independent experiments.

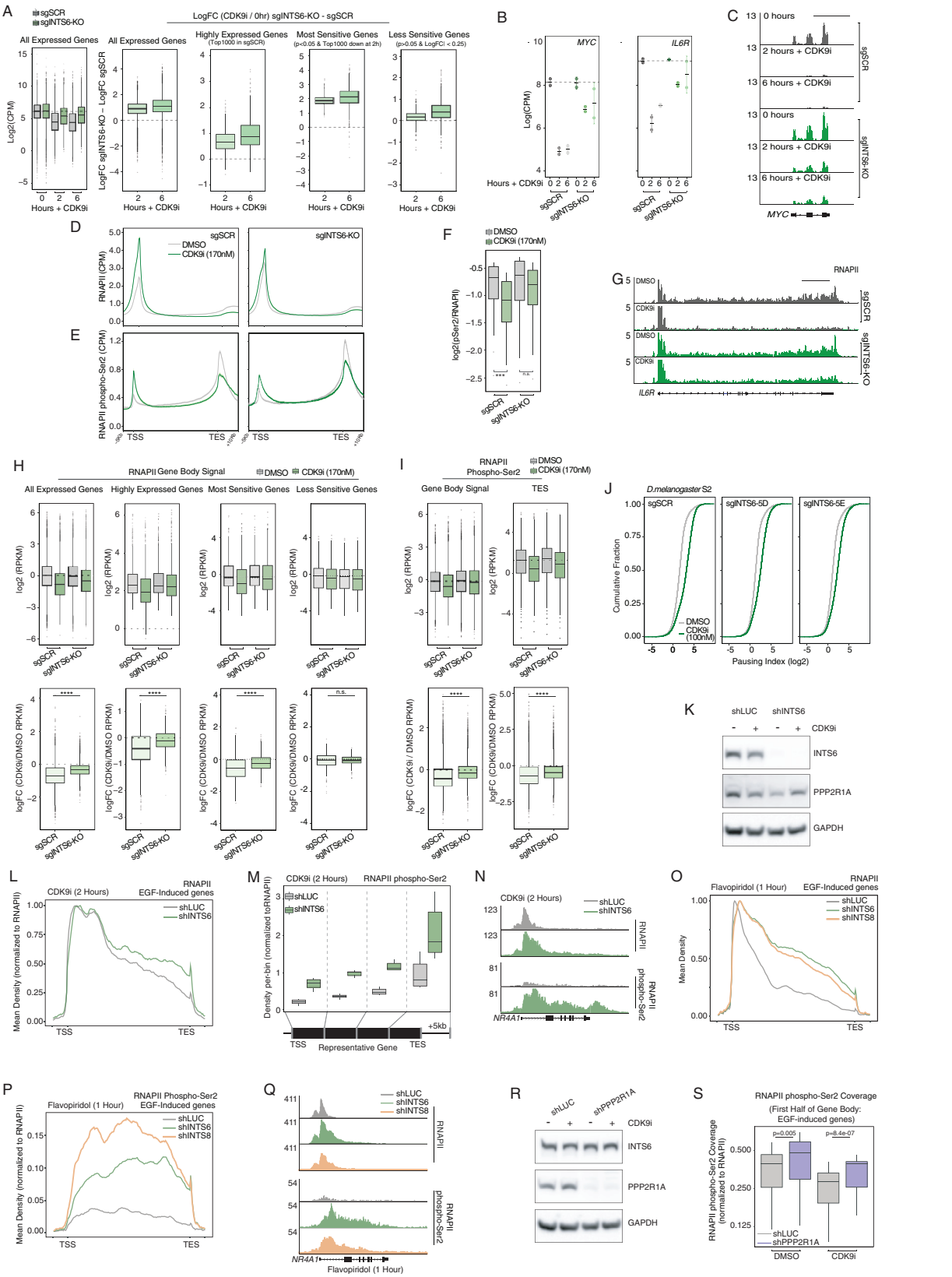

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Figure S5. INTS6/PP2A loss overrides CDK9i-induced transcriptional pausing, related to Figure 5

A. 4sU-seq signal (log2CPM) across all expressed genes and difference in log fold change of 4sU-seq signal (Figures 5B–5D) between THP-1-Cas9 sgINTS6-KO and sgSCR cells for all expressed genes, highly expressed genes and the most and least CDK9i-sensitive genes as indicated. B. 4sU-seq signal for *MYC* and *IL6R* genes in THP-1-Cas9 sgSCR and sgINTS6-KO cells treated as indicated. C. Example of 4sU-seq signal at the *MYC* locus under indicated conditions. Average profile of D. RNAPII and E. RNAPII pS2 ChIP-seq signal in THP-1-Cas9 sgSCR and sgINTS6-KO cells treated as indicated for 2 hours. F. Log2 ratio of RNAPII phosphor-Ser2 / total RNAPII ChIP-signal at TSS proximal regions (bin 51-75) of all expressed genes in THP-1-Cas9 sgSCR and sgINTS6-KO cells treated as indicated for 2 hours. G. Representative RNAPII ChIP-seq signal at the *IL6R* locus under the same conditions. H. Total and log fold change RNAPII ChIP-seq gene body signal at (i) all expressed, (ii) highly expressed, (iii) highly CDK9i sensitive and (iv) least CDK9i sensitive genes as defined by 4sU-seq analysis in THP-1-Cas9 sgSCR and sgINTS6-KO cells treated as indicated for 2 hours. I. Total and log fold change RNAPII pS2 gene body (left) and TES ChIP-seq signal (right) at all expressed genes for the same conditions as H. J. RNAPII pausing ratio in *D. melanogaster* S2-Cas9 cells expressing indicated sgRNAs and treated as indicated for 2 hours. K. western blot of shLUC and shINTS6 infected HeLa cells treated with CDK9i (AZ5576, 300nM) for 2 hours. L. Average profile of RNAPII ChIP-seq signal at EGF response genes (n = 50) in shLUC and shINTS6 infected HeLa cells treated with CDK9i (AZ5576, 300nM) for 2 hours and acutely treated with EGF (0.1μg/mL; 15 minutes). M. Quantification of Figure 5I, RNAPII pS2 ChIP-seq signal across gene body quartiles. N. RNAPII and RNAPII pS2 ChIP-seq signal at the *NR4A1* locus under the same conditions. Average profile of O. RNAPII and P. RNAPII pS2 ChIP-seq signal at EGF-response genes (n = 50) in shLUC, shINTS6 or shINTS8 infected HeLa cells treated with Flavopiridol (2μM) for 1 hour and acutely treated with EGF (0.1μg/mL; 15 minutes). Q. RNAPII and RNAPII pS2 ChIP-seq signal at the *NR4A1* locus under the same conditions. R. western blot of shLUC and shPPP2R1A infected HeLa cells treated with CDK9i (AZ5576, 300nM) for 2 hours. **S.** RNAPII pS2 ChIP-seq signal across the first half of the gene body for EGF-response genes (n = 50), in EGF-treated shLUC or shPPP2R1A HeLa cells, and treated as indicated for 2 hours. Scale bar for C represents 5kb. Scale bar for G represents 10kb. Figures F, H and I were analyzed by unpaired, two-sided Student's t test, ***$p < 0.001$, ****$p < 0.0001$.

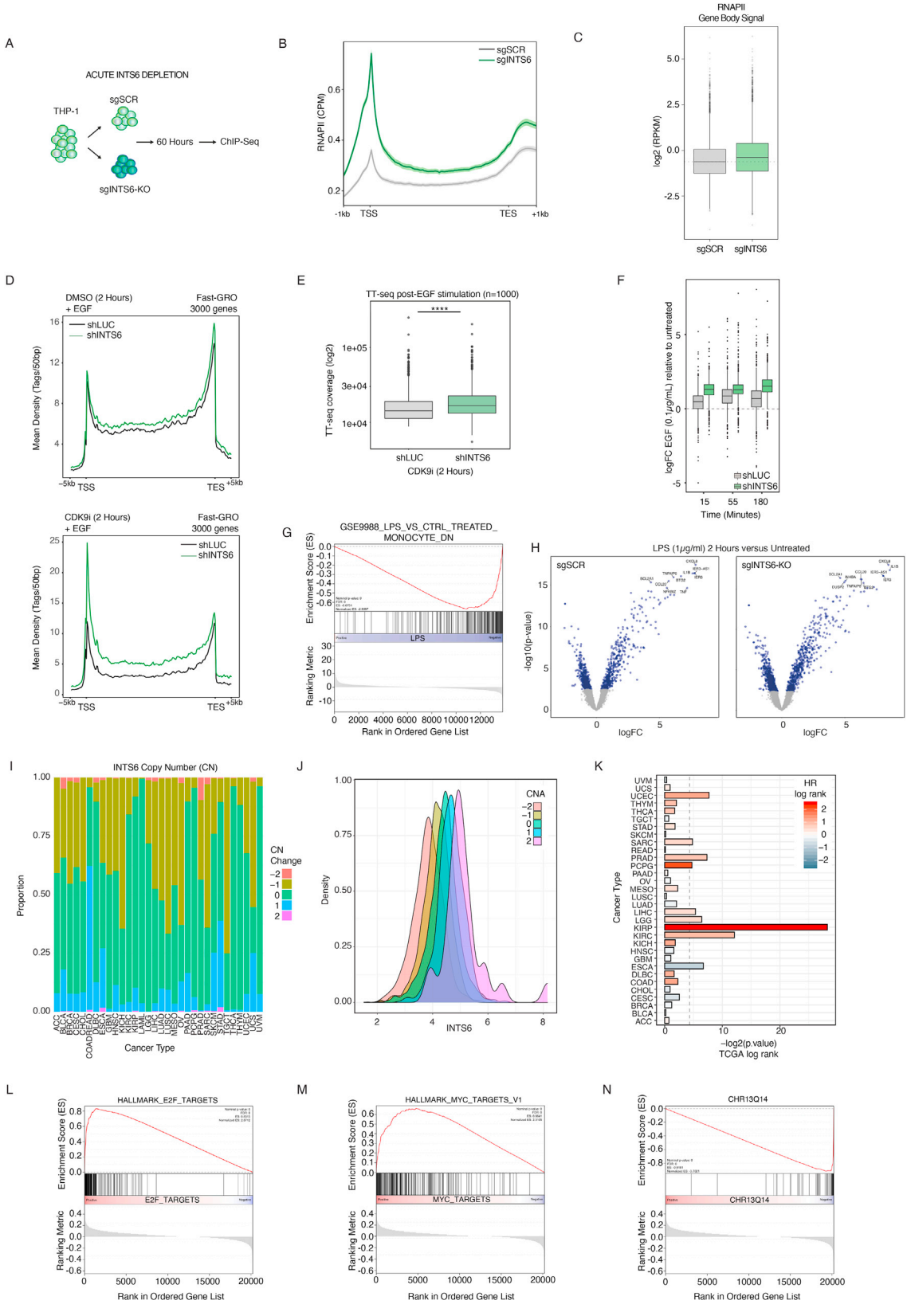

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Figure S6. The INTS6/PP2A axis fine-tunes acute transcriptional responses, related to Figure 6

A. Overview of acute INTS6 depletion; ChIP-seq for RNAPII was performed on THP-1 cells 60 hours following electroporation with recombinant Cas9 protein and SCR or *INTS6* targeting sgRNAs. B. Average ChIP-seq profile and C. gene body signal for RNAPII in THP-1 cells at 60 hours post-electroporation with Cas9 and SCR or *INTS6* sgRNAs. D. Average Fast-GRO signal for highly expressed genes (n = 2989) in shLUC and shINTS6 infected HeLa cells treated with EGF (0.1μg/mL; 15 minutes) and DMSO (top) or CDK9i (bottom) (AZ5576, 300nM) for 2 hours. E. TT-seq coverage in HeLa shLUC or shINTS6 cells for highest expressed genes (n = 990) after EGF treatment (0.1μg/mL; 15 minutes). F. Expression of EGF-induced genes (n = 50) in HeLa shLUC or shINTS6 cells treated with CDK9i (AZ5576, 300nM) for 2 hours followed by treatment with 0.1 μg/mL EGF for the indicated time-points. G. GSEA profile of THP-1-Cas sgSCR cells treated with LPS (2 hours versus 0 hours). H. Volcano plot of differentially expressed genes in THP-1-Cas9 sgSCR and sgINTS6-KO cells treated with LPS for 2 hours (relative to untreated control). I. Proportion of *INTS6* copy number (CN) gains and losses across indicated cancer types (TCGA). J. *INTS6* expression levels across cancer types (S4L) subset by copy number change (TCGA). K. Significance and hazard ratio (HR) ranking of INTS6 loss/mutation across indicated cancer types (TCGA). Gene set enrichment analysis of L. E2F and M. MYC targets as well as **N.** chromosome 13q14 in cancer cells exhibiting significant *INTS6* loss/mutation. Figure E was analyzed by unpaired, two-sided Student's t test, ****$p < 0.0001$.

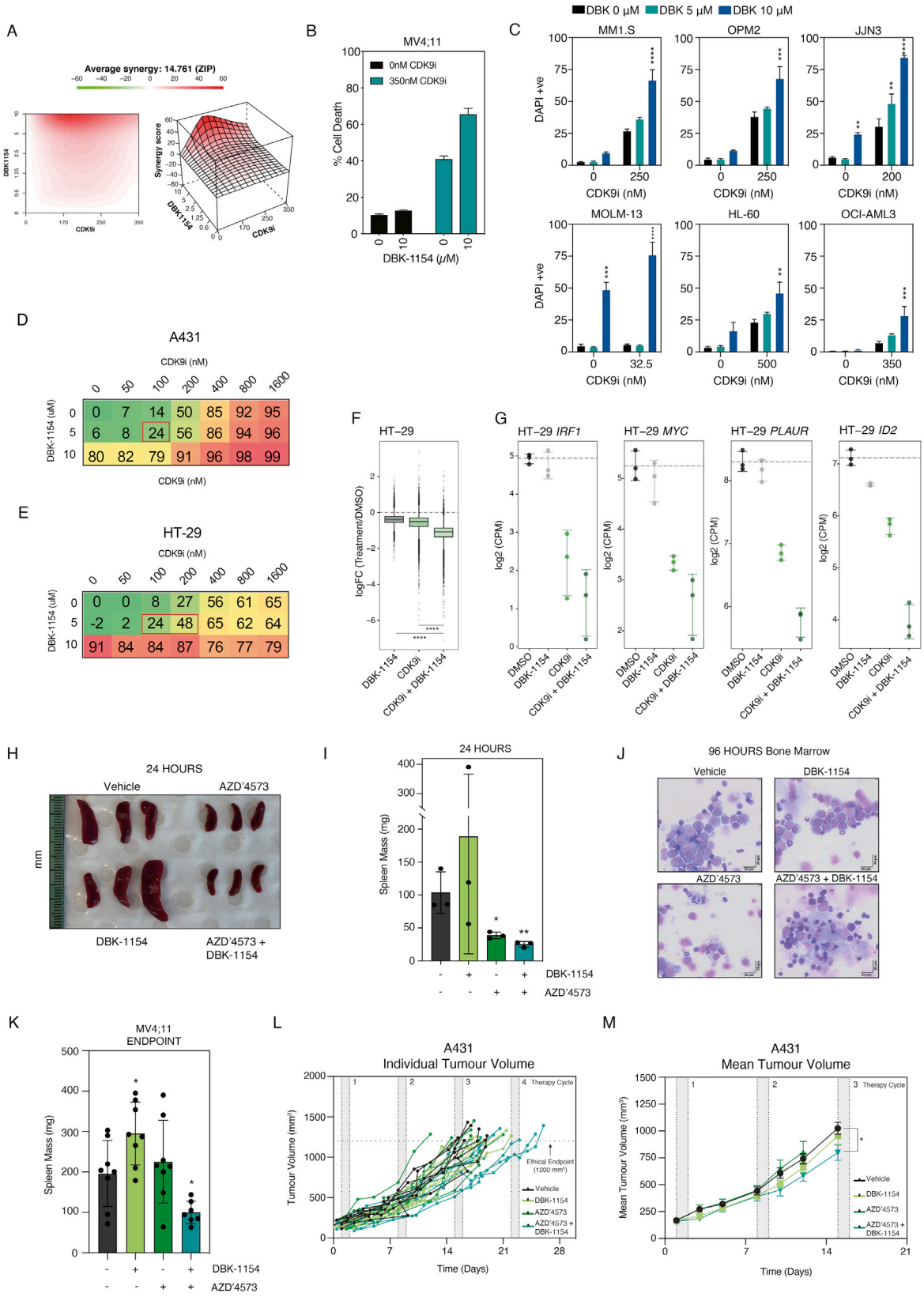

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Figure S7. Therapeutic and molecular synergy between PP2A agonist and CDK9i, related to Figure 7

A. Synergy for combined CDK9i and DBK-1154 treatment of THP-1-Cas9 sgSCR cells was determined using the SynergyFinder computational package and the ZIP synergy index and is denoted as regions of red in the graphs. B. Annexin-V analysis of MV4;11 cells treated as indicated for 72 hours. C. DAPI cell death analysis of indicated multiple myeloma (top) and AML (bottom) cell lines treated as indicated for 72 hours. Heatmaps of *in vitro* cell death in D. A431 and E. HT-29 cells treated with as indicated with AZ5576 and/or DBK-1154. Potential synergistic/additive interactions are highlighted by the red box. F. Log fold change relative to DMSO control of 3′RNA-seq signal (log2(CPM)) across all expressed genes for HT-29 cells treated as indicated for 2 hours. G. 3′-RNA-seq signal for *IRF1*, *MYC*, *PLAUR* and *ID2* transcripts in HT-29 cells treated with AZ-5576 (200nM), DBK-1154 (5 μM) or combination for 2 hours. H. Spleens and (I). sple*en masse*s from mice treated as indicated at 24 hours post-therapy. Scale bar in millimeters (mm). J. May-Grünwald Giemsa histological staining of bone marrow cytospins from mice treated as indicated 96 hours post therapy. Scale bar represents 20 μm. K. Sple*en masse*s of mice with disseminated MV4;11 leukemia at endpoint fof the long-term survival experiment. L. Individual tumor and M. mean tumor volumes of A431-transplanted mice treated as indicated. The dotted horizontal line indicates the ethical endpoint based on tumor volume (1200 mm^3). Figures B and C represents the mean ± SD of two independent experiments each containing two technical duplicates. Figure D and E respresents the mean of three independent experiments each containing three technical replicates. Figures F and G represent three biological replicates for each treatment condition. Figure I (mean ± SD) and J represents three mice per treatment group while Figures L and M represent seven mice per group. Figure K represents eight mice per group. Figure C was analyzed by 2-way ANOVA, and Figures F, I, K and M were analyzed by unpaired Student's t test, *$p < 0.05$ **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.