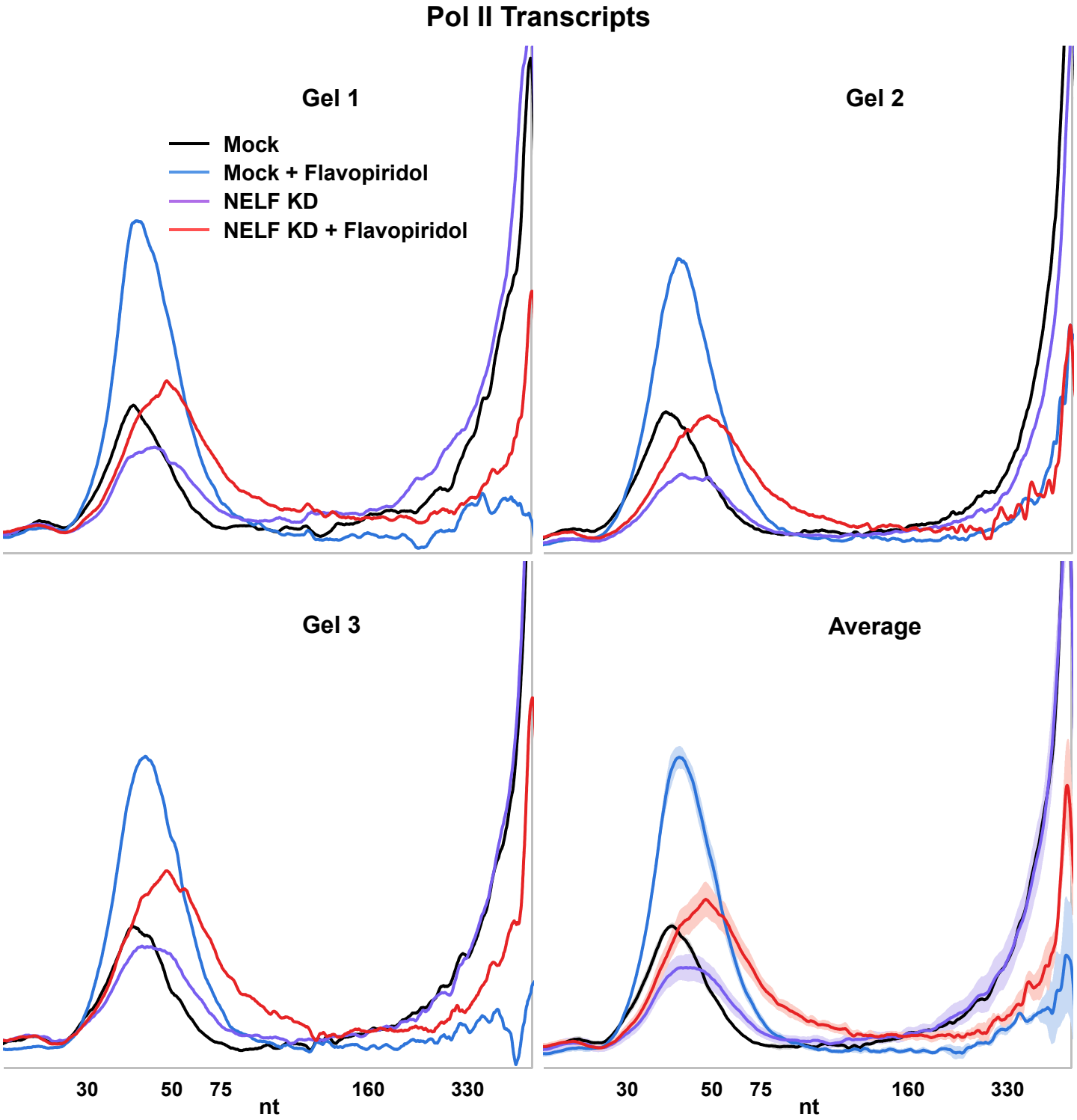

Figure S1. Reproducibility of the nuclear walk-on assay, related to Figure 1.
Adherent HeLa cells were transfected with 1.5 nM NELF-A siRNA (NELF KD) or lipid reagent only (Mock) for 48 h. Cells were treated with 0.1% DMSO or 1 μM flavopiridol 1 h prior to isolation of nuclei. Nuclear walk-ons were performed in triplicate using a 6 min α-^{32}P-CTP pulse ± 2 μg/ml α-amanitin. Pol II nascent transcript profiles were normalized to the area under the Mock curve in each gel and a 50 nt α-amanitin peak was used to adjust x-axes across all three gels. These triplicate profiles were then averaged and the standard error of the mean was plotted for all points. All plots have the same vertical scale.

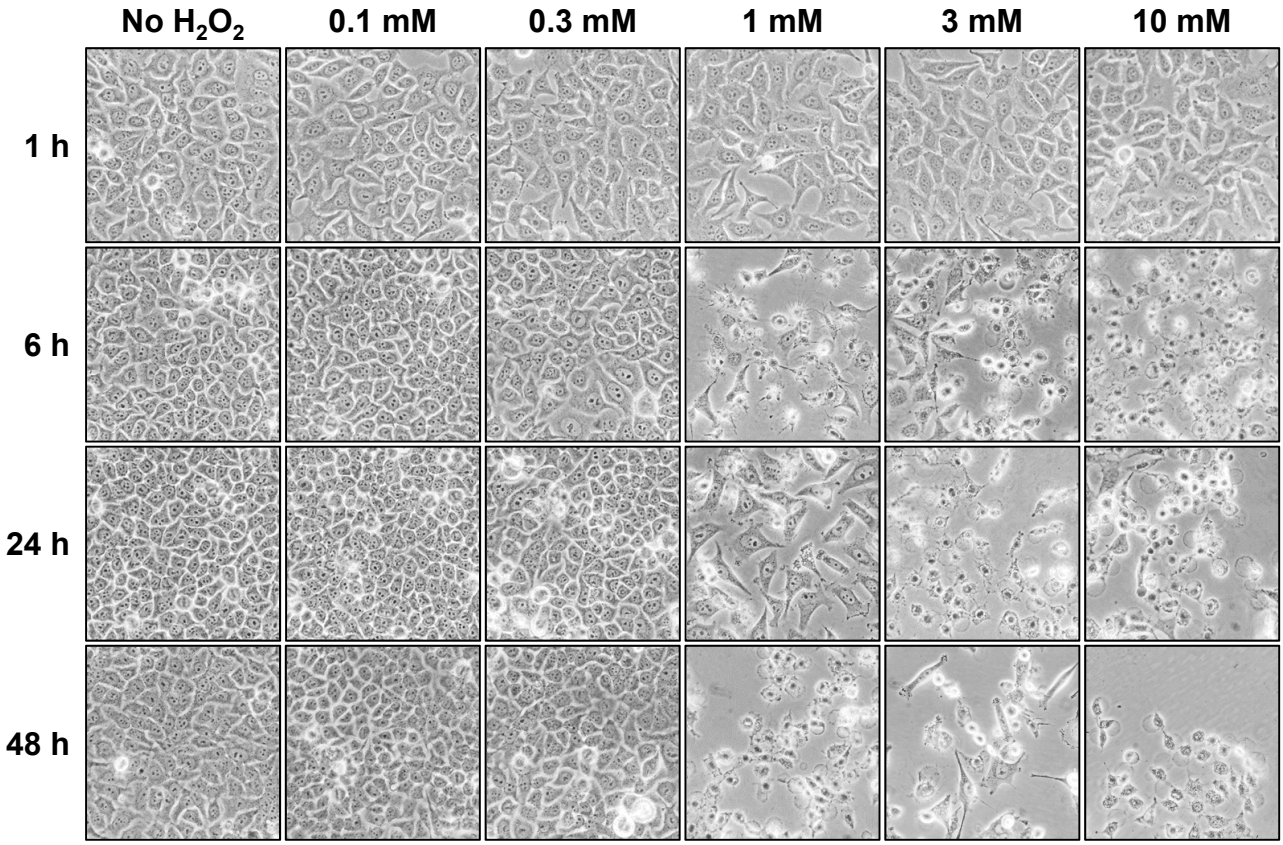

Figure S2. The effects of increasing H_2O_2 on adherent HeLa cell morphology, related to Figure 2. Adherent HeLa cells were seeded in 6-well plates and were treated at 80% confluency with indicated concentrations of H_2O_2. Cells were monitored using phase-contrast microscopy and images were captured at indicated times.

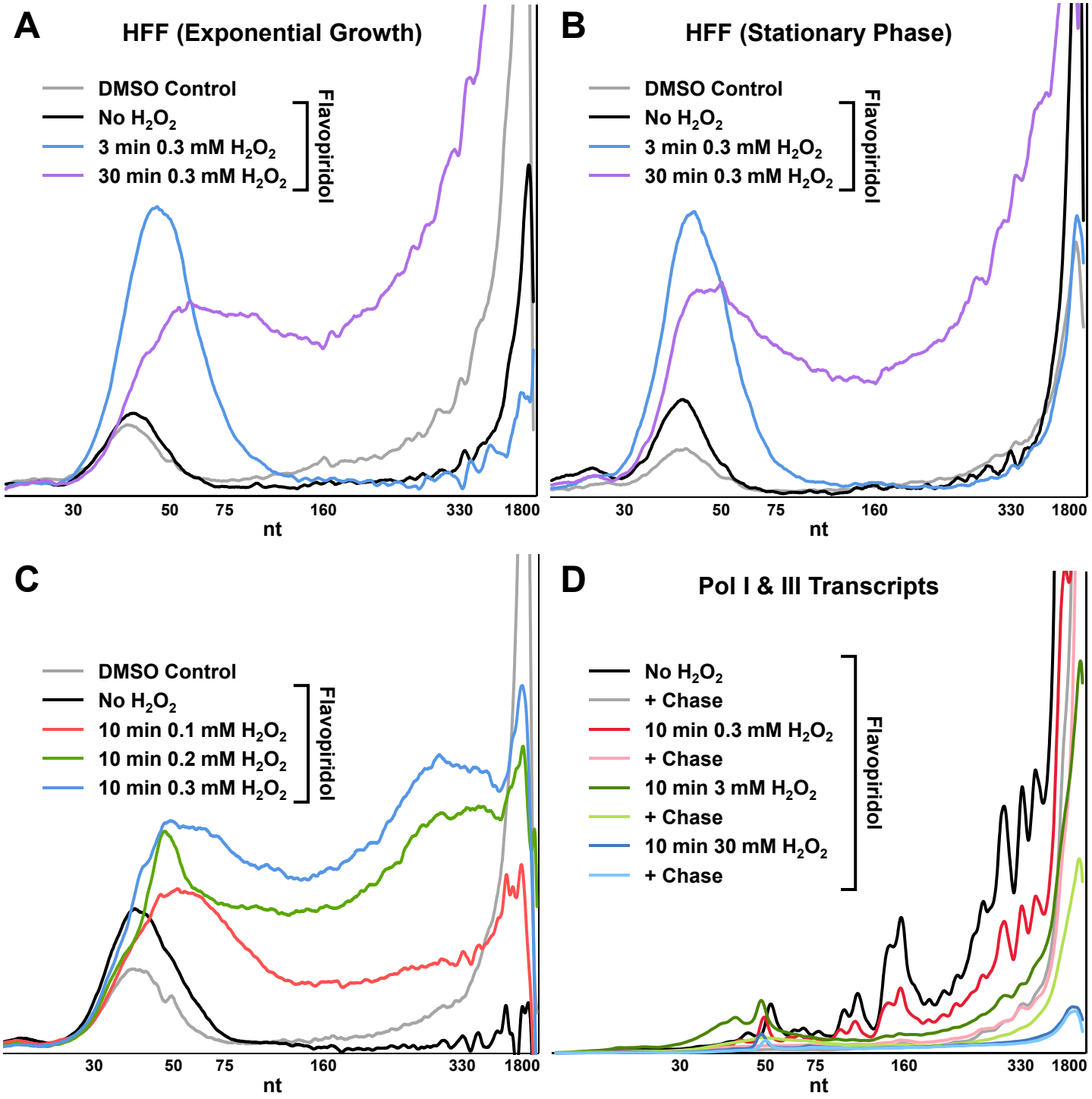

Figure S3. Additional effects of H_2O_2 on transcription, related to Figure 2.
(A) Amanitin-sensitive Pol II nascent transcript profiles from a nuclear walk-on using nuclei from 80% confluent, exponentially growing human foreskin fibroblasts (HFF) treated 1 h with 0.1% DMSO or 1 μM flavopiridol, with 0.3 mM H_2O_2 for the last 3 or 30 min.
(B) The experiment in (A), except HFF were grown at 100% confluency for 2 weeks prior to treatment.
(C) Amanitin-sensitive Pol II nascent transcript profiles from a nuclear walk-on using nuclei from adherent HeLa cells treated 1 h with 0.1% DMSO or 1 μM flavopiridol, with the indicated H_2O_2 for the last 10 min.
(D) Amanitin-insensitive Pol I and III nascent transcript profiles from a nuclear walk-on using nuclei from adherent HeLa cells treated 1 h with 1 μM flavopiridol. 0.3 mM, 3 mM, or 30 mM H_2O_2 was added during the last 10 min of flavopiridol treatment. After a 5 min α-^{32}P-CTP pulse, reactions were chased as indicated for 10 min with 500 μM cold ATP, UTP, GTP, and CTP.

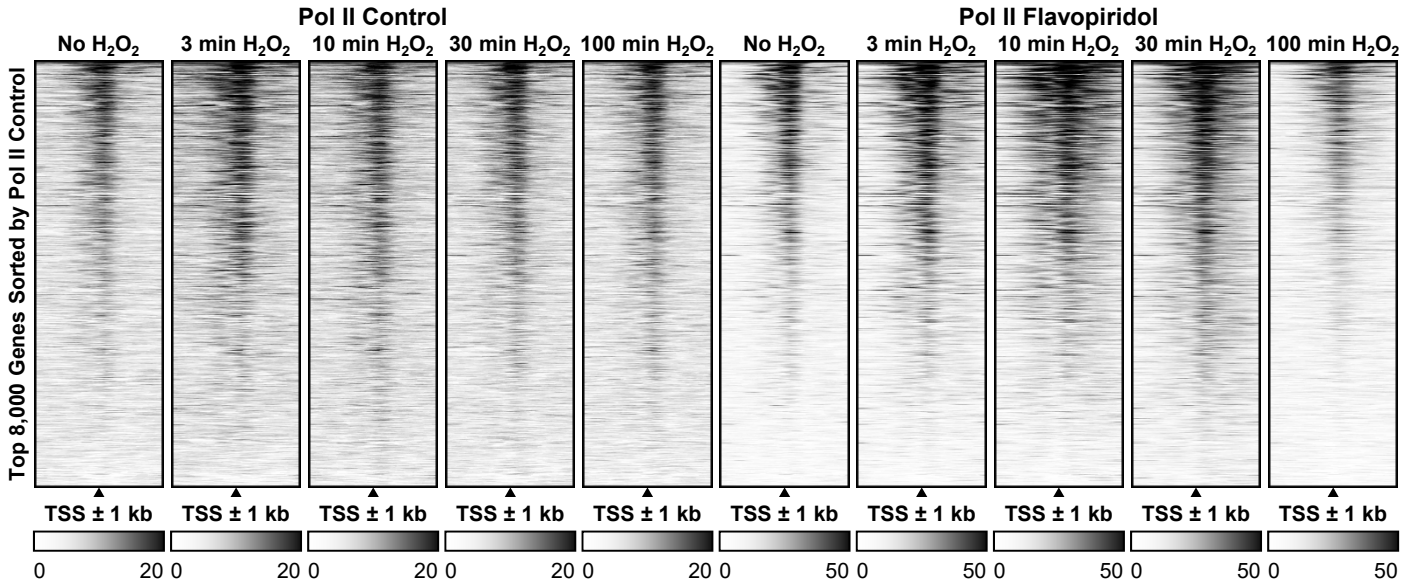

Figure S4. Heatmaps at top Pol II occupancy promoters after H_2O_2, related to Figure 3.
Pol II ChIP-Seq occupancies ± 1 kb around the top 8,000 EPDnew TSS with the highest median Pol II signal. Signals were depth-adjusted using the average number of mapped sequence reads in all ten samples. The heatmaps were sorted by TSS with decreasing Pol II occupancy in the no H_2O_2 control.

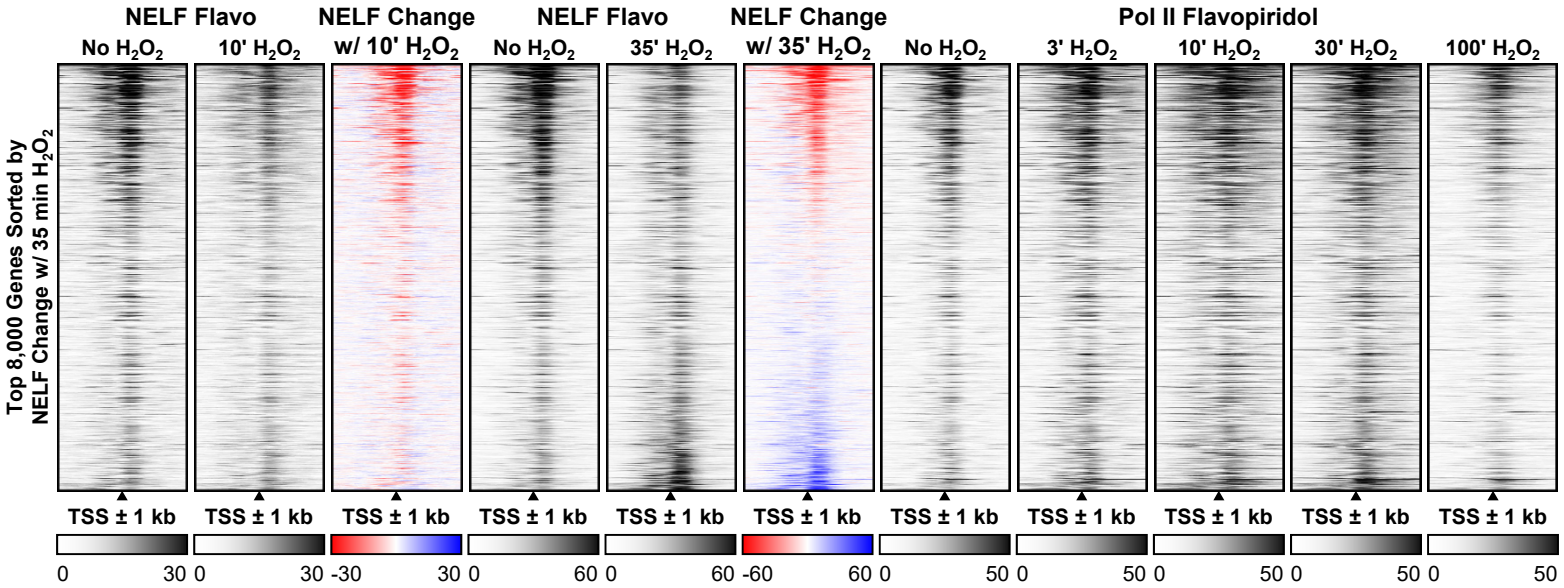

Figure S5. NELF is selectively lost from creeping elongation complexes over time after H_2O_2, related to Figure 4. Pol II and NELF ChIP-Seq occupancies ± 1 kb around the top 8,000 EPDnew TSS with the highest median Pol II signal. The heatmaps were sorted by the change in median NELF occupancy after 35 min 0.3 mM H_2O_2 treatment. Data is from three ChIP-Seq experiments: NELF Flavo ± 10 min H_2O_2, NELF Flavo ± 35 min H_2O_2, and the Pol II Flavopiridol H_2O_2 time course. Signals within each ChIP-Seq experiment were depth-adjusted using the average number of mapped sequence reads to enable occupancy comparisons within each experiment. Occupancy comparisons of samples between experiments are not valid.

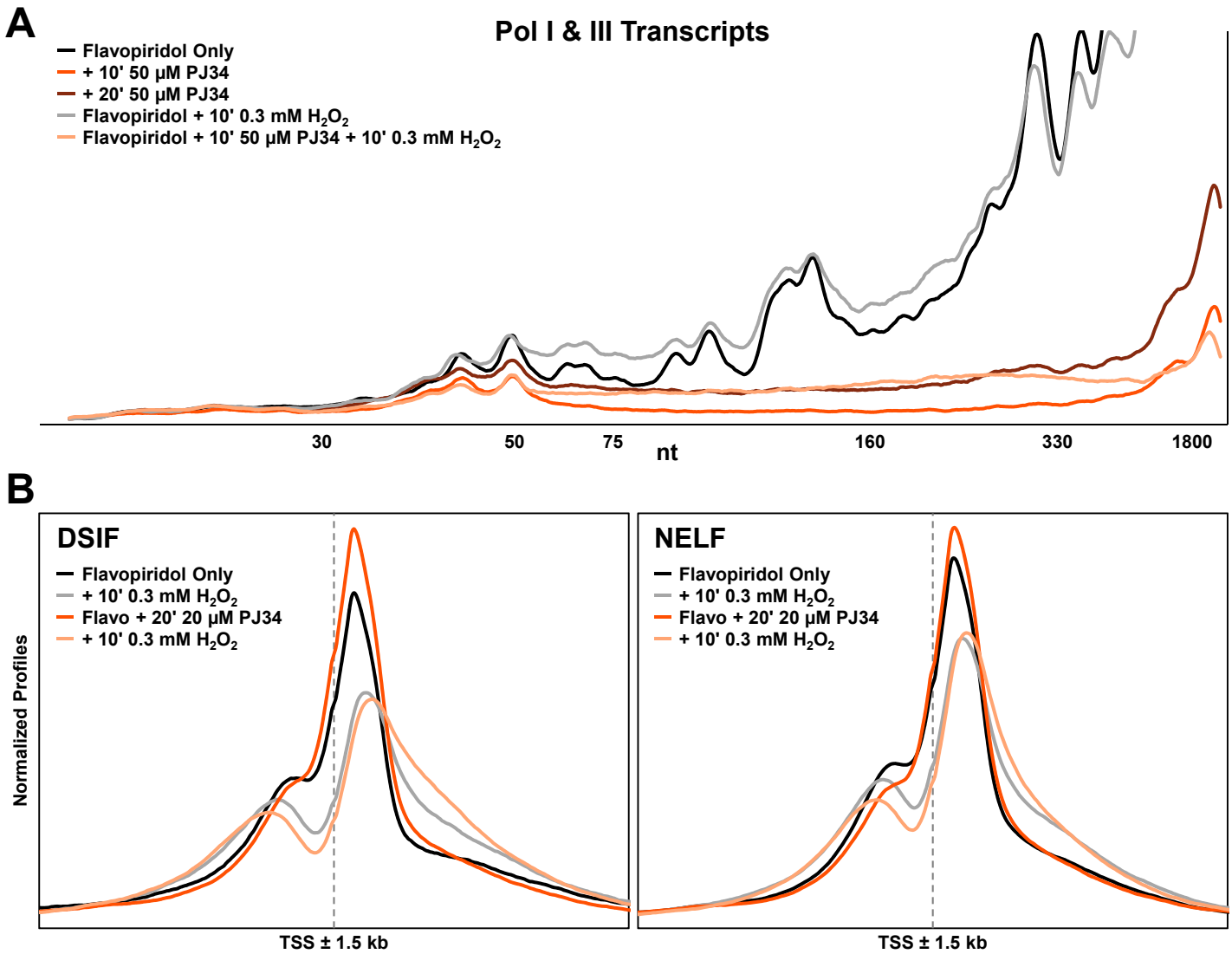

Figure S6. Effects of PJ34 on Pol I, Pol III, DSIF, and NELF, related to Figure 5.
(A) Amanitin-insensitive Pol I and III nascent transcript profiles from a nuclear walk-on using nuclei from adherent HeLa cells treated 50 min with 1 μM flavopiridol, then 10 min with 0.1% DMSO (Flavopiridol Only), 10 min with 50 μM PJ34, or 20 min with 50 μM PJ34. Cells were additionally treated 10 min with 0.3 mM H_2O_2 as indicated. DMSO was 0.2% in all conditions. (B) The experiment in (A), except HFF were grown at 100% confluency for 2 weeks prior to treatment.
(B) Average normalized DSIF ChIP-Seq occupancies ± 1.5 kb around 17,592 EPDnew TSS. Suspension HeLa cells were treated 40 min with 1 μM flavopiridol, then 20 min with either 0.05% DMSO (Flavopiridol Only) or 20 μM PJ34. Cells were additionally treated 10 min with 0.3 mM H_2O_2 as indicated. The vertical axis represents depth-adjusted signals that were background-subtracted and normalized using a window ± 10 kb around genes.
(C) The experiment in (B) except with average normalized NELF ChIP-Seq occupancies.