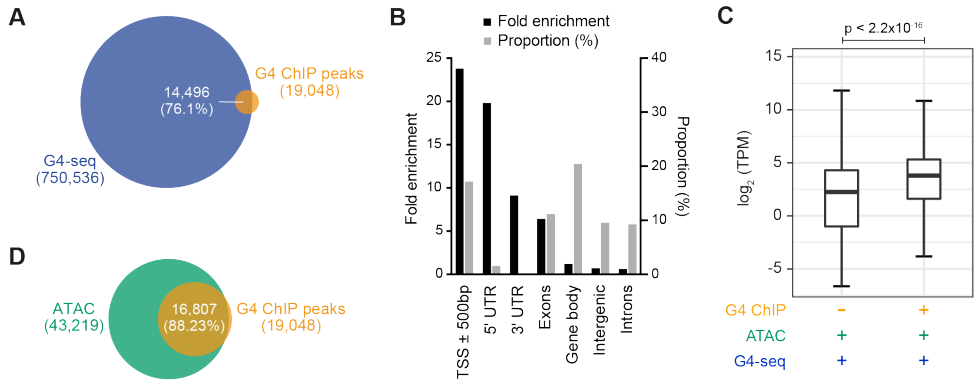


**Fig. S1: Endogenous G4 landscape in human K562 cells. (A)** Overlap of endogenous G4s (G4 ChIP peaks) found in K562 cells with sequence motifs that can fold into G4 structures *in vitro* (defined as G4-seq sites, see *ref 15*). **(B)** Enrichment and proportion of endogenous G4s by genomic feature. TSS, transcription start site; UTR, untranslated region. **(C)** Distribution of mRNA levels for genes with promoters in open chromatin (ATAC +) with an endogenous G4 (G4 ChIP peaks) or a G4 sequence motif that does not fold into a G4 (G4-seq sites) in K562 cells. $p < 2.2 \times 10^{-16}$, Wilcoxon test. TPM, transcripts per million. **(D)** Overlap of endogenous G4s (G4 ChIP-seq peaks) and open chromatin regions defined by ATAC-seq.

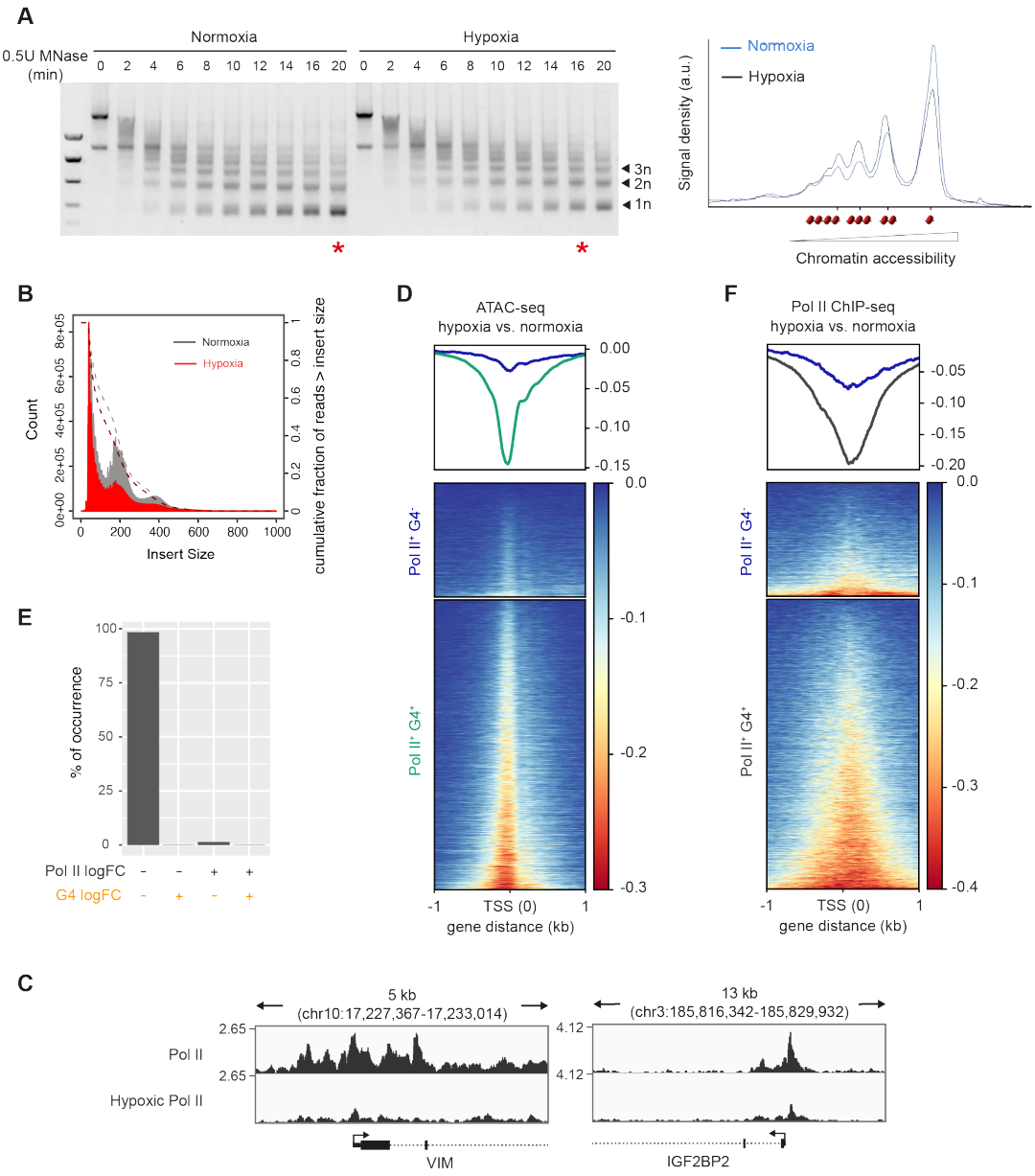


**Fig. S2: Induction of hypoxia decreases chromatin accessibility leading to G4 loss. (A)** Nucleosome distributions for cells cultured under normoxic (left) or hypoxic (right) conditions as assessed by micrococcal nuclease (MNase) digestion. 1n = mono-nucleosomes, 2n = di-nucleosomes, 3n = tri-nucleosomes. Asterisks indicate samples quantified by the graph on the right. Normoxia refers to cells cultured in 21% $O_2$ and hypoxia refers to cells exposed to 1% $O_2$ for 1 h. **(B)** Read counts and size distribution for different ATAC-seq fragment sizes for hypoxic (grey) and normoxic (red) chromatin. **(C)** Example genomic browser view showing

significantly reduced Pol II occupancy for cells cultured under hypoxic versus normoxic conditions. Genomic coordinates for *VIM* and *IGF2BP2* are indicated. **(D, F)** Signal differences in ATAC-seq signal or Pol II occupancy for promoters of active genes with or without a G4 (Pol $II^+$ $G4^-$ or Pol $II^+$ $G4^-$, respectively) under hypoxia or normoxia. Top panel, metagene plot of the normalised ATAC signal difference or Pol II ChIP-signal difference centered at the TSS in hypoxic versus normoxic condition. Bottom panel, data plotted for individual loci and represented by a heatmap plot. **(E)** Co-occurrence of loss of G4s and Pol II occupancy at promoters under hypoxic conditions. Genomic overlap of promoter G4s that have increases or reductions in G4 signal (G4 logFC + or – respectively), with increases or reductions in Pol II (Pol II logFC + or – respectively) at the same site graphed as percentage of co-occurrence.

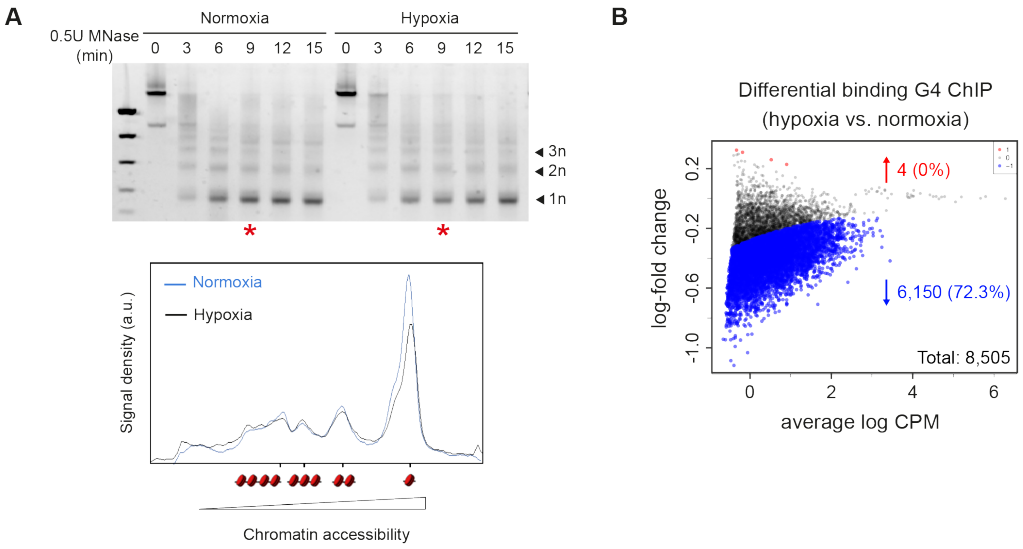


**Fig. S3: Hypoxia-induced chromatin compaction results in reduction of promoter G4 folding in U2OS cells. (A)** Nucleosome distributions for U2OS cells cultured under normoxic (left) or hypoxic (right) conditions as assessed by micrococcal nuclease (MNase) digestion. 1n = mono-nucleosomes, 2n = di-nucleosomes, 3n = tri-nucleosomes. Asterisks indicate samples quantified by the graph on the bottom. Normoxia refers to cells cultured in 21% $O_2$ and hypoxia refers to cells exposed to 1% $O_2$ for 1 h. **(B)** MA plot showing fold change in G4 ChIP-seq signal following hypoxia at promoters. Blue and red, sites with significantly reduced or increase signal respectively ($p < 0.05$). CPM, read count per million.

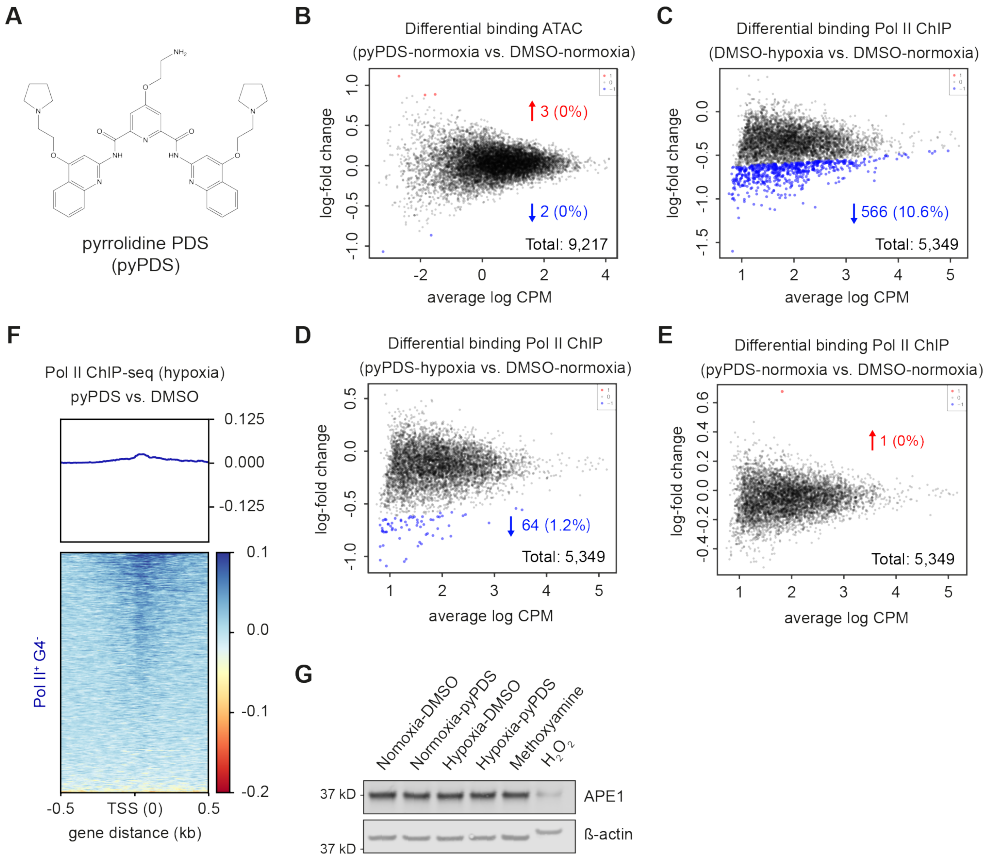


**Fig. S4: Response of endogenous G4s and Pol II following pre-treatment with G4-stablising ligands before and after hypoxia induction. (A)** Chemical structure of the G4-stabilising ligand, pyrrolidine PDS (pyPDS). **(B)** MA plot showing fold-change in ATAC-seq signal at promoters between pyPDS- and DMSO-treated cells under normoxic conditions. Significant increases or decreases ($p < 0.05$) in G4 ChIP-seq signal in pyPDS-treated cells relative to DMSO-treated cells are indicated in red and blue respectively. CPM, read count per million. **(C)** As in panel B but for changes in Pol II ChIP-seq signal ($p < 0.05$) between hypoxic and normoxic cells treated with control DMSO. **(D)** As in panel B but for changes in Pol II ChIP-seq signal for cells treated with pyPDS and subjected to hypoxia compared to DMSO-treated cells under normoxia. **(E)** As in panel B but for changes in Pol II ChIP-seq signal for cells treated with pyPDS or DMSO under normoxia. **(F)** Signal differences in Pol II occupancy for non-G4-marked promoters of active genes (Pol $II^+$ $G4^-$) under hypoxic condition treated

with DMSO or pyPDS. Top panel, metagene plot of Pol II signal centered at the TSS showing the signal difference between DMSO- and pyPDS-treated hypoxic cells. Bottom panel, data plotted for individual loci and represented by a heatmap plot. **(G)** Western blotting for APE1 under DMSO- or pyPDS-treated hypoxic and normoxic K562 cells. Methoxyamine and $H_2O_2$ treated cells represent negative and positive controls for APE1 respectively. Loading control, ß-actin.

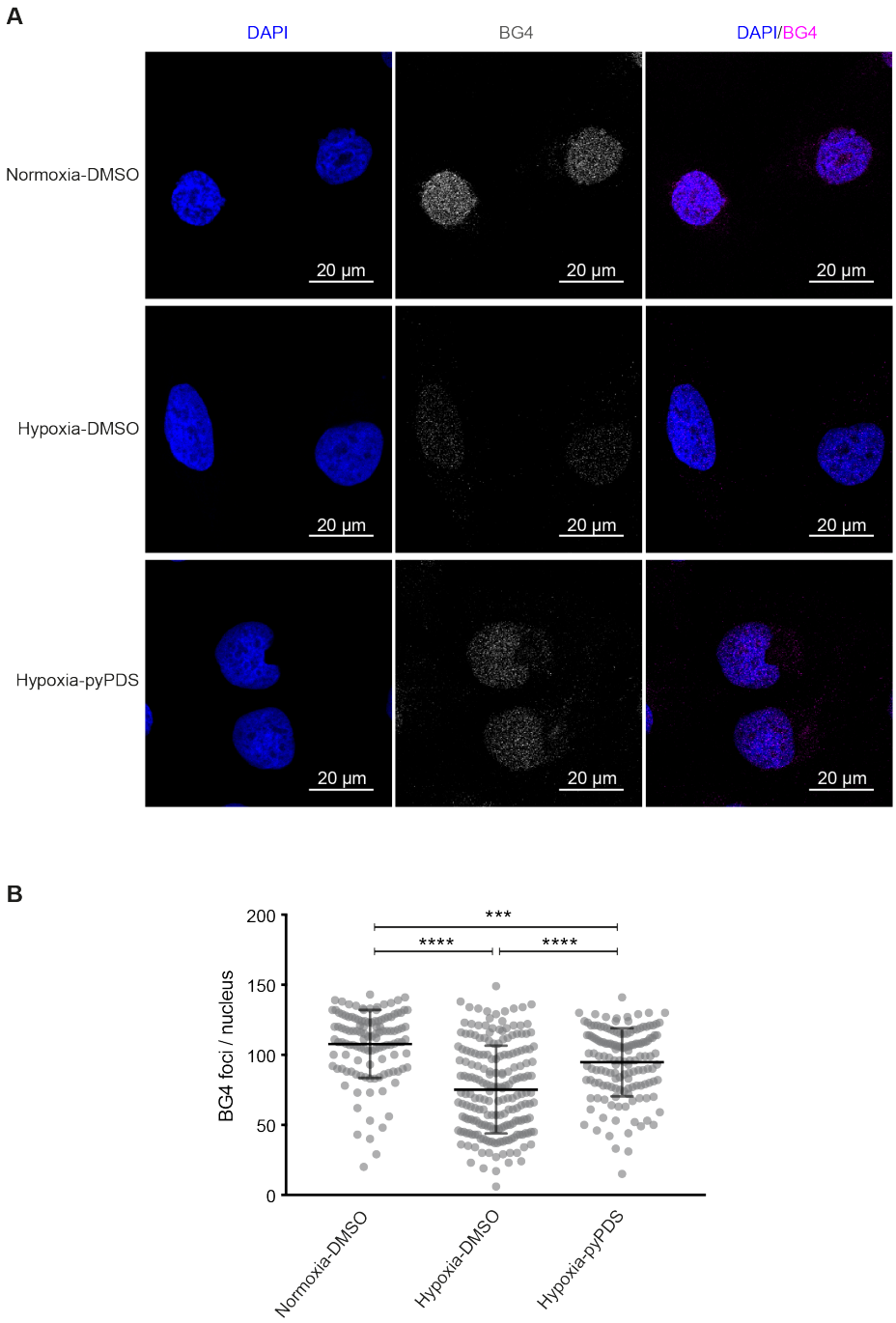


**Fig. S5: BG4 immunofluorescence (IF) staining for G4s in DMSO- or pyPDS-treated cells upon hypoxia treatment.** U2OS cells were fixed in paraformaldehyde, RNase A treated and stained with BG4 antibody. Confocal images were captured using a Leica SP5 microscope. **(A)** BG4 staining (grey/red) in the nuclei stained by DAPI (blue) of DMSO- or pyPDS-treated U2OS cells upon hypoxia treatment. DMSO-treated normoxic U2OS cells are controls. Scale bar = 20 µm. **(B)** Quantification of BG4 nuclear foci in individual cells. Each dot represents a

single nucleus. 150-200 nuclei were counted per condition and a one-way ANOVA test was performed for each condition across the mean values. Error bars represent the standard deviation (s.d.). ***: $p < 0.001$; ****: $p < 0.0001$.