Figure Legends:

Figure 1:

Western blot analysis shows the efficient separation of cell fractions and reveals that ≥99% of elongating Pol II (C-terminal domain (CTD) Ser2- and Ser5-phosphorylated forms, and the general CTD hyper-phosphorylated form (IIO) of Pol II) is captured in the chromatin fraction. Proteins with defined subcellular localization were also probed (Chromatin marker, Histone 2B; nucleoplasm marker, U1 snRNP70; cytoplasm marker, GAPDH). Data of a representative Western blot experiment is shown, using subcellular lysates generated from the same batch of CRL2097 cells. Sample volumes have been adjusted so that Western blot signals of the subcellular fractions can be compared. Further details are given in the Methods section.

Figure 2:

Western blot analysis reveals that ≥99% of the CTD hyper-phosphorylated form of Pol II (IIO) is present in the chromatin fraction. The CTD hypo-phosphorylated form of Pol II (IIA) is captured in all cellular fractions. Subcellular lysates were generated from the same batch of CRL2097 cells and probed with the F-12 antibody (Santa Cruz Biotechnology) that is directed against the N-terminal region of Rpb1, the largest subunit of Pol II. Sample volumes have been adjusted so that Western blot signals of the subcellular fractions can be compared. The percentage at the bottom is the amount of CTD hyper- and hypo-phosphorylated forms of Pol II in the different cellular fractions (as determined by image quantification). Further details are given in the Methods section.