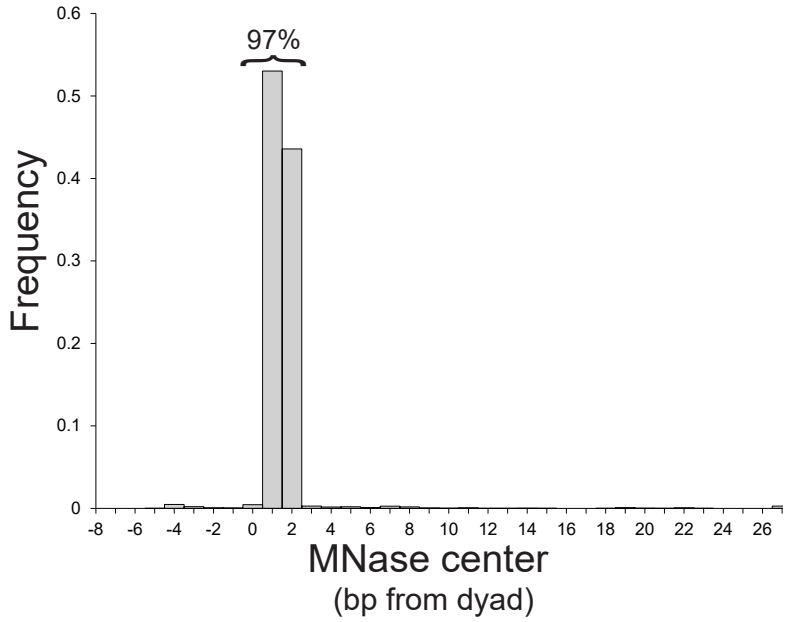
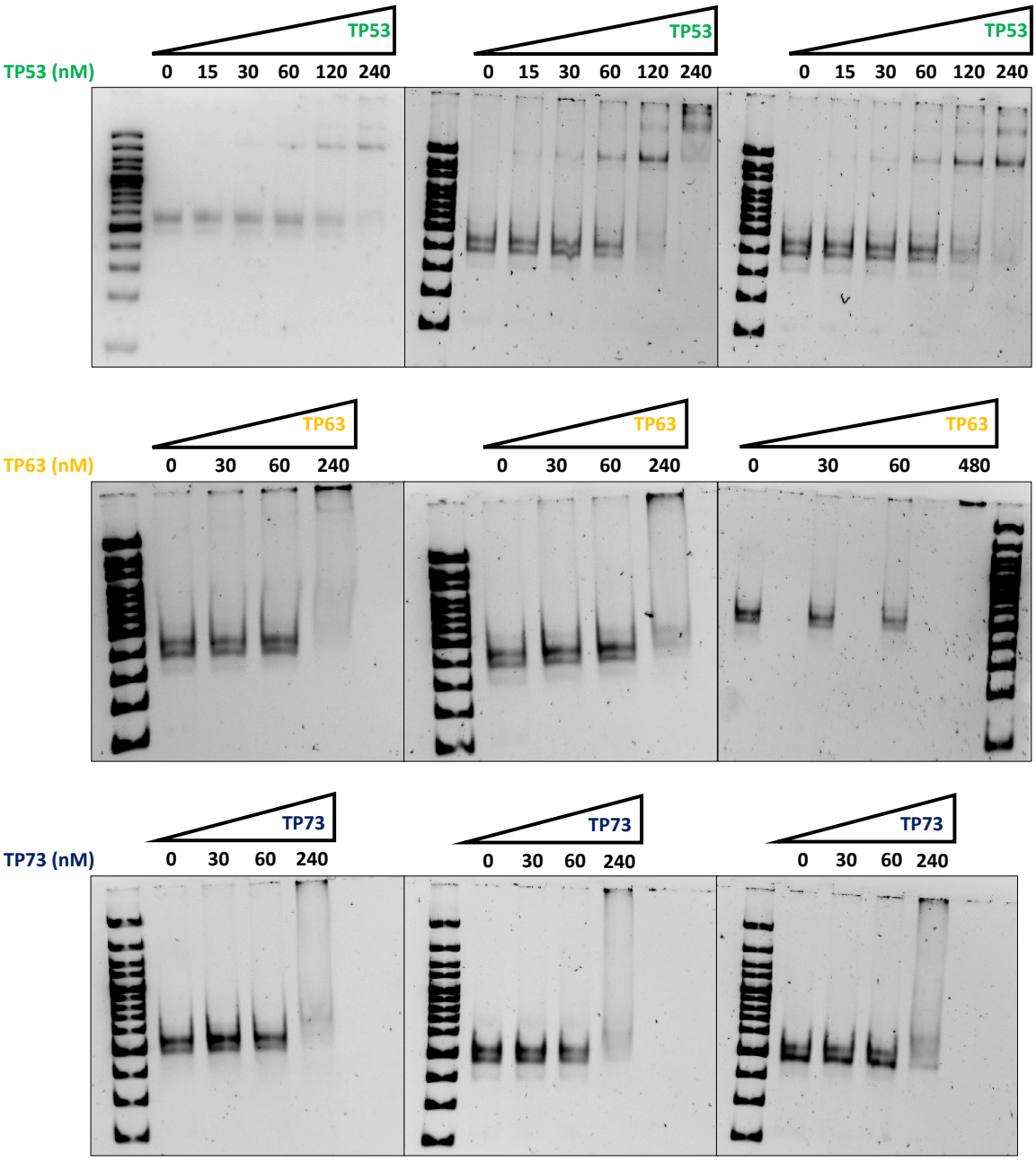
Supplemental Tables and Figures

Supplemental Table 1. TP53-family binding sites in nucleosome library

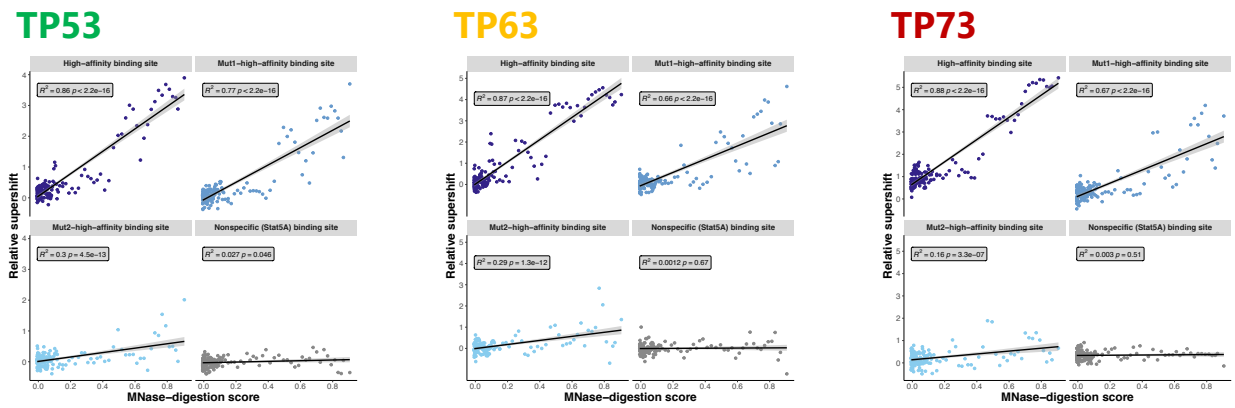
Name	**Sequence**
CDKN1A-promoter binding site	AGACTGGGCATGTCTGGGCA
High-affinity binding site	GGGCATGTCCGGGCATGTCC
Mut1-high-affinity binding site	GGGCATTTCCGGGCATGTCC
Mut2-high-affinity binding site	GGGCATTTCCGGGCATTTCC
MDR1-promoter binding site	GGGCAGGAACAGCGCCGGGGCGTGGGCTGAGCA
CHMP4C-promoter binding site	AAACAAGCCCAGTAGCAGCAGCTGCTCCGAGCTTGCCC
PUMA-promoter binding site	CTGCAAGTCCTGACTTGTCC
PPN1-promoter binding-site A	TGGCATGCTTCGGCTTGCTA
PPN2-promoter binding-site B	TGGCATGAGGCGTCTTGATA
Nonspecific (Stat5A) binding site	GTTTCTTCCTGAGAAGTACC



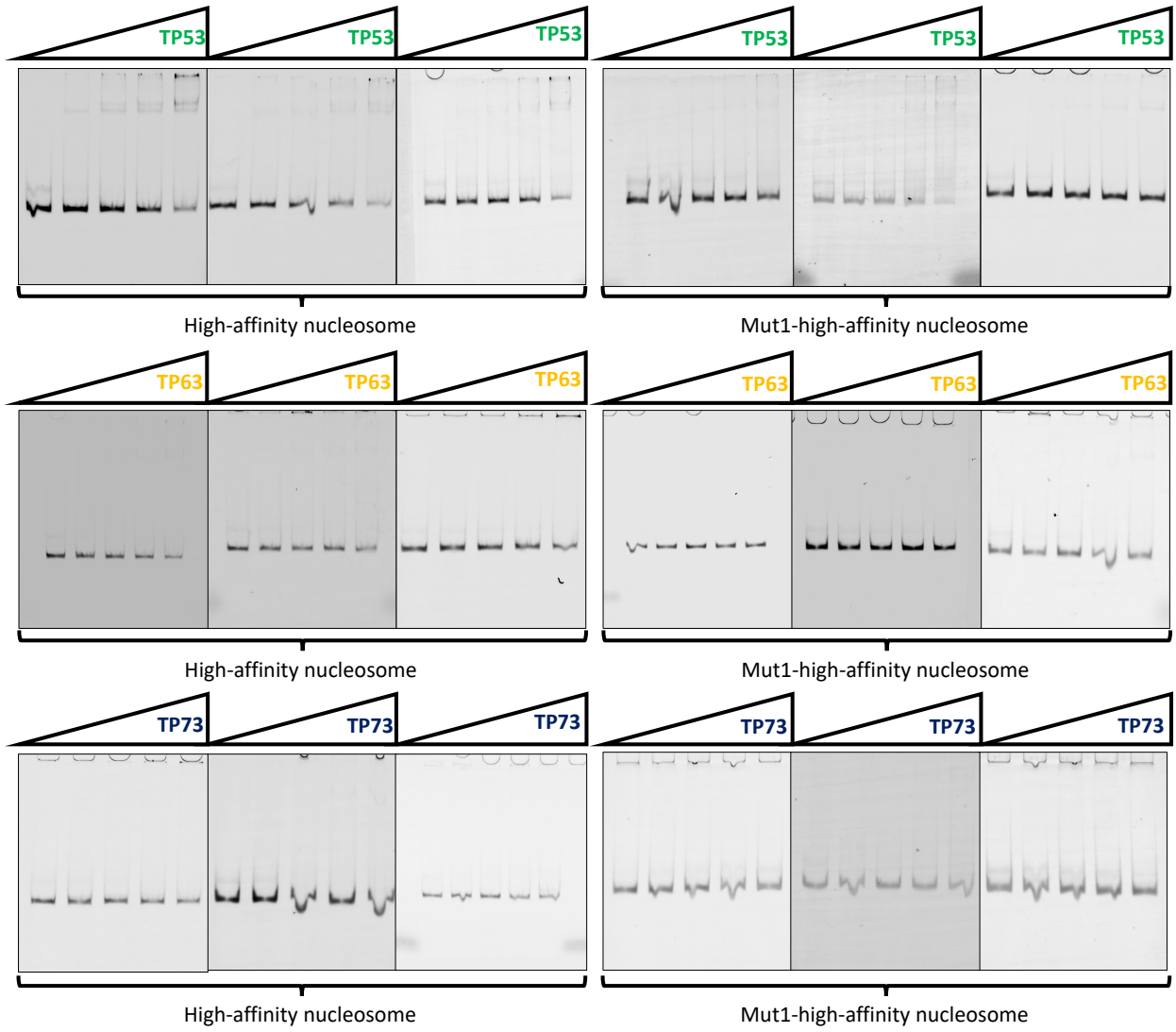
Supplemental Figure 1. MNase-defined centers of nucleosomes in the Pioneer-seq library. MNase-seq was performed on the Pioneer-seq library of nucleosomes. Following MNase digestion and sequencing, nucleosome-sized fragments (146-148 bp) were analyzed to determine their central positions. 97% of these central positions were within 2 bp of the dyad identified in the original Widom-601 nucleosome crystal structure (Luger et al. 1997).



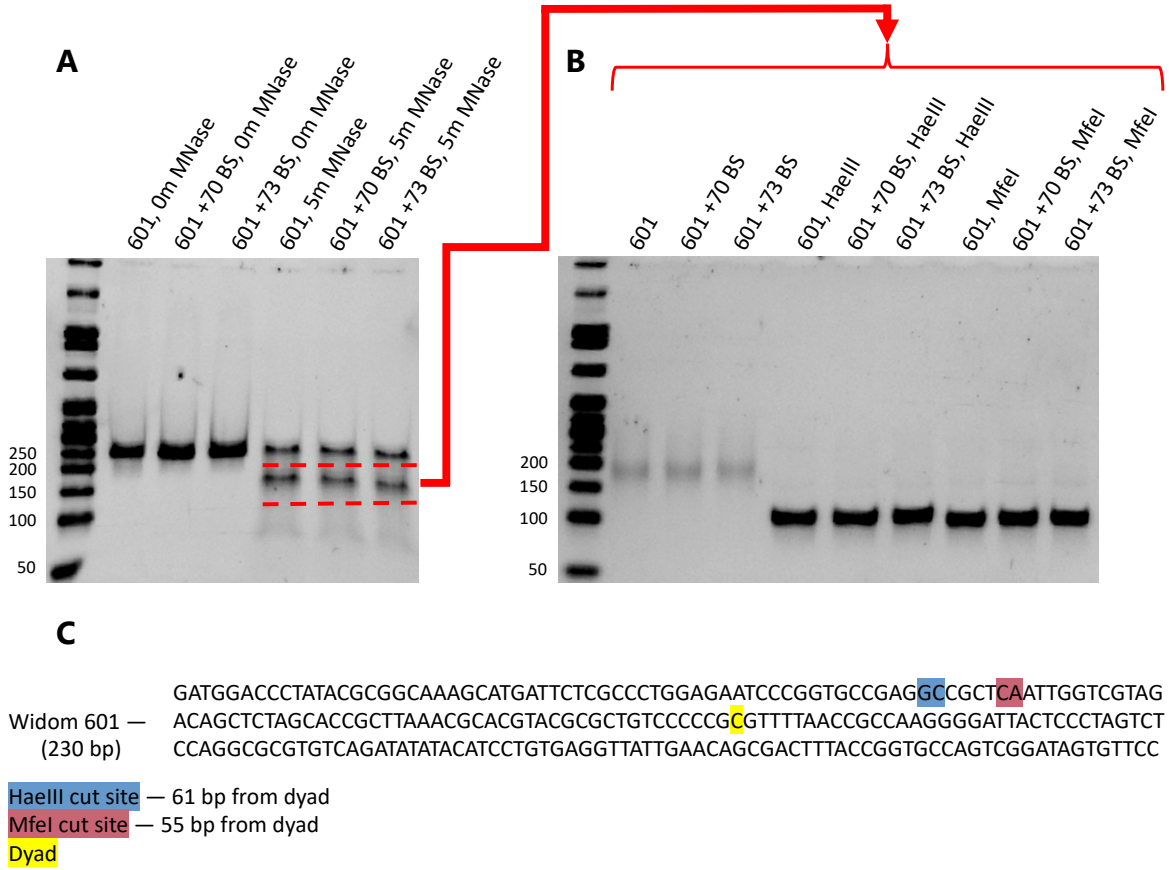
Supplemental Figure 2. Gel-shift assays of TP53, TP63, and TP73 with Pioneer-seq nucleosome library. Each protein was incubated with 30 nM Pioneer-seq nucleosome library. The concentration of protein added to each lane is indicated above the lane.



Supplemental Figure 3. Correlating the binding strength of TP53, TP63, or TP73 to three TP53-family binding sites and one nonspecific (Stat5A) binding site with the nucleosomal accessibility of these binding sites. TP53-family binding to each binding site is measured relative to TP53-family binding to nonspecific (non-TP53-family) binding sites and represented by relative-supershift values (**Eq. 1**). Nucleosomal accessibility of binding sites is measured by averaging the MNase-digestion scores of each base pair in the binding site. Shading around regression lines represents 95% confidence interval.



Supplemental Figure 4. Assessing the binding affinity between TP53, TP63, or TP73 and the +70 high-affinity nucleosome or the +70 mut1-high-affinity nucleosome. The concentrations of TP53-family member added in each lane were: 0 nM, 30 M, 60 M, 120 M, and 240 nM. 30 nM of +70 high-affinity nucleosome or +70 mut1-high-affinity nucleosome was added to each lane. Nucleosomal DNA was visualized via Cy5 labels.



Supplemental Figure 5. MNase digestion and restriction-enzyme treatment of nucleosomes from Pioneer-seq library. (**A**) 5% TBE gel showing Pioneer-seq-library nucleosomes—a Widom-601 nucleosome, a Widom-601 nucleosome with a TP53-family binding site 70 bp to the right of the Widom 601 dyad, or a Widom-601 nucleosome with this binding site 73 bp to the right of the dyad—digested with MNase for 0 or 5 min. (**B**) 5% TBE gel showing the purified nucleosome-sized fragments from (**A**) after further treatment with either no enzyme, HaeIII, or MfeI. (**C**) Sequence of the Widom-601 nucleosome, highlighting the two bases between which HaeIII cuts (blue), the two bases between which MfeI cuts (red), and the nucleosomal dyad (yellow) based on the crystal structure from Luger et al. 1997.