Electronic Supplementary Material (ESI) for Lab on a Chip

FIGURE S1

Flow schematic of HTChIP operation.

(i) Antibody-magnetic bead preparations are flowed into the immunoprecipitation ring.

(ii) The beads are trapped behind a sieve valve (dark grey), and after beads are loaded, sheared chromatin is introduced into the ring.

(iii) Ring is dead-end filled with chromatin and the ring is closed off from inputs and outputs for IP.

(iv) Valve-actuated peristaltic pumping mixes the antibody-conjugated beads with the chromatin very thoroughly for 2 hours, allowing antibody-target capture.

(v) After IP, wash buffer is introduced to push chromatin and beads out of the ring toward the outlet, where beads (now conjugated with antibodies that have captured the targets of interest) are trapped behind another sieve valve.

(vi) Beads stack into a column behind the sieve valve and wash buffer removes non-specific binding. After washing, beads are retrieved from the device and placed into microcentrifuge tubes for downstream PCR analysis.

FIGURE S1 (continued)

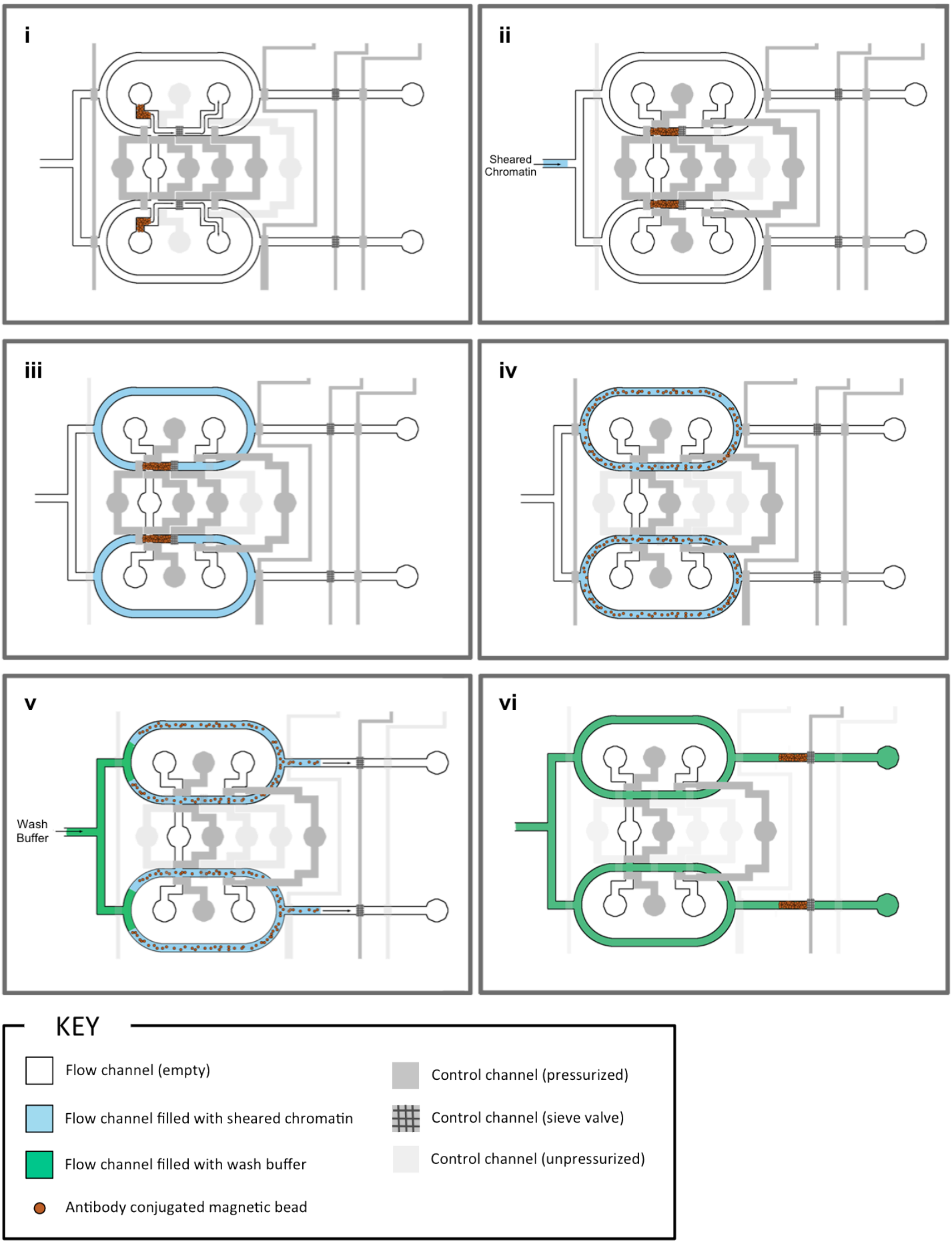

FIGURE S2

Bar graph comparing IP efficiency between IP performed in a microcentrifuge tube on the benchtop, and the microfluidic environment of HTChIP. ChIP was performed using anti-histone-3 (anti-PanH3), which targets all unmodified H3 histones. PanH3 levels are often used to normalization ChIP enrichments when comparing results across different experiments. Similar to Figure 3 in the main article, this figure shows that the HTChIP signal is higher than the signal from the microcentrifuge tube based method for each genomic loci tested. IgG is used as non-specific background control. Background noise is slightly higher for HTChIP than benchtop ChIP, but the magnitude of increase in background is lower than the magnitude of increase in signal from PanH3, resulting in net signal increase.

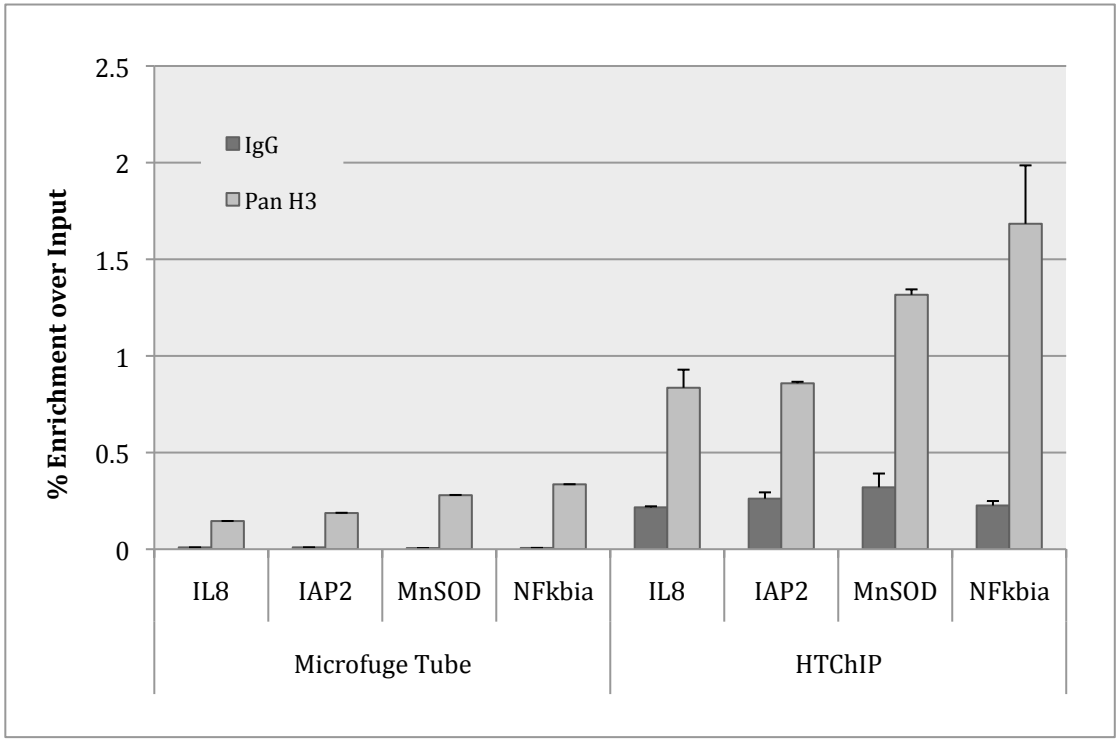

FIGURE S3

Bar graphs showing ChIP enrichment of H3K9Ac of HeLa cells with and without TNF-α stimulation, measured at NFκB target promoters. Left graphs show results obtained using HTChIP; right graphs show results obtained using conventional benchtop protocol. Each graph shows H3K9Ac enrichment at the loci of interest without TNF-α stimulation on the left, and with 1 hour of TNF-α treatment on the right. For benchtop ChIP, there is no significant difference between H3K9Ac levels at these gene promoters in untreated and treated cells; data from HTChIP leads to the same conclusion (Bonferroni corrected unpaired Student's T-test at α=0.01). Both sets of results concur with those previously reported in literature. In each ChIP, enrichments were normalized to PanH3 levels for comparability, and non-specific anti-IgG was used as background control.

These are results for two additional promoters to those already presented in Figure 4 of the main article.

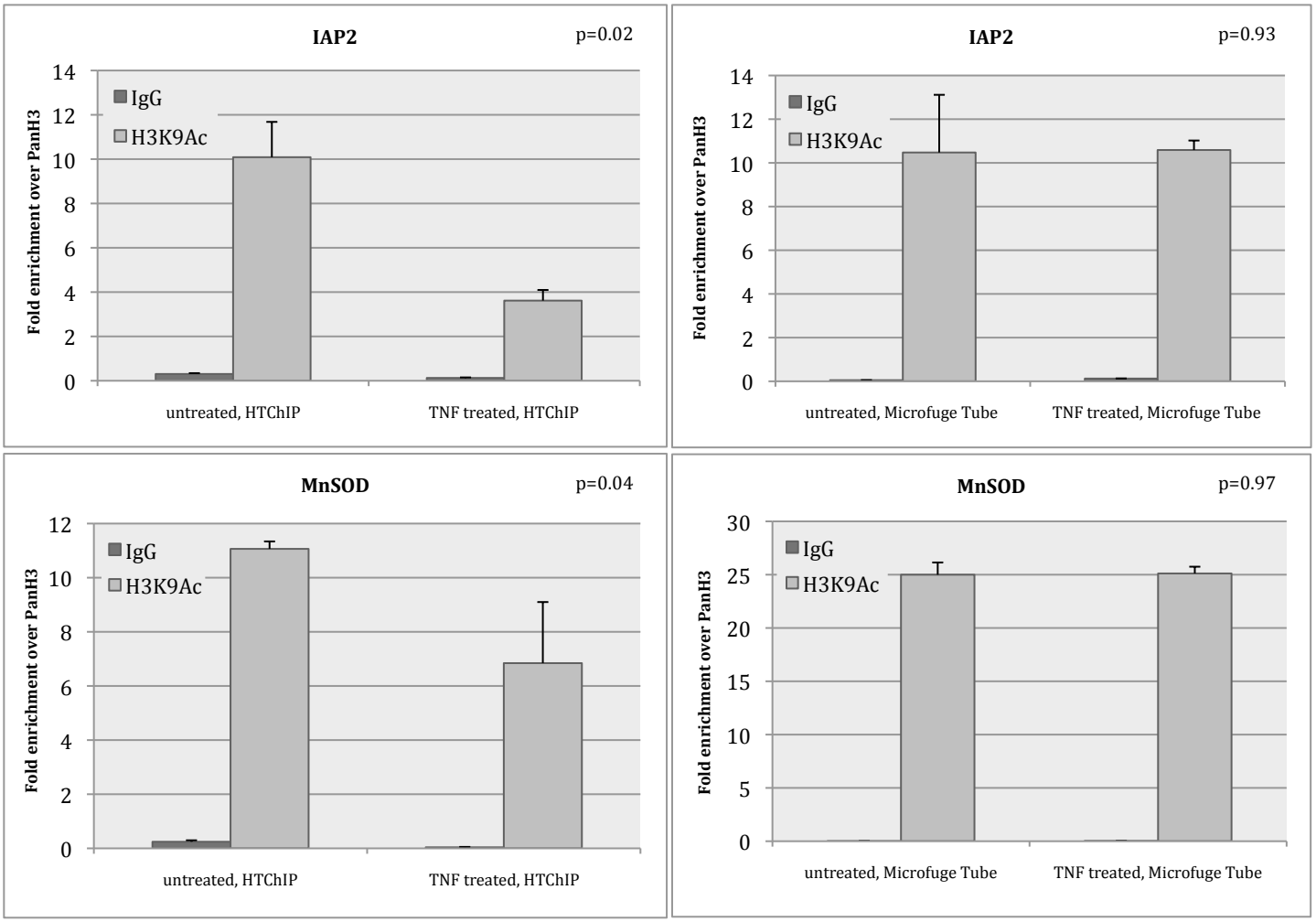

FIGURE S4

Bar graph of HTChIP results comparing ChIP enrichment obtained using three different anti-MYC antibodies from different vendors. *Odc1 Upstream* is the negative control region; *Odc1 E-box* is the positive control *Odc1* promoter. In addition to results presented in Figure 6 of the main article, this figure includes positive ChIP controls – unmodified H4 (PanH4) and acetylated H4 (H4Ac).