Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information for

A Droplet Microfluidic Platform for Efficient, Enzymatic Chromatin Digestion Enables Robust Determination of Nucleosome Positioning

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Videos of the device in operation were captured at 4000 fps and played at 10 fps. They are attached separately.

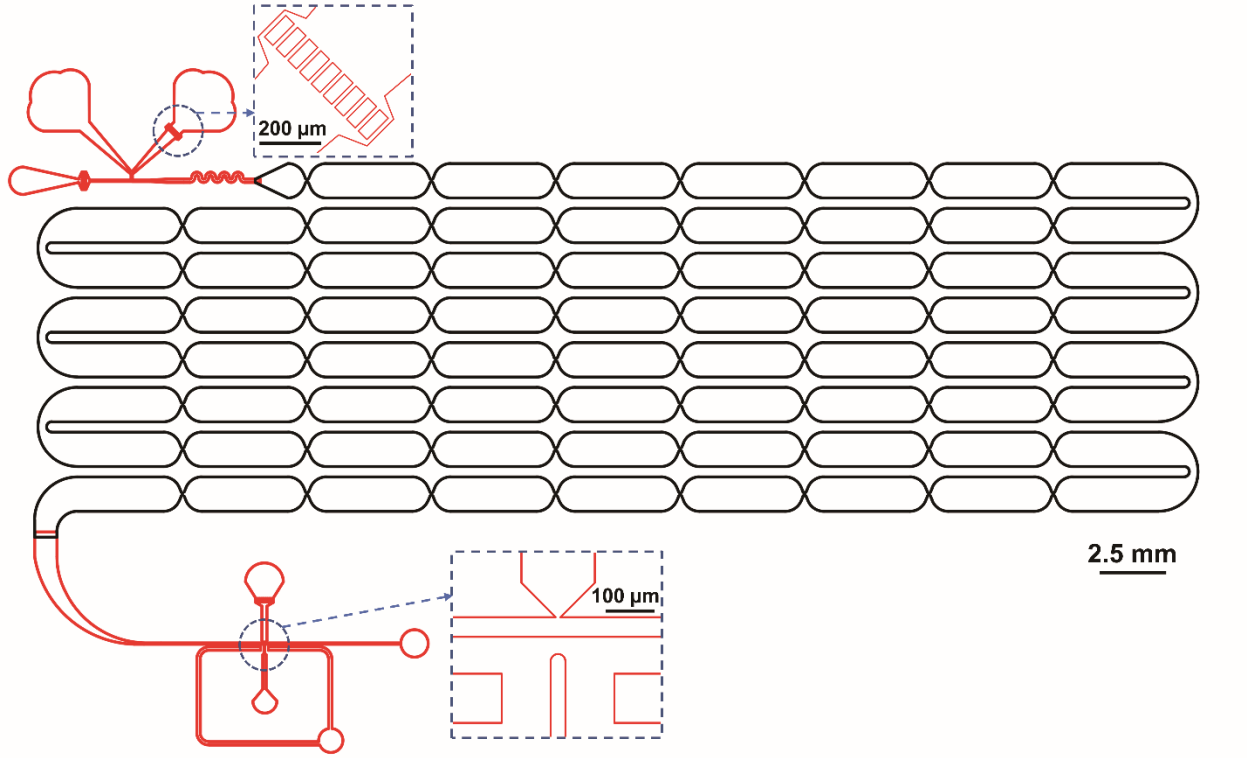

Fig. S1 Illustration of the photomask design of the device. The red parts are the first layer, and the black part the second. The two layers are printed on two individual masks. They should be aligned to ensure the connection of the channels (shown as the overlap between the red and black). Zoom-in insertions show the design for the filter and the picoinjector.

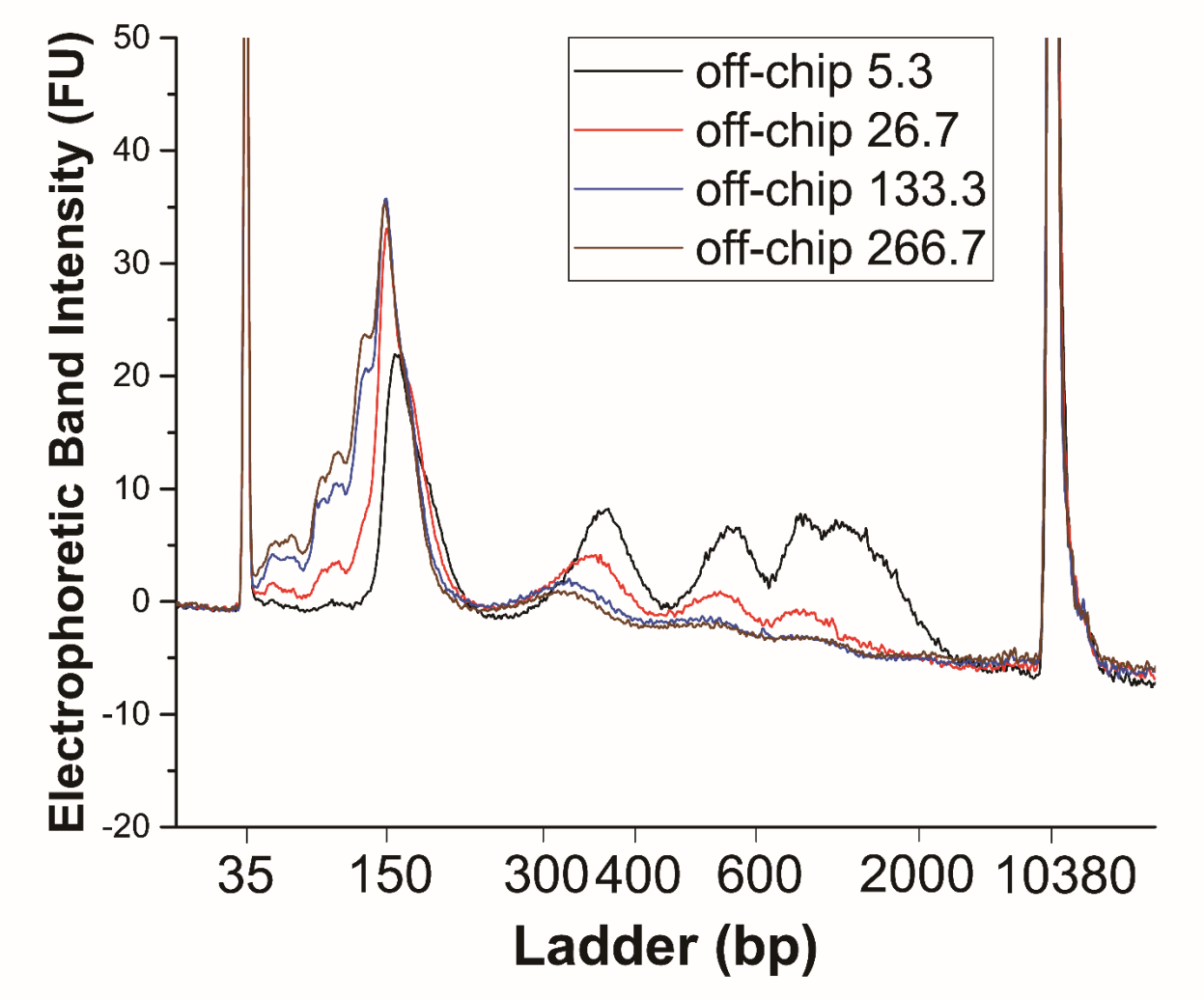

Fig. S2 Characterization of chromatin digestion efficiency in off-chip control experiments with different MNase concentrations with a common incubation time of 3.5 minutes. Electrophoretic fragment analysis shows increasing mononucleosome yields with higher MNase concentrations. The peaks at 35 bp and 10380 bp represent internal standards.

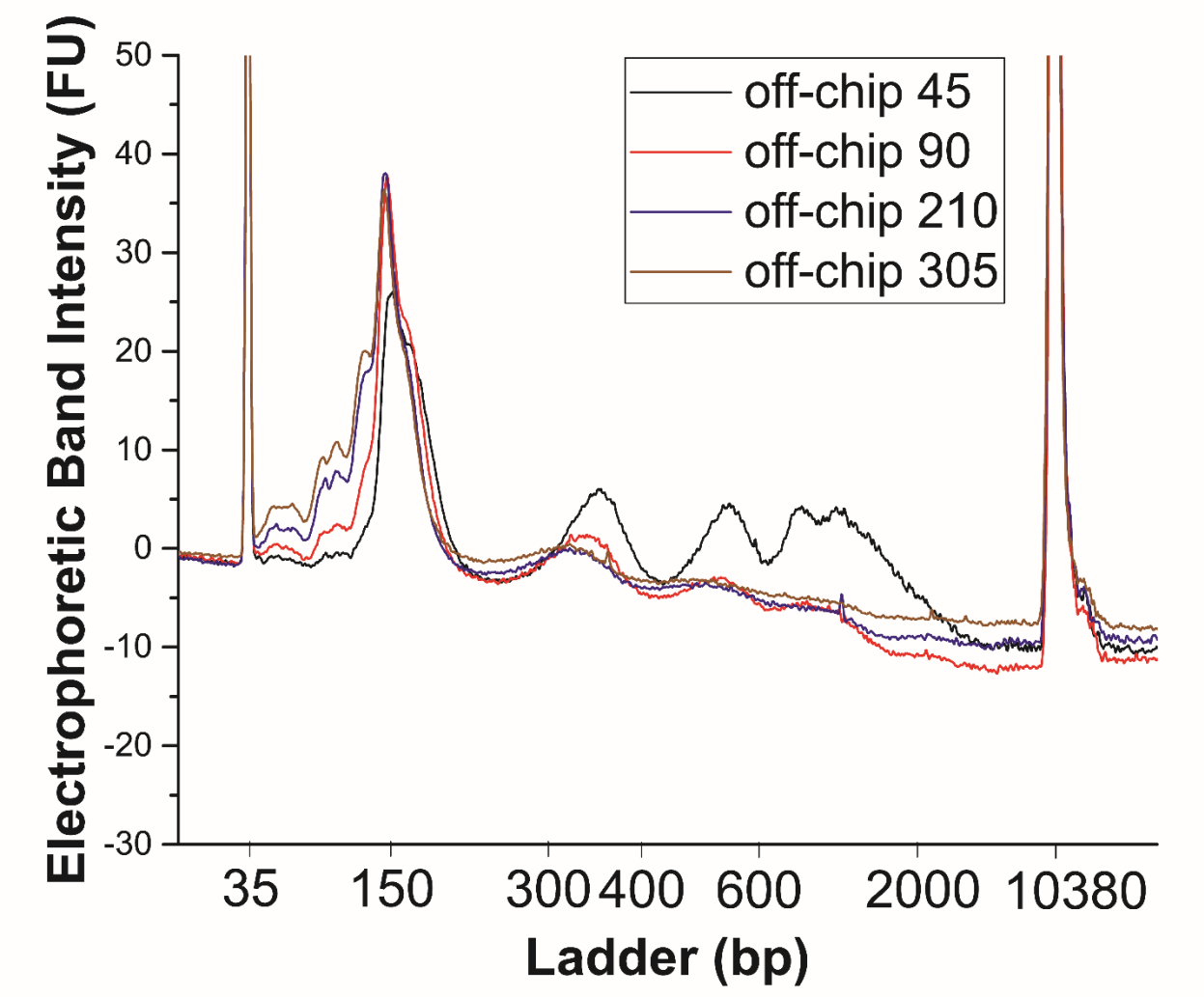

Fig. S3 Characterization of chromatin digestion efficiency in off-chip control experiments with different incubation periods with a common MNase concentration of 133.3 GU/μL. Electrophoretic fragment analysis shows increasing mononucleosome yields at longer incubation times. The peaks at 35 bp and 10380 bp represent internal standards.

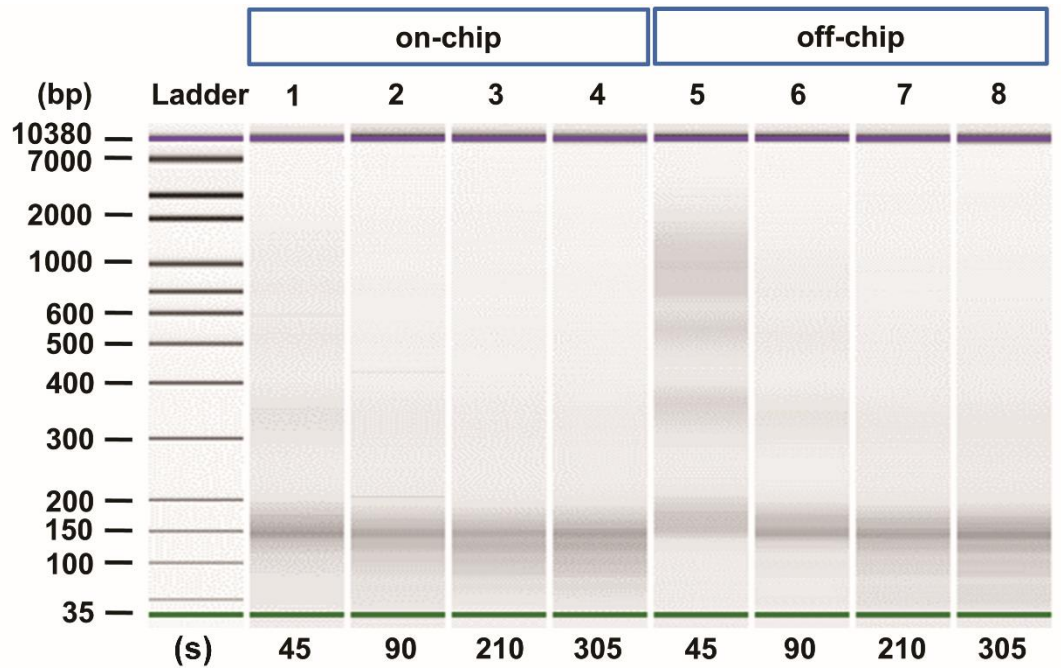

Fig. S4 Gel image of digested chromosomal DNA collected from cells processed on chip (lane 1-4) and off chip (lane 5-8). The incubation periods are listed on the bottom. More DNA fragments of approximately or smaller than 150 bp were generated with increasing incubation time.

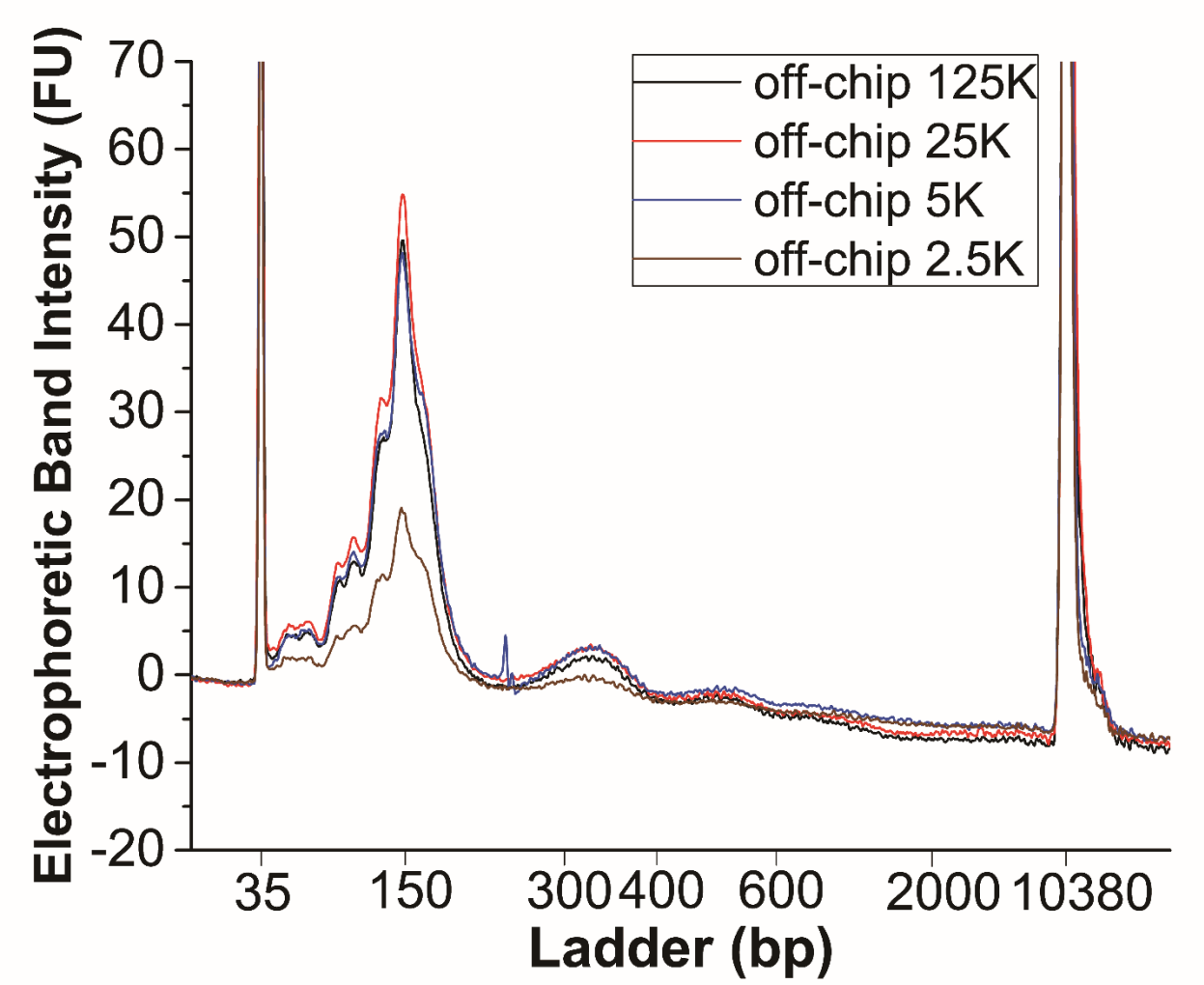

Fig. S5 Characterization of chromatin digestion efficiency in off-chip control experiments with flexible cell inputs. The electrophoretic band intensity profiles of off chip samples confirmed the consistent efficiency in mononucleosome yields at the optimal conditions, i.e. a common MNase concentration of 133.3 GU/μL and an incubation time of 210 s. The peaks at 35 bp and 10380 bp represent internal standards.

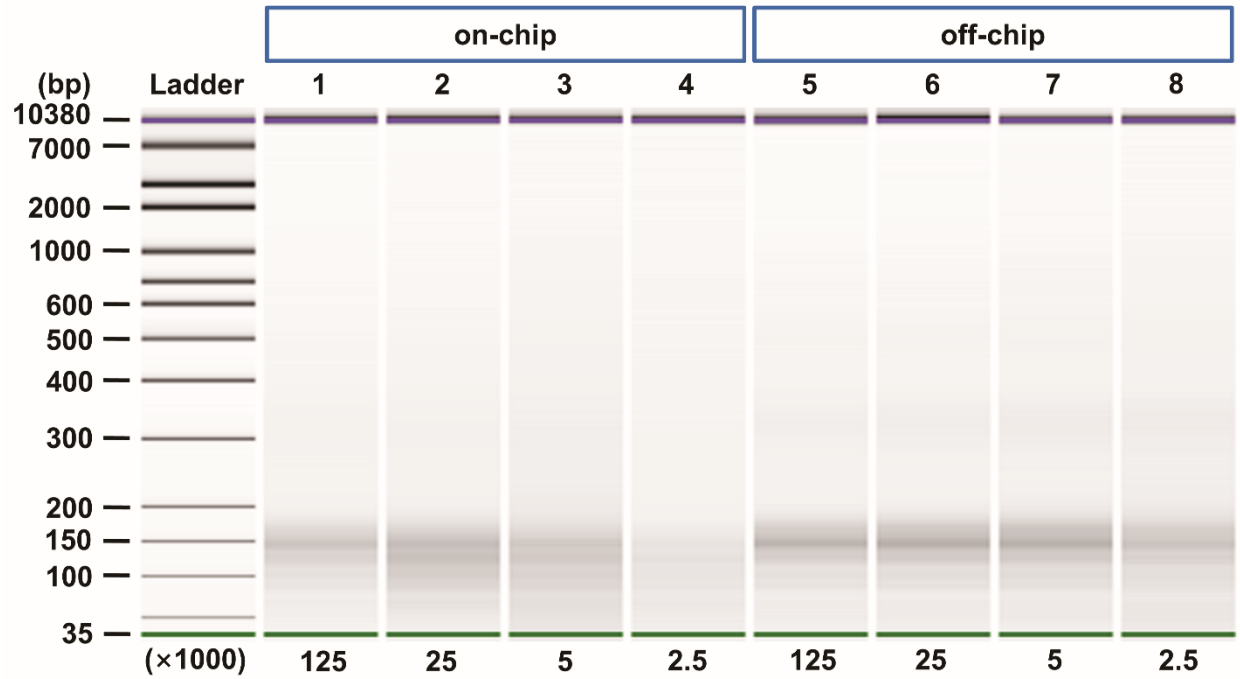

Fig. S6 The gel image of digested chromosomal DNA with flexible cell inputs processed on chip (lane 1-4) and off chip (lane 5-8). Starting input cell numbers were listed on the bottom. Notice more DNA fragments smaller than 150 bp were presented in on-chip processed samples, suggesting overdigestion that is desired in precise mapping of nucleosome positions.

Table S1 Statistical comparisons between sequenced samples

Comparing Type	Sample A	Sample B	Overlapping Peaks*	
	ID	ID	Sample A	Sample B
Biological replicates	off 1-Free	off 2-Free	97%	98%
Biological replicates	on 1-Free	on 2-Free	97%	97%
Biological replicates	off 1-PCR	off 2-PCR	98%	99%
Biological replicates	on 1-PCR	on 2-PCR	98%	98%
off vs. on	off 1-Free	on 1-Free	97%	98%
off vs. on	off 2-Free	on 2-Free	97%	97%
off vs. on	off 1-PCR	on 1-PCR	98%	99%
off vs. on	off 2-PCR	on 2-PCR	98%	99%
Free vs PCR	off 1-Free	off 1-PCR	99%	98%
Free vs PCR	on 1-Free	on 1-PCR	98%	97%
Free vs PCR	on 2-Free	off 2-PCR	99%	96%
Free vs PCR	on 2-Free	on 2-PCR	99%	97%

*Static and partially overlapping