Supporting Information

Probing Nucleosome Stability With a DNA Origami Nanocaliper

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Table of Contents **Page #**

1. Electromobility shift assay of reconstituted nucleosomes bound with neutravidin..... S3
2. TEM images of sucrose gradient purified nucleosomes bound with neutravidin…… S4
3. Sample TEM image of nanocalipers with integrated nucleosomes……... S5
4. Angular distributions and measured values for varying size nucleosome constructs… S6
5. Sample TEM image galleries for nanocalipers with nucleosomes and nucleosome location analysis……..... S7
6. Verifying calculation of end-to-end distances from individual nanocaliper angle measurements…… S8
7. Geometric analysis of DNA wrapping …….. S9
8. Nanocaliper angle measurements for increasing monovalent salt……..... S11
9. TEM of nanocalipers with 6L nucleosomes and increasing monovalent salt ……. S12
10. TEM of DNA control in increasing monovalent salt…….. S13
11. Nanocaliper measurements of transcription factor binding……..... S14
12. TEM of nanocalipers with 6L nucleosomes containing Gal4 site ……. S15
13. TEM of nanocalipers with 6L nucleosomes not containing Gal4 site …….. S16
14. FRET measurements of Gal4-VP16 binding to nucleosomes without the Gal4 target sequence …… S17

15. Fitting angular distributions to determine shift caused by Gal4 binding…… S18
16. caDNAno diagram of nanocaliper…….. S19
17. $MgCl_2$ screen for nanocaliper self-assembly……..… S20
18. Testing stability of nanocaliper in experimental buffer conditions……..… S21
19. Assessing error from manual measurement of angles and distances ……. S22
20. Staple sequences for DNA nanostructure……..… S23
21. Nucleosomal DNA sequences……..... S28
22. Oligonucleotides used for PCR……... S29

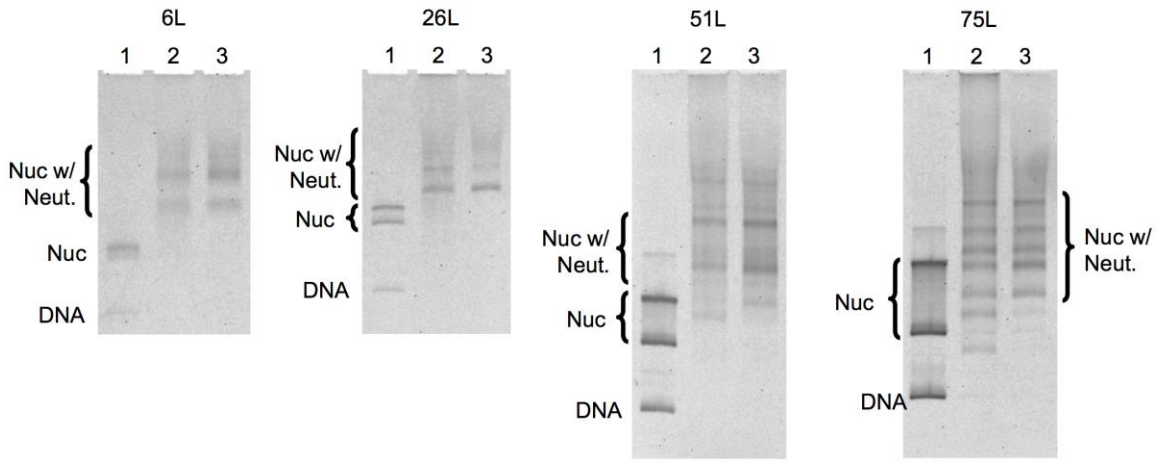


Figure S1: Electromobility Shift Assay of reconstituted nucleosomes bound with neutravidin. Each nucleosome sample (6L, 26L, 51L, and 75L) was analyzed by EMSA on a 5% polyacrylamide gel stained in ethidium bromide. Each image is of a different nucleosome sample. In each gel, lane 1 contains the sample after nucleosome reconstitution. Most of the DNA is shifted up into the nucleosome bands. The two nucleosome bands are due to different positions of the histone octamer on the DNA. The center nucleosome position is in the top band and the end nucleosome position is in the bottom band. Lane 2 contains the nucleosome sample following incubation with neutravidin. The binding of the neutravidin further reduces the electrophoretic mobility. Lane 3 contains the sucrose gradient purified neutravidin-bound nucleosomes. The range of mobilities is due to multimerization of nucleosomes by neutravidin cross linking. However, our TEM images reveal that a large fraction of molecules is composed of single nucleosomes bound to two neutravidins.

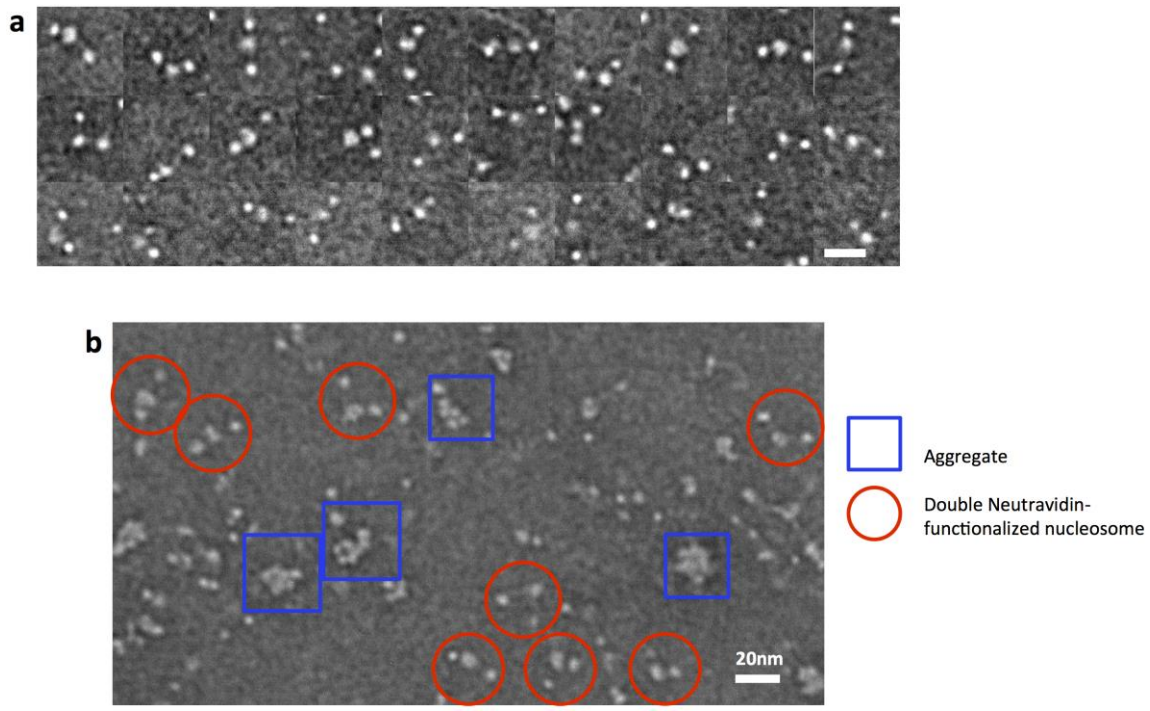


Figure S2: TEM images of sucrose gradient purified nucleosomes bound with neutravidin. The TEM images reveal that some nucleosomes are crosslinked by neutravidin to form small aggregates. However, the majority of purified nucleosomes are single nucleosomes bound by two neutravidins at each end of the nucleosome. (a) is a gallery of single nucleosome images. (b) is a representative image of the 6L nucleosomes with neutravidin. There are a number of small aggregates (blue squares). However, the majority of samples are located as a single nucleosome with two neutravidins bound at the DNA ends (red circles).

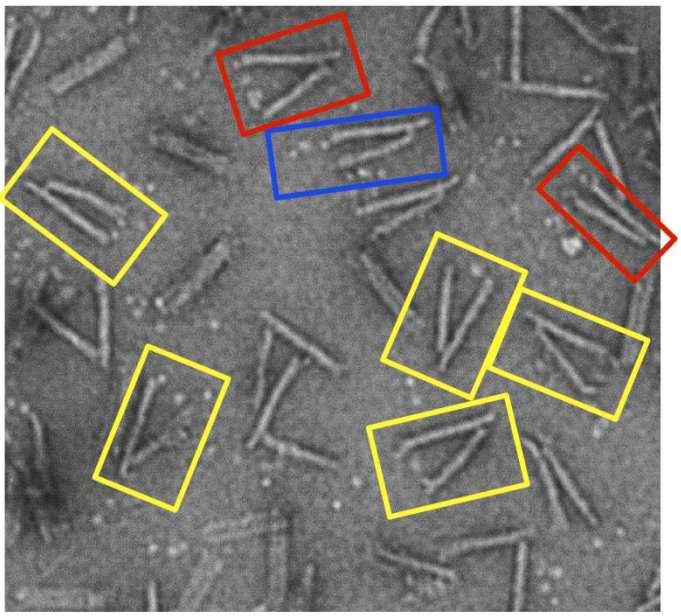
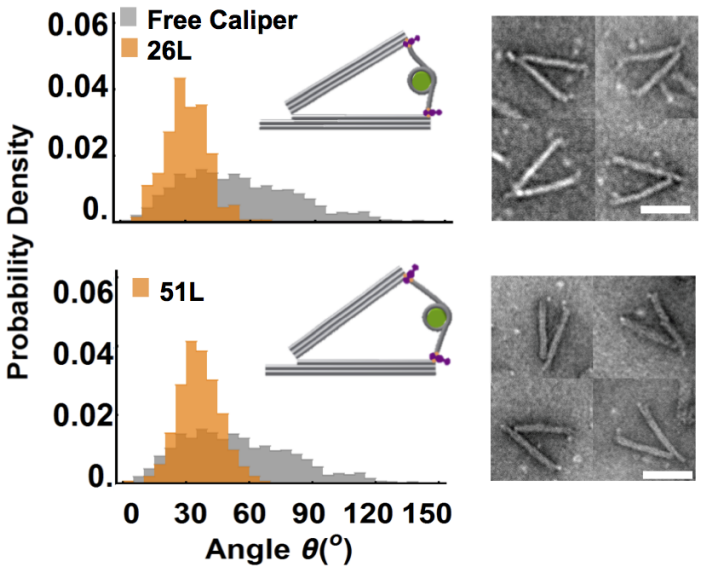


Figure S3: Sample TEM image of nanocalipers with integrated nucleosomes. TEM imaging of nanocalipers with integrated nucleosomes reveal a high efficiency of properly incorporated nucleosomes (yellow). We also occasionally see nanocalipers with a single nucleosome only bound to the end of a single arm (blue) or two nucleosomes incorporated, one of each arm via a single attachment point (red).



	6L	26L	51L	75L
Measured Distance (nm)	23.2 ± 0.4	32 ± 2	38 ± 2	41 ± 1
Calculated Distance (nm)	22 ± 3	32 ± 2	38 ± 3	42 ± 1
Angles(°)	19 ± 2	31± 3	36.4 ± 0.3	41 ± 1

Figure S4: Angular distributions and measured values for varying size nucleosome constructs. Angular distributions of the 26L and 51L construct reveal a shift and narrowing relative to the free nanocalipers suggesting the nucleosomes constrain the motion of the nanocaliper. Sample TEM images are shown at right (Scale bars = 50 nm), and the insets illustrate conformations that are consistent with the measured mean nanocaliper angle and end-to-end distance (additional details in **Figure S7**). The table shows the mean values for measured and calculated distances and the mean nanocaliper angle for all the constructs integrated in the nanocalipers.

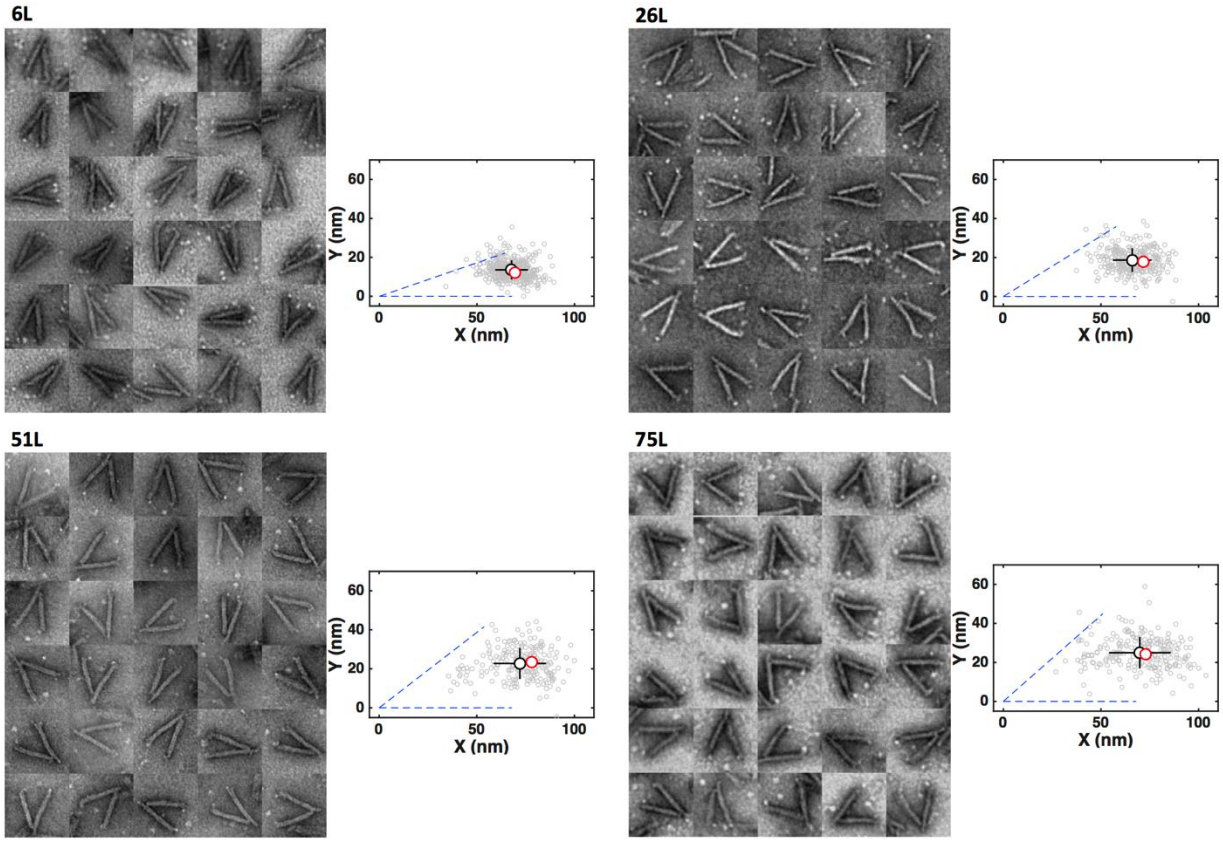
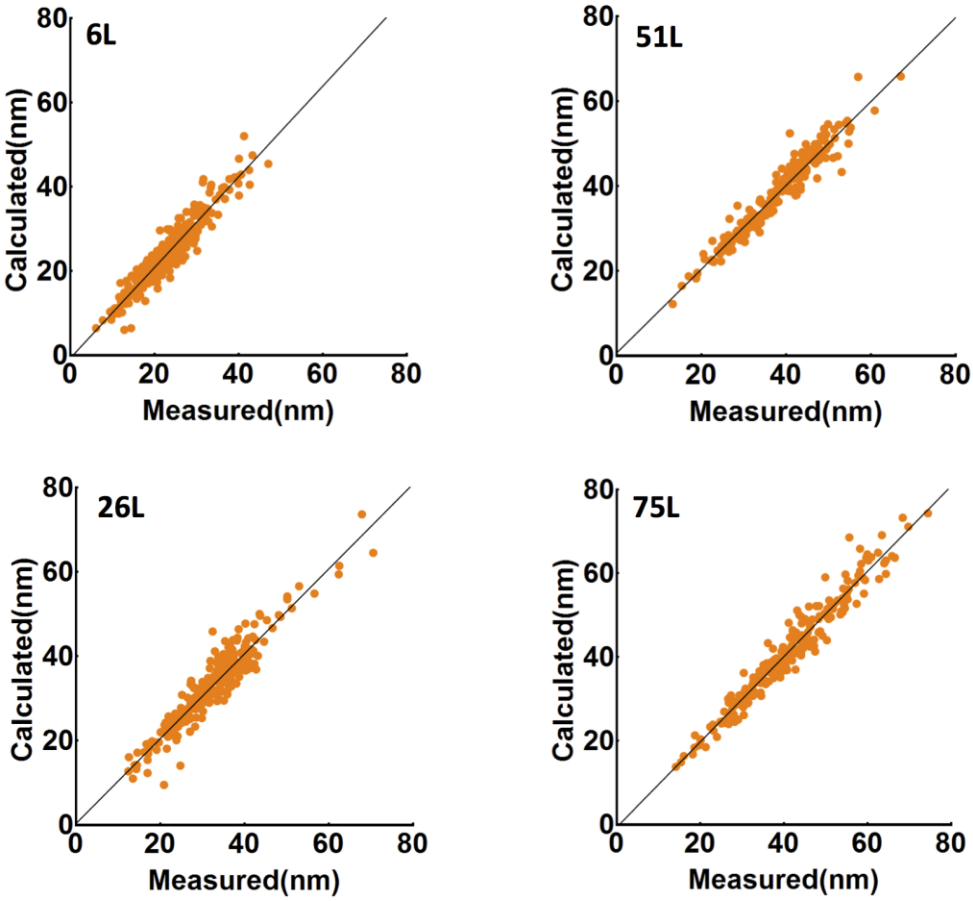


Figure S5: Sample TEM image galleries for nanocalipers with nucleosomes and nucleosome location analysis. Sample TEM image galleries for nanocalipers with the 6L, 26L, 51L, and 75L nucleosomes incorporated. The plots show blue dashed lines indicating the nanocaliper conformation at the measured mean angle. The gray circles show the locations of nucleosomes, measured relative to the neutravidin molecules. The black circle shows the average location of the nucleosome, and the solid black lines indicate the standard deviation in X and Y position. The red circle shows the location of the nucleosome, calculated by assuming that the nucleosomes adopt the conformations shown in **Figure 3c** further illustrating that these conformations agree well with experimental observations.



	FUNCTION y = mx + b	R^2	Standard Error (m)	Standard Error (b)
6L	1.08x - 0.7	0.90	0.02	0.5
26L	1.01x + 0.3	0.89	0.02	0.7
51L	0.99x + 0.7	0.93	0.02	0.7
75L	1.01x - 0.6	0.95	0.02	0.7

Figure S6: Verifying calculation of end-to-end distances from individual nanocaliper angle measurements. The end-to-end distance of nucleosomes was directly measured (x-axis) and also calculated (y-axis) from the measured nanocaliper angle using the equation $d = 2 \times L_{arm} \times \sin(\theta/2)$, where L_{arm} = 68 nm is the length of the nanocaliper arms from the hinge vertex to the tip where the biotins are located. Scatter plots show that the calculated distances very closely agree with the measured distances. Linear fits also reveal that the nanocaliper angle is a good measure of the end-to-end distance.

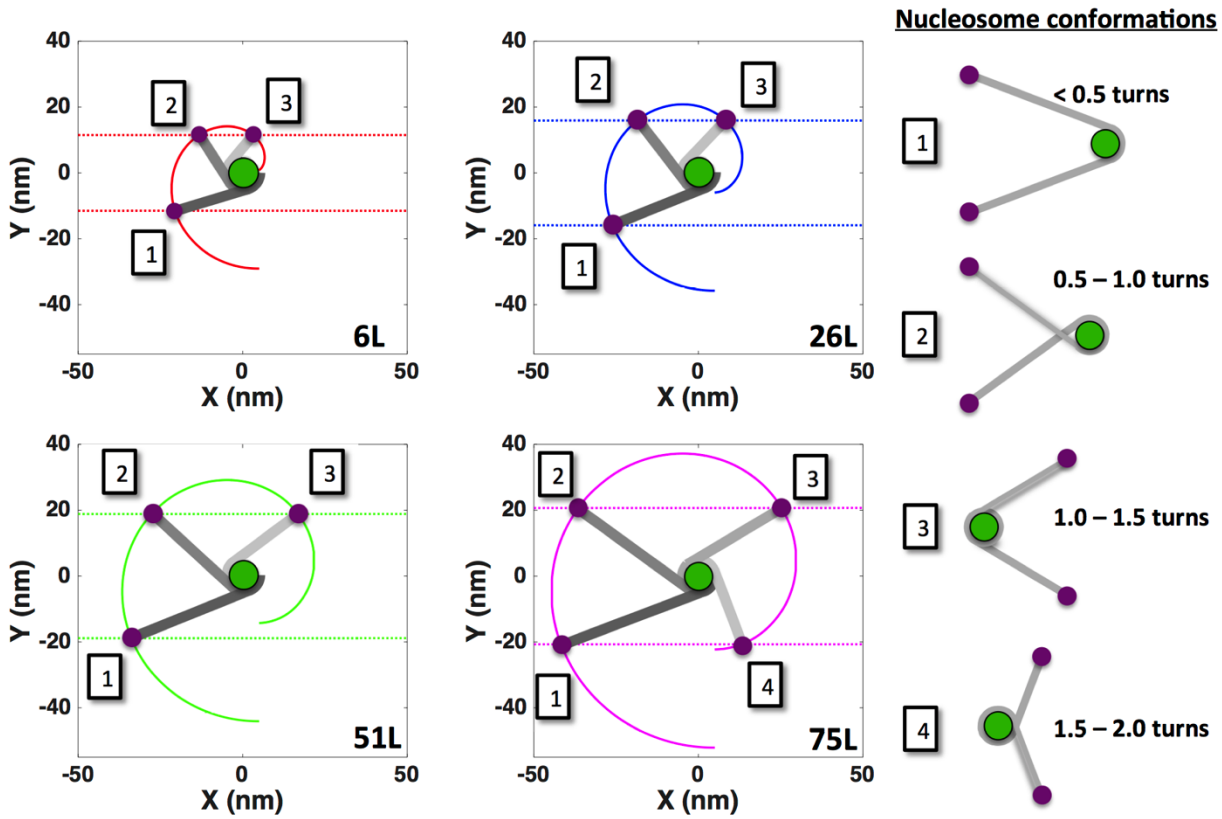
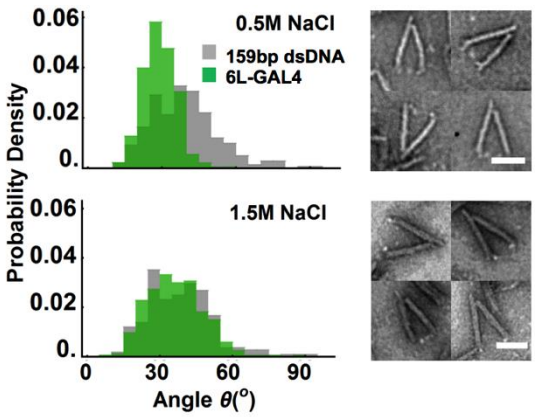


Figure S7: Geometric analysis of DNA wrapping for all nucleosome constructs. A geometric analysis of DNA wrapping around a histone core reveals the conformations that satisfy the respective end-to-end distances (dashed lines) of the nucleosome construct integrated within the nanocalipers. For this analysis, we assume the DNA exits the nucleosome tangentially and does not bend, twist, or stretch after exiting the nucleosome. In addition, the DNA is known to stretch when wrapped around the nucleosome. Hence, we used an average base pair length of 0.352 nm/bp for DNA wrapped around the histone core to satisfy 147 bp of DNA wrapped 1.65 turns around a histone core with a radius of 5 nm out to the center of the DNA. In general, there are four conformations that can satisfy integration within the nanocalipers (right) that correspond to 0 – 0.5 turns, 0.5 – 1.0 turns, 1.0 – 1.5 turns, and 1.5 – 2.0 turns. In the 6L (top left, red), 26L (top right, blue), and 51L (bottom left, green) the DNA is not long enough to reach the measured end-to-end distance in the fully wrapped configuration. For these constructs, the 1.0 – 2.5 turn configuration is the maximally wrapped configuration that satisfies the end-to-distances giving 20 bp, 19 bp, and 17 bp of unwrapped DNA per end, from the 1.65 turn configuration, for the 6L, 26L, and 51L nucleosomes, respectively. These conformations also agree well with the location of the nucleosome in TEM images as shown in **Figure S5**. The 75L nucleosome is long enough to satisfy the end-to-end distance. Our

geometric analysis suggests the 75L construct wraps an additional 10 bp per end around the histone core to satisfy the end-to-end distance, which also agrees well with the location of nucleosomes in TEM images (**Figure S5**).



159bp	0.2M	0.5M	1.0M	1.5M	2.0M
Calculated Distance (nm)	**50 ± 5**	**45.9 ± 0.7**	**46.9 ± 0.7**	**47 ± 2**	**47 ± 2**
Angles(°)	**43 ± 4**	**39.5 ± 0.6**	**40.3 ± 0.6**	**40 ± 1**	**40 ± 1**

6L	0.2M	0.5M	1.0M	1.5M	2.0M
Calculated Distance (nm)	**22 ± 3**	**34.4± 0.9**	**38 ± 2**	**43 ± 2**	**45 ± 1**
Angles(°)	**19 ± 2**	**29.3 ± 0.8**	**33 ± 2**	**37± 1**	**39 ± 1**

Figure S8: Nanocaliper angle measurements for increasing monovalent salt. Angular distributions the nanocalipers converge to the DNA only control with increasing monovalent salt concentrations. Sample TEM images are shown for the different salt condition (right). Scale bars = 50 nm. The tables indicate the mean values for the measured angles and corresponding mean values for the calculated distances.

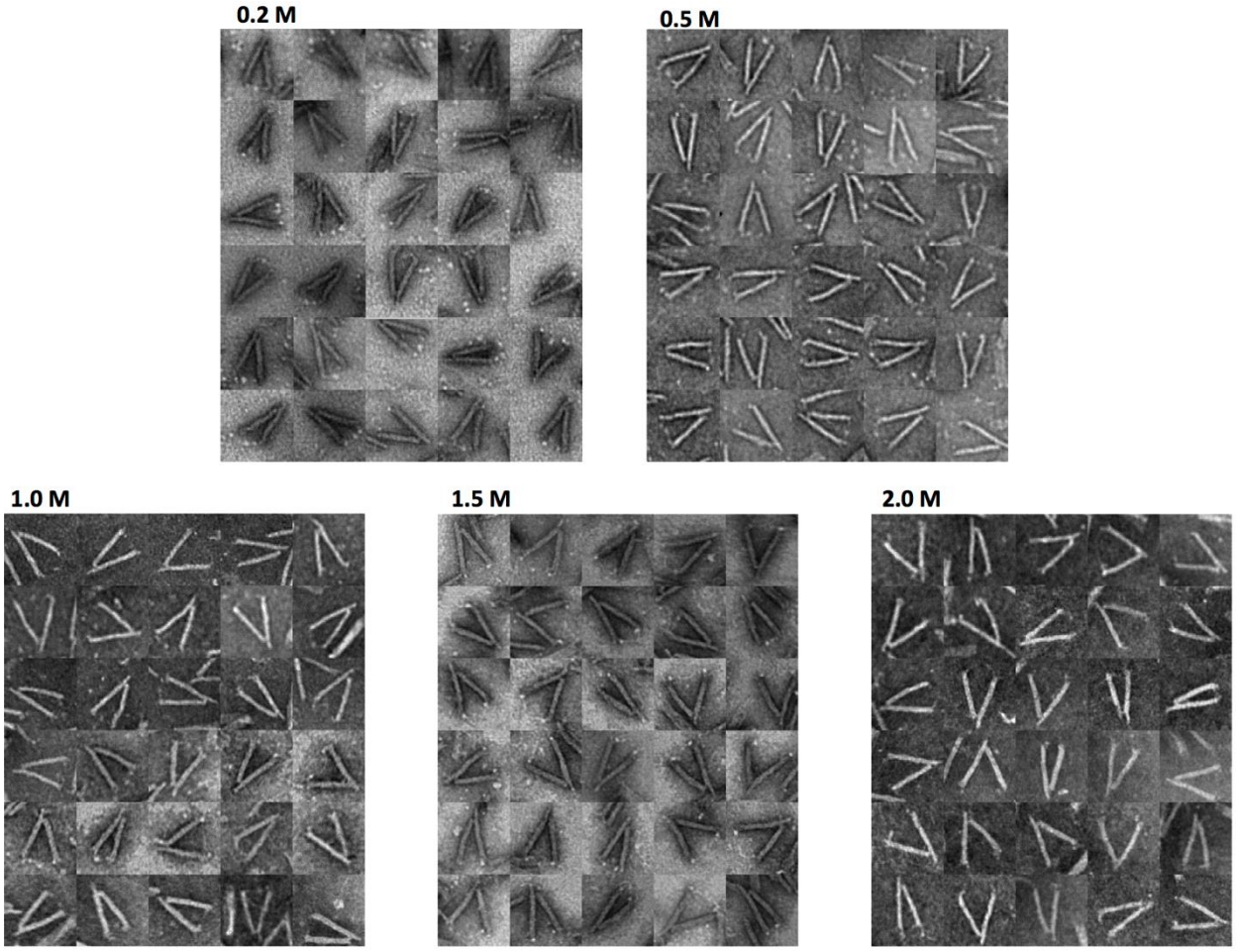


Figure S9: TEM images of nanocalipers with 6L nucleosomes and increasing monovalent salt. Sample TEM image galleries of the nanocalipers with the 6L nucleosome integrated and subjected to varying concentrations of NaCl. Hinge arms are ~70 nm long.

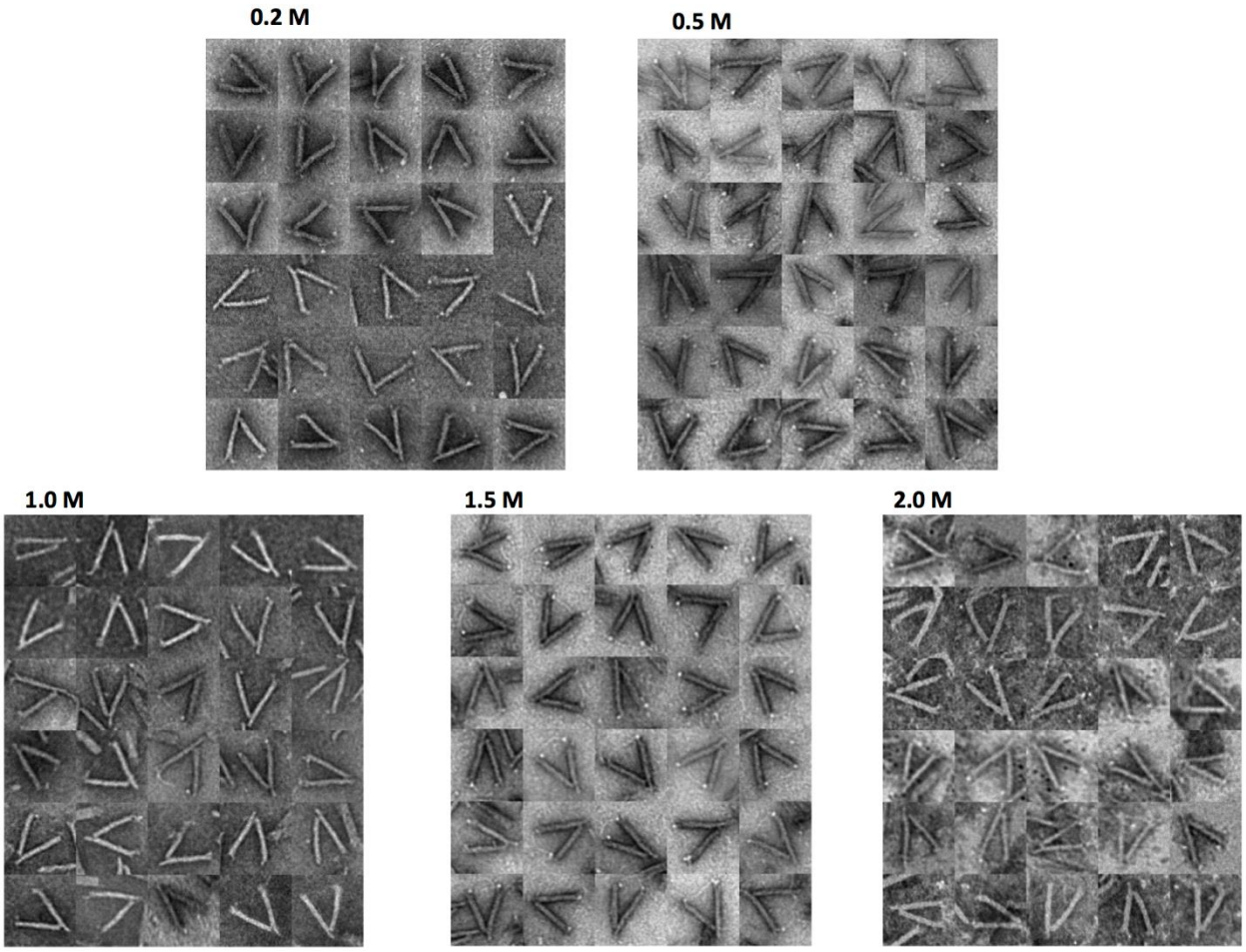
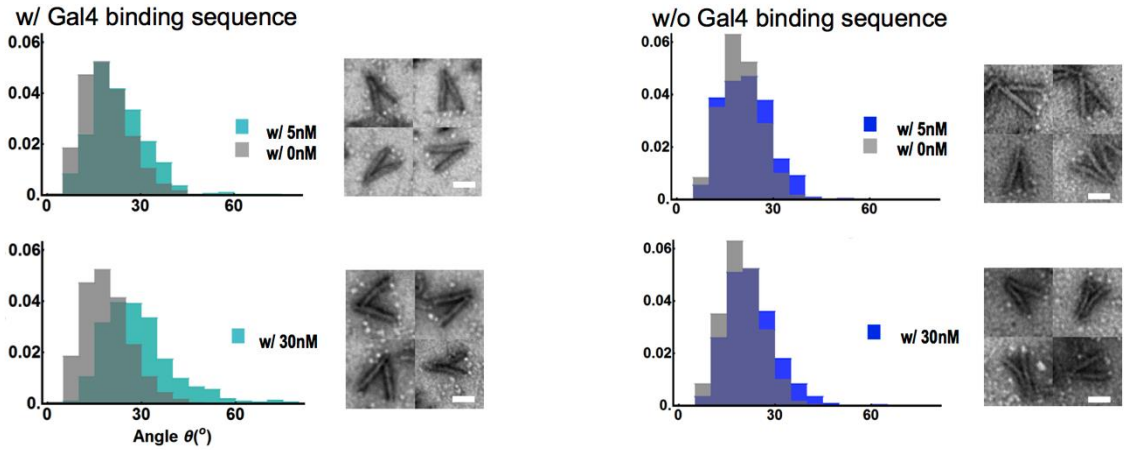


Figure S10: TEM images of DNA control in increasing monovalent salt. Sample TEM image galleries of the nanocalipers with a 159 bp DNA construct integrated and subjected to varying concentrations of NaCl. This construct is the same DNA as in the 6L nucleosome but without the histone core. Hinge arms are ~70 nm long.



With DNA binding site	0nM (GSB1)	1nM	5nM	10nM	30nM	100nM
Calculated Distance (nm)	**23 ± 3**	**26 ± 1**	**27 ± 0.6**	**30.86 ± 0.01**	**34.0 ± 0.4**	**33 ± 2**
Angles(°)	**19 ± 3**	**22 ± 1**	**23.2± 0.5**	**26.23 ±0.01**	**28.9 ±0.4**	**28 ±2**

Without DNA binding site	0nM (GSB1)	1nM	5nM	10nM	30nM	100nM
Calculated Distance (nm)	**24 ± 1**	**25 ± 1**	**25.6 ± 0.4**	**27 ± 3**	**26.8 ± 1.0**	**27 ± 1**
Angles(°)	**20.0 ± 0.9**	**21.5 ± 0.9**	**21.7 ± 0.4**	**23 ± 3**	**22.7 ± 0.8**	**22.5 ± 0.9**

Figure S11: Angle measurements for nanocalipers with 6L nucleosomes subjected to transcription factor binding. Increasing concentrations of the transcription factor Gal4-VP16 causes a significant shift in angular distributions of the nanocaliper resulting in a significant increase in the mean angle only when the Gal4 recognition site is present in the DNA (left) and not when the Gal4 recognition site is not contained in the DNA (right). Scale bars = 50 nm in sample TEM images. The tables show the average nanocaliper angle from the measured distributions and the corresponding calculated end-to-end distances.

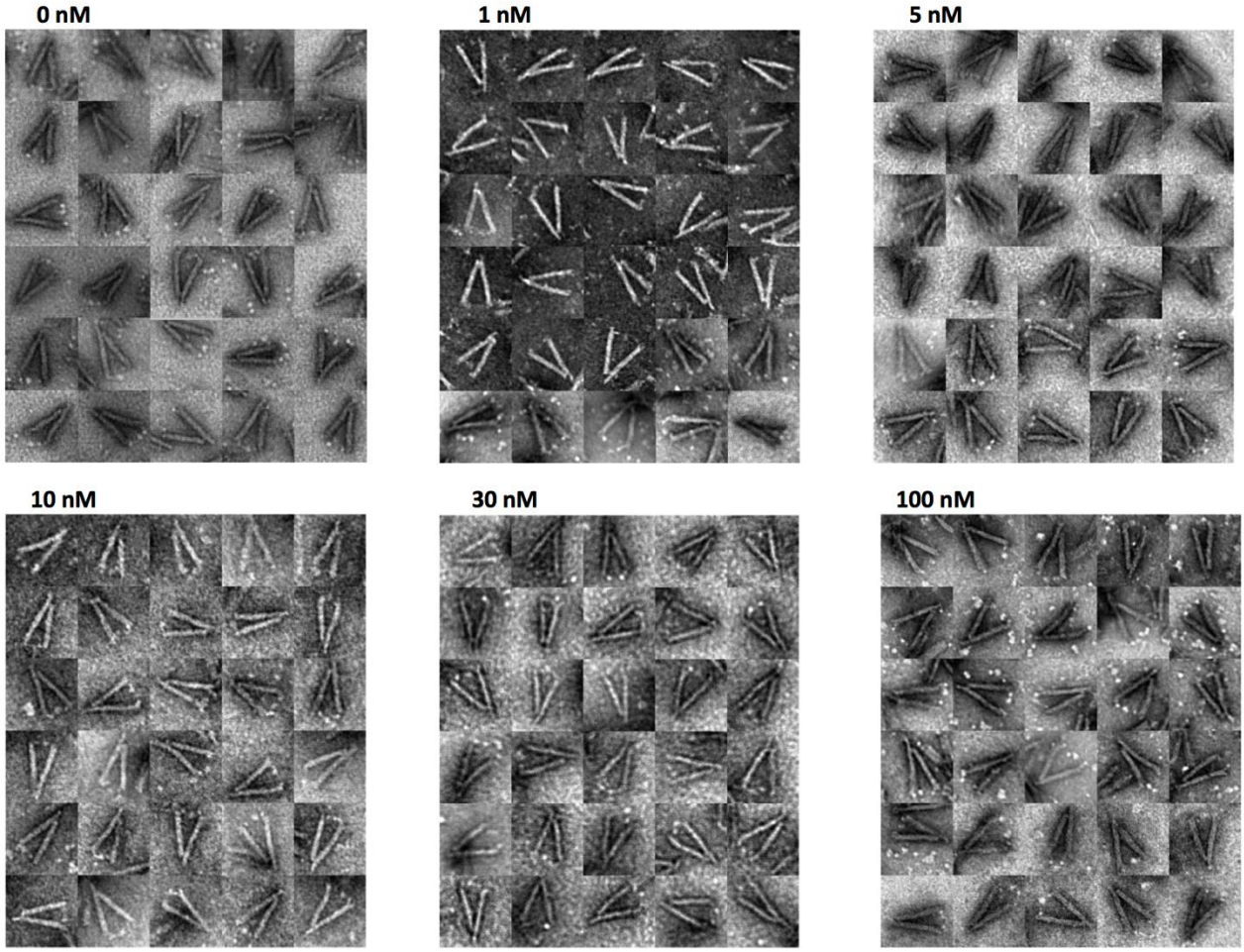


Figure S12: TEM of nanocalipers with 6L nucleosomes containing Gal4 site. Sample TEM image galleries of the nanocaliper integrated with the 6L nucleosome that contains the Gal4 site and subjected to increasing concentrations of Gal4-VP16 in solution. Hinge arms are ~70 nm long.

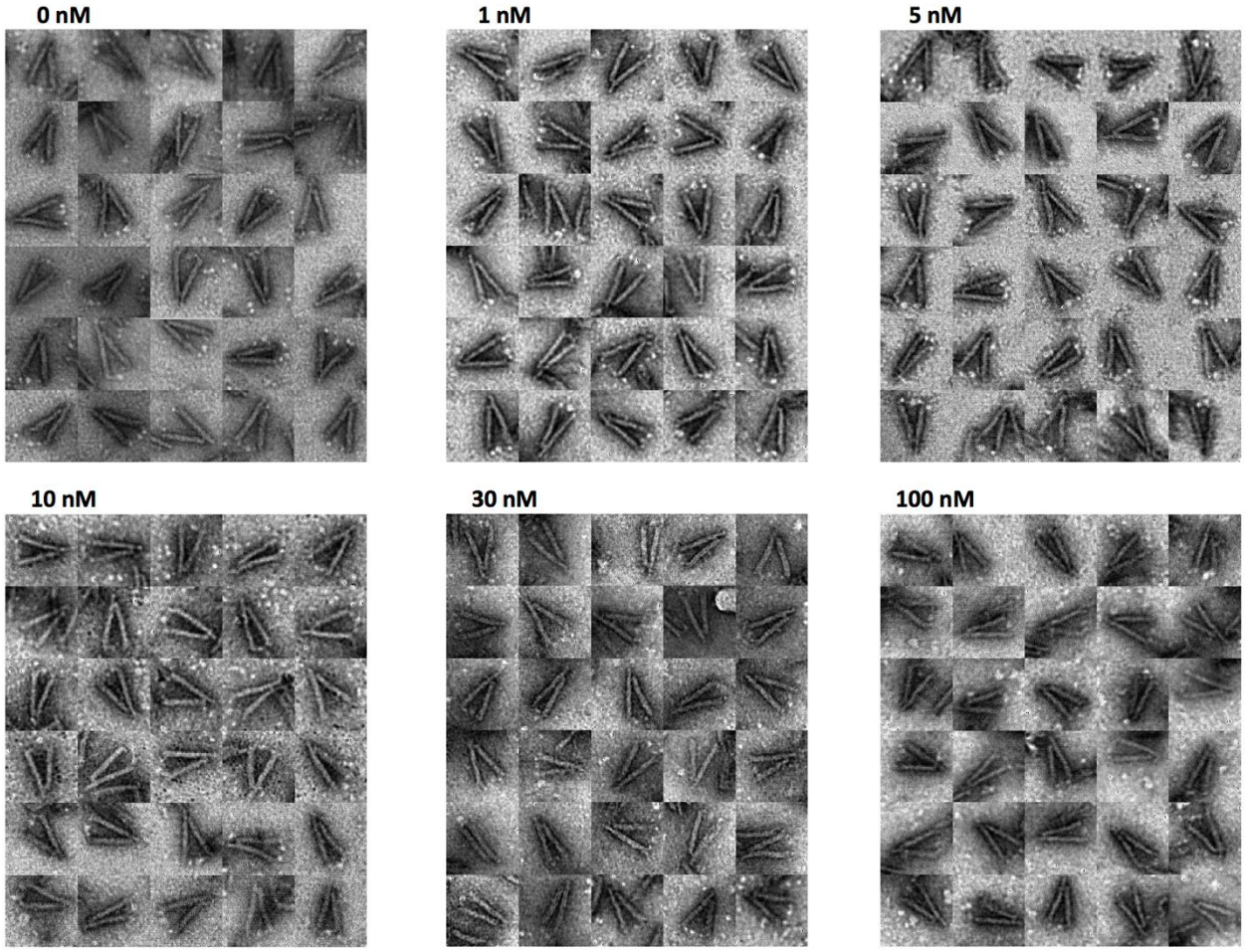


Figure S13: TEM of nanocalipers with 6L nucleosomes not containing Gal4 site. Sample TEM image galleries of the nanocaliper integrated with the 6L nucleosome that does not contain the Gal4 site and subjected to increasing concentrations of Gal4-VP16 in solution. Hinge arms are ~70 nm long.

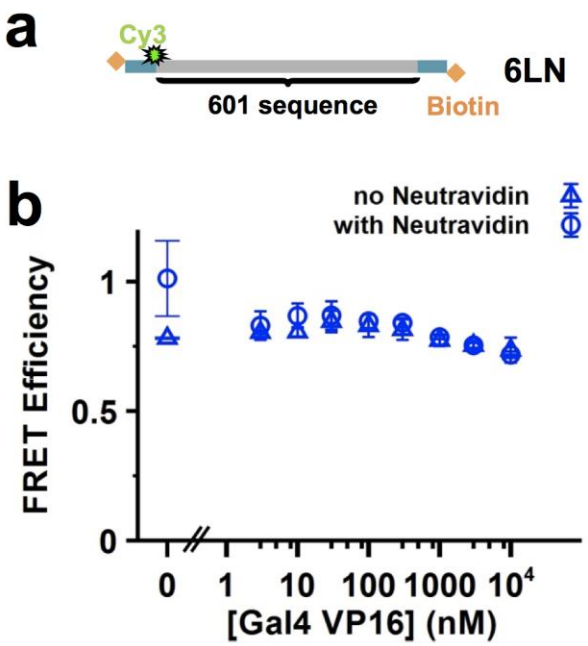
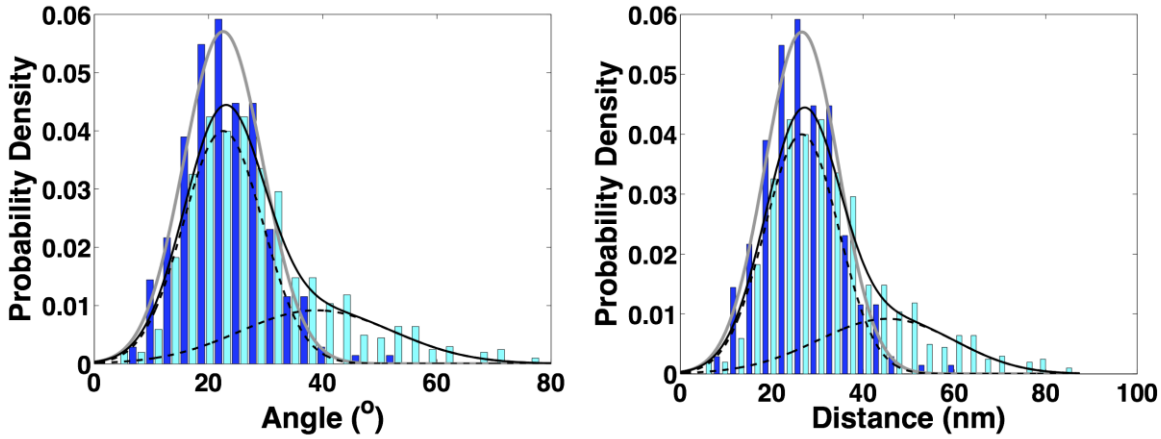


Figure S14: FRET measurements of Gal4-VP16 binding to nucleosomes without the Gal4 target sequence. (a) The DNA construct for detecting Gal4-VP16 without its site in a nucleosome using FRET. The DNA molecule is the 6L construct with a Cy3 fluorophore attached to the second base pair of the Widom 601 sequence. (b) The plot of the FRET efficiency for increasing concentrations of Gal4-VP16 without (triangles) and with (circles) neutravidin. There is no detectable change in the FRET efficiency as the concentration of Gal4-VP16 is increased. This demonstrates that the Gal4 binding site is required for the reduction of FRET efficiency.



	$A_{control}$ (peak max)	$x_{0,control}$	$S_{control}$	A_1 (peak 1 max)	A_2 (peak 2 max)	$x_{0,2}$	S_2
Angle, (°)	0.057 ±0.002	22.6 ± 0.2	6.9 ±0.2	0.040 ± 0.005	0.009 ± 0.001	39 ± 5	13 ± 4
Distance, (nm)	0.057 ±0.002	26.6 ± 0.3	8.2 ± 0.3	0.040 ± 0.005	0.009 ± 0.001	45 ± 6	16 ± 4

Figure S15: Fitting angular distributions to determine shift caused by Gal4 binding. The histograms show the nanocaliper angular (left) and 6L nucleosome end-to-end distance (right) distributions when subjected to 100 nM Gal4-VP16 in solution for both the 6L containing the Gal4 site (cyan) and not containing the site (blue). Binding of Gal4-VP16 to the Gal4 recognition site causes a shift in the angular distribution, which includes the appearance of a second population in the distribution at larger angles (left) or end-to-end distances (right). A single Gaussian distribution was fit to the control (no Gal4 site, blue data and gray distribution fit) to determine the peak height, $A_{control}$, the peak center, $x_{0,control}$, and the peak width or standard deviation, $S_{control}$, as shown in the table. The data for the case with the Gal4 binding site (cyan), can be represented by a similar peak to the control, with a different amplitude, and a second population that emerges at larger angles. The black line shows a fit of two Gaussian distributions where the control peak was maintained allowing the peak height, A_1, to vary, and a second Gaussian distribution was introduced (fit parameters A_2, $x_{0,2}$, and S_2) to capture the new population at larger angles or end-to-end distances. The dashed black lines show the individual peaks that additively make up the solid black line. The left graph shows all data and fits in terms of measured angles, and the right graph shows data and fits in terms of calculated end-to-end distances.

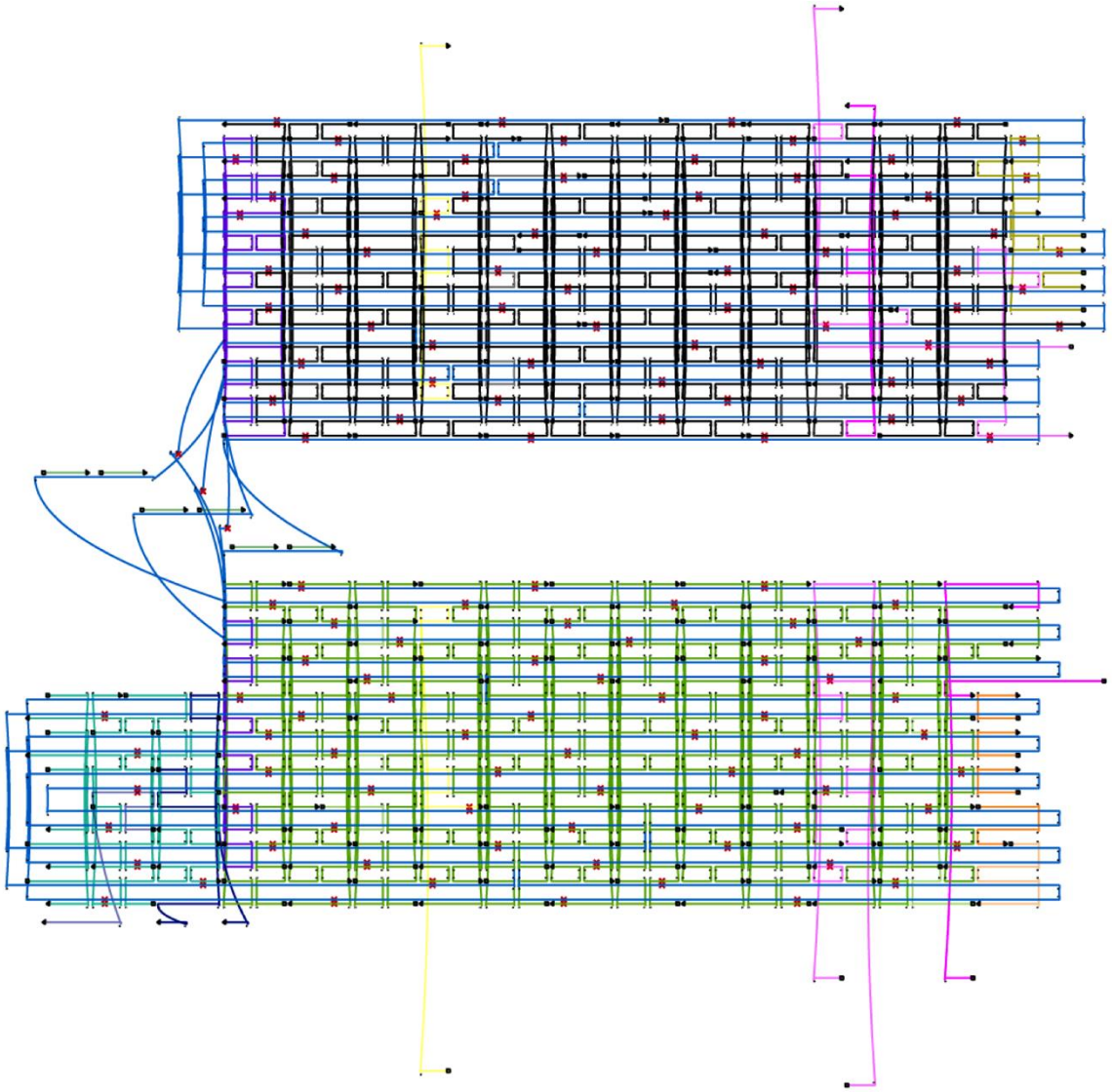


Figure S16: caDNAno diagram of the nanocaliper. caDNAno diagram for the design of the nanocaliper. The caDNAno file is available upon request.

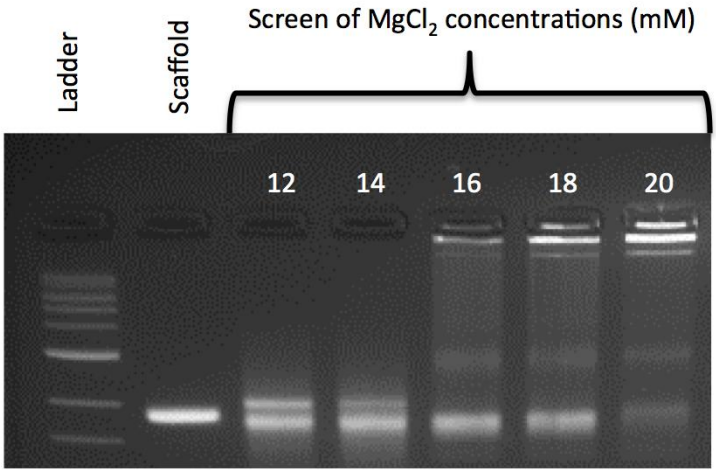


Figure S17: $MgCl_2$ screen for nanocaliper self-assembly. Representative results of agarose gel electrophoresis experiments testing for the optimal $MgCl_2$ folding concentrations for the nanocaliper self-assembly reactions. In the right 6 lanes, the nanocaliper is the bright band near the bottom of the gel. Based on these results, 18 mM $MgCl_2$ was used to fold the nanocaliper.

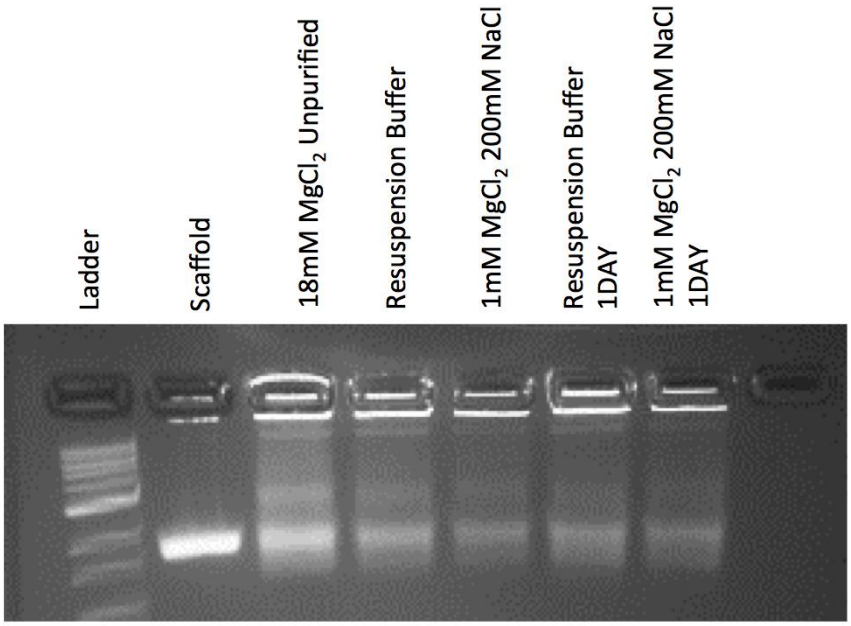
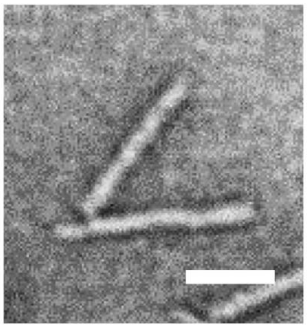
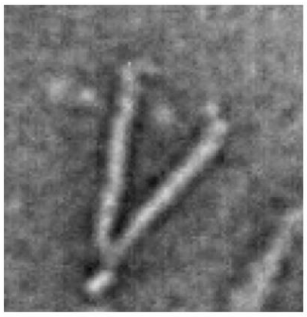


Figure S18: Testing stability of nanocaliper in experimental buffer conditions. Given that the desired buffer salt conditions for nucleosomes (200 mM NaCl, 1 mM $MgCl_2$) is different than the typical buffer conditions for DNA origami, we tested the stability of our nanocalipers in these buffer conditions by gel electrophoresis. These results show no significant change in the structure over the course of a day aside from some apparent increased aggregation.



Data Set	47.047, 46.676, 45.217, 44.825, 46.454, 45.005, 45.465, 45.465, 45.269, 44.788, 44.729, 45.414, 44.957, 45.094, 45.861
Standard Deviation, θ (^{O})	0.718
Standard Deviation (nm)	0.85



Data Set	40.771, 40.771 ,41.503, 41.503, 38.833, 40.224, 40.355, 39.528, 38.968, 39.815, 40.106, 41.21, 39.815, 39.3, 40.771
Standard Deviation (nm)	0.86

Figure S19: Assessing error from manual measurement of angles and distances. Several angle (top) measurements were carried out to assess the error, considered here as the standard deviation, of manual measurements and the corresponding error in the calculated distance. A similar analysis was carried out for end-to-end length measured directly from images (bottom). Scale bar = 50 nm.

Table S1: List of staple sequences for DNA nanostructure:

GAACCCTATGAGAGATCACCATCTATTTAATGCGCGAATTGGATTA
GAGGTGCCACCATTAGATACATTTAGTAGATTTAGTTTG
TTGGGGTCCCTGAGAGCAAAAGGGAAATTCGCCAAAGCGGGAACCTAC
ACTACGTGTGCCCTTCGCAATGCCCATTTTTTCAGGTCTTAGAGCAAC
GGCGAAAAGCAAGCGAAAATTTTAATTCGCCAGAAAACACGACGA
ACTATTAAGGCGAAAAGCGGGAGATTCATCAACATTGAATTTTGCAAA
TGAGTGTTTCGGCAAAACCAAAAACCGTCGGACGTCCAATTAGTAAAA
AAAACTAGTGCAAGGTGTTGGGATCCCGGAAAGTAACAGCATTCAGT
GCAAACATTTTCCCACAAAGCGCGTGGTGAAGATTTTCAACACCAGA
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CGCCAGGGAGAGAATCGATGAACGGGCTATCAGGTCATTG
GCTCGAATTCCGGCACACGCAGAGCATAGTAATGGTTTA
AGCTGTTTGGGGAGAATCAAAAATTAGAACCTTCGAGCTTACGGTG
TAAAGTGTACTGCCCGGTAACAACCATTATGTTAATTGCTCCTTTT
TCAGAGGTTTTCGCACTGCCATCCCACGCAAAGCTGCTTACCTTTT
ACGTTGTGTATGAGCCGCAAGAATGCCAACGGACGAGAAGGTTTAAC
CGTGCCTGTTCTTCGCGTAATGGGTCAGCGTG
TACCGGGGAACATCCCCTCATAACGGAACGTGTTGTGAATATAACGCC
GGTGCTGCGGCCAGAAGTTAACGGCGTCGCTG
CACTGCGCGCCAGCGGGTTCCGGCAAACGCGAAAATCTAATTACAGG
TGCGGCTGGTCCGTGAAAAAAGAGCGCTTCTGTGCACGTATACCCTGA
TCTTTGCTCGTCATAGTTTCTGTAAATTTC
AGCAAATCTGCGGCGGGCGGTTGGTTTGAGG
GTGCTGGTGGCTTGAGAAACAGAA
TCTCACGGGCCTCCTCACAGTACG
TGCTCATTGCAGATACTACCTTAT
GGAAGATCTTTACCAGGAGAATGA
GGACGACGTTCCTGTGCATTAATTGGTTTG
TTTTGTTATGAGAAAGACAATATTATTGCATC
GCTATTTTAAGGGAGCGAAGAAAGCGAAAGGATCACGCTGAAAGCGTA
CGGGCAACAGCTGATAACCATCACCTGTTTA
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TTCCGAAAGTTCCAGGCAAGGCAAAGAATTAGCAAAATTAAGCAATAAA
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CTGGCAAGTGTAGCGGGCGGGCGCAGTCAAATCTACAAAGTAATCGT
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CTTAATTGCTGAATACCAATAAAATAC
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GAATAAGGCTTGCCCTGCAGCACCGTCGGTGGTCAATCCGCCGGGCGC
ACGAGTAGTAAATTGCTGGTCA
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GCGATTTTAAGAACTCGGCCAGAGCAGTTGGGCCGTTTTATCCGCT
CAGGACGTTGGGAAGAGTCCGTTTAAAATCCCACTCTGTCGGAAGCA
GCAGGCGCGGAGCCGCTTTACCAGAGGGCGATCTGATTGTCTGATAGC
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CACAATTCTGCCAGCTAGCCAGCTAGCCTTTAAAACTCCAACAGGTCA
GCAGCCTCGGCTCATTTTAGGAAT
ACCACATTAAAATAGCGAGAGGCTCCCCCTCA
GGATGTGCCATGTCAATAAGCAAAAATATGATTGACCTGCGCGTAAC
AAGAGAATCTGCTCCATTGACCCCCAGCGATT
AAGAGAATCTGCTCCATTGACCCCCAGCGATT**CGCTTTGAAGCAGTTTGCTTCCGGTCA**
AGCAAGGCTACAGGAGTAAACAGTTAATGCCC
AGCAAGGCTACAGGAGTAAACAGTTAATGCCC**CGCTTTGAAGCAGTTTGCTTCCGGTCA**
GTCACCGATGCCCGTATGTACTGGTAATAAG
GTCACCGATGCCCGTATGTACTGGTAATAAG**CGCTTTGAAGCAGTTTGCTTCCGGTCAA**
ACATTCTGGCCA
CAGTAATAAAAG

CATATTCCTGAT
TCAGATGATGGC
TCTTGACAAGAA
GGATATTCATTA
ATCCGGTAAGGTAAAGTAATTCTGTCCAGAC
ACGCGAGGAAGAAAAGTGGCATGATTATCACC
ATAGAAGGCTTCCAATAGCAAG
CCCTTTTTCGTTTTAGTTTATTTTACAATAG
AAAATCACCAGCATTTGGGAAT
TTTGATGACGGAAACGAAGGTGAATTAAGACT
AGTAACAGCTTGAGCTAGCACCAATAGCCGAACAAAGTTACCAGAAG
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GAATCATAAAAGAACTAAGCAGTTACCATT
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TGTCGAAATCCGCGACATAAAGTAGTTTATCACATCGTAG
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GGCGCGTACTATGGTATTTACCGAGCACCGTGAAGGTAAACGTAGA
TATAACGTGCTTTCCTTTAAAGCCTGCCTTTACAAAAGGGATATAAAA
GGGAGCTAAACAGGAGGCCTTGAATCGGCATGAAAATTCAAGTTTAT
TTAGACAGGAACGGTATTGACAGGCGTTTGCACACTGAGGCATTCC
TATTTACAGAAGATACAATGAATTTTGAAGGTACCGCATATCCCAT
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CACCAGCATTGGCAGATCGATAGCTTCCAGTAACCTATTA
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CCAGTACAGCGGATAAGCAGGTCAGACGATTGGCCGATTAGCCAGCCA
CCAATAGGAACCCATAGTATAGCAGAGCCGC
CCCTCATTCAGGAGGCACCCTCAGAGCCGCCAGTGAGGCCTTGCCT
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ACAGACAGTTGCGAATCCAGTTACGTTTAGTAACAATTACGCTGAG
AAAGTTTTGAGTGAGACCCAATCCAACACCGGGAAACAGTCCTTGAAA
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AGCCTTTAAAAGACACGAGATAACACTGTAGCGCTCATGGTCAGAGC
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GCCCACGGCGAAAGATTCATCTTCTGACCTCTTAGAATACATAAAT
GGCGCATAGGCTGGCTACGAGGCGAGAGGCAAAAAATAACTCATCGA
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CCAACCTAACGAGCATACAACGCCCAAGATTATTATCATGTGAGGCG
CGGGTAAAATTGTATCAGTAGGGAATTTTATGAACGTTAAGTGCCAC

AAGACTTTGAATTTCAAATTCTTGCGTCTTTAATTCGACAAATCTAA
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ATCGTCACCCTCAGCACATAACCGTAAGAATAAAATAAGAATAGATTGGCAAATC
GCGTTAAAATATATTCTTCAACAG
CACCACACCCGCCGCGTGGCGAGACGTTCTAGAATGCCGGACCCCGG
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TTCATCAAAGAAACAAAACGTACACGGCTGGAATCAGCGGGGTCATT
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CCTGCCTATTCATTATCACCAATTACCGAAGAAAATACCGAATTATC
CGGAAATTATTTCGGAAGCGTCATACATGGCTTAATGCGCGTCACACG
CCTTATTAAAGCCGTTCGAACCTCCCAGTAATACGGATTCTCAAGAGT
TTGCGCTCAAAGCCTGGGGTGCCCGCAGTGT
AGGCGGCCTGATTGCCTGCCGGTG
CCGTAATGGGATGGGAACAAAC
ATAAAGCTAAATCGGTTGTATCCCTTTAATTGCG
TGATAAATAAGTGGTTTGAAAT
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CGCCACCCTCAGAACCGAACCGCCACCCTCAGGTTAGTAA
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CCCCAGCAAGAACGTGTAGTAGCATTAACATTAATGCTGTAGCTCAA**TTTTT***
CACACAACATCTGCCATGTACATCGTTGAGATATACCAGT**TTTTT***
TTCTCCGTAGGTCACGTTGGTGTAGCCGCACACAACATTCGTTAATAAAACGAA**TTTTT***
***TTTTT**GTCATTTTTGCGGATGGCTTAGAGCTTAATTGCTGAATACCAATAAAATAC
***TTTTT**AAGTGTTTTTATAATCACCAGAACGGAACCAGTAGCAAGCAACGATCT
***TTTTT**AAAAGAGTCTGTCCATCACGCAAATAGCAATA
***TTTTT**TGTAAATCGTCGCTATTAATTAATTTTCCAAATTTAA
***TTTTT**ACATAGCGATAGCTTATATATTTTTACTAGAAATGACAAGAACAAC
GGTTGCGAAAACGACCTTTCTCCCATTCGCCAAGGGTTATTTGAAT*
*GTCAGTATGACGCTCAATCAAGTTAGAATGGAGGCTGAGACTCCTCA

Table S2: DNA sequences for nucleosomal DNA

Color coding:
BOLD: 601 sequence
GREEN: Gal4-2C site (19bp long, from 8th – 26th bp of 601)

6LN	CATCGG**CTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGC ACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTC CCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGT**ATAATG
6L	CATCGG**CTGGAGA**CCGGAGGGCTGCCCTCCGG**TCAATTGGTCGTAGACAGCTCTAGC ACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTC CCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGT**ATAATG
26LN	AAGGAGGACACTGGGACATGCATCGG**CTGGAGAATCCCGGTGCCGAGGCCGCTCAAT TGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTT AACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGT** ATAATGCATAGGGCAGTGAGTTGACG
26L	AAGGAGGACACTGGGACATGCATCGG**CTGGAGA**CCGGAGGGCTGCCCTCCGG**TCAAT TGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTT AACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGT** ATAATGCATAGGGCAGTGAGTTGACG
51LN	AGCTTGTCGACGAATTCAGATTCATAAGGAGGACACTGGGACATGCATCGG**CTGGAGAA TCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCAC GTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCA CGTGTCAGATATATACATCCTGT**ATAATGCATAGGGCAGTGAGTTGACGCTACAATCAC GAATTCTGGATCCGA
51L	AGCTTGTCGACGAATTCAGATTCATAAGGAGGACACTGGGACATGCATCGG**CTGGAGA**C CGGAGGGCTGCCCTCCGG**TCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCA CGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGC ACGTGTCAGATATATACATCCTGT**ATAATGCATAGGGCAGTGAGTTGACGCTACAATCA CGAATTCTGGATCCGA
75LN	TCTAGAGCTAGCCTAGGCTCGAGAAGCTTGTCGACGAATTCAGATTCATAAGGAGGACA CTGGGACATGCATCGG**CTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGA CAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAG GGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGT**ATAATGCATAG GGCAGTGAGTTGACGCTACAATCACGAATTCTGGATCCGATACGTAACGCGTCTGCAGC ATGCG
75L	TCTAGAGCTAGCCTAGGCTCGAGAAGCTTGTCGACGAATTCAGATTCATAAGGAGGACA CTGGGACATGCATCGG**CTGGAGA**CCGGAGGGCTGCCCTCCGG**TCAATTGGTCGTAGA CAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAG GGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGT**ATAATGCATAG GGCAGTGAGTTGACGCTACAATCACGAATTCTGGATCCGATACGTAACGCGTCTGCAGC ATGCG

Table S3: Oligo sequences for PCR

Color coding:
BOLD: 601 sequence
GREEN: Gal4-2C site (19bp long, from 8th – 26th bp of 601)
RED: Cy3-label

6LN-forward	Bio-5'-CATCGG**CTGGAGAATCCCGGTGCCGAGGCC**-3'
6L-forward	Bio-5'-CATCGG**CTGGAGA**CCGGAGGGCTGCCCTCC-3'
6LN-Cy3-forward	Bio-5'-CATCGG**CT**GGAGAATCCCGGTGCCGAGGCC-3'
6L-Cy3-forward	Bio-5'-CATCGG**CT**GGAGACCGGAGGGCTGCCCTCC-3'
6LN-reverse	Bio-5'-CATTATACAGGATGTATATATCTGACACGT-3'
26N-forward	Bio-5'-AAGGAGGACACTGGGACATGCATCGG**CTGG**-3'
26N-reverse	Bio-5'-CGTCAACTCACTGCCCTATGCATTATACAG-3'
51N-forward	Bio-5'-AGCTTGTCGACGAATTCAGATTCATAAGGA-3'
51N-reverse	Bio-5'-TCGGATCCAGAATTCGTGATTGTAGCGTCA-3'
75N-forward	Bio-5'-TCTAGAGCTAGCCTAGGCTCGAGAAGCTTGTC-3'
75N-reverse	Bio-5'-CGCATGCTGCAGACGCGTTACGTATCGGATCC-3'