Supplementary Information

Clear, extended field-of-view single-molecule imaging by highly inclined swept illumination

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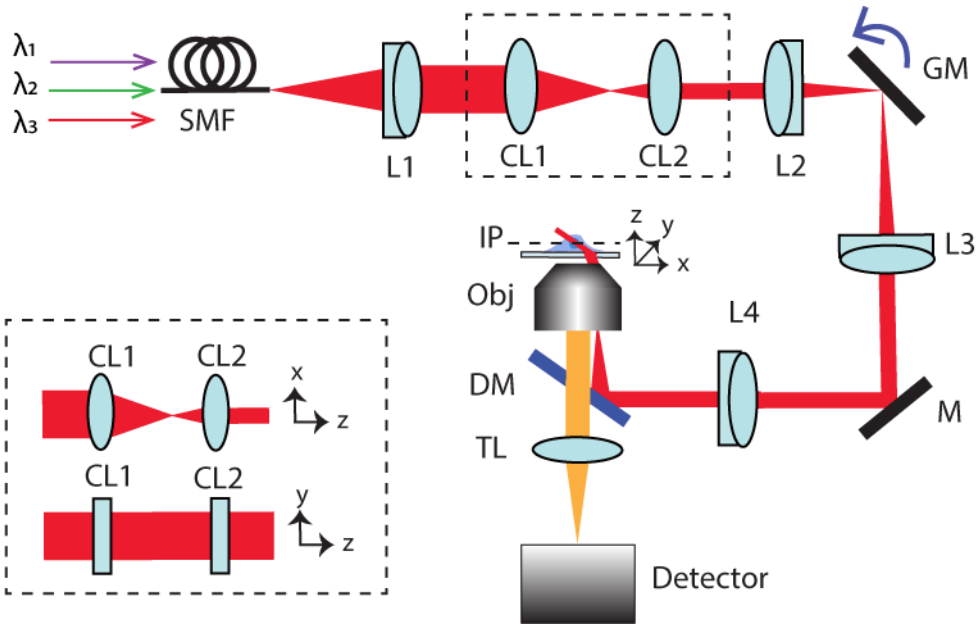
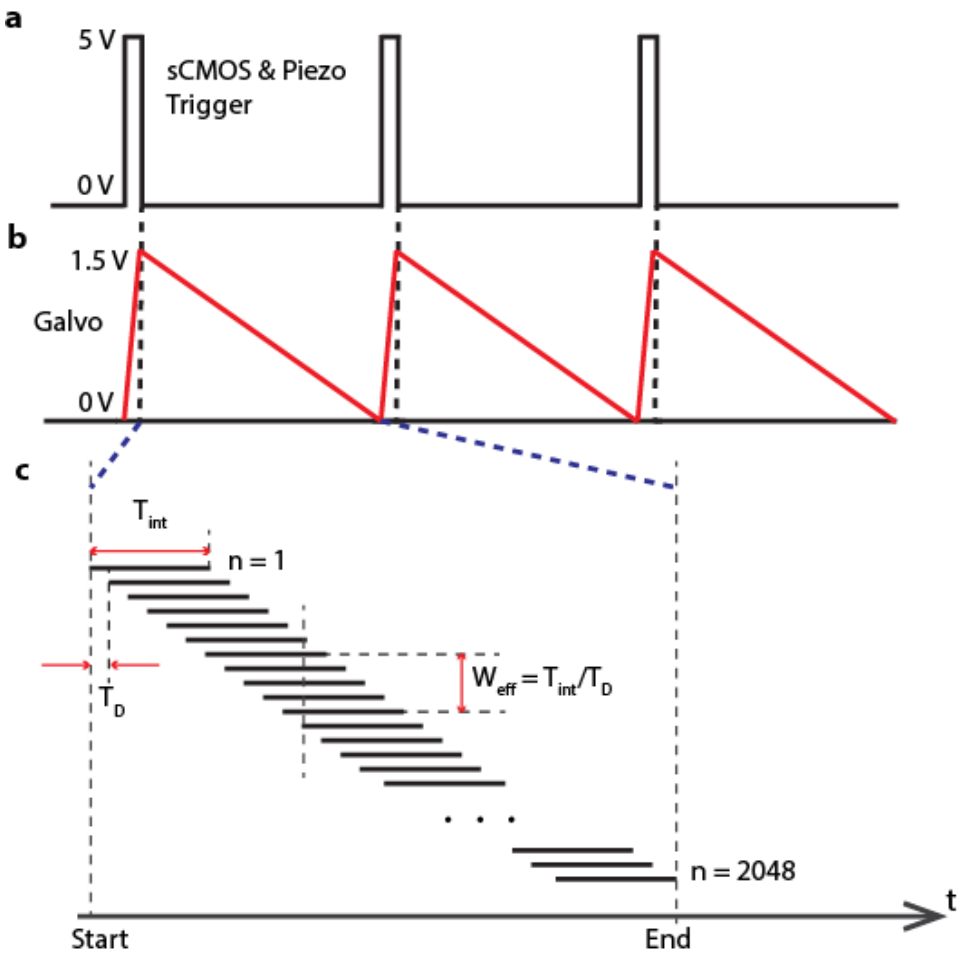
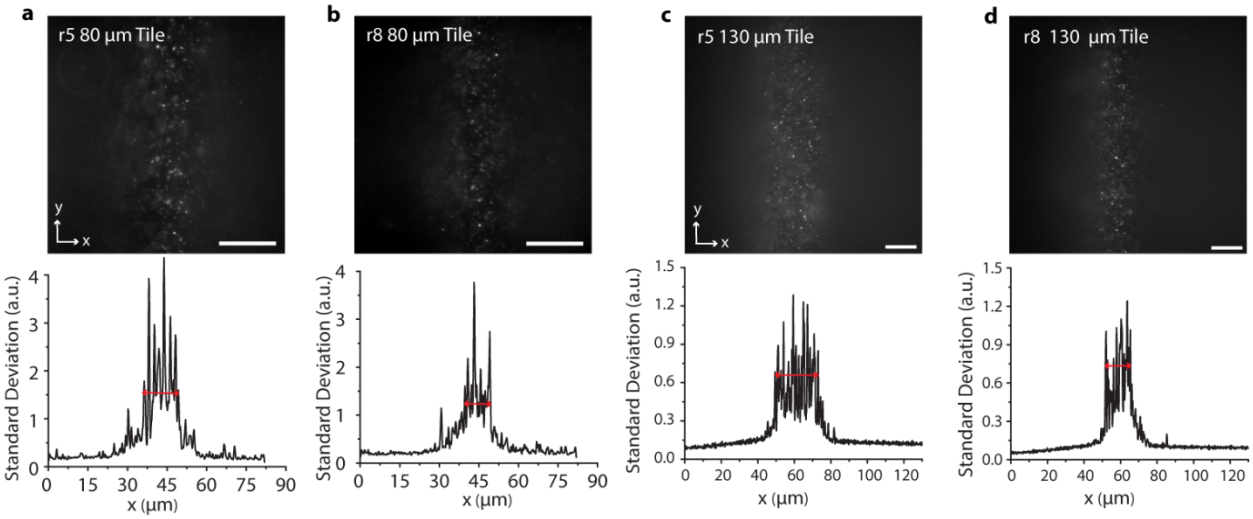
Supplementary Figures



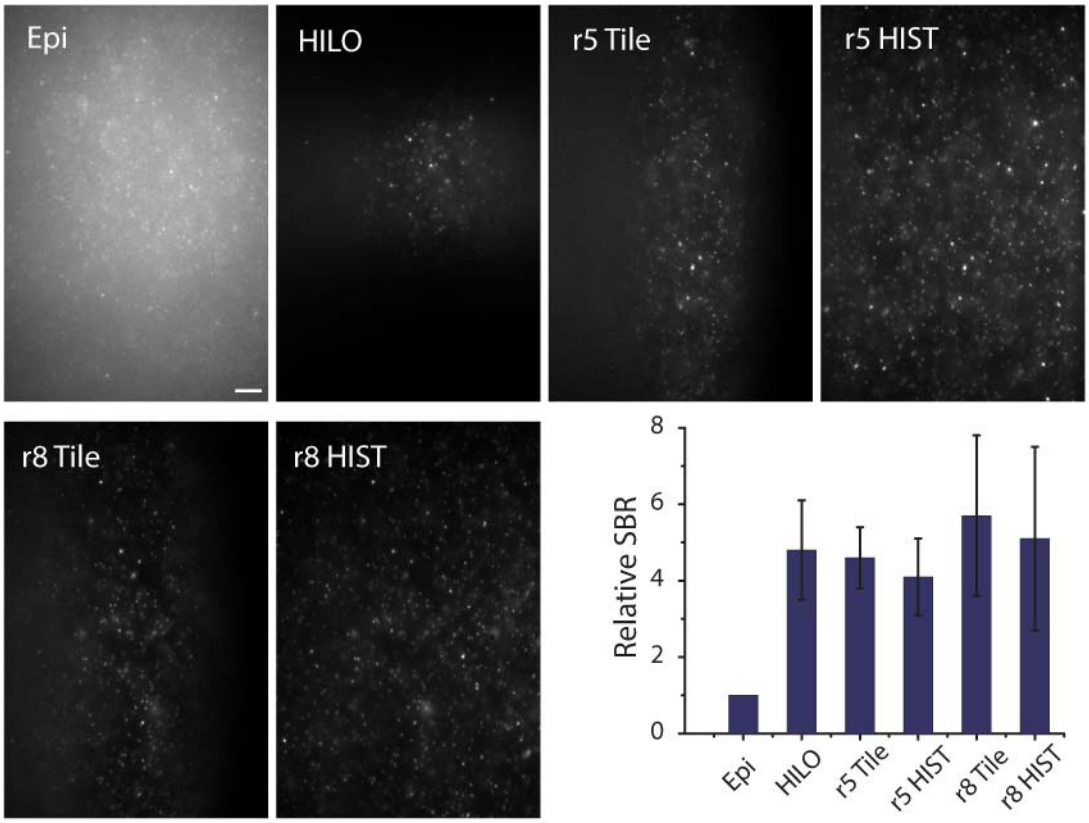
Supplementary Figure 1. Detailed experimental scheme of Highly Inclined Swept Tiles (HIST) microscopy. λ_1 = 405 nm, λ_2 = 561 nm, λ_3 = 638 nm; CL1-2, cylindrical lenses; DM, dichroic mirror; GM, galvo mirror; IP, imaging plane; L1-6, lenses; M, mirror; SMF, single mode fiber; TL, tube lens.



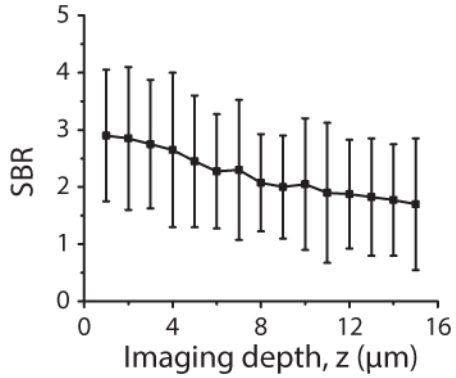
Supplementary Figure 2. DAQ timing diagram. (a,b) A trigger signal from a function generator starts the galvo mirror and sCMOS acquisition. (c) Details of light sheet rolling shutter mode control. T_{int} is the integration time of each pixel line, T_D is the delay time of consecutive pixels, W_{eff} is the effective acquisition width. In our experiments, we used T_{int} = 60 ms, T_D = 0.36 ms and W_{eff} = 166.



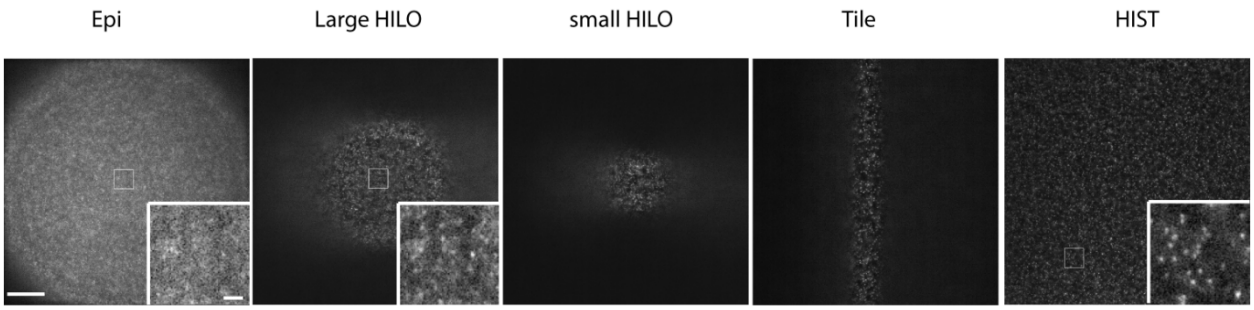
Supplementary Figure 3. Effective beam width of tile illumination. (top) Fluorescence images of 20 nm beads in 3D hydrogel using a compression ratio of 5 and 8 with a tile length of 80 µm or 130 µm, respectively. Scale bar, 20 µm. (bottom) Standard deviation projection along y direction for each image. The illumination widths of r = 5 and r = 8 for ~ 80 µm tile are ~10 µm and ~14 µm, and those of r = 5 and r = 8 for ~ 130 µm tile are ~14 µm and ~24 µm, indicated by the double sided red arrows.



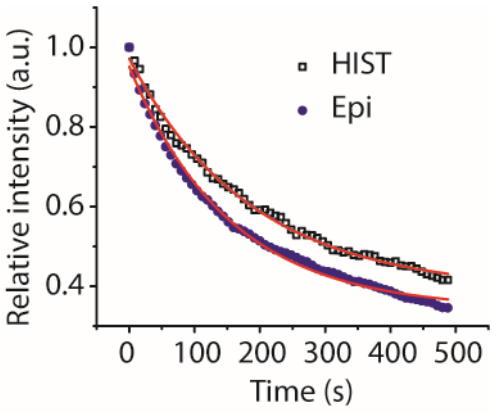
Supplementary Figure 4. Images of 20 nm beads in hydrogel and signal to background ratio (SBR) using different illumination methods at z = 5 μm. Scale bar, 5 μm.



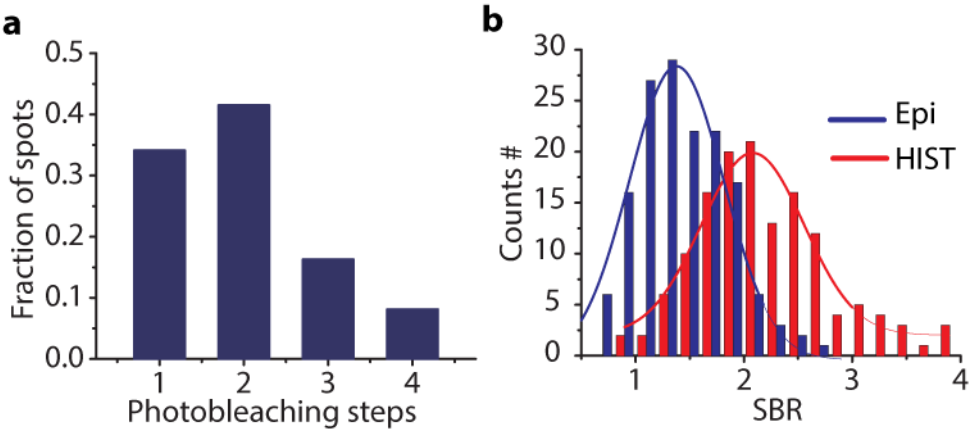
Supplementary Figure 5. SBR dependence on imaging depth using 20 nm beads in 3D hydrogel using r = 8 HIST illumination system.



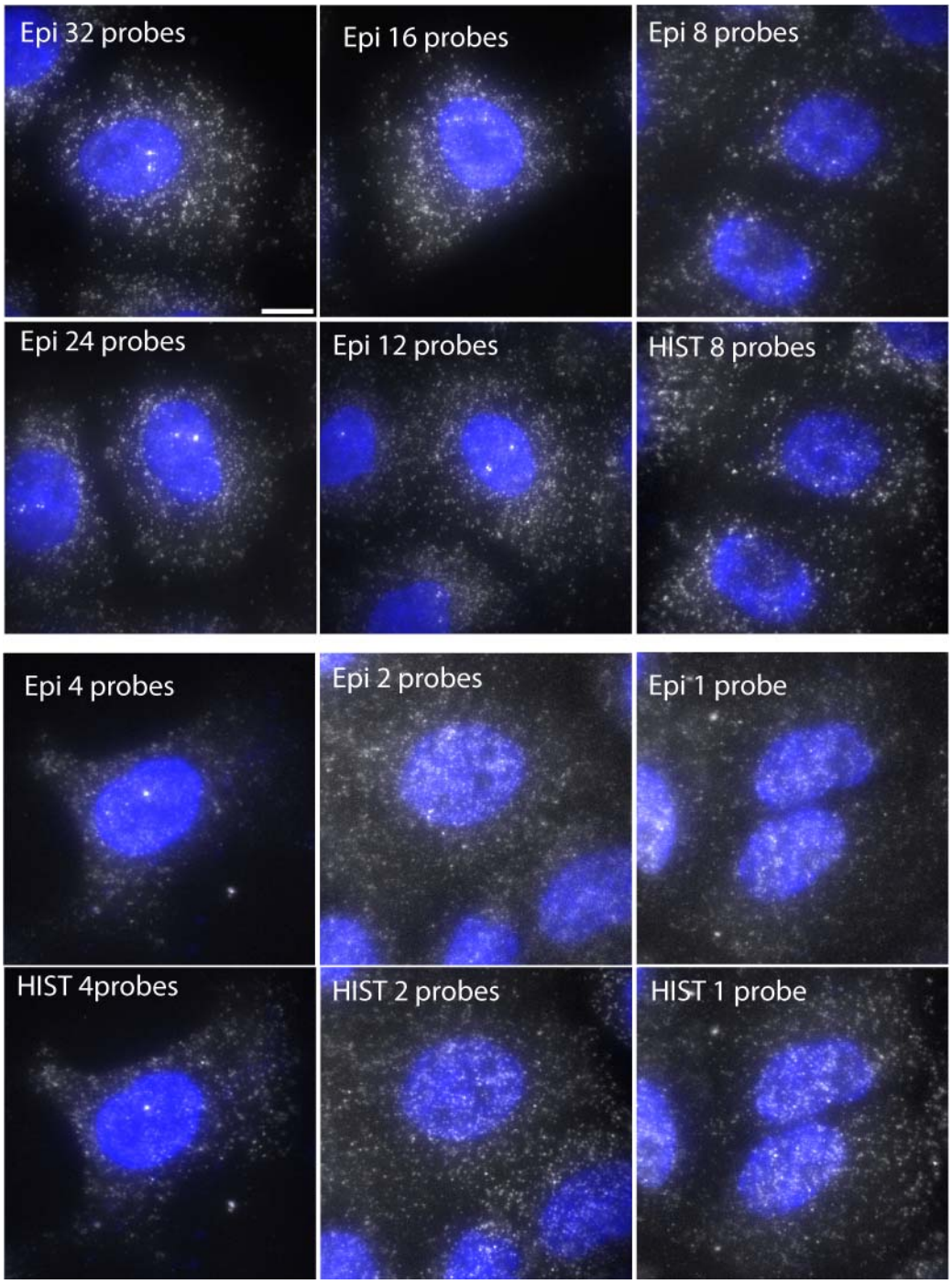
Supplementary Figure 6. Fluorescence background corrected images of single Atto647N DNA in 3D hydrogel by epi, large area HILO, small area HILO, tile and HIST illumination. The images were taken 5 μm above the surface. Scale bar, 20 μm and 2 μm (inset).



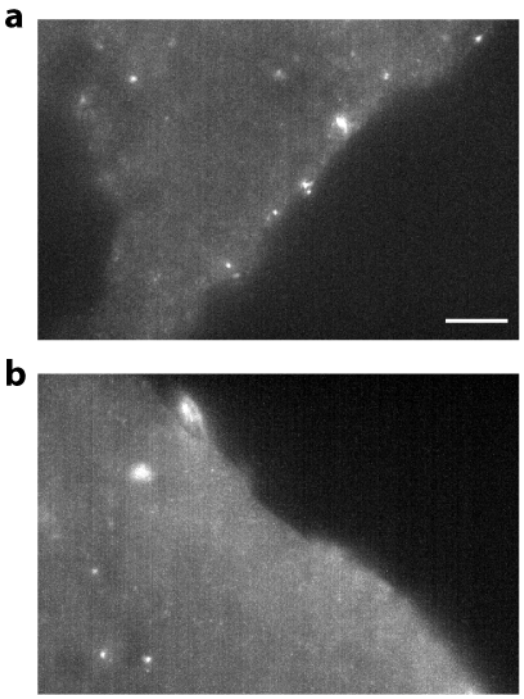
Supplementary Figure 7. Photobleaching curves for 130 x 130 x 5 μm^3 volume of Atto647N DNA in a hydrogel sample by epi and HIST illumination. The decay rates are 6.5 x 10^{-3} s^{-1} and 5.4 x 10^{-3} s^{-1}, respectively. Red curves are single exponential fits.



Supplementary Figure 8. Photobleaching step distribution (a) and SBR distribution (b) for 4 FISH probes in A549 cells. The blue and red curves are Gaussian fitting for the distributions. 211 mRNA spots were used.



Supplementary Figure 9. smFISH images in A549 cells with different number of probes using epi or HIST illumination. The DAPI stain is in blue and *EEF2* transcript is in white. A maximum intensity projection was performed on 20 z-stacks (5 µm thickness). The illumination power was 24 W/cm^2 and the integration time was 400 ms. Scale bar 10 µm.



Supplementary Figure 10. Control experiments for mouse brain tissue smFISH imaging. (a) A mouse brain tissue without any FISH probes. (b) Mouse brain smFISH image with 0.5% RNase treatment. Scale bar, 10 μm.

Supplementary Table 1. DNA sequences.

Name	Sequence	Experiment
probe1	5'-/ Acryd/GCCTCGCTGCCGTCGCCA/3ATTO647NN/-3'	Single probe hydrogel
P1	/5AmMC6/CCC AGG TAG AAC CGA AAG AA	*EEF2* A549 cells
P2	/5AmMC6/CTA CCG TGA AGT TCA CCA TG	
P3	/5AmMC6/CAG ACA TGT TGC GGA TGT TG	
p4	/5AmMC6/GTA TCA GTG AAG CGT GTC TC	
p5	/5AmMC6/GTT GAC TTG ATG GTG ATG CA	
P6	/5AmMC6/GCT CGT AGA AGA GGG AGA TG	
P7	/5AmMC6/ATG AGG TTG ATG AGG AAG CC	
P8	/5AmMC6/TCC GAG GAG AAG TCG ACA TG	
P9	/5AmMC6/CTT GTT CAT CAT CAG CAC AG	
P10	/5AmMC6/ACG ATG CGC TGG AAA GTC TG	
P11	/5AmMC6/GTA GGT GGA GAT GAT GAC GT	
P12	/5AmMC6/CGA GGA CAG GAT CGA TCA TG	
P13	/5AmMC6/AAA CTG CTT CAG GGT GAA GG	
P14	/5AmMC6/AAC TTG GCC ACA TAC ATC TC	
P15	/5AmMC6/TGG CTG ACT TGC TGA ACT TG	
P16	/5AmMC6/AAG ATG GGG TCC AGG ATC AG	
P17	/5AmMC6/CAT GAT CGC ATC AAA CAC CT	
P18	/5AmMC6/TGG ATG GTG ATC ATC TGC AA	
P19	/5AmMC6/TAG AAC CGA CCT TTG TCG GA	
P20	/5AmMC6/CCG AGA AGA CTC GTC CAA AG	
P21	/5AmMC6/TGG ATT GGC TTC AGG TAG AG	
P22	/5AmMC6/GCC CAT CAT CAA GAT TGT TC	
P23	/5AmMC6/AAT GTT CCC ACA AGG CAC AT	
P24	/5AmMC6/TGA ACT TCA TCA CCC GCA TG	
P25	/5AmMC6/ACT CTG ACA ACA GGG CTG AC	
P26	/5AmMC6/CGA TGA TGC ACT GCA CCA TG	
P27	/5AmMC6/ACG TTC GAC TCT TCA CTG AC	
P28	/5AmMC6/TTG TTG GGG GAC TTG GAG AG	
P29	/5AmMC6/CAA AGC ACC AGA TCT TGC GG	
P30	/5AmMC6/TGA TGT CGG TGA GGA TGT TG	
P31	/5AmMC6/CTT GAT CTC GTT GAG GTA CT	
P32	/5AmMC6/GTC AGC ACA CTG GCA TAG AG	
M1	/5AmMC6/GCT CGT AGA AGA GGG AGA TG	*EEF2* mouse brain
M2	/5AmMC6/ATG AGG TTG ATG AGG AAG CC	
M3	/5AmMC6/AAA CTG CTT CAG GGT GAA GG	
M4	/5AmMC6/AAC TTG GCC ACA TAC ATC TC	
M5	/5AmMC6/CAA AGC ACC AGA TCT TGC GG	