

PRMT5-mediated methylation of YBX1 regulates NF- κ B activity in colorectal cancer

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Running title: PRMT5 methylates YBX1 at R205

NON-PUBLISHED SUPPLEMENTAL INFORMATION

FIGURE 2A – WB: Anti-YBX1 and Anti-beta-actin

HEK293: Anti-YBX1 (left)

HT29: Anti-YBX1 (middle)

HT29 and HEK293, respectively: Anti-beta-actin (right)

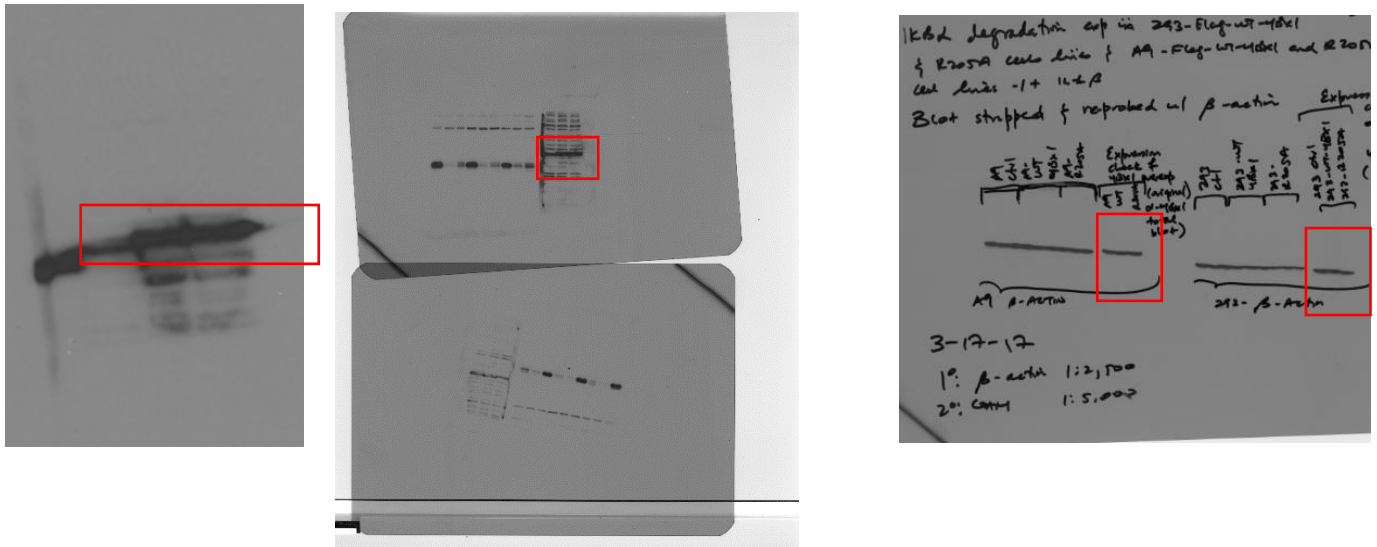


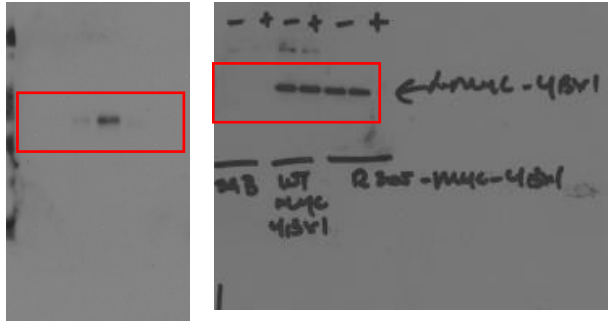
FIGURE 2B HEK293- IP: Anti-SDMA and IP: Anti-Myc (-/+ IL-1) were cropped from the same blot (10 sec exposure) stripped and reprobed with Anti-Myc. Input: Anti-Myc and Input: Anti-YBX1 were developed from different blots. Blots were developed along with multiple blots on the same film. Relevant parts of each blot are shown.

IP: Anti-SDMA (-/+ IL-1) (top left)

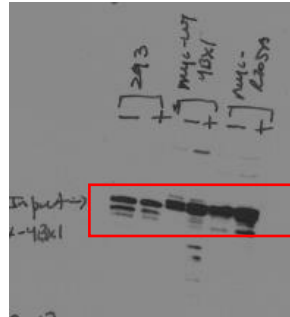
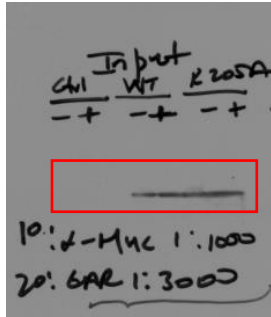
IP: Anti-Myc (-/+ IL-1) (top right)

Input: Anti-Myc (-/+ IL-1) (bottom left)

Input: Anti-YBX1 (-/+ IL-1) (bottom right)



Short exposure



Long exposure

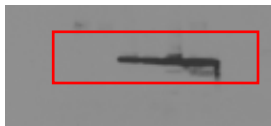


FIGURE 2B HT29 - IP: Anti-SDMA and IP: Anti-Myc (-/+ IL-1) were cropped from the same blot (10 sec exposure) stripped and reprobbed with Anti-Myc. Input: Anti-Myc and Input: Anti-YBX1 were developed from different blots. Blots were developed along with multiple blots on the same film. Relevant parts of each blot are shown.

IP: Anti-SDMA (-/+ IL-1) (top left)

IP: Anti-Myc (-/+ IL-1) (top right)

Input: Anti-Myc (-/+ IL-1) (bottom left)

Input: Anti-YBX1 (-/+ IL-1) (bottom right)

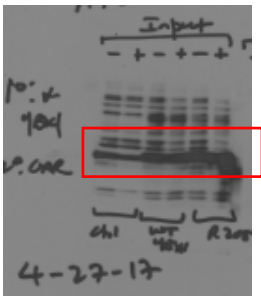
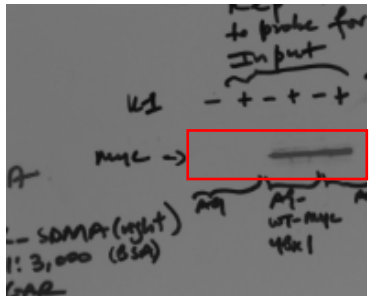
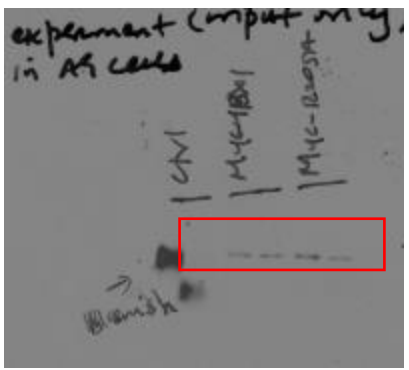
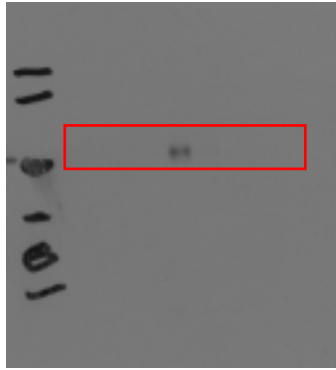


FIGURE 3A - IP: Anti-Myc and Input: Anti-Myc (-/+ IL-1) were cropped from the same blot (2 sec exposure) while the IP: Anti-Flag image was developed from the same blot that was stripped and re-probed with anti-Flag antibody (10sec exposure).

IP: Anti-Myc and Input: Anti-Myc (-/+ IL-1) (left)

IP: Anti-Flag (-/+ IL-1) (right)

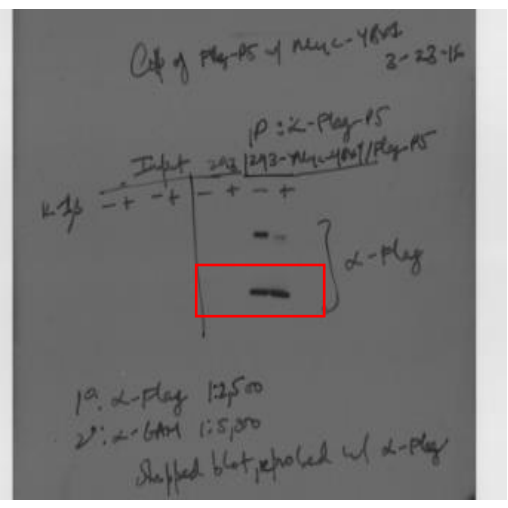
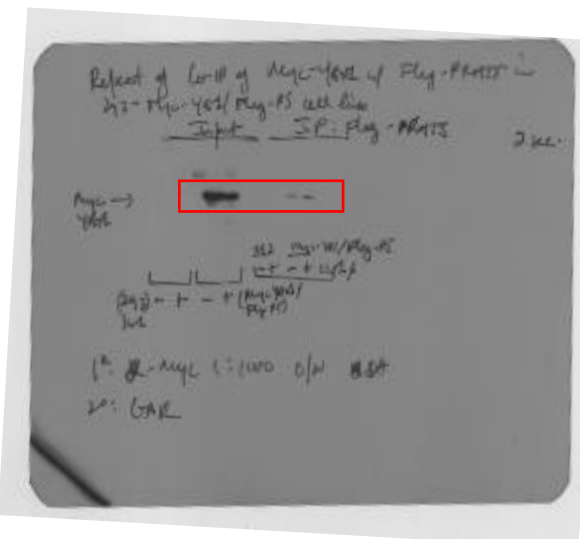


FIGURE 3B – Images were developed on blots with multiple other blots. Relevant part(s) of the blots shown. IP: Anti-Myc and Input: Anti-Myc (-/+ IL-1) were cropped from two different blots (1min exposure) while the IP: Anti-Flag image was developed from the same blot as IP: Anti-Myc that was stripped and re-probed with Anti-Flag antibody (1 min exposure). Note: samples were run alongside Myc-R205A samples which are shown in blots below but not in the main text Figures.

IP: Anti-Myc (-/+ IL-1) (left)

IP: Anti-Flag (-/+ IL-1) (middle)

Input: Anti-Myc (-/+ IL-1) (right)

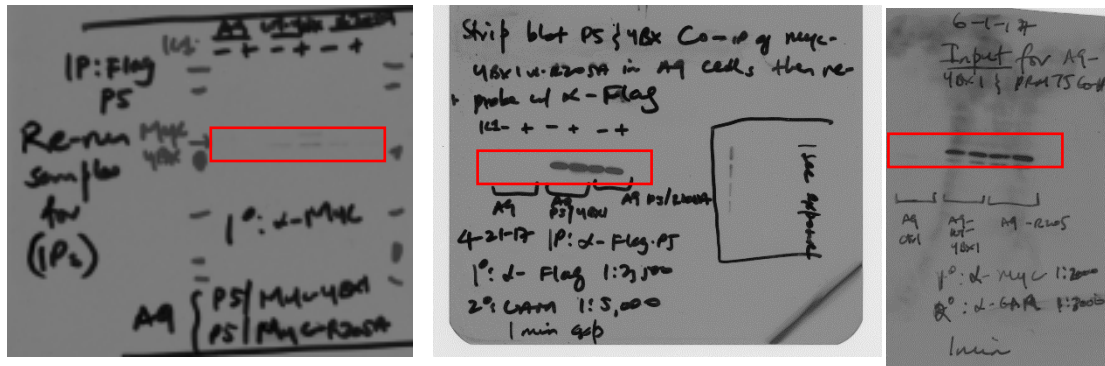


FIGURE 3C

(Left) Images were developed same blot as other samples. Relevant part(s) of the blots shown. WB: anti-PRMT5. The (right) WB for anti-actin was obtained from same blot stripped and re-probed.



FIGURE 3D

Images were developed on blots with multiple other blots. Relevant part(s) of the blots shown. IP: Anti-SDMA and Input: Anti-Myc (-/+ IL-1) were cropped from two different blots (1min) while the IP: Anti-YBX1 image was developed from the same blot as Input: Anti-Myc that was stripped and re-probed with Anti-YBX1 antibody (1 min exposure). The IP: Anti-Myc image was obtained from the IP: Anti-SDMA blot that was stripped and re-probed with Anti-Myc antibody.

IP: Anti-SDMA (-/+ IL-1) (top left)

IP: Anti-Myc (-/+ IL-1) (top right, long and short exposure)

Input: Anti-Myc (-/+ IL-1) (bottom left)

Input: Anti-YBX1 (-/+ IL-1) (bottom right)

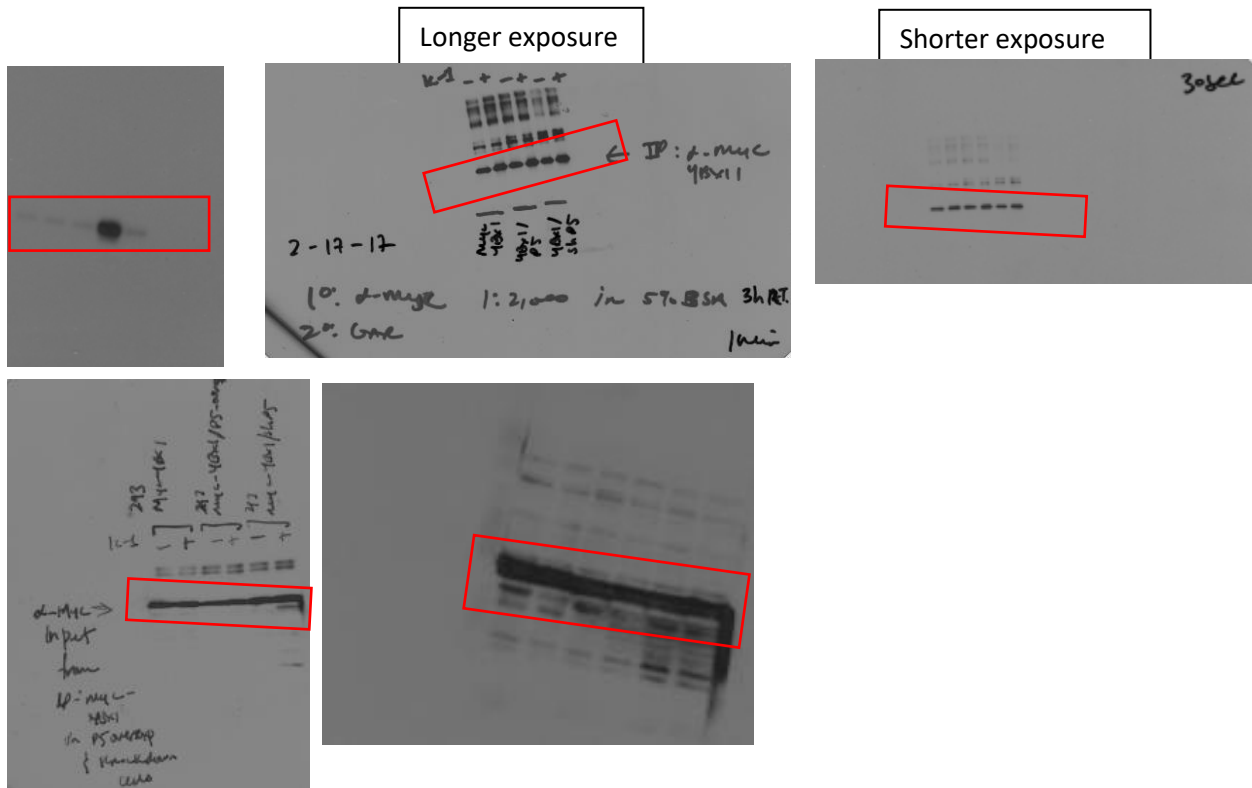


FIGURE 4B (HEK293) - IP: Anti-p65 and IP: Anti-Flag (-/+ IL-1) were cropped from the same blot (30 sec exposure) that was stripped and re-probed with Anti-Flag. Input: Anti-p65 image was developed from a different blot (10sec exposure). Blots were developed with other blots on the same film and the relevant part of each blot is shown.

IP: Anti-p65 (-/+ IL-1) (left)

IP: Anti-Flag (-/+ IL-1) (middle)

Input: Anti-p65 (-/+ IL-1) (right)

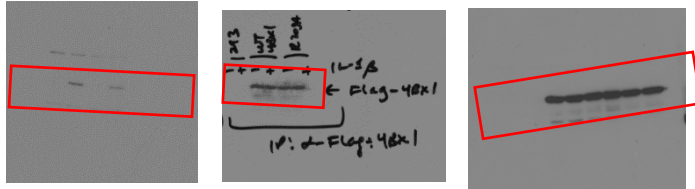


FIGURE 4B (HT29) - IP: Anti-p65 and IP: Anti-Flag (-/+ IL-1) were cropped from the same blot (30 sec exposure) that was stripped and re-probed with Anti-Flag. Input: Anti-p65 image was developed from a different blot (10sec exposure). Blots were developed with other blots on the same film and the relevant part of each blot is shown.

IP: Anti-p65 (-/+ IL-1) (left)

IP: Anti-Flag (-/+ IL-1) (middle)

Input: Anti-p65 (right)

