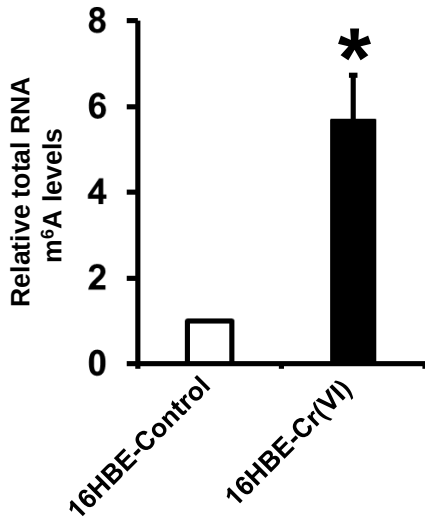
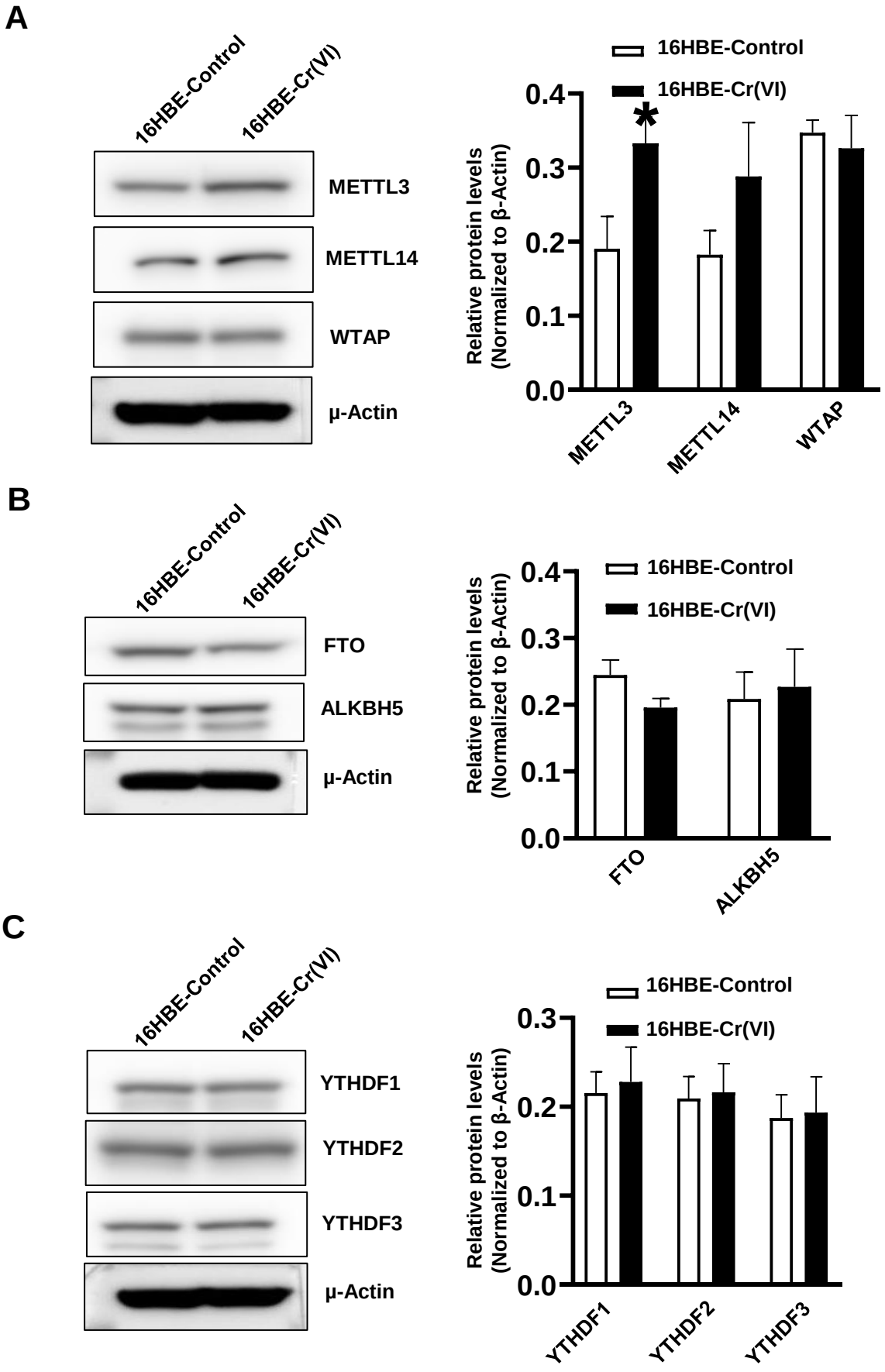
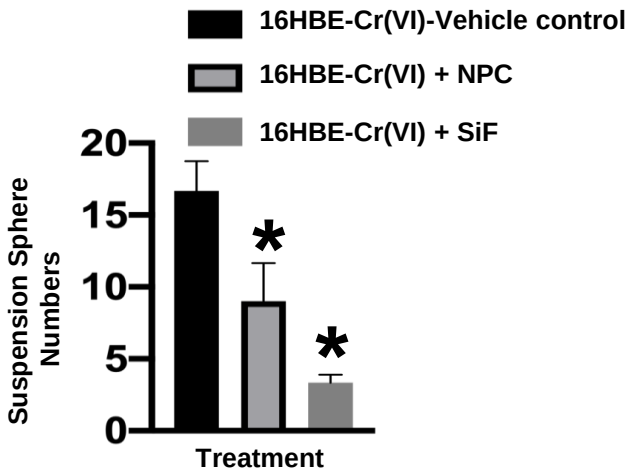


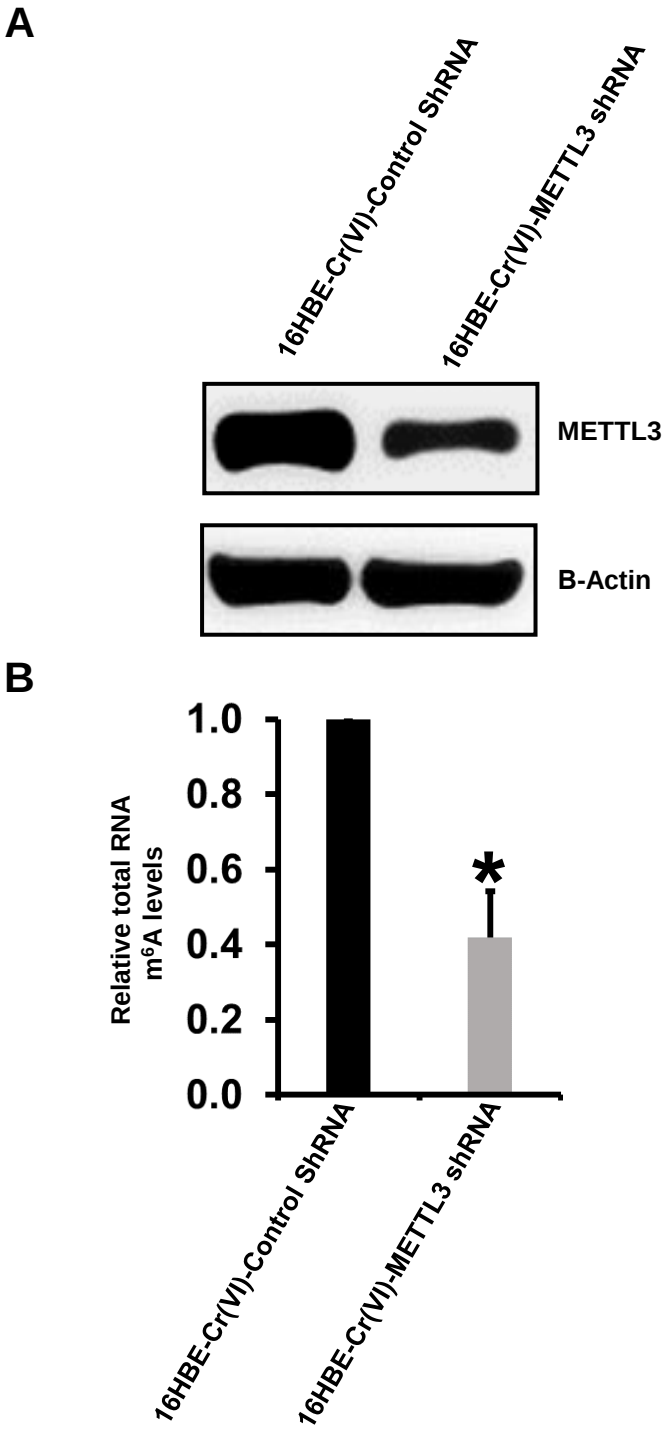
Supplementary Figure 1. Total RNA m^6A modification levels are significantly increased in chronic Cr(VI) exposure-transformed human bronchial epithelial16HBE cells. The immortalized 16HBE cells were continuously exposed to 0. 25 μM of Cr(VI) ($K_2Cr_2O_7$) for 40 weeks for cell transformation as described in Materials and Methods. At the end of cell transformation, passage-matched control cells (16HBE-Control) and Cr(VI)-exposed cells [16HBE-Cr(VI] were collected for total RNA m^6A level analysis by using the EpiQuik m^6A RNA Methylation Quantification Kit. The total RNA m^6A levels are expressed relative to the passage-matched control cells (means ± SD, n=3) * $p< 0.05$.


A
16HBE-Control
16HBE-Cr(VI)
METTL3
METTL14
WTAP
μ-Actin
16HBE-Control
16HBE-Cr(VI)
Relative protein levels (Normalized to β-Actin)
0.4
0.3
0.2
0.1
0.0
*
METTL3
METTL14
WTAP
B
16HBE-Control
16HBE-Cr(VI)
FTO
ALKBH5
μ-Actin
16HBE-Control
16HBE-Cr(VI)
Relative protein levels (Normalized to β-Actin)
0.4
0.3
0.2
0.1
0.0
FTO
ALKBH5
C
16HBE-Control
16HBE-Cr(VI)
YTHDF1
YTHDF2
YTHDF3
μ-Actin
16HBE-Control
16HBE-Cr(VI)
Relative protein levels (Normalized to β-Actin)
0.3
0.2
0.1
0.0
YTHDF1
YTHDF2
YTHDF3

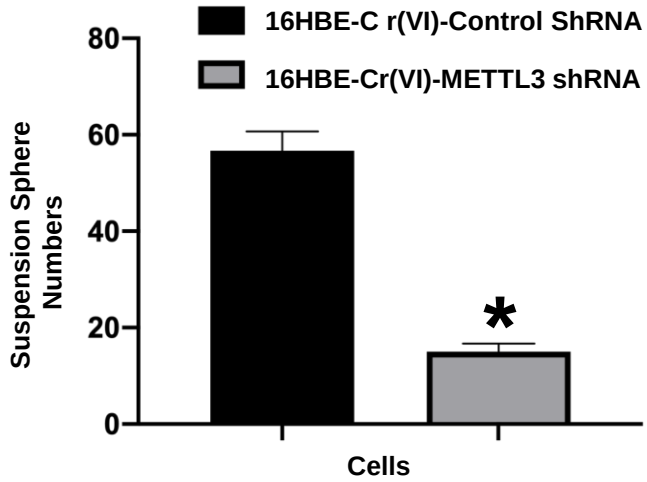
Supplementary Figure 2. The RNA methyltransferase METTL3 expression levels are significantly up-regulated in chronic Cr(VI) exposure-transformed human bronchial epithelial 16HBE cells. (A-C) Representative Western blot analysis images for the selected m^6A writers (A), erasers (B) and reader(C) proteins in passage-matched control cells and Cr(VI)-transformed 16HBE cells. The corresponding bar charts present the quantitative results of Western blot analysis of m^6A writers, erasers and reader proteins in passage-matched control cells and Cr(VI)-transformed cells. The corresponding Western blot protein band intensities were quantified using the ImageJ software and normalized by the intensity of the β-actin protein band (means ± SD, n=3). * $p< 0.05$.



Supplementary Figure 3. Pharmacological inhibitions of the methyltransferase activities significantly reduces the number of suspension spheres formed by chronic Cr(VI) exposure-transformed 16HBE cells. Cells were treated once with a vehicle control, NPC (Neplanocin A, 200 nM), or SiF (Sinefungin, 25 µM) when cells were seeded into the 24 well-plate. The suspension spheres were photographed and counted (if >100 µm) 10 days after the cell seeding. The results are presented as means ± SD (n=3). * $p< 0.05$.



Supplementary Figure 4. Stable knockdown of METTL3 in Cr(VI)-transformed 16HBE cells significantly reduces their total RNA m^{6}A modification levels. (A) Representative Western blot image showing the METTL3 knockdown efficiency in Cr(VI)-transformed 16HBE cells. (B) METTL3 knockdown significantly reduces total RNA m^{6}A levels determined by using the EpiQuik m^{6}A RNA Methylation Quantification Kit. The total RNA m^{6}A levels are expressed relative to the control shRNA cells (means ± SD, n=3). * $p< 0.05$.



Supplementary Figure 5. Stable knockdown of METTL3 significantly reduces the numbers of suspension spheres formed by Cr(VI)-transformed 16HBE cells. The results are presented as means ± SD (n=3). * $p< 0.05$.

Supplementary Figure 6. METTL3 expression levels in chronic Cr(VI)-exposed BEAS-2B-Control shRNA [BEAS-2B_Control shRNA-Cr(VI)] and BEAS-2B-METTL3 shRNA cells [BEAS-2B-METTL3 shRNA-Cr(VI)]. A representative Western blot analysis image for METTL3 expression levels in BEAS-2B-Control shRNA and BEAS-2B-METTL3 shRNA cells exposed to Cr(VI) for 20 weeks.