Downregulation of Stem-loop binding protein by nicotine *via* α7-nicotinic acetylcholine receptor and its role in nicotine-induced cell transformation

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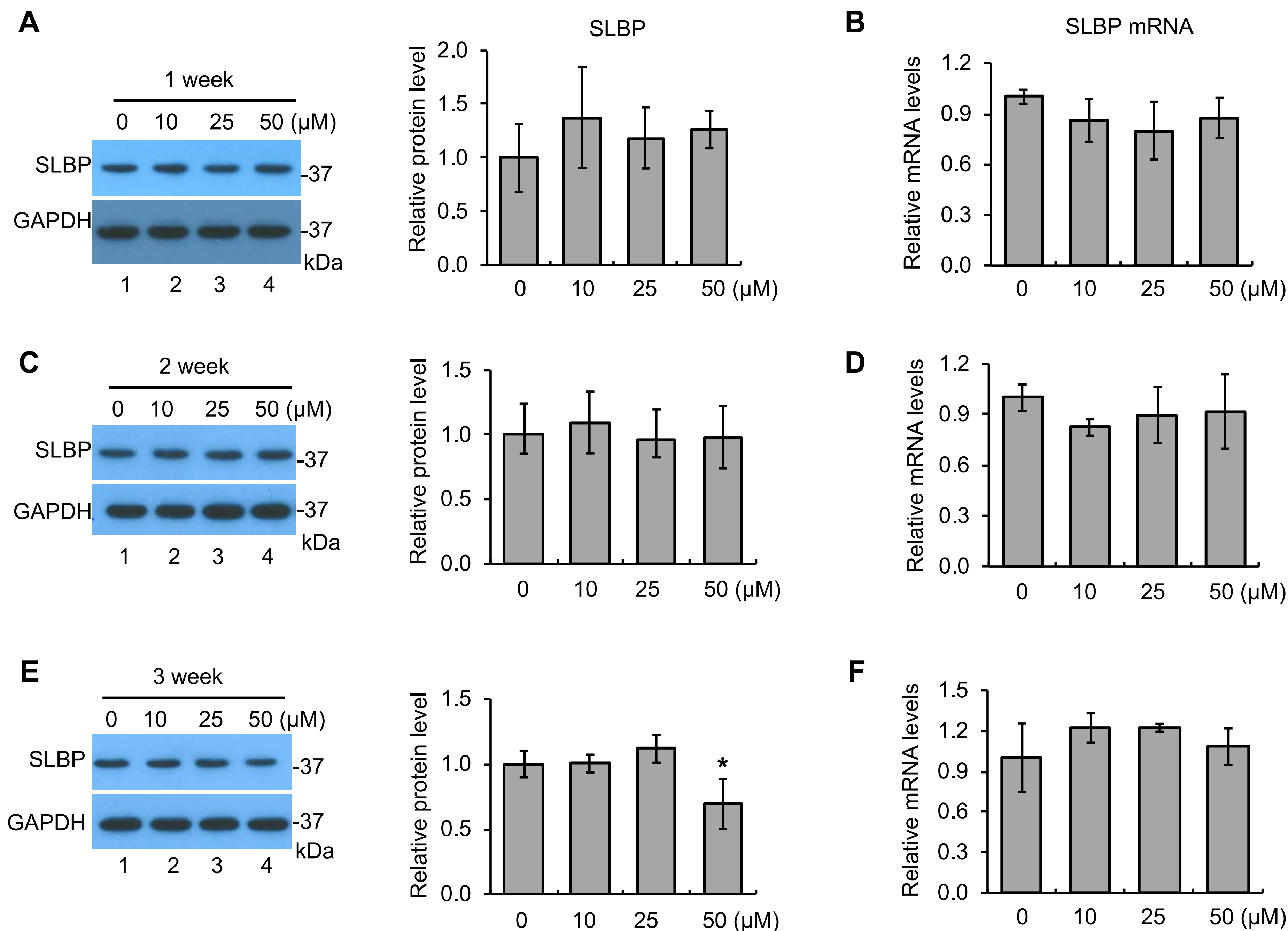
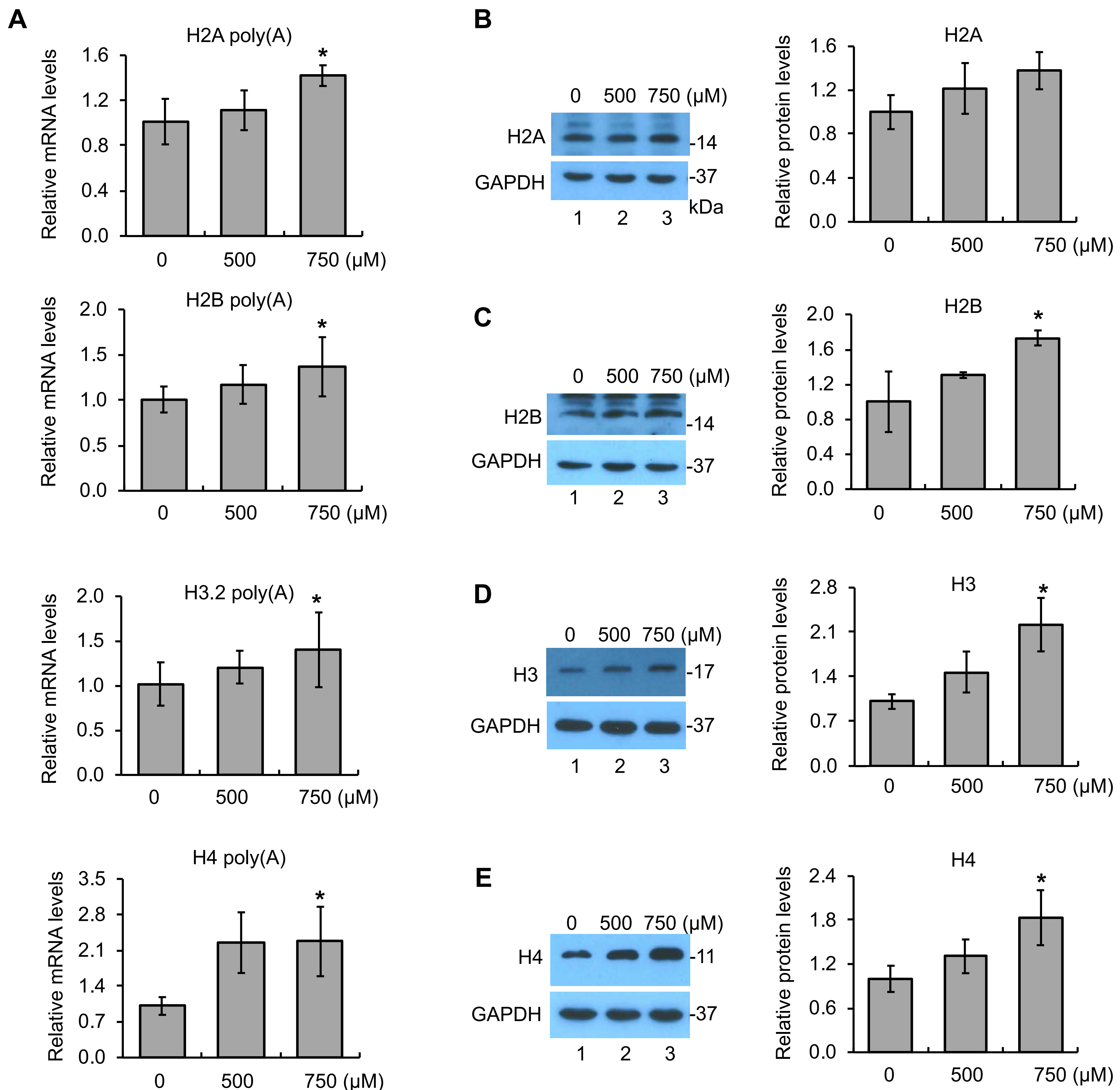
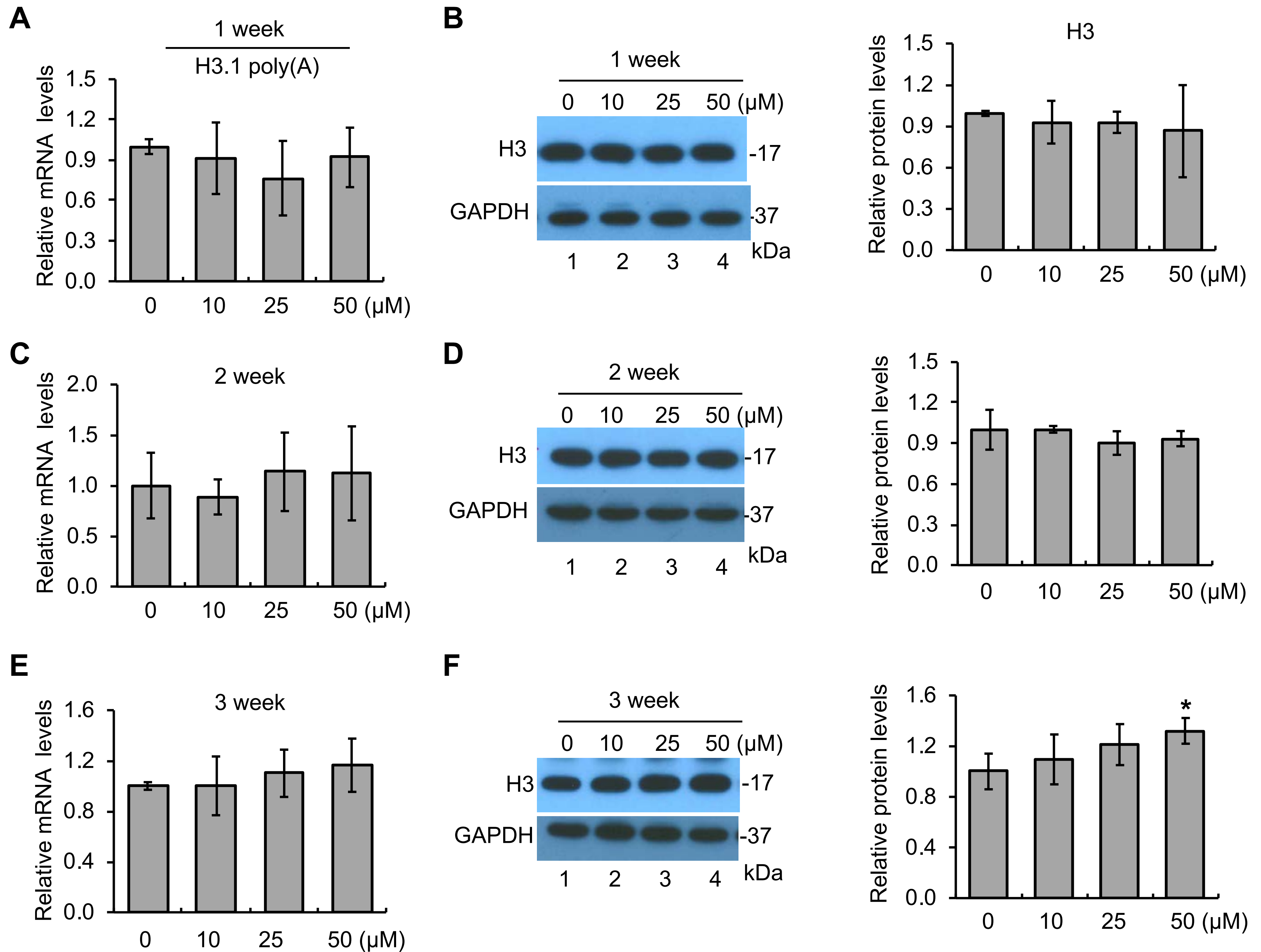
Supplementary Material:



Supplementary Figure 1. Changes in SLBP protein and mRNA levels in BEAS-2B cells following low-dose nicotine treatment for 1-3 weeks
The SLBP protein and mRNA levels were measured by Western blot (A, C, and E) and RT-qPCR (B, D, and F), respectively, in BEAS-2B cells treated with (10 μM, 25 μM, and 50 μM) or without nicotine for 1- (A and B), 2- (C and D), and 3-weeks (E and F). GAPDH was used as an internal control. The band intensities (left panels in A, C, and E) were quantified and presented as bar graphs (right panels in A, C, and E). The controls in lane 1 were used as references. The data shown are the mean ± S.D. (n = 3). *$p < 0.05$ vs. control group.

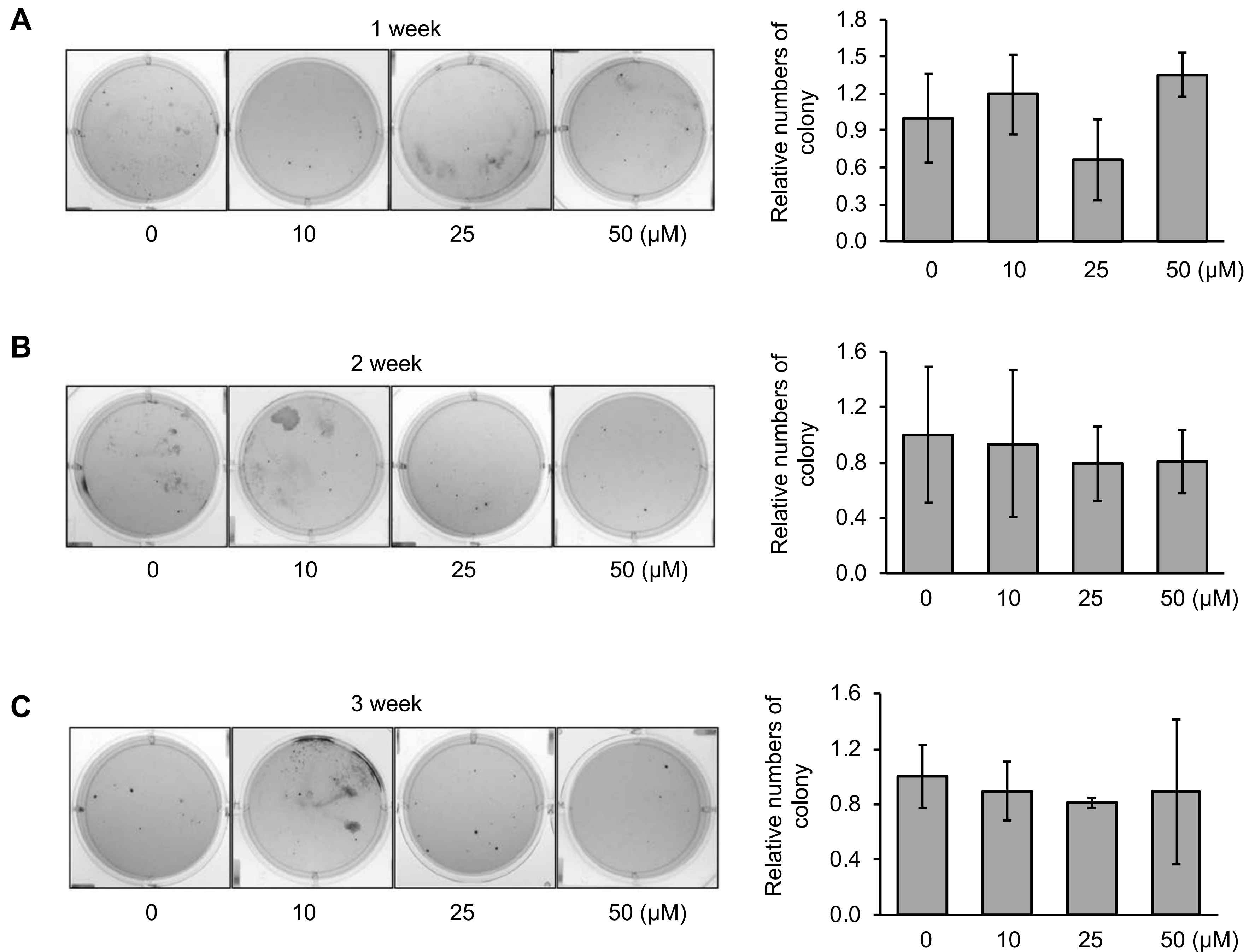


Supplementary Figure 2. Polyadenylation of canonical histone mRNAs by nicotine
The level of polyadenylated mRNAs for canonical histones H2A, H2B, H3.2, and H4 (A) as well as proteins for H2A, H2B, H3, and H4 (B-E) were determined by RT-qPCR and Western blot, respectively, in BEAS-2B cells treated with (500 μM and 750 μM) or without nicotine for 24 hrs. GAPDH was used as an internal control. The band intensities (left panels in B-E) were quantified and presented as bar graphs (right panels in B-E). The controls in lane 1 were used as references. The data shown are the mean ± S.D. (n = 3). *$p < 0.05$ vs. control group.



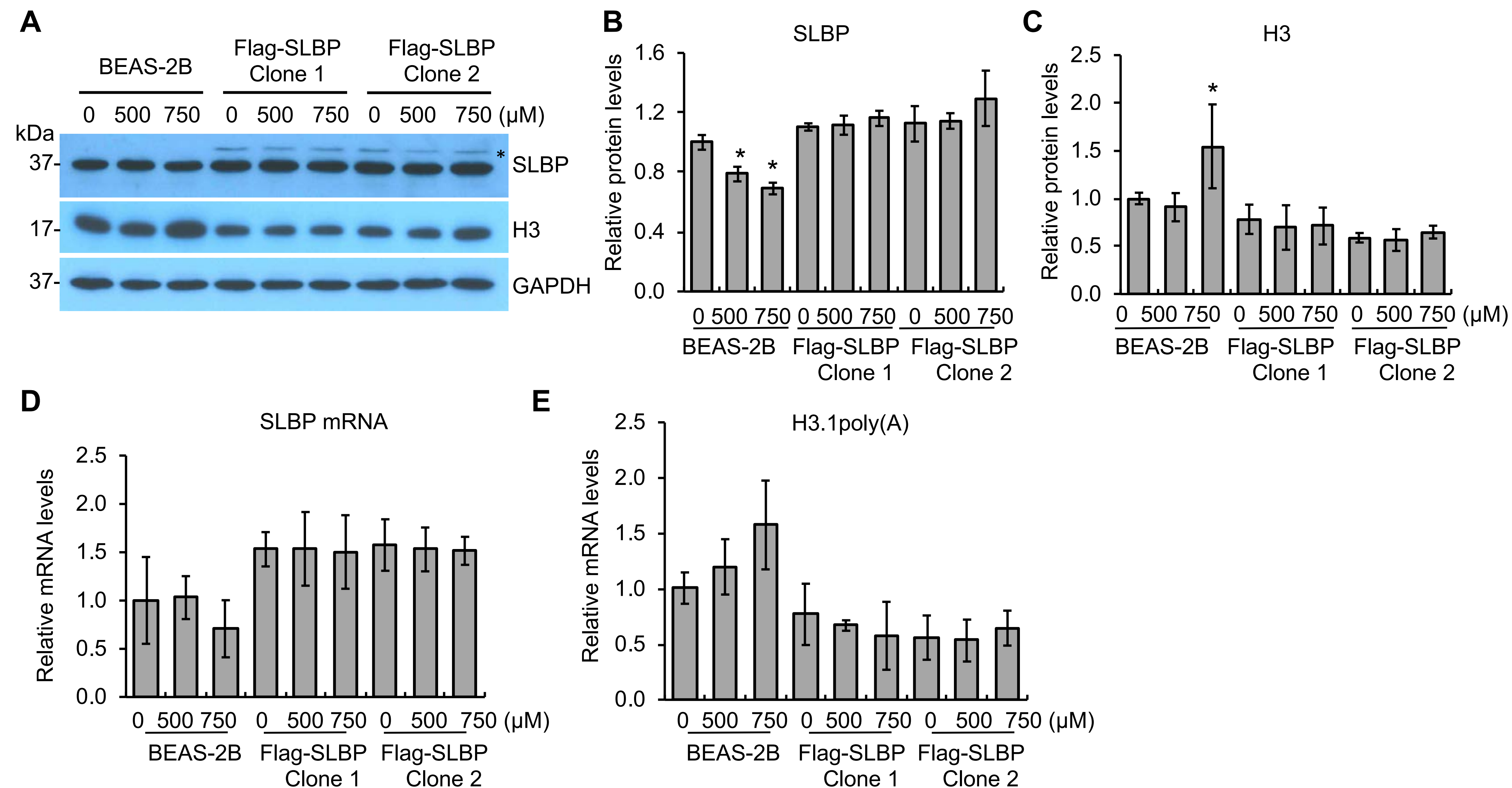
Supplementary Figure 3. Polyadenylation of canonical histone H3.1 mRNA by low-dose nicotine treatment for 1-3 weeks

The level of polyadenylated H3.1 mRNA (A, C, and E) and total H3 protein level (B, D, and F) were determined by RT-qPCR and Western blot, respectively, in BEAS-2B cells treated with (10 μM, 25 μM, and 50 μM) or without nicotine for 1- (A and B), 2- (C and D), or 3-weeks (E and F). GAPDH was used as an internal control. The band intensities (left panels in B, D, and F) were quantified and presented as bar graphs (right panels in B, D, and F). The controls in lane 1 were used as references. The data shown are the mean ± S.D. (n = 3). *$p < 0.05$ vs. control group.



Supplementary Figure 4. Anchorage-independent cell growth induced by low-dose nicotine treatment for 1-3 weeks

BEAS-2B cells treated with (10 μM, 25 μM, and 50 μM) or without nicotine for 1- (A), 2- (B), or 3-weeks (C) were subjected to soft-agar assays. The cells were plated in soft agar and cultured for 6 weeks. The data shown are the mean ± S.D. (n = 3). *p < 0.05. vs. control group.



Supplementary Figure 5. Overexpression of SLBP rescues downregulation of SLBP by nicotine exposure to 500 μM or 750 μM for 24 hrs

(A-C) BEAS-2B cells as well as two SLBP-overexpressing clones that have been stably transfected with Flag-tagged SLBP-expressing vector were treated with or without nicotine and then subjected to Western blot (A). The asterisk next to "SLBP" indicates the ectopic SLBP. The band intensities were quantified using ImageJ software and presented as bar graphs to show relative quantifications of SLBP (B) and H3 (C). GAPDH was used as an internal control. The controls in lane 1 were used as references. The data shown are the mean ± S.D. (n = 3). *$p < 0.05$ vs. control group.

(D and E) RT-qPCR detects changes in SLBP mRNA level and polyadenylation of H3.1 mRNA following nicotine exposure to 500 μM or 750 μM for 24 hrs in BEAS-2B as well as SLBP-overexpressing cells, i.e., Flag-SLBP clone 1 and clone 2. mRNA levels for SLBP and polyadenylated H3.1 were normalized to GAPDH. The data shown are the mean ± S.D. (n = 3). *$p < 0.05$ vs. control group.