

B cell adhesion to fibroblast-like synoviocytes is up-regulated by tumor necrosis factor-alpha via expression of human vascular cell adhesion molecule-1 mediated by B cell-activating factor

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Running title: Up-regulation of B cell adhesion to FLS by hVCAM-1 expression

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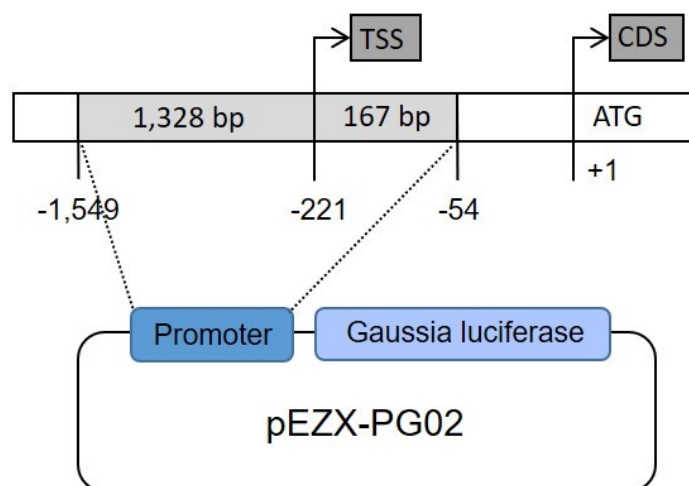
E-mail address: eunyimoon@sejong.ac.kr (E.Y. Moon)

A. hVCAM1 promotor (HPRM33454)

Length 1,496 bp

-1,539 ~ -54 from CDS

-1,328 ~ +167 from TSS

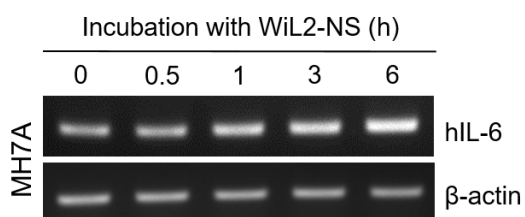


B.

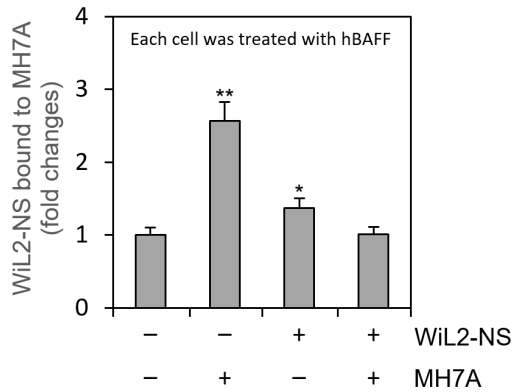
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-1,549      tccccacac cctagaacag catctccaac ttatttttcc ctccctgtct
-1,500 tttagtggga gccacatcag tatccaagag gagatccaga agcctctcca accaggtagg
-1,440 gacagttata gattccagac ctacagctat gcctttgita cagagtacaa atgttatata
-1,380 gtacaagttt attgtacaca tccattgag tctctgagct ttagaatttt ctgttagaat
-1,320 ttaacagttt ttcatgccc tatttacata ttattgctag tatttagaat ttcttctcc
-1,260 aaatgtataa cgtttattat tgcatttttt gtatccacta agtggaaaat catgcattag
-1,200 atattgtaga agtagataga acaatgaaca agaactggc ctgaccatga gaggaactga
-1,140 tcatccaatg ggggagatag acctgcacgt gttaataaaa aggaagtggc tattccggtt
-1,080 tctttttgat gggcaagcat ttgcaaggc ctgggctat gtgtgtgcaa ggctaagcca
-1,020 gttagttaat tgggattttt taaaaaggc acttcaactg ggggaaaagg aacatagagt
-960  tgggtattgt cccctgcct ataataaaaa cctattattt ttaattttt aactgggtt
-900  gcggttaaat ctacagccc aagagatttg ccactcaga tggattccat acacttgcct
-840  ttaagtatgc aaaaaaattc caattatcca gcaatttaac caaattattg gtaacttttc
-780  taaaacaaaa aaaaattgtt tcccttggtt tggcagcaat ttcagttaca gtcctttact
-720  ttctactcaa gaaaatagtt tcaaaaagtt gatgtttgtt gctaaaagaa ctatttttat
-660  gaataaatat aaaactaaga agttatgggt tccctttttt aaaaaatgac tcatcaaaaag
-600  aaataacttt ttcctttctc ttglaagaga aaaaaattaa tctcttttag aattgcaaac
-540  atatttcctt gatggagaaa atcaattcac atggcatagt cgttatttat ccagtcaaaa
-480  aaccagagta gaatttacta ctctgtctcc attttttctc tcccacccc cttaaccac
-420  attggattca gaaagcttca ttctgcaatc agcattgtcc ttatctttc cagtaaagat
-360  agccttttgg agtgaagat gaggaagagc ctgtatttta tagtcttggg agtgtcttct
-300  ttgccagga cagagagagg agcttcagca gtgagagcaa ctgaaggggt taatagtggg
-240  acttggctgg gtgtctgtta aactttttc cctggctctg ccctgggtt cccctgaag
-180  ggatttcct cgcctctgc aacaagacc ttataaagc acagacttc ttttacttc
-120  cgcggtatct gcatcgggcc tcaactggct caggagctga ataccctcc aggcacacac
-60  aggtgggaca caaataaggg ttttgaacc actattttct catcacgaca gcaactfaaa
+1  atgcctggga agatggtcgt gatccttggg gccctcaata tactttggat aatgttt
  
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Supplementary Figure S1. Schematic figures and sequences of pre-designed hVCAM1 (NM_001078) promoter. (A) hVCAMpromoter (HPRM33454) was 1,328 bp (-1,549 ~ -54) upstream from starting codon, ATG, of coding sequence (CDS) for hVCAM1 transcription. hVCAM1 promoter covers 1,328 bp upstream and 167 bp downstream from transcription starting site (TSS). (B) Sequence of hVCAM1 promoter includes AP-1 binding sites on -614 ~ -607 (black bold underlined) which is predicted by using TRANSFEC (version 8.3) database, TSS (black bold), and translation starting codon (italic black bold underlined) which is shown in between -53 and 57 bp (italic grey bold underlined).



Supplementary Figure S2. IL-6 expression in MH7A cells interacted with WiL2-NS cells. MH7A cells were plated overnight. Then, WiL2-NS cells were added and co-incubated to adhere for 0.5, 1, 3 and 6 h. WiL2-NS cells were separated and MH7A cells were collected at each time point. RNA was purified with Nucleozol[®]. The expression of hIL-6 in MH7A cells was respectively detected by RT-PCR.



Supplementary Figure S3. Interaction of MH7A cells with WiL2-NS cells was increased by the pre-treatment with hBAFF protein. MH7A cells were plated overnight. MH7A and WiL2-NS cells were pre-treated with hBAFF (20 ng/mL) for 30 min. Then, WiL2-NS cells were added and co-incubated to adhere for 30 min. Unbound WiL2-NS B cells were washed out and MTT assay was used to analyze bound B cells. Experiment was performed at least four times. Data in a bar graph represent the means \pm SD. * $p < 0.05$, ** $p < 0.01$; significantly different from hBAFF protein-untreated control group.