

**The insect repellent *N,N*-diethyl-*m*-toluamide (DEET) induces angiogenesis via  
allosteric modulation of M3 muscarinic receptor in endothelial cells**

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## Supplementary data

### Figure S1. DEET does not modify proliferation of B16F10 melanoma cell line

CyQUANT<sup>®</sup> assay reveals that both concentrations of DEET ( $10^{-8}$  M and  $10^{-5}$  M) do not increase proliferation of B16F10 cell line. Results are expressed at mean  $\pm$  SEM ; \* $p < 0.05$  compared to control (Kruskal-Wallis with Dunn's multiple comparison test).

### Figure S2. DEET does not induce neither cytotoxicity nor apoptosis in HUVEC

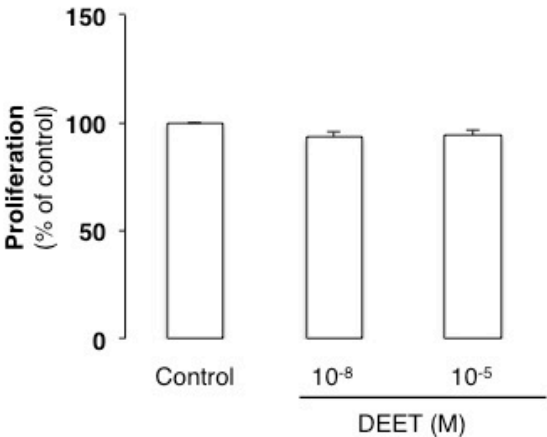
(a) *In vitro* cell viability was assessed by colorimetric analysis of MTT reduction after a treatment with  $10^{-8}$  M or  $10^{-5}$  M DEET for 24 h. (b) Flow cytometric DNA content histograms (fluorescence in arbitrary units, AU) of HUVEC exposed to  $10^{-8}$  or  $10^{-5}$  M DEET for 24 h, show that DEET does not induce apoptosis. Actinomycin D ( $10^{-6}$  M) was used as positive control. \* $p < 0.05$ ; \*\* $p < 0.01$  compared to control (Kruskal-Wallis with Dunn's multiple comparison test).

### Figure S3. *In vitro* capillary formation at increasing concentrations of DEET

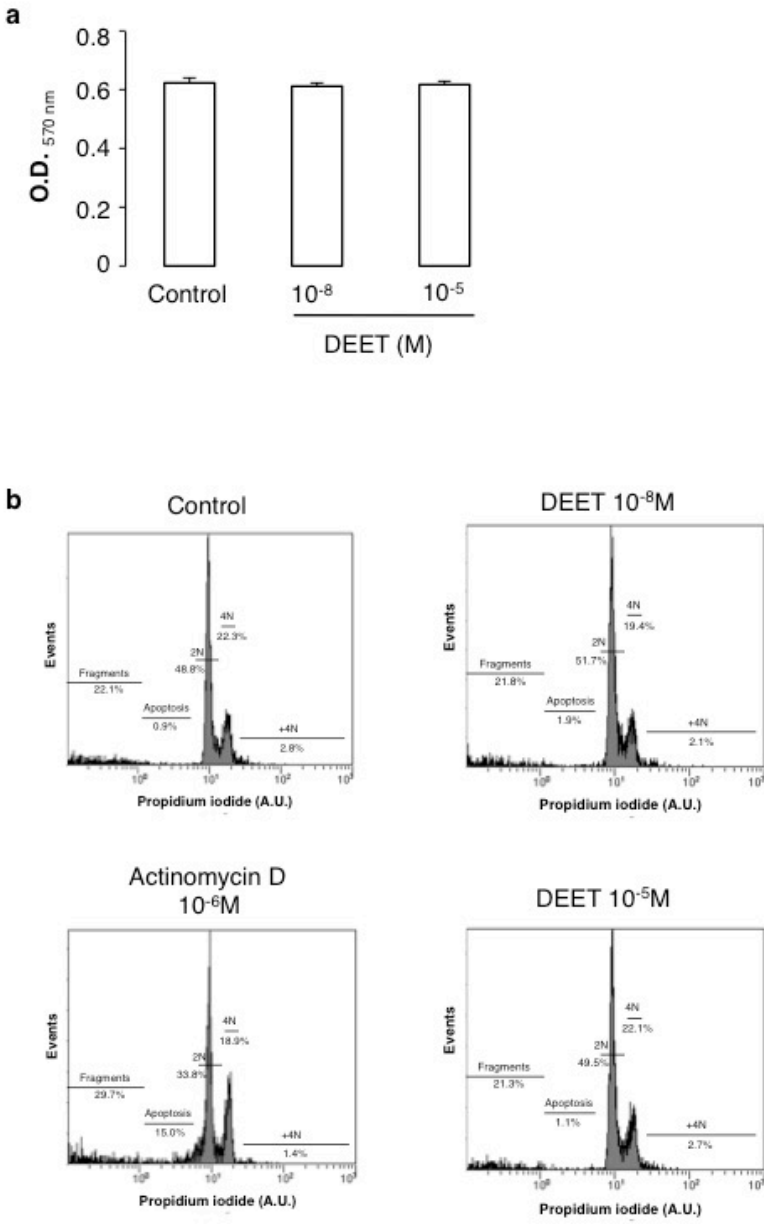
HUVEC were cultured on ECMgel<sup>®</sup> and treated for 24h with concentrations ranging from  $10^{-14}$  M to  $10^{-5}$  M DEET.  $10^{-8}$  M DEET allows reaching the plateau of capillaries formation. Reproducible data are obtained from five to six independent experiments. \* $p < 0.05$ ; \*\*\* $p < 0.001$  compared to control (Kruskal-Wallis with Dunn's multiple comparison test).

### Figure S4. Sequence alignment of rat and human M3 receptor

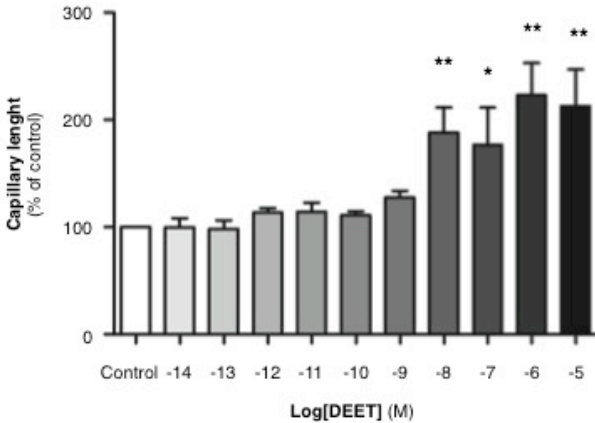
Supplemental data  
Figure S1



**Supplemental data**  
**Figure S2**



Supplemental data  
Figure S3



**Supplemental data  
Figure S4**

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ACM3_HUMAN  MTLHNNSTTSPLFPNIISSSWIHSPSDAGLP2GTVTTHFGSYNVSRAAGNFSSPDGTTDPL
ACM3_RAT    MTLHNSSTTSPLFPNIISSSWVHSPSEAGLP2GTVTQLGYSYNSQETGNFSSND-TSSDPL
Prim.cons.  MTLH2NSTTSPLFPNIISSSW2HSPS2AGLP2GTVT22GSYN2S222GNFSS2DGT22DPL

      70      80      90      100     110     120
ACM3_HUMAN  GGHTVWQVVFIAFLTGILALVTIIGNILVIVSFKVNRQLKTVNNYFLLSLACADLIIGVI
ACM3_RAT    GGHTIQQVVFIAFLTGFLALVTIIGNILVIVAFKVRQLKTVNNYFLLSLACADLIIGVI
Prim.cons.  GGHT2WQVVFIAFLTG2LALVTIIGNILVIV2FKVNRQLKTVNNYFLLSLACADLIIGVI

      130     140     150     160     170     180
ACM3_HUMAN  SMNLPTTYIIMNRWALGNLACDLWLAIIDYVASNASVMNLLVISFDRYFSTRPLTYRAKR
ACM3_RAT    SMNLPTTYIIMNRWALGNLACDLWLSIDYVASNASVMNLLVISFDRYFSTRPLTYRAKR
Prim.cons.  SMNLPTTYIIMNRWALGNLACDLWL2IDYVASNASVMNLLVISFDRYFSTRPLTYRAKR

      190     200     210     220     230     240
ACM3_HUMAN  TTKRAGVMIGLAWVISFVLWAPAILFWQYFVGKRTVPPGECFIQFLSEPTITFGTAIAAF
ACM3_RAT    TTKRAGVMIGLAWVISFVLWAPAILFWQYFVGKRTVPPGECFIQFLSEPTITFGTAIAAF
Prim.cons.  TTKRAGVMIGLAWVISFVLWAPAILFWQYFVGKRTVPPGECFIQFLSEPTITFGTAIAAF

      250     260     270     280     290     300
ACM3_HUMAN  YMFVVTIMTILYWRIYKETEKRTELAGLQASGTEAETENFVHPTGSSRS3SSYELQQQSM
ACM3_RAT    YMFVVTIMTILYWRIYKETEKRTELAGLQASGTEAETENFVHPTGSSRS3SSYELQQQGV
Prim.cons.  YMFVVTIMTILYWRIYKETEKRTELAGLQASGTEA2ENFVHPTGSSRS3SSYELQQQ22

      310     320     330     340     350     360
ACM3_HUMAN  KRSNRKRYGRCHFVFTTKSWKPSSEQMDQDHSSSDSWNNNDAAASLENSASSDEEDIGSE
ACM3_RAT    KRSNRKRYGRCHFVFTTKSWKPSAEQMDQDHSSSDSWNNNDAAASLENSASSDEEDIGSE
Prim.cons.  KRS2RRYGRCHFVFTTKSWKPS2EQMDQDHSSSDSWNNNDAAASLENSASSDEEDIGSE

      370     380     390     400     410     420
ACM3_HUMAN  TRAIYSIVLKLPGHSTILNSTKLPSSDNLQVPEELGMVDLERKADKLQAQKSVDDGGSF
ACM3_RAT    TRAIYSIVLKLPGHSSILNSTKLPSSDNLQVSNEDLGTVDVERNAHKLQAQKSMGDGDC
Prim.cons.  TRAIYSIVLKLPGHS2ILNSTKLPSSDNLQV2E2LG2VD2ER2A2KLQAQK2S22DG22

      430     440     450     460     470     480
ACM3_HUMAN  PKSFSKLP1QLESADVDTAKTSDVNSVVGKSTATLPLSFKEATLAKRFALKTRSQITKRRK
ACM3_RAT    QKDFTKLP1QLESADVDTGKTS2NNSADKTTATLPLSFKEATLAKRFALKTRSQITKRRK
Prim.cons.  2K2F2KLP1QLESADVDT2KTS2N2S22K2TATLPLSFKEATLAKRFALKTRSQITKRRK

      490     500     510     520     530     540
ACM3_HUMAN  MSLVKEKKAQTL5A1LLAF1ITWTFYNIMVLVNTFCDSKIPKTYWNLGYWLCYINSTVN
ACM3_RAT    MSLIKEKKAQTL5A1LLAF1ITWTFYNIMVLVNTFCDSKIPKTYWNLGYWLCYINSTVN
Prim.cons.  MSL2KEKKAQTL5A1LLAF1ITWTFYNIMVLVNTFCDSKIPK2WNLGYWLCYINSTVN

      550     560     570     580     590
ACM3_HUMAN  FVCYALCNKTFRTTFKMLLLCQCDK2KRRKQQYQQRQSVIFHKR2PEQAL
ACM3_RAT    FVCYALCNKTFRTTFKMLLLCQCDK2KRRKQQYQQRQSVIFHKR2PEQAL
Prim.cons.  FVCYALCNKTFRTTFK2LLCQCDK2KRRKQQYQQRQSVIFHKR2PEQAL

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**Alignment data :**

Alignment length : 590  
 Identity (\*) : 541 is 91.69 %  
 Strongly similar (:) : 26 is 4.41 %  
 Weakly similar (.) : 15 is 2.54 %  
 Different : 8 is 1.36 %  
 Sequence 0001 : ACM3\_HUMAN ( 590 residues).  
 Sequence 0002 : ACM3\_RAT ( 589 residues).