SUPPLEMENTAL DATA

VIP36 IS A TARGET OF ECTODOMAIN SHEDDING AND REGULATES PHAGOCYTOSIS IN MACROPHAGE Raw 264.7 CELLS

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Construction of various VIP36 mutants and chimeras

Cyto-Flag, -Lec, -Gly, ND, FN, LM, and SL mutants were constructed by two-step PCR. In the first step, N-terminal and C-terminal mutated fragments were amplified from wild-type VIP36 sequence using mutated primers and 5'- or 3'-primer. In the second step, mutated VIP36 were constructed from mixture of the fragments using 5'- and 3'-primers. Used mutated primers are: Cyto-Flag, tccagaaggattacaaggatgacgacgataagcggcaggagcggaacaa and tcctgccgcttatcgtcgtcatccttgtaatccttctggaacaccacggccc; -Lec, cctccatggaaacggcatcg and cgatgccgtttccatggagg; -Gly, tcggtgatggtgaacgatggctccctgtcctacgac and aggacagggagccatcgttcaccatcaccgagatgt; ND, atcgagcccagcgtcgacttcctcaagtcgcccaaa and gcgacttgaggaagtcgacgctgggctcgatcttgg; FN, gagcccagcgtcaacaacctcaagtcgcccaaagaca and ttgggcgacttgaggttgttgacgctgggctcgatct; LM, cccagcgtcaacttcatgaagtcgcccaaagacaacgt and gtctttgggcgacttcatgaagttgacgctgggctcga; SL, tcaacttcctcaagttgcccaaagacaacgtggacg and acgttgtctttgggcaacttgaggaagttgacgctg. KKFF, KRFF, KKFY, and KKAA mutants were constructed by one-step PCR. Those mutants were constructed from wild-type VIP36 using 5'- primer and mutated 3'-primers listed below. KKFF, gcggatcctcagaagaacttcttgttccgctcctgccgct; KRFF, gcggatcctcagaagaagcgcttgttccgctcc; KKFY, gcggatcctcagtagaacttcttgttccgctcctgccgct; KKAA, gcggatcctcaggcggccttcttgttccgctcctgccgct. Chimeras were constructed by two-step PCR from both VIP36 and VIPL sequences. N-terminal VIP36 fragments and C-terminal VIPL fragments were amplified using chimera primers and 5'- or 3'-primer, and then chimeras were constructed from mixture of the fragments using 5'- and 3'-primers. Used chimera primers are: Chimera #1, ggcccctgacggggtggcggctcttcctcatcgtcttttt and aaaaagacgatgaggaagagccgccaccccgtcaggggcc; Chimera #2, gtcaacttcctcaagtcgcctgagatgacagctccactgc and gcagtggagctgtcatctcaggcgacttgaggaagttgac; Chimera #3, actggaccaagatcgagccctcagtggacaatatgaa and ttcatattgtccactgagggctcgatcttggtccagt; Chimera #4, ctgatggtggagcacacgccagaagaggaaaagctccatc and gatggagcttttcctcttctggcgtgtgctccaccatcag; Chimera #5, acgagtggaagaactgcattgaagtgcccggagtccg and cggactccgggcacttcaatgcagttcttccactcgt. In all primers, the nucleotides different from wild-type VIP36 are underlined.

Preparation and transfection of murine bone marrow-derived macrophages

Bone marrow was collected from the tibiae and femurs of 8-wk-old $Tace^{flox/flox}/LysM\text{-}Cre^{-}$ mice (12). Red blood cells were removed with ACK lysing buffer, and the remaining cells were plated on tissue culture plates. Adherent cells were grown in IMDM supplemented with 20 % fetal bovine serum, 50 ng/ml recombinant human M-CSF (Peprotec), 2 mM L-Glutamine and antibiotics. At 8 days after harvesting, transfections were performed using Nucleofector™ (Lonza).

SUPPLEMENTAL FIGURE LEGEND

Fig. S1. Shedding of VIP36 in bone marrow-derived macrophages. N-terminally Myc-tagged VIP36 or VIPL was expressed in bone marrow-derived macrophages, and cell extracts (top panel) and anti-Myc immunoprecipitates from culture supernatants (bottom panel) were subjected to western blotting with an anti-Myc antibody.

Supplemental Figure S1