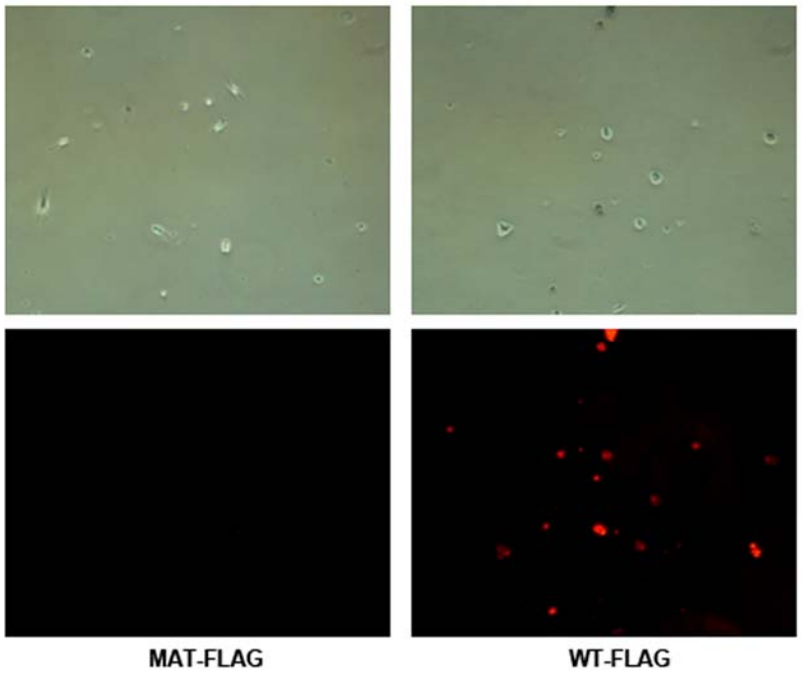
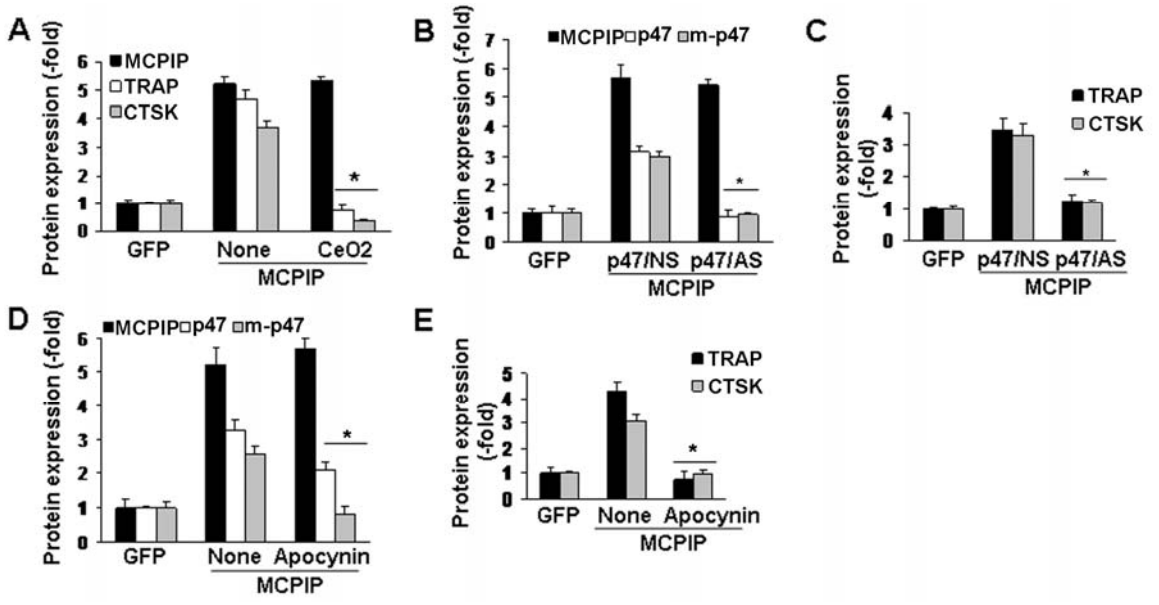
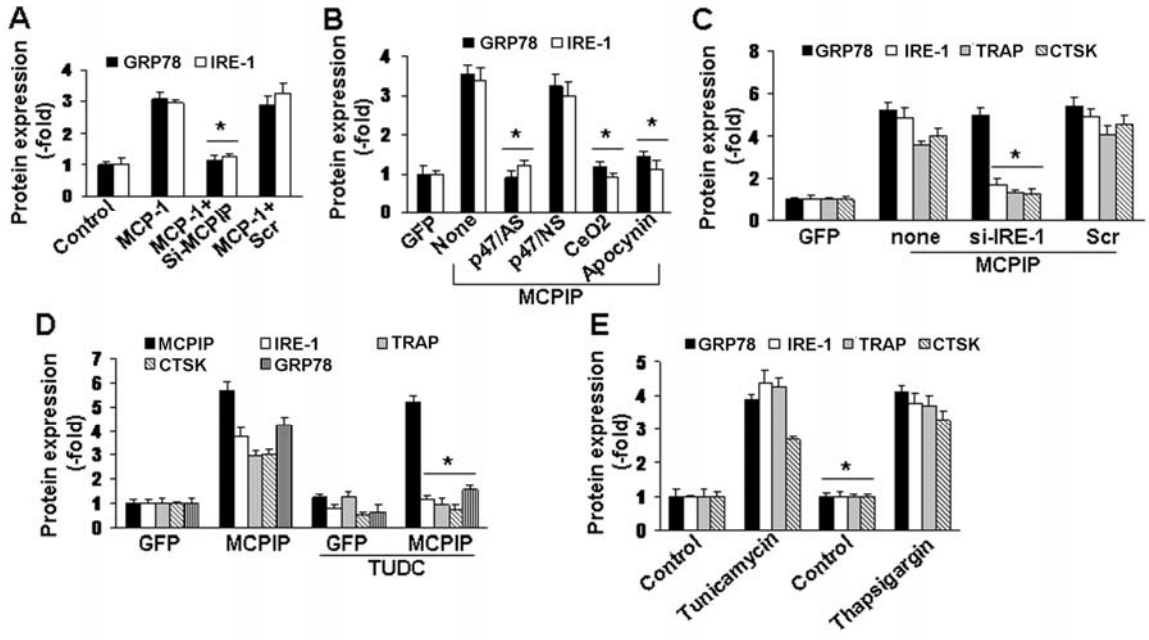


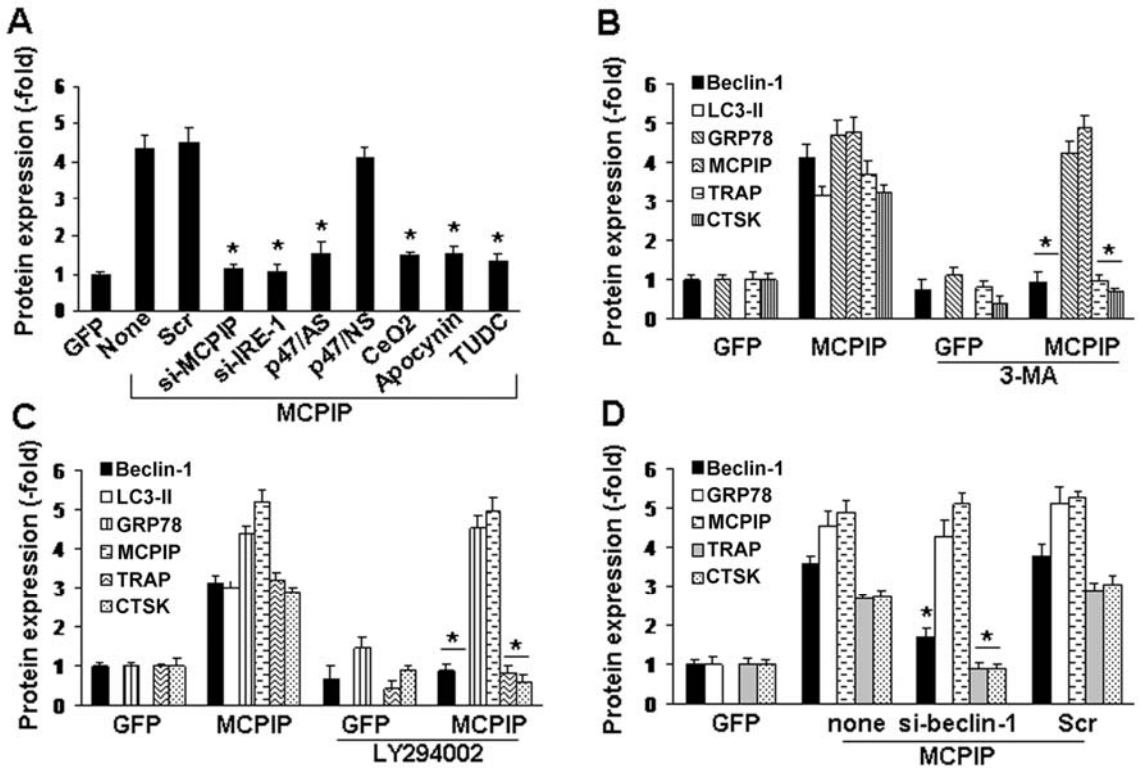
Supplementary Figure S1 MCPIP induced production of cellular ROS in bone marrow mononuclear cells (BBMN). Cells were transfected with empty vector (MAT-FLAG) or MCPIP-expression vector (WT-MCPIP) and after 24 hr stained with DHR123 that is a cell permeable nonfluorescent reagent that generates a fluorescent product (red) when oxidized by cellular ROS.



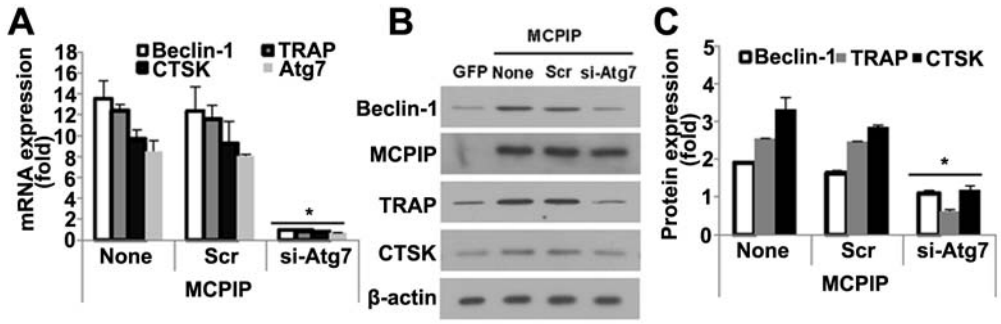
Supplementary Figure S2 ROS production involvement in MCPIP/MCP-1 induced OC precursor differentiation. Bone marrow monocytes were pretreated with CeO2 or apocynin for 6 h or $p47^{PHOX}$ antisense oligonucleotides for 24 h and then cells were transfected with MCPIP or GFP for 4 days. Cell lysate was collected and analysed using immunoblot with appropriate antibody and results were quantified against β-actin. ***A***, Effect of CeO2 on MCPIP-induced TRAP and CTSK expression [* $P<0.05$ compared to CeO2-untreated cells ("None")].***B***, Effect of $p47^{PHOX}$ antisense oligonucleotides on expression of $p47^{PHOX}$ and its translocation from cytoplasm to membrane [* $P<0.05$ compared to non-sense oligonucleotides treated cells ("p47/NS")]. ***C***, Effect of $p47^{PHOX}$ antisense oligonucleotides on MCPIP-induced TRAP and CTSK expression [* $P<0.05$ compared to non-sense oligonucleotides treated cells ("p47/NS")]. ***D***, Effect of apocynin on expression of $p47^{PHOX}$ and its translocation from cytoplasm to membrane [* $P<0.05$ compared to apocynin-untreated cells ("None")]. ***E***, Effect of apocynin on MCPIP-induced TRAP and CTSK expression [* $P<0.05$ compared to apoicynin-untreated cells ("None")].



Supplementary Figure S3 MCPIP induces ER stress via ROS production which involvement in OC precursor differentiation. After treatment, cell lysate was collected and analysed using immunoblot with appropriate antibody and results were quantified against β-actin. ***A***, MCP-1 induces ER stress marker GRP78 and IRE-1 expression via MCPIP (* $P<0.05$). ***B***, Effect of $p47^{PHOX}$ antisense oligonucleotides, CeO2 and apocynin on MCPIP-induced expression of GRP78 and IRE-1 (* $P<0.05$). ***C***, inhibition of ER stress by using knock-down of IRE-1 attenuates MCPIP-induced expression of TRAP and CTSK (* $P<0.05$). ***D***, pretreatment of TUDC inhibits MCPIP-induced expression of TRAP and CTSK and ER stress marker IRE-1 and GRP78 (* $P<0.05$). ***E***, ER stress inducer tunicamycin and thapsigargin induce expression of TRAP and CTSK (* $P<0.05$) .



Supplementary Figure S4 MCPIP induces autophagy via ROS production and ER stress which involvement in OC precursor differentiation. After treatment, cell lysate was collected and analysed using immunoblot with appropriate antibody and results were quantified against β-actin. ***A***, MCPIP induces autophagy characterized with the marker beclin-1 expression and ROS inhibitor or ER stress inhibitor inhibited MCPIP-induced expression of beclin-1. (* $P<0.05$). ***B***, autophagy blocker 3'-MA blocked MCPIP-induced osteoclast-related gene TRAP and CTSK expression but not GRP78 (* $P<0.05$). ***C***, autophagy blocker LY-294002 blocked MCPIP-induced osteoclast-related gene TRAP and CTSK expression but not GRP78. (* $P<0.05$). ***D***, blockage of autophagy by beclin-1 specific siRNA inhibited MCPIP-induced osteoclast-related gene *TRAP* and *CTSK* expression but not *GRP78* both in protein and mRNA level. * $P<0.05$.

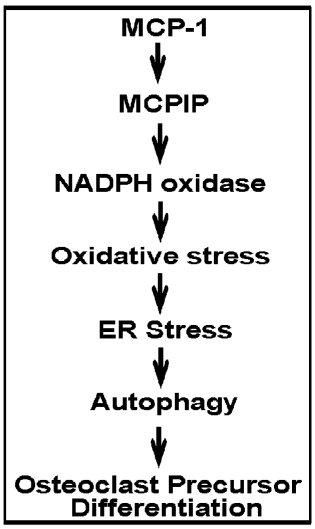


Supplementary Figure S5 MCPIP- induced autophagy marker Atg7 is necessary for induction of OC precursor differentiation. (A) qRT-PCR shows that transfection of MCPIP expression vector resulted in significantly increased mRNA levels of Atg7, Beclin-1, TRAP, and CTSK. Atg7-specific siRNA abolished MCPIP-induced expression of these genes. * $P<0.05$ compared with MCPIP alone. (B) Immunoblot shows that transfection with MCPIP expression vector caused induction of beclin-1, TRAP, and CTSK at the protein levels. Upregulation of these marker genes were inhibited by Atg7-specific siRNA but were not affected by scrambled siRNA. (C) The intensity of each protein was measured and normalized to β-actin of the corresponding group. * $P<0.05$ compared with MCPIP alone.

Supplementary Table S1 Primers for human genes tested in this study.

Product	Primer sequence	
MCPIP	sense	5’-GTTTCCAACGACACATACCGTGAC-3’
	antisense	5’-CTTCTTACG CAGGAAGTTGTCCAG-3’
TRAP,	sense	5’-GCAGATCCTGGGTGCAGACTTC-3’
	antisense	5’-GGGAGCGGTCAGAGAATACGTGC-3’
CTSK	sense	5’-GAGGGGGCTACATGACCAATGC-3’
	antisense	5’-CTGCCTTGCCTGTTGGGTTGA-3’
GRP78	sense	5’-ACAGCTTCTGATAATCAACCAA-3’
	antisense	5’-ACTTCAATCTGTGGGACCC-3’
IRE-1	sense	5’-ACACCATCACCATGTACGACACCA-3’
	antisense	5’-ATTCAC TGTCCACAGTCACCACCA-3’
IRE-1	sense	5’-ACACCATCACCATGTACGACACCA-3’
	antisense	5’-ATTCAC TGTCCACAGTCACCACCA-3’
MMP9	sense	5’-TACCACCTCGAACTTTGACAGCGA-3’
	antisense	5’-GCCATTCACGTCGTCCTTATGCAA-3’
αV integrin	sense	5’-T TCCAAACTGGGAGCACAAGGAGA-3’
	antisense	5’-TGTAAGGCCACTGAAGATG GAGCA-3’
β3 integrin	sense	5’-CTCCTGTGTCCGCTACAAGGG-3’
	antisense	5’-GTCCAGTCGGAGTCACACAGG-3’
beclin-1	sense	5’-CCGTGTCACCATCCAGGAACTC-3’
	antisense	5’-ACCATCCTGGCGAGGAGTTTC-3’
Atg7	sense	5’-ATGTGGTGGCCCCAGGAGAT3’
	antisense	5’-AGATACCATCAATTCCACGG-3’
β-actin	sense	5’-GAGGCACTCTTCCAGCCTTCC-3’
	antisense	5’-GCGGATGTCCACGTCACACTT-3’

Scheme 1


MCP-1
MCPIP
NADPH oxidase
Oxidative stress
ER Stress
Autophagy
Osteoclast Precursor Differentiation