

Supplementary materials

Table S1 Sequences of primer sets used in qRT-PCR

Gene	Sequence (5'-3')
<i>mCcl5</i>	forward, TTTGCCTACCTCTCCCTCG
	reverse, CGACTGCAAGATTGGAGCACT
<i>mCcl2</i>	forward, TAAAAACCTGGATCGGAACCAAA
	reverse, GCATTAGCTTCAGATTACGGGT
<i>mMmp-9</i>	forward, ATAGAGGAAGCCCATTACAGG
	reverse, GTGTACACCCACATTTGACG
<i>mArg-1</i>	forward, CAATGAAGAGCTGGCTGGTGT
	reverse, GTGTGAGCATCCACCCAAATG
<i>mCxcr4</i>	forward, GACTGGCATAGTCGGCAATG
	reverse, AGAAGGGGAGTGTGATGACAAA
<i>mVegf</i>	forward, GCACATAGAGAGAATGAGCTTCC
	reverse, CTCCGCTCTGAACAAGGCT
<i>mβ-actin</i>	forward, GGCTGTATCCCCTCCATCG
	reverse, CCAGTTGGTAACAATGCCATGT
<i>hCcl5</i>	forward, TACCATGAAGGTCTCCGC
	reverse, GACAAAGACGACTGCTGG
<i>hCcl2</i>	forward, AAGATCTCAGTGCAGAGGCTCG
	reverse, TTGCTTGCCAGGTGGTCCAT
<i>hMmp-9</i>	forward, GGGACGCAGACATCGTCATC
	reverse, TCGTCATCGTCGAAATGGGC
<i>hArg-1</i>	forward, TGGACAGACTAGGAATTGGCA
	reverse, CCAGTCCGTCAACATCAAACT
<i>hCxcr4</i>	forward, ACTACACCGAGGAAATGGGCT
	reverse, CCCACAATGCCAGTTAAGAAGA
<i>hVegf</i>	forward, TTGCCTTGCTGCTACCTC
	reverse, GTGATGATTGCTCCCTCTCTC
<i>hβ-actin</i>	forward, TGAAGTGTGACGTGGACATA
	reverse, GGAGGAGCAATGATCTTGAT

Table S2 The lists of PARP-1 binding proteins identified by IP/MS

Gene names	Intensity 1-IgG	Intensity 2-Ctrl	Intensity 3-A549
MYH9	498700000	460680000	513360000
PARP1	0	1256600000	1158400000
DSP	20713000	2337100	47391000
ACTB;ACTG2;ACTA2	295670000	275770000	804250000
ACTG1	0	0	10503000
PKM	62675000	33386000	57471000
ZYX	0	417530000	433190000
GAPDH	134540000	373240000	237870000
HIST1H2BK;H2BFS	2854500	0	0
HIST1H4A	271730000	56486000	137940000
HIST1H2BC;HIST1H2BM;HIST1H2BN;HIST1H2BH;HIST2H2BF;HIST1H2BD;HIST1H2BL	712130000	105850000	211400000
HIST1H1C	799740000	106660000	98439000
ACTA1;ACTC1	29662000	0	60537000
HIST1H1E;HIST1H1D;HIST1H1A	16332000	52472000	60740000
JUP	11250000	1180200	22664000
HIST2H2BE;HIST1H2BB;HIST1H2BO	9145000	1280900	10971000
ENO1	4348800	0	24403000
HIST1H1B	274540000	85963000	61872000
PPIA	55839000	34574000	43085000
HIST1H2AH;HIST1H2AG;HIST1H2AJ;H2AFJ;HIST1H2AD;H2AFX	376490000	66071000	147480000
LMNB1	16115000	4169800	3770200
RPL13	35864000	12043000	40298000
RPS16	9835200	12017000	28750000
EEF1A1;EEF1A1P5;EEF1A2	43373000	22541000	110300000
HIST1H3A;HIST2H3A;HIST3H3;H3F3A;H3F3C	329290000	28786000	134710000
PRDX1	8621500	3836300	56034000
LASP1	30933000	26832000	52871000
ZNF318	0	4200000	10910000
HIST1H2AC;HIST3H2A;HIST1H2AB	47205000	6466600	7647900
MYO1G	0	5550800	19121000
NCF1;NCF1B;NCF1C	18045000	10411000	20283000
CFL1	1567600	15047000	16183000
GRN	35377000	12720000	14879000
MYL6	58690000	49277000	63457000
RPS20	42359000	28760000	17766000

Table S2 Continued

Gene names	Intensity 1-IgG	Intensity 2-Ctrl	Intensity 3-A549
RPS13	19135000	9317700	13628000
RPS6	12698000	6845900	6058600
TUBA1C;TUBA1B;TUBA1A;TUBA4A;TUBA8	3829400	5481200	22869000
KPRP	4375600	0	8978200
HIST2H2AA3;HIST2H2AC	21796000	1298000	8163600
HSPA8	8192700	6677600	16021000
HSPA9	8219700	2990700	9205300
RPL35	41613000	13928000	43623000
LIG3	0	1649300	10340000
HNRNPM	10547000	3519400	13005000
TRIM21	0	10017000	11786000
CSRP1	0	14933000	26476000
RPS14	5845100	7249100	20018000
RPS18	10839000	9795100	9104600
RPS4X	7999300	7677400	8930000
GNB2L1	4140200	5666800	5552100
NPM1	4721900	4697300	8598400
TPM3	11692000	0	10462000
RPL4	4285100	2529700	7638900
RPL27A	16897000	30613000	19466000
RPS9	4997200	2504000	4788000
RPS3A	3680700	4374700	8410100
RPS23	8054500	6146200	10738000
RPS11	9411300	3589700	15688000
RPS28	31978000	14092000	45121000
RPL32	12036000	9023800	18993000
TUBB4B;TUBB;TUBB4A;TUBB2B;TUBB2A	0	4889800	3198100
RPL19	6267200	5936900	5332800
DSG1	9703300	0	5432900
RPL18	4611700	7624300	10762000
HIST2H2AB	4011300	0	0
SERPINA1	20123000	0	0
GH2;GH1;CSH2;CSH1;CSHL1	7661500	2899400	2186200
H2AFV;H2AFZ	19471000	0	2807400
RPL7	2630200	1828300	0

Table S2 Continued

Gene names	Intensity 1-IgG	Intensity 2-Ctrl	Intensity 3-A549
RPL30	6014600	0	6245200
RPL6	0	0	17959000
DSC1	3519500	0	1146500
SERBP1	0	2094600	6381100
RCC2	8517000	6239500	2263600
C11orf98	2303600	0	877290
LMNA	1277200	0	3145800
VIM	854360	0	3576700
HSPA5	4804900	4989000	4887200
DDX5	1245800	781670	1274100
RPL17	0	3433500	2148000
XRCC1	0	516290	4140000
PCMT1	2366500	1808100	5766900
PPIB	4467200	0	2404600
MSN	3202300	0	518220
PTPN6	0	0	4911700
HNRNPH1;HNRNPH2	0	1098900	1977600
STIP1	0	917910	998650
RPL9	6973400	0	4882300
LSP1	4286900	1126800	6636000
RPL3	2102800	4259700	3336200
RPL28	5793500	3490700	5923100
RPL29	30450000	0	0
TUFM	0	830720	3057300
LYZ	3035000	5123800	2105000
HNRNPK	1711500	883140	1420300
RPS8	4551400	5114500	5769100
RPS29	6926800	6514100	0
RPL11	4640400	0	2710800
RPL8	2570300	5674000	1378900
DCD	3994800	3632200	813760
RPL36AL;RPL36A	4279200	2556300	2164600
HNRNPU	1112200	3416000	0
AMPD2	0	0	3342700
PCBP1	0	0	4663400

Table S2 Continued

Gene names	Intensity 1-IgG	Intensity 2-Ctrl	Intensity 3-A549
ZNF609	0	0	1228100
ACTN4	0	0	1966100
ALDOA	1238700	0	993470
S100A8	585330	2702600	2079900
SLC25A5;SLC25A6;SLC25A4	1366700	6766400	2004100
S100A9	1507300	0	4454500
ANXA2;ANXA2P2	0	0	0
RPS17	0	636620	2287900
ALOX5	0	0	3020100
HSPD1	881190	0	720100
RPS2	2338800	0	1186800
FBL	0	1177300	0
SFPQ	2179400	585550	0
RPS3	0	1866400	1129100
ATP5A1	0	980930	1992400
CORO1A	3742200	979480	0
FUS;TAF15	1962100	0	1393000
GLRX	3046300	0	0
CAPZB	1132500	0	3325500
ATP5O	0	1114600	1309700
RPL34	0	0	852450
CAPZA1;CAPZA2	3815600	0	5767800
ATP5EP2;ATP5E	1535400	2733200	2151100
EIF4A1;EIF4A2	648330	1037200	2291100
RPL26;RPL26L1	1009100	1326100	0
RPS7	0	597080	602730
RPL7A	0	0	1802800
RAN	2128100	0	1213300
RPL23	0	3015200	0
RPS25	1899000	0	6097800
RPS26P11;RPS26	0	5751700	1450500
FAU	3278000	0	4486600
UBE2I	0	5292600	2315100
FABP5	0	0	6802500
PKP1	597640	0	0

Table S2 Continued

Gene names	Intensity 1-IgG	Intensity 2-Ctrl	Intensity 3-A549
ALYREF	467430	0	444860
H1FX	0	868160	1396700
MBNL1	0	390820	4216000
CCDC144CP;CCDC144A	2241600	0	0
HMGB1P1;HMGB1	0	0	632160
HMG4	1458300	0	0
ARPC1B	0	0	1607000
ARPC3	0	0	1628400
IGKC	2787700	0	0
IGHG1;IGHG3	0	0	2074900
HBZ	6195500	0	0
ARG1	1581700	0	0
HNRNPC	1923100	0	0
RPSA	0	1270600	0
HNRNPA1	854790	812750	0
UQCRRFS1;UQCRRFS1P1	0	0	2212600
U2AF1	0	0	427250
G6PD	0	0	978970
IMPDH2	0	0	0
LCP1;PLS3	0	0	737900
HSPA7;HSPA6	0	0	592020
PFKL	0	0	322810
PTBP1	605780	0	825150
RPL10L;RPL10	0	0	1997400
PRDX5	1232400	0	0
PDIA3	629040	0	0
RPL22	0	0	2405600
TAGLN3;TAGLN2	0	524350	0
MNDA	0	0	1553400
RPS27	512580	0	3668000
RPL21	2737000	0	2050300
RPL14	0	900190	0
NHP2L1	0	0	1790200
ARPC4	0	0	1410100
ACTR2	0	0	1338100

Table S2 Continued

Gene names	Intensity 1-IgG	Intensity 2-Ctrl	Intensity 3-A549
RPL37A	1641600	3471600	0
RPL37	0	0	0
CNBP	893320	0	0
RPS24	0	1635000	0
RPL39P5;RPL39	17361000	14246000	11903000
RPS27A	1314600	0	1315600
RPL38	3584400	4239400	0
YBX1	0	0	2593400
RPL24	2207700	0	0
SRSF3	0	0	747280
RPL18A	0	1692700	0
SSBP1	2538300	0	0
CLC	5357400	0	6014500
ACACA	6749000	0	0
PTPRK	0	0	0
ELAVL1	1636600	1696500	0
HP1BP3	1489700	0	0
KSR2	24414000	0	0
GSTA5	0	9124300	9774900
TTC21B	7694400	0	0
ABCA13	3771100	14851000	2479500
WDFY3	0	0	0
VPS8	0	1280700	0
SREK1IP1	0	0	659760
POMGNT2	0	1604800	0
RP9	504670	0	0
TTN	0	0	0
INPP5D	0	0	1316900
ISOC2	1362000	972770	0
TTC28	0	0	1804600
EFHD2	0	0	644470
PARK7	0	0	1193200
HSD17B10	860840	801910	0
LIMD2	0	896770	0
DNAH6	26480000	0	0

Table S2 Continued

Gene names	Intensity 1-IgG	Intensity 2-Ctrl	Intensity 3-A549
BOLA2	0	0	805580
WNK1	0	0	1501400
KIAA1551	0	0	0
ASH1L	0	5106000	0
THYN1	0	546070	0
RAB22A	3560300	0	0
SRRM2	0	537570	0
CHTOP	609020	0	363530

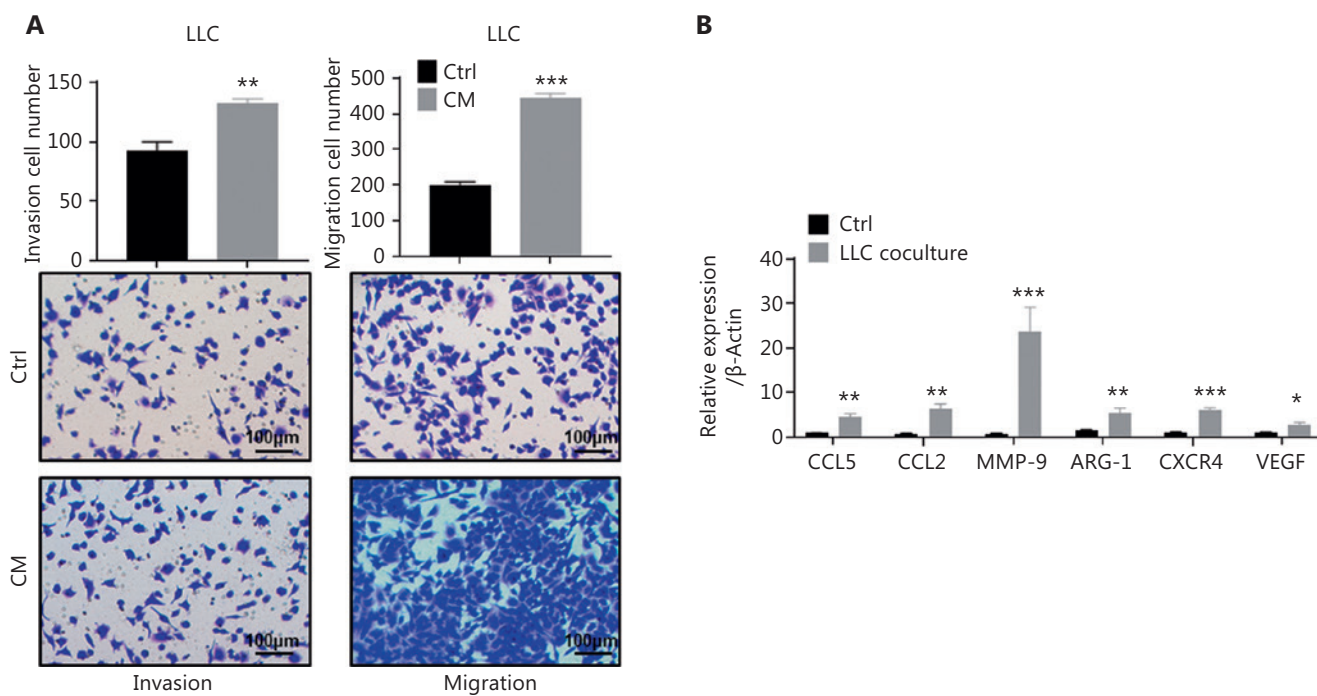


Figure S1 (related to result 2). Cancer-educated neutrophils promote lung cancer cell invasion, migration, proliferation, and lung cancer growth. (A) BMN-activated productions (50 µL) were added to Transwell upper chamber to stimulated LLC cells. Then, the invasion and migration cell numbers were analyzed (CM, condition medium as neutrophil-activated productions). Scale bar: 100 µm. (B) *ccl5*, *ccl2*, *mmp-9*, *arg-1*, *cxcr4*, and *vegf* mRNA in BMNs co-cultured with LLC cells were measured by real-time PCR. All data are expressed as the mean ± SEM ($n = 3-5$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

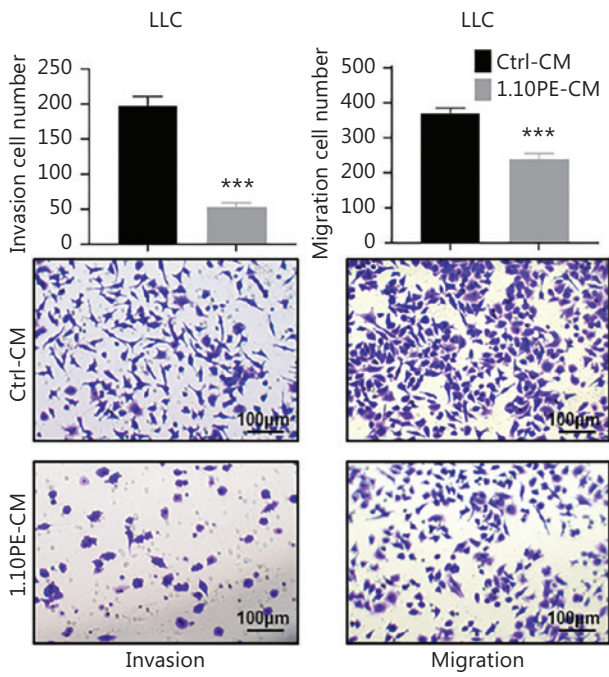


Figure S2 (related to result 3). Neutrophil-released MMP-9 promotes lung cancer cell invasion, migration, and proliferation. 1.10PE (200 μM) was used during BMN activation. Then, the neutrophil-activated productions (Ctrl-CM, 50 μL) with or without 1.10PE (1.10PE-CM) were added to the Transwell upper chamber to stimulated LLC cells. Then, the invasion and migration cell numbers were analysis. Scale bar: 100 μm. All data are expressed as the mean ± SEM ($n = 5$). *** $P < 0.001$.

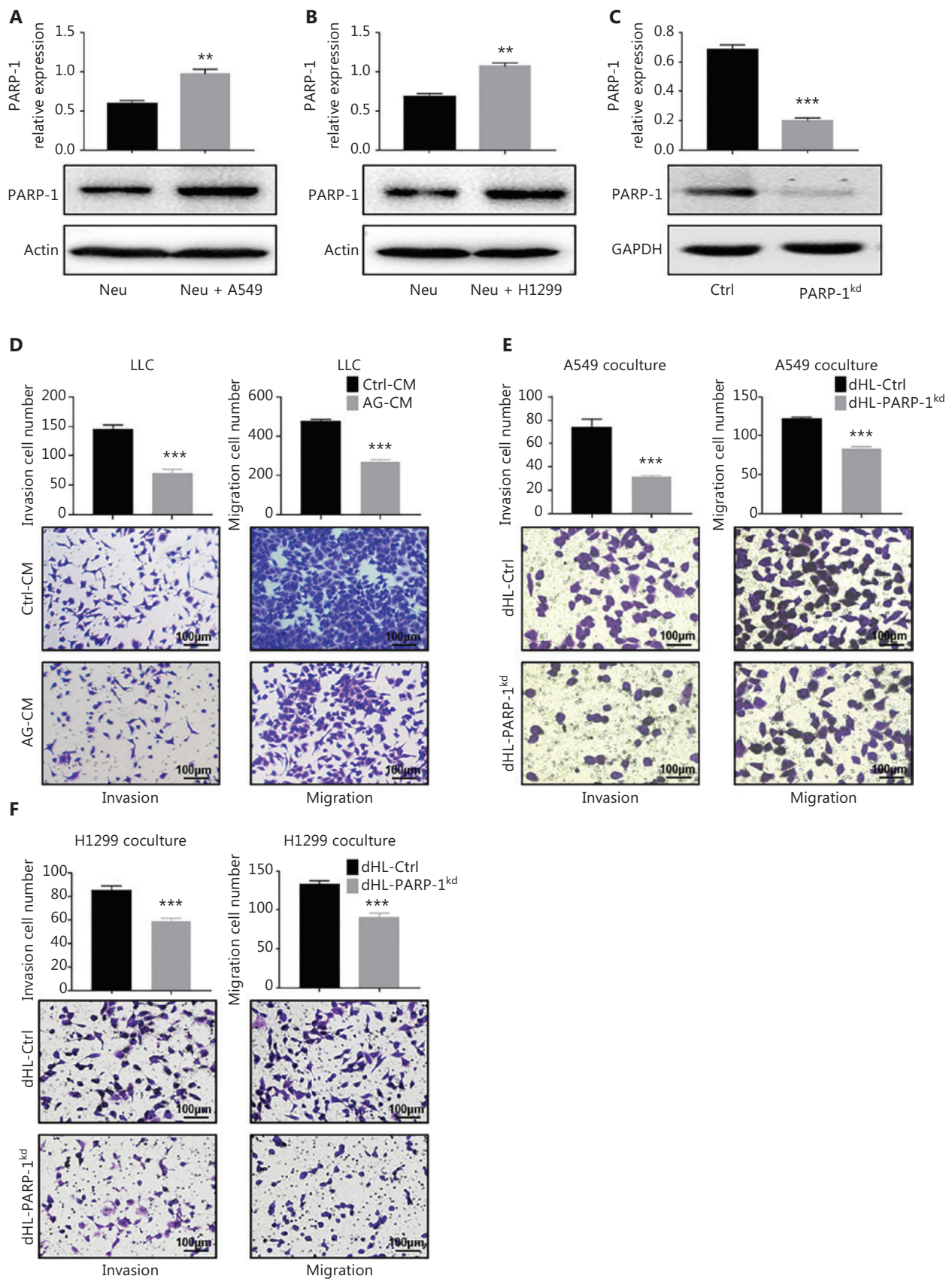


Figure S3 (related to result 4). PARP-1 regulates MMP-9 expression and neutrophil-induced lung cancer cell invasion, migration, and proliferation. (A and B) Neutrophil differentiated from HL-60 cells were co-cultured with A549 (A) and H1299 (B) cells for 4 h. Then, PARP-1 expression was measured by western blot analysis in neutrophils. (C) PARP-1 was knocked down by shRNA in HL-60 cells. PARP-1 expression was measured by western blot analysis. (D) AG14361 (1 μ M) was used during BMN activation. Then, the neutrophil-activated productions (50 μ L) with or without AG14361 (AG) were added to the Transwell upper chamber to stimulated LLC cells. Then, the invasion and migration cell numbers were analyzed. (E and F) For the co-culture Transwell system, the normal (dHL-Ctrl) and PARP-1 knockdown neutrophils (dHL-PARP-1^{kd}, 6×10^6 in 600 μ L of serum-free medium) were pre-seeded in the lower chamber. Then, A549 (E) or H1299 (F) cells were seeded in the upper chamber. The invasion and migration cell numbers were analyzed. Scale bar: 100 μ m. All data are expressed as the mean \pm SEM ($n = 3-5$). ** $P < 0.01$, *** $P < 0.001$.

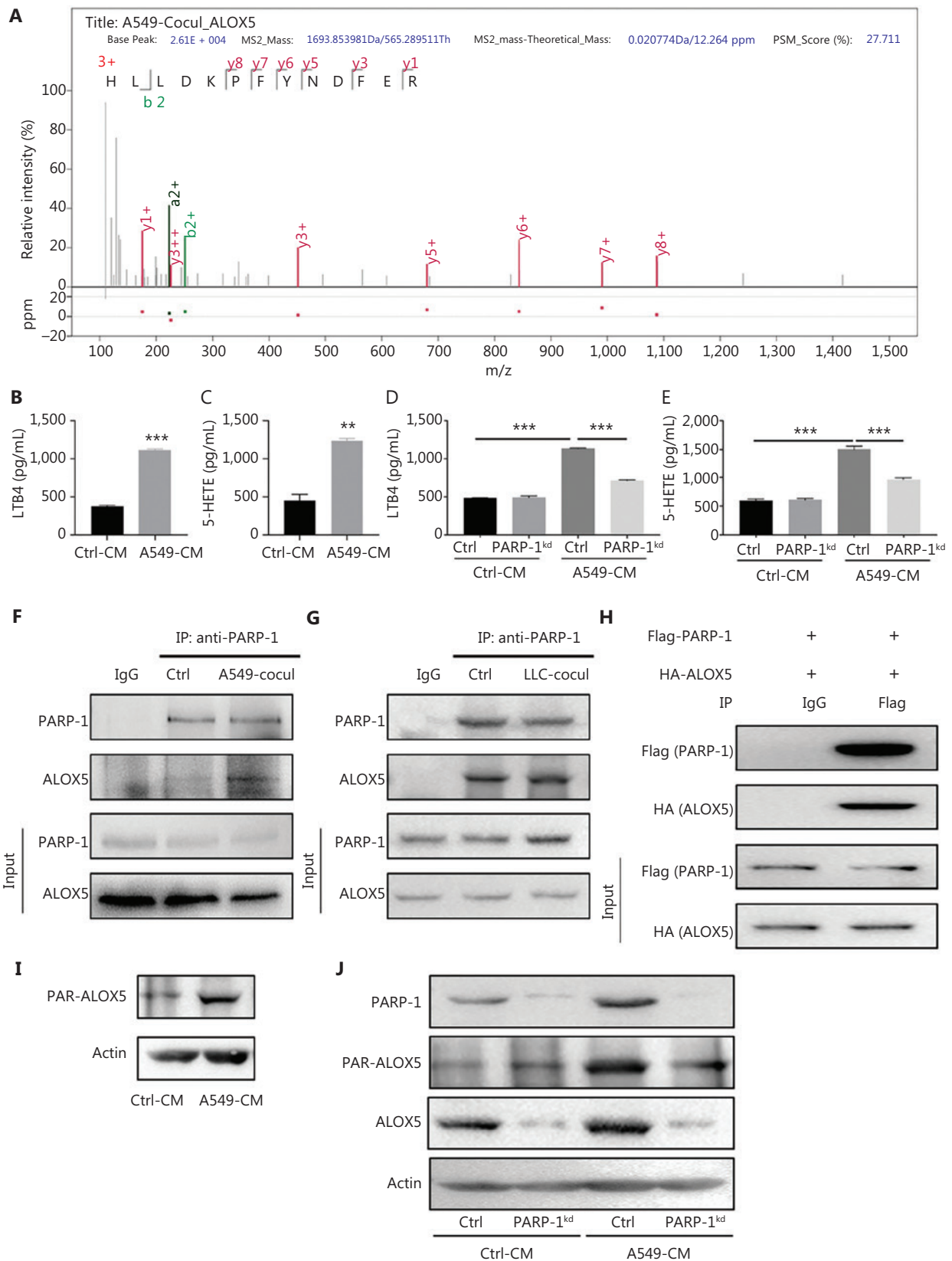


Figure S4 Continued

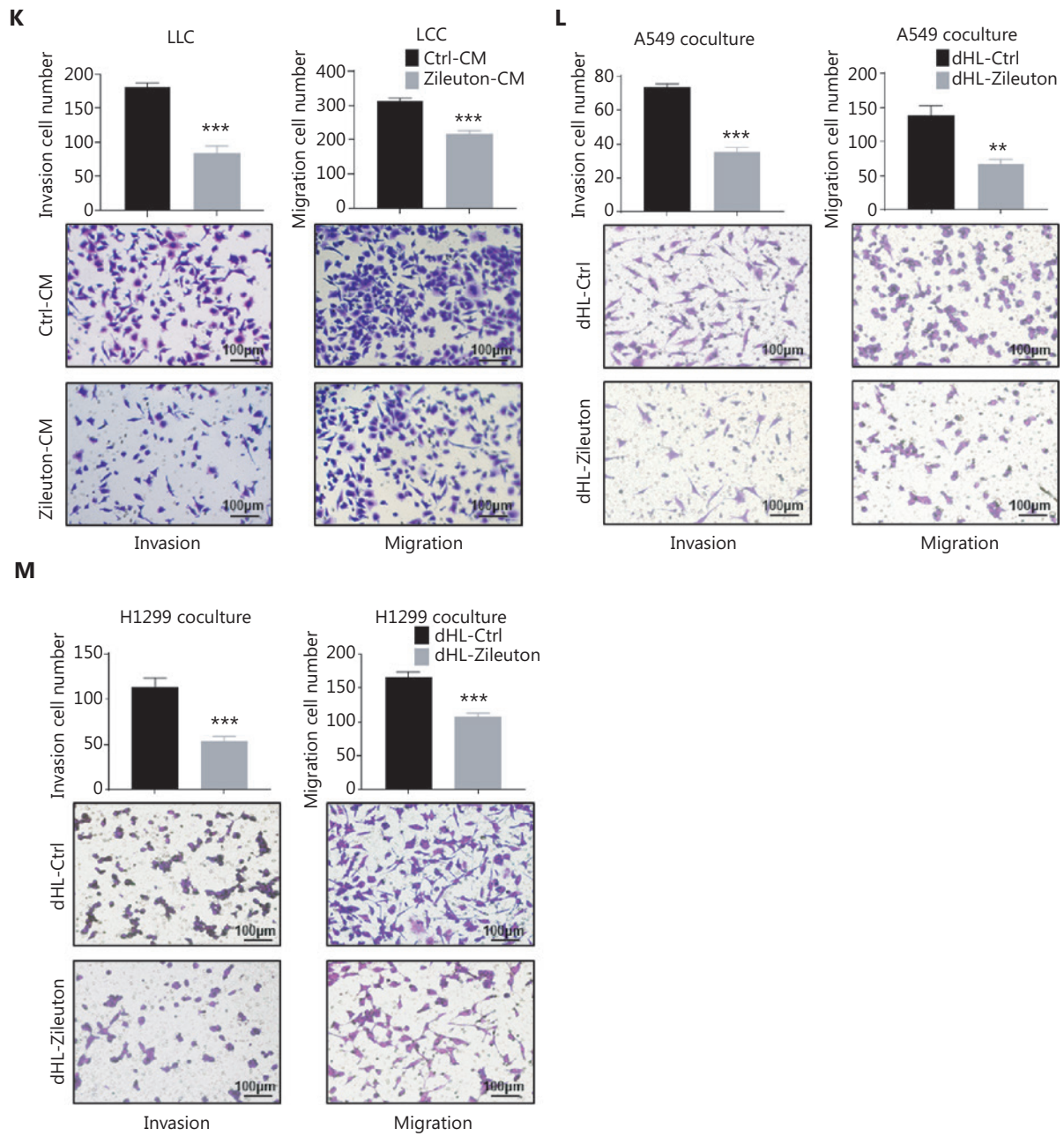


Figure S4 (related to result 5). PARP-1 binding to ALOX5 mediates MMP-9 expression in neutrophils. (A) IP/MS figure of ALOX5. (B and C) HL-60-differentiated neutrophils were co-cultured with A549 cells for 4 h. LTB4 (B) and 5-HETE (C) were measured by ELISA. (D and E) The normal and PARP-1 knockdown neutrophils were stimulated with control and A549-CM for 4 h. LTB4 (D) and 5-HETE (E) were measured by ELISA. (F) HL-60-differentiated neutrophils were co-cultured with A549 cells for 4 h. Co-immunoprecipitation of PARP-1 with ALOX5 in neutrophils was performed. (G) BMNs were co-cultured with LLC cells for 4 h. Co-immunoprecipitation of PARP-1 with ALOX5 in neutrophils was performed. (H) Flag-PARP-1 and HA-ALOX5 plasmids were transfected into 293T cells. After 48 h, ALOX5 was immunoprecipitated using anti-Flag antibody. (I) HL-60-differentiated neutrophils were co-cultured with A549 cells for 4 h. PARylation of ALOX5 was examined from immunoprecipitation by western blot analysis. (J) The normal and PARP-1 knockdown neutrophils were stimulated with control and A549-CM for 4 h. PARylation of ALOX5 was examined from immunoprecipitation by western blot analysis. (K) Zileuton was used during BMN activation. Then, the neutrophil-activated productions (50 μ L) with or without Zileuton were added to the Transwell upper chamber to stimulated LLC cells. Then, the invasion and migration cell numbers were analyzed. (L and M) For the co-culture Transwell system, the neutrophils differentiated from HL-60 cells (dHL-Ctrl, 6×10^6 in 600 μ L of serum-free medium) were pre-seeded in the lower chamber with or without Zileuton (50 μ M, dHL-Zileuton). Then, A549 (L) or H1299 (M) cells were seeded in the upper chamber. The invasion and migration cell numbers were analyzed. Scale bar: 100 μ m. All data are expressed as the mean \pm SEM ($n = 5$). ** $P < 0.01$, *** $P < 0.001$.