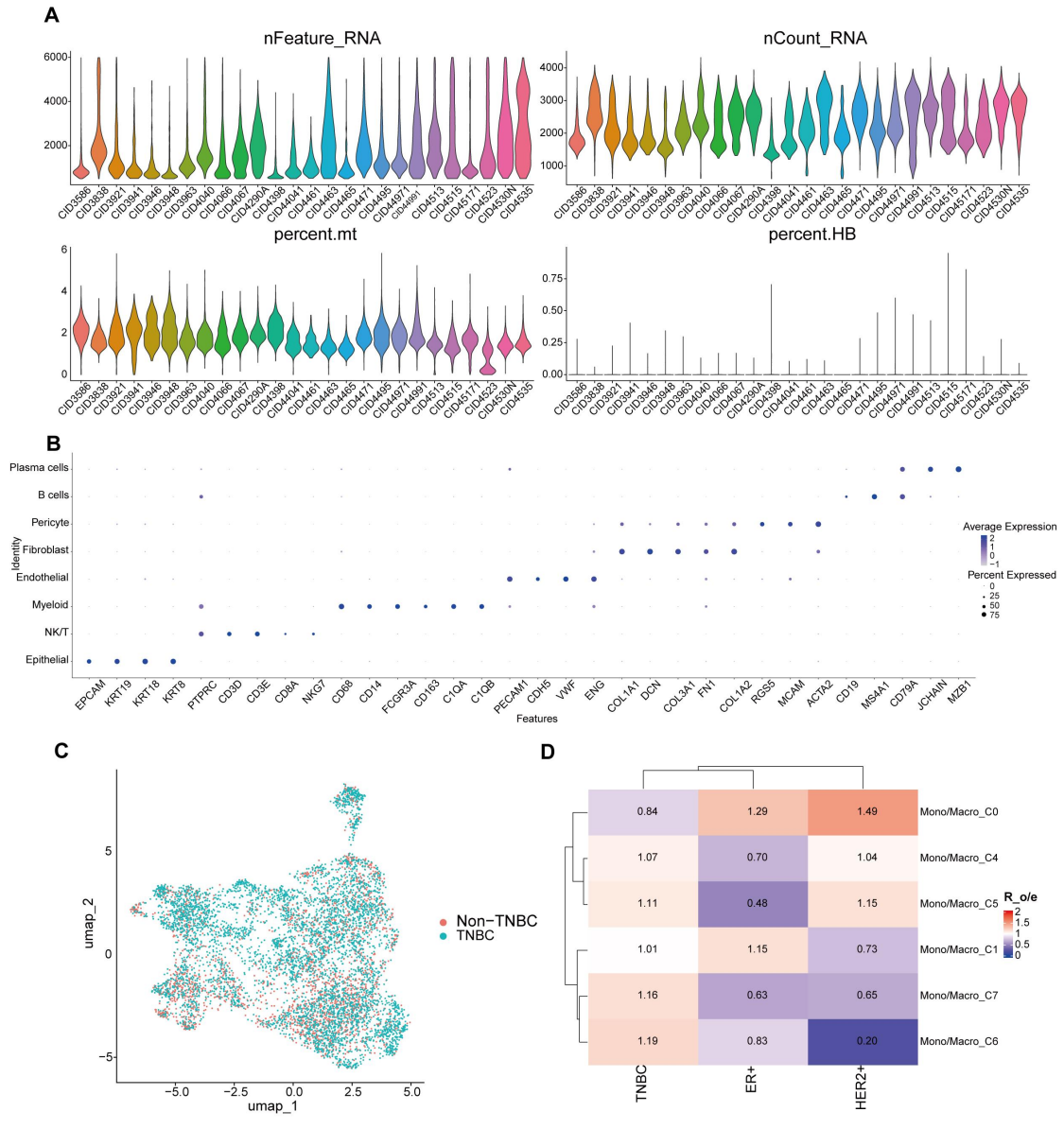
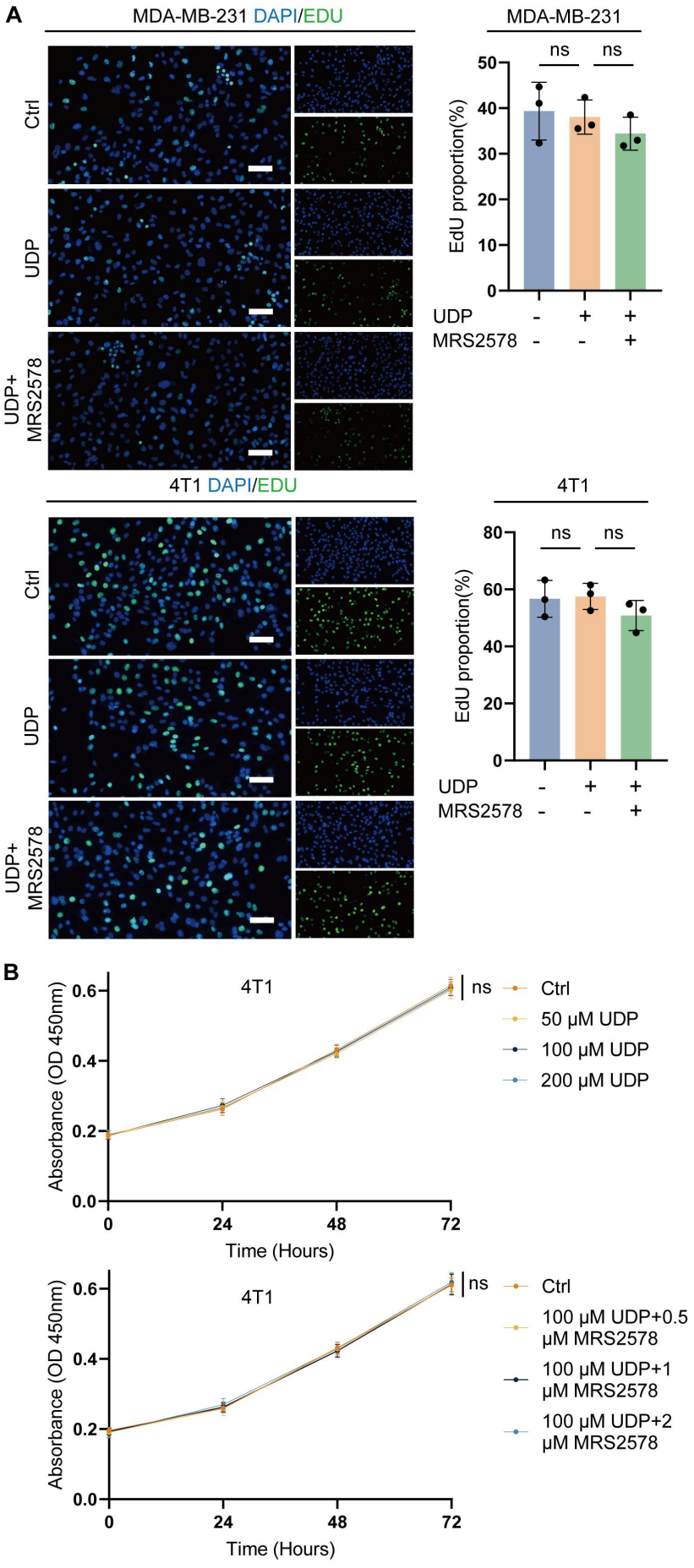
Figure legend



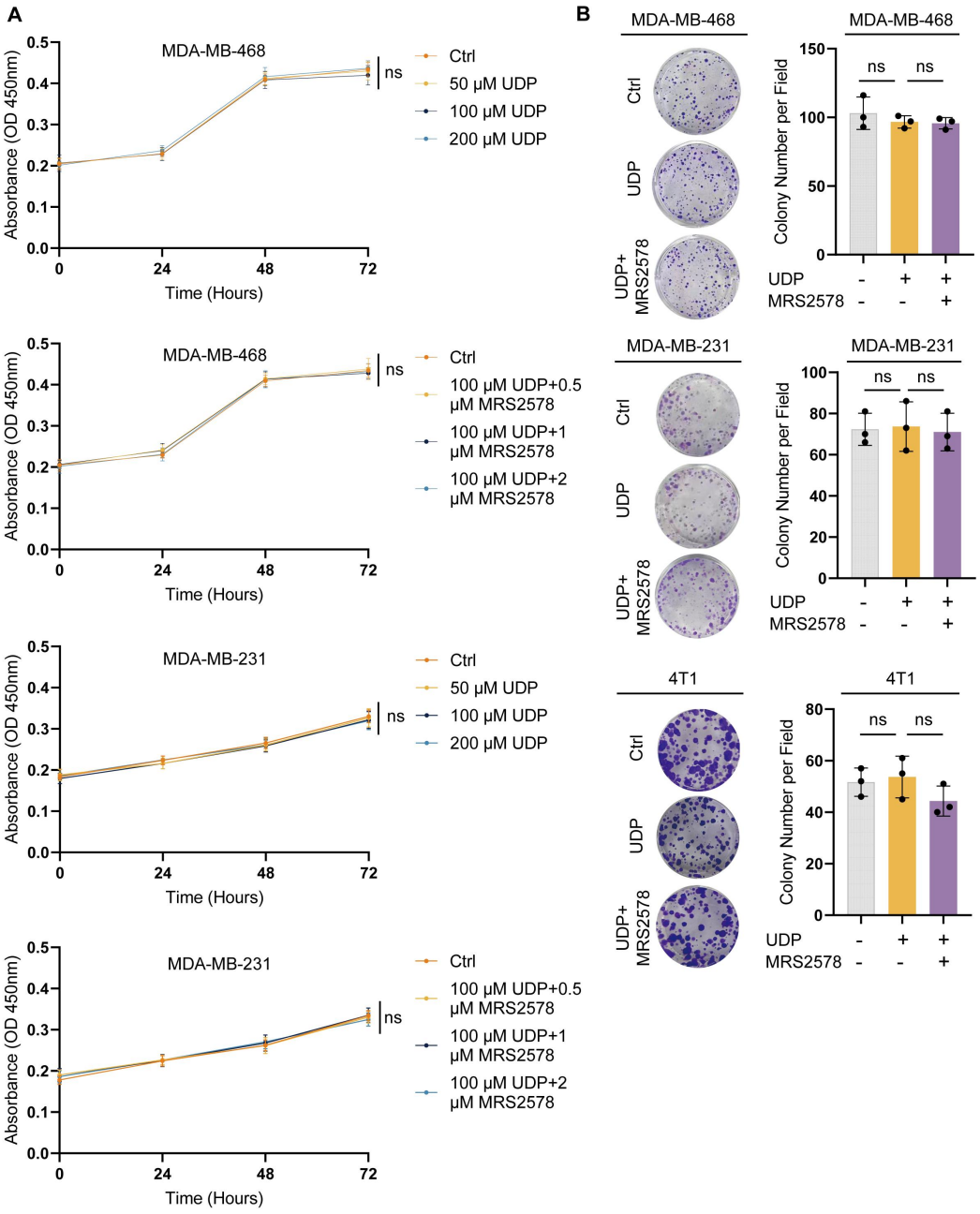
Supplementary Figure 1. **Marker-gene and differential-expression profiling across cell types in human breast cancer.**

A. Quality-filtered scRNA-seq data from the GSE176078 dataset. **B**. Bubble chart displaying canonical marker genes used for cell-type annotation in scRNA-seq analysis. **C**. Annotation of TNBC-specific cellular sub-clusters. **D**. Distribution of macrophage sub-populations among different molecular subtypes of breast cancer.


A
MDA-MB-231 DAPI/EDU
Ctrl
UDP
UDP+ MRS2578
MDA-MB-231
ns
ns
EdU proportion(%)
50
40
30
20
10
0
UDP - + +
MRS2578 - - +
4T1 DAPI/EDU
Ctrl
UDP
UDP+ MRS2578
4T1
ns
ns
EdU proportion(%)
80
60
40
20
0
UDP - + +
MRS2578 - - +
B
4T1
ns
Ctrl
50 μM UDP
100 μM UDP
200 μM UDP
Absorbance (OD 450nm)
0.6
0.4
0.2
0.0
0
24
48
72
Time (Hours)
4T1
ns
Ctrl
100 μM UDP+0.5 μM MRS2578
100 μM UDP+1 μM MRS2578
100 μM UDP+2 μM MRS2578
Absorbance (OD 450nm)
0.6
0.4
0.2
0.0
0
24
48
72
Time (Hours)

Supplementary Figure 2. Direct stimulation of tumor-cell P2RY6 does not alter TNBC growth *in vitro*.

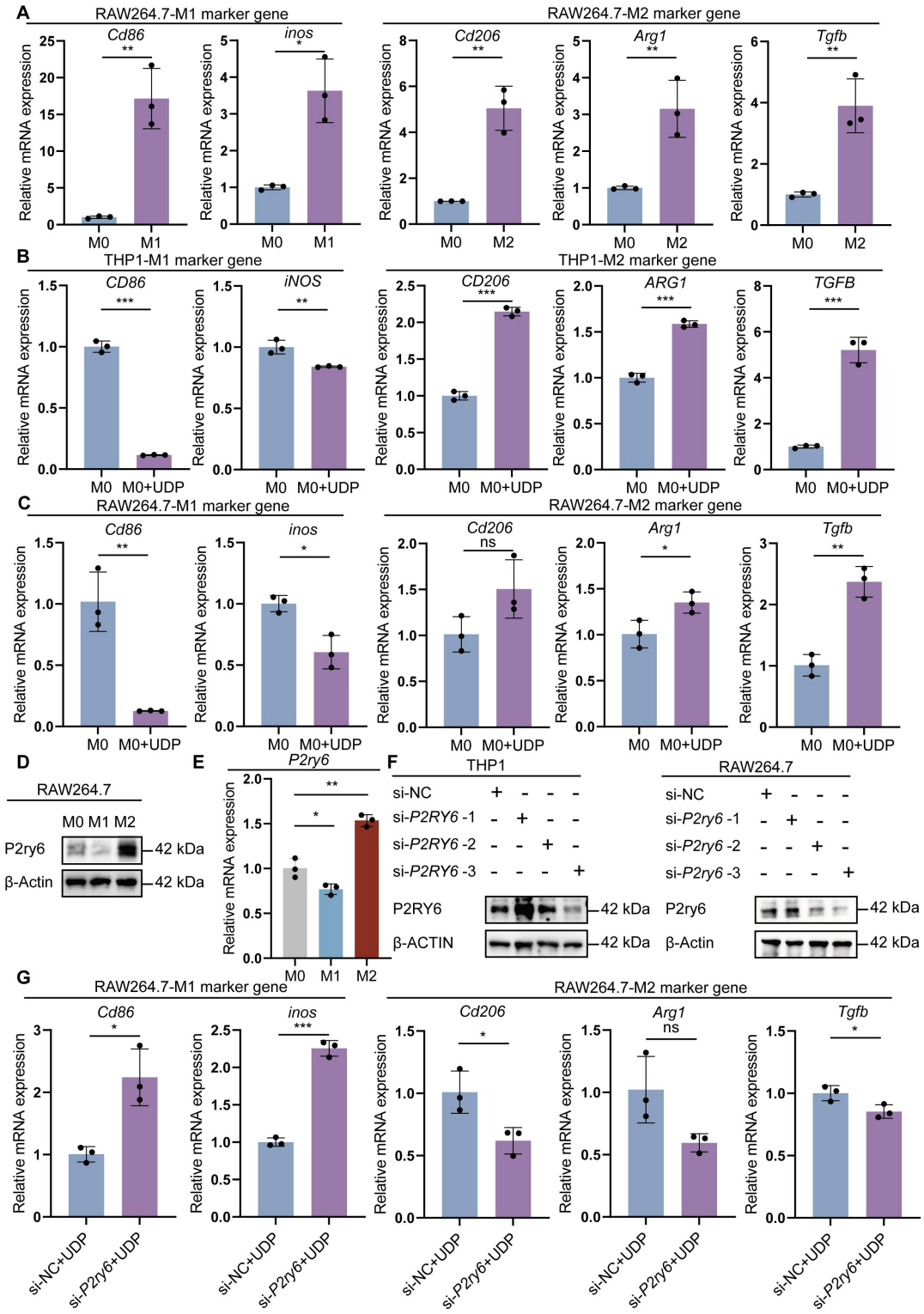
A. Proliferation of MDA-MB-231 and 4T1 cells following UDP (100 μM) -mediated P2RY6 activation or MRS2578 (1 μM) -mediated P2RY6 inhibition, measured by EDU assay. Scale bar: 80 μm. **B**. CCK-8 assay assessing the proliferative capacity of 4T1 cells upon UDP-mediated P2RY6 activation or MRS2578-mediated P2RY6 inhibition. ns, not significant.



19

20 **Supplementary Figure 3. Direct modulation of tumor-cell P2RY6 does not**
21 **influence TNBC growth *in vitro*.**

22 **A**. Effect of UDP-induced P2RY6 activation or MRS2578-mediated P2RY6 inhibition
23 on the proliferation of MDA-MB-468 and MDA-MB-231 cells, assessed by CCK-8
24 assay. **B**. Colony-formation assay evaluating the proliferative response of
25 MDA-MB-468, MDA-MB-231 and 4T1 cells following UDP (100 μM) - or MRS2578
26 (1 μM) -mediated modulation of P2RY6 activity. ns, not significant.



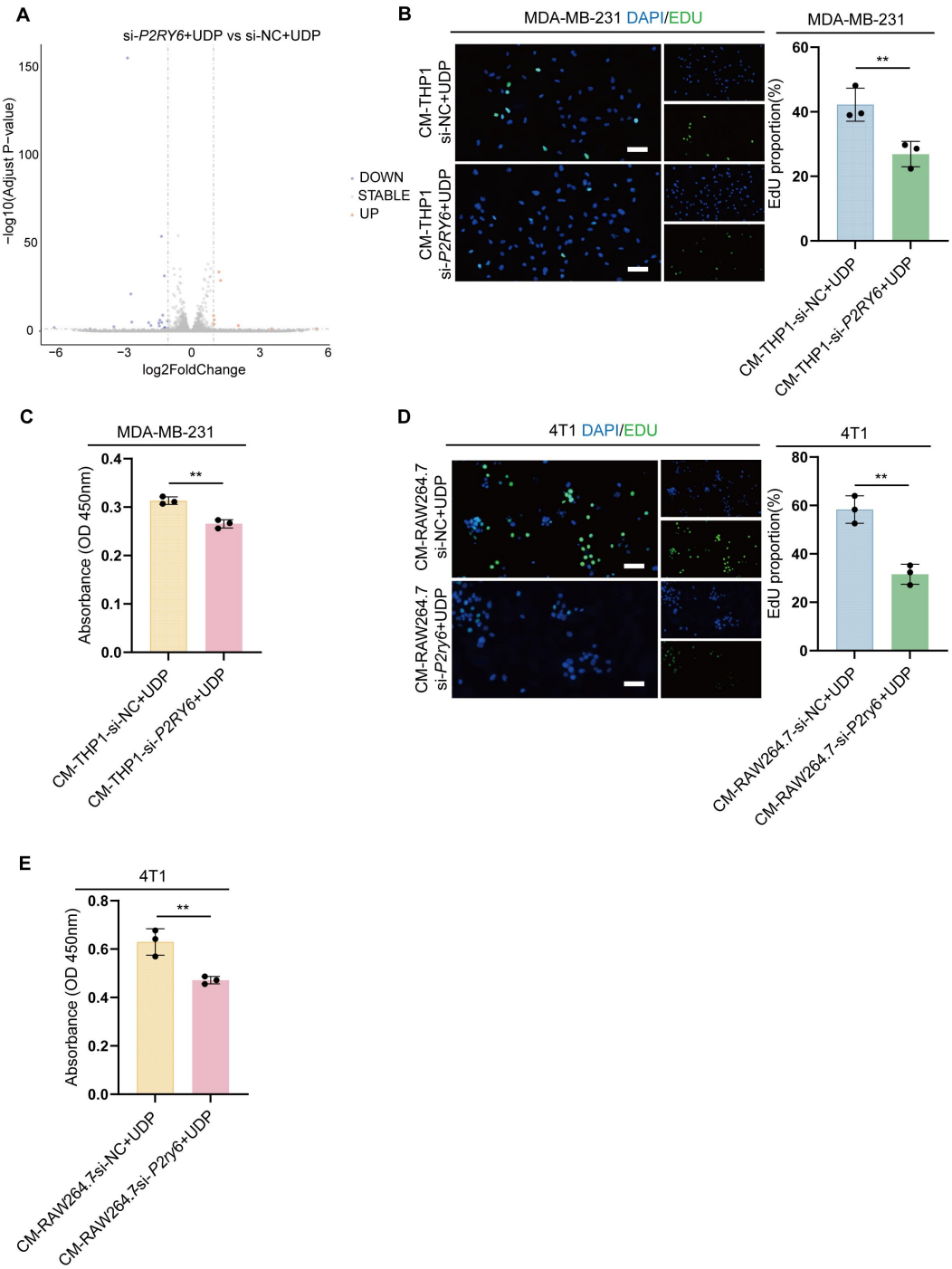
27

28 **Supplementary Figure 4. P2RY6 drives macrophage polarization toward an**

29 **M2-like phenotype.**

30 **A**. qRT-PCR analysis of macrophage polarization-associated markers under distinct

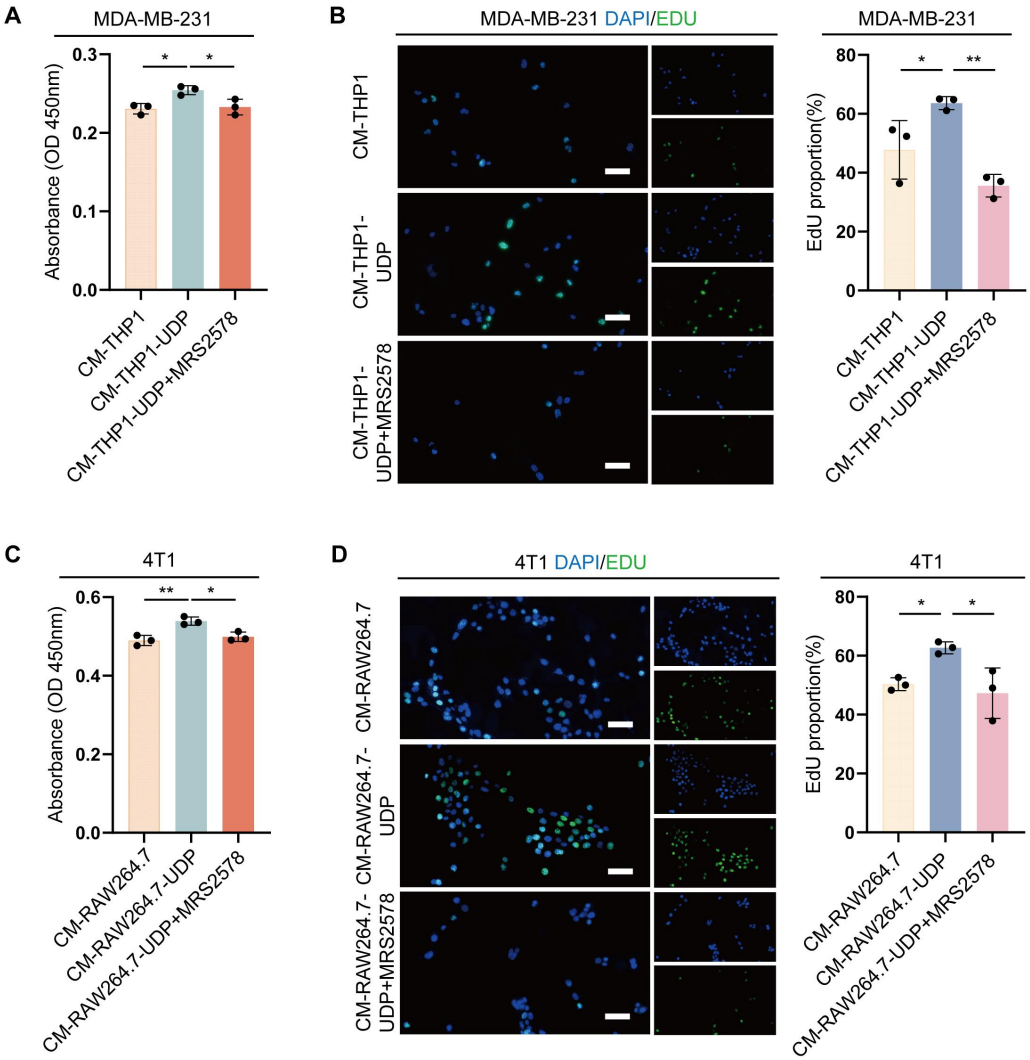
polarization stimuli in RAW264.7 cells. **B-C**. qRT-PCR measurement of macrophage marker expression after UDP (100 μM) -mediated activation of P2RY6 in macrophages. **D-E**. Western blot and qRT-PCR assessment of P2ry6 expression changes in polarized macrophages. **F**. Western blot validation of siRNA-mediated P2RY6 knockdown efficiency. **G**. qRT-PCR analysis of macrophage polarization markers following P2ry6 knockdown. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ns, not significant.



Supplementary Figure 5. P2RY6 knockdown in macrophages reduces the pro-proliferative effect of conditioned medium on TNBC cells.

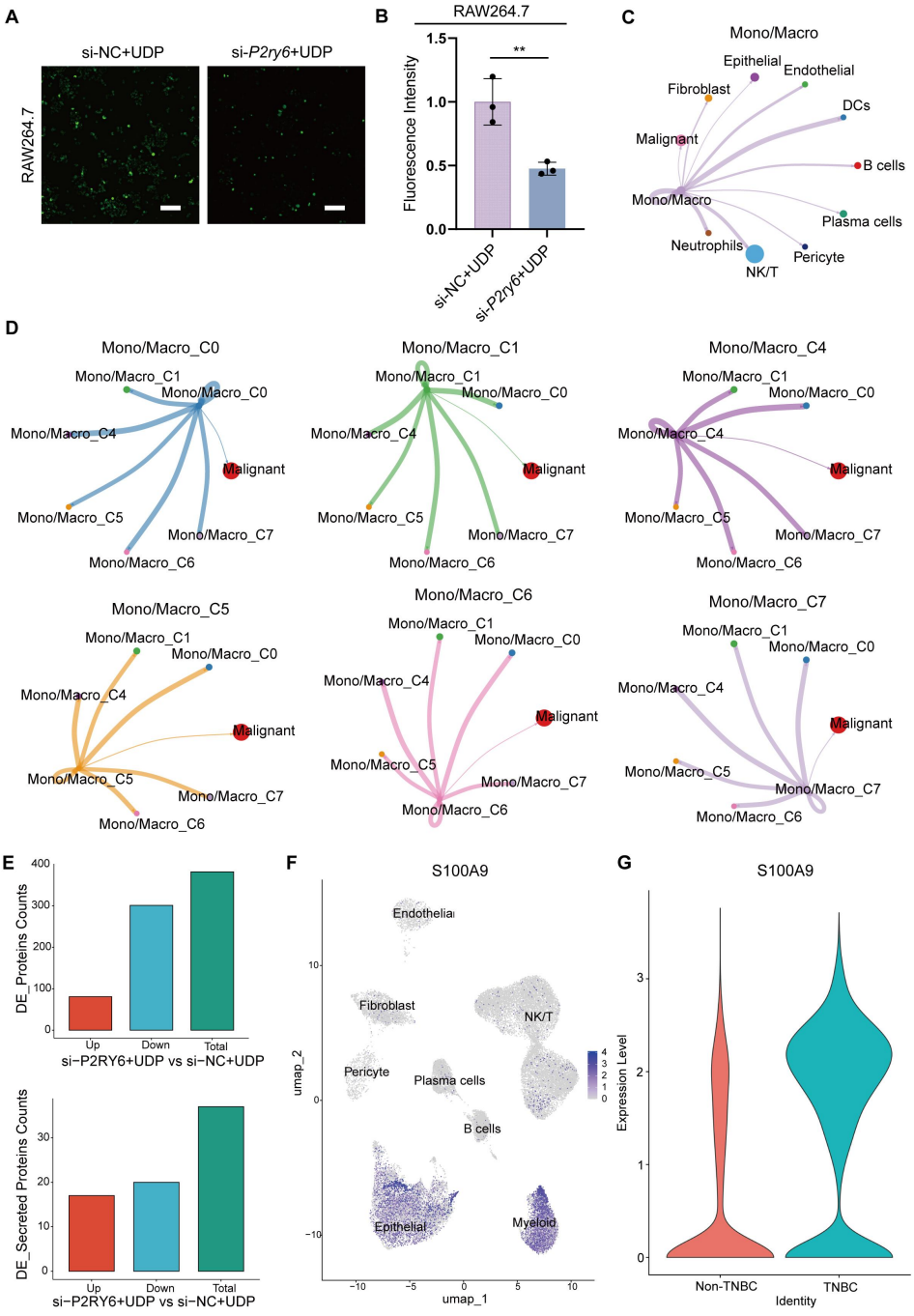
A. Volcano plot showing P2RY6-related DEGs. **B-C**. EdU and CCK-8 assays measuring the proliferation of MDA-MB-231 cells treated with conditioned medium from THP1 cells transfected with si-*P2RY6* or si-NC. Scale bar: 80 μm. **D-E**. EdU and

CCK-8 assays evaluating the proliferation of 4T1 cells treated with conditioned medium from RAW264.7 cells transfected with si-*P2ry6* or si-NC. Scale bar: 80 μm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



Supplementary Figure 6. Pharmacological inhibition of P2RY6 reduces the pro-proliferative effect of macrophage-conditioned medium on TNBC cells.

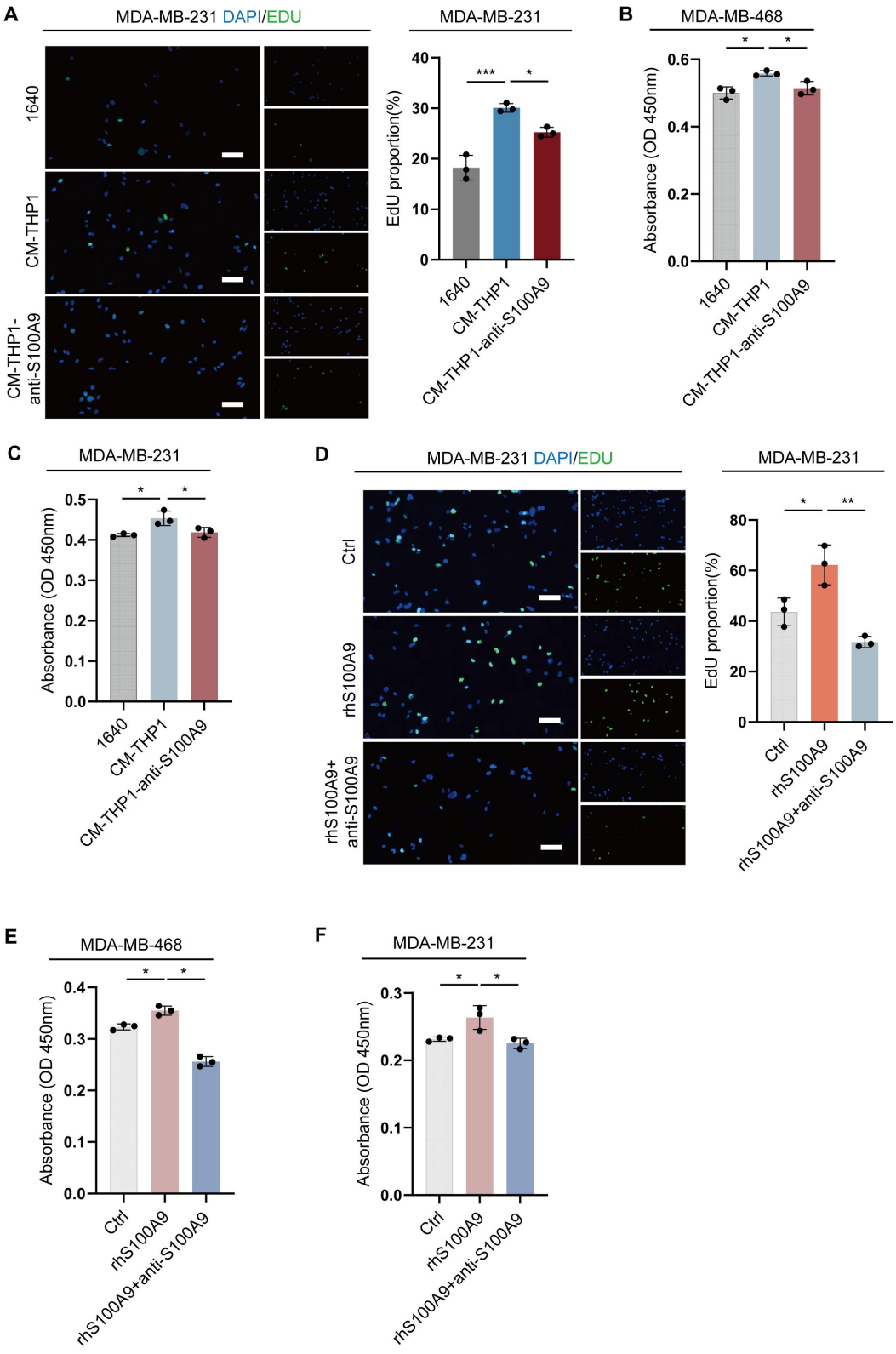
A-B. CCK-8 and EDU assays evaluating the proliferation of MDA-MB-231 cells exposed to conditioned medium from THP1 cells stimulated with UDP (100 μM) or UDP (100 μM) + MRS2578 (1 μM). Scale bar: 80 μm. **C-D**. CCK-8 and EDU assays evaluating the proliferation of 4T1 cells exposed to conditioned medium from RAW264.7 cells stimulated with UDP (100 μM) or UDP (100 μM) + MRS2578 (1 μM). Scale bar: 80 μm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



Supplementary Figure 7. P2RY6 modulates S100A9 production through the Ca^{2+} signaling cascade.

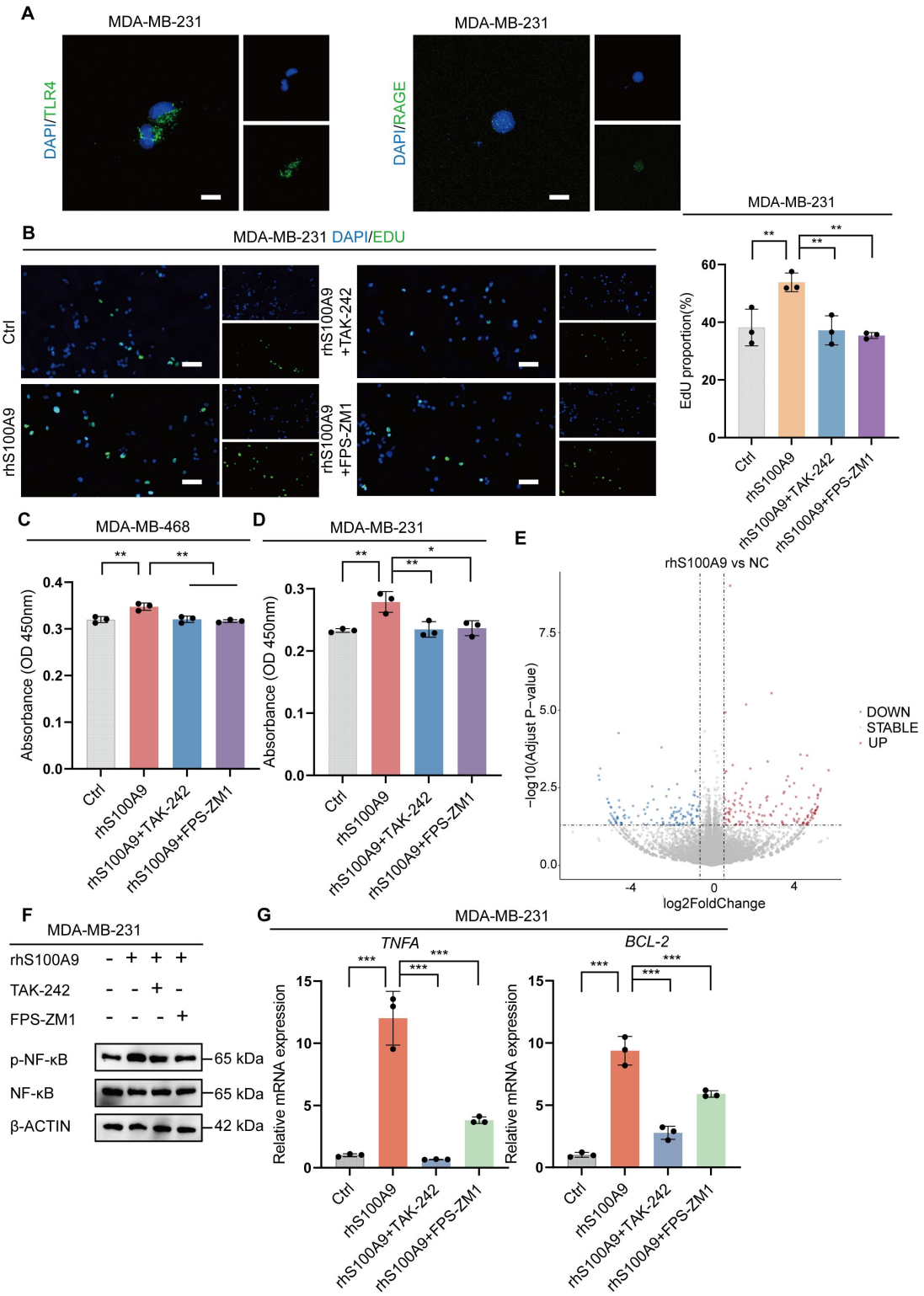
A. Intracellular Ca^{2+} levels were monitored using the fluorescent Ca^{2+} indicator Fluo-4 AM in RAW264.7 cells. Scale bar: 80 μm. **B**. Quantitative analysis of fluorescence intensity. **C**. Cell-cell communication network derived from scRNA-seq data

GSE176078, highlighting macrophage-originated signals to other cell types. **D**. Cell-cell communication network showing signaling from macrophage sub-clusters (senders) to other cellular sub-clusters and malignant cells, based on scRNA-seq analysis. **E**. The numbers of up-regulated and down-regulated secretory proteins in UDP (100 μM) -treated THP1 si-*P2RY6* cells compared with UDP (100 μM) -treated THP1 si-NC cells, based on mass spectrometry. **F**. Distribution of S100A9 expression among different cell populations in TNBC. **G**. Elevated S100A9 expression in macrophages from TNBC compared with non-TNBC samples. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



Supplementary Figure 8. P2RY6^{+} macrophages promote TNBC growth via S100A9 secretion.

A. Neutralization of S100A9 in THP1 conditioned medium with an anti-S100A9 antibody (2 μg/mL) abolishes its pro-proliferative effect on MDA-MB-231 cells, evaluated by EdU assay. Scale bar: 80 μm. **B-C.** Neutralization of S100A9 in THP1 conditioned medium with an anti-S100A9 antibody (2 μg/mL) abolishes its pro-proliferative effect on MDA-MB-468 and MDA-MB-231 cells, evaluated by CCK-8 assay. **D.** Proliferation of MDA-MB-231 cells treated with rhS100A9 (1 μg/mL) or an anti-S100A9 neutralizing antibody (2 μg/mL), evaluated by EdU assay. Scale bar: 80 μm. **E-F**. Proliferation of MDA-MB-468 and MDA-MB-231 cells treated with rhS100A9 (1 μg/mL) or an anti-S100A9 neutralizing antibody (2 μg/mL), evaluated by CCK-8 assay. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



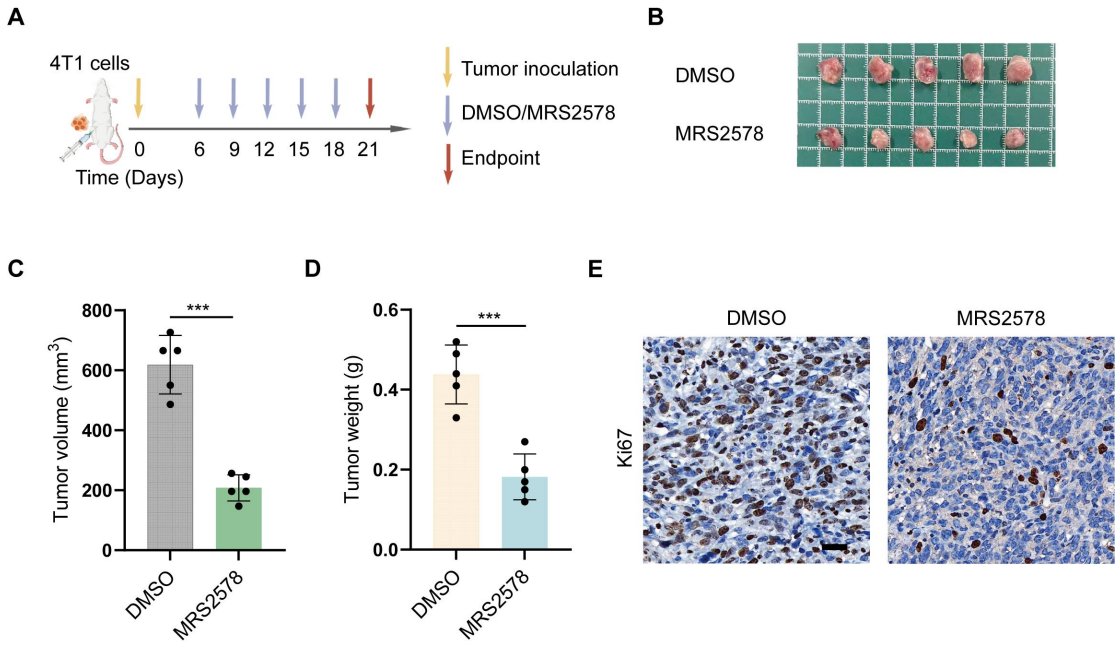
88

89 **Supplementary Figure 9. S100A9 promotes TNBC growth by activating the NF-κB**

90 **pathway through TLR4 and RAGE receptors.**

91 **A**. Immunofluorescence visualization of TLR4 and RAGE expression in

MDA-MB-231 cells. Scale bar: 40 μm. **B**. EdU assay demonstrating that pharmacological inhibition of TLR4 (TAK-242; 10 μM) or RAGE (FPS-ZM1; 10 μM) abrogates rhS100A9-induced proliferation of MDA-MB-231 cells. Scale bar: 80 μm. **C-D**. CCK-8 assay demonstrating that pharmacological inhibition of TLR4 (TAK-242; 10 μM) or RAGE (FPS-ZM1; 10 μM) abrogates rhS100A9-induced proliferation of MDA-MB-468 and MDA-MB-231 cells. **E**. Volcano plot showing S100A9-related DEGs. **F**. Western blot analysis showing that S100A9-induced activation of the NF-κB pathway in MDA-MB-231 cells is mediated by TLR4 and RAGE, and is attenuated by specific receptor inhibitors. **G**. qRT-PCR analysis confirming that S100A9 upregulates NF-κB target genes *TNFA* and *BCL-2* in MDA-MB-231 cells in a TLR4/RAGE-dependent manner, an effect reversed by receptor blockade. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



105

106 **Supplementary Figure 10. Pharmacological inhibition of P2RY6 suppresses**

107 **TNBC growth *in vivo*.**

108 **A**. Experimental timeline of the orthotopic mammary tumor model in BALB/c mice

109 treated with the P2RY6 inhibitor MRS2578 (3 mg/kg, every 3 days). **B**. Representative

110 gross images, measured volume, and weight of excised orthotopic tumors (n = 5). **C-D**.

111 Quantification of tumor volume and weight across treatment groups. **E**. IHC analysis

112 of Ki67 expression in tumors from MRS2578-treated mice. Scale bar: 50 μm. *$p < 0.05$,

113 **$p < 0.01$, ***$p < 0.001$.

114

115 **Supplementary Table 1. The sequences of the primers used in the study.**

Gene	Primer Sequence 5'-3'
human-*β-ACTIN*-F1	5′-CACCAGGGCGTGATGGTGGG-3′
human-*β-ACTIN*-R1	5′-GATGCCTCTCTTGCTCTGGGC-3′
human-*CD11b*-F1	5'-TGGCTCTCAGAGTCCTTCTGT-3'
human-*CD11b*-R1	5'-GATCCCTGAAGCTGGACCAC-3'
human-*KLF4*-F1	5'-GGGCCCAATTACCCATCCTT-3'
human-*KLF4*-R1	5'-GTGAGCATCATCCCGTGTGT-3'
human-*MAFB*-F1	5'-TGTATGAACCGCATGGTGCT-3'
human-*MAFB*-R1	5'-TTTCTGTTGCGGCAGGTTTG-3'
human-*CD86*-F1	5'-GCTTTGCTTCTCTGCTGCTG-3'
human-*CD86*-R1	5'-GGCAGGTCTGCAGTCTCATT-3'
human-*iNOS*-F1	5'-TCCCGAGTCAGAGTCACCAT-3'
human-*iNOS*-R1	5'-TCCATGCAGACAACCTTGGG-3'
human-*CD206*-F1	5'-CTGAATTGTACTGGTCTGTCCT-3'
human-*CD206*-R1	5'-GCTTAGATGTGGTGCTGTGG-3'
human-*ARG1*-F1	5'-ACTTAAAGAACAAGAGTGTGATGTG-3'
human-*ARG1*-R1	5'-CTCGCTTGCTTTTCCCACAG-3'
human-*TGFB*-F1	5'-GGAAATTGAGGGCTTTCGCC-3'
human-*TGFB*-R1	5'-CCGGTAGTGAACCCGTTGAT-3'
mouse-*β-actin*-F1	5'-CCTTCCAGCAGATGTGGATCA-3'
mouse-*β-actin*-R1	5'-TCAGTAACAGTCCGCCTAGA-3'
mouse-*Cd86*-F1	5'-CTTACGGAAGCACCCACGAT-3'
mouse-*Cd86*-R1	5'-TGTAAATGGGCACGGCAGAT-3'
mouse-*inos*-F1	5'-CAGCTGGGCTGTACAAACCTT-3'
mouse-*inos*-R1	5'-CATTGGAAGTGAAGCGTTTCG-3'

116 **Supplementary Table 1. (continued)**

Gene	Primer Sequence 5'-3'
mouse-*Cd206*-F1	5'-GTGGGGACCTGGCAAGTATC-3'
mouse-*Cd206*-R1	5'-CACTGGGGTTCCATCACTCC-3'
mouse-*Arg1*-F1	5'-AAGAATGGAAGAGTCAGTGTGG-3'
mouse-*Arg1*-R1	5'-GGGAGTGTTGATGTCAGTGTG-3'
mouse-*Tgfb*-F1	5'-CGTGGAAATCAACGCTCCAC-3'
mouse-*Tgfb*-R1	5'-GAAGTTGGCATGGTAGCCCT-3'
human-*P2RY6*-F1	5'-CATTGCACGCGACAGTTTCA-3'
human-*P2RY6*-R1	5'-GGCAGAGGTCTTGTTATCCAA-3'
mouse-*P2ry6*-F1	5'-CCAGTTCTCATGGCTTGCAG-3'
mouse-*P2ry6*-R1	5'-CACCTTGGGAAGCCATGTTC-3'
human-*S100A9*-F1	5'-GCCACTAATCAGGAGGCCAG-3'
human-*S100A9*-R1	5'-CCCCACAGCCAAGACAGTTT-3'
human-*TNFA*-F1	5'-ATCCTGGGGGACCCAATGTA-3'
human-*TNFA*-R1	5'-AAAAGAAGGCACAGAGGCCA-3'
human-*BCL-2*-F1	5'-AAAAATACAACATCACAGAGGAAGT-3'
human-*BCL-2*-R1	5'-GTTTCCCCCTTGGCATGAGA-3'

117

118

Supplementary Table 2. Neurotransmitter receptors and neuroactive ligand-receptors in the analysis.

Neurotransmitter	Neurotransmitter receptors and neuroactive ligand-receptors
Glutamate	GRIA1, GRIA2, GRIA3, GRIA4, GRIK1, GRIK2, GRIK3, GRIK4, GRIK5, GRID1, GRID2, GRIN1, GRIN2A, GRIN2B, GRIN2C, GRIN2D, GRIN3A, GRIN3B, GRM1, GRM2, GRM3, GRM4, GRM5, GRM6, GRM7, GRM8
Gamma aminobutyric acid (GABA)	GABRA1, GABRA2, GABRA3, GABRA4, GABRA5, GABRA6, GABRB1, GABRB2, GABRB3, GABRD, GABRE, GABRG1, GABRG2, GABRG3, GABRP, GABRQ, GABRR1, GABRR2, GABRR3, GABBR1, GABBR2
Glycine	GLRA1, GLRA2, GLRA3, GLRB
Histamine	HRH1, HRH2, HRH3, HRH4
Acetylcholine	CHRM1, CHRM2, CHRM3, CHRM4, CHRM5, CHRNA1, CHRNA2, CHRNA3, CHRNA4, CHRNA5, CHRNA6, CHRNA7, CHRNA9, CHRNA10, CHRNB1, CHRNB2, CHRNB3, CHRNB4, CHRND, CHRNE, CHRNG
Dopamine	DRD1, DRD2, DRD3, DRD4, DRD5
Epinephrine (E)	ADRA1A, ADRA1B, ADRA1D, ADRA2A, ADRA2B, ADRA2C,
Norepinephrine (NE)	ADRB1, ADRB2, ADRB3
Serotonin (5-HT)	HTR1A, HTR1B, HTR1D, HTR1F, HTR2A, HTR2B, HTR2C HTR3A, HTR3B, HTR3C, HTR3D, HTR3E, HTR4, HTR5A, HTR6, HTR7
Neuroactive ligand	TAAR, TACR1, TAKR, NPY1R, NPY2R, NPY4R, NPY5R, NPY6R, NPYNR, GPR83, OPRM1, OPRK1, OPRD1, OPRL1, AVPR1A, AVPR1B, AVPR2, CCKAR, CCKBR, CCKLR, OXTR, ATGR1, ATGR2, APLNR, NMBR, GRPR, BRS3, BDKRB1, BDKRB2, C3AR1, C5AR1, FPR1, FPRL, EDNRA, EDNRB, GALR1, GALR2, GALR3, GALRN, GHSR, KISS1R, MC1R, MC2R, MC3R, MC4R, MC5R, MLNR, NMUR1, NMUR2, NPFFR1, NPFFR2, NPBWR1, NPBWR2, NTSR1, NTSR2, HCRTR1, HCRTR2, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5, TACR2, TACR3, OPRM1, OPRK1, OPRD1, OPRL1, AVPR1A, AVPR1B, AVPR2, UTS2R, F2R, F2RL1, F2RL2, F2RL3, PARD3, PRLHR, MCHR1, MCHR2, FSHR, LHCGR, PTGDR, PTGER1, PTGER2, PTGER3, PTGER4, PTGFR, PTGIR, TBXA2R, ADORA1, ADORA2A, ADORA2B, ADORA3, P2RY1, P2RY2, P2RY4, P2RY6, P2RY8, P2RY10, P2RY11, P2RY13, P2RY14, LPAR4, LPAR6, GPR35, CNR1, CNR2, PTAFR, GNRHR, TRHR, MTNR1A, MTNR1B, GPR50, LPAR1, LPAR2, LPAR3, S1PR1, S1PR2, S1PR3, S1PR4, S1PR5, LTB4R1, LTB4R2, MAS1, RXFP1, RXFP2, RXFP3, RXFP4, CYSLTR1, CYSLTR2, CALCR, CALCRL, CRHR1, CRHR2, GIPR, GCGR, GLP1R, GLP2R, GHRHR, PTHR1, PTHR2, ADCYAP1R1, SCTR, VIPR1

Supplementary Table 3. Correlation of TAM-associated P2RY6 expression with clinicopathological features in TNBC.

Variables		Low expression		High expression		*p* value
		n=8	%	n=21	%	
Age	<49	1	12.5	7	33.33	0.262
	≥50	7	87.5	14	66.67	
Menstrual status	Pre-menopause	2	25	10	47.62	0.269
	Post-menopause	6	75	11	52.38	
Grade	I	0	0	0	0	0.045
	II	4	50	3	14.29	
	III	4	50	18	85.71	
Tumor size	≤2	4	50	7	33.33	0.408
(cm)	>2 and ≤5	4	50	14	66.67	
	>5	0	0	0	0	
TNM stage	I	4	50	6	28.57	0.278
	II	4	50	15	71.43	
	III	0	0	0	0	
	IV	0	0	0	0	
Lymph nodes	Negative	8	100	17	80.95	0.184
	Positive	0	0	4	19.05	
Poor prognosis	Negative	8	100	20	95.24	0.53
	Positive	0	0	1	4.76	

Supplementary Table 4. Differentially down-regulated proteins identified in UDP-treated THP1 cells of the si-*P2RY6* group compared to the untreated si-NC group.

Differentially down-regulated proteins
GMPR, BNIP2, ZNF407, DNAJC21, CALML5, STEAP2, FLG, SERPINB3, RNASE7, ACTN2, SCGB1D2, SPRR1B, RBM7, BRSK1, MROH9, MRPS6, SYNE2, DPYSL4, TGM3, SBSN, MIEF1, LGALSL, ZNF516, EIF4EBP3, DSP, FGD6, S100A7, CASP14, KRT16, GSDMA, SOAT1, CEP41, JUP, STMN2, CDK1, VARS2, MTIF3, CTNNBL1, KRT12, KITLG, MRPL3, ELOF1, CARHSP1, TNIK, CCDC88C, TARS2, HDAC5, H1-3, TRPA1, INTS1, JMJD7, KRT23, MOCS2, SARAF, KRT14, CPA4, INPP5F, KRT78, MMP10, PTBP2, C6orf120, TRAPPC2L, CIDEC, DST, NUDT16L1, EBP, MED28, CD47, CASKIN2, NDUFA7, NDUFA12, KPRP, HSCB, BCR, CREB1, PHYHIP, MKLN1, METAP1D, ACTN3, PPP2R3A, S100A9, FAM118B, PCIF1, KDM5C, AKAP12, NFATC4, YIF1B, GPD2, RPL34, NCDN, PGGHG, UBA52, NKRF, YARS2, POMK, DSC1, FLG2, ANKRD13A, LUM, SS18, FDXR, BST2, OSBPL9, UBE2E3, ASB1, RPS21, CTU2, ZNF146, CHTOP, DAP3, MTFR1L, NAA38, KRT10, MYO5A, DCTN6, FOXJ3, FER1L4, RGS4, NFATC2, YJU2, MAP1LC3B2;MAP1LC3B, HIP1R, KRT6A, COMMD8, TBCEL, SURF2, FAM169A, KATNB1, TGM1, BLOC1S3, DHRS4, NDUFA8, NSDHL, MRPS22, IFT74, TRNT1, FIS1, RAMAC, DPF3, NADK, UCK2, RPAP1, HERC5, VPS8, MAK16, UBE2D2, CD70, PCID2, GDPGP1, CTU1, CNRIP1, GRB10, ABCE1, SEC61A1;SEC61A2, C4BPA, PCDHGA11, TRAF1, CARD6, TUSC2, ITPKB, NUP107, HMGN4, EXOC2, MCMBP, MOCS2, PPP3CC, FAF2, DNAJB6, XIAP, CCDC50, WDR45B, CWC15, KLHL42, NCOA5, PPP1R35, PPOX, KRT17, ARRDC1, LMTK2, RSBN1, CIC, NCOA3, CDC73, MAPK13, PPP1R37, PDE1B, CASP10, RIPK1, ECHDC2, LRIF1, ATP6V0A1, SLC9A6, STK38, CRADD, FPGT, AP1S2, CDSN, LSM4, DISP1, TOR1A, VHL, NDUFAF3, RPL7L1, MRPS10, CD99, SMARCAL1, SFRP1, KRT1, BPTF, SELENOS, CCDC159, HIGD1A, IL18, DSG1, ARG2, ZC3H11B, GMDS, MMP3, SUGP1, DICER1, ABRACL, DPY19L3, RIOX1, DCD, ADI1, MYCBP, MTMR2, SLC1A4, GINS1, SPIRE1, MED6, FHIP2A, MAPK1IP1L, MEPCE, AKTIP, DDX49, COMMD10, FKBP3, NEDD8, UQCRH, PRKCI, ELOB, MIEN1, CSNK1G1, PIP4K2B, NCOR1, RTF1, THYN1, PIGR, FAHD2B, C11orf98, NT5C3A, CBR4, PSMG3, UTP18, KIF11, VPS50, SPRED2, SLC25A6, BRIX1, LYPLAL1, KIFBP, DGKQ, HSF1, WDFY3, CYP2W1, SEPHS2, WDR59, GUF1, TMEM59, ETHE1, GET3, GSTM1, BLOC1S5, ZNF687, MYL9, ADA2, C22orf31, PCNP, CRLF3, MAP7D3, RABGEF1, S100A8, KRT2, RNF14, THUMPD3, TUBGCP6, DOT1L, SYNJ1, FCGR1A, TELO2, COPS9, THOC3, LNPEP, BRPF1, PDE6D, AGO4, CRIM1, SP3, TRIAP1, MORC3

Supplementary Table 5. Differentially expressed secretory proteins identified in UDP-treated THP1 cells of the si-*P2RY6* group compared to the untreated si-NC group.

Differentially up-regulated secretory proteins	CXCL16, C1S, CLU, APOA1, CCDC134, HMGB1, COL4A2, ADM, XYLT1, IL1B, MMP7, SAA1, CCL2, CXCL2;CXCL3, ADAMTS12, SAA2, SERPINA1
Differentially down-regulated secretory proteins	RNASE7, SCGB1D2, SBSN, S100A7, CPA4, MMP10, C6orf120, S100A9, LUM, KRT10, C4BPA, CDSN, SFRP1, IL18, MMP3, DCD, PIGR, ADA2, S100A8, CRIM1