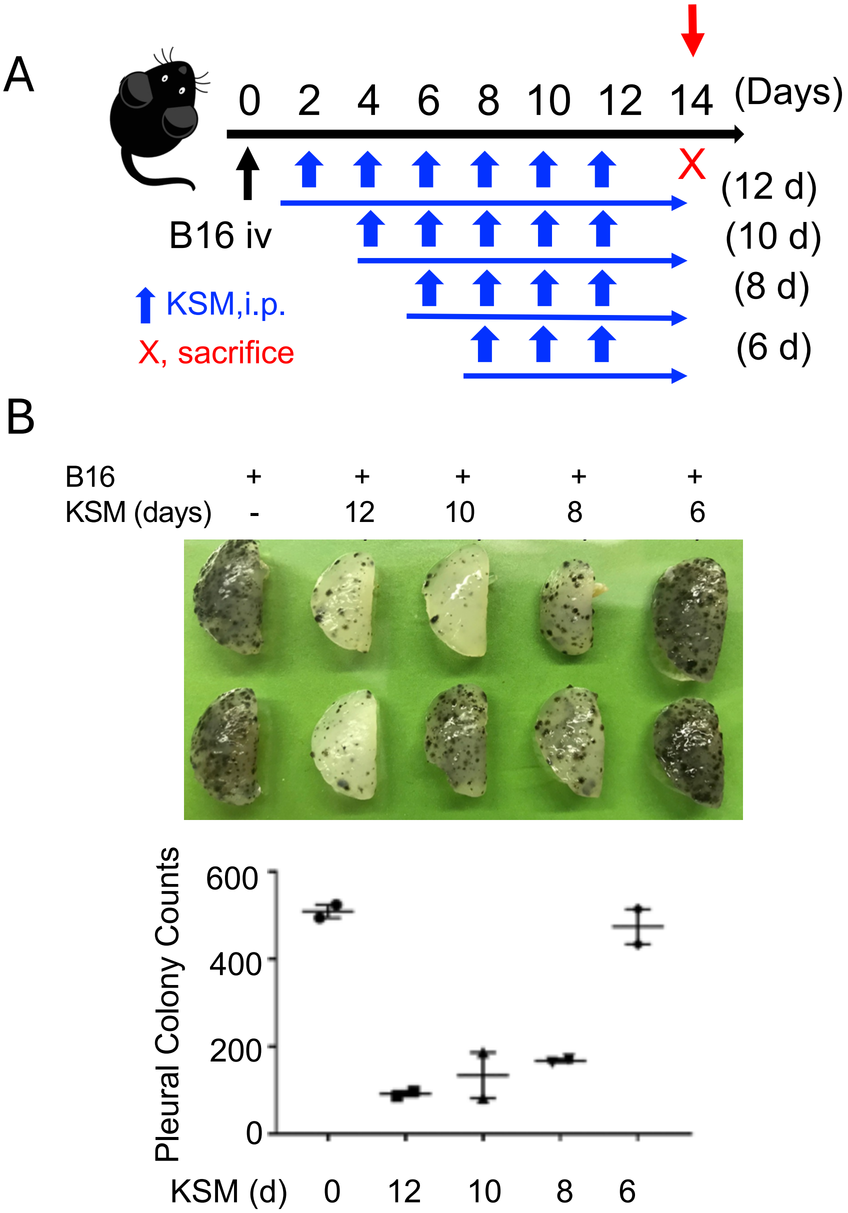
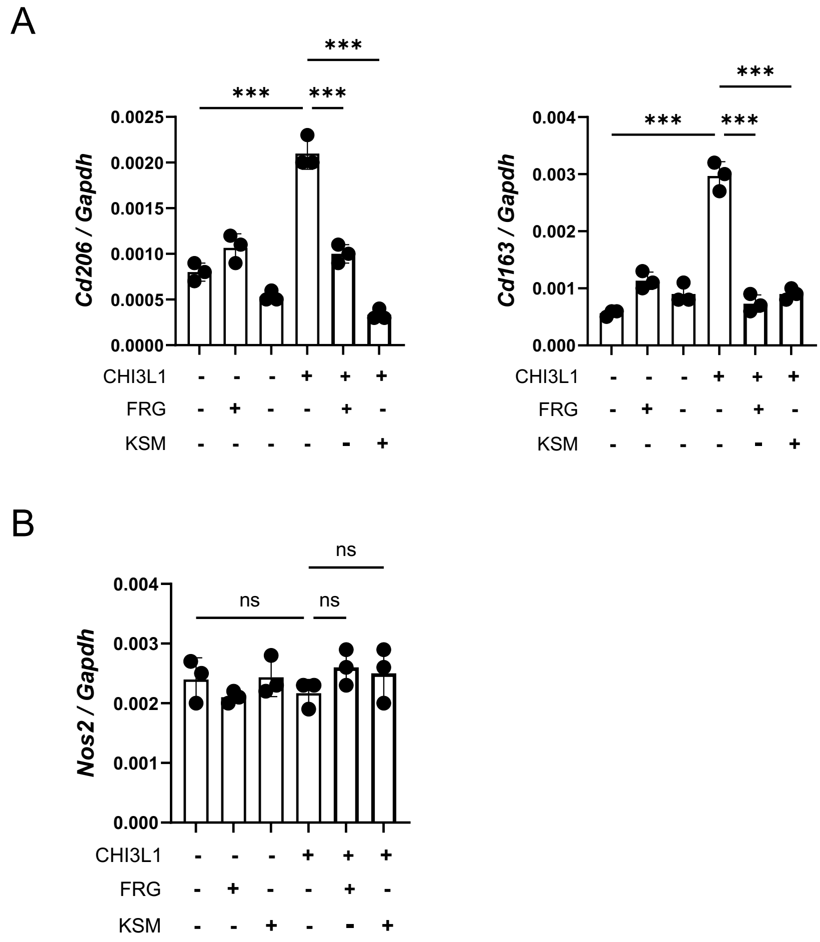
Supplemental Figures & Tables



<u>Figure S1</u>. Kasugamycin (KSM) inhibition of melanoma lung metastasis correlates positively with duration of KSM treatment. WT mice were challenged with B16-F10 (B16) melanoma cells, then the KSM (100 mg/kg) was delivered to the mice at different time points to see the time-kinetic effect of KSM. (A) KSM treatment protocol (B) Photograph of melanoma lung metastasis with different treatment time points.



<u>Figure S2</u>. Kasugamycin (KSM) inhibition of CHI3L1-driven M2 macrophage differentiation in AMJ2-C11 cells. AMJ2-C11 cells were stimulated with rCHI3L1 with or without FRG/KSM. The mRNA expressions of Cd206 and Cd163 (A) and Nos2 (B) were evaluated by qRT-PCR. The values are the mean ± SEM. ns, not significant, ***P<0.001 (One-Way ANOVA, multiple comparisons).

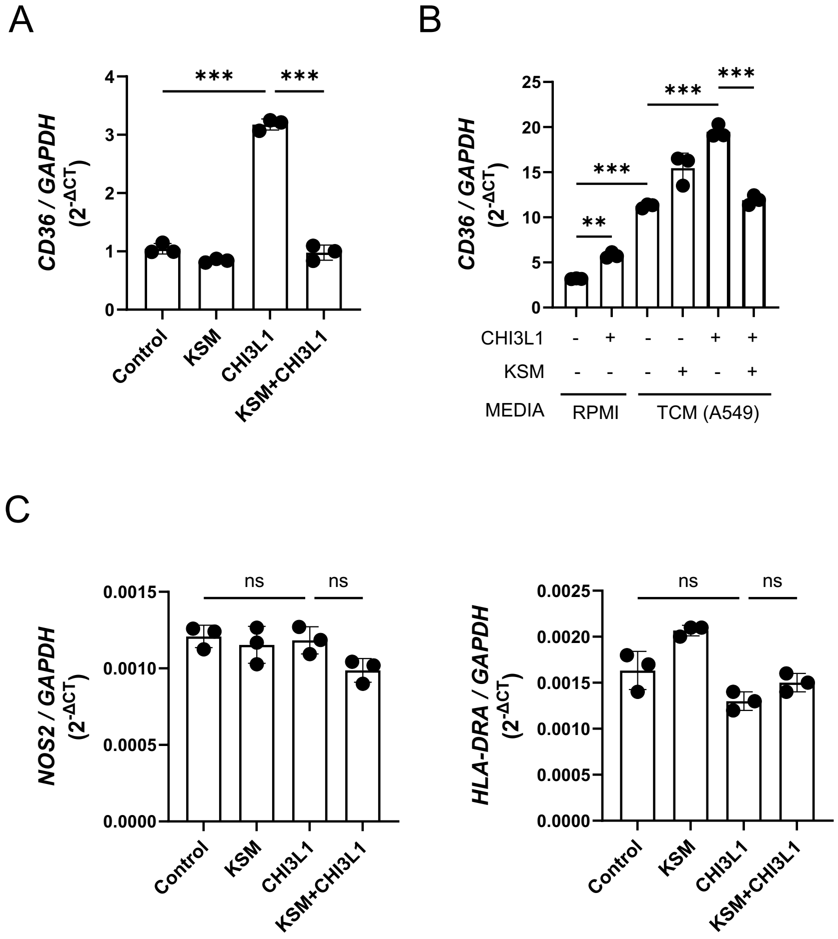
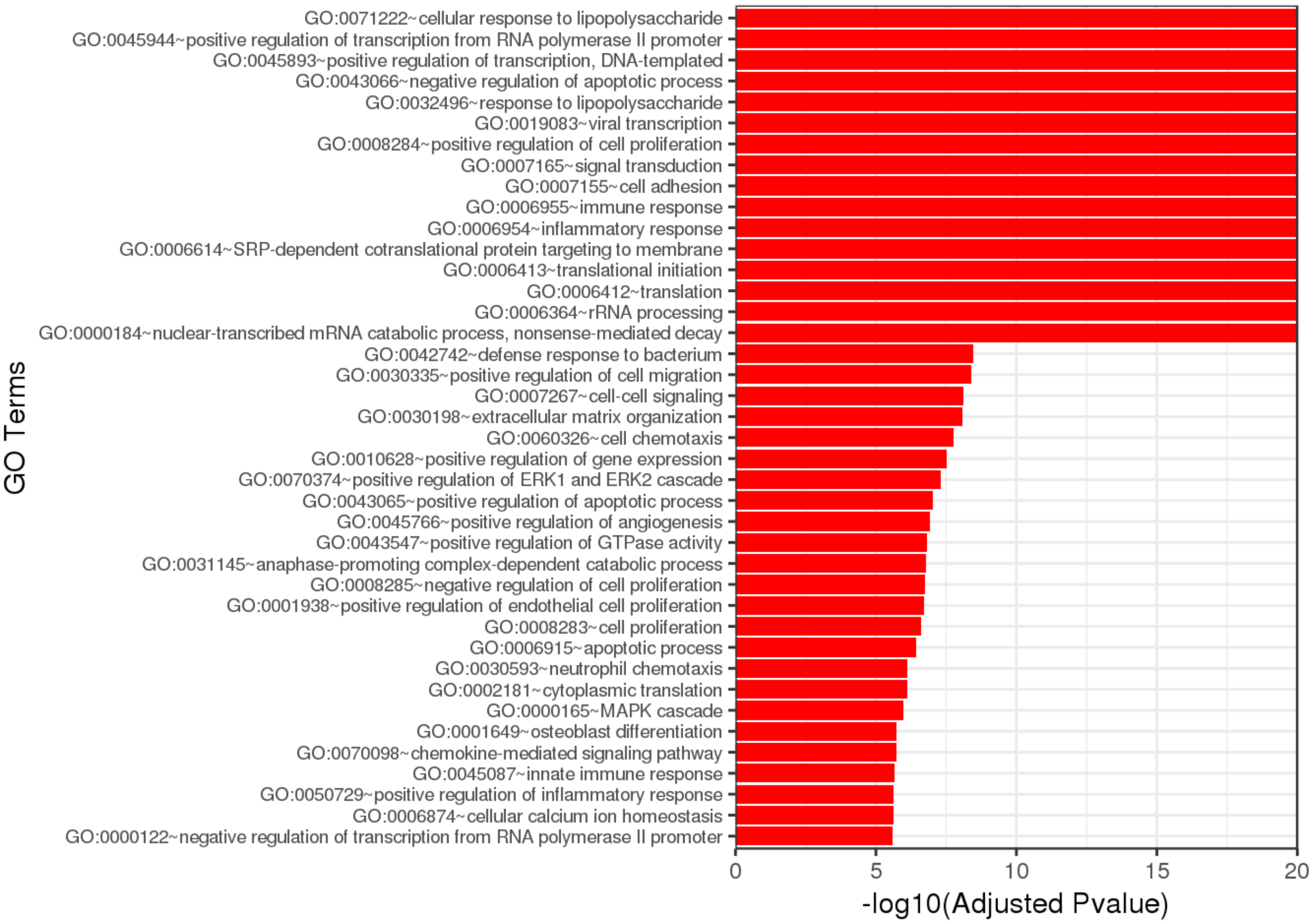
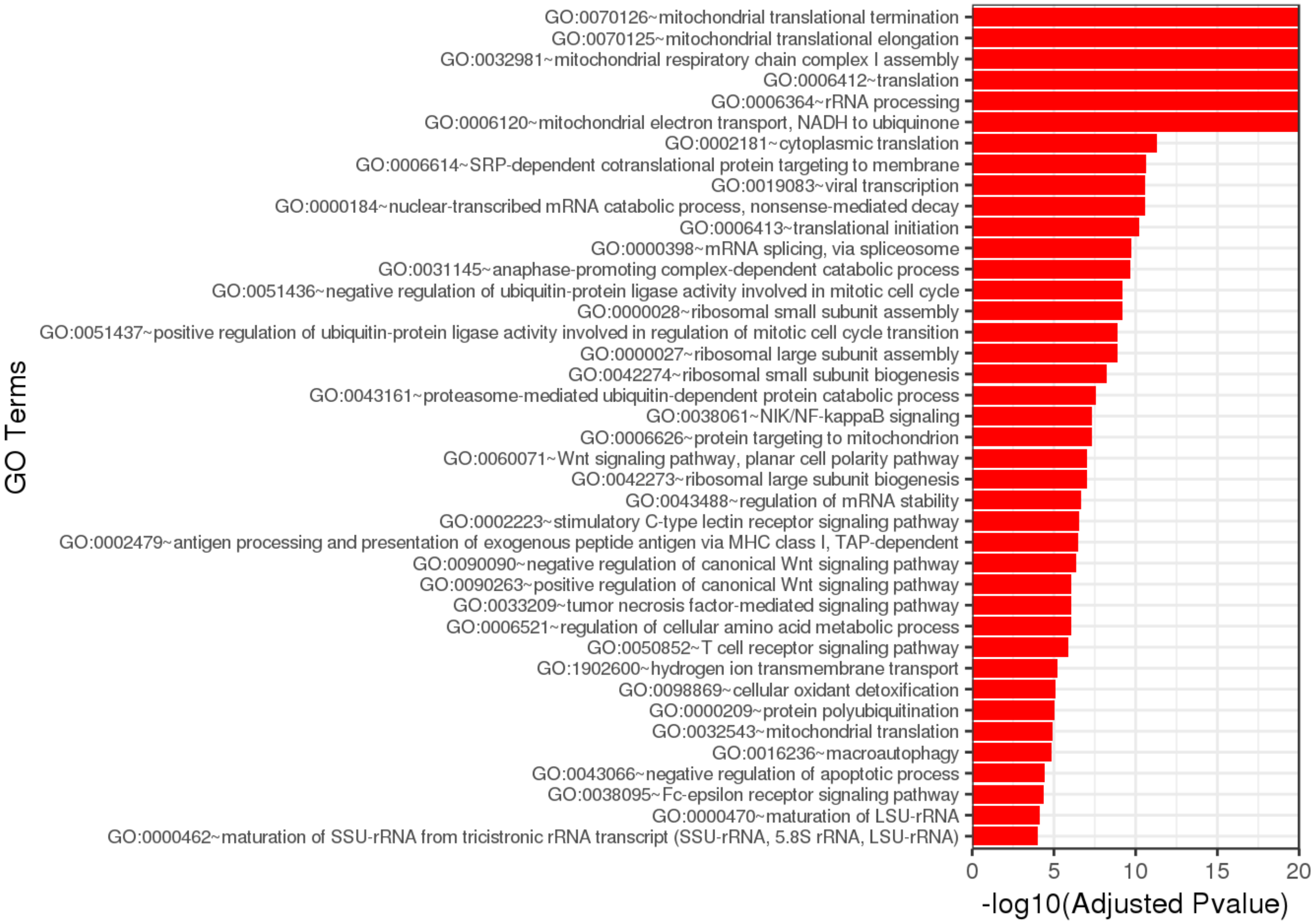


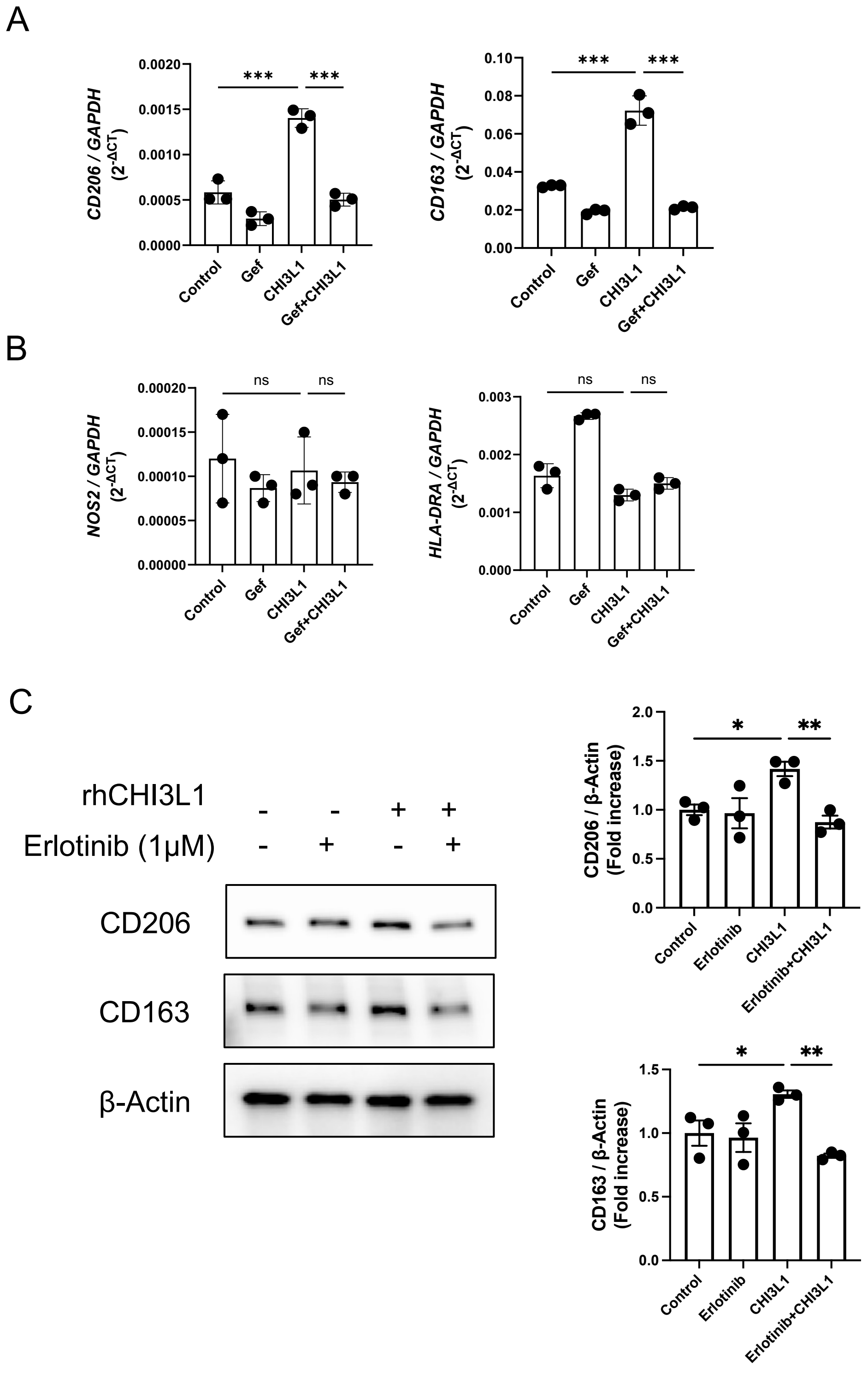
Figure S3. **Kasugamycin (KSM) inhibition of CHI3L1-driven M2 macrophage differentiation in Human monocytic THP-1 cells**. THP-1 cells were stimulated with CHI3L1 in the presence and absence of tumor-conditioned medium (TCM, A549 cell culture supernatant). The mRNA expressions of CD36 (A and B) (typical M2 macrophage differentiation marker, together with other markers such as CD206 and CD163, as described in the main text) and NOS2 and HLA-DRA (C) (typical M1 differentiation marker) were evaluated by qRT-PCR. The values are the mean ± SEM. ns, not significant, ***P*<0.01, ****P*<0.001 (One-Way ANOVA, multiple comparisons).



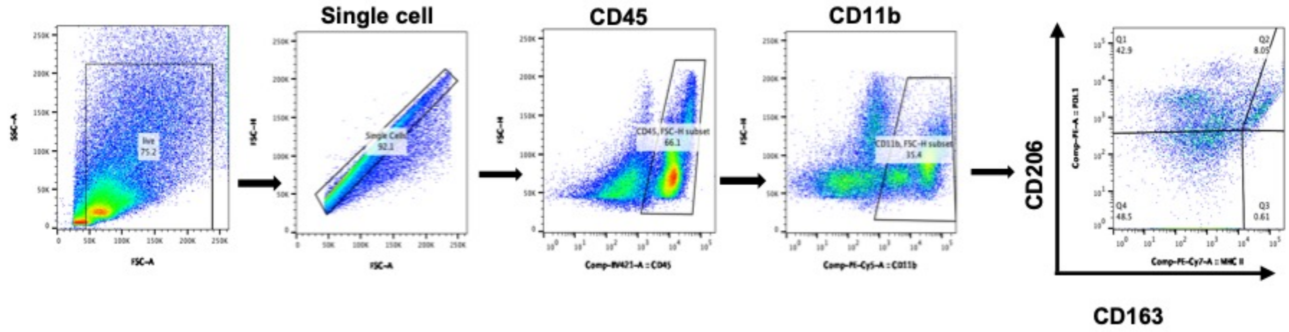
<u>Figure S4</u>. Gene ontology analysis on the significantly differentially expressed genes stimulated by CHI3L1 vs PBS control in the differentiated macrophages. The genes were clustered by their gene ontology, and the enrichment of gene ontology terms was tested using Fisher exact test (GeneSCF v1.1-p2). This figure shows gene ontology terms, if any, that are significantly enriched with an adjusted p-value less than 0.05 in the differentially expressed gene sets (up to 40 terms).



<u>Figure S5</u>. Gene ontology analysis on the significantly differentially expressed genes the differentiated macrophages with Kasugamycin (KSM) treatment. The genes were clustered by their gene ontology, and the enrichment of gene ontology terms was tested using Fisher exact test (GeneSCF v1.1-p2). This figure shows gene ontology terms, if any, that are significantly enriched with an adjusted p-value less than 0.05 in the differentially expressed gene sets (up to 40 terms).



<u>Figure S6</u>. EGFR inhibition of CHI3L1-driven M2 macrophage differentiation in Human monocytic THP-1 cells. THP-1 cells were stimulated with CHI3L1 with or without EGFR inhibitor (Gefitinib 1μM 48 hours). The mRNA expressions of CD206 and CD163 (A) and NOS2 and HLA-DRA (B) were evaluated by qRT-PCR. A structurally different EGFR inhibitor (Erlotinib, MilliporeSigma, CDS022564 1μM 72 hours) was used to confirm EGFR dependency. The values are the mean ± SEM. ns, not significant, **P*<0.05, ***P*<0.01, ****P*<0.001 (One-Way ANOVA, multiple comparisons).



<u>Figure S7</u>. Gating strategy of CD206(+)/CD163(+) macrophages in the lungs.

Supplementary Tables

Table S1. List of top 50 differentially expressed genes in the differentiated macrophages with CHI3L1-stimulation: Up-regulated genes (fold change; Log 2).

Gene Name	Signal A (PBS)	Signal B (CHI3L1)	Fold Change: Log2
CXCL13	10.4897611	5112.79519	8.92895648
GJA1	1.9668302	474.367334	7.91395383
ST18	1.31122014	314.21116	7.90464079
EGFR	1.31122014	271.502847	7.69387473
AC011294.1	3.27805034	594.865788	7.50355442
MMP8	4.58927048	626.897023	7.09379629
CCL18	0.65561007	88.4672198	7.0761078
RGS4	1.31122014	169.307955	7.01256
IGFBP3	156.690806	18762.6771	6.90379032
DIRAS2	1.31122014	144.903205	6.78800049
LRFN5	3.27805034	326.413535	6.63769539
FP671120.4	3.93366041	349.292989	6.47239979
MAPK10	1.31122014	112.87197	6.42759924
CCR7	8.52293089	730.617212	6.4216051
RASGEF1B	17.7014718	1415.47552	6.32125955
DPYSL3	3.27805034	227.269237	6.11539904
GLIS3	26.8800128	1786.12266	6.0541418
TFPI2	3.27805034	208.965674	5.99426305
MMP9	1025.37415	63710.1259	5.95729744
NRCAM	15.0790316	861.792745	5.83671276
AC026310.2	3.27805034	184.560924	5.81509483
ITGA1	114.076152	6304.05206	5.78820319
ADAMDEC1	664.788609	36713.8962	5.78728347
LINC01282	0.65561007	35.0818285	5.74168734
TNFRSF9	59.6605162	3050.59379	5.67616164
CLEC4E	3.27805034	161.681471	5.62415268
EBI3	113.420542	5588.68782	5.62274921
LINC01010	14.4234215	660.453555	5.51696042
AC120193.1	5.24488054	237.946315	5.50356649
ITGB8	14.4234215	651.301773	5.49682954
MACC1	8.52293089	376.748333	5.46609451

COL8A1	2.62244027	114.397267	5.44697724
NTRK3	13.7678114	593.340491	5.42947793
MET	38.680994	1433.77908	5.21204664
OLFML2B	22.9463524	846.539776	5.20523238
ST8SIA1	5.90049061	215.066862	5.18779246
TSPAN7	5.90049061	199.813893	5.08166447
CD80	7.86732082	253.199284	5.00824523
TNFRSF18	1.31122014	41.1830161	4.97303447
TNFAIP6	18.3570819	573.511632	4.9654056
XIRP1	1.9668302	61.0118757	4.95512103
NA	1.9668302	61.0118757	4.95512103
SGK1	713.303754	21714.1266	4.92797305
AP003354.2	3.93366041	117.447861	4.89998805
AC112496.1	7.86732082	218.117456	4.79307786
FAM216B	7.21171075	198.288596	4.78110481
RF00443	1.9668302	53.3853913	4.76247612
AL357874.2	1.31122014	35.0818285	4.74170864
TLR8	78.0175981	1982.88596	4.66765429
CCL20	46.5483148	1160.75094	4.64018068

Table S2. List of top 50 differentially expressed genes in the differentiated macrophages with KSM-stimulation: Up-regulated genes (fold change; Log 2).

Gene Name	Signal A (no KSM)	Signal B (KSM)	Fold Change: Log2
AC090498.1	3.03514764	131.789306	5.4403228
AC116347.1	1.51757382	52.7157222	5.1183947
AC009244.1	1.51757382	51.3978292	5.08186882
PVALB	1.51757382	48.1030965	4.98629116
RPL41P1	47.0447885	1267.15417	4.75141334
RPL21P16	3.03514764	80.3914764	4.72720394
AP000439.3	1.51757382	38.2188986	4.6544476
AL451165.2	1.51757382	37.5599521	4.62935662
GNG11	1.51757382	34.924166	4.52438706
AL355987.3	1.51757382	34.924166	4.52438706
TMEM191C	3.03514764	68.5304389	4.49690632
AC010343.1	1.51757382	30.9704868	4.35105546
BCYRN1	1.51757382	30.3115403	4.32002856
ROMO1	34.9041979	629.95288	4.17377146
NME1-NME2	1.51757382	27.0168076	4.15401861
MDP1	3.03514764	49.4209896	4.0252853
TCAF1P1	1.51757382	24.3810215	4.00591997
AC124067.2	1.51757382	23.722075	3.96639161
AC138761.1	3.03514764	44.8083639	3.88392945
RPL35	962.141803	13991.4116	3.86214818
C10orf143	1.51757382	21.0862889	3.79646661
TMEM183B	3.03514764	40.8546847	3.75066292
GCC2-AS1	1.51757382	20.4273424	3.75066292
AC005540.1	1.51757382	20.4273424	3.75066292
FRG1-DT	1.51757382	20.4273424	3.75066292
PLSCR3	1.51757382	19.7683958	3.7033572
AL512625.2	1.51757382	19.7683958	3.7033572
CDC20P1	1.51757382	19.7683958	3.7033572
TRIM74	3.03514764	38.8778451	3.67910966
AL451085.2	3.03514764	38.8778451	3.67910966
CCDC34	3.03514764	38.2188986	3.6544476
ITPKA	1.51757382	19.1094493	3.6544476
HIST1H2AI	1.51757382	19.1094493	3.6544476
AC064799.1	1.51757382	19.1094493	3.6544476

AL137779.2	3.03514764	37.5599521	3.62935662
RPL41	593.371364	7286.6307	3.61824466
TMED1	34.9041979	426.99735	3.61275465
AC241585.3	6.07029529	73.8020111	3.60382153
PIPSL	1.51757382	18.4505028	3.60382153
AL137782.1	4.55272146	54.6925618	3.58654354
RPS3AP26	10.6230167	126.517733	3.57407418
AC131206.1	6.07029529	71.166225	3.55135411
CD79A	1.51757382	17.7915562	3.55135411
H3F3AP6	1.51757382	17.7915562	3.55135411
FALEC	1.51757382	17.7915562	3.55135411
RPL7P23	1.51757382	17.7915562	3.55135411
AC087289.5	1.51757382	17.7915562	3.55135411
MTHFS	22.7636073	265.555451	3.54421204
CSKMT	4.55272146	52.7157222	3.5334322
YBX1P1	80.4314125	910.005155	3.50004376

Table S3. List of the genes that were significantly upregulated by CHI3L1 but downregulated by KSM treatment.

Gene Name	CHI3L1 (fold change: Log2)	KSM (fold change: Log2)
EGFR	7.69387473	-1.0139309
FP671120.4	6.47239979	-6.0427372
AC120193.1	5.50356649	-1.0459921
ITGB8	5.49682954	-1.2342665
MACC1	5.46609451	-1.0697516
AP003354.2	4.89998805	-1.2608666
AC112496.1	4.79307786	-1.134586
FAM216B	4.78110481	-2.3679202
RF00443	4.76247612	-4.0108883
AL357874.2	4.74170864	-1.5571703
HAUS1P1	4.43317949	-2.0966182
CFLAR-AS1	4.01403259	-2.468878
AC011944.1	3.77804715	-1.0140556
AL357055.3	3.60283837	-2.2986906
GUCY1A2	3.44815096	-1.2885389
ABCA13	3.14582767	-1.0474142
AC121761.1	3.02552794	-2.0848889
TDRD6	2.9067204	-1.2793648
HSPD1P11	2.8906014	-1.8759587
GREM1	2.80313303	-1.0722889
RHBDL2	2.4339082	-2.4192621
AL138895.1	2.42462738	-1.6509924
AC010422.3	2.34016853	-1.2837037
PDLIM1P4	2.29910081	-1.5419496
RN7SL2	2.22054775	-1.2980602
AC106865.1	2.17237465	-1.3503748
AL359752.1	2.07616223	-1.1707435
FGD5	2.04825421	-1.6799714
GXYLT2	2.02073516	-1.0060873
MT-TS1	1.95514449	-2.0400347
AC018410.1	1.8462091	-1.8315646
AC092301.1	1.55921758	-2.8664984
AC074032.1	1.46972068	-2.2624271
TNC	1.39618171	-2.4873264
QRICH2	1.3757239	-1.1073181
AC055822.1	1.36257213	-2.0108883
PLA2G2F	1.34746594	-1.2884223
AC007336.1	1.2852975	-1.9080775
H1FX-AS1	1.0066801	-1.7510212

Table S4. List of the genes that were significantly downregulated by CHI3L1 but upregulated by KSM treatment.

Gene Name	CHI3L1 (fold change: Log2)	KSM (fold change: Log2)
AL451085.2	-2.688671209	3.679109655
AC067735.1	-3.781763887	3.255898225
C2orf72	-3.17407816	3.118394701
AL023693.1	-3.563141835	2.654447601
CTSG	-6.300174259	2.554163642
PTGES3L-AARSD1	-3.2873255	2.328961687
GOLGA8J	-2.911063998	2.255898225
CCL23	-2.269069892	2.217930374
NKD2	-4.778028722	2.211504105
AC003070.1	-3.425638156	2.118394701
AC099568.2	-4.941652559	2.044394119
CD27	-2.208058332	1.966391607
TCHH	-3.305363523	1.897552731
GALR3	-2.437148045	1.796466606
NECAB2	-3.339794116	1.626541604
AL359853.1	-2.366751414	1.603821528
AL136116.3	-1.366762378	1.56905611
FOLR2	-1.688699115	1.56905611
AL121761.1	-1.735990364	1.551354108
HP	-3.899252939	1.543700536
NPAS1	-1.018846375	1.496906324
LAMP5	-3.826192565	1.451818435
PRR25	-2.208058332	1.440322796
CASC10	-4.046498959	1.381429107
LINC01624	-3.284289314	1.381429107
GPR55	-1.817427266	1.332519506
PNOC	-2.440011971	1.320028562
FAM72A	-2.468084126	1.249639234
AL033527.3	-3.007870648	1.237039197
ICAM3	-4.149365071	1.237039197
ANKRD34B	-1.817420861	1.229426013
SERPINB2	-4.965011457	1.165700416
KCNH2	-1.829111271	1.156362551
NCALD	-3.169818589	1.154018611
BSPRY	-2.895544588	1.154018611
LGALS12	-1.3053691	1.154018611
AC093616.1	-2.336392023	1.142241443
AC098934.1	-2.688693343	1.138858803
ANXA3	-1.208072131	1.136316609
TMEM139	-1.366760295	1.118394701

AZU1	-4.497323235	1.091070114
PLD4	-2.317727358	1.088393563
RIMS4	-1.43387797	1.076574525
HSD11B1	-5.819331711	1.044394119
CCNO	-2.336382346	1.044394119
AC016394.2	-1.781793989	1.044394119
MAGEB2	-3.455455295	1.014371636

Table S5. List of the primers used in qRT-PCR analysis

Human Gene	Sequence (5’ to 3’)
CD206-S	TCCGGGTGCTGTTCTCCTA
CD206-AS	CCAGTCTGTTTTTGATGGCACT
CD163-S	TTTGTCAACTTGAGTCCCTTCAC
CD163-AS	TCCCGCTACACTTGTTTTCAC
CX3CR1-S	CACAAAGGAGCAGGCATGGAAG
CX3CR1-AS	CAGGTTCTCTGTAGACACAAGGC
CD274-S	CAAAGAATTTTGGTTGTGGA
CD274-AS	AGCTTCTCCTCTCTCTTGGA
CD36-S	CAGGTCAACCTATTGGTCAAGCC
CD36-AS	GCCTTCTCATCACCAATGGTCC
GAPDH-S	GAAGGTCGGAGTCAACGGATT
GAPDH-AS	CGCTCCTGGAAGATGGTGAT
HLA-DRA-S	AGTCCCTGTGCTAGGATTTTTCA
HLA-DRA-AS	ACATAAACTCGCCTGATTGGTC
NOS2-S	GCTCTACACCTCCAATGTGACC
NOS2-AS	CTGCCGAGATTTGAGCCTCATG

Mouse Gene	Sequence (5’ to 3’)
Cd206-S	CTCTGTTCAGCTATTGGACGC
Cd206-AS	CGGAATTTCTGGGATTCAGCTTC
Cd163-S	GGTGGACACAGAATGGTTCTTC
Cd163-AS	CCAGGAGCGTTAGTGACAGC
Nos2-S	GTTCTCAGCCCAACAATACAAGA
Nos2-AS	GTGGACGGGTCGATGTCAC