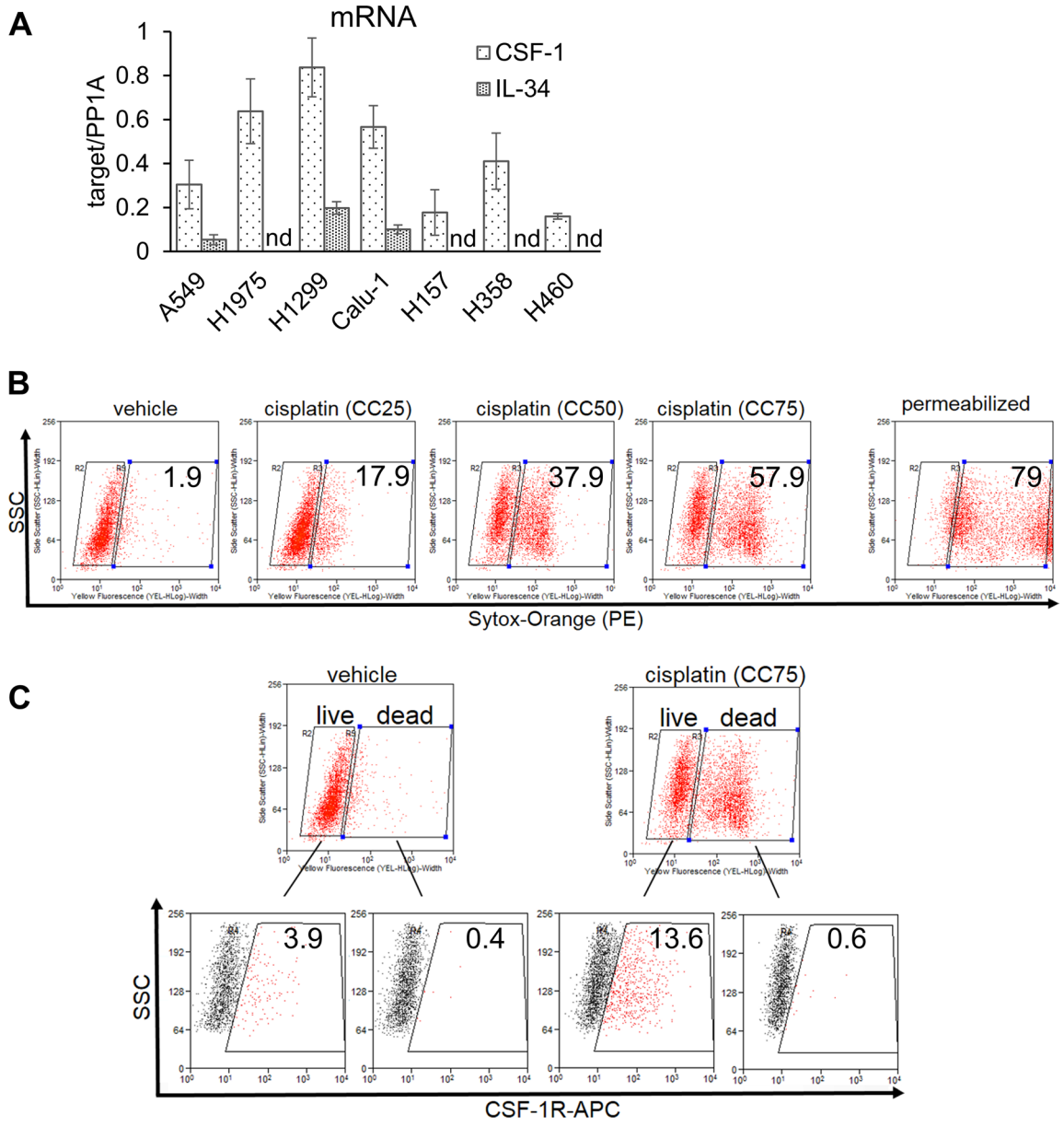
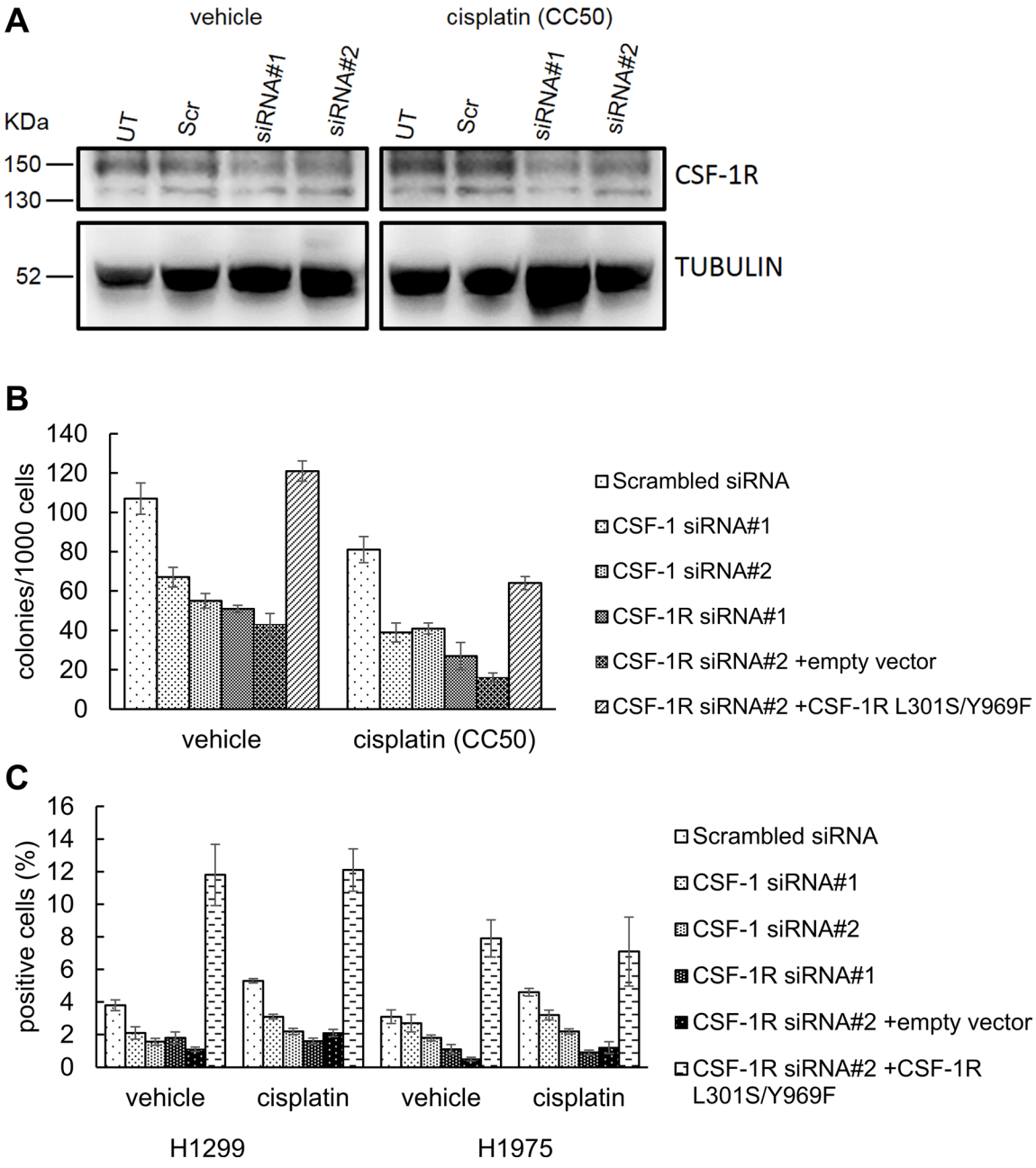
Inhibition of the colony-stimulating-factor-1 receptor affects the resistance of lung cancer cells to cisplatin

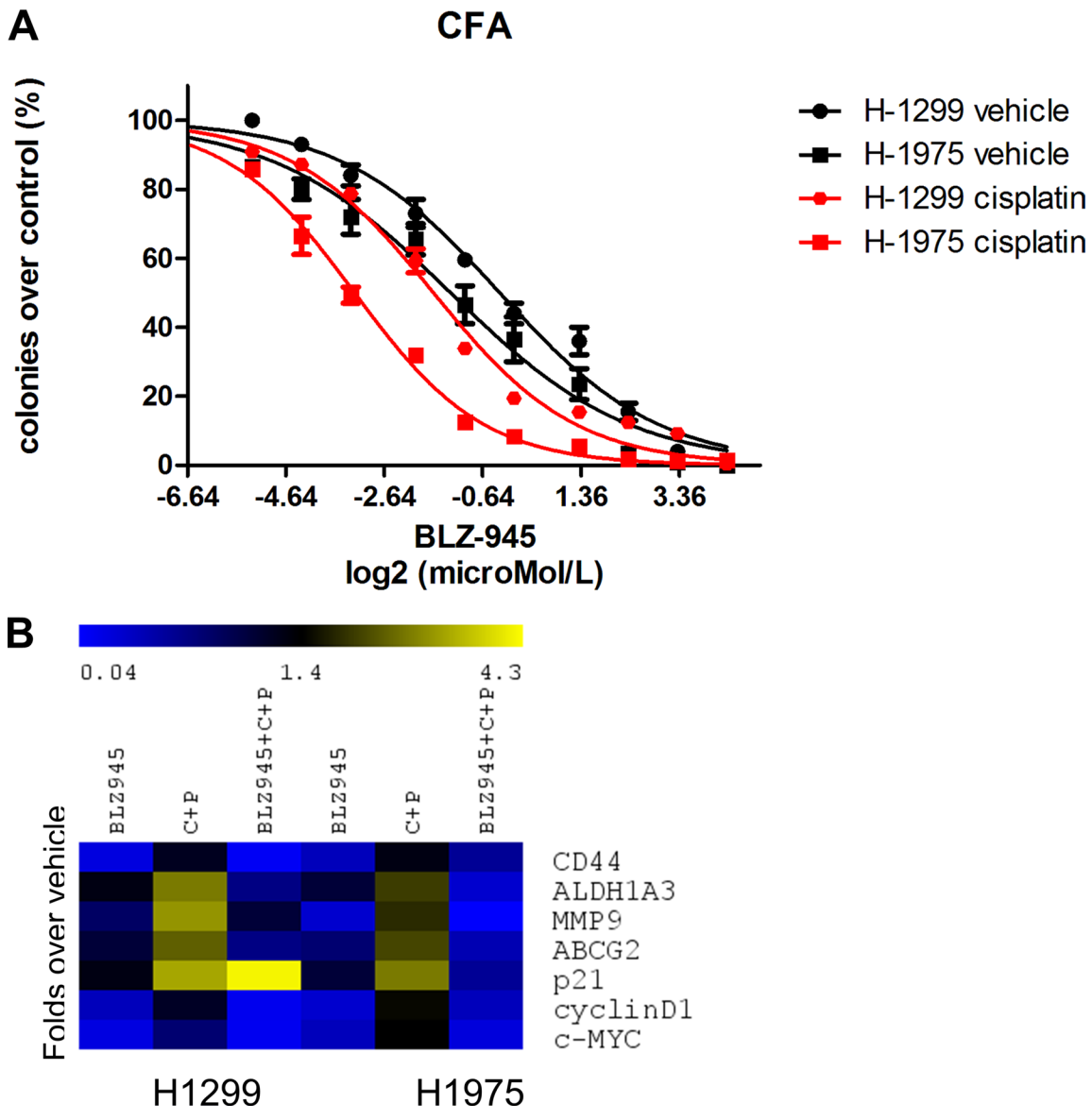
Supplementary Materials



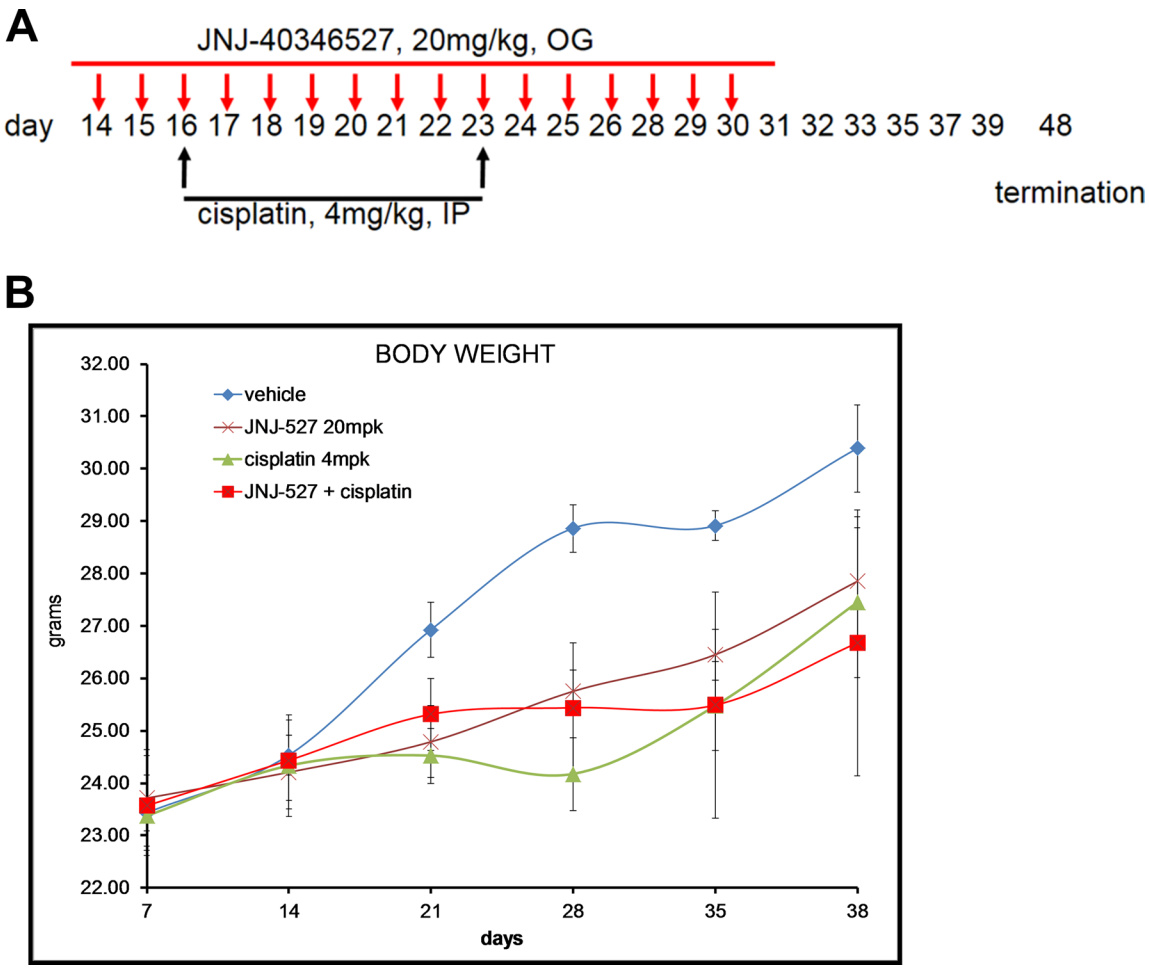
Supplementary Figure S1: (**A**) Lung cancer cell lines express CSF-1 and IL-34 mRNA. **(**A**)** Expression of CSF-1 and IL-34 mRNA (normalized to PP1A) as assessed by quantitative PCR. Mean ± SE of two experiments. (**B–C)** The CSF-1R^{pos} cells survive cisplatin treatment. **(**B**)** Representative dot plots of H1299 cells treated for 72 hrs with the indicated doses of cisplatin and stained with the cell impermeant dye Sytox-Orange before FACS analysis. As a positive control for the staining, H1299 treated with Triton X-100 for 10 min were stained (permeabilized). (C**)** Representative dot plots indicating the percentage of CSF-1R^{pos} cells in Sytox-negative (live) and Sytox-positive (dead) cells, respectively, after treatment with vehicle or cisplatin as from Supplementary Figure S1B.



Supplementary Figure S2: (**A**) Knocking down of CSF-1R affects the clonogenicity and resistance to cisplatin of lung cancer cell lines. (A) Representative western blottings of whole cell lysates from H1299 cells untransfected (UT) or transfected with a scrambled-control and CSF-1R targeting- siRNAs, and treated with vehicle or cisplatin for 72 hrs. Staining for tubulin was used as a loading control. (**B)** Briefly, H1299 cells transfected with CSF-1R targeting siRNAs, were treated 48 hrs later with vehicle- or cisplatin (CC_{25})-for 16 hrs, and seeded at clonal density. Additionally, cells transfected with the CSF-1R targeting siRNA#2 were co-transfected with either an empty vector or a vector coding for a constitutively active CSF-1R (L301S/Y969F). Histograms reporting the number of the formed colonies 7–10 days after seeding**.** Mean ± SE of three independent experiments. (**C)** Histograms reporting the number of $CSF\text{-}1R^{pos}$ cells in H1299 and H1975 cells treated as from Supplementary Figure S2B, as assessed by FACS. Mean ± SE of two independent experiments.



Supplementary Figure S3: (**A**) Treatment of lung cancer cell lines with BLZ-945 affects clonogenicity and expression of EMT genes. (A) Graph reporting the percentage of colonies (over vehicle-control) of H1299 and H1975 cells treated for 24 hrs with BLZ-945 at the indicated doses, in the presence of vehicle or cisplatin (CC_{25}), before seeding at clonal density. The formed colonies were stained 7-9dd later. Mean ± SE of two independent experiments. (**B**) Heatmap. Relative expression levels of proliferation- and EMT-associated genes in H1299 and H1975 cells treated with BLZ-945 (CC_{50}), C + P or both for 24 hrs, as assessed by quantitative PCR.



Supplementary Figure S4: (**A**) Scheme of the *in vivo* studies. Treatment started at day 14 (when the average tumor volume was ≥ 100 mm^3). Study was terminated at day 48, when tumors were excised, disaggregated and pooled before being processed for FACS. (**B**) A transient weight loss is induced by both cisplatin and JNJ-40346527 treatment. Graph reporting the average body weight of each group of mice as from Figure 4. The red bar indicates the period of treatment. Mean ± SE for each group of mice is reported.

Supplementary Table S1: List of the primers used in this study for the quantitative PCR assays

CSF-1R forward CSF-1R reverse	GAATGACTCCAACTACATTGTC GTGTAGACAGTCAAAGATG
CSF-1 forward CSF-1 reverse	GCTGTTGTTGGTCTGTCTC CATGCTCTTCATAATCCTTG
IL-34 forward IL 34 reverse	AAACAAAGCTCCGTCCTAAACTG GCCGCATACTGCAATGAGG
OCT4 forward OCT4 reverse	GGGAGATTGATAACTGGTGTGTT GTGTATATCCCAGGGTGATCCTC
SOX2 forward SOX2 reverse	TACAGCATGTCCTACTCGCAG GAGGAAGAGGTAACCACAGGG
NANOG forward NANOG reverse	TTTGTGGGCCTGAAGAAAACT AGGGCTGTCCTGAATAAGCAG
C-MYC forward C-MYC reverse	GTCAAGAGGCGAACACACAAC TTGGACGGACAGGATGTATGC
VIMENTIN forward VIMENTIN reverse	AGTCCACTGAGTACCGGAGAC CATTTCACGCATCTGGCGTTC
ABCG2 forward ABCG2 reverse	ACGAACGGATTAACAGGGTCA CTCCAGACACACCACGGAT
ALDH1A3 forward ALDH1A3 reverse	TCTCGACAAAGCCCTGAAGT TATTCGGCCAAAGCGTATTC
MMP9 forward MMP9 reverse	TGTACCGCTATGGTTACACTCG GGCAGGGACAGTTGCTTCT
CD44 forward CD44 reverse	AAGGTGGAGCAAACACAACC AACTGCAATGCAAACTGCAAG
P21 forward P21 reverse	TGTCCGTCAGAACCCATGC AAAGTCGAAGTTCCATCGCTC
PPIA forward PPIA reverse	TCTGAGCACTGGAGAGAAAGG GGAAAACATGGAACCCAAAGG
Cyclin D1 forward Cyclin D1 reverse	TTCGGGATGATTGGAATAGC TGTGAGCTGGCTTCATTGAG