Supplementary information

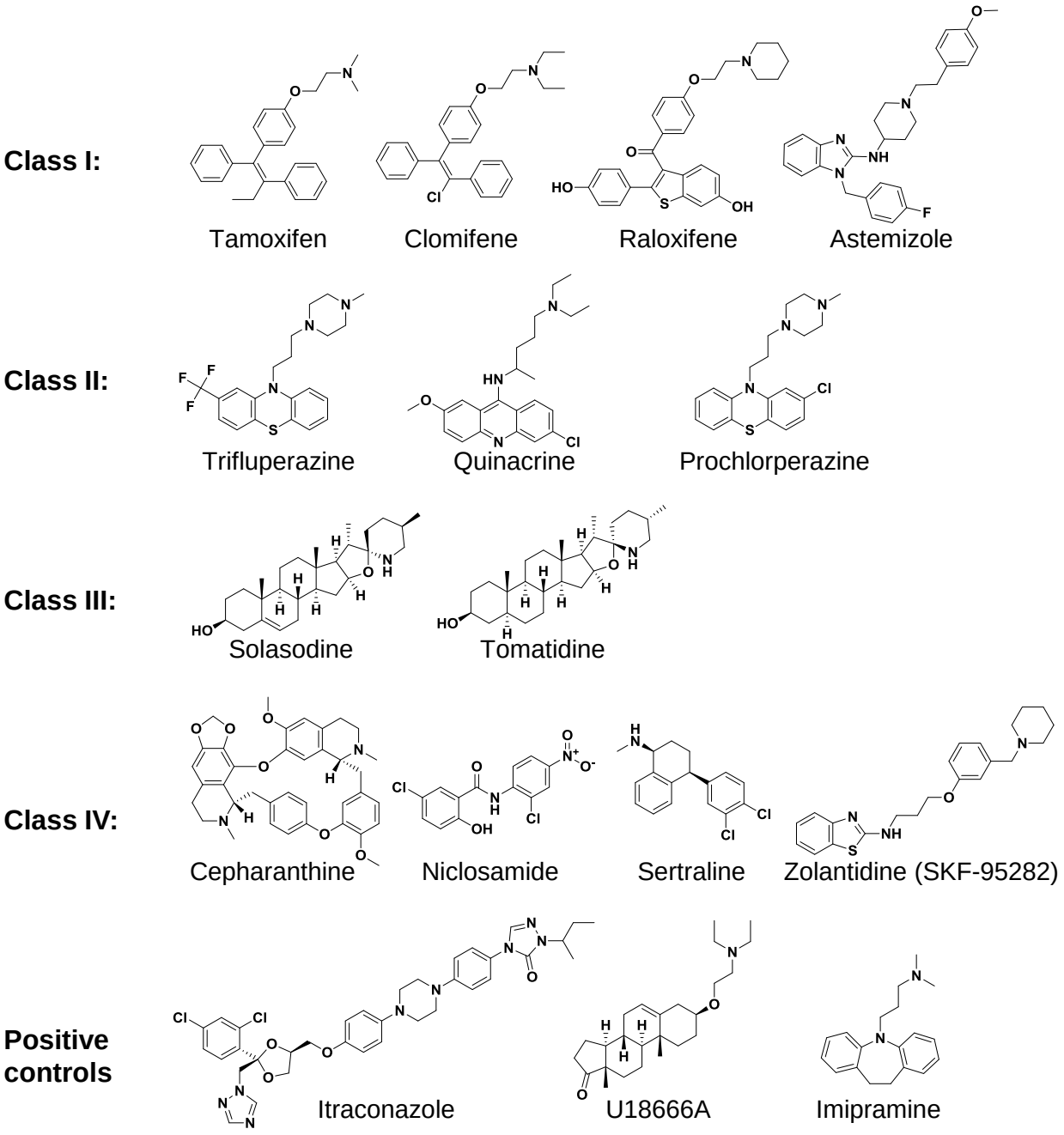


Figure S1. Chemical structures of the identified cholesterol trafficking inhibitors from the screening and their classification. The structures of the positive control compounds used for the screening are also shown.

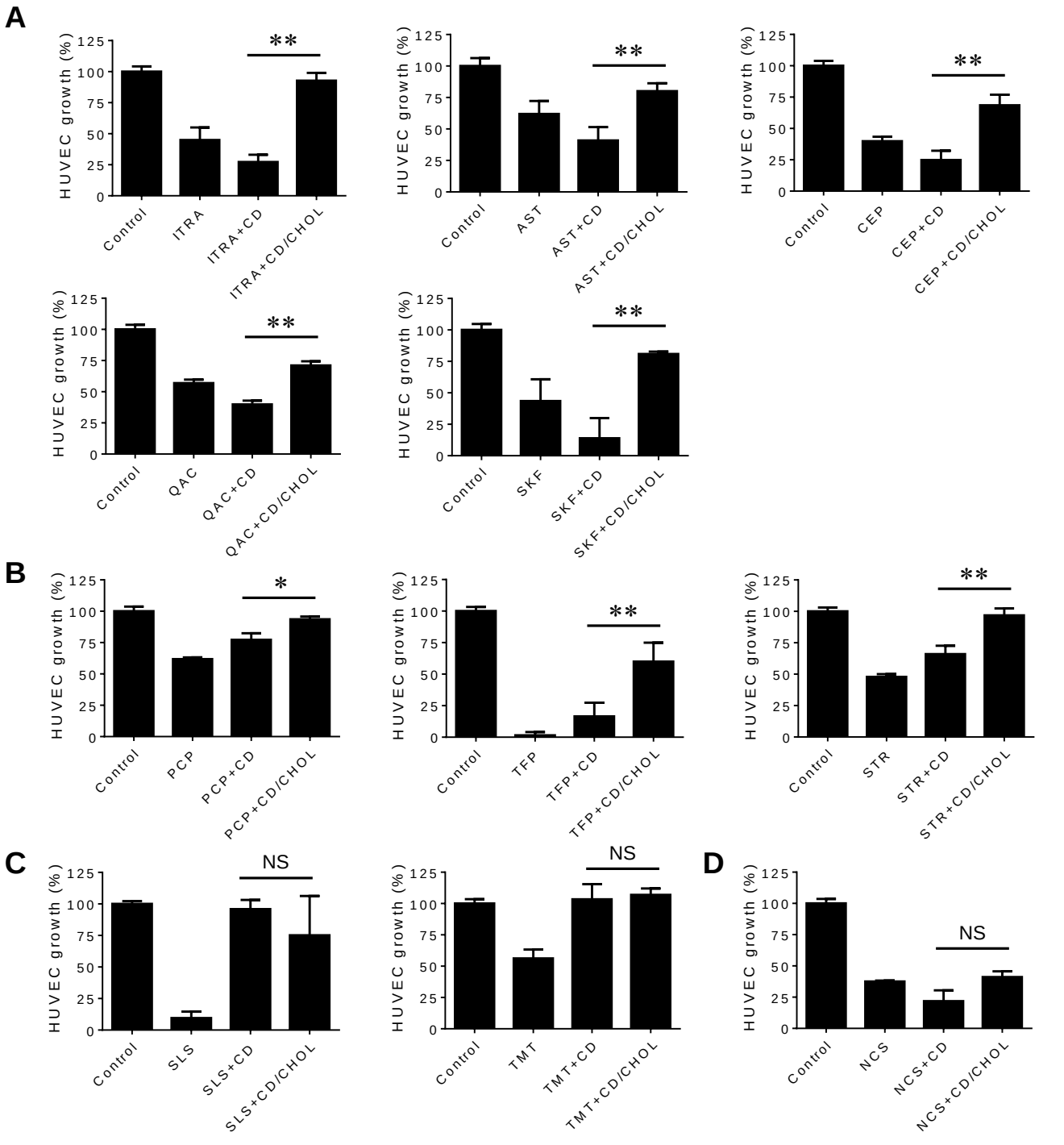


Figure S2. Effect of cyclodextrine (CD) or cyclodextrine-cholesterol complex (CD/CHOL) on anti-proliferative effect of cholesterol trafficking inhibitors in HUVEC. *P<0.05 and **P<0.01 between two indicated groups. NS denotes 'not significant'. ITRA, itraconazole; AST, astemizole; CEP, cepharanthine; QAC, quinacrine; SKF, zolantidine; PCP, prochlorperazine; TFP, trifluoperazine; STR, sertraline; SLS, solasodine; TMT, tomatidine; NCS, niclosamide.

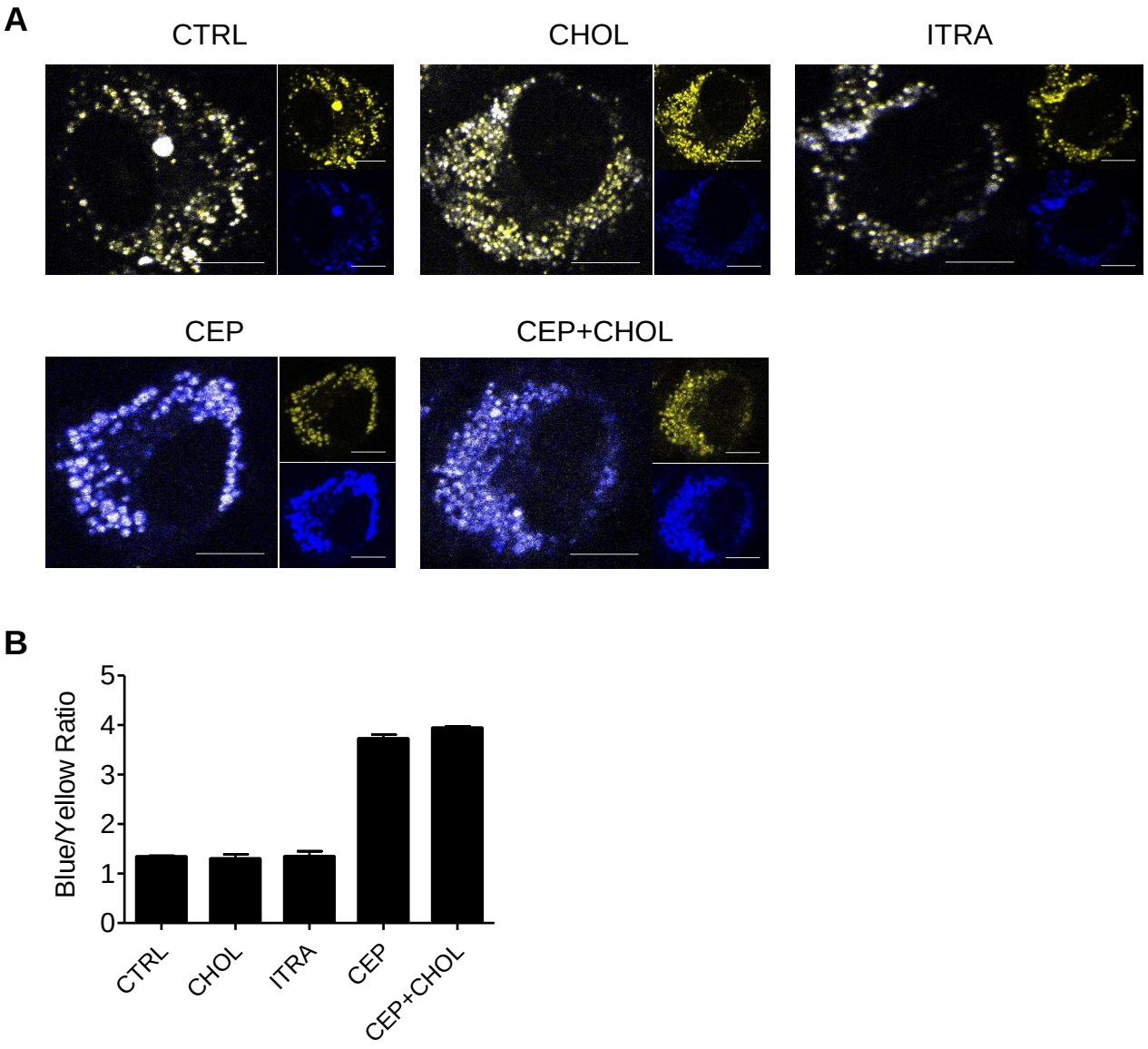


Figure S3. Effect of cholesterol on lysosomal pH change. HUVEC were treated with itraconazole (ITRA, 1 μM) or cepharanthine (CEP, 5 μM) for 1 h in the presence or absence of cyclodextrine-cholesterol complex (CHOL) prior to the measurement of lysosomal pH using LysoSensor Yellow/Blue DND-160. Representative confocal images of the LysoSensor Yellow/Blue DND-160-stained cells (A) and quantitative measurement of blue/yellow ratio, an indicative of lysosomal pH (B) are shown. Scale bar = 10 μm.

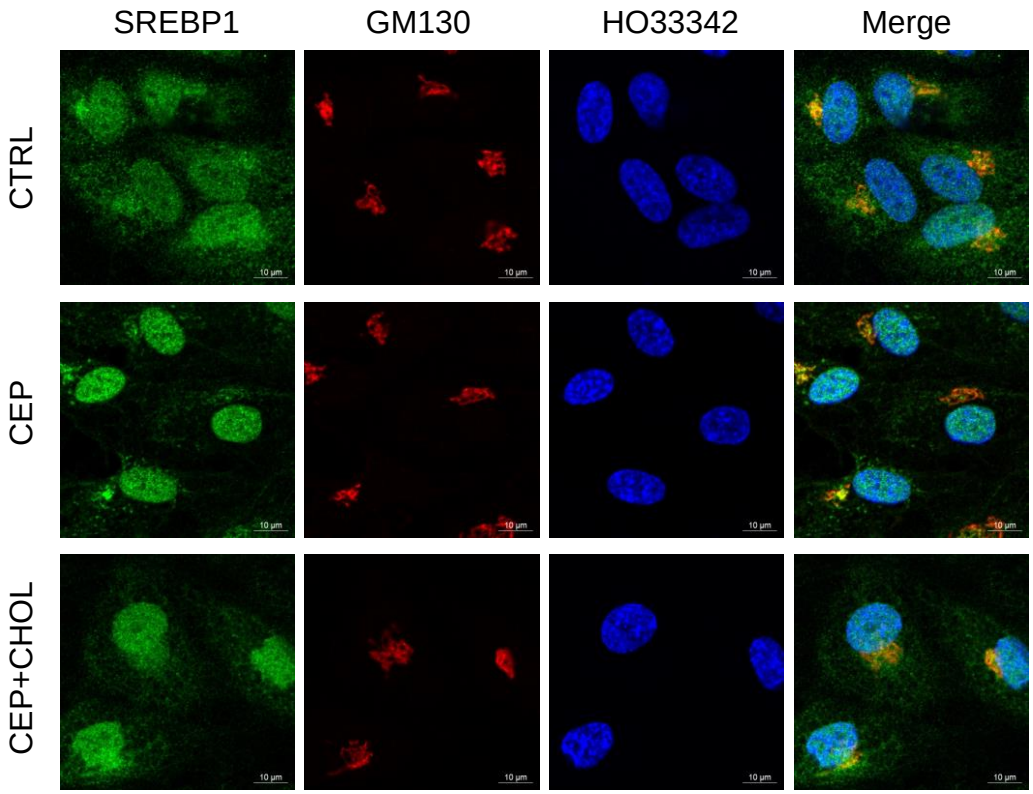


Figure S4. Effect of CEP and cholesterol on SREBP1 nuclear translocation. HUVEC were treated with CEP (5 μM) for 24 h with or without cyclodextrine-cholesterol complex (CHOL). The cells were then processed for immunofluorescence analysis of SREBP1 (cholesterol sensor), GM130 (Golgi marker) and Hoescht33342 (HO33342, nuclear marker). Scale bar = 10 μm.

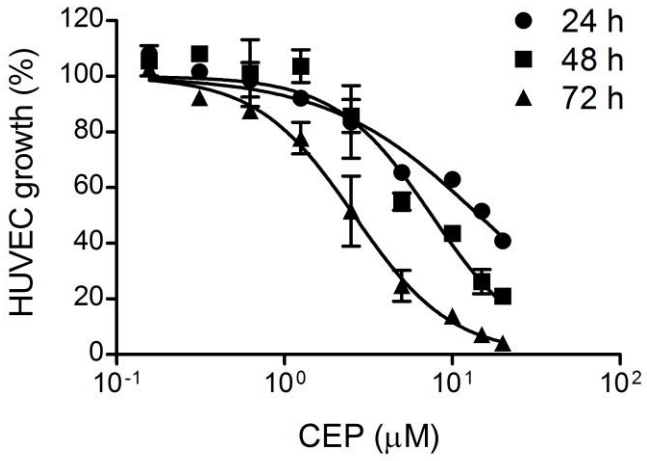
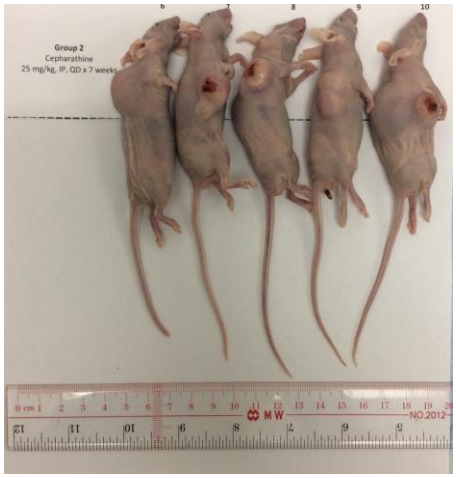


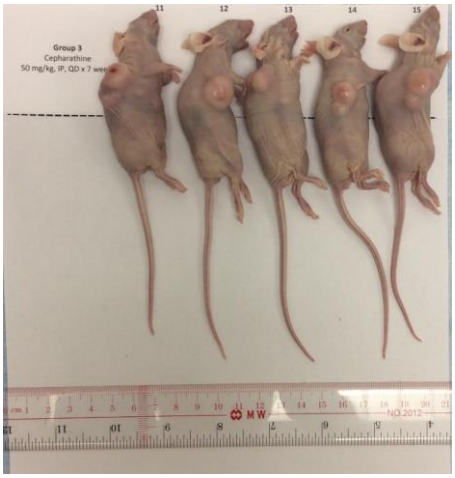
Figure S5. Effect of CEP on HUVEC proliferation in different time points. HUVEC were treated with various concentrations of CEP for indicated time points. Cell proliferation was measured with AlamarBlue fluorescence assay.

Vehicle

CEP (25 mg/kg)



CEP (50 mg/kg)



Cisplatin (5mg/kg)

CEP (50 mg/kg) +
Cisplatin (5mg/kg)

Figure S6. Breast cancer xenograft tumors and mice images. BALB/c athymic nude mice bearing MDA-MB-231 breast cancer were treated with vehicle (5% PEG-400, 5% tween-80 and 5% DMSO in sterile saline), CEP (25 and 50 mg/kg, daily), cisplatin (5 mg/kg, once a week) or combination of CEP (50 mg/kg, daily) and cisplatin (5 mg/kg, once a week) via i.p. injection. Mice images were taken at day-46 (at the end of the experiment).

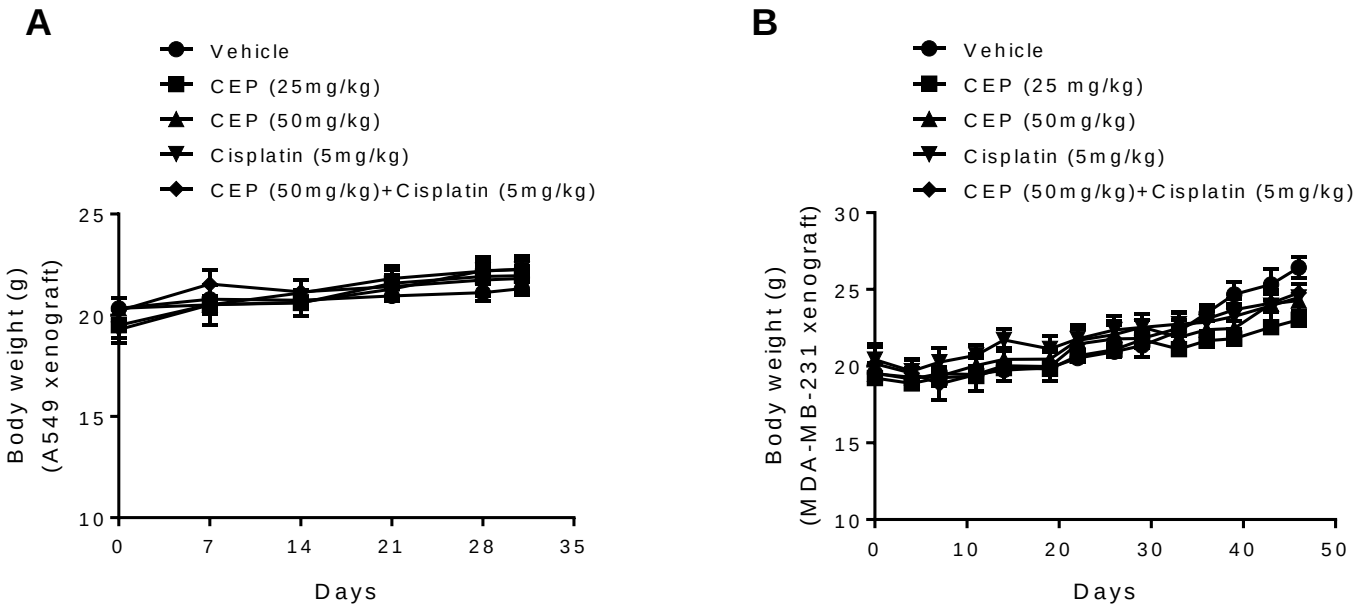


Figure S7. Mice body weight changes during the course of drug treatment. Body weight changes of the NOD/SCID mice bearing A549 lung cancer xenograft tumors (A) and BALB/c athymic nude mice bearing MDA-MB-231 breast cancer xenograft (B) are shown. No significant body weight change was observed in mice treated with CEP, cisplatin or drug combination.