

Supporting information

A new promising anticancer agent: a glycosaminoglycan-mimetic derived from the marine bacterial infernan exopolysaccharide

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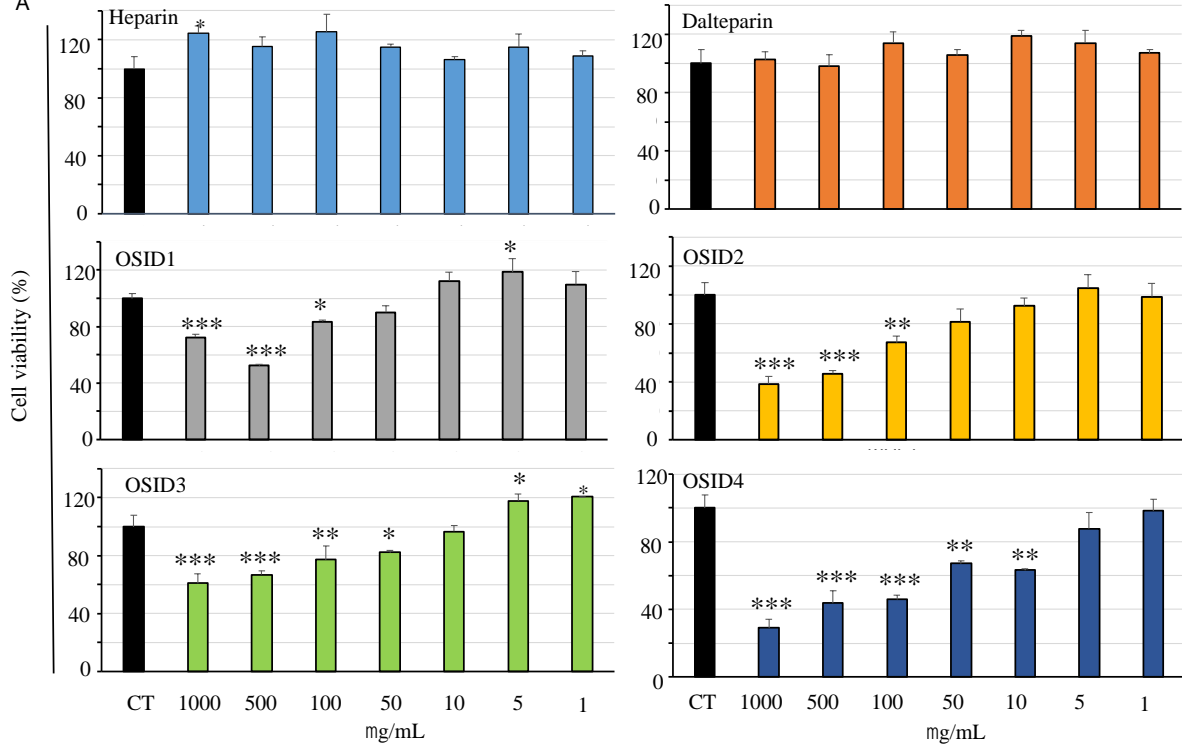
These authors contributed equally to the present work

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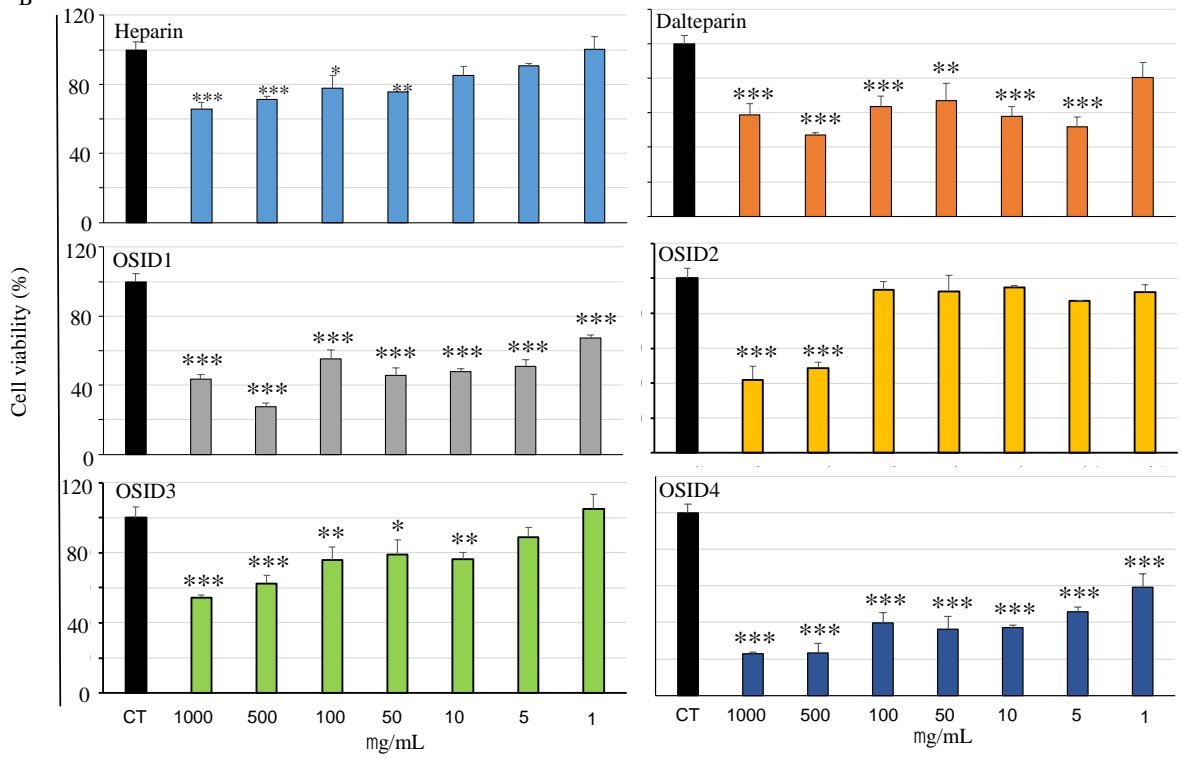
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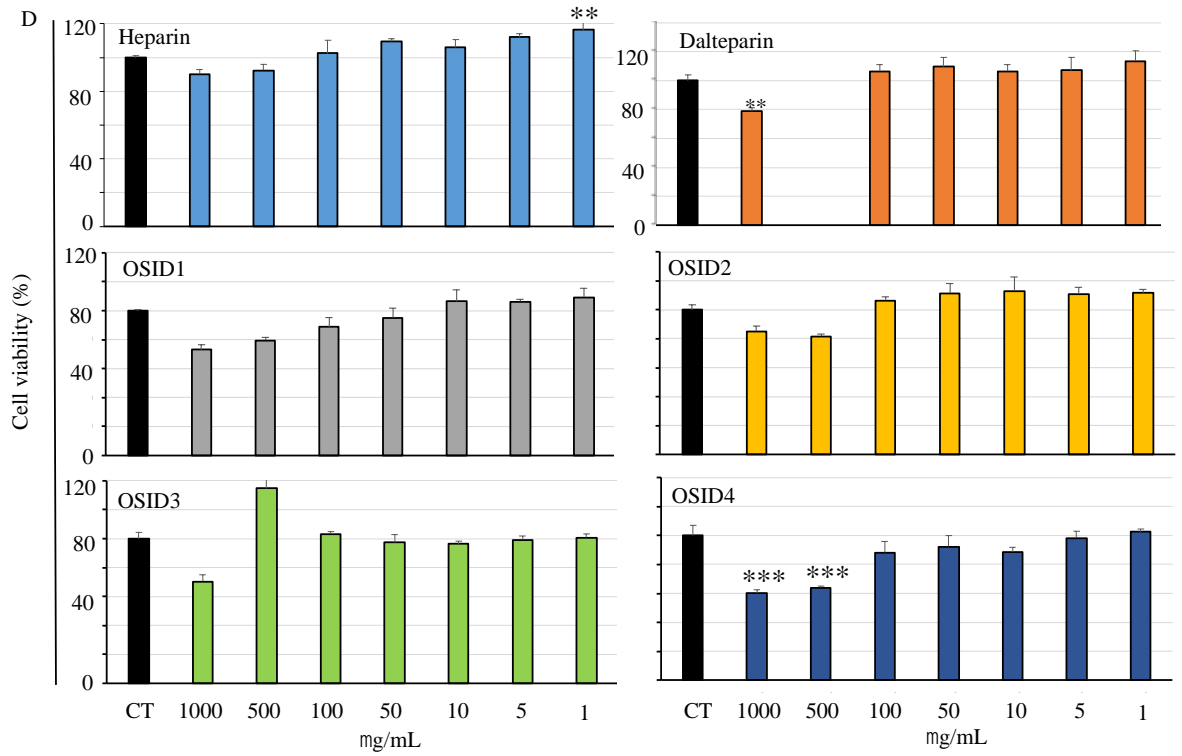
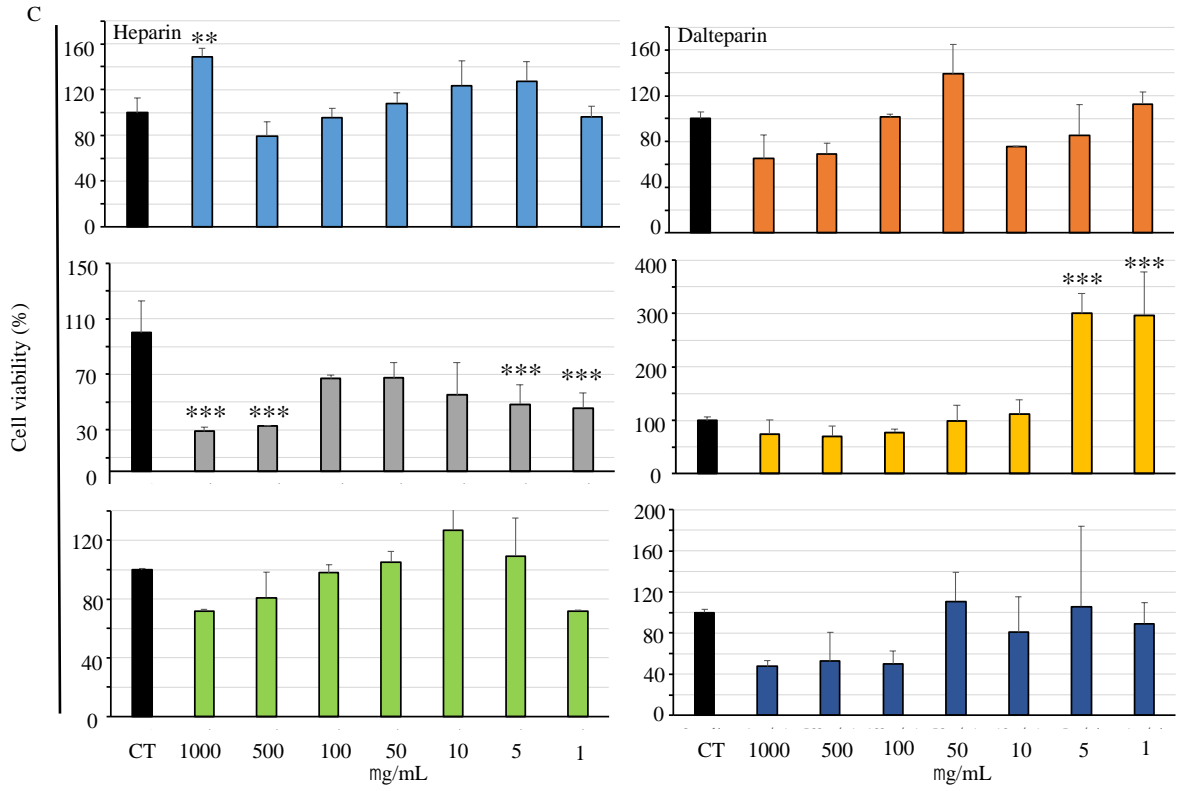
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A



B





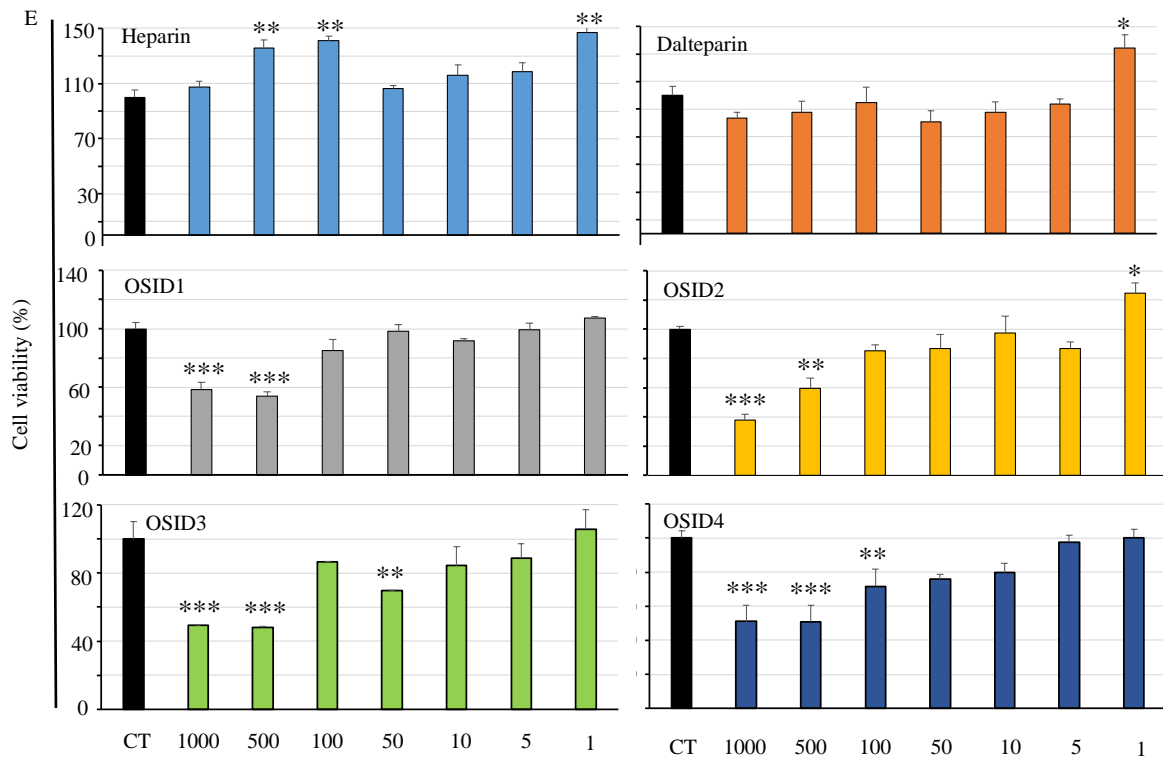


Fig. S1. Effect of heparin, dalteparin and OSIDs on the viability of various human cancer cell lines measured by MTT assay. (A) CaCO2 colorectal adenocarcinoma cells, (B) DU145 prostate adenocarcinoma cells, (C) LnCap prostate adenocarcinoma cells, (D) MDA-MB-231 breast adenocarcinoma cells, (E) A375 melanoma cells. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

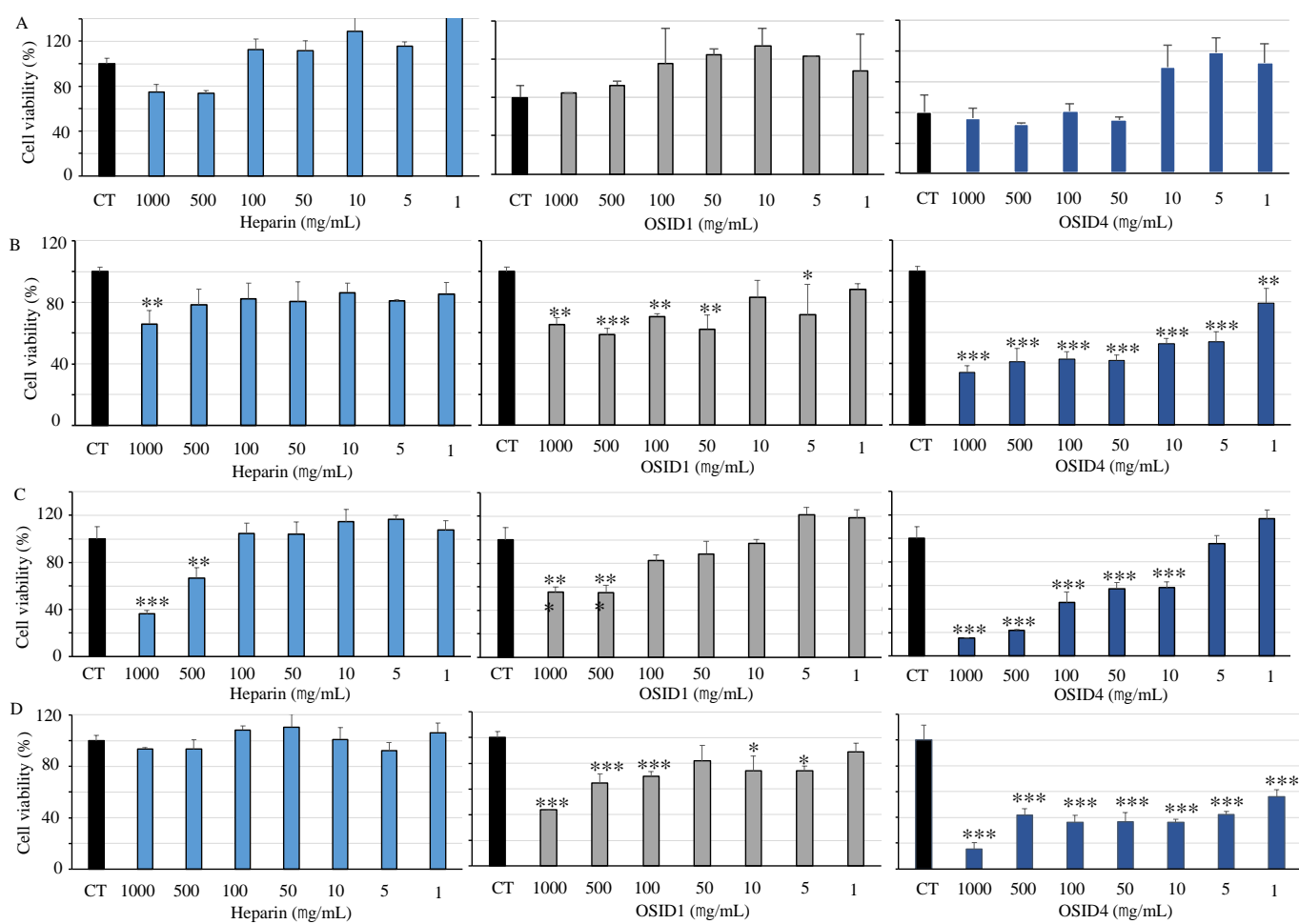


Fig. S2. Effect of heparin, OSID1 and OSID4 on the viability of various murine cancer cell lines measured by MTT assay: (A) 4T1 mammary cancer, (B) CMT-167 lung cancer, (C) B16-F10, and melanoma, (D) MOS-J osteosarcoma cell lines. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

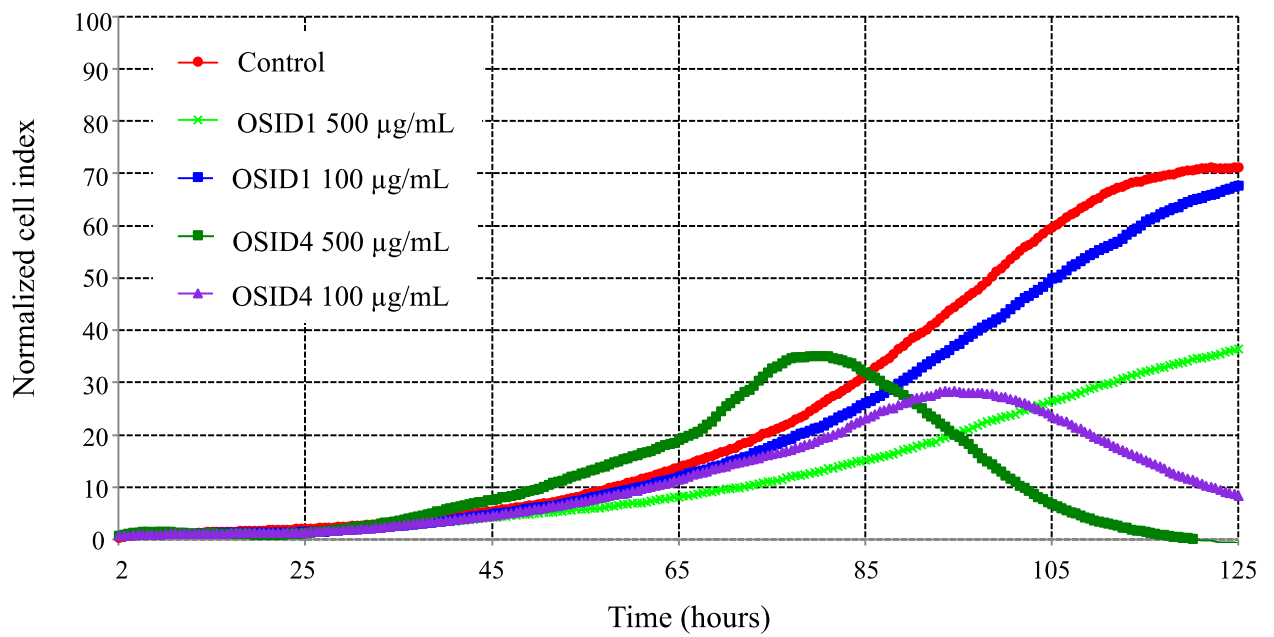


Fig. S3. OSID4 was more active than OSID1 in inhibiting proliferation of human prostate cancer cells. DU145 cancer cells were cultured with 100 or 500 mg/mL of OSID1 or OSID4. Cell proliferation was followed by the measurement of cell impedance in real time.

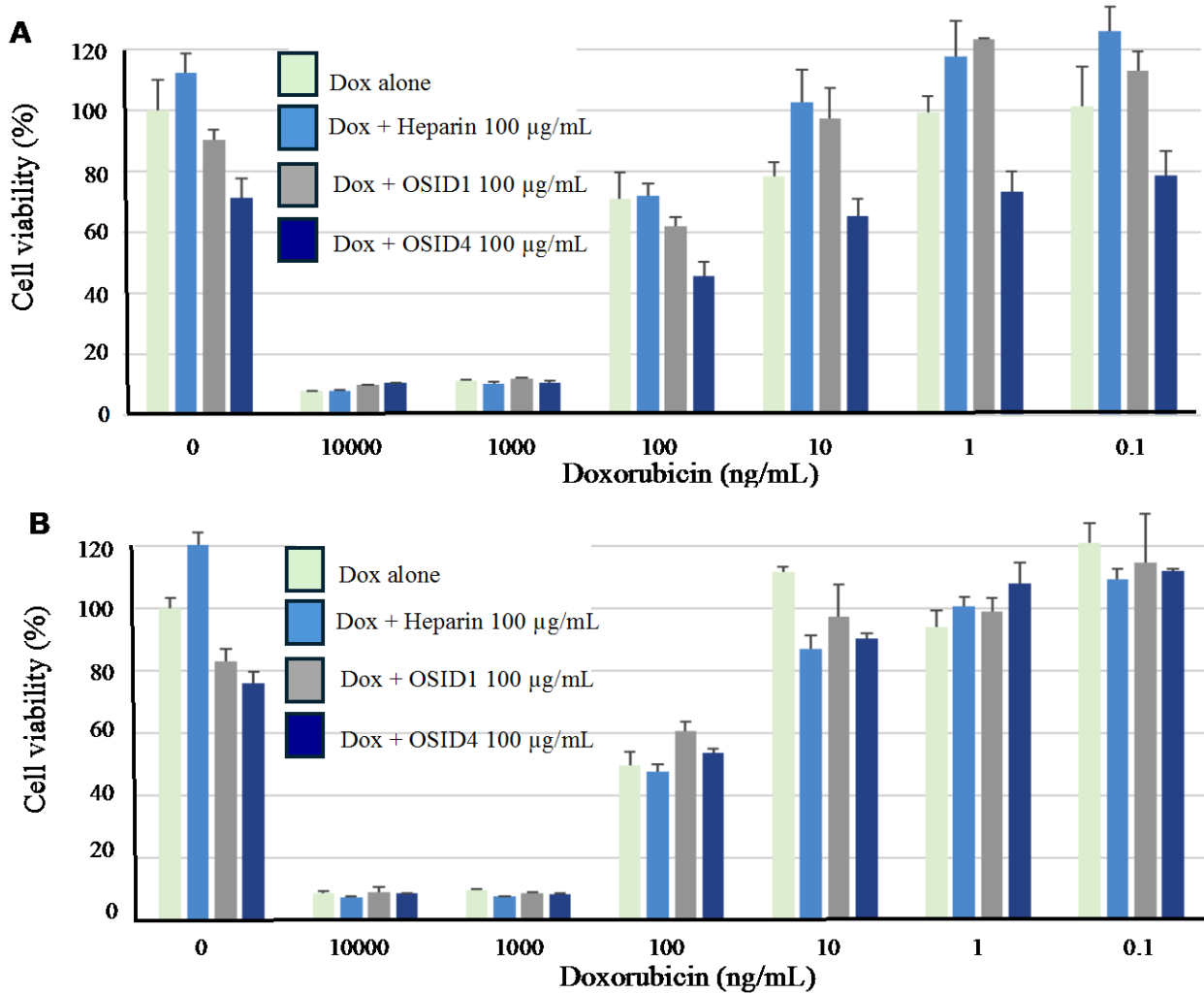


Fig. S4. Effects of heparin, OSID1 and OSID4 in the presence of absence of doxorubicin (Dox) on the viability of (A) MNGG-HOS human osteosarcoma cells, and (B) A375 human melanoma cell lines. After 96 hours of culture, cell viability was analyzed by MTT assay.

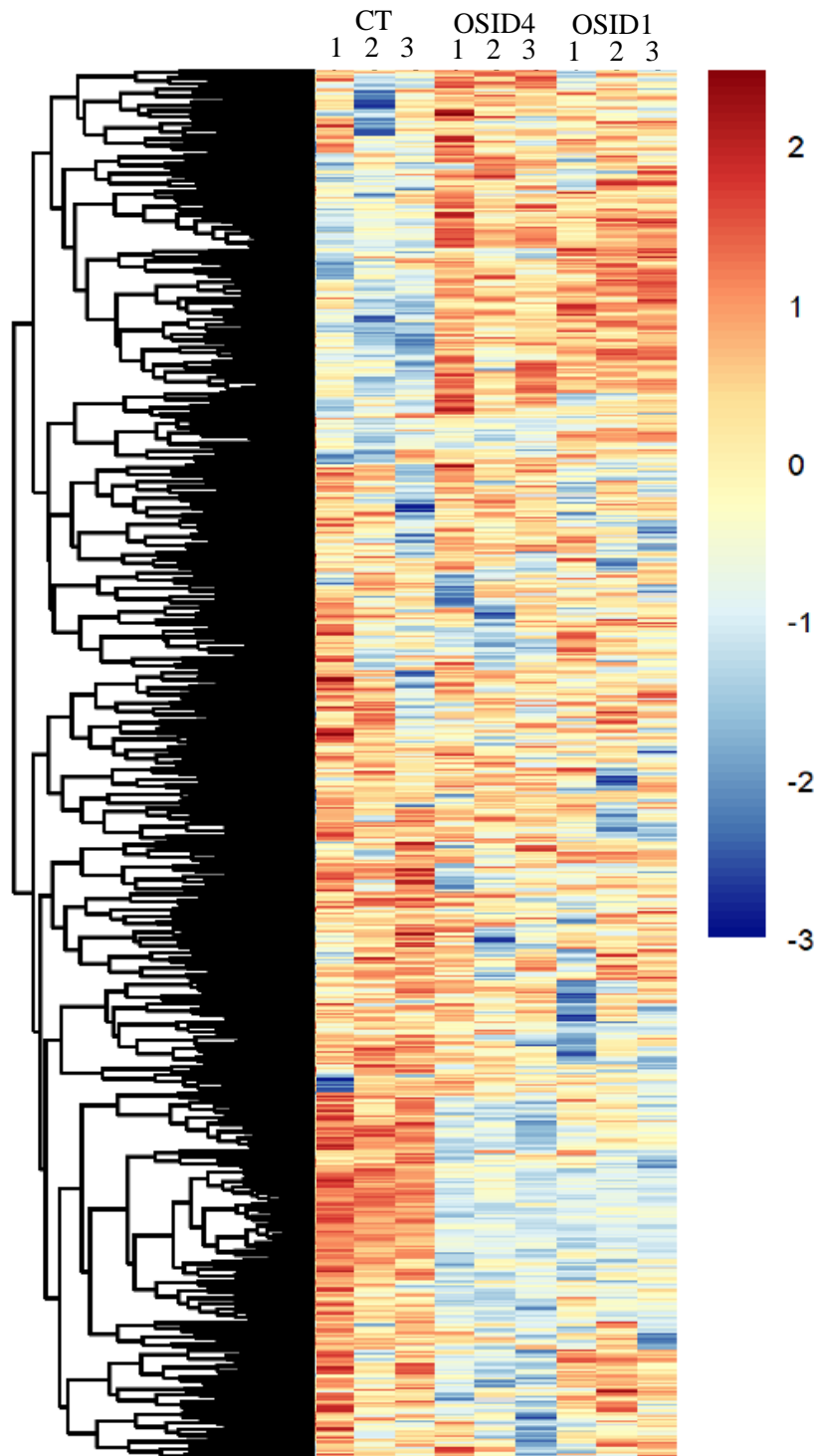
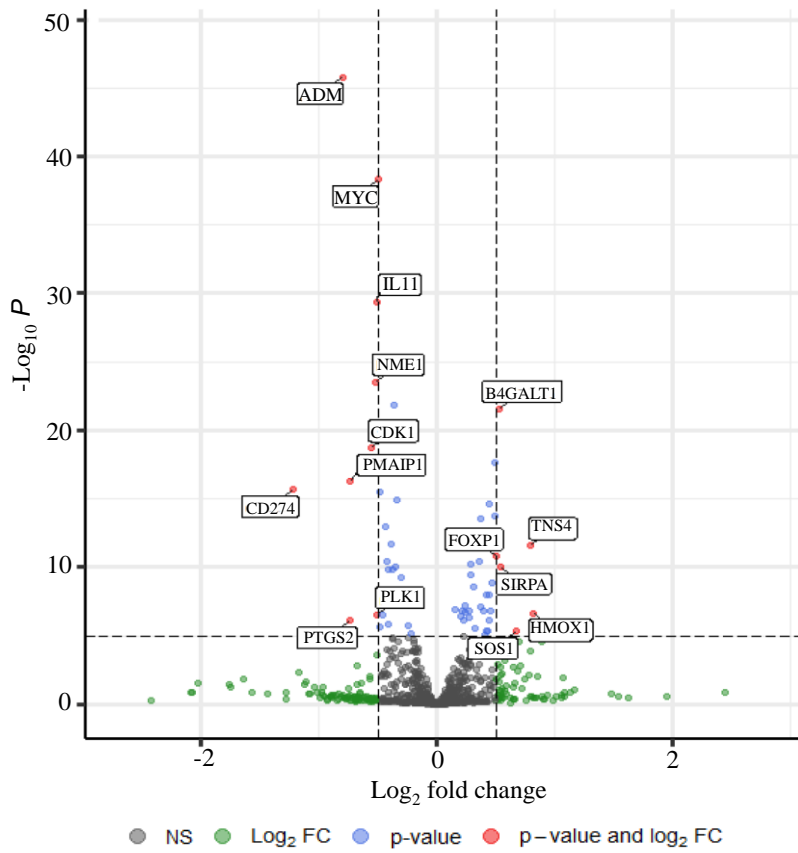


Fig. S5. Heatmap visualization of the z-scored expression of the genes ($|\log_2FC| > 1$, p value adjusted < 0.05) that were differentially expressed in OSID-treated cells compared to the untreated control group. Human A549 lung adenocarcinoma cells were treated or not with 100 $\mu\text{g}/\text{mL}$ of OSID1 or OSID4 for 24 hours before RNA extraction. 814 genes were analyzed by using the NH_Hs_TumorSig_v1.0 panel provided by NanoStringTM. The experiments were conducted in triplicate.

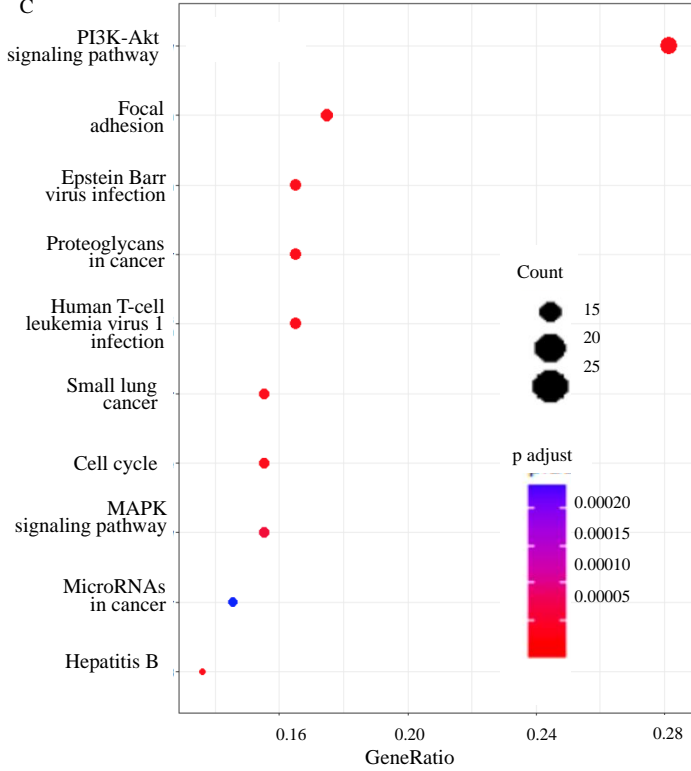
A



B

Genes	Fold change
CD274	-1.23
ADM	-0.80
PTGS2	-0.74
PMAIP1	-0.73
PLA2G4A	-0.67
CDK1	-0.56
NME1	-0.52
IL11	-0.51
PLK1	-0.51
HJURP	-0.51
MYC	-0.50
FOXP1	+0.50
ERBB2	+0.51
MSH2	+0.51
b4GALT1	+0.53
SIRP1	+0.54
AGO4	+0.54
PLEK2	+0.57
SOS1	+0.66
ALDH1A3	+0.69
FBN1	+0.70
TNS4	+0.79
PDGFb	+0.79
HMOX1	+0.81
PIK3CD	+0.88

C



D

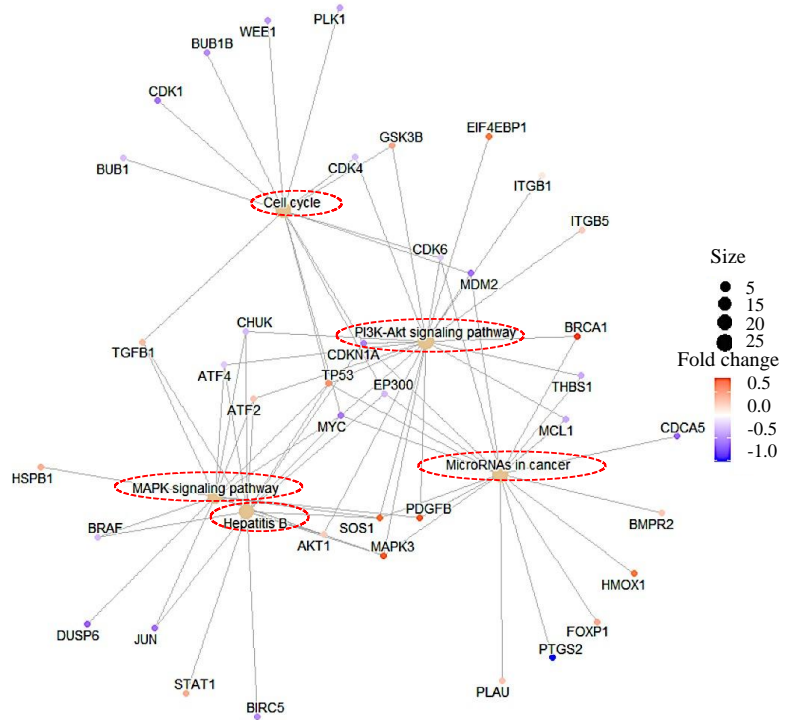


Fig. S6. OSID1 modulated the transcriptomic profile of human lung adenocarcinoma A549 cells. A549 cells were treated or not with 100 µg/mL of OSID1 for 24 hours before RNA extraction. 814 genes were analyzed by using the NH_Hs_TumorSig_v1.0 panel provided by NanoString™. The experiments were conducted in triplicate. (A) Volcano plot of shrunken \log_2 fold change and $-\log_{10} P$ of all tested genes between vehicle-control cells and treated cells with OSID1 at 24 hours post treatment. Red dots referred to genes significantly modulated by OSID1, (B) Main genes differentially expressed after OSID 1 treatment, (C) Functional predictive analysis of gene clusters modulated by OSID1. KEGG term enrichment analysis of cellular components, biological processes and molecular functions were performed on each gene cluster identified. (D) Enriched pathway plots. The size of colored circles was related to the number of genes identified. The color panel corresponded to the variation of the statistical significance.