

# **Functional characterization of RFC and PCFT for antifolates accumulation in non-small cell lung cancer cells**

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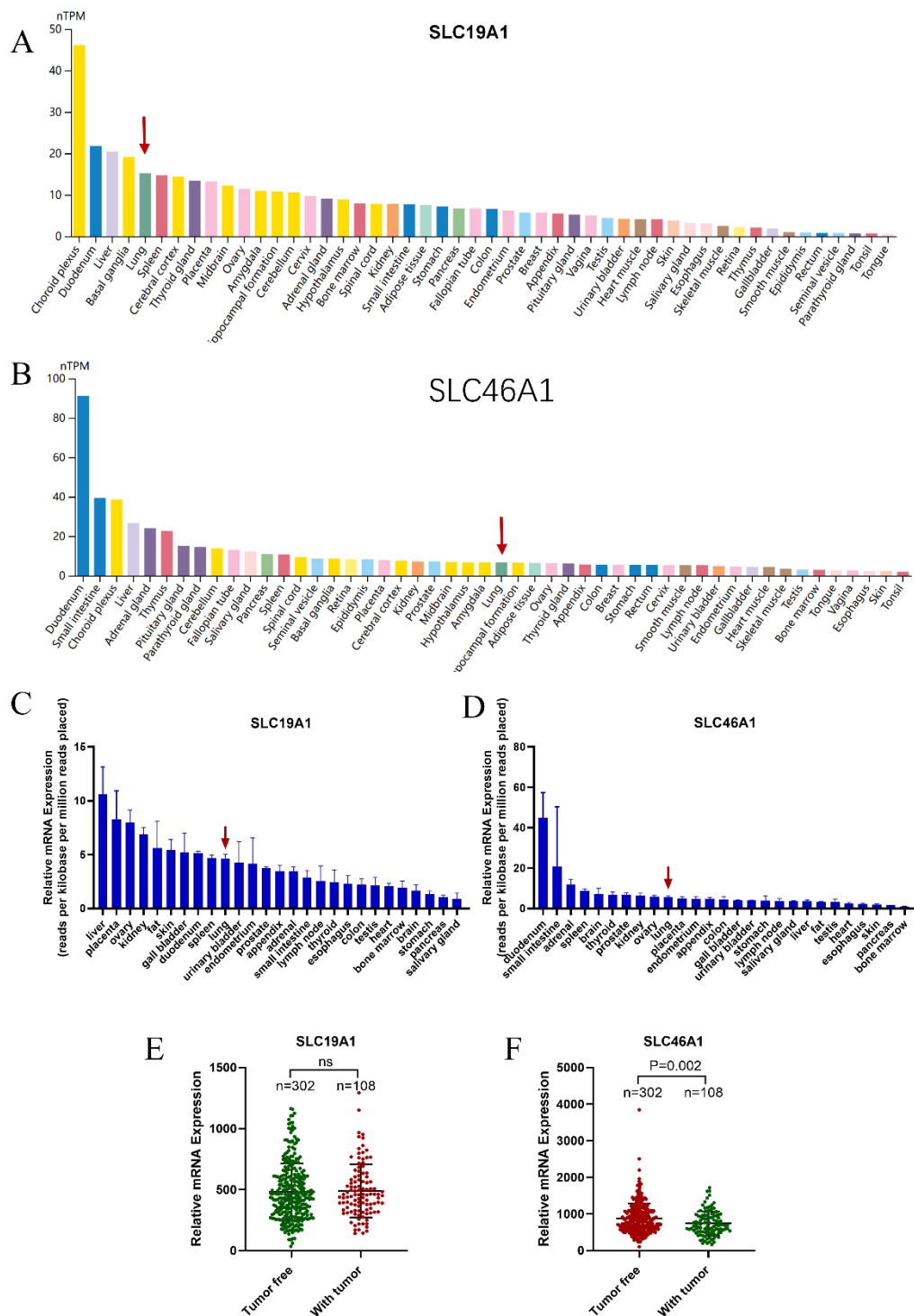
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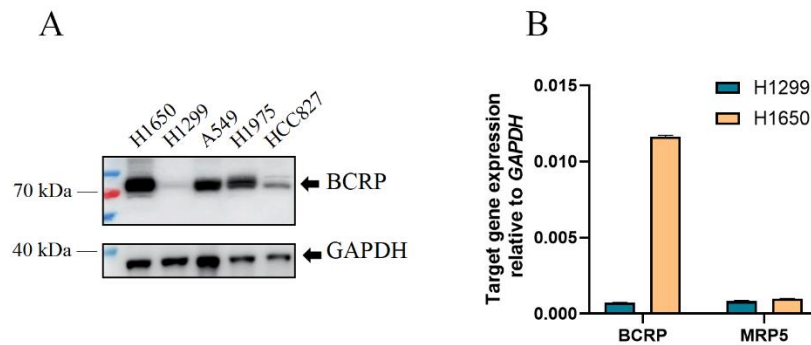
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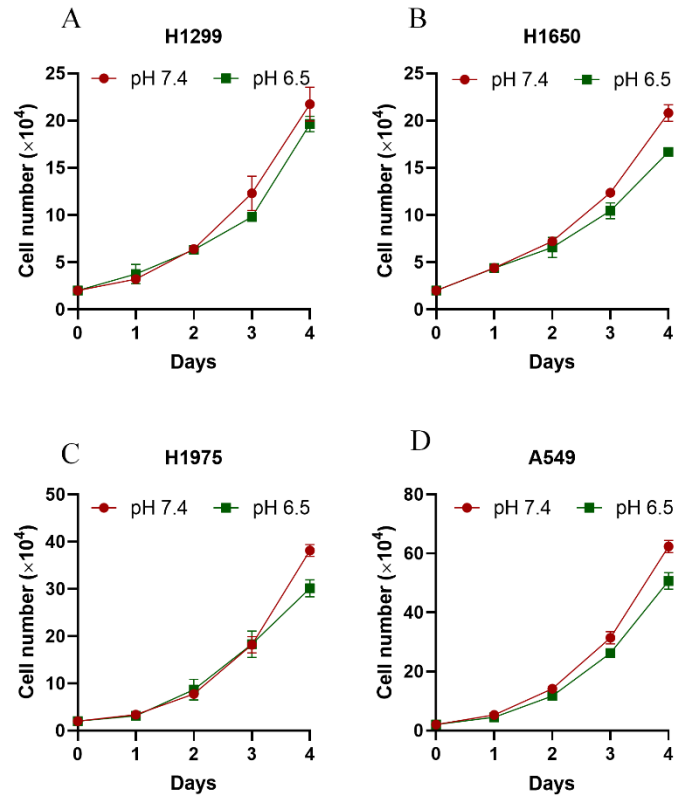


Supplementary Figure 1. The expression of *SLC19A1* and *SLC46A1* in human tissues and NSCLC. (A, B) Data are from the consensus dataset, which consists of normalized expression (nTPM) levels for 55 tissue types. The dataset and graphs are from Human

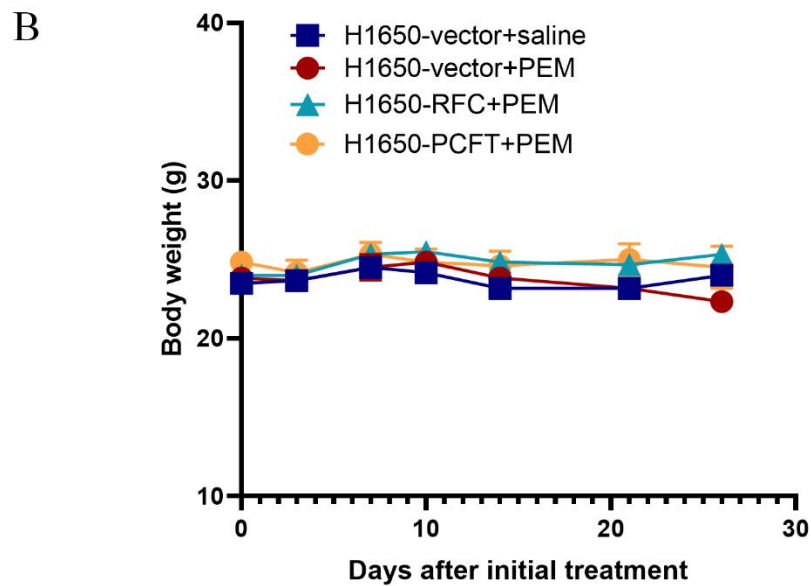
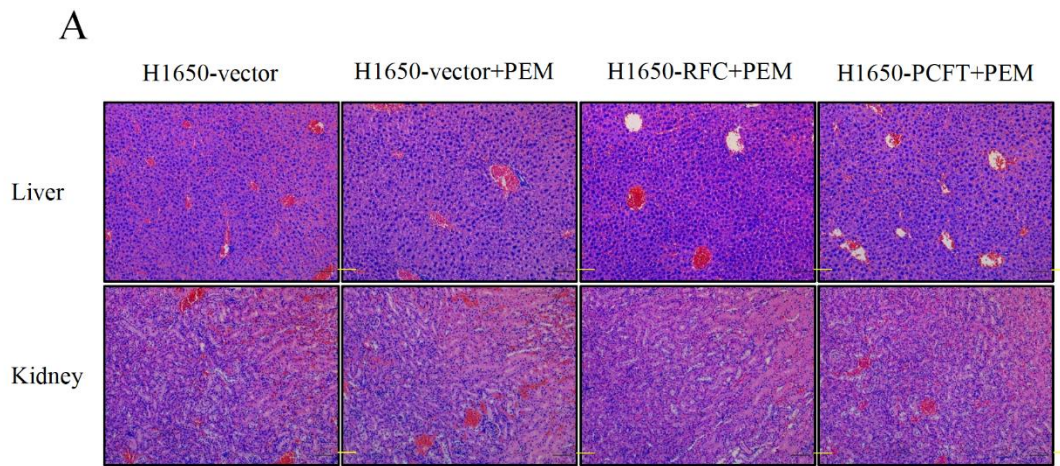
Protein Atlas (<https://www.proteinatlas.org/>). (C, D) Data are from BioProject: PRJEB4337 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJEB4337/>). RNA-seq was performed on tissue samples from 95 human individuals representing 27 different tissues. (E, F) Data are from cBioPortal for Cancer Genomics (Lung Adenocarcinoma (TCGA, PanCancer Atlas)). RNA-seq was performed of tissue samples from 302 normal lung tissues and 108 lung adenocarcinoma tissues.



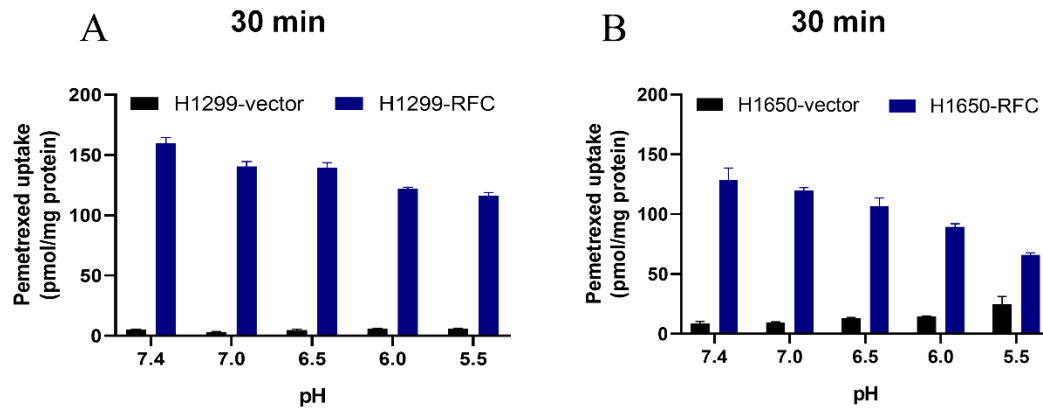
Supplementary Figure 2. BCRP and MRP5 expressions in NSCLC cells. (A) The protein expression levels of BCRP in H1650, H1299, A549, H1975 and HCC827 cells. (B) The mRNA expression of BCRP and MRP5 in H1299 and H1650 cells. Data are presented as the mean  $\pm$  S.D. for 3 independent experiments (n=3).



Supplementary Figure 3. Effect of pH on the growth of H1299, H1650, H1975 and A549 cells. A density of 20,000 cells/well was seeded in 24-well plates. Cells were cultured with different pH values of medium for 1 to 4 days and then counted. Data are presented as the mean  $\pm$  S.D. for 3 independent experiments (n=3).



Supplementary Figure 4. (A) Histological analysis was performed on kidney and liver sections collected from H1650-vector, H1650-RFC and H1650-PCFT xenografts. H&E staining shows neither nephrotoxicity nor hepatotoxicity in mice undergoing pemetrexed treatment. Scale bar = 50  $\mu$ m. PEM: pemetrexed (B) Body weight curves for H1650-vector, H1650-RFC and H1650-PCFT xenografts. Data are presented as the mean  $\pm$  S.D. from 6 mice.



Supplementary Figure 5. pH-dependent accumulation of pemetrexed in RFC-expressing cells for 30 min. pH-dependent accumulation of pemetrexed (20  $\mu$ M) was evaluated at 37°C for 30 min in H1299-RFC (A), H1650-RFC (B) and control cells. All the uptake buffer contained 25 mM HEPES for pH 6.5 and above and 10 mM MES for pH 6.0 and below. Data are presented as the mean  $\pm$  S.D. for 3 independent experiments (n=3).