

Therapeutic Effect of pHLIP-mediated CEACAM6 Gene Silencing in Lung Adenocarcinoma

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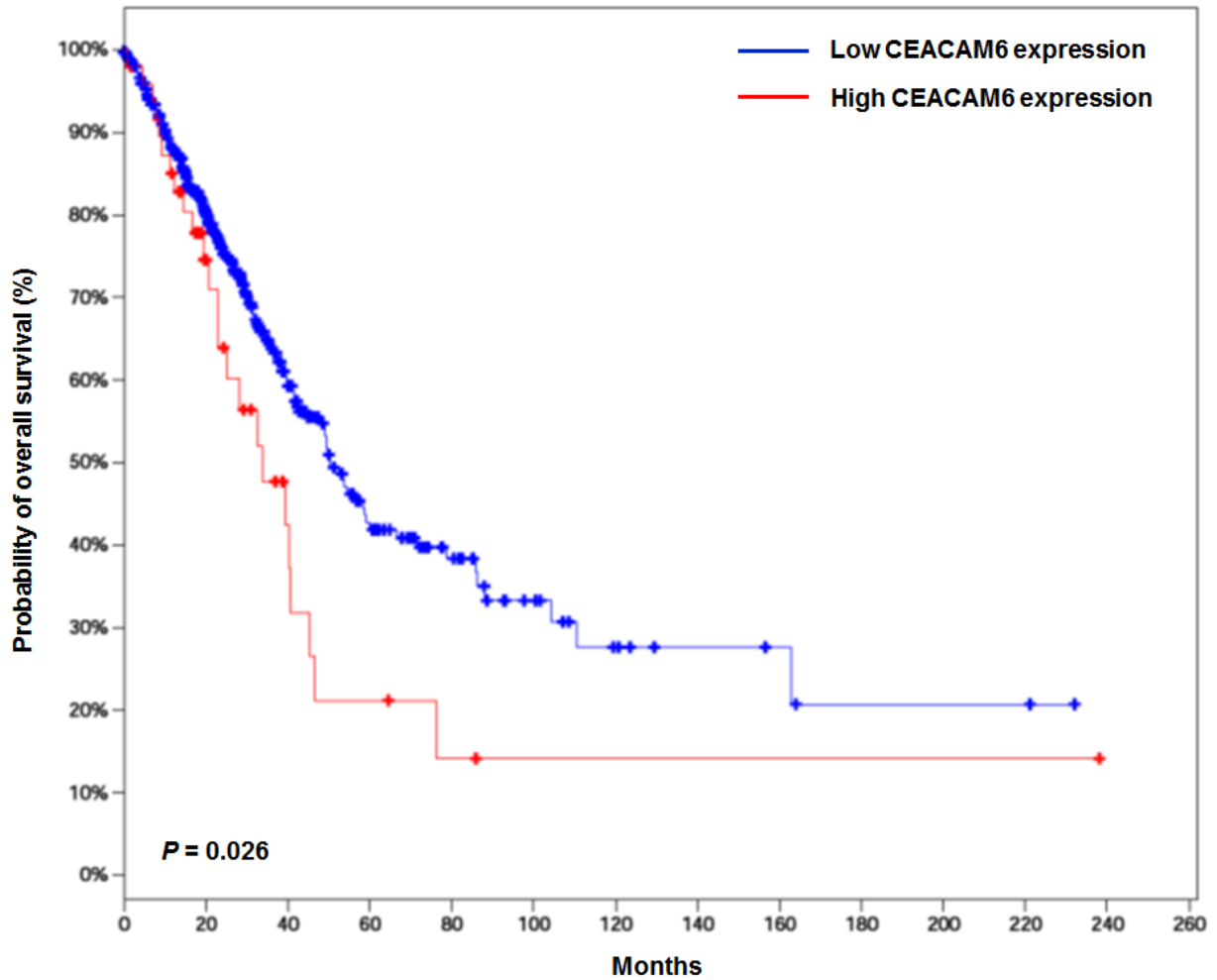
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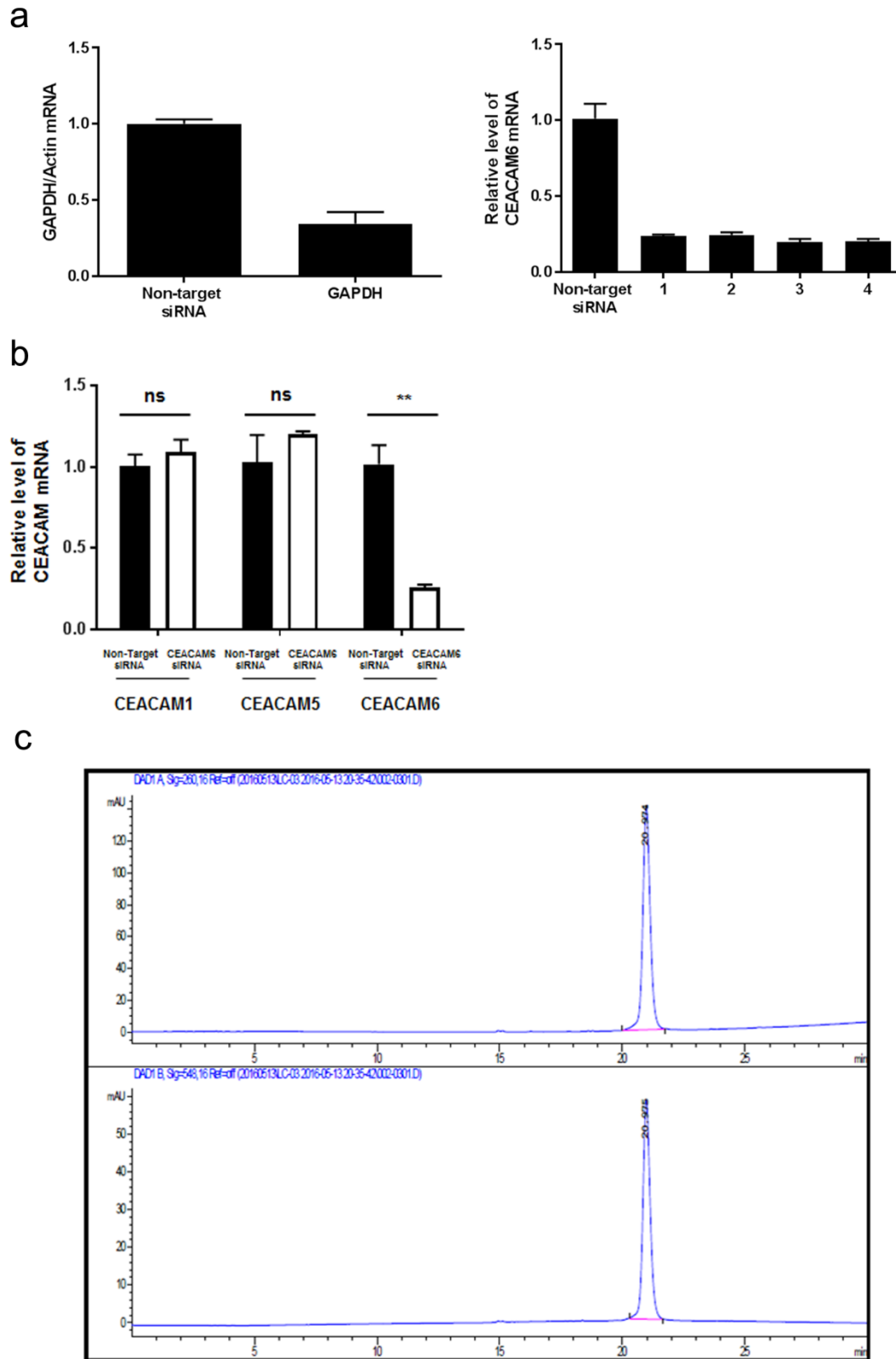
Supplementary Information

Supplementary Figure 1



Supplementary Figure 1. Kaplan-Meier survival analysis of CEACAM6 mRNA expression in lung adenocarcinoma.

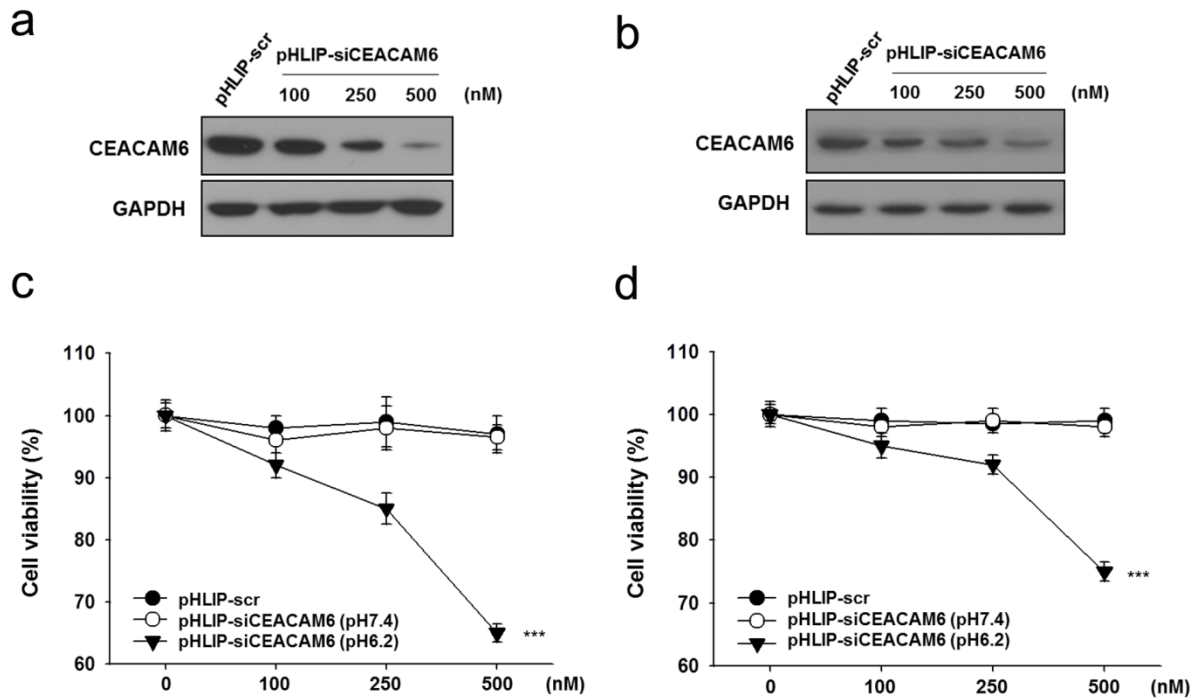
Supplementary Figure 2



Supplementary Figure 2. Generation of pHLIP- siCEACAM6

(a) Quantitative RT-PCR of CEACAM6 mRNA expression in A549 cells transfected with non-target siRNA, GAPDH siRNA, or one of four different siCEACAM6 sequences. (b) Effects of siCEACAM6 on relative mRNA expression of CEACAM1, CEACAM5, and CEACAM6. (c) HPLC elution profiles of a reaction mixture of PNA (siCEACAM6) and pHLIP. Data are shown as mean \pm s.d.; ns, not significant; $**P < 0.01$.

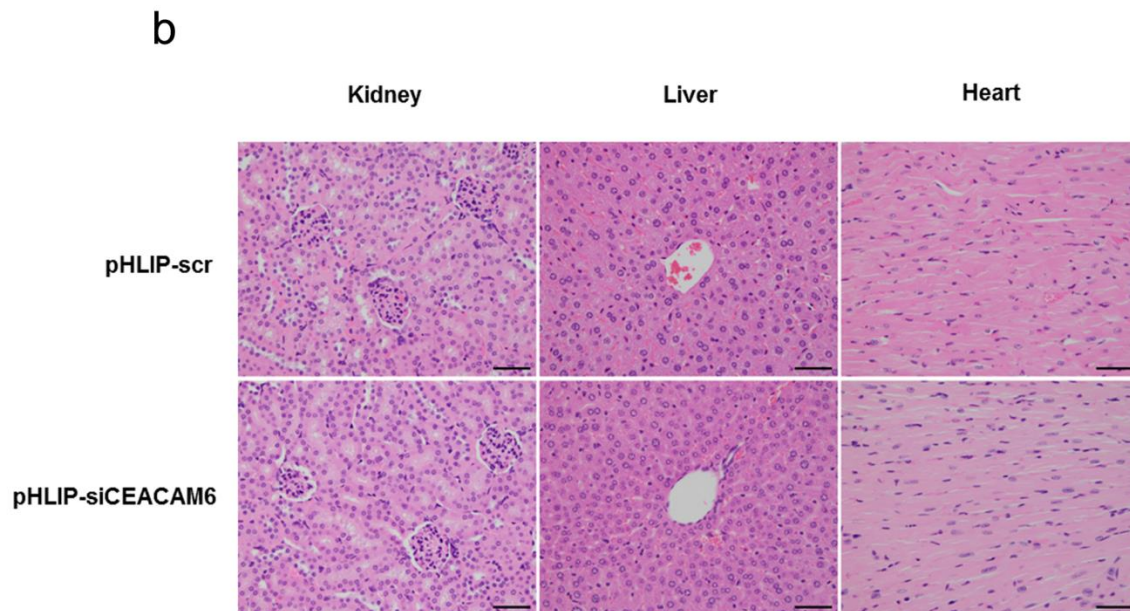
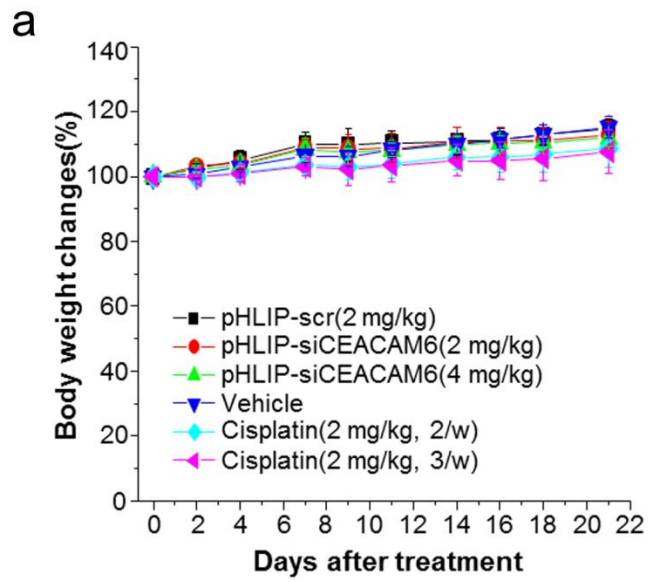
Supplementary Figure 3



Supplementary Figure 3. Assessment of the activity of pHLIP- siCEACAM6 in HT-29 and LoVo colon adenocarcinoma cells

(a, b) Western blotting analysis of CEACAM6 protein levels in HT-29 cells (a) and LoVo cells (b) incubated with pHLIP-siCEACAM6 at pH 6.2. (c, d) Effects of CEACAM6 inhibition on viability of HT-29 cells (c) and LoVo cells (d) at neutral and acidic pH values. Data are shown as mean \pm s.d.; *** $P < 0.001$.

Supplementary Figure 4

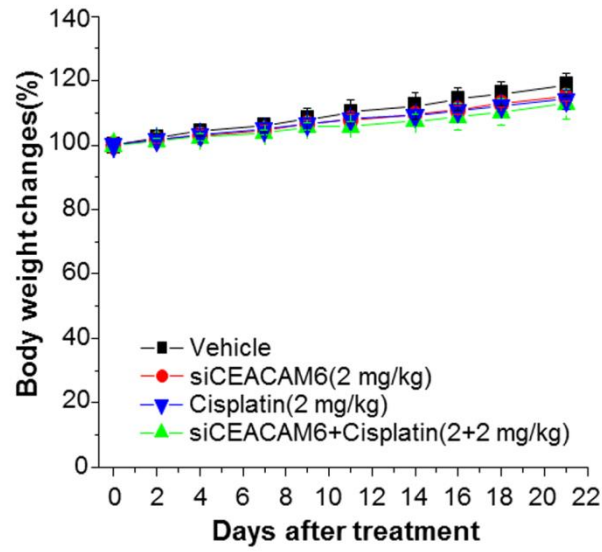


Supplementary Figure 4 Toxicity assessment of intravenously administered pHLIP-siCEACAM6

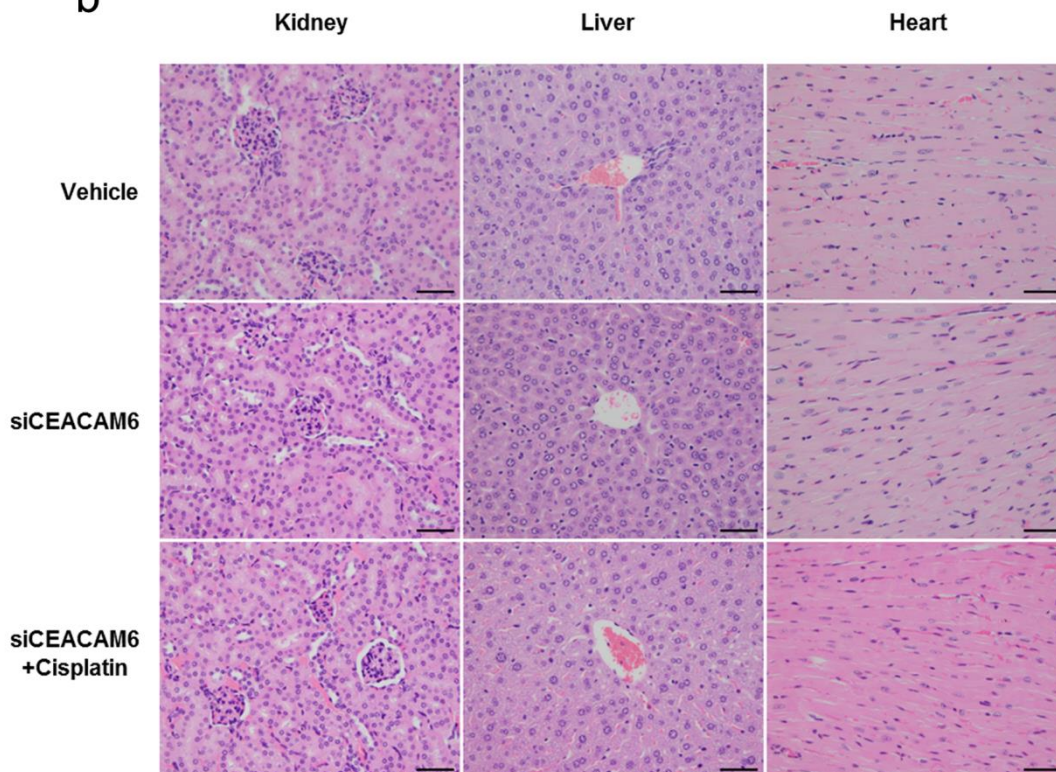
(a) Changes in body weight in experimental mice during the treatment period (n = 5 mice/group). Data are shown as mean \pm s.d. (b) Representative histological analyses of the kidney, liver, and heart (original magnification \times 400, scale bar = 50 μ m) harvested from mice. All sections reveal an absence of microscopic changes associated with toxicity in pHLIP-siCEACAM6-treated animals (compare with normal sections in pHLIP-scr control).

Supplementary Figure 5

a



b



Supplementary Figure 5 Toxicity assessment of combination treatment with pHLIP-siCEACAM6 and cisplatin

(a) Changes in body weight in experimental mice during the treatment period (n = 5 mice/group). Data are shown as mean \pm s.d. (b) Representative histological analysis of the kidney, liver, and heart (original magnification \times 400, scale bar = 50 μ m) harvested from mice. All sections reveal an absence of microscopic changes associated with toxicity in pHLIP-siCEACAM6-treated and pHLIP-siCEACAM6+cisplatin-treated animals (compare with normal sections in pHLIP-scr control).