

Effect of CEACAM6 on adenovirus infection. (**A**) CEACAM6 expression in stable CEACAM6overexpressing PaTu8988t cells after transfection with control siRNAs or CEACAM6-specific siRNAs and withdrawal of Zinc Sulphate from the medium for 3 weeks. (**B**) Ad-GFP expression in PaTu8988t-Hyg cells and PaTu8988t-CEACAM6 cells after down-regulation of CEACAM6 by siRNA or withdrawal of CEACAM6 expression-inducer Zinc Sulphate. The GFP expression was detected by FACS at 24 hours after infection with Ad-CMV-GFP adenovirus. The experiments were triplicated. \*\*p<0.01. (**C**) Cytotoxicity of oncolytic adenovirus in PaTu8988t-Hyg cells and PaTu8988t-CEACAM6 cells after down-regulation of CEACAM6 by siRNA or withdrawal of the CEACAM6 expression-inducer Zinc Sulphate. The experiments were duplicated. \*\*p<0.01.



#### **Supplementary Figure 2**

Effect of CEACAM6 on adenovirus infection. (**A**) CEACAM6 expression measured by Western blotting in CEACAM6-siRNA knockdown Suit-2 cells after transfection with a CEACAM6-expressing plasmid or control plasmid.  $1\times10^5$  of Suit-2 cells were transfected with CEACAM6-specific siRNA first, and 48 hours later the cells were transfected with CEACAM6-expressing plasmid pML1 or control plasmid. The cells were harvested for Western blot at 24 hours and 48 hours. (**B**) Adenovirus infectivity assayed by Ad-CMV-GFP virus in Suit-2 cells manipulated by siRNA and CEACAM6-expressing plasmid. (**C**) CEACAM6 expression in Suit-2 cells after transfection of CEACAM6-specific siRNA and control siRNAs over a time course. (**D**) Adenovirus infectivity assayed by Ad-CMV-GFP virus in Suit-2 cells after down-regulation of CEACAM6 by siRNA at different time points. (**E**) Adenovirus cytotoxicity in Suit-2 cells after down-regulation of CEACAM6 by siRNA at different time points. The GFP expression (**B** and **D**) was detected by FACAS at 24 hours after infection with Ad-CMV-GFP adenovirus. All experiments were duplicated. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.



CEACAM6 does not affect CAR expression. (**A**) FACS analysis showing the expression of CAR in stable clones PaTu8988t-Hyg and PaTu8988t-CEACAM6. (**B**) FACS analysis showing the expression of CAR in control and the CEACAM6-specific SMART<sup>™</sup> pool siRNA-treated Suit-2 cells.

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The expression of integrins in stable clones PaTu8988t-Hyg and PaTu8988t-CEACAM6 and in control and CEACAM6-specific SMART<sup>™</sup> pool siRNA-treated Suit-2 cells.

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