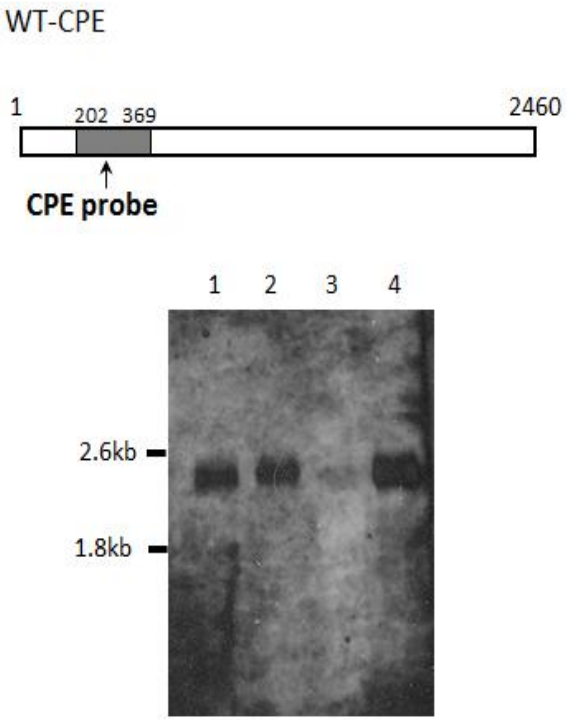
A novel 40kDa N-terminal truncated Carboxypeptidase E splice variant: Cloning, cDNA sequence analysis and role in regulation of metastatic genes in human cancers

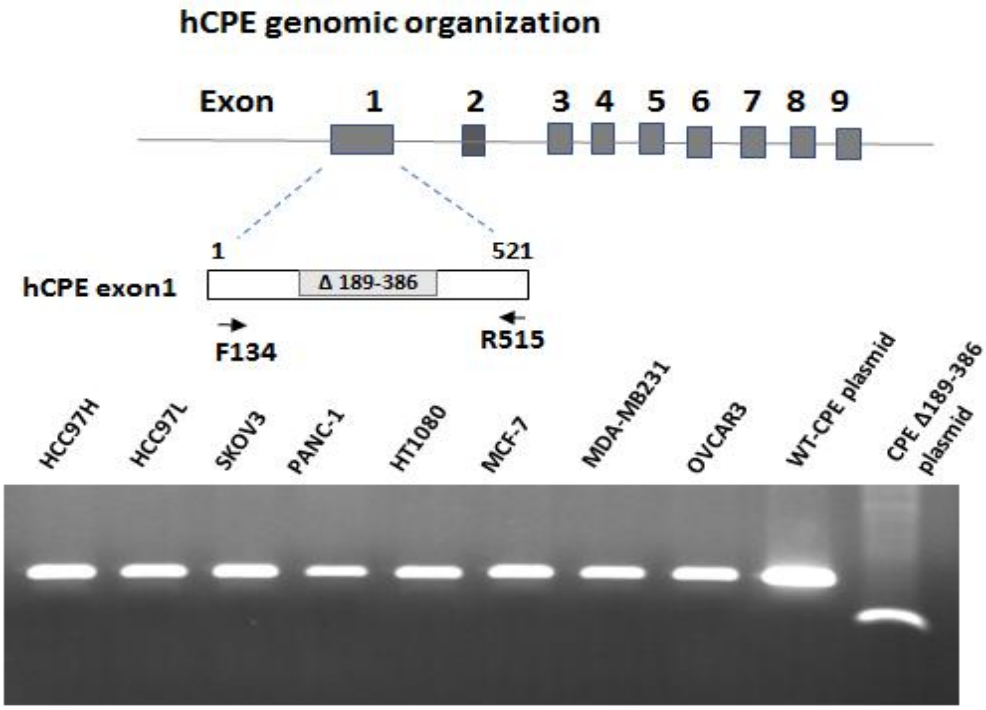
Supplementary Materials

Genomic PCR

Genomic PCR was performed to detect CPEΔ189-386 within exon1. Briefly, genomic DNA from the cells was extracted using a DNAeasy kit from Qiagen according to the instruction. Hot-start PCR protocol was used to amplify the CPE exon1 using primer set hCPE F134/ hCPE R515 (primer sequences are as following: CPE F134: 5'-CAT TCA GCC GGG GAA GGT G-3'; CPE R515:5'- CCA CCG TGT AAA TCC TGC TGA-3')). PCR amplification was carried out in a 50 µl volume consisting of 100 ng of genomic DNA, 1 U of SeqAmp DNA polymerase (Clontech, Palo Alto, CA, USA), 1 µM of ach primer, cycles involve an initial 'hot start' at 95 °C for 5min followed by 30 cycles of amplification (94°C 30 sec, 60°C 30 sec, 72°C 45 sec) with a final extension step of 72 °C for 5 min. PCR products were analyzed on 1.8% agarose gels. CPEΔ189-386 and wild type plasmids were used as control.



Supplementary Figure 1: Upper panel: schematic representation of the DIG-labeled CPE probe (shaded box) covering hCPE mRNA region 202-369nt. Numbers refer to the position of the probe in human CPE mRNA. Lower panel: Northern Blot, lane 1, LN18 mRNA; 2, CAOV3 mRNA; 3, U-118 mRNA; 4, pooled human hippocampus mRNA.



Supplementary Figure 2: Upper panel: schematic representation of the organization of human WT CPE gene. Primer set CPE F134/R515 was used for PCR to detect the CPEΔ 189-386 deletion within the 521bp long exon 1. Numbers of primer refer to the relative position to exon1. Lower panel: PCR was performed with genomic DNA derived from various cancer cell lines, a single PCR amplicon was amplified.