SUPPLEMENTARY

Figure S1: Receptor expression levels

To confirm CD44v6, Met and VEGFR-2 expression levels in cells used for FACS and ELISA experiments, cell lysates were subjected to Western blot analysis. **(A)** Expression level of Met and CD44v6 in AS and ASs6 cells was detected by means of specific antibodies against Met or CD44v6. For the loading control, the membranes were probed with a specific antibody against Erk**. (B)** Cell lysates from T47D and T47D/Met cells were subjected to Western blot analysis. Expression of Met and CD44v6 was detected by means of specific antibodies against Met or CD44v6. **(C)** ASs6 cells were transfected with the VEGFR-2 plasmid. The cell lysates were subjected to Western blot analysis and expression of VEGFR-2 was detected by means of a specific antibody against VEGFR-2.

Figure S2: The isolated purified protein is identified as the CD44v6 ectodomain.

(A) The purified ectodomain obtained from IMAC purification and used in MS was separated by SDS-Page and subjected to Western blot analysis. The CD44v6 ectodomain was detected by means of specific antibodies against CD44v6 or penta-histidine (His). **(B)** The purified ectodomain was digested with trypsin and the resulting peptides were analyzed by LC/MS-MS. The extracted peptide chromatogram was generated using database matching conducted by MassHunter BioConfirm software. Each peak corresponds to an identified peptide of the ectodomain. The CD44v6 specific peptide sequence WFENEWQGK is highlighted in green. **(C)** In MS six peptides were identified for the CD44v6 ectodomain and listed in the table. For each identified peptide sequence the measured mass was matched against the theoretical mass of the peptide sequence. A mass difference of 10 ppm was used for the positive peptide identification. **(D)** Spectra of the highlighted peptide sequence WFENEWQGK in (B). (top) Survey mass spectrum at 2.5 min showing the singly-charged precursor ion at m/z 993.4827 and the doubly-charged precursor ion at m/z 497.2457 (representing the complete peptide sequence). (bottom) Fragment ion mass spectrum of the doubly-charged precursor ion at m/z 497.2457. A mass tolerance of 50 ppm was used for positive peptide fragment identification. Annotations show m/z and ion species (representing the single amino acids corresponding to the sequence).

Figure S3: The CD44v6 ectodomain binds to HGF in anisotropy

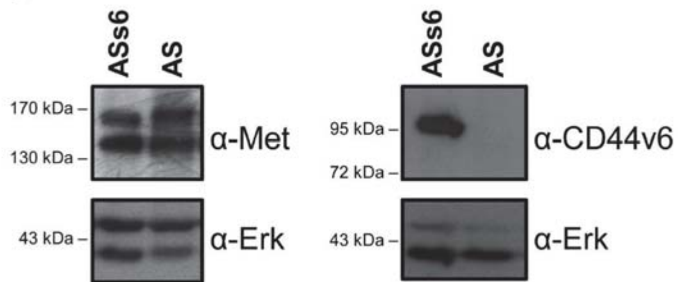
(A-B) In all anisotropy experiments, a 200 nM concentration of HGF-NT647 in PBS buffer was used. For labeled HGF alone, the anisotropy value was about 0.180. To test for direct protein interaction, **(A)** CD44v6 was added in increasing amounts and anisotropy was measured at each concentration (final CD44v6 or CD44s concentration was 16.3 μM). CD44v6 was able to bind directly to HGF-NT647 with a K_d of 3700 ± 600 nM. **(B)** The calculated K_d for CD44s was 10 ± 8 μM.

Figure S4: The CD44v6 peptide directly binds the CD44v6 ectodomain but does not bind HGF in MST.

(A-B) Using MST the Kd was determined whenever binding was observed (fitting curve in blue). (A) Fluorescently labeled CD44v6 ectodomain was titrated against varying concentrations of the CD44v6 peptide (Kd = 2.10 ± 0.2 nM) or (B) the fluorescently labeled HGF was titrated against varying concentrations of the CD44v6 peptide. A representative experiment is shown.

Figure S1

A



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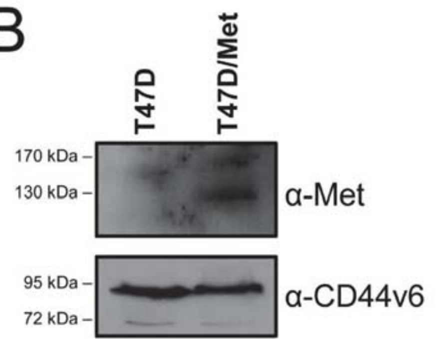

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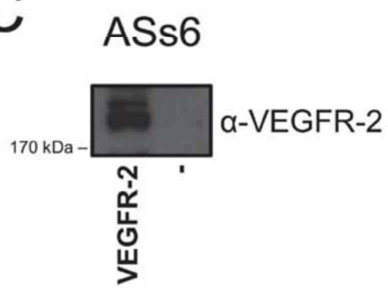
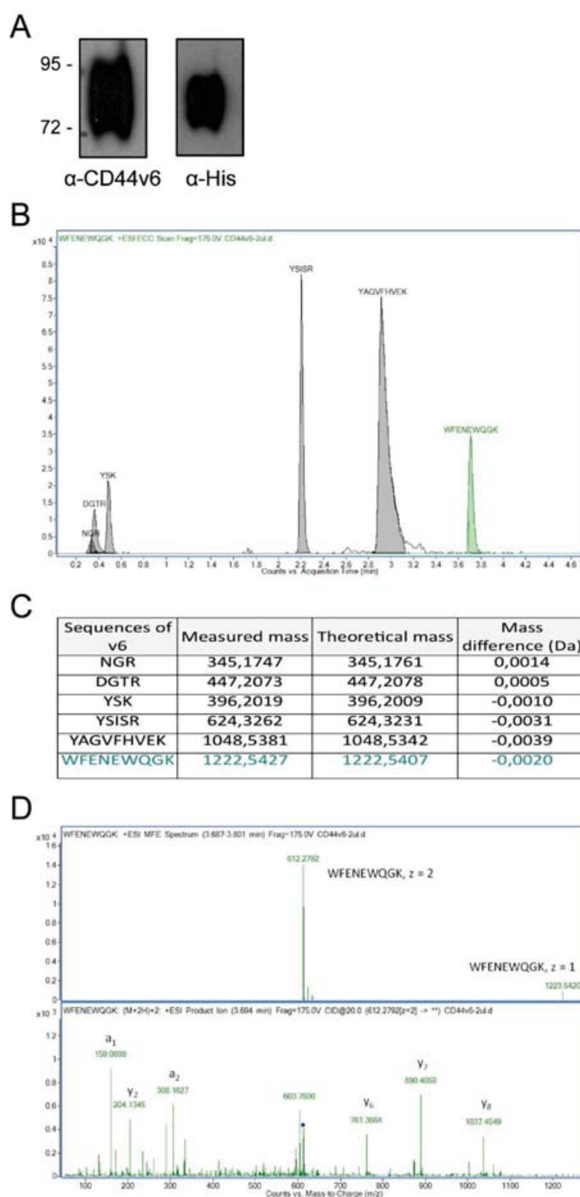


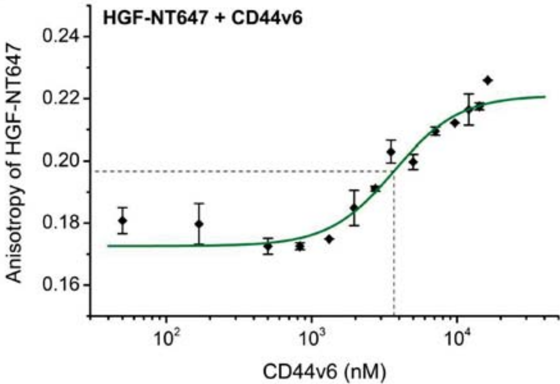
Figure S2



Sequences of v6	Measured mass	Theoretical mass	Mass difference (Da)
NGR	345,1747	345,1761	0,0014
DGTR	447,2073	447,2078	0,0005
YSK	396,2019	396,2009	-0,0010
YSISR	624,3262	624,3231	-0,0031
YAGVFHVEK	1048,5381	1048,5342	-0,0039
WFENEWQGK	1222,5427	1222,5407	-0,0020

Figure S3

A



B

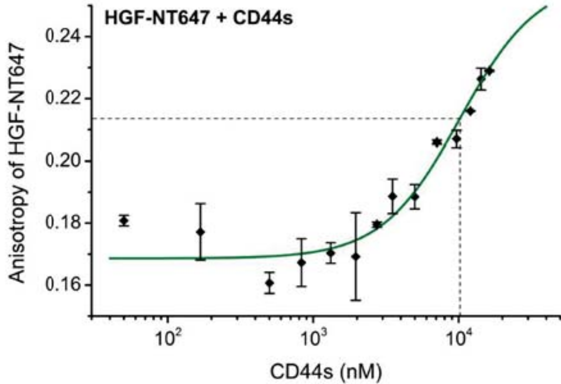


Figure S4