A novel human recombinant single-chain antibody targeting CD166/ ALCAM inhibits cancer cell invasion in vitro and in vivo tumour growth.

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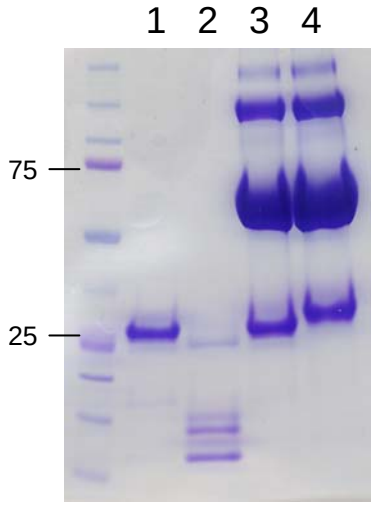
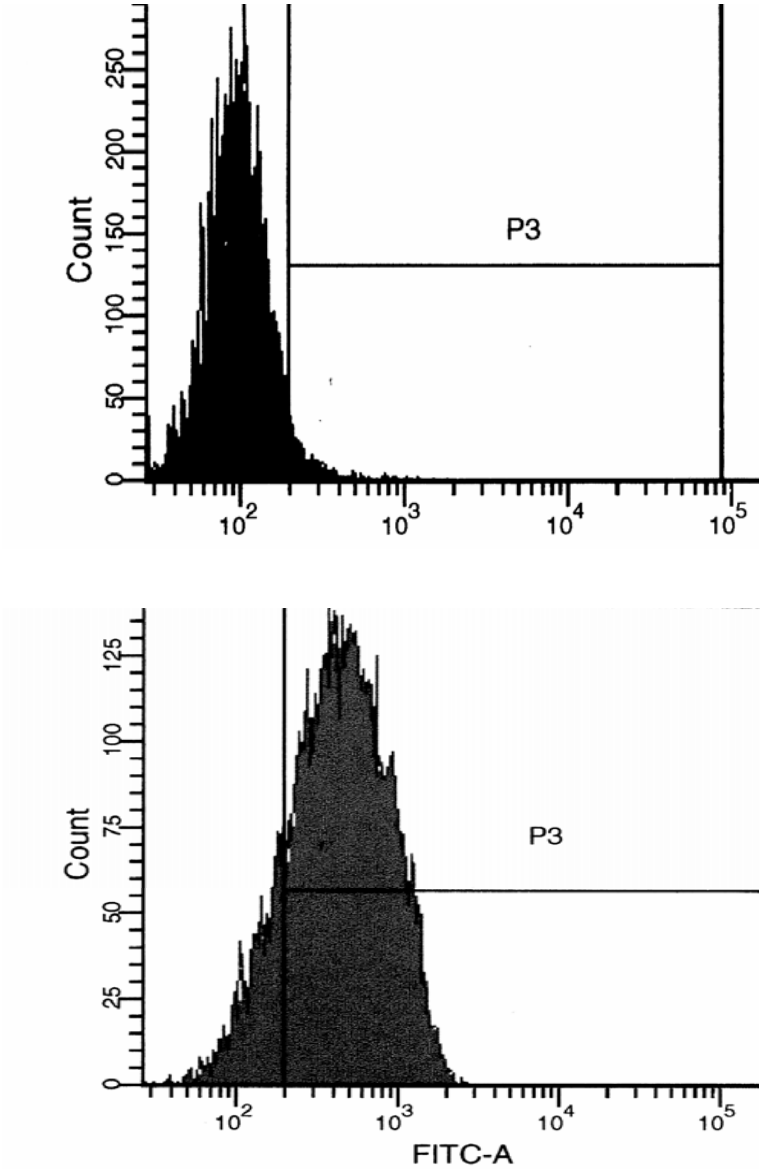
Online resource - figure 1



Coomassie Brilliant Blue stained SDS-PAGE of three different scFv173 batches and scFv141. To increase the antibody stability BSA was added to batches used for animal experiments.

1: scFv173 05.05.09
2: scFv173 (old batch stored > one year at 4°C)
3: scFv173 in storage buffer (animal exp)
4: scFv141 in storage buffer (animal exp)

Online resource – figure 2

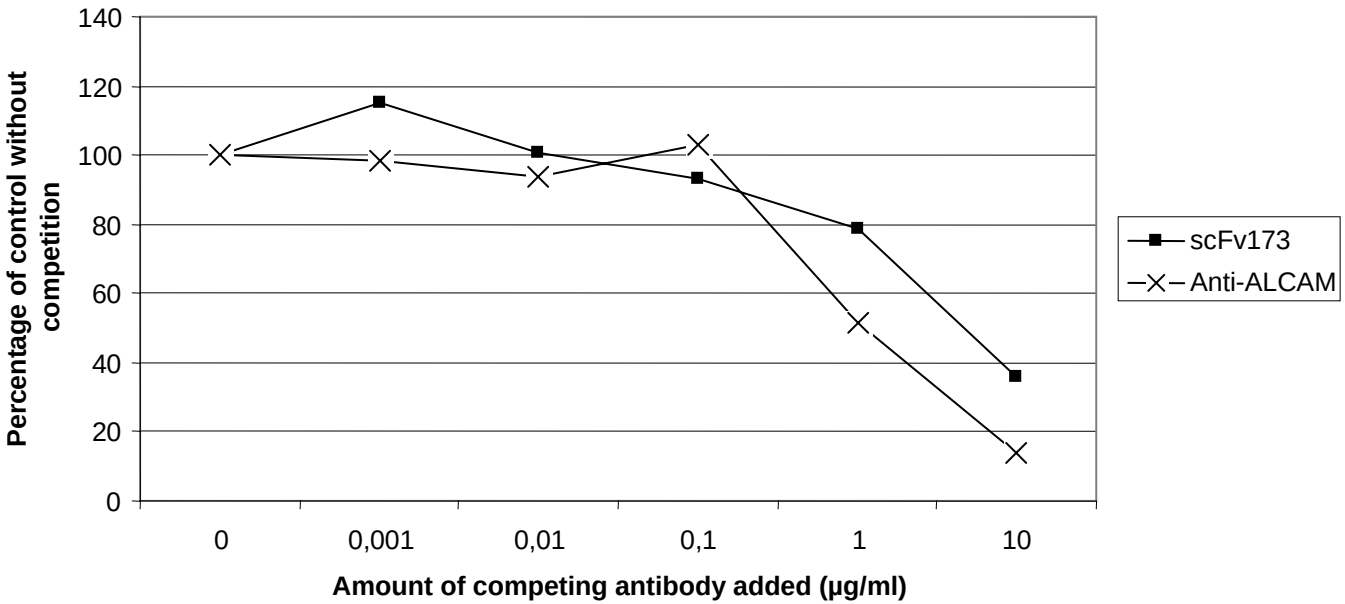


Flowcytometry analysis of DU145 cells

Histogram display of DU145 cells stained with streptavidin-FITC (upper) or scFv173 (biotinylated) + strept-FITC. In each case 10000 events were recorded and the P2 gated cells shown here contain 6514 and 6678 events.

Median fluorescense intensity shifted from 95 (negative control) to 426 (scFv173) i.e. 4.5x increase = ++ in table 1

Online resource figure 3

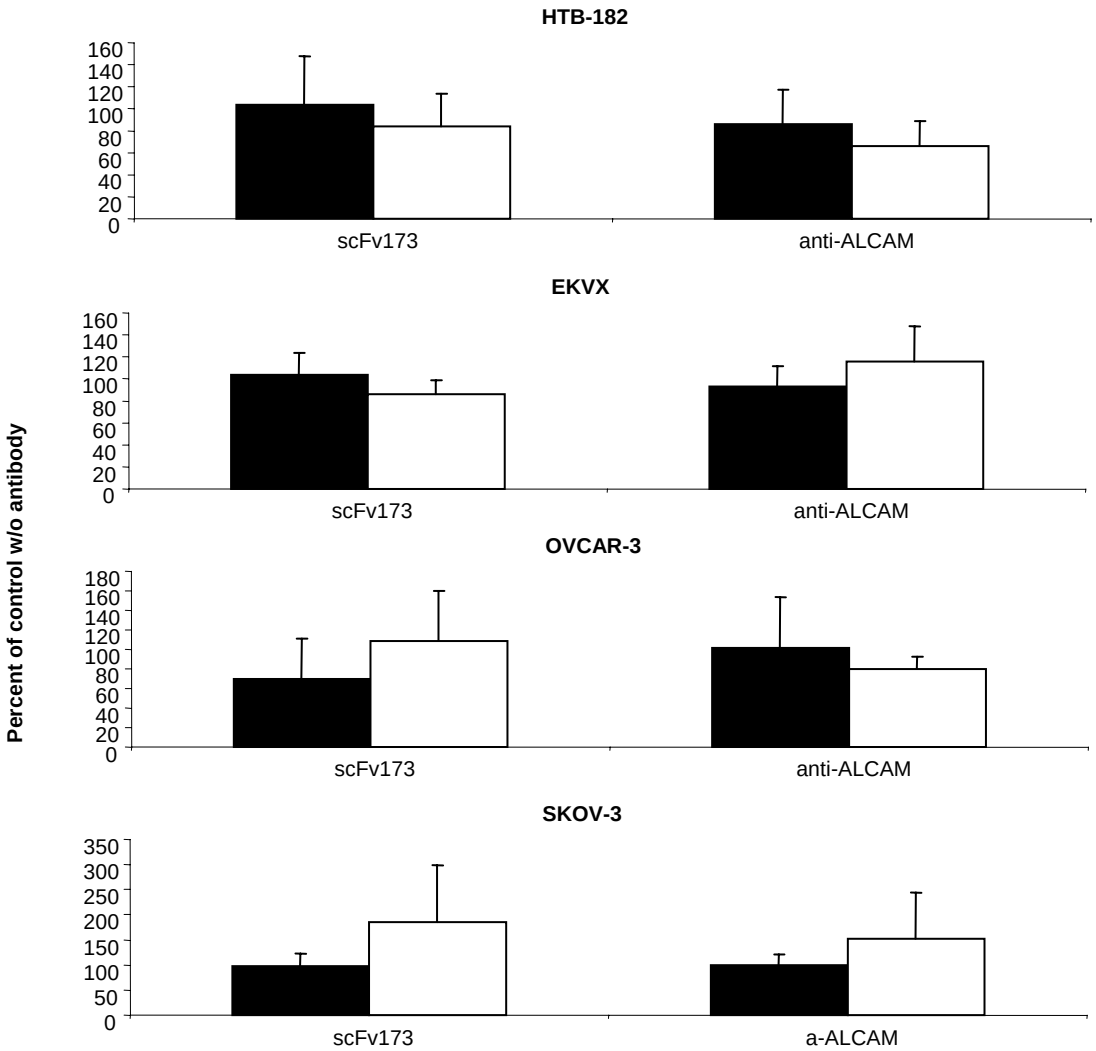


Antibody competition assay

Elisa plates were coated with recombinant ALCAM-Fc (5µg/ml, PBS) over night at 4°C and washed with PBS+0.05% Tween-20 prior to blocking with PBS added 1% BSA for 1 h at RT. Increasing amounts of competing antibodies were added and the plate incubated for 2 h at RT. Without washing the wells, biotinylated scFv173 (diluted to 1µg/ml in PBS) was added and the plate incubated for 1 h at RT.

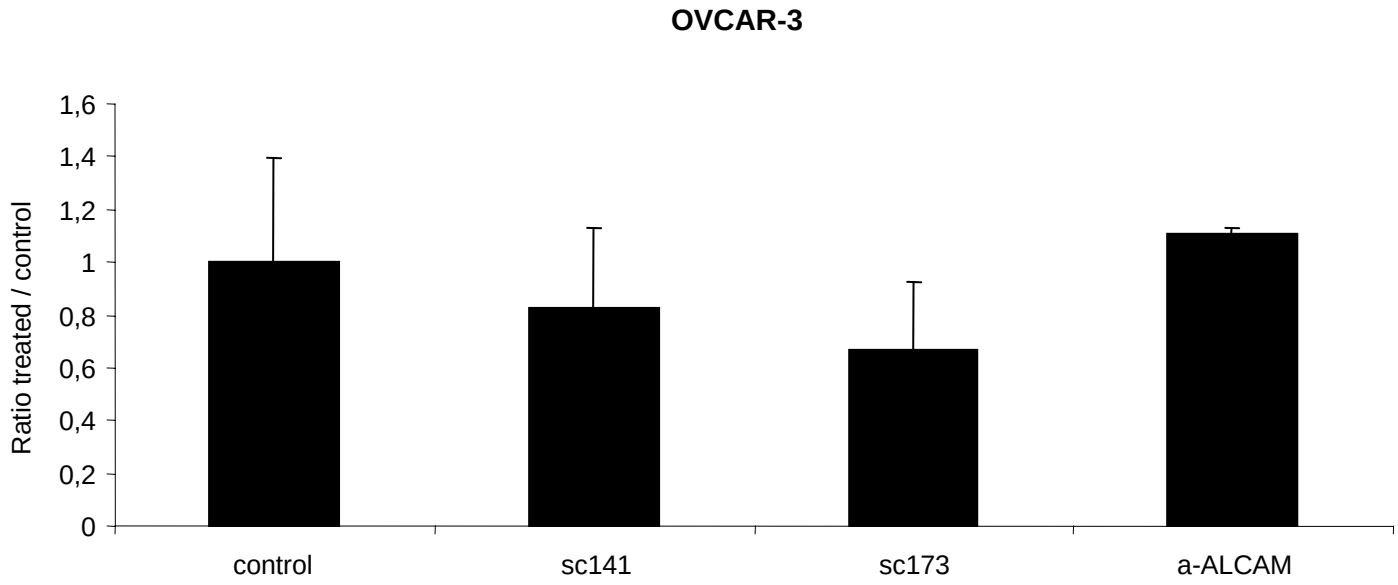
After washing, Neutravidin-HRP (Pierce, diluted 1µg to 5ml PBS) was added for 45 min at RT and the plate was washed, developed with TMB substrate (RnD systems) and quantified in a plate reader.

Online resource – figure 4



Adhesion assay of cells (HTB-182, EKVX, OVCAR-3 or SKOV-3) preincubated with antibodies and seeded in 96 wells tissue culture plates coated with fibronectin. Black bars shows adhesion at 4°C and white bars adhesion at 37°C.

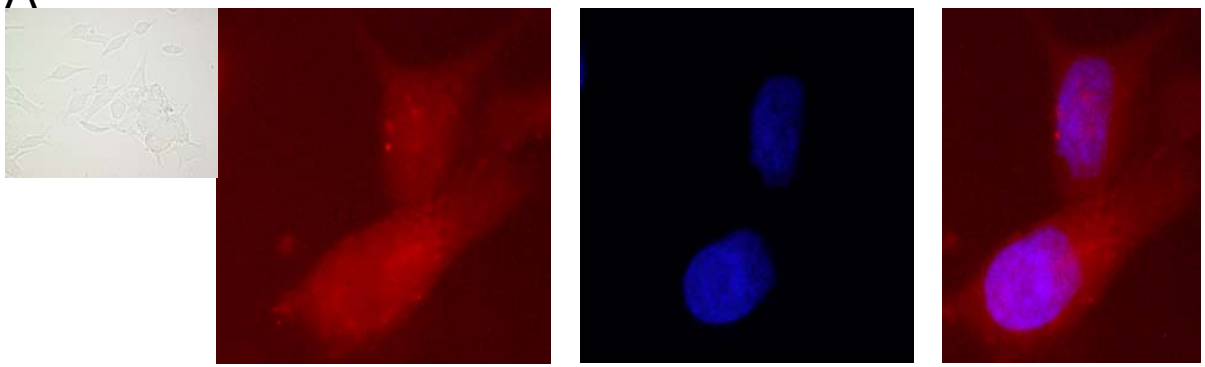
Online resource – figure 5



^{3}H-thymidine labelled OVCAR-3 cells were mixed with antibodies prior to seeding and harvested after 48 h.
The ratio of invading cells over non-invading cells in treated wells are normalised to the ratio in untreated wells.

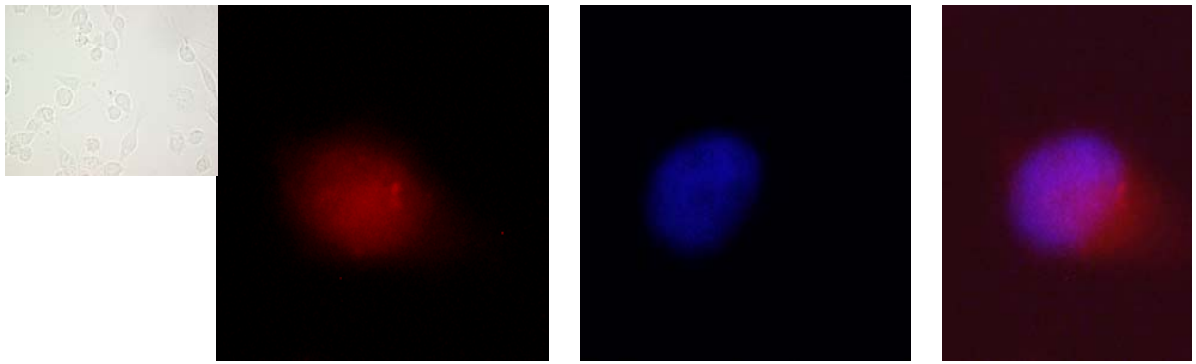
Online resource – fig 6

A



scFv173-Cy3 DAPI combined

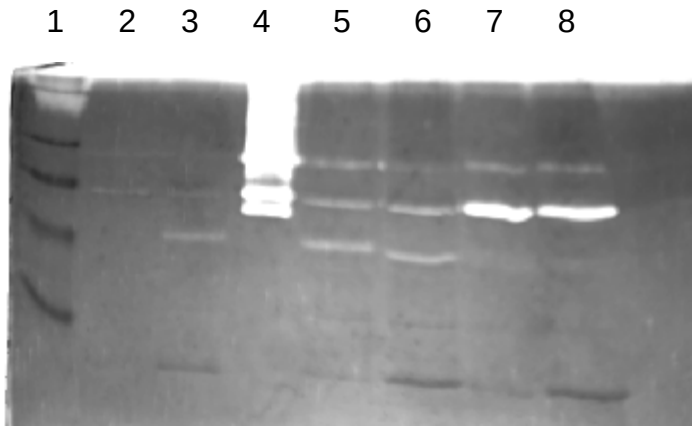
B



scFv173-Cy3 DAPI combined

Immunofluorescence of MDA-MB-231 cells labelled with 0.5µg/ml scFv173-Cy3 in serum-free medium. Panel A shows cells incubated with antibody for 1 h at 4°C, washed, and fixed with methanol for 15 min 4 °C and 1% formaldehyde in PBS/Ca/Mg at RT. Finally wash and mounting using Prolong Gold antifade reagent with DAPI (Invitrogen). In panel B the cells were treated as in A except for incubating labelled cells for 60 min at 37°C before fixation and mounting. Objectives: brightfield 20x with phase contrast and fluorescence 100x with oil

Online resource – figure 7

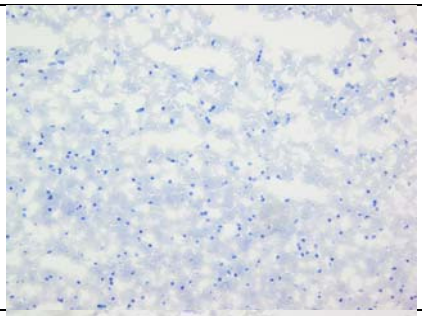
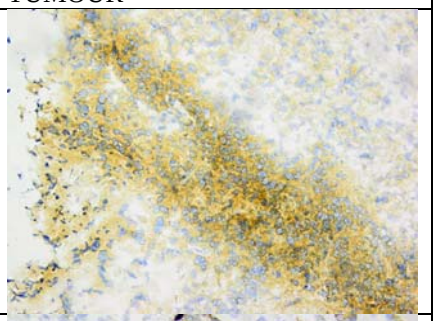
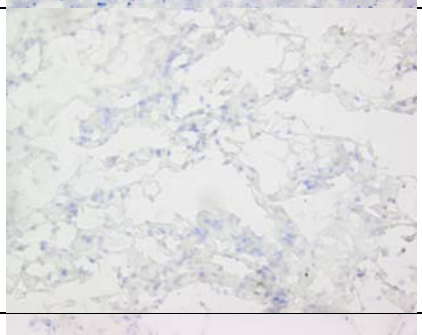
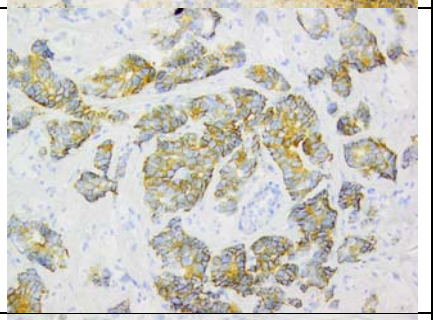
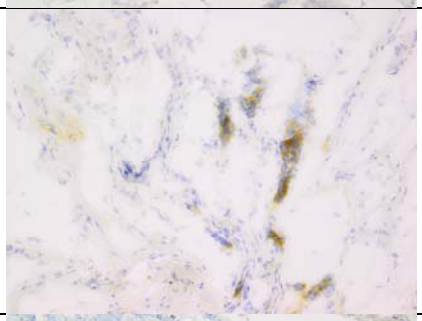
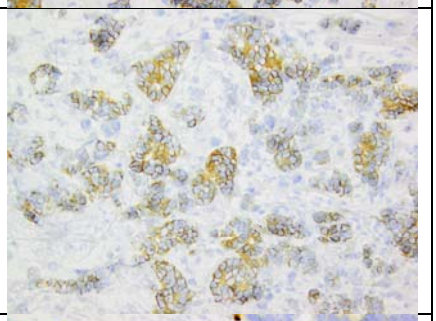
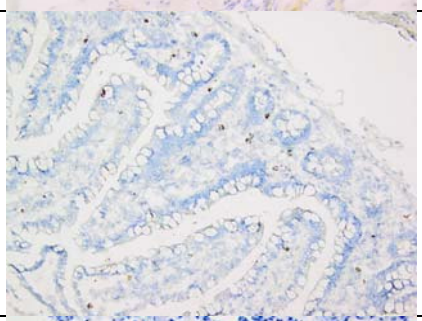
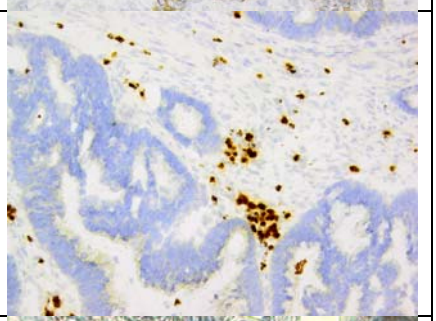
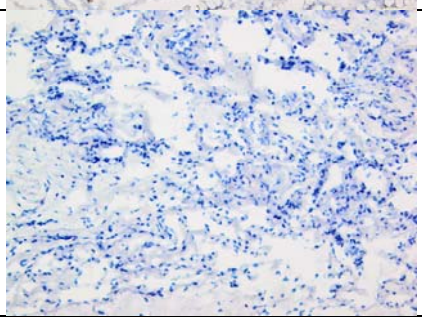
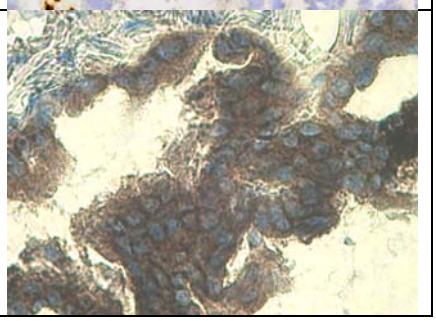


1. Mw standard
2. Medium untreated cells
3. Medium ConA treated cells
4. Zymography standard
5. Medium ConA
6. Medium ConA + scFv173
7. Medium ConA
8. Medium ConA + scFv173

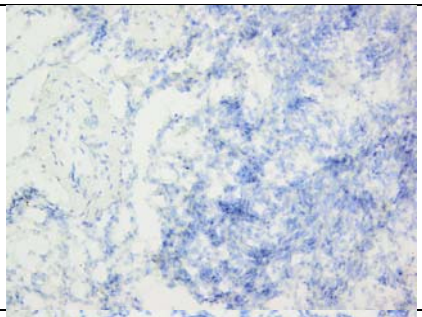
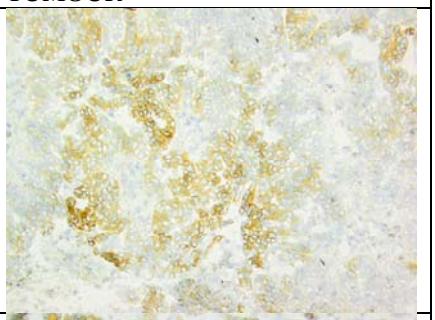
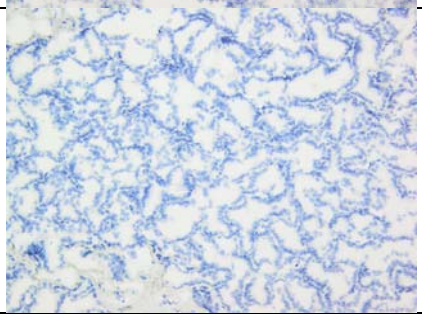
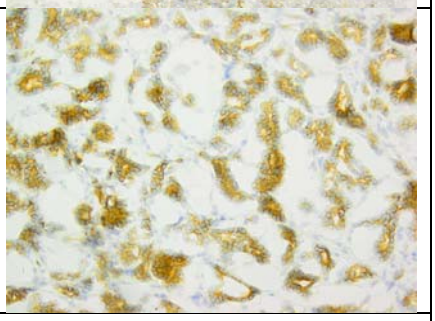
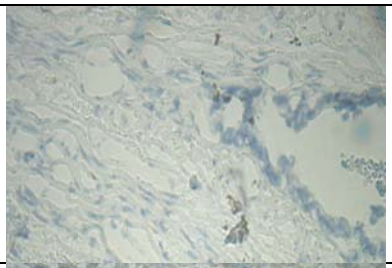
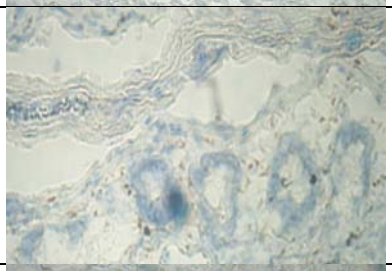
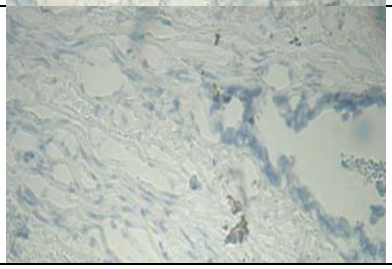
Gelatin zymography of MDA-MB-231 cells and OVCAR-3 cells (lanes 7 and 8).

Gelatinase-activity was hardly detetcted in medium from MDA-MB-231 cells (lane 2) and ConA was added to increase the amount of active MMP2 (lane 3). Comparing lanes 5 and 6 no inhibition of active MMP2 formation was observed adding scFv173. Samples were also tested in reverse zymography to look for changes in TIMP levels, but no reproducible changes were observed.
The effect of the antibody in invasion most likely do not involve gelatinases or TIMPs.

Online resource table 1

TISSUE	NORMAL	TUMOUR
Brain		
Breast[a]		
Breast		
Colon		
Lung		

[a] One negative and one positive normal breast are shown for comparison

TISSUE	NORMAL	TUMOUR
Prostate		
Ovary		
Kidney		N.A.[b]
Liver		N.A.
Small intestine		N.A.
Skin		N.A.

[b] N.A. not available