

## **Introduction to Additional file 1 – further information relating to Figures S1-S3**

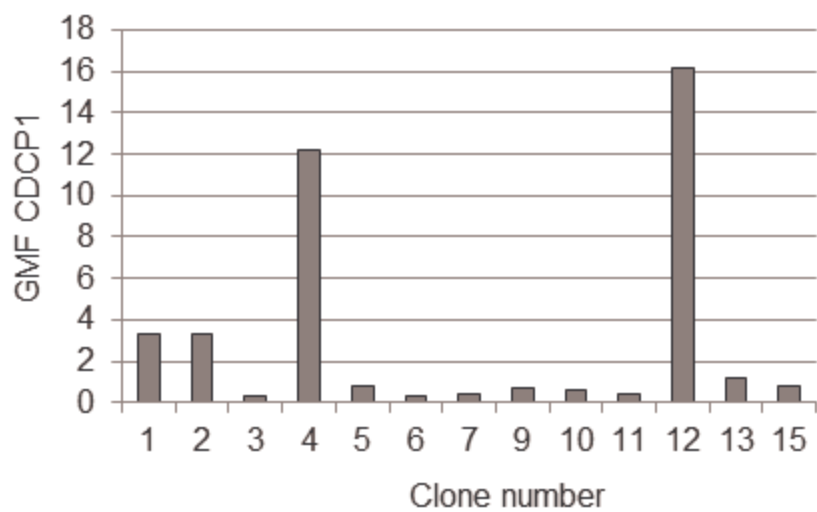
**Figure S1:** Production of a CDCP1-expressing Colo320 cell line. Clones of G418-resistant Colo320 cells were produced as described in Methods. A. Clonal cell populations were screened for cell-surface CDCP1 expression by flow cytometry using the mouse monoclonal antibody CUB1. B. A selection of clones was further analysed by Western blotting using the anti-FLAG M2 antibody in Western blotting of whole cell extracts. Clone 12 was used for all further experiments since it displayed the highest level of cell-surface CDCP1 (assessed by flow cytometry) and total cellular CDCP1 (assessed by Western blotting).

**Figure S2:** Reduction of CDCP1 by RNA interference in SW480 cells. SW480 cells were plated at  $2.2 \times 10^5$  cells per well in six well plates, cultured for approx. 15 h and transfected with 20pmoles of CDCP1 siRNA using Lipofectamine 2000. Three duplex siRNAs (at 8nM final concentration) were screened for reduction of CDCP1 (oligos 1–3). A) Cell-surface CDCP1 expression was assayed 48 hours post-transfection by flow cytometry using the CUB1 monoclonal antibody. B) To confirm the flow cytometry results shown in (A), total CDCP1 protein expression in SW480 cells at 48 hours post-transfection with 8nM siRNA (oligo 2) was analysed by Western blotting using the goat polyclonal anti-CDCP1 antibody. Oligo 2 (used in the remainder of this study) proved to be the most efficacious in reducing cell-surface and total cellular CDCP1.

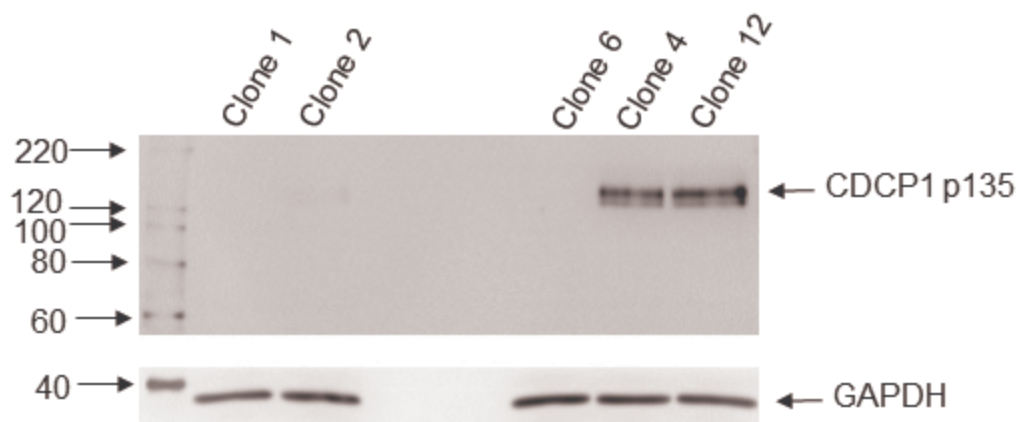
**Figure S3:** Reduction of SW480 colon cancer motility as a function of time after transfection of CDCP1 siRNA. The motility of SW480 cells through an 8  $\mu$ m pore membrane towards FBS following reduction of CDCP1 by RNA interference was measured using the Roche xCELLigence system. Data points at 1, 40, 60 and 72 h are shown. - = no addition; 10% serum = 10% FBS was present in the lower chamber; Lipofectamine = Lipofectamine 2000 only; Control siRNA = Lipofectamine 2000 and control siRNA; CDCP1 siRNA = Lipofectamine 2000 and CDCP1 siRNA.

# Supplementary Figure 1

A

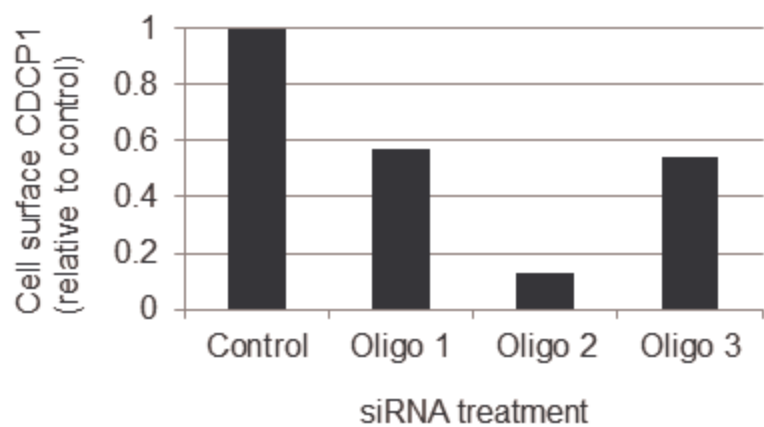


B

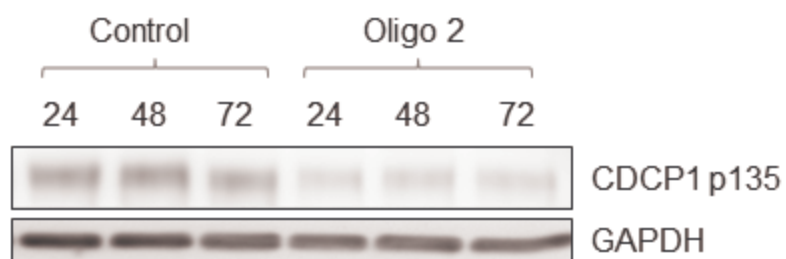


# Supplementary Figure 2

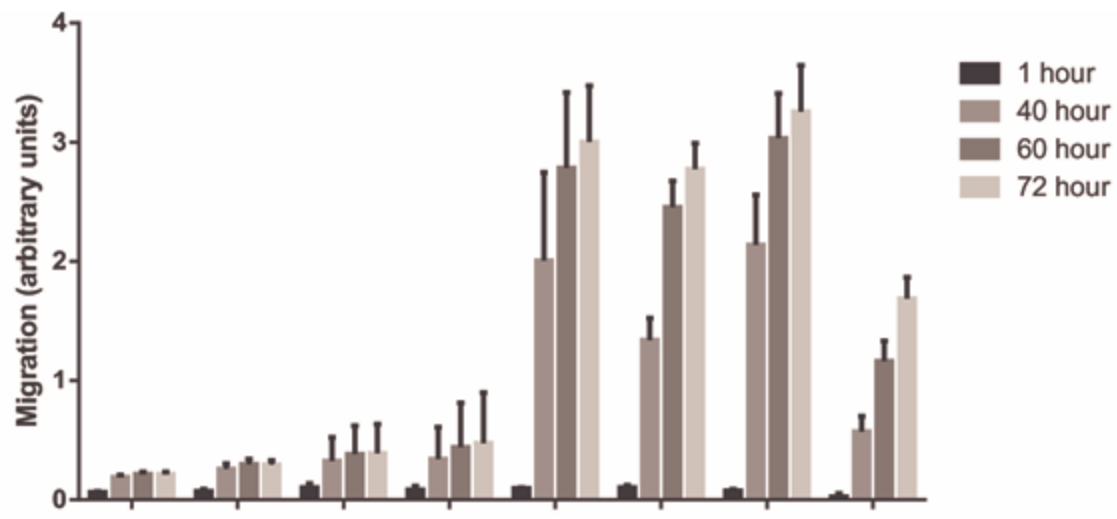
A



B



Supplementary Figure 3



Chemo-attractant:	10% Serum	Lipofectamine	Control siRNA	CDCP1 siRNA	1 hour	40 hour	60 hour	72 hour
	-	-	-	-	~0.1	~0.2	~0.2	~0.2
	-	+	-	-	~0.1	~0.3	~0.3	~0.3
	-	+	+	-	~0.1	~0.3	~0.4	~0.4
	-	+	-	+	~0.1	~0.4	~0.5	~0.5
	+	-	-	-	~0.1	~2.0	~2.8	~3.0
	+	+	-	-	~0.1	~1.4	~2.5	~2.8
	+	-	+	-	~0.1	~2.2	~3.0	~3.3
	+	+	-	+	~0.1	~0.6	~1.2	~1.7