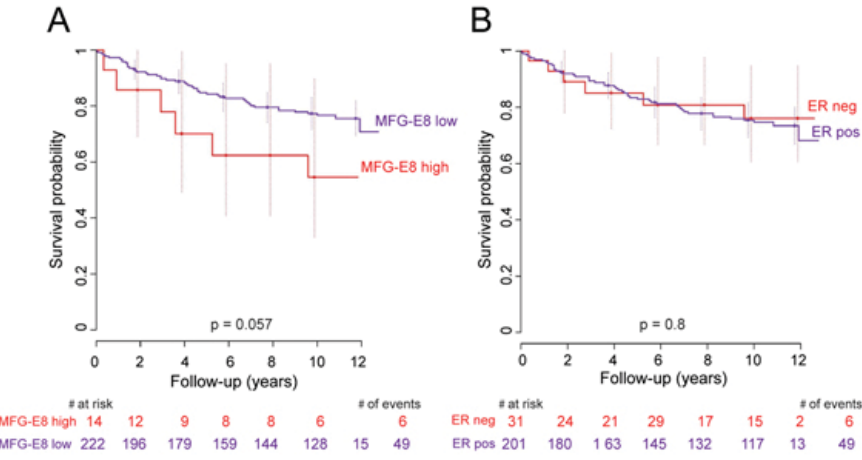
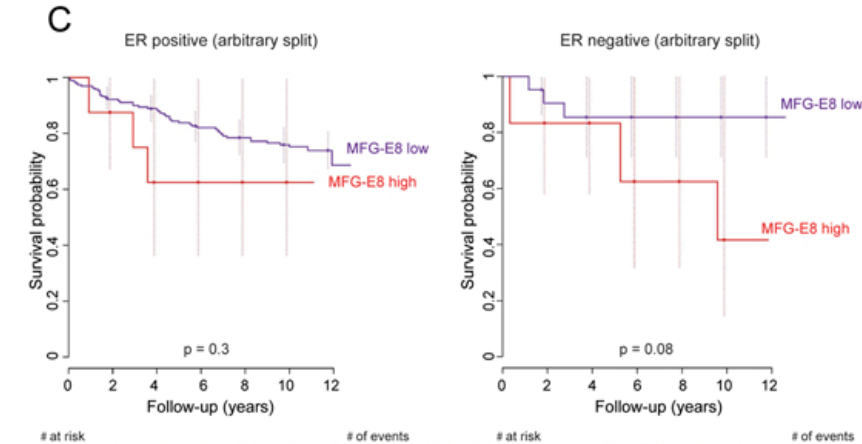
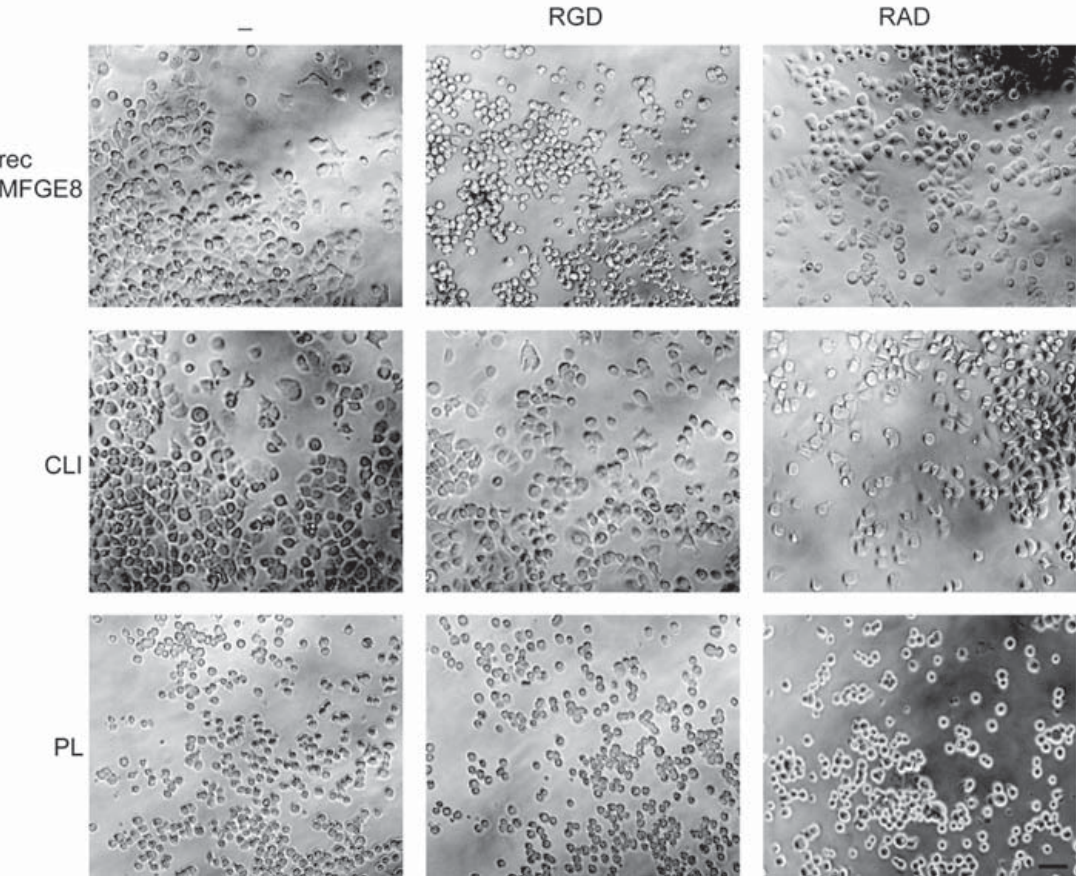
Suppl. Fig. 1

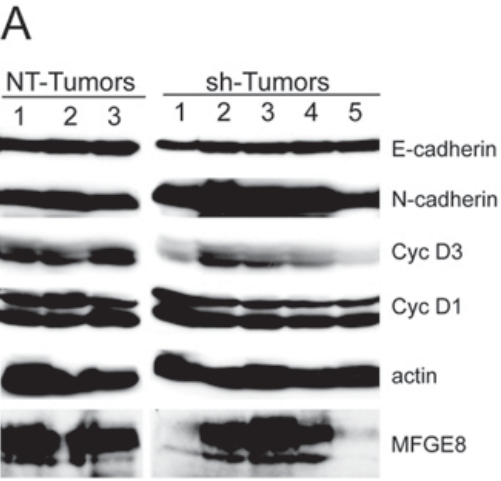

A
Survival probability
MFG-E8 low
MFG-E8 high
p = 0.057
Follow-up (years)
at risk
of events
MFG-E8 high 14 12 9 8 8 6 6
MFG-E8 low 222 196 179 159 144 128 15 49
B
Survival probability
ER neg
ER pos
p = 0.8
Follow-up (years)
at risk
of events
ER neg 31 24 21 29 17 15 2 6
ER pos 201 180 1 63 145 132 117 13 49

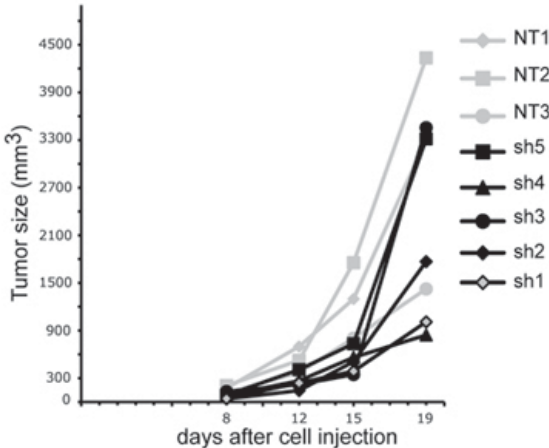

C
ER positive (arbitrary split)
Survival probability
MFG-E8 low
MFG-E8 high
p = 0.3
Follow-up (years)
at risk
of events
8 7 5 5 5 4 3 3
193 173 158 140 127 113 13 46
ER negative (arbitrary split)
Survival probability
MFG-E8 low
MFG-E8 high
p = 0.08
Follow-up (years)
at risk
of events
MFG-E8 high 6 5 4 3 3 2 3
MFG-E8 low 25 19 17 16 14 13 2 3

Suppl. Fig. 2

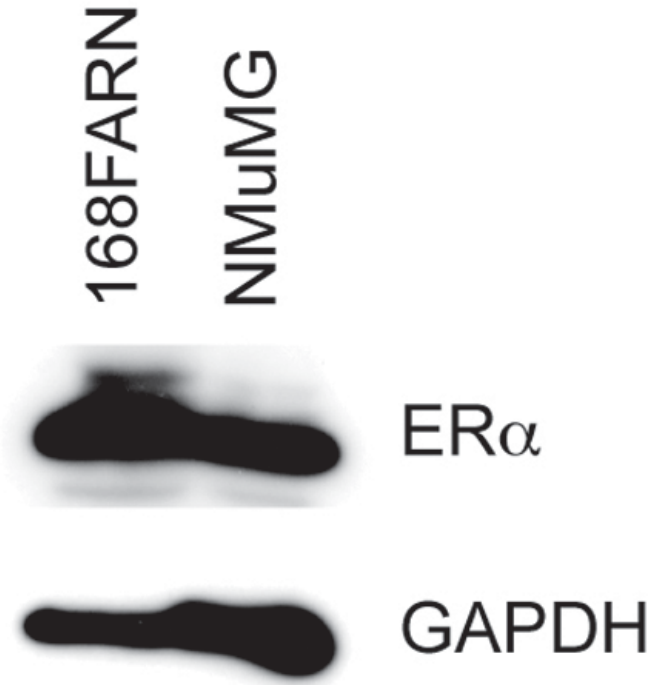


Suppl. Fig. 3


A
NT-Tumors
sh-Tumors
1 2 3
1 2 3 4 5
E-cadherin
N-cadherin
Cyc D3
Cyc D1
actin
MFGE8


B
Tumor size (mm3)
4500
3900
3300
2700
2100
1500
900
300
0
8
12
15
19
days after cell injection
NT1
NT2
NT3
sh5
sh4
sh3
sh2
sh1

Suppl. Fig. 4



Supplementary Table 1: Summary of the clinico-pahtological features of the samples contained in the exp

Dataset	**GSE20194**	**GSE3494**	**GSE6532**
N of Patients	230	251	327
Median Age (range)	51 (26 - 79)	63 (29 - 93)	60 (24 – 88)
Median Tumor Size (cm)	Unknown	2.0 (0.2 – 13)	2.1 (0.2 – 8.2)
ER status			
Positive	141	213	262
Negative	89	34	45
Unknown		4	20
PgR status			
Positive	104	191	121
Negative	126	61	11
Unknown			195
Lymph node status			
Positive	164	158	193
Negative	66	84	55
Unknown		9	79
Histological Grade			
I	13	67	68
II	94	128	143
III	123	54	64
Unknown		2	52
Survival			
OS at 10 yrs		75% (70 – 81%)	
N of events		55	
Median Follow-up (yrs)		10.6 (0.1 – 12.7)	

ression profile datasets

Suppl. Fig.1. Analysis of patients' survival in relation to MFG-E8 and ER expression. (A) Patients were arbitrarily divided in two groups depending on the expression levels of MFG-E8 and ErbB2 in their tumors. Patients within the rectangular area delimited by a dashed line in Fig. 1B (indicated as MFGE8 high) have high MFG-E8 expression (log intensity >9), and ErbB2 levels corresponding to log intensity <12. The overall survival of this group was tested with respect to survival of the remaining patients (MFGE8 low). The number of patients at risk at each time point is shown below the graph. The number of events represents deceased patients. MFGE8-high-patients show a tendency to shorter survival, however the difference in survival between the two groups is not statistically significant (p=0.057). (B) Survival of patients in relation to ER expression. The analysis was performed despite the low number of ER-negative patients: no significant difference in survival was observed between the two groups (p=0.8). (C) Survival of MFGE8 high patients in relation to ER expression. Survival of MFGE8-high-ER-positive (left) or -ER-negative (right) patients was tested with respect to survival of the remaining ER-positive or ER-negative patients respectively. MFGE8 high patients tend to have shorter survival in either group, but the differences are not statistically significant (p=0.3 and p=0.08). This analysis was performed on patients in the GSE3494 set which includes follow-up data.

Suppl. Fig. 2. RGD peptides prevent NMuMG spreading on recMFG-E8. NMuMG cells were plated for 3 hours in wells coated with mouse recMFG-E8, Collagen I (CLI), or poly-L-lysine (PL) without any peptides (first column on the left) or in the presence of RGD peptides or control RAD peptides. RGD peptides inhibit cell spreading on recMFG-E8 but not on CLI, while control RAD peptides have no

effect. Cells attach but do not spread on PL and this behavior is not affected by RGD/RAD peptides. Scale bar, 20μm.

Suppl. Fig. 3. MFG-E8 downregulation modulates cyclin D3 expression in experimental tumors. (A) Western blots of control- and sh-MFG-E8-tumor lysates. The expression of cyclin D3 correlates with that of MFG-E8 in sh-tumors, while cyclin D1 expression is not affected; N-cadherin levels appear to be slightly higher in sh-tumors re-expressing MFG-E8. (B) Growth curves of individual tumors as reported in Fig. 5E.

Suppl. Fig. 4. ERα is expressed in NMuMG and 168FARN cells. RIPA lysates of NMuMG and 168FARN cells were analyzed by SDS-PAGE and western blot for the expression of ERα. GAPDH expression is shown as a loading control.

Supplementary Materials and Methods

Survival analysis. Survival analysis was performed considering 236 patients from the GSE3494 set in which the follow-up data were provided. Two groups of patients were defined by arbitrary cut-off and compared for their survival: MFGE8-high patients characterized by log intensity MFGE8 >9 and log intensity ErbB2 <12, andMFGE8-low including all the remaining patients. To analyze patient survival in relation to ER expression, MFGE8-high patients were further subdivided depending on their ER status. Survival analyses were performed using Log-rank test.

Adhesion to recMFG-E8. Wells were coated o.n. at 4°C with 110 nM mouse recMFG-E8 (R&D Systems) or Collagen I (BD Biosciences); control wells were coated with 0.1 mg/ml poly-L-lysine (Sigma). Cells were plated in serum-free

medium in the presence of 100 μM RGD or control RAD peptides (Enzo Life Sciences) or without any peptides, and were incubated at 37°C for 3 h. Two replicates/condition were tested.

ER expression. Modified-RIPA lysates of NMuMG and 168FARN cells were analyzed by SDS-PAGE and western blot with anti-ERα antibody (Santa Cruz).