Holo-retinol-binding protein and its receptor STRA6 drive oncogenic transformation

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Supplemental Figures

Figure S1. a, b) Levels of STRA6 mRNA in samples of normal human breast and invasive breast carcinoma in two independent studies {Richardson, 2006 #2424}{Zhao, 2004 #2421}. Data were obtained from Oncomine™ (Compedia Bioscience, Ann Arbor, Michigan). *p<0.05. **c, d**) Levels of STRA6 mRNA in samples of normal colon and colon carcinoma in two independent studies ({Hong, 2010 #2451}{Kaiser, 2007 #2426} Oncomine™). *p<0.05. **e**) Levels of RBP mRNA in the 4T1 isogenic mouse cell line models of breast cancer, 67NR, 4T07, and 4T1, measured by Q-PCR. Mean±S.E.M. (n=3), #p<0.001 *vs.* 67NR cells. **f, g**) Levels of RBP mRNA in samples of normal colon and colon carcinoma in two independent studies ({Hong, 2010 #2451}{Kaiser, 2007 #2426} Oncomine™). *p<0.05.

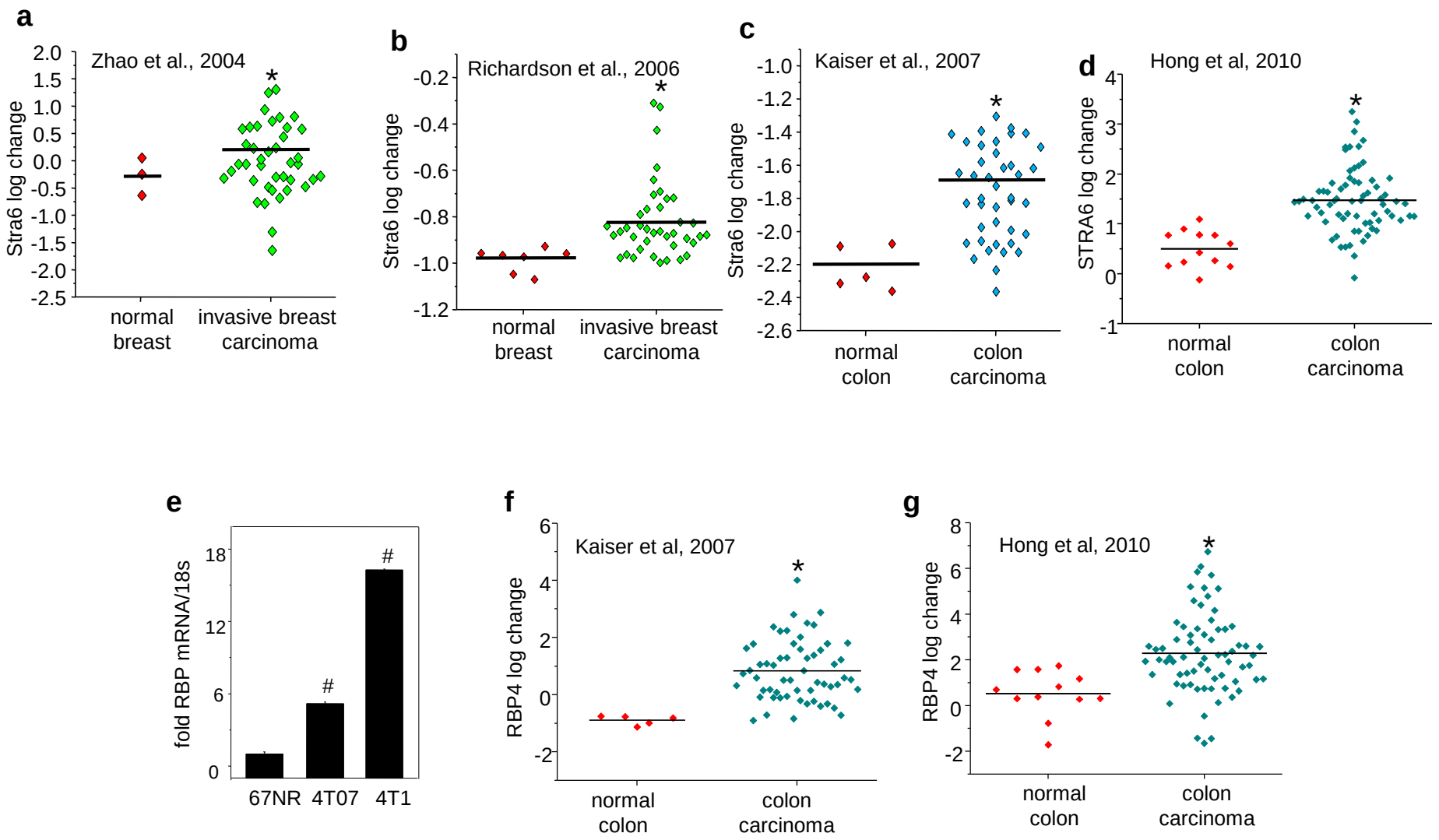
Figure S2. a) 24 h. BrdU incorporation in SW480 cells stably expressing GFPshRNA or two different STRA6shRNAs. Mean±S.E.M. (n=3). Inset: STRA6 mRNA in cells stably expressing lentiviral vectors encoding GFPshRNA, or the STRA6shRNA constructs TRCN0000128799 or TRCN0000129158 (Open Biosystems). **b**) Growth of MCF-7 cells expressing an empty vector (e.v.) or vector encoding STRA6shRNA. Cells were treated with vehicle or with 1 μM holo-RBP. Ligand was replenished every 24 h. Mean±S.E.M. (n=3), **p<0.03 *vs.* vehicle-treated cells, ##p<0.01 *vs.* e.v.-expressing, vehicle-treated cells. **c**) 24 h. BrdU incorporation in MCF-7 cells expressing e.v. or vector encoding STRA6shRNA treated with vehicle or 1 μM holo-RBP. Mean±S.E.M. (n=3), **p<0.03 *vs.* vehicle-treated cells. Inset: immunoblots demonstrating

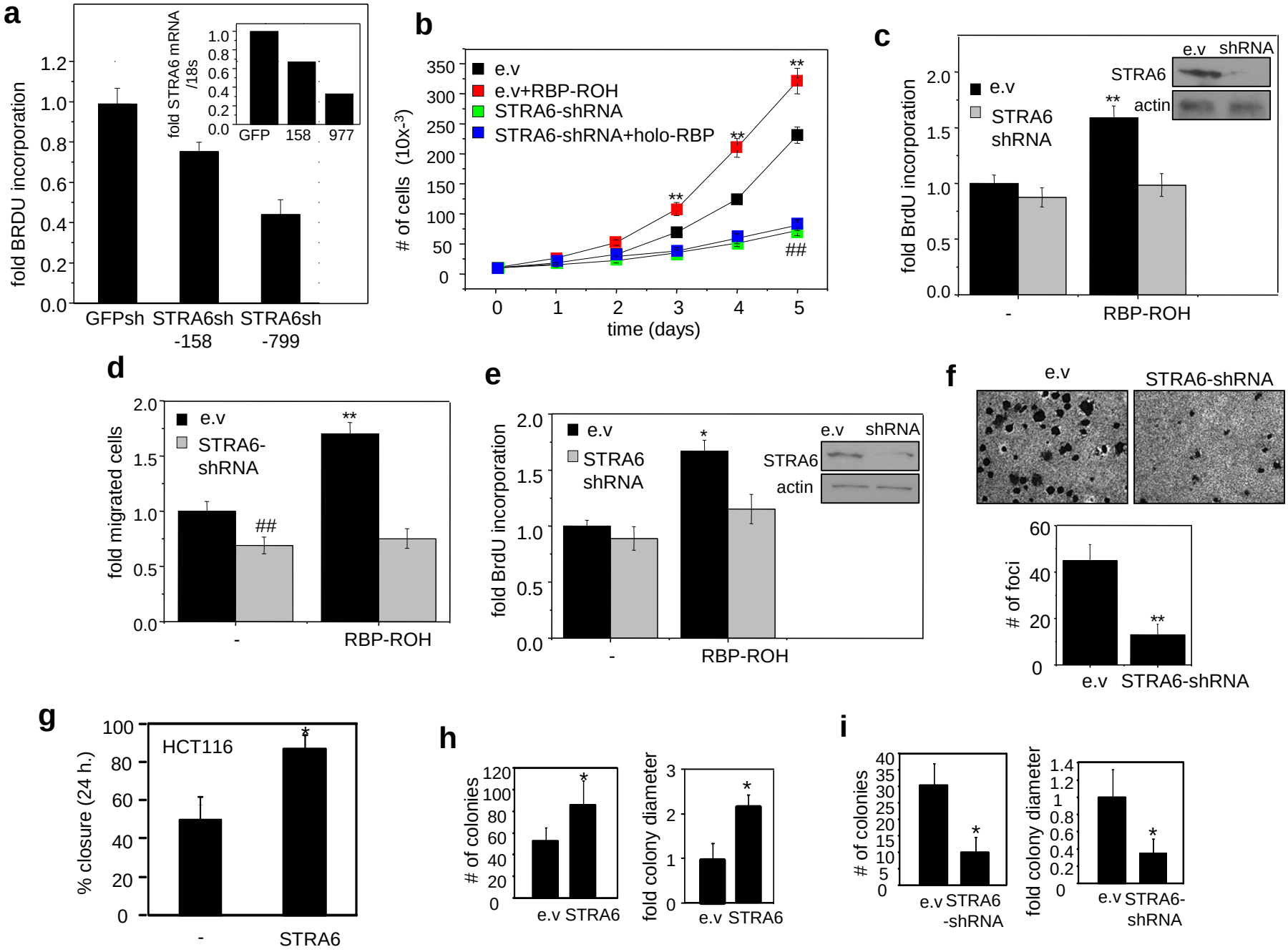
downregulation of STRA6 in MCF-7 cells. **d**) Migration assays using MCF-7 cells expressing e.v. or vector encoding STRA6shRNA treated with vehicle or 1 μM holo-RBP for 12 h. Mean±S.E.M. (n=3), **p<0.03 *vs.* e.v.-expressing, vehicle-treated cells. ##p<0.01 *vs.* e.v.-expressing, vehicle-treated cells. **e**) 24 h. BrdU incorporation in SW620 cells expressing e.v. or STRA6-shRNA and treated with 1 μM holo-RBP. Mean±S.E.M. (n=3), *p<0.01 *vs.* vehicle-treated cells. Inset: immunoblots demonstrating downregulation of STRA6 in SW620 cells. **f**) Top: secondary focus formation assays of SW620 stably expressing e.v. or STRA6-shRNA. Bottom: number of foci. Mean±S.E.M. (n=3). **p=0.0003 *vs.* e.v.-expressing cells. **g**) Quantitation of scratch assays in Fig. 3d. **h, i**) Quantitation of # of colonies (left panels) and colony sizes (right panels) in soft agar colony formation assays in Fig. 3e and 3j, respectively.

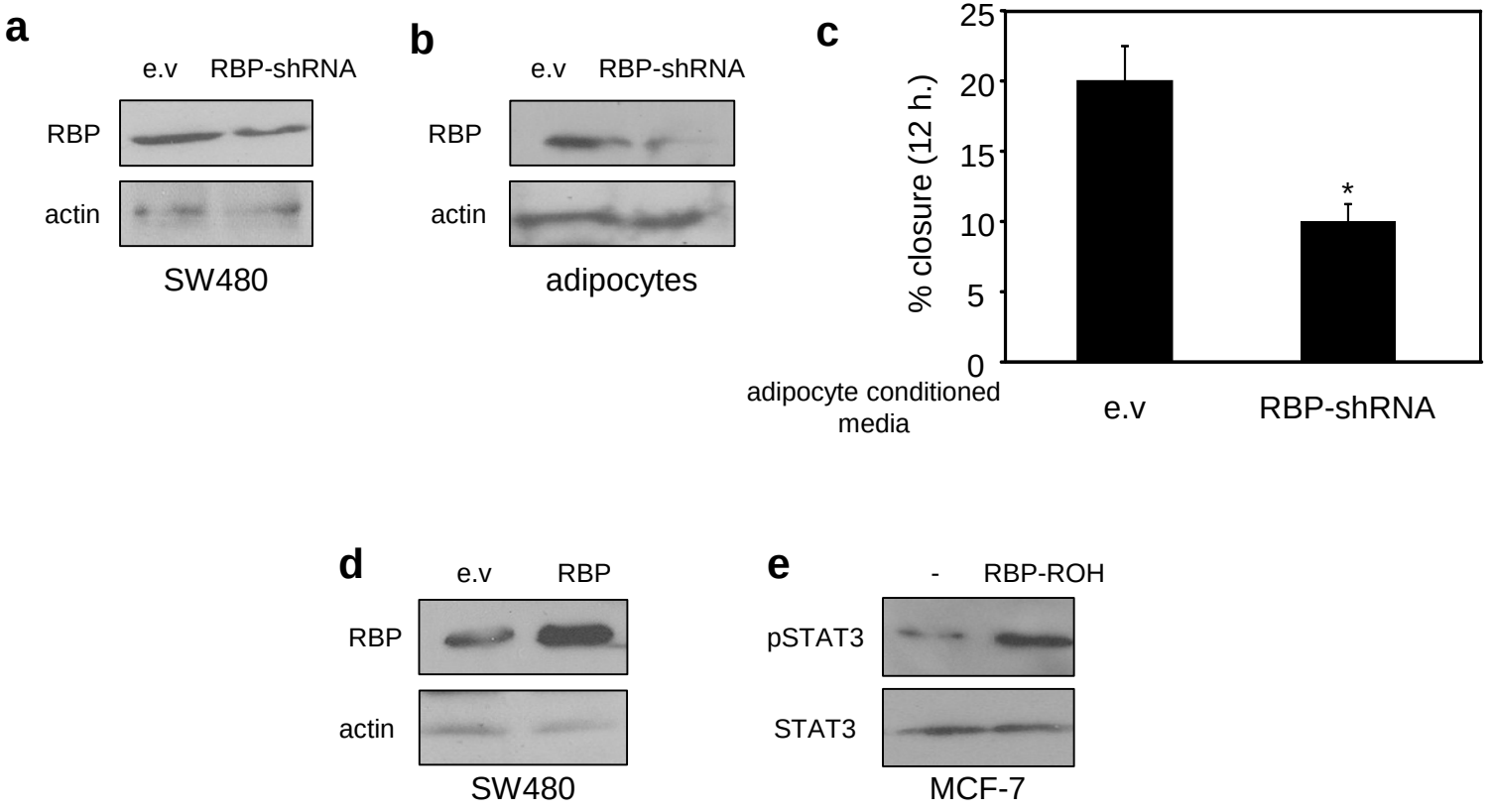
Figure S3. a, b) Immunoblots demonstrating downregulation of RBP in SW480 cells (**a**) and in NIH3T3-L1 adipoocytes expressing RBPshRNA (**b**). (**c**) Scratch assay using SW480 cells treated with conditioned media from NIH3T3-L1 adipocytes that ectopically express e.v. or RBP-shRNA. Mean±S.E.M. (n=3), *p<0.02 *vs* e.v.- expressing cells. **d)** Immunoblot demonstrating ectopic expression of RBP (in SW480 cells. **e**) Immunoblots of phosphorylated STAT3 (pSTAT3) in MCF-7 cells treated with vehicle or 1 μM holo-RBP for 15 min.

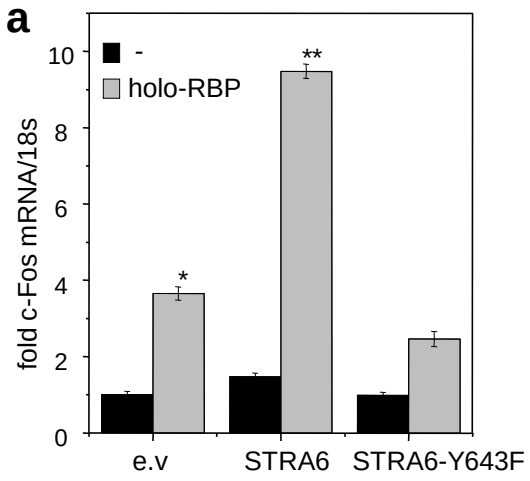
Figure S4. a) SW480 cells were transfected with an e.v. or with vectors encoding STRA6 or STRA6-Y643F. Cells were treated with 1 μM holo-RBP for 4 h. and c-Fos mRNA expression was assessed by Q-PCR. Mean±S.E.M. (n=3),*p<0.05 *vs.* vehicle-treated cells, **p<0.0001 *vs.* e.v.-expressing cells treated with holo-RBP. **b, c**) Levels of mRNA for denoted genes in SW480 cells expressing two different STRA6shRNAs (b) or RBPshRNAs (c). Mean±S.E.M.

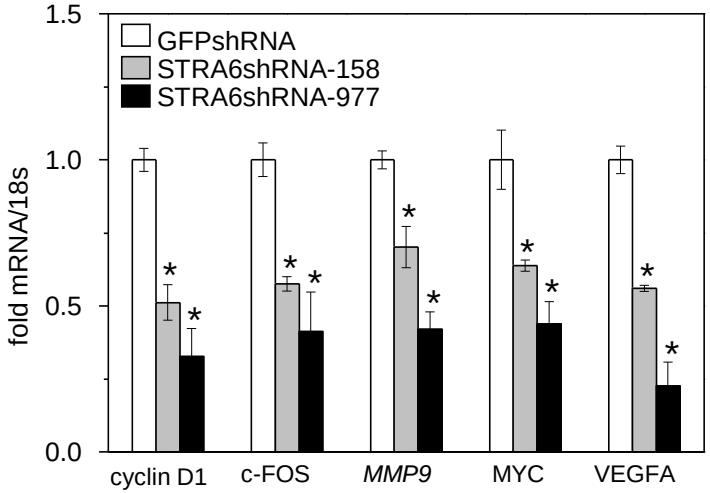
(n=3),*p<0.01 *vs.* GFPshRNA-expressing cells. **d**) SW480 cells were pre-treated with 20 µg/ml cycloheximide and then treated with 1 µM holo-RBP for 4 h. Levels of denoted mRNAs were measured by Q-PCR. Mean±S.E.M. (n=3),*p<0.05 *vs.* vehicle-treated cells. **e**) Immunoblots demonstrating reduced expression of STAT3 in HCT116 cells expressing STAT3-shRNA.

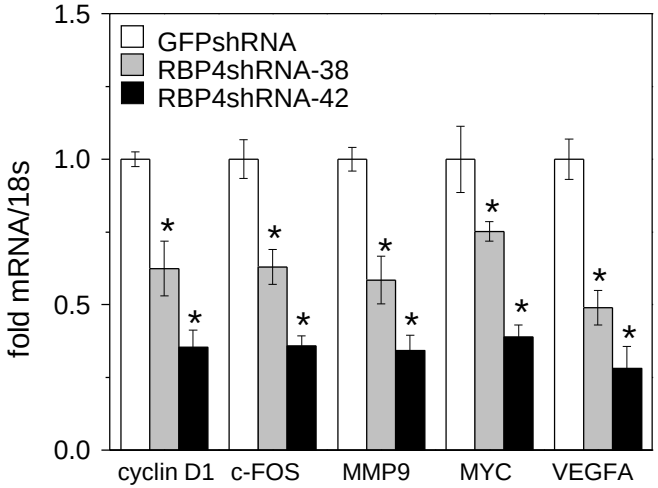

a
Zhao et al., 2004
Stra6 log change
normal breast
invasive breast carcinoma
b
Richardson et al., 2006
Stra6 log change
normal breast
invasive breast carcinoma
c
Kaiser et al., 2007
Stra6 log change
normal colon
colon carcinoma
d
Hong et al, 2010
STRA6 log change
normal colon
colon carcinoma
e
fold RBP mRNA/18s
67NR
4T07
4T1
f
Kaiser et al, 2007
RBP4 log change
normal colon
colon carcinoma
g
Hong et al, 2010
RBP4 log change
normal colon
colon carcinoma

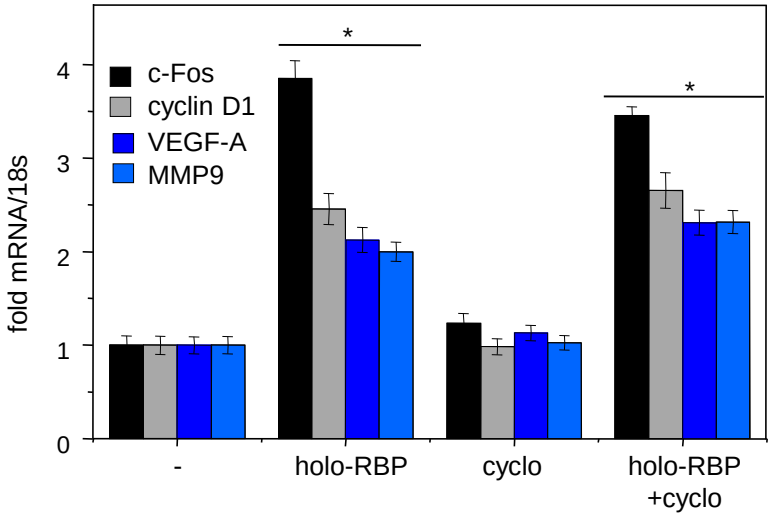

a
fold BRDU incorporation
GFPsh
STRA6sh -158
STRA6sh -799
fold STRA6 mRNA /18s
GFP
158
977
b
# of cells (10x-3)
time (days)
e.v
e.v+RBP-ROH
STRA6-shRNA
STRA6-shRNA+holo-RBP
c
fold BrdU incorporation
e.v
STRA6 shRNA
STRA6
actin
RBP-ROH
d
fold migrated cells
e.v
STRA6-shRNA
RBP-ROH
e
fold BrdU incorporation
e.v
STRA6 shRNA
STRA6
actin
RBP-ROH
f
e.v
STRA6-shRNA
# of foci
g
% closure (24 h.)
HCT116
STRA6
h
# of colonies
fold colony diameter
e.v STRA6
i
# of colonies
fold colony diameter
e.v STRA6 -shRNA
e.v STRA6-shRNA

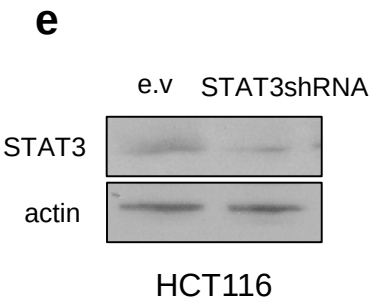

a
e.v
RBP-shRNA
RBP
actin
SW480
b
e.v
RBP-shRNA
RBP
actin
adipocytes
c
% closure (12 h.)
25
20
15
10
5
0
*
adipocyte conditioned media
e.v
RBP-shRNA
d
e.v
RBP
RBP
actin
SW480
e
-
RBP-ROH
pSTAT3
STAT3
MCF-7


a
fold c-Fos mRNA/18s
10
8
6
4
2
0
-
holo-RBP
**
*
e.v
STRA6
STRA6-Y643F


b
fold mRNA/18s
1.5
1.0
0.5
0.0
GFPshRNA
STRA6shRNA-158
STRA6shRNA-977
cyclin D1
c-FOS
MMP9
MYC
VEGFA


c
fold mRNA/18s
1.5
1.0
0.5
0.0
GFPshRNA
RBP4shRNA-38
RBP4shRNA-42
cyclin D1
c-FOS
MMP9
MYC
VEGFA


d
fold mRNA/18s
4
3
2
1
0
c-Fos
cyclin D1
VEGF-A
MMP9
*
*
-
holo-RBP
cyclo
holo-RBP
+cyclo


e
e.v
STAT3shRNA
STAT3
actin
HCT116