Supplementary Figure Legends

Figure S1. (a) Western blot of purified SRAP 1-236, purified SRAP 13-236 and whole cell lysates from HEK293 and HeLa cells. Probed with anti-SRAP antibody Abcam ab72552. (b) Western blot of purified SRAP 1-236, purified SRAP 13-236 and MCF-7 whole cell lysate probed with anti-SRAP antibody Abcam ab72407. (c) Histogram of RNA-seq reads around the two candidate SRAP start codons M1 and M13. Each black bar represents one nucleotide. The height of each bar is proportional to the number of reads mapped to that specific nucleotide. The positions of the two candidate start codons are marked in green.

Figure S2. Anomalous difference Fourier map. Map was computed with anomalous differences measured at 1.5 Å wavelength to 4.0 Å resolution and model phases. Cartoon drawing of B subunit of the SRAP carboxy-terminal domain, with Met and Cys side chains drawn as stick models. Sulfurs are orange. Anomalous map (magenta) contoured at 3 sigma. Molecular drawings were made with PyMOL (http://www.pymol.org).

Figure S3. EMSA of SRA RNA fragments by SRAP(V106M-215). Arrows show positions of unshifted RNAs. Experimental conditions same as in Figure 2 of main text.

Figure S4. Individual siRNAs in pool 1 have different effects on MCF-7 cell growth. In each case, approximately 200K MCF-7 cells were transfected with 25 nM corresponding siRNA. 3 days after transfection, cell number was examined by flow cytometry (n=2). * denotes the siRNA that has the most deleterious effect on cell growth.

Figure S5. Response of pS2 and PR expression to different levels of 17β-estradiol. Top graph, endogenous SRA RNA levels; middle and lower graphs, pS2 and PR mRNA levels, respectively. Lane 1: MCF-7 cells were grown in complete medium. Lane 2-11: MCF-7 cells were grown in hormone-free medium for 4 days and then allowed to continue for one day in hormone-free medium (Lane2), complete medium (Lane 3), hormone-free medium with 17β-estradiol and fulvestrant (Lane 4-7) or hormone-free medium with just 17β-estradiol (Lane 8-11). The RNA

levels of SRA, pS2 and PR were examined by RT-qPCR, using GAPDH mRNA as internal control (SRA primer: SRA-4).

Figure S6. Examination of the expression level of pS2 and PR at different time points after SRA knockdown. MCF-7 cells were transfected with 25 nM corresponding siRNA pool. Cells were harvested 1, 2 and 3 days after transfection. RNA level of pSR (left) and PR (right) was examined by RT-qPCR, with GAPDH mRNA as internal control (n=3).

Supplementary Tables

Table S1. Sequences of siRNAs in SRA siRNA pool 1 and their corresponding scrambled control siRNAs.

SRA siRNA	Forward (5' to 3')	Reverse (5' to 3')
Pool 1 #1 SRA siRNA	ucacuuggcucccuucuuatt	uaagaagggagccaagugatt
Pool 1 #1 control siRNA	cuuaccuuuuggucaccuctt	gaggugaccaaaagguaagtt
Pool 1 #2 SRA siRNA	agagggaguucauguguuatt	uaacacaugaacucccucutt
Pool 1 #2 control siRNA	uucguuaggagauguagagtt	cucuacaucuccuaacgaatt
Pool 1 #3 SRA siRNA	ggaaaguugucaauaccugtt	cagguauugacaacuuuccttt
Pool 1 #3 control siRNA	ccugaauaggaagucaguutt	aacugacuuccuauucaggtt
Pool 1 #4 SRA siRNA	gguaggaguuaaaagauuatt	uaaucuuuuaacuccuaccttt
Pool 1 #4 control siRNA	ggagaaaggguaauuauuatt	uaauaauuacccuuucucctt

Table S2. Sequences of RT-qPCR primers.

Gene	Forward (5' to 3')	Reverse (5' to 3')
GAPDH*	acagcaacagggtggtggac	gaccattgctggggctggtg
SRA-1 (Exon 2)	ccgccgcagttctcatac	cttggtaagcagcgagcg
SRA-2 (Exon 2,3 juction)	aggcgctcgctgcttac	tctctgatgcggggactc
SRA-3 (Exon 3,4 juction)	gtggccacacaaggaagc	tcctccagcccactgttc
SRA-4 (Exon 4)	gacatcagccgacgcc	cttgcaccagtagagccattc
SRA-5 (Exon 4,5 juction)	ctctactggtgcaagagctttc	ccatgagggagcggtg
SRA-6 (Exon 4,5 juction)	aggaacagtgggctggag	gtggcttgaaagctcttgc
SRA-7 (Exon 5)	acatccaccgctccctc	cattggctgcctcctctg
pS2	catcgacgtccctccagaagag	ctctgggactaatcaccgtgctg
PR	cgcgctctaccctgcactc	tgaatccggcctcaggtagtt
GREB1*	gcttgccgctctagaaggt	gtgcagctggaaagggttt
MSRB2	cgcctttcagtgggatctac	ccagtgccagagcagtacttt
RACGAP1	tctgtttgagcagcttgtgc	tcctctgccacttttttacgg
C3orf58	cttcgcaacctcaaggactc	aaatggccaaccttcatcag
NMD3	caaccaccaggaacttggat	aaagcctgcatctacaagcc
RMRP-1	ccaaagtccgccaagaag	gcactgcctgcgtaactagag
RMRP-2	gcatacgcacgtagacattcc	gagggagctgacggatgac

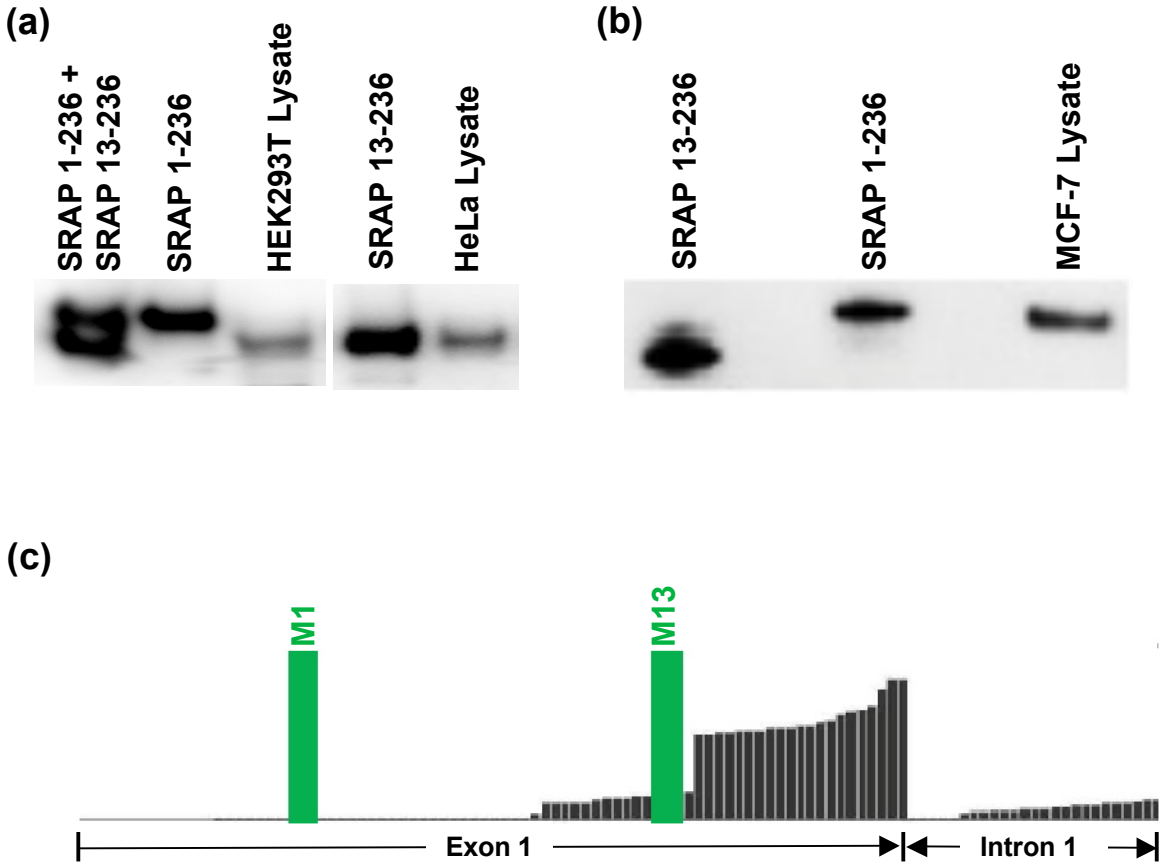
*The GAPDH primer listed was described in [1]. We also used Human GAPD (GAPDH) Endogenous Control (Applied Biosystems, 4326317E) for GAPDH RT-qPCR. The GREB1 primer listed was described in [2].

Table S3. Sequences of SRA cDNA cloning primers.

Gene	Forward (5’ to 3’)	Reverse (5’ to 3’)
Primer 1	ggcaatgcaagaggaggctgtagaagtc	gtggtaagaagggagccaagtgacagaag
Primer 2	gcggaagtggagatggcggag	gcataggagatggtgtccggtgagtc
Primer 3	agctgtacgtgaagccgggcaac	gcataggagatggtgtccggtgagtc

Supplementary References

1. Fleury, L., Gerus, M., Lavigne, A. C., Richard-Foy, H. & Bystricky, K. Eliminating epigenetic barriers induces transient hormone-regulated gene expression in estrogen receptor negative breast cancer cells. Oncogene 2008;27:4075-4085.
2. Foulds, C. E., Tsimelzon, A., Long, W., Le, A., Tsai, S. Y., Tsai, M. J. & O'Malley, B. W. Research resource: expression profiling reveals unexpected targets and functions of the human steroid receptor RNA activator (SRA) gene. Mol Endocrinol 2010;24:1090-1105.


(a)
SRAP 1-236 + SRAP 13-236
SRAP 1-236
HEK293T Lysate
SRAP 13-236
HeLa Lysate
(b)
SRAP 13-236
SRAP 1-236
MCF-7 Lysate
(c)
M1
M13
Exon 1
Intron 1

Figure_S1

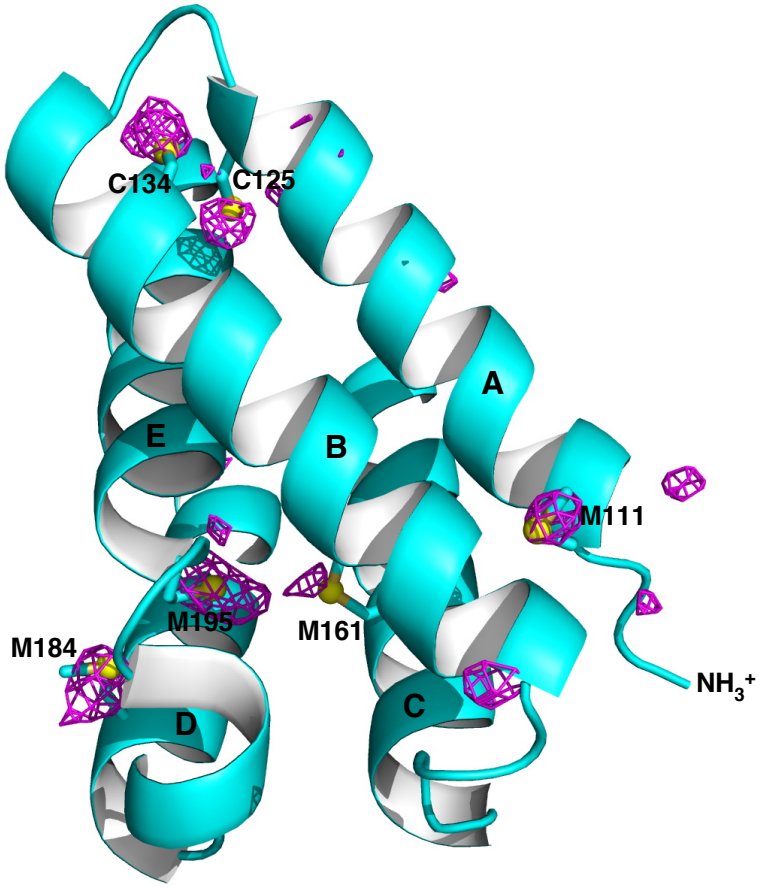
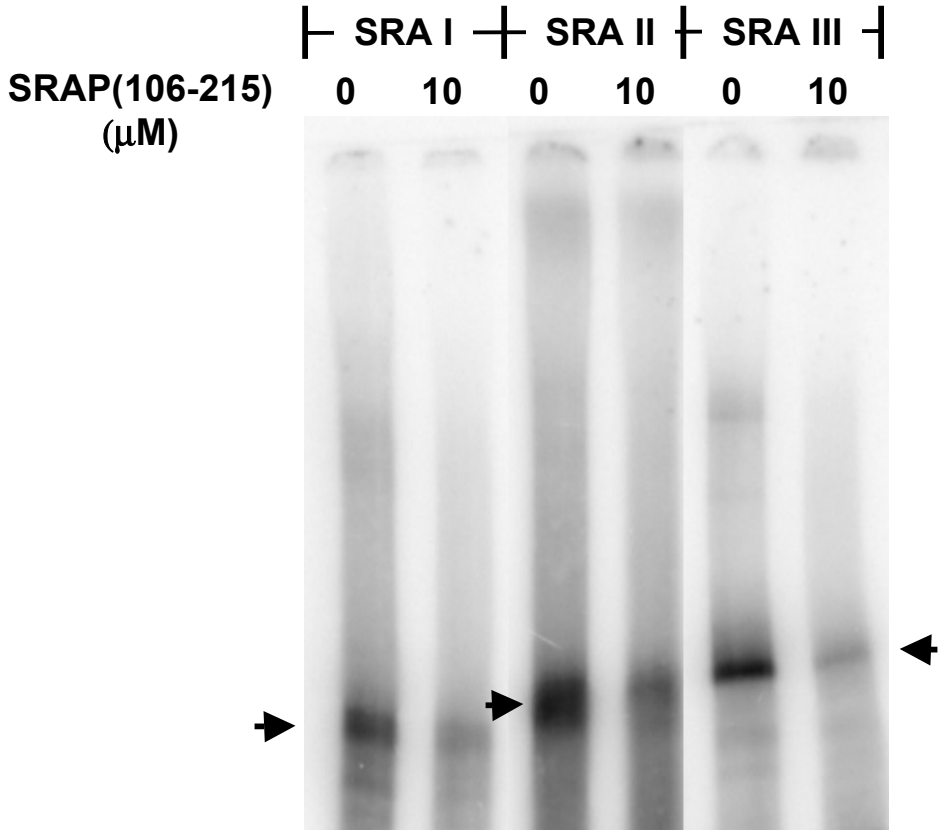


Figure S2



Fig_S3: SRA frag binding by SRAP C-terminal domain

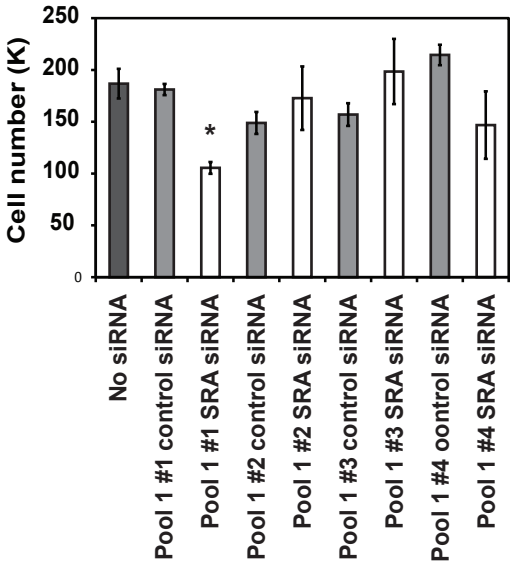

Cell number (K)
250
200
150
100
50
0
*
No siRNA
Pool 1 #1 control siRNA
Pool 1 #1 SRA siRNA
Pool 1 #2 control siRNA
Pool 1 #2 SRA siRNA
Pool 1 #3 control siRNA
Pool 1 #3 SRA siRNA
Pool 1 #4 oontrol siRNA
Pool 1 #4 SRA siRNA

Figure S4

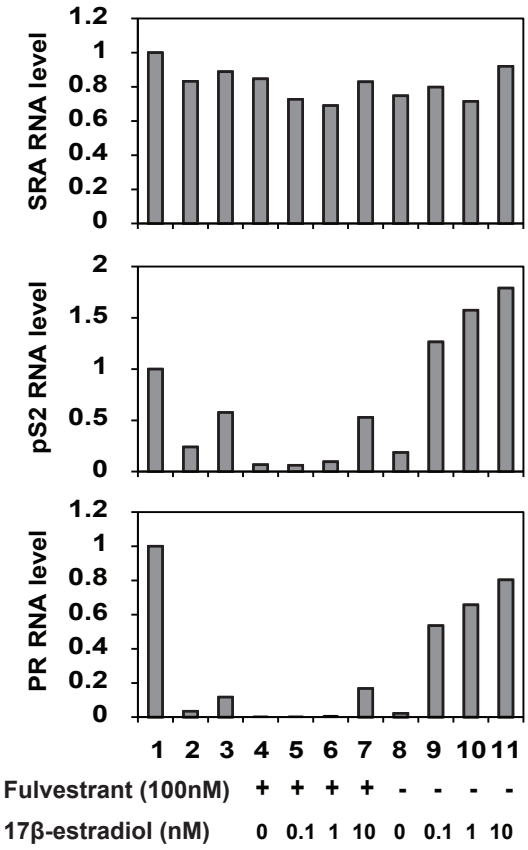

SRA RNA level
1.2
1
0.8
0.6
0.4
0.2
0
pS2 RNA level
2
1.5
1
0.5
0
PR RNA level
1.2
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11
Fulvestrant (100nM) + + + + - - - -
17β-estradiol (nM) 0 0.1 1 10 0 0.1 1 10

Figure S5

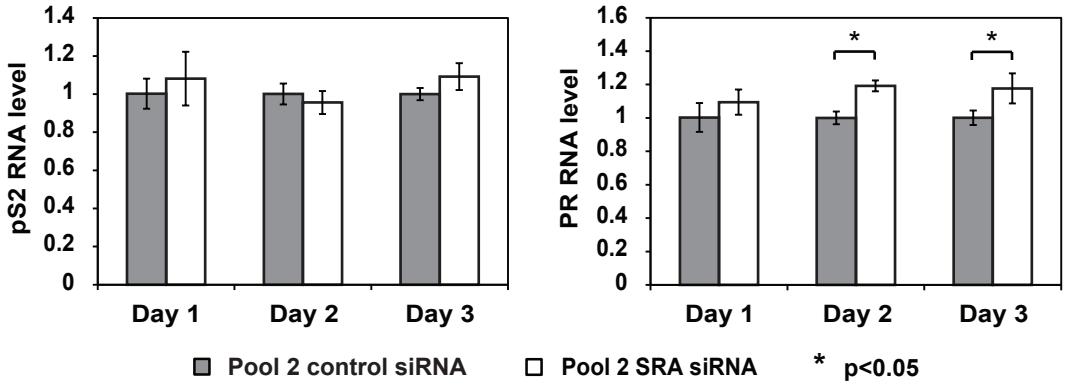

pS2 RNA level
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Day 1
Day 2
Day 3
PR RNA level
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
*
Day 1
Day 2
Day 3
Pool 2 control siRNA
Pool 2 SRA siRNA
* p<0.05

Figure S6