Supplement material

DIRECT RECRUITMENT OF ERK CASCADE COMPONENTS TO INDUCIBLE GENES IS REGULATED BY THE HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN K (HNRNP K)

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Running title: hnRNP K and ERK cascade at inducible genes

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Fig.S1

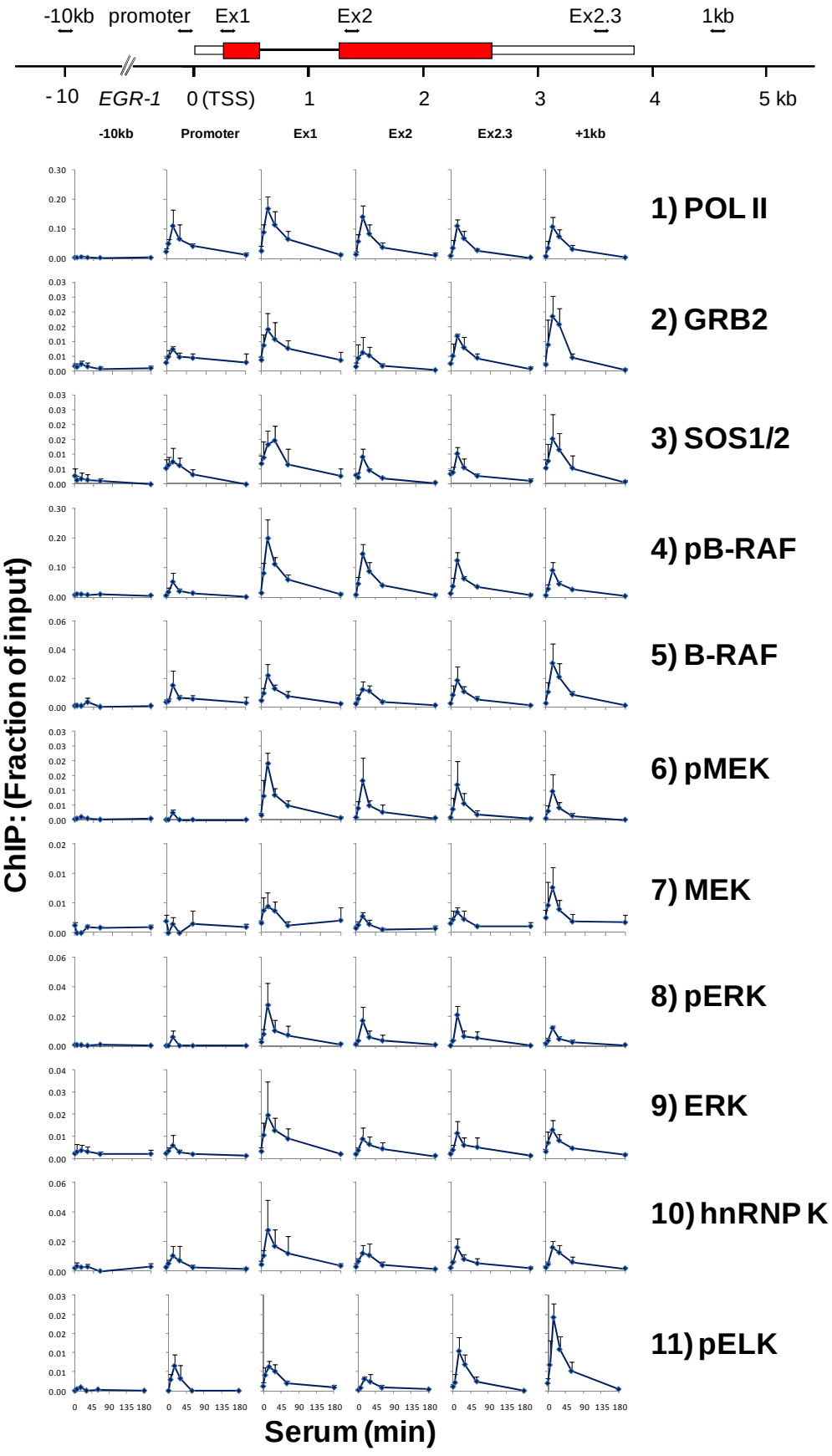

-10kb
promoter
Ex1
Ex2
Ex2.3
1kb
- 10
EGR-1
0 (TSS)
1
2
3
4
5 kb
-10kb
Promoter
Ex1
Ex2
Ex2.3
+1kb
ChIP: (Fraction of input)
1) POL II
2) GRB2
3) SOS1/2
4) pB-RAF
5) B-RAF
6) pMEK
7) MEK
8) pERK
9) ERK
10) hnRNP K
11) pELK
0 45 90 135 180
Serum (min)

Fig.S2

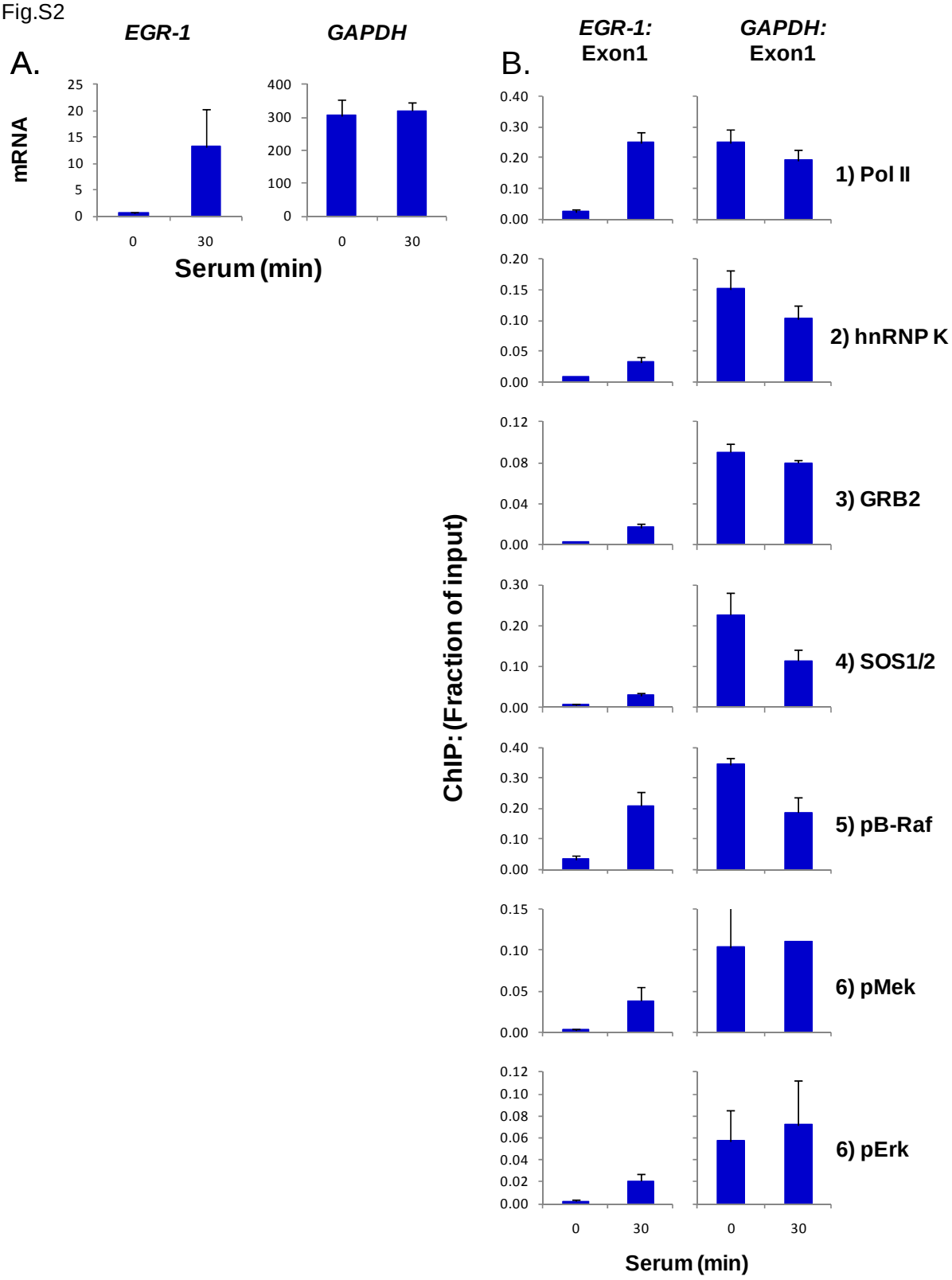

A.
EGR-1
GAPDH
mRNA
25
20
15
10
5
0
400
300
200
100
0
0
30
0
30
Serum (min)
B.
EGR-1: Exon1
GAPDH: Exon1
ChIP: (Fraction of input)
1) Pol II
2) hnRNP K
3) GRB2
4) SOS1/2
5) pB-Raf
6) pMek
6) pErk
0.40
0.30
0.20
0.10
0.00
0.15
0.05
0.12
0.08
0.04
0.06
0.02
0
30
Serum (min)

Fig.S3

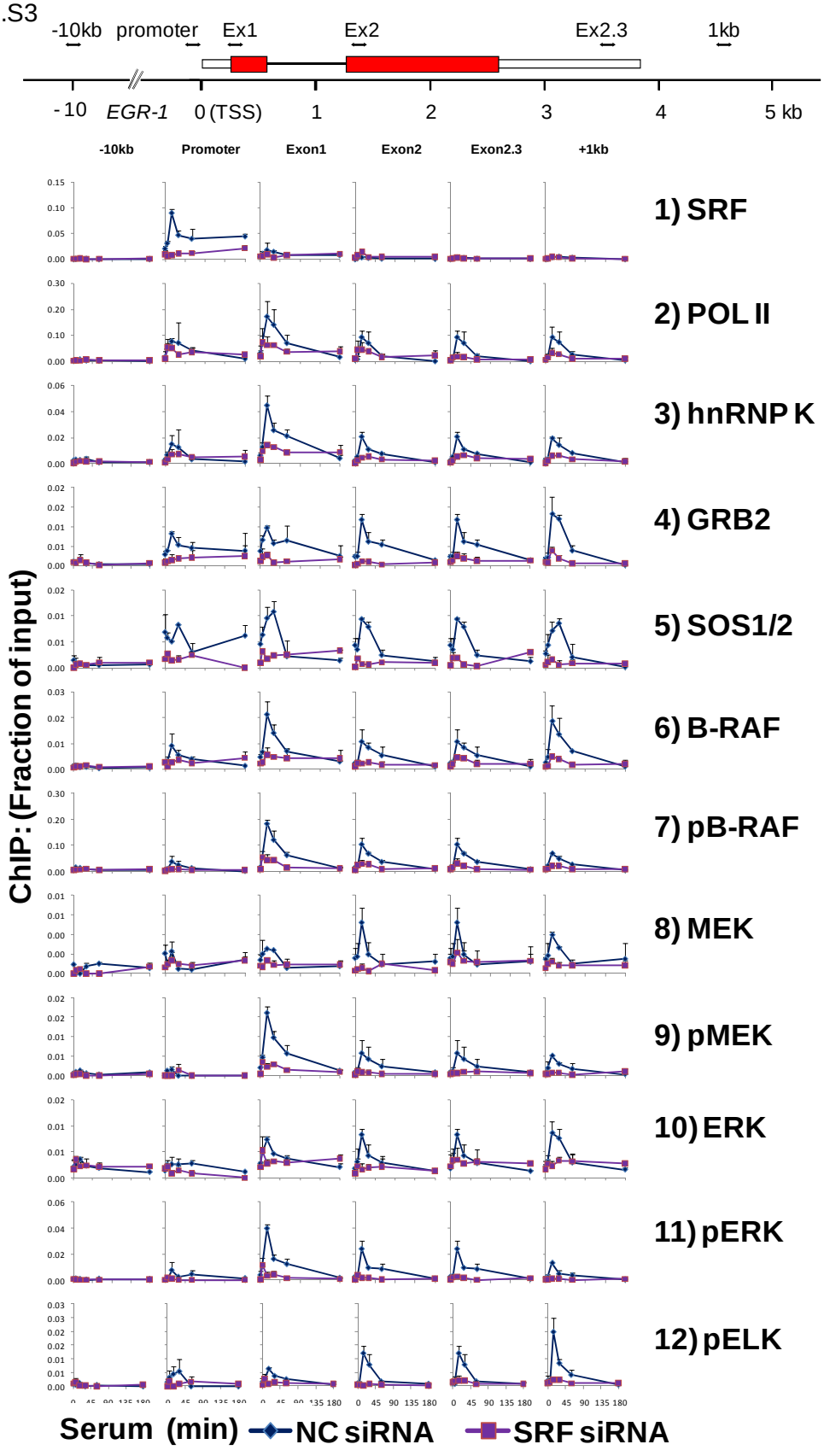

-10kb
promoter
Ex1
Ex2
Ex2.3
1kb
-10
EGR-1
0 (TSS)
1
2
3
4
5 kb
-10kb
Promoter
Exon1
Exon2
Exon2.3
+1kb
ChIP: (Fraction of input)
1) SRF
2) POL II
3) hnRNP K
4) GRB2
5) SOS1/2
6) B-RAF
7) pB-RAF
8) MEK
9) pMEK
10) ERK
11) pERK
12) pELK
0 45 90 135 180
Serum (min)
NC siRNA
SRF siRNA

Fig.S4

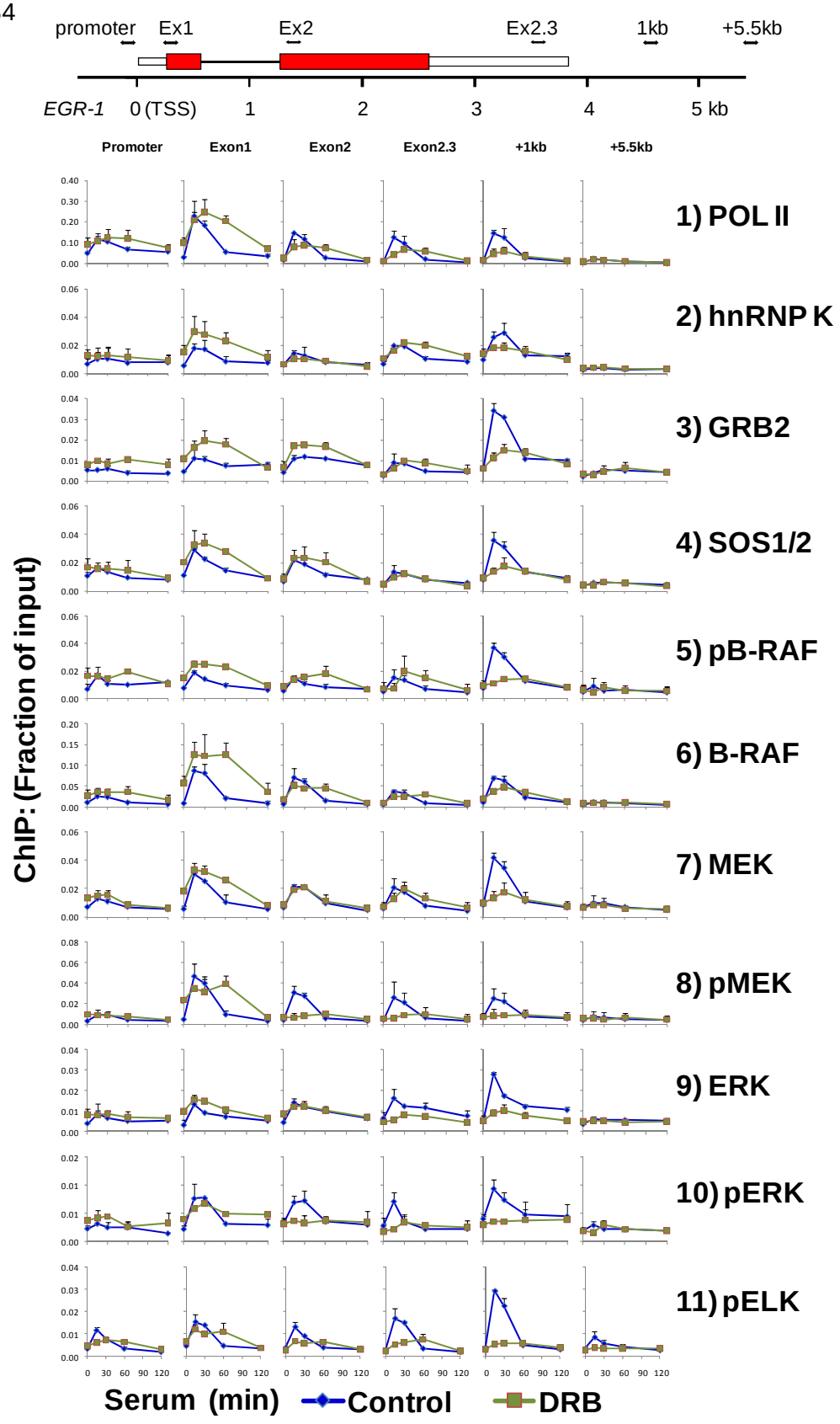
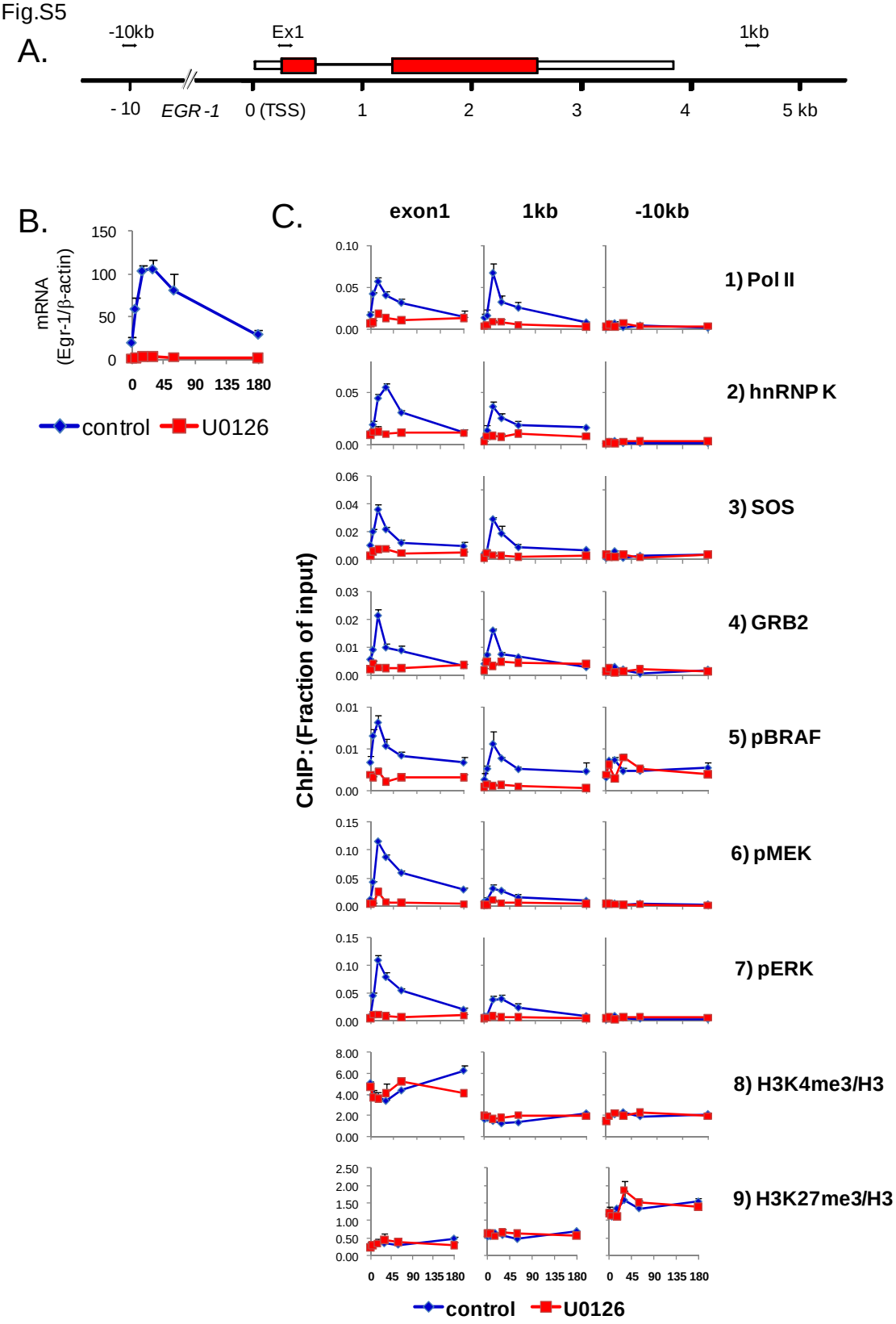

promoter
Ex1
Ex2
Ex2.3
1kb
+5.5kb
EGR-1
0 (TSS)
1
2
3
4
5 kb
Promoter
Exon1
Exon2
Exon2.3
+1kb
+5.5kb
ChIP: (Fraction of input)
1) POL II
2) hnRNP K
3) GRB2
4) SOS1/2
5) pB-RAF
6) B-RAF
7) MEK
8) pMEK
9) ERK
10) pERK
11) pELK
0 30 60 90 120
Serum (min)
Control
DRB

Fig.S5


A.
-10kb
Ex1
1kb
- 10
EGR-1
0 (TSS)
1
2
3
4
5 kb
B.
mRNA (Egr-1/β-actin)
150
100
50
0
0 45 90 135 180
control
U0126
C.
exon1
1kb
-10kb
ChIP: (Fraction of input)
1) Pol II
2) hnRNP K
3) SOS
4) GRB2
5) pBRAF
6) pMEK
7) pERK
8) H3K4me3/H3
9) H3K27me3/H3
0 45 90 135 180
control
U0126

Supplement figure legends

Fig.S1. Graph grid of time-course binding profile of ERK cascade components, hnRNP K and Pol II along the serum-induced *EGR-1* gene. Matrix ChIP analysis of sheared chromatin from a time-course of serum-treated (10%FBS for 0, 5, 15, 30, 60, 180 min) HCT116 WT human colon carcinoma cells was done using in-lab coated Protein A polypropylene 96-well plates. The antibodies used are listed in Table.1. Real-time PCR was done using primers to the regions shown in the diagram of the *EGR-1* gene (the two exons are shown as boxes). The primers are listed in Table. 2. The ChIP results are expressed as fraction of input DNA. The graphs represent mean±SEM from six experiments.

Fig.S2. High constitutive levels of Pol II, hnRNP and ERK cascade components at the highly expressed house-keeping GAPDH gene. Quantitative RT-PCR analysis of mRNA (A) and Matrix ChIP (B) was done in untreated and serum-treated (30min) HCT116 WT human colon carcinoma cells. The primers are listed in Table 2. The graphs represent mean±SEM from three experiments.

Fig.S3. siRNA SRF knockdown inhibits serum-induced co-recruitment of Pol II, hnRNP K and ERK signaling cassette along the *EGR-1* locus. Cells were transfected using Lipofectamine RNAiMAX with either SRF siRNA or non-complementary (NC) siRNA. 24hrs after transfection cells were switched to low serum medium and 24hrs after quiescence cells were treated with 10%FBS for 0, 5, 15, 30, 60 and 180 min. Matrix ChIP data are presented as a fraction of input DNA, means±SEM, n=3 experiments.

Fig.S4. DRB-inhibited Pol II elongation decreases serum-induced levels of hnRNP K and ERK signaling cassette along *EGR-1* regions distal to TSS. HCT116 WT cells grown to 40%–60% confluence were made quiescent overnight by lowering FBS concentration in the medium from 10% to 0.5 %. After 24hrs cells were pretreated with DRB (50μM dissolved in DMSO) in 0.5 % FBS media for 6h. DMSO alone was used as control. Cells were switched to 10%FBS media for 0, 15, 30, 60 and 120min, at which times they were cross-linked, chromatin was sheared. Matrix ChIP data are presented as fraction of input DNA, means±SEM, n=3 experiments.

Fig.S5. 1,4-Diamino-2,3-dicyano-1,4-bis(methylthio)butadiene (U0126) [1,2] blocks serum-induced EGR-1 mRNA expression and recruitment of Pol II, hnRNP K and ERK cascade components to the *egr-1* gene. HCT116 WT cells were grown in 6-well plates to 40%–60% confluence then made quiescent overnight by lowering FBS concentration in the medium to 1%. After 24hrs cells were pretreated with 10nM U0126 (Sigma; 10μM stock suspended in DMSO) in 1% FBS media for 1h. A 0.1% DMSO alone was used as a control. Cells were switched to 10% FBS media containing either DMSO or inhibitor for 0, 5, 15, 30, 60 and 180min, at which times they were immediately overlaid with Trizol (for RNA isolation) (***A***) or cross-linked with 1% formaldehyde followed by 125mM Glycine incubation for chromatin isolation (***B***). ***A.*** Total RNA was extracted from cells as described previously [3]. 1 μg of RNA was used in reverse transcription (RT) reaction carried out using random hexamers. Levels of EGR-1 mRNA were assessed by real- time RT-PCR, normalized for β-Actin transcript and expressed as fold change of a U0126 0' time point sample. Data represent mean+SEM, n=3 experiments. ***B.*** Matrix ChIP data are presented as fraction of input DNA or as ratios of modified histone marks to total H3 ChIP signals, means+SEM, n=2 experiments.

References:

1. Gupta P, Prywes R (2002) ATF1 Phosphorylation by the ERK MAPK Pathway Is Required for Epidermal Growth Factor-induced c-jun Expression. J Biol Chem 277: 50550-50556.
2. Kamakura S, Moriguchi T, Nishida E (1999) Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases. Identification and characterization of a signaling pathway to the nucleus. J Biol Chem 274: 26563-26571.
3. Ostrowski J, Kawata Y, Schullery DS, Denisenko ON, Bomsztyk K (2003) Transient recruitment of the hnRNP K protein to inducibly transcribed gene loci. Nucleic Acids Res 31: 3954-3962.