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Supplemental Information

ETS Proteins Bind with Glucocorticoid Receptors:

Relevance for Treatment of Ewing Sarcoma

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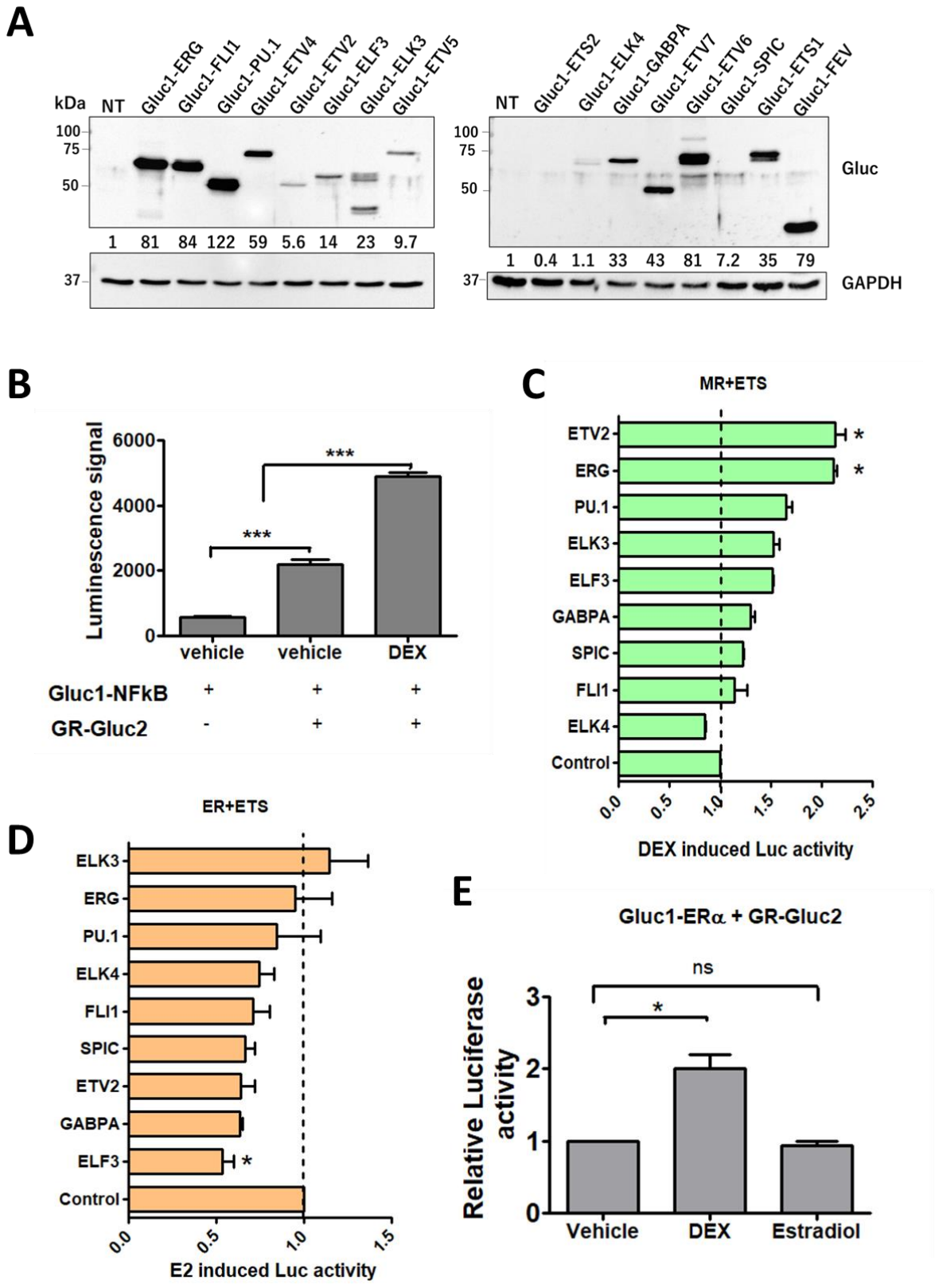
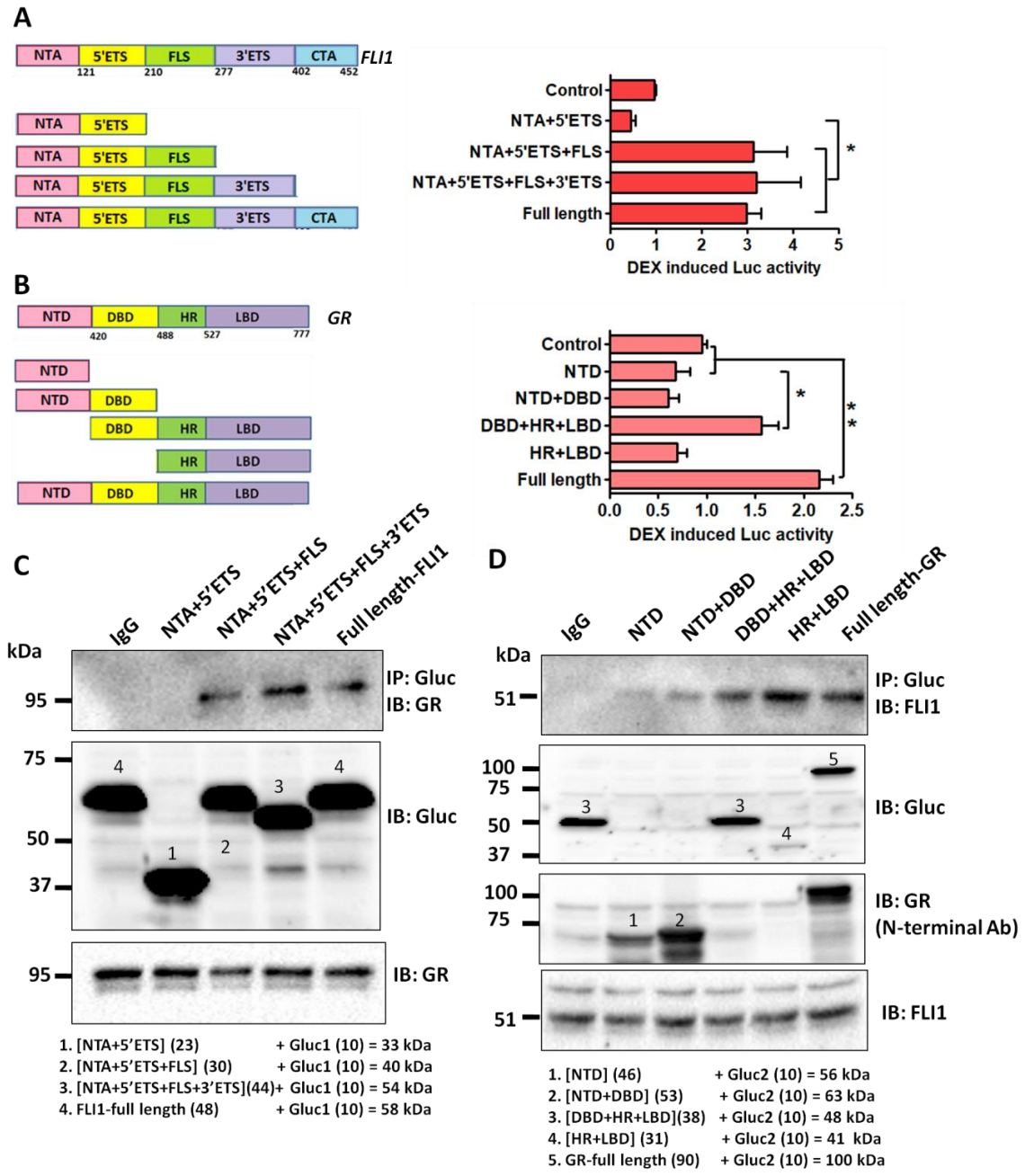


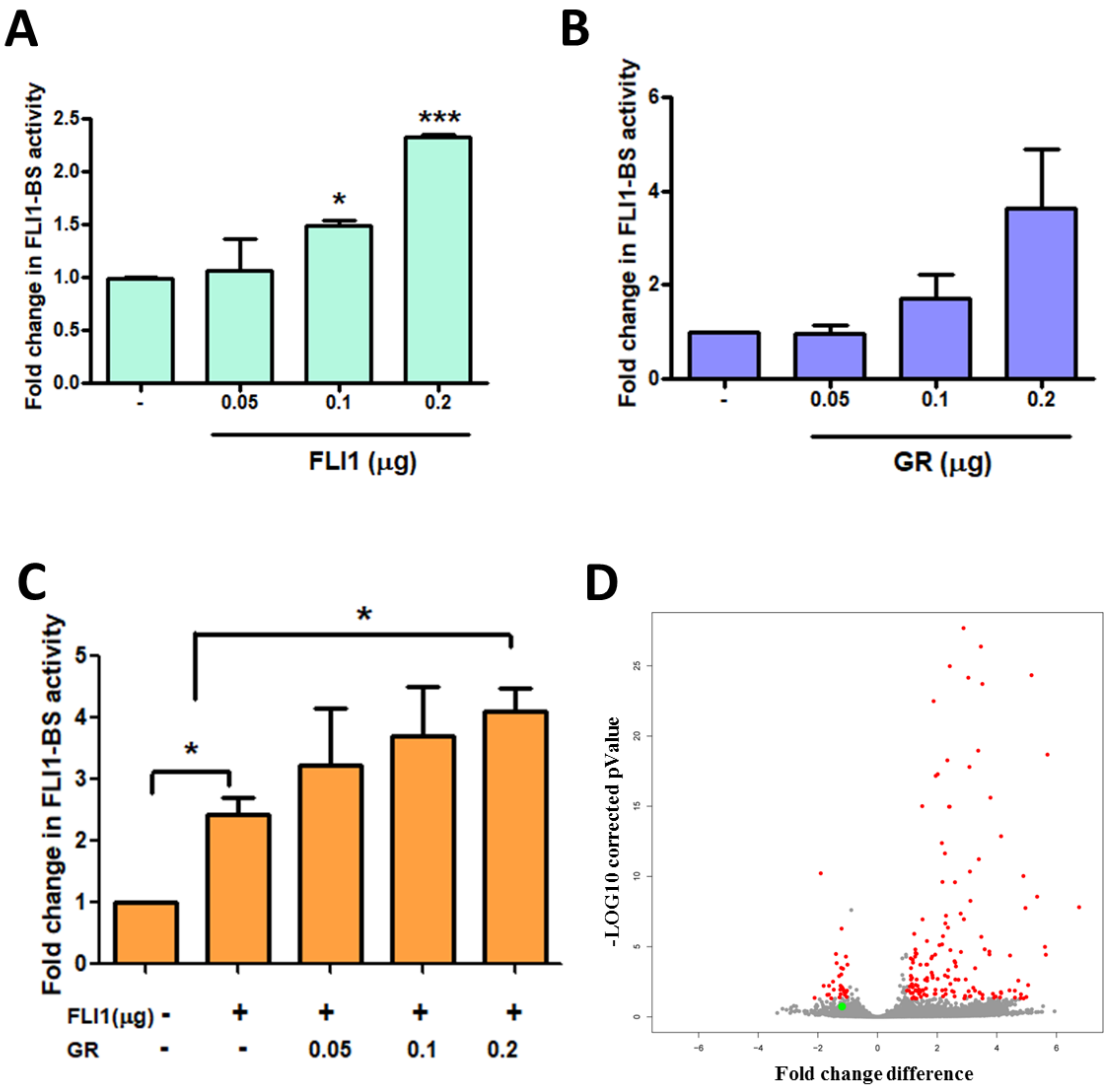
Supplementary Figure S1 (related to Figure 1): Specificity of interactions between steroid

hormone receptors and ETS family members. (**A**) HEK293T cells (10^6) were transfected with a Gluc1 plasmid encoding the indicated ETS fusion protein. Twenty-four hours later, cell extracts were subjected to immunoblotting with the indicated antibodies. Protein expression levels were quantified and normalized to GAPDH (numbers indicated under each lane). *NT*, un-transfected cells. (**B**) HEK293T cells ($6X10^3$) were seeded in 96-well plates. On the next day, cells were transfected with combinations of the Gluc1 plasmid encoding a fused, full length NF-κB and the Gluc2 plasmid encoding a fused GR protein. After 24 hours, cells were starved overnight for serum factors, and thereafter they were treated for 60 minutes with vehicle or with DEX (1 μM). The cells were later extracted and luminescence was determined in biological triplicates. The bar plot shows luciferase activity in arbitrary units. ***, $p<0.001$. (**C** and **D**) HEK293T cells ($6X10^3$) were seeded in 96-well plates. On the next day, cells were transfected with combinations of the Gluc1 plasmid encoding an ETS protein and a Gluc2 plasmid encoding either MR (C) or ERα (D). Twenty-four hours later, cells were starved overnight for serum factors and thereafter they were treated for 60 minutes with vehicle, DEX (1 μM) or estradiol (E2; 10 nM). Cells were then lysed and luminescence was determined. The bar plot shows the fold changes in luciferase activity in response to DEX or E2 (as compared to vehicle-treated cells) for each set of interactions between an ETS family TF and a steroid hormone receptor. Luminescence of treated cells was normalized to vehicle-treated cells. *, $p<0.05$. (**E**) HEK293T cells were co-transfected with Gluc1-ERα and GR-Gluc2. Twenty-four hours later, cells were treated for 60 minutes with either vehicle, DEX (1 μM) or estradiol (10 nM). Luminescence of extracted cells was determined in biological duplicates and normalized to cells treated with vehicle only. *, $p\leq0.05$; ns, not significant.



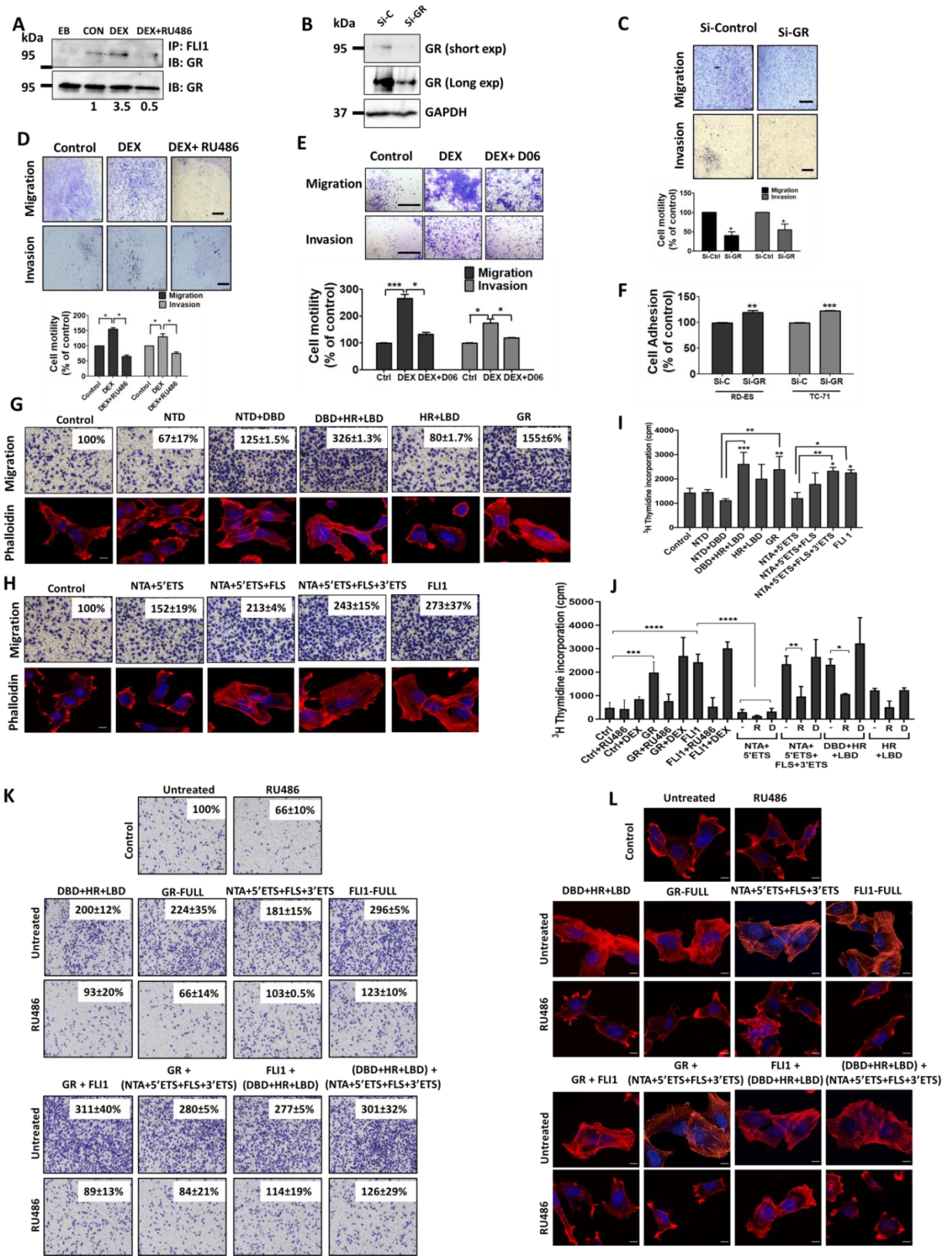
Supplementary Figure S2: **Mapping the mutually interacting domains of FLI1 and GR (related to Figures 1 and 2)**. (**A** and **B**) Schematic diagrams showing the various domains of FLI1 (A) and GR (B). Different domains of FLI1 were inserted C-terminally to GLuc1. Likewise, individual domains of GR were inserted N-terminally to Gluc2. HEK293T cells ($6X10^3$) were co-transfected with the Gluc1 plasmid encoding different domains of FLI1 and the Gluc2 plasmid encoding full length GR. Alternatively, we used the Gluc2 plasmid encoding

different domains of GR, and the Gluc1 plasmid encoding full length FLI1. After 24 hours, cells were starved overnight and then treated for 60 minutes with vehicle or with DEX (1 μM). Luminescence was determined in biological triplicates. The bar plots show the normalized fold changes in luciferase activity induced by DEX. *, $p \leq 0.05$; **, $p \leq 0.01$. (**C** and **D**) HEK293T cells were transfected with Gluc1 plasmids encoding different FLI1 domains (C), or Gluc2 plasmids encoding GR domains (D). Gluc proteins were immunoprecipitated (IP) using a specific antibody. Immunoblotting (IB) was performed using antibodies that detected the endogenous forms of GR or FLI1. Blots are representative of three or more biological replicates. Two different antibodies to GR were used. The lowermost panels present immunoblots of whole cell extracts (no prior IP) blotted for the respective endogenous protein, either GR (C) or FLI1 (D). The input of recombinant proteins is shown in the middle panels. Individual fusion proteins are identified by numbers and their molecular weights are shown below each panel. *IgG*, control immunoglobulin G used for IP. Note that the cells in the IgG panel were transfected with one of the positive interacting constructs, either full length FLI1 (C) or DBD+HR+LBD domain of GR (D).



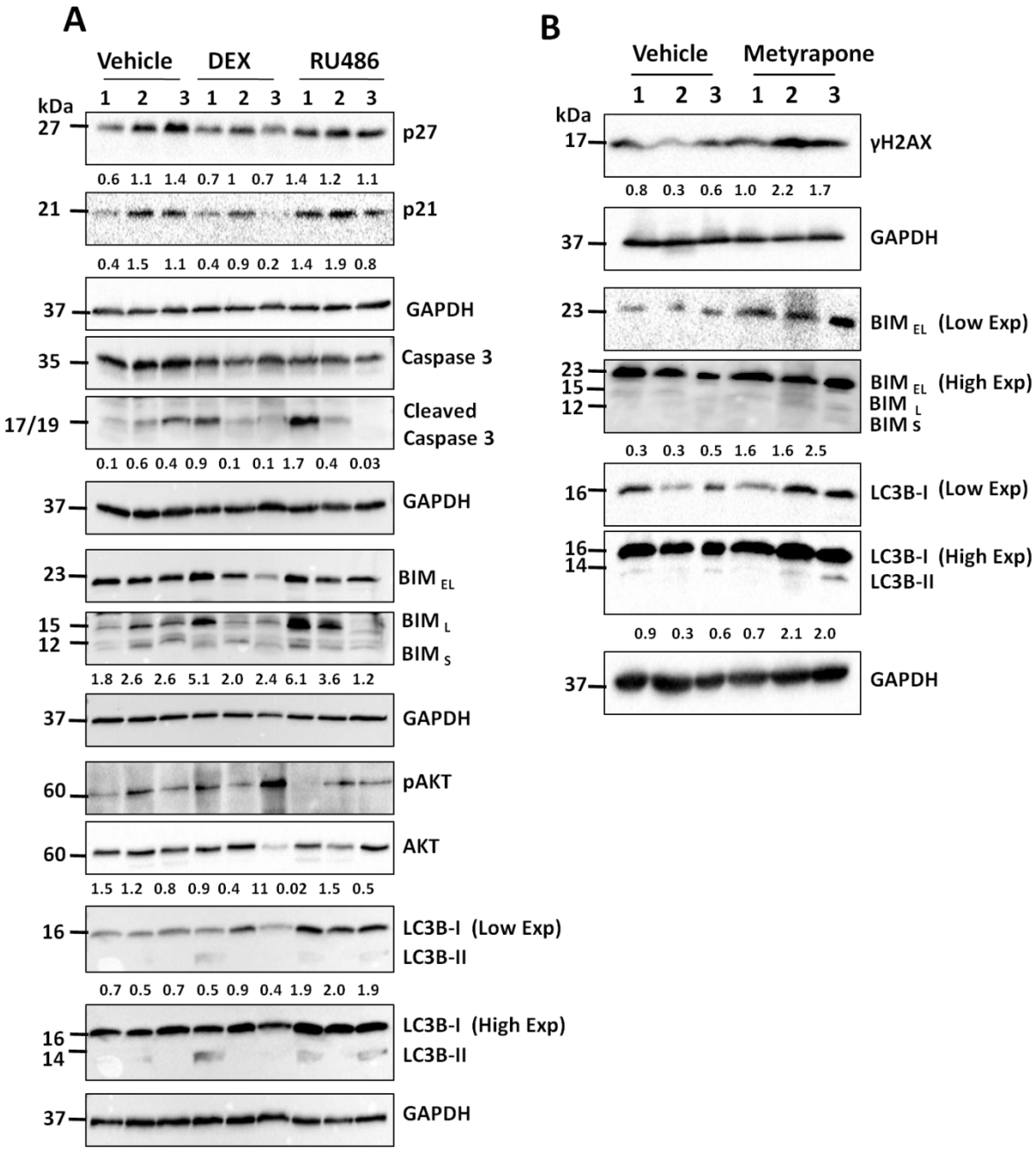
Supplementary Figure S3 (related to Figures 2 and 3): Transcription regulation by GR and FLI1. (**A-C**) HEK293T cells ($1.2X10^4$) were seeded in 48-well plates. On the next day, cells were transfected with the FLI1-BS-luciferase plasmid (2.5 µg), along with increasing amounts of either a FLI1 expression vector (A), a GR-encoding vector (B), or a combination of GR and FLI1 plasmids (C). Luciferase activity was determined in biological triplicates 48 hours later and presented in bar plots. Basal activity was determined in cells transfected only with the FLI1-BS reporter. *, $p \leq 0.05$; ***, $p < 0.001$. (**D**) A scatter plot (Volcano) of differentially expressed genes in FLI1-depleted Ewing sarcoma cells. A673 cells grown in 90-mm dishes were

transfected in triplicates with siRNA oligonucleotides specific to FLI1, or with control oligonucleotides. Forty-eight hours later, cells were harvested and processed for RNA isolation, which was followed by sequencing. Fold-change differences were plotted against statistical significance. Note the location of the FLI1 gene (in green; see Supplementary Table S3).

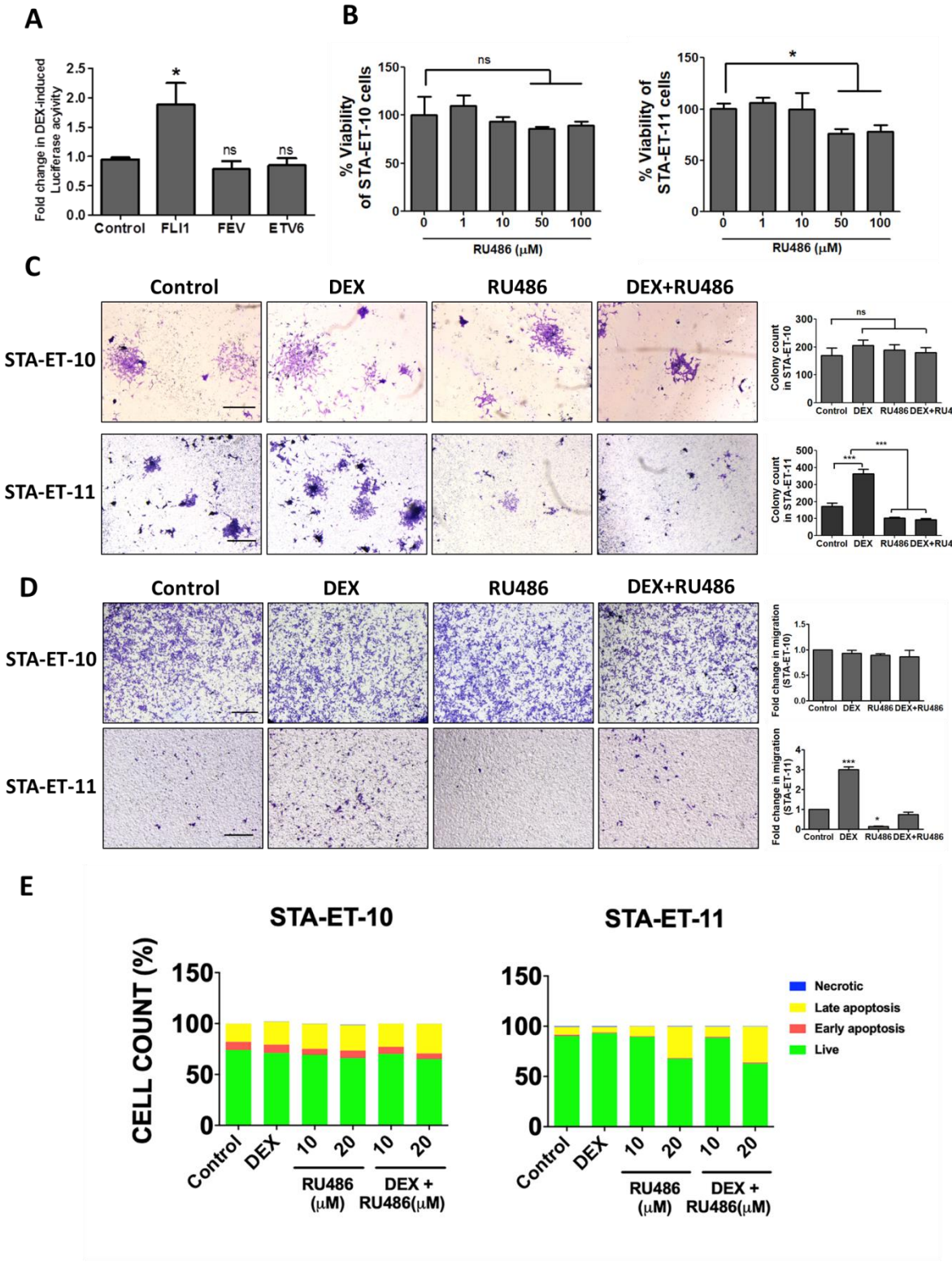

A
kDa
EB CON DEX DEX+RU486
95
IP: FLI1
IB: GR
95
IB: GR
1 3.5 0.5
B
kDa
Si-C Si-GR
95
GR (short exp)
GR (Long exp)
37
GAPDH
C
Si-Control
Si-GR
Migration
Invasion
Cell motility (% of control)
Migration
Invasion
Si-Ctrl Si-GR Si-Ctrl Si-GR
D
Control
DEX
DEX+ RU486
Migration
Invasion
Migration
Invasion
Cell motility (% of control)
Control DEX DEX+RU486 Control DEX DEX+RU486
E
Control
DEX
DEX+ D06
Migration
Invasion
Migration
Invasion
Cell motility (% of control)
Ctrl DEX DEX+D06 Ctrl DEX DEX+D06
F
Cell Adhesion (% of control)
Si-C Si-GR Si-C Si-GR
RD-ES
TC-71
G
Control
NTD
NTD+DBD
DBD+HR+LBD
HR+LBD
GR
Migration
Phalloidin
100%
67±17%
125±1.5%
326±1.3%
80±1.7%
155±6%
I
^{3}H Thymidine incorporation (cpm)
Control NTD NTD+DBD DBD+HR+LBD HR+LBD GR NTA+5'ETS NTA+5'ETS+FLS NTA+5'ETS+FLS+3'ETS FLI 1
H
Control
NTA+5'ETS
NTA+5'ETS+FLS
NTA+5'ETS+FLS+3'ETS
FLI1
Migration
Phalloidin
100%
152±19%
213±4%
243±15%
273±37%
J
^{3}H Thymidine incorporation (cpm)
Ctrl Ctrl+RU486 Ctrl+DEX GR GR+RU486 GR+DEX FLI1 FLI1+RU486 FLI1+DEX
- R D - R D - R D - R D
NTA+ 5'ETS
NTA+ 5'ETS+ FLS+3'ETS
DBD+HR +LBD
HR +LBD
K
Untreated
RU486
Control
100%
66±10%
DBD+HR+LBD
GR-FULL
NTA+5'ETS+FLS+3'ETS
FLI1-FULL
Untreated
RU486
200±12%
224±35%
181±15%
296±5%
93±20%
66±14%
103±0.5%
123±10%
GR + FLI1
GR + (NTA+5'ETS+FLS+3'ETS)
FLI1 + (DBD+HR+LBD)
(DBD+HR+LBD) + (NTA+5'ETS+FLS+3'ETS)
Untreated
RU486
311±40%
280±5%
277±5%
301±32%
89±13%
84±21%
114±19%
126±29%
L
Untreated
RU486
Control
DBD+HR+LBD
GR-FULL
NTA+5'ETS+FLS+3'ETS
FLI1-FULL
Untreated
RU486
GR + FLI1
GR + (NTA+5'ETS+FLS+3'ETS)
FLI1 + (DBD+HR+LBD)
(DBD+HR+LBD) + (NTA+5'ETS+FLS+3'ETS)
Untreated
RU486

Supplementary Figure S4 (related to Figure 4): GR of Ewing sarcoma cells physically associates with EWS-FLI1 and enhances cellular migration, DNA synthesis and actin filament reorganization. (**A**) Once RD-ES cells reached 70% confluence, they were starved overnight for serum factors. Thereafter, cells were treated in duplicates for 60 minutes with vehicle, DEX (1 μM) or the combination of DEX and RU486 (each at 1 μM). The cells were then extracted and lysates were processed for co-immunoprecipitation assays using an antibody against EWS-FLI1 and immunoblotting using an antibody specific to GR. The results shown are representative of two biological replicates. (**B**) RD-ES cells were seeded in 100-mm dishes. Once they reached 70% confluence, cells were transfected with siRNA oligonucleotides, either control siRNAs or oligonucleotides specific to GR. Cell extracts were prepared 24 hours later and probed for GR and GAPDH. Shown are short and long film exposures. (**C**) Control siRNAs or siRNAs specific to GR were added to RD-ES cells 24 hours prior to seeding in Transwell migration chambers or Matrigel invasion chambers. Cell migration/invasion was quantified 20 hours later. Representative microscope fields are shown along with the normalized signals. (**D**) RD-ES cells were seeded on the upper faces of migration/invasion chambers and incubated for 20 hours in full medium. DEX (1 μM) or the combination of DEX and RU486 (each at 1 μM) were added to the medium and 20 hours later we fixed and stained cells that migrated to the lower face of the intervening filters. Shown are representative images of the stained cells. The bar plots show quantification (using ImageJ) of areas covered by cells. *, p <0.05. Bars, 500 μm. (**E**) Migration and invasion assays of CHLA9 cells were performed as in D except for the use of a non-steroidal GR antagonist (DO6; 10 μM) instead of RU486. *, p <0.05; ***, p <0.001. Bars, 100 μM. (**F**) 48-well plates were coated with Cultrex® RGF BME prior to seeding RD-ES and TC-71 cells, which were pre-transfected with si-GR or si-C. Unattached cells were removed 8 hours later and adherent cells were fixed with paraformaldehyde (4%), stained with crystal violet (0.1%) and the optical density (550 nm) was quantified in triplicates. **, p < 0.01; ***, p <0.001. (**G** and **H**) A673 cells were transfected (or mock transfected, *Control*) with plasmids encoding the full-length forms of GR and FLI1, or the indicated deletion mutants (see schemes in Figs. S2). Twenty-four hours later, cells were subjected to migration assays (in duplicates) using Transwell chambers. Cells that migrated across the intervening filters of the chambers were counted in 5 non-overlapping microscope fields and the results are presented. Alternatively, transfected cells were fixed and stained with phalloidin and DAPI, to visualize actin fibers (red)

and nuclei (blue), respectively. (**I** and **J**) For thymidine incorporation assays, the medium of transfected cells was replaced with fresh serum-free medium containing ^{3}[H]-thymidine (1 μCi), along with vehicle, dexamethasone (*D*; 1 μM) or RU486 (*R*; 10 μM). Forty-eight hours later, cells were extracted and radioactivity incorporated into DNA was determined (in quadruplicates) using a scintillation counter. Results are presented as means ± S.D. *, $p < 0.05$; **, $p < 0.05$; ***, $p < 0.001$; ****, $p < 0.0001$. (**K** and **L**) Cells were transfected or co-transfected with the indicated plasmids and later incubated with RU486 (1 μM) or with vehicle, and their migration (K) and actin cytoskeleton (L) assayed as in G. Scale bars: 500 μm (migration assays) or 20 μm (phalloidin staining).

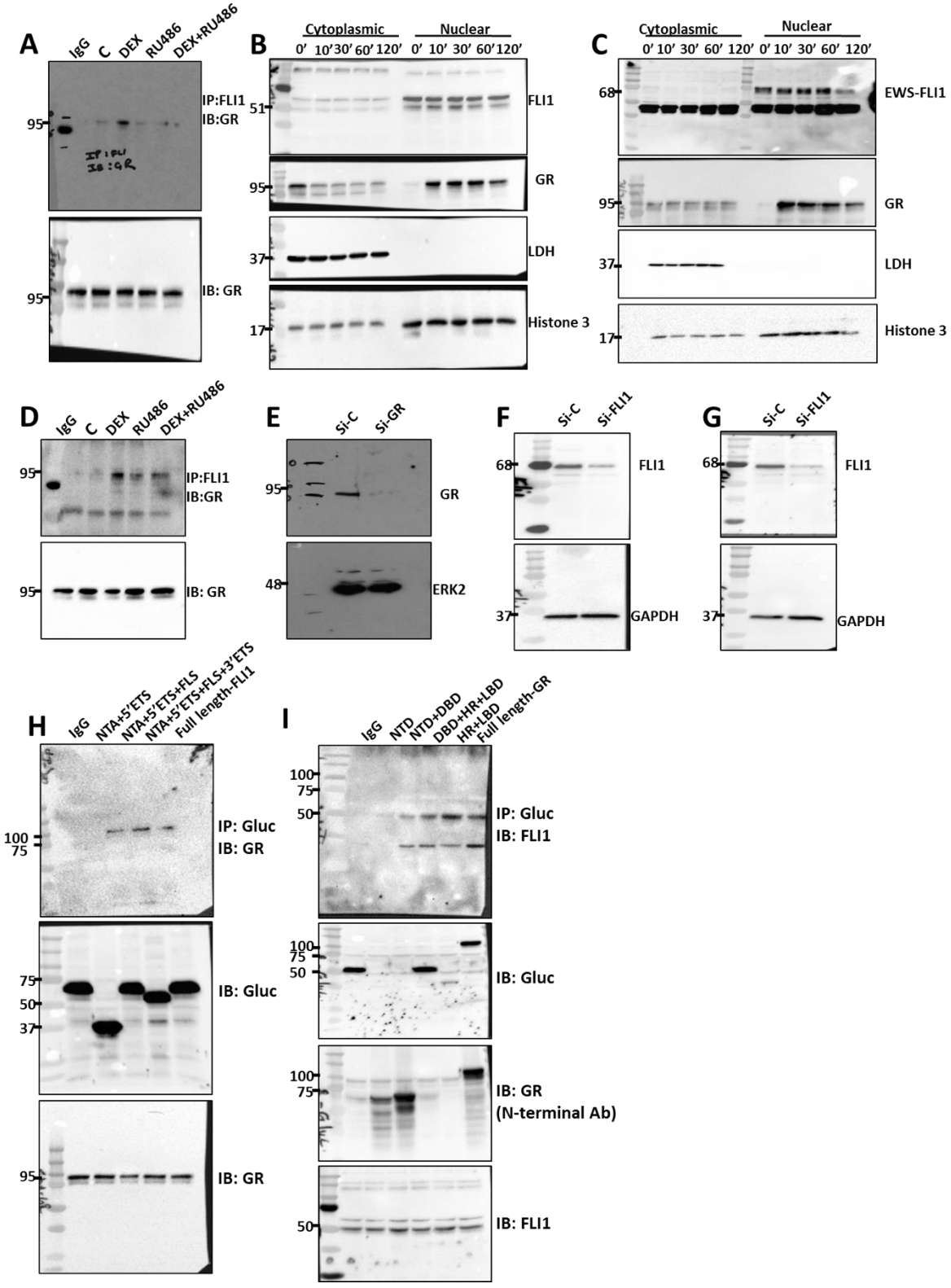


Supplementary Figure S5 (related to Figures 5 and 6): A GR antagonist and a cortisol-lowering drug induce markers of cell death in Ewing sarcoma animal models. (**A**) RD-ES tumors presented in Figure 6A were extracted and analyzed using immunoblotting with the indicated antibodies. (**B**) Whole extracts prepared from the tumors presented in Figure 6D were analyzed using immunoblotting with the indicated antibodies. Note that tumors from three different mice per group were analyzed.



Supplementary Figure S6 (related to Figures 4 and 5): Unlike EWS-ERG expressing cells, colony formation, viability and migration of Ewing sarcoma cells expressing EWS-FEV are

not regulated by GR. (**A**) HEK293T cells pre-transfected (in sextuplicates) with GR-Gluc2 and the indicated Gluc1-ETS plasmids (FLI1, FEV and ETV6), were treated with either vehicle or DEX (1 μM). Shown are normalized fold changes in luminescence (means ± S.E.). *, $p \leq 0.05$; ns, not significant. (**B**) Ewing sarcoma cells ($8X10^3$), either STA-ET-10 (expressing an EWS-FEV fusion protein) or STA-ET-11 cells (expressing EWS-ERG), were seeded in 96-well plates and treated with either vehicle or with the indicated concentrations of RU486. Cell survival assays (MTT) were performed after 48 hours of treatment. The assay was repeated twice in quadruplets. *, $p<0.05$. ns, non-significant. (**C**) The indicated Ewing sarcoma cells were sparsely seeded in 6-well plates. Cells were later treated every other day with either vehicle, DEX (1 μM), RU486 (10 μM) or the combination. Ten days later, cells were fixed and stained with crystal violet. Photos are shown along with bar plots presenting the quantification of colonies in 5 non-overlapping microscope fields. The experiment was repeated twice. ns, non-significant; ***, $p<0.001$. (**D**) The indicated cells were seeded in Transwell migration chambers. Cells were later treated with either DEX (1 μM), RU486 (1 μM) or the combination, and their ability to migrate was quantified 20 hours later. *, $p \leq 0.05$; ***, $p \leq 0.001$. Bars, 500 μm. (**E**) The indicated cells were grown in 100-mm dishes. Thereafter, cells were treated for 48 hours with DEX (1 μM) and/or RU486 (10 or 20 μM). Shown are results of an apoptosis assay performed using an annexin V/7-AAD kit (from BioLegend). Data shown are representative of two biological repeats.


A
IgG
C
DEX
RU486
DEX+RU486
95
IP:FLI1
IB:GR
95
IB: GR
B
Cytoplasmic
Nuclear
0' 10' 30' 60' 120' 0' 10' 30' 60' 120'
51
FLI1
95
GR
37
LDH
17
Histone 3
C
Cytoplasmic
Nuclear
0' 10' 30' 60' 120' 0' 10' 30' 60' 120'
68
EWS-FLI1
95
GR
37
LDH
17
Histone 3
D
IgG
C
DEX
RU486
DEX+RU486
95
IP:FLI1
IB:GR
95
IB: GR
E
Si-C
Si-GR
95
GR
48
ERK2
F
Si-C
Si-FLI1
68
FLI1
37
GAPDH
G
Si-C
Si-FLI1
68
FLI1
37
GAPDH
H
IgG
NTA+5'ETS
NTA+5'ETS+FLS
NTA+5'ETS+FLS+3'ETS
Full length-FLI1
100
75
IP: Gluc
IB: GR
75
50
37
IB: Gluc
95
IB: GR
I
IgG
NTD
NTD+DBD
DBD+HR+LBD
HR+LBD
Full length-GR
100
75
50
IP: Gluc
IB: FLI1
100
75
50
IB: Gluc
100
75
IB: GR
(N-terminal Ab)
50
IB: FLI1

Supplementary Figure S7 (related to Figures 1, 3, 4 and 5): Uncropped original blots of images shown in the main and supplementary figures of the manuscript. Panels labeled A, B, C, D, E, F, G, H and I show the original, uncropped blots corresponding to the following Figures: 1D, 1G, 3B, 4A, 4B, 4E, 5C, 2SC and 2SD, respectively.

Supplementary Tables

No.	Gene	Accession Number	X-GLuc1	X-GLuc2	GLuc1-X
1	GR	NM_000176.2	+	+	
2	MR	NM_000901.4	+	+	
3	ERβ	NM_001437.2	+	+	
4	ERα	NM_000125.3	+	+	
1	PU.1	NM_001080547.1			+
2	FLI1	NM_002017.4			+
3	ELK3	NM_005230.2			+
4	ERG	NM_001136154.1			+
5	ELF3	NM_001114309.1			+
6	SPIC	NM_152323.1			+
7	ETV2	NM_014209.3			+
8	ELK4	NM_001973.3			+
9	GABPA	NM_002040.3			+
10	ELK1	NM_001114123.2			+
11	ETV4	NM_001079675.2			+
12	ETV7	NM_016135.3			+
13	ETV5	NM_004454.2			+
14	ETV6	NM_001987.4			+
15	ETS1	NM_001143820.1			+
16	ETS2	NM_001256295.1			+
17	FEV	NM_017521.2			+

Supplementary Table S1: List of all constructs generated for PCA (related to Figure 1). Listed are seventeen cDNAs encoding either nuclear receptors (fused to the 5' end of *Gluc2*) or ETS family members (fused to the 3' end of *Gluc1*). The respective human genome accession numbers are indicated.

Gluc1-ETS primers	
PU.1-Fw	TGGTGGGTCCTCCGGATTACAGGCGTGCAAAATGGAAGGG
PU.1-Rev	AAACGGGCCCTCTAGATCAGTGGGGCGGGTGGC
FLI1-Fw	TGGTGGGTCCTCCGGAGAC GGG ACT ATT AAG GAG GCT CTG TCG
FLI1-Rev	AAACGGGCCCTCTAGACTA GTA GTA GCT GCC TAA GTG TGA AGG C
ELK3-Fw	TGGTGGGTCCTCCGGAGAG AGT GCA ATC ACG CTG TGG C
ELK3-Rev	AAACGGGCCCTCTAGATCA GGA TTT CTG AGA GTT TGA AGA AAG

	CAG TAC
ERG-Fw	TGGTGGGTCCTCCGGAATT CAG ACT GTC CCG GAC CCA GC
ERG-Rev	AAACGGGCCCTCTAGATTA GTA GTA AGT GCC CAG ATG AGA AGG CA
ELF3-Fw	TGGTGGGTCCTCCGGAGCT GCA ACC TGT GAG ATT AGC AAC A
ELF3-Rev	AAACGGGCCCTCTAGATCA GTT CCG ACT CTG GAG AAC CTC TTC C
SPIC-Fw	TGGTGGGTCCTCCGGAACG TGT GTT GAA CAA GAC AAG CTG GG
SPIC-Rev	AAACGGGCCCTCTAGATTA GCA ATC ATG GTG ATT TAG CTC ATG GTA ATT GG
ETV2-Fw	TGGTGGGTCCTCCGGAGAC CTG TGG AAC TGG GAT GAG GC
ETV-2Rev	AAACGGGCCCTCTAGATTA TTG TGT CTC TGC TCC CCG TCC G
ELK4-Fw	TGGTGGGTCCTCCGGAGAC AGT GCT ATC ACC CTG TGG CAG
ELK4-Rev	AAACGGGCCCTCTAGATTA TGT CTT CTG TAG GTC TGG GGA AAA TGG G
GABPA-Fw	TGGTGGGTCCTCCGGAACT AAA AGA GAA GCA GAG GAG CTG ATA GAA
GABPA-Rev	AAACGGGCCCTCTAGATCA ATT ATC CTT TTC CGT TTG CAG AGA AGC
ELK1-Fw	TGGTGGGTCCTCCGGAGAC CCA TCT GTG ACG CTG TGG C
ELK1-Rev	AAACGGGCCCTCTAGATCA TGG CTT CTG GGG CCC TGG
ETV4-Fw	TGGTGGGTCCTCCGGAGAG CGG AGG ATG AAA GCC GGA TAC
ETV4-Rev	AAACGGGCCCTCTAGACTA GTA AGA GTA GCC ACC CTT GGG GC
ETV7-Fw	TGGTGGGTCCTCCGGACAG GAG GGA GAA TTG GCT ATT TCT CCT
ETV7-Rev	AAACGGGCCCTCTAGATCA CGG AGA GAT TTC TGG CCT CTT GT
ETV5-Fw	TGGTGGGTCCTCCGGAGAC GGG TTT TAT GAT CAG CAA GTC CCT
ETV5-Rev	AAACGGGCCCTCTAGATTA GTA AGC AAA GCC TTC GGC ATA GGG G
ETV6-Fw	TGGTGGGTCCTCCGGATCT GAG ACT CCT GCT CAG TGT AGC ATT AAG
ETV6-Rev	AAACGGGCCCTCTAGATCA GCA TTC ATC TTC TTG GTA TAT TTG TTC ATC CAG
ETS1-Fw	TGGTGGGTCCTCCGGAAGC TAC TTT GTG GAT TCT GCT GGG AGC
ETS1-Rev	AAACGGGCCCTCTAGATCA CTC GTC GGC ATC TGG CTT GAC
ETS2-Fw	TGGTGGGTCCTCCGGAGGG TCG GCT CAA TTT CAG GGC
ETS2-Rev	AAACGGGCCCTCTAGATCA GTC CTC CGT GTC GGG C
FEV-Fw	TGGTGGGTCCTCCGGA AGA CAG AGC GGC GCC TCC CAG CCC CTG CTG ATC AAC ATG TAC CTG CCA GAT CCC GTC
FEV-Rev	AAACGGGCCCTCTAGA TTA GTG GTA ATG GCC CCC CAA GTG CGA GGC TGC GGC CAC GGC CCC GAA GGG CCC GGG
NFκBp65-Fw	TGGTGGGTCCTCCGGAGACGAACTGTTCCCCCTCATCTTCC
NFκBp65-Fw	AAACGGGCCCTCTAGATCACCCCCTTAGGAGCTGATCTGA
Primers for NR-Gluc2	
GR-Fw	AGCACAGTGGCGGCCGCATG GAC TCC AAA GAA TCA TTA ACT CCT GGT AGA G
GR-Rev	CCACCGCCACCATCGATCTT TTG ATG AAA CAG AAG TTT TTT GAT ATT TCC

MR-Fw	AGCACAGTGGCGGCCGCATG GAG ACC AAA GGC TAC CAC AGT CTC C
MR-Rev	CCACCGCCACCATCGATCTT CCG GTG GAA GTA GAG CGG C
ERα-Fw	AGCACAGTGGCGGCCGCATGACCATGACCCTCCACACCAAAGC
ERα-Rev	CCACCGCCACCATCGATGACCGTGGCAGGGAAACCCTCTGCCTCC
ERβ-Fw	AGCACAGTGGCGGCCGCATGGATATAAAAAACTCACCATCTAGCCTT AATTCTCCTTCC
ERβ-Fw	CCACCGCCACCATTCGATCTGAGACTGTGGGTTCTGGGAGCCCTCTTT GC
GR domains-GLuc2	
NTD-Fw	AGCACAGTGGCGGCCGC ATG GAC TCC AAA GAA TCA TTA ACT CCT GGT AGA GAA GAA AAC CCC
NTD-Rev	CCACCGCCACCATCGAT CTT GAA TAG CCA TTA GAA AAA ACT GTT CGA CCA GGG
NTD+DBD-Fw	same as NTD-Fw
NTD+DBD-Rev	CCACCGCCACCATCGAT CTT CCA GGT TCA TTC CAG CCT GAA GAC ATT
DBD+HR+LBD-Fw	AGCACAGTGGCGGCCGC ATG AGC CCC AGC ATG AGA CCA GAT GTA AGC TCT
DBD+HR+LBD-Rev	CCACCGCCACCATCGAT CTT TTG ATG AAA CAG AAG TTT TTT GAT ATT TCC ATT TGA ATA TTT TGG
HR+LBD-Fw	AGCACAGTGGCGGCCGC ATG GCT CGA AAA ACA AAG AAA AAA ATA AAA GGA ATT CAG CAG GC
HR+LBD-Rev	same as DBD+HR+LBD-Rev
Gluc1-FLI1 domains	
NTA+5'ETS-Fw	TGGTGGGTCCTCCGGAGACGGGACTATTAAGGAGGCTCTGTCGG
NTA+5'ETS-Rev	AAACGGGCCCTCTAGATTATTTCCCTGAGGTAACTGAGGTGTGACAAC AGC
NTA+5'ETS+FLS-Fw	same as NTA+5'ETS-Fw
NTA+5'ETS+FLS-Rev	AAACGGGCCCTCTAGATTAGGAGAGCAGCTCCAGGAGGAATTGCCAC AG
NTA+5'ETS+FLS+3'ETS-Fw	same as NTA+5'ETS-Fw
NTA+5'ETS+FLS+3'ETS-Rev	AAACGGGCCCTCTAGATTACTGCTGGTGGGCATGGTAGGA

Supplementary Table S2: List of all primers used for cloning and validation (related to Figure 1). Fw, forward primer; Rev, reverse primer.

Downregulated genes	**Upregulated genes**		
AC016612.1	ABI3BP	EIF3J-AS1	PLAT
AL365440.1	AC008938.1	ENC1	PLAU
AL844908.1	AC025857.2	EPGN	PLK2
AP000924.1	AC099673.1	EREG	PODXL
CALM1	ACTA2	ERRFI1	PPFIBP1
CD83	ADGRF5	EVA1A	PRSS23
CLDN1	ADGRG1	F3	PTGR1
CNTNAP2	ADM	FBN1	PTX3
CXCL10	AL050341.1	FCRL1	RAI14
DNAL4	ANKRD1	FN1	RHBDL2
FAM19A5	AP000753.1	FNDC3B	RN7SL125P
FAM84B	AREG	FOSL2	RNU6-1092P
FDX1	ARHGAP29	GNAI1	RUNX2
FEZF1	ATP1B1	GPC6	S100A10
FEZF1-AS1	ATP2B1	HHIPL2	S100A13
H2AFY2	BEND2	ID1	S100A16
HMGB1	BST1	IGFBP3	S100A2
HOOK1	C15orf48	IGFBP5	S100A4
IRS2	CALD1	IGFBP7	S100A5
KLHL23	CCL2	IL18	S100A6
LBH	CCL5	IL6	S1PR3
MCM4	CD44	ITPR2	SEC14L2
MYOM2	CDH13	KANK2	SERPINE1
NR0B1	CFI	KYNU	SH3BP5
OTX2	CH25H	L1CAM	SH3KBP1
PRKCB	CHI3L1	LAMA4	SIX1
SLAIN1	CLDN11	LAMC1	SMAD3
SLC5A6	COL12A1	LGALS3	SRGN
TRAV1-2	COL1A1	LOX	SYNJ2
UGT3A2	COL1A2	LOXL2	TAGLN
YPEL5	COL3A1	LRRC17	TFPI2
OTX2	COL4A1	MDGA2	TGFB2
PRKCB	COL6A1	MEST	TGFB2-OT1
SLAIN1	CPA4	MGP	TGFBI
SLC5A6	CREB3L1	MIR21	TGFBR2
TRAV1-2	CRYAB	MIR503	TIMP2
UGT3A2	CSF2	MIR503HG	TM4SF1

YPEL5	CTGF	MLPH	TMEM200A
	CTSB	MRPS6	TMSB4X
	CTSO	MYL6	TNFSF18
	CXCL1	NAMPT	TPM1
	CXCL6	NAV3	TRHR
	CYP1B1	NT5E	UGCG
	CYR61	P4HA2	VCAM1
	DCBLD2	PDP1	VEGFC
	DISP2	PENK	VIM
	DKK1	PHLDA1	ZFP36L1
	DKK3	PHLDB2	ZFP36L2
	DST		

Supplementary Table S3: List of down- or up-regulated genes in FLI1-depleted Ewing sarcoma cells (related to Supplementary Figure S3). A673 cells grown in 90-mm dishes were transfected in triplicates with siRNA oligonucleotides specific to FLI1, or with control oligonucleotides. Forty-eight hours later, cells were harvested and RNA was isolated and sequenced.