

Supplementary Fig. 1 SSO-induced ERG exon 4 skipping is confirmed by sequencing of PCR bands. a Schematic showing sequences of *ERG* mRNA targeted by E43' and E45' SSOs. b RT-PCR products from SSO-treated VCaP cells were sequenced and aligned with the *ERG* exon 4 sequence (from NM_182918.3) to assess the presence or absence of exon 4 sequence in the PCR product. Red areas show regions of sequenced PCR products outside the sequence of exon 4.

Supplementary Fig. 2 ERG exon 4 SSOs affect VCaP and MG63 cancer cell behaviour. a Representative Hoechst images of VCaP cells migrated in transwell assays after 48 h of E4 SSO treatment at 8 μ M. b Quantification of MG63 cells migrated in transwell assays after 48 h of E43' SSO treatment at 3 μ M by crystal violet residue absorbance (n = 3 for all groups). c Western blotting and quantification for regulators of cell cycle progression, cyclin D1 and c-Myc, following 72 h of 3 μ M E43' SSO treatment in MG63 cells. β -actin was used as loading control.*** = p<0.001, ** = p<0.01, * = p<0.05. Ctrl SSO: control SSO. Scale bar = 100 μ m.

Supplementary Fig. 3 ERG exon 4 SSOs do not affect endogenous *ERG* in vivo. a Alignment of exon 4 SSOs with mouse *ERG*. Note that the antisense sequence of the SSOs is shown in the alignments. Alignment shows very poor conservation between mouse and human, especially at the 3' SSO target site, making cross-reactivity highly unlikely. b Representative panels for CD31 (grey) and DAPI (blue) immunofluorescence on frozen sections of E43' SSO-treated MG63 subcutaneous tumours to assess tumour blood vessel density. c Quantification of CD31+ area per field of view in representative immunofluorescence images (n = 3 tumours per group, 2 sections per tumour analysed). Scale bars: 75 μ m. Scr ctrl SSO: Scrambled control SSO.

Supplementary Fig. 4 ERG exon 4 SSOs demonstrate efficacy in human prostate tumour samples cultured ex vivo. a Schematic showing ex vivo culture system for human radical prostatectomy tumour samples. b Left panel shows hematoxylin and eosin staining of an ERG+ tumour that was cultured ex vivo with a Gleason score of G4+3. Right panel shows representative ERG immunohistochemistry on a tumour section from patient PPL-0209. c ERG, PTEN and GAPDH (loading control) western blotting of lysates from prostate tumour samples cultured ex vivo. d Quantification of ERG protein levels in tumours treated with 10 μ M E43' SSO ex vivo (n = 1 for all). e Quantification of PTEN protein levels in tumours treated with 10 μ M E43' SSO ex vivo (n = 1 for all). For d and e, ERG and PTEN protein expression levels were normalised to GAPDH. Scale bars: left panel = 100 μ m, right panel = 250 μ m. Scr ctrl SSO: Scrambled control SSO.

a

5'

E43' SSO

E45' SSO

3'

.....gtgtttctttgggcattcag**GAAGC**.....**GGCTCAAG**gtaaggagacttccgcc.....

Intron 3

Exon 4

Intron 4

b

Untreated sequence

ERG Ex4 sequence

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      *      *      *      *      *      *      *      *      *      *
101 AGGACATGATTCAGACTGTCCCGGACCCAGCAGCTCATATCAAGGAAGCCTTATCAGTTGTGAGTGAGGACCAGTCGTTGTTTGAGTGTGCCTACGGAAC 200
    ~~~~~|
1   ~~~~~GAAGCCTTATCAGTTGTGAGTGAGGACCAGTCGTTGTTTGAGTGTGCCTACGGAAC 56
      *      *      *      *      *      *      *      *      *      *
201 GCCACACCTGGCTAAGACAGAGATGACCGCGTCCTCCTCCAGCGACTATGGACAGACTTCCAAGATGAGCCCACGCGTCCCTCAGCAGGATTGGCTGTCT 300
    ~~~~~|
57  GCCACACCTGGCTAAGACAGAGATGACCGCGTCCTCCTCCAGCGACTATGGACAGACTTCCAAGATGAGCCCACGCGTCCCTCAGCAGGATTGGCTGTCT 156
      *      *      *      *      *      *      *      *      *      *
301 CAACCCCCAGCCAGGGTCACCATCAAAATGGAATGTAACCCTAGCCAGGTGAATGGCTCAAGGAACCTCTCCTGATGAATGCAGTGTGGCCAAAGGCGGGA 400
    ~~~~~|
157 CAACCCCCAGCCAGGGTCACCATCAAAATGGAATGTAACCCTAGCCAGGTGAATGGCTCAAG~~~~~ 218
      *      *      *      *      *      *      *      *      *      *

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Ctrl SSO sequence

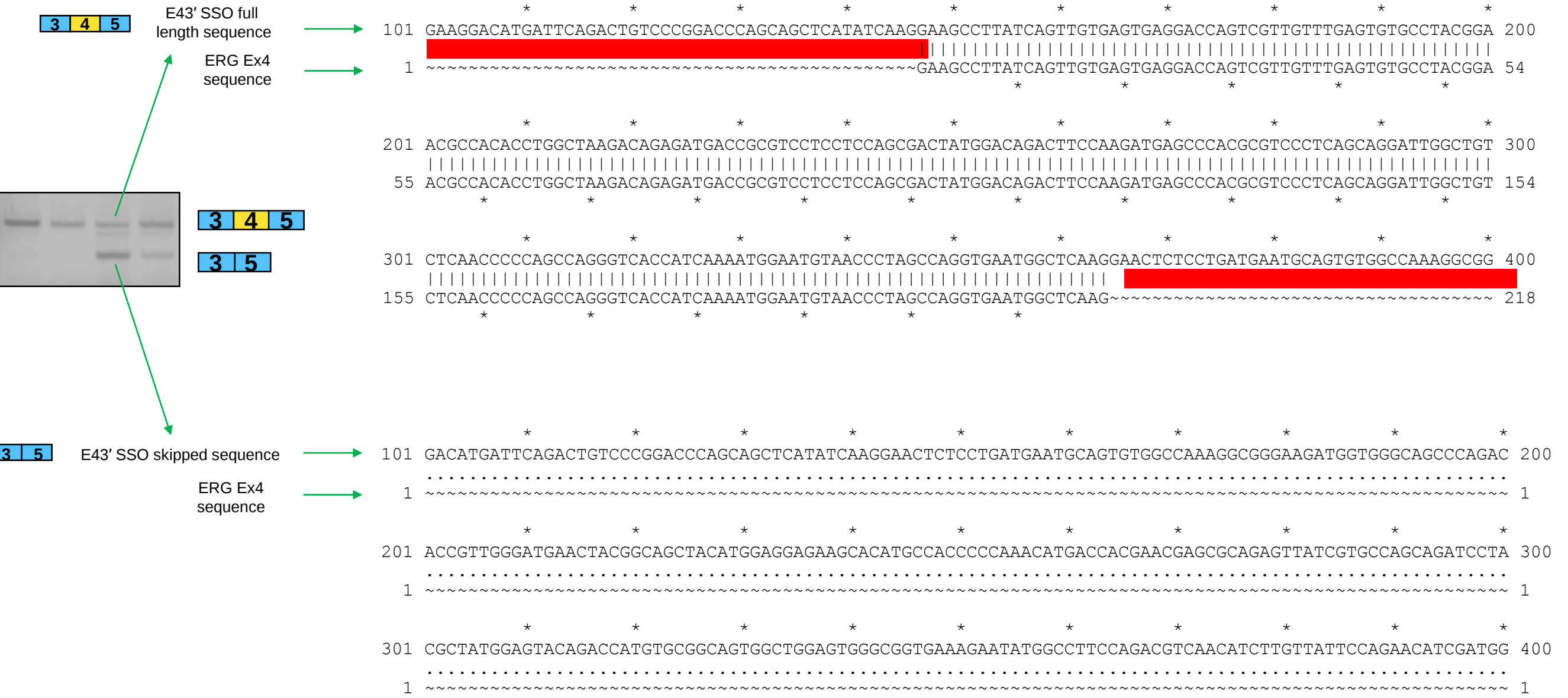
ERG Ex4
sequence

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      *      *      *      *      *      *      *      *      *      *
101 AAGGACATGATTCAGACTGTCCCGGACCCAGCAGCTCATATCAAGGAAGCCTTATCAGTTGTGAGTGAGGACCAGTCGTTGTTTGAGTGTGCCTACGGAA 200
    ~~~~~|
1   ~~~~~GAAGCCTTATCAGTTGTGAGTGAGGACCAGTCGTTGTTTGAGTGTGCCTACGGAA 55
      *      *      *      *      *      *      *      *      *      *
201 CGCCACACCTGGCTAAGACAGAGATGACCGCGTCCTCCTCCAGCGACTATGGACAGACTTCCAAGATGAGCCCACGCGTCCCTCAGCAGGATTGGCTGTC 300
    ~~~~~|
56  CGCCACACCTGGCTAAGACAGAGATGACCGCGTCCTCCTCCAGCGACTATGGACAGACTTCCAAGATGAGCCCACGCGTCCCTCAGCAGGATTGGCTGTC 155
      *      *      *      *      *      *      *      *      *      *
301 TCAACCCCCAGCCAGGGTCACCATCAAAATGGAATGTAACCCTAGCCAGGTGAATGGCTCAAGGAACCTCTCCTGATGAATGCAGTGTGGCCAAAGGCGGG 400
    ~~~~~|
156 TCAACCCCCAGCCAGGGTCACCATCAAAATGGAATGTAACCCTAGCCAGGTGAATGGCTCAAG~~~~~ 218
      *      *      *      *      *      *      *      *      *      *

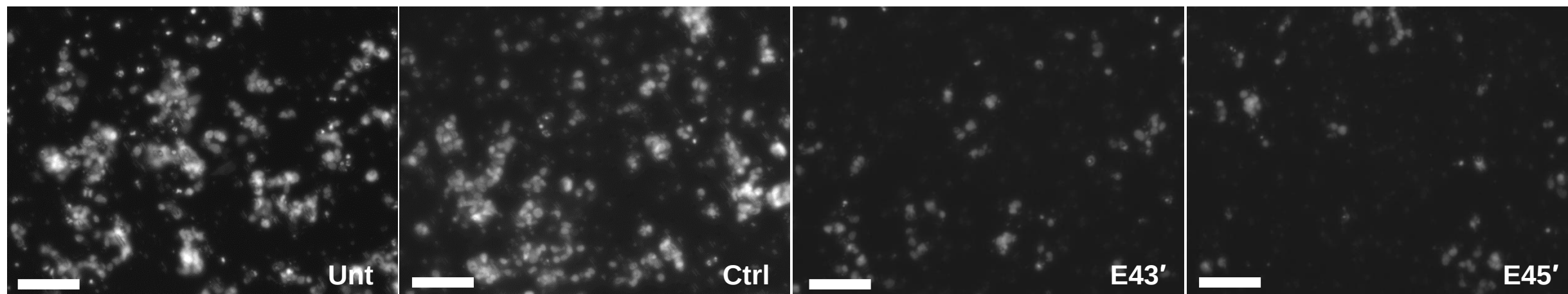
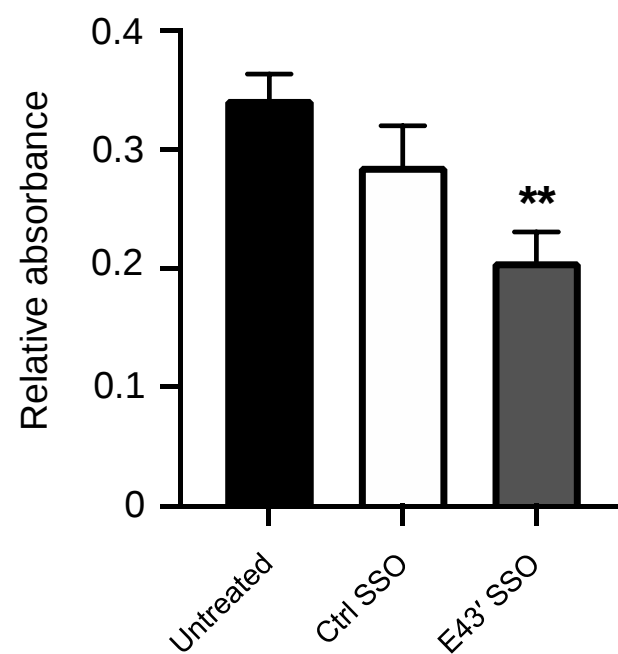
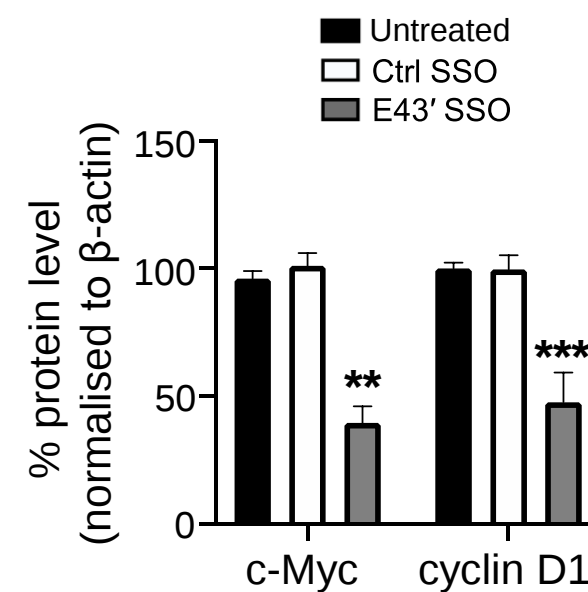
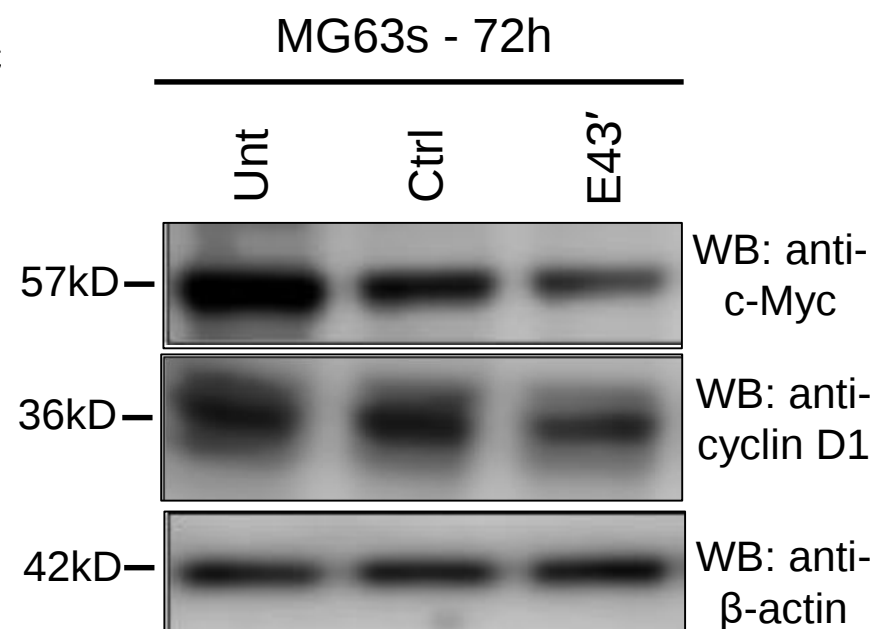
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b continued



a

Hoechst

**b****c**

a

E43' SSO: 5'-GCTTCCTGAATGCCCAAAGAAACAC-3'

E45' SSO: 5'-GGCGGAAGTCTCCTTACCTTGAGCC-3'

RED: EXON 4

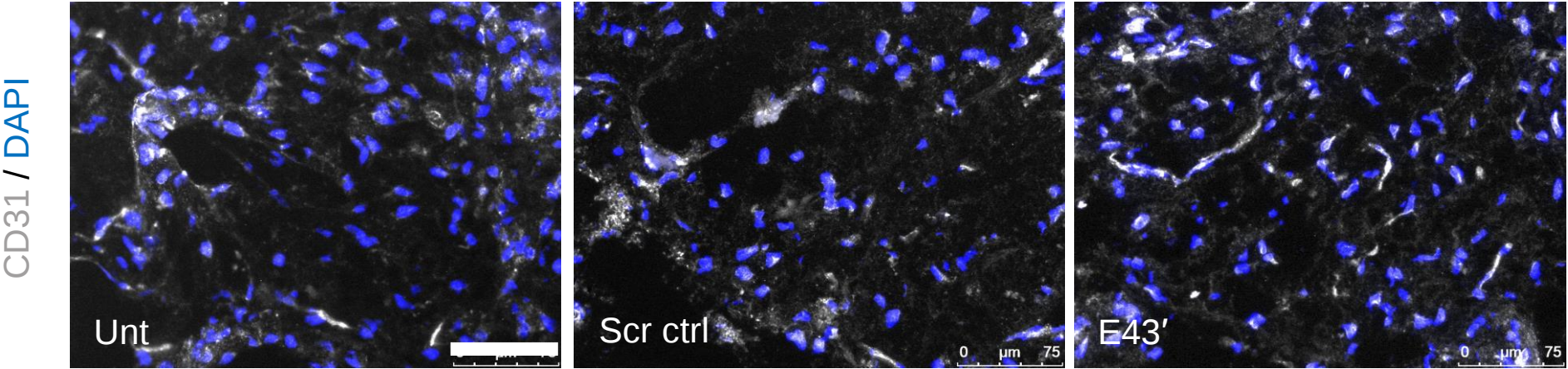
BLUE: SSOs

INTRON SEQUENCE: BLACK, ITALICS

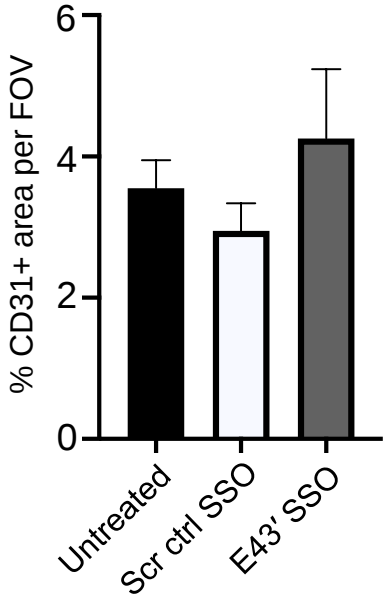
			E43' SSO	
			GTGTTTCTTTGGGCATTCA	
Human	-----	TTGGAGTGTCTTGAATTATGTGTTTCTTTGGGCATTCA		38
Mouse		TCAGCACCACTGCTTGTCTCTGGGATGTCCTGAGTCTGCGTTTCTGTTTCTTCGTCCA		60
		* *** ** * ** * * *** * * *		
		GGAAGC		
Human		GGAAGCCTTATCAGTTGTGAGTGAGGACCAGTCGTTGTTTGAGTGTGCCTACGGAACGCC		98
Mouse		GGAAGCCTTGTCTAGTTGTGAGCGAGGACCAGTCACTATTTGAGTGTGCCTACGGAACGCC		120
		*** *****		
Human		ACACCTGGCTAAGACAGAGATGACCGCGTCCTCCTCCAGCGACTATGGACAGACTTCCAA		158
Mouse		ACACCTGGCTAAGACAGAGATGACCGCATCCTCTTCCAGTGACTATGGCCAGACATCCAA		180

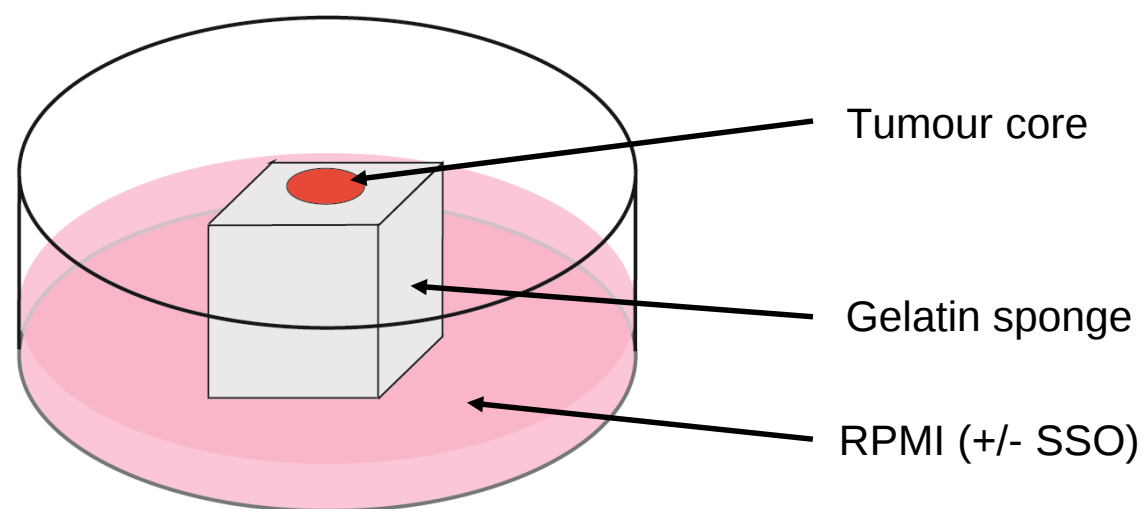
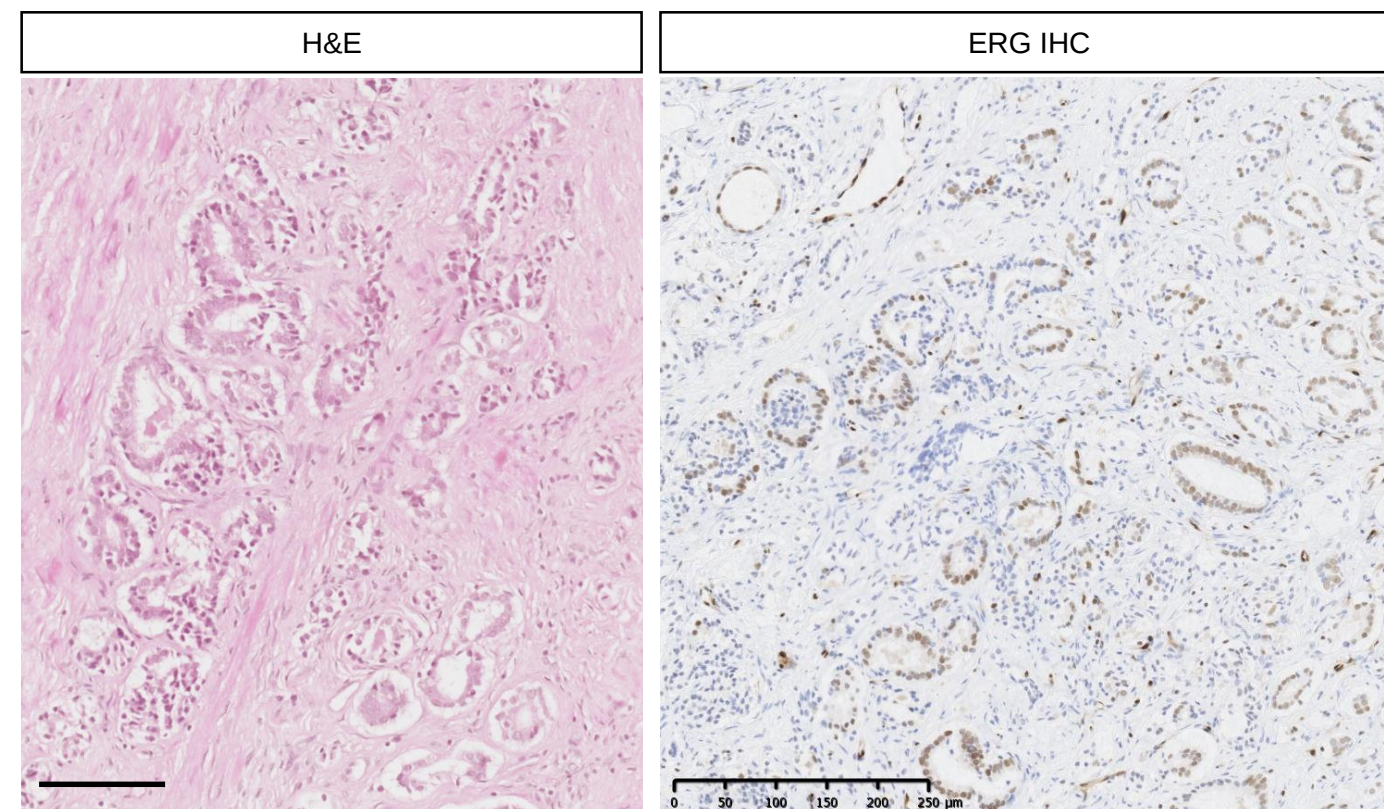
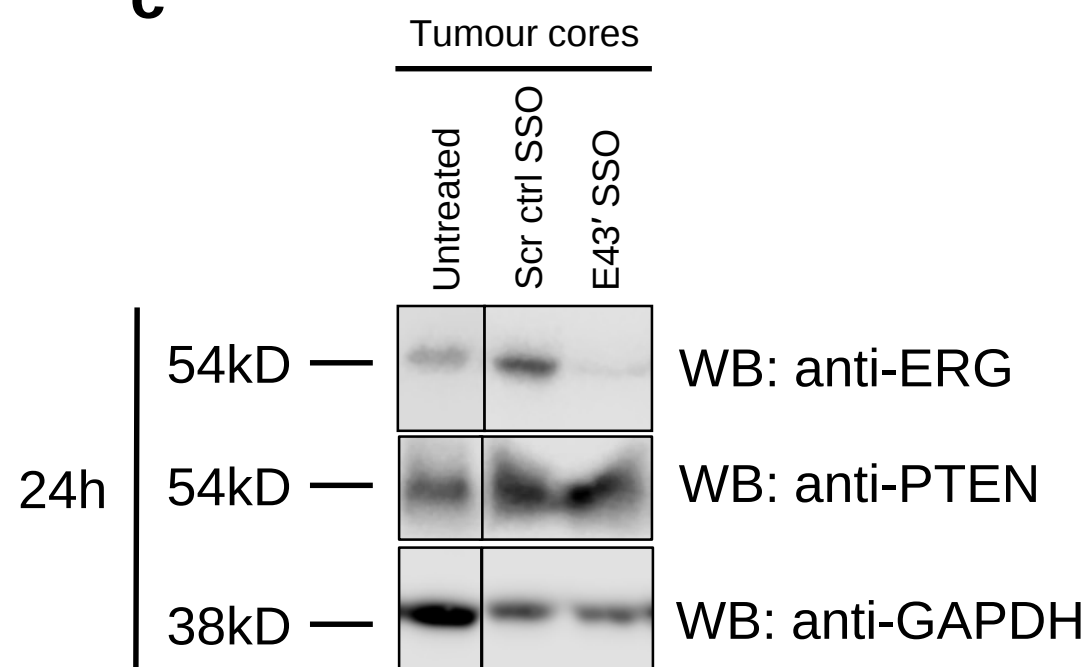
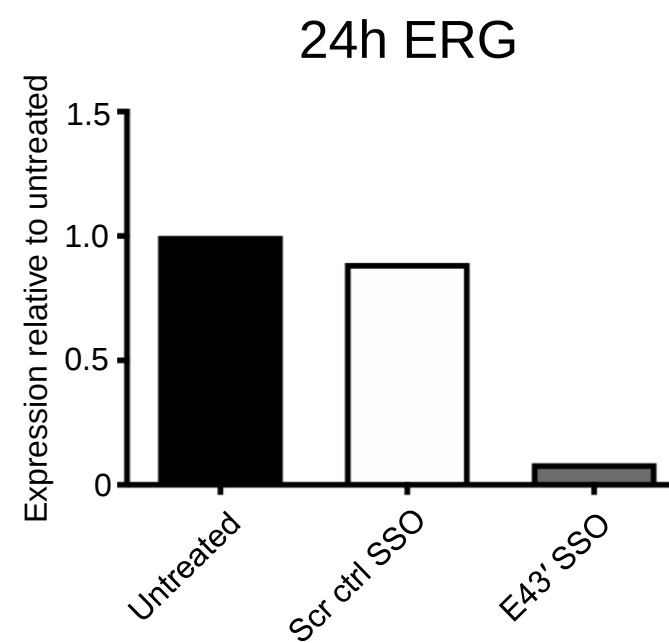
Human		GATGAGCCACGCGTCCCTCAGCAGGATTGGCTGTCTCAACCCCCAGCCAGGGTCACCAT		218
Mouse		GATGAGTCCCAGAGTCCCTCAGCAGGACTGGCTGTCTCAAGCCCCAGCCAGGGTCACCAT		240
		***** ** * *****		
			E45' SSO	
			GGCTCAAGGTAAGGAGACTTCCGCC	
Human		CAAAATGGAATGTAACCTAGCCAGGTGAATGGCTCAAG	GTAAGGAGACTTCCGCCCTT	278
Mouse		CAAGATGGAGTGCAACCTAGTCAGGTGAATGGTTCCAG	GTAAGCAATGCTGACCCTGCC	300
		*** ***** ** ***** ** ***** * * **		
Human		TCTCTCCCTGACCTCCCATTTT-----		300
Mouse		TGTCCTGG--ACCTCTATTCTTCCCATCGGTAGCTT		334
		* ** ***** * **		

b



c



aEx-vivo gelatin sponge culture**b****c****d****e**