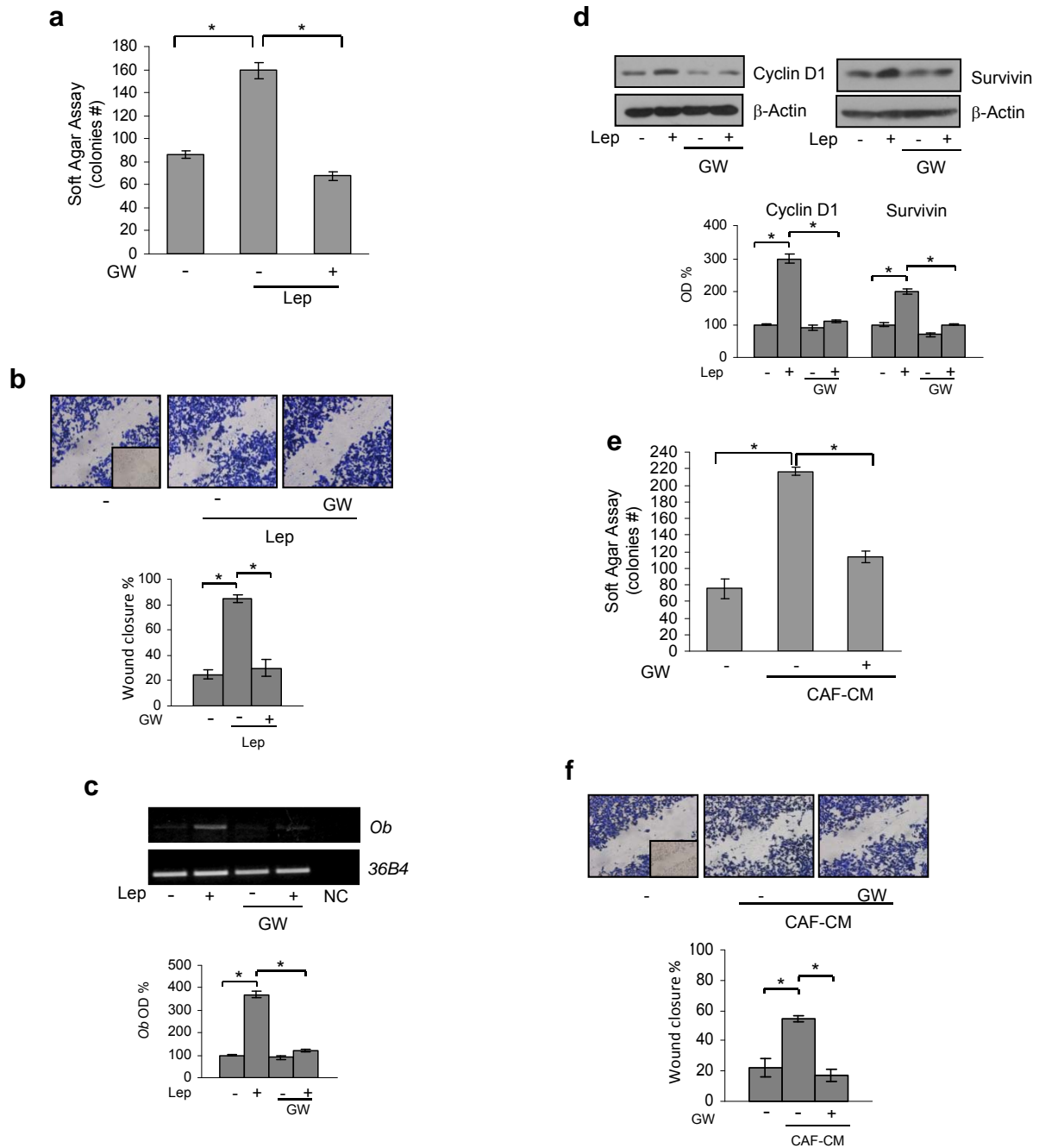


Activated FXR Inhibits Leptin Signaling and Counteracts Tumor-promoting Activities of Cancer-Associated Fibroblasts in Breast Malignancy.

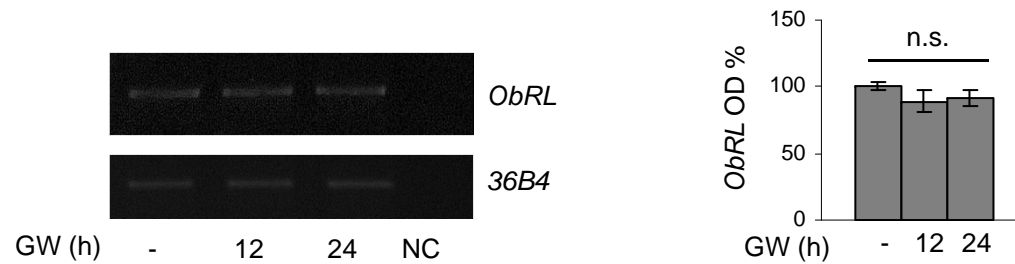
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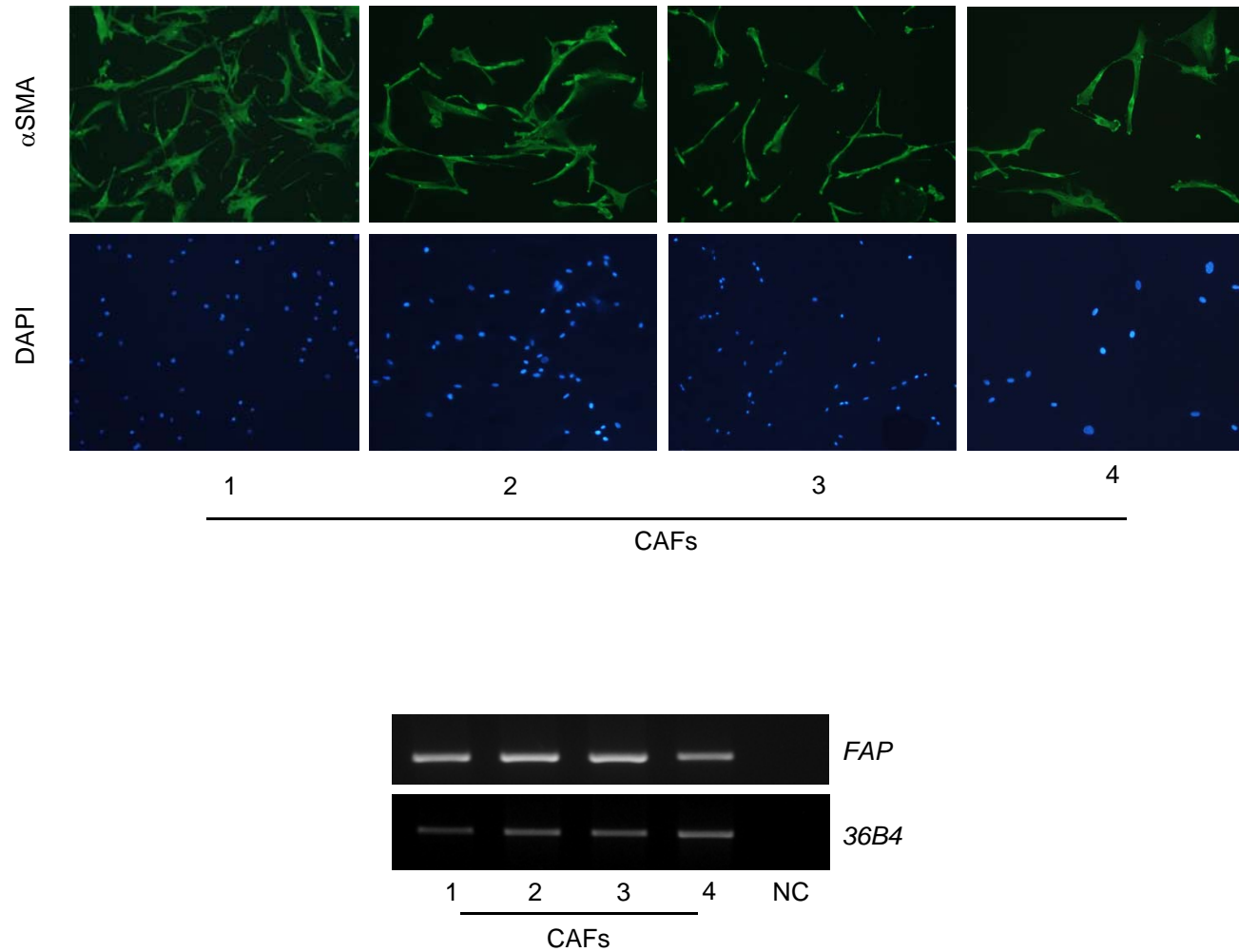
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Supplementary Figure S1. Effects of activated FXR in SKBR3 breast cancer cells. (a) Soft Agar growth assay in SKBR3 cells treated with Leptin (Lep, 500ng/ml) with/without GW4064 (6 μ M). **(b)** Wound-healing assay in cells treated as indicated for 24h. *Upper panel*, representative images from each condition (inset, time 0). *Lower panel*, relative percentage of wound closure. **(c)** RT-PCR analysis of leptin (*Ob*) mRNA levels. *36B4*, internal standard. NC, negative control. **(d)** Immunoblot analysis for Survivin, Cyclin D1, β -Actin (loading control) expression. *Lower panels* for **(c)** and **(d)**, the histograms represent the mean \pm SD of three separate experiments in which band intensities were evaluated in terms of optical density arbitrary units (OD), expressed as percentage of vehicle-treated samples assumed to be 100%. **(e)** Soft Agar growth assay in cells treated with CAF-CM with/without GW4064. **(f)** Wound-healing assay in cells treated as indicated. *Upper panel*, representative images from each condition (inset, time 0). *Lower panel*, relative percentage of wound closure. The values represent the mean \pm SD of three independent experiments. * $p < 0.05$.



Supplementary Figure S2. Effects of activated FXR on leptin receptor expression in MCF-7 breast cancer cells. Leptin receptor long isoform (*ObRL*) mRNA expression in MCF-7 cells treated for 12 or 24 hours with GW4064 (GW, 6 μ M) by RT-PCR analysis. *36B4* (internal standard). NC, negative control. The histograms represent the mean \pm SD of three separate experiments in which band intensities were evaluated in terms of optical density arbitrary units (OD) and expressed as percentage of vehicle-treated samples which were assumed to be 100%; n.s: nonsignificant.



Supplementary Figure S3. Characterization of Cancer-Associated Fibroblasts. Cancer-Associated Fibroblasts (CAFs) were isolated from four primary breast tumour biopsies by collagenase digestion. *Upper panel*, immunofluorescence of alpha-smooth muscle actin (α -SMA) and DAPI staining (for nuclei detection); *lower panel*, RT-PCR analysis of Fibroblast Activation Protein (*FAP*) mRNA levels. *36B4* (internal standard). NC, negative control.

Supplementary Table S1. Oligonucleotide primers used in this study.

Gene Symbol	Gene Name	Primer Sequences
<i>SOCS3</i>	Suppressor of cytokine signaling 3	For 5'-ATCCTGGTGACATGCTCCTC- 3' Rev 5' - GGCACCAGGTAGACTTTGGA-3'
<i>Ob</i>	Leptin	For 5' - CTGTGCCCATCCAAAAAGTCC-3' Rev 5' - CCCCAGGCTGTCCAAGGTC-3'
<i>ObRL</i>	Leptin Receptor Long Isoform	For 5'-CGAGAAACGTTTCAGCATCT-3' Rev 5' - CAAAAGCACACCACTCTCTC-3'
<i>FAP</i>	Fibroblast activation protein	For 5'-AGAAAGCAGAACTGGATGG-3' Rev 5'-ACACACTTCTTGCTTGGAGGAT-3'
<i>FXR</i>	Nuclear receptor subfamily 1, group H, member 4 (NR1H4)	For 5'-CGAGCCTGAAGAGTGGTACTGTC-3' Rev 5' - CATTGAGCCAACATTCCCATCTC-3'
<i>36B4</i>	36B4	For 5'-CTCAACATCTCCCCCTTCTC-3' Rev 5' - CAAATCCCATATCCTCGT -3'