

Inhibition of androgen receptor promotes CXCR7-chemokine receptor 7-mediated prostate cancer cell survival

James J. Hoy, Georgios Kallifatidis, Diandra K. Smith, and Bal L. Lokeshwar

Supplementary Table S1: primer sets and CRISPR gRNA sequences	
RT-q-PCR primers	
CXCR7 Forward	5'-GCACAGCACAGCCAGGAAGG-3'
CXCR7 Reverse	5'-TCGTTCTGAGGCGGGCAATC-3'
PPIA Forward	5'-CTTGGGCCGCGTCTCCTTTG-3'
PPIA Reverse	5'-ACCACCCTGACACATAAACCCCTG-3'
CXCR7 promoter amplification primers	
Forward (+ KpnI linker)	5'- CGTTGGTACCATGGGGTTTCACCATGTTGGC-3'
Reverse (+ XhoI linker)	5'- CGTTCTCGAGCTGGCTGGGCTGGCTG-3'
ChIP AR-pulldown target RT-q-PCR primers	
PSA Forward	5'-GCCTGGATCTGAGAGAGATATCATC-3'
PSA Reverse	5'-ACACCTTTTTTTTTCTGGATTGTTG-3'
ARE 1 Forward	5'-TACCCTCTCGTTTTCCCCCA-3'
ARE 1 Reverse	5'-CCTGAACTTGGCTGTCTCCT-3'
ARE 2 Forward	5'-CCCATGTGGCCTTTCTTCCA-3'
ARE 2 Reverse	5'GGGCCTGTCTTCAGAGAAC-3'
ARE 3 Forward	5'-CTACAGCCACAGAAAGCGGA-3'
ARE 3 Reverse	5'-CTTTCTTGTTGGGGGACCCTG-3'
ARE 4/5 Forward	5'-ATCCCCGTACATTTCCAGCG-3'
ARE 4/5 Reverse	5'-ACCTCGACTCTCCACAGCAT-3'
ARE 6 Forward	5'-CCGCTGGCAGTTTTCTTTC-3'
ARE 6 Reverse	5'-ACTCTTCGTGTCACATCGGC-3'
CRISPR gRNA sequences	
CXCR7-gRNA 1	5'-TTTGACGTGAGCTCGCCCA-3'
CXCR7-gRNA 2	5'-CATCTCGGCGTCCAGTGACC-3'
CXCR7-gRNA 3	5'-ACTGGACGCCGAGATGGCTC-3'
Supplementary Table S1: Sequences of primer sets utilized in this study and gRNA target sequences for CRISPR-Cas9 experiments.	

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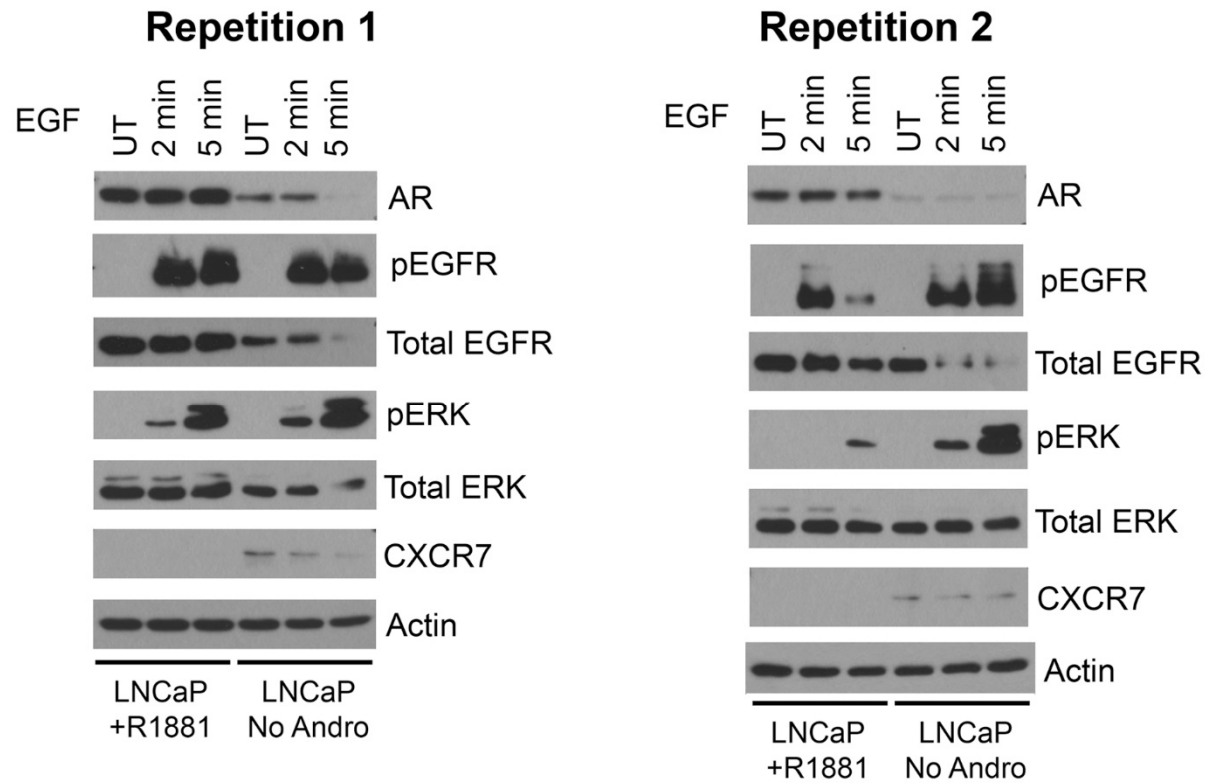
Supplementary Table S2: Antibody information

Western blot					
Target	Host species	Supplier	Catalog number	Dilution	Blocker
AR	Rabbit	Cell Signaling; Danvers, MA	5153	1:10,000	5% NFDM
Lamin A+C	Rabbit	Cambridge; United Kingdom	ab108595	1:5,000	5% NFDM
α-Tubulin	Rabbit	Abcam	ab4047	1:1,000	5% NFDM
CXCR7	Rabbit	Proteintech; Rosemont, IL	20423-1-AP	1:2,000	5% NFDM
phospho-Y1110 EGFR	Rabbit	Abcam	ab47370	1:5,000	5% BSA
EGFR (total)	Rabbit	Cell Signaling	2646S	1:5,000	5% BSA
phospho-ERK1/2	Mouse	Cell Signaling	9106S	1:5,000	5% NFDM
ERK1/2 (total)	Mouse	Cell Signaling	9107	1:5,000	5% NFDM
HRP-conjugated β-Actin	Rabbit	Cell Signaling	5125	1:10,000	5% NFDM
HRP-conjugated Rabbit	Goat	Cell Signaling	7074S	1:10,000	5% NFDM
HRP-conjugated Mouse	Horse	Cell Signaling	7076S	1:10,000	5% NFDM
Chromatin Immunoprecipitation (ChIP) Assay					
Target	Host species	Supplier	Catalog number	Dilution	Blocker
AR	Rabbit	EMD Millipore; Darmstadt, Germany	17-10489	2 µg / sample	N/A
Proximity Ligation Assay (PLA)					
Target	Host species	Supplier	Catalog number	Dilution	Blocker
CXCR7	Rabbit	GeneTex; Irvine, CA	GTX100027	1:300	PLA Kit supplied
ARRB1	Mouse	BioLegend; San Diego, CA	648602	1:15	PLA Kit supplied
ARRB2	Mouse	Santa Cruz Biotechnologies; Dallas, TX	sc-13140	1:60	PLA Kit supplied
Non-specific IgG	Mouse	Cell Signaling	5415	1:100	PLA Kit supplied

Supplementary Table S2: Details for antibodies used throughout this study. NFDM = non-fat dry milk, BSA = bovine serum albumin

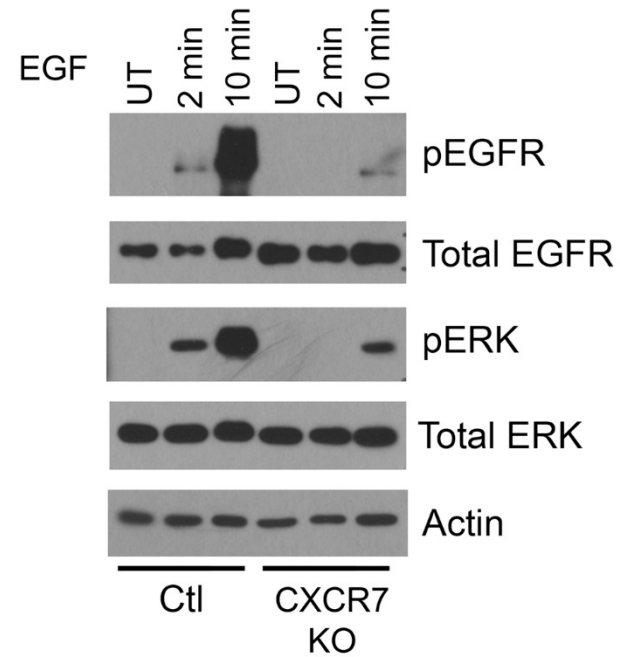
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LNCaP: +/- Androgen, EGF



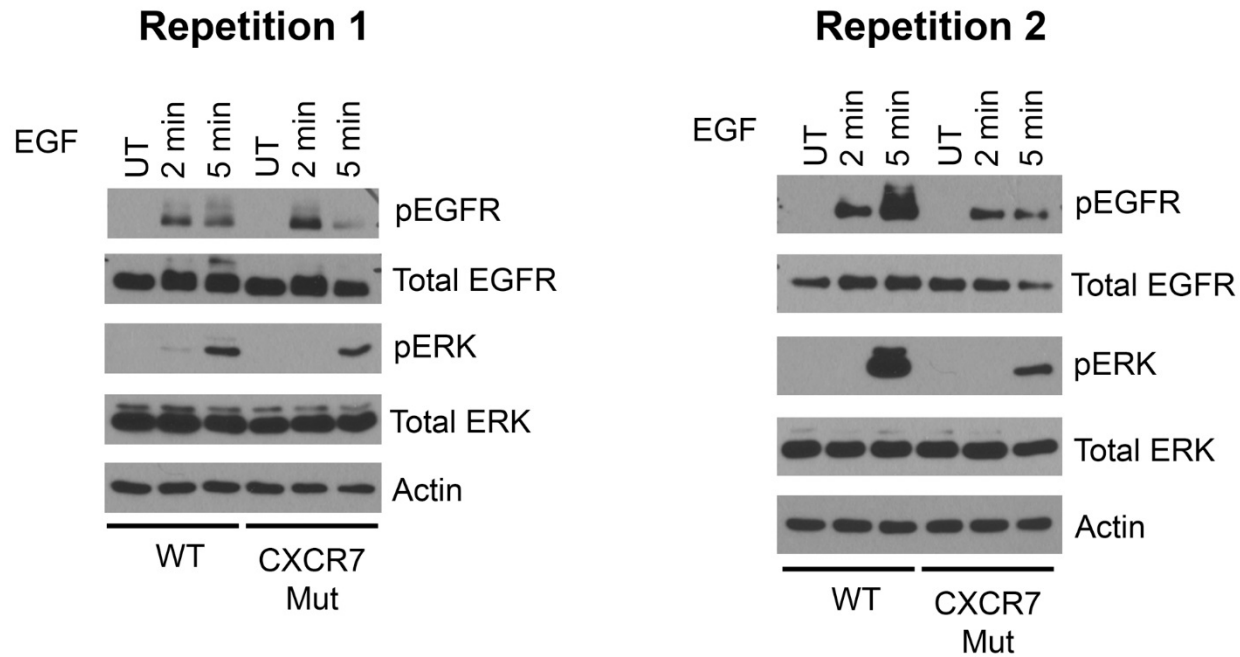
Supplementary Figure S1: Repetition of western blot experiment shown in figure 2a.

**C4-2B: Ctl vs. CXCR7 KO, EGF
Repetition**



Supplementary Figure S2: Repetition of western blot experiment shown in figure 4b.

LNCaP: WT CXCR7 vs. CXCR7 mutant, EGF



Supplementary Figure S3: Repetition of western blot experiment shown in figure 6b.

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