LIGAND-DEPENDENT COREPRESSOR LCoR IS AN ATTENUATOR OF PROGESTERONE-REGULATED GENE EXPRESSION

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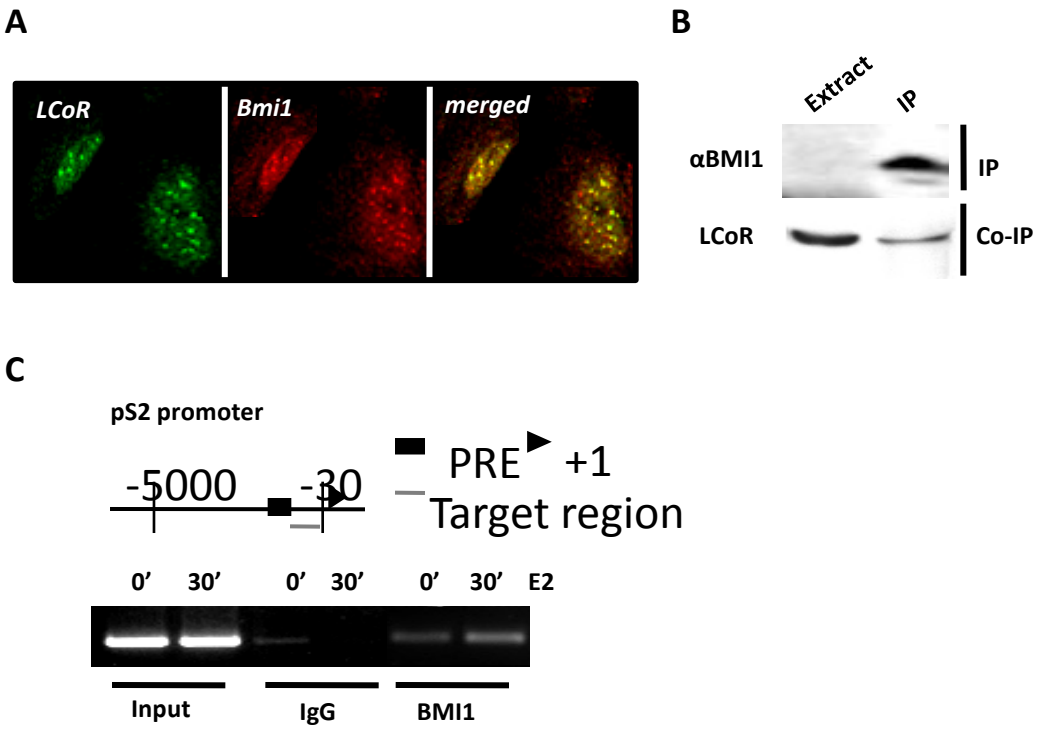
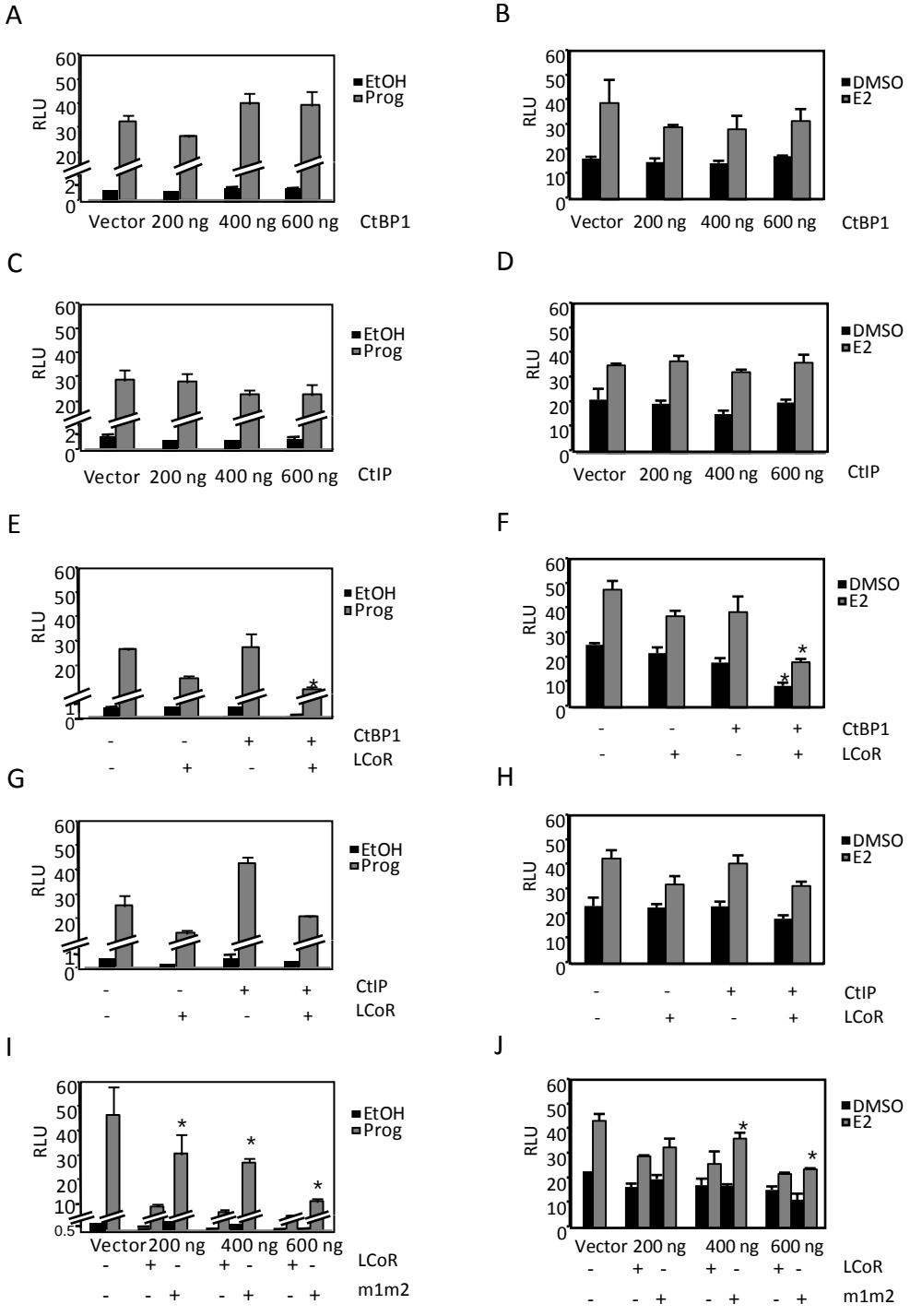
Supplemental Material



Supplemental Fig. 1. Association of LCoR with PRC1 complex marker BMI1. A. Confocal microscopic analysis of the subcellular colocalization of LCoR with BMI1 by immunocytochemistry (see Experimental Procedures for details). B. Analysis of the association of LCoR with BMI1 by coimmunoprecipitation (Co-IP). Extracts of MCF7 cells were immunoprecipitated (IP) with antibodies against BMI1, and probed by Western blotting for enrichment of target proteins in immunoprecipitates. Immunoprecipitates were also probed for the coimmunoprecipitation of LCoR. C. ChIP assays in extracts of MCF7 cells treated with E2 for 30 min. (10nM). The pS2 promoter region is depicted (upper panel). Cell extracts were collected and immunoprecipitated with IgG, or antibody against BMI1.



Palijan et al, Supplemental Fig. 2

Supplemental Fig. 2. Roles of CtBP1 and CtIP in LCoR-dependent corepression in T47D cells. Cells were transiently transfected with expression vectors for either PR (100ng) or ERα (100ng) and their corresponding reporter plasmids (250ng) for 18h. Media was then changed and cells were treated with vehicle or hormone for 30h. A and B. Dose-response curves analyzing the effects of CtBP1 on reporter gene expression in cells treated with P4 (10nM; A) or E2 (10nM; B). Increasing amounts of CtBP1 were transfected (200, 400 and 600ng). C and D. Dose-response curves analyzing the effects of CtIP on reporter gene expression in cells treated with P4 (C) or E2 (D). Increasing amounts of CtIP1 were transfected (200, 400 and 600ng). E and F. Analysis of the effects of coexpression of LCoR and CtBP1 on hormone-dependent gene expression. Cells were transiently transfected with either vector alone, LCoR alone (100ng), CtBP1 alone (200ng) or with both LCoR and CtBP1, and treated with P4 (E) or E2 (F). *, $P < 0.05$ for results of LCoR and CtBP1 coexpression versus empty vector control. G and H. Analysis of the effects of coexpression of LCoR and CtIP on hormone-dependent gene expression. Cells were transiently transfected with either vector alone, LCoR alone, CtIP1 alone or with both LCoR and CtIP1 and treated with P4 (G) or E2 (H). I and J. Dose response curves of either LCoR or m1m2 in cells treated with P4 (I) or E2 (J). Increasing amounts of wild-type or mutant LCoR were transfected (200, 400 and 600ng). *, $P < 0.05$ for results of corresponding wild-type LCoR versus mutant form m1m2.

ChIP and reChIP primer sequences

Name	Sequence
BMP7	Forward TGCAGACGACGAAAAATCAG
	Reverse AGGGGTGGGAGGTTTAGATG
BMP7 non-target control	Forward CGCTATCAGTCACCCCATTT
	Reverse CGAAAAGGCTTTGAGATTGC
IGFBP1	Forward GAGACGCTTTGCAGGAGA
	Reverse TTGCACCAGGAGGTTAATGA
IGFBP1 on-target control	Forward CTCCCTGATCACAGCTCTCC
	Reverse TCTGGAGGGGCAGTTAAGAA
pS2	Forward CTCTCACTATGAATCACTTCTGCAG
	Reverse AGATAACATTTGCCtAAGGAGGCC
pS2 non-target control	Forward CAGCCCCCAAGAACTTCCAG
	Reverse TGAGCAGGTTTGCAGCACACTT

Supplemental Table 1. ChIP and reChIP primer sequences.

QRT-PCR primer sequences

Name	Sequence	
BMP7	Forward	GGTCATGAGCTTCGTCAACC
	Reverse	GCAGGAAGAGATCCGATTCC
CYP26B1	Forward	ACATCCACCGCAACAAGC
	Reverse	GGATCTTGGGCAGGTAACTCT
FKBP51	Forward	AAAAGGCCAAGGAGCACAAC
	Reverse	TTGAGGAGGGGCCGAGTTC
GREB1	Forward	CCACAAAGGGTGGTCTCCAGAA
	Reverse	CACTGGCTTGGCCTTGCATATT
IGFBP1	Forward	GGGACGCCATCAGTACCTATG
	Reverse	GGCAGGGCTCCTTCCATTT
KRT4	Forward	GCCGACAATGACTTTGTGGT
	Reverse	CCTCCAACTCCACCTTGTTC
MUC1	Forward	GCAAGAGCACTCCATTCTCAATT
	Reverse	TGGCATCAGTCTTGGTGCTATG
pS2	Forward	ACCATGGAGACAAGGTGAT
	Reverse	AAATTCACACTCCTCTTCTG
SGK3	Forward	CAAAAGAAGATTCCACCACCA
	Reverse	TGTCAAAGTTTCTGATATCATCTC
β-actin	Forward	GGCATGGGTCAGAAGGATTCC
	Reverse	GCTGGGGTGTTGAAGGTCTC

Supplemental Table 2. QRT-PCR primer sequences.