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Supplemental Data

Ovaries and Female Phenotype in a Girl with 46,XY Karyotype and Mutations in the *Cbx2* Gene

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Figure 1: **: A**: qualitative end point reverse transcriptase PCR of RNA extracted form NT2-D1 cells that were either non transfected (control,) transfected with *CBX2* alone, *CBX2* in combination with two siRNAs (*CBX2* + siRNAs) or their scrambles (*CBX2* + scrambles). Whereas NT2-D1 cells do not express endogenous *CBX2*, overexpression of *CBX2* induces an increase in mRNA of *CBX2* itself (q.e.d.) and of other genes involved in sexual differentiation, *SRY*, *SF1* and *SOX9*. SiRNA treatment significantly down-regulated the expression of these genes and of *CBX2*, whereas treatment with scrambles does not. *GAPDH* was used as internal control. **B**: Western blot analysis of CBX2 protein in extracts of NT2-D1, transfected and treated as in A. An anti-M33/CBX2 commercially available antibody (αM33) was used. As for the mRNA, CBX2 protein expression is also significantly reduced under siRNA treatment, but not after scrambles transfection. Beta Actin was used as internal control. **C**: quantitative Real-Time PCR of mRNA extracted from NT2-D1 treated as in A. The results are the mean +/- SD of three measurements and are expressed as relative to control =1. The results mirror those of the RT-PCR experiments, i.e. siRNA treatment reduces *CBX2* expression to almost baseline. *SF1, SRY* and *SOX9* expression was also dowregulated by siRNA treatment, but not by transfection of scrambles. 1-way ANOVA: $p<0.0001$ for *CBX2, SRY* and *SF1*; $p<0.0343$ for *SOX9*. t-test: *CBX2* : control *vs CBX2* (and *CBX2* + scrambles) $p<0.0001$, CBX2 (and CBX2 + scrambles) *vs* CBX2+ siRNAs $p<0.0001$; SRY: control vs CBX2 (and *CBX2* + scrambles) $p<0.0001$, *CBX2* (and *CBX2* + scrambles) vs *CBX2* + siRNAs $p=0.0025$; *SF1* : control *vs CBX2* (and *CBX2* + scrambles) $p<0.0001$, *CBX2* (and *CBX2* + scrambles) *vs CBX2*+ siRNAs $p<0.0001$; *SOX9* : control *vs CBX2* (and *CBX2* + scrambles) $p=0.012$, *CBX2* (and *CBX2* + scrambles) *vs CBX2*+ siRNAs $p=0.05$.

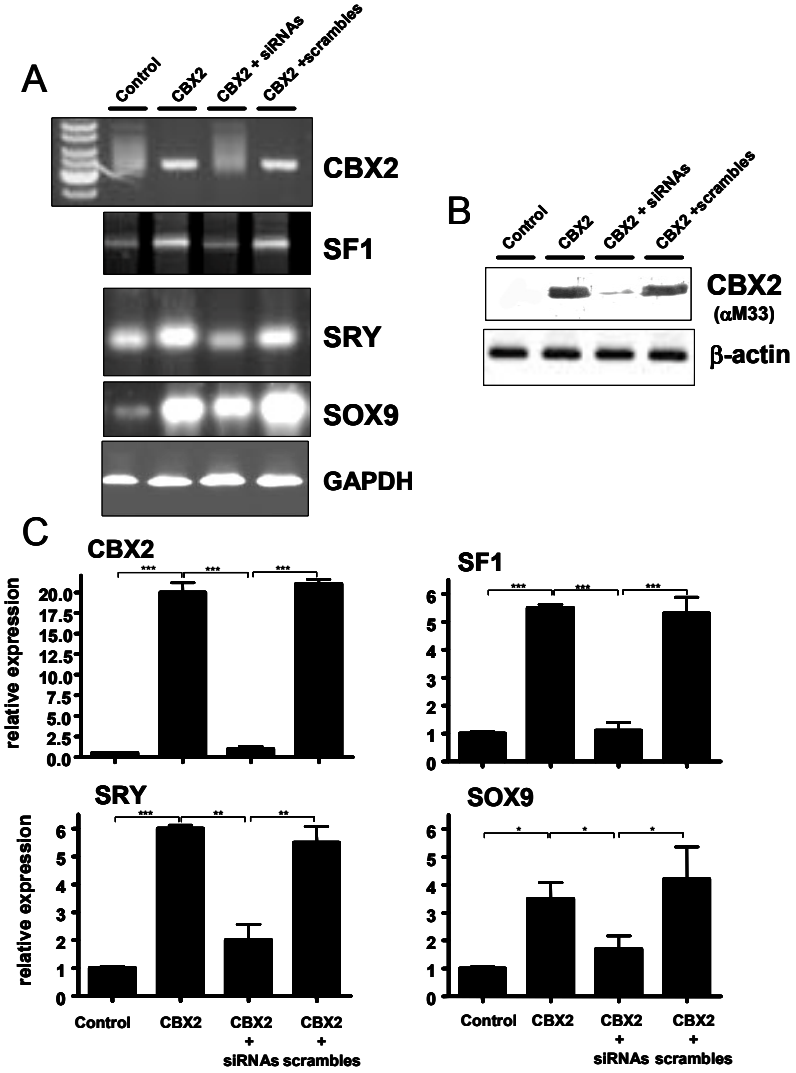

Table S1: CBX2 Primers

AMPLIFICATION

Oligo designation	**Isoform**	**Exon**	**Sequence**	**Size**
CBX2-ex1-2-5′ CBX2-ex1-2-3′	1 & 2	1-2	CCCCAGGCCCGAAGTCCCTA GCACCCGCGGGCGTGTAAAC	770
CBX2-ex3-5′ CBX2-ex3-3′	1 & 2	3	TTGGGTCTGAGGTTAGAGTG GATGGCCCTGGACACAATCT	414
CBX2-ex4.1/2-5′ CBX2-ex4.1-3′ CBX2-ex4.2-3′	1 & 2 1 2	4	TGATCTCTGCCTTGGGTATT GTCAGCCCTGCCTTTCGTGG TGACAGGACAGTCCCGAGAA	216 817
CBX2-ex5-1-5′ CBX2-ex5-1-3′ CBX2-ex5-2-5′ CBX2-ex5-2-3′ CBX2-ex5-3-5′ CBX2-ex5-3-3′ CBX2-ex5-4-5′ CBX2-ex5-4-3′	1	5 PCR1 PCR2 PCR3 PCR4	GAAACAGGGCTCCACTATGT GATCCTTCCGGGCGGTCTTC GCAACCCGAAGACCCGTGAG GGCATGCCATTCTTGACACT GCAGAAAGTGGGGAACACAG TGCCCTTGACACAGTCCCTC GTGTCAAGGGCAGTGCTACC TTGGGCTGGGAGTCACTTAG	427 575 347 391

Table S2: Real-Time PCR primers:

TARGET	**PRIMERS SEQUENCE**	**SIZE**
SF1	DIR: GCACCCACAGTCGCCACCGTCCC REV. AGCTCGTCCAGGTCCTCGTCGTA	154
WNT4	DIR: ACCTGGAAGTCATGGACTCGGTG REV: CCGCCTCCCGAGTCCCTTGCGTC	150
SRY	DIR: TACGGGCCTTTACCTGGTTACCTC REV: CTGGTGTGCTACTTACGCAAGTAC	302
WT1	DIR GGTGTCTTCAGAGGCATTCAGGA REV:GCAGCCTGGGTAAGCACACAT	151
SOX9	DIR: GGCTACGACTGGACGCTGGT REV: TGCTGAGCTCGGCGTTGTGC	163
DAX1	DIR: GTACCAAGGAGTACGCCTACCTC REV: CATGGGGCCCCTTGGTGCGTCATC	150
CBX2.1	DIR. AAGAGGGACTGTGTCAAGGG REV: GAGTCGGGGTCGGAGTCCGA	139

Table S3: Chromatin Immunoprecipitation primers

Fragment 1	dir	actggcctgtcctgactctactcc
	rev	ttgttggtgtttgtgttcatttacgtg
324 bp (-465/-85)		
Fragment 2	dir	caacaaagaacaccagggcg
	rev	atacacagcaagactcagaga
651 bp (-85/+ 540)		
Fragment 3	dir	ccgtgtgtctgtctccggcatcc
171 bp (+239/410)	rev	gtggatgcctggccagagaca
Fragment 4	dir	acaatcttggctcactgcaacctctgc
	rev	ggaggcaaggccatggcgacttgac
767 bp (-1232/-465)		
Fragment 5	dir	ggggtccgcgcgggggagc
	rev	gaggagctctgccgaccctgt
920 bp (exon 2 8761-9681)		