Supplementary Material

Cholesterol Triggers Nuclear Co-Association of Androgen Receptor, p160 Steroid Coactivators, and p300/CBP-Associated Factor Leading to Androgenic Axis Transactivation in Castration-Resistant Prostate Cancer

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Supplementary Fig. 1A. Profile Report PC3 Cell Line

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Diretoria Técnica de Apoio ao Ensino e Pesquisa

AUTENTICAÇÃO DE LINHAGENS
Laudo do Perfil de STR´s / STR Profile Report

INFORMAÇÃO DO CLIENTE / *CUSTOMER INFORMATION*

Nome / *Name*	IRAN AMPRIM DA SILVA
Instituição / *Institution*	HCFMUSP
Departamento / *Department*	CIRURGIA
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E-mail	iransilva@gmail.com

Nome da Linhagem / *Cell line name*	PC3 (ATCC CRL-1435)

Amostra recebida em / *Samples received in* 00-00-0000	**Data do laudo** / *Report date*: 16-12-2019

Loci	**Resultado do Teste** / *Test Result* (**amostra**/*sample*)				**Perfil da referência no Banco de Dados** / *Reference Database Profile*				
	Perfil da Amostra / *Query Profile* PC3				**Perfil da referência** / *Database Profile* PC3 (ATCC CRL-1435)				
TH01	6	7			6	7			
TPOX	8	9			8	9			
vWA	17				17				
CSF1PO	11				11				
D16S539	11				11				
D7S820	8	11			8	11			
D13S317	11				11				
D5S818	13				13				
D21S11									
Amelogenin	x				x				

Número de alelos iguais entre a amostra e referência / *Number of shared alleles between query sample and database profile*:	12
Total de alelos na amostra referência / *Total number of alleles in the database profile*:	12
Porcentagem de correspondência entre alelos da amostra e da referência / *Percent match between the submitted sample and the database profile*:	100

RESULTADO / *RESULT*
Linhagens celulares com uma porcentagem de correspondência >=80% são consideradas relacionadas, ou seja, derivada de um ancestral comum. Linhagens com porcentagem de correspondência entre 55% e 80% necessitam de novos testes (maior número de marcadores) para confirmar sua autenticidade. / *Cell lines with >=80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness. (ATCC - STR Profile Report)*

METODOLOGIA / *METHODOLOGY*
10 STR's, correspondendo aos loci TH01, TPOX, vWA, CSF1PO, D16S539, D7S820, D13S317, D5S818, Amelogenin e D21S11 (GenePrint 10 / Promega), foram amplificados e submetidos à eletroforese capilar para separação dos fragmentos (3730 DNA Analyzer - Applied Biosystems). Os dados foram analisados através do software XXX GeneMaker HDI v.1.1.0 (Softgenetics LCC). Controles positivos e negativos apropriados foram utilizados na validação de cada amostra. / *10 STR's (TH01, TPOX, vWA, CSF1PO, D16S539, D7S820, D13S317, D5S818, Amelogenin and D21S1-GenePrint 10 / Promega) are co-amplified and submitted to capillary electrophoresis (3730 DNA Analyzer - Applied Biosystems). An internal lane standard (ILS) and allelic ladder are provided for sizing and genotyping of amplified fragments, and the 2800M Control DNA is supplied as a positive control (GenePrint 10 / Promega). Data is analyzed using software GeneMaker HDI v.1.1.0 (Softgenetics LCC).*

INTERPRETAÇÃO DOS DADOS / *INTERPRETATION OF DATA*
Os resultados foram interpretados segundo as diretrizes do ANSI Standards 2011 (ASN-0002 / ATCC Standards Development Organization) / *Results were interpreted as described in ANSI Standards 2011 (ASN-0002 / ATCC Standards Development Organization / ATCC STR Profile Report)*

☐ **A linhagem enviada para análise é de origem humana, mas o seu perfil não corresponde a nenhuma linhagem padrão presente nos bancos de dados analisados** */ The submitted sample profile is of human origin, but not a match for any profile in the STR database*

☒ **A linhagem enviada para análise corresponde exatamente à seguinte linhagem celular humana** */ The submitted profile is an exact match for the following human cell lines)*:
PC-3 Prostate Adenocarcinoma Human (ATCC:CRL-1435)

☐ **O perfil de marcadores da linhagem enviada é similar à(s) seguinte(s) linhagem(s) celular(es) humana(s)** */ The submitted profile is similar to the following ATCC human cell line(s)*:

OBSERVAÇÕES */ Additional Comments*
https://www.atcc.org/search-str-database

Responsável Técnico: Christian Albert Merkel
Email: c.merkel@fm.usp.br

Supplementary Fig. 1B. Profile Report LNCAP Cell Line

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AUTENTICAÇÃO DE LINHAGENS
Laudo do Perfil de STR´s / STR Profile Report

INFORMAÇÃO DO CLIENTE / *CUSTOMER INFORMATION*

Nome / *Name*	IRAN AMPRIM DA SILVA
Instituição / *Institution*	HCFMUSP
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Nome da Linhagem / *Cell line name*	LNCAP (ATCC CRL-1740)

Amostra recebida em / *Samples received in* 00-00-0000	**Data do laudo** / *Report date*: 16-12-2019

Loci	**Resultado do Teste** / *Test Result* (**amostra**/*sample*)				**Perfil da referência no Banco de Dados** / *Reference Database Profile*				
	Perfil da Amostra / *Query Profile* LNCAP				**Perfil da referência** / *Database Profile* LNCaP clone FGC Prostate Carcinoma Human (ATCC:CRL-1740)				
TH01	9				9				
TPOX	8	9			8	9			
vWA	16	18			16	18			
CSF1PO	10	11			10	11			
D16S539	10	11			11				
D7S820	8	9	9.1	10.3	9	9.1	10.3		
D13S317	10	12			10	12			
D5S818	10	11	12		11	12			
D21S11									
Amelogenin	x	y			x	y			
Número de alelos iguais entre a amostra e referência / *Number of shared alleles between query sample and database profile*:									17
Total de alelos na amostra referência / *Total number of alleles in the database profile*:									17
Porcentagem de correspondência entre alelos da amostra e da referência / *Percent match between the submitted sample and the database profile*:									100

RESULTADO / *RESULT*
Linhagens celulares com uma porcentagem de correspondência >=80% são consideradas relacionadas, ou seja, derivada de um ancestral comum. Linhagens com porcentagem de correspondência entre 55% e 80% necessitam de novos testes (maior número de marcadores) para confirmar sua autenticidade. */ Cell lines with >=80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness. (ATCC - STR Profile Report)*

METODOLOGIA / *METHODOLOGY*
10 STR's, correspondendo aos loci TH01, TPOX, vWA, CSF1PO, D16S539, D7S820, D13S317, D5S818, Amelogenin e D21S11 (GenePrint 10 / Promega), foram amplificados e submetidos à eletroforese capilar para separação dos fragmentos (3730 DNA Analyzer - Applied Biosystems). Os dados foram analisados através do software XXX GeneMaker HDI v.1.1.0 (Softgenetics LCC). Controles positivos e negativos apropriados foram utilizados na validação de cada amostra. */ 10 STR's (TH01, TPOX, vWA, CSF1PO, D16S539, D7S820, D13S317, D5S818, Amelogenin and D21S1-GenePrint 10 / Promega) are co-amplified and submitted to capillary electrophoresis (3730 DNA Analyzer - Applied Biosystems). An internal lane standard (ILS) and allelic ladder are provided for sizing and genotyping of amplified fragments, and the 2800M Control DNA is supplied as a positive control (GenePrint 10 / Promega). Data is analyzed using software GeneMaker HDI v.1.1.0 (Softgenetics LCC).*

INTERPRETAÇÃO DOS DADOS */ INTERPRETATION OF DATA*
Os resultados foram interpretados segundo as diretrizes do ANSI Standards 2011 (ASN-0002 / ATCC Standards Development Organization) */ Results were interpreted as described in ANSI Standards 2011 (ASN-0002 / ATCC Standards Development Organization / ATCC STR Profile Report)*

☐ **A linhagem enviada para análise é de origem humana, mas o seu perfil não corresponde a nenhuma linhagem padrão presente nos bancos de dados analisados** */ The submitted sample profile is of human origin, but not a match for any profile in the STR database*

☒ **A linhagem enviada para análise corresponde exatamente à seguinte linhagem celular humana** */ The submitted profile is an exact match for the following human cell lines)*:
LNCaP clone FGC Prostate Carcinoma Human (ATCC:CRL-1740)

☐ **O perfil de marcadores da linhagem enviada é similar à(s) seguinte(s) linhagem(s) celular(es) humana(s)** */ The submitted profile is similar to the following ATCC human cell line(s)*:

OBSERVAÇÕES */ Additional Comments*
https://www.atcc.org/search-str-database

Responsável Técnico: Christian Albert Merkel
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Supplementary Table 1. TaqMan™ Assay tables were used in the study.

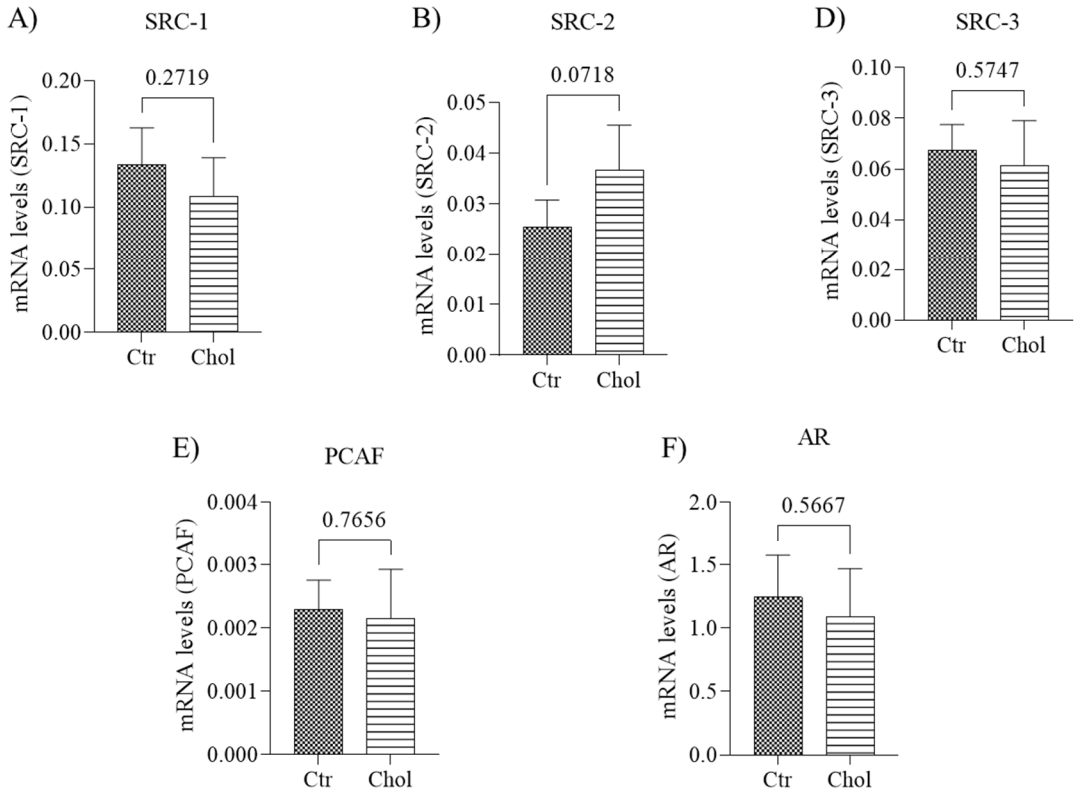
Genes	Assay	Company
SRC-1	Hs00186661_m1	Applied Biosystems
SRC-2	Hs00896109_m1	Applied Biosystems
SRC-3	Hs00180722_m1	Applied Biosystems
PCAF	Hs00918445_g1	Applied Biosystems
AR	Hs00171172-m1	Applied Biosystems
GAPDH	Hs99999905	Applied Biosystems

Supplementary Table 2. Antibody tables used in work.

Proteins	Cat	Company
SRC-1	128E7	Cell Signaling
SRC-2	D2X4M	Cell Signaling
SRC-3	5E11	Cell Signaling
PCAF	C14G9	Cell Signaling
AR*	SC-816	Santa Cruz Biotechnolgy
AR#	MA5-13426	Invitrogen
GAPDH	D16H11	Cell Signaling

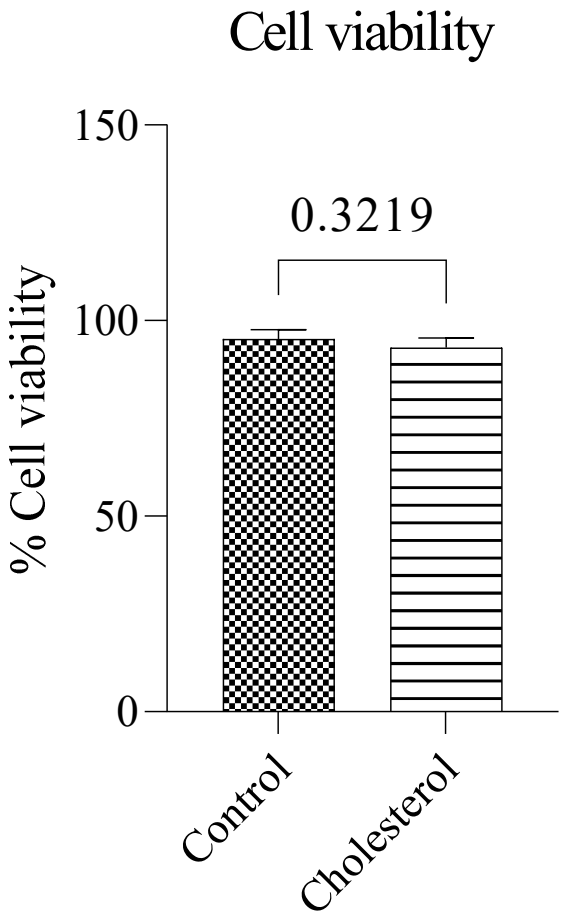
*Western Blot # Immunofluorescence

Supplementary Fig. 2. Effect of supplementation of LNCaP cells with cholesterol (Chol) (2µg/mL) for 8h on the expression of AR coactivator genes.



Effect of supplementation of LNCaP cells with cholesterol (Chol) (2µg/mL) for 8h on the expression of AR coactivator genes. **A)** Expression levels of SCR-1 genes in the absence and presence of Chol. **B)** Expression levels of SCR-2 gene in the absence and presence of Chol. **C)** SRC-3 gene expression levels in the absence and presence of Chol. **D)** PCAF gene expression levels in the absence and presence of Chol. **E)** Expression levels of the AR gene in the absence and presence of Chol. The p-values obtained from the statistical analyses are shown above the bars in each panel, and the error bar corresponds to the samples' standard deviation. T-test was used in all analyses.

Supplementary Fig. 3. Cell viability assay



Cell viability assay by flow cytometry. The cell viability assay demonstrated that supplementing PC-3 cells with 2ug of cholesterol for 8 hours does not alter cell viability ($p = 0.3219$). The p-values obtained from the statistical analyses are shown above the bars in each panel, and the error bar corresponds to the samples' standard deviation. The T-test was used in the analysis.

Supplementary Table 3. Weights of the animals.

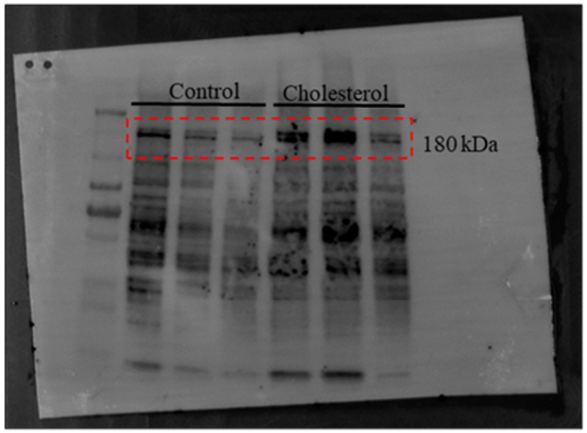
Measurements	CTR		HCOL		P-Value
	Mean	Mean rank	Mean	Mean rank	
1	23.98	11.55	23.82	11.45	>0.999
2	25.04	12.27	24.53	10.73	0.594
3	25.46	11.45	24.9	11.55	>0.999
4	25.68	12.68	25.16	10.32	0.410
5	25.78	11.95	25.46	11.05	0.761
6	25.17	11.23	25.45	11.77	0.860
7	25.12	10.64	25.67	12.36	0.561
8	25.11	9.409	26.43	13.59	0.137
9	24.92	9.909	26.15	13.09	0.270
10	25.04	10.09	26	12.91	0.323
11	25.3	10	26.02	13	0.299
12	25.13	10.5	25.79	12.5	0.488

Supplementary Table 4. Tumor Volume.

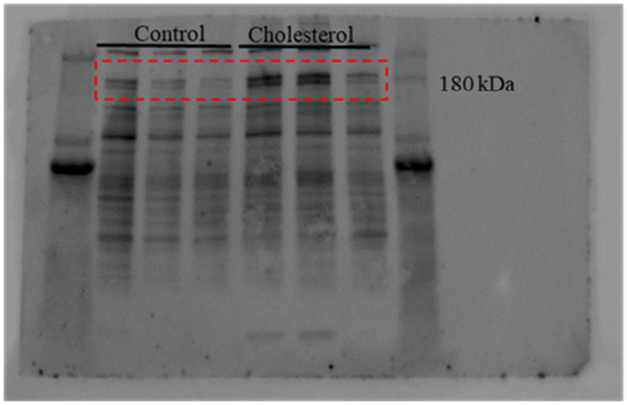
Measurements	CTR		HCOL		P-Value
	Mean	Mean rank	Mean	Mean rank	
1	6.28	8.18	16	14.8	**0.015**
2	9.34	7.18	27	15.8	**0.001**
3	15.8	7	39	16	**0.0005**
4	27.9	8.36	49.7	14.6	**0.023**
5	40.4	7.73	78.6	15.3	**0.005**
6	50.1	7.64	105	15.4	**0.004**
7	62.5	7.64	128	15.4	**0.004**
8	83.9	7.73	158	15.3	**0.005**
9	108	7.64	214	15.4	**0.004**
10	119	7	248	16	**0.0005**
11	138	7.27	300	15.7	**0.001**
12	149	6.73	346	16.3	**0.0001**

Supplementary Fig. 4. Raw data of AR coactivator's protein expression and the internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

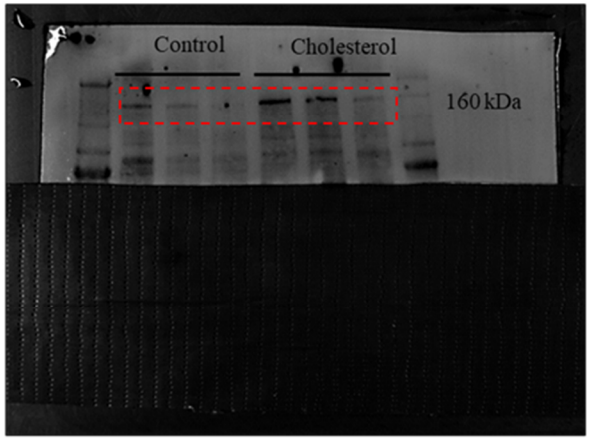
SRC-1



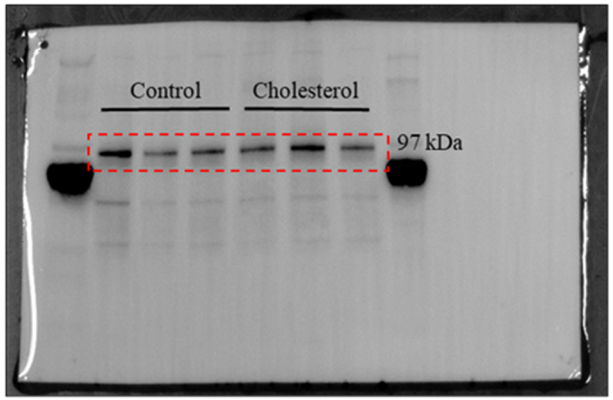
SRC-2



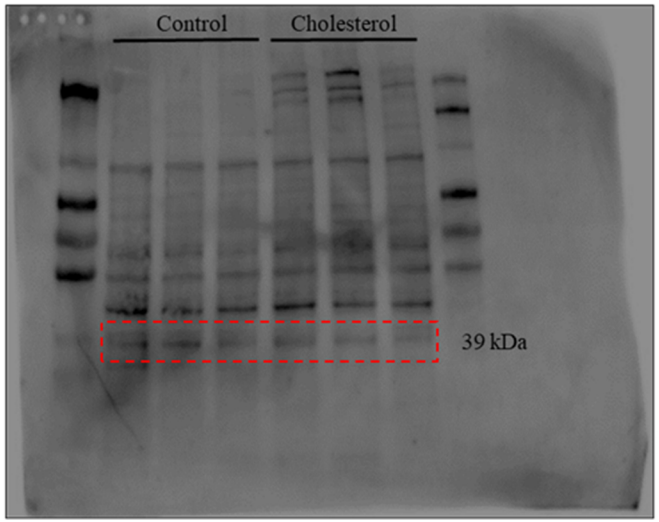
SRC-3



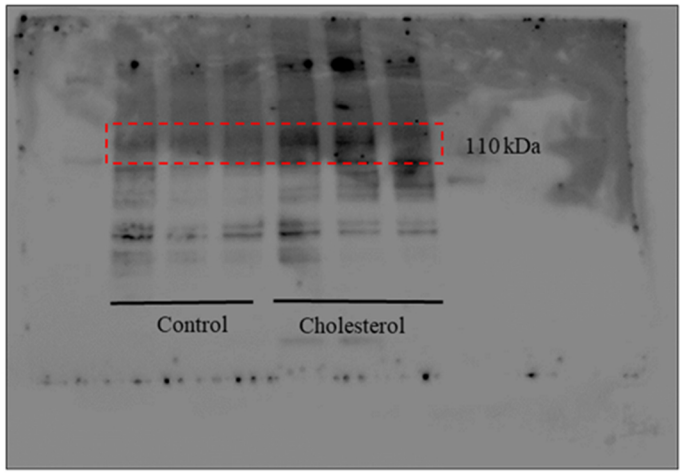
PCAF



SRD5A1



AR



GAPDH