Supporting Information

The CUE1 domain of the SNF2-like chromatin remodeler SMARCAD1 mediates its association with KRAB-associated protein 1 (KAP1) and KAP1 target genes

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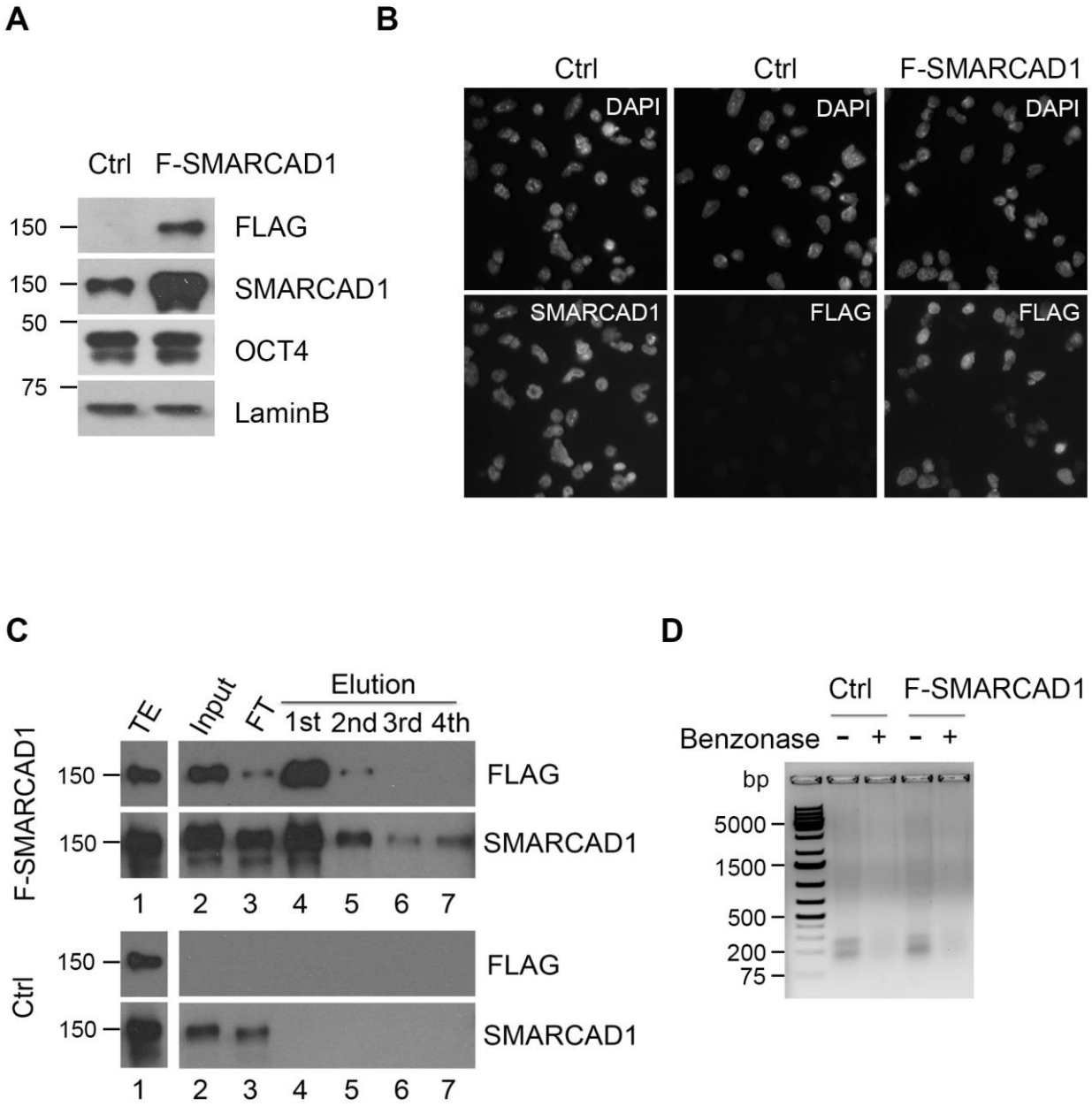
List of material included:

Figure S1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150
FLAG
150
SMARCAD1
50
OCT4
75
LaminB
B
Ctrl
Ctrl
F-SMARCAD1
DAPI
DAPI
DAPI
SMARCAD1
FLAG
FLAG
C
TE
Input
FT
Elution
1st 2nd 3rd 4th
F-SMARCAD1
150
FLAG
150
SMARCAD1
1 2 3 4 5 6 7
Ctrl
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FLAG
150
SMARCAD1
1 2 3 4 5 6 7
D
Ctrl
F-SMARCAD1
Benzonase - + - +
bp
5000
1500
500
200
75

Supplemental Fig. S1, related to Fig. 1: Characterization of FLAG-SMARCAD1 expressing ESCs and purification.

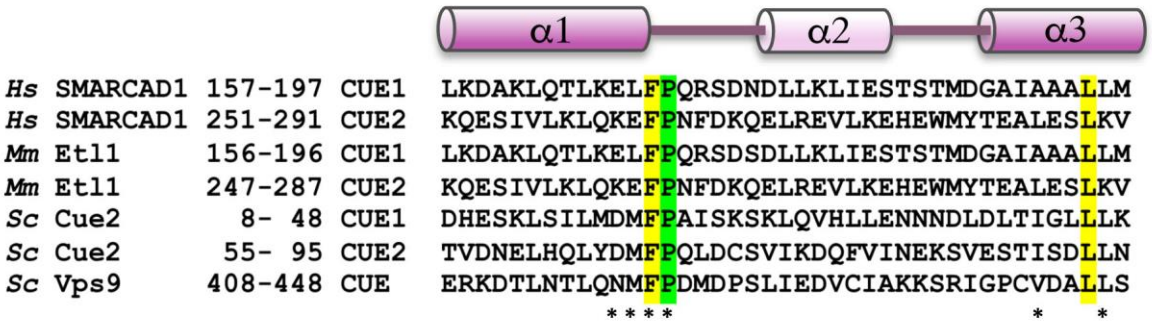
A, Levels of 1xFLAG tagged SMARCAD1 (F-SMARCAD1) compared to endogenous SMARCAD1 levels in a PGK12.1 ESC line transfected with the empty vector (Ctrl). Total extracts (8 μg) were analysed by Western blot with indicated antibodies. MW markers are indicated. Lamin B1 serves as a loading control. Pluripotency marker OCT4 levels are unchanged.

B, Immunofluorescence detection of the FLAG tag shows that epitope tagged SMARCAD1 is nuclear and expressed in most cells of the population. Localization of tagged SMARCAD1 is comparable to endogenous SMARCAD1. Nuclei were stained with DAPI.

C, FLAG-SMARCAD1 is purified from PGK12.1 ESC nuclear extract by anti-FLAG affinity chromatography and detected by immunoblotting. Lane 1, TE total extract, reference sample from FLAG SMARCAD1 ESCs; lane 2, input (0.1%); lane 3, FT flow through (0.1%); lanes 4-7, sequential elutions with FLAG peptide (7.5%). Lower panel: Control purification from cells not expressing FLAG-SMARCAD1 showing endogenous SMARCAD1. Lane 1, TE from FLAG SMARCAD1 ESCs. MW markers are indicated.

D, Benzonase treatment during FLAG affinity purification removed DNA. DNA was purified from aliquots of nuclear extracts (150 μg) before and after FLAG-purification to assess DNA degradation by benzonase incubation and analysed by agarose gel electrophoresis.

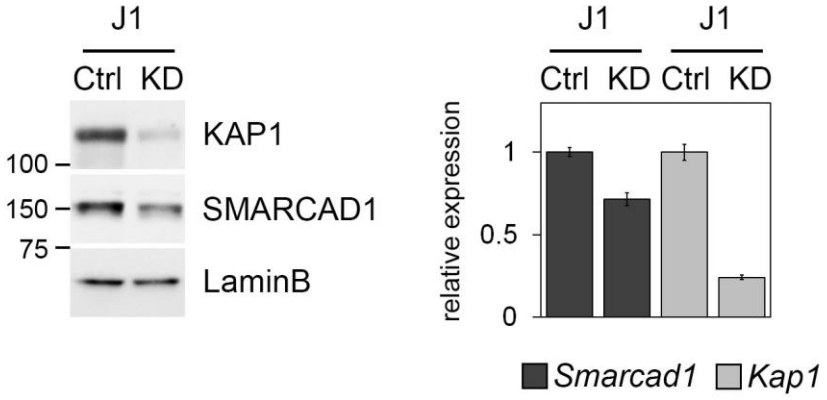
Figure S2



Supplemental Fig. S2, related to Fig. 2: Conserved CUE domain residues.

Alignment of CUE domain sequences, comparing human SMARCAD1, mouse SMARCAD1 (ETL1) with *S. cerevisae* ubiquitin binding protein CUE2 and vacuolar protein sorting-associated protein VSP9. The prototypical CUE secondary structure comprises three alpha helices as indicated above the sequence comparison. The consensus CUE motif of about 40 amino acids includes an invariant proline at the end of helix 1 and a di-leucine motif in helix 3 (1). Asterisks mark residues that make direct contact with ubiquitin in CUE2 and VPS9 (2,3). Conserved residues mutated in this study - P170, F169 and L196 in SMARCAD1 - are highlighted.

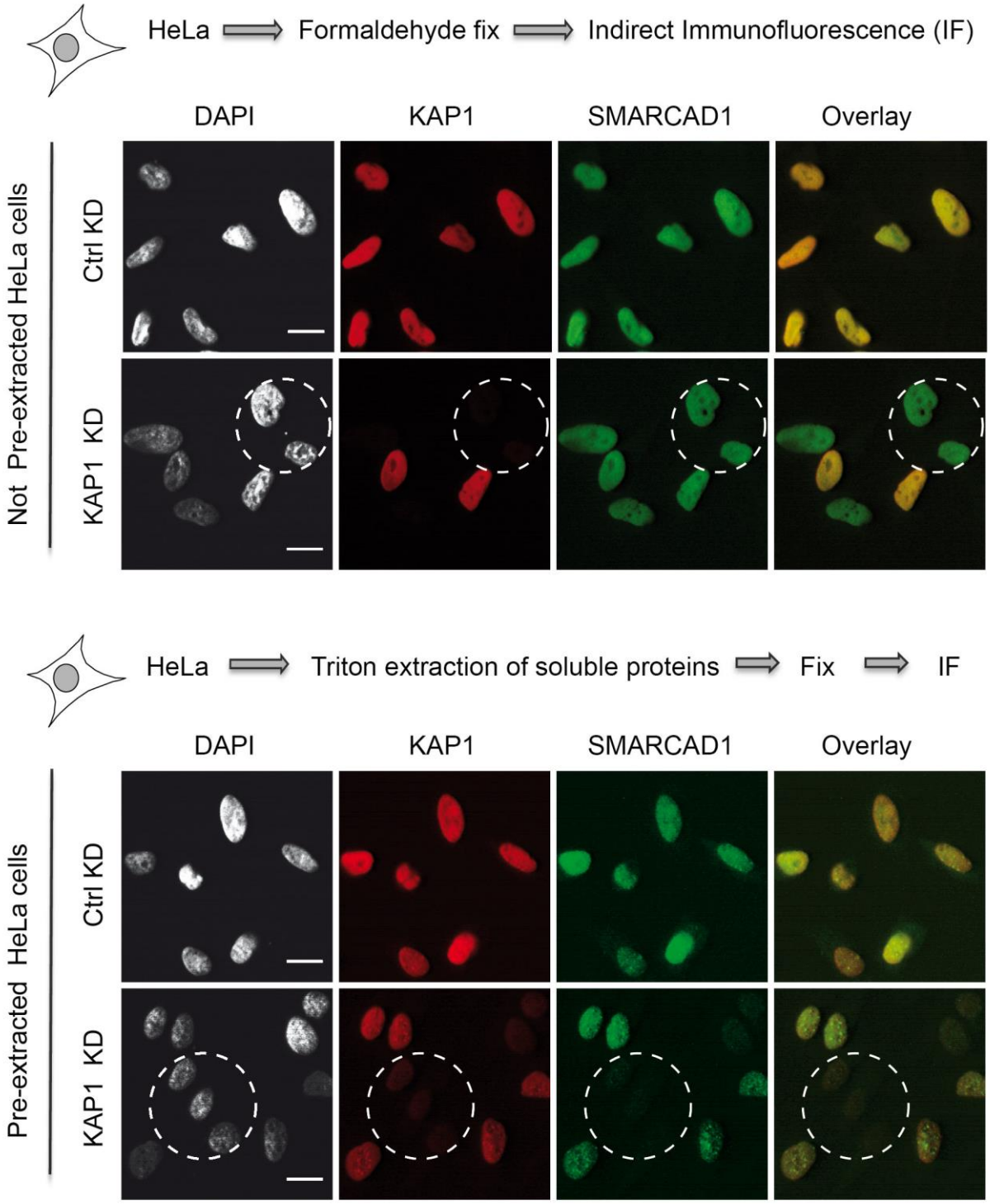
Figure S3



Supplement Fig. S3, related to Fig. 3: Depletion of KAP1 leads to reduced SMARCAD1 protein levels in J1 ESCs.

J1 ESCs were transfected with esiRNAs against FLUC (Ctrl) or Kap1 (KD) for 3 days prior to analysis by Western blot (left panel) or qRT-PCR (right panel). Clear reduction in KAP1 RNA and protein levels was observed. Quantification of SMARCAD1 protein revealed a 39% drop compared to the Ctrl knockdown. Right panel: qRT-PCR analysis of Smarcad1 and Kap1 in the same samples as to the left. Gene expression was normalized to the average of two housekeeping genes and data are presented as mean ± S.E. of three technical replicates.

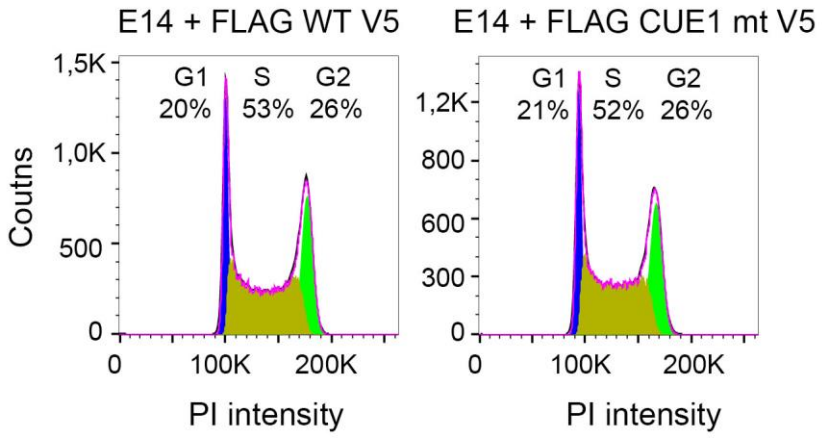
Figure S4


HeLa
Formaldehyde fix
Indirect Immunofluorescence (IF)
DAPI
KAP1
SMARCAD1
Overlay
Not Pre-extracted HeLa cells
Ctrl KD
KAP1 KD
HeLa
Triton extraction of soluble proteins
Fix
IF
DAPI
KAP1
SMARCAD1
Overlay
Pre-extracted HeLa cells
Ctrl KD
KAP1 KD

Supplemental Fig. S4, related to Fig. 4: SMARCAD1 protein becomes readily extractable upon KAP1 depletion in HeLa cells.

Stable depletion of KAP1 by shRNA in HeLa cells (KD) compared to cells expressing a non-specific shRNA (Ctrl). The KD pool allows for detection of untransfected and transfected cells in parallel. Indirect immunofluorescence for KAP1 and SMARCAD1 proteins as in Figure 4C. Top: Cells were formaldehyde fixed prior to permeabilisation (not pre-extracted). Bottom: Extraction of soluble proteins prior to fixation (pre-extracted). Dotted circle; example of SMARCAD1 localisation in nuclei depleted for KAP1. A representative example is shown, similar results were obtained with an independent KAP1 KD clone. Scale bar represents 10 μm.

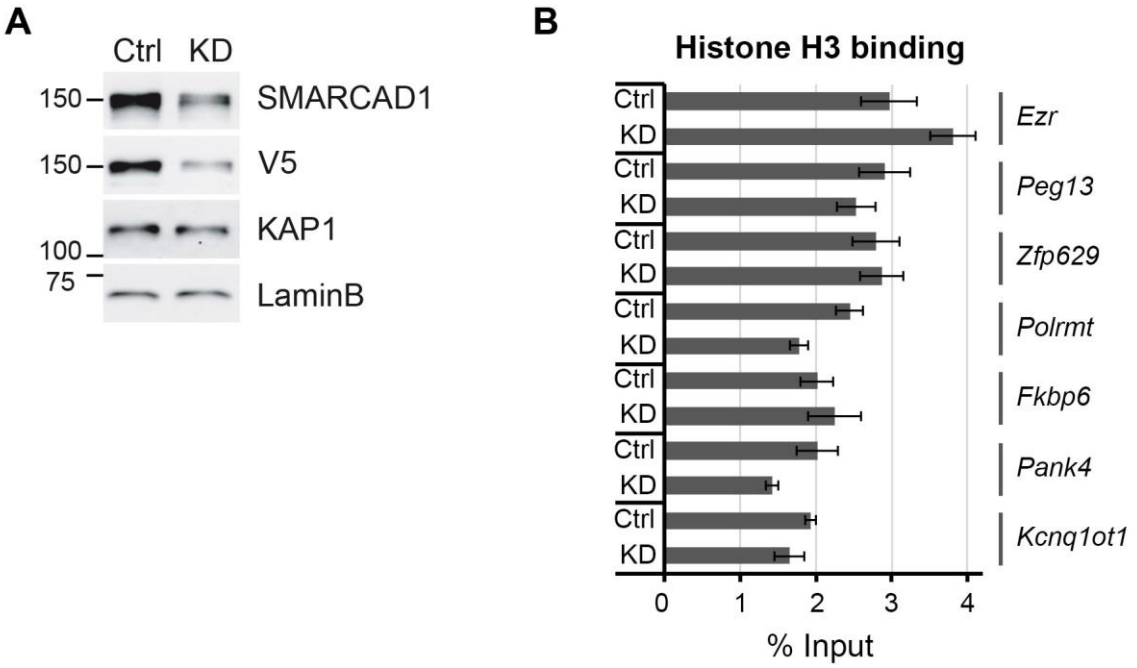
Figure S5



Supplemental Fig. S5, related to Fig. 5: ES cells with a mutated CUE1 domain of SMARCAD1 (F169K, L196K) show no apparent alterations in their cell cycle profile.

FACS analysis of E14 ES cells expressing tagged WT or CUE1 mutated SMARCAD1 shows similar cell cycle distribution. Cells were treated with doxycycline for 3 days to deplete endogenous SMARCAD1 levels.

Figure S6


A
Ctrl
KD
150
SMARCAD1
150
V5
KAP1
100
75
LaminB
B
Histone H3 binding
Ctrl
KD
Ezr
Peg13
Zfp629
Polrmt
Fkbp6
Pank4
Kcnq1ot1
0
1
2
3
4
% Input

Supplemental Fig. S6, related to Fig. 7: H3 occupancy in ES cells that lack or express SMARCAD1 protein or variants thereof.

A, Characterization of ESCs expressing epitope tagged wild-type SMARCAD1 after transient depletion of both endogenous and V5 epitope tagged SMARCAD1. Control FLUC esiRNAs (Ctrl) or specific esiRNAs targeting Smarcad1 (KD) were transfected into E14 ESCs expressing tagged SMARCAD1 WT protein. Western blot analysis of SMARCAD1 and KAP1 was performed 2 days after the transfection; Lamin B1 antibody was used as loading control.

B, Shown are the binding patterns of H3 on KAP1 target genes in E14 ESCs expressing tagged SMARCAD1. Chromatin analysed corresponds to samples from Fig.7, left panel; namely chromatin prepared from cells expressing tagged SMARCAD1 WT protein and transfected with a control esiRNAs (Ctrl) or specific esiRNAs targeting Smarcad1 (KD). ChIP results represent the mean ± S.E. of technical triplicates.

Supplemental Table 1 Primers used in this study

ChIP qPCR primers	**Sequence (5' to 3')**	**Reference**
Peg13 f	ACACTGGATGGAGACTTGCAG	(4)
Peg13 r	CGCGGTGTACCGAGTATTTG	
Peg3 f	CACCGCCACTGCGGCAAAAC	(5)
Peg3 r	GGCTGGCAGGGTCTTCGCAA	
Fkbp6 f	ATCTTGCCGCACAACTGTCT	(6)
Fkbp6 r	GATCACGTGCCGTTTCTCTC	
Intergenic ctrl f	CAGCATTCCAGGAGGTTAGC	(7)
Intergenic ctrl r	GTGCCTCATGTGCAGTCAGT	
ZFP629 f	TTGGCTCCTGGTGGCGGAGT	(5)
ZFP629 r	GCCCGGAGAGCTCAGGGTGA	
ZFP13 f	GGGTGCGATTATGTGCGATG	This study
ZFP13 r	GTCAAGCCCTACCCGTGC	
Polrmt f	GCTCGTGACTCCATACCGTC	This study
Polrmt r	GCCTCAAGAAGGGAGACTCG	
Pank4 f	TCAGCACACAGGCTGCTTTT	This study
Pank4 r	GAACACCAGGTCTCTCAGGC	
Ezr f	GGCCCCGTAACTGCTCTTTA	This study
Ezr r	AGTATAAGACGCTGCGGCAA	
Kcnq1ot1 f	ACCGAGCCGTAACTGCAAAA	This study
Kcnq1ot1 r	AAGCAGAGGTGATTCGTGCC	

RT qPCR primers	**Sequence (5' to 3')**	**Reference**
Gapdh f	TCCATGACAACTTTGGCATTG	(8)
Gapdh r	CAGTCTTCTGGGTGGCAGTGA	
Atp5b f	GGCCAAGATGTCCTGCTGTT	(9)
Atp5b r	GCTGGTAGCCTACAGCAGAAGG	
Hsp90ab1 f	GCTGGCTGAGGACAAGGAGA	(9)
Hsp90ab1 r	CGTCGGTTAGTGGAATCTTCA	
KAP1 f	CGGAAATGTGAGCGTGTTCTC	(8)
KAP1 r	CGGTAGCCAGCTGATGCAA	
Smarcad1 f	TTCCTGGCATACCTCTTTC	This study
Smarcad1 r	ATTTGCTTACGCTCTTCTTG	

Supplemental References

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