### SUPPLEMENTARY MATERIALS

#### Methods

#### **RNA** labeling and microarray hybridization

Total RNA was isolated using the RNEAsy mini kit from Qiagen. RNA was checked for quality and yield, measuring the RNA integrity number (RIN) (Agilent technologies). The RINs of the samples were between 9 and 10. The Ambion Illumina RNA amplification kit with biotin UTP (Life Technologies, Ca#AMIL1791) labeling was used to amplify the RNA. The cRNA was then checked for yield using the Bio-Rad Experion system. Illumina Mouse-6 V1 BeadChip (Illumina, Ca#BD-201-0202) mouse whole-genome expression arrays were used in this study. Two samples were hybridized twice and used as technical replicates. A total of 1.5 µg of cRNA was hybridized for each array using standard Illumina protocols with streptavidin-Cy3 (Sigma-Aldrich, Ca#PA43001) being used for detection.

#### **Proliferation assays**

Cells were seeded at  $1 \times 10^5$  cells per well in 6-well plates. Cells were infected with the UHRF1 shRNA lentiviruses and let grow to achieve efficient silencing for three days. Efficiency of knockdown and overexpression of UHRF1 was assessed by RT-qPCR and Western blots, and further triplicate counts taken on days 4, 6, and 8, with medium being replaced on days 2 and 4.

### **Decoy construct generation**

Complimentary oligos harboring two tandem miR-145 or miR-143 complementary sequences were annealed in vitro and placed at the 3' to the GFP gene as previously described (1, 2).

### Luciferase reporter assay

UHRF1-3'-UTR was cloned downstream of the Renilla report gene in the psiCHECK-2 plasmid (Promega) and transfected in miR-145-overexpressing A7r5 cells and relative controls, using Lipofectamine 2000 (Life Technologies). 3'UTR site-specific mutagenesis at the predicted sites for each target was performed using the QuikChange Site-Directed Mutagenesis Kit, as described by the manufacturer (Stratagene, Ca#200523). Cells were harvested 48 h after transfection and analyzed with a dual-luciferase reporter assay kit (Promega), according to manufacturer's protocol.

### REFERENCES

- Bonci D, Coppola V, Musumeci M, Addario A, Giuffrida R, Memeo L, D'Urso L, Pagliuca A, Biffoni M, Labbaye C, et al. The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. *Nat Med.* 2008;14(11):1271-7.
- Climent M, Quintavalle M, Miragoli M, Chen J, Condorelli G, and Elia L. TGFbeta Triggers miR-143/145 Transfer From Smooth Muscle Cells to Endothelial Cells, Thereby Modulating Vessel Stabilization. *Circ Res.* 2015;116(11):1753-64.

### **Supplementary Figures**



3

### Figure S1. Gene expression modulation in treated VSMCs.

(A) RT-qPCR gene expression analysis of VSMCs treated with different stimuli. Control samples (0.1% FBS) were set to 1. (B) *Uhrf1* RNA expression in VSMCs treated with rapamycin. (C) Representative Western blot showing MYH11 and UHRF1 levels in VSMCs treated with PDGF-BB (25 ng/mL), TGF- $\beta$  (10 ng/mL), or cultured in 10% and 0.1% of FBS. (D and E) RT-qPCR analysis of VSMC differentiation markers and *Uhrf1* to doses of PDGF-BB. Control samples (0.1% FBS) were set to 1. (F and G) RT-qPCR analysis of VSMC differentiation markers and *Uhrf1* to control samples (0.1% FBS) were set to 1. (F and G) RT-qPCR analysis of VSMC differentiation markers and *Uhrf1* at different times after PDGF-BB treatment (o.n.: overnight). Control samples (0.1% FBS) were set to 1. PDGF was used at 12.5 ng/ml.

The results are the average of at least 3 independent experiments and error bars indicate SD. To compare means unpaired two-tailed Student's t-test was used;  $^{\#}P < 0.05$ .



Figure S2. TGF-β effects on VSMC differentiation and *Uhrf1* expression.

(A and B) TGF- $\beta$  time-course: RT-qPCR analysis of VSMC differentiation markers and *Uhrf1*. Control samples (0.1% FBS) were set to 1. (C) *UHRF1* RNA expression in human VSMCs treated with PDGF-BB (GSE69637). (D) RT-qPCR analysis of VSMCs treated with a miR-145 inhibitor oligo (i145) cultured in 10% FBS.

The results are the average of at least 3 independent experiments and error bars indicate SD. To compare means, unpaired two-tailed Student's t-test was used;  $^{\#}P < 0.05$ .



Figure S3. UHRF1 cell tracing in vivo.

(A) Uhrf1 RNA expression in vessels of mice undergoing TAC (tissues collected two weeks after the procedure); immunostaining showing UHRF1 expression in Tomato<sup>+</sup> cells (UHRF1 in green, dtTomato in red and DAPI in blue; sections on aortas above the constriction). N: 4 animals. Size bar: 10  $\mu$ m. (**B** and **C**) Representative immunostaining for UHRF1 on aortic sections of ApoE<sup>-/-</sup> mice fed respectively with Chow and Western diet (colorimetric images showing UHRF1 in brown, size bar 1mm; insets: immunofluorescence showing UHRF1 in green and ACTA2 in red, size bar 100  $\mu$ m). N: 4 chow and 4 western fed animals (**D**) Immunostaining showing ACTA2 colocalization with Tomato reporter (ACTA2 in green and tdTomato in red). Size bar: 50  $\mu$ m. (**E**-**F**) UHRF1 expression analysis on: cerebral aneurysm tissue versus superficial temporal artery (GSE66238) – Y axis indicates number of RNA-seq reads falling into UHRF1 gene normalized for the total number of uniquely mapped reads for all samples (**E**); and in infrarenal aortic tissue from 10-week-old male C57BL/6J mice after AAA-induction (GSE51227) – Y axis indicates normalized Uhrf1 probe intensity (ID 7560), measured by Agilent SurePrint G3 Mouse GE 8x60K Microarray (**F**). (**G**) Representative immunostaining for UHRF1 on carotid sections of ApoE<sup>-/-</sup> mice sham-operated (UHRF1 in green and ACTA2 in red, size bar 20  $\mu$ m). N: 4 chow, 4 western fed and 4 sham-operated animals.

Error bars indicate SD. To compare means, unpaired two-tailed Student's t-test was used in A, E and F.





(A) DNMT1 expression analysis on atheroma plaque versus paired macroscopically intact tissue of 32 patients (GSE43292). Y axis indicates normalized DNMT1 probe intensity (ID 8033912), measured by Affymetrix Human Gene 1.0 ST Array. (B) DNMT1 RNA expression in cerebral aneurysm tissue versus superficial temporal artery (GSE66238). Y axis indicates number of RNA-seq reads falling into DNMT1 gene normalized for the total number of uniquely mapped reads for all samples. (C) Dnmt1 expression analysis in infrarenal aortic tissue from 10-week-old male C57BL/6J mice after AAA

induction (GSE51227). Y axis indicates normalized DNMT1 probe intensity (ID 38313), measured by Agilent SurePrint G3 Mouse GE 8x60K Microarray. (D) Positive weak to moderate correlation between UHRF1 and DNMT1 RNA expression on atheroma plaque versus paired macroscopically intact tissue of 32 patients (GSE43292). Plaque versus Intact ratio was calculated in each of the 32 patients. Linear Regression:  $R^2 = 0.3586$ , P<0.001; No clear correlation was observed between UHRF1 and HDAC1. Linear Regression:  $R^2 = 0.2455$ , P=0.624. (E) Positive strong correlation between UHRF1 and DNMT1/HDAC1 RNA expression in cerebral aneurysm tissue (GSE66238). Linear Regression:  $R^2 = 0.9075$ , P=0.003 (DNMT1); Linear Regression:  $R^2 = 0.9255$ , P=0.002 (HDAC1). (F) Negative strong correlation between UHRF1 and HDAC1 RNA expression in infrarenal aortic tissue from 10week-old male C57BL/6J mice after AAA induction (GSE51227). Linear Regression:  $R^2 = 0.8299$ , P=0.031. No correlation was observed between UHRF1 and DNMT1. Linear Regression:  $R^2 = 0.0129$ , P=0.855. (G) HFAC1 expression analysis on atheroma plaque versus paired macroscopically intact tissue of 32 patients (GSE43292). Y axis indicates normalized HDAC1 probe intensity (ID 7899774), measured by Affymetrix Human Gene 1.0 ST Array. (H) HDAC1 RNA expression in cerebral aneurysm tissue versus superficial temporal artery (GSE66238). Y axis indicates number of RNA-seq reads falling into HDAC1 gene normalized for the total number of uniquely mapped reads for all samples. (I) HDAC1 expression analysis in infrarenal aortic tissue from 10-week-old male C57BL/6J mice after AAA induction (GSE51227). Y axis indicates normalized Hdac1 probe intensity (ID 60327), measured by Agilent SurePrint G3 Mouse GE 8x60K Microarray.

Data represent mean  $\pm$  SD. Unpaired two-tailed Student's t test was used to compare means in D, E, F, G, H, and I, and paired two-tailed Student's t test in A, B, and C.



### Figure S5. *Uhrf1*<sup>fl/fl</sup>cre/ER<sup>T2</sup> mouse generation.

(A) RT-qPCR analysis of mouse carotids transduced with a shUHRF1 lentivirus. (B) Schematic of the mouse generation strategy and PCR method to evaluate the genomic deletion. (C) PCR results showing

the genomic excision following tamoxifen induction *in vivo*. (D) RT-qPCR analysis of KO mouse aortas showing reduction of *Uhrf1* expression. (E to I) Additional measurements of the restenosis experiment shown in Figure 4. (J) Immunostaining showing ACTA2 colocalization with Ki67 and TUNEL<sup>+</sup> cells (ACTA2 in red, Ki67 and TUNEL in green). N: 5 WT animals. Size bar: 100  $\mu$ m. Error bars indicate SD. To compare means, unpaired two-tailed Student's t-test was used; NS: not statistically significant.





(A) Proliferation curve of VSMCs transfected with an empty vector (E.V.) or *UHRF1* vector (UH..V.). Growth curves:  $2x10^4$ /ml cells were plated in 6-well plates and cultured with 10% FBS. The number of

viable cells was counted for 3 days. (**B**) Proliferation curve of *Uhrf1*-silenced VSMCs transfected with an empty vector (E.V.) or *UHRF1* vector (UH.V.). Growth curves:  $2x10^4$ /ml cells were plated in 6-well plates and cultured with 10% FBS. The number of viable cells was counted for 3 days. (**C**) Proliferation measured by BrdU incorporation in shSCR and shUHRF1 VSMCs at 16 h after PDGF-BB treatment. (**D**) Proliferation curve of VSMCs stable over-expressing miR-145 transfected with an empty vector (E.V.) or *UHRF1* vector (UH.V.). Growth curves:  $2x10^4$ /ml cells were plated in 6-well plates and cultured with 10% FBS. The number of viable cells was counted for 3 days. (**E**) RT-qPCR analysis of *Cdkn1a* and *Cdkn1b* in VSMCs transfected with a *UHRF1* expressing vector (UH.V.). (**F**) RT-qPCR analysis of *Cdkn1a* and *Cdkn1b* in *Uhrf1*-silenced VSMCs transfected with a *UHRF1* expressing vector (UH.V.). (**G**) Densitometric quantification of the western blot showed in figure 5E (Analysis performed using ImageJ software). (**H**) Wound assay on *Uhrf1*-silenced VSMCs. The quantification graph shows the residual wounded area 24h post-scratch compared to shSCR controls. The results are the average of at least 3 independent experiments and error bars indicate SD. To compare means, unpaired two-tailed Student's t-test was used; <sup>#</sup>P<0.05.



### Figure S7

### Figure S7. Gene expression modulation in *Uhrf1*-silenced VSMCs.

(A) Representative Western blot showing MYH11 and UHRF1 levels in *Uhrf1*-silenced VSMCs (referred to Figure 6). (B) Expression of *Opn* in *Uhrf1*-silenced VSMCs compared to shSCR cells, measured by RT-qPCR. (C) Expression of different genes associated with VSMC differentiation in *Uhrf1*-silenced VSMCs compared to shSCR cells, measured by RT-qPCR. (D) RT-qPCR gene expression analysis of *UHRF1* overexpressing VSMCs (UH.V.) compared to cells transduced with an empty vector (E.V.). (E) RT-qPCR gene expression analysis of miR-145 overexpressing VSMCs transfected with a *UHRF1* vector (UH.V.) compared to cells transduced with an empty vector (E.V.). (F) Expression of genes associated with the TGF- $\beta$  pathway in *Uhrf1*-silenced VSMCs compared to

shSCR cells, measured by RT-qPCR. The results are the average of at least 3 independent experiments and error bars indicate SD (Control set to 1). To compare means, unpaired two-tailed Student's t-test was used;  $^{\#}P < 0.05$ .



# Figure S8. Gene expression modulation of ECM genes and *Uhrf1* target genes in *Uhrf1*-silenced VSMCs at the basal level and upon TGF-β inhibition.

(A) RT-qPCR gene expression analysis of silenced VSMCs compared to shSCR transduced cells (set to 1). (B) Representative Western blot showing UHRF1 levels in *Uhrf1*-silenced VSMCs (referred to Figure 8). (C) RT-qPCR gene expression analysis of silenced VSMCs compared to shSCR cells treated with the TGF- $\beta$  inhibitor SB431542 (control set to 1).

The results are the average of at least 3 independent experiments and error bars indicate SD. To compare means, unpaired two-tailed Student's t-test was used;  $^{\#}P < 0.05$ .



## Figure S9. Inverse correlation analysis between genes regulated by UHRF1 and modulated in aortic aneurysm

(A) Dot plot showing the weak to-moderate anti-correlation of genes differentially modulated between PPE versus Sham and shUHRF1 versus shSCR. R and P-Value were calculated with Spearman's Rank-Order Correlation. (B) Pie chart showing the degree of positive or negative correlation between genes differentially modulated in the two datasets. (C) Heatmap showing the 119 negatively correlated protein coding genes in the two datasets.













### Figure S10

### Figure S10. Uhrf1 KO mouse characterization in post-natal and 3-week-old animals.

Representative aorta cross-sections from WT and KO mice (tamoxifen injection starting at p7, and sacrifice at P17) with Masson trichrome (A), elastin (B), and ACTA2 (brown color) (C) staining. N: 4

KO and 4 WT animals. Scale bar:  $30\mu m$ . Representative aorta cross-sections of WT and KO mice (tamoxifen injection starting at p21, and sacrifice at P31) with Masson trichrome (**D**), elastin (**E**), and ACTA2 (brown color) (**F**) staining. N: 6 KO and 6 WT animals. Scale bar:  $40\mu m$ .





Figure S11

Figure S11. Uhrf1 KO mouse characterization in adulthood.

Representative aorta cross-sections from adult WT and KO mice (tamoxifen injection starting at 8 weeks of age, and sacrifice at 10 weeks of age) with Masson trichrome (A), elastin (B), ACTA2 (brown color) (C), and CD31 (brown color) (D) staining; Scale bar:  $60\mu$ m. (E) Representative fluorescent images of WT and KO aorta cross-sections labeled with phalloidin and DAPI and relative quantifications of media layer cell numbers (calculated by counting nuclei of the medial layer) and media thickness. Scale bar:  $20\mu$ m. (F) Representative confocal images of WT and KO aorta cross-sections labeled with fluorescent Wheat Germ Agglutinin and relative quantification of cell areas and cell perimeters (measurements were obtained using ImageJ software evaluating the cytoplasmic back area surrounded by the fluorescent signal due to the cytoplasmic labeling). N: 6 KO and 6 WT animals. Scale bar:  $10\mu$ m.



## Figure S12

### Figure S12. Effects of Uhrf1 KO in vivo.

(A) Quantitative echodoppler analysis of the aortas diameters in diastole and systole of WT and KO mice. Error bars indicate SD. To compare means, two-way ANOVA test was used. CSD: cross-sectional diameter. (B) Representative echodoppler images of transversal abdominal aortas of Ang-II-infused ApoE<sup>-/-</sup>UHRF1<sup>fl/fl</sup>cre/ER<sup>T2</sup> mice compared to control (ApoE<sup>-/-</sup>UHRF1<sup>wt/wt</sup>cre/ER<sup>T2</sup>) (Vessel

lumens are highlighted with dot lines). (C) P-Smad-2/3 staining on WT e KO mice following Ang-II stimulation and relative quantification (Scale bar:  $60\mu$ m). Error bars indicate SD. (D) Representative M-mode echodoppler images of abdominal aortas of WT and KO mice at the basal level. To compare means, unpaired two-tailed Student's t-test was used. N: 11 KO and 10 WT animals. NS: not statistically significant.

### Supplementary Tables

Primers			
Gene	Forward	Reverse	
Cdkn1b	GGCCTGTAGTAGAACTCGGG	CCGCCTGCAGAAATCTCTTC	
Acta2	GGGCTCATAGTCATGGAGGA	CCCACAGAGATCACCAGTCA	
Myh11	CAGGGTGCCCTACTGCTTAC	CCAAGACTGCCTTGAGTTCC	
Sm22	ACAAAGAGAAGGCCGAGGAC	TCCCTCATTGCTTGCTTCTT	
Cnn1	GGCCAGTGAATGGATATTGG	GGAATAGGCAGGATGCTCAG	
Tgfβ2	AAAGGGACGAGACGAGAAGG	CATCCACACGCACACTCATC	
Tgfβr1	GTGCAACGGTCACAGTCTTT	GTTTCTGCCTCTAACCACGC	

### Table S1. Primers used in this study for ChIP experiments

Primers				
Gene	Forward	Reverse		
Uhrfl	CTAGAACCAGGCGTTCCAAG	TCCACATGATGCCGATGTA		
Uhrf1 Ex4/5	CATGTGGCAGGCATCCATG	GGACTGGCCTGCTGTACG		
Suv39h2	CCAAATGGTTTAGCCTTGGA	AAAGGATGTGGAAGGGAACC		
Acta2	CTGACAGAGGCACCACTGAA	CATCTCCAGAGTCCAGCACA		
Cnnl	GGCAGGAACATCATTGGACT	GACCTGGCTCAAAGATCTGC		
Sm22	CCTGCCTCACAAATGCCTAT	CCCTGCTTACTCCAGGATGA		
Exh2	ATCTGAGAAGGGACCGGTTT	TGTGCACAGGCTGTATCCTC		
Dnmt1	CGAGGACAACAAGCACAAGTT	CCTTGGGCATTTCTTTTGT		
Myh11	GACAACTCCTCTCGCTTTGG	GCTCTCCAAAAGCAGGTCAC		
<i>U6</i>	CGCTTCGGCAGCACATATAC	TTCACGAATTTGCGTGTCAT		
Opn	TCTGATGAGACCGTCACTGC	AGGTCCTCATCTGTGGCATC		
Chpa	CACCGTGTTCTTCGACATC	ATTCTGTGAAAGGAGGAACC		
Cdkn1a	CCACGTGGCCTTGTCGCTGT	TGCCTCCGTTTTCGGCCCTG		
Cdkn1b	GCGGAGCAGTGTCCAGGGAT	GCGTCTGCTCCACAGTGCCA		
Cdkn2a	GCTCTGGCTTTCGTGAACAT	CGAATCTGCACCGTAGTTGA		
Cdkn2b	GGCCCTCTACCTTTCAGGAC	TTTCAGTTCACAGGGGAAGG		
Cdkn2c	CACTGTACAGGCTTTGCTGG	CAACCCCATTTGCCTCCATC		
Cdkn2d	CAATGTCCAAGATGCCTCCG	TATGGCCCTCTCTTATCGCC		
Srf	CTCACCTACCAGGTGTCGGAAT	CTGCTGACTTGCATGGTGGTAG		
Tet2	CCTTGCATTGGAGGGGGGGGGCT	GTGGGGTGATTCCGGTCGGG		
Myocd	TTCCAAAGATCCCTGGGTCC	CGTTGGCGTAGTGATCGAAG		

 Table S2. Primers used in this study for RT-qPCR experiments.

Hat	AGAGTGCCGTGGAGAAGAAA	AGCAAGATCTTCAGGCCCTT
Setdb	TAGGAGAGCTCACGAGGACT	GCAGCTCCTCATCACACAAG
Prmt3	ACAACAGGGTCGTGTTCTCT	AGTCAGGGTCACAATGAGGG
Tgfβ2	GGAGCGACGAGGAGTACTAC	AGACCCTGAACTCTGCCTTC
Tgfβlr	CGACGCTGTTCTATTGGTGG	TCACTCTCAAGGCCTCACAG
Collal	GCCTGGACTTCCTGGTCC	AGCAAAGTTTCCTCCAAGGC
Ddr1	AGGAGTACTTGCAGGTGGAC	GAAATCACCTCCTGTCCCCA
Sparc	GTCCTGGTCACCTTGTACGA	CCAGTGGACAGGGAAGATGT
Cd151	GATCATTGCTGGCATCCTGG	GAGAGTTGTTGCTGCCACAA
Ptprs	CCGCTATGTCCTCTTTGTGC	TGGCAATCACGATGCATG
Pdgfa	CCATTAACCATGTGCCCGAG	AGATCAGGAAGTTGGCCGAT
Ltbp4	TGTACTGTGGGAGAAGGCTG	TAGTAGAACCCGTTGCTGCA
Tnfrsf11	ATGAACAAGTGGCTGTGCTG	TAAGAGTGGTCAGGGCAAGG
Cst3	AGTACAACAAGGGCAGCAAC	TGCCTTCCTCATCAGATGGG
Dagl	TGACCTCACCAAGATGACCC	TTCTTTGCATTTCCTGGGCC
Crtap	CGAGCCCTACAAGTTTCTGC	ACAAACAGGCTCTCGTACGA
Lamp2	TGAACATGCAGGGGAAGCTA	AGTTCTCATCCTGCACAGCT
Efemp	ACCTTCCTGTGTCGCTGTAA	AAGTTGACACAGGTTTGGGC
Ctsb	CCTTCTTTCTTGCCTGCTGG	GTTCCCGTGCATCAAAGGTT
Mmp2	CGATGTCGCCCCTAAAACAG	GCATGGTCTCGATGGTGTTC
Mmo11	GTTTCCACCATCCGAGGAGA	CCAGTACTGAGCACCTTGGA
Col4a5	GGAAACCCTGGAGCAAAAGG	CTTGACCTGGCTTGCCTTTT
Agrn	TGATGTGGCCAGTCTTGCTA	AGTTGGAGCCTTCTGAGTCC

Full uncut gels



Full unedited gel for Figure 1C Acquired with Films



Full unedited gel for Figure 2E Acquired with ChemiDoc (BioRad)



UHRF1 (91 kDa)

Full unedited gel for Figure 5E, Panel 1 Acquired with ChemiDoc (BioRad)



CDKN1A (21 KDa)

Full unedited gel for Figure 5E, Panel 2 Acquired with ChemiDoc (BioRad)



CDKN1B (27 kDa)

Full unedited gel for Figure 5E, Panel 3 Acquired with ChemiDoc (BioRad)



CDKN2B (15 kDa)

Full unedited gel for Figure 5E, Panel 4 Acquired with ChemiDoc (BioRad)



Full unedited gel for Figure 5E, Panel 5 Acquired with ChemiDoc (BioRad)



Full unedited gel for Figure 5E, Panel 6 Acquired with ChemiDoc (BioRad)



Full unedited gel for Figure 6B, Panel 1 Acquired with ChemiDoc (BioRad)



Full unedited gel for Figure 6B , Panel 2 Acquired with ChemiDoc (BioRad)



Full unedited gel for Figure 6B, Panel 3 Acquired with ChemiDoc (BioRad)



Full unedited gel for Figure 6B, Panel 4 Acquired with ChemiDoc (BioRad)



Full unedited gel for Figure 8E, Panel 1,2,3 and 4 Acquired with ChemiDoc (BioRad)



Full unedited gel for Figure 8E, Panel 5,6 and 7 Acquired with ChemiDoc (BioRad)



Full unedited gel for Figure S1C Acquired with ChemiDoc (BioRad)



Full unedited gel for Figure S5C, Acquired with GelDoc (BioRad)



Full unedited gel for Figure S7A Acquired with ChemiDoc (BioRad)



Full unedited gel for Figure S8B Acquired with ChemiDoc (BioRad)