

## Supplemental Information

Title: **Intermittent hypoxia mediates paraspeckle protein-1 upregulation in sleep apnea**

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**Table S1.** qPCR Primer sequences are shown.

<i>PSPC1</i>	Forward primer	GACGCTTGGAAGAACTCAGA
	Reverse primer	TTGGAGGAGGACCTTGGTTAC
<i>TGF<math>\beta</math></i>	Forward primer	CTAATGGTGGAAACCCACAACG
	Reverse primer	TATCGCCAGGAATTGTTGCTG
<i>SMAD3</i>	Forward primer	AACGGCCAGGAGGAGAAATG
	Reverse primer	ATCCAGGGACCTGGGGATG
<i>SMAD4</i>	Forward primer	TGCATTCCAGCCTCCCATT
	Reverse primer	CTCTCCTACCTGAACGTCCATT
<i>GSDMD</i>	Forward primer	GTGTGTCAACCTGTCTATCAAGG
	Reverse primer	CATGGCATCGTAGAAGTGGAAG
<i>MMP2</i>	Forward primer	GATACCCCTTTGACGGTAAGGA
	Reverse primer	CCTTCTCCCAAGGTCCATAGC
<i>MMP9</i>	Forward primer	TTCCAAACCTTTGAGGGCGA
	Reverse primer	CAAAGGCGTCGTCAATCACC
<i>HIF1<math>\alpha</math></i>	Forward primer	TTCCAGTTACGTTCTTCGATCA
	Reverse primer	TTTGAGGACTTGCGCTTTCA
<i>TWIST1</i>	Forward primer	GGAGTCCGCAGTCTTACGAG
	Reverse primer	TCTGGAGGACCTGGTAGAGG
<i>SLUG</i>	Forward primer	GGGGAGAAGCCTTTTTCTTG
	Reverse primer	TCCTCATGTTTGTGCAGGAG
<i>SNAIL</i>	Forward primer	CCAGGCTGAGGTATTCCTTG
	Reverse primer	CCTCCCTGTCAGATGAGGAC
<i>NANOG</i>	Forward primer	TGAACCTCAGCTACAAACAGGTC
	Reverse primer	AACTGCATCCAGGACTGCAGAG
<i>OCT3/4</i>	Forward primer	CTTGCTGGAGAAGTGGGTGGAA
	Reverse primer	CTGGAGTGTGGGTTTCGGGCA
<i>SOX2</i>	Forward primer	AGAACCCCAAGATGCACAAC
	Reverse primer	CGGGGCCGGTATTTATAATC
<i>E-cadherin</i>	Forward primer	TGCCCAGAAAATGAAAAAGG
	Reverse primer	GTGTATGTGGCAATGCGTTC
<i>18S</i>	Forward primer	CGGCGACGACCCATTCTGAAC
	Reverse primer	GAATCGAACCCTGATTCCCCGTC

**Table S2.** Position, Matrix Score and Sequence from the EBOX and HRE motifs\*

	<b>Moti f</b>	<b>Core Similarit y</b>	<b>Matrix Similarit y</b>	<b>Start Position</b>	<b>End Position</b>	<b>Sequence</b>
PSPC 1	HRE	0.997	0.985	1978308 3	1978309 0	CGCGTGCA
	HRE	0.997	0.970	1978571 7	1978572 4	GGCACGCT
	HRE	0.825	0.806	1978795 0	1978795 7	AGTGTGCC
TGF $\beta$	HRE	0.828	0.791	4135397 7	4135399 0	AAGCGCACGGGGC G
	HRE	0.793	0.773	4136257 2	4136258 5	TTCTAAGTGCTGTT
MMP 2	HRE	0.835	0.803	5547084 7	5547086 0	CCCTGCGTGCCACC
	HRE	0.835	0.825	5548054 5	5548055 8	GTGAGCGTGCGCG C

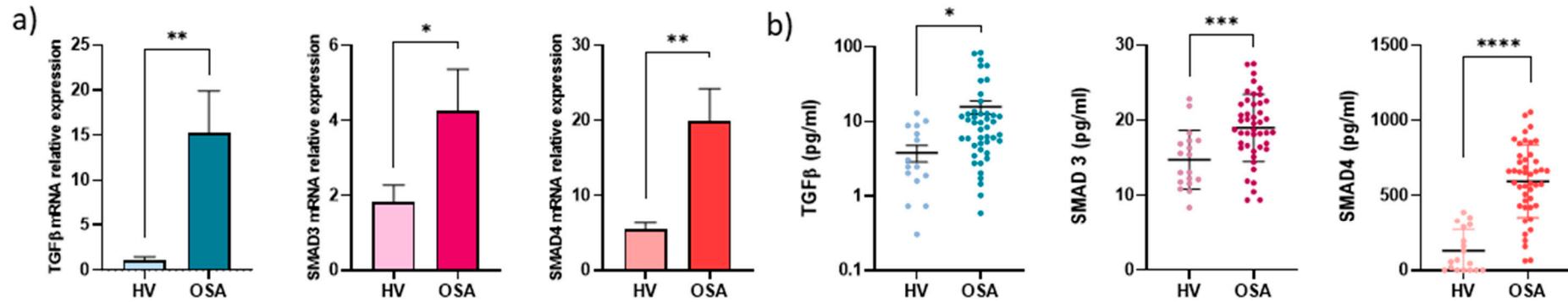
\* These data were obtained TRANSFAC® database

**Table S3.** Correlation of mRNA expression of PSPC1, TGF $\beta$  and markers of EMT and CSC in OSA patients

Intermittent hypoxia							
	TGF $\beta$	TWIST	SNAIL	SLUG	OCT3/4	SOX2	NANO G
<b>PSPC 1</b>	r=0.761 p<0.001	r=0.509 p=0.016	r=0.561 p=0.015	r=0.539 p=0.010	r=0.491 p=0.028	r=0.030 p=0.916	r=0.777 p<0.001
<b>TGF<math>\beta</math></b>		r=0.577 p=0.005	r=0.693 p=0.001	r=0.544 p=0.009	r=0.399 p=0.082	r=0.237 p=0.395	r=0.696 p<0.001
Normoxia							
	TGF $\beta$	TWIST	SNAIL	SLUG	OCT3/4	SOX2	NANO G
<b>PSPC 1</b>	r=0.285 p=0.199	r=0.251 p=0.261	r=0.246 p=0.296	r=0.225 p=0.315	r=0.143 p=0.536	r=0.240 p=0.307	r=0.223 p=0.320
<b>TGF<math>\beta</math></b>		r=0.198 p=0.376	r=0.659 p=0.002	r=0.136 p=0.545	r=0.231 p=0.313	r=0.334 p=0.150	r=0.566 p=0.006

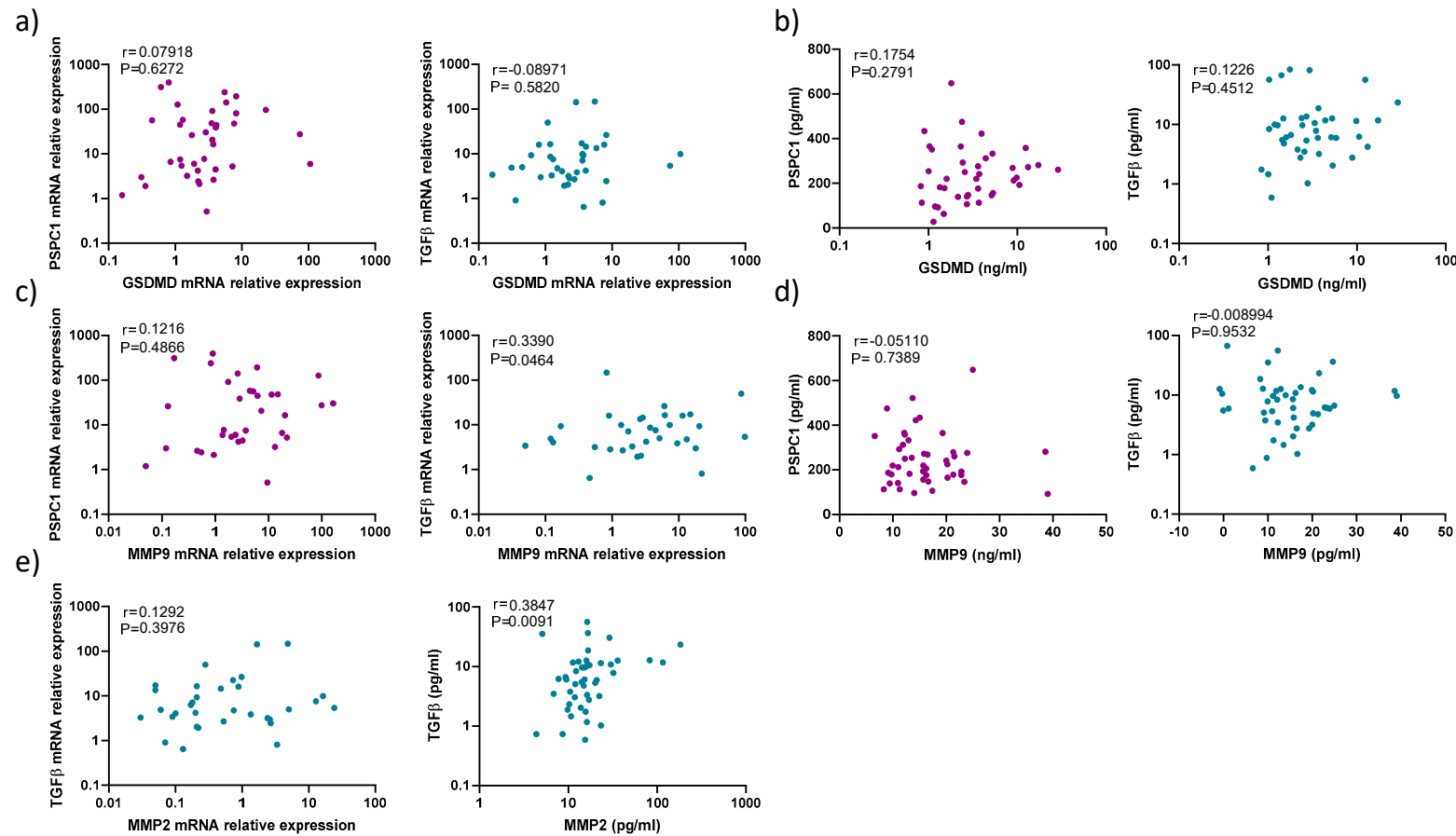
Correlation values between PSPC1, TGF $\beta$  and SMAD3 mRNA with EMT-TF or CSC-TF mRNA Melanoma cell line was cultured under IH and normoxia conditions with plasma from OSA patients (10% concentration) during 16h. Pearson's correlation coefficient (r) and p-values from correlations between PSPC1, TGF $\beta$  or SMAD3 mRNA expression and the different EMT-TFs and CSC-TFs mRNA expression are shown.

## FIGURES



**Figure S1. TGFβ, SMAD3 and SMAD4 expression in monocytes from severe OSA patients.**

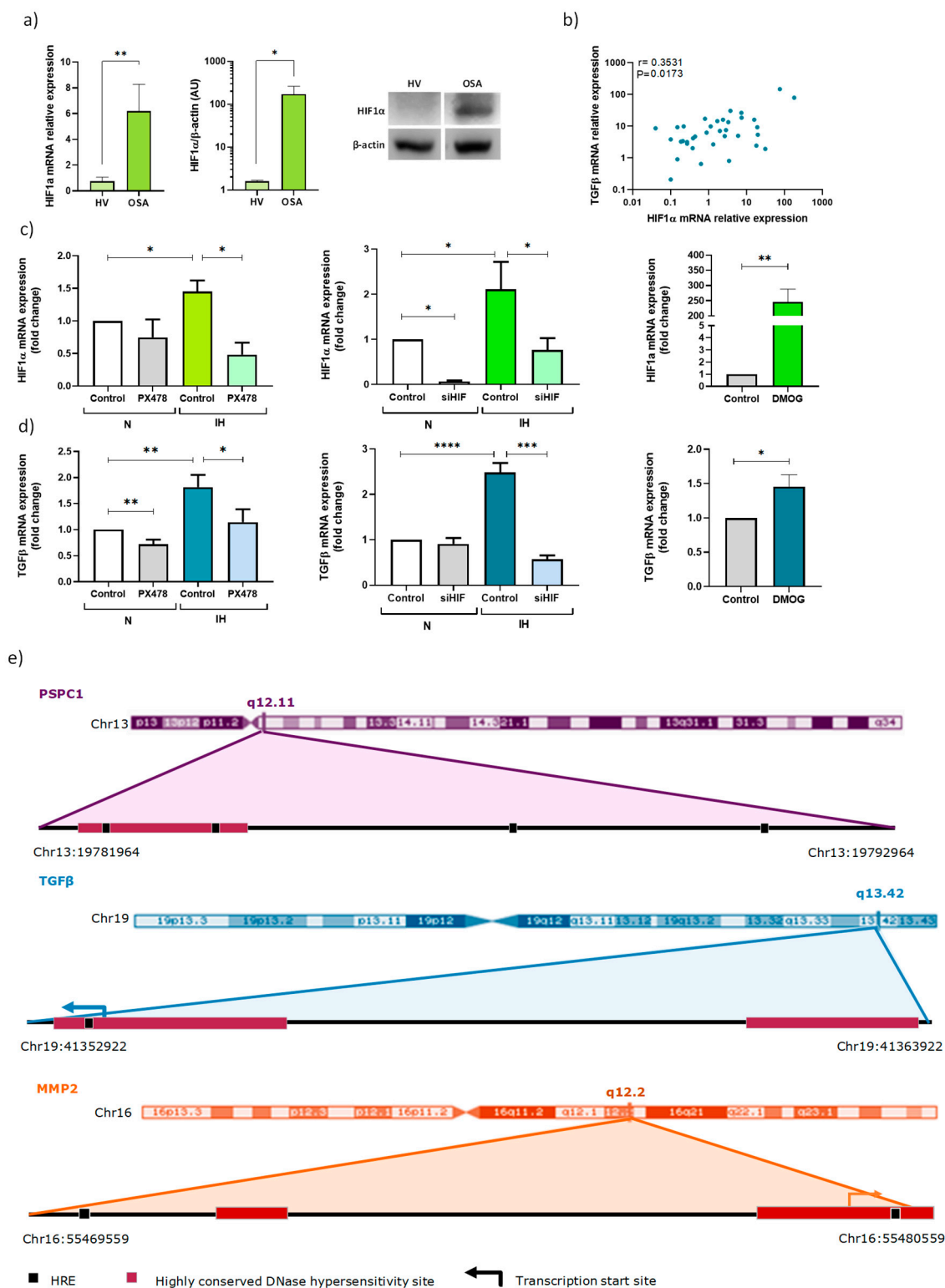
a) The TGFβ, SMAD3 and SMAD4 mRNA expression analysis by qPCR from healthy volunteers (HV) (n=18) and severe OSA patients (n=45) are shown. b) The TGFβ, SMAD3 and SMAD4 protein expression analysis by ELISA from HV (n=18) and severe OSA (n=45) plasma are shown. Comparisons between groups were performed by paired t-test. Error bars: SEM. \*:p<0.05, \*\*:p<0.01, \*\*\*:p<0.001; \*\*\*\*:p<0.0001.



**Figure S2. The PSPC1 and TGFβ expression correlations with GSDMD and MMP9**

Correlation between GSDMD with PSPC1 and TGFβ mRNA expression in monocytes (a) and protein level from plasma (b) from severe OSA patients (n=40) that were randomly selected. Correlation between MMP9 with PSPC1 and TGFβ mRNA in monocytes (c, n=35) and protein level from plasma (d, n=45) from severe OSA patients that were randomly selected. Correlation between MMP2 with TGFβ mRNA in monocytes (e, n=45) and protein level from plasma (f, n=45)

from severe OSA patients that were randomly selected. Pearson correlation coefficients (R) and p-values are shown.



**Figure S3. Hypoxemia is associated with TGF $\beta$  and MMP2 expression in OSA monocytes.**

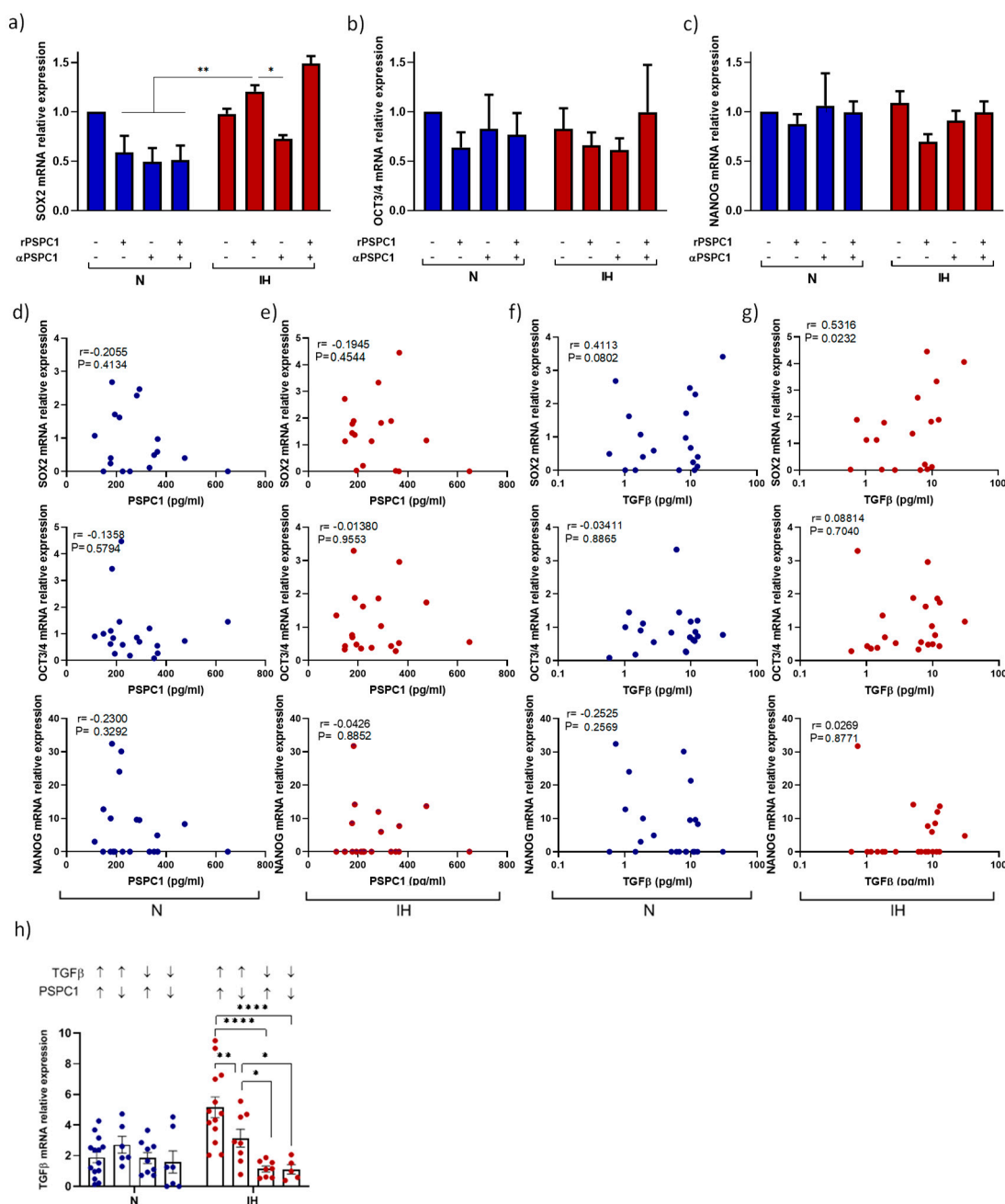
a) Comparison of HIF1 $\alpha$  mRNA expression in monocytes from healthy volunteers (HV, n=20) and patients with severe OSA (n=41) (left panel). Representative western blot of monocytes from HV (n=3) and OSA (n=3) of HIF-1 $\alpha$  and  $\beta$ -actin expression analysis. (right panel).

b) Correlation between HIF1 $\alpha$  and TGF $\beta$  mRNA expression on monocytes from randomly selected OSA patients (n=40). Pearson correlation coefficients (r) and P-values are shown.



(c) HIF1 $\alpha$  and (d) TGF $\beta$  mRNA expression estimation by qPCR in monocytes from HV subjects (n=7) treated with a specific inhibitor of HIF1 $\alpha$  PX478 30 $\mu$ M or not and exposed to IH or normoxia conditions for 16 hours. (left panel), monocytes from HV subjects (n=3) treated with or not siHIF1 $\alpha$  and exposed to IH or normoxia conditions for 16 hours (middle panel), monocytes from HV subjects (n=3) treated or not with DMOG for 2 hours (right panel). Comparisons between groups were performed by Two-way-ANOVA. Error bars: SEM.\*:p<0.05, and \*\*:p<0.01 versus non treated cells are shown.

e) Schematic representation of the in-silico analysis of the potential HRE binding sites, in the PSpC1, TGF $\beta$  and MMP2 promoter sequence (Ensemble reference sequence ENS), based on the consensus sequence. Location of these and HREs are shown. Data obtained from TRANSFAC® data base.



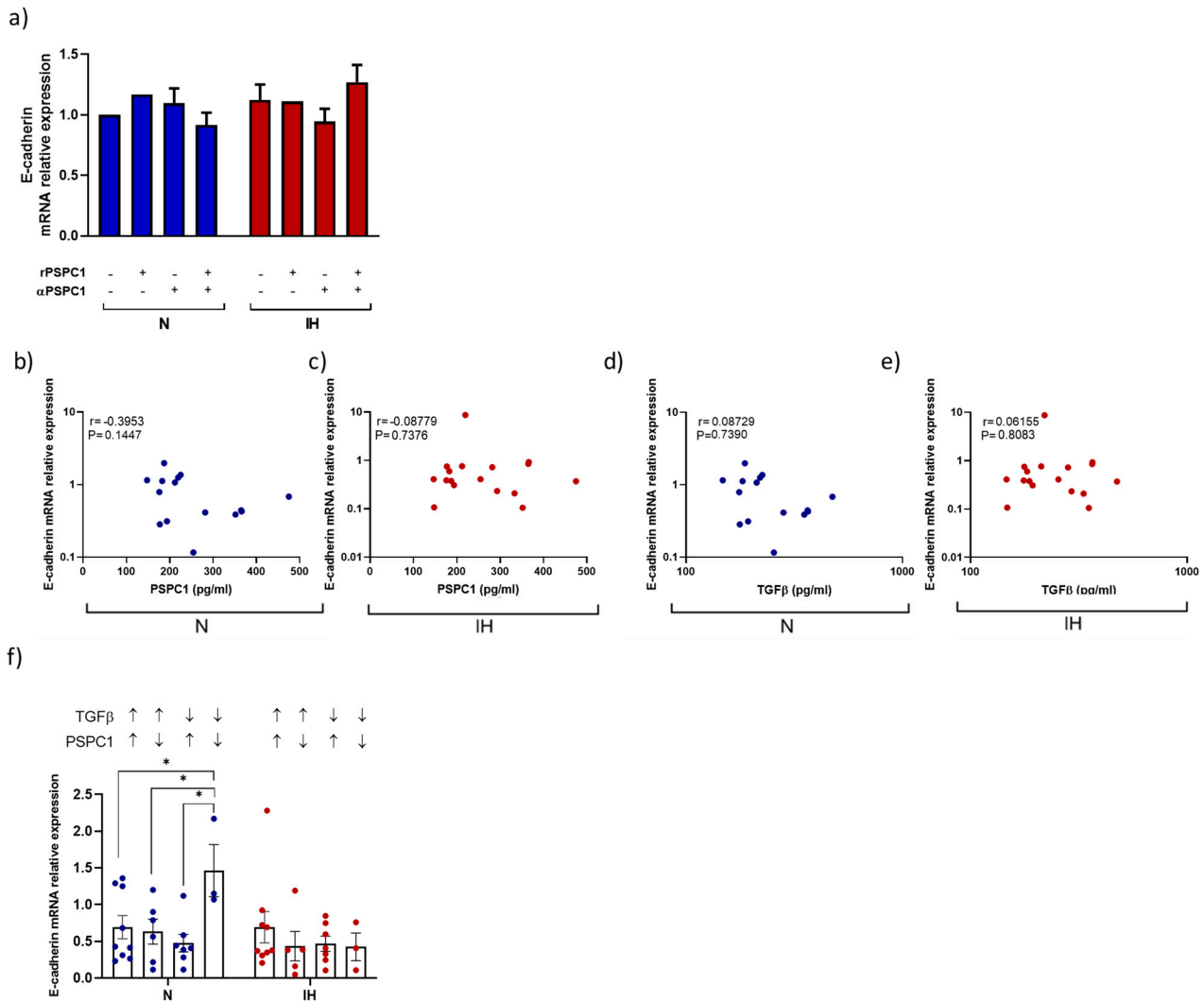
**Figure S4. PSC1 expression in OSA patients increase the effect on CSC.**

The C-8161 melanoma cell line was cultured under normoxia and intermittent hypoxia (IH) conditions with rPSPC1 protein (2,5ng/ml) with or without  $\alpha$ -PSPC1 (1ug/ml) during 16h. Then, melanoma cells were harvested to analyze the SOX2 (a), OCT3/4 (b) and NANOG (c) mRNA expression by qPCR. Comparisons between groups were performed by Two-way-ANOVA. Error bars: SEM.\*: $p < 0.05$  \*\*:  $p < 0.01$  versus treated group respectively shown.

The melanoma cell line was cultured under IH and normoxia conditions with plasma from randomly selected severe OSA patients (10% concentration) during 16h. Then, melanoma cells were harvested to analyze the SOX2, OCT3/4 and NANOG mRNA expression by qPCR analysis. The correlation between PSC1 protein concentrations from OSA plasma added to cell culture with SOX2, OCT3/4 and NANOG mRNA expression from melanoma cells cultured in normoxia (d) and IH (e) conditions are shown. The correlation between TGFβ protein concentrations from OSA plasma added to cell culture with SOX2, OCT3/4 and NANOG mRNA expression from melanoma cells cultured in normoxia (f) and IH (g) conditions is shown. Pearson correlation coefficients (r) and

P-values are shown.

h) The melanoma cell line was cultured under normoxia and IH conditions with plasma from OSA patients (10% concentration) during 16h. OSA plasma was selected according to the PSPC1 and TGF $\beta$  protein concentration; we performed four groups, PSPC1-high (200-2000pg/ml) with TGF $\beta$ -high (5-100pg/ml) as group 1, PSPC1-low (30-200pg/ml) with TGF $\beta$ -high as group 2, PSPC1-high (200-2000pg/ml) with TGF $\beta$ -low (0-5pg/ml) as group 3, PSPC1-low (30-200pg/ml) with TGF $\beta$ -low(0-5pg/ml) as group 4. Then, melanoma cells were harvested to analyze the mRNA expression by qPCR analysis. The TGF $\beta$  mRNA expression was analyzed by qPCR. The comparison between groups was performed with two-way ANOVA. Error bars: SEM. \*:p<0.05, \*\*:p<0.01, \*\*\*:p<0.0001 versus treated group respectively shown.



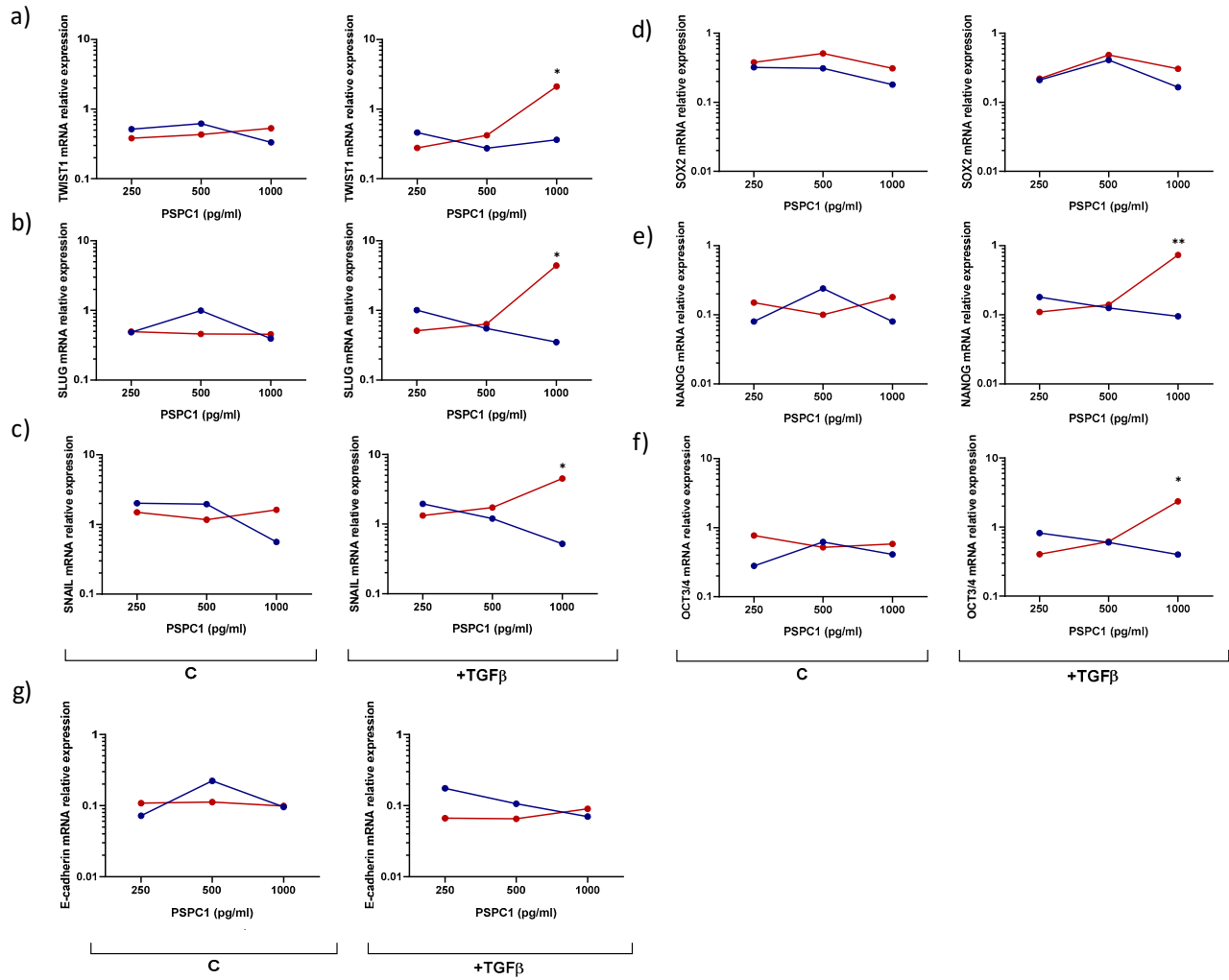
**Figure S5. PSpC1 and TGFβ expression in OSA patients effect on E-cadherin expression.**

a) The C-8161 melanoma cell line was cultured under normoxia and intermittent hypoxia (IH) conditions with rPSpC1 protein (2,5ng/ml) with or without α-PSpC1 (1ug/ml) during 16h. Then, melanoma cells were harvested to analyze the E-cadherin. Comparisons between groups were performed by Two-way-ANOVA. Error bars: SEM. \*:  $p < 0.05$  \*\*:  $p < 0.01$  versus treated group respectively shown.

The melanoma cell line was cultured under IH and normoxia conditions with plasma from randomly selected severe OSA patients (10% concentration) during 16h. Then, melanoma cells were harvested to analyze the E-cadherin mRNA expression by qPCR analysis. The correlation between PSpC1 protein concentrations from OSA plasma added to cell culture with cadherin mRNA expression from melanoma cells cultured in normoxia (b) and IH (c) conditions are shown. The correlation between TGFβ protein concentrations from OSA plasma added to cell culture with E-cadherin mRNA expression from melanoma cells cultured in normoxia (d) and IH (e) conditions is shown. Pearson correlation coefficients (r) and P-values are shown.

f) The melanoma cell line was cultured under normoxia and IH conditions with plasma from OSA patients (10% concentration) during 16h. OSA plasma was selected according to the PSpC1 and TGFβ protein concentration; we performed four groups, PSpC1-high (200-2000pg/ml) with TGFβ-high (5-100pg/ml) as group 1, PSpC1-low (30-200pg/ml) with TGFβ-high as group 2, PSpC1-high (200-2000pg/ml) with TGFβ-low (0-5pg/ml) as group 3, PSpC1-low (30-200pg/ml) with TGFβ-low (0-5pg/ml) as group 4. Then, melanoma cells were harvested to analyze the E-cadherin mRNA expression by qPCR analysis. The comparison between groups was performed with two-way ANOVA. Error bars: SEM. \*:  $p < 0.05$





**Figure S6. rPSPC1 effect on EMT.**

The melanoma cell line (C-8161) was cultured under normoxia or IH conditions with different PSPC1 concentrations and with or without TGFβ during 16h. Then, melanoma cells were harvested to analyze the mRNA expression by qPCR analysis. a) TWIST b) SLUG c) SNAIL d) SOX2 e) NANOG, f) OCT3/4 g) E-cadherin mRNA expressions are shown. Mean values for two different experiments are shown. The comparison between groups was performed with two-way ANOVA. \*:p<0.05 \*\*:p<0.01, Mean values for two different experiments are shown.