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# Supplementary Materials for

## Increased internalization of complement inhibitor CD59 may contribute to endothelial inflammation in obstructive sleep apnea

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**The PDF file includes:**

**Supplementary Materials**

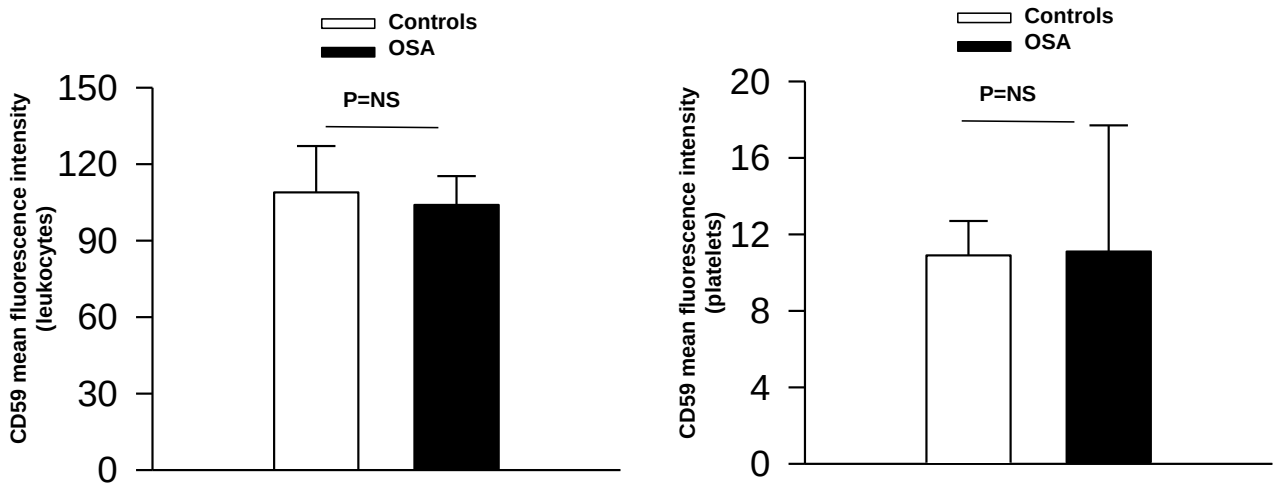


**Fig. S1. Expression of CD59 in leukocytes and platelets.** Bar graphs quantitate the expression of CD59 in unpermeabilized leukocytes and platelets of OSA patients (n=7) and controls (n=7) (flow cytometry, mean ± SE, 2-sided Exact permutation test). NS=not significant.

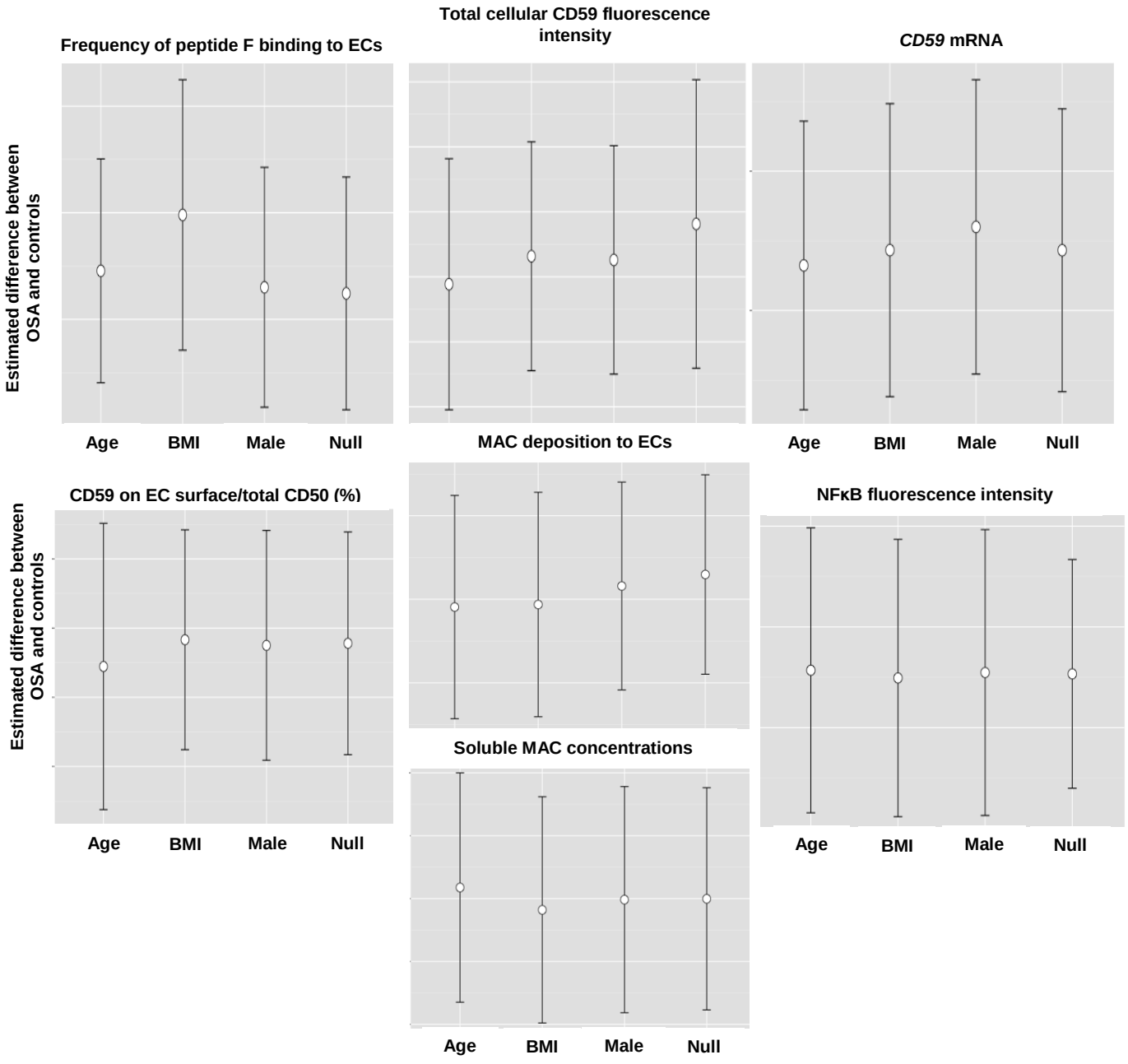


**Fig. S2. Linear regression analysis.** The graphs show estimated differences in measured variables between OSA patients and controls and their 95% confidence intervals from the following 4 models: null = model without adjustment for confounders; age = model adjusted for age; BMI = model adjusted for BMI; male = model adjusted for gender.

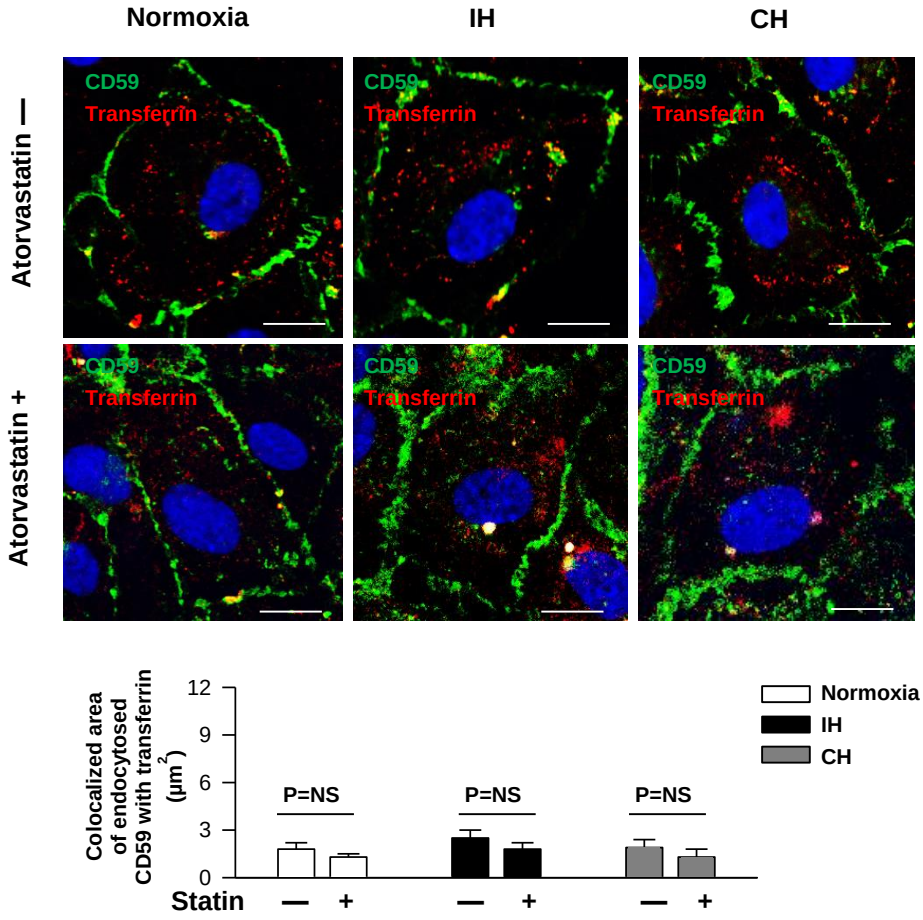


**Fig S3. Endocytosis of CD59 with transferrin**. Representative confocal images of CD59 endocytosis in HUVECs in normoxia, intermittent hypoxia (IH), or continuous hypoxia (CH) with or without atorvastatin (n=4). Scale bar 10 μm. Bar graph quantitates colocalized area of endocytosed CD59 with transferrin ($\mu m^2$). All data throughout the figure are shown as the mean ± SE, 2-sided Exact permutation test.

CH = Continuous Hypoxia; HUVEC = Human Umbilical Vein Endothelial Cells; IH = Intermittent Hypoxia. NS=not significant.

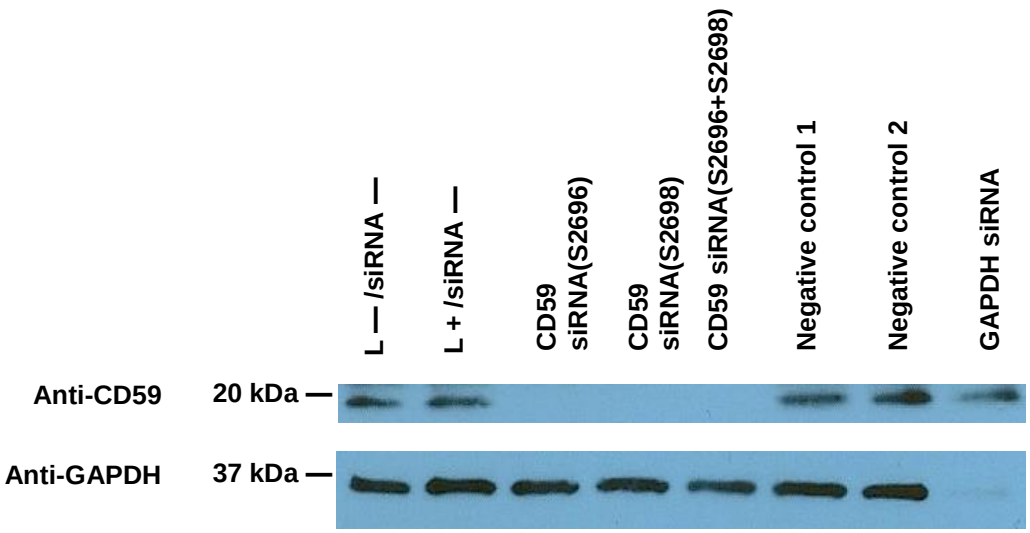


**Fig S4. Confirmation of HUVEC transfection with CD59 siRNA.** Western blots probed with antibodies directed against CD59 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Transfection of HUVECs was carried out for 48 hours using lipofectamine (L) and siRNAs s2696, s2698. and a combination of s2696 and s2698 to knock down CD59. Two scrambled siRNAs served as a negative control, and siRNA against GAPDH served as a positive control (n=3). The expression of CD59 was almost completely knocked down by either siRNA (s2696 or s2698).

GAPDH = glyceraldehyde 3-phosphate dehydrogenase; HUVEC = Human Umbilical Vein Endothelial Cells.

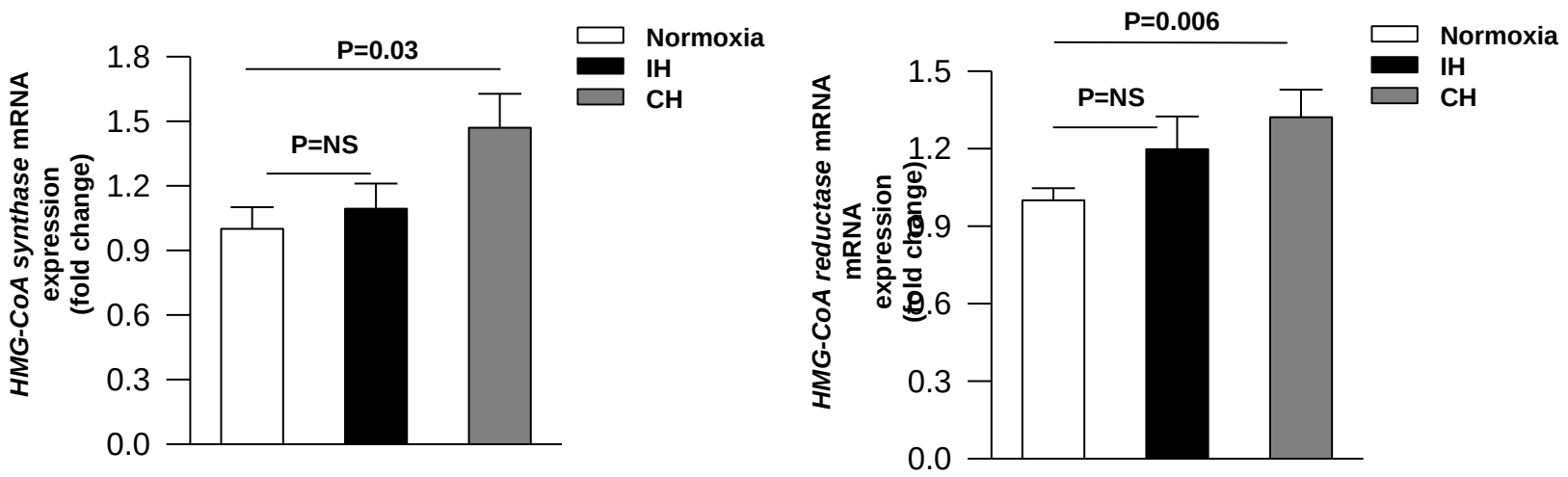


**Fig S5. Cholesterol biosynthesis in IH.** Quantitation of the *3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase* and *synthase* mRNA expression in HUVECs in normoxia, intermittent hypoxia (IH), and continuous hypoxia (CH) expressed as fold change over normoxia (n=4). All data throughout the figure are shown as the mean ± SE, 2-sided Exact permutation test. CH = Continuous Hypoxia; HUVEC = Human Umbilical Vein Endothelial Cells; IH = Intermittent Hypoxia; NS=not significant.

**Table. S1. Baseline characteristics of patients with OSA and control subjects.**

| | **OSA patients** (n=76) | **Control subjects** (n=52) | **p value** |
|---|---|---|---|
| **Age** (years) | 45±1.6 | 39±1.6 | 0.01 |
| **Body mass index** ($kg/m^2$) | 37±1.0 | 34±1.4 | NS |
| **Apnea-hypopnea index** (events/hour of sleep) | 26±2.6 | 1.6±0.2 | <0.001 |
| **Oxygen Desaturation Index** (events/hour of sleep) | 16±2.8 | 1.0±0.2 | <0.001 |
| **Epworth sleepiness scale score** | 11±0.7 | 9±0.8 | NS |
| **Systolic blood pressure** (mmHg) | 121±2.1 | 123±2.3 | NS |
| **Diastolic blood pressure** (mmHg) | 77±1.3 | 77±1.5 | NS |

Data are presented as mean ± SE

| | **OSA patients** (n=76) | **Control subjects** (n=52) | **p value** |
|---|---|---|---|
| **Gender** (% female) | 50 | 51 | NS |
| **$SaO_2$ nadir** (%) | 85±0.8 | 91±0.8 | <0.001 |
| **t < $SaO_2$ 90%** (% of the total sleep time) * | 1.4±0.5 | 0±0.0 | <0.001 |
| **Hypertension, n** (%) | 17 (22) | 9 (17) | NS |
| **Dyslipidemia, n** (%) | 10 (13) | 5 (10) | NS |

Data are presented as %.

$SaO_2$= arterial oxyhemoglobin saturation.

* Time spent below $SaO_2$ of 90% during sleep.

NS= not significant.

**Table. S2. Phages isolated after panning without target cells and with bovine serum albumin in polystyrene wells.**

| Negative template |
| --- |
| NWKPYPT |
| IVLPYPI |
| QFQHSHP |
| TPITQLL |
| IVLPYPI |
| THPPNAL |
| YLPLYEL |