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ARG2 impairs endothelial autophagy through regulation of MTOR and PRKAA/AMPK signaling in advanced atherosclerosis

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Supplementary Materials

Legends to Supplementary Figures

Figure S1. ARG2 impairs autophagy and enhances TP53, MTORC2-AKT-MTORC1-RPS6KB1 and impairs PRKAA signaling independently of its enzymatic activity in HAECs. The young HAECs were transduced with empty vector rAd/CMV-*V5* as control (*V5*), rAd/CMV-*Arg2* (*Arg2*) or rAd/CMV–*Arg2-H160F* (*H160F*, an inactive *Arg2* mutant). 48 h post transduction, cells were starved in serum-free medium for 16 h. During the last 1 h of incubation prior to the experiment, the cells were either untreated or treated with Baf A1 (20 nmol/L, 1 h) as indicated. 64 h post transduction, the cells were subjected to immunoblotting analysis for the parameters as indicated. TUBULIN served as a loading control. The bar graph presents the quantification of the signals (n=3). *p<0.05, **p<0.01, ***p<0.001 vs. *V5*-control group within the corresponding experimental condition.

Figure S2. ARG1 is not detectable in young or senescent HUVEC. The young and senescent HUVECs were subjected to immunoblotting for ARG1 expression. P: positive control of human ARG1 expressed from plasmid pBudCE4.1-*hARG1*. TUBULIN served as a loading control.

Figure S3. ARGINASE inhibitors do not restore impaired autophagy nor reduce augmented RPS6KB1 signaling in senescent HUVEC**.** The senescent HUVECs were either untreated (Con) or treated with ARGINASE inhibitors BEC (200 μmol/L) or nor-NOHA (50 μmol/L) overnight in serum-free medium and then subjected to immunoblotting for LC3-I/-II, ATG12–ATG5 conjugate and SQSTM1, RPS6-S235/236 and RPS6. TUBULIN served as a loading control. The quantification of the signals is shown in the graphs in the right panels.