Supplementary Figure 1

H Alganga, T.A.M Almabrouk, O.J. Katwan, C.J. Daly, S Pyne, N J Pyne, & S Kennedy. Short periods of hypoxia upregulate Sphingosine Kinase 1 and increase vasodilation of arteries to Sphingosine 1-Phosphate *via* S1P₃ Journal of Pharmacology & Experimental Therapeutics.



Supplementary Figure 1 Representative immunofluorescent images showing expression of S1P₃ receptors (panels A and B) and S1P₁ receptors (panels C and D) under normoxia (A and C) or hypoxia (B and D) in rat aortic endothelium. Receptor-associated fluorescence appears red and nuclei appear blue due to addition of Syto 82 nuclear stain. S1P₃ was expressed at a lower level under normoxic conditions compared to S1P₁ while hypoxia increased expression

of both receptors on the endothelium as determined by measuring integrated density (for all conditions at least four images were captured and analysed by a blinded observer).

Supplementary Figure 2

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Supplementary Figure 2 Representative immunofluorescent images showing: (A) expression of SK1 (red) in rat coronary artery endothelium and (B) following pre-treatment with cycloheximide (10 μ M, 30 min) under normoxic conditions. (C) Expression of SK1 in rat coronary artery subjected to 30 min hypoxia. (D) preincubation of rat coronary artery with

cycloheximide reduced the hypoxia-induced increase in SK1 expression. Nuclei appear blue due to addition of Syto 61 nuclear stain. (E) Quantitative fluorescence measurement of SK1 in the presence and absence of cycloheximide. *p<0.05 vs hypoxia alone as determined by one way ANOVA with Bonferroni multiple comparison post-tests. Results are representative of 3-5 separate experiments. Scale bar is 30 μ m.