Supplementary Figure Legends

PPARγ Activation Increases HUWE1 to Attenuate NF-κB/p65 and Sickle Cell Disease with Pulmonary Hypertension

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Supplementary Figure S1. PH develops spontaneously in SCD mice with aging. Right ventricular systolic pressure (RVSP) was recorded in anesthetized mice with a pressure transducer. Each bar represents the mean RVSP in mm Hg ± SE. n=3-5. Each bar represents the mean ± SE. * $p<0.05$ vs. AA (age 7 weeks). + $p<0.05$ vs. SS (age 7 weeks), n=3-5.

Supplementary Figure S2. Hemin increases HPAEC VCAM1 expression, monocyte adhesion, and proliferation. Whole lung homogenates were collected from littermate control (AA) and sickle cell (SS) mice at age 15-17 weeks. In (**A**), lung VCAM1 mRNA or protein levels were measured with qRT-PCR or Western blotting and expressed relative to lung mRNA (9S mRNA) or protein (GAPDH). *$p<0.05$ vs AA, n=4. In (**B**), human pulmonary artery endothelial cells (HPAECs) were treated with dimethyl sulfoxide vehicle (CON) or hemin (HEM, 5.0 μM) for 72 hours. Mean HPAEC VCAM1 mRNA and protein levels were measured with qRT-PCR or Western blotting. Each bar represents the mean VCAM1 level ± SE relative to GAPDH expressed as fold-change vs. CON. *$p<0.05$ vs. CON, n=11-12. In (**C-D**) HPAECs in a 96-well plate were treated with vehicle (CON) or hemin (HEM, 2.5, 5.0, and 10.0 μM) for 72 hours. (**C**) THP1 monocytes labeled with calcein were then added (5×10^4 monocytes / well) and incubated for 30 min. Monolayers were then washed 3 times to remove unbound monocytes, and scanned with a plate reader to measure total fluorescence / well. Each bar represents the mean ± SE fluorescence intensity of bound monocytes expressed as % CON. n=6. *$p<0.05$ vs. CON. (**D**) hemin-induced HPAEC proliferation measured with BrdU assays. Each bar represents the mean ± SE proliferation as fold change vs. CON. n=6. *$p<0.05$ vs. CON.

Supplementary Figure S3. Screening of siRNAs of HUWE1. Human pulmonary artery endothelial cells (HPAECs) were treated with 10 nM scrambled (SCR) or siRNA duplexes to HUWE1 (siHUWE-V1 which targeted exon 44-45 region on HUWE1 protein coding sequence (CDS), siHUWE-V2 which targeted exon 36-37 region on HUWE1 CDS, or siHUWE-V2 which targeted exon 36-37 region on HUWE1 3' untranslated region (UTR)) for 72 hours. HUWE1 relative to GAPDH expressed as fold change vs. SCR.

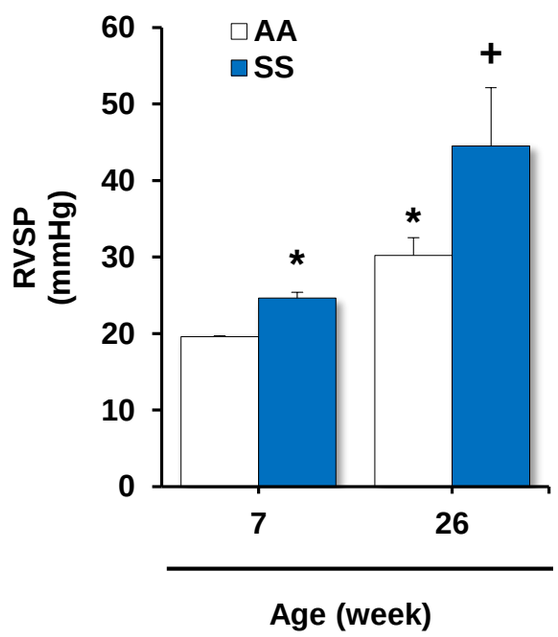
Supplementary Figure S4. Loss of p65 reduces ET-1 and VCAM1 levels. In (**D**-**G**), HPAECs were treated with scrambled (SCR) or p65 (si-p65, 10 nM) siRNAs for 6 h then incubated for an additional 72 h**.** qRT-PCR was performed for p65 (**A**), ET-1 (**B**), or VCAM1 (**C**) mRNAs. Each bar represents mean ± SE mRNA level relative to GAPDH expressed as fold-change vs cells treated with scrambled siRNA (SCR). n=3-6, *p<0.05 vs SCR**.**

Supplementary Figure S5. Loss of PPARγ function reduces HUWE1 and miR-98 levels. In (**A**-**C**), human pulmonary artery endothelial cells (HPAECs) were treated with scrambled (SCR) or siRNA duplexes (10 and 20 nM) to PPARγ for 6 h then incubated for an additional 72 h**.** qRT-PCR was performed for PPARγ (**A**), HUWE1 (**B**) or miR-98 (**C**). Each bar represents mean ± SE relative to GAPDH or RNU6B expressed as fold-change vs cells treated with scrambled siRNA (SCR). n=4-6, *p<0.05 vs SCR.

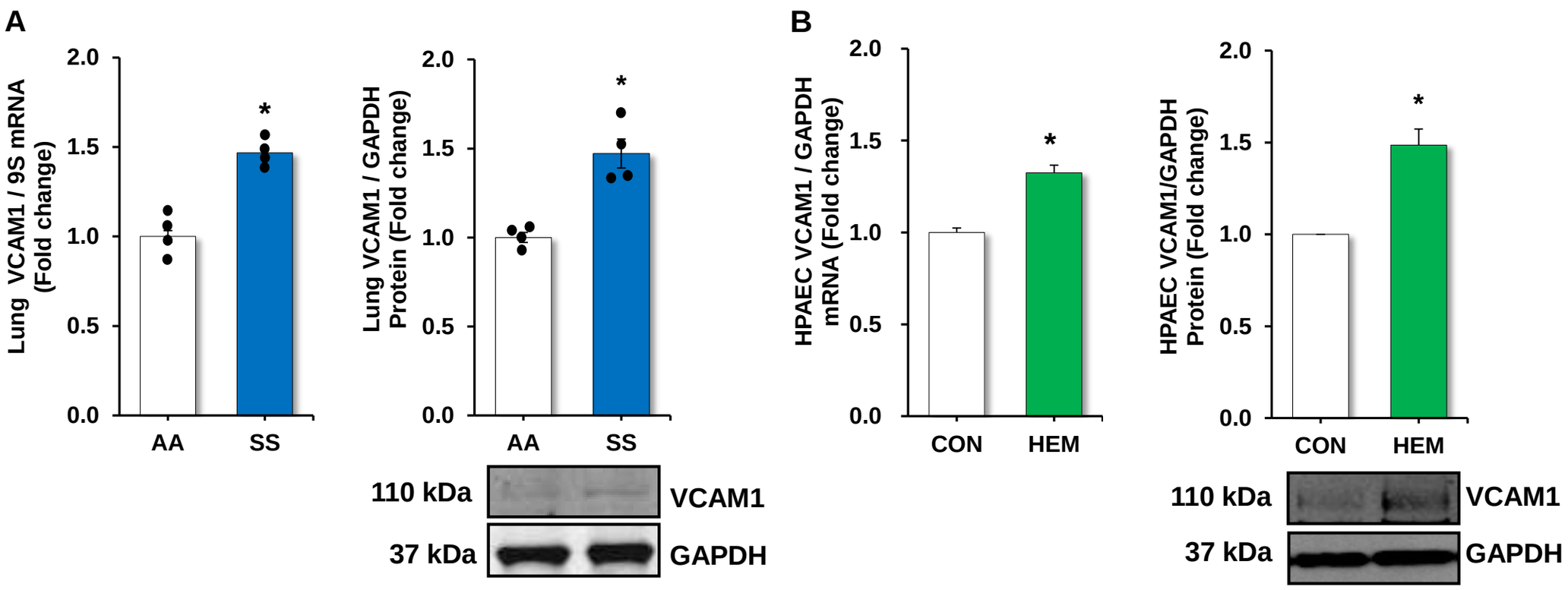
Supplementary Figure S6. The PPARγ ligand, RSG, attenuates increases in VCAM1 and endothelial dysfunction in hemin-treated HPAECs. Overexpression of miR-98 reduces increases in ET-1 in hemin-treated HPAECs. Human pulmonary

artery endothelial cells (HPAECs) were treated with hemin (HEM, 5 μM) for 72 h. During the final 24 h of hemin exposure, selected HPAECs were treated ± rosiglitazone (RSG, 10 μM). In (**A**), qRT-PCR was performed for VCAM1 levels. Each bar represents the mean ± SE relative to GAPDH as indicated. *p<0.05 vs. HEM/RSG(-). n=6. In (**B**), THP1 monocytes labeled with calcein were then added (5 X 10^4 monocytes / well) and incubated for 30 min. Monolayers were then washed 3 times to remove unbound monocytes, and scanned with a plate reader to measure total fluorescence / well. Each bar represents the mean ± SE fluorescence intensity of bound monocytes expressed as % CON. n=6. *p<0.05 vs. HEM/RSG(-). In (**C**), hemin-induced HPAEC proliferation measured with BrdU assays. Each bar represents the mean ± SE proliferation as fold change vs. CON. n=6. *p<0.05 vs. HEM/RSG(-). In (**D**), HPAECs were transfected with mimic-miR-98 (10 nM) or an equivalent amount of scrambled mimic-miR negative control using lipofectamine RNAiMax (Qiagen). After transfection for 6 hours, media were replaced with endothelial growth medium (EGM) containing 5% FBS. HPAECs were treated with HEM (5 μM) and then cultured for 72 hours. Alterations in ET-1 levels were examined using qRT-PCR. Each bar represents the mean ± SE as fold change vs. HEM/mimic miR-98(-). n=6. *p<0.05 vs. HEM/mimic miR-98(-).

Supplementary Fig. S1



Supplementary Fig. S2



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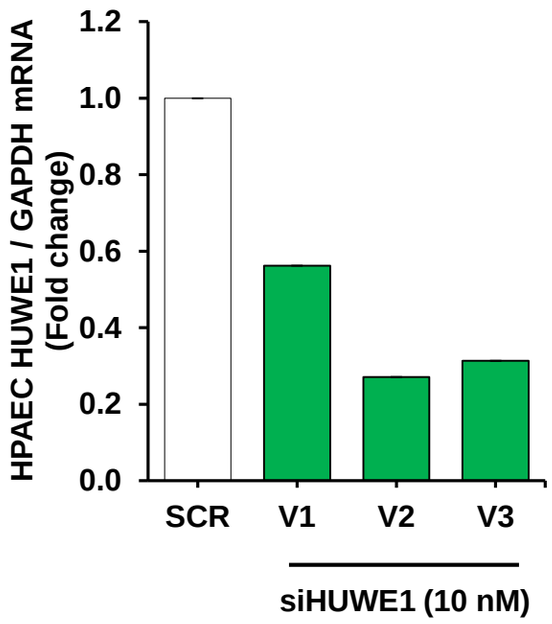

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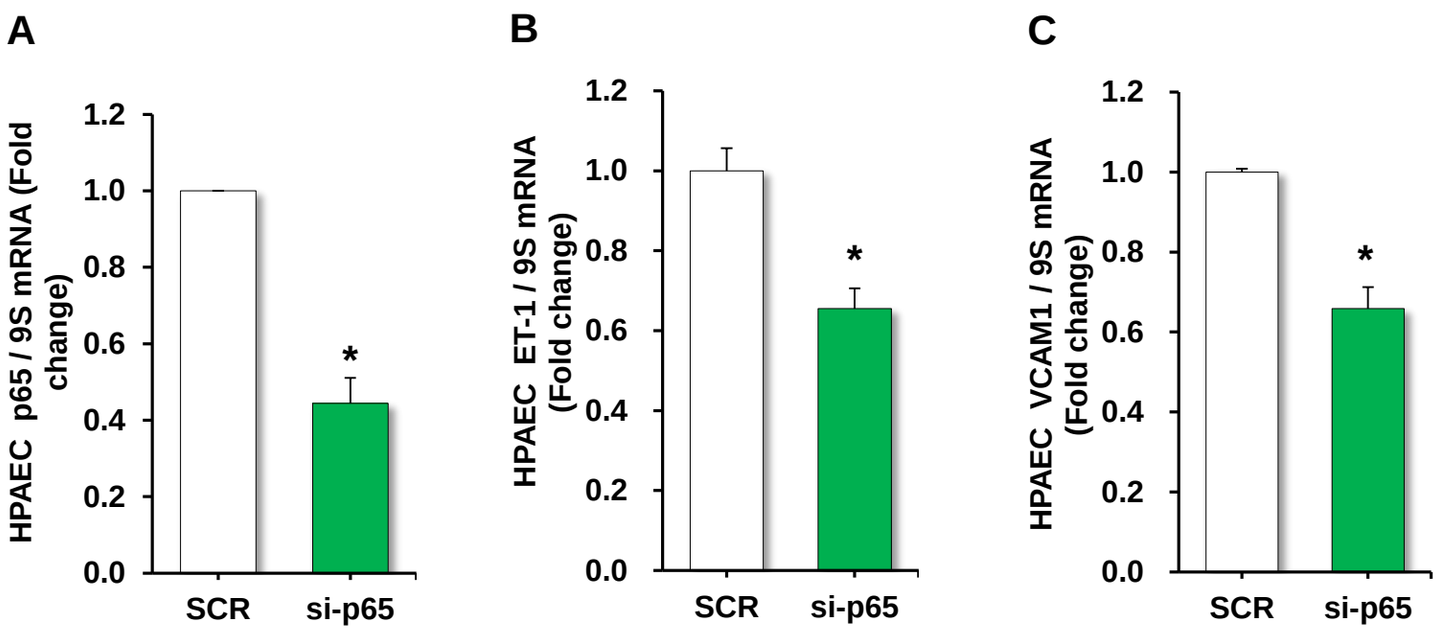

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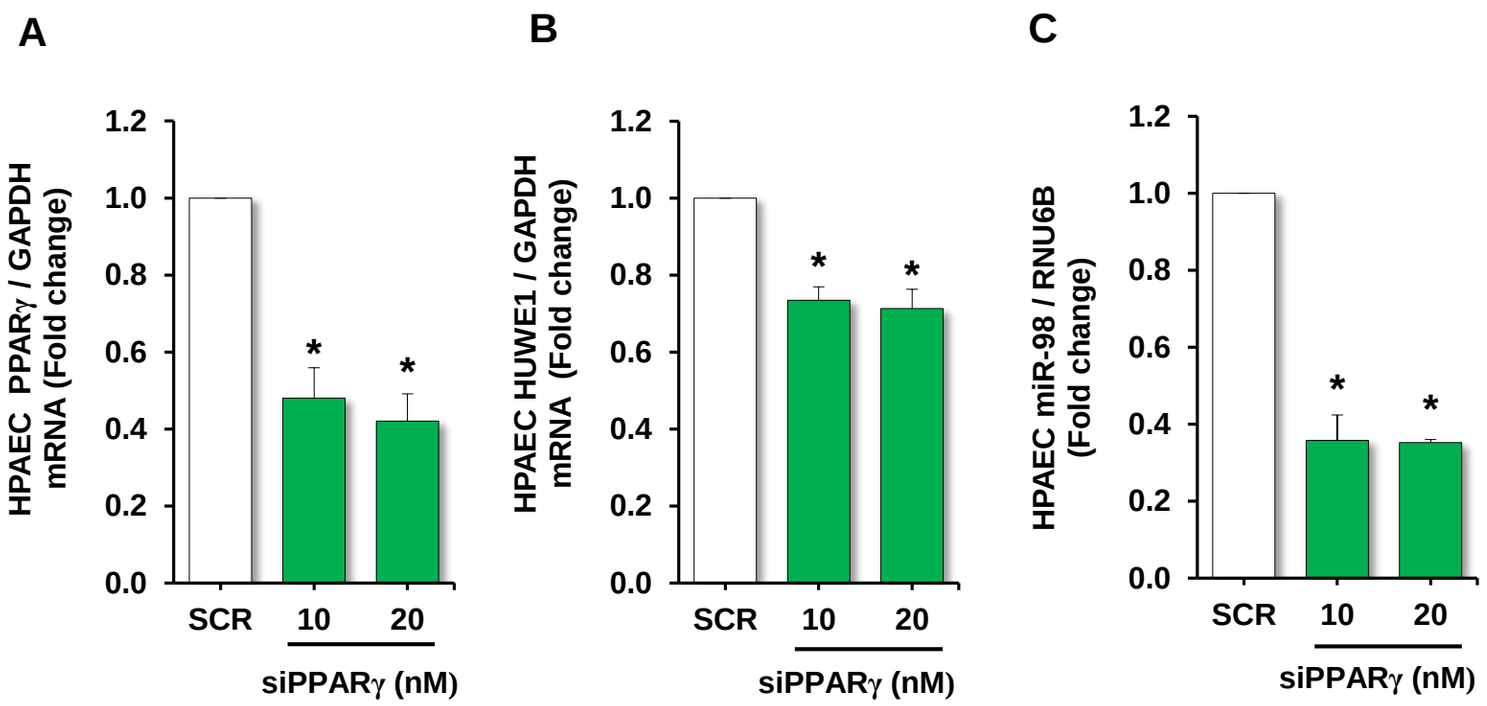

Supplementary Fig. S6