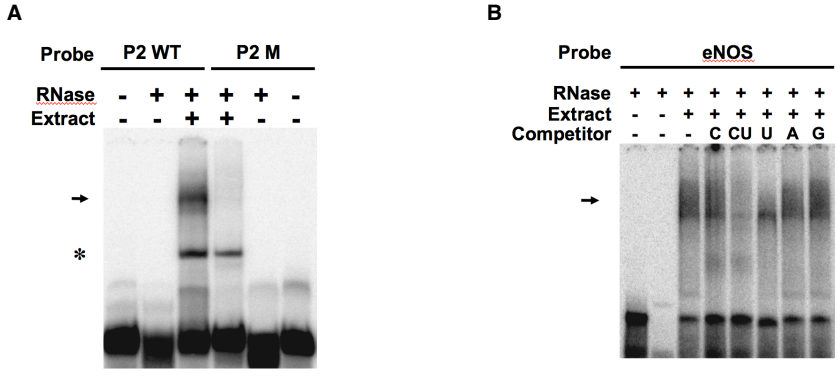
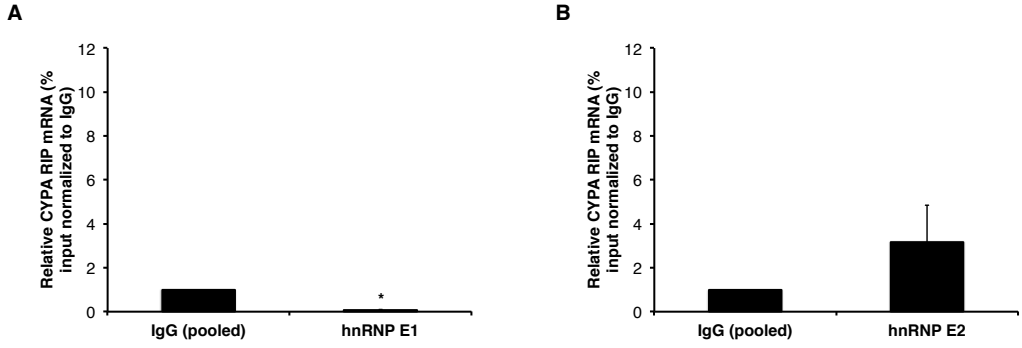
Name	Primer Sequence	Region Mutated*
Main-S	5'-TAC GTG CAG GAC ATC CTG-3'	
Main-AS	5'-GAT CAC GCC ACT GCA CTC CAG C-3'	
PyrI-MS	5'-**GTC GAA GCT TCC AGG** GAG GTG GTG CCT TCT CAC ATC T-3'	+75→+89
PyrI-MAS	5'-**TCG CAA GCT TGT GC**G GGC TGA TCC TGG TCG GGC GGA-3'	
PyrII-MS	5'-**GTC GAA GCT TCC AGG** AGG CCT GTT GCC TCG GGC CTG G-3'	+164→+187
PyrII-MAS	5'-**TCG CAA GCT TGT GC**G CGT TTT GCT CCT TCC TGG AGG-3'	
PyrIII-MS	5'-**GTC GAA GCT TCC AGG** AGC AGC GGT ACC CCA GGG CCT A-3'	+214→+228
PyrIII-MAS	5'-**TCG CAA GCT TGT GC**G ATT AAG GCG GAC CCA GGC CCG-3'	
PyrIV-MS	5'-**GTC GAA GCT TCC AGG** GCC TCT CTC AGG AGT ATC TTA C-3'	+280→+295
PyrIV-MAS	5'-**TCG CAA GCT TGT GCT** TCG ACT AAG AAA CAG GAA GCG-3'	
PyrV-MS	5'-**GTC GAA GCT TCC AGG** ATC TTA CCT GTA AAG TCT AAT C-3'	+296→+310
PyrV-MAS	5'-**TCG CAA GCT TGT GC**A AGA GGA ATC TAA CAT TCG ACT-3'	
PYRIII-MS-Acl I	5'-**GTC GAA CGT TCC AGG** AGC AGC GGT ACC CCA GGG-3'	+164→+187 +214→+228 +280→+295
PYRIII-MAS-Acl I	5'-**TCG CAA CGT TGT GC**G ATT AAG GCG GAC CCA GGC CCG-3'	
ΔARE-MS	5'-**GTC GAA GCT TGC** GAA GAT TTA CCA TAA GGG-3'	+313→+333
ΔARE-MAS	5'-**TCG CAA GCT TCG** ACT TGA TTT AGA GAT TAG-3'	

*Relative to the first nucleotide of the translation stop codon

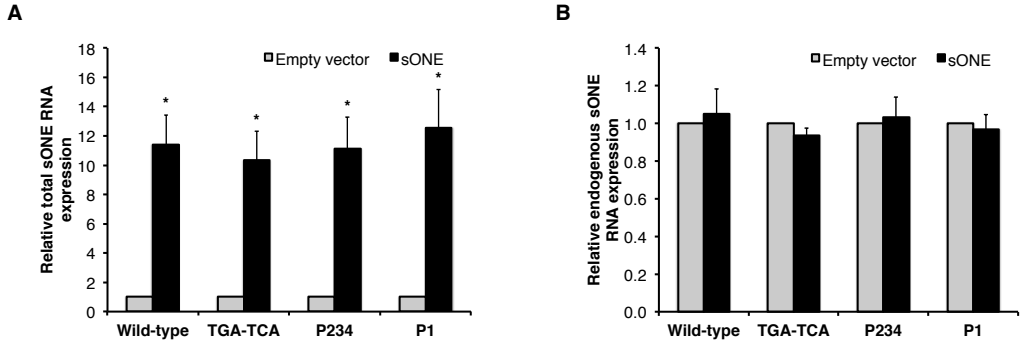
Supplemental Table S1. Primers used for linker mutagenesis of the eNOS 3'-UTR.



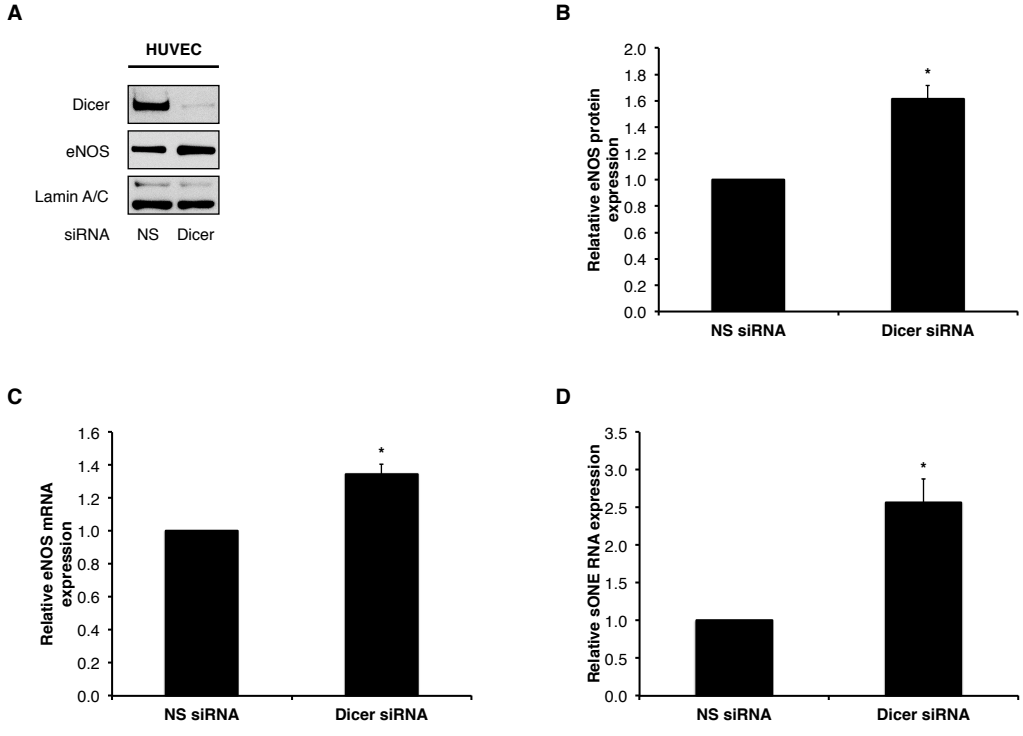
Supplemental Figure S1. Formation of hnRNP E1-containing RNP complexes on functionally important elements of the eNOS 3'-UTR. (A) *In vitro* transcribed, ^{32}P-labeled riboprobes containing wild-type eNOS P2 element (P2 WT) or a 15 nt P2 (P2 M) mutation were incubated with or without S100 cytoplasmic extract from HUVEC. Formation of a specific RNP complex indicated by an arrow was observed. Formation of a second, faster migrating, non-specific RNP complex was variably observed with these probes (indicated by *). (B) Effect of competing poly(A), poly(C), poly(CU), poly(U), or poly(G) sequences on eNOS RNP complex formation on the eNOS P2 element. Specific RNP complex formation is indicated by an arrow.



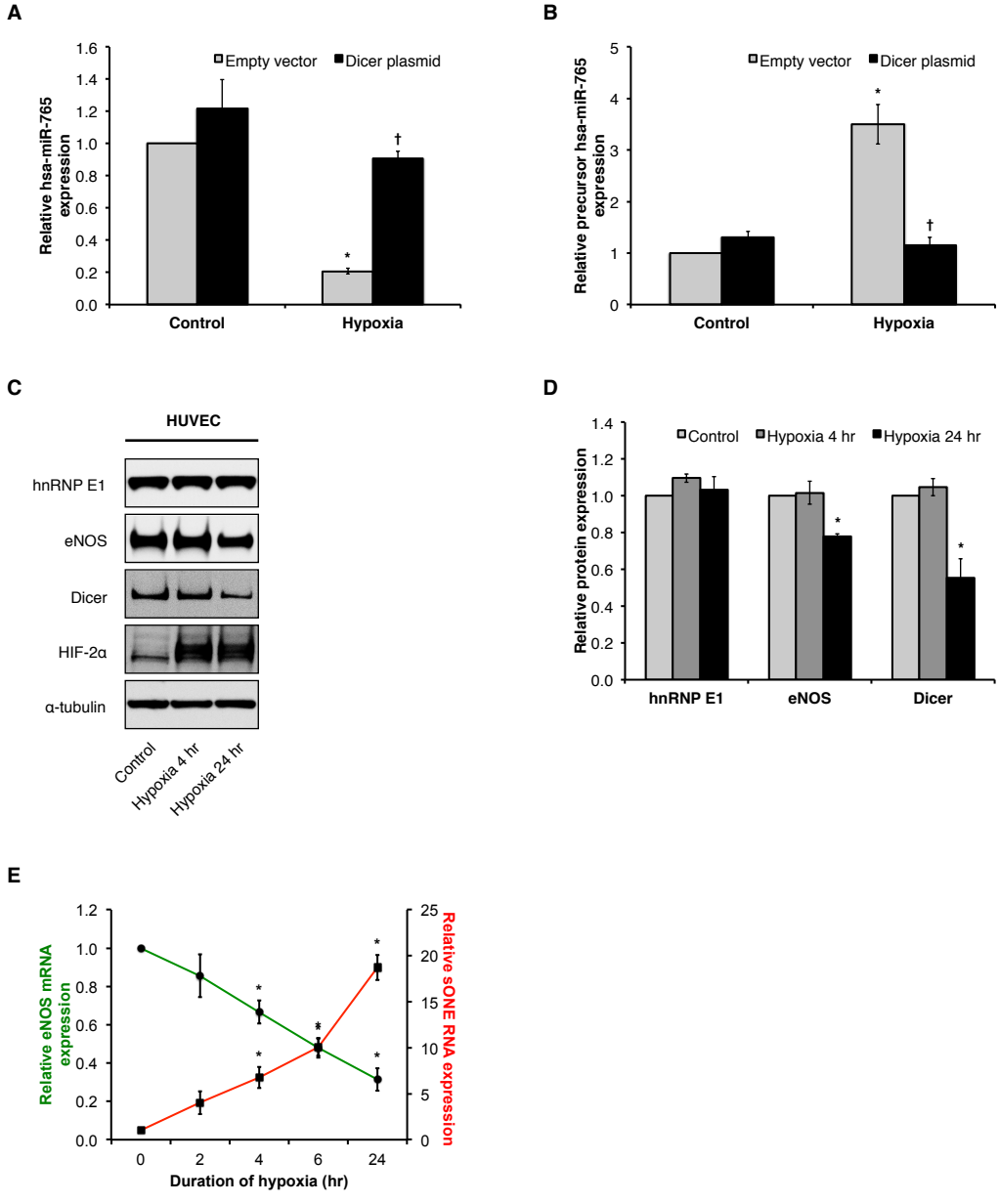
Supplemental Figure S2. CYPA mRNA does not associate with hnRNP E1 or E2. hnRNP E1 or E2 and associated RNAs were immunoprecipitated using an hnRNP E1-specific or hnRNP E2-specific antibody in HUVEC. (A) hnRNP E1-associated and (B) hnRNP E2-associated CYPA mRNA levels. Data is presented as percent (%) input relative to measurements of control IgG (pooled) pull-down. Data represent mean +/- SEM (n=3). * indicates statistical significance ($p<0.05$) compared to control IgG (pooled).



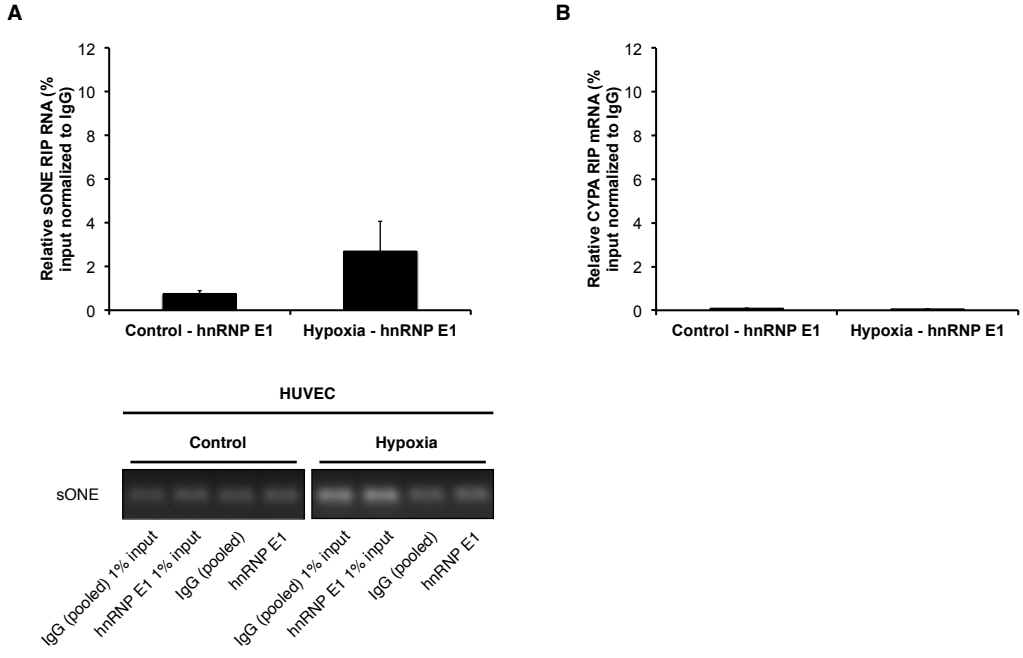
Supplemental Figure S3. sONE overexpression in stably transfected eNOS-expressing HepG2 cell lines. (A) Total and (B) endogenous sONE RNA levels were measured following transient transfection of a plasmid encoding 1555nt RNA corresponding to the region of sONE RNA that overlaps with eNOS mRNA, or empty vector. Data represent mean +/- SEM (n=3). * indicates statistical significance ($p<0.05$) compared to corresponding Empty vector.



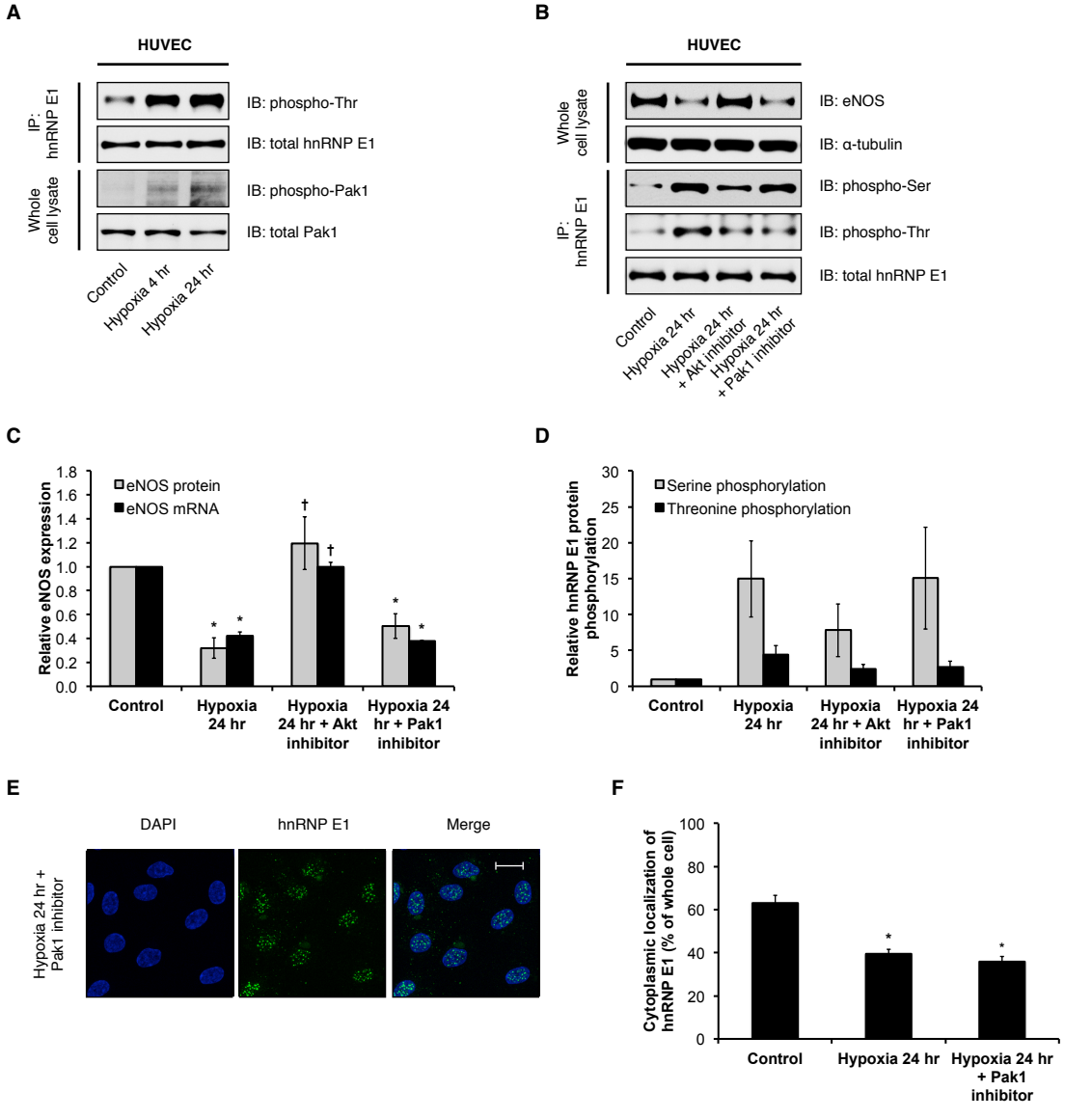
Supplemental Figure S4. Effects of Dicer knockdown on basal eNOS and sONE expression. (A) Representative immunoblots of HUVEC under Dicer-specific siRNA knockdown conditions. (B) Quantification of eNOS immunoblots in (A). (C) eNOS mRNA and (D) sONE RNA levels in HUVEC under Dicer-knockdown conditions. Data represent mean +/- SEM (n=3). * indicates statistical significance ($p<0.05$) compared to non-silencing siRNA (NS siRNA).



Supplemental Figure S5. Effects of hypoxia on miR-765, hnRNP E1, eNOS, and sONE expression. Relative levels of (A) mature and (B) precursor hsa-miR-765 species in control versus hypoxic Dicer-overexpressing and control vector-transfected HUVEC. Data represent mean +/- SEM (n=3). * and † indicate statistical significance ($p<0.05$) compared to Control and Hypoxia-empty vector, respectively. (C) Representative immunoblots of control (21% O_2) versus hypoxic (1% O_2) HUVEC. (D) Quantification of immunoblots in (C). (E) eNOS mRNA and sONE RNA levels in hypoxic HUVEC. Data represent mean +/- SEM (n=3/4). * indicates statistical significance ($p<0.05$) compared to corresponding Control.



Supplemental Figure S6. sONE RNA does not associate with hnRNP E1 under basal or hypoxic conditions. hnRNP E1 and associated RNAs were immunoprecipitated using an hnRNP E1-specific antibody in control (21% O_2) versus hypoxic (1% O_2, 24 hr) HUVEC. hnRNP E1-associated (A) sONE RNA and (B) CYPA mRNA levels in control versus hypoxic HUVEC. Data is presented as percent (%) input relative to measurements of control IgG (pooled) pull-down. Data represent mean +/- SEM (n=3). Bottom panels represent real-time PCR products obtained from measurements in the respective top panels. The association of sONE RNA with hnRNP E1in hypoxic HUVEC is not significant even though the total amount of sONE RNA was significantly up-regulated (Supplemental Figure S5E).



Supplemental Figure S7. Effects of hnRNP E1 threonine phosphorylation on hnRNP E1 subcellular localization and eNOS expression. (A) Representative immunoblots of control versus hypoxic HUVEC hnRNP E1 immunoprecipitation (IP) samples (top two panels), and whole cell lysates of control versus hypoxic HUVEC (bottom two panels). (B) Representative immunoblots of whole cell lysates (top two panels) and IP samples (bottom three panels) from control HUVEC, hypoxic HUVEC, and hypoxic HUVEC treated with 5 μM of Akt inhibitor IV or Pak1 inhibitor IPA-3. Levels of (C) eNOS mRNA and protein (immunoblot quantification in (B)), and (D) phosphorylated serine and threonine in control HUVEC, hypoxic HUVEC, and hypoxic HUVEC treated with Akt inhibitor IV or IPA-3. Data represent mean +/- SEM (n=3). * and † indicate statistical significance ($p<0.05$) compared to corresponding Control and Hypoxia 24 hr, respectively. (E) Subcellular localization of hnRNP E1 was determined by immunofluorescence using confocal microscopy in hypoxic HUVEC treated with IPA-3 (representative image shown). Left column: DAPI stain (blue); Middle column: hnRNP E1 (green); Right column: Merge of left and middle columns. Scale bar at top right represents 20 μm. (F) Quantification of cytoplasmic hnRNP E1 based on images in (E), expressed as a percentage (%) of whole cells. Data represent mean +/- SEM (n=3). * indicates statistical significance ($p<0.05$) compared to Control.