ANGIOPOIETIN 2 IS A PARTIAL AGONIST/ANTAGONIST OF TIE2 SIGNALING IN ENDOTHELIUM

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Running title:

Ang2 is a partial agonist of Tie2

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Supplementary Data

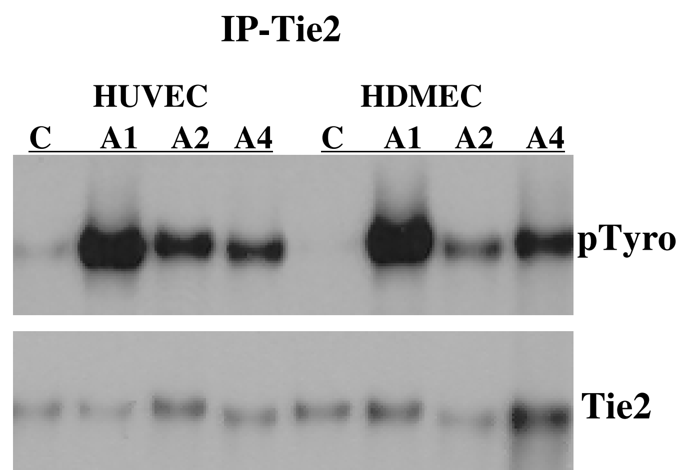
Figure 1. Tie2 phosphorylation in human dermal microvascular endothelial cells (HDMEC). Confluent HDMECs or HUVECs were stimulated with 400ng/ml of Ang1, Ang2 or Ang4 for 30 minutes and cell lysates were prepared as described and immunoprecipitated with anti-Tie2. After IP, Western blot for phospho-tyrosine and total Tie2 was performed.

Figure 2. Ang2 neutralizing antibody blocks Ang2-, but not Ang1-induced phosphorylation of Tie2. Confluent HUVECs were stimulated with Ang2 and Ang1 (400ng/ml) for 30 minutes in the presence or absence of Ang2 neutralizing antibody (20µg/ml). Cell lysates were prepared as described and immunoprecipitated with anti-Tie2. After IP, Western blot for phospho-tyrosine and total Tie2 was performed sequentially.

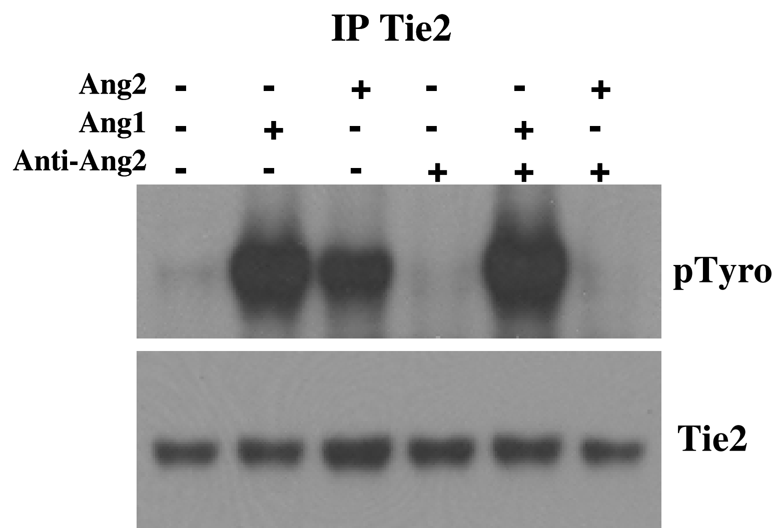
Figure 3. Matrigel-induced tube formation of HUVECs treated with Ang2 siRNAs
Matrigel-induced tube formation assay was performed as previously described (48). 48 hours after HUVECs were transfected with Ang2-82, Ang2-84 or NC siRNA, 2.5×10^4 cells in 300µl of full medium were inoculated into 48-well plate pre-coated with 100µl Matrigel. The images of tubes formed were taken 24hours after cell inoculation and 3 representative images were depicted (**A**) alone with the percentage of areas (**B**) covered by tubes formed by HUVECs treated with different siRNAs (n=6). ** indicates a P value < 0.01 comparing cells treated with NC siRNA to those treated with Ang2 specific siRNAs.

Figure 4. Ang1-dependent Tie2 phosphorylation in HUVECs treated with Ang2 siRNAs
80% confluent HUVECs were transfected with NC, Ang2-82 or Ang2-84 siRNA and 48 hours later, cells were stimulated 100ng/ml of Ang1 for 30 min before cell lysates were

prepared and immunoprecipitated with anti-Tie2. After IP, Western blot for phospho-tyrosine and total Tie2 was performed sequentially. Representative blots of three independent experiments (**A**) are depicted alone with Phospho/Total Tie2 (P/T) ratio (**B**) for bands in A (mean ± SD, n=3). The P/T ratio of Tie2 is expressed in arbitrary units with those treated with individual siRNA without Ang1 stimulation (Con) accepted as being equal to 1.0. * indicates a P value < 0.05 and ** indicates a P < 0.01 respectively comparing between the cells stimulated with or without Ang1. ## indicates a P < 0.01 comparing the cells treated Ang2 specific siRNA to those treated with NC siRNA.

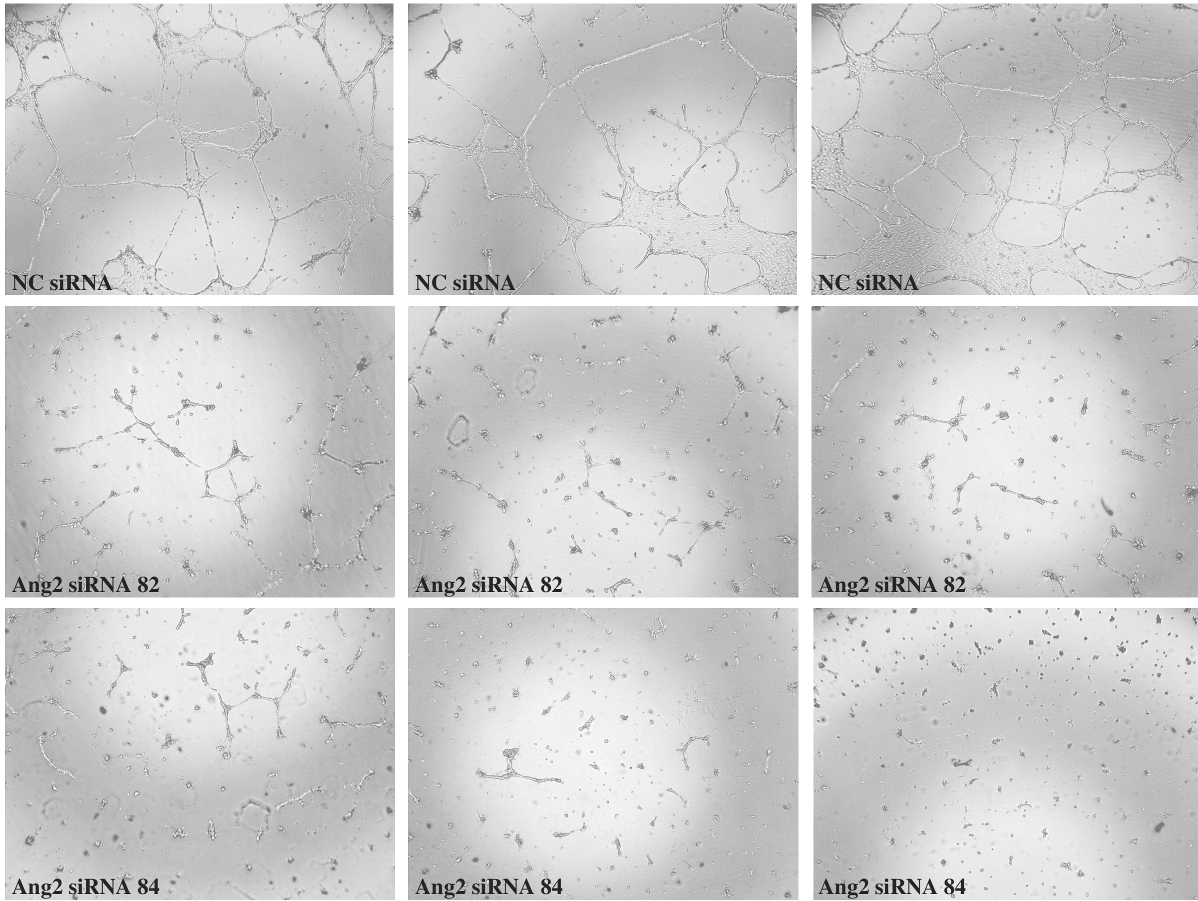


Supplementary Figure 1. Yuan, HT et al

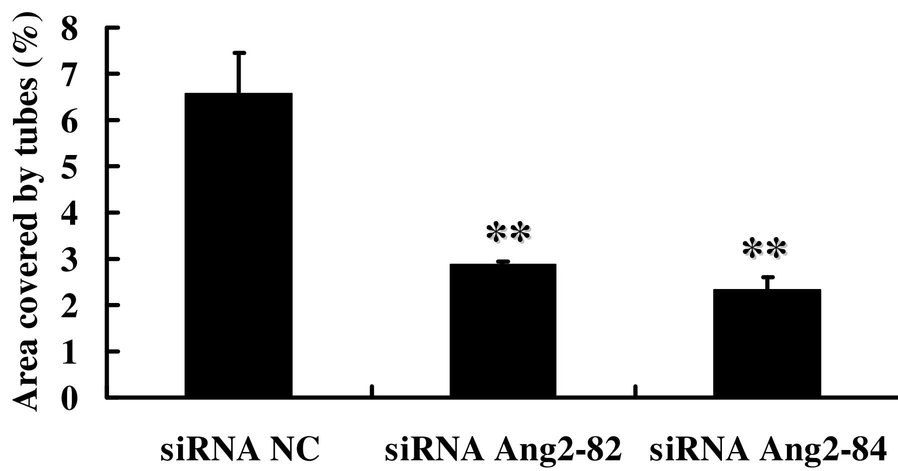


Supplementary Figure 2. Yuan, HT et al

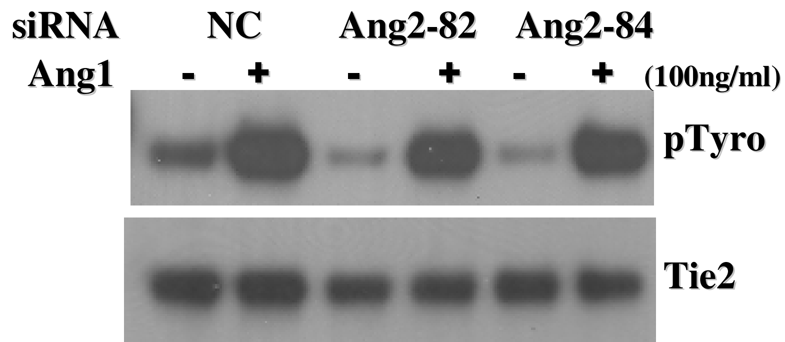
A

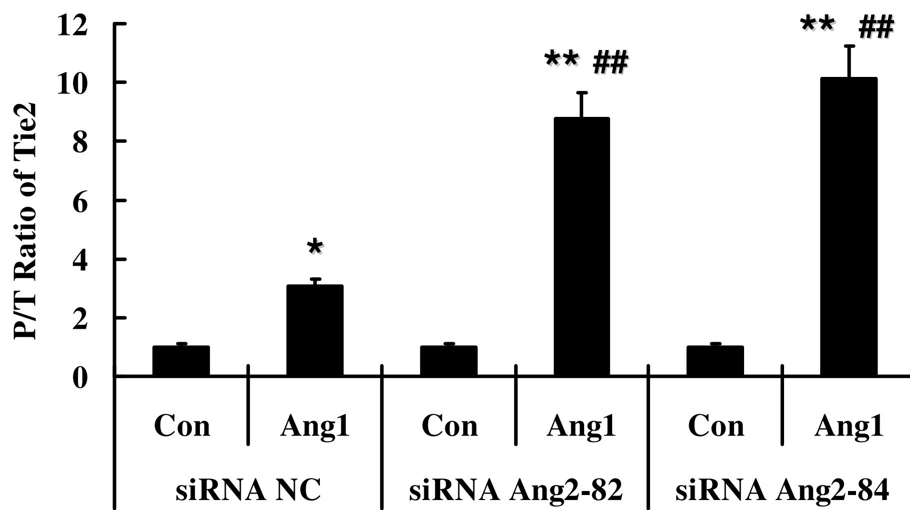


B



Supplementary Figure 3. Yuan, HT et al





Supplementary Figure 4. Yuan, HT et al