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Supplemental Information

Improving the Angiogenic Potential of EPCs via Engineering with Synthetic Modified mRNAs

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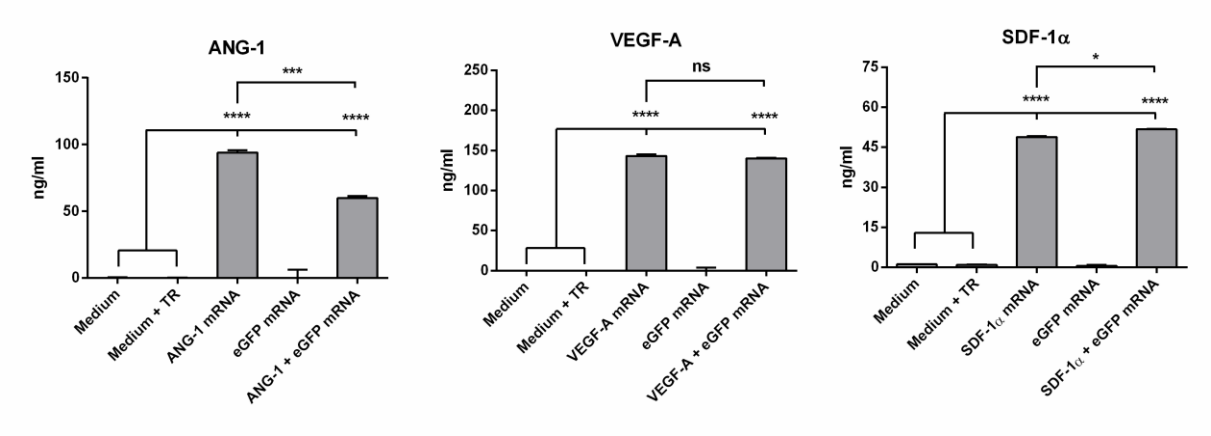
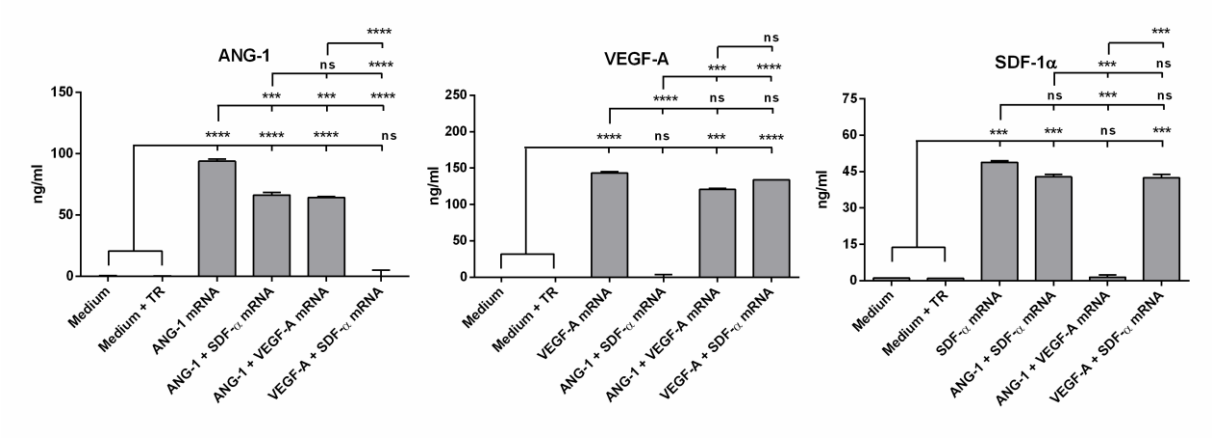
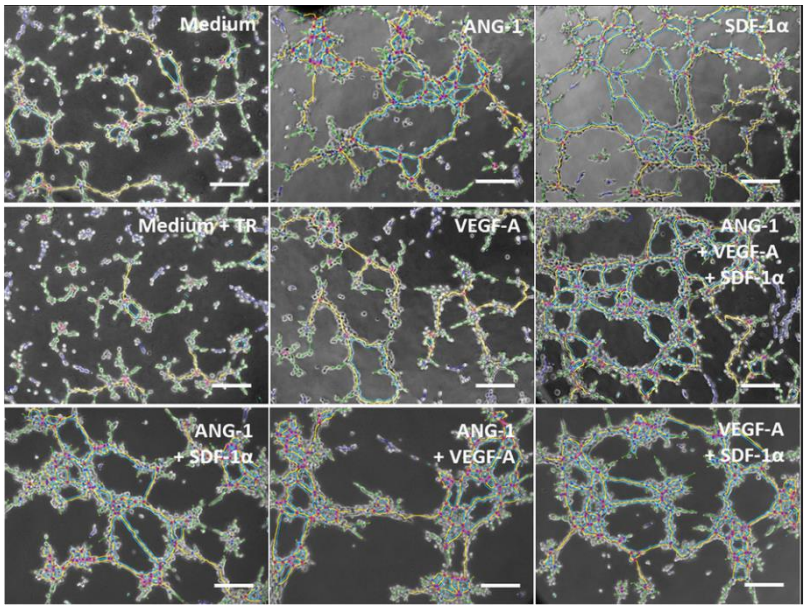
Supplementary Figures

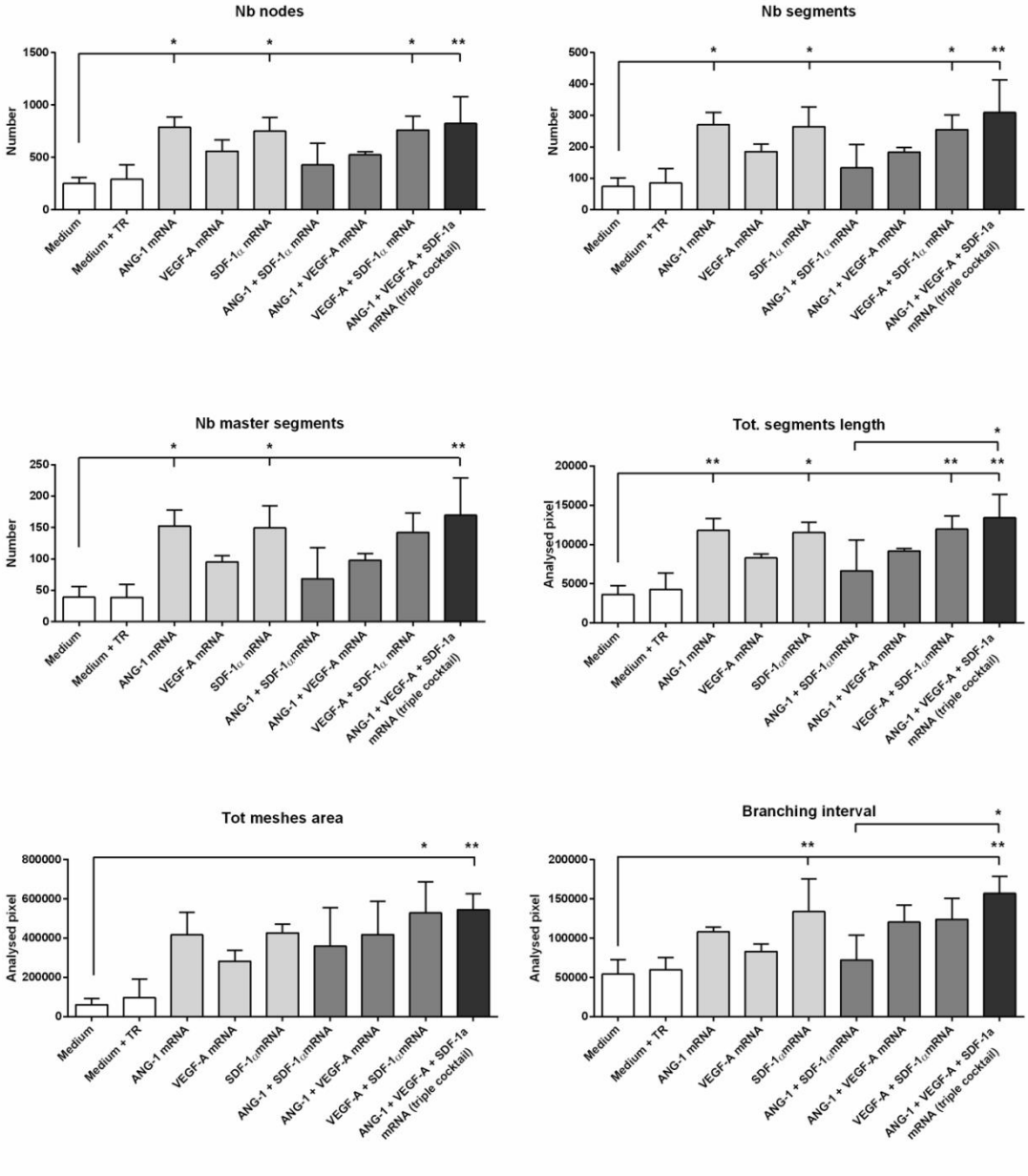


Supplementary Figure 1: Influence of mRNA amount on protein translation. 1 x10^5 murine EPCs were seeded and transfected the next day with 1.6 µg ANG-1, 0.8 µg VEGF-A, 0.5 µg SDF-1α, or 2.9 µg eGFP mRNA. Additionally, cells were transfected with proangiogenic mRNAs and eGFP mRNA, which was used as filler mRNA, to reach a total amount of 2.9 µg mRNA. The protein expression was analyzed in supernatants 24 h after the transfection using ELISA. Cells treated with only medium, eGFP mRNA, or medium and transfection reagent (TR) served as negative controls. Results are shown as mean + SEM (n=3). Statistical differences were determined using one-way ANOVA followed by Bonferroni's multiple comparison test (*p<0.05, ***p<0.001, ****p<0.0001, n.s. not significant).



Supplementary Figure 2: Influence of double proangiogenic mRNA combinations on protein synthesis. 1 x 10^5 murine EPCs were seeded and transfected the next day with 1.6 µg ANG-1, 0.8 µg VEGF-A, 0.5 µg SDF-1α mRNA, or with double mRNA combination cocktails: 1.6 µg ANG-1 + 0.5 µg SDF-1α, 1.6 µg ANG-1 + 0.8 µg VEGF-A, or 0.8 µg VEGF-A + 0.5 µg SDF-1α. The protein expression was analyzed in supernatants 24 h after the transfection using ELISA. Cells treated with only medium, or medium and transfection reagent (TR) served as negative controls. Results are shown as mean + SEM (n=3). Statistical differences were determined using one-way ANOVA followed by Bonferroni's multiple comparison test (***p<0.001, ****p<0.0001, n.s. not significant).


Medium
ANG-1
SDF-1α
Medium + TR
VEGF-A
ANG-1
+ VEGF-A
+ SDF-1α
ANG-1
+ SDF-1α
ANG-1
+ VEGF-A
VEGF-A
+ SDF-1α


Nb nodes
Number
Nb segments
Number
Nb master segments
Number
Tot. segments length
Analysed pixel
Tot meshes area
Analysed pixel
Branching interval
Analysed pixel
Medium
Medium + TR
ANG-1 mRNA
VEGF-A mRNA
SDF-1α mRNA
ANG-1 + SDF-1α mRNA
ANG-1 + VEGF-A mRNA
VEGF-A + SDF-1α mRNA
ANG-1 + VEGF-A + SDF-1a mRNA (triple cocktail)

Supplementary Figure 3: Influence of double proangiogenic mRNA combinations on tube formation. 1 x 10^5 murine EPCs were seeded and transfected the next day with 1.6 µg ANG-1, 0.8 µg VEGF-A, 0.5 µg SDF-1α mRNA, or in double mRNA combination cocktails: 1.6 µg ANG-1 + 0.5 µg SDF-1α, 1.6 µg ANG-1 + 0.8 µg VEGF-A, 0.8 µg VEGF-A + 0.5 µg SDF-1α. The angiogenic potential was assesed by tube formation assay and analyzed with ImageJ software. Cells treated with only medium, or medium and transfection reagent (TR) served as negative controls. Results are shown as mean + SD (n=3). Statistical differences were determined using one-way ANOVA followed by Bonferroni's multiple comparison test (*p<0.05, **p<0.01).