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# **AngiomiR-126 expression and secretion from circulating CD34<sup>+</sup> and CD14<sup>+</sup> PBMCs: role for proangiogenic effects and alterations in type 2 diabetics**

Pavani Mocharla, Sylvie Briand et al

# Angiogenesis

- AngiomiR-126 expression in PBMCs of type 2 diabetic patients and healthy controls
- Differences between CD34<sup>+</sup>/CD14<sup>+</sup>, CD34<sup>+</sup>/CD14<sup>-</sup>, CD34<sup>-</sup>/CD14<sup>+</sup>, and CD34<sup>-</sup>/CD14<sup>-</sup> PBMC subsets
- miR-126 levels in supernatant, microparticles and exosomes

- PBMCs are suggested to raise angiogenesis in ischemia
- Diabetic patients have a reduced cardiovascular repair function and an impaired neovascularization

- CD34<sup>+</sup> - hematopoietic progenitor cells, tumor cells
- CD14<sup>+</sup>-monocytes, macrophages
- microRNAs (miRs) are non-coding RNA molecules, which regulate gene expression
- AngiomiRs are microRNAs regulating angiogenesis

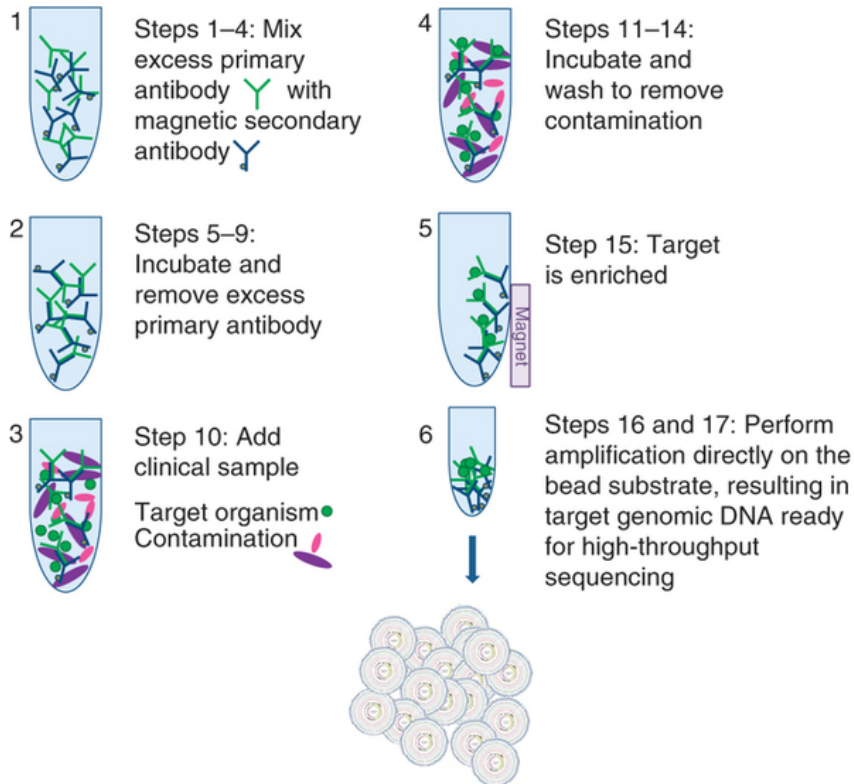
# Methods in vitro

- Positive and negative selection of CD34/CD14 cells
- MicroRNA RT PCR array
- Anti-miR-126 and miR-mimic-126 transfection
- In vitro tube formation assay (matrigel plugs, co-culture with Human aortic endothelial cells)

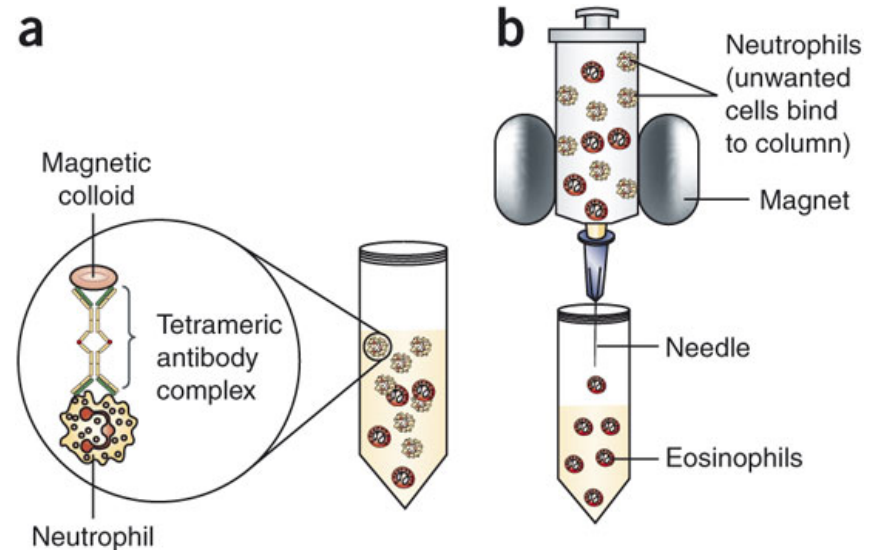
# Methods in vivo

- Matrigel basement membrane matrix with
  - miR-mimic-126,
  - anti-miR-126,
  - scrambled RNA,
  - supernatant,
  - microvesicles and
  - exosomeswas injected subcutaneously in mice along the abdominal midline
- Male NRM1 nu/v mice were used for transplantation of human PBMC subpopulation
- Diabetes was induced in C57BL/6 mice with streptozotocin

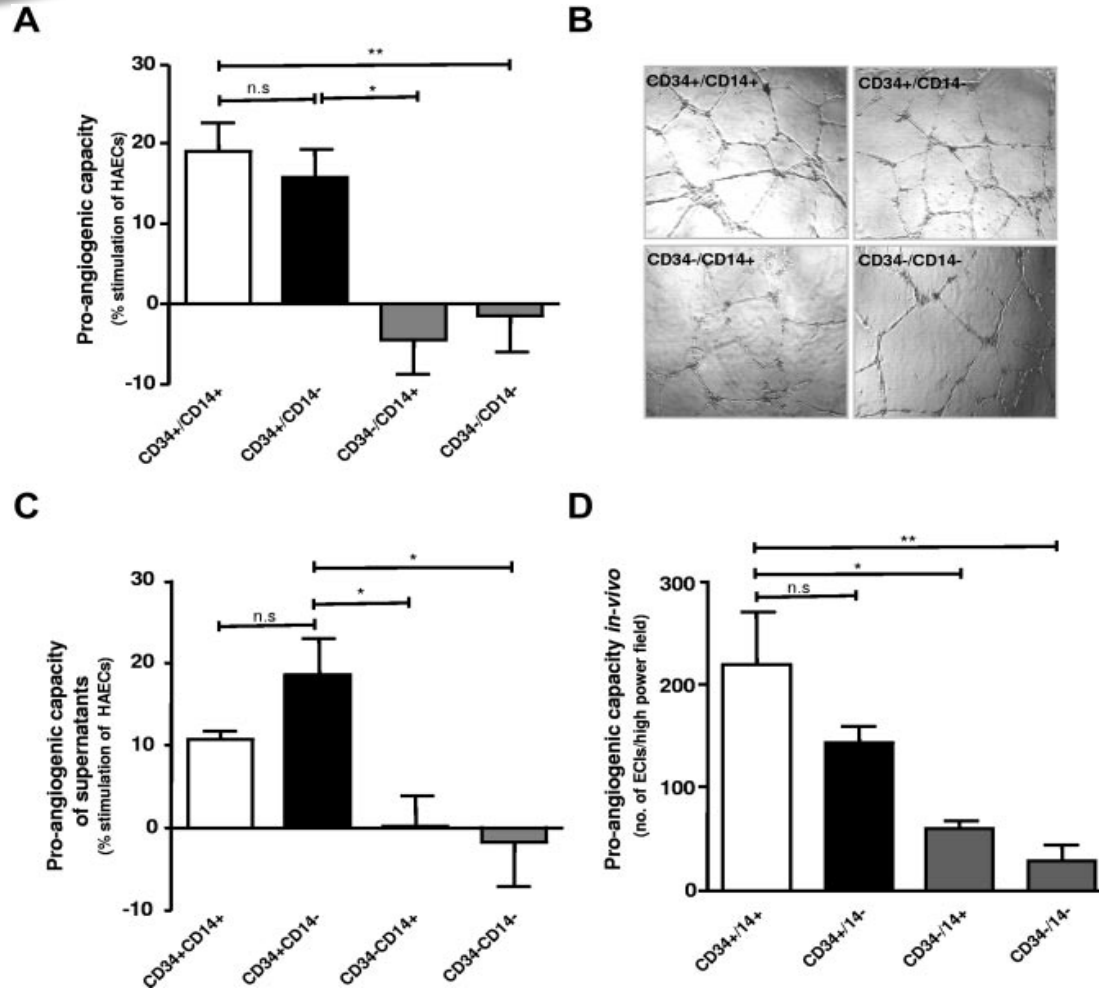
## Positive selection



## Negative selection

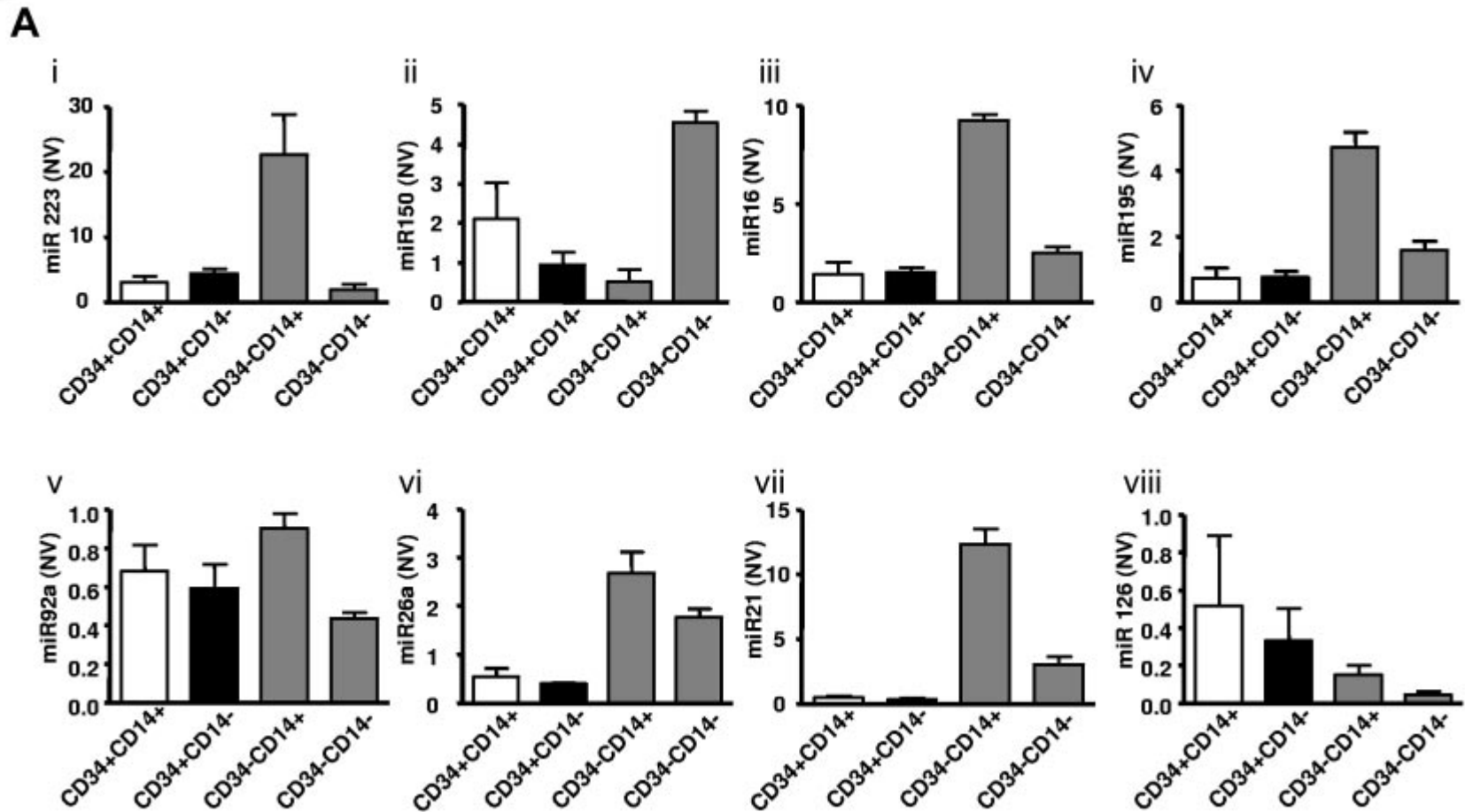


# Results

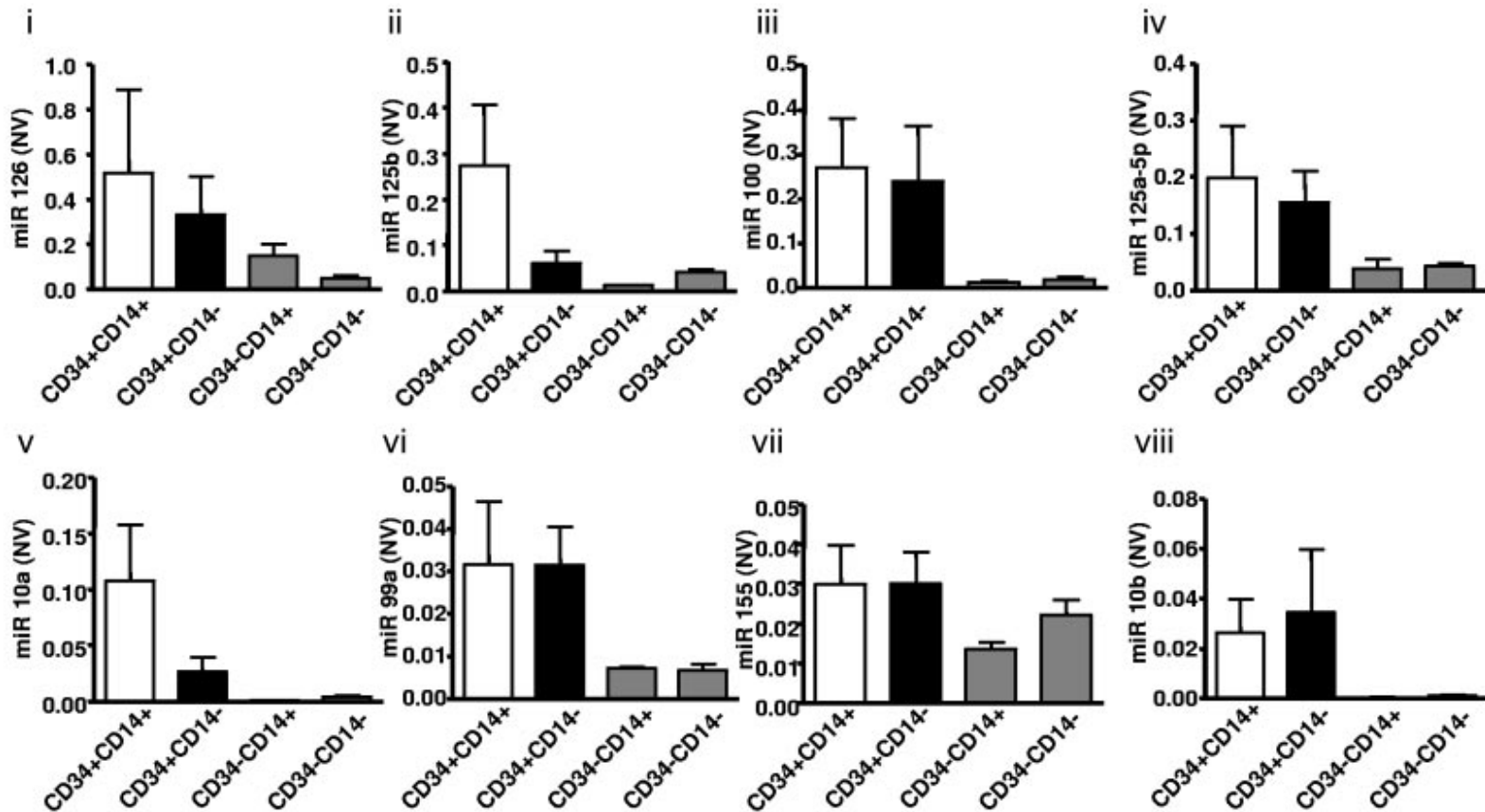


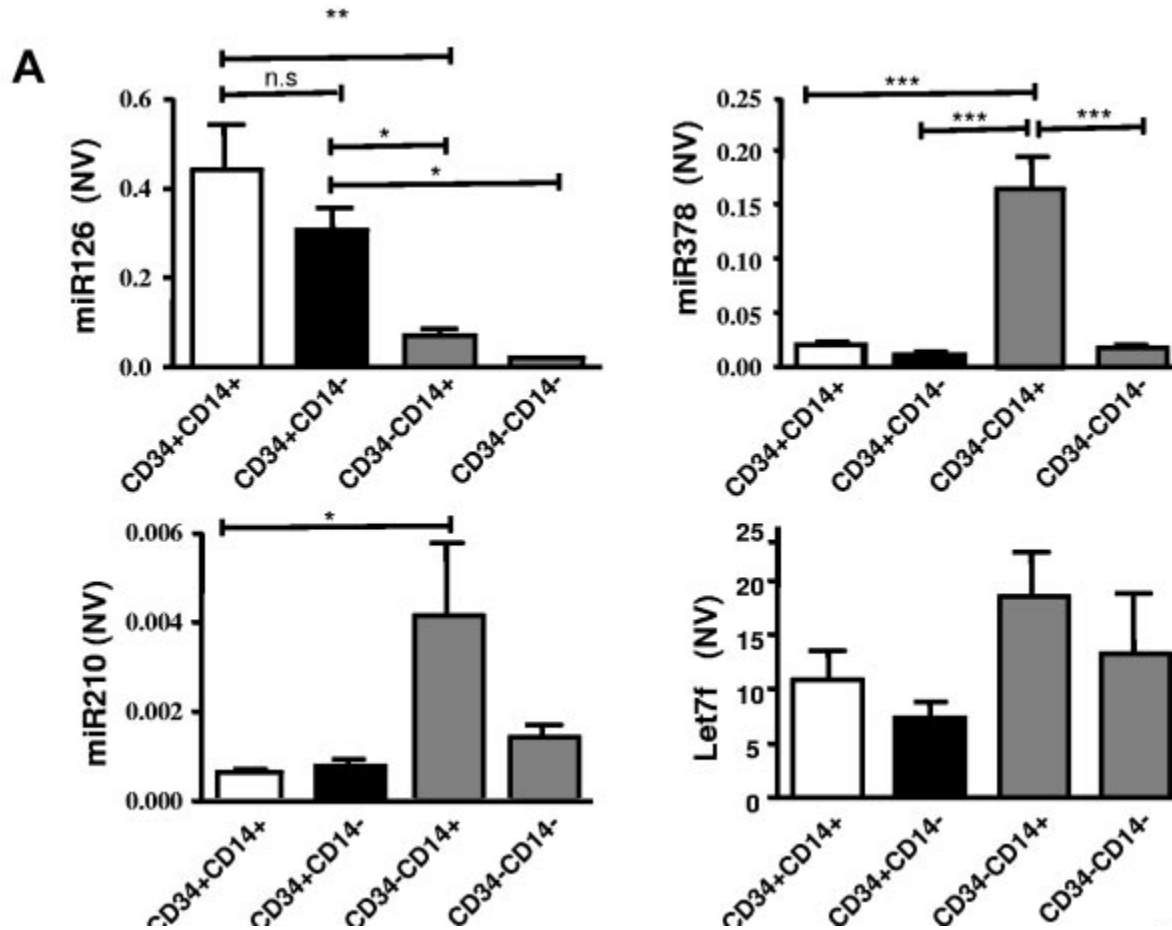


# MicroRNA expression

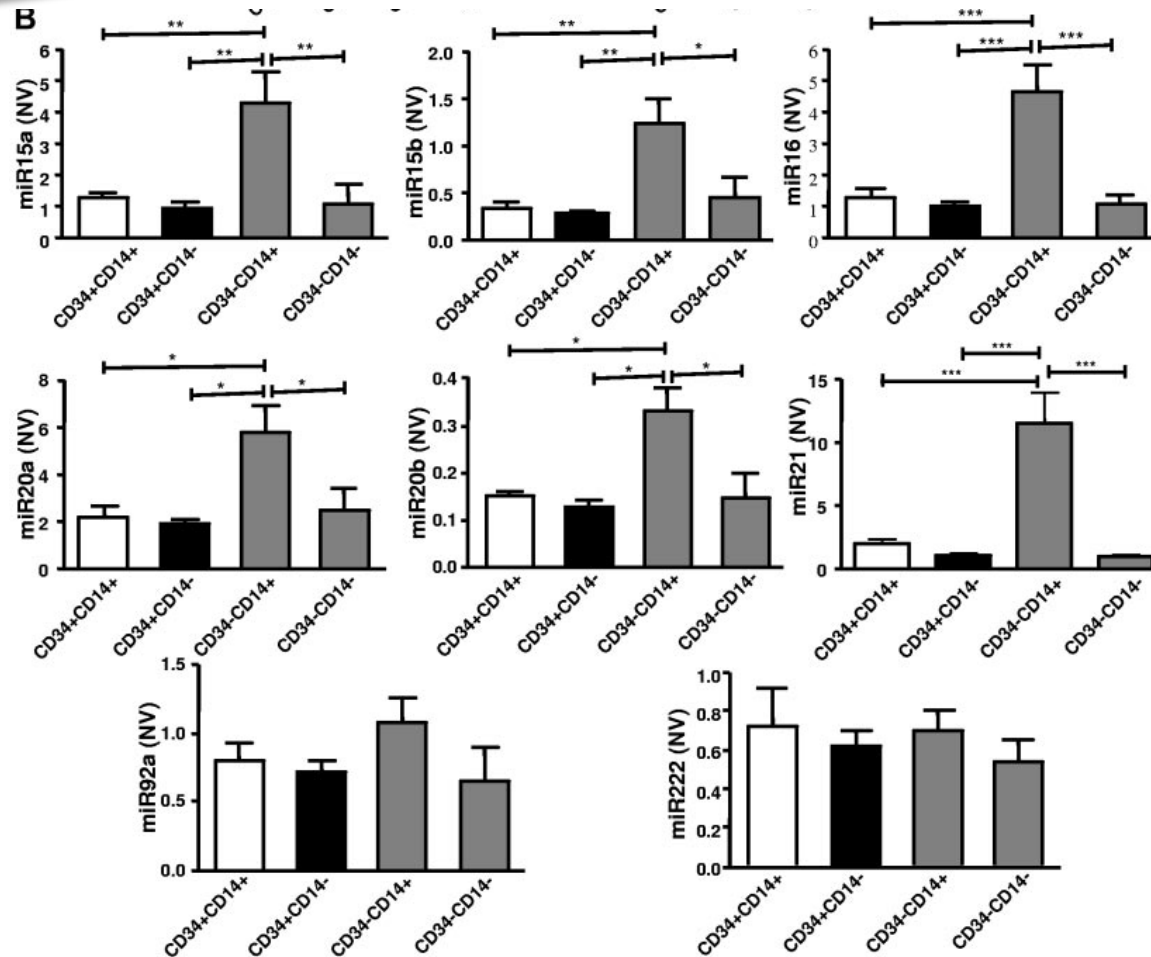


**B**

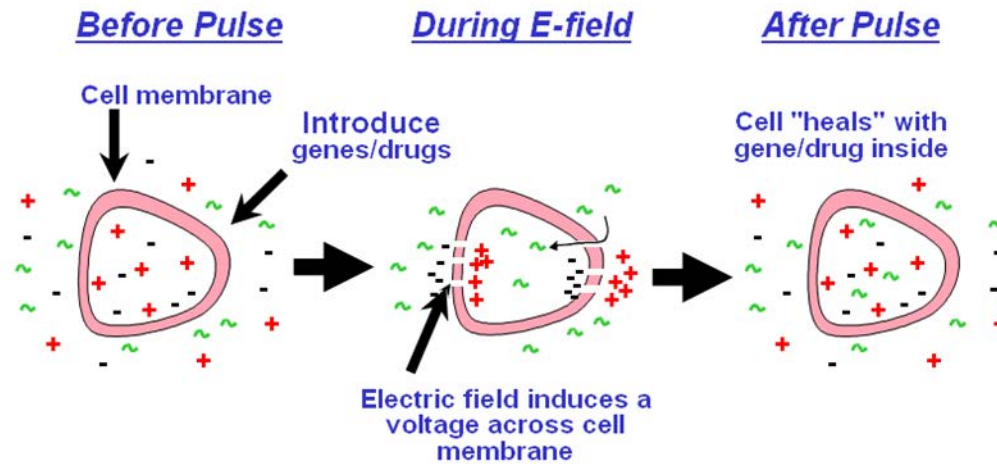


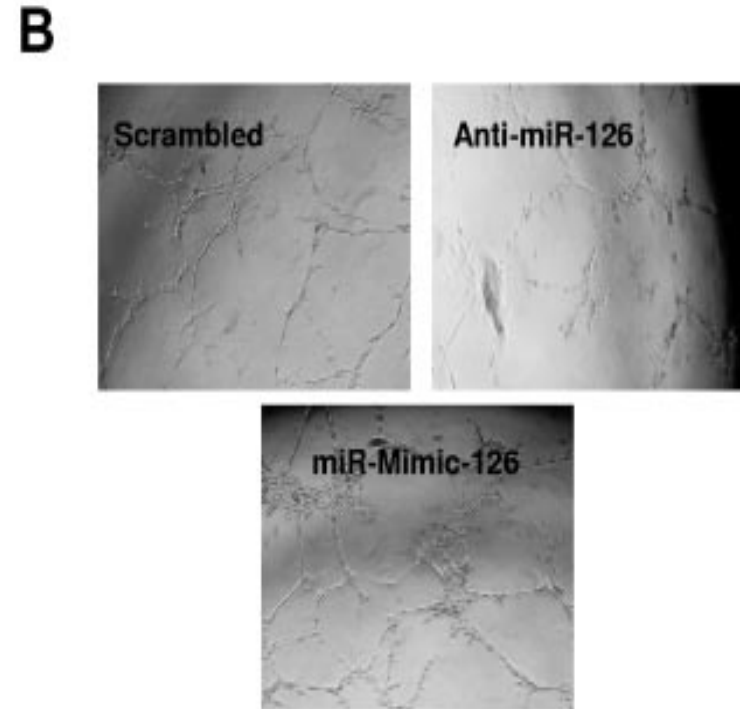
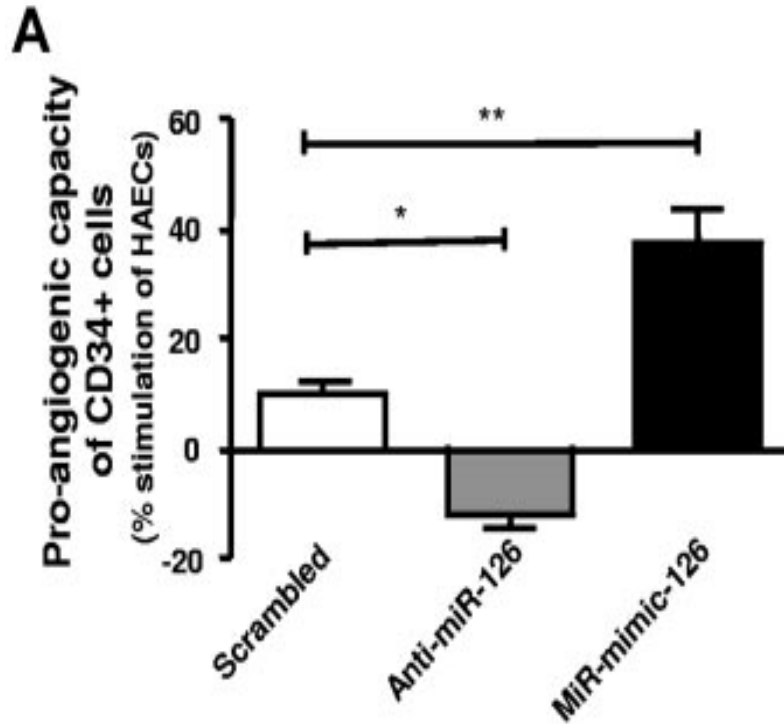


# Antiangiogenic miR



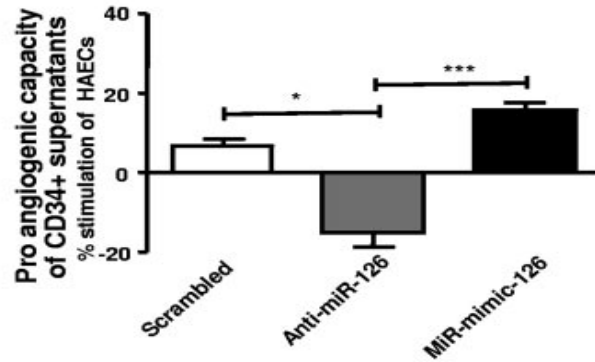
# Transfection



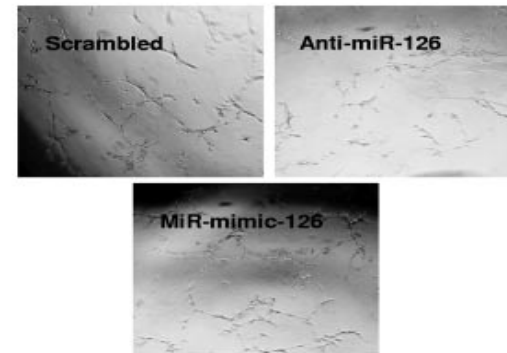




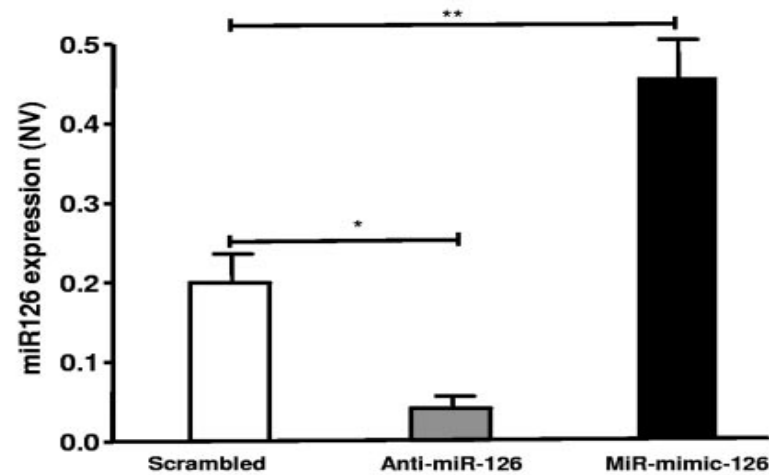
**C**



**D**



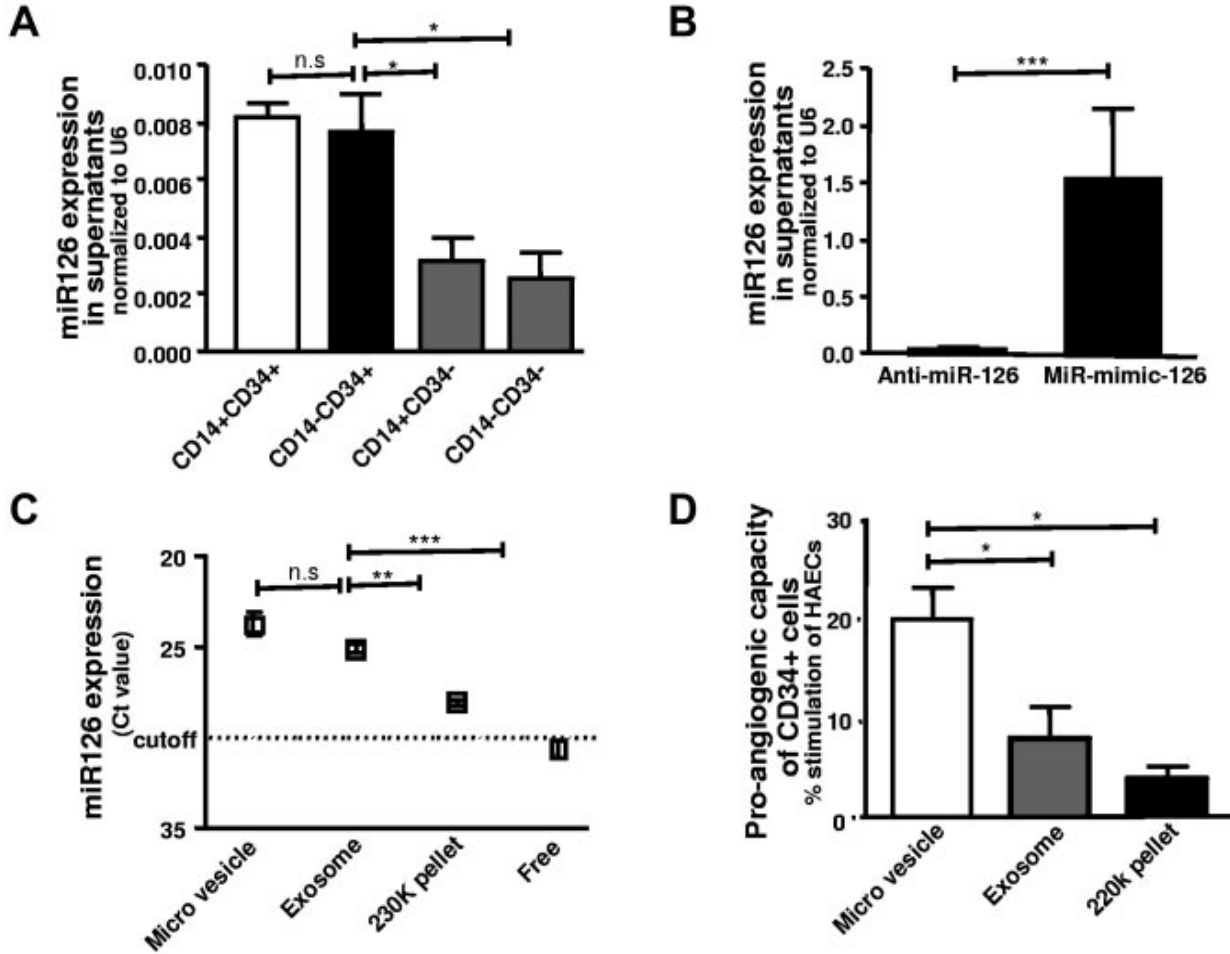
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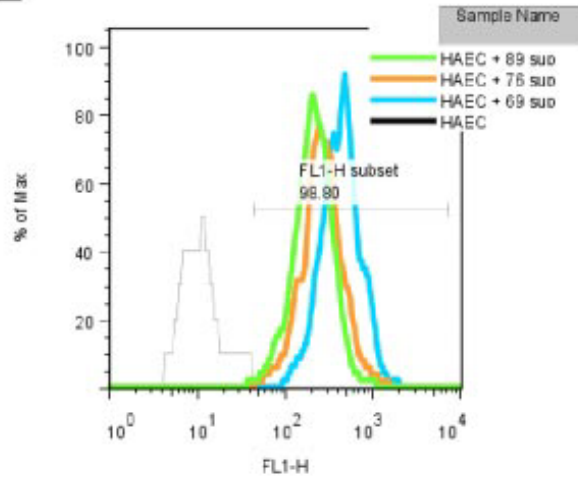
# Microvesicles and Exosomes

- Supernatant:
  - Centrifugation of PBMCs in cell culture for 1000g for 10min
- Microvesicles:
  - Pellet of centrifugated supernatant (16 000g for 60min)
- Exosomes:
  - Pellet of centrifugated microvesicle-supernatant (120000g for 60min)
- 220 000g-pellet:
  - Pellet of centrifugated exosome-supernatant (220000g for 60min)

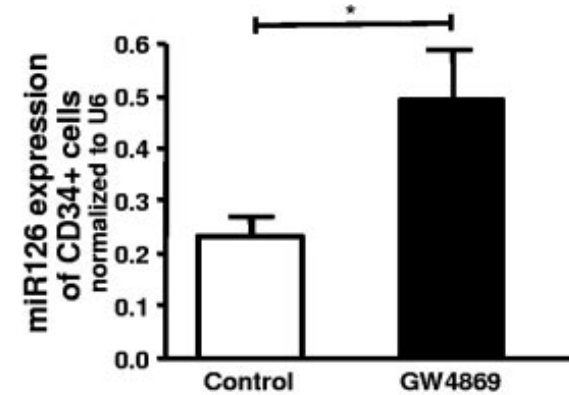




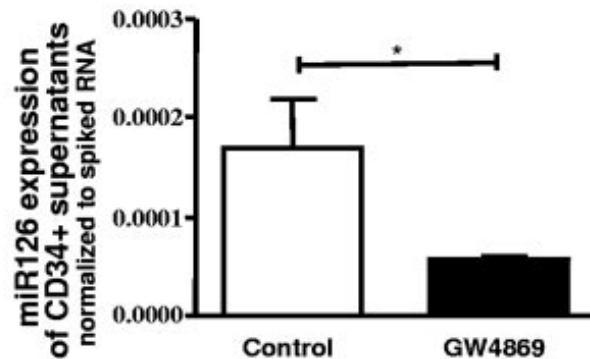
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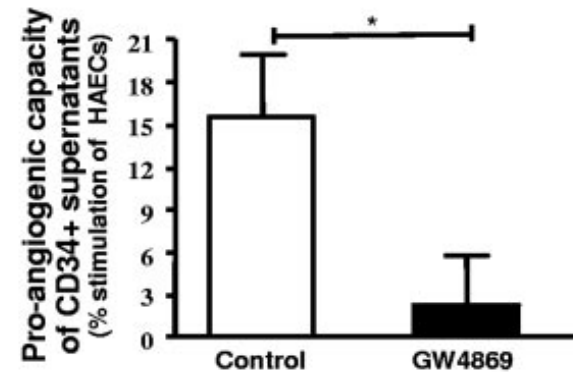
**F**



**G**

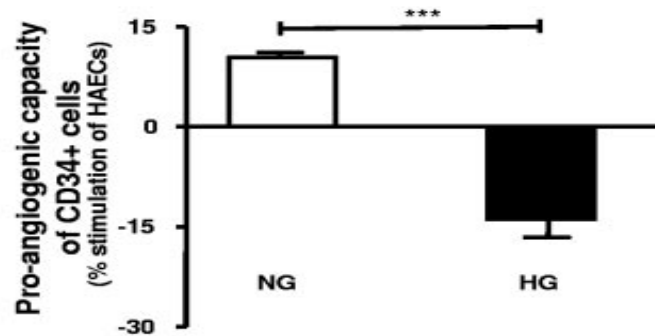


**H**

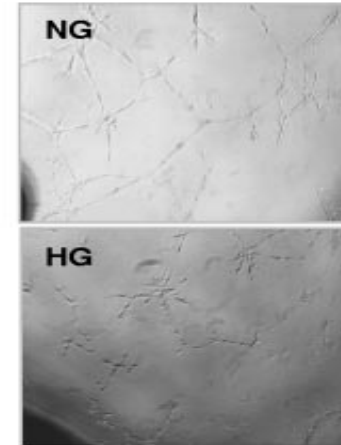


# Effects of High Glucose/Diabetes

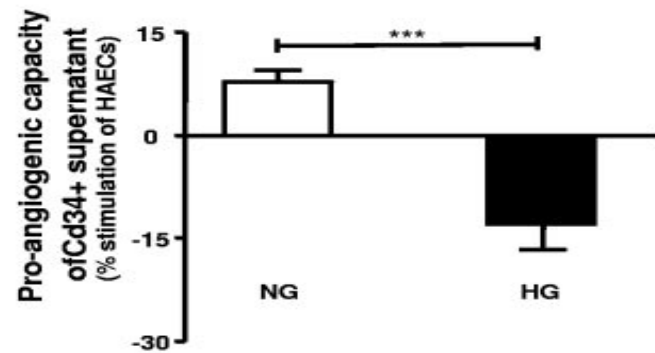
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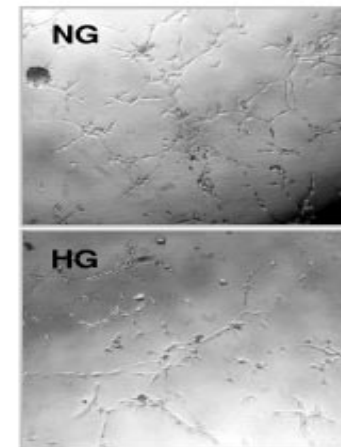
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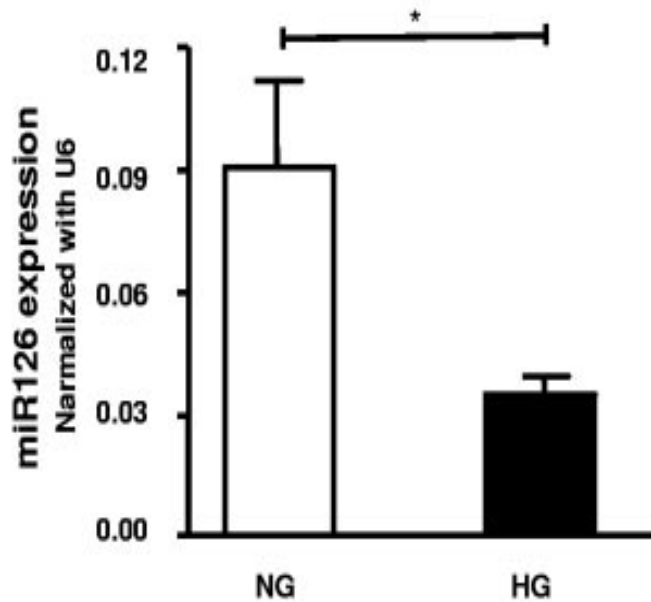
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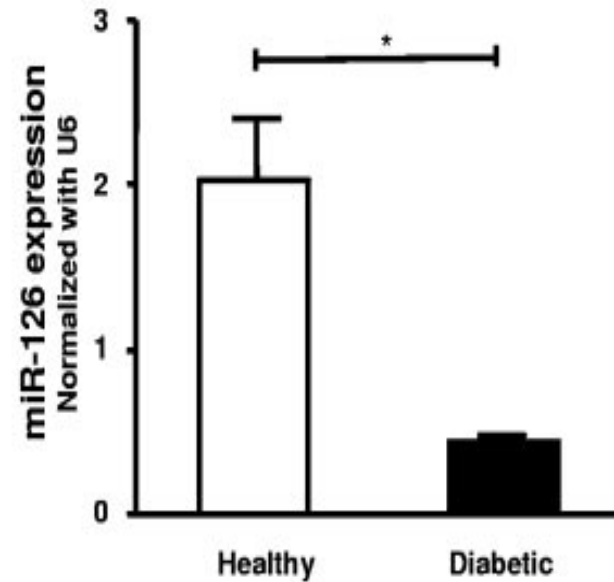
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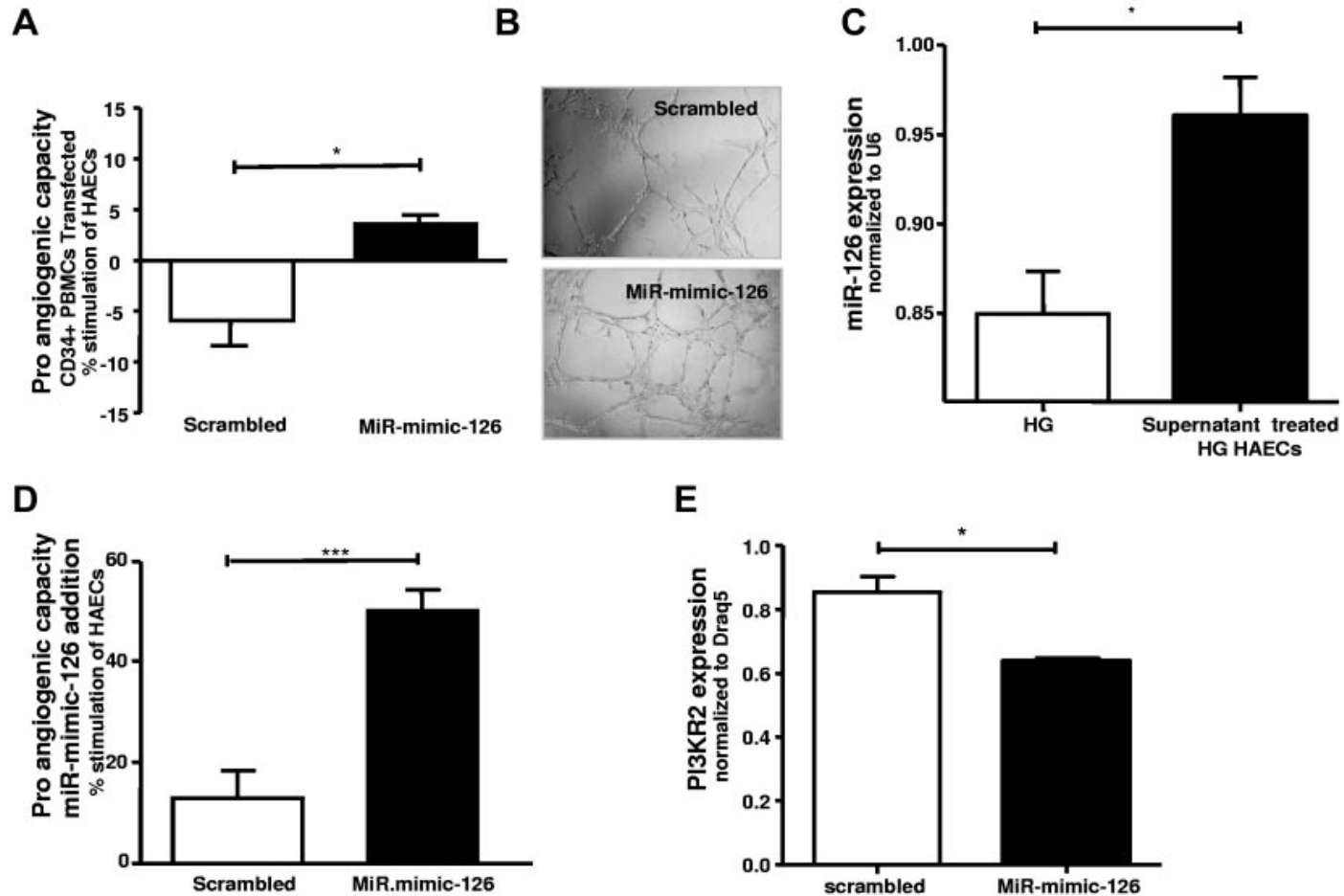


**E**

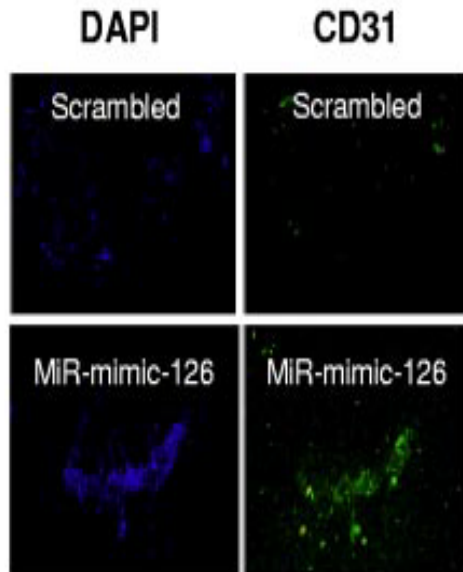


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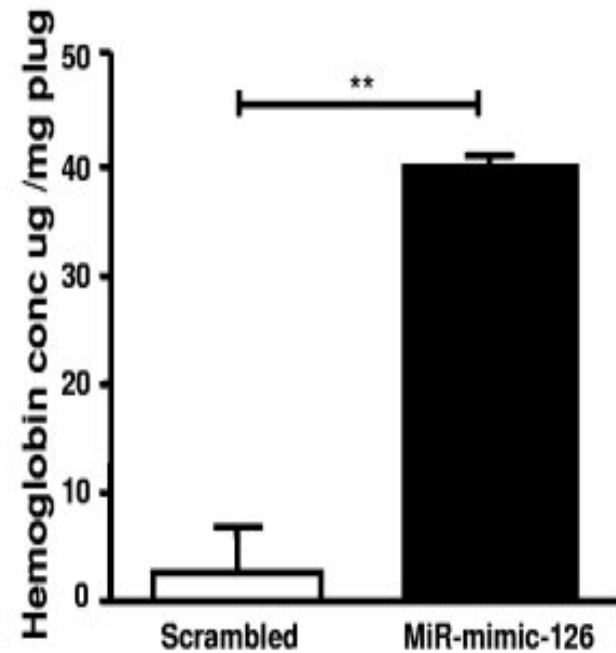




**F**



**G**



## Discussion

- Higher AngiomiR-126 expression and secretion in microvesicles and exosomes in CD34<sup>+</sup> cells
- Modulation of AngiomiR-126 expression has a critical effect on proangiogenic capacity
- Reduced AngiomiR-126 expression in patients with Diabetes

- After myocardial infarction in Angiomir-126 knock out mice, decreased vascular growth in the border zone
- Proangiogenic Angiomir-126 is enriched in microvesicles and exosomes, which leads to increased tube formation capacity
- MiR-100 expression inhibits proliferation of endothelial cells and tube formation
- MiR-10b promotes endothelial cell migration and tube formation



Thank you for your attention!