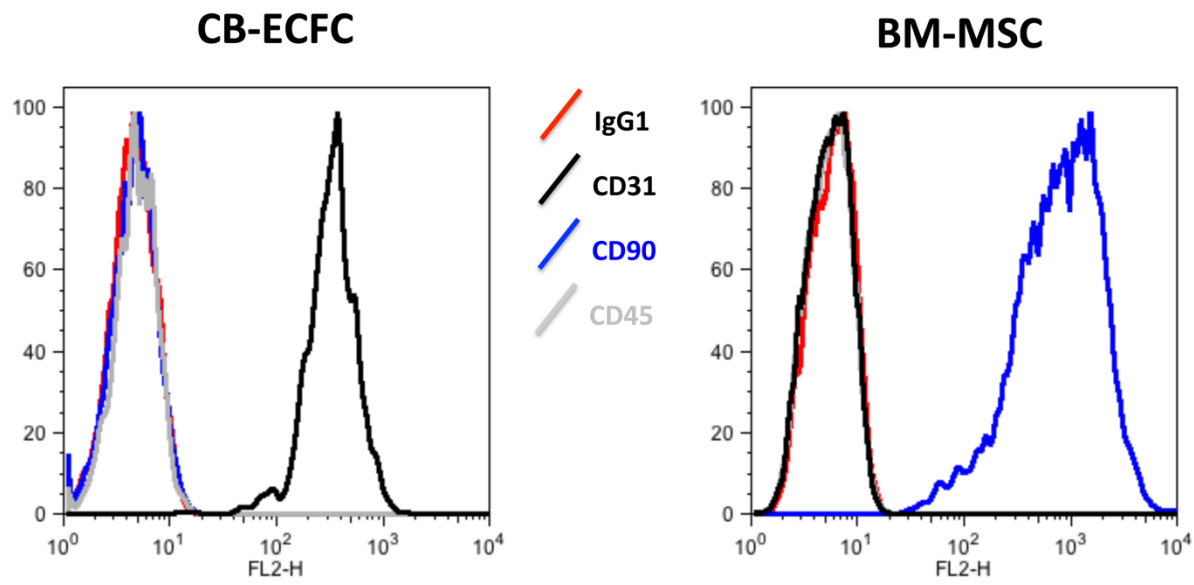
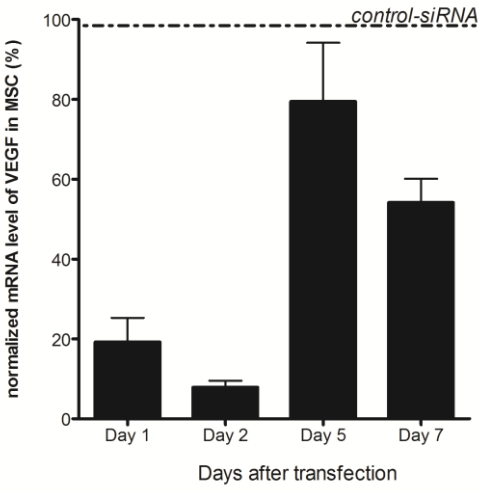
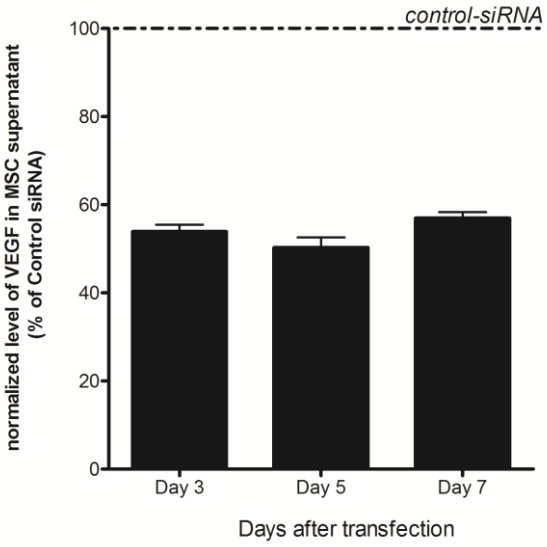
Supplementary Material to Smadja et al. 'Treprostinil indirectly regulates endothelial colony forming cell angiogenic properties by increasing VEGF-A produced by mesenchymal stem cells' (Thromb Haemost 2015; 114.4)



Suppl. Figure 1: Expression of endothelial (CD31), mesenchymal (CD90) and leukocytes (CD45) markers in CB-ECFC and BM-MSC. Cytometric analyses were carried out by labeling with phycoerythrin (PE)-conjugated mouse anti-human CD31 (Becton Dickinson), PE- conjugated mouse anti-human CD90 (Chemicon International), PE-conjugated mouse anti-human CD45 (Beckman Coulter). Antibody labeling was carried out for 20 minutes on ice followed by 3 washes with PBS/10% FBS. Flow cytometric analyses were performed using a Becton Dickinson FACScan flow cytometer and FlowJo software (Tree Star Inc., Ashland, OR).

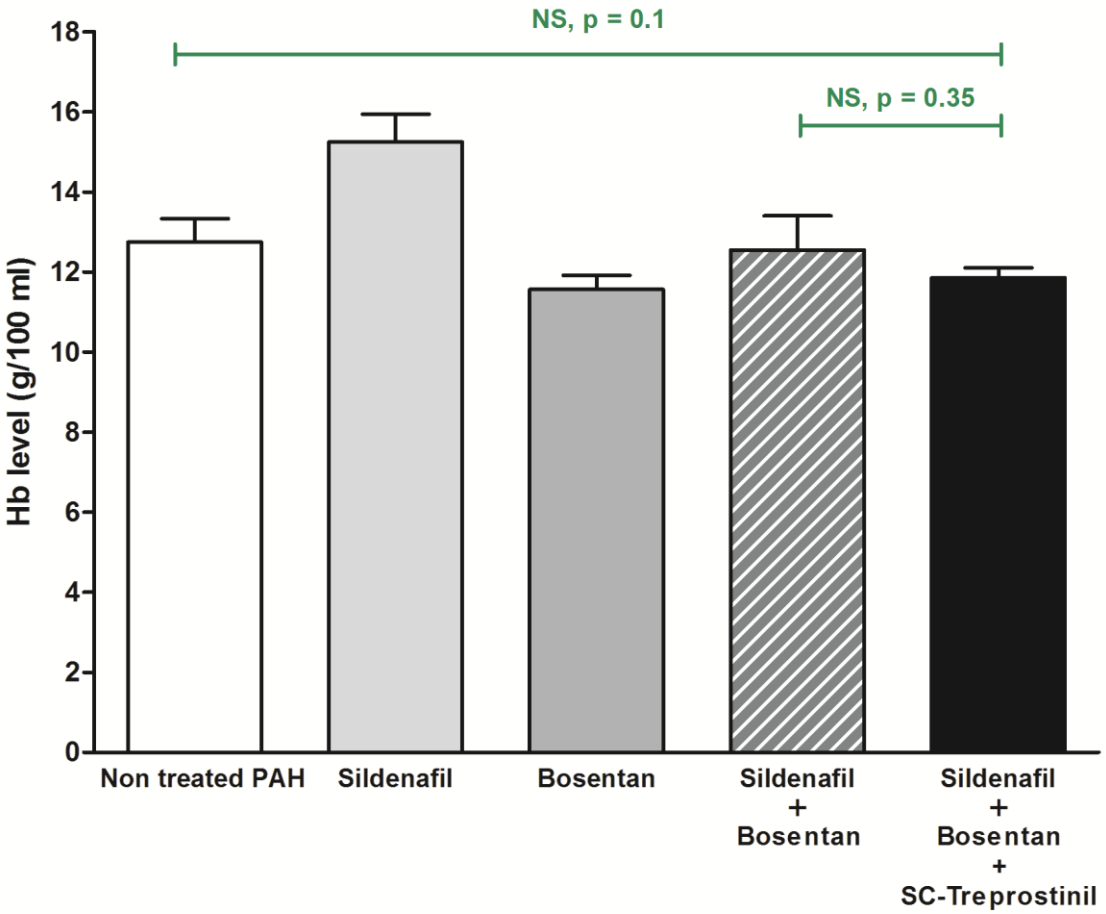


A



B

<u>Suppl. Figure 2</u>: Validation of *VEGF* gene silencing in siRNA-transfected-MSC. A) RT-qPCR: VEGF-siRNA-transfected MSC shows a 80 and 90% inhibition of *VEGF* mRNA expression level, compared to control-siRNA-transfected MSC at day 2. VEGF-siRNA-transfected MSC shows an increase of *VEGF* mRNA expression level, compared to control-siRNA-transfected MSC with a % of inhibition around 60 at day 5 and 7. **B)** VEGF quantification in MSC supernatant after transfection. VEGF-siRNA-transfected MSC shows a 50% inhibition of VEGF in supernatant from day 3 to 7.



<u>Suppl. Figure 3:</u> Hemoglobin levels in Treprostinil treated patients are not different from non-treated PAH or patient with oral treatment (sildenafil and/or bosentan). Compared to untreated PAH patients, no differences were observed in Hb level after subcutaneous treprostinil treatment (respectively with a p=0.1 *vs* none treated PAH patients and p=0.35 *vs* oral sildenafil and bosentan treated patients).