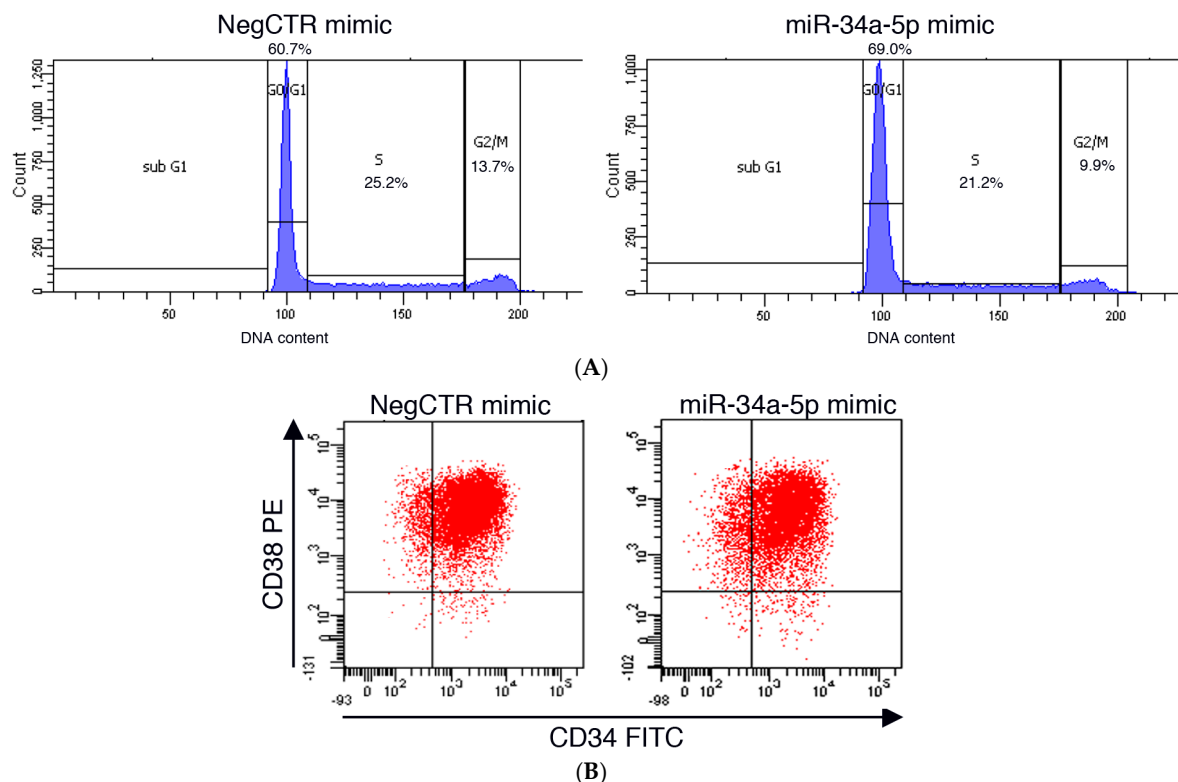
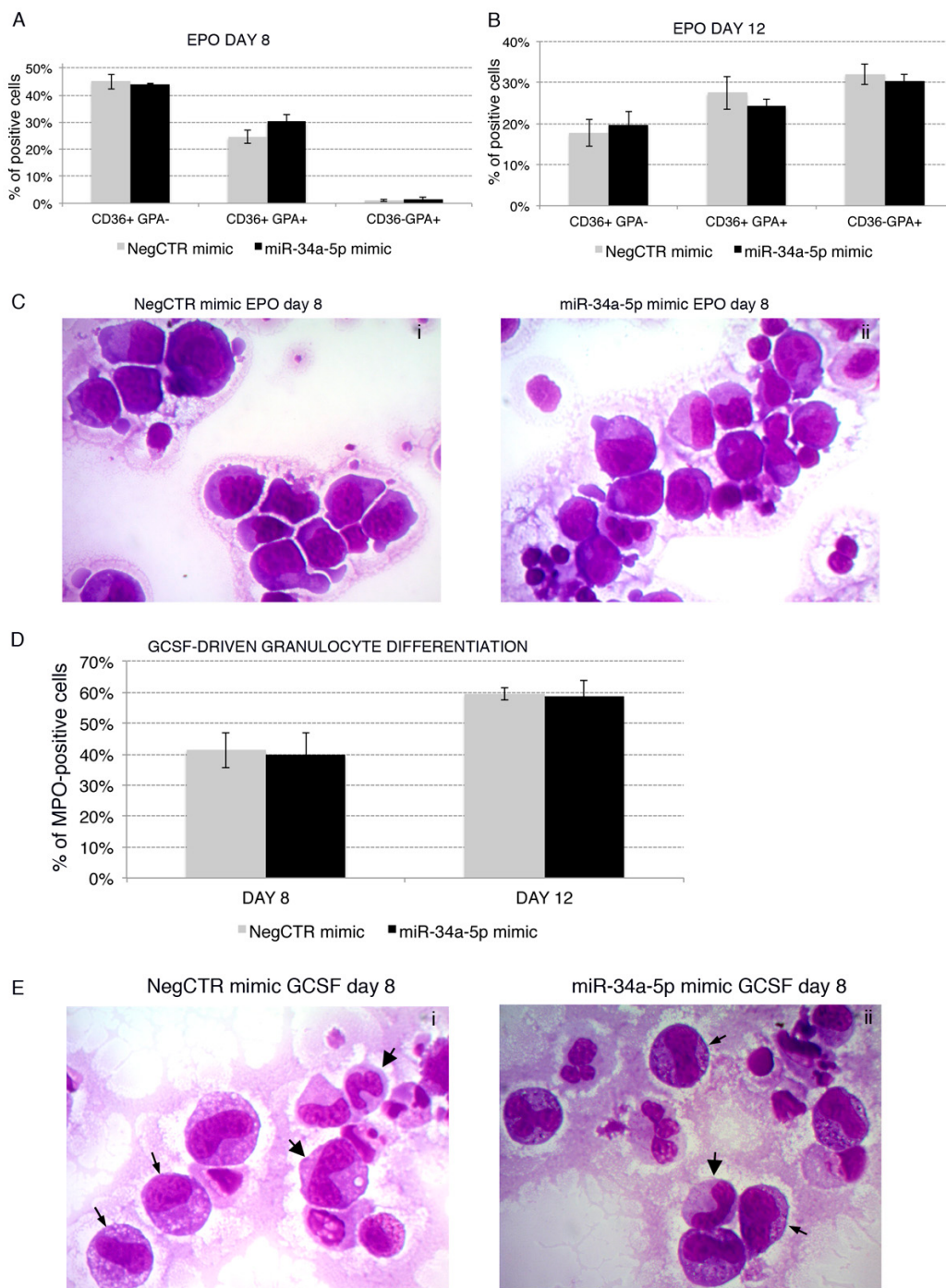


# Supplementary Materials: Role of miR-34a-5p in Hematopoietic Progenitor Cells Proliferation and Fate Decision: Novel Insights into the Pathogenesis of Primary Myelofibrosis

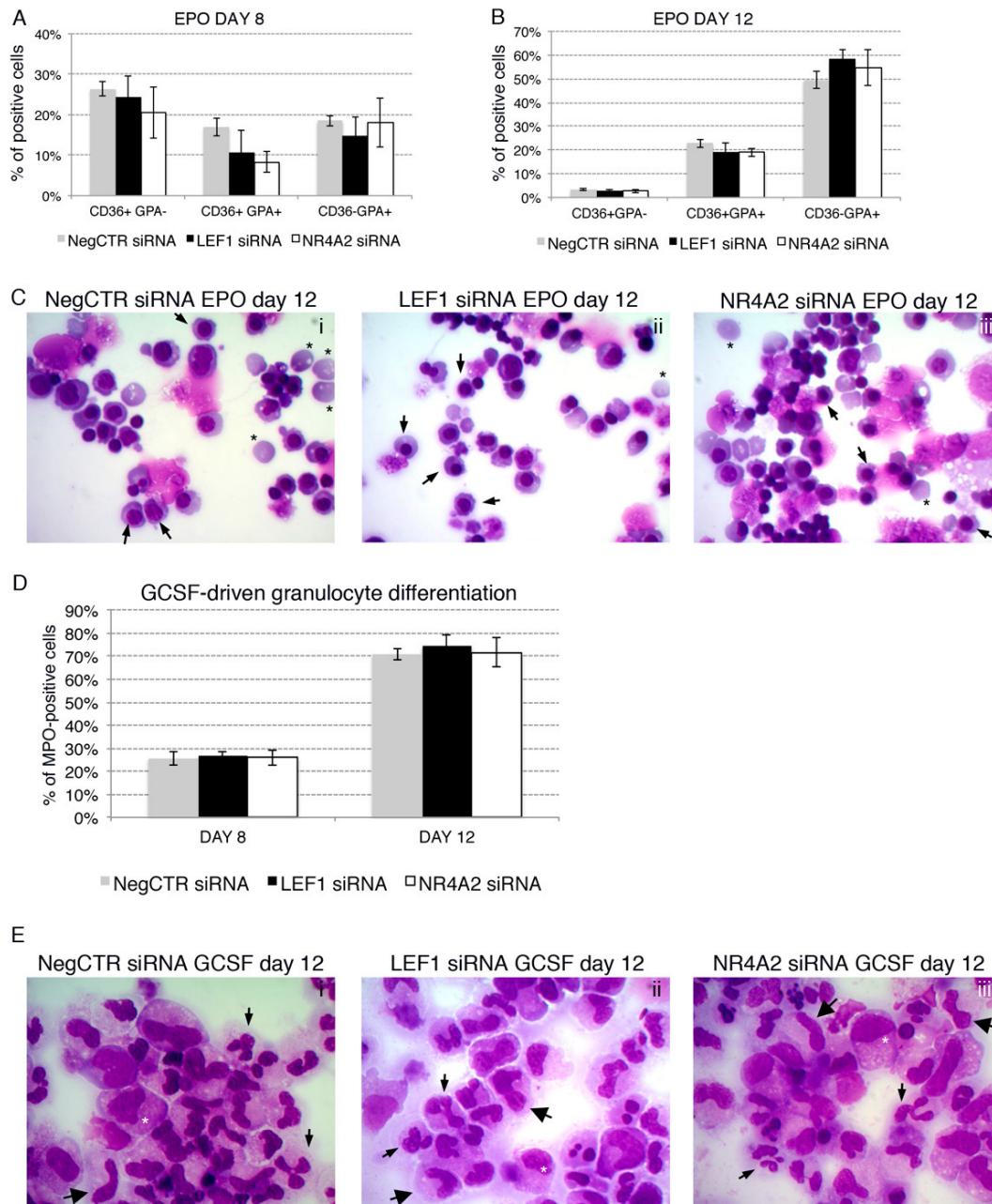
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**Figure S1.** Effects of miR-34a-5p overexpression on CD34<sup>+</sup> hematopoietic progenitor cells proliferation and commitment. **(A)** Representative histograms for the flow cytometric analysis of cell cycle distribution by propidium iodide staining in NegCTR mimic and miR-34a-5p mimic CD34<sup>+</sup> cells; **(B)** Representative dot plots for the flow cytometric detection of CD34 and CD38 markers at 24 h post-nucleofection. No significant differences in the earliest CD34<sup>+</sup>CD38<sup>−</sup> stem cell population, the intermediate CD34<sup>+</sup>CD38<sup>+</sup> hematopoietic progenitor cells fraction and the most committed CD34<sup>−</sup>CD38<sup>+</sup> population were detected between NegCTR mimic and miR-34a-5p samples.



**Figure S2.** Effects of miR-34a-5p overexpression on erythroid and granulocyte differentiation. (A,B) Flow cytometric analysis (mean  $\pm$  SEM;  $n = 3$ ) of CD36 and glycophorin A (GPA) expression at day 8 (A) and day 12 (B) of EPO-driven erythroid unilineage culture post-nucleofection; (C) Morphological analysis of NegCTR mimic (i) and miR-34a-5p mimic-transfected cells (ii) after May–Grünwald–Giemsa staining at day 8 of EPO-induced erythroid differentiation post-nucleofection in a representative experiment. Magnification,  $\times 1000$ ; (D) Flow cytometric detection (mean  $\pm$  SEM;  $n = 3$ ) of the myeloperoxidase (MPO) marker at day 8 and day 12 of GCSF-driven granulocyte unilineage culture post-nucleofection; (E) Morphological analysis of NegCTR mimic (i) and miR-34a-5p mimic-transfected cells (ii) after May–Grünwald–Giemsa staining at day 8 of GCSF-driven granulocyte differentiation post-nucleofection in a representative experiment. Thin arrows indicate myelocytes; thick arrows indicate band neutrophils. Magnification,  $\times 1000$ . Abbreviations: EPO, erythropoietin; GCSF, granulocyte colony stimulating factor.



**Figure S3.** Effects of LEF1 and NR4A2 silencing on erythroid and granulocyte differentiation. (**A,B**) Flow cytometric analysis (mean  $\pm$  SEM;  $n = 3$ ) of CD36 and glycophorin A (GPA) expression at day 8 (**A**) and day 12 (**B**) of EPO-driven erythroid unilineage culture post-nucleofection; (**C**) Morphological analysis of NegCTR siRNA (**i**), LEF1 siRNA (**ii**) and NR4A2 siRNA-transfected cells (**iii**) after May–Grünwald–Giemsa staining at day 8 of EPO-induced erythroid differentiation post-nucleofection in a representative experiment. Arrows indicate erythroblasts; asterisks indicate reticulocytes that have already extruded the nucleus. Magnification,  $\times 1000$ ; (**D**) Flow cytometric detection (mean  $\pm$  SEM;  $n = 3$ ) of the myeloperoxidase (MPO) marker at day 8 and day 12 of GCSF-driven granulocyte unilineage culture post-nucleofection; (**E**) Morphological analysis of NegCTR siRNA (**i**), LEF1 siRNA (**ii**) and NR4A2 siRNA-transfected cells (**iii**) after May–Grünwald–Giemsa staining at day 8 of GCSF-driven granulocyte differentiation post-nucleofection in a representative experiment. Thick arrows indicate band neutrophils; thin arrows indicate two-lobed neutrophils or mature neutrophils; white asterisks indicate promyelocytes. Magnification,  $\times 1000$ . Abbreviations: EPO, erythropoietin; GCSF, granulocyte colony stimulating factor.

**Table S1.** Ambion Silencer Select siRNA Sequences.

| Target Transcript | RefSeq ID                                                   | Sequence (Sense)            | Targeted Exon(s) | Ambion Silencer Select siRNA Product |
|-------------------|-------------------------------------------------------------|-----------------------------|------------------|--------------------------------------|
| LEF1              | NM_001130713.2; NM_001130714.2; NM_001166119.1; NM_016269.4 | 5'-GUUGCUGAGUGUACUCUAAtt-3' | Exons 7,8        | Silencer Select Pre-designed siRNA   |
| NR4A2             | NM_006186.3                                                 | 5'-GGCGAACCCUGACUAUCAAtt-3' | Exon 6           | Silencer Select Validated siRNA      |

**Table S2.** Differentially expressed genes between miR-34a-5p mimic and NegCTR mimic-transfected CD34+ cells.

| Probeset ID   | Gene Symbol                                     | Gene Title                                                                                    | RefSeq Transcript ID                                                          | Fold Change miR-34a-5p Mimic vs. NegCTR Mimic-Transfected CD34+ Cells | p-Value miR-34a-5p Mimic vs. NegCTR Mimic-Transfected CD34+ Cells |
|---------------|-------------------------------------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|-----------------------------------------------------------------------|-------------------------------------------------------------------|
| 11753714_a_at | HBE1                                            | hemoglobin, epsilon 1                                                                         | NM_005330                                                                     | 4.17                                                                  | 0.0023                                                            |
| 11755537_s_at | HBG1 /// HBG2 /// LOC100653006 /// LOC100653319 | hemoglobin, $\gamma$ A /// hemoglobin, $\gamma$ G /// uncharacterized LOC100653006 /// unchar | NM_000184 /// NM_000559 /// XR_132577 /// XR_132954                           | 3.47                                                                  | 0.0013                                                            |
| 11744478_s_at | HBG1 /// HBG2 /// LOC100653006 /// LOC100653319 | hemoglobin, $\gamma$ A /// hemoglobin, $\gamma$ G /// uncharacterized LOC100653006 /// unchar | NM_000184 /// NM_000559 /// XR_132577 /// XR_132954                           | 3.44                                                                  | 0.0006                                                            |
| 11754267_x_at | HBA1 /// HBA2                                   | hemoglobin, $\alpha$ 1 /// hemoglobin, $\alpha$ 2                                             | NM_000517 /// NM_000558                                                       | 3.40                                                                  | 0.0142                                                            |
| 11715494_s_at | HBG1 /// HBG2 /// LOC100653006 /// LOC100653319 | hemoglobin, $\gamma$ A /// hemoglobin, $\gamma$ G /// uncharacterized LOC100653006 /// unchar | NM_000184 /// NM_000559 /// XR_132577 /// XR_132954                           | 3.31                                                                  | 0.0004                                                            |
| 11729582_s_at | CA1                                             | carbonic anhydrase I                                                                          | NM_001128829 /// NM_001128830 /// NM_001128831 /// NM_001164830 /// NM_001738 | 3.06                                                                  | 0.0062                                                            |
| 11758685_s_at | CEACAM8                                         | carcinoembryonic antigen-related cell adhesion molecule 8                                     | NM_001816                                                                     | 2.97                                                                  | 0.0371                                                            |
| 11753823_a_at | S100A8                                          | S100 calcium binding protein A8                                                               | NM_002964                                                                     | 2.85                                                                  | 0.0021                                                            |
| 11758696_x_at | HBG1 /// HBG2 /// LOC100653006 /// LOC100653319 | hemoglobin, $\gamma$ A /// hemoglobin, $\gamma$ G /// uncharacterized LOC100653006 /// unchar | NM_000184 /// NM_000559 /// XR_132577 /// XR_132954                           | 2.77                                                                  | 0.0017                                                            |
| 11720541_at   | LGALS1                                          | lectin, galactoside-binding-like                                                              | NM_014181                                                                     | 2.39                                                                  | 0.0111                                                            |
| 11716523_at   | S100A9                                          | S100 calcium binding protein A9                                                               | NM_002965                                                                     | 2.37                                                                  | 0.0002                                                            |
| 11729583_x_at | CA1                                             | carbonic anhydrase I                                                                          | NM_001128829 /// NM_001128830 /// NM_001128831 /// NM_001164830 /// NM_001738 | 2.27                                                                  | 0.0297                                                            |
| 11758545_s_at | RHAG                                            | Rh-associated glycoprotein                                                                    | NM_000324                                                                     | 2.09                                                                  | 0.0305                                                            |
| 11725897_at   | TUBB1                                           | tubulin, beta 1 class VI                                                                      | NM_030773                                                                     | 2.06                                                                  | 0.0468                                                            |
| 11756809_a_at | EFHC2                                           | EF-hand domain (C-terminal) containing 2                                                      | NM_025184                                                                     | 2.06                                                                  | 0.0228                                                            |
| 11758577_s_at | ELOVL7                                          | ELOVL fatty acid elongase 7                                                                   | NM_001104558 /// NM_024930                                                    | 2.06                                                                  | 0.0038                                                            |
| 11720608_a_at | S100B                                           | S100 calcium binding protein B                                                                | NM_006272                                                                     | 2.05                                                                  | 0.0261                                                            |
| 11751647_a_at | IL7R                                            | interleukin 7 receptor                                                                        | NM_002185                                                                     | 2.01                                                                  | 0.0058                                                            |
| 11725632_at   | NR4A2                                           | nuclear receptor subfamily 4, group A, member 2                                               | NM_006186 /// NM_173171 /// NM_173172 /// NM_173173                           | -2.03                                                                 | 0.0245                                                            |

Table S2. Cont.

| Probeset ID   | Gene Symbol    | Gene Title                                                                  | RefSeq Transcript ID                                            | Fold Change miR-34a-5p Mimic vs.<br>NegCTR Mimic-Transfected<br>CD34+ Cells | p-Value miR-34a-5p Mimic vs.<br>NegCTR Mimic-Transfected<br>CD34+ Cells |
|---------------|----------------|-----------------------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------|
| 11729641_a_at | TPD52          | tumor protein D52                                                           | NM_001025252 /// NM_001025253 /// NM_005079                     | -2.06                                                                       | 0.0036                                                                  |
| 11715245_s_at | IGLL1          | immunoglobulin lambda-like polypeptide 1                                    | NM_020070 /// NM_152855                                         | -2.15                                                                       | 0.0303                                                                  |
| 11718479_x_at | STAR           | steroidogenic acute regulatory protein                                      | NM_000349 /// NM_001007243                                      | -2.17                                                                       | 0.0057                                                                  |
| 11720051_at   | SPOCK1         | sparc/osteonectin, cwcv and kazal-like domains<br>proteoglycan (testican) 1 | NM_004598                                                       | -2.17                                                                       | 0.0183                                                                  |
| 11718477_a_at | STAR           | steroidogenic acute regulatory protein                                      | NM_000349 /// NM_001007243                                      | -2.24                                                                       | 0.0164                                                                  |
| 11726333_s_at | LEF1           | lymphoid enhancer-binding factor 1                                          | NM_001130713 /// NM_001130714 ///<br>NM_001166119 /// NM_016269 | -2.29                                                                       | 0.0042                                                                  |
| 11723339_at   | CTSG           | cathepsin G                                                                 | NM_001911                                                       | -2.29                                                                       | 0.0436                                                                  |
| 11745205_s_at | TPD52          | tumor protein D52                                                           | NM_001025252 /// NM_001025253 /// NM_005079                     | -2.59                                                                       | 0.0027                                                                  |
| 11715306_s_at | AREG /// AREGB | amphiregulin /// amphiregulin B                                             | NM_001657 /// XM_001125684                                      | -2.61                                                                       | 0.0045                                                                  |
| 11727965_at   | ELANE          | elastase, neutrophil expressed                                              | NM_001972                                                       | -2.65                                                                       | 0.0095                                                                  |
| 11754659_x_at | TPD52          | tumor protein D52                                                           | NM_001025252 /// NM_001025253 /// NM_005079                     | -2.69                                                                       | 0.0079                                                                  |
| 11729643_s_at | TPD52          | tumor protein D52                                                           | NM_001025252 /// NM_001025253 /// NM_005079                     | -2.89                                                                       | 0.0069                                                                  |
| 11756600_a_at | TPD52          | tumor protein D52                                                           | NM_001025252 /// NM_001025253 /// NM_005079                     | -2.93                                                                       | 0.0047                                                                  |
| 11728026_x_at | IGLL1          | immunoglobulin lambda-like polypeptide 1                                    | NM_020070 /// NM_152855                                         | -3.22                                                                       | 0.0157                                                                  |
| 11734171_at   | PRTN3          | proteinase 3                                                                | NM_002777                                                       | -3.81                                                                       | 0.0004                                                                  |