# Supplemental Information for Suppression of Androgen Receptor, A Key Factor in Male Sexual Phenotype, Enhances the Self-renewal of Mesenchymal Stem Cells Through Elevated Expression of EGFR

## Supplemental Figure legends

**Supplemental Figure 1. BM-MSCs AR functional validation.** MMTV luciferase containing strong androgen response element was transfected to WT and ARKO BM-MSCs. WT BM-MSCs luciferase activity was normalized to ARKO BM-MSCs basal level. **, $p<0.001$ when compared to control.

**Supplemental Figure 2 Osteogenic differentiation in WT and ARKO BM-MSCs.** Expressions of Dmp1 were determined using qRT-PCR in undifferentiated WT and ARKO BM-MSCs, and differentiated WT and ARKO BM-MSCs at day 21.

**Supplemental Figure 3 WT and ARKO BM-MSCs retain multipotency.** CFU-alkaline phosphatase (CFU-ALP), CUF-osteoblast (CFU-O), and CFU-adipocyte, which represented osteoprogentior, osteogenesis, and adipogenesis respectively, were used to measure their multi-lineage

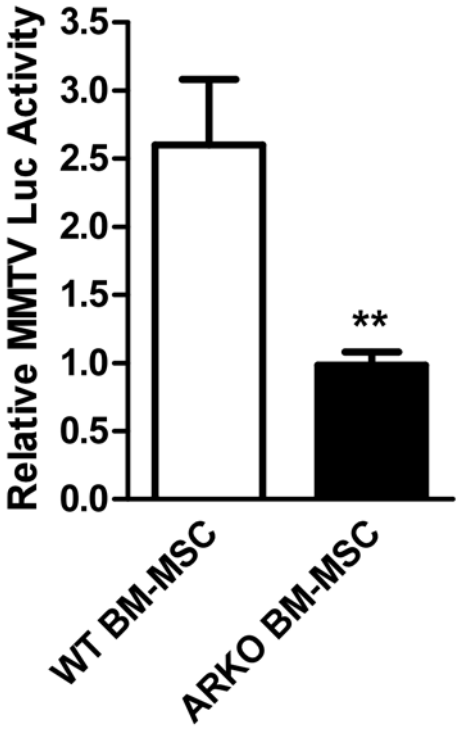
differentiation capacities.

**Supplemental Figure 4 AR and pEGFR localizations upon androgen treatment.** AR, pEGFR, and merged images were shown in MSCs treated either with vehicle (EtOH) or androgen (DHT) for 10 minutes, 30 minutes, and 24 hours.

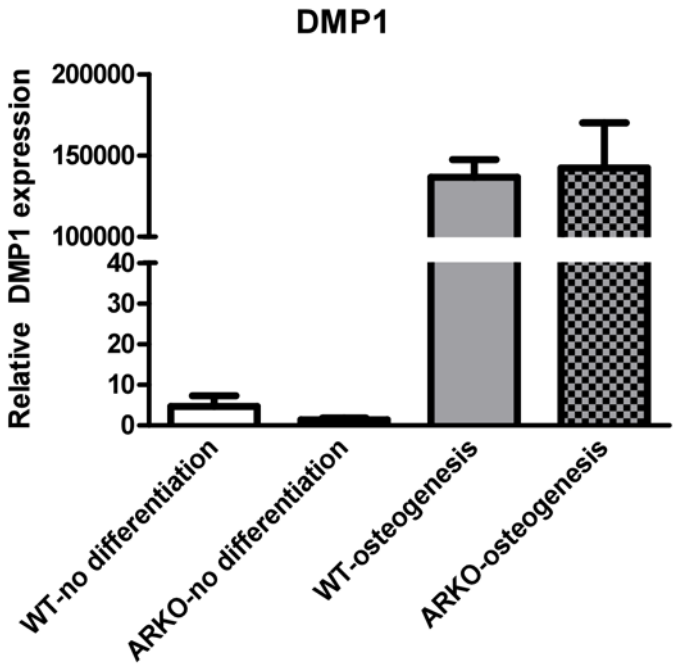
**Supplemental Figure 5 AR negatively regulates EGFR promoter activity.** (A) Illustration of the EGFR promoter region which contains 2 putative androgen receptor response elements (ARE). (B) pGL3-luciferase plasmids hooked with EGFR promoter regions from -2396 to +3 and from -831 to +3, and MMTV-luciferase were transfected into HEK-293T cells with or without AR to test the AR effect in regulating EGFR promoter transcriptional activity.

**Supplemental Figure 6 Targeting AR effects on BM-MSCs self-renewal.** (A) WT and (B) ARKO BM-MSCs were cultured with or without hydroxyflutamide (HF) plus exogenous ligand, DHT, to test HF effect on BM-MSCs self-renewal. ASC-J9 was used to treat (C) WT and (D) ARKO BM-MSCs in the presence of DHT to determine its effect on BM-MSC self-renewal. (E) WT and (F) ARKO BM-MSCs was infected with lentivirus which contain either scramble control or siRNA-AR and then performed CFU-f assays to test siRNA-AR effects on BM-MSCs self-renewal. *, $p<0.05$; ***, $p<0.0001$, when compared with control.

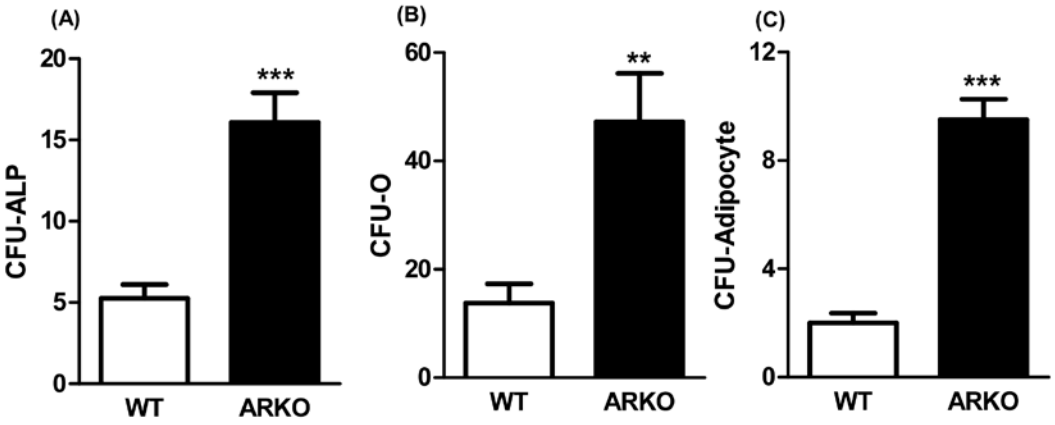
**Supplemental Figure 1**



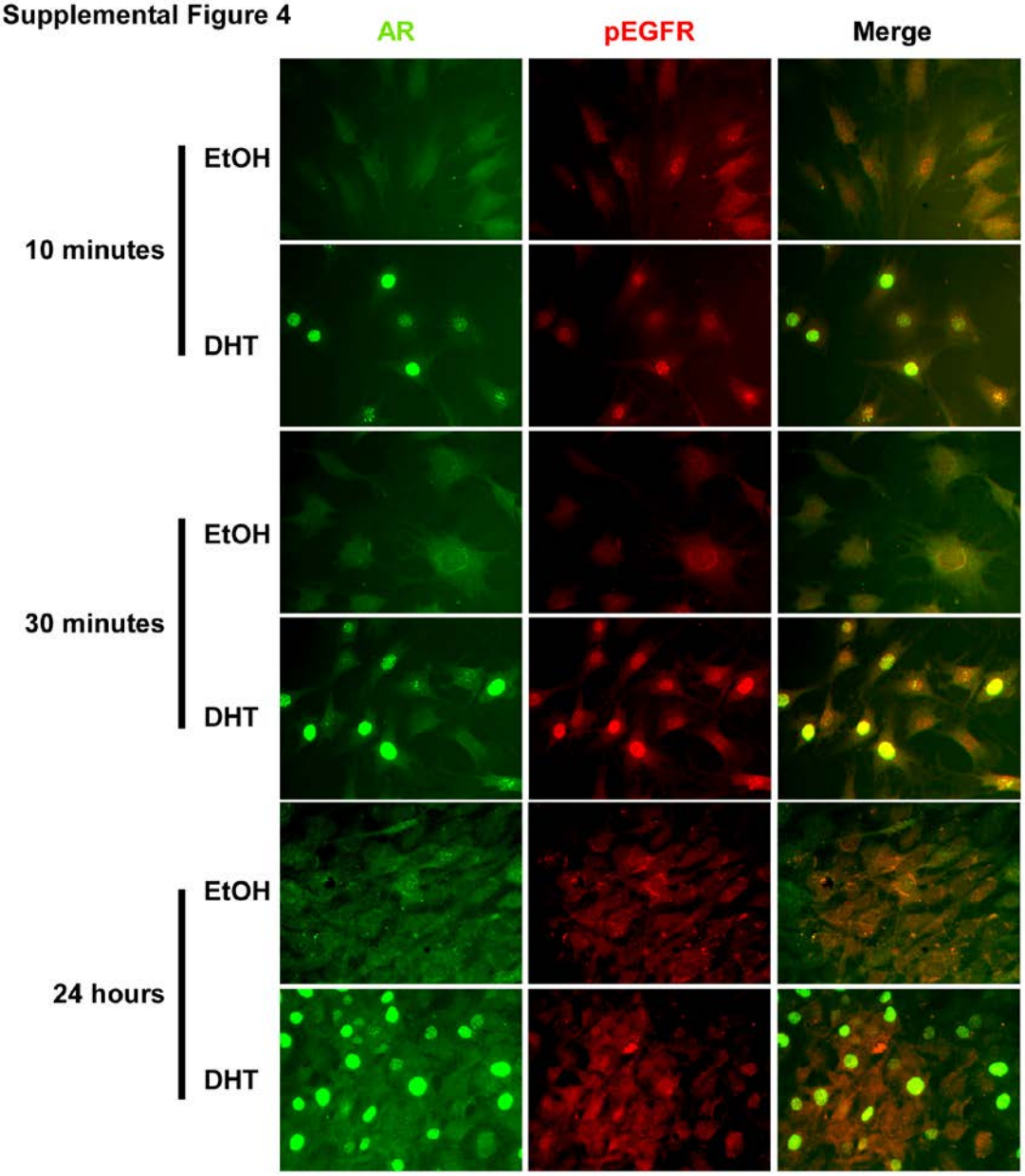
**Supplemental Figure 2**



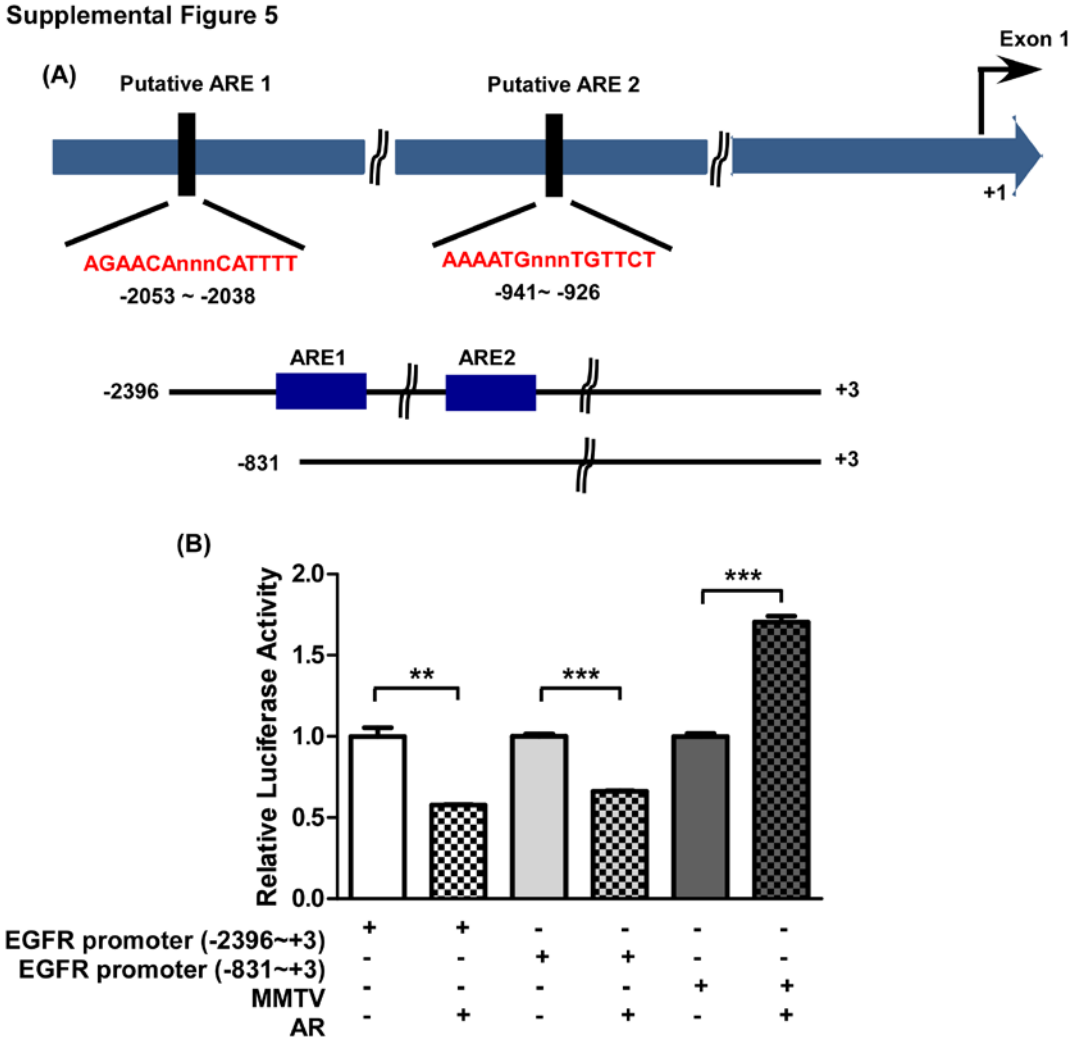
**Supplemental Figure 3**



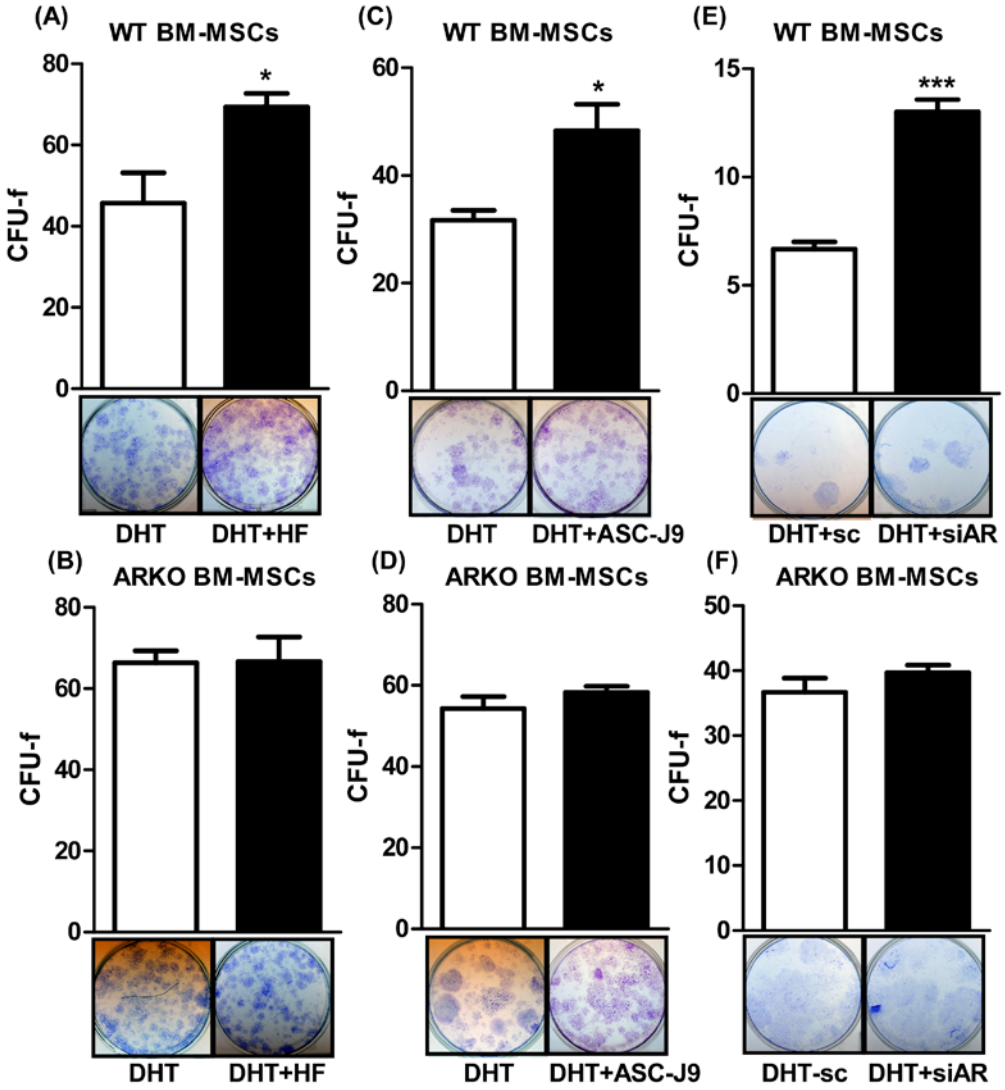
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



**Supplemental Table 1 Surface markers expressions in BM-MSCs and ADSCs**

| | BM-MSCs | ADSCs |
|---|---|---|
| **CD44** | 99.0% | 99.3% |
| **CD29** | 98.5% | 98.0% |
| **CD117** | 29.4% | 27.2% |
| **CD106** | 32.5% | 33.4% |
| **CD45** | 1.28% | 0.85% |
| **CD34** | 0.59% | 1.37% |