Supplementary Fig. 1. Secreted VEGF from young male fibroblasts with increasing concentrations of DHT. Young fibroblasts were treated with ethanol (EtOH), 4 nM, 40 nM and 400 nM DHT for 48 h. Conditioned media (CM) were collected after 24 h incubation with fresh phenol red-free DMEM + 10% csFBS. VEGF levels in CM were analyzed by ELISA. * P<0.05 *vs.* vehicle EtOH control

Supplementary Fig. 2. Fibroblast cell proliferation in response to LY294002 concentrations. **A**, Cell density standard curve of proliferation assay. Fibroblasts were plated in a 24-well plate at seeding densities (625, 1250, 2500, 5000, 10000 and 20000 cells/well). **B**, Fibroblasts were seeded in 24-well plates (5000 cells/well) and were treated with 5, 10, 20, 50 and 100 μM LY294002 in phenol red-free DMEM + 10% csFBS. Cell proliferation was assessed after 48 h using crystal violet staining. Data are presented as mean ± SEM, n=3 performed in triplicate.

Supplementary Fig. 3. Standard curve of VEGF ELISA. **A**, Standard VEGF (100,000 pg/ml) provided in the ELISA kit was serially diluted according to the manufacture's protocol. Data represent the mean of technical triplicates. **B**, Standard VEGF145 (1000 pg/ml) was serially diluted according to the manufacture's protocol. Data represent the mean of technical triplicates. Standard curves were used to estimate concentrations of secreted VEGF and VEGF145 in the conditioned media from DHT-treated young fibroblasts.

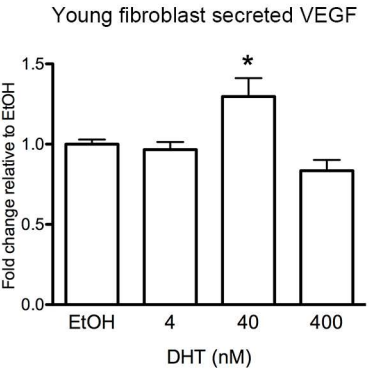
Supplementary Fig. 4. The mRNA expression of VEGF isoforms in young fibroblasts treated with 40 nM DHT for 24 h, relative to vehicle control. **A**, Level of VEGF121 mRNA. **B**, Level of VEGF165 mRNA. **C**, Level of VEGF189 mRNA. **D**, Level of VEGF206 mRNA. n=3 donors performed in triplicate over 3 individual experiments (means ± SEM).

Supplementary Fig. 5. The VEGF mRNA levels in fibroblasts treated 48 hours with vehicle control or 40 nM DHT in the presence of 10 μM PI3-kinase inhibitor LY294002. n=3 donors performed in triplicate. Data are presented at mean ± SEM.

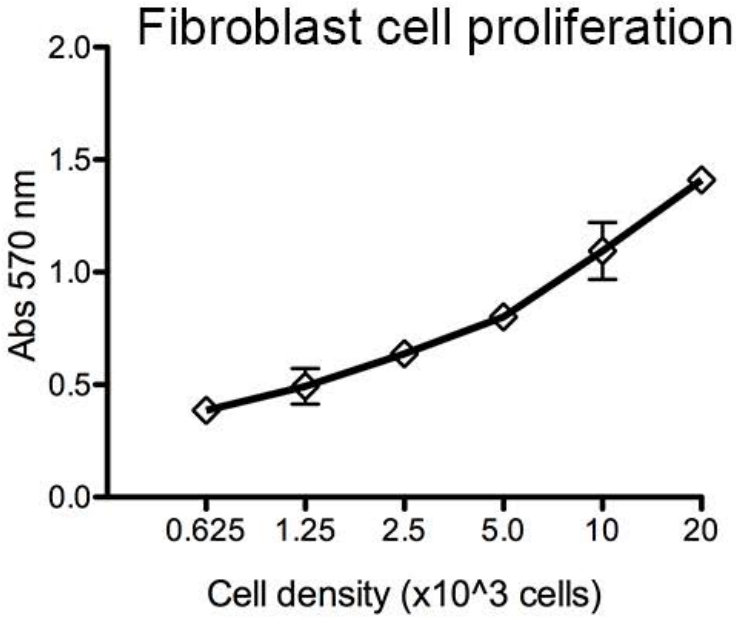
Supplementary Fig. 6. A, Basal pAKT(ser473)/AKT levels in old fibroblasts, relative to young fibroblasts. n=5. Statistical analysis was performed by Students T-test. *, P<0.05. **B**, Relative fold change of pAKT(ser473)/AKT levels in young and old fibroblasts treated with 500 nM insulin for 15

min, normalised to untreated cells. Fibroblasts were serum starved for 2 h prior to the addition of insulin. Levels of pAKT(ser473) and AKT were analysed by western blotting. Statistic analysis was performed by 2-way ANOVA using 2-way ANOVA with Bonferroni correction. *, $P < 0.05$ insulin-treated young *vs.* untreated-young fibroblasts. n=3 performed in triplicate. Data are presented at mean ± SEM.

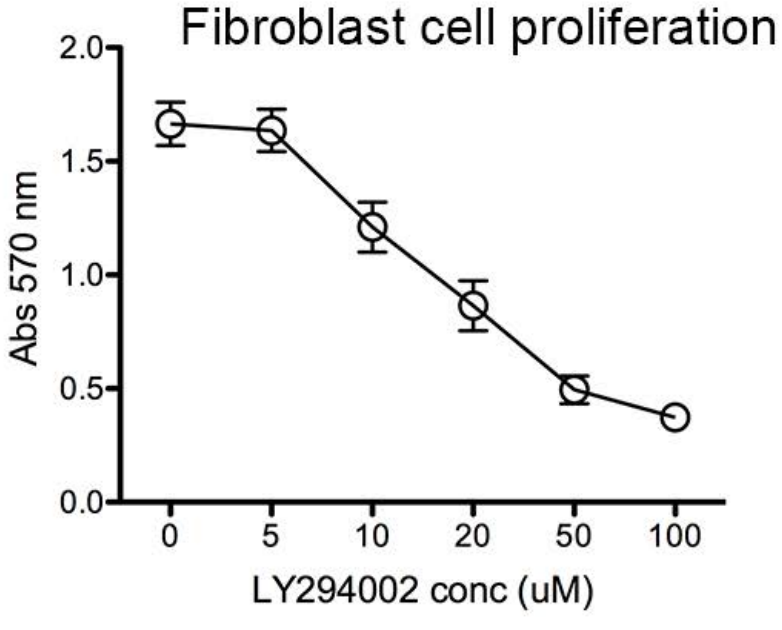
Supplementary Fig. 7. The effect of DHT treatment on AR localization in fibroblasts from young men. Longer exposure of western blot demonstrating cytoplasmic (C) and nuclear (N) expression of AR following sub-cellular fractionation of fibroblasts from young men subjected to 6 h treatment with 40 nM DHT. Red pixels represent overexposure. Nuclear marker LSD1, Lamin A/C and cytoplasmic marker tubulin were used to loading control of sub-cellular fractionation of fibroblasts.

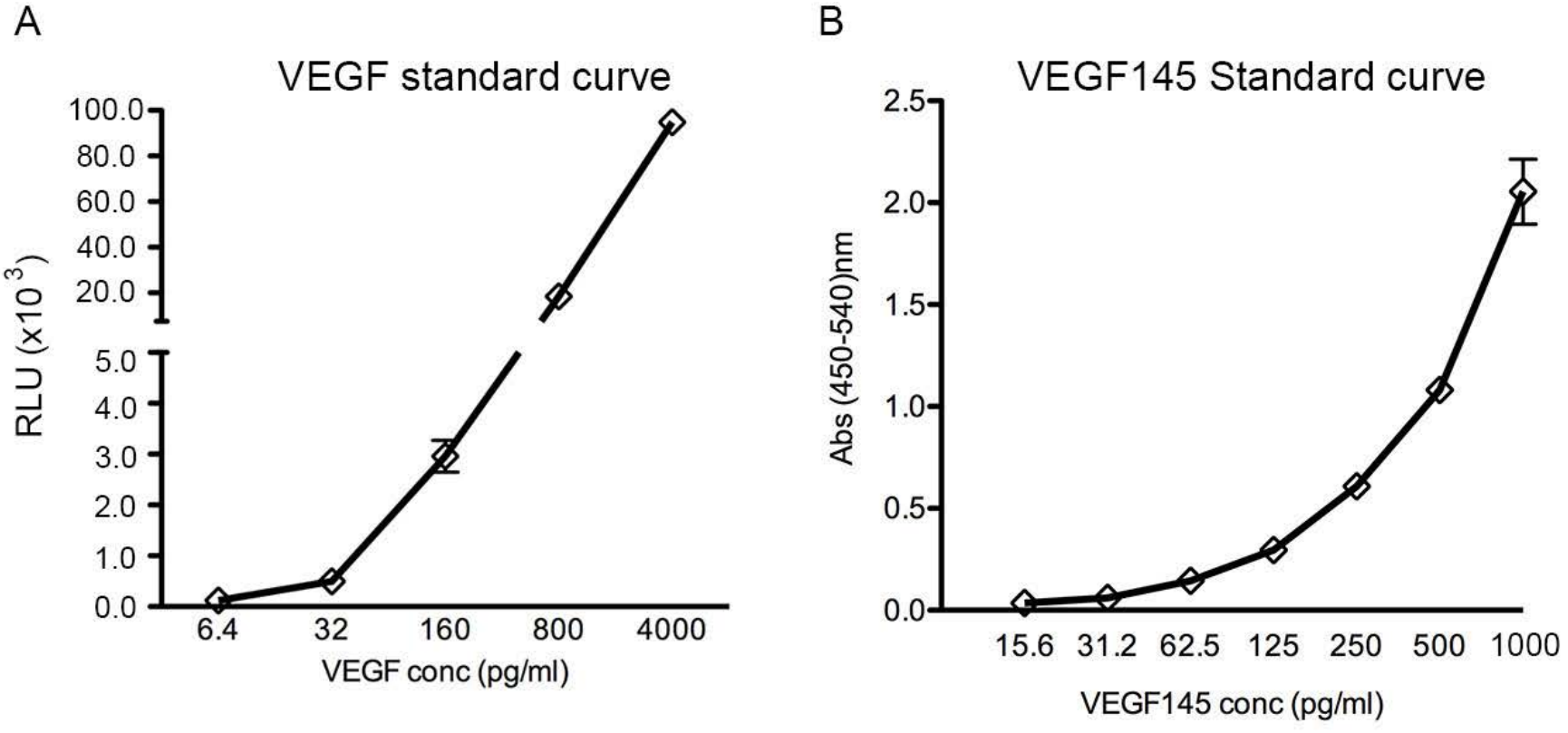

Young fibroblast secreted VEGF
Fold change relative to EtOH
1.5
1.0
0.5
0.0
*
EtOH
4
40
400
DHT (nM)

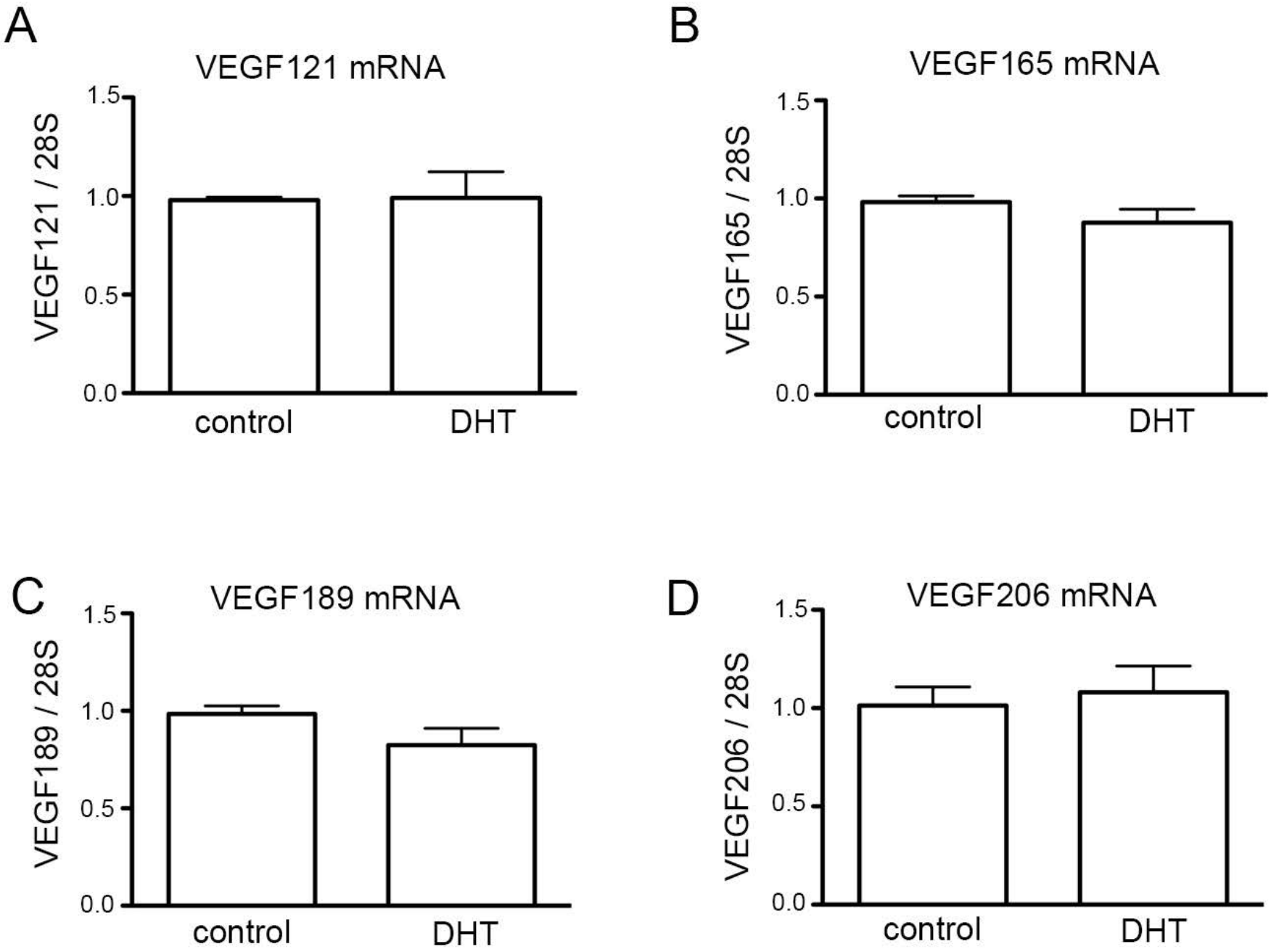
A

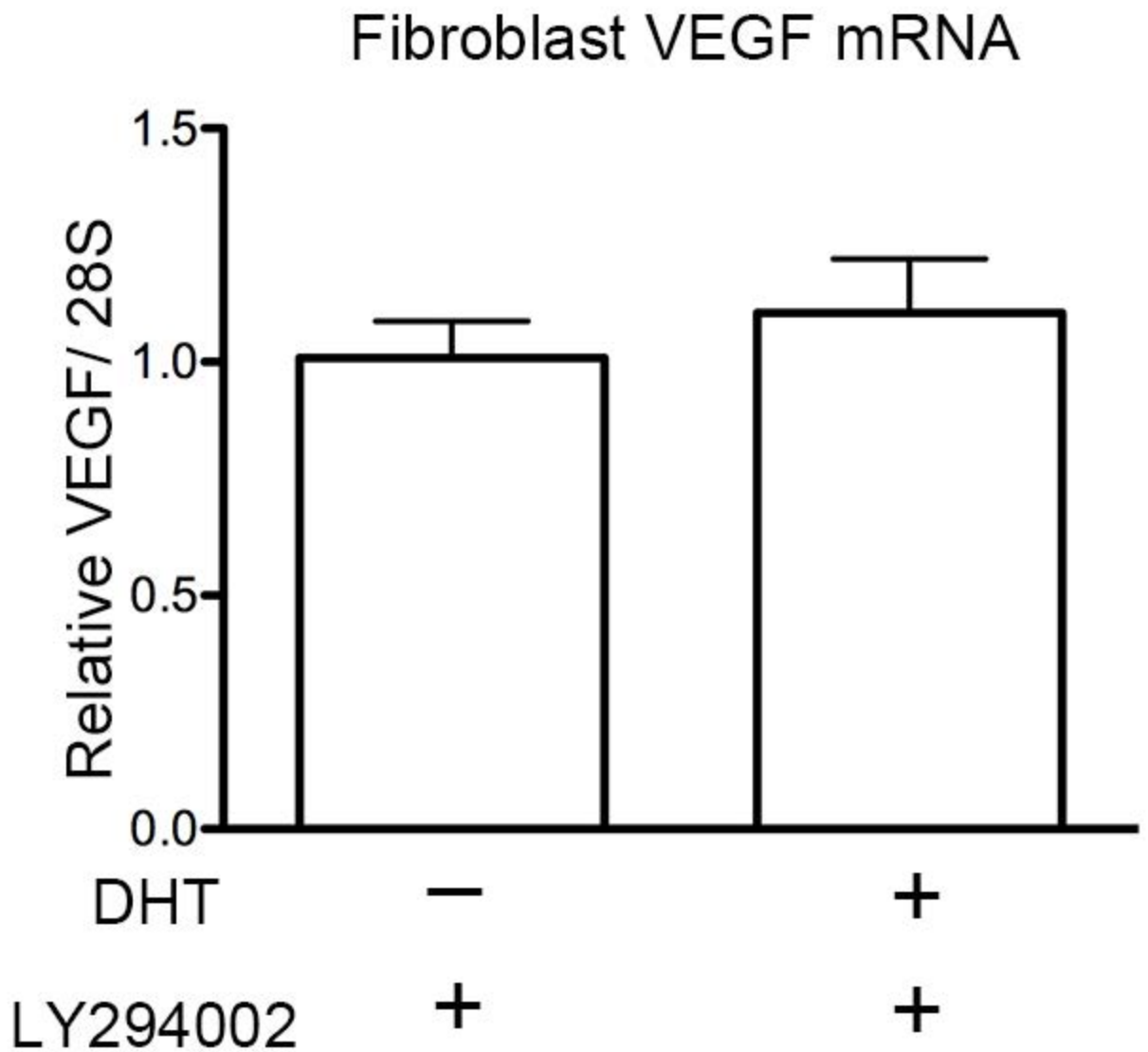

Fibroblast cell proliferation
Abs 570 nm
2.0
1.5
1.0
0.5
0.0
0.625
1.25
2.5
5.0
10
20
Cell density (x10^3 cells)

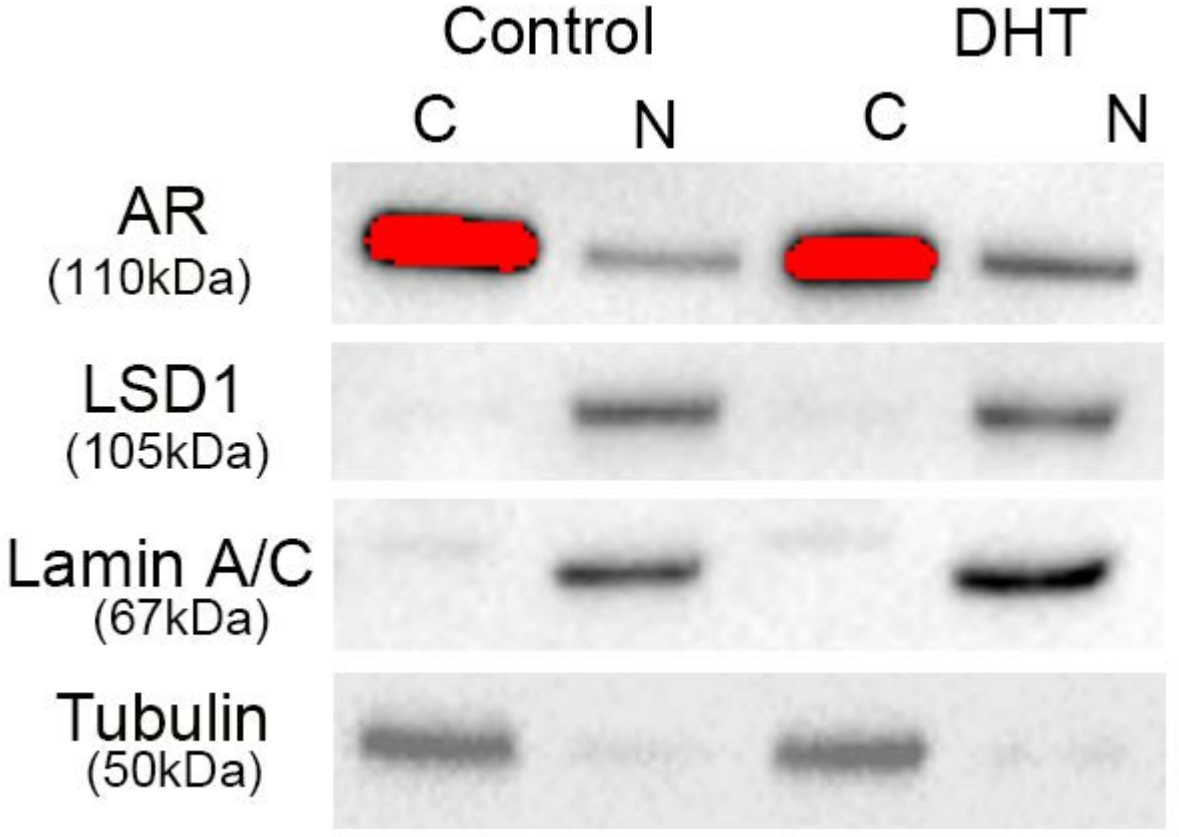
B

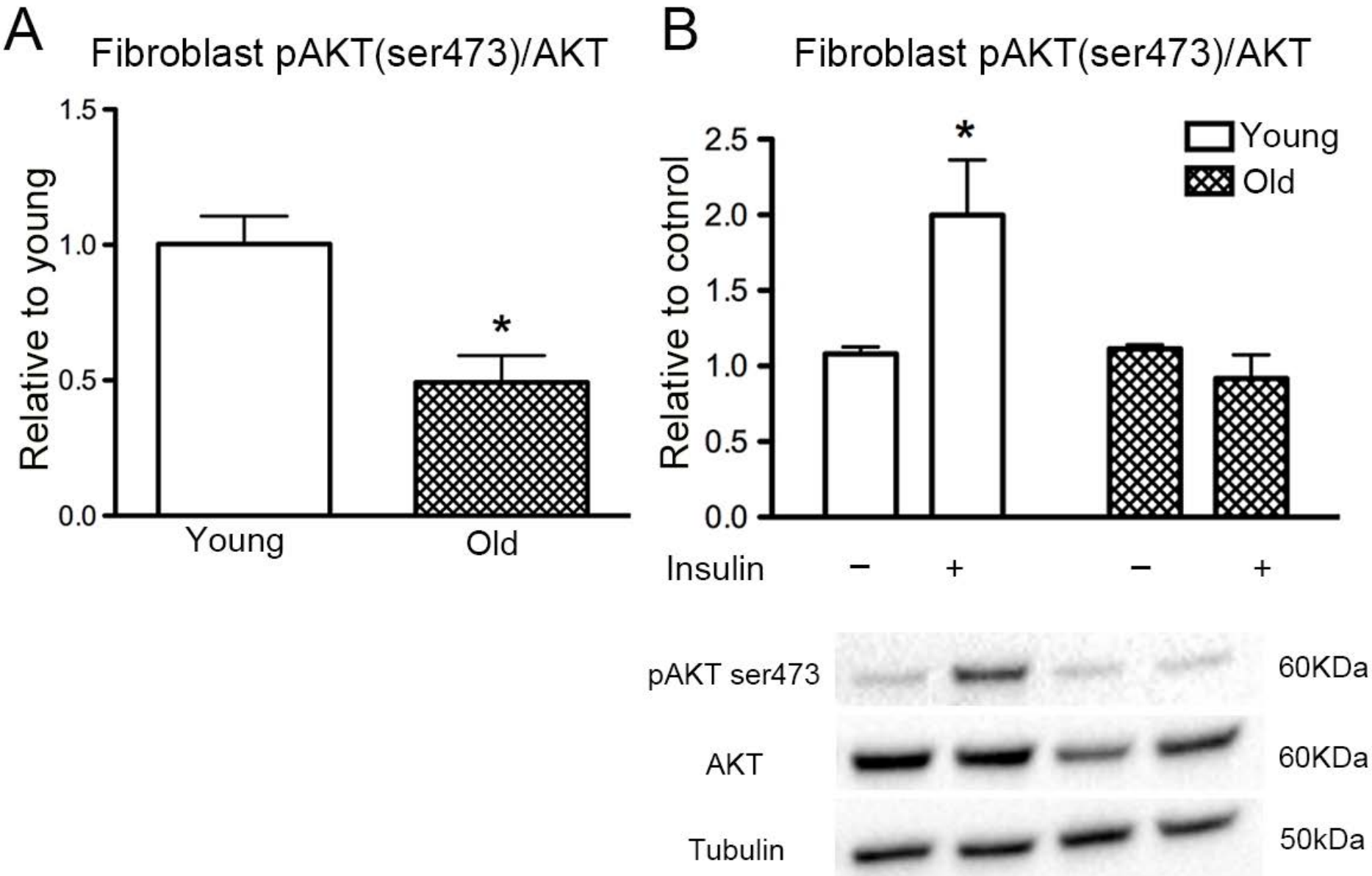

Fibroblast cell proliferation
Abs 570 nm
2.0
1.5
1.0
0.5
0.0
0
5
10
20
50
100
LY294002 conc (uM)


A
VEGF standard curve
RLU (x10^3)
100.0
80.0
60.0
40.0
20.0
5.0
4.0
3.0
2.0
1.0
0.0
6.4
32
160
800
4000
VEGF conc (pg/ml)
B
VEGF145 Standard curve
Abs (450-540)nm
2.5
2.0
1.5
1.0
0.5
0.0
15.6
31.2
62.5
125
250
500
1000
VEGF145 conc (pg/ml)


A
VEGF121 mRNA
VEGF121 / 28S
1.5
1.0
0.5
0.0
control
DHT
B
VEGF165 mRNA
VEGF165 / 28S
1.5
1.0
0.5
0.0
control
DHT
C
VEGF189 mRNA
VEGF189 / 28S
1.5
1.0
0.5
0.0
control
DHT
D
VEGF206 mRNA
VEGF206 / 28S
1.5
1.0
0.5
0.0
control
DHT


Fibroblast VEGF mRNA
Relative VEGF/ 28S
1.5
1.0
0.5
0.0
DHT
−
+
LY294002
+
+


Control
DHT
C
N
C
N
AR
(110kDa)
LSD1
(105kDa)
Lamin A/C
(67kDa)
Tubulin
(50kDa)


A
Fibroblast pAKT(ser473)/AKT
Relative to young
1.5
1.0
0.5
0.0
*
Young
Old
B
Fibroblast pAKT(ser473)/AKT
Relative to cotnrol
2.5
2.0
1.5
1.0
0.5
0.0
*
Young
Old
Insulin
−
+
−
+
pAKT ser473
60KDa
AKT
60KDa
Tubulin
50kDa

Supplementary Table 1. Concentrations of VEGF (pg/ml) secretion in the conditioned media of fibroblasts isolated from each young donor after DHT treatment

Donor (Age)	**Ethanol Control**	**40nM DHT**
Donor 1 (30)	835.37±15.05	1114.10±70.32
Donor 2 (30)	463.24±7.63	513.03±52.42
Donor 3 (30)	175.43±22.85	276.86±19.08
Medium only	10.16±0.13	

Unit is expressed as picogram of VEGF presented in milliliter of fibroblast conditioned medium. “Medium only” represents phenol-free DMEM + charcoal stripped FBS basal medium. Data presented as mean ± SD

Supplementary Table 2. Concentrations of VEGF145 (pg/ml) secretion in the conditioned media of fibroblasts isolated from each young donor after DHT treatment

Donor (Age)	**Ethanol Control**	**40nM DHT**
Donor 1 (30)	45.13±7.55	59.82±3.83
Donor 2 (30)	62.19±7.55	71.72±9.22
Donor 3 (24)	52.61±9.71	75.02±6.15
Donor 4 (31)	76.88±2.78	88.10±7.27
Average of 4 donors	52.9±13.70	73.7±11.63

Unit is expressed as picogram of VEGF presented in milliliter of fibroblast conditioned medium. Data presented as mean ± SD

Supplementary Table 3 Nuclear fluorescence intensity of young and old fibroblasts after 6 h DHT treatment.

	Young		**Old**	
	Control	**DHT**	**Control**	**DHT**
Donor 1	57.33	73.04	67.41	60.36
Donor 2	33.56	66.77	56.13	54.15
Donor 3	56.26	70.55	47.21	56.93
Mean ± SEM	48.72 ± 6.00	70.12 ± 2.71	56.92 ± 4.64	57.14 ± 2.64

Data represent average fluorescence intensity per pixel of all nuclei measured in each image taken for each donor.