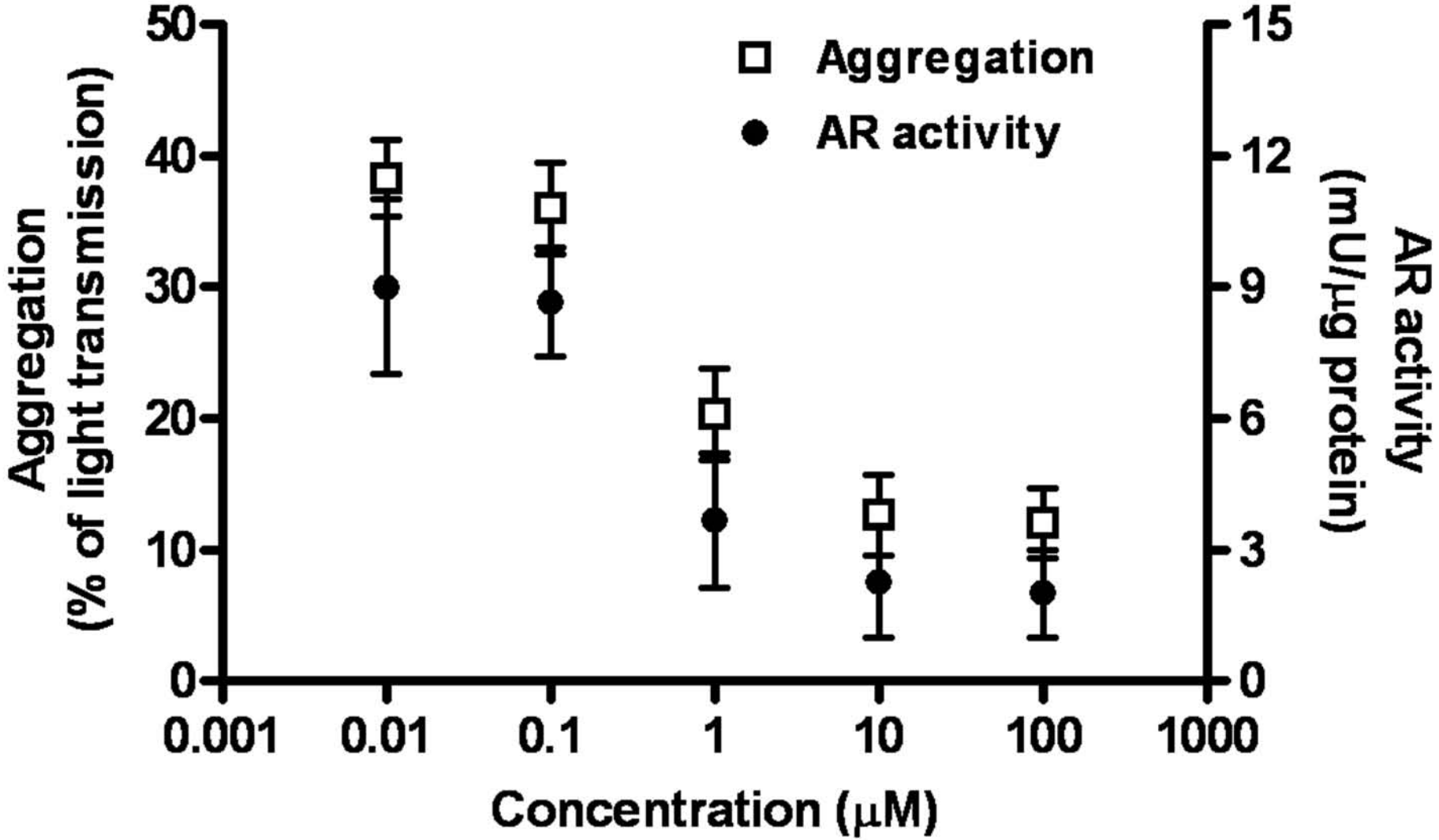
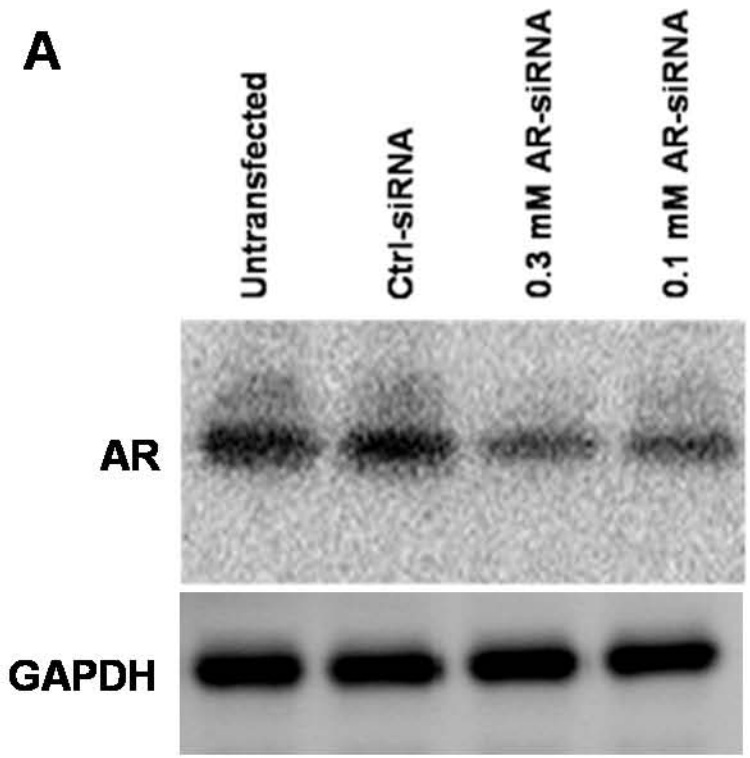
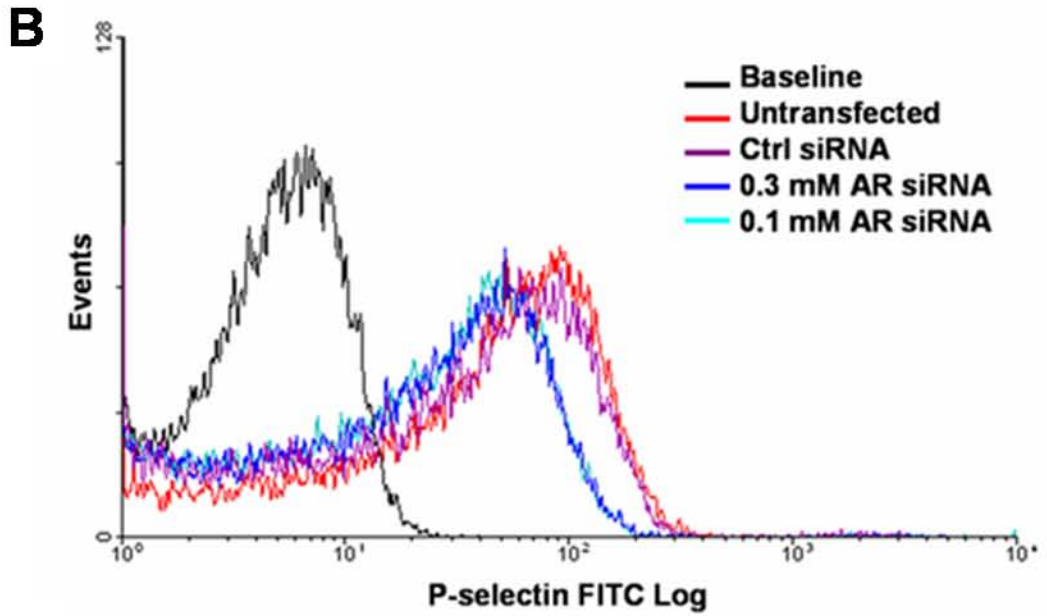
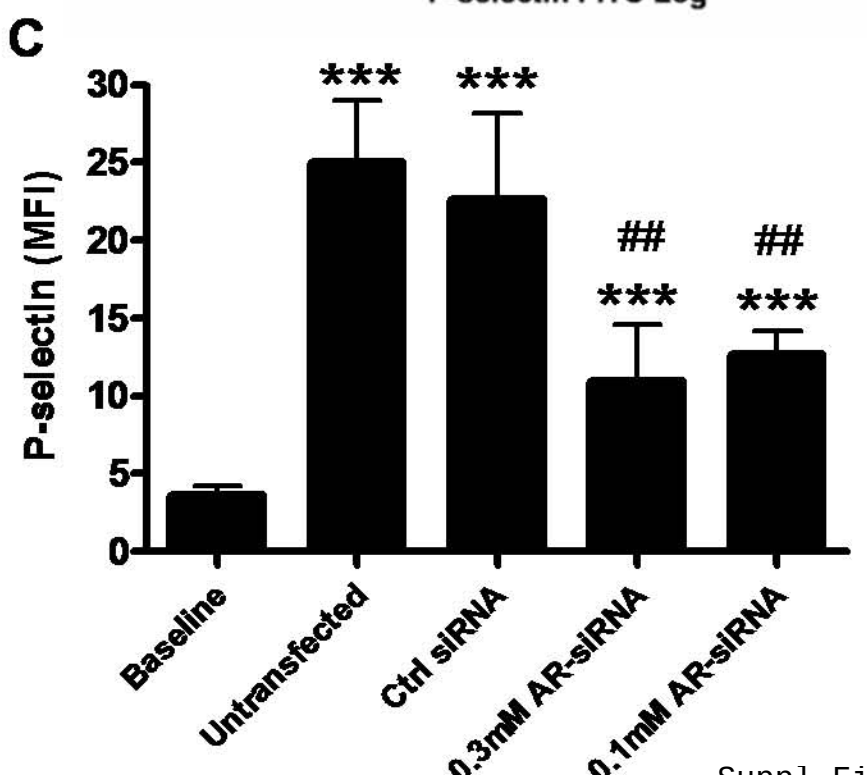

Aggregation
AR activity
Aggregation (% of light transmission)
AR activity (mU/μg protein)
50
40
30
20
10
0
15
12
9
6
3
0
0.001
0.01
0.1
1
10
100
1000
Concentration (μM)

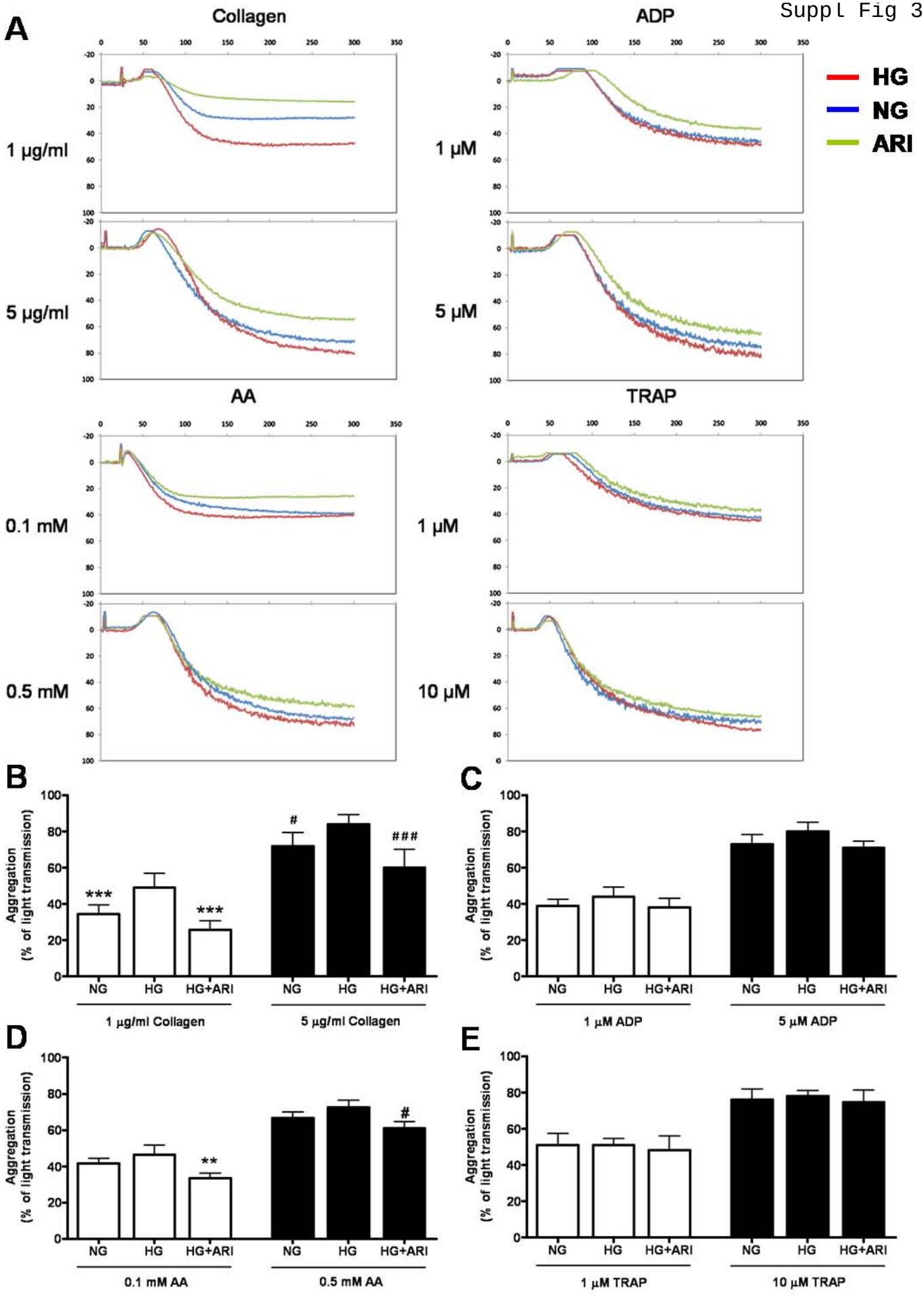
Suppl Fig 1

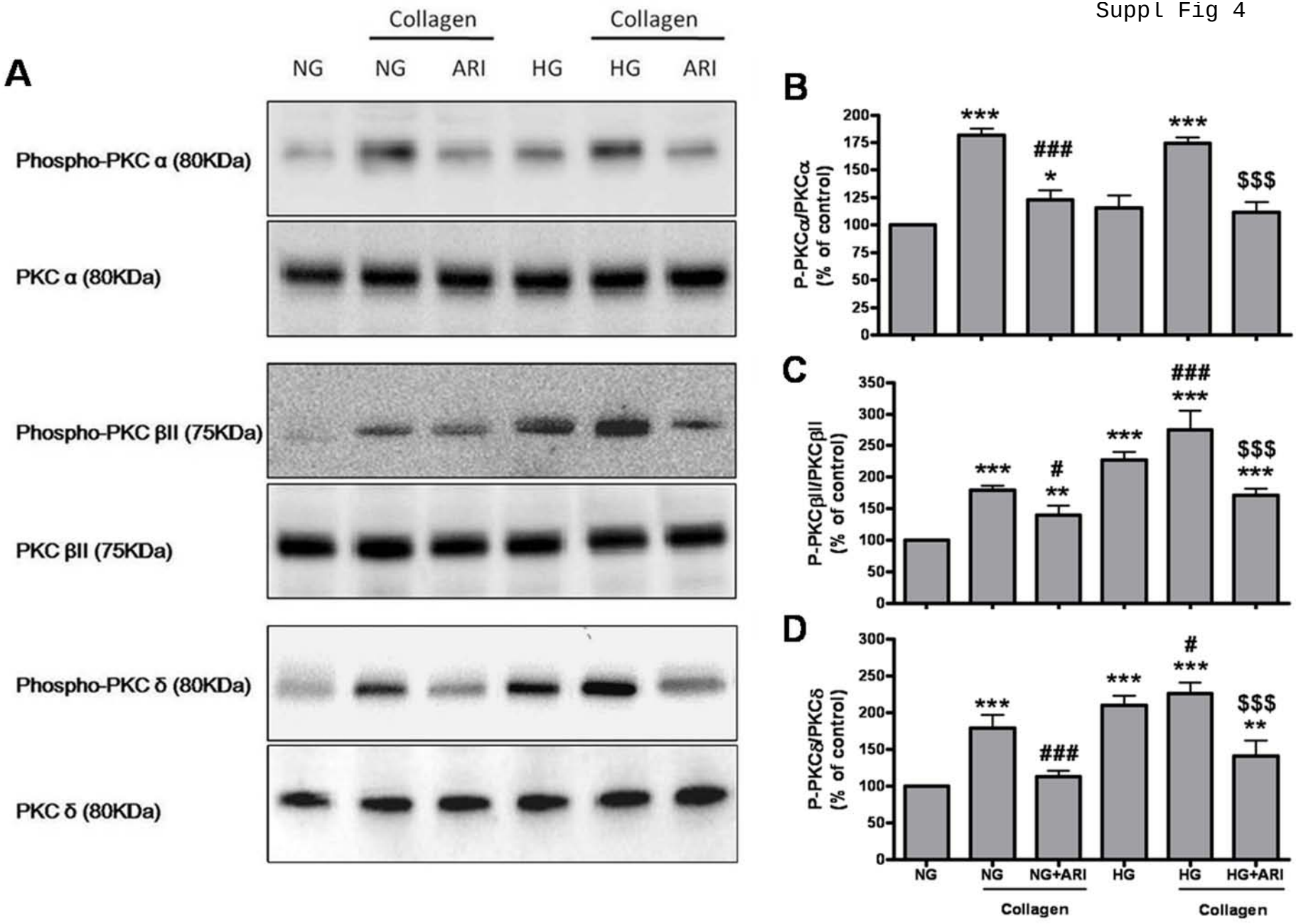

A
Untransfected
Ctrl-siRNA
0.3 mM AR-siRNA
0.1 mM AR-siRNA
AR
GAPDH

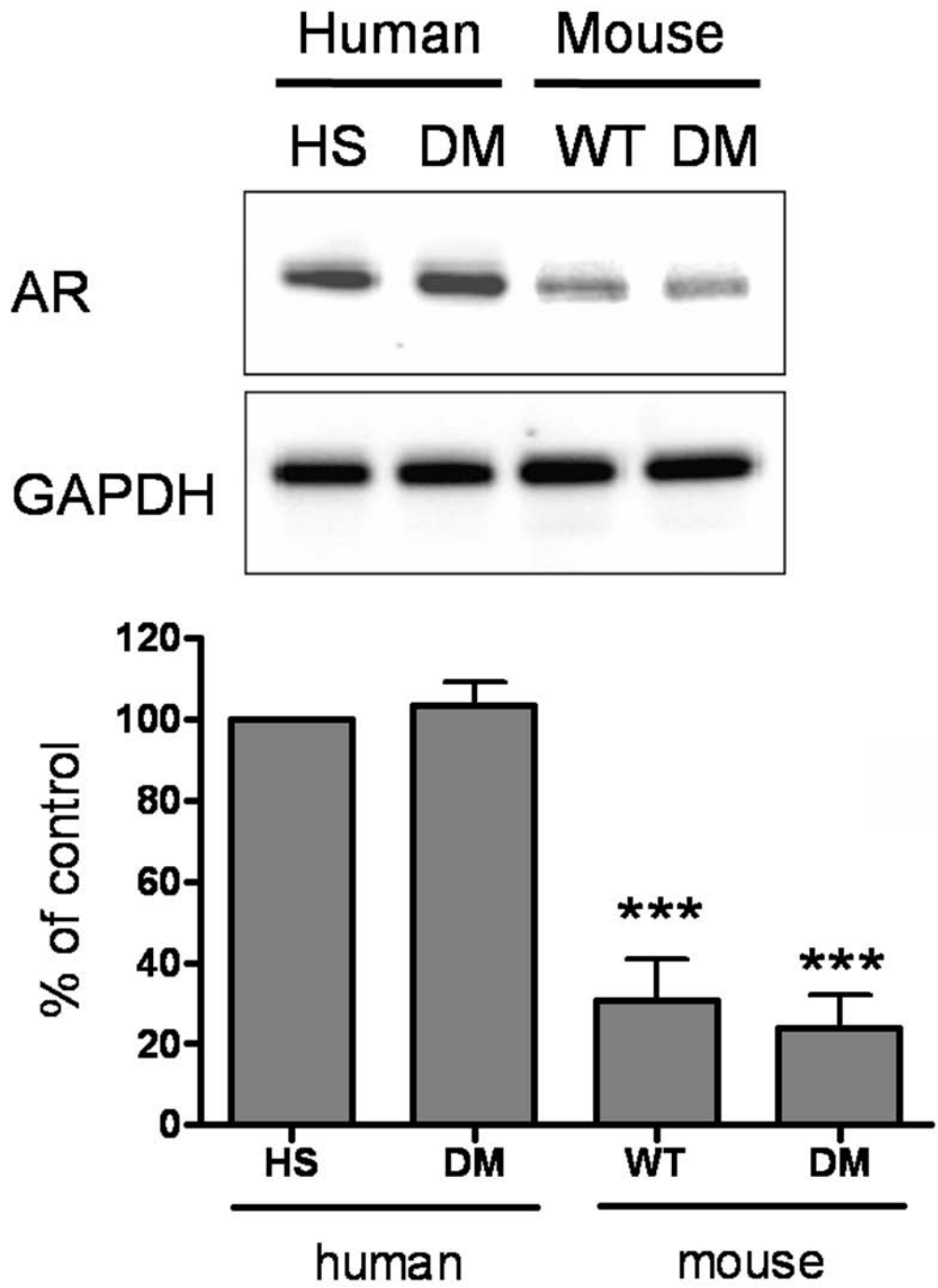

B
Baseline
Untransfected
Ctrl siRNA
0.3 mM AR siRNA
0.1 mM AR siRNA
Events
P-selectin FITC Log


C
P-selectin (MFI)
***
***
##
***
##
***
Baseline
Untransfected
Ctrl siRNA
0.3mM AR-siRNA
0.1mM AR-siRNA

Suppl Fig 2


A
Collagen
ADP
HG
NG
ARI
1 µg/ml
5 µg/ml
1 µM
5 µM
AA
TRAP
0.1 mM
0.5 mM
1 µM
10 µM
B
Aggregation (% of light transmission)
***
***
#
###
NG
HG
HG+ARI
1 µg/ml Collagen
5 µg/ml Collagen
C
1 µM ADP
5 µM ADP
D
**
#
0.1 mM AA
0.5 mM AA
E
1 µM TRAP
10 µM TRAP


A
Collagen
Collagen
NG
NG
ARI
HG
HG
ARI
Phospho-PKC α (80KDa)
PKC α (80KDa)
Phospho-PKC βII (75KDa)
PKC βII (75KDa)
Phospho-PKC δ (80KDa)
PKC δ (80KDa)
B
P-PKCα/PKCα (% of control)
C
P-PKCβII/PKCβII (% of control)
D
P-PKCδ/PKCδ (% of control)
NG
NG
NG+ARI
HG
HG
HG+ARI
Collagen
Collagen


Human
Mouse
HS
DM
WT
DM
AR
GAPDH
120
100
80
60
40
20
0
% of control
***
***
HS
DM
WT
DM
human
mouse

Suppl Fig 5

Supplementary figure legends

Supp. Figure 1 Dose response of epalrestat on collagen-induced aggregation AR activity in human platelets. Platelet suspensions were incubated with NG for 90 min in the presence or absence of different concentrations of epalrestat (0.01-100 μmol/l), prior to stimulation by 1 μg/ml collagen for 10 min. Data are expressed as mean ± SD (n=5 healthy subjects).

Supp. Figure 2 Silencing of AR reduces the P-selectin translocation to membrane in megakaryocytes (MEG-01) culture. The MEG-01 cells were transfected with 0.1 or 0.3 mM AR siRNA. After 48 hour NG incubation, the P-selectin translocation to membrane was assessed by flow cytometry in response to 1 μg/ml collagen. **(A)** The level of AR knockdown by siRNA was determined by Western blot analysis, and the AR expression is normalized to GAPDH expression. **(B)** The representative overlay plots were presented as the number of events over the log of associated fluorescence (Baseline refers to the untransfected MEG-1 cells without collagen stimulation). **(C)** Quantification of data was presented as mean fluorescent intensity (MFI). Data are expressed as mean ± SD (n=5). ***P<0.001 compared with the baseline; $^{\#\#}P$<0.01 compared with the control siRNA group.

Supp. Figure 3 Effect of AR inhibition on glucose-induced platelet aggregation in response to various agonists. The platelet suspensions were incubated with 5.5 mmol/l (NG) or 25 mmol/l glucose (HG) for 90 min, in the presence or absence of 10 μM epalrestat (ARI). The percentage of light transmission, an index of platelet aggregation, was measured in platelet suspensions in response to 1 or 5 μg/ml collagen, 1 or 5 μM ADP, 0.1 or 0.5 mM arachidonic acid (AA), and 1 or 10 μM thrombin receptor activator peptide (TRAP) for 5 min. **(A)** The representative aggregation curves were shown. Quantification of data was presented as the percentage of light transmission in response to **(B)** collagen (n=6), **(C)** ADP (n=6), **(D)** AA (n=4) and **(E)** TRAP (n=3). Data are expressed as mean ± SD. ***P<0.001 & **P<0.01 compared with HG group with low-dose agonist stimulation; $^{\#\#\#}P$<0.001 & $^{\#}P$<0.05 compared with high-dose agonist stimulation.

Supp. Figure 4 AR is required for the phosphorylation of PKC isoforms (α, βII & δ) in collagen-stimulated platelets. (A) Platelet suspensions were incubated with NG or HG for 90 min in the presence and absence of 10 μmol/l epalrestat (ARI), prior to stimulation by 1 μg/ml collagen for 10 min, and the total cellular extract was harvested for experiments. The representative blots were shown. The quantitative results of **(B)** PKCα, **(C)** PKCβII and **(D)** PKCδ phosphorylation were plotted in histogram. Data are expressed as mean ± SD (n=5 healthy subjects). ***P<0.001, **P<0.01 & *P<0.05 compared with values incubated in NG alone; $^{###}P$<0.001 & $^{\#}P$<0.05 compared with values in NG with the addition of 1 μg/ml collagen; $^{\$\$\$}P$<0.001 compared with values in HG with the addition of 1 μg/ml collagen.

Supp. figure 5 Expression of AR in human and mouse platelets. Human platelets were isolated from healthy subjects (HS) and type-1 diabetic patients (DM), and mouse platelets were isolated from the wild-type (WT) and non-obese diabetic mice (type-1 diabetes). The total cellular extract was collected for experiments. The expression of AR was determined by Western blot, and it was normalized to GAPDH expression. Data are expressed as mean ± SD (n=5 HS; n=5 DM patients; n=6 WT mice; n=3 DM mice). ***P<0.001 compared with HS.