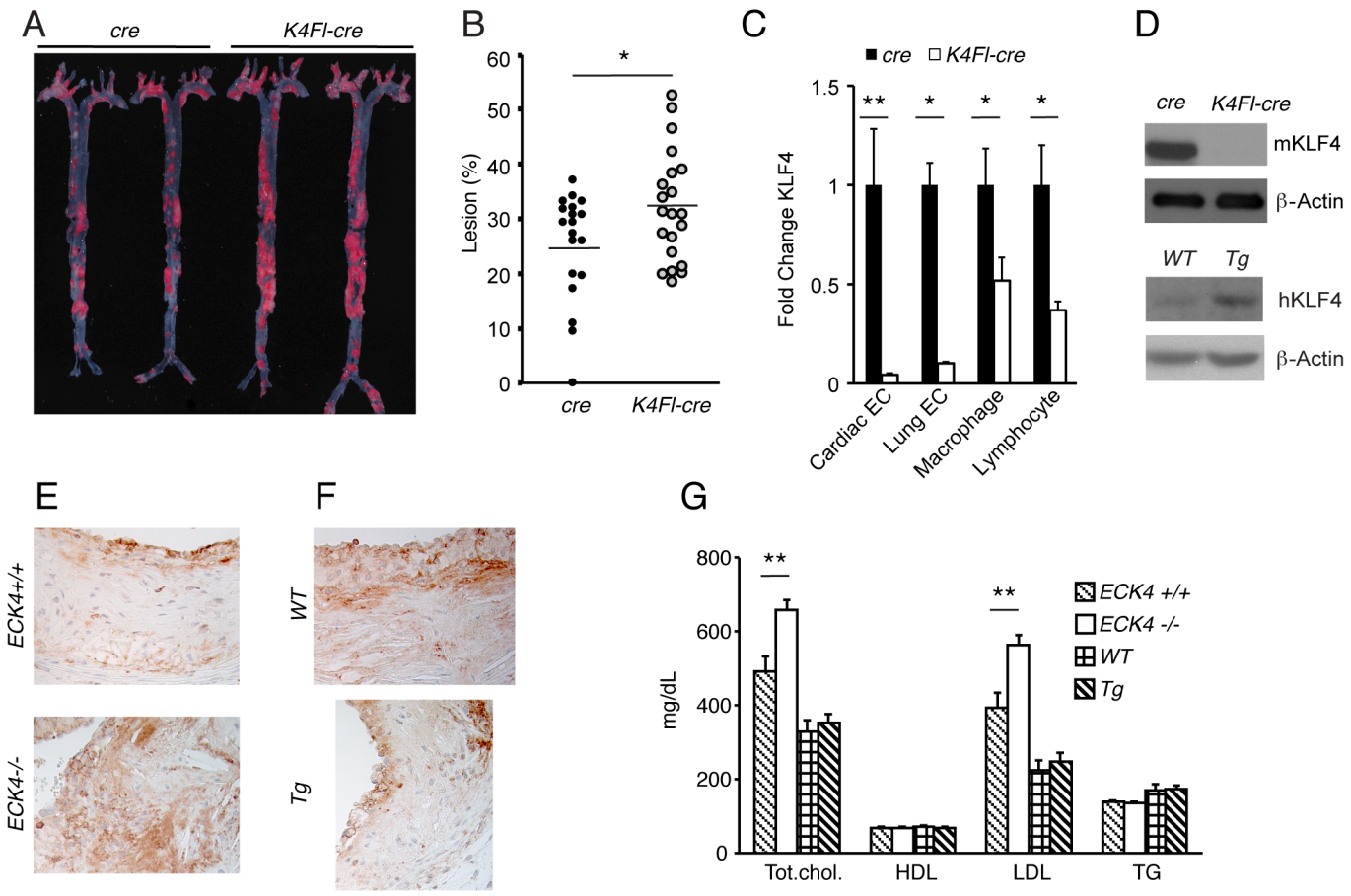
Supplemental Table 1 Complete Blood Counts

	WT	*Tg*	*ECK4+/+*	*ECK4-/-*
Leukocytes				
WBC (K/uL)	9.16 ± 4	7.91 ± 4	12.78 ± 5	13.69 ± 3
Neutrophils (K/uL)	1.68 ± 1	1.55 ± 1	2.74 ± 1	3.75 ± 1
Lymphocytes (K/uL)	6.91 ± 3	5.79 ± 3	9.19 ± 3	8.73 ± 2
Monocytes (K/uL)	0.43 ± 0.2	0.44 ± 0.2	0.45 ± 0.4	0.38 ± 0.1
Eosinophils (K/uL)	0.11 ± 0.1	0.11 ± 0.04	0.29 ± 0.3	0.49 ± 0.3
Basophils (K/uL)	0.02 ± 0.02	0.03 ± 0.02	0.11 ± 0.1	0.17 ± 0.1
Erythrocytes				
RBC (M/uL)	7.91 ± 2	7.28 ± 3	9.04 ± 1	8.79 ± 1
Hb (g/dL)	11.12 ± 3	10.00 ± 4	12.46 ± 1	11.97 ± 2
HCT (%)	33.08 ± 10	30.62 ± 11	39.67 ± 4	38.00 ± 5
MCV (fL)	41.68 ± 1	41.9 ± 1	43.94 ± 1	43.20 ± 1
MCH (pg)	14.02 ± 0.4	13.68 ± 0.7	13.80 ± 0.6	13.58 ± 0.4
MCHC (g/dL)	33.64 ± 01	32.68 ± 1	31.43 ± 1	31.50 ± 1
RDW (%)	19.06 ± 2	18.83 ± 1	17.99 ± 1	17.85 ± 0.3
Thrombocytes				
PLT (K/ul)	842 ± 200	650 ± 200	174 ± 100	261 ± 200
PMT (fL)	4.56 ± 0.1	4.82 ± 1	5.66 ± 1	5.43 ± 0.3

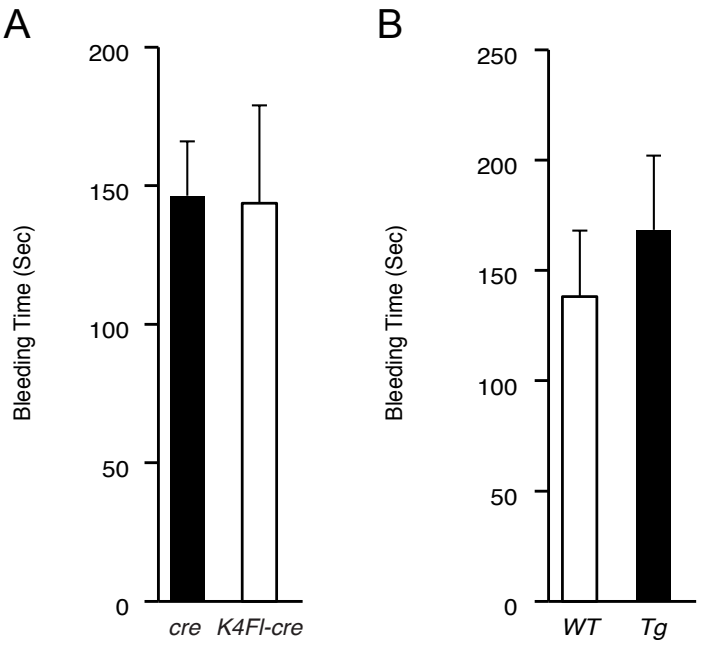
WT vs *Tg*, n=5-6; *ECK4+/+* vs *ECK4-/-,* n=6-7

Supplemental Figure 1



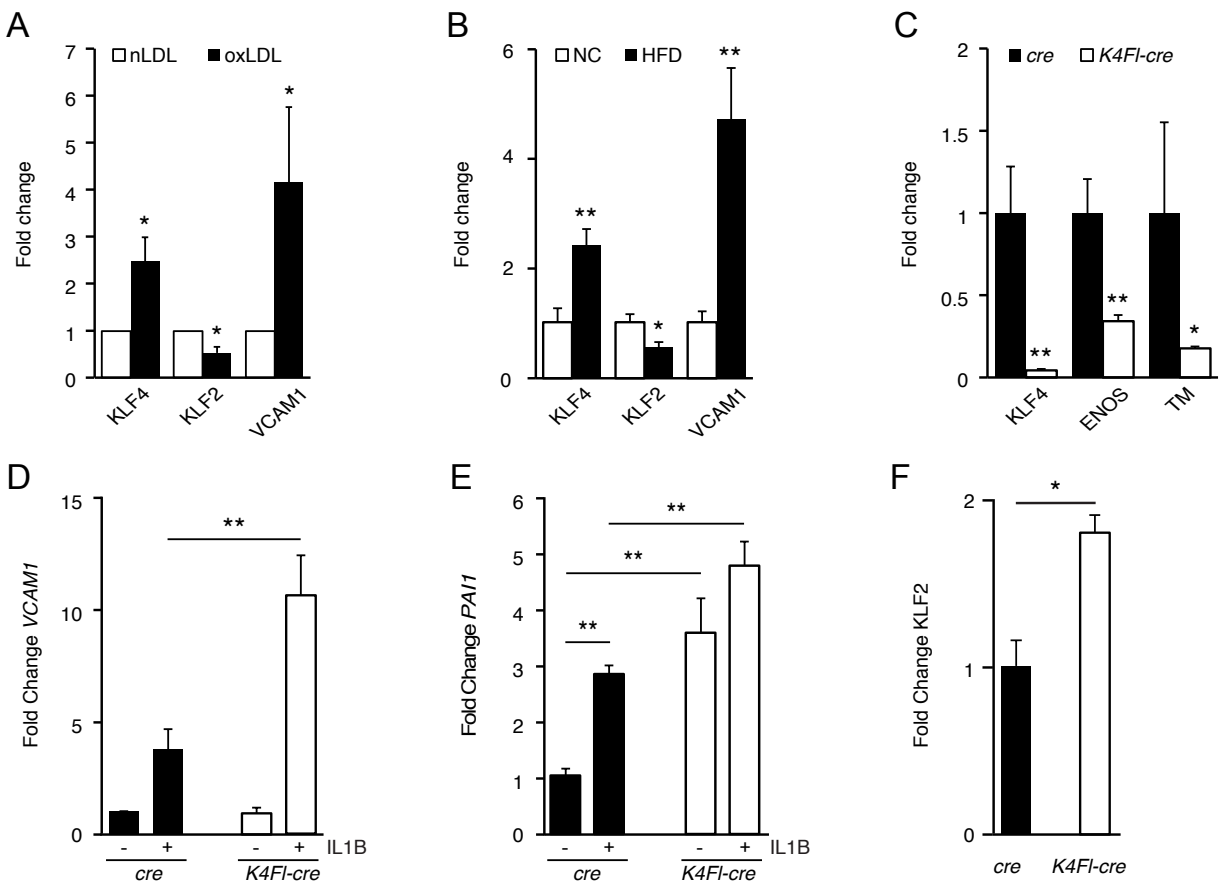
Phenotypic changes in endothelial KLF4 knockdown and transgenic mice. A) After HFD, aortas from *K4Fl-cre* mice (no BMT, *ApoE-/-* background) demonstrate greater atherosclerosis by Sudan IV staining of the entire aorta (A,B). C) Primary ECs from the heart and lungs of floxed KLF4 x cre mice (*K4Fl-cre*) demonstrate ≥80% loss of *Klf4* expression. Decreased *Klf4* expression was also seen in macrophages and lymphocytes. (EC experiments, n=3; macrophages and lymphocytes, 5-11 animals per experiment, n=3 experiments. D) ECs isolated from mouse hearts demonstrate loss of KLF4 protein in the *K4Fl-cre* mice, and enhanced KLF4 protein in the *ECKLF4 Tg* mice. A representative experiment with pooled cells is shown. E-F) 400x magnification of boxed insets from Figure 1C and 1G, respectively. G) After BMT, *ECK4-/-* mice have enhanced serum total cholesterol and LDL. Serum was collected from 30 week-old mice, after 20 weeks of HFD (n= 9 pairs). There is no difference in the lipid panel between *WT* and *ECKLF4Tg* mice (25 week old mice, n=9 pairs).

Supplemental Figure 2



Modulation of endothelial KLF4 expression does not alter bleeding times. A) Control (*cre*) versus floxed KLF4 x cre (*K4Fl-cre*) mice (n= 6 pairs). B) *WT* versus *ECKLF4 Tg* mice (n= 6 pairs).

Supplemental Figure 3



KLF4 is upregulated by proinflammatory stimuli, and modulates expression of markers of inflammation. A). *KLF4* expression is upregulated by oxLDL in HAECs exposed to atheroprone flow (n= 8; nLDL, nonoxidized LDL; oxLDL, oxidized LDL). B) Aortic expression of *KLF4*, *KLF2*, and *VCAM1* in animals exposed to HFD mimics that seen in in vitro assays with oxLDL. These mice were *WT* for *KLF4*, on an *ApoE-/-* background. (NC, normal chow, n=4 individual aortas; HFD, high fat diet, n=5 individual aortas) C) KLF4 deficiency in EC isolated from mouse hearts decreases *ENOS* and *TM* expression (n=3 experiments). D) KLF4 deficiency in EC isolated from mouse hearts leads to enhanced expressed of the pro-inflammatory mediator *VCAM1*. After 2 hour treatment with 5nM IL1B, RNA was and assessed by QPCR (n=2 experiments). E) Increased expression of the pro-coagulant *PAI1* in KLF4 deficient cells isolated from mouse hearts (experiments performed as in D). F) *KLF2* expression is enhanced in KLF4-deficient ECs derived from heart or lung (n=3 experiments).

Supplemental Methods:

Atherosclerosis Studies

Animals were sacrificed after 16-20 weeks of normal chow or high fat diet. After sacrifice, mouse aortas were fixed and stained with Sudan IV. Digital images of *en face* preps were quantitated using Image-Pro software (Media Cybernetics). Results are expressed as percent Sudan IV positive area relative to the total aorta area. For HE and CD45 staining, paraffin-embedded sections of the aortic root were used. Plasma lipid profiles were performed on fasted animals using the Cholesterol Reagent Set (Pointe Scientific, Inc.).

Thrombosis Studies

In vitro clotting assays were performed as previously described (1). Confluent murine primary cardiac ECs cultured in 96 well plates were stimulated with TNFA or vehicle for 5 hours. Cells were rinsed twice with warm phosphate-buffered saline and 100 μl 37 C human plasma (Sigma-Aldrich) added to each well. Immediately thereafter, 100 μl of 25 mM $CaCl_2$ was added, and plates were placed in a prewarmed V_{max} kinetic plate reader (Molecular Devices) and read at 405 nm every 20 seconds for 30 minutes. Fibrin clot formation is indicated when a maximum absorbance is reached. Clotting times are reported at half-maximal absorbance, as determined using the V_{max} software.

Isolation of Mouse Cells

Microvascular cardiac ECs were isolated from 4-6 week old mice as previously described (2). ECs were pooled from 5 mice and purified using two rounds of sorting with antibodies conjugated to Dynal beads (Invitrogen). Anti-PECAM was used for the first round of purification, anti-ICAM2 for the second. Antibodies were obtained from BD Biosciences-Pharmingen.

RT-PCR and Immunoprecipitation

Total RNA from cultured cells or mouse tissue samples was isolated using either TRIzol reagent (Invitrogen) or an RNeasy kit (QIAGEN). QPCR was performed using either SYBR green or Roche Universal probe reagents and a StepOnePlus Real-Time PCR System (Applied Biosystems). For immunoprecipitation experiments, cultured 293 cells were transfected with Flag-KLF4 or Flag KLF4ΔTAD constructs using Fugene HD (Promega). Nuclear proteins were extracted using the NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce Biotechnology). M2 beads (Sigma) were used to immunoprecipitate the Flag-KLF4 complex. The KLF4 antibody was obtained from R&D Systems and the p300 antibody from Santa Cruz Biotechnology.

References for Supplemental Methods:

1. Hamik, A., Lin, Z., Kumar, A., Balcells, M., Sinha, S., Katz, J., Feinberg, M.W., Gerzsten, R.E., Edelman, E.R., and Jain, M.K. 2007. Kruppel-like factor 4 regulates endothelial inflammation. J Biol Chem 282:13769-13779.
2. Lim, Y.C., Garcia-Cardena, G., Allport, J.R., Zervoglos, M., Connolly, A.J., Gimbrone, M.A., Jr., and Luscinskas, F.W. 2003. Heterogeneity of endothelial cells from different organ sites in T-cell subset recruitment. Am J Pathol 162:1591-1601.