Supporting Information

Keramati et al. 10.1073/pnas.1019443108

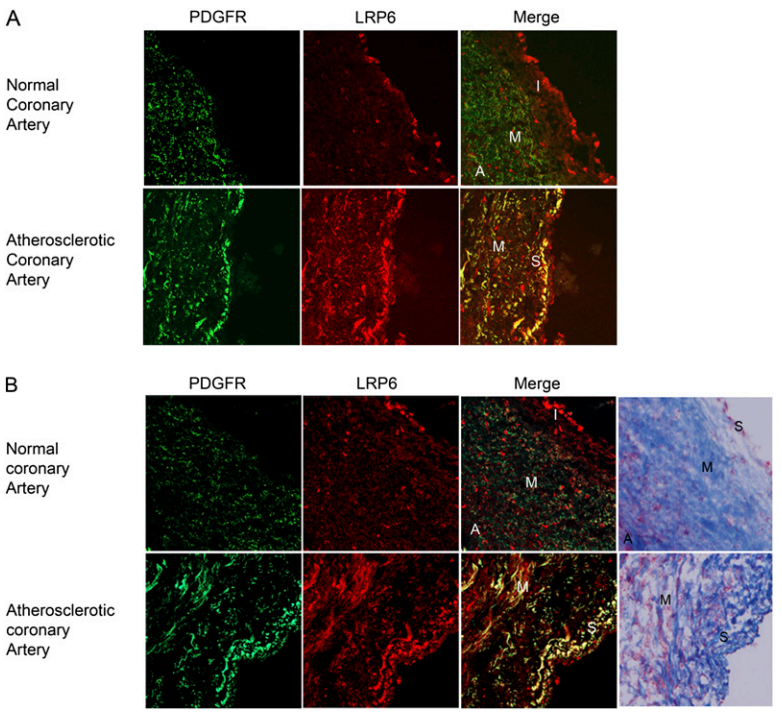

Fig. S1. (*A* and *B*) PDGF receptor β (PDGFR-β) and LDL receptor-related protein 6 (LRP6) expression in normal and atherosclerotic coronary arteries. Cross-sections of normal arteries (*Upper*) and atherosclerotic lesions (*Lower*) stained for PDGFR and LRP6 are shown. *B* also shows the Sudan staining of the normal and the atherosclerotic vessels for better orientation. To show PDGFR/LRP6 expression, only a cell-rich segment of the atherosclerotic lesion that lacks lipid core is shown. A, adventicia; I, intima; M, muscularis; S, subintima.

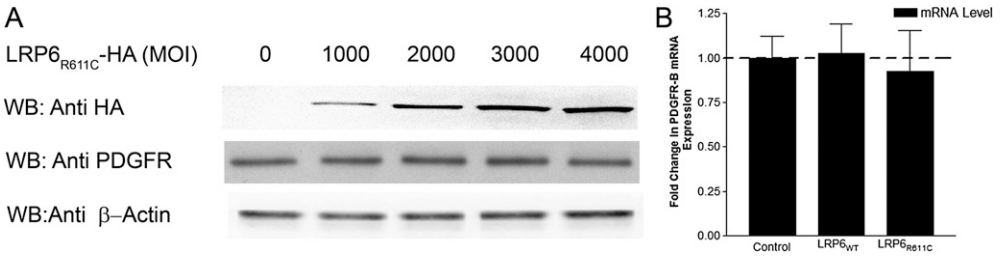

Fig. S2. Dose effect of $LRP6_{R611C}$ on endogenous PDGFR-β expression. (*A*) No significant changes in PDGFR-β expression levels were noted with $LRP6_{R611C}$. (*B*) PDGFR-β mRNA expression levels. There was no difference in mRNA expression levels of PDGFR-β in cells infected with empty vector or with adenovirus vector expressing $LRP6_{WT}$ or $LRP6_{R611C}$.

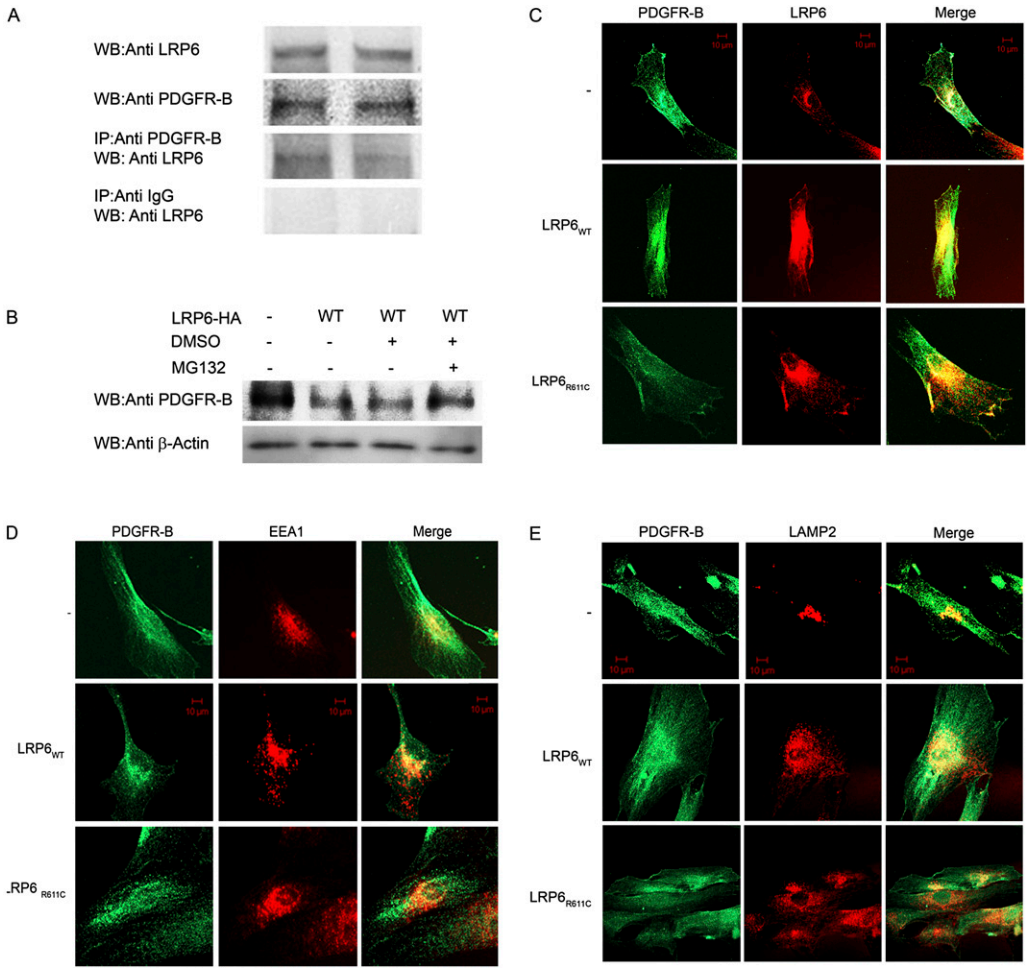

Fig. S3. (*A*) Coimmunoprecipitation of the native LRP6 and PDGFR in human aortic smooth muscle cells. (*B*) Inhibition of the proteosomal degradation with MG132 partially rescues reduced expression of PDGFR-β ($P = 0.02$). (*C–E*) Colocalization of LRP6, PDGFR-β, early endosomal antigen 1 (EEA1), and (lysosomal-associated membrane protein 2) Lamp2 in unstimulated primary aortic smooth muscle cells infected with plasmids expressing $LRP6_{WT}$ and $LRP6_{R611C}$ and empty plasmids. $LRP6_{WT}$ and $LRP6_{R611C}$ both colocalize with PDGFR-β (*C*) and EEA1 (*D*). Colocalization of PDGFR-β with the lysosomal marker LAMP2 (*E*) was observed in more than two-thirds of the cells overexpressing $LRP6_{WT}$ and was significantly higher than in cells overexpressing $LRP6_{R611C}$ and cells infected with empty vector.

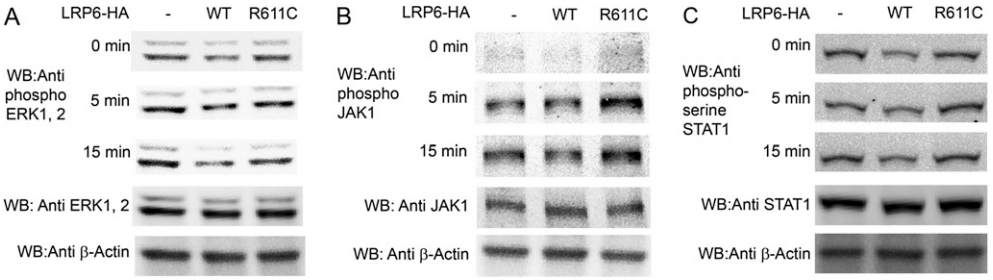

Fig. S4. Expression levels of PDGF signal peptides. Phosphorylation of (*A*) ERK1/2, (*B*) JAK1, and (*C*) STAT1 is significantly lower in cells expressing $LRP6_{WT}$ than in uninfected cells after stimulation with PDGF ($P = 0.02$, 0.03, and 0.01, respectively). In comparison, JAK1 phosphorylation is increased considerably in cells expressing $LRP6_{R611C}$ as compared with $LRP6_{WT}$ ($P < 0.01$) and uninfected cells ($P < 0.05$).