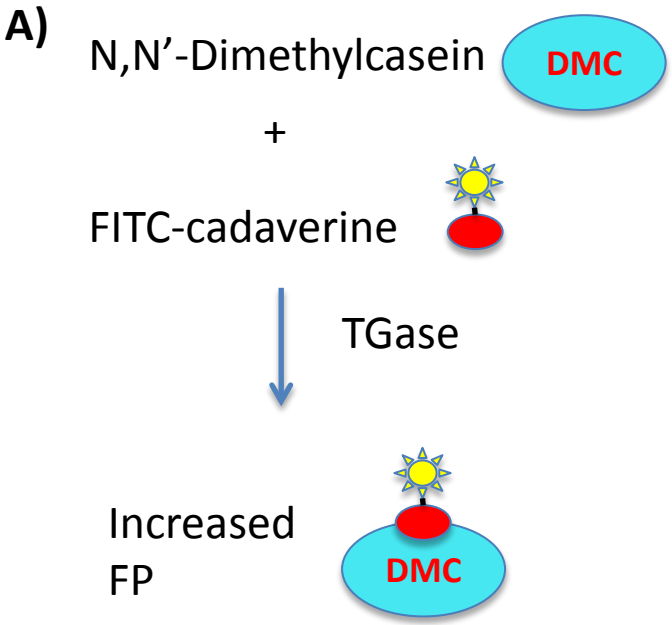
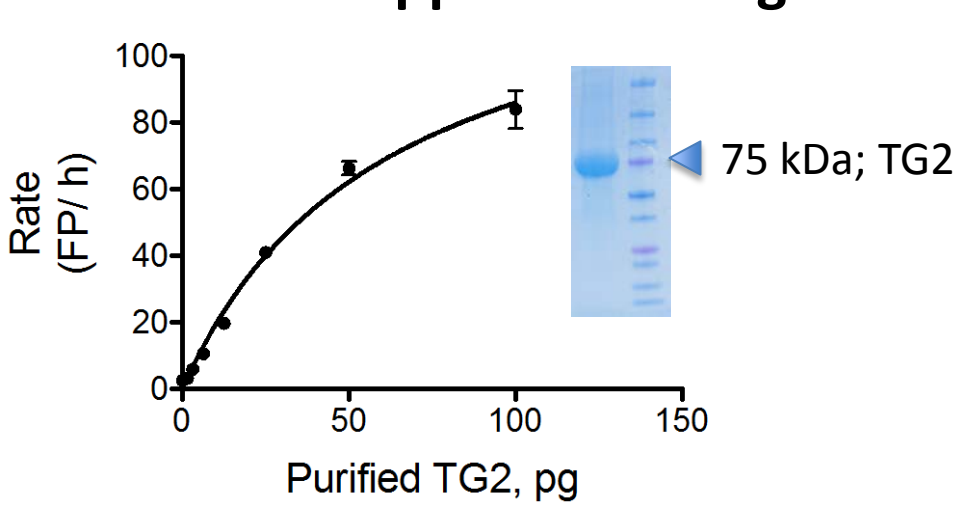
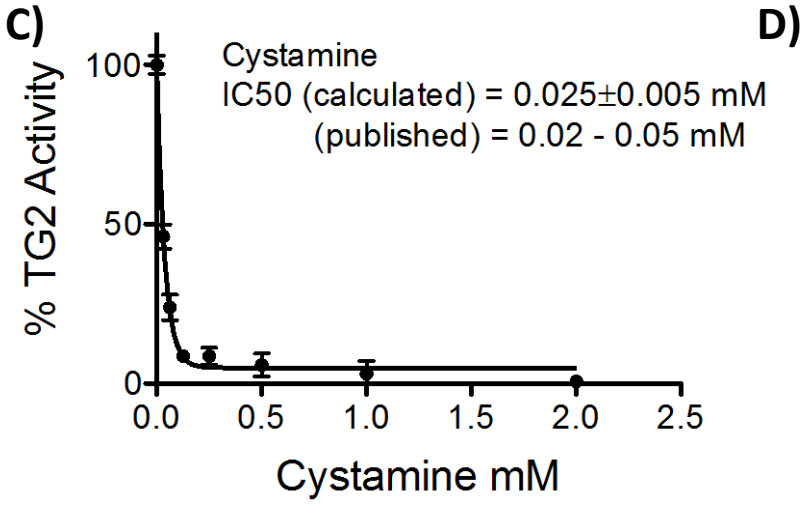
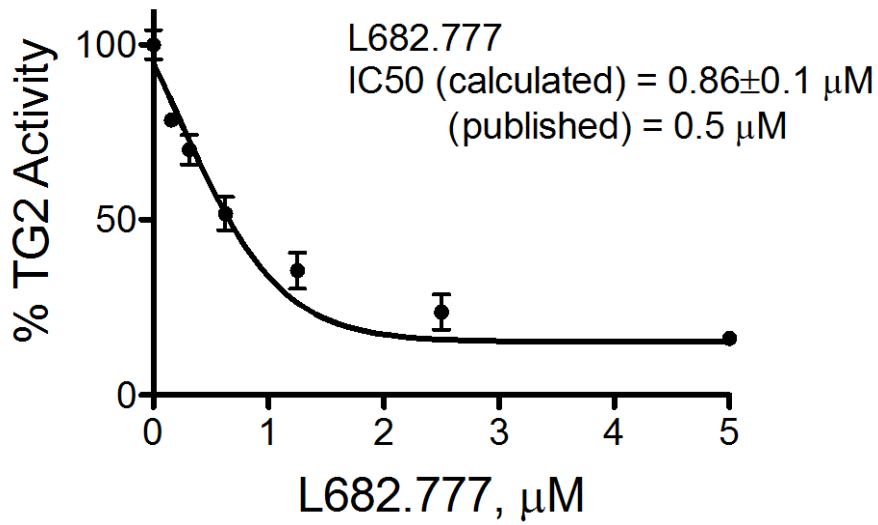
Supplemental Figure I

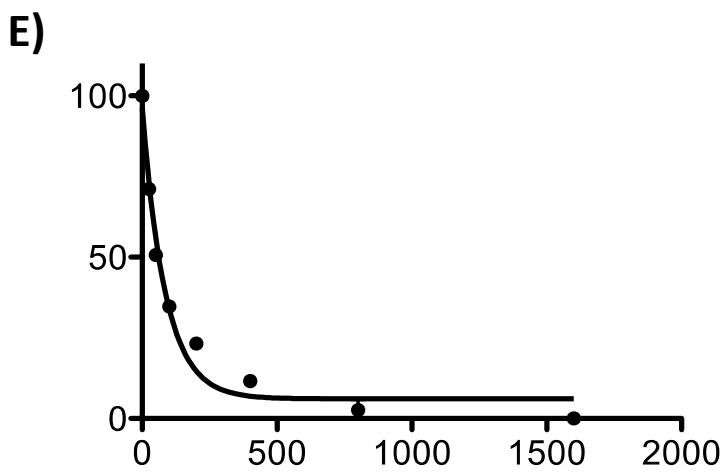


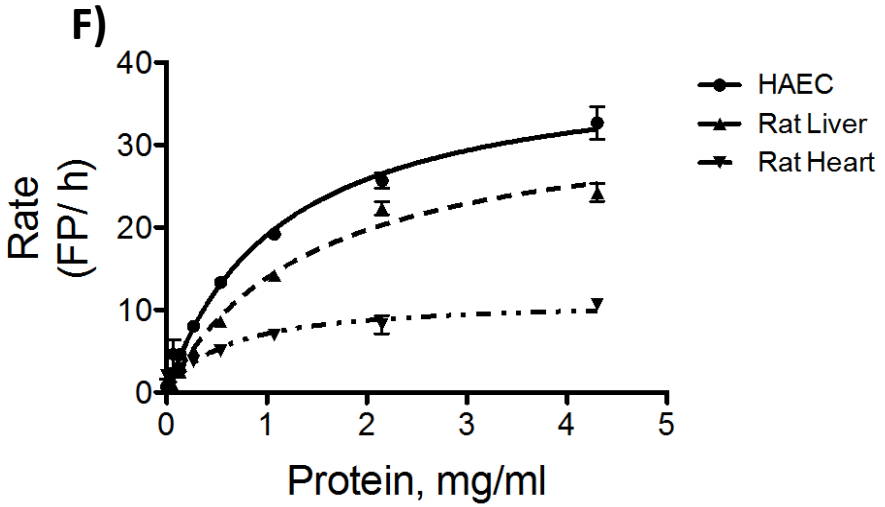


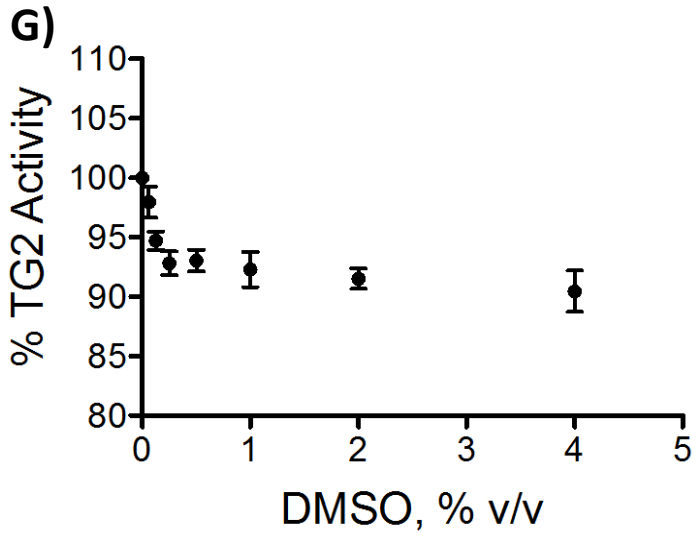


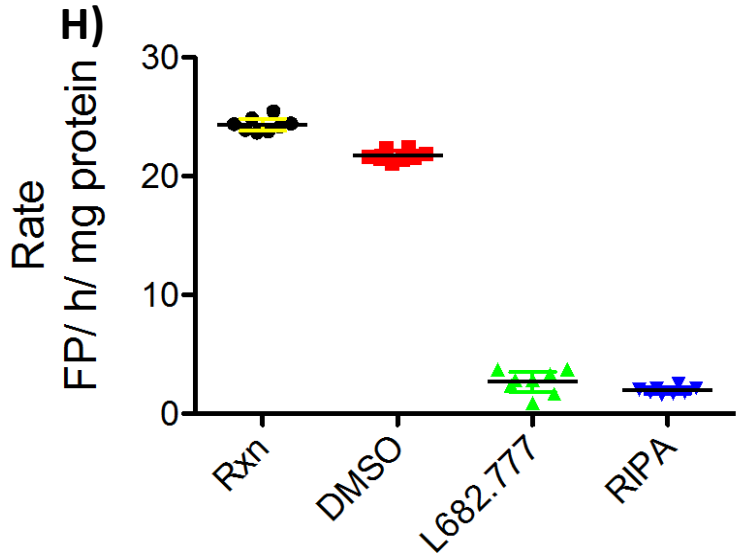


Supplemental Figure I





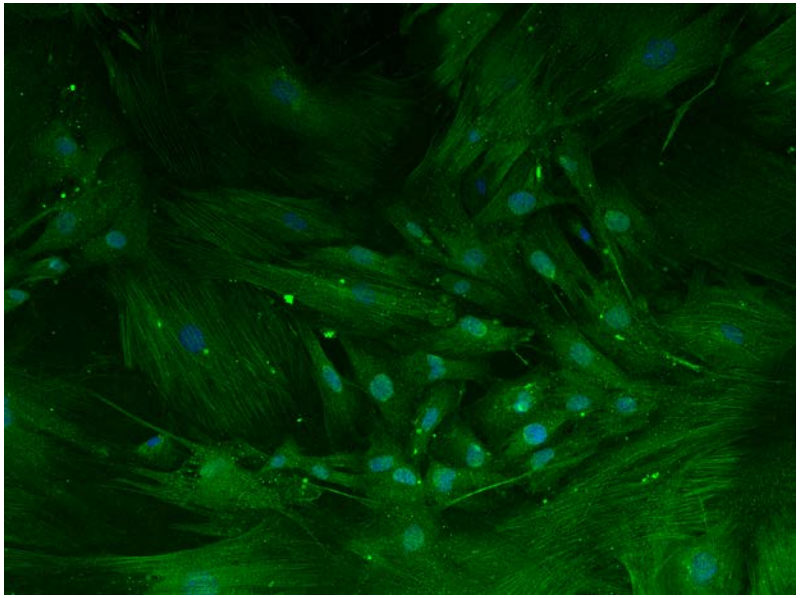




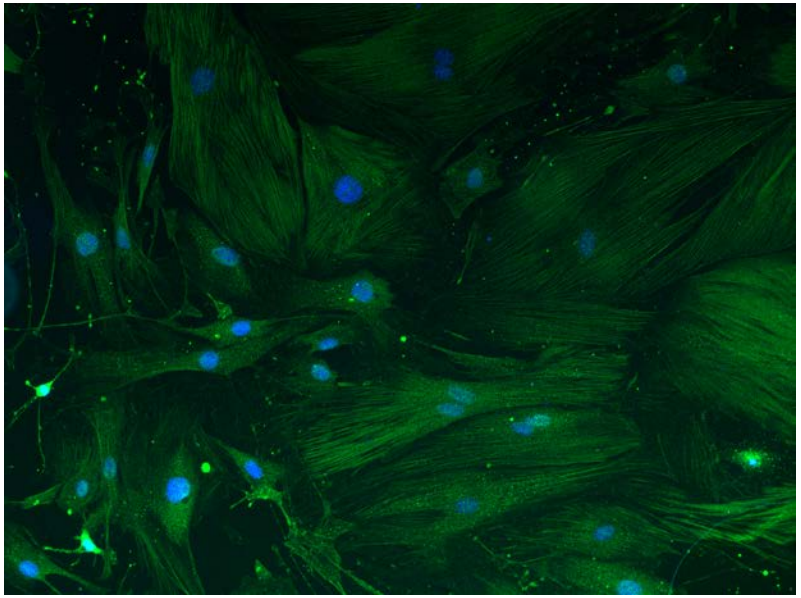
Supplemental Figure I. Fluorescence polarization (FP)-based assay for transglutaminase (TG) activity. A, The principle of the assay is that as the low molecular weight fluorescent molecule FITC-cadaverine is incorporated by transamidation into the high molecular weight N,N'-dimethylcasein, the FP signal of FITC-cadaverine increases. **B,** His-tagged recombinant human TG2 was expressed in HEK293 cells and purified with a BD-Talon purification kit before use. Representative Coomassie blue staining of the purified product is shown in the inset. FP signal increased with increasing TG2 protein in the reaction mixture. **C,** The IC_{50} of cystamine was calculated to be 0.025±0.005 mmol/L in the FP assay. This value is in good agreement with the published value of 0.02±0.05 mmol/L (n=12 per group). **D,** The IC_{50} of L682.777 was calculated to be 0.86±0.1 μmol/L, which is in good agreement with the value of 0.5 μmol/L provided by the vendor (Zedira; n=12 per group). **E,** Unlabeled cadaverine, which was used as a competitive inhibitor for FITC-cadaverine, had an IC_{50} of 56±0.1 nmol/L (n=12 per group). **F,** The assay was used to examine activity in the protein lysates/homogenates obtained from human aortic endothelial cells (HAEC), rat liver, and rat heart prepared in 1x radioimmunoprecipitation assay (RIPA) lysis buffer with protease inhibitors. FP signal increased in a protein concentration-dependent manner in all of the samples (n=8 per group). **G**, The assay tolerated DMSO up to 4% v/v with ~5-7% decrease in activity when compared with 0% DMSO. **H,** We evaluated the assay by carrying out 24 replicates each of baseline reaction (Rxn); 4% DMSO, 10 μmol/L L682.777, and RIPA buffer negative control. The Z' values were 0.76 (Rxn vs. RIPA), 0.71 (Rxn vs. L682.777), 0.71 (DMSO vs. RIPA), and 0.62 (DMSO vs. L682.777). A Z' value of 0.5-1 represents a robust assay (n=24 per group).

Supplemental Figure II

WT MASMC

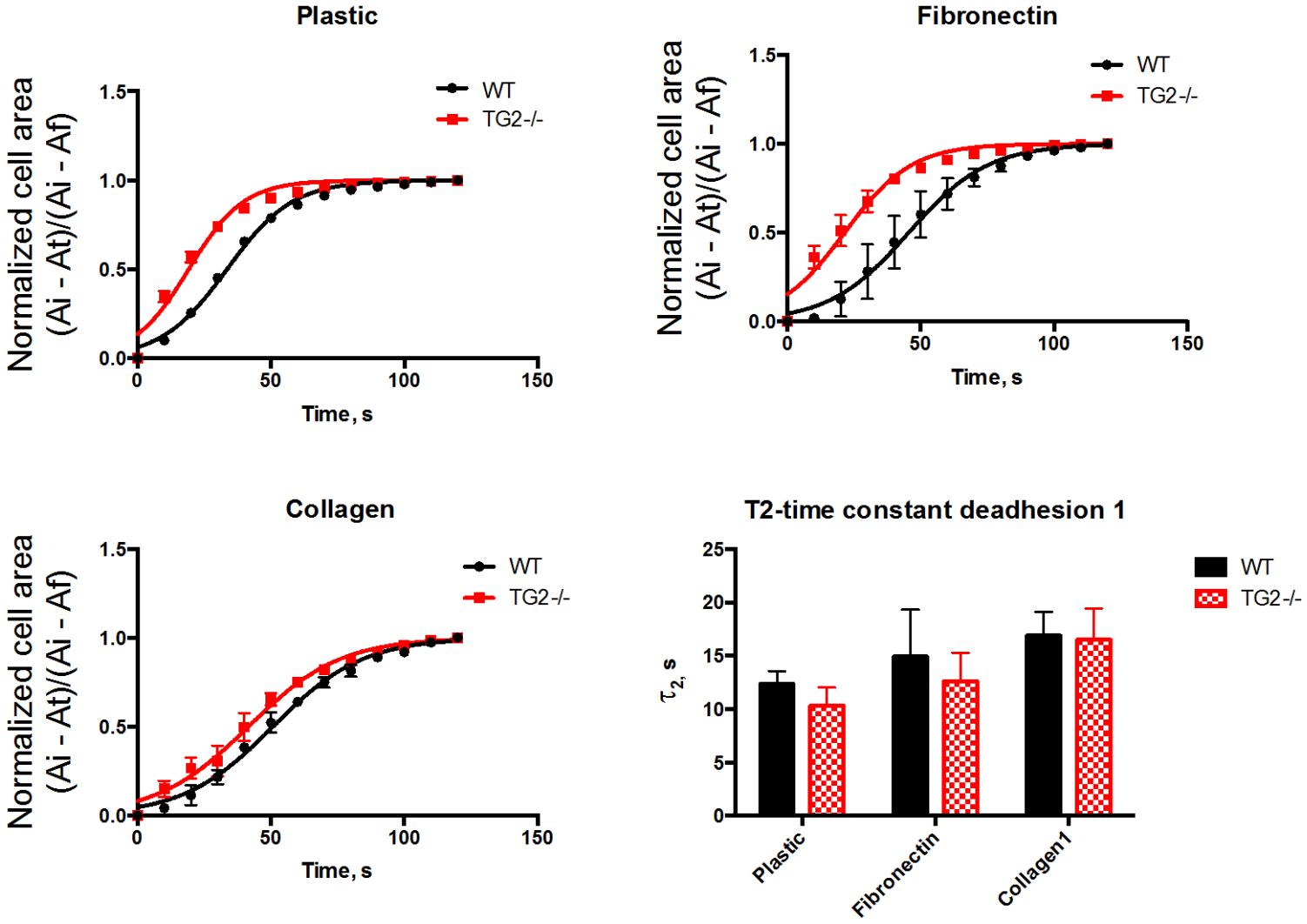


TG2-/- MASMC



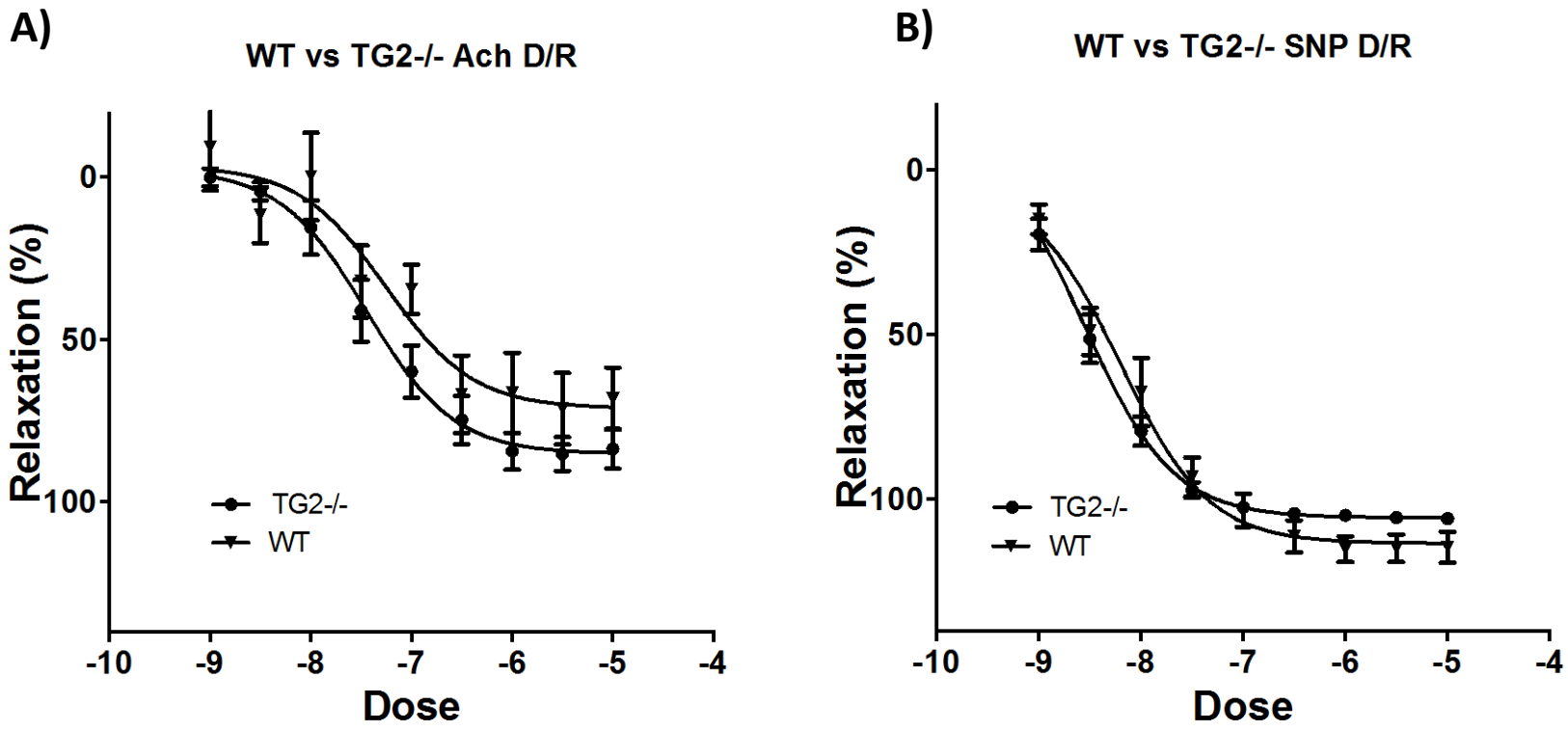
Supplemental Figure II. Smooth muscle cells (SMCs) isolated from TG2-/- and wild-type (WT) mouse aortae (MASMC) were stained with smooth muscle actin to verify the quality of SMC isolation. More than 98% of the cells stained positive for smooth muscle actin. Images are representative of 10 SMC preparations.

Supplemental Figure III



Supplemental Figure III. De-adhesion dynamics of TG2-/- and wild-type (WT) smooth muscle cells (SMCs) were examined by using 0.05% trypsin to sever the cell-matrix interactions. Time course of de-adhesion on **A,** tissue culture plastic; **B,** fibronectin; and **C,** collagen I-coated dishes. **D,** the τ2 time constant of the de-adhesion curves was similar for TG2-/- and WT SMCs on all supports. A_i = initial spread area; A_t = spread area at time t, A_f = final spread area.

Supplemental Figure IV



Supplemental Figure IV. Dose response (D/R) curves for induction of vasorelaxation by acetylcholine (Ach: **A**) and sodium nitroprusside (SNP; **B**) in aortic rings from wild-type (WT) and TG2-/- mice preconstricted with phenylephrine. No difference is apparent between the two groups (n=8 per group). **C,** gpTG2 (10001) shares 81% sequence identity with rat TG2 (10003) and 82% with mouse TG2 (10002); rat and mouse TG2 share 93% sequence identity.

C)

```
10001   1    MAEDLILERCDLQLEVNGRDHRTADLCRERLVLRRGQPFWLTLHFEGRGYEAGVDTLTFNAVTGPDPSEEAGTMARFSLS  80
10002   1    MAEELLLERCDLEIQANGRDHHTADLCQEKLVLRRGQRFRLTLYFEGRGYEASVDSLTFGAVTGPDPSEEAGTKARFSLS  80
10003   1    MAEELNLERCDLEIQANGRDHHTADLCQEKLVLRRGQRFRLTLYFEGRGYEASVDRLTFGAVTGPDPSEEAGTKARFSLS  80

10001  81    SAVEGGTWSASAVDQQDSTVSLLLSTPADAPIGLYRLSLEASTGYQGSSFVLGHFILLYNPRCPADAVYMDSDQERQEYV 160
10002  81    DNVEEGSWSASVLDQQDNVLSLQLCTPANAPIGLYRLSLEASTGYQGSSFVLGHFILLYNAWCPADDVYLDSEEERREYV 160
10003  81    DDVEEGSWSASVLDQQDNVLSLQLCTPANAPVGQYRLSLETSTGYQGSSFMLGHFILLFNAWCPADDVYLDSEAERREYV 160

10001 161    LTQQGFIYQGSAKFINGIPWNFGQFEDGILDICLMLLDTNPKFLKNAGQDCSRRSRPVYVGRVVSAMVNCNDDQGVLQGR 240
10002 161    LTQQGFIYQGSVKFIKSVPWNFGQFEDGILDTCLMLLDMNPKFLKNRSRDCSRRSSPIYVGRVVSAMVNCNDDQGVLLGR 240
10003 161    LTQQGFIYQGSVKFIKSVPWNFGQFEDGILDACLMLLDVNPKFLKDRSRDCSRRSSPIYVGRVVSGMVNCNDDQGVLLGR 240

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10003 401    VVDWIRQSDGSVLKSINNSLVVGQKISTKSVGRDDREDITYTYKYPEGSPEEREVFTRANHLNKLAEK----EETGVAMR 476

10001 480    IRVGQNMTMGSDFDIFAYITNGTAESHECQLLLCARIVSYNGVLGPVCSTNDlLNLTLDPFSENSIPLHILYEKYGDYLT 559
10002 477    IRVGDSMSMGNDFDVFAHIGNDTSETRECRLLLCARTVSYNGVLGPECGTED-INLTLDPYSENSIPLRILYEKYSGCLT 555
10003 477    IRVGDGMSLGNDFDVFAHIGNDTSESRECRLLLCARTVSYNGVLGPECGTED-INLTLDPYSENSIPLRILYEKYSGCLT 555

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10002 636    EVSDPVPAGDLVKARVDLFPTDIGLHKLVVNFQCDKLKSVKGYRNVIIGPA 686
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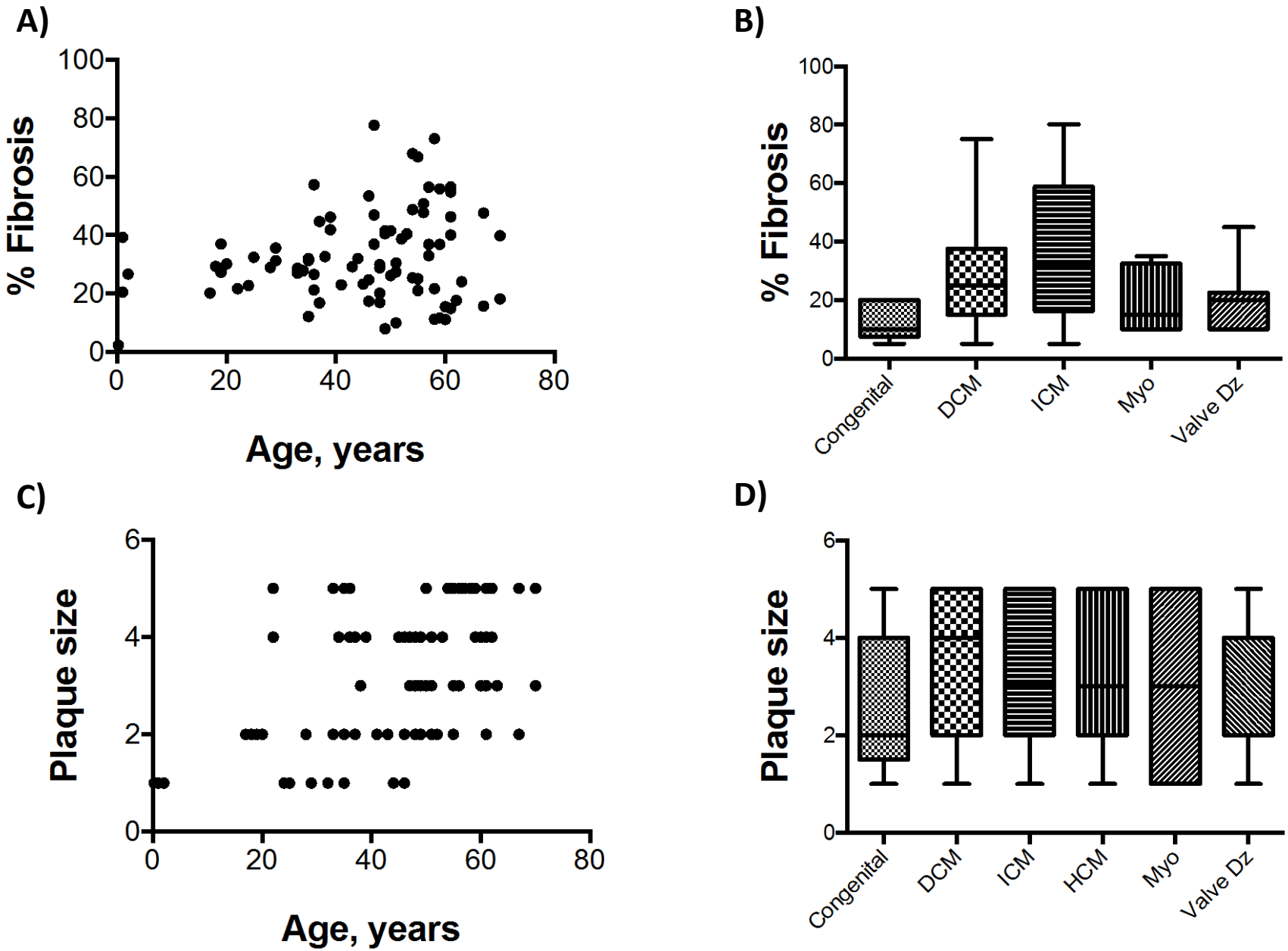
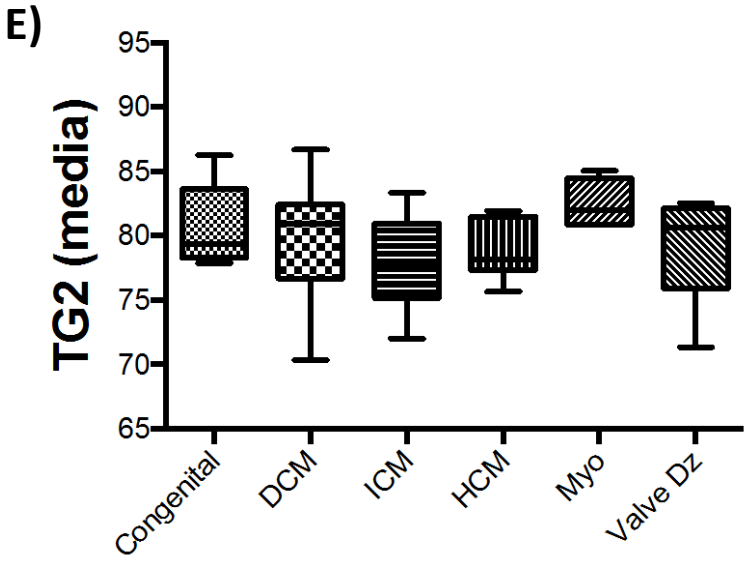
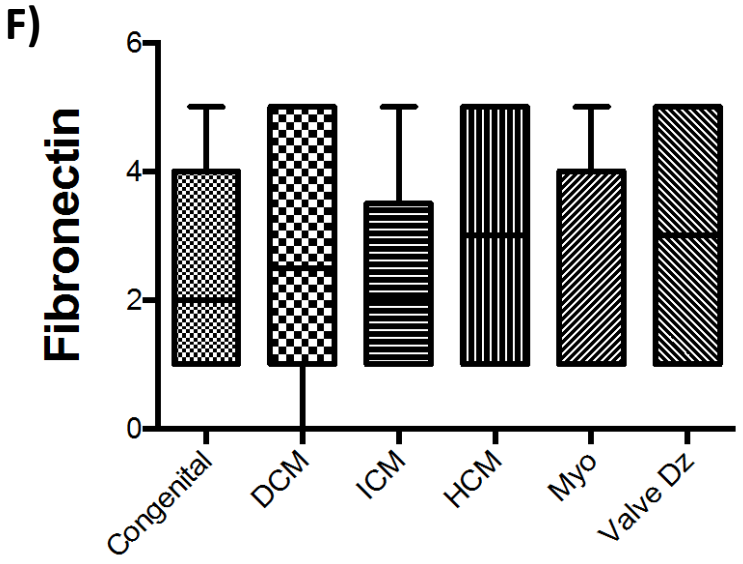
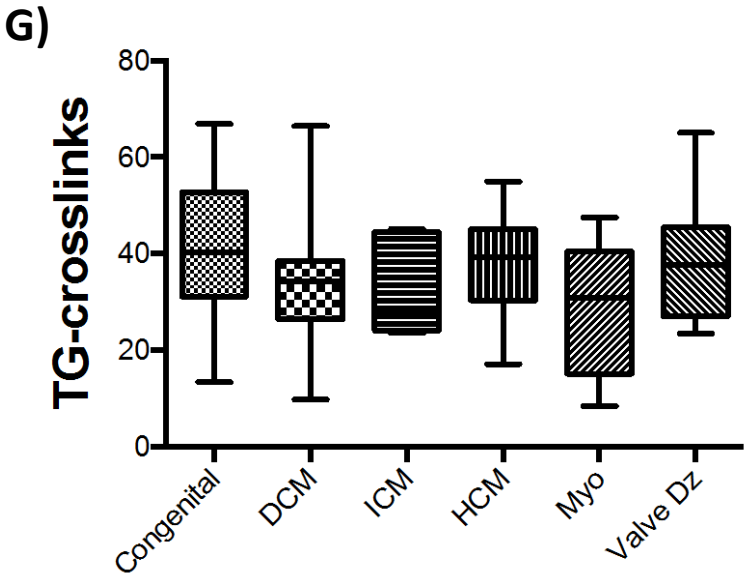
Supplemental Figure V



Supplemental Figure V







Supplemental Figure V. Results from human coronary artery TMA. **A,** Significant fibrosis was present in samples and %Fibrosis (scored using the Aperio software) did not correlate with age or **B,** disease; **C,** Plaque was present in all the samples; plaque size was scored by a pathologist at the Johns Hopkins University School of Medicine and did not correlate with age or **D,** disease; **E,** TG2; **F,** Fibronectin; and **G**, TG-crosslinks were highly expressed in the vascular media and did not correlate with disease.