SUPPLEMENTAL MATERIAL

EXPANDED METHODS

Artery Procurement

Human epicardial coronary arteries were procured from explanted hearts in the operating room. The vessels were exposed to minimal warm ischemia by the use of intra-arterial and/or topical perfusion with ice-cold saline. Cold ischemic times required for coronary artery excision after rapid cardiectomy were <15 min. The right coronary artery from ostium to crux was used for organ culture experiments since this vessel segment tapers minimally in its course and yielded relatively uniform size artery rings. Variability of clinical specimens was taken into account by the use of multiple replicates, randomly distributed between experimental groups, as well as by the use of multiple donors. The left coronary artery and the root, ascending, and descending segments of the thoracic aorta were minced for explant outgrowth of SMC.

Cell Culture

Isolated cells were serially cultured and expanded using M199 supplemented with 20% FBS (Invitrogen) for SMC and venous EC, EGM-2-MV with 5% FBS for arterial EC, and DMEM with 10% FBS for fibroblasts. Venous EC cultures were also supplemented with 50 μg/mL ECGS (BD Biosciences) and 100 μg/mL heparin (Sigma-Aldrich). The cells were used for experiments after 2-3 passages.

Histology and Immunohistochemistry

Artery segments were embedded in OCT compound and 5 μm-thick transverse sections were stained with hematoxylin and eosin or elastin-Van Gieson using standard techniques to identify the vascular layers. Immunolabeling was performed with the following primary antibodies: mouse anti-human LAP-β1 (clone 27235, R&D Systems) and rabbit anti-pan TGF-β (mixture of human TGF-β1, porcine TGF-β1.2, porcine TGF-β2, and amphibian TGF-β5, cat. # AB-100-NA, R&D Systems). Isotype-matched, irrelevant IgG were used as negative controls. Binding of secondary antibodies (Jackson ImmunoResearch, West Grove, PA) was detected with vectastain ABC reagent and AEC peroxidase substrate kits (Vector Laboratories, Burlingame, CA). The sections were counter-stained with hematoxylin (Sigma-Aldrich). Images were acquired using an Axioskop2 plus microscope (Carl Zeiss MicroImaging, Thornwood, NY).

Immunofluorescence Microscopy

Immunolabeling of 5 μm-thick, OCT-embedded artery sections was performed with mouse anti-human LAP-β1 (clone 27235, R&D Systems), rabbit anti–human CD45 (Santa Cruz Biotechnology), rabbit anti-human CD3 (DAKO), rabbit anti-human α-smooth muscle actin (Abcam), and goat anti-human CD31 (Santa Cruz Biotechnology). Detection of primary antibodies was visualized with Alexa Fluor 594-conjugated donkey anti-mouse IgG, Alexa Fluor 488-conjugated donkey anti-goat IgG, and Alexa Fluor 488-conjugated donkey anti-rabbit IgG (Invitrogen). The specimens were mounted with ProLong Gold antifade reagent with DAPI for nuclei labeling (Invitrogen). Images were acquired using an Axiovert 200M microscopy system and processed using AxioVision 4.6 software (Carl Zeiss MicroImaging).

Apoptosis Assay and Flow Cytometry

SMC were pretreated with IFN-γ (Invitrogen) at 50 ng/mL for 72 hr, washed, and then treated with IL-11 (R&D Systems) at 5 ng/mL for 24 hr. To augment apoptosis, the cells were treated with multimeric, recombinant TRAIL (PeproTech) at 40 ng/mL for 24 hr. To evaluate apoptosis, adherent and floating SMC were collected and labeled using a buffer solution and PEconjugated annexin V (BD Pharmingen) according to the manufacturer's instructions. Analysis was performed using a LSR II (BD Biosciences).

ELISA

Cytokine levels in culture supernatants and artery/cell lysates were determined using sandwich ELISA Duoset kits (R&D Systems) for TGF-β1, IL-6, IL-8, IL-10, IL-11, and IFN-γ according to the manufacturer's instructions. Cell lysates were prepared after washing in PBS, adding RIPA lysis buffer (Sigma-Aldrich) and detaching the cells with a scraper, centrifuging the mixture at 14,000 g for 15 min at 4 °C, and the supernatant was removed for analysis. Lysates were prepared from coronary artery rings by crushing the specimens on dry ice, adding RIPA lysis buffer, briefly sonicating the sample followed by overnight agitation at 4 °C on a rocker, centrifuging at 14,000 g for 15 min at 4 °C, and the supernatant was removed for analysis.

Immunoblotting

Protein was extracted from cells using RIPA lysis buffer with a protease inhibitor cocktail (Thermo Scientific, Rockford, IL) and boiled in SDS sample buffer for 5 min. Equal amounts of protein per sample were separated by SDS-polyacrylamide gel electrophoresis, transferred electrophoretically to a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA), and blotted with rabbit anti-human TGF-β1 (clone 56E4, Cell Signaling Technology, Beverly, MA), rabbit anti-human Ser465/467 phospho-SMAD2 (cat. #3101, Cell Signaling Technology), mouse anti-human SMAD2 (clone L16D3, Cell Signaling Technology), or mouse anti-β-actin (clone AC-15, Sigma-Aldrich), followed by horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch). Bound antibody was detected with Western Lightning Plus-ECL (Perkin Elmer, Waltham, MA).

Real-time Quantitative RT-PCR

Serial sections of OCT-embedded arteries or centrifuged cell pellets were immersed in RLT lysis buffer (QIAGEN, Valencia, CA), vigorously vortexed, and total RNA was isolated using RNeasy Mini kits (QIAGEN) according to the manufacturer's protocol. RT with random hexamer and oligo-dT primers was performed according to the Multiscribe RT system protocol (Applied Biosystems, Foster City, CA). RT-PCR reactions were prepared with TaqMan PCR Master Mix, predeveloped assay reagents, and probes (Applied Biosystems). Samples were analyzed on an iCycler (Bio-Rad Laboratories). RNA samples processed without the RT enzyme were used as negative controls.

Microarray Analysis

SMC were cultured from 3 different thoracic aortic segments of 4 donors, deprived of serum in M199 medium for 48 hr, and remained untreated (n=12) or were treated with TGF-β (n=12) at 1 ng/mL for 6 hr. Preparation of sscDNA and hybridization to the human genechip set (1.0 ST Array) were performed according to the manufacturer's protocol (Affymetrix). The stained chips were read and analyzed with GeneChip Scanner 3000 (Affymetrix). Expression intensities were quantified and compared using Partek Genomics Suite v6.4 (Partek Inc., St. Louis, MO) with Robust Multi-Array Average for normalization and fold-change calculations. For the purposes of this study, genes that were regulated by TGF-β more than 2-fold relative to untreated controls with P values <0.05 by t test, after filtering for False Discovery Rate to correct for multiple comparisons, were considered significant. The primary microarray data have been deposited in the Gene Expression Omnibus under accession no. GSE30004.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. LAP expression by intrinsic vascular cells and infiltrating leukocytes. Human coronary arteries were analyzed by immunofluorescence analysis using antibodies to LAP (red color) and (*A*) the EC marker CD31 (green color), (*B*) the SMC marker smooth muscle α-actin (SMA) (green color), (*C*) the pan-leukocyte marker CD45 (green color), or (*D*) the T cell marker CD3. Examples of double labeled cells in the merged panels are marked with arrows (bar=30 μm).

Supplemental Figure 2. Altered cytokine production after artery procurement and *ex vivo* culture. Human coronary arteries were divided into 3 mm rings and either immediately frozen (0 hr) or cultured in serum-free medium for 72 hr. (*A*) Total TGF-β1 and (*B*) IL-6 levels of artery lysates were measured by ELISA (*n*=5) at 72 hr. $^{*}P<0.05$, 72 hr vs. 0 hr, t-test.

Supplemental Figure 3. Arterial expression of TGF-β is not modulated by treatment with pro-inflammatory cytokines. Human coronary arteries were divided into 3 mm rings and either immediately frozen (0 hr) or cultured in serum-free medium and treated with IL-1α at 10 ng/mL, TNF-α at 10 ng/mL, IFN-γ at 30 ng/mL, or IL-17 at 30 ng/mL. (*A*) Total TGF-β1 and (*B*) IL-6 levels were measured by ELISA (*n*=6) at 72 hr. $^{+}P<0.05$, vs. 0 hr, one-way ANOVA.

Supplemental Figure 4. SMC differentiation markers are decreased after artery procurement and *ex vivo* culture. Human coronary arteries were divided into 3 mm rings and either immediately frozen (0 hr) for which treatment was not applicable (NA) or cultured in serum-free medium and treated or not with TGF-β1 at 10 ng/mL for 6-24 hr. (*A*) Smooth muscle myosin heavy chain 11 (MYH11) and (*B*) smooth muscle α-actin (SMA) transcript expression (normalized to GAPDH) were measured by quantitative RT-PCR (*n*=2-4). $^{+}P<0.05$, Cultured vs. Not cultured (0 hr), one-way ANOVA.

Supplemental Figure 5. TGF-β expression in cultured cells. Arterial EC from multiple donors were cultured in serum-free medium and treated or not with PMA/ionomycin for 6 hr. Total TGF-β1 and β-actin of cell lysates was determined by western blotting and the relative protein expression was assessed by densitometry (*n*=3); **P*<0.05, PMA/ionomycin vs. Untreated, ANOVA.

Supplemental Figure 6. TGF-β responses in cultured cells. (*A*) Arterial EC, SMC, and fibroblast were treated with TGF-β1 at the concentrations indicated for 30 min under serum-free conditions and phospho-SMAD2 and β-actin expression were assessed by western blotting.

Supplemental Figure 7. TGF-β induces IL-11 production in cultured arteries and correlates with IL-11 expression in non-diseased and arteriosclerotic arteries. (*A*) Human coronary arteries were divided into 3 mm rings and either immediately frozen (0 hr) for which treatment was not applicable (NA) or cultured in serum-free medium and treated or not with TGF-β1 at 10 ng/mL for 6-18 hr. IL-11 transcript expression (normalized to GAPDH) was measured by quantitative RT-PCR (*n*=2-4). $^{+}P<0.05$, Cultured vs. Not cultured (0 hr), one-way ANOVA and **P*<0.05, TGF-β1-treated vs. Untreated, one-way ANOVA. (*B*) Quantitative RT-PCR analysis of IL-11 transcripts normalized to GAPDH in non-arteriosclerotic (*n*=4 donors) and arteriosclerotic (*n*=9 donors) coronary arteries.

SUPPLEMENTAL TABLES

Supplemental Table I. Clinical characteristics of individuals with non-arteriosclerotic and arteriosclerotic coronary arteries*

Characteristic	Non-arteriosclerotic	Arteriosclerotic	*P* value
Age (yr)	44.0±8.4	57.4±5.0	0.1755
Gender (# male:female)	2:2	6:3	1.0000
Race (# Caucasian:other)	2:2	7:2	0.2028
Operation (# donor:recipient)	2:2	4:5	1.0000

*Human coronary arteries were procured at donor and recipient cardiac transplantation operations from 13 individuals and classified as non-arteriosclerotic or arteriosclerotic based on macroscopic characteristics. The age, gender, race, and type of operation were compared by t test or Fisher's exact test.

Supplemental Table II. TGF-β-inducible genes in cultured SMC*

Transcript ID	Gene Assignment	Gene Symbol	Fold-Change (T	*P* -value (TGF-β-	FDR-adj *P* -valu	TGF-β-treated (I	Untreated (Mea
8156919	NM_001018116 // MURC // muscle-related coiled-coil protein // 9q31.1 // 347273 /	**MURC**	**4.72**	0.000000	0.000000	**369.2**	**78.3**
8109159	NR_027180 // LOC728264 // hypothetical LOC728264 // 5q32 // 728264	**LOC728264**	**4.37**	0.000034	0.000049	**521.7**	**119.4**
7918857	NM_005725 // TSPAN2 // tetraspanin 2 // 1p13.2 // 10100 /// ENST00000369516 // T	**TSPAN2**	**3.88**	0.000000	0.000000	**1538.2**	**396.4**
7950933	NR_026571 // NOX4 // NADPH oxidase 4 // 11q14.2-q21 // 50507 /// NM_016931 // N	**NOX4**	**3.88**	0.000001	0.000002	**370.3**	**95.5**
8039484	NM_000641 // IL11 // interleukin 11 // 19q13.3-q13.4 // 3589 /// ENST00000264563	**IL11**	**3.50**	0.000391	0.000415	**2503.6**	**715.7**
8095043	NM_023940 // RASL11B // RAS-like, family 11, member B // 4q12 // 65997 /// ENST0	**RASL11B**	**3.07**	0.000123	0.000145	**237.7**	**77.4**
8029693	NM_006732 // FOSB // FBJ murine osteosarcoma viral oncogene homolog B // 19q1:	**FOSB**	**3.06**	0.000000	0.000000	**512.3**	**167.6**
8004691	NM_203411 // TMEM88 // transmembrane protein 88 // 17p13.1 // 92162 /// ENST00(	**TMEM88**	**3.06**	0.000006	0.000009	**1377.8**	**450.8**
8114805	NM_000800 // FGF1 // fibroblast growth factor 1 (acidic) // 5q31 // 2246 /// NM_	**FGF1**	**3.00**	0.000002	0.000003	**346.2**	**115.6**
8175256	NR_024607 // MGC16121 // hypothetical protein MGC16121 // Xq26.3 // 84848	**MGC16121**	**2.98**	0.000000	0.000001	**727.8**	**243.9**
7942064	NM_015973 // GAL // galanin prepropeptide // 11q13.3 // 51083 /// ENST0000026564	**GAL**	**2.98**	0.000128	0.000147	**1000.7**	**335.9**
8123246	NM_021977 // SLC22A3 // solute carrier family 22 (extraneuronal monoamine transp	**SLC22A3**	**2.97**	0.000105	0.000127	**885.6**	**297.8**
7951284	NM_002422 // MMP3 // matrix metallopeptidase 3 (stromelysin 1, progelatinase) //	**MMP3**	**2.96**	0.000130	0.000147	**298.4**	**100.9**
8063382	NM_005985 // SNAI1 // snail homolog 1 (Drosophila) // 20q13.1-q13.2 // 6615 ///	**SNAI1**	**2.91**	0.000001	0.000001	**2702.2**	**927.2**
8177222	NM_013230 // CD24 // CD24 molecule // 6q21 // 100133941 /// ENST00000382840 //	**CD24**	**2.91**	0.000238	0.000258	**197.1**	**67.7**
8038890	NM_001523 // HAS1 // hyaluronan synthase 1 // 19q13.4 // 3036 /// ENST000002221	**HAS1**	**2.85**	0.000022	0.000033	**433.5**	**152.3**
7984802	NM_001130136 // ISLR2 // immunoglobulin superfamily containing leucine-rich repe	**ISLR2**	**2.80**	0.000001	0.000002	**284.4**	**101.5**
7924977	NM_024554 // PGBD5 // piggyBac transposable element derived 5 // 1q42.13 // 7960	**PGBD5**	**2.78**	0.000000	0.000000	**327.1**	**117.7**
7968556	NM_004795 // KL // klotho // 13q12 // 9365 /// ENST00000380099 // KL // klotho /	**KL**	**2.68**	0.000000	0.000001	**333.3**	**124.6**
7989750	NM_003613 // CILP // cartilage intermediate layer protein, nucleotide pyrophosph	**CILP**	**2.66**	0.000002	0.000004	**209.4**	**78.7**
7908312	NM_005807 // PRG4 // proteoglycan 4 // 1q25-q31 // 10216 /// NM_001127708 // PR(	**PRG4**	**2.65**	0.000010	0.000015	**2602.9**	**983.5**
8114572	NM_001945 // HBEGF // heparin-binding EGF-like growth factor // 5q23 // 1839 ///	**HBEGF**	**2.64**	0.000000	0.000000	**6299.0**	**2381.7**
8004671	NM_001080424 // KDM6B // lysine (K)-specific demethylase 6B // 17p13.1 // 23135	**KDM6B**	**2.54**	0.000000	0.000000	**1808.2**	**711.4**
8024485	NM_015675 // GADD45B // growth arrest and DNA-damage-inducible, beta // 19p13.	**GADD45B**	**2.53**	0.000000	0.000000	**931.9**	**367.9**
8112045	NM_007036 // ESM1 // endothelial cell-specific molecule 1 // 5q11.2 // 11082 ///	**ESM1**	**2.52**	0.001844	0.001880	**325.8**	**129.1**
8067233	NM_020182 // PMEPA1 // prostate transmembrane protein, androgen induced 1 // 20	**PMEPA1**	**2.51**	0.000000	0.000000	**1614.6**	**642.9**
8070665	NM_173354 // SIK1 // salt-inducible kinase 1 // 21q22.3 // 150094 /// ENST000002	**SIK1**	**2.51**	0.000000	0.000000	**805.7**	**321.6**
7930482	NM_000681 // ADRA2A // adrenergic, alpha-2A-, receptor // 10q24-q26 // 150 /// A	**ADRA2A**	**2.50**	0.000048	0.000065	**756.9**	**302.6**
8077499	AF086709 // LOH3CR2A // loss of heterozygosity, 3, chromosomal region 2, gene A	**LOH3CR2A**	**2.50**	0.000001	0.000001	**166.4**	**66.6**
8129562	NM_001901 // CTGF // connective tissue growth factor // 6q23.1 // 1490 /// ENST0	**CTGF**	**2.49**	0.000104	0.000127	**8171.6**	**3283.2**
8004360	NM_001002914 // KCTD11 // potassium channel tetramerisation domain containing 1	**KCTD11**	**2.44**	0.000000	0.000000	**1313.9**	**538.7**
7962000	NM_198965 // PTHLH // parathyroid hormone-like hormone // 12p12.1-p11.2 // 5744	**PTHLH**	**2.43**	0.000077	0.000100	**468.3**	**192.8**
7951259	NM_002425 // MMP10 // matrix metallopeptidase 10 (stromelysin 2) // 11q22.3 // 4	**MMP10**	**2.40**	0.000194	0.000215	**328.0**	**136.9**
8092839	NM_001135057 // LRRC15 // leucine rich repeat containing 15 // 3q29 // 131578 //	**LRRC15**	**2.39**	0.000000	0.000001	**910.0**	**380.9**
8077490	NM_014583 // LMCD1 // LIM and cysteine-rich domains 1 // 3p26-p24 // 29995 /// E	**LMCD1**	**2.38**	0.000000	0.000000	**2143.7**	**900.3**
7962375	NM_153026 // PRICKLE1 // prickle homolog 1 (Drosophila) // 12q12 // 144165 /// N	**PRICKLE1**	**2.34**	0.000051	0.000068	**1471.2**	**628.7**
8133360	NM_001305 // CLDN4 // claudin 4 // 7q11.23 // 1364 /// ENST00000435050 // CLDN4	**CLDN4**	**2.34**	0.000000	0.000000	**490.6**	**209.7**
8121257	NM_001198 // PRDM1 // PR domain containing 1, with ZNF domain // 6q21-q22.1 // 6	**PRDM1**	**2.34**	0.000002	0.000004	**373.2**	**159.6**
8063634	ENST00000419204 // MGC4294 // hypothetical protein MGC4294 // 20q13.32 // 7916	**MGC4294**	**2.33**	0.000002	0.000003	**480.4**	**205.9**
8123936	NM_001142393 // NEDD9 // neural precursor cell expressed, developmentally down-	**NEDD9**	**2.29**	0.000002	0.000004	**326.1**	**142.1**
8026047	NM_002229 // JUNB // jun B proto-oncogene // 19p13.2 // 3726 /// ENST0000030275	**JUNB**	**2.28**	0.000000	0.000000	**3551.0**	**1559.6**
8021169	NM_006033 // LIPG // lipase, endothelial // 18q21.1 // 9388 /// ENST00000261292	**LIPG**	**2.26**	0.000088	0.000112	**402.7**	**178.2**
8092707	NM_018192 // LEPREL1 // leprecan-like 1 // 3q28 // 55214 /// NM_001134418 // LEP	**LEPREL1**	**2.26**	0.000006	0.000010	**192.7**	**85.3**
8104663	NM_004932 // CDH6 // cadherin 6, type 2, K-cadherin (fetal kidney) // 5p15.1-p14	**CDH6**	**2.23**	0.004330	0.004330	**925.1**	**415.7**
8122734	NM_030949 // PPP1R14C // protein phosphatase 1, regulatory (inhibitor) subunit 1	**PPP1R14C**	**2.19**	0.000038	0.000053	**799.6**	**365.7**
8041206	NM_030915 // LBH // limb bud and heart development homolog (mouse) // 2p23.1 //	**LBH**	**2.11**	0.000000	0.000001	**1353.8**	**640.3**
7929145	NM_182765 // HECTD2 // HECT domain containing 2 // 10q23.32 // 143279 /// NM_1	**HECTD2**	**2.11**	0.000003	0.000005	**829.9**	**393.3**
8023995	NM_005860 // FSTL3 // follistatin-like 3 (secreted glycoprotein) // 19p13 // 102	**FSTL3**	**2.06**	0.000000	0.000000	**2331.3**	**1133.8**
8083876	NM_005414 // SKIL // SKI-like oncogene // 3q26 // 6498 /// NM_001145098 // SKIL	**SKIL**	**2.03**	0.000000	0.000000	**2732.5**	**1345.2**
7979710	NM_016445 // PLEK2 // pleckstrin 2 // 14q23.3 // 26499 /// ENST00000216446 // PL	**PLEK2**	**2.01**	0.000000	0.000000	**948.9**	**472.0**
8112202	NM_006622 // PLK2 // polo-like kinase 2 (Drosophila) // 5q12.1-q13.2 // 10769 //	**PLK2**	**-2.12**	0.001467	0.001525	**436.9**	**924.9**
8129937	NM_006079 // CITED2 // Cbp/p300-interacting transactivator, with Glu/Asp-rich ca	**CITED2**	**-2.27**	0.000004	0.000006	**1141.8**	**2588.9**

*Microarray analysis of SMC ± TGF-β1 at 1 ng/mL x6 hr (*n* =12) in serum-free medium. Mean, fold-change (> or <2x), *P* value (<0.05 t test) after false discovery rate (FDR) adjustment were calculated.

MC-TGF	MC-TGF	MC-TGF	MC-TGF	MC-TGF	MC-TGF	MC-TGF	MC-TGF	MC-TGF	MC-TGF	MC-TGF	MC-TGF	SMC-Ur	SMC-Ur	SMC-Ur	SMC-Ur	SMC-Ur	SMC-Ur	SMC-Ur	SMC-Ur	SMC-Ur	SMC-Ur	SMC-Ur	SMC-Ur
220	227	922	529	254	461	851	656	154	272	222	431	174	87	96	59	66	75	49	65	74	60	91	95
616	845	639	332	778	1174	906	260	267	890	364	198	469	186	95	60	230	201	216	72	52	196	57	40
2027	1125	2232	1644	1615	1509	2225	2052	1158	1036	1072	1466	1656	382	567	337	365	376	247	227	481	277	387	315
476	336	462	421	248	186	656	460	214	382	507	371	419	98	67	103	60	67	73	57	87	172	95	86
2959	4243	1692	1751	3121	2614	2151	1241	5593	5346	1672	1498	2607	1564	408	536	559	376	347	236	3239	2095	585	297
581	266	110	189	269	234	363	292	148	460	73	310	359	64	65	79	72	52	66	64	71	75	59	78
316	509	728	541	429	357	716	495	482	542	638	568	270	143	168	173	153	127	167	145	203	144	182	175
918	1609	1519	987	2105	1421	1527	701	2802	1956	1226	982	795	422	464	300	625	413	385	273	1054	501	365	288
380	314	297	425	214	376	294	213	360	608	304	588	397	98	76	116	68	89	112	87	95	133	116	188
772	531	1389	716	463	758	1041	557	529	885	874	649	648	168	314	236	205	266	243	205	196	252	242	168
768	655	1137	1088	2395	942	1940	741	1187	748	732	769	709	197	364	251	1322	166	384	284	642	200	247	211
330	936	1173	705	1492	1145	1413	527	1604	1062	643	653	310	376	281	164	625	483	313	151	667	383	143	173
346	466	236	154	163	109	326	147	273	698	1169	450	306	121	111	68	76	45	66	71	80	135	338	66
2124	3552	2265	2170	2629	3220	2471	2186	3997	3667	2453	2486	1761	1475	577	690	732	692	618	433	2165	1681	934	848
103	203	114	125	268	78	248	149	290	666	154	504	77	77	34	56	32	52	25	45	50	225	96	419
175	418	604	334	538	577	741	220	969	736	354	234	146	134	175	120	134	143	157	129	322	195	129	120
119	246	400	294	280	410	583	385	259	362	152	220	119	78	123	87	88	160	101	91	143	113	85	69
244	313	469	323	338	205	348	274	411	363	384	342	235	120	126	100	111	91	107	80	156	107	144	96
197	272	441	422	646	626	588	468	268	208	136	222	192	113	136	122	259	157	112	101	134	95	73	92
200	151	135	207	364	167	382	422	244	183	80	240	141	58	70	62	112	69	92	109	77	66	65	61
2954	1884	3980	3969	3519	4382	4471	2241	1994	1394	1848	1388	2828	694	1818	1186	1473	1992	1539	761	640	324	689	394
2791	6278	7975	6350	6145	5089	7725	5444	8008	8493	7354	6683	2301	2550	3027	2151	2176	1620	1637	1129	4829	3820	3399	2137
1389	1846	1720	1772	2320	1664	2102	1526	2710	2105	1465	1510	1243	682	655	593	887	554	556	508	1308	667	669	630
880	716	1050	908	991	876	1250	977	1025	764	828	1038	712	301	368	307	351	300	334	327	486	392	368	314
388	884	195	885	119	264	408	155	263	765	243	247	339	550	84	583	63	70	46	54	93	276	77	112
1543	1547	1408	1583	1716	1521	2206	1714	1823	2014	1074	1517	1380	624	496	479	605	597	617	527	856	779	521	597
437	778	849	591	826	560	988	854	1492	1173	748	858	345	295	336	230	296	220	297	281	627	443	312	329
1093	529	717	710	1032	942	1436	1600	683	425	323	573	1021	326	230	239	207	499	191	433	337	185	210	288
169	210	189	146	137	226	179	116	126	277	144	143	158	84	71	63	42	60	36	42	77	109	57	71
8190	9674	9088	8559	5288	8843	9324	9029	3778	12153	8431	9437	7581	5898	3355	3081	1347	3139	2616	2076	1419	8775	3945	3012
1014	1362	1312	1050	1176	1584	1826	1164	1315	1911	1227	1139	850	592	538	447	500	550	427	393	647	593	519	537
387	505	267	902	310	620	306	299	454	669	823	537	372	135	139	439	140	204	188	149	190	151	334	113
578	331	289	335	267	126	313	219	299	347	844	413	569	142	99	132	132	71	84	101	121	106	227	179
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261	370	645	489	461	588	706	541	486	426	591	502	227	187	254	190	140	204	259	213	270	182	237	194
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244	385	320	313	190	285	442	343	253	598	294	421	222	184	119	118	106	120	113	97	154	281	130	149
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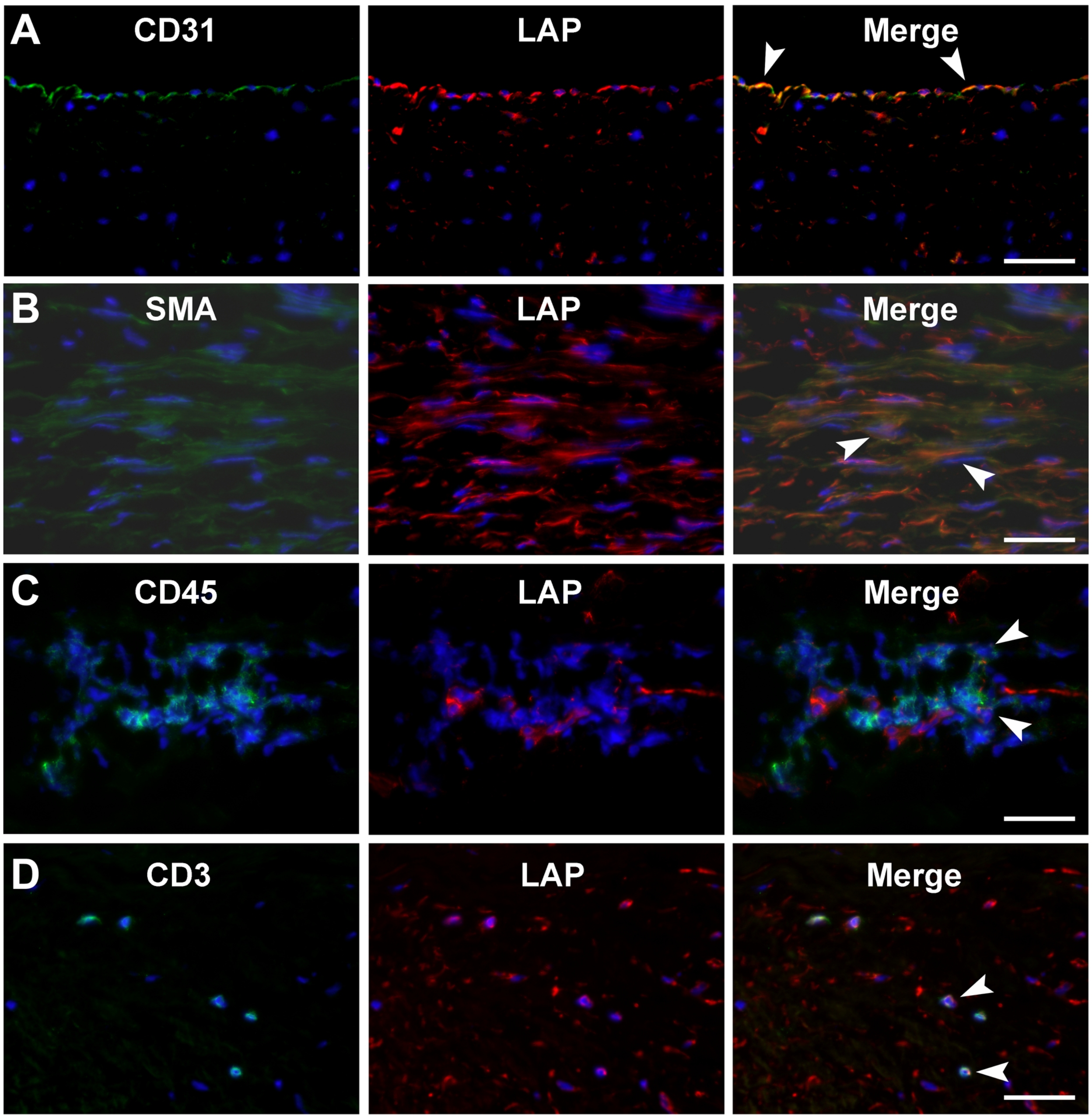
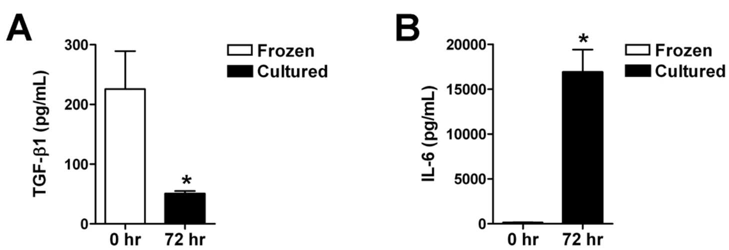
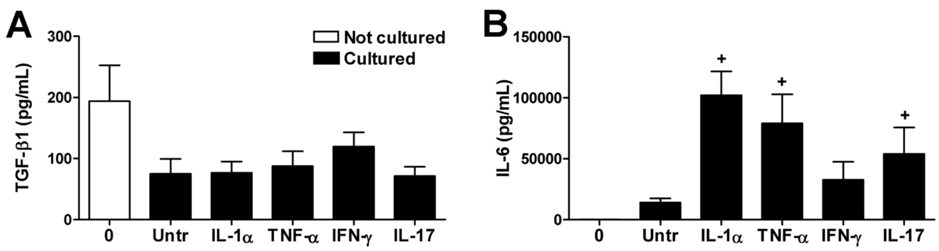
Supplemental Figure 1



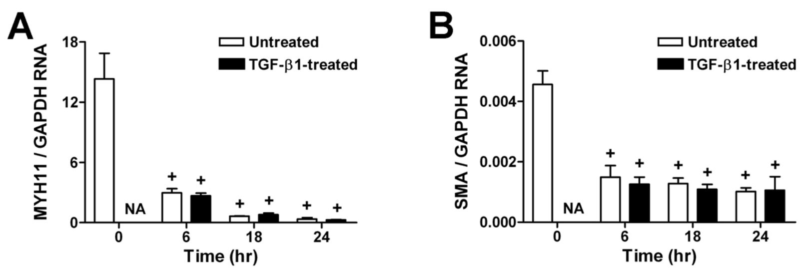
Supplemental Figure 2



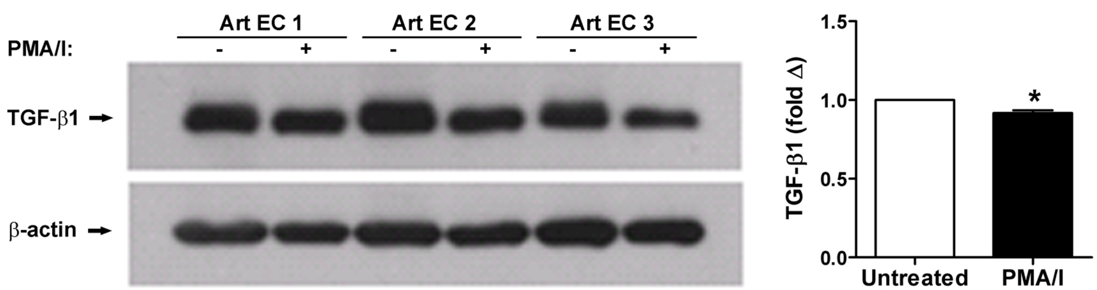
Supplemental Figure 3



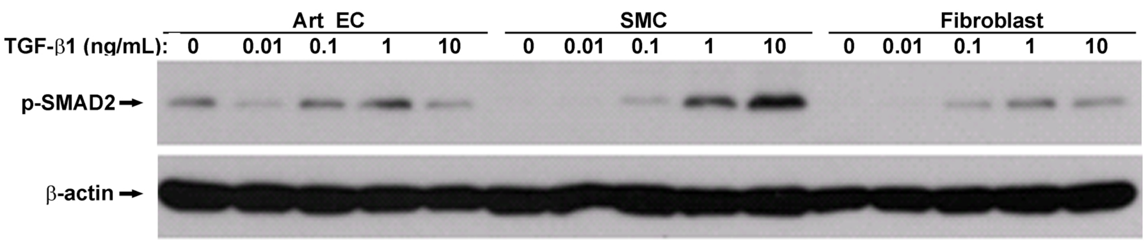
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7