

Partial carotid ligation and flow pattern validation by high resolution ultrasound

All animal studies were performed with Male C57Bl/6 mice according to the approved IACUC protocol by Emory University. Mice (Jackson Laboratories) were partially ligated between 6 to 8 weeks of age as we recently described¹. Briefly, three of four caudal branches of left common carotid artery (LCA) - left external carotid, internal carotid, and occipital artery - were ligated with 6-0 silk suture while the superior thyroid artery was left intact in anesthetized mice. Six hours post-surgery, each animal was examined by VEVO 770 High-resolution *in vivo* micro-imaging ultrasound system whether the ligation induced low and oscillatory shear stress in LCA with the contralateral RCA as a control¹.

Intimal RNA isolation from carotid arteries

Total RNA from intima were separately obtained from LCA and RCA at 12, 24 and 48 hr post-ligation as we described previously¹. Briefly, LCA and RCA were quickly flushed (few seconds) with 150 μ l of QIAzol lysis reagent (QIAGEN) using 29G insulin syringe into a microfuge tube. The eluate was then used for total intimal RNA isolation using miRNeasy mini kit (QIAGEN).

Microarray Procedures

Total intimal RNAs were obtained from LCA and RCA at 12hr and 48hr post-ligation. Intimal RNAs from three LCAs or RCAs were pooled to obtain ~30 ng total RNA. All RNA samples used for the microarray study passed a quality control test using Agilent BioAnalyze NanoChip. Each sample was linearly amplified by WT-Ovation RNA amplification system (NuGEN) and used for the microarray study using MouseWG-6 v2 Expression BeadChip array with 45,281 probes (Illumina) at the Emory Biomarker Service Center. After hybridization, BeadChips are scanned on the Illumina BeadArray Reader to determine the probe fluorescence intensity. The raw probe intensities were then normalized by the quantile normalization algorithm² using the GenomeStudio software from Illumina.

Microarray Data Analysis and Bioinformatics

The microarray data was statistically analyzed by Significance Analysis of Microarrays software (SAM)³. The differentially expressed genes between LCA and RCA were identified for those which showed more than 1.5 fold-changes at <10% false discovery rate. The lists of differentially expressed genes were interrogated for statistically significant overrepresented cellular functions and

disorders using DAVID analysis and Ingenuity Pathway (IPA) Analysis (Ingenuity Systems).

Quantitative real time PCR (qPCR) validation

Total RNA of each sample was reverse transcribed into cDNA using SuperScript III and random primers (Invitrogen) as we described¹. Briefly, qPCR was performed on selected genes using Brilliant II SYBR Green QPCR Master Mix (Stratagene) with custom designed primers on a Real-Time PCR System (ABI StepOne Plus). Predesigned TaqMan Gene Expression Assay probes (Applied Biosystems) were also used for some selected genes. All qPCR results were normalized based on 18S RNA expression in each sample. Fold changes between LCA and RCA were determined using the $\Delta\Delta C_t$ method⁴.

Immunohistochemical staining

Paraffin section immunostaining – Mice were euthanized by CO₂ inhalation and then were pressure-perfused at 100 mmHg with normal saline followed by pressure fixation with a 10% formalin solution. LCA and RCA were collected *en block* with the trachea and esophagus. Paraffin sections (5 μ m) were then microwaved for 20 min in citrate buffer (0.1 M, pH 6.0) for BMP4 and LMO4 staining or in Tris buffer (0.1 M, pH 9.0) for Angpt2 and Jam2 staining. Sections were blocked with 10% donkey serum for 1 hour at room temperature and incubated with primary antibodies specific to BMP4 (5 μ g/ml, Biovision), Lmo4 (5 μ g/ml,⁵⁻⁷), Jam2 (2 μ g/ml, R&D System), and Angpt2 (0.4 μ g/ml, Santa Cruz) overnight at 4°C in a humidified chamber⁸. To visualize primary antibodies, rhodamine-conjugated secondary antibodies (donkey anti-goat, anti-rat IgG, Jackson) were used for one hour at room temperature. Nuclei were counter stained with Hoechst #33258. All photographs were taken using a Zeiss epifluorescent microscope. Paraffin sections of human coronary arteries from patients undergoing heart transplants were obtained with the patients' consent according to the IRB protocol approved at Emory as described previously⁸. The same staining method used for mouse carotids as described above was used for Lmo4 staining.

En Face staining - Mice were euthanized by CO₂ inhalation and the aortas were pressure-perfused at 100 mmHg with normal saline followed by pressure fixation with a 10% formalin solution. The aortas were carefully dissected *in situ* and the aortic arches and thoracic aortas were dissected and stained with Lmo4 antibody⁵⁻⁷, followed by rhodamine-conjugated secondary antibodies for 2 hours at room temperature. The aortas were then mounted on glass slides using Vectashield containing DAPI (Vector Laboratories). They were then opened and lesser curvature and the greater curvature of the arch were separated. *En face* images were obtained using a Zeiss LSM 510 META confocal microscope.

Ex vivo tissue culture

Mice were euthanized by CO₂ inhalation and then pressure-perfused with heparinized normal saline. Under sterile conditions, common carotid arteries were harvested and carefully cleaned of perivascular fat. Carotid artery rings (~3 mm) and incubated for 3 to 5 days at 37 °C and 5% CO₂ in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 100 U/mL of penicillin and 100 µg/mL of streptomycin and 10% of heat-inactivated fetal bovine serum.

Statistical analysis

Data are presented as mean±SEM. Paired Student's t-test was carried out for all qPCR results of each gene to compare LCA vs. RCA and $p < 0.05$ ($n = 3-5$) was considered statistically significant.

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Table S.1 The list of gene probes identified as significant expressed in microarray

12hr Down-regulated gene probes (LCA/RCA) (38)							12hr Up-regulated gene probes (LCA/RCA) (29)						
Gene ID	Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change	q-value(%)	Gene ID	Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change	q-value(%)
ILMN_1232928	<u>Timp3</u>	25.53	347.03	13.59	0.61	0.00	ILMN_1235698	<u>Bmp4</u>	-17.05	-285.04	16.72	1.54	0.00
ILMN_2604029	<u>Klf2</u>	15.76	204.23	12.96	0.30	0.00	ILMN_2593496	<u>Got2</u>	-16.37	-103.56	6.33	1.68	0.00
ILMN_2686883	<u>Gnaq</u>	13.56	112.33	8.28	0.66	0.00	ILMN_2909150	<u>Ctgf</u>	-13.70	-1531.93	111.85	4.81	0.00
ILMN_2697304	<u>Eln</u>	12.97	664.84	51.24	0.56	0.00	ILMN_2932964	<u>Ctsp</u>	-12.10	-190.35	15.73	2.03	0.00
ILMN_1235077	<u>Capn2</u>	12.97	113.98	8.79	0.55	0.00	ILMN_1239386	<u>GaInt2</u>	-12.01	-188.83	15.73	2.56	0.00
ILMN_2595664	<u>Dhh</u>	12.33	467.52	37.91	0.50	0.00	ILMN_2451036	<u>LOC100047093</u>	-11.79	-736.61	62.46	1.91	0.00
ILMN_2672190	<u>Id1</u>	12.05	131.11	10.88	0.59	0.00	ILMN_1244612	<u>GaInt2</u>	-11.75	-547.54	46.58	1.82	0.00
ILMN_2880906	<u>Pdlim2</u>	10.44	167.75	16.07	0.50	2.54	ILMN_2694569	<u>LOC631037</u>	-9.82	-156.24	15.90	1.59	9.02
ILMN_2750053	<u>Ptprj</u>	10.43	459.92	44.08	0.53	2.54	ILMN_1228475	<u>Ulk1</u>	-9.32	-176.19	18.91	1.64	9.02
ILMN_2424721	<u>Pdgfra</u>	9.84	183.84	18.68	0.54	4.78	ILMN_1236958	<u>Gabarapl1</u>	-9.29	-639.61	68.86	1.54	9.02
ILMN_2634083	<u>Cdkn1a</u>	9.17	164.57	17.95	0.54	7.17	ILMN_2642403	<u>Lmp4</u>	-8.85	-133.04	15.03	1.82	9.02
ILMN_2618408	<u>Icam2</u>	9.01	325.97	36.18	0.53	7.17	ILMN_1231490	<u>2410006H16Rik</u>	-8.50	-66.77	7.86	2.17	9.37
ILMN_2745876	<u>BC0200353</u>	8.54	228.48	26.75	0.60	7.17	ILMN_2595660	<u>Phactr1</u>	-8.46	-368.96	43.59	2.10	9.37
ILMN_1220170	<u>Tek</u>	8.09	271.04	33.50	0.64	7.17	ILMN_1213034	<u>2010312A17Rik</u>	-8.34	-60.24	7.22	1.52	9.37
ILMN_2498731	<u>E030024M20Rik</u>	8.01	200.79	25.05	0.48	7.17	ILMN_2741621	<u>Birc2</u>	-8.12	-48.14	5.93	1.79	9.37
ILMN_2950503	<u>Dab2ip</u>	7.94	82.96	10.45	0.66	7.17	ILMN_2790373	<u>Snn</u>	-7.89	-86.61	10.98	1.63	9.56
ILMN_2999439	<u>Klifa</u>	7.57	162.86	21.52	0.48	7.17	ILMN_1258158	<u>Aldh6a1</u>	-7.63	-89.06	11.67	1.76	9.56
ILMN_2675760	<u>2310046K01Rik</u>	7.52	181.95	24.20	0.46	7.17	ILMN_2888552	<u>Slc1a4</u>	-7.48	-106.66	14.25	1.90	9.56
ILMN_2976129	<u>Tinagl</u>	7.30	300.71	41.21	0.60	7.17	ILMN_2977558	<u>Dapk2</u>	-7.47	-145.95	19.53	4.43	9.56
ILMN_2773211	<u>Kras</u>	7.16	51.76	7.23	0.54	7.17	ILMN_2909336	<u>Gpm6a</u>	-7.42	-248.37	33.47	2.23	9.56
ILMN_2608133	<u>Rhpn2</u>	7.14	358.28	50.18	0.48	7.17	ILMN_1238215	<u>Ctgf</u>	-7.21	-716.25	99.35	6.13	9.56
ILMN_1216781	<u>Rab11fp5</u>	7.10	173.70	24.45	0.57	7.17	ILMN_2471996	<u>Al317223</u>	-7.12	-69.95	9.83	2.52	9.56
ILMN_2587084	<u>C230099C22Rik</u>	6.96	63.54	9.13	0.60	7.17	ILMN_1234487	<u>Angpt2</u>	-7.07	-58.39	8.26	1.97	9.56
ILMN_2866267	<u>F2r1</u>	6.88	128.94	18.75	0.56	7.17	ILMN_1213034	<u>Ier3</u>	-7.02	-447.96	63.85	1.65	9.56
ILMN_2789562	<u>P4ha2</u>	6.83	141.49	20.71	0.66	7.17	ILMN_1252481	<u>Fosl2</u>	-6.95	-303.04	43.62	1.79	9.56
ILMN_1247916	<u>Lims2</u>	6.73	96.99	14.40	0.51	7.17	ILMN_1215136	<u>Scn3b</u>	-6.74	-45.96	6.82	1.73	9.56
ILMN_1231445	<u>lnmt</u>	6.57	77.88	11.85	0.35	7.17	ILMN_1223313	<u>Fn3k</u>	-6.64	-51.35	7.73	1.63	9.56
ILMN_2683586	<u>Capn2</u>	6.49	130.67	20.13	0.63	7.45	ILMN_2711966	<u>Mrpl1</u>	-6.62	-62.59	9.46	1.87	9.56
ILMN_2710274	<u>Slc9a3r2</u>	6.41	572.81	89.38	0.48	7.45	ILMN_1230596	<u>E030033D05Rik</u>	-6.34	-240.87	38.00	1.64	10.00
ILMN_1229745	<u>Sertad4</u>	6.39	163.24	25.54	0.62	7.45							
ILMN_3140516	<u>Rapgef1</u>	6.32	103.56	16.39	0.58	7.45							
ILMN_2641228	<u>Hspa12b</u>	6.28	300.72	47.88	0.46	7.45							
ILMN_2738345	<u>Lims2</u>	5.76	81.77	14.20	0.65	9.70							
ILMN_2699637	<u>Lsr</u>	5.74	113.06	19.69	0.51	9.70							
ILMN_1243407	<u>Klkl10</u>	5.73	931.40	162.41	0.31	9.70							
ILMN_2689207	<u>Col6a3</u>	5.70	47.59	8.35	0.49	9.70							
ILMN_2774056	<u>Cmklr1</u>	5.59	34.09	6.10	0.60	9.70							
ILMN_1212703	<u>Kras</u>	5.57	181.54	32.58	0.50	9.70							

48hr Down-regulated gene probes (LCA/RCA) (338)							48hr Up-regulated gene probes (LCA/RCA) (250)						
Gene ID	Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change	q-value(%)	Gene ID	Gene Name	Score(d)	Numerator(r)	Denominator(s+s0)	Fold Change	q-value(%)
ILMN_2672190	<u>Id1</u>	48.65	122.48	2.52	0.45	0.00	ILMN_2743013	<u>Ncf4</u>	-52.17	-92.58	1.77	3.20	0.00
ILMN_2588249	<u>S3-12</u>	31.40	73.58	2.34	0.38	0.00	ILMN_2939681	<u>Lyzs</u>	-39.30	-135.27	3.44	3.10	0.00
ILMN_3113303	<u>Atp2b2</u>	29.64	181.01	6.11	0.32	0.00	ILMN_2878071	<u>Lyz</u>	-24.78	-302.22	12.20	3.08	0.00
ILMN_2619136	<u>Pthlh</u>	28.04	145.42	5.19	0.24	0.00	ILMN_2867147	<u>Tyrobp</u>	-24.36	-443.39	18.20	3.64	0.00
ILMN_2687547	<u>Sdpr</u>	22.68	568.94	25.08	0.52	2.91	ILMN_2935386	<u>63300548G22Rik</u>	-22.59	-112.80	4.99	1.82	3.14
ILMN_2765224	<u>Bcam</u>	22.64	569.06	25.13	0.23	2.91	ILMN_1245129	<u>Ifitm1</u>	-21.94	-43.43	1.98	2.14	3.14
ILMN_1237224	<u>Kctd12</u>	22.01	115.55	5.25	0.46	2.91	ILMN_2767918	<u>Ifi30</u>	-21.45	-28.37	1.32	1.61	3.14
ILMN_3037580	<u>Rbms2</u>	20.05	96.76	4.83	0.48	3.26	ILMN_3120652	<u>Smao2</u>	-20.12	-224.65	11.17	1.56	3.14
ILMN_1228233	<u>Gstm1</u>	19.53	66.42	3.40	0.55	3.26	ILMN_2511249	<u>scf0002785.1_4f</u>	-20.10	-49.58	2.47	1.64	3.14
ILMN_1240938	<u>AW212394</u>	19.07	279.74	14.67	0.48	3.26	ILMN_1223257	<u>Ccl4</u>	-19.59	-42.79	2.18	2.41	3.14
ILMN_2595664	<u>Dhh</u>	19.01	621.90	32.72	0.36	3.26	ILMN_2718801	<u>Fosl2</u>	-18.65	-30.83	1.65	1.71	3.40
ILMN_2728539	<u>Exd12</u>	17.83	30.51	1.71	0.65	3.26	ILMN_3009860	<u>Sell</u>	-18.51	-58.24	3.15	2.82	3.40
ILMN_2630182	<u>Syp</u>	16.62	42.12	2.53	0.48	3.26	ILMN_2712986	<u>Chl3l3</u>	-18.35	-191.36	10.43	6.37	3.40
ILMN_1222685	<u>1200016G03Rik</u>	16.40	91.80	5.60	0.46	6.00	ILMN_3127739	<u>Sf3b4</u>	-16.11	-82.72	5.13	1.75	7.51
ILMN_1245307	<u>Fbln2</u>	16.40	53.44	3.25	0.62	6.00	ILMN_2737713	<u>Efn1</u>	-15.56	-444.25	28.56	2.86	7.51
ILMN_2628567	<u>Phlda3</u>	15.95	22.37	1.40	0.63	6.00	ILMN_1252076	<u>Lyz2</u>	-15.32	-205.83	13.44	6.70	7.51
ILMN_1236168	<u>C030034J23Rik</u>	15.77	190.89	12.11	0.34	6.00	ILMN_2470131	<u>6720475I19Rik</u>	-14.90	-37.43	2.51	2.11	7.51
ILMN_2710159	<u>MGC41689</u>	15.70	278.76	17.75	0.35	6.00	ILMN_2888834	<u>Apob48r</u>	-14.71	-126.35	8.59	4.83	7.51
ILMN_1253178	<u>Aldh3a1</u>	15.41	149.83	9.72	0.40	6.00	ILMN_2920849	<u>Pir4a</u>	-14.64	-46.79	3.20	2.38	7.51
ILMN_2576994	<u>C230099G16Rik</u>	14.88	67.10	4.51	0.66	6.00	ILMN_1240256	<u>Slc9a3r1</u>	-14.01	-130.32	9.90	1.75	7.51
ILMN_2697304	<u>Eln</u>	14.82	934.16	63.02	0.57	6.00	ILMN_1251066	<u>BC067047</u>	-13.79	-42.11	3.05	1.90	7.51
ILMN_2904686	<u>Cyb5f3</u>	14.36	163.32	11.37	0.60	6.87	ILMN_3117876	<u>Chl3l3</u>	-13.67	-211.31	15.45	5.78	7.51
ILMN_1212935	<u>Fzd4</u>	14.35	37.25	2.60	0.66	6.87	ILMN_2714796	<u>Coro1a</u>	-13.61	-318.14	23.38	6.70	7.51
ILMN_1258578	<u>Ahnak</u>	14.33	221.71	15.47	0.62	6.87	ILMN_1249242	<u>Dnajc2</u>	-13.27	-113.51	8.55	1.59	7.51
ILMN_2658804	<u>Rras</u>	13.77	78.66	5.71	0.59	6.87	ILMN_1221354	<u>9330156H06Rik</u>	-13.19	-18.72	1.42	1.57	8.59
ILMN_2441501	<u>Ctstn1</u>	13.77	30.38	2.21	0.62	6.87	ILMN_1225192	<u>Nfkbid</u>	-12.91	-65.80	5.10	3.07	8.59
ILMN_2769567	<u>F2r1</u>	13.13	125.14	9.53	0.60	6.87	ILMN_2699531	<u>Rgs10</u>	-12.58	-71.58	5.69	1.52	8.59
ILMN_1238603	<u>Pcolce2</u>	13.11	71.99	5.49	0.48	6.87	ILMN_1217928	<u>C230067006Rik</u>	-12.58	-77.27	6.14	1.80	8.59
ILMN_3066763	<u>Ar14a</u>	12.46	171.95	13.80	0.38	6.87	ILMN_1217849	<u>Laptm5</u>	-12.52	-773.19	61.77	4.84	8.59
ILMN_2557319	<u>D530030K12Rik</u>	12.34	111.85	9.06	0.63	6.87	ILMN_3013874	<u>EG434858</u>	-12.45	-44.72	3.59	1.77	8.59
ILMN_2598103	<u>Emp2</u>	12.34	656.62	53.23	0.40	6.87	ILMN_2731760	<u>Myo1f</u>	-12.09	-64.59	5.34	2.76	8.59
ILMN_2605539	<u>Sgcd</u>	12.12	47.08	3.88	0.52	6.87	ILMN_3089584	<u>Cd74</u>	-11.69	-301.56	25.80	3.80	8.59
ILMN_2772855	<u>Plek2</u>	12.07	45.53	3.77	0.50	6.87	ILMN_1221817	<u>Cd74</u>	-11.59	-223.38	19.28	3.42	8.59
ILMN_2736783	<u>Kctd12</u>	12.04	380.66	31.61	0.47	6.87	ILMN_2652511	<u>Hist1h2bj</u>	-11.44	-125.50	10.97	1.62	8.59
ILMN_2712873	<u>Cyb5f3</u>	12.00	101.47	8.46	0.61	6.87	ILMN_2571683	<u>9830169E20Rik</u>	-11.43	-166.80	14.59	2.12	8.59
ILMN_2625920	<u>Aoc3</u>	11.95	115.86	9.69	0.30	6.87	ILMN_1259561	<u>Prep</u>	-11.40	-40.08	3.52	1.84	8.59
ILMN_3061923	<u>Rbms2</u>	11.63	89.70	7.71	0.59	6.87	ILMN_2659151	<u>Thbs1</u>	-11.34	-387.28	34.15	3.36	8.59
ILMN_2691641	<u>Gja5</u>	11.56	678.83	58.73	0.58	6.87	ILMN_1248139	<u>Gp49a</u>	-11.16	-128.29	11.49	4.54	8.59
ILMN_1240266	<u>Ankrd25</u>	11.24	195.29	17.38	0.52	6.87	ILMN_1254513	<u>4930553M18Rik</u>	-11.12	-178.39	16.04	2.15	8.59
ILMN_2765101	<u>Nkx6-2</u>	11.19	112.90	10.09	0.66	6.87	ILMN_2637165	<u>3110001H17Rik</u>	-11.09	-72.58	6.55	1.90	8.59
ILMN_2639809	<u>Nucb1</u>	11.10	78.22	7.05	0.								

ILMN_2502860	Ern1	10.27	116.17	11.31	0.62	6.87	ILMN_2686244	Rassf4	-9.81	-74.07	7.55	3.21	8.67
ILMN_3139103	Adam15	10.24	160.88	15.71	0.34	6.87	ILMN_2609323	Lst1	-9.80	-71.92	7.34	2.85	8.67
ILMN_1215859	Serpina1b	10.20	23.75	2.33	0.59	6.87	ILMN_1226525	H2-Ab1	-9.24	-370.46	40.10	3.83	9.45
ILMN_1213456	Dhrs7	10.10	103.87	10.29	0.50	6.87	ILMN_3102376	Fcgr2b	-9.17	-23.48	2.56	1.70	9.45
ILMN_2725493	2700078K21Rik	10.06	61.98	6.16	0.41	6.87	ILMN_1258723	Bop1	-9.16	-36.00	3.93	1.76	9.45
ILMN_2713835	Nos3	10.04	149.12	14.85	0.46	6.87	ILMN_2766780	Lyzs	-9.15	-20.06	2.19	1.56	9.45
ILMN_1214275	Ddef2	9.65	43.46	4.50	0.66	7.51	ILMN_2644587	Bzw2	-9.12	-35.95	3.94	1.57	9.45
ILMN_2596117	Kctd10	9.65	190.39	19.73	0.61	7.51	ILMN_2669404	Lmbb2	-8.93	-294.56	33.00	1.89	9.45
ILMN_2953751	Sec14l1	9.60	81.62	8.50	0.59	7.51	ILMN_2416628	Pcsd4	-8.87	-201.17	22.68	2.99	9.45
ILMN_2715195	Stxbp3a	9.43	52.44	5.56	0.63	7.51	ILMN_2712151	1810033817Rik	-8.85	-23.08	2.61	1.65	9.45
ILMN_2801891	Cymb	9.41	49.01	5.21	0.43	7.51	ILMN_1247377	Mpeg1	-8.79	-57.43	6.53	2.46	9.45
ILMN_1236917	Tmem59	9.41	88.83	9.44	0.58	7.51	ILMN_2692960	Ero1b	-8.73	-42.71	4.89	1.84	9.45
ILMN_1224018	Foxk1	9.19	58.55	6.37	0.46	7.51	ILMN_1241302	Csf3r	-8.69	-33.12	3.81	2.03	9.45
ILMN_3022252	Arhgef15	9.15	171.08	18.69	0.60	7.51	ILMN_2606144	Cd300f	-8.68	-221.92	25.56	7.18	9.45
ILMN_2646052	C4a	9.01	23.42	2.60	0.67	7.51	ILMN_2685023	Hmba1	-8.65	-55.09	6.37	2.72	9.45
ILMN_1217742	Atp2b4	8.98	190.99	21.26	0.39	7.51	ILMN_1229530	Csk	-8.64	-97.86	11.32	1.53	9.45
ILMN_1221146	Cvtl1	8.98	765.84	85.29	0.41	7.51	ILMN_2820260	Wdr77	-8.59	-57.58	6.71	2.07	9.45
ILMN_1230578	LOC100045421	8.97	34.39	3.83	0.50	7.51	ILMN_2607880	Tkt	-8.53	-99.97	11.72	1.67	9.45
ILMN_2710274	Slc9a3r2	8.86	728.41	82.22	0.32	8.11	ILMN_2956092	Rassf4	-8.48	-79.40	9.36	3.33	9.45
ILMN_2630039	E130014J05Rik	8.77	58.00	6.61	0.58	8.11	ILMN_2909150	Ctgef	-8.48	-1891.21	223.12	6.00	9.45
ILMN_2738345	Lims2	8.64	162.65	18.82	0.40	8.11	ILMN_2687586	Cxcl16	-8.46	-282.89	33.45	2.20	9.45
ILMN_1253182	Hs3st1	8.64	64.10	7.42	0.66	8.11	ILMN_2924831	Gas7	-8.44	-56.56	6.70	2.08	9.45
ILMN_1245446	5730405J09Rik	8.63	47.55	5.51	0.63	8.11	ILMN_1238215	Ctgef	-8.37	-755.01	90.15	7.29	9.45
ILMN_2970167	Wwp2	8.52	58.26	6.83	0.61	8.11	ILMN_2685393	CCR5	-8.36	-69.17	8.27	3.39	9.45
ILMN_2942674	Lims2	8.52	188.91	22.18	0.34	8.11	ILMN_2607675	LOC641240	-8.35	-268.72	32.18	3.29	9.45
ILMN_1243080	Taf9b	8.51	61.14	7.19	0.62	8.11	ILMN_2619861	Nipsnap1	-8.35	-48.60	5.82	1.54	9.45
ILMN_1243407	Klk10	8.48	840.82	99.17	0.10	8.11	ILMN_2673776	Ezf2	-8.34	-69.27	8.31	1.89	9.45
ILMN_2416218	5530400B01Rik	8.45	84.43	10.00	0.40	8.11	ILMN_2910934	Cd52	-8.33	-214.32	25.72	5.07	9.45
ILMN_2511768	Ttc17	8.40	60.34	7.18	0.60	8.11	ILMN_2694857	Gpatct3	-8.33	-23.57	2.83	1.53	9.45
ILMN_2940642	St6galnac2	8.29	199.26	24.04	0.37	8.11	ILMN_1256883	Rad51	-8.23	-43.04	5.23	2.15	9.45
ILMN_2659879	Adcy6	8.22	66.22	8.06	0.54	8.11	ILMN_2559943	A230055O06Rik	-8.14	-56.18	6.90	2.59	9.45
ILMN_2744414	Nme3	8.21	50.86	6.20	0.58	8.11	ILMN_2629971	Fzr1	-8.03	-20.23	2.52	1.51	9.45
ILMN_2778181	Plekha6	8.10	47.16	5.82	0.44	8.11	ILMN_1213364	LOC638892	-8.03	-112.73	14.04	1.68	9.45
ILMN_1213850	Col4a3	8.00	394.35	49.30	0.22	8.11	ILMN_1223317	Lgals3	-8.01	-37.83	4.72	2.16	9.45
ILMN_2638114	Ptn	7.99	18.90	2.36	0.62	8.11	ILMN_2748875	Fcer1g	-8.00	-85.61	10.70	3.09	9.45
ILMN_1225825	LOC100039175	7.99	104.11	13.03	0.49	8.11	ILMN_1235392	LOC668183	-7.91	-76.35	9.65	2.58	9.45
ILMN_1248397	Smarcd3	7.98	41.30	5.18	0.56	8.11	ILMN_1239569	Lsg1	-7.89	-44.59	5.65	1.64	9.45
ILMN_1239381	Klf3	7.96	295.85	37.19	0.35	8.11	ILMN_1230157	Rnd3	-7.89	-113.47	14.38	2.15	9.45
ILMN_2633386	LOC100044190	7.91	163.23	20.63	0.53	8.11	ILMN_2787257	Coro1a	-7.86	-275.12	35.02	6.06	9.45
ILMN_2669602	Pit6	7.87	839.18	106.64	0.27	8.11	ILMN_2781030	Napsa	-7.80	-85.04	10.90	3.00	9.45
ILMN_2479359	Tmod3	7.87	67.27	8.55	0.58	8.11	ILMN_1230440	1700041B20Rik	-7.79	-65.43	8.40	2.16	9.45
ILMN_2433213	Klf7	7.87	116.57	14.82	0.59	8.11	ILMN_1252804	Map4k1	-7.77	-26.02	3.35	1.66	9.45
ILMN_1259554	Marveld1	7.86	253.40	32.24	0.48	8.11	ILMN_1236387	BC024537	-7.72	-178.09	23.07	1.76	9.45
ILMN_3094506	Arhgef15	7.78	171.53	22.04	0.54	8.11	ILMN_2887986	Cd300a	-7.72	-252.37	32.71	4.31	9.45
ILMN_2755833	Lrrc3b	7.70	26.91	3.49	0.58	8.11	ILMN_1227434	Itgb7	-7.71	-42.06	5.45	2.10	9.45
ILMN_2723024	BC004044	7.66	20.95	2.73	0.66	8.11	ILMN_2749063	Dock10	-7.67	-63.52	8.28	2.52	9.45
ILMN_1234318	Ubx1	7.65	154.31	20.18	0.60	8.11	ILMN_2460168	Wdr1	-7.65	-71.49	9.34	1.52	9.45
ILMN_2699637	Lsr	7.61	175.35	23.04	0.38	8.11	ILMN_1244123	Slc38a2	-7.54	-275.69	36.58	1.82	9.45
ILMN_2999439	Klf4	7.57	230.54	30.46	0.28	8.11	ILMN_1219712	Ctsp	-7.50	-88.49	11.80	1.98	9.45
ILMN_1231439	Aat1	7.51	168.67	22.46	0.51	8.11	ILMN_1222471	Gmfj	-7.49	-64.19	8.57	2.23	9.45
ILMN_2675760	2310046K01Rik	7.49	316.15	42.21	0.33	8.11	ILMN_2816180	Lbh	-7.48	-169.34	22.65	2.13	9.45
ILMN_2661422	Ramp2	7.48	563.57	75.38	0.44	8.11	ILMN_2769285	Sema6b	-7.42	-204.00	27.49	2.45	9.45
ILMN_1248994	4933407C03Rik	7.38	107.64	14.59	0.47	8.11	ILMN_2642403	Lmo4	-7.40	-105.47	14.25	1.70	9.45
ILMN_1260405	D330008E13Rik	7.36	35.13	4.77	0.65	8.11	ILMN_2657409	Rps18	-7.39	-48.59	6.58	1.79	9.45
ILMN_2675232	Klk8	7.29	118.36	16.23	0.43	8.11	ILMN_3113420	Ptpn6	-7.38	-71.35	9.66	2.87	9.45
ILMN_2608133	Rhopn2	7.29	599.12	82.24	0.26	8.11	ILMN_2656666	Pstpip1	-7.34	-40.93	5.58	2.26	9.45
ILMN_1248740	Sema3f	7.27	637.08	87.58	0.49	8.11	ILMN_2747456	Ivns1abp	-7.33	-546.74	74.58	4.23	9.45
ILMN_2609025	Elmo1	7.21	59.82	8.30	0.66	8.11	ILMN_2690603	Spp1	-7.32	-31.52	4.30	2.11	9.45
ILMN_1230129	Adamts1	7.21	71.65	9.94	0.65	8.11	ILMN_1218123	Aifi	-7.28	-106.31	14.60	3.00	9.45
ILMN_1231802	Tbc1d9b	7.20	18.54	2.57	0.66	8.11	ILMN_2633062	9130422G05Rik	-7.25	-25.89	3.57	1.59	9.45
ILMN_2790842	Jam2	7.15	151.84	21.23	0.40	8.11	ILMN_2787785	Aknk	-7.23	-33.42	4.62	1.75	9.45
ILMN_1242787	4930557M22Rik	7.13	23.00	3.22	0.64	8.11	ILMN_1224876	Znhit1	-7.22	-60.25	8.35	1.77	9.45
ILMN_1228031	Dusp8	7.13	323.32	45.34	0.27	8.11	ILMN_2685392	Ccr5	-7.21	-59.36	8.23	2.87	9.45
ILMN_1224589	Tmem77	7.11	35.33	4.97	0.65	8.11	ILMN_1255766	Sh3bp2	-7.20	-146.71	20.37	1.96	9.45
ILMN_2485594	B130005J07Rik	7.10	20.17	2.84	0.65	8.11	ILMN_2524817	Dnahc17	-7.14	-40.68	5.70	1.82	9.45
ILMN_1237264	Trspap1	7.10	37.17	5.23	0.59	8.11	ILMN_1228657	Fcgr2b	-7.12	-25.28	3.55	1.77	9.45
ILMN_2678355	Amigo2	7.10	226.95	31.98	0.44	8.11	ILMN_2628629	Cdh1	-7.03	-24.99	3.56	1.58	9.45
ILMN_1218934	Rdm1	7.05	64.87	9.20	0.54	8.11	ILMN_2671984	Ptpcr	-7.01	-60.84	8.67	2.59	9.45
ILMN_2777082	P4ha2	7.01	468.20	66.75	0.40	8.11	ILMN_2619961	4933429F08Rik	-6.99	-22.30	3.19	1.61	9.45
ILMN_2430542	Nos3	6.94	136.32	19.64	0.46	8.11	ILMN_2663930	Sfnf1	-6.95	-144.45	20.79	5.35	9.45
ILMN_3003864	Cgnt1	6.93	320.81	46.26	0.39	8.11	ILMN_2675223	Cd33	-6.91	-77.32	11.18		

ILMN_1228245	Prickle1	6.46	27.93	4.32	0.64	8.11	ILMN_1221736	Samhd1	-6.31	-108.70	17.22	2.13	9.45
ILMN_3137804	Pbx1	6.41	40.53	6.32	0.63	8.11	ILMN_2634248	Synchron	-6.31	-80.52	12.76	1.71	9.45
ILMN_2618408	Icam2	6.38	476.29	74.65	0.48	8.11	ILMN_2957167	4931417G12Rik	-6.30	-27.27	4.33	1.68	9.45
ILMN_1249888	Adcy6	6.36	80.57	12.66	0.62	8.11	ILMN_2963974	Gemin4	-6.30	-34.03	5.40	1.63	9.45
ILMN_1215879	Pkh1l1	6.36	31.44	4.94	0.40	8.11	ILMN_2742152	Gadd45a	-6.28	-138.42	22.03	4.87	9.45
ILMN_1218241	Slc9a3r2	6.35	33.07	5.20	0.55	8.11	ILMN_1219333	9830134K01Rik	-6.28	-25.26	4.02	1.61	9.45
ILMN_3049559	C4b	6.34	65.15	10.28	0.54	8.11	ILMN_3157568	Bcl2l1l	-6.28	-59.58	9.49	1.84	9.45
ILMN_1255416	Ly6a	6.30	685.75	108.79	0.53	8.11	ILMN_2601155	Frbz	-6.28	-70.68	11.26	2.01	9.45
ILMN_1257193	Ppm1a	6.30	47.33	7.51	0.64	8.11	ILMN_2529254	LOC223653	-6.25	-26.41	4.23	1.64	9.45
ILMN_1241605	LOC383884	6.30	20.62	3.27	0.64	8.11	ILMN_1249486	Mgl1	-6.21	-295.82	47.66	4.25	9.45
ILMN_2700166	Cnd2	6.30	165.71	26.30	0.63	8.11	ILMN_2894211	8430408G22Rik	-6.18	-32.44	5.25	2.00	9.45
ILMN_1225657	2410095820Rik	6.30	27.46	4.36	0.63	8.11	ILMN_2896805	Psmid12	-6.17	-62.78	10.17	1.57	9.45
ILMN_2896843	Cd248	6.29	45.73	7.27	0.49	8.11	ILMN_2906473	Gbi	-6.17	-88.64	14.36	1.53	9.45
ILMN_2513922	Pdm16	6.27	25.09	4.00	0.63	8.11	ILMN_2821148	Serhl	-6.12	-96.50	15.77	2.29	9.45
ILMN_2614380	Map3k1	6.25	141.98	22.72	0.60	8.11	ILMN_2685194	Lass6	-6.10	-44.65	7.32	2.43	9.45
ILMN_1248895	Cachd1	6.21	24.29	3.91	0.60	8.11	ILMN_3139875	Aco1l	-6.09	-46.77	7.67	1.79	9.45
ILMN_1260571	Spsa2	6.18	81.47	13.18	0.65	8.11	ILMN_1254577	Al607873	-6.07	-63.12	10.39	2.81	9.45
ILMN_2640570	Pak4	6.12	267.69	43.75	0.42	8.11	ILMN_2526163	OC380753	-6.07	-23.63	3.90	1.61	9.45
ILMN_1243249	2810410A03Rik	6.08	242.39	39.89	0.51	8.11	ILMN_2593496	Got2	-6.05	-127.90	21.14	1.62	9.45
ILMN_2733887	Mknk2	6.06	230.14	37.96	0.43	8.11	ILMN_2618714	Pdgrfb	-6.00	-192.47	32.06	3.27	9.45
ILMN_1239673	LOC672215	6.04	41.20	6.82	0.53	8.11	ILMN_1255419	Zfpn1a1	-5.99	-32.61	5.44	2.04	9.45
ILMN_2963704	Sfnx3	6.01	410.52	68.36	0.66	8.11	ILMN_1259488	Mgea6	-5.93	-86.89	14.66	1.78	9.45
ILMN_2513570	AW123240	5.99	48.15	8.04	0.64	8.11	ILMN_2836137	E2f2	-5.89	-70.10	11.90	1.96	9.45
ILMN_2670375	Irm2b	5.98	254.84	42.59	0.64	8.11	ILMN_2734729	H2-Aa	-5.89	-60.77	10.32	2.27	9.45
ILMN_1219447	Zmym3	5.97	77.53	12.98	0.57	8.11	ILMN_2742592	Hist1h2be	-5.87	-169.99	28.97	1.74	9.45
ILMN_2761918	Mmnm2	5.96	506.28	84.98	0.54	8.11	ILMN_1254035	Myo10	-5.84	-31.72	5.43	2.00	9.45
ILMN_2789562	P4ha2	5.95	219.30	36.88	0.41	8.11	ILMN_1256359	Smox	-5.82	-23.62	4.06	1.72	9.45
ILMN_2493521	Trnc6c	5.94	52.82	8.89	0.62	8.11	ILMN_1262606	Tmem132a	-5.81	-127.84	22.01	1.54	9.45
ILMN_2728538	Exdl2	5.93	29.64	5.00	0.63	8.11	ILMN_1247832	Cd74	-5.80	-67.15	11.57	2.67	9.45
ILMN_2658407	Elmo1	5.91	52.73	8.92	0.62	8.11	ILMN_2704919	Ube2t	-5.79	-19.63	3.39	1.64	9.45
ILMN_1223049	Tns1	5.89	137.96	23.43	0.49	8.11	ILMN_2494707	OC381232	-5.73	-15.62	2.73	1.50	9.45
ILMN_2855515	Pnplg6	5.86	69.34	11.82	0.62	8.11	ILMN_2727663	Tgfb	-5.69	-61.32	10.78	2.82	9.45
ILMN_2913222	Efcabada	5.84	136.41	23.35	0.50	8.11	ILMN_2435584	scf0001978.1_6	-5.67	-196.97	34.71	1.83	9.45
ILMN_2577853	Rw1-pending	5.82	29.72	5.11	0.63	8.11	ILMN_1214071	Ifft1	-5.66	-211.63	37.41	4.60	9.45
ILMN_1224866	Ptgs1	5.80	45.80	7.90	0.51	8.36	ILMN_2552295	Vcam1	-5.65	-34.00	6.01	1.53	9.45
ILMN_2933431	Pps	5.78	25.83	4.47	0.62	8.36	ILMN_2814974	Klra2	-5.64	-72.50	12.85	3.25	9.45
ILMN_2750053	Ptprj	5.78	573.00	99.14	0.33	8.36	ILMN_2922899	Pibcb2	-5.64	-21.05	3.73	1.57	9.45
ILMN_2641228	Hspa12b	5.77	267.94	46.45	0.46	8.36	ILMN_1247540	Vcan	-5.64	-21.42	3.80	1.62	9.45
ILMN_2947526	Ecm1	5.76	102.54	17.79	0.55	8.36	ILMN_2810405	Myo1g	-5.64	-80.61	14.30	3.19	9.45
ILMN_2757019	She	5.72	105.35	18.41	0.66	8.36	ILMN_2655336	Vcan	-5.61	-56.36	10.05	2.22	9.45
ILMN_2509327	Wipf3	5.71	25.26	4.42	0.60	8.36	ILMN_1231012	Lcp2	-5.60	-24.11	4.31	1.74	9.45
ILMN_2633897	Pde6d	5.70	29.69	5.20	0.64	8.36	ILMN_2915232	Cotl1	-5.58	-147.47	26.41	3.69	9.45
ILMN_2615035	Mgst3	5.70	156.45	27.43	0.56	8.36	ILMN_1222059	Thbs1	-5.58	-1097.52	196.84	3.77	9.45
ILMN_3126277	Palmd	5.70	375.82	65.94	0.58	8.36	ILMN_2936380	Sgpl1	-5.56	-27.63	4.97	1.59	9.45
ILMN_2743320	Myst4	5.66	35.97	6.35	0.63	8.36	ILMN_2585233	Selpl	-5.56	-20.65	3.71	1.57	9.45
ILMN_2592823	Cdc42ep5	5.65	46.78	8.28	0.60	8.36	ILMN_1242661	Itgb2	-5.50	-38.79	7.06	1.89	9.45
ILMN_1241293	Cldn5	5.64	905.91	160.54	0.39	8.36	ILMN_3072427	Iltm	-5.49	-26.03	4.74	1.67	9.45
ILMN_2727687	Numb	5.64	40.69	7.21	0.63	8.36	ILMN_2577664	Fcgr2b	-5.48	-38.40	7.00	2.12	9.45
ILMN_3111877	Rbms2	5.62	204.42	36.35	0.62	8.36	ILMN_2715234	Rnmt1l	-5.45	-40.18	7.37	1.75	9.45
ILMN_2727309	LOC100044204	5.60	98.74	17.62	0.40	8.36	ILMN_3043669	Sla	-5.44	-29.67	5.45	1.77	9.45
ILMN_3159275	Ahnak	5.60	146.55	26.17	0.62	8.36	ILMN_2957862	Noc4l	-5.42	-105.05	19.37	1.71	9.45
ILMN_2877069	Tspo	5.60	108.53	19.38	0.60	8.36	ILMN_1222803	Hspa9	-5.41	-121.18	22.38	1.65	9.45
ILMN_2678477	Gja5	5.60	81.70	14.60	0.44	8.36	ILMN_2547840	220005K02Rik	-5.39	-96.34	17.86	2.08	9.45
ILMN_3052632	Epas1	5.59	1006.29	179.97	0.50	8.36	ILMN_1227907	Gmfg	-5.39	-97.65	18.13	2.59	9.45
ILMN_2592881	Jam2	5.58	56.34	10.10	0.53	8.36	ILMN_1242457	Fpr2	-5.37	-102.16	19.04	4.03	9.45
ILMN_3132223	C630004H02Rik	5.55	98.03	17.66	0.61	8.36	ILMN_2752224	Mrps28	-5.35	-36.40	6.80	5.59	9.45
ILMN_2466164	Wfdc1	5.54	36.49	6.59	0.48	8.36	ILMN_2657478	Cd53	-5.33	-45.50	8.53	2.30	9.45
ILMN_3115796	Cd40	5.54	63.12	11.40	0.43	8.36	ILMN_1249498	Plek	-5.27	-34.74	6.59	1.95	9.45
ILMN_27120083	Bace2	5.54	155.25	28.05	0.44	8.36	ILMN_1251669	Evi2a	-5.26	-72.52	13.79	2.87	9.45
ILMN_2705128	Muted	5.53	212.07	38.32	0.65	8.36	ILMN_1220418	Hcst	-5.25	-81.31	15.48	3.13	9.45
ILMN_1220234	Serpin1e	5.52	64.66	11.71	0.37	8.36	ILMN_2746501	Csfr	-5.23	-31.19	5.97	1.93	9.45
ILMN_2599008	Kirrel3	5.51	37.01	6.71	0.53	8.36	ILMN_2859847	Pygl	-5.21	-26.85	5.15	1.71	9.45
ILMN_2588295	Rarres2	5.48	47.39	8.64	0.46	8.36	ILMN_2666487	Ruvbl1	-5.21	-59.50	11.42	2.08	9.45
ILMN_2881681	Trnc6c	5.47	157.45	28.76	0.47	8.36	ILMN_2745425	Rcc1	-5.14	-123.41	24.01	1.51	9.45
ILMN_2865335	Krt80	5.46	205.30	37.58	0.47	8.36	ILMN_1248604	D030029G14Rik	-5.12	-26.35	5.14	1.62	9.45
ILMN_2432550	Trib2	5.46	148.79	27.26	0.36	8.36	ILMN_2485839	Trnfrs1b	-5.12	-48.65	9.50	1.75	9.45
ILMN_2756665	Cbr2	5.46	96.44	17.68	0.42	8.36	ILMN_3155245	Arhgap25	-5.10	-67.34	13.20	1.76	9.45
ILMN_2736379	Nfia	5.45	164.72	30.21	0.65	8.36	ILMN_2495068	scf000854.1_75	-5.09	-39.51	7.76	1.82	9.45
ILMN_2572849	C920007D24Rik	5.44	46.49	8.54	0.53	8.36	ILMN_2720634	Prmt5	-5.08	-67.20	13.22	1.84	9.45
ILMN_1225988	Zdhc3	5.44	49.76	9.15	0.44	8.36	ILMN_2653619	Ctag5	-5.03	-99.04	19.69	1.70	9.45
ILMN_2862179	Ccl11	5.44	18.10	3.33	0.64	8.36	ILMN_2637714	Rasa3	-5.02	-154.22	30.73	1.96	9.45
ILMN_1257077	Jag												

ILMN_1238936	D130063P19Rik	5.02	29.32	5.84	0.61	8.65	ILMN_2633275	Golt1b	-4.70	-20.69	4.41	1.52	9.92
ILMN_3112526	Ldb2	5.02	75.50	15.04	0.49	8.65	ILMN_1257019	BC037034	-4.69	-178.07	37.97	1.66	9.92
ILMN_1233340	Pkp4	5.00	332.80	66.59	0.64	8.65	ILMN_2507890	Ddx27	-4.66	-59.63	12.79	1.79	9.92
ILMN_2454786	Tpcn1	4.99	92.71	18.58	0.52	8.65							
ILMN_3163020	Klc1	4.98	54.81	11.01	0.66	8.65							
ILMN_1232123	Traf3ip2	4.97	33.68	6.77	0.60	8.65							
ILMN_1231520	Trpv4	4.97	66.51	13.39	0.51	8.65							
ILMN_2449620	S830427D02Rik	4.96	23.51	4.74	0.61	8.65							
ILMN_2740628	Ndr3	4.95	28.58	5.78	0.58	8.65							
ILMN_2931918	4432416J03Rik	4.95	29.33	5.93	0.56	8.65							
ILMN_2834370	Cutc	4.94	67.50	13.65	0.63	8.65							
ILMN_2610442	Wscd1	4.91	90.57	18.44	0.56	8.65							
ILMN_2663211	Stbd1	4.88	61.45	12.58	0.58	8.65							
ILMN_2688236	Atp2a3	4.87	1787.36	366.78	0.63	8.65							
ILMN_1212703	Kras	4.87	110.37	22.66	0.63	8.65							
ILMN_2870522	Plekha6	4.86	249.03	51.28	0.20	8.65							
ILMN_2790839	Jam2	4.85	246.15	50.75	0.40	8.65							
ILMN_2621038	Hoxa7	4.84	33.50	6.92	0.64	8.65							
ILMN_2745367	Myo1c	4.84	54.10	11.18	0.64	8.65							
ILMN_1253304	Stmn2	4.81	137.74	28.63	0.27	9.10							
ILMN_2473692	1110059G02Rik	4.81	31.28	6.51	0.64	9.10							
ILMN_2923607	Phlda3	4.80	107.05	22.30	0.54	9.10							
ILMN_1238331	Rom1	4.79	154.73	32.29	0.46	9.10							
ILMN_1250469	Bcl9l	4.78	650.33	136.03	0.46	9.10							
ILMN_2507400	9330180L10Rik	4.77	53.34	11.19	0.62	9.10							
ILMN_3161897	Dync1l2	4.76	80.60	16.93	0.62	9.10							
ILMN_2686087	Cutc	4.74	50.46	10.64	0.55	9.10							
ILMN_1229828	Adamts10	4.72	32.02	6.79	0.57	9.10							
ILMN_2700408	Mgl1	4.71	45.83	9.72	0.46	9.10							
ILMN_2624451	4933407C03Rik	4.71	193.57	41.08	0.45	9.10							
ILMN_2474515	9430020K01Rik	4.68	42.93	9.17	0.66	9.10							
ILMN_1259753	Sp4	4.67	31.44	6.73	0.59	9.10							
ILMN_2727481	Palmd	4.66	261.30	56.11	0.57	9.10							
ILMN_2597769	Igf2	4.65	190.26	40.93	0.29	9.10							
ILMN_2697760	Nkx2-3	4.65	63.00	13.56	0.58	9.10							
ILMN_2622354	Arf4	4.65	30.19	6.50	0.57	9.10							
ILMN_2838317	Pqlc3	4.63	32.01	6.91	0.55	9.10							
ILMN_2604029	Klf2	4.62	118.69	25.69	0.27	9.10							
ILMN_1246346	8230107H12Rik	4.62	37.68	8.16	0.63	9.10							
ILMN_1222365	2610200O14Rik	4.61	34.86	7.56	0.62	9.10							
ILMN_1251524	Them4	4.58	27.08	5.91	0.66	9.10							
ILMN_2993109	Ddit4	4.58	343.37	75.01	0.63	9.10							
ILMN_2646166	Ndr1	4.57	110.74	24.23	0.64	9.10							
ILMN_2729153	Nos3	4.56	129.94	28.49	0.45	9.45							
ILMN_1237671	Setmar	4.56	43.57	9.56	0.50	9.45							
ILMN_2634689	Itgb4	4.55	122.74	26.97	0.40	9.45							
ILMN_1217061	Casp9	4.54	95.47	21.03	0.64	9.45							
ILMN_2833781	Pwwp2b	4.54	76.92	16.96	0.52	9.45							
ILMN_2857957	Mgl1	4.53	41.95	9.26	0.48	9.45							
ILMN_1232928	Tim3	4.52	366.95	81.19	0.55	9.45							
ILMN_2622500	Zbtb7c	4.52	209.94	46.49	0.19	9.45							
ILMN_1249637	Peg13	4.51	25.91	5.75	0.64	9.45							
ILMN_2765047	Chrd	4.50	62.48	13.88	0.37	9.45							
ILMN_1217606	1500005K14Rik	4.50	75.17	16.71	0.39	9.45							
ILMN_2683095	Ap1g2	4.48	89.31	19.91	0.67	9.45							
ILMN_2498731	E030024M20Rik	4.48	198.90	44.37	0.33	9.45							
ILMN_1232295	Sort1	4.47	75.52	16.89	0.66	9.45							
ILMN_2614889	B3gnt8	4.46	250.68	56.27	0.28	9.45							
ILMN_2602185	9/9/2009	4.44	141.84	31.92	0.50	9.45							
ILMN_2661299	Pmp22	4.44	20.82	4.68	0.66	9.45							
ILMN_2504268	Gcap26	4.40	113.00	25.68	0.24	9.45							
ILMN_2620233	Fmo5	4.38	28.06	6.41	0.57	9.45							
ILMN_2876579	Ubx1	4.37	94.21	21.57	0.57	9.45							
ILMN_3144575	Itgb4	4.36	182.41	41.81	0.43	9.45							
ILMN_2755424	Bcor1	4.36	50.23	11.52	0.60	9.45							
ILMN_2880906	Pdlim2	4.36	201.44	46.22	0.29	9.45							
ILMN_2798993	Nr1d2	4.35	76.58	17.59	0.66	9.45							
ILMN_2702547	4930519N16Rik	4.34	48.97	11.29	0.40	9.45							
ILMN_2759563	2410008K03Rik	4.34	54.93	12.66	0.54	9.45							
ILMN_2583163	D430023I21Rik	4.33	34.78	8.03	0.62	9.45							
ILMN_2674367	Agrn	4.31	206.43	47.88	0.49	9.45							
ILMN_3138157	Arf6ip2	4.30	34.42	8.00	0.55	9.92							
ILMN_2615557	Dab2ip	4.30	277.58	64.54	0.62	9.92							
ILMN_1238479	Mrst3	4.30	40.69	9.47	0.66	9.92							
ILMN_1228942	Cd59a	4.28	193.36	45.16	0.45	9.92							
ILMN_2846812	Sp100	4.28	85.94	20.08	0.57	9.92							
ILMN_2664224	Ephx1	4.28	122.70	28.70	0.51	9.92							
ILMN_2419858	E230020D15Rik	4.27	33.59	7.87	0.61	9.92							
ILMN_2416876	Gm967	4.26	77.39	18.17	0.44	9.92							
ILMN_2913089	Brd9	4.25	55.60	13.08	0.65	9.92							
ILMN_2741464	Fgd5	4.23	74.06	17.50	0.50	9.92							
ILMN_2702997	Thap7	4.23	58.84	13.92	0.42	9.92							
ILMN_1242571	Pkn3	4.21	369.99	87.85	0.54	9.92							
ILMN_1227126	Ppp2r3a	4.21	20.51	4.88	0.65	9.92							
ILMN_2790188	4921533L14Rik	4.20	143.07	34.07	0.41	9.92							
ILMN_2742627	Six2	4.19	54.10	12.91	0.57	9.92							
ILMN_1256136	Nme7	4.19	34.40	8.22	0.56	9.92							
ILMN_1222004	Rbbp9	4.17	32.90	7.89	0.55	9.92							
ILMN_2418725	Zdhhc3	4.17	35.63	8.54	0.54	9.92							
ILMN_2706268	Scara3	4.17	84.36	20.24	0.48	9.92							
ILMN_1225835	Mfap5	4.17	206.03	49.44	0.33	9.92							

Table S2. Common mechanosensitive genes between 12hr and 48hr

Up-regulated (LCA/RCA)

Down-regulated (LCA/RCA)

Ctgf

2310046K01Rik

Ctps

BC020535

Fosl2

Dab2ip

Got2

Dhh

Lmo4

E030024M20Rik

Eln

F2r1

Icam2

Id1

Inmt

Klf2

Klf4

Klk10

Kras

Lims2

Lsr

P4ha2

Pdlim2

Ptprj

Rab11fip5

Rhpn2

Slc9a3r2

Tek

Timp3

Table S3. Taqman qPCR probes

Assay Type	Gene Name	Assay ID
Angpt2,mCG1200	angiopoietin 2	Mm00545822_m1
Bcam,mCG4887	basal cell adhesion molecule	Mm00522338_m1
Bmp4,mCG4634	bone morphogenetic protein 4	Mm00432087_m1
Cd300a,mCG13614	CD300A antigen	Mm00468054_m1
Col4a3,mCG118161	collagen, type IV, alpha 3	Mm00483656_m1
Ctgf,mCG6745	connective tissue growth factor	Mm01192931_g1
Cxcl12,mCG133006	chemokine (C-X-C motif) ligand 12	Mm00445553_m1
Cxcl16,mCG21161	chemokine (C-X-C motif) ligand 16	Mm00469712_m1
Dhh	desert hedgehog	Mm03053542_s1
Dusp8,mCG130915	dual specificity phosphatase 8	Mm00456230_m1
Emp2,mCG123874	epithelial membrane protein 2	Mm00801709_m1
Epas1,mCG20417	endothelial PAS domain protein 1	Mm00438717_m1
Hdc,mCG2100	histidine decarboxylase	Mm00456104_m1
Icam1,mCG14043	intercellular adhesion molecule 1	Mm00516023_m1
Igf2,mCG11082	insulin-like growth factor 2	Mm00439565_g1
Jam2,mCG129050	junction adhesion molecule 2	Mm00470197_m1
Klf2,mCG18931	Kruppel-like factor 2 (lung)	Mm00500486_g1
Klk10,mCG22144	kallikrein related-peptidase 10	Mm00505112_m1
Lat2,mCG16701	linker for activation of T cells family, member 2	Mm00499104_m1
Mif, mCG3124	macrophage migration inhibitory factor,macrophage migration inhibitory factor-like	Mm01611157_gH
Nos3,mCG16477	nitric oxide synthase 3, endothelial cell	Mm00435204_m1
Pprc1,mCG10300	peroxisome proliferative activated receptor, gamma, coactivator-related 1	Mm00521078_m1
Pthlh,mCG7104	parathyroid hormone-like peptide	Mm00436057_m1
Ramp2,mCG20228	receptor (calcitonin) activity modifying protein 2	Mm00490256_g1
Rhpn2,mCG113678	rhopilin, Rho GTPase binding protein 2	Mm00518451_m1
Tgfb1,mCG7649	transforming growth factor, beta 1	Mm03024053_m1
Tyrobp,mCG22805	TYRO protein tyrosine kinase binding protein	Mm00449152_m1
Vcam1,mCG19764	vascular cell adhesion molecule 1	Mm01320970_m1

Table S4. SYBR Green qPCR probes

Gene Symble	Gene ID	Gene Name	Forward primer 5'-3'	Reverse primer 5'-3'
Ankrd25	NM_145611.4	Mus musculus KN motif and ankyrin repeat domains 2	CCAGGTCCTGCATGTGCCCG	TCCAGGTCCAGGCGGTAGCC
Arhgef15	NM_177566.3	Mus musculus Rho guanine nucleotide exchange factor (GEF) 15	GGCCCAGCAGGTTCTGACC	ACCTGGGGTGGGAAGGCTC
Ctps	NM_016748.2	Mus musculus cytidine 5'-triphosphate synthase	TCCCTGGGGTGCCAGGACTC	ATGGCGAGGGCAACCACAGC
Cyb5r3	NM_029787.2	Mus musculus cytochrome b5 reductase 3	GTGCGTGAGGCCACCGTCTC	GGTGATGGCCGGTGTGGAGC
ICAM2	NM_010494.1	Mus musculus intercellular adhesion molecule 2	CACGGTGTCCCCTGTGCAGC	CGTGGCTGTGGCCTCTTGGG
KLF4	NM_010637.3	Mus musculus Kruppel-like factor 4 (gut	GCAGGTGCCCCGACTAACCG	CTGCACCAGCTCCGCCACTC
Lims2	NM_144862.3	Mus musculus LIM and senescent cell antigen like domains 2	ACGCCAACTGGCATCCTGGC	TTGTGGCAAGGCCGGCAGAG
Lmo4	NM_010723.3	Mus musculus LIM domain only 4 (Lmo4)	GCCGGCTCCCTCTCCTGGAA	GGACGTGCCAATGTGCGCCA
Mfap5	NM_015776.2	Mus musculus microfibrillar associated protein 5	GGCCACCGGCAGACAGATCG	CCGCGTTGACCACTGACCCC
Pak4	NM_027470.3	Mus musculus p21 protein (Cdc42/Rac)-activated kinase 4	CCAGGAGGACCCCAGGAGGC	GTGGTCCGTGTCAGCCCGTG
Plec1	NM_201394.2	Mus musculus plectin 1 (Plec1), transcript variant 11	TCAGAGCCTCCGAGGGCAAGA	GGTTGTGGCCATCACGGAGGTC
Plek2	NM_013738.3	Mus musculus pleckstrin 2 (Plek2)	ACGGCGTGCTCAAGGAAGGC	CCTTGGGCGGGGTTACTCGC
Ptprj	NM_008982.5	Mus musculus protein tyrosine phosphatase, receptor type, J	TGCCCCACAGTCCCCTTCCC	CTTCCTCCCCACCCCCACCC
Rab11fip5	NM_001003955.2	Mus musculus RAB11 family interacting protein 5 (class I)	AGTGGGATCCTGGCCCCTGC	TCTCCCTGGGGCTCTGTGCGC
Sgcd	NM_011891.4	Mus musculus sarcoglycan, delta (dystrophin-associated glycoprotein)	CTGCGTCTGCGCCAATGGGA	TGCTGCCGGCAATTGTCCACT
Slc9a3r2	NM_023055.2	Mus musculus solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 2	GGGCGAGACGCATCACCAGG	AGTGCAGGTCAGTTGCCGCC
Tek	NM_013690.2	Mus musculus endothelial-specific receptor tyrosine kinase	ACTTGCCGCATGCTCAGCCC	TCGGGCCCCCACTTCTGAGC
Timp3	NM_011595.2	Mus musculus tissue inhibitor of metalloproteinase 3	GCTGGAGCCTTGGGCACTGG	AGGGCCCCTCCTTCACCAGC

Table S5. Overrepresented Gene Ontology categories regulated by flow-disturbance in mouse carotid endothelium

<i>12hr post-ligation</i>	<i>No. of Genes</i>	<i>48hr post-ligation</i>	<i>No. of Genes</i>
Diseases and Disorders		Diseases and Disorders	
Developmental Disorder	10	Immunological Disease	40
Cancer	9	Inflammatory Response	75
Immunological Disease	4	Connective Tissue Disorders	22
Cardiovascular Disease	6	Inflammatory Disease	36
Respiratory Disease	4	Skeletal and Muscular Disorders	34
Molecular and Cellular Functions		Molecular and Cellular Functions	
Cellular Growth and Proliferation	12	Cellular Movement	70
Cellular Development	20	Cell-To-Cell Signaling and Interaction	76
Cell Morphology	12	Antigen Presentation	30
Cellular Function and Maintenance	6	Cellular Function and Maintenance	44
Cell Cycle	4	Cellular Growth and Proliferation	73

Table S6. Comparison of flow-sensitive genes found in vivo mouse carotid endothelium to cultured HUVEC

Gene Symbol	Gene Name	Carotid 48hr (LCA/RCA) microarray	Carotid 48hr (LCA/RCA) qPCR	HUVEC 24hr (OS/LS) microarray	Congruency
Downregulated (LCA/RCA)					
KLK10	kallikrein related-peptidase 10	0.10	0.01	ND	N
Col4a3	collagen, type IV, alpha 3	0.22	0.11	ND	N
Bcam	basal cell adhesion molecule	0.23	0.10	0.81	Y
Pthlh	parathyroid hormone-like peptide	0.24	0.08	0.06	Y
Rhpn2	rhopilin, Rho GTPase binding protein 2	0.26	0.12	1.52	N
KLF2	kruppel-like factor 2	0.27	0.06	0.37	Y
Dusp8	dual specificity phosphatase 8	0.27	0.12	0.70	Y
KLF4	kruppel-like factor 4	0.28	0.06	0.12	Y
IGF2	insulin-like growth factor 2	0.29	0.15	0.78	Y
Slc9a3r2	solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 2	0.32	0.11	0.22	Y
Ptprj	protein tyrosine phosphatase, receptor type, J	0.33	0.13	ND	N
Mfap5	microfibrillar associated protein 5	0.33	0.13	0.94	N
Dhh	desert hedgehog	0.36	0.11	0.16	Y
Emp2	epithelial membrane protein 2	0.40	0.22	1.09	N
Lims2	LIM and senescent cell antigen like domains 2	0.40	0.09	0.45	Y
Jam2	junction adhesion molecule 2	0.40	0.17	0.45	Y
Pak4	p21 (CDKN1A)-activated kinase 4	0.42	0.36	0.78	Y
Ramp2	receptor (calcitonin) activity modifying protein 2	0.44	0.16	0.33	Y
NOS3	nitric oxide synthase 3	0.46	0.28	0.25	Y
ICAM2	intercellular adhesion molecule 2	0.48	0.19	1.04	N
Plek2	pleckstrin 2	0.50	0.24	0.52	Y
Epas1	endothelial PAS domain protein 1	0.50	0.20	0.59	Y
Ankrd25	ankyrin repeat domain 25	0.52	0.22	ND	N
Sgcd	sarcoglycan, delta	0.52	0.16	0.97	N
Tek	endothelial-specific receptor tyrosine kinase	0.53	0.23	0.30	Y
Timp3	tissue inhibitor of metalloproteinase 3	0.55	0.30	1.26	N
Arhgef15	Rho guanine nucleotide exchange factor (GEF) 15	0.60	0.29	1.12	N
Cyb5r3	cytochrome b5 reductase 3	0.61	0.40	0.71	Y
Plec1	plectin 1	0.62	0.36	0.50	Y
Rab11fip5	RAB11 family interacting protein 5 (class I) (Rab11fip5), transcript variant 1	0.65	0.56	0.73	Y
Upregulated (LCA/RCA)					
Angpt2	angiopoietin 2	8.44	7.81	4.53	Y
Ctgf	connective tissue growth factor	6.00	22.88	3.87	Y
Cd300a	CD300A antigen	4.31	11.36	ND	N

Tyrobp	TYRO protein tyrosine kinase binding protein	3.64	37.05	ND	N
Cxcl12	chemokine (C-X-C motif) ligand 12	3.43	4.33	1.14	N
Cxcl16	chemokine (C-X-C motif) ligand 16	2.20	1.74	0.56	N
Ctps	cytidine 5'-triphosphate synthase 2	1.98	1.64	1.63	Y
Lmo4	LIM domain only 4	1.70	1.87	1.18	N
VCAM1	vascular cell adhesion molecule 1	1.53	1.90	0.87	N
BMP4	Bone morphogenetic protein 4	1.37	1.25	4.57	Y

Table S7. Comparison of gene expressions between different microarray studies

Gene Symbol	Gene Name	Mouse Carotid 48hr (LCA/RCA) microarray	HUVEC 24hr (OS/LS) microarray	HUVEC 24hr (RF/HSS) microarray	Pig Aorta (DF/UF) microarray	Category *
Downregulated (LCA/RCA)						
KLK10	kallikrein related-peptidase 10	0.10	ND	ND	NA	-
Col4a3	collagen, type IV, alpha 3	0.22	ND	ND	NA	-
Bcam	basal cell adhesion molecule	0.23	0.81	1.60	NA	b
Pthlh	parathyroid hormone-like peptide	0.24	0.06	0.23	NA	b,c
Rhpn2	rhopilin, Rho GTPase binding protein 2	0.26	1.52	1.56	NA	-
KLF2	kruppel-like factor 2	0.27	0.37	0.69	NA	b,c
Dusp8	dual specificity phosphatase 8	0.27	0.70	0.57	NA	b,c
KLF4	kruppel-like factor 4	0.28	0.12	0.45	0.59	a,b,c
IGF2	insulin-like growth factor 2	0.29	0.78	0.63	2.18	b,c
Slc9a3r2	solute carrier family 9 (sodium/hydrogen exchanger), member 3	0.32	0.22	0.63	NA	b,c
Ptprj	regulator 2 protein tyrosine phosphatase, receptor type, J	0.33	ND	0.98	NA	-
Mfap5	microfibrillar associated protein 5	0.33	0.94	ND	NA	-
Dhh	desert hedgehog	0.36	0.16	1.37	NA	b
Emp2	epithelial membrane protein 2	0.40	1.09	0.76	NA	c
Lims2	LIM and senescent cell antigen like domains 2	0.40	0.45	0.45	NA	b,c
Jam2	junction adhesion molecule 2	0.40	0.45	0.59	NA	b,c
Pak4	p21 (CDKN1A)-activated kinase 4	0.42	0.78	1.12	NA	b
Ramp2	receptor (calcitonin) activity modifying protein 2	0.44	0.33	0.73	NA	b,c
NOS3	nitric oxide synthase 3	0.46	0.25	0.44	0.78	b,c
ICAM2	intercellular adhesion molecule 2	0.48	1.04	0.75	NA	c,
Plek2	pleckstrin 2	0.50	0.52	0.53	NA	b,c
Epas1	endothelial PAS domain protein 1	0.50	0.59	0.71	NA	b,c
Ankrd25	ankyrin repeat domain 25	0.52	ND	0.58	NA	c
Sgcd	sarcoglycan, delta	0.52	0.97	ND	NA	-
Tek	endothelial-specific receptor tyrosine kinase	0.53	0.30	0.36	NA	b,c
Timp3	tissue inhibitor of metalloproteinase 3	0.55	1.26	ND	NA	-
Arhgef15	Rho guanine nucleotide exchange factor (GEF) 15	0.60	1.12	0.82	NA	-
Cyb5r3	cytochrome b5 reductase 3	0.61	0.71	1.75	NA	b

Plec1	plectin 1 RAB11 family interacting protein 5 (class I)	0.62	0.50	1.69	NA	b
Rab11fip5	(Rab11fip5), transcript variant 1	0.65	0.73	0.70	NA	b,c

Upregulated (LCA/RCA)

Angpt2	angiopoietin 2	8.44	4.53	1.69	0.61	b,c
Ctgf	connective tissue growth factor	6.00	3.87	0.79	1.59	b
Cd300a	CD300A antigen	4.31	ND	2.02	NA	c
Tyrobp	TYRO protein tyrosine kinase binding protein	3.64	ND	2.04	NA	c
Cxcl12	chemokine (C-X-C motif) ligand 12	3.43	1.14	0.65	NA	-
Cxcl16	chemokine (C-X-C motif) ligand 16	2.20	0.56	1.84	NA	c
Ctps	cytidine 5'-triphosphate synthase 2	1.98	1.63	1.06	NA	b
Lmo4	LIM domain only 4	1.70	1.18	0.97	NA	-
VCAM1	vascular cell adhesion molecule 1	1.53	0.87	ND	NA	-
BMP4	Bone morphogenetic protein 4	1.37	4.57	0.91	NA	b

-
- * **a** : gene expression is consistent between mouse and pig (2)
b : gene expression is consistent between mouse and HUVEC (OS/LS) (23)
c : gene expression is consistent between mouse and HUVEC (RF/HF) (21)

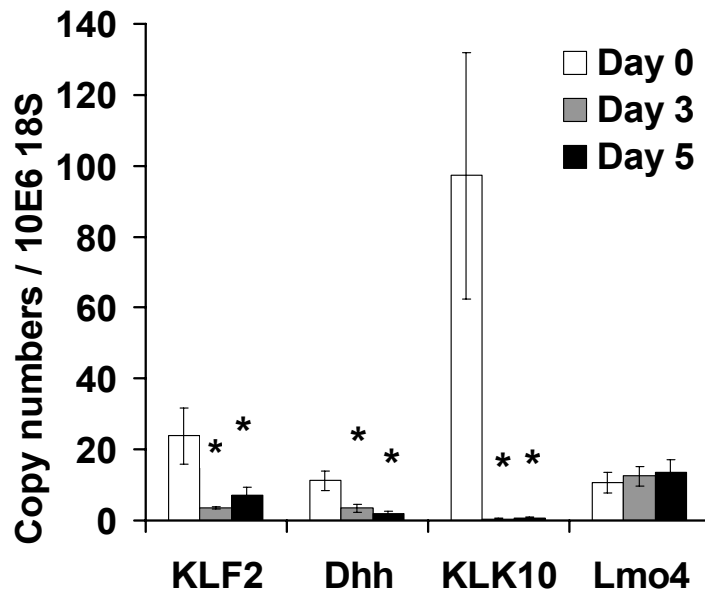


Fig. S1 Endothelial expression of *KLF2*, *Dhh*, and *KLK10*, but not *Lmo4*, decreased during *ex vivo* tissue culture. Mouse carotid rings were incubated *ex vivo* in a growth medium. Intimal RNAs were collected after 0, 3, and 5 days during culture. qPCR analyses were carried out to examine the mRNA levels of *KLF2*, *Dhh*, *KLK10* and *Lmo4*. mRNA copy numbers were normalized against 18S and were shown as mean \pm SEM (n=3), * p<0.05 (vs. Day 0).

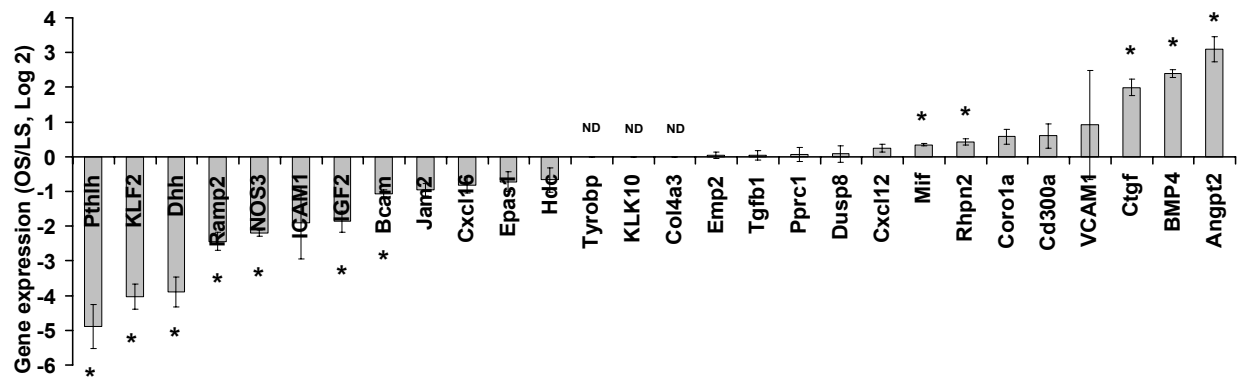


Fig. S2 Validation of shear-sensitive mRNAs in HUVEC by qPCR. Total RNAs were collected from HUVECs exposed to OS or LS for 24hr. qPCR analysis was then performed using SYBR green with custom design primers. mRNA copy numbers were normalized against 18S and are shown as mean \pm SEM (n=4). $p^* < 0.05$ (OS vs, LS). ND is not detectable in qPCR.

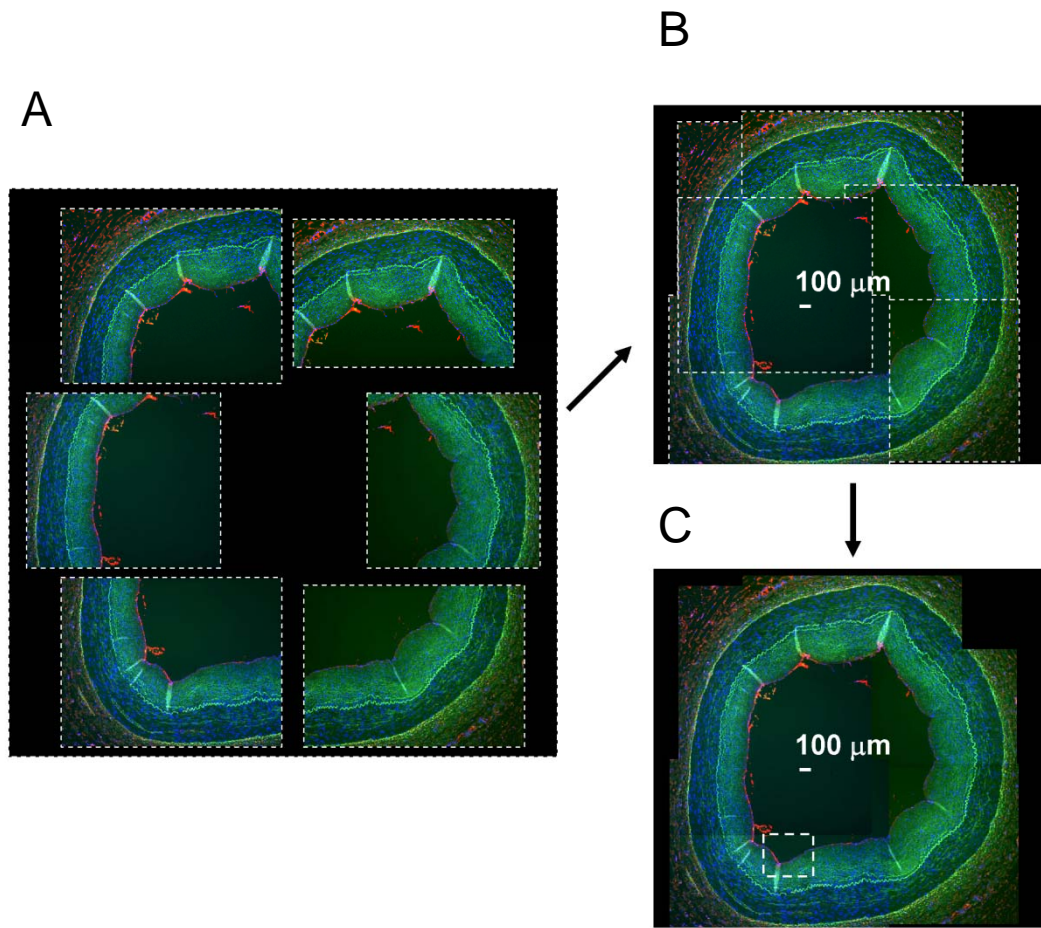


Fig. S3. The raw images of the composite figure shown in Fig 4B. Paraffin sections of human left anterior descending coronary artery were stained for Lmo4 protein expression. A) Fluorescence photomicroscopic images were taken from six different but overlapping regions of the stained section. B) The six images were then overlapped together to make a composite image in order to show an overall staining pattern of the entire coronary artery section. C) White dashed lines indicate the edges of each image. C. White dashed lines were removed for a cleaner view and the final picture is shown in Figure 4B.