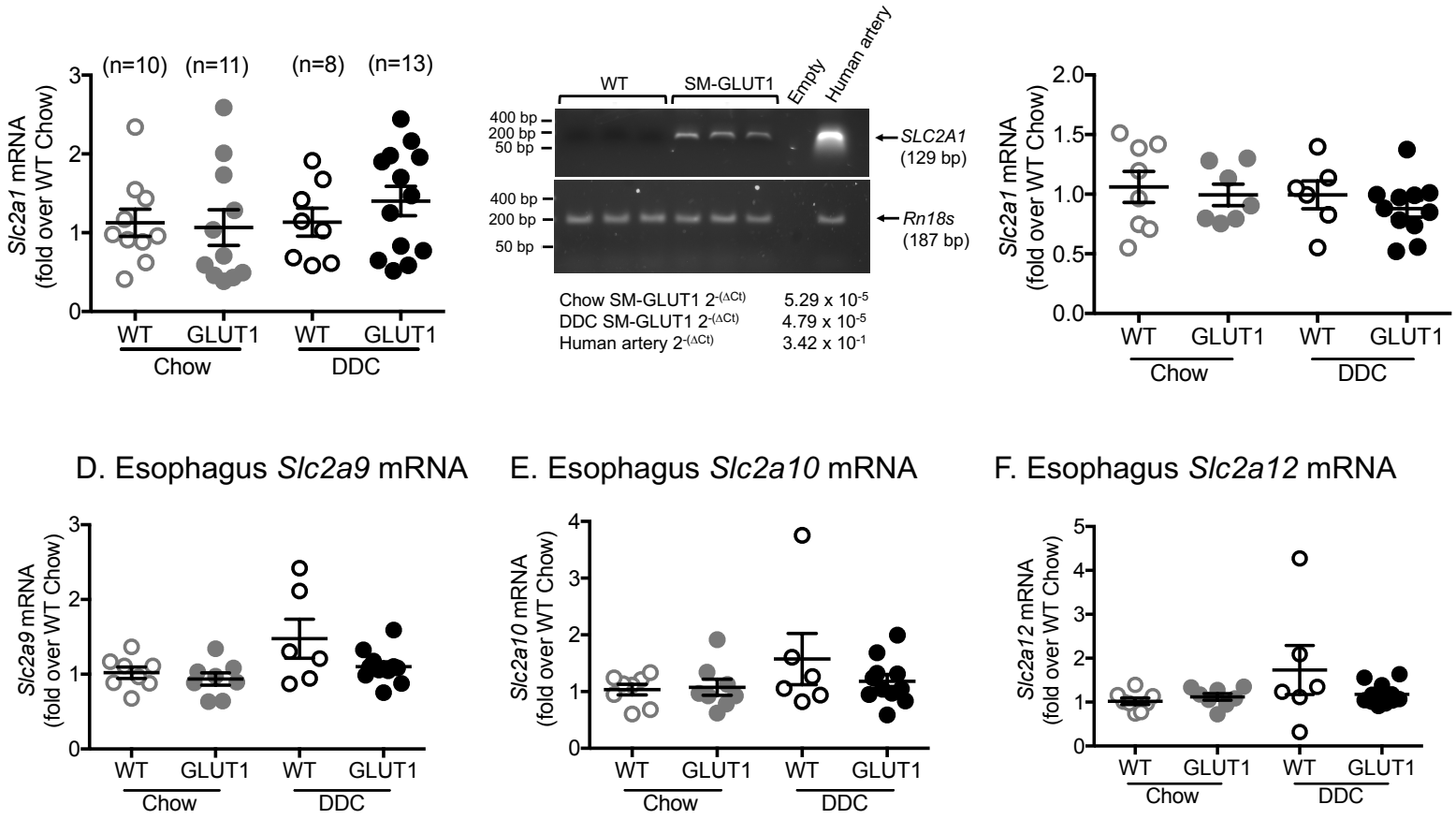

Figure S1. Expression of the human GLUT1 transgene does not alter mouse GLUT1 or other GLUTs expressed in SMCs. Aortic mRNA from SM-GLUT1 mice does not exhibit altered levels of mouse GLUT1 (*Slc2a1*) (A), but shows the human GLUT1 (*SLC2A1*) band of expected size by PCR (B). Note the low levels of human *SLC2A1* in mouse aortas versus a human coronary artery sample. Average $2^{(-\Delta Ct)}$ values are shown below the gels. *Rn18s* was used as a loading control. Esophagi were isolated from a subset of mice and used for measurements of mouse GLUTs expressed in SMCs; GLUT1 (*Slc2a1*) (C), GLUT9 (*Slc2a9*) (D), GLUT10 (*Slc2a10*) (E), and GLUT12 (*Slc2a12*) (F). Results are expressed as mean ± SEM (n=8-13 in A; in C-E, n=8 in WT and SM-GLUT1 chow groups, n=6 in WT DDC group, and n=11 in SM-GLUT1 DDC group).

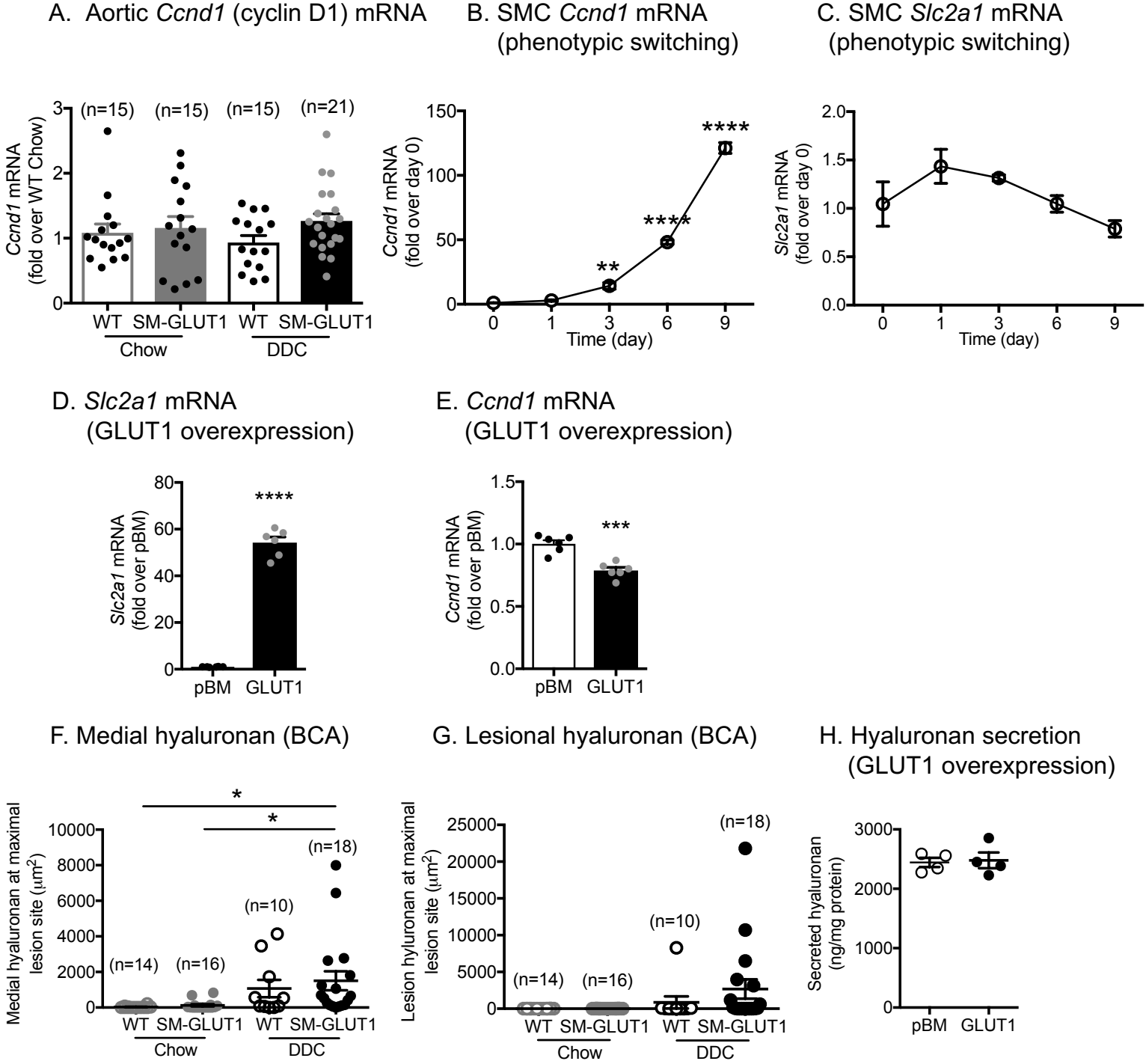

Figure S2. SMC proliferation and hyaluronan production do not appear to be directly mediated by GLUT1 expression. Aortic mRNA from SM-GLUT1 mice has a very modest, non-significant, increase in *Ccnd1* mRNA (A). Mouse aortic SMCs in culture in the presence of 20% fetal bovine serum adopt a synthetic, proliferative phenotype (B), which does not associate with *Slc2a1* mRNA upregulation (C). Overexpression of GLUT1 in mouse aortic SMCs was achieved with a retroviral vector (D). GLUT1 overexpression did not alter the proliferative status of these cells in response to 10% fetal bovine serum, as measured by *Ccnd1* mRNA (E). Presence of medial and lesional hyaluronan in BCA cross sections was detected using a biotinylated hyaluronan-binding protein (F-G). Hyaluronan secretion from SMCs transduced with an empty pBM virus or GLUT1 virus (H). Results are expressed as mean ± SEM (n=15-21 in A; n=3 in B-C; n=6 in D-E; n=10-18 in F-G; n=4 in H). Statistical analysis was performed by ANOVA with Tukey's post hoc test (A-C; F-G), or two-tailed unpaired Student's t-test (D-E); *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

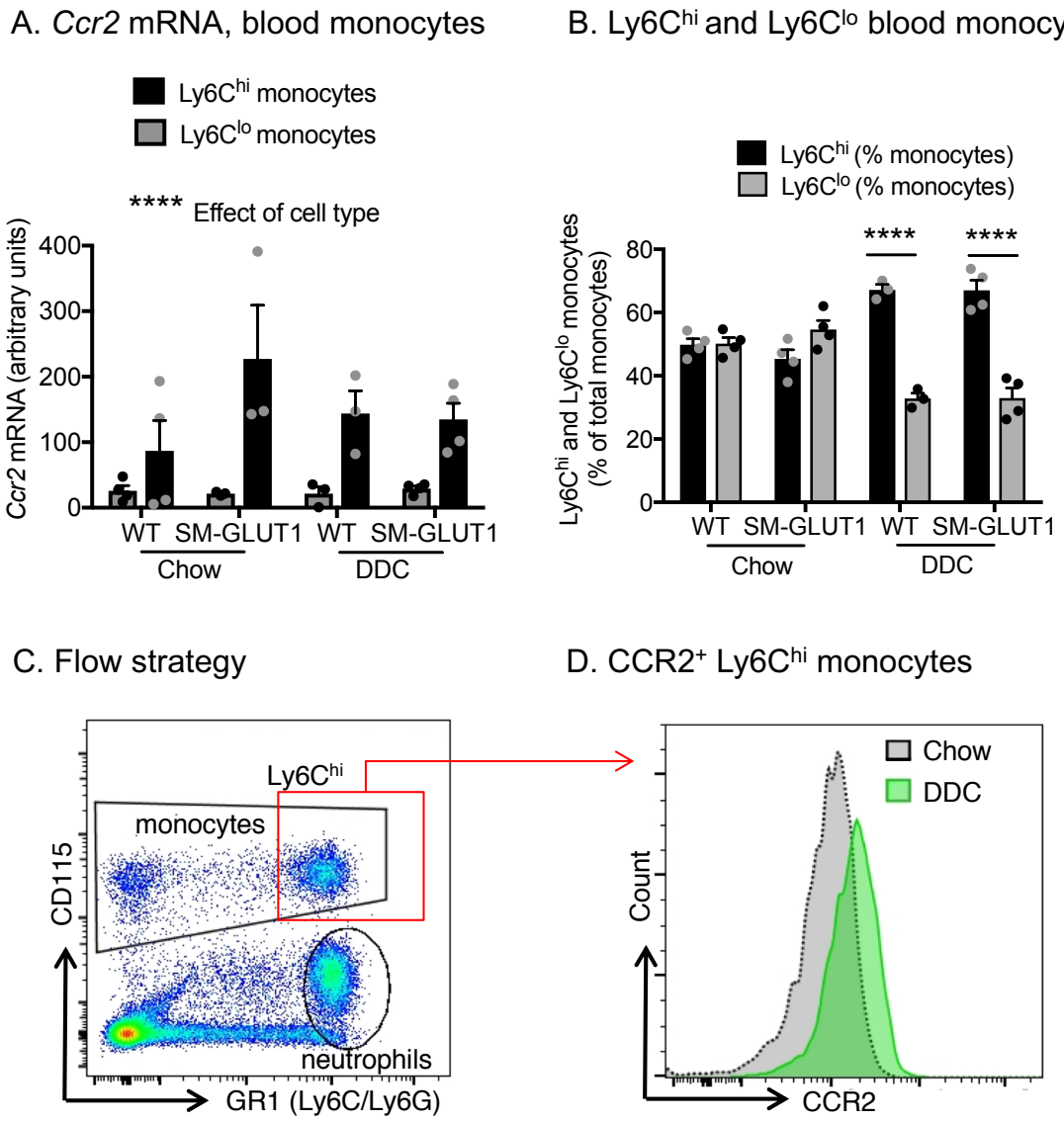

Figure S3. Smooth muscle GLUT1 overexpression does not alter Ly6C^{hi} or Ly6C^{lo} monocytes or *Ccr2* mRNA in monocytes. SM-GLUT1 *Ldlr*$^{-/-}$ mice and *Ldlr*$^{-/-}$ littermates were fed DDC for 4 weeks. (A) *Ccr2* mRNA levels were measured by real-time PCR in Ly6C^{hi} and Ly6C^{lo} monocytes in mice fed DDC or chow for 4 weeks. *Ccr2* levels were similar in SM-GLUT1 mice and controls, and were significantly higher in Ly6C^{hi} monocytes, as compared with Ly6C^{lo} monocytes. (B) Blood Ly6C^{hi} and Ly6C^{lo} monocyte populations were assessed by flow cytometry and results were expressed as % of total CD115^{+} monocytes. SM-GLUT1 expression had no effect, but DDC-feeding increased the relative amount of Ly6C^{hi} monocytes. (C) Flow strategy for analysis of cell surface CCR2 levels in myeloid cells. (D) Representative flow data showing CCR2-positive Ly6C^{hi} monocytes from chow-fed and DDC-fed mice. Results are expressed as mean ± SEM. Statistical analysis was performed by ANOVA with Tukey's post hoc test; n=3-4; ****p<0.0001

Supplemental Table 2. BCA lesion morphology. The BCAs from all chow-fed mice and from DDC-fed mice with plasma cholesterol levels above 1000 mg/dl were serial sectioned and Movat's stained, and every 6th section was scored for the presence or absence of the morphological features below. The results are expressed as frequencies ± SEM. Statistical analysis was performed by Kruskal-Wallis test for non-parametric data followed by Dunn's multiple comparison tests. * $p<0.05$ versus WT chow group; $^{\#}$ $p<0.05$ versus SM-GLUT1 chow group. One animal in the chow-fed SM-GLUT1 group was excluded as a statistical outlier by Grubbs' test. The lower part of the table shows Ki-67 immunopositive medial SMCs, lesional SMCs and lesional macrophages in BCA cross-sections, based on co-localization. CLC, chondrocyte-like cells

Feature	**WT Chow (n=21)**	**SM-GLUT1 Chow (n=23)**	**WT DDC (n=18)**	**SM-GLUT1 DDC (n=18)**
Medial acellularity	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.02	0.09 ± 0.03*
Medial glycosaminoglycans	0.15 ± 0.05	0.06 ± 0.02	0.31 ± 0.06$^{\#}$	0.62 ± 0.09*$^{\#}$
Medial collagen	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.02	0.04 ± 0.02*
Medial CLCs	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lesional glycosaminoglycans	0.01 ± 0.01	0.02 ± 0.02	0.17 ± 0.06	0.29 ± 0.08*$^{\#}$
Lesional collagen	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.02	0.04 ± 0.03
SMC droplets	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.04	0.21 ± 0.7*$^{\#}$
Lesional SMCs	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.04	0.21 ± 0.07*$^{\#}$
Lesional CLCs	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02
Cholesterol clefts	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.04*
Calcification	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Feature	**WT Chow (n=12)**	**SM-GLUT1 Chow (n=13)**	**WT DDC (n=11)**	**SM-GLUT1 DDC (n=16)**
Ki-67-positive medial SMCs (cells/BCA cross-section)	0.7 ± 0.7	0.8 ± 0.6	1.3 ± 0.8	2.2 ± 1.4
Ki-67-positive lesional SMCs	0.1 ± 0.1	0.0 ± 0.0	0.4 ± 0.3	4.0 ± 2.2
Ki-67-positive macrophages	0.6 ± 0.5	0.3 ± 0.2	9.3 ± 4.9	8.8 ± 3.1

Supplemental Table 3. Primer sequences (mouse and human)

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
Mouse		
Abca1	CTCAGAGGTGGCTCTGATGAC	CCCATACAGCAAGAGCAGAAG
Acta2	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Ccnd1	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
Ccr2	ATCCACGGCATACTATCAACATC	TCGTAGTCATACGGTGTGGTG
Cd11b	TGTGAGCAGCACTGAGATC	ATGGCTCCACTTTGGTCTC
Cnn1	GACGGGATCATTCTTTGCGAA	CCCCATACTTGGTAATGGCTTTG
Cxcl1	TGGCTGGGATTCACCTCAAG	CCGTTACTTGGGGACACCTT
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Has1	CTTTCAAGGCACTGGGCGACTCC	TCCAGCAGTGCCATGGGGTCTAG
Has2	GCCTTCAGAGCACTGGGGCGAA	TCCACAGATGAGGCAGGGTCAAGC
Has3	CCTGGAGCACCGTCGAATG	CCTTGAGGTTTGGAAAGGCAA
Hk2	ATGATCGCCTGCTTATTCACG	CGCCTAGAAATCTCCAGAAGGG
Pfkfb3	TCACCGACCCTCGTTGCCCA	TGCTTCACAGCCTCACGCCG
Rn18s	CATTAAATCAGTTATGGTTCCTTTGG	CCCGTCGGCATGTATTAGCT
Slc2a1	CAGTTCGGCTATAACACTGGTG	GCCCCCGACAGAGAAGATG
Scl2a9	CCCTCCTTCCTGTGGACTCT	ACGAGCGAGAAGGACCATTT
Slc2a10	CGGGGATTTGACCAGGACAC	CATATCCGAAGGTCAGGCCC
Slc2a12	CCCAGCTGCTTACAAATGGC	CACTAAGCACCAACCAAGGC
Slc16a4	CCTTACACCAAACCCCTTGATG	CCAATCCAACCAATTTGCTCTG
Tnfa	CCTGTAGCCCACGTCGTAG	GGGAGTAGACAAGGTACAA
Txnip	GGCCGGACGGGTAATAGTG	AGCGCAAGTAGTCCAAAGTCT
Human		
SLC2A1	CTGCTCATCAACCGCAAC	CTTCTTCTCCCGCATCATCT