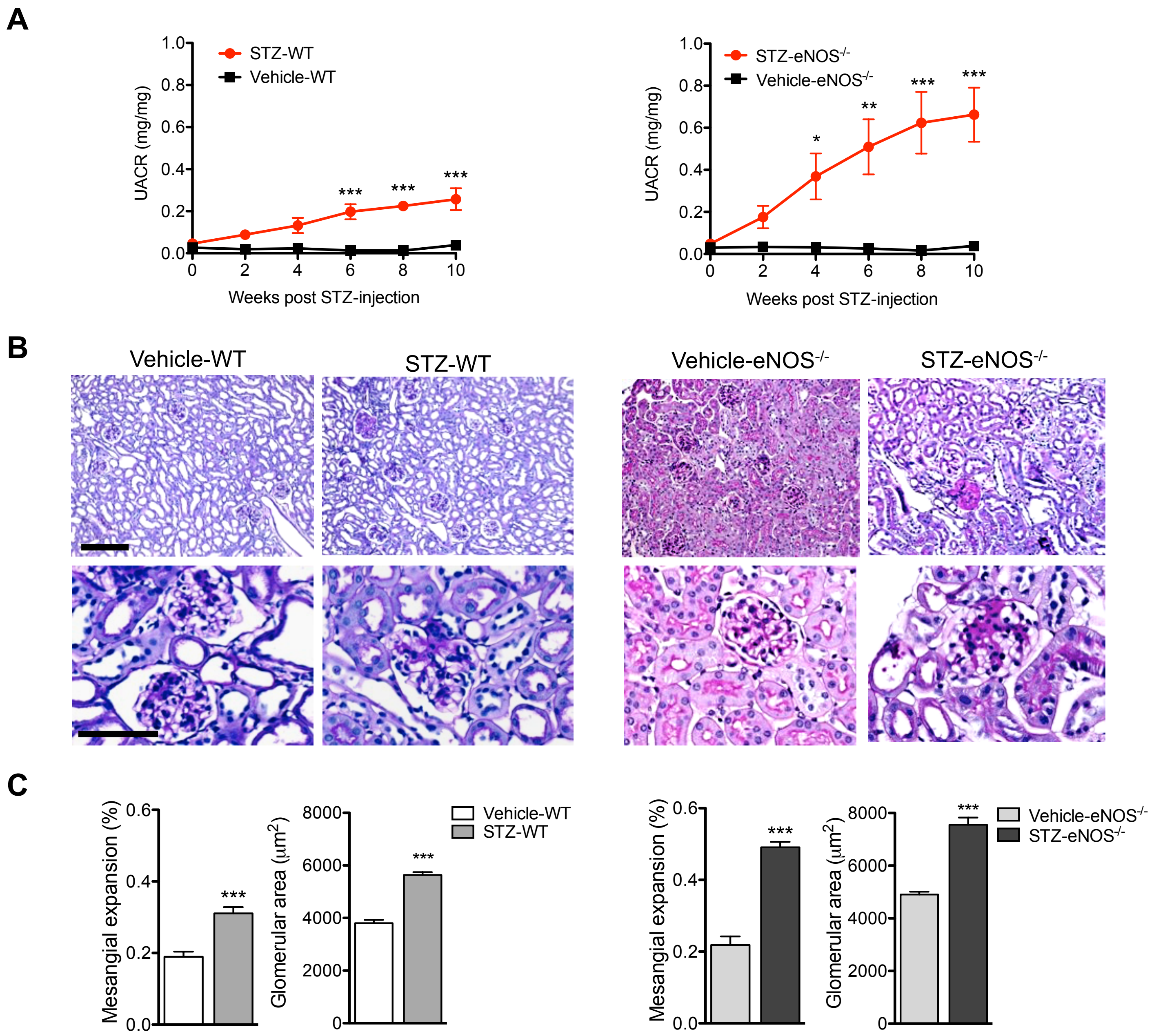
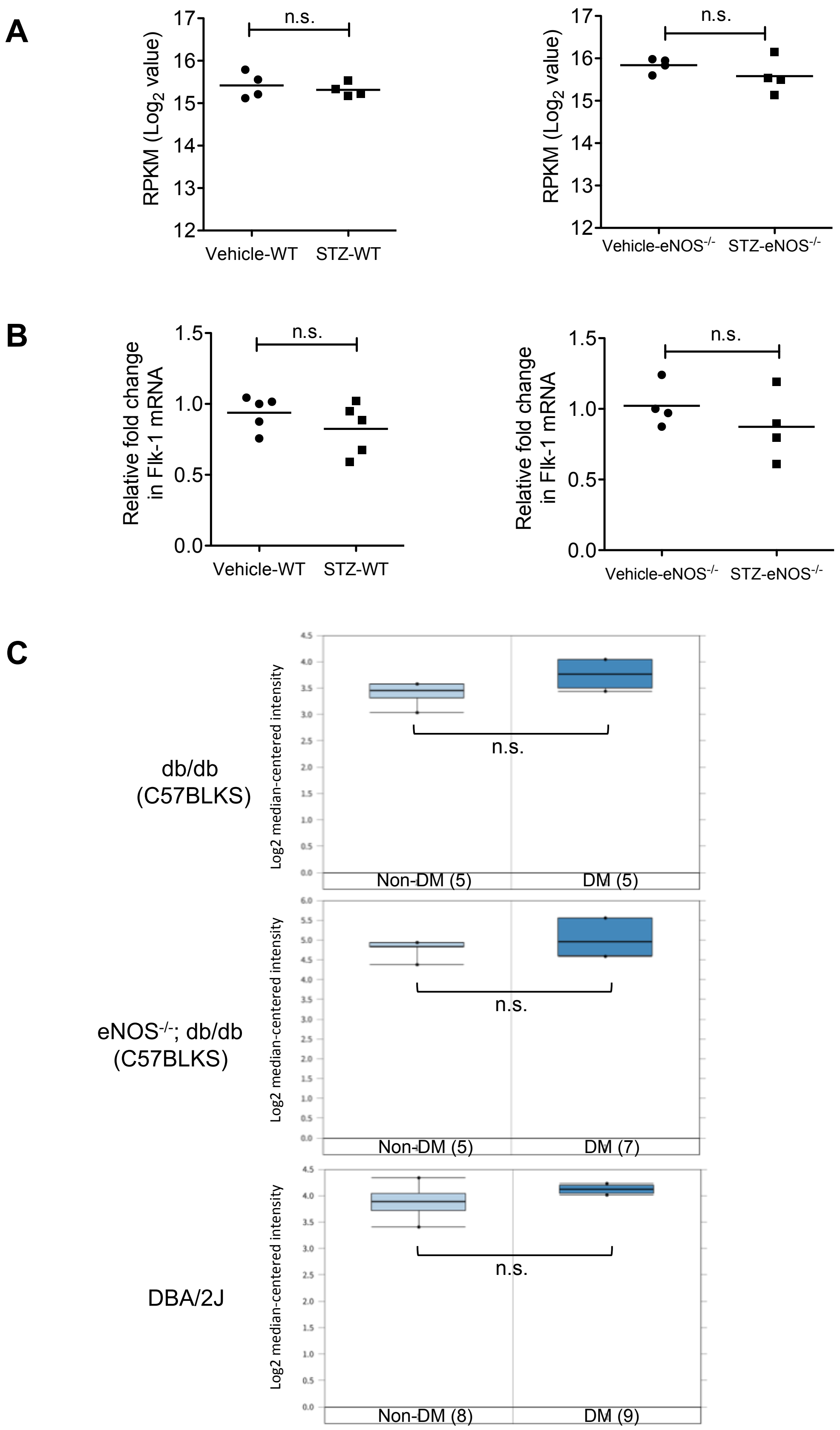
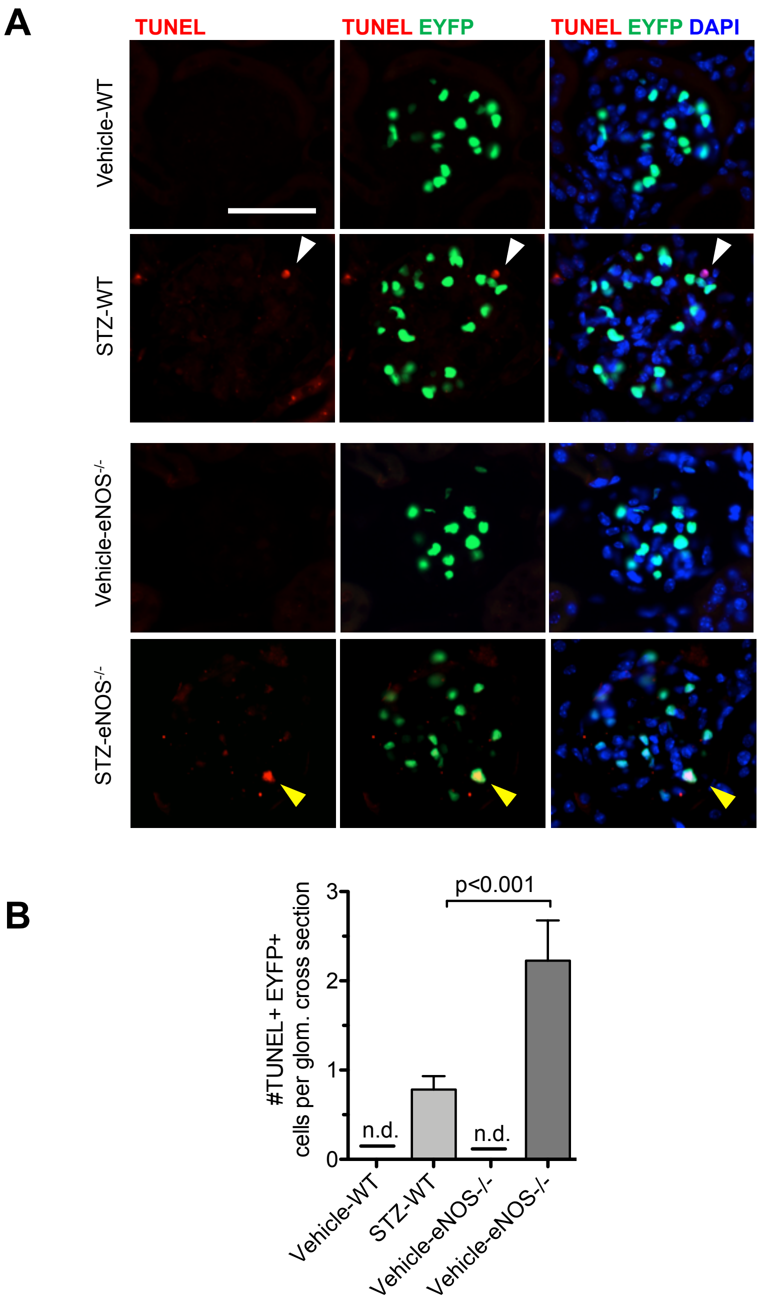
Supplementary Figures



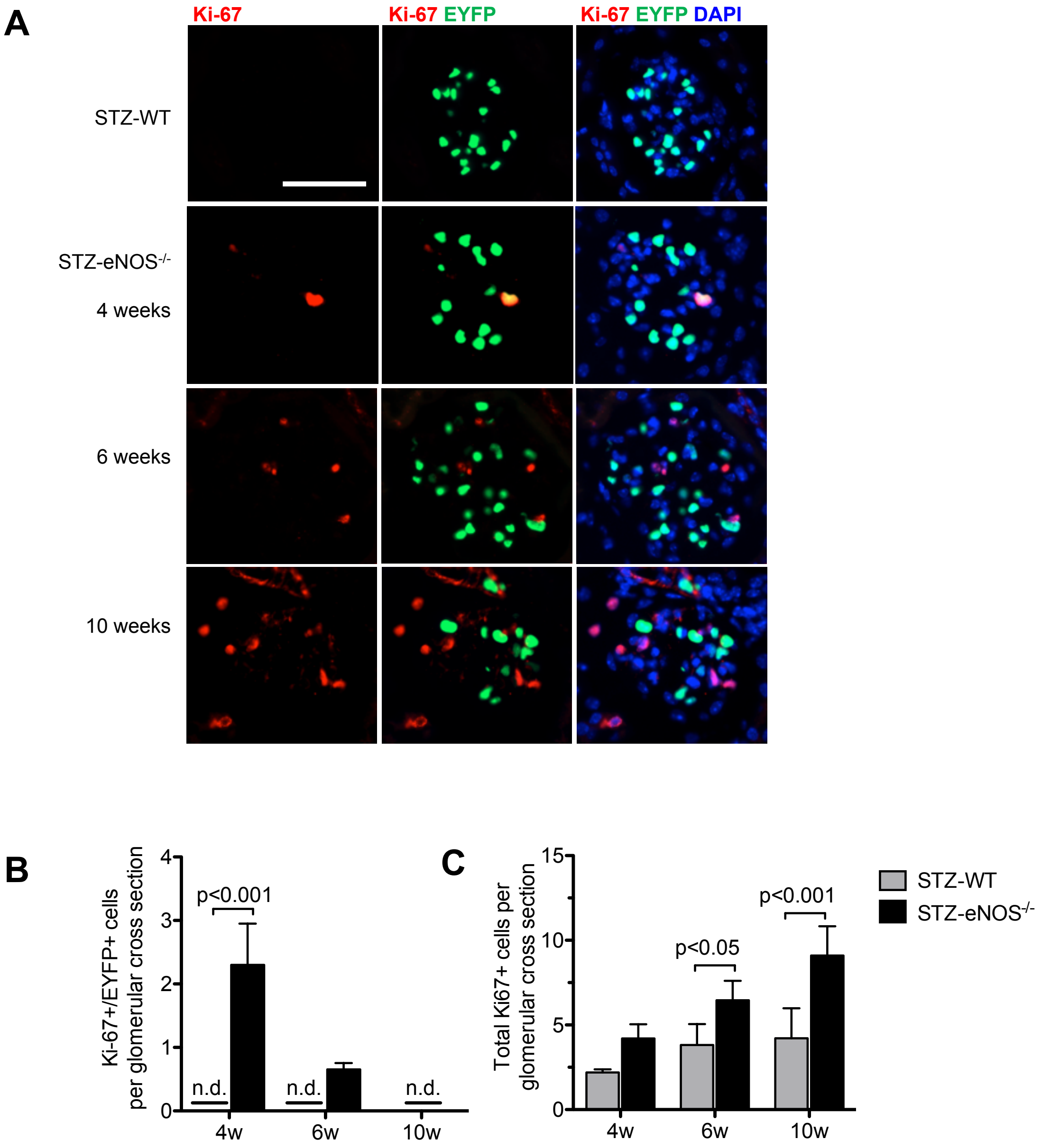
Supplementary Figure 1: Proteinuria and glomerular injury is worsened in diabetic eNOS$^{-/-}$ mice compared to diabetic wildtype mice. (A) Urinary albumin to creatinine ratio (UCAR) shows the development of proteinuria in diabetic mice. (B) Periodic-acid Schiff-stained kidney sections at 100x (top) and 200x (bottom) magnification. Scale bar: 50μm. (C) Mesangial area and glomerular volume is increased in STZ-diabetic mice. *P<0.05, **P<0.01, and ***P<0.001 compared to respective vehicle-control.



Supplementary Figure 2: Flk1 (KDR) Expression in glomeruli did not change between normal and diabetic mice. A) Relative Flk1 expression between vehicle and STZ-injected mice by qPCR. (B) Log2 value of Flk1 based on mRNA sequencing reads. (C) Expression of Flk1 from 3 datasets of mouse diabetic glomeruli in Nephroseq. RPKM, Reads Per Kilobase of transcript per Million mapped reads; DM, diabetes mellitus; n.s., not significant.

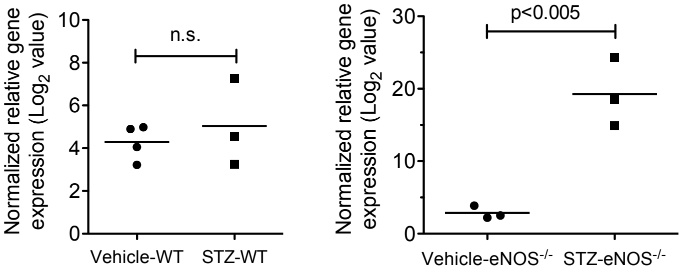


Supplementary Figure 3: Determination of apoptosis in GECs by TUNEL assay. (A) Representative fluorescent images of EYFP and TUNEL-positive cells in mouse glomeruli. White arrows shows TUNEL+, EYFP- cells in STZ-WT glomeruli. Yellow arrow show TUNEL+, EYFP+ cell in the STZ-eNOS$^{-/-}$ glomeruli. Scale Bar: 50µm. (B) Quantification of # of TUNEL+, EYFP+ cells per glomerular cross section. Results are mean±SEM of at least 20 glomeruli evaluated per group (n=3 mice per group). n.d., not detected.

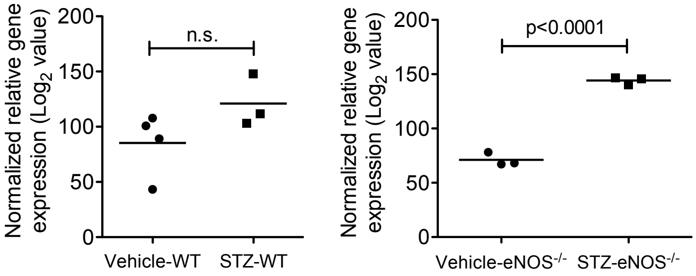


Supplementary Figure 4: Determination of GEC proliferation by Ki-67 immunofluorescence. (A) Representative fluorescent images of EYFP+ and Ki-67+ cells in mouse glomeruli. (B) Quantification of # of Ki-67+, EYFP+ cells per glomerular cross section. (C) Quantification of total # of Ki-67+ cells per glomerular cross section. Results are mean±SEM of at least 20 glomeruli evaluated per group (n=3 mice per group). n.d., not detected.

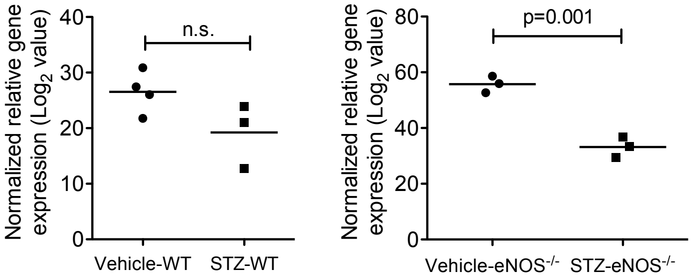
***Lrg1* expression:**



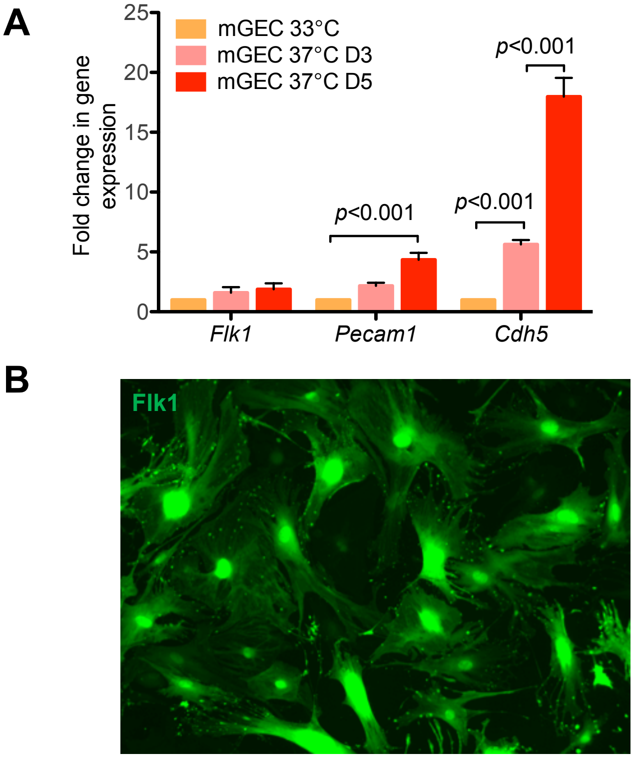
***Gpr56* expression:**



***Zfp57* expression:**



Supplementary Figure 5: mRNA expression of *Lrg1*, *Gpr56*, and *Zfp57* in GECs. Expression of *Lrg1*, *Gpr56*, and *Zfp57* levels from mRNA sequencing results are shown as RPKM (Reads Per Kilobase of transcript, per Million mapped reads) as Log2 values.



Supplementary Figure 6: mGEC characterization and overexpression lentivectors. Immortalized mGECs established from Flk1-H2B-EYFP mice were cultivated at 33°C and further subcloned by either limiting dilution or by single cell sorting into 96-well plates. All in vitro experiments were performed with a single clone (Clone 1) obtained from the outgrowth of a clonal population from single cell sorting into a 96 well plate. (A) Expression of endothelial marker expression by qPCR analysis of mGECs cultivated at 33°C (with IFN-γ), at 37°C for three days (D3), or at 37°C for 5 das (D5) (n=3). (B) Immunofluorescence of Flk-1 (membrane-localized green) is shown in mGEC cultivated for 5 days at 37°C. Robust H2B-EYFP (nucleus-localized green) is still visible in clone1 mGEC.

Supplementary Tables

Supplementary Table 1: Top 10 upregulated GO pathways (Enrichr) in STZ-WT vs. Vehicle-WT GECs

Term	p-value	Genes
apoptotic mitochondrial changes (GO:0008637)	1.54E-05	GGCT;MAPK9;JUN;GCLC;ERBB4;BNIP3;PMAIP1;GCLM;ATP7A;BCL2L1;MCL1;IFIT2
mitochondrion organization (GO:0007005)	1.66E-04	MGME1;JUN;HSP90AA1;EPAS1;BNIP3;TFB2M;IFIT2;GGCT;MAPK9;GCLC;COL4A3BP;ERBB4;PMAIP1;MPV17;LPIN1;GCLM;ATP7A;BCL2L1;MCL1
response to unfolded protein (GO:0006986)	1.73E-04	GSK3A;HSP90AA1;FKBP14;GFPT1;DAB2IP;HSPB1;PARP16;YOD1;HSPE1;HSPD1;DNAJA1;HSPH1;CASP12;DDIT3;CREBRF;HSPA1A
apoptotic signaling pathway (GO:0097190)	3.83E-04	CDKN1A;GSK3A;CUL5;ROCK2;RASGRF2;CUL2;XPA;FOXO3;EDA2R;CASP12;APH1B;PMAIP1;JAK2;PHLDA3;MCL1;PDPK1;SORT1;BNIP3;DAPK3;DAB2IP;AEN;CFLAR;DDIT3;DDIT4;JMY;ITGB3BP;BCL2L1
developmental maturation (GO:0021700)	6.58E-04	FZD5;CBFB;IRX5;EPAS1;NTN4;SYP;FOXO3;KLF2;CLN5;DLG4;DDIT3;GNAQ;S1PR1;PPARG;CFTR;HOXA5
pattern specification process (GO:0007389)	9.35E-04	BMPR2;LEF1;GLI1;CXXC4;YY1;EFNB1;SOX17;ERBB4;BASP1;TMEM107;DVL2;HOXA5;BPTF;EDN1;FZD5;TCF15;IRX3;PCGF2;PBX3;FBXL15;PALB2;TTC8;HOXB9;HOXB4;HOXB2;HOXB8;HOXB7;HOXD8;HOXB6
regionalization (GO:0003002)	1.18E-03	EDN1;BMPR2;TCF15;PCGF2;LEF1;PBX3;FBXL15;GLI1;PALB2;YY1;HOXB9;SOX17;BASP1;TMEM107;HOXB4;HOXB2;HOXB8;HOXB7;HOXA5;HOXB6;BPTF
response to virus (GO:0009615)	1.36E-03	RSAD2;DDX58;BNIP3;MX2;NLRC5;IFI44;HSPB1;IFIT1;IFIT3;IFIT2;IFIH1;OAS2;HYAL1;DDIT4;IRF7;TRIM25;PMAIP1;BCL2L1;TRIM56;GBP3;HSPA1A
intracellular receptor signaling pathway (GO:0030522)	1.67E-03	DDX58;HNF4G;NR1D2;NR1D1;NR2F6;PIAS1;DCBLD2;IFIH1;DNAJA1;IRAK2;CRY2;KLF9;IRF7;TXNIP;PPARG;JAK2;BCL2L1
negative regulation of cell growth (GO:0030308)	1.37E-03	CDKN1A;GSK3A;BMPR2;DAB2IP;JADE1;ADIPOR2;DCBLD2;SOX17;BCL6;HYAL1;SERTAD2;CCDC85B;SLIT1;PPARG;HSPA1A

Supplementary Table 2: Top 10 upregulated GO pathways (Enrichr) in STZ-eNOS$^{-/-}$ vs. Vehicle-eNOS$^{-/-}$ GECs

Term	p-value	Genes
platelet activation (GO:0030168)	4.08E-07	PROS1;ITGB3;SERPINE1;ITGA2B;ARRB1;RASGRP2;GNG2;RAP1A;TBXA2R;LAMP2;CFL1;MAPK1;CD36;RAC1;SRGN;VAV3;ABCC4;ENTPD1;FCER1G;HSPA5;RHOG;MERTK;SOD1;RHOB;F8;PECAM1;MGLL;F2RL3
apoptotic signaling pathway (GO:0097190)	2.85E-06	CDKN1A;PPP1R13B;YWHAB;CUL2;CIB1;BRCA2;EDA2R;PPM1F;POLB;APH1B;MCF2L;MKNK2;UBC;CD38;PMAIP1;HMOX1;RAC1;PHLDA3;SRGN;VAV3;SORT1;BNIP3;AEN;HIC1;SGPP1;P2RX7;OBSCN;P2RX4;DDIT4;SELK;BAX;FAS;FGFR3;ITGB3BP;BCL2L1
protein folding (GO:0006457)	4.47E-06	FKBP10;HSP90AB1;FKBP14;ITGB3;DNAJC24;HSP90B1;BAG5;PSMD5;MLEC;QSOX1;B2M;PDIA3;HSPA8;TOR3A;RIC3;FKBPL;ST13;TUBB4B;PDIA6;PDIA4;FKBP1A;DNAJA4;CANX;FKBP7;PPIH;CALR;FKBP5
angiogenesis (GO:0001525)	4.95E-06	ACVRL1;COL18A1;RAMP2;CLIC4;ECM1;NOTCH1;EPAS1;ITGB3;FLT4;SERPINE1;CIB1;CX3CL1;HEY1;SOX18;S1PR1;KDR;HMOX1;EPHB4;HTATIP2;VAV3;JUN;ANXA2;GPR56;RHOB;EFNA1;ID1;MMRN2;ANGPTL4
regulation of angiogenesis (GO:0045765)	1.09E-05	ACVRL1;RAMP2;ECM1;NOTCH1;SERPINE1;LECT1;HSPB1;CIB1;ADM;CX3CL1;AQP1;RHOB;EFNA1;CXCL10;LRG1;HYAL1;TBXA2R;ID1;MMRN2;KDR;HMOX1;ANGPTL4;HTATIP2
response to drug (GO:0042493)	1.39E-05	COL18A1;CDKN1A;LPL;ATP1A1;PPM1F;ICAM1;AQP1;PNP;CCND1;TBXA2R;PDE4A;CD38;B2M;SNCA;VAV3;MEF2A;ABCC4;JUN;EDN1;SLC12A5;PDE2A;GGH;FOS;DUSP6;FOSL2;SOD1;ACER2;CDK1;TXNIP;PPARG;CALR;UMPS;CFTR;ENG;ABCG2
negative regulation of protein modification process (GO:0031400)	1.96E-05	ERRFI1;CDKN1A;YWHAB;GBA;HSPB1;ARRB1;CIB1;PTPRJ;PPM1F;SOCS3;UCHL1;BAG5;PSMD5;CAMK2N1;HYAL2;UBC;INPP5K;XDH;FBXO7;SNCA;JUN;DUSP3;DUSP1;PDE4D;LIMK1;ISG15;SMAD6;DUSP6;SMAD7;PER2;ACER2;FABP4;PPAP2B;DDIT4;CRY1;BAX;TRIB1;ENG
regulation of endothelial cell proliferation (GO:0001936)	2.11E-05	ACVRL1;ECM1;JUN;BMPR2;FLT4;ITGB3;LECT1;HMGB2;BMP6;NR4A1;LRG1;KDR;FGFR3;XDH;ENG
positive regulation of defense response (GO:0031349)	2.16E-05	SERPINE1;GIMAP5;CX3CL1;COCH;HSP90B1;IKBKB;CTSL;HYAL2;UBC;MAPK1;CD36;CTSB;TGM2;MAP2K3;MEF2A;JUN;FCER1G;DUSP3;IL15;PDE2A;IRAK4;FOS;OSMR;DUSP6;NFKBIA;GPRC5B;FABP4;CDK1;LGMN
response to oxygen levels (GO:0070482)	2.37E-05	CDKN1A;RAMP2;ALAS2;NOTCH1;EPAS1;CUL2;ADM;PLAT;PLOD1;AQP1;ICAM1;HSP90B1;UBC;UCP2;CAPN2;PMAIP1;HMOX1;CD38;EDN1;NOS2;BNIP3;SMAD9;FNDC1;DDIT4;TACC3;FAS;ANGPTL4;ENG

Supplementary Table 3: Top 10 downregulated GO pathways (Enrichr) in STZ-WT vs. Vehicle-WT GECs

Term	**p-value**	**Genes**
electron transport chain (GO:0022900)	4.76E-08	NDUFA8;NDUFA13;NDUFB8;NDUFB7;NDUFA11;NDUFB11;NDUFB4;NDUFA2;NDUFA1;UQCR11;UQCR10;ATP5H;ATP5O;ATP5G1;COX7C;UQCRH;COX6B1;TBXA2R;ATP5E;CYC1;NDUFV3;CYB561
generation of precursor metabolites and energy (GO:0006091)	6.49E-07	GLP1R;NDUFA13;NDUFB8;NDUFB7;NDUFA11;NDUFB11;NDUFB4;AQP7;UQCR11;UQCR10;ATP5H;PYGL;ATP5O;COX7A1;ATP5G1;COX7C;PRKAG3;UQCRH;LDHA;GNG2;TBXA2R;BLOC1S1;ATP5E;CYC1;NDUFV3;NDUFA8;KCNB1;ATPIF1;PGAM2;NDUFA2;NDUFA1;CACNA2D2;GNG11;PHKA2;COX6B1;PPP1R1A;PPP1R3C;PPP1R3D;CYB561;RAPGEF3
extracellular matrix organization (GO:0030198)	3.57E-06	COL15A1;RAMP2;COL16A1;SPARC;ITGB5;LAMA4;ITGA2B;ILK;TNFRSF11B;OLFML2A;NID2;FBLN5;HAPLN1;CAPNS1;CASP3;ABI3BP;ITGB7;JAM2;ANXA2;ITGA3;FN1;LAMB1;GDF5;HSPG2;MMP10;BMP2;ADAM15;LOX;MMP15;COL4A1;COL5A3;COL5A2;MFAP2;COL6A1;VWA1;ITGA6;TGFBI
chemotaxis (GO:0006935)	7.83E-05	HMGB2;DEFB1;TREM1;PIK3CG;TYMP;EDNRB;FLRT2;FLRT3;CCL5;NTF3;CCRL2;DPYSL3;CMKLR1;MAP2K1;EGR3;CMTM3;HGF;RHOG;VEGFC;IL1B;RAB13;KIT;PLXNB3;ACKR3;ACKR2;MET;EPHA2
hydrogen ion transmembrane transport (GO:1902600)	5.42E-05	CYB5A;UQCR11;ATP5G3;UQCR10;ATP5H;ATP5G2;ATP5O;COX7A1;ATP5G1;COX7C;UQCRH;COX6B1;SLC9A5;ATP5E;ATP6V1E1;ATP6V1F
protein localization to membrane (GO:0072657)	1.63E-04	NDUFA13;RAMP2;RAMP3;RPL32;RPL11;RPL36A;NKD2;RPL7;RPS4X;RPS15;RPS14;RPS18;SEC61G;ITGB7;RPL39;RAB8A;PEX16;ANXA2;SSR2;TIMM22;HPS6;ANK3;RPS27;SPCS1;DOK7;MYADM;VAMP5
angiogenesis (GO:0001525)	2.19E-04	EGFL7;COL15A1;RAMP2;ANXA2;NOS3;ATPIF1;FN1;VEGFC;VASH1;HSPG2;SAT1;PLXDC1;PIK3R6;PIK3CG;TYMP;DLL4;EFNA1;TMEM100;ADAM15;ACKR3;TGFBI;CD34;RAPGEF3;EPHA2
cell-substrate adhesion (GO:0031589)	2.86E-04	ITGB5;ITGA3;ITGA2B;FN1;ILK;LAMB1;ADAMTS12;NID2;FBLN5;EFNA1;TIAM1;ADAM15;COL5A3;MICALL2;ITGA6;ITGB7;CD34
cellular protein complex disassembly (GO:0043624)	2.73E-04	RPL32;RPL11;RPL36A;MICAL2;RPL7;RPS4X;RPS15;RPS14;RPS27;KIF18B;DNAJC6;RPS18;STMN1;KIF2C;RPL39
extracellular matrix organization (GO:0030198)	3.37E-04	PEX16;RPL32;ANXA2;SSR2;RPL11;RPL36A;ANK3;NKD2;RPL7;RPS4X;RPS15;RPS14;RPS27;SPCS1;RPS18;SEC61G;MYADM;RPL39

Supplementary Table 4: Top 10 downregulated GO pathways (Enrichr) in STZ-eNOS$^{-/-}$ vs. Vehicle-eNOS$^{-/-}$ GECs

Term	**p-value**	**Genes**
ncRNA metabolic process (GO:0034660)	1.60E-11	ZCCHC11;VARS;TSEN15;WDR4;MTO1;RRP8;TSEN54;RRP9;FBL;NARS2;DUS4L;UTP14A;UTP15;PRMT5;NCBP1;TYW5;DIMT1;DIS3;NSUN5;TYW3;PUS1;ELAC2;NSUN2;DDX56;ELAC1;TRPT1;IMP4;TFB2M;UTP11L;QTRTD1;QRSL1;FTSJ1;UTP18;GTPBP3;WDR77;FTSJ3;QTRT1;CTU2;DALRD3;SARS2;CIRH1A;PPA2;RRNAD1;DKC1;PAPD5;TUT1;GEMIN5;EEF1E1;DUS3L;TRUB1;NOLC1;LAGE3;RBFA;NOL6;EXOSC7;EXOSC5;EMG1;PDCD11;WBSCR22;UTP20;SMN1;RPP14;PUSL1;TRDMT1;EXOSC1;DCAF13;FDXACB1;SMAD2;NOP56;NOP14;UTP6;NOP58;SMAD3;GATC;TRMT1;RRP1B;PA2G4;PUS7L;HARS2;TARBP2;WARS2;BOP1;LAS1L;AGO2;TRMT13;INTS6;FARSA;NOP10
rRNA processing (GO:0006364)	2.66E-07	NOLC1;RBFA;RRP8;RRP9;NOL6;EXOSC7;FBL;EXOSC5;EMG1;PDCD11;WBSCR22;UTP20;UTP14A;EXOSC1;DCAF13;NOP56;UTP15;NOP14;UTP6;NOP58;DIMT1;DIS3;NSUN5;DDX56;IMP4;TFB2M;PA2G4;UTP11L;RRP1B;UTP18;FTSJ3;BOP1;CIRH1A;LAS1L;RRNAD1;DKC1;PAPD5;NOP10
chromatin modification (GO:0016568)	8.94E-07	KDM5A;SUV420H2;CRTC2;KDM5C;SETD8;HDAC10;LDB1;PHF1;SETD7;HDAC11;JADE1;DPY30;ARID4A;PRDM2;JADE3;CHD4;CHD3;BMI1;RRP8;MECP2;ELK4;ING5;FBL;ING2;ING3;RPS6KA5;EPC1;HMG20A;EPC2;JAK2;JARID2;HMGN3;CSRP2BP;KDM6A;TSPYL2;NCOA1;PRMT6;PRMT5;SMARCC1;KDM2A;PRMT7;USP49;KDM2B;PRMT2;RING1;DTX3L;PRMT3;DNMT3A;TET2;MSL3;TET1;PRKCA;CHD1L;ARID1B;TERF2;DDB2;JMJD6;DNAJC2;KAT2B;WDR82;KAT6B;CLOCK;KDM7A;HDAC5;ENY2;PRKAA2;L3MBTL2;MEAF6;LEF1;DOT1L;HDAC6;TAF5L;BAG6;HLCS;LEO1;SMYD2;WBSCR22;PCGF1;BRD9;APBB1;DMAP1;CBX2;SS18L1;PCGF2;ELP4;TDRD3;HOPX;ASXL1;TADA2A;KANSL2;CDK2;TADA1;TCF3;OGT
RNA modification (GO:0009451)	4.25E-06	TRUB1;RNMT;WDR4;TRMT2A;MTO1;EMG1;PUSL1;DUS4L;TRDMT1;WTAP;TYW5;DIMT1;PUS1;NSUN2;METTL3;TRMT1;TFB2M;QTRTD1;PUS7L;FTSJ1;FTSJ3;GTPBP3;QTRT1;RPUSD4;CTU2;RRNAD1;DKC1;DUS3L;NOP10
tRNA metabolic process (GO:0006399)	6.22E-06	TRUB1;VARS;TSEN15;WDR4;MTO1;LAGE3;TSEN54;FBL;NARS2;PUSL1;RPP14;DUS4L;TRDMT1;FDXACB1;TYW5;PUS1;TYW3;ELAC2;NSUN2;ELAC1;GATC;TRPT1;TRMT1;QTRTD1;PUS7L;QRSL1;FTSJ1;HARS2;WARS2;GTPBP3;QTRT1;CTU2;DALRD3;SARS2;PPA2;TRMT13;EEF1E1;FARSA;DUS3L
methylation (GO:0032259)	2.45E-05	N6AMT1;SUV420H2;SETD8;SETD7;WDR4;ARID4A;DPY30;PRDM2;RRP8;GCSH;MECP2;TRMT112;FBL;COMTD1;METTL13;METTL16;KDM6A;PRMT6;HEMK1;PRMT5;PRMT7;PRMT2;DIMT1;NSUN5;TYW3;NSUN2;PRMT3;DNMT3A;METTL3;TET2;NSUN6;TFB2M;FTSJ1;FTSJ3;METTL23;RRNAD1;WDR82;PICK1;GART;RNMT;SMYD5;DOT1L;TRMT2A;PRDM10;EMG1;ZFP57;SMYD2;WBSCR22;DPH5;DMAP1;TRDMT1;ATF7IP;WTAP;TRMT1;TRMT13;ZCCHC4;OGT
positive regulation of GTPase activity (GO:0043547)	4.77E-05	BCAR3;USP6NL;CD40;DOCK3;FAM13B;DENND1A;DOCK7;ARHGEF10L;ARHGAP6;RCBTB2;ARHGAP4;ARHGAP44;DOCK10;RGS4;DOCK11;RGS5;SYDE1;ARHGAP42;SYNGAP1;RASGEF1A;AKT2;ARHGDIB;EVI5L;ARHGEF40;GARNL3;PSD;PLEKHG3;PLEKHG1;PLEKHG2;ARHGEF15;DIS3;ARHGEF17;DENND2A;AXIN1;ARAP1;ARFGAP1;VAV2;BCR;ARHGAP10;TIAM1;TBC1D1;ARHGEF9;RABEP1;RASA3;SPATA13;RASA2;TBC1D25;PTPLAD1;DENND6B;DOCK2;SOS1;RAPGEF6;ARHGEF5;LLGL1;ARHGEF6;TBC1D19;ARHGEF25;ARHGAP18;RASAL3;KALRN;FGD1;FGD6;CHN2;NTF3;ERBB2;NCKAP1L;EPS8L2;WNT4;FARP1;STARD13;EIF2B3;DENND4B;DENND4A;RAB3IP;MYO9A;SGSM1;ARHGAP33;TBCK;ARHGAP32;SNX18;ARHGAP30;SGSM2;RGL3;PLXNB1;AGRN;SMAP2;RGL1
regulation of cell adhesion (GO:0030155)	7.67E-05	DDR1;PTPRR;ECM2;LDB1;CITED2;PDE3B;ILK;PTPN23;ARHGAP6;PPP1CB;FLCN;C1QBP;ARHGDIB;PIEZO1;ITGAV;JAK2;CYTIP;HOXA7;PRKG1;PPP1R12A;SYK;PRKCD;PRKCA;ANK3;DUSP26;GTPBP4;SFRP2;MYADM;COL8A1;PRKD2;UTRN;KANK1;ROCK2;LAMA4;LEF1;ALOX12;PRKX;PIK3R1;FBLN2;THBS1;ATXN3;NUAK1;BAG4;MAPK7;ERBB2;NCKAP1L;JAM2;WNT4;TGFB2;SMAD3;DMTN;ANGPT2;MYO10;FZD7;VEGFC;LAMB1;BRAF;ASS1;DAB2;BMP2;CXCL12;PTPRC;SNAI2;PLXNB1;SERPINI1
regulation of Ras GTPase activity (GO:0032318)	7.31E-05	USP6NL;CD40;DENND1A;DOCK7;ARHGEF10L;ARHGAP6;RCBTB2;ARHGAP4;DOCK10;DOCK11;SYDE1;SYNGAP1;RASGEF1A;AKT2;EVI5L;ARHGEF40;PLEKHG3;EPHA4;PLEKHG1;PLEKHG2;ARHGEF15;ARHGEF17;DENND2A;ARAP1;ARFGAP1;VAV2;BCR;ARHGAP10;TIAM1;TBC1D1;ARHGEF9;RASA3;SPATA13;RASA2;TBC1D25;DENND6B;SOS1;DOCK2;ARHGEF5;LLGL1;ARHGEF6;TBC1D19;ARHGEF25;RASAL3;KALRN;FGD1;FGD6;NTF3;ERBB2;NCKAP1L;EPS8L2;RICTOR;WNT4;FARP1;NTRK2;DENND4B;DENND4A;RAB3IP;SGSM1;ARHGAP33;TBCK;SGSM2;RGL3;PLXNB1;AGRN;RGL1;SMAP2
positive regulation of Rho GTPase activity (GO:0032321)	4.08E-05	CD40;ARHGEF25;DOCK7;ARHGEF10L;ARHGAP6;KALRN;FGD1;ARHGAP4;DOCK10;DOCK11;SYDE1;FGD6;ERBB2;EPS8L2;NCKAP1L;ARHGEF40;WNT4;PLEKHG3;FARP1;PLEKHG1;PLEKHG2;ARHGEF15;ARHGEF17;ARAP1;VAV2;BCR;ARHGAP33;ARHGAP10;TIAM1;ARHGEF9;SPATA13;PLXNB1;DOCK2;SOS1;AGRN;ARHGEF5;ARHGEF6

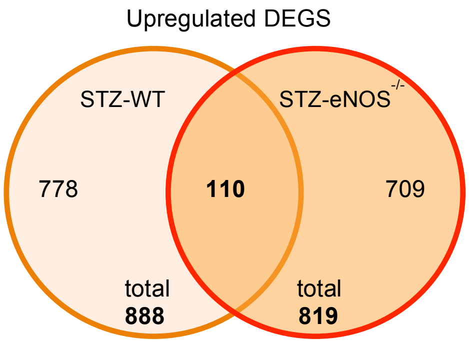
Supplementary Table 5: Top 10 upregulated GO pathways (Enrichr) in STZ-eNOS$^{-/-}$ vs. Vehicle-eNOS$^{-/-}$ GECs

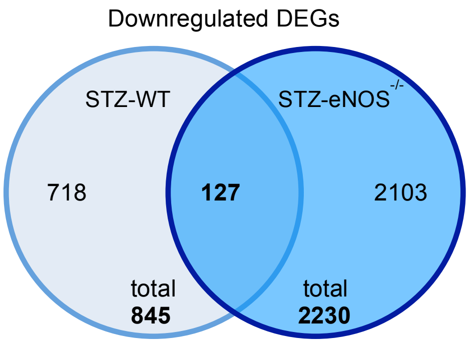
Term	**p-value**	**Genes**
positive regulation of inflammatory response (GO:0050729)	2.26E-08	NLRP12;SEMA7A;SERPINE1;TNFAIP3;LPL;S100A9;SELE;PLA2G7;S100A8;CX3CL1;TLR2;BIRC3
neutrophil degranulation (GO:0043312)	3.63E-08	ITGAM;ATP8A1;GMFG;PYGL;CNN2;ADGRG3;TRPM2;HK3;ALDH3B1;CXCR2;QSOX1;ACE;FCER1G;NFAM1;RHOG;PLAUR;AMPD3;TUBB4B;MMP8;MMP9;LRG1;CLEC4D;FABP5;LCN2;ADAM8;S100A9;S100A8;TLR2;LTF
inflammatory response (GO:0006954)	4.11E-05	NRROS;NFAM1;F2R;CXCR4;FOS;SELE;CXCL2;ADORA2A;IL1B;CXCR2;NLRP3;ADAM8;S100A9;S100A8
negative regulation of Notch signaling pathway (GO:0045746)	4.69E-05	DLL4;GALNT11;NLRP12;SRMS;IGFBP3;CHAC1
negative regulation of lipopolysaccharide-mediated signaling pathway (GO:0031665)	5.39E-05	NLRP12;SRMS;IGFBP3;TRIB1;LTF
negative regulation of insulin receptor signaling pathway (GO:0046627)	1.00E-04	SOCS3;NLRP12;SRMS;CISH;IGFBP3;IL1B
dendritic cell chemotaxis (GO:0002407)	1.76E-04	CCR1;TRPM2;CXCR2;CXCR4
negative regulation of CD40 signaling pathway (GO:2000349)	2.27E-04	NLRP12;SRMS;IGFBP3;TNFAIP3
positive regulation of actin cytoskeleton reorganization (GO:2000251)	2.41E-04	TRPM2;NTF3;HCLS1;BAIAP2;SORBS3
negative regulation of cell adhesion mediated by integrin (GO:0033629)	2.45E-04	EFNA1;ACER2;SERPINE1;TGFBI;EPHA2;RND1

Supplementary Table 6: Top 10 downregulated GO pathways (Enrichr) in STZ-eNOS$^{-/-}$ vs. Vehicle-eNOS$^{-/-}$ GEC

Term	**p-value**	**Genes**
synapse assembly (GO:0007416)	3.95E-08	CHRM3;PCDHGB6;PCDHGB5;FUT10;PCDHGB2;EFNB3;ST8SIA4;PCDHGA8;GDF11;PCDHGA7;PCDHGA6;PCDHGA5;FZD5;PCDHGA4;PCDHGA3;PCDHGC5;PCDHGA2;PCDHGC4;PCDHGA1;PCDHGA9;PCDHGA10;DNM3;PCDHGA11;OLFM1;PCDHGA12;ZEB2;MPPED2;PCDHGB1;FGF11
response to unfolded protein (GO:0006986)	1.80E-07	DNAJA1;DNAJB2;HSPA8;DNAJB1;HSP90AA1;HSPH1;HSPA4L;HSPA1A;HSPD1
ATP generation from ADP (GO:0006757)	1.36E-04	HSPA8;AK1;MYH8;CMPK2;HSPA1B;HSPA1A
renal system development (GO:0072001)	1.45E-04	PAX8;SOX17;IFT140;ITGB4;PTCH1
negative regulation of protein ubiquitination (GO:0031397)	4.42E-04	DNAJA1;DNAJB2;HSP90AA1;CHORDC1;HSPA1B;SOX4;HSPA1A
membrane fusion (GO:0061025)	5.42E-04	DNM3;NAPB;UBXN2A;MX2;CHORDC1;DNM1
regulation of cellular response to heat (GO:1900034)	8.20E-04	HSPA8;DNAJB1;HSP90AA1;HSPH1;PTGES3;CHORDC1;CRYAB;HSPA1B;HSPA1A
positive regulation of cell-cell adhesion (GO:0022409)	9.77E-04	EPHA7;CXCL12;CITED2;LEF1;CHRD;CCR7;DUSP26
kidney development (GO:0001822)	1.24E-03	TGFB2;PAX8;SOX17;IFT140;ITGB4;PTCH1;PCSK5
canonical Wnt signaling pathway involved in positive regulation of epithelial to mesenchymal transition (GO:0044334)	1.28E-03	OLFM1;TGFB2;SFRP1;FZD3;FZD5;EGF;LEF1;SOX4;BCL9L

Supplementary Tables 7-10: Analysis of DEGs[STZ-eNOS$^{-/-}$ vs. Vehicle-eNOS$^{-/-}$] vs. DEGs[STZ-WT vs. vehicle-WT]





Supplementary Table 7: Top 10 GO pathways of *shared* upregulated DEGs between [STZ-eNOS$^{-/-}$ vs. Vehicle-eNOS$^{-/-}$] and [STZ-WT vs. vehicle-WT] (110 genes)

Term	**p-value**	**Genes**
intrinsic apoptotic signaling pathway by p53 class mediator (GO:0072332)	9.12E-07	CDKN1A;DDIT4;PMAIP1;AEN;EDA2R;PHLDA3
apoptotic signaling pathway (GO:0097190)	1.02E-06	CDKN1A;APH1B;SORT1;BNIP3;DDIT4;CUL2;PMAIP1;AEN;ITGB3BP;EDA2R;BCL2L1;PHLDA3
regulation of cell growth (GO:0001558)	3.50E-05	CDKN1A;BMPR2;BCL6;HYAL1;FAM107A;ISLR2;PPARG;ADIPOR2;PLXNA4;CXCL16
positive regulation of cellular component movement (GO:0051272)	1.03E-04	EDN1;BMPR2;BCL6;HYAL1;SERPINE1;S1PR1;HSPB1;MAPK1;CXCL16
white fat cell differentiation (GO:0050872)	1.18E-04	PER2;FABP4;PPARG
regulation of chemotaxis (GO:0050920)	1.22E-04	EDN1;BMPR2;SERPINE1;S1PR1;HSPB1;PLXNA4
regulation of endothelial cell proliferation (GO:0001936)	2.63E-04	ECM1;JUN;BMPR2;LECT1;XDH
positive regulation of response to external stimulus (GO:0032103)	2.86E-04	EDN1;BMPR2;FABP4;SERPINE1;S1PR1;HSPB1;OSMR
negative regulation of intracellular signal transduction (GO:1902532)	3.14E-04	TLE1;PIK3IP1;BCL6;DDIT4;HSPB1;RASAL2;XDH;BCL2L1;PHLDA3
release of cytochrome c from mitochondria (GO:0001836)	6.53E-04	JUN;PMAIP1;BCL2L1

Supplementary Table 8: Top 10 GO pathways of *shared* downregulated DEGs between [STZ-eNOS$^{-/-}$ vs. Vehicle-eNOS$^{-/-}$] and [STZ-WT vs. vehicle-WT] (127 genes)

Term	**p-value**	**Genes**
positive regulation of cell migration (GO:0030335)	7.46E-05	BMP2;SEMA6A;NTF3;MYADM;ILK;VEGFC;LAMB1;MET;HBEGF
positive regulation of cell development (GO:0010720)	9.13E-05	CAMK2B;TIAM1;BMP2;SEMA6A;VDR;NTF3;MYADM;ILK;VEGFC;RARB
extracellular matrix organization (GO:0030198)	9.33E-05	BMP2;COL15A1;COL16A1;MMP15;CASP3;LAMA4;COL5A2;ILK;LAMB1;JAM2
muscle cell differentiation (GO:0042692)	1.25E-04	BMP2;EDNRB;IGFBP5;NTF3;RARB;MET
positive regulation of MAP kinase activity (GO:0043406)	2.72E-04	CD40;BMP2;DBNL;NTF3;ILK;MET;PIK3R6
cell morphogenesis (GO:0000902)	3.34E-04	FAM101B;BMP2;VDR;MICALL2;ILK;LAMB1;MET
positive regulation of neurogenesis (GO:0050769)	4.42E-04	CAMK2B;TIAM1;BMP2;SEMA6A;NTF3;ILK;VEGFC;RARB
neuron projection guidance (GO:0097485)	5.29E-04	SEMA6A;FLRT2;FLRT3;COL5A2;NTF3;EVL;LAMB1;ANK3;MET
morphogenesis of an epithelium (GO:0002009)	5.90E-04	ADAMTS16;BMP2;HHEX;VDR;ILK;VEGFC;MET;MTSS1
filopodium assembly (GO:0046847)	6.05E-04	SPATA13;MTSS1L;MTSS1

Supplementary Table 9: Top 10 GO pathways of upregulated DEGs *only* in [STZ-eNOS$^{-/-}$ vs. Vehicle-eNOS$^{-/-}$] and not in [STZ-WT vs. vehicle-WT] (709 genes)

Term	**p-value**	**Genes**
platelet activation (GO:0030168)	2.38E-06	SRGN;VAV3;ABCC4;ENTPD1;FCER1G;HSPA5;ITGB3;ITGA2B;RHOG;ARRB1;RASGRP2;MERTK;SOD1;RHOB;GNG2;RAP1A;TBXA2R;LAMP2;CFL1;PECAM1;CD36;RAC1;MGLL;F2RL3
cell morphogenesis involved in differentiation (GO:0000904)	2.51E-05	MEF2A;COL18A1;CLIC4;NOTCH1;ARHGEF26;FZD4;ITGB3;NOTCH4;PXN;MERTK;EFNA1;RELN;HEY1;ID1;HES1;RAC1;FERMT3;VAMP3
angiogenesis (GO:0001525)	5.65E-05	ACVRL1;VAV3;COL18A1;RAMP2;CLIC4;NOTCH1;ANXA2;GPR56;ITGB3;FLT4;CIB1;CX3CL1;RHOB;EFNA1;HEY1;SOX18;ID1;MMRN2;KDR;HMOX1;ANGPTL4;EPHB4;HTATIP2
protein folding (GO:0006457)	6.12E-05	PDIA3;FKBP10;HSPA8;TOR3A;HSP90AB1;FKBPL;ST13;ITGB3;TUBB4B;PDIA6;HSP90B1;PDIA4;FKBP1A;BAG5;PSMD5;CANX;FKBP7;MLEC;QSOX1;PPIH;CALR;B2M
regulation of blood pressure (GO:0008217)	8.47E-05	ACVRL1;KCNK6;RAMP2;ACE;NOS2;ECE1;ATP1A1;ADH5;MANF;SOD1;P2RX4;TBXA2R;ID2;HMOX1;SGK1;ENG
establishment of endothelial barrier (GO:0061028)	9.20E-05	RAP1A;SOX18;PDE4D;PDE2A;MSN;ICAM1
vasculogenesis (GO:0001570)	9.63E-05	GJC1;RAMP2;NOTCH1;HEY1;SOX18;FZD4;ZMIZ1;KDR;ADM;PTPRJ;ENG
regulation of cytoskeleton organization (GO:0051493)	9.81E-05	CLIC4;ARPC1B;CIB1;SKA3;PPM1F;ICAM1;CAPZB;CFL1;CAPN2;INPP5K;SH3BGRL3;RAC1;SNCA;MEF2A;PLK4;ARHGEF10;LIMK1;SHROOM2;BAIAP2;SORBS3;BST2;P2RX7;TPX2;CLIP1;ARPC3;DLC1;CDC42EP2;MAPT;MYO1F
regulation of vasculature development (GO:1901342)	1.02E-04	ACVRL1;RAMP2;NOTCH1;CIB1;ADM;CX3CL1;AQP1;RHOB;EFNA1;CXCL10;RAP1A;LRG1;HEY1;TBXA2R;ID1;MMRN2;KDR;HMOX1;ANGPTL4;HTATIP2
toll-like receptor signaling pathway (GO:0002224)	1.12E-04	MEF2A;MAP2K3;DUSP3;IRAK4;FOS;DUSP6;HSP90B1;IKBKB;NFKBIA;CTSL;UBC;CDK1;CD36;CTSB;LGMN

Supplementary Table 10: Top 10 GO pathways of downregulated DEGs *only* in [STZ-eNOS$^{-/-}$ vs. Vehicle-eNOS$^{-/-}$] and not in [STZ-WT vs. vehicle-WT] (2103 genes)

Term	**p-value**	**Genes**
ncRNA metabolic process (GO:0034660; GO0034470)	2.15E-12	ZCCHC11;VARS;TSEN15;WDR4;MTO1;RRP8;TSEN54;RRP9;FBL;NARS2;DUS4L;UTP14A;UTP15;PRMT5;NCBP1;TYW5;DIMT1;DIS3;NSUN5;TYW3;PUS1;ELAC2;NSUN2;DDX56;ELAC1;TRPT1;IMP4;TFB2M;UTP11L;QTRTD1;QRSL1;FTSJ1;UTP18;GTPBP3;WDR77;FTSJ3;QTRT1;CTU2;DALRD3;SARS2;CIRH1A;PPA2;RRNAD1;DKC1;PAPD5;TUT1;GEMIN5;EEF1E1;DUS3L;TRUB1;NOLC1;LAGE3;RBFA;NOL6;EXOSC7;EMG1;PDCD11;WBSCR22;UTP20;SMN1;RPP14;PUSL1;TRDMT1;EXOSC1;DCAF13;FDXACB1;SMAD2;NOP56;NOP14;UTP6;NOP58;SMAD3;GATC;TRMT1;RRP1B;PA2G4;PUS7L;HARS2;TARBP2;WARS2;BOP1;LAS1L;AGO2;TRMT13;INTS6;FARSA;NOP10
chromatin modification (GO:0016568)	1.52E-07	KDM5A;SUV420H2;CRTC2;KDM5C;SETD8;HDAC10;LDB1;PHF1;SETD7;HDAC11;JADE1;DPY30;ARID4A;PRDM2;JADE3;CHD4;CHD3;BMI1;RRP8;MECP2;ELK4;ING5;FBL;ING2;ING3;RPS6KA5;EPC1;HMG20A;EPC2;JAK2;JARID2;HMGN3;CSRP2BP;TSPYL2;NCOA1;PRMT6;PRMT5;SMARCC1;KDM2A;PRMT7;USP49;KDM2B;PRMT2;RING1;DTX3L;PRMT3;DNMT3A;TET2;MSL3;TET1;PRKCA;CHD1L;ARID1B;TERF2;DDB2;JMJD6;DNAJC2;KAT2B;WDR82;KAT6B;CLOCK;KDM7A;HDAC5;ENY2;PRKAA2;L3MBTL2;MEAF6;LEF1;DOT1L;HDAC6;TAF5L;BAG6;HLCS;LEO1;SMYD2;WBSCR22;PCGF1;BRD9;APBB1;DMAP1;CBX2;SS18L1;PCGF2;ELP4;TDRD3;HOPX;ASXL1;TADA2A;KANSL2;CDK2;TADA1;TCF3;OGT
rRNA processing (GO:0006364)	1.80E-07	NOLC1;RBFA;RRP8;RRP9;NOL6;EXOSC7;FBL;EMG1;PDCD11;WBSCR22;UTP20;UTP14A;EXOSC1;DCAF13;NOP56;UTP15;NOP14;UTP6;NOP58;DIMT1;DIS3;NSUN5;DDX56;IMP4;TFB2M;PA2G4;UTP11L;RRP1B;UTP18;FTSJ3;BOP1;CIRH1A;LAS1L;RRNAD1;DKC1;PAPD5;NOP10
RNA modification (GO:0009451)	1.51E-06	TRUB1;RNMT;WDR4;TRMT2A;MTO1;EMG1;PUSL1;DUS4L;TRDMT1;WTAP;TYW5;DIMT1;PUS1;NSUN2;METTL3;TRMT1;TFB2M;QTRTD1;PUS7L;FTSJ1;FTSJ3;GTPBP3;QTRT1;RPUSD4;CTU2;RRNAD1;DKC1;DUS3L;NOP10
tRNA metabolic process (GO:0006399)	1.78E-06	TRUB1;VARS;TSEN15;WDR4;MTO1;LAGE3;TSEN54;FBL;NARS2;PUSL1;RPP14;DUS4L;TRDMT1;FDXACB1;TYW5;PUS1;TYW3;ELAC2;NSUN2;ELAC1;GATC;TRPT1;TRMT1;QTRTD1;PUS7L;QRSL1;FTSJ1;HARS2;WARS2;GTPBP3;QTRT1;CTU2;DALRD3;SARS2;PPA2;TRMT13;EEF1E1;FARSA;DUS3L
methylation (GO:0032259)	1.87E-05	N6AMT1;SUV420H2;SETD8;SETD7;WDR4;ARID4A;DPY30;PRDM2;RRP8;GCSH;MECP2;TRMT112;FBL;COMTD1;METTL13;METTL16;PRMT6;HEMK1;PRMT5;PRMT7;PRMT2;DIMT1;NSUN5;TYW3;NSUN2;PRMT3;DNMT3A;METTL3;TET2;TFB2M;FTSJ1;FTSJ3;METTL23;RRNAD1;WDR82;PICK1;GART;RNMT;SMYD5;DOT1L;TRMT2A;PRDM10;EMG1;ZFP57;SMYD2;WBSCR22;DPH5;DMAP1;TRDMT1;ATF7IP;WTAP;TRMT1;TRMT13;ZCCHC4;OGT
positive regulation of Ras GTPase activity (GO:0032320)	4.15E-05	USP6NL;DENND1A;DOCK7;ARHGEF10L;ARHGAP6;ARHGAP4;RCBTB2;DOCK10;DOCK11;SYDE1;SYNGAP1;RASGEF1A;AKT2;EVI5L;PLEKHG3;PLEKHG1;PLEKHG2;ARHGEF15;ARHGEF17;DENND2A;ARAP1;VAV2;BCR;ARHGAP10;TBC1D1;ARHGEF9;RASA3;RASA2;TBC1D25;DENND6B;DOCK2;SOS1;ARHGEF5;LLGL1;ARHGEF6;TBC1D19;ARHGEF25;RASAL3;KALRN;FGD1;FGD6;ERBB2;NCKAP1L;EPS8L2;WNT4;FARP1;DENND4B;DENND4A;RAB3IP;SGSM1;ARHGAP33;TBCK;SGSM2;PLXNB1;RGL3;AGRN;RGL1
protein acetylation (GO:0006473)	4.46E-05	CRTC2;LDB1;MEAF6;LEF1;JADE1;JADE3;TAF5L;BAG6;MECP2;ING5;ING3;EPC1;ASL;DMAP1;APBB1;CSRP2BP;NCOA1;NAA10;PCGF2;MSL3;ELP4;KAT2B;TADA2A;KANSL2;KAT6B;TADA1;NAA16;TCF3;NAA15;CLOCK;OGT
positive regulation of GTPase activity (GO:0043547)	1.22E-04	BCAR3;USP6NL;DOCK3;FAM13B;DENND1A;DOCK7;ARHGEF10L;ARHGAP6;RCBTB2;ARHGAP4;ARHGAP44;DOCK10;RGS4;DOCK11;RGS5;SYDE1;ARHGAP42;SYNGAP1;RASGEF1A;AKT2;ARHGDIB;EVI5L;GARNL3;PSD;PLEKHG3;PLEKHG1;PLEKHG2;ARHGEF15;DIS3;ARHGEF17;DENND2A;AXIN1;ARAP1;VAV2;BCR;ARHGAP10;TBC1D1;ARHGEF9;RABEP1;RASA3;RASA2;TBC1D25;PTPLAD1;DENND6B;DOCK2;SOS1;RAPGEF6;ARHGEF5;LLGL1;ARHGEF6;TBC1D19;ARHGEF25;ARHGAP18;RASAL3;KALRN;FGD1;FGD6;CHN2;ERBB2;NCKAP1L;EPS8L2;WNT4;FARP1;STARD13;EIF2B3;DENND4B;DENND4A;RAB3IP;MYO9A;SGSM1;ARHGAP33;TBCK;ARHGAP32;SNX18;ARHGAP30;SGSM2;RGL3;PLXNB1;AGRN;SMAP2;RGL1
positive regulation of Rho GTPase activity (GO:0032321)	2.43E-04	ARHGEF25;DOCK7;ARHGEF10L;ARHGAP6;KALRN;FGD1;ARHGAP4;DOCK10;DOCK11;SYDE1;FGD6;ERBB2;EPS8L2;NCKAP1L;WNT4;PLEKHG3;FARP1;PLEKHG1;PLEKHG2;ARHGEF15;ARHGEF17;ARAP1;VAV2;BCR;ARHGAP33;ARHGAP10;ARHGEF9;PLXNB1;DOCK2;SOS1;AGRN;ARHGEF5;ARHGEF6

Supplementary Table 11: Primer sequences for real-time PCR

Gene	Forward	Reverse
Gapdh	5’-GCCATCAACGACCCCTTCAT-3’	5’-ATGATGACCCGTTTGGCTCC-3’
Nphs1	5’-GTGCCCTGAAGGACCCTACT-3’	5’-CCTGTGGATCCCTTTGACAT-3’
Nphs2	5’-CTTGGCACATCGATCCCTCA-3’	5’-CGCACTTTGGCCTGTCTTTG-3’
Synpo	5’-CTTTGGGGAAGAGGCCGATTG-3’	5’-GTTTTCGGTGAAGCTTGTGC-3’
Wt1	5’-GAGAGCCAGCCTACCATCC-3’	5’-GGGTCCTCGTGTTTGAAGGAA-3’
Pecam-1	5’-AGCCTAGTGTGGAAGCCAAC-3’	5’-AGCCTTCCGTTCTCTTGGTG-3’
Cdh5	5’-GTCGATGCTAACACAGGGAATG-3’	5’-AATACCTGGTGCGAAAACACA-3’
Pax8	5’-ATGCCTCACAACTCGATCAGA-3’	5’-ACAATGCGTTGACGTACAACTT-3’
Angptl4	5’-CATTGGCTTGACTCCCAACG-3’	5’-AAGTCCACAGAGCCGTTCAG-3’
Ecm1	5’-ACCAGTGACCAGTTCTTGCC-3’	5’-CATGGAGGTGCTGGAAGAGG-3’
Serpine1	5’-TTCAGCCCTTGCTTGCCTC-3’	5’-ACACTTTTACTCCGAAGTCGGT-3’
Itgb3	5’-CCACACGAGGCGTGAACTC-3’	5’-CTTCAGGTTACATCGGGGTGA-3’
Ramp2	5’-GCAGGCATTACAGCGACCT-3’	5’-GGAGCAGTTCGCAAAGTGTATC-3’
Adm	5’-CACCCTGATGTTATTGGGTTCA-3’	5’-TTAGCGCCCACTTATTCCACT-3’
Epas1	5’-CTGAGGAAGGAGAAATCCCGT-3’	5’-TGTGTCCGAAGGAAGCTGATG-3’
Cib1	5’-CCGAGTACCAGGCTAACCCTT-3’	5’-TCCCGTGAGGCAATTCACAAG-3’
Gpr56	5’-CTGCGGCAGATGGTCTACTTC-3’	5’-ATAGTGGAGGGTGCTCTGTTG-3’
Kdm6a	5’-AAGGCTGTTCGCTGCTACG-3’	5’-GGATCGACATAAAGCACCTCC-3’
Prkaa2	5’-CAGGCCATAAAGTGGCAGTTA-3’	5’-AAAAGTCTGTCGGAGTGCTGA-3’
Met	5’-GTGAACATGAAGTATCAGCTCCC-3’	5’-TGTAGTTTGTGGCTCCGAGAT-3’
Ddb2	5’-AAATGCCCAGAAACCCAGAAG-3’	5’-GTCCTGCTAGAAACGGGACC-3’
Zfp57	5’-ACACCAGTCAGTTATGAGGACG-3’	5’-TCCACCTTTGAATACCCCTGT-3’