

725 **Supplemental Figure 1.**

726 (A) Southern blot analysis of targeted ES cell clone used to generate *Stox1* KO mouse.

727 (B) PCR genotyping of *Stox1* mice. WT allele, 559 bp; KO, 448 bp.

728 (C) Tissue expression of *Stox1* and β -actin by RT-PCR.

729

730 **Supplemental Figure 2.**

731 (A) Immunofluorescence for nidogen-1 (AKA entactin) and Col IV α 1 in the placenta. Scale bar:

732 50 μ m. D, decidua; JZ, junctional zone; L, labyrinth. Solid line marks border between the

733 decidua and junctional zone; dotted line marks border between junctional zone and labyrinth.

734 (B-C) Expression of genes involved in hypertension, vascular integrity, and inflammation in the
735 placenta (RT-PCR) reveals increased renin mRNA.

736 (D) Renin expression increased in *Stox1* KO placenta but not *Stox1* heterozygotes. *Stox1*
737 genotypes of female and male mice shown. F, female; M, male.

738 (E-F) Renin expression in the kidney no increased in *Stox1* KO by RT-PCR (E) or Western blot
739 analysis (F). Prorenin (44 kD) was the predominant form detected in the kidney.

740

741 **Supplemental Figure 3.**

742 (A) Expression of markers genes (defined by Nelson et al. 2016) in *Stox1*⁺ cells. NK, natural
743 killer. SpA-TGC, spiral artery trophoblast giant cell.

744

745 **Supplemental Figure 4.**

746 (A) DNA sequencing results from HTR-8/SVneo human trophoblast cell line showing that this
747 line contains the STOX1 variant allele, Y153H. *NCBI Reference Sequence: NG_012975.2,
748 Homo sapiens storkhead box 1.

752 **Supplemental Table 1: Comparison of systolic blood pressure (mmHg) between groups.**

	Gestational time point				
Group	Non-pregnant	E13.5	E14.5	E17.5	Postpartum
WT	116 ± 10	111 ± 9	107 ± 12	109 ± 9 ^A	--
Stox1 KO	108 ± 10 ^A	114 ± 8	116 ± 12	124 ± 10 ^B	109 ± 9 ^C
Stox1 KO + Losartan	--	--	116 ± 3	106 ± 9 ^C	--

753 Data are mean ± SD. WT, wild type; KO, knockout; E, embryonic day.

754 The following numbers of mice were used (group, n):

755 Non-pregnant: WT, 8; KO, 12

756 Pregnant E13.5: WT, 10; KO, 16

757 Pregnant E14.5: WT, 9; KO, 14; KO + Losartan, 3

758 Pregnant E17.5: WT, 5; KO, 15; KO + Losartan, 7

759 Postpartum (day 10): KO, 7

760 Statistical significance determined using one-way ANOVA with Bonferroni correction.

761 ^ANot significant when compared with non-pregnant WT.

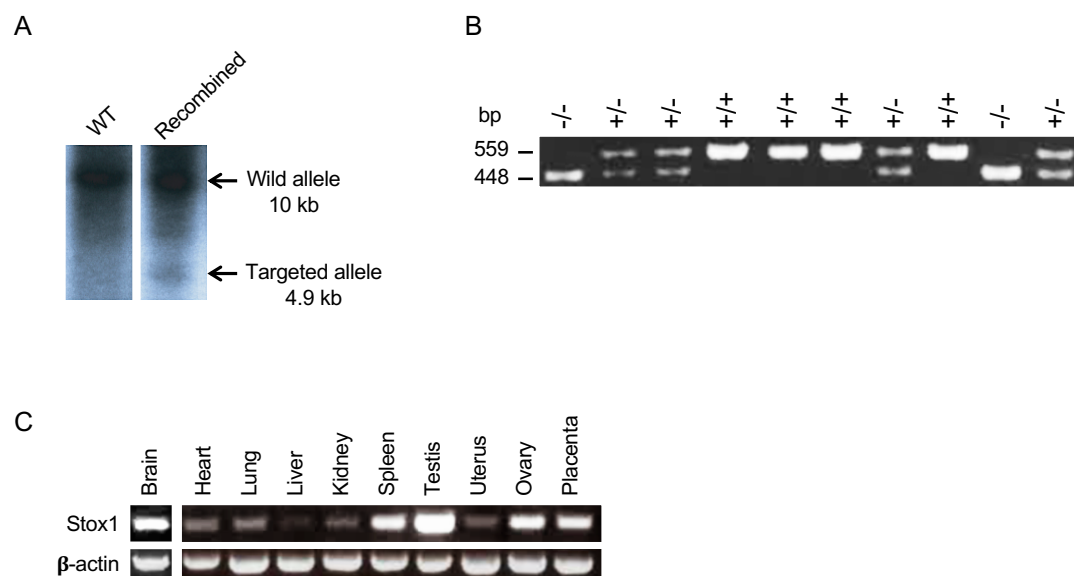
762 ^BSignificant difference when compared with non-pregnant Stox1 KO (adjusted $P \leq 0.01$).

763 ^CSignificant difference when compared with E17.5 Stox1 KO; adjusted $P \leq 0.01$ for E17.5 Stox1

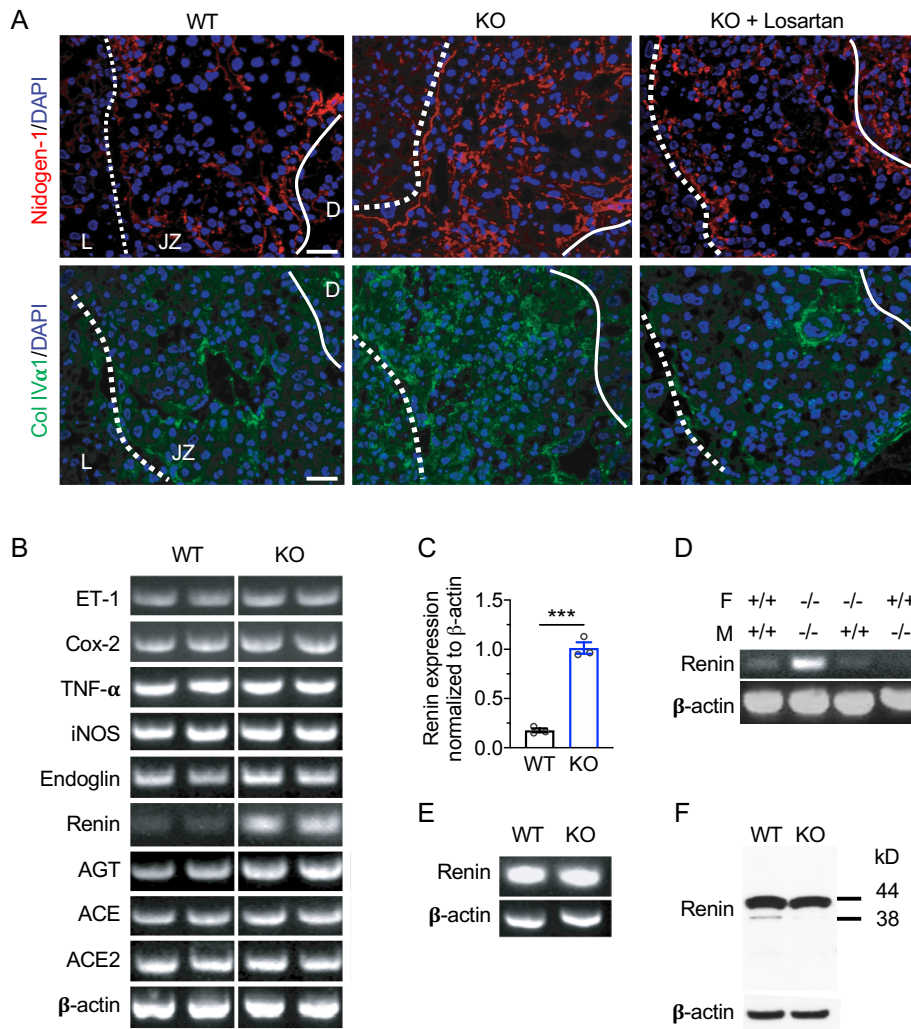
764 KO + Losartan, adjusted $P \leq 0.05$ for postpartum Stox1 KO.

765 **Supplemental Table 2: PCR primer sequences**

	Forward	Reverse
Genotyping		
Mouse	TTGCAGCAGCTCACATGCAGAG	GCGTTGGCTACCCGTGATATTG (WT allele) GAGGTCCTTAGAGTCGCACATG (KO allele)
Human (HTR-8/SVneo)	TTAGGTGATGTCTTTCCAGTGC	CCAAACACATACAGTGAAGTACAAATAG
Mouse, RT-PCR		
ET1	AACTCAGCACCGGAGCTGAGAATGG	CACTTCTTTCCCAACTTGGAACAGGG
COX-2	ACCGTGGGGAATGTATGAGCACAGG	CAGGTCCTCGCTTATGATCTGTCTTG
TNF α	CTTGGGCAGATTGACCTCAG	GTCTCAGCCTCTTCTCATTC
iNOS	CTGATCTTGTGTTGGAGGTG	TCTGCCAGATGTGGGTCTTC
ENG	CAGGTCTCGCAGAAAGAGTC	ACTGGAGGACGATGCTTTGG
Renin	CCGCTACCTTTGAACGAATC	CTGAACCCGTGTCAAAGATG
AGT	AGCTAGAGGATGAGGACCGGAAGCG	AGTGGCAAGTTCATCTTCCACCCTG
ACE	GAGTCTGACAACCTGGAGCAAGACC	CACAGAGGTACACTGCTTGATCCTG
ACE2	GCAATGATGAATCAGGGCTGGGATG	TTCAGATGCTTGGGGGTAGCTGCAG
Stox1	CCCACATCTGTGTGAACCCC	CATTAGGGCAGTGTGCTTGG
β -actin	TGGGCATTGTTACCAACTGGG	AGTTTCATGGATGCCACAGG
Human, RT-PCR		
Renin	GAACGAGGTGTGGACATGGCCAGGC	GCTGGAGGAATCCGAAGCATCGAAG
Stox1, primer 1	TGTGTACAGGCCTCAGCACCTGCTG	CTGTCCCTTCCAAGTGAAGTGTGTTT
Stox1, primer 2	GATTACTATAGCGCAAGAAAAGCC	TTATTAGATCCCAGACTCTGTGTC
β -actin	ACCACACCTTCTACAATGAGCTGCG	CGACGTAGCACAGCTTCTCCTTAATG
Luciferase constructs		
Renin promoter	TATGAGCTCTAGCTGGTCTGTGTACAGAGCTAAGC	ATACTCGAGGCTCTCTCTGAGATCCACTGAGGTTT
Renin 3'UTR	ATATCTAGAATTGGCTTCGCCTTGGCCCGCTGAGG	ATAGGATCCCGCAGCCCCTCCCTCTGTTCTAAACCC
CMV promoter	ATAACGCGTGAGTTCGAGCTTGCATGCC	ATACTCGAGGCGGGTACAATTCCGCAGC

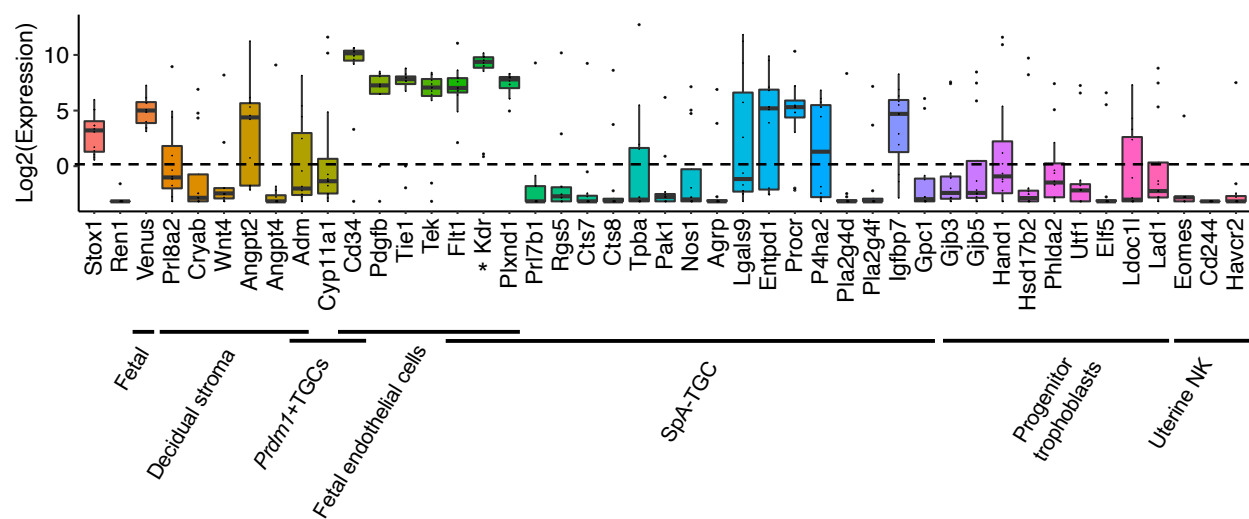


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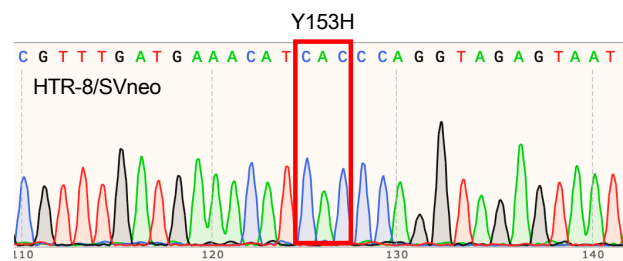
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A



	Genomic DNA sequence	153
HTR-8/SVneo	CGTTTGATGAAACATCACCGGTAGAGTAAT	H
Reference*	CGTTTGATGAAACATTACCGGTAGAGTAAT	Y

Supplemental Figure 4. (A) DNA sequencing results from HTR-8/SVneo human trophoblast cell line showing that this line contains the *STOX1* variant allele, Y153H. *NCBI Reference Sequence: NG_012975.2, Homo sapiens storkhead box 1.