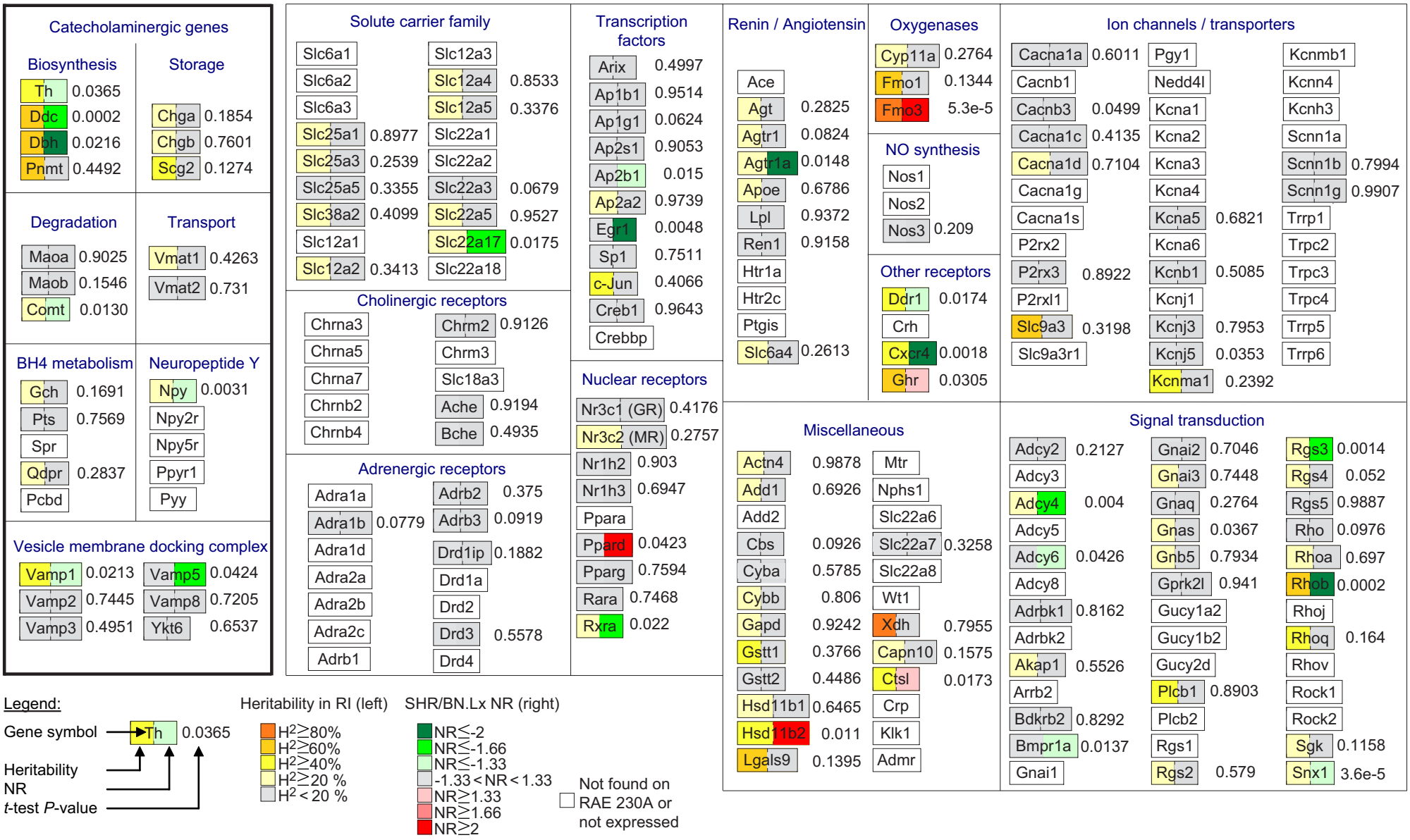
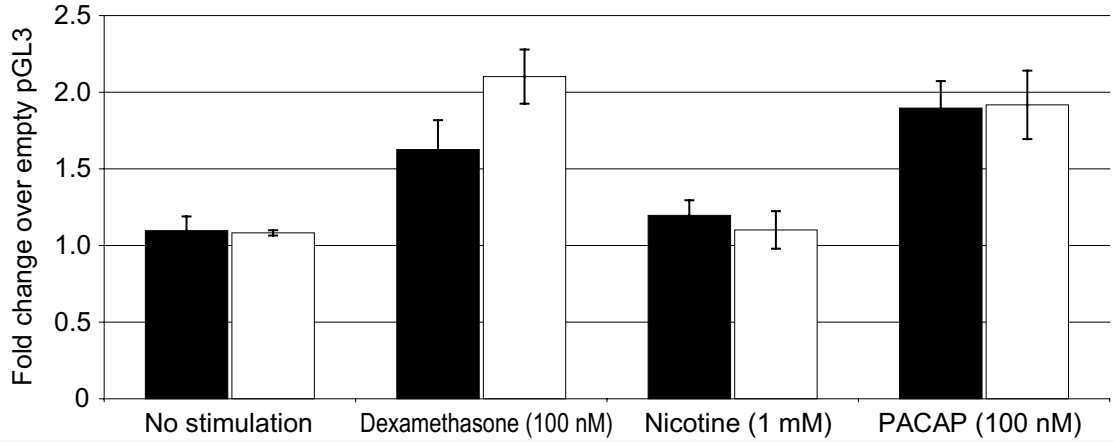
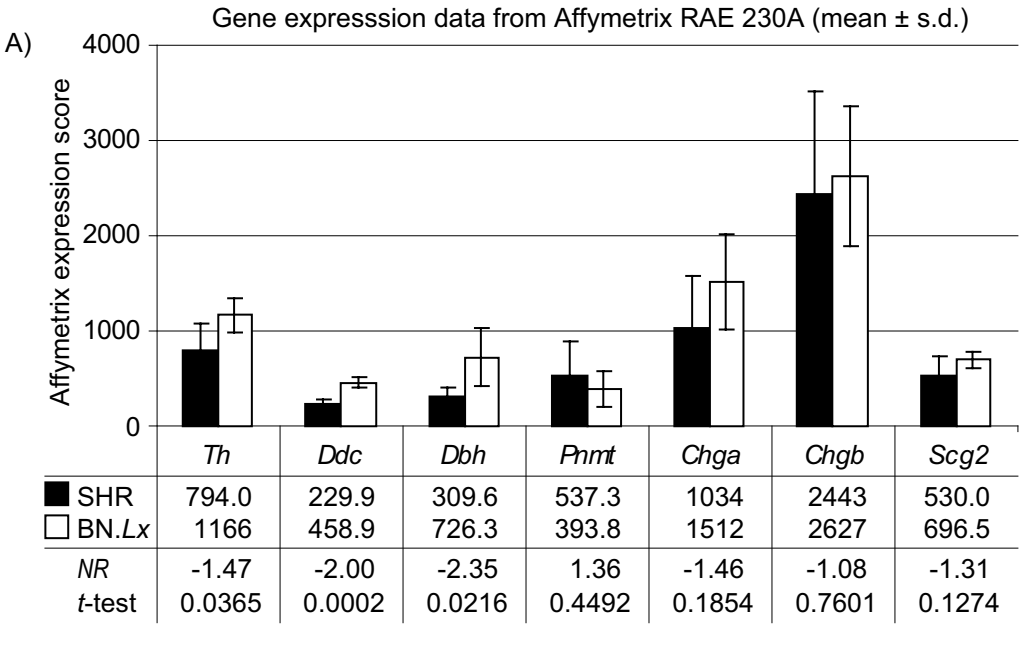


Supplementary Material, Figure S1 Hypertension candidate genes: heritability of gene expression in the RI strains and differential expression in the SHR vs. BN.Lx progenitors. Each box represents a candidate gene referred to by its official symbol. Two different parameters are color-coded: the transcript level heritability (H^2) as computed from the RI panel is shown on the left, and the normalized ratio (*NR*) for differences between the progenitors is shown on the right side of each box. Numbers next to boxes are *t*-test *P*-values obtained from progenitor data. Genes are grouped according to their function. Genes were selected with the help of the UCSD NHLBI PPG Program website <elcapitan.ucsd.edu/hyper/index.html>.

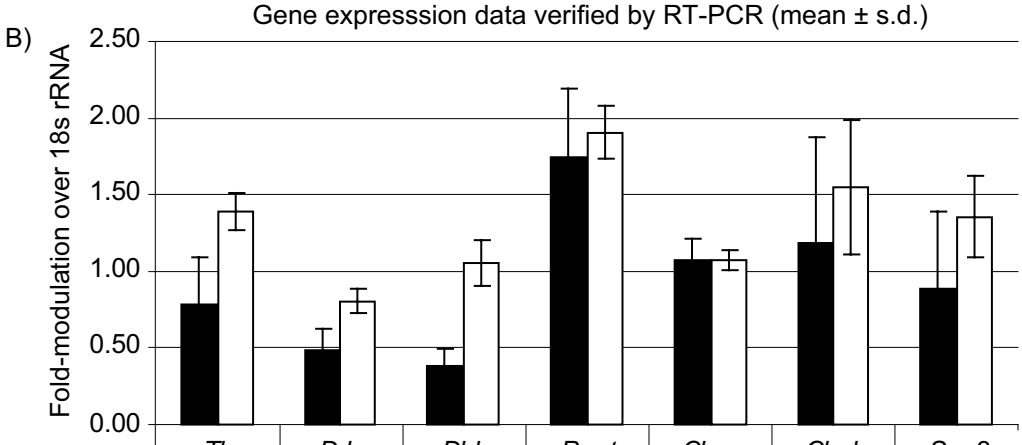


	No stimulation	Dexamethasone (100 nM)	Nicotine (1 mM)	PACAP (100 nM)
■ SHR	1.10	1.63	1.20	1.90
□ BN.*Lx*	1.08	2.10	1.10	1.92
NR	1.01	-1.29	1.09	-1.01
t-test *P*-value	0.782	**0.004**	0.276	0.888

Supplementary Material, Figure S6 Functional studies on the single nucleotide polymorphisms identified in the *Pnmt* promoter. Bioluminescent activity of luciferase was measured in rat PC12 pheochromocytoma cells transfected with ~1 kbp segment of *Pnmt* promoter/luciferase reporter in pGL3-Basic vector (Promega) after 16 hour incubation with or without stimulus (dexamethasone, PACAP and nicotine). Each experiment was conducted in 4 replicates, with luciferase results normalized to cell protein in each plate. Results are presented as fold-augmentation (by stimulus) over the signal from cells transfected with a promoterless (empty) pGL3-Basic vector. Only dexamethasone stimulation elicited significant differences in SHR versus BN.*Lx* promoter activity.



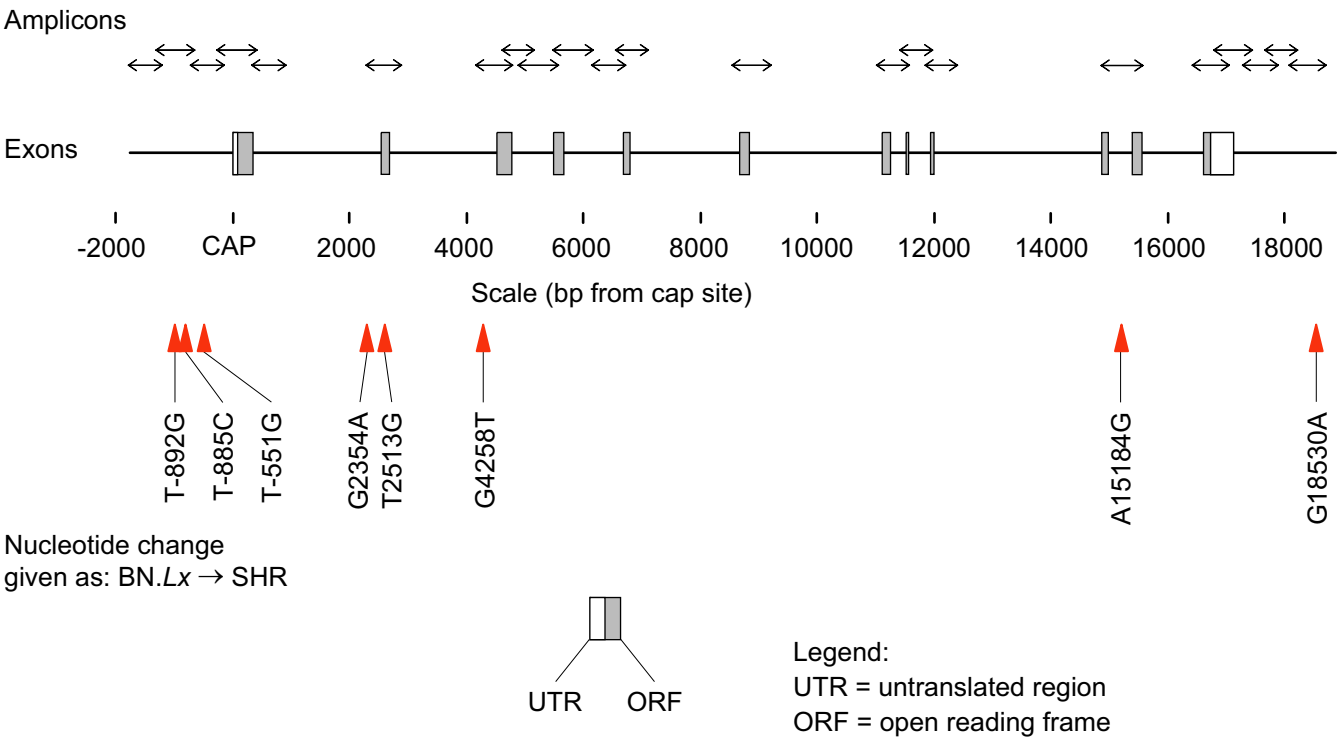
	Th	*Ddc*	*Dbh*	*Pnmt*	*Chga*	*Chgb*	*Scg2*
SHR	794.0	229.9	309.6	537.3	1034	2443	530.0
BN.*Lx*	1166	458.9	726.3	393.8	1512	2627	696.5
NR	-1.47	-2.00	-2.35	1.36	-1.46	-1.08	-1.31
t-test	0.0365	0.0002	0.0216	0.4492	0.1854	0.7601	0.1274



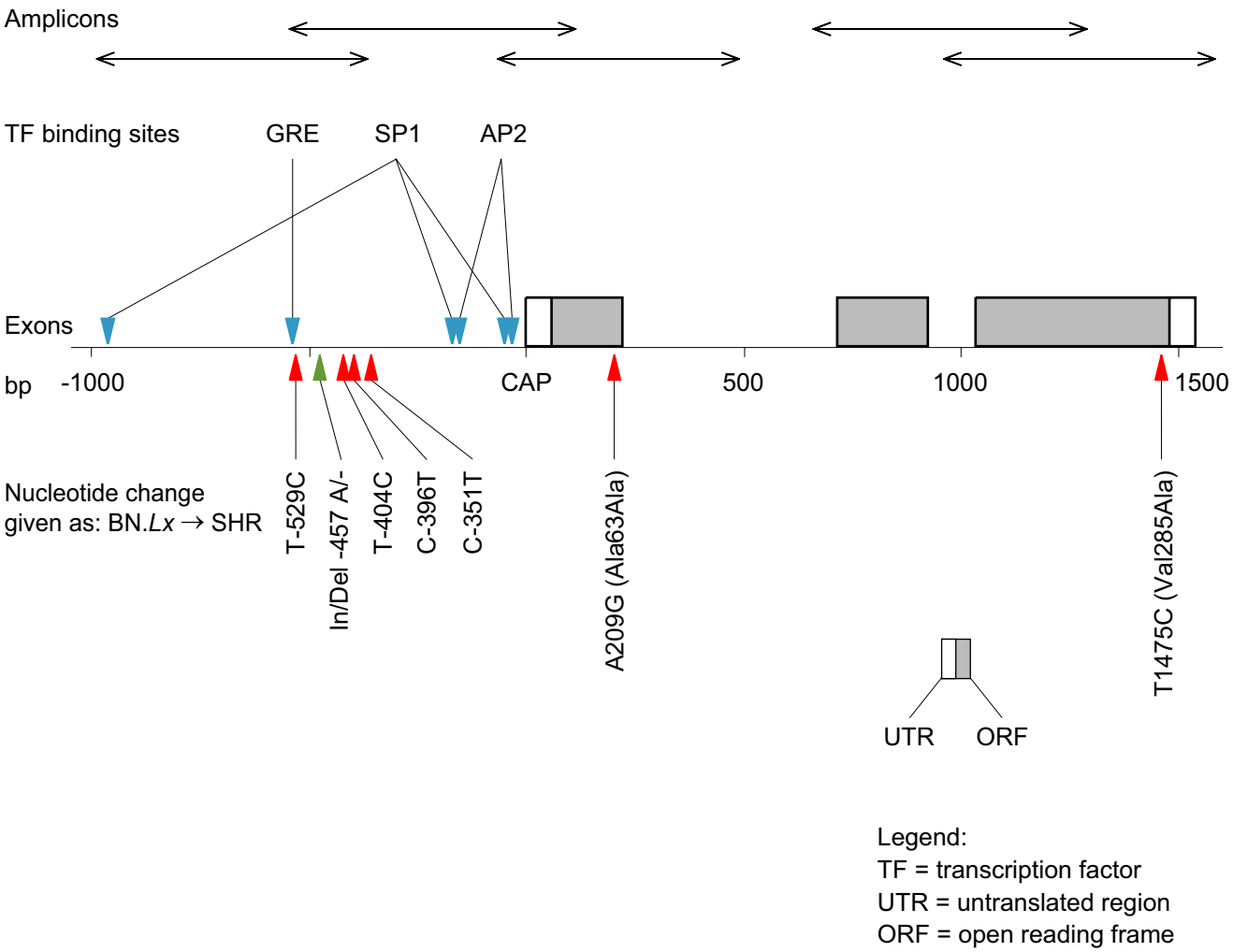
	Th	*Ddc*	*Dbh*	*Pnmt*	*Chga*	*Chgb*	*Scg2*
SHR	0.79	0.48	0.38	1.75	1.07	1.19	0.88
BN.*Lx*	1.39	0.80	1.05	1.91	1.07	1.55	1.36
NR	-1.76	-1.66	-2.76	-1.09	-1.00	-1.30	-1.54
t-test	0.0145	0.0086	0.0001	0.5781	0.9781	0.4416	0.1823

Legend: s.d. = standard deviation

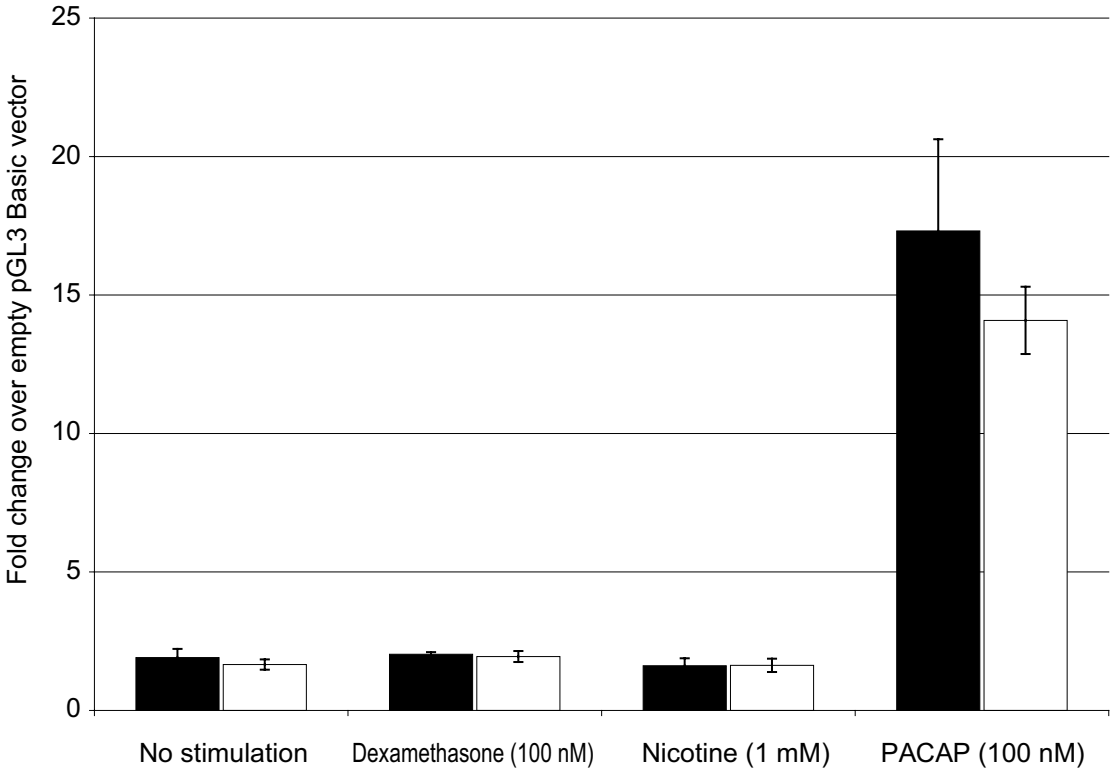
Supplementary Material, Figure S2 Adrenal gene expression levels of genes vital for catecholamine biosynthesis and storage: Affymetrix data validated by RT-PCR. Data obtained from Affymetrix RAE 230A microarray (A), were subsequently validated by real time-PCR in tissues from different age and sex-matched genetically identical animals (B). Tables underneath the graphs provide the mean values for gene expression in each progenitor, normalized ratios (*NR*) computed from those means, and *t*-test *P*-values calculated from the progenitor strain data.



Supplementary Material, Figure S3 Single nucleotide polymorphism discovery at the *Dbh* locus in SHR vs. BN.*Lx* strains. The bp distances refer to the distance from the CAP (transcription initiation) site. Exons are rendered as boxes: grey portions are translated, while empty portions are 5'- and 3'-untranslated. The extent of each of the 22 amplicons (spanning each exon, exon/intron border, ~1.75 kbp of proximal promoter, and ~1.75 kbp of 3' [downstream] sequence) is represented by double-headed arrows. The solid red arrowheads indicate the SNP positions (all variants discovered were SNPs). The nucleotide change is given as BN.*Lx* → SHR.



Supplementary Material, Figure S4 Single nucleotide polymorphism discovery at the *Pnmt* locus in SHR vs. BN.*Lx* strains. The bp distances refer to the distance from the CAP (transcription initiation) site. Exons are rendered as boxes: grey portions are translated, while empty portions are 5'- and 3'-untranslated. The span of each of the 6 amplicons is represented by double-headed arrows. The solid red arrowheads indicate the SNP positions, the green arrowhead indicates an insertion/deletion polymophism. The nucleotide change is given as BN.*Lx* → SHR. Amino acid changes in coding region SNPs are given in parentheses. Blue arrowheads indicate important trascription factor binding sites.



	No stimulation	Dexamethasone (100 nM)	Nicotine (1 mM)	PACAP (100 nM)
■ SHR	1.91	2.03	1.61	17.3
□ BN.*Lx*	1.66	1.95	1.63	14.1
NR	1.15	1.04	-1.01	1.23
t-test *P*-value	0.23	0.56	0.94	0.14

Supplementary Material, Figure S5 Functional studies on the single nucleotide polymorphisms identified in the *Dbh* promoter. Bioluminescent activity of luciferase was measured in rat PC12 pheochromocytoma cells transfected with ~1.1 kbp segment of *Dbh* promoter/luciferase reporter in pGL3-Basic vector (Promega) after 16 hour incubation with or without stimulus (dexamethasone, PACAP and nicotine). Each experiment was conducted in 4 replicates, with luciferase results normalized to cell protein in each plate. Results are presented as fold-augmentation (by stimulus) over the signal from cells transfected with a promoterless (empty) pGL3-Basic vector. No significant differences between SHR and BN.*Lx* were observed.

Supplementary Material, Table S1 Correlations among biochemical, physiological and gene expression phenotypes. Spearman rank order correlations among cardiovascular phenotypes and adrenal biochemical and gene expression phenotypes were computed. Enzymes catalyzing the last two steps of catecholamine biosynthesis, Dbh and Pnmt, and their respective substrates and products (dopamine, norepinephrine and epinephrine) were assayed in adrenal tissue at the age of 6 weeks. *Dbh* and *Pnmt* gene expression levels were measured by Affy RAE230A at the age of 6 weeks. Cardiovascular physiological phenotypes were measured telemetrically at the age of 12 weeks. Correlation coefficients (ρ) and *P*-values (*P*) are given for all phenotype pairs. Significant correlations are flagged with asterisks (* = significant at the 0.05 level; ** = significant at the 0.01 level (2-tailed)).

Spearman's rho		DA tissue conc.	Dbh enz. activity	NE tissue coc.	Pnmt enz. activity	EPI tissue conc.	Dbh gene expression	Pnmt gene expression	SBP	HR
DA tissue concentration	ρ		-.408*	.423*	.594**	.633**	-.551**	.345	.250	-.382*
	P		.031	.025	.001	.000	.002	.072	.199	.045
	N		28	28	28	28	28	28	28	28
Dbh enzymatic activity	ρ	-.408*		.095	-.119	.011	.570**	.046	-.476*	.050
	P	.031		.632	.545	.954	.002	.816	.010	.801
	N	28		28	28	28	28	28	28	28
NE tissue concentration	ρ	.423*	.095		.059	.463*	-.330	-.031	-.257	-.205
	P	.025	.632		.764	.013	.086	.875	.187	.295
	N	28	28		28	28	28	28	28	28
Pnmt enzymatic activity	ρ	.594**	-.119	.059		.429*	-.119	.436*	.094	-.383*
	P	.001	.545	.764		.023	.547	.020	.634	.044
	N	28	28	28		28	28	28	28	28
EPI tissue concentration	ρ	.633**	.011	.463*	.429*		-.386*	.047	.013	-.550**
	P	.000	.954	.013	.023		.042	.814	.949	.002
	N	28	28	28	28		28	28	28	28
Dbh gene expression	ρ	-.551**	.570**	-.330	-.119	-.386*		.294	-.148	.187
	P	.002	.002	.086	.547	.042		.121	.443	.332
	N	28	28	28	28	28		29	29	29
Pnmt gene expression	ρ	.345	.046	-.031	.436*	.047	.294		.209	-.276
	P	.072	.816	.875	.020	.814	.121		.277	.147
	N	28	28	28	28	28	29		29	29
SBP	ρ	.250	-.476*	-.257	.094	.013	-.148	.209		-.079
	P	.199	.010	.187	.634	.949	.443	.277		.680
	N	28	28	28	28	28	29	29		29
HR	ρ	-.382*	.050	-.205	-.383*	-.550**	.187	-.276	-.079	
	P	.045	.801	.295	.044	.002	.332	.147	.680	
	N	28	28	28	28	28	29	29	29	

Legend:
Dbh = dopamine beta-hydroxylase, Pnmt = phenylethanolamine N-methyltransferase, DA = dopamine, NE = norepinephrine, EPI = epinephrine, SBP = systolic blood pressure, HR = heart rate

Supplementary Material, Table S2 Analysis of the *Dbh* region for known physiological QTLs. The conflated 95% CI's for *Dbh* transcript, Dbh activity and dopamine concentration QTLs, and harboring the *Dbh* gene itself (RNO 3p:1-14 Mbp, see Figure 2A), was examined for known cardiovascular and cardiovascular-related physiological QTLs (data from http://rgd.mcw.edu). The numbers in the first column correspond to the bars in Figure 2D. Progenitor strains used in the various crosses, in which QTLs were mapped, are shown. LOD scores and *P*-values are given where available.

Number in figure 2D	Cardiovascular pQTLs mapping to the *Dbh* region RNO 3p:1-14 Mbp. (source: http://rgd.mcw.edu)		Position (Mbp)		Progenitors of crosses in which QTL was mapped		LOD	P-val
	QTL symbol	QTL name (trait measured)	start	end	Progenitor 1	Progenitor 2		
1	*Cm10*	Cardiac mass (LV) QTL 10	0.00	19.01	SHR/FubRkb	SS/JrRkb	7.3	10^{-4}
2	*Bp15*	BP (salt loaded systolic) QTL 15	0.00	21.37	SHRSP	WKY	4.4	-
3	*Bp85*	BP (systolic) QTL 85	0.00	21.37	SHRSP/Izm	WKY/Izm	3.1	-
4	*Bp140*	BP (systolic) QTL 140	0.18	32.05	SHR/Snk	WKY/Snk	2.5	-
5	*Bp92*	BP (salt loaded systolic) QTL 92	0.18	38.71	SHR	SS/Jr	2.7	-
6	*Arunc3*	Aerobic running capacity QTL 3	3.48	30.25	COP/OlaHsd	DA/OlaHsd	3.3	-
7	*BpQTLcluster4*	BP (systolic) QTL cluster 4	6.27	47.58	SHR	BN	2.2	-
8	*Cm43*	Cardiac mass (BW adjusted) QTL 43	6.37	26.67	SHRSP/Tkyo	WKY/Tkyo	6.7	$2x10^{-5}$
9	*Bw56*	Body weight QTL 56	6.37	26.67	SHRSP/Tkyo	WKY/Tkyo	4.5	$3x10^{-8}$
10	*Cm46*	Cardiac mass (BW adjusted) QTL 46	6.37	26.67	SHRSP/Tkyo	WKY/Tkyo	6.6	$6x10^{-6}$
11	*Cm48*	Cardiac mass (BW adjusted) QTL 48	6.37	26.67	SHRSP/Tkyo	WKY/Tkyo	5.4	$4x10^{-4}$
12	*Bp264*	BP (mean arterial) QTL 264	10.27	121.62	HTG	LEW	4,0	-
13	*Bp251*	BP (mean arterial) QTL 251	10.27	121.62	HTG	LEW	2.8	-
14	*Hrtrt17*	Heart rate (salt loaded) QTL 17	11.14	88.65	SHRSP	WKY	3.8	-
15	*Alc19*	Alcohol consumption QTL 19	12.91	23.76	P	NP	4.4	-
16	*Alc8*	Alcohol consumption QTL 8	12.91	29.75	P	NP	5.9	$5x10^{-5}$
17	*Bp151*	BP (decreased) QTL 151	13.85	44.55	SS	LEW	-	$4x10^{-2}$

Legend: BP = blood pressure, BW = body weight, LV left ventricle, BN = Brown Norway, COP = Curtiss rat, DA = "d" blood group and agouti color, HTG = Prague hypertriglyceridemic (from WKY), LEW = Lewis rat, NP = Alcohol-nonpreferring, P = Alcohol-preferring, SHR = Spontaneously hypertensive rat, SHRSP = Stroke prone spontaneously hypertensive rat, SS = Salt Sensitive, WKY = Wistar Kyoto rat

Supplementary Material, Table S3 Analysis of the *Pnmt* region for known physiological QTLs. The conflated 95% CI's for Pnmt pQTL and *Pnmt*, *Chga* and *Vmat1* eQTLs, which also harbors the *Pnmt* gene itself (RNO 10q:81-104 Mbp, see Figure 3A), was examined for known cardiovascular and cardiovascular-related physiological QTLs (data from http://rgd.mcw.edu). The numbers in the first column correspond to the bars in Figure 3C. Progenitor strains used in the various crosses, in which QTLs were mapped, are shown. LOD scores and p-values are given where available.

Number in figure	Cardiovascular pQTLs mapping to the *Pnmt* region RNO 10q:81-104 Mbp. (source: *http://rgd.mcw.edu)*		Position (Mbp)		Progenitors of crosses in which QTL was mapped		LOD	*P*-val
3C	QTL symbol	QTL name (trait measured)	start	end	Progenitor 1	Progenitor 2		
1	*Bp12*	BP QTL 12	50.04	80.04	SS/Jr	LEW	6.3	10^{-4}
2	*Bp57*	BP QTL 57	21.51	84.56	MHS/Gib	MNS/Gib	5.0	-
3	*Bp71*	BP QTL 71	55.50	85.50	SS	LEW	-	$4x10^{-2}$
4	*BpQTLcluster9*	BP QTL cluster 9	21.51	91.48	SHR	BN	2.9	-
5	*Bp186*	BP QTL 186	5.92	94.98	SS/JrHsdMcwi	BN/SsNHsd	3.6	-
6	*Cm31*	CM QTL 31	29.97	95.37	SS/JrHsdMcwi	BN/SsNHsd	3.9	-
7	*Bp76*	BP QTL 76	36.42	95.38	SS/Jr	MNS	-	10^{-4}
8	*Bp87*	BP QTL 87	65.71	95.71	SHRSP/Izm	WKY/Izm	4.5	-
9	*Bp168*	BP QTL 168	27.09	102.70	SS/Jr	LEW	5.5	-
10	*Bp82*	BP QTL 82	27.09	103.67	SS/Jr	MNS	6.8	-
11	*Cm51*	CM QTL 51	53.79	96.59	SS/Jr	MNS	3.0	-
12	*Stresp5*	SR QTL 5 (corticosterone)	43.37	108.89	F344/NHsd	LEW/NHsd	3.0	$3x10^{-4}$
13	*Cm33*	CM QTL 33	56.93	97.59	LH/Mav	LN/Mav	2.8	-
14	*Bp1*	BP QTL 1	53.78	101.85	WKY	SHRSP	5.1	-
15	*Cm44*	CM QTL 44	69.26	99.26	WKY/Tkyo	SHRSP/Tkyo	4.8	$4x10^{-5}$
16	*Hrtrt21*	HR QTL 21	68.53	101.29	SHR/Ola	BN.Lx/Cub	2.4	-
17	*Bp72*	BP QTL 72	70.65	100.65	SS	LEW	-	-
18	*Bp249*	BP QTL 249	69.99	102.59	SS	MNS	-	10^{-4}
19	*Bp91*	BP QTL 91	71.96	101.96	SS	MNS	-	10^{-4}
20	*Bp150*	BP QTL 150	77.01	82.00	SS	LEW	-	10^{-4}
21	*Bp45*	BP QTL 45	77.25	91.18	SHR/Mol	BB/OK	23.2	-
22	*Bp9*	BP QTL 9	80.36	110.36	SS/Jr	MNS	4.8	10^{-4}
23	*Bp134*	BP QTL 134	84.26	95.69	SHRPS	WKY	-	10^{-3}
24	*Stresp7*	SR QTL 7 (catecholamines)	90.46	92.46	HTG	BN	3.52	-
25	*Bp149*	BP QTL 149	94.98	99.70	SS	LEW	-	10^{-4}
26	*Bp137*	BP QTL 137	99.11	101.47	SS	MNS	-	10^{-2}
27	*Bp250*	BP QTL 250	101.85	110.72	SS	MNS	-	10^{-4}

Legend: BP = blood pressure, CM = Cardiac mass, HR = heart rate, SR = stress response, BB = Diabetic strain from outbred Wistar rats, BN = Brown Norway, F344 = Fischer rat, HTG = Prague hypertriglyceridemic (from WKY), LEW = Lewis rat, LH = Lyon Hypertensive, LN = Lyon normotensive, MHS = Milan hypertensive strain, MNS = Milan normotensive strain, SHR = Spontaneously hypertensive rat, SHRSP = Stroke prone spontaneously hypertensive rat, SR = Salt Resistant (from a Sprague-Dawley outbred colony), SS = Salt Sensitive, WKY = Wistar Kyoto rat

Supplementary Material, Table S4 qRT-PCR primers used to validate microarray results. Each primer name consists of gene symbol, NCBI reference sequence (in parentheses), primer position in the reference sequence and primer direction/function (F = forward, R = reverse, T = tagged).

Primer name	*Primer sequence*
Th-(NM_013158)-1725F	5'-AGCGCCCATTCTCTGTGAAG-3'
Th-(NM_013158)-17292R	5'-GGTGTGAGGGCTGTCCAGTAC-3'
Th-(NM_013158)-1746T	5'-TTGACCCGTACACACTGGCCATTGA-3'
Ddc-(NM_012545)-799F	5'-CCCTCGCTTTGAAATTTGCA-3'
Ddc-(NM_012545)-867R	5'-AACTGGTTGGAGCCCTTTAGC-3'
Ddc-(NM_012545)-820T	5'-TCATCCTCGGGTTGGTCTGCTTC-3'C
Dbh-(NM_013158)-1730F	5'-GCTTCCCGGGTAACTGGAA-3'
Dbh-(NM_013158)-1800R	5'-GCGTGGGTCGGGTTCTT-3'
Dbh-(NM_013158)-1750T	5'-CTGCAGCCTCTGCCTAATATCACTTCCG-3'
Pnmt-(X14211)-340F	5'-GAGTCCTGGCAGGAGAAAGAAC-3'
Pnmt-(X14211)-407R	5'-TGCACATCAATGGGCAAGAC-3'
Pnmt-(X14211)-363T	5'-CCAGCTCCGAGCGAGGGTGAAG-3'
Chga-(NM_021655)-765F	5'-AGAAGGCTGGGCCTAAAGAAGT-3'
Chga-(NM_021655)-838R	5'-CTGGATCTTCTTGTAGCCTGAATAGA-3'
Chga-(NM_021655)-788T	5'-CCCACGGCAGCATCCAGTTCTCA-3'
Chgb-(NM_012526)-1877F	5'-TGGACCAGCTCCTTCACTACAG-3'
Chgb-(NM_012526)-1948R	5'-CCATCTGCTCCTCTGAATCGTA-3'
Chgb-(NM_012526)-1900T	5'-AAGAAGGCAGCCGAATTTCCCGACT-3'
Scg2-(NM_022669)-1668F	5'-GCAGGCCATCAAGGAGCAT-3'
Scg2-(NM_022669)-1735R	5'-TGCTCACCTTGGCCAGTTTC-3'
Scg2-(NM_022669)-1688T	5'-TGGGTCAAGGAAGCTCCCAGGAAATG-3'