

Finding a disease gene – how to do it

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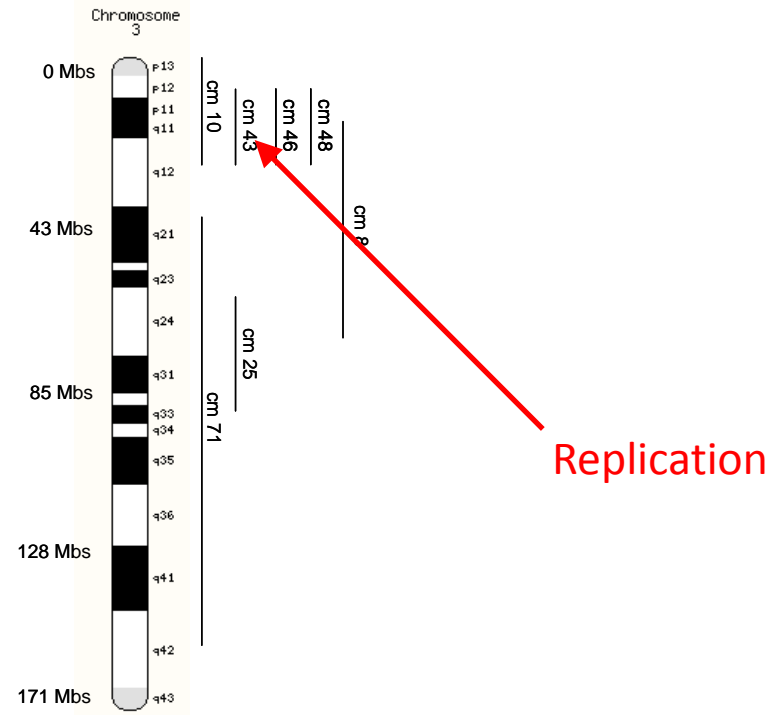


Left ventricular mass (LVM)

Linkage and GWAS of LVM in humans have not been informative

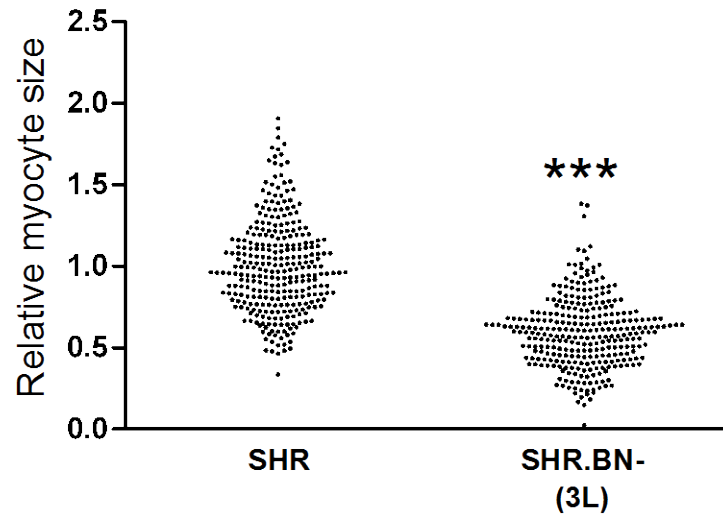
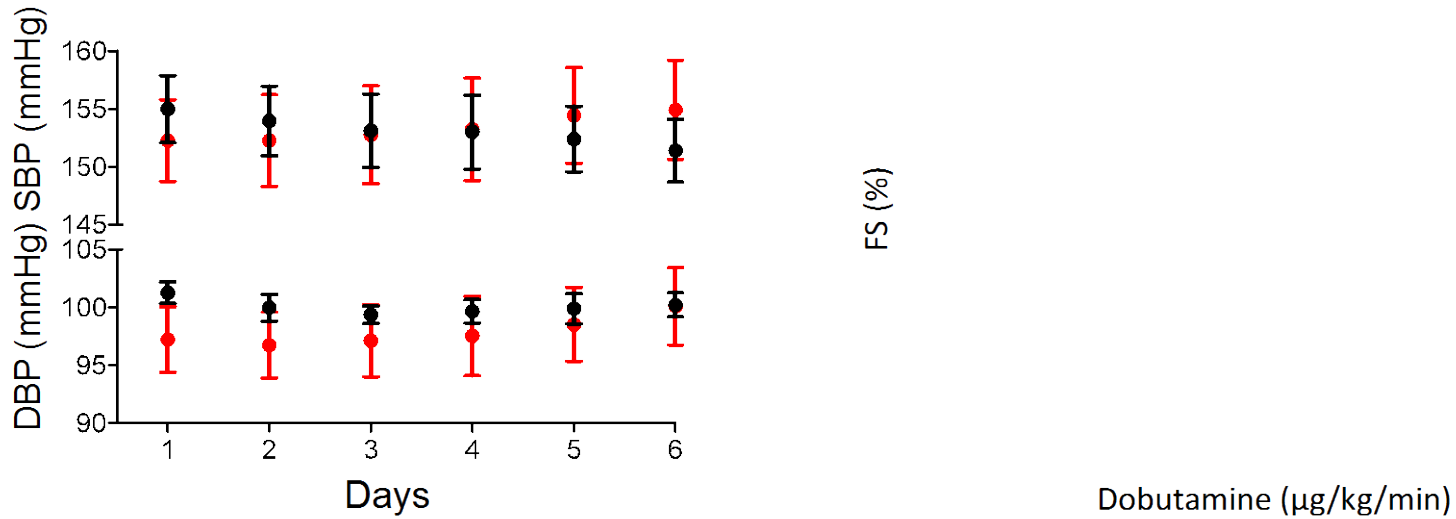
The mouse has few (<10) LVM loci

The rat has over 75 LVM loci, many replicated and some blood pressure independent



Further replication of the Chr 3 locus in a BNxSHR F2 cross

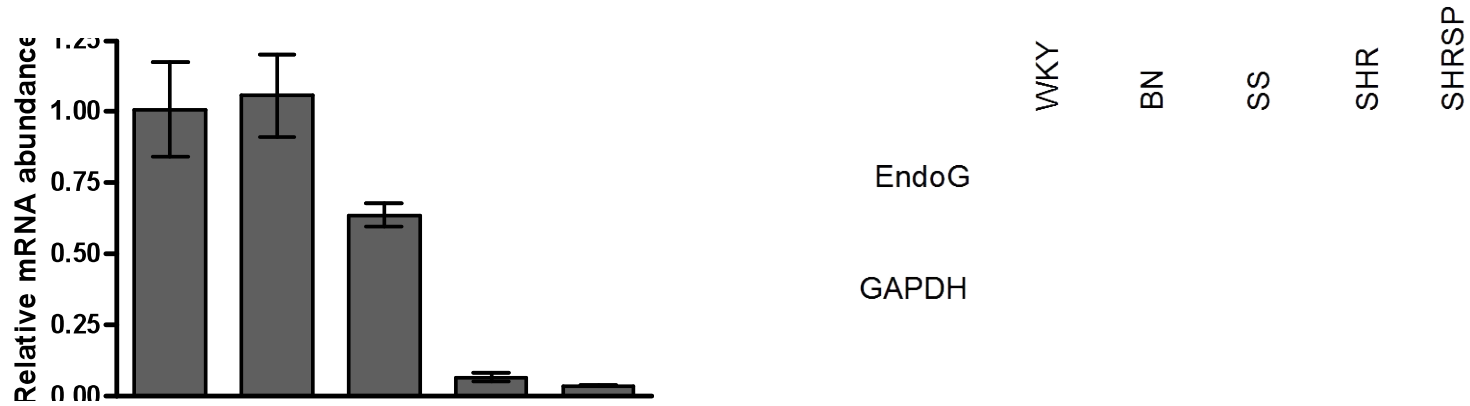
Physiology of the SHR.BN-(3L) congenenic strain



Candidate prioritisation using haplotypes and expression

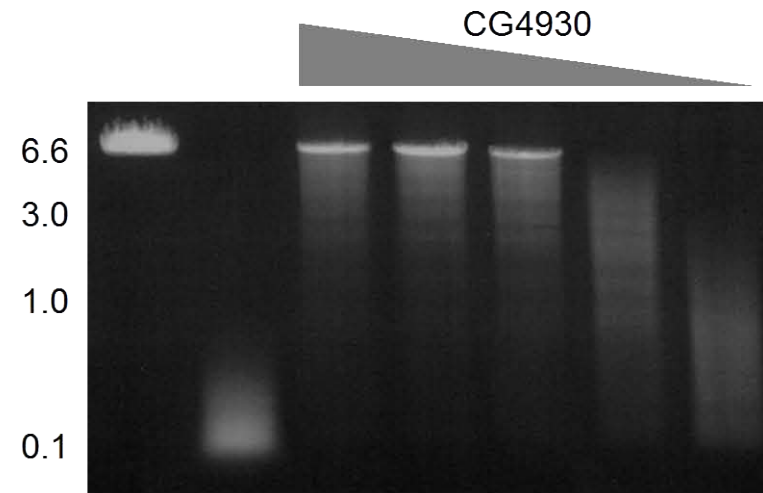
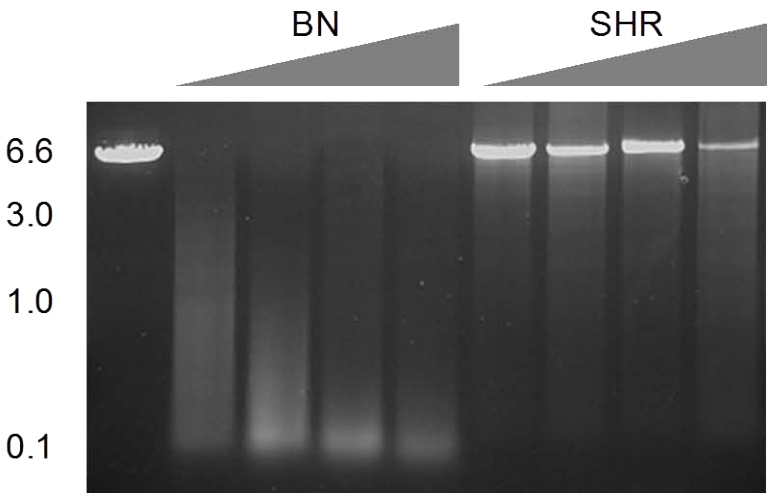
Ensembl SNP ID	Position (Mbp)	Low LVM		High LVM		Haplotype block	Probe ID	Gene symbol	Fold change		P value				
		BN-LX		SHRSP					SHR vs WKY	SHRSP vs WKY	SHR vs WKY	SHRSP vs WKY			
		WKY	BN-LX	SS	SHR				SHRSP						
ENSRNOSNP2787453	6.445	T	C	C	C	C	1: 8870982-9022111	1388019_at	<i>Odf2</i>	-1.32	1.00	<0.05	n.s.		
ENSRNOSNP2787454	6.641	A	A	A	A	A		1368466_a_at	<i>Odf2</i>	2.13	-1.00	<0.01	n.s.		
ENSRNOSNP2787455	6.709	G	A	A	G	G		1399142_at	--	2.19	1.07	<0.0001	n.s.		
ENSRNOSNP2787456	6.972	C	T	T	C	C		1393357_at	<i>Gle1</i>	-1.17	1.06	n.s.	n.s.		
ENSRNOSNP2787458	7.117	T	C	C	T	T		1374690_at	<i>Gle1</i>	1.41	1.18	n.s.	n.s.		
ENSRNOSNP2787460	7.158	G	T	T	G	G		1397752_at	--	-1.26	1.02	<0.01	n.s.		
ENSRNOSNP2787462	7.446	C	C	T	C	C		1370838_s_at	<i>Spna2</i>	4.65	1.03	<0.0001	n.s.		
ENSRNOSNP2787463	7.552	A	A	G	A	A		1379524_at	<i>Wdr34</i>	1.87	1.21	n.s.	n.s.		
ENSRNOSNP2787464	7.652	T	T	C	T	T	2: 9126115-9195508	1389642_at	--	3.11	-1.05	<0.01	n.s.		
ENSRNOSNP2787465	7.759	C	T	C	C	C			1389816_at	<i>Endog</i>	-1.75	-3.57	<0.01	<0.0001	
ENSRNOSNP2787467	7.914	G	A	G	G	G			1373782_a_at	<i>LOC499770</i>	-1.39	1.01	n.s.	n.s.	
ENSRNOSNP2787468	7.914	A	G	A	A	A			1373667_at	<i>Ccbl1</i>	1.49	-1.51	<0.05	<0.05	
ENSRNOSNP2787469	7.959	A	A	G	A	A		3: 9815960-9980785	1393913_at	<i>RGD1311084</i>	-1.38	-1.00	n.s.	n.s.	
ENSRNOSNP2787470	8.124	T	A	A	A	A				1381152_at	<i>Mettl11a</i>	1.88	-1.03	<0.01	n.s.
ENSRNOSNP2787471	8.136	A	G	A	A	A				1372185_at	<i>Mettl11a</i>	2.53	-1.23	<0.01	n.s.
ENSRNOSNP2787472	8.279	C	T	C	C	C				1371497_at	<i>Asb6</i>	1.56	1.06	n.s.	n.s.
ENSRNOSNP2787473	8.336	G	G	A	G	G				1390772_at	--	-1.21	1.03	n.s.	n.s.
ENSRNOSNP2787474	8.422	G	T	T	T	T				1374477_at	<i>Prrx2</i>	1.05	1.22	n.s.	n.s.
ENSRNOSNP2787475	8.592	C	C	T	C	C			1368015_at	<i>Ptges</i>	-2.29	-1.08	<0.01	n.s.	
ENSRNOSNP2787476	8.674	T	T	C	T	T			1368014_at	<i>Ptges</i>	-3.96	-1.02	<0.0001	n.s.	
ENSRNOSNP2787477	8.818	G	G	A	G	G	4: 10039939-10197334		1378309_at	<i>Usp20</i>	1.52	1.07	n.s.	n.s.	
ENSRNOSNP2787478	8.871	N	C	T	C	C				1376656_at	<i>Usp20</i>	1.85	-1.03	n.s.	n.s.
ENSRNOSNP2787479	8.949	A	A	A	G	G			1377941_at	--	-1.65	1.01	<0.0001	n.s.	
ENSRNOSNP2787480	9.022	T	C	T	T	T			1376784_at	<i>Fnbp1</i>	-1.39	-1.10	n.s.	n.s.	
ENSRNOSNP2787481	9.096	C	T	C	C	C			1372825_at	<i>Fnbp1</i>	2.51	-1.26	<0.0001	n.s.	
ENSRNOSNP2787482	9.126	T	C	C	T	T			1373537_at	--	1.03	1.03	n.s.	n.s.	
ENSRNOSNP2787483	9.160	G	G	G	C	C			1377342_s_at	<i>Fnbp1</i>	-1.72	1.06	<0.01	n.s.	
ENSRNOSNP2787484	9.196	T	C	C	C	C			1390682_at	<i>Fnbp1</i>	-4.95	1.09	<0.0001	n.s.	
ENSRNOSNP2787485	9.279	C	C	T	C	C			1369471_at	<i>Fnbp1</i>	1.12	1.18	n.s.	n.s.	
ENSRNOSNP2787486	9.320	G	G	G	G	G			1395365_at	--	-1.54	1.09	n.s.	n.s.	
ENSRNOSNP2787487	9.325	T	C	C	C	C	5: 10697268-10944869	1379947_at	--	-2.13	1.05	<0.0001	n.s.		
ENSRNOSNP2787488	9.325	G	G	G	G	G			1375406_s_at	<i>Fnbp1</i>	-1.62	-1.04	<0.05	n.s.	
ENSRNOSNP2787490	9.472	G	A	G	A	A			1381483_at	--	-1.03	1.06	n.s.	n.s.	
ENSRNOSNP2787491	9.492	C	C	T	C	C			1382390_at	--	-1.06	-1.08	n.s.	n.s.	
ENSRNOSNP2787492	9.816	A	C	A	C	C			1393006_at	--	1.11	1.51	n.s.	n.s.	
ENSRNOSNP2787493	9.965	T	T	T	C	C			1375614_at	<i>Prdm12</i>	-2.28	-1.04	<0.01	n.s.	
ENSRNOSNP2787494	9.981	T	T	A	T	T			1379348_at	<i>Exosc2</i>	-1.05	1.04	n.s.	n.s.	
ENSRNOSNP2787495	10.023	C	T	T	C	C			1379348_at	<i>Exosc2</i>	-1.05	1.04	n.s.	n.s.	
ENSRNOSNP2787496	10.026	G	T	G	G	G			1371713_at	<i>Abl1</i>	2.84	-1.11	<0.01	n.s.	
ENSRNOSNP2787497	10.040	A	A	A	A	A			1390677_at	<i>Fibcd1</i>	-1.12	-1.08	n.s.	n.s.	
ENSRNOSNP2787498	10.041	T	T	T	C	C									
ENSRNOSNP2787500	10.197	G	A	G	G	G									
ENSRNOSNP2787501	10.213	C	C	T	C	C									
ENSRNOSNP2787502	10.272	G	A	G	G	G									
ENSRNOSNP2787504	10.372	C	T	T	C	C									
ENSRNOSNP2787505	10.586	T	C	T	T	T									
ENSRNOSNP2787506	10.697	G	A	G	A	A									
ENSRNOSNP2787507	10.796	A	A	A	T	T									
ENSRNOSNP2787508	10.945	T	C	T	T	T									
ENSRNOSNP2787509	11.015	C	T	C	C	C									
ENSRNOSNP2787510	11.058	G	T	G	G	G									
ENSRNOSNP2787511	11.182	T	C	T	T	T									

Endog expression and sequencing

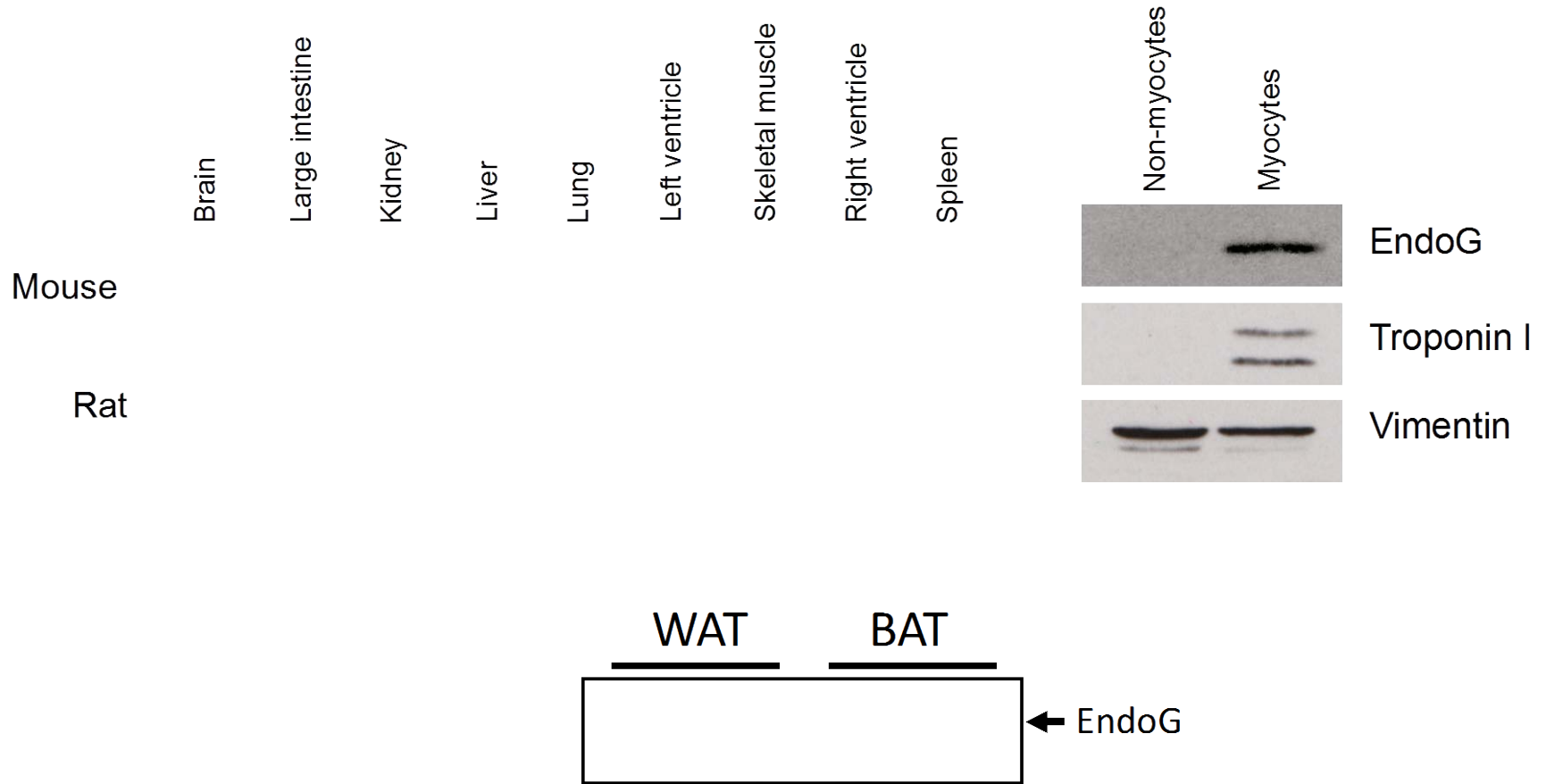


	Putative promoter		Exon I
	-313	-278	+454
SS/Jr	T	-	-
WKY/Tkyo	T	-	-
SHR/Tkyo	C	18 bp del	37 bp repeat
SHRSP/Tkyo	C	18 bp del	37 bp repeat

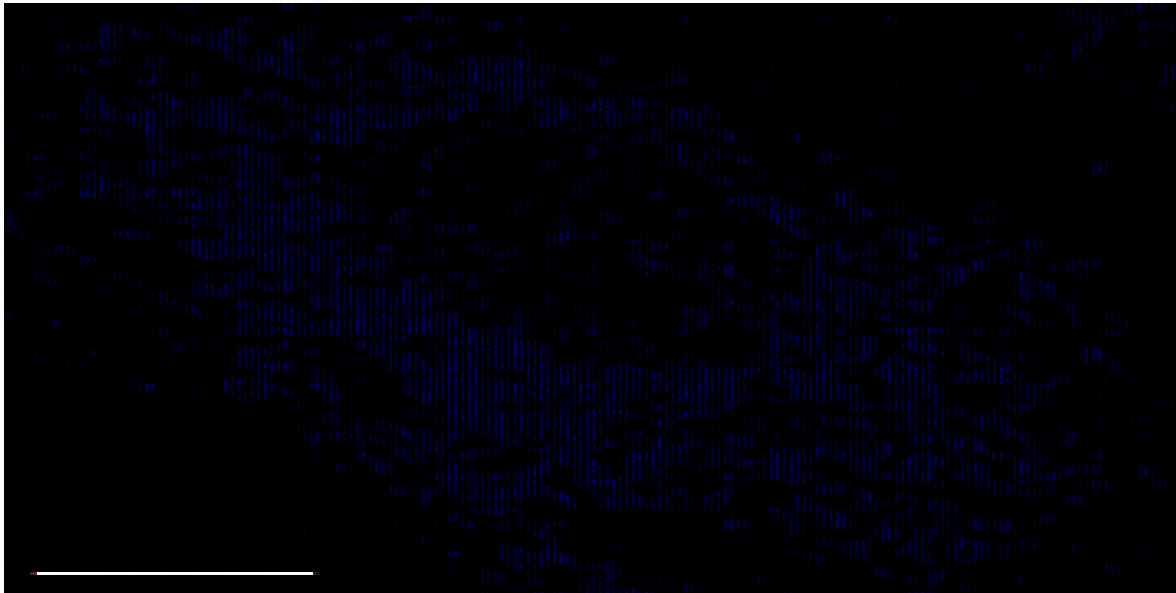
Endog – a major heart nuclease



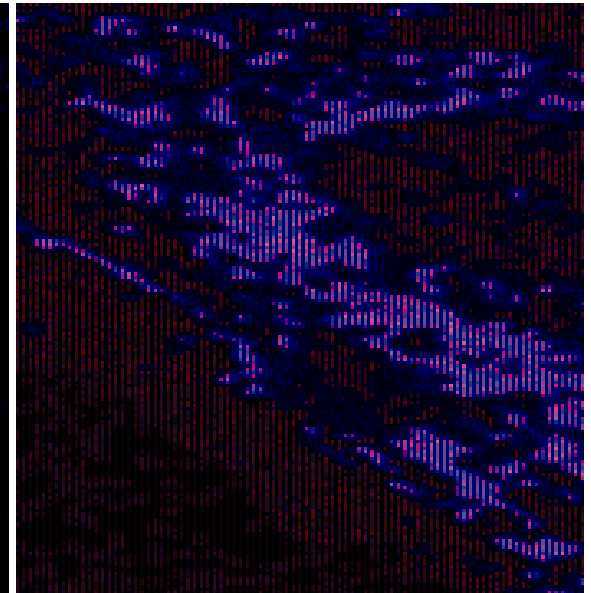
Endog expression



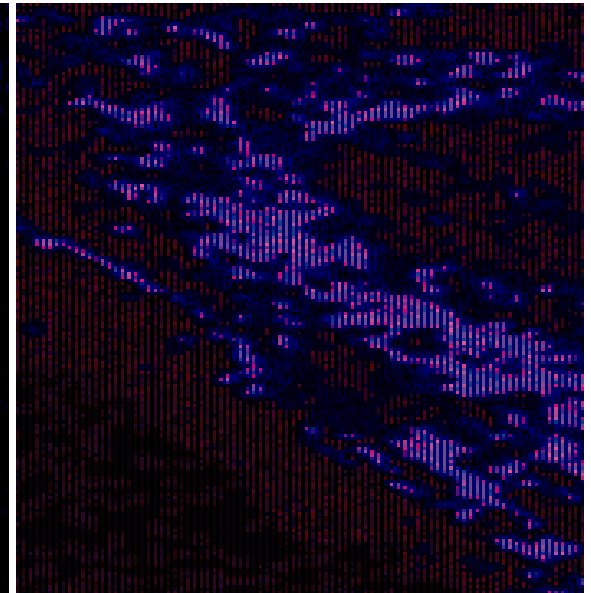
Endog localisation in cardiac myocytes



Endog

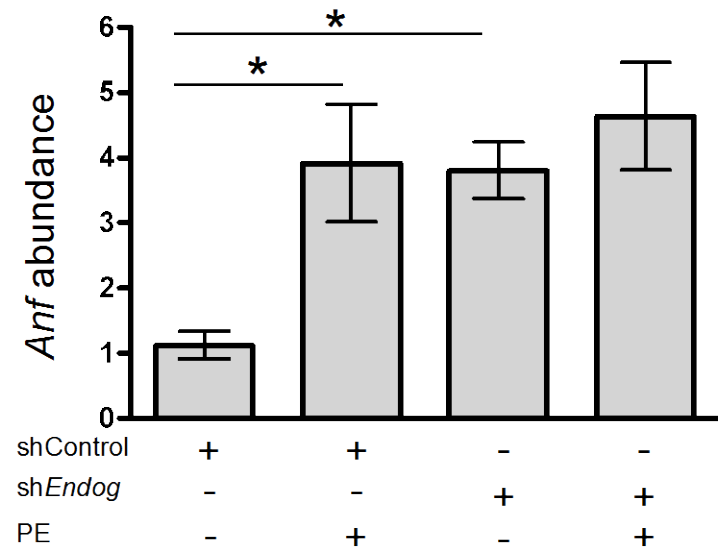
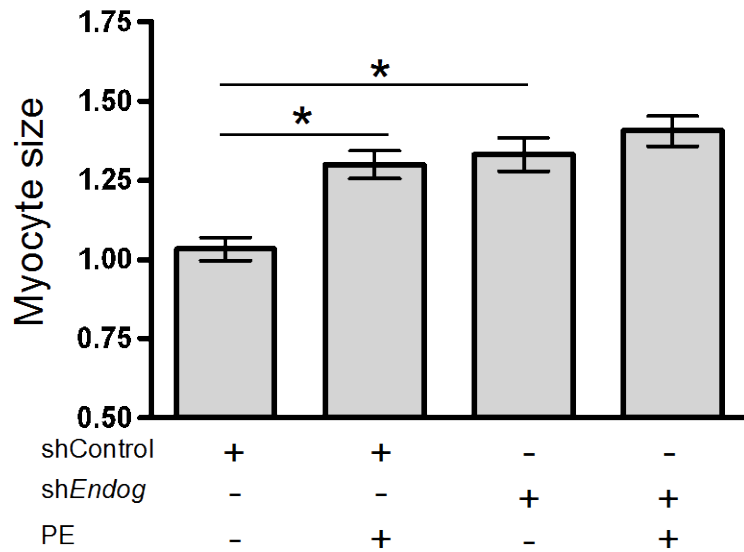


Mitotracker

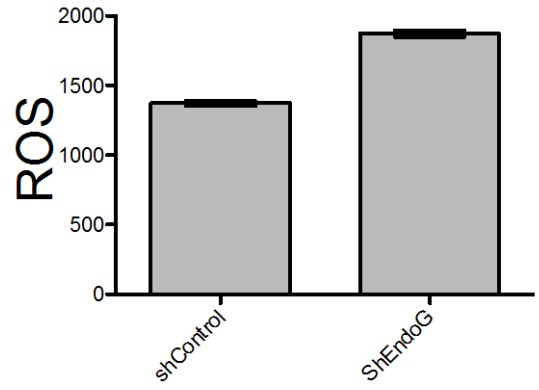
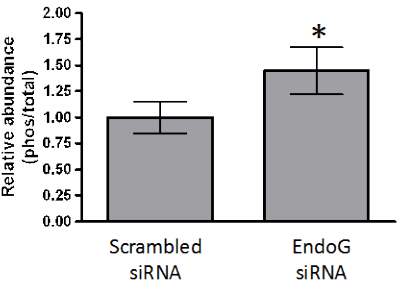
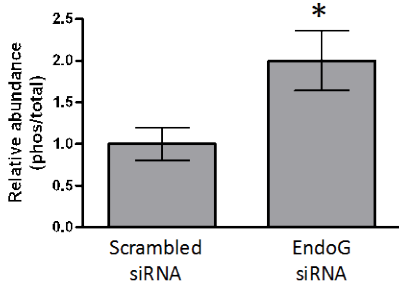
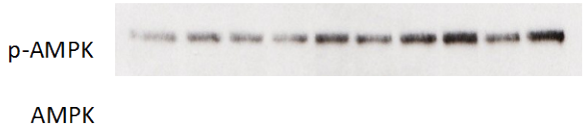
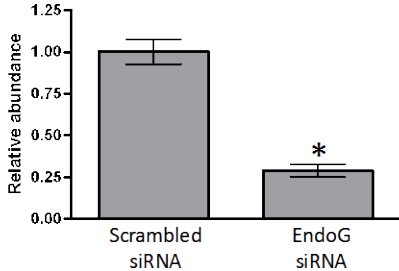
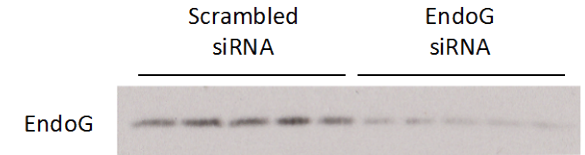


Merge

Endog loss-of-function induces cardiac hypertrophy *in vitro*



Endog loss-of-function activates AMPK and increases ROS



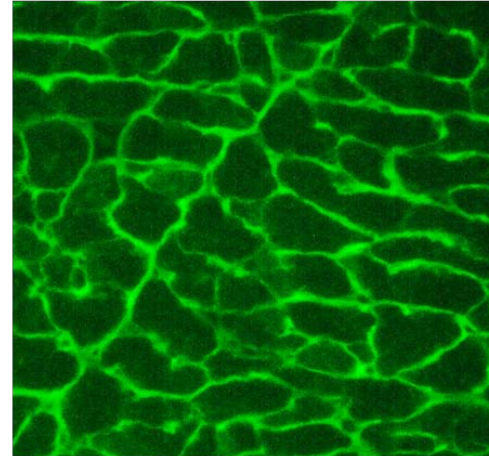
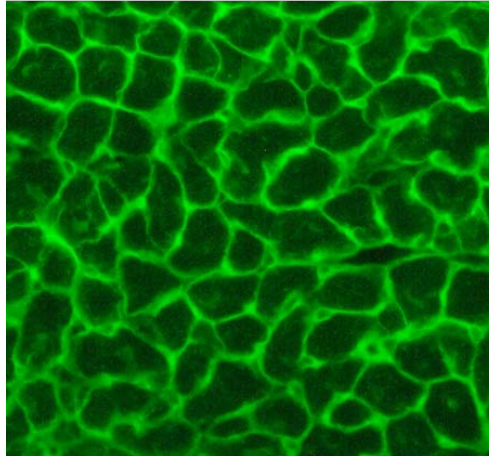
Endog loss-of-function induces cardiac hypertrophy *in vivo*

AngII

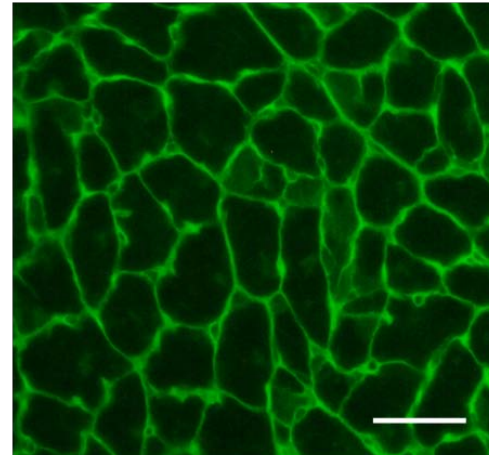
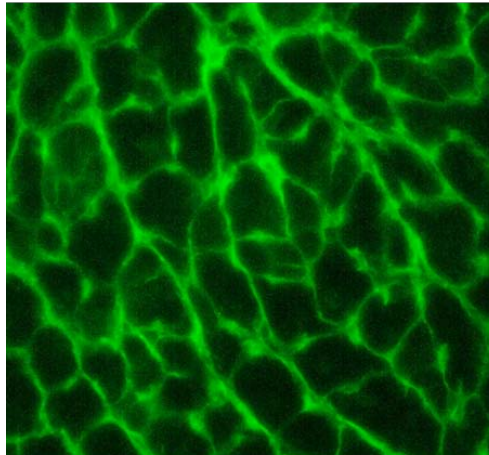
WT

Endog^{-/-}

-



+



Endog – what does it do?

Endog is important for apoptosis

- Li, L. Y., Luo, X. & Wang, X. Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature* **412**, 95-99, (2001)

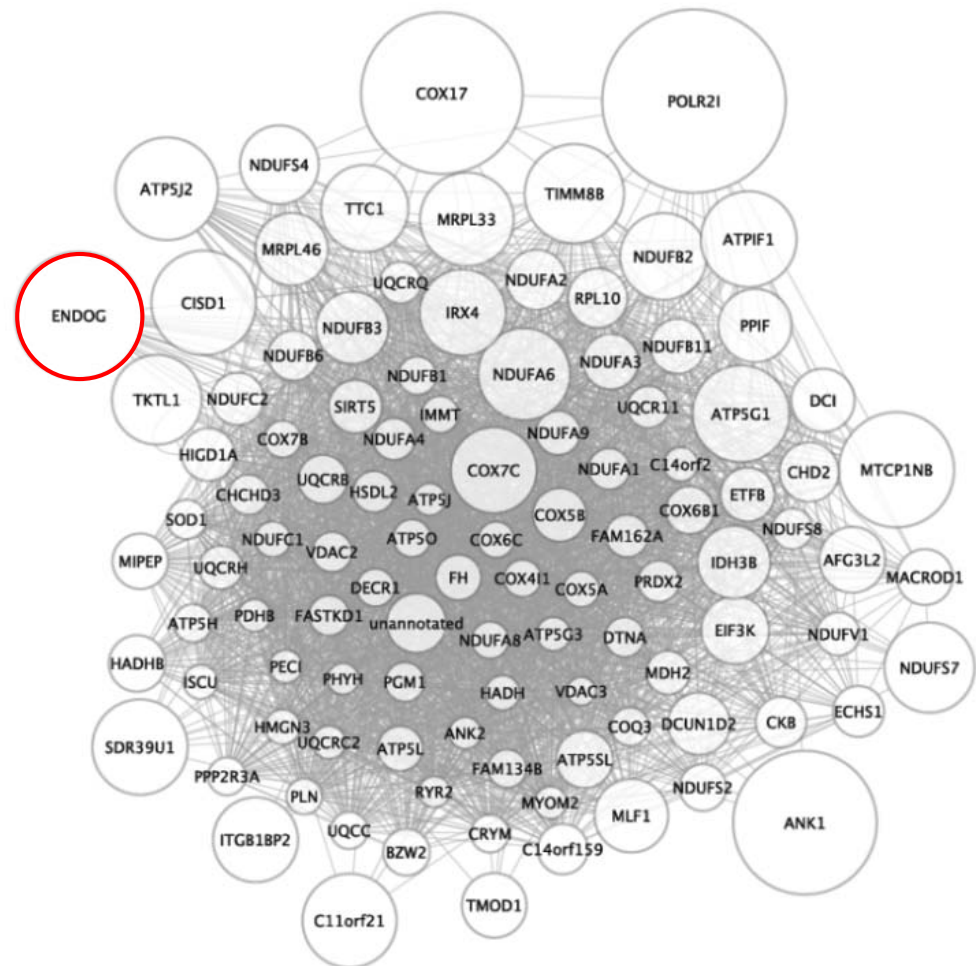
Endog has no effect on apoptosis

- Irvine, R. A. *et al.* Generation and characterization of endonuclease G null mice. *Mol Cell Biol* **25**, 294-302, (2005)
- David, K. K., Sasaki, M., Yu, S. W., Dawson, T. M. & Dawson, V. L. EndoG is dispensable in embryogenesis and apoptosis. *Cell Death Differ* **13**, 1147-1155, (2006)

Endog is important for cell proliferation

- Buttner, S. *et al.* Endonuclease G regulates budding yeast life and death. *Mol Cell* **25**, 233-246, (2007)

Endog belongs to a an oxidative phosphorylation network



□

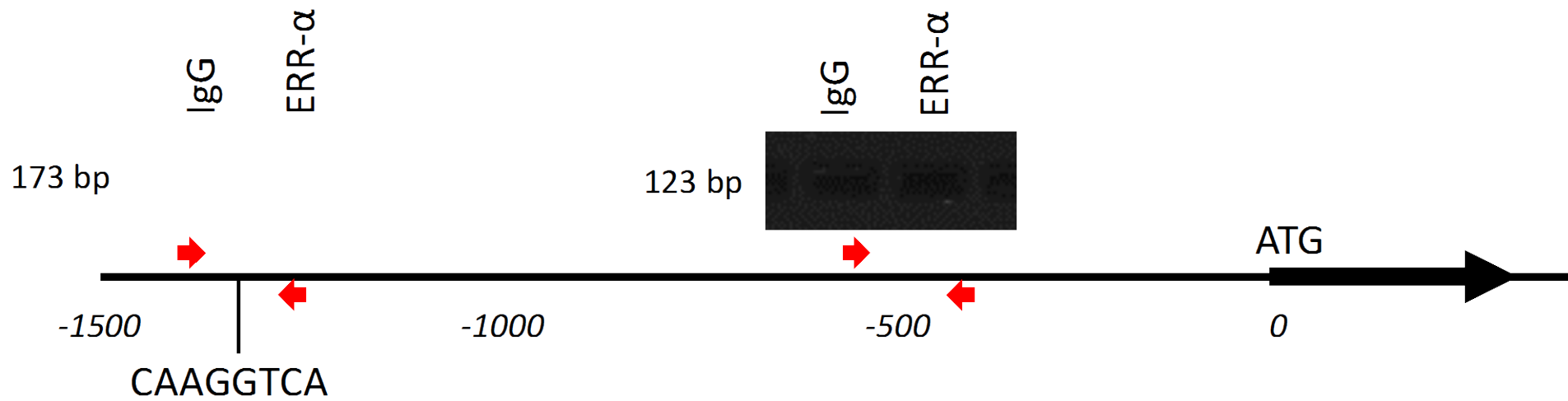
Zhang, B. & Horvath, S. A general framework for weighted gene co-expression network analysis. *Stat Appl Genet Mol Biol*4, (2005)

GO component: Mitochondrion, $P=2 \times 10^{-58}$

GO function: Oxidative phosphorylation, $P=2 \times 10^{-37}$

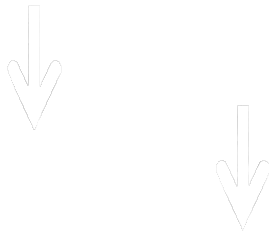
PGC1 α and *ERR α* regulate *Endog* expression

ChIP *ERR* α : Endog promoter PCR

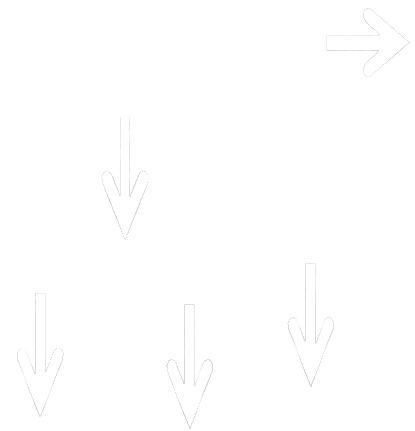


Mitochondrial-associated “droplets” in *Endog* KO hearts

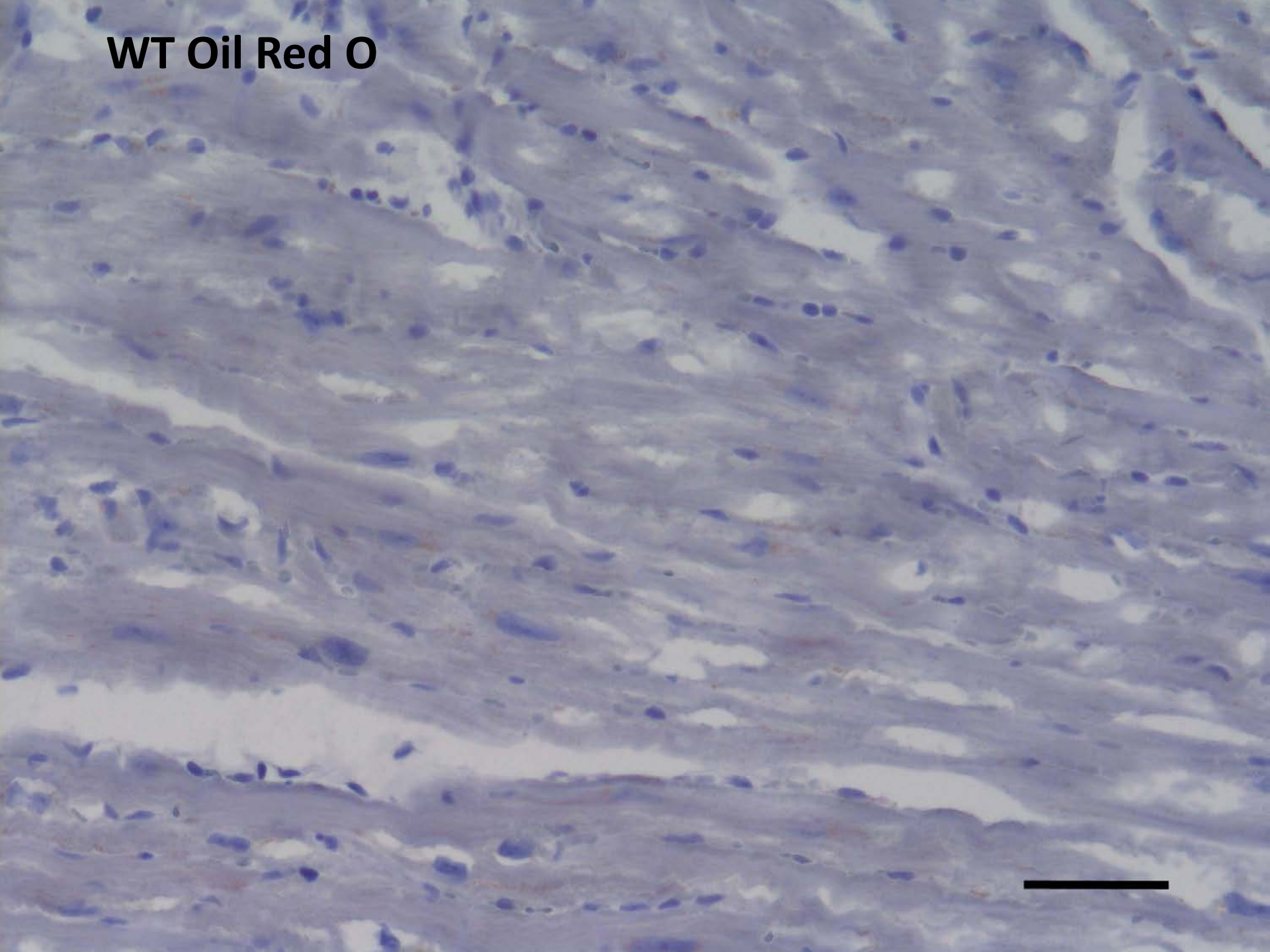
WT



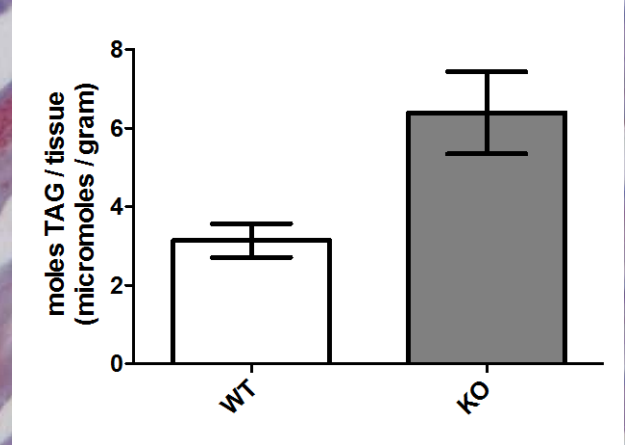
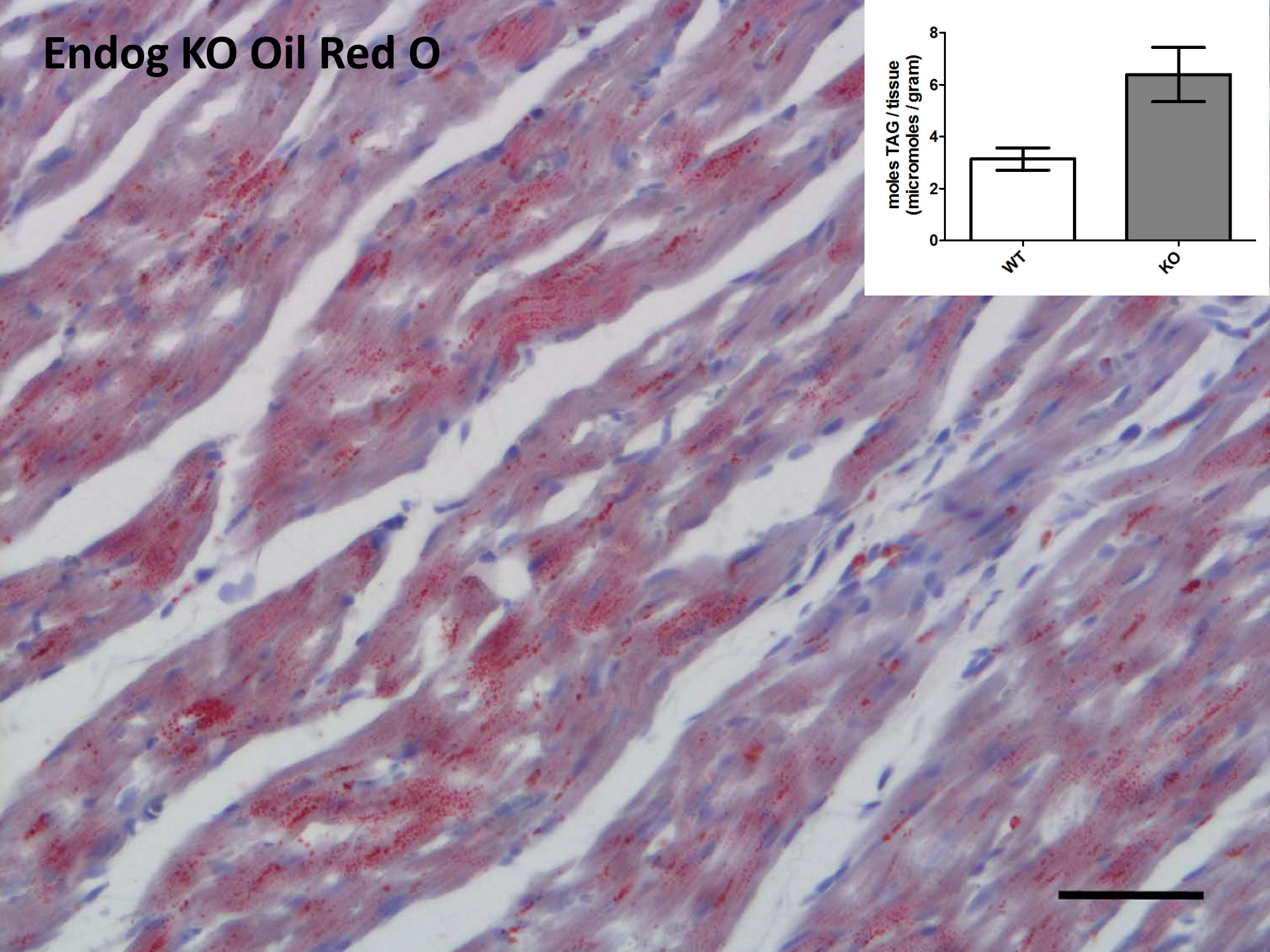
Endog^{-/-}



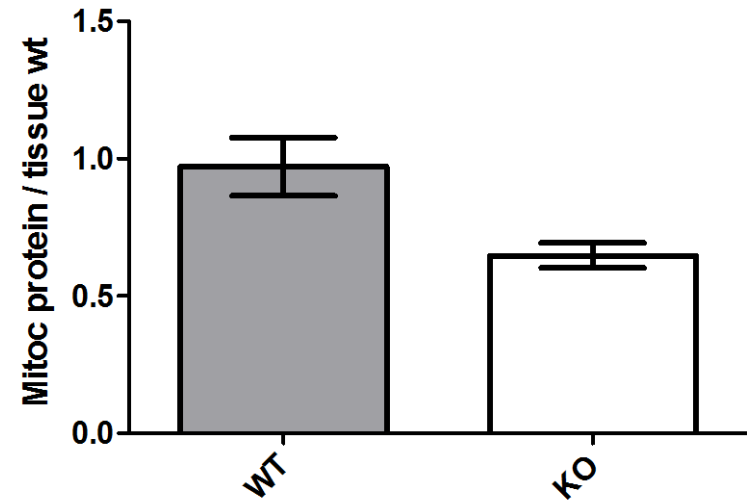
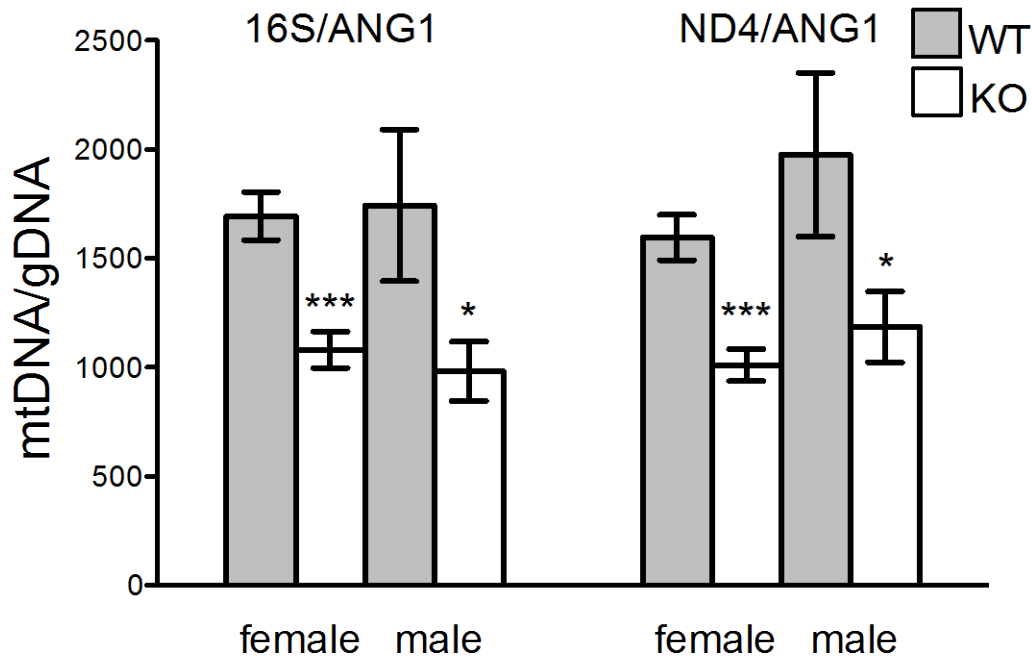
WT Oil Red O



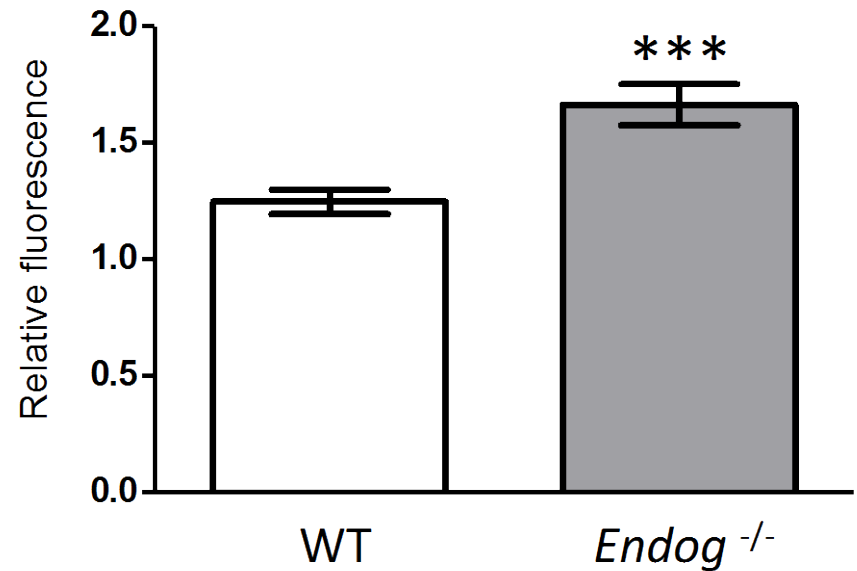
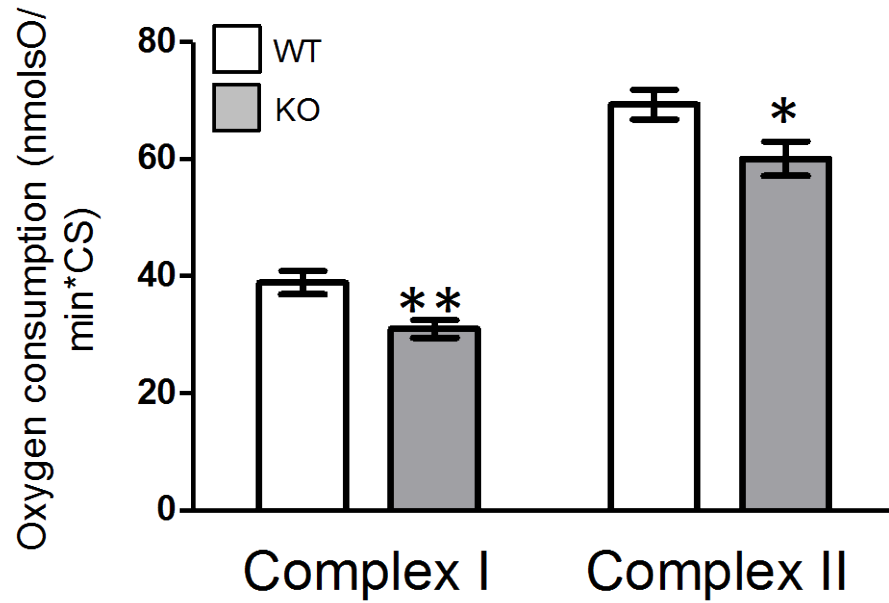
Endog KO Oil Red O



Depletion of mitochondrial DNA and protein



Mitochondrial dysfunction and ROS production

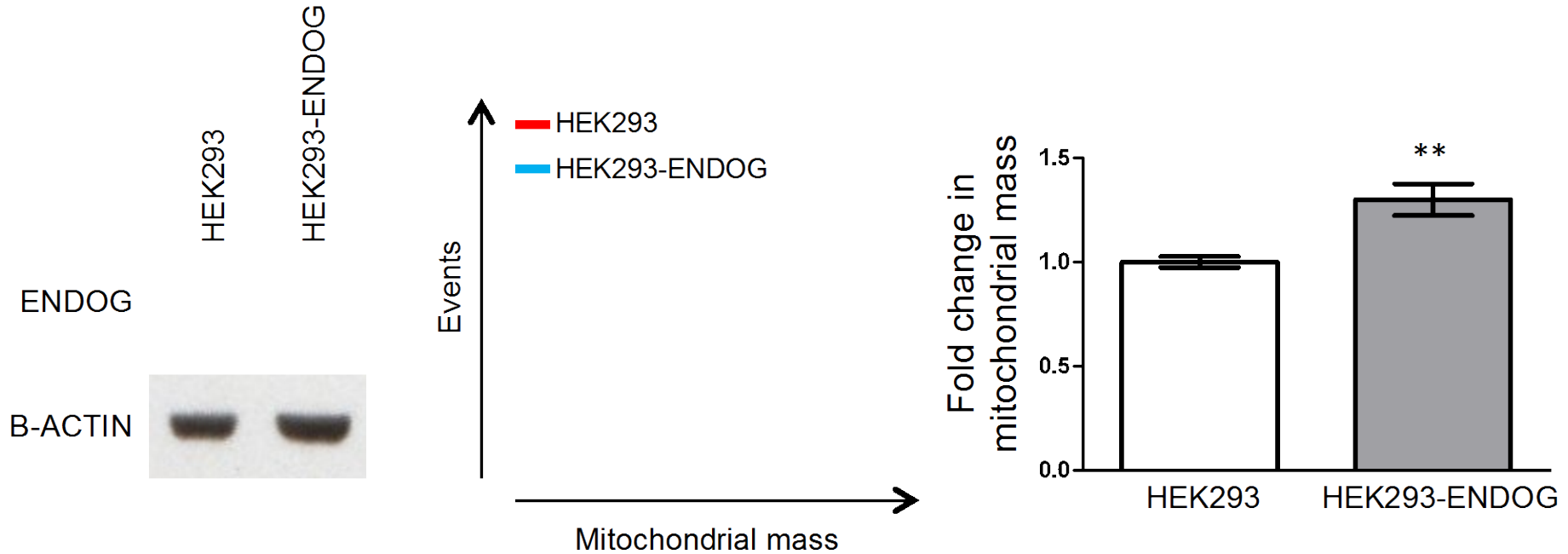


A role for *Endog* in mitochondrial biogenesis?

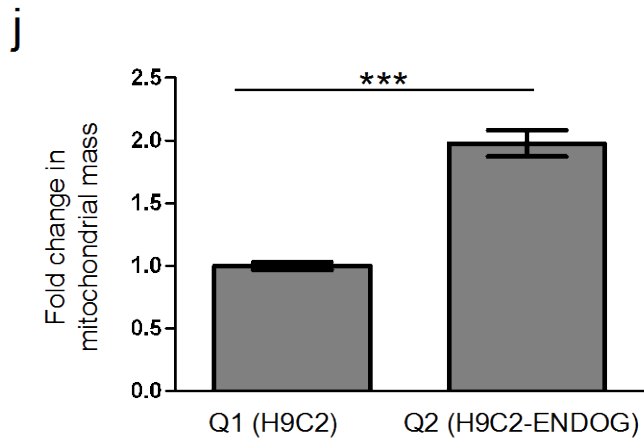
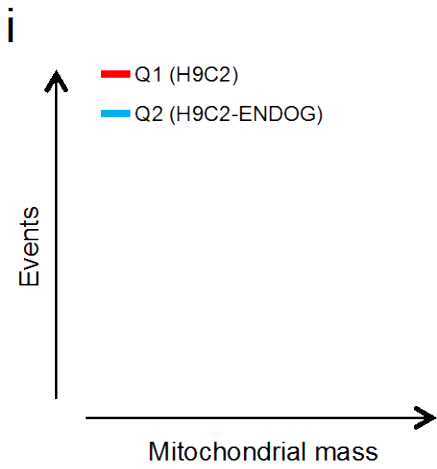
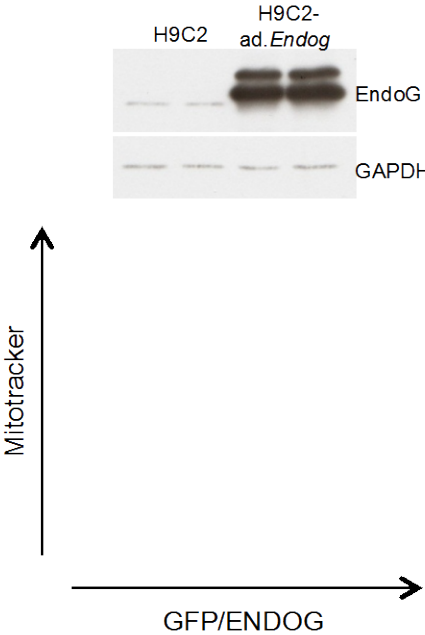
Cote, J. & Ruiz-Carrillo, A. Primers for mitochondrial DNA replication generated by endonuclease G. *Science* **261**, 765-769, (1993).

Tiranti, V. *et al.* Chromosomal localization of mitochondrial transcription factor A (TCF6), single-stranded DNA-binding protein (SSBP), and endonuclease G (ENDOG), three human housekeeping genes involved in mitochondrial biogenesis. *Genomics* **25**, 559-564, (1995).

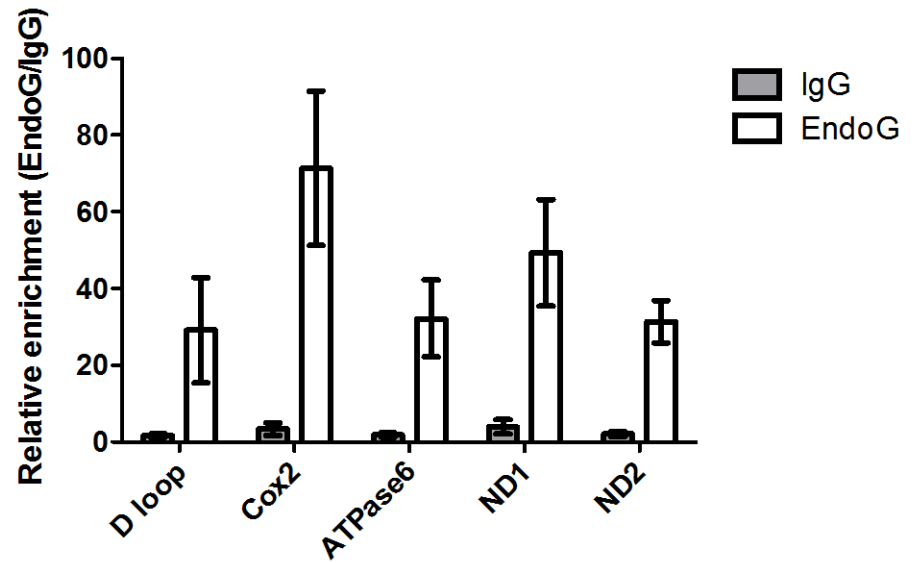
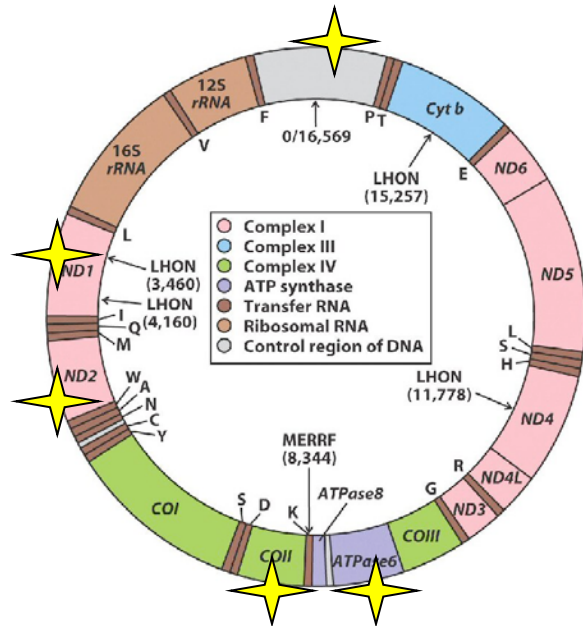
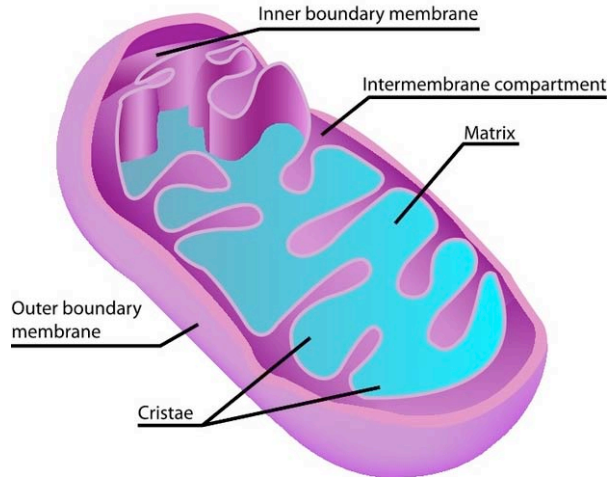
Endog expression regulates mitochondrial biogenesis in HEKs



Endog regulates mitochondrial biogenesis in H9C2 cells

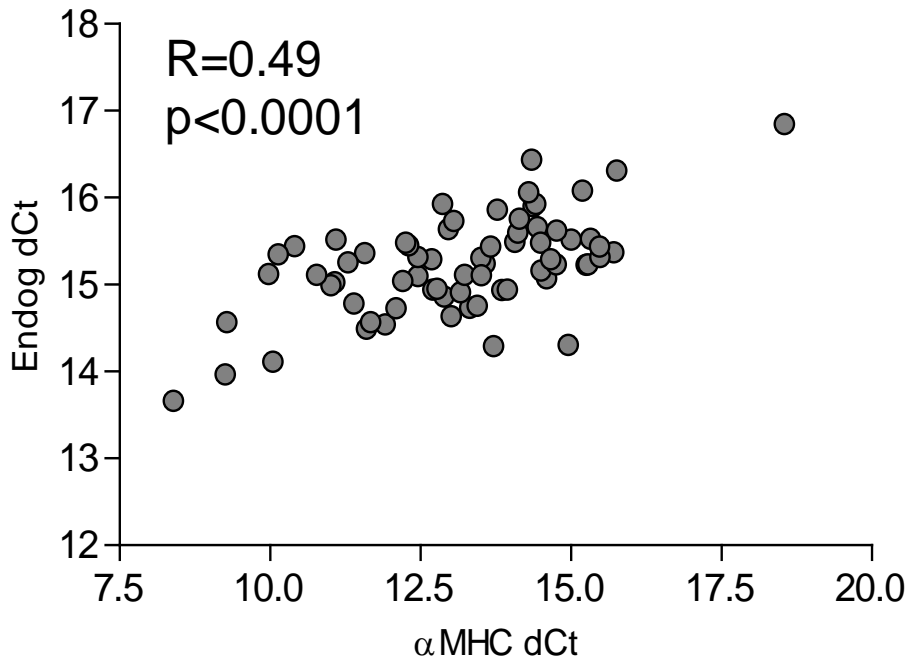


EndoG ChIP + mitochondrial gene PCR

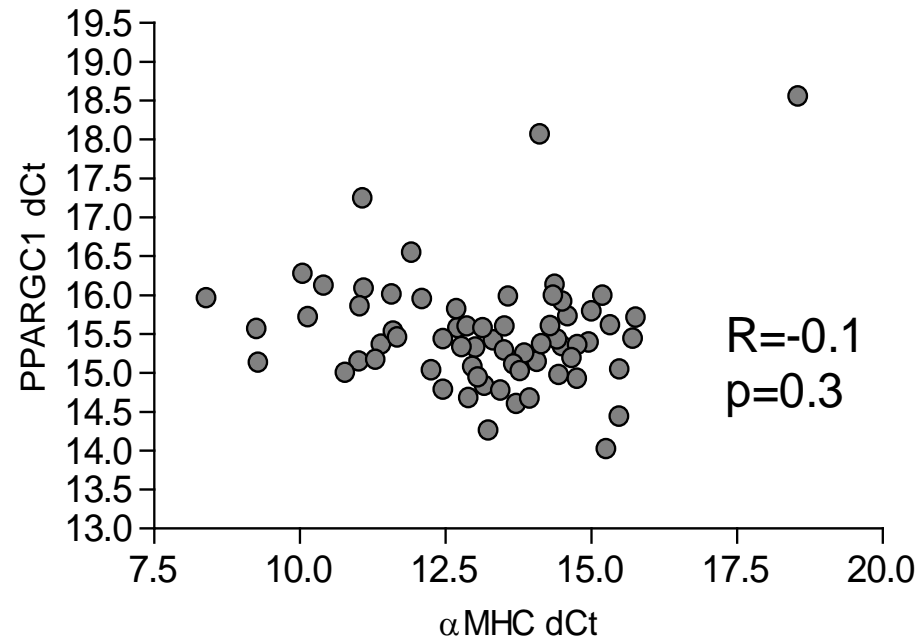


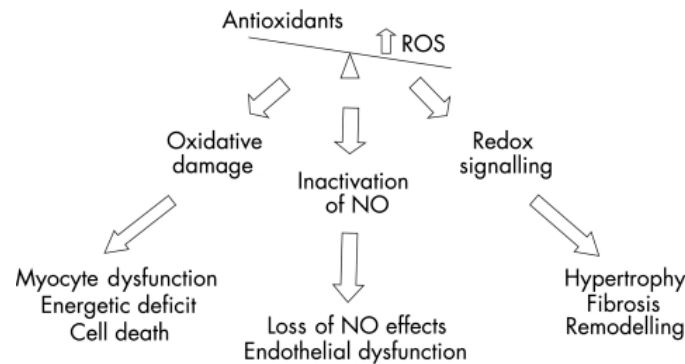
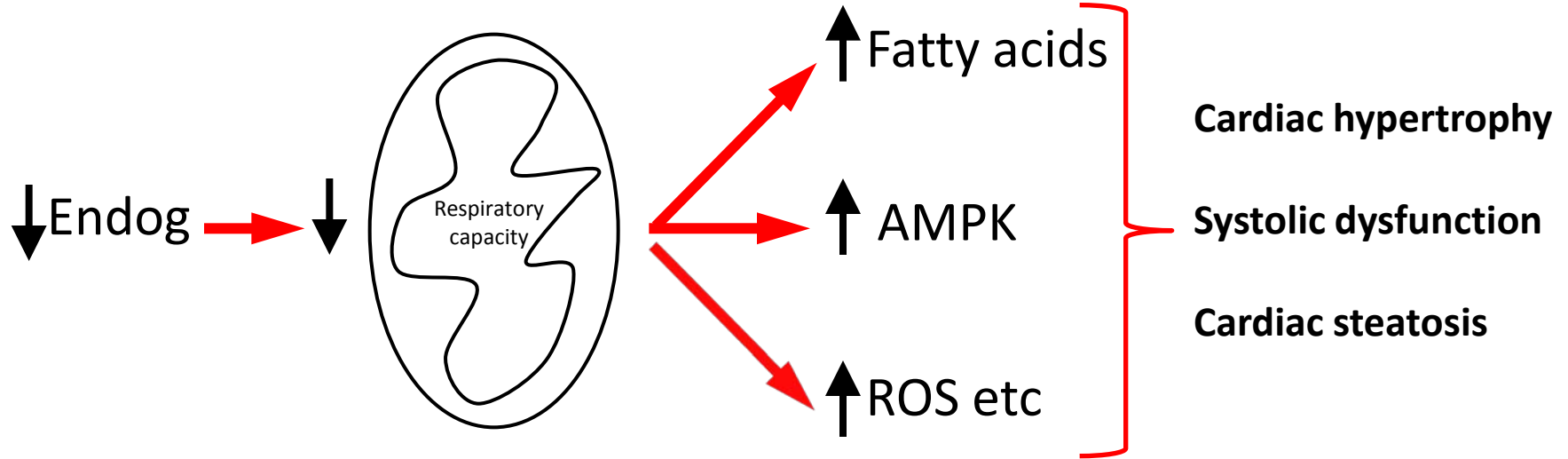
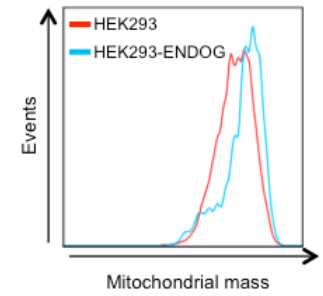
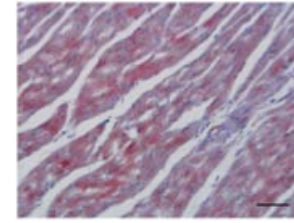
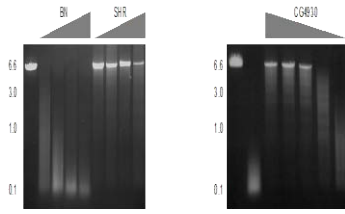
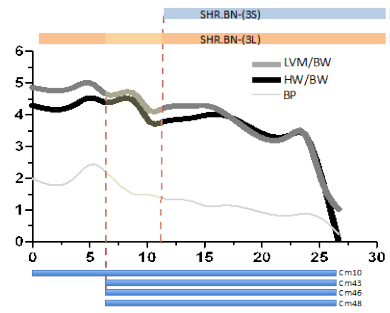
EndoG expression in the failing human heart

α MHC vs. Endog



α MHC vs. PPARGC1





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Xi-Ming Sun
Rizwan Ahmed
James Ware
Paul Barton
Enrico Petretto

University Lleida, Spain
Daniel Sanchis

Institute of Physiology, Czech Rep
Michal Pravenec

MDC, Germany
Norbert Hubner

Harvard, USA
Zolt Arany