SUPPORTING INFORMATION

Role of cellular retinol-binding protein, type 1 and retinoid homeostasis in the adult mouse heart: a multi-omic approach

Stephanie Zalesak-Kravec¹+, Weiliang Huang¹+, Jace W. Jones¹+, Jianshi Yu¹, Jenna Alloush¹, Amy E. Defnet¹, Alexander R. Moise², Maureen A. Kane^{1*}

¹University of Maryland, School of Pharmacy, Department of Pharmaceutical Sciences, Baltimore, MD;

²Medical Sciences Division, Northern Ontario School of Medicine, Sudbury, ON, Canada; Departments of

Chemistry and Biochemistry, and Biology and Biomolecular Sciences Program, Laurentian University,

Sudbury, ON, Canada.

+Co-first authors

*Corresponding Author

Correspondence: Maureen A. Kane University of Maryland, School of Pharmacy Department of Pharmaceutical Sciences 20 N. Pine Street, Room N731 Baltimore, MD 21201 Phone: (410) 706-5097 Fax: (410) 706-0886 Email: makne@rx.umaryland.edu



Supplementary Figure 1. Multivariate analysis and hierarchical clustering of *Rbp1*-/- and WT mice tissues.

A. PCA plot comparing *Rbp1*^{-/-} Atria (Red), *Rbp1*^{-/-} LV (Green), *Rbp1*^{-/-} RV (Blue), WT Atria (Cyan), WT LV (Magenta), WT RV (Yellow), with n=5 per group.

B. PCA plot comparing all $Rbp1^{-/-}$ samples (black) and WT samples (gray). n=15 per group. PCA displayed a total of 54.2% variance, attributed to PC1 (35.3%) and PC2 (18.9%) components. The 95% confidence intervals are indicated by elliptical patterns per group. C. Heatmap displaying the top 25 metabolites of $Rbp1^{-/-}$ (black) and WT (gray) based on t-test/ANOVA, Euclidean distancing, and Ward clustering.



Supplementary Figure 2. Multivariate analysis and hierarchical clustering displays statistical metabolite differences between atria for WT (cyan) and *Rbp1^{-/-}* (red) mice. A. PLS-DA plot R2=0.99. Q2=0.91. N=5 per group. Each point represents a data set from an individual animal tissue. The 95% confidence intervals are indicated by elliptical patterns per group.

B. PCA plot. PCA displayed a total of 56.9% variance, attributed to PC1 (34%) and PC2 (22.9%) components. The 95% confidence intervals are indicated by elliptical patterns per group.

C. Heatmap displaying the top 25 metabolites based on t-test/ANOVA, Euclidean distancing, and Ward clustering.



Supplementary Figure 3. Multivariate analysis and hierarchical clustering displays statistical metabolite differences between Left Ventricle of WT (magenta) and *Rbp1*-/- (green) mice.

A. PLS-DA plot R2=0.99. Q2=0.82. N=5 per group. Each point represents a data set from an individual animal tissue. The 95% confidence intervals are indicated by elliptical patterns per group.

B. PCA plot. PCA displayed a total of 60.6% variance, attributed to PC1 (39.3%) and PC2 (21.3%) components. The 95% confidence intervals are indicated by elliptical patterns per group.

C. Heatmap displaying the top 25 metabolites based on t-test/ANOVA, Euclidean distancing, and Ward clustering.



Supplementary Figure 4. Multivariate analysis and hierarchical clustering displays statistical metabolite differences between Right Ventricle (RV) for WT (blue) and *Rbp1*^{-/-} (yellow) mice.

A. PLS-DA plot R2=0.99. Q2=0.83. N=5 per group. Each point represents a data set from an individual animal tissue. The 95% confidence intervals are indicated by elliptical patterns per group.

B. PCA plot. PCA displayed a total of 66.8% variance, attributed to PC1 (48.2%) and PC2 (18.6%) components. The 95% confidence intervals are indicated by elliptical patterns per group.

C. Heatmap displaying the top 25 metabolites based on t-test/ANOVA, Euclidean distancing, and Ward clustering.



Supplemental Figure 5. Multivariate analysis and hierarchical clustering displays metabolite differences between WT LV (dark purple) and RV (light purple).

A. PLS-DA plot R2=0.53. Q2=0.07. N=5 per group. Each point represents a data set from an individual animal tissue. The 95% confidence intervals are indicated by elliptical patterns per group.

B. PCA plot. PCA displayed a total of 71.2% variance, attributed to PC1 (55.3%) and PC2 (15.9%) components. The 95% confidence intervals are indicated by elliptical pattens per roup.

C. Heatmap displaying the top 25 metaoblites based on t-test/ANOVA, Euclidean distancing, and Ward clustering.

Genes	Left	Right	Genes	Left	Right	Genes	Left	Right	Genes	Left	Right	Genes	Left	Right	Genes	Left	Right	
A1bg			Cnn1			Fkbp4			Krt1			Nenf			RpI34			
Abcb10			Cnn3			Flot1			Krt16			Nfe2l2			Rps16			
Abhd14b			Cobll1			Flot2			Krt2			Nhp2			Rps23			
Actr10			Col4a1			Galm			Krt6a			Nmnat3			S100a10			
Acy1			Col6a5			Ganab			Krt76			Nnt			Scamp1			
Асу3			Col6a6			Gbp2			L3hypdh			Nppa			Sept9			
Ago2			Comtd1			Gbp6			Lactb			Obsl1			Serpina1e			
Ahnak2			Cops6			Gfm2			Lamp2			Orm1			Serpinb1a			
Akap12			Cops8			Gm17190			Lancl1			Osbp			Serpinf1			
Alox12			Cotl1			Gm20547			Lbr			Osbpl1a			Serpinf2			
Ambp			Cpn2			Gng2			Lifr			Pafah1b2			Serping1			
Anp32b			Cpne1			Gng5			Lman2			Pakap			Sh2d4a			
Ap1g1			Cpq			Gstm1			Lmcd1			Pam16			SIc35f6			
Ap2a2			Creld2			Gypc			Lrrc10			Parl			Slirp			
Ap2b1			Crkl			H2-Q10			Lsm2			Parvb			Smim26			
Apoc1			Ctbp1			H3f3c			Lsm8			Pcdhb2			Smtn			
Apoc3			Cul5			Hadhb			Lyrm4			Pdha2			Snrnp70			
Apoe			Cutc			Hbb-b2			March1			Pdk4			Snrpd2			
Arhgef28			Ddi2			Hbb-y			Mcrip2			Pgm2			Stat1			
Arpp19			Ddo			, Hdac5			Metap2			Phldb1			Stx7			
Atad5			Ddx19a			Hdgf			Mgst1			Pkia			Surf1			
Atp1b3			Ddx51			Hip1			Mic13			Plbd1			Svngap1			
B2m			Dek			Hmgb3			Mmab			Pon1			Tapbp			
Bckdk			Dmtn			Hmgn2			1aqM			Pobo			Tfrc			
Bves			Dmxl2			Hnrnph2			Mrpl28			Ppip5k2			Tmem256			
Bzw1			Dnah10			Hsna12b			MrnI45			Pome1			Tmlhe			
C4h			Dnah17			Huwe1			Mrns18h			Pnn2r2a			Tmx1			
C9			Dnaic11			Ide			Mrns23			Pnn3r1			Top1			
Cald1			Dnen1			løhø2h			Mrns7			Prns113			Tor1ain1			
Cann8			Dot			laha2c			Munq			Pthn1			Tnd52			
Cav2			Dynlt1			lghv1-15			Myhhn1a			Ptads			Tpd52			
Chr2			Eof1a1			laby1-21			Mybopia			Dtnn12			Toop			
Code/7			Fif2h2			laby1-30			Myb11			Durb			Trot1			
CdE02			EITZDZ Eif2b			Ighv1 25			N/vb7			Puid			Tubb1			
CdEl			EII SII			IgKV1-33						Pyuri Pah2E						
			EII SI			IgKV15-05			N/14			RdU33			Tup2			×0/WTT
			EIT4e			1gKV4-5/									IXIIZ			>10
Ceopzos			EIT5D			IgKV4-58						картр			UDe 3a			5
Ces1b			Emi2			igkv9-124			My K3			Rap1gds1			Ubqin2			2
Ces1d			Epb42			igics			Nyot			Rasip1			Uggt1			1
Chmp5			F12			lrgm1			Naalad2			Rbm3			Vamp8			0.5
Chpt1			Fars2			lsg15			Nadk2			Rcn3			Vma21			0.2
Clasp1			Fasn			ltgb1			Nbeal1	_		Rpia			Vps26b			< 0.1
Clta			Fbxo40			Klkb1			Ncoa5			Rpl24						
Cmc1			Fitm1			Knstrn			Ndufaf8			RpI32						

Supplementary Figure 6. Expression of proteins most changed in either left or right ventricles of *Rbp1^{-/-}* **mice compared to WT mice.** Minimum 2-fold change of expression and FDR adjusted p-value <0.05 were criteria for inclusion.

Genes	wт	ко	Genes	wт	ко	Genes	wт	ко	Genes	wт	ко	Genes	wт	ко
Abcb7			Cops8			Hadhb			Mybbp1a			Rps23		
Abhd14b			Coro1c			Hbb-b2			Mylk			Rufy1		
Acin1			Cotl1			Hip1			Mypn			Safb		
Actbl2			Cox15			Hist2h3c1			Naprt			Sars		
Actr10			Срq			Hmgn2			Ncoa5			Scamp1		
Add1			Creld2			Hsd17b11			Ndufaf8			Selenop		
Agfg2			Crkl			Huwe1			Nfe2l2			Serpinb1a		
Ago2			Cutc			lgbp1			Nhp2			Serpinf1		
Ahsp			Ddi2			lghv1-15			Nppa			Sf3b1		
Aldh1l1			Ddx19b			lghv1-31			Nudt17			Sf3b2		
Alox12			Dek			lghv1-39			Obsl1			Sh2d4a		
Ambp			Denr			Igkv4-58			Osbpl1a			Sh3kbp1		
Anp32b			Dmxl2			lgkv9-124			Oxsr1			SIc35f6		
Ap1g1			Dnajc11			Iglc3			Pafah1b2			Slirp		
Ap2a2			Dynlt1			lrgm1			Pakap			Smarcc2		
Apmap			Efemp1			ltgb1			Parl			Smim26		
Arhgef28			Eif2b2			ltsn2			Parvb			Smtn		
Arpp19			Eif3h			Klkb1			Pcdhb2			Snca		
Atad5			Eif3i			Kpna3			Pf4			Snrnp70		
Atp2a3			Eif4b			Krt2			Pgm2			Snrpd2		
Bckdk			Eif4e			Krt6a			Phldb1			Spcs2		
Bin2			Eif5b			Lama3			Pkia			Surf1		
Camk2a			Etl4			Lamp2			Plek			Svil		
Capn8			F12			Lamtor2			Ppbp			Syngap1		
Cav2			Fbxo40			Larp4b			Ppm1g			Tbc1d4		
Cbr2			Fitm1			Lima1			Ppp1r12a			Tgfb1i1		
Cbr4			Fkbp15			Lrrc10			Ppp3r1			Top1		
Ccdc127			Flot1			Lsm2			Prps1l3			Tor1aip1		
Cd59a			Flot2			Lsm8			Ptbp1			Tpd52		
Cd5l			Fmo2			March1			Ptms			Treml1		
Cdc42bpb			Ganab			Mcrip2			Ptpn12			Triobp		
Cds2			Gfm2			MrpI40			Pyurf			Trnt1		
Chmp5			Gm17190			Mrpl45			Rab11fip5			U2af1		
Chpt1			Gng2			Mrpl46			Rab5b			Ube3a		
Ckap4			Gng5			Mrps18b			Rab8b			Ubqln2		
Clasp1			Gp1ba			Mrps2			Ranbp3			Uggt1		
Clptm1			Gp1bb			Mrps33			Rdh5			Vamp8		
Cnn3			Gp9			Mt1			Rpl28			Vma21		
Col1a2			Gypc			Mtco1			Rpl32			Vps26b		
Col6a5			H3f3c			Mtnd5			Rpl34			7mpste24		

Supplementary Figure 7. Expression of proteins most changed in left vs. right ventricle in either *Rbp1*^{-/-} **or WT mice.** Minimum 2-fold change of expression and FDR adjusted p-value <0.05 were criteria for inclusion.