SUPPLEMENTARY INFORMATION

Inter-individual variability and differential tissue abundance of mitochondrial amidoxime reducing component (mARC) in humans

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Table S1: Optimized MS/MS parameters used for the quantification of mARC enzymes. Light peptides obtained from digestion of recombinant mARC proteins were used as the calibrators and the corresponding heavy peptides containing terminal labeled [$^{13}C_6$ $^{15}N_2$]-lysine or $^{13}C_6$ $^{15}N_4$]-arginine residues served as the internal standards.

Protein	Peptide Sequence	Peptide label	Parent ion (m/z)	Product ion (m/z)	CE (eV)	CV (V)
mARC1	DLLPIK	light	406.2680 (+2)	583.4178 (+1)	20	35
		light	406.2680 (+2)	470.3337 (+1)	20	35
		light	406.2680 (+2)	357.2496 (+1)	20	25
		heavy	410.2751 (+2)	591.4320 (+1)	20	35
		heavy	410.2751 (+2)	478.3479 (+1)	20	35
		heavy	410.2751 (+2)	365.2638 (+1)	20	25
	VGDPVYLLGQ	light	530.7873 (+2)	430.2660 (+1)	20	35
		light	530.7873 (+2)	204.0979 (+1)	20	35
		heavy	532.2892 (+2)	433.2698 (+1)	20	35
		heavy	532.2892 (+2)	207.1016 (+1)	20	35
mARC2	LSPLFGIYYSVE	light	758.4083 (+2)	958.4880 (+1)	25	35
		light	758.4083 (+2)	788.3825 (+1)	25	35
		light	758.4083 (+2)	625.3192 (+1)	25	35
		heavy	762.4154 (+2)	966.5022 (+1)	25	35
		heavy	762.4154 (+2)	796.3967 (+1)	25	35
		heavy	762.4154 (+2)	633.3334 (+1)	25	35
	WFTNFL	light	478.2554 (+2)	769.4243 (+1)	25	35
		light	478.2554 (+2)	622.3559 (+1)	25	35
		heavy	482.2685 (+2)	777.4385 (+1)	25	35

		heavy	482.2685 (+2)	630.3701 (+1)	25	35

Table S2: Demographic information of samples used in mARC enzymes quantification		
Paired adult and pediatric liver and kidney samples		
Sample ID	**Age(yrs)**	**Sex**
1078	17.1	Female
5387	12.8	Male
1670	13.3	Male
612	17.0	Male
1409	18.1	Male
1429	18.7	Male
4548	20.2	Female
4790	203	Female
1475	20.3	Male
1712	20.4	Male
695	21.5	Male
5602	22.0	Female
777	22.9	Male
4917	22.5	Male
289	25.0	Male
1737	35.0	Female
1380	36.0	Female
Pediatric liver samples		
86	0.15	Male
195	0.34	Male
260	2.00	Male
271	0.05	Male
283	0.54	Male
322	1.00	Male
432	0.01	Male
435	0.75	Male
551	4.56	Male
569	0.36	Male
617	1.95	Female
620	14.00	Male
671	0.10	Male
677	1.97	Male
738	8.92	Female
759	0.10	Male
771	2.75	Male
774	0.75	Male
776	4.00	Female
780	0.00	Male

781	15.00	Female
811	16.00	Male
825	0.37	Male
845	0.03	Male
872	2.00	Male
1053	5.00	Male
1055	0.26	Male
1144	12.64	Female
1157	0.05	Female
1261	13.86	Male
1281	8.17	Male
1296	13.86	Male
1325	0.50	Female
1443	0.91	Female
1482	0.67	Female
1547	0.71	Male
1904	0.27	Male
1908	13.99	Male
4787	12.87	Male
4925	13.16	Male
5077	16.71	Female
8703	10.00	Female
8804	14.00	Male
8910	14.00	Male
8912	12.00	Female
8917	6.00	Female
8920	11.00	Male
8926	0.73	Female
9005	17.00	Male
9006	10.00	Male
9011	3.20	Female
9013	11.00	Male
9022	5.00	Unknown
9023	2.58	Female
9028	8.00	Female
9101	2.00	Male
9105	17.00	Male
9127	15.00	Male
9507	14.00	Male
9608	4.00	Male
9611	9.00	Male
9612	3.00	Male
70622	4.00	Female

70650	1.90	Female
70684	1.50	Female
70685	10.00	Male
70690	13.00	Female
70701	3.00	Male
70706	11.00	Female
70851	1.00	Female
71017	1.00	Male
71032	15.00	Male
71047	17.00	Male
71065	18.00	Male
71077	1.00	Male
71414	8.00	Male
71187	4.00	Male
71188	1.90	Male
71512	4.00	Male
71616	9.00	Female
70919	3.00	Female

Pooled S9 fractions			
Organ	Total number of samples	Age (year)	Sex
Liver	10	15-64	Male (6), Female (4)
Intestine	15	33-67	Male (10), Female (5)
Kidney	12	47-76	Male (8), Female (4)
Lung	11	2-66	Male (8), Female (3)
Heart	17	65-78	Male (10), Female (7)
In vitro models			
CHIM	8	32-60	Male
	7	16-54	Female
Hepatocytes	10	24-58	Male

Table S3: Chromatographic conditions for the separation of mARC1 and mARC2 surrogate peptides			
Trap column: Symmetry C18 column (100Å, 1.7 µm, 150µm * 50 mm			
iKey BEH C18 column (130Å, 5 µm, 300µm * 50 mm)			
Injection volume: 1 µL			
LC gradient program			
Time (min)	Flow Rate (µL)	A (Water with 0.1% formic acid, %)	B (Acetonitrile with 0.1% formic acid, %)
0	3	97	3
4	3	97	3
8	3	87	13
18	3	70	30
20.5	3	65	35
21.1	3	40	60
23.1	3	20	80
23.2	3	97	3
27	3	97	3

Table S4: Analytical validation parameters of µLC-MS/MS method used for quantitative analysis of mARC enzymes.

Protein	Peptide	LLOQ (fmol on column)	ULOQ (fmol on column)	Linearity (R^2)	Intraday precision (%CV for QC)	Interday precision (%CV for QC)
mARC1	DLLLPIK	14.2	934223	0.99	20.03	18.05
mARC2	LSPLFGIYYSVEK	2.8	44862	0.99	10.29	16.97

LLOQ: lower limit of quantification; **ULOQ**: upper limit of quantification; **CV**: coefficient of variance; **QC**: quality control. The calibration curve range of mARC1 (84-675 fmol on-column) and mARC2 (115-925 fmol on-column) was linear and within LLOQ and ULOQ limit.

Table S5: Protein recovery (mg of membrane or S9 protein per gram of tissue) in different organ and scaled abundance of mARC1 and mARC2 (pmol/gm tissue).

		Liver	Intestine	Kidney	Heart	Lung
MS9PPGT (mg S9/gram tissue)		101.05	38.60	59.40	26.50	156.59
Abundance (pmol/gm tissue)	mARC1	3900.53	255.42	296.45	BLQ	465.07
	mARC2	3793.31	753.57	3544.38	BLQ	500
TM-PPGT (mg total membrane/gram tissue)		49	-	38	-	-
Abundance (pmol/gm tissue)	mARC1	3675		494		
	mARC2	3283		4560		

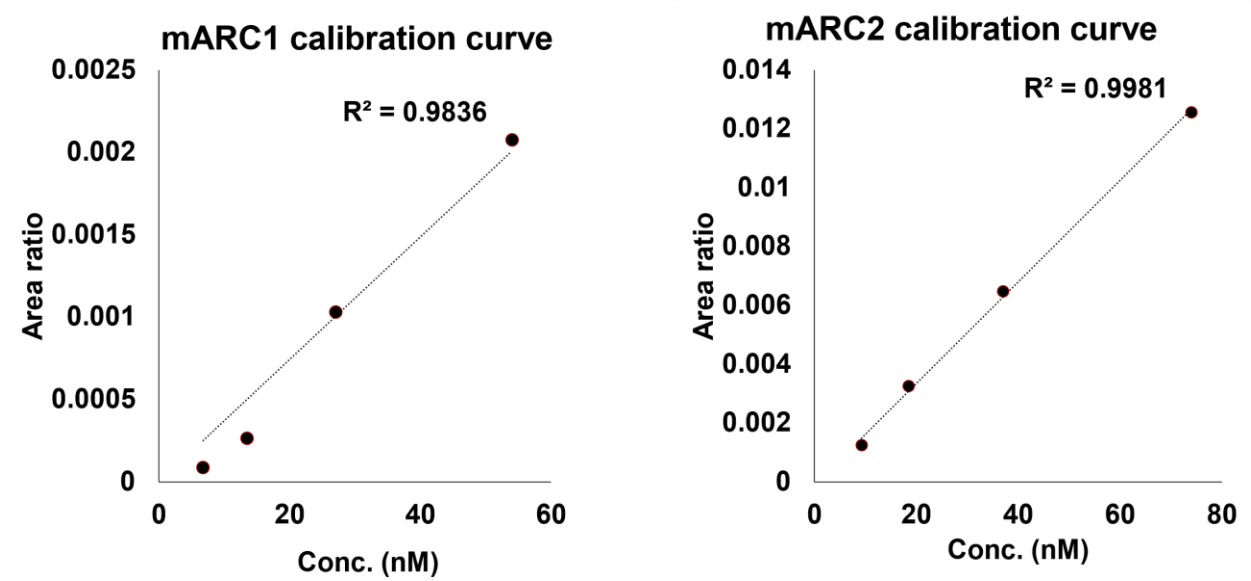

Figure S1: mARC1 and mARC2 calibration curve in which digests of recombinant protein served as calibrators. The dynamic range of the method was linear between 6.74 to 54 nM for mARC1 (R^2=0.98) and 9.25 to 74 nM for mARC2 (R^2=0.99), respectively.

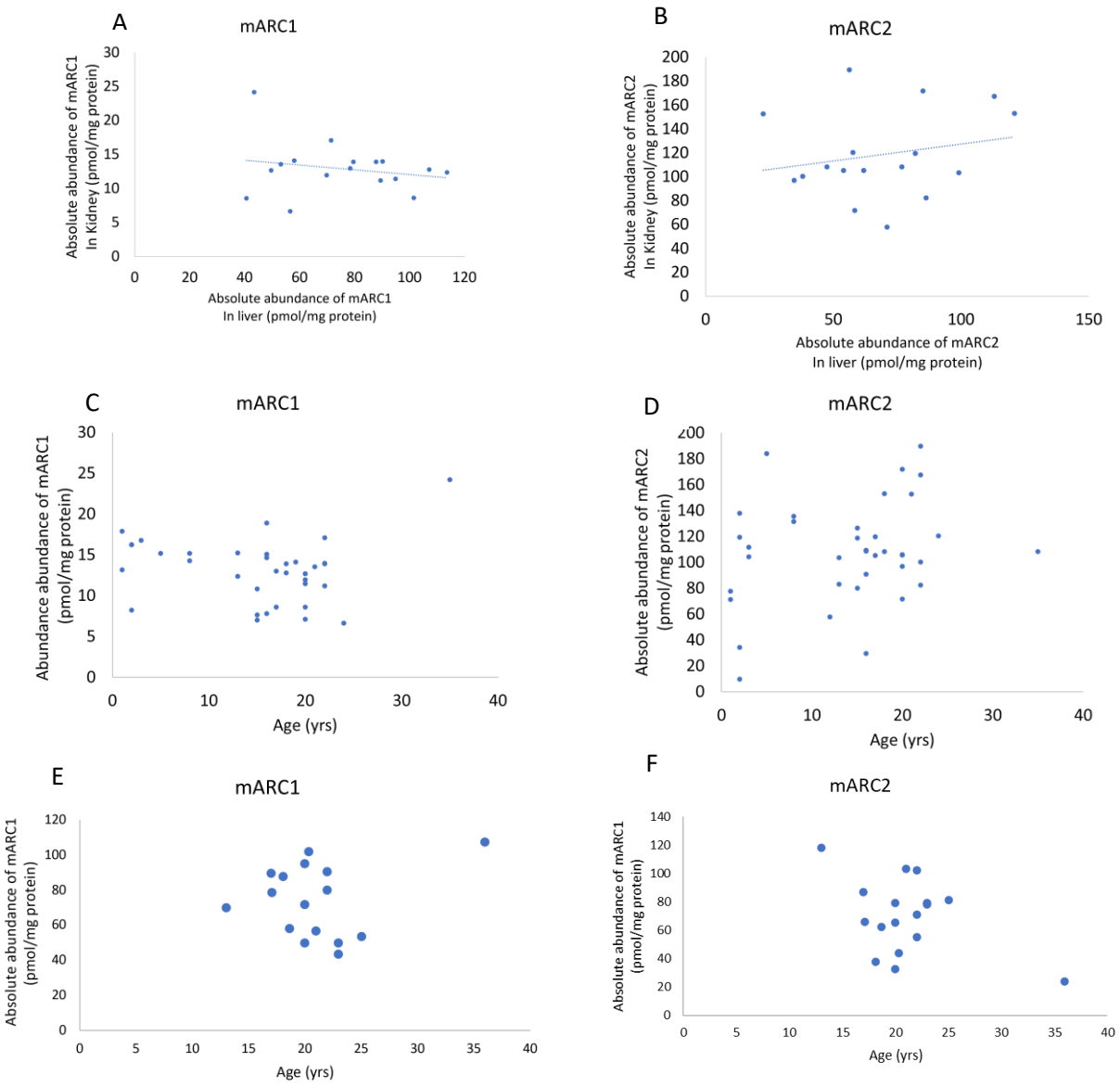

Figure S2: Correlation of mARC1 and mARC2 abundance in paired liver and kidney samples (A and B) and age vs. mARC1 and mARC2 abundance in kidney samples (C and D) and liver samples (E and F).

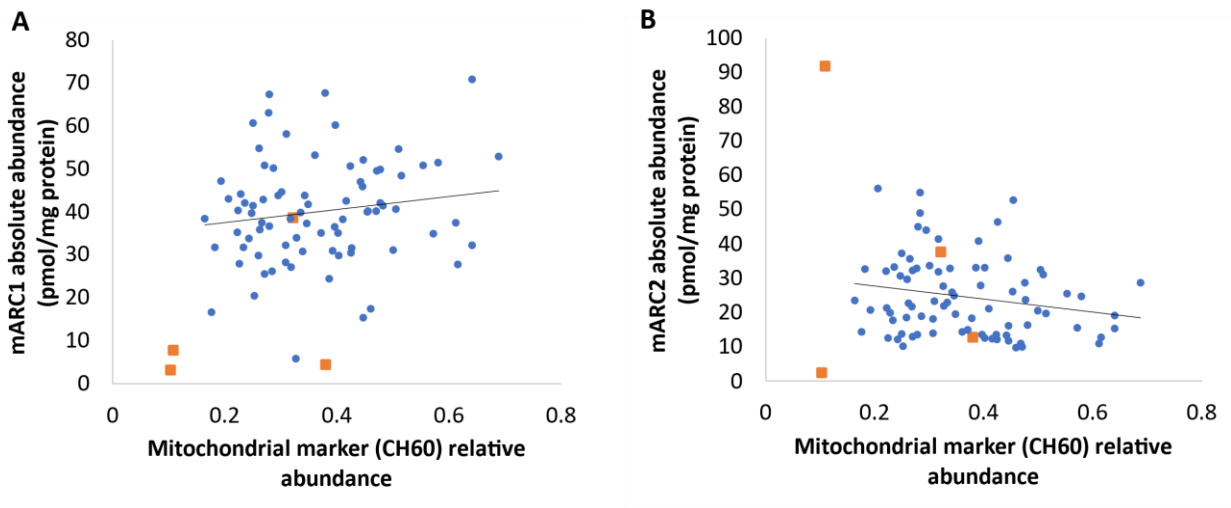

Figure S3: Correlation of mitochondrial marker (CH60) and mARC1 (A) and mARC2 (B) abundance in differential tissue (liver, kidney, intestine, and lungs) (orangesquares) and paired liver and kidney samples (blue dots). These data suggest that technical variability in the sample preparation is not a confounder in mARC quantification.

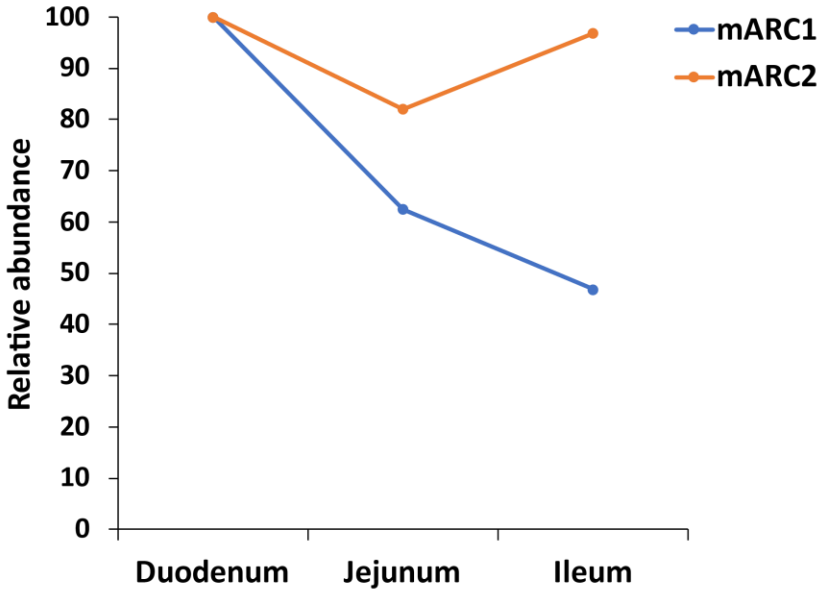

Figure S4: Relative abundance of mARC enzymes in CHIM samples isolated from duodenum, jejunum, and ileum sections of human intestine. The relative abundance data were first normalized by the marker levels (villin and FABP2). These marker-normalized data were then expressed as % relative to the duodenum levels.