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Protein-Protein Interactions of Human Mitochondrial Amidoxime-Reducing Component in Mammalian Cells

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PROTEIN-PROTEIN INTERACTIONS OF HUMAN MITOCHONDRIAL AMIDOXIME-REDUCING COMPONENT IN MAMMALIAN CELLS

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By

John A. Thomas

December 2016

PROTEIN-PROTEIN INTERACTIONS OF HUMAN MITOCHONDRIAL

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Approved October 24, 2016

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ABSTRACT

PROTEIN-PROTEIN INTERACTIONS OF HUMAN MITOCHONDRIAL AMIDOXIME-REDUCING COMPONENT IN MAMMALIAN CELLS

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December 2016

Dissertation supervised by Partha Basu

Newly discovered pterin-molybdoenzymes mammalian proteins, mARC1, and mARC2, are though to activate pro-drug, metabolize mutated base pairs, and producing nitric oxide from nitrite. However, the physiological function of the mARC proteins remain unclear. It is hypothesized that identifying partner proteins through protein-protein interactions can provide evidence to their physiological function. This study aimed to further investigate the potential function of mARC proteins through the identification of protein-protein interaction in human cell lysate, utilizing co-immunoprecipitation, crosslinking reagent, and pull-down assays. Several proteins (e.g. mitogen-activated protein kinase 5, phosphatidylinositol 4-phospahte 3-kinase C2 domain-containing subunit beta, ADP/ATP translocase 1, and myotubularin) were identified as interacting partners of mARC1, and mARC2. These interacting proteins are affiliated with various KEGG signaling pathways. From the interacting proteins we infer, mARC1 and mARC2 may be involved in the regulation of nitric oxide signaling under hypoxic. Reverse co-immunoprecipitation experiments using select proteins from the pathways detected mARC proteins. The investigation was then expanded to include pig liver mitochondrial fraction and rat liver cell lysate, in which all experimental conditions identified mARC proteins using reverse co-immunoprecipitation.

DEDICATION

This dissertation is dedicated to:

- My mother, Suzanne Baker, father, John Thomas, step-mother, Susie Thomas, and step-father, David Baker who have always been there to support and encouraged me through this journey.
- My sisters, Marlena and Angela Thomas, and my step-sisters, Ashley and Mackenzie Messer who have kept me motivated throughout the years.
- My Grandfather, Frank Apicella, who kept me focused and motivated throughout the years.
- My family and friends for their unwavering support over the years.

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system, and experimental techniques were critical in this project. I cannot thank you all enough for all the help you have offered me over the year, and I truly am grateful.

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LIST OF ABBREVIATIONS

ADP: adenosine diphosphate

Akt: protein kinase B

ATP: adenosine triphosphate

cAMP: cyclic adenosine monophosphate

CBP: calmodulin binding peptide tag

cGMP: cyclic guanosine monophosphate

CID: collision induced dissociation

CRAPome: Contaminant Repository for Affinity Purification

DAVID: Database for Annotation, Visualization, and Integrated Discovery

DMSOR: dimethyl sulfoxide reductase

DTT: dithiothreitol

EC: enzyme commission number

eNOS: endothelial NOS

ERK: extracellular signal-regulated kinase

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

GFP: green fluorescent protein

GPHN: gephyrin

GOTerm: gene ornithology term

GST: glutathione S-transferase tag

HEK-293: human embryonic kidney cell line 293

FeMoco: iron-molybdenum cofactor

iNOS: inducible NOS

K-BLoC: site specific mARC compound with lysine sidechain

KEGG: Kyoto Encyclopedia of Genes and Genomes

LC-MS/MS: liquid chromatography-mass spectrometry

LTR-K-BLoC: mARC specific crosslinking complex

MAPK: mitogen-activated protein kinase

mARC: mitochondrial amidoxime-reducing component

MBP: maltose-binding protein tag

Moco: pterin-based molybdenum cofactor

MOCS1: molybdenum cofactor biosynthesis protein 1

MOCS2: molybdenum cofactor biosynthesis protein 2

MOSC: moco sulfurase C-terminal

MPT: molybdopterin

mRNA: messenger RNA

nNOS: neuronal NOS

NOS: nitric oxide synthase

PDC: pyruvate dehydrogenase complex

PKG: protein kinase G

Q-TOF: quadrupole-time-of-flight

sGC: soluble guanylate cyclase

SO: sulfite oxidase

TBS: tris-buffered saline

XO: xanthine oxidase

Chapter 1: Introduction

1.1 Molybdenum-containing Enzymes and their Classes

Molybdenum is an essential trace element in all forms of life.[1] Molybdenum-containing enzymes play important roles in a vast array of metabolic processes in all forms of life. Enzymes requiring molybdenum cofactor for full functionality are capable of redox reactions involving carbon, sulfur, and nitrogen metabolism.[2-4] These enzymes require molybdenum to be ligated by a cofactor, resulting in two general categories for molybdenum cofactors, iron-molybdenum cofactor (FeMoco) and pterin-based molybdenum cofactor (Moco).[5-9] Reactions involving Moco-containing enzymes can be characterized into either the transformation of species utilizing hydroxylation or oxygen atom transfer to/from a substrate involving two electrons.[2, 5, 10-12] Moco is commonly comprised of a molybdenum atom directly coordinated by a dithiolene ligand which is appended to a pyranopterin (**Figure 1.1**) or metal binding pterin.[13]

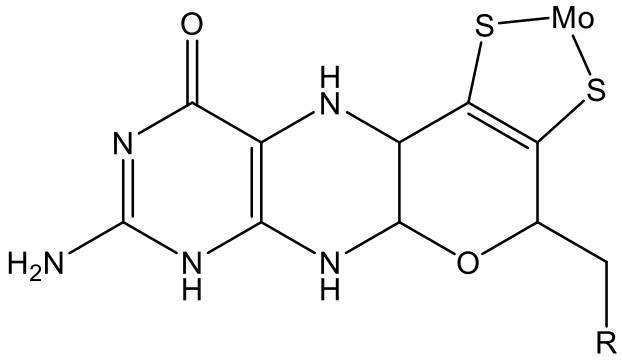

Figure 1.1: The general structure of pyranopterin cofactor for molybdenum containing enzymes. Molybdenum atom is covalently bound to the ene-dithiolate. R = H, monophosphate, guanidine dinucleotide, or cytosine dinucleotide.

The two molybdenum-containing enzyme categories, FeMoco, and Moco can be further divided into different families based on active site structure. An example of a FeMoco containing enzyme is nitrogenase, the enzyme is involved in dinitrogen fixation.[14-17] Moco-containing enzymes, can further be divided into three families; sulfite oxidases (SO), xanthine oxidases (XO), and dimethyl sulfoxide reductases (DMSOR).[18] SO and XO family proteins contain a simple molybdopterin (MPT)-type cofactor.[5] They differ in the third Mo-S ligand in which SO contains an enzyme-derived cysteine and XO contains a sulphido ligand.[5] In DMSOR family of enzymes, two pyranopterin cofactors coordinate the molybdenum center, each having an additional guanosine monophosphate.[5] It has been proposed that there may be a fourth family in this category, mitochondrial amidoxime reducing-component (mARC), which may contain a structurally unique active site when compared to the other three families.[19] However, it has been recently accepted that mARC is indeed a part of the sulfite oxidase family.[8] Pterin-based molybdenum enzymes are generally mononuclear, where the molybdenum atom is coordinated by the pterin. In addition, the molybdenum is also coordinated by a sulfur, oxygen or selenium donor. The pterin-based molybdenum cofactor families typically function via oxygen atom transfer reactions or hydroxylation, and during catalysis the molybdenum center shifts between a fully oxidized and a reduced state. **Figure 1.2** shows a comprehensive tree of the categories and families of molybdenum containing enzymes as well as the corresponding enzyme commission (EC)

number and the Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways involved.

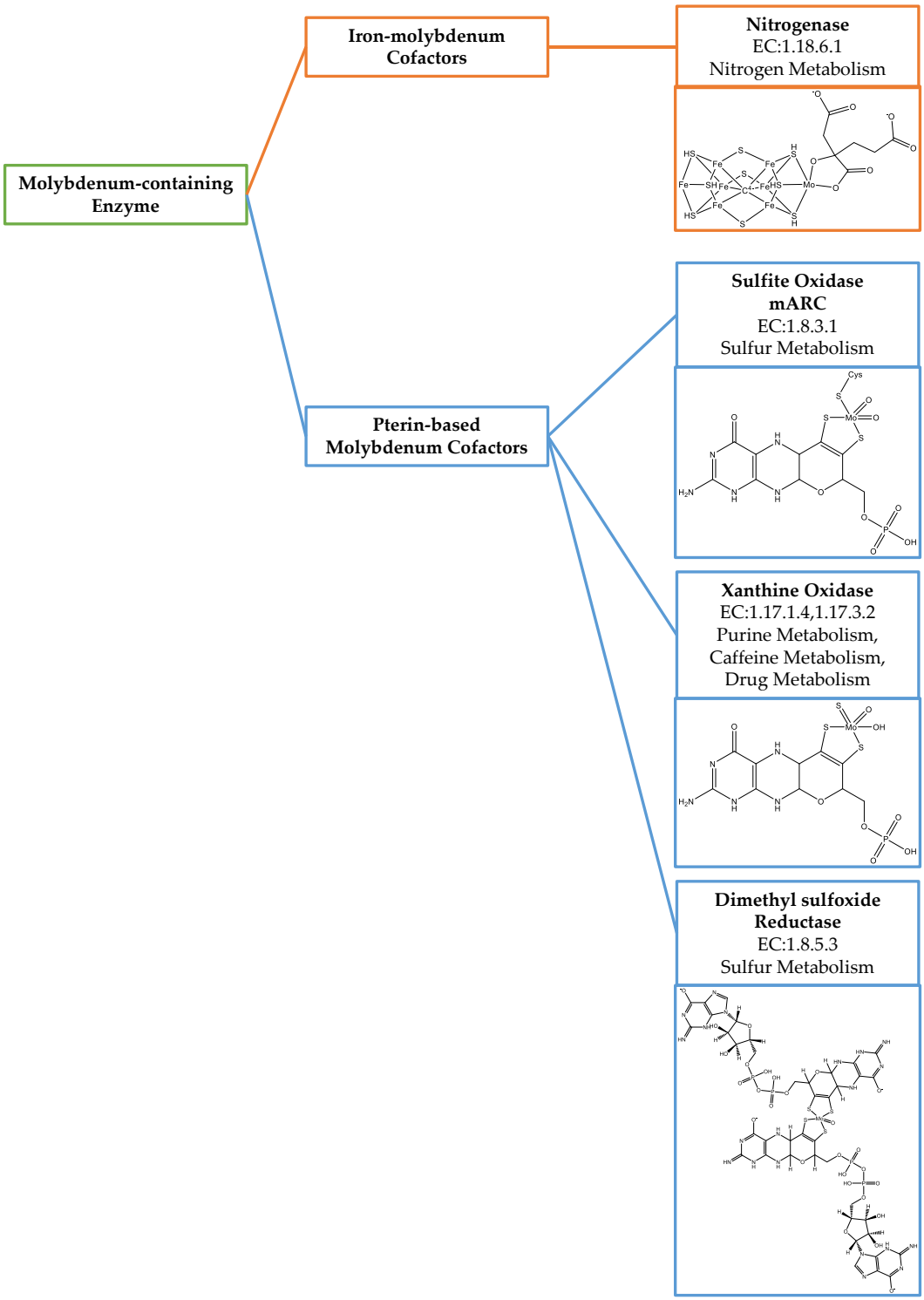

Figure 1.2: Categories of molybdenum containing enzymes. The families, enzyme function and corresponding metabolisms are listed.

1.2 Mammalian Molybdenum-containing Enzymes

There are four mammalian molybdenum containing enzymes known. They include SO, XO, aldehyde oxidase (AO), and mARC.[11, 18, 20] The structural differences of the cofactor in these enzymes can be seen in **Figure 1.3**. The four proteins can be categorized into two or potentially three families. SO belongs to the SO family which contains a conserved cysteine ligated to the molybdenum center.[18] Xanthine oxidoreductase and AO belong to the xanthine oxidase family which contains a sulfido group bound the to the molybdenum center.[18] The active site in AO and XO can be superimposed, demonstrating a high degree of similarity.[21] The Moco-containing

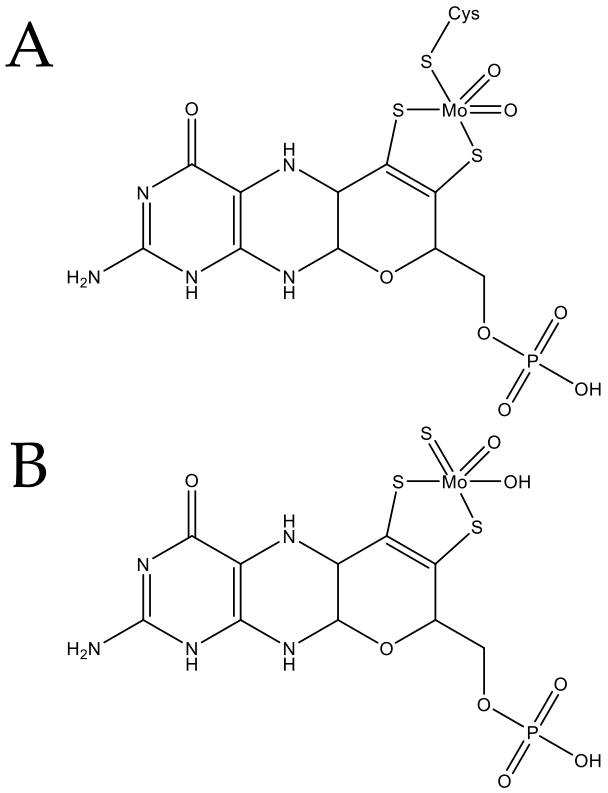

Figure 1.3: The structural differences in the mammalian molybdenum enzymes cofactors. A. Moco for sulfite oxidase family enzymes, B. Moco for xanthine oxidase family enzymes.

enzyme, mARC, is suggested to belong to the SO family based on the conserved cysteine residue or may potentially represent a new type of molybdenum-containing enzyme.[22] SO and XO are among the more well-known mammalian molybdenum containing enzymes with defined physiological roles in cysteine and purine metabolism.[2] The physiological roles of AO and mARC enzymes remain unclear; both proteins have been proposed to function in pro-drug activation.[23, 24] Aldehyde oxidases have a broad substrate specificity corresponding to compounds containing carboxylic acids and heterocycles, transforming substrates into hydroxylated derivatives.[23] The mARC enzymes have been shown to reduce substrates containing amidoxime groups into their active drug forms; these include antiprotozoal, anti-inflammatories, anti-cancer, and antidepressants.[24]

The four mammalian molybdenum containing enzymes all have markedly different catabolic properties. SO catalyzes the oxidation of sulfite to sulfate. XO catalyzes the formation of hypoxanthine to xanthine and xanthine to uric acid.[18] AO catalyzes the oxidation of aldehydes to carboxylic acids.[2] Recently, mARC proteins have been found to metabolize compounds with N-hydroxylated side groups.[24, 25] These types of compounds are regularly used as pro-drugs (**Figure 1.4** shows three pro-drugs. A more complete set of examples can be found in **Figure 1.8**). Interestingly, mARC proteins can

reduce nitrite to nitric oxide, which is a potent signaling molecule in mammalian systems.[26]

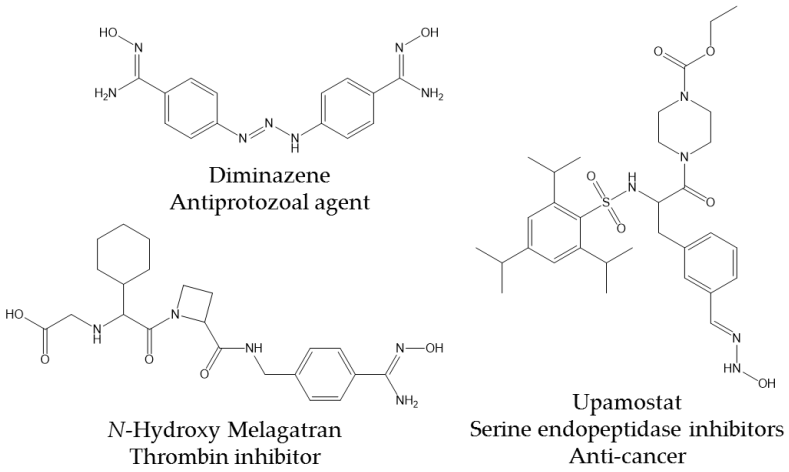

Figure 1.4: Three example pro-drugs that mARC has been shown to be able to catalyze the activation by reducing the amidoxime functional groups.

1.3 Molybdenum-containing Enzymes and their Involvement in Mammalian Nitric Oxide

There are two distinct nitric oxide synthesis pathways in mammalian systems. The first pathway is the nitric oxide synthase (NOS)-dependent pathway in which arginine is metabolized to form nitric oxide. The NOS pathway requires oxygen to catalyze the reaction. Under oxygen-limiting conditions, however, the body can produce nitric oxide using a second pathway involving the conversion of nitrite to nitric oxide. Nitrate is more available than nitrite in mammalian systems and requires the aid of oral commensal bacteria to generate nitrite from nitrate.[27] The subsequent reduction of nitrite to nitric oxide occurs in the mitochondria of cells. Nitric oxide is an important signaling molecule in multiple pathways. **Figure 1.5** demonstrates two different pathways for nitric oxide

production. The first is the normal oxygen conditions under which NOS can catalyze the formation of nitric oxide from arginine. The second pathway is under the hypoxic conditions when mARC proteins can be used to reduce nitrite into nitric oxide and the subsequent pathways associated with nitric oxide. Investigating the mammalian nitrite reductase pathways is of great interest for human health and disease, as the effects of nitrite are largely mediated by its reduction to nitric oxide.

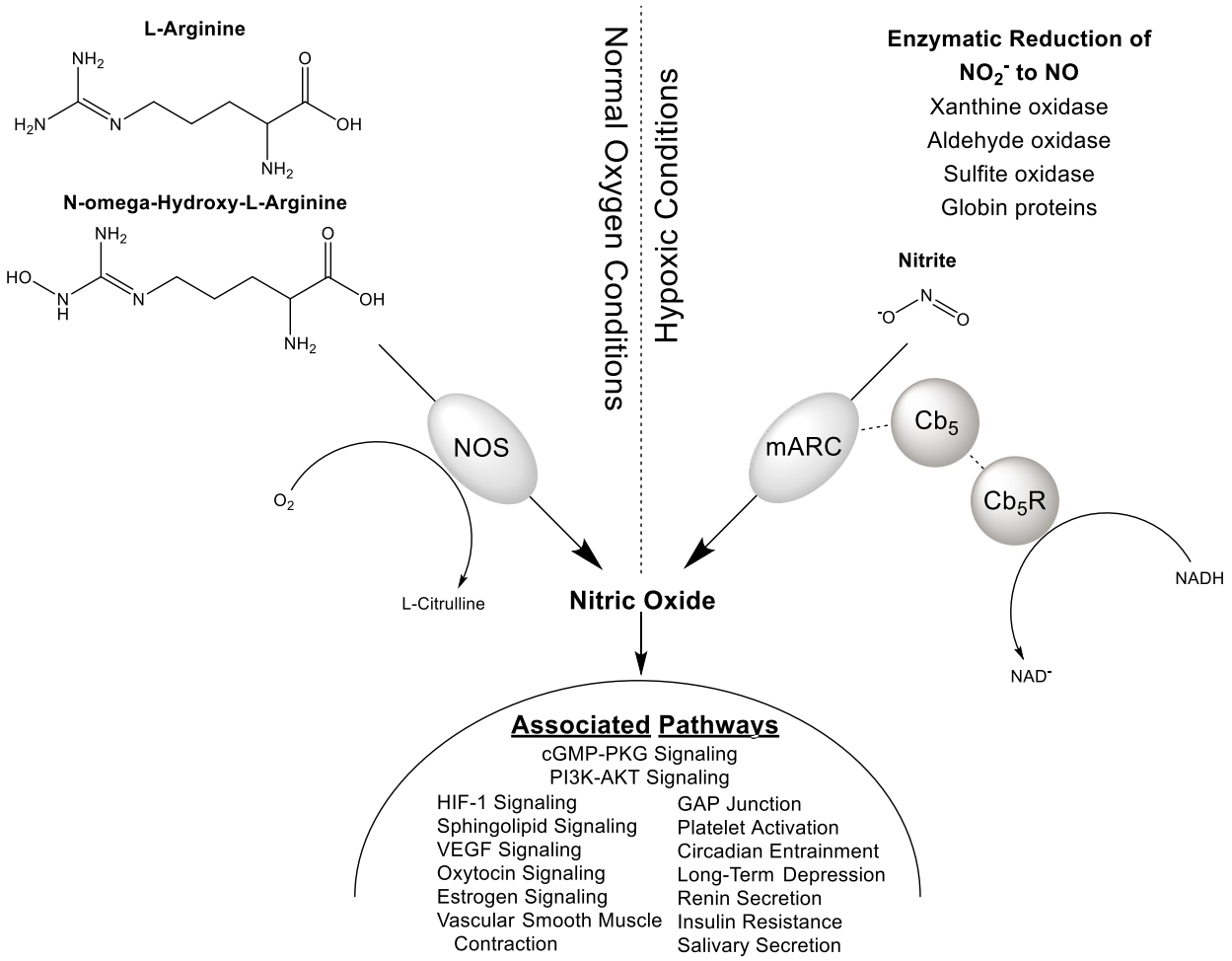

Figure 1.5: (Left) NOS pathway for nitric oxide production in which L-arginine or N-omega-hydroxy-L-arginine can be oxidized by nNOS, iNOS, or eNOS to form L-citrulline and nitric oxide under normal oxygen conditions. (Right) Formation of nitric oxide by mARC proteins; nitrite is reduced by mARC proteins but requires cytochrome b_5 (Cb_5) and cytochrome b_5 reductase (Cb_5R) as well as NADH. Nitric oxide has been found to be associated with different pathways in mammalian systems.

In mammals there are a several sources of nitric oxide namely, L-arginine, S-nitrosothiols, nitrate and nitrite. The oxidation of L-arginine catalyzed by NOS is a well-characterized pathways for nitric oxide production.[28-31] There are three distinct isozymes that have been identified in mammalian systems, neuronal NOS (nNOS or NOS-1), inducible NOS (iNOS or NOS-2), and endothelial NOS (eNOS or NOS-3).[32-34] It was found that if all three proteins were knocked out in mice, they displayed a shorter life expectancy, with increased risk of cardiovascular disease including hypertension, cardiac hypertrophy, diastolic heart failure, arteriosclerosis, and myocardial infarction; demonstrating the importance of nitric oxide production in mammalian systems.[35] The full functioning NOS proteins require five cofactors to effectively catalyze the formation of nitric oxide from L-arginine; tetrahydrobiopterin, flavin adenine dinucleotide, flavin mononucleotide, calmodulin, and iron protoporphyrin IX.[32, 36] The monomeric NOS isozyme is incapable of producing nitric oxide, producing instead oxygen.[36] NOS enzymes require substrates NADPH, L-arginine, and oxygen in order to produce $NADP^+$, L-citrulline, and nitric oxide.[32] It is imperative that the NOS proteins form dimers in order to bind the tetrahydrobiopterin cofactor and the substrate L-arginine subsequently forming nitric oxide.[37]

In addition to the oxidation of L-arginine to form nitric oxide, S-nitrosothiols are utilized to regulate protein expression for nitric oxide production and can also provide a source of nitric oxide.[38] S-nitrosothiols can be formed two ways; exogenously by a S-

nitrosothiol donor (i.e., S-nitrosoglutathione) or endogenously by NOS proteins.[39] Furthermore, donor S-nitrosylated proteins can transfer the nitric oxide group to acceptor S-nitrosylation protein complexes.[40] While nitric oxide has a very short half-life (2 ms) in plasma, S-nitrosoproteins have half-lives of approximately one hour.[39] The release of nitric oxide from S-nitrosothiols have been shown to be achieved several ways *in vitro*; trace amounts of transition metal ions (Cu^{2+} or Fe^{2+}), flash photolysis, as well as the addition of dithiothreitol, or vitamin C.[41-43]

Nitrate can undergo a reductive process in which nitrite is formed by microbial nitrate reductase, and subsequently nitrite can be reduced to nitric oxide by different mammalian enzymes. Although many proteins and catalysts are known, this latter pathway is not well understood in mammalian cells at a molecular level. Nitrate and nitrite are now considered to be biological reservoir of nitric oxide, however, all the key enzymes influencing the process have yet to be understood.[38, 44] There is no known mammalian nitrate reductase, although several mammalian enzymes can reduce nitrate to nitrite with millimolar (mM) binding, which is orders of magnitude lower than the micromolar (μM) K_m determined for bacterial and plant enzymes.[45] Thus, mammalian nitrate reduction is catalytically less efficient. Our assertion is that the nitrate to nitrite transformation is likely to be achieved by commensal bacterial nitrate reductase, a process we have been investigating for years.[46, 47] Several *in vitro* studies have indicated that nitrite to nitric oxide reduction is achieved by essentially three different

types of biomolecules: members of the globin family (e.g., hemoglobin, myoglobin and neuroglobin), pterin-containing molybdenum enzymes (e.g., XO; AO; SO) and nitrite anhydrase enzymes (e.g., carbonic reductase). Under specific conditions, these pterin-containing enzymes have been reported to be able to catalyze the reduction of nitrate and nitrite to nitric oxide (**Table 1.1**).[26, 48-50] Hemoglobin and myoglobin are iron and

Table 1.1: The *in vitro e*nzymatic properties of some known mammalian proteins capable of reducing nitrite to nitric oxide.

Protein Name	Reducing Substrate, pH	$k_{cat}^{NO_2^-}$ (s^{-1})	$k_m^{NO_2^-}$ (mM)	$k^{NO_2^-}$ ($M^{-1}s^{-1}$)
Aldehyde oxidase [48]	Aldehyde, pH 7.4	0.47	4.1	115
Mitochondrial amidoxime reducing-component [26]	NADH, pH 7.4	0.1	9.5	11
Sulfite oxidase [50]	Sulfite, pH 7.4 Phenosafranine, pH 7.4	0.002 1.9	1.6 80	1.3 26
Xanthine oxidase [48,49]	Aldehyde, pH 7.4	0.41	2.2	186

oxygen-binding proteins are capable of catalyzing the formation of produce nitric oxide. Hemoglobin, which is used in oxygen transport, has demonstrated potential to produce nitric oxide from nitrite at a max rate under the conditions of 40-60% saturation at a pH of 6.4.[51-53] The second protein, myoglobin, is capable of producing nitric oxide from nitrite; deoxymyoglobin has been shown to reduce nitrite at a rate of 36 times faster than deoxyhemoglobin.[54] Hemoglobin and myoglobin are oxygen-sensing proteins that aid in the scavenging of nitric oxide, but under conditions of hypoxia the roles change to nitric oxide producers; inducing vasodilation and increasing blood flow to tissue.[55-57] Xanthine oxidoreductase is capable of producing nitric oxide from both nitrate and nitrite in anoxic tissue.[58, 59] Under hypoxic conditions mitochondrial cytochrome *c* oxidase

has been shown to produces nitric oxide depending on the pH of the system.[60] Xanthine oxidoreductase and mitochondrial cytochrome *c* oxidase potentially demonstrate a role in increasing blood flow to ischemic tissue.[60-62]

Organic nitro compounds have been shown to be reduced to nitric oxide by aldehyde dehydrogenase 2 under normoxic conditions.[62, 63] Although aldehyde dehydrogenase 2 has been shown to reduce nitroglycerin, the proteins physiological function have eluded researchers.[62, 63] Interestingly, the activity and expression of aldehyde dehydrogenase 2 in the vascular system when there is a tolerance to organic nitrates.[64, 65] The final proteins that have demonstrated their capabilities to reduce nitrate and nitrite to nitric oxide are cytochrome P450 reductase and cytochrome P450.[66]

Nitric oxide is an important signaling molecule in the vascular endothelium, nervous and immune systems, aiding in the regulation of behavior, gastrointestinal motility, and defense mechanisms against infectious disease and tumors.[67-73] The signaling molecule stimulates soluble guanylyl cyclase (sGC) and promotes the formation of cyclic guanosine monophosphate (cGMP) through a signaling cascade referred to as cGMP-PKG signaling pathway.[74-78] The signaling cascade is used in smooth muscle cell relaxation by activating protein kinase G (PKG) and increases the reuptake of calcium into the sarcoplasmic reticulum, reducing cytosolic calcium concentrations, resulting in the activation of potassium channels.[79-83] Myosin light

chain kinase is a calcium/calmodulin-dependent enzyme, the decrease concentration of cytosolic calcium prevents the phosphorylation of myosin and subsequently causes the relaxation of smooth muscle.[84-88] In addition to being a key signaling molecule in muscle relaxation, in the presence of electron acceptors, nitric oxide can form S-nitrosylated proteins by reacting with deprotonated cysteines.[89] S-nitrosylations aids in the regulation and function of G protein-coupled receptors, as well as help to modulate pathways within vascular cells.[40, 90] The activity of G protein-coupled receptors kinase 2 regulates the phosphorylation of β-adrenoceptors, this system can be regulated by nitric oxide and S-nitrosylation. In some cases S-nitrosylation helps prevent the reduction of β-adrenergic signaling *in vivo* by mediating the phosphorylation of β-adrenoceptors and avoiding the receptors desensitization.[90, 91] Activation of β_1 and β_2-adrenoceptors in heart tissue increases muscular contraction; while the opposite occurs in blood vessels, activation of β-adrenoceptors results in vasodilation.[92-98] Thus, NO-cGMP signaling cascade is therapeutically relevant for treating cardiovascular disorders with therapies targeting sGC.[99]

1.4 Novel Mammalian Enzyme: mARC

The newly discovered pterin molybdenum-containing enzyme, mitochondrial amidoxime-reducing component (mARC) has been shown *in vitro* to reduce nitrite to nitric oxide more efficiently than any other human molybdenum containing enzymes.[26] As previously stated, mARC proteins are proposed to belong to the SO family of

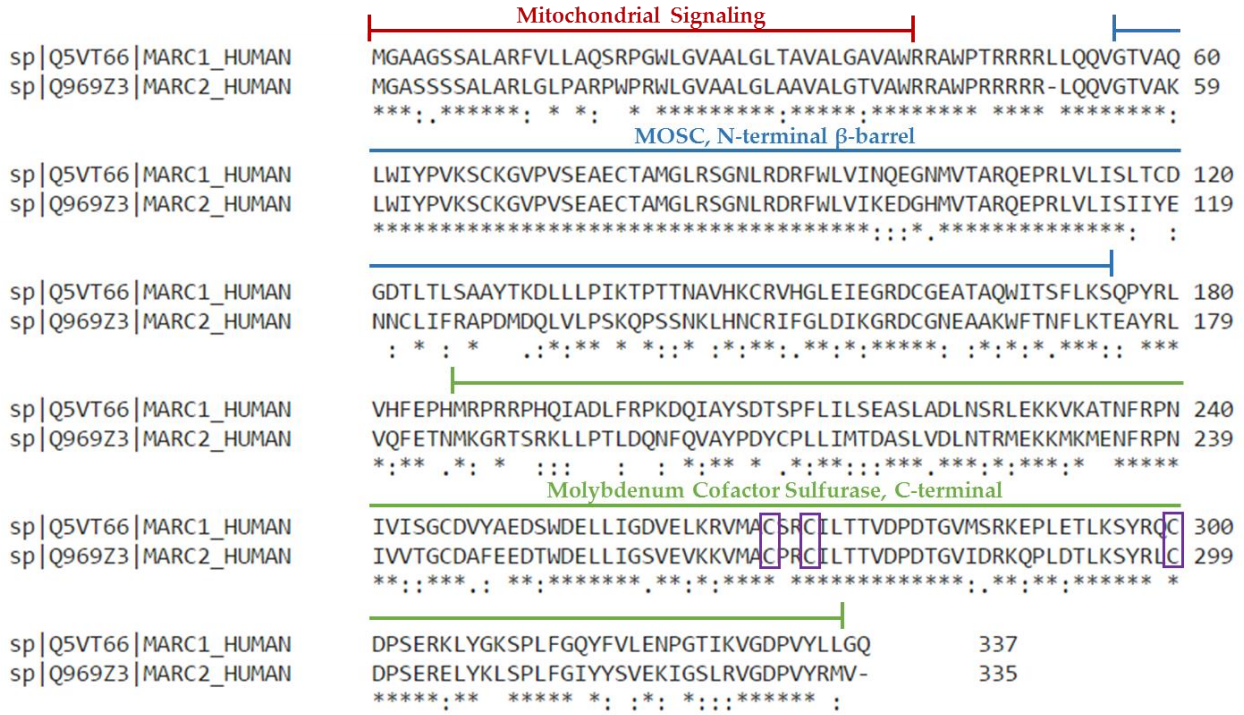

Figure 1.6: Sequence alignment of human mARC1 and mARC2 proteins. Similar domains in mARC proteins are highlighted; mitochondrial signaling sequence (red), MOSC, N-terminal β-barrel sequence (blue), molybdenum cofactor sulfurase, C-terminal (green), and conserved cysteines (purple) which may coordinate to Moco.

molybdenum containing enzymes.[2, 100] There are two isoforms of mARC proteins that have been annotated in humans with an overall sequence identity/similarity of 66/80%, mARC1 and mARC2 (UniProt ID: Q5VT66, Q969Z3) (**Figure 1.6**).[19] The number of amino acids in both mARC proteins is very similar (mARC1: 337 amino acids, and mARC2: 335 amino acids).[22] The proteins contain similar domains; amino-terminal mitochondrial signal sequence, a predicted β-barrell domain, and a MOSC domain near the carboxyl terminus.[101] Even though mARC is a mitochondrial protein it has been found through cellular staining to be also located outside the mitochondria, as it is anchored to the outer mitochondrial membrane and may detach.[102, 103] Both mARC

proteins are capable of reducing *N*-oxygenated species, although the rate of the reaction are not high. These proteins require cytochrome b_5, and cytochrome b_5 reductase ancillary proteins to perform reduction.[24, 25, 104, 105] The physiological function of mARC enzymes is uncertain at a cellular level, but an *in vitro* study demonstrated that they may be utilized in the pro-drug metabolism by catalyzing the reduction of amidoxime functional groups to amidines.[46, 106-109] There is also evidence that mARC proteins can function in lipid synthesis, nitrite reduction to for nitric oxide and the detoxification of *N*-hydroxylated nucleotides.[19, 26, 105, 110] The expression of mARC1 and mARC2 is found throughout the body with varying degrees of expression of each protein in selected tissue (**Figure 1.7**). Using a method referred to as intensity based absolute quantitation (iBAQ) in which the sum of all peptide peak intensities are divided by the number of theoretically observed tryptic peptides the relative abundances of mARC1 and mARC2 proteins were quantitated in different tissues.[111] **Figure 1.7** shows that mARC2 is expressed to a higher degree in similar tissues as mARC1. This may indicate that the physiological roles of mARC1 and mARC2 may be different. In the kidneys and liver, both involved in detoxification of harmful metabolites, mARC2 showed a higher expression than mARC1; indicating that one potential role of these proteins is the catabolism of harmful substrates. In addition, mARC2 was found to be expressed in the heart while mARC1 was not. To this end, the physiological function of mARC proteins

in humans is unknown and this investigation seeks to provide clarity in the physiological role of mARC.

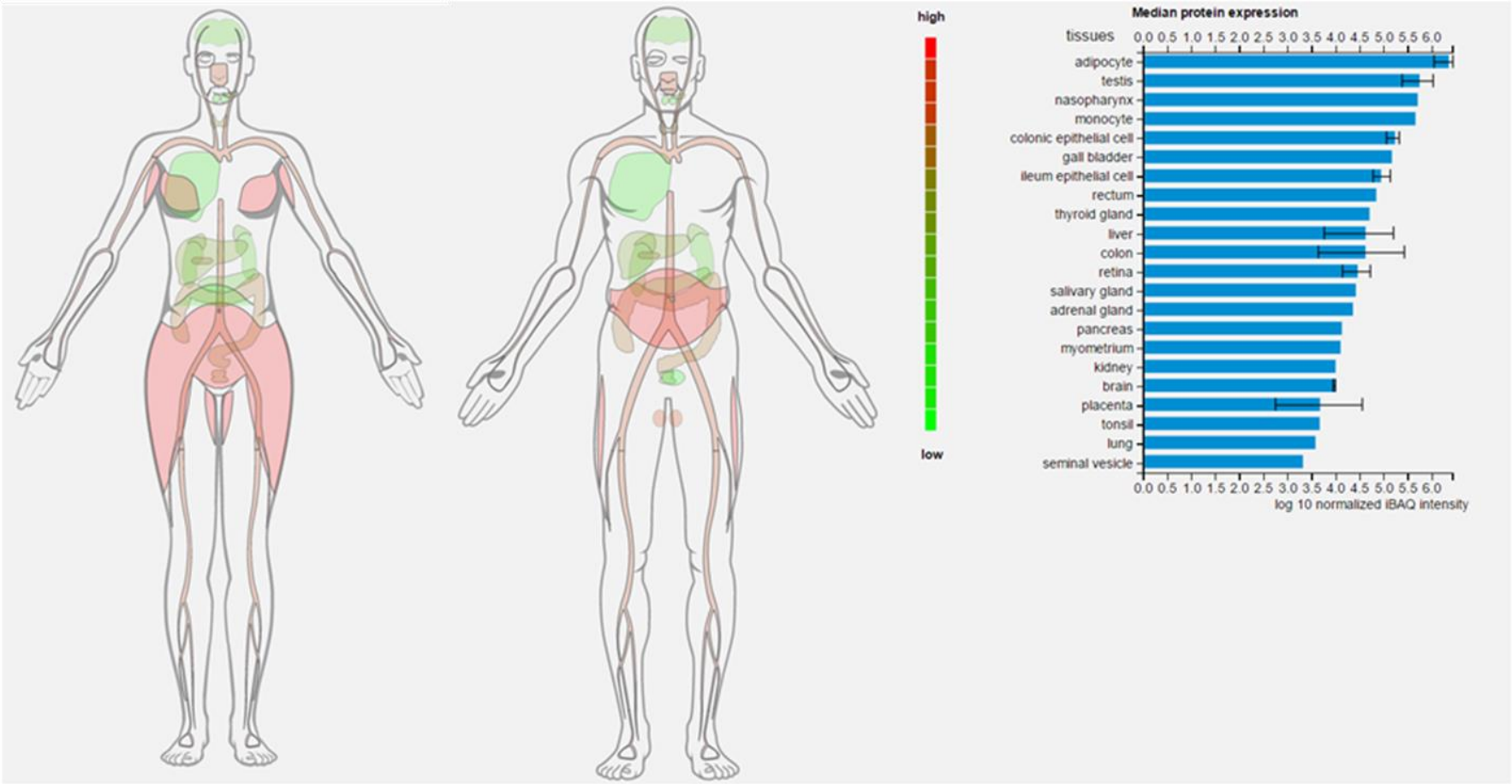

Figure 1.7: Protein expression of mARC1. Expression of mARC1 in females (left), males (center), and the iBAQ intensities for tissue (right). (proteomicsdb.org/)[112]

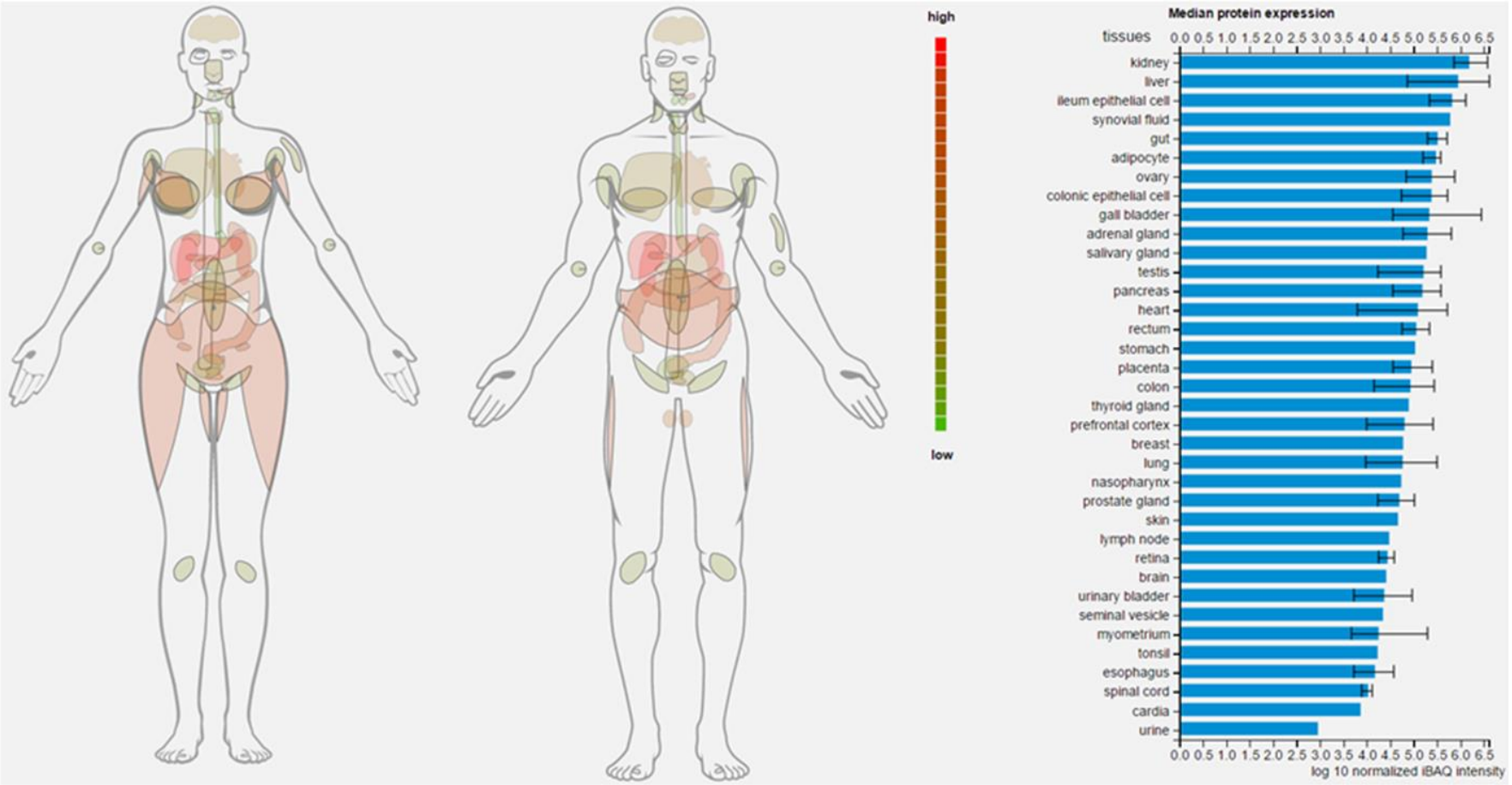

Figure 1.8: Protein expression of mARC2 in humans. Expression of mARC2 in females (left), males (center), and the iBAQ intensities for tissue (right). (proteomicsdb.org/)[112]

The presence of NADH, cytochrome b_5, and cytochrome b_5 reductase has been shown to be important to the activity of mARC proteins.[19, 25, 105, 108, 113] In *in vitro* studies, during the regeneration step, the catalytic center of the mARC enzyme receives electrons from NADH via electron transfer partner proteins, cytochrome b_5, and cytochrome b_5 reductase, without which mARC proteins' function is impaired. Given the slow rate of the reaction, it is not clear whether cytochrome b_5, and cytochrome b_5 reductase are the physiological electron transfer partners with mARC. There are two isoforms of cytochrome b_5 encoded by different genes, type A and type B.[114, 115] Type A cytochrome b_5 is localized as membrane-bound in microsomes as well as a soluble form.[114, 116] The type B isoform is localized to the mitochondrial outer membrane.[117-119] The catalytic properties of mARC have been achieved *in vitro* using both types of cytochrome b_5.[120] However, a knockout study demonstrated that type B-cytochrome b_5 is required for the *N*-reductive pathways.[25, 105]

As previously stated mARC proteins may have an involvement in several different pathways, but the exact physiological role of mARC proteins is not well understood.[22] There is the potential that mARC proteins may be involved in nitric oxide production in humans. Under aerobic conditions, mitochondrial fractions of different tissue containing heterologously expressed mARC1 and mARC2 have been shown to produce nitric oxide from N^{ω}-hydroxy-L-arginine.[113] Under hypoxic conditions mARC proteins coupled with the ancillary proteins cytochrome b_5, and cytochrome b_5 reductase have been

identified to reduce nitrite into nitric oxide.[26] As previously stated nitric oxide is a potent signaling molecule and affects several different mammalian pathways, suggesting that mARC proteins may be involved in signaling cascades.

Human mARC proteins may also participate in the metabolic detoxification of *N*-hydroxylated nucleobases and nucleosides such as *N*-hydroxycytosine, *N*-hydroxyadenine, cytidine, and adenine.[19, 110] The down-regulation of mARC2 in colon tumors demonstrated a significant role of mARC-mediated detoxification of mutagenic DNA bases.[121] This suggests that mARC proteins may be involved in the detoxification and prevention of accumulation of mutagenic substances within the cells.

It has also been found that the expression of mARC2 and *N*-reductive activity were increased in adipose tissue, suggesting that mARC2 may be regulated under lipogenic conditions within differentiation of murine cells into mature adipocytes.[22, 105, 122, 123] The down-regulation of mARC2 was found to impact lipid synthesis in cells negatively.[105] It has also been observed that the expression of mARC proteins and *N*-reductive activity were affected by fasting and a high-fat-diet in mice, suggesting that mARC proteins may be involved lipid metabolism within cells.[116]

Currently, mARC proteins are exploited for their potential in pro-drug metabolism to reduce amidoxime to amidines.[22] Compounds containing amidine functional groups are used in several types of treatment including; factor Xa inhibitors, thrombin inhibitors, factor VIIa inhibitors, urokinase-type plasminogen activators, anti-

microbial agents (i.e. parasitic, bacterial, malaria), and antiplatelet GPIIb/IIIa-receptor antagonist.[124-131] The issue with compounds containing amidines is their strong basicity and under physiological conditions can readily be protonated, resulting in a low bioavailability within the body.[132, 133] By converting the amidines to amidoxime functional groups the compounds can no longer be protonated, and the basicity is also greatly reduced, allowing for a bioreversible compound that utilizes enzymes like mARC proteins to catalyze the reduction of the pro-drug into the active form.[22] The mARC protein system has been tested and showed success in several model substrates, pro-drugs including anti-cancer and antiprotozoal agents, transforming drug metabolites, and the reduction of mutated nucleotides and harmful metabolites (**Figure 1.8**).[19, 26, 107-110, 113, 116, 126, 134, 135] Thus, the physiological function of mARC proteins remains unknown in human systems, but studies have demonstrated that they may be involved in nitric oxide production, detoxification of mutated base pairs, and lipid metabolism. By understanding the metabolic properties of the mARC system, physiological functions may be proposed.

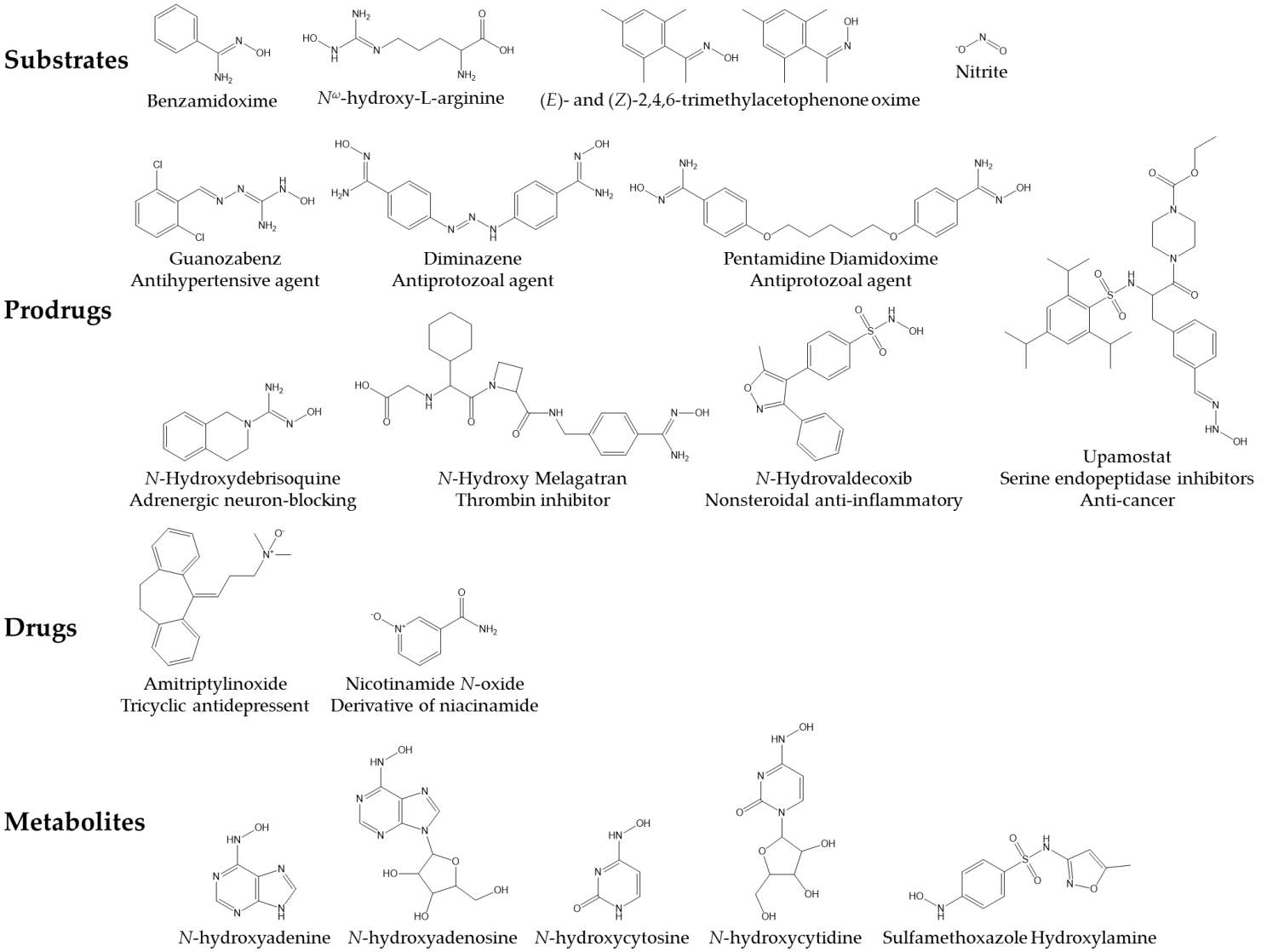

Figure 1.9: Substrates that have been evaluated using the mARC system.

1.5 Specific Aims of Study

The global objective of this study is to understand potential the physiological role of mARC1 and mARC2 through identifying interacting proteins through protein-protein interaction *in vitro* experiments. Protein-protein interactions can provide insight into the physiological function of mARC proteins. One of the issues with performing protein-protein interaction studies is the number of falsely identified proteins (false positives).[136] The experimental conditions, cell types, and reagents used affect the protein-protein interactions. To collect a more comprehensive the data set for this study, three different methods to identify protein-protein interactions were used; chemical/photoactivated crosslinking, co-immunoprecipitation, and pull-down assay. Interactions were probed in human embryonic kidney (HEK-293) cell lysate using purified mARC1 and mARC2 overexpressed in *Escherichia coli*. Proteins were selected for reverse co-immunoprecipitation specifically looking for mARC1 and mARC2 for the selected proteins. Reverse co-immunoprecipitation experiments were carried out in HEK-293 cells lysate as well rat liver tissue lysate and pig mitochondrial lysate. By identifying potential interacting partners of mARC proteins, a better understanding of the physiological function can be probed.

Chapter 2: Sample Preparation and Experimental Methods

All reagents, materials, and kits used in the subsequent experiments can be found in **Appendix I, Table A1**.

2.1 Lysate Preparation and Recombinant mARC Protein

HEK-293 cells were grown, harvested, and lysed at the University of Pittsburgh, Pittsburgh, PA, by Dr. Courtney Sparacino-Watkins. The cells used were modified to overexpress mARC2 with a green fluorescent protein (GFP) tag, as reported in the literature.[26] Rat liver tissue was supplied by Dr. Courtney Sparacino-Watkins University of Pittsburgh, Pittsburgh, PA. Purified mARC1 and mARC2 were supplied by Dr. Courtney Sparacino-Watkins University of Pittsburgh, Pittsburgh, PA, following a procedure previously published.[26] Pig liver mitochondrial fractions were supplied by Dr. Antje Havemeyer of the Institute of Pharmaceutical Chemistry, Christian-Albrechts University of Kiel, Kiel.

HEK-293 cells were lysed by resuspending the cell pellet in 5 mL of Pierce Lysis Buffer and sonicating them with 5 cycles of 3-second pulses on ice. The lysis buffer contains a mild detergent capable of releasing proteins in the membrane fraction. The soluble cell fraction was collected by centrifuging at 11,250x*g* for 15 minutes at 4°C. Rat liver tissue was washed using the Pierce™ Lysis Buffer (**Appendix I**). The liver was then diced into smaller pieces using a stainless steel razor blade. The pieces were resuspended

in 5 mL of lysis buffer (**Appendix I**) and sonicated for 5 cycles of 3-second pulses on ice. The soluble cell fraction was collected by centrifuging at 11,250x*g* for 15 minutes at 4°C.

2.2 Experimental Methods to Identify Interacting Proteins with mARC Protein

Three different methods to identify protein-protein interactions were used to identify potentially affiliated partners with mARC1 and mARC2. Each type of experiment had experimental and control conditions to improve the results interpreted. The experimental conditions used purified mARC proteins and HEK-293 cell lysate to identify interacting partners. The control conditions were to ensure there were no false positives identified by the affinity purification methods and used either purified mARC proteins or HEK-293 cell lysate. In **Table 3.3** the experimental and control conditions are listed. All experimental and control conditions (co-immunoprecipitation, crosslinking,

Table 2.1: Experimental and control conditions for each method used to identify protein-protein interactions.

	Experimental	Control
Co-immunoprecipitation	† mARC1 + HEK-293 Lysate ‡ mARC2 + HEK-293 Lysate	†‡ HEK-293 Lysate † Purified mARC1 ‡ Purified mARC2
Crosslinking	† mARC1 + HEK-293 Lysate ‡ mARC2 + HEK-293 Lysate † Reduced mARC1 + HEK-293 Lysate ‡ Reduced mARC2 + HEK-293 Lysate	†‡ HEK-293 Lysate
Pull-down	† mARC1 + HEK-293 Lysate ‡ mARC2 + HEK-293 Lysate	†‡ HEK-293 Lysate † Purified mARC1 ‡ Purified mARC2
Reverse Co-Immunoprecipitation	†‡ HEK-293 Lysate †‡ Pig Liver †‡ Rat Liver	†‡ HEK-293 Lysate

† Conditions used for mARC1
‡ Conditions used for mARC2
†‡ Conditions used for mARC1 and mARC2

pull-down assay and associated mass spectrometric analyses) were performed in triplicate to improve the robustness of the data.

2.2.1 Co-immunoprecipitation of Interacting proteins with mARC Proteins

The mARC antibodies used for co-immunoprecipitation can be found in **Table 2.1**.

Table 2.2: Antibodies that were used in the co-immunoprecipitation and reverse co-immunoprecipitation.

Antibody Gene Name	Type	Company	Catalog Number
MAP3K5 (ASK1)	Monoclonal	Santa Cruz Biotechnology, Santa Cruz, California	sc-5194
PIK3C2B	Monoclonal	Santa Cruz Biotechnology, Santa Cruz, California	sc-100407
SLC25A4 (ANT 1/2)	Polyclonal	Santa Cruz Biotechnology, Santa Cruz, California	sc-9299
MTM1	Monoclonal	Santa Cruz Biotechnology, Santa Cruz, California	sc-377309
GAPDH	Monoclonal	Invitrogen, Waltham, Massachusetts	39-8600
mARC1 (MOSC1)	Polyclonal	Abcam, Cambridge, United Kingdom	ab103191
mARC2 (MOSC2)	Polyclonal	Abcam, Cambridge, United Kingdom	ab111685
mARC2 (MOSC2)*	Polyclonal	Abcam, Cambridge, United Kingdom	ab197195

*Used previously and was not able to purify mARC2 based on mass spectrometry data.

A stock of 1X coupling buffer (0.01 M sodium phosphate, 0.15 M sodium chloride, pH 7.2) was prepared from the 20X coupling buffer (0.20 M sodium phosphate, 3 M sodium chloride, pH 7.2)(**Appendix I**) by diluting 3 mL of buffer into 57 mL of ultra-pure water. The AminoLink® Plus Coupling Resin, aldehyde-activated agarose beads, and coupling buffer were equilibrated to room temperature. The AminoLink® Coupling Resin was resuspended, and 50 µL was pipetted into labeled spin column tubes. Using a table top centrifuge (1,000x*g*), the supernatant was removed. The resin was washed with 200 µL aliquots of 1X coupling buffer, centrifuged to remove supernatant and repeated once

more. Antibody (50 μg) was added to the resin, and the volume was adjusted to 200 μL using 1X coupling buffer. The antibody was coupled to the AminoLink® Coupling Resin by adding 3 μL of 5 M sodium cyanoborohydride solution in the fume hood. The reaction was allowed to proceed for 120 minutes at room temperature on a rotator. The supernatant was removed using centrifugation at 1,000x*g*. The reaction mixture was rinsed with 200 μL 1X coupling buffer, centrifuged, and the process was repeated once more. The reaction was quenched by adding 200 μL of quenching buffer (**Appendix I**), the supernatant was removed through centrifuging. Quenching buffer was again added (200 μL) with 3 μL of sodium cyanoborohydride and incubated for 15 minutes at room temperature on a rotator. The supernatant was removed by centrifuging, and the resin was washed twice with 200 μL of 1X coupling buffer. The resin was then washed a total of 6 times with 150 μL of the wash buffer (**Appendix I**). The bait/prey interaction mixture was prepared by adding 50 μg of purified mARC protein to 1 mg of protein in HEK-293 cell lysate. The resin was washed twice using 200 μL of IP lysis/wash buffer (**Appendix I**), and centrifuged to remove supernatant. The reaction was added to the resin and incubated overnight at 4°C on a rotator to ensure the mixture remains suspended. The flow through was removed by centrifuging and the resin was washed three times with 200 μL of IP lysis/wash buffer. The bait/prey proteins were eluted from the resin by adding 25 μL of the elution buffer (**Appendix I**) and incubating at room temperature for 5 minutes. Elution was repeated a total of 3 times resulting in ~75 μL if flow-through.

2.2.2 Crosslinking of Interacting proteins with mARC Proteins

The Sulfo-SBED Label Transfer Reagent (**Appendix I, Figure A.1A**) was purchased from Thermo Fisher Scientific, Waltham, MA. The No-Weigh™ microtubes contained 1.12 mg of Sulfo-SBED Label Transfer Reagent and were reconstituted in 22 μL of DMSO. The K-BLoC (**Appendix I, Figure A.1B**) compound was a customized to bind to the active site in mARC and bind the non-specific crosslinking reagent transforming it into a site specific complex. was reconstituted in phosphate buffer (0.01 M sodium phosphate, 0.15 M sodium chloride, pH 7.2). K-BLoC and Sulfo-SBED crosslinker were reacted by adding 5 mg of K-BLoC and 11 μL of the reconstituted Sulfo-SBED crosslinker and incubated for 30 minutes at room temperature; the resulting complex will be referred to as LTR-K-BLoC complex (**Appendix I, Figure A.1C**). The precipitate was seperated from the soluble fraction by centrifugation. To bind the LTR-K-BLoC complex to purified mARC proteins, 200 μg was added to 50 μg of protein. They were incubated together for 15 minutes at room temperature. The LTR-K-BLoC complex binding to mARC1 was confirmed after buffer exchange, using intact protein mass spectrometry. The buffer exchange was carried out using Zeba™ Micro Spin Desalting Columns. The storage solution was removed by centrifuging using a table top centrifuge. The resin was then rinsed 3 times with 50 μL of 50 mM triethylammonium acetate. The sample was added to the final volume of 12 μL and 3 μL of 50 mM triethylammonium acetate was added as a stacking layer to aid in flow-through. The column was centrifuged at 1000x*g* for 2 minutes, and

the sample was collected. Excess LTR-K-BLoC complex was removed using Zeba™ Micro Spin Desalting Columns. The storage solution was removed by centrifuging using a table top centrifuge. The sample was added to the final volume of 12 μL and 3 μL of ultrapure water was added as a stacking layer to aid in flow-through. The column was centrifuged at 1,000x*g* for 2 minutes, and the sample was collected. Once the mARC complex was desalted, 1 mg of HEK-293 cell lysate was incubated for 5 minutes at room temperature. The sample was then placed on ice under a long-wave UV lamp for 15 minutes to bond interacting partners through the photoactive aryl azide covalently. Three biological replicates from mARC1 and mARC2 interacting with HEK-293 cell lysate were reduced by adding 10 μL of 50 mM dithiothreitol (DTT) and incubated for 15 minutes to reduce the disulfide bond present in the Sulfo-SBED crosslinker. Immobilized Streptavidin was resuspended, and 50 μL aliquots were placed into each spin column. The resin was rinsed using 250 μL of TBS buffer and inverted several times. The supernatant was removed using centrifugation at 1,000xg for 30 seconds. This process was repeated a total of three times. The bait/prey crosslinked samples were then incubated with the streptavidin resin for 1 hour at 4°C. The unbound proteins were rinsed from the resin using centrifugation at 1,000x*g* for 30 seconds. The resin was washed by adding 250 μL of wash buffer and inverted several times to resuspended the slurry. The eluent was removed using centrifugation at 1,000x*g* for 30 seconds. This process was repeated a total of four times. Proteins were eluted from the Streptavidin resin by first neutralizing the pH of the sample

by adding 10 μL of the neutralization buffer. Then 250 μL of elution buffer was added to the samples and inverted several times. Samples were incubated for 5 minutes at room temperature and then collected using centrifugation at 1,000x*g* for 30 seconds.

2.2.3 Pull-down Assay of Interacting proteins with mARC Proteins

The bait proteins, i.e., mARC1 and mARC2, were overexpressed and purified using a polyhistidine tag as previously discussed in **Chapter 2.1**.[26] The bait protein was immobilized onto HisPur™ Cobalt Resin by pipetting 50 uL of the slurry resin into spin columns. The resin was rinsed using 400 uL of wash solution (1:1 tris-buffered saline:lysis buffer, 10 mM imidazole)(**Appendix I**) and inverting the spin columns. The caps were then removed and placed into a tabletop centrifuge to remove buffer. There was a total of 5 washing steps to prepare the cobalt resin. The purified mARC1 and mARC2 were added to the Pierce™ spin columns to the final concentration of 50 μg of protein. The samples were then incubated at 4°C for 60 min using a rotator to maintain suspension of the resin. Both caps were removed, and the supernatant was removed using a table top centrifuge. The resin was rinsed using 400 μL of wash buffer and inverted several times. Again, the supernatant was removed using a table top centrifuge. This wash process was repeated a total of 5 times. The HEK-293 cell lysate was added to the spin columns to the final amount of 1 mg of proteins. Samples were then incubated at 4°C overnight on a rotating platform. The supernatant was then removed using a table top centrifuge. The samples were then washed with 400 μL of the wash buffer a total of 5 times. Bait and prey

protein were then eluted from the resin by adding 250 μL of 290 mM imidazole elution buffer (**Appendix I**). Samples were incubated at room temperature for 5 min on a rotating platform. The bait and prey protein was collected in sample tubes using a table top centrifuge.

2.3 Mass Spectrometry Method for Protein Analysis

All mass spectrometric data collection and analyses was performed at Duquesne University, Pittsburgh, PA at the Agilent Technologies Center of Excellence, supported by NSF DBI 0821401

2.3.1 In-solution Trypsin Digestion

Tryptic peptides were produced by first adding equal volumes of 2,2,2-trifluoroethanol (TFE) to samples with 5 μL of 200 mM dithiothreitol (DTT). Samples were then incubated at 56°C for 1 hour to denature and reduce proteins. A 10 μL aliquot of 200 mM iodoacetamide (IAM) was added to each sample and incubated at room temperature and covered for 1 hour. Samples were dried using a Forma Speed Vac and resuspended in 50 μL of 100 mM ammonium bicarbonate buffer. Digestion occurred by adding 5 μg of trypsin and incubated at 36°C overnight. The pH of the solution was adjusted by adding 2 μL of formic acid and vortexed to cease the digestion. The samples were again dried and then reconstituted in 95% water, 5% acetonitrile, and 0.1% formic acid for LC-MS/MS analysis.

2.3.2 Liquid Chromatography Mass Spectrometry (LC-MS/MS) Peptide Analysis

The protein mass spectrometric analysis was performed using a quadrupole-time-of-flight (Q-TOF) LC-MS system (Agilent Technologies, Santa Clara, CA) equipped with a 1200 Series binary liquid chromatography, Dual AJS ESI source, and a 6530 Q-TOF mass spectrometer. The instrument was operated under the MassHunter Data Acquisitions software (version B.05.01 build 5.01.5125.1, Agilent Technologies, Santa Clara, CA). Tryptic digested peptides were separated on a Aeris™ 3.6 μm WIDEPORE XB-C18 200 Å, LC Column 150 x 2.1 mm (00F-4482-AN, Phenomenex, Torrance, CA) using a gradient of 5% acetonitrile, 95% water, and 0.1% formic acid to 80% acetonitrile 10% water, 10% 2,2,2-trifluoroethanol, and 0.1% formic acid using the time table and flow rates in **Table 2.2**. The source parameters were set to the values in **Table 2.3**. Fragmentor voltage, skimmer voltage, and OCT RF Vpp were set to 200 V, 65 V, and 750 V, respectively. The MS settings used were the following: precursor ion selection, medium (4 m/z); mass range, 120−1700 m/z; acquisition rate MS, 7 scans/sec; acquisition rate MS/MS, 4 scans/s (m/z 59-3000). The collision energy was adjusted based on the charge of the precursor (**Table 2.4**). Reference mass correction was enabled using reference mass of 121.05087300, and 922.00979800 m/z.

2.3.2 Intact Protein Mass Spectrometry

The intact protein mass spectrometric analysis was performed using a quadrupole-time-of-flight (Q-TOF) LC-MS system (Agilent Technologies, Santa Clara,

Table 2.3: Binary pump gradient table used for LC-MS/MS analysis.

Time	A [%]	B [%]	Flow [mL/min]	Max Pressure [bar]
0.00	100.0	0.0	0.450	400.00
35.00	70.0	30.0	0.450	400.00
40.00	0.0	100.0	0.450	400.00
42.00	0.0	100.0	0.450	400.00
43.00	100.0	0.0	0.450	400.00
45.00	100.0	0.0	0.450	400.00

CA) equipped with a 1200 Series binary liquid chromatography, Dual AJS ESI source, and a 6530 Q-TOF mass spectrometer. The instrument was operated under the MassHunter Data Acquisitions software (version B.05.01 build 5.01.5125.1, Agilent Technologies, Santa Clara, CA). Intact proteins were injected using a 10 µL injection volume with a 3-second needle wash and set with an isocratic gradient of 5% acetonitrile, 95% water, 0.1% formic acid with 5 minutes run time using a flow rate of 0.450 mL/min. The source parameters were set to the values in **Table 2.3**. Fragmentor voltage, skimmer voltage, and OCT RF Vpp were set to 400 V, 65 V and 750 V, respectively. The MS settings used were the mass range, 100−3200 m/z; acquisition rate MS, 7.00 scans/sec.

Table 2.4: Dual AJS ESI source parameters for LC-MS/MS analysis.

Dual AJS ESI	Value
Gas Temperature	340°C
Drying Gas	10 L/min
Nebulizer	45 psig
Sheath Gas Temperature	400°C
Sheath Gas Flow	11 L/min
Voltage Capillary	3500 V
Nozzle Voltage	1000 V

Table 2.5: Collision energy to generate product ions.

Charge	Slope	Offset
2	3	2
3	3.6	-4.8
>3	3.6	-4.8
Unknown	3.6	-4.8
1	3.5	6

2.4 Bioinformatics Software for Peptide and Protein Identification

2.4.1 Spectrum Mills Proteomic Workbench (Rev B.04.01.141)

Our research group has previously published a proteomic research paper in a peer-reviewed journal using the similar parameters as will be discussed.[137] Collision-induced dissociation (CID) data were searched against a custom homo sapiens databases that were created using the UniProt (uniprot.org) database, and all protein identifiers used in this investigation were denoted by their UniProt numbers and gene name. Peak lists were created with the Spectrum Mills Proteomic Workbench Data Extractor program with the following attributions. Scans with the same precursor ±1.4 m/z were merged within a time frame of ±30 seconds. Precursor ions needed to have a minimum signal to noise value of 25. Charges up to a maximum of 7 were assigned to the precursor ion, and the ^{12}C peak was determined by the Data Extractor. The custom database was searched for tryptic peptides with a mass tolerance of ±1.2 Da for the precursor ions and a tolerance of ±0.7 Da for the product ions. Two missed cleavages were allowed. A Spectrum Mills auto validation was performed first in the protein details followed by peptide mode using a.) in protein details mode; protein score ≥20, peptide score (scored percent intensity

[SPI]) charge +2 (>6.0, >60.0%), peptide charge +1 (>6.0, >70.0%), peptide charge +3 (>8.0, >70.0%), peptide charge +4 (>8.0, >70%), peptide charge +5 (>12.0, >70.0%), peptide charge +2 (>6.0, >90.0%), and rank 1 minus rank 2 score threshold >2.0 for all rules the last which was set to >1.0; b.) in peptide mode: precursor charge +2 (>11.0, >60.0%), peptide charge +1 (>13.0, >70%), peptide charge +3 (>13.0, >70.0%), peptide charge +4 (>13.0, >70.0%), peptide charge +5 (>15.0, >70.0%), and rank 1 minus rank 2 score threshold >2. All protein hits found in a distinct database search by Spectrum Mill are non-redundant.

2.4.2 Agilent MassHunter Qualitative Analysis with BioConfirm (Version B.06.00, Build 6.0.633.0)

Once proteins were identified using Spectrum Mills Proteomic Workbench the BioConfirm software package was used to investigate the identified proteins further using high-resolution mass spectrometry data. Once the files were loaded into the software "Find by Molecular Feature" was used to identify the compound in the samples meeting the criteria found in **Table 2.5**. The protein sequences from each protein identified were loaded into the software using trypsin as the digestion reagent allowing

Table 2.6: BioConfirm "Find by Molecular Feature" parameters used to identify compounds from mass spectrometry data.

Find by molecular Feature	Parameter
Target Data type	Small molecules (chromatographic)
Restrict m/z to	350.00-2000.00 m/z
Use Peaks with height	≥100 counts
Ion Species	H^+, Na^+, K^+
Peak Space tolerance	0.0025 m/z, plus 7.0 ppm
Isotope Model	Peptides
Maximum charge state	15

for up to 2 missed cleavages, global modifications were set to alkylation (iodoacetamide), and variable modifications were set to deamidation and oxidation. The compounds identified from "Find by Molecular Feature" were then analyzed using "Define and Match Sequences" with the MS tolerance of 10.00 ppm, and the MS/MS tolerance of 50.00 ppm.

Chapter 3: Results and Discussion for Proteins Interacting with mARC1 and mARC2

3.1 K-BLoC, LTR-K-BLoC Complex, and mARC1 Native Intact Protein Mass Spectrometry

Mass spectrometric analysis was used to identify LTR-K-BLoC complex binding to mARC1. A bottom-up approach was used first to identify the starting materials K-BLoC, and Sulfo-SBED reagent, then the LTR-K-BLoC complex, and finally intact mARC1 with LTR-K-BLoC bound.

The K-BLoC compound (179.22 MW) was identified by mass spectrometry as seen in **Figure 3.1**. The highest abundant peak at 180.1105 m/z $(M+H)^+$ suggests the protonated form of K-BLC. The second most abundant peak at 163.0838 m/z indicates a loss of a –OH group suggesting the reduced form of K-BLoC may also be present.

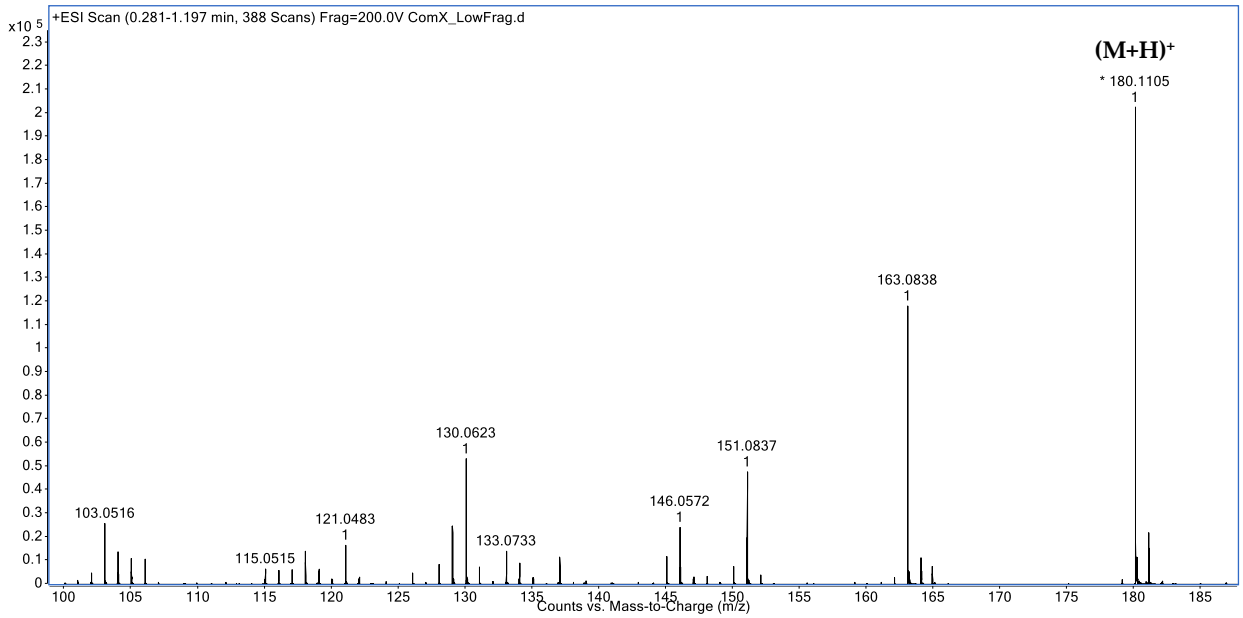

Figure 3.1: Mass spectrum for K-BLC compound.

Analysis of Sulfo-SBED label transfer reagent was performed under the same instrument parameters. Upon the addition of DMSO the NHS-ester side chain is

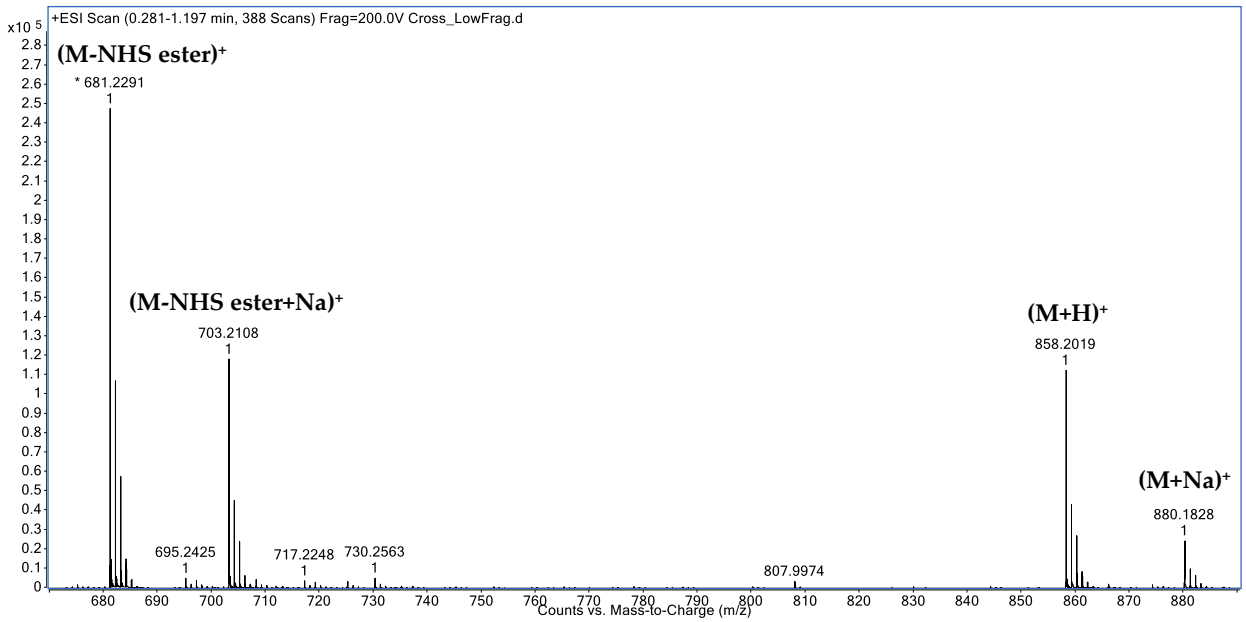

Figure 3.2: Mass spectrum for Sulfo-SBED label transfer reagent.

removed, activating one of the reactive group. In **Figure 3.2** of the mass spectrometry analysis of Sulfo-SBED label transfer reagent, there are four compounds present from the m/z range of 675 to 885. The most abundant peak at 681.2291 m/z is proposed to be activated Sulfo-SBED reagent with the loss of the NHS-ester. The peak present at 703.2108 m/z denotes a mass shift of ~22 m/z from the activated Sulfo-SBED reagent suggesting a sodium adduct. The peaks present at 858.2019 and 880.1828 m/z are proposed to be the starting/unactivated form of Sulfo-SBED, without and with a sodium adduct.

Once the Sulfo-SBED label transfer reagent was activated by removal of the NHS-ester side chain, it was anticipated to bind to the lysine side chain of the K-BLoC compound. **Figure 3.3** is the mass spectrometry analysis of the LTR-K-BLoC complex. The peaks present at 681.2266 and 703.2084 m/z are the activated Sulfo-SBED without and with sodium adduct. The peaks of interest are present at 842.3227 and 864.3047 m/z which

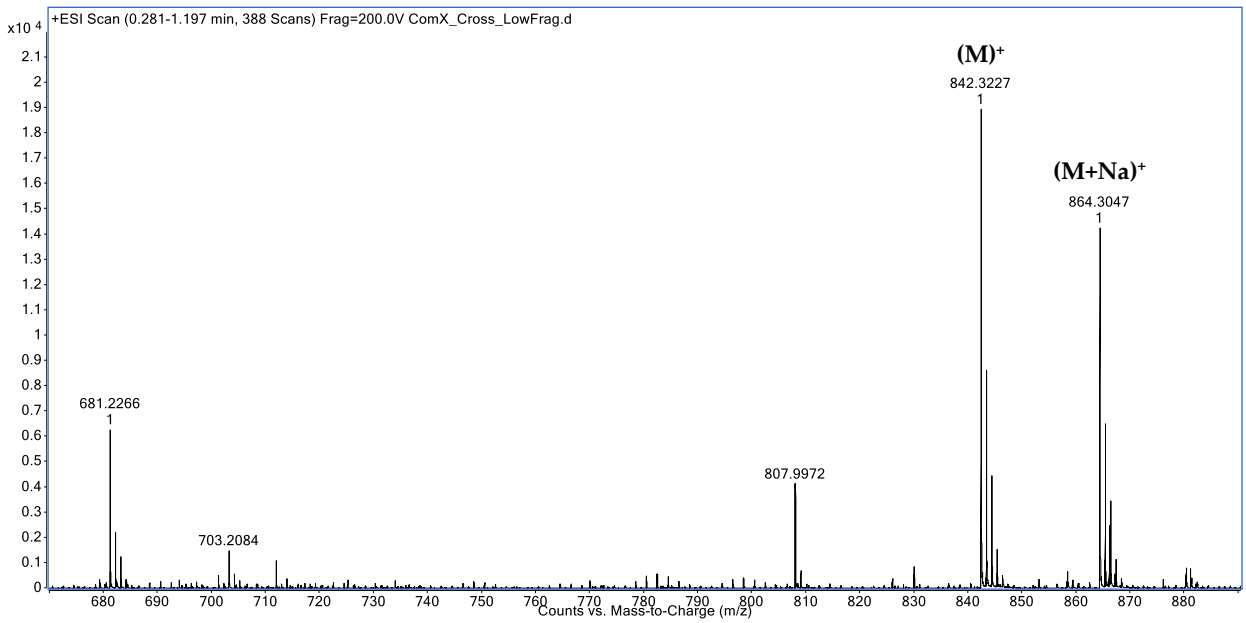

Figure 3.3: Mass spectrum for LTR-K-BLC complex.

are suggested to be the bound Sulfo-SBED and K-BLoC compound (LTR-K-BLoC complex).

To identify the interaction of the LTR-K-BLoC complex and mARC proteins, native intact protein mass spectrometry was used. **Figure 3.4** show the purified mARC1 analyzed intact (**A**) and the deconvoluted results (**B**). The calculated MW of the cytoplasmic portion (41-337 AA) of mARC1 is 33,635.80 Da, and the molybdopterin cofactor is 519.26 Da. Adding the protein and the cofactor the calculated mass is 34,155.06 Da. The calculated value and observed (34,146.42 Da) from the deconvoluted results agree reasonably with a 0.025% difference. After incubating with the LTR-K-BLoC complex and deconvoluting the results (**Figure 3.5**) there is a noticeable split of the intact mARC1 peak. The unreacted mARC1 appears at 34,144.47 Da, but four other peaks appear to have emerged. The calculated MW of mARC1, the cofactor, and the LTR-K-

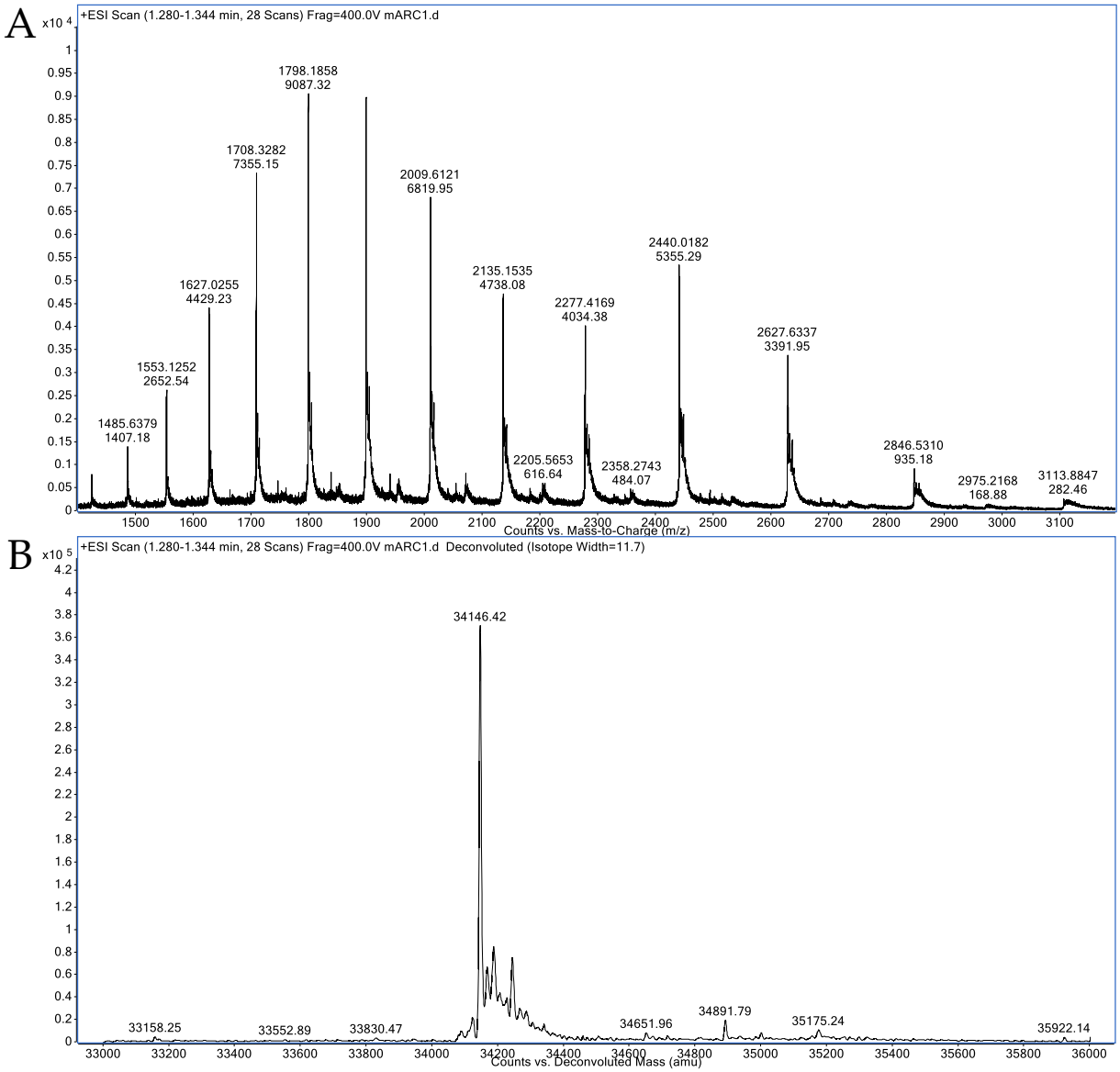

Figure 3.4: Mass spectrum for intact mARC1 unreacted with LTR-K-BLoC complex. A. Mass spectrum gathered from the analysis of intact mARC1, B. the deconvoluted results of intact mARC1.

BLoC complex is 34,997.38 Da. The peak of interest is 34,982.86 Da; the difference is approximately 0.041% difference (or ~14.5 Da) which may be a loss of nitrogen or possibly oxygen in the complexed protein.

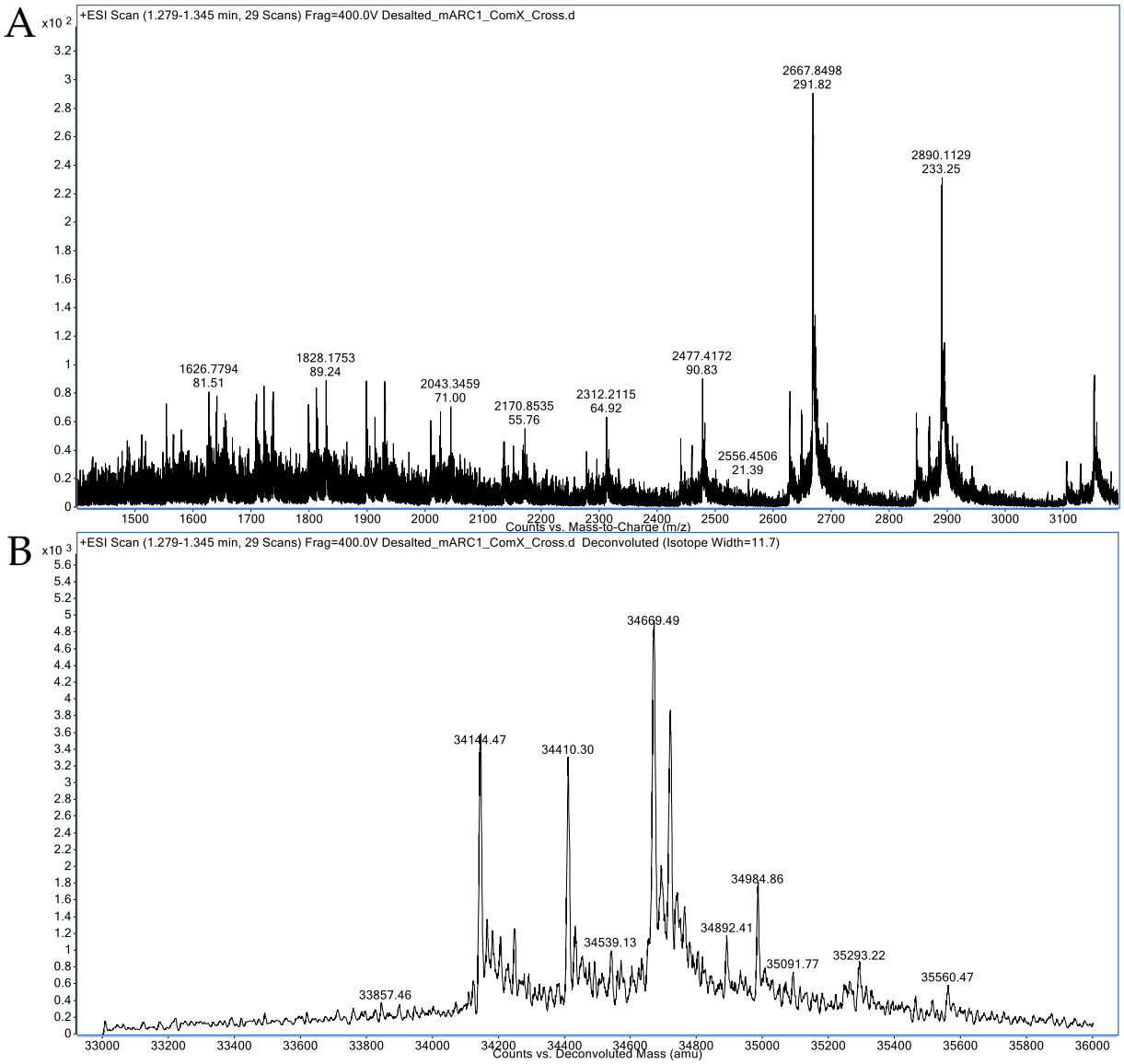

Figure 3.5: Mass spectrum for intact mARC1 reacted with LTR-K-BLoC complex. A. Mass spectrum gathered from the analysis of intact mARC1 reacted with LTR-K-BLoC complex, B. the deconvoluted results of intact mARC1 reacted with LTR-K-BLoC complex.

3.2 Data Mining mARC1 and mARC2 Protein-protein Interactions

Very few interaction studies have been performed using mARC1 and mARC2. Using STRING database (string-db.org/), reported protein-protein interactions were compiled for mARC1 and mARC2.[138] Interactions in the database classified under 8 categories (neighborhood, gene fusion, co-occurrence, experiments, databases, data

mining, and homology). Each interaction is scored with a confidence score, which was set to ≥0.5 for mARC1 and mARC2 interactions. The confidence score is calculated by combining the probabilities of various evidence and correcting for the random observations of interaction.[139] STRING database also categorizes the interacting proteins into functional networks (*e.g.*, GOTerm biological process, GOTerm molecular function, and KEGG pathways). The categories are ranked by false discovery rate; nothing was reported with a score of ≥0.03 for biological process, ≥0.01 molecular function, and ≥0.01 KEGG pathway. The false discovery rate was calculated using a statistical enrichment test referred to as LRpath.[140, 141] This form of statistical comparison uses logistic regression so the data can remain on a continuous scale and results can be interpreted similarly to the Fisher's Exact test.[140, 141]

There were 20 proteins that were reported in the STRING database to be shown as interacting with mARC1. There were 8 of the 20 were identified from data mining, and 12 identified from experiments (i.e. co-immunoprecipitation, and affinity capture-MS assay) for mARC1. The 20 proteins can be found in **Table 3.1** and labeled according to their associated categories, functional networks, and scores.

There are 12 GOTerm biological processes involving the interacting proteins. Interestingly, the three biological processes with the lowest false discovery rate are: small molecules metabolic, single-organism metabolic, and oxidation-reduction processes. Based on this information, 8 of the interacting proteins are involved in the oxidation-

reduction process, and mARC1 is known to reduce compound with amidoxime groups suggesting its potential physiological involvement. There are also 3 biological processes, respiratory electron transport chain, mitochondrial electron transport, and mitochondrial ATP synthesis coupled electron transport. All of which are involved in electron transfer processes, suggesting mARC may function as a redox protein.

There are 4 GOTerm molecular functions identified for the interacting proteins. The top two molecular functions are oxidoreductase activity, and oxidoreductase activity acting on NAD(P)H. Again strengthening the proposal that mARC1 may be involved in reductive processes as its biological function.

Of the 20 interacting proteins identified with mARC1, 9 were affiliated with 7 different KEGG pathways identified for interacting proteins with mARC1. The pathway with the lowest false discovery score is oxidative phosphorylation. Interestingly enough the next 4 pathways identified are different disease pathways, non-alcoholic fatty liver disease, Parkinson's disease, Alzheimer's disease, and Huntington's disease. There were 8 potential interacting proteins with mARC2 predicted through data mining. The 8 proteins can be found in Table 3.2 and labeled according to their associated categories, functional networks, and scores. There were 4 GOTerm biological processes that were reported for interacting proteins with mARC2, molybdopterin cofactor biosynthetic proteins, small molecule, water-soluble vitamin, and single-organism metabolic process. The interactions of molybdopterin proteins (MOCS1, MOCS2, GPHN) may be involved

in the maturation process of mARC2. Similar to mARC1, cytochrome b5 reductase has also been proposed to interact with mARC proteins because it is involved in the reductive system.

The information provided regarding the GOTerm molecule function and KEGG pathways provides almost no information regarding the potential physiological function of mARC2. Xanthine dehydrogenase contains a molybdopterin cofactor binding domain similar to that in mARC2. The MOCS1 and MOCS2 proteins are both involved in the sulfur relay system and the folate biosynthesis pathways. The sulfur relay system is a signaling system involved in cell cycles processes like cell proliferation, apoptosis, and DNA repair.

Table 3.1: Proteins identified in the STRING database as interacting proteins with mARC1.

UniProt ID (Gene Name), Protein Name	Category (Score)	GOTerm Biological Process (False Discovery Rate)	GOTerm Molecular Function (False Discovery Rate)	KEGG Pathway (False Discovery Rate)
P15313 (ATP6V1B1), V-type proton ATPase subunit B, kidney isoform**[20]**	Experiments (0.644)	small molecule metabolic process (0.00878); single-organism metabolic process (0.00878); purine nucleotide metabolic process (0.024); ATP metabolic process (0.0252); organonitrogen compound metabolic process (0.0341)		Oxidative phosphorylation (0.0000516); Metabolic pathways (0.00512); Phagosome (0.0163)
Q9UKI2 (CDC42EP3), Cdc42 effector protein 3**[142-152]**	Data mining (0.675)			
Q6BCY4 (CYB5R2), NADH-cytochrome b5 reductase 2**[105]**	Data mining (0.567)	small molecule metabolic process (0.00878); single-organism metabolic process (0.00878); oxidation-reduction process (0.00878)	oxidoreductase activity (0.0055); oxidoreductase activity, acting on NAD(P)H (0.0055)	
Q52LR7 (EPC2), Enhancer of polycomb homolog 2**[49, 153]**	Data mining (0.526)	single-organism metabolic process (0.00878)		
Q96DN0 (ERP27), Endoplasmic reticulum resident protein 27**[105]**	Data mining (0.517)			
Q15031 (LARS2), Probable leucine--tRNA ligase, mitochondrial**[154]**	Data mining (0.596)	small molecule metabolic process (0.00878); single-organism metabolic process (0.00878); organonitrogen compound metabolic process (0.0341)		
Q9P015 (MRPL15), 39S ribosomal protein L15, mitochondrial**[20]**	Experiments (0.644)	single-organism metabolic process (0.00878); organonitrogen compound metabolic process (0.0341)		

Table 3.1: Continued

UniProt ID (Gene Name), Protein Name	Category (Score)	GOTerm Biological Process (False Discovery Rate)	GOTerm Molecular Function (False Discovery Rate)	KEGG Pathway (False Discovery Rate)
Q9NYK5 (MRPL39), 39S ribosomal protein L39, mitochondrial**[20]**	Experiments, Data mining (0.695)	single-organism metabolic process (0.00878); organonitrogen compound metabolic process (0.0341)		
O96000 (NDUFB10), NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10**[20, 155]**	Experiments, Data mining (0.676)	small molecule metabolic process (0.00878); single-organism metabolic process (0.00878); oxidation-reduction process (0.00878); respiratory electron transport chain (0.0202); mitochondrial electron transport, NADH to ubiquinone (0.024); purine nucleotide metabolic process (0.024); energy derivation by oxidation of organic compounds (0.024); mitochondrial ATP synthesis coupled electron transport (0.0252); cellular respiration (0.0252); ATP metabolic process (0.0252); oxidative phosphorylation (0.0286); organonitrogen compound metabolic process (0.0341)	oxidoreductase activity (0.0055); oxidoreductase activity, acting on NAD(P)H (0.0055); NADH dehydrogenase (ubiquinone) activity (0.0114)	Oxidative phosphorylation(0.0000516); Non-alcoholic fatty liver disease (0.00133); Parkinson s disease (0.00133); Alzheimer s disease (0.00154); Huntington s disease (0.00181); Metabolic pathways (0.00512)
O95168 (NDUFB4), NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 4**[5, 14, 20]**	Experiments (0.644)	small molecule metabolic process (0.00878); single-organism metabolic process (0.00878); oxidation-reduction process (0.00878); respiratory electron transport chain (0.0202); mitochondrial electron transport, NADH to ubiquinone (0.024); purine nucleotide metabolic process (0.024); energy derivation by oxidation of organic compounds (0.024); mitochondrial ATP synthesis coupled electron transport (0.0252); cellular respiration (0.0252); ATP metabolic process (0.0252); oxidative phosphorylation (0.0286); organonitrogen compound metabolic process (0.0341)	oxidoreductase activity (0.0055); oxidoreductase activity, acting on NAD(P)H (0.0055); NADH dehydrogenase (ubiquinone) activity (0.0114)	Oxidative phosphorylation (0.0000516); Non-alcoholic fatty liver disease (0.00133); Parkinson s disease (0.00133); Alzheimer s disease (0.00154); Huntington s disease (0.00181); Metabolic pathways (0.00512)

Table 3.1: Continued

UniProt ID (Gene Name), Protein Name	Category (Score)	GOTerm Biological Process (False Discovery Rate)	GOTerm Molceular Function (False Discovery Rate)	KEGG Pathway (False Discovery Rate)
O43181 (NDUFS4), NADH dehydrogenase [ubiquinone] iron-sulfur protein 4, mitochondrial**[20]**	Experiments (0.644)	small molecule metabolic process (0.00878); single-organism metabolic process (0.00878); oxidation-reduction process (0.00878); respiratory electron transport chain (0.0202); mitochondrial electron transport, NADH to ubiquinone (0.024); purine nucleotide metabolic process (0.024); energy derivation by oxidation of organic compounds (0.024); mitochondrial ATP synthesis coupled electron transport (0.0252); cellular respiration (0.0252); ATP metabolic process (0.0252); oxidative phosphorylation (0.0286); organonitrogen compound metabolic process (0.0341)	oxidoreductase activity (0.0055); oxidoreductase activity, acting on NAD(P)H (0.0055); NADH dehydrogenase (ubiquinone) activity (0.0114)	Oxidative phosphorylation(0.0000516); Non-alcoholic fatty liver disease (0.00133); Parkinson s disease (0.00133); Alzheimer s disease (0.00154); Huntington s disease (0.00181); Metabolic pathways (0.00512)
Q9H0N0 (RAB6C), Ras-related protein Rab-6C**[14, 20]**	Experiments (0.644)			
P31040 (SDHA), Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial**[20]**	Experiments, Data mining (0.655)	small molecule metabolic process (0.00878); single-organism metabolic process (0.00878); oxidation-reduction process (0.00878); respiratory electron transport chain (0.0202); energy derivation by oxidation of organic compounds (0.024); cellular respiration (0.0252)	oxidoreductase activity (0.0055)	Oxidative phosphorylation (0.0000516); Non-alcoholic fatty liver disease (0.00133); Parkinson s disease (0.00133); Alzheimer s disease (0.00154); Huntington s disease (0.00181); Metabolic pathways (0.00512)
Q8IWL2 (SFTPA1), Pulmonary surfactant-associated protein A1**[156, 157]**	Data mining (0.53)			Phagosome (0.0163)

Table 3.1: Continued

UniProt ID (Gene Name), Protein Name	Category (Score)	GOTerm Biological Process (False Discovery Rate)	GOTerm Molceular Function (False Discovery Rate)	KEGG Pathway (False Discovery Rate)
O00330 (PDHX), Pyruvate dehydrogenase protein X component, mitochondrial**[11, 20]**	Experiments, Data mining (0.662)	small molecule metabolic process (0.00878); single-organism metabolic process (0.00878)		Metabolic pathways (0.00512)
Q8IWL1 (SFTPA2), Pulmonary surfactant-associated protein A2**[156, 157]**	Data mining (0.527)			Phagosome (0.0163)
Q96AG3 (SLC25A46), Solute carrier family 25 member 46**[20]**	Experiments (0.644)			
P0CG48 (UBC), Polyubiquitin-C**[158]**	Experiments (0.86)	small molecule metabolic process (0.00878); single-organism metabolic process (0.00878); oxidation-reduction process (0.00878); energy derivation by oxidation of organic compounds (0.024)		
P47989 (XDH), Xanthine dehydrogenase/oxidase**[49, 105, 159-161]**	Data mining (0.52)	small molecule metabolic process (0.00878); single-organism metabolic process (0.00878); oxidation-reduction process (0.00878); purine nucleotide metabolic process (0.024); organonitrogen compound metabolic process (0.0341)	oxidoreductase activity (0.0055); molybdopterin cofactor binding (0.0114)	Metabolic pathways (0.00512)
Q9UPT8 (ZC3H4), Zinc finger CCCH domain-containing protein 4**[20]**	Experiments (0.644)			

Table 3.2: Proteins identified in the STRING database as interacting proteins with mARC2.

UniProt ID (Gene Name), Protein Name	Category (Score)	GOTerm Biological Process (False Discovery Rate)	GOTerm Molecular Function (False Discovery Rate)	KEGG Pathway (False Discovery Rate)
Q15031 (LARS2), Probable leucine-tRNA ligase, mitochondrial**[154]**	Texting mining (0.636)	small molecule metabolic process (0.00489), single-organism metabolic process (0.0147)		
Q96DN0 (ERP27), Endoplasmic reticulum resident protein 27**[105]**	Texting mining (0.500)			
Q6BCY4 (CYB5R2), NADH-cytochrome b5 reductase 2**[105]**	Texting mining (0.560)	small molecule metabolic process (0.00489), single-organism metabolic process (0.0147)		
Q52LR7 (EPC2), Enhancer of polycomb homolog 2**[162]**	Texting mining (0.566)	single-organism metabolic process (0.0147)		
O96007 (MOCS2), Molybdopterin synthase catalytic subunit**[49, 160, 163]**	Texting mining (0.842)	Mo-molybdopterin cofactor biosynthetic process (0.00000325), small molecule metabolic process (0.00489), water-soluble vitamin metabolic process (0.0138), single-organism metabolic process (0.0147)		Sulfur relay system (0.00222), Folate biosynthesis (0.00224)
Q9NP27 (MOCS1), Molybdenum cofactor synthesis-step 1 protein A splice type I**[49]**	Texting mining (0.882)	Mo-molybdopterin cofactor biosynthetic process (0.00000325), small molecule metabolic process (0.00489), water-soluble vitamin metabolic process (0.0138), single-organism metabolic process (0.0147)		Sulfur relay system (0.00222), Folate biosynthesis (0.00224)
P47989 (XDH), Xanthine dehydrogenase oxidase**[49, 105, 160, 164, 165]**	Texting mining (0.505)	small molecule metabolic process (0.00489), single-organism metabolic process (0.0147)	molybdopterin cofactor binding (0.00707)	
Q9NQX3 (GPHN), Gephyrin**[49]**	Neighborhood, Data mining (0.758)	Mo-molybdopterin cofactor biosynthetic process (0.00000325), small molecule metabolic process (0.00489), water-soluble vitamin metabolic process (0.0138), single-organism metabolic process (0.0147)		

3.3 Experimental and Control Conditions to Identify Interacting Partners with mARC Proteins

3.3.1 Co-immunoprecipitation

Co-immunoprecipitation experiments utilize antigen-antibody reactions to precipitate the targeted bait protein as well as interacting partners.[166] Monoclonal and polyclonal antibodies have their advantages and challenges in co-immunoprecipitation experiments. While monoclonal antibodies target specific epitopes within protein sequences resulting in reduced background noise and cross-reactivity, polyclonal antibodies allow for sequence flexibility in the target site tolerating sequence variability leading to a more robust detection of the target proteins.[166] The advantages of co-immunoprecipitation include high specificity based on the characteristics of the antibody, and compatibility with downstream analysis, in which mass spectrometric techniques will be used.[167, 168] In contrast the disadvantages to co-immunoprecipitation include the limitation of the specificity of the antibody used, and often a considerable amount of protein extract (100-1000 μg) of protein is required for identification downstream.[166-168] Co-immunoprecipitation experiments have been used in several instances to identify interacting partners. Luethy *et al.* used co-immunoprecipitation to study protein-protein interactions of pyruvate dehydrogenase complex (PDC) in pea seedlings.[169] PDC is a large (5000-7000 kDa) multi-component complex comprised of three major enzymes (pyruvate dehydrogenase, E1 dihydrolipoamide transacetylase, E2; and

dihydrolipoamide dehydrogenase, E3) with multiple subunits of each enzyme.[169] A highly specific antibody to PDC-E1α subunit was found to precipitate subunits E1β, E2, and E3 when separated on SDS-PAGE.[169] Using a novel approach in which co-immunoprecipitation was linked with crosslinking Huang *et al.* examined interacting proteins with protein kinase B (Akt) in mouse neuroblastoma cells.[170] Akt is a serine/threonine-specific protein kinase that is essential for multiple cellular processes.[170] They were able to crosslink the Akt antibody to agarose bead, incubate with cell lysate containing Akt protein, then chemically crosslinking interacting proteins to Akt using dithiobis[succinimidylpropionate].[170] Using this method they were able to identify ten interacting proteins with Akt, with only one of which that was able to be detected without the added crosslinking step.[170] The previous examples are only a few of the successful experiments utilizing co-immunoprecipitation to studying interacting partners with target proteins.

3.3.2 Crosslinking

Chemical crosslinking involves the process of covalently binding interacting proteins together using a reagent with two reactive groups separated by a spacer/linker region.[171] The benefits of crosslinking allow for transient interactions between proteins to be probed by "locking" the interactions.[166] Crosslink reagents have been customized to meet the users' needs by varying the chemical activity, length of that spacer, water solubility, cell membrane permeability, and cleavability.[167] Crosslinking is coupled

with including, but not limited to affinity purification, mass spectrometric identification, and SDS-PAGE.[172, 173] A disadvantage to using crosslinking reagents is the nonspecific interactions that may occur when other proteins are in the vicinity of the bait protein.[166] These nonspecific interactions may not be in direct contact with the bait protein. Downstream analysis of proteins or peptides can often be more difficult by the addition of chemical crosslinking reagents.[167] One such example of chemical crosslinking utilizes formaldehyde as the crosslinking reagent to investigate protein-protein interactions within yeast cells.[174] In this study Hall *et al.* wished to develop a way of investigating interactions within the cells using formaldehyde to crosslink transcriptional activator proteins chemically.[174] They were able to demonstrate the direct interaction of VP16 activation domain and TATA-binding protein, transcription factor II B, and SAGA histone acetylase complex.[174] Li *et al.* performed a comprehensive protein-protein interaction study of DmsD maturation protein in *E. coli* strains by utilizing affinity chromatography, co-precipitation, and crosslinking.[175] The study used the same crosslinking reagent as was used in this investigation, Sulfo-SBED label transfer reagent. They were able to identify six chaperones and molybdenum cofactor biosynthesis proteins through this method.[175] The use of crosslinking reagents aids in the stabilization of protein interactions by covalently linking partners together, allowing for the strong and weak interacting partners to be identified.

3.3.3 Pull-down

The pull-down assay usually utilizes a fusion tagged (i.e. GST, MBP, CBP, poly-His, or biotin) bait protein which demonstrates a high affinity for an enzyme and substrate, receptor and ligand, antibody and antigen.[166] The bait protein can often be overexpressed and purified using the resin compatible with the fusion tag. The drawback to pull-down assays is a significant amount of DNA manipulation is required to overexpress tagged bait protein.[167] Matsunami *et al.* utilized pull-down assays to identify interacting partners of FlgA-FlgI complex in *E. coli* Origami™ 2.[176] FlgI protein was overexpressed with an N-terminal poly-histidine tag to investigate the isolation of the complex using metal affinity chromatography.[176] Pull-down assays are not limited to poly-histidine tags, Wei *et al.* utilized a GST-tag to affinity purify interacting proteins in the ATP-binding cassette transport system in *Bifidobacterium longum* NCC2705.[177] There are four subunits in the system, FruE, FruK, FruF, and FruG. Using the GST-tag they were able to identify interactions between external-side membrane subunits FruF and FruG with FruE which shows a high correlation with the proposed model for bacterial ABC sugar transporters.[177] Pull-down assays can utilize a wide array of affinity tags to affinity purify bait and target proteins to identify interacting partners with high compatibility with downstream analysis methods.

3.3.4 Protein-protein Interactions of mARC1 and mARC2 Results

Utilizing the antibodies listed in **Table 2.1** a total of 176 proteins were identified in mARC1 and 83 proteins were identified in mARC2 control and in the co-immunoprecipitation experiments. **Figure 3.6A** show the proteins that were identified in control samples as well as in experimental conditions in mARC1 and mARC2 samples. In mARC1 samples, 85 proteins were identified as unique when compared to the controls conditions, and 23 proteins were identified as unique in mARC2 conditions.

Using the trifunctional crosslinking reagent (**Appendix I, Figure A.1A)** a total of 124 proteins were identified in mARC1 and 25 proteins identified in mARC2 control and in experimental conditions in the crosslinking experiments. **Figure 3.6B** show the proteins that were identified in control, and experimental conditions in mARC1 and mARC2 samples. In mARC1 samples 106 proteins were identified as unique when compared to the controls conditions and in mARC2 13 proteins were identified as unique.

A total of 181 proteins were identified in mARC1 and 112 proteins identified in mARC2 control and experimental conditions in the crosslinking experiments. **Figure 3.6C** show the proteins that were identified in control, and experimental conditions in mARC1 and mARC2 samples. In mARC1 samples 27 proteins were identified as unique when compared to the control conditions, and in mARC2 samples 18 proteins were identified as unique.

Pull-down experiments including mARC1 and mARC2 proteins had the most proteins identified from other protein-protein interaction methods. This may suggest an increase number of false positives interacting with the Co-NTA resin. The crosslinking experiments in both mARC1 and mARC2 yielded the lowest number of proteins identified, suggesting a reduced number of false positive interactions and potentially a

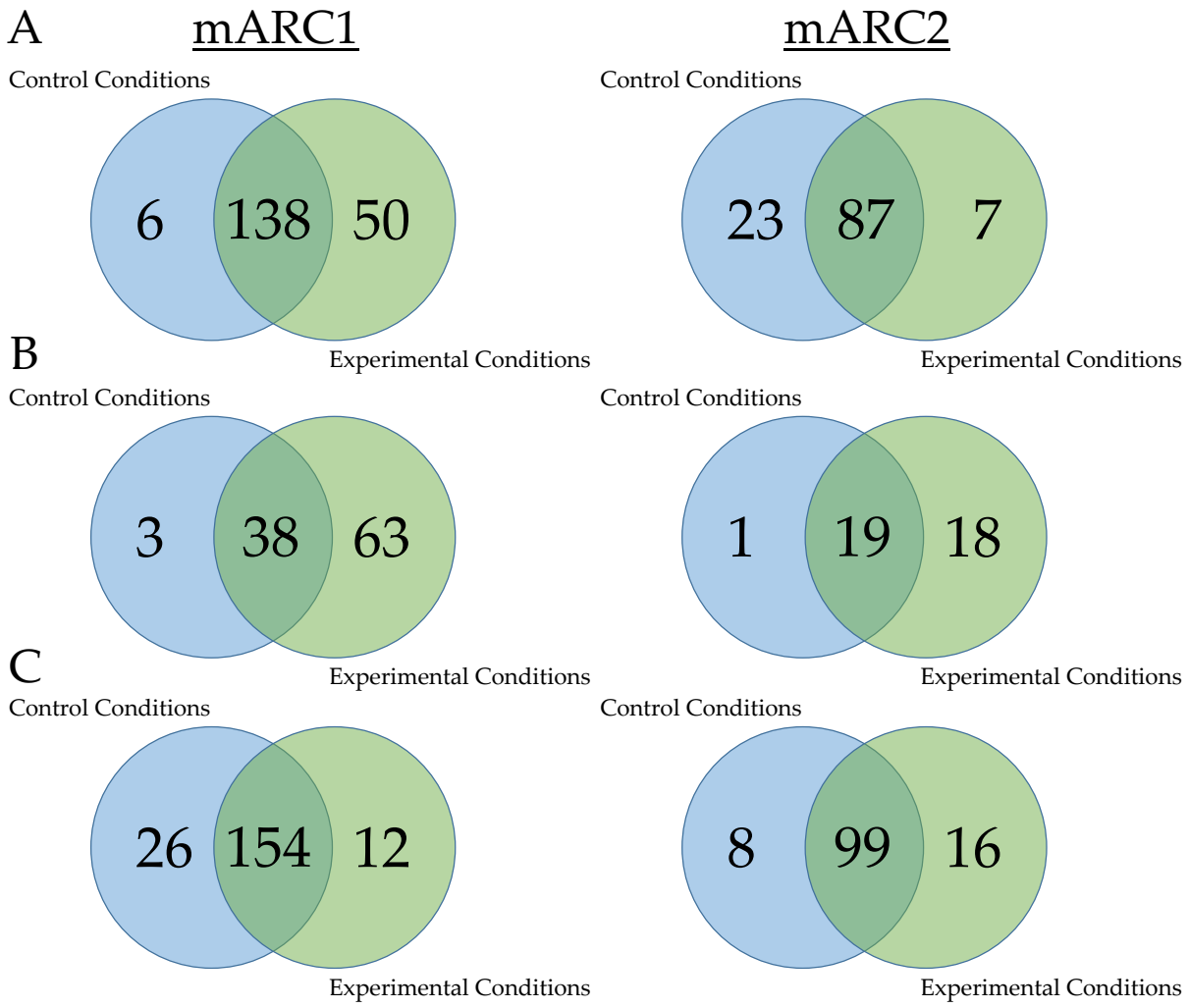

Figure 3.6: Venn diagrams depicting the number of proteins that were identified in control conditions compared to experimental conditions. A. Co-immunoprecipitation experiments, B. Crosslinking experiments, C. Pull-down experiments. The complete list of proteins identified for all experiments can be found in **Appendix II, Table A.1** for mARC1 samples and **Appendix II, Table A.2** for mARC2 samples.

more reliable method for analyzing protein-protein interactions using the method used for this investigation. The co-immunoprecipitation experiments yielded a number of proteins between the pull-down crosslinking experiments, but there was difficulty disguising interacting partners due to the lack of specificity of the mARC1 and mARC2 antibodies.

3.4 Bioinformatics Results and Discussion for Protein-protein Interactions

3.4.1 Reducing Falsely Identified Interacting Proteins with mARC Proteins

One of the issues with performing any protein-protein interaction studies is false positives identified from the mass spectrometric analysis. To minimize the number of false positives, the following criteria were set: BioConfirm score of ≥10, ≥2 MS/MS spectra per peptide, ≥2 unique peptides per protein, and absent in the controls (**Figure 3.7**). The number of total proteins meeting these criteria significantly reduced the number of

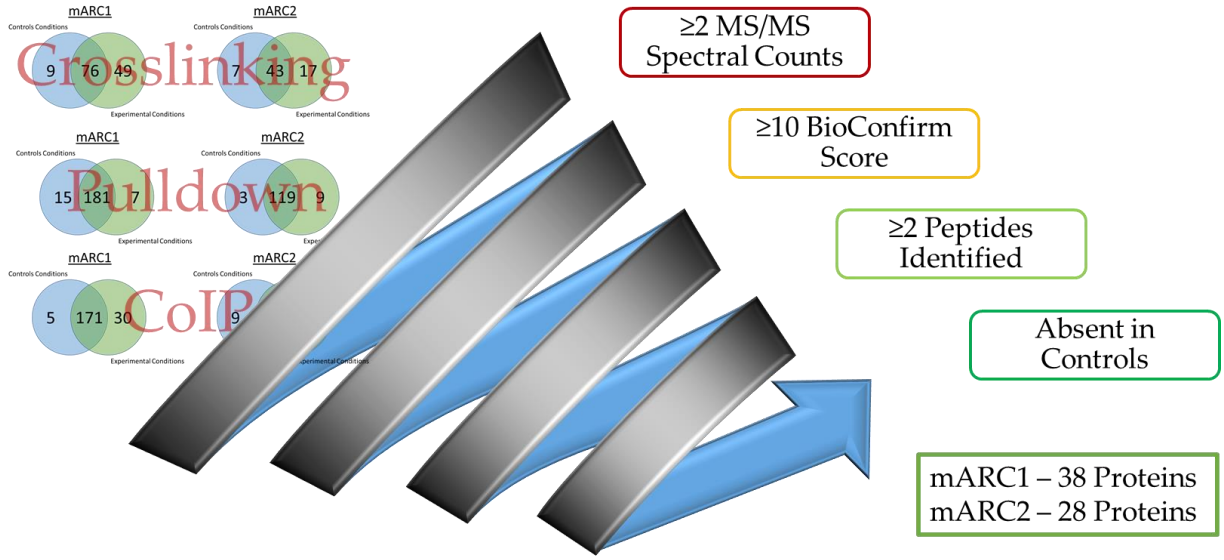

Figure 3.7: Workflow for reducing the potential false positive interactions in all three experimental conditions.

identified proteins. In mARC1 samples, 38 proteins were identified and 28 proteins identified for mARC2. **Figure 3.8** represents the number of proteins found in each of the different experimental conditions. The proteins identified in the crosslinking experiment seemed to demonstrate a higher similarity with proteins identified in both pull-down and co-immunoprecipitation. Upon further analysis of the co-immunoprecipitation experiments, it was determined that the mARC1 and mARC2 antibodies used were not specific and subsequently also purified both mARC proteins. Therefore, we included a single circle in the middle of the Venn diagram. In mARC1 samples, 12 proteins were found in all three experimental types and mARC2 6 proteins were found in all three types

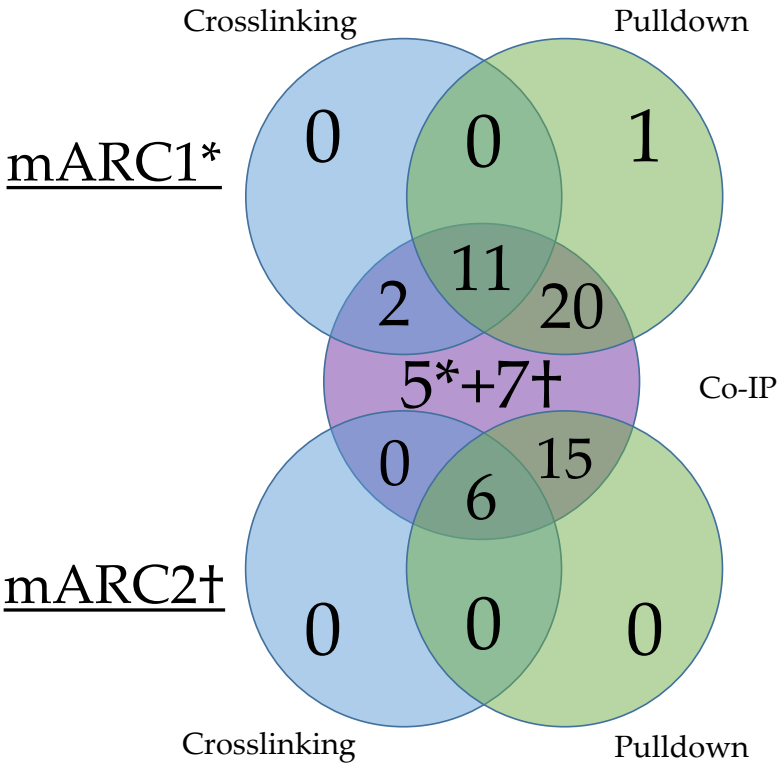

Figure 3.8: Venn diagram depicting the number of proteins identified comparing the experimental conditions. Proteins identified in co-immunoprecipitation for mARC1 (*) and mARC2 (†)

(**Table 3.4**). The proteins in **Table 3.5** were identified in two experimental conditions and **Table 3.6** were identified one experimental condition for mARC1 and mARC2 respectively.

Table 3.3: The proteins identified in all three experimental conditions for mARC1 and mARC2 proteins.

UniProt ID	Protein Name	Interacting mARC Protein
Q8N187	Calcium-responsive transcription factor	mARC1
Q8IYX3	Coiled-coil domain-containing protein 116	mARC1
Q7L0Q8	Rho-related GTP-binding protein RhoU	mARC1
P02768	Serum albumin	mARC1
P31040	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	mARC1
Q15431	Synaptonemal complex protein 1	mARC1
Q9BZE0	Zinc finger protein GLIS2	mARC1
Q5VT66	Mitochondrial amidoxime-reducing component 1	mARC1
R4GMW8	Protein BIVM-ERCC5 (Fragment)	mARC1
Q9P218	Collagen alpha(XX) chain	mARC1
P27816	Microtubule-associated protein 4	mARC1
Q04726	Transducin-like enhancer protein 3	mARC1
O75891	Cytosolic 10-formyltetrahydrofolate dehydrogenase	mARC2
O94927	HAUS augmin-like complex subunit 5	mARC2
O95696	Bromodomain-containing protein 1	mARC2
Q6UB35	Monofunctional C1-tetrahydrofolate synthase, mitochondrial	mARC2
Q86XP3	ATP-dependent RNA helicase DDX42	mARC2
Q969Z3	Mitochondrial amidoxime reducing component 2	mARC2

Table 3.4: Proteins identified in at least two experimental conditions interacting partners with mARC1 and mARC2 in HEK-293 cell lysate.

UniProt ID	Protein Name	Interacting mARC Protein; Technique
Q32M45	Anoctamin-4	mARC1; 1, 3
P06727	Apolipoprotein A-IV	mARC1; 1, 3
Q8IYE1	Coiled-coil domain-containing protein 13	mARC1; 1, 3
P51160	Cone cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha	mARC1; 1, 3
P78334	Gamma-aminobutyric acid receptor subunit epsilon	mARC1; 1, 3
P04908	Histone H2A type 1-B/E	mARC1; 1, 3
Q6UXK5	Leucine-rich repeat neuronal protein 1	mARC1; 1, 3
E5KNQ5	Mitochondrial thymidine kinase 2	mARC1; 1, 3
Q969R2	Oxysterol-binding protein 2	mARC1; 1, 3
Q8IUH5	Palmitoyltransferase ZDHHC17	mARC1; 1, 3
Q8NFW9	Rab effector MyRIP	mARC1; 1, 3
Q3MIN7	Ral guanine nucleotide dissociation stimulator-like 3	mARC1; 1, 3
Q6MZT1	Regulator of G-protein signaling 7-binding protein	mARC1; 1, 3
P21549	Serine--pyruvate aminotransferase	mARC1; 1, 3
Q549N5	Signal recognition particle receptor beta subunit	mARC1; 1, 3
Q8NCR6	Spermatid-specific manchette-related protein 1	mARC1; 1, 3
Q9BT88	Synaptotagmin1	mARC1; 1, 3
O95049	Tight junction protein ZO-3	mARC1; 1, 3
Q8IYS4	Uncharacterized protein C16orf71	mARC1; 1, 3
Q3SY52	Zinc finger protein interacting with ribonucleoprotein K	mARC1; 1, 3
Q14684	Ribosomal RNA processing protein 1 homolog B	mARC1; 1, 2
P46977	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase	mARC1; 1, 2
Q9NUT2	ATP-binding cassette sub-family B member 8, mitochondrial	mARC1; 1, 3
Q8TCU4	Alstrom syndrome protein 1	mARC1; 1, 3
Q6P2H3	Centrosomal protein of 85 kDa	mARC1; 1, 3
P62269	40S ribosomal protein S18	mARC2; 1, 3
P62701	40S ribosomal protein S4, X isoform	mARC2; 1, 3
P46782	40S ribosomal protein S5	mARC2; 1, 3
Q9C0H9	SRC kinase signaling inhibitor 1	mARC2; 1, 3
Q71U36	Tubulin alphaA chain	mARC2; 1, 3
Q9UKX5	Integrin alpha1	mARC2; 1, 3
Q6ZU35	Uncharacterized protein KIAA1211	mARC2; 1, 3
P54764	Ephrin type-A receptor 4	mARC2; 1, 3
P00748	Coagulation factor XII	mARC2; 1, 3
Q5T0W9	Protein FAM83B	mARC2; 1, 3
Q6PJ61	F-box only protein 46	mARC2; 1, 3
Q06787	Fragile X mental retardation protein 1	mARC2; 1, 3

Technique:
1. Co-immunoprecipitation
2. Crosslinking reagent
3. Pull-down assay

Table 3.5: Proteins identified in one experimental conditions interacting partners with mARC1 and mARC2 in HEK-293 cell lysate.

UniProt ID	Protein Name	Interacting mARC Protein; Technique
Q53EZ4	Centrosomal protein of 55 kDa	mARC1; 1
Q9BTM1	Histone H2A.J	mARC1; 1
A2A2F5	Protein TMEPAI	mARC1; 1
J3QRV1	Sodium/hydrogen exchanger 5	mARC1; 1
Q9H2M3	S-methylmethionine-homocysteine S-methyltransferase BHMT2	mARC1; 3
Q13496	Myotubularin	mARC2; 1
O76074	cGMP-specific 3',5'-cyclic phosphodiesterase	mARC2; 1
C9JH25	Proline-rich transmembrane protein 4	mARC2; 1
Q5W0Q7	SUMO-specific isopeptidase USPL1	mARC2; 1
Q8N7Q3	Zinc finger protein 676	mARC2; 1
O75128	Protein cordon-bleu	mARC2; 1
O75947	ATP synthase subunit d, mitochondrial	mARC2; 1

Technique:
1. Co-immunoprecipitation
2. Crosslinking reagent
3. Pull-down assay

Samples were cross-compared searching to see if the other mARC protein was present. To this end, co-immunoprecipitation experiments seemed to have both mARC1 and mARC2 present in the analysis, this data was represented in the center circle of the Venn diagram (**Figure 3.8**) as it could not be distinguished if the proteins identified were potentially interacting with mARC1 or mARC2. The chemical crosslinking reagent appeared to have the greatest overlap in protein identified when compared to the co-immunoprecipitation and pull-down assay. There were no proteins identified in mARC1 and mARC2 samples that were uniquely identified using the LTR-K-BLoC complex, suggesting that this method may have a lower false discovery rate.

To be able to propose a physiological function of mARC proteins, relationships of the interacting proteins based on their functions and involvement need to be determined.

To aid in the analysis two bioinformatics software will be used to help form relationships of proteins.

3.4.2 Bioinformatics Platforms used to Form Relationships for Interacting Protein

To aid in the analysis of the proteins identified two different bioinformatics platforms were used to develop connections of identified proteins (DAVID, **D**atabase for **A**nnotation, **V**isualization, and **I**ntegrated **D**iscovery), and to help reduce contaminating background proteins identified (CRAPome, **C**ontaminant **R**epository for **A**ffinity **P**urification). DAVID was used by uploading the UniProt Accession numbers and proteins were clustered based on similar annotations. CRAPome utilized the proteins identified and the spectral counts for each protein within a sample. This software platform uses experimental conditions and control conditions and applies a SAINT probability score for each protein identified.

3.4.3 DAVID Bioinformatics Results using the Reduced Protein List

DAVID bioinformatics software was used to help compare the identified proteins by comparing annotations found in several databases. To compare the proteins, GOTerm function was used from The European Bioinformatics Institute (http://www.ebi.ac.uk/). The initial analysis using DAVID were grouped into functional annotation clustering using the parameters similarity term overlap of 3 with a threshold of 0.50, classified with at least 3 proteins per group and multiple linkage thresholds of 0.50. The analysis of proteins interacting with mARC1 showed 12 clusters with 70 biological process and

molecular function annotations present. The identified proteins observed in mARC2 samples had 9 clusters with 76 biological process and molecular function annotations. The full GOTerm trees with the cluster number, number of proteins annotated, and similar annotation for proteins identified in mARC1 and mARC2 conditions can be found in **Appendix III, Figure A.2 to A.13**. There is evidence that mARC proteins are found outside of the mitochondria of cells, but our interest is potential physiological roles of mARC proteins. To aid in the narrowing the scope to viable candidates, annotations were required to be part of metabolic, and/or cellular function not including cell cycle type functions to reduce the complexity and highlight features of interest. A truncated table with a few interesting candidate annotations can be found in **Table 3.7**; the complete table can be found in **Appendix III, Table A.3, and A.4**. A complete table of the identified clusters of interacting proteins with mARC1 and mARC2 with their associated GOTerm annotations can be found in **Appendix III, Table A.8, and A.7**. The annotations of interest that were found in both mARC1 and mARC2 interacting proteins were several regulatory process proteins both cellular and metabolic, and metabolism of various nitrogen-containing compounds (amine-containing, amino acid, and nucleic acid). The current research for both mARC proteins has demonstrated their abilities to reduce several amidoxime compounds, mutated base-pairs, and nitrite; which would suggest why proteins annotated in nitrogen-containing metabolic processes are being identified as well as nucleobase-containing compound metabolic process.[24, 26, 104, 109] More

interestingly, however, is the number of proteins with regulatory annotations. With a list of proteins that were identified under stringent conditions, using more than one method of probing protein-protein interactions, and a potential relationship of proteins based on clusters of GOTerm annotations the list of proteins was expanded to include all proteins that were identified in the experimental conditions and not in the controls.

Table 3.6: Select GOTerm annotations for proteins interacting with mARC1 and mARC2. The number of interacting proteins identified for mARC1 and mARC2 are listed under the annotation tag with a short description that was found from http://www.ebi.ac.uk/

Annotated GOTerm (# of Proteins)	**Description of Term**
Cellular protein modification process (mARC1 : 0) (mARC2 : 3)	The covalent alteration of one or more amino acids occurring in proteins, peptides and nascent polypeptides (co-translational, post-translational modifications) occurring at the level of an individual cell. Includes the modification of charged tRNAs that are destined to occur in a protein (pre-translation modification).
Nitrogen compound metabolic process (mARC1 : 8) (mARC2 : 5)	The chemical reactions and pathways involving organic or inorganic compounds that contain nitrogen, including (but not limited to) nitrogen fixation, nitrification, denitrification, assimilatory/dissimilatory nitrate reduction and the interconversion of nitrogenous organic matter and ammonium.
Nucleobase-containing compound metabolic process (mARC1 : 4) (mARC2 : 3)	Any cellular metabolic process involving nucleobases, nucleosides, nucleotides and nucleic acids.
Regulation of biological process (mARC1 : 12) (mARC2 : 8)	Any process that modulates the frequency, rate or extent of a biological process. Biological processes are regulated by many means; examples include the control of gene expression, protein modification or interaction with a protein or substrate molecule.
Signal transduction (mARC1 : 0) (mARC2 : 3)	The cellular process in which a signal is conveyed to trigger a change in the activity or state of a cell. Signal transduction begins with reception of a signal (e.g. a ligand binding to a receptor or receptor activation by a stimulus such as light), or for signal transduction in the absence of ligand, signal-withdrawal or the activity of a constitutively active receptor. Signal transduction ends with regulation of a downstream cellular process, e.g. regulation of transcription or regulation of a metabolic process. Signal transduction covers signaling from receptors located on the surface of the cell and signaling via molecules located within the cell. For signaling between cells, signal transduction is restricted to events at and within the receiving cell.

3.4.4 CRAPome Bioinformatics and KEGG Pathway Results for Proteins

By expanding the list to include all proteins not identified in the controls, there is an increased likelihood of identifying false positive results. To help strengthen the results a unique bioinformatics software was used. CRAPome utilizes all proteins identified in a study (both control and experimental) and compares the spectral count of those proteins to generate a SAINT score which is the likelihood of that protein being a positive interactor or a background contaminant.[178] The spectral counts for each protein were consolidated from the spectral count of each identified peptide in the replicates. By expanding the search from the reduced list to the full list, we anticipate expanding the list of proteins involved in pathways of interest to aid in the proposition of mARC physiological function. **Figure 3.9** shows the number of proteins compared to their SAINT scores for all the proteins in the study (orange) and the proteins identified in the reduced protein list (blue) for mARC1 (**Figure 3.9A**) and mARC2 (**Figure 3.9B**). The majority of the total proteins had a SAINT score between 0 and 20% meaning the likelihood of those proteins interacting with mARC proteins is reduced, and they may be background or contaminant. In the reduced protein list the majority of the proteins had a SAINT score between 20 and 40% allowing for greater confidence in these interacting partners with mARC proteins. As the SAINT score increases the confidence of the interacting protein increases. To aid in the analysis of the total proteins, KEGG annotated pathways were investigated to see if any relationships could be generated.

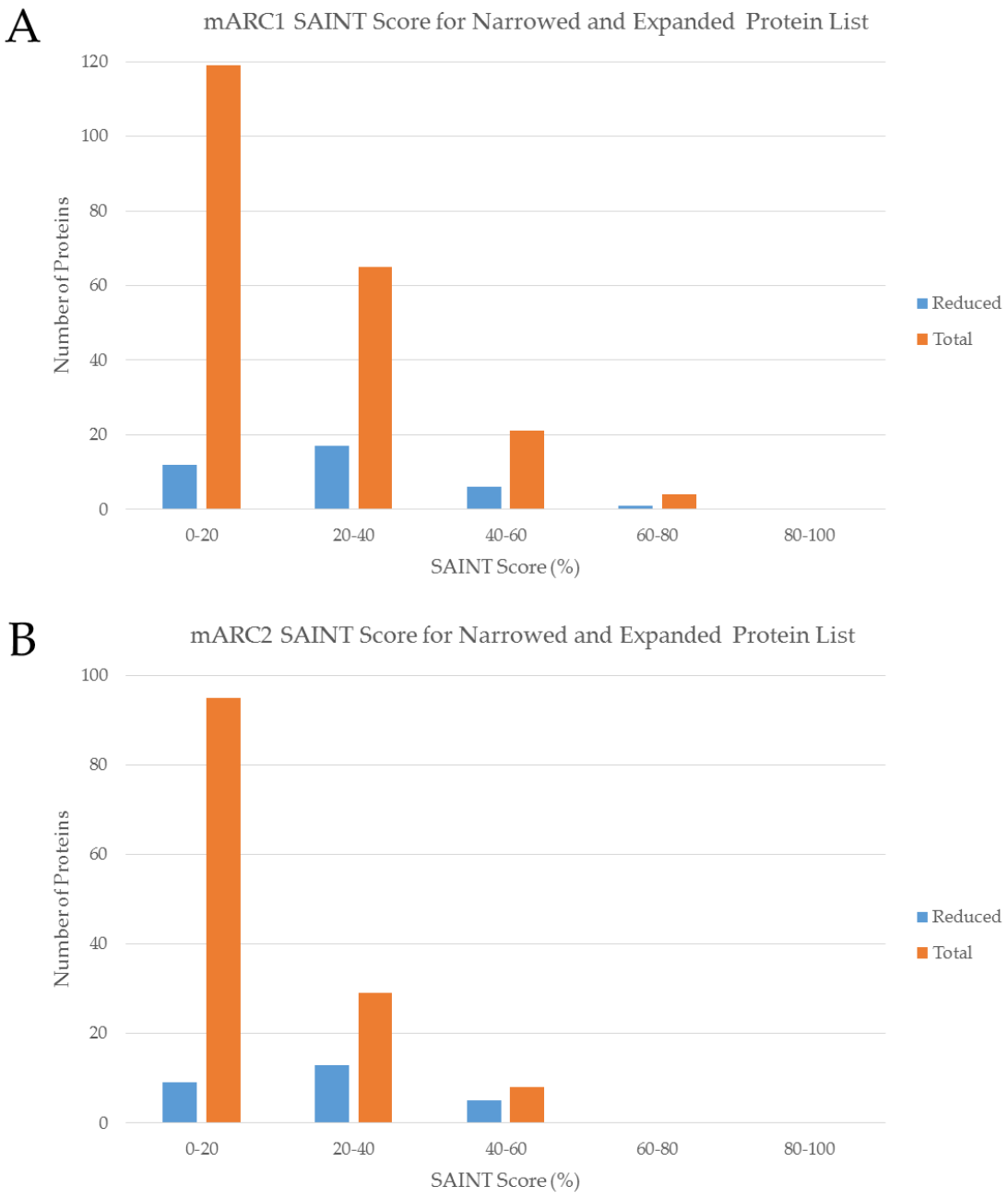

Figure 3.9: CRAPome SAINT score comparison between the proteins identified in the reduced list and the total proteins identified in the study. Blue bars represent the number of proteins identified and SAINT scores with the reduced protein list and the orange bars represent all the proteins identified in the study. A. SAINT score comparison of identified proteins in mARC1 experiments, B. SAINT score comparison of identified proteins in mARC2 experiments

The interacting proteins were compared using KEGG pathway annotations to elucidate relationships of proteins further. The full list of proteins used in the CRAPome analysis was used to compare pathways to attempt to capture a more complete overview of interacting partners. To summarize the KEGG annotated proteins, two plots were generated with this information for each mARC protein (**Figure 3.10**, and **Figure 3.11**).

When examining the pathway categories for mARC1 (**Figure 3.10A**), proteins annotated in human disease and metabolism have the most identified. The number of proteins identified in the category metabolism appears to have many proteins identified, but when it is divided into the pathways, the proteins are annotated with "Global and overview maps" which is a general pathway annotation. The pathways for human disease and organism system categories can be found in **Appendix III, Figure A.14A**. In **Figure 3.10B** the categories are divided into their pathways and the number of proteins identified affiliated with each pathway. A complete list of proteins with their annotated KEGG pathways can be found in **Appendix III, A.8, and A.9**. Interestingly enough, the most proteins were identified as part of signal transduction pathways. The signal transduction pathways can be further divided into specific pathways of involvement. Investigating these pathways may be important in understanding the physiological role of mARC1 within the cell. The proteins that were present in the signal transduction pathways can be found in **Table 3.8** with their specific pathway(s) of involvement. There

is not a significant number of proteins identified for one pathway, with MAPK signaling pathway having the most, but several of the pathways can be connected to one another

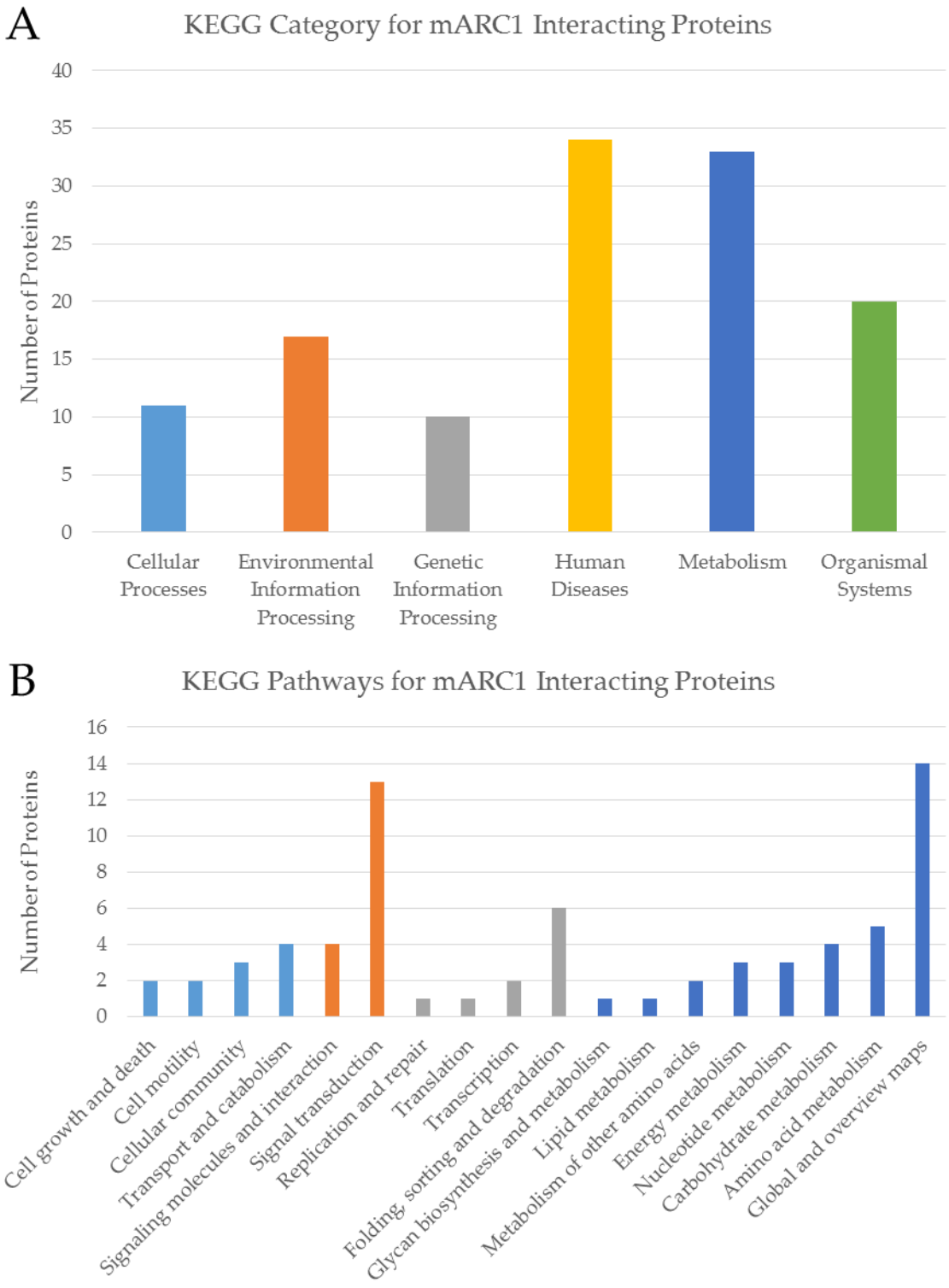

Figure 3.10: KEGG categories and pathways for proteins interacting with mARC1. A. KEGG categories for interacting proteins with mARC1, B. KEGG pathways for interacting proteins with mARC1. Human disease, and organismal systems pathways can be found in **Appendix III, A.14A**. Pathways in plot B are color coordinated with the category they are related to.

Nitric oxide is an important second messenger molecule in several regulatory processes. In fact, it has been reported that some cytokines including tumor necrosis factors and interleukins are regulated by nitric oxide.[179, 180] Cytokines are important signaling proteins which aid in immune response. The protein identified as an interacting partner with mARC1 is tumor necrosis factor superfamily member 11 (O14788) which may be involved in the modulation of intracellular concentration of calcium. More interesting is that tumor necrosis factor-alpha has been shown to greatly impact the nitric oxide synthase mRNA levels inside the endothelium cell.[181] In fact, it was reported that the half-life of nitric oxide synthase mRNA was reduced from 48 hours to 3 hours.[181] With the declining stability of nitric oxide synthase mRNA, it is possible that mARC1 may aid in the control of cytokines and promote homeostasis within the cell through the production of nitric oxide from nitrite.

One of the predicted interactions with mARC1 is ubiquitin C which labels proteins for degradation, DNA repair, cell cycle regulation, kinase modification, endocytosis, and regulation of other cell signaling pathways. Two different E3 ubiquitin protein ligases were identified as interacting with mARC1, mitogen-activated protein kinase 1, E3 ubiquitin protein ligase (Q13233) and E3 ubiquitin-protein ligase parkin (O60260). Although there is no exact reason for the ubiquitination, we suggest it may be involved in regulating mARC1 involvement in cell signaling.

Mammalian signaling pathways aid the cellular response to external changes in the cell to elicit a response inside the cell. Some signal responses are activated by receptors or secondary messenger molecules resulting in several changes within the cell. It is of interest that mARC1 was identified as interacting with several proteins in signaling pathways because one of the predicted functions of mARC proteins is the reduction of nitrite to nitric oxide under hypoxic conditions. Nitric oxide is known as a very potent signaling molecule within cells. In the calcium signaling pathway, an efflux of calcium into the cell binds calmodulin protein and subsequently activating the enzyme. Once calmodulin is activated it targets proteins downstream within the NOS proteins, MAPK and phosphatidylinositol signaling pathways, as well as other pathways. Interestingly, the protein that was identified as interacting with mARC1, guanine nucleotide binding protein (P30679), is upstream of calmodulin. Through a series of activations promoted by

Table 3.7: Signal transduction proteins and pathways identified as interacting proteins with mARC1.

Signal Transduction Pathway	Proteins Names (UniProt ID)
Calcium signaling	Guanine nucleotide binding protein (P30679)
Hippo signaling	Par-6 family cell polarity regulator (Q9BYG4)
MAPK signaling	Mitogen-activated protein kinase 5 (Q99683) Mitogen-activated protein kinase 6 (O95382) Mitogen-activated protein kinase 1, ubiquitin protein ligase (Q13233)
NF-kappa B signaling	Tumor necrosis factor 11 (O14788)
Phosphatidylinositol signaling	Phosphatidylinositol-4-phosphate 3-kinase (O00750)
PI3K-Akt signaling	Integrin 6 (P18564)
Rap1 signaling	Par-6 family cell polarity regulator (Q9BYG4)
Sphingolipid signaling	Mitogen-activated protein kinase 5 (Q99683)
TGF-beta signaling	Retinoblastoma-like 1 (P28749)
TNF signaling	Mitogen-activated protein kinase 5 (Q99683)
Wnt signaling	LDL receptor related protein 6 (O75581)

guanine nucleotide binding protein, calcium is released from the endoplasmic reticulum. The release of calcium then activates calmodulin, promoting downstream processes. The MAPK signaling pathway is a highly conserved cascade that is capable of controlling several cellular processes. Three different mitogen-activated kinases (Mitogen-activated protein kinase 1 (Q13233), Mitogen-activated protein kinase 5 (Q99683), Mitogen-activated protein kinase 6 (O95382)) were identified as interacting with mARC1. It has been reported that the phosphorylation of eNOS occurs through interactions with MAPK proteins.[182] Bernier *et al.* were able to isolate a complex containing eNOS, ERK1/2, Raf1, and Akt through co-immunoprecipitation; through a Brandykinin treatment, they were able to determine that eNOS is phosphorylated by extracellular signal-regulated kinase (ERK) proteins.[182] The MAPK signaling pathway can be divided into three cascades, ERK1/2 module, JNK/p38 module, and ERK5 module, each affecting different downstream processes. The proteins that were identified as interacting with mARC1 are affiliated with the JNK/p38 module of the MAPK signaling cascade. Suggesting that mARC1 may be phosphorylated for activation or deactivation within the cell. The MAPK signaling pathway has involvement in the phosphorylation of proteins in the Wnt, PI3K-Akt, and the sphingolipid signaling pathways. Nitric oxide has been determined to promote the regulation of the Wnt signaling pathway by inhibiting Dickkopf-1 gene expression.[183] The Dickkopf family of proteins negatively inhibits the Wnt pathways by binding the same receptor that is used to stimulate the pathway.[184] The PI3K-Akt

and the sphingolipid signaling pathways promote the production of nitric oxide through the phosphorylation of NOS proteins. It is unclear why mARC1 would interact with integrin 6 (P18564), which is a β-subunit in the receptor complex; but Murohara *et al.* report that nitric oxide aids in maintaining the functional expression of integrin $\alpha v\beta 3$ in endothelial cells.[185] The potential physiological involvement of mARC1 appears to be extremely complex, but we propose it is involved in the regulation of signal transduction pathways through nitric oxide production.

Comparing the annotated functions of mARC1 and mARC2 it is clear that fewer proteins are annotated in mARC2 as affiliated with different metabolic functions. Examining this information more closely, it can be noted that there are no proteins annotated as "Global and overview maps" identified as interacting with mARC2 (**Figure 3.11B**). The general categories for mARC2 proteins (Figure 3.6A) exhibit a similar relationship to mARC1 about "Environmental Information Processing," and it is under this category that the signaling pathways can be identified. The pathways for human disease and organism system categories can be found in **Appendix III, Figure A.14B**. A new third pathway list in **Figure 3.11B** was identified in proteins interacting with mARC2 ("Membrane transport") when compared to mARC1 pathways.

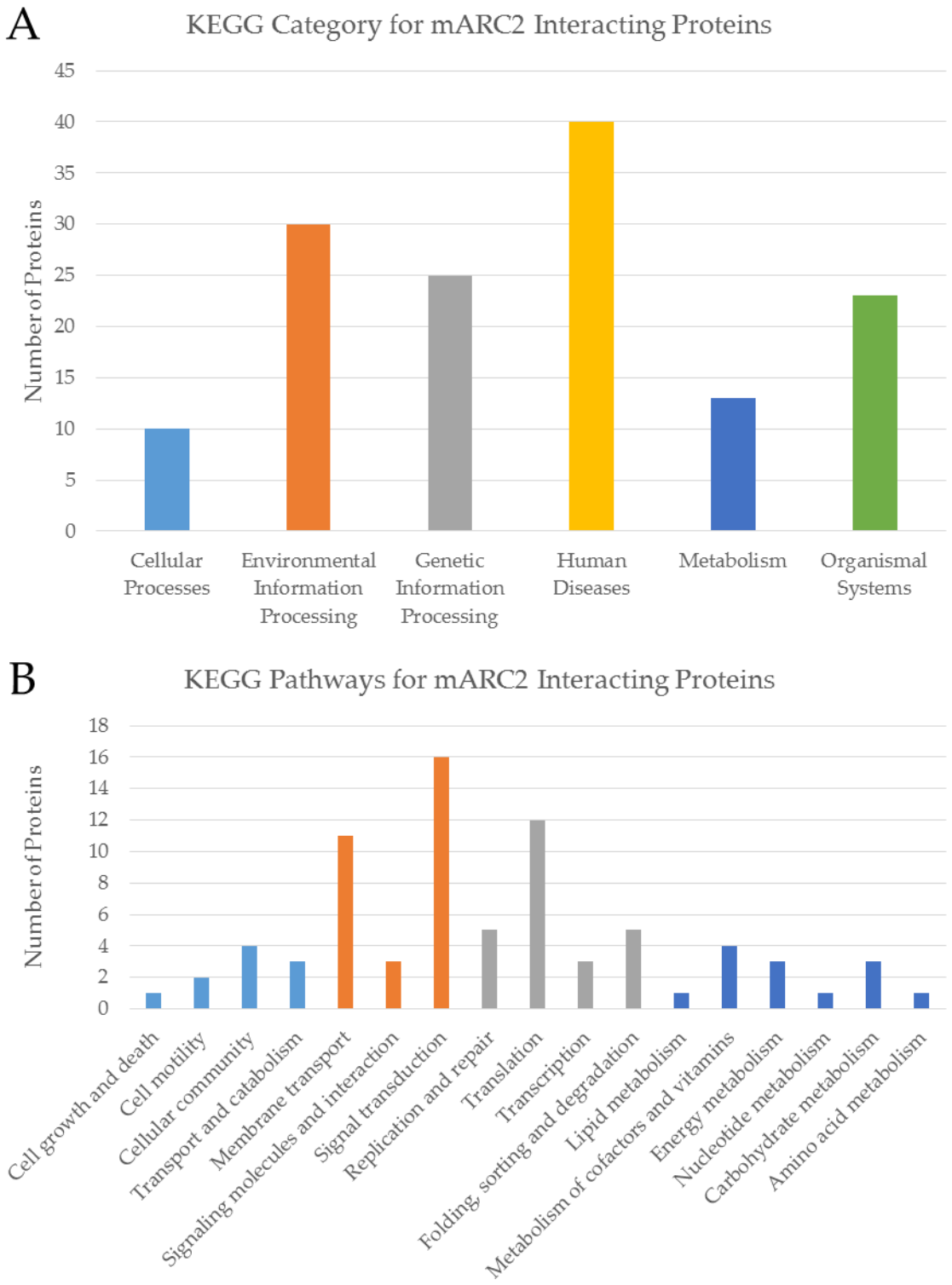

Figure 3.11: KEGG categories and pathways for proteins interacting with mARC2. A. KEGG categories for interacting proteins with mARC1, B. KEGG pathways for interacting proteins with mARC2. Human disease, and organismal systems pathways can be found in **Appendix III, A.14B**. Pathways in plot B are color coordinated with the category they are related to.

The annotated "Membrane transport" proteins are of interest to investigating the potential physiological role of mARC2 because they can be related to what is currently know about the enzyme. A complete list of annotated "Membrane transport" proteins can be found in **Appendix III, Table A.3**. Three different ATP synthase subunits (P36542, P48047, and O75947) were identified as being mitochondrial, similar to the known location of the mARC2 protein. ATP synthase is a protein complex that utilizes a proton gradient to produce ATP from ADP and inorganic phosphate. One cell process, in particular, requires ATP to drive the uptake of calcium into the cell and results in muscle contractions. Previously reported an increase in nitric oxide levels caused an inhibition of ATP synthase in rat muscle cells.[186] Similar to mARC1, mARC2 is capable of producing nitric oxide from nitrite.[26] Suggesting that mARC2 may aid in the relaxation of muscle cell by generating nitric oxide to inhibit ATP synthase and as a result decreasing the calcium uptake allowed through the energy-dependent calcium transport proteins.

A sequence analysis of mARC2 protein demonstrates the presence of a molybdenum cofactor sulfurase (MOSC) domain, which is a highly conserved region in other molybdenum containing enzymes (AO, and xanthine dehydrogenase). The three molybdenum cofactor maturation proteins, MOCS1, MOCS2, and GPHN, were also identified as interacting partners with mARC2. This would suggest that a complex between the four proteins may take place in the maturation of mARC2.

Similar to the mARC1 proteins, interacting proteins with mARC2 were identified as being affiliated with several signaling pathways. The pathways and proteins for interacting proteins with mARC2 can be found in **Table 3.9**. Comparable to the pathways identified in mARC1 interacting proteins, the calcium, MAPK, phosphatidylinositol,

Table 3.9: Signal transduction proteins and pathways identified as interacting proteins with mARC2.

Signal Transduction Pathway	Proteins Names (UniProt ID)
Calcium signaling	Solute carrier family 25 (P05141) Calcium channel, voltage-dependent (Q13698)
cAMP signaling	Glutamate receptor, AMPA 2 (P42262) Calcium channel, voltage-dependent (Q13698)
cGMP-PKG signaling	Phosphodiesterase 5A (O76074) Solute carrier family 25 (P05141) Calcium channel, voltage-dependent (Q13698)
MAPK signaling	Platelet derived growth factor (P01127) Calcium channel, voltage-dependent (Q13698)
Phosphatidylinositol signaling	Myotubularin 1 (Q13496)
Phospholipase D signaling	Platelet derived growth factor (P01127)
PI3K-Akt signaling	Integrin subunit alpha 11 (Q9UKX5) Platelet derived growth factor (P01127)
Rap1 signaling	Platelet derived growth factor (P01127)
Ras signaling	Platelet derived growth factor (P01127)
Wnt signaling	LDL receptor related protein 6 (O75581)

PI3K-Akt, Rap1 and Wnt signaling pathways seem to be present. Interestingly the cGMP-PKG pathway is also present, which has involvement in nitric oxide and natriuretic peptides regulation. Nitric oxide binds to soluble guanylate cyclase catalyzing the conversion of cGTP to cGMP.[75] The resulting cGMP molecule activates protein kinase G, subsequently influencing several downstream pathways including the reduction of calcium within the cell causing muscle relaxation. The increase of cGMP within the cell prevents the hydrolysis of cAMP by cGMP-inhibited phosphodiesterases.[187-189] The cAMP signaling pathway also regulates downstream processes like metabolism, calcium

homeostasis, and muscle contraction through the direct activation of protein kinase A, Rap guanine nucleotide exchange factor, and cyclic nucleotide-gated ion channels. Protein kinase A regulates ion channels, transporters, intracellular Ca^{2+}-handling proteins, and the contractile machinery, to name a few, through phosphorylation. Guanine nucleotide exchange factor, on the other hand, modulates phospholipases and Rap signaling downstream. Phospholipase D catalyzes the hydrolysis of phosphatidylcholine to phosphatidic acid, which is an invaluable second messenger in the activation of MAPK signaling pathway. In fact, nitric oxide has been reported as stimulating the activation of phospholipase D and subsequently activating the MAPK signaling cascade.[190-194] Nitric oxide is a potent second messenger that has an immense impact on several pathways throughout the cells and the data presented suggests that mARC2 may be involved as a proximity pathway activator through the production of nitric oxide.

3.5 Protein-protein Interactions Summary for mARC1 and mARC2

Three methods of investigating protein-protein interactions were used to understand the potential physiological role of mARC1 and mARC2 in mammalian cells. The co-immunoprecipitation experiments were carried out using commercially available antibodies and were able to identify 194 potential interacting proteins with mARC1 and 117 with mARC2. The crosslinking reagent experiments utilized a unique method involving a substrate (K-BLoC) specifically designed to bind mARC proteins and "lock"

into the binding pocket bound to a commercially available non-specific crosslinking reagent. There was a total of 104 potential interacting proteins with mARC1 and 38 proteins with mARC2. The final experiment, pull-down assay, utilized recombinant poly-his tagged mARC1 and mARC2. There was a total of 192 potential interacting proteins with mARC1 and 123 proteins with mARC2. To fortify the data collected from the three methods, thresholds were applied to reduce the protein list and increase the validity. The reduced protein list resulted in 38 proteins identified as interacting with mARC1 and 28 proteins with mARC2. There were 11 proteins that were identified in all three experiments for mARC1 and 6 proteins for mARC2. The crosslinking reagent experiments appeared to have the least number of potential false positive identified proteins, as there were no unique proteins identified in the experiment.

The bioinformatics software DAVID and CRAPome were used to analysis and group proteins together based on their GOTerm and KEGG pathway annotations. The proteins identified as interacting with mARC1 and mARC2 proteins were clustered into GOTerm biological and molecular function processes. There were 12 clusters of proteins with 70 biological and molecular function processes for interacting proteins with mARC1 and 9 clusters with 76 biological and molecular function processes with mARC2. There were 3 processes identified in proteins interacting with mARC1 and mARC2 that are of potential importance based on what is currently known about mARC proteins and 2 processes identified only in mARC2 interacting proteins of interest. Nitrogen compound

metabolic, regulation of biological, and nucleobase-containing compound metabolic process were three processes had multiple identified interacting proteins with mARC1 and mARC2. The mARC proteins have been reported previously to reduce nitrogen containing compounds, namely nitrite and compounds containing amidoxime components as well as reducing mutated nucleobases. Interestingly, the regulation of biological process and the other 2 processes identified in mARC2 interacting proteins, signal transduction, and cellular proteins modification processes, may be related to mARC proteins capabilities to produce nitric oxide from nitrite.

The bioinformatics software CRAPome was used to statistically compare the proteins identified in the control and experimental conditions and scored for the likelihood of positive identification. The CRAPome data was then analyzed and compared using KEGG pathway annotations. It was found that when the interacting proteins were grouped into their subcategories that the majority of the proteins were grouped into signal transduction pathways. Upon further investigation of the interacting proteins, it was found that mARC1 interacted with a tumor necrosis factor type protein (O14788) and 2 ubiquitin-protein ligase (Q13233, O60260). We were unable to confirm a connection between the ubiquitination of and the potential role of signaling cascades, but we propose that mARC1 may it is possible that mARC1 may aid in the control of cytokines and promote homeostasis within the cell through the production of nitric oxide from nitrite. The effects of nitric oxide in several of the signaling pathways including

calcium, Wnt, PI3K-Akt, and the sphingolipid. There is a chance that mARC1 may be phosphorylated through the JNK/p38 module of the MAPK signaling cascade, and subsequently affecting its reactivity or activation. We propose that the potential physiological involvement of mARC1 appears to be involved in the regulation of signal transduction pathways through nitric oxide production and a potential safety net for cells if nitric oxide synthase proteins become functionally impaired. A very similar trend was able to be followed with mARC2 protein. The signal transduction subcategory had the majority of the proteins annotated when compared to the other categories. In addition, there was a great deal of membrane transport proteins that were identified as interacting with mARC2. Three subunits, in particular, that are part of the ATP synthase protein (P36542, P48047, and O75947) may demonstrate the role of mARC2 in inhibiting the calcium reuptake by nitric oxide and subsequently allowing muscle relaxation. The three molybdenum cofactor maturation proteins, MOCS1, MOCS2, and GPHN, were also identified as interacting partners with mARC2. Suggesting that a complex between the four proteins may take place in the maturation of mARC2. The signaling pathways identified in mARC2 are comparable to the ones identified in mARC1, except for the cAMP, and cGMP-PKG signaling pathways that were found in mARC2. Nitric oxide aids in the catalysis of cGTP to cGMP in the cGMP-PKG signaling pathways. The generation of cGMP has been found to inhibit the hydrolysis of cAMP, promoting that cAMP pathway which affects muscle contraction through calcium transport regulation. Nitric

oxide is a potent second messenger that has an immense impact on several pathways throughout the cells and the data presented suggest that mARC2 may be involved as a proximity pathway activator through the production of nitric oxide.

3.6 Reverse Co-immunoprecipitation of mARC Proteins in HEK-293 Cells

Using the data derived from DAVID, and CRAPome analysis two proteins for mARC1 and mARC2 were selected for reverse co-immunoprecipitation to support evidence of the interactions. Reverse co-immunoprecipitation uses the same principles as co-immunoprecipitation, except the intended target. The prey protein from the original co-immunoprecipitation becomes the target of the antibody with the goal of precipitating the proteins of interest. The objective of this reverse co-immunoprecipitation is to select interacting proteins with mARC1 and mARC2, and using their antibodies to immunoprecipitate the interacting protein with mARC proteins. The proteins selected were of interest to signaling pathways and were identified in multiple experiments. This was used as a confirmatory method to see if the mARC proteins interacted with the proteins selected.

Reverse co-immunoprecipitation has been used previously by researchers to help support their data from co-immunoprecipitation experiments. Denuc *et al.* used this method to support evidence of connexin 43, a gap junction related protein found in cardiomyocyte mitochondria, interacting with two new proteins that were identified through co-immunoprecipitation.[195] A total of 80 candidate proteins were identified in

three co-immunoprecipitation experiments using connexin 43 antibody.[195] Of those proteins identified two proteins were selected for reverse co-immunoprecipitation experiments to support evidence of protein-protein interactions, apoptosis-inducing factor, and electron-transfer flavoprotein subunit beta.[195] They were able to determine that connexin 43 preferentially interacts with apoptosis-inducing factor and electron-transfer flavoprotein subunit beta.[195] Using a similar strategy, Vandermoere *et al.* used co-immunoprecipitation of serine/threonine kinase Akt and identified ten interacting partners.[196] They were able to identify a 14-fold increase in the interaction of activated-Akt with cytoskeleton protein actin when compared to the non-activated form.[196] They confirmed the interaction of Akt and cytoskeleton protein actin using reverse co-immunoprecipitation and confocal microscopy, in which they used the cytoskeleton protein actin antibody to isolate Akt.[196] Reverse co-immunoprecipitation helps to support evidence of protein-protein interactions when coupled with previous methods (i.e., co-immunoprecipitation, crosslinking reagents, and pull-down assays).

The reverse co-immunoprecipitation followed the same method as co-immunoprecipitation except different antibodies were used (**Table 2.1**), and no purified mARC1 and mARC2 protein were added to the sample, meaning all analysis was carried out under native protein concentrations. The control antibody for the reverse co-immunoprecipitation experiments was an antibody for Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). GAPDH was not identified in any of the mARC experiments

and the commercially available antibodies are specific. During the LC-MS/MS analysis, a preferential auto MS/MS method was established in which specific mARC1 and mARC2 peptides that were previously identified consistently were scanned for and fragmented using the parameters present in **Table 3.10 and Table 3.11**. Utilizing the preferred function in auto MS/MS allows for the selected precursor ions to search for, while also performing MS/MS on other peptides.

Table 3.9: Selected ions for preferential MS/MS for analysis of mARC1 protein.

Sequence (Amino Acid Location)	Precursor m/z	Z	Retention Time (min)
DLLLPIK (133-139)	811.52	1	17
GVPVSEAECTAMGLR (78-85)	788.87	2	15.3
KEPLETLK (288-295)	479.46	2	5.7
KEPLETLKSYR (288-298)	682.38	2	9.6
RPHQIADLFRPK (191-202)	493.28	3	11.1
VGDPVYLLGQ (328-337)	530.78	2	18.3
VHGLEIEGR (151-159)	505.46	2	7.2

Table 3.10: Selected ions for preferential MS/MS for analysis of mARC2 protein

Sequence (Amino Acid Location)	Precursor m/z	Z	Retention Time (min)
DRFWLVIK (90-97)	538.81	2	18.5
GVPVSEAECTAMGLRSGNLR (70-89)	788.87	2	15.3
IFGLDIK (150-156)	805.48	1	18.2
IFGLDIKGR (150-158)	509.8	2	15
IGSLRVGDPVYR (322-333)	444.58	3	11.6
KQPLDTLK (287-294)	471.96	2	5.1
LCDPSERELYK (298-308)	705.33	2	8.3
LSPLFGIYYSVEK (309-321)	758.4	2	23.4
LVQFETNMK (179-187)	555.28	2	11.1
LVQFETNMKGR (179-189)	661.84	2	9.5
LWIYPVK (60-66)	459.95	2	16.3
LWIYPVKSCK (60-69)	647.35	2	13.6
SGNLRDRFWLVIK (85-97)	802.87	2	19.6
VGDPVYR (327-333)	403.21	2	4.7

Agilent MassHunter Qualitative Analysis with BioConfirm software was used to identify peptides in the spectrum data for each condition. In the control condition using GAPDH antibody, no mARC1 or mARC2 peptides were identified, establishing it as a viable negative control. For mARC1, mitogen-activated protein kinase 5 (Q99683) and phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta (O00750) were the target bait proteins. **Table 3.12** is the number of unique mARC1 peptides and the number of spectral counts for peptides using reverse co-immunoprecipitation in

HEK-293 cells. In both replicates, mARC1 peptides were identified supporting the evidence that the interactions of mARC1 with mitogen-activated protein kinase 5 and phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta may exist. It should be noted that in the LC-MS/MS method seven peptides were scanned for but in the second replicate using mitogen-activated protein kinase 5 there were 8 unique peptides identified, this is the benefits of using preferential auto MS/MS.

Table 3.11: The number of unique peptides and spectral counts for mARC1 in HEK-293 cells lysate using reverse co-immunoprecipitation of selected proteins.

UniProt ID	Protein Name	Unique Peptides (Spectral Counts)	
		Replicate 1	Replicate 2
Q99683	Mitogen-activated protein kinase 5	5 (8)	8 (17)
O00750	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta	3 (6)	4 (7)
P04406	Glyceraldehyde-3-phosphate dehydrogenase	0 (0)	0 (0)

For mARC2 ADP/ATP translocase 1 (P12235) and myotubularin (Q13496) were the target bait proteins. **Table 3.13** is the number of unique mARC2 peptides and the number of spectral counts for peptides using reverse co-immunoprecipitation in HEK-293 cells. In both replicates, mARC2 peptides were identified supporting the evidence that the

Table 3.12: The number of unique peptides and spectral counts for mARC2 in HEK-293 cells lysate using reverse co-immunoprecipitation of selected proteins.

UniProt ID	Protein Name	Unique Peptides (Spectral Counts)	
		Replicate 1	Replicate 2
P12235	ADP/ATP translocase 1	3 (3)	7 (8)
Q13496	Myotubularin	5 (5)	4 (4)
P04406	Glyceraldehyde-3-phosphate dehydrogenase	0 (0)	0 (0)

interactions of mARC2 with ADP/ATP translocase 1 and myotubularin may exist.

It needs to be reiterated that all proteins were at natural abundances within the experiments. In proteomic studies, it is accepted that proteins identified with 2 or more

unique peptides with multiple spectral counts for the identified proteins to suggest a high probability that the identified protein was confirmed. In mARC1 and mARC2 reverse co-immunoprecipitation experiments, more than 2 unique peptides were identified in each experimental condition, and no mARC protein was identified in the GAPDH control condition. This information helps to support that mARC1 and mARC2 interact with the selected proteins.

3.7 Reverse Co-immunoprecipitation Results in Tissue Samples

The study was expanded further to include pig liver mitochondrial fraction and rat liver tissue. The pig liver mitochondrial fractions were supplied by Dr. Antje Havemeyer of the Institute of Pharmaceutical Chemistry, Christian-Albrechts University of Kiel, Kiel and rat liver tissue was supplied by Dr. Courtney Sparacino-Watkins University of Pittsburgh, Pittsburgh, PA. Sample preparation and further information can be found in Chapter 2.1. The reverse co-immunoprecipitation of tissue cell lysate followed the same method and antibodies as previously described in Chapter 3.6. The protein sequences for mARC1 and mARC2 are not well annotated in pig and rat, a protein BLAST using human mARC1 and mARC2 sequences was used to identify proteins in both organisms. In rat mARC1 (UniProt ID: G3V6I4) had a 77.4% sequence identity to human mARC1 and mARC2 (UniProt ID: O88994) had a 74.2% sequence identity to human mARC2. After performing the protein sequence BLAST on the pig proteome, mARC1 could not be identified but mARC2 (UniProt ID: F1S9I9) with a 74.1% sequence

identity to human mARC2. Even though mARC1 was not identified in pig, a sequence alignment using human mARC1 and pig mARC2 showed a sequence similarity of 70.0%. Mass spectrometric analysis was performed using the method described in **Chapter 2.3.1** and not the preferential auto MS/MS as employed in the previous reverse co-immunoprecipitation method.

Agilent MassHunter Qualitative Analysis with BioConfirm was again used to identify peptides in the spectrum data for each condition using the mARC1 and mARC2 protein sequences for each species. Since mARC1 was not identified in pig, but there was a high sequence similarity to human mARC1 and pig mARC2, it was in both mARC1 and mARC2 analysis. Again, GAPDH antibody was used as a control as no mARC1 or mARC2 peptides were not identified.

In rat liver samples mARC1was identified in both reverse co-immunoprecipitation experiments involving mitogen-activated protein kinase 5 and phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta proteins. However, the number of unique peptides and spectral counts for rat mARC2 was much lower. Only 1 unique peptide for ADP/ATP translocase 1 and 2 unique peptides for myotubularin, potentially questioning if rat mARC2 interacts with these two proteins. It also may be caused to a low reactivity between the antibody and rat proteins. In **Table 3.14** and **Table 3.15,** the number of unique peptides can be seen for mARC1 and mARC2 analysis of rat liver cell lysate.

In pig liver, mitochondrial fractions samples were analyzed for the presence of pig mARC2. Interestingly, the experiments involving mitogen-activated protein kinase 5 and phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta proteins that were intended to immunoprecipitate mARC1 had unique peptides and spectral count in all experiments. Pig mARC2 was also identified in proteins predicted to interact with mARC2, ADP/ATP translocase, and myotubularin. In **Table 3.14** and **Table 3.15,** the number of unique peptides can be seen for mARC1 and mARC2 analysis of pig liver mitochondrial fractions.

Table 3.13: The number of unique peptides and spectral counts for mARC1 in pig liver mitochondria fractions and rat liver cell lysate using reverse co-immunoprecipitation of selected proteins.

UniProt ID	Protein Name	Unique Peptides (Spectral Counts)	
		Pig Liver	Rat Liver
Q99683	Mitogen-activated protein kinase 5	14 (20)	7 (16)
O00750	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta	11 (15)	5 (10)
P04406	Glyceraldehyde-3-phosphate dehydrogenase	0 (0)	0 (0)

Table 3.14: The number of unique peptides and spectral counts for mARC2 in pig liver mitochondria fractions and rat liver cell lysate using reverse co-immunoprecipitation of selected proteins.

UniProt ID	Protein Name	Unique Peptides (Spectral Counts)	
		Pig Liver	Rat Liver
P12235	ADP/ATP translocase 1	8 (8)	1 (2)
Q13496	Myotubularin	8 (11)	2 (3)
P04406	Glyceraldehyde-3-phosphate dehydrogenase	0 (0)	0 (0)

In the rat liver cell lysate samples mARC1 samples demonstrated a high relationship with the interactions seen in human mARC1 and HEK-293 cell lysate experiments. However, the mARC2 information was less supportive. This could be due to a low reactivity of antibody to selected proteins or a low abundance of rat mARC2

protein. Although there was no pig mARC1 identified from BLAST searches, pig mARC2 was identified in all four reverse co-immunoprecipitation experiments and thus supporting the evidence of mARC proteins interacting with the four selected proteins. The data collected from reverse co-immunoprecipitation experiments is not conclusive because only single replicates were prepared for all conditions.

3.8 Protein-protein interactions of mARC1 and mARC2 summary and potential opportunities for future work

The human mARC1 and mARC2 enzymes are pterin-based molybdenum enzymes part of the SO family of molybdenum enzymes. Although the physiological function of mARC proteins inside the cell remains elusive, studies have shown their potential in the activation of pro-drugs, metabolism of mutated base pairs, and producing nitric oxide from nitrite under hypoxic conditions. The latter is of interest to many researchers as it provides another pathway for production of nitric oxide under oxygen limiting conditions. The goal of these experiments was to investigate the protein-protein interactions of mARC1 and mARC2 proteins in HEK-293 cell lysate that can corroborate with the information obtained previously from co-immunoprecipitation. For this purpose reverse co-immunoprecipitation experiments using HEK-293 cell lysate, pig liver mitochondrial fractions, and rat liver cells were performed. Three methods were used to investigate protein-protein interactions; co-immunoprecipitation, crosslinking reagents, and pull-down assays.

The crosslinking reagent experiments involved a novel approach of using a commercially available non-specific crosslinking reagent (Sulfo-SBED) and creating a compound (K-BLoC) that could bind crosslinking reagent and lock into the active site of mARC proteins. Mass spectrometric analysis demonstrated that the crosslinking reagent and compound were able to bind forming the complex referred to as LTR-K-BLoC. Using intact protein mass spectrometry we were able to confirm that mARC1 is about to bind the LTR-K-BLoC complex. This demonstrates not only a new way to transform non-specific crosslinking reagents into site-specific complexes but also potentials a novel method for the purification of mARC proteins from lysate samples.

Three methods were used to investigate protein-protein interactions and were performed in triplicate with appropriate controls; co-immunoprecipitation, crosslinking reagents, and pull-down assays. Using Agilent MassHunter Qualitative Analysis with BioConfirm software a total of 176 proteins were identified in mARC1 co-immunoprecipitation experiments, 124 proteins in mARC1 crosslinking reagent experiments, 181 protein in mARC1 pull-down assays, 83 proteins were identified in mARC2 co-immunoprecipitation experiments, 25 proteins in mARC2 crosslinking reagent experiments, and 112 protein in mARC2 pull-down assays. To aid in the reduction of false positive identification, the original list of peptides needed to meet certain criteria to be considered positive potential interacting partners with mARC

proteins. There were 38 proteins identified as interacting with mARC1 and 28 proteins identified as interacting with mARC2.

To aid in the formation of relationships in the identified proteins bioinformatics software was used. The proteins from the reduced list of interacting partners were clustered using GOTerm biological process and molecular function. Five GOTerm annotations were interesting to proteins identified as interacting with mARC proteins; cellular protein modification process, nitrogen compound metabolic process nucleobase-containing metabolic process, regulation of biological process, and signal transduction.

The identified proteins were then compared using KEGG annotated pathways to investigate relationships. Interestingly, both mARC1 and mARC2 had several interacting partners that were identified as part of signaling pathways. There were 11 signaling pathways that were affiliated with proteins identified as interacting with mARC1 and 10 signaling pathways for mARC2. In both cases, relationships were able to be connected based on effects of nitric oxide production, and nitric oxide synthase involvement. Based on the analysis of the data we suggest that mARC1 and mARC2 proteins may potentially be involved in the production of nitric oxide under oxygen limiting conditions to aid in maintaining cell homeostasis through signaling pathways.

Reverse co-immunoprecipitation was used to support the evidence of interactions between mARC1 and mARC2 and selected proteins. This included probing interactions in HEK-293 cell lysate, pig liver mitochondrial fractions, and rat liver cell lysate.

Interestingly mARC1 and mARC2 proteins were identified in all experimental conditions. The information gathered from the reverse co-immunoprecipitation experiments supported the evidence of mARC proteins interacting with mitogen-activated protein kinase 5, phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta, ADP/ATP translocase, and myotubularin.

The experiments that were compiled together in this study were not driven by a common hypothesis but were used to help propel the mARC system research by generating new testable hypotheses. A few testable hypotheses we were able to generate from this study include:

1. Post-translational modification of mARC1 occurs through JNK/p38 signaling cascade, modulating downstream processes of mARC1.

2. The mARC1 and mARC2 proteins aid in the regulation of the calcium signaling pathway in mammalian systems through the production of nitric oxide from nitrite under hypoxic conditions.

3. The maturation of mARC2 involves the formation of a complex consisting of MOCS1, MOCS2, and GPHN.

4. Through the production of nitric oxide from nitrite under hypoxic conditions, mARC2 is involved in the activity of phosphodiesterase 5A, subsequently affecting the conversion cGMP to 5'GMP.

The hypotheses that preceded are only examples of future direction for research into mARC1 and mARC2 systems. We anticipate the information provided by this investigation stimulates further research into mARC proteins and their potential physiological roles in mammalian cells.

Although there were relationships that could be formed from the interacting proteins with mARC1 and mARC2 through this study, the exact physiological function of mARC1 and mARC2 can only be inferred from the data collected. There is still a great deal of work that needs to be accomplished to determine their roles in the mammalian system. The identified interacting partners with mARC proteins must be confirmed through other methods to establish their requirements in the signaling pathways. After the interactions have been confirmed, under what conditions is mARC proteins most active, potentially under oxygen limiting or nitric oxide synthase inhibited conditions. The protein-protein interactions identified in this study have not only raised questions but have also allowed for the study of mARC proteins to be expanded outside the realm of normal metabolic functions.

Appendix I: Reagents, Kits, Materials and Buffers

Table A1: Reagents, kits, materials, companies, and catalog numbers for chemicals used.

Reagent/Kit	Company	Catalog Number
Pierce™ His Protein Interaction Pull-Down Kit	Thermo Fisher Scientific	21277
Sulfo-SBED Biotin Label Transfer Reagent	Thermo Fisher Scientific	33033
Pierce™ Biotinylated Protein Interaction Pull-Down Kit	Thermo Fisher Scientific	21115
Pierce™ Co-Immunoprecipitation Kit	Thermo Fisher Scientific	26149
Pierce™ Trypsin Protease, MS Grade, Frozen	Thermo Fisher Scientific	90305
Zeba™ Micro Spin Desalting Columns, 7K MWCO, 75 μL	Thermo Fisher Scientific	89877
Ammonium Bicarbonate	Sigma Aldrich	A6141
Dithiothreitol	Acros	16568
2,2,2-trifluoroethanol	Oakwood Chemical	001273
Pierce™ Dimethylsulfoxide (DMSO), LC-MS Grade	Thermo Fisher Scientific	85190
Formic Acid, Optima™ LC/MS Grade, Fisher Chemical	Fischer Scientific	A117
Iodoacetamide	Amresco	M216
Triethylammonium acetate buffer	Fluka	90357
Acetonitrile (Optima™), Fisher Chemical	Fischer Scientific	A996-4
Water-0.1% Formic Acid	JT Baker	9834-03

Buffers

IP Lysis/Wash Buffer

- 25 mM tris hydrochloride
- 150 mM sodium chloride
- 1 mM EDTA (Edetic Acid)
- 1% NP-40 (4-Nonylphenyl poly(ethylene glycol))
- 5% glycerol
- pH 7.4

Coupling Buffer (20X)

- 0.01 M sodium phosphate
- 0.15 M sodium chloride
- pH 7.2

Quenching Buffer

- 1 M tris hydrochloride
- pH 7.4

Wash Solution

- 1 M sodium chloride

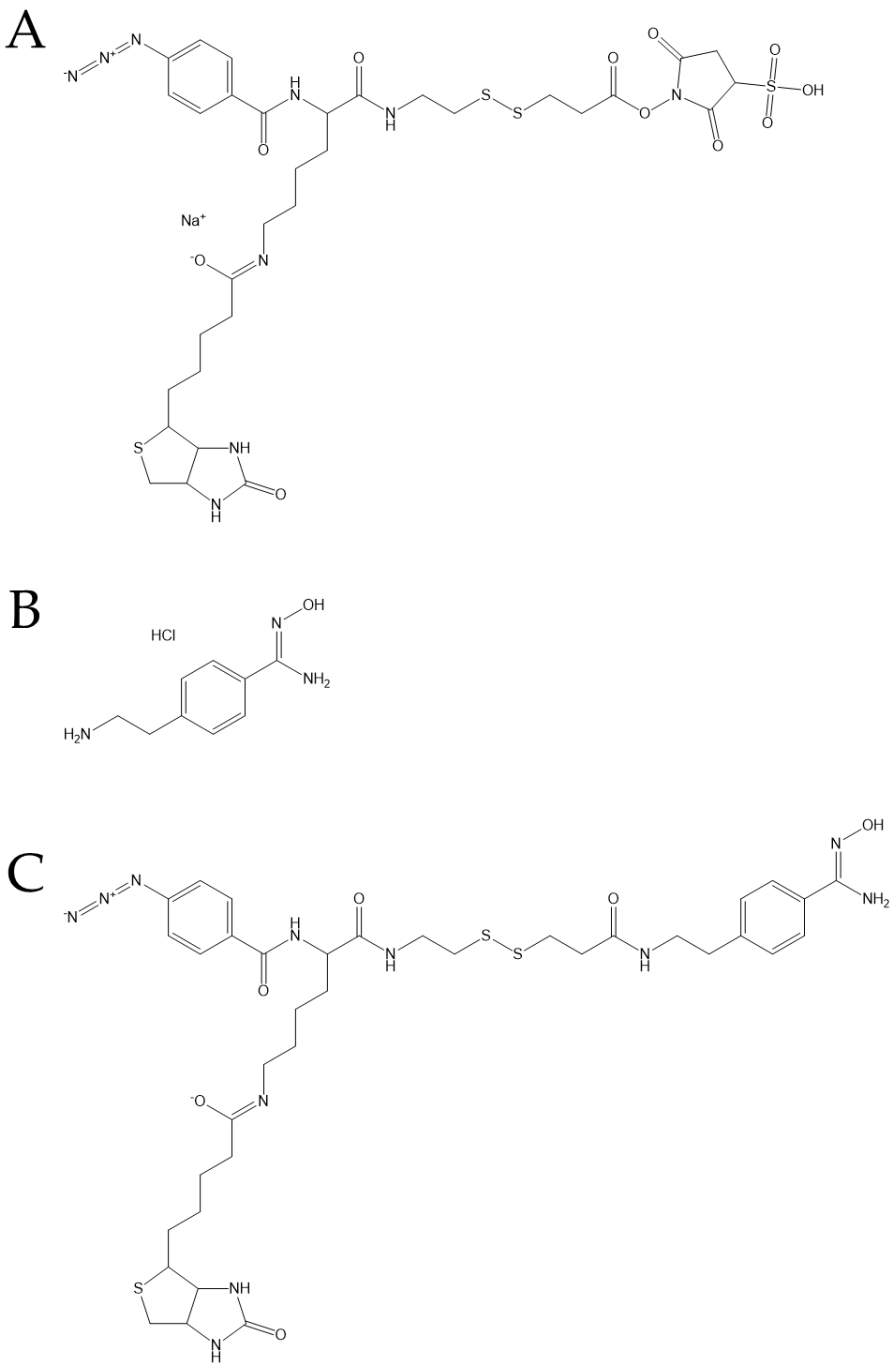

Figure A.1: Reagents and chemical structures of the compounds used in chemical crosslinking. A. Sulfo-SBED Label Transfer Reagent (MW: 879.97, MW-Na: 856.98), B. K-BLoC compounds (MW: 215.68, MW-HCl: 179.22), C. LTR-K-BLoC complex (MW: 841.06)

Appendix II: Protein Lists for Interacting Proteins with mARC1 and mARC2

Table A.2: Protein identified as interacting with mARC1 proteins in HEK-293 cell lysate. The technique in which proteins were identified in are; 1–co-immunoprecipitation, 2–crosslinking reagent, 3–pull-down assay. The condition in which the proteins were identified in are; C–control condition, E–experimental condition, CE–control and experimental condition.

UniProt ID	Protein Name	Technique and Condition
A0A075B6Z2	Protein TRAJ56	1C, 2CE, 3CE
A2A2F5	Protein TMEPAI	1E, 2E, 3C
A8K761	NADH dehydrogenase (Ubiquinone) 1 beta subcomplex, 10, 22kDa, isoform CRA_b	3C
A8MPX8	Protein phosphatase 2C-like domain-containing protein 1	1E, 3CE
C9J069	Uncharacterized protein C9orf172	1CE, 2E, 3CE
E5KNQ5	Mitochondrial thymidine kinase 2	1E, 3CE
E7EMC7	Sequestosome-1	2E, 3CE
F5H2Z5	Complement C1r subcomponent-like protein	3C
F8W1A0	Serine/threonine-protein phosphatase PP1-gamma catalytic subunit	3C
F8W1K5	Protein canopy homolog 2	1E
F8W6H6	Unconventional myosin-Va	1CE, 2E, 3CE
H0Y8F3	Folliculin-interacting protein 2	3E
H0YE05	Retinoblastoma-like protein 1	1E, 3CE
H3BP21	Nuclear factor of-activated T-cells 5	1E, 2E
H3BP40	Mitochondrial Rho GTPase 2	1E, 3E
H7C2G6	Probable 28S rRNA (cytosine-C(5))-methyltransferase	1E, 3E
J3KNJ8	DnaJ homolog subfamily C member 4	1CE, 2E, 3C
J3QRV1	Sodium/hydrogen exchanger 5	1E
O00267	Transcription elongation factor SPT5	1E, 2E, 3CE
O00330	Pyruvate dehydrogenase protein X component, mitochondrial	1E, 3CE
O00425	Insulin-like growth factor 2 mRNA-binding protein 3	1CE, 2E, 3C
O00750	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta	1CE, 2E, 3CE
O14730	Serine/threonine-protein kinase RIO3	1E, 2E, 3CE
O14788	Tumor necrosis factor ligand superfamily member 11	2E, 3CE
O15079	Syntaphilin	1CE, 2CE, 3C
O15240	Neurosecretory protein VGF	1E, 2E, 3C
O43181	NADH dehydrogenase [ubiquinone] iron-sulfur protein 4, mitochondrial	1E, 2E, 3C
O43312	Metastasis suppressor protein 1	1CE
O43314	Inositol hexakisphosphate and diphosphoinositol-pentakisphosphate kinase 2	1CE, 2E, 3CE
O60260	E3 ubiquitin-protein ligase parkin	1E, 3CE
O60518	Ran-binding protein 6	3CE

Table A.2: Continued

UniProt ID	Protein Name	Technique and Condition
O60683	Peroxisome biogenesis factor 10	1E, 2E
O75061	Putative tyrosine-protein phosphatase auxilin	1CE, 2E, 3CE
O75128	Protein cordon-bleu	1E, 2E, 3CE
O75530	Polycomb protein EED	1E, 3CE
O75581	Low-density lipoprotein receptor-related protein 6	1CE, 2E, 3CE
O75891	Cytosolic 10-formyltetrahydrofolate dehydrogenase	1E, 2E, 3CE
O94761	ATP-dependent DNA helicase Q4	1CE, 2E, 3CE
O94806	Serine/threonine-protein kinase D3	1CE, 2E, 3C
O94983	Calmodulin-binding transcription activator 2	1CE, 2E, 3CE
O95049	Tight junction protein ZO-3	1CE, 2C, 3E
O95199	RCC1 and BTB domain-containing protein 2	1E, 2E, 3CE
O95382	Mitogen-activated protein kinase kinase kinase 6	1CE, 2E, 3CE
O95447	Lebercilin-like protein	1CE, 2E, 3C
O95602	DNA-directed RNA polymerase I subunit RPA1	1CE, 2E, 3CE
O95954	Formimidoyltransferase-cyclodeaminase	3C
P00734	Prothrombin	1E, 2E, 3CE
P01036	Cystatin-S	1E, 3C
P02768	Serum albumin	1E, 2E, 3E
P04406	Glyceraldehyde-3-phosphate dehydrogenase	1E, 3E
P04818	Thymidylate synthase	1C, 2E
P04908	Histone H2A type 1-B/E	1E, 3E
P06727	Apolipoprotein A-IV	1CE, 3C
P0C870	JmjC domain-containing protein 7	1E, 2E, 3C
P11216	Glycogen phosphorylase, brain form	1CE, 2E, 3CE
P12273	Prolactin-inducible protein	3C
P15313	V-type proton ATPase subunit B, kidney isoform	1E, 3CE
P18564	Integrin beta-6	1CE, 2E, 3CE
P20810	Calpastatin	1E, 3CE
P21549	Serine-pyruvate aminotransferase	1E, 3CE
P22059	Oxysterol-binding protein 1	1CE, 2CE, 3CE
P23396	40S ribosomal protein S3	1CE, 2CE, 3CE
P23467	Receptor-type tyrosine-protein phosphatase beta	1CE, 3CE
P25705	ATP synthase subunit alpha, mitochondrial	2E, 3E
P27816	Microtubule-associated protein 4	1E, 2E, 3CE
P30050	60S ribosomal protein L12	1CE, 2E, 3CE
P30679	Guanine nucleotide-binding protein subunit alpha-15	3E
P31040	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	1CE, 2E, 3CE
P31937	3-hydroxyisobutyrate dehydrogenase, mitochondrial	1CE, 2E, 3CE
P43355	Melanoma-associated antigen 1	1C, 3CE

Table A.2: Continued

UniProt ID	Protein Name	Technique and Condition
P46977	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit STT3A	1E, 2E, 3C
P49862	Kallikrein-7	1E, 2E, 3C
P50479	PDZ and LIM domain protein 4	1C, 3CE
P51160	Cone cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha	1E, 2E, 3CE
P52788	Spermine synthase	1CE, 2CE, 3C
P55056	Apolipoprotein C-IV	2E, 3E
P62330	ADP-ribosylation factor 6	1E
P62701	40S ribosomal protein S4, X isoform	1CE, 2E, 3C
P78334	Gamma-aminobutyric acid receptor subunit epsilon	1E, 3CE
P85037	Forkhead box protein K1	1CE, 2E, 3CE
P98088	Mucin-5AC	1CE, 2CE, 3CE
Q01970	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-3	1CE, 2E, 3CE
Q02383	Semenogelin-2	1CE, 2E, 3E
Q03113	Guanine nucleotide-binding protein subunit alpha-12	1C, 2E
Q04726	Transducin-like enhancer protein 3	1E, 2E, 3CE
Q07021	Complement component 1 Q subcomponent-binding protein, mitochondrial	1E, 3C
Q12879	Glutamate receptor ionotropic, NMDA 2A	1CE, 2E, 3CE
Q13112	Chromatin assembly factor 1 subunit B	1CE, 2E, 3C
Q13233	Mitogen-activated protein kinase kinase kinase 1	1CE, 2E, 3CE
Q13884	Beta-1-syntrophin	1E
Q14103	Heterogeneous nuclear ribonucleoprotein D0	1CE, 2E, 3CE
Q14141	Septin-6	1E, 2E, 3CE
Q14684	Ribosomal RNA processing protein 1 homolog B	1E, 2E, 3C
Q14789	Golgin subfamily B member 1	1CE, 2E, 3CE
Q15431	Synaptonemal complex protein 1	1CE, 2E, 3CE
Q155Q3	Dixin	1E, 2E, 3CE
Q15637	Splicing factor 1	1E, 3E
Q16555	Dihydropyrimidinase-related protein 2	1E, 2E, 3C
Q17R98	Zinc finger protein 827	1E, 3CE
Q2TAC6	Kinesin-like protein KIF19	1CE, 2E, 3CE
Q32M45	Anoctamin-4	1E, 3E
Q3MIN7	Ral guanine nucleotide dissociation stimulator-like 3	1CE, 3E
Q3SY52	Zinc finger protein interacting with ribonucleoprotein K	1E, 3C
Q49A26	Putative oxidoreductase GLYR1	1E, 2E, 3CE
Q53EZ4	Centrosomal protein of 55 kDa	1CE
Q53RY4	Keratinocyte-associated protein 3	3E
Q549N5	Signal recognition particle receptor beta subunit	3E
Q5JQC9	A-kinase anchor protein 4	1CE, 2E, 3CE
Q5T749	Keratinocyte proline-rich protein	1E, 2E, 3CE

Table A.2: Continued

UniProt ID	Protein Name	Technique and Condition
Q5TEU4	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 5	1E, 3E
Q5VVJ2	Histone H2A deubiquitinase MYSM1	1CE, 2CE, 3CE
Q66K64	DDB1- and CUL4-associated factor 15	2E, 3C
Q66K74	Microtubule-associated protein 1S	1CE, 2CE, 3CE
Q6BAA4	Fc receptor-like B	1E, 2E, 3CE
Q6IQ32	ADNP homeobox protein 2	1CE, 2E, 3CE
Q6MZT1	Regulator of G-protein signaling 7-binding protein	1CE, 3CE
Q6P1R4	tRNA-dihydrouridine(16/17) synthase [NAD(P)(+)]-like	1E, 2E, 3CE
Q6P3S6	F-box only protein 42	1CE, 2E, 3C
Q6P996	Pyridoxal-dependent decarboxylase domain-containing protein 1	3CE
Q6PF15	Kelch-like protein 35	1E, 2E, 3CE
Q6Q0C0	E3 ubiquitin-protein ligase TRAF7	1E, 2E, 3CE
Q6SJ96	TATA box-binding protein-like protein 2	1E, 3C
Q6TGC4	Protein-arginine deiminase type-6	1E, 3C
Q6UXK5	Leucine-rich repeat neuronal protein 1	1E, 2E, 3E
Q6UXU4	Germ cell-specific gene 1-like protein	1E
Q6ZNA1	Zinc finger protein 836	1CE, 2E, 3CE
Q6ZS94	Putative uncharacterized protein C1orf229	1C, 2E
Q7L0Q8	Rho-related GTP-binding protein RhoU	1E, 2E
Q7L590	Protein MCM10 homolog	1CE, 2E, 3C
Q7L8A9	Vasohibin-1	1CE
Q7Z4P5	Growth/differentiation factor 7	2E
Q7Z5M5	Transmembrane channel-like protein 3	1CE, 2E, 3E
Q7Z7G8	Vacuolar protein sorting-associated protein 13B	1CE, 2E, 3CE
Q86WI1	Fibrocystin-L	1E, 2E, 3CE
Q86YV0	RAS protein activator like-3	2E, 3CE
Q8IUH5	Palmitoyltransferase ZDHHC17	1CE, 3CE
Q8IWV8	E3 ubiquitin-protein ligase UBR2	1CE, 2E, 3CE
Q8IXY8	Peptidyl-prolyl cis-trans isomerase-like 6	1E, 3CE
Q8IYE1	Coiled-coil domain-containing protein 13	1CE, 2E, 3CE
Q8IYS4	Uncharacterized protein C16orf71	3E
Q8IYX3	Coiled-coil domain-containing protein 116	1CE, 2CE, 3CE
Q8IZ73	RNA pseudouridylate synthase domain-containing protein 2	1CE, 3CE
Q8N0U4	Protein FAM185A	1E
Q8N187	Calcium-responsive transcription factor	1CE, 2E
Q8N4H5	Mitochondrial import receptor subunit TOM5 homolog	3C
Q8N7S6	Uncharacterized protein ARIH2OS	1E
Q8N7X4	Melanoma-associated antigen B6	2C, 3CE
Q8NCR6	Spermatid-specific manchette-related protein 1	1E, 3CE
Q8ND04	Protein SMG8	1E, 2E, 3CE

Table A.2: Continued

UniProt ID	Protein Name	Technique and Condition
Q8NDV3	Structural maintenance of chromosomes protein 1B	1CE, 2E, 3CE
Q8NEE6	F-box/LRR-repeat protein 13	1CE, 2E, 3CE
Q8NFW9	Rab effector MyRIP	1CE, 3CE
Q92616	eIF-2-alpha kinase activator GCN1	1E, 2CE, 3CE
Q92945	Far upstream element-binding protein 2	1CE, 2CE, 3CE
Q969R2	Oxysterol-binding protein 2	1CE, 3CE
Q96AG3	Solute carrier family 25 member 46	1E
Q96B01	RAD51-associated protein 1	1CE, 3CE
Q96BH3	Epididymal sperm-binding protein 1	3E
Q96F45	Zinc finger protein 503	1CE, 3CE
Q96FV2	Secernin-2	1E, 3CE
Q96GN5	Cell division cycle-associated 7-like protein	1E, 2E
Q96N96	Spermatogenesis-associated protein 13	1CE, 2E, 3CE
Q96QB1	Rho GTPase-activating protein 7	1CE, 2E, 3CE
Q99489	D-aspartate oxidase	3E
Q99683	Mitogen-activated protein kinase kinase kinase 5	1CE, 2E, 3CE
Q9BPX6	Calcium uptake protein 1, mitochondrial	1E, 3E
Q9BT88	Synaptotagmin-11	1E, 3E
Q9BTM1	Histone H2A.J	1E
Q9BYG4	Partitioning defective 6 homolog gamma	3E
Q9BZE0	Zinc finger protein GLIS2	1E, 2E, 3CE
Q9C0F0	Putative Polycomb group protein ASXL3	1CE, 2E, 3CE
Q9H0N0	Ras-related protein Rab-6C	1E, 2E, 3C
Q9H2D6	TRIO and F-actin-binding protein	1CE, 2E, 3CE
Q9H2M3	S-methylmethionine-homocysteine S-methyltransferase BHMT2	3E
Q9H490	Phosphatidylinositol glycan anchor biosynthesis class U protein	3C
Q9H5N1	Rab GTPase-binding effector protein 2	1E, 2E, 3CE
Q9H9Z2	Protein lin-28 homolog A	1E, 3CE
Q9HD20	Manganese-transporting ATPase 13A1	1E, 2E, 3CE
Q9NQW6	Actin-binding protein anillin	1CE, 2E, 3E
Q9NU02	Ankyrin repeat and EF-hand domain-containing protein 1	1E, 2E, 3CE
Q9NUQ3	Gamma-taxilin	1E, 3CE
Q9NY59	Sphingomyelin phosphodiesterase 3	1CE, 3CE
Q9NYK5	39S ribosomal protein L39, mitochondrial	1CE, 2CE, 3C
Q9P015	39S ribosomal protein L15, mitochondrial	1CE, 2E, 3CE
Q9P218	Collagen alpha-1(XX) chain	1E, 2E, 3CE
Q9P266	Junctional protein associated with coronary artery disease	1E, 2E, 3CE
Q9P2J5	Leucine--tRNA ligase, cytoplasmic	1CE, 3CE
Q9P2N5	RNA-binding protein 27	1CE, 2E, 3CE
Q9UBW7	Zinc finger MYM-type protein 2	1CE, 2CE, 3CE

Table A.2: Continued

UniProt ID	Protein Name	Technique and Condition
Q9UHN6	Transmembrane protein 2	1E, 2E, 3CE
Q9UHV7	Mediator of RNA polymerase II transcription subunit 13	1CE, 3CE
Q9UKS7	Zinc finger protein Helios	1CE, 2CE, 3CE
Q9UPT8	Zinc finger CCCH domain-containing protein 4	1CE, 2E, 3CE
Q9UPU5	Ubiquitin carboxyl-terminal hydrolase 24	1E, 2E, 3CE
Q9UQ10	Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase	1CE, 3C
Q9UQ84	Exonuclease 1	1CE, 2E, 3CE
Q9Y2P5	Bile acyl-CoA synthetase	1E, 3CE
Q9Y2X0	Mediator of RNA polymerase II transcription subunit 16	1CE, 2CE, 3CE
Q9Y426	C2 domain-containing protein 2	1C, 2E, 3CE
Q9Y466	Nuclear receptor subfamily 2 group E member 1	1CE, 2CE, 3CE
Q9Y6X6	Unconventional myosin-XVI	1E, 2E, 3CE
R4GMW8	Protein BIVM-ERCC5	1E, 2E, 3CE

Table A.3: Protein identified as interacting with mARC2 proteins in HEK-293 cell lysate. The technique in which proteins were identified in are; 1–co-immunoprecipitation, 2–crosslinking reagent, 3–pull-down assay. The condition in which the proteins were identified in are; C–control condition, E–experimental condition, CE–control and experimental condition.

UniProt ID	Protein Name	Technique and Condition
A0A075B6Z2	Protein TRAJ56	1CE, 2CE, 3CE
A5YKK6	CCR4-NOT transcription complex subunit 1	1E, 2E, 3CE
A6NE02	BTB/POZ domain-containing protein 17	1C, 3CE
B1AL46	NUT family member 2E	3CE
B1ANS9	WD repeat-containing protein 64	1C, 3CE
C9IYN7	Elongator complex protein 6	1C
C9JH25	Proline-rich transmembrane protein 4	1C, 3CE
E9PIH7	Solute carrier family 26 member 10	3E
E9PKF4	Four and a half LIM domains protein 2	3E
G8JLL9	MYH14 protein	1CE, 2CE, 3CE
O14730	Serine/threonine-protein kinase RIO3	1CE, 3CE
O15020	Spectrin beta chain, non-erythrocytic 2	1CE, 2CE, 3CE
O43312	Metastasis suppressor protein 1	1CE, 3CE
O43708	Maleylacetoacetate isomerase	3E
O75061	Putative tyrosine-protein phosphatase auxilin	1CE, 3CE
O75128	Protein cordon-bleu	3CE
O75581	Low-density lipoprotein receptor-related protein 6	1CE, 3CE
O75695	Protein XRP2	3CE
O75891	Cytosolic 10-formyltetrahydrofolate dehydrogenase	1E, 3CE
O75947	ATP synthase subunit d, mitochondrial	3CE
O76074	cGMP-specific 3',5'-cyclic phosphodiesterase	3CE
O94927	HAUS augmin-like complex subunit 5	1CE, 2E, 3CE
O95696	Bromodomain-containing protein 1	1CE, 2E, 3CE
O95870	Protein ABHD16A	3CE
O96007	Molybdopterin synthase catalytic subunit	3E
P00748	Coagulation factor XII	1E, 3CE
P01127	Platelet-derived growth factor subunit B	3E
P05141	ADP/ATP translocase 2	1E, 2CE, 3CE
P06727	Apolipoprotein A-IV	1CE
P0C870	JmjC domain-containing protein 7	3E
P14410	Sucrase-isomaltase, intestinal	1E, 3CE
P14678	Small nuclear ribonucleoprotein-associated proteins B and B'	1CE, 2E, 3CE
P23396	40S ribosomal protein S3	1E, 2CE, 3CE
P27694	Replication protein A 70 kDa DNA-binding subunit	1C, 3C
P36542	ATP synthase subunit gamma, mitochondrial	1CE, 3CE
P39019	40S ribosomal protein S19	1E
P40855	Peroxisomal biogenesis factor 19	1E, 3C

Table A.3: Continued

UniProt ID	Protein Name	Technique and Condition
P42262	Glutamate receptor 2	3CE
P42285	Superkiller viralicidic activity 2-like 2	1CE, 3CE
P46782	40S ribosomal protein S5	1C, 2C, 3CE
P48047	ATP synthase subunit O, mitochondrial	2E, 3C
P50454	Serpin H1	3CE
P54259	Atrophin-1	1C
P54764	Ephrin type-A receptor 4	3E
P62249	40S ribosomal protein S16	1E, 3CE
P62269	40S ribosomal protein S18	3CE
P62273	40S ribosomal protein S29	3CE
P62701	40S ribosomal protein S4, X isoform	1CE, 3CE
P62750	60S ribosomal protein L23a	1E, 3CE
P85037	Forkhead box protein K1	1CE, 2E, 3CE
Q02952	A-kinase anchor protein 12	1CE, 3CE
Q06787	Synaptic functional regulator FMR1	1CE, 3CE
Q07021	Complement component 1 Q subcomponent-binding protein, mitochondrial	1C
Q13404	Ubiquitin-conjugating enzyme E2 variant 1	1E
Q13496	Myotubularin	3CE
Q13698	Voltage-dependent L-type calcium channel subunit alpha-1S	1E, 2CE, 3CE
Q15185	Prostaglandin E synthase 3	1C, 3E
Q15390	Mitochondrial fission regulator 1	1C, 2E, 3CE
Q2M2I8	AP2-associated protein kinase 1	1CE, 3CE
Q32P51	Heterogeneous nuclear ribonucleoprotein A1-like 2	1C, 2CE, 3CE
Q5BKY1	Leucine-rich repeat-containing protein 10	1CE
Q5S007	Leucine-rich repeat serine/threonine-protein kinase 2	1CE, 2E, 3CE
Q5T0W9	Protein FAM83B	3CE
Q5U651	Ras-interacting protein 1	1CE, 3E
Q5W0Q7	SUMO-specific isopeptidase USPL1	3CE
Q6EEV4	DNA-directed RNA polymerase II subunit GRINL1A, isoforms 4/5	1C
Q6NUQ1	RAD50-interacting protein 1	1C, 3E
Q6P2H3	Centrosomal protein of 85 kDa	3CE
Q6PD62	RNA polymerase-associated protein CTR9 homolog	1CE, 2CE, 3CE
Q6PI77	Protein BHLHb9	3CE
Q6PJ61	F-box only protein 46	3CE
Q6UB35	Monofunctional C1-tetrahydrofolate synthase, mitochondrial	1C, 2CE, 3CE
Q6ZU35	Uncharacterized protein KIAA1211	1CE, 3E
Q71U36	Tubulin alpha-1A chain	1E, 3CE
Q7Z2K8	G protein-regulated inducer of neurite outgrowth 1	1E, 3CE
Q7Z388	Probable C-mannosyltransferase DPY19L4	1C, 2E, 3CE

Table A.3: Continued

UniProt ID	Protein Name	Technique and Condition
Q7Z7M0	Multiple epidermal growth factor-like domains protein 8	1CE, 3CE
Q86WI3	Protein NLRC5	1CE, 3CE
Q86XP3	ATP-dependent RNA helicase DDX42	1E, 2C, 3E
Q8IUR0	Trafficking protein particle complex subunit 5	3C
Q8IY51	Tigger transposable element-derived protein 4	1CE, 3CE
Q8IYE1	Coiled-coil domain-containing protein 13	1C, 3C
Q8N0U4	Protein FAM185A	3CE
Q8N187	Calcium-responsive transcription factor	3C
Q8N4Q0	Zinc-binding alcohol dehydrogenase domain-containing protein 2	1C, 3C
Q8N7Q3	Zinc finger protein 676	3E
Q8NBS9	Thioredoxin domain-containing protein 5	1CE, 2E, 3CE
Q8NBU5	ATPase family AAA domain-containing protein 1	1C, 3E
Q8NEM8	Cytosolic carboxypeptidase 3	1CE, 2E, 3CE
Q8TBB5	Kelch domain-containing protein 4	1CE, 3E
Q8TCU4	Alstrom syndrome protein 1	1E, 3CE
Q8TD16	Protein bicaudal D homolog 2	1CE, 3CE
Q8TD57	Dynein heavy chain 3, axonemal	1CE, 3CE
Q8WUY1	Protein THEM6	3CE
Q8WWN9	Interactor protein for cytohesin exchange factors 1	1E, 3C
Q8WXS8	A disintegrin and metalloproteinase with thrombospondin motifs 14	1CE, 3CE
Q96AE7	Tetratricopeptide repeat protein 17	1CE, 3CE
Q96F45	Zinc finger protein 503	1CE, 3C
Q96JH8	Ras-associating and dilute domain-containing protein	1C, 3C
Q96RP9	Elongation factor G, mitochondrial	1E, 3CE
Q96RS0	Trimethylguanosine synthase	3E
Q9BRT2	Ubiquinol-cytochrome-c reductase complex assembly factor 2	1E, 3CE
Q9BT88	Synaptotagmin-11	1E, 2E, 3C
Q9C0D5	Protein TANC1	1C, 3CE
Q9C0H9	SRC kinase signaling inhibitor 1	3CE
Q9HCE9	Anoctamin-8	1CE, 3CE
Q9NQW6	Actin-binding protein anillin	1CE, 3E
Q9NQX3	Gephyrin [Includes: Molybdopterin adenylyltransferase	3CE
Q9NUE0	Palmitoyltransferase ZDHHC18	1E, 3C
Q9NUT2	ATP-binding cassette sub-family B member 8, mitochondrial	1E, 3CE
Q9NY43	BarH-like 2 homeobox protein	3CE
Q9NZB8	Molybdenum cofactor biosynthesis protein 1	3E
Q9P2P1	Protein NYNRIN	1CE, 3CE
Q9P2Y5	UV radiation resistance-associated gene protein	3C
Q9UHD8	Septin-9	1E, 3C
Q9UKX5	Integrin alpha-11	3CE

Table A.3: Continued

UniProt ID	Protein Name	Technique and Condition
Q9UPA5	Protein bassoon	1CE, 2E, 3CE
Q9Y285	Phenylalanine-tRNA ligase alpha subunit	1CE, 3CE
Q9Y2G9	Protein strawberry notch homolog 2	3CE
R4GMW8	Protein BIVM-ERCC5	1C, 2CE, 3C

Appendix III: GOTerm and KEGG Pathway Annotations

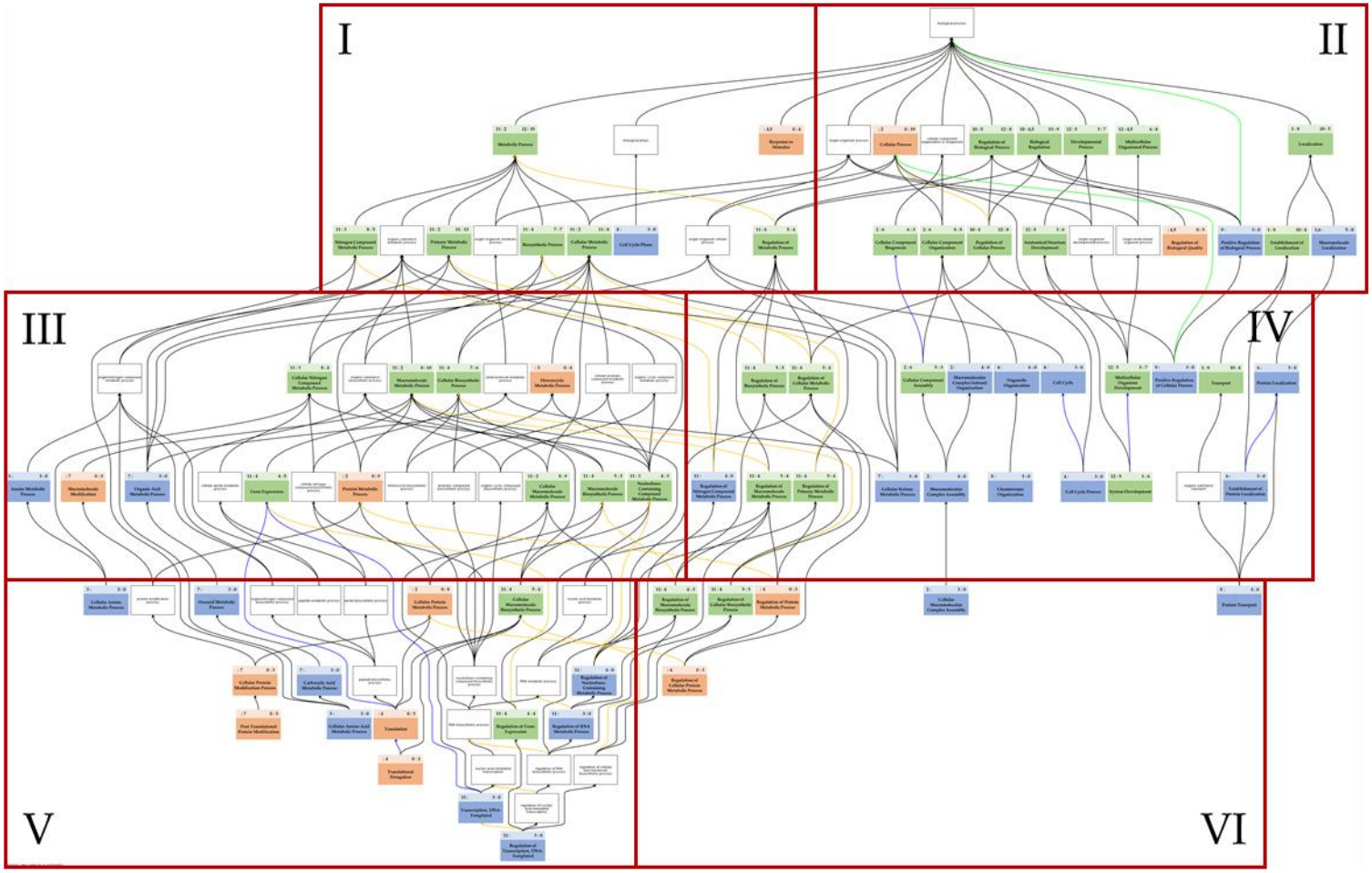

Figure A.2: Biological processes GOTerm annotations for proteins that were identified as interacting partners with mARC1 and mARC2. Green boxes depict annotations found in proteins identified in both mARC1 and mARC2, blue boxes depict annotations found in proteins identified in both mARC1, and orange boxes depict annotations found in proteins identified in both mARC2. The DAVID cluster number is in the top right corner of each box (mARC1:mARC2). The number of proteins with that annotation are found in the top left of each box (mARC1:mARC2). **Figures A.3** through **Figure A.8** are expanded portions of the individual sections.

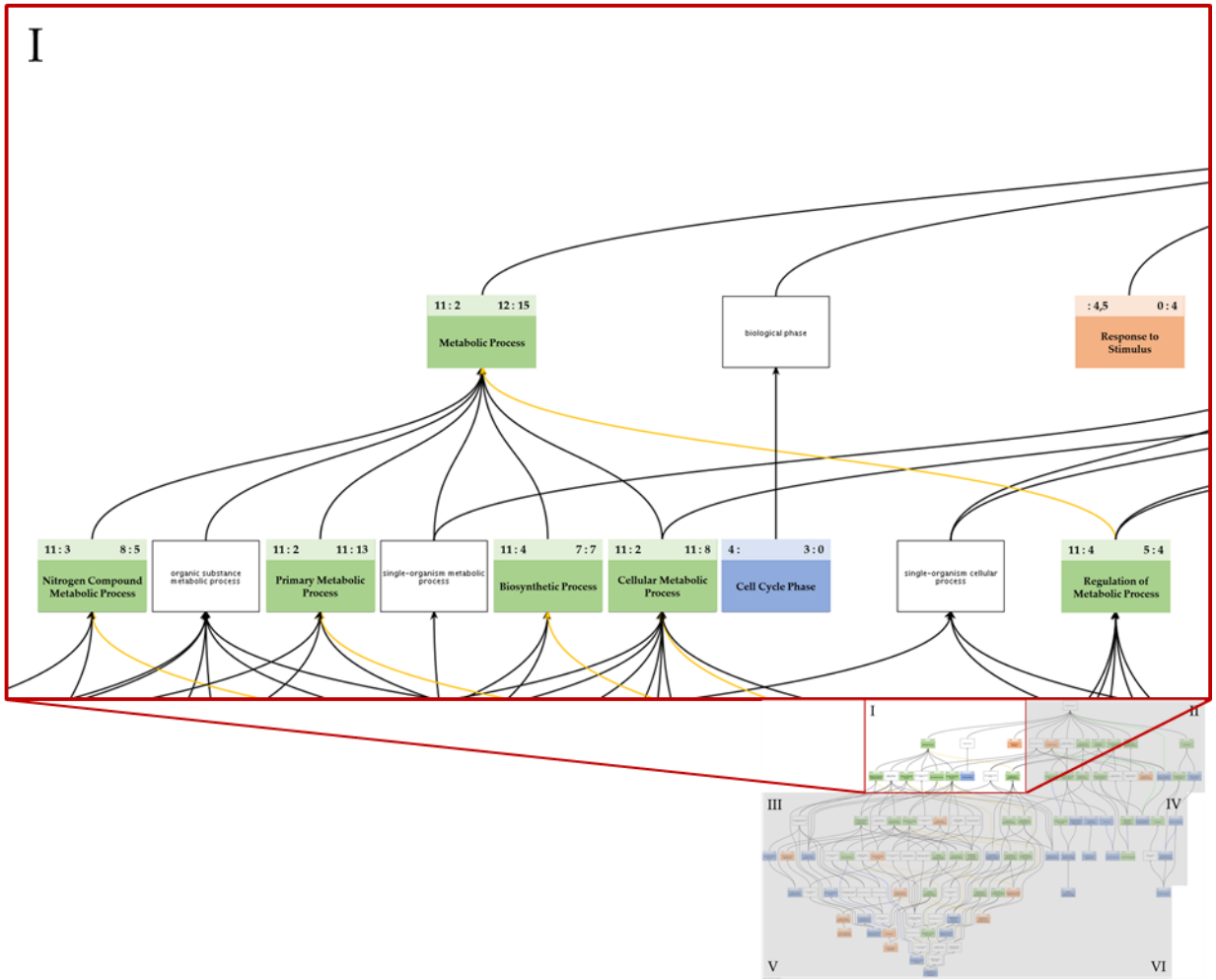

Figure A.3: Expanded Section I of the GOTerm annotations tree for proteins that were identified as interacting partners with mARC1 and mARC2.

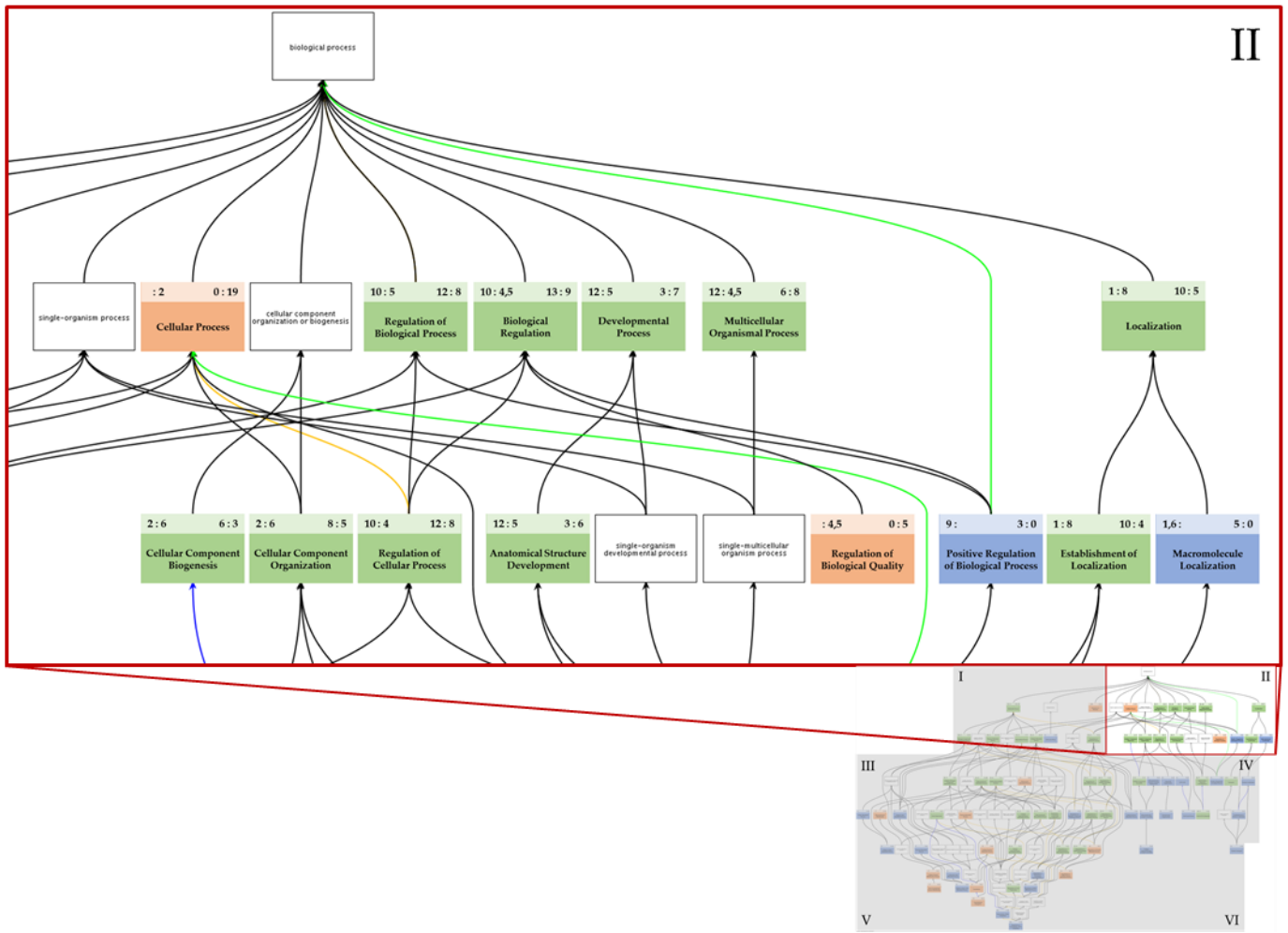

Figure A.4: Expanded Section II of the GOTerm annotations tree for proteins that were identified as interacting partners with mARC1 and mARC2.

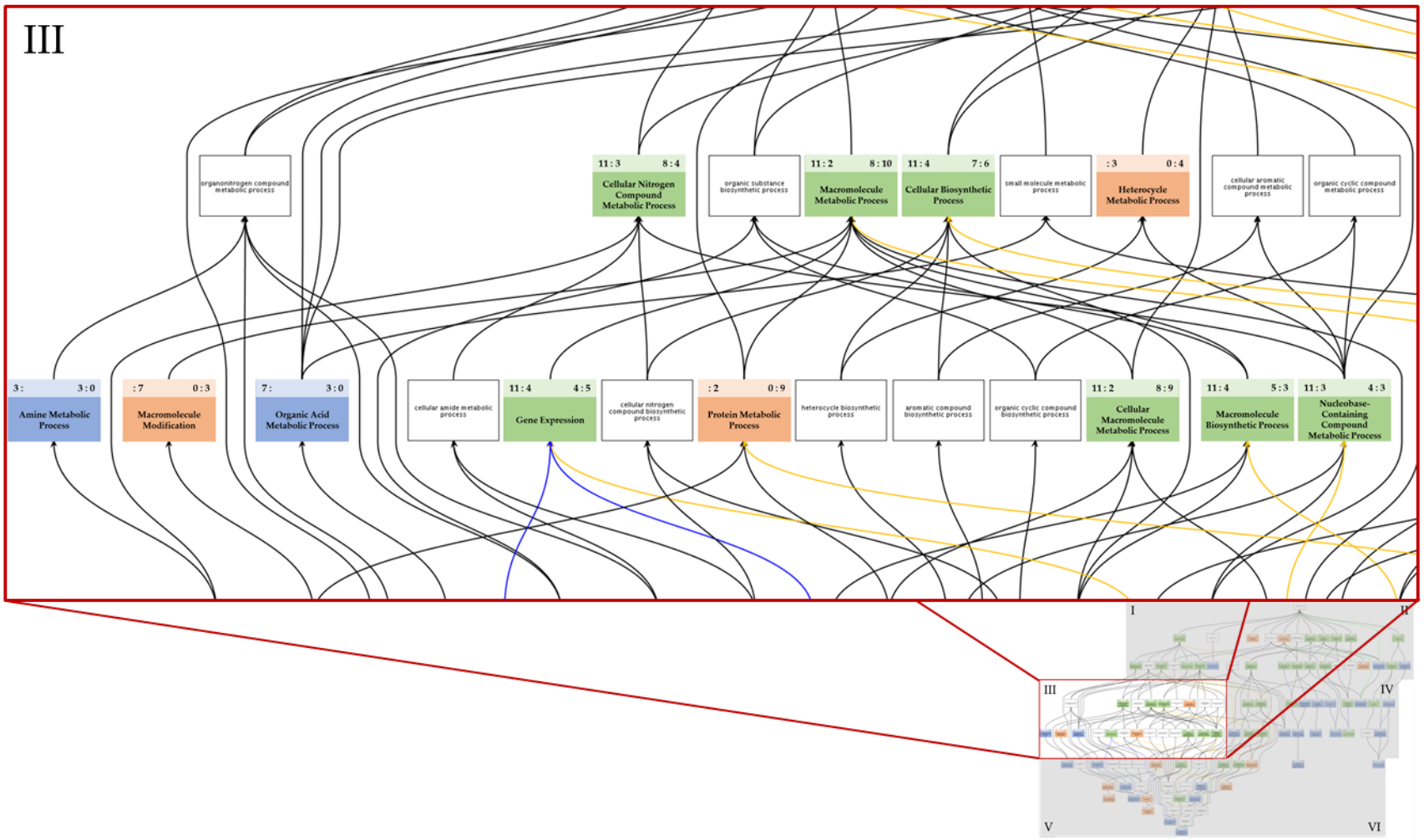

Figure A.5: Expanded Section III of the GOTerm annotations tree for proteins that were identified as interacting partners with mARC1 and mARC2.

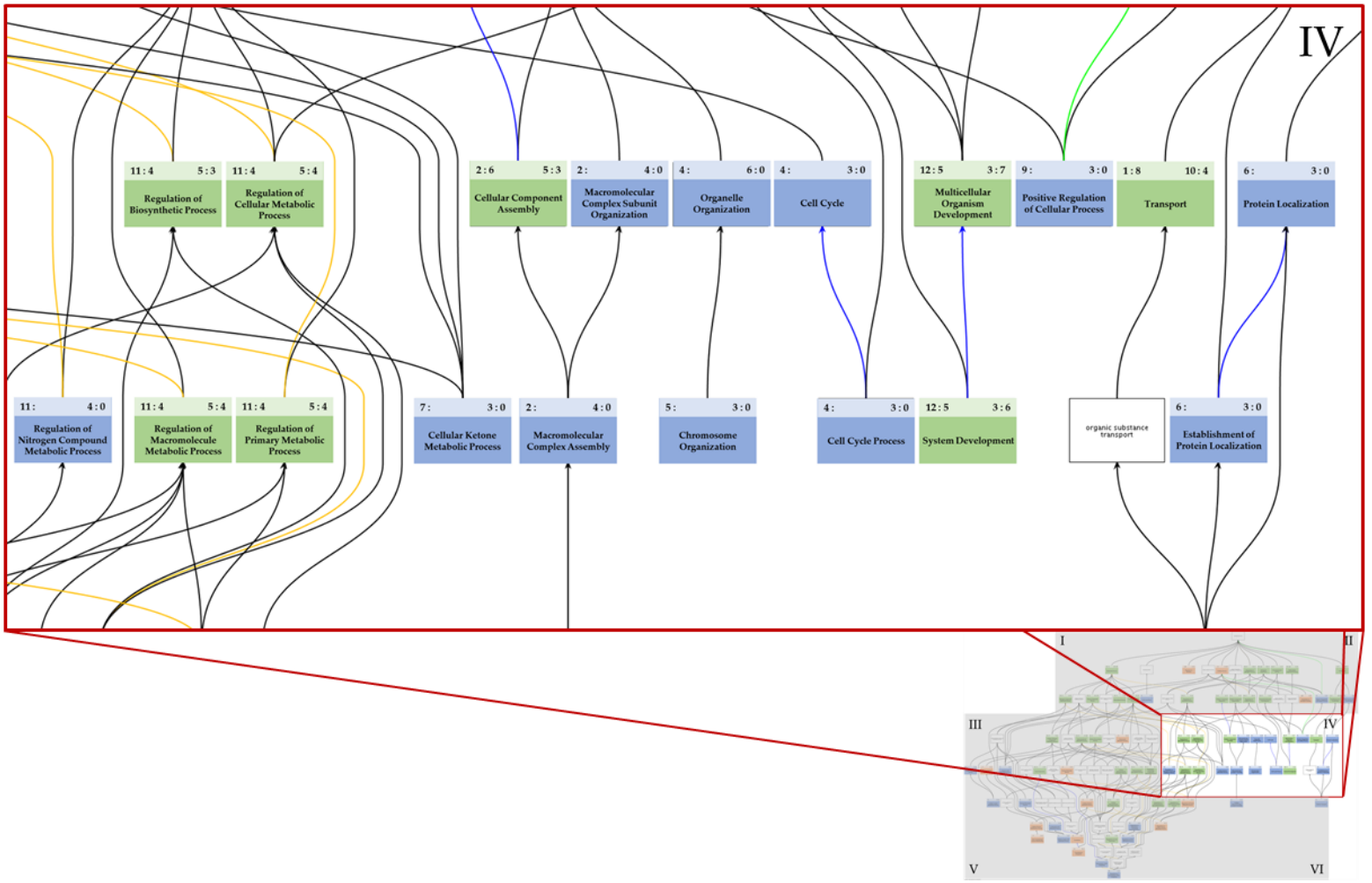

Figure A.6: Expanded Section IV of the GOTerm annotations tree for proteins that were identified as interacting partners with mARC1 and mARC2.

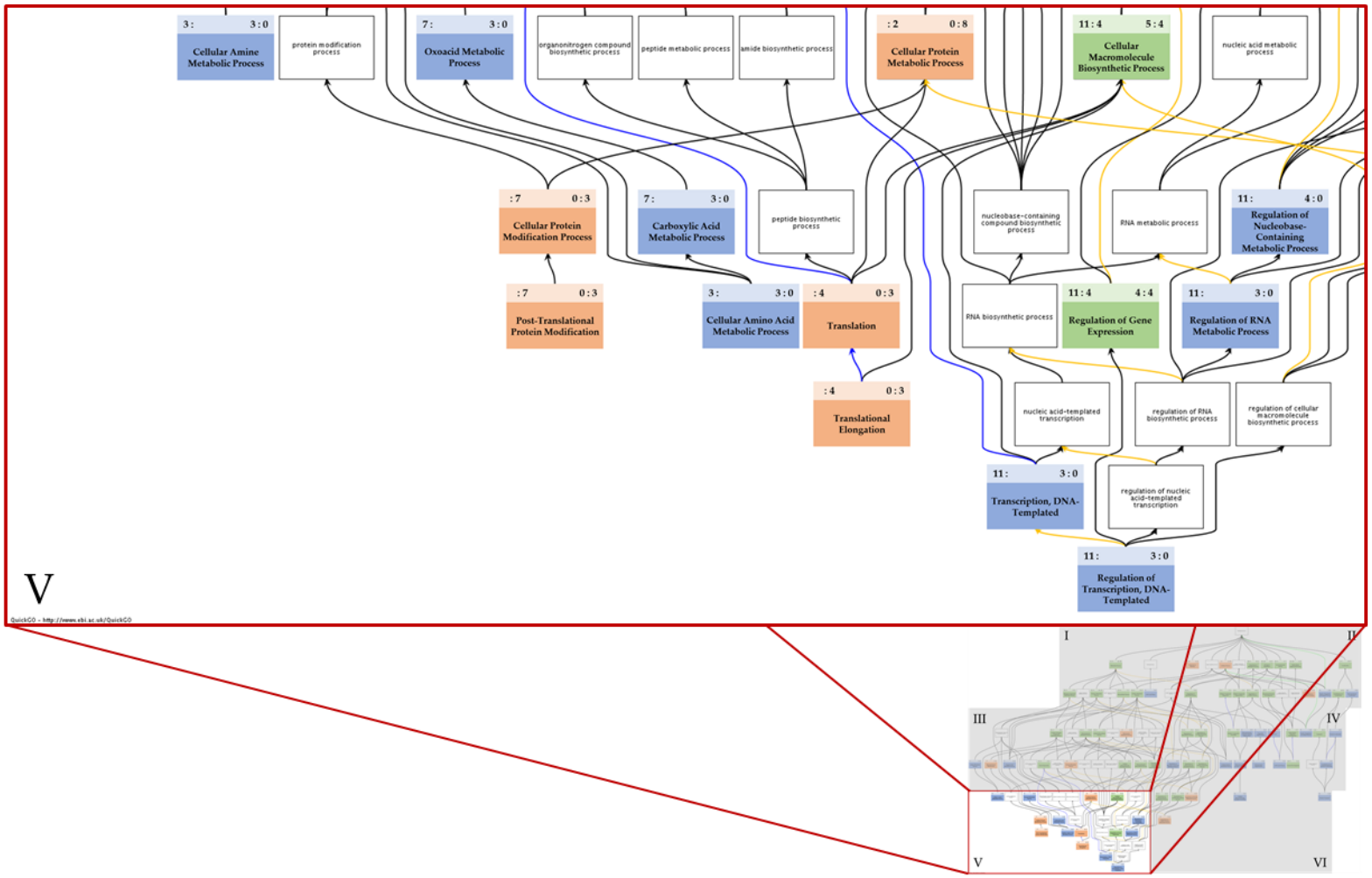

Figure A.7: Expanded Section V of the GOTerm annotations tree for proteins that were identified as interacting partners with mARC1 and mARC2.

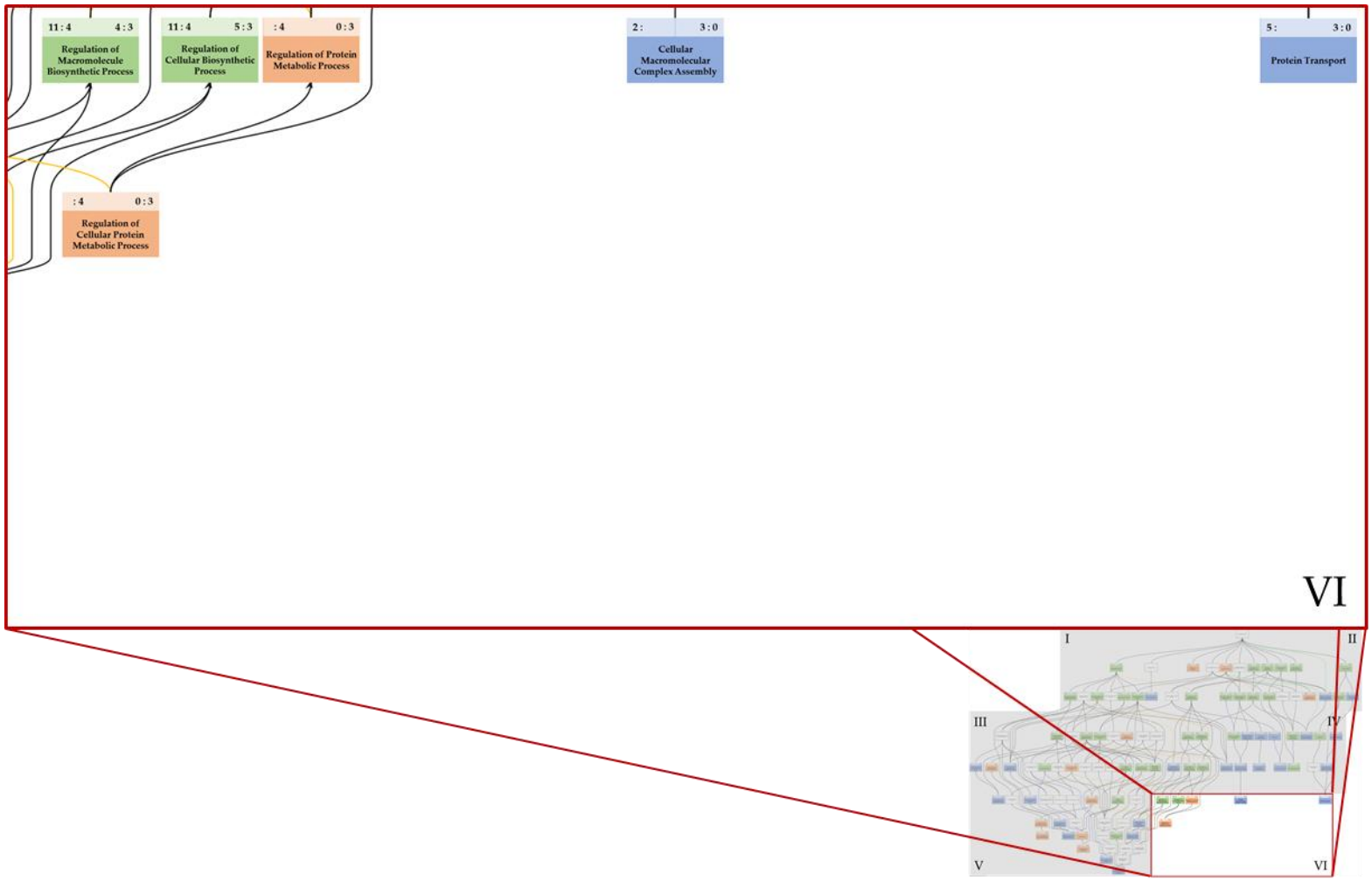

Figure A.8: Expanded Section VI of the GOTerm annotations tree for proteins that were identified as interacting partners with mARC1 and mARC2.

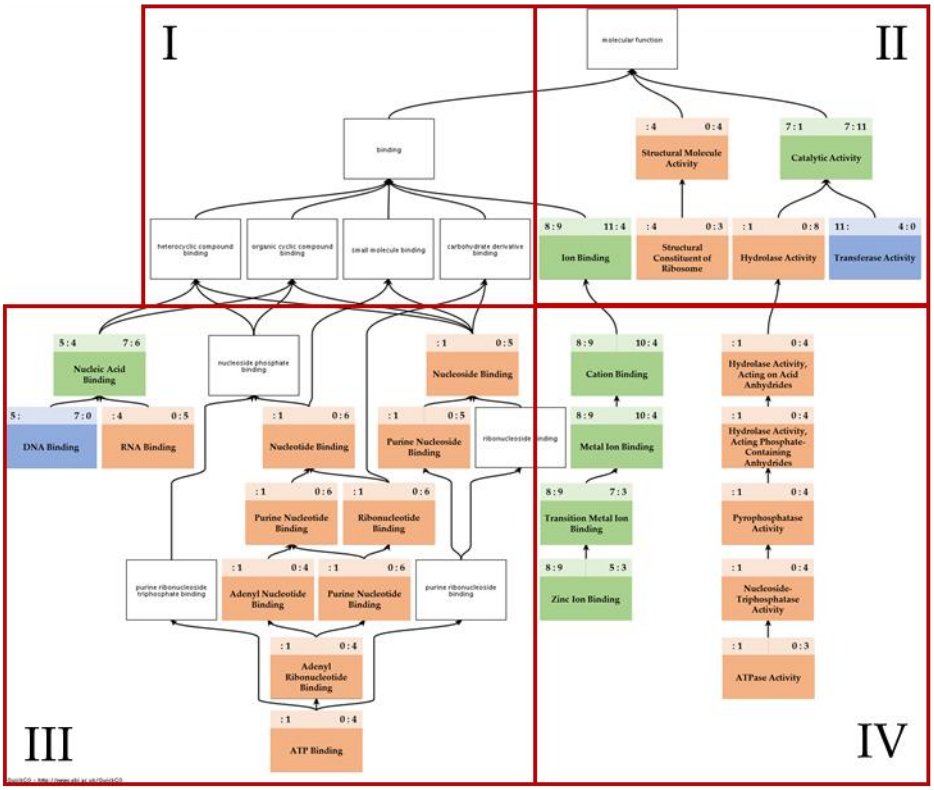

Figure A.9: Molecular functions GOTerm annotations for proteins that were identified as interacting partners with mARC1 and mARC2. Green boxes depict annotations found in proteins identified in both mARC1 and mARC2, blue boxes depict annotations found in proteins identified in both mARC1, and orange boxes depict annotations found in proteins identified in both mARC2. The DAVID cluster number is in the top right corner of each box (mARC1:mARC2). The number of proteins with that annotation are found in the top left of each box (mARC1:mARC2). **Figures A.10** through **Figure A.13** are expanded portions of the individual quadrants.

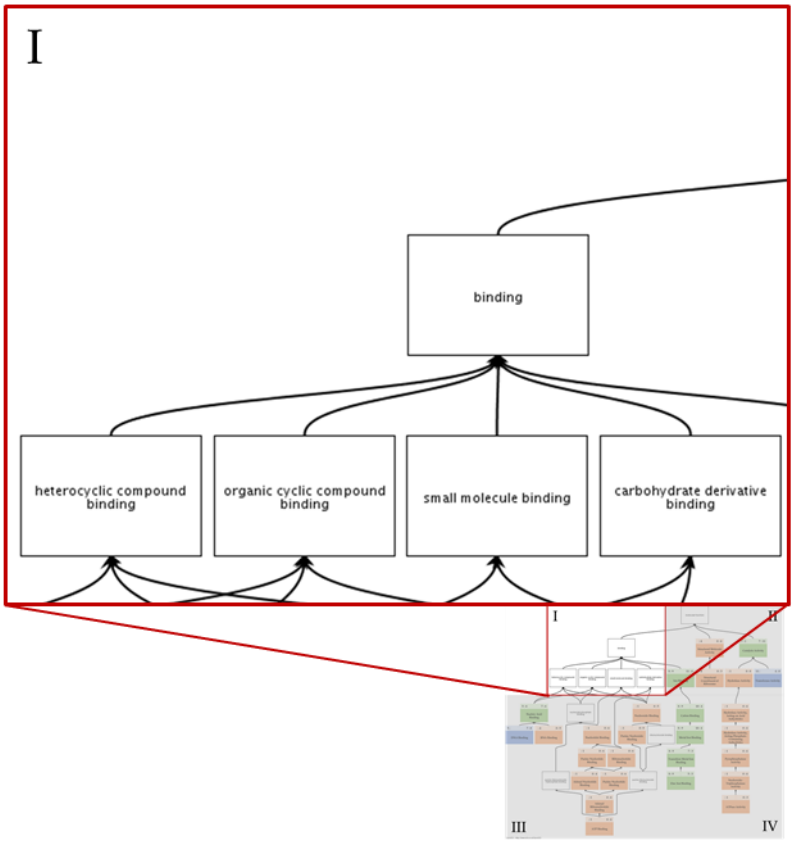

Figure A.10: Expanded Quadrant I of the GOTerm annotations tree for proteins that were identified as interacting partners with mARC1 and mARC2.

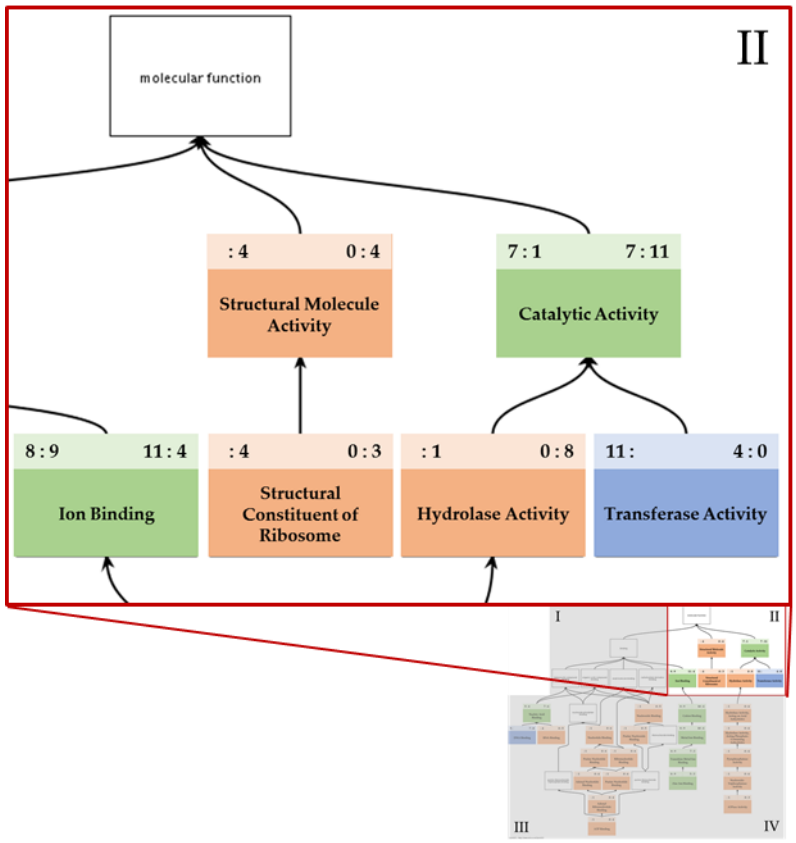

Figure A.11: Expanded Quadrant II of the GOTerm annotations tree for proteins that were identified as interacting partners with mARC1 and mARC2.

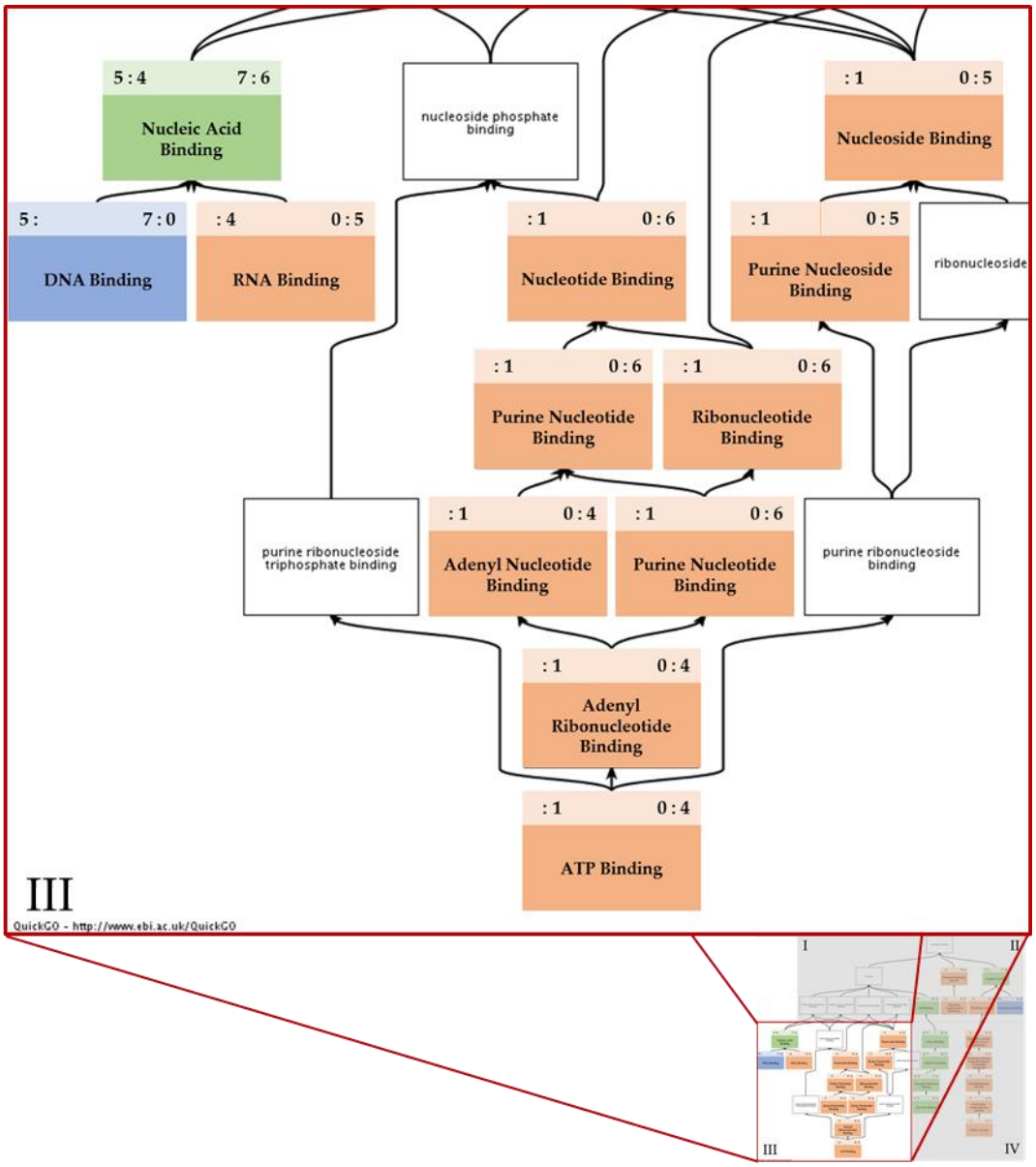

Figure A.12: Expanded Quadrant III of the GOTerm annotations tree for proteins that were identified as interacting partners with mARC1 and mARC2.

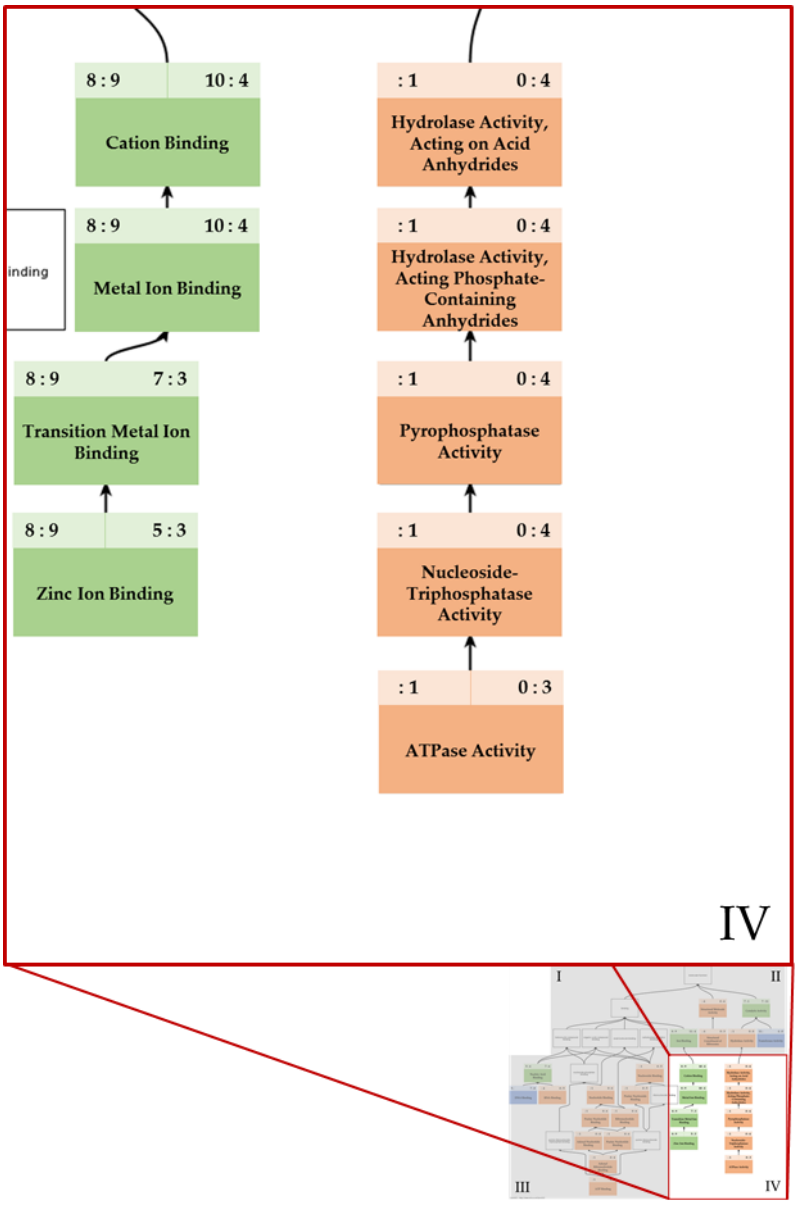

Figure A.13: Expanded Quadrant IV of the GOTerm annotations tree for proteins that were identified as interacting partners with mARC1 and mARC2.

Table A.4: GOTerm biological and molecular process annotations for proteins interacting with mARC1 and mARC2. The number of interacting proteins identified for mARC1 and mARC2 are listed under the annotation tag with a short description that was found from http://www.ebi.ac.uk/

Annotated GOTerm (# of Proteins)	**Description of Term**
Amine metabolic process (mARC1 : 3) (mARC2 : 0)	The chemical reactions and pathways involving any organic compound that is weakly basic in character and contains an amino or a substituted amino group, as carried out by individual cells. Amines are called primary, secondary, or tertiary according to whether one, two, or three carbon atoms are attached to the nitrogen atom.
Biosynthetic process (mARC1 : 7) (mARC2 : 7)	The chemical reactions and pathways resulting in the formation of substances; typically the energy-requiring part of metabolism in which simpler substances are transformed into more complex ones.
Carboxylic acid metabolic process (mARC1 : 3) (mARC2 : 0)	The chemical reactions and pathways involving carboxylic acids, any organic acid containing one or more carboxyl (COOH) groups or anions (COO-).
Cellular amino acid metabolic process (mARC1 : 3) (mARC2 : 0)	The chemical reactions and pathways involving amino acids, carboxylic acids containing one or more amino groups, as carried out by individual cells.
Cellular biosynthetic process (mARC1 : 7) (mARC2 : 6)	The chemical reactions and pathways resulting in the formation of substances, carried out by individual cells.
Cellular ketone metabolic process (mARC1 : 3) (mARC2 : 0)	The chemical reactions and pathways involving any of a class of organic compounds that contain the carbonyl group, CO, and in which the carbonyl group is bonded only to carbon atoms, as carried out by individual cells. The general formula for a ketone is RCOR, where R and R are alkyl or aryl groups.
Cellular macromolecule biosynthetic process (mARC1 : 5) (mARC2 : 4)	The chemical reactions and pathways resulting in the formation of a macromolecule, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass, carried out by individual cells.
Cellular macromolecule metabolic process (mARC1 : 8) (mARC2 : 9)	The chemical reactions and pathways involving macromolecules, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass, as carried out by individual cells.
Cellular metabolic process (mARC1 : 11) (mARC2 : 8)	The chemical reactions and pathways by which individual cells transform chemical substances.
Cellular nitrogen compound metabolic process (mARC1 : 8) (mARC2 : 4)	The chemical reactions and pathways involving various organic and inorganic nitrogenous compounds, as carried out by individual cells.

Table A.4: Continued

Annotated GOTerm (# of Proteins)	Description of Term
Cellular process (mARC1 : 0) (mARC2 : 19)	Any process that is carried out at the cellular level, but not necessarily restricted to a single cell. For example, cell communication occurs among more than one cell, but occurs at the cellular level.
Cellular protein metabolic process (mARC1 : 0) (mARC2 : 8)	The chemical reactions and pathways involving a specific protein, rather than of proteins in general, occurring at the level of an individual cell. Includes cellular protein modification.
Cellular protein modification process (mARC1 : 0) (mARC2 : 3)	The covalent alteration of one or more amino acids occurring in proteins, peptides and nascent polypeptides (co-translational, post-translational modifications) occurring at the level of an individual cell. Includes the modification of charged tRNAs that are destined to occur in a protein (pre-translation modification).
Heterocycle metabolic process (mARC1 : 0) (mARC2 : 4)	The chemical reactions and pathways involving heterocyclic compounds, those with a cyclic molecular structure and at least two different atoms in the ring (or rings).
Macromolecule biosynthetic process (mARC1 : 5) (mARC2 : 3)	The chemical reactions and pathways resulting in the formation of a macromolecule, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass.
Macromolecule metabolic process (mARC1 : 8) (mARC2 : 10)	The chemical reactions and pathways involving macromolecules, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass.
Macromolecule modification (mARC1 : 0) (mARC2 : 3)	The covalent alteration of one or more monomeric units in a polypeptide, polynucleotide, polysaccharide, or other biological macromolecule, resulting in a change in its properties.
Metabolic process (mARC1 : 12) (mARC2 : 15)	The chemical reactions and pathways, including anabolism and catabolism, by which living organisms transform chemical substances. Metabolic processes typically transform small molecules, but also include macromolecular processes such as DNA repair and replication, and protein synthesis and degradation.
Nitrogen compound metabolic process (mARC1 : 8) (mARC2 : 5)	The chemical reactions and pathways involving organic or inorganic compounds that contain nitrogen, including (but not limited to) nitrogen fixation, nitrification, denitrification, assimilatory/dissimilatory nitrate reduction and the interconversion of nitrogenous organic matter and ammonium.
Nucleobase-containing compound metabolic process (mARC1 : 4) (mARC2 : 3)	Any cellular metabolic process involving nucleobases, nucleosides, nucleotides and nucleic acids.
Organic acid metabolic process (mARC1 : 3) (mARC2 : 0)	The chemical reactions and pathways involving organic acids, any acidic compound containing carbon in covalent linkage.

Table A.4: Continued

Annotated GOTerm (# of Proteins)	Description of Term
Oxoacid metabolic process (mARC1 : 3) (mARC2 : 0)	The chemical reactions and pathways involving any oxoacid; an oxoacid is a compound which contains oxygen, at least one other element, and at least one hydrogen bound to oxygen, and which produces a conjugate base by loss of positive hydrogen ion(s) (hydrons).
Primary metabolic process (mARC1 : 11) (mARC2 : 13)	The chemical reactions and pathways involving those compounds which are formed as a part of the normal anabolic and catabolic processes. These processes take place in most, if not all, cells of the organism.
Post-translational protein modification (mARC1 : 0) (mARC2 : 3)	The process of covalently altering one or more amino acids in a protein after the protein has been completely translated and released from the ribosome.
Protein metabolic process (mARC1 : 0) (mARC2 : 9)	The chemical reactions and pathways involving a specific protein, rather than of proteins in general. Includes protein modification.
Signal transduction (mARC1 : 0) (mARC2 : 3)	The cellular process in which a signal is conveyed to trigger a change in the activity or state of a cell. Signal transduction begins with reception of a signal (e.g. a ligand binding to a receptor or receptor activation by a stimulus such as light), or for signal transduction in the absence of ligand, signal-withdrawal or the activity of a constitutively active receptor. Signal transduction ends with regulation of a downstream cellular process, e.g. regulation of transcription or regulation of a metabolic process. Signal transduction covers signaling from receptors located on the surface of the cell and signaling via molecules located within the cell. For signaling between cells, signal transduction is restricted to events at and within the receiving cell.

Table A.5: GOTerm regulation process annotations for proteins interacting with mARC1 and mARC2. The number of interacting proteins identified for mARC1 and mARC2 are listed under the annotation tag with a short description that was found from http://www.ebi.ac.uk/

Annotated GOTerm (# of Proteins)	**Description of Term**
Regulation of cellular metabolic process (mARC1 : 5) (mARC2 : 4)	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways by which individual cells transform chemical substances.
Biological regulation (mARC1 : 13) (mARC2 : 9)	Any process that modulates a measurable attribute of any biological process, quality or function.
Positive regulation of biological process (mARC1 : 3) (mARC2 : 0)	Any process that activates or increases the frequency, rate or extent of a biological process. Biological processes are regulated by many means; examples include the control of gene expression, protein modification or interaction with a protein or substrate molecule.
Positive regulation of cellular process (mARC1 : 3) (mARC2 : 0)	Any process that activates or increases the frequency, rate or extent of a cellular process, any of those that are carried out at the cellular level, but are not necessarily restricted to a single cell. For example, cell communication occurs among more than one cell, but occurs at the cellular level.
Regulation of biological process (mARC1 : 12) (mARC2 : 8)	Any process that modulates the frequency, rate or extent of a biological process. Biological processes are regulated by many means; examples include the control of gene expression, protein modification or interaction with a protein or substrate molecule.
Regulation of Biological Quality (mARC1 : 0) (mARC2 : 5)	Any process that modulates a qualitative or quantitative trait of a biological quality. A biological quality is a measurable attribute of an organism or part of an organism, such as size, mass, shape, color, etc.
Regulation of biosynthetic process (mARC1 : 5) (mARC2 : 3)	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways resulting in the formation of substances.
Regulation of cellular biosynthetic process (mARC1 : 5) (mARC2 : 3)	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways resulting in the formation of substances, carried out by individual cells.
Regulation of cellular protein metabolic process (mARC1 : 0) (mARC2 : 3)	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving a protein, occurring at the level of an individual cell.
Regulation of cellular process (mARC1 : 12) (mARC2 : 8)	Any process that modulates the frequency, rate or extent of a cellular process, any of those that are carried out at the cellular level, but are not necessarily restricted to a single cell. For example, cell communication occurs among more than one cell, but occurs at the cellular level.

Table A.5: Continued

Annotated GOTerm (# of Proteins)	Description of Term
Regulation of macromolecule biosynthetic process (mARC1 : 4) (mARC2 : 3)	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving macromolecules, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass.
Regulation of macromolecule metabolic process (mARC1 : 5) (mARC2 : 4)	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving macromolecules, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass.
Regulation of metabolic process (mARC1 : 5) (mARC2 : 4)	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways within a cell or an organism.
Regulation of nitrogen compound metabolic Process (mARC1 : 4) (mARC2 : 0)	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving nitrogen or nitrogenous compounds.
Regulation of nucleobase-containing metabolic process (mARC1 : 4) (mARC2 : 0)	Any cellular process that modulates the frequency, rate or extent of the chemical reactions and pathways involving nucleobases, nucleosides, nucleotides and nucleic acids.
Regulation of primary metabolic process (mARC1 : 5) (mARC2 : 4)	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways within a cell or an organism involving those compounds formed as a part of the normal anabolic and catabolic processes. These processes take place in most, if not all, cells of the organism.
Regulation of protein metabolic process (mARC1 : 0) (mARC2 : 3)	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving a protein.
Regulation of RNA metabolic process (mARC1 : 3) (mARC2 : 0)	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving RNA.

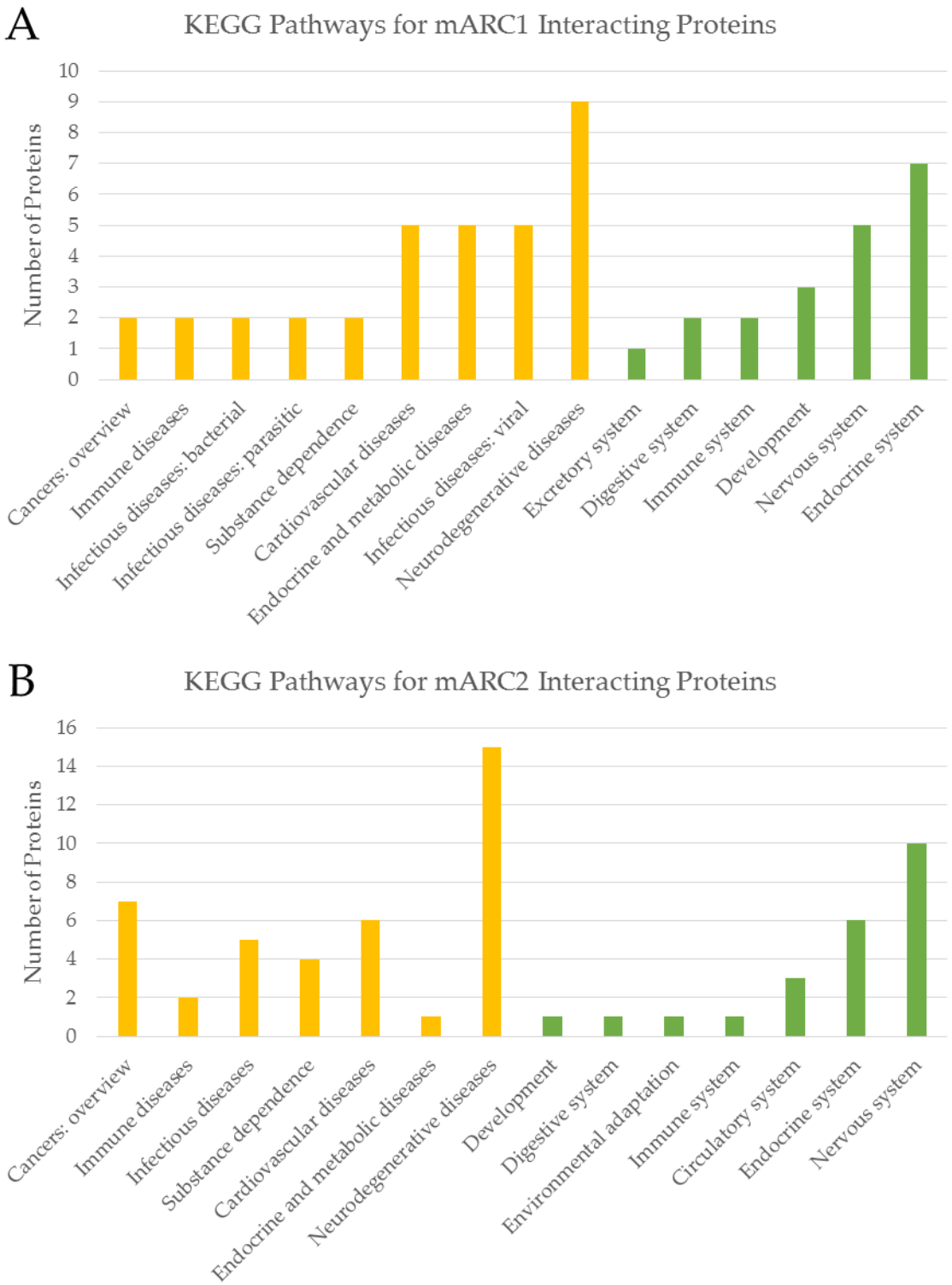

Figure A.14: KEGG pathways for proteins interacting with mARC proteins for human diseases and organismal system categories. A. KEGG human diseases and organismal system categories for proteins interacting with mARC1, B. KEGG human diseases and organismal system categories for proteins interacting with mARC2.

Table A.6: Annotated membrane transport proteins for proteins identified as interacting with mARC2.

UniProt ID	Proteins Names
Q9NUT2	ATP binding cassette subfamily B member 8
P36542	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1
P48047	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit
O75947	ATP synthase, H+ transporting, mitochondrial Fo complex subunit D
O43708	Glutathione S-transferase zeta 1
Q6UB35	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like
Q9NZB8	Molybdenum cofactor synthesis 1
O96007	Molybdenum cofactor synthesis 2
Q13496	Myotubularin 1
Q15185	Prostaglandin E synthase 3
P14410	Sucrase-isomaltase

Table A.7: GOTerm annotations and clusters for proteins identified as interacting with mARC1.

Cluster	UniProt ID	Protein Name	GOTerm Annotations
1	P02768	albumin	establishment of localization, ion binding, localization, transport
	P06727	apolipoprotein A-IV	establishment of localization, ion binding, localization, macromolecule localization, transport
	P21549	alanine-glyoxylate aminotransferase	establishment of localization, localization, macromolecule localization, transport
	P31040	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	establishment of localization, localization, transport
	P78334	gamma-aminobutyric acid (GABA) A receptor, epsilon	establishment of localization, ion binding, localization, transport
	Q32M45	anoctamin 4	establishment of localization, ion binding, localization, transport
	Q3SY52	zinc finger protein interacting with K protein 1 homolog	ion binding
	Q7L0Q8	ras homolog gene family, member U	ion binding
	Q8IUH5	zinc finger, DHHC-type containing 17	establishment of localization, ion binding, localization, macromolecule localization, transport
	Q8NFW9	myosin VIIA and Rab interacting protein	establishment of localization, ion binding, localization, macromolecule localization, transport
	Q969R2	oxysterol binding protein 2	establishment of localization, localization, macromolecule localization, transport
	Q9BT88	synaptotagmin XI	establishment of localization, ion binding, localization, transport
	Q9BZE0	GLIS family zinc finger 2	ion binding
	Q9H2M3	betaine-homocysteine methyltransferase 2	ion binding
2	P04908	histone cluster 1, H2ae; histone cluster 1, H2ab	cellular component assembly, cellular component biogenesis, cellular component organization, cellular macromolecular complex assembly, cellular macromolecular complex subunit organization, macromolecular complex assembly, macromolecular complex subunit organization
	P06727	apolipoprotein A-IV	cellular component assembly, cellular component biogenesis, cellular component organization, macromolecular complex assembly, macromolecular complex subunit organization
	P21549	alanine-glyoxylate aminotransferase	cellular component organization
	Q14684	ribosomal RNA processing 1 homolog B	cellular component biogenesis
	Q15431	synaptonemal complex protein 1	cellular component assembly, cellular component biogenesis, cellular component organization

Table A.7: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
2	Q53EZ4	centrosomal protein 55kDa	cellular component organization
	Q7L0Q8	ras homolog gene family, member U	cellular component organization
	Q8NCR6	chromosome 9 open reading frame 24	cellular component assembly, cellular component biogenesis, cellular component organization, cellular macromolecular complex assembly, cellular macromolecular complex subunit organization, macromolecular complex assembly, macromolecular complex subunit organization
	Q9BTM1	H2A histone family, member J	cellular component assembly, cellular component biogenesis, cellular component organization, cellular macromolecular complex assembly, cellular macromolecular complex subunit organization, macromolecular complex assembly, macromolecular complex subunit organization
	P04908	histone cluster 1, H2ae; histone cluster 1, H2ab	cellular component assembly, cellular component biogenesis, cellular component organization, cellular macromolecular complex assembly, cellular macromolecular complex subunit organization, macromolecular complex assembly, macromolecular complex subunit organization
	P06727	apolipoprotein A-IV	cellular component assembly, cellular component biogenesis, cellular component organization, macromolecular complex assembly, macromolecular complex subunit organization
	P21549	alanine-glyoxylate aminotransferase	cellular component organization
	Q14684	ribosomal RNA processing 1 homolog B	cellular component biogenesis
	Q15431	synaptonemal complex protein 1	cellular component assembly, cellular component biogenesis, cellular component organization
3	P06727	apolipoprotein A-IV	amine metabolic process, cellular amine metabolic process, cellular amino acid and derivative metabolic process
	P21549	alanine-glyoxylate aminotransferase	amine metabolic process, cellular amine metabolic process, cellular amino acid and derivative metabolic process
	Q9H2M3	betaine-homocysteine methyltransferase 2	amine metabolic process, cellular amine metabolic process, cellular amino acid and derivative metabolic process
4	P04908	histone cluster 1, H2ae; histone cluster 1, H2ab	organelle organization
	P21549	alanine-glyoxylate aminotransferase	organelle organization
	Q15431	synaptonemal complex protein 1	cell cycle, cell cycle phase, cell cycle process, organelle organization
	Q53EZ4	centrosomal protein 55kDa	cell cycle, cell cycle phase, cell cycle process, organelle organization

Table A.7: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
4	Q7L0Q8	ras homolog gene family, member U	cell cycle, cell cycle phase, cell cycle process, organelle organization
	Q9BTM1	H2A histone family, member J	organelle organization
5	P02768	albumin	DNA binding, nucleic acid binding
	P04908	histone cluster 1, H2ae; histone cluster 1, H2ab	chromosome organization, DNA binding, nucleic acid binding
	Q15431	synaptonemal complex protein 1	chromosome organization, DNA binding, nucleic acid binding
	Q3SY52	zinc finger protein interacting with K protein 1	DNA binding, nucleic acid binding
	Q8N187	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 8	DNA binding, nucleic acid binding
	Q9BTM1	H2A histone family, member J	chromosome organization, DNA binding, nucleic acid binding
	Q9BZE0	GLIS family zinc finger 2	DNA binding, nucleic acid binding
6	P06727	apolipoprotein A-IV	macromolecule localization
	P21549	alanine-glyoxylate aminotransferase	establishment of protein localization, macromolecule localization, protein localization, protein transport
	Q8IUH5	zinc finger, DHHC-type containing 17	establishment of protein localization, macromolecule localization, protein localization, protein transport
	Q8NFW9	myosin VIIA and Rab interacting protein	establishment of protein localization, macromolecule localization, protein localization, protein transport
	Q969R2	oxysterol binding protein 2	macromolecule localization
7	P21549	alanine-glyoxylate aminotransferase	carboxylic acid metabolic process, catalytic activity, cellular ketone metabolic process, organic acid metabolic process, oxoacid metabolic process
	P31040	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	carboxylic acid metabolic process, catalytic activity, cellular ketone metabolic process, organic acid metabolic process, oxoacid metabolic process
	P46977	STT3, subunit of the oligosaccharyltransferase complex, homolog A	catalytic activity
	P51160	phosphodiesterase 6C, cGMP-specific, cone, alpha prime	catalytic activity
	Q7L0Q8	ras homolog gene family, member U	catalytic activity
	Q8IUH5	zinc finger, DHHC-type containing 17	catalytic activity

Table A.7: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
7	Q9H2M3	betaine-homocysteine methyltransferase 2	carboxylic acid metabolic process, catalytic activity, cellular ketone metabolic process, organic acid metabolic process, oxoacid metabolic process
8	P02768	albumin	cation binding, ion binding, metal ion binding, transition metal ion binding
	P06727	apolipoprotein A-IV	cation binding, ion binding, metal ion binding, transition metal ion binding
	P78334	gamma-aminobutyric acid (GABA) A receptor, epsilon	ion binding
	Q32M45	anoctamin 4	cation binding, ion binding, metal ion binding
	Q3SY52	zinc finger protein interacting with K protein 1	cation binding, ion binding, metal ion binding, transition metal ion binding, zinc ion binding
	Q7L0Q8	ras homolog gene family, member U	cation binding, ion binding, metal ion binding
	Q8IUH5	zinc finger, DHHC-type containing 17	cation binding, ion binding, metal ion binding, transition metal ion binding, zinc ion binding
	Q8NFW9	myosin VIIA and Rab interacting protein	cation binding, ion binding, metal ion binding, transition metal ion binding, zinc ion binding
	Q9BT88	synaptotagmin XI	cation binding, ion binding, metal ion binding
	Q9BZE0	GLIS family zinc finger 2	cation binding, ion binding, metal ion binding, transition metal ion binding, zinc ion binding
	Q9H2M3	betaine-homocysteine methyltransferase 2	cation binding, ion binding, metal ion binding, transition metal ion binding, zinc ion binding
9	P02768	albumin	transition metal ion binding
	P06727	apolipoprotein A-IV	positive regulation of biological process, positive regulation of cellular process, transition metal ion binding
	Q3SY52	zinc finger protein interacting with K protein 1 homolog	transition metal ion binding
	Q8IUH5	zinc finger, DHHC-type containing 17	positive regulation of biological process, positive regulation of cellular process, transition metal ion binding
	Q8NFW9	myosin VIIA and Rab interacting protein	transition metal ion binding
	Q9BZE0	GLIS family zinc finger 2	positive regulation of biological process, positive regulation of cellular process, transition metal ion binding
	Q9H2M3	betaine-homocysteine methyltransferase 2	transition metal ion binding

Table A.7: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
10	P02768	albumin	biological regulation, regulation of biological process, regulation of cellular process
	P06727	apolipoprotein A-IV	biological regulation, regulation of biological process, regulation of cellular process
	P27816	microtubule-associated protein 4	biological regulation, regulation of biological process, regulation of cellular process
	P51160	phosphodiesterase 6C, cGMP-specific, cone, alpha prime	biological regulation, regulation of biological process, regulation of cellular process
	Q04726	transducin-like enhancer of split 3	biological regulation, regulation of biological process, regulation of cellular process
	Q15431	synaptonemal complex protein 1	biological regulation, regulation of biological process
	Q3MIN7	ral guanine nucleotide dissociation stimulator-like 3	biological regulation, regulation of biological process, regulation of cellular process
	Q3SY52	zinc finger protein interacting with K protein 1 homolog	biological regulation, regulation of biological process, regulation of cellular process
	Q6MZT1	regulator of G-protein signaling 7 binding protein	biological regulation, regulation of biological process, regulation of cellular process
	Q7L0Q8	ras homolog gene family, member U	biological regulation, regulation of biological process, regulation of cellular process
	Q8IUH5	zinc finger, DHHC-type containing 17	biological regulation, regulation of biological process, regulation of cellular process
	Q8N187	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 8	biological regulation, regulation of biological process, regulation of cellular process
	Q9BZE0	GLIS family zinc finger 2	biological regulation, regulation of biological process, regulation of cellular process
11	P06727	apolipoprotein A-IV	cellular macromolecule metabolic process, cellular metabolic process, cellular nitrogen compound metabolic process, macromolecule metabolic process, metabolic process, nitrogen compound metabolic process, nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, primary metabolic process, regulation of biosynthetic process, regulation of cellular biosynthetic process, regulation of cellular metabolic process, regulation of macromolecule metabolic process, regulation of metabolic process

Table A.7: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
11	P21549	alanine-glyoxylate aminotransferase	biosynthetic process, cellular biosynthetic process, cellular metabolic process, cellular nitrogen compound metabolic process, metabolic process, nitrogen compound metabolic process, primary metabolic process, transferase activity
	P31040	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	cellular metabolic process, metabolic process
	P46977	STT3, subunit of the oligosaccharyltransferase complex	biosynthetic process, cellular biosynthetic process, cellular macromolecule biosynthetic process, cellular metabolic process, macromolecule biosynthetic process, macromolecule metabolic process, metabolic process, primary metabolic process, regulation of macromolecule metabolic process, transferase activity
	Q04726	transducin-like enhancer of split 3	biosynthetic process, cellular biosynthetic process, cellular macromolecule biosynthetic process, cellular macromolecule metabolic process, cellular metabolic process, cellular nitrogen compound metabolic process, gene expression, macromolecule biosynthetic process, macromolecule metabolic process, metabolic process, nitrogen compound metabolic process, nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, primary metabolic process, regulation of biosynthetic process, regulation of cellular biosynthetic process, regulation of cellular metabolic process, regulation of gene expression, regulation of macromolecule biosynthetic process, regulation of macromolecule metabolic process, regulation of metabolic process, regulation of nitrogen compound metabolic process, regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, regulation of primary metabolic process, regulation of RNA metabolic process, regulation of transcription, regulation of transcription, DNA-dependent, transcription
	Q14684	ribosomal RNA processing 1 homolog B	cellular metabolic process, cellular nitrogen compound metabolic process, gene expression, macromolecule metabolic process, metabolic process, nitrogen compound metabolic process, primary metabolic process, regulation of macromolecule metabolic process, regulation of primary metabolic process
	Q15431	synaptonemal complex protein 1	cellular metabolic process, cellular nitrogen compound metabolic process, macromolecule metabolic process, metabolic process, nitrogen compound metabolic process, primary metabolic process, regulation of macromolecule metabolic process, regulation of primary metabolic process

Table A.7: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
11	Q3SY52	zinc finger protein interacting with K protein 1	biosynthetic process, cellular biosynthetic process, cellular macromolecule biosynthetic process, cellular macromolecule metabolic process, cellular metabolic process, cellular nitrogen compound metabolic process, gene expression, macromolecule biosynthetic process, macromolecule metabolic process, metabolic process, nitrogen compound metabolic process, nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, primary metabolic process, regulation of biosynthetic process, regulation of cellular biosynthetic process, regulation of cellular metabolic process, regulation of gene expression, regulation of macromolecule biosynthetic process, regulation of macromolecule metabolic process, regulation of metabolic process, regulation of nitrogen compound metabolic process, regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, regulation of primary metabolic process, regulation of RNA metabolic process, regulation of transcription, regulation of transcription, DNA-dependent, transcription
	Q8IUH5	zinc finger, DHHC-type containing 17	biosynthetic process, cellular biosynthetic process, cellular macromolecule biosynthetic process, cellular metabolic process, macromolecule biosynthetic process, macromolecule metabolic process, metabolic process, primary metabolic process, regulation of macromolecule metabolic process, transferase activity
	Q8N187	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 8	cellular macromolecule metabolic process, nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, regulation of biosynthetic process, regulation of cellular biosynthetic process, regulation of cellular metabolic process, regulation of gene expression, regulation of macromolecule biosynthetic process, regulation of metabolic process, regulation of nitrogen compound metabolic process, regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, regulation of RNA metabolic process, regulation of transcription, regulation of transcription, DNA-dependent
	Q969R2	oxysterol binding protein 2	metabolic process, primary metabolic process

Table A.7: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
11	Q9BZE0	GLIS family zinc finger 2	biosynthetic process, cellular biosynthetic process, cellular macromolecule biosynthetic process, cellular macromolecule metabolic process, cellular metabolic process, cellular nitrogen compound metabolic process, gene expression, macromolecule biosynthetic process, macromolecule metabolic process, metabolic process, nitrogen compound metabolic process, nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, primary metabolic process, regulation of biosynthetic process, regulation of cellular biosynthetic process, regulation of cellular metabolic process, regulation of gene expression, regulation of macromolecule biosynthetic process, regulation of macromolecule metabolic process, regulation of metabolic process, regulation of nitrogen compound metabolic process, regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, regulation of primary metabolic process, regulation of transcription, transcription
	Q9H2M3	betaine-homocysteine methyltransferase 2	biosynthetic process, cellular biosynthetic process, cellular metabolic process, cellular nitrogen compound metabolic process, metabolic process, nitrogen compound metabolic process, primary metabolic process, transferase activity
12	P06727	apolipoprotein A-IV	multicellular organismal process
	P31040	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	anatomical structure development, developmental process, multicellular organismal development, multicellular organismal process, system development
	P51160	phosphodiesterase 6C, cGMP-specific, cone, alpha prime	multicellular organismal process
	Q04726	transducin-like enhancer of split 3	anatomical structure development, developmental process, multicellular organismal development, multicellular organismal process, system development
	Q15431	synaptonemal complex protein 1	multicellular organismal process
	Q9BZE0	GLIS family zinc finger 2	anatomical structure development, developmental process, multicellular organismal development, multicellular organismal process, system development

Table A.8: GOTerm annotations and clusters for proteins identified as interacting with mARC2.

Cluster	UniProt ID	Protein Name	GOTerm Annotations
1	O75891	aldehyde dehydrogenase 1 family, member L1	catalytic activity
	O75947	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d	ATPase activity, catalytic activity, hydrolase activity, hydrolase activity, acting on acid anhydrides, hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides, nucleoside-triphosphatase activity, pyrophosphatase activity
	O76074	phosphodiesterase 5A, cGMP-specific	catalytic activity, hydrolase activity, nucleoside binding, nucleotide binding, purine nucleoside binding, purine nucleotide binding, purine ribonucleotide binding, ribonucleotide binding
	P00748	coagulation factor XII	catalytic activity, hydrolase activity
	P54764	EPH receptor A4	adenyl nucleotide binding, adenyl ribonucleotide binding, ATP binding, catalytic activity, nucleoside binding, nucleotide binding, purine nucleoside binding, purine nucleotide binding, purine ribonucleotide binding, ribonucleotide binding
	Q13496	myotubularin 1	catalytic activity, hydrolase activity
	Q5W0Q7	ubiquitin specific peptidase like 1	catalytic activity, hydrolase activity
	Q6UB35	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like	adenyl nucleotide binding, adenyl ribonucleotide binding, ATP binding, catalytic activity, nucleoside binding, nucleotide binding, purine nucleoside binding, purine nucleotide binding, purine ribonucleotide binding, ribonucleotide binding
	Q71U36	tubulin, alpha 1a	catalytic activity, hydrolase activity, hydrolase activity, acting on acid anhydrides, hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides, nucleoside-triphosphatase activity, nucleotide binding, purine nucleotide binding, purine ribonucleotide binding, pyrophosphatase activity, ribonucleotide binding
	Q86XP3	DEAD (Asp-Glu-Ala-Asp) box polypeptide 42	adenyl nucleotide binding, adenyl ribonucleotide binding, ATP binding, ATPase activity, catalytic activity, hydrolase activity, hydrolase activity, acting on acid anhydrides, hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides, nucleoside binding, nucleoside-triphosphatase activity, nucleotide binding, purine nucleoside binding, purine nucleotide binding, purine ribonucleotide binding, pyrophosphatase activity, ribonucleotide binding

Table A.8: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
1	Q9NUT2	ATP-binding cassette, sub-family B (MDR/TAP), member 8	adenyl nucleotide binding, adenyl ribonucleotide binding, ATP binding, ATPase activity, catalytic activity, hydrolase activity, hydrolase activity, acting on acid anhydrides, hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides, nucleoside binding, nucleoside-triphosphatase activity, nucleotide binding, purine nucleoside binding, purine nucleotide binding, purine ribonucleotide binding, pyrophosphatase activity, ribonucleotide binding
2	O75891	aldehyde dehydrogenase 1 family, member L1	cellular metabolic process, cellular process, metabolic process
	O75947	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d	cellular metabolic process, cellular process, metabolic process, primary metabolic process
	O76074	phosphodiesterase 5A, cGMP-specific	cellular metabolic process, cellular process, metabolic process, primary metabolic process
	O94927	HAUS augmin-like complex, subunit 5	cellular process
	O95696	bromodomain containing 1	cellular macromolecule metabolic process, cellular metabolic process, cellular process, cellular protein metabolic process, macromolecule metabolic process, metabolic process, primary metabolic process, protein metabolic process
	P00748	coagulation factor XII	macromolecule metabolic process, metabolic process, primary metabolic process, protein metabolic process
	P46782	ribosomal protein S5	cellular macromolecule metabolic process, cellular metabolic process, cellular process, cellular protein metabolic process, macromolecule metabolic process, metabolic process, primary metabolic process, protein metabolic process
	P54764	EPH receptor A4	cellular macromolecule metabolic process, cellular metabolic process, cellular process, cellular protein metabolic process, macromolecule metabolic process, metabolic process, primary metabolic process, protein metabolic process
	P62269	ribosomal protein S18 pseudogene 12; ribosomal protein S18 pseudogene 5; ribosomal protein S18	cellular macromolecule metabolic process, cellular metabolic process, cellular process, cellular protein metabolic process, macromolecule metabolic process, metabolic process, primary metabolic process, protein metabolic process

Table A.8: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
2	P62701	ribosomal protein S4X pseudogene 6; ribosomal protein S4X pseudogene 13; ribosomal protein S4, X-linked	cellular macromolecule metabolic process, cellular metabolic process, cellular process, cellular protein metabolic process, macromolecule metabolic process, metabolic process, primary metabolic process, protein metabolic process
	Q13496	myotubularin 1	cellular macromolecule metabolic process, cellular metabolic process, cellular process, cellular protein metabolic process, macromolecule metabolic process, metabolic process, primary metabolic process, protein metabolic process
	Q5W0Q7	ubiquitin specific peptidase like 1	cellular macromolecule metabolic process, cellular metabolic process, cellular process, cellular protein metabolic process, macromolecule metabolic process, metabolic process, primary metabolic process, protein metabolic process
	Q6PJ61	F-box protein 46	cellular macromolecule metabolic process, cellular metabolic process, cellular process, cellular protein metabolic process, macromolecule metabolic process, metabolic process, primary metabolic process, protein metabolic process
	Q6UB35	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like	cellular metabolic process, cellular process, metabolic process
	Q71U36	tubulin, alpha 1a	cellular process
	Q8N7Q3	zinc finger protein 676	cellular macromolecule metabolic process, cellular metabolic process, cellular process, macromolecule metabolic process, metabolic process, primary metabolic process
	Q8TCU4	Alstrom syndrome 1	cellular metabolic process, cellular process, metabolic process, primary metabolic process
	Q9C0H9	hypothetical LOC100132856; hypothetical LOC100128100; hypothetical LOC100133017; SNAP25-interacting protein	cellular process
	Q9NUT2	ATP-binding cassette, sub-family B (MDR/TAP), member 8	cellular process
	Q9UKX5	integrin, alpha 11	cellular process
3	O75891	aldehyde dehydrogenase 1 family, member L1	biosynthetic process
	O75947	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d	biosynthetic process, cellular biosynthetic process

Table A.8: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
3	O76074	phosphodiesterase 5A, cGMP-specific	biological regulation, multicellular organismal process, regulation of biological process, regulation of biological quality, regulation of cellular process, response to stimulus
	Q6UB35	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like	heterocycle metabolic process, nitrogen compound metabolic process
	Q8N7Q3	zinc finger protein 676	cellular nitrogen compound metabolic process, nitrogen compound metabolic process, nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
4	O75891	aldehyde dehydrogenase 1 family, member L1	biosynthetic process
	O75947	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d	biosynthetic process, cellular biosynthetic process
	O76074	phosphodiesterase 5A, cGMP-specific	biological regulation, multicellular organismal process, regulation of biological process, regulation of biological quality, regulation of cellular process, response to stimulus
	P00748	coagulation factor XII	biological regulation, gene expression, multicellular organismal process, regulation of biological process, regulation of biological quality, regulation of cellular metabolic process, regulation of cellular process, regulation of cellular protein metabolic process, regulation of gene expression, regulation of macromolecule metabolic process, regulation of metabolic process, regulation of primary metabolic process, regulation of protein metabolic process, response to stimulus
	P46782	ribosomal protein S5	biological regulation, biosynthetic process, cellular biosynthetic process, cellular macromolecule biosynthetic process, gene expression, macromolecule biosynthetic process, nucleic acid binding, regulation of biological process, regulation of biological quality, regulation of biosynthetic process, regulation of cellular biosynthetic process, regulation of cellular metabolic process, regulation of cellular process, regulation of cellular protein metabolic process, regulation of gene expression, regulation of macromolecule biosynthetic process, regulation of macromolecule metabolic process, regulation of metabolic process, regulation of primary metabolic process, regulation of protein metabolic process, RNA binding, structural constituent of ribosome, structural molecule activity, translation, translational elongation

Table A.8: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
4	P54764	EPH receptor A4	biological regulation, multicellular organismal process, regulation of biological process, regulation of cellular process, response to stimulus
	P62269	ribosomal protein S18 pseudogene 12; ribosomal protein S18 pseudogene 5; ribosomal protein S18	biosynthetic process, cellular biosynthetic process, cellular macromolecule biosynthetic process, gene expression, macromolecule biosynthetic process, nucleic acid binding, RNA binding, structural constituent of ribosome, structural molecule activity, translation, translational elongation
	P62701	ribosomal protein S4X pseudogene 6; ribosomal protein S4X pseudogene 13; ribosomal protein S4, X-linked	biological regulation, biosynthetic process, cellular biosynthetic process, cellular macromolecule biosynthetic process, gene expression, macromolecule biosynthetic process, nucleic acid binding, regulation of biological process, regulation of biological quality, regulation of biosynthetic process, regulation of cellular biosynthetic process, regulation of cellular metabolic process, regulation of cellular process, regulation of cellular protein metabolic process, regulation of gene expression, regulation of macromolecule biosynthetic process, regulation of macromolecule metabolic process, regulation of metabolic process, regulation of primary metabolic process, regulation of protein metabolic process, RNA binding, structural constituent of ribosome, structural molecule activity, translation, translational elongation
	Q06787	fragile X mental retardation 1	multicellular organismal process, nucleic acid binding, RNA binding
	Q13496	myotubularin 1	biological regulation, multicellular organismal process, regulation of biological quality
	Q6UB35	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like	biosynthetic process, cellular biosynthetic process
	Q71U36	tubulin, alpha 1a	structural molecule activity
	Q86XP3	DEAD (Asp-Glu-Ala-Asp) box polypeptide 42	nucleic acid binding, RNA binding

Table A.8: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
4	Q8N7Q3	zinc finger protein 676	biological regulation, biosynthetic process, cellular biosynthetic process, cellular macromolecule biosynthetic process, gene expression, macromolecule biosynthetic process, nucleic acid binding, regulation of biological process, regulation of biosynthetic process, regulation of cellular biosynthetic process, regulation of cellular metabolic process, regulation of cellular process, regulation of gene expression, regulation of macromolecule biosynthetic process, regulation of macromolecule metabolic process, regulation of metabolic process, regulation of primary metabolic process
	Q8TCU4	Alstrom syndrome 1	biological regulation, multicellular organismal process, regulation of biological process, regulation of biological quality, regulation of cellular process, response to stimulus
	Q9C0H9	hypothetical LOC100132856; hypothetical LOC100128100; hypothetical LOC100133017; SNAP25-interacting protein	biological regulation, regulation of biological process, regulation of cellular process
	Q9UKX5	integrin, alpha 11	multicellular organismal process
5	O76074	phosphodiesterase 5A, cGMP-specific	anatomical structure development, biological regulation, developmental process, multicellular organismal development, multicellular organismal process, nervous system development, regulation of biological quality, response to stimulus, signal transduction, system development
	P00748	coagulation factor XII	biological regulation, multicellular organismal process, regulation of biological quality, response to stimulus
	P46782	ribosomal protein S5	biological regulation, regulation of biological quality
	P54764	EPH receptor A4	anatomical structure development, biological regulation, developmental process, multicellular organismal development, multicellular organismal process, nervous system development, response to stimulus, signal transduction, system development
	P62701	ribosomal protein S4X pseudogene 6; ribosomal protein S4X pseudogene 13; ribosomal protein S4, X-linked	biological regulation, developmental process, multicellular organismal development, multicellular organismal process
	Q06787	fragile X mental retardation 1	anatomical structure development, developmental process, multicellular organismal development, multicellular organismal process, nervous system development, system development
	Q13496	myotubularin 1	anatomical structure development, biological regulation, developmental process, multicellular organismal development, multicellular organismal process, organ development, regulation of biological quality, system development

Table A.8: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
5	Q8N7Q3	zinc finger protein 676	biological regulation
	Q8TCU4	Alstrom syndrome 1	anatomical structure development, biological regulation, developmental process, multicellular organismal development, multicellular organismal process, nervous system development, organ development, regulation of biological quality, response to stimulus, signal transduction, system development
	Q9C0H9	hypothetical LOC100132856; hypothetical LOC100128100; hypothetical LOC100133017; SNAP25-interacting protein	biological regulation
	Q9UKX5	integrin, alpha 11	anatomical structure development, developmental process, multicellular organismal development, multicellular organismal process, organ development, system development
6	O94927	HAUS augmin-like complex, subunit 5	cellular component assembly, cellular component biogenesis, cellular component organization
	O95696	bromodomain containing 1	cellular component organization
	P54764	EPH receptor A4	cellular component organization
	Q71U36	tubulin, alpha 1a	cellular component assembly, cellular component biogenesis, cellular component organization
	Q8TCU4	Alstrom syndrome 1	cellular component assembly, cellular component biogenesis, cellular component organization
7	O95696	bromodomain containing 1	biopolymer modification, post-translational protein modification, protein modification process
	P54764	EPH receptor A4	biopolymer modification, post-translational protein modification, protein modification process
	Q13496	myotubularin 1	biopolymer modification, post-translational protein modification, protein modification process
8	O75947	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d	establishment of localization, localization, transport
	Q06787	fragile X mental retardation 1	establishment of localization, localization, transport
	Q9C0H9	hypothetical LOC100132856; hypothetical LOC100128100; hypothetical LOC100133017; SNAP25-interacting protein	establishment of localization, localization, transport
	Q9NUT2	ATP-binding cassette, sub-family B (MDR/TAP), member 8	establishment of localization, localization, transport
	Q9UKX5	integrin, alpha 11	localization

Table A.8: Continued

Cluster	UniProt ID	Protein Name	GOTerm Annotations
9	O76074	phosphodiesterase 5A, cGMP-specific	cation binding, ion binding, metal ion binding, transition metal ion binding, zinc ion binding
	O95696	bromodomain containing 1	cation binding, ion binding, metal ion binding, transition metal ion binding, zinc ion binding
	Q8N7Q3	zinc finger protein 676	cation binding, ion binding, metal ion binding, transition metal ion binding, zinc ion binding
	Q9UKX5	integrin, alpha 11	cation binding, ion binding, metal ion binding

Table A.9: KEGG pathways for proteins identified as interacting with mARC1 in HEK-293 cell lysate.

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
O00330	pyruvate dehydrogenase complex component X	**Metabolism** *Global and overview maps* -Metabolic pathways	0.39
O00750	phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 beta	**Environmental Information Processing** *Signal transduction* -Phosphatidylinositol signaling system **Metabolism** *Carbohydrate metabolism* -Inositol phosphate metabolism *Global and overview maps* -Metabolic pathways	0.26
O14788	tumor necrosis factor superfamily member 11	**Environmental Information Processing** *Signal transduction* -NF-kappa B signaling pathway ***Signaling molecules and interaction*** -Cytokine-cytokine receptor interaction **Human Diseases** *Immune diseases* -Rheumatoid arthritis **Organismal Systems** *Development* -Osteoclast differentiation *Endocrine system* -Prolactin signaling pathway	0.37
O43181	NADH:ubiquinone oxidoreductase subunit S4	**Human Diseases** *Endocrine and metabolic diseases* -Non-alcoholic fatty liver disease (NAFLD) *Neurodegenerative diseases* -Alzheimer's disease -Huntington's disease -Parkinson's disease **Metabolism** *Energy metabolism* -Oxidative phosphorylation *Global and overview maps* -Metabolic pathways	0.2
O60260	parkin RBR E3 ubiquitin protein ligase	**Genetic Information Processing** *Folding, sorting and degradation* -Protein processing in endoplasmic reticulum -Ubiquitin mediated proteolysis **Human Diseases** *Neurodegenerative diseases* -Parkinson's disease	0.17
O60683	peroxisomal biogenesis factor 10	**Cellular Processes** *Transport and catabolism* -Peroxisome	0.28
O75581	LDL receptor related protein 6	**Environmental Information Processing** *Signal transduction* -Wnt signaling pathway	0.18

Table A.9: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
O95049	tight junction protein 3	**Cellular Processes** *Cellular community* -Tight junction	0.33
O95382	mitogen-activated protein kinase 6	**Environmental Information Processing** *Signal transduction* -MAPK signaling pathway	0.22
O95602	polymerase (RNA) I polypeptide A	**Genetic Information Processing** *Transcription* -RNA polymerase **Metabolism** *Global and overview maps* -Metabolic pathways *Nucleotide metabolism* -Purine metabolism -Pyrimidine metabolism	0.51
P00734	coagulation factor II, thrombin	**Cellular Processes** *Cell motility* -Regulation of actin cytoskeleton **Environmental Information Processing** *Signaling molecules and interaction* -Neuroactive ligand-receptor interaction **Organismal Systems** *Immune system* -Complement and coagulation cascades	0.51
P11216	phosphorylase, glycogen	**Human Diseases** *Endocrine and metabolic diseases* -Insulin resistance **Metabolism** *Carbohydrate metabolism* -Starch and sucrose metabolism *Global and overview maps* -Metabolic pathways **Organismal Systems** *Endocrine system* -Glucagon signaling pathway -Insulin signaling pathway	0.32

Table A.9: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
P15313	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B1	**Cellular Processes** *Transport and catabolism* -Phagosome **Human Diseases** *Immune diseases* -Rheumatoid arthritis *Infectious diseases: Bacterial* -Epithelial cell signaling in Helicobacter pylori infection -Vibrio cholerae infection **Metabolism** *Energy metabolism* -Oxidative phosphorylation *Global and overview maps* -Metabolic pathways **Organismal Systems** *Excretory system* -Collecting duct acid secretion *Nervous system* -Synaptic vesicle cycle	0.16
P18564	integrin subunit beta 6	**Cellular Processes** *Cell motility* -Regulation of actin cytoskeleton *Cellular community* -Focal adhesion **Environmental Information Processing** *Signal transduction* -PI3K-Akt signaling pathway *Signaling molecules and interaction* -ECM-receptor interaction **Human Diseases** *Cardiovascular diseases* -Arrhythmogenic right ventricular cardiomyopathy (ARVC) -Dilated cardiomyopathy -Hypertrophic cardiomyopathy (HCM)	0.37
P21549	alanine-glyoxylate aminotransferase	**Cellular Processes** *Transport and catabolism* -Peroxisome **Metabolism** *Amino acid metabolism* -Alanine, aspartate and glutamate metabolism -Glycine, serine and threonine metabolism *Carbohydrate metabolism* -Glyoxylate and dicarboxylate metabolism *Global and overview maps* -Carbon metabolism -Metabolic pathways	0.35

Table A.9: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
P28749	retinoblastoma-like 1	**Cellular Processes** *Cell growth and death* -Cell cycle **Environmental Information Processing** *Signal transduction* -TGF-beta signaling pathway **Human Diseases** *Cancers: Overview* -Viral carcinogenesis	0.11
P30679	guanine nucleotide binding protein (G protein), alpha 15	**Environmental Information Processing** *Signal transduction* -Calcium signaling pathway **Human Diseases** *Infectious diseases: Parasitic* -Amoebiasis -Chagas disease (American trypanosomiasis)	0.17
P31040	succinate dehydrogenase complex subunit A, flavoprotein (Fp)	**Human Diseases** Endocrine and metabolic diseases -Non-alcoholic fatty liver disease (NAFLD) *Neurodegenerative diseases* -Alzheimer's disease -Huntington's disease -Parkinson's disease **Metabolism** *Carbohydrate metabolism* -Citrate cycle (TCA cycle) *Energy metabolism* -Oxidative phosphorylation *Global and overview maps* -Carbon metabolism -Metabolic pathways	0
P46977	STT3A, subunit of the oligosaccharyltransferase complex	**Genetic Information Processing** *Folding, sorting and degradation* -Protein processing in endoplasmic reticulum **Metabolism** *Global and overview maps* -Metabolic pathways *Glycan biosynthesis and metabolism* -N-Glycan biosynthesis	0.18
P51160	phosphodiesterase 6C	**Metabolism** *Nucleotide metabolism* -Purine metabolism	0.3

Table A.9: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
P52788	spermine synthase	**Metabolism** *Amino acid metabolism* -Arginine and proline metabolism -Cysteine and methionine metabolism *Global and overview maps* -Metabolic pathways *Metabolism of other amino acids* -beta-Alanine metabolism -Glutathione metabolism	0.29
P78334	gamma-aminobutyric acid (GABA) A receptor, epsilon	**Environmental Information Processing** *Signaling molecules and interaction* -Neuroactive ligand-receptor interaction **Human Diseases** *Substance dependence* -Morphine addiction -Nicotine addiction **Organismal Systems** *Nervous system* -GABAergic synapse -Retrograde endocannabinoid signaling	0.3
Q13233	mitogen-activated protein kinase 1, E3 ubiquitin protein ligase	**Environmental Information Processing** *Signal transduction* -MAPK signaling pathway **Genetic Information Processing** *Folding, sorting and degradation* -Ubiquitin mediated proteolysis **Human Diseases** *Infectious diseases: Viral* -Hepatitis B -HTLV-I infection **Organismal Systems** *Endocrine system* -GnRH signaling pathway *Immune system* -RIG-I-like receptor signaling pathway *Nervous system* -Neurotrophin signaling pathway	0.16
Q16555	dihydropyrimidinase like 2	**Organismal Systems** *Development* -Axon guidance	0.17
Q549N5	SRP receptor beta subunit	**Genetic Information Processing** *Folding, sorting and degradation* -Protein export	0.51

Table A.9: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
Q6SJ96	TATA-box binding protein like 2	**Genetic Information Processing** *Transcription* -Basal transcription factors **Human Diseases** *Cancers: Overview* -Viral carcinogenesis *Infectious diseases: Viral* -Epstein-Barr virus infection -Herpes simplex infection -HTLV-I infection	0.14
Q8WZ42	titin	**Human Diseases** *Cardiovascular diseases* -Dilated cardiomyopathy -Hypertrophic cardiomyopathy (HCM)	0.47
Q99683	mitogen-activated protein kinase 5	**Cellular Processes** *Cell growth and death* -Apoptosis **Environmental Information Processing** *Signal transduction* -MAPK signaling pathway -Sphingolipid signaling pathway -TNF signaling pathway **Genetic Information Processing** *Folding, sorting and degradation* -Protein processing in endoplasmic reticulum **Human Diseases** *Endocrine and metabolic diseases* -Non-alcoholic fatty liver disease (NAFLD) *Neurodegenerative diseases* -Amyotrophic lateral sclerosis (ALS) **Organismal Systems** *Nervous system* -Neurotrophin signaling pathway	0.47
Q9BYG4	par-6 family cell polarity regulator gamma	**Cellular Processes** *Cellular community* -Tight junction *Transport and catabolism* -Endocytosis **Environmental Information Processing** *Signal transduction* -Hippo signaling pathway -Rap1 signaling pathway **Organismal Systems** *Development* -Axon guidance	0.34
Q9H2M3	betaine--homocysteine S-methyltransferase 2	**Metabolism** *Amino acid metabolism* -Cysteine and methionine metabolism *Global and overview maps* -Metabolic pathways	0.33

Table A.9: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
Q9P2J5	leucyl-tRNA synthetase	**Genetic Information Processing** *Translation* -Aminoacyl-tRNA biosynthesis	0.15
Q9UHV7	mediator complex subunit 13	**Organismal Systems** *Endocrine system* -Thyroid hormone signaling pathway	0.19
Q9UQ84	exonuclease 1	**Genetic Information Processing** *Replication and repair* -Mismatch repair	0.13
Q9Y2P5	solute carrier family 27 (fatty acid transporter), member 5	**Human Diseases** *Endocrine and metabolic diseases* -Insulin resistance **Metabolism** *Global and overview maps* -Metabolic pathways *Lipid metabolism* -Primary bile acid biosynthesis **Organismal Systems** *Digestive system* -Bile secretion *Endocrine system* -PPAR signaling pathway	0.15
Q9Y2X0	mediator complex subunit 16	**Organismal Systems** *Endocrine system* -Thyroid hormone signaling pathway	0.13

Table A.10: KEGG pathways for proteins identified as interacting with mARC2 in HEK-293 cell lysate.

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
A5YKK6	CCR4-NOT transcription complex subunit 1	**Genetic Information Processing** *Folding, sorting and degradation* -RNA degradation	0.35
O43708	hsa:2954 GSTZ1; glutathione S-transferase zeta 1	**Environmental Information Processing** *Membrane transport* -Metabolic pathways **Metabolism** *Amino acid metabolism* -Tyrosine metabolism	0.08
O75061	DnaJ heat shock protein family (Hsp40) member C6	**Cellular Processes** *Transport and catabolism* -Endocytosis	0.05
O75581	LDL receptor related protein 6	**Environmental Information Processing** *Signal transduction* -Wnt signaling pathway	0.4
O75891	aldehyde dehydrogenase 1 family member L1	**Metabolism** *Metabolism of cofactors and vitamins* -One carbon pool by folate	0.23
O75947	ATP synthase, H+ transporting, mitochondrial Fo complex subunit D	**Environmental Information Processing** *Membrane transport* -Metabolic pathways **Human Diseases** *Neurodegenerative diseases* -Alzheimer's disease -Huntington's disease **Metabolism** *Energy metabolism* -Oxidative phosphorylation	0.28
O76074	phosphodiesterase 5A	**Environmental Information Processing** *Signal transduction* -cGMP-PKG signaling pathway **Metabolism** *Nucleotide metabolism* -Purine metabolism	0.11
O95685	protein phosphatase 1 regulatory subunit 3D	**Human Diseases** *Endocrine and metabolic diseases* -Insulin resistance **Organismal Systems** *Endocrine system* -Insulin signaling pathway	0
O96007	molybdenum cofactor synthesis 2	**Environmental Information Processing** *Membrane transport* -Metabolic pathways **Genetic Information Processing** *Folding, sorting and degradation* -Sulfur relay system **Metabolism** *Metabolism of cofactors and vitamins* -Folate biosynthesis	0.25

Table A.10: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
P00748	coagulation factor XII	**Organismal Systems** *Immune system* -Complement and coagulation cascades	0.18
P01127	platelet derived growth factor subunit B	**Cellular Processes** *Cellular community* -Focal adhesion -Gap junction *Cell motility* -Regulation of actin cytoskeleton **Environmental Information Processing** *Signal transduction* -MAPK signaling pathway -Phospholipase D signaling pathway -PI3K-Akt signaling pathway -Rap1 signaling pathway -Ras signaling pathway *Signaling molecules and interaction* -Cytokine-cytokine receptor interaction **Human Diseases** *Cancers* -Choline metabolism in cancer -Glioma -Melanoma -MicroRNAs in cancer -Pathways in cancer -Prostate cancer -Renal cell carcinoma *Infectious diseases* -HTLV-I infection	0.17
P05141	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5	**Environmental Information Processing** *Signal transduction* -Calcium signaling pathway -cGMP-PKG signaling pathway **Human Diseases** *Infectious diseases* -HTLV-I infection *Neurodegenerative diseases* -Huntington's disease -Parkinson's disease	0.03
P14410	sucrase-isomaltase (alpha-glucosidase)	**Environmental Information Processing** *Membrane transport* -Metabolic pathways **Metabolism** *Carbohydrate metabolism* -Galactose metabolism -Starch and sucrose metabolism **Organismal Systems** *Digestive system* -Carbohydrate digestion and absorption	0.08

Table A.10: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
P14678	small nuclear ribonucleoprotein polypeptides B and B1	**Genetic Information Processing** *Transcription* -Spliceosome **Human Diseases** *Immune diseases* -Systemic lupus erythematosus	0.04
P23396	ribosomal protein S3	**Genetic Information Processing** Translation -Ribosome	0
P27694	replication protein A1	**Genetic Information Processing** *Replication and repair* -DNA replication -Fanconi anemia pathway -Homologous recombination -Mismatch repair -Nucleotide excision repair	0
P36542	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	**Environmental Information Processing** *Membrane transport* -Metabolic pathways **Human Diseases** *Neurodegenerative diseases* -Alzheimer's disease -Huntington's disease -Parkinson's disease **Metabolism** *Energy metabolism* -Oxidative phosphorylation	0.12
P39019	ribosomal protein S19	**Genetic Information Processing** *Translation* -Ribosome	0
P40855	peroxisomal biogenesis factor 19	**Cellular Processes** *Transport and catabolism* -Peroxisome	0.1

Table A.10: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
P42262	glutamate receptor, ionotropic, AMPA 2	**Environmental Information Processing** *Signal transduction* -cAMP signaling pathway *Signaling molecules and interaction* -Neuroactive ligand-receptor interaction **Human Diseases** *Neurodegenerative diseases* -Amyotrophic lateral sclerosis (ALS) *Substance dependence* -Amphetamine addiction -Cocaine addiction **Organismal Systems** *Environmental adaptation* -Circadian entrainment *Nervous system* -Dopaminergic synapse -Glutamatergic synapse -Long-term depression -Long-term potentiation -Retrograde endocannabinoid signaling	0.01
P42285	Ski2 like RNA helicase 2	**Genetic Information Processing** *Folding, sorting and degradation* -RNA degradation	0.08
P46782	ribosomal protein S5	**Genetic Information Processing** *Translation* -Ribosome	0.07
P48047	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit	**Environmental Information Processing** *Membrane transport* -Metabolic pathways **Human Diseases** *Neurodegenerative diseases* -Alzheimer's disease -Huntington's disease -Parkinson's disease **Metabolism** *Energy metabolism* -Oxidative phosphorylation	0.01
P54764	EPH receptor A4	**Organismal Systems** *Development* -Axon guidance	0.26
P62249	ribosomal protein S16	**Genetic Information Processing** *Translation* -Ribosome	0.01
P62269	ribosomal protein S18	**Genetic Information Processing** *Translation* -Ribosome	0
P62273	ribosomal protein S29	**Genetic Information Processing** *Translation* -Ribosome	0.04

Table A.10: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
P62701	ribosomal protein S4, X-linked	**Genetic Information Processing** *Translation* -Ribosome	0
P62750	ribosomal protein L23a	**Genetic Information Processing** *Translation* -Ribosome	0.01
P62979	ribosomal protein S27a	**Genetic Information Processing** *Translation* -Ribosome	0
Q06787	fragile X mental retardation 1	**Genetic Information Processing** *Translation* - RNA transport	0.29
Q07021	complement component 1, q subcomponent binding protein	**Human Diseases** *Infectious diseases* -Herpes simplex infection	0
Q13496	myotubularin 1	**Environmental Information Processing** *Membrane transport* -Metabolic pathways *Signal transduction* -Phosphatidylinositol signaling system **Metabolism** *Carbohydrate metabolism* -Inositol phosphate metabolism	0.29

Table A.10: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
Q13698	calcium channel, voltage-dependent, L type, alpha 1S subunit	**Environmental Information Processing** *Signal transduction* -Calcium signaling pathway -cAMP signaling pathway -cGMP-PKG signaling pathway -MAPK signaling pathway **Human Diseases** *Cardiovascular diseases* -Arrhythmogenic right ventricular cardiomyopathy (ARVC) -Dilated cardiomyopathy -Hypertrophic cardiomyopathy (HCM) *Neurodegenerative diseases* -Alzheimer's disease **Organismal Systems** *Circulatory system* -Adrenergic signaling in cardiomyocytes -Cardiac muscle contraction -Vascular smooth muscle contraction *Endocrine system* -Aldosterone synthesis and secretion -GnRH signaling pathway -Insulin secretion -Oxytocin signaling pathway -Renin secretion *Nervous system* -Cholinergic synapse -GABAergic synapse -Retrograde endocannabinoid signaling -Serotonergic synapse	0.24
Q15185	prostaglandin E synthase 3	**Environmental Information Processing** *Membrane transport* -Metabolic pathways **Metabolism** *Lipid metabolism* -Arachidonic acid metabolism	0.05
Q32P51	heterogeneous nuclear ribonucleoprotein A1-like 2	**Genetic Information Processing** *Transcription* -Spliceosome	0
Q5S007	leucine-rich repeat kinase 2	**Human Diseases** *Neurodegenerative diseases* -Parkinson's disease	0.27
Q6UB35	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like	**Environmental Information Processing** *Membrane transport* -Metabolic pathways **Metabolism** *Metabolism of cofactors and vitamins* -One carbon pool by folate	0.14

Table A.10: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
Q71U36	tubulin alpha 1a	**Cellular Processes** *Cell growth and death* -Apoptosis *Cellular community* -Gap junction *Transport and catabolism* -Phagosome **Human Diseases** *Infectious diseases* -Pathogenic Escherichia coli infection	0
Q86XP3	DEAD-box helicase 42	**Genetic Information Processing** *Transcription* -Spliceosome	0.54
Q8NBS9	thioredoxin domain containing 5	**Genetic Information Processing** *Folding, sorting and degradation* -Protein processing in endoplasmic reticulum	0.08
Q8TD57	dynein, axonemal, heavy chain 3	**Human Diseases** *Neurodegenerative diseases* -Huntington's disease	0.19
Q96RS0	trimethylguanosine synthase 1	**Genetic Information Processing** *Translation* -RNA transport	0.41
Q9BTM1	H2A histone family member J	**Human Diseases** *Immune diseases* -Systemic lupus erythematosus *Substance dependence* -Alcoholism	0
Q9NQX3	gephyrin	**Organismal Systems** *Nervous system* -GABAergic synapse	0.3
Q9NUT2	ATP binding cassette subfamily B member 8	**Environmental Information Processing** *Membrane transport* -ABC transporters	0.43
Q9NZB8	molybdenum cofactor synthesis 1	**Environmental Information Processing** *Membrane transport* -Metabolic pathways **Genetic Information Processing** *Folding, sorting and degradation* -Sulfur relay system **Metabolism** *Metabolism of cofactors and vitamins* -Folate biosynthesis	0.1
Q9UHD8	septin 9	**Human Diseases** *Infectious diseases* -Bacterial invasion of epithelial cells	0.02

Table A.10: Continued

UniProt ID	Protein Name	KEGG Category, Pathway Class, and Pathway	SAINT Score
Q9UKX5	integrin subunit alpha 11	**Cellular Processes** *Cell motility* -Regulation of actin cytoskeleton *Cellular community* -Focal adhesion **Environmental Information Processing** *Signal transduction* -PI3K-Akt signaling pathway *Signaling molecules and interaction* -ECM-receptor interaction **Human Diseases** *Cardiovascular diseases* -Arrhythmogenic right ventricular cardiomyopathy (ARVC) -Dilated cardiomyopathy -Hypertrophic cardiomyopathy (HCM	0.3
Q9Y285	phenylalanyl-tRNA synthetase alpha subunit	**Genetic Information Processing** *Translation* -Aminoacyl-tRNA biosynthesis	0

Appendix IV: Protein sequence comparison for mARC1 and mARC2 in human, rat, and pig

A

```
77.4% identity in 314 residues overlap; Score: 1292.0; Gap frequency: 0.3%

Q5VT66|MAR    22 WLGVAALGLTAVALGAVAWRRAWPTRRRRLLQQVGTVAQLWIYPVKSCKGVPVSEAECTA
G3V6I4|G3V    26 WVCVAALGLAAVTLGTVAWRRARPRRRRRL-QQVGTVAQLWIYPIKSCKGVSVTEAECTA
                  *  ****** ** ** ****** * ***** ************ ****** * ******

Q5VT66|MAR    82 MGLRSGNLRDRFWLVINQEGNMVTARQEPRLVLISLTCDGDTLTLSAAYTKDLLLPIKTP
G3V6I4|G3V    85 MGLRCGHLRDRFWLVVNEEGNMVTARQEPRLVQISLTCEDDNLTLSAAYTKDLLLPITPP
                  **** * ******** * ************** *****  * ***************  *

Q5VT66|MAR   142 TTNAVHKCRVHGLEIEGRDCGEATAQWITSFLKSQPYRLVHFEPHMRPRRPHQIADLFRP
G3V6I4|G3V   145 ATNPLLQCRVHGLEVQGRDCGEDAAQWISGFLKTQRCRLVHFEPHMHPRSSQKMRASFRP
                   **    *******  ******  ****  *** *  ********* **        ***

Q5VT66|MAR   202 KDQIAYSDTSPFLILSEASLADLNSRLEKKVKATNFRPNIVISGCDVYAEDSWDELLIGD
G3V6I4|G3V   205 TDQVAYADASPFLVLSEASLEDLNSRLERRVKAANFRPNIVISGCGIYAEDSWNEVLIGD
                   ** ** * **** ****** *******  *** ***********  ****** * ****

Q5VT66|MAR   262 VELKRVMACSRCILTTVDPDTGVMSRKEPLETLKSYRQCDPSERKLYGKSPLFGQYFVLE
G3V6I4|G3V   265 VELKRVMPCTRCLLTTVDPDTGIMDKKEPLETLKSYRLCEPSEQALCGKLPTFGQYFALE
                  ******* * ** ********* *  ********** * ***  * ** * ***** **

Q5VT66|MAR   322 NPGTIKVGDPVYLL
G3V6I4|G3V   325 NPGTIKVGDPVYLL
                  **************
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B

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74.2% identity in 337 residues overlap; Score: 1367.0; Gap frequency: 0.6%

Q969Z3|MAR     1 MGASSSSALARLGLPARPWPRWLGVAALGLAAVALGTVAWRRAWPRRRRRLQQVGTVAKL
O88994|MAR     1 MGSSSSTALARLGLPGQPRSTWLGVAALGLAAVALGTVAWRRARPRRRRQLQQVGTVSKV
                  ** *** ********  *   ********************** ***** ******* *

Q969Z3|MAR    61 WIYPVKSCKGVPVSEAECTAMGLRSGNLRDRFWLVIKEDGHMVTARQEPRLVLISIIYEN
O88994|MAR    61 WIYPIKSCKGVSVCETECTDMGLRCGKVRDRFWMVVKEDGHMITARQEPRLVLVTITLEN
                  **** ****** * * *** **** *  ***** * ****** **********  *  **

Q969Z3|MAR   121 NCLIFRAPDMDQLVLPSKQPSSNKLHNCRIFGLDIKGRDCGNEAAKWFTNFLKTEAYRLV
O88994|MAR   121 NYLMLEAPGMEPIVLPIKLPSSNKIHDCRLFGLDIKGRDCGDEVARWFTSYLKTQAYRLV
                  * *   ** *   *** * ***** * ** *********** * * ***  *** *****

Q969Z3|MAR   181 QFETNMKGRTSRKLLPTLD--QNFQVAYPDYCPLLIMTDASLVDLNTRMEKKMKMENFRP
O88994|MAR   181 QFDTKMKGRTTKKLYPSESYLQNYEVAYPDCSPIHLISEASLVDLNTRLQKKVKMEYFRP
                  ** * *****  ** *     **  *****  *      *********  ** *** ***

Q969Z3|MAR   239 NIVVTGCDAFEEDTWDELLIGSVEVKKVMACPRCILTTVDPDTGVIDRKQPLDTLKSYRL
O88994|MAR   241 NIVVSGCEAFEEDTWDELLIGDVEMKRVLSCPRCVLTTVDPDTGIIDRKEPLETLKSYRL
                  **** ** ************* ** * *  **** ********* **** ** *******

Q969Z3|MAR   299 CDPSERELYKLSPLFGIYYSVEKIGSLRVGDPVYRMV
O88994|MAR   301 CDPSVKSLYQSSPLFGMYFSVEKIGSLRVGDPVYRMV
                  ****   **  ***** * ******************
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Figure A.15: Protein sequence comparison for mARC1 and mARC2 in human and rat. A. mARC1 (Q5VT66) in human compared to mARC1 (G3V6I4) in rat, B. mARC2 (Q969Z3) in human compared to mARC2 (O88994) in rat.

Figure A.16: Protein sequence comparison for mARC1 and mARC2 in human and pig. A. mARC1 (Q5VT66) in human compared to mARC2 (F1S9I9) in pig, B. mARC2 (Q969Z3) in human compared to mARC2 (F1S9I9) in pig.

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