

Supplemental Material

Conserved Gene Microsynteny Unveils Functional Interaction Between Protein Disulfide Isomerase and Rho Guanine-Dissociation Inhibitor Families

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Supplemental Figure Legends

Suppl. Fig. S1 – The maximum likelihood phylogeny of the PDI protein family.

The tree was reconstructed using the LG model of protein sequence evolution, estimating the amino acid equilibrium frequencies from the data. The color code indicates that at least three PDI sub-families that can be traced back to the last eukaryotic common ancestor (LECA), indicated by the filled circles. Open circles denote the position of the last common ancestor of the animals. Black branches represent PDI orthologs that cannot be associated with any of the main PDI lineages. Branch labels denote percent bootstrap support.

Suppl. Fig. S2 – The maximum likelihood phylogeny of the RhoGDI protein family.

The tree was reconstructed using the LG model of protein sequence evolution. The color code indicates the RhoGDI subfamilies that can be traced back to the last common ancestor of the vertebrates (open circles). The filled circle represents the last common ancestor of the animals. Note that the RhoGDI β lineage (represented in green) has been lost in ray finned fish (actinopterygii) and the shark *C. milii*. Branches in black represent RhoGDI lineages outside the vertebrates. Branch labels denote percent bootstrap support.

Suppl. Fig. S3: Alignment of genomic regions adjacent to RhoGDI genes in selected species in vertebrates.

Gene names and positioning are based upon the Genomicus database (see Methods). All species are aligned against *Homo sapiens*. The map is centralized in RhoGDI genes, focusing on species (particularly ray-finned fish) in which the PDI/RhoGDI arrangement is altered (vs. *Homo sapiens*) in some aspects (gene duplications, displacement of microsyntenic arrangement or absence of the clusters). Data are shown for RhoGDI α (ARHGDI A) (A and B), RhoGDI β (ARHGDI B) (in C) and RhoGDI γ (ARHGDI G) (in D). Genes are aligned in columns and kept in the order in which they appear in chromosomes (Chr) and scaffolds (Scf), without consideration for distance, while the transcriptional sense is represented by the pentagon tip. All orthologs are drawn with the same color and the lettering on the top refers to the *Homo sapiens* genes. In addition to the RhoGDIs, additional neighboring genes are included for reference.

Suppl. Fig. S4: Identity and similarity (between brackets) of amino acid sequence from human PDIs (A) and RhoGDIs (B) addressed in this study. Proteins are identified by UNIPROT ID.

Suppl. Fig. S5: Effects of PDIA1 or RhoGDI α silencing on the expression of the corresponding pair.

PDIA1 or RhoGDI α were silenced via siRNA transfection and their reduced level (48 h after transfection) was unassociated with alterations in their respective partner.

Suppl. Fig. S6: Physical interaction between PDIA1 and RhoGDI α by co-immunoprecipitation.

RhoGDI α were immunoprecipitated with anti-RhoGDI clone A-20 sc360, rabbit IgG. Epitope: N-terminus – against peptide derived of the 25 aminoacids from N-terminus. Western blots: Anti-PDI clone RL90-MA3019, Thermo; anti-RhoGDI clone A-20 sc360, rabbit IgG; anti-actin-B A5441, Sigma Aldrich, mouse IgG.

Suppl. Fig. S7: Physical interaction between PDIA1 and RhoGDI α in endothelial cells.

Confocal microscopic images depicting PDIA1 and RhoGDI α co-localization. In upper and lower panels, antibodies were targeted to the N- and C-terminus of RhoGDI α , respectively. Magnification 40x.

Suppl. Fig. S8: Uncropped western blottings.

A) Western blotting against PDI and RhoGDI α in rabbit VSMC transducing Tet-on system. Representative of 3 samples from three independent experiments (From fig. 6A); **B)** Western blotting against PDI and RhoGDI α in VSMC from PDI transgenic mice. Representative of 9 samples from three independent experiments (From fig. 6C); **C)** Western blotting against PDI and RhoGDI α in HUVEC transducing CRISPR activation system dCas9-VP64 to PDI promoter region (From fig. 7A); **D)** Western blotting against PDI and RhoGDI α in HUVEC transducing CRISPR activation system dCas9-VP64 to RhoGDI α promoter region (From fig. 7B);

Suppl. Fig. S9: Uncropped western blottings

A – E) Effects of PDIA1 overexpression upon RhoGDI α levels in a transgenic mouse model. Tissue from transgenic PDIA1-overexpressing mice: brain (A), kidney (B), liver (C), aortae(D)and heart (E). Data representative of n \geq 3, 2 (heart) or 1 (brain),.

Suppl. Fig. S10: Uncropped western blottings.

A) RhoGDI α were immunoprecipitated with anti-RhoGDI, ab5385, ABCAM, rabbit IgG. Epitope: N-terminus – against peptide derived of the 25 aminoacids from N-terminus. Western blots: Anti-PDI clone RL90-MA3019, Thermo; anti-RhoGDI ab5385, ABCAM, rabbit IgG; anti-actin-B A5441, Sigma Aldrich, mouse IgG (From Fig. 8A); **B)** RhoGDI α was immunoprecipitated with anti-RhoGDI clone B1- sc-13120, Santa Cruz, mouse IgG. Epitope: C-terminus - aminoacids 166-198. Western blots: Anti-PDI clone RL90-MA3019, Thermo; anti-RhoGDI clone A-20 sc360, rabbit IgG; anti-actin-B A5441, Sigma Aldrich, mouse IgG (From Fig. 8B); **C)** Western blotting against PDI and GST-tag (RhoGDI α) pulldown assay in HUVEC homogenate. Representative of 3 independent experiments (From fig. 8C); **D)** RhoGDI α was immunoprecipitated with anti-RhoGDI clone A-20 sc360, rabbit IgG. Epitope: N-terminus – against peptide derived from the N-terminal 25 aminoacids. Western blots: Anti-PDI clone RL90-MA3019, Thermo; anti-RhoGDI clone A-20 sc360, rabbit IgG; anti-actin-B A5441, Sigma Aldrich, mouse IgG (From Suppl fig. S6);

Supplementary Table S1: PDI/RhoGDI synteny in other organisms.

Protein	Organism	Protein name	Gene ID	mRNA and protein IDs	Genomic sequence	Location	Sense reading	Intergenic distance PDI vs GDI (bases)	Protein Identity/similarity vs human sequences ⁽¹⁾		
									RhoGDI α	RhoGDI β	RhoGDI γ
RhoGDI	<i>Trichoplax adhaerens</i>	Putative uncharacterized protein	TRIADDRAFT_53598	XM_002109678.1 XP_002109714.1 ⁽²⁾	NW_002060944.1	180,991.....185,870 ⁽³⁾	reverse	6,180	53% / 67%	51% / 69%	47% / 65%
	<i>Trichoplax adhaerens</i>	Putative uncharacterized protein	TRIADDRAFT_20518	XM_002109679.1 XP_002109715.1	NW_002060944.1	189,570.....193,770	reverse	14,759	52% / 68%	51% / 69%	46% / 63%
	<i>Nematostella vectensis</i>	Predicted Protein	NEMVEDRAFT_v1g170702	XM_001628841.1 XP_001628891.1	NW_0018342601	150,219...157,089	sense	7,246	47% / 64%	50% / 67%	40% / 62%
	<i>Strongylocentrotus purpuratus</i>	Sp-Rhogdi/Rho GDP dissociation inhibitor	SPU_025771	SPU_025771.1	-----	Scaffold569: 461576...467249	reverse	4,909	52% / 67%	49% / 67%	46% / 65%
	<i>Patiria miniata</i>	Sp-Rhogdi_1/Rho GDP dissociation inhibitor	PMI_007749	PMI_007749.1	-----	Scaffold6534: 65864...90774	sense	47,978	34% / 44%	34% / 43%	30% / 38%
	<i>Patiria miniata</i>	Sp-Rhogdi_2/Rho GDP dissociation inhibitor	PMI_007751.1	PMI_007751.1	-----	Scaffold6534: 99795...101554	sense	37,198	49% / 72%	55% / 71%	55% / 70%
PDI	<i>Trichoplax adhaerens</i>	Protein Disulfide Isomerase	TRIADDRAFT_52862	XM_00210977.1 XP_002109713.1	NW_002060944.1	169,664...174,811	reverse	-----	49% / 69%	24% / 47%	37% / 59%
	<i>Nematostella vectensis</i>	Protein Disulfide Isomerase	NEMVEDRAFT_v1g118540	XM_001628843.1 XP_001628893.1	NW_001834260.1	142,973...147,036	sense	-----	58% / 76%	31% / 51%	42% / 61%
	<i>Strongylocentrotus purpuratus</i>	Sp-Calseq/Protein Disulfide Isomerase ⁽⁴⁾	SPU_025772	SPU_05772.1	-----	Scaffold569: 443358...456667	reverse	-----	46% / 58%	22% / 41%	35% / 52%
	<i>Patiria miniata</i>	Pm-Calseq/Protein Disulfide Isomerase ⁽⁵⁾	PMI_007752	PMI_007752.1	-----	Scaffold6534: 138752...167074	sense	-----	38% / 53%	29% / 49%	35% / 58%

⁽¹⁾Uniprot IDs, GDI α , GDI β and GDI γ respectively, P52562, P52566 and Q99819; and for PDIA1, PDIA8 and PDIA2 are P07237, Q96DN0 and Q13087, respectively.

⁽²⁾XP_002109714.1 vs XP_002109715.1 = 65% and 77% of identity and similarity, respectively

⁽³⁾Intergenic distance between TRIADDRAFT_53598 and TRIADDRAFT_20517 = 3,700 pb.

⁽⁴⁾Sp-Calseq vs human calsequestrins - Casq1 (UNIPROT ID P31415) and Casq2 (UNIPROT ID O14958): identity/similarity are respectively, 21%/37% and 19%/33%

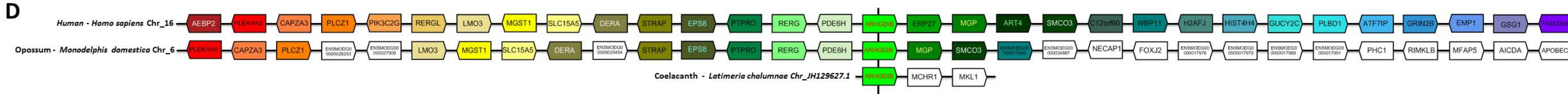
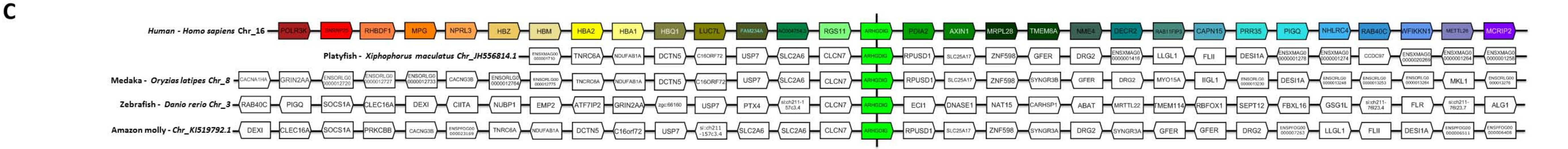
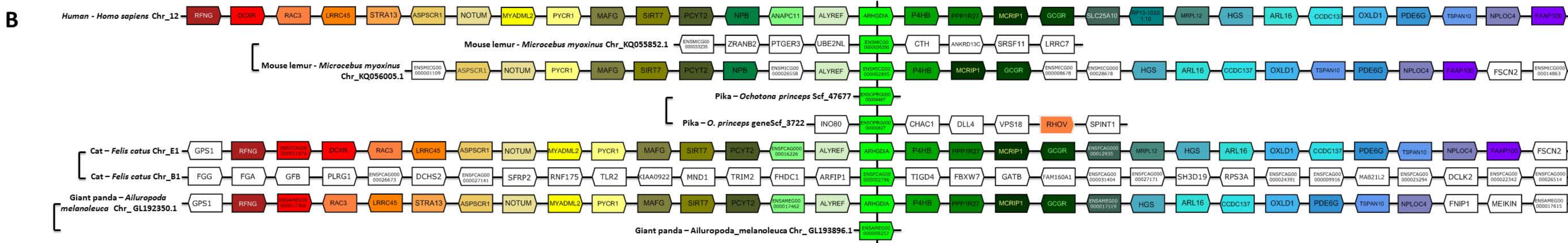
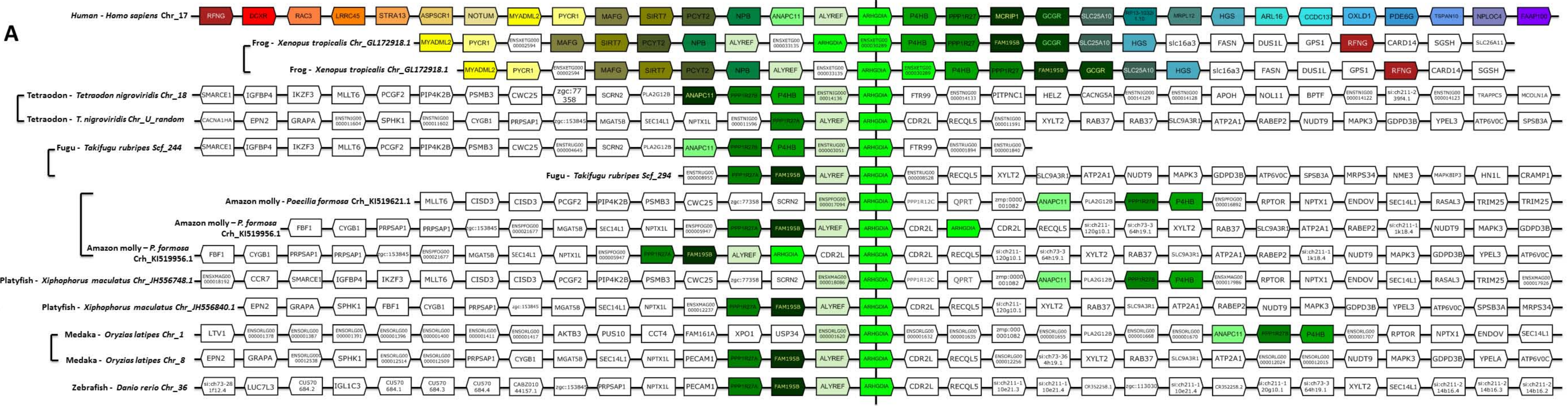
⁽⁵⁾Pm-Calseq vs human calsequestrins - Casq1 (UNIPROT ID P31415) and Casq2 (UNIPROT ID O14958): identity/similarity are respectively, 21%/39% and 21%/39%

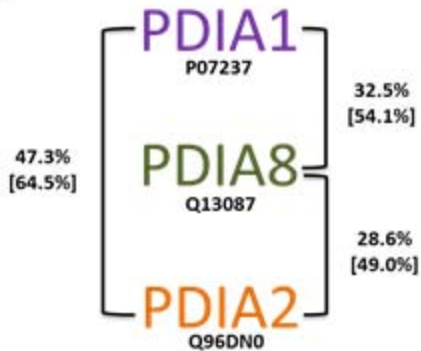
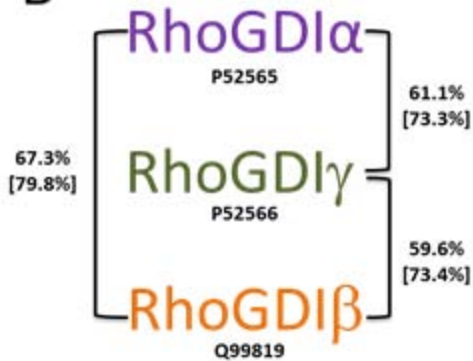
T.Adhaerens and N. vectensis source of data: NCBI gene and protein database, UNIPROT.

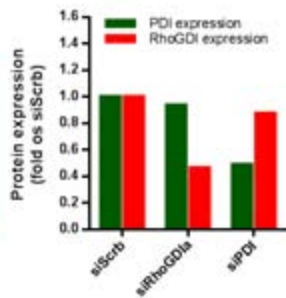
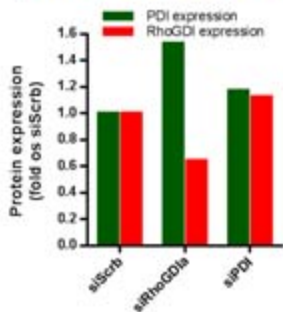
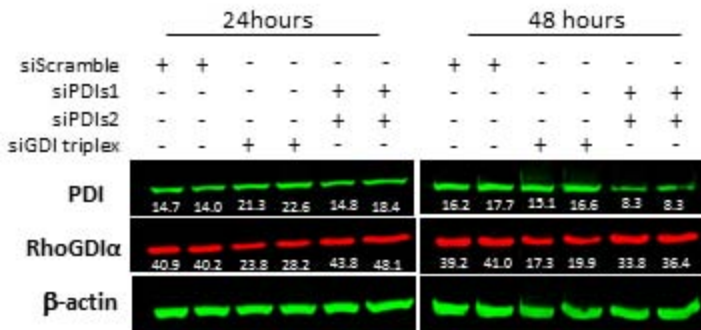
S. Purpuratus and P. miniata source of data: EchnoBase

Supplementary Table S2: Polycistronic gene transcripts in different organisms

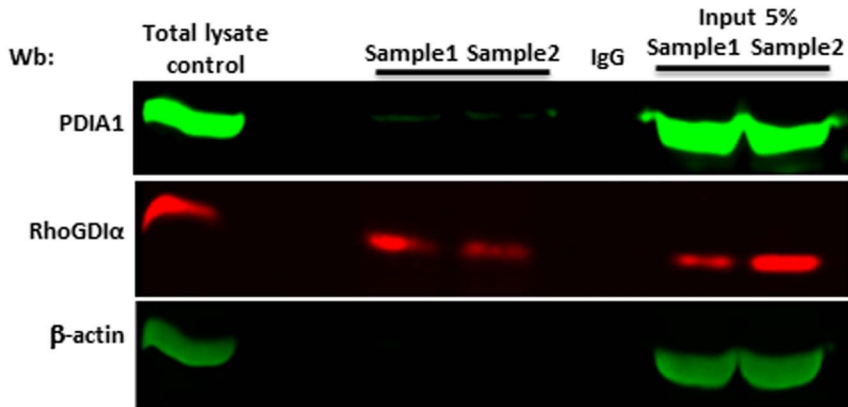
Organism	Name	ID	Source
<i>Homo sapiens</i>	ARHGDIG, PDIP polycistronic mRNA for Rho GDP dissociation inhibitor gamma, pancreatic protein disulfide isomerase, complete cds	AB127078.1	GeneBank
<i>Mus musculus</i>	Rho GDP dissociation inhibitor (GDI) gamma (strand-) contains the whole PDIA2 transcript	ENSMUST00000176961.7	Ensembl
<i>Macaca mulatta</i>	Axin1 or PPP1R49 (strand -) contains exons of PDIA2	ENSMMUT00000000876.3	Ensembl
<i>Callithrix jacchus</i>	PDIA2 contains Axin1 in the intronic region	ENSCJAT00000057730.1	Ensembl
<i>Canis familiaris</i>	ARHGDIB contains exons of ERP27/PDIA8	ENSRAFT00000020458.3	Ensembl
<i>Meleagris gallopavo</i>	PDIA2 (strand -) and part of an exon exhibit sequences of the gene RGS11, regulator of G-protein signaling (strand +)	ENSMGAG00000008956	Ensembl



A**B**



Ipp RhoGDI α (anti-RhoGDI α clone A-20 – against N-terminus)

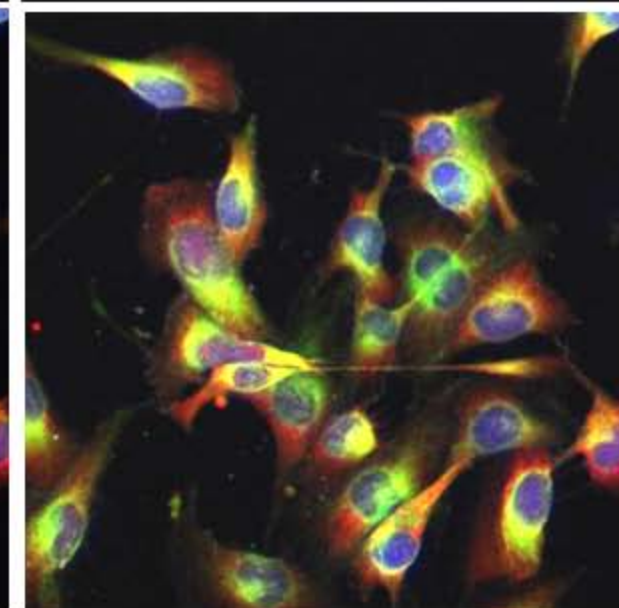
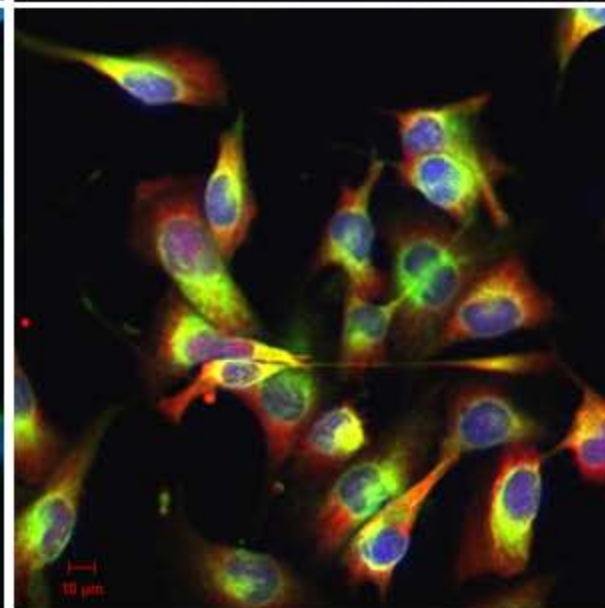
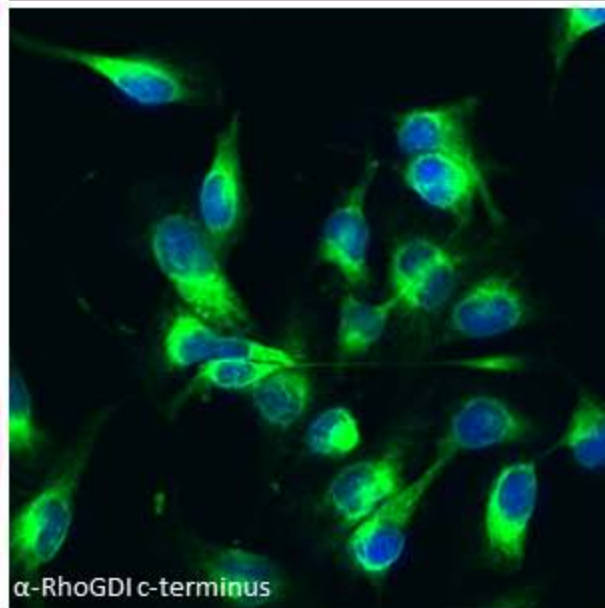
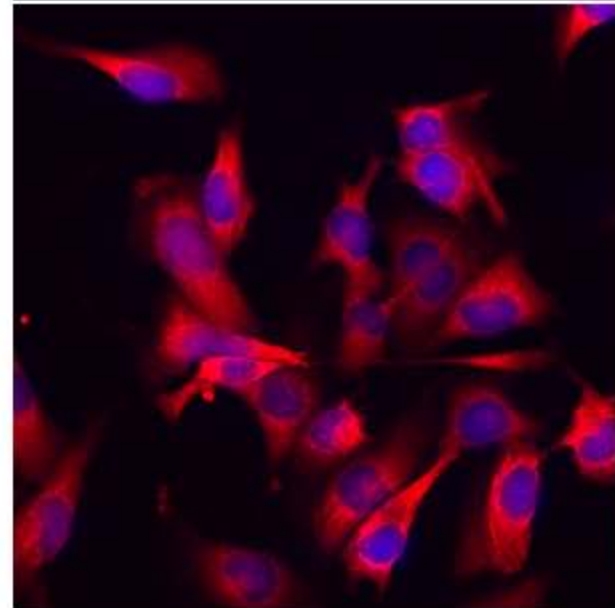
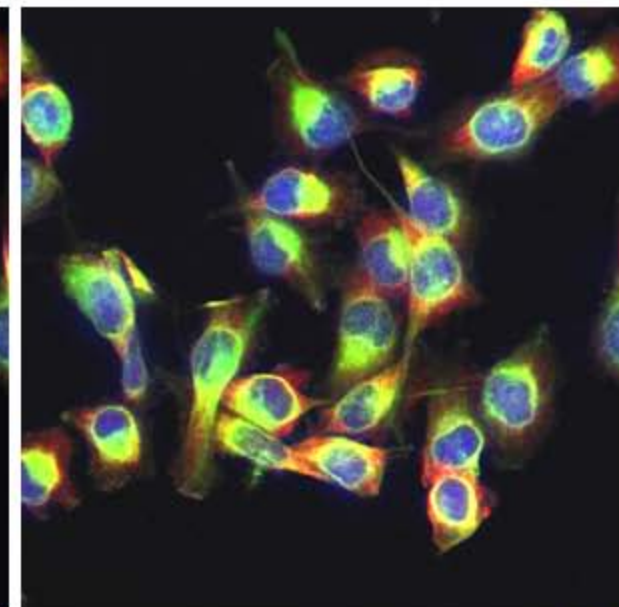
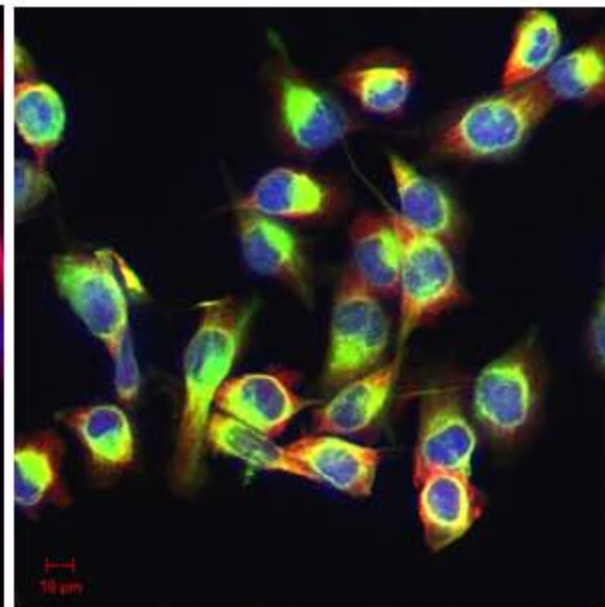
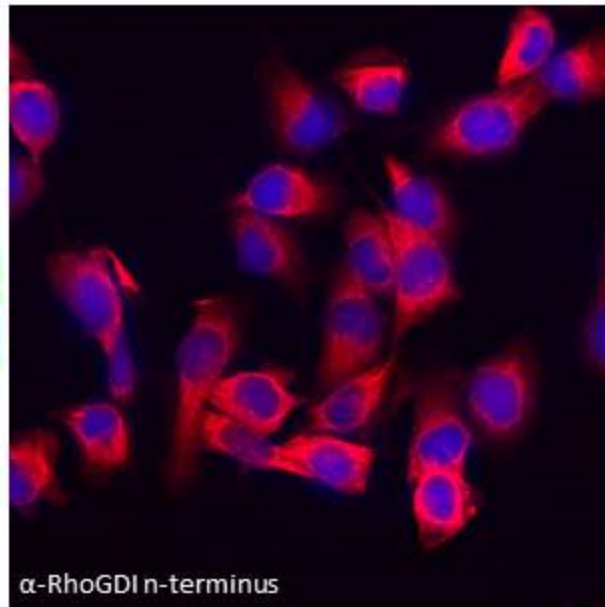
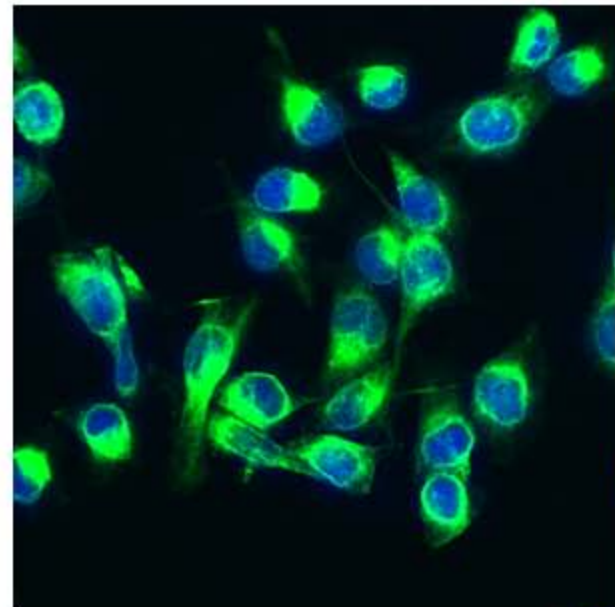


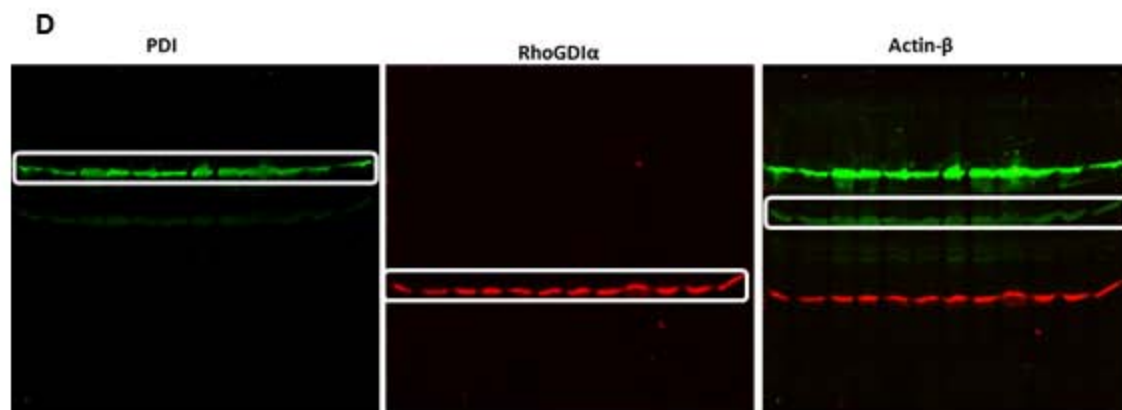
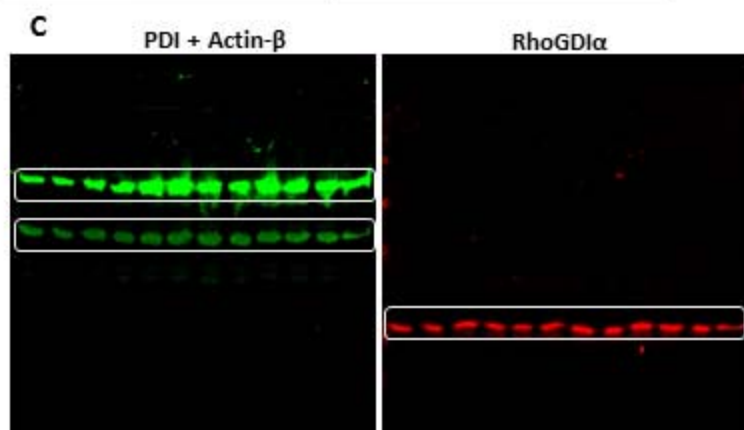
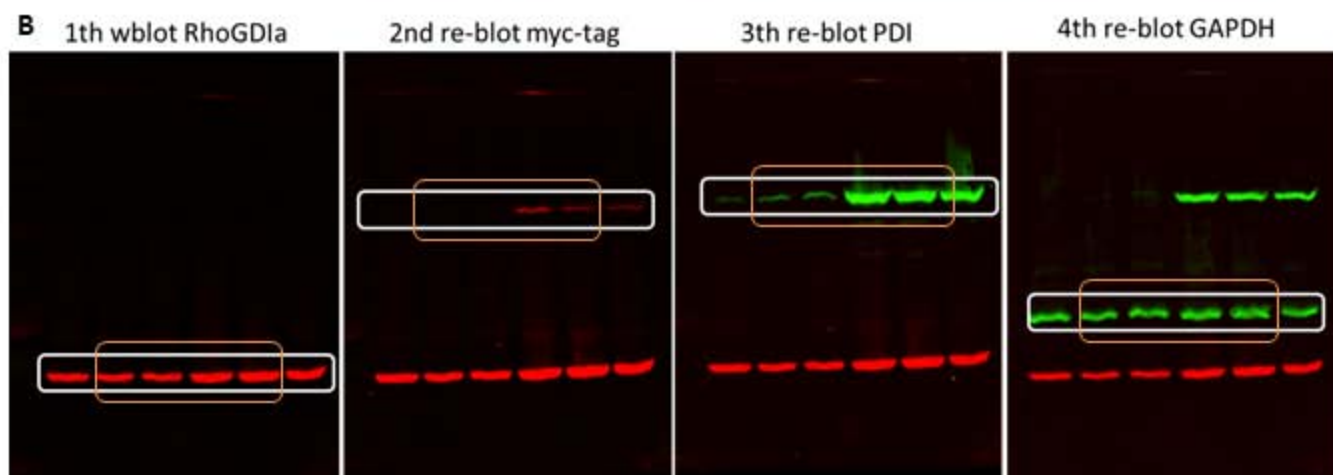
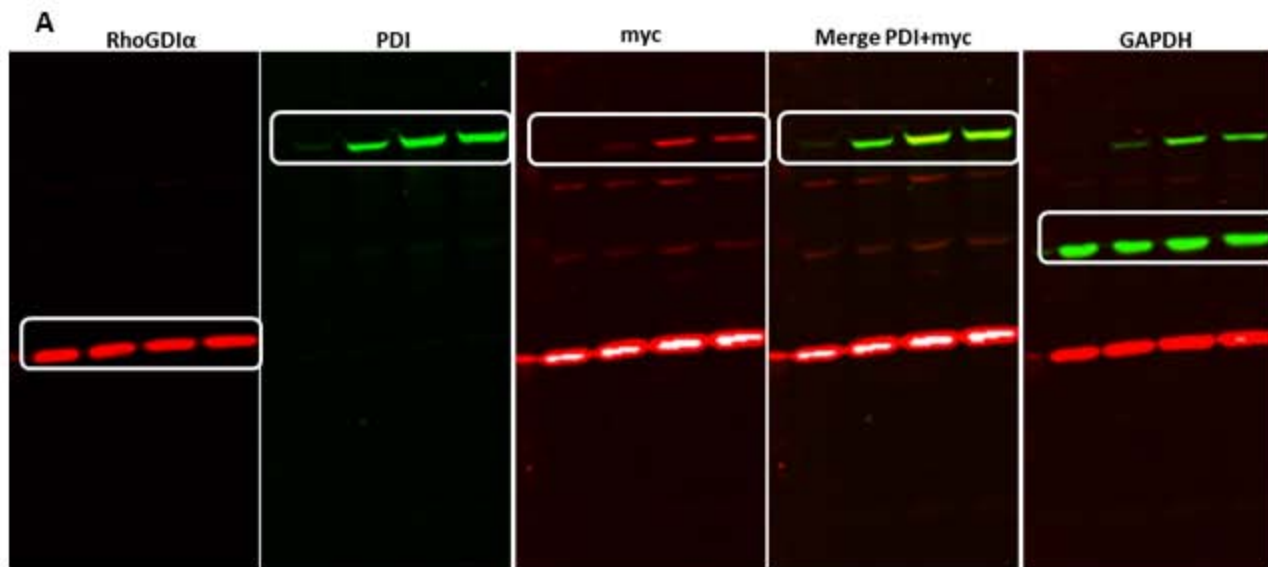
PDI

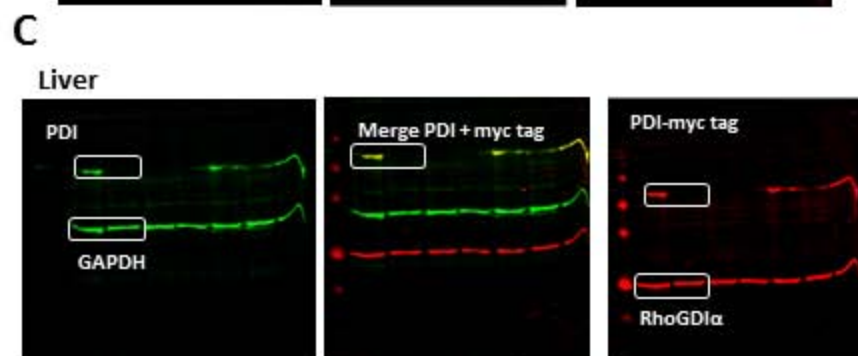
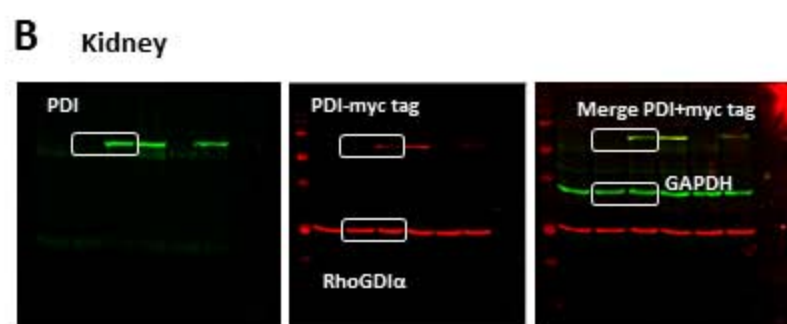
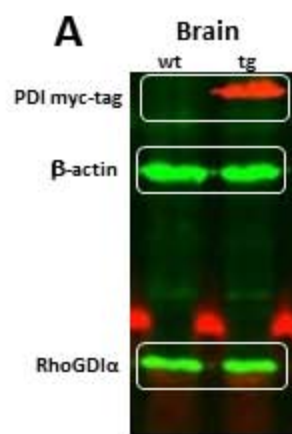
RhoGDI α

Merge

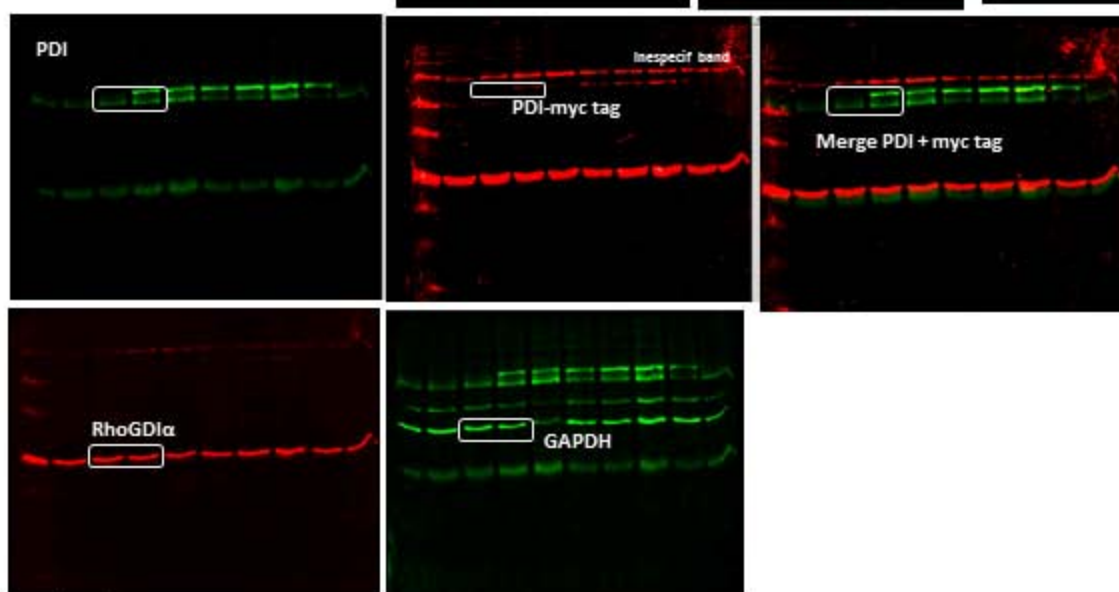
Phalloidin







D Aortae



E Heart

