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Functions and therapeutic potential of protein phosphatase 1: Insights from mouse genetics[☆]



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ABSTRACT

Protein phosphatase 1 (PP1) catalyzes more than half of all phosphoserine/threonine dephosphorylation reactions in mammalian cells. *In vivo* PP1 does not exist as a free catalytic subunit but is always associated with at least one regulatory PP1-interacting protein (PIP) to generate a large set of distinct holoenzymes. Each PP1 complex controls the dephosphorylation of only a small subset of PP1 substrates. We screened the literature for genetically engineered mouse models and identified models for all PP1 isoforms and 104 PIPs. PP1 itself and at least 49 PIPs were connected to human disease-associated phenotypes. Additionally, phenotypes related to 17 PIPs were clearly linked to altered PP1 function, while such information was lacking for 32 other PIPs. We propose structural reverse genetics, which combines structural characterization of proteins with mouse genetics, to identify new PP1-related therapeutic targets. The available mouse models confirm the pleiotropic action of PP1 in health and diseases.

1. Introduction

In a typical mammalian cell, more than three-quarters of all proteins are phosphorylated at serine, threonine and/or tyrosine residues during their lifetime [1]. While phosphorylation of these residues (i.e. transfer of the γ -phosphate of ATP to the hydroxyl group side chain) is catalyzed by protein kinases, protein phosphatases catalyze the hydrolytic removal of phosphate groups [2, 3]. Thus, the spatiotemporal phosphorylation state of a protein is the result of a balance between counteraction of protein kinases and protein phosphatases. Disturbance of this balance, which causes aberrant phosphorylation, is implicated in various human diseases, including cancer, cardiac hypertrophy, diabetes and neurodegeneration [4]. Therapeutic strategies for protein phosphorylation diseases have mainly focused on drugs that target protein kinases, although protein phosphatases are equally attractive targets [4, 5]. To date, more than two dozen small-molecule protein kinase inhibitors and six therapeutic antibodies against protein tyrosine kinases have been approved by the US Food and Drug Administration (FDA) for clinical use, mainly for targeted cancer therapies [6, 7]. Along with these FDA-approved drugs, hundreds of other protein kinase

inhibitors are being tested in clinical trials [6, 7].

In contrast, protein phosphatases have been neglected as potential drug targets because they were classified as ‘undruggable’, evoking the misconception that it is impossible to target a particular protein phosphatase [4, 5, 8]. In fact, multiple studies by various research groups revealed that protein phosphatases are unequivocally ‘druggable’ targets [5, 9]. Various small-molecule effectors that modulate (activate or inhibit) protein phosphatase activity and exhibit therapeutic potential for various human diseases have been generated [5, 9]. For example, the immunosuppressants cyclosporine A and FK506, which are successfully used to treat acute organ transplant rejection, rheumatoid arthritis, psoriasis and Crohn's disease, target and inhibit protein serine/threonine phosphatase PP2B, also known as PP3 or calcineurin [4, 10, 11]. Their mode of action, however, was only elucidated after their approval by the US FDA. These PP2B inhibitors do not block the active site but bind and block one of the substrate binding sites of PP2B [12, 13]. Various protein tyrosine phosphatase (PTP) inhibitors, including distinct small molecule inhibitors such as orthosteric (binds at the enzyme active site), allosteric (binds outside the enzyme active site) and competitive (binds to an enzyme at the site of substrate binding)

Abbreviations: gPIP, guiding PIP; iPIP, inhibitory PIP; KO, knockout; PIP, PP1-interacting protein; PP, protein phosphatase; PTP, protein tyrosine phosphatase; SLiM, short linear motif

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inhibitors and biologics [8, 9, 14] have also been developed for therapeutic purposes. A few are currently undergoing clinical trials. For example, small-molecule inhibitors of vascular endothelial PTP and PTP1B are being tested for treatment of diabetic macular edema and metastatic breast cancer, respectively [8, 9, 14].

Protein phosphatase 1 (PP1) is a widely expressed and highly conserved phosphatase of ~38 kDa that is active only towards phosphoserine/threonine residues and belongs to the phosphoprotein phosphatase (PPP) family of the eukaryotic protein phosphatome [15]. Based on biochemical data, it is estimated that PP1 is responsible for more than half of all phosphoserine/threonine dephosphorylation reactions in eukaryotic cells [2]. PP1 itself shows a rather broad substrate specificity *in vitro*, but exhibits in a cellular context a much more narrow and tightly regulated substrate selectivity. This is mainly achieved through association with a wide array of PP1-interacting proteins (PIPs), thereby forming > 200 distinct multisubunit PP1:PIP(s) complexes, each of which dephosphorylates only a small subset of PP1 substrates [2, 16]. Consistent with its numerous substrates, PP1 is a key regulator of several cellular processes, including cell cycle progression, protein synthesis, pre-mRNA splicing and transcription. It is therefore not surprising that PP1 plays a crucial role in human pathologies such as cancer, heart disease, memory loss, type 2 diabetes and viral infections, suggesting a great therapeutic potential for PP1-directed drugs [17, 18]. Genetically engineered mouse models have significantly contributed to our understanding of the physiological role of PP1 holoenzymes in health and diseases as they can determine *in vivo* gene function and identify disease-causing target genes [19, 20]. Also, mouse models are often crucial for discovering therapeutic targets as demonstrated by the history of the development of PTP-targeted drugs [21,

22]. The first breakthrough was the discovery of the phenotype of mice with a global deletion of protein tyrosine phosphatase PTP1B. Because these mice were hypersensitive to insulin and resistant to obesity, academics and pharmaceutical companies began to search for PTP1B inhibitors to treat type 2 diabetes and obesity [21, 22].

In this review, we first summarize the current knowledge about the structure and diversity of PP1 holoenzymes, and the way in which their specificity is achieved. Next, we address genetically modified mouse models of PP1 isoforms and their PIPs. Since most PIPs are multifunctional and/or interact with multiple proteins, we focus on mouse models of PIPs with a phenotype that is connected to the PP1 holoenzyme function. We review the discoveries made with the mouse models regarding the *in vivo* function of PP1 holoenzymes and their therapeutic potential. Finally, we examine mouse models for phenotypes that are associated with human diseases. Abbreviations of protein names and the mouse genetics nomenclature [23] are defined in **Box 1** and **2**, respectively.

2. Structure of PP1 holoenzymes

Each PP1 holoenzyme consists of PP1, the catalytic subunit, and one or two regulatory PIPs (Fig. 1). While the mammalian genome contains only three PP1 genes, > 200 PIP-encoding genes have been identified, and it is estimated that hundreds have yet to be discovered [2]. The mammalian PP1 isoforms are about 90% identical at the amino acid level, whereas PIPs are mainly structurally unrelated proteins that define where and when a PP1 holoenzyme is active, and towards which set of substrates. The binding of PIPs to PP1 is facilitated by short linear motifs (SLIMs) of 4–8 residues long that bind to specific surface grooves

Box 1

Abbreviations of protein names.

Abbreviation	Full protein name
CPI-17	protein kinase C-potentiated protein phosphatase-1 inhibitor Mr. 17 kDa
CREB1	cAMP responsive element binding protein 1
CReP	constitutive repressor of eIF2 α phosphorylation
DARPP32	dopamine and cyclic AMP-regulated phosphoprotein of Mr. 32,000
DRD1	dopamine receptor D1
DRD2	dopamine receptor D2
E2f1	E2F transcription factor 1
eIF2 α	eukaryotic initiation factor 2 α
GADD34	growth arrest and DNA damage-inducible protein
GBP-1	gut and brain phosphatase inhibitor 1
GL	hepatic glycogen-targeting protein phosphatase 1 regulatory subunit
GM	skeletal muscle glycogen-targeting protein phosphatase 1 regulatory subunit, alias R _{GL}
HSP20	heat shock protein 20
I1	inhibitor 1
I2	inhibitor 2
KEPI	kinase C-enhanced PP1 inhibitor
MYPT1	myosin phosphatase targeting subunit 1
MYPT2	myosin phosphatase targeting subunit 2
MYL2	myosin light chain 2V
MYBPC3	cardiac myosin binding protein C
NIPP1	nuclear inhibitor of protein phosphatase 1
PHACTR4	phosphatase and actin regulator 4
PNL	phospholamban
PP1	protein phosphatase 1
Rb	retinoblastoma
SDS22	suppressor 2 of dis2–11 mutation
SPZ1	spermatogenic leucine zipper protein 1

Box 2

Mouse genetics nomenclature (Mouse Genome Informatics consortium [23]).

Conditional genotype: A genotype that is dependent on the presence of a certain factor. The term often refers to Cre-mediated excision of genomic sequences flanked by loxP sites.

Cre recombinase: A site-specific recombination enzyme that recognizes the 34-base pair long DNA sequence, known as a loxP site.

Cre/loxP system: Transgenic technology based on the ability of Cre recombinase to excise the DNA sequence between two loxP sites. The promoter of the Cre-expressing transgene determines the spatiotemporal expression of Cre recombinase. Cre recombinase can be expressed wide-spread, tissue-specific and/or cell-type specific.

Genotype: A description of the genetic information carried by an organism. It can also refer to specific alleles and their variants.

Genetically engineered mice: All mice with an altered genome compared to wild-type mice, including knockouts, knockins and transgenic mice.

Knockout (KO): A casual term for a targeted disruption of a gene of interest in which a loss-of-function allele is produced. KOs can be created by deleting portions of the gene to make it nonfunctional or by replacing the gene with an altered sequence. Because the creation of KOs involves directed homologous recombination, KOs are here not considered to be transgenic.

Knockin (KI): A casual term for targeted mutation of a gene of interest in which an alteration in gene function other than a loss-of-function allele is produced. Because the creation of KIs involves directed homologous recombination, KIs are here not considered to be transgenic.

Phenotype: Any measurable and observable characteristic of mice that is the result of the interaction between the genetic background and the environment.

Transgenic mice: Mice that carry a transgene in its genome.

Transgene (Tg): Any DNA sequence that has been introduced into the germ line of mice by random integration. Example, Tg (Myh6-PP1 α): PP1 α -expressing transgene, which expression is driven by the Myh6 promoter.

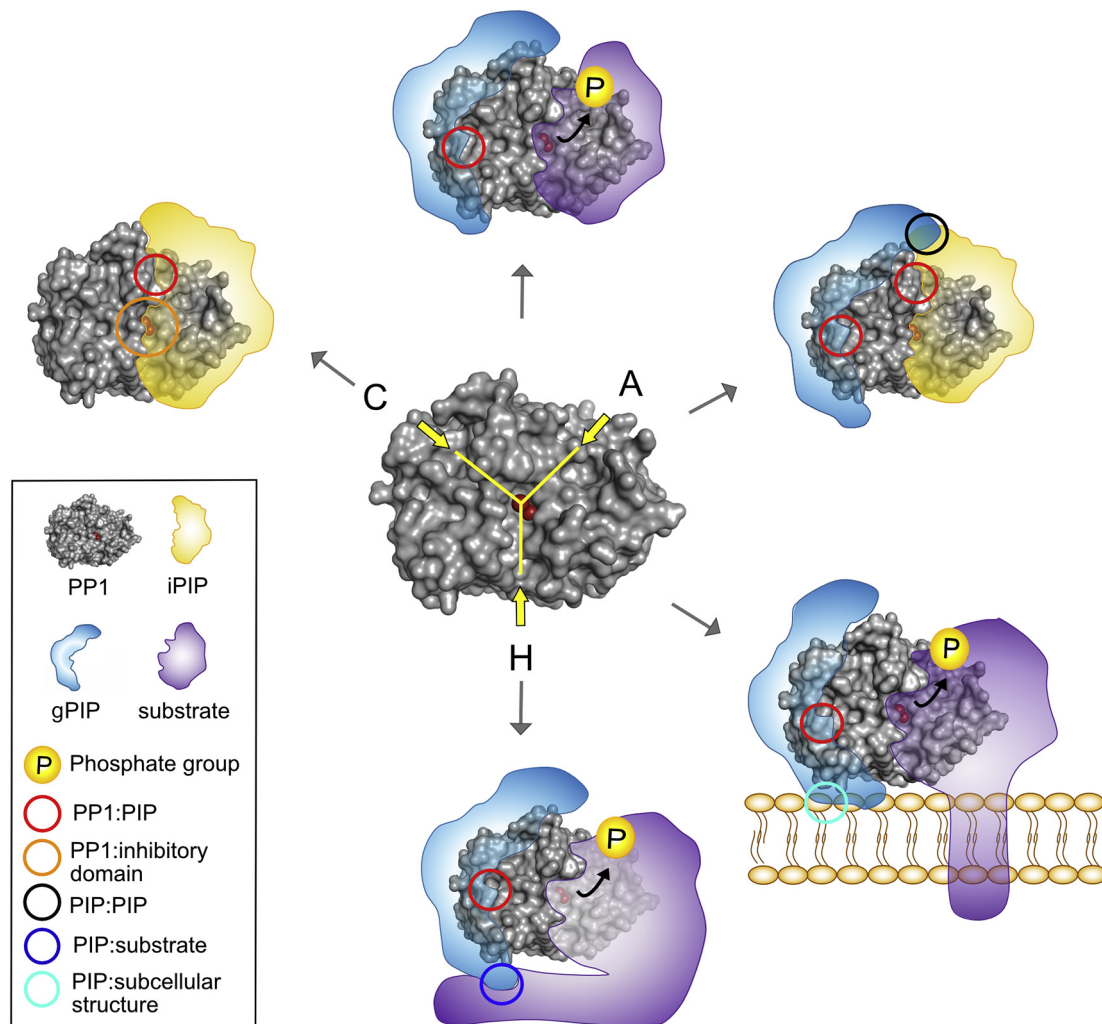


Fig. 1. Structure of PP1 holoenzymes. Surface representation of the PP1 catalytic domain (*center*). Indicated are the two metals in the active site of PP1 (red spheres) that lies at the Y-shaped intersection of three substrate-binding grooves; the acidic groove (A), the C-terminal groove (C) and the hydrophobic groove (H). Inhibitory PIP (iPIP) is depicted in yellow, a guiding PIP (gPIP) in blue and substrates in purple. Various interaction sites that can be considered for therapeutic targeting are represented by colored circles as indicated in the inset box. Bended black arrows indicate dephosphorylation of the substrate. PP1 surface structure was made using PyMOL (www.pymol.com). PIPs and substrates are schematically placed on PP1. PP1, Protein Phosphatase 1; PIP, PP1-Interacting Protein. See text for references.

of PP1 [2, 10, 16, 24]. Multiple SLiMs, also known as PP1 docking motifs, are often clustered to form an intrinsically disordered PP1-anchoring domain [13, 16, 25]. The combined binding of SLiMs establishes a high-affinity binding between PP1 and its PIP, which is often associated with (partial) structural folding of the PP1-binding domain [26]. The best-studied motif for PP1:PIP interactions is the so-called RVxF-motif, which docks into a hydrophobic groove that is remote from the catalytic pocket of PP1 (Fig. 1) [27]. This motif is present in about 70% of all known PIPs, and mutation of the valine and/or phenylalanine into an alanine is often sufficient to at least partially disrupt the interaction with PP1. However, some SLiMs, such as the SILK-motif, are present in far fewer PIPs [13]. In addition, some binding sites are highly structured, such as leucine-rich repeats of SDS22 or ankyrin repeats of MYPT1 [28, 29]. To date, about a dozen distinct PP1-binding sites have been characterized, enabling a huge combinatorial potential for generating unique PP1 holoenzymes [2]. The number and combination of PP1-binding sites differ between PP1 holoenzymes, as confirmed by several crystal structures, explaining how PP1 can interact with numerous structurally unrelated PIPs [16].

From a therapeutic viewpoint, the most promising approach for developing PP1-directed drugs does not involve interference with the catalytic site but with specific interaction sites within the PP1 holoenzymes [13]. Drugs that bind to the catalytic site will non-selectively target all PP1 holoenzymes, possibly even other structurally related phosphatases such as PP2A and PP2B holoenzymes. In contrast, drugs that disturb specific PP1 holoenzymes will selectively modulate PP1 activity towards a limited number of substrates [13, 16].

3. Diversity and specificity of PP1:PIP complexes

PP1 shows extreme phylogenetic and functional conservation, even though the number of PP1 genes differ between species (e.g. 7–11 genes in plants, 1 gene in *S. cerevisiae*, 4 genes in *D. melanogaster*, and 3 genes in vertebrates) [30]. The functional conservation of PP1 is illustrated by sequence identity (> 80%) between PP1 from yeast and humans, and by the observation that the lethality of PP1 loss in *S. cerevisiae* can be rescued by any human PP1 isoform [31]. Together, the three mammalian PP1 genes *Ppp1ca*, *Ppp1cb* and *Ppp1cc* encode four nearly identical PP1 isoforms (PP1 α , β , γ 1 and γ 2) that differ primarily at their extremities [32]. PP1 γ 1 and γ 2 isoforms arise from alternatively spliced transcripts that only differ in terms of retention or deletion of the last intron of the *Ppp1cc* gene. This results in PP1 γ 1 and γ 2 isoforms with a unique C-terminal tail of 9 or 23 amino acids in mice, respectively [33]. At the biochemical level, there are no substantial differences between the PP1 isoforms [34], but genetic ablation of individual PP1 genes in mice suggests distinct and overlapping functions, as discussed in Section 4 (Table 1).

Generally, PIPs are structurally unrelated, but they share and combine various functional domains involved in PP1-anchoring, PP1-inhibition, substrate-recruitment and/or subcellular-targeting [16]. First, all PIPs contain a PP1-anchoring domain, as discussed in Section 2 (Figs. 1 and 2). Second, some PIPs have an additional PP1-inhibitory domain that covers the active site of PP1, thereby preventing substrates from binding at the catalytic site. A PP1-inhibitory domain can bind to the active site of PP1 constitutively or only after phosphorylation [35, 36]. Third, many PIPs have a specific targeting domain that mediates binding to a particular subcellular compartment or structure (e.g. sarcoplasmic reticulum or glycogen particles) [37]. This enhances the local concentration of PP1 and promotes dephosphorylation of phospho-substrates that are specifically associated with that subcellular structure [16, 37]. Fourth, some PIPs contain a binding domain for a small subset of PP1 substrates [38]. The combination of PP1 and PIP substrate-binding sites dramatically increases the affinity for these substrates, allowing their dephosphorylation at physiological levels [16, 38]. Fifth, some PIPs prevent dephosphorylation of certain substrates by occluding one of the substrate-binding grooves of PP1 [25]. Finally, it should be

noted that PIPs often combine these different strategies and that multiple PIPs serve themselves as substrates for associated PP1 [2, 16].

From the list of 189 biochemically validated PIPs [37], we found genetically modified mouse models for 104 distinct PIPs (~55%), and often multiple models for one PIP, bringing the total number of PP1/PIP mouse models to > 170. Since most PIPs are multifunctional and/or do interact with multiple proteins, it is critical for the development of potential PP1-directed drugs to examine whether the observed phenotype depends on associated PP1. Therefore, we screened these mouse models to identify phenotypes connected to altered PP1 function as determined by changed PP1 activity, altered phosphorylation levels of substrates, and/or a phenotype associated with the expression of a PP1 dysfunctional mutant of a PIP. Based on these criteria, we selected 39 mouse models linked to 17 distinct PIPs (Tables 2 and 3). We functionally classify PIPs as inhibitory PIPs (iPIPs) or guiding PIPs (gPIPs) (Figs. 1 and 2). iPIPs block dephosphorylation of substrates by occupying the active site of PP1. Thus, ablation of an iPIP in mice will lead to increased dephosphorylation of physiological substrates of the PP1:iPIP holoenzyme. In contrast, gPIPs are defined as PIPs that guide PP1 towards a specific subset of substrates within a cell. Ablation of a gPIP in mice will lead to increased phosphorylation of the *in vivo* substrates of PP1:gPIP complex. We analyzed genetically altered mouse models of 7 iPIPs and 10 gPIPs, which are described in Table 2 and 3, respectively.

4. Mouse models of PP1 isoforms

The global ablation of PP1 α or PP1 γ isoforms in mice, did not cause an overt phenotype, except that male PP1 γ knockout (KO) mice were infertile due to impaired spermiogenesis (Table 1) [Agnus Nairn, personal communication, 39–41]. This indicates that other PP1 isoforms compensate for the loss of PP1 α or PP1 γ in all tissues except the testis, which could be explained by the distinct expression patterns of PP1 isoforms in the testis [41, 42]. More specifically, PP1 α , β and γ 1, but not PP1 γ 2, are expressed in somatic testicular cells and spermatogonia. Conversely, in (post-) meiotic germ cells only PP1 γ 2 is detected [41, 43, 44]. Thus, the germ-cell specific deletion of the *Ppp1cc* gene results in male infertility and sperm with abnormal morphology or teratospermia due to the loss of entire cellular pool of PP1 in the (post-) meiotic germ cells (Table 1) [41]. Accordingly, the testis-specific transgenic expression of PP1 γ 2 restored the fertility of *Ppp1cc* null mice in two independent mouse models (Table 1) [43]. Together, the various *Ppp1cc* mouse models demonstrate that PP1 γ 2 has a unique spermiogenic function. Along with the testis-enriched PP1 γ 2 isoform, there are testis/sperm-enriched PIPs isoforms such as NIPP1-T (also known as NIPP1 ϵ) [45] and SPZ1 [46], accounting for the formation of unique PP1 γ 2:PIP complexes in testis or sperm [47]. Such complexes are attractive candidate-targets for pharmacological intervention to combat male infertility since these drugs will likely not affect cellular processes in other tissues [47].

In contrast to total PP1 α and PP1 γ KO mice, the global deletion of PP1 β led to pre-weaning mortality (i.e. homozygotes died before an age of 3–4 weeks) as described by the International Mouse Phenotyping Consortium¹ (Table 1) [48]. This lethal phenotype suggests essential PP1 β -specific functions that cannot be compensated for by the remaining PP1 isoforms, likely because of the existence of PP1 β selective PIPs. For example, MYPT1 has an ankyrin domain that specifically interacts with the C-terminal domain of PP1 β [49]. However, biochemical data with PP1 chimera revealed that the central and N-terminal parts of PP1 β also contribute to its isoform-specific functions [31, 49, 50].

The selective deletion of individual PP1 isoforms in hearts

¹ International Mouse Phenotyping Consortium produces KO mice and phenotypes them according to fully validated and standardized protocols [48].

Table 1
Mouse models of PP1 isoforms.

Protein gene	Description of mouse model	Genotype of mouse model	Alterations in PP1/substrates [§]	Phenotype [reference]
PP1 α <i>Ppp1ca</i>	KO	<i>Ppp1ca</i> ^{-/-}	nd	No overt phenotype [A. Nairn, personal communication]
	Heart-specific Tg PP1 α	Tg (<i>Myh6</i> -PP1 α)	PP1 \uparrow , PNL-pS16 \downarrow	Decreased heart function [51]
	Embryonic heart-specific KO	<i>Ppp1ca</i> ^{fl/fl}	no	Viable, normal lifespan [42]
	Inducible heart-specific KO	Tg (<i>Nkx2.5</i> -Cre)	no	Viable, normal lifespan [42]
	Inducible heart-specific KO	Tg (<i>Myh6</i> -MerCreMer)	no	Viable, normal lifespan [42]
PP1 β <i>Ppp1cb</i>	KO	<i>Ppp1cb</i> ^{-/-}	nd	Preweaning lethality [48]
	Embryonic heart-specific KO	<i>Ppp1cb</i> ^{fl/fl}	pMYL2 \uparrow	Promoted heart failure [42]
	Inducible heart-specific KO	Tg (<i>Nkx2.5</i> -Cre)	MYBPC3-pS273/282/302 \uparrow	Promoted heart failure [42]
	Inducible heart-specific KO	<i>Ppp1cb</i> ^{fl/fl}	pMYL2 \uparrow	Promoted heart failure [42]
	Inducible heart-specific KO	Tg (<i>Myh6</i> -MerCreMer)	MYBPC3-pS282/302 \uparrow	Promoted heart failure [42]
PP1 γ 1/ γ 2 <i>Ppp1cc</i>	KO	<i>Ppp1cc</i> ^{-/-}	PP1 α \uparrow	Viable but only males are infertile [39, 40, 44]
	Embryonic heart-specific KO	<i>Ppp1cc</i> ^{fl/fl}	no	Viable, normal lifespan [42]
	Inducible heart-specific KO	Tg (<i>Nkx2.5</i> -Cre)	no	Viable, normal lifespan [42]
	Inducible heart-specific KO	<i>Ppp1cc</i> ^{fl/fl}	no	Viable, normal lifespan [42]
	Inducible heart-specific KO	Tg (<i>Myh6</i> -MerCreMer)	no	Viable, normal lifespan [42]
	Germ-cells specific KO	<i>Ppp1cc</i> ^{fl/fl}	PP1 γ 2 \downarrow PP1 γ 1 =	Male infertility and oligo-teratospermia [41]
	Testis-specific Tg PP1 γ 2 in PP1 γ KO	Tg (<i>Stra8</i> -Cre)	PP1 γ 2 \downarrow PP1 γ 1 =	Restoration of spermatogenesis [43]
	Testis-enriched Tg PP1 γ 2 in PP1 γ KO	<i>Ppp1cc</i> ^{-/-} Tg (<i>Pgk2</i> -PP1 γ 2)	nd	Restoration of spermatogenesis [43]

Cre, Cre recombinase; fl, floxed; KO, knockout; MerCreMer, Cre recombinase that is fused to two mutated estrogen receptor domains (Mer); MYBPC3 (cMYBPC), cardiac myosin binding protein C; *Myh6* (α MHC), cardiac muscle α isoform myosin heavy chain; MYL2 (MLC2V or RLC), cardiac ventricular myosin regulatory light chain 2; nd, not determined; no, no alteration detected in the analysed substrates, *Nkx2.5*, NK2 homolog 5; *Pgk2*, phosphoglycerate kinase 2; p-, phospho; PNL, cardiac phospholamban; PP, protein phosphatase; Ppp, phosphoprotein phosphatase; *Stra8*, stimulated by retinoic acid 8; Tg, transgene. §, numbers of phospho-amino acids correspond to mouse sequence according to <https://www.phosphosite.org/>.

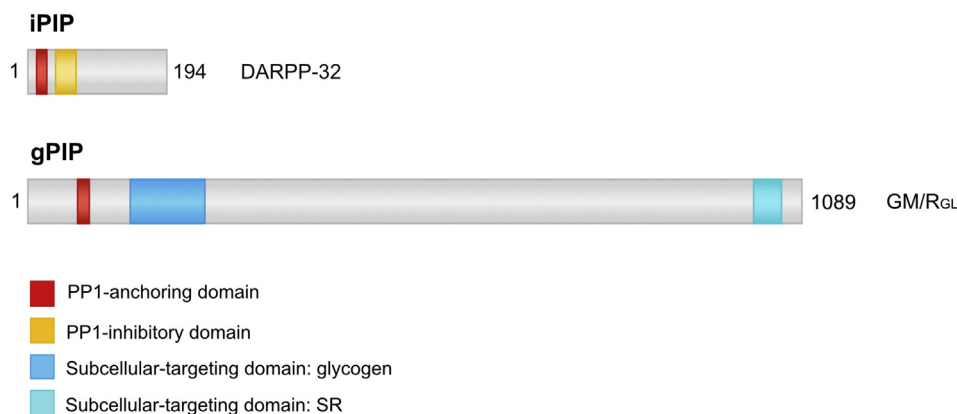


Fig. 2. Schematic representation of domain structure of an inhibitory PIP (iPIP) and a guiding PIP (gPIP). DARPP32 and GM from *Mus musculus* are depicted on scale. DARPP-32 contains a PP1-anchoring domain (red box) and a PP1-inhibitory domain (yellow box) in its N-terminal third. DARPP32 inhibits PP1 potently after phosphorylation of the PP1-inhibitory domain at threonine 34 residue. GM contains N-terminal a PP1-anchoring and a glycogen-targeting domain, while a second subcellular targeting domain that binds to sarcoplasmic reticulum (SR) is located at its C-terminus. DARPP-32, dopamine and cyclic AMP-regulated phosphoprotein of 32 kDa; GM, skeletal muscle glycogen targeting protein phosphatase 1 regulatory subunit; PIP, PP1-Interacting Protein.

See text for references.

confirmed the existence of PP1 β -specific functions. More specifically, the cardiac-specific deletion of PP1 α and PP1 γ by either embryonic or adult-heart specific Cre-mediated gene deletion revealed no major heart phenotype, while ablation of the PP1 β isoform using the same conditional Cre/lox targeting approaches induced heart failure (Table 1) [42]. The heart-specific deletion of PP1 β was associated with increased myofilament phosphorylation (myosin light chain 2V (MYL2) and cardiac myosin binding protein C (MYBPC3)). However, it did not cause changes in the phosphorylation of phospholamban (PLN), a key regulator of cardiac contractility, hinting at redundancy between PP1 isoforms in the dephosphorylation of PLN, but not of MYL2 and MYBPC3 [42]. Accordingly, the heart-specific overexpression of PP1 α resulted in diminished phosphorylation of PLN at serine 16 [51]. Phenotypically, transgenic mice with overexpressed PP1 α also showed reduced heart function, indicating that PP1 α is a negative regulator of cardiac function (Table 1) [51]. This aligns with various studies indicating that increased PP1 activity is associated with human and

experimental heart failure (reviewed in [52]). Increased PP1 activity due to increased PP1 levels and/or a decreased expression of PP1 inhibitory proteins is associated with less PLN phosphorylation at serine 16 and threonine 17 [52–55]. Collectively, the mouse models of PP1 isoforms revealed functional overlap but unique functions of PP1 γ 2 in testis and of PP1 β in multiple tissues, including the heart.

5. Mouse models of PP1 inhibitory PIPs

5.1. Diversity of iPIPs

Various iPIPs, including inhibitor 1 (I1) and its homolog dopamine and cyclic AMP-regulated phosphoprotein of Mr. 32,000 (DARPP32), Inhibitor 2 (I2), C-kinase potentiated protein phosphatase-1 inhibitor M, 17 kDa (CPI-17) and its homologs phosphoprotein holoenzyme inhibitor-1 (PHI-1), kinase C-enhanced PP1 inhibitor (KEPI) and gut and brain phosphatase inhibitor 1 (GBP1), contain a PP1-inhibitory region.

Table 2
Mouse models of PP1 inhibitory PIPs.

Protein gene	Description of mouse model	Genotype of mouse models	Alterations in PP1/substrate [§]	Phenotype [reference]
Inhibitor 1 <i>Ppp1r1a</i>	KO	<i>Ppp1r1a</i> ^{-/-}	PLN-pS16 ↓ RZR2-pS2813 ↓ PP1 level ↑	No obvious phenotype, some neuro-logical and heart alterations [51, 64, 65] Cardiac hypertrophy and mild cardiac dysfunction [64]
	Heart-specific Tg I1	Tg (Myh6-I1)		Enhanced cardiac function in long term and protection against pressure-overload-induced hypertrophy [66]
	Heart-specific Tg I1 ^{1-65, T35D} (constitutive active PP1 inhibitor)	Tg (Myh6-I1 ^{1-65, T35D})	PP1 activity ↓, PLN-pS16/T17 ↑	Improved cardiac contractility in young mice, but lethal after catecholaminergic stress and with aging [76]
	Inducible heart-specific Tg I1 ^{1-65, T35D} in I1 KO Heart-specific Tg I1 ^{G109E} (PP1 binding mutant) Inducible brain-specific Tg I1 ^{9-54, T35D} (constitutive active PP1 inhibitor)	<i>Ppp1r1a</i> ^{-/-} Tg (TetO-I1 ^{1-65, T35D}) Tg (Myh6-tTA), Tg (Myh6-I1 ^{G109E}) Tg (TetO-I1 ^{9-54, T35D}) Tg (Camk2a-rtTA)	PLN-pS16 ↑, RZR2-pS2813 ↑ PP1 activity ↑, PLN-pS16/T17 ↓ RZR2-pS2813 ↑ PP1 activity ↓, CREB1-pS133 ↑ CAMK2A-pT286 ↑ GLUR1-pS849 ↑	Improved learning and enhanced memory, facilitated potentiation, impaired recovery from ischemia [77–79]
DARPP32 <i>Ppp1r1b</i>	KO	<i>Ppp1r1b</i> ^{-/-}	*pGLUN1 ↓	Diminished responses to dopamine, psy-chotomimetic and antipsychotic drugs [81]
	DARPP32 ^{T34A} (constitutive inactive PP1 inhibitor)	<i>Ppp1r1b</i> ^{T34A}	*CREB1-pS133 ↓ *GSK3β-pS9 ↓ *H3-pS10 ↓ #pERK2 ↓ #H3-pS10/acK14 ↓ #GLUR1-pS849 ↓ #pRPS6 ↓ #pERK1/2 ↓ #H3-pS10/acK14 ↓ #GLUR1-pS849 ↓ #pRPS6 ↓	Impaired response to psychotomimetic (dopaminergic agonists, serotonergic and glutamatergic antagonist) [84–86]
	Striatonigral neuron specific KO	<i>Ppp1r1b</i> ^{fl/fl} Tg (Drd1-Cre)	#pERK1/2 ↓ #H3-pS10/acK14 ↓ #GLUR1-pS849 ↓ #pRPS6 ↓	Decreased motor behavior, abolished dyskinetic behavior in response to Parkinson's disease drug L-DOPA [83, 84]
Striatopallidal neuron specific KO	<i>Ppp1r1b</i> ^{fl/fl} Tg (Drd2-Cre)	#phosphorylation of ERK1/2, H3, GLUR1, RPS6: not altered	Increased motor behavior, reduced cataleptic response to antipsychotic drug [83, 84]	
Inhibitor 2 <i>Ppp1r2</i>	KO	<i>Ppp1r2</i> ^{-/-}	Ho: PP1α-pT320 ↑ He: CREB1-pS133↑ He: PP1 activity ↓	Ho: embryonic lethal He: viable, no overt phenotype; increased memory formation [87]
	Heart-specific Tg I2 ¹⁻¹⁴⁰ (constitutive active PP1 inhibitor)	Tg (Myh6-I2 ¹⁻¹⁴⁰)	PP1 level ↑ PP1 activity ↓ PLN-pS16 ↑	Enhanced cardiac contractility [68], but deleterious under conditions of pressure overload [73]
CPI-17 <i>Ppp1r14a</i>	Double heart-specific Tg I2 ¹⁻¹⁴⁰ and Tg PP1α	Tg (Myh6-I2 ¹⁻¹⁴⁰) Tg (Myh6-PP1α)	Normalized PP1 activity	Normalized heart morphology and heart function [69]
	KO	<i>Ppp1r14a</i> ^{-/-}	**MYPT1-pT852 ↓ **MYPT1-pT694 ↓ **MYL2-pS19 ↓ **MYPT1-pT852 ↓ **MYL2-pS19 ↓	Decreased main blood pressure [70]
KEPI <i>Ppp1r14c</i>	CPI-17 ^{T38A} (constitutive inactive PP1 inhibitor)	<i>Ppp1r14a</i> ^{T38A}	**MYPT1-pT852 ↓ **MYL2-pS19 ↓	Decreased main blood pressure [70]
	KO	<i>Ppp1r14c</i> ^{-/-}	##PP1 activity ↑ in thalamus	Decreased response to morphine after chronic morphine injections, increased SARS coronavirus pathogenesis [88, 89]
GBPI-1 <i>Ppp1r14d</i>	KO	<i>Ppp1r14d</i> ^{-/-}	nd	Abnormal heart morphology [48]
HSP20 <i>Hspb6</i>	Cardiac-specific Tg HSP20	Tg (Myh6-HSP20)	PP1 activity ↓ PLN pS16/T17 ↑	Improved cardiac function and recovery reduced infarction [63, 71], improved angiogenesis in diabetic hearts [72]

Ac, acetyl; CAMK2A (*Camk2a*), Calcium/calmodulin-dependent protein kinase type II subunit alpha; CPI-17, protein kinase C-potiated protein phosphatase-1 Inhibitor M, 17 kDa; CREB1, cAMP responsive element-binding protein 1; DARPP32, dopamine and cyclic AMP-Regulated PhosphoProtein of Mr. 32,000; *Drd1*, dopamine receptor D1; *Drd2*, dopamine receptor D2; ERK1/2 (*Mapk3/1*), extracellular signal-regulated kinase 1/2; GBPI1, gut and brain phosphatase inhibitor 1; GLUN1 (*Gria1*), glutamate ionotropic receptor NMDA type subunit 1; GLUR1 (*Gria1*), glutamate ionotropic receptor AMPA type subunit 1; H3, histone 3; He, heterozygous; Ho, halberomozygous; HSP20 (*Hspb6*), heat shock protein 20; KEPI, Kinase C-enhanced PP1 inhibitor; KO, knockout; *Myh6*, cardiac muscle α isoform myosin heavy chain; nd, not determined; p, phospho; PLN, cardiac phospholamban; RPS6, ribosomal protein S6; RZR2, ryanodine receptor 2; rtTA, reverse tetracycline-controlled transactivator; SARS, severe acute respiratory syndrome, TetO, tetracycline operator; Tg, transgene; tTA, tetracycline-controlled transactivator; L-DOPA, L-3,4-dihydroxy-phenylalanine. *Psychotomimetic drug-induced, **, phorbol ester-induced; #, L-DOPA-induced; ##, morphin-induced §, numbers of phospho-amino acids correspond to mouse sequence according to <https://www.phosphosite.org/>.

I1, CPI-17 and their homologs, are converted into a potent PP1 inhibitor upon phosphorylation of their PP1-inhibitory domain, thereby presumably acting as a pseudosubstrate [56–58]. I2, on the other hand, is inhibitory without prior phosphorylation [36]. The iPIPs form stable complexes with PP1 alone but can also form stable trimeric complexes with various heterodimeric PP1:PIPs (Fig. 1). Various trimeric PP1 complexes containing I1 or I2 have been described, including I1:PP1:GADD34 [59], I1:PP1:AKAP18 [60], I2:PP1α:Spinophilin [61] and I2:PP1α/γ1:Neurabin [62]. In contrast, CPI-17 and its homologs

specifically inhibit MYPT1/2 containing PP1 complexes [58]. In addition to these well-known inhibitors, we also classified heat shock protein (HSP20) as an iPIP based on its reported *in vivo* inhibitory function for PP1 [63]. The genetically engineered mouse models of the selected iPIPs disclosed their importance for heart and brain function and the response to viral infections (Table 2). This suggests that PP1 holoenzymes with an associated iPIP may serve as a therapeutic target for neurologic and psychiatric disorders, heart diseases and viral infections, as described below.

5.2. Heart-specific functions of iPIPs

Various mouse models of iPIPs support the notion that inhibition of PP1 in the heart is an attractive therapeutic approach to treat heart failure and cardiac arrhythmia (Table 2). First, the global KO of I1 resulted in impaired heart function [51, 64, 65]. Second, cardiac-specific expression of a phosphomimetic I1 mutant (I1^{1-65, T35D}) that acts as a constitutively active PP1 inhibitor, was associated with decreased PP1 activity, increased phosphorylation of PLN at serine 16 and threonine 17 and enhanced heart function [66]. Additionally, I1^{1-65, T35D} expressing mice prevented cardiac dysfunction or hypertrophy under transverse aortic constriction conditions, suggesting that I1^{1-65, T35D} may protect against heart failure progression [66]. A heart-specific transgenic overexpression of wild-type I1 in mice resulted in a compensatory increased level of PP1 and, hence, was associated with mild heart dysfunction [64]. Third, the heart-specific expression of a transgene that encodes a human PP1 binding mutant of I1 (I1^{G109E}) in mice also resulted in higher PP1 activity, impaired heart function and increased arrhythmia [67]. Fourth, heart-specific transgenic expression of a constitutively inhibitory fragment of I2 (I2¹⁻¹⁴⁰, a C-terminal truncated form of I2) in mice showed enhanced cardiac contractility. This was associated with severely decreased PP1 activity and increased phosphorylation of PLN at serine 16, despite an increased level of PP1 [68]. Importantly, the heart-specific transgenic expression of PP1 α in I2¹⁻¹⁴⁰ expressing mice, rescued its phenotype and showed a normal PP1 activity, heart morphology and heart function [69]. Although transgenic expression of I1^{1-65, T35D} and I2¹⁻¹⁴⁰ acted similarly in the heart based on reduced PLN phosphorylation, the global KO of I2, but not of I1, was embryonically lethal. This suggests that I1 and I2 have distinct functions in other tissues. Fifth, global KOs of CPI-17 or its homolog GBP1, were associated with decreased blood pressure and abnormal heart morphology, respectively (Table 2) [48, 70]. Decreased blood pressure was also observed in mice expressing only a non-phosphorylatable CPI-17 mutant (CPI-17^{T38A}) that acts as a constitutively inactive PP1 inhibitor, confirming the importance of PP1:CPI-17 interaction for this phenotype. Sixth, heart-specific transgenic expression of HSP20 was cardioprotective and associated with increased phosphorylation of PLN at serine 16 and threonine 17 and enhanced sarcoplasmic reticulum calcium cycling [63, 71]. Elevated levels of HSP20 also improved cardiac function and angiogenesis in diabetic mice [72].

Together, these different mouse models suggest that PP1 inhibition holds great potential for the treatment of heart failure. However, this approach should be considered cautiously. First, a long-term inhibition of PP1 with I2¹⁻¹⁴⁰ worsened heart failure under conditions of pressure overload (i.e. an experimental model for cardiac hypertrophy) [73, 74]. Second, ablation of I1 was cardioprotective under conditions of increased adrenergic drive [64, 75]. Third, the inducible heart-specific expression of I1^{1-65, T35D} in I1-deficient mice improved heart function in young mice but was deleterious after catecholaminergic stress and with increased age [76]. Thus, it is still uncertain whether I1 is beneficial or detrimental to the development of heart disease [74].

5.3. Brain-specific functions of iPIPs

Four iPIPs (I1, DARPP32, I2 and KEPI) are associated with neurological phenotypes (Table 2). Inducible expression of the constitutively active PP1 inhibitor I1^{9-54, T35D} in the brain improved learning and memory but impaired recovery from an ischemic stroke [77–79]. Thus, PP1 constrains learning and memory formation but positively regulates recovery of brain damage [77, 78]. DARPP32, which is a homolog of I1 that is highly enriched in the dopaminergic medium spiny neurons in the striatum, is turned into a potent PP1 inhibitor upon phosphorylation at T34 e.g. by protein kinase A. DARPP32 plays a pivotal role in various neurological processes, including dopamine neurotransmission. Medium spiny neurons are classified as striatonigral and striatopallidal neurons, which are morphologically similar but differ in their

expression of different subtypes of dopamine receptors. Striatonigral neurons primarily express dopamine receptors of type 1 (DRD1), while striatopallidal neurons primarily express dopamine receptors of type 2 (DRD2) [80]. Various neurological and psychiatric disorders such as drug addiction, schizophrenia and Parkinson's disease are characterized by a disturbed balance between DRD1 and DRD2 signalling, which is often associated with altered DARPP32 function [80]. Global deletion of DARPP32 in mice led to alterations in dopaminergic neurotransmission and an impaired behavioral response to psychostimulants (e.g. cocaine), and antipsychotic drugs (e.g. haloperidol) [81]. Cell type-specific deletion of DARPP32 or expression of differentially-tagged DARPP32 in DRD1- or DRD2-expressing neurons of mice revealed neuron-specific pathways in the brain coupled to opposite functional outputs [80, 82–85]. Psychostimulants and antipsychotic drugs exert differential effects on DARPP32 phosphorylation in striatonigral and striatopallidal neurons, explaining their opposing behavioural and clinical effects [80, 82–85]. Mice that carried the T34A mutation in the endogenous DARPP32 gene, and thus express a constitutively inactive inhibitor of PP1, largely phenocopied DARPP32 KO both functionally and biochemically. For example, DARPP32^{T34A} mice or mice with DARPP32 specifically ablated in striatonigral neurons featured phospho-substrates with the same alterations (Table 2). This underscores the importance of the PP1:DARPP32 holoenzyme for the observed phenotypes [86].

While genetic deletion of both I2 alleles was embryonic lethal, I2 heterozygous mice exhibited no overt phenotype but showed increased memory formation, decreased PP1 activity and increased phospho-CREB1 [87]. Importantly, this model showed that, despite its presumed function as a PP1 inhibitor, I2 positively regulates PP1 in memory function *in vivo* [87]. KEPI is yet another iPIP linked to a neurological phenotype. KEPI inhibits PP1 after phosphorylation by protein kinase C. Global deletion of KEPI was associated with increased PP1 activity and decreased responsiveness to morphine after chronic morphine injections [88], suggesting that the selective inhibition of PP1 enhances morphine analgesia. Collectively, the neurological phenotypes associated with the different iPIPs revealed that PP1 constrains learning and memory formation and plays a key role in dopamine neurotransmission and morphine signaling. Therefore, targeting a particular PP1:iPIP holoenzyme can be a valuable therapeutic approach for the treatment of various neurological disorders. However, a cell type-selective method may be required to target, for example PP1:DARPP32 [80].

5.4. The role of iPIPs in viral infections

A global KO of KEPI disclosed the importance of iPIPs for the treatment of viral infections (Table 2) [89]. KEPI-null mice were more susceptible to the pathogenesis of severe acute respiratory syndrome coronavirus (SARS-CoV), suggesting that KEPI may protect against this virus [89]. Independently, some studies identified the importance of PP1 in other types of viral infections, including Human Immunodeficiency Virus (HIV) 1, hepatitis B and adeno-associated virus [90–92]. However, these findings have not yet been validated by mouse models and have been coupled to other PIPs such as trans-activator of transcription (TAT), hepatitis B regulatory protein x (HBx) and nuclear inhibitor of PP1 (NIPP1), respectively [90–92].

6. Mouse models of guiding PIPs

6.1. Diversity of gPIPs

gPIPs employ various strategies to guide PP1 to a subset of substrates (Fig. 1 and 2). Many gPIPs contain subcellular-targeting domains that bind to specific organelles or macromolecular complexes, such as glycogen particles, plasma membranes or the endoplasmic reticulum [16, 37]. For example, various glycogen-targeting G subunits (e.g. hepatic glycogen-targeting protein phosphatase 1 regulatory subunit (GL),

Table 3
Mouse models of guiding PIPs.

Protein/ gene	Description of mouse model	Genotype of mouse model	Alterations in PP1/ substrate [§]	Phenotype [reference]
GM (R_{GL}) <i>Ppp1r3a</i>	KO	<i>Ppp1r3a</i> ^{-/-}	PP1 level/ activity ↓ GS-pS641/645 ↑ GP- pS15 ↑ PP1 level ↑	Decreased glycogen content; prediabetic phenotype in 129/Ola derived background but no obvious phenotype in C57BL/6, 129s2/ sV and 129/SvJ background [97–100]
	Muscle-specific Tg GM GM ^{ΔC} (SR binding mutant)	Tg (Ckm-GM) <i>Ppp1r3a</i> ^{ΔC}	GS activity ↓ GP activity ↑	Increased muscle glycogen content, abolished GS activation in response to exercise [101] Decreased muscle glycogen content [102]
GL <i>Ppp1r3b</i>	Liver-specific KO	<i>Ppp1r3b</i> ^{R/R} Tg (Alb-Cre) <i>Ppp1r3b</i> ^{Y284F/Y284F}	GS level ↓ GS-pS641 ↑ GS-pS641/645 ↓ GP-pS15 ↑	Reduced hepatic glycogen content, impaired whole body glucose homeostasis [104] Improved glucose tolerance [103, 105]
	GL ^{Y284F/Y284F} (phospho-GP binding mutant)			
NIPP1 <i>Ppp1r8</i>	KO	<i>Ppp1r8</i> ^{-/-}	Pan-pThr ↑ Nuclear	Early embryonic lethality [107, 113]
	Inducible brain-specific Tg NIPP1 ^{143–224}	Tg (biTetO-NIPP1 ^{143–224} - LacZ), Tg (Camk2a-rtTA2)	PP1 activity ↓ H3-pS10 ↑	Improved memory performance, enhanced long-term potentiation and alteration in gene transcription [116, 117]
Neurabin I <i>Ppp1r9a</i>	KO	<i>Ppp1r9a</i> ^{-/-}	PP1 levels ↓ GluR1-pS849 ↓ [§] GluR1-pS849 ↑	Abnormal psychostimulant response and dopamine signaling transduction, reduced anxiety- and depression-related behaviors in young adult mice, impaired contextual fear memory [145–147]
Spinophilin <i>Ppp1r9b</i>	KO	<i>Ppp1r9b</i> ^{-/-}	PP1 levels ↓ GluR1-pS849 ↓ [§] GluR1-pS849 ↑	Abnormal psychostimulant response and dopamine signaling transduction, reduced brain size, reduced anxiety- and depression- related behaviors in middle-aged mice and associative learning ability [145, 147–149]
MYPT1 <i>Ppp1r12a</i>	KO	<i>Ppp1r12a</i> ^{-/-}	nd	Embryonic lethality [150]
	Smooth muscle-specific KO	<i>Ppp1r12a</i> ^{R/R} Tg (Acta2-Cre)	[§] PP1β level ↓ [§] CPI-17-pT38 ↓ [§] pMYL2 ↑	Altered contractile responses in intestinal [151] and vascular smooth muscle, hypertension [152]
MYPT2 <i>Ppp1r12b</i>	Inducible smooth muscle heavy chain-specific KO	<i>Ppp1r12a</i> ^{R/R} Tg (Myh11-CreERT2)	PP1β level ↓ [§] CPI-17-pT38 ↓	Moderate alteration in bladder contractile responses [153]
	Cardiac specific- Tg MYPT2	Tg (Myh6-MYPT2)	PP1β level ↑ PP1 activity ↑ pMYL2 ↓	Left ventricular heart enlargements with heart dysfunctions [154]
GADD34 <i>Ppp1r15a</i>	KO	<i>Ppp1r15a</i> ^{-/-}	[#] eIF2α-pS52 ↑	No overt phenotype, reduced hepatocarcinogenesis in chemical- induced tumorigenesis [122, 126]
	Early-embryonic specific KO	<i>Ppp1r15a</i> ^{R/R} Tg (eIIA-Cre)	[#] eIF2α-pS52 ↑	Hypersplenism, erythrocyte abnormalities, resembling mild thalassemia syndromes [125]
	GADD34 ^{1–549} (PP1 binding mutant)	<i>Ppp1r15a</i> ^{1–549/1–549}	(*) eIF2α-pS52 ↑	No overt phenotype [123]
CReP <i>Ppp1r15b</i>	KO	<i>Ppp1r15b</i> ^{-/-}	(*) eIF2α-pS52 ↑	Perinatal lethality, impaired erythropoiesis [123]
	GADD34 ^{1–549} and CReP KO	<i>Ppp1r15a</i> ^{1–549/1–549} <i>Ppp1r15b</i> ^{-/-}	nd	Early embryonic lethality [123]
	GADD34 ^{1–549} , CReP KO and eIF2α ^{S52A}	<i>Ppp1r15a</i> ^{1–549/1–549} <i>Ppp1r15b</i> ^{-/-} <i>Eif2α</i> ^{S52A}	Unphosphorylatable eIF2α ^{S52A}	Rescue of the early embryonic lethality by the eIF2α ^{S52A} mutation [123]
PHACTR4 <i>Phactr4</i>	PHACTR4 ^{R650P} (PP1 binding mutant)	<i>Phactr4</i> ^{R650P/R650P}	PP1 activity ↓ PP1-pT320 ↑ Rb-pS601 ↑ Rb-pS800/804 ↑	Lethality at birth; neuronal tube, eye and gastrointestinal defects in embryos; resembles human Hirschsprung disease [131, 132]
	PHACTR4 ^{R650P} in E2f1 KO	<i>Phactr4</i> ^{R650P/R650P} <i>E2f1</i> ^{-/-}	nd	Rescue of the <i>Phactr4</i> ^{R650P/R650P} phenotype by loss of E2f1 [131]

Acta2, Actin aortic smooth muscle; *alb*, albumin; GADD34, growth arrest and DNA damage-inducible transcript 34; Camk2a, Calcium/calmodulin-dependent protein kinase type II subunit alpha; *Ckm*, creatine kinase muscle; cre, site specific recombinase; CReP, Constitutive repressor of eIF2alpha phosphorylation; E2f1, transcription factor E2f1; eIF2α, eukaryotic translation initiation factor 2α; *eIIA*, adenovirus promoter directs Cre expression in preimplantation embryos; GluR1 (*Gria1*), glutamate ionotropic receptor AMPA type subunit 1; GS, glycogen synthase (*Gys1*, muscle isoform; *Gys2*, liver isoform); GL, hepatic glycogen-targeting protein phosphatase 1 regulatory subunit; GM, skeletal muscle glycogen-targeting protein phosphatase 1 regulatory subunit; GP, glycogen phosphorylase muscle isoform (*Pygm*) and liver isoform (*Pygl*); KO, knockout; LacZ, beta-galactosidase; *Myh6*, cardiac muscle alpha isoform myosin heavy chain; *Myh11*, smooth muscle isoform myosin heavy chain; MYL2 (RLC), cardiac ventricular myosin regulatory myosin light chain; nd, not determined; p, phospho; Rb, retinoblastoma; SR, sarcoplasmic reticulum; Tg, transgene. *, Thapsigargin/DTT (inducer of ER stress)-induced; #, hemin-induced; %, KCl/norepinephrine/carbachol-induced contraction; \$, dopamine D1 agonist-induced; §, numbers of phospho-amino acids correspond to mouse sequence according to <https://www.phosphosite.org/>.

skeletal muscle glycogen-targeting protein phosphatase 1 regulatory subunit (GM), protein targeting to glycogen (PTG)) target PP1 toward glycogen, thereby enhancing the dephosphorylation of glycogen-associated substrates such as glycogen synthase and glycogen phosphorylase [93]. Other gPIPs have substrate-recruitment domains that bind to specific subsets of substrates. For example, the forkhead-associated domain of NIPP1 binds a specific subset of proteins that are phosphorylated at threonine-proline dipeptide motifs, for regulated dephosphorylation by associated PP1 [94]. One PIP alone can combine multiple subcellular-targeting and/or substrate-recruitment domains.

For example, GM has separate glycogen and membrane-targeting domains (Fig. 2) [95], while the stress-induced protein GADD34 binds to the endoplasmic reticulum and eIF2α via its subcellular-targeting and substrate-recruitment domains, respectively [96]. Various gPIPs have a number of isoforms with often a different tissue expression pattern. For example, the mammalian genome harbors seven *Ppp1r3* genes (*Ppp1r3a-g*), which each encode an isoform of the glycogen-targeting G subunit. The *Ppp1r3a*, *Ppp1r3b* and *Ppp1r3c* genes encode GM, GL and PTG isoforms, respectively. GM is primarily expressed in muscle and GL in the liver, while PTG is ubiquitously expressed.

We identified 10 gPIP-encoding genes (*Ppp1r3a/b*, *Ppp1r8*, *Ppp1r9a/b*, *Ppp1r12a/b*, *Ppp15a/b*, *Phactr4*) that have been functionally studied using genetically modified mouse models and showed PP1-dependent phenotypes (Table 3). While all mouse models showed alteration in PP1 activity and/or altered phosphorylation levels of substrates, six gPIPs (GM, GL, GADD34, CREP, PHACTR4 and NIPP1) had at least one mouse model with the expression of a PIP mutant that alters associated PP1 function. Phenotypical analyses of expression of these mutants in mice enable the identification of interaction sites with huge therapeutic potential. We selected these six gPIPs for further review.

6.2. PP1-dependent functions of glycogen-targeting subunits GM and GL

GM- and GL-deficient mice showed increased phosphorylation of their substrates glycogen synthase and glycogen phosphorylase, resulting in the inactivation of glycogen synthase but activation of glycogen phosphorylase and thus decreased glycogen content (Table 3) [97–100]. Furthermore, the striated-muscle specific transgenic expression of GM was associated with increased muscle glycogen content and the abolition of glycogen synthase activation in response to exercise (Table 3) [101]. A third GM mouse model expressed a C-terminally truncated form of GM (GM^{ΔC}) with intact glycogen-targeting and PP1-anchoring domains, but lacking its sarcoplasmic-targeting domain (Fig. 2). These mice demonstrated impaired glycogen synthesis associated with the inactivation of glycogen synthase (i.e. hyperphosphorylation), thus phenocopying the GM-null mice (Table 3) [102]. This result suggests that a disturbance of the binding of a gPIP to its subcellular structure can lead to a nonfunctional PP1 holoenzyme. Therefore, interference with the subcellular targeting of a gPIP is a potential therapeutic target for altering PP1 function (Fig. 1, Table 3). Interestingly, phenylalanine mutation of GL at tyrosine 284 prevented the allosteric inhibitory binding of phospho-glycogen-phosphorylase to GL, resulting in the dephosphorylation and activation of glycogen synthase [103, 104]. Thus, knockin mice that express mutant GL^{Y284F} displayed an enhanced hepatic glycogen synthase activity and improved glucose tolerance, supporting the notion that pharmacological inhibition of this particular GL:phospho-glycogen-phosphorylase interaction can be used to combat diabetic hyperglycaemia [93, 103, 105, 106].

6.3. Cellular-context dependent functions of NIPP1

NIPP1, encoded by the *Ppp1r8* gene, is a major nuclear PIP that is ubiquitously expressed, but its expression level is cell-type dependent [107, 108]. The N-terminal forkhead-associated domain of NIPP1 recruits PP1 substrates that are phosphorylated at threonine-proline dipeptide motifs, including the pre-mRNA splicing factor SAP155 and the methyltransferase EZH2 [38, 94, 109, 110]. NIPP1 binds PP1 via a central PP1-anchoring and a C-terminal PP1-inhibitory domain [26, 111]. The global deletion of NIPP1 in mice resulted in early embryonic lethality associated with reduced cell proliferation (Table 3) [107]. Similarly, the postnatal tamoxifen-induced deletion of NIPP1 in the testis resulted in a progressive loss of germ cells, culminating in a Sertoli-cell-only phenotypes [108]. This can be attributed to the decreased proliferation and survival capacity of cells of the spermatogenic lineage. In contrast, the liver-specific NIPP1 KO showed a rather mild phenotype associated with bile-duct hyperplasia through enhanced biliary epithelial cell proliferation [112]. Collectively, the NIPP1 mouse models indicate that the functions of NIPP1 may be cell-type dependent [107, 108, 112]. NIPP1-null embryos at embryonic day E6.5 showed increased global phospho-threonine signals [113], in line with a substrate-specifying role of NIPP1 (Table 3) [26, 110]. There are no data available regarding alterations in PP1 activity and/or substrate phosphorylation for the inducible and conditional NIPP1 KOs. However, various biochemical and cellular assays have revealed that known cellular NIPP1 functions (pre-mRNA splicing and transcription) are largely

dependent on both association with PP1 and recruitment of substrates [38, 110, 114]. Mice that inducibly express the central part of NIPP1 (NIPP1^{143–224}) in the forebrain showed a decreased nuclear PP1 activity and increased histone H3 phosphorylation at serine 10 (Table 3). Since NIPP1^{143–224} mainly comprises the central PP1-anchoring domain but does not include the substrate-recruitment domain, it seems likely that this effect of NIPP1^{143–224} stems from a PP1 titration effect resulting in the competitive disruption of PP1:PIP holoenzymes that are important for the forebrain [115–117]. Transgenic NIPP1^{143–224} mice demonstrated improved long-term memories associated with decreased nuclear PP1 activity. In agreement, the same phenotype was observed in transgenic mice expressing a constitutively inhibitory mutant of I1 (Table 2). Likewise, a decreased phospho-H3 was detected in mice expressing the inactive DARPP32^{T34A} mutant (Table 2) [86].

6.4. PP1-dependent functions of GADD34 and CREP

Ppp1r15a and *Ppp1r15b* genes encode for growth-arrest and DNA-damage-inducible transcript 34 (GADD34) and constitutive repressor of eIF2 α phosphorylation (CREP), respectively. They both target the eukaryotic initiation factor (eIF) 2 α for dephosphorylation of phosphoserine 52 by associated PP1. The phospho-serine 52 in mouse corresponds to the well-known phospho-serine 51 site of eIF2 α . GADD34 and CREP share structural homology in their C-termini which harbors their PP1-binding domain [118]. The expression of GADD34 is mainly induced in stress conditions, while CREP is constitutively expressed and its levels are unchanged by stress [119–121]. Stress signaling in eukaryotic cells depends critically on the phosphorylation of eIF2 α at serine 52, which occurs for example in response to the accumulation of misfolded proteins in the endoplasmic reticulum (also known as the unfolded protein response UPR), amino acid deprivation and viral infections [120, 121]. Upon stress, eIF2 α gets hyperphosphorylated at serine 52, attenuates global protein synthesis and induces the expression of GADD34 (but not CREP) at the transcript and protein levels. GADD34 expression generates a feedback loop that reverses the stress-induced phosphorylation of eIF2 α , thereby restoring normal protein synthesis. Thus, under basal conditions, the dephosphorylation of eIF2 α -pS52 is tightly regulated by PP1:CREP, while PP1:GADD34 is responsible for the dephosphorylation of eIF2 α -pS52 in stress condition.

Mice lacking either GADD34 or CREP exhibited enhanced basal and/or stress-induced eIF2 α -pS52 signals, indicating that both isoforms are crucial for eIF2 α dephosphorylation at serine 52 (Table 3) [122, 123]. Two other genetically engineered GADD34 mice models confirmed the increased stress-induced hyperphosphorylation of eIF2 α at serine 52 (Table 3). First, a conditional KO model in which GADD34 was ablated at the early embryonic stages using the Cre/loxP system confirmed the altered phosphorylation of eIF2 α . Second, mice that expressed a truncated form of GADD34 (GADD34^{1–549}) that was no longer able to form a functional phosphatase complex but still interacted with eIF2 α , displayed the similar effect on eIF2 α -pS52 [123–125]. The latter mouse model established the importance of the PP1:GADD34 interaction for eIF2 α dephosphorylation [96, 123]. Phenotypically, mice lacking functional GADD34 exhibited mild phenotypes with no visible abnormalities but demonstrated enhanced obesity when fed a high-fat diet, impaired hepatocarcinogenesis in chemically-induced tumorigenesis models and erythrocyte defects that resembled mild thalassemia syndromes [122, 125, 126]. In contrast, the global deletion of CREP causes perinatal lethality associated with severe anemia [123]. Furthermore, mice expressing GADD34^{1–549} and lacking CREP displayed early embryonic lethality, demonstrating that GADD34 and CREP have overlapping as well as distinct functions [123]. Together, this suggests functional redundancy between the two isoforms during embryonic development, but not throughout the neonatal stage. Importantly, early embryonic lethality could be rescued by alanine mutation of eIF2 α at serine 52 (eIF2 α ^{S52A}), indicating that eIF2 α is the

only important substrate for PP1:CREP and PP1:GADD34 [123].

Some viruses encode proteins (e.g. ICP34.5) that are structurally related to the C-terminal domain of GADD34/CREP and also promote PP1-mediated dephosphorylation of eIF2 α -pS52 [96, 118, 127]. This enables these viruses to reverse the translational block that is instigated when they enter the host cell [96, 127]. Finally, there is evidence at the biochemical level for the existence of trimeric complexes such as PP1:GADD34:I1 and PP1:GADD34:ACTIN, hinting at additional levels of regulation of eIF2 α phosphatases [59, 128, 129]. To gain further insight into the physiological importance of I1 in eIF2 α -pS52 dephosphorylation, it would be worthwhile to analyze the stress-induced eIF2 α phosphorylation in I1-null mice. From a therapeutic perspective, drugs that maintain eIF2 α phosphorylation by inhibiting eIF2 α phosphatases hold great promise for the treatment of protein misfolding disorders (e.g. neurodegenerative diseases) and viral infections [5].

6.5. PP1-dependent functions of PHACTR4

The family of phosphatase and actin regulators (PHACTR) comprises four structurally related members (PHACTR1-4) [130]. These isoforms exhibit different spatiotemporal expression patterns in the brain of mice, but contain each a highly conserved C-terminal PP1-anchoring domain that is preceded by an actin-targeting domain. Mice with a missense mutation in their *Phactr4* gene, known as *Phactr4^{humdy}*, express the PHACTR4^{R650P} mutant, which binds actin, but not PP1 (Table 3) [131]. This indicates that the phenotype of *Phactr4^{humdy}* is

caused by deficient targeting of PP1. The *Phactr4^{humdy}* mutant mouse embryos had defective neural tubes and optic fissure closures, resulting in exencephaly (fetal malformation with protruding brain tissues) and retinal coloboma (eye abnormalities). This fetal neural phenotype accords with the specific expression of PHACTR4 in the neural stem cells of developing and adult mouse brains. This phenotype was rescued by a loss of the transcription factor E2F1, indicating that PHACTR4 regulates PP1 during E2F1-regulated cell cycle progression in neurulation and eye development [131]. At the molecular level, disruption of the PP1:PHACTR4 interaction led to increased inhibitory phosphorylation of PP1 at threonine 320, resulting in retinoblastoma (Rb) hyperphosphorylation and the derepression of E2F targets [131]. The *Phactr4^{humdy}* phenotype also seems to mimic Hirschsprung disease in humans, which is a congenital disorder of the distal colon characterized by the absence of the enteric nervous system [132, 133]. Indeed, the *Phactr4^{humdy}* mutants showed intestinal hypoganglionosis (a reduced number of nerves in the intestinal wall) as well as defects in cell migration and lamellipodia of the enteric neural crest cells. This suggests that the PP1:PHACTR4 holoenzyme plays a role in the directional migration of enteric neural crest cells.

7. Mouse models with human disease-associated phenotypes

Lastly, we screened the > 170 mouse models that were connected to 3 PP1- and 104 PIP-encoding genes for their association with human diseases. First, we examined the PP1 and PIP-related mouse models that

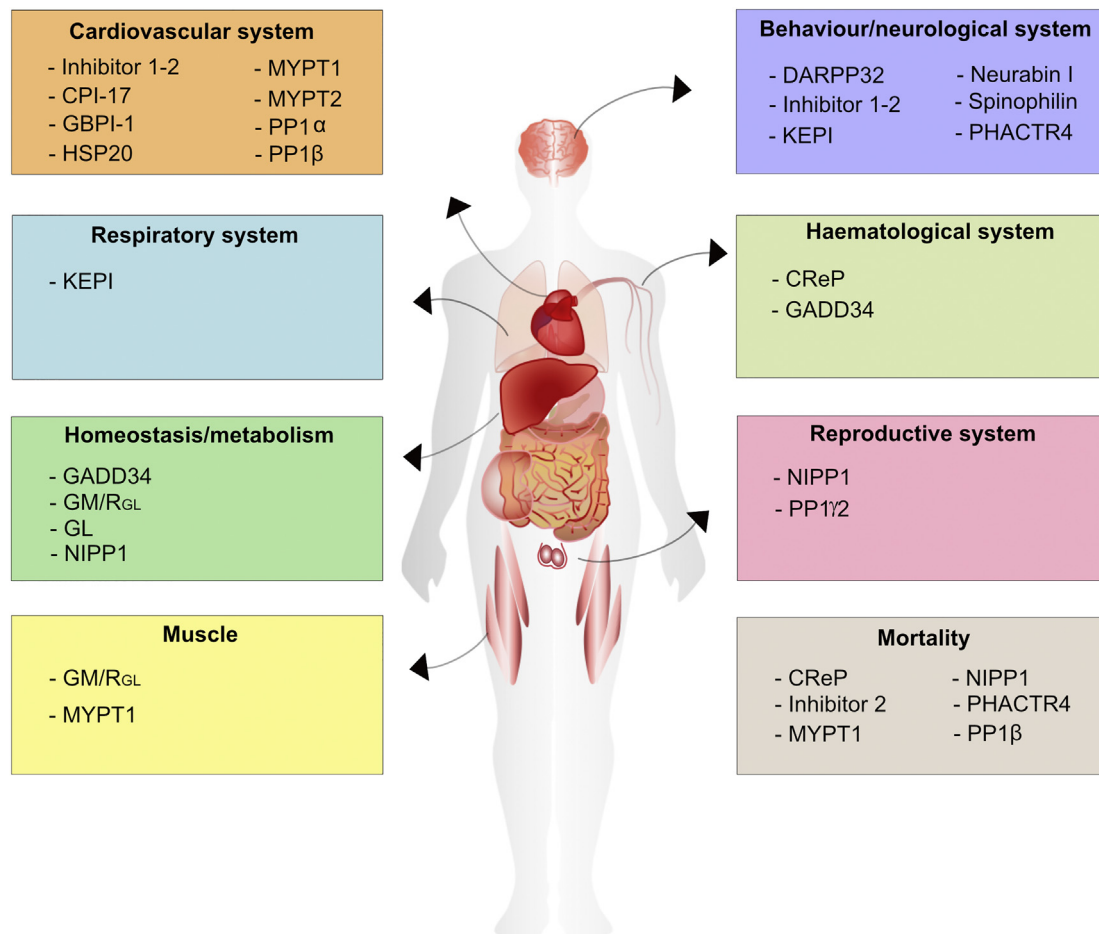


Fig. 3. Classification of PP1 and PIPs according to their biological functions and their connection to at least one human disease-associated phenotypes. The figure shows PP1 isoforms and PIPs that are linked to specific pathologies reminiscent of several human diseases: cardiovascular systems to heart failure, heart arrhythmia and/or hypertension; behavioural/neurological to drug addiction, schizophrenia, and/or Parkinson's disease, homeostatic/metabolic to type 2 diabetes and/or bile duct hyperplasia; reproductive to male infertility; haematological to thalassemia and respiratory system to SARS-CoV pathogenesis. See text for references. PP1, protein phosphatase 1; PIPs, PP1 interacting proteins; SARS-CoV, severe acute respiratory syndrome coronavirus.

are functionally linked to PP1 (3 PP1s, 7 iPIPs and 10 gPIPs; Tables 1–3) for human disease-associated phenotypes. The annotation was based on the Mouse Genome Informatics database (<http://www.informatics.jax.org/>) [23] as well as scientific literature (Fig. 3). We categorized PP1 and PIP proteins according their biological functions and their connection to at least one human disease: cardiovascular (heart failure, heart arrhythmia and hypertension); behavioural/neurological (drug addiction, schizophrenia, Parkinson's disease and human Hirschsprung disease); homeostatic/metabolic (type 2 diabetes and bile duct hyperplasia); reproductive (male infertility); haematological (thalassemia); respiratory system (SARS-CoV pathogenesis); mortality and muscle systems (Fig. 3). Next, we screened the mouse models of the other 87 PIPs for their connection to human diseases using only the Mouse Genome Informatics database. We found 32 PIPs that were affiliated to human disease-associated phenotypes and connected to eight different disease areas: 2 in cardiovascular, 15 in behavioural/neurological, 3 in homeostasis/metabolism, 3 in reproductive, 1 in haematological, 2 in renal, 1 in the immune system, 5 in muscle and 5 in cancer predisposition disorders, as documented in Table S1. A few PIPs were associated with two human disease areas. Although these PIPs are biochemically proven PP1 interactors, their phenotypes are not yet connected to PP1 functions, indicating that we cannot exclude that the molecular basis of human disease phenotypes is PP1-independent.

None of the mouse models that are functionally linked to PP1 (Tables 1–3) displayed a spontaneous cancer phenotype, although various PP1:PIP complexes have been linked to cell cycle progression and cancer [17]. However, mouse models from five non-functionally validated PIPs (tumor suppressors APC, BRCA1 and Rb; protein kinase Aurora A and chromatin remodeling factor SNF5) are connected to cancer predisposition (Table S1). Various genetically engineered mouse models of these PIPs, showed increased susceptibility to develop tumors, hinting at an opportunity to target PP1:PIP complexes also for cancer therapy [134–140].

Since most of the PP1 binding sites of these 32 PIPs have been mapped [37, 141], their connection to PP1 can be explored by structural reverse genetics, i.e. a genetically engineered mouse model that expresses a PIP mutant that alters associated PP1 function but does not affect the interaction with other ligands [142]. A PP1-binding mutant can be generated through the deletion of the PP1-binding domain (e.g. GADD34^{1–549}) or the introduction of a point mutation in its PP1-binding domain (e.g. PHACTR4^{R650P}), while non-inhibitory (e.g. DARPP32^{T34A}, CPI-17^{T38A}) or constitutively-inhibitory PP1 mutants (e.g. I1^{1–65}, T35D) can be generated by mutation of a critical residue in the PP1-inhibitory domain. Generation of these mutants in mice endorses the functional involvement of PP1 in that particular mouse's phenotype (Tables 2 and 3). Importantly, other interactions within functional PP1 holoenzyme complexes such as the interaction of PIPs with subcellular structures can also be exploited as targets for therapeutically intervention as example by GM^{ΔC} mutant (Table 3, Figs. 1 and 2) [102, 143]. Together with the recent expansion of structural biological methods and genome editing technologies, it is expected that structural reverse genetics will increase in popularity and lead to the identification of PP1 holoenzymes with a therapeutical potential.

8. Conclusion

PP1 does not exist alone in a mammalian cell; it is always associated with at least one iPIP or gPIP. It generates numerous distinct multi-subunit PP1 complexes, each of which dephosphorylates a small set of PP1 substrates [16]. The diversity of the PP1 holoenzymes increases through different isoforms of PP1 (PP1 α , β , γ 1 and γ 2), iPIPs (e.g. I1 and DARPP32) and gPIPs (e.g. seven isoforms of glycogen-targeting G subunits). Most isoforms derive from distinct genes, resulting from gene duplication during evolution [144]. The isoforms are structurally related, but they often differ in their spatiotemporal expression pattern (GM and GL, for instance), and have overlapping and distinct functions

(e.g. GADD34 and CREP).

The examination of > 170 mice connected with 3 PP1- and 104 PIP-encoding genes revealed that the disease-associated phenotypes of 20 proteins were dependent on PP1 function (Tables 1–3 and Fig. 3), while the 32 others have not yet been functionally linked to PP1 (Table S1). We propose the use of structural reverse genetics, which combines the structural characterization of proteins with mouse genetics, as a powerful and attractive tool to establish whether human-disease-associated phenotypes are physiologically dependent on PP1 functions [142]. Various interaction sites, including PP1:PIP, PP1:substrate and PP1:subcellular interaction site, can be considered for therapeutic targeting (see colored circles in Fig. 1). We believe that structural reverse genetics provides exciting opportunities for new discoveries as an initial step to identify novel PP1-related therapeutic targets.

In conclusion, mice models have contributed enormously to understanding the *in vivo* functions of PP1 holoenzymes and have confirmed the pleiotropic role of PP1s in health and diseases, especially in the heart and brain. The discovery that PP1 itself and at least 49 PIPs proteins are associated with human disease-associated phenotypes suggests a huge potential for targeting PP1 holoenzymes and indicates that PP1:PIP-directed drugs hold great potential for treatment of human diseases.

Conflict of interest

The authors declare no conflict of interests.

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Transparency document

The Transparency document associated with this article can be found, in online version.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbamcr.2018.07.019>.

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