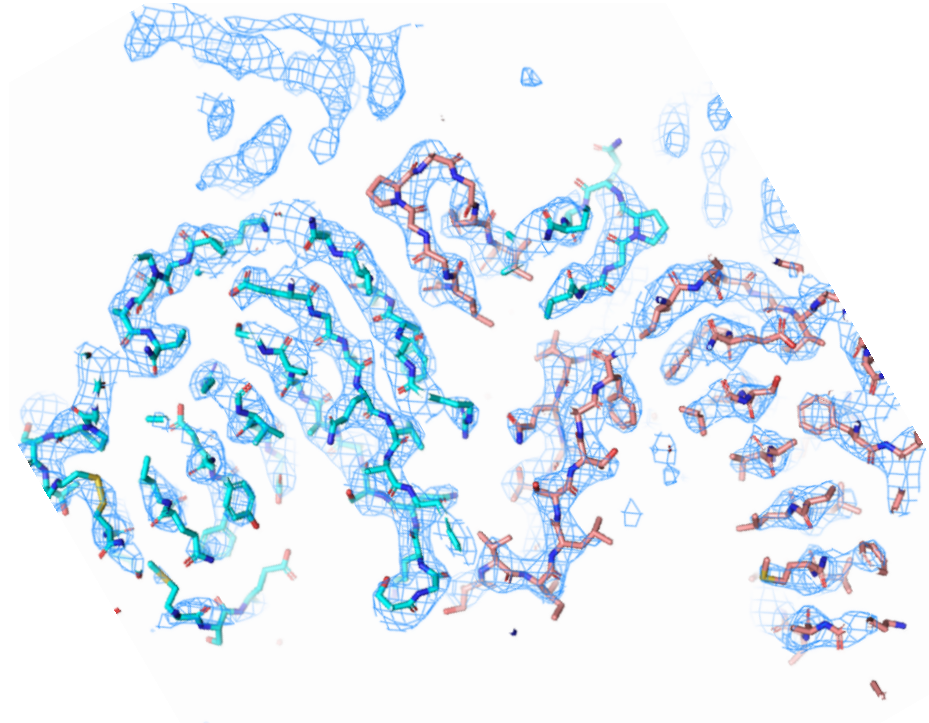
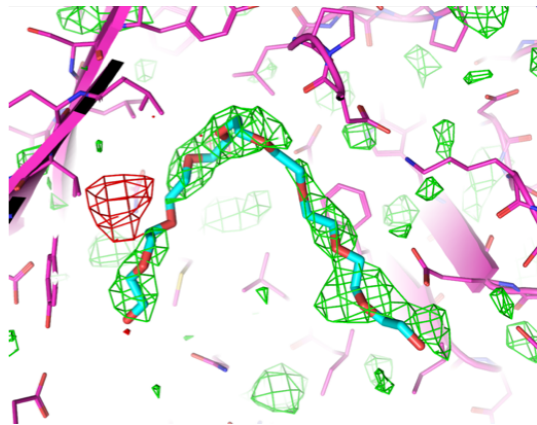
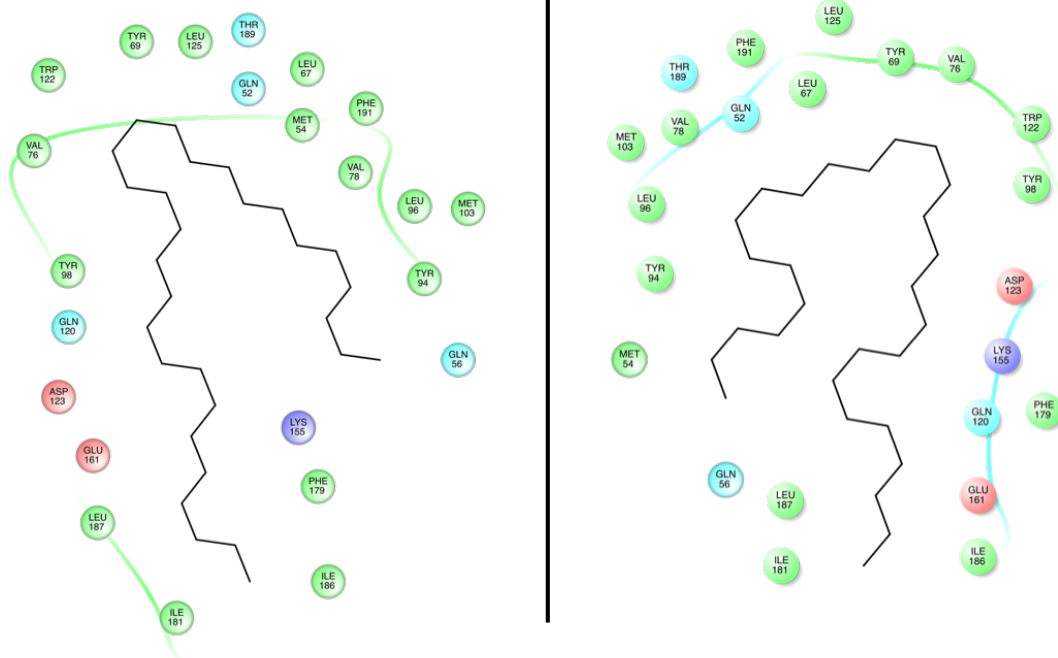
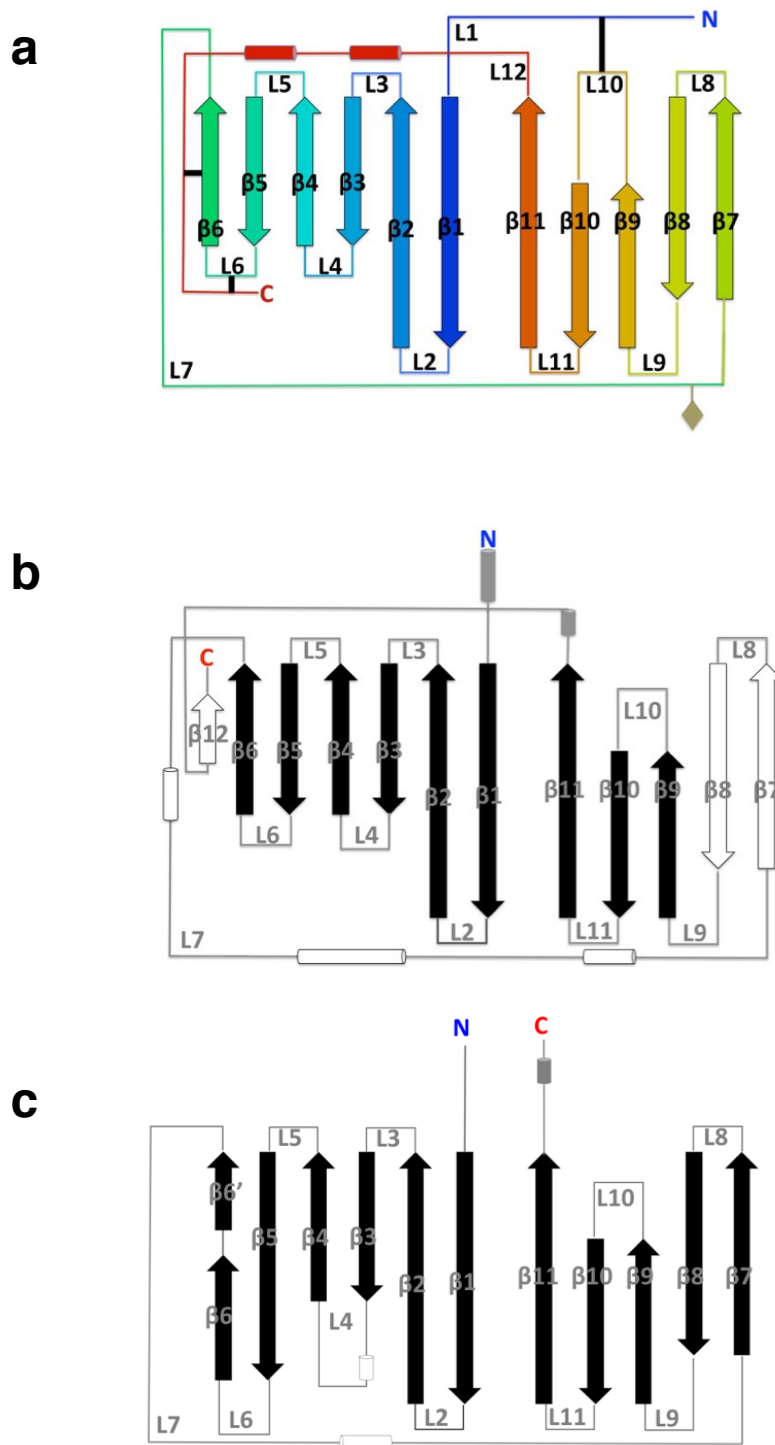


a**b**

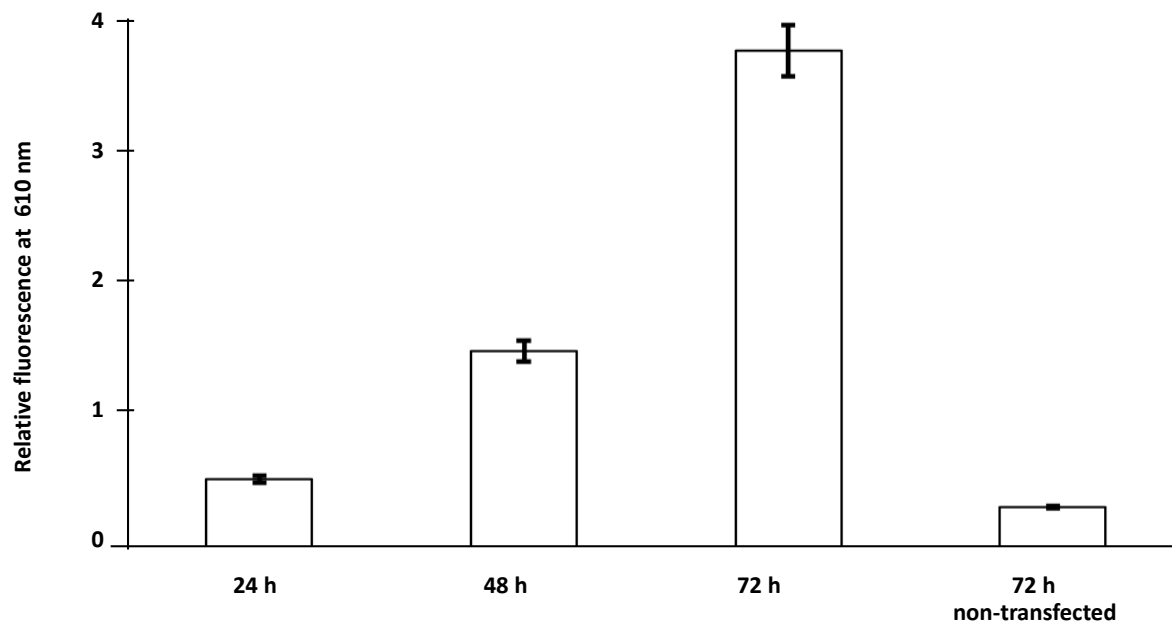
Supplementary Figure 1. a. 2Fo-Fc electron density map near the dimer interface of glycosylated EPDR1. The carbons of the two chains are colored blue and pink, respectively. **b.** Difference electron density map following removal of the ligand (modeled as a PEG molecule) and 3 cycles of Phenix torsional angle simulated annealing refinement. The map was contoured at 2.0 sigma. The negative peak to the left (red) is approximately 4 Å from the ligand.



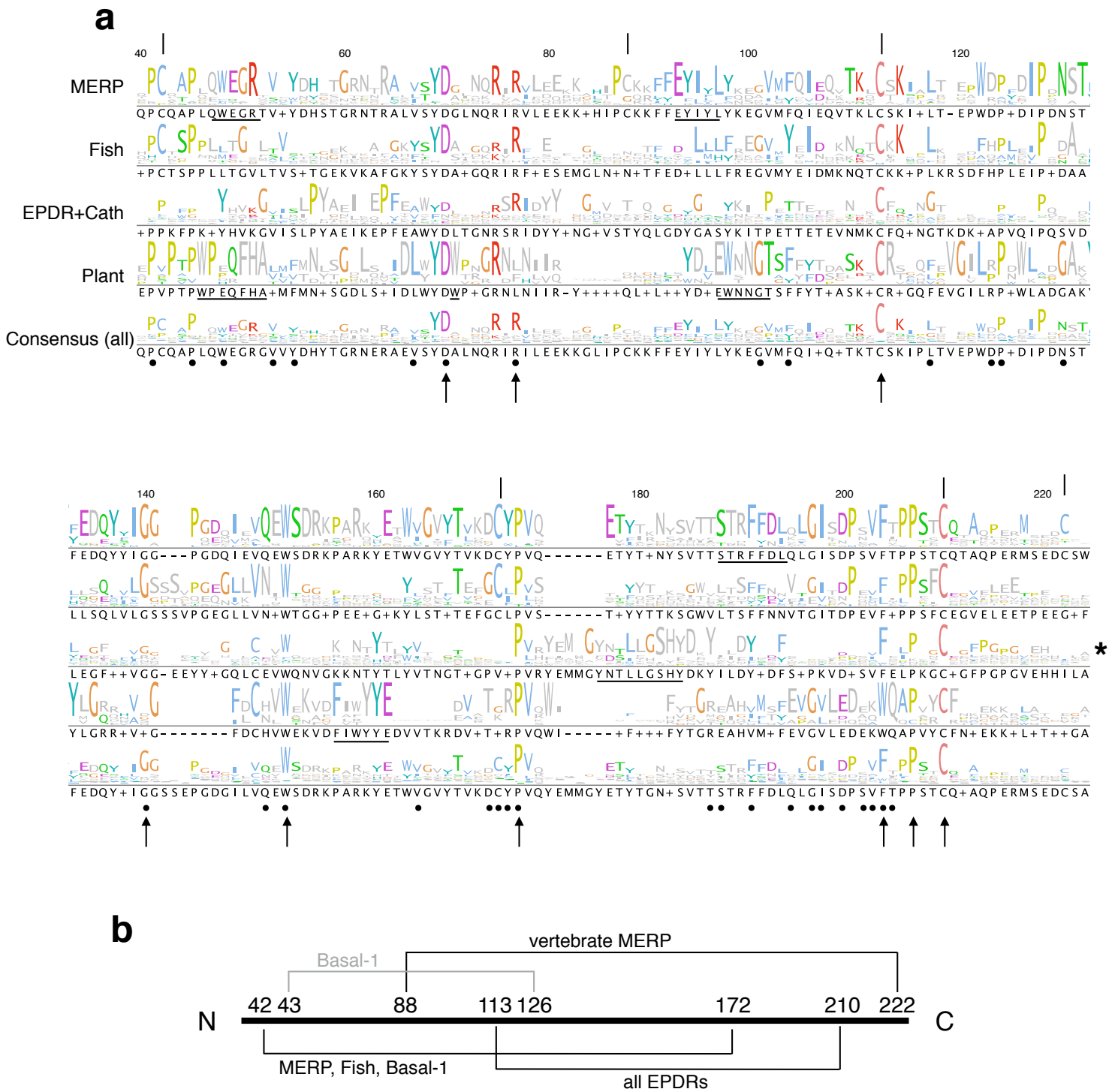
Supplementary Figure 2. Residue contacts of the two ligands in the glycosidated EPDR1 homodimer structure based on a 4 Å cutoff distance. Hydrophobic residues are colored green, polar residues are colored cyan, acidic residues are colored red, and basic residues are colored blue.



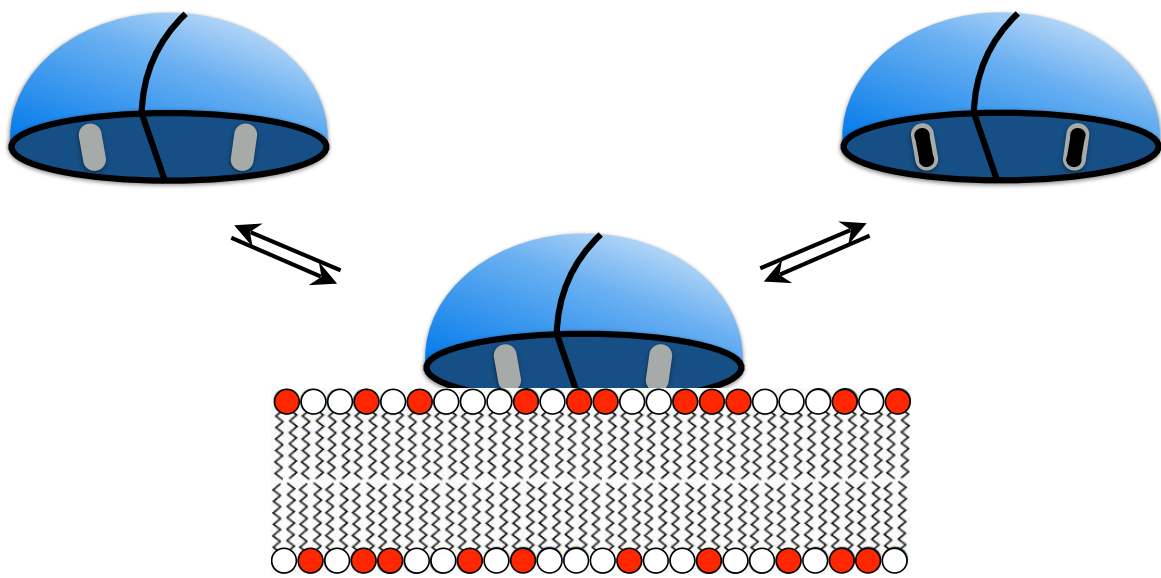
Supplementary Figure 3. Topology diagrams of (a) EPDR1, (b) LolA (PDB 1IWL) and (c) VioE (2ZF4).



Supplementary Figure 4 Secretion of EPDR1-mCherry fusion protein into the culture medium as measured by fluorescence.

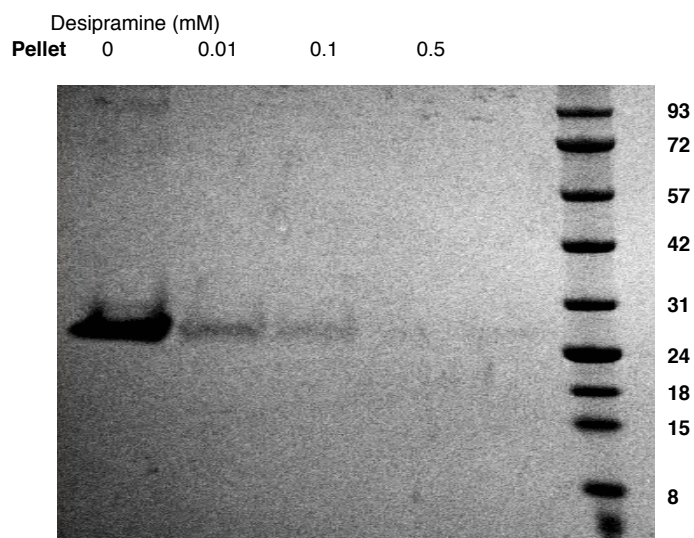
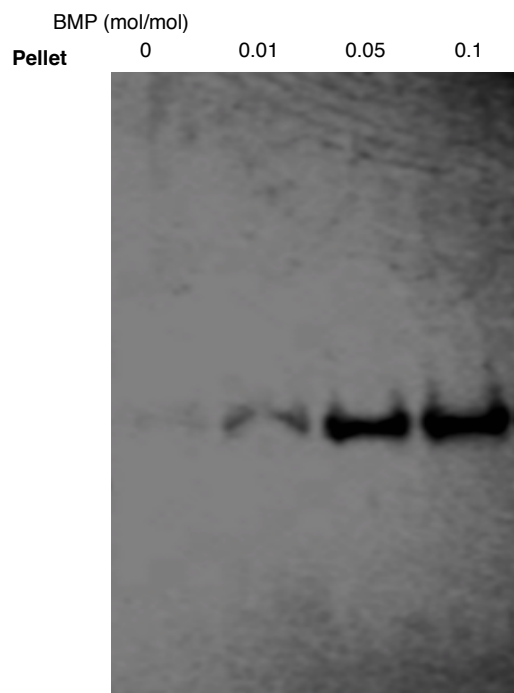
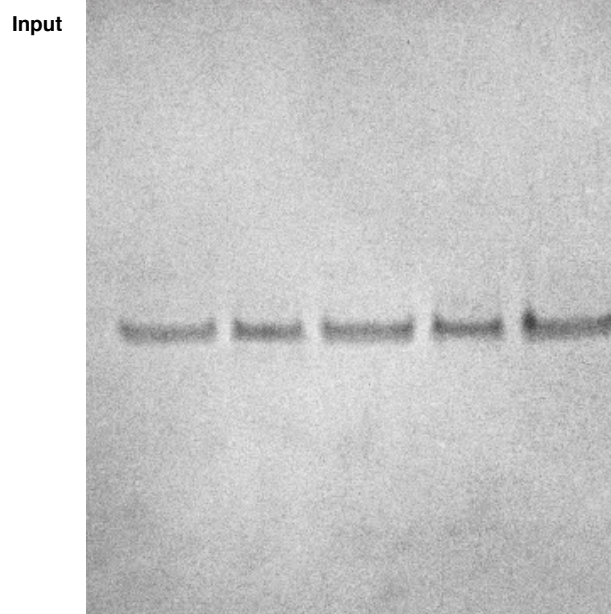
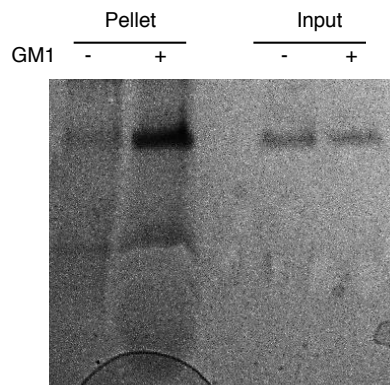
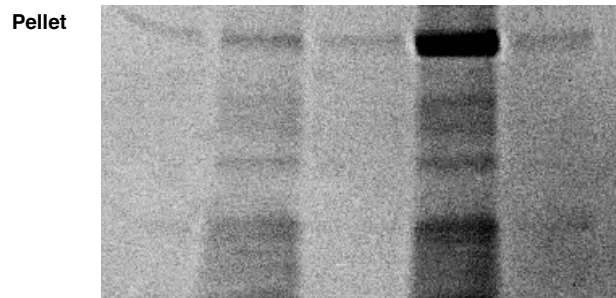


Supplementary Figure 5. a. Sequence logos of selected subgroups of the EPDR family (see also Fig. 9 in the main text). Residue numbering is based on human EPDR1. Sequence logos for the basal-1 group can be found in Hall et al., *Nature* **544**, 231-234 (2017). Key cysteine residues are marked with vertical bars above the logos, selected diagnostic sequence motifs are underlined, and sites conserved across the broader family are indicated with upward arrows. Positions that are conserved between the eukaryotic EPDR and bacterial members of the LolA family are marked with black dots as in Figs. 2 and 5 of the main text. The alignments from these clades represent the full length sequences of the predicted mature forms of the proteins following the cleavage of the signal sequences, except for the EPDR+Cath class, which continues into the cathepsin domain (asterisk).
b. Disulfide conservation in the eukaryotic EPDRs.



Supplementary Figure 6. Schematic of EPDR1 membrane binding and lipid extraction. The EPDR1 dimer contains two lipid-binding pockets on the flat side of a hemisphere. At acidic pH, the electropositive surface of EPDR1 (blue) interacts with the negatively charged lipids (red headgroups) on membrane surfaces.

EPDR1	+	+	+	+	+
PC	-	+	+	+	+
BMP	-	-	-	+	+
pH	4.5	4.5	7	4.5	7



Supplementary Figure 7. Full size images of the Coomassie-stained SDS-PAGE gels shown in Fig. 7 of the main text.

species name	common name	# of proteins	# of domains identified	Subgroup (# of domains)						
				MERP	Fish-specific / Ependymin	Basal-1	Basal-2	EPDR+Cath	Plant-specific	Other
<i>Trichomonas vaginalis</i>		4	4	0	0	0	0	4	0	0
<i>Dictyostelium discoideum</i>	slime mould	2	2	0	0	1	0	1	0	0
<i>Giardia intestinalis</i>		2	2	0	0	0	0	2	0	0
<i>Leishmania major</i>		1	1	0	0	1	0	0	0	0
<i>Chlamydomonas reinhardtii</i>	alga	2	2	0	0	0	0	2	0	0
<i>Zea mays</i>	maize	4	4	0	0	0	0	0	4	0
<i>Arabidopsis thaliana</i>	thale cress	3	3	0	0	0	0	0	3	0
<i>Naegleria gruberi</i>	amoeba	10	10	0	0	4	3	0	0	3
<i>Schizosaccharomyces pombe</i>	fission yeast	0	0	0	0	0	0	0	0	0
<i>Saccharomyces cerevisiae</i>	budding yeast	0	0	0	0	0	0	0	0	0
<i>Aspergillus niger</i>		0	0	0	0	0	0	0	0	0
<i>Capsaspora owczarzaki</i>		6	7	0	0	1	6	0	0	0
<i>Monosiga brevicollis</i>	Choanoflagellate	7	9	1	0	5	2	1	0	0
<i>Trichoplax adhaerens</i>	Placozoa	16	16	0	0	13	0	3	0	0
<i>Amphimedon queenslandica</i>	sponge	4	6	0	0	0	5	1	0	0
<i>Nematostella vectensis</i>	starlet sea anemone	3	3	0	0	0	1	2	0	0
<i>Lottia gigantea</i>	owl limpet	22	30	1	0	15	13	1	0	0
<i>Crassostrea gigas</i>	Pacific oyster	21	29	1	0	15	13	0	0	0
<i>Helobdella robusta</i>	California leech	1	1	0	0	0	0	1	0	0
<i>Capitella teleta</i>	bristle worm	0	0	0	0	0	0	0	0	0
<i>Caenorhabditis elegans</i>	nematode	0	0	0	0	0	0	0	0	0
<i>Anopheles gambiae</i>	African malaria mosquito	1	1	0	0	0	0	1	0	0
<i>Drosophila melanogaster</i>	fruit fly	1	1	0	0	0	0	1	0	0
<i>Strongylocentrotus purpuratus</i>	purple sea urchin	10	11	1	0	5	4	1	0	0
<i>Branchiostoma floridae</i>	Florida lancelet (amphioxus)	27	37	0	0	17	13	1	0	6
<i>Ciona intestinalis</i>	vase tunicate, sea squirt	5	6	0	0	4	2	0	0	0
<i>Lepisosteus oculatus</i>	spotted gar	2	2	1	0	0	0	1	0	0
<i>Danio rerio</i>	zebrafish	11	11	1	3	0	0	1	0	6
<i>Salmo salar</i>	Atlantic salmon	7	7	1	5	0	0	1	0	0
<i>Takifugu rubripes</i>	Japanese pufferfish	5	5	1	3	0	0	1	0	0
<i>Oryzias latipes</i>	Japanese medaka	6	6	1	4	0	0	1	0	0
<i>Latimeria chalumnae</i>	coelacanth	3	3	1	1	0	0	1	0	0
<i>Xenopus tropicalis</i>	tropical clawed frog	1	1	1	0	0	0	0	0	0
<i>Anolis carolinensis</i>	green anole	2	2	1	0	0	0	1	0	0
<i>Gallus gallus</i>	chicken	2	2	1	0	0	0	1	0	0
<i>Chelonia mydas</i>	green sea turtle	2	2	1	0	0	0	1	0	0
<i>Ornithorhynchus anatinus</i>	platypus	0	0	0	0	0	0	0	0	0
<i>Monodelphis domestica</i>	gray short-tailed opossum	1	1	1	0	0	0	0	0	0
<i>Homo sapiens</i>	human	1	1	1	0	0	0	0	0	0
<i>Mus musculus</i>	house mouse	1	1	1	0	0	0	0	0	0
	domain count:		229	17	16	81	62	31	7	15
	protein count:	196		17	16	75	36	31	7	14

Supplementary Table 1. Distribution of eukaryotic EPDR proteins in selected species. No EPDR proteins were identified in the bold face entries. See also Supplementary Dataset 1.