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### Supplemental information

#### Popeye domain containing proteins

#### modulate the voltage-gated

#### cardiac sodium channel Nav1.5

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**Figure S1.** Current-voltage (IV) and conductance-voltage (GV) relationships of Nav1.5 in the absence or presence of POPDC proteins, Related to Figure 2, Related to STAR Methods.

(A) Current-voltage relationships (IVs) of Nav1.5 in the absence (black) or presence of mPOPDC1, mPOPDC2 or mPOPDC3. The number of technical replicates is given in parentheses. Data are presented as mean ± s.e.m..

(B) Voltage-dependence of activation curves (GVs) derived from (A) for Nav1.5 in the absence (black) or presence of mPOPDC1, mPOPDC2 or mPOPDC3. The number of technical replicates is given in parentheses. Data are presented as mean ± s.e.m..

(C) Current-voltage relationships (IVs) as in (A). Here, to avoid clamping artifacts and an artificial shift in the GV as observed in (A) for wild-type, only measurements with current amplitudes smaller than 6  $\mu$ A and a Boltzmann constant k of > 4 in the corresponding GVs were considered. The number of technical replicates is given in parentheses.

(D) Voltage-dependence of activation curves (GVs) derived from (C) with current amplitudes smaller than 6  $\mu$ A and a Boltzmann constant k of > 4. Here the apparent difference between Nav1.5 and Nav1.5 co-expressed with the three POPDC isoforms as observed in (B) is not present and POPDC proteins do not alter the voltage-dependence of Nav1.5 activation. The number of technical replicates is given in parentheses.



**Figure S2.** Current-voltage (IV) and conductance-voltage (GV) relationships of Nav1.4 in the absence or presence of human POPDC2, Related to Figure 2, Related to STAR Methods.

(A) Current-voltage relationships (IVs) of Nav1.4 in the absence (black) or presence of POPDC2 (blue). The number of technical replicates is given in parentheses. Data are presented as mean ± s.e.m..

(B) Current-voltage relationships (IVs) of Nav1.4 as in (A). Here, to avoid clamping artifacts only measurements with current amplitudes smaller than 6  $\mu$ A and a Boltzmann constant k of > 4 in the corresponding GVs were considered.

(C) Voltage-dependence of activation curves (GVs) of Nav1.4 in the absence (black) or presence of POPDC2 (blue). Note, that only measurements with current amplitudes smaller than 6  $\mu$ A and a Boltzmann constant k of > 4 were considered. The number of technical replicates is given in parentheses. Data are presented as mean ± s.e.m..



**Figure S3.** Current-voltage (IV) and conductance-voltage (GV) relationships of Nav1.5 in the absence or presence of human POPDC2, Related to Figure 3, Related to STAR Methods.

(A) Current-voltage relationships (IVs) of Nav1.5 in the absence (black) or presence of human POPDC2 (blue). The number of technical replicates is given in parentheses. Data are presented as mean  $\pm$  s.e.m.. Note, that only measurements with current amplitudes smaller than 6  $\mu$ A and a Boltzmann constant k of > 4 in the corresponding GVs were considered.

(B) Conductance-voltage relationships (GVs) of Nav1.5 in the absence (black) or presence of human POPDC2 (blue). Note, that only measurements with current amplitudes smaller than 6  $\mu$ A and a Boltzmann constant k of > 4 were considered. The number of technical replicates is given in parentheses. Data are presented as mean ± s.e.m..



**Figure S4.** Current amplitudes of Nav1.5 co-expressed with different amounts of human POPDC2 in the absence or presence of theophylline, Related to Figure 4, Related to STAR Methods.

(A) Current amplitudes of Nav1.5, Nav1.5 co-injected with 50% POPDC2 or 100% POPDC2 analyzed at -20 mV. The number of technical replicates is given in parentheses. Data are presented as mean ± s.e.m..

(B) Current amplitudes determined by experiments as in (A), but from oocytes incubated with theophylline in the storage solution. The number of technical replicates is given in parentheses. Data are presented as mean ± s.e.m..