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Supplementary Materials for

Neuronal and Intestinal Protein Kinase D Isoforms Mediate Na^+ (Salt Taste)–Induced Learning

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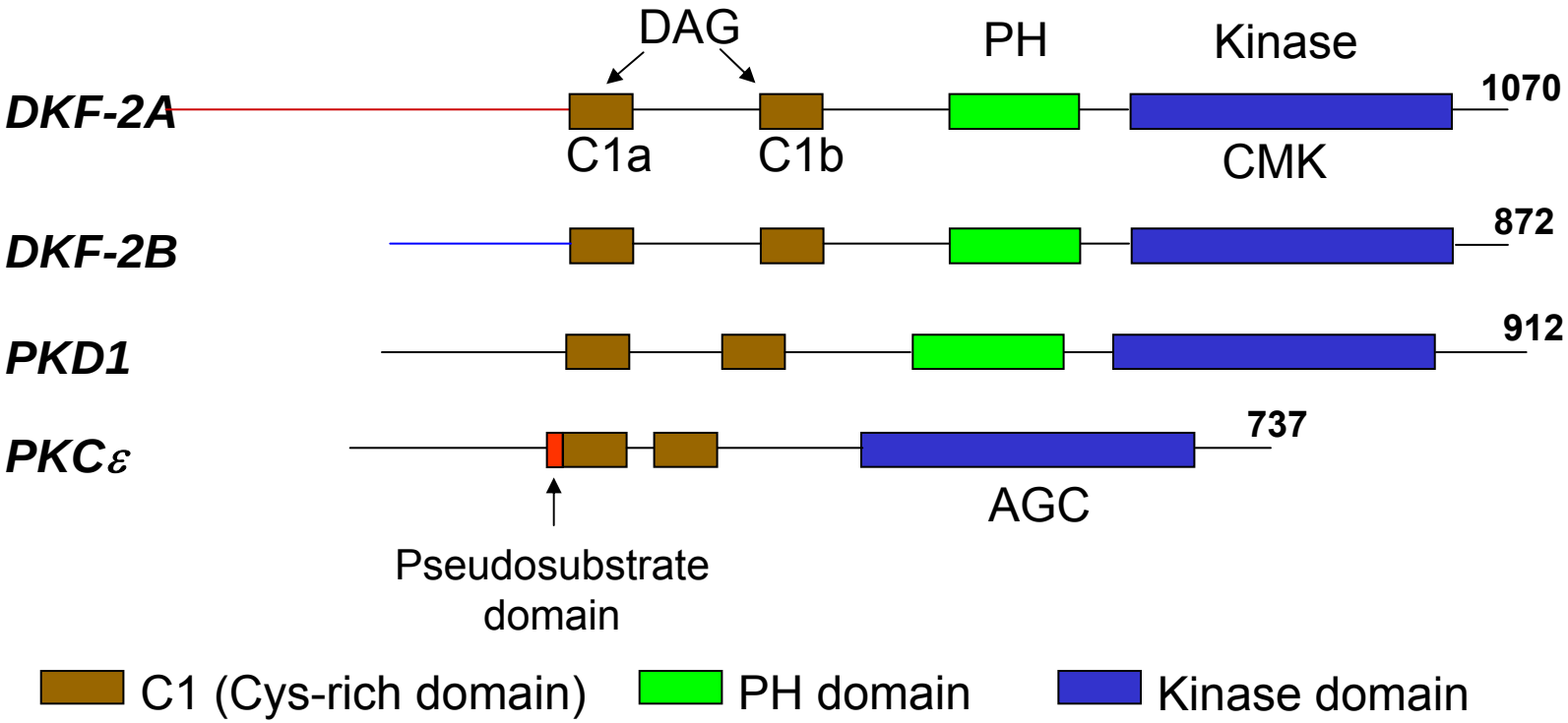

Figure S1. Domain organization of PKDs and PKCε. A diagram shows the distribution of regulatory (C1) and kinase domains in *C. elegans* PKDs (DKF-2A and 2B), mammalian PKD1 and mammalian PKCε. Amino acid sequences of PKD C1 and kinase domains are highly conserved (~75% identity) between *C. elegans* and mammals. Kinase domains of PKDs and calmodulin activated protein kinase I are homologous. Thus, PKDs are assigned to the CMK (calmodulin kinase) branch of the kinome. C1 domains of PKCs and PKDs share substantial homology (~50% identity) across species. Amino acid sequences of kinase domains of PKCs and PKDs are divergent. PKC kinase modules are homologous with catalytic domains of protein kinases A and G. Thus, PKCs are included in the AGC (for A, G and C kinases) super-family in the kinome. PKCs and PKDs phosphorylate distinct, non-overlapping groups of substrates. PKCs contain an autoinhibitory pseudosubstrate site; PKDs lack a pseudosubstrate site, but contain a distinctive PH domain. The PH domain does not mediate translocation of PKDs to plasma membrane. Roles of PH domains are discussed in E. Rozengurt *et. al.*, *J. Biol. Chem*. **280**, 13205 (2005); H. Feng, *et al.*, *J. Biol. Chem*. **282**, 31273 (2007).

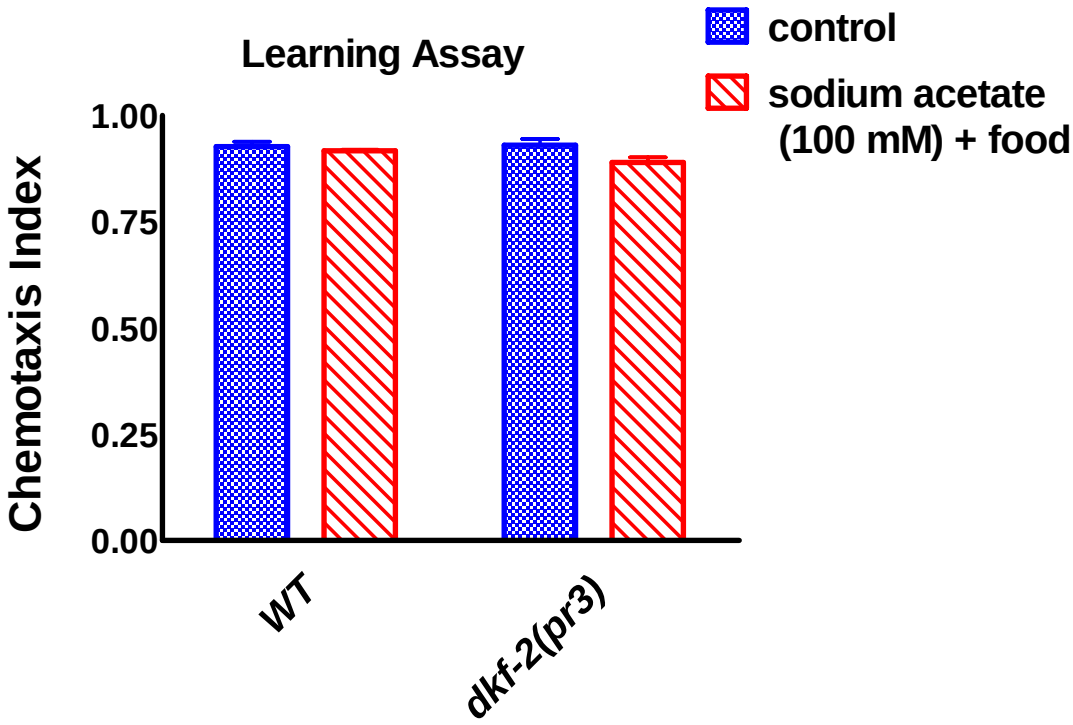

Figure S2. Food suppresses Na^+-induced learning. Learning assays were performed and the chemotaxis index (CI) was calculated as described under "Materials and Methods". WT and DKF-2 depleted animals were assayed for chemotaxis to 25 mM sodium acetate after 1h preincubation in the presence or absence of 100 mM sodium acetate buffer containing *E. coli* OP50 (food). Error bars represent SEM; Similar results were obtained in three replications of the experiment. Typical data are shown.

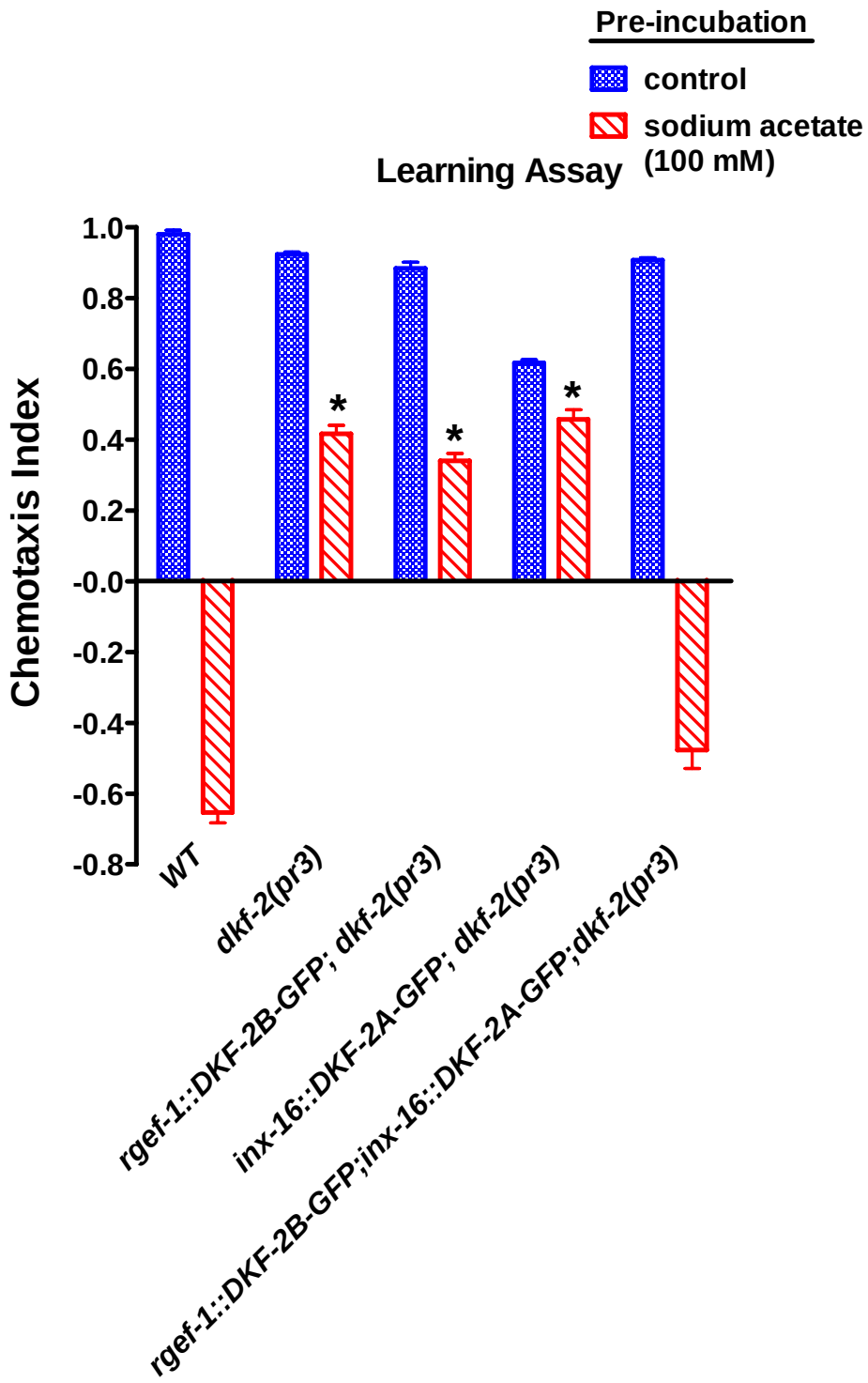

Figure S3. Both neuronal DKF-2B and intestinal DKF-2A are essential for Na^+-induced, aversive learning. Learning assays were performed as described under "Materials and Methods". After preincubation with 100 mM sodium acetate in the absence of food, the indicated *C. elegans* strains were assayed for chemotaxis to 25 mM sodium acetate. The *inx-16* promoter drives expression of DKF-2A-GFP exclusively in intestinal cells; the *rgef-1* promoter enables synthesis of DKF-2B-GFP mRNA and protein only in neurons. Cell specific accumulation of DKF-2A-GFP and DKF-2B-GFP was verified by monitoring GFP fluorescence in living animals via microscopy. Error bars represent SEM. * $p < 0.001$ compared with WT animals pre-incubated with 100 mM sodium acetate, Dunnett's t test.

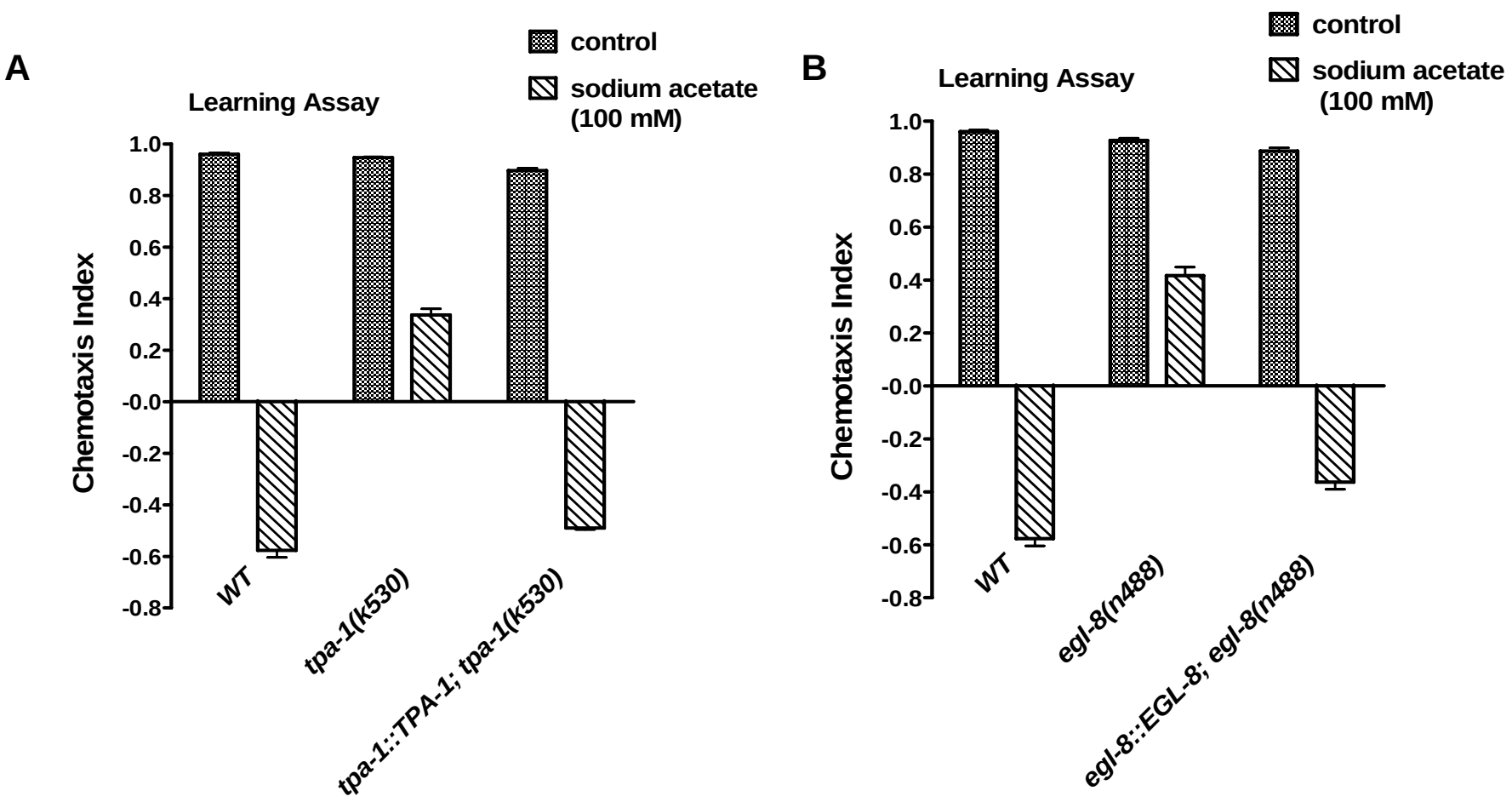

Figure S4. WT transgenes rescue learning defects in TPA-1 and EGL-8 deficient animals - Transgenic animals that express WT TPA-1 in the *tpa-1(k530)* background and WT EGL-8 in the *egl-8(n488)* background were generated by standard procedures (see "Materials and Methods"). Learning assays were performed as described under "Materials and Methods Procedures". After preincubation with 100 mM sodium acetate in the absence of food, the indicated *C. elegans* strains were assayed for chemotaxis to 25 mM sodium acetate. The chemotaxis index (CI) was calculated as described under "Materials and Methods". **A** shows the effect of WT TPA-1 expression on Na^+-dependent learning. **B** shows the effect of WT EGL-8 on aversive learning. Error bars represent SEM. Similar results were obtained in three replications of the experiments. Typical data are shown.

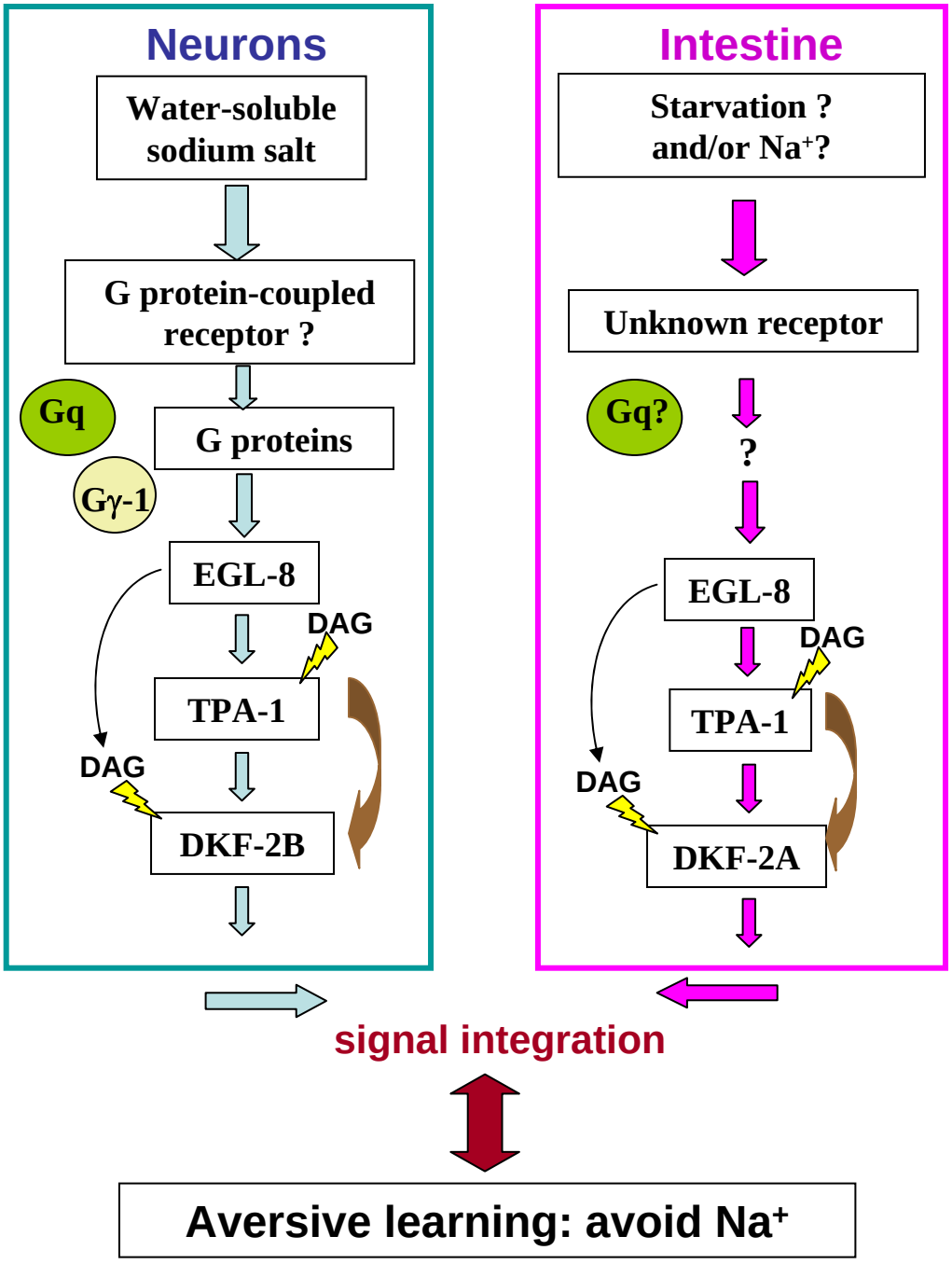

Figure S5. PLC/DAG/PKC/PKD signaling modules in neurons and intestinal cells cooperatively mediate Na^+-induced, associative learning. Similar, DAG-controlled signaling pathways are activated by Na^+ (and potentially starvation) in neurons and intestinal cells. Cross-talk between the nervous system and intestine occurs downstream from PKDs. Signal integration in interneurons elicits learning and behavioral plasticity.

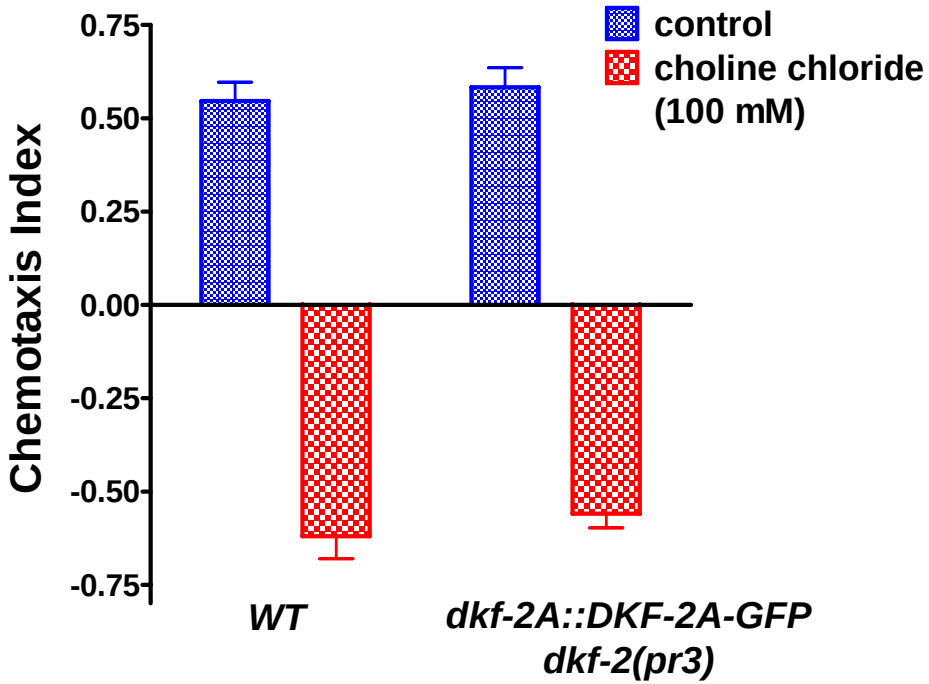

Figure S6. Animals differentially expressing DKF-2A are not generally compromised in sensing or learning. WT *C. elegans* and animals expressing a *dkf-2A*::DKF-2A-GFP transgene in a *dkf-2(pr3)* null background were preincubated in the presence or absence (control) of 100 mM choline chloride. Subsequently, animals were assayed for chemotaxis to 25 mM choline chloride. The data show that animals expressing only the DKF-2A isoform are not generally compromised in sensing or learning. They detect and learn to avoid Cl^- in a normal manner, but have specific deficits in Na^+ sensing and Na^+-induced aversive learning.

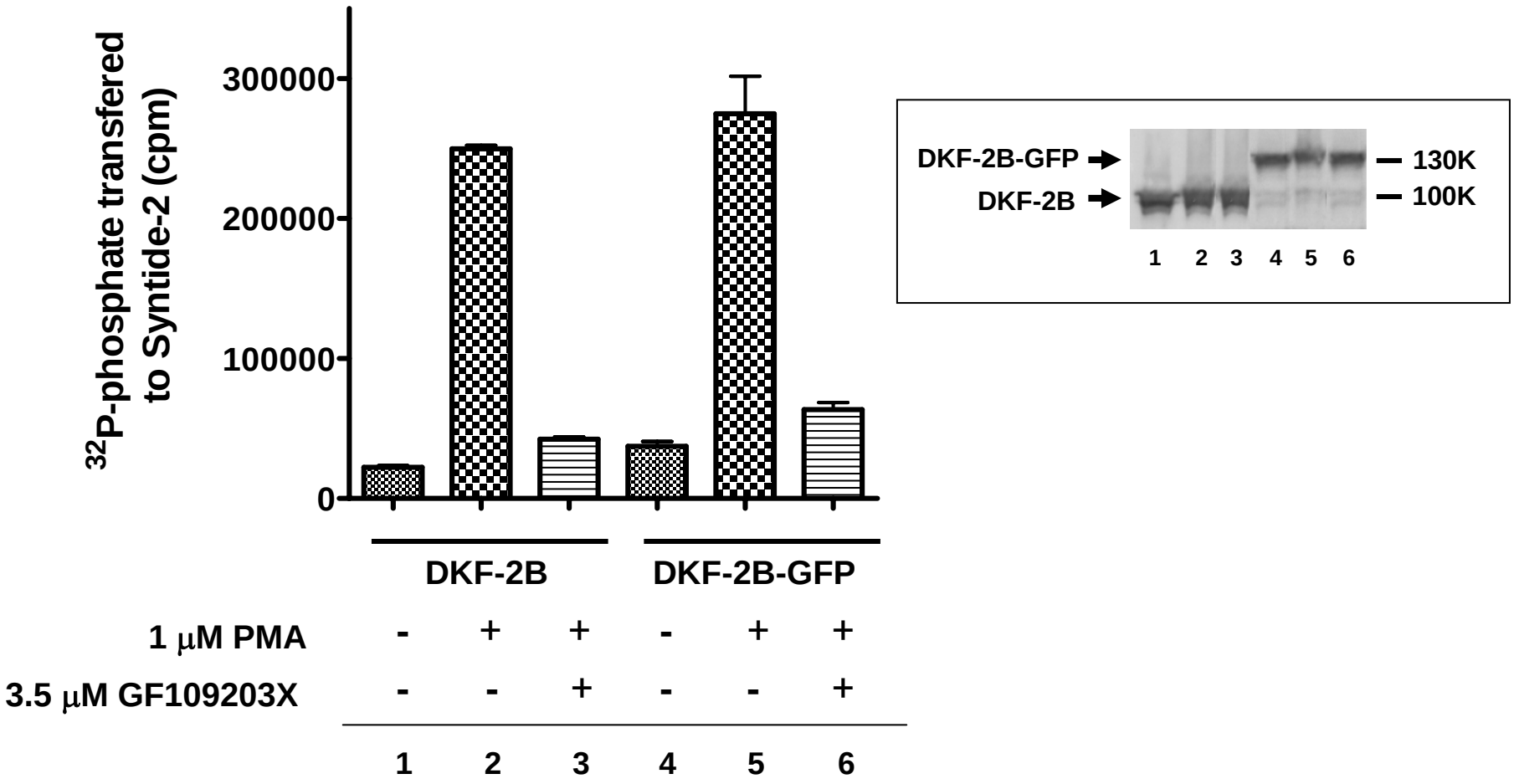

Figure S7. DKF-2B-GFP and WT DKF-2B have similar properties. Transfected cells expressing either DKF-2B or DKF-2B-GFP were treated with PMA or vehicle for 10 min before lysis. Duplicate samples of cells were incubated with GF109203X for 1 h prior to PMA addition. DKF-2B proteins were immunoprecipitated and assayed for catalytic activity. An immunoblot shows that similar amounts of DKF-2 and DKF-2B-GFP were used in each assay (*inset*). DKF-2B-GFP and DKF-2B exhibited similar basal levels of catalytic activity and both kinases were potently activated by PMA. The PKC inhibitor GF109203X efficiently suppressed PMA-induced activation of both enzymes. Thus, the GFP tag does not alter regulatory and enzymatic properties of DKF-2B. Experiments were repeated three times and similar results were obtained.