Supporting Information

Hoshi et al. 10.1073/pnas.1221997110

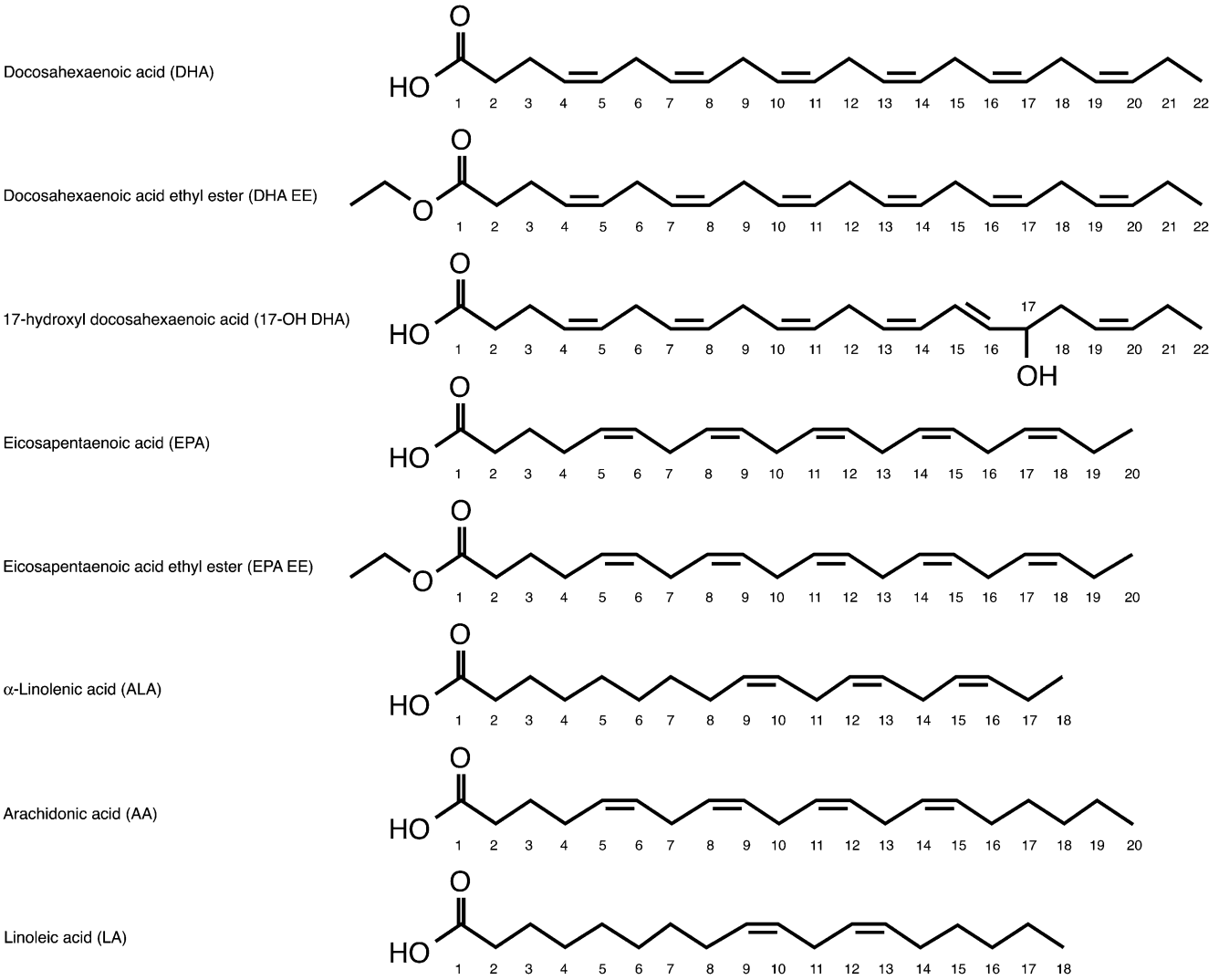

Fig. S1. Structures of the omega-3 fatty acids, omega-6 fatty acids, and their derivatives used in the study.

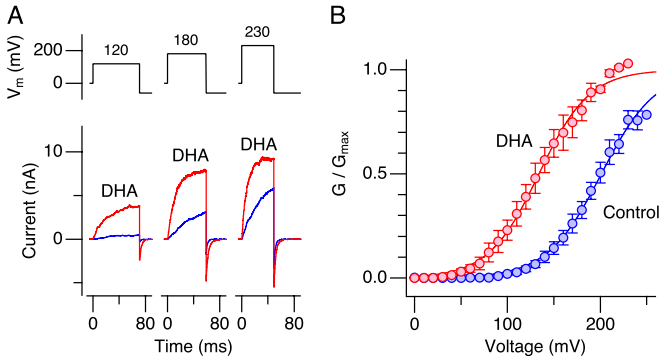

Fig. S2. Docosahexaenoic acid (DHA) enhances currents through divalent-insensitive Slo1+β1 channels. (*A*) Representative currents through Ca^{2+}- and Mg^{2+}-insensitive Slo1 D362A:D367A:E399A:Δ894–895+β1 channels before and after application of DHA (3 μM). (*B*) Normalized conductance in Slo1 D362A:D367A:E399A:Δ894–895+β1 channels. Curves represent Boltzmann fits with $V_{0.5} = 201.1 \pm 1.5$ mV and $Q_{app} = 0.85 \pm 0.04$ (control) and $V_{0.5} = 133.9 \pm 1.9$ mV and $Q_{app} = 0.92 \pm 0.06$ (DHA, 3 μM). $n = 5$. All results were obtained without Ca^{2+}.

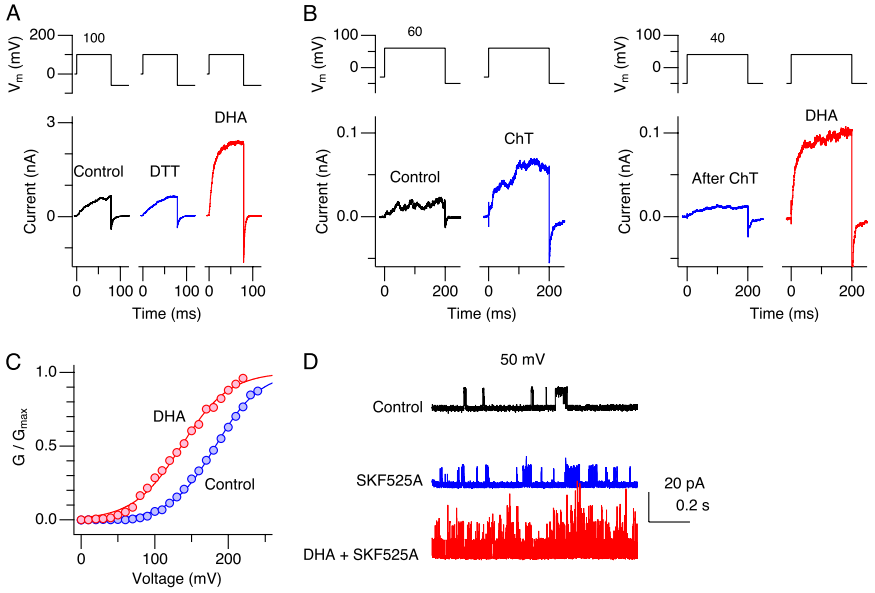

Fig. S3. Neither a redox change or an intracellular signaling cascade is likely to be involved in the stimulatory effect of DHA on Slo1+β1 channels. (*A*) Pretreatment with the reducing agent DTT does not antagonize the effect of DHA. Channels were treated with DTT (2 mM) for 4.5 min before DHA (3 μM) was applied. (*B*) Treatment with the oxidant chloramine-T (ChT) (100 μM) increases currents (*Left*) but does not impair the stimulatory effect of subsequent application of DHA (3 μM; *Right*). Note that the test voltages are different to account for the shift in normalized conductance by ChT. (*C*) DHA (3 μM) remains effective in shifting $V_{0.5}$ in a patch taken from a cell preincubated with the P450 epoxygenase inhibitor SKF525A (Enzo; 30 μM) for 1 h. (*D*) The presence of SKF525A (10 μM) does not impair the ability of DHA (3 μM) to increase P_o. P450 epoxygenase generates epoxyeicosatrienoic acids from long-chain polyunsaturated fatty acids. All results were obtained without Ca^{2+}.

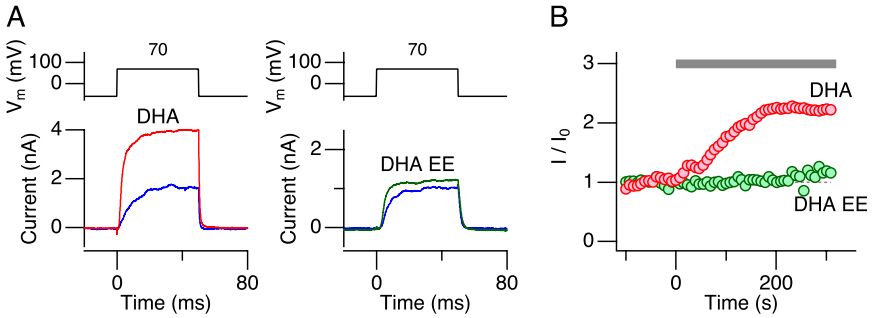

Fig. S4. Contrasting effects of DHA and DHA EE applied to the extracellular side in the whole-cell configuration. (*A*) Representative currents at 70 mV before and after application of DHA (3 μM; *Left*) or DHA EE (3 μM; *Right*). Mouse neuroblastoma cells (Neuro-2a from ATCC) were transfected with Slo1+β1. (*B*) Fractional changes in peak outward current size by DHA (red) and DHA EE (green). Currents were elicited by pulses from −60 to −70 mV every 8 s. Extracellular solution contained (in millimolars): 134 NaCl, 6 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 Hepes, pH 7.4 [with *N*-methyl-D-glucamine (NMG)]. Intracellular solution contained (in millimolars): 110 K aspartate, 30 KCl, 10 NaCl, 2 $MgCl_2$, 10 Hepes, pH 7.2 (with NMG). Similar results were obtained from three cells with each compound.