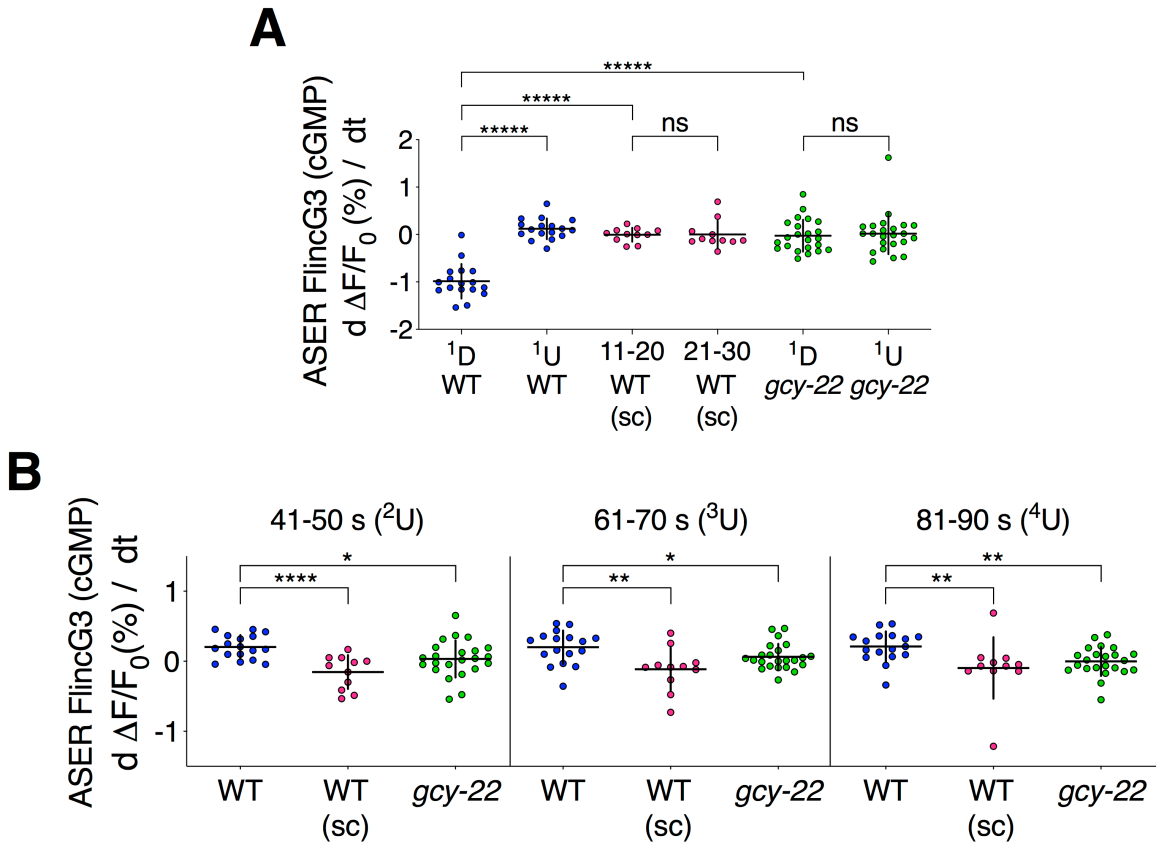

Figure S5 The slope of FlincG3 fluorescence in the ASER cell body changes in response to 50 mM NaCl step changes and depends on the receptor guanylyl cyclase GCY-22.

(A) FlincG3 fluorescence in the ASER cell body decreases in response to a 50 to 0 mM NaCl downstep and stops decreasing in response to a 0 to 50 mM NaCl upstep in wild-type animals. The slopes of FlincG3 fluorescence for the first 50 to 0 mM NaCl downstep between wild-type and *gcy-22(tm2364)* animals are different (n=17 (first set, blue; wild-type), n=23 (fifth set, green; *gcy-22*); permutation test p<0.00001). In wild-type animals, the slopes in response to the first 50 to 0 mM NaCl downstep are also different from those exposed to the switch control (n=17 (first set, blue; wild-type), n=11 (third set, pink; switch

control); permutation test $p<0.00001$). The slopes of the first 50 to 0 mM NaCl downstep and 0 to 50 mM NaCl upstep are different in wild-type animals (n=17; first pair, blue; permutation test $p<0.00001$). By contrast, the slopes of the first 50 to 0 mM NaCl downstep and 0 to 50 mM NaCl upstep are not different in wild-type animals exposed to switch control and *gcy-22* animals (n=11; second pair, pink and n=23; third pair, green, respectively). Regression analysis was applied to the data for the first 50 to 0 mM NaCl downstep. $R^2 = 0.99$, $R^2 = 0.04$ and $R^2 = 0.15$ for wild-type, *gcy-22 (tm2364)* and wild-type switch control, respectively. Individual dots are the slopes calculated for each animal. sc = switch control. Horizontal bars indicate mean; vertical error bars indicate ±SD. See Materials and Methods for details of statistical analysis. (B) FlincG3 fluorescence in the ASER cell body increases in response to the second, third and fourth 0 to 50mM NaCl upstep in wild-type animals. The slopes for the second 0 to 50 mM NaCl upstep between wild-type and *gcy-22(tm2364)* animals are different (n=17 (first set, blue; wild-type), n=23 (third set, green; *gcy-22*); permutation test $p<0.05$). In wild-type animals, the slopes in response to the second 0 to 50 mM NaCl upstep are also different from those of the switch control (n=17 (first set, blue; wild-type), n=11 (second set, pink; switch control); permutation test $p<0.0001$). The difference in slopes for the third and fourth 0 to 50 mM NaCl upstep between wild-type and *gcy-22(tm2364)* animals is also significant (n = 17 (fourth set, blue; wild-type for third upstep and seventh set, blue; wild-type for fourth upstep), n=23 (sixth set, green; *gcy-22* for third upstep and ninth set, green; *gcy-22* for fourth upstep); permutation test $p<0.05$ for third upstep and $p<0.01$ for fourth upstep). In wild-type animals, the slope values in response to the third and fourth 0 to 50 mM NaCl

upstep are also different from those of the switch control (n=17 (fourth set, blue; wild-type for third upstep and seventh set, blue; wild-type for fourth upstep), n=11 (fifth set, pink; switch control for third upstep and eighth set, pink; switch control for fourth upstep); permutation test $p<0.01$). Individual dots are the slopes calculated for each animal. sc = switch control. Horizontal bars indicate mean; vertical error bars indicate ±SD. See Materials and Methods for details of statistical analysis.