SUPPLEMENTAL MATERIAL

SUPPLEMENTAL NOTE 1. Components of *Wnt* signaling pathway and GJ-mediated neural effect act synergistically

To better understand signals regulating GJ-mediated neural effect along the A/P axis, we first asked whether anatomical or structural limitations minimize octanol effectiveness in anterior areas (head and pre-pharyngeal). Bipolar *S. mediterranea* worms can be obtained from head fragments if *Smed-ßcatenin-1* is down-regulated (Gurley et al., 2008; Iglesias et al., 2008; Petersen and Reddien, 2008), suggesting that spatial dependence of GJ blockade is probably not related to anatomical restrictions, but rather to additional regulatory mechanisms or planarian specie-specific differences (*S. mediterranea vs D. japonica*). Despite recent attempts aimed at characterizing *Wnt* signaling (Kobayashi et al., 2007), bipolar phenotypes from inhibition of *Smed-ßcatenin-1* homologs have not been reported in *D. japonica*; this is an important issue because of potential species-specific differences in A/P polarity establishment. In the *D. japonica* EST database (Mineta et al., 2003), we identified a homolog of *Smed-ßcatenin-1* and termed it *Dj-ßcatenin-B* (Supplementary Figure S8A). *Dj-ßcatenin-B (RNAi)* worms exhibited similar phenotypes during regeneration and tissue maintenance to those previously reported in *S. mediterranea* after *Smed-ßcatenin-1(RNAi),* indicating that in both species ß-catenin is similarly relevant for A/P polarity establishment. Further, since regenerating head and pre-pharyngeal fragments can give rise to bipolar head with high penetrance (Supplementary Figure S8B), brain inhibitory signals require ß-catenin

function. Interestingly, post-pharyngeal regenerating fragments subjected to *Dj-ßcatenin-B (RNAi)* did not reach maximum penetrance for bipolar regenerates except when treated with octanol, suggesting that these two pathways are additive (Supplementary Figure S8B). The time required to observe phenotypes associated with either RNAi of *Dj*-ß-catenin-*B* or *Dj-Inx-5+13, 12* is different (it requires only one week before amputation in the former while for the latter it requires continuous RNAi for several weeks -about a month). Also, the phenotype associated with innexins involve multiple genes while in ß-catenin downregulation of just one gene is required. This together makes combinatorial analyses by RNAi of these two pathways somewhat difficult to evaluate. We conclude that spatial differences in bipolar regeneration after octanol treatment are unlikely to be due to structural limitations associated with anterior (i.e.: head or pre-pharyngeal) fragments and that ß-catenin and GJ might complement each other. Our identification of *Dj*-ß-catenin-*B* in *D. japonica* and the induction of similar RNAi phenotypes as those reported for *Smed-ßcatenin-1* in *S. mediterranea,* argue against specie-specific differences and instead suggest that indeed both *Wnt* and GJ-mediated signals coexist within the same animal and are relevant for establishment and maintenance of polarity

SUPPLEMENTAL NOTE 2. Treatment with different n-alcohols induce transitory behavioral changes.

Treatment with n-alcohols (i.e. hexanol, heptanol and octanol) in intact animals does not alter polarity even if worms are treated for more than one month. However, we

did observe that after exposure to these compounds intact animals displayed abnormal behavior characterized by random contractions, motility problems that lasted for few minutes after exposure. Interestingly, no microscopic effect was noticed in these animals suggesting that the effect of these drugs was transitory and did not affect noticeable internal structures.

TABLE S1. Summary of screen of RNAi innexin genes and phenotypes during tissue maintenance and regeneration.

dsRNA microinjections of different *DJ-Inx* genes were administered individually or in combination of up to four with an scheme similar to the one shown in Figures 3A and 3C. In all cases but one [*Dj-Inx-5+12, 2+4(RNAi)],* multiple RNAi led to behavioral changes in intact animals; however, in most cases this was not followed by abnormalities during regeneration. These behavioral signs were mostly observed about 30 days after first dsRNA microinjection, when intact planarians began to show behavioral changes characterized by slow movements and random contractions. The strongest behavioral changes were observed in worms subjected to *Dj-Inx-5+13, -12(RNAi)* (~95%, n=137). Behavioral changes in this group were mostly followed by fissioning in pre or post-pharyngeal areas (~43%, n=137), mortality (~ 37% mostly from small fissioning fragments. i.e.: generalized lyses), or development of ectopic photoreceptor-like pigmentation and pharynxes (~ 50%, n=30) (Figure 3B and supplemental Movie M1). Note: RNAi was performed in all cases by microinjection in