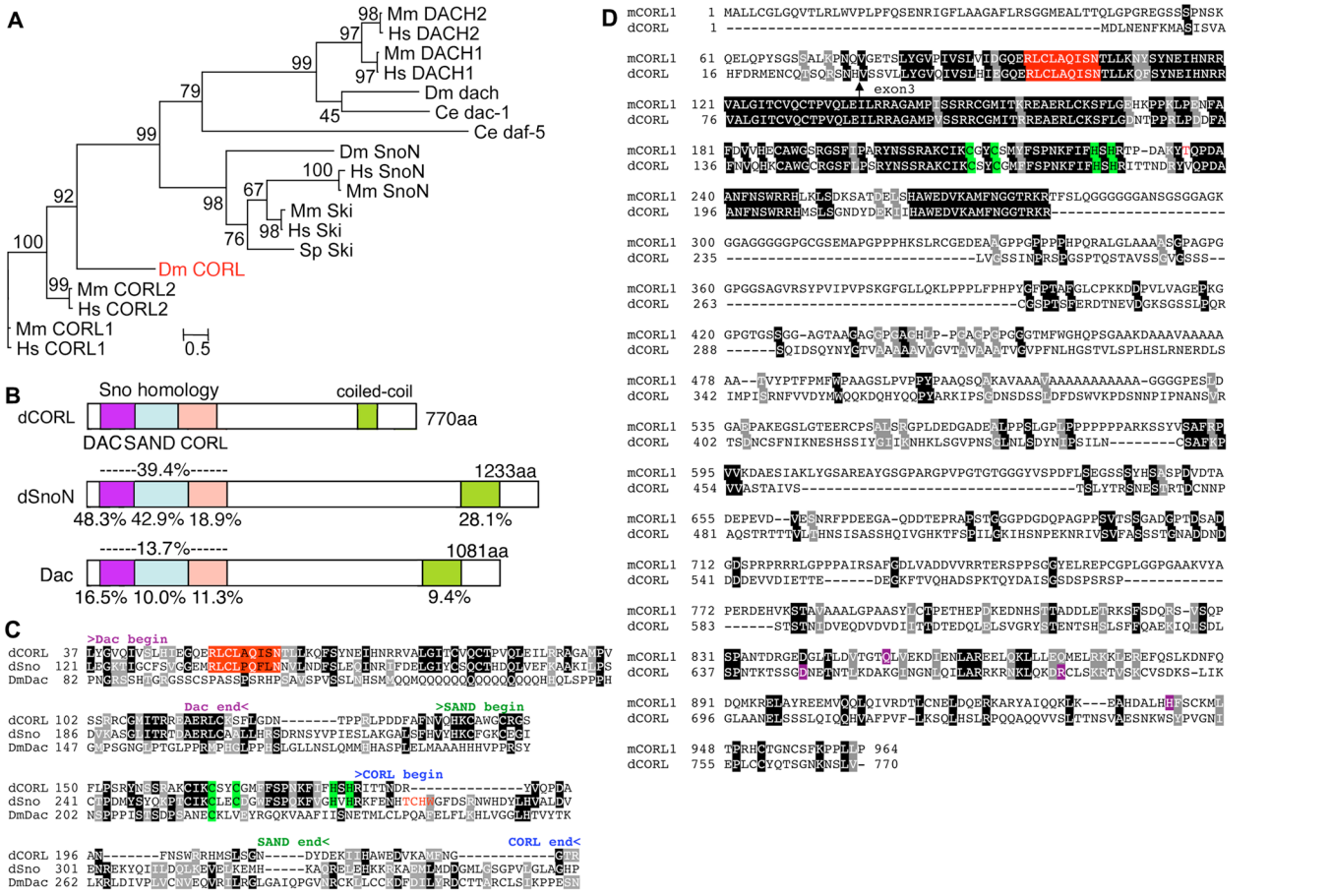

Fig. S1. dCORL belongs to a highly conserved family of proteins. (**A**) Phylogenetic tree of the Sno/Dac/CORL family. Accession numbers: DmCORL (in red; JX 126878); DmDach NP_723971; Cedac-1 NP_1021129; Cedaf-5 NP_496941; DmSnoN NP_1097115; SpSki XP_1185880; MmCORL1 NP_766034; MmCORL2 A7M7C7; MmDACH1 NP_31852; MmDACH2 NP_291083; MmSnoN Q60665; MmSki NP_35515; HsCORL1 NP_1026977; HsCORL2 Q2VWA4; HsDACH1 NP_542937; HsDACH2 NP_444511; HsSnoN CAA33289; HsSki NP_3027. HsCORL1 and HsCORL2 are also known as Fussel15 and Fussel18, respectively. (**B**) dCORL, dSno and Dac are compared as in Fig. 1D. The level of amino acid similarity between the indicated protein and dCORL is shown for all domains. (**C**) Sno homology domain of dCORL aligned with dSnoN and Dac as in Fig. 1C. The red TCHW motif that binds Smad4 in dSno (beginning with Thr^{280}) is absent in dCORL and Dac. (D) The ORF of *dCORL* aligned with mCORL1 annotated as above. The boundaries of each protein's coiled-coil domain is indicated by purple shading.

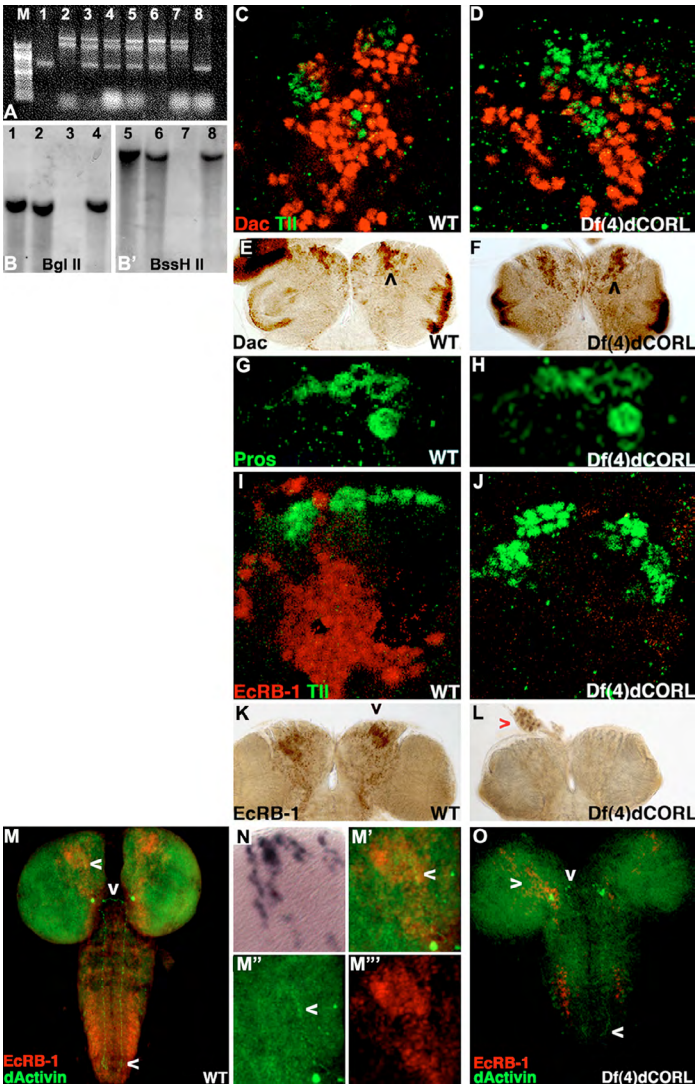

Fig. S2. Tll, Dac, Pros and dAct are unaffected in *dCORL* mutants. (**A**) Two-sided PCR from single flies balanced over *In(4)Ci*^{D} amplifying genomic DNA at the 3′ end of *Pbac{WH}f07015* (718 bp) and genomic DNA at the 5′ end of *Pbac{WH}f06253* (1988 bp) in the same reaction. Lanes contain DNA templates as follows: M, marker; 1, *f07015*; 2, *f06253*; 3, mixed *f07015+f06253*; 4, *Df(4)37*; 5, *Df(4)59*a; 6, *Df(4)59*b; 7, *Df(4)36*; 8, *Df(4)58*. Note that *Df(4)37* and both samples of *Df(4)59* contain the 5′ and 3′ amplicons on the non-*Ci*^{D} chromosome. (**B**,**B′**) Southern blot of genomic DNA cut with *Bgl*II or *Bss*HII from wild type (lanes 1, 4), *Df(4)dCORL*37/*Ci*^{D} (lanes 2, 5), *Df(4)dCORL*37 homozygous (lanes 3, 7) and *Bt*^{D}/*Ci*^{D} (lanes 4, 8) flies analyzed with a *dCORL* DNA probe. No hybridization is seen in either of the *Df(4)dCORL* homozygous lanes but is present in all others. (**C**,**D**) Single confocal slice of wild-type and *Df(4)dCORL* MBs in anterior/dorsal view (anterior up and optic lobe to the left) stained for Dac (red) and Tll (green). *Df(4)dCORL* larvae were age matched to wild-type larvae by the number of ommatidial rows in their eye disks. Tll NB/GMC clusters (three of four are shown) are near Dac in MB neurons and unaffected in *Df(4)dCORL* (the appearance of Tll overexpression in *Df(4)dCORL* is due to differences in slice depth as it is not visible in the respective stacks). (**E**,**F**) Whole-brain view confirms that Dac MB neuron expression (black arrowheads) is unaffected in *Df(4)dCORL*. (**G**,**H**) Anterior/dorsal slice of MB cells stained for Pros (green; nuclear in GMC and cortical in a subset of neurons) shows that Pros is unaffected in *Df(4)dCORL*. In both genotypes, a GMC and its associated neurons are observed with the oldest neuron (at left) beginning to extend axons. (**I**,**J**) Anterior/dorsal slice of MB cells showing EcR-B1 (red) and Tll (green). In wild-type, Tll NB/GMC clusters are near EcR-B1 in MB neurons. In *Df(4)dCORL*, Tll is normal but EcR-B1 is absent. (**K**,**L**) Whole-brain view confirms that EcR-B1 MB expression is lost (black arrowhead) in *Df(4)dCORL* whereas ring gland EcR-B1 is normal (red arrowhead). (**M**) dAct (green) in wild-type larvae analyzed by antibody staining shown as a confocal stack and compared with EcR-B1 (red). dAct is secreted and low level ubiquitous staining is evident throughout in the CNS. Significant expression is present in a single pair of subesophageal cells and their axons extending in three directions (white arrowheads). (**N**) High magnification view of *dCORL* expression in the brain focusing on the anterior/dorsal region. (**M′-M‴**) High magnification view of a dAct anterior directed axon revealing its termination in the center of the cluster of EcR-B1 MB neurons in comparison to *dCORL*. (**O**) In *Df(4)dCORL*, dAct in the pair of subesophageal cells and their axons are unaffected (single slices reveal the intercellular axon and the axon extending to the MB are intact; data not shown). In *Df(4)dCORL,* EcR-B1 in the MB is absent and EcR-B1 in the VC is severely reduced.

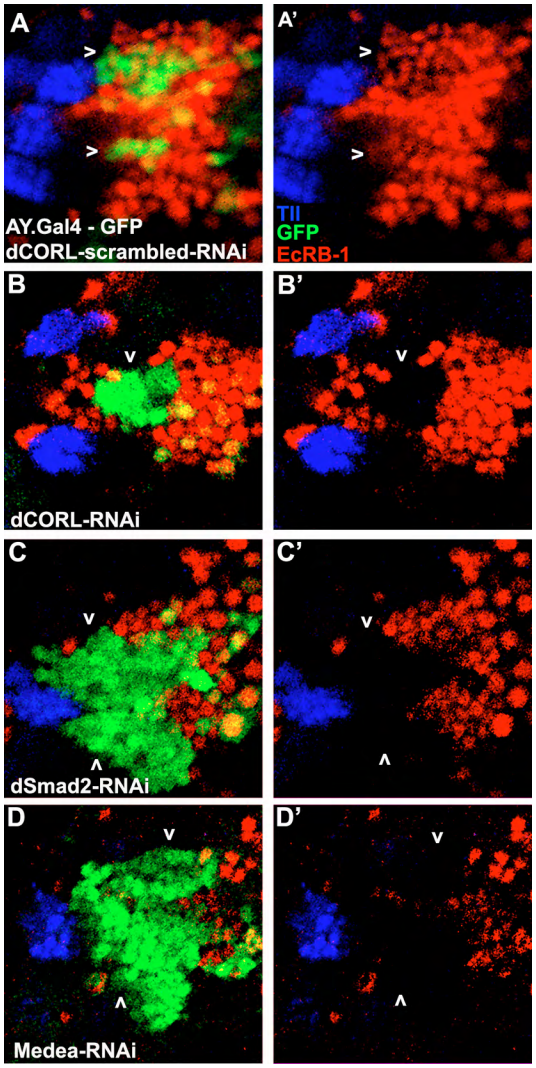

Fig. S3. dCORL-, dSmad2- and Medea-RNAi eliminate EcR-B1 in anterior/dorsal MB clones. Single confocal slices of flip-out clones in the anterior/dorsal region of the MB. Tll (blue), EcR-B1 (red) and GFP (green) are shown with the left column in three-color and the right column in two-color with Tll (blue) and EcR-B1 (red). (**A**,**A′**) dCORL-scrambled-RNAi has two medium clones (white arrowheads) and two single cell clones inside the domain of EcR-B1. EcR-B1 is not affected any of the clones. (**B**,**B′**) dCORL-RNAi has one medium clone near the Tll clusters (white arrowhead) that is surrounded by EcR-B1 cells. Several single cell clones inside the domain of EcR-B1 are also visible. In the medium-sized clone EcR-B1 is absent. EcR-B1 in the single cell clones is not affected. (**C**,**C′**) dSmad2-RNAi has two large clones (white arrowheads) and several single cells clones inside the domain of EcR-B1. In both large clones EcR-B1 is absent but EcR-B1 is present in the single cell clones – a phenocopy of dCORL-RNAi. (**D**,**D′**) Medea-RNAi has two large clones (white arrowheads) and several single cells clones inside the domain of EcR-B1. In both large clones EcR-B1 is absent but EcR-B1 in present in the single cell clones – a phenocopy of dCORL-RNAi and dSmad2-RNAi.

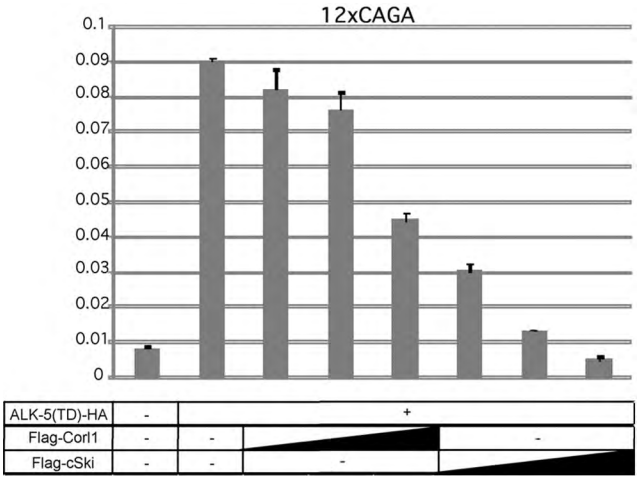

Fig. S4. mCorl1 overexpression represses TGF-β/Activin signaling. Ability of mCORL1 and c-Ski to repress 12xCAGA-Luc reporter stimulation by CA-ALK-5 (TGF-β/Activin Type I Receptor) is shown. Increasing amounts of mCORL1 or c-Ski reduced reporter expression proportionately and dramatically. Results here are for 293T cells and similar results were obtained in HepG2 cells (not shown). Error bars indicate standard deviation.