Supplemental Information

Concerted action of CB1 cannabinoid receptor and Deleted in Colorectal Cancer (DCC) in axon guidance

Abbreviated title: CB1R and RGC axon guidance

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Supplementary figure legends

Figure 1. Expression of the eCB system. ***A***, primary cortical neurons immunolabeled for DAGLα, MGL and NFL. ***B***, Western blot analysis of CB1R expression in primary neuron cultures at several DIVs. Molecular weight markers are indicated on the right side of the panel. ***C***, Photomicrographs of retinal cross-sections showing CB1R, eCB synthesizing (NAPE-PLD, DAGLα) and eCB degrading (FAAH, MGL) enzyme expression (magenta) during early postnatal development. Syntaxin was used to label retinal projections (green). ONL, Outer nuclear layer; OPL, outer plexiform layer; INL, Inner nuclear layer; IPL, Inner plexiform layer; GCL, Ganglion cell layer; GCFL, Ganglion cell fiber layer. ***D***, Photomicrographs of retinal tissues from $CB1R^{-/-}$ and $FAAH^{-/-}$ mice and matched wild type animals showing CB1R and FAAH antibodies specificity.

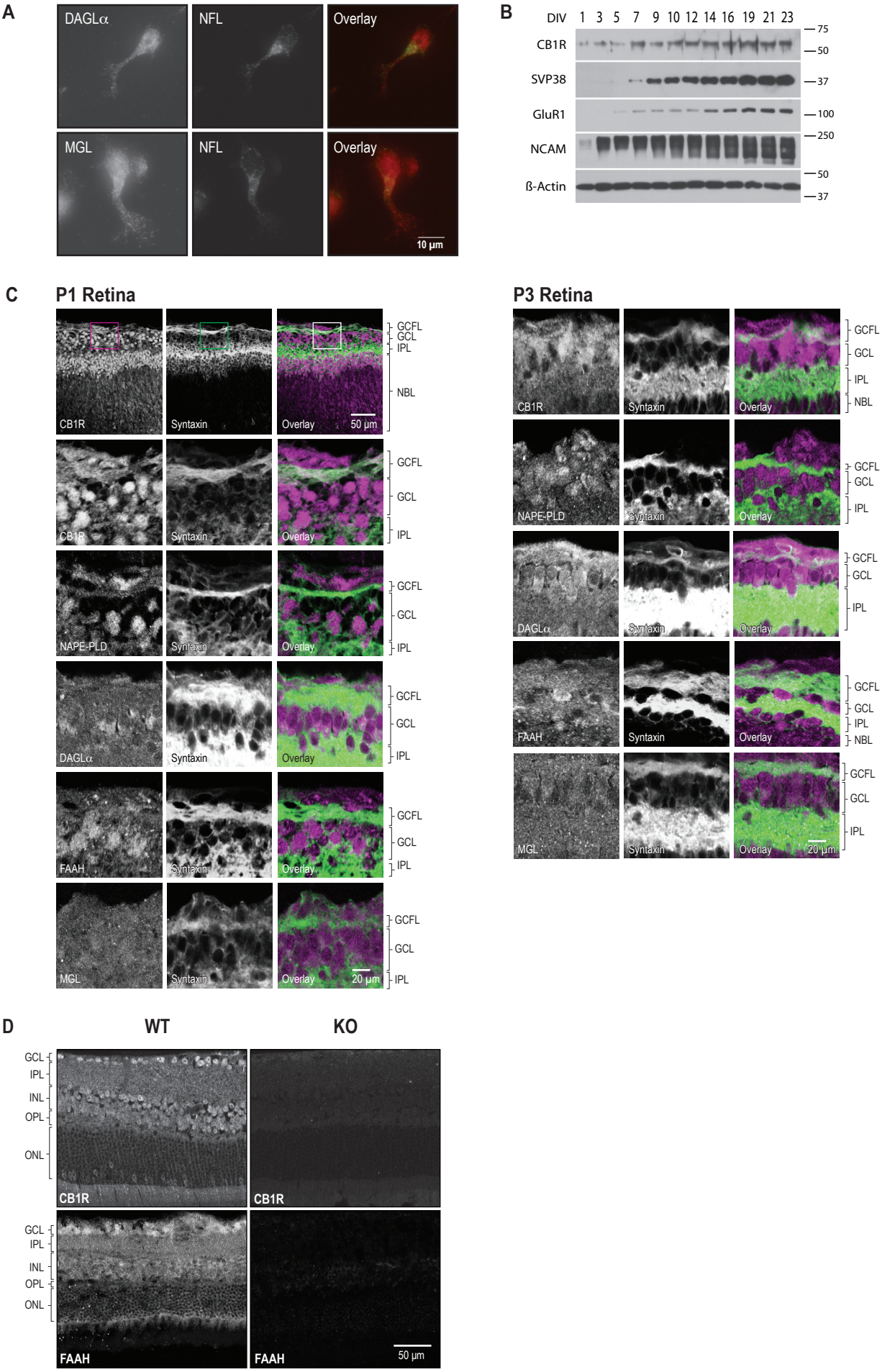
Figure 2. CB1R activity and second-messenger cascades. CB1R agonist, inverse agonist and antagonist did not activate the PI3K, ERK1/2 or mTOR pathways following a 20 min treatment (***A***). Molecular weight markers are indicated on the right side of the panel. Quantification of the optical density for P-AKT (***B***), P-ERK1/2 (***C***) and P-S6 (***D***). CB1R stimulation following KCl induced depolarisation (***E***) or insulin treatment (***F***) failed to recruit PI3K, ERK1/2 or mTOR second messenger cascades. Molecular weight markers are indicated on the right side of the panel.

Figure 3. DCC regulates CB1R induced reorganization of the GC. ***A*,** Photomicrographs of primary neuron cultures treated with αDCCfb followed by the

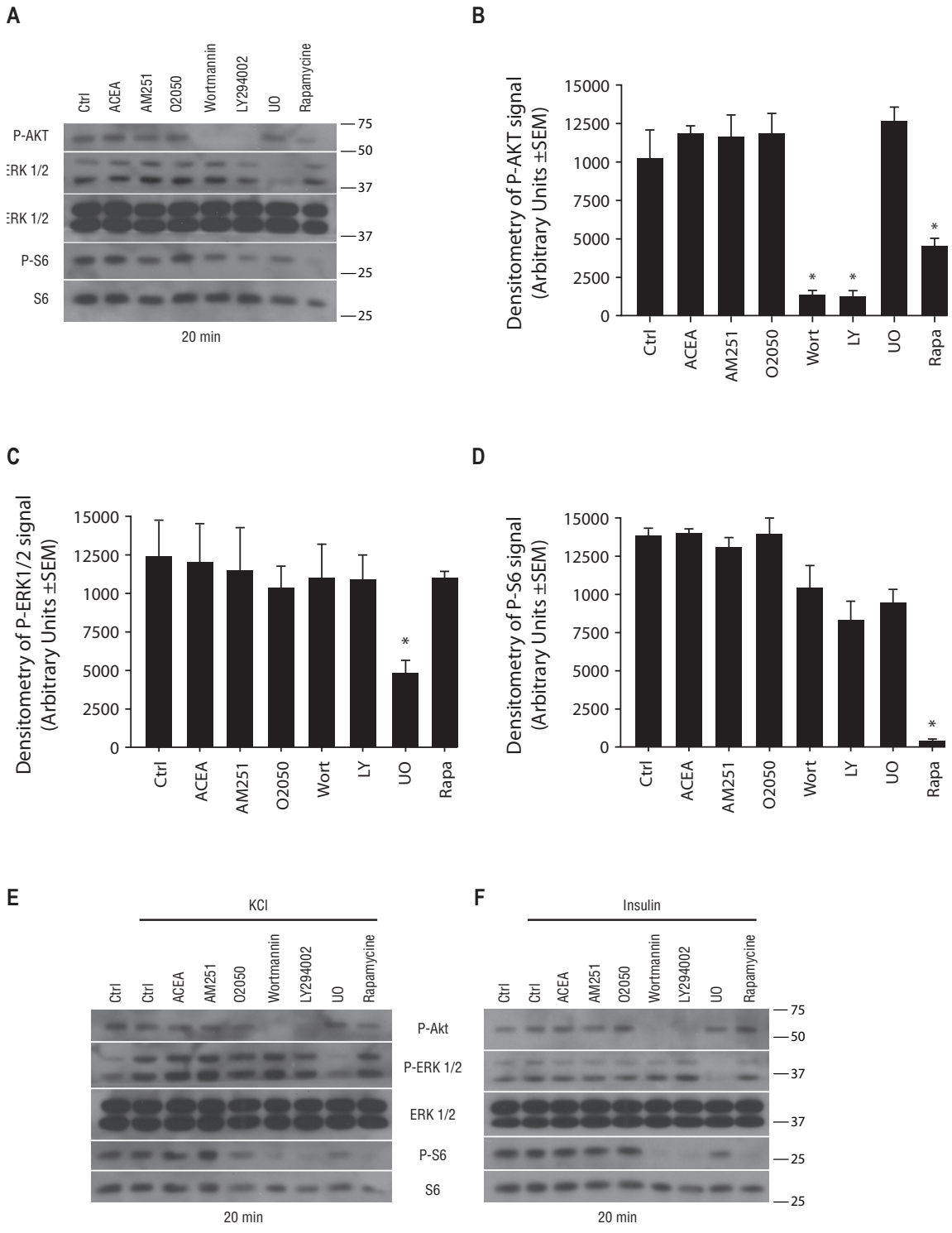
addition of either a CB1R inverse agonist or antagonist (AM251 or O2050, respectively) or FSK. GC photomicrographs of *dcc*$^{-/-}$ (***B***) and *dcc*$^{+/+}$ (***C***) primary neuron cultures treated with either ACEA or AM251.

Figure 4. Intraocular injections and mechanism by which cannabinoids modulate GC steering. ***A***, Schema illustrating vitreal injection and CB1R, FAAH and MGL expression analysis sites during retinal projection development. ***B*** and ***C*****,** Illustrations of the methods used to quantify retinal projection branches length (***B***) and the number of retinal axon branches (***C***) in the DTN. Arrowed dotted lines indicate the distance between the lateral border of the thalamus and the tip of the farthest projections (***B***). ***D***, Photomicrographs of optic nerves following vitreal injections of CTb-546 and CTb-488 in to the left and right eye, respectively. ***E***, A model illustrating the interactions between the CB1R and DCC during axon navigation. Antagonizing the CB1R increases intracellular cAMP levels, triggering a PKA-dependent translocation of DCC to the plasma membrane and resulting in GC expansion, whereas CB1R agonists induce the opposite resulting in GC collapse.

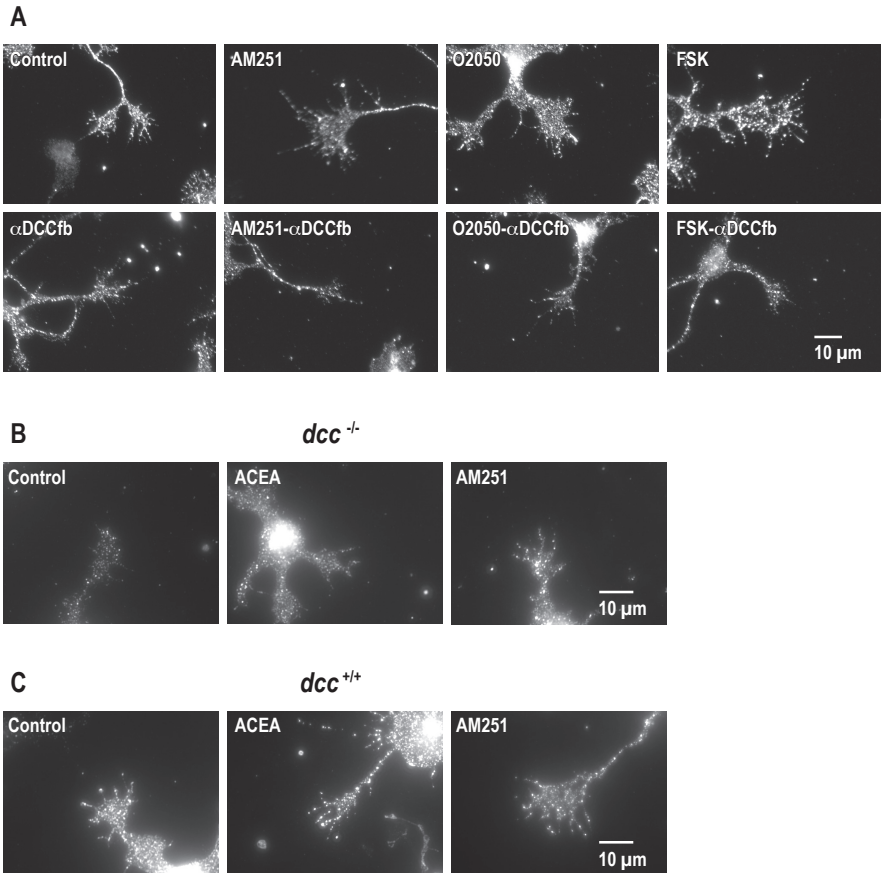
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4