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Supplemental Data

EphB Receptors Couple Dendritic Filopodia Motility to Synapse Formation

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Supplemental Text

Live imaging of synaptic puncta with EphB2 shRNA.

To examine how loss of EphB affects synaptic contacts and synaptic puncta in live neurons, we studied the stability of synaptic puncta in EphB2 knockdown cells at the time when defects in synaptogenesis are first observed. Wild type cortical neurons were transfected at 0 or 3DIV with vector control or EphB2 shRNA + PSD-95-GFP, and time lapse images were taken at 9DIV every 5 minutes for 1-2 hours. As has been observed previously (Gerrow et al., 2006; Okabe et al., 1999; Prange and Murphy, 2001), most puncta were very stable throughout the imaging period. In control cells, we found that ~18% of PSD-95 puncta moved (n = 205 puncta, 7 cells), defined as a total distance >2.5µm or average distance between frames of >0.25µm. However, with knockdown of EphB2 the percent of moving PSD-95-GFP increased to over 30% (n = 156 puncta, 5 cells; $P < 0.01$; chi square). These results indicate that PSD-95 puncta are less stable when EphB2 expression is reduced, possibly due to fewer potential synaptic anchoring sites for non-synaptic clusters of PSD-95 (Gerrow et al., 2006; Gray et al., 2006; Prange and Murphy, 2001). Our findings also raise the possibility that the decrease in synapse number with loss of EphB might result from a change in stability of synaptic contacts that occur. To address whether synapses that have formed in EphB2 knockdown cells are more transient than those in control conditions, we transfected neurons in suspension with PSD-95-GFP ± EphB2 shRNA,

and infected these cultures at 2DIV with lentivirus encoding Synaptophysin-RFP (SynRFP) to visualize presynaptic specializations in axons along with the postsynaptic PSD-95-GFP clusters in dendrites. Although we observed few instances of pre- and postsynaptic contacts becoming co-localized during the 2 hour imaging period in neurons at this age, the overwhelming majority of puncta positive for both PSD-95-GFP and SynRFP at the start of imaging were stable throughout the duration under either condition (Figure S2). Thus, the reduction in synapse density with EphB2 knockdown is not due to a large increase in turnover of synaptic contacts. Consistent with the idea that EphBs are required for only a subset of synapses to form, these data indicate that the contacts remaining with EphB2 knockdown are stable.

Supplemental Data References

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Gray, N. W., Weimer, R. M., Bureau, I., and Svoboda, K. (2006). Rapid redistribution of synaptic PSD-95 in the neocortex in vivo. PLoS Biol *4*, e370.

Okabe, S., Kim, H. D., Miwa, A., Kuriu, T., and Okado, H. (1999). Continual remodeling of postsynaptic density and its regulation by synaptic activity. Nat Neurosci *2*, 804-811.

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Supplemental Figure Legends

Figure S1. Effects of EphB2 shRNA on synapse formation are specific. (A) Average synapse density on 21DIV wild type rat neurons transfected with the indicated constructs + GFP at 10DIV (n = 30, 15, 27, and 15 cells from left to right; *P < 0.0001; ANOVA). **(B)** Average synapse density on 21DIV DKO or TKO mouse neurons transfected with GFP and either vector control or EphB2 shRNA at 10DIV (n = 20, 17, 23, and 16 cells from left to right; *P < 0.0001 compared to DKO + control; ANOVA). **(C)** To control for whether knockdown is at equal magnitude at all tested time points, we determined the percent change in EphB2 puncta (synaptic and non-synaptic) density in neurons transfected with GFP + control or EphB2 shRNA and fixed at the indicated times, immunostained with two different EphB2 antibodies (n = 11, 10, 11, 10, 9, 10, 9, 10, 10, 6, 10, 6, 9, 8, 9, and 8 cells from left to right; *P < 0.003; ANOVA). Error bars indicate SEM. Immunostaining indicates that EphB2 puncta are not present at every synapse, and that EphB2 puncta are not present only at synapses. Here we measure knockdown of both synaptic and nonsynaptic EphB2 puncta, and thus if anything underestimate the reduction in synaptic EphB2.

Figure S2. Reduced expression of EphB does not result in changes in synapse stability. Representative frames from time-lapse images of 9DIV neurons transfected at 0DIV with PSD-95-GFP and either control or EphB2 shRNA, and infected at 2DIV with SynRFP. White arrowheads indicate synapses (clusters of co-localized PSD-95-GFP and SynRFP) that are stable throughout the 2 hour imaging period. Open arrowheads indicate mobile PSD-95-GFP puncta (control: 26/29 stable for 2 hours, average = 117.2±1.96 minutes; n = 6 cells; EphB2 shRNA: 18/19 stable for 2 hours, average = 115.3±4.74 minutes; n = 6 cells; P = 0.7; t test). Scale bar, 2µm.

Figure S3. Knockdown of EphB2 from 14-25DIV results in dendritic protrusion abnormalities but no synapse loss. (A) Cortical neurons cultured from E18 rats were transfected at 14DIV with GFP and either control (n = 15 cells) or EphB2 shRNA (n = 17 cells), fixed at 25DIV, and immunostained with antibodies recognizing GFP, PSD-95 (red), and vGlut1 (blue). Representative image of immunostained dendrite from a neuron expressing the EphB2 knockdown construct from 14-25DIV. Scale bar, 2μm. Error bars indicate SEM. **(B)** Expression levels of EphB2 in dissociated cortical neurons cultured for various lengths of time. Neurons were cultured and allowed to survive for 3, 5, 7, 10, 14, or 28 days *in vitro*, subsequently lysed, and the total protein concentration determined using a Bradford assay. Equal amounts of total protein were run on a gel, and the resulting Western blot probed with anti-EphB2 antibodies. The same blots were probed with an anti-tubulin antibody (Developmental Studies Hybridoma Bank) as additional loading control (n = 2).

Figure S1

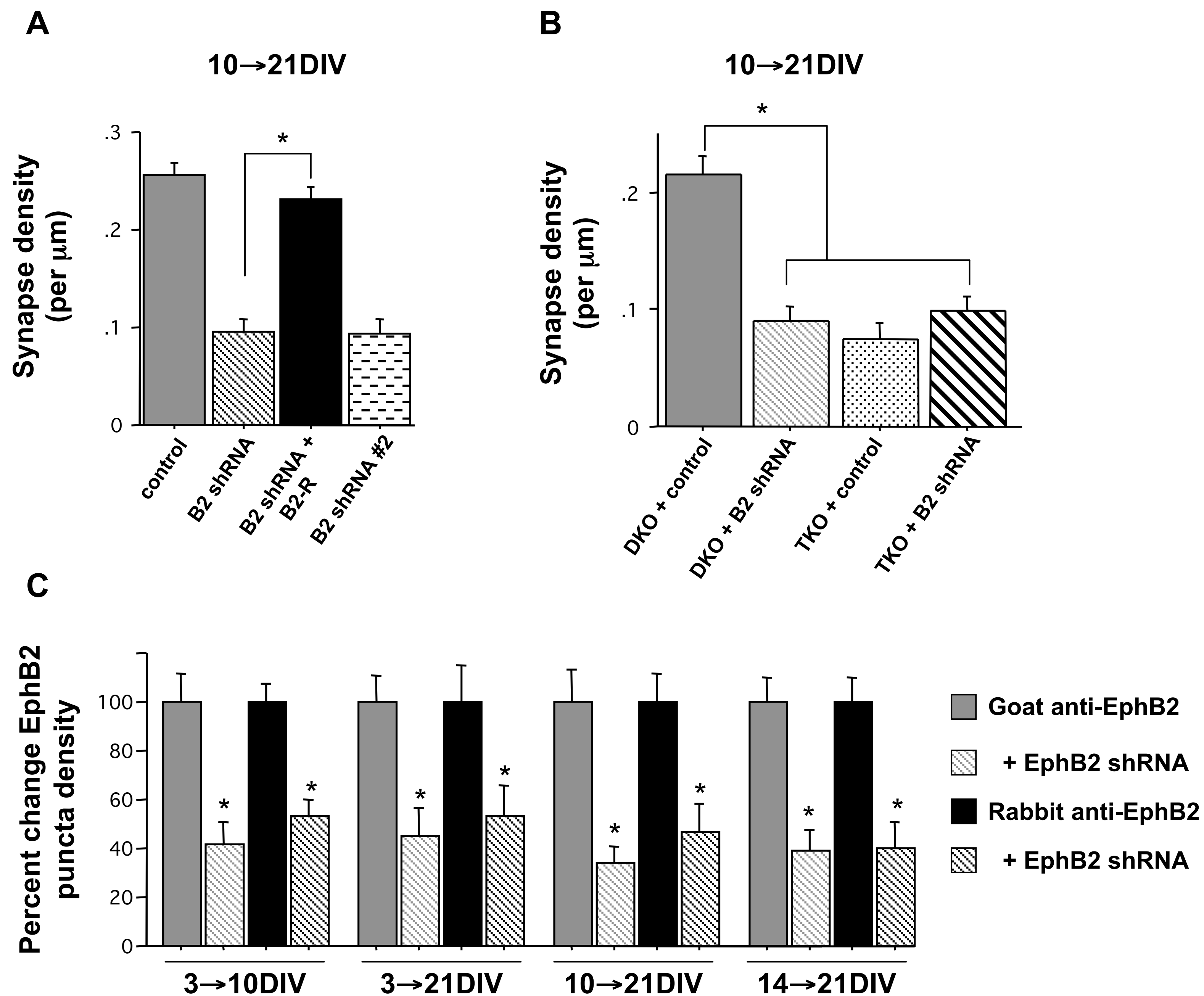

Figure S2

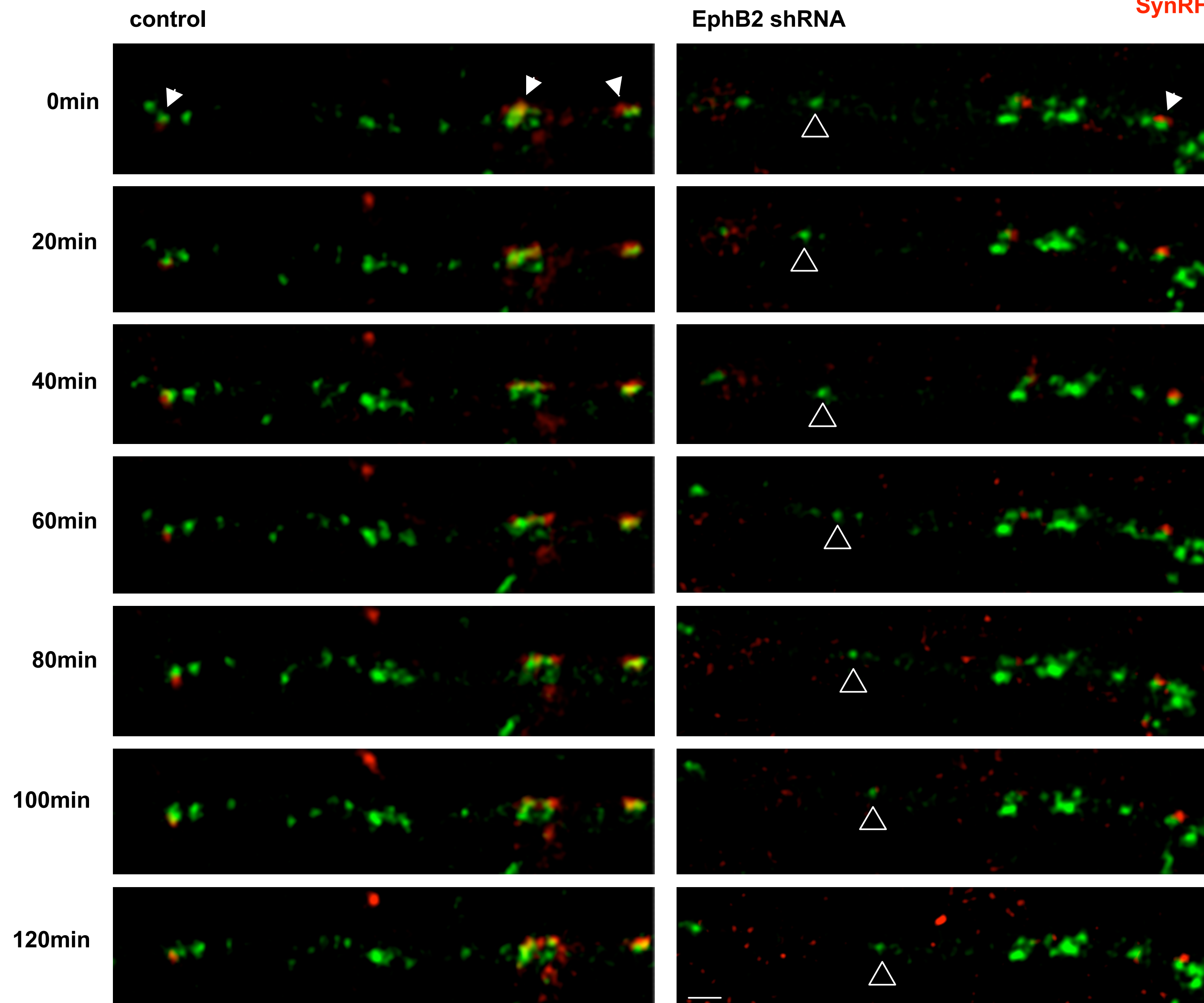

Figure S3

A

Synapse density (per μm)

.3
.2
.1
0

14→25DIV

control
EphB2 shhRNA

PSD-95
vGlut1

B

DIV:

kDa 3 5 7 10 14 28 Blot:

120 α-EphB2

50 α-tubulin