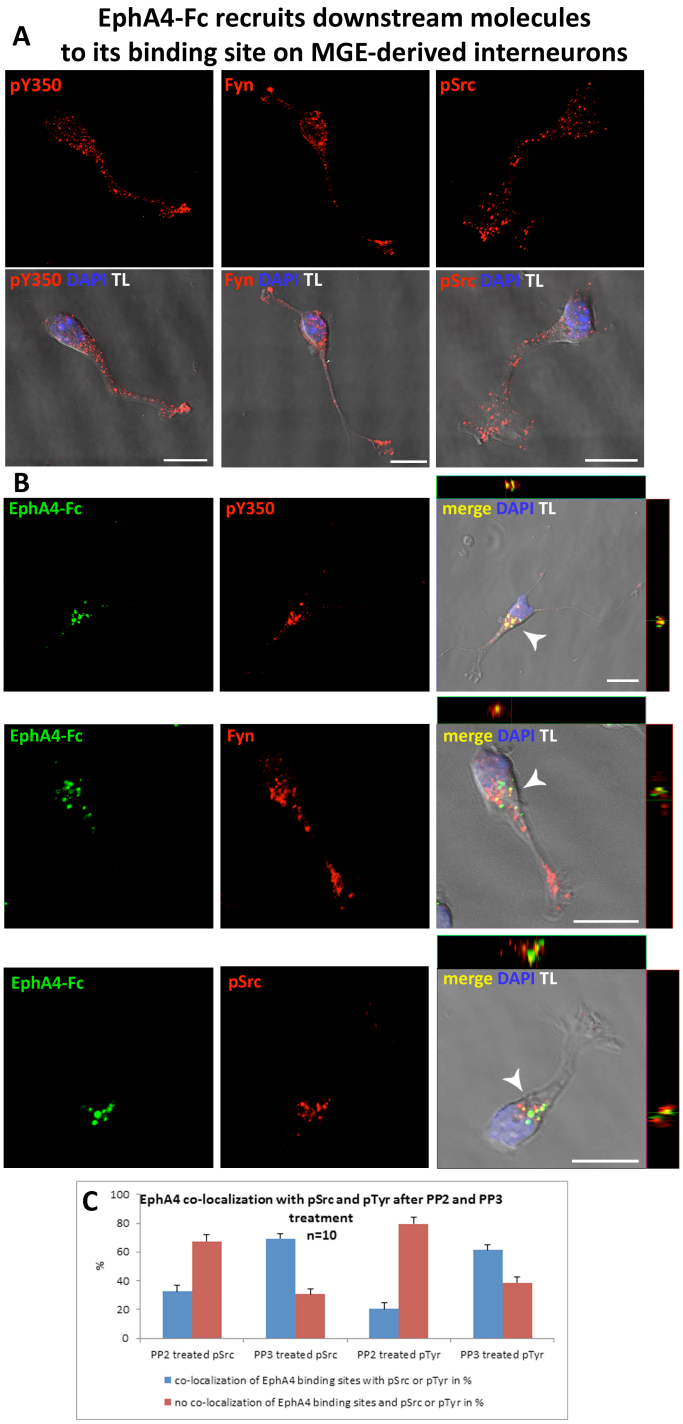

Fig. S1: Recruitment of downstream effectors to the EphA4 binding site

Earlier studies demonstrated that multiple mediators are involved in the transmission of Eph/ephrin reverse signaling, such as the transmembrane receptors TrkB or p75NTR, which are co-expressed with ephrin ligands in lipid rafts. In (A) confocal micrographs of MGE-derived interneurons immunostained with antibodies against pY350, Fyn, and pSrc are shown. The pSrc, Fyn, and pY350 immuno signals are equally distributed throughout the cell. In (B) confocal micrographs with x-y line scans in a single optical section of MGE-derived

interneurons treated with recombinant EphA4-Fc (green) and immuno stained with pY150, Fyn, and pSrc antibodies (red) are presented. Arrowheads indicate the initial segments of the leading processes, where fluorescence labeled EphA4-Fc overlapped with pY350, Fyn, and pSrc immuno signals, while the rest of the cell showed only weak immuno signals. TL: transmitted light. Scale bars: 10 μm. This co-labeling was reduced when the cells were treated with the inhibitor of Src family kinases PP2, a chemical compound that is known to block activation of SFKs, including Src and Fyn (Hanke et al., 1996). In comparison to the treatment with the control substance PP3 the co-localization of EphA4 with pSrc or pY350 signals decreased from 69 ± 4% to 33 ± 4% for pSrc and from 61 ± 4% to 20 ± 4% for pY350 signals (C).

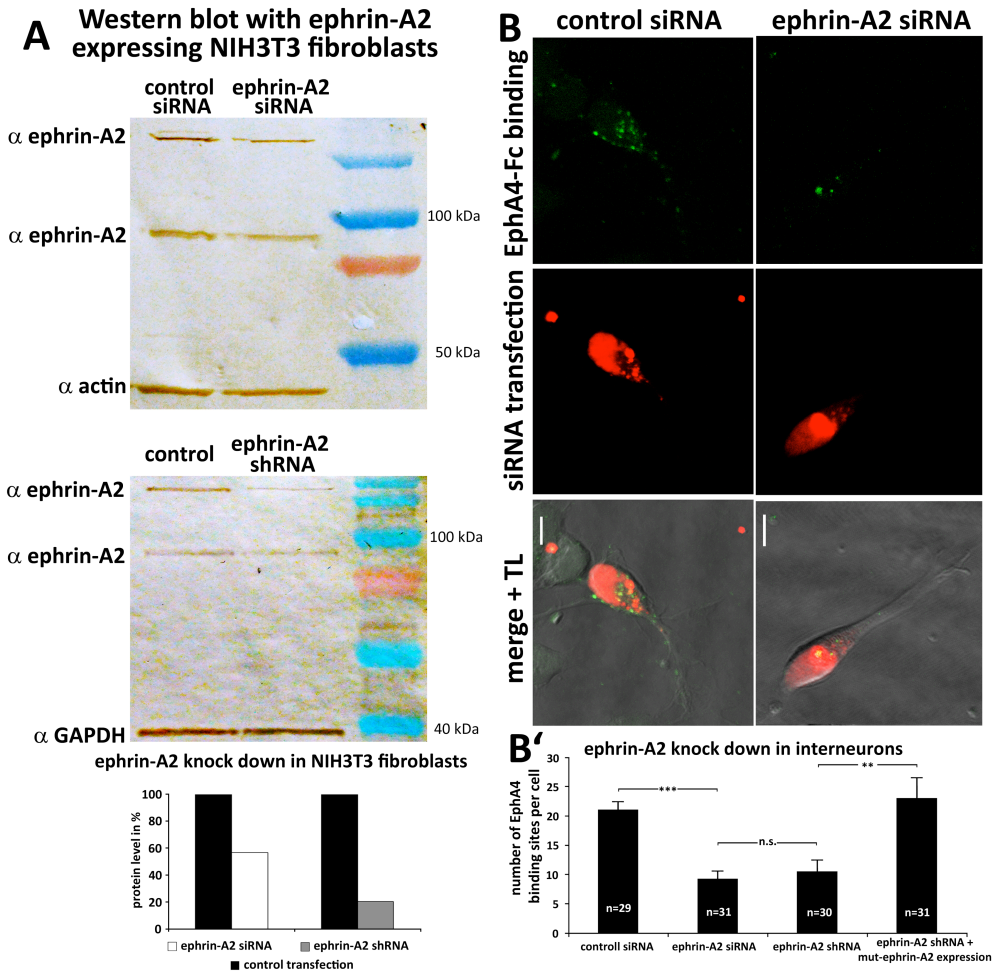

Fig. S2: KD efficiency of ephrin-A2-siRNA and -shRNA

(A), Ephrin-A2-siRNA and -shRNA decrease the ephrin-A2-level in ephrin-A2 expressing NIH3T3 fibroblasts as shown by western blot analysis. To quantify the KD efficiency we calculated the grey value of ephrin-A2-bands relative to the control bands (Actin or GAPDH). Additionally this ratio was calculated relative to the transfection efficiency of NIH3T3 fibroblasts that was about 35%. Ephrin-A2-siRNA decreased the level of ephrin-A2-protein by 43% compared to a control transfection with scrambled siRNA and the ephrin-A2-shRNA showed an 80% decrease of ephrin-A2. (B), Confocal micrograph of MGE-derived cells that were transfected with control or ephrin-A2-siRNA and treated with pre clustered EphA4-Fc. (B'), Binding assays on ephrin-A2-siRNA or -shRNA but not double transfected shRNA + mut-ephrin-A2-expression-vectors resulted in a decreased number of binding sites compared to control transfection. Student's t-test: *** $p<0.001$, ** $p<0.01$, n.s. $p\geq0.05$. Error bars: SEM.

Movie 1. Time-lapse video microscopy of EGFP^{+} MGE-derived cells on WT-slices treated with Fc-protein as a control. Asterisks indicate tracked cells. Time is presented in minutes. (see main text for details)

Movie 2. Time-lapse video microscopy of EGFP^{+} MGE-derived cells on WT-slices treated with EphA4-Fc-protein. Asterisks indicate tracked cells. Time is presented in minutes. (see main text for details)

recombinant protein	proportion of total MGE cells (%)	proportion of calbindin positive MGE cells (%)
ephrinA5-Fc	55 ± 2	85 ± 2
ephrinA3-Fc	54 ± 3	75 ± 2
EphA3-Fc	10 ± 1	38 ± 3
EphA4-Fc	10 ± 1	36 ± 3
EphA6-Fc	10 ± 1	43 ± 4
ephrinB1-Fc	34 ± 2	40 ± 3
ephrinB3-Fc	54 ± 2	77 ± 4
EphB1-Fc	81 ± 2	98 ± 1
EphB3-Fc	85 ± 2	96 ± 2

Table S1: Binding study with recombinant Eph/ephrin proteins on MGE-derived cells

Analysis of the binding assay indicated that almost all CB^+ interneurons bind EphBs and about 55% of the CB^+ neurons bind EphAs. Thus most CB^+ interneurons from the MGE express ephrin-B ligands and a large proportion ephrin-A ligands. As the MGE generates various cell types, including also striatal and globus pallidus neurons, the present data indicate that different populations of neurons express different sets of Eph receptors and ephrin ligands and to certain degree a co-expression of ephrin ligands and Eph receptors in the same cohort of cells. Results are presented as a percentage ± SEM.