



**Supplementary information, Figure S2** Expression of recombinant human Slit2 and Robo1 proteins and characterization of R5, S1 and R4 mAbs.

(A) Silver staining of recombinant human Slit2 (hSlit2) and human Robo1-Fc (hRobo1-Fc) isolated from the supernatants of Sf9 insect cells infected with the corresponding viral stocks. (B) Effect of R5 mAb on hRobo1-Fc binding to hSlit2. Biotinylated hRobo1-Fc was added to hSlit2-immobilized 96-well microtiter plate, in the absence or presence of mIgG or R5, followed by streptavidin-conjugated horseradish peroxidase and its substrate. (C-D) Specificity of S1 and R4 mAbs. HEK293 cells were transfected with the plasmids of Slit1-3 with the myc tag (A) and Robo1-4 with the myc or His tag (B) and cell lysates were immunoblotted with S1, R4, 9E10 for the myc tag and M2 for the His tag. Results represent at least three separate experiments (A, C and D) or the mean  $\pm$  S.D. of triplicate measurements from three independent experiments (B).