showed staining patterns apparently distinct from that of hepatoblasts, which were stained with anti-Dlk antibody (Figure 1A, C and D).

Supplementary Figure 3

FCM of E11.5 liver cells with anti-p75NTR (25-8) antibody and an endothelial lineage marker, anti-PECAM1 antibody or anti-Flk-1 antibody.

Supplementary Figure 4

Double immunofluorescence of *in situ* TUNEL (green) and anti-p75NTR antibody (red) in E12.5, E14.5 and E16.5 liver. (Original magnification; X200: upper panels, X400: lower panels)

References

1. Hautekeete ML, Geerts A. The hepatic stellate (Ito) cell: its role in human liver disease. Virchows Arch 1997;430:195-207.
2. Li Z, Dranoff JA, Chan EP, Uemura M, Sevigny J, Wells RG. Transforming growth factor-beta and substrate stiffness regulate portal fibroblast activation in culture. Hepatology 2007;46:1246-56.
3. Knittel T, Kobold D, Piscaglia F, Saile B, Neubauer K, Mehde M, Timpl R, Ramadori G. Localization of liver myofibroblasts and hepatic stellate cells in normal and diseased rat livers: distinct roles of (myo-)fibroblast subpopulations in hepatic tissue repair. Histochem Cell Biol 1999;112:387-401.
4. Taub R. Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol 2004;5:836-47.
5. Friedman SL, Wei S, Blaner WS. Retinol release by activated rat hepatic lipocytes: regulation by Kupffer cell-conditioned medium and PDGF. Am J Physiol 1993;264:G947-52.
6. Tsukamoto H, Cheng S, Blaner WS. Effects of dietary polyunsaturated fat on ethanol-induced Ito cell activation. Am J Physiol 1996;270:G581-6.
7. Schmitt-Graff A, Kruger S, Bochard F, Gabbiani G, Denk H. Modulation of alpha smooth muscle actin and desmin expression in perisinusoidal cells of normal and diseased human livers. Am J Pathol 1991;138:1233-42.
8. Yamaoka K, Nouchi T, Marumo F, Sato C. Alpha-smooth-muscle actin expression in normal and fibrotic human livers. Dig Dis Sci 1993;38:1473-9.
9. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 2000;275:2247-50.
10. Kinnman N, Francoz C, Barbu V, Wendum D, Rey C, Hultcrantz R, Poupon R, Housset C. The myofibroblastic conversion of peribiliary fibrogenic cells distinct from hepatic stellate cells is stimulated by platelet-derived growth factor during liver fibrogenesis. Lab Invest 2003;83:163-73.
11. Guyot C, Lepreux S, Combe C, Doudnikoff E, Bioulac-Sage P, Balabaud C, Desmouliere A. Hepatic fibrosis and cirrhosis: the (myo)fibroblastic cell subpopulations involved. Int J Biochem Cell Biol 2006;38:135-51.
12. Burt AD, Robertson JL, Heir J, MacSween RN. Desmin-containing stellate cells in rat liver; distribution in normal animals and response to experimental acute liver injury. J Pathol 1986;150:29-35.
13. Neubauer K, Knittel T, Aurisch S, Fellmer P, Ramadori G. Glial fibrillary acidic protein--a cell type specific marker for Ito cells in vivo and in vitro. J Hepatol 1996;24:719-30.
14. Niki T, Pekny M, Hellemans K, Bleser PD, Berg KV, Vaeyens F, Quartier E, Schuit F, Geerts A. Class VI intermediate filament protein nestin is induced during activation of rat hepatic stellate cells. Hepatology 1999;29:520-7.
15. Knittel T, Aurisch S, Neubauer K, Eichhorst S, Ramadori G. Cell-type-specific expression of neural cell adhesion molecule (N-CAM) in Ito cells of rat liver. Up-regulation during in vitro activation and in hepatic tissue repair. Am J Pathol 1996;149:449-62.
16. Cassiman D, van Pelt J, De Vos R, Van Lommel F, Desmet V, Yap SH, Roskams T. Synaptophysin: A novel marker for human and rat hepatic stellate cells. Am J Pathol 1999;155:1831-9.
17. Cassiman D, Barlow A, Vander Borght S, Libbrecht L, Pachnis V. Hepatic stellate cells do not derive from the neural crest. J Hepatol 2006;44:1098-104.

18. Baba S, Fujii H, Hirose T, Yasuchika K, Azuma H, Hoppo T, Naito M, Machimoto T, Ikai I. Commitment of bone marrow cells to hepatic stellate cells in mouse. J Hepatol 2004;40:255-60.

19. Geerts A. On the origin of stellate cells: mesodermal, endodermal or neuro-ectodermal? J Hepatol 2004;40:331-4.

20. Duncan SA. Mechanisms controlling early development of the liver. Mech Dev 2003;120:19-33.

21. Matsumoto K, Yoshitomi H, Rossant J, Zaret KS. Liver organogenesis promoted by endothelial cells prior to vascular function. Science 2001;294:559-63.

22. Knook DL, Seffelaar AM, de Leeuw AM. Fat-storing cells of the rat liver. Their isolation and purification. Exp Cell Res 1982;139:468-71.

23. Geerts A, Vrijsen R, Rauterberg J, Burt A, Schellinck P, Wisse E. In vitro differentiation of fat-storing cells parallels marked increase of collagen synthesis and secretion. J Hepatol 1989;9:59-68.

24. Blomhoff R, Berg T. Isolation and cultivation of rat liver stellate cells. Methods Enzymol 1990;190:58-71.

25. Greenwel P, Schwartz M, Rosas M, Peyrol S, Grimaud JA, Rojkind M. Characterization of fat-storing cell lines derived from normal and CCl4-cirrhotic livers. Differences in the production of interleukin-6. Lab Invest 1991;65:644-53.

26. Friedman SL, Rockey DC, McGuire RF, Maher JJ, Boyles JK, Yamasaki G. Isolated hepatic lipocytes and Kupffer cells from normal human liver: morphological and functional characteristics in primary culture. Hepatology 1992;15:234-43.

27. Bibel M, Barde YA. Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. Genes Dev 2000;14:2919-37.

28. Campagnolo L, Russo MA, Puglianiello A, Favale A, Siracusa G. Mesenchymal cell precursors of peritubular smooth muscle cells of the mouse testis can be identified by the presence of the p75 neurotrophin receptor. Biol Reprod 2001;64:464-72.

29. Cassiman D, Denef C, Desmet VJ, Roskams T. Human and rat hepatic stellate cells express neurotrophins and neurotrophin receptors. Hepatology 2001;33:148-58.

30. Trim N, Morgan S, Evans M, Issa R, Fine D, Afford S, Wilkins B, Iredale J. Hepatic stellate cells express the low affinity nerve growth factor receptor p75 and undergo apoptosis in response to nerve growth factor stimulation. Am J Pathol 2000;156:1235-43.

31. Oakley F, Trim N, Constandinou CM, Ye W, Gray AM, Frantz G, Hillan K, Kendall T, Benyon RC, Mann DA, Iredale JP. Hepatocytes express nerve growth factor during liver injury: evidence for paracrine regulation of hepatic stellate cell apoptosis. Am J Pathol 2003;163:1849-58.

32. Asai K, Tamakawa S, Yamamoto M, Yoshie M, Tokusashi Y, Yaginuma Y, Kasai S, Ogawa K. Activated hepatic stellate cells overexpress p75NTR after partial hepatectomy and undergo apoptosis on nerve growth factor stimulation. Liver Int 2006;26:595-603.

33. Passino MA, Adams RA, Sikorski SL, Akassoglou K. Regulation of hepatic stellate cell differentiation by the neurotrophin receptor p75NTR. Science 2007;315:1853-6.

34. Geerts A. The simple truth is seldom true and never simple: dual role for p75(NTR) in transdifferentation and cell death of hepatic stellate cells. Hepatology 2007;46:600-1.

35. Tanimizu N, Nishikawa M, Saito H, Tsujimura T, Miyajima A. Isolation of hepatoblasts based on the expression of Dlk/Pref-1. J Cell Sci 2003;116:1775-86.

36. Nonaka H, Tanaka M, Suzuki K, Miyajima A. Development of murine hepatic sinusoidal endothelial cells characterized by the expression of hyaluronan receptors. Dev Dyn 2007;236:2258-67.

37. Lemaigre FP. Development of the biliary tract. Mech Dev 2003;120:81-7.

38. Li L, Krantz ID, Deng Y, Genin A, Banta AB, Collins CC, Qi M, Trask BJ, Kuo WL, Cochran J, Costa T, Pierpont ME, Rand EB, Piccoli DA, Hood L, Spinner NB. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. Nat Genet 1997;16:243-51.

39. Oda T, Elkahloun AG, Pike BL, Okajima K, Krantz ID, Genin A, Piccoli DA, Meltzer PS, Spinner NB, Collins FS, Chandrasekharappa SC. Mutations in the human Jagged1 gene are responsible for Alagille syndrome. Nat Genet 1997;16:235-42.

40. McCright B, Lozier J, Gridley T. A mouse model of Alagille syndrome: Notch2 as a genetic modifier of Jag1 haploinsufficiency. Development 2002;129:1075-82.

41. Tanimizu N, Miyajima A. Notch signaling controls hepatoblast differentiation by altering the expression of liver-enriched transcription factors. J Cell Sci 2004;117:3165-74.

42. Enzan H, Himeno H, Hiroi M, Kiyoku H, Saibara T, Onishi S. Development of hepatic sinusoidal structure with special reference to the Ito cells. Microsc Res Tech 1997;39:336-49.

43. Sasaki K, Sonoda Y. Histometrical and three-dimensional analyses of liver hematopoiesis in the mouse embryo. Arch Histol Cytol 2000;63:137-46.

44. Shiojiri N. Development and differentiation of bile ducts in the mammalian liver. Microsc Res Tech 1997;39:328-35.

45. Clotman F, Jacquemin P, Plumb-Rudewiez N, Pierreux CE, Van der Smissen P, Dietz HC, Courtoy PJ, Rousseau GG, Lemaigre FP. Control of liver cell fate decision by a gradient of TGF beta signaling modulated by Onecut transcription factors. Genes Dev 2005;19:1849-54.

46. Kamiya A, Kinoshita T, Ito Y, Matsui T, Morikawa Y, Senba E, Nakashima K, Taga T, Yoshida K, Kishimoto T, Miyajima A. Fetal liver development requires a paracrine action of oncostatin M through the gp130 signal transducer. Embo J 1999;18:2127-36.

47. Hara T, Nakano Y, Tanaka M, Tamura K, Sekiguchi T, Minehata K, Copeland NG, Jenkins NA, Okabe M, Kogo H, Mukouyama Y, Miyajima A. Identification of podocalyxin-like protein 1 as a novel cell surface marker for hemangioblasts in the murine aorta-gonad-mesonephros region. Immunity 1999;11:567-78.

48. Kitamura T, Onishi M, Kinoshita S, Shibuya A, Miyajima A, Nolan GP. Efficient screening of retroviral cDNA expression libraries. Proc Natl Acad Sci U S A 1995;92:9146-50.

49. Chida D, Miura O, Yoshimura A, Miyajima A. Role of cytokine signaling molecules in erythroid differentiation of mouse fetal liver hematopoietic cells: functional analysis of signaling molecules by retrovirus-mediated expression. Blood 1999;93:1567-78.

50. Nakamura K, Nonaka H, Saito H, Tanaka M, Miyajima A. Hepatocyte proliferation and tissue remodeling is impaired after liver injury in oncostatin M receptor knockout mice. Hepatology 2004;39:635-44.

51. Miyaoka Y, Tanaka M, Naiki T, Miyajima A. Oncostatin M inhibits adipogenesis through the RAS/ERK and STAT5 signaling pathways. J Biol Chem 2006;281:37913-20.

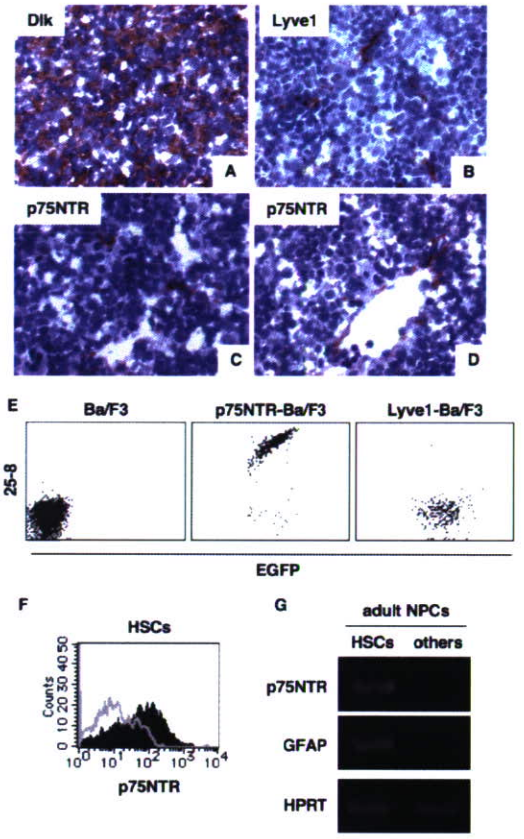


Fig.1 Suzuki et.al

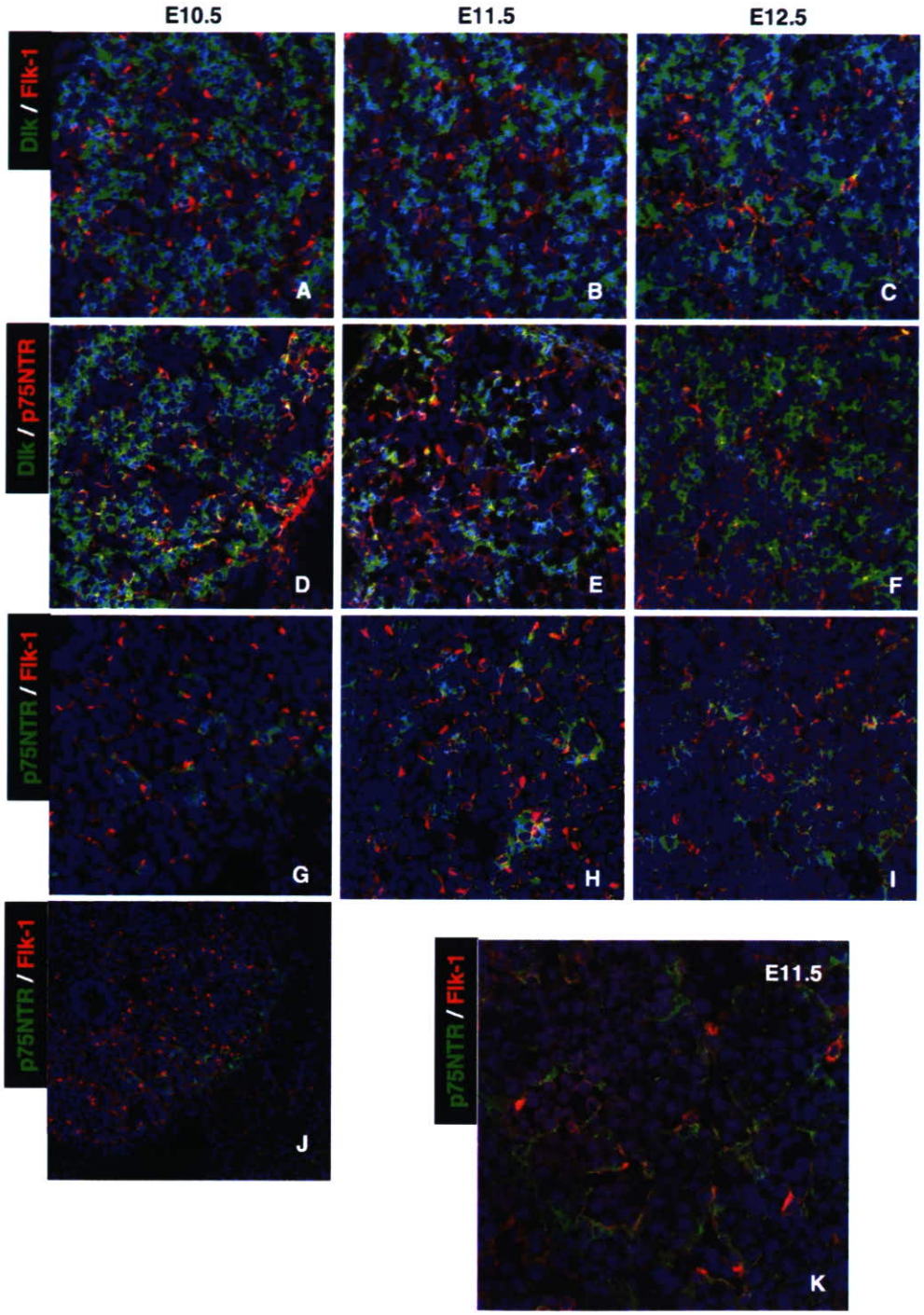
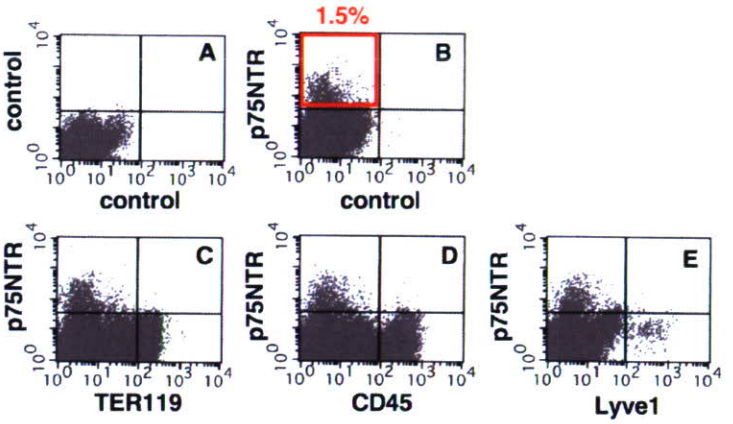


Fig.2 Suzuki et.al



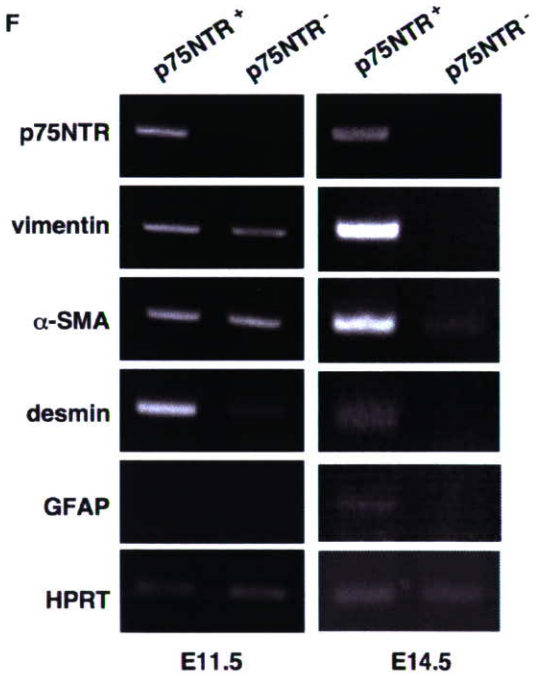


Fig.3 Suzuki et.al

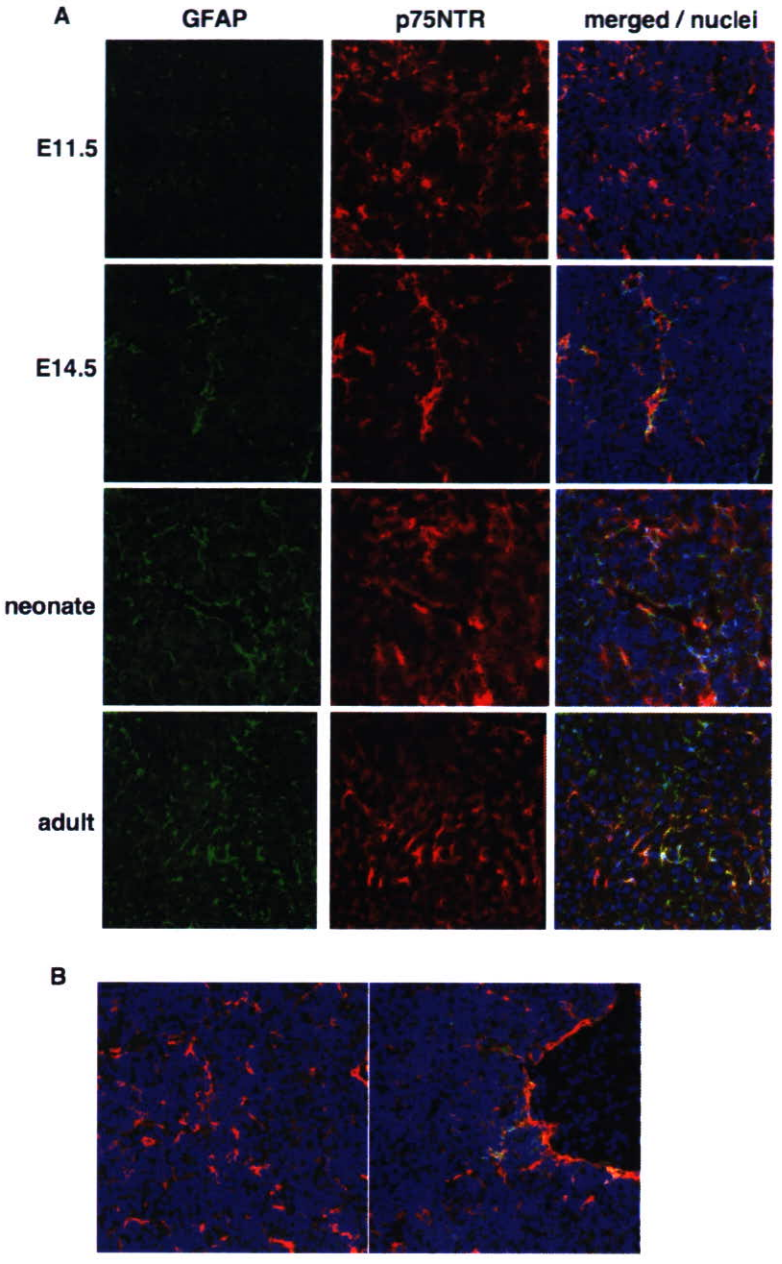
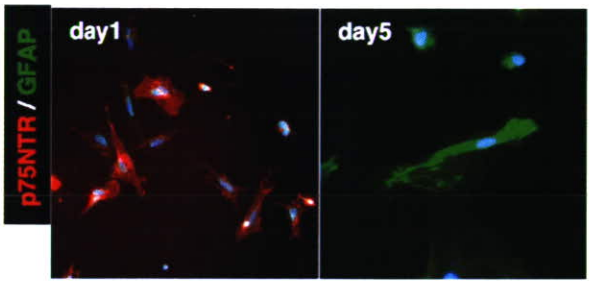


Fig.4 Suzuki et.al

C



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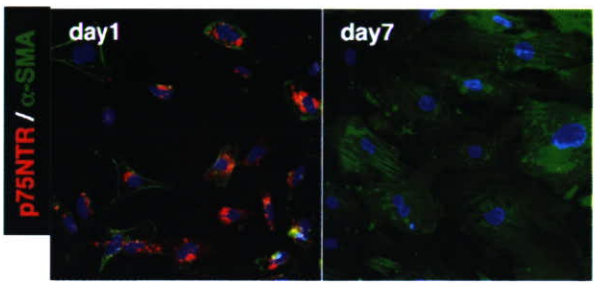
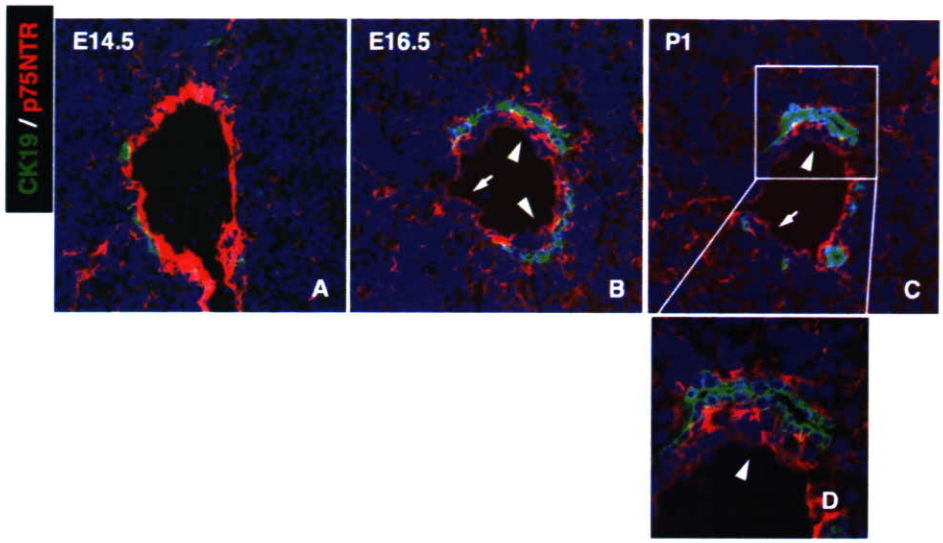
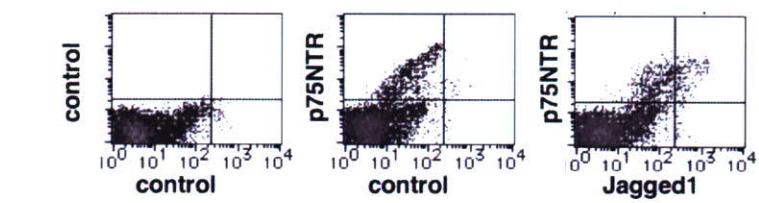


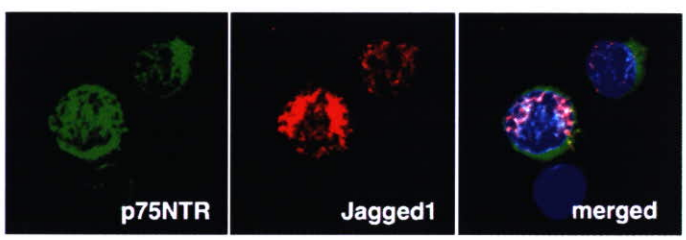
E

	E11.5	E14.5	E18.5	neonate
ratio of lipid-containing cells	3.1 %	11.8 %	32.8 %	27.1 %

Fig.4 Suzuki et.al







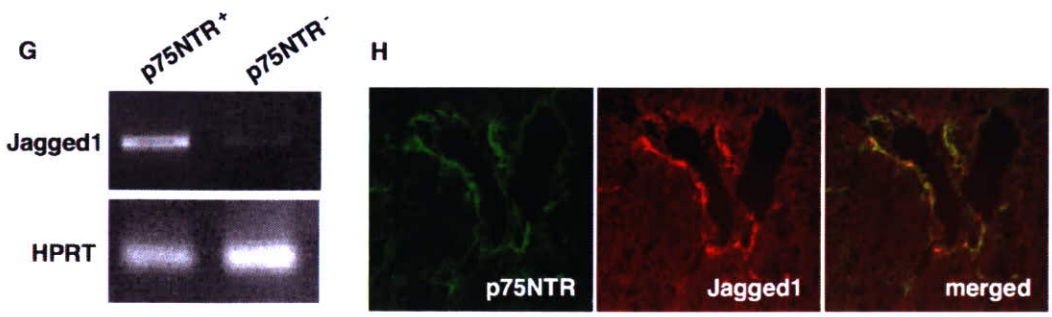
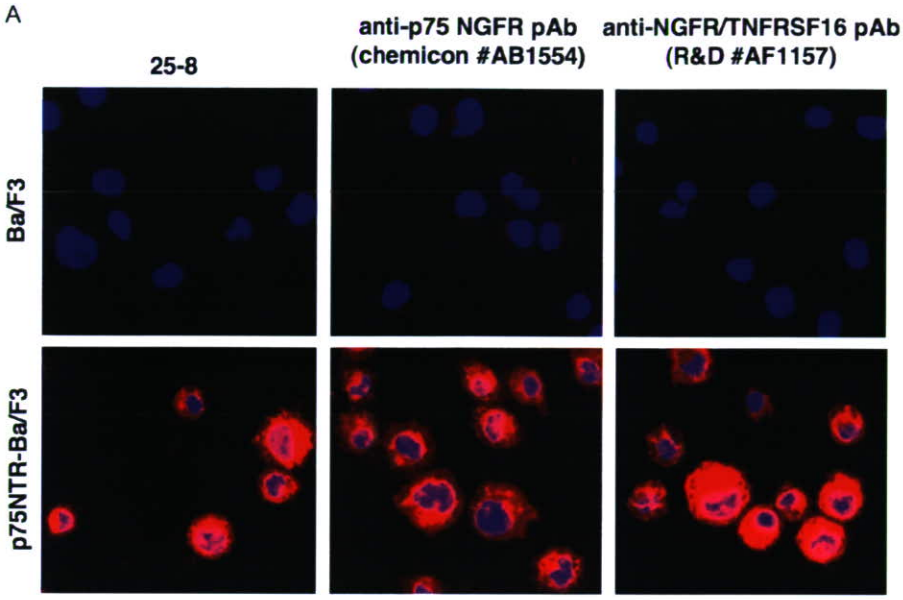
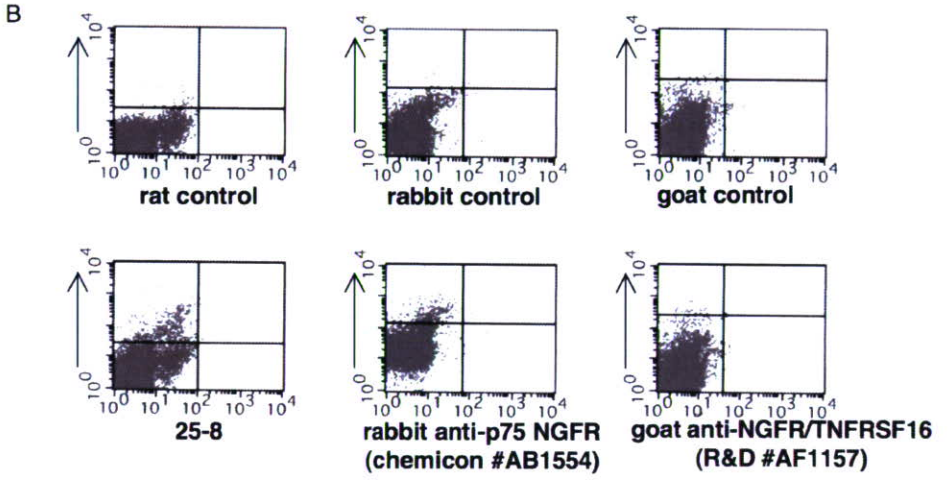
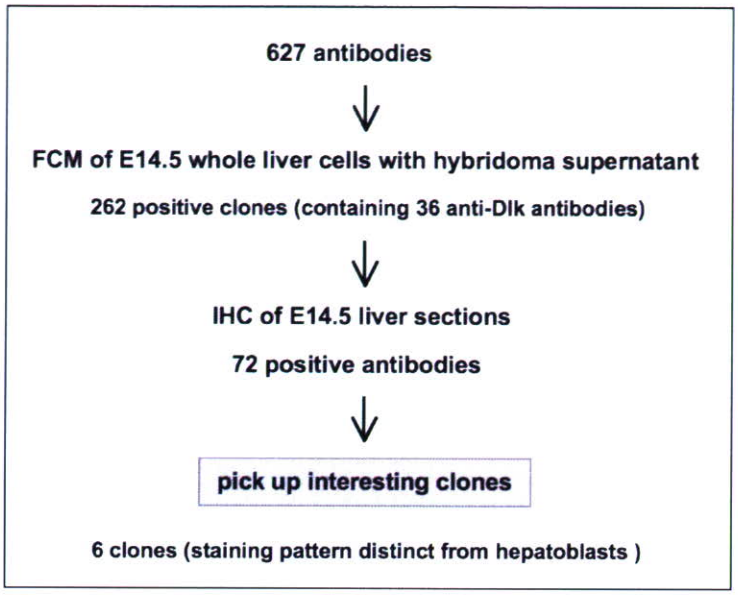


Fig.5 Suzuki et.al



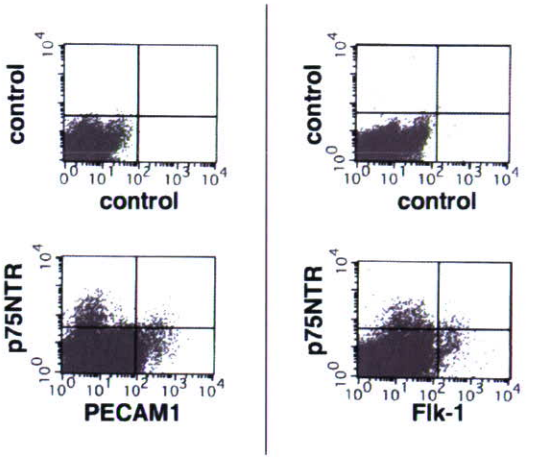


Supplemental Figure 1 Suzuki et.al

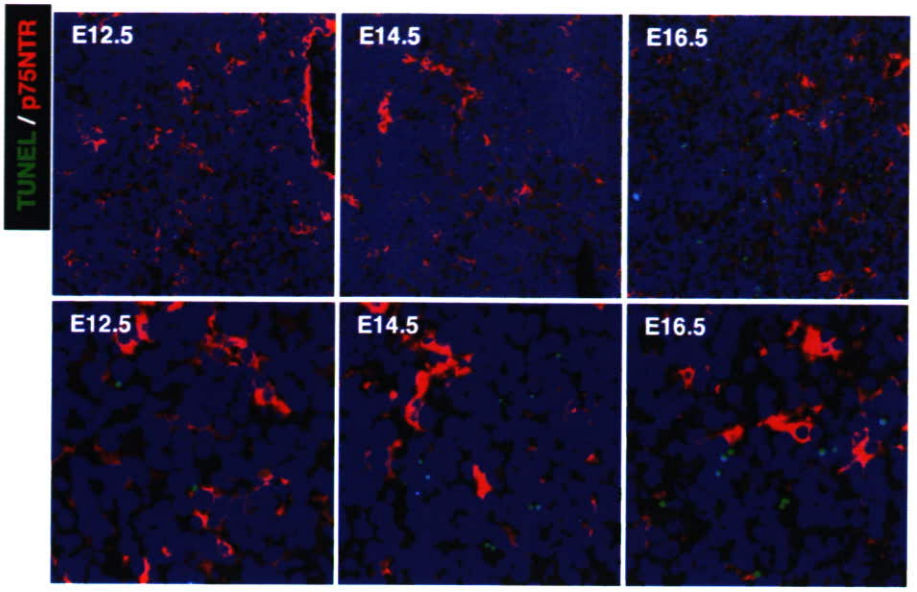


Flow chart for screening of antibodies

Supplemental Figure 2 Suzuki et.al



Supplemental Figure 3 Suzuki et.al



Supplemental Figure 4 Suzuki et.al