Table W1. Primers Used in This Study.

Gene	Purpose	Direction	Sequence
Human β-actin	RT-PCR	Forward	5′-TGGCACCCAGCACAATGAA
		Backward	5′-CTAAGTCATAGTCCGCCTAGAAGCA
Human Hes1	RT-PCR	Forward	5′-TGGAAATGACAGTGAAGCACCTC
		Backward	5′-TCGTTCATGCACTCGCTGAAG
Human Hey1	RT-PCR	Forward	5′-CATGAAGAGAGCTCACCCAGA
		Backward	5′-CGCCGAACTCAAGTTTCC
Mouse β-actin	RT-PCR	Forward	5′-CATCCGTAAAGACCTCTATGCCAAC
		Backward	5′-ATGGAGCCACCGATCCACA
Mouse Hes1	RT-PCR	Forward	5′-AAAGACGGCCTCTGAGCAC
		Backward	5′-GGTGCTTCACAGTCATTTCCA
Mouse VEGFR1	RT-PCR	Forward	5′-TAATGACGATGGCAACAGGGTAGA
		Backward	5′-TGTGCACGACCTAAGCACACAG
Mouse VEGFR2	RT-PCR	Forward	5′-GGGATGGTCCTTGCATCAGAA
		Backward	5′-ACTGGTAGCCACTGGTCTGGTTG
hD1S	Cloning	Forward	5′-CGCCATGGTCCACACAGATTCTCCTG
		Backward	5′-CGCTCGAGGATCGGCTCTGTGCAGTAG
hD1D	Cloning	Forward	5′-CGCCATGGTCCACACAGATTCTCCTG
		Backward	5′-CGCTCGAGTTAGTATCTAACGCCGCATCTGCCATCGCAGATCGGCTCTGTGCAGTAG
hD1R	Cloning	Forward	5′-CGCCATGGTCCACACAGATTCTCCTG
		Backward	5′-CGCTCGAGTTAGTATCTAACGCCGCAATCGCCTCTGCAGATCGGCTCTGTGCAGTAG

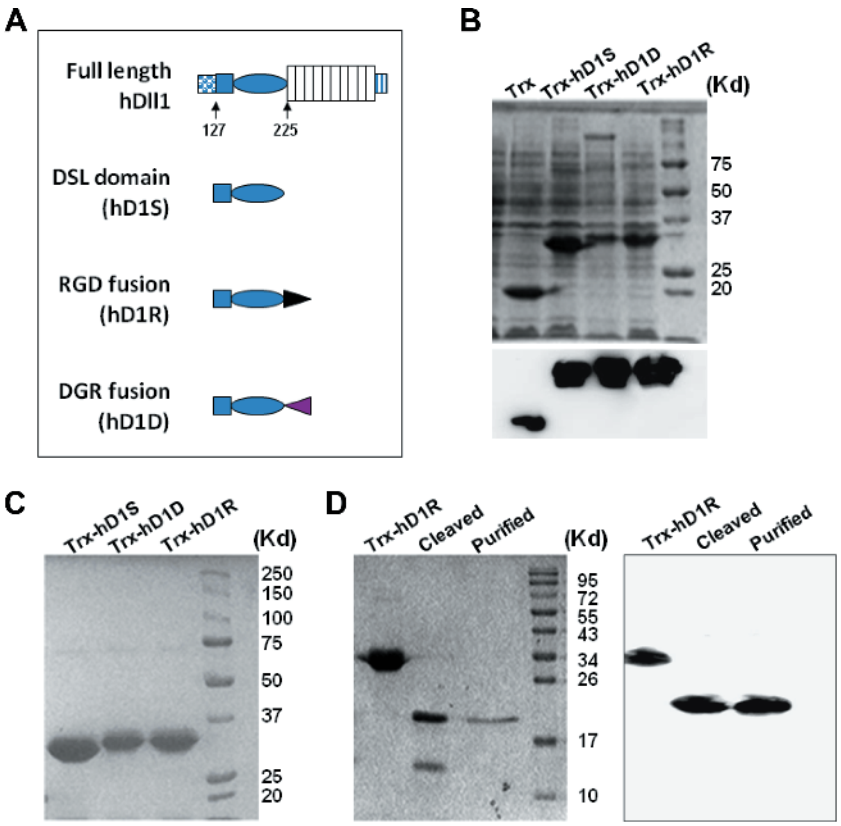

Figure W1. Expression of hD1R, an EC-targeted DSL domain of hDll1. (A) Schematic illustrations of the full-length hDll1, the DSL domain of hDll1 (hD1S), hDll1 fused with the RGD peptide (hD1R), and hDll1 fused with the DGR peptide (hD1D). (B) *E. coli* (BL21) was transformed with pET32(a), pET32a-hD1S, pET32a-hD1D, or pET32a-hD1R. Positive transformants were induced with isopropyl β-D-thiogalactoside. Total cell lysates were analyzed by SDS-PAGE (upper). The gel was blotted onto PVDF membrane and probed with the anti-His antibody (lower). (C) The recombinant Trx-hD1S, Trx-hD1D, and Trx-hD1R proteins were purified with Ni^{+}-NTA columns and analyzed by using SDS-PAGE. (D) Trx-hD1R was cleaved with thrombin and purified by using an Ni^{+}-NTA column. The eluent was analyzed by SDS-PAGE (left), followed by Western blot by using anti–S-Tag.

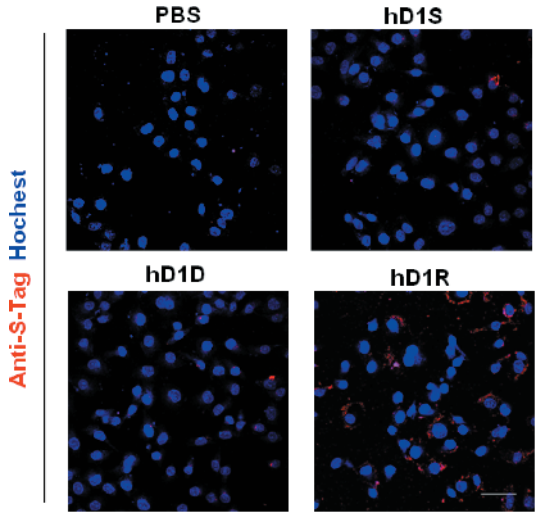

Figure W2. hD1R could efficiently bind to ECs. HUVECs were incubated with PBS, hD1S, hD1D, or hD1R for 2 hours. Cells were washed with PBS and fixed with 4% paraformaldehyde. Samples were then stained with anti–S-Tag plus Cy3-conjugated secondary antibody and observed under a confocal microscope.

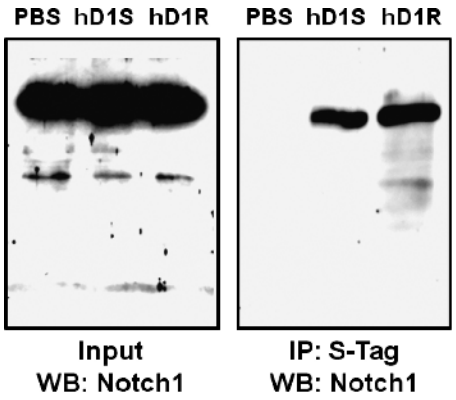

Figure W4. hD1S and hD1R could interact with the extracellular domain of Notch1 receptor. HUVECs were lysed with cell lysis buffer, and the supernatants were incubated with PBS, hD1S, or hD1R. Immunoprecipitation was performed with anti–S-Tag, and the presence of Notch1 extracellular domain was detected by immunoblot analysis with anti-Notch1. For methodology, HUVECs were washed once with ice-cold PBS and lysed in IP buffer (Beyotime, Haimen, China). Cell lysates were incubated with PBS, S-tagged hD1S, or S-tagged hD1R for 2 hours at 4°C and then incubated with protein G-Sepharose beads (Invitrogen) precoated with anti–S-Tag antibody (1:200; Abcam) overnight. The beads were washed extensively, and co-precipitated proteins were detected by immunoblot analysis with an anti-Notch1 (Santa Cruz Biotechnology, Inc) as the primary antibody.

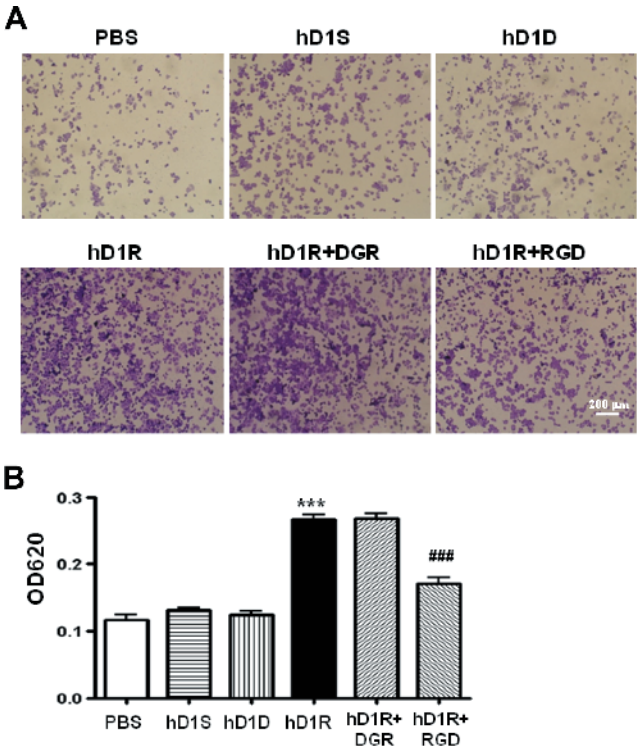

Figure W3. hD1R bound to ECs through the RGD motif. (A) Culture dishes were coated with PBS, hD1S, hD1D, or hD1R. HUVECs were distributed into different wells and incubated for 1.5 hours. In some wells, synthetic DGR or RGD nonapeptide was included. After incubation, wells were washed with medium and nonadherent cells were discarded. Adherent cells were stained with crystal violet and photographed. (B) Cells in A were lysed, and A620 in the supernatants was detected and compared between different groups. Bars, means ± SD. $^{***, ###}P < .001$, $n = 4$.

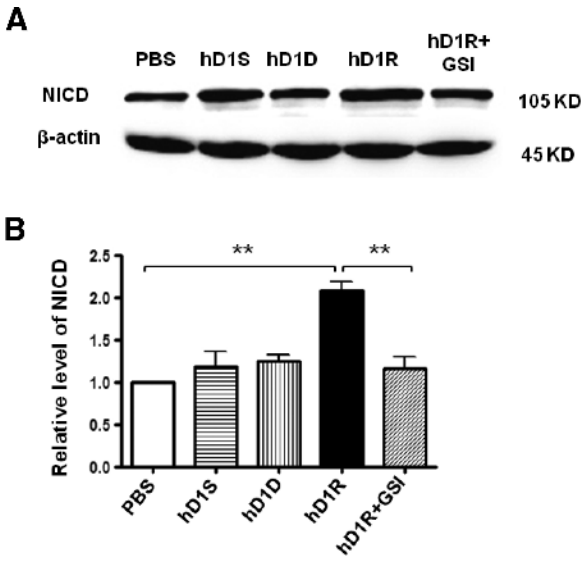

Figure W5. Western blot. (A) HUVECs were incubated with PBS, hD1S, hD1D, or hD1R for 24 hours. GSI was included in some of the cultures as indicated. Total cell lysates were prepared and analyzed by SDS-PAGE. The gel was blotted onto PVDF membrane and probed with the anti-NICD antibody, with β-actin as an internal control. (B) Each band was quantified by grayscale scanning, and the relative level of NICD was compared. Bars, means ± SD. $^{**}P < .01$, $n = 3$.

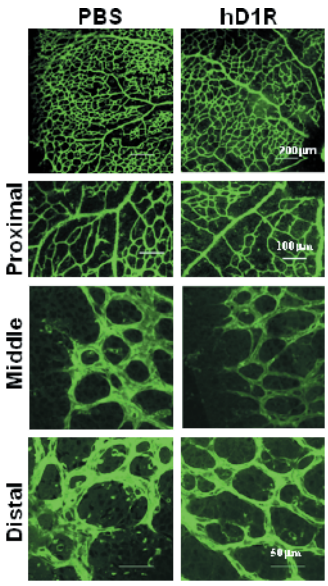

Figure W6. hD1R repressed angiogenesis *in vivo*. P3 pups were injected daily s.c. with PBS or hD1R. On P7, the retinas of the pups were collected, flat-mounted, and stained with fluorescein-labeled *Griffonia simplicifolia* Lectin I. The structures of the whole retinal vasculature and the proximal, middle, and distal areas were shown.

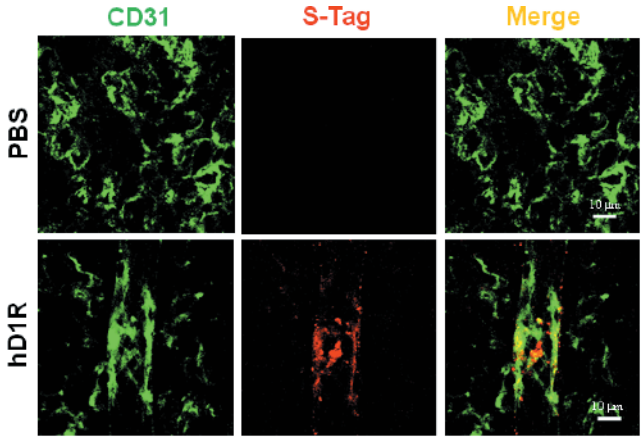

Figure W7. hD1R bound to tumor vessels *in vivo*. Nude mice were inoculated s.c. with U87 glioma cells (5×10^6) on the back. Mice were injected i.p. with PBS or hD1R twice a week from the 7th day of inoculation. At the ending day of the experiments, the tumors were dissected 30 minutes after the last injection, sectioned, and stained by using anti-CD31 (green) and anti–S-Tag (red).

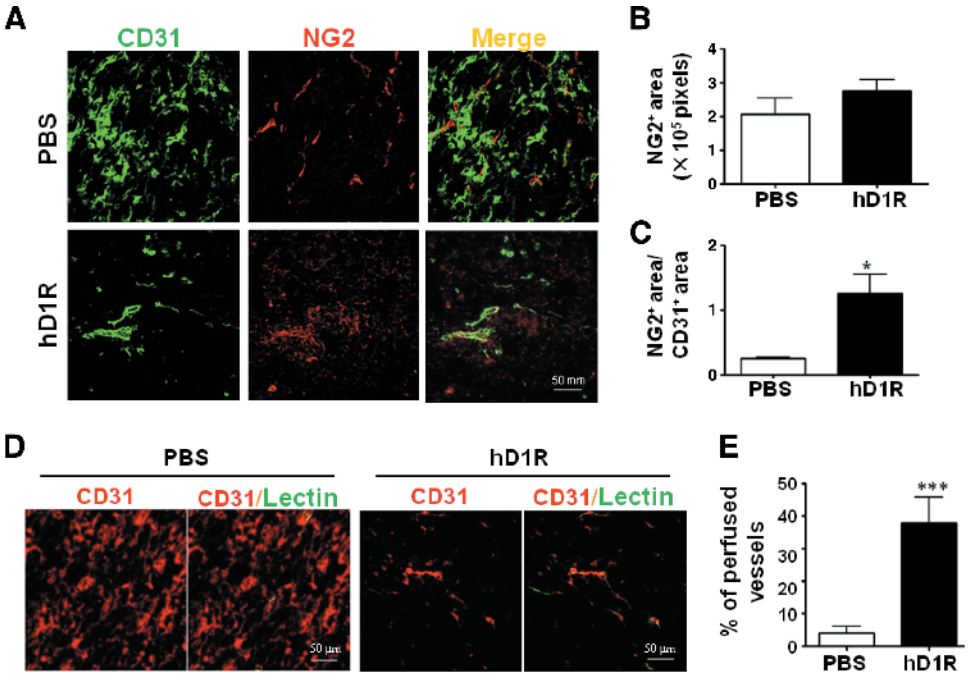

Figure W8. hD1R enhanced the recruitment of perivascular cells. LLC cells were inoculated s.c. in nude mice, and the mice were treated with PBS or hD1R. (A–C) Tumors were stained with anti-CD31 (green) and anti-NG2 (red). The $NG2^+$ areas (B) and the ratio of $NG2^+$ areas *versus* $CD31^+$ areas (C) were compared. (D, E) On the last day of the experiments, mice were injected with lectin (green) through the tail vein. Tumors were dissected 30 minutes after the injection and were stained with anti-CD31 (red). The percentage of the perfused vessels (lectin and CD31 double positive) was counted and compared (E). Bars, means ± SD. $^*P < .05$, $^{***}P < .001$, $n = 8$.

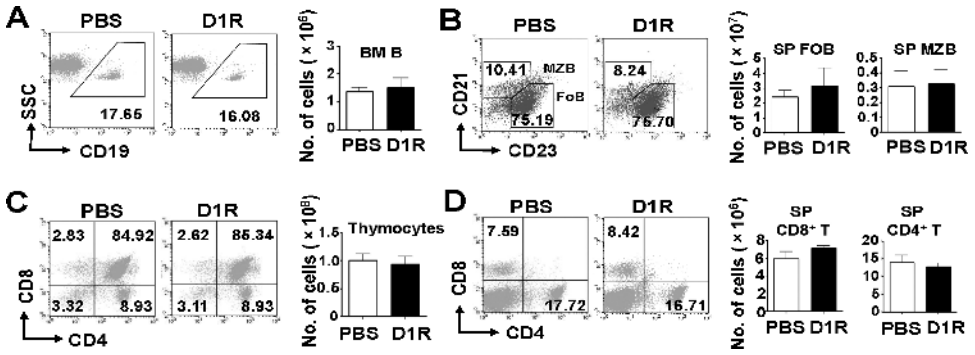

Figure W9. mD1R did not affect lymphocyte differentiation and homeostasis. Mice were injected i.p. with PBS or mD1R twice a week for 4 weeks. On day 28 after treatment, lymphoid organs were collected. Single-cell suspensions were prepared and analyzed by flow cytometry with the indicated staining settings. (A) Bone marrow (BM) B cell analysis shown by representative $CD19^+$ B cell FACS plots along with cell numbers on the side. (B) Spleen follicular (Fo) B and marginal zone (MZ) B cell distribution displayed by B220CD21CD23 profiles of splenocytes. (C) Thymocyte subset analysis shown by CD4CD8 profiles of thymocytes. (D) Spleen T cell subset analysis presented by CD4CD8 profiles of splenocytes. Bars, mean ± S.D. $n = 5$.