SUPPLEMENTARY INFORMATION

Fig. S1. Suppression of Asef and APC expression by siRNAs. *A.* HAECs were transfected with Asef- and APC-specific or control siRNAs, respectively. Lysates prepared from transfected cells were subjected to immunoblot analysis with antibodies against Asef and APC, respectively. Anti-α-tubulin antibody was used as a control. *B.* HAECs transfected with the indicated siRNAs were stained with antibody against APC (green). Nuclei were counterstained with TO-PRO3 (blue).

Fig. S2. Effects of Asef-ΔDH on cell migration of HAECs. HAECs infected with the indicated adenoviruses were added to the upper compartment of the Transwell chamber and allowed to migrate to the underside of the top chamber for 4.5 h in the presence or absence of bFGF or VEGF, respectively. Cell migration was determined by counting the cells that had migrated to the lower side of the polycarbonate filters. Infection efficiency was more than 90%. Results are expressed as the means ± SEM of at least three independent experiments. * $P<0.05$.

Fig. S3. Effects of β-catenin S33Y on migration of HAECs. *A.* Expression of adenoviral β-catenin S33Y in HAECs. Lysates prepared from infected cells were analysed by immunoblot with antibody against FLAG. An adenovirus encoding LacZ was used as a negative control. *B.* Cells were infected with the indicated adenoviruses, and subjected to migration assays using Transwell migration chambers. Cells were allowed to migrate for 4.5 h in the presence or absence of bFGF or VEGF, respectively. Results are expressed as the means ± SEM of three independent experiments. * $P<0.05$.

Fig. S4. Immunohistochemistry of Matrigel plugs from Asef+/+ and Asef-/- mice. *A.* Cross-sections of Matrigel plugs were double-stained with antibobies against Ki67 (red) and CD31 (green). *B.* Cross-sections of Matrigel plugs were stained with TUNEL reagents (green) and antiboby to CD31 (red).

Fig. S5. Immunohistochemistry of subcutaneous melanoma tumors from Asef+/+ and Asef-/- mice. *A-B.* Tumor cross-sections were stained with anti-F4/80 antibody (brown) and counterstained with nuclear fast red. Arrows indicate F4/80-positive macrophages. Scale bar, 50 μm. The number of macrophages within tumors were counted (B). Results are expressed as the mean ± SEM. *C.* Absence of Asef expression in Asef-/- macrophages. RT-PCR analysis was performed on total RNA samples from macrophages of Asef+/+ and Asef-/- mice using primers located in exons 2 and 5. The 484-bp fragment represents the full-length transcript. Expression of GAPDH was examined as an internal control. *D.* Tumor cross-sections were stained with anti-VEGF antibody (brown) and counterstained with hematoxylin.

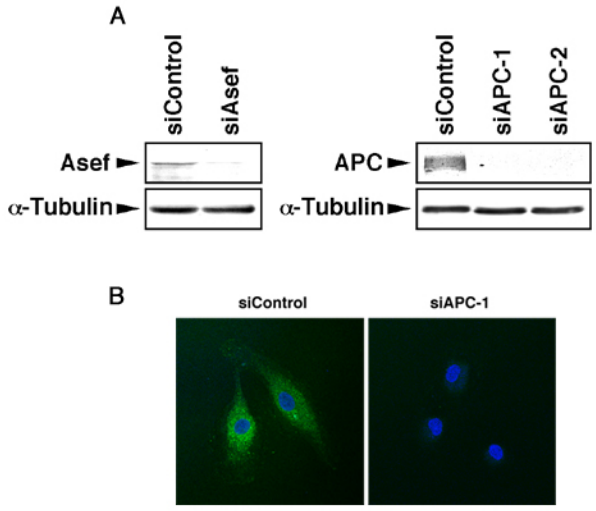
A
siControl
siAsef
siControl
siAPC-1
siAPC-2
Asef
APC
α-Tubulin
α-Tubulin
B
siControl
siAPC-1

Fig. S1.

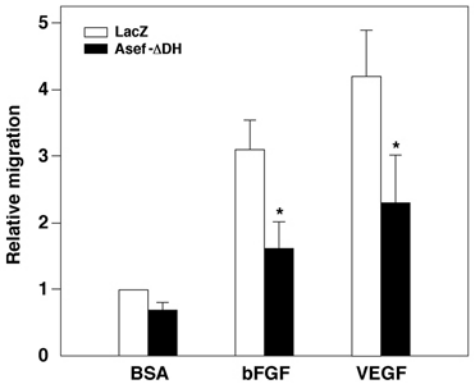

Fig. S2.

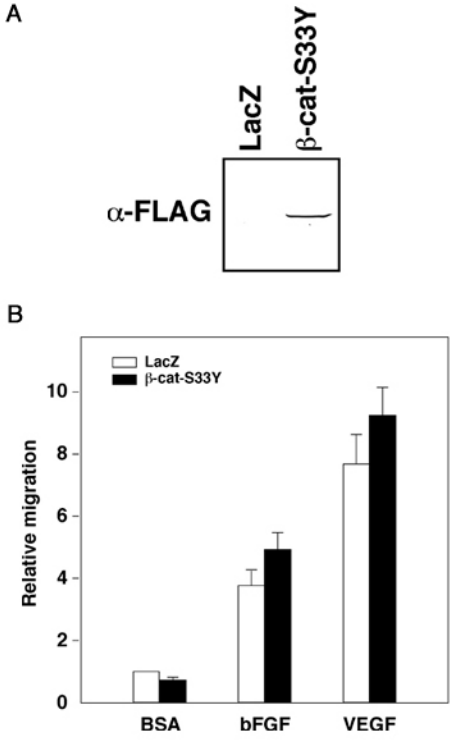
A
LacZ
β-cat-S33Y
α-FLAG
B
LacZ
β-cat-S33Y
Relative migration
0
2
4
6
8
10
BSA
bFGF
VEGF

Fig. S3.

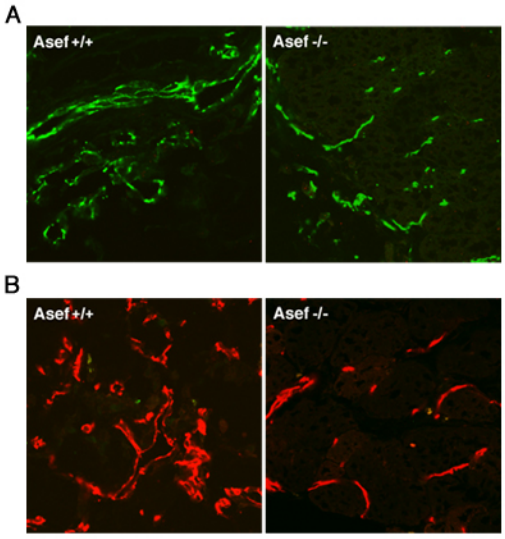

Fig. S4.

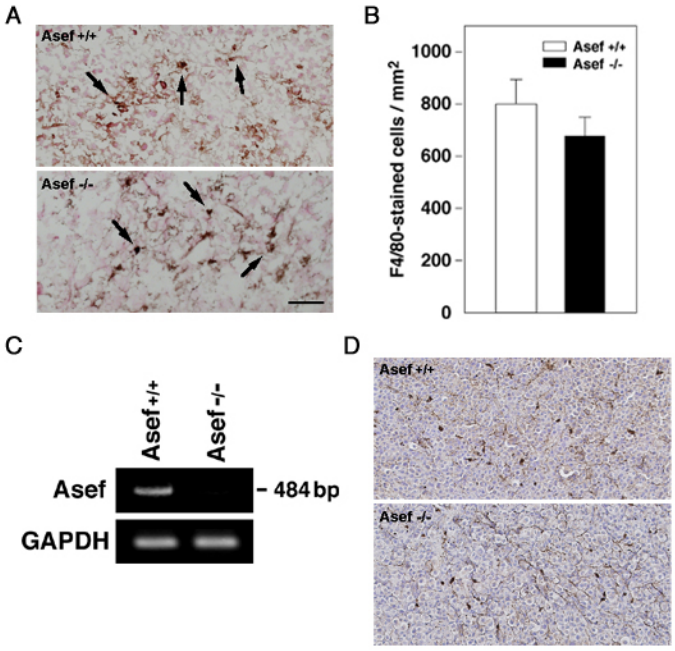
A
Asef +/+
Asef -/-
B
Asef +/+
Asef -/-
F4/80-stained cells / mm2
1000
800
600
400
200
0
C
Asef +/+
Asef -/-
Asef
GAPDH
484 bp
D
Asef +/+
Asef -/-

Fig. S5.