SUPPLEMENTARY INFORMATION

MATERIALS AND METHODS

Generation of recombinant AdsFlt-4. cDNA fragment encoding sFlt-4 was subcloned between the cytomegalovirus immediate early promoter and the rabbit β-globin intron/polyadenylation sequences. The sFlt-4 construct and linearized adenoviral backbone containing the cytomegalovirus early promoter and the rabbit β-globin intron/polyadenylation sequences for homologous recombination were transformed into recombination competent E. coli BJ5183, colonies containing recombinant plasmids were isolated, and plasmid DNA was used for transformation of E. coli DH5α. The genome of the recombined virus (AdsFlt-4) was confirmed by sequencing, and virus cultures were initiated by polyethyleneimine transfection (1) of the linearized AdsFlt-4 genome into 293//TetR/GFP cells (2). After amplification, virus was purified by banding twice on CsCl gradients, transferred into HEPES-buffered saline (HBS)/40% glycerol by passage over a Nick Column Sephadex G-50 gel filtration column (Amersham Biosciences, New Jersey, USA) and stored at –80°C.

REFERENCES

FIGURE LEGENDS

Fig. S1. Tumor tissue disaggregation and lymph node metastasis in Rip1Tag2;NCAM-deficient mice. Hematoxylin & eosin staining of pancreata from Rip1Tag2 (A), Rip1Tag2;NCAM-/-(B) and Rip1Tag2;NCAM+/- (C, D) mice, as indicated. Hemorrhagic lacunae of Rip1Tag2;NCAM-/- and Rip1Tag2;NCAM+/- mice contain floating tumor cell clusters (arrows in B) and disseminating tumor cells (arrow in C). Lymph node metastasis in a Rip1Tag2;NCAM+/- mouse (D). MET, metastasis; LN, lymph node. Scale bar, 50 µm.

Fig. S2. Anaplastic phenotype of a lymph node metastasis of a Rip1Tag2;NCAM+/- mouse injected with AdsFlt-4. Immunohistochemical staining of tissue sections with antibodies against insulin reveals rare insulin-expressing cells (brown staining) within the primary tumor (B) and a lymph node metastasis (A) of an AdsFlt-4-treated mouse. MET, metastasis; LN, lymph node; T, tumor. Scale bar, 100 µm.