Supplemental data for Lyck et al.

**Generation of the ICAM-1 mutants: Detailed description of the cloning strategy:** The cytoplasmic deletion mutant pICAM-1(M1) was generated by introducing a stop codon into the reading frame of the cDNA for murine ICAM-1 by PCR using the oligonucleotides RL01 and RL04. The resulting PCR fragment was cloned *Eco RI/Sal I* into pBluescript. In order to reduce the fragment size generated by PCR a *Spe I/Sal I* insert from pBluescript was cloned into pBABEpuro (*Spe I/Sal I*) to obtain a shuttle construct (pRL202). Finally, a *Hind III* fragment from pBABEpuro-ICAM-1 was substituted for the *Hind III* fragment of pRL202. The ICAM-1 mutant M2 where the tyrosines 507 and 509 were replaced by phenylalanines was derived by PCR mutagenesis using the oligonucleotides RL02, RL04 and RL06. The PCR fragment was cloned into *Hind III/Sal I* cut pBluescript. To complete the ICAM-1 cDNA and to reduce the PCR fragment size a *Eco RI/Nco I* insert from pICAM-1(M1) was substituted for the corresponding insert of the construct created resulting in pBluescriptICAM-1(M2). Full length mutated ICAM-1 was shifted into pBABEpuro *Eco RI/Sal I* to give the final pICAM-1(M2) cDNA construct. PCR mutagenesis to obtain pICAM-1(M3) was performed using the oligonucleotides RL03, RL04 and RL06. The PCR fragment was cloned into pBluescript *Hind III/Sal I*. To complete the ICAM-1 cDNA and to minimize the PCR fragment size a *Eco RI/Nco I* insert from pICAM-1(M1) was substituted for the corresponding insert of pBluescript resulting in pICAM-1(M3). Full length mutated ICAM-1 was shifted I into pBABEpuro *Eco RI/Sal I* to give the final pICAM-1(M3) construct. In order to obtain pICAM-1(M4) the *Eco RI/Sna BI* insert from pBluescript ICAM-1(M2) was ligated with the *Sna BI/Sal I* insert from pBluescript ICAM-1 (M3) and cloned into pBluescript (*Eco RI/ Sal I*). Full length mutated ICAM-1 was shifted into pBABEpuro *Eco RI/Sal I* to give the final pICAM-1(M4) retroviral construct. To
obtain pICAM-1(M5), a PCR fragment was generated using the oligonucleotides RL11 and RL13 and pBluescript-ICAM-1(M2) as a template. After restriction digest of this PCR fragment with \textit{Nco} I/\textit{Sal} I it was substituted for the corresponding insert of pBluescript ICAM-1(M3). Full length mutated ICAM-1 was shifted into pBABEpuro \textit{Eco} RI/\textit{Sal} I to give the final pICAM-1(M5) retroviral construct.