Supporting Information

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SI Materials and Methods

Engineering of R-LM249. BAC LM249 was constructed by 2-step replacement in *Escherichia coli*, as described (1–4). The pS249 shuttle vector carries the coding sequence for a chimeric glycoprotein gD engineered with a single chain antibody (scFv) anti HER-2 flanked by serine-glycine linkers (upstream 8 aa residues: HSSGGGSG; downstream 12 aa residues: SSGGGSGSGGSG) in place of gD amino acid residues 61 to 218. Mutagenesis and cloning was performed on pLM5, a plasmid containing WT-gD plus ≈500 bp upstream and downstream flanking sequences (4, 5). First, 2 NdeI sites were inserted at the coding sequence for gD amino acid residues 61–62 and 218–219 of mature gD with mutagenic primers gD_61/62_NdeI_f (5′-ACG GTT TAC TAC GCC CAT ATG GAG CGC GCC TGC C-3′) and gD_218/219_NdeI_f (5′-GAC GGT GGA CAG CAT CCA TAT GCT GCC CCG CTT C-3′). Next, an oligo encoding a 9 aa serine-glycine linker was inserted in place of the sequence encoding amino acid 61–218 of gD by annealing and ligating into the NdeI cut vector the 2 phosphorylated oligos P-SG9Bam7/Nde_f (5′-TAG TAG TGG CGG TGG CTC TGG ATC CGG-3′) and P-SG9Bam7/Nde_r (5′-TAC CGG ATC CAG AGC CAC CGC CAC TAC-3′), containing a silent BamHI site. The scFv to HER-2 was amplified from pS2019a (6) with primers scFv_Bam_f (5′-GGC TTA TGG ATC CGA TAT CCA GAT GAC CCA GTC CCC-3′) and scFv_SG_x37_BamH_r (5′-CGG AGG ATC CAC CGG AAC CAG AGC CAC CGC CAC TCG AGG-3′) and inserted into the BamHI site of the serine-glycine linker. Last, the cassette containing the engineered gD, plus gD genomic upstream and downstream flanking sequences was subcloned to pST76KSR shuttle vector to obtain pS249 for homologous recombination in *E. coli*. The receiving genome gDminus-EGFP-HSV-BAC, carrying EGFP as reporter gene, was described in ref. 4.

The recombinant virus, designated R-LM249, was reconstituted as described in ref. 4. Briefly, the BAC-DNA was transfected into WT-gD-expressing and complementing R6 cells (7); next, a single passage in BHK cells was performed to remove any virus pseudotyped with WT-gD; finally the virus was serially passaged in SK-OV-3 cells. Infection was monitored by observation of the cythopathic effect and EGFP. Viral stocks were prepared from lysates of infected SK-OV-3 cells collected at low speed centrifugation. Extracellular virions were harvested from infected cell supernatants by ultracentrifugation (215,000 × *g* for 75 min) on a sucrose cushion (25% in PBS). The titer of viral stocks and extracellular virion preparations was determined in SK-OV-3 cells monolayers, overlaid with 1% SeaPlaque Agarose in RPMI+Glutamax-I, supplemented with 6% FBS.

HER-2 Expression. HER-2 expression in J, J-HER-2, SJ-Rh4, MCF-7, MDA-MB-453, SK-BR-3, and SK-OV-3 cell lines was evaluated by indirect immunofluorescence with monoclonal antibody MGR-3 (kindly given by S. Pupa, National Cancer Institute, Milan) (8), followed by Alexa-Fluor 488-conjugated anti-mouse IgG (Invitrogen). After final washings, cells were resuspended in PBS containing 1 μg/mL of ethidium bromide to gate out dead cells, and analyzed by FACScan flow cytometry (Becton Dickinson).

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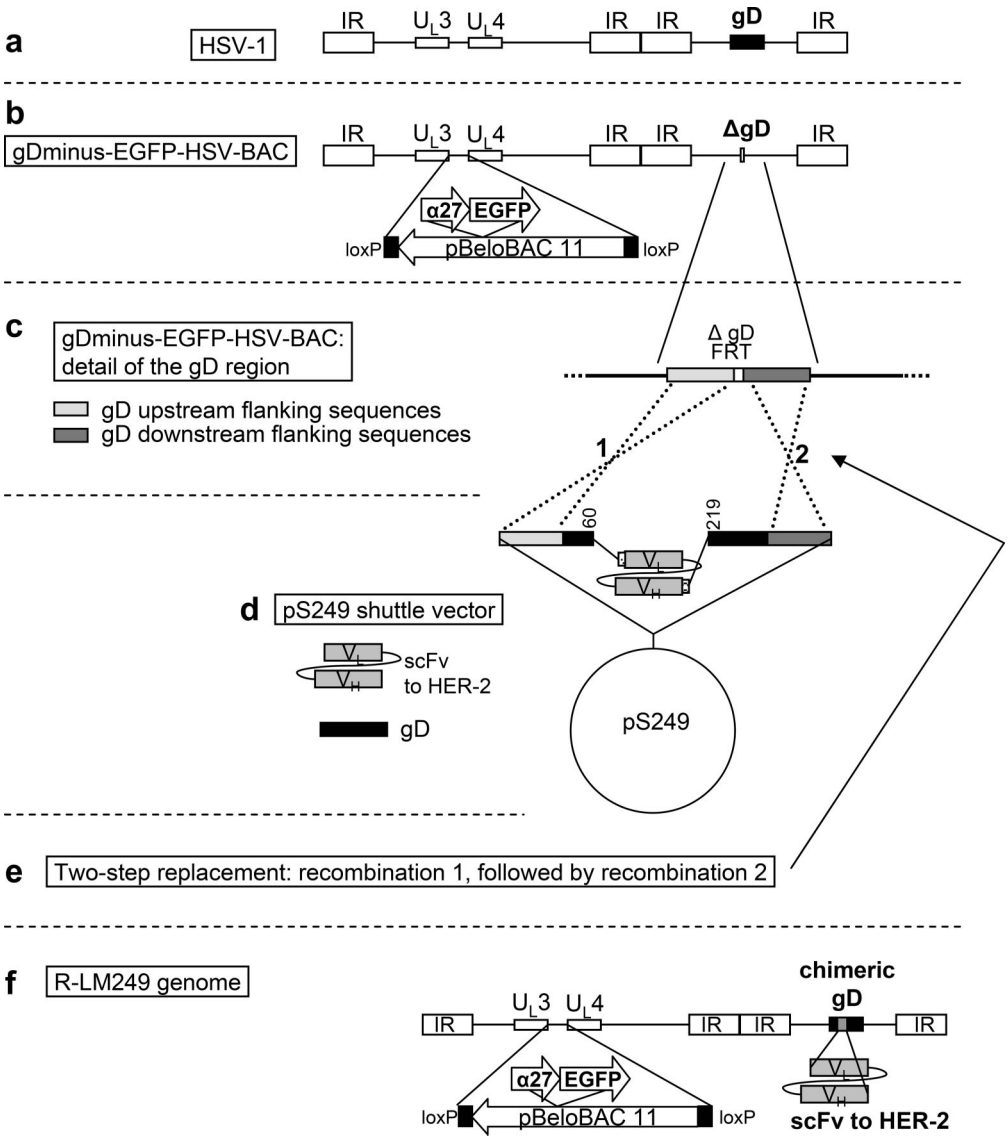

Fig. S1. Schematic representation of the genome of WT HSV-1 and of recombinants relevant to this study used for the generation of R-LM249. (*a*) WT HSV-1 genome linear map. (*b*) Genomic organization of gD-minus-EGFP-HSV-BAC, the recipient genome for BAC mutagenesis. BAC sequences are located between UL3 and UL4 genes, and contain EGFP reporter gene under control of viral immediate-early α27 promoter; gD is deleted (Δ). (*c*) Enlarged view of the gD region. Genomic sequences upstream and downstream of gD are represented by boxes with different grayscale filling, respectively; gD ORF is replaced by a FRT site. (*d*) Schematic representation of pS249 shuttle vector. The cassette contains, in order: gD upstream genomic sequence (≈500 bp), the coding sequences for gD aa residues 1–60, the 8-aa serine-glycine linker, the scFv to HER-2, the 12-aa serine-glycine linker, gD aa residues 219–369, followed by gD downstream genomic region (≈500 bp). The elements are depicted as boxes with different grayscale filling. Numbers indicate the amino acid residues of gD maintained at the boundary of the deletion. (*e*) Two sequential recombination events in *E. coli* (arrow, and nos. 1 and 2) between homologous sequences present in the recipient HSV genome and in the shuttle vector yield the final recombinant R-LM249. (*f*) R-LM249 genome organization. Δ, deletion; FRT, FLP recombinase recognition target; gD, glycoprotein D; IR, inverted repeats; scFv, single-chain antibody; UL, unique long genomic region.

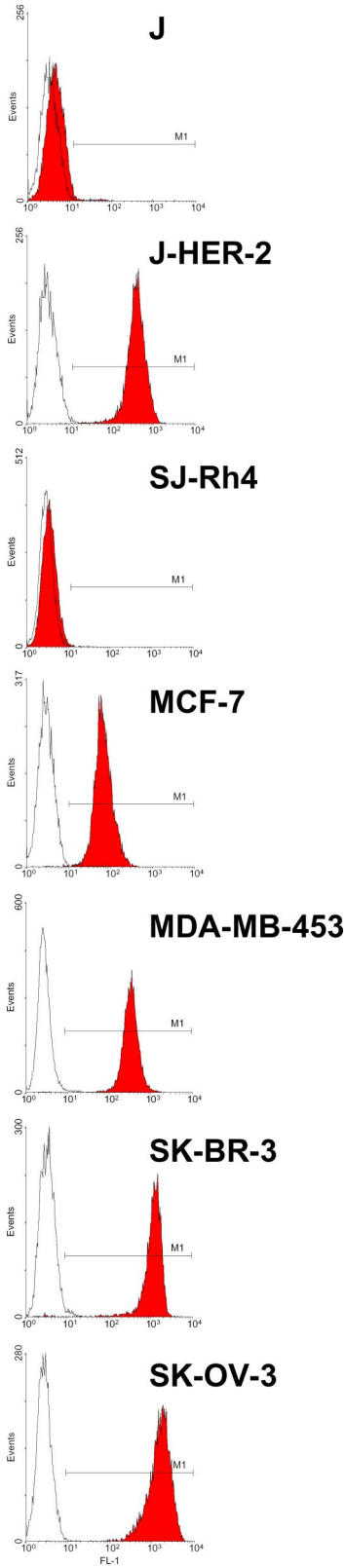

Fig. S2. Membrane expression of HER-2, determined by flow cytometry after indirect immunofluorescence with anti-HER-2 monoclonal antibody (red profiles) or secondary antibody alone (empty profiles). HER-2 median fluorescence intensities were: J, 4; J-HER-2, 407; SJ-Rh4, 4; MCF-7, 72; MDA-MB-453, 340; SK-BR-3, 1241; and SK-OV-3, 1655.