SUPPLEMENTAL DATA #1

Lentiviral vector construction protocol. Non-replicating lentiviral vectors expressing short-hairpin RNAs (shRNAs) for stable RNAi knock down and EGFP to enhance detection were produced using the pLenti6/V5-D-TOPO and ViraPower Lentivirus Expression system supplied by Invitrogen (Carlsbad, CA). The manufacturers manual was followed for TOPO cloning and production of viral particles; however, modifications of the pLenti6/V5-D-TOPO vector were performed to generate a U6 driven shRNA and EGFP expression. Initially, EGFP (BD Biosciences, La Jolla, CA) was inserted into the CMV driven expression cassette by TOPO cloning. After the viability of this construct was established, the parent construct was modified. The CMV driven expression cassette was replaced with the pSilencer human U6 driven shRNA cassette supplied by Ambion (Austin, Texas), the blasticidin resistance gene was replaced with EGFP remaining under the control of the SV40 promoter, and the HIV central polypurine tract (cPPT) was inserted to enhance transduction efficiency (Follenzi A, Ailles LE, Bakovic S, et al. Nat Genet 2000;25:217-22). The cPPT and pSilencer U6 shRNA cassette were inserted simultaneously by 3-way ligation using Cla I- Eco RI- Xho I from 5' to 3' respectively. The cPPT was PCR amplified from the pLP1 construct included in the ViraPower packaging mix and inserted 5' to the U6 expression cassette by including Cla I and Eco RI sites at the ends. The blasticidin resistance gene downstream of the SV40 promoter was replaced using a PCR amplified EGFP with Xma I and Kpn I ends. For generation of knockdown shRNAs with pSilencer, manufacturer's instructions were followed with the exception of including an Xho I site immediately 5' to the Hind III site in

the hairpin-oligo for transfer into our lentiviral vector. This lentiviral construct allows the one-step exchange of shRNAs using the unique Bam HI and Xho I sites immediately downstream of the U6 promoter. Lentiviral particles in the supernatants from transfected 293T producer cells typically reached > 5 x 10^6 transforming units (TU)/ml and were easily quantified by flow cytometry or fluorescence microscopy.