



## **Supplemental Material to:**

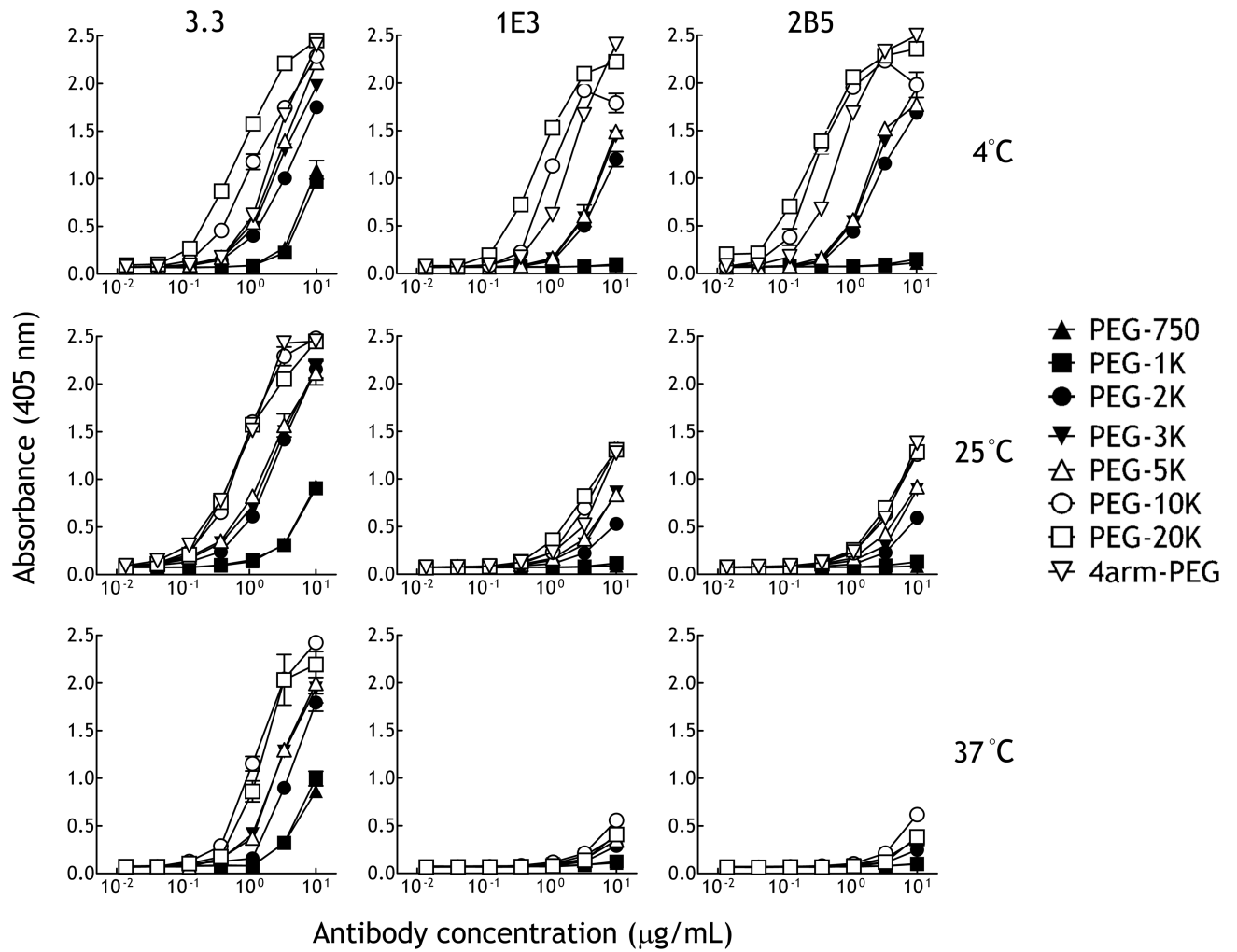
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and Steve R Roffler**

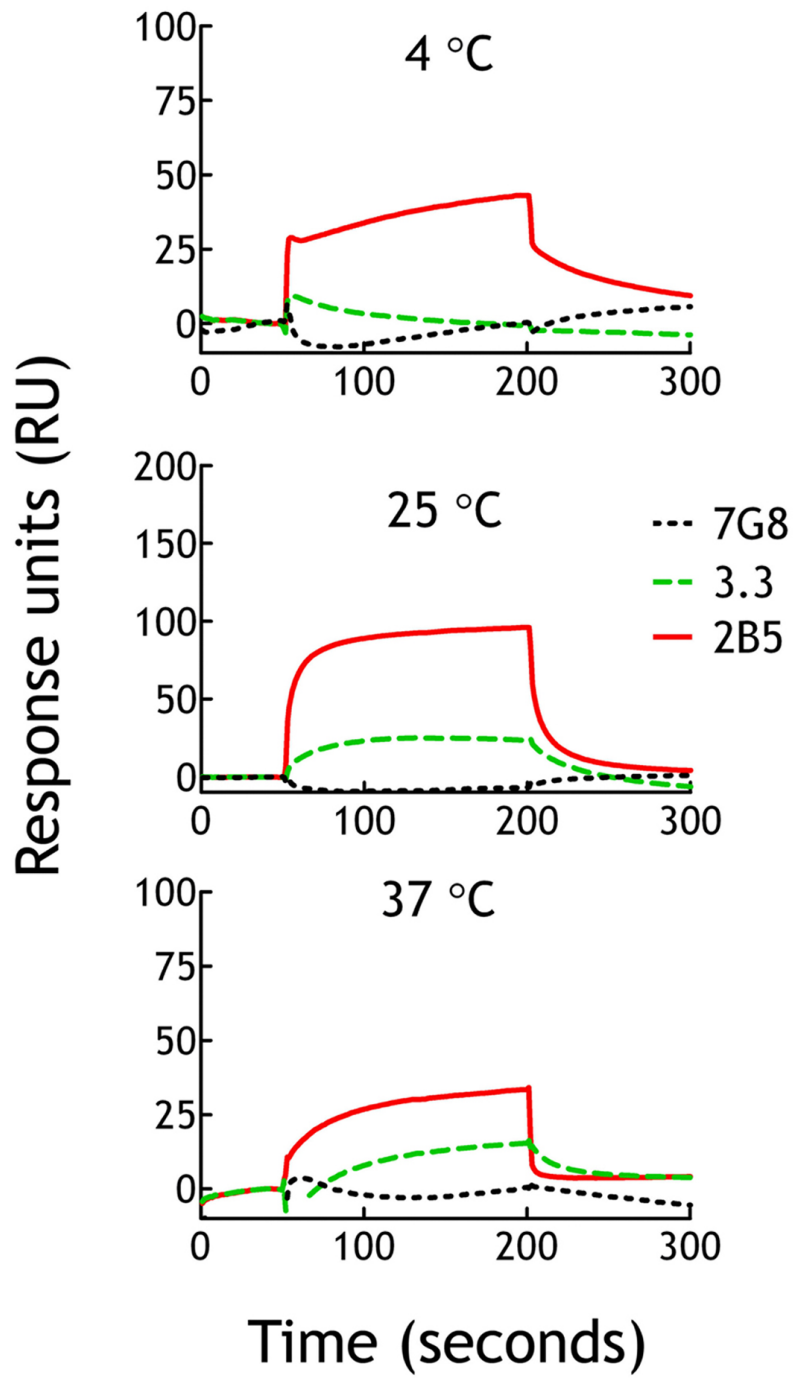
**Mimicking the germinal center reaction in hybridoma cells  
to isolate temperature-selective anti-PEG antibodies**

**mAbs 2014; 6(4)**

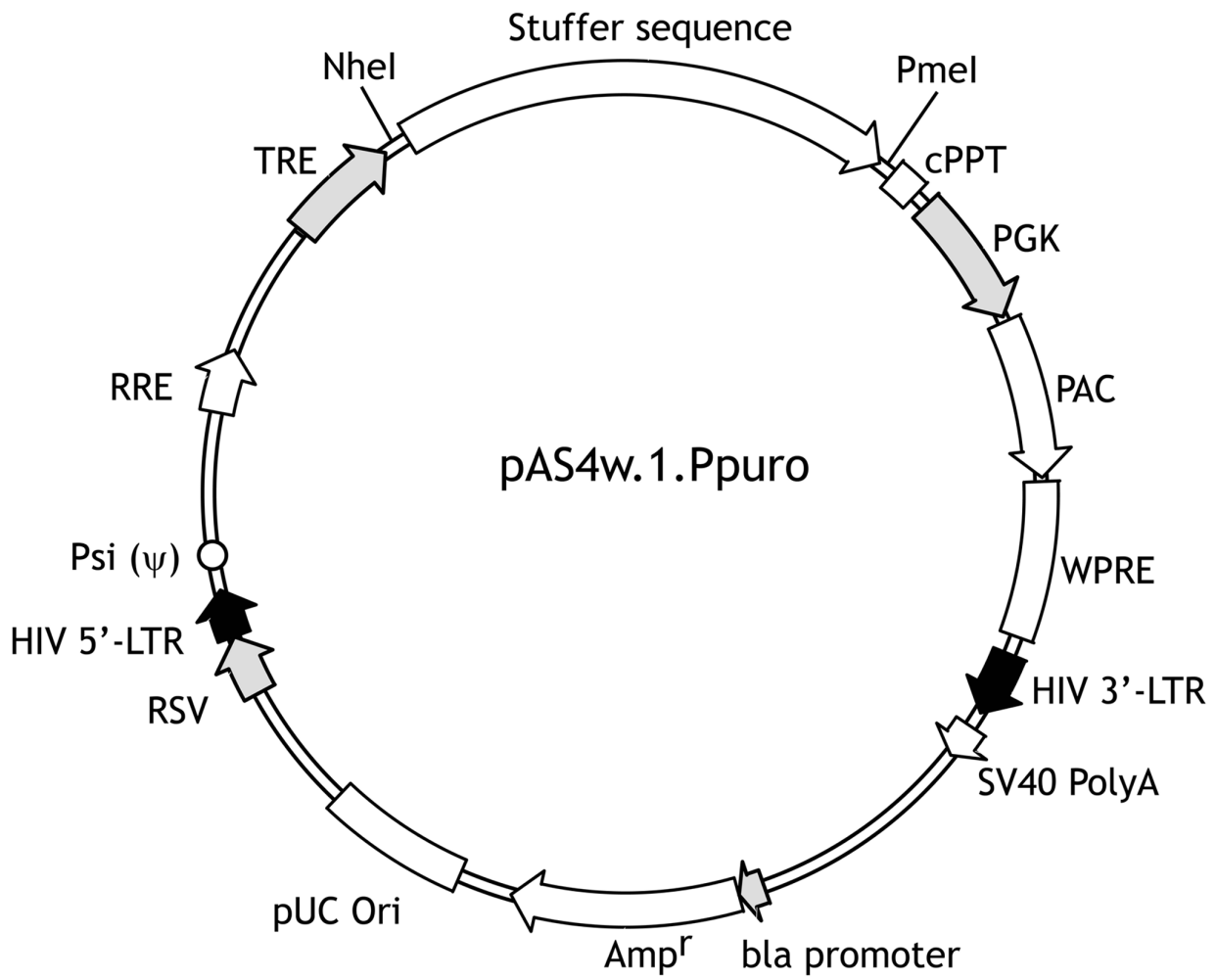
**<http://dx.doi.org/10.4161/mabs.29124>**

**<http://www.landesbioscience.com/journals/mabs/article/29124/>**

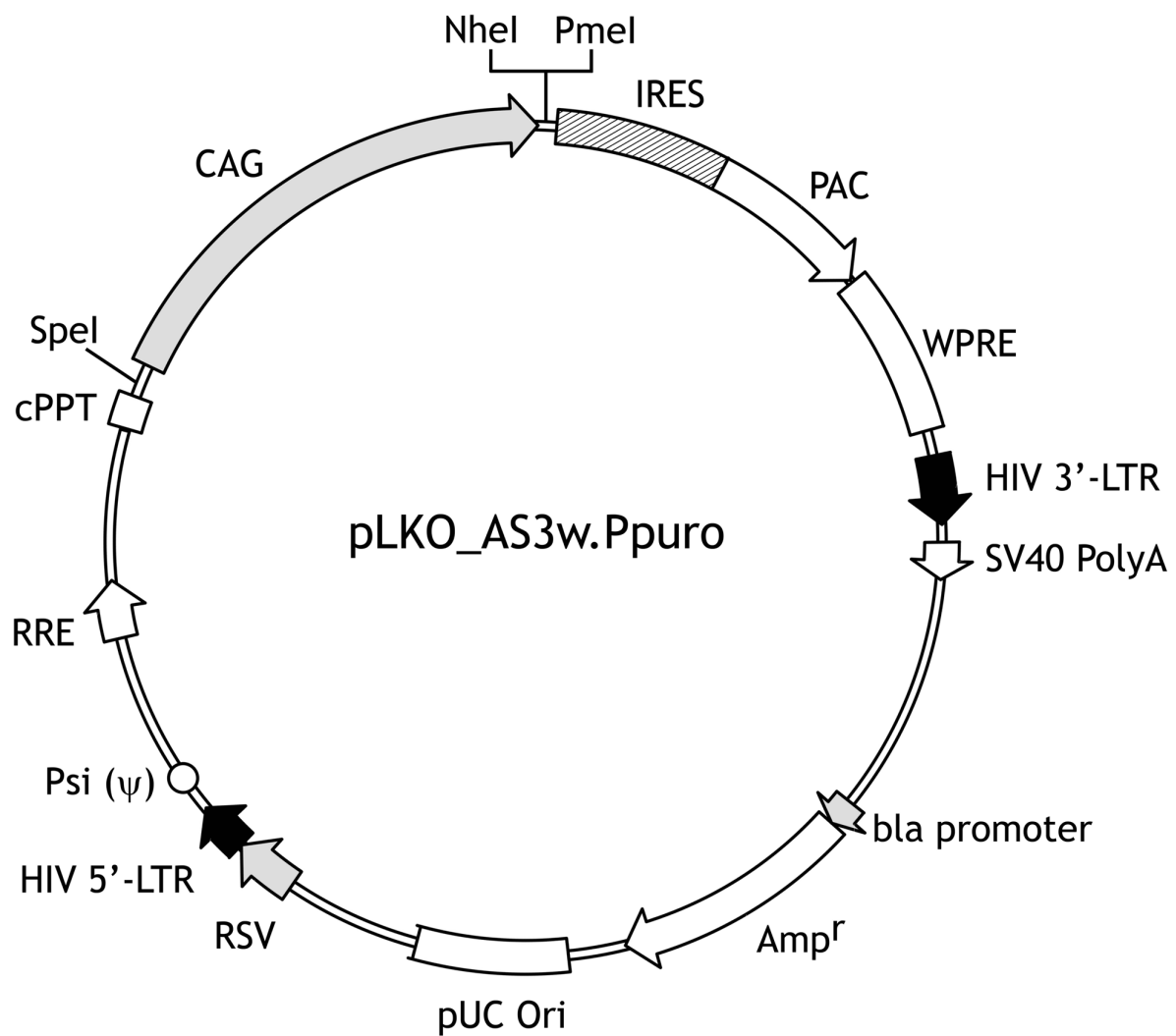




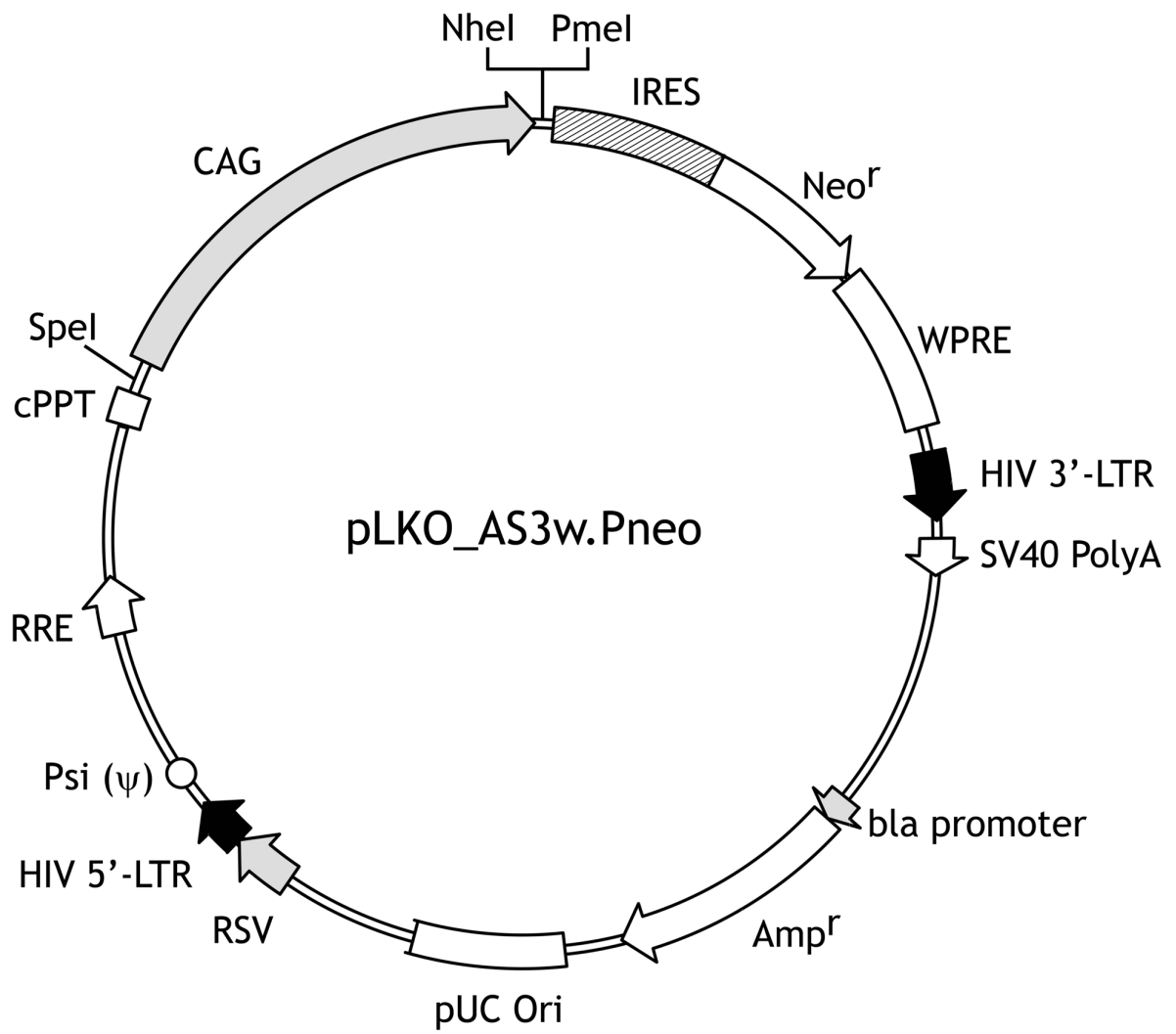
Supplemental Figure 2



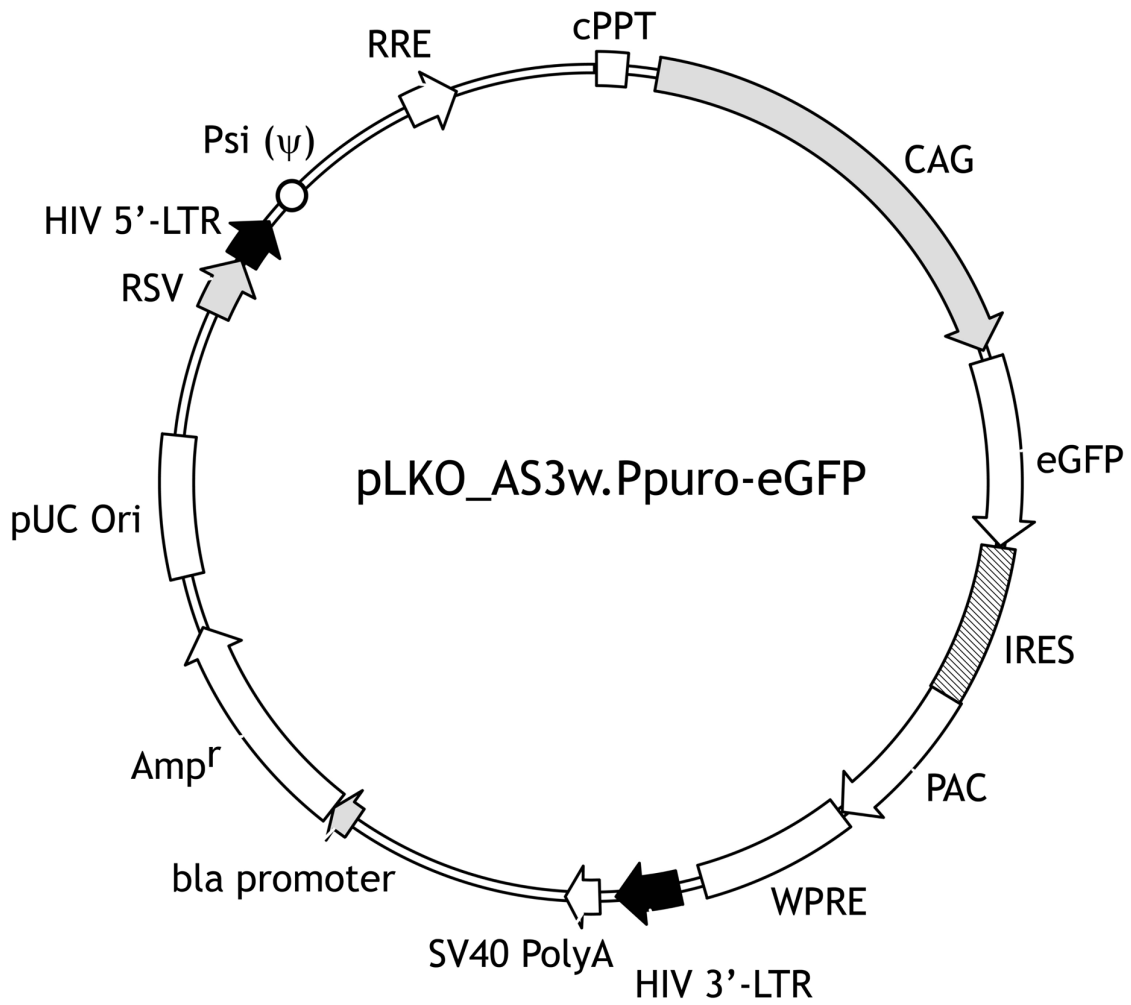
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 4



Supplemental Figure 5

## Supplemental Figure Legends

**Supplemental Figure 1.** Temperature and PEG length-dependent binding of anti-PEG antibody variants. Graded concentrations of purified 3.3, 1E3, or 2B5 antibodies were added to microplate wells coated with linear amino-PEG (MW 750, 1000, 2000, 3000, 5000, 10,000 or 20,000 Da) or branched 4-arm PEG (10,000 Da) at the indicated temperatures. After 1 h, the wells were washed and antibody binding was determined by adding HRP-conjugated donkey anti-mouse IgG Fc antibodies, followed by ABTS substrate. The mean absorbance values (405 nm) of triplicate determinations are shown. Bars, SD.

**Supplemental Figure 2.** Biacore sensorgrams of antibody binding to crown ether. Binding of 3.3 (dashed green line), 2B5 (solid red line) or 7G8 (dotted black line) antibodies to 2-aminomethyl-18-crown-6 immobilized on a CM5 chip was analyzed on a Biacore T-200 at 4°C, 25°C or 37°C.

**Supplemental Figure 3.** pAS4w.1.Ppuro lentiviral vector map. The vector backbone contains elements for efficient viral packaging, including Psi ( $\Psi$ ), the lentiviral packaging site; cPPT, the central polypurine tract sequence from HIV-1 to improve vector integration and transduction efficiency; RRE, a Rev-responsive element that increases lentivirus titers by promoting the nuclear export of unspliced viral genomic RNA; RSV-HIV 5'-LTR, a hybrid of the Rous Sarcoma virus promoter and the HIV 5'-LTR; HIV 3'-LTR, a self-inactivating U3 deletion; WPRE, a woodchuck hepatitis virus posttranscriptional regulatory element that promotes RNA processing and maturation; SV40 PolyA (Simian virus 40 PolyA), a DNA element that possesses the activity of transcription termination and can add a PolyA tail to mRNA. The lentiviral vector contains a tetracycline response element promoter (TRE) for doxycycline-inducible gene



expression. The vector contains the puromycin resistance gene (PAC) under the control of the PGK promoter. Amp<sup>r</sup> is the ampicillin resistance gene under the control of the bla promoter. pUC ori is the bacterial origin of replication of the plasmid. The vector contains a 1.9 kb DNA stuffer fragment of inserted in between the NheI and PmeI cloning sites.

**Supplemental Figure 4.** pLKO\_AS3w.Ppuro lentiviral vector map. The vector backbone contains elements for efficient viral packaging, including Psi ( $\Psi$ ), cPPT, RRE, RSV-HIV 5'-LTR, HIV 3'-LTR, WPRE and SV40 PolyA elements. Gene expression is driven by a CMV early enhancer/chicken  $\beta$  actin (CAG) promoter. The puromycin resistance gene (PAC) is expressed from an internal ribosome entry site (IRES) as part of the bicistronic transcript. Amp<sup>r</sup> is the ampicillin resistance gene under the control of the bla promoter. pUC ori is the bacterial origin of replication of the plasmid. The CAG promoter is located between SpeI and NheI cloning sites followed by a PmeI cloning site.

**Supplemental Figure 5.** pLKO\_AS3w.Pneo lentiviral vector map. The vector backbone contains elements for efficient viral packaging, including Psi ( $\Psi$ ), cPPT, RRE, RSV-HIV 5'-LTR, HIV 3'-LTR, WPRE and SV40 PolyA elements. Gene expression is driven by a CAG promoter. The neomycin resistance gene (Neo<sup>r</sup>) is expressed from an internal ribosome entry site (IRES) as part of the bicistronic transcript. Amp<sup>r</sup> is the ampicillin resistance gene under the control of the bla promoter. pUC ori is the bacterial origin of replication of the plasmid. The CAG promoter is located between SpeI and NheI cloning sites followed by a PmeI cloning site.

**Supplemental Figure 6.** pLKO\_AS3w.Ppuro-eGFP lentiviral vector map. The vector backbone contains elements for efficient viral packaging, including Psi ( $\Psi$ ), cPPT, RRE, RSV-HIV 5'-LTR, HIV 3'-LTR, WPRE and SV40 PolyA elements. Expression of the eGFP gene is driven by a CAG promoter. The puromycin resistance gene (PAC) is expressed from an internal ribosome

entry site (IRES) as part of the bicistronic transcript. Amp<sup>r</sup> is the ampicillin resistance gene under the control of the bla promoter. pUC ori is the bacterial origin of replication of the plasmid.

## Supplemental Table

**Supplemental Table 1.** Mutations in the 3D8 V<sub>H</sub> domain occurring during CSR

V<sub>H</sub> // ----- FR1 ----- // ----- CDR1 ----- //

3D8 // A E L V M P G // Y T F T S C W //

clone 1 // ● ● ● L ● ● ● // ● ● ● ● ● ● ● //

clone 2 // ● ● ● ● ● ● ● // ● ● ● I ● ● ● //

clone 3 // ● ● V ● ● ● ● // ● ● ● ● ● Y ● //

Antibody framework regions and CDRs were assigned according to the Kabat numbering system<sup>61</sup> using the website <http://www.bioinf.org.uk/abysis>.