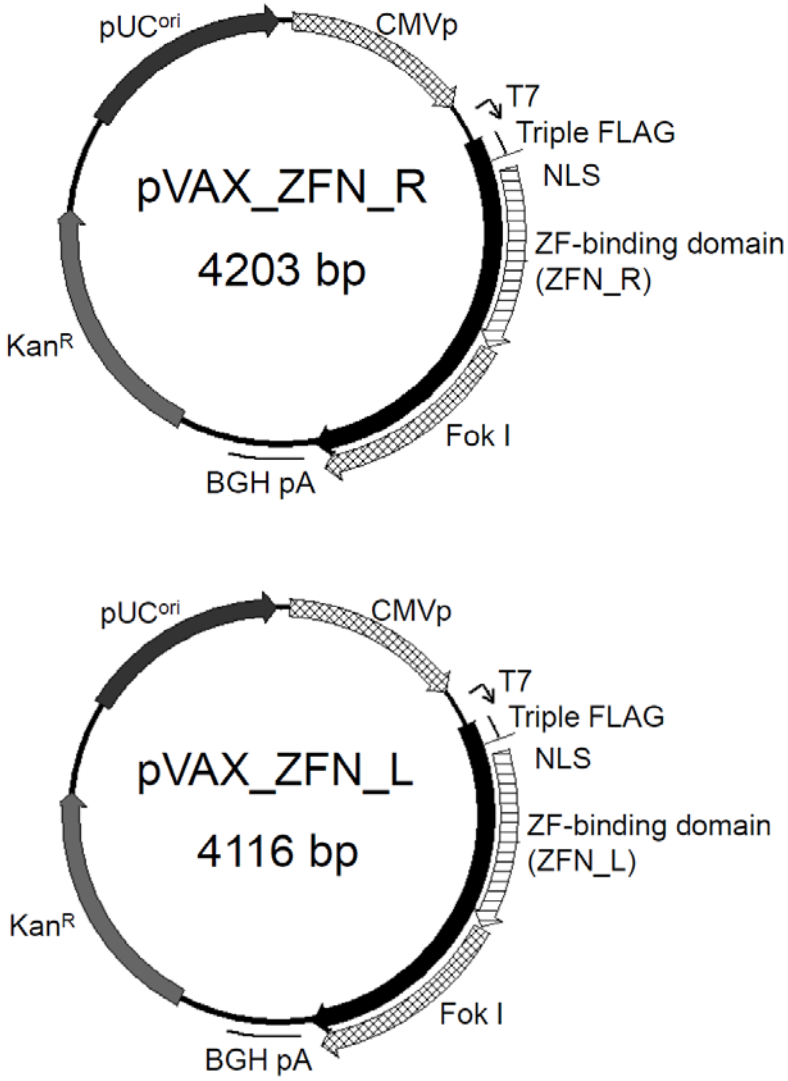


Supplementary Figure 1: Schematic of the plasmids encoding ZFN-R and ZFN-L. CMVp: CMV promoter, T7: T7 RNA polymerase promoter, Triple FLAG: three repeats of FLAG sequence, NLS: nuclear localization signal, BGH pA: bovine growth hormone polyadenylation signal, Kan^R: kanamycin resistant gene, pUC^{ori}: origin of replication

A3 disrupted clone, 18-1

```
HLA-A*03:01, WT:     GTTCTCACACCATCCAGATAATGTATGGCTGCGACGTGGGGTCGGACGGGCGCTTCCTCC
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
HLA-A*03:01,18-1:    GTTCTCACACCATCCAGA-------TGGCTGCGACGTGGGGTCGGACGGGCGCTTCCTCC
```

(Predicted amino acid sequence)

```
HLA-A*03:01 WT:      GSHTIQIMYGCDVGSDGRFLRGYRQDAYDGKDYIALNEDLRSWTAA
                     ||||||||||||||||||||||||||||||||||||
HLA-A*03:01, 18-1:   GSHTIQMAATWGRTGASSAGTGRTPTTARITSP*
```

A2 disrupted clone, 8.18

```
HLA-A*02:01 WT:      GTTCTCACACCGTCCAGAGG----ATGTATGGCTGCGACGTGGGGTCGGACTGGCGCTTCCTCC
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
HLA-A*02:01, 8.18:   GTTCTCACACCGTCCAGAGGATGTATGTATGGCTGCGACGTGGGGTCGGACTGGCGCTTCCTCC
```

(Predicted amino acid sequence)

```
HLA-A*02:01 WT:      GSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHV
                     |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
HLA-A*02:01, 8.18:   GSHTVQRMYVWLRRGVGLALPPRVPPVRLRRQGLHRPERGPALLDRGGHGSSDHQAQVGGGP

HLA-A*02:01 WT:      AEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEI
                     ||||||||||||||||||||||||||||||||||||||||||||||||
HLA-A*02:01, 8.18:   CGGAVESLPGGHVRGVAPQIPGEREGDAAAHGRPQNAYDSPRCL*
```

Both A2 and A3 disrupted clone, 83

HLA-A3

```
HLA-A*03:01 WT:      GTTCTCACACCATCCAGATAATGTATGGCTGCGACGTGGGGTCGGACGGGCGCTTCCTCC
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
HLA-A*03:01, 83:     GTTCTCACACCATCCAGAT-ATGTATGGCTGCGACGTGGGGTCGGACGGGCGCTTCCTCC
```

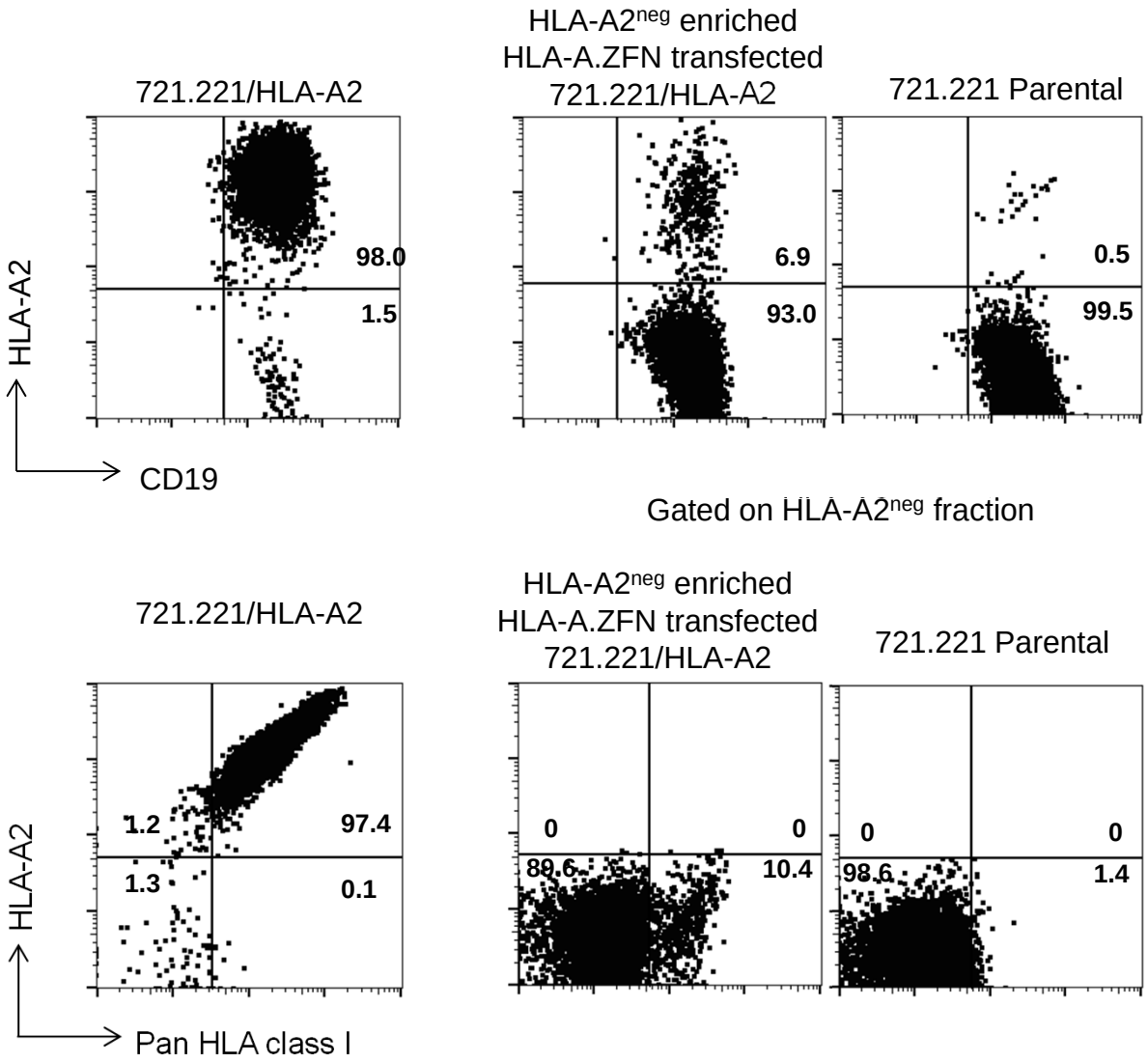
(Predicted amino acid sequence)

```
HLA-A*03:01 WT:      GSHTIQIMYGCDVGSDGRFLRGYRQDAYDGKDYIALNEDLRSWTAA
                     ||||||||||||||||||||||||||||||||||||
HLA-A*03:01, 83:     GSHTIQICMAATWGRTGASSAGTGRTPTTARITSP*
```

HLA-A2

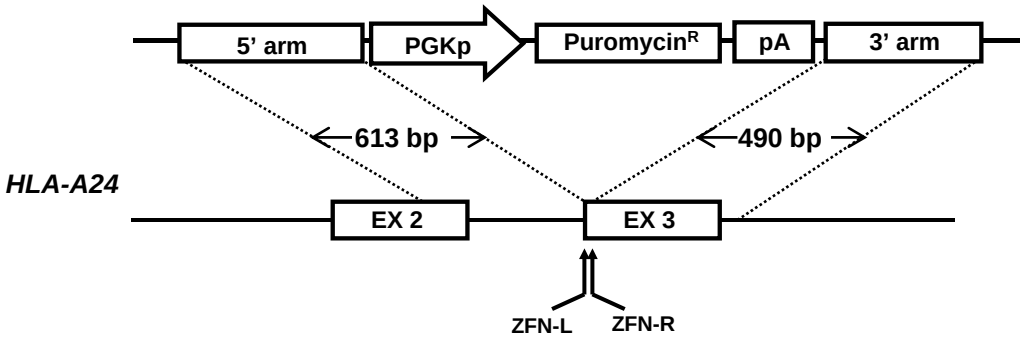
Same 4bp duplication as clone 8.18

Supplementary Figure 2: Genotype of HLA-A disrupted HEK293 clones. Sequencing of HLA-disrupted clones was performed on TOPO cloned (Invitrogen) PCR products containing the ZFN target region. Primers used in the PCR were the same as those used in the Surveyor nuclease assay. Deleted bases are indicated by hyphens, inserted bases by bold type. The C-terminal part of the predicted amino acid sequences of the mutated alleles is also shown, with changes in the amino acids sequence indicated by bold type and the stop codon by *.



Supplementary Figure 3: HLA-A2 is eliminated from cell surface after ZFN-mediated disruption of HLA-A locus. HLA-A2 expression after genetic editing by ZFN was evaluated by introducing ZFN into HLA-A*02:01 transduced HLA class I^{null} 721.221 B cells. HLA-A2 expression in parental 721.221, HLA-A2 transduced 721.221 (721.221/HLA-A2), and HLA-A target ZFN-transfected and $A2^{neg}$ fraction-enriched 721.221/HLA-A2 are shown. Also shown is staining with monomorphic domain-specific pan-anti-HLA class I antibody, G46-2.6 (BD Bioscience, #555555). All cells were gated in PI^{neg} population

Donor: HLA-A24-PGK-Puro



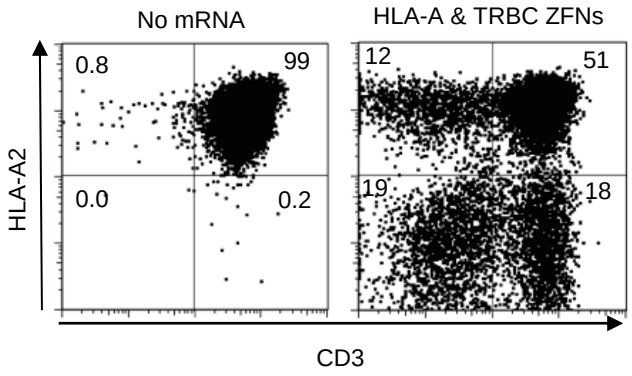
5' homology-arm sequence

GGCCCGCCTGGCGGGGGCGCAAGACCCGGGAAGCCGCGCCGGGAGGAGGGTCGGGCGGGTCTCAGCCACTCCTCGTCCCCAGGCTCCCA
CTCCATGAGGTATTTCTCCACATCCGTGTCCCGGCCCGGCCGCGGGGAGCCCCGCTTCATCGCCGTGGGCTACGTGGACGACACGCAGT
TCGTGCGGTTCGACAGCGACGCCGCGAGCCAGAGGATGGAGCCGCGGGCGCCGTGGATAGAGCAGGAGGGGCCGGAGTATTGGGACGAG
GAGACAGGGAAAGTGAAGGCCCACTCACAGACTGACCGAGAGAACCTGCGGATCGCGCTCCGCTACTACAACCAGAGCGAGGCCGGTGA
GTGACCCCGGCCCGGGGCGCAGGTCACGACCCCTCATCCCCCACGGACGGGCCGGGTCGCCCACAGTCTCCGGGTCCGAGATCCACCCC
GAAGCCGCGGGACCCCGAGACCCTTGCCCCGGGAGAGGCCCAGGCGCCTTAACCCGGTTTCATTTTCAGTTTAGGCCAAAAATCCCCCC
GGGTTGGTCGGGGCCGGGCGGGGCTCGGGGGACTGGGCTGACCGCGGGGTCGGGGCCAGGTTCTCACACCCTCCAGATG

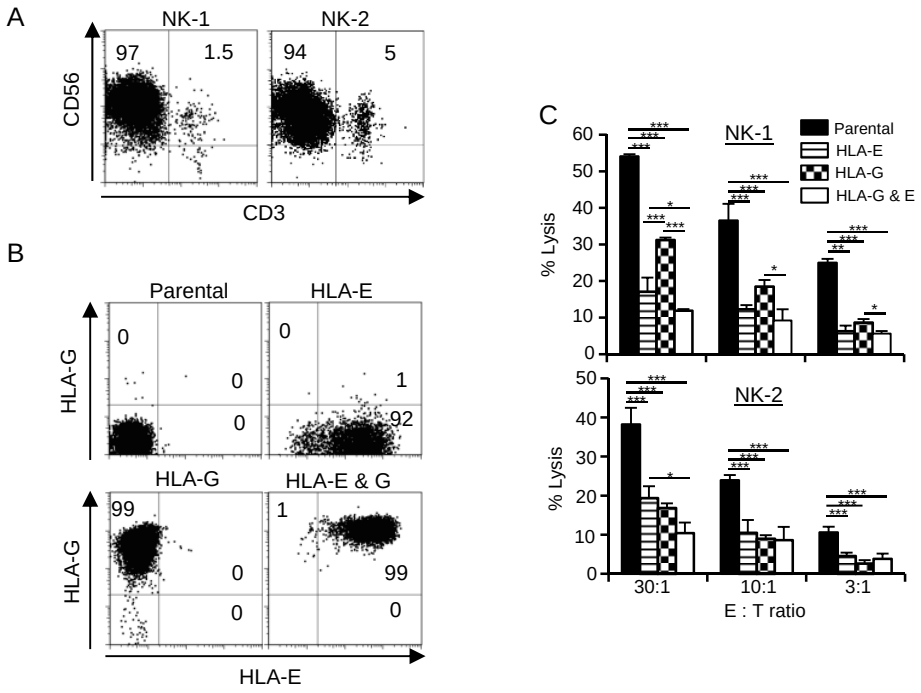
3' homology-arm

TTGGCTGCGACGTGGGGTCGGACGGGCGCTTCCTCCGCGGGTACCACCAGTACGCCTACGACGGCAAGGATTACATCGCCCTGAAAGA
GGACCTGCGCTCTTGGACCGCGGCGGACATGGCGGCTCAGATCACCAAGCGCAAGTGGGAGGCGGCCCATGTGGCGGAGCAGCAGAGA
GCCTACCTGGAGGGCACGTGCGTGGACGGGCTCCGCAGATACCTGGAGAACGGGAAGGAGACGCTGCAGCGCACGGGTACCAGGGGCC
ACGGGGCGCCTACCTGATCGCCTGTAGGTCTCCCGGGCTGGCCTCCCACAAGGAGGGGAGACAATTGGGACCAACACTAGAATATCGC
CCTCCCTCTGGTCCTGAGGGAGAGGAATCCTCCTGGGTTTCCAGATCCTGTACCAGAGAGTGACTCTGAGGTTCCGCCCTGCTCTCTG
ACACAATTAAGGGATAAAATCTCTGACGGAATGACGGAAAGACGATCCCT

Supplementary Figure 4: Schematic depiction of the donor construct for ZFN-mediated targeted integration within the HLA-A24 allele. A puromycin resistance gene under control of the PGK promoter flanked 5' 613 bp and 3' 490 bp by arms homologous to the putative ZFN binding region within HLA-A24. The sequences of 5'- and 3'- homology arms are shown with the intended ZFN binding sites underlined.



Supplementary Figure 5: Synchronous modification of T-cells with ZFNs targeting HLA-A and T-cell receptor β constant (TRBC) regions. T-cells were co-transfected with two ZFN species that target HLA-A and TRBC loci. HLA-A2 and CD3 expression within $CD4^+CD8^+$ cells are shown. The numbers are the percentage per quadrant.



Supplementary Figure 6: Enforced Expression of HLA-E and/or HLA-G Prevents NK-cell Mediated Lysis of HLA class I^{null} cells. A. Isolation of NK cells from PBMC. Immunophenotype of NK cells obtained from two healthy donor PBMC (NK-1 and NK-2 represents each donor). Flow cytometry data shown are gated for PI^{neg} population. The numbers represent percentage of each upper quadrant. **B. Genetic modification of 721.221 cells to express HLA-E and/or HLA-G.** SB transposon/transposase system was used to homogenously express HLA-E and/or HLA-G in three clones of 721.221 cells. Each number represents percentage expression of HLA-G, HLA-E, or both HLA-G and HLA-E as detected by flow cytometry. **C. Specific lysis by circulating NK cells targeting 721.221 cells.** The relative ability of NK cells to kill parental (HLA class I^{null}), HLA-E^{+}, HLA-G^{+}, and both HLA-E^{+}HLA-G^{+} 721.221 cells. Each column represents the mean ± standard deviation (SD) * .01< P < 0.05, **P < .01; and ***P < .001