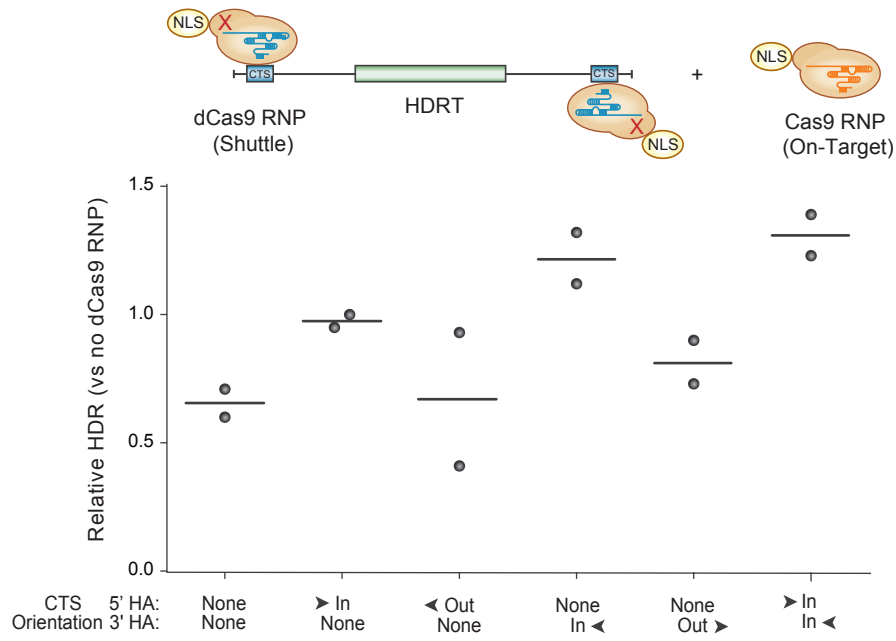


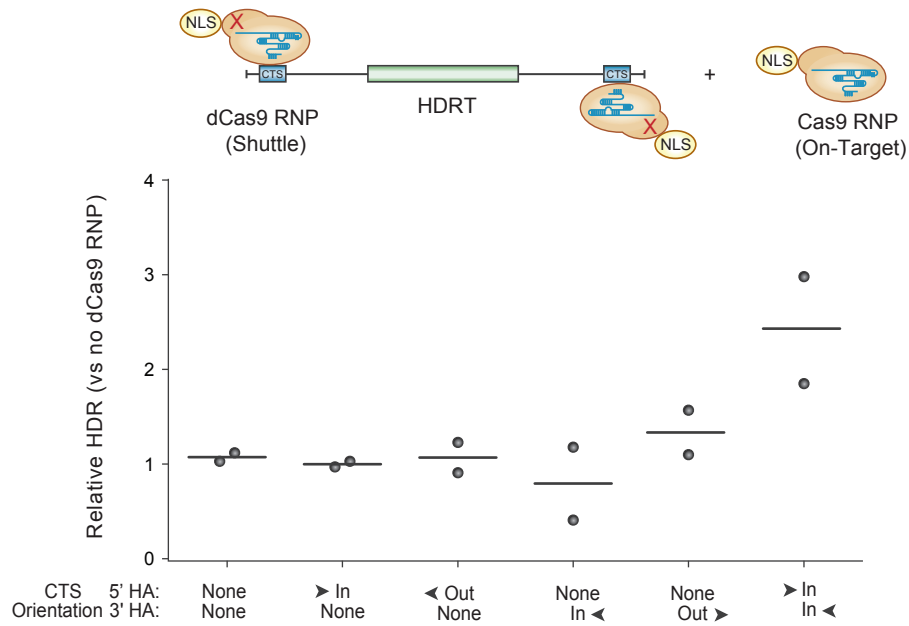
SUPPLEMENTARY INFORMATION

a

Shuttle gRNA different from cutting gRNA

**b**

Shuttle gRNA same as cutting gRNA

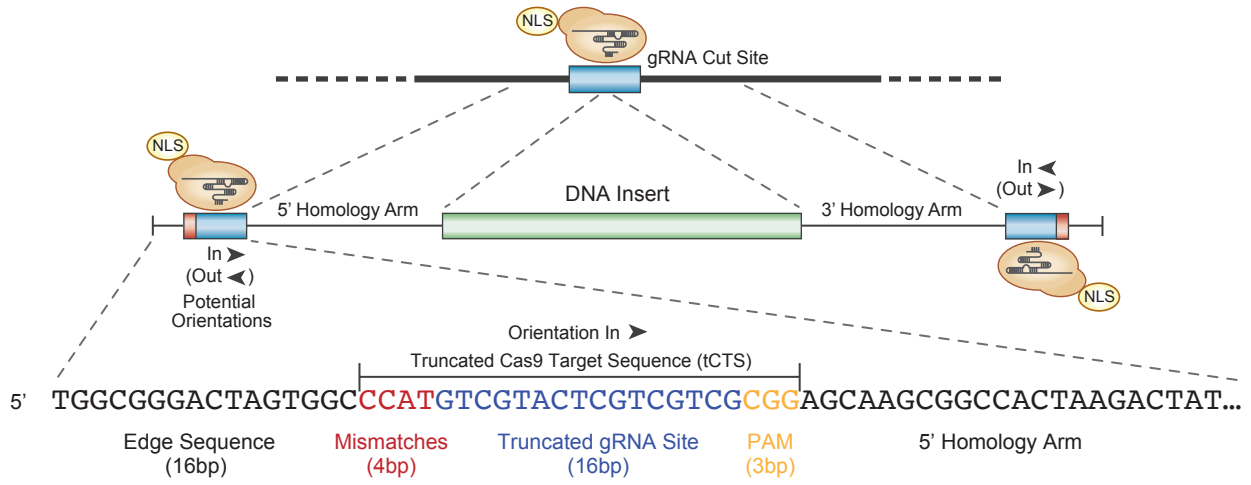
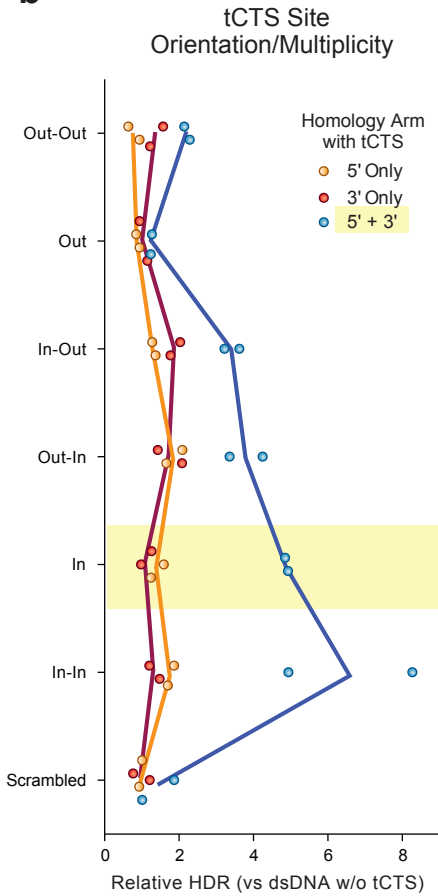
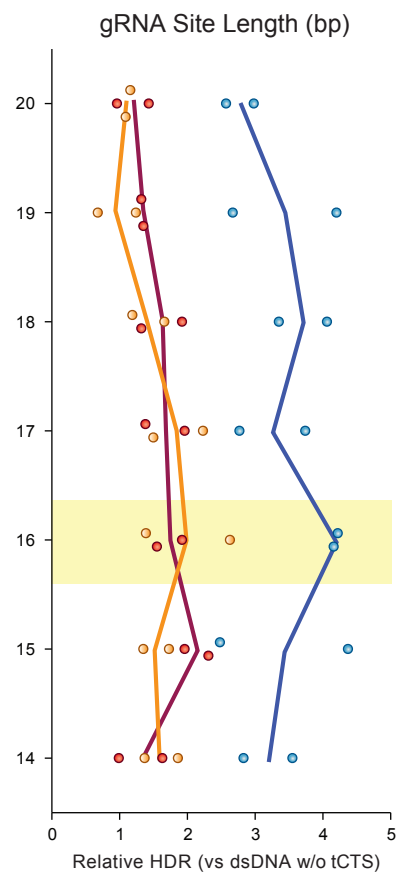
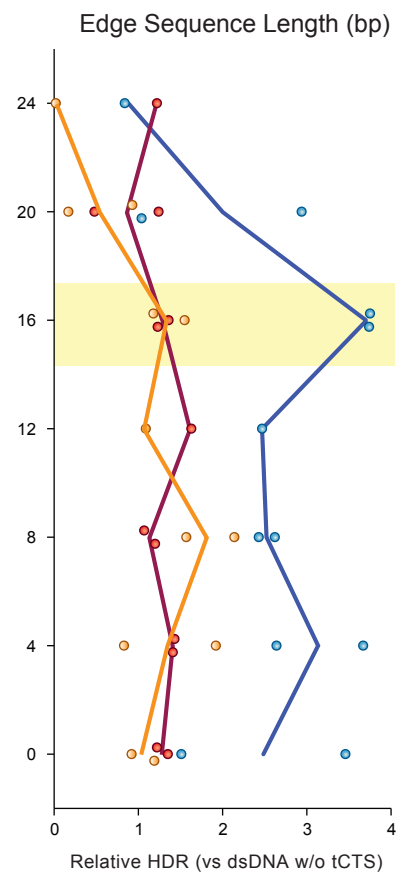


Supplementary Fig. 1: dCas9 complexing to DNA HDR templates moderately improved knockin efficiencies.

(a) Initial testing of a dCas9 shuttle. A full 20bp gRNA target sequence was added to the edge of a dsDNA HDR template that inserts a new TCR specificity (1G4 TCR clone, specific for the NY-ESO 1 tumor antigen) at the endogenous TCR locus⁵. A dCas9 RNP was formed with a gRNA specific for the sequence attached to the ends of the HDR template, incubated with the dsDNA HDR template for 5 min at room temperature, and then electroporated into primary human T cells along with a separate Cas9 RNP with a gRNA specific for the target locus, in this case the TCR alpha constant region exon 1. The orientation of the gRNA target sequence added to the ends of the HDR template appeared to matter, with an “In” facing orientation (PAM sequence towards the center of the insert) on both the 5’ and 3’ homology arms showed the greatest improvement in knockin efficiency.

(b) The dCas9 shuttle RNP could be complexed with the same gRNA sequence as the on-target cutting Cas9 RNP by appending the same gRNA target sequence as in the on-target genomic locus to the ends of the HDR template. Similarly, an “In” facing orientation of the sequence on both edges of the homology arm were important for improved knockin efficiency.

The relative rates of HDR are displayed at four days post electroporation when including the dCas9 ‘shuttle’ RNP compared to electroporation of the unmodified HDR template and Cas9 for n=2 biologically independent blood donors (a, b). Center lines indicate mean (a, b).

a**b****c****d**

Supplementary Fig. 2: Optimization of Cas9 ‘shuttle’ end modification DNA sequence parameters.

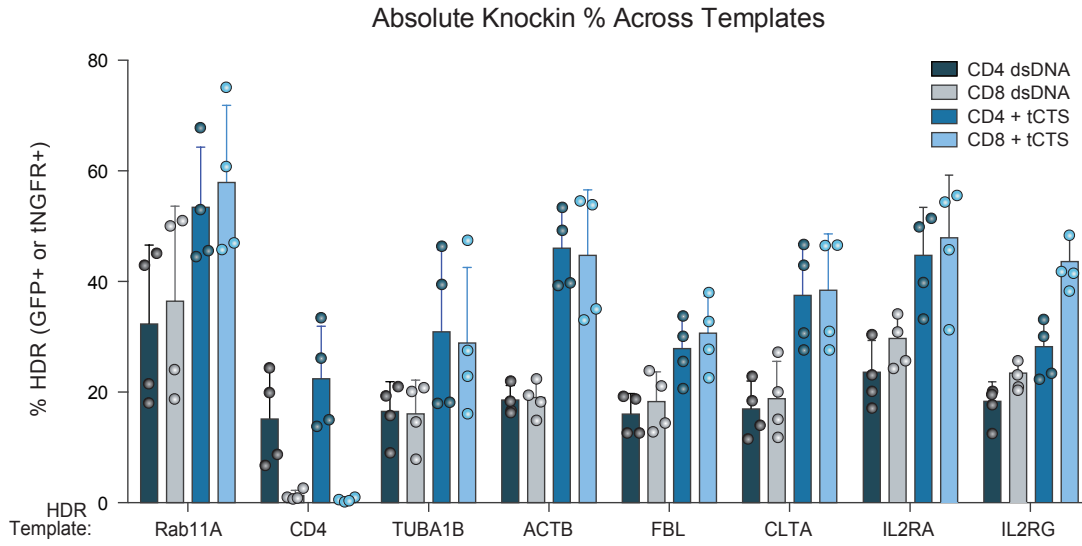
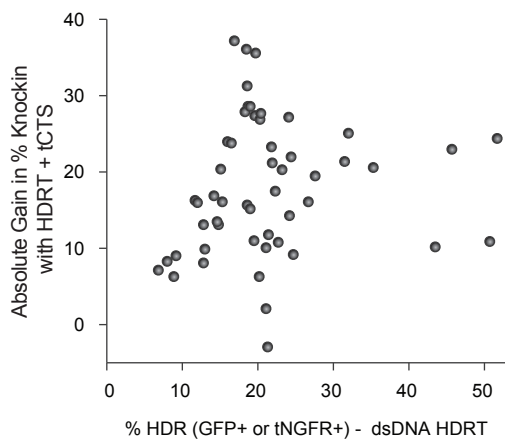
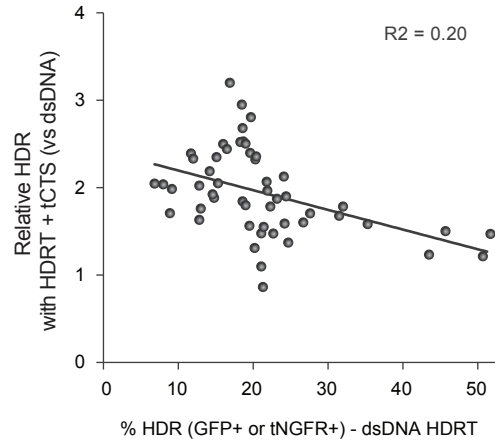
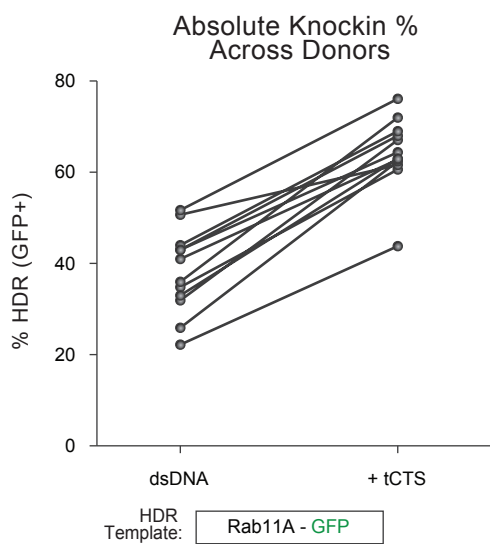
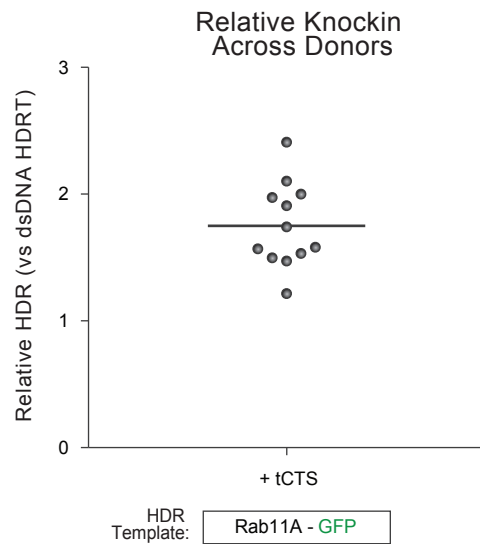
(a) DNA sequence design for the Cas9 ‘shuttle’ system. A short DNA sequence can be easily added to the edges of a dsDNA HDR template by PCR. To mediate binding of a Cas9-NLS RNP to the HDR template but prevent cutting, a truncated 16 bp Cas9 target sequence is added to either or both of the 5’ and 3’ homology arms, along with a 3 bp PAM sequence (NGG for spCas9) and 4 bp of gRNA mismatches. An additional 16 bps of random DNA edge spacer sequence is added outside of the gRNA binding site. The gRNA site can have two orientations, one “In” facing with the PAM directed towards the center of the HDR template, and another “Out” facing with the PAM directed towards the edge of the HDR template.

(b) Optimization of the orientation and multiplicity of the Cas9 ‘shuttle’ DNA sequence. Single or multiple 16 bp gRNA binding sites with PAMs were added to the 5’ homology arm, 3’ homology arm, or both homology arms of an HDR template that replaces the endogenous T cell receptor with a new specificity (insertion size ~1.5kb). Inwards facing orientations on both edges of the HDR template showed the greatest improvements in HDR efficiency, either with a single copy of the tCTS site (“In”) or two copies in parallel (“In-In”). For simplicity a single copy was used in all subsequent experiments.

(c) Optimization of the length of the truncated gRNA binding site. Note that a full-length gRNA was used in all experiments, while the length of the CTS binding site varied. A DNA sequence with 16 base pairs matching the gRNA sequence proved optimal, corresponding to previously demonstrated gRNA target site lengths that mediate Cas9 binding but not cutting⁸. Again, presence of the tCTS-binding site on both homology arms increased HDR efficiency.

(d) Optimization of the edge spacer length showed that a 16 bp random DNA sequence outside of the tCTS yielded the greatest improvements in HDR efficiency. Maximal gains in HDR were again dependent on presence of the Cas9 ‘shuttle’ sequence on both homology arms.

The relative rates of HDR (1.5 kb knockin) at the TCR locus with the indicated ‘shuttle’ sequence modifications compared to unmodified dsDNA HDR template for n=2 biologically independent blood donors are displayed with a line connecting means (**b-d**).

a**b****c****d****e**

Supplementary Fig. 3: Cas9 'shuttle' tCTS HDR template modifications improved large knockin efficiency across donors and genomic target loci.

(a) Percentage of primary human CD4 or CD8 T cells expressing a knocked in GFP or tNGFR after non-viral genome targeting of eight different genomic sites with and without Cas9 'shuttle' tCTS-modifications to the dsDNA HDR template. Across all eight tested loci the Cas9 shuttle improved knockin percentages in both cell types. Note that knockin of a GFP to the CD4 locus did not show GFP expression in CD8 T cells.

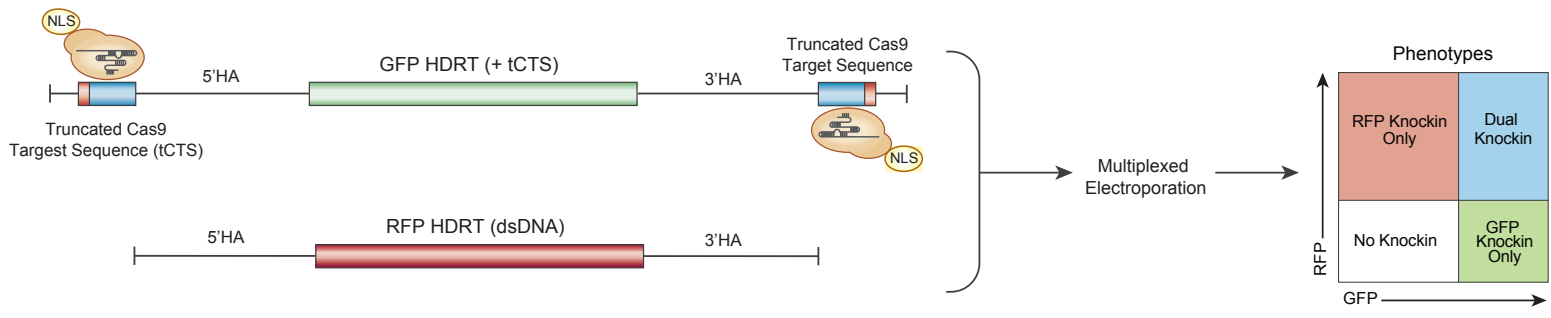
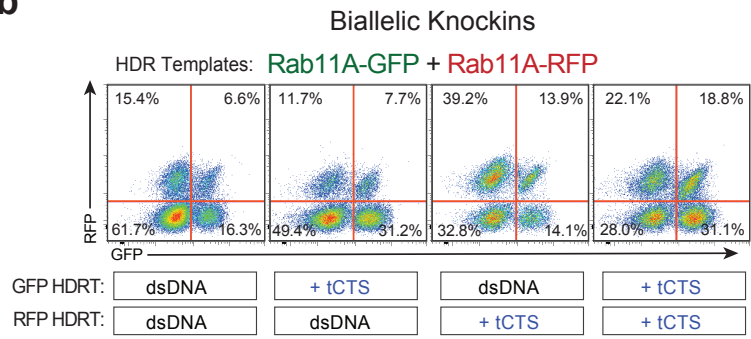
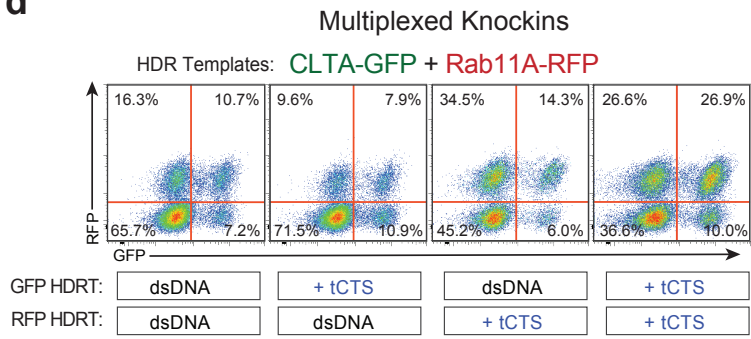
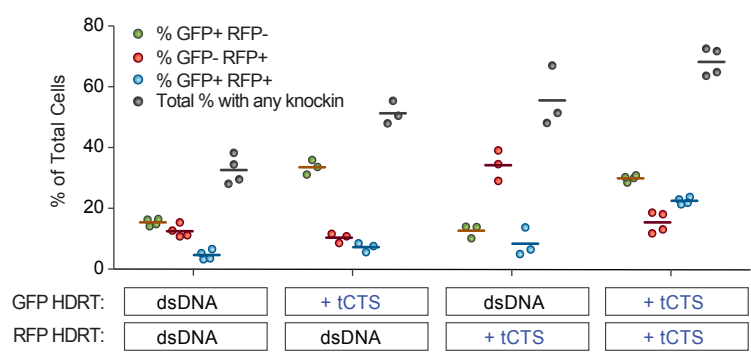
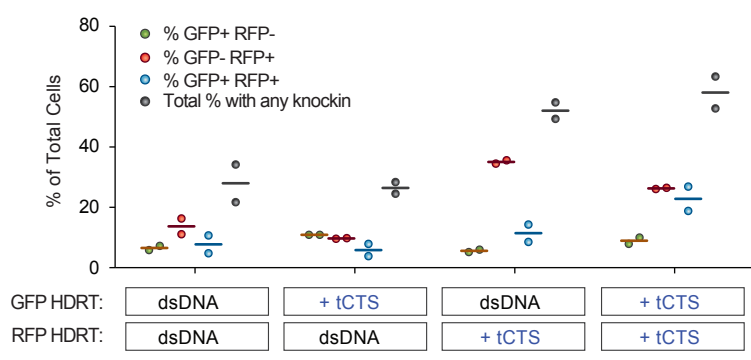
(b) Aggregation of data across donors and target sites shows that the tCTS modification enabled large increases in the % of cells with an observed large knockin of GFP or tNGFR across initial knockin efficiencies with an unmodified dsDNA HDR template.

(c) Relative improvement in HDR efficiency is dependent on the initial knockin efficiency with an unmodified dsDNA HDR template. At lower initial knockin efficiencies, the relative gain in HDR with the Cas9 shuttle was higher than at target sites where the unmodified template efficiency was higher. Linear regression with a goodness of fit R^2 value of 0.20 is overlaid.

(d) In a cohort of 12 healthy primary T cell donors, all donors showed an improved knockin efficiency with the Cas9 'shuttle' compared to unmodified dsDNA HDR template for knockin of a GFP fusion at the *RAB11A* locus. Lines connect values from the same blood donor.

(e) Relative improvement in HDR efficiency at the *RAB11A* locus with a Cas9 shuttle across 12 donors.

Knockins were assayed 4 days after electroporation in n=4 (a-c) or n=12 (d-e) biologically independent blood donors. Bars (a) or center lines (e) indicate mean.

a**b****d****c****e**

Supplementary Fig. 4: Improved bi-allelic and multiplexed knockin efficiency with Cas9 shuttle.

(a) Diagram of multiplexing experiments with two HDR templates, encoding either a GFP or RFP integration, simultaneously electroporated into the same cells. Neither, one, or both HDR templates had a Cas9 shuttle DNA sequence added to the ends of the homology arms, and the percentages of single and dual knockin positive cells were assayed by flow cytometry.

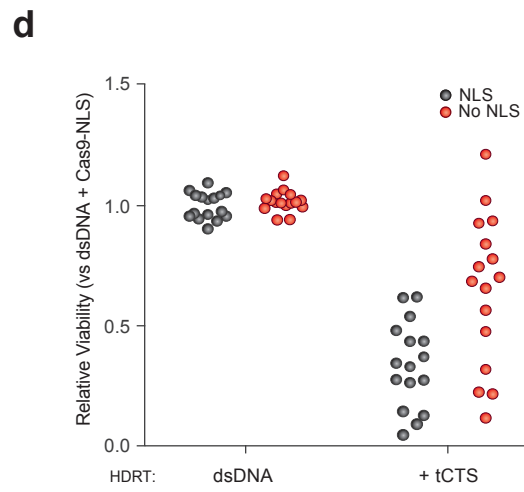
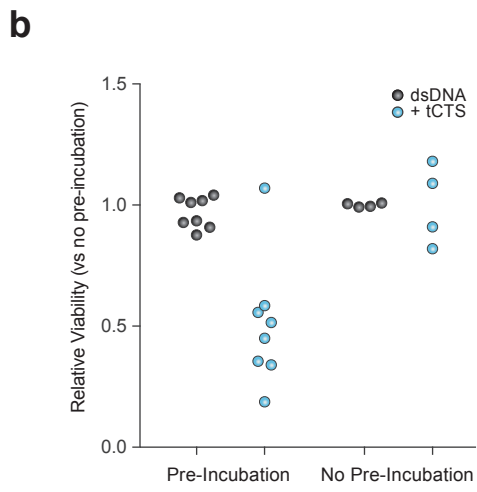
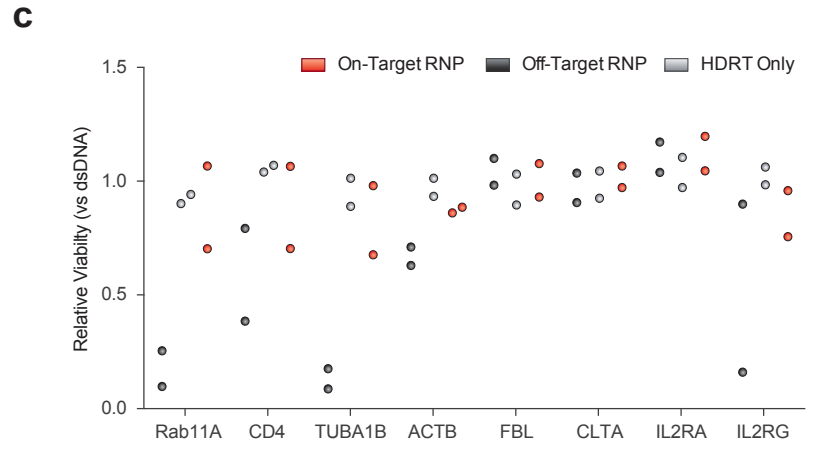
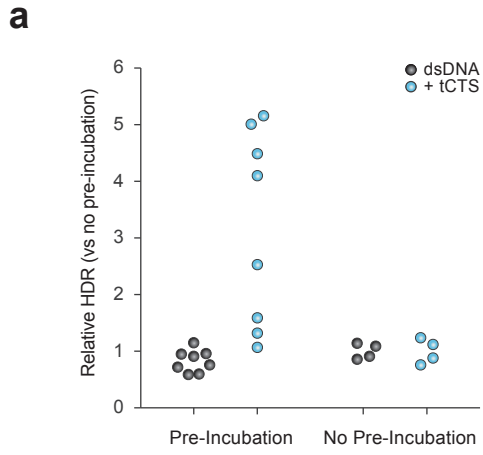
(b) Representative flow cytometric plots for bi-allelic knockin of GFP and RFP to the *RAB11A* housekeeping gene. When one HDR template possessed tCTS 'shuttle' modifications, the knockin rates for that fluorescent protein (either GFP or RFP) increased, whereas the knockin rates for the other fluorescent protein remained largely unaffected. Knockins with both templates possessing Cas9 shuttle sequences increased the percentage of bi-allelic targeting. Note that the percentage of GFP+RFP+ cells is only approximately half of the true bi-allelic knockin percentage, as knockins of either GFP or RFP at both alleles still yields a single positive at the protein level⁵.

(c) Quantification of percent knockin efficiency across bi-allelic knockin experiments.

(d) Representative flow cytometric plots for multiplexed knockin experiments at two genomic loci, the *CLTA* locus encoding Clathrin and the *RAB11A* housekeeping gene. Again, the greatest improvements in multiplexed knockin efficiency were observed when both templates possess the Cas9 shuttle sequence.

(e) Quantification of percent knockin efficiency across multiplexed knockin experiments.

Knockins were assayed 4 days after electroporation. 1-2 technical replicates from each of n=2 biologically independent blood donors shown (c,e). Center lines indicate mean (c, e).



Supplementary Fig. 5: Viability loss with Cas9 shuttle requires pre-incubation with HDR template, an on-target gRNA, and partially an NLS peptide.

(a) Pre-incubation of the Cas9 RNP along with dsDNA HDR template containing tCTS on the ends of its homology arms was required for the improvement in knockin efficiency. Pre-incubation was performed for a minimum of 30 seconds at room temperature. In no pre-incubation conditions, the dsDNA HDR template was first mixed with cells, followed by mixing with Cas9 RNP immediately prior to electroporation.

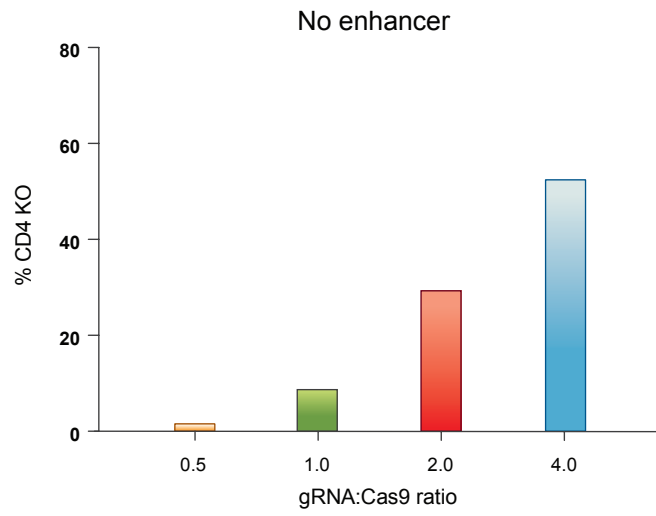
(b) Pre-incubation was also necessary for the observed drop in viability when comparing tCTS-modified HDR template to unmodified dsDNA.

(c) Use of an on-target gRNA in the Cas9 RNP (specific for both cutting at the desired genomic locus and for binding to the tCTS-binding site introduced on the edges of the HDR template homology arms) was necessary for the observed drop in viability with the Cas9 'shuttle' at certain genomic sites. Note that there was no decreased viability compared to unmodified dsDNA HDR template when electroporating the tCTS-modified HDR template by itself or with an off-target scrambled RNP, indicating that decreased viability was not an inherent property of the tCTS-modifications but rather due to the specific complexing of the tCTS-modified HDR template with the on-target Cas9 RNP. This reduced viability was not observed at all genomic loci tested.

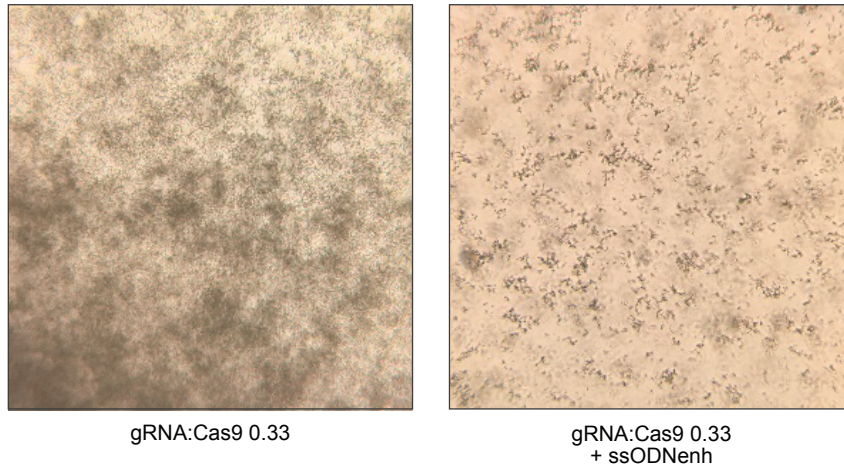
(d) Use of a Cas9 RNP that did not possess an NLS sequence still showed some reduced viability together with a tCTS-modified HDR template when compared to unmodified dsDNA HDR template.

The relative rates of knockin (a) or viability (b-d) with the various forms of the Cas9 'shuttle' are displayed compared to unmodified dsDNA HDR template (a-d) for TCR replacement at the *TRAC* locus (1.5 kb knockin, a-b, d) or GFP or tNGFR knockin at the indicated locus (c) in multiple technical replicates from n=2 biologically independent blood donors (a-d). Knockin was measured 4 days post electroporation and viability (total number of live cells relative to no electroporation control) at 2 days post electroporation.

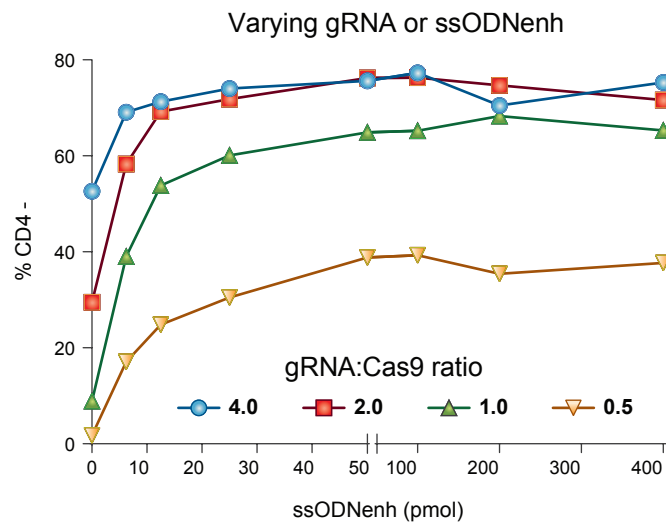
a



b



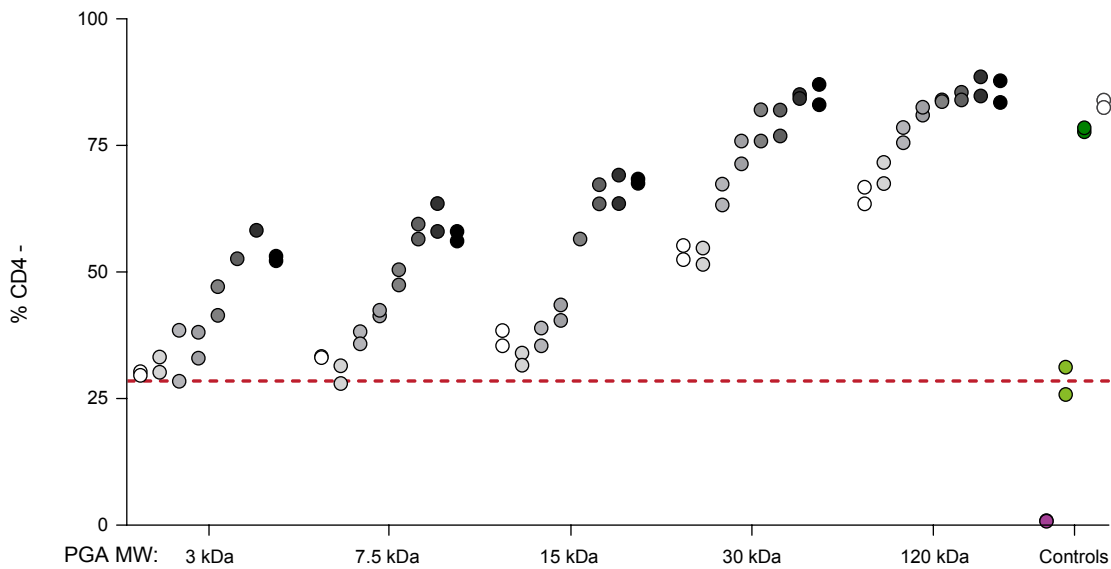
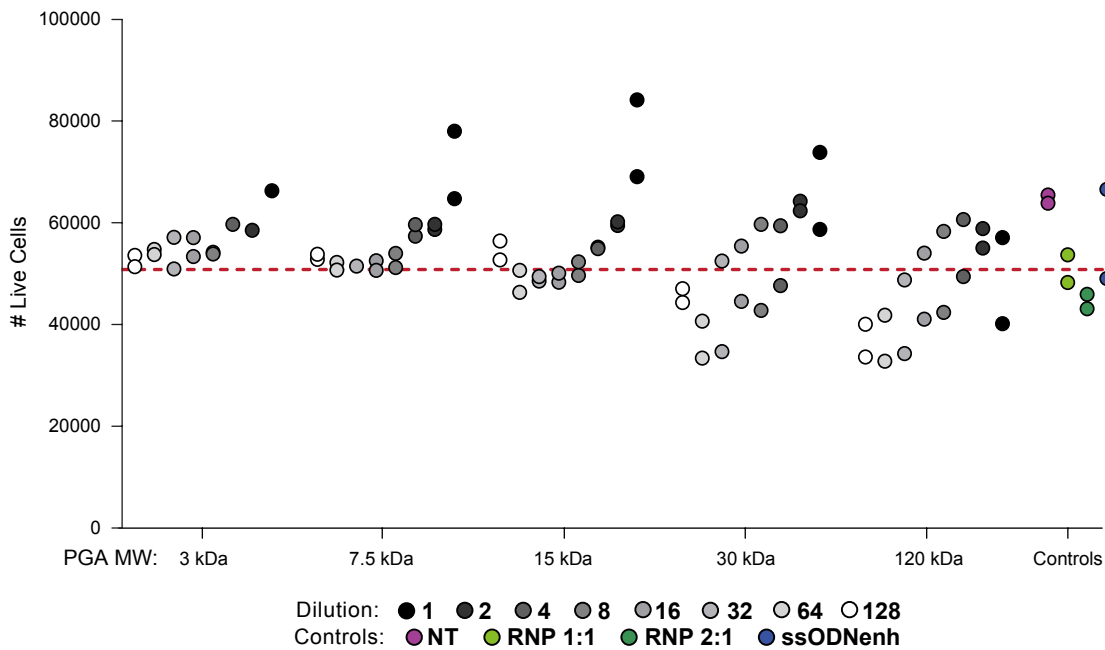
c



Supplementary Fig. 6: Increasing gRNA or ssODNenh improved editing efficiency.

Cas9 RNPs targeting *CD4* gene knock-out were electroporated into primary human CD4+ T cells at the indicated gRNA:protein ratio **(a)** without enhancer, or **(c)** with indicated doses of ssODNenh. Loss of surface CD4 expression at 3 days was assessed by flow cytometry. With the exception of 0.5:1 ratio RNPs, which contain only 25pmol of gRNA and thus maximum 50% less functional RNPs; all other gRNA:protein ratios achieved maximal editing with addition of >100pmol of ssODNenh.

(b) Photograph of 20x light microscopy showing RNP aggregates at low 0.33:1 ratio RNPs before (left) and within 1 minute (right) after addition of ssODNenh. Representative of 3 independent experiments with similar results.

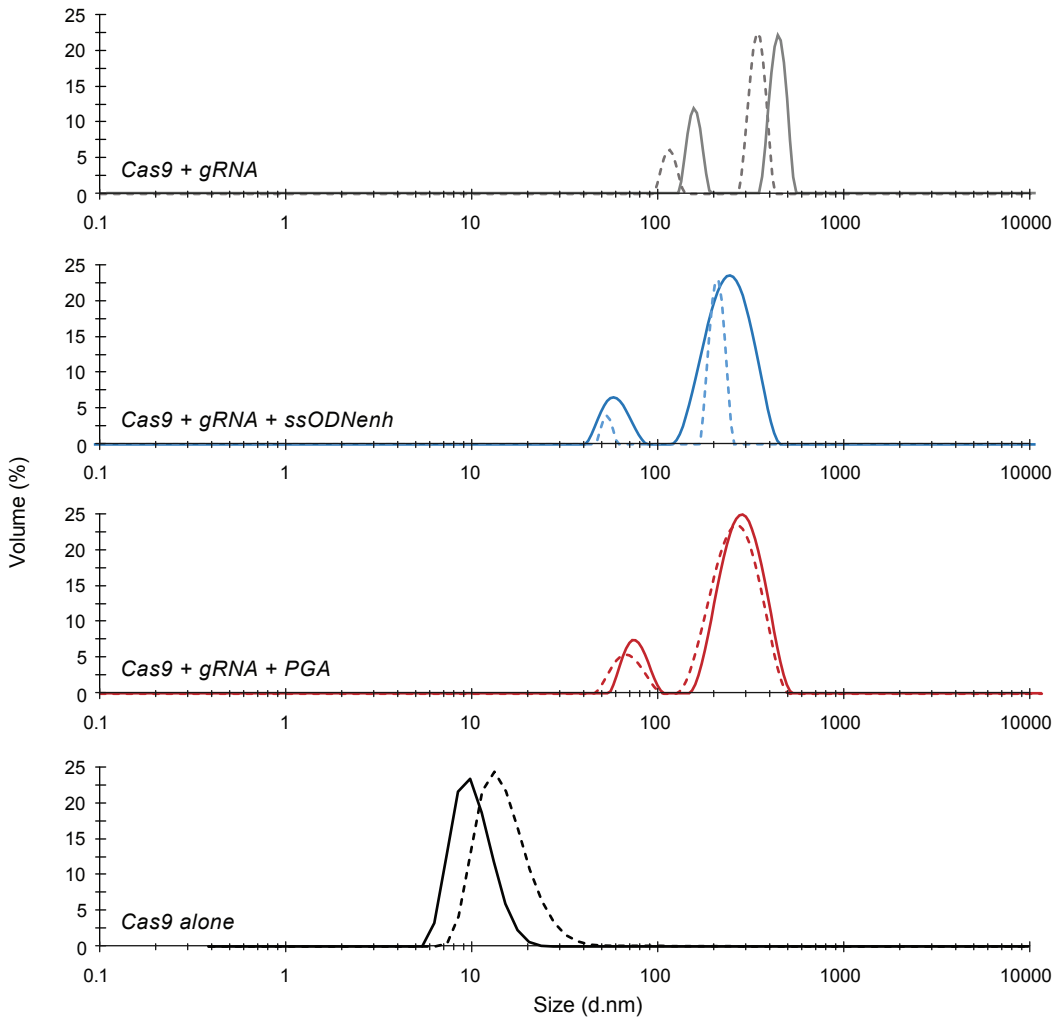
a**b**

Supplementary Fig. 7: Poly(glutamic acid) enhanced editing at higher molecular weights

PGA samples with narrowly-defined molecular weight were assessed for the ability to enhance *CD4* gene knockout editing. Polymers were reconstituted to 100mg/mL then serially diluted as indicated, mixed with RNPs formulated at a 1:1 gRNA:protein ratio, and electroporated into primary human CD4+ T cells.

(a) Loss of surface CD4 expression and **(b)** live cell count at 3 days post electroporation were assessed by flow cytometry. Higher molecular weight resulted in improved editing but reduced cell viability at the highest MW. Data shown for each of n=2 biologically independent blood donors.

Size Distribution by Volume

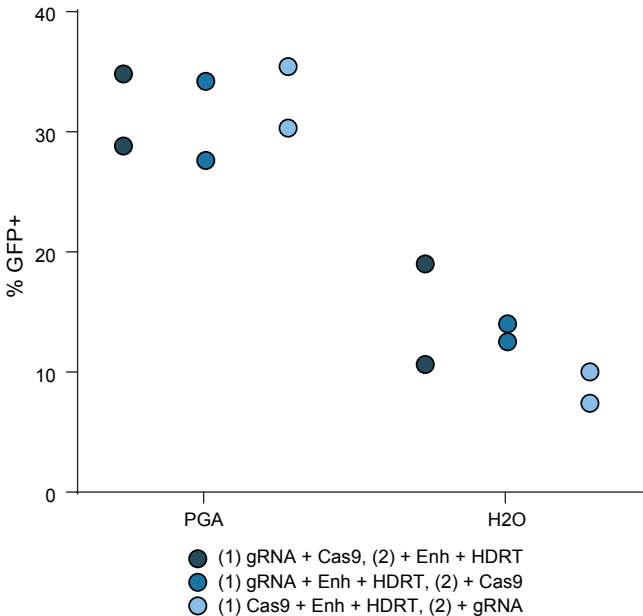


Sample	Z-avg (nm)	PDI	Peak 1 (nm)	Peak 1 (Volume %)	Peak 2 (nm)	Peak 2 (Volume %)
Cas9 + gRNA 1	177.50	0.343	217.90	68.10	62.80	31.90
Cas9 + gRNA 2	195.90	0.277	173.20	80.80	46.76	19.20
Cas9 + gRNA + ssODNenh 1	90.76	0.357	115.30	85.90	21.14	14.10
Cas9 + gRNA + ssODNenh 2	115.00	0.183	87.88	89.50	17.04	10.50
Cas9 + gRNA + PGA 1	77.87	0.317	116.40	87.10	23.21	12.90
Cas9 + gRNA + PGA 2	81.31	0.317	127.00	85.90	25.89	14.10
Cas9 alone 1	25.21	0.633	10.66	99.80	309.40	0.10
Cas9 alone 2	31.83	0.44	15.80	99.50	131.00	0.50

Supplementary Fig. 8: Anionic polymers stabilized and reduced the hydrodynamic size of RNP nanoparticles.

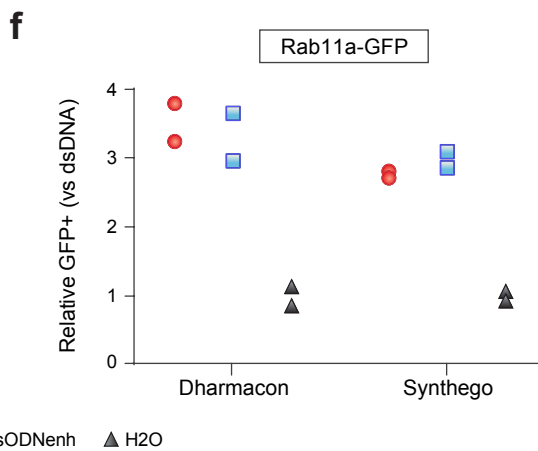
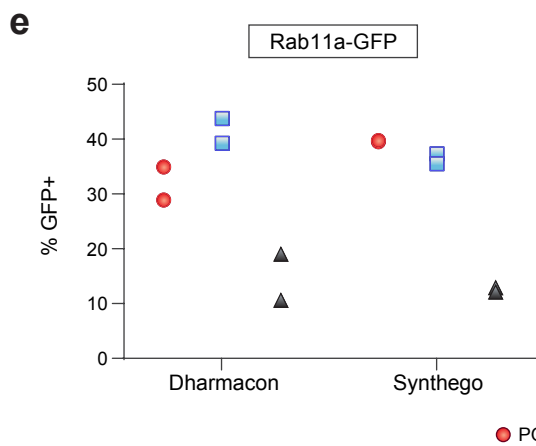
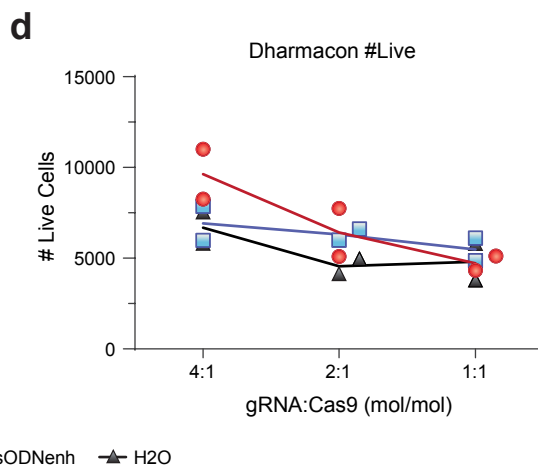
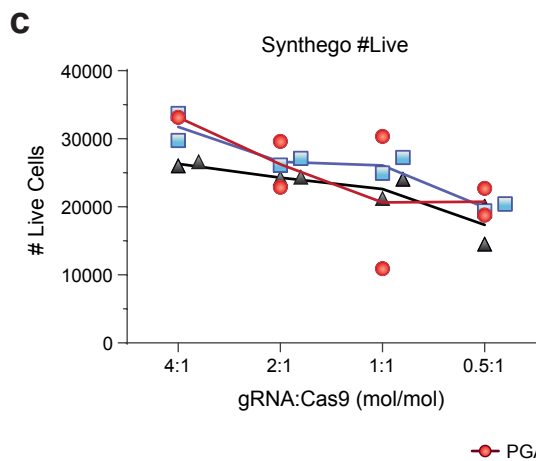
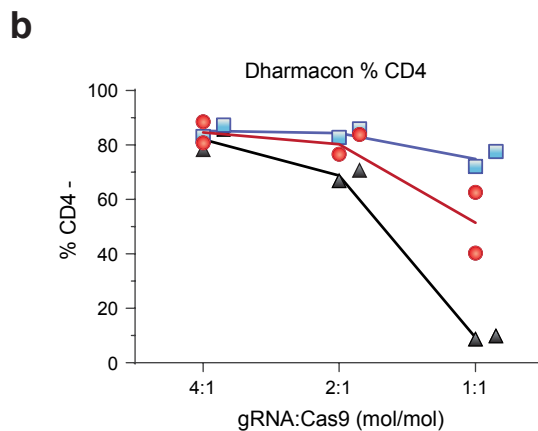
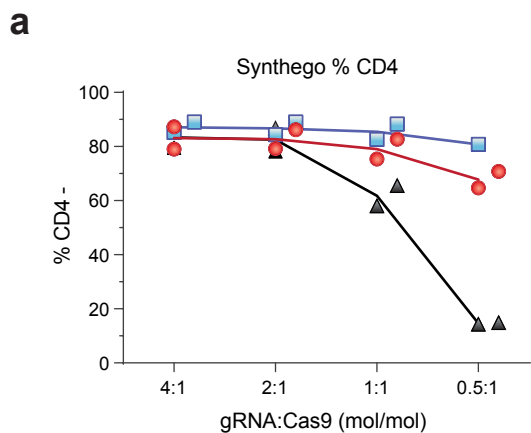
Cas9 RNPs were prepared at a 2:1 gRNA:protein ratio, mixed with PGA or ssODNenh, then assessed for particle size by dynamic light scattering. Size distribution by volume % and a summary table of statistics are shown for each of n=2 independent preparations averaged over ten repeated measurements (PDI = polydispersity index).

Order of addition



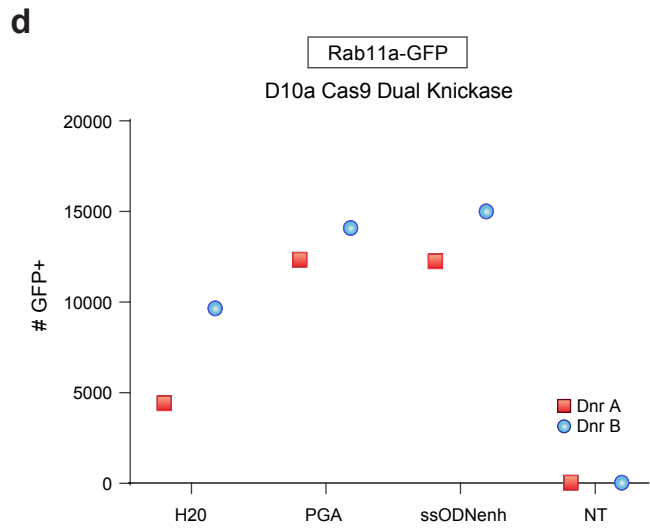
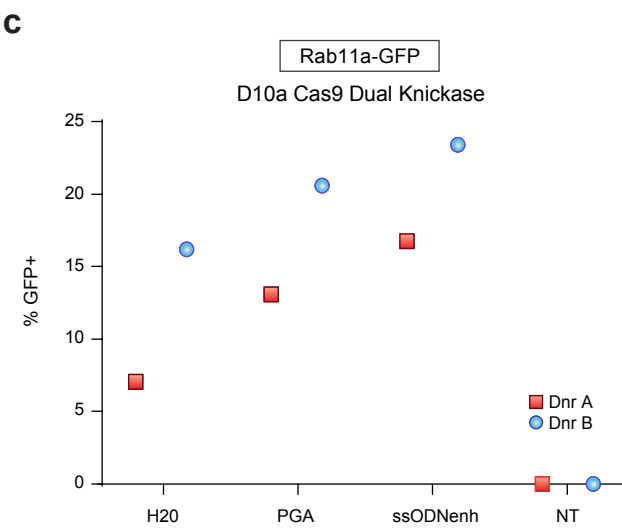
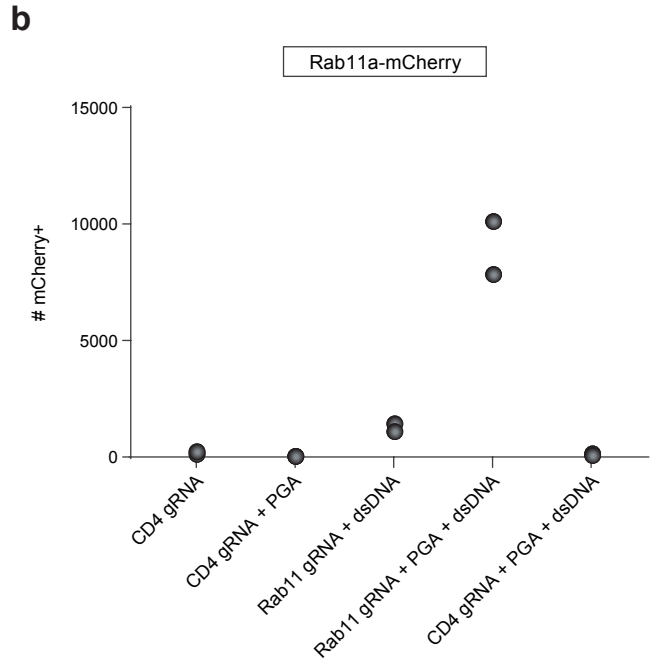
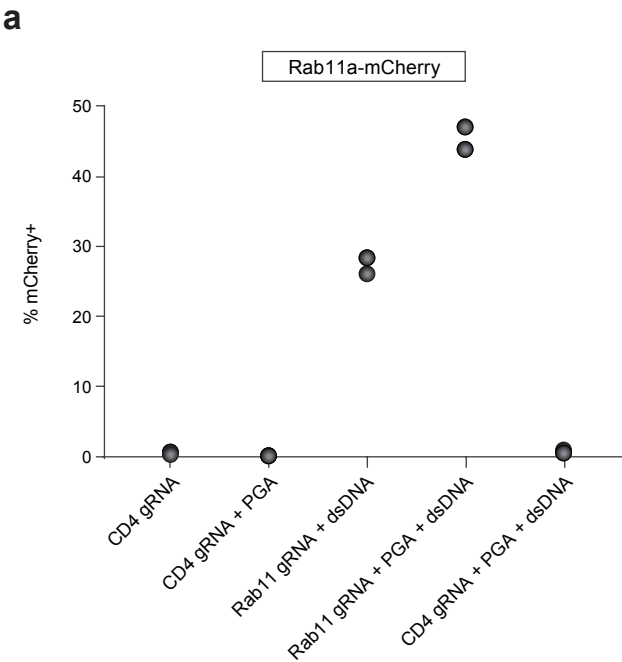
Supplementary Fig. 9: Order of addition did not affect editing efficiency.

Cas9 RNP were formulated at 2:1 gRNA:protein ratio with regular dsDNA HDR template targeting insertion of an N-terminal fusion of GFP to *RAB11A*. PGA polymer or water was added after RNP formation (navy), after gRNA formation but prior to adding Cas9 (blue), or mixed with the protein prior to adding gRNA (light blue). In all three cases, PGA improved editing efficiency but order of addition had no immediately discernable impact. However, we noted improved consistency and workflow when mixing PGA with gRNA first then adding Cas9 protein, and this protocol was adopted for subsequent studies. Data shown for each of n=2 biologically independent blood donors.



Supplementary Fig. 10: Both PGA and ssODNenh improved editing independent of guide RNA source.

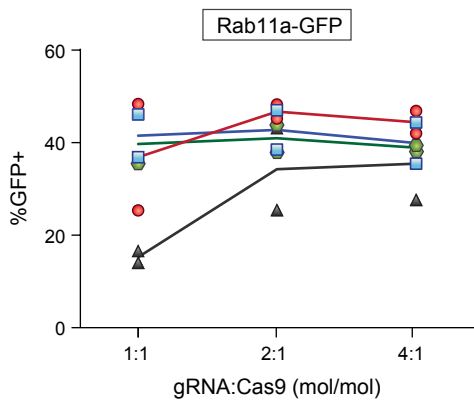
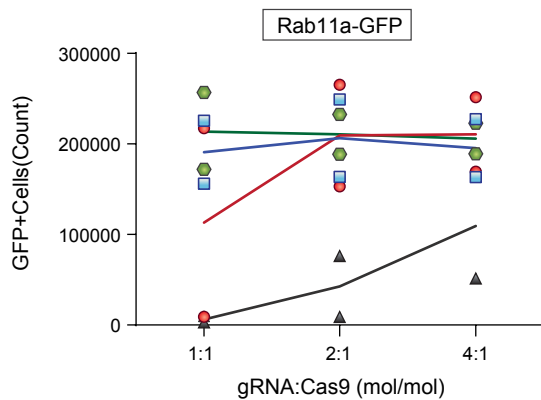
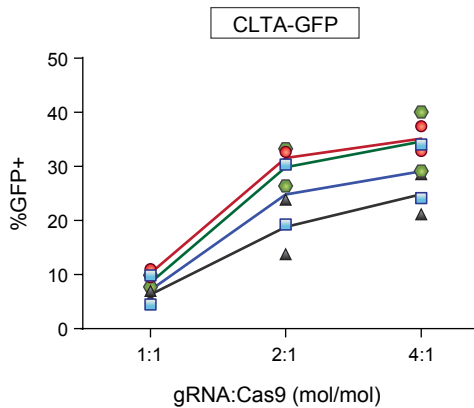
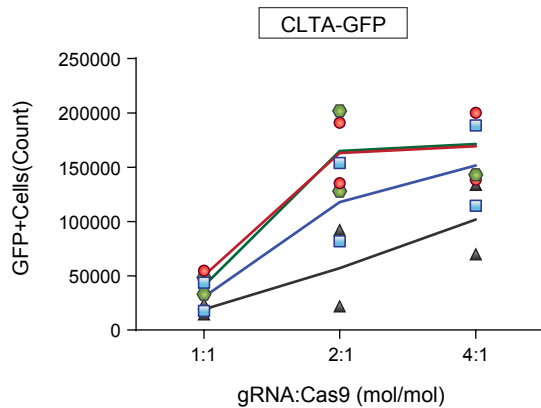
Cas9 RNPs were generated at the indicated RNA:protein ratio plus no enhancer (H₂O) or with either PGA or ssODNenh and electroporated into primary human CD4⁺ T cells. RNPs targeted *CD4* gene knock-out (**a-d**), or targeted insertion of an N-terminal fusion of GFP to *RAB11A* when mixed with 1ug of dsDNA HDR template (**e-f**). Loss of surface CD4 expression or GFP positivity and live cell count at 3 days was assessed by flow cytometry. Regardless of guide RNA type either single strand chemically synthesized sgRNA from Synthego (**a,c,e**), or chemically synthesized duplexed tracrRNA:crRNA gRNA from Dharmacon Horizon (**b,d,f**), the addition of either PGA or ssODNenh improved both knock-out and knockin editing efficiency assessed by flow cytometry at day 3. Data shown for each of n=2 biologically independent blood donors with a line connecting means (**a-d**).



Supplementary Fig. 11: Polyanions improved editing efficiency with variant Cas9 RNPs

(a-b) PGA improved editing efficiency with HiFi SpyCas9. A novel Cas9 variant, HiFi Cas9, was recently developed with reduced off-target activity but slightly reduced on-target efficiency¹⁴. HiFiCas9 RNPs were formulated with a guide RNA targeting either the *CD4* gene or *RAB11A* gene with or without PGA polymer at a 2:1 gRNA:protein ratio and electroporated into primary human bulk (CD3+) T cells together with an unmodified dsDNA HDR template for targeted integration of an N-terminal fusion of mCherry to *RAB11A*. **(a)** mCherry-expressing cells and **(b)** live cell count were quantified at day 3 by flow cytometry. PGA improved on-target editing efficiency while maintaining minimal detection of mCherry+ cells when using an off-target gRNA. Data shown for each of n=2 biologically independent blood donors.

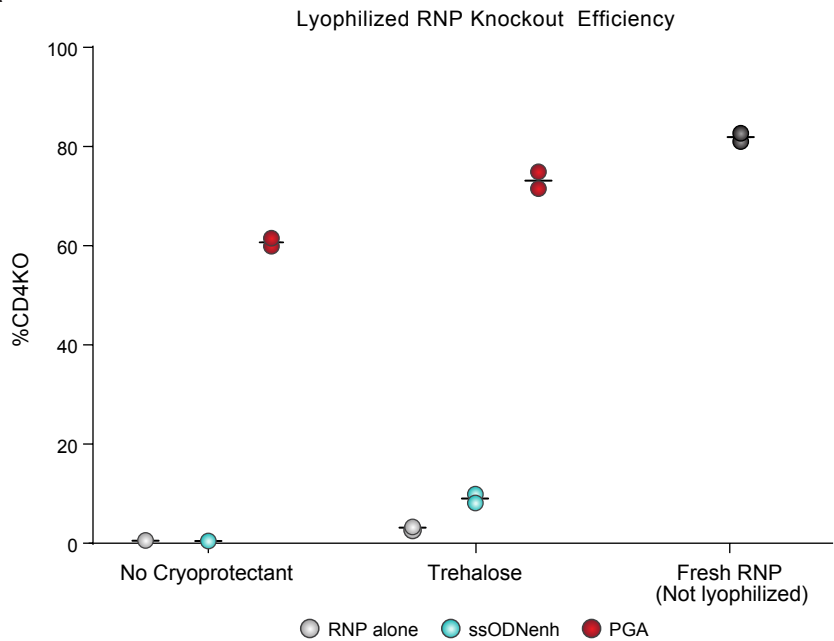
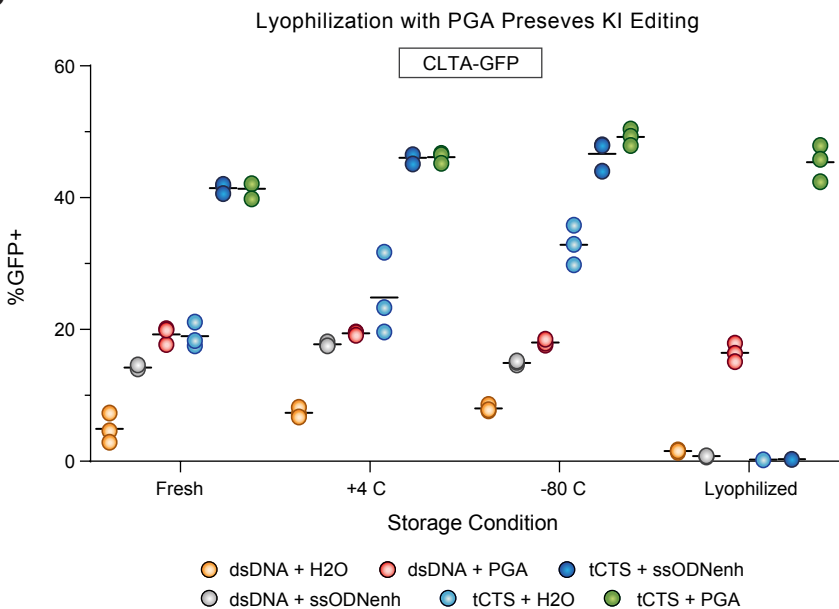
(c-d) PGA or ssODNenh improved editing efficiency with D10a 'nickase' variant Cas9 RNPs. A dual nickase approach to induce neighboring opposed single strand breaks has been proposed as a strategy to minimize off-target editing¹⁷. D10a Cas9 RNPs were formulated with guide RNAs as previously described⁵ targeting the *RAB11A* gene exon 1 with or without polymer at a 2:1 gRNA:protein ratio, and 50 pmol RNP were electroporated into primary human bulk (CD3+) T cells together with 1ug of regular dsDNA HDR template encoding insertion of an N-terminal fusion of GFP to *RAB11A*. **(c)** GFP-expressing cells and **(d)** live cell count were quantified at day 3 by flow cytometry. Data shown for each of n=2 biologically independent blood donors.

a**b****c****d**

■ ssODN
 ● PGA
 ● ssODN+PGA
 ▲ H2O

Supplementary Fig 12: Addition of ssODNenh to PGA did not further increase knockin efficiency at 2:1 or greater gRNA:Cas9 ratios.

Cas9 RNPs were generated at the indicated RNA:protein ratio plus no enhancer (H₂O), with either PGA or ssODNenh, or with a combination of PGA and ssODNenh at equivalent total dose, and electroporated into primary human CD3⁺ Bulk T cells along with 1ug of a standard dsDNA HDR template targeting insertion of an N-terminal fusion of GFP to *RAB11A* (**a-b**) or *CLTA* (**c-d**). Expression of GFP positivity and live cell count was assessed by flow cytometry at 4 days. Data shown for each of n=2 biologically independent blood donors with a line connecting means.

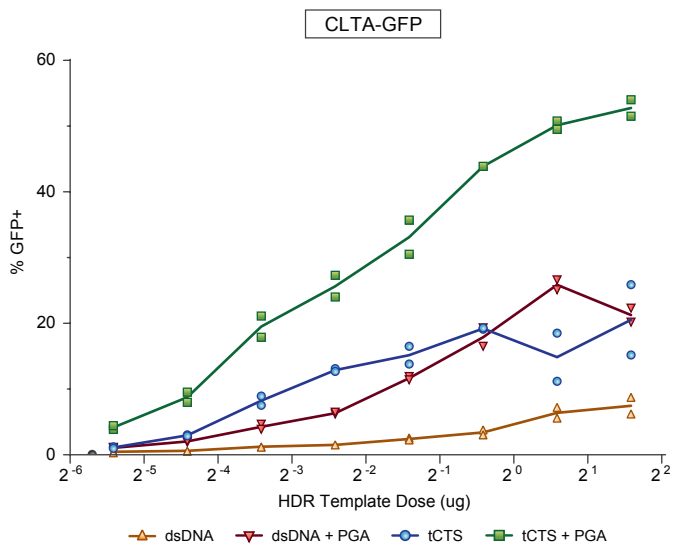
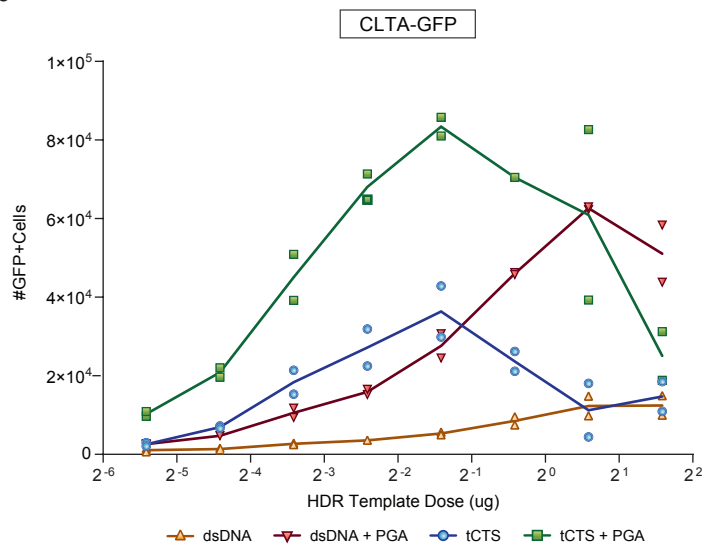
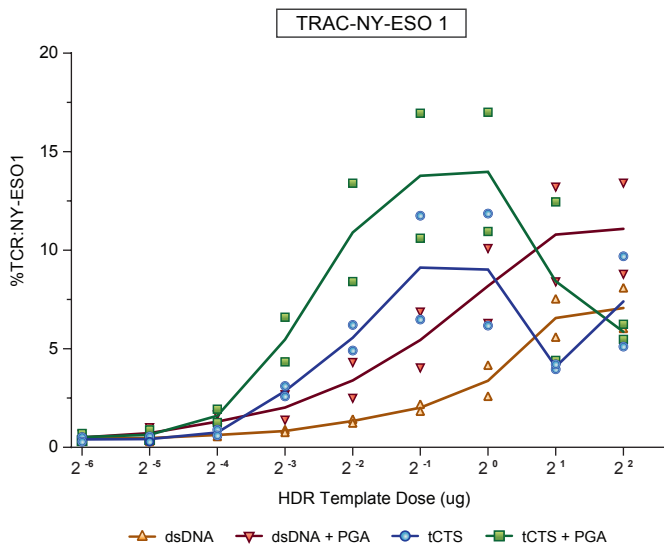
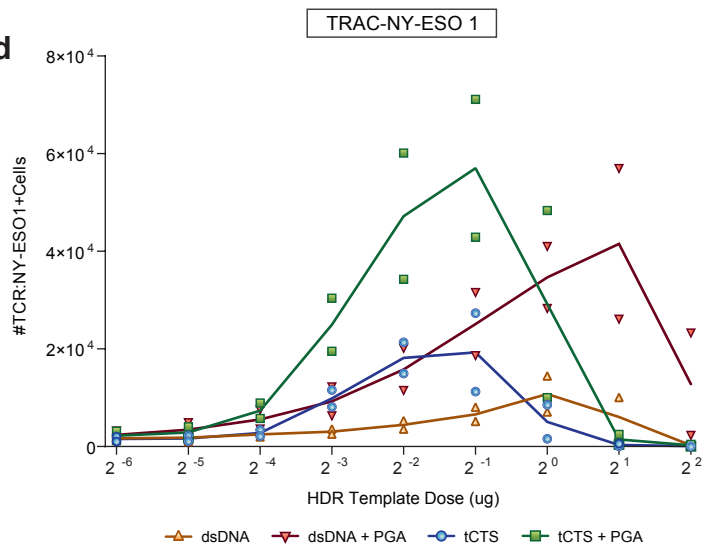
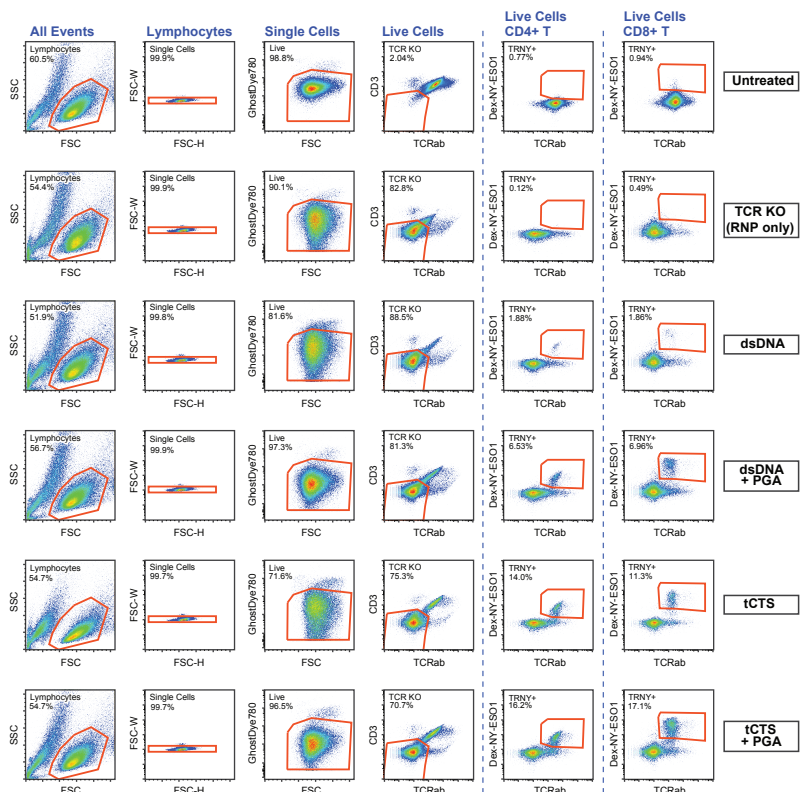
a**b**

Supplementary Fig. 13: PGA-stabilized Cas9 RNP nanoparticles could be lyophilized with retained knockout and knockin editing efficiency.

Cas9 RNP nanoparticles (with or without HDR template) that are stabilized with PGA, but not ssODNenh, were protected through lyophilization-reconstitution and retained robust knockout (a) and knockin (b) editing efficiency compared to freshly prepared RNPs. We hypothesize this is attributable to the capability of polypeptides to crystallize and form powders while long nucleic acids tend to form amorphous viscous solids at lower temperature.

(a) Cas9 RNPs targeting the *CD4* gene were prepared at 2:1 ratio of gRNA:protein without or with PGA or ssODNenh then lyophilized with or without Trehalose cryoprotectant. RNPs were later reconstituted in water, and a fresh batch of RNP was made for comparison prior to electroporating into primary human CD4+ T cells. Two technical replicates shown for n=1 blood donor, representative of three repeated independent experiments; center lines indicate mean.

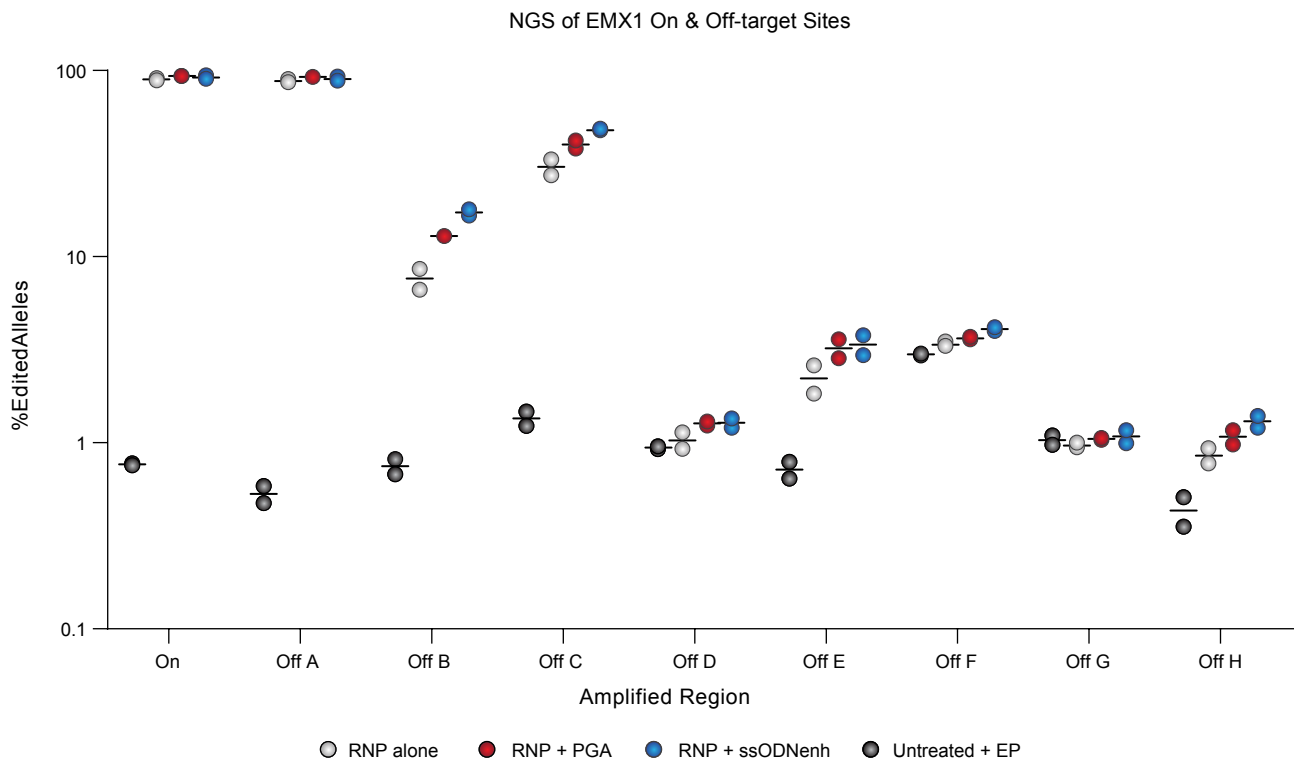
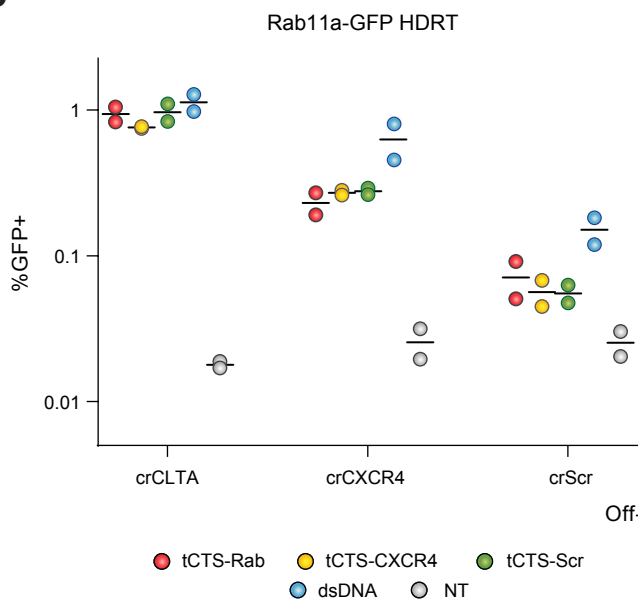
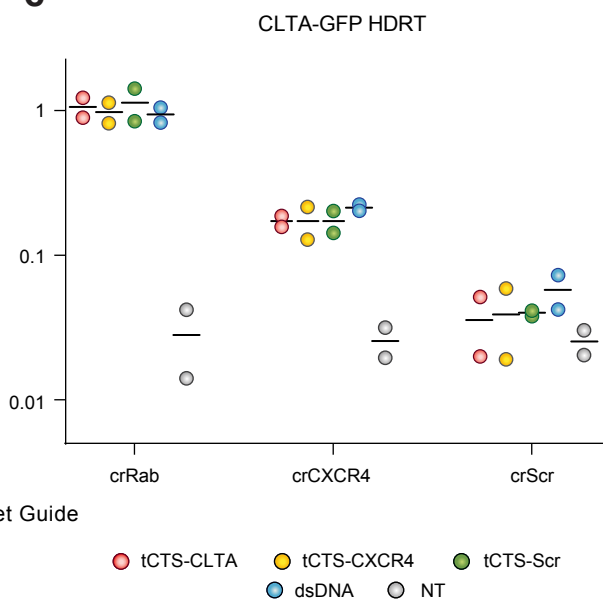
(b) Cas9 RNPs were prepared at 2:1 ratio gRNA:protein together with extra H₂O, PGA, or ssODNenh, then mixed with regular dsDNA HDR template (ds) or tCTS-modified HDR template (tCTS) targeting insertion of an N-terminal fusion of GFP to *RAB11A*. Samples were incubated at 37C to enhance RNP-HDR template interaction prior to storage at +4C or -80C for 2 days or immediate lyophilization. Lyophilized RNP-HDR templates were later reconstituted in water, and a fresh batch of RNP and HDR template were made for comparison prior to electroporating into primary human bulk (CD3+) T cells. Three technical replicates shown for n=1 blood donor, representative of two repeated independent experiments; center lines indicate mean.

a**b****c****d****e**

Supplementary Fig. 14: PGA-stabilized Cas9 RNP nanoparticles improved knockin.

A series of doses of unmodified HDR template or tCTS-modified HDR template were combined with 50 pmol of regular Cas9 RNP or PGA-stabilized Cas9 RNP then electroporated into primary human bulk (CD3+) T cells targeting insertion of a N-terminal fusion of GFP to *CLTA* (**a-b**) or replacement of the endogenous T cell receptor with a TCR targeting the NY-ESO 1 tumor antigen (**c-d**). The fraction of successful knockin edited cells (**a,c**) and the knockin edited cell count yield (**b,d**) were measured at day 3 (**a-b**) or day 4 (**c-d**) by flow cytometry. Data shown for each of n=2 biologically independent blood donors with a line connecting means.

(e) Flow cytometry analysis and gating strategy shown for NY-ESO 1 TCR replacement. The TCR-/CD3- population is gated for all T-cells, indicating a shift from knockout phenotype (resulting from NHEJ indel formation similar to the RNP alone TCR KO group) towards more successful HDR with tCTS-modified HDR templates and PGA-stabilized RNPs. Dextramer-NY-ESO 1 staining is shown separately gated on CD4+ or CD8+ T cells owing to the differential expression levels between the two cell types.

a**b****c**

Supplementary Fig. 15: Effects of anionic polymers and tCTS-modified HDR templates on off-target genome modifications

(a) The anionic polymers PGA or ssODNenh can increase NHEJ editing efficiency, including at off-target sites. CD3⁺ T cells isolated from two healthy donors were treated with regular, PGA-stabilized, or ssODNenh-stabilized RNPs incorporating a gRNA targeting the *EMX1* gene previously identified by CIRCLE-Seq to create multiple off-target double-strand breaks^{14,15}. Control cells were electroporated without RNP. On- and off-target genomic regions were PCR-amplified, and editing outcomes were quantified by deep sequencing with reads aligned and indels identified by CRISPresso2 (ignoring substitutions arising from PCR or sequencing errors)¹⁶. Off-target region A is a duplication of the entire gRNA target (with high efficiency editing), while off-target regions B-H have varying amounts of gRNA target homology.

(b-c) CD3⁺ T cells were treated with PGA-stabilized off-target gRNA RNPs and tCTS-modified HDR templates designed for targeting insertion of an N-terminal fusion of GFP to **(b)** *RAB11A* or **(c)** *CLTA*. Appending a tCTS designed to bind off-target gRNA sequences (as indicated in the legend) did not exacerbate off-target transgene expression regardless of which off-target gRNA was used (indicated on the x-axis). Transgene expression was measured as percentage of live cells expressing GFP at 4 days post electroporation; NT = background signal from non-treated cells.

Data shown for each of n=2 biologically independent blood donors; center lines indicate mean.

Supplementary Table 1 - List of HDR Template, DNA primers, gRNA sequences, and target amplicons used in this study.

For sequences please see uploaded MS Excel file.

Supplementary Table 2 - Polymers Tested

<u>Polymer</u>	<u>Abbreviation</u>	<u>MW (kDa)</u>	<u>Concentration (mg/mL)</u>	<u>Manufacturer</u>
polyethylenimine (branched)	bPEI	2	5	Sigma
poly(L-arginine) HCL	PLA	15-70	100	Sigma
poly(L-lysine) HBr	PLL	15-30	100	Sigma
poly(L-ornithine) HBr	PLO	30-70	100	Sigma
Protamine Sulfate	PS	>100 U/mg	10	Fresenius Kabi
Poly(ethylene glycol)	PEG-35	35	44	Sigma
Poly(L-glutamic acid)	PGA	15-50	100	Sigma
Poly(L-glutamic acid)	PLE20	3	100	Alamanda Polymers
Poly(L-glutamic acid)	PLE50	7.5	100	Alamanda Polymers
Poly(L-glutamic acid)	PLE100	15	100	Alamanda Polymers
Poly(L-glutamic acid)	PLE200	30	100	Alamanda Polymers
Poly(L-glutamic acid)	PLE800	120	100	Alamanda Polymers
Heparin	Hep	>180 U/mg	60	Sigma
Hyaluronic acid	HA-150	100-150	75	Lifecore Biomedical
Poly(acrylic acid)	PAA-5	5	180	Sigma
Poly(acrylic acid)	PAA-25	25	100	Sigma
Poly(acrylic acid)	PAA-250	250	72	Sigma
poly(L-aspartic acid)	PLD	27	13.3	Alamanda Polymers
ssODNenh electroporation enhancer*	ssODNenh	31	3.1	IDT Technologies

* ssODNenh sequence:

TTAGCTCTGTTTACGTCCCAGCGGGCATGAGAGTAACAAGAGGGTGTGGTAATATTACGG
TACCGAGCACTATCGATACAATATGTGTCATACGGACACG

Positively-charged polymers shaded in yellow, neutral polymers shaded in green, negatively-charged polymers shaded in blue.

Supplementary Table 3 – Antibodies Used in Study

Target	Fluor	Vendor	Clone	Catalog Number
Fc Receptor	-	Biolegend	Human TruStain FcX	422302
GhostDye Red 780	-	Tonbo	-	13-0865-T500
GhostDye Violet 510	-	Tonbo	-	13-0870-T500
LIVE/DEAD® Fixable Aqua Dead Cell Stain	-	ThermoFisher Scientific	-	L34966
CD19	PacBlue	Biolegend	HIB19	302223
CD271 (tNGFR)	APC	Biolegend	ME20.4	345108
CD3	AlexaFluor 700	Becton-Dickson	UCHT1	557943
CD3	PE	Biolegend	UCHT1	300408
CD34	PE-Cy7	Becton-Dickson	8G12	348791
CD4	PE-Cy7	Biolegend	OKT4	317414
CD4	FITC	Biolegend	SK3	344604
CD4	PerCP	Tonbo	SK3	67-0047-T500
CD56	PerCP	Biolegend	HCD56	318342
CD8	APC	Tonbo	OKT8	20-0086-T100
CD8	PE-Cy7	Becton-Dickson	SK1	335787
TCR- 1G4	PE	Immudex	HLA-A*0201/SLLMWITQV	WB3247-PE
TCR-gd	PE-Cy7	Biolegend	B1	331221
TCR-αβ	BV-421	Biolegend	IP26	306722

Supplementary Table 4 – Statistics for Figure 1

For individual p values please see uploaded MS Excel file.