APPENDIX

Appendix Figure S1. Donor CD4+ cells engraftment in lymphoid organs after adoptive transfer of \textit{ex vivo} cultured T cells.

Appendix Table S1. Off-target analysis of the selected \textit{CD40LG} gRNA.

Appendix Table S2. Detection of \textit{P. murina} infection by immunofluorescence, immunohistochemistry and ddPCR after HSPC therapy

Appendix Table S3. Detection of \textit{P. murina} infection by immunofluorescence, immunohistochemistry and ddPCR after T-cell therapy.

Appendix Table S4. List of antibodies used in this study.

Appendix Table S5. List of primers used in this study.

Appendix Table S6. List of gRNAs tested in this study.

Supplementary Statistical Methods. Details of statistical analyses.
Appendix Figure S1. Donor CD4+ cells engraftment in lymphoid organs after adoptive transfer of *ex vivo* cultured T cells.

A Percentage of engrafted donor CD45.1+ cells in spleen and lymph nodes of mice from Fig 6B (n=4 NC, 4 CPA 300, 4 CPA 200, 5 ALS, 6 ANTI-CD4). Median ± IQR.

B Percentage of engrafted primed donor CD45.1+ cells in spleen and lymph nodes of mice from Fig 6F (n=6 NC, 11 CPA 300, 9 CPA 200). Kruskal-Wallis test followed by post-hoc analysis with Dunn’s test. P-values were adjusted with Bonferroni’s correction to account for multiple comparisons (*p=0.0213 and *** p=0.0007). Median ± IQR.

Appendix Table S1. Off-target analysis of the selected *CD40LG* gRNA.

| OT#*   | Location                  | Guide-Seq | Digenome | In silico | Amp-seq | Average off-target editing |
|--------|---------------------------|-----------|----------|-----------|---------|----------------------------|
| OT-1   | Chr8: ~300 kb from gene   | Yes       | Yes      | No        | 3/3 donors | 0.24%                      |
| OT-2   | Chr5: Intron of DMXL1     | Yes       | Yes      | Yes       | 1/3 donors | 0.13%                      |
| OT-3   | Chr8: Intron of SPIDR     | Yes       | Yes      | No        | No      | n.d.                       |
| OT-4   | Chr6: >50 kb from gene    | Yes       | Yes      | No        | No      | n.d.                       |
| OT-5   | Chr15: ~30 kb from gene   | No        | Yes      | No        | No      | n.d.                       |
| OT-6   | Chr7: ~200 kb from gene   | No        | Yes      | No        | No      | n.d.                       |
Top six of 93 analyzed putative off-targets of S.p. g1 gRNA. Full list in Dataset S1. N.d.: not detectable.

**Appendix Table S2.** Detection of *P. murina* infection by immunofluorescence, immunohistochemistry and ddPCR after HSPC therapy

| Group  | IF for *P. murina* on lung homogenates | IHC for *P. murina* on histological sections of lungs | ddPCR | Histopathology (H&E): *P. murina*-associated interstitial pneumonia |
|--------|----------------------------------------|-----------------------------------------------------|-------|---------------------------------------------------------------|
| 100% WT | -                                      | -                                                   | 0.046 | -                                                             |
|        | -                                      | -                                                   | 0     | -                                                             |
|        | -                                      | -                                                   | 0     | -                                                             |
|        | -                                      | -                                                   | 0.009 | -                                                             |
|        | -                                      | -                                                   | 0     | -                                                             |
|        | -                                      | -                                                   | 0.039 | -                                                             |
|        | +                                      | -                                                   | 0.029 | -                                                             |
|        | -                                      | -                                                   | 0     | -                                                             |
| 10% WT  | -                                      | -                                                   | 0.03  | -                                                             |
|        | +++                                    | +++                                                 | 14.987| +                                                             |
|        | +                                      | +                                                   | 0.567 | -                                                             |
|        | +                                      | -                                                   | 0.013 | -                                                             |
|        | -                                      | -                                                   | 0.005 | -                                                             |
|        | -                                      | -                                                   | 0     | -                                                             |
| 25% WT  | -                                      | -                                                   | 0.089 | -                                                             |
|        | +++                                    | +++                                                 | 38.564| +++                                                           |
|        | -                                      | -                                                   | 0.167 | -                                                             |
|        | +                                      | +                                                   | 0.238 | -                                                             |
|        | +                                      | -                                                   | 0.272 | -                                                             |
|        | -                                      | -                                                   | 0.007 | -                                                             |
|        | +                                      | +                                                   | 0.419 | +                                                             |
| 0% WT   | +                                      | -                                                   | 1.943 | -                                                             |
|        | +                                      | +                                                   | 4.379 | +                                                             |
|        | +++                                    | +++                                                 | 91.03 | +++                                                           |
|        | +                                      | +                                                   | 0.064 | -                                                             |
|        | -                                      | -                                                   | 0.01  | -                                                             |
|        | +++                                    | +++                                                 | 121   | +++                                                           |
|        | +                                      | -                                                   | 0.512 | -                                                             |
|        | +                                      | +                                                   | 0.856 | +                                                             |

**Appendix Table S3.** Detection of *P. murina* infection by immunofluorescence, immunohistochemistry and ddPCR after T-cell therapy.
Group | IF for *P. murina* on lung homogenates | IHC for *P. murina* on histological sections of lungs | ddPCR | Histopathology (H&E): *P. murina* associated interstitial pneumonia
---|---|---|---|---
+ T cells | - | - | 0.008 | -
| - | - | 0.013 | -
| - | - | 0.003 | -
| - | - | 0.001 | -
| - | - | 0.003 | -
| - | - | 0.002 | -
| - | - | 0.023 | -
| - | - | 0.002 | -
| - | - | 0.015 | -

CD40LG | +++ | +++ | 18.498 | +++
| - | - | 0.013 | -
| ++ | +++ | 28 | +++
| - | - | 1.555 | -
| +++ | ++ | 10.616 | -
| nd | +++ | 180 | +++
| +++ | ++ | 6.826 | +
| - | + | 0.036 | -

* Immunofluorescence on homogenate from lung tissue
**amount of positive material with morphological pattern compatible with *P. murina*

Scoring system: - absent; + minimal; ++ moderate; +++ severe (abundant); nd: not determined because of artifacts

Appendix Table S4. List of antibodies used in this study.

| Antibody | Fluorochrome | Clone | Company |
|---|---|---|---|
| **Anti-human antibodies** |
| CD16/32 | none | | Miltenyi Biotec |
| CD271 (LNGFR) | APC | ME20.4-1.H4 | Miltenyi Biotec |
| CD271 (LNGFR) | PE | ME20.4-1.H4 | Miltenyi Biotec |
| CD271 (LNGFR) | PB | ME20.4-1.H4 | Miltenyi Biotec |
|---------------|----|-------------|-----------------|
| Anti-hEGFR    | Biotin conjugated | #HU1 | R&D Systems |
| Anti-biotin   | APC | Bio3-18E7 | Miltenyi Biotec |
| Anti-biotin   | FITC | Bio3-18E7 | Miltenyi Biotec |
| CD154         | PE | 24-31 | Invitrogen |
| CD62L         | PE | DREG-56 | BD |
| CD62L         | APC | DREG-56 | Biolegend |
| CD45RA        | PB | T6D11 | Miltenyi Biotec |
| CD45RA        | APC | HI100 | Biolegend |
| CD45RA        | FITC | L48 | BD |
| CD133/2       | PE | 293C3 | Miltenyi Biotec |
| CD34          | PB | AC136 | Miltenyi Biotec |
| CD34          | PECy7 | 8G12 | BD |
| CD90          | APC | 5E10 | BD |
| CD45          | PB | HI30 | Biolegend |
| CD45          | APCH7 | HI30 | eBioscience |
| CD19          | PE | HIB19 | BD |
| CD19          | FITC | 4G7 | BD |
| CD19          | PECy7 | HIB19 | Biolegend |
| CD19          | APC | HIB19 | Biolegend |
| CD19          | PE-Vio770 | SJ25C1 | BD |
| Antigen | Fluorescent Label | Clone | Manufacturer |
|---------|------------------|-------|--------------|
| CD3     | PEcy7            | HIT3a | Biolegend    |
| CD3     | APC              | UCHT1 | BD           |
| CD3     | FITC             | BW264/56 | Miltenyi Biotec |
| CD13    | APC              | WM15  | BD           |
| CD33    | PEcy7            | P67.6 | BD           |
| CD38    | PerCP            | HB7   | Biolegend    |
| CD4     | PB               | RPA-T4 | BD           |
| CD4     | PerCP            | VIT4  | Miltenyi Biotec |
| CD8     | APCH7            | SK1   | BD           |
| CD69    | APC-Vio770       | FN50  | Miltenyi Biotec |
| CD25    | APC              | M-A251 | Biolegend    |
| CD197 (CCR7) | FITC         | REA546 | Miltenyi Biotec |
| CD45RO  | PEC7             | UCHL1 | Biolegend    |
| CD95    | APC-Vio770       | REA738 | Miltenyi Biotec |
| IgM     | PB               |       | Biolegend    |
| IgG     | PE               |       | Jackson ImmunoResearch |

**Anti-mouse antibodies**

| Antigen | Fluorescent Label | Clone | Manufacturer |
|---------|------------------|-------|--------------|
| CD16/32 | none             | 2.4G2 | BD           |
| CD11b   | APC-Cy7          | M1/70 | Biolegend    |
| CD150   | APC              | TC15-12F12.2 | Biolegend    |
| Description                  | Orientation | Sequence (5’-3’)          |
|------------------------------|-------------|---------------------------|
| dsDNA donor template linearization | FW          | GCAGGAACAAAAGAGCAGAG      |
|                              | RV          | ACCATTTTCTTGTCTTTAAGAGTAG |

**Appendix Table S5.** List of primers used in this study.
| NHEJ CD40LG | FW | CTCACAAGCACTGATTGTAGTTGC |
|-------------|----|--------------------------|
| RV         |    | CCAACACAAATAACCAACCAGACC |

| CD40LG integrations with 3’ UTR as right homology arm | FW | TACATCTTTCCGACTGCTGAAG |
|------------------------------------------------------|----|------------------------|
| RV                                                  |    | ACTGAATAGGCTAGCACATCATC |

| CD40LG 5’ HDR integration junction ddPCR (except for different splice acceptors constructs) | FW | TTAGGAGGGGGTCTGATACA |
|------------------------------------------------------------------------------------------------|----|---------------------|
| RV                                                                                           |    | TCCTCGATCTGTGGGAGGAAGAGAA |

| CD40LG 5’ HDR integration junction ddPCR (different splice acceptors constructs) | FW | TCCGCTGCCAGATCTCTCGA |
|---------------------------------------------------------------------------------|----|---------------------|
| RV                                                                              |    | TCCTCGATCTGTGGGAGGAAGAGAA |

| CD40LG wild-type mRNA detection ddPCR | FW | GCACATGTCATAAGTGAGGC |
|-------------------------------------|----|---------------------|
| RV                                 |    | CCCATTTTCCAGGGTTACCA |

| CD40LG codon optimized mRNA (edited) detection ddPCR | FW | CCAGATGATTGGGTCAGCA |
|-----------------------------------------------------|----|---------------------|
| RV                                                  |    | TCTTCATGAACACGAGATCCT |

| P. Murina Ribosomal RNA Detection | FW | ATGAGGTGAAAAGTCGAAAGGG |
|----------------------------------|----|------------------------|
| RV                               |    | TGAGGTCTCAGATGAAAAACCTCTT |

| OT chr8 – First step PCR | FW | GTTAATGGGTGTTTATTTCACTATTCTTGC |
|-------------------------|----|--------------------------------|
| RV                      |    | TGAAACTATCTCTGTGGTTATAAAAAGGC |
| OT chr8 - P5 NGS 1   | FW  | AATGATACGCGACCACCCGAGATCTACACTAGA TCGCNNWNNWNNACACTCTTCTTCCCTACAGCAGC GCTCTTTCCGATCT GTTAATGGGGTTTATTTCACCTATTTCTTGC |
| OT chr8 - P5 NGS 2   | FW  | AATGATACGCGACCACCCGAGATCTACACCTCT CTATNNWNNWNNACACTCTTCTTCCCTACACGAC GCTCTTTCCGATCT GTTAATGGGGTTTATTTCACCTATTTCTTGC |
| OT chr8 - P5 NGS 3   | FW  | AATGATACGCGACCACCCGAGATCTACACTATC CTCTNNWNNWNNACACTCTTCTTCCCTACACGAC GCTCTTTCCGATCT GTTAATGGGGTTTATTTCACCTATTTCTTGC |
| OT chr8 - P5 NGS 4   | FW  | AATGATACGCGACCACCCGAGATCTACACAGA GTAGANNWNNWNNACACTCTTCTTCCCTACACGAC CGCTCTTTCCGATCTGTTAATGGGGTTTATTTCACCTATTTCTTGC |
| OT chr8 – P7 NGS 1   | RV   | CAAGCAGAAGACGCGATACGAGATTCGCTCTTA GTGACTGGAGTCTCTCTATGGGCAGTCGGTGATGAAACTATCTCTGTTGTTTATAAAGC |
| OT chr8 – P7 NGS 2   | RV   | CAAGCAGAAGACGCGATACGAGATCTAGTACG GTGACTGGAGTCTCTCTATGGGCAGTCGGTGATGAAACTATCTCTGTTGTTTATAAAGC |
| OT chr8 – P7 NGS 3   | RV   | CAAGCAGAAGACGCGATACGAGAATTCTGCTG TGACTGGAGTCTCTCTATGCGGAGTCGGTGATGAAACTATCTCTGTTGTTTATAAAGC |
### Appendix Table S6. List of gRNAs tested in this study.

| Name | Sequence | Cas9 |
|------|----------|------|
| g1   | TGGATGATTGCACCTTTATCA | Pyogenes |
| g2   | TTTTCTAACAGGATAAGGTG | Pyogenes |
| g3   | CAGTGGACTGATATTACC | Pyogenes |
| g4   | AGTGAGGGCTGAAGTCATCCA | Aureus |
| g5   | TGGGTTATCCAAATATTAGGT | Aureus |
| g6   | CAATGAGAAATGTGACAATTA | Aureus |
| g7   | AGAATAGCTCTGATTTCTACC | Aureus |

### Supplementary Statistical Methods. Details of statistical analyses.

**Nonlinear mixed-effects model analysis of longitudinal data in Fig 5B and 5C**

Due to the type of longitudinal trajectories of data in Fig 5B and 5C, the comparison of the longitudinal trend of the two groups was performed with a nonlinear mixed-effects (NLME) regression with the following asymptotic model:

\[ \sqrt{Y} = \text{Asym} + (R_0 - \text{Asym}) e^{(-\text{Time}\cdot e^{lrc})}, \]

where the time was considered as continuous with the origin shifted at day 1 (for a better interpretation of the parameters of the model), while the dependent variable \( Y \) was transformed in the square root scale to meet the assumption of normality of the residuals. In the model, the parameter \( \text{Asym} \) represents the horizontal asymptote (i.e. the value of the plateau reached by \( \sqrt{Y} \)). The parameter \( R_0 \) represents the value at day 1 and the \( lrc \) parameter is the natural logarithm of
the rate constant. For evaluating differences between the two groups, in the full model, all parameters were allowed to depend on the group variable:

\[ Asym = \beta_0 + \beta_1(NC), R0 = \gamma_0 + \gamma_1(NC), lrc = \delta_0 + \delta_1(NC) \]

where \( NC = 1 \), for NC group, and \( NC = 0 \) for CPA 300 group. For each dependent variable, the final model was obtained with a backward variable selection of fixed-effects covariates (where p-values less than 0.05 were considered significant). Random effects were set on the asymptote to account for mice heterogeneity. When necessary few observations were excluded from the analysis since they were outliers for the full model.

**Final NLME model for n. recipient CD3+ cells data in Fig 5B (excluded 11 outlier observations from the analysis):**

\[ Asym = \beta_0 + \beta_1(NC), R0 = \gamma_0 + \gamma_1(NC), lrc = \delta_0 \]

| Parameter | Estimate | p-value |
|-----------|----------|---------|
| \( \beta_0 \) | 1.6549 | <0.0001 |
| \( \beta_1 \) | -0.3933 | 0.0297 |
| \( \gamma_0 \) | 0.2328 | 0.0001 |
| \( \gamma_1 \) | 1.0624 | <0.0001 |
| \( \delta_0 \) | -4.3562 | <0.0001 |

**Final NLME model for n. donor T cells data in Fig 5C (excluded 8 outlier observations from the analysis):**

\[ Asym = \beta_0 + \beta_1(NC), R0 = \gamma_0 + \gamma_1(NC), lrc = \delta_0 + \delta_1(NC) \]

| Parameter | Estimate | p-value |
|-----------|----------|---------|
| \( \beta_0 \) | 0.4649 | <0.0001 |
| \( \beta_1 \) | -0.2992 | <0.0001 |
| \( \gamma_0 \) | 0.1608 | <0.0001 |
| \( \gamma_1 \) | 0.1079 | 0.0001 |
| \( \delta_0 \) | -2.2529 | <0.0001 |
| \( \delta_1 \) | -2.3329 | 0.0130 |

**Linear mixed-effects model analysis of data in Fig 5F, 6E, 6H and 7C**

For evaluating differences of IgG concentration among groups overall and, within each group, between pre and post values, a full linear mixed-effects (LME) model was estimated, followed by a post-hoc analysis with the R package phia. In each post-hoc analysis, p-values were adjusted with Bonferroni’s correction to account for multiple comparisons or testing, depending on the analysis. The full LME model included the following terms: time (pre vs post), group and an interaction term between time and group. When necessary, to meet the assumptions of the LME model, an adequate transformation of the dependent variable was used (the natural logarithmic transformation for data of first boost in Fig 5F and 6E and data of Fig 7C and the square root transformation for data of second boost in Fig 6H). Random effects of the LME model were set on the intercept term and they were defined either to account only for the variability among mice in case of a single experiment (data of Fig 6H), or as nested to account for the variability among
experiments and among mice within each experiment (data of Fig 5F, 6E and 7C). Only the results of post-hoc analysis are reported.

**Post-hoc analysis of IgG concentration of first boost in Fig 5F:**

**Post-hoc analysis testing the overall difference among groups:**

| Group comparison   | Estimated difference | P-value   |
|--------------------|----------------------|-----------|
| NC vs CPS 300      | -2.0480              | <0.0001   |
| NC vs CD40LG       | 1.4230               | <0.0001   |
| NC vs WT           | -4.4195              | <0.0001   |
| CPA 300 vs CD40LG  | 3.4711               | <0.0001   |
| CPA 300 vs WT      | -2.3714              | <0.0001   |
| CD40LG vs WT       | -5.8425              | <0.0001   |

**Post-hoc analysis testing the differences between pre vs post values for each group:**

| Group   | Estimated difference | P-value |
|---------|----------------------|---------|
| NC      | 0.8318               | <0.0001 |
| CPA 300 | -0.1799              | 1.0000  |
| CD40LG  | 0.6498               | 0.0201  |
| WT      | -0.7004              | 0.0100  |

Post-hoc analysis of IgG concentration of first boost in Fig 6E (excluded group CPA 200 from the analysis because n=4):

**Post-hoc analysis testing the overall difference among groups:**

| Group comparison   | Estimated difference | P-value   |
|--------------------|----------------------|-----------|
| NC vs ALS          | -0.7029              | 0.3639    |
| NC vs ANTI-CD4     | -1.2905              | 0.0002    |
| NC vs CPA 300      | -1.2066              | <0.0001   |
| NC vs CD40LG       | 1.9892               | <0.0001   |
| NC vs WT           | -4.0915              | <0.0001   |
| ALS vs ANTI-CD4    | -0.5876              | 1.0000    |
| ALS vs CPA 300     | -0.5037              | 1.0000    |
| ALS vs CD40LG      | 2.6921               | <0.0001   |
| ALS vs WT          | -3.3886              | <0.0001   |
| ANTI-CD4 vs CPA 300| 0.0840               | 1.0000    |
| ANTI-CD4 vs CD40LG | 3.2797               | <0.0001   |
| ANTI-CD4 vs WT     | -2.8010              | <0.0001   |
| CPA 300 vs CD40LG  | 3.1957               | <0.0001   |
| CPA 300 vs WT      | -2.8850              | <0.0001   |
| CD40LG vs WT       | -6.0807              | <0.0001   |

**Post-hoc analysis testing the differences between pre vs post values for each group:**
| Group  | Estimated difference | P-value |
|--------|----------------------|---------|
| UT     | 0.8956               | <0.0001 |
| ALS    | 1.0212               | <0.0001 |
| ANTI-CD4 | 0.6644             | 0.0022  |
| CPA 300| -0.5643              | <0.0001 |
| CD40LG | 1.0779               | <0.0001 |
| WT     | -1.0489              | <0.0001 |

Post-hoc analysis of IgG concentration of first boost in Fig 6H:

Post-hoc analysis testing the overall difference among groups:

| Group comparison | Estimated difference | P-value |
|------------------|----------------------|---------|
| NC vs CD40LG     | 494.41               | 0.2545  |
| NC vs CPA 300    | -167.56              | 1.0000  |
| NC vs CPA 200    | -307.53              | 0.0787  |
| CD40LG vs CPA 300| -661.96              | 0.0282  |
| CD40LG vs CPA 200| -801.94              | 0.0037  |
| CPA 300 vs CPA 200| -139.98              | 1.0000  |

Post-hoc analysis testing the differences between pre vs post values for each group:

| Group  | Estimated difference | P-value |
|--------|----------------------|---------|
| NC     | -141.570             | 0.5027  |
| CD40LG | 10.608               | 1.0000  |
| CPA 300| -126.274             | 0.2576  |
| CPA 200| -133.687             | 0.2009  |

Post-hoc analysis of IgG concentration of second boost in Fig 6H:

Post-hoc analysis testing the overall difference among groups:

| Group comparison | Estimated difference | P-value |
|------------------|----------------------|---------|
| NC vs CPA 300    | -3.0329              | 0.7276  |
| NC vs CPA 200    | -3.4306              | 0.6091  |
| CPA 300 vs CPA 200| -0.3977              | 1.0000  |

Post-hoc analysis testing the differences between pre vs post values for each group:

| Group  | Estimated difference | P-value |
|--------|----------------------|---------|
| NC     | -10.028              | <0.0001 |
| CPA 300| -11.853              | <0.0001 |
| CPA 200| -11.768              | <0.0001 |

Post-hoc analysis of IgG concentration of first boost in Fig 7C:
Post-hoc analysis testing the overall difference among groups:

| Group comparison | Estimated difference | P-value |
|------------------|----------------------|---------|
| 0% vs 1%         | -0.3086              | 1.0000  |
| 0% vs 10%        | -2.8160              | <0.0001 |
| 0% vs 25%        | -3.3936              | <0.0001 |
| 0% vs 100%       | -4.4820              | <0.0001 |
| 1% vs 10%        | -2.5074              | <0.0001 |
| 1% vs 25%        | -3.0850              | <0.0001 |
| 1% vs 100%       | -4.1733              | <0.0001 |
| 10% vs 25%       | -0.5776              | 0.0511  |
| 10% vs 100%      | -1.6660              | <0.0001 |
| 25% vs 100%      | -1.0884              | <0.0001 |

Post-hoc analysis testing the differences between pre vs post values for each group:

| Group | Estimated difference | P-value |
|-------|----------------------|---------|
| 0%    | 0.7441               | <0.0001 |
| 1%    | -0.3287              | 0.9528  |
| 10%   | -0.0793              | 1.0000  |
| 25%   | -0.6546              | 0.0001  |
| 100%  | -1.0919              | <0.0001 |

Linear mixed-effects model analysis of data in Fig 6B and 6C

For evaluating differences among groups at a fixed time-point accounting for data belonging to different experiments, a linear mixed-effects (LME) model with the only group term was estimated, followed by a post-hoc analysis comparing all pairs of groups with the R package phia. In each post-hoc analysis, p-values were adjusted with Bonferroni’s correction to account for multiple comparisons. To meet the assumptions of the model, an adequate transformation of the dependent variable was used in the corresponding LME model (for data in Fig 6B the natural logarithmic scale, while for data in Fig 6C the square root transformation) and few observations were excluded from the analysis since they were outliers for model. Random effects of the LME model were set on the intercept term to account for the variability between experiments. Only the results of post-hoc analysis are reported.

Post-hoc analysis of n. recipient CD3+ cells at day 3\(\frac{1}{4}\) of Fig 6B (excluded 1 outlier observation from the analysis):

| Group comparison | Estimated difference | P-value |
|------------------|----------------------|---------|
| NC vs CPA 300    | 3.3077               | <0.0001 |
| NC vs CPA 200    | 2.1906               | <0.0001 |
| NC vs ANTI-CD4   | 1.1168               | <0.0001 |
| NC vs ALS        | 1.8642               | <0.0001 |
| CPA 300 vs CPA 200 | -1.1171         | <0.0001 |
| CPA 300 vs ANTI-CD4 | -2.1910         | <0.0001 |
Post-hoc analysis of n. donor CD4+ cells at day 18\24 of Fig 6C (excluded group CPA 200, because n=4, and 1 outlier observation from the analysis):

| Group comparison         | Estimated difference | P-value |
|--------------------------|----------------------|---------|
| NC vs CPA 300            | -0.4122              | <0.0001 |
| NC vs ANTI-CD4           | -0.1379              | <0.0001 |
| NC vs ALS                | -0.1376              | 0.0002  |
| CPA 300 vs ANTI-CD4      | 0.2743               | <0.0001 |
| CPA 300 vs ALS           | 0.2746               | <0.0001 |
| ANTI-CD4 vs ALS          | 0.0003               | 1.0000  |

Nonlinear mixed-effects model analysis of data in Fig 6D

The nonlinear relationship between n. recipient CD3+ cells at day 3\4 and n. donor CD4+ cells at day 18\24 was analyzed with a nonlinear mixed-effects (NLME) model analysis by using an asymptotic model. The standard NLME model with horizontal right asymptote, was reparametrized in the following way to enhance the interpretation of the results with respect to the aim of the analysis:

\[
\sqrt{Y} = R_0 - \delta + \delta e^{-X \cdot e^{lrc}}.
\]

where the X was n. recipient CD3+ cells at day 3\4, while the dependent variable Y (n. donor CD4+ cells at day 18\24) was transformed in the square root scale to meet the assumption of normality of the residuals. In the model, the parameter \(R_0\) represents the value at \(X=0\) and the \(lrc\) parameter is the natural logarithm of the rate constant. The parameter \(\delta\) represents the difference between \(R_0\) and the value of the horizontal asymptote (i.e. the value of the plateau reached by \(\sqrt{Y}\) for higher values of \(X\)). Thus, a positive value of the \(\delta\) parameter denotes that a decrease of \(X\) corresponds to an increase of \(Y\). Random effects were set on the \(\delta\) parameter to account for heterogeneity among groups and among experiments. One observation was excluded from the analysis since it was an outlier for the full model.

| Parameter | Estimate | P-value |
|-----------|----------|---------|
| \(R_0\)   | 0.7132   | <0.0001 |
| \(\delta\) | 0.3274   | 0.0006  |
| \(Lrc\)   | 2.4347   | 0.0003  |

Linear mixed-effects model analysis for longitudinal data in Fig 6F, 6G, 1H, 2F, EV4A, EV1J

When the nonlinear trajectories over time could not be modeled with known nonlinear mixed-effects models or data were not adequate for applying this kind of models, for evaluating
differences among groups over time, a full linear mixed-effects (LME) model was estimated, which included the following terms: time (treated as categorical variable), group and an interaction term between time and group. In case of two groups, results of the comparisons at each time-points were retrieved directly from the estimated LME model. In case of more than three groups, a post-hoc analysis with the R package phia was performed for testing differences between all pairs of groups at each time-point. In case of data in Fig 2F, also a post-hoc analysis for testing the overall differences among groups was performed. In each post-hoc analysis, p-values were adjusted with Bonferroni’s correction to account for both multiple testing and comparisons. In this case, only the results of post-hoc analysis are reported. When necessary, to meet the assumptions of the model, an adequate transformation of the dependent variable was used in the corresponding LME model (the square root transformation for data in Fig 6F, the cubic root transformation for data in Fig 2F, the natural logarithmic transformation for RFI data in Fig 1H, RFI data in Fig EV1J, the log(x+0.01) transformation for data in Fig 6G due to the presence of zeros) and, eventually, few observations were excluded from the analysis since they were outliers for model. Random effects of the LME model were set on the intercept term and they were defined either to account only for the variability among mice (data of Fig 6F, 6G, 2F and EV4A), or as nested to account for the variability among donors and among samples with the same donor (data of Fig 1H and EV1J).

LME model of % donor T cells comparing CD40LG vs WT 2x10⁶ doses in Fig EV4A (time-points 19, 26, 33 days were excluded from the analysis since n=3 for WT):

| Parameter                        | Estimate | P-value |
|----------------------------------|----------|---------|
| Intercept                        | 0.0640   | <0.0001 |
| at day 4 vs 1                    | -0.0100  | 0.1751  |
| at day 7 vs 1                    | -0.0200  | 0.0119  |
| group CD40LG vs WT               | -0.0080  | 0.5209  |
| at day 4:group CD40LG vs WT      | 0.0000   | 1.0000  |
| at day 7:group CD40LG vs WT      | 0.0080   | 0.4339  |

LME model of % donor T cells comparing CD40LG vs WT 20x10⁶ doses in Fig EV4A:

| Parameter                        | Estimate | P-value |
|----------------------------------|----------|---------|
| Intercept                        | 0.5700   | <0.0001 |
| at day 4 vs 1                    | -0.1040  | 0.2310  |
| at day 7 vs 1                    | -0.0060  | 0.9444  |
| at day 19 vs 1                   | -0.0960  | 0.2682  |
| at day 26 vs 1                   | -0.2420  | 0.0072  |
| at day 33 vs 1                   | -0.2000  | 0.0244  |
| group CD40LG vs WT               | 0.0760   | 0.4358  |
| at day 4:group CD40LG vs WT      | -0.0460  | 0.7056  |
| at day 7:group CD40LG vs WT      | -0.1860  | 0.1319  |
| at day 19:group CD40LG vs WT     | -0.1760  | 0.1533  |
| at day 26:group CD40LG vs WT     | -0.0900  | 0.4610  |
| at day 33:group CD40LG vs WT     | -0.1460  | 0.2344  |
Post-hoc analysis testing the differences between all pairs of groups at each time-point of n.
recipient CD3+ cells data in Fig 6F:

| Day  | Group comparison   | Estimated difference | P-value  |
|------|--------------------|----------------------|----------|
| 4    | NC vs CPA 300      | 1.2606               | <0.0001  |
| 4    | NC vs CPA 200      | 0.8920               | <0.0001  |
| 4    | CPA 300 vs CPA 200 | -0.3687              | <0.0001  |
| 10   | NC vs CPA 300      | 0.8721               | <0.0001  |
| 10   | NC vs CPA 200      | 0.7295               | <0.0001  |
| 10   | CPA 300 vs CPA 200 | -0.1426              | 0.8994   |
| 25   | NC vs CPA 300      | -0.4551              | <0.0001  |
| 25   | NC vs CPA 200      | -0.3542              | 0.0003   |
| 25   | CPA 300 vs CPA 200 | 0.1009               | 1.0000   |
| 52   | NC vs CPA 300      | 0.1225               | 1.0000   |
| 52   | NC vs CPA 200      | 0.0280               | 1.0000   |
| 52   | CPA 300 vs CPA 200 | -0.0945              | 1.0000   |
| 128  | NC vs CPA 300      | -0.1935              | 0.3338   |
| 128  | NC vs CPA 200      | 0.0194               | 1.0000   |
| 128  | CPA 300 vs CPA 200 | 0.2129               | 0.0618   |
| 215  | NC vs CPA 300      | -0.4329              | <0.0001  |
| 215  | NC vs CPA 200      | 0.1655               | 1.0000   |
| 215  | CPA 300 vs CPA 200 | 0.5984               | <0.0001  |

Post-hoc analysis testing the differences between all pairs of groups at each time-point of n.
donor CD4+ cells data in Fig 6G:

| Day  | Group comparison   | Estimated difference | P-value  |
|------|--------------------|----------------------|----------|
| 4    | NC vs CPA 300      | 0.6067               | 0.0882   |
| 4    | NC vs CPA 200      | 0.1950               | 1.0000   |
| 4    | CPA 300 vs CPA 200 | -0.4117              | 0.4151   |
| 10   | NC vs CPA 300      | -1.8595              | <0.0001  |
| 10   | NC vs CPA 200      | -1.2032              | <0.0001  |
| 10   | CPA 300 vs CPA 200 | 0.6563               | 0.0086   |
| 25   | NC vs CPA 300      | -1.9468              | <0.0001  |
| 25   | NC vs CPA 200      | -1.6638              | <0.0001  |
| 25   | CPA 300 vs CPA 200 | 0.2830               | 1.0000   |
| 52   | NC vs CPA 300      | -1.8021              | <0.0001  |
| 52   | NC vs CPA 200      | -1.2322              | <0.0001  |
| 52   | CPA 300 vs CPA 200 | 0.5699               | 0.0498   |
| 128  | NC vs CPA 300      | -1.5464              | <0.0001  |
| 128  | NC vs CPA 200      | -0.8890              | 0.0010   |
| 128  | CPA 300 vs CPA 200 | 0.6574               | 0.0124   |
LME model of RFI in Fig 1H:

| Parameter                        | Estimate | P-value |
|----------------------------------|----------|---------|
| Intercept                        | 6.4269   | <0.0001 |
| at hour 3 vs 0                   | 1.2568   | <0.0001 |
| at hour 8 vs 0                   | 1.3998   | <0.0001 |
| at hour 24 vs 0                  | 0.2025   | 0.0016  |
| at hour 48 vs 0                  | 0.1870   | 0.0034  |
| Group HD GFP+ vs GFP-            | 0.0000   | 1.0000  |
| at hour 3:Group HD GFP+ vs GFP-  | -0.3459  | 0.0002  |
| at hour 8:Group HD GFP+ vs GFP-  | -0.4433  | <0.0001 |
| at hour 24:Group HD GFP+ vs GFP- | -0.1609  | 0.0692  |
| at hour 48:Group HD GFP+ vs GFP- | -0.2029  | 0.0230  |

LME model of %CD40LG+ cells in Fig 1H (excluded 3 outlier observations from the analysis):

| Parameter                        | Estimate | P-value |
|----------------------------------|----------|---------|
| Intercept                        | 3.7723   | 0.0135  |
| at hour 3 vs 0                   | 69.2857  | <0.0001 |
| at hour 8 vs 0                   | 76.5891  | <0.0001 |
| at hour 24 vs 0                  | 16.9704  | <0.0001 |
| at hour 48 vs 0                  | 17.4771  | <0.0001 |
| Group HD GFP+ vs GFP-            | -2.9604  | 0.1033  |
| at hour 3:Group HD GFP+ vs GFP-  | -6.0205  | 0.0090  |
| at hour 8:Group HD GFP+ vs GFP-  | -4.4848  | 0.0450  |
| at hour 24:Group HD GFP+ vs GFP- | -7.7445  | 0.0008  |
| at hour 48:Group HD GFP+ vs GFP- | -9.3037  | 0.0001  |

Post-hoc analysis testing the differences between all pairs of groups at each time-point of CD4+ RFI data of case (i) in Fig EV1J:

| Hour | Group comparison      | Estimated difference | P-value |
|------|-----------------------|----------------------|---------|
| 0    | CO GFP+ vs WT GFP+    | 0.1461               | 1.0000  |
| 0    | CO GFP+ vs GFP-       | 0.0763               | 1.0000  |
| 0    | WT GFP+ vs GFP-       | -0.0698              | 1.0000  |
| 3    | CO GFP+ vs WT GFP+    | 0.2019               | 0.2179  |
| 3    | CO GFP+ vs GFP-       | -0.3724              | <0.0001 |
Post-hoc analysis testing the differences between all pairs of groups at each time-point of RFI data of case (iv) in Fig EV1J:

| Hour | Group comparison                  | Estimated difference | P-value |
|------|-----------------------------------|----------------------|---------|
| 0    | CD40LG GFP+ vs GFP-               | -0.0037              | 1.0000  |
| 0    | CD40LG GFP+ vs SV40 GFP+          | -0.0378              | 1.0000  |
| 0    | GFP- vs SV40 GFP+                 | -0.0341              | 1.0000  |
| 3    | CD40LG GFP+ vs GFP-               | -0.4446              | <0.0001 |
| 3    | CD40LG GFP+ vs SV40 GFP+          | 0.0334               | 1.0000  |
| 3    | GFP- vs SV40 GFP+                 | 0.4780               | <0.0001 |
| 6    | CD40LG GFP+ vs GFP-               | -0.6614              | <0.0001 |
| 6    | CD40LG GFP+ vs SV40 GFP+          | 0.0049               | 1.0000  |
| 6    | GFP- vs SV40 GFP+                 | 0.6662               | <0.0001 |
| 8    | CD40LG GFP+ vs GFP-               | -0.6120              | <0.0001 |
| 8    | CD40LG GFP+ vs SV40 GFP+          | 0.0193               | 1.0000  |
| 8    | GFP- vs SV40 GFP+                 | 0.6312               | <0.0001 |
| 24   | CD40LG GFP+ vs GFP-               | -0.0990              | 1.0000  |
| 24   | CD40LG GFP+ vs SV40 GFP+          | 0.0424               | 1.0000  |
| 24   | GFP- vs SV40 GFP+                 | 0.1414               | 1.0000  |
| 48   | CD40LG GFP+ vs GFP-               | -0.0280              | 1.0000  |
| 48   | CD40LG GFP+ vs SV40 GFP+          | 0.0236               | 1.0000  |
| 48   | GFP- vs SV40 GFP+                 | 0.0516               | 1.0000  |

Post-hoc analysis testing the differences between all pairs of groups at each time-point of %hCD45+ cells data in Fig 2F:

| Week | Group comparison                  | Estimated difference | P-value |
|------|-----------------------------------|----------------------|---------|
| 0    | CD40LG GFP+ vs GFP-               | -0.0037              | 1.0000  |
| 0    | CD40LG GFP+ vs SV40 GFP+          | -0.0378              | 1.0000  |
| 0    | GFP- vs SV40 GFP+                 | -0.0341              | 1.0000  |
| 3    | CD40LG GFP+ vs GFP-               | -0.4446              | <0.0001 |
| 3    | CD40LG GFP+ vs SV40 GFP+          | 0.0334               | 1.0000  |
| 3    | GFP- vs SV40 GFP+                 | 0.4780               | <0.0001 |
| 6    | CD40LG GFP+ vs GFP-               | -0.6614              | <0.0001 |
| 6    | CD40LG GFP+ vs SV40 GFP+          | 0.0049               | 1.0000  |
| 6    | GFP- vs SV40 GFP+                 | 0.6662               | <0.0001 |
| 8    | CD40LG GFP+ vs GFP-               | -0.6120              | <0.0001 |
| 8    | CD40LG GFP+ vs SV40 GFP+          | 0.0193               | 1.0000  |
| 8    | GFP- vs SV40 GFP+                 | 0.6312               | <0.0001 |
| 24   | CD40LG GFP+ vs GFP-               | -0.0990              | 1.0000  |
| 24   | CD40LG GFP+ vs SV40 GFP+          | 0.0424               | 1.0000  |
| 24   | GFP- vs SV40 GFP+                 | 0.1414               | 1.0000  |
| 48   | CD40LG GFP+ vs GFP-               | -0.0280              | 1.0000  |
| 48   | CD40LG GFP+ vs SV40 GFP+          | 0.0236               | 1.0000  |
| 48   | GFP- vs SV40 GFP+                 | 0.0516               | 1.0000  |
| Group comparison          | Estimated difference | P-value |
|--------------------------|----------------------|---------|
| UT vs NGFR+              | -0.2223              | 1.0000  |
| UT vs NGFR bulk          | -0.1432              | 1.0000  |
| NGFR+ vs NGFR bulk       | 0.0791               | 1.0000  |

**Post-hoc analysis testing the overall differences between all pairs of groups of %hCD45+ cells data in Fig 2E:**

**Linear mixed-effects model analysis of longitudinal data in Fig 2G and 4E**

Due to the linear longitudinal trajectories of data in Fig 2G and 4E, the comparison of the longitudinal trend among groups was performed with a linear mixed-effects (LME) with time treated as a continuous variable. In order to test eventual differences among the groups, firstly a full model was estimated including the following terms: time, group and an interaction term between time and group (in case of the data of Fig 4E, the origin of the time was shifted at week 6 for a better interpretation of the parameters of the model). Then, the final model was obtained with a backward variable selection of fixed-effects covariates (where p-values less than 0.05 were considered significant). Random effects were set on the intercept to account for mice heterogeneity. In case of the analysis of the data in Fig 4E, also a post-hoc analysis was performed.
with the R package phia for testing the differences between all pairs of groups both at 20 weeks and with respect to the slope of the model.

**Final LME model of % editing by ddPCR (called $Y$) in Fig 2G:**

$$Y = \beta_0 + \beta_1(Bulk),$$

where $Bulk = 1$, for NGFR bulk group, and $Bulk = 0$ for NGFR+ group.

| Parameter | Estimate | p-value  |
|-----------|----------|----------|
| $\beta_0$ | 75.3772  | <0.0001  |
| $\beta_1$ | -45.1728 | <0.0001  |

**Final LME model of % HDR (called $Y$) in Fig 4E:**

$$Y = \beta_0 + \beta_1(NGFR) + \beta_2(NGFR + GSE56) + \gamma_0 Time + \gamma_1 Time \times (NGFR)$$

$$+ \gamma_2 Time \times (NGFR + GSE56),$$

where $NGFR = 1$, for NGFR group, and $NGFR = 0$ otherwise, $NGFR + GSE56 = 1$, for NGFR+GSE56 group, and $NGFR + GSE56 = 0$ otherwise.

| Parameter | Estimate | P-value |
|-----------|----------|---------|
| $\beta_0$ | 33.5432  | <0.0001 |
| $\beta_1$ | -10.5454 | 0.0019  |
| $\beta_2$ | -12.3534 | 0.0008  |
| $\gamma_0$ | -0.3305  | 0.0588  |
| $\gamma_1$ | -0.7397  | 0.0028  |
| $\gamma_2$ | 0.4782   | 0.0590  |

Post-hoc analysis testing the differences between all pairs of groups at time-point 20 weeks of % HDR data in Fig 4E:

| Group comparison          | Estimated difference | P-value |
|---------------------------|----------------------|---------|
| NR+GSE56 vs NGFR+GSE56    | 5.6582               | 0.0812  |
| NR+GSE56 vs NGFR          | 20.9006              | <0.0001 |
| NGFR+GSE56 vs NGFR        | 15.2424              | <0.0001 |

Post-hoc analysis testing the differences between all pairs of groups in the slope of the LME model of % HDR data in Fig 4E:

| Group comparison          | Estimated difference | P-value |
|---------------------------|----------------------|---------|
| NR+GSE56 vs NGFR+GSE56    | -0.4782              | 0.1624  |
| NR+GSE56 vs NGFR          | 0.7397               | 0.0054  |
| NGFR+GSE56 vs NGFR        | 1.2179               | <0.0001 |

**Nonlinear mixed-effects model analysis of longitudinal data in Fig 4D**
Due to the type of longitudinal trajectories of data in Fig 4D, the comparison of the longitudinal trend among the groups was performed with a nonlinear mixed-effects (NLME) model regression with the following asymptotic model:

$$\%h\text{CD45}+ \text{cells} = Asym + (R0 - Asym)e^{(-\text{time}\cdot e^{lrc})},$$

where the time was considered as continuous with the origin shifted at week 6 (for a better interpretation of the parameters of the model). In the model, the parameter $Asym$ represents the horizontal asymptote (i.e. the value of the plateau reached by the dependent variable). The parameter $R0$ represents the value at week 6 and the $lrc$ parameter is the natural logarithm of the rate constant. For evaluating differences among groups, in the full model, all parameters were allowed to depend on the group variables:

$$Asym = \beta_0 + \beta_1(NGFR + GSE56) + \beta_2(NR + GSE56),$$

$$R0 = \gamma_0 + \gamma_1(NGFR + GSE56) + \gamma_2(NR + GSE56), lrc$$

$$\begin{align*}
\delta_0 + \delta_1(NGFR + GSE56) + \delta_2(NR + GSE56)
\end{align*}$$

where $NGFR + GSE56 = 1$, for NGFR+GSE56 group, and $NGFR + GSE56 = 0$ otherwise, $NR + GSE56 = 1$, for NR+GSE56 group, and $NR + GSE56 = 0$ otherwise. The final model was obtained with a backward variable selection of fixed-effects covariates (where p-values less than 0.05 were considered significant). Random effects were set on the asymptote to account for mice heterogeneity.

**Final NLME model:**

$$Asym = \beta_0,$$

$$R0 = \gamma_0 + \gamma_1(NGFR + GSE56) + \gamma_2(NR + GSE56), lrc$$

$$\begin{align*}
\delta_0 + \delta_1(NGFR + GSE56) + \delta_2(NR + GSE56)
\end{align*}$$

| Parameter | Estimate | P-value |
|----------|----------|---------|
| $Asym$   | 74.5959  | <0.0001 |
| $\gamma_0$ | 3.8121   | 0.3570  |
| $\gamma_1$ | 14.9799  | 0.0213  |
| $\gamma_2$ | 17.7147  | 0.0069  |
| $\delta_0$ | -1.7940  | <0.0001 |
| $\delta_1$ | 1.0127   | 0.0034  |
| $\delta_2$ | 0.9707   | 0.0080  |