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Supplementary Materials for

In vivo correction of cystic fibrosis mediated by PNA nanoparticles

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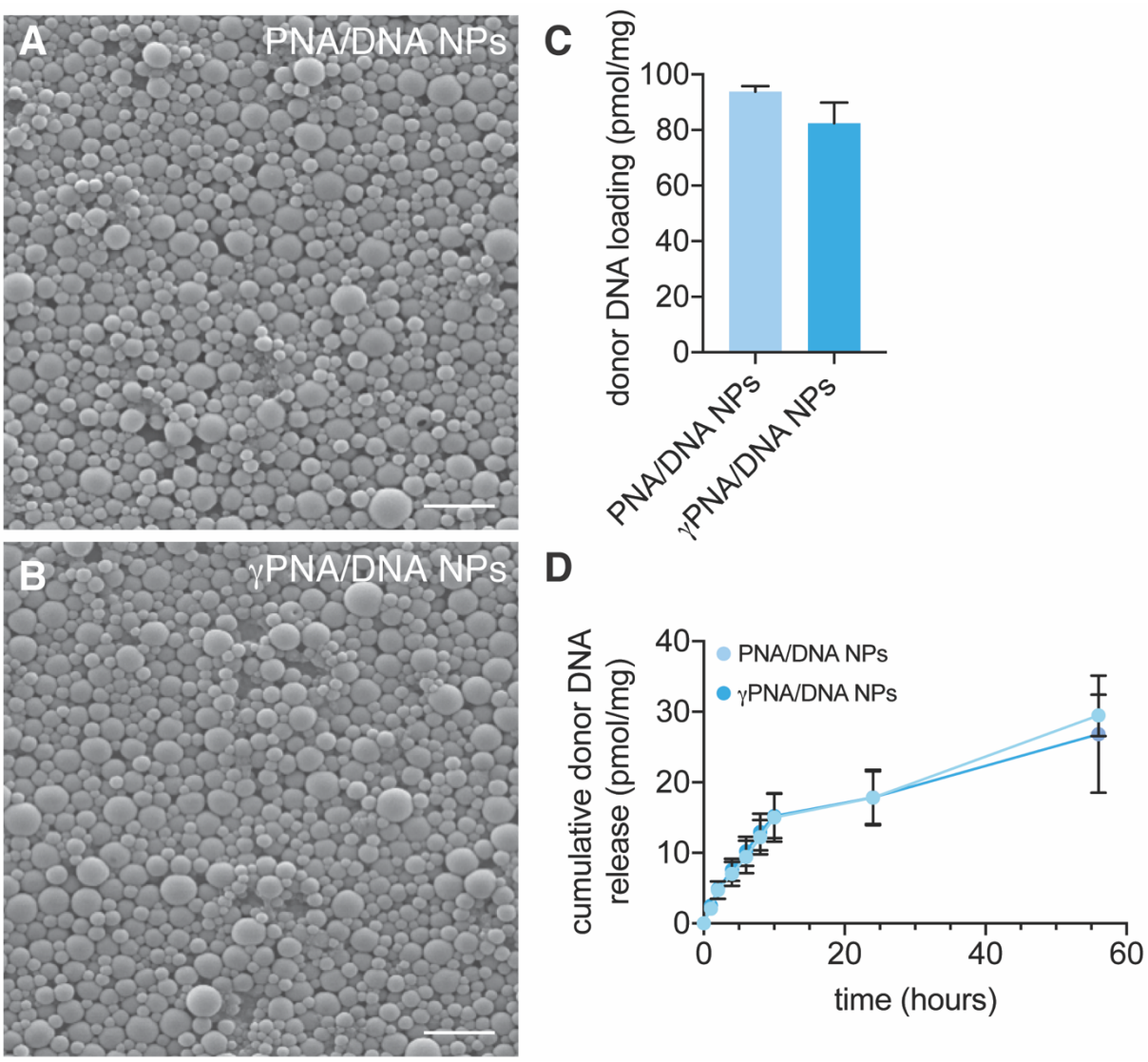

Figure S1. Characterization of NP formulations. SEM images of PLGA NPs containing (**A**) PNA and donor DNA and (**B**) γPNA and donor DNA. Scale bars, 1 mm. (**C**) Total donor DNA loading of PNA/DNA and γPNA/DNA PLGA NPs as measured by the Oligreen assay. (**D**) Cumulative donor DNA release from PNA/DNA and γPNA/DNA PLGA NPs as measured by the Oligreen assay.

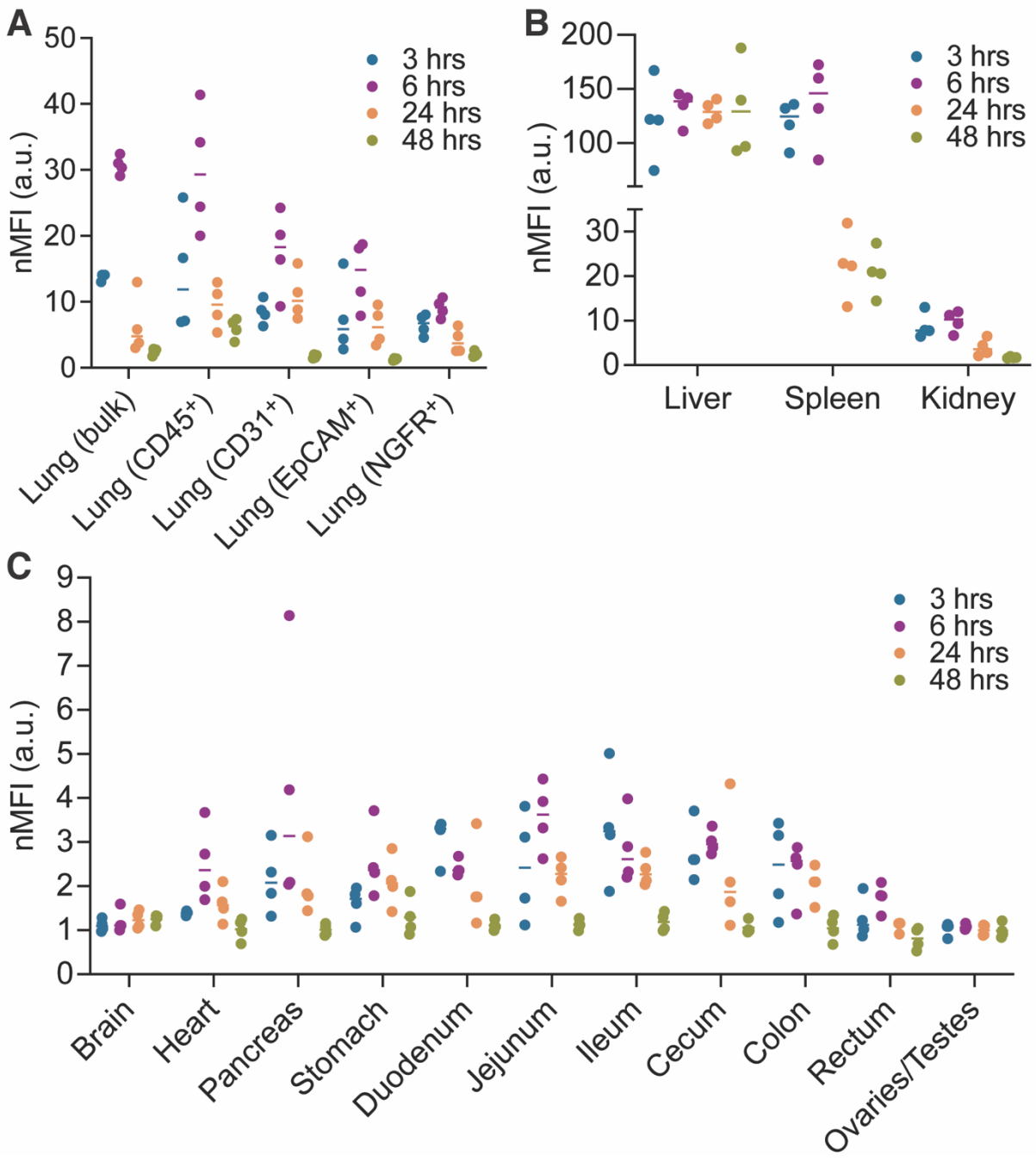

Figure S2. PLGA NPs accumulate in multiple organs including the lung and GI tract following systemic IV administration. (**A**) Flow cytometry median fluorescence intensity values normalized to untreated control animals (nMFI) for homogenized bulk lung and specific cell types ($CD45^+$ macrophages, $CD31^+$ endothelial cells, $EpCAM^+$ epithelial cells, and $NGFR^+$ basal cells) at 3, 6, 24, and 48 hours post-IV administration of Cy5-conjugated PLGA NPs *in vivo*. Each dot represents data from one mouse. (**B**) Flow cytometry median fluorescence intensity values normalized to untreated control animals (nMFI) for homogenized bulk liver, spleen and kidney at 3, 6, 24, and 48 hours post-IV administration of Cy5-conjugated PLGA NPs *in vivo*. Each dot represents data from one mouse. (**C**) Flow cytometry median fluorescence intensity values

normalized to untreated control animals (nMFI) for homogenized bulk brain, heart, pancreas, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, and ovaries/testes at 3, 6, 24, and 48 hours post-IV administration of Cy5-conjugated PLGA NPs *in vivo*. Each dot represents data from one mouse.

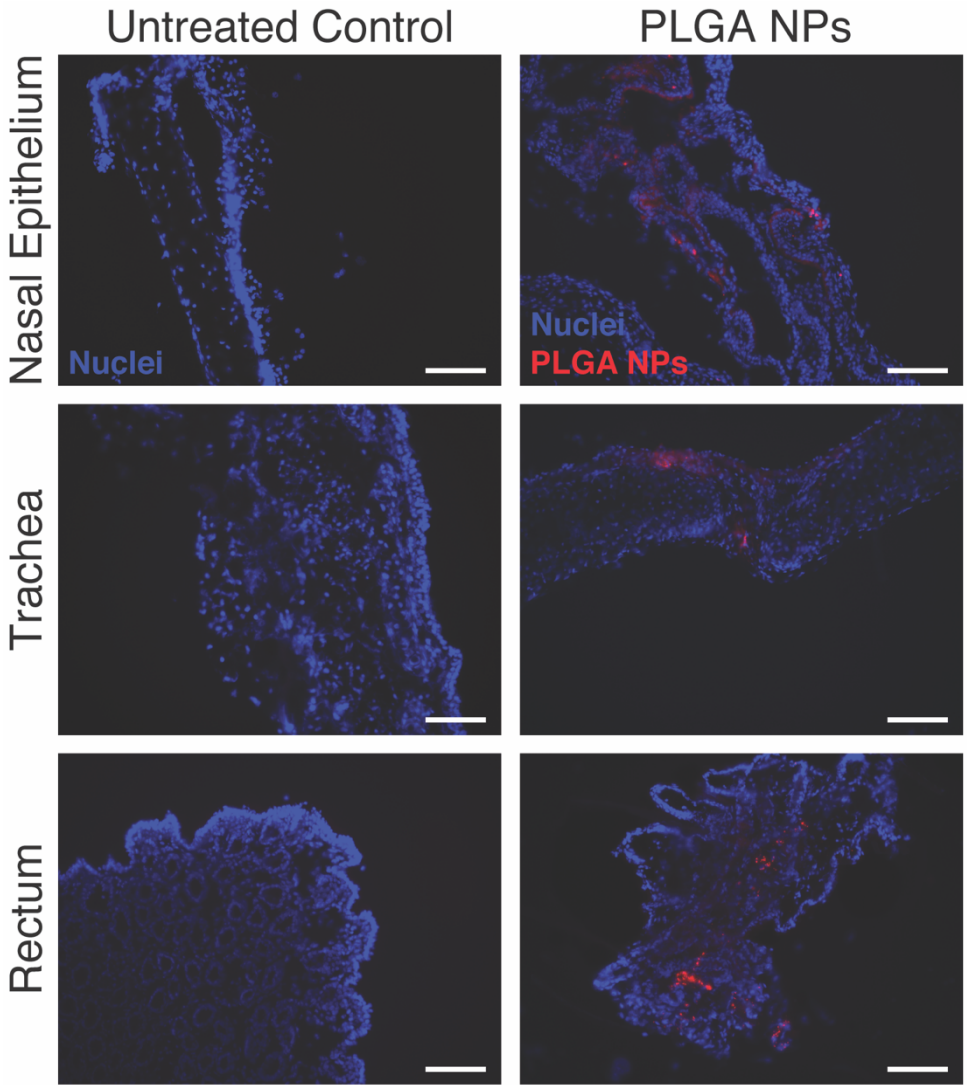

Figure S3. Biodistribution of PLGA NPs to the nasal epithelium, trachea, and rectum. Representative fluorescence microscopy images indicating biodistribution of DiD-loaded PLGA NPs (red) to the nasal epithelium, trachea, and rectum, at 24 hours post-IV administration *in vivo*, with untreated control images provided for comparison. Cell nuclei are shown in blue. Scale bars, 100 μm.

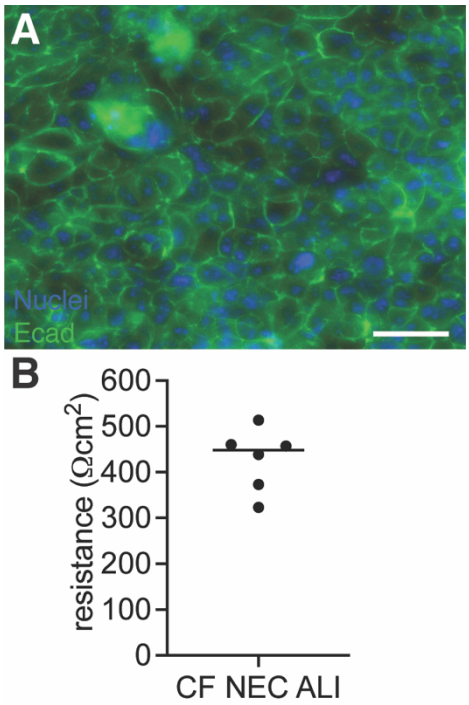

Figure S4. Integrity and resistance of NEC ALI cultures. (**A**) Representative epifluorescence image of a mature NEC ALI culture. Nuclei are shown in blue and E-cadherin is shown in green. Scale bar, 100 μm. (**B**) Representative resistance values of NEC ALI cultures in the Ussing chamber assay.

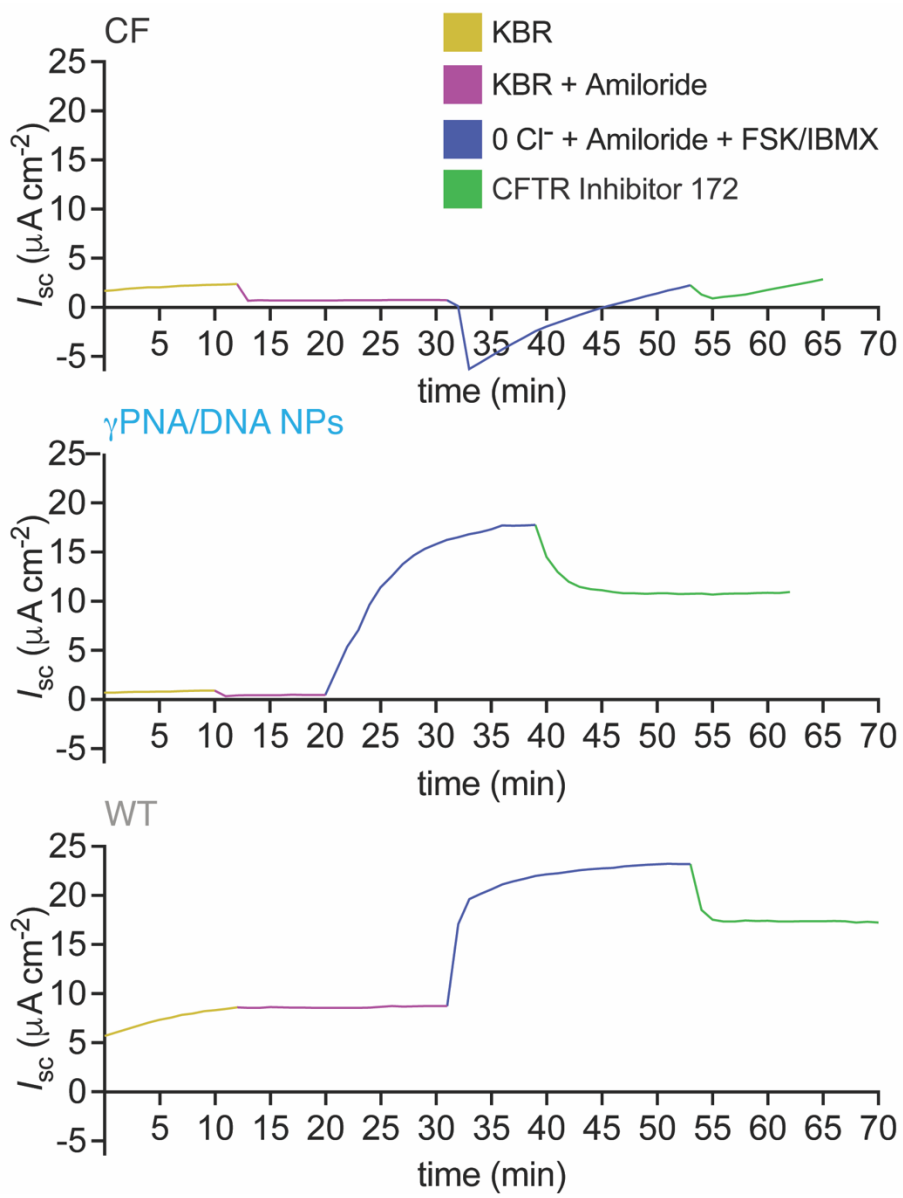

Figure S5. Representative NEC ALI Ussing traces. Primary NEC ALI cultures were loaded into Ussing chambers and short-circuit current (I_{sc}) was recorded during the addition of amiloride, forskolin/IBMX, and a CFTR inhibitor (172). Representative traces of CF NEC ALI cultures, CF NEC ALI cultures treated with γPNA/DNA NPs, and wild-type NEC ALI cultures are shown.

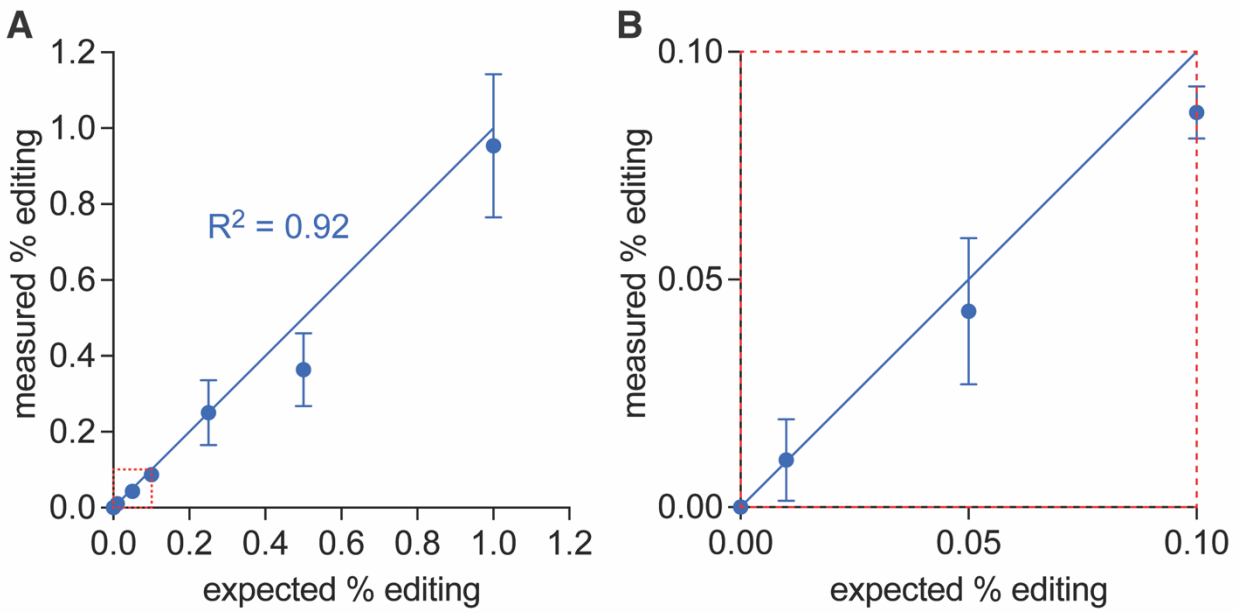

Figure S6. CF F508del droplet digital PCR (ddPCR) assay validation. A ddPCR assay was designed in which fluorescent probes differentiating two alleles (wild-type or mutant (F508del) are specific for the γDNA template present in the PCR reaction. (**A**) The expected percent fractional abundance of the wild-type allele (wild-type/wild-type + mutant) is plotted against the measured fractional abundance for an experiment in which increasing known amounts (0.01% to 1% by weight) of wild-type γDNA were spiked into samples of mutant F508del γDNA. The observed correlation is linear with an R^2 value of 0.92. (**B**) An expanded view of the lower end of the analysis range indicated by the red dashed box in panel (**A**). Error bars indicate SD.

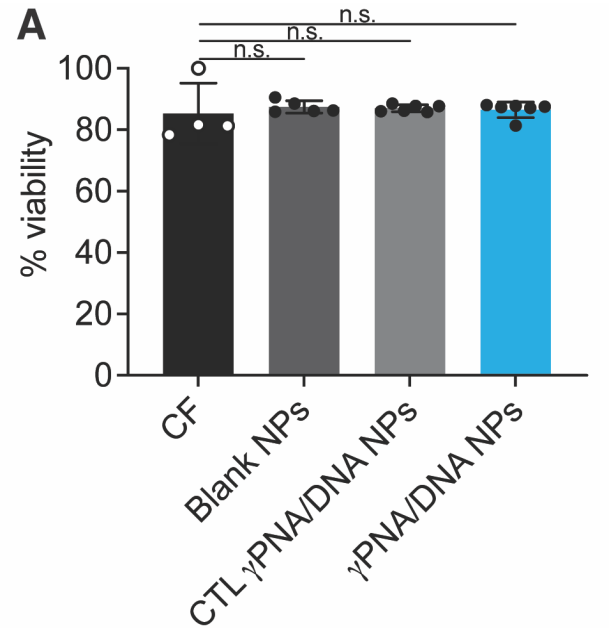

Figure S7. PLGA NPs do not affect cell viability in NEC ALI cultures. (**A**) Flow cytometry results indicating the percentage of viable cells isolated from primary mouse NEC ALI cultures following either no treatment, or treatment with 1 mg blank NPs, CTL (non-specific) γPNA/DNA NPs, or γPNA/DNA NPs for 24 hours.

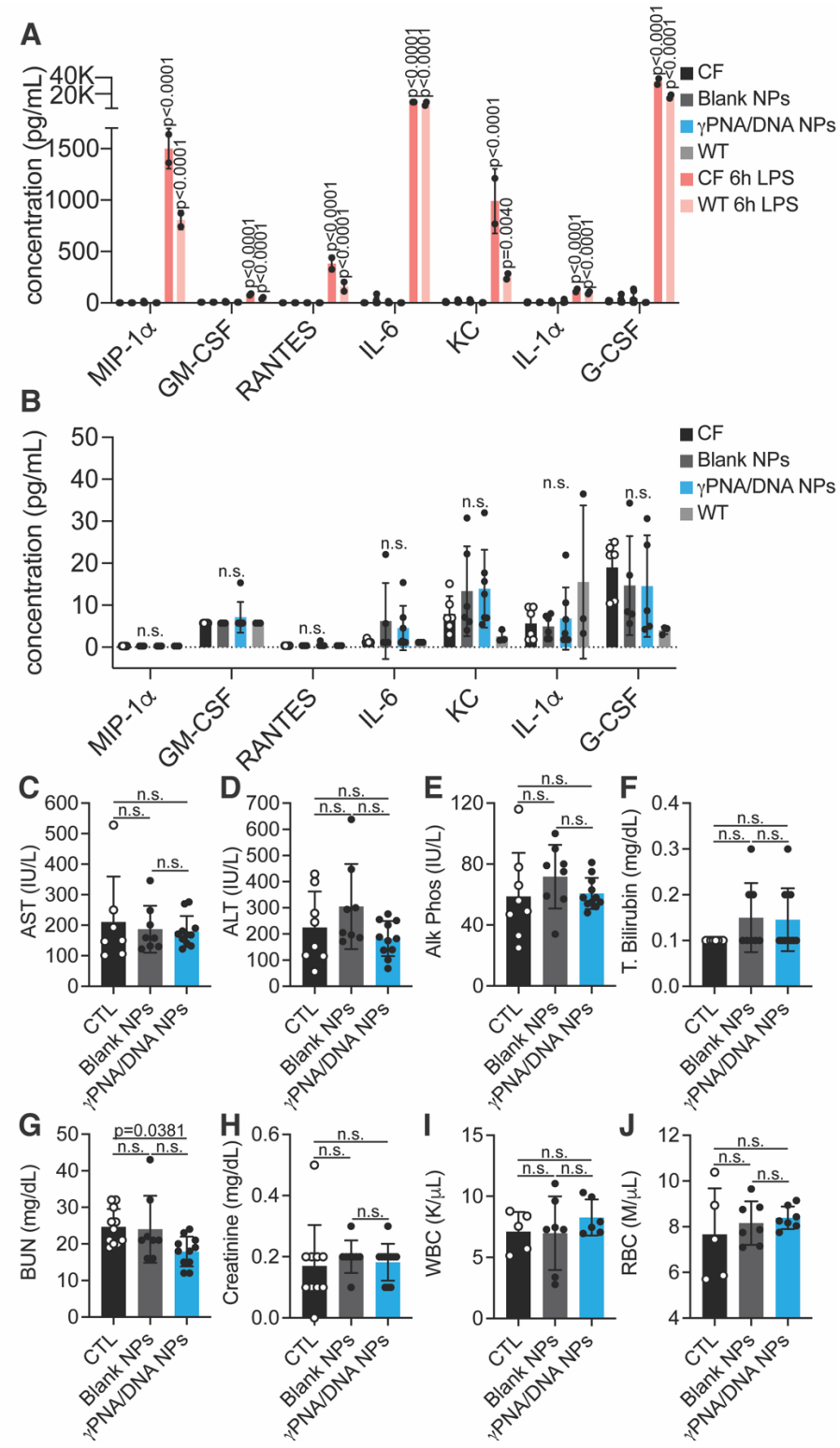

Figure S8. PNA NPs are non-toxic and do not elicit an immune response. (**A**) Cytokine production in BAL fluid of CF (untreated control), blank NP-treated mice, γPNA NP-treated mice, CFTR KO mice nebulized with LPS (positive control), and WT mice nebulized with LPS (positive

control). (**B**) Same plot as in (**A**) without positive LPS controls. (**D**)-(**K**) Blood serum chemistry analyses for untreated control, blank NP-treated, and γPNA NP-treated mice.

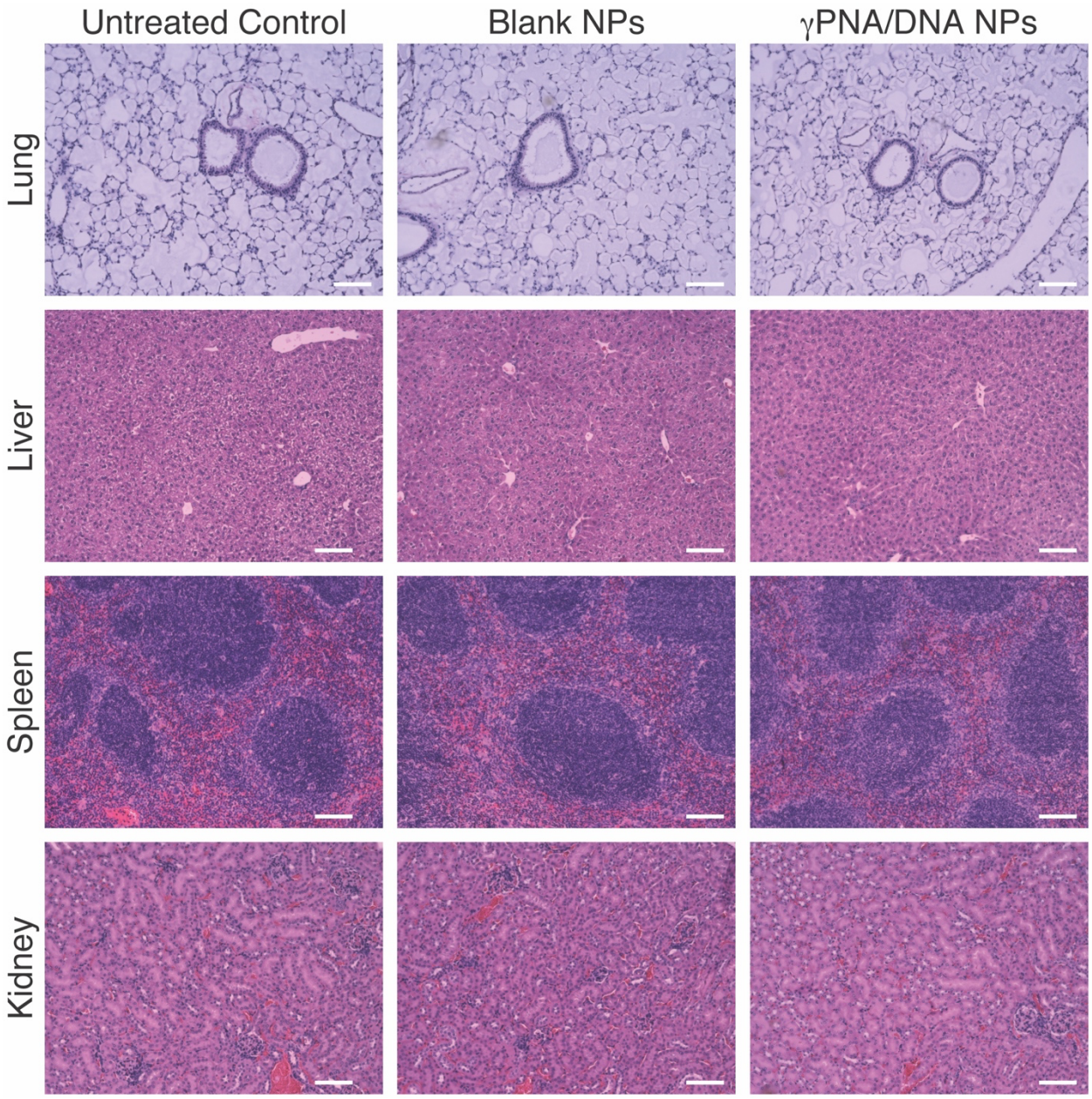

Figure S9. Blank and γPNA/DNA NP-treated mice exhibit normal histology in the lung, liver, spleen, and kidneys. Histology images of untreated, Blank NP-treated, and γPNA/DNA NP-treated mouse lungs, livers, spleens, and kidneys that were paraffin-embedded and stained with haematoxylin and eosin. Scale bars, 100 μm.

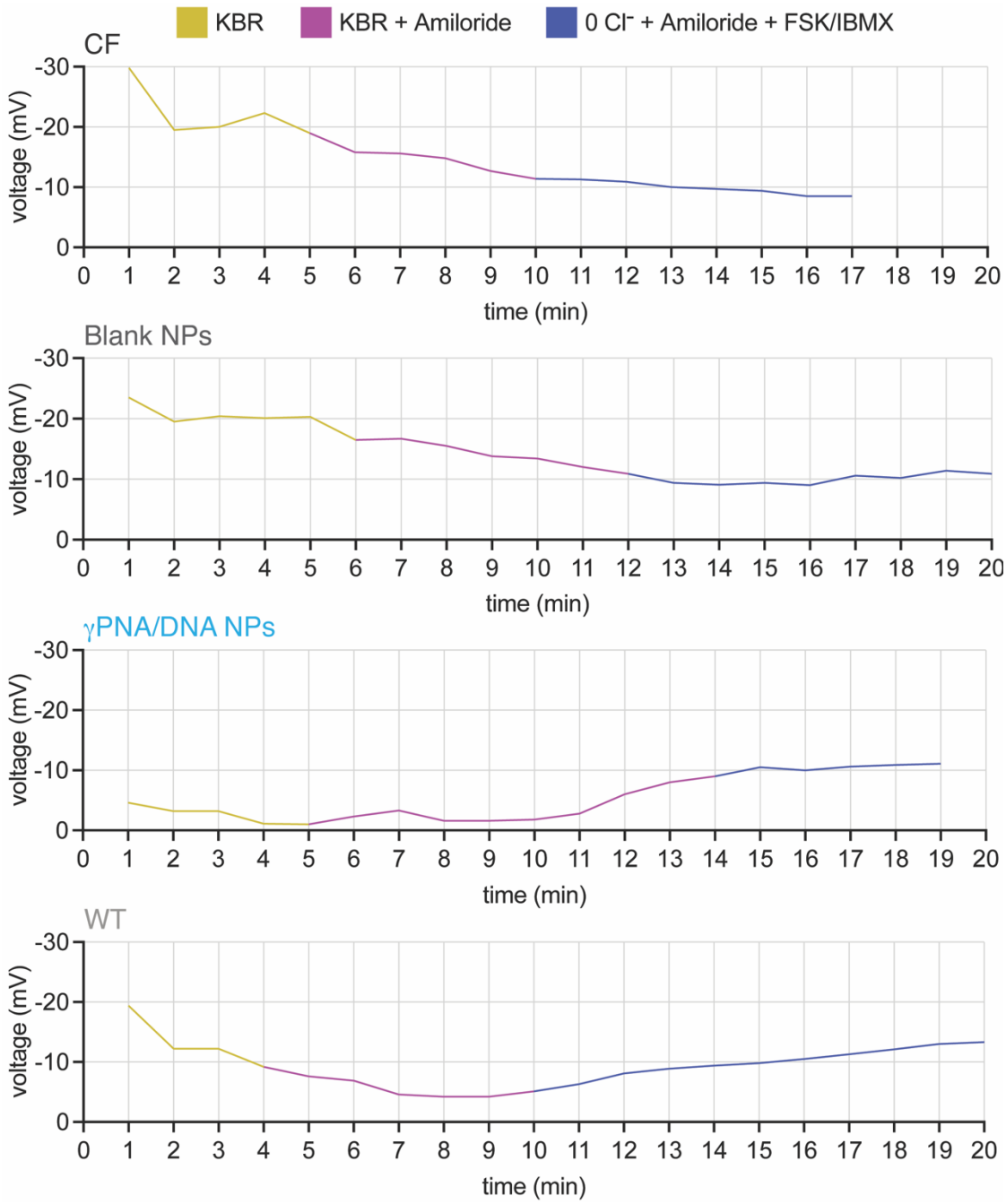

Figure S10. Representative Raw NPD traces. Nasal epithelia of F508del-CFTR mice were probed with an electrodes connected to a voltmeter to measure potential differences recorded following a course of solutions: Ringer's (KBR), KBR with amiloride, and chloride-free solution with amiloride and forskolin/IBMX. Representative traces of untreated CF mice, CF mice treated with Blank NPs or γPNA/DNA NPs, and wild-type mice shown.

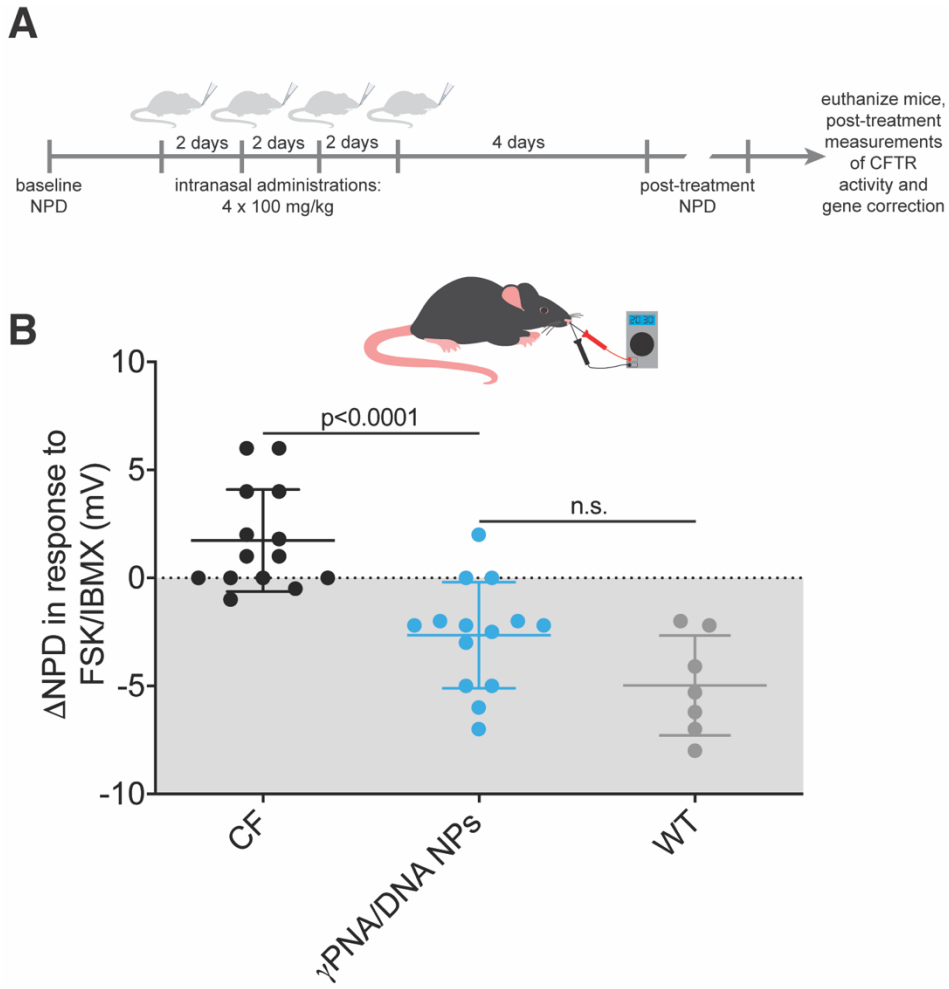

Figure S11. Local intranasal γPNA NP treatment results in robust *in vivo* NPD response. (**A**) Schematic of *in vivo* intranasal dosing scheme for PLGA/PBAE/MPG NPs loaded with γPNA and donor DNA. (**B**) NPD measurements following γPNA/DNA NP treatment with CF (black circles) and wildtype (light grey circles) controls. Grey region indicates wild-type range.

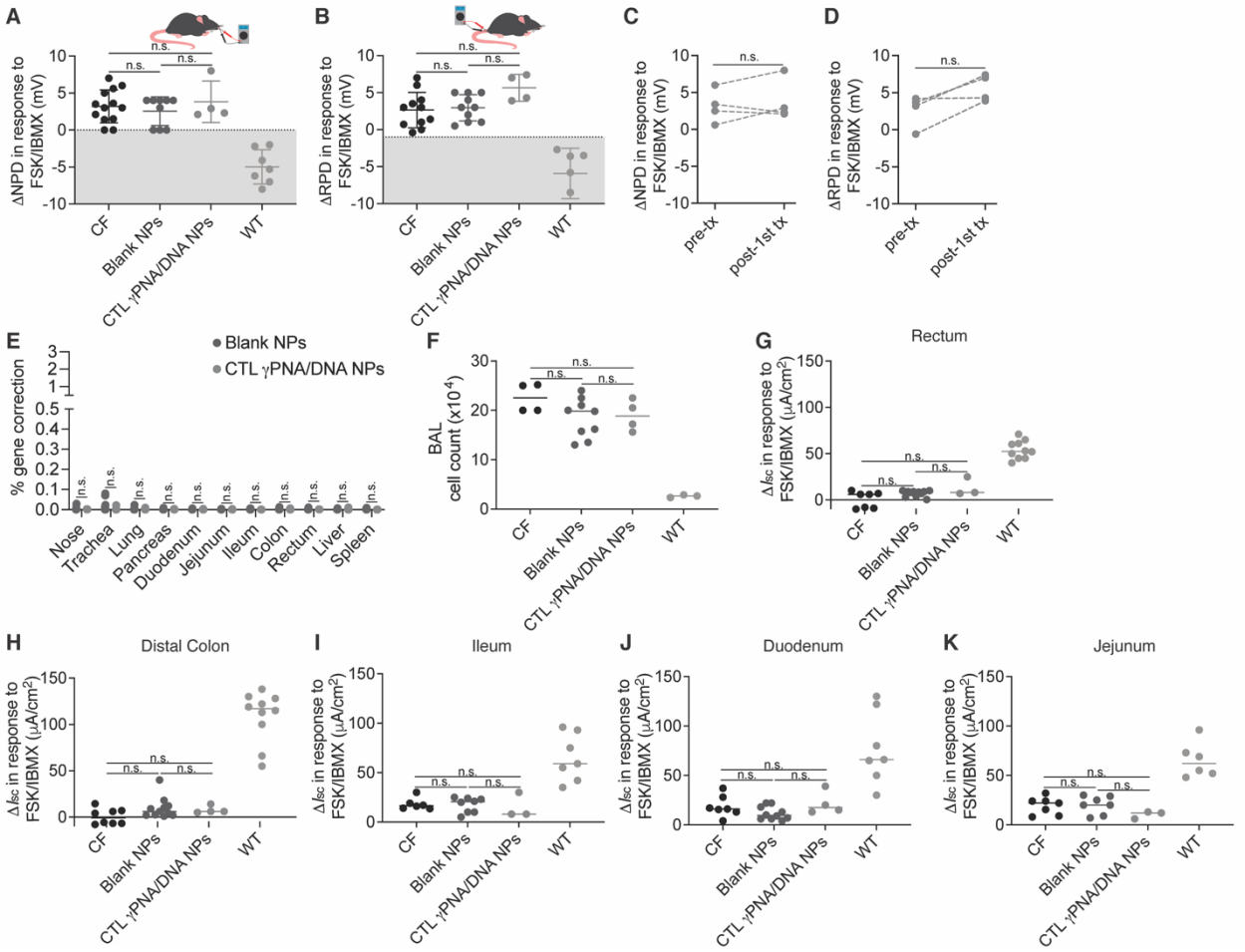

Figure S12. Assessment of phenotypic and genotypic correction of non-specific γPNA/DNA NPs *in vivo*. (**A**) Nasal potential difference (NPD) measurements following either 4 x 2 mg blank NP (dark grey circles) or 4 x 2 mg CTL (non-specific) γPNA/DNA NP (medium grey circles) treatment with CF (black circles) and wildtype (light grey circles) controls. CTL γPNA/DNA NPs contain ~2 μg/0.2 nmol of PNA and ~2 μg/0.1 nmol DNA per mg; each animal received ~0.2 mg/kg of PNA and donor DNA per dose. Grey region indicates wild-type range. (**B**) Rectal potential difference (RPD) measurements following either blank NP (dark grey circles) or CTL γPNA/DNA NP (medium grey circles) treatment with CF (black circles) and wildtype (light grey circles) controls. Grey region indicates wild-type range. (**C**) Pre- and post-treatment NPD measurements for F508del-CFTR mice treated with CTL γPNA/DNA NPs. (**D**) Pre- and post-treatment RPD measurements for F508del-CFTR mice treated with CTL γPNA/DNA NPs. (**E**) Gene correction levels measured by ddPCR at the F508del locus for multiple organs from mice treated with either blank NPs or CTL γPNA/DNA NPs. (**F**) BAL cell counts following either blank NP (dark grey circles) or CTL γPNA/DNA NP (medium grey circles) treatment with CF (black circles) and wildtype (light grey circles) controls. Phenotypic ΔI_{sc} measurements in (**G**) rectum, (**H**) distal colon, (**I**) ileum, (**J**) duodenum, and (**K**) jejunum following either 4 x 2 mg blank NP (dark grey circles) or 4 x 2 mg CTL γPNA/DNA NP (medium grey circles) treatment with CF (black circles) and wildtype (light grey circles) controls.

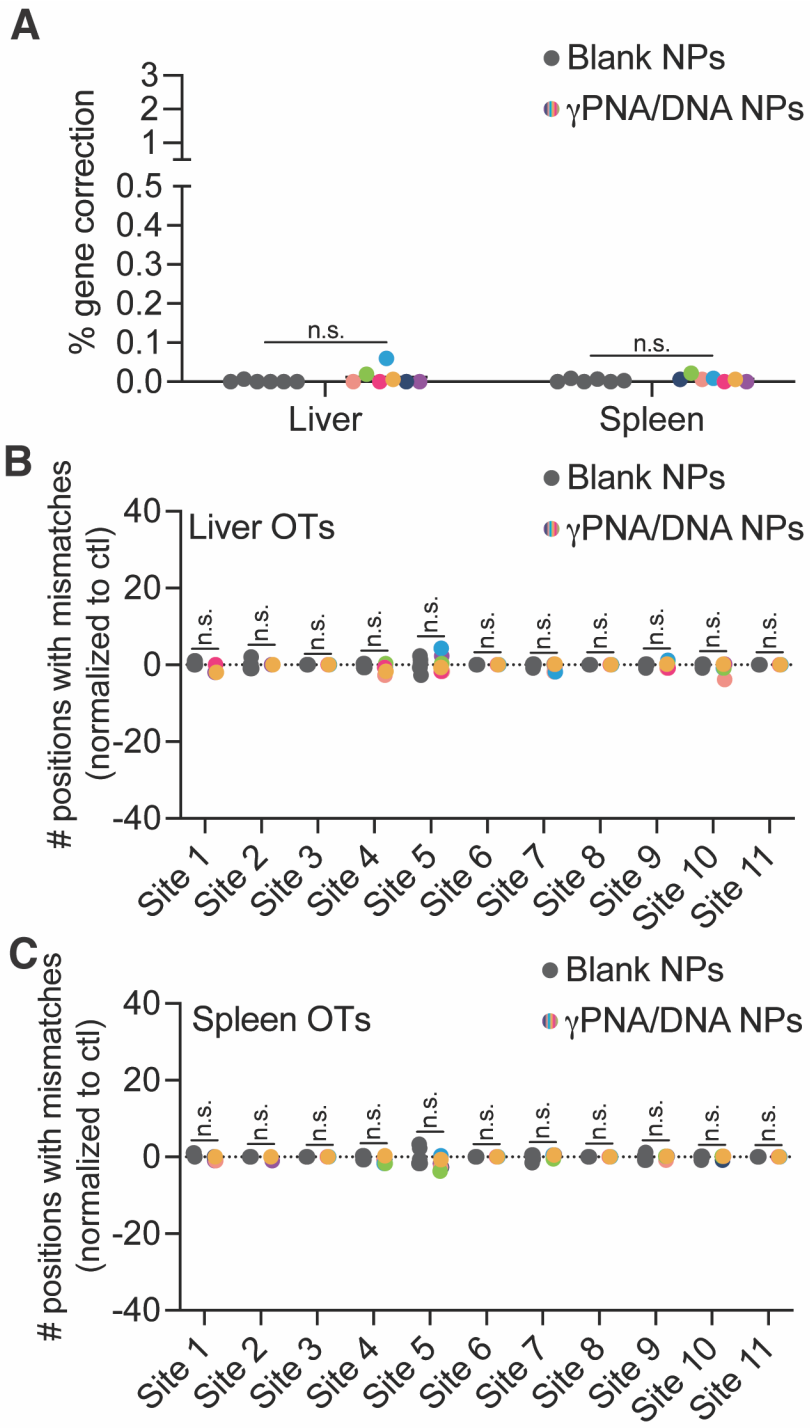

Figure S13. Assessment of gene correction and off-target effects in the liver and spleen. (**A**) Gene correction levels measured by ddPCR at the F508del locus for airway and GI organs from mice treated with either blank NPs or γPNA/DNA NPs. Deep sequencing off-target analysis of Blank or γPNA/DNA NP-treated F508del mice (**B**) livers and (**C**) spleens at 11 genomic sites with partial PNA binding site homology displayed as the number of positions with mismatches to the reference sequence normalized to untreated control samples.

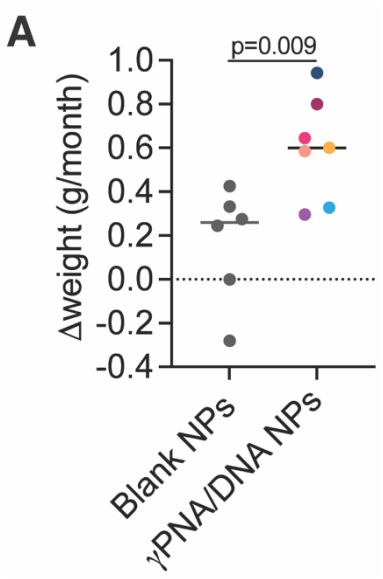

Figure S14. Weight gain per month of blank NP- or γPNA/DNA NP-treated mice. (**A**) Weight gain in g/month for representative blank NP- or γPNA/DNA NP-treated F508del/F508del mice. Color coding of γPNA/DNA NP-treated animals is consistent with Figures 3 and 4. Each color represents a different mouse.

Table S1. Characterization data for PLGA NP formulations.

Formulation	Diameter (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
PLGA-Cy5	257 ± 3	0.050	-10.7 ± 1.4
PLGA DiD	273 ± 2	0.190	-17.2 ± 0.7
PLGA Blank	240 ± 3	0.110	-23.4 ± 0.5
PLGA PNA/DNA	265 ± 6	0.110	-27.6 ± 1.0
PLGA γPNA/DNA	265 ± 3	0.130	-28.2 ± 0.3

Table S2. Primer Sequences for PNA and donor DNA off-target analysis.

Off-Target Site #	PNA or donor DNA	Chromosome	Primer Sequences
0 (on-target)	PNA	6	F: TTGTCAAAGCTTGCCAACTA R: ACGGTATCATCCCTGAAAAG
1	PNA	3	F: TACCTTCGGTATCCCAAATCTC R: GCCTGTGATATGATAGACACCT
2	PNA	1	F: GAGCCTACTGGGAGGTAAAAT R: GGACCTGATTACCTTGGGTAT
3	PNA	1	F: ATGTGAGAGGACTCTGTGAA R: ACCTGTACTGGTTTATAGGG
4	PNA	1	F: TATCACATTGGCCATCTCAG R: GGTACAAGGATAGCAGTAGC
5	PNA	13	F: TGGTACAAGGATGGCAGTA R: CCATTACCTCGGGAAGATTT
6	PNA	X	F: AATGCCCAATACAACAGATTT R: GAGCCATCTTTTGATGTTCAG
7	PNA	7	F: CCTGACTGATGGATGACGAGTTA R: TCAGTCCTGGTTGGAAAAGC
8	PNA	7	F: CCTCACCTTAACGAGCAAA R: TCAATGGACTCTCCCTAGAC
9	PNA	9	F: TGGTACAAGGATGGCAGTA R: GGATCTTCTTGGCTATCACA
10	PNA	15	F: GTCTCAGTCCTGGTAGGAAA R: AATACCCTACTGCCCTACTC
11	donor DNA	X	F: TGGATCTTCCTGGTGATTTTG R: TTATAAATTTCCCAGACTAGGCTATAA
12 (on-target)	donor DNA	6	F: TCTGCTCTCAATTTTCTTGGA R: GGCAAGCTTTGACAACACTC

Table S3. Blinded histopathology analysis of key organs following blank NP or γPNA/DNA NP treatment in C57BL/6J mice. 1 = no lymphoid aggregates, 2 = at least 1 lymphoid aggregate. N=3 animals per group.

	Average Score			
Treatment Group	**Lungs**	**Liver**	**Spleen**	**Kidneys**
Untreated Control	1.33	2	1	1
Blank NPs	1	1.33	1	1
γPNA/DNA NPs	1.67	1	1	1

Table S4. ΔAmiloride NPD responses for a representative subset of Blank NP- and γPNA/DNA NP-treated F508del/F508del animals.

Treatment Condition and Mouse Number	Baseline NPD ΔAmiloride (mV)	Post-treatment NPD ΔAmiloride (mV)
Blank NP-treated mouse 1	10	10
Blank NP-treated mouse 2	8	8
Blank NP-treated mouse 3	10.6	10.1
Blank NP-treated mouse 4	12.5	14.4
Blank NP-treated mouse 5	7.4	12
Blank NP-treated mouse 6	12.5	14
Blank NP-treated mouse 7	16.3	14
Blank NP-treated mouse 8	13	12.2
Blank NP-treated mouse 9	8	8
Blank NP-treated mouse 10	10	10
γPNA/DNA NP-treated mouse 1	18	10
γPNA/DNA NP-treated mouse 2	15	3
γPNA/DNA NP-treated mouse 3	7	2
γPNA/DNA NP-treated mouse 4	16.3	10.3
γPNA/DNA NP-treated mouse 5	7.5	5.4
γPNA/DNA NP-treated mouse 6	6.8	14.1
γPNA/DNA NP-treated mouse 7	11.6	4.7
γPNA/DNA NP-treated mouse 8	15	9
γPNA/DNA NP-treated mouse 9	12	10
γPNA/DNA NP-treated mouse 10	12.7	6