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Supplemental information

Restoring hematopoietic stem and progenitor cell function in *Fancc*$^{-/-}$ mice by *in situ* delivery of RNA lipid nanoparticles

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Table S1 – List of Materials

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Alexa Fluor® 700 anti-CD48 (HM48-1)	Biolegend	Cat# 103426, RRID: AB_10612755
APC/Cyanine7 anti-Sca-1 (D7)	Biolegend	Cat# 108126, RRID: AB_10645327
Brilliant Violet 650™ anti-CD45.2 (104)	Biolegend	Cat# 109836, RRID: AB_2563065
Brilliant Violet 711™ anti-CD150 (TC15-12F12.2)	Biolegend	Cat# 115941, RRID: AB_2629660
BUV395 Anti-Mouse CD117 (2B8)	BD Horizon™	Cat# 564011, RRID: AB_2738541
FITC anti-mouse Lineage Cocktail with Isotype Ctrl	Biolegend	Cat# 133302, AB_10697030
APC/Cyanine7 anti-mouse CD45.2 Antibody	Biolegend	Cat# 109824, RRID: AB_830789
Alexa Fluor® 700 anti-mouse CD31 Antibody	Biolegend	Cat# 102444, RRID: AB_2832289
CD51 antibody | RMV-7	Bio-Rad	Cat# MCA2461, RRID: AB_931725
FITC anti-mouse TER-119 Antibody	Biolegend	Cat# 116206, RRID: AB_313707
Pacific Blue™ anti-mouse Ly-6A/E (Sca-1) Antibody	Biolegend	Cat# 108120, RRID: AB_493273
PE/Cyanine5 anti-mouse CD135 Antibody	Biolegend	Cat# 135312, RRID: AB_2263031
PE/Cyanine7 anti-mouse CD45.1 Antibody	Biolegend	Cat# 110730, RRID: AB_1134168
APC/Cyanine7 anti-mouse CD117 (c-kit) Antibody	Biolegend	Cat# 105826, RRID: AB_1626278
Chemicals, Peptides, and Recombinant Proteins		
DAPI (4',6-Diamidino-2-Phenylindole, Dilactate)	Biolegend	Cat# 422801
Recombinant murine thrombopoietin	Peprotech	Cat# 315-14
Recombinant murine stem cell factor	Peprotech	Cat# 250-03
Insulin–transferrin–selenium–ethanolamine	Gibco	Cat# 51500-056

HEPES	Gibco	Cat# 15630-080
Polyvinyl alcohol	Sigma	Cat# P8136
10X RBC lysis buffer	eBioscience™	Cat# 00-4300-54
Fibronectin human plasma	Sigma Aldrich	Cat# F0895
Critical Commercial Assays		
EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit	StemCell Technologies	Cat# 19856
Experimental Models: Organisms/Strain		
Mouse: C57BL/6-Ly5.2 Fancc(+/-)	Dr. Manuel Buchwald's Laboratory	https://doi.org/10.1038/ng0496-448
Mouse: B6.SJL-*Ptprc*^{a} *Pepc*^{b}/BoyJ	Jackson laboratories	https://www.jax.org/strain/002014
Software and Algorithms		
Prism 9	GraphPad	https://www.graphpad.com/scientific-software/prism/
Matlab (R2021a)	Mathworks	https://www.mathworks.com/

Table S2 – LNP Formulation Method

Figure	AcuitasTX	In-house
1	**a-c**	**d-f**
2	✓	
3	✓	
4		✓
5	**a-d**	**e-h**
6		✓
7		✓
S1		
S2		✓
S3		✓
S4		✓
S5		✓
S6		✓
S7	✓	
S8	**c-e**	**a, b, f**

Figure S1

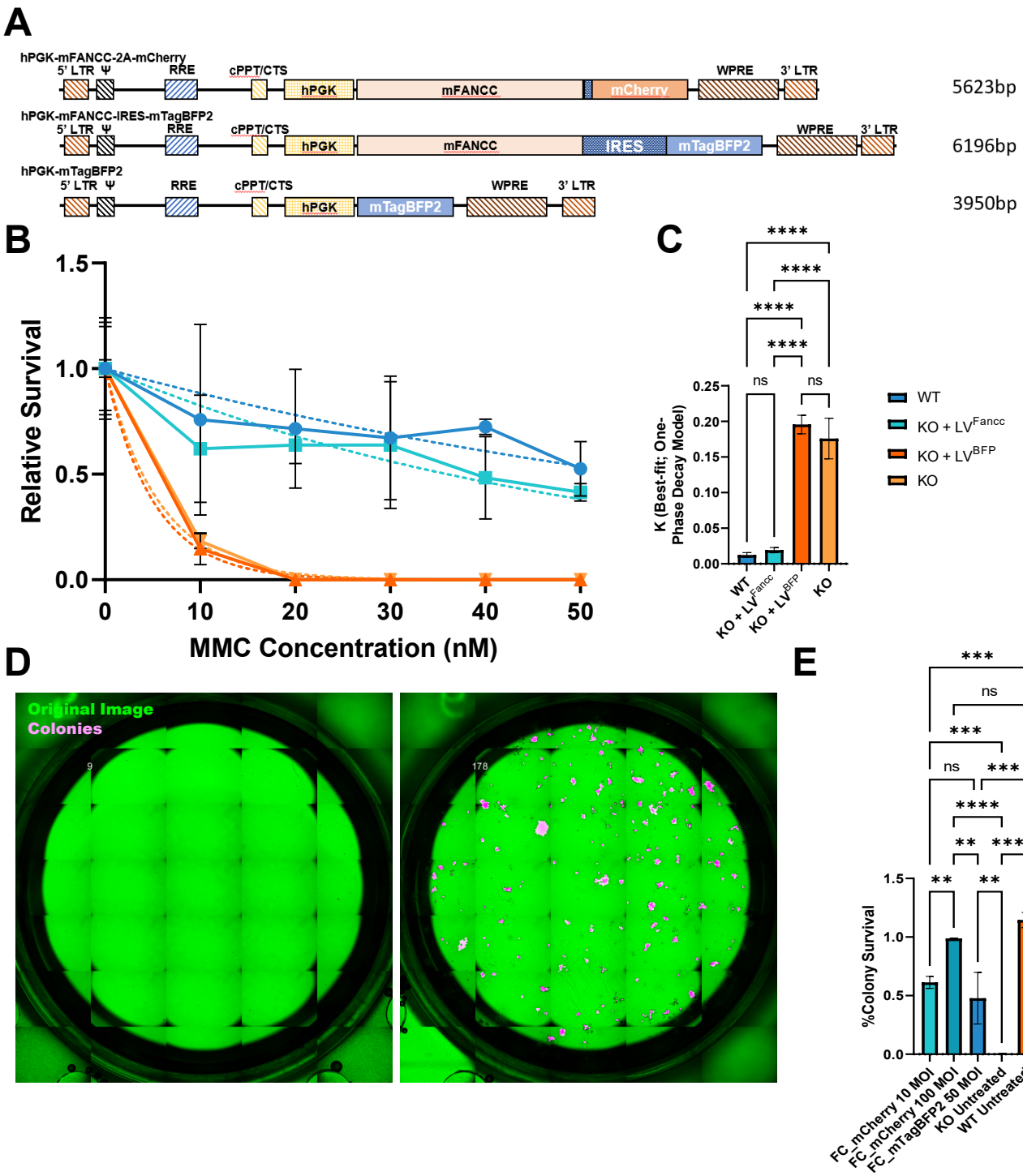

Figure S1 - Lentiviral Transduction of Fancc$^{(-/-)}$ mice to restore MMC tolerance. (A) The murine Fancc gene was cloned into a 3rd generation lentiviral transfer plasmid under control of the human PGK promoter. (B-C) Plot of the relative colony survival of Fancc$^{(-/-)}$ or wild-type KSL populations in methylcellulose media with or without 0-50nM MMC. Data are fit with exponential decay model constrained to full survival at 0nM MMC and plateau at 0% relative survival. (D) Representative images of 30nM CFU assay (green) with detected colonies (magenta) overlayed for non-transduced Fancc$^{(-/-)}$ cells (left) and LVFancc-transduced Fancc$^{(-/-)}$ cells (right). (E) Colony survival under various transduction schemes.

Figure S2

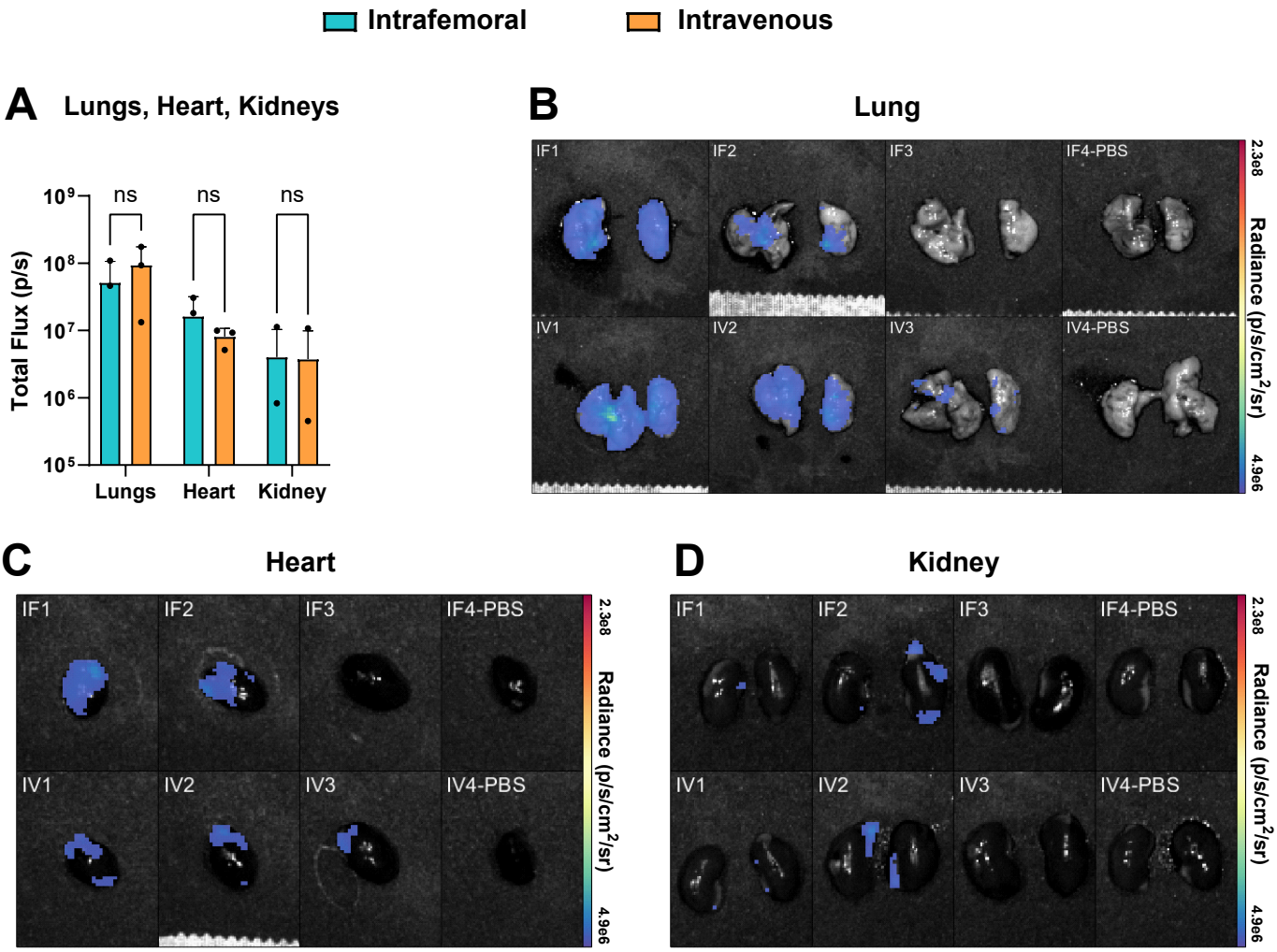

Figure S2 - LNP^{LFluc} expression in lungs, heart, and kidneys after intrafemoral or intravenous delivery. (A) The total luminescent flux measured 10 minutes after D-luciferin administration in LNP^{LFluc} treated mice is shown. (B-D) Heatmaps of radiance in organs corresponding to those in (A), reveal the distribution of radiance displayed as an overlay on reflected light images of the organs.

Figure S3

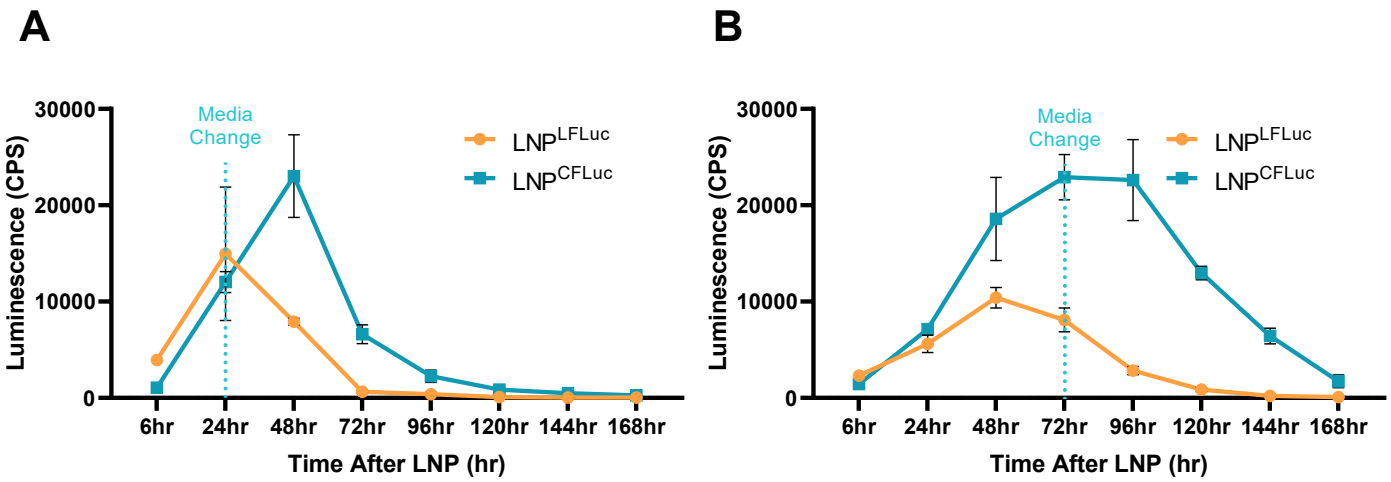

Figure S3 - Increased exposure time of LNPs increases total expression and peak expression time.
(A,B) Time-series plots of luminescence as a function of time post exposure to either linear or circular Luciferase mRNA delivered with LNPs in MEFs. Excess extracellular LNPs were removed from cells either 24 hours (A) or 72 hours (B) post-exposure by a media change.

Figure S4

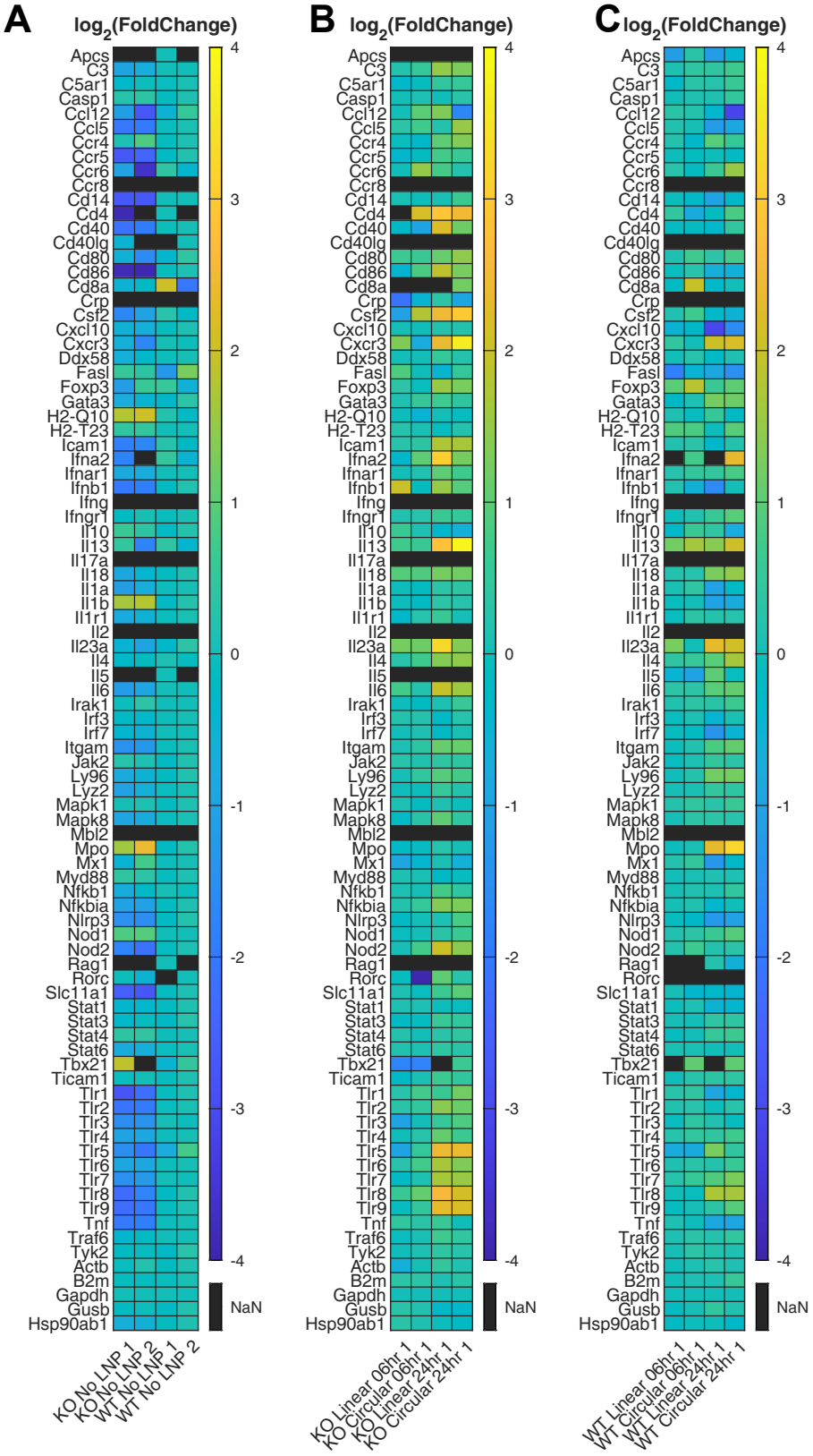

Figure S4 - qRT-PCR Array Screening of LNP-treated *ex vivo* expanded wildtype and Fancc$^{(-/-)}$ LT-HSC. *Ex vivo* expanded LT-HSCs were treated with either LNP^{LFancc} or LNP^{CFancc} for 6 or 24 hours and RNA content was analyzed by qRT-PCR. (A) A heatmap of relative transcript concentrations highlighting baseline differences between Fancc$^{(-/-)}$ and wildtype cells. Values are expressed as the binary log of fold change difference from the mean value of the wildtype cells. Additional heatmaps highlighting differences in relative transcript concentrations of the mutant cells (B) or wildtype cells(C) after treatment with Fancc mRNA from the respective mean of untreated controls. All fold change values are calculated as the relative concentration difference to the untreated control after normalization to Gapdh.

Figure S5

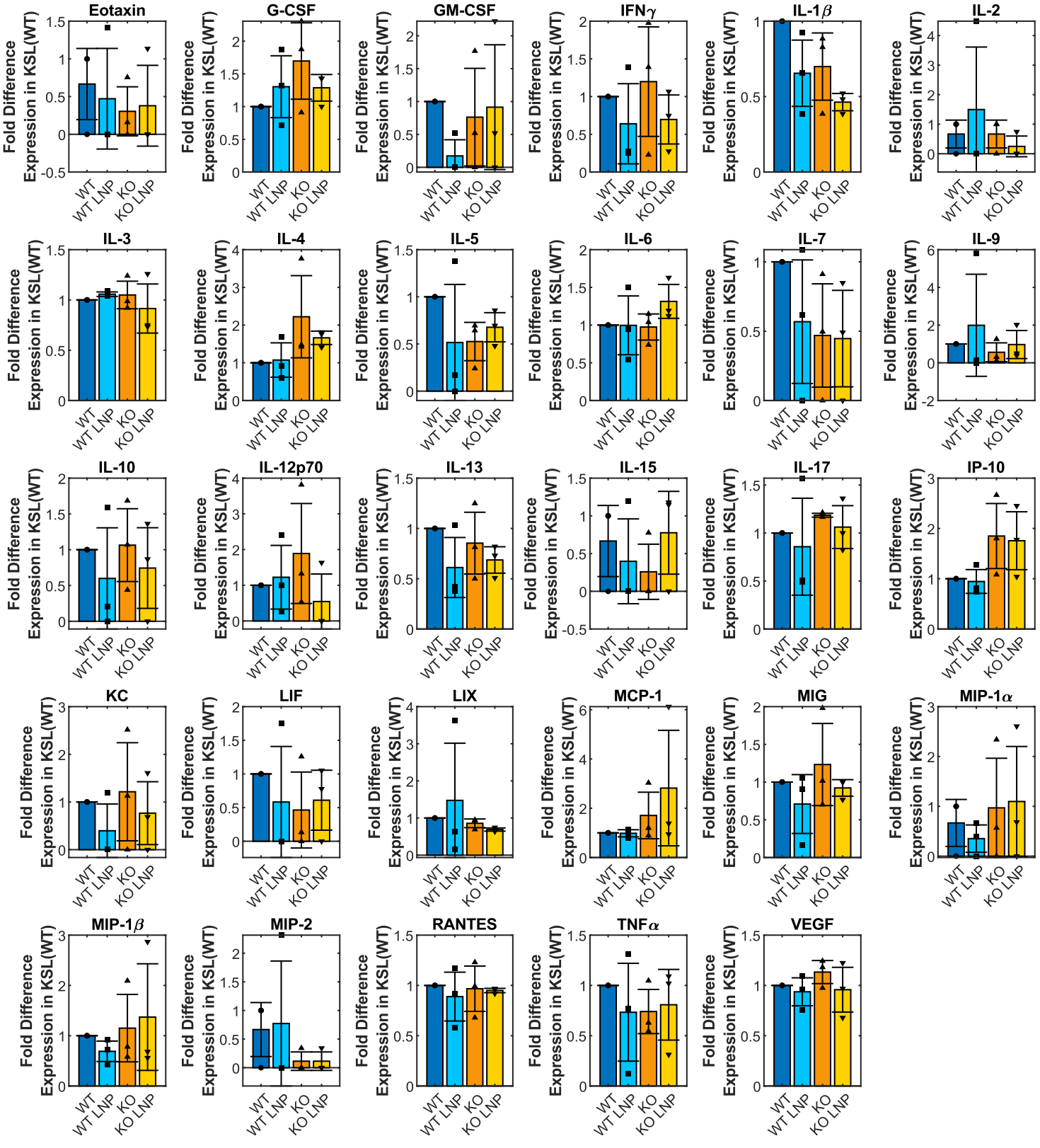

Figure S5 – Immune cytokine expression of KSL populations after LNP^{CFancc} exposure. Cytokine expression in KSL media supernatant was measured by Mouse Cytokine 32-Plex Discovery Assay (Eve Technologies Corporation) after LNP^{CFancc} treatment and a 24hr incubation period. Each data point reflects pooled supernatants from an individual cohort. Values are displayed as the fold difference expression measured in the untreated wildtype from the respective cohort.

Figure S6

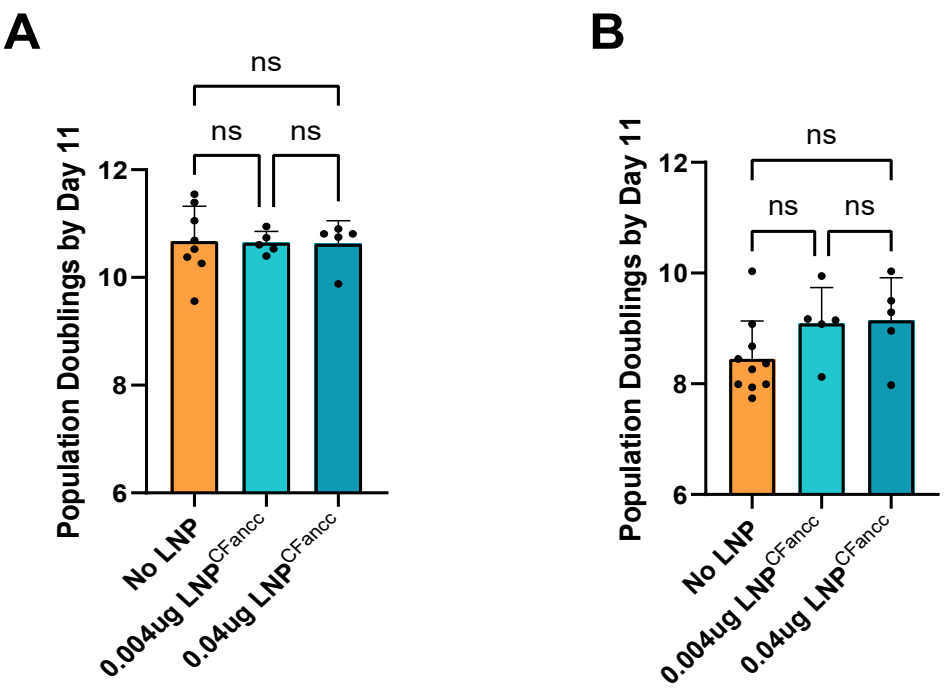

Figure S6 – Impact of a single dose of LNP^{CFancc} on proliferation of *ex vivo* expanded LT-HSCs.

LT-HSCs sorted from freshly isolated bone marrow were treated with a single dose (0.004ug or 0.04ug RNA) of LNP^{CFancc} and expanded over 11 days. Cells were counted after 11 days of culture to determine the total number of population doublings in (A) wild-type and (B) Fancc(-/-) populations.

Figure S7

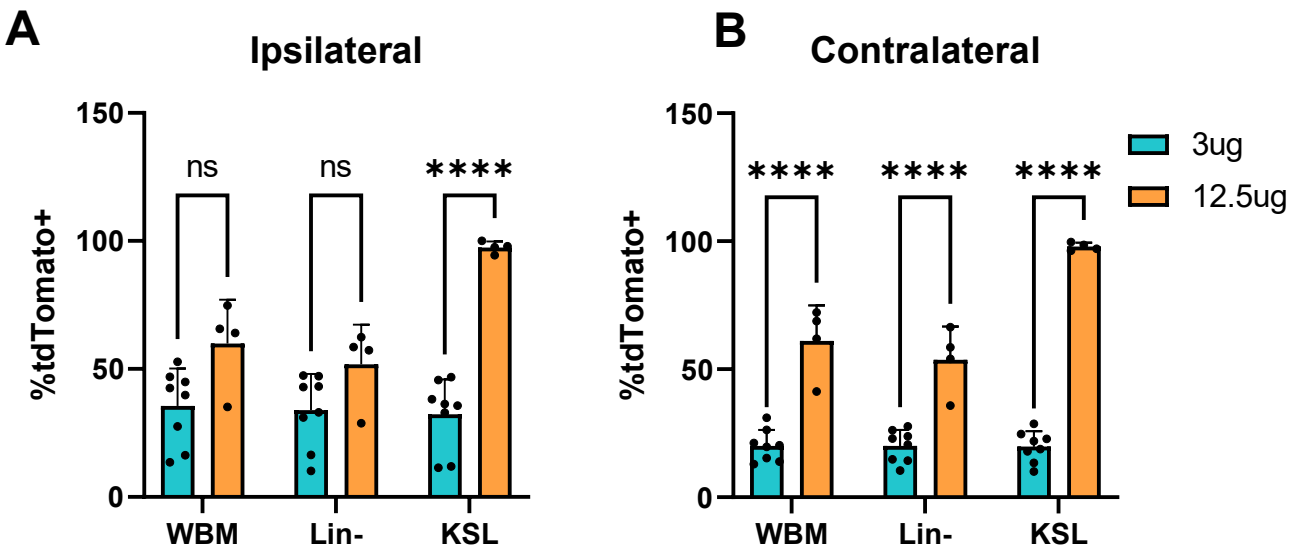

Figure S7 - tdTomato expression after LNP^{Cre} Treatment is correlated to LNP dose. Displayed are positivity rates of tdTomato in bone marrow populations after either 3ug or 12.5ug doses of LNP^{Cre} administered intrafemorally measured in (A) ipsilateral, or (B) contralateral femurs.

Figure S8

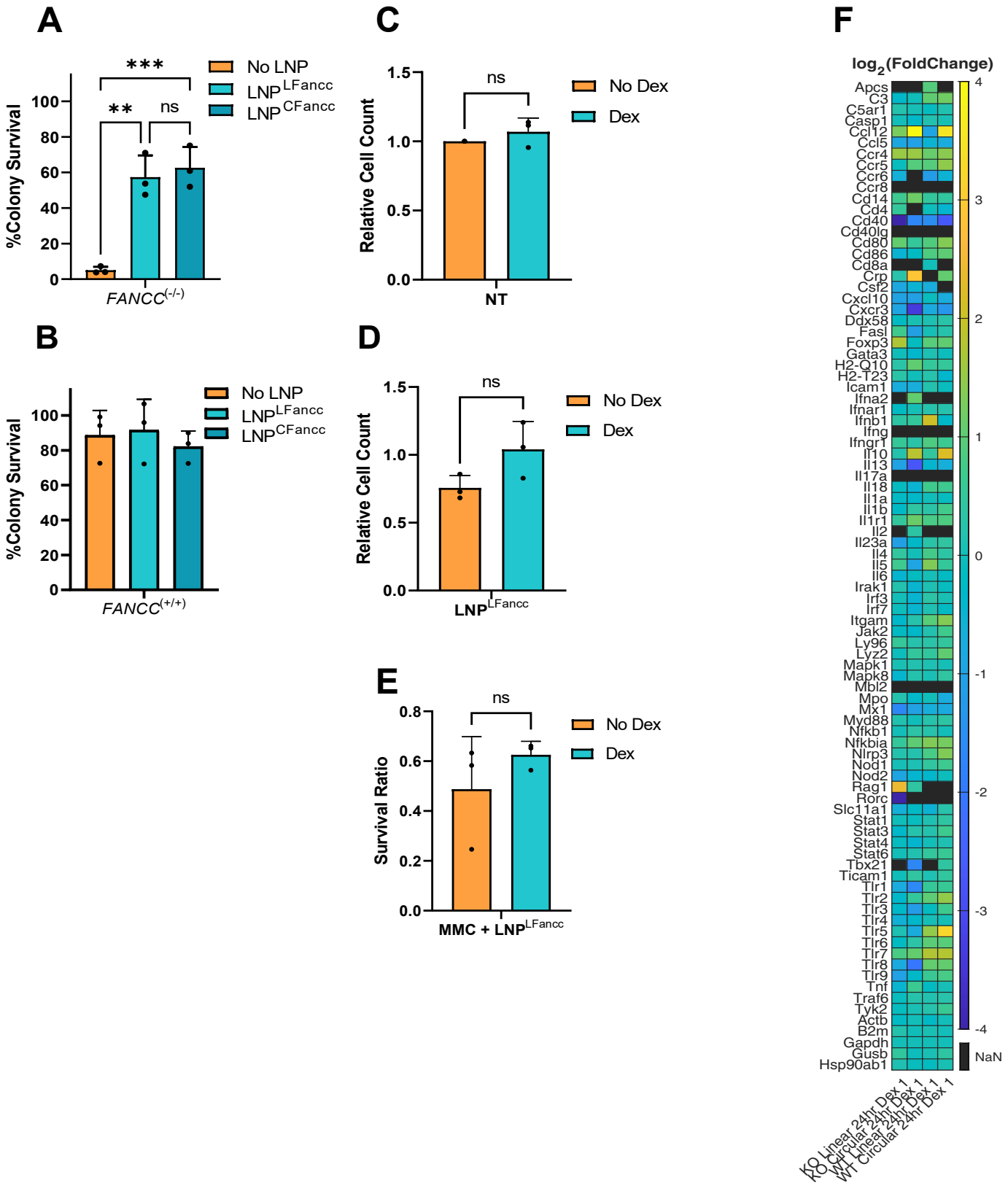

Figure S8 - Addition of dexamethasone to BM populations in vitro does not affect conferral of Fancc functionality. (A,B) *Ex vivo* expanded HSCs plated into CFU assay as in Figure 1 after exposure to 100nM dexamethasone during LNP treatment and MMC exposure. No impact of dexamethasone on colony survival was observed. (C-E) Similarly, no impact is measured on in vitro proliferation as a function of cell counts or in the survival rate after MMC exposure in Lin- populations. (F) Screening by qRT-PCR highlighting differences in immune marker expression when treated with LNPs in the presence of dexamethasone. All values are normalized to the respective LNP treated cells without dexamethasone.