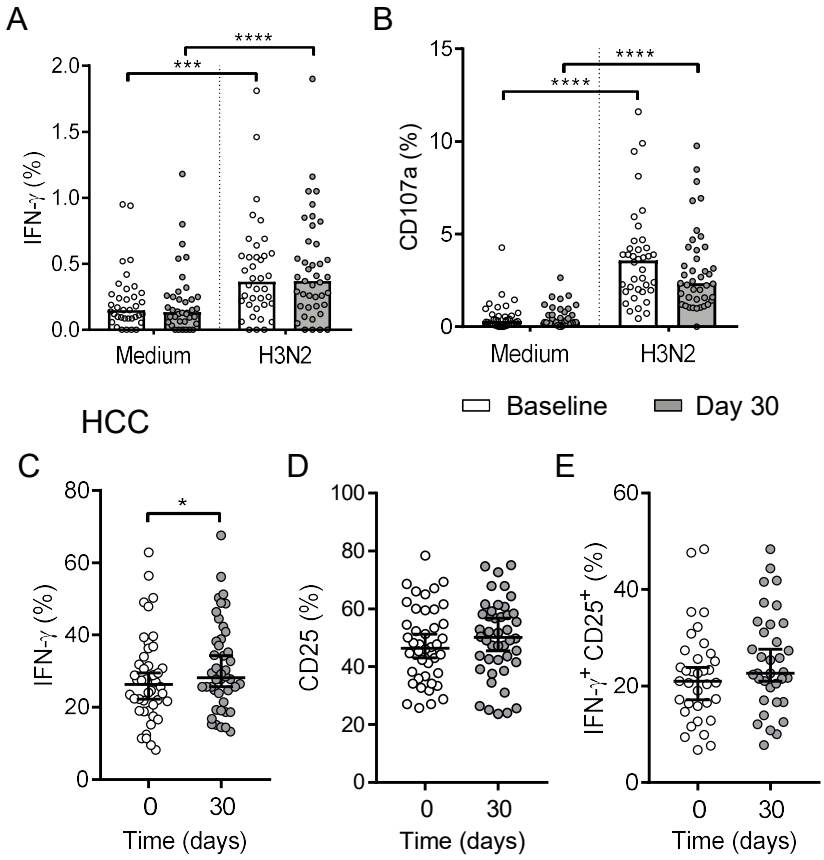
Supplementary Figure 1

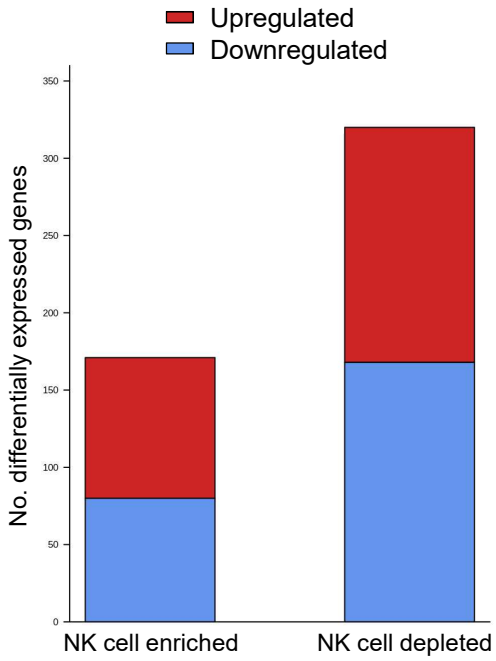


Post-vaccination NK cell responses to H3N2 alone or a high concentration of cytokines (HCC).

Baseline and 30 day post-vaccination PBMC samples were stimulated *in vitro* with medium alone, H3N2 alone or a high concentration of IL-12 and IL-18 (HCC). IFN-γ, CD107a and CD25 expression of $CD56^{+}CD3^{-}$ NK cells was measured after 18 hours by flow cytometry. IFN-γ (a) and CD107a expression (b) in response to medium and H3N2 alone is shown as one dot per donor with a bar at the median percentage. IFN-γ (c), CD107a (d) and IFN-γ+CD25+ expression (e) in response to HCC is also shown with a line representing the median and interquartile range. Comparisons were made using Wilcoxon signed-rank test. *p < 0.05, ***p < 0.001, ****p < 0.0001.

Supplementary Figure 2

A



B

NK cell enriched

Fold Change	P Value	Gene Symbol
6.43190721	0.000170821	ANKMY2
5.355390706	4.98692E-05	TMTC4
3.816643993	0.00033623	NEK2
3.292438988	0.001694043	MIS12
-2.414231737	0.001438823	MYL12B
-4.197723471	0.000212728	ALDH1A3
-4.276269007	0.001877481	KRT14
-4.335515006	0.00052463	S100A2
-4.358778881	0.001507239	SPRR2G
-4.840941282	0.000736282	SPRR2E
-5.204932251	0.000391761	DEFB4B
-5.209462057	0.000387381	DEFB4A
-5.564447038	7.12657E-05	SPRR2A
-5.853781345	1.69684E-05	S100A7

NK cell depleted

Fold Change	P Value	Gene Symbol
4.329536445	7.2356E-05	NDUFB11
4.298929479	0.000403647	MTIF2
4.241901944	4.23996E-05	DSTN
3.992520393	0.001539383	TAF11
3.783450721	0.000313461	RPS27L
3.576370618	0.000353145	HELLS
3.57110555	0.000298696	DDX1
3.547422619	0.000368178	ARMC1
3.465053564	0.000493905	MAP7
3.311301535	0.001391633	CHID1
3.247626837	0.001065012	RBM15B
3.233765505	0.000741021	STYXL1
3.190237932	0.000663372	SQSTM1
2.966848594	0.001826702	HDAC7
2.710011762	0.001128226	DHTKD1
-3.076998984	0.001839859	ZFP36L1
-3.589496013	0.001897364	SOCS3
-4.191728976	2.22091E-05	RHOXF2
-4.19269165	7.3451E-05	RHOXF2B
-4.288219474	0.001727109	NFKBIZ
-4.428849115	0.000711537	ALPL
-4.566206763	0.000261361	PROK2
-6.1396533	1.50688E-06	AC025627.3

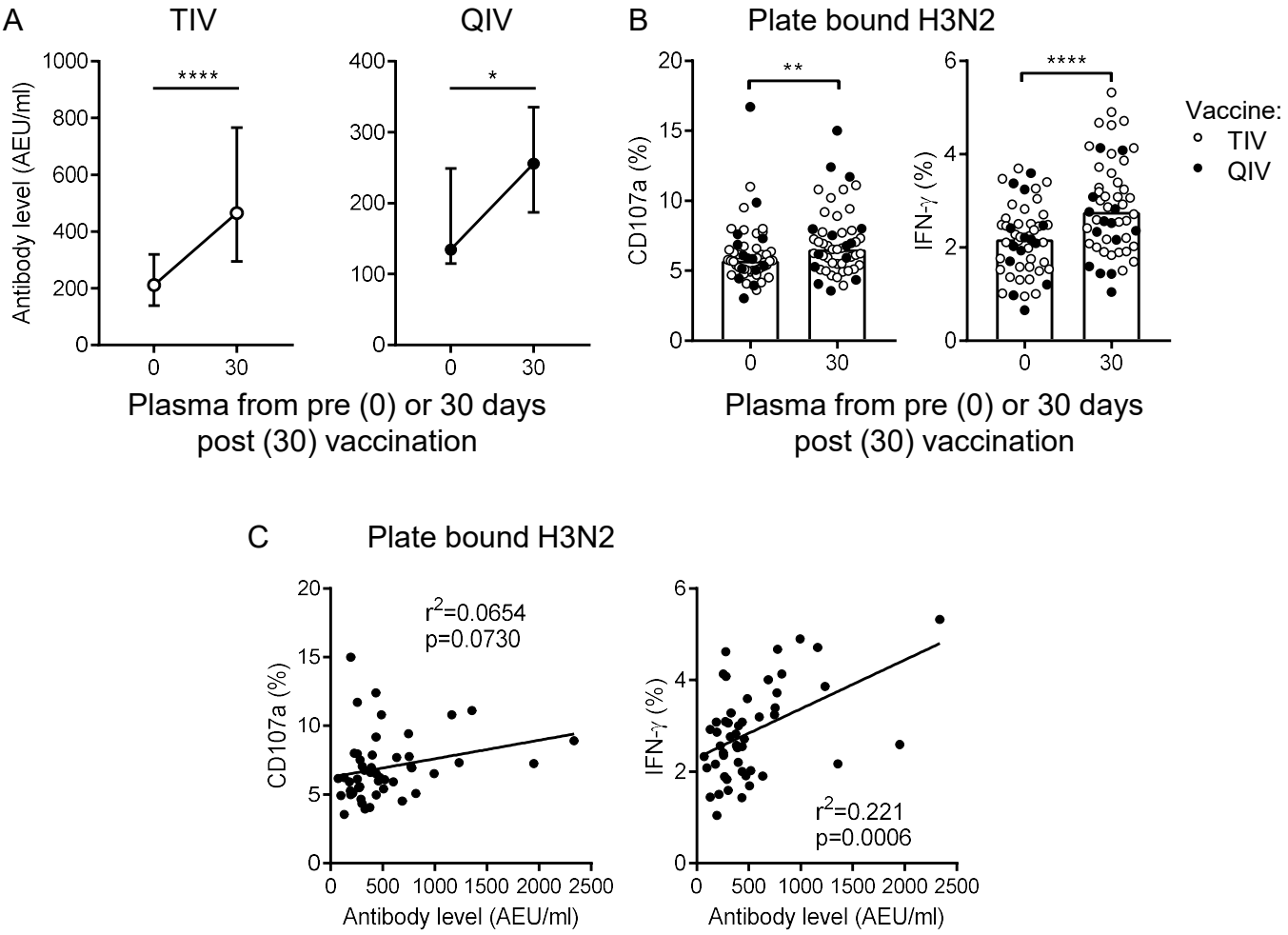
RNA sequencing analysis of IL-15 stimulated baseline and 30 day post-vaccination PBMC samples.

Baseline and 30 day post-vaccination PBMC samples were stimulated *in vitro* with 0.75ng/ml IL-15 for 18 hours, PBMC were separated in to NK cell enriched and NK cell depleted fractions using NK cell isolation kit (Miltenyi Biotec). RNA was extracted from cell pellets using Norgen RNA/DNA purification kits (Norgen Biotek) following the manufacturer's instructions.

Library preparation was performed using QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen GmbH, Austria), following the manufacturer's instructions. 3' RNA sequencing generates one read per transcript at the 3' end of polyadenylated mRNA and maintains strand-specificity. Sample RNA concentration was measured using Qubit HS RNA kits (Thermo Fisher Scientific) and RNA quality was tested using Agilent RNA 6000 Pico chips (Agilent Technologies, USA). 5 µl RNA was used for library preparation from sample concentrations ranging between 600pg/µl to 70ng/µl. The PCR Add-on kit (Lexogen GmbH) was used during sample preparation to generate libraries that were not under or over-cycled. Final library concentration was assessed using Agilent DNA chips and Qubit before libraries were normalised to 6nM prior to pooling. Libraries were shipped to Lexogen GmbH for quality control and sequencing on two lanes of an Illumina HiSeq (Illumina). Bam files were converted to FastQ using Picard, FastQ files were uploaded to BlueBee Genomics Platform (BlueBee). FastQ files were trimmed and quality filtered using the default Integrated QuantSeq FWD workflow. In brief, Bbduk was used to trim low-quality tails, adapter contamination and polyA read-through, reads were aligned to human genome version GRCh38.77 using STAR Aligner and reads were counted using HTSeq-count with kit-specific options.

Read counts were normalised by variance-stabilising transformation (R package *DESeq2*). A linear mixed-effects model with normalised read counts as the outcome and including individual ID as a random effect was used to identify differentially expressed (DE) genes at 30 days post-vaccination vs baseline (R package *lmer*). A cut off P value of <0.05 and fold-change of >2/<-2 were used to define DE (a), the DE with a p value of <0.001 and fold-change of >2/<-2 are listed as gene symbols in a table (b). Raw and processed sequencing data are deposited in the NCBI GEO public database (GSE133478).

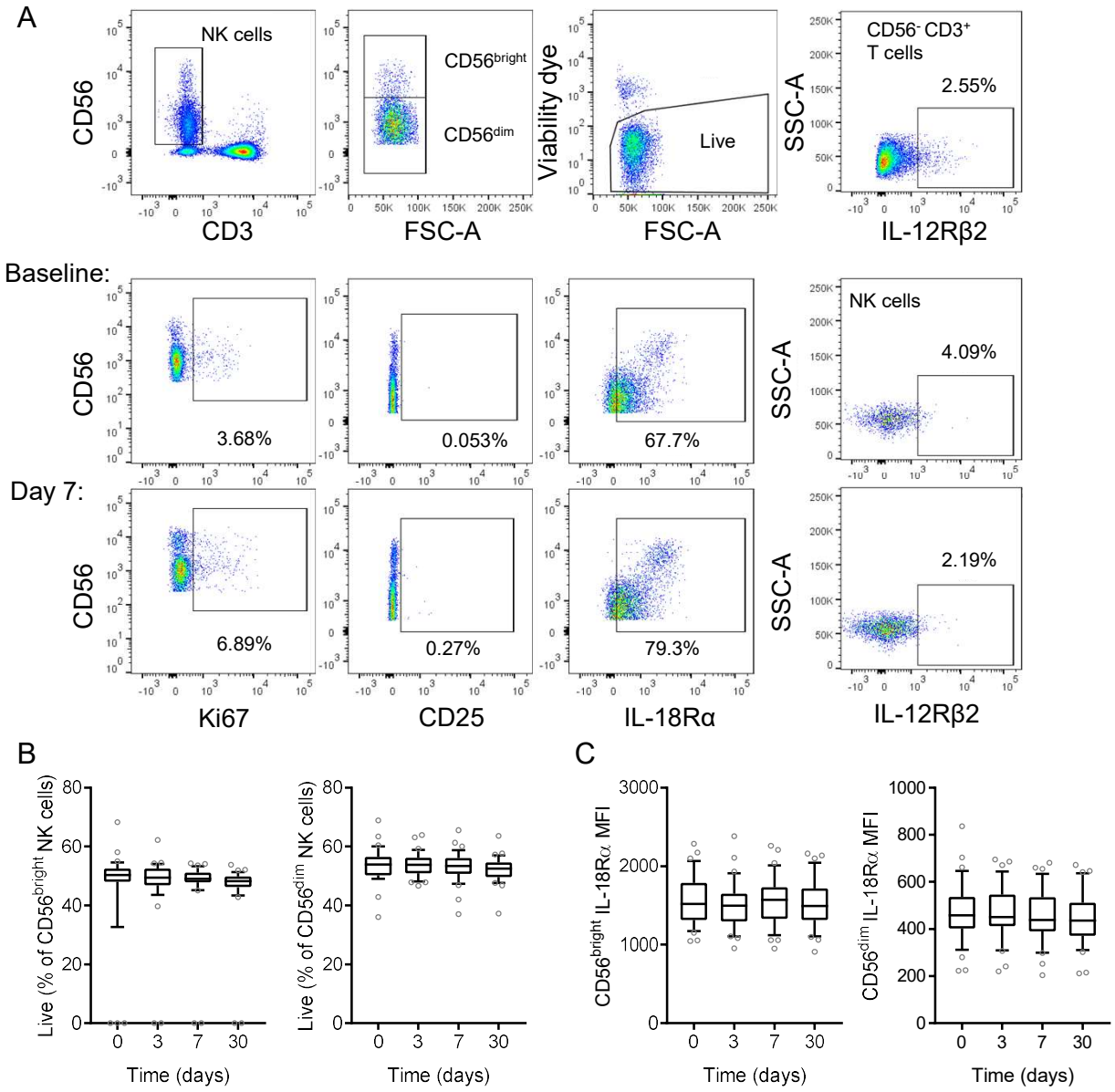
Supplementary Figure 3



Antibody-dependent NK cell responses post-vaccination.

Antibody level in baseline (0) and 30 day post-vaccination (30) plasma samples of participants vaccinated with TIV (n=37) and QIV (n=14) measured by ELISA (a). NK cell CD107a and IFN-γ expression in response to pre or post-vaccination plasma and plate bound inactivated H3N2 virus, one non-vaccinated control donor (b). Linear regression analysis showing correlation between antibody level and NK cell CD107a and IFN-γ expression at 30 days post-vaccination. Goodness of fit was determined using r^2 and significance was determined by Pearson coefficient as p value below 0.05 (c). Comparisons were made using one-way ANOVA with Dunn's correction for multiple comparisons. *p < 0.05, **p < 0.01, ****p < 0.0001. AEU; arbitrary ELISA units.

Supplementary Figure 4



***Ex vivo* flow cytometry gating strategy.**

NK cell phenotype was measured after 2015-2016 TIV vaccination (n=37) at baseline, day 3, day 7 and day 30 post-vaccination by flow cytometry. The flow cytometry gating strategy for CD56, Ki67 and IL-12Rβ2 expression from one representative donor is shown (a). Percentage of live $CD56^{bright}$ and $CD56^{dim}$ NK cells (viability dye negative) (b) and $CD56^{bright}$ and $CD56^{dim}$ IL-18Rα MFI (c) across vaccination visits is shown as box and whisker plots with 10^{th}-90^{th} percentile. Comparison were determined by one-way ANOVA with Dunn's correction for multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.