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Supplemental Information

Heterogeneous Escape from X Chromosome Inactivation Results in Sex Differences in Type I IFN Responses at the Single Human pDC Level

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Supplemental Figure S1

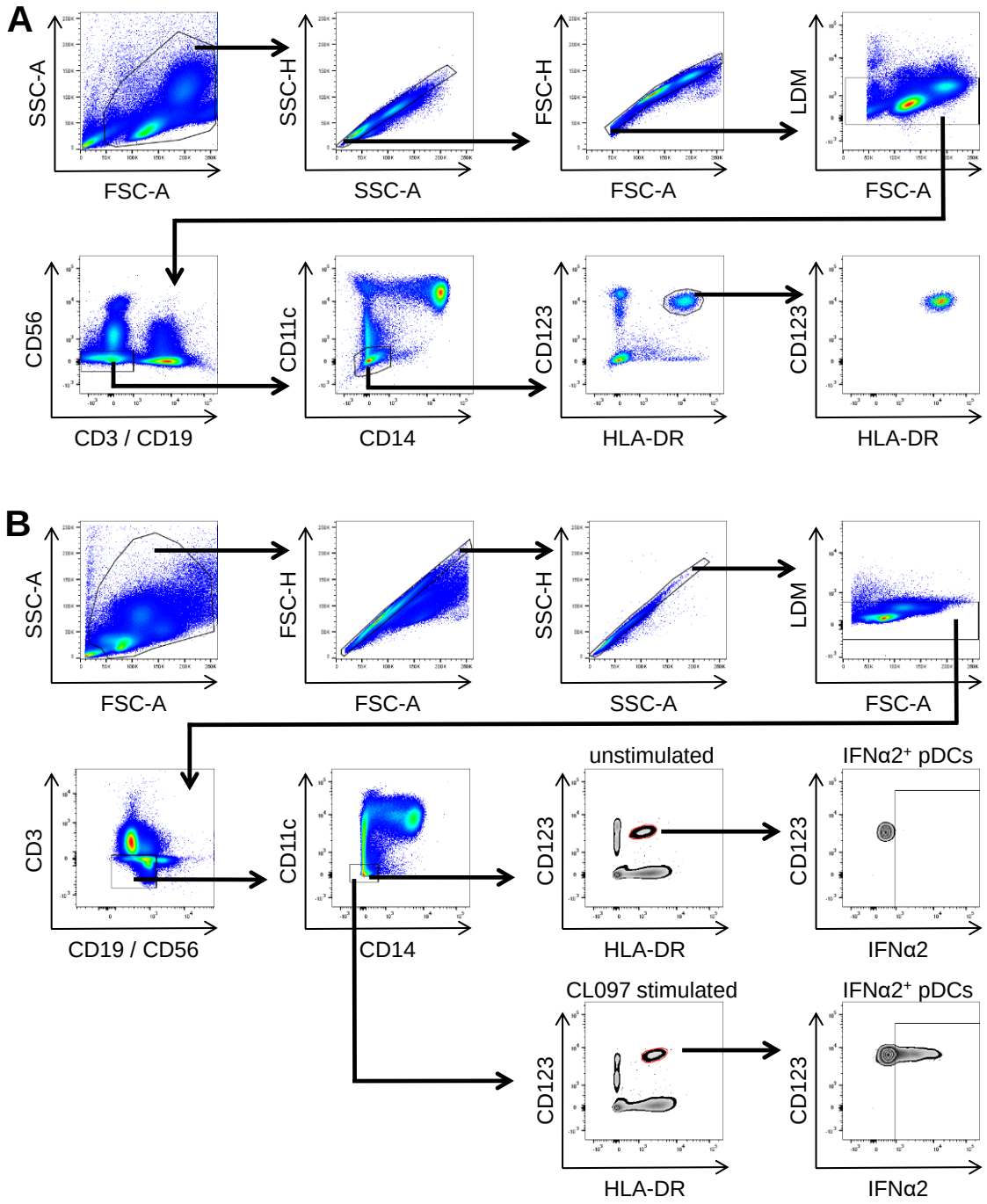

Figure S1. Gating strategy for identifying pDCs. Related to Figures 1B and S4.
(A) Gating strategy for identification of pDCs is shown in one representative plot. Using FSC-H und SSC-H single cells were selected. pDCs were defined as live and CD3-CD19-CD56-CD11c-CD14-CD123+HLA-DR+. pDCs were sorted from PBMCs or isolated pDCs based on this gating. This gating was also used for the assessment of TLR7 protein levels in pDCs. LDM: live-dead marker. **(B)** Gating strategy for identification of cytokine^{+} pDCs is shown in one representative plot. Using FSC-H und SSC-H single cells were selected. pDCs were defined as live and CD3-CD19-CD56-CD11c-CD14-CD123+HLA-DR+. An unstimulated sample (but BFA treated sample) was used to define the threshold for cytokine^{+} pDCs, with the example showing IFNα2 that was quantified with the clone 7N4-1 (BD) that primarily detects IFNα2. LDM: live-dead marker.

Supplemental Figure S2

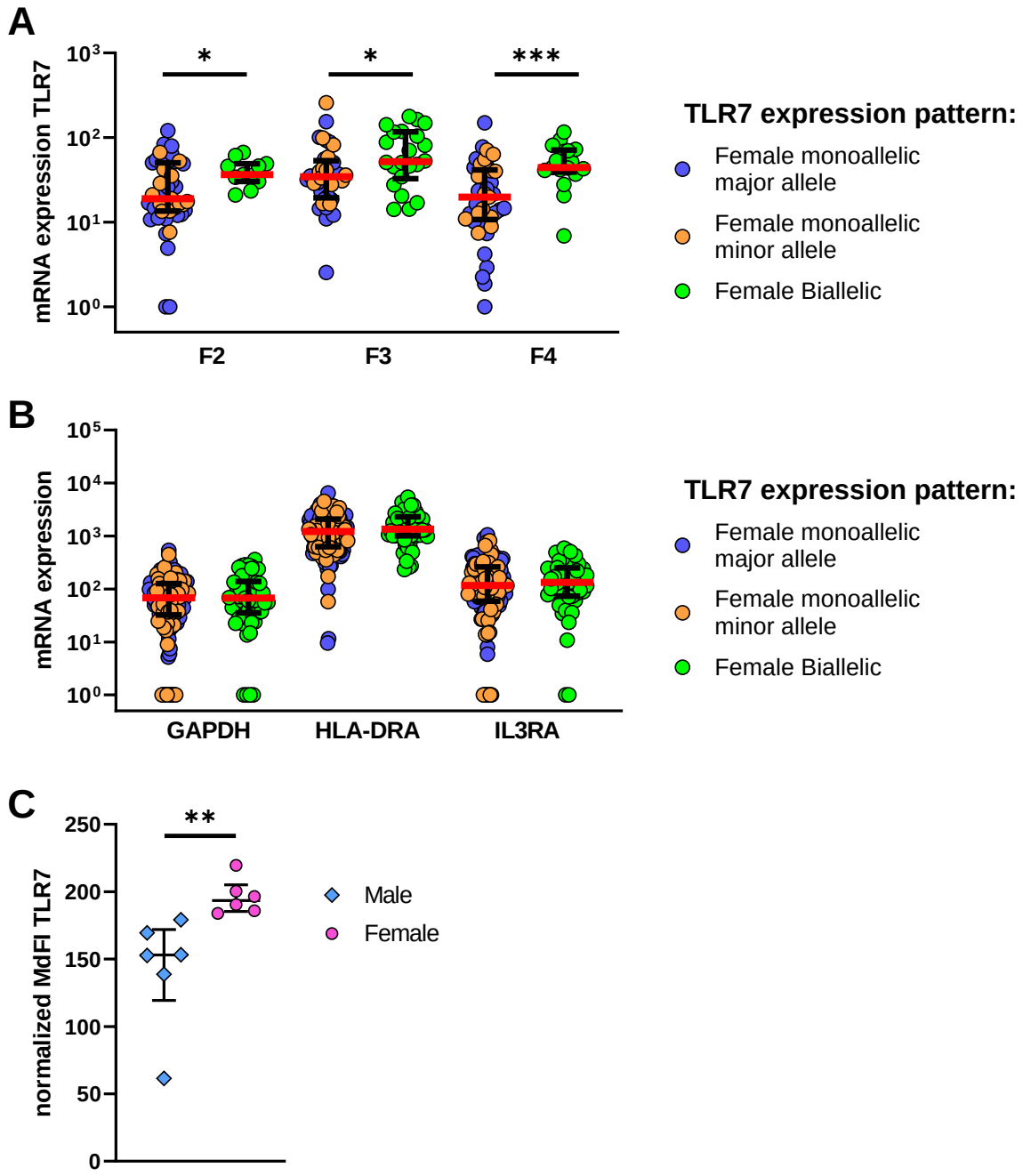

Figure S2. mRNA expression levels of TLR7 and mRNA expression of GAPDH, HLA-DR, IL3RA comparing monoallelic and biallelic TLR7-expressing pDCs. Related to Figure 4. **(A)** mRNA expression levels of TLR7 between female monoallelic (orange and blue circles) and female biallelic expressing pDCs (green circles) separately for F2, F3 and F4. Median (red bar) with interquartile range (black bars) is shown. Mann–Whitney test was used for statistical analysis. F = female. *$p < 0.05$; ***$p < 0.001$ **(B)** mRNA expression levels of GAPDH, HLA-DR and IL3RA (CD123) between female monoallelic (orange and blue circles) and female biallelic TLR7-expressing pDCs (green circles). n = 3 females. Median (red bars) with interquartile range (black bars) is shown. A mixed effects linear regression model with a random intercept was used to take into account the intra-sample correlations. **(C)** PBMCs from females (n = 6) and males (n = 6) were stained and pDCs were identified according to gating strategy in **Figure S1A**. TLR7 protein levels were determined via intracellular staining. The

median fluorescent intensity (MdFI) of TLR7 from pDCs was normalized to the MdFI of a corresponding FMO control of the same donor (MdFI TLR7 / MdFI FMO). Median with interquartile range is shown. Mann–Whitney test was used for statistical analysis. **$p < 0.01$.

Supplemental Figure S3

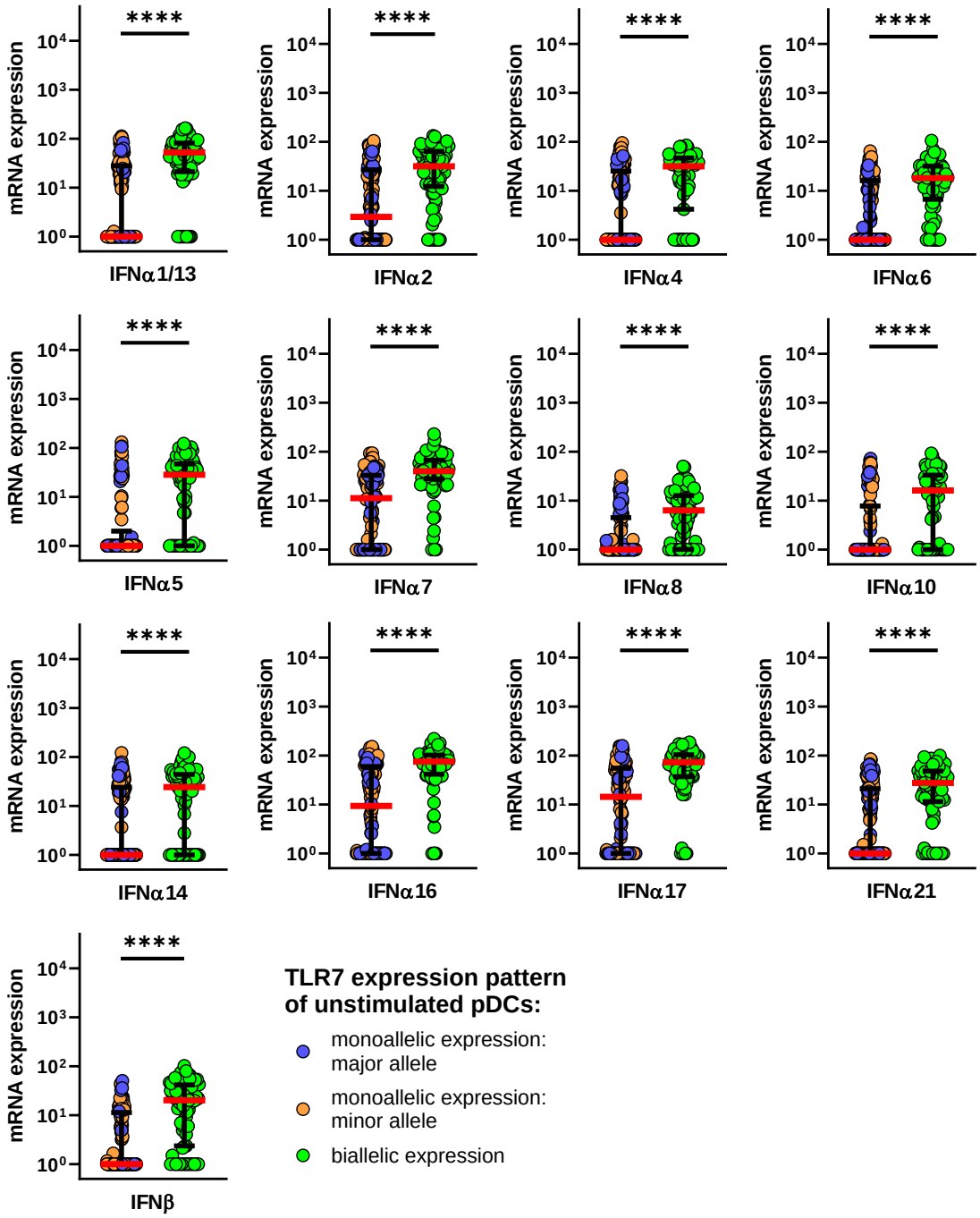

Figure S3. pDCs with biallelic expression of TLR7 have significantly higher mRNA levels of all IFNα subtypes and IFNβ. Related to Figure 5A.
Comparison of mRNA expression levels of isolated, unstimulated pDCs for all IFNα subtypes and IFNβ separately. Female monoallelic TLR7-expressing pDCs (blue circles = monoallelic expression of the major allele; orange circles = monoallelic expression of the minor allele) and female biallelic TLR7-expressing pDCs (green circles = pDCs with escape of *TLR7* from XCI) are shown. Expression patterns were determined using the following TLR7 SNP: rs3853839. Individual pDCs from n = 3 females are displayed. Median (red bar) with interquartile range (black bars) is shown. A mixed effects linear regression model with a random intercept was used to take into account intra-sample correlations. ****$p < 0.0001$.

Supplemental Figure S4

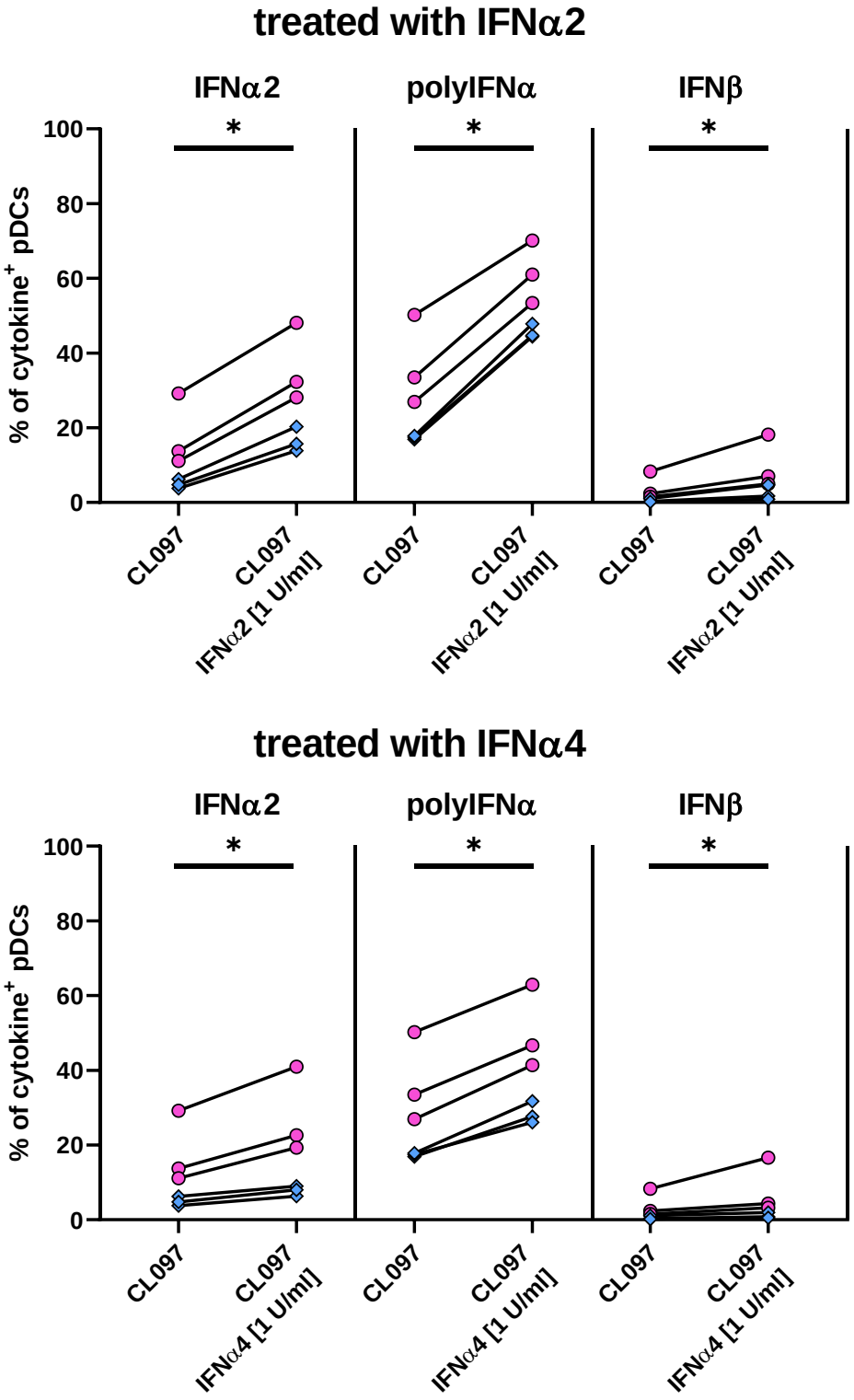

Figure S4. Effect of pre-treatment of PBMCs with IFNα2 and IFNα4 protein on IFNα/β protein production by human pDCs. Related to Figure 5E.
Comparing the percentage of cytokine$^+$ pDCs determined through intracellular cytokine staining (ICS). Gating strategy is shown in **Figure S1B**. PBMCs were incubated for 2 h in R10 (CL097) or in R10 with the indicated U of IFNα2 or IFNα4, before being stimulated with

CL097. IFNα2 was quantified with clone 7N4-1 (BD) which primarily detects IFNα2. PolyIFNα was quantified with clone LT27:295 (Miltenyi Biotec), which detects IFNα2 and several other IFNα subtypes. n = 3 females (pink circles) and n = 3 males (blue squares) were used. Female results are shown independent of TLR7 expression pattern. Wilcoxon signed rank test was used for statistical analysis. *$p < 0.05$.

Supplemental Tables

Table S1. Overview of genes and corresponding SNPs used to investigate escape from XCI in this study. Related to Figure 1A.

Information was obtained from the Ensembl genome database project (ensembl.org).
SNP = single nucleotide polymorphism; F = female; MAF = minor allele frequency.

Gene	Location of the gene [Mbp]	refSNP ID	Nucleotide Exchange	Location of SNP in mRNA	MAF	n	Individuals
TLR7	12.867 – 12.890	rs3853839	C > G	3‘ UTR	40.2%	3	F2, F3, F4
RPS6KA3	20.150 – 20.268	rs7051161	T > A	3‘ UTR	25.0%	3	F3, F4, F5
CYBB	37.780 – 37.813	rs5964151	T > G	3‘ UTR	20.4%	2	F1, F3
BTK	101.349 – 101.391	rs700	T > G	3‘ UTR	26.9%	5	F1, F2, F3, F4, F5
IL13RA1	118.727 – 118.795	rs2495636	A > G	3‘ UTR	25.6%	2	F1, F5

Table S2. Oligonucleotides used for pre-amplification of SNP regions. **Related to Key resources table for oligonucleotides.**

Gene	Forward Primer	Reverse Primer
BTK	GGAGCCCTGGAGCCTT	TCAGTCTGTCTTAATTCTCTCGGG
CYBB	AAGGAAATTTTCCAGATCATTAGGACA	CCCAGTTACCCTGCTGTATTAGTA
IL13RA1	CACTGTGACCTTGAGAAGATTC	GCTCTTATGAGCTGCCTGTTTT
RPS6KA3	GTAGAAAGCCTTCCATTTTGTGAAC	TCGAAGATAATTGCCTTCTTTGCC
TLR7	TGGGCACCACACAGGT	CTGTTTCCCTATGGAACCCAGAA

Table S3. Oligonucleotides used for pre-amplification and real-time quantitative PCR. Related to Key resources table for oligonucleotides.

Gene	Forward Primer	Reverse Primer
B2M	TTAGCTGTGCTCGCGCTAC	CTCTGCTGGATGACGTGAGTAA
BTK	CCTCTCTACATCTGGGAATGCA	TGCTCAGAAGCCACTATCCC
CYBB	GAGAGTGTCTCAACACTTATTAGTGAC	CCCAGTTACCCTGCTGTATTAGTA
GAPDH	GAACGGGAAGCTTGTCATCAA	ATCGCCCCACTTGATTTTGG
HLA-DRA	CGCTCAGGAATCATGGGCTA	CGCCTGATTGGTCAGGATTCA
IFNA1/13	GCCTCGCCCTTTGCTTTAC	TGTGGGTCTCAGGGAGATCA
IFNA10	CTATAACCACGACGCGTTGAA	AGTGCCTGCACAGGTATACA
IFNA14	CAAGTCAAGCTGCTCTCTGG	TGCCATGAGCATCAAAGTCC
IFNA16	CCATCCTGGCTGTGAGGAAA	GCACAAGGGCTGTATTTCTTCC
IFNA17	ACCACCACGAGTTGAATCAAAA	ACTAGTGCCTGCACAGGTAT
IFNA2	CCTGGCACAGATGAGGAGAA	CCAAACTCCTCCTGGGGAAA
IFNA21	TGGAAGCCTGCGTGATACA	CCAGGATGGAGTCCACATTCA
IFNA4	CACTTCTATAACCACCACGAGTTG	TGCACAGGTATACACCAAGCT
IFNA5	GTGGAAGACACTCCTCTGATGAA	CTCTGACAACCTCCCATGCA
IFNA6	GGAGGAGTTTGATGGCAACC	AGGTCTGCTGAATCACCTCA
IFNA7	CTCCTGCTTGAAGGACAGACA	TGGAACTGGTGGCCATCAAA
IFNA8	TGGTGCTCAGCTACAAGTCA	ATCAAGGCCCTCCTGTTACC
IFNB1	GCTTGAATACTGCCTCAAGGAC	GAACTGCTGCAGCTGCTTAA
IL13RA1	CACTGTGACCTTGAGAAGATTC	GGTGCAGTAGTTTCAGTTTCC
IL3RA	CTGGTCTGTGTCTTCGTGATCT	GTGAGGGATGCGGGGAAA
RPL13A	GAGGCCCCTACCACTTCC	GCCGTCAAACACCTTGAGAC
RPS6KA3	GTAGAAAGCCTTCCATTTTGTGAAC	ATTGCCTTCTTTGCCTAGCC
TLR7	ACAGGTGGTTGCTGCTTCA	CTGTTTCCCTATGGAACCCAGAA
XIST	TTGGATGGGTTGCCAGCTA	TCTCCACCTAGGGATCGTCAA

Table S4. Oligonucleotides used for SNPtying of gDNA and mRNA. Related to Key resources table for oligonucleotides.

Gene	SNP	Forward Primers	Reverse Primer
BTK	rs700	CTTTGTGCTCCCACTCAATACAA CTTTGTGCTCCCACTCAATACAC	TGCTCAGAAGCCACTATCCCAG
CYBB	rs5964151	ACATGTTGAGAGTGTCTCAACACTTAT ACATGTTGAGAGTGTCTCAACACTTAG	GGAGTATGCTCAGATGTCAATACTGTCA
IL13RA1	rs2495636	GGTGCAGTAGTTTCAGTTTCCATT GGTGCAGTAGTTTCAGTTTCCATC	CCCATTCTCCATTTGTTATCTGGGAAC
RPS6KA3	rs7051161	GCCTAGCCAAGCAGCCAA GCCTAGCCAAGCAGCCAT	GCCTTCCATTTTGTGAACATATAACTTGCT
TLR7	rs3853839	CTTCAGTGCTTCCTGCTCTTTTTC CTTCAGTGCTTCCTGCTCTTTTTG	CTATGGAACCCAGAAGCAGGC