Supplemental Materials

Discordant genotype calls across technology platforms elucidate variants with systematic errors in next-generation sequencing

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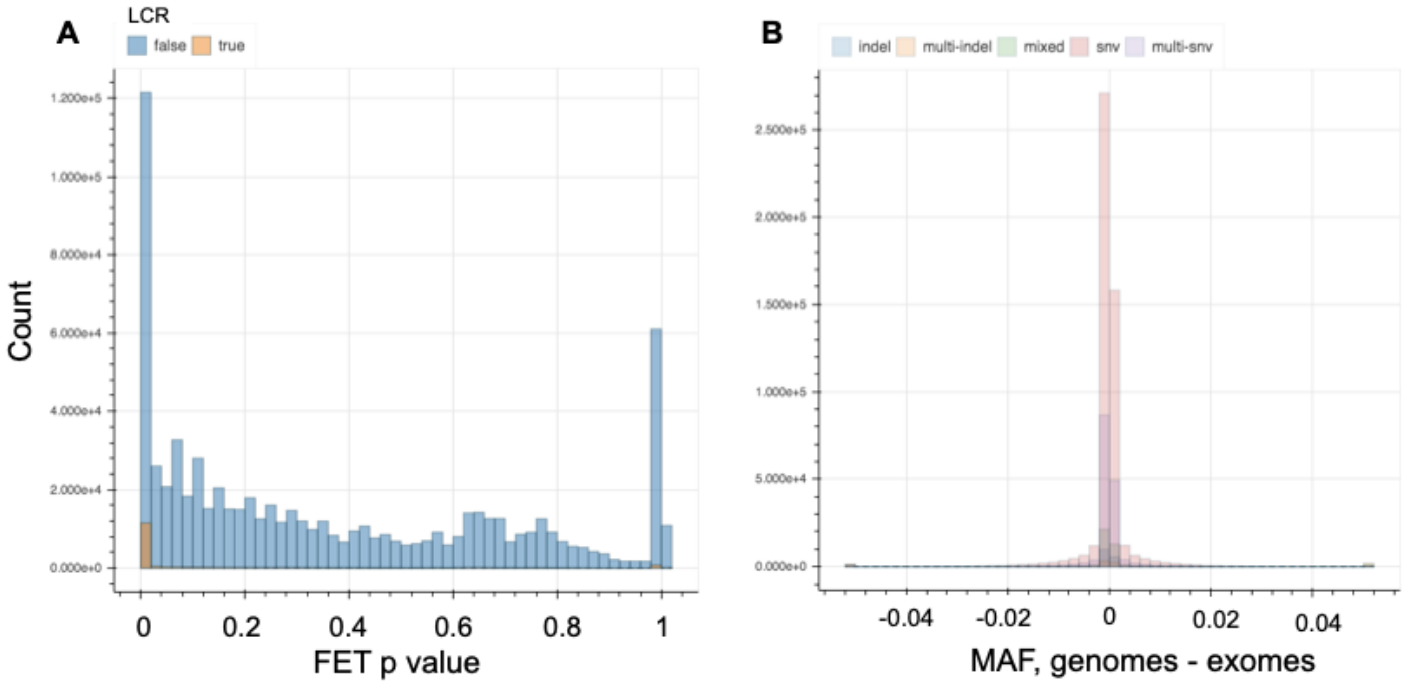
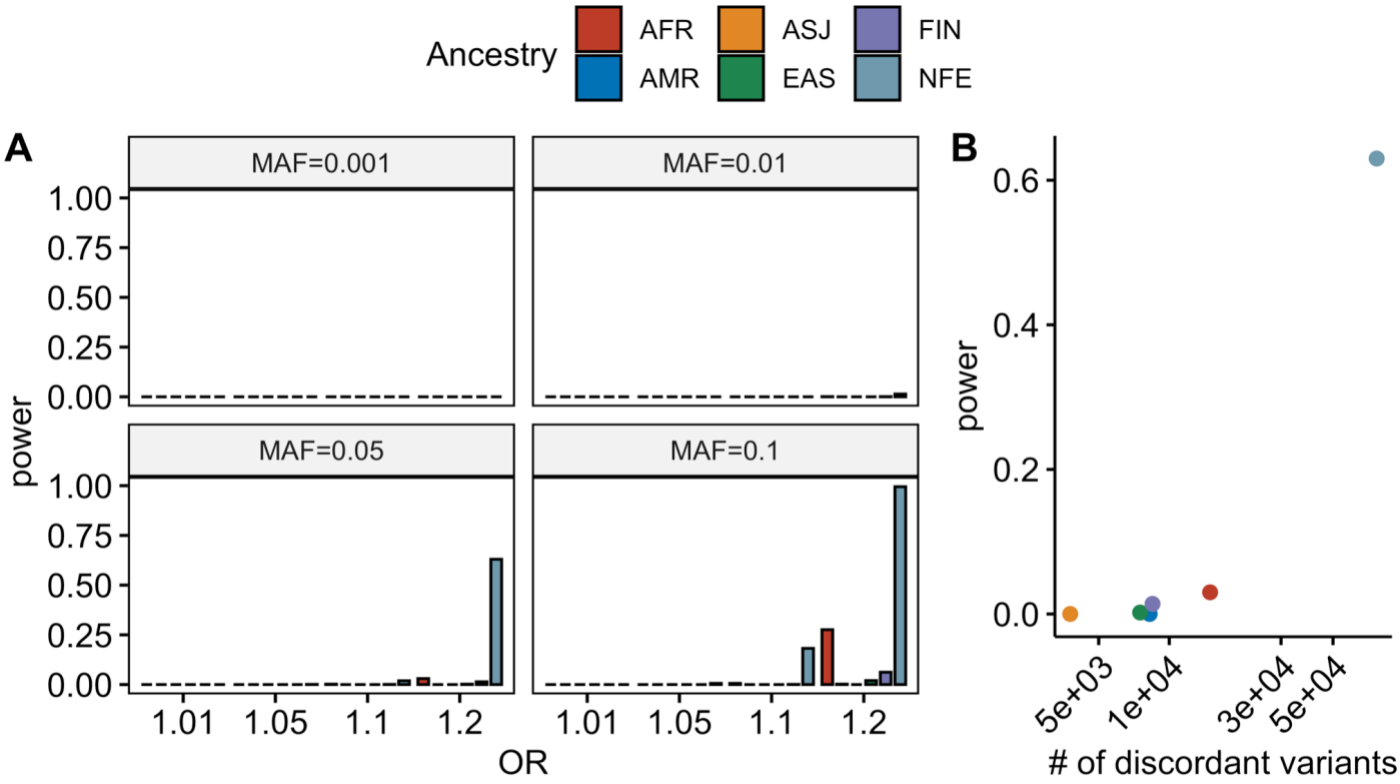
Supplemental Figures



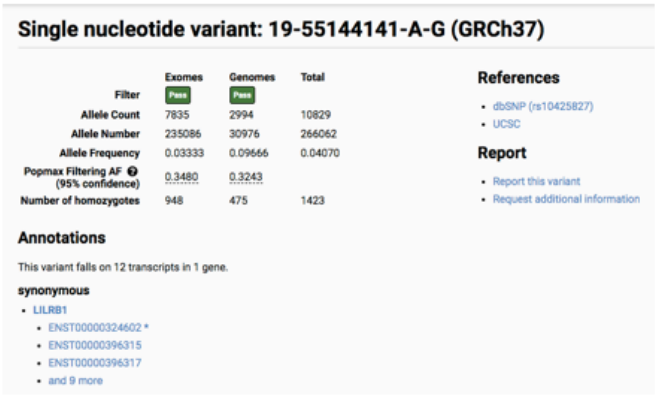
Supplemental Figure S1. The signal of discordance is consistent across AC thresholds. (A) FET p value for variants with at AC>1. Variants falling in the low complexity region (LCR) are indicated with orange and are enriched in the worst performing bin. **(B)** Distribution of allele

frequencies in the NFE exomes vs genomes at AC>5. Variants are colored by variant type. Both panels show results for the NFE.



Supplemental Figure S2. Power to detect variants with discordant allele frequencies between sequencing platforms. (A) Fisher's exact test power evaluated for the number of samples in gnomAD WES and WGS datasets for each ancestry given a particular MAF cutoff; **(B)** Fisher's exact test power for MAF=0.05 and OR=1.2 and the observed number of discordant variants ($p<1x10^{-5}$) for each ancestry. Note that due to sample size the NFE are the most highly powered for identifying discordant variants.

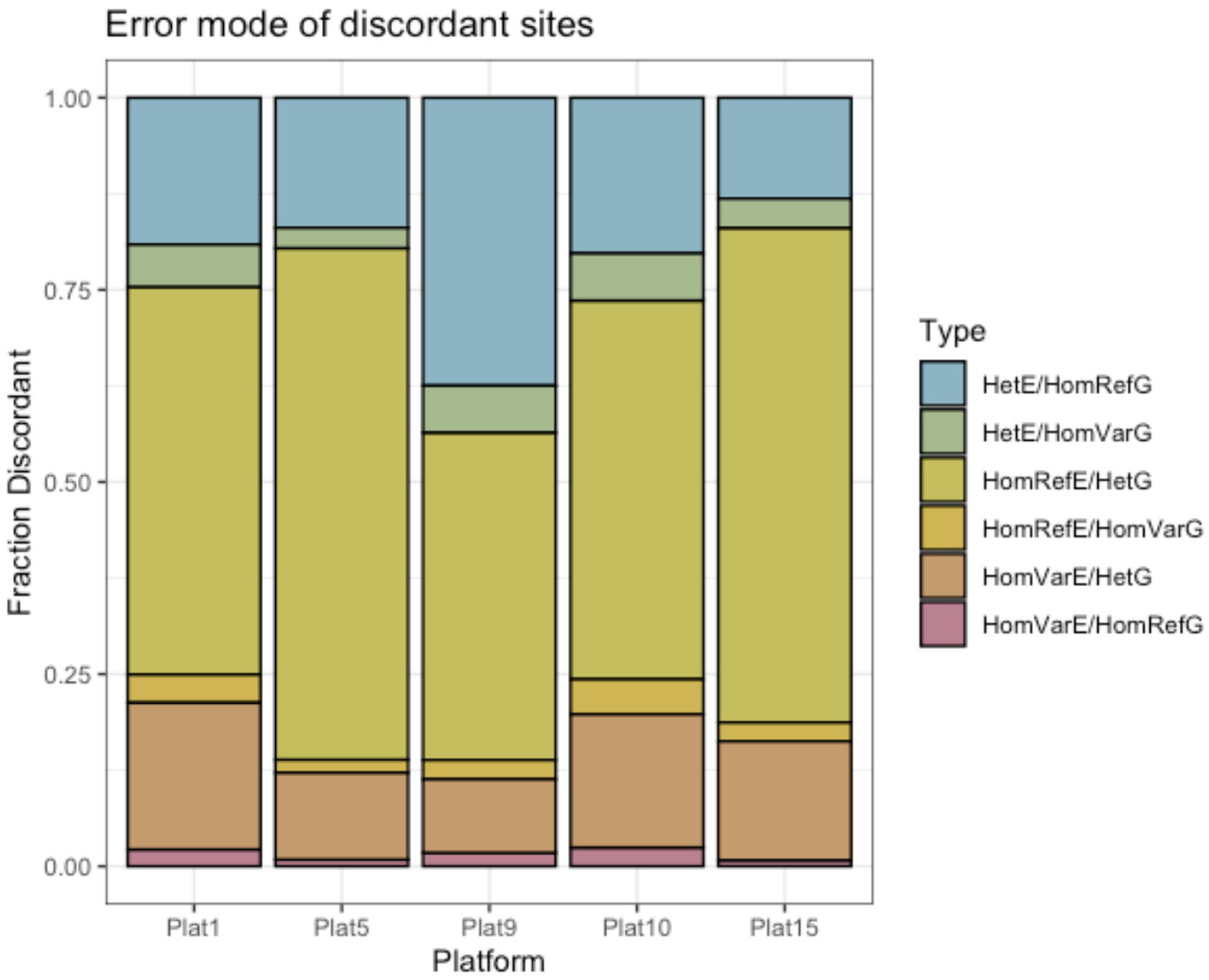
A



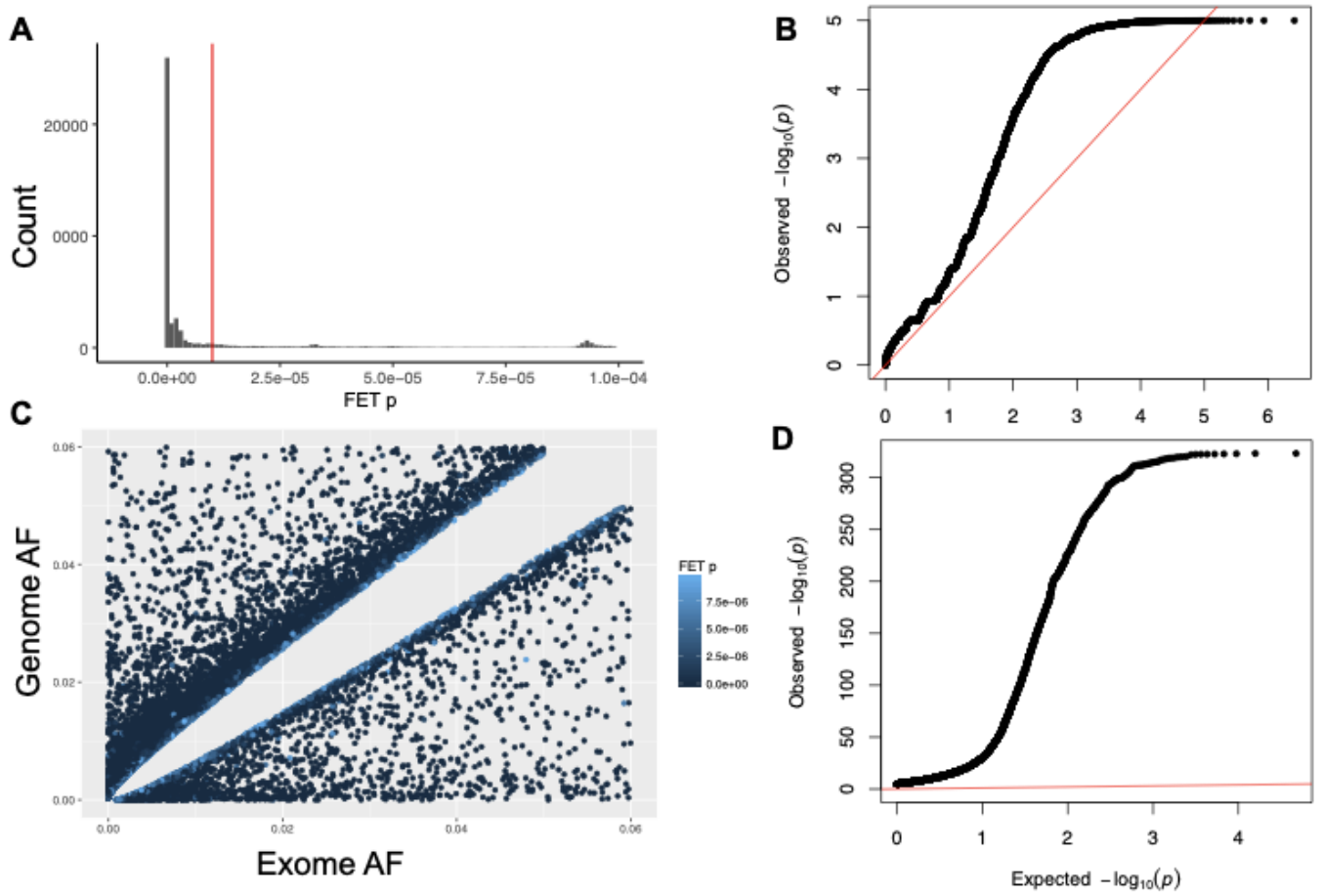
B

	0	1	2	3	4
0	Missing variant both	Missing variant G	Missing variant G	Missing variant G	Missing variant G
1	Missing variant E	No call both	No call G	No call G	No call G
2	Missing variant E	No call E	ref/ref both	Hom ref G / Het E	Hom ref G / Hom Var E
3	Missing variant E	No call E	Het G / Hom ref E	het both	Het G / Hom var E
4	Missing variant E	No call E	Hom var G / hom ref E	Hom var G / het E	hom var both

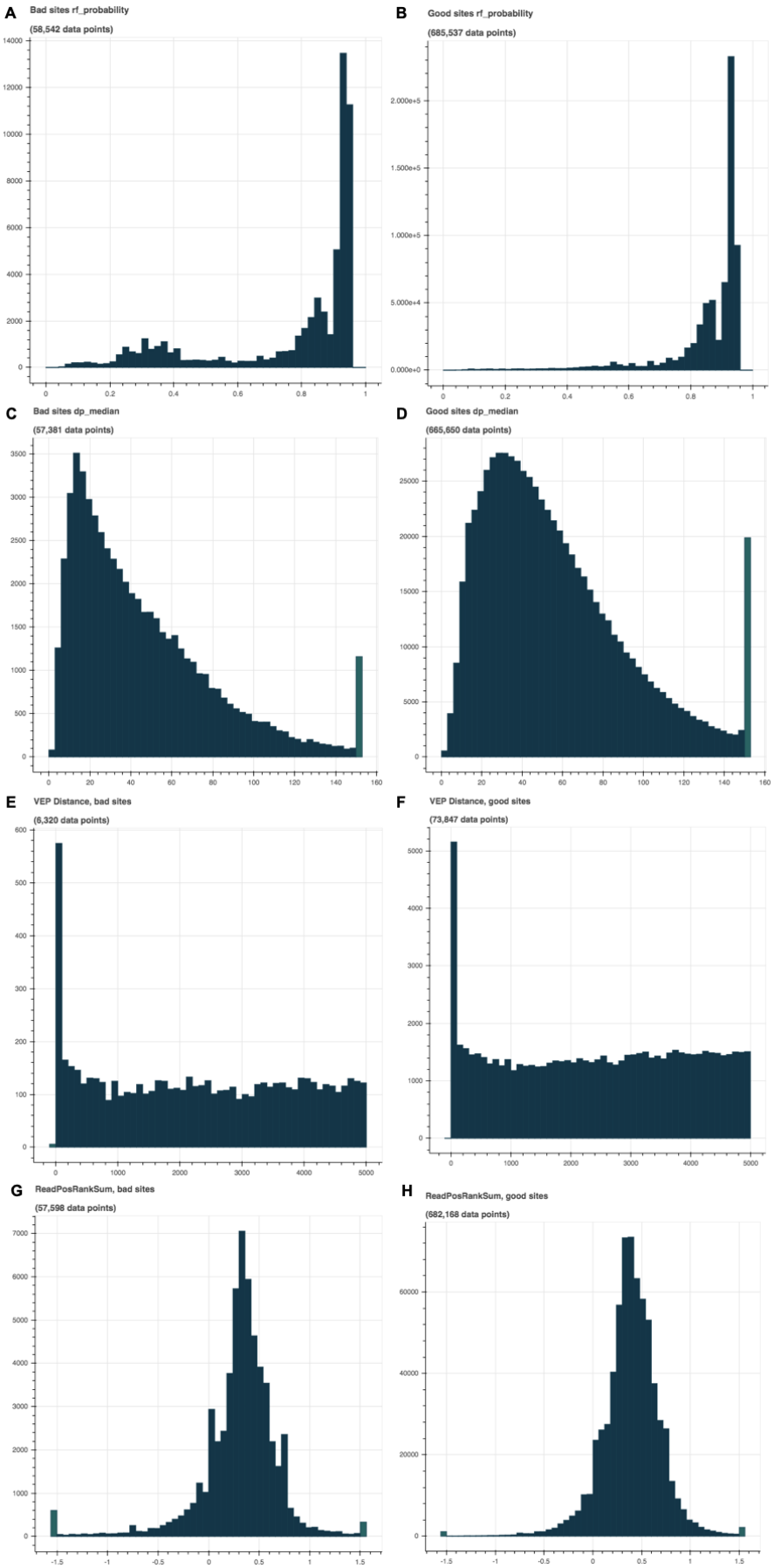
Supplemental Figure S3. Examples of discordance. (A) Example of a discordant variant as seen in the gnomAD browser. Note that this variant is PASS in both the Exomes and Genomes, but that there is a sizable MAF difference depending on technology. **(B)** Concordance table. The miscall categories considered as discordant here are shown in white. Gray indicates variants that were excluded from the concordance test due to missing information in one of the two datasets. Red indicates no alternative alleles were observed in either dataset. Green indicates concordant calls when alternative alleles were observed.



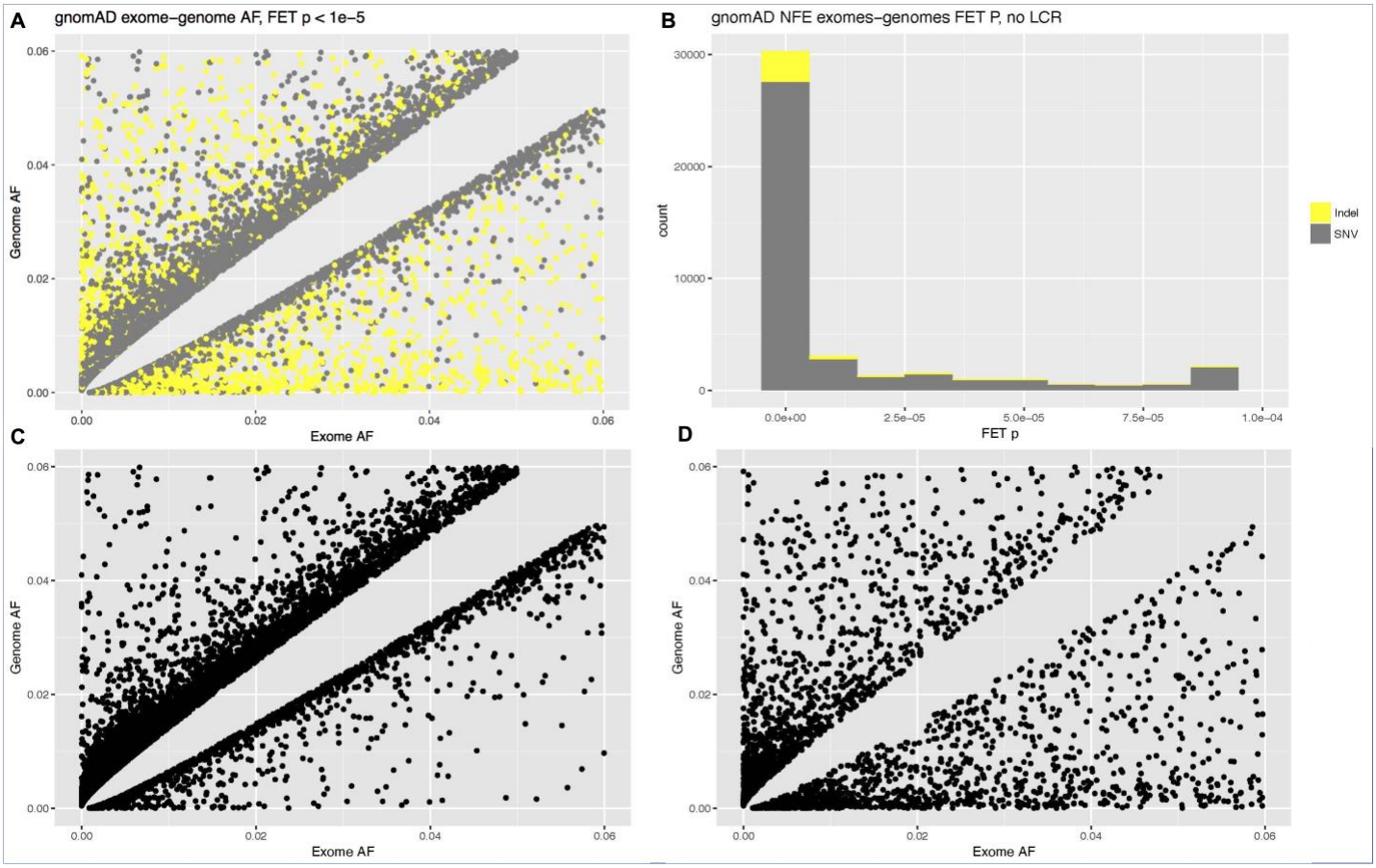
Supplemental Figure S4. Wrong call error mode by technology platform. The proportion of wrong calls in each error mode category are shown for each gnomAD sequencing platform for which overlapping data was available.



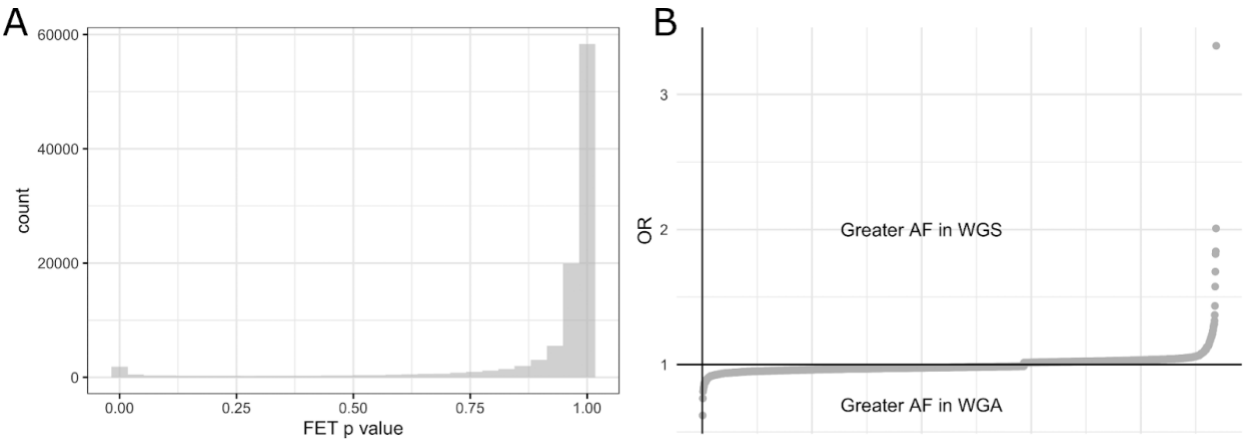
Supplemental Figure S5. Selection of 1e-5 as the threshold for 'good' vs 'bad' variants. (A) Concordance FET *p* value for AC>10 highlighting 1e-5, indicated with the red line, which was chosen as the threshold for NFE variants considered to be discordant. **(B)** QQ plot for the concordance test for all NFE variants. **(C)** Exomes vs genomes AF for bad NFE variants failing the 1e-5 threshold. **(D)** QQ plot for just the bad variants.


A
Bad sites rf_probability
(58,542 data points)
B
Good sites rf_probability
(685,537 data points)
C
Bad sites dp_median
(57,381 data points)
D
Good sites dp_median
(665,650 data points)
E
VEP Distance, bad sites
(6,320 data points)
F
VEP Distance, good sites
(73,847 data points)
G
ReadPosRankSum, bad sites
(57,598 data points)
H
ReadPosRankSum, good sites
(682,168 data points)

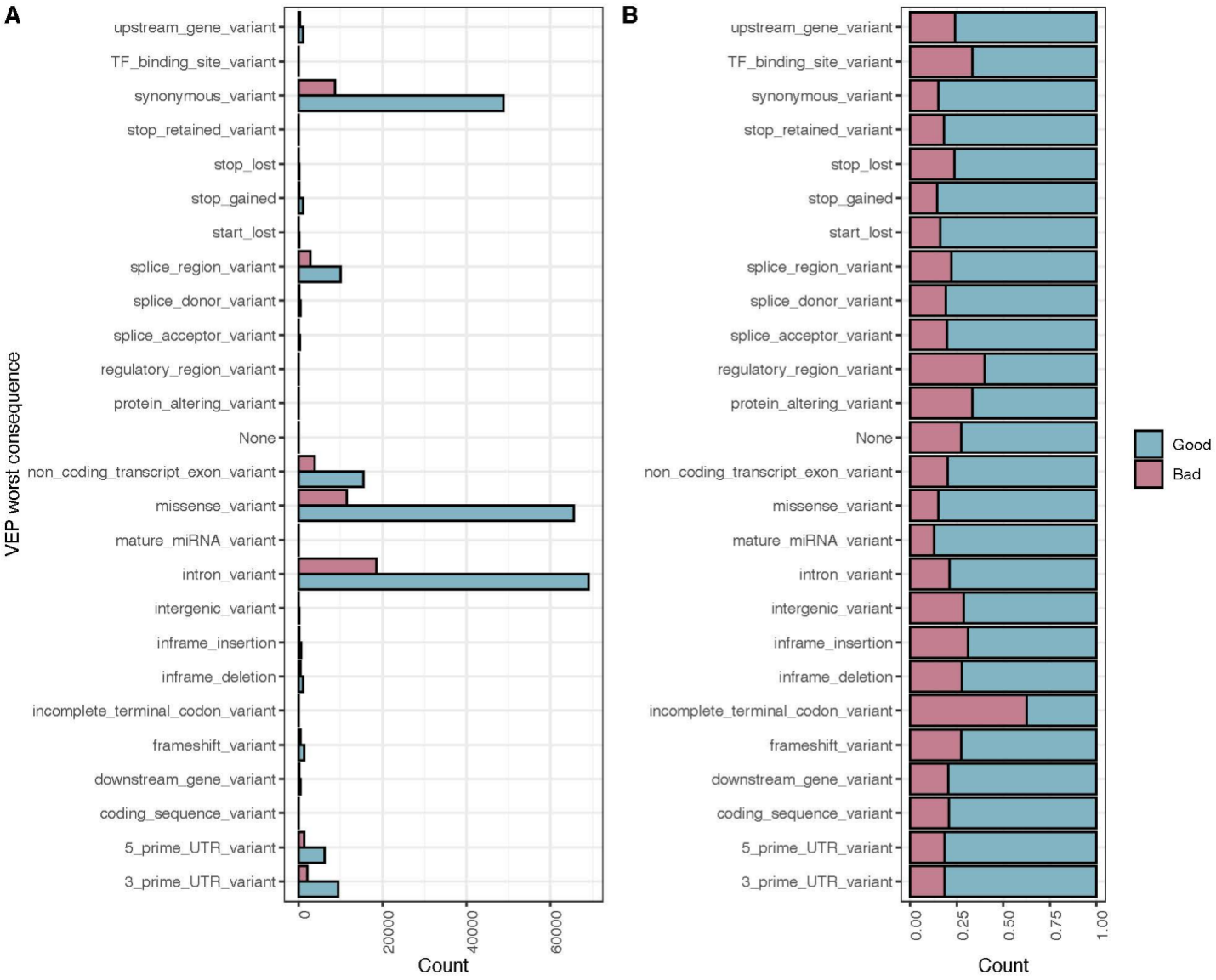
Supplemental Figure S6. **Distribution of gnomAD exomes metadata features for good versus bad variants**. Bad variant distributions are presented on the left, good on the right. **(A,B)** RF_Probability, the confidence of the random forest genotyper implemented with gnomAD. **(C,D)** DP_Median, the median depth of exomes. **(E,F)** VEP Distance, the distance to the closest canonical gene. **(G,H)** ReadPosRankSum, how far along sequencing reads the variant is falling.



Supplemental Figure S7. Different patterns of discordance in SNVs as compared to indels. (A) Allele frequencies in the exomes vs genomes for SNVs vs indels. Note an enrichment for indels at particularly discordant allele frequencies and a trend for SNVs to have higher AF in the genomes as compared to exomes. **(B)** FET *p* value for variants after excluding the LCR region. **(C)** Exomes vs genomes AF for bad NFE SNV variants failing the 1e-5 threshold. **(D)** Exomes vs genomes AF for indel bad NFE indels failing the 1e-5 threshold.



Supplemental Figure S8. Analysis of variant allele frequency concordance between whole genome sequencing and microarray data in the All of Us cohort. (A) Distribution of the Fisher's exact test *P*-values for the allele frequency comparison between the whole genome sequencing and microarray genotyping for the same individuals from All of Us cohort. The distribution is centered at 1 as the analysis cohort was subsampled to the individuals with both whole genome sequencing (WGS) and whole genome array (WGA) data available. (B) Odds ratios for allele frequency concordance analysis between WGS and WGA datasets.



Supplemental Figure S9. **VEP predicted worst consequence for 'good' versus 'bad' variants with MAF>0.01 after an AC > 10 filter.** Note the presence of many bad variants that are predicted to have severe functional consequences. (A) Absolute count; (B) proportion of total in each category

Supplemental Tables

Population	Fraction bad sites shared with NFE
AFR	0.745
AMR	0.865
ASJ	0.992
EAS	0.803
FIN	0.938
Average	**0.869**

Supplemental Table S1. Large overlap in discordant sites between NFE and other continental ancestry groups.

Low complexity region membership
segdup
nonpar
variant_type
allele_type
was_mixed
has_star
qd
info_SOR
rf_probability
was_split
score
qual
BaseQRankSum
ClippingRankSum
FS
InbreedingCoeff
MQ
MQRankSum
ReadPosRankSum

Supplemental Table S2. Features of the gnomADv2 variant annotations used in the random forest prediction model.

Ancestry	AFR	AMR	EAS	EUR	SAS
#Samples	296	222	422	351	102

Supplemental Table S3. 1000 Genomes samples per ancestry included into testing dataset.

VEP_worst_consequence	All	Bad	Good
3_prime_UTR_variant	11519	2130	9380
5_prime_UTR_variant	7602	1399	6175
coding_sequence_variant	35	7	27
downstream_gene_variant	596	121	475
frameshift_variant	1833	494	1312
incomplete_terminal_codon_variant	8	5	3
inframe_deletion	1485	410	1075
inframe_insertion	791	244	547
intergenic_variant	263	75	188
intron_variant	87799	18589	69116
mature_miRNA_variant	95	12	83
missense_variant	77243	11536	65588
non_coding_transcript_exon_variant	19353	3858	15463
regulatory_region_variant	50	20	30
splice_acceptor_variant	467	91	370
splice_donor_variant	554	106	446
splice_region_variant	12916	2842	10053
start_lost	277	44	232
stop_gained	1257	180	1077
astop_lost	195	46	148
stop_retained_variant	72	13	59
synonymous_variant	57514	8703	48755
TF_binding_site_variant	3	1	2
upstream_gene_variant	1341	323	1013
None	10	3	8
protein_altering_variant	9	3	6

Supplemental Table S4. **Counts for good and bad variants within VEP categories (AC >10)**.

Note the substantial numbers of bad variants with severe predicted consequences.

Chromosome	Position
4	3494956
5	33963745
6	31852866
6	151687847
9	712766
9	4576774
9	5126343
9	5185581
11	308180
11	308290
11	308314
11	309127
11	828916
18	618124
18	662103
19	844020
19	913048

Supplemental Table S5. Discordant variants seen at genome-wide significance in the GWAS catalog.

Supplemental File Legends

Supplemental File S1. List of the 2,344 variants which were found to have Fisher's exact test P<0.05 in the All of Us Research Program dataset.

Supplemental File S2. Source code for the *DNAdiscover* package described in this manuscript for prediction of the presence of technical bias in variants coming from high-throughout sequencing. This code, alongside a user manual, is also available at https://github.com/na89/DNAdiscover.