Samples

Matched lymphocyte controls

DNA extraction

QC

Amplicon-based PCR amplification, library preparation

Multiplexed Sequencing[a]

Alignment[b]

Aligned data (*.bam)

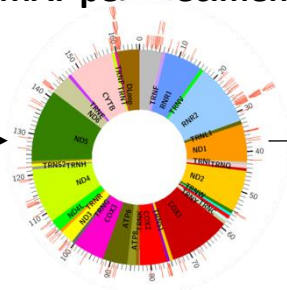
MT-extracted reads (mito*.bam)

Pre - processing

Post - processing

MitoSeek

Mutation positions and MAF per specimen



- Group-level statistics
- Intra-patient sample comparisons (tumor vs. margin, e.g)
- Clonal mutations across samples/patients

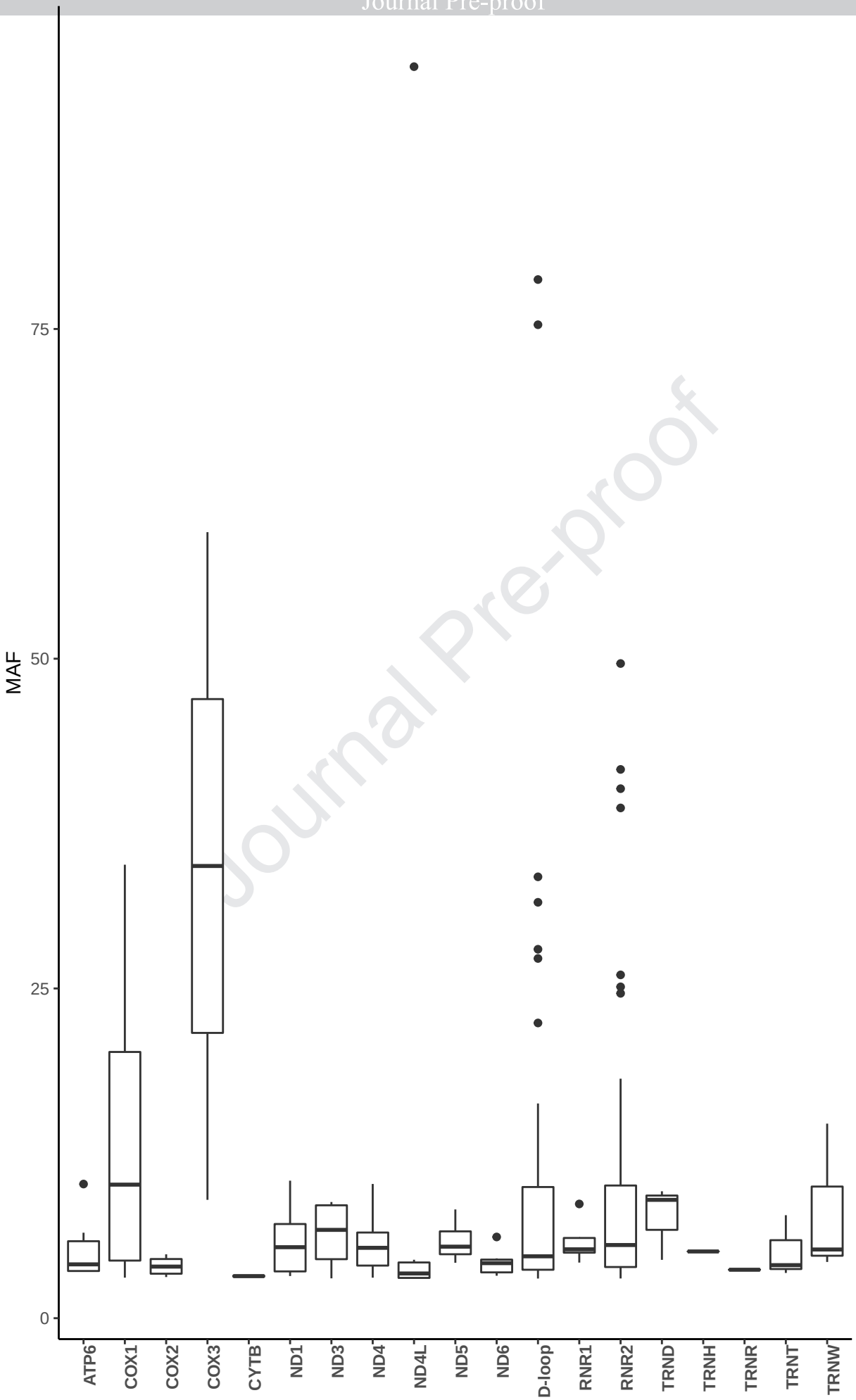
CN calculations using reference MT and nuclear genes

[a] includes barcoding and demultiplexing, as described in Methods
[b] using Bowtie2
[c] Read quality control (using FastQC), depth/coverage check
[d] Annotation of somatic mutations with sample data (sample type, patient, etc), check for missing values, annotation of variants, pathogenicity prediction

Samples collection and processing
Library preparation and sequencing
Data processing and analysis

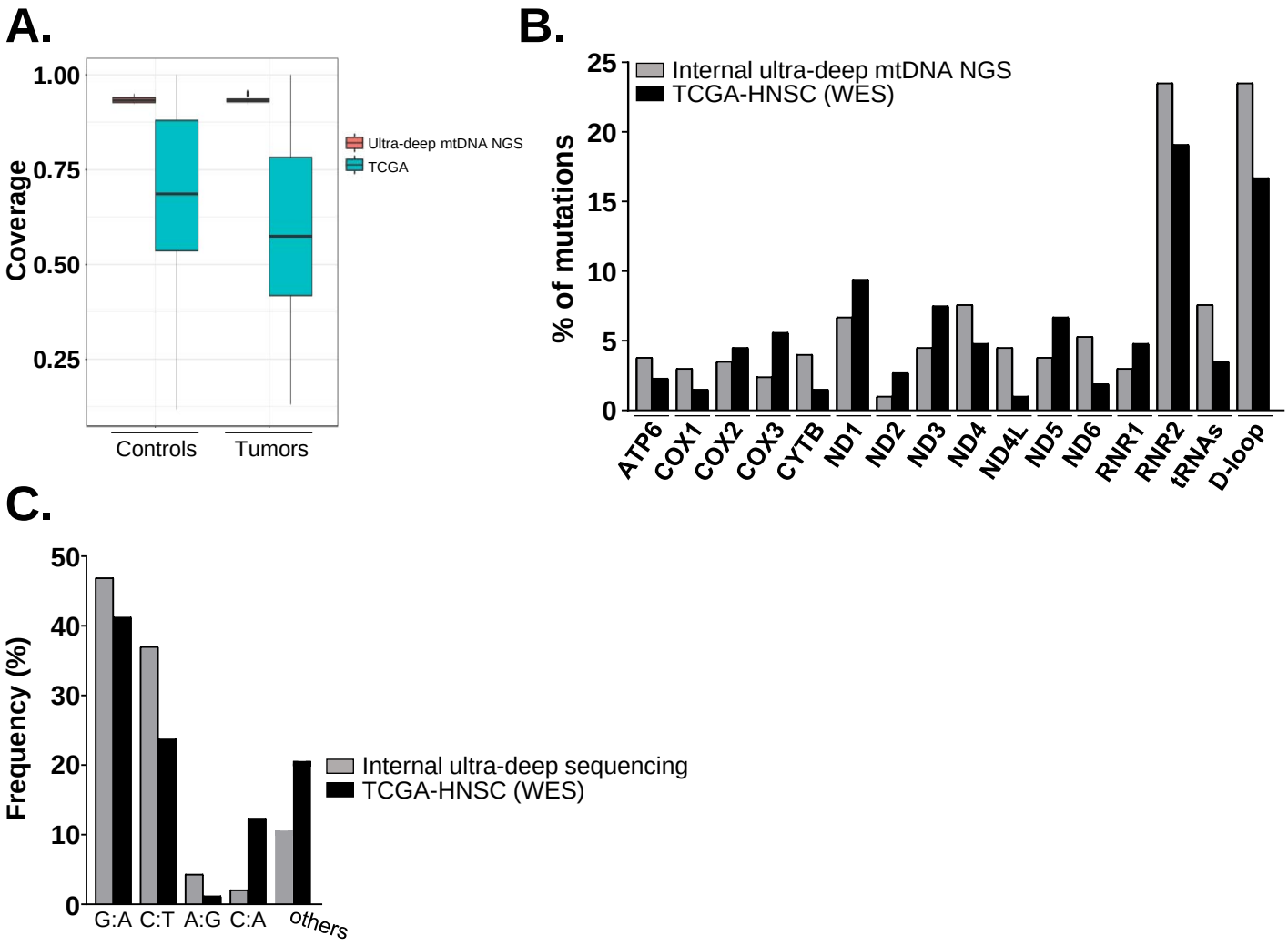
Supplementary Figure 1

Journal Pre-proof



Journal Pre-proof

Supplementary Figure 2



Supplementary Figure 3