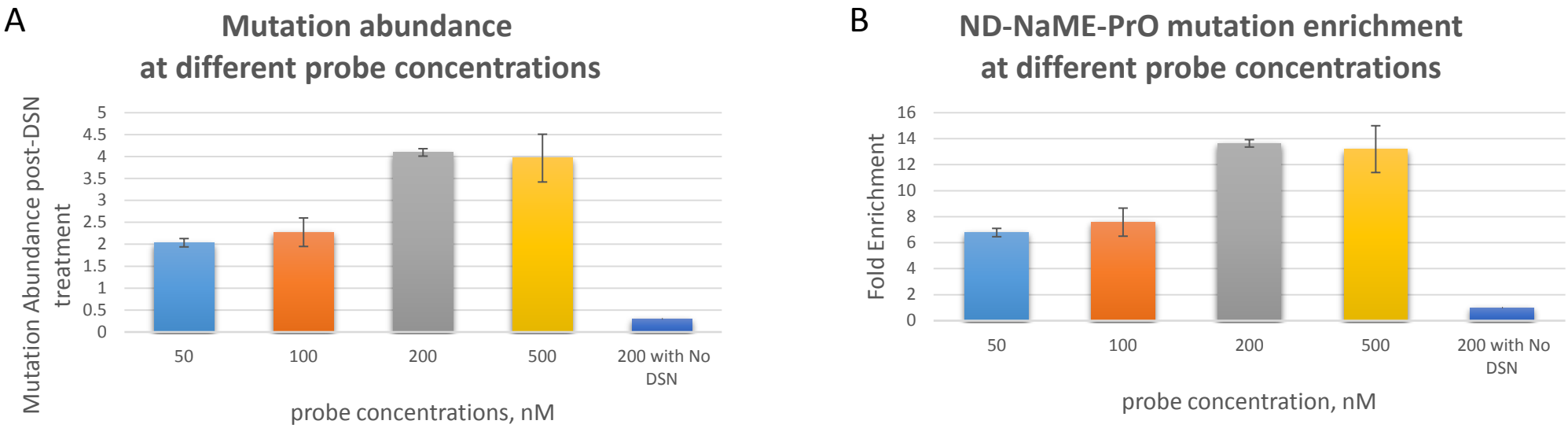
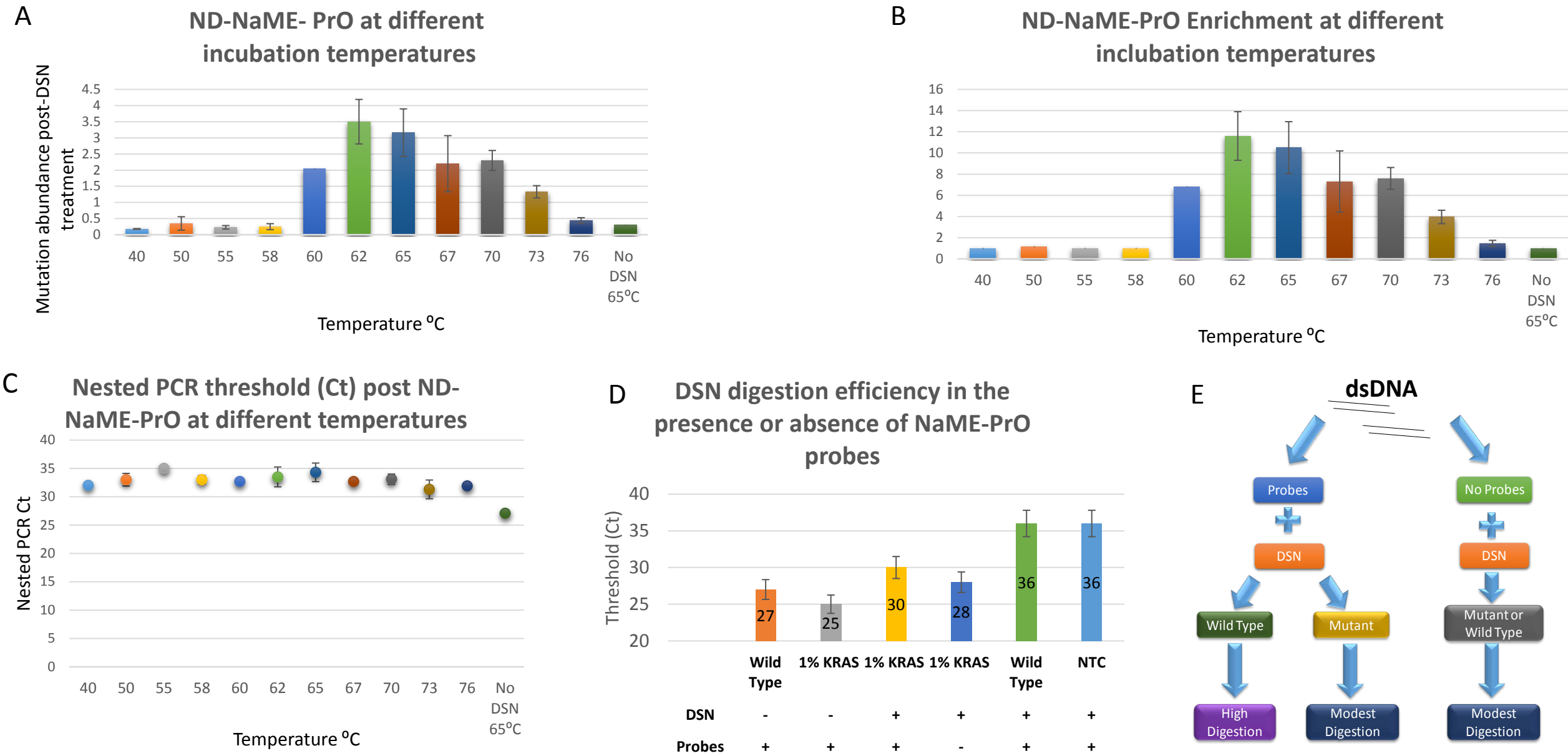
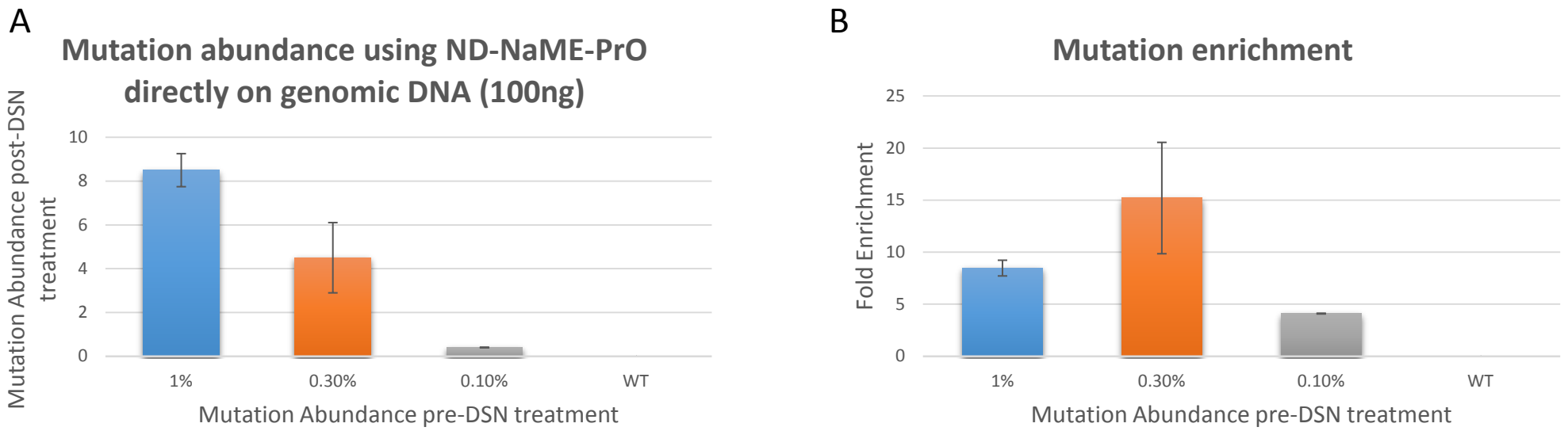


Supplementary Figure 1. ND-NaME-PrO performed at different probe concentrations. A. mutation enrichment assessment of an initial 0.3% mutation abundance at a range of different ND-NaME-PrO overlapping probe concentrations. B. Fold mutation enrichment for an initial 0.3% mutation abundance at a range of different ND-NaME-PrO overlapping probe concentrations.

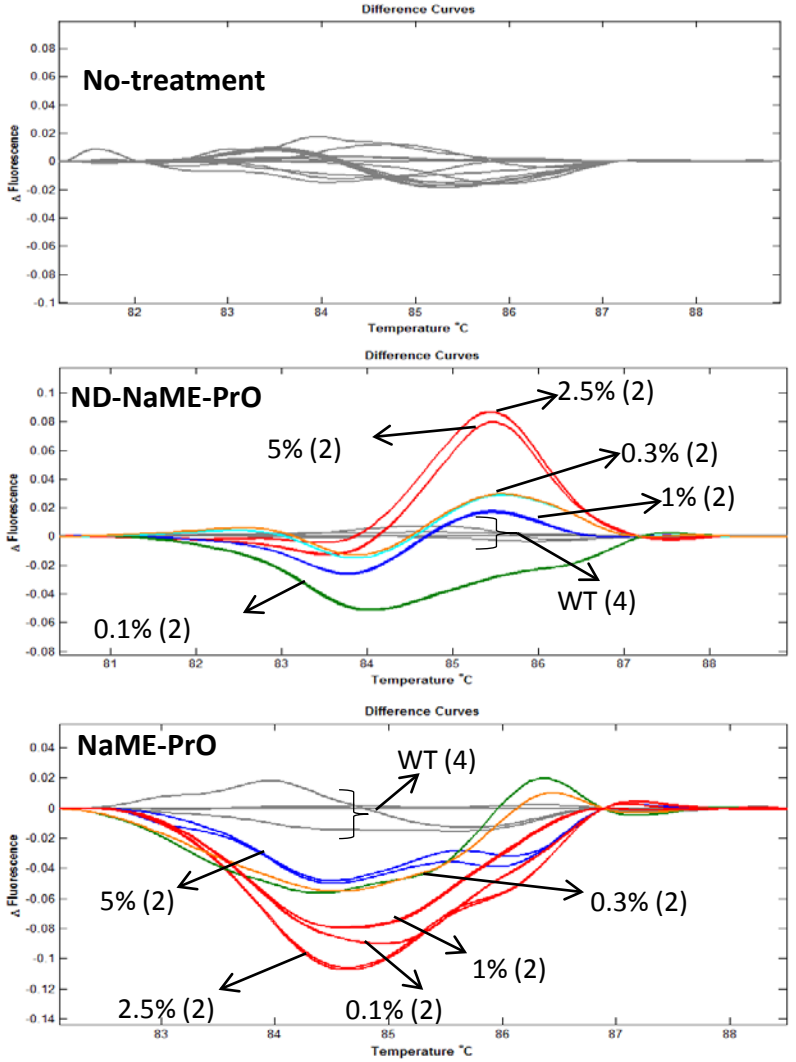


Supplementary Figure 2. ND-NaME-PrO performed under different conditions. **A**. Post-DSN treatment mutation abundance starting from an original 0.3% mutation abundance, for a range of DSN incubation temperatures during ND-NaME-PrO. **B**. fold-enrichment of mutations measured via ddPCR application before and after ND-NaME-PrO starting from an original 0.3% mutation abundance. **C**. DSN enzymatic activity at different temperatures assessed via nested real time PCR (Ct) performed after ND-NaME-PrO. **D**. Influence of the presence or absence of NaME-PrO probes on the degree of DSN digestion, for WT DNA or DNA containing 1% mutation (*KRAS*). WT DNA is almost eliminated (Ct equal to NTC control) in the presence of probes, while it is only modestly digested in the absence of probes, or if there is a mutation at the probe binding position. **E**. A layout consistent with the current data is depicted, highlighting the influence of NaME-PrO probes on DNA digestion by DSN during ND-NaMe-PrO.

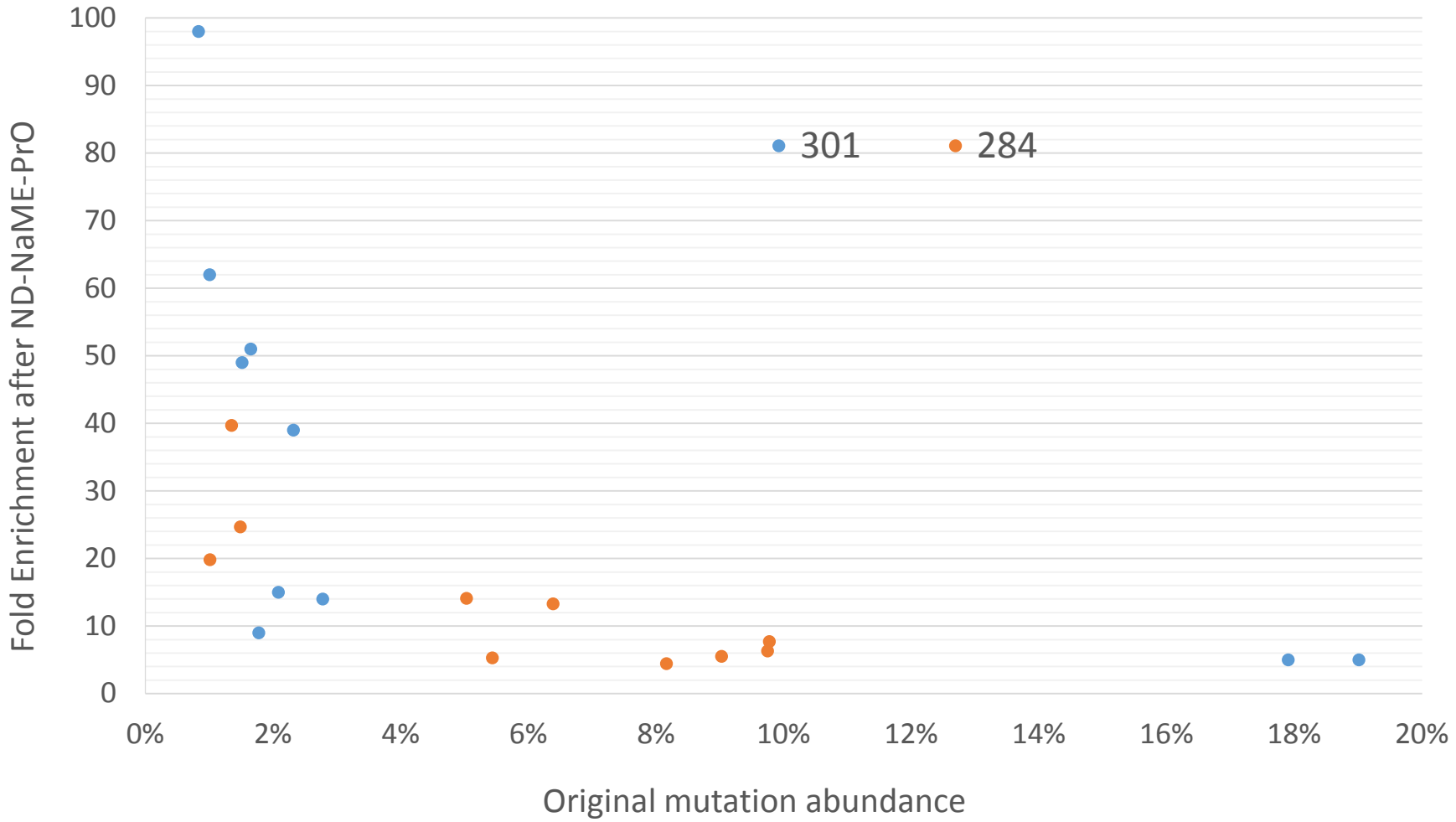


Supplementary Figure 3. ND-NaME-PrO performed directly on unamplified genomic DNA containing *KRAS* mutations, following serial dilution in WT DNA, at 100ng total DNA input. Mutation abundance before and after ND-NaME PrO was assessed via ddPCR.

20plex



Supplementary Figure 4. ND-NaME-PrO-HRM was performed following a 20-plex PCR reaction containing serial mutation dilutions for 8 mutated targets. The 20-plex PCR-HRM scanning was also performed for no-treatment samples in parallel, where DSN was omitted. For comparison, NaME-PrO (non-homogenous format) was also performed.



Supplementary Figure 5. Dependence of the amount of mutation enrichment following ND-NaME-PrO, on the original mutation abundance. Mutations in plasma-circulating DNA obtained from patients 301 or 284 were quantified via targeted re-sequencing (x-axis, original mutation abundance) or following mutation enrichment via ND-NaME-PrO prior to targeted re-sequencing (y-axis). Lower level mutations are enriched more than higher level mutations.