### Supplementary table S1 The optional parameters of the tools in the bioinformatics analysis

| Tools                      | Optional parameters                                                                 |
|----------------------------|-------------------------------------------------------------------------------------|
| fastp (v0.20.0)            | `-f 10 -t 0 -F 10 -T 0 -w 8 --length_required 35 -q 15 -u 40 -n 5 -3 1`            |
| BWA mem2(v2.1)             | mem -Y -M -R "@RG\tID:"..."tSM:"..."tLB:"..."tPU:"..."tPL:"ILLUMINA"               |
| SAMtools (v1.9)            | sort --reference hs37d5.fa                                                           |
| MarkDuplicates (packed in GATK 4.1.2.0) | --VALIDATION_STRINGENCY SILENT                      |
|                            | --OPTICAL_DUPLICATE_PIXEL_DISTANCE 2500                                             |
|                            | --ASSUME_SORT_ORDER "coordinate"                                                   |
|                            | --CLEAR_DT false                                                                    |
|                            | --CREATE_MD5_FILE true                                                              |
| BaseRecalibrator (packed in GATK 4.1.2.0) | -R hs37d5.fa                        |
|                            | --use-original-qualities                                                            |
|                            | --known-sites Mills_and_1000G_gold_standard.indels.b37.vcf                           |
|                            | --known-sites dbsnp_138.b37.del100.vcf.gz                                           |
|                            | --known-sites 1000G_phase1.indels.b37.vcf.gz                                        |
| ApplyBQSR (packed in GATK 4.1.2.0) | -R hs37d5.fa                        |
|                            | --add-output-sam-program-record                                                     |
|                            | --use-original-qualities                                                            |
| Mutect2 (packed in GATK 4.1.2.0) | --germline-resource af-only-gnomad.raw.sites.b37.vcf.gz                         |
|                            | --af-of-alleles-not-in-resource 0.0000025                                           |
|                            | -A ClippingRankSumTest                                                             |

### Supplementary table S2 The distribution of TP53 status in 92 GI-NENs

| TP53 Mutations | NET | NECs |
|----------------|-----|------|
| Nonsense       | 0   | 8    |
| LOF            |     |      |
| Frameshift indels | 0 | 4    |
| Splicing       | 0   | 5    |
| Wild type      | 34  | 4    |
| GOF            |     |      |
| Missense       | 1   | 31   |
| In-frame indels | 0 | 5    |
Supplementary table S3 Comparison between the percentage of p53 positive cells and \( TP53 \) mutation status in 92 GI-NEN cases

| Percentage of p53 positive cells (%) | Mutational Analysis |       |       |       |       |       |       |       |       |       |
|-------------------------------------|---------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                                     | LOF                 | Nonsense | Frameshift | Splicing | Wild | Type | Missense | In-Frame | Total |       |
| 0                                  |                     | 5 | 3 | 3 | 2 | 0 | 0 | 13 |       |       |
| 1-10                               |                     | 1 | 0 | 1 | 28 | 0 | 0 | 30 |       |       |
| 11-20                              |                     | 0 | 0 | 0 | 5 | 0 | 0 | 5 |       |       |
| 21-30                              |                     | 1 | 0 | 1 | 0 | 2 | 0 | 4 |       |       |
| 31-40                              |                     | 0 | 0 | 0 | 0 | 0 | 0 | 0 |       |       |
| 41-50                              |                     | 0 | 0 | 0 | 0 | 1 | 1 | 2 |       |       |
| 51-60                              |                     | 0 | 0 | 0 | 0 | 0 | 0 | 0 |       |       |
| 61-70                              |                     | 0 | 1 | 0 | 1 | 1 | 0 | 3 |       |       |
| 71-80                              |                     | 0 | 0 | 0 | 1 | 2 | 0 | 3 |       |       |
| 81-90                              |                     | 0 | 0 | 0 | 1 | 3 | 2 | 6 |       |       |
| 91-100                             |                     | 1 | 0 | 0 | 0 | 23 | 2 | 26 |       |       |

Supplementary table S4 The inter-pathologist concordance for p53 IHC patterns in GI-NENs

| Original interpretation | Interpretation from the third pathologist | Pattern A | Pattern B | Pattern C1 | Pattern C2 |
|-------------------------|-------------------------------------------|-----------|-----------|------------|------------|
| Pattern A               |                                           | 13 | 0 | 0 | 0 |
| Pattern B               |                                           | 1 | 34 | 0 | 0 |
| Pattern C1              |                                           | 0 | 1 | 5 | 0 |
| Pattern C2              |                                           | 0 | 0 | 0 | 38 |
Supplementary figure S1. The examples of uninterpretable cases. p53 expression was completely absent throughout the entire section including both tumor cells and non-tumor internal control cells (40×) in one case with wild-type TP53 (A) and another case with TP53 missense mutation (B).