Supporting Information

SI materials and methods

Cell culture. The NPC cell lines, HONE1, HK1, C666 and the immortalized nasopharyngeal (NP) epithelial cell lines, NP69 and NP460, were obtained from the Cell Line Repository of the Area of Excellence, Center for NPC Research (CNPCR). All the NPC and immortalized cell lines were cultured as previously described (1). Growth medium for all cell lines consisted of DMEM supplemented with 10% (vol/vol) fetal calf serum.

Real-time quantitative reverse transcription PCR (QPCR). The QPCR was performed by utilizing the FastStart Universal SYBR green master mix as described (2). The QPCR primers are forward: 5'-TAT CCT GCA GGT GGA GCT G-3' and reverse: 5'-ATG AAA TGC CAT GCC CTT AG-3' for full-length *MST1R* (MST1R FL) (3) and forward: 5'-CCT CAT GAC CCT CTC TGC AGT-3' and reverse: 5'- CTG GCA GCT CTC ACC ACC-3' for *MST1R* short isoform (MST1R SF). The primer set for short isoform was designed to target a 90 bp region from intron 10 to exon 11.

Immunohistochemical staining (IHC). NPC tissue microarray (TMA) construction and IHC were performed using the standard steptavidin-biotin-peroxidase complex method as previously described (1). The TMA slides were incubated with rabbit anti-RON β polyclonal antibody (C-20, Santa Cruz, CA, USA, 1:100 dilution) overnight. The TMA results were reviewed by a pathologist (F.C.).

Illumina Infinium assay for methylation profiling. The NPC methylome data assayed on Illumina HumanMethylation450 BeadChip were processed as described previously (4) and are available in the NCBI GEO database with accession ID GSE62336 (4). The normalized methylation data of the selected locus in the morphologically normal tissues obtained from breast, kidney, liver, lung, prostate, head and neck and bladder cancer patients were generated by The Cancer Genome Atlas (TCGA) studies and obtained in the MARMAL-AID database (5). The normalized methylation data of the selected locus in peripheral blood mononuclear cells (PBMCs) were obtained from MARMAL-AID database.

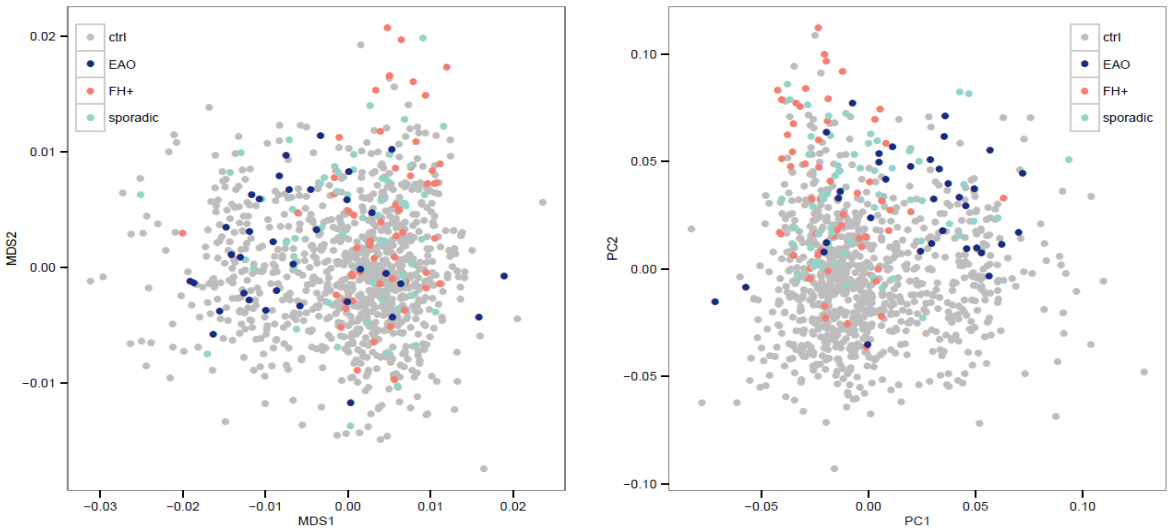

Fig. S1: The multi-dimensional scaling and principal component analysis of the samples sequenced by WES. The graph includes 39 EAO patients, 63 FH+ patients from 52 families, 59 sporadic patients, and 895 non-cancer controls.

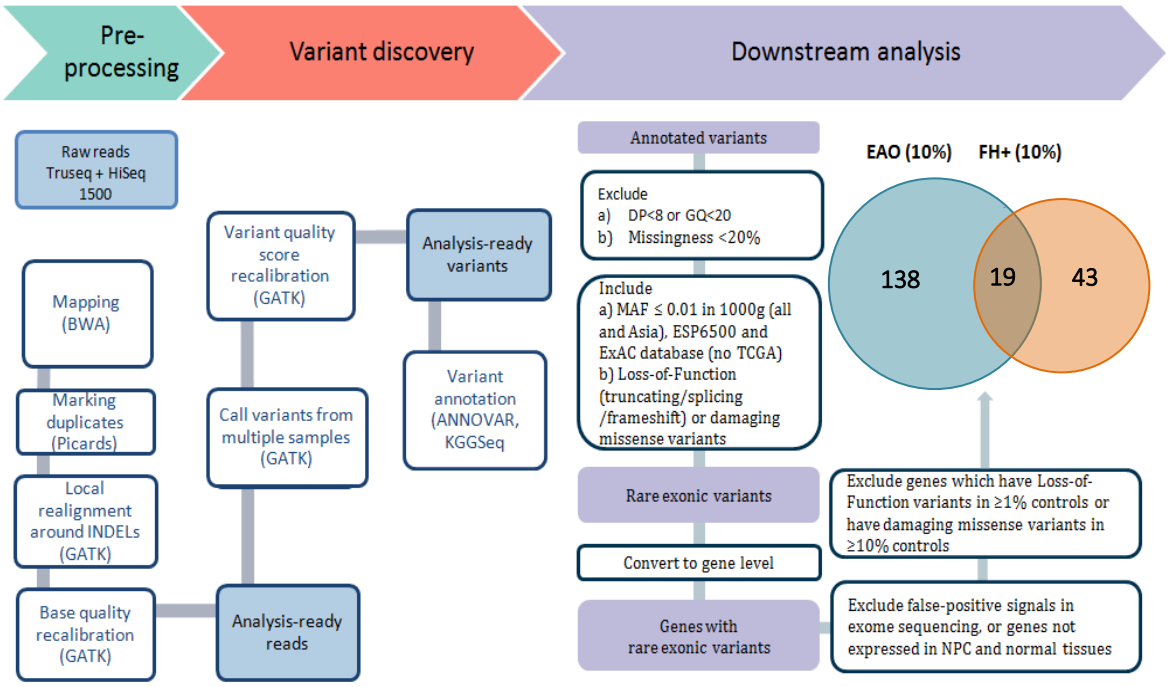

Fig. S2: Workflow for identification of the genes with deleterious variants in at least 10% of the cases in either EAO or FH+ group using filtering strategy.

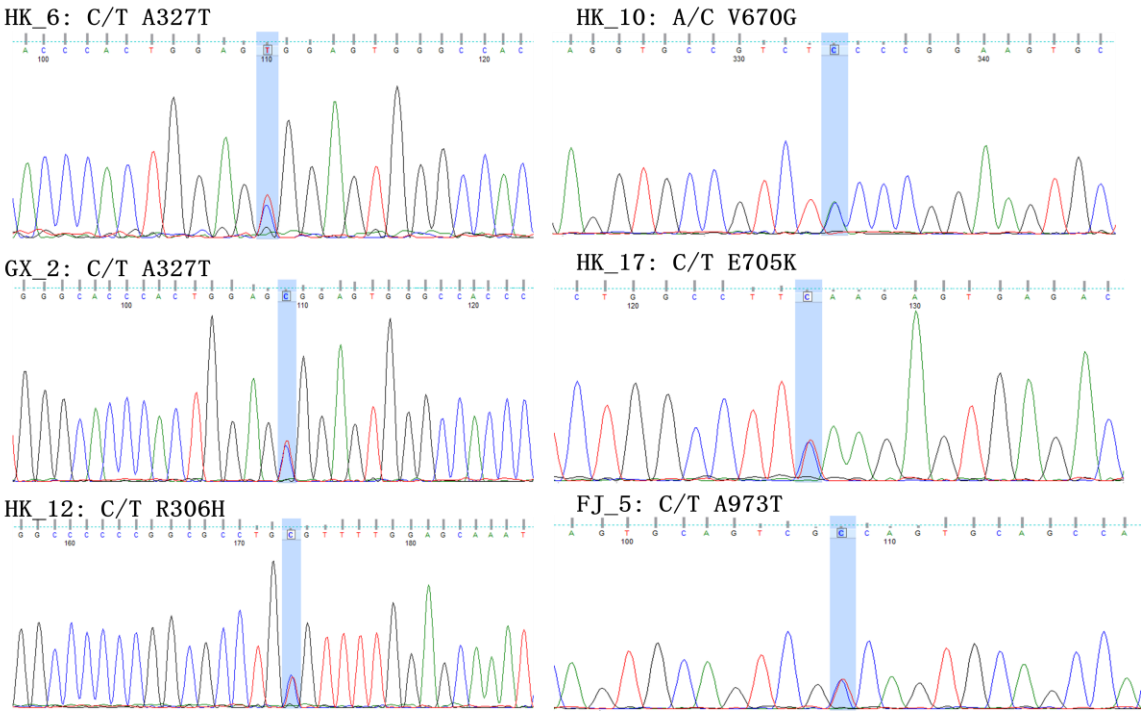

Fig. S3: Validation of the deleterious variants at *MST1R* in the EAO cases by Sanger sequencing

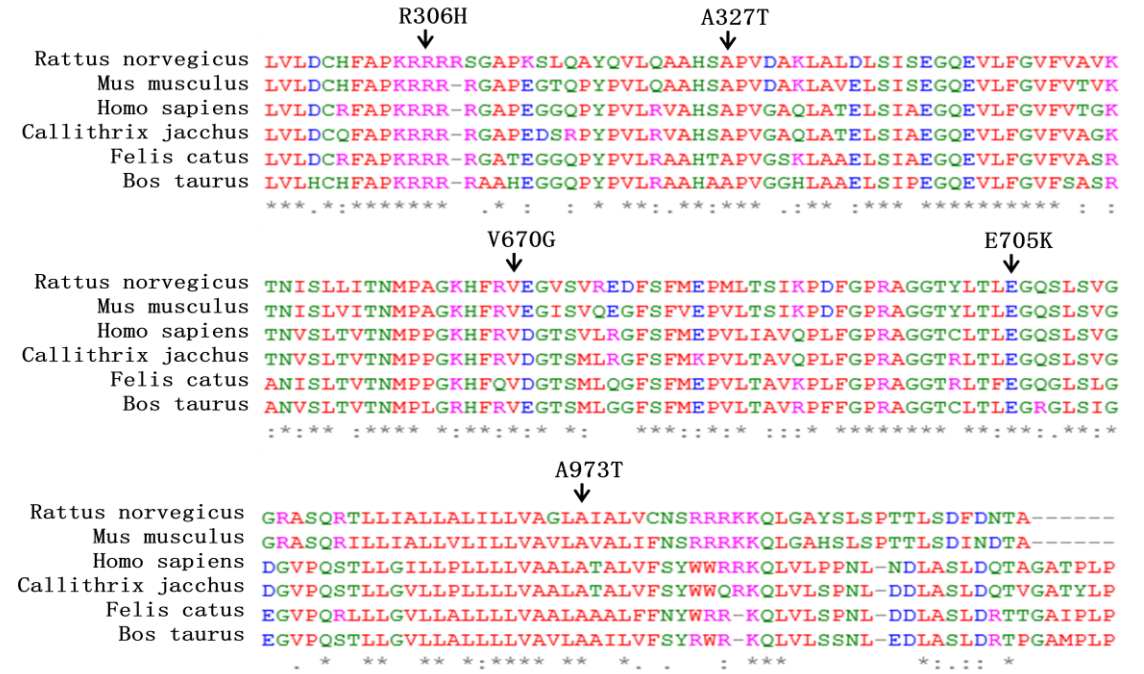

Fig. S4: Multiple alignment showed the evolutionary conservation of residues, where the rare deleterious variants in the EAO cases occurred. Multiple alignment was done by Clustal Omega (EBI).

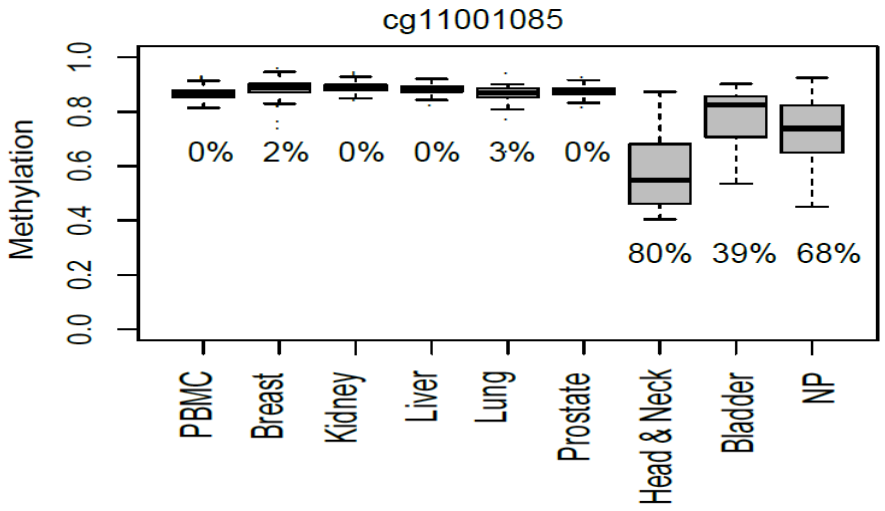

Fig. S5: Methylation of the CpG site (cg11001085, chr3: 49940126) within the CGI 2 in the peripheral blood mononuclear cell (PBMC) (n=400) and the morphologically normal tissues from breast (n=98), kidney (n=185), liver (n=50), lung (n=75), prostate (n=45), head and neck (n=50), bladder (n=18), and nasopharynx (NP) (n=25). The cytosine in this CpG site is located at the same genomic location as the variant c.G917A:p.R306H. A dramatic decrease of methylation of this CpG site was observed in head and neck, bladder, and NP tissues, indicating there is a tissue-specific methylation pattern at this locus. The percentage of the samples with reduced methylation level (<0.8) at this locus is listed on the graph. The solid points are the outliers.

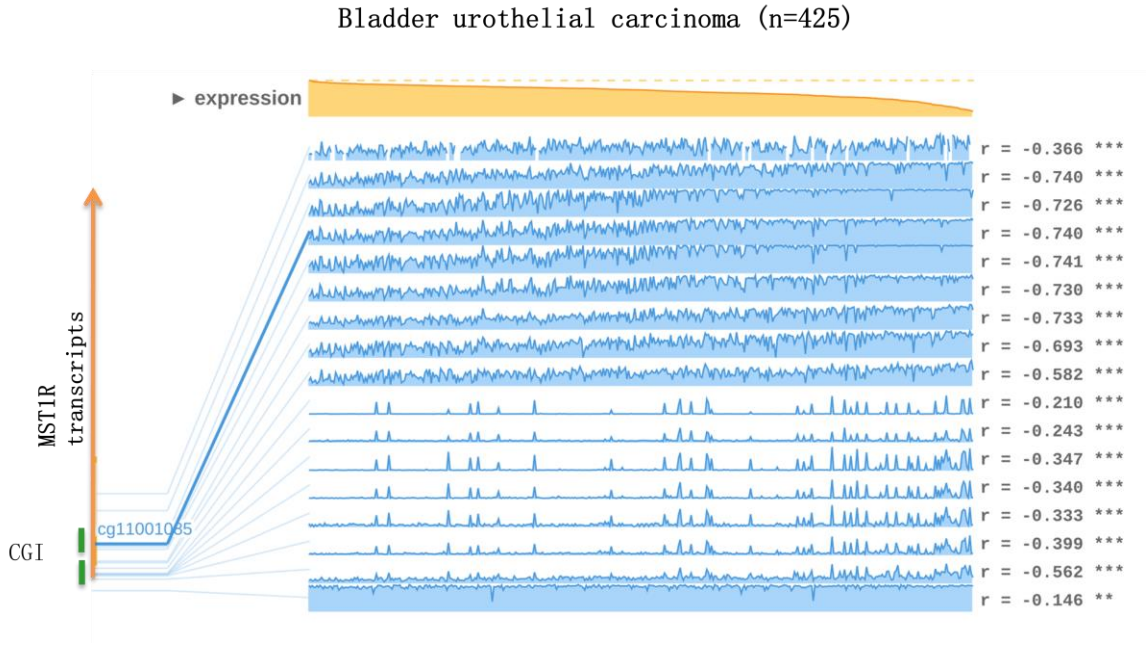

Fig. S6: Correlation between *MST1R* methylation and expression in bladder urothelial carcinoma in TCGA study (n=425). Increased methylation of the CpG site at cg11011085 was correlated with decreased *MST1R* expression.

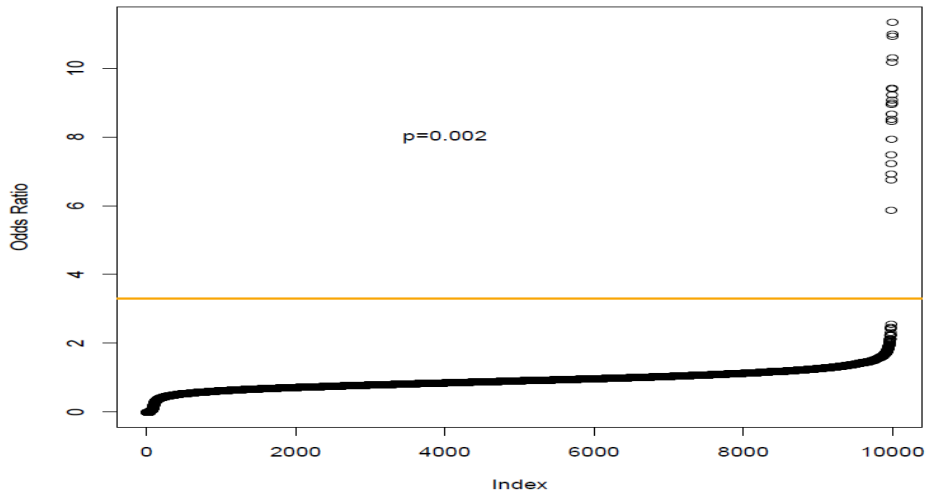

Fig. S7: Permutation test by Monte Carlo method for estimating the probability of observing the association of deleterious variants in 10 randomly selected genes with NPC by chance. The orange line indicates the odds ratio of 3.3 as we observed in the MST1R interaction network. The permutation test was done for 10000 times. The chance that we observed odd ratios>3.3 for 10 randomly selected genes is only 0.002 (20/10000).

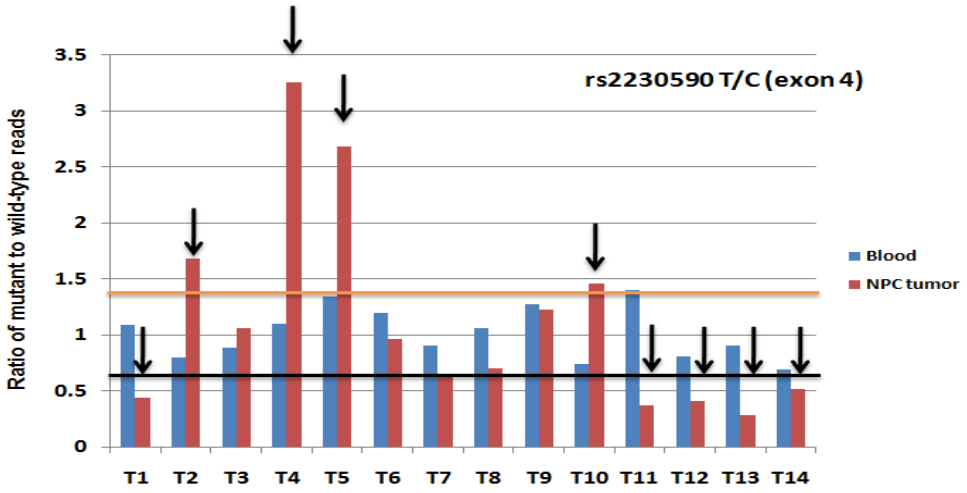

Fig. S8: The ratio of the reads for the mutant allele to the wild-type allele of a common missense variant c.A1568G:p.Q523R (rs2230590) at *MST1R*. This variant was found in 14 out of 59 NPC patients. The average coverage at this locus is 78X in tumors and 58X in blood, respectively. The cut-off for the allelic imbalance was determined by the ratios in the blood samples (mean ±2SD). The orange line is the upper cut-off (1.43) and the black line is the lower cut-off (0.59). The NPC tumors with the ratio higher or lower than the cut-off are highlighted by an arrow. The increased ratio of the reads for the mutant to wild-type allele indicates loss of the wild-type allele or gain of the mutant allele and *vice-versa*. In total, 9/14 (64%) patients have an allelic imbalance of copy numbers at this selected locus.

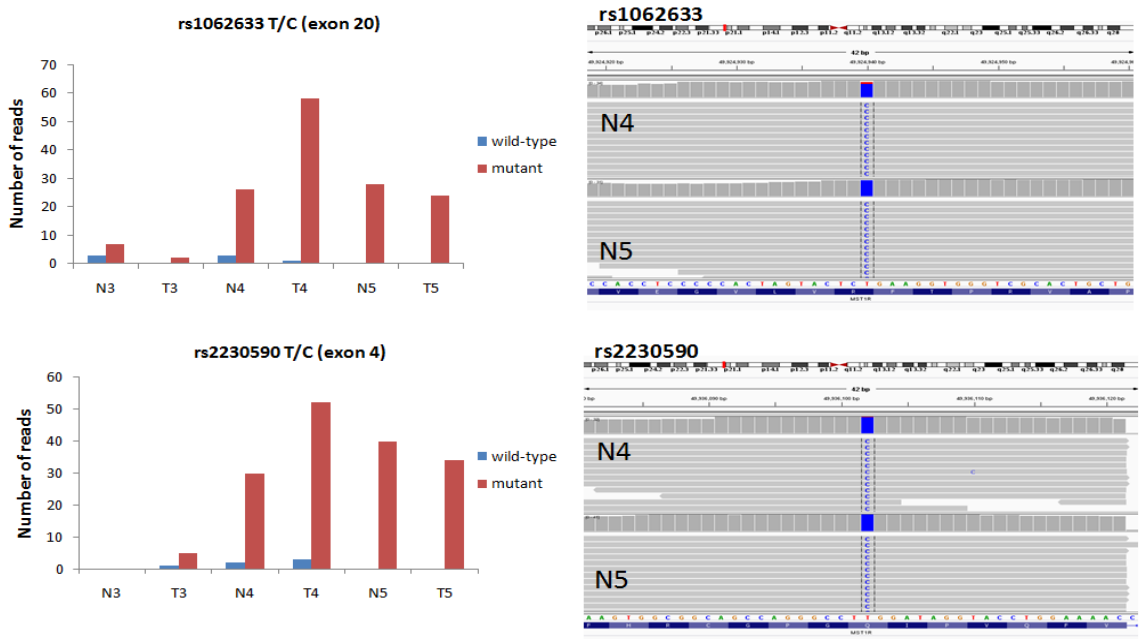

Fig. S9: Expression of mutant and wild-type *MST1R* alleles at two *MST1R* common variants c.A1568G:p.Q523R (rs2230590) and c.A4003G:p.R1335G (rs1062633). Left: Barplot of the number of reads from wild-type and mutant alleles in RNASeq data. T is the wild-type allele and C is the mutant allele. Only cases 3, 4 and 5 carry the variant rs1062633 and rs102230590 out of 10 patients in RNASeq. Right: The visualization of the reads near rs1062633 and rs2230590 in RNASeq data by Integrative Genomics Viewer (IGV). The mutant allele C is predominantly expressed in the normal surrounding tissues from two cases. N: non-tumor surrounding samples. T: NPC tumors.

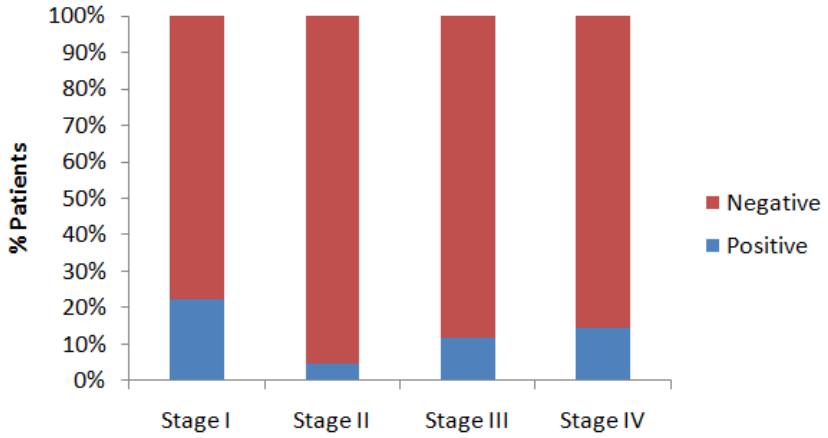

Fig. S10: MST1R expression in NPC by IHC staining using rabbit anti-RON β polyclonal antibody (C-20, 1:100 dilution). Negative: no MST1R expression. Positive: weak (+) to moderate (++) MST1R expression

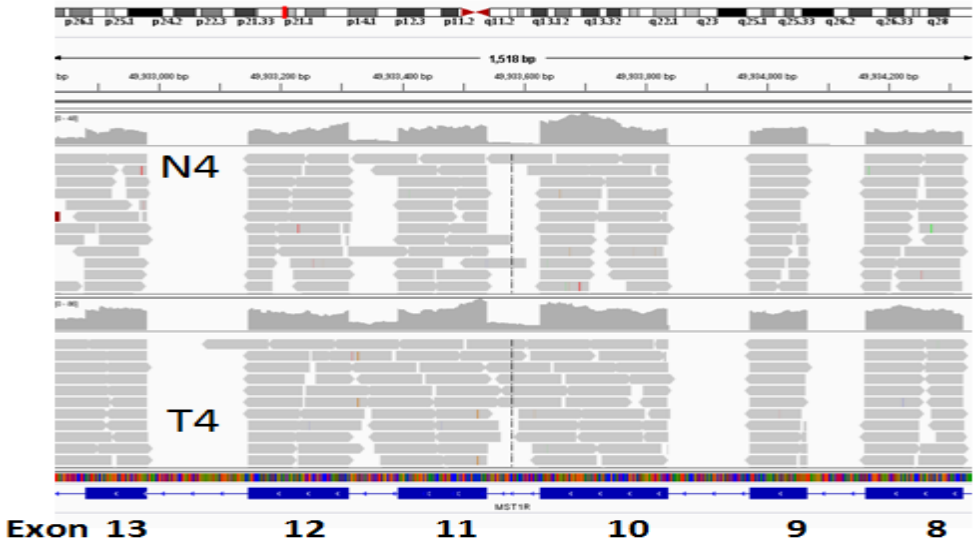

Fig. S11: Visualization of the reads in *MST1R* from exon 8 to 13 by Integrative Genomics Viewer (IGV) in one pair of samples (N4 vs. T4). The *MST1R* short-isoform transcript starts from intron 10. We detected reads in both introns 10 and 11 for the short-isoform transcript, which is consistent with previous study (3).

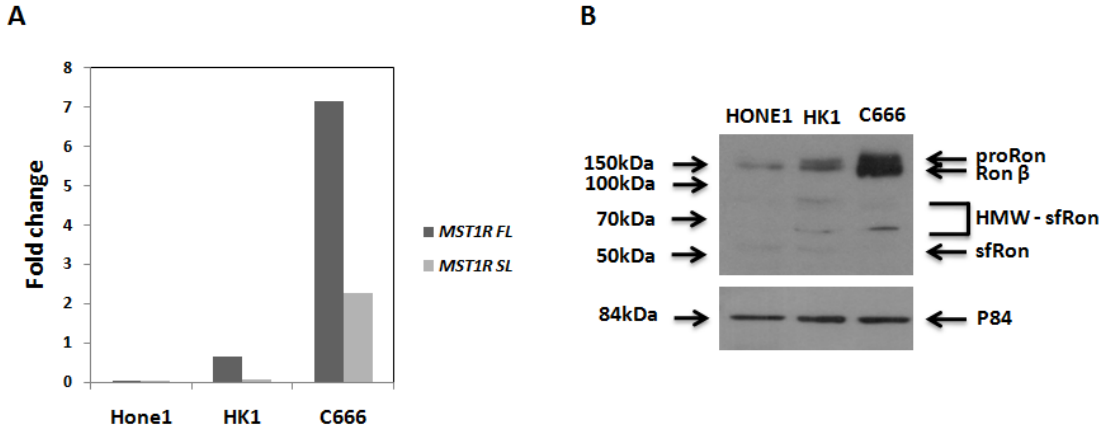

Fig. S12: Expression of two *MST1R* isoforms measured by QPCR and Western blotting in NPC cell lines HONE1, HK1, and C666. A: Expression of two MST1R transcripts measured by QPCR. *MST1R FL* is the full-length *MST1R* and *MST1R SF* is the *MST1R* short isoform. The average expression in the immortalized NP cell lines NP69 and NP460 was used as the reference for calculating the fold change. Overexpression of two transcripts were detected in C666. The experiments were done in triplicates. B: Expression of MST1R at the protein level detected by anti-RON β polyclonal antibody (C-20, 1:500 dilution) in NPC cell lines HONE1, HK1, and C666. The putative ubiquitinated sfRON (HMW-sfRON) is also noted. The p84 was used as a loading control.

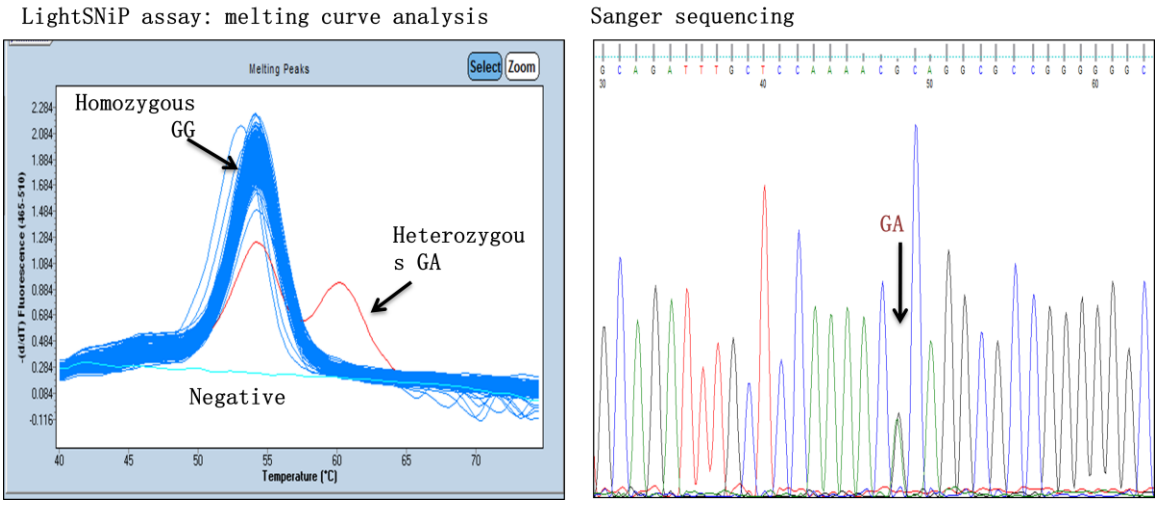

Fig. S13: The LightSNiP assay designed for detecting the variant c.G917A:p.R306H in the validation study (left). Water was used as a negative control. The variant was further validated by Sanger sequencing (right).

Table S1: Clinical information of NPC patients and overview of WES statistics

Index	Sample ID	Group	% unique reads	% off-target bases	Average coverage	% Coverage 10X	% Coverage 20X	% Coverage 30X	% Coverage 50X	DNA input (ng)	Gender	Age	Stage	Source
1	ST_1	EAO	88.8	19.2	55.4	90.5	80.2	65.8	39.6	250	F	15	IV	Shantou, China
2	ST_2	EAO	91.0	17.9	47.6	90.9	81.5	67.3	37.0	250	M	15	II	Shantou, China
3	ST_3	EAO	90.4	18.4	43.6	90.3	79.8	64.4	32.7	250	M	15	IV	Shantou, China
4	ST_4	EAO	90.1	17.8	46.2	90.6	81.4	67.6	37.0	250	M	18	IV	Shantou, China
5	ST_5	EAO	90.2	18.2	48.4	90.9	82.3	68.9	38.5	250	F	17	IV	Shantou, China
6	ST_6	EAO	91.4	17.5	50.5	90.5	80.6	65.7	37.5	250	F	16	III	Shantou, China
7	ST_7	EAO	91.4	18.0	57.1	90.9	80.9	66.6	41.0	250	M	20	III	Shantou, China
8	GX_1	EAO	91.9	17.9	55.5	90.1	79.3	64.4	39.3	250	M	19	IV	Guangxi, China
9	GX_2	EAO	92.3	19.2	52.6	89.7	78.5	63.4	37.7	250	M	19	III	Guangxi, China
10	HK_1	EAO	84.3	15.1	63.1	86.7	81.2	74.3	56.7	1000	F	20	III	Hong Kong, China
11	HK_2	EAO	86.0	15.1	64.9	88.6	83.6	77.0	58.9	1000	F	12	IV	Hong Kong, China
12	HK_3	EAO	95.2	23.0	52.1	89.1	81.7	70.1	42.5	250	M	16	I	Hong Kong, China
13	HK_4	EAO	90.1	21.8	46.1	87.6	70.5	52.5	30.0	250	M	18	III	Hong Kong, China
14	HK_5	EAO	90.1	18.8	48.8	86.7	69.4	52.6	31.5	250	M	19	II	Hong Kong, China
15	HK_6*	EAO	90.3	19.2	41.3	86.8	68.4	49.4	26.4	250	F	19	III	Hong Kong, China
16	HK_7	EAO	89.1	19.7	47.3	86.8	69.3	52.0	30.5	250	M	12	III	Hong Kong, China
17	HK_8*	EAO	89.6	22.5	47.8	86.6	69.6	52.9	31.4	250	M	19	II	Hong Kong, China
18	HK_9	EAO	94.6	19.0	41.2	87.5	72.8	54.8	27.4	250	M	16	IV	Hong Kong, China
19	HK_10	EAO	94.2	20.0	43.9	88.2	73.7	55.9	29.7	250	F	19	III	Hong Kong, China
20	HK_11	EAO	94.6	19.5	41.8	87.3	70.7	52.2	27.3	250	M	19	III	Hong Kong, China
21	HK_12	EAO	85.0	15.2	59.6	88.4	82.9	75.3	55.1	1000	F	17	IV	Hong Kong, China
22	HK_13	EAO	89.2	17.3	57.0	91.8	85.2	75.0	48.2	250	F	18	III	Hong Kong, China
23	HK_14	EAO	86.3	17.2	54.9	88.1	74.3	58.6	36.0	250	M	13	III	Hong Kong, China
24	HK_15*	EAO	86.3	17.1	49.1	88.1	73.5	56.9	33.1	250	M	13	IV	Hong Kong, China
25	HK_16*	EAO	86.3	17.1	49.5	87.7	72.9	56.3	33.1	250	M	18	III	Hong Kong, China
26	HK_17	EAO	82.8	14.8	47.5	87.5	79.3	68.1	41.9	1000	M	20	III	Hong Kong, China
27	HK_18	EAO	86.7	15.5	56.2	88.0	81.4	72.7	50.8	1000	M	14	III	Hong Kong, China
28	HK_19	EAO	85.6	14.8	40.5	86.0	75.3	60.8	31.1	1000	M	15	III	Hong Kong, China
29	HK_20	EAO	84.8	15.1	55.6	85.7	78.8	70.2	49.7	1000	M	16	IV	Hong Kong, China
30	HK_21	EAO	83.8	15.9	47.4	84.4	75.9	65.1	41.2	1000	M	17	IV	Hong Kong, China
31	HK_22	EAO	88.3	16.6	65.2	87.5	82.1	75.2	57.4	1000	M	17	III	Hong Kong, China
32	HK_23	EAO	80.4	15.2	44.8	86.7	77.9	65.8	38.2	1000	M	12	III	Hong Kong, China
33	FJ_1	EAO	90.9	17.9	44.9	87.7	72.0	54.1	29.9	250	M	18	III	Fujian, China
34	FJ_2	EAO	90.5	17.6	49.4	88.8	75.6	59.0	33.8	250	F	17	II	Fujian, China
35	FJ_3	EAO	90.4	18.0	46.7	88.5	74.9	57.9	32.0	250	M	16	III	Fujian, China
36	FJ_4	EAO	90.9	18.3	45.7	88.1	73.7	56.2	30.8	250	M	11	IV	Fujian, China
37	FJ_5	EAO	90.8	18.6	46.8	87.9	73.1	55.7	31.3	250	M	12	III	Fujian, China
38	FJ_6	EAO	90.9	17.5	46.1	88.2	73.7	56.1	31.0	250	F	20	III	Fujian, China
39	FJ_7	EAO	91.3	18.4	47.6	88.8	75.7	59.2	33.2	250	M	20	II	Fujian, China
40	HK1_FH_1	FH+	88.2	15.8	75.9	88.7	84.6	79.3	64.9	1000	F	55	N/A	Hong Kong, China
41	HK1_FH_2	FH+	90.3	15.8	35.7	84.4	62.8	43.0	21.5	1000	F	77	III	Hong Kong, China
42	HK1_FH_3	FH+	94.9	16.8	45.2	89.1	80.4	66.7	35.1	1000	M	40	I	Hong Kong, China
43	HK1_FH_4	FH+	95.5	16.9	51.9	89.9	82.7	70.9	41.5	1000	M	33	II	Hong Kong, China
44	HK2_FH_1	FH+	88.7	17.1	54.9	86.5	79.1	69.7	47.9	1000	M	59	III	Hong Kong, China
45	HK2_FH_2	FH+	95.2	17.7	56.0	90.1	83.9	73.9	47.6	1000	M	59	N/A	Hong Kong, China
46	HK3_FH_1	FH+	94.8	14.9	56.5	90.5	84.2	73.6	45.4	1000	M	39	III	Hong Kong, China
47	HK3_FH_2	FH+	95.1	17.3	51.2	89.9	83.3	72.1	42.2	1000	F	48	III	Hong Kong, China
48	HK4_FH_1	FH+	95.3	14.7	52.2	90.3	83.3	71.7	42.0	1000	M	40	III	Hong Kong, China
49	HK4_FH_2	FH+	95.0	14.0	58.7	90.6	85.3	76.2	49.5	1000	F	34	IIb	Hong Kong, China
50	HK5_FH_1	FH+	95.6	16.6	55.3	90.5	84.8	74.7	46.1	1000	F	39	III	Hong Kong, China
51	HK5_FH_2	FH+	95.5	16.4	53.0	90.4	83.6	72.5	43.6	1000	M	39	IVA	Hong Kong, China
52	HK6_FH_2	FH+	95.3	24.6	59.1	90.0	84.6	75.3	49.4	1000	F	62	III	Hong Kong, China
53	HK7_FH_1	FH+	94.8	14.4	50.3	89.9	82.4	70.2	40.2	1000	M	41	III	Hong Kong, China
54	HK7_FH_2	FH+	95.0	15.8	54.3	90.2	83.4	72.1	43.2	1000	M	40	II	Hong Kong, China
55	HK8_FH_1	FH+	91.5	23.0	36.1	84.8	64.2	44.4	21.7	1000	M	55	II	Hong Kong, China
56	HK8_FH_2	FH+	90.4	18.4	42.6	76.9	56.2	42.2	26.5	1000	M	46	III	Hong Kong, China
57	ZS1_FH_1	FH+	84.4	15.9	47.5	85.0	76.5	65.6	41.4	1000	M	40	N/A	Zhongshan, China
58	ZS1_FH_2	FH+	83.7	16.1	46.0	84.6	75.5	64.0	39.1	1000	M	44	N/A	Zhongshan, China
59	ZS2_FH_1	FH+	85.5	17.1	45.7	86.7	78.1	66.2	38.1	1000	F	47	N/A	Zhongshan, China
60	HK9_FH_1	FH+	91.3	19.8	53.7	87.5	80.7	71.1	47.3	1000	M	40	IV	Hong Kong, China
61	HK10_FH_1	FH+	91.8	17.2	49.2	86.6	78.9	68.0	42.2	1000	F	43	III	Hong Kong, China
62	HK11_FH_1	FH+	88.3	14.9	60.5	87.8	81.8	73.7	53.3	1000	F	44	III	Hong Kong, China
63	HK_FH_12	FH+	91.1	18.5	39.7	86.5	68.1	48.7	25.1	250	M	36	IV	Hong Kong, China
64	HK_FH_13	FH+	92.2	22.9	35.6	83.3	60.2	41.0	21.2	250	F	32	I	Hong Kong, China
65	HK_FH_14	FH+	90.6	16.3	38.5	82.3	60.3	42.5	23.7	250	M	37	II	Hong Kong, China
66	HK_FH_15	FH+	90.7	18.5	45.5	88.0	72.5	54.5	30.2	250	F	34	III	Hong Kong, China
67	HK_FH_16	FH+	92.2	20.6	35.3	84.3	62.5	42.4	20.7	250	M	53	I	Hong Kong, China
68	HK_FH_17	FH+	91.8	19.8	41.8	85.9	67.2	48.6	26.4	250	M	45	III	Hong Kong, China
69	HK_FH_18	FH+	90.6	20.6	46.3	85.3	66.5	49.5	29.4	250	M	36	III	Hong Kong, China
70	HK_FH_19	FH+	90.8	19.5	39.6	85.8	66.6	47.5	24.9	250	M	39	III	Hong Kong, China
71	HK_FH_20	FH+	91.1	17.7	34.8	82.9	60.7	41.3	20.4	250	M	37	III	Hong Kong, China
72	HK_FH_21	FH+	90.0	18.8	48.0	87.8	71.8	54.2	31.4	250	M	23	II	Hong Kong, China
73	HK_FH_22	FH+	90.9	16.3	38.2	83.4	61.8	43.2	23.4	250	F	41	III	Hong Kong, China
74	HK_FH_23	FH+	90.6	16.4	41.8	85.0	65.2	47.0	26.3	250	F	42	IV	Hong Kong, China
75	HK_FH_24	FH+	90.2	16.3	36.6	84.0	63.1	44.0	22.4	250	M	33	II	Hong Kong, China
76	HK_FH_24	FH+	91.9	19.2	44.3	82.3	61.2	45.0	27.4	250	M	31	III	Hong Kong, China
77	HK_FH_26	FH+	89.9	16.3	40.7	84.8	64.9	46.7	25.9	250	M	36	III	Hong Kong, China
78	HK_FH_28	FH+	91.0	16.5	42.9	80.9	59.5	43.9	26.7	250	M	38	IV	Hong Kong, China
79	HK_FH_29	FH+	90.5	17.0	40.7	75.3	54.6	40.7	25.2	250	M	38	III	Hong Kong, China
80	HK_FH_30	FH+	91.4	19.3	31.5	82.9	59.2	38.3	17.0	250	M	36	III	Hong Kong, China
81	HK_FH_31	FH+	91.3	18.3	42.1	82.7	62.4	45.8	26.6	250	F	28	N/A	Hong Kong, China
82	HK_FH_32	FH+	90.5	20.7	58.6	87.4	72.6	58.0	37.9	250	M	36	III	Hong Kong, China
83	HK_FH_33	FH+	90.9	18.0	36.0	83.7	61.5	42.1	21.5	250	M	27	III	Hong Kong, China
84	HK_FH_34	FH+	90.6	18.7	45.4	84.2	64.2	47.3	28.3	250	M	35	III	Hong Kong, China
85	HK_FH_35	FH+	91.7	20.4	42.7	83.8	63.3	45.9	26.6	250	M	34	III	Hong Kong, China
86	HK_FH_36	FH+	89.8	16.1	43.6	86.0	75.8	62.1	34.0	1000	M	58	IVA	Hong Kong, China
87	HK_FH_37	FH+	88.6	14.8	62.7	87.4	81.2	73.3	54.2	1000	F	39	III	Hong Kong, China
88	HK_FH_38	FH+	88.7	15.1	51.1	85.9	77.8	67.3	44.0	1000	F	45	III	Hong Kong, China
89	HK_FH_39	FH+	88.1	15.7	48.4	87.2	78.7	66.9	40.5	1000	M	58	III	Hong Kong, China
90	HK_FH_40	FH+	87.9	17.8	52.1	87.4	80.1	70.1	45.8	1000	M	54	II	Hong Kong, China
91	HK_FH_41	FH+	85.3	16.2	44.6	85.0	75.7	63.6	36.9	1000	M	43	II	Hong Kong, China
92	HK_FH_42	FH+	87.3	17.0	42.1	84.5	74.5	61.3	33.0	1000	F	45	IV	Hong Kong, China
93	HK_FH_43	FH+	89.1	15.7	55.3	86.9	79.9	70.7	49.0	1000	M	34	III	Hong Kong, China
94	HK_FH_44	FH+	85.0	15.0	48.5	86.1	78.5	67.9	43.0	1000	F	59	I	Hong Kong, China
95	HK_FH_45	FH+	86.8	15.3	52.9	86.9	79.9	70.4	47.6	1000	M	40	II	Hong Kong, China
96	HK_FH_46	FH+	86.2	18.3	49.7	86.5	79.2	68.9	44.4	1000	F	42	II	Hong Kong, China
97	HK_FH_47	FH+	90.8	16.2	63.9	89.1	84.1	77.0	57.1	1000	F	40	III	Hong Kong, China

98	HK_FH_48	FH+	90.0	14.8	65.6	88.4	83.0	75.8	57.5	1000	M	39	III	Hong Kong, China
99	HK_FH_49	FH+	91.3	17.2	66.0	89.2	84.3	77.6	58.7	1000	M	55	II	Hong Kong, China
100	HK_FH_50	FH+	92.1	17.2	53.5	88.3	81.7	72.0	46.9	1000	F	34	II	Hong Kong, China
101	HK_FH_51	FH+	88.1	20.1	59.1	87.5	81.7	73.7	52.4	1000	F	60	IV	Hong Kong, China
102	ZS_FH_3	FH+	81.8	16.6	44.8	86.2	77.8	66.0	38.0	1000	F	27	N/A	Zhongshan,China
103	NPCHKE1_N	sporadic	87.7	20.6	40.4	87.5	73.2	55.6	26.8	250	F	40	I	Hong Kong, China
104	NPCHKE2_N	sporadic	89.7	19.3	48.5	89.2	76.7	59.9	33.4	250	F	57	I	Hong Kong, China
105	NPCHKE3_N	sporadic	88.4	17.6	49.6	88.0	73.1	56.1	32.8	250	M	48	I	Hong Kong, China
106	NPCHKE4_N	sporadic	92.5	17.8	57.9	90.1	79.5	65.0	40.4	250	M	40	I	Hong Kong, China
107	NPCHKE5_N	sporadic	88.7	20.1	43.3	88.8	76.1	59.5	30.5	250	M	58	I	Hong Kong, China
108	NPCHKE6_N	sporadic	86.3	17.0	50.0	88.0	74.3	58.2	34.2	250	M	63	I	Hong Kong, China
109	NPCHKE7_N	sporadic	86.4	16.8	58.6	88.5	75.2	60.0	38.0	250	F	69	II	Hong Kong, China
110	NPCHKE8_N	sporadic	90.3	16.7	48.3	90.8	81.5	66.9	36.6	250	F	55	II	Hong Kong, China
111	NPCHKE9_N	sporadic	92.7	18.6	54.1	90.0	79.4	64.7	39.1	250	M	64	II	Hong Kong, China
112	NPCHKE10_N	sporadic	89.6	19.0	43.3	86.9	68.8	50.4	27.9	250	M	82	II	Hong Kong, China
113	NPCHKE11_N	sporadic	89.9	20.7	52.6	88.0	72.1	55.1	33.4	250	M	62	II	Hong Kong, China
114	NPCHKE12_N	sporadic	89.6	22.5	47.8	86.6	69.6	52.9	31.4	250	M	20	II	Hong Kong, China
115	NPCHKE13_N	sporadic	94.3	19.6	43.9	87.9	72.2	54.2	29.2	250	M	38	II	Hong Kong, China
116	NPCHKE14_N	sporadic	94.3	18.9	49.0	89.2	76.4	60.2	34.3	250	M	67	II	Hong Kong, China
117	NPCHKE15_N	sporadic	86.2	16.7	53.6	88.6	75.3	59.6	36.2	250	M	55	II	Hong Kong, China
118	NPCHKE16_N	sporadic	94.5	20.1	42.4	88.0	72.9	54.6	28.1	250	F	39	III	Hong Kong, China
119	NPCHKE17_N	sporadic	93.5	19.1	51.2	89.2	77.2	61.6	36.1	250	F	38	III	Hong Kong, China
120	NPCHKE18_N	sporadic	88.1	20.8	45.2	88.6	76.2	59.8	31.8	250	F	42	III	Hong Kong, China
121	NPCHKE19_N	sporadic	89.2	18.6	44.2	87.2	70.7	52.5	28.7	250	M	58	III	Hong Kong, China
122	NPCHKE20_N	sporadic	90.1	18.6	39.4	87.7	71.6	52.2	25.2	250	M	48	III	Hong Kong, China
123	NPCHKE21_N	sporadic	89.7	18.8	43.3	87.5	71.1	52.6	28.2	250	M	64	III	Hong Kong, China
124	NPCHKE22_N	sporadic	89.1	19.5	50.3	89.1	75.5	58.7	33.7	250	M	60	III	Hong Kong, China
125	NPCHKE23_N	sporadic	89.8	22.1	46.5	88.3	72.9	54.9	30.4	250	M	56	III	Hong Kong, China
126	NPCHKE24_N	sporadic	88.8	19.9	51.2	86.2	69.4	53.1	32.5	250	M	64	III	Hong Kong, China
127	NPCHKE25_N	sporadic	93.8	19.3	48.3	88.5	74.8	58.2	33.2	250	M	49	III	Hong Kong, China
128	NPCHKE26_N	sporadic	93.8	18.6	52.7	89.6	78.5	63.8	38.0	250	M	51	III	Hong Kong, China
129	NPCHKE27_N	sporadic	88.0	19.0	57.5	90.8	81.6	68.4	42.3	250	M	48	III	Hong Kong, China
130	NPCHKE28_N	sporadic	94.5	22.6	39.9	86.9	70.7	52.1	25.7	250	M	43	III	Hong Kong, China
131	NPCHKE29_N	sporadic	89.3	17.5	49.8	88.5	74.6	57.9	33.5	250	M	58	III	Hong Kong, China
132	NPCHKE30_N	sporadic	94.2	18.9	47.5	88.0	73.4	56.7	32.3	250	M	65	III	Hong Kong, China
133	NPCHKE31_N	sporadic	94.1	21.7	47.9	89.0	76.0	59.5	33.3	250	M	47	III	Hong Kong, China
134	NPCHKE32_N	sporadic	94.2	17.8	48.6	89.3	77.2	61.4	34.7	250	M	63	III	Hong Kong, China
135	NPCHKE33_N	sporadic	89.5	23.6	39.1	87.1	70.2	50.6	24.5	250	M	37	III	Hong Kong, China
136	NPCHKE34_N	sporadic	89.1	19.5	51.5	89.7	78.1	62.5	36.2	250	M	48	III	Hong Kong, China
137	NPCHKE35_N	sporadic	90.2	18.7	45.6	88.3	73.0	54.7	29.9	250	M	61	III	Hong Kong, China
138	NPCHKE36_N	sporadic	90.1	17.8	51.8	89.5	76.9	60.8	35.3	250	M	37	III	Hong Kong, China
139	NPCHKE37_N	sporadic	94.3	21.0	47.0	88.3	74.0	57.1	32.1	250	M	52	IV	Hong Kong, China
140	NPCHKE38_N	sporadic	94.4	19.1	46.4	88.9	76.2	59.5	32.5	250	F	64	IV	Hong Kong, China
141	NPCHKE39_N	sporadic	93.8	18.3	52.8	89.8	79.2	64.7	38.5	250	F	38	IV	Hong Kong, China
142	NPCHKE40_N	sporadic	85.4	16.2	73.6	93.1	88.9	82.6	63.8	250	M	49	IV	Hong Kong, China
143	NPCHKE41_N	sporadic	87.3	19.2	53.0	90.4	80.5	66.5	39.3	250	M	62	IV	Hong Kong, China
144	NPCHKE42_N	sporadic	88.9	18.7	47.2	89.6	78.1	62.4	34.1	250	M	46	IV	Hong Kong, China
145	NPCHKE43_N	sporadic	89.5	19.4	52.0	89.4	76.6	60.3	35.2	250	M	55	IV	Hong Kong, China
146	NPCHKE44_N	sporadic	89.1	18.9	49.9	88.8	74.6	57.4	33.0	250	M	60	IV	Hong Kong, China
147	NPCHKE45_N	sporadic	82.8	13.7	57.9	85.8	73.8	61.6	42.1	250	M	29	IV	Hong Kong, China
148	NPCHKE46_N	sporadic	88.9	18.3	49.4	89.2	77.0	61.1	34.7	250	M	49	IV	Hong Kong, China
149	NPCHKE47_N	sporadic	94.3	26.6	34.3	85.0	64.9	44.3	19.9	250	M	68	IV	Hong Kong, China
150	NPCHKE48_N	sporadic	88.8	18.5	47.2	87.9	72.9	55.3	31.2	250	F	58	IV	Hong Kong, China
151	NPCHKE49_N	sporadic	90.0	19.1	55.7	91.8	84.1	72.1	44.5	250	M	62	IV	Hong Kong, China
152	NPCHKE50_N	sporadic	90.3	18.8	55.6	88.8	74.7	58.7	36.4	250	M	50	IV	Hong Kong, China
153	NPCHKE51_N	sporadic	89.9	17.8	65.8	92.5	86.8	78.0	54.7	250	M	48	IV	Hong Kong, China
154	CASE17_N	sporadic	94.7	17.4	37.2	85.2	65.9	46.5	22.9	250	M	NA	N/A	Hong Kong, China
155	CASE20_N	sporadic	93.5	19.5	43.2	85.3	66.8	49.3	27.9	250	M	NA	N/A	Hong Kong, China
156	CASE21_N	sporadic	94.2	21.9	38.2	84.2	63.9	45.5	23.9	250	M	NA	N/A	Hong Kong, China
157	CASE22_N	sporadic	94.1	16.8	42.7	87.1	71.6	53.7	28.5	250	M	NA	N/A	Hong Kong, China
158	CASE24_N	sporadic	94.5	16.5	37.7	84.5	64.7	45.9	23.5	250	M	NA	N/A	Hong Kong, China
159	CASE27_N	sporadic	94.3	17.2	38.8	86.2	69.3	50.6	24.8	250	M	NA	N/A	Hong Kong, China
160	CASE35 _N	sporadic	92.1	19.5	52.3	89.9	78.9	63.8	37.7	250	M	NA	N/A	Hong Kong, China
161	CASE36_N	sporadic	92.3	18.6	51.3	89.6	78.2	62.7	36.7	250	M	NA	N/A	Hong Kong, China

* EAO cases with family history of NPC

N/A: Information is not available

Table S2: Non-cancer controls used in gene-based burden test

Study	# patients	Origin
Degenerative disc disease	713	Hong Kong Chinese
Congenital disorders	111	Hong Kong Chinese
Epilepsy	50	Hong Kong Chinese
Healthy individuals from Red Cross	10	Hong Kong Chinese
Healthy individuals from FH+ NPC families	6	Hong Kong Chinese
Healthy individuals from Fujian	5	Southern Chinese
Total	895	

Table S3: Enrichment of the gene sets with deleterious variants in the EAO cases

Pathway Name	Set Size	# Genes (%)	Genes*	p-value	q-value	Pathway Source
Extracellular matrix organization	264	10 (3.8%)	SERPINH1, COL6A1, ITGAD, FBN2, TNC, COL7A1, DMD, COL18A1, ADAMTS9, **COL12A1**	5.71E-05	0.00526	Reactome
ABC transporters - Homo sapiens (human)	44	4 (9.1%)	ABCA1, ABCA2, ABCC11, ABCG2	0.000451	0.0207	KEGG
Ligand-receptor interactions	8	2 (25.0%)	BOC, **PTCH1**	0.00181	0.0555	Reactome
Proteoglycans in cancer - Homo sapiens (human)	204	6 (2.9%)	**PTCH1**, ITPR3, FZD6, **FLNC**, **ANK2**, **PLCE1**	0.00662	0.1	KEGG
DNA Repair	147	5 (3.4%)	ERCC2, FANCA, LIG3, POLE, FANCI	0.00718	0.1	Reactome
Transmembrane transport of small molecules	579	11 (1.9%)	SLC15A1, TTYH3, ATP7B, ATP1A4, ABCA2, RYR3, ABCC11, ANO9, ABCG2, **CLCNKA**, SLC9A3	0.00761	0.1	Reactome

*Genes with somatic mutation(s) in both the Hong Kong and Singapore cohorts are in **BOLD.** Genes with somatic mutation(s) found in only one cohort are underlined.

Table S4: Genes with deleterious variants relevant to NPC genetic susceptibility and/or tumor development

Gene	EAO patients % (n=39)	FH+ patients % (n=56*)	Sporadic patients % (n=59)	Function in NPC	Reference
ERCC2 (XPD)	10.3%	3.6%	1.7%	Single nucleotide polymorphism p.K751Q associated with decreased risk of NPC in Sichuan population and Malaysian population	(6, 7)
ADAMTS9	10.3%	1.8%	0%	Inhibition of tumor growth and colony formation in NPC cell lines; inhibition of angiogenesis by downregulation of MMP9 and VEGF	(8, 9)
PTPRG	10.3%	7.1%	8.4%	Inhibition of tumor growth by induction of G0/G1 cell cycle arrest through downregulation of cyclin D1	(1)

***** Four EAO cases with family history of NPC were included in the analysis making a total of 56 independent FH+ families

Table S5: Candidate genes in gene-based burden test with p<0.001 in two out of three tests

Index	Chromosome	Start	End	Gene	Direction*	All controls n=895			Controls without healthy individuals from NPC families n=889		
						VT	SKAT	CMC	VT	SKAT	CMC
1	3	49924751	49940666	*MST1R*	+	0.00092	0.02051	0.00026	0.00150	0.02995	0.00037
2	14	23528501	23549346	*ACIN1*	+	0.00046	0.00021	0.00017	0.00026	0.00005	0.00005
3	16	19020565	19067997	*TMC7*	+	0.00089	0.00224	0.00031	0.00064	0.00209	0.00025
4	5	140593726	140595791	*PCDHB13*	+	1.40E-06	0.00332	2.24E-06	8.00E-07	0.00351	3.76E-06
5	3	46871967	46874502	*PRSS42*	+	0.00002	0.00034	0.00140	0.00002	0.00084	0.00162
6	17	7977074	7984039	*ALOX12B*	+	0.00005	0.01855	0.00019	0.00001	0.00727	0.00006
7	11	64664250	64681911	*ATG2A*	+	0.00240	0.00008	0.00085	0.00670	0.00062	0.00283
8	7	112090763	112102405	*IFRD1*	+	0.00056	0.00260	0.00032	0.00140	0.00541	0.00052
9	16	85711859	85721107	*GINS2*	+	0.00060	0.00193	0.00033	0.00002	0.00003	0.00001
10	8	6728214	6728230	*DEFB1*	+	0.00074	0.00535	0.00073	0.00110	0.00590	0.00075
11	14	55836545	55844598	*ATG14*	+	0.00019	0.00004	0.00006	0.00092	0.00046	0.00031
12	17	79870317	79872517	*SIRT7*	+	0.00120	0.00002	0.00044	0.00180	0.00006	0.00112
13	17	73124834	73125065	*ARMC7*	+	0.00014	0.00006	0.00005	0.00036	0.00037	0.00014
14	4	47901069	47912981	*NFXL1*	+	0.00150	0.00080	0.00054	0.00240	0.00133	0.00088
15	9	37762066	37776324	*TRMT10B*	+	0.00090	0.00024	0.00030	0.00150	0.00036	0.00046

*direction +: the rare deleterious variants are more frequently in NPC cases than in controls

Table S6: Non-reference discrepancy rate of 0.996 in duplicates with 250 ng and 1000 ng input DNA

	A/A (250 ng)	A/B (250 ng)	B/B (250 ng)	no call (250 ng)
A/A (1000 ng)		54	2	18350
A/B (1000 ng)	108	34592	28	1362
B/B (1000 ng)	2	39	27233	1250
no call (1000 ng)	29834	2949	2576	

A: wild-type allele. B: mutant allele

Table S7: PCR primers and conditions for Sanger sequencing for MST1R variants

Target	Assay	PCR primers	Size of amplicon (bp)	PCR condition
R306H A327T	Assay 1	forward 5'-GTCAATGGGGAAGGCACAGA-3' (sequencing primer) reverse 5'-GGGTGACTATCGGGAGCTG-3'	247	95^{o}C 5 min
V670G E705K	Assay 2	forward 5'-AAGCACTCTTCCCTCCATCC-3' (sequencing primer) reverse 5'-TGCCCCGGAAAGACTTTGTA-3'	488	(95^{o}C 30 sec, 60^{o}C 30 sec, 72^{o}C 30 sec)x30 cycles
A973T	Assay 3	forward 5'-CCTAAGTCCTGGGCAGAGAG-3' (sequencing primer) reverse 5'-CCTTGGTATCCTGCTGCCTT-3'	188	72^{o}C 7 min

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