

## APPENDIX

### **Leukocytes carrying *Clonal Hematopoiesis of Indeterminate Potential* (CHIP) Mutations invade Human Atherosclerotic Plaques**

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#### **Content**

**Supplemental Table 1** – List of CHIP mutations in MISSION

**Supplemental Table 2** – List of CHIP mutations in STARNET

**Supplemental Table 3** – STARNET patient characteristics TET2 macrophages

**Supplemental Table 4** – STARNET patient characteristics ASXL1 macrophages

**Supplemental Figure 1** – VAF and distribution of CHIP mutations in MISSION

**Supplemental Figure 2** – DeepDNAseq identifies CHIP mutations in atherosclerotic coronary and carotid samples, and left ventricular myocardium

**Supplemental Figure 3** – Overview plaque of interest – different stainings

**Supplemental Figure 4** – Visualization of CD68<sup>+</sup> CHIP mutated macrophage

**Supplemental Figure 5** – DNMT3A CHIP mutation (c.2245C>T) in human atherosclerotic plaque

**Protocol** – Adapted mutaFISH™ protocol

**Supplemental Table 1** – List of 445 unique CHIP mutations identified in MISSION based on deep-DNA-sequencing. Provided are gene name, confirmation of CHIP mutation – polymorphisms, variants, synonymous and uncertain mutations were excluded, change on DNA level, change on amino acid (AA) level and variant allele frequency (VAF).

Gene	CHIP result	DNA result	AA result	VAF (%)
ASXL1	mutated	c.1534C>T	p.Gln512*	4.1
ASXL1	mutated	c.1564C>T	p.Gln522*	4.7
ASXL1	mutated	c.1585C>T	p.Gln529*	16.3
ASXL1	mutated	c.1720-2A>G	p.splice site mutation	6.3
ASXL1	mutated	c.1749G>A	p.Trp583*	31.5
ASXL1	mutated	c.1762C>T	p.Gln588*	5.5
ASXL1	mutated	c.1772dup	p.Tyr591*	5.2
ASXL1	mutated	c.1772dup	p.Tyr591*	3.0
ASXL1	mutated	c.1900_1922del	p.Glu635Argfs*15	7.6
ASXL1	mutated	c.1900_1922del	p.Glu635Argfs*15	6.6
ASXL1	mutated	c.1900_1922del	p.Glu635Argfs*15	1.5
ASXL1	mutated	c.1900_1922del	p.Glu635Argfs*15	11.0
ASXL1	mutated	c.1900_1922del	p.Glu635Argfs*15	19.6
ASXL1	mutated	c.1934dup	p.Gly646Trpfs*12	18.6
ASXL1	mutated	c.1934dup	p.Gly646Trpfs*12	16.6
ASXL1	mutated	c.1934dup	p.Gly646Trpfs*12	12.4
ASXL1	mutated	c.1934dup	p.Gly646Trpfs*12	10.8
ASXL1	mutated	c.1934dup	p.Gly646Trpfs*12	7.0
ASXL1	mutated	c.1934dup	p.Gly646Trpfs*12	11.2
ASXL1	mutated	c.1934dup	p.Gly646Trpfs*12	16.0
ASXL1	mutated	c.2069_2075del	p.Asp690Glyfs*11	1.5
ASXL1	mutated	c.2077C>T	p.Arg693*	2.1
ASXL1	mutated	c.2083C>T	p.Gln695*	2.6
ASXL1	mutated	c.2290del	p.Leu764Tyrfs*8	5.5
ASXL1	mutated	c.2302C>T	p.Gln768*	22.6
ASXL1	mutated	c.2324T>G	p.Leu775*	14.1
ASXL1	mutated	c.2387G>A	p.Trp796*	16.3
ASXL1	mutated	c.2468del	p.Leu823*	6.1
ASXL1	mutated	c.2528_2529insCT	p.Thr844*	2.2
ASXL1	mutated	c.2676del	p.Asn893Thrfs*15	9.6
ASXL1	mutated	c.2989G>T	p.Glu997*	7.8
ASXL1	mutated	c.3554dup	p.Thr1186Hisfs*7	2.7
BCOR	mutated	c.4616dup	p.Asn1540Glufs*16	7.0
CALR	mutated	c.1154_1155insTTGTC	p.Lys385Asnfs*47	28.8
CBL	mutated	c.1009del	p.Tyr337Ilefs*13	5.0
CBL	mutated	c.1102T>C	p.Tyr368His	11.5
CBL	mutated	c.1129A>G	p.Thr377Ala	8.0
CBL	mutated	c.1139T>C	p.Leu380Pro	1.7
CBL	mutated	c.1145A>G	p.Lys382Arg	4.3
CBL	mutated	c.1175A>G	p.Lys392Arg	1.3

CBL	mutated	c.1211G>A	p.Cys404Tyr	29.2
CBL	mutated	c.1211G>A	p.Cys404Tyr	9.1
CBL	mutated	c.1211G>A	p.Cys404Tyr	3.0
CBL	mutated	c.1211G>A	p.Cys404Tyr	4.2
CBL	mutated	c.1254C>A	p.Phe418Leu	6.7
CBL	mutated	c.1268T>A	p.Ile423Asn	23.0
CBL	mutated	c.1694del	p.Leu565Cysfs*50	3.5
DNMT3A	mutated	c.1014+1G>T	p.splice site mutation	4.0
DNMT3A	mutated	c.1021_1022del	p.Val341*	1.9
DNMT3A	mutated	c.1040T>C	p.Leu347Pro	9.6
DNMT3A	mutated	c.1058_1066del	p.Ala353_Gln356delinsGlu	11.0
DNMT3A	mutated	c.1069_1086dup	p.Ala357_Gln362dup	1.2
DNMT3A	mutated	c.1077_1078dup	p.Asn360Thrfs*48	2.1
DNMT3A	mutated	c.1136G>A	p.Arg379His	3.3
DNMT3A	mutated	c.1138_1147del	p.Ala380Cysfs*24	1.8
DNMT3A	mutated	c.1152_1155del	p.Phe384Leufs*22	8.0
DNMT3A	mutated	c.1156del	p.Val386Cysfs*21	3.0
DNMT3A	mutated	c.1223_1226del	p.Glu408Glyfs*242	5.1
DNMT3A	mutated	c.1226G>A	p.Trp409*	1.7
DNMT3A	mutated	c.1229C>T	p.Ala410Val	5.5
DNMT3A	mutated	c.1234_1235insA	p.Gly412Glufs*9	1.1
DNMT3A	mutated	c.1238dup	p.Phe414Leufs*7	2.7
DNMT3A	mutated	c.1410del	p.Ile471Leufs*180	1.4
DNMT3A	mutated	c.1428del	p.Glu477Serfs*174	1.5
DNMT3A	mutated	c.1429G>C	p.Glu477Gln	5.5
DNMT3A	mutated	c.1481G>A	p.Cys494Tyr	2.9
DNMT3A	mutated	c.1489T>C	p.Cys497Arg	2.2
DNMT3A	mutated	c.1498del	p.Leu500Serfs*151	2.3
DNMT3A	mutated	c.1507dup	p.Thr503Asnfs*43	14.2
DNMT3A	mutated	c.1517A>G	p.His506Arg	2.6
DNMT3A	mutated	c.1523T>C	p.Leu508Pro	1.5
DNMT3A	mutated	c.1543C>T	p.Gln515*	19.4
DNMT3A	mutated	c.1543del	p.Gln515Lysfs*136	1.8
DNMT3A	mutated	c.1551_1552delinsG	p.Cys517Trpfs*134	1.3
DNMT3A	mutated	c.1555-2A>T	p.splice site mutation	4.8
DNMT3A	mutated	c.1555-8_1555del	p.splice site mutation	1.7
DNMT3A	mutated	c.1591G>A	p.Asp531Asn	1.4
DNMT3A	mutated	c.1592A>G	p.Asp531Gly	6.1
DNMT3A	mutated	c.1628G>C	p.Gly543Ala	21.6
DNMT3A	mutated	c.1637T>A	p.Val546Glu	3.8
DNMT3A	mutated	c.1640T>A	p.Leu547His	4.1
DNMT3A	mutated	c.1640T>A	p.Leu547His	1.4
DNMT3A	mutated	c.1657_1659del	p.Asn553del	8.9
DNMT3A	mutated	c.1713_1724del	p.Ala572_Ala575del	1.4
DNMT3A	mutated	c.1726_1729delinsC	p.Ile576_Lys577delinsGln	22.6
DNMT3A	mutated	c.1742G>A	p.Trp581*	1.8
DNMT3A	mutated	c.1903C>T	p.Arg635Trp	2.8

DNMT3A	mutated	c.1906G>A	p.Val636Met	1.1
DNMT3A	mutated	c.1969G>A	p.Val657Met	5.3
DNMT3A	mutated	c.1969G>A	p.Val657Met	1.4
DNMT3A	mutated	c.1972G>T	p.Asp658Tyr	10.0
DNMT3A	mutated	c.1976G>A	p.Arg659His	1.4
DNMT3A	mutated	c.1979A>G	p.Tyr660Cys	4.5
DNMT3A	mutated	c.1998_1999del	p.Cys666*	2.2
DNMT3A	mutated	c.1998T>G	p.Cys666Trp	2.9
DNMT3A	mutated	c.2007dup	p.Ile670Hisfs*43	20.6
DNMT3A	mutated	c.2023G>A	p.Val675Met	1.1
DNMT3A	mutated	c.2024_2026dup	p.Val675_Arg676insLeu	4.7
DNMT3A	mutated	c.2032del	p.Gln678Argfs*27	1.1
DNMT3A	mutated	c.2053G>C	p.Gly685Arg	15.0
DNMT3A	mutated	c.2056del	p.Asp686Thrfs*19	1.9
DNMT3A	mutated	c.2057A>G	p.Asp686Gly	2.3
DNMT3A	mutated	c.2062C>T	p.Arg688Cys	1.7
DNMT3A	mutated	c.2063G>A	p.Arg688His	1.1
DNMT3A	mutated	c.2063G>A	p.Arg688His	2.3
DNMT3A	mutated	c.2084T>C	p.Ile695Thr	2.0
DNMT3A	mutated	c.2095G>C	p.Gly699Arg	16.7
DNMT3A	mutated	c.2095G>C	p.Gly699Arg	2.3
DNMT3A	mutated	c.2098C>A	p.Pro700Thr	4.7
DNMT3A	mutated	c.2099C>T	p.Pro700Leu	2.4
DNMT3A	mutated	c.2104G>T	p.Asp702Tyr	11.8
DNMT3A	mutated	c.2108del	p.Leu703Argfs*2	1.1
DNMT3A	mutated	c.2114T>C	p.Ile705Thr	1.3
DNMT3A	mutated	c.2171A>G	p.Tyr724Cys	2.2
DNMT3A	mutated	c.2171A>G	p.Tyr724Cys	5.7
DNMT3A	mutated	c.2177G>T	p.Gly726Val	1.7
DNMT3A	mutated	c.2183G>A	p.Gly728Asp	5.6
DNMT3A	mutated	c.2185C>T	p.Arg729Trp	30.8
DNMT3A	mutated	c.2185C>T	p.Arg729Trp	13.9
DNMT3A	mutated	c.2192T>A	p.Phe731Tyr	5.0
DNMT3A	mutated	c.2204A>C	p.Tyr735Ser	8.5
DNMT3A	mutated	c.2204A>G	p.Tyr735Cys	1.1
DNMT3A	mutated	c.2204A>G	p.Tyr735Cys	11.3
DNMT3A	mutated	c.2204A>G	p.Tyr735Cys	22.8
DNMT3A	mutated	c.2204A>G	p.Tyr735Cys	1.4
DNMT3A	mutated	c.2204A>G	p.Tyr735Cys	1.9
DNMT3A	mutated	c.2206C>T	p.Arg736Cys	9.6
DNMT3A	mutated	c.2206C>T	p.Arg736Cys	4.8
DNMT3A	mutated	c.2206C>T	p.Arg736Cys	9.5
DNMT3A	mutated	c.2228C>T	p.Pro743Leu	12.2
DNMT3A	mutated	c.2245C>T	p.Arg749Cys	5.6
DNMT3A	mutated	c.2245C>T	p.Arg749Cys	6.3
DNMT3A	mutated	c.2245C>T	p.Arg749Cys	4.4
DNMT3A	mutated	c.2254T>G	p.Phe752Val	1.0

DNMT3A	mutated	c.2259G>A	p.Trp753*	5.2
DNMT3A	mutated	c.2261T>C	p.Leu754Pro	1.8
DNMT3A	mutated	c.2261T>G	p.Leu754Arg	1.8
DNMT3A	mutated	c.2264T>C	p.Phe755Ser	4.3
DNMT3A	mutated	c.2302delG	p.Asp768Thrfs*11	2.3
DNMT3A	mutated	c.2309C>T	p.Ser770Leu	2.9
DNMT3A	mutated	c.2311C>G	p.Arg771Gly	3.6
DNMT3A	mutated	c.2311C>T	p.Arg771*	4.9
DNMT3A	mutated	c.2311C>T	p.Arg771*	15.3
DNMT3A	mutated	c.2311C>T	p.Arg771*	1.3
DNMT3A	mutated	c.2311C>T	p.Arg771*	1.8
DNMT3A	mutated	c.2312G>T	p.Arg771Leu	23.7
DNMT3A	mutated	c.2320G>T	p.Glu774*	11.3
DNMT3A	mutated	c.2330C>G	p.Pro777Arg	1.3
DNMT3A	mutated	c.2332G>A	p.Val778Met	1.3
DNMT3A	mutated	c.2333T>G	p.Val778Gly	6.5
DNMT3A	mutated	c.2333T>G	p.Val778Gly	2.8
DNMT3A	mutated	c.2339T>C	p.Ile780Thr	5.4
DNMT3A	mutated	c.2339T>C	p.Ile780Thr	1.1
DNMT3A	mutated	c.2339T>C	p.Ile780Thr	2.2
DNMT3A	mutated	c.2347A>T	p.Lys783*	1.6
DNMT3A	mutated	c.2377T>G	p.Tyr793Asp	1.3
DNMT3A	mutated	c.2387del	p.Gly796Valfs*6	2.2
DNMT3A	mutated	c.2387G>A	p.Gly796Asp	1.5
DNMT3A	mutated	c.2389A>T	p.Asn797Tyr	2.7
DNMT3A	mutated	c.2390A>G	p.Asn797Ser	17.0
DNMT3A	mutated	c.2395C>T	p.Pro799Ser	5.0
DNMT3A	mutated	c.2404A>T	p.Asn802Tyr	5.1
DNMT3A	mutated	c.2462del	p.His821Leufs*4	3.0
DNMT3A	mutated	c.2471dup	p.Ala825Serfs*30	1.4
DNMT3A	mutated	c.2524C>T	p.Gln842*	2.4
DNMT3A	mutated	c.2524C>T	p.Gln842*	3.7
DNMT3A	mutated	c.2533del	p.Asp845Thrfs*8	3.0
DNMT3A	mutated	c.2550del	p.Phe851Serfs*2	3.1
DNMT3A	mutated	c.2578T>C	p.Trp860Arg	6.2
DNMT3A	mutated	c.2617C>T	p.His873Tyr	1.1
DNMT3A	mutated	c.2644C>T	p.Arg882Cys	2.6
DNMT3A	mutated	c.2644C>T	p.Arg882Cys	32.2
DNMT3A	mutated	c.2645G>A	p.Arg882His	2.2
DNMT3A	mutated	c.2645G>A	p.Arg882His	2.5
DNMT3A	mutated	c.2645G>A	p.Arg882His	3.2
DNMT3A	mutated	c.2645G>A	p.Arg882His	1.5
DNMT3A	mutated	c.2666T>C	p.Leu889Pro	2.2
DNMT3A	mutated	c.2666T>C	p.Leu889Pro	0.9
DNMT3A	mutated	c.2679G>C	p.Trp893Cys	3.5
DNMT3A	mutated	c.2695C>T	p.Arg899Cys	1.9
DNMT3A	mutated	c.2695C>T	p.Arg899Cys	2.0

DNMT3A	mutated	c.2695C>T	p.Arg899Cys	4.7
DNMT3A	mutated	c.2695C>T	p.Arg899Cys	2.2
DNMT3A	mutated	c.2705del	p.Phe902Serfs*4	8.2
DNMT3A	mutated	c.2710C>T	p.Pro904Ser	1.2
DNMT3A	mutated	c.2714T>G	p.Leu905Arg	1.6
DNMT3A	mutated	c.2723A>G	p.Tyr908Cys	1.9
DNMT3A	mutated	c.2726T>C	p.Phe909Ser	1.8
DNMT3A	mutated	c.719_725del	p.Glu240Alafs*74	1.8
DNMT3A	mutated	c.814A>T	p.Lys272*	1.1
DNMT3A	mutated	c.884T>A	p.Leu295Gln	2.3
DNMT3A	mutated	c.890G>A	p.Trp297*	1.5
DNMT3A	mutated	c.893G>A	p.Gly298Glu	2.6
DNMT3A	mutated	c.901C>T	p.Arg301Trp	2.2
DNMT3A	mutated	c.905G>T	p.Gly302Val	13.9
DNMT3A	mutated	c.914G>A	p.Trp305*	2.5
DNMT3A	mutated	c.915G>A	p.Trp305*	26.4
DNMT3A	mutated	c.929T>C	p.Ile310Thr	3.0
DNMT3A	mutated	c.976C>T	p.Arg326Cys	20.5
DNMT3A	mutated	c.976C>T	p.Arg326Cys	3.6
DNMT3A	mutated	c.976C>T	p.Arg326Cys	8.4
DNMT3A	mutated	c.98G>A	p.Arg33His	1.8
EZH2	mutated	c.875A>G	p.Tyr292Cys	1.1
GNAS	mutated	c.2531G>A	p.Arg844His	2.9
GNB1	mutated	c.169A>G	p.Lys57Glu	13.9
IDH1	mutated	c.395G>A	p.Arg132His	43.7
JAK2	mutated	c.1849G>T	p.Val617Phe	2.0
JAK2	mutated	c.1849G>T	p.Val617Phe	2.6
JAK2	mutated	c.1849G>T	p.Val617Phe	5.5
JAK2	mutated	c.1849G>T	p.Val617Phe	2.3
JAK2	mutated	c.1849G>T	p.Val617Phe	4.5
JAK2	mutated	c.1849G>T	p.Val617Phe	38.7
JAK2	mutated	c.1849G>T	p.Val617Phe	1.5
JAK2	mutated	c.2569A>G	p.Lys857Glu	1.2
KRAS	mutated	c.35G>A	p.Gly12Asp	4.3
PPM1D	mutated	c.1270G>T	p.Glu424*	5.9
PPM1D	mutated	c.1297A>T	p.Lys433*	1.0
PPM1D	mutated	c.1349del	p.Leu450*	4.4
PPM1D	mutated	c.1372C>T	p.Arg458*	17.4
PPM1D	mutated	c.1382del	p.Val461Alafs*4	2.4
PPM1D	mutated	c.1434C>A	p.Cys478*	8.8
PPM1D	mutated	c.1528C>T	p.Gln510*	2.8
PPM1D	mutated	c.1535del	p.Asn512Ilefs*2	0.7
PPM1D	mutated	c.1535del	p.Asn512Ilefs*2	0.9
PPM1D	mutated	c.1566dup	p.Ala523Serfs*5	10.0
PPM1D	mutated	c.1609del	p.Thr537Hisfs*2	34.0
PPM1D	mutated	c.1649del	p.His550Leufs*6	6.9
PPM1D	mutated	c.1654C>T	p.Arg552*	2.7

PPM1D	mutated	c.1654C>T	p.Arg552*	13.6
PPM1D	mutated	c.1714C>T	p.Arg572*	2.8
RAD21	mutated	c.144+1G>T	p.splice site mutation	6.1
SETBP1	mutated	c.2572G>A	p.Glu858Lys	1.8
SF3B1	mutated	c.1868A>T	p.Tyr623Phe	1.3
SF3B1	mutated	c.1876A>G	p.Asn626Asp	1.3
SF3B1	mutated	c.1985A>T	p.His662Leu	1.0
SF3B1	mutated	c.1986C>G	p.His662Gln	1.2
SF3B1	mutated	c.1996A>C	p.Lys666Gln	4.8
SF3B1	mutated	c.1997A>G	p.Lys666Arg	7.8
SF3B1	mutated	c.1998G>C	p.Lys666Asn	8.1
SF3B1	mutated	c.1998G>C	p.Lys666Asn	1.0
SF3B1	mutated	c.1998G>T	p.Lys666Asn	5.4
SF3B1	mutated	c.1998G>T	p.Lys666Asn	27.1
SF3B1	mutated	c.1998G>T	p.Lys666Asn	23.9
SF3B1	mutated	c.1998G>T	p.Lys666Asn	1.7
SF3B1	mutated	c.1998G>T	p.Lys666Asn	26.7
SF3B1	mutated	c.2098A>G	p.Lys700Glu	13.8
SF3B1	mutated	c.2098A>G	p.Lys700Glu	23.0
SMC1A	mutated	c.2131C>T	p.Arg711Trp	4.7
SMC3	mutated	c.3353G>T	p.Gly1118Val	0.7
SMC3	mutated	c.3598G>A	p.Val1200Met	6.0
SRSF2	mutated	c.170T>A	p.Phe57Tyr	2.0
SRSF2	mutated	c.170T>A	p.Phe57Tyr	2.1
SRSF2	mutated	c.284C>A	p.Pro95His	32.0
SRSF2	mutated	c.284C>A	p.Pro95His	23.1
SRSF2	mutated	c.284C>G	p.Pro95Arg	1.2
SRSF2	mutated	c.284C>G	p.Pro95Arg	18.2
SRSF2	mutated	c.284C>T	p.Pro95Leu	41.8
SRSF2	mutated	c.287C>T	p.Pro96Leu	7.4
TET2	mutated	c.1028_1046del	p.Thr343Metfs*23	2.4
TET2	mutated	c.1061C>A	p.Ser354*	17.5
TET2	mutated	c.1061C>G	p.Ser354*	1.3
TET2	mutated	c.1201_1203del	p.Pro401del	2.7
TET2	mutated	c.1201_1203del	p.Pro401del	2.8
TET2	mutated	c.1212del	p.Leu404Phefs*23	1.9
TET2	mutated	c.1219del	p.Ser407Leufs*20	27.7
TET2	mutated	c.1249C>T	p.Gln417*	4.4
TET2	mutated	c.1259del	p.Ser420*	1.6
TET2	mutated	c.1430del	p.Ser477Leufs*9	3.2
TET2	mutated	c.1469_1470del	p.Ile490Thrfs*13	1.7
TET2	mutated	c.1588C>T	p.Gln530*	5.9
TET2	mutated	c.1630C>T	p.Arg544*	25.0
TET2	mutated	c.1630C>T	p.Arg544*	3.8
TET2	mutated	c.1699_1703del	p.Leu567Glyfs*14	2.7
TET2	mutated	c.1800_1801dup	p.Thr601Argfs*39	6.3
TET2	mutated	c.1803del	p.Ser602Profs*37	16.5

TET2	mutated	c.1842del	p.Leu615Serfs*24	3.0
TET2	mutated	c.1863_1879del	p.Gln622Glyfs*10	3.8
TET2	mutated	c.2167_2170del	p.Pro723Ilefs*27	1.8
TET2	mutated	c.2255_2261del	p.Asn752Argfs*59	2.1
TET2	mutated	c.2276del	p.Thr759Ilefs*54	5.5
TET2	mutated	c.2370_2382dup	p.Ser795Valfs*11	6.6
TET2	mutated	c.2375C>G	p.Ser792*	17.4
TET2	mutated	c.2662C>T	p.Gln888*	2.6
TET2	mutated	c.2662C>T	p.Gln888*	6.0
TET2	mutated	c.2674C>T	p.Gln892*	7.6
TET2	mutated	c.2746C>T	p.Gln916*	4.6
TET2	mutated	c.2746C>T	p.Gln916*	5.1
TET2	mutated	c.2746C>T	p.Gln916*	1.5
TET2	mutated	c.2749C>T	p.Gln917*	27.0
TET2	mutated	c.2757C>A	p.Tyr919*	1.8
TET2	mutated	c.2839C>T	p.Gln947*	19.2
TET2	mutated	c.2884C>T	p.Gln962*	29.5
TET2	mutated	c.2896C>T	p.Gln966*	2.0
TET2	mutated	c.2905C>T	p.Gln969*	1.3
TET2	mutated	c.2926C>T	p.Gln976*	2.0
TET2	mutated	c.2926C>T	p.Gln976*	16.3
TET2	mutated	c.2944A>T	p.Lys982*	38.9
TET2	mutated	c.3119T>G	p.Leu1040*	2.9
TET2	mutated	c.3127del	p.His1043Ilefs*12	21.0
TET2	mutated	c.3287del	p.Thr1096Lysfs*10	2.6
TET2	mutated	c.3339_3340del	p.Thr1114Serfs*15	1.3
TET2	mutated	c.3344del	p.Pro1115Leufs*2	1.9
TET2	mutated	c.3344del	p.Pro1115Leufs*2	1.0
TET2	mutated	c.3369del	p.Val1124Serfs*13	2.0
TET2	mutated	c.3404G>A	p.Cys1135Tyr	1.9
TET2	mutated	c.3404G>A	p.Cys1135Tyr	1.8
TET2	mutated	c.3409+1G>A	p.splice site mutation	2.6
TET2	mutated	c.3415del	p.Ile1139Leufs*13	6.9
TET2	mutated	c.3415del	p.Ile1139Leufs*13	3.1
TET2	mutated	c.3491T>G	p.Met1164Arg	1.0
TET2	mutated	c.3500G>A	p.Arg1167Lys	1.1
TET2	mutated	c.3522_3523insG	p.Ile1175Aspfs*2	19.0
TET2	mutated	c.3523A>T	p.Ile1175Phe	18.7
TET2	mutated	c.3524_3526delinsCTT	p.Ile1175_Arg1176delinsThrTrp	5.9
TET2	mutated	c.3530T>G	p.Ile1177Ser	7.4
TET2	mutated	c.3578G>A	p.Cys1193Tyr	2.6
TET2	mutated	c.3637G>A	p.Val1213Met	1.4
TET2	mutated	c.3640C>T	p.Arg1214Trp	1.4
TET2	mutated	c.3640C>T	p.Arg1214Trp	4.7
TET2	mutated	c.3646C>T	p.Arg1216*	1.5
TET2	mutated	c.3656A>C	p.His1219Pro	1.4
TET2	mutated	c.3661T>G	p.Cys1221Gly	1.7



TET2	mutated	c.3662G>C	p.Cys1221Ser	1.5
TET2	mutated	c.3732_3733del	p.Tyr1245Leufs*22	4.8
TET2	mutated	c.3732_3733del	p.Tyr1245Leufs*22	1.5
TET2	mutated	c.3733_3737del	p.Tyr1245Glyfs*21	1.2
TET2	mutated	c.3734A>G	p.Tyr1245Cys	39.6
TET2	mutated	c.3755T>C	p.Leu1252Pro	4.3
TET2	mutated	c.3781C>T	p.Arg1261Cys	1.6
TET2	mutated	c.3782G>A	p.Arg1261His	2.0
TET2	mutated	c.3785G>A	p.Arg1262Gln	2.0
TET2	mutated	c.3788G>C	p.Cys1263Ser	1.5
TET2	mutated	c.3788G>C	p.Cys1263Ser	1.6
TET2	mutated	c.3821_3822del	p.Gln1274Argfs*25	1.5
TET2	mutated	c.3822G>C	p.Gln1274His	5.7
TET2	mutated	c.3863G>A	p.Gly1288Asp	2.0
TET2	mutated	c.3863G>A	p.Gly1288Asp	3.3
TET2	mutated	c.3866G>T	p.Cys1289Phe	2.2
TET2	mutated	c.3894dup	p.Lys1299*	13.5
TET2	mutated	c.3904A>G	p.Arg1302Gly	5.7
TET2	mutated	c.3968del	p.Glu1323Glyfs*40	37.8
TET2	mutated	c.4015A>T	p.Lys1339*	23.3
TET2	mutated	c.4021dup	p.Ala1341Glyfs*3	1.6
TET2	mutated	c.4030G>A	p.Ala1344Thr	8.0
TET2	mutated	c.4042del	p.Gln1348Argfs*15	7.6
TET2	mutated	c.4075C>A	p.Arg1359Ser	4.7
TET2	mutated	c.4075C>A	p.Arg1359Ser	1.2
TET2	mutated	c.4076G>A	p.Arg1359His	2.9
TET2	mutated	c.4081G>C	p.Gly1361Arg	4.9
TET2	mutated	c.4082G>A	p.Gly1361Asp	2.9
TET2	mutated	c.4103_4116del	p.Phe1368Cysfs*28	19.2
TET2	mutated	c.4126G>A	p.Asp1376Asn	1.9
TET2	mutated	c.4131_4132del	p.Phe1377Leufs*23	21.5
TET2	mutated	c.4132T>C	p.Cys1378Arg	19.6
TET2	mutated	c.4133G>A	p.Cys1378Tyr	26.3
TET2	mutated	c.4136C>T	p.Ala1379Val	36.6
TET2	mutated	c.4138C>T	p.His1380Tyr	7.6
TET2	mutated	c.4138C>T	p.His1380Tyr	1.5
TET2	mutated	c.4140T>G	p.His1380Gln	5.9
TET2	mutated	c.4193T>G	p.Leu1398Arg	6.4
TET2	mutated	c.4234G>T	p.Asp1412Tyr	19.3
TET2	mutated	c.4256C>G	p.Pro1419Arg	7.7
TET2	mutated	c.4354C>T	p.Arg1452*	5.0
TET2	mutated	c.4393C>T	p.Arg1465*	2.1
TET2	mutated	c.4393C>T	p.Arg1465*	26.9
TET2	mutated	c.4399del	p.Arg1467Glyfs*3	14.6
TET2	mutated	c.4481C>G	p.Ser1494*	3.6
TET2	mutated	c.4546C>T	p.Arg1516*	29.7
TET2	mutated	c.4546C>T	p.Arg1516*	3.9

TET2	mutated	c.4570C>T	p.Gln1524*	12.3
TET2	mutated	c.4589_4618del	p.Pro1530_Gln1539del	57.0
TET2	mutated	c.4621C>T	p.Gln1541*	18.4
TET2	mutated	c.4624C>T	p.Gln1542*	6.6
TET2	mutated	c.4639C>T	p.Gln1547*	1.8
TET2	mutated	c.4757C>G	p.Ser1586*	3.0
TET2	mutated	c.4854C>G	p.Tyr1618*	5.0
TET2	mutated	c.4879C>T	p.Gln1627*	2.5
TET2	mutated	c.506_508delinsC	p.His169Profs*6	2.4
TET2	mutated	c.5220dup	p.Pro1741Thrfs*12	2.9
TET2	mutated	c.5271_5272dup	p.Ser1758Phefs*6	28.7
TET2	mutated	c.532G>T	p.Glu178*	29.3
TET2	mutated	c.5413_5420del	p.Asn1805*	3.7
TET2	mutated	c.5454_5458del	p.Leu1819*	9.8
TET2	mutated	c.5467_5472delinsCC	p.Asn1823Profs*9	25.7
TET2	mutated	c.5482C>T	p.Gln1828*	2.1
TET2	mutated	c.5500C>T	p.Gln1834*	4.2
TET2	mutated	c.5541G>A	p.Trp1847*	3.1
TET2	mutated	c.5541G>A	p.Trp1847*	2.1
TET2	mutated	c.5543C>A	p.Ser1848*	4.2
TET2	mutated	c.5551_5554del	p.Glu1851Argfs*35	4.0
TET2	mutated	c.5551G>T	p.Glu1851*	0.8
TET2	mutated	c.5603A>G	p.His1868Arg	2.1
TET2	mutated	c.5615T>A	p.Leu1872His	3.3
TET2	mutated	c.561dup	p.Lys188Glufs*4	18.9
TET2	mutated	c.5621A>T	p.Glu1874Val	2.1
TET2	mutated	c.5636A>C	p.Glu1879Ala	11.5
TET2	mutated	c.5642A>G	p.His1881Arg	2.5
TET2	mutated	c.5650A>G	p.Thr1884Ala	1.4
TET2	mutated	c.5690T>G	p.Ile1897Ser	4.1
TET2	mutated	c.5720T>A	p.Met1907Lys	11.1
TET2	mutated	c.651del	p.Val218Trpfs*32	1.4
TET2	mutated	c.661_667del	p.Thr221Valfs*27	5.8
TET2	mutated	c.662_663insTC	p.Gly223Metfs*28	2.2
TET2	mutated	c.763C>T	p.Gln255*	6.5
TET2	mutated	c.822del	p.Asn275Ilefs*18	2.0
TET2	mutated	c.822del	p.Asn275Ilefs*18	1.3
TET2	mutated	c.840_841insTT	p.Asn281Leufs*13	4.1
TET2	mutated	c.897dup	p.Asp300*	4.9
TP53	mutated	c.223C>G	p.Pro75Ala	4.7
TP53	mutated	c.329G>C	p.Arg110Pro	5.2
TP53	mutated	c.332T>G	p.Leu111Arg	1.7
TP53	mutated	c.376T>C	p.Tyr126His	5.9
TP53	mutated	c.464C>T	p.Thr155Ile	3.2
TP53	mutated	c.473G>T	p.Arg158Leu	4.4
TP53	mutated	c.530C>T	p.Pro177Leu	2.5
TP53	mutated	c.533A>C	p.His178Pro	2.9

TP53	mutated	c.541C>T	p.Arg181Cys	45.9
TP53	mutated	c.584T>C	p.Ile195Thr	11.4
TP53	mutated	c.586C>T	p.Arg196*	24.2
TP53	mutated	c.658T>C	p.Tyr220His	2.4
TP53	mutated	c.668C>T	p.Pro223Leu	2.1
TP53	mutated	c.731G>T	p.Gly244Val	1.9
TP53	mutated	c.734G>A	p.Gly245Asp	4.6
TP53	mutated	c.745A>G	p.Arg249Gly	2.6
TP53	mutated	c.817C>T	p.Arg273Cys	1.7
TP53	mutated	c.997del	p.Arg333Valfs*12	2.5
U2AF1	mutated	c.101C>T	p.Ser34Phe	7.0
U2AF1	mutated	c.470A>C	p.Gln157Pro	9.8
U2AF1	mutated	c.470A>C	p.Gln157Pro	3.2
U2AF1	mutated	c.470A>G	p.Gln157Arg	1.6
U2AF1	mutated	c.470A>G	p.Gln157Arg	23.6
U2AF1	mutated	c.470A>G	p.Gln157Arg	4.6
U2AF1	mutated	c.470A>G	p.Gln157Arg	37.1
ZRSR2	mutated	c.1017del	p.Trp340Glyfs*?	20.9
ZRSR2	mutated	c.1141dup	p.Arg381Lysfs*4	16.9
ZRSR2	mutated	c.1223dup	p.His408Glnfs*20	5.7
ZRSR2	mutated	c.195_198del	p.Glu67Glyfs*10	1.8
ZRSR2	mutated	c.376C>T	p.Arg126*	17.5
ZRSR2	mutated	c.398_399del	p.Glu133Glyfs*11	8.3
ZRSR2	mutated	c.593del	p.Pro198Leufs*40	4.5
ZRSR2	mutated	c.706T>A	p.Phe236Ile	1.8
ZRSR2	mutated	c.80G>T	p.Arg27Leu	6.8
ZRSR2	mutated	c.83dup	p.Lys29Glnfs*26	4.6
ZRSR2	mutated	c.860_864delinsAAT	p.Phe287*	2.4
ZRSR2	mutated	c.988C>T	p.His330Tyr	1.6

**Supplemental Table 2** – List of 159 unique CHIP mutations identified in STARNET in the genes ASXL1, DNMT3A, JAK2 and TET2 based on whole-genome-sequencing with limited depth (35-fold). Provided are gene name, confirmation of CHIP mutation – polymorphisms, variants, synonymous and uncertain mutations were excluded, change on DNA level, change on amino acid (AA) level and variant allele frequency (VAF).

Gene	CHIP result	DNA result	AA result	VAF (%)
ASXL1	mutated	c.1211C>T	p.Arg404*	29.5
ASXL1	mutated	c.1550C>T	p.Gln517*	20.8
ASXL1	mutated	c.2238C>T	p.Gln575*	17.0
ASXL1	mutated	c.2331C>T	p.Arg606Trp	43.9
ASXL1	mutated	c.2364A>G	p.Ile617Val	42.9
ASXL1	mutated	c.2443G>T	p.Gly643Val	67.6
ASXL1	mutated	c.2469G>A	p.Gly652Ser	38.9
ASXL1	mutated	c.2469G>A	p.Gly652Ser	46.7
ASXL1	mutated	c.2469G>A	p.Gly652Ser	45.0
ASXL1	mutated	c.2469G>A	p.Gly652Ser	53.3
ASXL1	mutated	c.2469G>A	p.Gly652Ser	42.0
ASXL1	mutated	c.2469G>A	p.Gly652Ser	53.3
ASXL1	mutated	c.2469G>A	p.Gly652Ser	45.7
ASXL1	mutated	c.2469G>A	p.Gly652Ser	59.3
ASXL1	mutated	c.2469G>A	p.Gly652Ser	51.2
ASXL1	mutated	c.2469G>A	p.Gly652Ser	68.8
ASXL1	mutated	c.2469G>A	p.Gly652Ser	55.9
ASXL1	mutated	c.3223C>A	p.Ser903*	15.0
ASXL1	mutated	c.3598C>T	p.Ser1028Leu	72.7
ASXL1	mutated	c.3650G>C	p.Lys1045Asn	61.2
ASXL1	mutated	c.3732C>T	p.Arg1073Cys	28.8
ASXL1	mutated	c.4207C>T	p.Ser1231Phe	57.4
ASXL1	mutated	c.4260A>G	p.Met1249Val	31.5
ASXL1	mutated	c.4260A>G	p.Met1249Val	50.0
ASXL1	mutated	c.4260A>G	p.Met1249Val	43.2
ASXL1	mutated	c.4260A>G	p.Met1249Val	60.6
ASXL1	mutated	c.4260A>G	p.Met1249Val	48.1
ASXL1	mutated	c.4260A>G	p.Met1249Val	56.7
ASXL1	mutated	c.4260A>G	p.Met1249Val	52.7
ASXL1	mutated	c.4260A>G	p.Met1249Val	53.2
ASXL1	mutated	c.4260A>G	p.Met1249Val	47.4
ASXL1	mutated	c.4260A>G	p.Met1249Val	47.5
ASXL1	mutated	c.4260A>G	p.Met1249Val	55.4
ASXL1	mutated	c.4260A>G	p.Met1249Val	60.0
ASXL1	mutated	c.4260A>G	p.Met1249Val	39.0
ASXL1	mutated	c.4260A>G	p.Met1249Val	53.1

ASXL1	mutated	c.4260A>G	p.Met1249Val	50.0
ASXL1	mutated	c.4260A>G	p.Met1249Val	63.3
ASXL1	mutated	c.4630C>G	p.Thr1372Ser	60.8
ASXL1	mutated	c.4630C>G	p.Thr1372Ser	41.2
ASXL1	mutated	c.4630C>G	p.Thr1372Ser	46.3
ASXL1	mutated	c.4698C>G	p.Leu1395Val	40.4
ASXL1	mutated	c.4698C>G	p.Leu1395Val	45.7
ASXL1	mutated	c.4704G>A	p.Gly1397Ser	50.8
ASXL1	mutated	c.4890A>G	p.Ser1459Gly	47.5
ASXL1	mutated	c.4894C>T	p.Ser1460Phe	48.8
ASXL1	mutated	c.4980T>C	p.Ser1489Pro	35.6
ASXL1	mutated	c.5137G>A	p.Arg1541Lys	40.5
ASXL1	mutated	c.9387T>C	p.Val295Ala	48.5
DNMT3A	mutated	c.0498G>A	p.Arg326Cys	20.0
DNMT3A	mutated	c.0516G>A	p.Arg320*	36.5
DNMT3A	mutated	c.0555G>A	p.Pro307Ser	34.1
DNMT3A	mutated	c.0597C>A	p.Gly293Trp	60.0
DNMT3A	mutated	c.2050G>C	p.Ser786*	50.0
DNMT3A	mutated	c.2050G>A	p.Ser786Leu	50.0
DNMT3A	mutated	c.3054G>A	p.Thr44Met	55.8
DNMT3A	mutated	c.3568A>G	p.Ile705Thr	16.7
DNMT3A	mutated	c.4543A>G	p.Val657Ala	25.5
DNMT3A	mutated	c.7140C>T	p.Asp579Asn	15.6
DNMT3A	mutated	c.7290C>T	p.splice site mutation	20.5
DNMT3A	mutated	c.7290C>G	p.splice site mutation	20.5
DNMT3A	mutated	c.7476G>A	p.Gln534*	36.4
DNMT3A	mutated	c.8382G>C	p.Ser160Cys	46.6
DNMT3A	mutated	c.8648T>C	p.Gln842Arg	44.4
DNMT3A	mutated	c.9073G>A	p.Ala462Val	43.5
DNMT3A	mutated	c.9073G>A	p.Ala462Val	62.7
DNMT3A	mutated	c.9098C>A	p.Ala454Ser	14.8
DNMT3A	mutated	c.9529insC	p.Gly413fs*	22.9
DNMT3A	mutated	c.9529insC	p.Gly413fs*	13.1
DNMT3A	mutated	c.9529delG*	p.Gly413fs*	12.0
JAK2	mutated	c.0232T>C	p.Leu712Pro	46.5
JAK2	mutated	c.1803A>G	p.Asp838Gly	47.7
JAK2	mutated	c.1828G>C	p.Glu846Asp	50.0
JAK2	mutated	c.1828G>C	p.Glu846Asp	53.8
JAK2	mutated	c.1828G>C	p.Glu846Asp	46.5
JAK2	mutated	c.1828G>C	p.Glu846Asp	61.4
JAK2	mutated	c.1828G>C	p.Glu846Asp	48.6
JAK2	mutated	c.1828G>C	p.Glu846Asp	48.6
JAK2	mutated	c.1828G>C	p.Glu846Asp	50.9

JAK2	mutated	c.2130G>A	p.Gly48Leu	54.2
JAK2	mutated	c.2561G>A	p.Gly571Ser	66.7
JAK2	mutated	c.2561G>A	p.Gly571Ser	41.5
JAK2	mutated	c.2561G>A	p.Gly571Ser	47.7
JAK2	mutated	c.2576A>G	p.Thr576Ala	43.8
JAK2	mutated	c.2576A>G	p.Thr576Ala	68.8
JAK2	mutated	c.3770G>T	p.Val617Phe	32.6
JAK2	mutated	c.3770G>T	p.Val617Phe	12.5
JAK2	mutated	c.3770G>T	p.Val617Phe	34.1
JAK2	mutated	c.4997G>C	p.Ala391Pro	56.4
JAK2	mutated	c.6715A>G	p.Asn1108Ser	51.6
JAK2	mutated	c.6715A>G	p.Asn1108Ser	55.3
JAK2	mutated	c.6715A>G	p.Asn1108Ser	54.5
JAK2	mutated	c.6776C>G	p.Thr438Ser	57.1
JAK2	mutated	c.7524A>C	p.Asn646His	44.1
JAK2	mutated	c.7569A>G	p.Met661Val	51.5
JAK2	mutated	c.9976C>G	p.Thr522Ser	63.3
TET2	mutated	c.0785C>A	p.Cys1271*	30.8
TET2	mutated	c.0798G>A	p.Arg1359His	36.5
TET2	mutated	c.0843G>T	p.Cys1374Phe	21.6
TET2	mutated	c.0852T>C	p.Tyr1294His	50.0
TET2	mutated	c.4880G>A	p.Glu1250Lys	64.5
TET2	mutated	c.4897C>G	p.Tyr1255*	16.3
TET2	mutated	c.5620C>A	p.Pro14His	46.7
TET2	mutated	c.5620C>A	p.Pro14His	61.5
TET2	mutated	c.5620C>A	p.Pro14His	48.8
TET2	mutated	c.5620C>A	p.Pro14His	54.7
TET2	mutated	c.5620C>A	p.Pro14His	59.6
TET2	mutated	c.5620C>A	p.Pro14His	43.2
TET2	mutated	c.5620C>A	p.Pro14His	47.6
TET2	mutated	c.5620C>A	p.Pro14His	47.4
TET2	mutated	c.5620C>A	p.Pro14His	54.2
TET2	mutated	c.5620C>A	p.Pro14His	42.9
TET2	mutated	c.5620C>A	p.Pro14His	45.5
TET2	mutated	c.5620C>A	p.Pro14His	45.9
TET2	mutated	c.5620C>A	p.Pro14His	64.3
TET2	mutated	c.5620C>A	p.Pro14His	81.1
TET2	mutated	c.5620C>A	p.Pro14His	51.8
TET2	mutated	c.5620C>A	p.Pro14His	53.1
TET2	mutated	c.5800A>G	p.Tyr234Cys	49.0
TET2	mutated	c.5843C>A	p.His248Gln	38.6
TET2	mutated	c.5843C>A	p.His248Gln	57.5
TET2	mutated	c.5890T>A	p.Ile264Asn	51.7

TET2	mutated	c.6209T>G	p.Tyr370*	35.9
TET2	mutated	c.6213C>T	p.Arg1516*	19.6
TET2	mutated	c.6384G>A	p.Gly429Arg	47.2
TET2	mutated	c.6384G>A	p.Gly429Arg	57.5
TET2	mutated	c.6770G>A	p.Met1701Ile	50.0
TET2	mutated	c.6770G>A	p.Met1701Ile	64.9
TET2	mutated	c.6770G>A	p.Met1701Ile	50.0
TET2	mutated	c.6770G>A	p.Met1701Ile	45.5
TET2	mutated	c.6770G>A	p.Met1701Ile	58.1
TET2	mutated	c.6770G>A	p.Met1701Ile	40.0
TET2	mutated	c.6770G>A	p.Met1701Ile	44.7
TET2	mutated	c.6770G>A	p.Met1701Ile	42.5
TET2	mutated	c.6770G>A	p.Met1701Ile	60.5
TET2	mutated	c.6819C>G	p.Gln574Glu	28.3
TET2	mutated	c.6819G>T	p.Val1718Leu	56.4
TET2	mutated	c.6819G>T	p.Val1718Leu	56.0
TET2	mutated	c.6819G>T	p.Val1718Leu	51.2
TET2	mutated	c.6834C>T	p.Pro1723Ser	44.9
TET2	mutated	c.6834C>T	p.Pro1723Ser	52.6
TET2	mutated	c.6834C>T	p.Pro1723Ser	64.3
TET2	mutated	c.6834C>T	p.Pro1723Ser	48.8
TET2	mutated	c.6834C>T	p.Pro1723Ser	48.6
TET2	mutated	c.6834C>T	p.Pro1723Ser	60.4
TET2	mutated	c.7243G>GGTAA	p.splice site mutation	43.8
TET2	mutated	c.7434G>T	p.Glu1923*	51.4
TET2	mutated	c.7600T>C	p.Val1978Ala	59.1
TET2	mutated	c.7600T>C	p.Val1978Ala	46.5
TET2	mutated	c.7698T>C	p.Tyr867His	59.1
TET2	mutated	c.7698T>C	p.Tyr867His	52.9
TET2	mutated	c.7698T>C	p.Tyr867His	42.5
TET2	mutated	c.7698T>C	p.Tyr867His	50.0
TET2	mutated	c.7698T>C	p.Tyr867His	57.4
TET2	mutated	c.7698T>C	p.Tyr867His	50.0
TET2	mutated	c.7703T>G	p.Phe868Leu	43.2
TET2	mutated	c.7785C>A	p.Leu896Ile	55.7
TET2	mutated	c.8332C>T	p.Thr1078Ile	49.1
TET2	mutated	c.8350A>C	p.Gln1084Pro	56.3

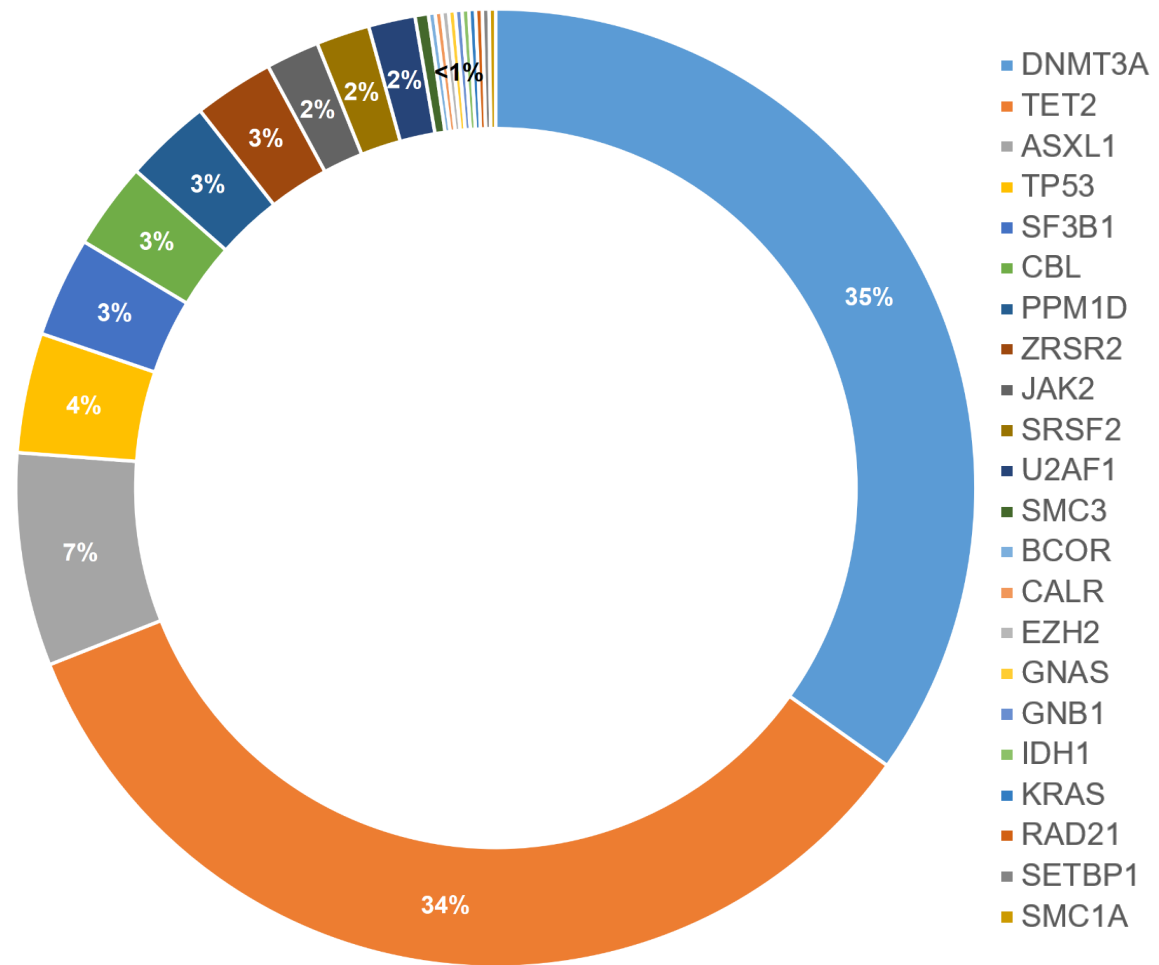
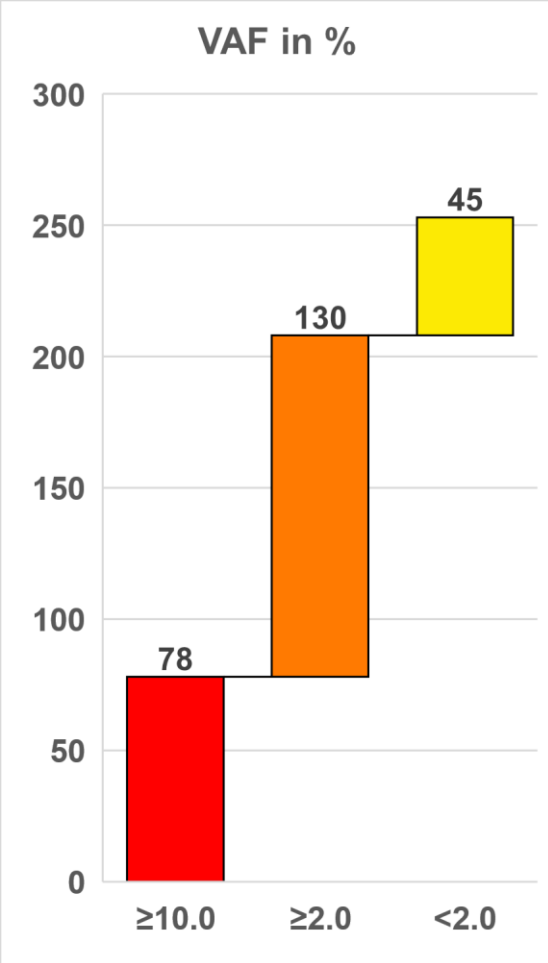
**Supplemental Table 3** – Patient characteristics of TET2 mutation carriers with CHIP-affected macrophages and controls without CHIP mutation based on STARNET. Matching between cases and controls was based on age and sex. Cardiovascular-relevant phenotype data are indicated. \*: p<0.05.

<b>Baseline characteristics</b>	<b>TET2 CHIP (n=3)</b>	<b>TET2 non-CHIP (=21)</b>	<b>p-value</b>
Age, mean (sd)	60.3 (12.0)	61.8 (8.2)	0.82
Sex (male)	3	21	1
BMI, mean (sd)	28.0 (4.9)	28.6 (5.0)	0.86
Arterial hypertension	2	14 (n=20)	1
Hypercholesterolemia	0	14 (n=20)	<0.05*
Smoking (ever)	2	7 (n=20)	0.54
Diabetes	0	4 (n=20)	1
Prior myocardial infarction	1	7 (n=20)	1
Prior stroke	0	0 (n=20)	1

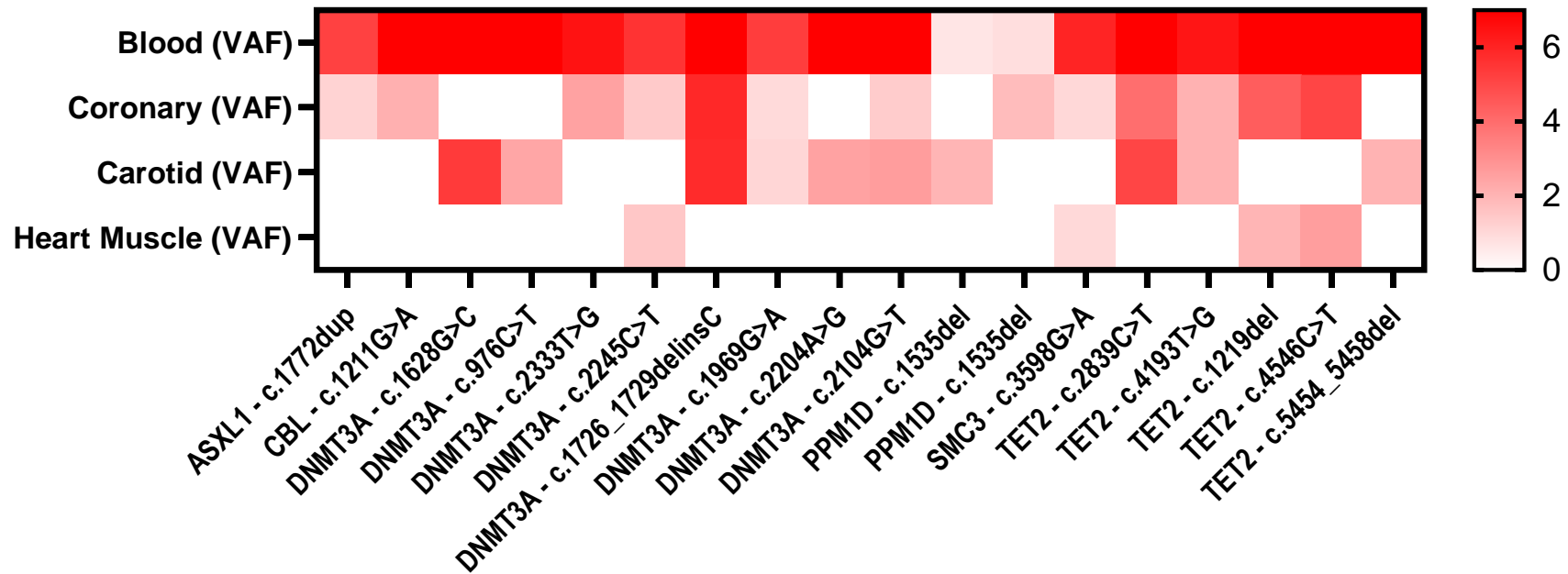


**Supplemental Table 4** – Patient characteristics of ASXL1 mutation carriers with CHIP-affected macrophages and controls without CHIP mutation based on STARNET. Matching between cases and controls was based on age and sex. Cardiovascular-relevant phenotype data are indicated. \*: p<0.05.

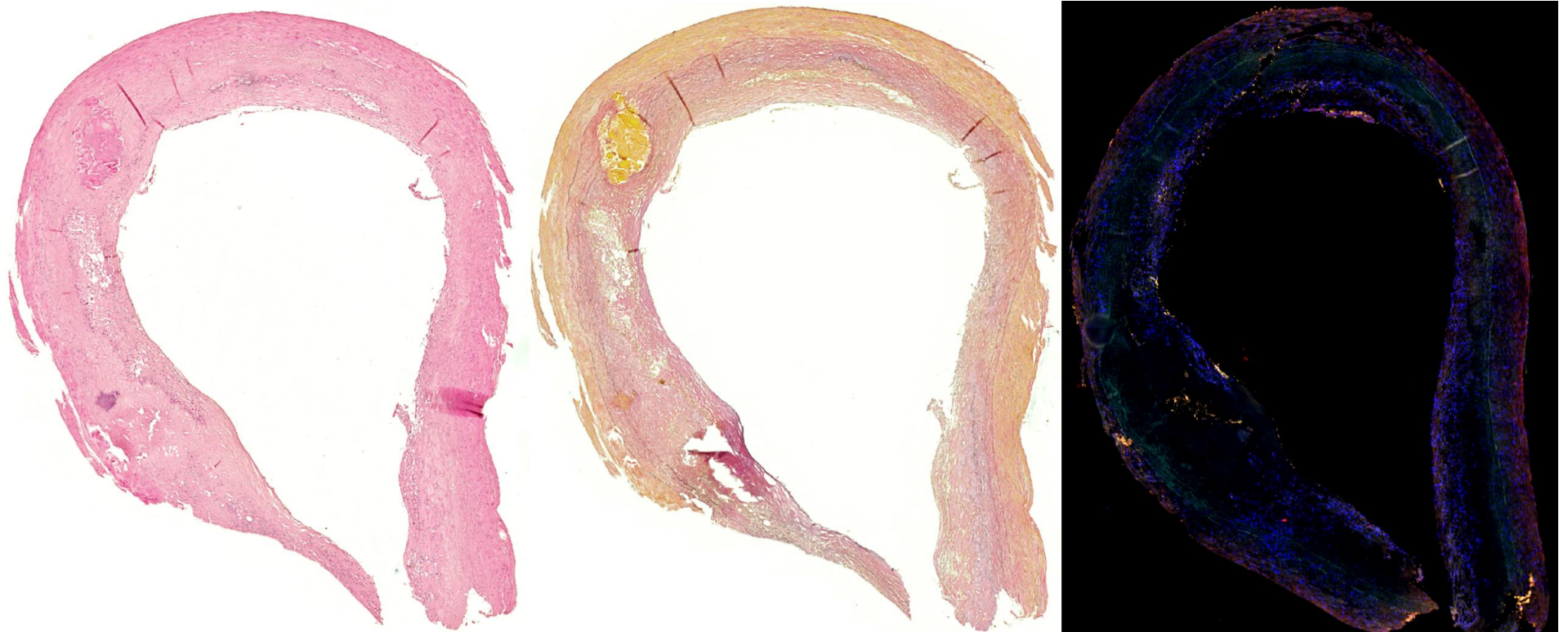
<b>Baseline characteristics</b>	<b>ASXL1 CHIP (n=3)</b>	<b>ASXL1 non-CHIP (n=27)</b>	<b>p-value</b>
Age, mean (sd)	67.3 (5.5)	67.3 (5.6)	0.98
Sex (male)	3	27	1
BMI, mean (sd)	26.4 (0.9)	29.3 (3.6)	<0.05*
Arterial hypertension	1	23 (n=26)	0.07
Hypercholesterolemia	2	14 (n=26)	1
Smoking (ever)	1	4 (n=26)	0.45
Diabetes	0	4 (n=26)	1
Prior myocardial infarction	1	10 (n=26)	1
Prior stroke	0	4 (n=26)	1



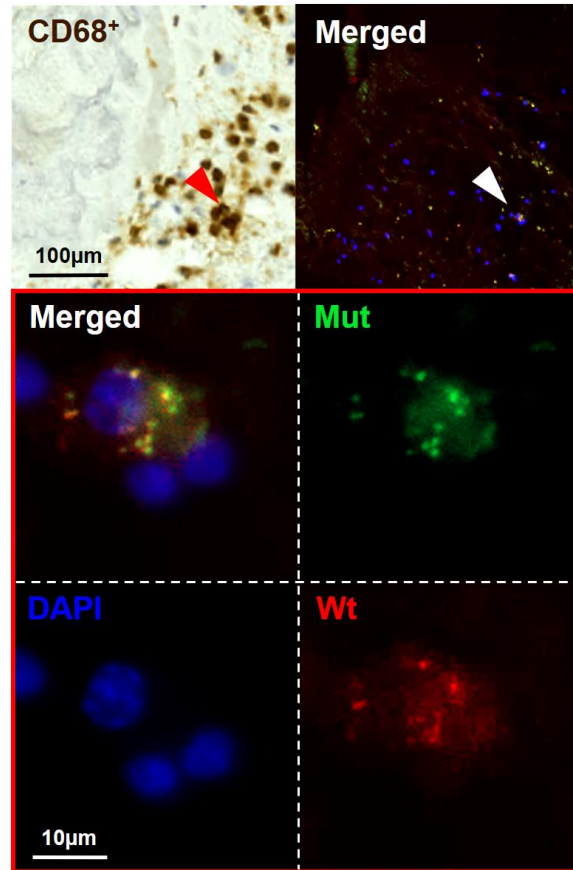
**Supplemental Figure 1** – VAF and distribution of CHIP mutations in MISSION. The left panel shows the distribution of VAF in 253 CHIP mutation carriers. For individuals with multiple CHIP mutations, the mutation with the highest VAF was considered. The right panel shows the percentage of genes affected by CHIP mutations. All 445 mutations identified by the 56-gene or 13-gene panel (Material and Methods Section) were considered. The color coding of the individual genes is indicated on the right.



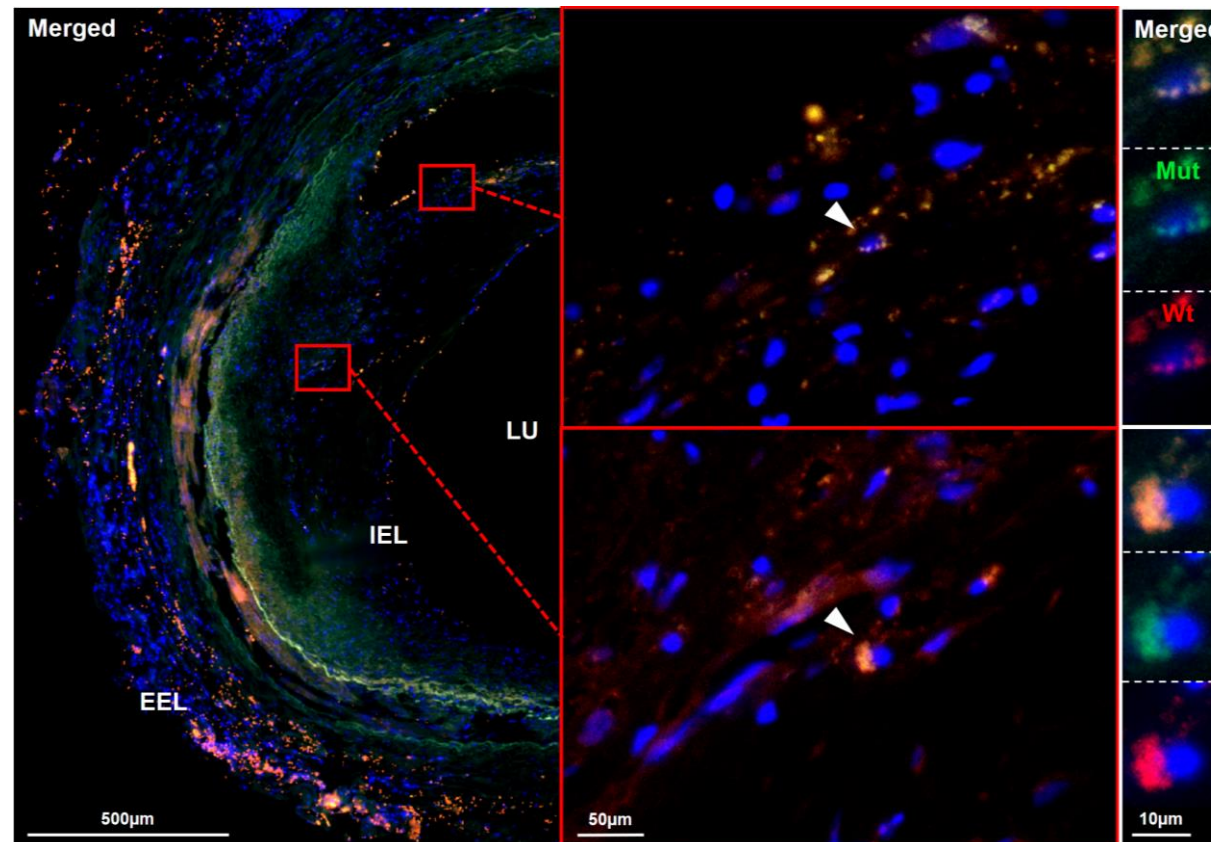
**Supplemental Figure 2** – DeepDNaseq identifies CHIP mutations in atherosclerotic coronary and carotid samples, and left ventricular myocardium. To confirm the identified CHIP mutations from whole blood in cardiovascular relevant tissues at the DNA level, coronary artery and carotid artery affected by atherosclerosis as well as myocardium of the left ventricle of CHIP mutation carriers were examined. The representation is in the format of a heat map. VAF >6 is presented in dark red. Tissues without mutation evidence are presented in white.



**Supplemental Figure 3** – Overview plaque of interest – different stainings. Coronary arteries and carotids in MISSION are processed in a standardized fashion using different staining methods to enable optimal histological characterization. As an example, an overview of a proximal LAD affected by atherosclerosis is shown here of a CHIP mutation carrier. The three images have been prepared from three immediate follow-up sections. **Left panel** - staining with HE. **Middle panel** - staining with EvG. **Right panel** - mutaFISH™ to screen for specific CHIP mutations.



**Supplemental Figure 4** – Visualization of a single CHIP affected macrophage in the shoulder region of a human atherosclerotic plaque. **Upper panel** – the area of interest in this atherosclerotic plaque is stained for CD68<sup>+</sup> macrophages. Using mutaFISH™, staining for the specific DNMT3A mutation (Mut) c.2333T>G and wild type (Wt) at the RNA level was performed *in situ* in this atherosclerotic plaque of human FFPE tissue. **Lower panel** – provided are the merged and individual signals on single cell resolution. The DNMT3A mutation c.2333G>T was detected via the green signal (Mut), the DNMT3A wild type via the red (Wt) signal and the cell nuclei (DAPI) via the blue signal. DAPI: 4',6-Diamidin-2-phenylindole. mutaFISH: mutation-specific Fluorescence In Situ Hybridization.



**Supplemental Figure 5** – DNMT3A CHIP mutation (c.2245C>T) in human atherosclerotic plaques. Staining for the specific DNMT3A mutation (Mut) c.2245C>T and wild type (Wt) at the RNA level was performed *in situ* in this advance atherosclerotic plaque of human FFPE tissue. **Left panel** – merged overview of the left part of the atherosclerotic plaque. Red boxes highlight the areas of interest. **Middle panel** – CHIP carrying macrophages are highlighted with white arrows. **Right panel** – provided are the merged and individual signals on single cell resolution. The DNMTA3 mutation c.2245C>T was detected via the green signal (Mut), the DNMT3A wild type via the red (Wt) signal and the cell nuclei (DAPI) via the blue signal. DAPI: 4',6-Diamidin-2-phenylindole; EEL: external elastic lamina; FFPE: formalin-fixed paraffin embedded; IEL: internal elastic lamina; LU: lumen; mutaFISH: mutation-specific Fluorescence In Situ Hybridization.

## Adapted mutaFISH™ protocol

### Used Kits

- mutaFISH RNA Probes KIT (Abnova Corporation, Taiwan)
- mutaFISH™ RNA Accessory KIT (KA4915, Abnova Corporation, Taiwan)

### Preparation of RNase free buffers

All buffers for the mutaFISH protocol have to be nuclease free.

- **Poly-L-Lysine 1:10:** Prepare 1:10 Poly-L-Lysine in ddH<sub>2</sub>O.
- **DEPC H<sub>2</sub>O:** Use 1 ml DEPC for 1000 ml ddH<sub>2</sub>O incubate for 1h at RT and autoclave.
- **DEPC PBS\*:** Use 1 ml DEPC for 1000 ml PBS pH 7.4 incubate for 1h at RT and autoclave.
- **PBST\*:** Use autoclaved DEPC PBS and add 1 ml Tween20 after autoclaving. If you use ready-to-use PBST Use DEPC and filter after 1h of incubation at RT.
- **Permeable Protease buffer:** Use 3mg/ml Pepsin to 0.5 M HCL.
- **Nuclease Free 1x Citric acid buffer pH6:** dilute nuclease free 10x ready-to-use Citric acid buffer in DEPC H<sub>2</sub>O or prepare 1x nuclease free buffer pH6.
- **2x SSC Buffer\*\*:** Dilute ready-to-use nuclease free 20x SSC buffer in DEPC H<sub>2</sub>O or prepare 2x nuclease free buffer.
- **70% and 85% EtOH:** Dilute EtOH absolute to 70% and 85%.
- **3-4% Paraformaldehyde:** Paraformaldehyde solution has to prepared methanol free. Dilute in DEPC-PBS

\* here Roti@fair PBST 7.4 and Roti@ PBS 7.4 (CarlRoth GmbH&CoKG, Karlsruhe) were used

\*\* here here Roti@-Stock 20x SSC (CarlRoth GmbH&CoKG, Karlsruhe) was used

### Protocol – Coating with Poly-L-Lysine

1. Let Poly-L-Lysine (1:10 in ddH<sub>2</sub>O) come to room temperature.
2. Incubate Slides 7min at RT in **1:10 Poly-L-Lysine** for coating.
3. Remove slides from the rack and tap off water droplets.
4. Incubate slides at 56 °C for at least 1h.

### Protocol – mutaFISH

#### Tissue preparation

1. Prepare 3-5 µm thick FFPE sections, air dry sections at heating plate (40 °C).
2. Incubate FFPE sections for 1h at 56 °C.

#### Deparaffinization and rehydration

1. Rinse slides 2 times in xylene substitute for each 5 min.
2. Immerse slides 2 times in 100% EtOH for each 3 min.
3. Immerse slides 2 times in 85% EtOH for each 3 min.
4. Immerse slides 2 times in 70% EtOH for each 3 min.
5. Wash slides in DEPC-H<sub>2</sub>O for 1 min and dry shortly at RT.

#### Target retrieval

1. Preheat heating plate to 75 °C.
2. Create secure bond with wax pen around the tissue sections.
3. Wash slides in DEPC-PBS for 2 min
4. Incubate slides with 1x citric buffer at 75-85 °C on heating plate for 20 min.  
*CAVE: Renew citric buffer every 10-15 min – sample should not dry out.*

5. Wash twice with 2x SSC buffer for 5 min.

*CAVE: If costaining with an antibody should be done perform permeabilization and immunostaining prior to metaFISH and end up with 20 min fixation in 4% formaldehyde at RT.*

#### Fixiation and permeabilization

1. Incubate in 3-4% paraformaldehyde (provided) for 20 min at RT.
2. Immerse slides in 2x SSC buffer for 5 min.
3. Prewarm permeable buffer (provided in the kit or self-made) to 37 °C.
4. Use permeable buffer or 3 mg/mL Pepsin to 0.1 M HCl) at 37 °C for 30 min.  
*TIPP: RNAscope®\* Protease III & Protease IV<sup>3</sup> reagents can be used. Here no prewarming is necessary.*
5. Wash slides in 2x SSC buffer for 5 min.

\* RNAscope® Protease IV (Advanced Cell Diagnostics, Inc., Canada) was used in this case

#### Dehydration

1. Immerse slides in 70% EtOH for 1 min.
2. Immerse slides in 85% EtOH for 1 min.
3. Immerse slides in 100% EtOH for 1 min.
4. Immerse slides in fresh PBST for 1 min.

#### In situ reverse transcription

1. prepare the following mixture on ice and use 100 µl of the mixture per slide.

<b>Component</b>	<b>Amount per slide [µL]</b>
DEPC-H <sub>2</sub> O (Kit)	60.5
5x RT Buffer (Kit)	20.0
BSA (Kit)	1.0
dNTP Mix (Kit)	5.0
<b>RT Primer (individual)</b>	1.0
RNase Inhibitor (Kit)	2.5
RT Enzyme (Kit)	10.0
<b>Total volume:</b>	<b>100.0</b>

2. Incubate Slides at 37 °C in humidity oven over night.  
*CAVE: Take care that there is enough humidity. Slides should not dry out.*
3. Wash shortly in PBST.
4. Immerse slides with fresh PBST 2 times for each 2 min.

#### Postfixiation and probe hybridization

1. Cover tissue with 3-4% paraformaldehyde (provided) and incubate at 37 °C in humidity Box for 45 min.  
*CAVE: Check formaldehyde every 10-15 min – sample should not dry out.*
2. Wash slides with fresh PBST 2 times for 2 min.
3. prepare the following mixture on ice and use 100µl per slide.

<b>Component</b>	<b>Amount per slide [µL]</b>	<b>Amount for negative control [µl]</b>
DEPC-H <sub>2</sub> O (Kit)	32.5	34.5
Formamide (Kit)	20.0	20.0
10x Hybrid Enzyme Buffer (Kit)	10.0	10.0
1 M KCl (Kit)	5.0	5.0



<b>mutaFISH probe wt (individual)</b>	1.0	
<b>mutaFISH probe mutation (individual)</b>	1.0	
RNase Inhibitor (Kit)	2.5	2.5
RNaseH (Kit)	8.0	8.0
Hybrid Enzyme (Kit)	20.0	20.0
<b>Total volume:</b>	<b>100.0</b>	<b>200.0</b>

4. Incubate at 37 °C in humidity oven for 60 min.
5. Heat up to 45 °C and incubate slides for another 90 min.
6. Immerse slide with fresh PBST 2 times for 2 min.

#### Amplification

1. Prepare the following mixture on ice and use 100 µl per slide.

<b>Component</b>	<b>Amount per slide [µL]</b>
DEPC-H2O (Kit)	61.5
50% Glycerol (Kit)	10.0
10x DNA Polymerase Buffer (Kit)	10.0
BSA (Kit)	1.0
dNTP mix (Kit)	5.0
RNase Inhibitor (Kit)	2.5
DNA Polymerase (Kit)	10.0
<b>Total volume:</b>	<b>100.0</b>

2. Incubate slides at 37 °C in humidity oven for 120 min.
3. Wash shortly in PBST.
4. Immerse slide with fresh PBST two times for 1 min.

#### Detection and counterstain

1. Use 100 µl of the following mixture per slide (prepare on ice).

<b>Component</b>	<b>Amount per slide [µL]</b>
Detection Buffer (Kit)	98.0
<b>Detection probe for wt (individual)</b>	1.0
<b>Detection probe for mutation (individual)</b>	1.0
<b>Total volume:</b>	<b>100.0</b>

2. Incubate at 37 °C in humidity oven for 60 min.
3. Wash shortly in PBST.
4. Immerse slide with fresh PBST 2 times for 2 min.
5. Immerse the slide in 70% EtOH for 0.5 min.
6. Immerse the slide in 85% EtOH for 0.5 min.
7. Immerse the slide in 100% EtOH for 0.5 min.
8. Mix 4 µl DAPI with 664 µl DEPC-PBS and apply 100 µl to the sample for 2-3 min at RT.

#### Sealing

- Immerse slide two times in fresh DEPC-PBS for 1 min.
- Cover slide with Prolong-Gold-Anti-Fade let it dry for 15 min and seal with nail-polish.
- Let dry slides for 1h and proceed with microscopy.