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## Supplemental Information

# Germline *PIK3CA* and *AKT1* Mutations in Cowden and Cowden-like Syndromes

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### Supplemental Inventory

### 1. Supplemental Figures and Tables

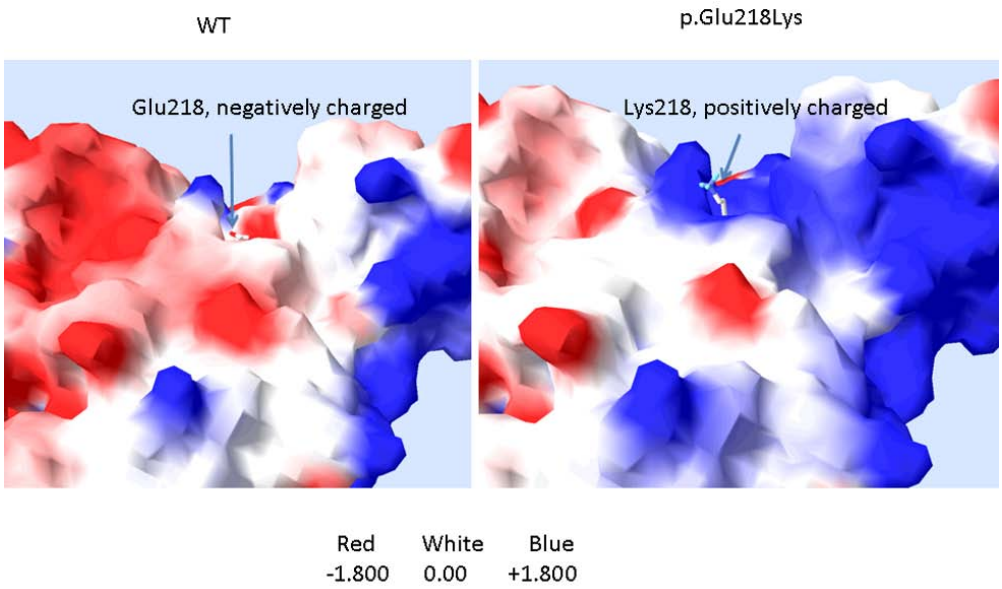


**Figure S1. Three-Dimensional Modeling of PIK3CA–Glu218Lys**

p.Glu218Lys change alters the overall surface charge of the area from negative (red) to positive charge (blue)

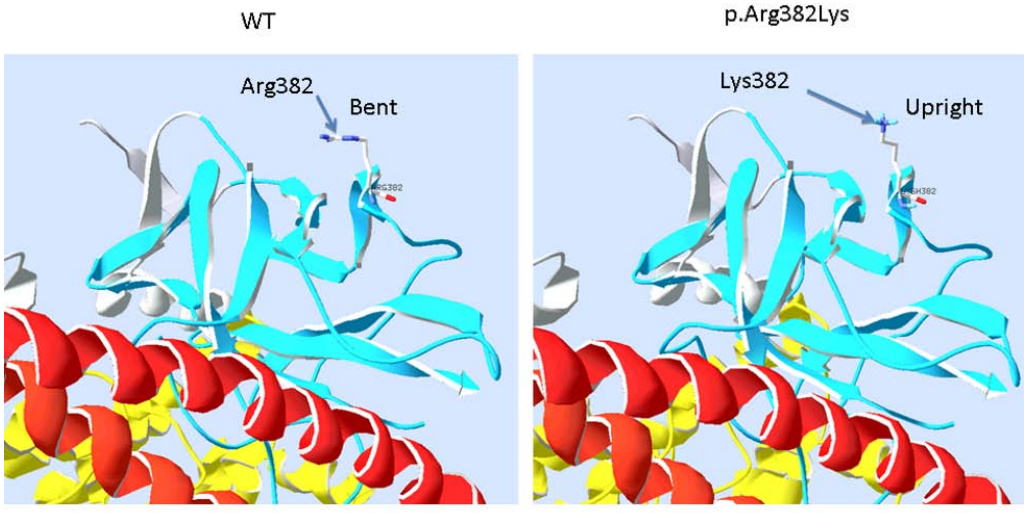


**Figure S2. Three-Dimensional Modeling of PIK3CA–Arg382Lys Mutation**

p.Arg382Lys change exposes its positive charge upright on the C2 domain, which may alter the protein or lipid layer binding

**Table 1. List of Primers Designed for *AKT1, PIK3CA, PIK3R1* and *PIK3R2***

| Gene | Primer Name | Primers 5--->3 |
|---|---|---|
| **AKT1** | Exon 1-Forward | TGGGGGTCAGAGAGCTTAGA |
| | Exon 1-Reverse | ACCAGGAAGCCACTCAGATG |
| | Exon 2-Forward | TGTCCATGGTACTCCATCCC |
| | Exon 2-Reverse | CAGCCAGTGCTTGTTGCTT |
| | Exon 3-Forward | CGAGAAACTGAGGCTTGGAG |
| | Exon 3-Reverse | GTTTCCAAACTGGGCTCTGA |
| | Exon 4-Forward | TGACCCTGAGTGTATGTGGC |
| | Exon 4-Reverse | CTACATGGAAAACCGGCCTA |
| | Exon 5-Forward | GTCCTCTTCCCCATGTCAGA |
| | Exon 5-Reverse | AGTCCACGGTGTGTAAAGCC |
| | Exon 6-Forward | GGTGATCCTGGTGAAGGAGA |
| | Exon 6-Reverse | AGTCCACGGTGTGTAAAGCC |
| | Exon 7-Forward | GGTATCAGGCGACGTGGTAT |
| | Exon 7-Reverse | AGGAACAAGTCACCCCACAG |
| | Exon 8-Forward | CTGTGGGGTGACTTGTTCCT |
| | Exon 8-Reverse | CTTAATGTGCCCGTCCTTGT |
| | Exon 9-Forward | TCTATGGCGCTGAGATTGTG |
| | Exon 9-Reverse | CATCTCGTACATGACCACGC |
| | Exon 10-Forward | ACAAGGACGGGCACATTAAG |
| | Exon 10-Reverse | CCTGAGGCTTTGGAGATCAG |
| | Exon 11-Forward | GCCCTACATCACAGGAGGAA |
| | Exon 11-Reverse | AGGGGAGGAGGAAACTCAGA |
| | Exon 12-Forward | CTGTTGAGGGTTGTCTCCGT |
| | Exon 12-Reverse | ACAGCTCCAGTAGGAAGCCA |
| | Exon 13-Forward | TGGCTTCCTACTGGAGCTGT |
| | Exon 13-Reverse | CTCAAATGCACCCGAGAAAT |
| **PIK3CA** | Exon 1-Forward | TGCTTTGGGACAACCATACA |
| | Exon 1-Reverse | AGAGCAAAGGCAGCAAACAT |
| | Exon 2-Forward | ATGTTTGCTGCCTTTGCTCT |
| | Exon 2-Reverse | ATAAGCAGTCCCTGCCTTCA |
| | Exon 3-Forward | GCAGCCCGCTCAGATATAAA |
| | Exon 3-Reverse | CATGGTGCAAAACCTGTCTC |
| | Exon 4-Forward | TTTTTGCCTCCAGTTAAGGG |
| | Exon 4-Reverse | AGGTCTTTCTGCCAAACGAA |
| | Exon 5-Forward | TAAGGGGATTGTGGGCCTAT |
| | Exon 5-Reverse | CTGGCCAGTGCCTAGCTAAT |
| | Exon 6-Forward | CCCTTGGCATCATCAGTCTT |
| | Exon 6-Reverse | TGAACCAAAGCAAGCATGAG |
| | Exon 7-Forward | CTCATGCTTGCTTTGGTTCA |
| | Exon 7-Reverse | TTGGCATGCTCTTCAATCAC |
| | Exon 8-Forward | AAGGAACACTGTCCATTGGC |
| | Exon 8-Reverse | TAGTGCCTTTCCCAGTGCTT |
| | Exon 9-Forward | CTGTGAATCCAGAGGGGAAA |
| | Exon 9-Reverse | TGCTGAGATCAGCCAAATTC |
| | Exon 10-Forward | GGGGTTCCCTTTCATTTTTC |

| | | |
|---|---|---|
| | Exon 10-Reverse | GGCCAATCTTTTACCAAGCA |
| | Exon 11-Forward | GGCAGTGTTTTAGATGGCTCA |
| | Exon 11-Reverse | CAAATCAGGGTCAGTTTCTGC |
| | Exon 12-Forward | CATGCAGAAACTGACCCTGA |
| | Exon 12-Reverse | GGAAAACTCTTCCAGCCAAA |
| | Exon 13-Forward | CAGGAACTACCTGAAACTCATGG |
| | Exon 13-Reverse | TTGGCCACCTTCTATGTTCC |
| | Exon 14-Forward | CCCTGCTCTACAGCCAAAAG |
| | Exon 14-Reverse | TGAGGGTAGGAGAATGAGAGAGA |
| | Exon 15-Forward | TGGATGCTCTACAGGGCTTT |
| | Exon 15-Reverse | AGAGGGCAGGGATTCTGTTT |
| | Exon 16-Forward | CATGCTTGTGATCCCAGTTG |
| | Exon 16-Reverse | AATGGTGACACTCCAGAGGC |
| | Exon 17-Forward | GGGGAAAGGCAGTAAAGGTC |
| | Exon 17-Reverse | CACAAACACCGACAGACTCA |
| | Exon 18-Forward | AAATGGAAACTTGCACCCTG |
| | Exon 18-Reverse | GTCAAAACAAATGGCACACG |
| | Exon 19-Forward | TCATGGTGAAAGACGATGGA |
| | Exon 19-Reverse | GCTGGTTTCAATTCCTGAGC |
| | Exon 20-Forward | CAGGCATTGTTGTAGGTGCT |
| **PIK3R1** | Exon1-Forward | GCCAGTCACCTCTCCTCTTAAA |
| | Exon1-Reverse | CAGAACATAACGACTCAACCCA |
| | Exon2-Forward | AGACCCTAGGTGAGAAGCAGTG |
| | Exon2-Reverse | AACAGTTTGAGTGTCCAGCAGA |
| | Exon3-Forward | ACTGGATGGAAACTGGAATGTC |
| | Exon3-Reverse | CTGAAACAGAAAGCCAAGACCT |
| | Exon4-Forward | GAGACCTCCACAGGAAAAATTG |
| | Exon4-Reverse | GCAAATGAGAATCTGGGTCTTC |
| | Exon5-Forward | GTACTGTGTGCTTCTCCCAACA |
| | Exon5-Reverse | TACCCTCATCACCCCAAAATAC |
| | Exon6-Forward | AGATTCTCAGCAGCCAGGTAAG |
| | Exon6-Reverse | TTGAAGGGTACAGGGAACTCTC |
| | Exon7-Forward | GTAGTTGGGATTGCGAACAACT |
| | Exon7-Reverse | ACTTAGCAAGCTGGTGCTTTTC |
| | Exon8-Forward | ATCTATGTGGGCAGGAGGAATA |
| | Exon8-Reverse | CAGAGAAGCCATATTTCCCATC |
| | Exon9-Forward | GCGTCTACTAAAATGCATGGTG |
| | Exon9-Reverse | GGGATGTGCGGGTATATTCTT |
| | Exon10-Forward | CCAGTATCCAAATACCAACAGG |
| | Exon10-Reverse | CCCACCTCATTCGTAAAAACTC |
| | Exon11-Forward | CTGGTCACTCATGTATCTGGGA |
| | Exon11-Reverse | CAGAGTGATATTCCCCTTCCTG |
| | Exon12-Forward | GAAAACTGCTGGGAAACCATAG |
| | Exon12-Reverse | ACACCTTTTTGAGTCAACCACC |
| | Exon13-Forward | ATCCAGCTGAGAAAGACGAGAG |
| | Exon13-Reverse | GGGACATCATTATGGACACAGA |
| | Exon14-Forward | AGGTACCTGAGTGTGGTTGCTT |

| | | |
|---|---|---|
| | Exon14-Reverse | ACAGCTGCTTTGGTTTCTCTTC |
| | Exon15-Forward | CCCAAGTTGAGACTGCACAATA |
| | Exon15-Reverse | TCACAGATCAGACTGGAGAGGA |
| **PIK3R2** | Exon1-Forward | CCCTTGTAAGGGTCATGGAATA |
| | Exon1-Reverse | ACTCTCATCCCTTCTTGAACCA |
| | Exon2-Forward | CTAACCCAGAAAATTGTGTCCC |
| | Exon2-Reverse | CCTATTCAACATAGAGGCCCAG |
| | Exon3-Forward | GCCTCTATGTTGAATAGGGCAC |
| | Exon3-Reverse | ACGTCCAACTCATGTGTACTGG |
| | Exon4-Forward | GCCTCTATGTTGAATAGGGCAC |
| | Exon4-Reverse | CCCCTCCCTTCTTAGGGTCTA |
| | Exon5-Forward | CAGTACACATGAGTTGGACGTG |
| | Exon5-Reverse | CATAGATTCTCACACAAAGGCG |
| | Exon6-Forward | GTGCCTGTATCATCTCCTCCTC |
| | Exon6-Reverse | CTCAGGGATCAGTATTTACGGC |
| | Exon7-Forward | GCCGTAAATACTGATCCCTGAG |
| | Exon7-Reverse | CTTGCTAGAAGCATCTCGGACT |
| | Exon8-Forward | TATACAGTCGGTGCTCAGGATG |
| | Exon8-Reverse | GATTACAGGTGTAAGCCACCGT |
| | Exon9-Forward | GAGCAGCAAGACTCTGTCTCAA |
| | Exon9-Reverse | TCTATGTCTCGGTTTCCCTCAT |
| | Exon10-Forward | GGGAACAATAAGCTGATCAAGG |
| | Exon10-Reverse | TCTATGTCTCGGTTTCCCTCAT |
| | Exon11-Forward | GAGTTGAGATGTGCCTTTACCC |
| | Exon11-Reverse | CCTAAAGTGCTGGGATTACAGG |
| | Exon12-Forward | CTTGATCCCAGGAGTTTGAGAC |
| | Exon12-Reverse | GCACACACCTCTGACTAAGCTG |
| | Exon13-Forward | CGGAGATATTGTACCCAGAACC |
| | Exon13-Reverse | TCCCTGGTGAACTCCTACTCAT |
| | Exon14-Forward | ATGAGTAGGAGTTCACCAGGGA |
| | Exon14-Reverse | CATTCAATGAGAGCTCTGGGAG |
| | Exon15-Forward | AGAGCTCTCATTGAATGCCTG |
| | Exon15-Reverse | CAGAGAAAAGGAGACAGAGGGA |

**Table S2. PTEN Mutation Negative Patients Harboring *AKT1* (**NM_001014431.1) **and *PIK3CA* (**NM_006218.2) **Mutation Positives with their Respective Phenotypes**

| | ***Malignancies - age at diagnosis*** | | | | | ***Benign tumors/other findings*** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Missense Mutations** | **Breast** | **Thyroid** | **Renal/renal pelvis** | **Endometrial - age at diagnosis** | **Macrocephaly** | **Lhermitte-Duclos disease** | **Breast - other** | **Thyroid nodule/goiter** | **Hashimoto's thyroiditis** | **Trichilemmoma** | **Skin papilloma** | **Oral papilloma** | **Lipoma** | **Oral fibroma** | **Oral mucosa hyperpigmentation** | **Skin hemangioma** | **Visceral hemangioma** | **GI polyps - variable** | **Uterine leiomyoma** |
| c.73C>T, p.Arg25Cys | Y - 37 | Y - 30 | | | | | | | | | | | | | | | | | |
| c.1303A>C, p.Thr435Pro | | | Y - 47 | | Y | | Y | Y | | | Y | | | | | | | | |
| c.353G>A, p.Gly118Asp | | Y - 28 | Y - 32 | | Y | | | | | | | | Y | | | | | | |
| c.403G>A, p.Glu135Lys | Y - 51 | | | | Y | | Y | Y | | | | | Y | | | Y | | | |
| c.652G>A, p.Glu218Lys | Y - 38 | Y - 27 | | | Y | | Y | | | Y | | | Y | Y | | | | Y | Y |
| c.1145G>A , p.Arg382Lys | | | | | Y | Y - 31 | Y | Y | | Y | Y | Y | | | | Y | | Y | |
| c.1066G>A, p.Val356Ile | | Y - 27 | | | | | Y | | Y | | | | | | | | | Y | Y |
| c.1634A>C, p.Glu545Ala & c.1658_1659delGTinsC; p. Ser553Thrfs*7 | Y - 70 | Y - 72 | | | Y | | Y | Y | | | | | | | | | | Y | |
| c.1634A>C, p.Glu545Ala & c.1658_1659delGTinsC; p. Ser553Thrfs*7 | | | | | Y | | | | | | | | | | | | | Y | |
| c.1895T>G, p.Leu632* | | | Y - 59 | | Y | | | | | | | | | | | | | Y | |