# Hedgehog pathway dysregulation contributes to the pathogenesis of human gastrointestinal stromal tumors *via* GLI-mediated activation of *KIT* expression

## SUPPLEMENTARY TABLES S1-S8

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**Supplementary Table S1. Representation of the "Development_Hedgehog Signaling" network (MetaCore™) in FACS-purified human gastric ICC, mouse small intestinal ICC and mouse gastric ICC-SC**.

| **Cells**[a] | **Species** | **Rank**[b] | ***P***[c] | **FDR**[c] | **Ratio**[d] | |
|---|---|---|---|---|---|---|
| | | | | | **Genes in set** | **Genes in network** |
| gastric ICC | *Homo sapiens* | 29 | 1.046E-02 | 0.0566 | 139 | 254 |
| ICC-MY | *Mus musculus* | 14 | 1.009E-03 | 0.0113 | 34 | 254 |
| ICC-DMP | *Mus musculus* | 9 | 3.192E-06 | 0.0000560 | 46 | 254 |
| 2xSCS2F10 | *Mus musculus* | 47 | 7.910E-05 | 0.000265 | 105 | 254 |

[a], FACS-purified and cultured cells analyzed by Affymetrix Human Genome U133 Plus 2.0 and Mouse Genome 430 2.0 microarrays: human gastric ICC purified by FACS (n=6) and compared to dissociated, unfractionated gastric *tunica muscularis* source tissue samples (n=4) (see details in main text); FACS-purified ICC-MY, mouse small intestinal ICC associated with the myenteric plexus (n=3) and ICC-DMP, mouse small intestinal ICC associated with the deep muscular plexus (n=3) compared to unfractionated BALB/c small intestinal *tunica muscularis* tissues (n=2) [1]; 2xSCS2F10, mouse gastric ICC-SC line (n=3) compared to unfractionated C57BL/6J gastric *tunica muscularis* tissues (n=2) [2]. [b], Ranking of the "Development_Hedgehog Signaling" network among biological process networks detected by MetaCore™ analysis of gene sets with significantly higher expression than controls ($\log_2$ fold change >1, Benjamini-Hochberg $Q<0.05$). See top 50 process networks for each cell type in **Supplementary Table S8**. [c], $P$ values and false discovery rates (FDR) from the network analysis. [d], Ratio of differentially expressed genes and genes in the "Development_Hedgehog Signaling" network.

**Supplementary Table S2. Sources of human GIST microarray data.**

| **NCBI GEO[a] Series: GSE17743** [3][b] | | **NCBI GEO Series: GSE8167** [4] | | **NCBI GEO Series: GSE20708** [5] | |
|---|---|---|---|---|---|
| **Label[c]** | **Sample ID** | **Label** | **Sample ID** | **Label** | **Sample ID** |
| GIST_KIT_Ex11_1 | GSM442936 | GIST_WT_8 | GSM202198 | GIST_KIT_Ex11_15 | GSM519700 |
| GIST_KIT_Ex11_2 | GSM442937 | GIST_WT_9 | GSM202199 | GIST_KIT_Ex9_2 | GSM519701 |
| GIST_KIT_Ex11_3 | GSM442938 | GIST_KIT_Ex11_22 | GSM202200 | GIST_PDGFRA_Ex12_3 | GSM519702 |
| GIST_PDGFRA_Ex18_1 | GSM442939 | GIST_KIT_Ex11_23 | GSM202204 | GIST_WT_4 | GSM519703 |
| GIST_KIT_Ex11_4 | GSM442940 | GIST_KIT_Ex11_24 | GSM202205 | GIST_KIT_Ex11_16 | GSM519704 |
| GIST_KIT_Ex11_5 | GSM442941 | GIST_KIT_Ex11_25 | GSM202206 | GIST_KIT_Ex11_17 | GSM519705 |
| GIST_KIT_Ex9_1 | GSM442942 | GIST_WT_10 | GSM202207 | GIST_WT_5 | GSM519706 |
| GIST_WT_1 | GSM442943 | GIST_KIT_Ex11_26 | GSM202208 | GIST_KIT_Ex11_18 | GSM519707 |
| GIST_WT_2 | GSM442944 | GIST_WT_11 | GSM202209 | GIST_PDGFRA_Ex14_1 | GSM519708 |
| GIST_KIT_Ex11_6 | GSM442945 | GIST_KIT_Ex11_27 | GSM202210 | GIST_KIT_Ex11_19 | GSM519710 |
| GIST_KIT_Ex11_7 | GSM442946 | GIST_KIT_Ex11_28 | GSM202211 | GIST_PDGFRA_Ex18_10 | GSM519711 |
| GIST_PDGFRA_Ex18_2 | GSM442947 | GIST_KIT_Ex11_29 | GSM202212 | GIST_KIT_Ex11_20 | GSM519712 |
| GIST_PDGFRA_Ex12_1 | GSM442948 | GIST_KIT_Ex11_30 | GSM202213 | GIST_WT_6 | GSM519715 |
| GIST_PDGFRA_Ex18_3 | GSM442949 | GIST_KIT_Ex11_31 | GSM202216 | GIST_PDGFRA_Ex18_11 | GSM519716 |
| GIST_PDGFRA_Ex18_4 | GSM442950 | GIST_KIT_Ex11_32 | GSM202217 | GIST_KIT_Ex11_21 | GSM519717 |
| GIST_WT_3 | GSM442951 | GIST_KIT_Ex11_33 | GSM202218 | GIST_WT_7 | GSM519718 |
| GIST_PDGFRA_Ex18_5 | GSM442952 | GIST_KIT_Ex11_34 | GSM202220 | GIST_PDGFRA_Ex12_4 | GSM519720 |
| GIST_PDGFRA_Ex12_2 | GSM442953 | GIST_KIT_Ex11_35 | GSM202221 | | |
| GIST_KIT_Ex11_8 | GSM442954 | GIST_WT_12 | GSM202223 | | |
| GIST_PDGFRA_Ex18_6 | GSM442955 | GIST_WT_13 | GSM202225 | | |
| GIST_PDGFRA_Ex18_7 | GSM442956 | GIST_KIT_Ex11_36 | GSM202226 | | |
| GIST_KIT_Ex11_9 | GSM442957 | GIST_WT_14 | GSM202227 | | |
| GIST_KIT_Ex11_10 | GSM442958 | GIST_KIT_Ex11_37 | GSM202228 | | |
| GIST_KIT_Ex11_11 | GSM442959 | | | | |
| GIST_KIT_Ex11_12 | GSM442960 | | | | |
| GIST_PDGFRA_Ex18_8 | GSM442961 | | | | |
| GIST_KIT_Ex11_13 | GSM442962 | | | | |
| GIST_PDGFRA_Ex18_9 | GSM442963 | | | | |
| GIST_KIT_Ex11_14 | GSM442964 | | | | |

[a], NCBI GEO, National Center for Biotechnology Information Gene Expression Omnibus.

**[b], References:**

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[c], Labels used in **Figure 3A**. Mutated gene, exon number and serial number are indicated.

**Supplementary Table S3. Antibodies and other reagents used for the isolation of ICC, *NOT* ICC and hematopoietic cells from human gastric *tunica muscularis.***

| Target | Supplier | Host/Source | Clone/ID | Isotype | Label | Final conc. or amount/$10^6$ cells/100 µL |
|---|---|---|---|---|---|---|
| KIT[a] | BioLegend | Mouse mc[b] | 104D2 | $IgG_1$, κ | APC[c] | 0.5 µg |
| CD34[d] | BD Pharmingen | Mouse mc | 581 | $IgG_1$, κ | PE[e] | 0.0625 µg |
| FCER1A[f] | BioLegend | Mouse mc | AER-37 | $IgG_{2b}$, κ | PE-Cy7[g] | 0.25 µg |
| CD11B[h] | BD Pharmingen | Mouse mc | ICRF44 | $IgG_1$, κ | PE-Cy7 | 0.25 µg |
| CD45[i] | BD Pharmingen | Mouse mc | HI30 | $IgG_1$, κ | PE-Cy7 | 0.1 µg |
| Dead cells[j] | eBioscience | | Fixable Viability Dye | | APC-eFluor 780 | 1 µL/mL |
| Fcγ receptors | BD Pharmingen | | Purified NA/LE Human BD Fc Block™ | | | 20 µL |

[a], v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (CD117); [b], mc, monoclonal; [c], APC, allophycocyanin; [d], CD34 molecule; [e], PE, phycoerythrin; [f], FCER1A, Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide; [g], Cy7, cyanine 7; [h], CD11B, integrin, alpha M (complement component 3 receptor 3 subunit) (ITGAM, MAC-1); [i], CD45, protein tyrosine phosphatase, receptor type, C (PTPRC), alias: leukocyte common antigen; [j], Fixable Viability Dye. Suppliers: BioLegend, Inc., San Diego, CA; BD Pharmingen, Becton, Dickinson and Company, Franklin Lakes, NJ; eBioscience, Inc., San Diego, CA.

**Supplementary Table S4. Configuration of the Becton Dickinson LSR II flow cytometer.**

| Laser | Excitation wavelength (nm) | Dichroic filter (nm) | Emission filter (nm; peak/bandwidth) | Detector type | Light scatter or fluorochrome used |
|---|---|---|---|---|---|
| Coherent® Sapphire™ 20 mW | 488 | | | Photodiode | Forward scatter |
| | | | 488/10 | PMT[a] | Side scatter |
| | | 505 LP[b] | 530/30 | PMT | Unused |
| | | 550 LP | 575/26 | PMT | PE[c] |
| | | 595 LP | 610/20 | PMT | Unused |
| | | 685 LP | 695/40 | PMT | Unused |
| | | 735 LP | 780/60 | PMT | PE-Cy7[d] |
| Coherent® CUBE 100 mW | 407 | | 450/50 | PMT | Unused |
| | | 505LP | 525/50 | PMT | Unused |
| | | 535 LP | 590/40 | PMT | Unused |
| | | 595 LP | 610/20 | PMT | Unused |
| | | 630 LP | 670/30 | PMT | Unused |
| | | 670 LP | 710/50 | PMT | Unused |
| Coherent® CUBE 40 mW | 640 | | 660/20 | PMT | APC[e] |
| | | 685 LP | 712/20 | PMT | Unused |
| | | 735 LP | 780/60 | PMT | APC-eFluor 780 |

[a], PMT, photomultiplier tube; [b], LP, long-pass; [c], PE, phycoerythrin; [d], Cy7, cyanine 7; [e], APC, allophycocyanin.

**Supplementary Table S5. Patient, tissue and harvested cell information.**

| Age (yr) | Sex | Surgery | Sample | ICC count[a] | *NOT* ICC count[b] | HP$^+$ count[c] |
|---|---|---|---|---|---|---|
| 33 | M | Duodenal switch | ICC_1 | 1,009 | | |
| 32[d] | F | Sleeve gastrectomy | ICC_2 | 5,528 | | |
| 23[d] | F | Sleeve gastrectomy | | | | |
| 60 | F | Sleeve gastrectomy | ICC_3 | 4,929 | | |
| 51 | F | Sleeve gastrectomy | ICC_4 | 10,448 | | |
| 52 | M | Sleeve gastrectomy | ICC_5 | 4,584 | | |
| 51 | F | Sleeve gastrectomy | NOT_ICC | | 428,676 | |
| 31 | F | Sleeve gastrectomy | TISSUE_1 | | | |
| 51 | M | Sleeve gastrectomy | TISSUE_2 | | | |
| 51 | F | Sleeve gastrectomy | TISSUE_3 | | | |
| 23 | F | Sleeve gastrectomy | TISSUE_4 | | | |
| 57 | F | Sleeve gastrectomy | HP_1 | | | 127,186 |
| 47 | F | Sleeve gastrectomy | ICC_6 | 2,541 | | |
| | | | HP_2 | | | 397,368 |
| 27 | M | Sleeve gastrectomy | HP_3 | | | 552,154 |

[a], ICC, interstitial cells of Cajal: KIT$^+$CD34$^-$ cells excluding the Fixable Viability Dye allophycocyanin (APC)-eFluor 780 (live cells) and not labeled with antibodies against the hematopoietic markers CD45, FCER1A, and anti-CD11B (HP$^-$); [b], *NOT* ICC: live, HP$^-$KIT$^-$CD34$^-$ cells; [c], HP$^+$: live HP$^+$ cells. [d], Samples combined for processing due to small size.

**Supplementary Table S6.** RT-PCR primers for human samples.

| Gene | Direction | Sequence | Amplicon size (bp) |
|---|---|---|---|
| *SHH* | Forward | GCTCGGTGAAAGCAGAGAAC | 109 |
| | Reverse | CTCAGGTCCTTCACCAGCTT | |
| *IHH* | Forward | TCCGTCAAGTCCGAGCAC | 109 |
| | Reverse | GCCTCACGGCTGACAAGG | |
| *PTCH1* | Forward | CCACCAGACGCTGTTTAGTCA | 72 |
| | Reverse | CGATGGAGTCCTTGCCTACAA | |
| *SMO* | Forward | CAGTTCCAAACATGGCAAACAG | 200 |
| | Reverse | TGCTATGTCAGGCCAATGTGA | |
| *GLI1* | Forward | TGCAGTAAAGCCTTCAGCAATG | 132 |
| | Reverse | TTTTCGCAGCGAGCTAGGAT | |
| *GLI2* | Forward | CGAGAAACCCTACATCTGCAAGA | 88 |
| | Reverse | GTGGACCGTTTTCACATGCTT | |
| *GLI3* | Forward | AAACCCCAATCATGGACTCAAC | 98 |
| | Reverse | TACGTGCTCCATCCATTTGGT | |
| *KIT* | Forward | TGTCATCAGCCACCATCCTA | 156 |
| | Reverse | GCAGAATTGGAGAAGCCTTG | |
| *KIT*[a] | Forward | GGGATTTTCTCTGCGTTCTG | 99 |
| | Reverse | GATGGATGGATGGTGGAGAC | |
| *ANO1* | Forward | TGTCAGAGCCAAAGACATCG | 183 |
| | Reverse | AAGGAGGAGAGGGTGTGGTT | |
| *ACTB* | Forward | AATGTGGCCGAGGACTTTGATTGC | 93 |
| | Reverse | AGGATGGCAAGGGACTTCCTGTAA | |

[a], Used for microarray validation.

**Supplementary Table S7.** RT-PCR primers for mouse samples.

| **Gene** | **Direction** | **Sequence** | **Amplicon size (bp)** |
|---|---|---|---|
| *Shh* | Forward | CTGGCCAGATGTTTTCTGGT | 117 |
| | Reverse | TAAAGGGGTCAGCTTTTTGG | |
| *Ihh* | Forward | CCGAACCTTCATCTTGGTG | 124 |
| | Reverse | ACAGATGGAATGCGTGTGAA | |
| *Ptch1* | Forward | ATGCTCCTTTCCTCCTGAAACC | 168 |
| | Reverse | TGAACTGGGCAGCTATGAAGTC | |
| *Smo* | Forward | GCCTGGTGCTTATTGTGG | 75 |
| | Reverse | GGTGGTTGCTCTTGATGG | |
| *Gli1* | Forward | AACTCCACAGGCACACAGG | 79 |
| | Reverse | GCTCAGGCTTCTCCTCTCTC | |
| *Gli2* | Forward | CCATTCATAAGCGGAGCAAG | 105 |
| | Reverse | CCAGGTCTTCCTTGAGATCG | |
| *Gli3* | Forward | GCTCTTCAGCAAGTGGTTCC | 122 |
| | Reverse | CTGTCGGCTTAGGATCTGTTG | |
| *Kit* | Forward | GGCTAGCCAGAGACATCAGG | 159 |
| | Reverse | AGGAGAAGAGCTCCCAGAGG | |
| *Actb* | Forward | GCTCTGGCTCCTAGCACC | 74 |
| | Reverse | CCACCGATCCACACAGAGTACTTG | |

**Supplementary Table S8.** Top 50 process networks for each cell type (in attached MS Excel file).