Supplemental table 1. Primer sequences for real-time RT-PCR

Human	
GLI1E1/4-qF	5'-GCGCCCAGACAGAGGCCCACT
GLI1E3-qF	5'-CCACAGTTATGGGCCAGCCAGAGAG
GLI1E4-qR	5'-GGCATCCGACAGAGGTGAGATGGAC
RPLP0-qF	5'-CCTTCTCCTTTGGGCTGGTCATCCA
RPLP0-qR	5'-CAGACACTGGCAACATTGCGGACAC
HPRT1-qF	5'-TGCAGACTTTGCTTTCCTTGGTCAGG
HPRT1-qR	5'-CCAACACTTCGTGGGGTCCTTTTCA
GAPDH-qF	5'-CTTCGCTCTCTGCTCCTCCTGTTCG
GAPDH-qR	5'-ACCAGGCGCCCAATACGACCAAAT
ACTB-qF	5'-CAAGGCCAACCGCGAGAAGATGAC
ACTB-qR	5'-GCCAGAGGCGTACAGGGATAGCACA
TBP-qF	5'-GCCAGCTTCGGAGAGTTCTGGGATT
TBP-qR	5'-CGGGCACGAAGTGCAATGGTCTTTA
Mouse	
Gli1-qF	5'-CCCATAGGGTCTCGGGGTCTCAAAC
Gli1-qR	5'-GGAGGACCTGCGGCTGACTGTGTAA
Ptch1-qF	5'-TGCTGTGCCTGTGGTCATCCTGATT
Ptch1-qR	5'-CAGAGCGAGCATAGCCCTGTGGTTC
Ptch2-qF	5'-CCCGTGGTAATCCTCGTGGCCTCTAT
Ptch2-qR	5'-TCCATCAGTCACAGGGGCAAAGGTC
Sfrp-qF	5'-GCAAGCGAGTTTGCACTGAGGATGA
Sfrp-qR	5'-GGCCCCAGCTTCAAGGGTTTCTTCT
Arp-qF	5'-TGCACTCTCGCTTTCTGGAGGGTGT
Arp-qR	5'-AATGCAGATGGATCAGCCAGGAAGG
Gapdh-qF	5'-GGTGTGAACGGATTTGGCCGTATTG
Gapdh-qR	5'-CCGTTGAATTTGCCGTGAGTGGAGT
Hprt1-qF	5'-CAACGGGGGACATAAAAGTTATTGGTGGA
Hprt1-qR	5'-TGCAACCTTAACCATTTTGGGGCTGT

SUPPLEMENTAL FIGURE LEGENDS

Supplemental figure 1. Western blot analysis of GLI1 isoform expression. SAG-treated Daoy-EGFP cells, the RMS13 cell line and transfected Hek293 cells with FLAG-tagged expression constructs of GLI1FL or GLI1ΔN (see Experimental Procedures) were used. Arrows indicate the GLI1 isoforms. Molecular weight markers are also shown.

Supplemental figure 2. Comparison of the luciferase activity of the FLAG-tagged pCMV5 constructs of GLI1ΔN and GLI1FL in C3H10T1/2 cell lines. Fifty ng of the constructs were co-transfected with 12xGLIBS-luc and pRL-SV reporter plasmids into C3H10T1/2 cells. The luciferase activity was measured at days 1 and 2 after transfection. The error bars indicate the standard deviation. The differences between the GLI1FL and GLI1ΔN values were statistically significant ($P < 0.001$, ANOVA – Bonferroni test).

Supplemental figure 3. Inhibition of GLI1ΔN and GLI1FL activity by the PTCH1 variants and PTCH2. Fifty ng of the FLAG-tagged GLI1ΔN or GLI1FL constructs in pCMV5 were co-transfected with 25 or 100 ng of PTCH2, PTCH1-1 or PTCH1-1C expression constructs, and the luciferase activity of the 12xGLIBS-luc reporter was measured. The error bars indicate the standard deviation. The differences between the GLI1FL and GLI1ΔN values were not statistically significant (ANOVA – Bonferroni test).

Supplemental figure 4. Cytoplasmic localization of GLI1FL and GLI1ΔN in the presence of PTCH1. NIH3T3 cells were co-transfected with 200 ng of the Myc-tagged PCTH1-1B (red signal) and 100 ng of the GLI1 constructs or a pCMV5 empty vector. The red scale bars represent 20 μm.

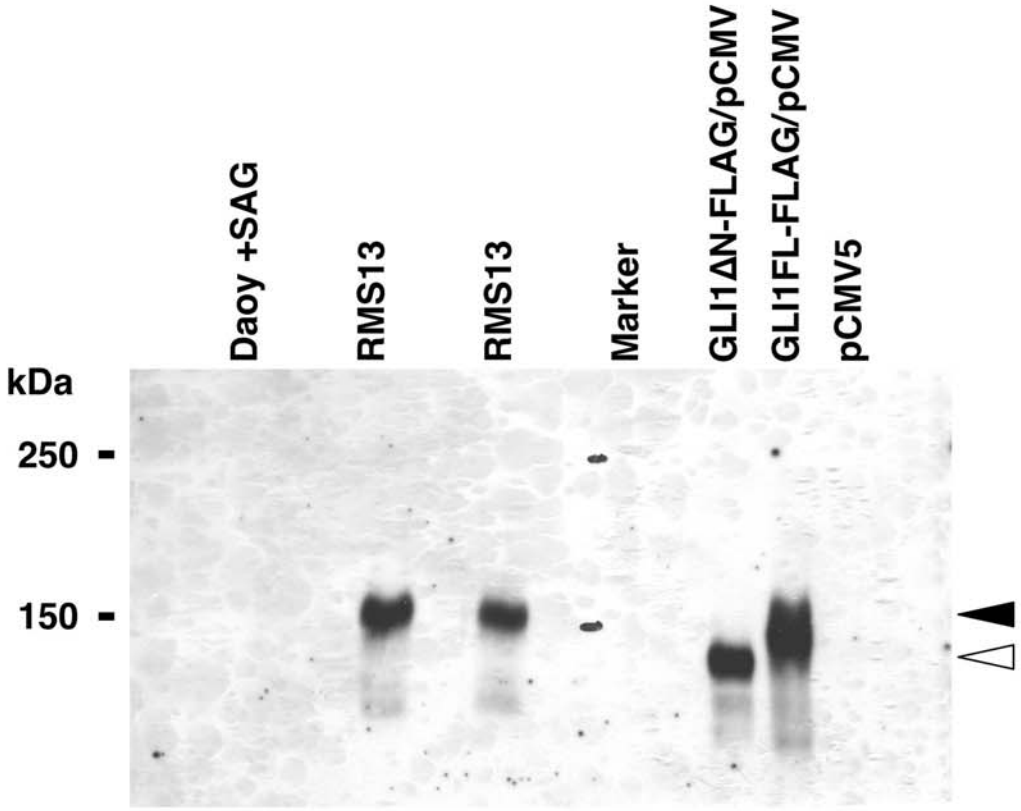
Supplemental figure 5. Subcellular localization of the EGFP-tagged GLI1 isoforms. NIH3T3 cells were transfected with 100 ng of the EGFP-tagged GLI1FL and GLI1ΔN constructs or the pEGFP empty vector. Expressed proteins (green signal) and nuclei stained with the DRAQ5 (blue signal) were visualized by immunofluorescence microscopy. The red scale bars represent 20 μm.

Supplemental figure 6. Subcellular localization of the FLAG-tagged GLI1 isoforms co-transfected with Dyrk1 constructs. FLAG-tagged GLI1 constructs (100 ng) were co-transfected with 200 ng of Dyrk1WT (A) or Dyrk1KR (B) constructs into NIH3T3 cells. Expressed GLI1 (green signal) and Dyrk1 (red signal) proteins and nuclei stained with the DRAQ5 (blue signal) were visualized by immunofluorescence microscopy. The red scale bars represent 20 μm.

Supplemental figure 7. Subcellular localization of the EGFP-tagged GLI1 isoforms co-transfected with Dyrk1 constructs. EGFP-tagged GLI1 constructs (100 ng) were co-transfected with 200 ng of Dyrk1WT (A) or Dyrk1KR (B) constructs into NIH3T3 cells. Expressed GLI1 protein (green signal) and nuclei stained with the DRAQ5 (blue signal) were visualized by immunofluorescence microscopy. The red scale bars represent 20 μm.

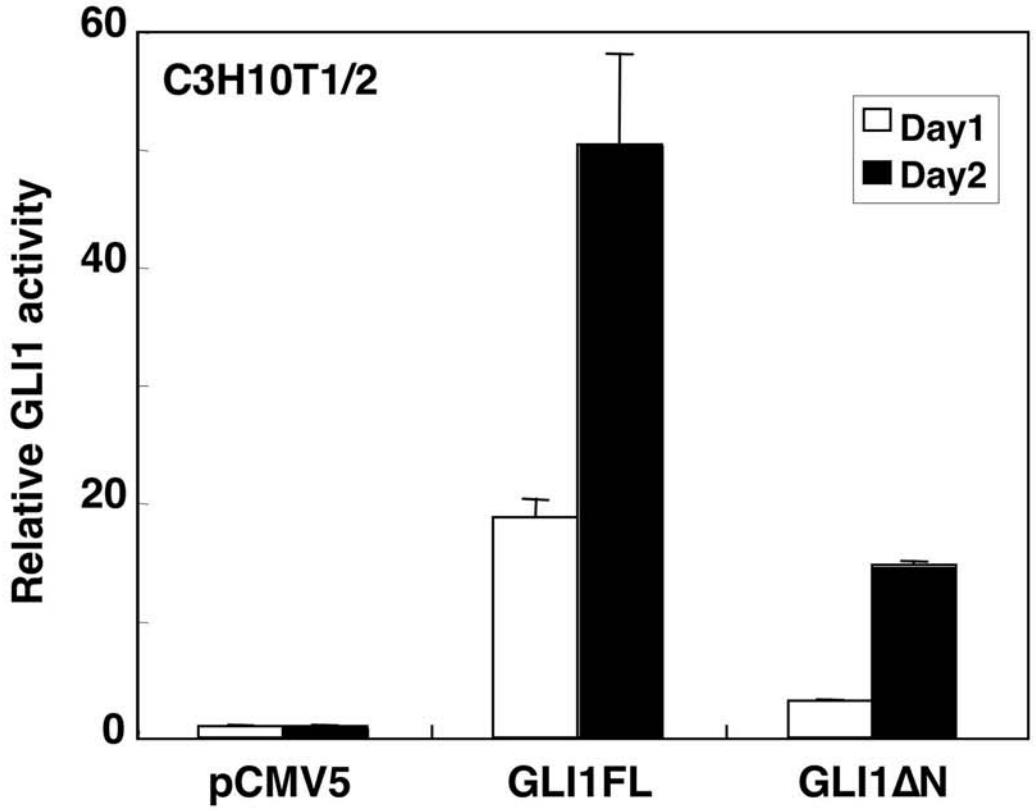
Supplemental figure 1

Western blot analysis of GLI1 isoform expression

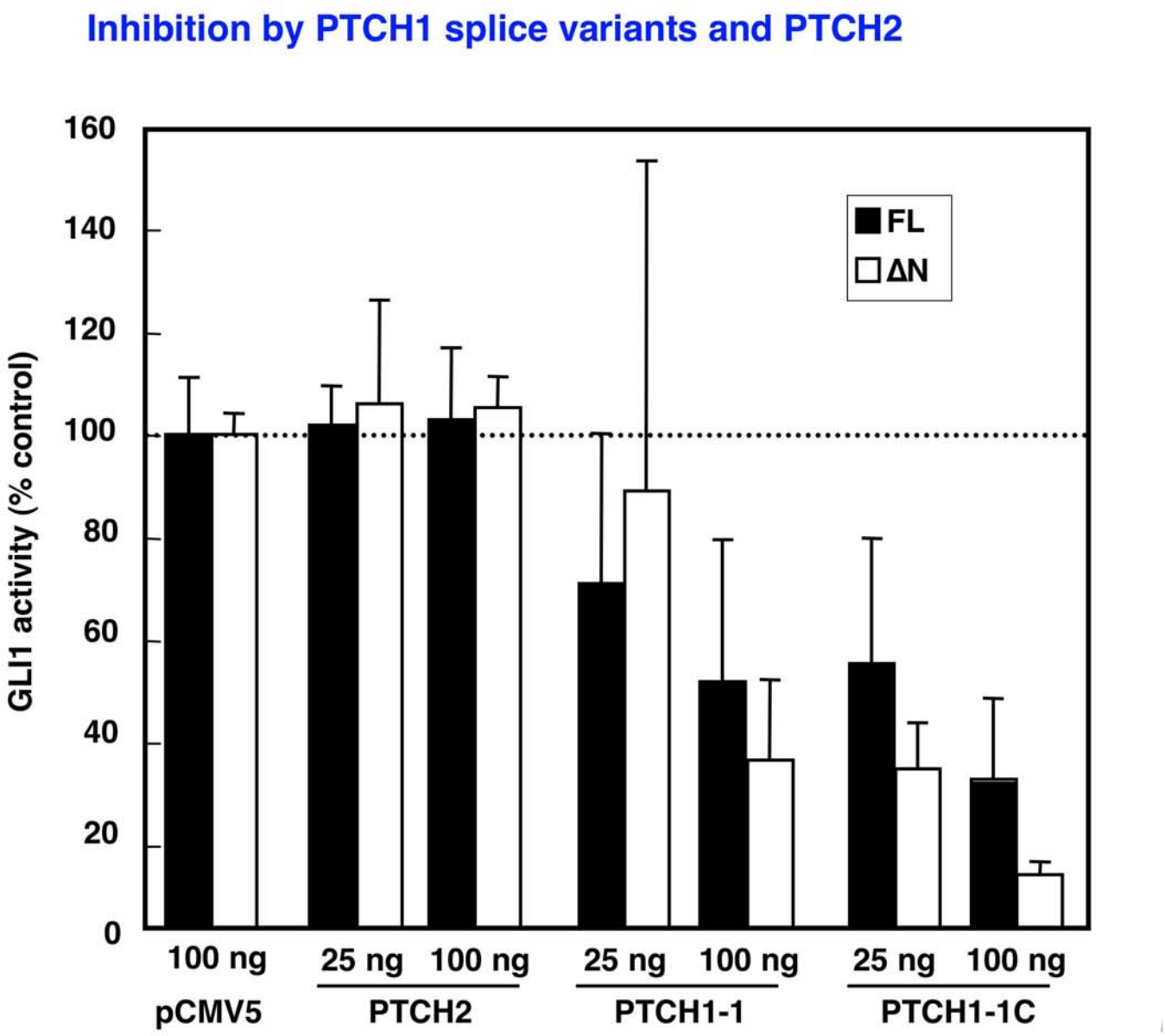


Supplemental figure 2

Overexpression and comparison of transcriptional activity

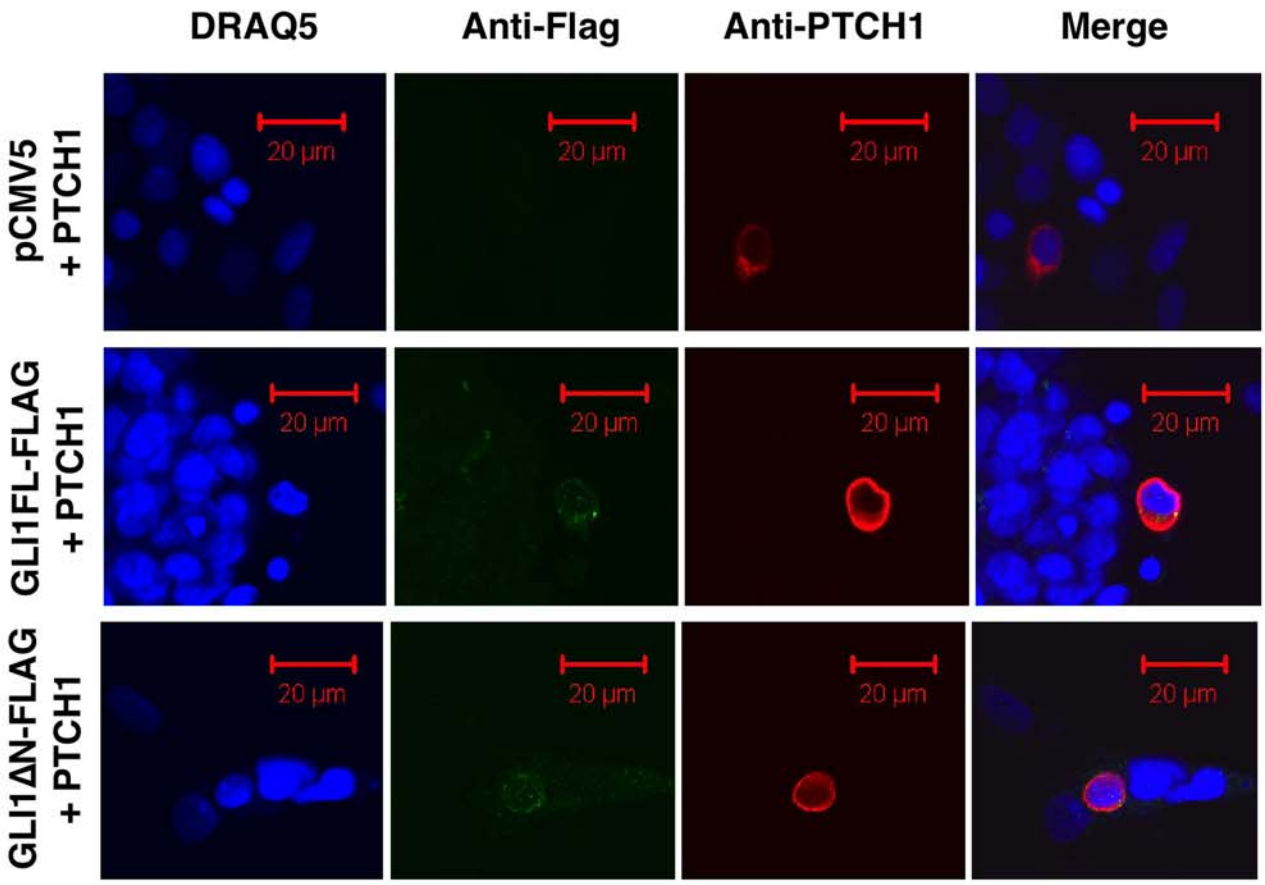


Supplemental figure 3



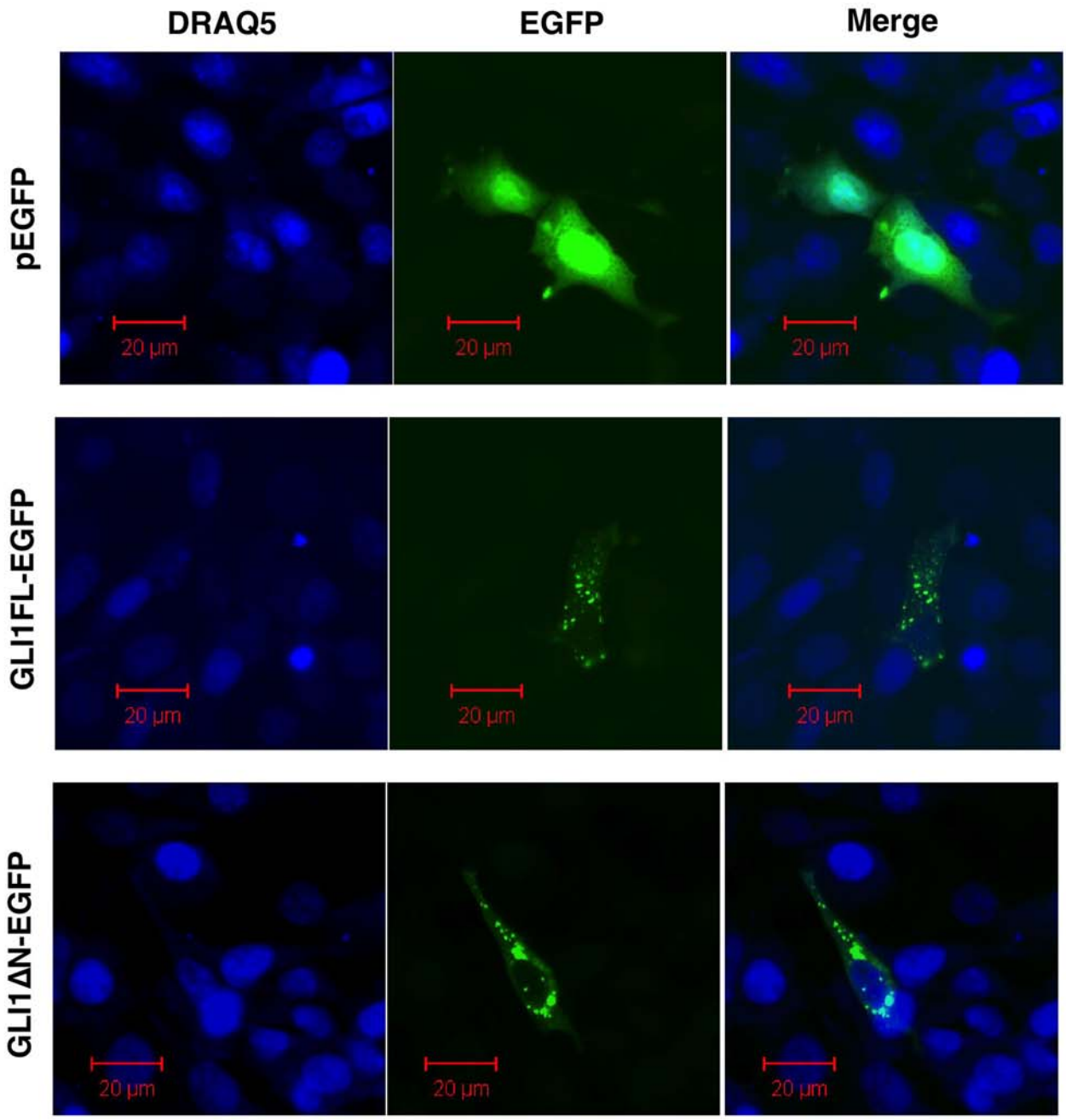
Supplemental figure 4

Cytoplasmic localization of GLI1FL and GLI1ΔN in the presence of PTCH1



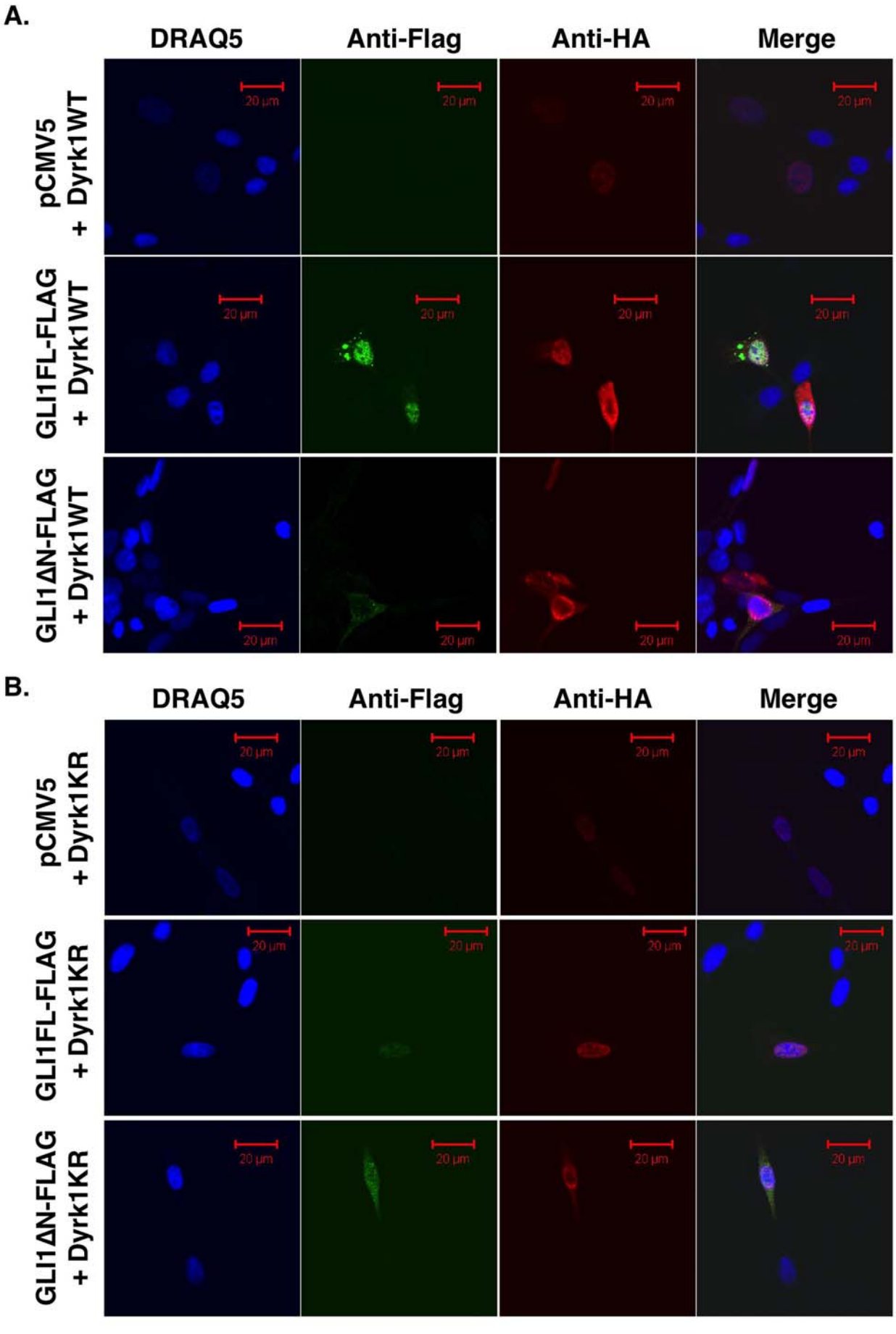
Supplemental figure 5

Subcellular localization of the EGFP-tagged GLI1 isoforms



Supplemental figure 6

Subcellular localization of the FLAG-tagged GLI1 isoforms co-transfected with Dyrk1 constructs



Supplemental figure 7

Subcellular localization of the EGFP-tagged GLI1 isoforms co-transfected with Dyrk1 constructs