

Supplementary Information

SLITRK5 is a negative regulator of hedgehog signaling in osteoblasts

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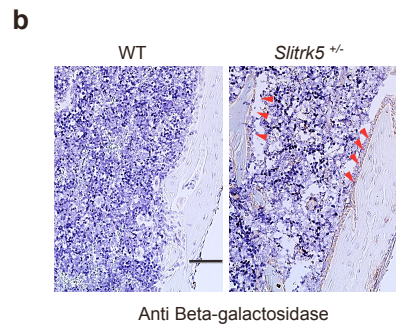
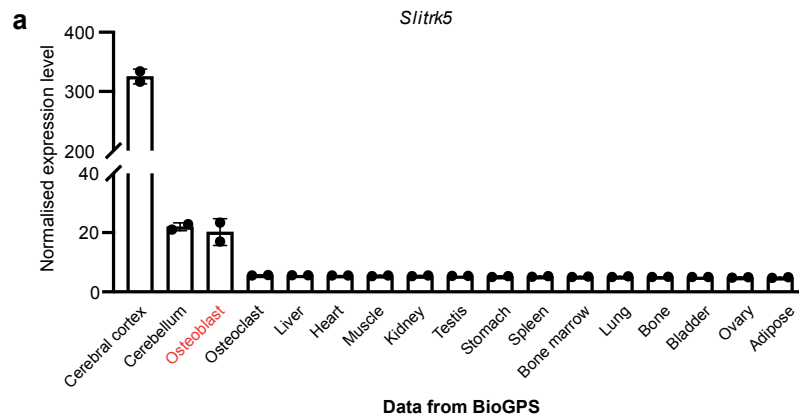
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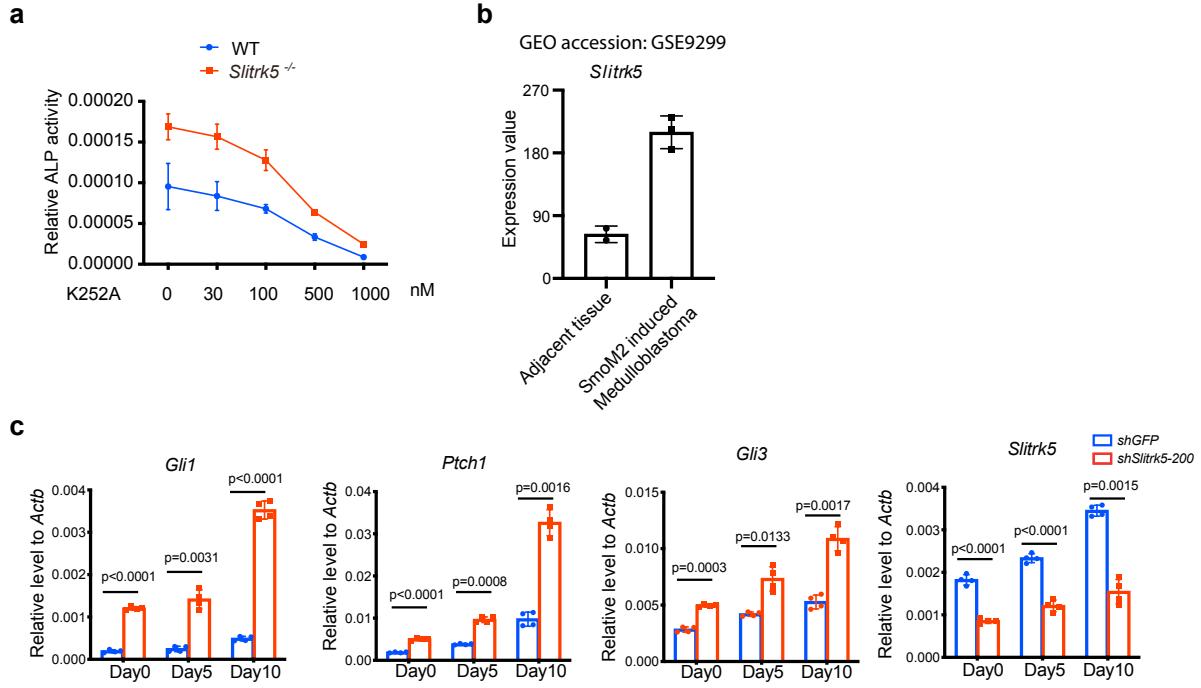
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Supplementary Figure 1

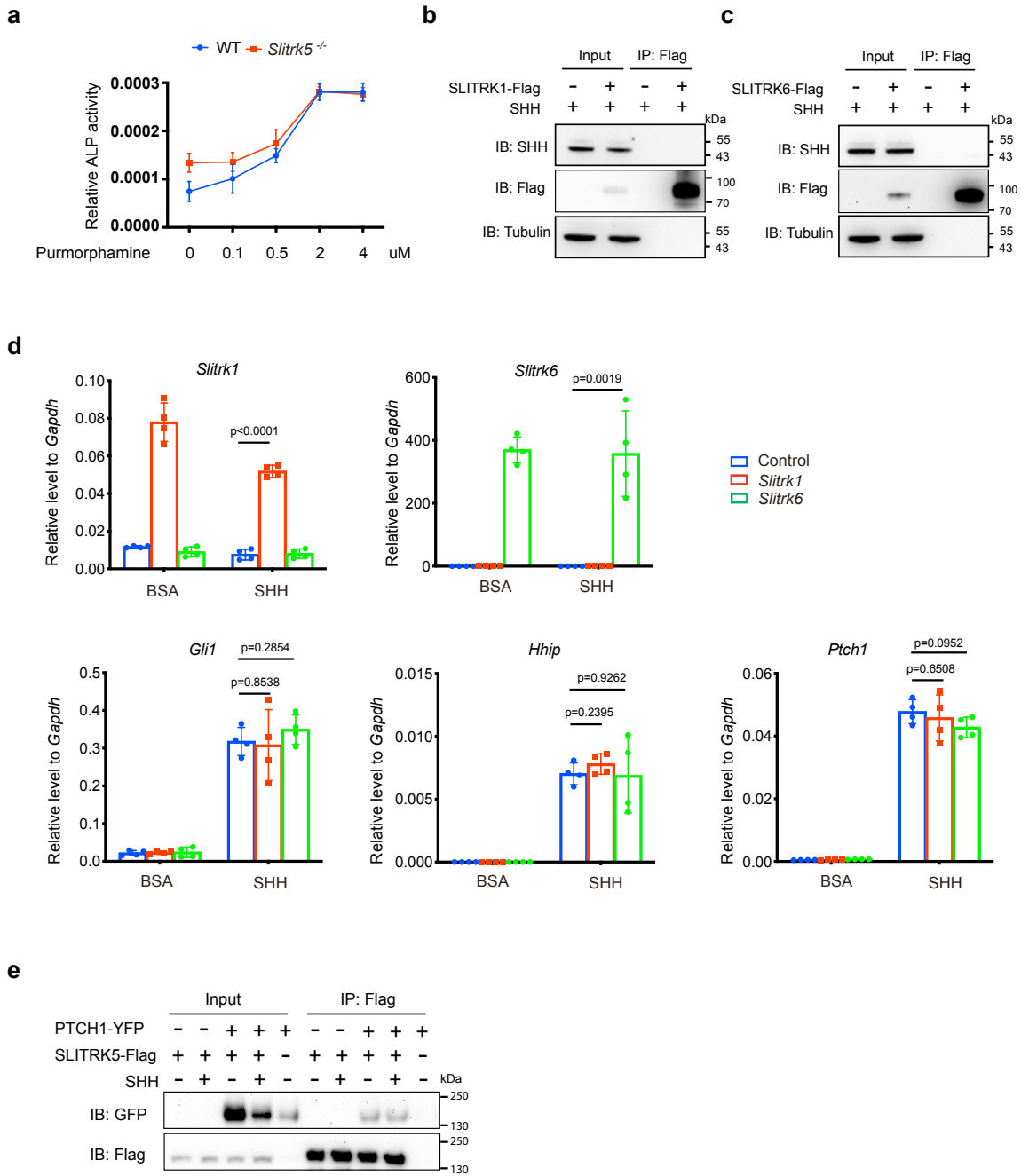


Supplementary Figure 1. a *Slitrk5* mRNA level in different cells and tissues. The raw expression data were obtained from BioGPS (<http://biogps.org/>). Data are presented as mean±s.d., n=2 biologically independent samples. **b** Immunostaining of mouse femur sections with beta-galactosidase antibody, indicating the expression of *Slitrk5* in osteoblasts. Data are representative of two independent experiments, scale bar=100 μ m.

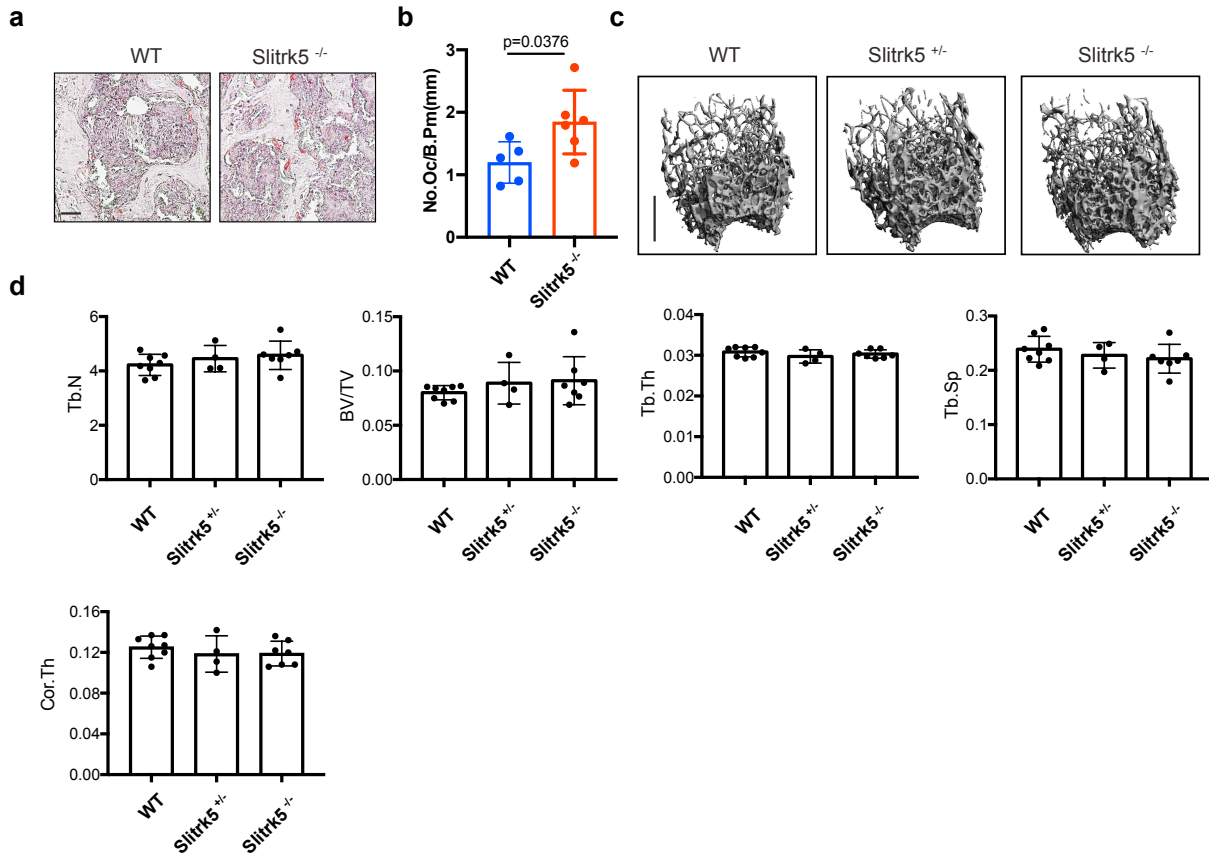


Supplementary Figure 2. **a** Primary osteoblasts from WT and *Slitrk5*^{-/-} mice were treated with the indicated doses of K252a and cultured in osteoblast differentiation medium. ALP activity was measured at day 8 of osteoblast differentiation. Data are presented as mean±s.d. n=10 biologically independent samples. **b** *Slitrk5* mRNA level in SmoM2 induced medulloblastoma and adjacent tissue (GEO: GSE9299). Data are presented as mean±s.d., n=3 biologically independent samples. **c** Human osteoblast-like cells (Saos-2) infected with *Slitrk5* or GFP shRNA and cultured in osteoblast differentiation medium. mRNA level of *Slitrk5*, *Gli1*, *Ptch1* and *Gli3* at 0, 5 and 10 days of differentiation was assessed by RT-PCR. Data are presented as mean±s.d., n=4 biologically independent samples, 2-tailed unpaired t-test.

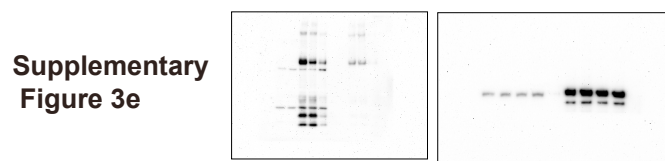
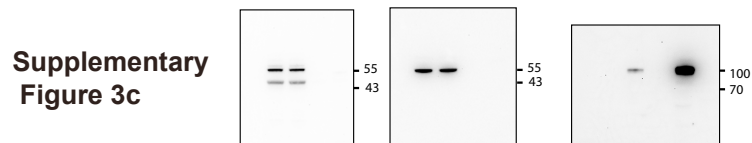
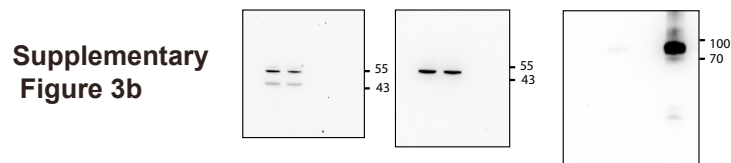
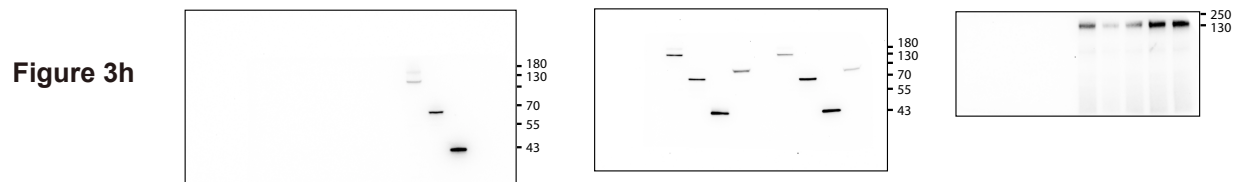
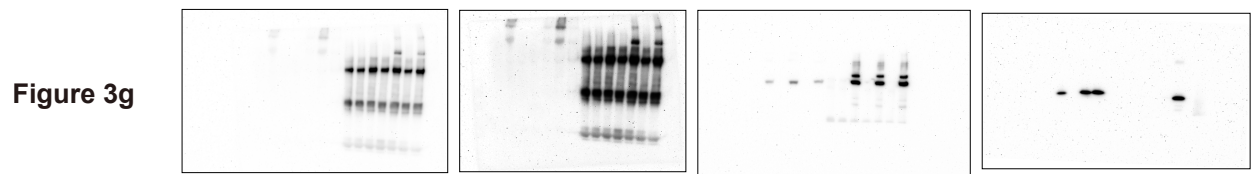
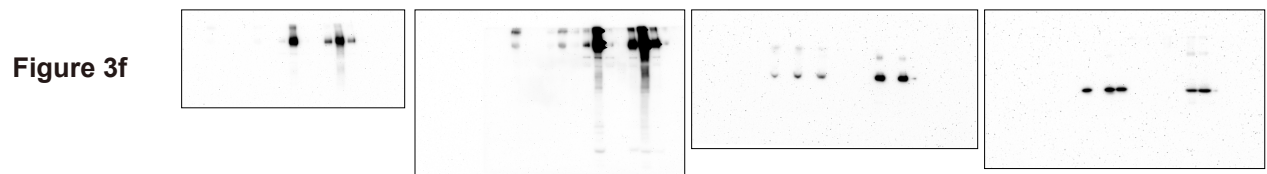
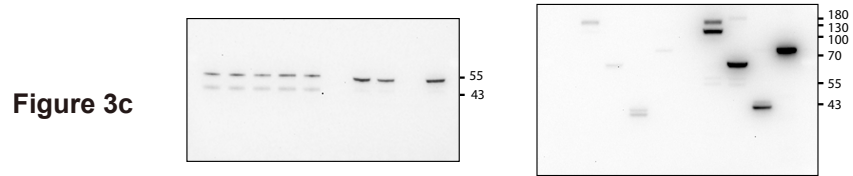
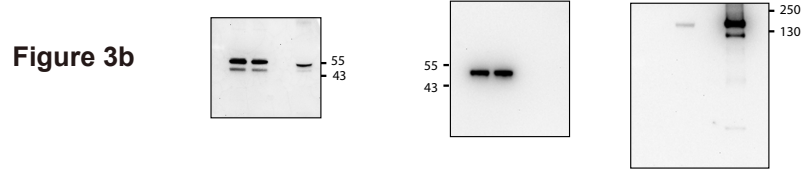
Supplementary Figure 3



Supplementary Figure 3. **a** Primary osteoblasts from WT and *Slitrk5*^{-/-} mice were treated with the indicated doses of Purmorphamine and cultured in osteoblast differentiation medium. ALP activity was measured at day 8 of differentiation. Data are presented as mean±s.d. n=10 biologically independent samples. **b** Co-immunoprecipitation of Flag-SLITRK1 and SHH in HEK293T cells. Data are representative of two independent experiments. **c** Co-immunoprecipitation of Flag-SLITRK6 and SHH in HEK293T cells. Data are representative of two independent experiments. **d** C3H10T1/2 cells transfected with either control, *Slitrk1* or *Slitrk6* overexpression vectors were treated with BSA or 100ng/ml SHH in serum free medium for 48h. *Gli1*, *Ptch1*, *Hhip*, *Slitrk1* and *Slitrk6* mRNA levels were measured by RT-PCR. n=4 biologically independent samples, 2-tailed unpaired t-test. **e** Co-immunoprecipitation of Flag-SLITRK5 and PTCH1-YFP in C3H10T1/2 cells. Data are representative of two independent experiments.



Supplementary Figure 4. a&b Representative images of TRAP staining (a) and quantification of No.Oc./B.Pm (b) of the L3 vertebrae in 7-week-old WT and Slitrk5^{-/-} female mice. osteoclast number/bone perimeter (No.Oc./B.Pm) . N=5 or 6 per group. Data are presented as mean±s.d, 2-tailed unpaired t-test, scale bar=100um. **c&d** Representative images of uCT (c) and quantification of bone volume/total volume (BV/TV), trabecular number per cubic millimeter (Tb.N), trabecular thickness (Tb.Th) , trabecular separation (Tb.Sp) and cortical bone thickness (Cor.Th)(d) of femurs in WT, Slitrk5^{+/-} and Slitrk5^{-/-} female mice at 7-week-old. N=4-7 per group. Data are presented as mean±s.d, 2-tailed unpaired t-test, scale bar=1mm.



Supplementary Table

Name	Species	shRNA primers
shSlitrk5-199	Mouse	CCGGCCTCCTGCACCTGGGTAATAACTCGAGTTATTACCCAGGTGCAGGAGGTTTTTG
shSlitrk5-200	Mouse	CCGGTGCAGAAACCATCGATTACTCGAGTAATAATCGATGGTTTCTGCATTTTTG
sh-control	Mouse	CCGGGCGCGATAGCGCTAATAATTTCTCGAGAAATTATTAGCGCTATCGCGCTTTTT
		qPCR primers
Slitrk5-forward	Mouse	CGCAGAACAGACTTCCTGGAAG
Slitrk5-reverse	Mouse	TCAGCCTCTCAATCCTGTTGCC
Slitrk6-forward	Mouse	AACAACCCACCATAACAACGGA
Slitrk6-reverse	Mouse	TTCCTCAACTTCTTCCAAATGCT
Bsg-forward	Mouse	AGAGGACACAGGCACTTACGAG
Bsg-reverse	Mouse	GACTTCCTGGACAGAGGTTTGG
Hhip-forward	Mouse	GAGAGATCCTGTGTGGTGGCTT
Hhip-reverse	Mouse	GCTGCATTCTGAGTTGTTGGTG
Hprt-forward	Mouse	TCAGTCAACGGGGACATAAA
Hprt-reverse	Mouse	GGGGCTGTAAGCTTAACCAG
Gli1-forward	Mouse	CCAAGCCAACCTTTATGTCAGGG
Gli1-reverse	Mouse	AGCCCGCTTCTTTGTTAATTTGA
Ptch1-forward	Mouse	AAAGAACTGCGGCAAGTTTTTG
Ptch1-reverse	Mouse	CTTCTCCTATCTTCTGACGGGT
Ptch2-forward	Mouse	GGAAGTACATCCGTCAACAAC
Ptch2-reverse	Mouse	GAAGACGAGCATTACCGCTGCA
Ocn-forward	Mouse	TGCTTGTGACGAGGTATCAG
Ocn-reverse	Mouse	GTGACATCCATACTTGCAGG
Alp-forward	Mouse	CGGGACTGGTACTCGGATAA
Alp-reverse	Mouse	ATTCCACGTCGGTTCTGTTC
Sp7-forward	Mouse	TCACTTGCCTGCTCTGTTCC
Sp7-reverse	Mouse	GCGGCTGATTGGCTTCTTCT
Runx2-forward	Mouse	CCAACCGAGTCATTTAAGGCT
Runx2-reverse	Mouse	GCTCACGTCGCTCATCTTG
Gli2-forward	Mouse	AACTGTGGAGGACTGCCTACA
Gli2-reverse	Mouse	GGCATCTCCATGCCACTGTCAT
Col1a1-forward	Mouse	CCTCAGGGTATTGCTGGACAAC
Col1a1-reverse	Mouse	CAGAAGGACCTTGTGGCCAGG
Gli3-forward	Human	TCAGCAAGTGGCTCCTATGGTC
Gli3-reverse	Human	GCTCTGTTGTCCGGCTTAGGATC
Gli1-forward	Human	AGCCTTCAGCAATGCCAGTGAC
Gli1-reverse	Human	GTCAGGACCATGCACTGTCTTG
Ptch1-forward	Human	GCTGCACTACTTCAGAGACTGG
Ptch1-reverse	Human	CACCAGGAGTTTGTAGGCAAGG
Slitrk5-forward	Human	CCTCCAGTACAATCTCATCCGC
Slitrk5-reverse	Human	TCAAGCCAGAGAAGACGCCTGA