

**Fig. S1.** LRP4 is a specific facilitator of sclerostin-mediated inhibition of Wnt/ $\beta$ -catenin signaling: LRP4 cDNA expression **A.** LRP4 cDNA expression specifically facilitates sclerostin's inhibitory action on Wnt/ $\beta$ -catenin signaling. C28a2 cells were transiently transfected with LRP4, STF-LUC reporter plasmid and Wnt signaling inducing (Wnt1 + LRP5) plasmids. Five hours after transfection, sclerostin or DKK1 was added in a dose-dependent manner for additional 19h. Cells were resuspended in lysis buffer and luciferase levels measured. **B.** LRP4 cDNA expression specifically facilitates sclerostin inhibitory action on Wnt/ $\beta$ -catenin signaling. HEK293 cells were transiently transfected with LRP4, STF-LUC reporter plasmid and Wnt signaling inducing (Wnt1) plasmids. Five hours after transfection, sclerostin or DKK1 was added in a dose-dependent manner for additional 19h. Cells were resuspended in lysis buffer and luciferase levels measured. **C.** LRP4 cDNA expression specifically facilitates sclerostin inhibitory action on Wnt/ $\beta$ -catenin signaling. Experiments were performed as described in B but in (Wnt1 + LRP6) Wnt signaling inducing conditions.

**Fig. S2.** LRP4 is a specific facilitator of sclerostin-mediated inhibition of Wnt/ $\beta$ -catenin signaling: LRP4 knockdown **A.** Downregulation of LRP4 diminishes sclerostin action on Wnt signaling. Stable HEK 293-Wnt1-STF were transfected with control siRNA or 4 different siRNA against *LRP4* for 48h prior to 24h incubation with sclerostin. Cells were resuspended in lysis buffer and luciferase levels measured. **B.** Downregulation of LRP4 reduces *LRP4* RNA levels. Stable HEK 293-Wnt1-STF were transfected with control siRNA, 4 different siRNA (A,C,D,E) or combination (A +D) against *LRP4* for 48h prior to RNA expression determination by qPCR (GAPDH normalized values). The corresponding effects of LRP4 downregulation on the sclerostin mediated inhibition of Wnt signaling are shown in FigureS2A for individual siRNA and in Figure 2B for the combination (A+D). **C.** Downregulation of *Lrp4* reduces *Lrp4* RNA levels. Kusa-A1 were transduced with lentiviral particles harboring shRNA against *Lrp4*. Reduction of *Lrp4* RNA levels were assessed by realtime qPCR (18S normalized values). The corresponding effects of LRP4 downregulation on the sclerostin mediated inhibition of in vitro bone mineralization are shown in Figure 3C.

**Fig. S3.** LRP4 expression data **A.** LRP4 protein is expressed in human osteocytes (arrows). Immunohistochemistry of LRP4 in human femoral neck from a female subject aged 71. Scale bar corresponds to 50  $\mu$ m. **B.** *Lrp4* protein is expressed in 4 month old male mice osteoblasts (arrowheads) and osteocytes (arrows). Immunohistochemistry of *Lrp4* in femora from skeletally mature mice. Scale bar corresponds to 20  $\mu$ m.

**Fig. S4.** LRP4 sequence alignment and structural model **A.** High species conservation of both affected residues. CLUSTAL W protein sequence alignment of a part of the LRP4 protein, encoded by exon 24-26 (the numbering refers to the transcript with ID ENST00000378623), in several species and in the affected individuals. The mutations are highlighted in dark grey while the wildtype residues that are affected by these mutations are highlighted in light grey. '\*' indicates positions which have a single, fully conserved residue; ':' indicates that one of the following 'strong' groups is fully conserved: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW; and '.' indicates that one of the following 'weaker' groups is fully conserved: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, FVLIM, HFY. **B.** The structural model of LRP4 predicts four 5-bladed  $\beta$ -propellers. Propeller 1 is shown in top and lateral view.

Figure S1

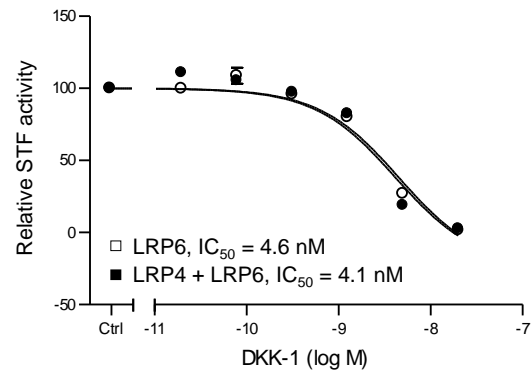
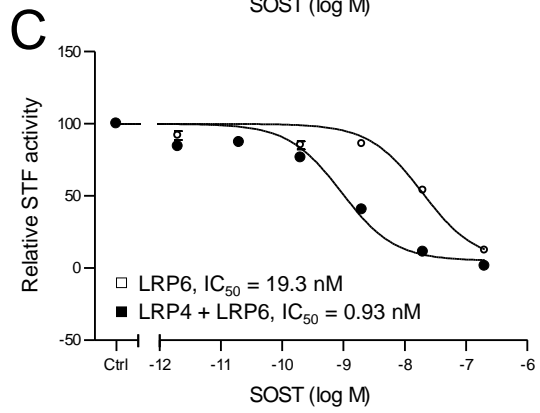
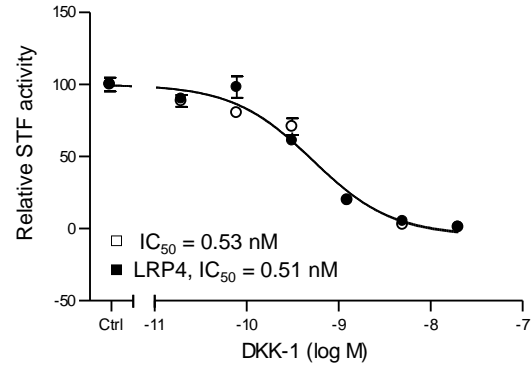
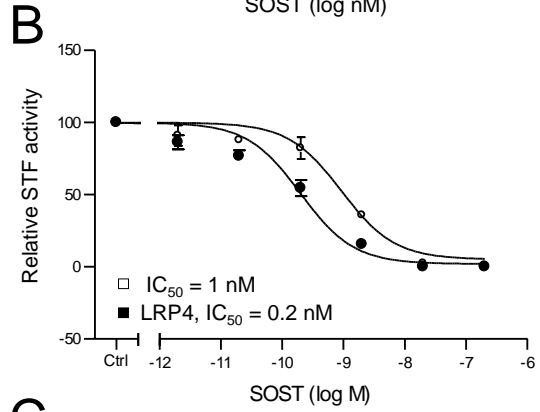
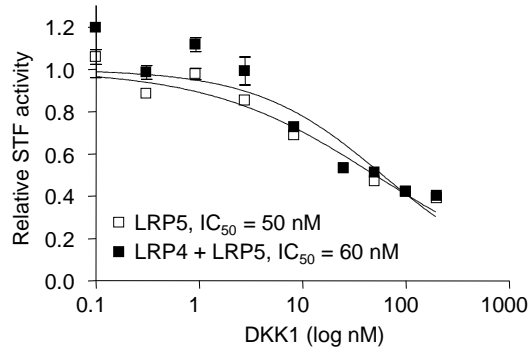
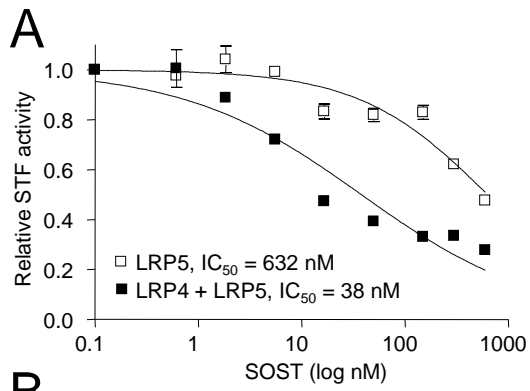


Figure S2

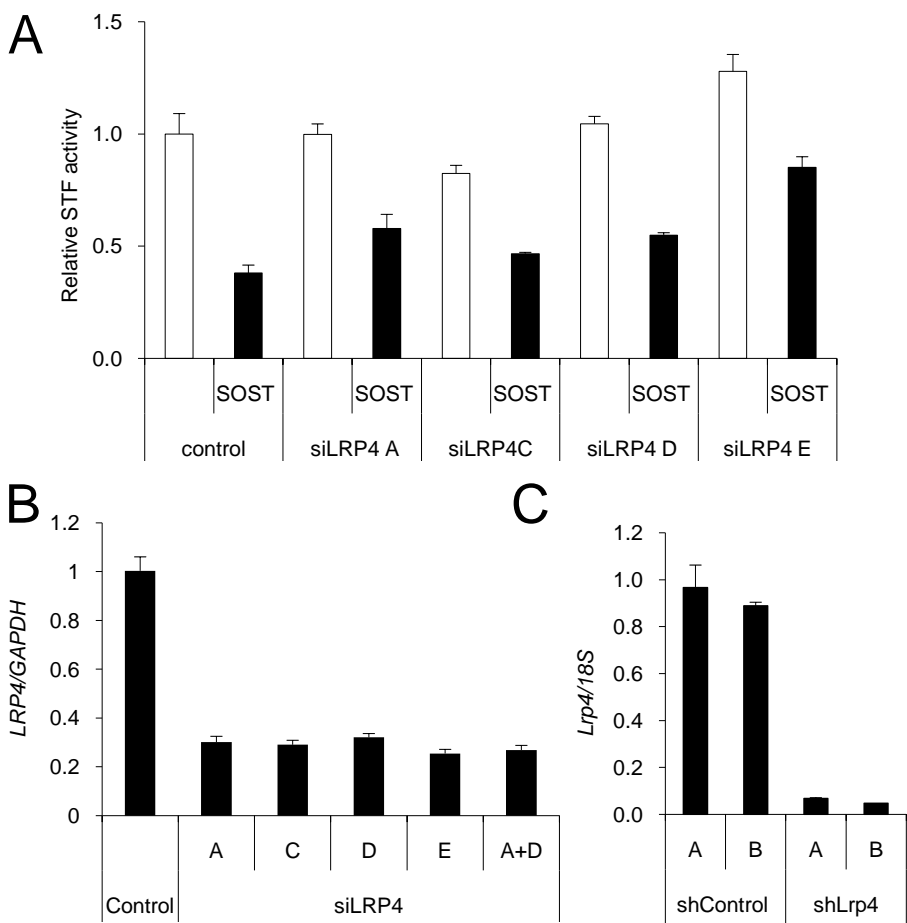
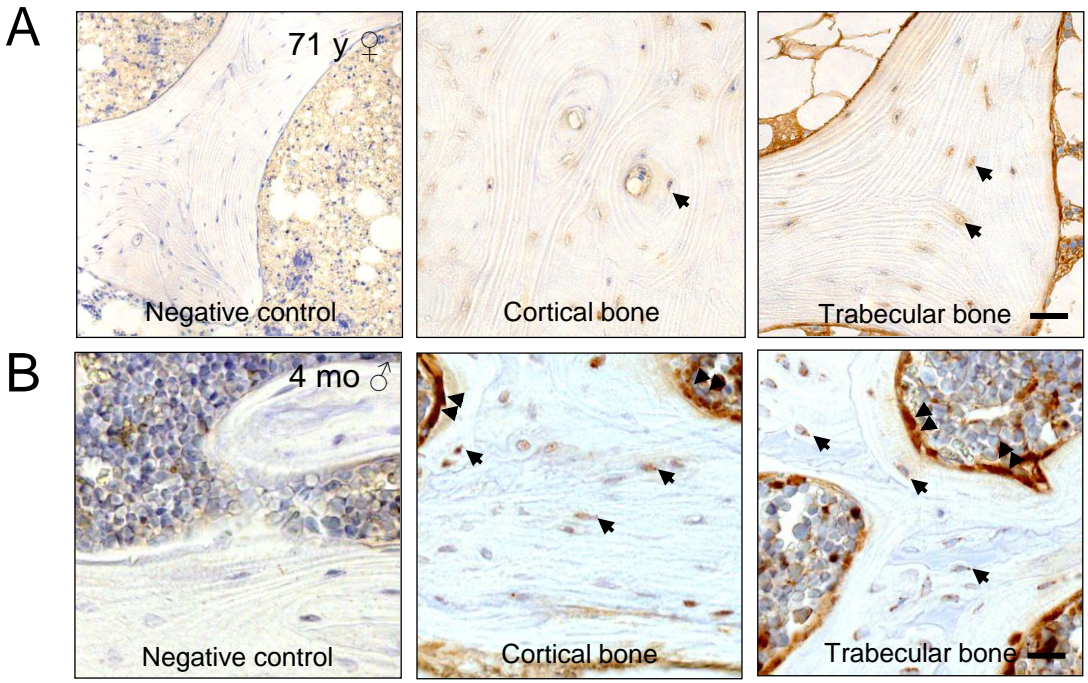


Figure S3



# Figure S4

## A

Mus_musculus	QHEDIITTGLQTTDGLAVDAIGRKVYWTDGTGNRIEVGNLDGSMRKVLVW
Rattus_norvegicus	QHEDIITTGLQTTDGLAVDAIGRKVYWTDGTGNRIEVGNLDGSMRKVLVW
Pan_troglodytes	QHEDIITTGLQTTDGLAVDAIGRKVYWTDGTGNRIEVGNLDGSMRKVLVW
Pongo_pygmaeus	QHENIITTGLQTTDGLAVDAIGRKVYWTDGTGNRIEVGNLDGSMRKVLVW
Homo_Sapiens-Wildtype	QHEDIITTGLQTTDGLAVDAIGRKVYWTDGTGNRIEVGNLDGSMRKVLVW
Gorilla_gorilla	QHADIVTTGLQTTDGLAVDAIGRKVYWTDGTGNRIEVGNLDGSMRKVLVW
Macaca_mulatta	QHEDIITTGLQTTDGLAVDAIGRKVYWTDGTGNRIEVGNLDGSMRKVLVW
Xenopus_tropicalis	NHQDIITTGLVTTDGLAVDAIGRKLYWTDGTGNRIEVANLDGTMRKVLVW
Drosophila_melanogaster	HVQKIVGDSLENPDGLVVDSIGRTIYWADAGRHTIEVASLDGSMRHVIAY
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Homo_sapiens-p.Arg1170Trp	QHEDIITTGLQTTDGLAVDAIGRKVYWTDGTGNRIEVGNLDGSMRKVLVW
Homo_sapiens-p.Trp1186Ser	QHEDIITTGLQTTDGLAVDAIGRKVYWTDGTGNRIEVGNLDGSMRKVLVW
Mus_musculus	QNLDSPRAIVLYHEMGFMYWTDWGENAKLERSGMDGSDRTVLINNNLGWP
Rattus_norvegicus	QNLDSPRAIVLYHEMGFMYWTDWGENAKLERSGMDGSDRTVLINNNLGWP
Pan_troglodytes	QNLDSPRAIVLYHEMGFMYWTDWGENAKLERSGMDGSDRAVLINNNLGWP
Pongo_pygmaeus	QNLDSPRAIVLYHEMGFMYWTDWGENAKLERSGMDGSDRAVLINNNLGWP
Homo_Sapiens-Wildtype	QNLDSPRAIVLYHEMGFMYWTDWGENAKLERSGMDGSDRAVLINNNLGWP
Gorilla_gorilla	QNLDSPRAIVLYHEMGFMYWTDWGENAKLERSGMDGSDRAVLINNNLGWP
Macaca_mulatta	QNLDSPRAIVLYHEMGFMYWTDWGENAKLERSGMDGSDRTVLISNNLGWP
Xenopus_tropicalis	QNLGSPRAIALYHEMGYMYWSDWGENAKLERAGMDGSERMVLISLNLGWP
Drosophila_melanogaster	KDLESPRGLALDYEAGLLFWTDWGHYRKIERSHLDGNERSRIVTANLGWP
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Homo_sapiens-p.Arg1170Trp	QNLDSPRAIVLYHEMGFMYWTDWGENAKLERSGMDGSDRAVLINNNLGWP
Homo_sapiens-p.Trp1186Ser	QNLDSPRAIVLYHEMGFMYWTDWGENAKLERSGMDGSDRAVLINNNLGWP

## B

