

SUPPLEMENTARY DATA

Sympathetic Regulation of Bone Metabolism: Mechanisms across Homeostasis, Aging and Disease

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Supplementary Table 1. Operational criteria for two-domain evidence classification and intervention-specific causal attribution

Panel A1. Operational criteria for mechanistic or causal support

Grade	Operational definition	Core evidence considered	Typical qualifying studies	Downgrade/boundary triggers
M1—Strong causal support	Direct in vivo causal support at the stated node and skeletal endpoint, with selective perturbation and complementary validation	Cell- or lineage-specific or highly selective manipulation; validated target engagement; structural, histomorphometric, mechanical, or fracture endpoint; specificity controls; replication or rescue	Conditional or inducible KO/knock-in; validated bone-targeted neuromodulation; genetic rescue; convergent selective pharmacology and genetics	Nonspecific systemic intervention; surrogate-only endpoints; unvalidated targeting; single unreplicated study; unexplained reversal across site or time
M2—Moderate causal or intervention support	In vivo intervention supports pathway involvement, but cellular specificity, generalizability, replication, or confounder control is incomplete	Verified systemic or regional pharmacology, denervation, whole-body genetics, or transmitter-handling intervention with at least one structural or robust histomorphometric endpoint	β -blockade, sympathectomy, whole-body receptor manipulation, NET intervention, disease-model pathway inhibition	Major unmeasured systemic effects; incomplete intervention validation; single-site finding generalized broadly; inconsistent direction without prespecified boundaries
M3—Mechanistic or biological plausibility	Evidence supports anatomy, receptor engagement, downstream signaling, or cellular function, but in vivo necessity or sufficiency for the skeletal claim is not established	Cellular or ex vivo functional assays; pathway readouts; anatomical mapping; spatial or single-cell profiles; correlative in vivo activation	Osteoblast, osteocyte, or osteoclast cultures; marrow cultures; receptor-expression maps; TH/DBH anatomy; downstream signaling assays	Expression-only inference; non-bone-cell extrapolation; absence of functional validation; indirect transmitter measurement treated as direct dynamics

Panel A2. Operational categories for direct human evidence

Category	Operational definition	Typical evidence	Major limitations
H2—Human interventional evidence	A prospective human intervention directly evaluates an adrenergic or sympathetic exposure with predefined skeletal or target-engagement endpoints	Randomized or controlled pharmacological studies; prospective mechanistic challenge studies	Small sample size; short follow-up; surrogate endpoints; limited target engagement; no microarchitecture, strength, falls, or fracture endpoint
H1—Human observational or human-tissue evidence	Human cohorts, case-control studies, registries, meta-analyses, clinical tissue studies, or ex vivo human-bone	Beta-blocker cohorts; fracture or BMD registries; human bone receptor-	Indication bias; residual confounding; selection bias; drug-class pooling; no direct causal attribution

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	observations provide evidence relevant to the claim	expression or functional observations	
H0—No direct human evidence	No human study directly addresses the specific claim; human relevance is inferred from animal, cellular, or ex vivo nonhuman evidence	Preclinical-only claim	No inference regarding human mechanism, effect size, efficacy, or safety should be made

Note: M and H classifications are independent dimensions. H2, H1, and H0 describe the presence and design of direct human evidence rather than overall study importance or therapeutic efficacy. A claim may have strong mechanistic support and little human evidence, or substantial human observational relevance and limited causal certainty.

Panel B. Intervention-specific interpretation of direct skeletal, local microenvironmental, and systemic effects

Intervention or evidence type	Inference directly supported	Inference not independently supported	Minimum accompanying measurements or controls
Cell- or lineage-specific adrenergic receptor manipulation	Direct receptor–cell contribution within the targeted lineage, provided targeting and receptor engagement are validated	Whole-organism therapeutic efficacy; absence of developmental, compensatory, or paracrine effects	Targeting specificity, recombination efficiency, receptor engagement, developmental compensation, off-target recombination, and site-specific structural and mechanical endpoints
Local bone-targeted neural or pharmacological intervention	Local neural or adrenergic pathway involvement within the treated skeletal compartment	Exclusive receptor-subtype or skeletal-cell attribution; absence of vascular, inflammatory, sensory, or systemic effects	Local and systemic exposure, intervention extent, perfusion, inflammatory indices, sensory-fiber integrity, activity, and mechanical loading
Whole-body receptor or signaling manipulation	Organism-level contribution of the manipulated receptor or pathway	Dominant skeletal cell source; absence of developmental or systemic compensation	Body weight and composition, food intake, activity, blood pressure or hemodynamics, energy metabolism, receptor compensation, and cell-specific follow-up
Systemic adrenergic pharmacology or chemical/surgical sympathectomy	Pharmacological or neural-pathway responsiveness at the organism level	Direct bone-cell causality; exclusive local skeletal mechanism; unique receptor-subtype attribution	Dose and exposure, heart rate and blood pressure, activity and loading, body weight and food intake, perfusion, energy-metabolic indices, stress-axis measures, inflammation, and intervention selectivity
Human observational drug exposure	Population-level association between drug exposure and skeletal outcomes	Causality, receptor subtype, target-cell attribution, or therapeutic efficacy	Cardiovascular indication, adherence, dose and duration, comorbidity, concomitant medications, frailty and activity, blood pressure, falls, and separate assessment of BMD, microarchitecture, turnover, and fracture outcomes

Note: Panel B defines the level of causal attribution supported by each intervention or evidence type and does not rank overall biological or clinical importance. Direct skeletal attribution requires selective manipulation of the relevant receptor, neural input, or target-cell population together with adequate assessment of local microenvironmental and systemic consequences.

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BMD, bone mineral density. **Endpoint interpretation:** Bone-turnover markers and dynamic histomorphometry are remodeling-process endpoints; BMD and imaging-derived microarchitecture are density and structural endpoints, respectively; biomechanical testing provides direct strength-related evidence; and fracture incidence is a hard clinical outcome influenced by skeletal strength, falls, trauma exposure, follow-up duration, and competing risks. Improvement in one endpoint category should not be assumed to establish improvement in another. **Measurement hierarchy for intraosseous NE signaling:** TH/DBH immunolabeling, neural tracing, and three-dimensional neural mapping establish sympathetic-fiber distribution and neurovascular proximity but do not measure extracellular norepinephrine (NE) exposure. Bulk tissue NE assays quantify average tissue abundance but do not resolve spatial gradients, temporal dynamics, or cell-specific exposure. Adrenergic receptor expression and downstream signaling readouts indicate receptor availability or pathway engagement but do not establish extracellular NE concentration. Direct demonstration of an intraosseous NE microdomain requires spatially and temporally resolved measurement of extracellular NE in living bone. Accordingly, anatomical proximity, bulk tissue NE content, receptor/pathway activation, and direct extracellular NE dynamics should be treated as distinct and non-interchangeable evidence domains.