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Supplementary information

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The hallmarks of skeletal muscle health

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Supplementary Table 1: Hallmark Measures and Modulators - Metabolism & Bioenergetics

Characteristic	Biomarkers / measures (examples)	Modulators (examples)	Refs
Metabolism & Bioenergetics			
Metabolic flexibility and fuel use	**Biological measures:** • Substrate oxidation rates (indirect calorimetry; respiratory exchange ratio) • Lipid vs. carbohydrate oxidation during fasting, feeding, and exercise • Fiber type composition (type I predominantly oxidative vs. type II predominantly glycolytic) • Insulin sensitivity **Biomarkers:** • Circulating acylcarnitine profiles • Fasting and postprandial substrate utilization (indirect calorimetry) • Fasting insulin concentration	• Endurance and resistance exercise training • Nutritional strategies (caloric restriction, time-restricted feeding, dietary composition)	1–5
Mitochondrial quality control and bioenergetic capacity	**Biological measures:** • Mitochondrial oxidative capacity: ^{31}P-MRS (PCr recovery kinetics), near-infrared spectroscopy • High-resolution respirometry of biopsy specimens (ex vivo) • Mitochondrial volume density and cristae density (electron microscopy) **Biomarkers:** • Circulating GDF-15, FGF-21 (mitochondrial stress markers) • Citrate synthase activity (biopsy-based mitochondrial mass proxy)	• Exercise (acute: fission/mitophagy; chronic: fusion/biogenesis) • PGC-1α pathway activation • NAD^+ precursors (NMN, NR) • AMPK activators (e.g., metformin, AICAR) • Cardiolipin stabilizers • Mitophagy enhancers	6–12
Aerobic capacity and oxidative function	**Biological measures:** • Maximal oxygen uptake (VO_2max; cycle or treadmill ergometry) • VO_2max as an integrated measure of skeletal muscle capillary density, oxygen extraction, and mitochondrial respiration, rate-limiting in sedentary individuals, small muscle group exercise, and pathological states including type 2 diabetes, peripheral artery disease, mitochondrial myopathies, and aging **Biomarkers:** • Peak VO_2 relative to body mass (mL/kg/min) • Ventilatory threshold	• Endurance exercise training • Combined endurance and resistance training	13–15
Vascularization and insulin signaling	**Biological measures:** • Capillary-to-fiber ratio and capillary density (histological biopsy) • Flow-mediated dilation and contrast-enhanced ultrasound • Euglycemic hyperinsulinemic clamp (gold standard for insulin sensitivity) • Insulin signaling in muscle and fat biopsies	• Endurance and resistance exercise (VEGF-mediated angiogenesis) • Weight loss • Myostatin/activin pathway inhibitors (lean	16–22

	Biomarkers: • Fasting plasma insulin, HOMA-IR • ^{18}F-FDG PET/MRI (regional muscle glucose uptake) • GLUT4 and hexokinase expression (biopsy)	mass preservation during weight loss)	

Supplementary Table 2: Hallmark Measures and Modulators - Proteostasis

Characteristic	Biomarkers / measures (examples)	Modulators (examples)	Refs
Proteostasis			
Anabolic signaling and protein synthesis	**Biological measures:** • mTOR pathway phosphorylation (4EBP1, S6K1, ribosomal protein S6) • Ribosomal content / ribosome biogenesis markers • Labeled amino acid incorporation (puromycin/SUnSET, azidonorleucine) **Biomarkers:** • AKT and FoxO phosphorylation status • Fractional synthetic rate (stable isotope tracer methods)	• Resistance exercise and mechanical loading • Nutritional support (protein timing, leucine enrichment, essential amino acids) • IGF-1/insulin signaling • Rapamycin (mTORC1 inhibition to restore autophagic balance in aging)	23–28
Autophagy–lysosome system	**Biological measures:** • Autophagic flux: LC3-II, p62/SQSTM1 accumulation with lysosomal inhibitors (colchicine, bafilomycin, pepstatin A/leupeptin) • Mitophagy markers: BNIP3, PINK1/Parkin • Immunofluorescent detection of p62- or ubiquitin-positive puncta/aggregates **Biomarkers:** • LC3/Gabarap transcript and protein ratios (steady-state vs. flux) • FoxO target gene expression (transcriptional readout of catabolic drive)	• Exercise (activates AMPK–ULK1, AMPK–FoxO pathways) • Caloric restriction and fasting • Mitophagy enhancers • Spermidine (autophagy inducer) • Herbal terpenoids (autophagy/mitophagy modulation)	29–35
Ubiquitin–proteasome system (UPS)	**Biological measures:** • E3 ubiquitin ligase expression (MuRF1, Atrogin-1/MAFbx) at transcript and protein level • Polyubiquitinated protein accumulation (with/without proteasome inhibition: MG262, bortezomib) **Biomarkers:** • MuRF1/Atrogin-1 mRNA (note: protein may not mirror transcript due to autoubiquitination) • K48-linked polyubiquitin conjugates in biopsy	• FoxO transcription factor regulation • Proteasome activity modulation • Nutritional and hormonal optimization to reduce catabolic drive	36–39
Chaperone networks and protein folding	**Biological measures:** • HSP70, HSP90, small HSP expression • Unfolded protein response markers (PERK, eIF2α phosphorylation, ATF6) • Protein aggregate burden (immunohistochemistry) **Biomarkers:** • ER/SR stress markers (spliced XBP1, CHOP)	• Exercise-induced chaperone upregulation • Pharmacological chaperones (under investigation)	40–42

Supplementary Table 3: Hallmark Measures and Modulators - Genomics

Characteristic	Biomarkers / measures (examples)	Modulators (examples)	Refs
Genomics			
Transcriptional programs and fiber identity	**Biological measures:** • Fiber type–specific transcriptional profiles (MEF2, FoxO, PGC-1α, Six1/4, Maf) • Enhancer and super-enhancer activity (H3K27ac ChIP-seq) • Chromatin accessibility mapping (ATAC-seq) **Biomarkers:** • Myosin heavy chain isoform expression (MYH7, MYH2, MYH1, MYH4) • Fiber type proportion shifts on biopsy	• Exercise training (widespread enhancer remodeling) • Activity-responsive kinase signaling (CaMK, calcineurin) • Endocrine inputs (thyroid hormone, sex steroids)	5,43–46
Myonuclear specialization and multicellular regulation	**Biological measures:** • Single-nucleus RNA-seq and multiomics (snRNA-seq, snATAC-seq) • Spatial transcriptomics of myonuclear domains (NMJ, MTJ, body nuclei) • FAP and immune cell transcriptional trajectories **Biomarkers:** • Subsynaptic vs. extrasynaptic nuclear gene signatures • FAP fibrogenic vs. supportive state markers ($PDGFR\alpha^+$ subtypes)	• Neural input and mechanical load • Innervation status • Niche-derived signals (FAP, immune, endothelial transcriptional states)	47–51
Genetic architecture of muscle traits	**Biological measures:** • GWAS loci associated with lean mass and strength (HLA-DQA1, GDF5, IRS1, FTO, VCAN) • Polygenic risk scores for muscle traits • ACTN3 R577X genotyping **Biomarkers:** • Heritability estimates of strength/mass phenotypes • Pathogenic variant identification (WES/WGS for myopathies)	• Genetic counseling and diagnosis (for monogenic disorders) • CRISPR-based genome editing for monogenic muscle diseases • Antisense oligonucleotides / exon skipping	52–56
Epigenetic regulation and muscle aging	**Biological measures:** • Genome-wide DNA methylation profiling (450K/EPIC arrays, WGBS) • Muscle-specific epigenetic clocks (biological age estimation) • Enhancer–promoter interaction mapping (Hi-C, HiChIP) **Biomarkers:** • Circulating myomiRs (miR-1, miR-133, miR-206) • Cell-free DNA methylation patterns • Epigenetic age acceleration (Δ biological – chronological age) • DNA methylation–based "muscle memory" signatures	• HDAC inhibitors (preclinical dystrophy models) • Partial epigenetic reprogramming (OSKM factors) • Senolytic clearance of epigenetically aged cells	57–63

Supplementary Table 4: Hallmark Measures and Modulators - Excitability

Characteristic	Biomarkers / measures (examples)	Modulators (examples)	Refs
Excitability			
Neuromuscular junction (NMJ) structure and signaling	**Biological measures:** • NMJ morphology (pre-/post-synaptic apposition, endplate area, fragmentation index) • Miniature endplate potential amplitude and quantal content (intracellular electrophysiology) **Biomarkers:** • Repetitive nerve stimulation EMG (decremental CMAP) • Fluorescent α-bungarotoxin / immunofluorescence of pre-synaptic markers in biopsy • Circulating C-terminal agrin fragment (CAF)	• Agrin–LRP4–MuSK signaling axis • MuSK agonist antibodies (e.g., ARGX-119) • PGC-1α / mitochondrial–NMJ signaling • HDAC6 inhibitors (preclinical DMD models)	64–68
Excitation–contraction (EC) coupling and ion channel function	**Biological measures:** • DHPR density and DHPR–RyR1 coupling ratio at triads • Sarcoplasmic reticulum Ca^{2+} release kinetics • Ca^{2+} imaging (Fluo-4, Fura-2) for real-time Ca^{2+} dynamics **Biomarkers:** • RyR1 calstabin1 (FKBP12) binding stoichiometry • Voltage-gated sodium channel expression / function	• RyR1 stabilizers • Strategies restoring DHPR expression • Exercise-induced improvements in Ca^{2+} handling	69–72
Motor unit integrity and age-related denervation	**Biological measures:** • Motor unit number estimation (MUNE) • Decomposition-based motor unit electrophysiology • Fiber type grouping and type II fiber loss on biopsy **Biomarkers:** • Compound muscle action potential (CMAP) amplitude • Motor unit firing rate and recruitment patterns	• Resistance and endurance exercise training • IGF-1 overexpression / signaling enhancement • Collateral sprouting / reinnervation support	73–77

Supplementary Table 5: Hallmark Measures and Modulators - Structure

Characteristic	Biomarkers / measures (examples)	Modulators (examples)	Refs
Structure			
Sarcomeric scaffold and force generation	**Biological measures:** • Sarcomere ultrastructure (electron microscopy) • Thin filament length (nebulin-dependent) • Titin isoform expression and passive tension • Single-fiber and whole-muscle force measurements (ex vivo, permeabilized fibers) Biomarkers: • Nebulin and titin isoform profiling (SDS-PAGE, mass spectrometry) • Desmin intermediate filament integrity (immunofluorescence)	• Gene-based correction (exon skipping, gene replacement therapy) • Utrophin upregulation • Chaperone support (HSP70/HSP90)	55,78–81
Force transmission: costameres and ECM	**Biological measures:** • Dystrophin and dystrophin-glycoprotein complex expression (immunofluorescence, Western blot) • Costameric protein integrity (vinculin, talin, integrins) • ECM composition: collagen content, crosslinking, alignment (Sirius red, Masson's trichrome) **Biomarkers:** • Serum creatine kinase (sarcolemmal damage marker) • MRI (fat/fibrotic infiltration), ultrasound shear-wave elastography (tissue stiffness) • Advanced glycation end-product (AGE) accumulation • Circulating P3NP as a collagen-turnover signal	• Anti-TGF-β approaches and antifibrotic agents • Integrin-based strategies for lateral force transmission • Microdystrophin gene therapy • Exercise to maintain ECM remodeling balance	68,82–86
Myosin energy states and structural–energetic coupling	**Biological measures:** • Super-relaxed (SRX) state proportion (mant-ATP chase assay) • Basal myosin ATPase activity • ATP turnover rates in resting muscle **Biomarkers:** • ^{31}P-MRS resting energetics (PCr/ATP ratio) • Indirect calorimetry (resting metabolic rate)	• Myosin modulators targeting SRX↔DRX equilibrium • Glycogen sparing strategies • Mitochondrial support (NAD^+ precursors) • AMPK–mTORC1 axis tuning	87–90
Contractile function and fatigue	**Biological measures:** • Maximum voluntary contraction (MVC) / peak torque (isokinetic dynamometry) • Grip strength dynamometry (applicable in both rodents and humans) • In vivo nerve stimulation force, ex vivo direct stimulation	• Resistance and combined exercise training • Nutritional support (protein, creatine) • Combination therapies addressing structure + metabolism	74,91,92

	• Fatigue index (force decline during repeated stimulation) **Biomarkers:** • Handgrip strength, knee extension torque • Fiber type distribution and cross-sectional area (biopsy)		

Supplementary Table 6: Hallmark Measures and Modulators - Regeneration

Characteristic	Biomarkers / measures (examples)	Modulators (examples)	Refs
Regeneration			
Satellite cell intrinsic regenerative capacity	**Biological measures:** • Satellite cell quiescence and injury-induced activation • Myogenic progenitor proliferation, differentiation, and self-renewal • Myogenic lineage fidelity during regeneration **Biomarkers:** • Quiescence/activation: Pax7, Notch3, FoxO, mTORC1 • Proliferation/differentiation: Myf5, MyoD, Ki67, myogenin • Regenerating fibers: embryonic myosin heavy chain (eMHC)	• Exercise • Nutritional interventions (fasting, calorie restriction) • Signaling and metabolic pathways (Notch, p38MAPK, AMPK–mTOR–FoxO) • Epigenetic regulators (HDACs, SIRT1)	93–96
Extrinsic regulation of regeneration	**Biological measures:** • Satellite cell–niche crosstalk • Transient ECM remodeling and FAP dynamics • Inflammatory response and resolution during repair **Biomarkers:** • Niche components: PDGFRα^{+} FAPs, ECM deposition (Sirius red staining) • Immune cells: macrophage polarization (M1 → M2), Treg abundance • Cytokines: TNFα, IL-10, TGFβ	• Growth factors and morphogens (FGF, Wnt, prostaglandins) • Immune-modulating pathways • Anti-fibrotic and anti-inflammatory interventions • Senescence-associated secretory phenotype (SASP) modulation	97–101
Muscle regenerative capacity and recovery	Biological measures: • Muscle fiber repair and architectural restoration • Resolution of inflammation and fibrosis • Functional recovery after injury **Biomarkers:** • Number and size of newly formed myofibers (eMHC^{+} regenerating fibers) • Fibrotic content (Sirius red staining); collagen content and inflammatory markers	• Exercise and myokines • Nutritional modulation • Mitochondrial and autophagy enhancers • Senolytics / senomorphics • Partial reprogramming strategies (OSKM)	102–106

Supplementary Table 7: Hallmark Measures and Modulators - Crosstalk

Characteristic	Biomarkers / measures (examples)	Modulators (examples)	Refs
Crosstalk			
Myokine secretion and inter-organ signaling	**Biological measures:** • Circulating myokine profiles: IL-6, irisin, meteorin-like, BDNF, myostatin, GDF-15 • Muscle-derived extracellular vesicle cargo analysis **Biomarkers:** • Plasma IL-6 kinetics during/after exercise • Circulating irisin, FGF-21, GDF-15 levels • Myostatin / follistatin ratio	• Resistance and endurance exercise (distinct myokine signatures) • PGC-1α–dependent myokine regulation	107–111
Endocrine regulation of muscle (hormones, adipokines, hepatokines)	**Biological measures:** • Serum testosterone, estradiol, IGF-1, insulin, cortisol • Adipokine profiles (adiponectin, leptin) • Hepatokine profiles (FGF-21, follistatin) **Biomarkers:** • IGF-1 / IGFBP-3 ratio • Sex steroid levels relative to age/sex norms • Insulin sensitivity indices (HOMA-IR)	• Hormone replacement therapy (testosterone, estrogen) • GLP-1 receptor agonists (systemic metabolic improvement) • Myostatin/activin pathway inhibitors • Nutritional optimization	22,112–115
Immune–muscle interactions and inflammatory status	**Biological measures:** • Intramuscular immune cell composition (macrophage M1/M2 ratio, Treg abundance) • Systemic inflammatory markers: CRP, TNFα, IL-1β, IL-10 • Muscle biopsy: immune cell infiltration, SASP factor expression **Biomarkers:** • hs-CRP, IL-6/IL-10 ratio (systemic inflammation index) • Muscle-specific inflammatory gene expression	• Exercise (anti-inflammatory myokine release) • Anti-inflammatory interventions (IL-6 pathway modulation) • Senolytic/senomorphic therapies (SASP reduction) • Omega-3 fatty acids	99–101,116,117
Metabolite-mediated crosstalk (muscle–liver–adipose–brain axis)	**Biological measures:** • Lactate, succinate, β-aminoisobutyric acid (BAIBA), kynurenine pathway metabolites • Metabolomic profiling (targeted and untargeted) **Biomarkers:** • Circulating lactate, BAIBA levels • Kynurenine / tryptophan ratio	• Exercise-induced metabolite flux • Kynurenine aminotransferase modulation (PGC-1α–dependent) • Nutritional interventions	118–120

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Supplementary Table 8: Clinical trial endpoints for muscle health

Endpoint category	Measure	How it's measured	What it captures	Best-fit indications / notes	Refs
Function & performance					
Composite performance	**SPPB**	Standardized battery (balance, chair stands, gait speed)	Global lower-extremity function; strongly linked to disability risk	Sarcopenia, frailty, aging trials; useful co-primary with PRO	[1]
Gait function	**Usual gait speed**	Timed walk over short distance	Mobility, survival/disability risk marker	Sarcopenia/aging; interpret with sex/height norms	[2]
Submax endurance	**6MWT**	6-minute walk distance	Integrated functional capacity (cardiopulmonary + muscle)	Sarcopenia, cachexia supportive; DMD trials commonly use	[3,4]
Longer-distance capacity	**400m walk**	Timed 400-m walk	Higher-demand mobility endpoint	Aging/sarcopenia; helpful for "real-world" mobility	[5,6]
Transfer/mobility	**TUG**	Timed up-and-go	Balance + transitional mobility	Older/frail; pragmatic clinic endpoint	[7,8]
Timed functional tasks	**Chair-rise, stair-climb power**	Standardized timing/power	Leg power and functional reserve	Sarcopenia/cachexia; sensitive to meaningful change	[1,9–11]
Performance status (oncology)	**ECOG / Karnofsky**	Clinician-rated scales	Global functional impact	Cachexia/oncology programs; pairs well with PROs	[12–14]
Disease-specific function (dystrophy)	**NSAA**	17-item ambulatory scale	Motor performance in ambulatory DMD	DMD therapeutic trials; complements 6MWT	[15–17]
Disease-specific motor (SMA)	**HFMSE**	Standardized motor scale	Motor function in SMA II/III	SMA trials; clinically meaningful change established	[18,19]
Upper limb function (SMA)	**RULM**	Standardized upper-limb module	Upper limb trajectory and treatment response	SMA trials (esp non-ambulant)	[20,21]
Strength					
Global strength	**Handgrip**	Dynamometry	Convenient proxy for overall strength and outcomes	Sarcopenia definitions/diagnosis; interpret with sex-specific cut-points	[22–25]
Lower-limb strength	**Knee extensor torque**	Isokinetic/ isometric dynamometry	More specific, responsive strength endpoint	Sarcopenia/aging; rehab/exercise and anabolic programs	[25–27]
Myopathy strength	**MMT (incl. MMT-8 variants)**	Standardized manual testing	Multimuscle strength profile	Myositis/myopathies (often paired with function + biomarkers)	[28]
Muscle quantity (mass)					

Lean mass (clinical standard)	**DXA ALM / ALMI**	DXA appendicular lean mass	Widely used quantity endpoint	Sarcopenia consensus definitions; not equivalent to strength/function	[29–32]
Estimation (field/clinic)	**BIA → ASMI**	Bioimpedance equations	Estimated lean/appendicular skeletal muscle mass	Screening/large studies; more variability vs DXA/MRI/CT	[33–35]
Anatomical bulk	**MRI/CT CSA (thigh, L3)**	Imaging cross-sectional area	Regional muscle size	Sarcopenia/cachexia; oncology CT routinely available	[36–39]
Bedside imaging	**Ultrasound thickness/ pennation**	Standardized US	Local muscle size, architecture, echogenicity	ICU, geriatrics, pragmatic trials; operator dependent	[38,40–42]
Direct contractile mass	**D3-creatine dilution muscle mass**	Stable isotope + urine sampling	More "muscle-specific" mass than DXA	Aging/sarcopenia; correlates with function/outcomes better than DXA in some cohorts	[43–45]
Muscle quality					
Myosteatosis proxy	**CT muscle attenuation (HU)**	CT density of muscle	Fat infiltration/"low density" muscle linked to outcomes	Cachexia/oncology, aging; predicts risk beyond mass	[46–48]
Fat fraction	**MRI PDFF / fat fraction**	Quantitative MRI	Intramuscular fat burden	Sarcopenia, metabolic disease; harmonizable across sites with protocols	[49–51]
Fibrosis/ECM	**MRI (T1/T2 mapping), elastography (emerging)**	Imaging-based tissue characterization	Fibrosis/ architecture changes (emerging)	Early-stage trials; needs standardization	[52–54]
Histology/ omics (subset)	**Muscle biopsy**	Fiber CSA, fibrosis, myonuclei, proteostasis markers	Mechanistic depth, target engagement	Myopathies/rare disease; feasibility limits for broad sarcopenia trials	[55–59]
Patient-reported outcomes					
Sarcopenia-specific QoL	**SarQoL**	Questionnaire	QoL impact specific to sarcopenia	Sarcopenia trials; pairs well with performance co-primary	[60]
Cancer QoL	**EORTC QLQ-C30**	Questionnaire	Cancer QoL domains incl physical function, fatigue	Cachexia/oncology trials	[61]
Fatigue/ function (oncology)	**FAACT / anorexia-cachexia modules**	Questionnaire	Appetite/anorexia + cachexia-related QoL	Cachexia programs where appetite/weight loss are key	[62]

Generic function	**PROMIS Physical Function / Fatigue**	Questionnaire	Cross-disease, comparable function/fatigue	Useful for mixed myopathies/longitudinal cohorts	[63,64]
Metabolism & Muscle Metabolic Health					
Whole-body insulin sensitivity	**Hyperinsulinemic–euglycemic clamp (M value)**	Clamp physiology	Gold-standard insulin sensitivity	Metabolic-flexibility interventions; higher burden but clean signal	[65–68]
Tissue-specific glucose uptake	**Clamp + 18F-FDG PET (± PET/MRI) or leg balance for glucose**	Imaging during clamp	Regional skeletal muscle glucose uptake	Mechanism-focused trials; bridges systemic to muscle-specific effects	[68–70]
Substrate utilization	**Indirect calorimetry (RER, oxidation rates)**	Metabolic cart	Metabolic flexibility (lipid↔carb switching)	Useful across sarcopenia/metabolic disease; interpret with sex differences	[39,71,72]
Pragmatic glycemic endpoints	**OGTT/ITT (or GTT/ITT)**	Standard tests	Global glucose handling (not muscle-specific)	Supportive endpoints; best when paired with clamp/PET if muscle claim is central	[73–75]

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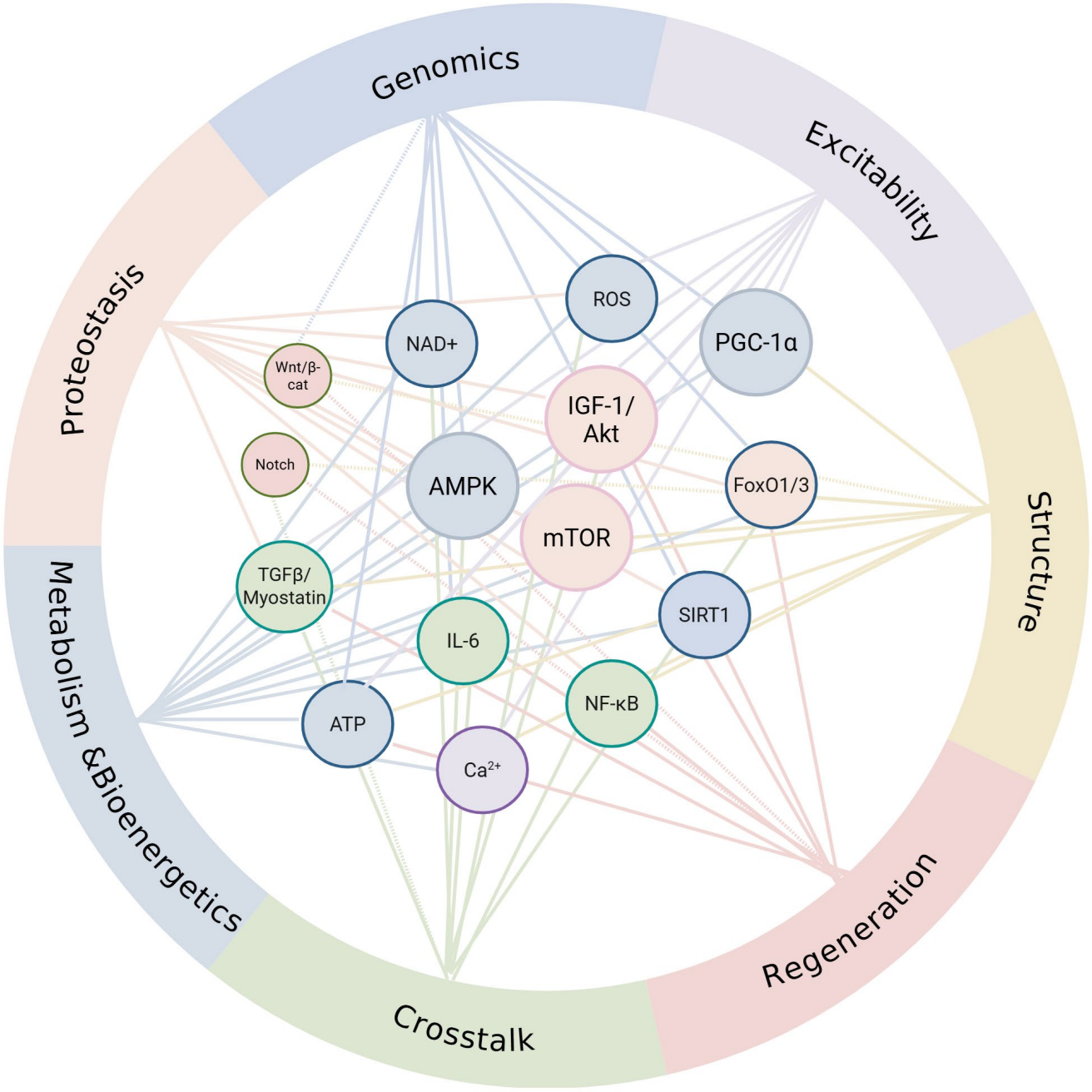

Supplementary Figure 1. Molecular nodes bridging the seven hallmarks of skeletal muscle health. Colored arcs on the outer ring represent the seven hallmarks. Molecular integrators are shown as circles colored according to the hallmark from which they primarily signal, with node size reflecting tier ranking. Master regulators (mTORC1, AMPK, PGC-1α, IGF-1/Akt) and key integrators (FoxO1/3, SIRT1, NF-κB, TGFβ/Myostatin, NAD^+, ATP, Ca^{2+}, ROS) are connected to the hallmarks they regulate by solid lines (direct signaling axes) or dashed lines (context-dependent modulation). Pathway modulators Notch and Wnt/β-catenin (dashed connections) denote myogenic-to-fibrogenic fate switching. The network illustrates that no hallmark operates in isolation; signals propagate across domains through shared molecular mediators. **Abbreviations:** AMPK, AMP-activated protein kinase; Ca^{2+}, Calcium; FoxO, Forkhead box O; IGF-1, insulin-like growth factor 1; mTORC1, mechanistic target of rapamycin complex 1; NAD^+, nicotinamide adenine dinucleotide; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; ROS, reactive oxygen species; SIRT1, sirtuin 1; TGFβ, transforming growth factor beta; Wnt, Wingless/integrated signaling pathway. Figure created in BioRender.