

Conserved biological response states organize nanoparticle biosafety across neurological systems

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1. SUPPLEMENATRY METHODS

1.1. Literature survey and data acquisition

We conducted a literature survey to identify studies reporting nanoparticle-associated biosafety and recovery responses in neurological systems such as Brain injury, Brain disease, Spinal cord injury, Peripheral nerve injury. Articles published between 1994 and 2025 were retrieved from PubMed, Web of Science, and Scopus using combinations of nanomaterial-related terms (“nanoparticle”, “nanomaterial”, “quantum dot”, and “nanoformulation”), biological-response terms (“toxicity”, “biosafety”, “inflammation”, “cytokines”, “apoptosis”, and “recovery”), and neurological context terms (“brain”, “spinal cord”, and “peripheral nerve”). The search was supplemented by screening reference lists and citing articles and by manual searches of relevant journals, including *ACS Nano*, *Advanced Materials*, *Nature Nanotechnology*, *Nature Communications*, *Biomaterials*, and *Nano Letters*. Study identification and eligibility screening were performed manually without the use of AI-based tools.

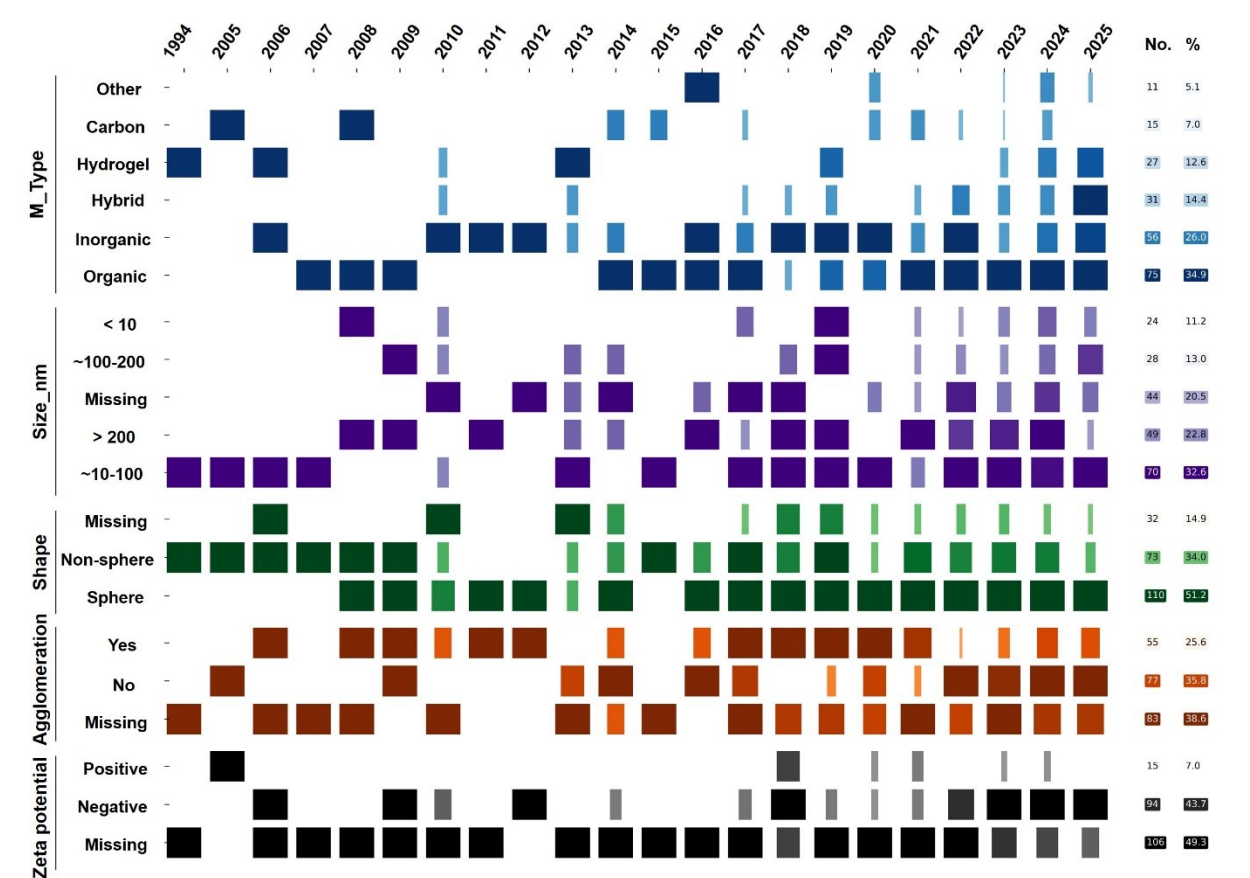
After eligible studies were selected, ChatGPT-4 and SciSpace were used only to assist retrieval of relevant physicochemical, exposure, and biological information from article PDFs. These tools were used only to facilitate information retrieval and were not used to determine study eligibility, interpret experimental findings, or assign biological-response programme states, biosafety labels or recovery labels. All extracted information was independently checked by two domain experts against the corresponding information reported in the original publications before inclusion in the final dataset. Verified variables included nanoparticle characteristics and concentration, stimulant conditions, exposure parameters, administration route, dosing regimen, biological model characteristics, including animal age and sex where applicable, and reported biological responses and phenotypic or functional outcome relevant to biosafety and recovery.

Studies were eligible if they investigated engineered nanomaterials with reported physicochemical characteristics and provided sufficient experimental evidence to extract biological-response features and/or annotate biosafety- or recovery-related outcomes according to the predefined criteria. Studies were

excluded if they lacked primary experimental data, basic nanoparticle characterization, or sufficient biological information for the intended response and outcome annotations.

1.2. Dataset extraction and annotation

Reporting of nanoparticle characteristics and experimental conditions varied considerably among studies. We therefore harmonized the extracted information into common feature categories before analysis. Extracted variables included nanoparticle physicochemical characteristics, exposure conditions, biological context, and measured biological responses. Physicochemical descriptors were standardized into predefined categories, including material type, particle size, morphology, agglomeration status, and zeta-potential characteristics (Supplementary Fig. S1). A single publication could contribute multiple experimental contexts when it reported distinct nanoparticle formulations, exposure conditions, or biological systems. The final dataset comprised 893 experimental contexts derived from 599 independent studies.



Supplementary Fig. S1. Distribution of nanoparticle physicochemical descriptors across the dataset.

Distribution of harmonized physicochemical descriptors used for model development across 893 experimental contexts derived from 599 independent studies, including material type (M_Type), particle size (Size_nm), shape, agglomeration state, and zeta potential. Numbers and percentages indicate the number and relative representation of each descriptor category.

For each experimental context, we recorded nanoparticle characteristics, exposure conditions and biological context, literature-derived biological responses, and separately annotated outcome labels. Variables included nanoparticle concentration, experimental model, age, sex, weight, administration route and dosing regimen, together with pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated response features. Biological-response features were annotated according to the procedures described in Supplementary Methods Section 1.4, whereas biosafety and recovery were separately annotated as “Good” or “Bad” outcomes based on phenotypic and functional evidence according to the criteria described in Supplementary Methods Section 1.5. Biological-response features were therefore treated as model inputs and were not themselves used as outcome labels. Variables or outcomes that could not be supported by the reported evidence were retained as missing rather than inferred.

1.3. Nanomaterial classification and standardization

Nanomaterials were classified according to their composition and structural characteristics into five broad groups: inorganic, organic and polymeric, carbon-based, hybrid or composite, and hydrogel-based systems. More specific material and physicochemical information was retained within each group (Supplementary Table S1). Materials comprising more than one structural component, such as core–shell nanoparticles or polymer-coated inorganic nanoparticles, could be assigned to more than one category and were represented using separate binary variables.

Physicochemical descriptors were converted into predefined categories to allow comparison across studies. Particle size was classified using the reported core or hydrodynamic diameter, and morphology was grouped as spherical, rod-like, tubular, sheet-like, fibrous, or irregular. Agglomeration was recorded as present or absent based on dynamic light scattering (DLS) or electron microscopy data reported in the original study. Surface charge was classified from the reported zeta potential as positive ($> +10$ mV), neutral (-10 to $+10$ mV), or negative (< -10 mV).

1.4. Literature-based biological response annotation and CRA feature construction

Each eligible experimental context was manually reviewed to identify the biological responses reported following nanoparticle exposure. For each context, the biological responses reported in the original publication were evaluated and assigned to one or more of three higher-order biological programmes: pro-inflammatory, anti-inflammatory (resolution-associated), or apoptosis-associated. Literature-assisted tools, including SciSpace, were used only to facilitate identification of relevant passages and descriptions of biological responses; final interpretation and CRA assignment were performed through manual review of the original publication.

Programme-level states were assigned according to the direction of the biological response reported in the original study. Responses described as increased, elevated, activated, induced, or enhanced were classified as high, whereas responses described as decreased, reduced, suppressed, inhibited, or attenuated were classified as low. Directional assignments were based on the author's reported experimental findings relative to the corresponding control or reference condition, where available. The reported biological response and its supporting experimental evidence were considered together when assigning the programme and its state. When the available information was insufficient to support a programme-level assignment, the value was recorded as missing rather than inferred.

Table S1. Standard labeling of heterogeneous nanomaterials and associated physicochemical descriptors

Inorganic	Organic	Carbon	Composite nanomaterials	Hydrogel	Others	Size (nm)	Shape	Agglomerat-ion	Zeta Potential
Metal nanomaterial	Protein-based material	Carbon nanostructures	Lipid composite	Base hydrogels	Extracellular vesicle systems	>10	Sphere	Yes	Positive
Metal oxide nanomaterial	Polymer-based material	Carbon dots	Polymer composite	Biopolymer hydrogels	Carbon-based systems	~10-100	Non-sphere	No	Negative
Bimetallic nanomaterial	Co-polymer material	Carbon nanotubes	Polysaccharide composite	Polymer hydrogels	Core-shell nanostructures	~100-200			
Polymer-associated metal	Polysaccharide-based material	Graphene-based	Hydrogel composite	Nanoparticle-incorporated hydrogels	Hydrogel-based systems	>200			
Biopolymer-associated metal	Dendrimer-based material	Carbon-metal hybrid	Carbon composite	Carbon / nanostructure hydrogels	Silica-based systems				
Protein/ECM-associated metal	Lipid-based material	Carbon-polymer hybrid	Metal composite	Metal / metal-oxide hydrogels	Metal / metal oxide systems				
Peptide-associated metal	Hydrogel-based polymer	Carbon-biopolymer hybrid	Biomimetic composite	Composite / multi-component hydrogels	Lipid-based systems				
Carbon-associated metal	Scaffold / biomaterial systems	Carbon-hydrogel hybrid	Biofunctional composite	Biomimetic hydrogels	Magnetic / hybrid polymer systems				
Conductive polymer-associated metal	Carbon-associated polymer	Carbon-silica hybrid	Engineered composite	Drug-loaded / bioactive hydrogels	Peptide-functionalized systems				
Functionalized metal	Metal-associated polymer	Carbon-protein hybrid	Functionalized composite	Functionalized hydrogels	Drug-loaded systems				
Nanofiber/scaffold-associated metal	Silica / inorganic-associated polymer	Carbon-carrier hybrid	2D composite	Coordination / hybrid hydrogels	Bimetallic systems				

Hydrogel-integrated metal	Drug-loaded polymer systems	Carbon-metal oxide hybrid	Enzyme-mimic / catalytic hydrogels	Nanocomplex systems
Drug-loaded metal	Functionalized polymer systems	Functionalized carbon	Specialized engineered hydrogels	
Silicate/clay-based systems	Nanotube-associated systems Micelle / mesoporous systems Biomimetic systems			

Notes:

Inorganic materials include a wide range of metal-based and hybrid nanomaterials. Metal nanomaterials comprise silver nanoparticles (AgNPs), gold nanoparticles (AuNPs), magnetic nanoparticles (MNPs), iron oxide nanoparticles (IONPs) and superparamagnetic iron oxide nanoparticles (SPIONs). Metal oxide nanomaterials include cerium oxide nanoparticles (CeO₂ NPs), titanium dioxide nanoparticles (TiO₂ NPs), zinc oxide nanoparticles (ZnO NPs), silicon dioxide nanoparticles (SiO₂ NPs) and metal oxide nanostructures (MONs). Bimetallic nanomaterials include cerium–manganese nanoparticles (Ce–Mn NPs) and other bimetallic nanoparticle systems (BMNPs). Polymer-associated metal nanomaterials include poly(lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), PLGA–PEG copolymers (PLGA–PEG), poly(ε-caprolactone-co-lactide) (PLCL), poly(glycolic acid) (PGA) and polyurethane (PU). Biopolymer-associated metal systems include chitosan (CS), alginate (Alg), hyaluronic acid (HA), heparin (Hep), cellulose (Cel) and keratin (Ker). Protein or extracellular matrix-associated systems include collagen (Col), type I collagen (Col I), silk fibroin (SF), elastin-like protein (ELP), fibronectin (FN) and Matrigel (MG). Peptide-associated systems include polypeptides (PPs), RAD16-I peptide systems (RAD16-I) and DNA-peptide systems (DNA-Pep). Carbon-associated metal nanomaterials include graphene oxide (GO), graphene sheets (GNs), single-walled carbon nanotube-PEG systems (SWNT-PEG) and graphene oxide-based systems (GO-based). Conductive polymer-associated systems include polyaniline (PANI), polypyrrole (PPy) and polyaniline–cellulose composites (PANI-Cel). Functionalized metal systems include thiolated hyaluronic acid (SH-HA), hydroxypropyl-β-cyclodextrin (HP-β-CD) and PEGylated systems. Nanofiber or scaffold-associated systems include electrospun silk fibroin (ES-SF), nanofibers (NFs) and collagen fibers. Hydrogel-integrated systems include hyaluronic acid–methylcellulose hydrogel (HAMC), gelatin methacryloyl (GelMA) and alginate hydrogels. Drug-loaded metal systems include drug-loaded nanoparticles (Drug-NPs) and betulinic acid-loaded nanoparticles (BA-NPs). Silicate or clay-based systems include Laponite (Lap) and mesoporous bioactive glass (MBG).

Organic materials comprise diverse biomolecular and polymeric systems. Protein-based materials include zein (Zein), general proteins (Prot), pectin–zein composites (Pec–Zein), chitin hydrogels (Chitin) and amygdalin–glycyrrhizic acid systems (Amy–GA). Polymer-based materials include poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLA), poly(2-hydroxyethyl methacrylate) (PHEMA), polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), poly(L-lactide-co-ε-caprolactone) (PLCL) and cross-linked hydrophilic polymers (CLHPs). Co-polymer materials include PLGA–PEG, polypyrrole-PLA (PPy–PLA) and PLA-b-PHEMA. Polysaccharide-based materials include chitosan (CS), β-chitosan (β-CS), alginate (Alg), hyaluronic acid (HA) and hyaluronic acid–methylcellulose (HAMC). Dendrimer-based systems include dendrimer–drug complexes (Dendrimer–Drug) and collagen-associated dendrimers (Col–Dendrimer). Lipid-based systems include solid lipid nanoparticles (SLNs). Hydrogel-based polymers include hydrogels (Hydrogel), PHEMA hydrogels (PHEMA-HG) and HAMC hydrogels. Scaffold or biomaterial systems include alginate scaffolds (Alg-Scaf), collagen scaffolds (Col-Scaf), silk fibroin systems (SF) and elastin-like biomaterials (ELP). Carbon-associated polymers include single-walled carbon nanotube–poly(aminobenzenesulfonic acid) (SWNT-PABS), graphene (Gr), graphene/CNT composites (Gr-CNT) and graphene oxide-based systems (GO-based). Metal-associated polymers include titanium dioxide (TiO₂), silver (Ag), gold (Au), copper oxide (CuO), iron oxide (Fe₃O₄, IONP) and manganese-based systems (Mn-based). Silica-associated polymers include silicon dioxide nanoparticles (SiO₂ NPs), silica-based systems (SiO₂-based) and Laponite (Lap).

Drug-loaded polymer systems include curcumin (Cur), rapamycin (Rap) and multi-drug systems (e.g., celastrol (Cel), metformin (Met)). Functionalized polymers include HA-BCN + PEG click systems (HA-BCN-PEG), ROS-responsive β -cyclodextrin (β -CD) and polydopamine-modified systems (PDA). Nanotube-associated systems include polymer nanotubes (Poly-NT), protein nanotubes (Prot-NT) and silk nanotubes (SF-NT). Micelle and mesoporous systems include micelles (Micelle) and mesoporous materials (Meso). Biomimetic systems include platelet-functionalized PLGA (PLT-PLGA) and membrane-coated systems (Mem-Coated).

Carbon-based nanomaterials include **Carbon nanostructures**: Nanostructured carbon, generic carbon systems; **Carbon dots**: Carbon dots (CDs), graphene quantum dots (GOQDs), GOQD-polymer systems; **Carbon nanotubes**: Single-walled and multiwalled carbon nanotubes; **Graphene-based**: Graphene, graphene oxide, reduced graphene derivatives; **Carbon-metal composite**: AuNPs, PEG-AuNPs, iron oxide (Fe_3O_4), maghemite ($\gamma\text{-Fe}_2\text{O}_3$), Fe-gallic acid systems; **Carbon-polymer composite**: PPy-PCL, PTMAc-PEG-PTMAc, PCL, PPy-coated systems; **Carbon-biopolymer composite**: Collagen, alginate, cellulose, chitosan, genipin-crosslinked systems; **Carbon-hydrogel composite**: HAMC, iECM, carbon nanoparticle hydrogels; **Carbon-silica composite**: Silicon dioxide, silica-polycaprolactone systems; **Carbon-protein composite**: Fibrin-based systems; **Carbon-carrier composite**: Dendrosome-based systems; Functionalized carbon: PEGylated and surface-modified carbon systems; Other: Unspecified or unclear materials.

Composite nanomaterials namely **Lipid composite**: Targeted liposome, Lipid-polymer, Polymer-lipid, Membrane-lipid, Lecithin-chitosan, Exosome-liposome; **Polymer composite**: PLGA, PLGA-hydrogel, Polymer-hydrogel, Polymer-peptide, Ppy-PLLA-ECM; **Polysaccharide composite**: Chitosan, Chitosan-lipid, Polysaccharide-chitosan, Chitosan-coated, Ch-DS; **Hydrogel composite**: Hydrogel-AgNPs, Carbon-hydrogel, Peptide hydrogel, Graphene oxide hydrogel, Highly superporous hydrogel; **Carbon composite**: Graphene oxide hydrogel, Carbon-hydrogel, Carbon nanotubes, Carbon dots (CDs), Human hair-derived CDs; **Metal composite**: Gold, TiO_2 , SiNP30, Metal-polymer, Protein-metal, AgNPs-hydrogel; **Biomimetic composite**: Platelet-CCR2 membrane, Exosome-liposome, Membrane-lipid; **Biofunctional composite**: Polymer-peptide, CAQK peptide, Protein-metal, Peptide hydrogel; **Engineered composite**: Composite, ADH/EDC crosslinked systems; **Functionalized composite**: Carboxylated, NAC-functionalized; **2D composite**: Graphene-based materials, Nanosheets, Sheets;

Hydrogel-based materials such as **Base hydrogels**: Hydrogel, collagen, collagen I, 2D/3D collagen; **Biopolymer hydrogels**: Chitosan, alginate, silk fibroin, laminin-based systems; **Polymer hydrogels**: PEG (polyethylene glycol), PLGA, PLCL, PAMAM dendrimers, CMC-PAMAM systems; **Nanoparticle-incorporated hydrogels**: SiO_2 nanoparticles, TiO_2 , silver (Ag), gold (Au), Zn-based systems, layered double hydroxides; **Carbon/nanostructure hydrogels**: Carbon nanotubes, PDA-based systems, mPDA-based systems; **Metal/metal-oxide hydrogels**: Titanium dioxide, MgO, CeO_2 , Cu/Se-based systems; **Composite hydrogels**: Multi-component systems including HAMC + PLGA, phenolic chitosan + Pluronic F, PEG-based polymer blends; **Biomimetic hydrogels**: Membrane-coated systems, extracellular vesicles (e.g., Müller glia-derived sEVs), liposome-integrated systems; **Bioactive/drug-loaded hydrogels**: Luteolin, quercetin, rutin, mangiferin, gallic acid, TMP, Rg1, edaravone, melatonin (MEL), bFGF, GDNF; **Functionalized hydrogels**: Mannitol-modified, biotinylated, folate-conjugated, glutathione-functionalized systems; **Coordination hydrogels**: Metal-ligand coordinated systems (e.g., Eu(III), NADH/AAL systems); **Catalytic/enzyme-mimic hydrogels**: DNase1 mimic, Ptzyme-based systems; **Engineered hydrogels**: Structurally designed systems (e.g., FNBCs-based, RSC-PLGA-MEL, T@RA@M).

Others includes **Extracellular vesicle systems**: Exosomes, EV, extracellular vesicles; **Carbon-based systems**: MWNT- NH_3^+ , oxMWNT- NH_3^+ , SWNTs; **Core-shell nanostructures**: Core-shell systems; **Hydrogel-based systems**: HA hydrogel, type I collagen hydrogel, GelMA-based systems; **Silica-based systems**: FITC- SiO_2 , SiNP100, SiNP400; **Metal/metal oxide systems**: Silver (Ag), TiO_2 , magnesium ions; **Lipid-based systems**: Liposome; Hybrid polymer systems: Thixotropic hybrid polymers; Peptide-functionalized systems: RGD (Arg-Gly-Asp) functionalized systems; **Drug-loaded systems** namely Curcumin-loaded systems; Bimetallic systems: Bimetallic nanostructures; Nanocomplex systems: Polymer/nanocomplex assemblies

Size

<10 nm: Ultra-small nanoparticles with dimensions below 10 nm; ~10-100 nm: Small nanoparticles ranging from approximately 10 to 100 nm; ~100-200 nm: Intermediate-sized nanoparticles ranging from approximately 100 to 200 nm; >200 nm: Large nanoparticles with sizes greater than 200 nm.

Shape

Non-sphere: needle, rod, nanosheets, nanofiber

Table S2. Harmonized Classification of Cell Types, Nanoparticle Concentrations, and Stimulus Categories

Glial	Neuronal	Primary	Macrophage	Other	NPs_Conc (ug/mL)	Stimulant	Stimulant_Conc(μg/ml)
BV2	SH-SY5Y	Primary microglia	RAW 264.7	THP-1	1-10	Inflammatory stimuli	1-10
Astrocytes	PC12	NSCs		J774A.1	10-100	Oxidative stress	10-100
Microglia	N2a	NPCs		Fibroblast	100-500	Parkinsonism stimuli	100-500
N9	HT22	hNSCs		nHDF	>500	Neurodegenerative peptide	>500
C6	NE-4C	mNSCs		L929		Growth/DF	
U373	NT2	iNSCs		HUVECs		Neurotoxins	
RSC96	Spiral ganglion	hNPCs		bEnd3		Drug/metabolic modulators	
SCs	C17.2	It-NES		rBMEC		NPs stimuli	
	FMCC	MSCs		BMVECs		Physical stimuli	
	Motoneurons	BMSCs		endothelial cells		Ischemia models	
		GMSCs		Cos-7			
		PDLSCs		HEK293T			
		Pre-MSCs		HSCs			
		iPSCs		PBECs			
		Mouse-iPS		Murine dendritic cells			
		Cortical neurons					
		Hippocampal neurons					
		DRG neurons					
		Primary E18					

Notes: **Inflammatory stimuli** include lipopolysaccharide (LPS), LPS combined with hydrogen peroxide (LPS + H₂O₂), LPS with tert-butyl hydroperoxide (LPS + tBHP), LPS with spinal cord injury (LPS + SCI), hydrogen peroxide (H₂O₂), LPS with adenosine triphosphate (LPS + ATP), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), LPS with IFN-

γ (LPS + IFN- γ), cytokine mixtures (Cytokines), hemin (Hemin), CpG oligodeoxynucleotides (CpG ODN) and double-stranded DNA (dsDNA). Oxidative stress stimuli (OxStress) include hydrogen peroxide (H_2O_2) and peroxy-TCM (peroxyTCM). Ischemia models (Ischemia) include oxygen–glucose deprivation (OGD), cobalt chloride ($CoCl_2$), OGD combined with LPS (OGD + LPS), oxygen–glucose deprivation/reperfusion (OGD/R) and OGD/R with repetitive transcranial magnetic stimulation (OGD/R + rTMS). Neurodegenerative peptide stimuli (NeuroPep) include amyloid beta ($A\beta$) and LPS combined with $A\beta$ (LPS + $A\beta$). Parkinsonism-related stimuli (Parkinsonism) include 1-methyl-4-phenylpyridinium (MPP^+) and combined oxidative–Parkinsonian stress (H_2O_2 + MPP^+). Neurotoxic stimuli (Neurotoxins) include glutamate (Glu) and N-methyl-D-aspartate (NMDA). Growth and differentiation factors (Growth/DF) include beta-nerve growth factor (β -NGF). Drug and metabolic modulators (Drug/Metabolic) include retinoic acid (RA), niclosamide (Niclo), okadaic acid (OA) and combinations such as OA with LPS (OA + LPS). Nanoparticle-induced stimuli (NP stimuli) include multi-walled carbon nanotubes (MWCNTs) and graphene (Gr).

Table S3. Harmonized Classification of Animal Models, Exposure Conditions, and Injury Models

Small rodents	Large mammals	Fish	Other vertebrates	Gender	Age_Weeks	NPs_Dose_mg/Kg	Dose_Regimen	Ad_route	Injury_Model
Mouse	Cat	Shark	<i>C.elegans</i>	Male	0-5	1-50	Single	Intravenous	Brain disease
Rat	Dog	Zebra		Female	5-10	>50	Multiple	Intranasal	Brain injury
	Rabbit			Other	>10			Intraperitoneal	Spinal cord injury
								Intracerebral	Peripheral nerve injury
								Oral gavage	
								Others	

Notes:

Ad_route_Others: Abdominal, aquatic, conduit, culture medium, dietary foods, environmental exposure, inhalation, intrahippocampal, microinjection, water borne exposure

Brain disease: Neurodegenerative diseases: Alzheimer/Alzheimer's disease/Alzheimer, Parkinson/Parkinson's disease, neuroinflammation, chronic stress, neurotoxicity (chemical-induced disease-like conditions), neuroprotective/neuroprotection studies, neuron degeneration, neuron inflammation, neuron differentiation (disease context), neurodevelopmental, neurodegenerative (general); Other CNS diseases: autoimmune encephalomyelitis, encephalopathy, Krabbe's/demyelination, neural damage (disease context), 3D neural culture (disease-adjacent), neurodifferentiation, neurovascular dysfunction (disease context).

Brain injury: traumatic brain, brain trauma model, focal brain injury, acutely injured brain, brain impairment, brain lesion, hippocampal injury, fetal brain impairment, spatial impairment, cerebral ischemia, ischemic stroke, cerebral infarction, ischemia–reperfusion, stroke, post-stroke, hemorrhagic stroke, intracerebral hemorrhage, brain neurotoxicity (injury-related), ischemic brain injury, cortex/stroke (injury context), brain model (injury context), BBB/neurovascular unit model (injury context).

Spinal injury: Spinal cord: spinal cord injury, spinal cord, spinal cord transection, spinal cord lesion, dorsal column transection, lateral hemisection, injured spinal cord, severe spinal cord injury, spinal cord recovery, spinal cord regeneration, spinal cord model, ONC injury (CNS/spinal regeneration context).

Peripheral nerve: sciatic nerve, sciatic nerve injury, sciatic nerve transection, sciatic nerve lesion, sciatic functional deficit, sciatic nerve regeneration, sciatic nerve defect, sciatic nerve models, sural nerve defect, tibial nerve, facial nerve defect, cavernous nerve transection, optic tract lesion (PNS context), nerve injury, nerve transection, nerve growth, nerve tissue engineering, peripheral nerve growth, peripheral nerve regeneration.

Examples of biological responses considered within the three programmes included inflammatory activation or suppression for the pro-inflammatory programme, anti-inflammatory or resolution-associated responses for the anti-inflammatory programme, and experimentally reported apoptotic responses for the apoptosis-associated programme. Annotation was not restricted to a fixed list of biomarkers; rather, the biological response reported in each publication was interpreted within its experimental context and mapped to the corresponding higher-order programme according to its reported biological function and direction of change.

Because the underlying studies used heterogeneous experimental approaches and biological systems, the programme-level annotation was designed as a qualitative biological abstraction of experimentally reported responses programmes rather than a quantitative normalization of individual measurements. *In vitro*, *ex vivo*, and *in vivo* contexts were retained separately in the dataset, while convergent biological responses were represented within the corresponding higher-order programme. This representation captures response identity and direction across heterogeneous experimental contexts without implying quantitative equivalence among assays, measurement platforms, or biological systems.

The resulting biological response programmes were encoded as pro-inflammatory, anti-inflammatory, and apoptosis-associated features and incorporated as the cellular-response (CR) layer in the hierarchical machine-learning analyses. Biological-response feature assignment was based exclusively on reported biological-response evidence and was performed without using the corresponding biosafety or recovery label as an annotation criterion. CR feature construction was therefore kept separate from the biosafety and recovery outcome annotations to minimize circularity and prevent target leakage.

1.5. Biosafety and recovery annotation criteria

Biosafety and recovery were annotated as separate binary outcomes for *in vitro* and *in vivo* experimental systems, following the criteria described in the main Methods. Biosafety reflected the presence or absence

of experimentally demonstrated adverse biological effects associated with nanoparticle exposure, whereas recovery described evidence of attenuation of an experimentally induced perturbation, restoration of biological function, or maintenance of function under the challenged condition. Outcome assignments were based on phenotypic or functional experimental evidence together with the biological interpretation reported in the original studies.

Each annotation was checked against the corresponding publication before inclusion in the dataset. Molecular or signalling changes alone were not used to infer biosafety or recovery outcomes in the absence of supporting evidence of cellular, tissue, physiological, or functional effects. Importantly, pro-inflammatory, anti-inflammatory, and apoptosis-associated programme states were not themselves used to define the biosafety or recovery labels. Outcome annotations were completed independently of the CR feature-assignment process and were not used as criteria for assigning CR programme states. When an experimental outcome or phenotypic measure was not reported clearly enough to support an assignment, the value was recorded as missing rather than inferred.

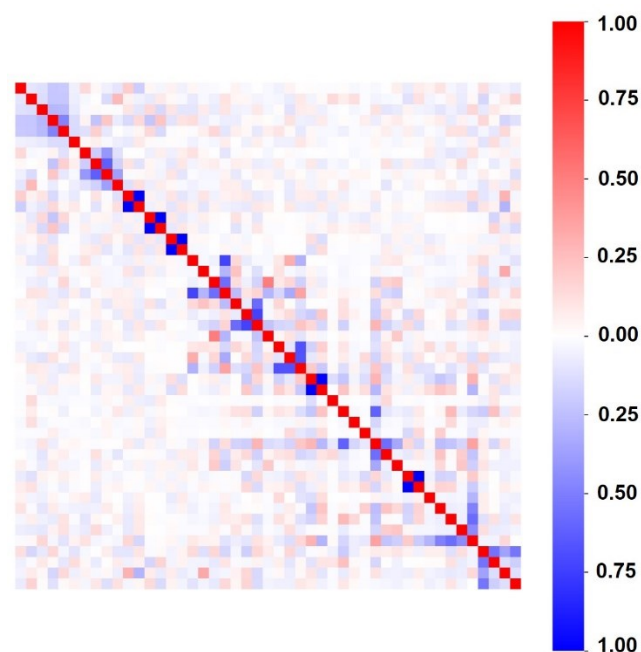
For *in vitro* contexts, Biosafety = Good was assigned when no nanoparticle-associated cellular injury or sustained functional impairment was reported based on the available phenotypic or functional evidence. Biosafety = Bad required experimentally demonstrated cellular injury, impaired viability or proliferation, persistent functional dysfunction, or another adverse phenotypic effect attributable to nanoparticle exposure. Recovery = Good required evidence of attenuation or improvement of an experimentally induced adverse state, whereas Recovery = Bad reflected persistence of the impaired state without meaningful improvement within the reported experimental timeframe.

For *in vivo* contexts, Biosafety = Good was assigned when nanoparticle exposure produced no measurable adverse physiological, histological, behavioural or functional effects, whereas Biosafety = Bad required evidence of tissue injury, organ dysfunction, systemic adverse effects or behavioural abnormalities supported by appropriate experimental assessments. Recovery = Good required reported improvement,

attenuation or reversal of an experimentally induced pathological or functional phenotype, whereas Recovery = Bad reflected persistence or progression of the impaired state within the reported timeframe.

1.6. Machine learning preprocessing and feature engineering

The compiled dataset contained missing values because physicochemical, exposure, and biological variables were not consistently reported across studies. The preprocessing and feature-engineering workflow is summarized in Supplementary Fig. S2.



Supplementary Fig. S2 | Correlation among features used for machine-learning analysis. Heatmap showing Pearson correlation coefficients for pairwise combinations of features included in model training and evaluation.

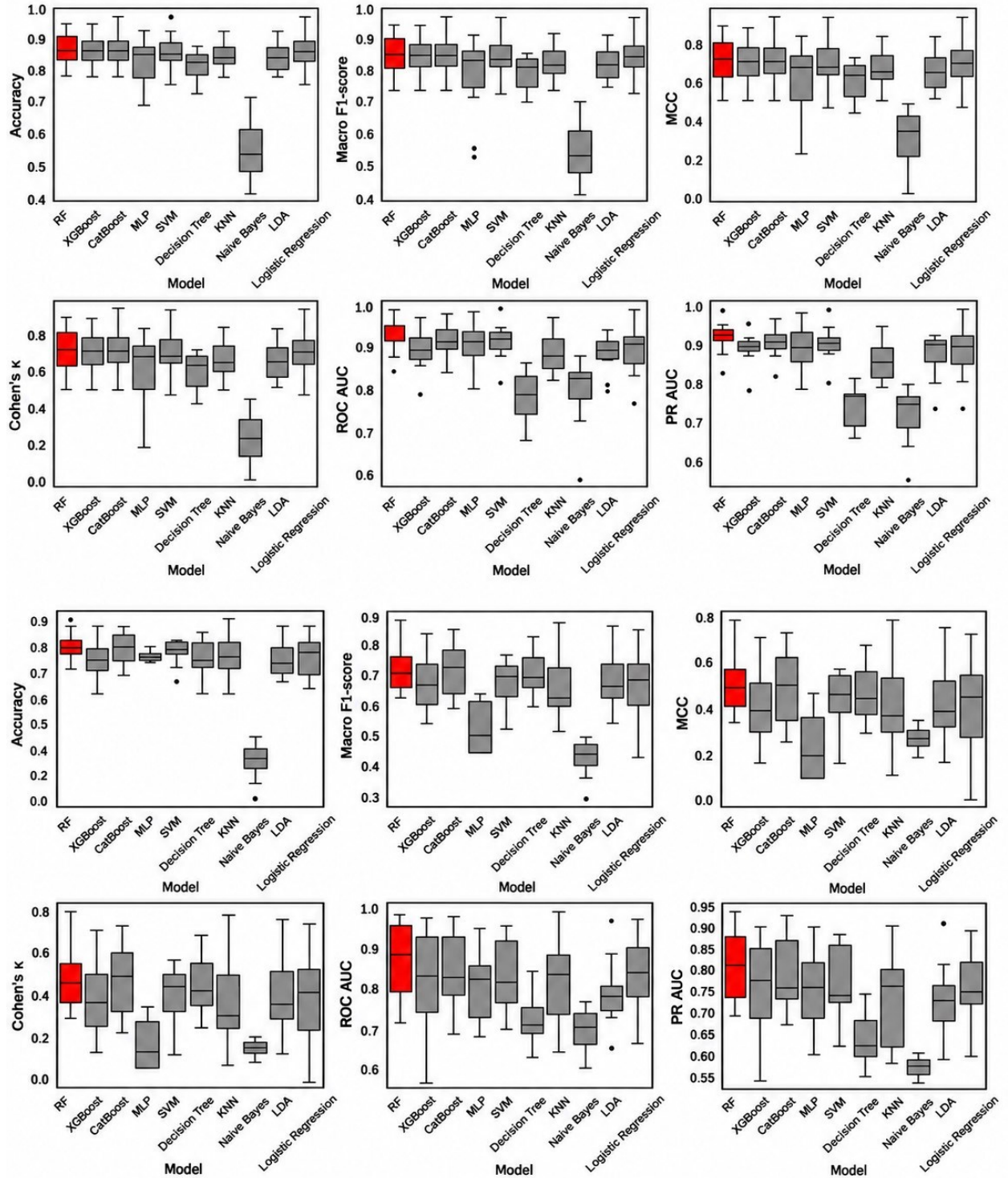
The data were divided into training and test sets before imputation, scaling, or other data-dependent transformations. Missing numerical values were replaced with the median calculated from the training set, whereas missing categorical values were replaced with the most frequent category. The resulting imputation

parameters were then applied unchanged to the test set. We did not use iterative or model-based imputation methods.

Categorical variables without an inherent order were represented by one-hot encoding. Numerical variables were standardized to zero mean and unit variance using parameters estimated from the training data. The same fitted preprocessing transformations were subsequently applied to the test data, thereby preventing information from the test set from influencing model development.

1.7. Machine learning models and evaluation

We evaluated machine-learning algorithms spanning different modeling approaches: Naive Bayes (GaussianNB), k -nearest neighbors (KNeighborsClassifier), support vector machine (SVC), decision tree (DecisionTreeClassifier), random forest (RandomForestClassifier), extreme gradient boosting (XGBClassifier), adaptive boosting (AdaBoostClassifier), and a multilayer perceptron neural network (MLPClassifier). Models were implemented in scikit-learn (v1.2.1), except XGBoost, which was implemented using xgboost (v1.7.4).



Supplementary Fig. S3. Comparative model performance reveals robustness differences across heterogeneous datasets. Boxplots of cross-validation performance for multiple machine learning models evaluated across diverse metrics, including accuracy, F1-score, MCC, Cohen's kappa, ROC-AUC, and PR-

AUC. Models assessed include RF, XGBoost, AdaBoost, MLP, SVM, DT, kNN, NB, LDA, and logistic regression. Boxes indicate interquartile ranges with medians, and whiskers represent the spread of the data. The results demonstrate consistent performance of ensemble-based models and greater variability in simpler classifiers, underscoring the importance of model selection in heterogeneous biological datasets.

Features reported in fewer than 60% of the training samples were excluded, and samples without target annotations were not included in the corresponding prediction task. Categorical variables were encoded using one-hot or ordinal encoding according to whether the categories were nominal or ordered. After preprocessing, the data were represented by 130 numerical features across 397 distinct nanoparticle formulations.

The data were split at the study level into training (80%) and independent test (20%) sets using stratified sampling. All experimental entries originating from the same publication were assigned to the same partition to prevent information from a single study appearing in both sets. Model development was performed within the training set using repeated stratified tenfold cross-validation with shuffling. Hyperparameters were selected by grid search within the training folds. The selected models were then fitted to the complete training set and evaluated on the independent test set, which was not used during preprocessing, feature selection, or hyperparameter tuning. The model-development and evaluation workflow is summarized in Supplementary Fig. S3.

1.8. Experimental procedures

1.8.1. Material synthesis and characterization

HPMC solutions (1% or 3%) containing cobalt nitrate (1 or 5 mM) were subjected to solution plasma processing (SPP). Plasma was generated between cobalt electrodes separated by 1 mm using a pulsed unipolar power supply operated at 800 V and 30 kHz with a pulse width of 2 μ s. The treatment was applied for 30 s. The resulting three-dimensional scaffolds were freeze-dried and crosslinked by UV irradiation at

254 nm for 30 min. The composites were designated C_xCo_y, where *x* represents the HPMC concentration (%) and *y* represents the cobalt nitrate concentration (mM). UV–visible absorption spectra were recorded over 200–900 nm, and Fourier-transform infrared (FTIR) spectra were acquired over 800–4000 cm⁻¹. Surface morphology and elemental distribution were examined by scanning electron microscopy (SEM). For transmission electron microscopy (TEM), nanoparticles were extracted from the composites by sonication and filtration before imaging.

1.8.2. Cell culture and treatments

BV2 microglial cells were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and antibiotics. To induce an inflammatory phenotype, cells were stimulated with IFN- γ (50 ng/mL). Cells were treated with the cellulose–cobalt biocomposites C1Co5 or C3Co5 at 500 μ g/mL for 24 h.

For cellular uptake experiments, methyl- β -cyclodextrin (MC; 20 μ M) and chlorpromazine (CH; 20 μ M) were used to inhibit caveolae- and clathrin-mediated endocytosis, respectively.

Unless otherwise stated, the following treatment groups were used:

1. Control: untreated cells
2. IFN- γ : cells stimulated with IFN- γ (50 ng/mL)
3. C1Co5: cells treated with C1Co5 (500 μ g/mL)
4. C3Co5: cells treated with C3Co5 (500 μ g/mL)
5. C1Co5 + IFN- γ and C3Co5 + IFN- γ : cells treated with the respective biocomposite in the presence of IFN- γ
6. MC: cells treated with MC (20 μ M)
7. CH: cells treated with CH (20 μ M)
8. C1Co5 + IFN- γ $\pm$ MC/CH: cells treated with C1Co5 and IFN- γ in the presence or absence of the indicated endocytosis inhibitor

9. C3Co5 + IFN- γ $\pm$ MC/CH: cells treated with C3Co5 and IFN- γ in the presence or absence of the indicated endocytosis inhibitor

Treatment groups 1–5 were used for ROS measurements, apoptosis, lysosomal and mitochondrial assays, ICP-MS analysis, and immunofluorescence analysis of BV2 spheroids. Groups 1–9 were included in the cellular uptake experiments, including flow cytometry and cell-cycle analysis.

1.8.3. Cellular uptake and imaging analyses

Cell viability was assessed using the MTT assay, with absorbance measured at 560 nm. Nitric oxide (NO) production was quantified using the Griess assay as described previously¹

Intracellular reactive oxygen species (ROS) were measured using H₂DCFDA fluorescence and visualized by live-cell fluorescence microscopy. Apoptosis-associated changes were assessed by Hoechst/propidium iodide staining. Lysosomal and mitochondrial responses were examined using LysoTracker and MitoTracker probes, respectively.

Cellular cobalt uptake was quantified by inductively coupled plasma mass spectrometry (ICP-MS) after 24 h of treatment, following the previously described procedure²

1.8.4. Flow cytometry analysis

Apoptosis-associated cellular responses were examined by flow cytometry using FACSCalibur and FACS Aria II instruments. Cells were stained with either PI/RNase or Annexin V–FITC/PI according to the respective experimental protocol and subsequently subjected to flow cytometric analysis.

1.8.5. 3D spheroid culture

BV2 cells were seeded into ultra-low-attachment plates at a density of 1×10^4 cells per well and cultured for 72 h in the presence of ROCK inhibitor to generate spheroids. The resulting spheroids were subjected to the indicated stimulation and nanocomposite treatments. At 24 h after treatment, spheroids were fixed and processed for immunofluorescence microscopy.

1.8.6. RNA-seq datasets and transcriptomic analyses

Publicly available transcriptomic datasets were obtained from the Gene Expression Omnibus (GEO) to examine CRA-associated response organization across neurological disease and nanoparticle exposure

models. The datasets included Alzheimer's disease (GSE142066), multiple sclerosis (GSE244966), traumatic brain injury (GSE306006), and nanoparticle-related datasets GSE43515, GSE55286, GSE259384 and GSE280933. GSE246159 was included as an independent validation dataset. Expression data and corresponding sample metadata were curated and processed using dataset- and platform-appropriate procedures before downstream analyses.

1.8.7. Construction of CRA

To determine whether common biological response patterns were retained across disease and nanoparticle exposure models, the transcriptomic datasets were mapped onto the three biological programmes underlying the CRA: pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated responses. Genes representing these programmes were selected based on established biological pathways and literature evidence related to inflammatory activation, immune regulation/resolution and apoptosis. The resulting gene sets were used to characterize the relative activity and coordinated behaviour of the three response programmes within each independent transcriptomic dataset.

1.8.8. CRA mapping and pathway scoring

Expression profiles were mapped to the CRA using gene sets corresponding to the pro-inflammatory, anti-inflammatory and apoptosis-associated programmes. RNA-seq and microarray datasets were processed separately using platform-appropriate normalization procedures. Within each dataset, normalized expression values were converted to gene-wise Z -scores across samples, such that each gene was standardized relative to its mean and standard deviation within that dataset.

For each sample, the activity of an individual CRA programme was estimated as the mean standardized expression of the genes assigned to that programme:

$$S_{p,s} = \frac{1}{n_p} \sum_{g=1}^{n_p} Z_{g,s}$$

where $S_{p,s}$ represents the score for programme p in sample s , n_p is the number of genes assigned to programme p , and $Z_{g,s}$ is the standardized expression value of gene g in sample s . Higher programme scores

therefore indicate relatively greater relative expression of genes assigned to the corresponding programme within that dataset. Programme scores were interpreted as relative, dataset-specific measures and were not treated as directly equivalent quantitative values across independent datasets.

Injury-, biosafety- and recovery-associated conditions were assigned independently from the experimental conditions, phenotypic outcomes and biological interpretations reported in the original studies. These annotations were not derived from CRA programme scores or expression patterns. Transcriptomic CRA mapping was subsequently used to examine how the three response programmes were organized within these independently defined biological contexts.

1.8.9. Biosafety and recovery state analysis

Experimental conditions in the independent transcriptomic datasets were annotated as injury-, biosafety- or recovery-associated contexts based on the experimental conditions, phenotypic outcomes and biological interpretations reported in the original studies. These annotations were assigned independently of the pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated CRA programme scores. Phenotypic or functional evidence was used to support biosafety- and recovery-associated assignments, whereas molecular-expression patterns alone were not used to define these states. When the reported evidence was insufficient or ambiguous, no outcome-associated state was assigned. All annotations were manually cross-checked against the corresponding original publications before CRA mapping and programme-level comparison.

1.8.10. Experimental validation

CRA-associated response patterns were evaluated in three-dimensional BV2 microglial spheroids subjected to IFN γ -induced inflammatory stimulation followed by nanocomposite treatment. NF- κ B p65 and NOTCH2 were assessed as inflammation-associated signalling markers, whereas Caspase-3 and cytochrome *c* were examined as apoptosis-associated markers. Fluorescence intensities were quantified from confocal microscopy images using ImageJ. Marker expression patterns were compared across experimental conditions to determine whether coordinated inflammatory and apoptosis-associated response changes observed in two-dimensional cultures were retained in the three-dimensional spheroid model. These

measurements were interpreted as experimental readouts associated with CRA response programmes rather than direct measurements of functional tissue recovery.

1.8.11. Statistical analysis

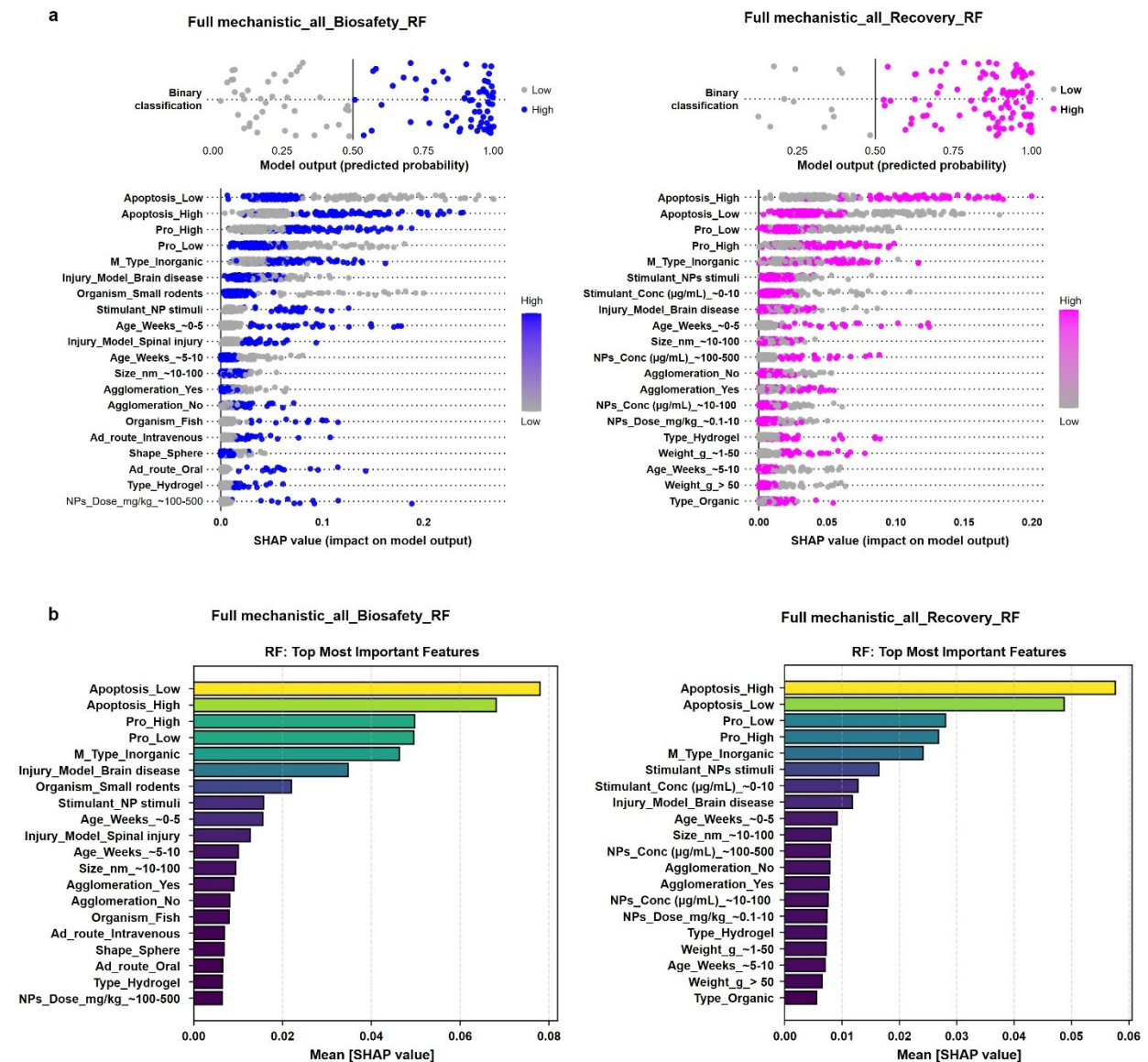
For transcriptomic datasets, differential expression analysis was performed using DESeq2 for RNA-seq data or limma for microarray data, as appropriate to the dataset and platform. *P* values were adjusted for multiple testing using the Benjamini–Hochberg procedure, and adjusted *P* values < 0.05 were considered statistically significant. For experimental validation, statistical comparisons were performed using two-sided Student's *t*-tests or one-way ANOVA with appropriate post hoc correction, as specified for each experiment. Data are presented as mean ± s.d. unless otherwise indicated. Exact sample sizes and statistical tests are provided in the corresponding figure legends and Source Data.

2. SUPPLEMENTARY RESULTS & DISCUSSION

2.1. SHAP-based interpretation of integrated models

SHAP analysis was used to interpret feature contributions in the integrated Step 3 biosafety and recovery models (Supplementary Fig. S4a–d). Across both outcomes, biological-response features ranked prominently alongside nanoparticle, exposure and biological-context descriptors. In particular, pro-inflammatory and apoptosis-associated responses were among the leading contributors, indicating that biological-response information remained influential despite substantial heterogeneity in nanoparticle characteristics and experimental conditions. Feature-level SHAP distributions showed that both the magnitude and direction of individual contributions varied across experimental contexts, rather than reflecting uniform effects across samples (Supplementary Fig. S4a,b). Global feature-importance analysis further showed that biological-response variables contributed predictive information comparable to, and in some cases greater than, conventional material and exposure-related descriptors (Supplementary Fig. S4c,d). The relative feature rankings differed between biosafety and recovery models, indicating related but non-identical predictive structures for the two outcomes. Overall, these analyses identify inflammatory and

apoptosis-associated responses as prominent components of the integrated models and provide the basis for examining their coordinated organization across biological systems.



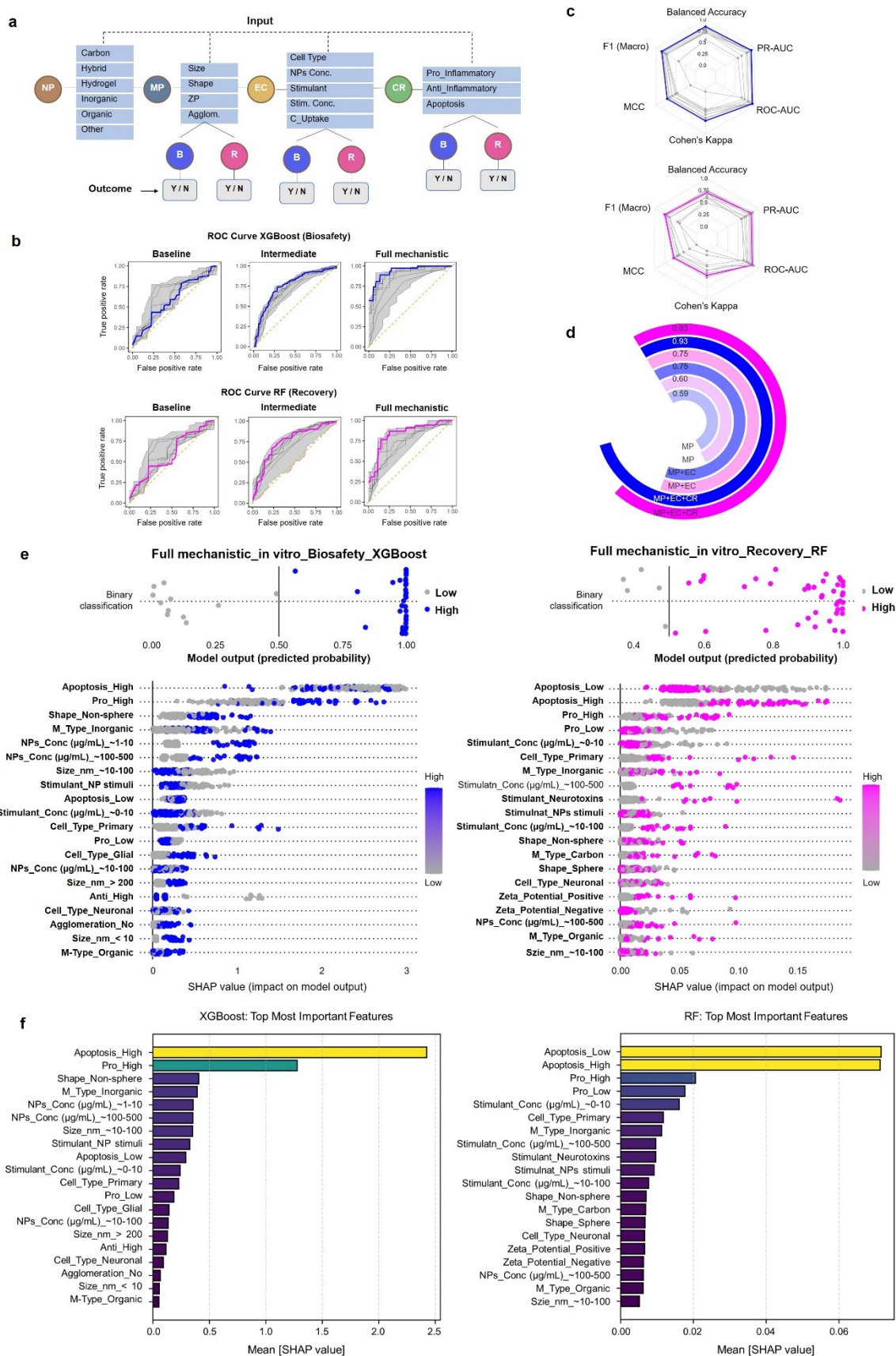
Supplementary Fig. S4. a, SHAP-based interpretation and feature importance analysis of Step 3 integrated modeling across heterogeneous nanoparticle datasets. SHAP (SHapley Additive exPlanations) summary plots and global feature importance analyses for Step 3 machine-learning models integrating spinal material properties, exposure context, biological system information, and conserved biological-response features for prediction of nanoparticle toxicity (left) and recovery (right). Models were evaluated across 893 experimental contexts from 599 studies, spanning multiple nanoparticle classes and

heterogeneous *in vitro* and *in vivo* neurological systems. SHAP analyses show the direction and magnitude of feature contributions (**a**), whereas mean absolute SHAP values summarize global feature importance. **b**, highlighting prominent inflammatory- and apoptosis-associated contributors to biosafety and recovery predictions.

2.2. *In vitro* modeling framework analysis

Analysis of the *in vitro* modeling framework ($n = 405$ experimental contexts) showed progressive improvement in predictive performance as contextual and biological-response information was incorporated (Supplementary Fig. S5a–d). Models based on nanoparticle material properties (MP) alone showed comparatively limited and variable performance across heterogeneous experimental contexts, whereas addition of experimental and exposure conditions (EC) substantially improved prediction. Incorporation of cellular-response (CR) features representing pro-inflammatory, anti-inflammatory and apoptosis-associated programmes provided further improvement in both biosafety and recovery prediction. ROC and comparative performance analyses similarly supported the added predictive contribution of contextual and cellular-response information.

SHAP analyses of the best-performing integrated models showed that biological-response features were among the leading contributors to prediction (Supplementary Fig. S5e,f). Pro-inflammatory High and Apoptosis High were the leading determinants of biosafety prediction, whereas Apoptosis High and Apoptosis Low were dominant for recovery prediction. Despite variation in nanoparticle composition, exposure conditions and cellular systems, cellular-response features remained prominent, with their relative contributions differing between biosafety and recovery outcomes *in vitro*.



Supplementary Fig. S5 | *In vitro* modeling of nanoparticle biosafety and recovery. **a**, *In vitro* modeling framework integrating material properties (MP), experimental conditions (EC), and cellular responses (CR) to predict biosafety (B) and recovery (R). **b**, ROC curves comparing progressive MP, MP+EC, and MP+EC+CR models for biosafety (top) and recovery (bottom). **c**, Radar plots summarizing performance of the integrated MP+EC+CR models for biosafety (top) and recovery (bottom). **d**, Concentric summary of predictive performance across progressive feature configurations. **e**, SHAP plots for the best-performing biosafety (left) and recovery (right) models. Pro-inflammatory High and Apoptosis High were the leading determinants of biosafety, whereas Apoptosis High and Apoptosis Low were dominant for recovery. **f**, Global feature importance based on mean absolute SHAP values, confirming distinct determinants of biosafety and recovery predictions.

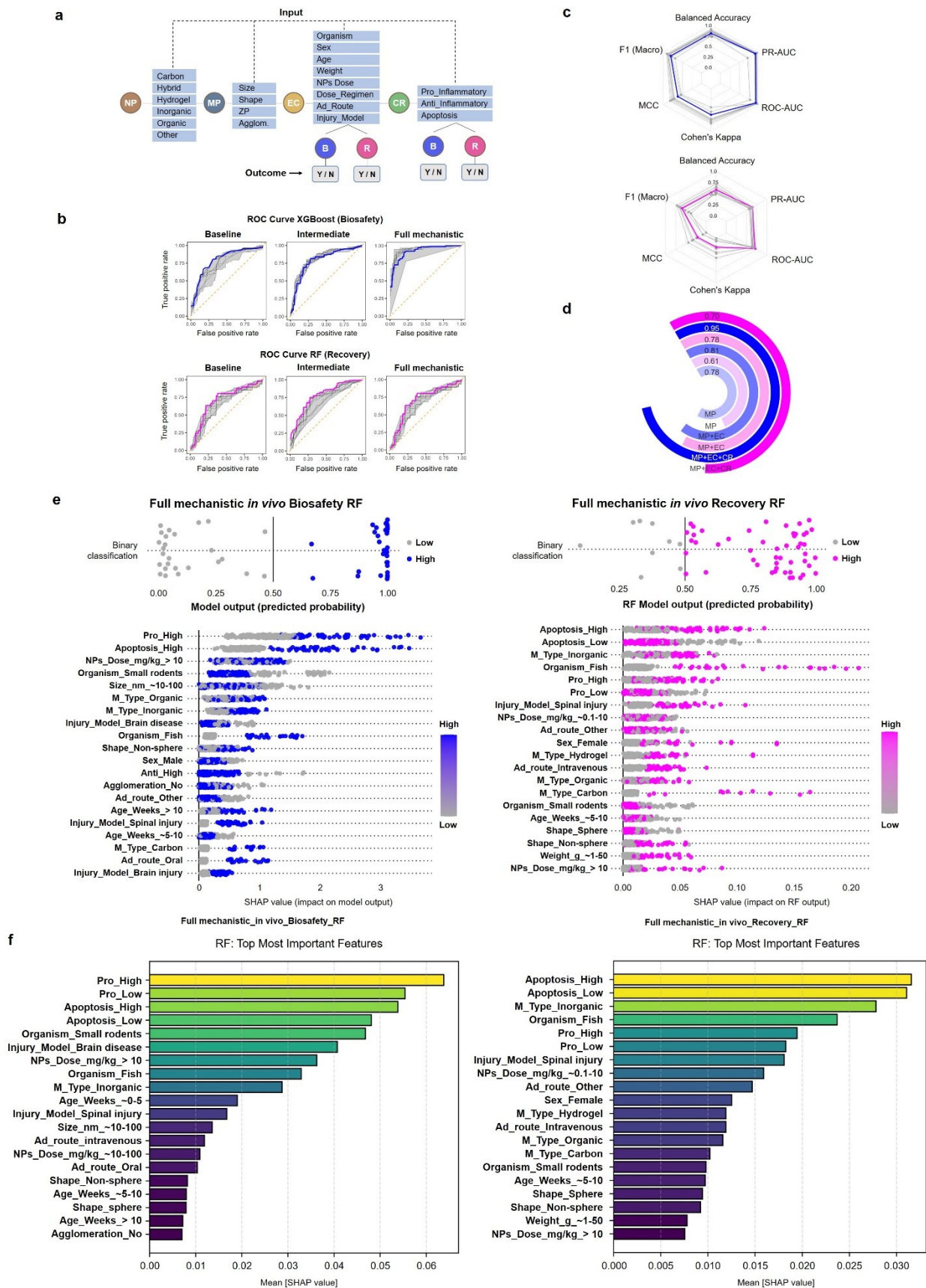
2.3. *In vivo* modelling framework analyses

Analysis of the *in vivo* modeling framework ($n = 488$ experimental contexts) similarly showed incorporation of exposure-context information substantially improved predictive performance, whereas the additional contribution of cellular-response information differed between biosafety and recovery (Supplementary Fig. S6a–f). The integrated framework encompassed heterogeneous *in vivo* experiments spanning multiple nanoparticle classes, organisms, administration routes, dosing regimens and injury models.

Similar to *in vitro* modeling, models based primarily on nanoparticle physicochemical descriptors showed comparatively limited performance across heterogeneous *in vivo* contexts. Incorporation of organism- and exposure-related variables improved predictive performance, with recovery prediction showing substantial improvement after inclusion of administration route, dosing regimen, organism and injury-context information. These results indicate that exposure and biological context captured a substantial component of the predictive information associated with *in vivo* recovery.

For biosafety, incorporation of cellular-response features representing pro-inflammatory, anti-inflammatory and apoptosis-associated programmes provided additional predictive information. In contrast, the MP+EC+B configuration marginally outperformed the fully integrated MP+EC+B+CR configuration for recovery, indicating that addition of CR features did not uniformly improve prediction across outcomes. The relative gains across feature configurations therefore differed between biosafety and recovery, highlighting the context-dependent contribution of biological-response information.

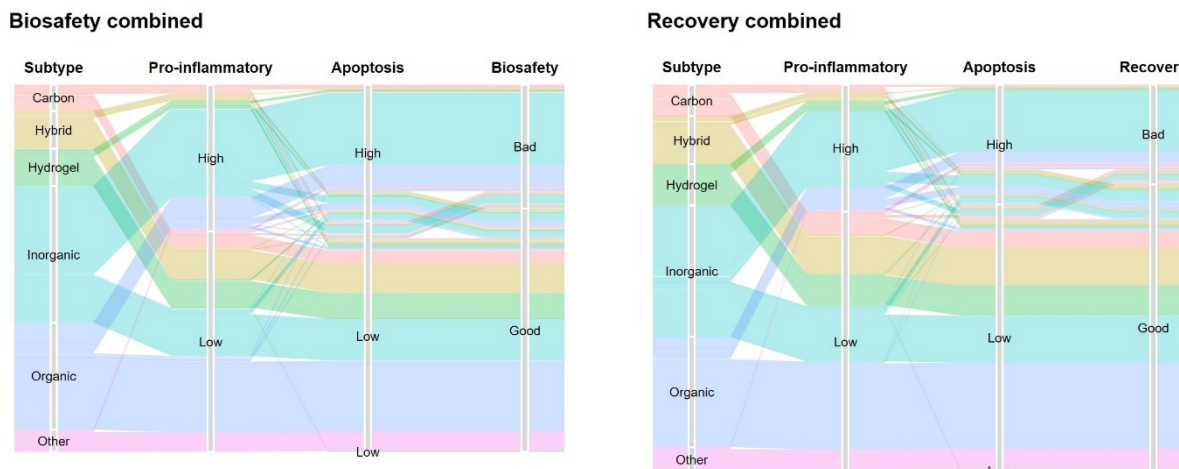
However, SHAP analyses and feature importance further showed that pro-inflammatory, and apoptosis-associated features were repeatedly represented among the influential contributors to model predictions (Supplementary Fig. S6e). Their relative importance differed between biosafety and recovery and occurred alongside contributions from material, organism and exposure-context features.



Supplementary Fig. S6 | *In vivo* modeling reveals contextual and biological-response contributions to nanoparticle biosafety and recovery. a, Schematic of the *in vivo* modeling framework integrating material properties (MP), experimental and exposure conditions (EC), and cellular-response features (CR) to predict biosafety (B) and recovery (R). b, ROC curves comparing progressive MP, MP+EC and MP+EC+CR models for biosafety (top) and recovery (bottom). c, Radar plots summarizing performance of the integrated MP+EC+CR models for biosafety (top) and recovery (bottom). d, Concentric summary comparing predictive performance across the progressive feature configurations. e, SHAP plots for the best-performing biosafety (left) and recovery (right) models, showing the direction and magnitude of individual feature contributions. f, Global feature importance based on mean absolute SHAP values, highlighting the leading determinants of biosafety and recovery predictions.

2.4. Alluvial and feature-transition analyses

Alluvial analysis was used to visualize how heterogeneous nanoparticle classes were distributed across biological-response states and biosafety- and recovery-associated outcomes (Supplementary Fig. S7). Despite differences in material class, multiple nanoparticle categories converged on overlapping high- and low-response states, indicating that these response states were not restricted to individual nanoparticle types. Distinct material classes contributed to both favorable and adverse outcome categories through different combinations of response states. The dominant flows further showed recurrent associations between biological-response states and biosafety or recovery outcomes, although their relative distributions varied across nanoparticle classes. Thus, outcome-associated response organization was not explained by material identity alone. Together with the hierarchical modeling and SHAP analyses, these transition patterns show that heterogeneous nanoparticle classes can map onto a shared biological-response space, providing a basis for subsequent evaluation of the Conserved Response Axis (CRA).



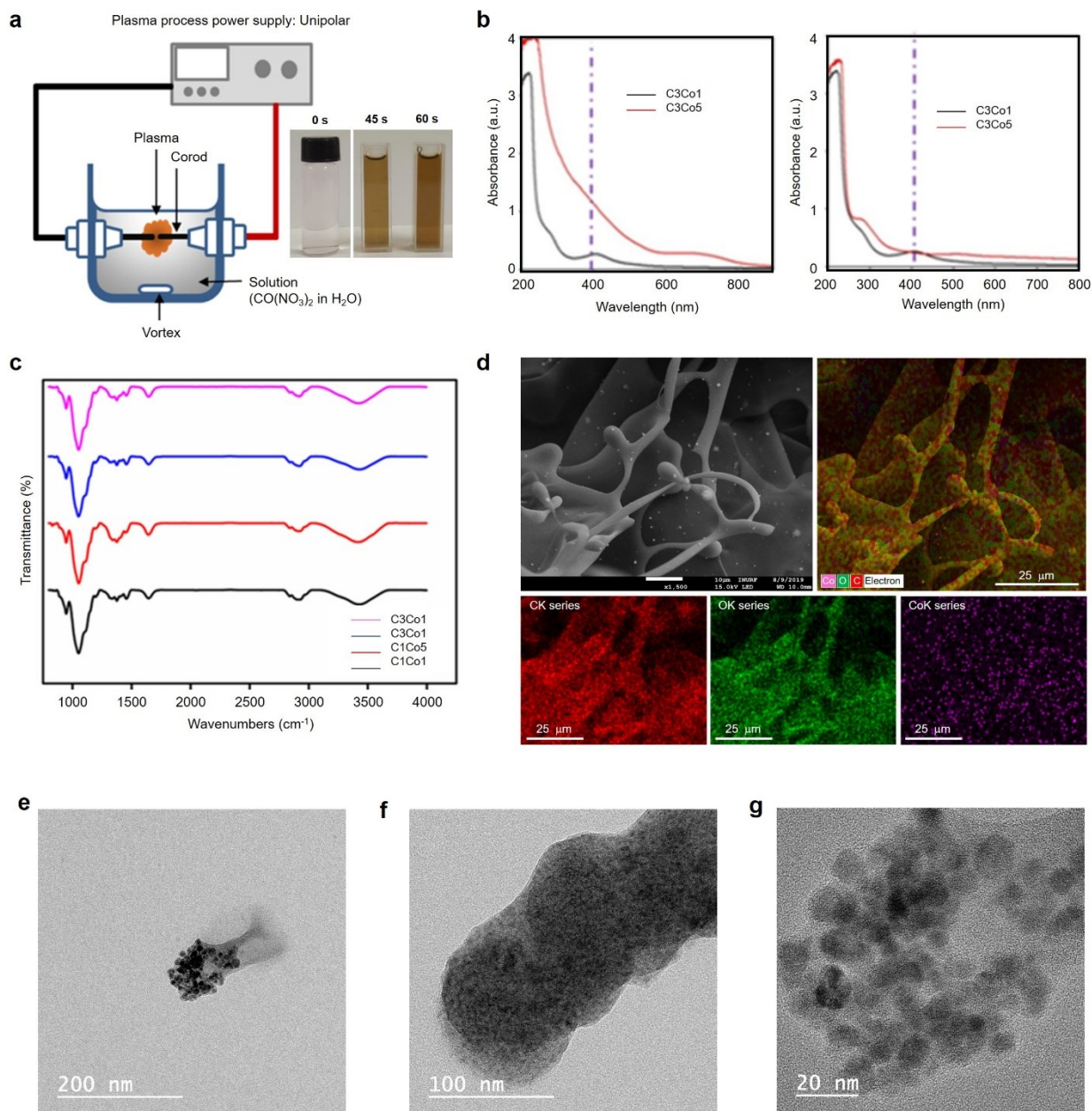
Supplementary Fig. S7 | Alluvial analysis reveals shared biological-response states across heterogeneous nanoparticle classes. Alluvial plots visualize transitions from nanoparticle class through biological-response states to biosafety (left) and recovery (right) outcomes across the integrated dataset. Multiple physiochemically distinct nanoparticle classes converged on overlapping high- and low-response states before reaching “good or bad” outcome categories. Flow widths represent the relative frequencies of experimental contexts following each transition. The recurrent convergence of distinct material classes onto shared response states illustrates biological-response organization beyond nanoparticle class and provides a basis for subsequent CRA analysis.

2.5. Material synthesis and characterization

Cobalt-containing cellulose-based nanocomposites (C1Co1, C1Co5, C3Co1 and C3Co5) were synthesized using a plasma-assisted approach and characterized to establish their physicochemical and structural properties (Supplementary Fig. S8). The resulting formulations showed visible differences in colour and distinct optical profiles, consistent with formulation-dependent differences in the local physicochemical environment of cobalt. C3Co1 and C3Co5 exhibited different absorption characteristics, including differences in spectral broadening and intensity, indicating that variation in cobalt content altered the optical properties of the nanocomposites. FTIR spectra retained characteristic features of the cellulose-derived matrix while showing formulation-dependent spectral changes following cobalt incorporation. These

observations indicate that incorporation of cobalt modified the chemical environment of the matrix without eliminating its principal structural features. Electron microscopy revealed a heterogeneous, interconnected matrix containing nanoscale domains. SEM imaging showed the broader matrix architecture, whereas TEM resolved nanoscale cobalt-containing structures associated with the composite material. Elemental mapping further confirmed the presence and spatial distribution of cobalt within the matrix, together with the constituent elements of the cellulose-based structure.

Collectively, these analyses demonstrate successful incorporation of cobalt-containing nanoscale structures within the cellulose-derived matrix and distinguish the physicochemical characteristics of the resulting formulations. These structural and compositional differences provided experimentally defined materials for subsequent evaluation of cellular uptake, inflammatory responses, apoptosis-associated signalling and recovery-associated responses.



Supplementary Fig. S8. Synthesis, characterization, and structural analysis of cobalt-based nanomaterials. **a**, Schematic illustration of the synthesis setup, along with representative photographs showing the appearance of the prepared samples. **b,c**, UV-vis spectra of different samples (C3Co1 and C3Co5), showing changes in absorbance profiles across the wavelength range. The dashed line indicates a reference position for comparison. **d**, FTIR spectra of the samples (C1Co1, C3Co1, C1Co5, and C3Co5), highlighting differences in characteristic vibrational peaks associated with chemical bonding and functional

groups. **e**, Scanning electron microscopy (SEM) image showing the surface morphology of the synthesized material. **f**, Elemental mapping images (C, O, and Co) confirming the spatial distribution of elements across the structure. **g–i**, Transmission electron microscopy (TEM) images at increasing magnifications, revealing the morphology and internal structure of the synthesized nanoparticles. At lower magnification (**g**), particles appear as aggregated clusters associated with a supporting matrix. Higher magnification images (**h,i**) show nanoscale particles with relatively uniform distribution embedded within the matrix.

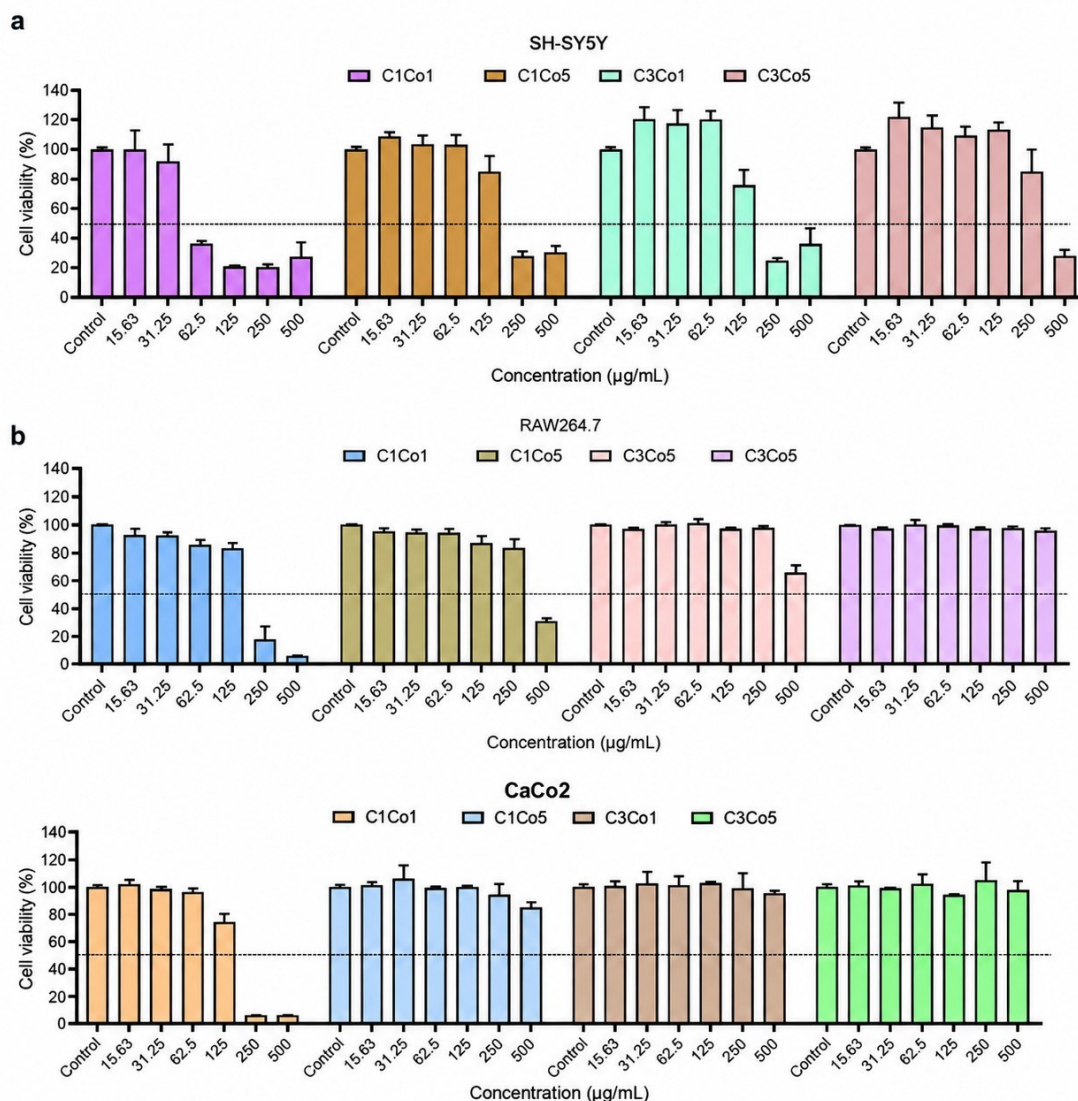
2.6. Experimental biological validation and mechanistic analyses

2.6.1. Cell viability and Biocompatibility

Complementary cell-viability assays revealed nanocomposite-, concentration- and cell-type-dependent responses in SH-SY5Y neuronal cancer cells and normal cell lines (Supplementary Fig. S9). In SH-SY5Y cells, the C1Co1 nanocomposite maintained approximately 100% cell viability at lower concentrations, followed by a concentration-dependent decline to approximately 36.2%, 20.5%, 19.9% and 27.5% at 62.5, 125, 250 and 500 $\mu\text{g mL}^{-1}$, respectively. C1Co5 similarly maintained approximately 108.5–103.2% viability at 15.63, 31.25, 62.5 $\mu\text{g mL}^{-1}$, before decreasing to approximately 84.8% and 27.5–30.1% at 250, 500 $\mu\text{g mL}^{-1}$ respectively. In comparison, the C3Co1 nanocomposite maintained approximately 100–125% viability across low-to-intermediate concentrations, with viability decreasing to approximately 20–35% at the highest concentrations, whereas the fourth formulation retained approximately 122.3–84.7% viability upto 250 $\mu\text{g mL}^{-1}$ range before declining to approximately 24.8 at 500 $\mu\text{g mL}^{-1}$.

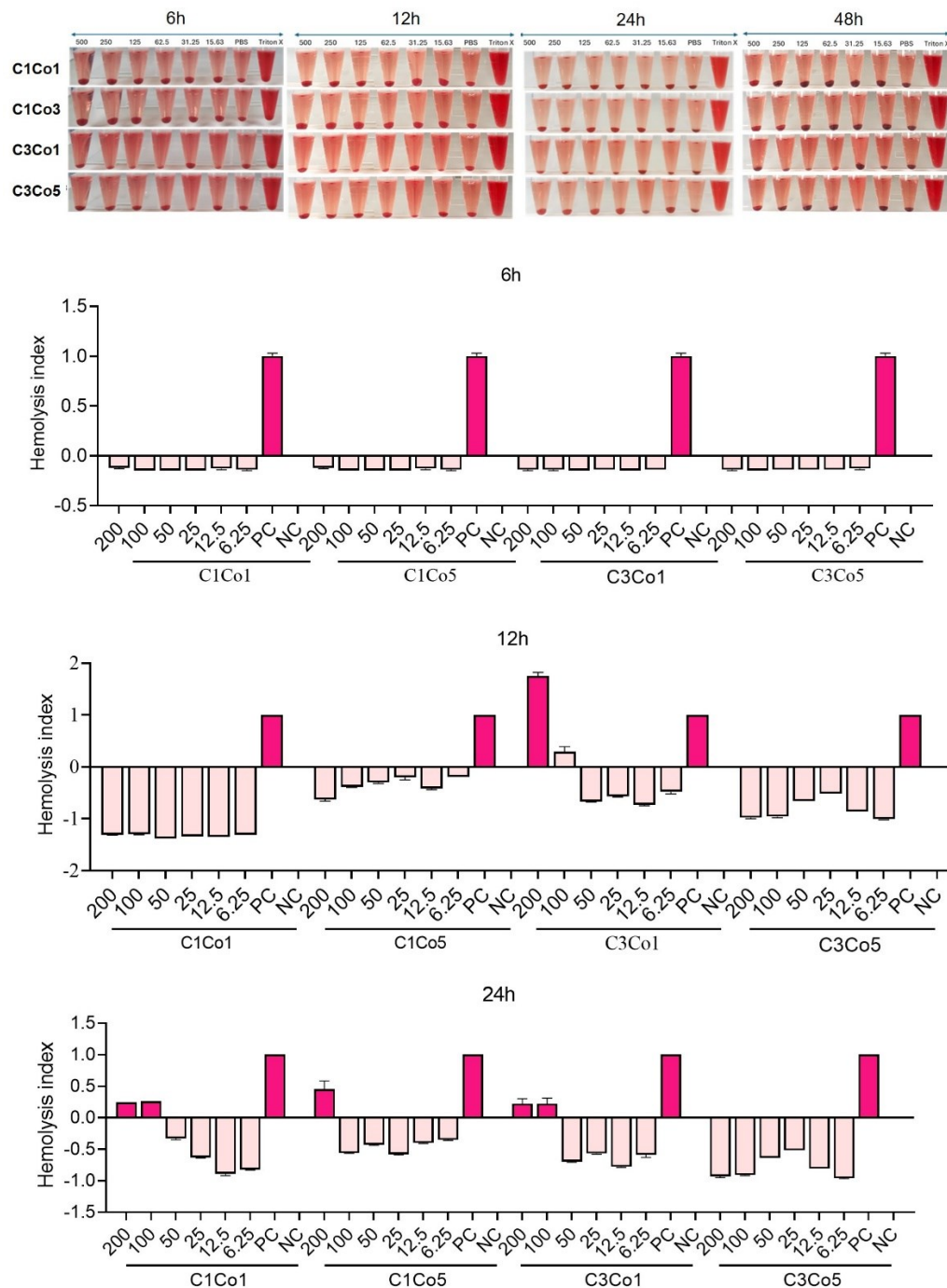
In RAW 264.7 cell models, viability was generally maintained across a broader range of concentrations from 15.63–250 $\mu\text{g mL}^{-1}$, although at higher concentrations of 250–500 $\mu\text{g mL}^{-1}$ cell viability reductions were evident. Additionally, CaCo2 normal cell model, the first formulation maintained approximately 102.1 - 96.5 % viability at 15.63–62.5 $\mu\text{g mL}^{-1}$ concentrations, followed by a decrease to approximately 74.5% and then to 5–6% levels at 250–500 $\mu\text{g mL}^{-1}$ concentrations. In contrast, the remaining formulations showed comparatively stable viability profiles, with values remaining approximately 101.7–85.9%, 100.7–95.5% and 101.8 – 98.5%, respectively, across most or all of the tested concentration range.

Together, these complementary assays showed that pronounced loss of viability occurred primarily in specific formulation–concentration–cell combinations, whereas several formulations maintained comparatively high viability across broad exposure ranges. These findings extend the BV2 screening results and demonstrate that nanocomposite-associated cellular effects depend on the interaction between material formulation, exposure concentration and cellular context (Supplementary Fig. S9). The observed viability profiles further supported the selection of exposure conditions for subsequent mechanistic analyses of inflammatory, oxidative-stress and apoptosis-associated responses without relying solely on overt cytotoxicity as the biological endpoint.



Supplementary Fig. S9 | Cell viability across neuronal cancer and normal cell models following nanocomposite treatment. Cell viability of SH-SY5Y neuronal cancer cells and normal cell lines following exposure to the indicated nanocomposite formulations across increasing concentrations. Selected formulation–concentration combinations produced concentration-dependent reductions in viability, whereas other conditions maintained comparatively high viability across the tested range. Bar plots show relative cell viability (%) normalized to untreated controls; dashed lines indicate the reference control level.

Hemolysis assays showed generally low responses across the tested nanocomposites at 6 h, with hemolysis indices remaining close to baseline across 6.25–200 $\mu\text{g mL}^{-1}$ (Supplementary Fig. S10). At 12 h, most formulation–concentration conditions similarly showed no pronounced positive hemolytic response. A notable exception was C3Co1 at 200 $\mu\text{g mL}^{-1}$, which showed a marked increase in the hemolysis index to approximately 1.75, whereas the response decreased to approximately 0.29 at 100 $\mu\text{g mL}^{-1}$ and remained below baseline at lower concentrations. These findings indicate generally limited hemolytic responses across the tested conditions, with a distinct time- and concentration-dependent response for high-dose C3Co1.



Supplementary Fig. S10 | Hemocompatibility of cellulose-cobalt nanobiocomposites across concentration and exposure time. Representative photographs and quantitative analysis of erythrocyte hemolysis following exposure to C1Co1, C1Co5, C3Co1 and C3Co5 at the indicated concentrations and exposure times, together with positive and negative controls. Supernatant coloration reflects hemoglobin

release following erythrocyte lysis. Hemolytic responses were generally low across most tested conditions, whereas C3Co1 at 200 $\mu\text{g mL}^{-1}$ after 12 h showed a pronounced increase in hemolysis index. Overall, the results show formulation-, concentration- and time-dependent differences in erythrocyte compatibility.

2.6.2. Nanoparticle modulation of inflammatory stress, organelle responses and gene expression

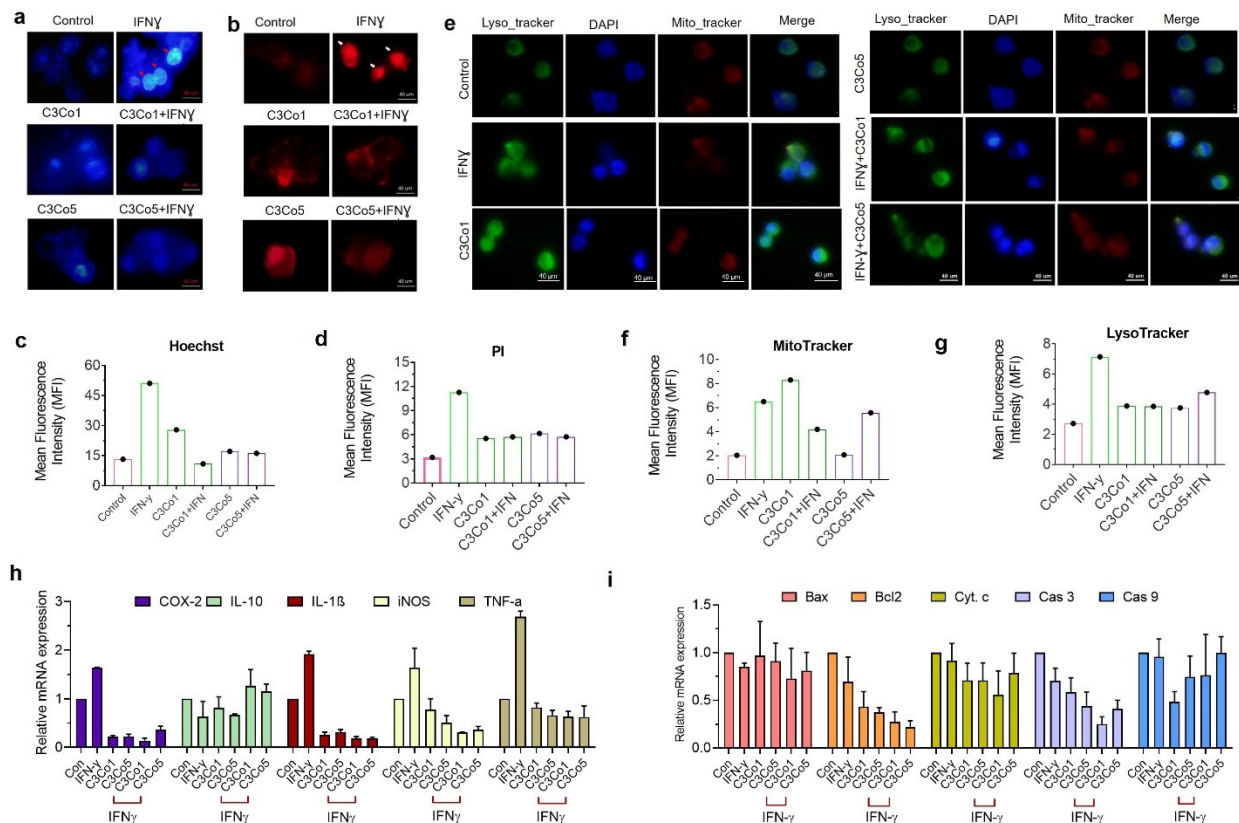
Hoechst staining showed a marked change in nuclear-associated fluorescence following $\text{IFN}\gamma$ stimulation, with the signal increasing from approximately 13.5 MFI in control cells to 49.4 MFI following $\text{IFN}\gamma$ stimulation (Supplementary Fig. S11a,c). Under $\text{IFN}\gamma$ challenge, C3Co1 and C3Co5 reduced Hoechst fluorescence to approximately 10.5 and 18.9 MFI, respectively, indicating attenuation of the $\text{IFN}\gamma$ -associated increase in nuclear staining.

PI staining showed increased membrane-compromise-associated fluorescence following $\text{IFN}\gamma$ stimulation, with mean fluorescence intensity increasing from approximately 5.48 MFI in control cells to 10.65 MFI following $\text{IFN}\gamma$ stimulation (Supplementary Fig. S11b,d). Under $\text{IFN}\gamma$ challenge, C3Co1 and C3Co5 reduced PI fluorescence to approximately 6.4 and 6.13 MFI, respectively, similar to that of control. These reductions are consistent with decreased membrane-compromise-associated staining under both treatment conditions.

MitoTracker and LysoTracker staining further showed modulation of organelle-associated responses following $\text{IFN}\gamma$ challenge (Supplementary Fig. S11e-g). MitoTracker fluorescence increased from approximately 2.0 MFI in control cells to 6.5 MFI following $\text{IFN}\gamma$ stimulation. Under $\text{IFN}\gamma$ challenge, C3Co1 reduced MitoTracker fluorescence to approximately 4.2 MFI, whereas C3Co5 produced a value of approximately 5.5 MFI. LysoTracker fluorescence similarly increased from approximately 2.7 MFI in control cells to 7.1 MFI following $\text{IFN}\gamma$ stimulation. Under $\text{IFN}\gamma$ challenge, fluorescence decreased to approximately 3.8 MFI with C3Co1 and 4.7 MFI with C3Co5. Thus, both formulations attenuated the $\text{IFN}\gamma$ -associated increase in lysosomal fluorescence, with a larger reduction observed for C3Co1.

Gene-expression analyses provided complementary molecular evidence of nanocomposite-dependent response modulation (Supplementary Fig. S11h-i). $\text{IFN}\gamma$ increased several inflammatory transcripts,

including COX-2 (~1.64-fold), IL-1 β (~1.92-fold), iNOS (~1.64-fold) and TNF- α (~2.68-fold) relative to their respective controls. Under IFN γ challenge, C3Co1 and C3Co5 reduced these inflammatory responses, with COX-2 decreasing to ~0.14 and ~0.36-fold, IL-1 β to ~0.18 and ~0.18-fold, iNOS to ~0.3 and ~0.36-fold, and TNF- α to ~0.62 and ~0.62-fold, respectively. In contrast, the anti-inflammatory marker IL-10 increased to ~1.26 and ~1.14-fold with C3Co1+IFN γ and C3Co5+IFN γ , respectively. Together, C3Co1 and C3Co5 reduced the IFN γ -associated increases in mitochondrial and lysosomal fluorescence, although the magnitude and baseline effects differed between nanocomposites. These findings complement the Hoechst, PI and molecular measurements and support nanocomposite-dependent modulation of cellular-stress responses under inflammatory challenge.



Supplementary Fig. S11 | Nanocomposite modulation of cellular stress, mitochondrial and lysosomal-

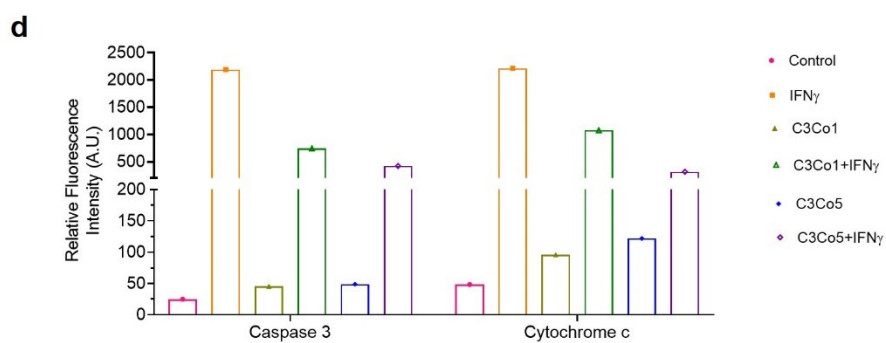
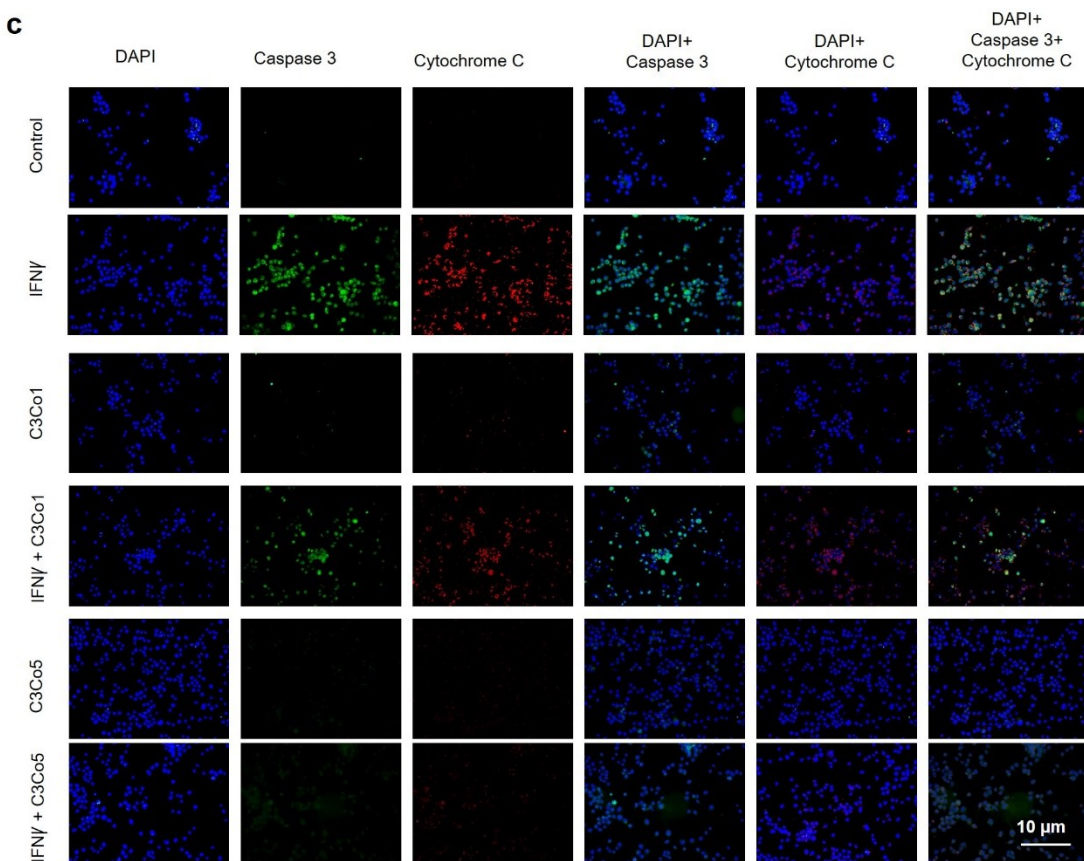
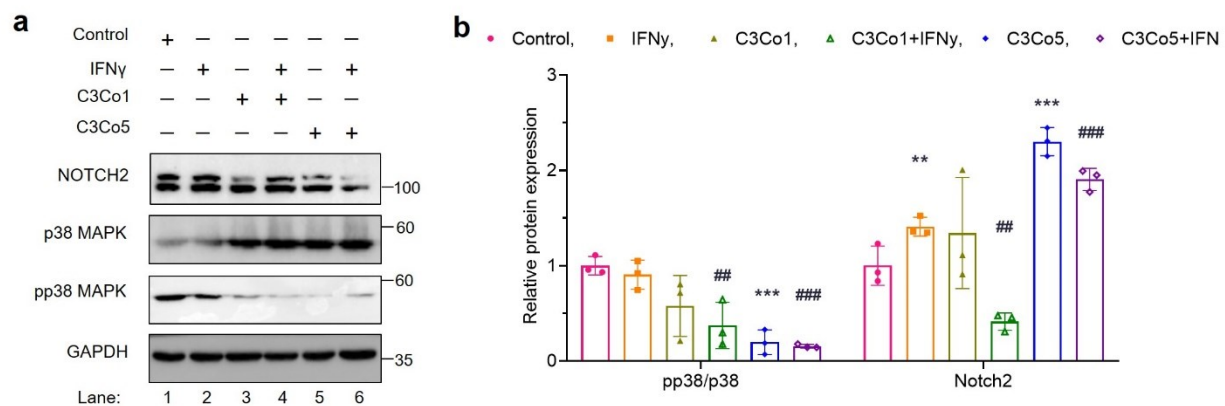
associated responses and gene expression. a,b, Representative Hoechst and PI fluorescence images of SH-SY5Y cells under control, IFN γ and C3Co1/C3Co5 treatment conditions. Scale bars, 40 μ m. **c,d,** Quantification of Hoechst (c) and PI (d) fluorescence showing IFN γ -associated increases that were

attenuated by C3Co1 and C3Co5 under IFN γ challenge. **e**, Representative LysoTracker, MitoTracker and DAPI images of BV2 cells under the indicated conditions. Scale bars, 40 μ m. **f,g**, Quantification of MitoTracker (f) and LysoTracker (g) fluorescence showing IFN γ -associated increases and formulation-dependent modulation by C3Co1 and C3Co5. **h,i**, Relative expression of apoptosis-associated (h) and inflammatory/anti-inflammatory (i) genes, showing coordinated but gene-specific responses to IFN γ and nanocomposite treatment. Gene expression was normalized to control; biological triplicates, $n = 3$.

2.6.3. Nanoparticle regulation of inflammatory signaling pathways

Western blot quantification showed differential modulation of p38 MAPK and NOTCH2 signalling following nanocomposite treatment (Supplementary Fig. S12a-b). The pp38/p38 ratio was approximately 1.0- fold in control cells and 0.9- fold following IFN γ stimulation. Under IFN γ challenge, C3Co1 and C3Co5 reduced the pp38/p38 ratio to approximately 0.35-and 0.12-fold, respectively. NOTCH2 protein expression increased from approximately 1.0-fold in control cells to 1.35-fold following IFN γ stimulation. Under IFN γ challenge, C3Co1 reduced NOTCH2 expression to approximately 0.4-fold, whereas C3Co5+IFN γ showed approximately 1.85-fold expression. Thus, unlike the consistent reduction in pp38/p38, NOTCH2 showed a formulation-dependent response, with C3Co1 attenuating the IFN γ -associated signal while C3Co5 was associated with higher NOTCH2 expression.

Immunofluorescence staining further showed pronounced modulation of apoptosis-associated proteins following IFN γ challenge (Supplementary Fig. S12c-d). Caspase-3 fluorescence increased markedly from approximately 24.9 AU in control cells to 2,192 AU following IFN γ stimulation. Under IFN γ challenge, C3Co1 and C3Co5 reduced Caspase-3 fluorescence to approximately 742.8 and 421.4 AU, respectively. A similar pattern was observed for cytochrome c, with fluorescence increasing from approximately 48.3 AU in control cells to 2,216 AU following IFN γ stimulation. Under IFN γ challenge, C3Co1 reduced the signal to approximately 1,077 AU, whereas C3Co5 produced a stronger reduction to approximately 315.5 AU. C3Co1 and C3Co5 alone showed approximately 96 and 121.8 AU, respectively. Together, the marked IFN γ -associated increases in Caspase-3 and cytochrome c and their reduction following C3Co1 and C3Co5 treatment support attenuation of apoptosis-associated responses under inflammatory challenge, with the stronger reduction observed for C3Co5.

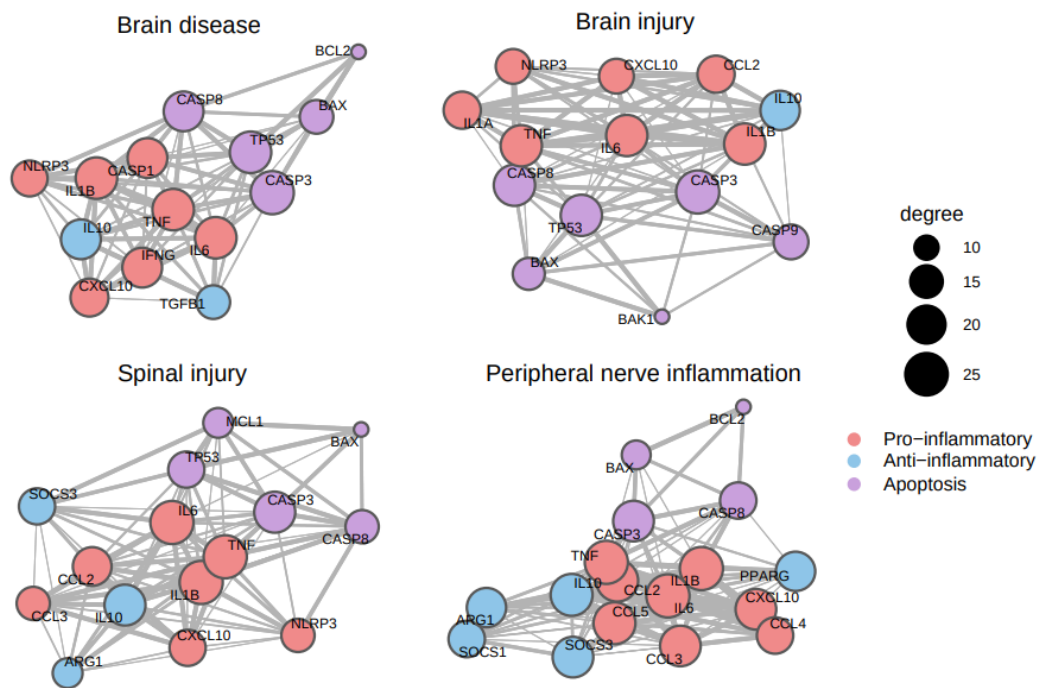


Supplementary Fig. S12 | Nanocomposite modulation of inflammatory and apoptosis-associated protein responses under IFN γ challenge. **a-b**, Representative western blot and quantitative analysis of pp38/p38, NOTCH2, Caspase-9 and Bcl2 under control, IFN γ , C3Co1, C3Co5 and combined treatment conditions. C3Co1 and C3Co5 reduced pp38/p38 under IFN γ challenge, whereas NOTCH2 showed nanocomposite-dependent regulation. Caspase-9 showed comparatively modest changes, while Bcl2 increased under C3Co1+IFN γ and C3Co5+IFN γ conditions. **c,d**, Representative immunofluorescence images and quantification of Caspase-3 and cytochrome c. IFN γ markedly increased both apoptosis-associated signals, whereas C3Co1 and C3Co5 attenuated these responses, with a stronger reduction observed for C3Co5. Data are presented as mean $\pm$ variation from independent experiments ($n = 3$). Statistical significance is indicated by *, **, ***, and # symbols as defined in the Methods.

2.6.4. Network-level integration of inflammatory and apoptosis-associated responses

Network analysis showed interconnected pro-inflammatory, anti-inflammatory and apoptosis-associated genes across brain disease, brain injury, spinal injury and peripheral nerve inflammation (Supplementary Fig. S13). Pro-inflammatory mediators, including *TNF*, *IL6*, *IL1B*, *NLRP3* and *CXCL10*, formed highly connected nodes across multiple contexts and were linked to apoptosis-associated genes including *CASP3*, *CASP8*, *TP53* and *BAX*. Anti-inflammatory and regulatory genes, including *IL10*, *SOCS1*, *SOCS3* and *ARG1*, were also incorporated within these networks, indicating integration of inflammatory regulation with apoptosis-associated responses. Network organization nevertheless differed among biological contexts. Brain disease and brain injury showed prominent connectivity between pro-inflammatory and apoptosis-associated components, whereas spinal injury and peripheral nerve inflammation showed greater representation of anti-inflammatory and regulatory nodes. Despite these differences, connections among the three response programmes were evident across all four contexts. Degree-based analysis further showed that no single molecular hub dominated across all contexts. Instead, highly connected nodes varied among disease and injury settings, indicating a distributed response architecture in which different molecular components can contribute to a similar programme-level organization. Together, these networks

demonstrate recurrent integration of pro-inflammatory, anti-inflammatory and apoptosis-associated programmes across distinct neurological contexts, while retaining context-specific molecular configurations. This network-level organization complements the predictive, experimental and transcriptomic analyses and is consistent with the programme-level response architecture represented by the CRA.



Supplementary Fig. S13 | Network organization of inflammatory, anti-inflammatory and apoptosis-associated genes across neurological conditions. Gene-interaction networks for brain disease, brain injury, spinal injury and peripheral nerve inflammation. Nodes represent genes, edges indicate functional or regulatory interactions, and node size is proportional to degree. Genes are grouped into pro-inflammatory (red), anti-inflammatory (blue) and apoptosis-associated (purple) response programmes. Pro-inflammatory mediators, including *TNF*, *IL6*, *IL1B*, *NLRP3* and *CXCL10*, showed prominent connectivity with apoptosis-associated genes such as *CASP3*, *CASP8*, *TP53* and *BAX*, while anti-inflammatory and regulatory genes including *IL10*, *SOCS1*, *SOCS3* and *ARG1* were integrated within the same networks. Although individual

gene connectivity varied among conditions, recurrent interactions among the three response programmes were observed across all four neurological contexts.

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