

Supplemental material legends

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Supplemental Figure 3. Physical and biochemical characterization of osteosarcoma exosomes. (A) Scanning electron micrographs of OSCA-40 exosomes (B) Immunoblotting of exosome preparations documenting enrichment of tetraspanins (CD63) and depletion of β -actin in OSCA-8, OSCA-32, OSCA-40, and OSCA-78 dog osteosarcoma cell lines. (C) NanoSight particle tracking analysis of triplicate samples each of osteosarcoma cell lines OSCA-8, OSCA-32, OSCA-40, and OSCA-78, showing modal diameters of approximately 130 nm.

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Figure 10. Machine learning performance after data randomization based on repeated 10-fold cross-validations. (A) Original data. (B) Randomized data. (C) Sample distribution was equalized by adding 100 of simulated data points to each group. (D) Randomized equalized data. T-test analysis was performed between original and randomized data. *p = 0.049; ***p < 0.0001

Supplemental Table S1

		Osteosarcoma (n=28)	Non-neoplastic disease (n=10)	Other neoplasia of bone (n=2)	No apparent disease (n=13)
Breed	Golden retriever	n=6	n=1		
	Labrador retriever	n=3	n=1	n=1	n=4
	Greyhound	n=3	n=1		
	Doberman	n=2			
	Great Dane	n=2			
	German Shepherd				n=2
	Other purebred	n=6	n=6		n=4
Mixed breed	n=6	n=1	n=1	n=3	
Tumor location	Humerus	n=8			
	Radius	n=7		n=1	
	Ulna	n=2			
	Femur	n=2	n/a	n=1	n/a
	Tibia	n=7			
	Fibula	n=1			
	Mandible	n=1			
Sex	Intact female	n=1			
	Spayed female	n=15	n=7		n=6
	Intact male		n=1		n=1
	Castrated male	n=12	n=2	n=2	n=6
Age (years)	Mean	7.2	10.6		6.1
	Median	7.4	11.0		4.8
	Range	2.3 - 11.0	7.0 - 14.0	3.1, 11.1*	2.3 - 14.3
Body weight (kg)	Mean	39.0	30.3		29.8
	Median	34.3	29.7		26.7
	Range	19.5 - 72.0	16.0 - 46.3	31.2, 35.0*	14.9 - 45.0
Time between pre- and post-tx samples (days)	Mean	162			
	Median	39			
	Range	2 - 984	n/a	n/a	n/a
	n/a	n=5			
Non-osteosarcoma disease	Benign splenic lesion		n=5		
	Lipoma	n/a	n=2	n/a	n/a
	Other benign lesion		n=3		
Non-osteosarcoma neoplasia of bone	Metastatic carcinoma	n/a	n/a	n=1	n/a
	Hemangiosarcoma			n=1	

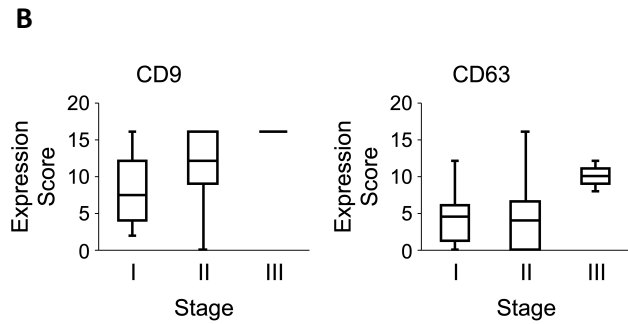
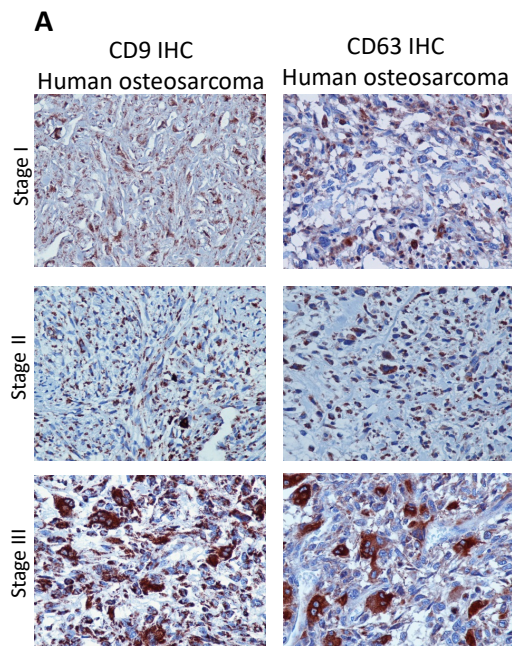
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Supplemental Table S2

Sample	Diagnosis	Post-treatment classification	Days till relapse
Dog 01	Osteosarcoma	Osteosarcoma - NOT detectable	518
Dog 07	Osteosarcoma	Osteosarcoma - detectable	244
Dog 11	Osteosarcoma	Osteosarcoma - NOT detectable	74
Dog 13	Osteosarcoma	Osteosarcoma - NOT detectable	465
Dog 14	Osteosarcoma	Osteosarcoma - detectable	215
Dog 15	Osteosarcoma	Osteosarcoma - NOT detectable	252
Dog 16	Osteosarcoma	Osteosarcoma - NOT detectable	286
Dog 17	Osteosarcoma	Osteosarcoma - detectable	98
Dog 20	Osteosarcoma	Osteosarcoma - NOT detectable	77
Dog 23	Osteosarcoma	Osteosarcoma - NOT detectable	168
Dog 26	Osteosarcoma	Osteosarcoma - NOT detectable	57
Dog 29	Osteosarcoma	Osteosarcoma - NOT detectable	472
Dog 32	Osteosarcoma	Osteosarcoma - NOT detectable	722
Dog 35	Osteosarcoma	Osteosarcoma - detectable	98
Dog 36	Osteosarcoma	Osteosarcoma - detectable	287
Dog 37	Osteosarcoma	Osteosarcoma - detectable	991
Dog 38	Osteosarcoma	Osteosarcoma - detectable	309
Dog 39	Osteosarcoma	Osteosarcoma - detectable	149
Dog 42	Osteosarcoma	Osteosarcoma - detectable	103
Dog 45	Osteosarcoma	Osteosarcoma - detectable	76
Dog 48	Osteosarcoma	Osteosarcoma - NOT detectable	681
Dog 49	Osteosarcoma	Osteosarcoma - detectable	663
Dog 50	Osteosarcoma	Osteosarcoma - detectable	15
Dog 53	Osteosarcoma	Osteosarcoma - detectable	66

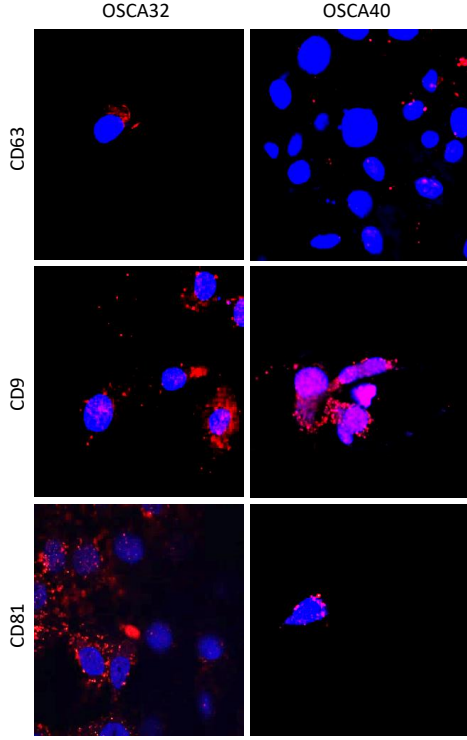
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Supplemental Figure 1



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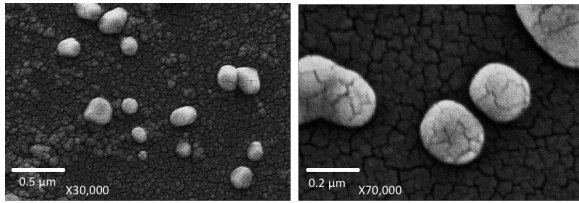
Supplemental Figure 2



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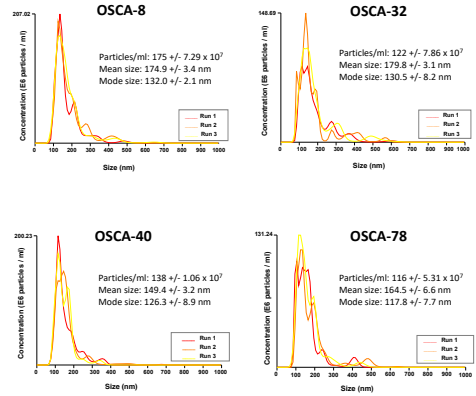
Supplemental Figure 3

A

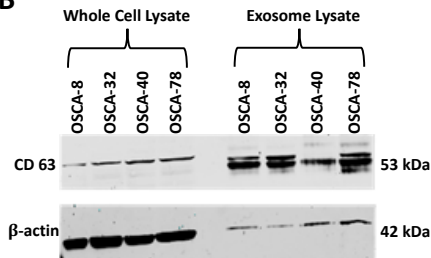


C

Nanoparticle Size Analysis of Isolated Exosomes



B

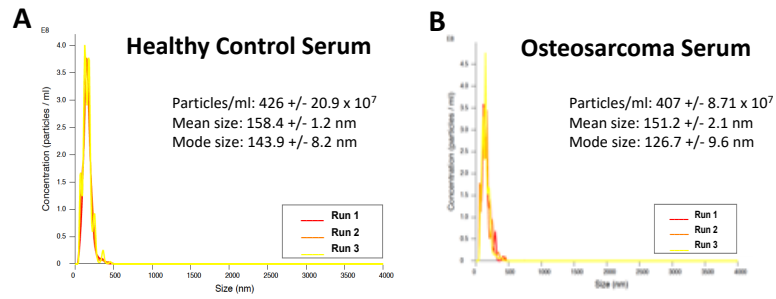


Supplemental Figure 3. Physical and biochemical characterization of osteosarcoma exosomes.

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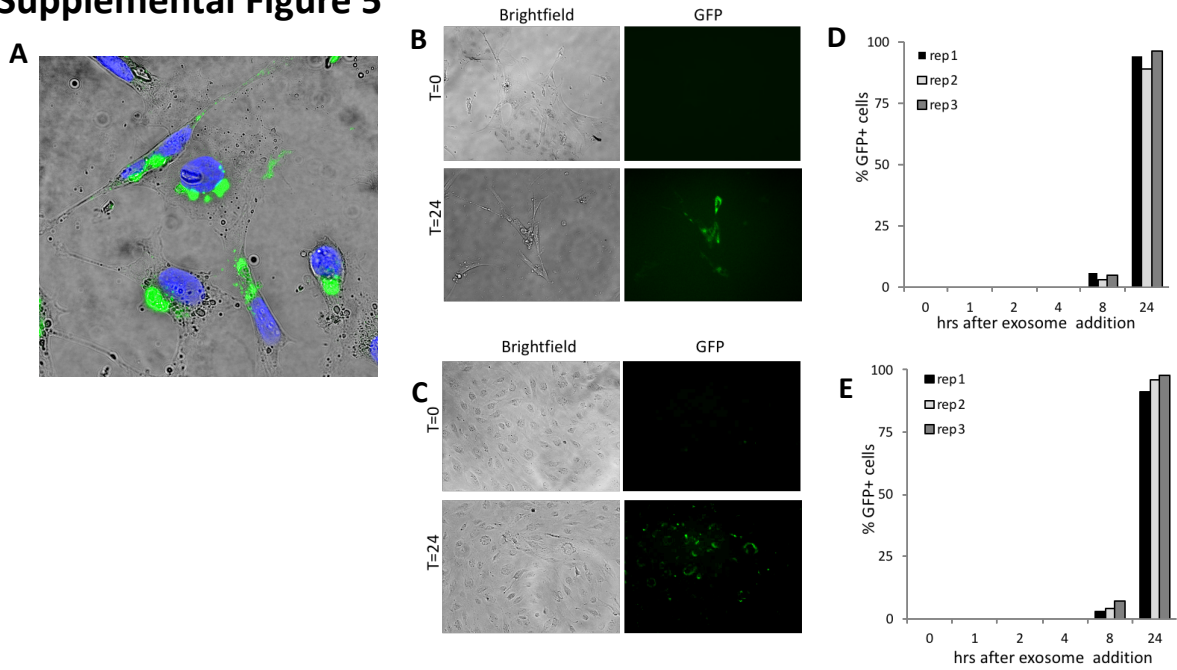
Supplemental Figure 4

Nanoparticle Size Analysis of Isolated Exosomes



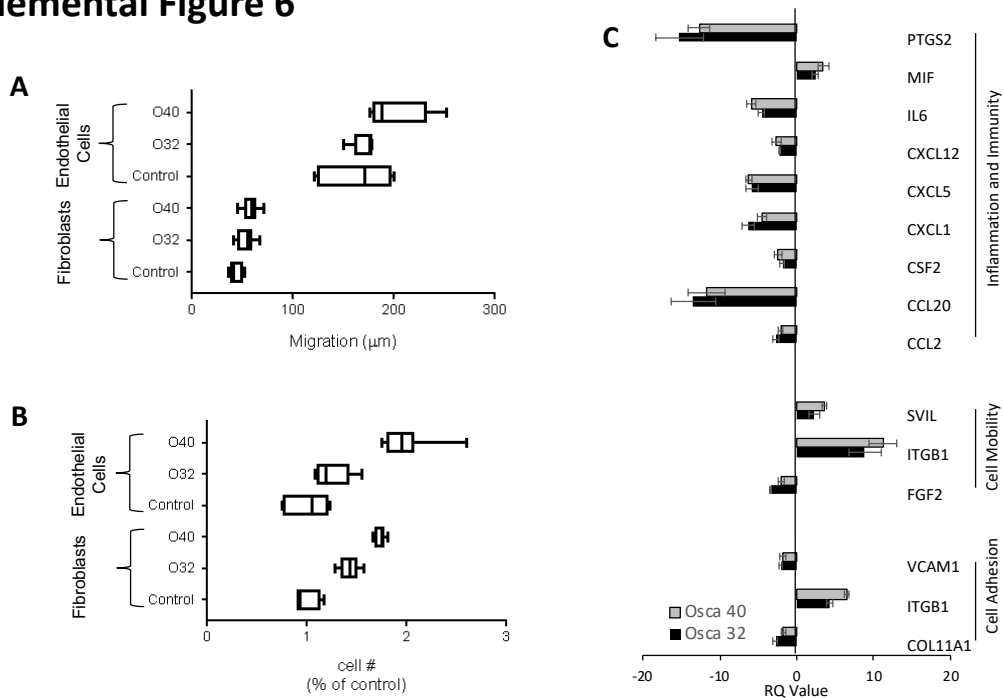
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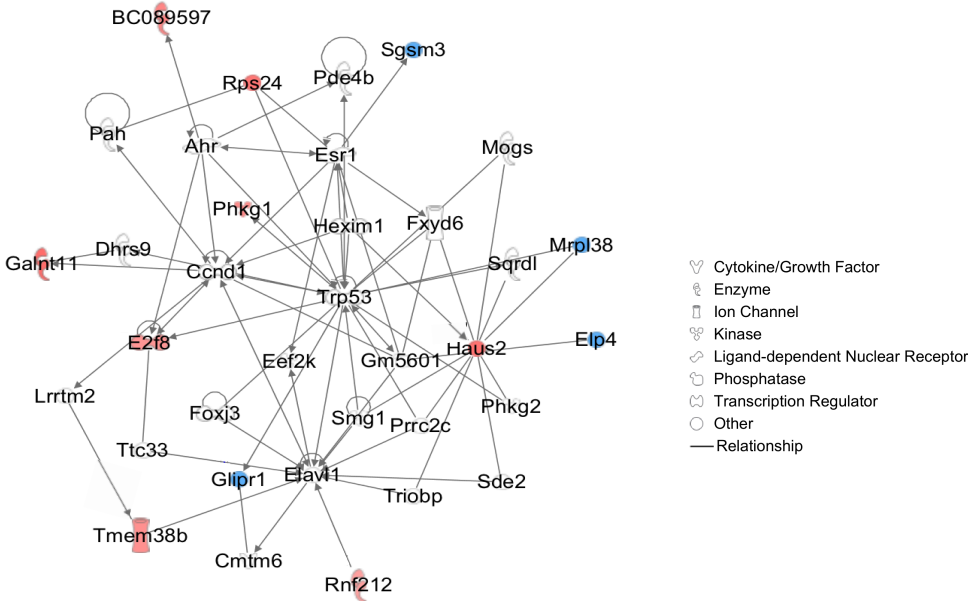
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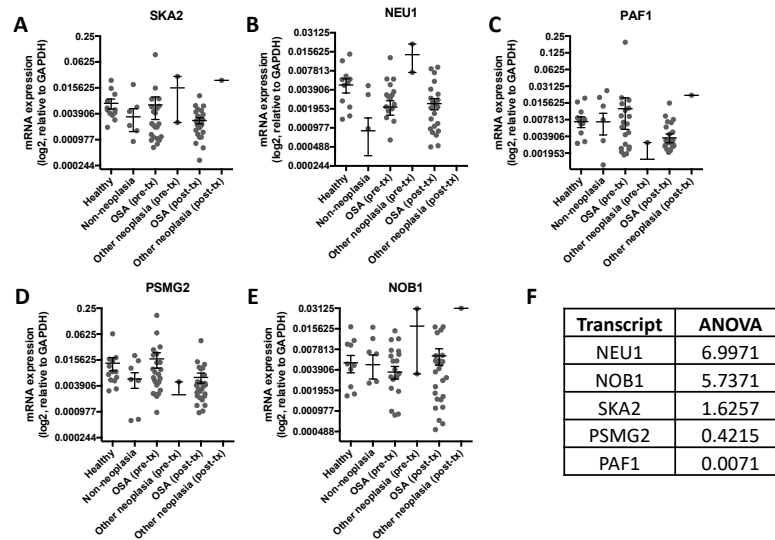
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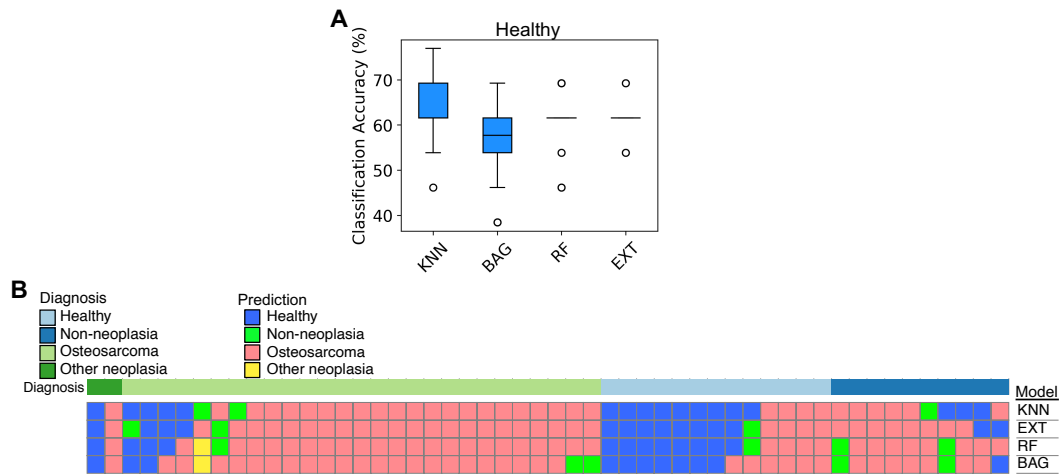
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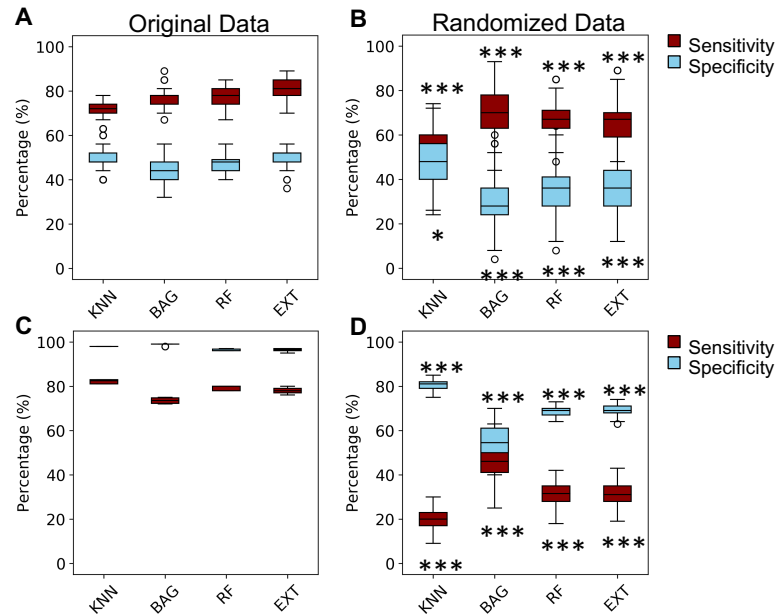


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