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Supplemental Information

Local administration of IL-12 with an HC vector results in local and metastatic tumor control in pediatric osteosarcoma

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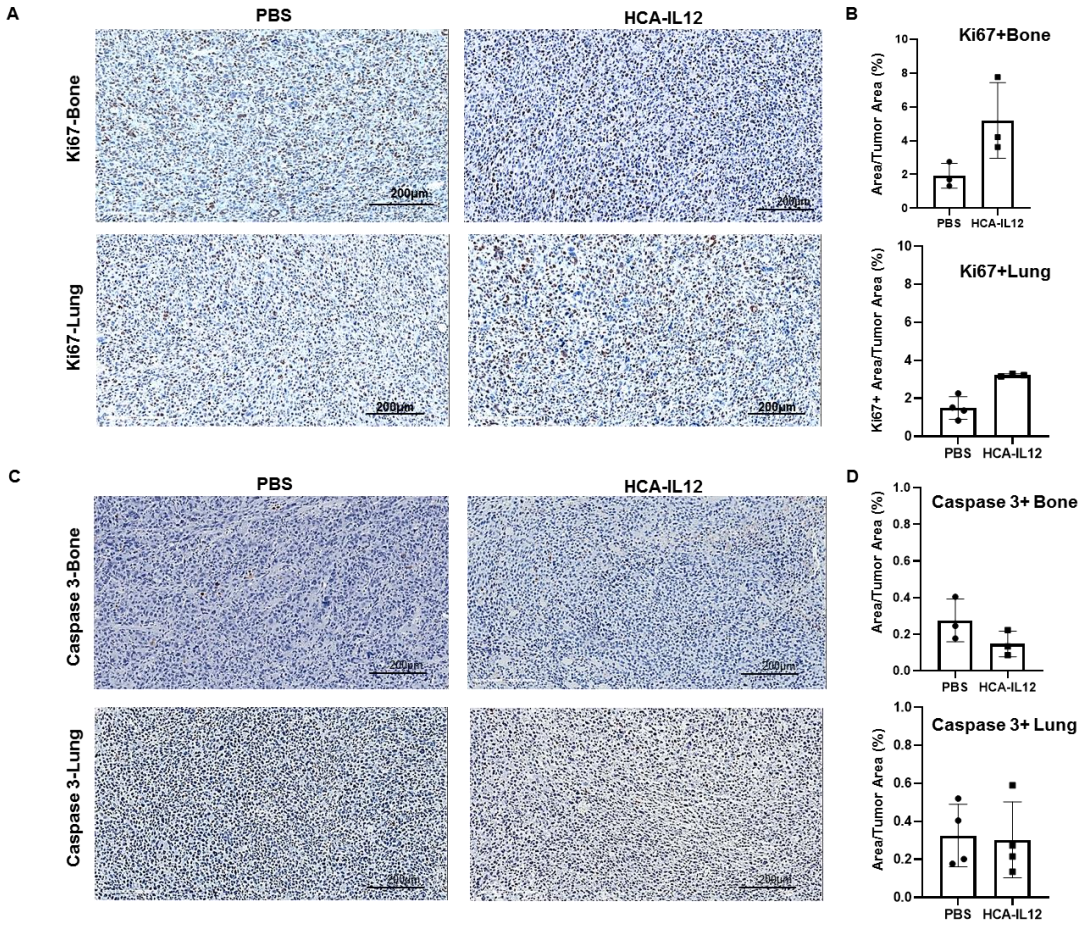
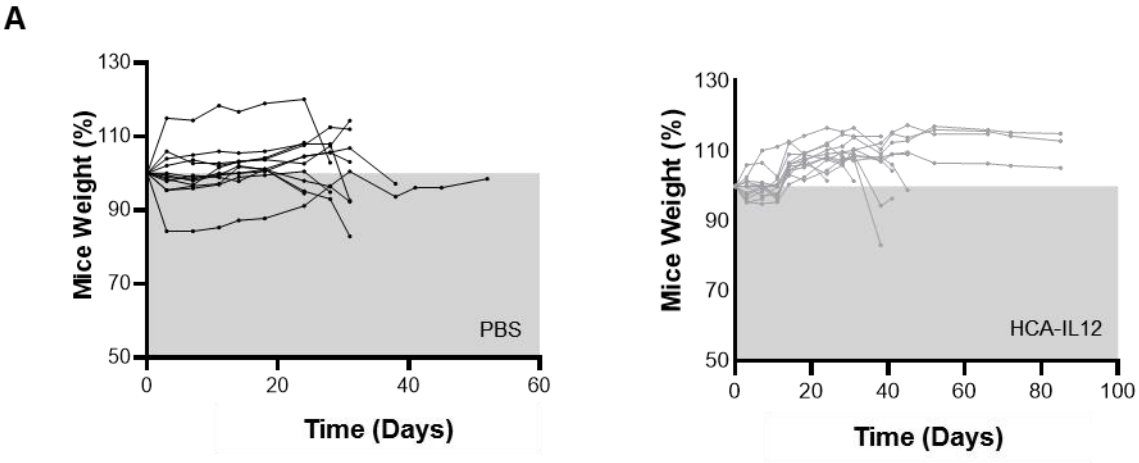
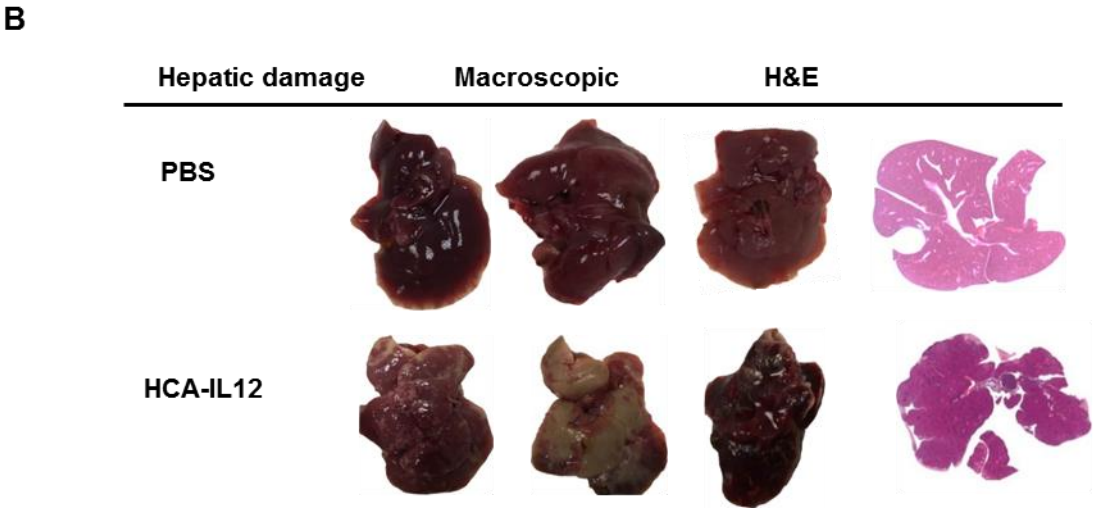
Supplementary Text



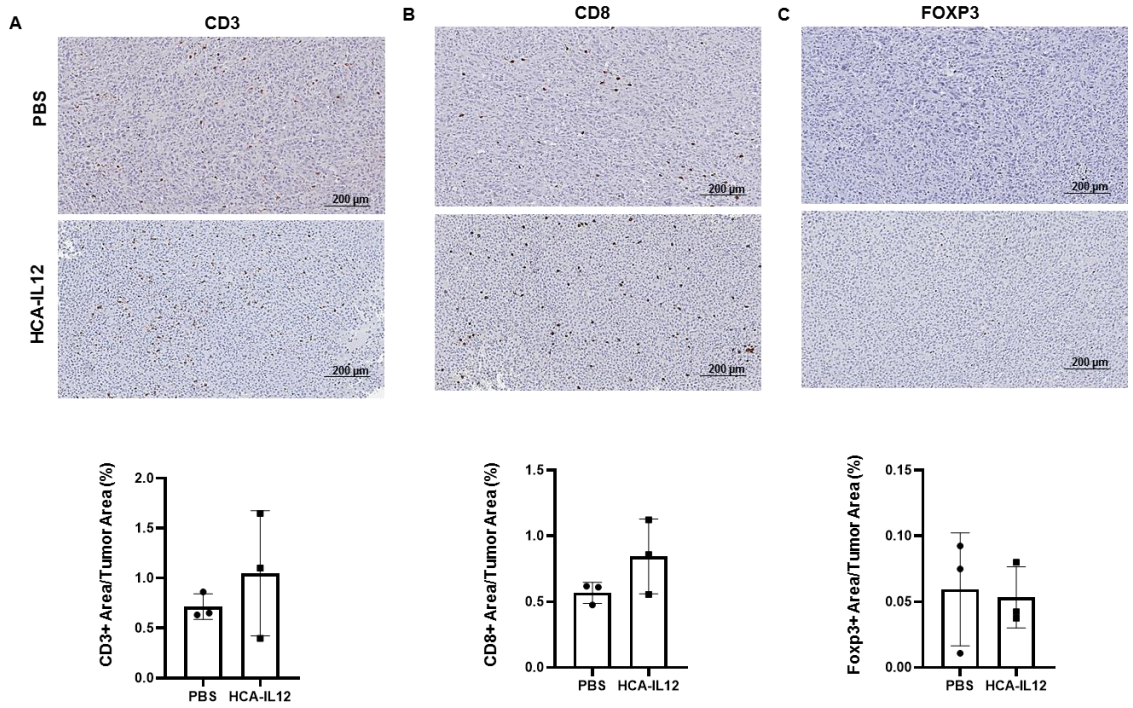
Supplementary Figure 1. (A) Representative images of Ki67 immunostaining of osteosarcoma bone tumors (upper panel) and lung metastasis (lower panel) from control (PBS) mice and HCA-EFZP-IL12-treated mice (scale bar, 200 µm). **(B)** Quantification of Ki67 staining in tibia and lung tumors. Graph showing the quantification of cell infiltration represented as the stained area/total tissue area ratio (%) of bone tumors in PBS-treated mice or HCA-EFZP-IL12-treated mice (N = 3-4 mice from each group). Mann Whitney test was used to calculate the significance. **(C)** Representative images of caspase 3 immunostaining of osteosarcoma bone tumors (upper panel) and lung metastasis (lower panel) from control (PBS) mice and HCA-EFZP-IL12-treated mice (scale bar, 200 µm). **(D)** Quantification of caspase 3 staining in tibia and lung tumors. Graph showing the quantification of cell infiltration represented as the stained area/total

tissue area ratio (%) of bone tumors in PBS-treated mice or HCA-EFZP-IL12-treated mice (N = 3-4 mice from each group). Mann Whitney test was used to calculate the significance.



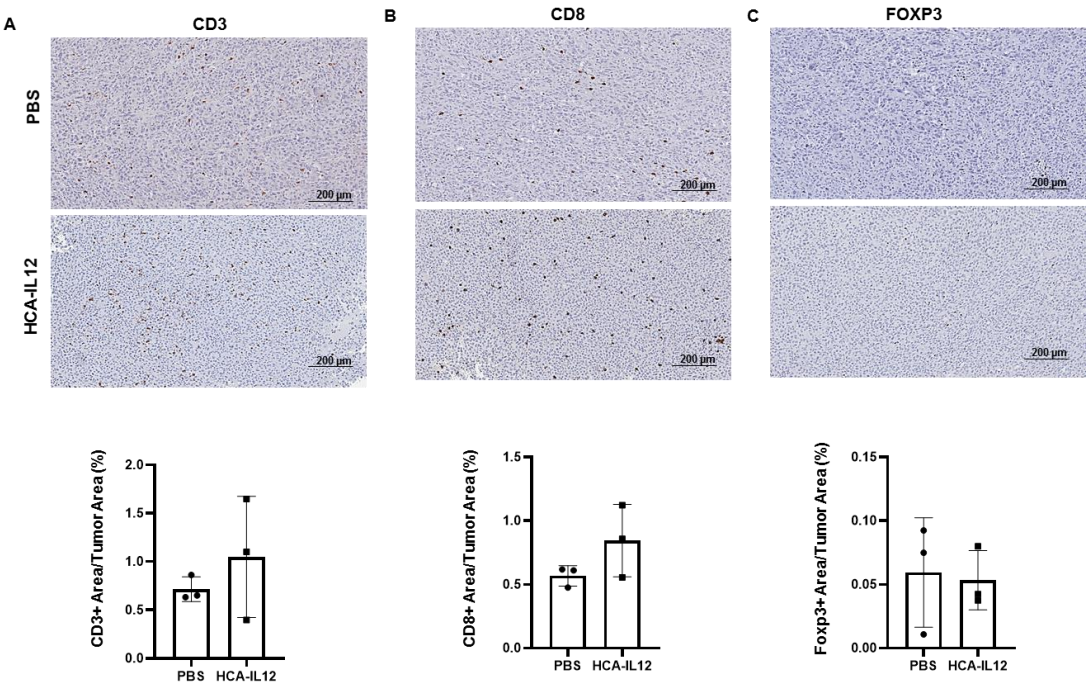


Supplementary Figure 2 (A) Evolution of mouse weights after Delta-24-RGD treatment. Each line represents a single mouse and comparison between PBS (black) and HCA-IL12 (grey) was performed by a matched two-way ANOVA (*P<0.001*). The grey area represents the values under the reference weight. **(B)** Representative macroscopic and histopathological images of livers from the control group (PBS) or treated animals (HCA-IL12).



Supplementary Figure 3. (A) Representative images of CD3 immunostaining of osteosarcoma bone tumors from control (PBS) mice and HCA-EFZP-IL12-treated mice (upper panel; scale bar, 200 µm). Quantification of CD3 staining in tibia (lower panel). Graph showing the quantification of cell infiltration represented as the stained area/total tissue area ratio (%) of bone tumors in PBS-treated mice or HCA-EFZP-IL12-treated mice (N = 3 mice from each group). Mann Whitney test was used to calculate the significance. **(B)** Representative images of CD8 immunostaining of osteosarcoma bone tumors from control (PBS) mice and HCA-EFZP-IL12-treated mice (upper panel; scale bar, 200 µm). Quantification of CD8 staining in tibia (lower panel). Graph showing the quantification of cell infiltration represented as the stained area/total tissue area ratio (%) of bone tumors in PBS-treated mice or HCA-EFZP-IL12-treated mice (N = 3 mice from each group). Mann Whitney test was used to calculate the significance. **(C)** Representative images of FOXP3 immunostaining of osteosarcoma bone tumors from

control (PBS) mice and HCA-EFZP-IL12-treated mice (upper panel; scale bar, 200 μm). Quantification of FOXP3 staining in tibia (lower panel). Graph showing the quantification of cell infiltration represented as the stained area/total tissue area ratio (%) of bone tumors in PBS-treated mice or HCA-EFZP-IL12-treated mice (N = 3 mice from each group). Mann Whitney test was used to calculate the significance.



Supplementary Figure 4. (A) Representative images of CD3 immunostaining of osteosarcoma lung metastases (PBS) mice and HCA-EFZP-IL12-treated mice (upper panel; scale bar, 200 μm). Quantification of CD3 staining in osteosarcoma lung metastases (lower panel). Graph showing the quantification of cell infiltration represented as the stained area/total tissue area ratio (%) of lung metastases in PBS-treated mice or HCA-EFZP-IL12-treated mice (N = 3 mice from each group). Mann Whitney test was used to calculate the significance. **(B)** Representative images of CD8 immunostaining of osteosarcoma lung metastases from control (PBS) mice and HCA-EFZP-IL12-treated mice (upper panel; scale bar, 200 μm). Quantification of CD8 staining in osteosarcoma lung metastases (lower panel). Graph showing the quantification of cell infiltration

represented as the stained area/total tissue area ratio (%) of osteosarcoma lung metastases in PBS-treated mice or HCA-EFZP-IL12-treated mice (N = 3 mice from each group). Mann Whitney test was used to calculate the significance. **(C)** Representative images of FOXP3 immunostaining of osteosarcoma lung metastases from control (PBS) mice and HCA-EFZP-IL12-treated mice (upper panel; scale bar, 200 μm). Quantification of FOXP3 staining in osteosarcoma lung metastases (lower panel). Graph showing the quantification of cell infiltration represented as the stained area/total tissue area ratio (%) of osteosarcoma lung metastases in PBS-treated mice or HCA-EFZP-IL12-treated mice (N = 3 mice from each group). Mann Whitney test was used to calculate the significance.