

NTR lentiviral packaging plasmids. *In vitro* analysis of the levels of expression of NTR in the MDA-MB-231 NTR<sup>+</sup> cell line and in the MDA-MB-231 after transduction and sorting with the lentiviral NTR particles. Similar levels of expression were observed in both cell lines.



(A) Example of HIF1 $\alpha$  IHC in NTR<sup>-</sup> and NTR<sup>+</sup> MDA-MB-231 xenografts. (B) No significant difference was observed in the normalised area positive for HIF1 $\alpha$  between NTR<sup>-</sup> and NTR<sup>+</sup> MDA-MB-231 xenografts (n = 3, per group).



(A) NTR<sup>+</sup>/NTR<sup>-</sup> ratio was notably lower for NCI-H460 than for MDA-MB-231 cell line (B) Representative <sup>18</sup>F-FMISO PET/CT MIP images of NCI-H460 xenografts. NTR<sup>-</sup> tumour (right flank) and NTR<sup>+</sup> tumour (left flank). (C) SUV<sub>max</sub> values were significantly higher in NTR<sup>+</sup> xenografts. (\*, p < 0.05, n = 4)

The p-values are represented as indicated: \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 and \*\*\*\* p < 0.0001.



 $SUV_{max}$ ,  $SUV_{p5}$  and  $SUV_{p10}$  analyses show similar results in the *in vivo* transduced tumours. The p-values are represented as indicated: \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 and \*\*\*\* p < 0.0001.



(A) The spatial pattern of HIF1 $\alpha$  accumulation was similar in both intratumorally injected and non-injected tumours MDA-MB-231 NTR<sup>-</sup> xenografts. (B) The normalised HIF1 $\alpha$  positive area was significantly lower in intratumorally injected xenografts (n = 4 per group).