**RG7386, a novel tetravalent FAP-DR5 antibody, effectively triggers FAP-dependent, avidity-driven DR5 hyperclustering and tumor cell apoptosis**

Brünker et al Supplementary Results 1

- Supplementary Figure 1 and associated figure legend

- Supplementary Tables 1 and 2



**Figure S1. Related to Figure 1. Characterization of FAP-drozitumab BsAbs and anti-tumor efficacy in the presence of FAP-expressing fibroblasts in *in vitro* co-culture models.** (A) Simultaneous binding of mAb007\_drozitumab to huDR5 followed by huFAP or muFAP recombinant proteins was assessed by surface plasmon resonance at the indicated antibody concentrations. (B) FAP cell-surface expression in GM05389 human fibroblasts and in NIH3T3 cells following stable transfection of muFAP was assessed by flow cytometry using fluorescently-labeled mAb082 anti-FAP antibody. (C-D) Analysis of DLD-1 tumor cell apoptosis in the presence of control-transfected (C) or stable muFAP-expressing NIH3T3 fibroblasts (D) in response to the FAP-DR5 BsAb, mAb082\_drozitumab, or drozitumab coupled to the non-targeting DP47GS IgG (N.D. = not determined).

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|  | **Binding to huDR5 Ki (nM)\*** | **Binding to huFAP Ki (nM)\*\*** |
| mAb082\_drozitumab VHCL | 2.1 | 0.32 |
| mAb007\_drozitumab VHCL | 1.58 | 0.53 |
| mAb007\_drozitumab VLCH1 | 1.43 | 5.96 |
| mAb007\_drozitumab 2+1  | 1.99 | 8.56 |

**Table S1. BsAb binding to DR5 and FAP antigens.** Binding avidities of FAP-drozitumab bispecific molecules to DR5 and FAP was analyzed in cells expressing human DR5 (huDR5) or huFAP extracellular domains using a TagLite competition assay. \* Drozitumab: 0.98 nM, \*\* anti-FAP mAb007: 0.61 nM and anti-FAP mAb082: 0.29 nM.

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|  | **Apoptosis induction EC50 values (nM)** |
|  | **MDA-MB-231 cells - fibroblasts** | **MDA-MB-231 cells + fibroblasts** |
| Drozitumab alone | 15.2 | 11.06 |
| Drozitumab + anti-Fc | 0.14 | 0.33 |
| mAb082\_drozitumab VHCL | 25.4 | 0.09 |
| mAb007\_drozitumab VHCL | 4.46 | 0.014 |
| mAb007\_drozitumab VLCH1 | 3.4 | N.D |
| mAb007\_drozitumab 2+1  | 11.17 | 0.57 |

**Table S2. Anti-tumor efficacy of FAP-drozitumab bispecific molecules is FAP-dependent.** EC50: half-maximal effective concentration; N.D.: value not determined