



**Figure 1 - figure supplement 1. GFP transfer requires direct GFP-αGFP interaction.**

**a.** GFP protein is transferred to receiver cells. Western blot analysis of FACS purified Control and αGFP receiver 293 cells cultured in isolation or co-culture with sGFP Tomato-positive sender cells (mouse KPT lung cancer cells) for 24 hours. sGFP but not Tomato is present in touched αGFP receiver cells. Human mitochondrial antigen (hu-Mito) is a marker for receiver cells. GAPDH shows loading. Rightmost lane is sGFP sender cells.

**b.** GFP cannot be transferred from fixed sGFP sender cells to live αGFP receiver cells. sGFP sender cells were fixed in 1% PFA for 5 minutes and washed with PBS before co-cultured with αGFP receiver cells at a 1:1 ratio for 24 hours. Receivers were defined as Tomato<sup>neg</sup>PI<sup>neg</sup>BFP<sup>pos</sup> cells.

**c.** GFP transfer to 293 receiver cells required sGFP-αGFP recognition. GFP is transferred from sGFP sender cells to αGFP-receiver cells but not from sGFP sender cells to amCherry-receiver cells. Control receiver cells do not express any nanobody. Sender and receiver cells were co-cultured at a 1:1 ratio for 24 hours. Receivers were defined as Tomato<sup>neg</sup>PI<sup>neg</sup>BFP<sup>pos</sup> cells.

**d.** GFP transfer to receiver cells is accompanied by a reduction of GFP on the sender cells. GFP expression on sender cells after 24 hour co-culture with control or αGFP-expressing 293 receiver cells at a 1:1 ratio. Co-culture with αGFP expressing but not control receiver cells reduced GFP on sGFP sender cells. Senders were defined as Tomato<sup>pos</sup>DAPI<sup>neg</sup>BFP<sup>neg</sup> cells.

**e.** GFP transfer is accompanied with αGFP internalization on receiver cells. Analysis of surface αGFP (Myc-tag) on 293 receiver cell co-cultured for 24 hours with sGFP sender cells. Receivers were defined as Tomato<sup>neg</sup>PI<sup>neg</sup> cells.

**f.** GFP transfer to 293 receiver cells is partially dependent on membrane dynamics of endocytosis. Both a clathrin inhibitor (Pitstop, 20 μM) and a dynamin inhibitor (Dyngo 4a, 10 μM) partially inhibit GFP transfer from sGFP sender cells to αGFP receivers. Inhibitors were present during the 24 hour co-culture. Receivers were defined as Tomato<sup>neg</sup>PI<sup>neg</sup> cells.

**g.** Analysis of GFP Mean Fluorescence Intensity (MFI) of αGFP receiver cells (marked by intracellular BFP) co-cultured with sGFP sender cells (marked by intracellular tdTomato) co-cultured for the indicated amount of time in the first 12 hrs. Sender and receiver cells were seeded at a 1:1 ratio. Receivers were defined as Tomato<sup>neg</sup>PI<sup>neg</sup>BFP<sup>pos</sup> cells.

**h.** GFP transfer to αGFP 293 receiver cells can be very rapid. A shift in GFP MFI was detected 5 minutes after mixing sGFP sender cells with αGFP receiver cell. Receivers were defined as Tomato<sup>neg</sup>PI<sup>neg</sup>BFP<sup>pos</sup> cells. MFI mean ± SD of triplicate cultures is shown.

**i.** Rapid GFP degradation in touched receiver cells after removal of the sGFP sender cells. After 6 hours co-culture, GFP positive receiver cells were purified by FACS followed by culture without sender cells. Receivers were defined as mCherry<sup>neg</sup>PI<sup>neg</sup>BFP<sup>pos</sup> cells. GFP MFI in receiver cells reduced rapidly ( $T_{1/2}$  approximately 2 hours). MFI mean ± SD of triplicate cultures is shown.