Primer design parameters
Design in CDS- preferably towards 3’ end if possible and to be exon spanning. Product Size: Min-100bp Opt-120bp Max- 200bp. Primer Size: Min-20bp Opt-22bp Max-25bp. Primer Tm: Min- 58°C Opt-60°C Max-65°C (so that Ta will be ca. 60°C). Max Self Complementary: 5,00. Max 3’Self Complementarity: 3,00. Max. Poly-X: 3. GC Clamp: 1. Use mfold (http://mfold.rna.albany.edu/?q=mfold/DNA-Folding-Form) to check for secondary structures. Folding Temperature: 60°C. Ionic Conditions of SYRB in mM: 50Na⁺, 3Mg²⁺. Structures: few loops, best if none, no loops in primer attachment region, ΔG ok until -4. Use Primer BLAST to check for specificity.

Figure 1: CAF metabolic activity and “CAF treatment”. Metabolic activity of CAF samples compared to HDFs. VE-Cadherin protein expressed into media after “CAF treatment”. ACTA2 gene levels in tumouroids after “CAF treatment” for HT29 and HCT116 tumouroids.
Figure 2: Full western blots for alpha smooth muscle actin and beta tubulin for HDF and CAF samples.
Figure 3: Additional genes tested on CAF samples from primer Table 1