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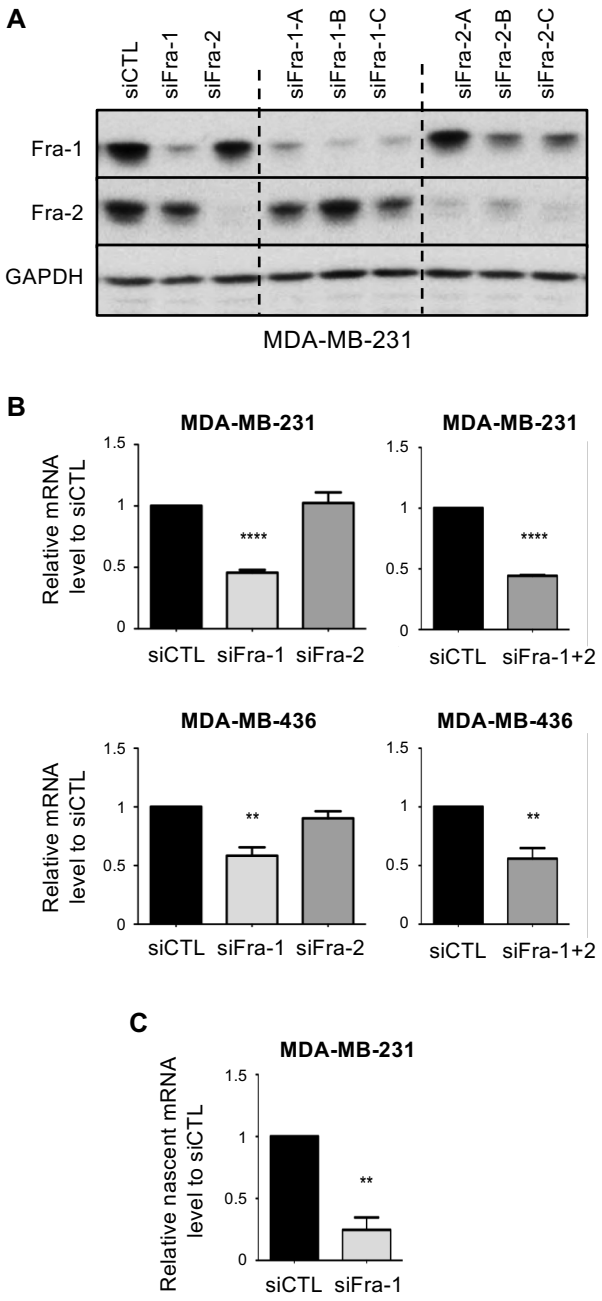

Supplementary Figure S2: Fra-1 regulates HMGA1 expression. (A) *Optimization of RNAi conditions for elimination of Fra-1 and Fra-2 while minimizing undesirable off-target effects of individual siRNAs*. Individual siRNAs targeting mRNA sequences strictly specific for either Fra-1 (siFra-1-A to -C) or Fra-2 (siFra-2-A to -C) mRNAs were designed. When transfected individually at a concentration of 4.5 nM in MDA-MB-231 cells, they led to efficient decreases of Fra-1 and Fra-2 proteins, respectively. However, for unelucidated reasons, certain of them (siFra-1-A and -C and siFra-2-B and C) affected to some extent the abundance of the other Fra protein (middle and right panels). To overcome these undesirable side-effects, we turned to pooling the 3 siRNAs directed to either Fra-1 or Fra-2 to form siFra-1 and siFra-2 pools, respectively, and transfected them under conditions where each siRNA was present at a lower concentration (1.5 nM per siRNA, i.e. a total of 4.5 nM siRNA per pool) as recommended in reference 51. This limited alterations in levels of each Fra protein by siRNAs directed against the other one (left panel). (B) *HMGA1 mRNA steady-state level in MDA-MB-231- and MDA-MB-436 cells 72 h after transfection of control siRNA (siCTL) or of siRNAs against Fra-1 or/and against Fra-2*. The experiments are the same as those presented in Figure 2F and 2G, except that another set of primers was used to amplify HMGA1 cDNA by RT-qPCR. The primer sequences are described in Supplementary Table S1B. (C) *Nascent HMGA1 mRNA quantified by RT-qPCR. Same experiment as that presented in Figure 2H except that another set of primers allowing amplification of an amplicon located +3.23 kb from the TSS was used. Primer sequences are given in Supplementary table S1C*. Errors bars indicate SEM. The results of the Students 's unpaired *t*-test are indicated on the graphs. Sequences of the different siRNA are presented in Supplementary Table S1D. *P-value ≤0.05, **P≤0.01, ***P≤0.005, ****P≤0.0001